From the Max-Delbrück-Center for Molecular Medicine Berlin

Head of the research group: Prof. Dr. M. Bader

"Individual functions and substrate specificities of importin α subtypes"

Dissertation

for Fulfillment of

Requirements

for the Doctoral Degree

of the University of Lübeck

from the Department of Natural Sciences

Submitted by

Stefanie Hügel

from Hennigsdorf

Lübeck 2013

First referee: Prof. Dr. M. Bader

Second referee: Prof. Dr. N. Tautz

Date of oral examination: 21.02.13

Approved for printing. Lübeck, 22.02.2013

1 TABLE OF CONTENTS

2 ABSTRACT

The importin α:β complex is responsible for the nuclear import of proteins bearing classical nuclear localization signals. Several importin α subtypes are known to exist in mammals and although each subtype shares a high degree of sequence identity, individual importin α subtypes do not appear functionally redundant. Importin α7 has been shown to play a crucial role in early embryonic development in mice. Embryos from importin α7 depleted females stop at the two-cell stage and show disturbed zygotic genome activation. Since there is evidence that individual importin α subtypes possess cargo specificities, it has been hypothesized that importin α7 binds a unique set of intracellular proteins. This study therefore set out to screen for importin α7 candidate cargoes to shed light on the molecular mechanisms underlying the importin α7 KO mice phenotype. By using a collection of *in vitro* and *in vivo* binding assays not only an interesting set of importin α7 binding partners has been identified but also it could be shown that importin α cargo sets differ among individual subtypes. Hence, individual members of the importin α protein family can be seen as molecular hubs for different cellular pathways.

Furthermore, murine embryonic fibroblasts (MEFs) have been generated from the different importin α knockout mice available in the lab of Prof. M. Bader (Max-Delbrück Center Berlin) in order to check the intracellular localization of known importin α cargoes. Thereby, some postulated cargo specificities for a certain importin α could be disproved in MEFs, like the importin α7 dependent nuclear import of Keap1 or the importin α5 mediated translocation of STAT1 upon IFN-γ stimulation. On the other hand, it could be confirmed that the RCC1 nuclear import is preferentially mediated by importin α3.

This study also provides valuable insights into the *in vivo* role of importin α3, an importin α subtype that has been largely uncharacterized. Importin α3 appears to play a key role in fertility and embryonic development in mice. Moreover, mice that lack importin α3 exhibit right ventricular hypertrophy possibly caused by a disturbed pulmonary function. Furthermore, there is first evidence of importin α3 playing a role in the kidney, impacting the glomerular shape. However, the underlying mechanisms by which this importin α subtype influences reproduction, pulmonary as well as kidney physiology are yet to be determined.

Taken together, this work provides valuable insights into the physiological roles and cargo specificities of the importin α subtypes 3 and 7.

3 ZUSAMMENFASSUNG

Der Kernimport von Proteinen mit klassischen nukleären Lokalisationssignalen wird von Importin α und Importin β durchgeführt. In Säugern existiert eine Vielzahl an Importin α Subtypen, von denen vermutet wird, dass sie individuelle Funktionen erfüllen. Tatsächlich konnte im Mausmodell gezeigt werden, dass Importin α7 eine essentielle Rolle in der Entwicklung von Embryonen spielt. So stoppen Embryonen von Importin α7 Knockout Mäusen im Zwei-Zell-Stadium und weisen eine gestörte zygotische Genomaktivierung auf. Da es Hinweise auf Substratspezifitäten innerhalb der Importin α Familie gibt, wurde angenommen, dass Importin α7 ein einzigartiges Set von intrazellulären Proteinen transportiert. Ziel dieser Arbeit war es daher, nach Importin α7 Substraten zu suchen, welche zur Aufklärung der dem Phänotyp zugrunde liegenden molekularen Mechanismen beitragen sollen. Mit Hilfe eines kombinierten Ansatzes aus *in vitro* und *in vivo* Bindungsexperimenten konnten nicht nur interessante Importin α7 Interaktionspartner identifiziert werden, sondern es wurde ebenfalls deutlich, dass sich die Cargo Sets von individuellen Importin α Subtypen unterscheiden. Diese Ergebnisse deuten somit darauf hin, dass die einzelnen Mitglieder der Importin α Familie als molekulare Netzknoten für unterschiedliche zelluläre Signalwege angesehen werden können.

Ein weiteres Ziel dieser Studie war es neben den neu identifizierten Importin α7 Bindungspartnern auch bereits publizierte Importin α Substratspezifitäten in einem Zellkulturmodell *in vivo* zu überprüfen. Hierfür wurden murine embryonale Fibroblasten (MEFs) präpariert, welche von Importin α Knockout Mäusen stammten, die im Labor von Prof. M. Bader (Max-Delbrück Center Berlin) generiert wurden. Während einige postulierte Importin α Substratspezifitäten nicht verifiziert werden konnten - wie der Importin α7–vermittelte Transport von Keap1 oder der Importin α5–abhängige Kernimport von STAT1 nach IFN-γ Stimulation - scheint sich die Hypothese über die Importin α3–abhängige Translokation von RCC1 in den Kern bestätigt zu haben.

Darüber hinaus ermöglicht die hier beschriebene Arbeit erste Einblicke in die physiologische Bedeutung von Importin α3, über welches bislang noch wenig bekannt ist. Im Mausmodell konnte gezeigt werden, dass Importin α3 eine wichtige Rolle in der Embryonalentwicklung und Fertilität spielt. Des Weiteren führte der Verlust von Importin α3 in der Maus zu einer rechtsventrikulären Hypertrophie, welche vermutlich durch eine gestörte Lungenfunktion verursacht wurde. Ferner liefern die hier generierten Daten erste Hinweise auf eine Importin α3 vermittelten biologischen Prozess in der Niere, welcher die Form von Glomeruli beeinträchtigt. Die zugrunde liegenden molekularen Mechanismen, durch welche Importin α3 Einfluss auf die Reproduktion sowie Lungen- und Nierenphysiologie nimmt, müssen erst noch aufgeklärt werden.

Zusammenfassend ermöglicht die vorliegende Arbeit wertvolle Einblicke in die physiologischen Rollen sowie Substratspezifitäten der Importin α Proteine 3 und 7.

4 INTRODUCTION

4.1 Nucleocytoplasmic protein transport

Only in eukaryotic cells, the genetic information is preserved in its own cellular compartment. Here, it is protected from external influences, which contribute to genomic stability enabling eukaryotes to handle more genetic information than prokaryotes. The nucleus is the site of DNA replication, mRNA transcription and DNA repair. Protein synthesis takes place in the cytoplasm, requiring mRNA, ribosomal subunits and tRNA, all of which must be exported from the nucleus. On the other hand, the nucleus needs to import all nuclear proteins synthesized in the cytoplasm like RNA and DNA polymerases, transcription factors or histones (**Figure 1**). The spatial separation of biological processes leads to an accumulation of specific factors making catalytic processes more effective. Moreover, it enables the cell to handle different biological actions in a certain chronological order. So, mRNA translation takes place in the cytoplasm not before primary transcripts have been spliced in the nucleus and mRNA is fully maturated. Furthermore, the physical separation of cytoplasm and nucleoplasm is used to regulate and control gene expression.

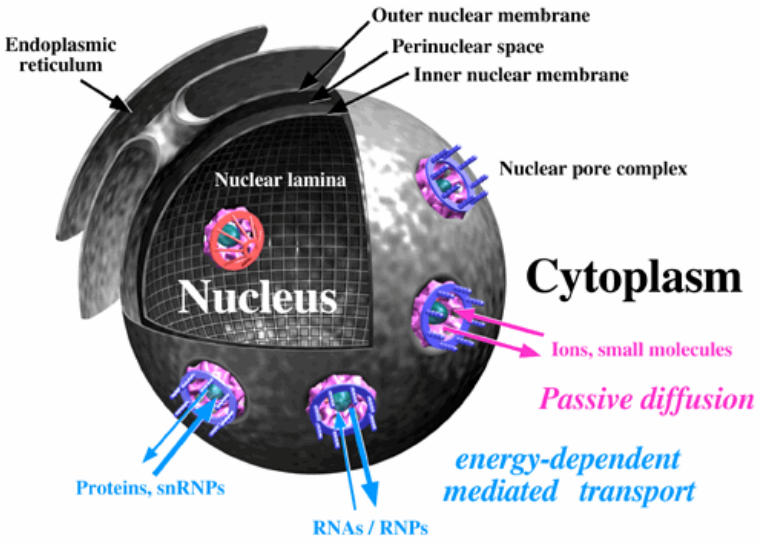


Figure 1: Schematic overview of nucleocytoplasmic transport of different molecules. Taken from [1].

4.1.1 The nuclear pore complex – gateway to the nucleus

Nucleocytoplasmic transport across the nuclear membrane takes place via embedded nuclear pores. The vertebrate pores are roughly 125 MDa large complexes and composed of 30-50 different nucleoporines. The nuclear pore complex (NPC) exhibit an eightfold rotational symmetry thus each nucleoporin exists at least in eight copies per pore. All in all, one nuclear pore contains approximately 1000 polypeptides. The composition of NPCs is asymmetric to the membrane surface. The main structures of NPCs are cytoplasmic filaments, the central core, and the nuclear basket (**Figure 2**). Cytoplasmic fibrils branch from the cytoplasmic ring and are about 50 nm long. The central core is also called "central gated channel" (CGC) and consists of eight spokes sandwiched between the nuclear and cytoplasmic rings. From the nuclear ring, nuclear fibrils are branching that are 100 nm long. These fibrils merge at distal ends and form a basket-like structure. The number of pores per nucleus differs according to its size. In yeast, approximately 200 pores are embedded in the nuclear membrane, whereas in human cell nuclei ~3000 nuclear pores are found and in *Xenopus* oocytes even ~50,000,000 nuclear pores are present in the nuclear membrane.

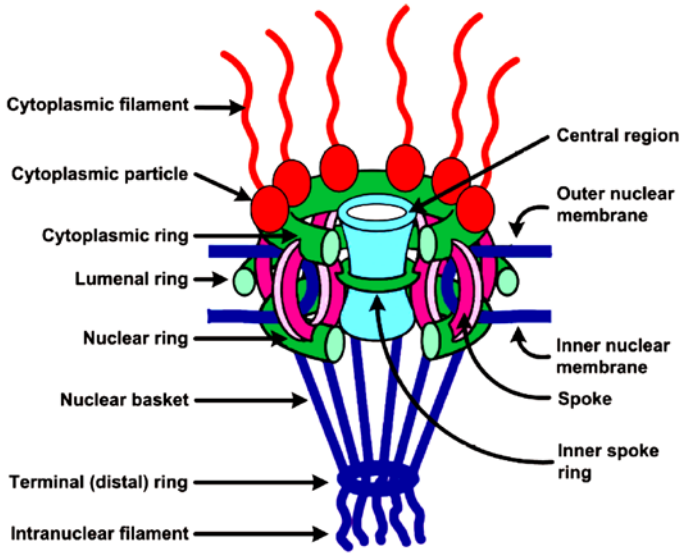


Figure 2: Schematic representation of the nuclear pore complex. Taken from [2].

In contrast to transport systems of other organelles, the NPC allows passive diffusion of small molecules such as ions and metabolites. The molecular size limit for this passive influx is, however, not consistently described. It is supposed that everything bigger than 40 kDa needs the help of transport factors to enter or leave the nucleus (for reviews see [2, 3]). Exceptions of this rule are proteins that directly interact with nucleoporins [4, 5] or proteins of the inner nuclear membrane that have a cytoplasmic domain smaller than 40 kDa and translocate through the NPC independently of energy and transport factors.

4.1.1 Transport factors

Nucleocytoplasmic transport is mediated by specialized transport factors. The largest group of nuclear transport factors is the superfamily of importin β-related proteins, also known as karyopherins. According to the direction of transport, they are further called importins or exportins. Only very few transport factors carry proteins in both directions, like exportin 5 (kpn142p), which imports the Replication protein A (RPA) complex into the nucleus and exports for example Pho4p. Some karyopherins transport only a certain set of proteins [6]. Karyopherin β3 (kpnb3) for example, imports ribosomal proteins; exportin-t (kpnb 127p) exports tRNAs. Yet, many transport factors have overlapping substrates.

A second group of transport receptors are closely related to the nuclear transport factor 2 (NTF2). NTF2 mediates the transport of RanGDP into the nucleus. All these transport receptors have in common that they recognize their substrates by a certain nuclear transport signal. Most signals share rather general features such as length, charge, or hydrophobicity and spacing of key residues than a defined consensus (for reviews see [2, 3]). In addition to these two large groups of transport factors, further carrier proteins may exist. The protein Hikeshi, for example is evolutionary conserved and has just recently been discovered to mediate a different mechanism of nuclear import than importin β and NTF2 [7].

4.1.2 Nothing happens without RanGTP

All active transport mechanisms need metabolic energy. The nucleocytoplasmic transport, however, is rather described as a process of facilitated diffusion. Nevertheless, nuclear import and export of proteins mediated by importins and exportins requires GTP but not in the conventional way. The coupling of facilitated transport to a RanGTP gradient determines the directionality and allows accumulation of cargo against its own chemical gradient. Ran is a preferably nuclear GTPase that, like most GTPases, hydrolyzes GTP very slowly. Thus, its activity and nucleotide binding are regulated by interactions of different regulatory proteins. The Ran GTPase activating protein 1 (RanGAP1) is found only in the cytoplasm or at the cytoplasmic fibrils of the NPC. It amplifies Ran's activity about 10,000 fold. Ran binding protein 1 (RanBP1) in turn, activates RanGAP. Together, the GTP hydrolysis function of Ran is amplified about 100,000 fold by these regulators. In addition to RanBP1, RanBP2, which is a major component of the NPC in higher eukaryotes, can aid in the conversion of RanGTP to RanGDP. In the nucleus, Ran guanine nucleotide exchange factor (RanGEF) is found that triggers the exchange of Ran bound GDP to GTP. RanGEF, in turn is activated by the Ran binding protein 3 (RanBP3). Since RanGAP1 is localized to the cytoplasm and RanGEF to the nucleus, RanGTP concentration in the nucleus is high, whereas RanGDP is high in the cytoplasm. This leads to the establishment of a RanGTP gradient that ensures the directionality of nucleocytoplasmic transport (**Figure 3**, for reviews see [2, 3]).

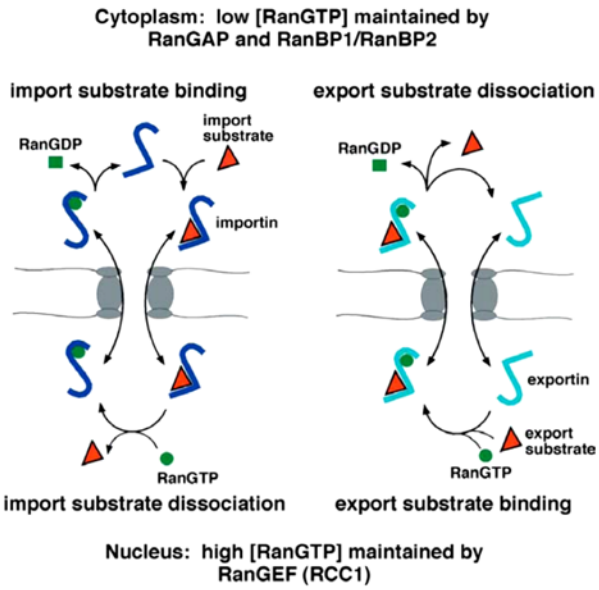


Figure 3: The RanGTP gradient ensures directionality of nucleocytoplasmic transport. Importins (dark blue) bind a substrate (triangle) in the cytoplasm where the RanGTP concentration is low. In the nucleus, RanGEF leads to a high RanGTP concentration. RanGTP binding to importins leads to release of the cargo. Exportins (light blue) bind substrate and RanGTP in the nucleus. After export to the cytoplasm, the export complex is disassembled by RanGAP assisted by RanBP1 and/or RanBP2 [3].

4.2 The classical nuclear protein import pathway

The best studied nuclear import mechanism is the classical nuclear protein import pathway mediated by karyopherin β1 (kpnb1) and karyopherin α (kpna), also known as importin β and importin α. Despite the fact that many cargoes can bind directly to an importin β-like transport receptor, in this classical pathway, cargoes bind directly to importin α, which serves as an adaptor protein to tether the cargo to importin β.

4.2.1 Structure and function of importin α

Importin α recognizes the classical NLS (cNLS), which consist of either one (monopartite) or two (bipartite) stretches of basic amino acids [8]. Importin αs are composed of a flexible N-terminal importin β binding (IBB) domain, ten armadillo (ARM) motifs, and an acidic C-terminal domain (**Figure 4**). The cellular apoptosis susceptibility (CAS) protein that exports importin α from the nucleus, binds to the tenth ARM repeat of the adapter protein [9]. Monopartite cNLS like the one from SV40 large T antigen ($_{126}$PKKKRRV$_{132}$) are bound in the major pocket formed by the

curved ARM repeats domain next to the N-terminus (**Figure 5**). Here, also the larger stretch of classical bipartite NLS is bound. The smaller stretch of cNLS exemplified by the nucleoplasmin NLS ($_{155}$KRPAATKKAGQAKKKK$_{170}$) binds to the minor groove of the ARM repeats domain more C-terminal. Although many NLSs that interact with importin α are part of the primary amino acid sequence in a given cargo, some non-classical NLSs are only functional when presented in a defined 3-dimensional structure. Phosphorylated STAT1, for example, is imported by importin α only in its homodimerized form. Here, importin α binds between two STAT1 monomers which form a non-classical NLS [10].

The IBB domain of importin α does not only serve as the binding site for importin β. Moreover, it has an auto-inhibitory function covering the NLS binding site of importin α in the absence of importin β. Hereby, cargo binding is not blocked, however, the binding affinity of importin α to a cargo is lowered significantly [9].

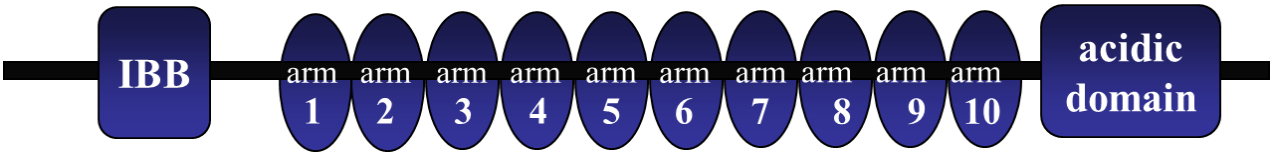


Figure 4: Schematic domain structure of importin α. Importin α consists of an N-terminal importin β binding domain (IBB), 10 armadillo repeats (arm) and a C-terminal acidic domain.

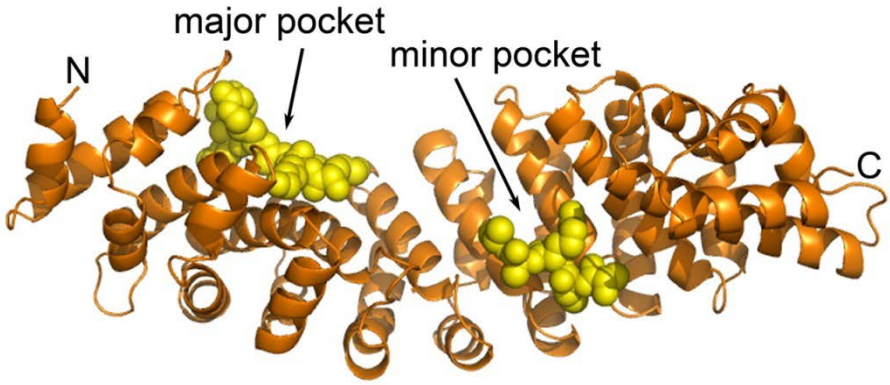


Figure 5: Importin α: cNLS interaction. cNLS peptides bound to the major and minor binding pockets of yeast importin α lacking the IBB domain (Protein Data Bank entry 1BK6), adapted from [8].

4.2.2 Importin α:β mediated nuclear protein import

Importin α:β mediated nuclear import of proteins is, like most nucleocytoplasmic transport mechanisms, a Ran dependent process. In the cytoplasm, where RanGTP is low, importin α binds its substrate by the NLS and links it to importin β. The trimeric complex moves the initial distance from the cytoplasmic fibrils to the surface of the central channel by bending of the fibrils. Then, the complex crosses the central channel by the interaction of importin β with phenylalanine-glycine (FG) repeats of nucleoporins [11]. The RanGTP gradient across the NPC determines the directionality of the transport. The guanine nucleotide exchange factor (RanGEF) RCC1 converts RanGDP to RanGTP providing the high RanGTP concentration in the nucleus. Leaving the NPC, the transport complex comes in contact with RanGTP. The binding of RanGTP causes a conformational change in the importin β molecule leading to the dissociation of the importin α:β complex. Thereby, the IBB domain of importin α is exposed that lowers the affinity of importin α to its cargo by auto-inhibition. Additionally, nucleoporins like the mobile nuclear basket nucleoporin Npap60 (also known as Nup50) can affect the release of cargo proteins from importin α [12-14]. The long Npap60 isoform (60L) competes with the cargo for the NLS-binding pocket of importin α, promoting the release of the NLS-cargo [15]. Finally, importin α is exported via its export receptor CAS in conjunction with RanGTP whereas importin β is recycled from the nucleus in a Ran-independent manner [16]. In the cytoplasm, RanGAP together with RanBP1 and/or RanBP2 facilitates rapid dissociation of export complexes (**Figure 6**, for reviews see [2, 3, 17]).

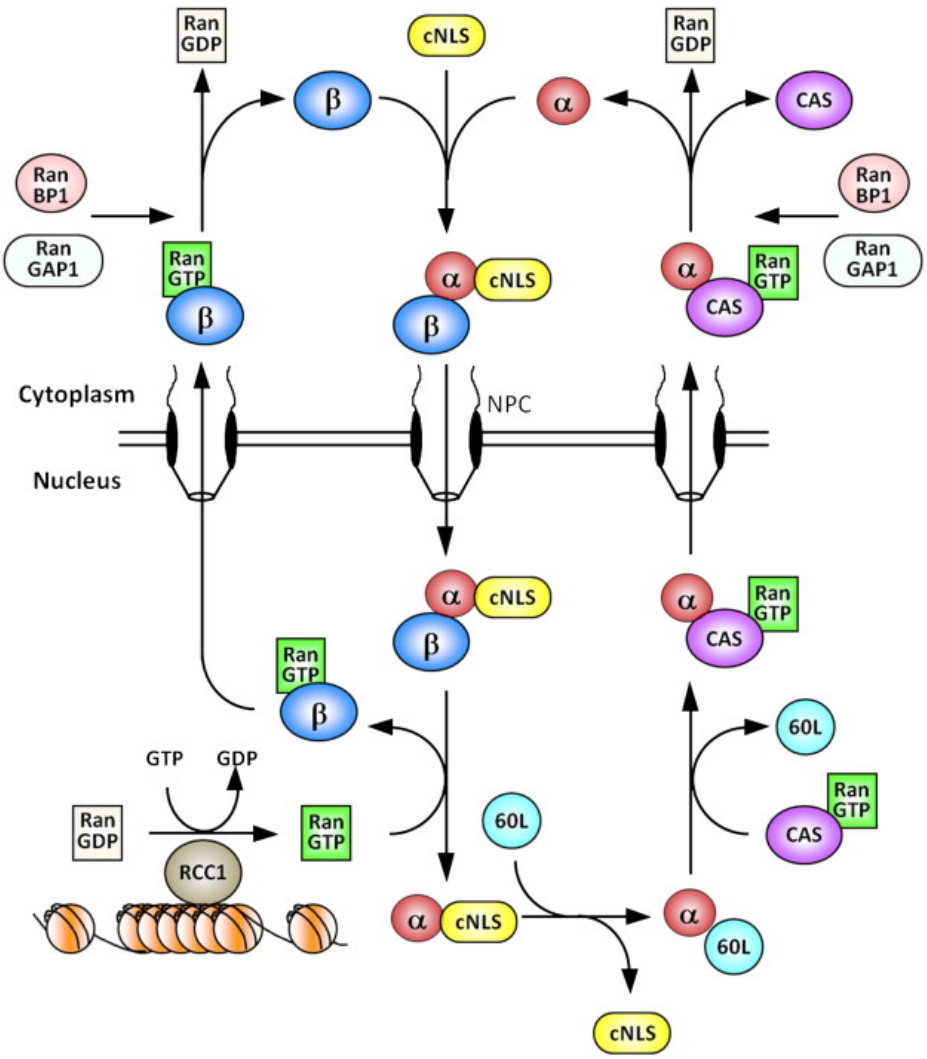


Figure 6: Schematic representation of importin α:β mediated nuclear transport. α: importin α, β: importin β, cNLS: cargo with classical NLS, NPC: nuclear pore complex, 60L: Npap60; example of a nucleoporin that can facilitate the cargo release [18]. For description see text above.

4.2.3 The importin α protein family

During evolution, the family of importin α adapter proteins expanded a lot. In yeast, there appears to be only one importin α and one importin β protein present, together these two proteins serve to import all proteins that carry classical NLSs. However in humans, seven different importin α paralogs have been identified [19-27]. According to sequence homologies, α importins can be divided into three subfamilies (**Figure 7**). Isoforms of one subfamily share 50 – 80 % sequence homology and mainly differ in the regions outside of their NLS-binding pockets

(**Table 1**) [20, 21, 27]. While most importin α proteins are ubiquitously expressed in different organs, they differ in expression levels and some are even restricted to specific tissues [20, 27] and particular time points of development [28], respectively.

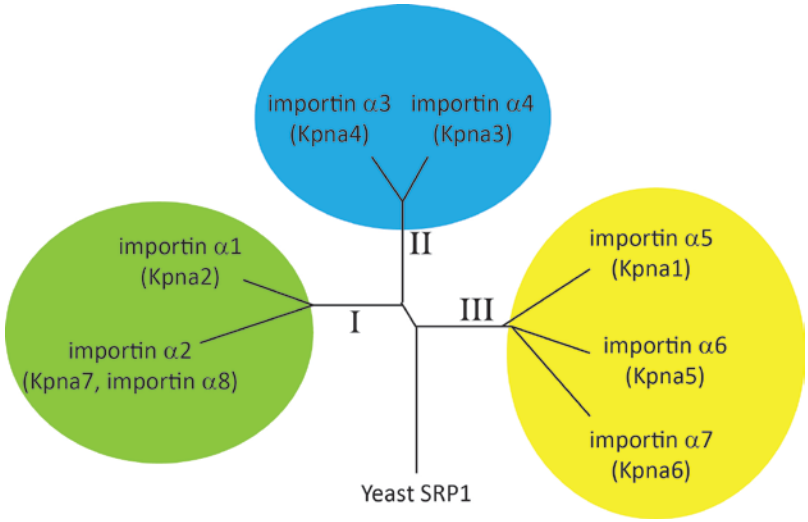


Figure 7: Schematic view of importin α subfamilies. The three subfamilies are based on sequence homologies of human importin α genes. The first subfamily (I) is shaded in green, the second (II) in blue and the third (III) in yellow. The sole *S. cerevisiae* importin α, SRP1 (Yeast Kpna) is included as a common ancestor. Adapted from [26].

Table 1: Importin α family identity matrix. The number shown is percentage identity between the respective importin α (impa) proteins. Adapted from [26].

	impa1	**impa2**	**impa3**	**impa4**	**impa5**	**impa6**	**impa7**
impa1	**100**	54.7	50.9	49.5	45.7	48.1	47.3
impa2		**100**	43.7	43.7	42.6	41.4	42.5
impa3			**100**	85.5	47.5	48.7	48.2
impa4				**100**	47.9	48.4	47.8
impa5					**100**	80.7	82.1
impa6						**100**	84.5
impa7							**100**

Invertebrates have at least three variants of importin α, one of each subfamily. The specific physiological role of invertebrate importin α paralogs has been studied, and distinct functions of single importin α genes in gametogenesis and early development have been revealed. RNAi knockdown of the *C. elegans* importin α IMA-3 results in disturbed oogenesis with incomplete

meiosis and disruption of the nuclear pore complex [29]. Over-expression of the remaining two α importins could not rescue this phenotype. Similar impact on *D. melanogaster* gametogenesis was reported from the depletion of the drosophila importin α2 [30]. Functional characterizations of α importins in vertebrate development, however, are scarce.

4.2.4 Alternative roles of importin α proteins

Despite its known role in the classical nuclear protein import, importin α has been reported to be involved in diverse alternative processes in the cell. Different studies showed that importin α is required for correct assembly of the nuclear envelope as well as for spindle formation [9, 31-33]. During mitosis, when the nuclear envelope breaks down, importin α binds spindle assembly factors (SAF) such as TPX2, NuMA and the kinesin XCTK2. Complexed with importin α:β, SAFs are kept inactive. The formation of RanGTP by chromosome-bound RCC1 releases SAFs from that complex, allowing them to promote spindle formation (**Figure 8**). After mitosis, the absence or excess of importin α inhibits nuclear envelope assembly and nucleoporin embedding (**Figure 9**).

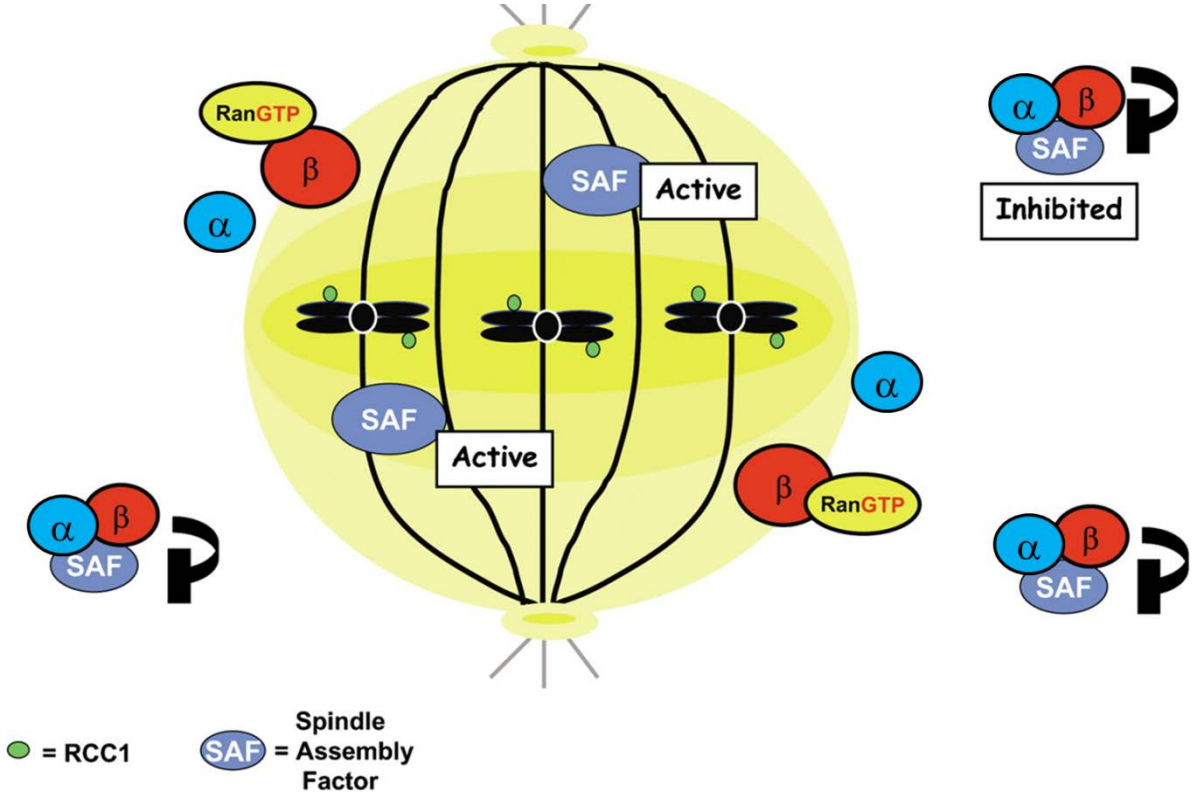


Figure 8: Spindle assembly requires importin α and β. RanGTP is produced only in the region of the mitotic chromosomes (yellow circle and yellow zone around chromosomes), because the RanGEF RCC1 (small circle on chromosomes) remains chromosome-bound at mitosis. Adapted from [34]. For description see text.

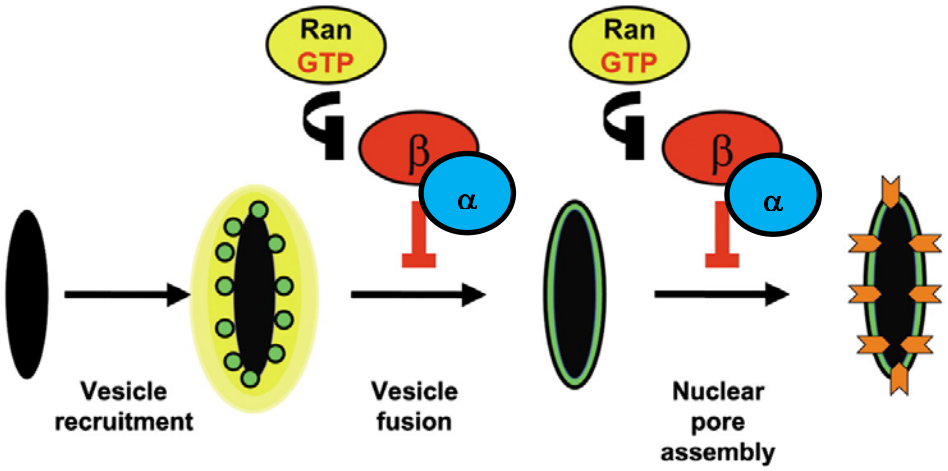


Figure 9: Nuclear membrane assembly and nuclear pore embedding is regulated by importin α and β. Adapted from [34]. Chromatin (black oval) recruits membrane vesicles (green circles). These vesicles fuse to form double nuclear membranes. Not till then, nuclear pore complexes (orange chevrons) can assemble and are embedded into the membrane.

Furthermore, importin α has been reported to act as a cytoplasmic chaperone for highly basic molecules [35]. Ribosomal proteins, histones and many other nuclear proteins are extremely basic. Upon translation, these factors are immediately in danger of aggregation with polyanions in the cytoplasm. Importin α and β can bind such proteins and shield their basic domains until they are imported into the nucleus where they are released to fulfill their tasks.

Besides that, importin α:β mediate retrograde injury signaling, as well [36]. In injured nerves, axoplasmic proteins harboring a NLS are transported by importin α and newly synthesized importin β via microtubules and the motor protein dynein from the side of injury over a long distance to the cell body (For reviews see [9, 34]).

Current studies even indicate importin α acting as a transcription factor during stress response. Upon stress, importin α localizes to RNA stress granules (SG) that form large bodies in the cytoplasm [37]. Importin α not only regulates the dynamics of SG assembly but also promotes cell survival during stress response. It was found that nuclear-retained importin α binds with DNase I-sensitive nuclear component(s) and exhibits selective upregulation of mRNA encoding Serine/threonine kinase 35 (STK35) [38].

4.2.5 Importin α7 is essential for zygotic genome activation and early mouse development

Disruption of the importin α7 (Kpna6) gene in mice leads to female infertility due to a very early embryonic developmental stop [39]. Embryos from importin α7 knockout females display a reduced ability for the first cleavage and arrest completely at the two-cell stage. The analysis of pre-implantation embryos from females lacking importin α7 did not reveal problems with pronuclear membrane formation or DNA replication. But the developmental block of importin α7-deficient embryos coincides with the onset of zygotic genome activation in mice (ZGA). The analysis of ZGA marker genes revealed that embryos from importin α7 knockout females cannot activate the embryonic genome. However, the detailed molecular mechanism underlying this phenotype is still unclear.

4.2.6 Importin α3

The specific physiological role of importin α3 (Kpna4) in mammals has not been analyzed. Importin α3 belongs to the second subfamily of importin α proteins and shares most sequence identity with importin α4 (Kpna3). Whereas importin α4 is one of the maternally expressed importin α proteins in mice, importin α3 is only detected from the two-cell stage on [39]. The human importin α3 gene locates to chromosome 3 and consists of 17 exons that result in an approximately 58 kDa big protein. Importin α3 seems to be ubiquitously expressed in human tissues but only at very low levels in spleen and kidney [20].

4.2.7 Substrate specificities of α importins

Many hints from different studies lead one to assume that importin α paralogs transport individual cargo sets *in vivo*. In fact, there seems to be a high redundancy in cargo transport capacity of importin α proteins [21, 40-44] however, several importin α interaction studies indicated different import efficiencies among importin α paralogs [21, 42, 45, 46].

4.2.7.1 Importin α5 and STAT1

One of the best characterized importin α interactions are the binding studies between importin α paralogs and members of the signal transducer and activator of transcription (STAT) protein family. Interestingly, *in vitro* binding assays and *in vivo* co-immunoprecipitation of STATs interacting with importin α paralogs led to partially different results implicating the existence of factors in living cells that influence the specificity of importin α interactions (**Table 2**).

STAT proteins are transcription factors that regulate the development, differentiation, proliferation and survival of cells. They are controlled by different signals like cytokines, growth factors and hormones. Upon binding of these signaling molecules to specific trans-membrane receptors the Janus kinases (JAK) get active which in turn phosphorylate and by that activate the STAT proteins. The family of STAT proteins consists of seven members that form homo- and heterodimers and translocate to the nucleus within minutes upon activation. STAT1, for example, responds to type I (α, β) and type II (γ) interferons (IFN) and is also activated by the interleukin-6 (IL-6) family of cytokines. STAT1 acts in a pro-inflammatory and anti-proliferatory way. Interestingly, STAT1 does not possess a classical monopartite or bipartite NLS. Instead, a

structure dependent NLS has been identified, which is recognized by importin α only after dimerization of STAT1 [43, 45, 47, 48]. STAT1 has been reported to bind to importin α5 and not to importin α1, 3 or 7 [47]. Therefore, STAT1 has been suggested to be transported exclusively by importin α5 in living cells. However, the STAT1 binding assays above did not involve an exhaustive screen of importin α subtypes and importin α7 has since been shown to bind STAT1 as well [44]. Whether or not STAT1 is preferentially imported into the nucleus by importin α5 and if importin α7 is able to substitute for importin α5 in living cells has not been analyzed, so far.

Table 2: STAT proteins are bound by different importin α paralogs.

	in vitro binding assay [43, 45, 47-49]	***in vivo co-immunoprecipitation [44]***
STAT1	importin α5	importin α5 and -7
STAT2	importin α5	*not analyzed*
STAT3	importin α1, -3, -5, -6, -7	importin α5 and -7
STAT4	*not analyzed*	*not analyzed*
STAT5	*not analyzed*	no interaction found
STAT6	*not analyzed*	*not analyzed*

4.2.7.2 Importin α3 and RCC1

In the following years, an *in vivo* study was published reporting importin α3 dependent import of the regulator of chromosome condensation 1 (RCC1). These findings strengthened the hypothesis of substrate specificities of importin α proteins in living cells [50]. RCC1 belongs to a family of highly conserved proteins that exists in all eukaryotic cells [51]. RCC1 binds to the chromatin in the cell nucleus and is the only known guanine-nucleotide exchange factor for the small GTPase Ran [52]. The exchange of RanGDP to RanGTP is essential not only for the nucleocytoplasmic transport [53] but also for the correct progression of the cell cycle, as well as for the formation of the spindle and the nuclear membrane [54, 55]. In digitonin permeabilized HeLa cells, RCC1 has been shown to be imported into the nucleus preferentially by importin α3

[21]. Silencing of importin α3 expression led to a strong delay of RCC1 nuclear import. This change in RCC1 import could be rescued by the co-injection of recombinant importin α3 but not by another importin α protein [50].

4.2.7.3 Importin α7 and Keap1

Within the time of this study, another very interesting analysis was published demonstrating an importin α7 dependent nuclear import of the E3 ubiquitin ligase subunit Keap1 in living cells [56]. Keap1 targets the nuclear factor erythroid 2-related factor 2 (Nrf2), a transcription factor *trans*-acting on the antioxidant response element (ARE) controlling genes involved in cellular redox homeostasis [57, 58]. Under normal oxygen levels, Nrf2 is bound by the Keap1-Cul3-Rbx1 complex, leading to ubiquitination and degradation of Nrf2 [59, 60]. Nrf2 is released from this complex under oxidative stress. Upon release, Nrf2 increases in stability and accumulates in the nucleus where it activates genes involved in the anti-oxidative stress response. Keap1 serves as a key regulator of this process. Nuclear Keap1 binds Nrf2 and attenuates Nrf2 signaling by targeting Nrf2 for export and subsequent degradation in the cytoplasm. Due to its strong nuclear export signal, Keap1 turns off Nrf2 signaling by exporting Nrf2 back to the cytoplasm where it is subsequently degraded [61, 62]. By *in vitro* binding studies, Sun et al. could show that Keap1 is bound by importin α7 but not by importin α1, -3, -5 or -6 [56]. This importin α7/Keap1 interaction has been confirmed by immunoprecipitation experiments *in vivo*. Moreover, silencing of importin α7 expression decreased nuclear Keap1 contents and prolonged Nrf2 signaling which led to subsequent upregulation of genes involved in oxidative stress response.

5 OBJECTIVES OF THIS STUDY

The relevance of the importin α diversity for the function of cells in metazoans is still not fully understood. The principal aim of this study was to analyze individual functions and substrate specificities of importin α paralogs.

The specific objectives of this study were to:

A. Identify and analyze importin α7 binding partners to shed light on the molecular mechanisms of the developmental arrest of embryos from importin α7 knockout females.

B. Generate a new tool to examine importin α cargo specificities *in vivo*. For this purpose, cell lines from importin α knockout mice should be isolated and the following questions should be addressed:

   Is STAT1 nuclear translocation upon IFN-γ stimulation importin α5 dependent?
   Does RCC1 use importin α3 for its nuclear import?
   Is importin α7 needed for the nuclear import of Keap1?

C. Elucidate the roles of importin α3 in mammals by characterizing an importin α3 knockout mouse model.

6 RESULTS & DISCUSSION

1. Binding partners of importin α7 (Part A)

Published reports strongly suggest that individual importin α subtypes possess differing affinities for NLS-bearing cargoes [21, 48, 50, 63]. Therefore, the first aim of this study was to use *in vivo* and *in vitro* binding assays to screen for cargoes that uniquely bind specific importin α subtypes. Of particular interest was to identify cargoes for importin α7. Mice that lack functional importin α7, although viable, display a unique reproductive phenotype in both male and female mice that results in infertility [39].

1.1. Identification of importin α7 binding partners from ovary tissue

Proteomic screening approaches can be a powerful tool to search for proteins that uniquely interact with individual importin α subtypes. One objective of this study was to identify importin α7 binding partners that could be involved in the female infertility phenotype. For this purpose, the open reading frame of murine importin α7 was cloned into a bacterial expression vector leading to a C-terminal GST-tagged fusion protein. The importin α7 GST pull-down experiment was performed using mouse ovary lysate as a source of prey proteins and binding partners were identified by high resolution mass spectrometry. The high sensitivity of this system allows the identification of peptides in the femtomolar range. Therefore, identified proteins need to be quantitatively examined according to intensity parameters. For this purpose, a label-free quantification (LFQ) has been performed [64] that allows a comparison of importin α7-GST binders relative to proteins found in the GST-control sample. Importin α7 binding partners were ranked according to their fold change (log2 LFQ intensity importin α7–GST/ LFQ intensity GST-control). 757 proteins did bind only to importin α7 and not to the GST-control protein. To complete the list of importin α7 binding partners, the top 5 % of importin α7 binding partners found to bind also to the GST-control were included as well (see Appendix Table 1; 807 proteins).

1.2. Involvement of importin α7 binding partners from ovary tissue in RNA processing, chromosome organization and chromatin modification

Candidate importin α7 binding partners identified by GST pull-down experiments were further examined according to their cellular localization and biological processes based on Gene Ontology (GO) terms. For this purpose, the result list of 807 proteins was analyzed using the enrichment analysis program ToppFun from the ToppGene Suite service [65]. Genes found to bind to importin α7 were compared to all annotated genes in the genome. The GO-term "nucleoplasm", for example will be found for 1342 out of ~ 19,000 genes (7%) in the genome. In the list of importin α7 binding partners 167 out of 807 (21%) genes were found to be annotated with the GO-term "nucleoplasm". Thus, the GO-term enrichment analysis revealed a significant enrichment ($p<0.01$) of nuclear and nucleolar proteins present in the list of importin α7-GST binding partners (**Table 3, B**). The top 5 most important biological processes importin α7 binding partners were found to be involved in, are displayed in **Table 3, A** ($p<0.01$).

Table 3: Top 5 enriched GO-terms among importin α7 binding partners identified by GST pull-down from ovary lysate. 807 proteins were analyzed using the ToppFun analysis software from the ToppGene Suite. List entries are ranked according to p-value; $p<0.01$.

A) Biological Process

	ID	Name	P-value	Term in Query	Term in Genome
1	GO:0006396	RNA processing	4.07E-27	99	626
2	GO:0051276	chromosome organization	1.09E-18	87	631
3	GO:0016071	mRNA metabolic process	7.75E-16	74	520
4	GO:0006397	mRNA processing	1.26E-14	61	382
5	GO:0016568	chromatin modification	1.86E-14	60	373

B) Cellular Component

	ID	Name	P-value	Term in Query	Term in Genome
1	GO:0005654	nucleoplasm	2.50E-38	167	1342
2	GO:0005730	nucleolus	1.31E-28	137	1133
3	GO:0016585	chromatin remodeling complex	5.58E-19	35	99
4	GO:0044451	nucleoplasm part	1.90E-17	94	783
5	GO:0030529	ribonucleoprotein complex	3.98E-12	68	544

1.3. Identification of importin α7 binding partners from fibroblast cells

In order to identify proteins that require physiological conditions to form a complex with importin α7, another proteomic screening approach was performed. For this purpose, the open reading frame of murine importin α7 was cloned into a mammalian expression vector leading to a C-terminal mycHis-tagged fusion protein. Importin α7-mycHis was overexpressed in murine fibroblast cells (NIH3T3) and bound proteins were co-immunoprecipitated via the His tag. Correct cellular cytoplasmic and nuclear localization of importin α7-mycHis protein was verified by Western Blot in advance (**Figure 10**).

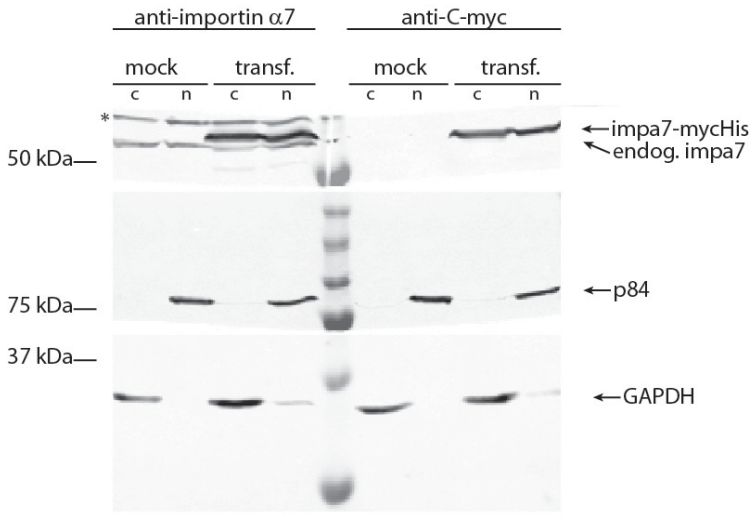


Figure 10: Expression and localization of importin α7-mycHis in NIH3T3 cells. Cytoplasmic (c) and nuclear (n) fractions were prepared of NIH3T3 cells. Cells were treated with the transfection mix without vector (mock) or including the importin α7-mycHis construct (transf.). P84 served as nuclear marker, GAPDH served as cytoplasmic marker. The anti-importin α7 antibody detects endogenous importin α7 as wells overexpressed importin α7-mycHis. The anti-c-myc antibody detects only the mycHis tagged version of importin α7. *: cross reactivity with importin α5.

The LFQ-intensities of identified proteins were determined and importin α7-mycHis binding partners were again ranked according to their fold change (log2 LFQ intensity importin α7–mycHis/ LFQ intensity mycHis-control). 267 proteins were identified to bind importin α7-mycHis and not to the mycHis control. The top 5% of importin α7 bound proteins found to bind

also to the control were again added to the result list of importin α7-mycHis binding partners (see Appendix Table 2, 299 proteins).

1.4. Involvement of importin α7 binding partners from fibroblast cells in RNA processing, chromosome organization and chromatin modification

GO-term enrichment analysis of cellular components revealed a significant ($p<0.01$) accumulation of nuclear and nucleolar proteins in the result list of importin α7mycHis co-immunoprecipitated proteins (**Table 4, B**). The top 5 enriched biological process GO-terms found in the list of importin α7-mycHis binding partners are displayed in **Table 4, A** ($p<0.01$).

Table 4: Top 5 enriched GO-terms among importin α7mycHis binding partners identified by co-immunoprecipitation from NIH3T3 cells. 299 proteins were analyzed using the ToppFun analysis software from the ToppGene Suite. List entries are ranked according to p-value;$p<0.01$

A) Biological Process

	ID	Name	P-value	Term in Query	Term in Genome
1	GO:0006396	RNA processing	1.44E-19	54	626
2	GO:0051276	chromosome organization	3.45E-15	49	631
3	GO:0016568	chromatin modification	1.60E-14	38	373
4	GO:0006325	chromatin organization	1.72E-13	41	473
5	GO:0006397	mRNA processing	2.38E-12	36	382

B) Cellular Component

	ID	Name	P-value	Term in Query	Term in Genome
1	GO:0005654	nucleoplasm	1.21E-39	101	1342
2	GO:0005730	nucleolus	2.51E-34	88	1133
3	GO:0016585	chromatin remodeling complex	8.73E-26	30	99
4	GO:0044451	nucleoplasm part	6.04E-20	59	783
5	GO:0016514	SWI/SNF complex	4.34E-10	10	15

1.5. 36 % overlap of importin α7 binding partners identified by co-immunoprecipitation from fibroblast cells with GST pull-down results from ovary

Out of 299, 107 proteins identified by importin α7mycHis co-immunoprecipitation from fibroblast cells did also show up in the list of potential importin α7 interaction partners

identified by GST pull-down from ovary (**Table 5**). These proteins seem to be robust importin α7 binding partners and hence interesting candidates.

Table 5: Overlay of importin α7 binding partners from ovary and fibroblast cells. Comparison of 807 importin α7 binding partners identified by GST pull-down from ovary lysate and 299 proteins identified by co-immunoprecipitation from fibroblast cells. Candidate genes were ranked according to their LFQ intensity from the importin α7-GST pull-down experiment using ovary lysate. Protein information about cellular localization (Cell. Loc.) and association to certain protein complexes was extracted using the UniProt webpage (http://www.uniprot.org/); Nuc: nucleus, Cyt: cytoplasm, secr.Ves: secretory vesicle. Proteins with NLS are marked by an asterisk, information taken from *The Nuclear Protein Database* (http://npd.hgu.mrc.ac.uk/user/). Listed biological information is not experimentally proven in all cases.

	Gene name (Protein name)	Cell. Loc.	Complex association	Function	LFQ intensity
1	Lmnb1 (Lamin B1) *	Nuclear membrane		scaffolding, interaction with chromatin	3.288E+10
2	Chd4 (Chromodomain-helicase-DNA-binding protein 4) *	Nuc/Cyt	NuRD	remodeling of chromatin	2.119E+10
3	Lmn1 (lamin 1) *	Nuclear membrane			1.281E+10
4	Kiaa0398 (mRNA cap guanine-N7 methyltransferase)	Nuc		mRNA-capping	1.044E+10
5	Mta1l1 (Metastasis-associated protein MTA2)*	Nuc		modification of chromatin	6.97E+09
6	Mcm3 (DNA replication licensing factor MCM3) *	Nuc	MCM	replicative helicase essential for 'once per cell cycle' DNA replication	6.731E+09
7	Taf2s (Transcription elongation regulator 1) *	Nuc	TATA box	Transcription factor that binds RNA polymerase II and inhibits elongation of transcripts	5.097E+09
8	Cdc46 (DNA replication licensing factor MCM5) *	Nuc	MCM	replicative helicase essential for 'once per cell cycle' DNA replication	3.8E+09
9	Ars2 (Serrate RNA effector molecule homolog) *	Nuc/Cyt		mediator between cap-binding complex and primary microRNAs processing machinery during cell proliferation	3.668E+09
10	Baf170 (SWI/SNF complex subunit SMARCC2) *	Nuc	SWI/SNF	remodeling of chromatin	3.45E+09
11	Npap60 (Nuclear pore complex protein Nup50)	Nuc	NPC	facilitates disassembly of importin-alpha:beta-cargo complex	3.189E+09
12	Hdac2 (Histone deacetylase 2)	Nuc	HDAC	modification of chromatin	2.921E+09
13	Nup153 (Nuclear pore complex protein Nup153) *	Nuc	NPC	Possible DNA-binding subunit of the NPC	2.92E+09
14	Gatad2b (Transcriptional repressor p66-beta)	Nuc		transcriptional repressor activity, targets MBD3 to discrete loci in nucleus	2.483E+09
15	Ddx21 (Nucleolar RNA helicase 2) *	Nuc		rRNA processing	2.438E+09
16	Dhm1 (5'-3' exoribonuclease 2) *	Nuc		termination of transcription by RNA polymerase II	2.438E+09

17	Baf190a (Transcription activator BRG1) *	Nuc	CREST-BRG1, nBAF, WINAC	remodeling of chromatin	2.209E+09
18	Rbap48 (Histone-binding protein RBBP4)	Nuc	CAF-1, HDAC, NuRD, PRC2/EED-EZH2, NURF	remodeling of chromatin	1.996E+09
19	Rbap46 (Histone-binding protein RBBP7)	Nuc	HAT, HDAC, NuRD, PRC2/EED-EZH2, NURF	remodeling of chromatin	1.946E+09
20	Hnrnpc (Heterogeneous nuclear ribonucleoproteins C1/C2) *	Nuc		Binds pre-mRNA and nucleates assembly of 40S hnRNP particles	1.917E+09
21	Tceb3 (Transcription elongation factor B polypeptide 3)	Nuc	elongin BC	increases RNA polymerase II transcription elongation	1.757E+09
22	Mta1 (Metastasis-associated protein MTA1) *	Nuc/Cyt		modification of chromatin	1.6E+09
23	Ctnnbl1 (Beta-catenin-like protein 1)	Nuc		Induces apoptosis in CHO cells	1.375E+09
24	Smarca5 (SWI/SNF-related matrix-associated actin-dependent regulator of chromatin subfamily A member 5) *	Nuc	SWI/SNF, WICH, NoRC	Helicase, remodeling of chromatin	1.276E+09
25	Kiaa1113 (E3 ubiquitin-protein ligase TRIM33) *	Nuc		Promotes SMAD4 ubiquitination, nuclear exclusion and degradation via ubiquitin proteasome pathway	1.224E+09
26	Edd1 (E3 ubiquitin-protein ligase UBR5)	Nuc		ubiquitination and subsequent degradation	1.188E+09
27	Anp32e (Acidic leucine-rich nuclear phosphoprotein 32 family member E) * [66]	Nuc/Cyt		Inhibits activity of protein phosphatase 2A (PP2A)	1.144E+09
28	Adnp (ADNP homeobox protein 2)	Nuc		transcriptional regulation	1.099E+09
29	Aprin (Sister chromatid cohesion protein PDS5 homolog B)	Nuc		Regulator of sister chromatid cohesion in mitosis which may stabilize cohesin complex association with chromatin	1.083E+09
30	Hdac1 (Histone deacetylase 1)	Nuc	HDAC, BRG1-RB1-HDAC1, RCOR/GFI/KDM1A/HDAC	modification of chromatin	1.055E+09
31	Baf60b (SWI/SNF-related matrix-associated actin-dependent regulator of chromatin subfamily D member 2) *	Nuc	SWI/SNF (?)	remodeling of chromatin	873570000
32	Abh5 (Probable alpha-ketoglutarate-dependent dioxygenase ABH5)	?		dioxygenase, may repair alkylated DNA and RNA by oxidative demethylation	853660000

33	Ase1 (DNA-directed RNA polymerase I subunit RPA34) *	Nuc	RNA polymerase I	transcription of DNA into RNA	847580000
34	Baf47 (SWI/SNF-related matrix-associated actin-dependent regulator of chromatin subfamily B member 1)	Nuc	SWI/SNF, npBAF, WINAC	remodeling of chromatin	744640000
35	Nol5 (Nucleolar protein 58) *	Nuc		Required for 60S ribosomal subunit biogenesis	705270000
36	Gatad2a (Transcriptional repressor p66-alpha)	Nuc (speckle)		Transcriptional repressor	620640000
37	Nol5a (Nucleolar protein 56)	Nuc/Cyt		early to middle stages of 60S ribosomal subunit biogenesis	597470000
38	Arid1a (AT-rich interactive domain-containing protein 1A) *	Nuc	SWI/SNF, npBAF, WINAC	remodeling of chromatin	529030000
39	Myo1c (Unconventional myosin-Ic)	Membrane, Nuc	myosin, NPC	intracellular movements, regulation of transcription	528030000
40	Fbl (rRNA 2'-O-methyltransferase fibrillarin)	Nuc	snoRNP	pre-rRNA processing	501870000
41	Bod1l (Biorientation of chromosomes in cell division protein 1-like 1)	?		?	449740000
42	Alpha-CP2 (Poly(rC)-binding protein 2)	Nuc/Cyt		Major cellular poly(rC)-binding protein. Negatively regulates cellular antiviral responses mediated by MAVS signaling.	448310000
43	Arid1b (AT-rich interactive domain-containing protein 1B)	Nuc	SWI/SNF, npBAF	remodeling of chromatin	407070000
44	Mbd3 (Methyl-CpG-binding domain protein 3) *	Nuc		Recruits histone deacetylases and DNA methyltransferases. Acts as transcriptional repressor and plays a role in gene silencing.	392860000
45	Tex10 (Testis-expressed sequence 10 protein)	Nuclear membrane	MLL1/MLL	?	379460000
46	Mbd2 (Methyl-CpG-binding domain protein 2) *	Nuc		Demethylase, binds CpG islands in promoters where the DNA is methylated, recruits histone deacetylases and DNA methyltransferases	342200000
47	Mta3 (Metastasis-associated protein MTA3)	Nuc/Cyt		maintenance of normal epithelial architecture through the repression of SNAI1 transcription in a histone deacetylase-dependent manner, and thus the regulation of E-cadherin levels	340580000
48	Paf53 (DNA-directed RNA polymerase I subunit RPA49) *	Nuc	RNA polymerase I	DNA-dependent RNA polymerase	336870000
49	Pcif1 (Phosphorylated CTD-interacting factor 1)	Nuc		transcription elongation or in coupling transcription to pre-mRNA processing	283540000

50	Carf (CDKN2A-interacting protein) *	Nuc		Activates p53/TP53 by CDKN2A-dependent and CDKN2A-independent pathways	280170000
51	Nolc1 (Nucleolar and coiled-body phosphoprotein 1) *	Nuc/Cyt		maintenance of fundamental structure of fibrillar center and dense fibrillar component in the nucleolus, transcription catalyzed by RNA polymerase I	238570000
52	Nup121 (Nuclear envelope pore membrane protein POM 121) *	Nuclear membrane	NPC	anchoring components of the pore complex to the pore membrane	228630000
53	Hnrnpu (Heterogeneous nuclear ribonucleoprotein U) *	Nuc/Cyt	CRD	promotes MYC mRNA stabilization	219240000
54	Nipbl (Nipped-B-like protein) *	Nuc		structural role in chromatin, sister chromatid cohesion	167550000
55	Baf45d (Zinc finger protein ubi-d4)	Nuc/Cyt	SWI/SNF (?)	transcription factor required for apoptosis response following survival factor withdrawal from myeloid cells, role in the development and maturation of lymphoid cells	166780000
56	Cspg6 (Structural maintenance of chromosomes protein 3) *	Nuc	cohesin	chromosome cohesion during the cell cycle	154610000
57	Baf155 (SWI/SNF complex subunit SMARCC1) *	Nuc	SWI/SNF, npBAF, WINAC	remodeling of chromatin	151100000
58	Mnar (Proline-, glutamic acid- and leucine-rich protein 1) *	Nuc/Cyt		Plays a role in estrogen receptor (ER) genomic activity	145240000
59	Cwc15 (Pre-mRNA-splicing factor CWC15)	Nuc		Involved in pre-mRNA splicing	140130000
60	Maged1 (Melanoma-associated antigen D1)	Membrane/Nuc [67]		apoptotic response after nerve growth factor (NGF) binding in neuronal cells, regulator of the function of DLX family members	121080000
61	Hnrnpl (Heterogeneous nuclear ribonucleoprotein L) *	Nuc/Cyt	hnRNP	pre-mRNAs processing	116680000
62	Gnl3 (Guanine nucleotide-binding protein-like 3) *	Nuc		regulating cellular proliferation	112710000
63	Smc1a (Structural maintenance of chromosomes protein 1A) *	Nuc	cohesin	chromosome cohesion during cell cycle and in DNA repair	110390000
64	Baf60a (SWI/SNF-related matrix-associated actin-dependent regulator of chromatin subfamily D member 1) *	Nuc	SWI/SNF, npBAF, WINAC	remodeling of chromatin	107760000
65	Metap1 (Methionine aminopeptidase 1)	?		Removes the N-terminal methionine from nascent proteins. Required for normal progression through the cell cycle.	100370000
66	Ppil1 (Peptidyl-prolyl cis-trans isomerase-like 1)	?		PPIases accelerate the folding of proteins	97006000

67	Chd3 (Chromodomain-helicase-DNA-binding protein 3) *	Nuc	NuRD	remodeling of chromatin	96858000
68	Wdr18 (WD repeat-containing protein 18)	?		May play a role during development	96488000
69	Ash2l (Set1/Ash2 histone methyltransferase complex subunit ASH2) *	Nuc	HMT	modification of chromatin	94731000
70	Baf60c (SWI/SNF-related matrix-associated actin-dependent regulator of chromatin subfamily D member 3) *	Nuc	SWI/SNF, npBAF, WINAC	remodeling of chromatin	94429000
71	Kiaa0425 (Zinc finger MYM-type protein 4)	?		regulation of cell morphology and cytoskeletal organization	87907000
72	Rps27l (40S ribosomal protein S27-like)	?	Ribosom	?	82910000
73	Clk2 (Dual specificity protein kinase CLK2) *	Nuc (speckle)		Dual specificity kinase acting on both serine/threonine and tyrosine-containing substrates	77581000
74	RAM (RNMT-activating mini protein)	Nuc		mRNA cap methylation	76178000
75	Baf180 (Protein polybromo-1) *	Nuc	SWI/SNF ?	remodeling of chromatin	75851000
76	Trrap (Transformation/transcription domain-associated protein) *	Nuc	HAT	adapter protein, remodeling and modification of chromatin	69640000
77	Dhx16 (Putative pre-mRNA-splicing factor ATP-dependent RNA helicase DHX16) *	Nuc		RNA helicase involved in pre-mRNA splicing	68796000
78	Dinb1 (DNA polymerase kappa)	Nuc		DNA repair	60927000
79	Abra1 (BRCA1-A complex subunit Abraxas)	Nuc	BRCA1-A	DNA repair	58297000
80	Nhp2l1 (NHP2-like protein 1)	Nuc		late stage of spliceosome assembly	56352000
81	Brcc3 (Lys-63-specific deubiquitinase BRCC36)	Nuc	BRCA1-A, BRISC	Metalloprotease, DANN repair	56226000
82	Rbbp5 (Retinoblastoma-binding protein 5) *	Nuc	MLL1/MLL	modification of chromatin	54128000
83	Aimp1 (Aminoacyl tRNA synthase complex-interacting multifunctional protein 1)	Nuc/Cyt/ER/ secr.Ves./secreted	multisynthase	Stimulates the catalytic activity of cytoplasmic arginyl-tRNA synthase, …	54077000
84	Anp32b (Acidic leucine-rich nuclear phosphoprotein 32 family member B) *	Nuc/Cyt		Multifunctional protein working as cell cycle progression factor, cell survival factor, histone chaperone, stimulating core histones to assemble into nucleosome	54077000

85	Bptf (Nucleosome-remodeling factor subunit BPTF) *	Nuc/Cyt	NURF	remodeling of chromatin	40443000
86	Csnk1d (Casein kinase I isoform delta)	Nuc/Cyt/Membrane	circadian clock	Essential serine/threonine-protein kinase that regulates diverse cellular growth and survival processes including Wnt signaling, DNA repair and circadian rhythms	39791000
87	Dsp (High mobility group protein DSP1) *	Nuc		Binds preferentially single-stranded DNA and unwinds double stranded DNA	36318000
88	Tada1 (Transcriptional adapter 1)	Nuc		transcriptional regulation	32645000
89	Carp1 (Cell division cycle and apoptosis regulator protein 1)	Cyt/Nuc [68]	Mediator, p160 coactivator	transduces regulatory signals from upstream transcriptional activator proteins to basal transcription machinery at the core promoter, p53 coactivator, cell cycle progression and/or cell proliferation	30571000
90	Rsbn1l (Round spermatid basic protein 1-like protein)	Nuc		?	30055000
91	Kifc1 (Kinesin-like protein KIFC1)	Nuc/Cyt/early Endosome		spindle formation, May contribute to movement of early endocytic vesicles	29510000
92	Emp2 (Epithelial membrane protein 2)	Membrane		?	26632000
93	Ylpm1 (YLP motif-containing protein 1) *	Nuc (speckle)		reduction of telomerase activity during differentiation of embryonic stem cells by binding to core promoter of TERT and controlling its down-regulation	20613000
94	Baf57 (SWI/SNF-related matrix-associated actin-dependent regulator of chromatin subfamily E member 1) *	Nuc	SWI/SNF, npBAF, WINAC	remodeling of chromatin	18988000
95	Nvl (Putative ribosome biogenesis ATPase nvl) *	Nuc		ribosome biogenesis	17584000
96	H1f3 (Histone H1.3) *	Nuc		forms chromatin fiber, remodeling of chromatin	17142000
97	Spty2d1 (Protein SPT2 homolog)	?		?	15244000
98	Polr2e (DNA-directed RNA polymerases I, II, and III subunit RPABC1) *	Nuc	RNA polymerases I, II and III	synthesize ribosomal RNA precursors, mRNA precursors and many functional non-coding RNAs, and small RNAs	10855000
99	Paf65b (TAF5-like RNA polymerase II p300/CBP-associated factor-associated factor 65 kDa subunit 5L)	Nuc	PCAF	modification of chromatin	10681000
100	Gtf2h4 (General transcription factor IIH subunit 4) *	Nuc	core-TFIIH	involved in nucleotide excision repair of DNA and, when complexed to CAK, in RNA transcription by RNA polymerase II	9828500

101	Paf65a (TAF6-like RNA polymerase II p300/CBP-associated factor-associated factor 65 kDa subunit 6L)	Nuc	PCAF	modification of chromatin	7735300
102	Lyar (Cell growth-regulating nucleolar protein)	Nuc		?	5280200
103	Qk (Protein quaking)	Nuc/Cyt		RNA-binding protein that plays a central role in myelinization, regulating pre-mRNA splicing, mRNA export, mRNA stability and protein translation	2825800
104	Atxn2l (Ataxin-2-like protein)	Membrane (?)		?	2188200

1.6. Nuclear localization of candidate proteins despite the absence of importin α7

Importin α7 binding partners identified in both screens, the GST pull-down from ovary and the co-immunoprecipitation from fibroblast cells (see **Table 5**) were chosen for further analysis. Six candidate importin α7 substrates that play a role in transcription, chromatin modification or in regulation of the cell cycle were analyzed in more detail (**Table 6**).

Table 6: Selection of candidate importin α7 cargoes. Proteins found in ovary and fibroblast cells that bound to importin α7.

Candidate	Subcellular localization	Biological process	Function
Anp32e / LANPL / PHAP III	nuclear, cytoplasmic	regulation of transcription	similar to Anp32a → i.e. inhibition of acetyltransferases as part of the INHAT (inhibitor of histone acetyltransferases) complex
Brg1 / Smarca4 / Baf190a	nuclear	chromatin regulator	ATP-dependent helicase, transcription activator as part of the SWI/SNF chromatin remodeling complex
Ddx21	nucleolus	regulation of transcription	Nucleolar RNA helicase
Gatad2a	nuclear	regulation of transcription	Transcriptional repressor
HDAC1	nuclear	chromatin regulator	Histone deacetylase, epigenetic repression
Delangin / Nip-BL	nuclear	cell cycle	Probably plays a structural role in chromatin, involved in sister chromatid cohesion

To examine whether candidate cargoes depend on the presence of importin α7, their cellular localization was analyzed in importin α7 knockout MEFs. For this purpose, MEFs were prepared from importin α7-/- embryos [39] and the absence of importin α7 expression was verified by RT-PCR (**Figure 11**). Initial characterization of importin α7-/- MEFs showed that these MEFs grow more slowly than wild type MEFs. The reason for this phenotype, however, is unclear and was not further examined, yet. Moreover, whereas importin α3 levels do not differ between wildtype and importin α7 KO MEFs, importin α4 seems to be decreased ($p<0.01$) and importin

α1 ($p<0.01$) and α5 ($p=0.056$) were increased as measured in initial Western Blot experiments (**Figure 12**).

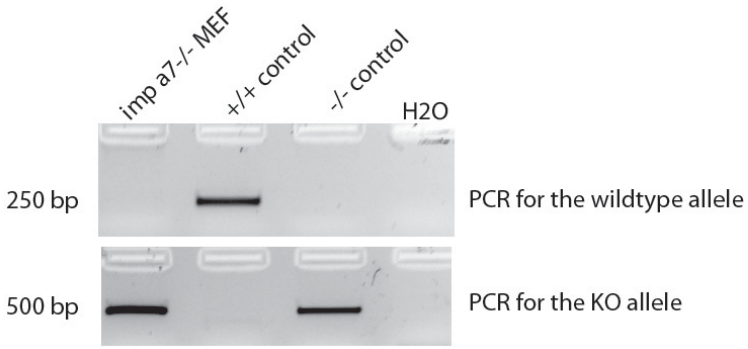


Figure 11: Genotyping PCR of importin α7 KO MEFs. MEFs were genotyped by PCR amplification of a DNA fragment spanning the insertion site of the importin α7 KO (imp a7 -/-) gene trap cassette for detection of the wild type allele (upper panel). To detect the importin α7 KO allele, a DNA fragment was amplified spanning an intronic part of the importin α7 gene and the inserted KO cassette (lower panel). Wildtype (+/+ control) and importin α7 KO (-/- control) tissue DNA served as controls. Water (H2O) was used to ensure the absence of DNA contaminations in the PCR mix.

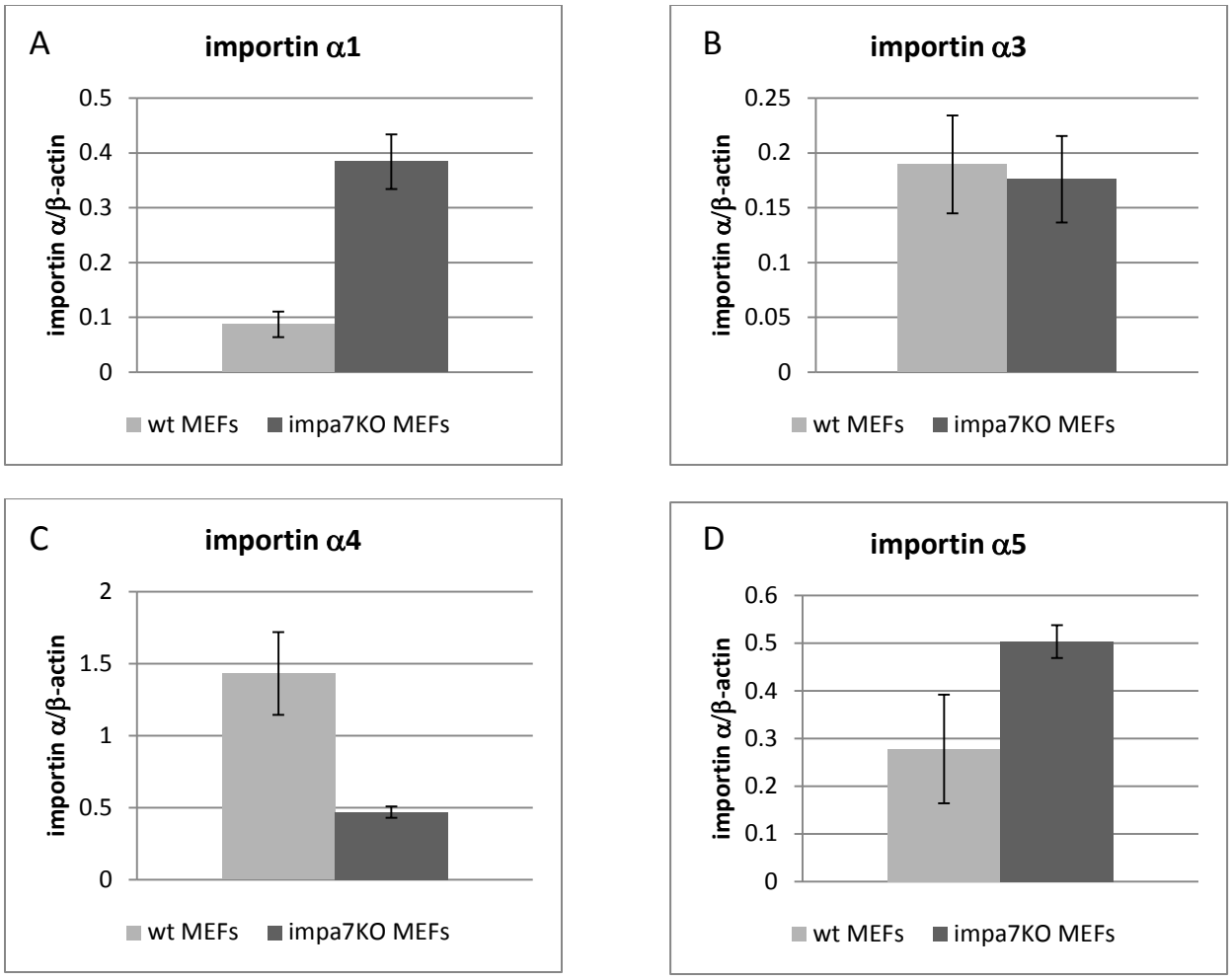


Figure 12: Quantification of importin α proteins in importin α7-/-/ MEFs. Importin α7-/- MEF (impa7 KO) and wildtype (wt) MEF protein extract were analyzed by Western Blot. Western Blot signals of importin α1 (A), importin α3 (B), importin α4 (C) and importin α5 (D) were quantified using the Odyssey scanner software. Importin α signals were normalized to β-actin signals. The diagrams display the mean of three independent protein preparations. Error bar: SD.

Almost all candidates checked showed nuclear staining in wild type MEFs as well as in importin α7-/- MEFs (**Figure 13 - Figure 17**). Only the immunocytochemical staining of Delangin revealed different results. In many importin α7-/- MEF nuclei Delangin staining was spared out (**Figure 18 and Figure 19**), however, not in all cells. It was hypothesized that Delangin subcellular localization may change during the cell cycle and is only nuclear during certain stages. Importin α7-/- MEFs proliferate much slower than wild type MEFs which implicates a high proportion of resting cells within this population. Hence, differences in nuclear Delangin staining may be based on different cell cycle phases of importin α7-/- and wild type MEFs. In order to check the

subcellular localization of Delangin in resting cells, different amounts of wild type MEFs were serum starved for 48 hours to increases the proportion of cells at the G0/G1 phase of the cell cycle. Under serum starvation, many wild type cells showed cytoplasmic Delangin staining (**Figure 20 and Figure 21**).

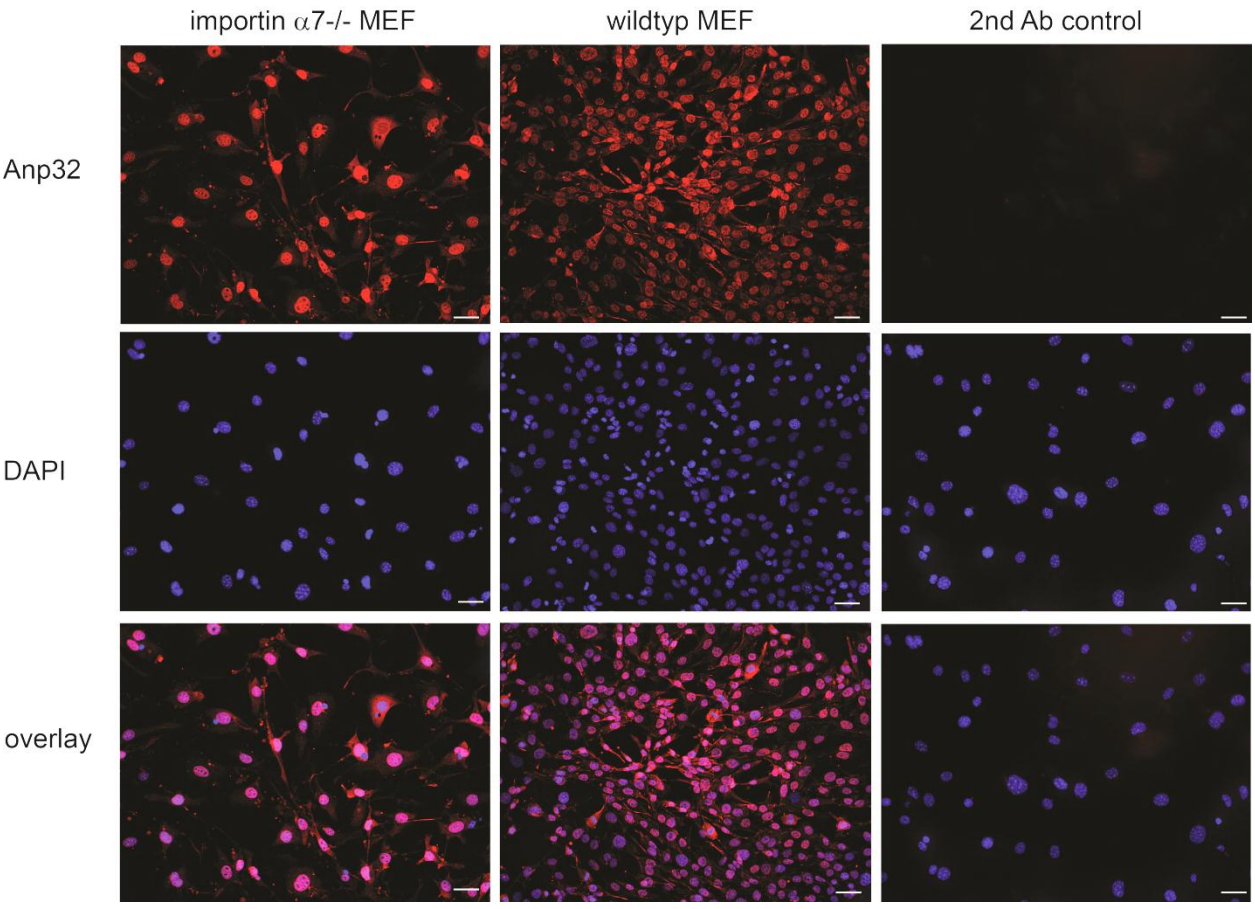


Figure 13: Subcellular localization of Anp32 in importin α7-/- MEFs. Wildtype and importin α7-/- MEFs were probed with anti-Anp32 antibody. DAPI served as nuclear marker. For the secondary antibody control (2nd Ab control), no primary antibody was used; scale bar: 50 µm.

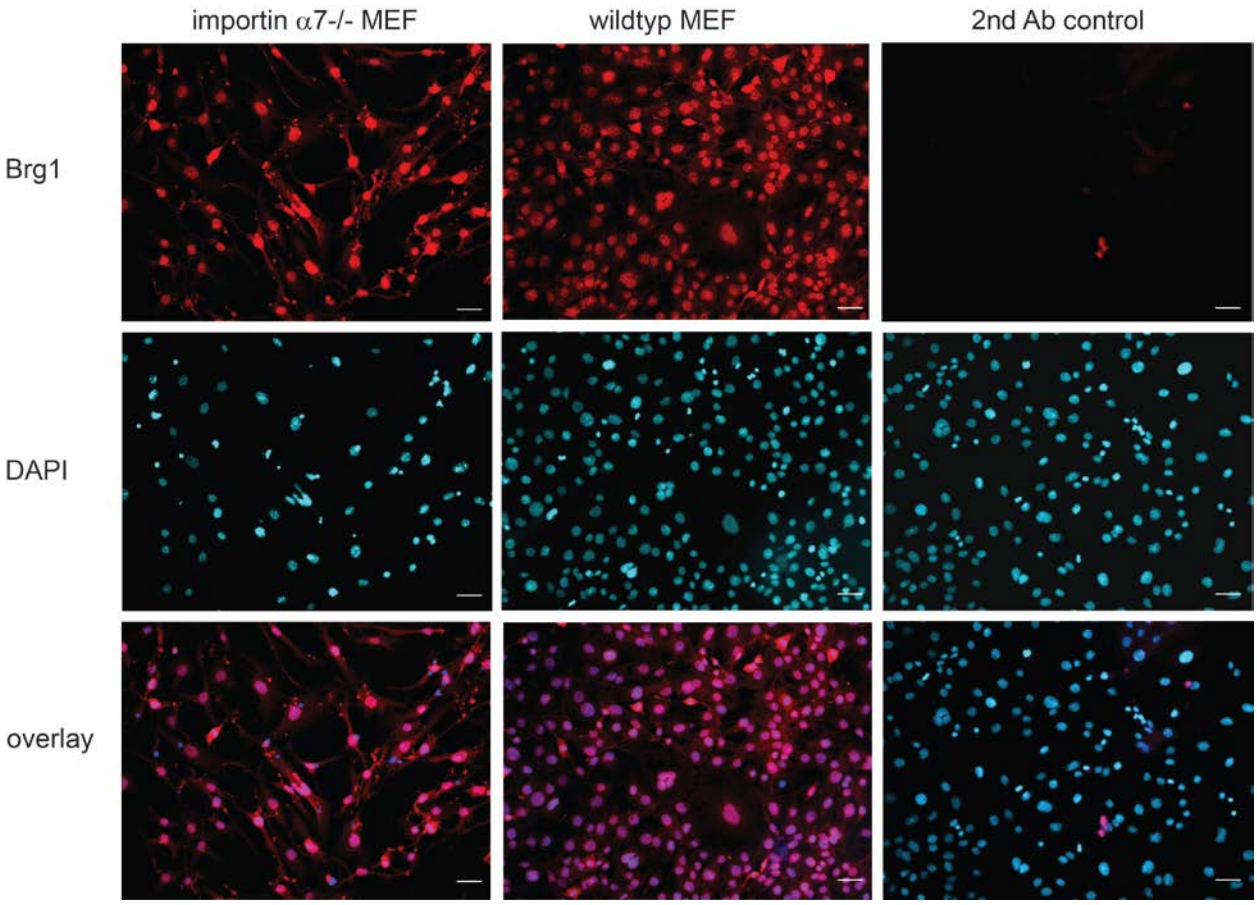


Figure 14: Subcellular localization of Brg1 in importin α7-/- MEFs. Wildtype and importin α7-/- MEFs were probed with anti-Brg1 antibody. DAPI served as nuclear marker. For the secondary antibody control (2nd Ab control), no primary antibody was used. 20x magnification; scale bar: 50 µm.

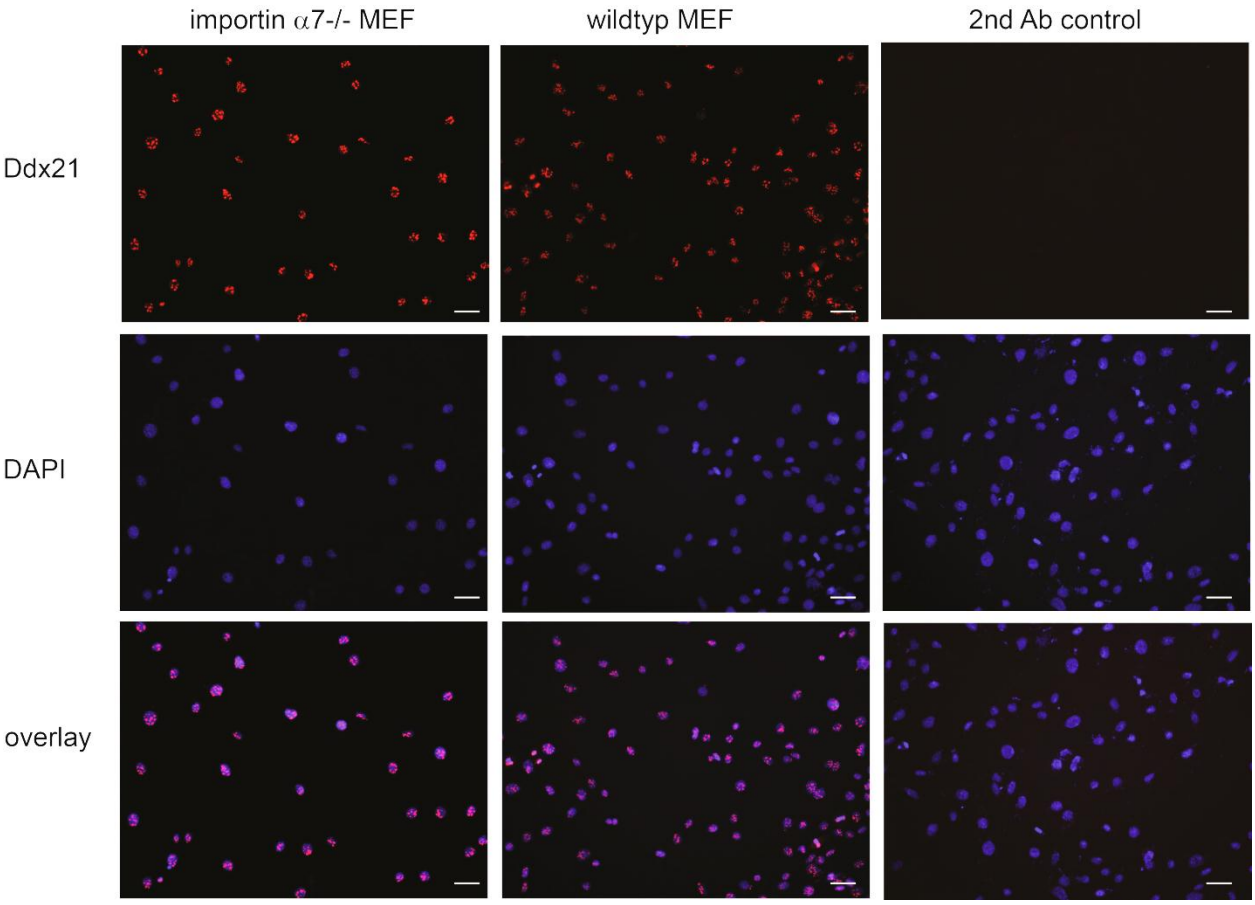


Figure 15: Subcellular localization of Ddx21 in importin α7-/- MEFs. Wildtype and importin α7-/- MEFs were probed with anti-Ddx21 antibody. DAPI served as nuclear marker. For the secondary antibody control (2nd Ab control), no primary antibody was used. 20x magnification; scale bar: 50 µm.

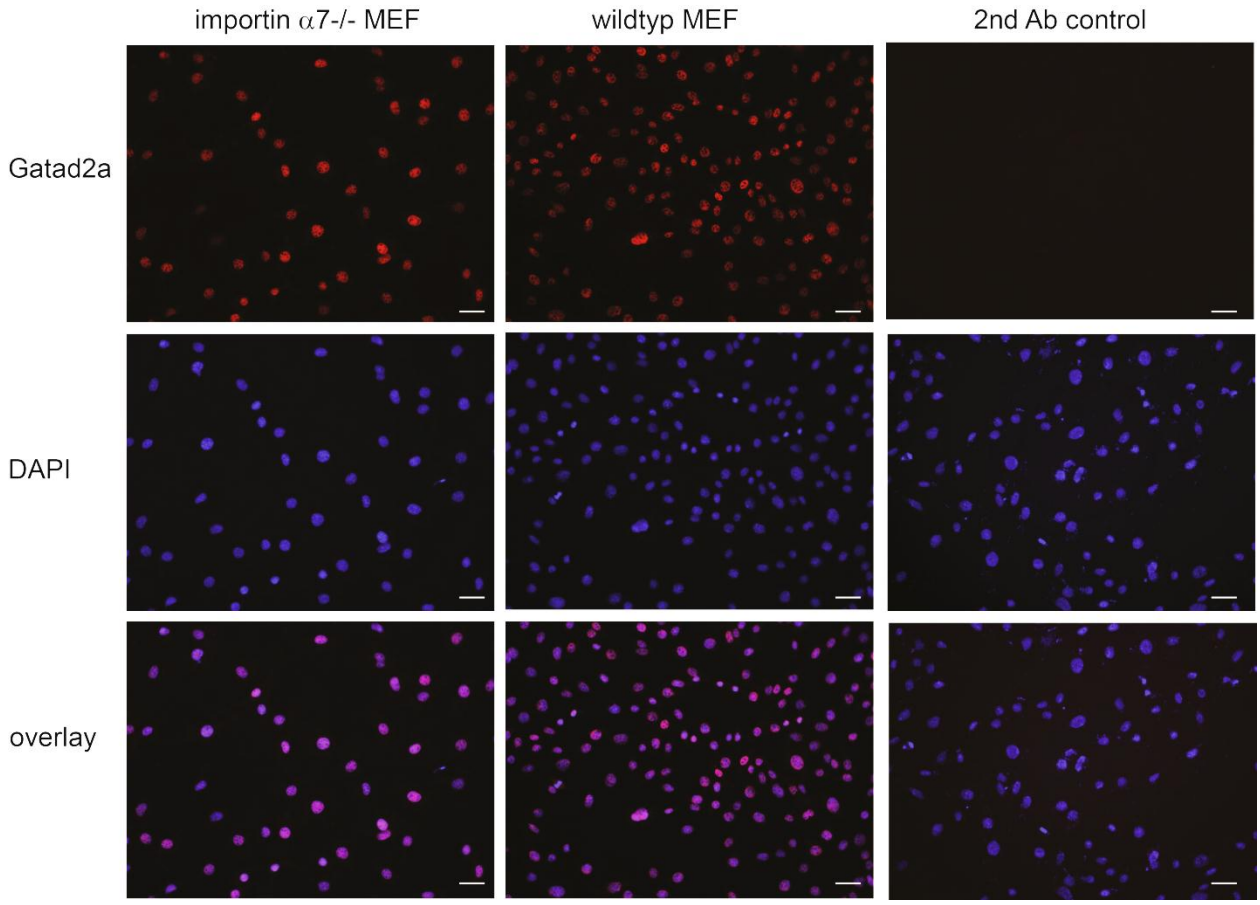


Figure 16: Subcellular localization of Gatad2a in importin α7-/- MEFs. Wildtype and importin α7-/- MEFs were probed with anti-Gatad2a antibody. DAPI served as nuclear marker. For the secondary antibody control (2nd Ab control), no primary antibody was used. 20x magnification; scale bar: 50 µm.

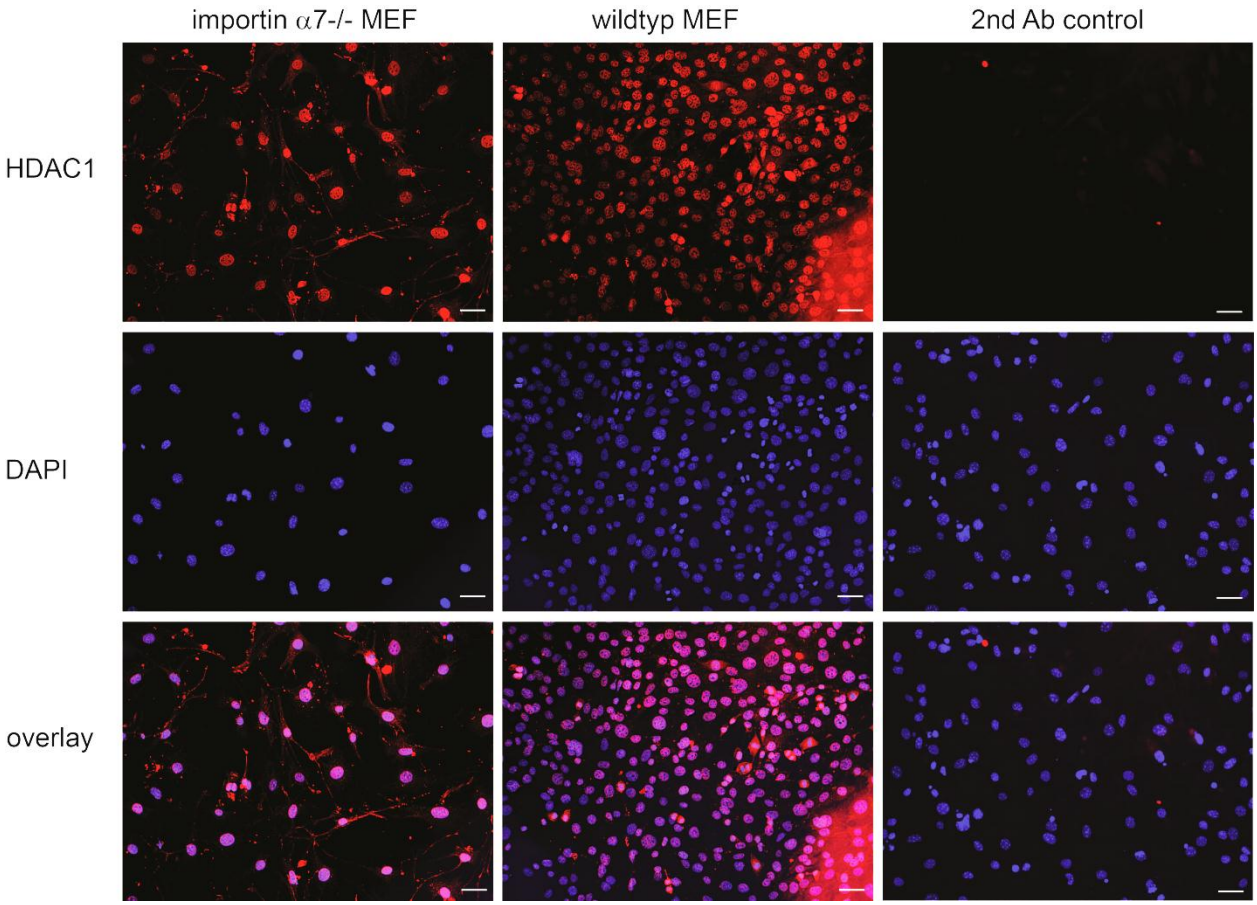


Figure 17: Subcellular localization of HDAC1 in importin α7-/- MEFs. Wildtype and importin α7-/- MEFs were probed with anti-HDAC1 antibody. DAPI served as nuclear marker. For the secondary antibody control (2nd Ab control), no primary antibody was used. 20x magnification; scale bar: 50 µm.

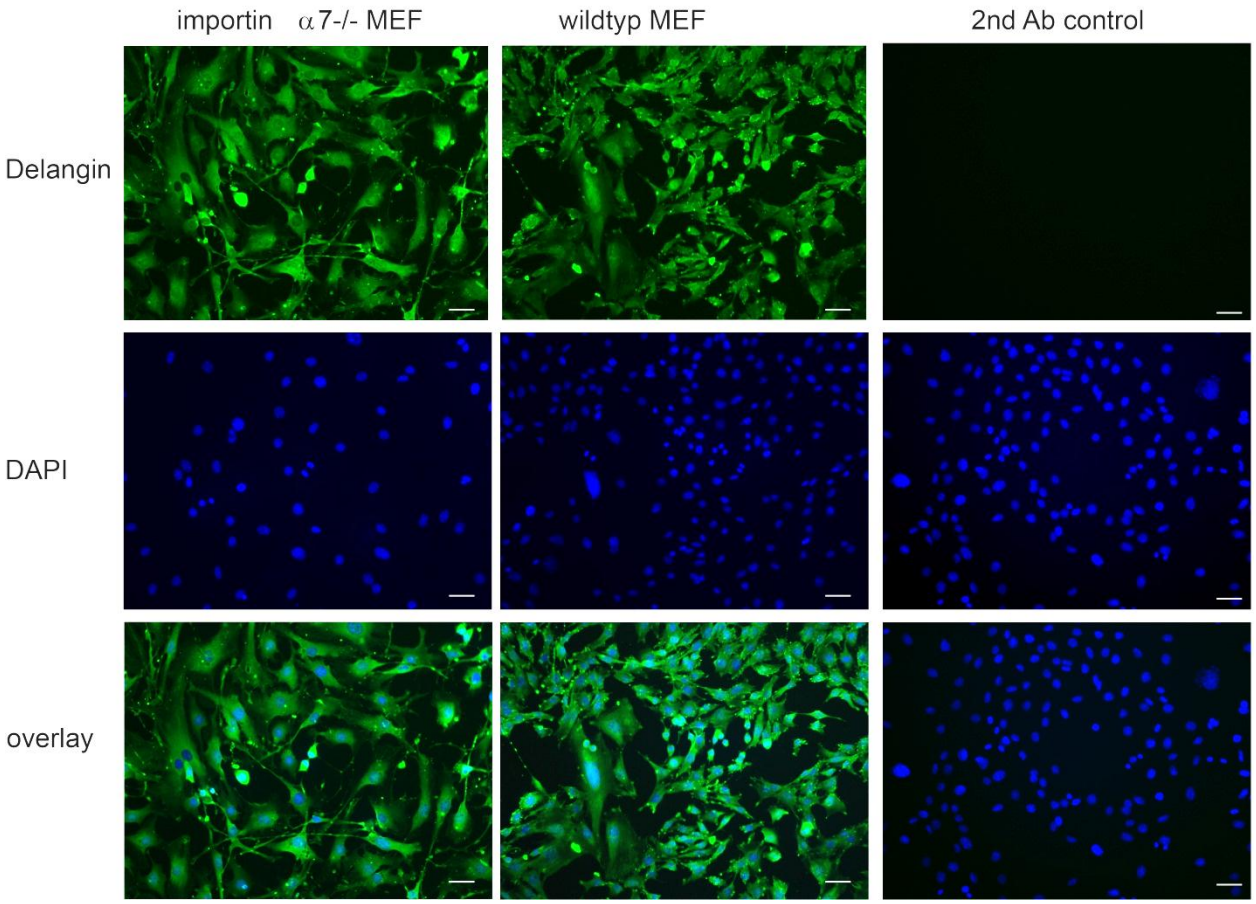


Figure 18: Subcellular localization of Delangin in importin α7-/- MEFs. Wildtype and importin α7-/- MEFs were probed with anti-Delangin antibody. DAPI served as nuclear marker. For the secondary antibody control (2nd Ab control), no primary antibody was used. 20x magnification; scale bar: 50 µm.

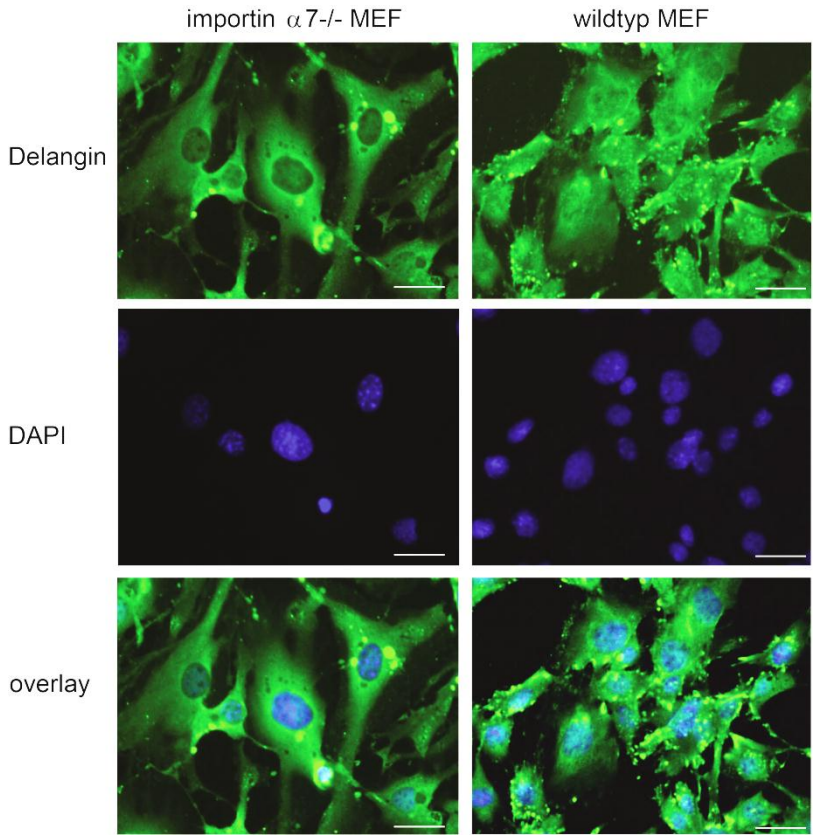


Figure 19: DETAIL: Subcellular localization of Delangin in importin α7-/- MEFs. Wildtype and importin α7-/- MEFs were probed with anti-Delangin antibody. DAPI served as nuclear marker. 40x magnification; scale bar: 50 µm.

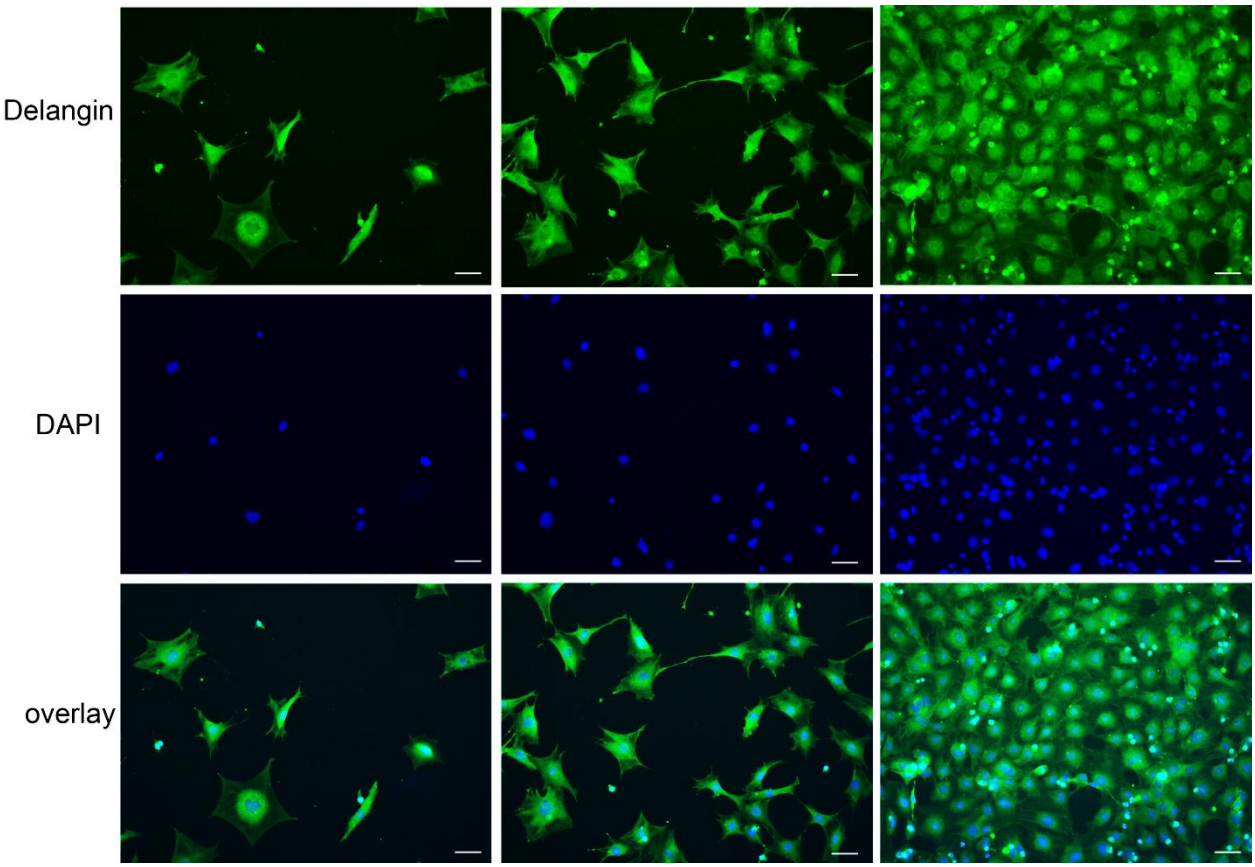


Figure 20: Subcellular localization of Delangin in starved wildtype MEFs. Wildtype MEFs were seeded in low and medium and high densities (pictures from left to right). After serum starvation for 48 hours, cells were probed with anti-Delangin antibody. DAPI served as nuclear marker. For the secondary antibody control (2nd Ab control), no primary antibody was used. 20x magnification; scale bar: 50 µm.

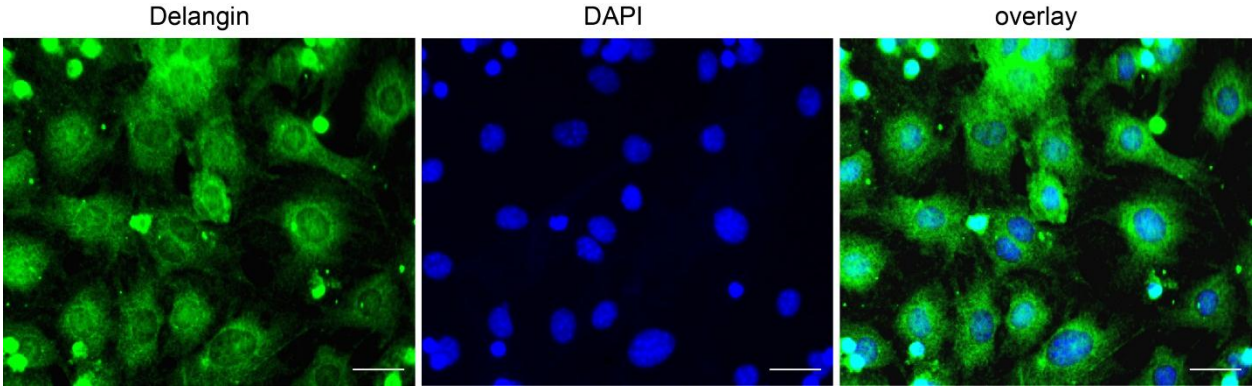


Figure 21: DETAIL (high density): Subcellular localization of Delangin in starved wildtype MEFs. After serum starvation for 48 hours, cells were probed with anti-Delangin antibody. DAPI served as nuclear marker. For the secondary antibody control (2nd Ab control), no primary antibody was used. 40x magnification; scale bar: 50 µm.

1.7. Differences between importin α7 binding partners and importin α2 and α3 substrates

Another important question this study wanted to address was whether identified importin α7 binding partners differ from that of other importin α subtypes. For this purpose, NIH3T3 cells were transfected not only with the expression construct for importin α7, but also with constructs carrying mycHis tagged versions of murine importin α1 (Kpna2), importin α2 (Kpna7), importin α3 (Kpna4), importin α4 (Kpna3) and importin α5 (Kpna1). To verify the expression and correct cellular localization of importin α-mycHis proteins, cytoplasmic and nuclear fractions of transfected cells were analyzed by Western Blot (**Figure 22**). Unexpectedly, the transfection of importin α1, 4 and 5 led to toxic effects in NIH3T3 cells and an appropriate expression of fusion proteins was not possible in that case. Therefore, further co-immunoprecipitation experiments were done with importin α2 and - α3, only.

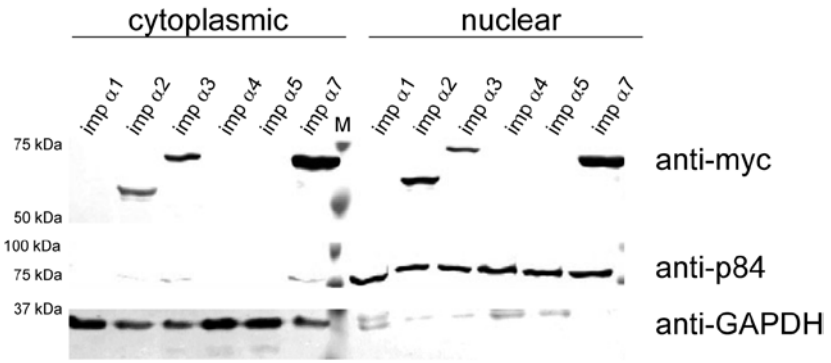


Figure 22: Expression and localization of mycHis-tagged importin α subtypes in NIH3T3 cells. Cells were transfected with importin α-mycHis constructs and expression of fusion proteins was detected by anti-c-myc antibody. p84 served as nuclear marker, GAPDH served as cytoplasmic marker.

Identified proteins were ranked according to their fold change (log2 LFQ intensity importin α2–mycHis/ LFQ intensity mycHis-control and log2 LFQ intensity importin α3–mycHis/ LFQ intensity mycHis-control, respectively) and the top 5% of importin α2 or importin α3 binders found to bind the control as well were included in the final result lists. 137 proteins bound to importin α2-mycHis and whereas 434 proteins were found to bind importin α3 (Appendix Table 3 and 4).

In order to determine proteins that bind importin α7 more effective than the other two importin α subtypes, peptides with a negative log2 fold change impa7/impa2 ratio and -impa7/impa3 ratio were excluded from the importin α7 binding partner list. Hereby, proteins were determined that bind importin α7 preferentially (Appendix Table 5). Potential importin α7 binding partners were ranked according to their intensity and the top 10 importin α7 candidate cargoes are displayed in **Table 7**.

Based on GO-term analysis, nuclear factors were significantly ($p<0.01$) enriched in all result lists (**Table 8** - **Table 12**, **B**). The analysis of importin α7 binding partners deleted of preferentially importin α2 and –α3 binders covered the same enriched biological processes as the first analysis of identified importin α7 binders from ovary (**Table 8, A**). Proteins bound by importin α2 or importin α3, however, seem to be involved in different biological processes than importin α7 (**Table 10 and Table 12, A**). Moreover, among importin α7 binding partners, factors are significantly enriched that are associated with embryonic lethality (**Table 9**) as detected by ToppFun (ToppGene Suite) whereas proteins binding to importin α3 are rather linked to abnormal skeletal and muscle fiber morphology (**Table 11**, Appendix Table 8). No common pathways have been revealed by ToppFun enrichment analysis of proteins bound to importin α2.

Table 7: Top 10 proteins that bound importin α7 preferentially and overlap with importin α7 binding partners from ovary. Importin α7 binding partners identified from fibroblast cells by co-immunoprecipitation were compared with proteins found by importin α7-GST pull-down from ovary lysate. Proteins were ranked according to their fold change and the top 5 % of importin α7 binding partners found to bind also to the GST-control were included as well. Importin α2 and – α3 binding partners identified by co-immunoprecipitation were arranged in the same way and excluded from the list of importin α7 binding partners. Displayed are the top 10 importin α7 binding partners identified in ovary and fibroblast cells that were absent in the list of importin α2 and -3 binding partners (ranked according to LFQ-intensity).

	Gene name	Protein name	Localization	Description	Biological process	LFQ-intensity
1	**Hnrnpu**	Heterogeneous nuclear ribonucleoprotein U	cytoplasm, nucleus, spliceosome	component of coding region determinant (CRD)-mediated complex	mRNA processing	3.76E+10
2	**Lmnb1**	Lamin-B1	intermediate filament, membrane, nucleus	components of the nuclear lamina	positive regulation of JNK cascade	3.29E+10
3	**Hnrnpl**	Heterogeneous nuclear ribonucleoprotein L	cytoplasm, nucleus, spliceosome	component of the heterogeneous nuclear ribonucleoprotein (hnRNP) complexes	mRNA processing	9.71E+09
4	**Mcm3**	DNA replication licensing factor MCM3	nucleus	component of MCM complex, putative replicative helicase essential for 'once per cell cycle' DNA replication initiation and elongation in eukaryotic cells	cell cycle, DNA replication	6.73E+09
5	**Anp32b**	Acidic leucine-rich nuclear phosphoprotein 32 family member B	cytoplasm, nucleus	required for the progression from the G1 to the S phase	chaperone	6.58E+09
6	**Taf2s**	Transcription elongation regulator 1	nucleus	transcription factor that binds RNA polymerase II and inhibits the elongation of transcripts from target promoters	Transcription regulation	5.1E+09
7	**Cdc46**	DNA replication licensing factor MCM5	nucleus	component of MCM complex, putative replicative helicase essential for 'once per cell cycle' DNA replication initiation and elongation in eukaryotic cells	cell cycle, DNA replication	3.8E+09
8	**Dhm1**	5'-3' exoribonuclease 2	nucleus	may promote termination of transcription by RNA polymerase II	Transcription regulation, mRNA processing	3.73E+09
9	**Smarcc2**	SWI/SNF complex subunit SMARCC2	nucleus	chromatin remodeling	Transcription regulation	3.45E+09

10	**Smarca4**	Transcription activator BRG1	nucleus	chromatin remodeling	Transcription regulation	2.21E+09

Table 8: Top 5 enriched GO-terms among preferential importin α7mycHis binding partners identified by co-immunoprecipitation from NIH3T3 cells. 260 proteins analyzed using the ToppFun analysis software from the ToppGene Suite. Proteins that were more abundant among the list of importin α2 or -3 binders were excluded. List entries are ranked according to p-value; p<0.01.

A) Biological Process

	ID	Name	P-value	Term in Query	Term in Genome
1	GO:0016568	chromatin modification	8.41E-20	38	373
2	GO:0051276	chromosome organization	2.64E-19	47	631
3	GO:0006325	chromatin organization	5.56E-18	40	473
4	GO:0006396	RNA processing	9.86E-18	45	626
5	GO:0006397	mRNA processing	9.35E-14	32	382

B) Cellular Component

	ID	Name	P-value	Term in Query	Term in Genome
1	GO:0005654	nucleoplasm	8.99E-40	91	1342
2	GO:0005730	nucleolus	3.01E-37	82	1133
3	GO:0016585	chromatin remodeling complex	1.04E-30	30	99
4	GO:0044451	nucleoplasm part	6.51E-23	55	783
5	GO:0016514	SWI/SNF complex	9.42E-14	10	15

Table 9: Mouse phenotype of importin α7 binding partners. 299 importin α7 binding partners from fibroblast cells were analyzed using the ToppFun analysis software from the ToppGene Suite according to enriched proteins associated with a certain mouse phenotype. List entries are ranked according to p-value; p<0.01.

	ID	**Name**	**P-value**	**Term in Query**	**Term in Genome**
1	MP:0008762	embryonic lethality	9.08E-08	48	1245
2	MP:0001672	abnormal embryogenesis/ development	3.39E-04	43	1319
3	MP:0005380	embryogenesis phenotype	3.39E-04	43	1319
4	MP:0006205	embryonic lethality before somite formation	4.73E-04	18	286
5	MP:0009850	embryonic lethality between implantation and placentation	4.73E-04	21	385

Table 10: Top 5 enriched GO-terms among importin α3mycHis binding partners identified by co-immunoprecipitation from NIH3T3 cells. 434 proteins were analyzed using the ToppFun analysis software from the ToppGene Suite. List entries are ranked according to p-value; p<0.01.

A) Biological Process					
	ID	Name	P-value	Term in Query	Term in Genome
1	GO:0034660	ncRNA metabolic process	6.16E-07	26	276
2	GO:0030029	actin filament-based process	9.47E-06	29	383
3	GO:0006412	translation	4.47E-05	31	461
4	GO:0044085	cellular component biogenesis	1.37E-04	61	1396
5	GO:0051276	chromosome organization	2.09E-04	36	631
B) Cellular Component					
	ID	Name	P-value	Term in Query	Term in Genome
1	GO:0005730	nucleolus	2.85E-11	65	1133
2	GO:0044430	cytoskeletal part	2.93E-04	49	1121
3	GO:0015629	actin cytoskeleton	6.62E-04	22	327
4	GO:0043292	contractile fiber	1.08E-03	14	147
5	GO:0044449	contractile fiber part	1.57E-03	13	131

Table 11: Mouse phenotype of importin α3 binding partners. 434 importin α3 binding partners from fibroblast cells were analyzed using the ToppFun analysis software from the ToppGene Suite according to enriched proteins associated with a certain mouse phenotype. List entries are ranked according to p-value; p<0.01.

	ID	**Name**	**P-value**	**Term in Query**	**Term in Genome**
1	MP:0003084	abnormal skeletal muscle fiber morphology	2.68E-03	15	144
2	MP:0004087	abnormal muscle fiber morphology	6.75E-03	21	287

Table 12: Top 5 enriched GO-terms among importin α2mycHis binding partners identified by co-immunoprecipitation from NIH3T3 cells. 137 proteins were analyzed using the ToppFun analysis software from the ToppGene Suite. List entries are ranked according to p-value; p<0.01.

A: Biological Process					
	ID	Name	P-value	Term in Query	Term in Genome
1	GO:0022415	viral reproductive process	3.99E-05	12	225
2	GO:0030048	actin filament-based movement	1.23E-04	7	57
3	GO:0016032	viral reproduction	8.08E-04	14	416
4	GO:0034621	cellular macromolecular complex subunit organization	1.12E-03	17	634
5	GO:0030029	actin filament-based process	1.91E-03	13	383
B: Cellular Component					
	ID	Name	P-value	Term in Query	Term in Genome
1	GO:0015629	actin cytoskeleton	9.88E-11	19	327
2	GO:0031967	organelle envelope	1.23E-10	27	768
3	GO:0016459	myosin complex	1.23E-10	11	66
4	GO:0031975	envelope	1.77E-10	27	780
5	GO:0005643	nuclear pore	2.31E-07	9	71

1.8. Limited availability of importin α proteins in murine oocytes and zygotes

Little is known as to why multiple importin α isoforms exist in higher eukaryotes, but there is evidence that importin α subtypes have tissue specific expression patterns and distinct physiological roles, especially in fertility and embryonic development [25, 27, 29, 30]. On the transcript level, it has been shown that only a subset of importin α proteins is present in pre-implantation embryos before zygotic genome activation [39]. In order to verify the expression of importin α1, 3, 4, 5 and 7 in murine ovary as well as in oocytes and zygotes on protein level, a Western Blot analysis was performed. Expression of importin α2 in ovaries and pre-implantation embryos has already been reported in mice [25]. All importin α proteins were detected in murine ovary except importin α1 (**Figure 23**). Furthermore, importin α3 and α5

could not be detected in murine oocytes and zygotes whereas importin α1, 4 and 7 are maternally expressed (**Figure 24**).

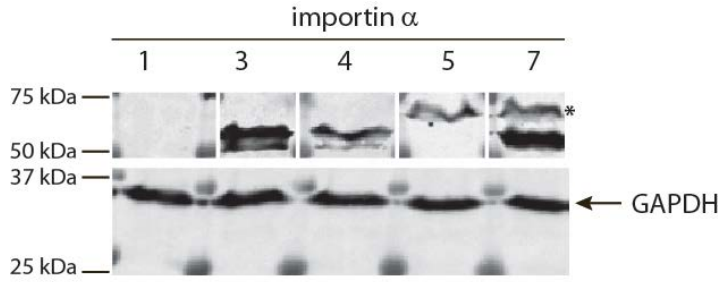


Figure 23: Importin α expression in ovary. Importin α subtypes expressed in murine ovary. GAPDH served as loading control. *: antibody cross-reactivity with importin α5.

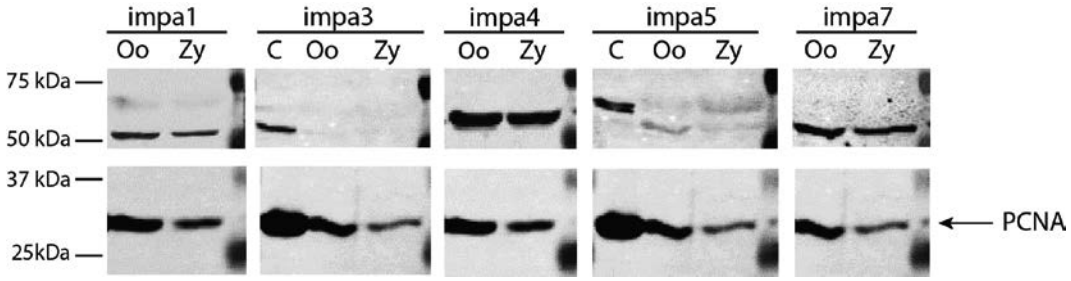


Figure 24: Importin α expression in oocytes and zygotes. Importin α subtype expression in murine oocytes (Oo) and zygotes (Zy). Pcna served as loading control; C: positive control = NIH3T3 cell lysate; impa1-7: importin α1-7.

1.9. Brg1 bound importin α7, but not other maternally expressed importin α subtypes *in vitro*

Among candidate proteins, Brg1 (also known as smarca4 or baf190a) was identified as an importin α7 interacting protein in both the GST pull-down and co-immunoprecipitation experiments (**Table 5**). Like importin α7, Brg1 was reported to be a maternal effect gene whose depletion leads to a two-cell arrest in murine embryos with disturbed zygotic genome activation [69]. Brg1 appeared to be much more abundant in the eluate of importin α7 co-immunoprecipitated proteins than compared to importin α2 or 3 co-immunoprecipitated proteins (**Table 7**). Hence, Brg1 represents a potential importin α7 specific cargo and was selected for further analysis.

Besides importin α7, importin α1, 2 and 4 have been shown to be expressed in oocytes and zygotes (**Figure 24**) [27, 39]. To figure out whether one of the other maternally expressed importin α subtypes can bind to Brg1 as well, an *in vitro* binding assay has been performed. The data confirmed binding of Brg1 to importin α7-GST but no binding of Brg1 was observed to importin α1-, 2- or 4-GST (**Figure 25**).

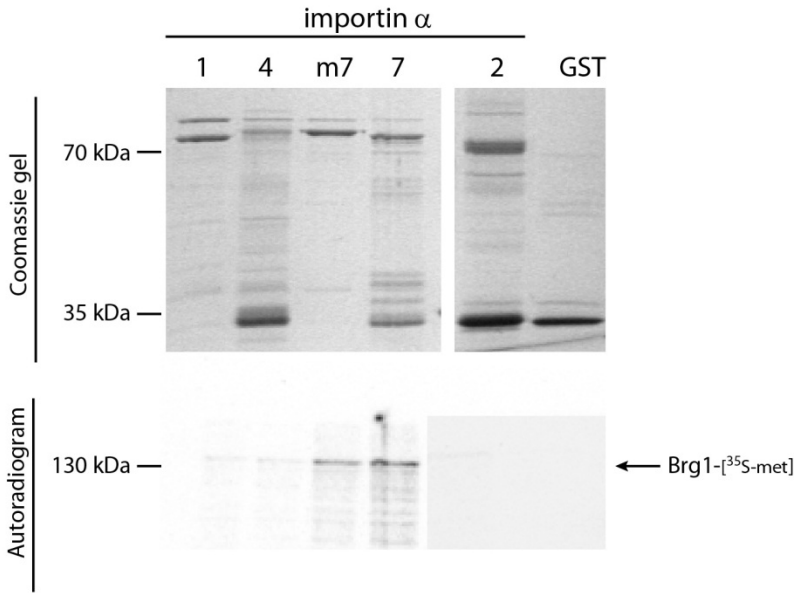


Figure 25: Importin α-GST *in vitro* binding assay. *In vitro* transcribed and translated Brg1 was incubated with human recombinant importin α-GST proteins immobilized on glutathione sepharose beads. In addition, murine importin α7 (m7) was examined as well. Detection of bound radioactively labeled Brg1 (Brg1-[^{35}S-met]) was performed by autoradiography. Only maternally expressed importin α subtypes were analyzed. Recombinant GST served as negative control.

1.10. Normal Brg1 nuclear localization in importin α7 -/- oocytes

In murine fibroblast cells, immunocytochemical staining revealed normal nuclear staining of Brg1 (**Figure 14** - **Figure 26**). However, nuclear import of Brg1 may be taken over by another importin α protein in these cells. In murine oocytes and zygotes, only certain importin α subtypes are expressed (**Figure 24**) [27, 39]. Based on the developmental phenotype of fetuses from importin α7-/- mice, an importin α7 dependent nuclear import of Brg1 in pre-

implantation embryos was considered. Therefore, the subcellular localization of Brg1 in unfertilized germinal vesicle (GV) oocytes was examined by immunocytochemistry. However, in GV-oocytes from importin α7 null females, Brg1 staining was clearly detected in the nucleus and did not differ from control cells (**Figure 26**).

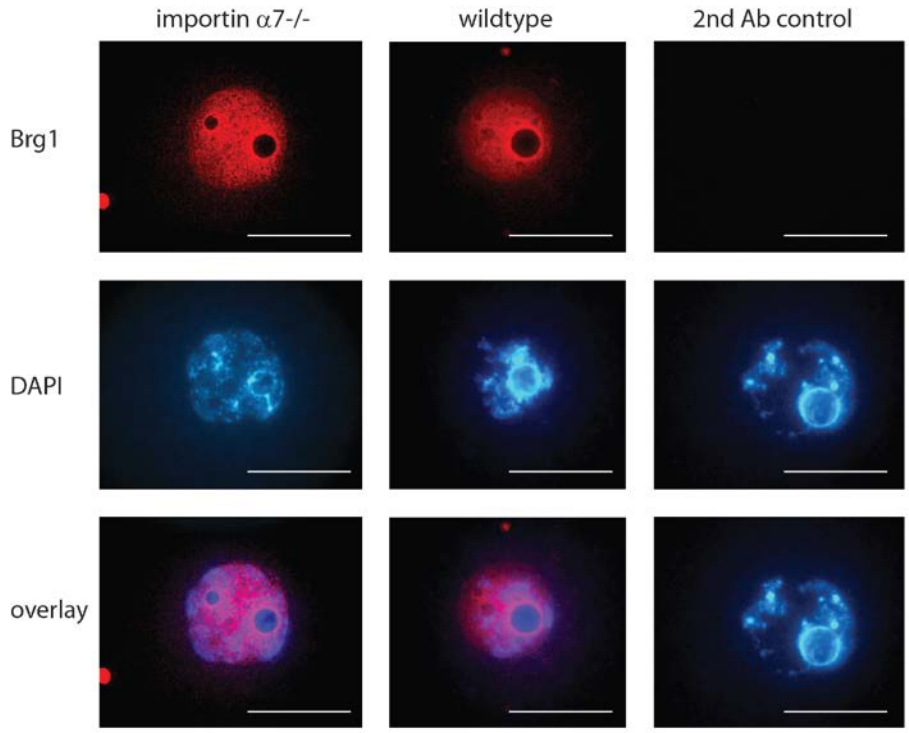


Figure 26: Nuclear Brg1 staining in importin α7-/- GV-oocytes. Germinal vesicle (GV) oocytes from wild type and importin α7-/- embryos were probed with anti-Brg1 antibody. DAPI staining served as nuclear marker. For the secondary antibody control (2nd Ab control), no primary antibody was used; scale bar: 50 µm.

1.11. Proteomic analysis of importin α7 depleted MEFs – SILAC approach

Another approach to identify importin α7 dependent cargos is to examine the nuclear proteome of importin α7-/- MEF via stable isotope labeling by amino acids in cell culture (SILAC). Hereby, proteins will be identified that are abundant in the nucleus of wild type cells but decreased in nuclear fractions of importin α7-/- MEFs. For this purpose, wild type MEF were cultured for several passages in medium with heavy amino acids whereas importin α7-/- MEF were grown in light amino acid containing medium. After verifying the complete labeling status of wild type MEF and absence of heavy amino acids in importin α7-/- MEF by

mass spectrometric analysis, cytoplasmic and nuclear fractions were prepared and analyzed. Identified proteins were ranked according to heavy / light signal intensities. Proteins which were quantified with less than 3 SILAC counts were excluded. The analysis resulted in 536 proteins that were at least 1.5 fold decreased in the nuclear fractions of importin α7-/- MEFs (Appendix Table 6) and 483 proteins that were at least 0.5 fold increased (Appendix Table 7). Further GO-term analysis revealed a significant enrichment of proteins functioning in cell cycle processes or chromosome organization (**Table 13, A**).

Out of that list, NLS cargoes were identified via ToppFun whose mouse phenotypes are associated with embryonic lethality (**Table 14**) and hence may help to elucidate the phenotype of importin α7 knockout mice.

Furthermore, the result lists that were generated by all three different approaches, importin α7-GST pull-down, importin α7 co-immunoprecipitation and SILAC were compared and resulted in 5 proteins listed in **Table 15**. These proteins represent candidates that were identified to bind importin α7 in ovary and fibroblast cells and moreover, were decreased in the nuclei of MEFs depleted from importin α7.

Table 13: Top 5 enriched GO-terms among nuclear proteins decreased in importin α7-/- MEFs. 536 proteins analyzed using the ToppFun analysis software from the ToppGene Suite; p<0.01

A) Biological Process

	ID	Name	P-value	Term in Query	Term in Genome
1	GO:0007049	cell cycle	4.47E-14	91	1249
2	GO:0006259	DNA metabolic process	1.38E-12	61	676
3	GO:0022402	cell cycle process	3.10E-12	73	934
4	GO:0000278	mitotic cell cycle	3.84E-12	61	691
5	GO:0051276	chromosome organization	5.02E-11	56	631

B) Cellular Component

	ID	Name	P-value	Term in Query	Term in Genome
1	GO:0005654	nucleoplasm	8.35E-16	96	1342
2	GO:0044427	chromosomal part	9.07E-16	53	472
3	GO:0005694	chromosome	2.84E-15	57	555
4	GO:0000228	nuclear chromosome	1.24E-12	33	224
5	GO:0044454	nuclear chromosome part	1.03E-11	29	184

Table 14: Potential importin α7 dependent NLS-cargoes with lethal phenotypes in the mouse. 536 proteins were analyzed using ToppFun analysis software from the ToppGene Suite. Proteins were selected according to associated mouse phenotype "embryonic lethality; MP:0008762". This table includes only proteins with known NLS (extracted from *The Nuclear Protein Database* (http://npd.hgu.mrc.ac.uk/user/).

	Gene Name	Protein name	Localization	Function	Decreased by factor
1	**Terf2**	telomeric repeat binding factor 2	Nuc	central role in telomere maintenance and protection against end-to-end fusion of chromosomes	2.551
2	**Ikbkap**	inhibitor of kappa light polypeptide gene enhancer in B-cells, kinase complex-associated protein	Nuc/Cyt	May act as a scaffold protein that may assemble active IKK-MAP3K14 complexes. cts as subunit of the RNA polymerase II elongator complex, which is a histone acetyltransferase component of the RNA polymerase II	2.390
3	**Rpa1**	replication protein A1, 70kDa	Nuc	DNA-dependent RNA polymerase	2.052
4	**Apex1**	APEX nuclease (multifunctional DNA repair enzyme) 1	Nuc/ER/Cyt/ Mito	Multifunctional protein that plays a central role in the cellular response to oxidative stress	1.975
5	**Gtf2i**	general transcription factor IIi	Nuc/Cyt	Interacts with the basal transcription machinery by coordinating the formation of a multiprotein complex at the C-FOS promoter, and linking specific signal responsive activator complexes	1.970
6	**Rbpj**	recombination signal binding protein for immunoglobulin kappa J region	Nuc/Cyt	Transcriptional regulator that plays a central role in Notch signaling, a signaling pathway involved in cell-cell communication that regulates a broad spectrum of cell-fate determinations.	1.954
7	**Uhrf1**	ubiquitin-like with PHD and ring finger domains 1	Nuc	Putative E3 ubiquitin-protein ligase. May participate in methylation-dependent transcriptional regulation. Important for G1/S transition. May be involved in DNA repair and chromosomal stability. May have E3 ubiquitin ligase activity specific for histone H3	1.902
8	**Usp7**	ubiquitin specific peptidase 7 (herpes virus-	Nuc/Cyt	Hydrolase that deubiquitinates target proteins such as FOXO4, p53/TP53, MDM2, ERCC6, PTEN and DAXX	1.863

		associated)			
9	**Chaf1a**	chromatin assembly factor 1, subunit A (p150)	Nuc	mediate chromatin assembly in DNA replication and DNA repair	1.851
10	**Atr**	ataxia telangiectasia and Rad3 related	Nuc	DNA damage sensor	1.818
11	**Mcm4**	minichromosome maintenance complex component 4	Nuc	cts as component of the MCM2-7 complex (MCM complex) which is the putative replicative helicase essential for 'once per cell cycle' DNA replication initiation and elongation in eukaryotic cells	1.792
12	**Dnmt1**	DNA (cytosine-5-)-methyltransferase 1	Nuc	modification of chromatin	1.755
13	**Chd8**	chromodomain helicase DNA binding protein 8	Nuc	remodeling of chromatin	1.605
14	**Rif1**	RAP1 interacting factor homolog (yeast)	Nuc	Required for checkpoint mediated arrest of cell cycle progression in response to DNA damage during S-phase	1.603
15	**Cabin1**	calcineurin binding protein 1	Nuc	replication-independent chromatin assembly	1.578
16	**Fen1**	flap structure-specific endonuclease 1	Nuc	Structure-specific nuclease with 5'-flap endonuclease and 5'-3' exonuclease activities involved in DNA replication and repair.	1.573
17	**Csda**	cold shock domain protein A	Nuc/Cyt	Binds to the GM-CSF promoter. Seems to act as a repressor. Binds also to full length mRNA and to short RNA sequences containing the consensus site 5'-UCCAUCA-3'. May have a role in	1.559

				translation repression	
18	**Ash2l**	ash2 (absent, small, or homeotic)-like (Drosophila)	Nuc	modification of chromatin	1.525
19	**Upf1**	UPF1 regulator of nonsense transcripts homolog (yeast)	Chromatin/ Cyt/P-body	RNA-dependent helicase and ATPase required for nonsense-mediated decay (NMD) of mRNAs containing premature stop codon	1.522
20	**Parp1**	poly (ADP-ribose) polymerase 1	Nuc	base excision repair	1.517

Table 15: Overlay of nuclear proteins less abundant in importin α7-/- MEF that bind importin α7 in ovary and fibroblast cells. Comparison of proteins identified by SILAC to be less abundant in MEFs depleted of importin α7 and importin α7 binding partners identified by GST pull-down from ovary lysate and co-immunoprecipitation from fibroblast cells.

	Gene name	Protein name	Localization	Description	Biological process	Decreased by factor
1	**Anp32e**	Acidic leucine-rich nuclear phosphoprotein 32 family member E	cytoplasm, nucleus	Inhibits activity of protein phosphatase 2A (PP2A)	phosphatase inhibitor activity	1.6786
2	**Ash2l**	Set1/Ash2 histone methyltransferase complex subunit ASH2	nucleus	Component of Set1/Ash2 histone methyltransferase (HMT) complex that specifically methylates 'Lys-4' of histone H3, but not if the neighboring 'Lys-9' residue is already methylated. As part of the MLL1/MLL complex it is involved in methylation and dimethylation at 'Lys-4' of histone H3.	Chromatin regulator / Transcription regulation	1.525
3	**Chd3**	Chromodomain-helicase-DNA-binding protein 3	nucleus, cytoplasm, cytoskeleton, centrosome	Component of histone deacetylase NuRD complex which participates in remodeling of chromatin by deacetylating histones.	centrosome organization / transcription regulation /	1.6931

				Required for anchoring centrosomal pericentrin in both interphase and mitosis, for spindle organization and centrosome integrity.	spindle organization	
4	**Mcm3**	DNA replication licensing factor MCM3	nucleus	component of MCM complex, putative replicative helicase essential for 'once per cell cycle' DNA replication initiation and elongation in eukaryotic cells	cell cycle, DNA replication	1.9935
5	**Smarcc1**	SWI/SNF complex subunit SMARCC1	nucleus	ATP-dependent helicase, transcription activator as part of the SWI/SNF chromatin remodeling complex	chromatin regulator	1.5334

1.12. Summary and Discussion Part A

Substrate specificities among importin α subtypes have been postulated to allow the cell a more flexible control over nuclear cargo import in response to different environmental stimuli [70].

While most importin α isoforms are ubiquitously expressed in different organs, their expression levels differ and the expression of some importin α subtypes are even restricted to specific tissues [20, 27] and particular time points of development [28], respectively. It is therefore likely that importin α subtypes import individual cargoes. However, the identities of these cargoes remain largely unknown.

The specific use of single importin α subtypes has also been described for viruses, such as the influenza virus whose polymerase subunit PB2 and the nucleoprotein (NP) are specifically imported to the nucleus by importin α7 offering novel opportunities for antiviral therapies [71].

The depletion of importin α7 in mice strongly supports the hypothesis that importin α isoforms transport unique subsets of intracellular cargoes in higher eukaryotic organisms. Since embryos derived from female mice lacking importin α7 show a developmental arrest at the two-cell stage despite the presence of other α importins, there may be intracellular cargoes that rely on this particular adapter protein for nuclear import. Therefore, one aim of this study was to use *in vitro* and *in vivo* screening approaches to identify possible importin α7 specific binding partners.

1.12.1. Importin α7 binding partners play a role in RNA processing, chromosome organization and chromatin remodeling

Since importin α7 knockout females have a severe fertility problem, the first approach to identify importin α7 interaction partners was comprised of a GST pull-down experiment using ovary lysate as source of prey proteins. As expected for a nuclear transport receptor, GO-term analysis regarding cellular localization of identified importin α7 binders revealed a high proportion of nuclear proteins. Interestingly, the majority of these nuclear proteins appeared to be involved in RNA processing, chromosome organization and chromatin remodeling; such

processes are known to be dynamically regulated during early embryonic development and during zygotic genome activation.

To get a more comprehensive picture of importin α7 binding partners, importin α7 cargoes were searched from a cultured murine cell line using a co-immunoprecipitation screening approach. Importin α7-/- MEFs would have been a suitable system to perform an importin α7-mycHis co-immunoprecipitation experiment due to the absence of possibly disturbing endogenous importin α7 protein. However, first transfection attempts showed that the transfection efficiency of importin a7-/- MEFs is very low (< 10%, using Lipofectamine LTX) and hence the established murine fibroblast cell line NIH3T3 was used. The functional enrichment analysis revealed the same biological processes among importin α7-mycHis binders as in the first result list from importin α7-GST pull-down with murine ovary lysate. 36% of identified proteins match the list of importin α7-GST interaction partners from ovary lysate. Since both screening approaches can cover only a subset of proteins present in a cell or tissue, 36% overlapping proteins display a relatively high degree of congruency. Almost all overlapping proteins are known to localize to the nucleus. Moreover, approximately half of these factors own a NLS and are therefore likely to be imported by importin α7. Among NLS cargoes, also nuclear membrane components like Lmnb1 or Lmn1 appeared. These proteins are supposed to be targeted to the inner nuclear membrane via the classical nuclear protein import pathway [72, 73]. Furthermore, the importin α7 binding partner list contained Npap60 (**Table 5**; 11) which is known to support the cargo release from importin α [12-14].

Protein Database entries are not complete and many genes have not yet been examined according to the existence of a potential NLS. Therefore, the real number of NLS-cargoes among candidate genes may be even higher. Nevertheless, proteins without NLS should not be excluded for further investigations since NLS patterns can be very complex and may only form in a certain three dimensional structure as it is the case for STAT1 [43, 45, 47, 48].

In the list of importin α7 binding partners, also Emp2 and Atxn2l appeared that seem to be membrane proteins (**Table 5**; 92, 104). These proteins may be isolation artifacts or they were part of a protein complex which contained a NLS bearing cargo that bound to importin α7.

The ranking of importin α7 candidate cargoes according to their LFQ intensities from the ovary lysate pull-down is supposed to mirror their abundance and maybe even importance as importin α7 interaction partners in the mouse ovary. However, since the protein expression profiles between individual mice can differ, the composition and abundance of peptides does not need to be equal in each experiment. Therefore, this ranking of candidate importin α7 cargoes can help to provide a first indication to select proteins that will be examined in more detail; but it should not be taken as selection criterion on its own.

All candidates analyzed did enter the nucleus despite the absence of importin α7 in MEFs. The reasons for these results can be manifold. Remaining importin α proteins may be able to take over the nuclear import of cargoes. Especially importin α5 or importin α1 may compensate the loss of importin α7 since both proteins seemed to be upregulated in importin α7 KO MEFs. The decreased importin α4 levels may be based on the reduced proliferation of importin α7 KO MEFs. In other cells and tissues, the situation can be completely different and substrate specificities may become visible according to the availability of importin α subtypes. Furthermore, importin α7 candidate cargoes may be transported pickaback by another importin α7 cargo as part of a bigger protein complex. On the other hand, alternative transport factors might exist that can be used by the cargoes, as well. Hence, the analysis of identified importin α7 candidate cargoes need to be further validated in importin α7 deficient oocytes and embryos.

1.12.2. Brg1 is one representative cargo in pre-implantation embryos that preferentially binds importin α7

Brg1 was found in both the GST pull-down and co-immunoprecipitation screen as a protein that interacted with importin α7. Interestingly, Brg1 is one of the first identified maternal effect genes in mice and part of the ATP-dependent chromatin remodeling SWItch/Sucrose Non Fermentable (SWI/SNF) complex. This complex uses the energy of ATP hydrolysis to locally disrupt or alter the association of histones with DNA. Like embryos from importin α7 knockout females, zygotes of Brg1 depleted females display a two-cell arrest [69]. Furthermore, it has been shown that Brg1 regulates zygotic genome activation and controls the expression of genes involved in transcription, RNA processing, and cell cycle regulation. Hence, Brg1 was chosen to

be analyzed in more detail in order to determine if depletion of importin α7 perturbs the intracellular localization of endogenous Brg1. However, nuclear detection of Brg1 in importin α7 depleted MEFs shows that Brg1 is not solely dependent on the nuclear import by this particular transport receptor. Furthermore, although oocytes and zygotes lack at least some of the importin α dependent pathways, also here Brg1 can enter the nucleus. Since Brg1 did specifically bind to importin α7 *in vitro* and not to the remaining maternally expressed importin α subtypes, Brg1 is suggested to have a distinct nuclear import mechanism in pre-implantation embryos. Brg1 may enter the nucleus in complex with other NLS-bearing importin α7 substrates or Brg1 is transported by an alternative nuclear import receptor. Nevertheless, importin α7 may serve a role in regulating the amount of Brg1 that enters the nucleus at critical times in development, or individual splice variants of Brg1 are trafficked specifically by importin α7 *in vivo.* For this reason, further investigations of Brg1 in different stages of ovarian follicle development are planned.

1.12.3. Importin α7 cargo sets differ from that of other importin α subtypes

A disadvantage of the importin α7 binding partner screening approach is that it is not clear whether the identified proteins bind importin α7 subtype specifically. The examination of importin α7 candidate cargoes in importin α7 knockout MEFs showed that all proteins selected were able to enter the nucleus. Apparently, other transport receptors, most probably remaining importin α proteins, are able to compensate for the loss of importin α7. Therefore, the cargo sets of all importin α subtypes were set out to be analyzed by the co-immunoprecipitation approach. However, the expression of importin α1, 4 and 5 was not possible in NIH3T3 cells. After transfection of the cells, a massive toxic effect was noted that was most probably caused by the overexpression of the α importins. Recently it was reported that importin β negatively regulates multiple aspects of mitosis and its overexpression inhibited the GTPase-activating protein RANGAP1 recruitment to mitotic kinetochores in HeLa cells [74]. It will be interesting to study, if similar defects are elicited by the overexpression of certain α importins.

Nevertheless, importin α2 and importin α3 binding partners were identified from fibroblast cells. Excitingly, the biological process enrichment analysis revealed that proteins binding importin α7 differ from binding partners of the other two α importins. Not only the biological processes differed among cargo sets of individual importin α subtypes but also associated mouse phenotypes. Consistent with the developmental arrest of embryos from importin α7 knockout females, importin α7 binding partners were found to be linked to embryonic lethality. Importin α3 binding cargoes, in contrast, seem to be associated with abnormal skeletal and muscle fiber morphology. It has to be mentioned that the analysis of gene sets using enrichment algorithms like ToppFun is limited by the quality of underlying online databases. Annotations of genes are incomplete or redundant and not in all cases experimentally proven. That means, results from functional enrichment analysis do not play an absolute, decision making role; they rather play an advisory role. Nevertheless, it could be shown that the patterns of cargoes from individual importin α subtypes differ. These data support a molecular model in which importin α transport receptors represent molecular hubs for different cellular pathways.

1.12.4. Loss of importin α7 leads to disturbed nuclear import of cell-cycle regulators and chromosome organization factors in MEFs

Another attempt to identify importin α7 dependent cargoes was the quantitative analysis of nuclear proteins from MEFs depleted of importin α7 via a SILAC approach. Interestingly, factors were less abundant in nuclei of importin α7 knockout MEF that play a role in cell-cycle regulation and chromosome organization. These findings fit well to the suggested alternative roles of α importins during mitosis and also would help to explain the proliferation phenotype of importin α7-/- MEFs. This difference in growth behavior between wildtype and importin α7 knockout MEFs, on the other hand, may also lead to a certain fuzziness of results. Hence, it is planned to perform an additional SILAC approach that includes samples from wildtype MEFs adjusted to the slow growth conditions of importin α7 knockout MEFs.

Among proteins decreased in importin α7-/- MEFs, five proteins appeared also in the list of importin α7 binding partners from murine fibroblasts and ovary (**Table 15**): Mcm3, Anp32e,

Smarcc1, Chd3 and Ash2l (ranked according to their LFQ intensities from ovary lysate **Table 5**). All proteins contain at least one NLS and are therefore likely to interact with importin α7.

Mcm3 belongs to the evolutionary conserved minichromosome maintenance (MCM) complex consiting of six subunits (MCM2 to MCM7) that are required for the initiation and elongation of DNA replication. The MCM complex ensures that DNA is replicated only once per cell cycle and promotes genome stability [75]. Thereby, it is part of the pre-replication complex at origins and furthermore it functions as a helicase associated with replication forks. Mcm3 depletion is embryonic lethal in mice [76, 77]. In general, all importin α7 binding partners being essential genes represent good candidate cargoes that might play a role in the phenotype of importin α7 mice. It is not possible to analyze the role of essential proteins in fertility by targeted gene deletion. However, the nuclear import of such proteins (like Mcm3) may be dependent on importin α7 at certain time points of development and in particular tissues like ovaries. Further investigations of the Mcm3 subcellular distribution in MEFs, embryos and ovaries are planned.

Smarcc1 and Ash2l are essential for murine embryonic development, as well (**Table 14**). Smarcc1, also known as BRG1-associated factor 155, is as a core subunit of the SWI/SNF complex. Mice with a null mutation in the Smarcc1 locus develop to the early implantation stage but undergo rapid degeneration thereafter [78]. Furthermore, heterozygous mutant embryos display defects in brain development with abnormal organization of the brain. Ash2I, on the other hand, is a component of the Set1/Ash2 histone methyltransferase (HMT) complex that epigenetically regulates transcription via methylation of histone lysine residues. Ash2l knockout mice die early during gestation [79]. Like in the case of Mcm3, it is planned to further examine the cellular distribution of Smarcc1 and Ash2l in MEFs, embryos and ovaries

The leucine-rich acidic nuclear protein (LANP)-like Anp32e belongs to a big Anp32 protein family and shares 58% sequence homology to its founding member Anp32a (LANP, also known as PHAP I) [66]. As shown in part 1.6, Anp32e is able to enter the nucleus independently of importin α7 (**Figure 13**). However, the nomenclature of Anp32 proteins is ambiguous and therefore it was not easy to select the proper antibody for immunocytochemical stainings. The antibody used in this experiment did not distinguish between Anp32a (PHAP I), Anp32b (PHAP l2a) and Anp32e (also known as Cpd1/PHAP III). Therefore, the staining should be repeated

with an isoform specific antibody. Anp32e seems to be involved in the signaling pathway that directs cerebellar neuron differentiation [80]. Moreover, Anp32e is thought to fulfill similar functions in the cell as Anp32a like for example the inhibition of the protein phosphatase 2A (PP2A). However, Anp32e knockout mice are viable and fertile but display subtle neurological clasping phenotype and mild motor deficits [81]. These data do not really fit to the phenotype of importin α7 deficient mice. However, one has to point out that the characterization of importin α7-/- mice has been focused on the female infertility so that mild deficits in fine motor functions may have been overlooked. Furthermore, Anp32e has been reported to bind to importin α1 and importin α5 but not to the third subfamily of importin α proteins [66]. Based on these data, Anp32e is supposed to be able to use several importin α subtypes for nuclear import. Nevertheless, it needs to be clarified whether Anp32e is specifically imported by importin α7 in living cells.

The chromodomain helicase DNA (CHD) binding protein 3 (Chd3) belongs to the CHD family of ATP-dependent chromatin remodelers which is further divided into three subfamilies according to the presence of structural motifs [77]. Chd3 (also known as Mi-2α) belongs to the third subfamily of CHD proteins and lacks a DNA binding domain but instead it contains two plant homeodomains (PHD) that are zinc-finger-like motifs. It is part of the Nucleosome Remodeling and histone Deacetylation (NuRD) complex that leads to transcriptional repression and couples ATP-depended chromatin remodeling by Chd3/4 (Mi-2α/β) with histone deacetylation catalyzed by HDAC1/2 (reviewed in [82]); proteins that were likewise identified as importin α7 binding partners (**Table 5**). Moreover, Chd3 is needed to anchor pericentrin to centrosoms in both interphase and mitosis, for spindle organization and centrosome integrity [83]. Mi-2 homologues (Chd3 and Chd4) play essential roles in development of *C. elegans* and *D. melanogaster [84, 85]*. Both proteins are involved in the determination of vulval cell fate during development and the maternal absence of the worm Mi-2 protein LET-418 (ortholog of murine Chd4) let to sterility and developmental stop of *C. elegans* larvae. In the fruit fly, *drosophila* Mi-2 (ortholog of murine Chd4) appears to be essential for viability whereas *drosophila* Chd3 (ortholog of murine Chd3) deletion had no effect on viability or fertility [84]. Preliminary data of a gene deletion study in mice showed that loss of Chd4 is embryonic lethal [86]. Gene deletion

studies of Chd3 in mice do not seem to be performed, yet. According to invertebrate data, Chd3 gene deletion might not be embryonic lethal in mice. However, Mi-2 genes share only approximately 50-60% sequence similarity to the murine orthologs (WormBase web site, http://www.wormbase.org, release WS233; euGenes, version 3.204 [87]). Thus, it can't be ruled out that Chd3 plays an essential role in murine embryo development. Therefore, Chd3/Chd4 will be examined in more detail according to their subcellular distribution in MEFs, embryos and ovaries, as well.

1.12.5. Conclusion and Future Perspectives

In conclusion, an interesting set of potential importin α7 dependent cargoes could be identified by using a combined approach of *in vitro* and *in vivo* binding partner screens supplemented by the quantitative proteome analysis via SILAC. Unfortunately, it was not possible to demonstrate an importin α7 specific nuclear import of a cargo, in this study. The examination of Brg1, that binds importin α7 and no other maternally expressed importin α subtypes in oocytes and zygotes, demonstrated that alternative transport pathways may compensate for the loss of one importin α protein. However, the examination of potential importin α7 candidate cargoes has just begun and is part of ongoing research. Future experiments will clarify the dependence of Mcm3, Anp32e and the Mi-2 proteins Chd3 / Chd4 to be imported into the nucleus of oocytes and embryos lacking importin α7. Moreover, upcoming studies will have the aim to clarify the composition of cargo sets from remaining importin α subtypes.

2. Analysis of published importin α substrate specificities (Part B)

The probably best way to proof that a cargo is imported into the nucleus by a specific importin α subtype is to check its nuclear transport in a cell line depleted of the corresponding importin α protein. Therefore, further homozygous importin α knockout MEF cell lines were generated in addition to importin α7-/- MEF. Since the knockout mouse models for importin α4 [39], α5 [88] and recently α3 (unpublished data) have been generated in our lab, importin α4-/-, α5-/- and α3-/- MEFs were generated and the absence of the corresponding importin α subtype was confirmed by PCR (**Figure 27**). As observed by common cell cultivation, all importin α KO MEFs showed a slightly reduced growth behavior when compared to wildtype MEFs. The reason for these phenotypes, however, is unclear and was not further examined, yet.

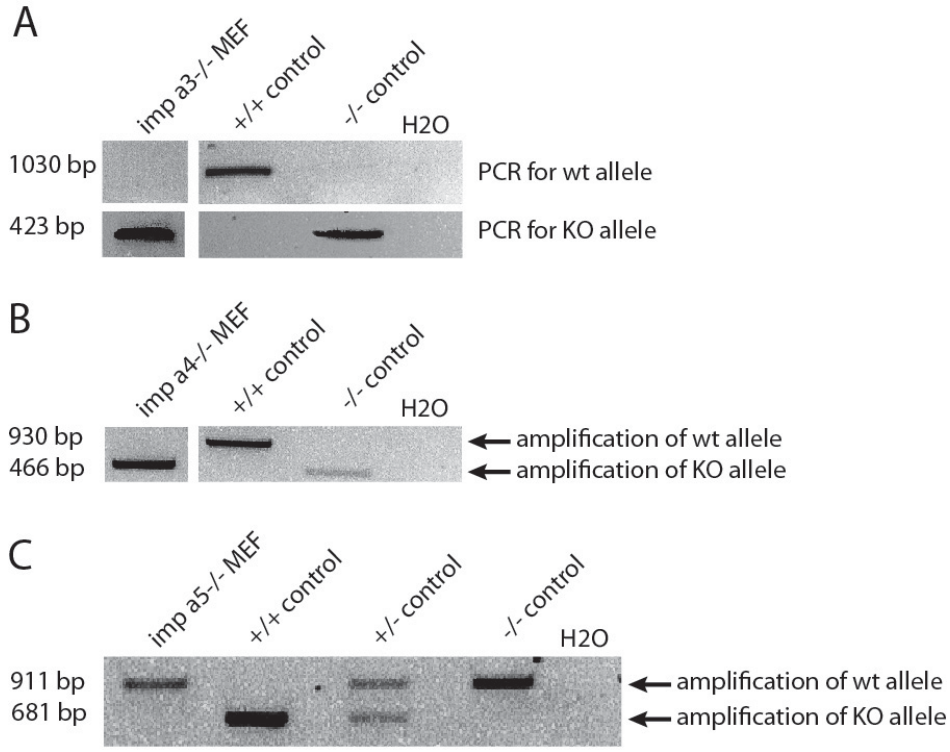


Figure 27: Genotyping PCR of importin α3, -4, and 5 KO MEFs. MEFs were genotyped by PCR amplification of a DNA fragment spanning the insertion site of the (A) importin α3 KO (imp a3 -/-) cassette for detection of the wild type (wt) allele (upper panel). To detect the importin α3 KO allele, a DNA fragment was amplified spanning an intronic part of the importin α3 gene and the inserted KO cassette (lower panel). In order to genotype importin α4 (B) and α5 KO MEFs (C), three primer PCRs were performed allowing the detection of the wt and the KO allele in one reaction. Wildtype (+/+ control) and corresponding importin α KO (-/- control) and importin α heterozygous KO (+/- control) tissue DNA served as controls. Water (H2O) was used to ensure the absence of DNA contaminations in the PCR mix.

2.1. Nuclear localization of Keap1 despite the absence of importin α7

Upon oxidative stress, Nrf2 accumulates in the nucleus where it activates genes involved in anti-oxidative stress response. The key regulator of Nrf2 signaling, Keap1 was reported to be specifically imported into the nucleus by importin α7 [56]. These findings were based on human cells silenced for importin α7 gene expression by RNAi. To verify this importin α substrate specificity, Keap1 nuclear level were analyzed in importin α7 knockout MEFs. Keap1 contains a very strong NES leading to a quick export of Keap1 from the nucleus. Hence, cells need to be treated with leptomycin B (LMB) that blocks the classical nuclear export of proteins. Wild type and importin α7-/- MEFs were treated with 50 nM LMB and cytoplasmic and nuclear fractions were analyzed by Western Blot. In both cell lines, the nuclear export of Keap1 was blocked and Keap1 was detectable in the nuclear fraction (**Figure 28, A**). Quantification of the infrared antibody signals from Western Blots was done using the Odyssey scanner software. However, no difference in nuclear Keap1 level was detectable between wild type and importin α7-/- MEFs (**Figure 28, B**). For this reason, Nrf2 signaling was not further analyzed.

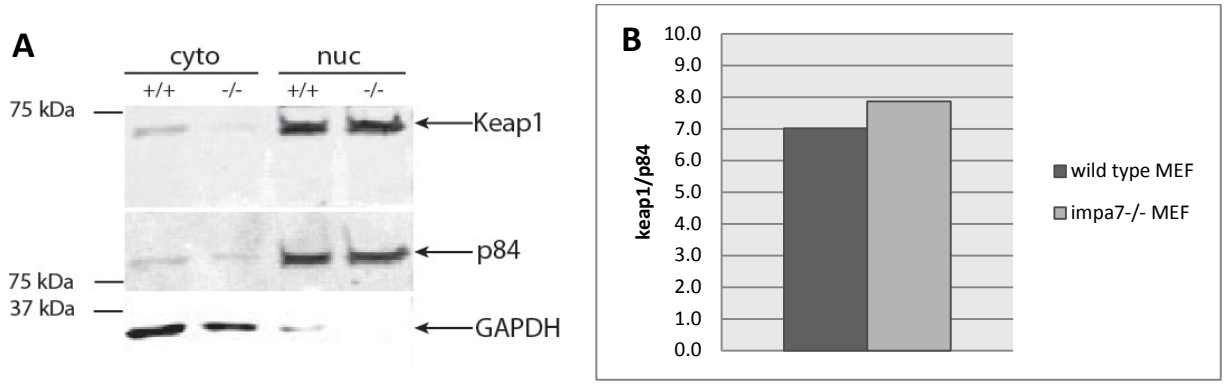


Figure 28: Nuclear Keap1 protein in importin α7-/- and wild type MEFs. A) Western Blot analysis of subcellular Keap1 localization. B) Signal quantification of nuclear Keap1 normalized to the nuclear matrix protein p84; n=1.

2.2. Decreased nuclear RCC1 levels in importin α3 depleted MEFs

The first evidence of cargo preferences for individual importin α isoforms *in vivo* came from a cell culture assay showing that importin α3 is responsible for the classical nuclear import of the Ran guanine nucleotide exchange factor RCC1 [50]. RNAi mediated silencing of importin α gene expression in human cells showed a strong decrease of RCC1 nuclear import in the case of importin α3 and α5 depletion. This effect could be rescued only by re-addition of recombinant importin α3 not by importin α5. In order to verify the RCC1 specificity for importin α3 *in vivo*, importin α3 knockout MEFs were examined regarding its nuclear RCC1 level. In Western Blot experiments, RCC1 was detected merely nuclear and not in cytoplasmic fractions of cells (**Figure 29, A**). In importin α3-/- MEFs the RCC1 signal appeared to be much weaker than in wild type cells. Indeed, a signal quantification revealed a significant (p=0.02) lower nuclear RCC1 level in importin α3-/- MEFs which was decreased to 30% of the wild type control levels (**Figure 29, B**).

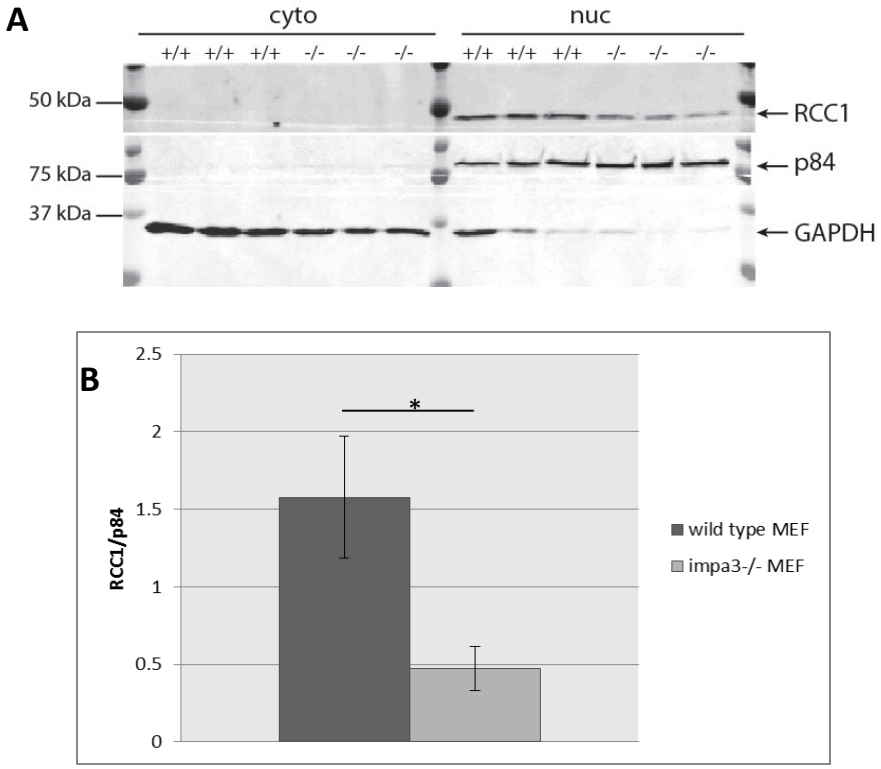


Figure 29: Nuclear RCC1 protein in importin α3-/- and wild type MEFs. A) Western Blot analysis of subcellular RCC1 localization. B) Signal quantification of nuclear RCC1 normalized to the nuclear matrix protein. The diagram shows the mean of RCC1/p84 ratio from 3 different cytoplasmic and nuclear fraction preparations in arbitrary units; error bar: SD; *: p<0.05.

Based on these data, importin $\alpha 3$ knockout mice (see part C) were expected to have decreased RCC1 tissue levels. In order to examine the nuclear RCC1 content in different tissues, nuclear fractions were prepared from major organs. In nuclear samples from brain, RCC1 was hardly detectable at all and hence not analyzable (**Figure 30**). RCC1 organ levels varied very much between individual animals of the same group. However, in all organs analyzed, no significant differences were detectable in nuclear RCC1 levels (**Figure 31**).

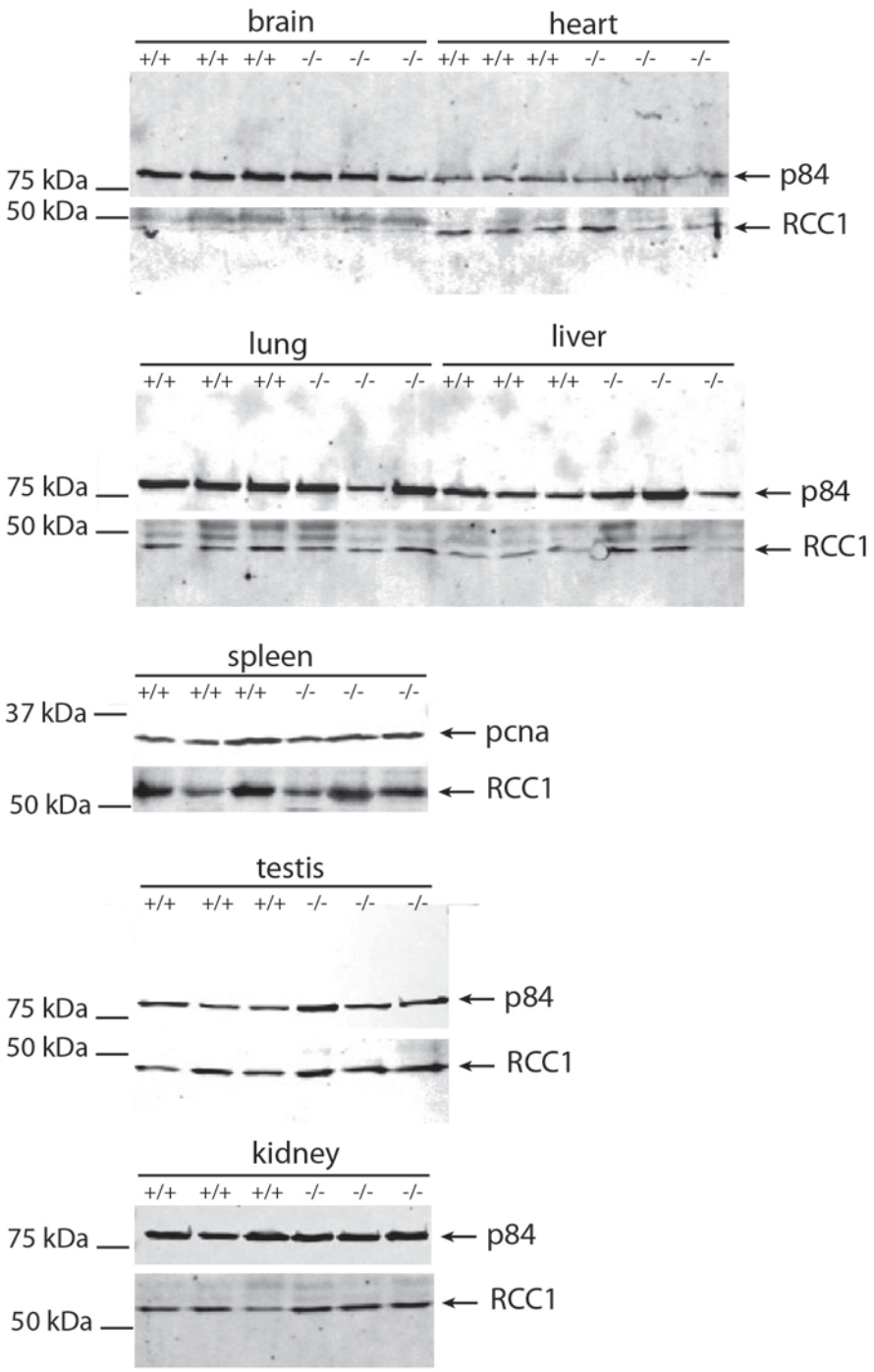
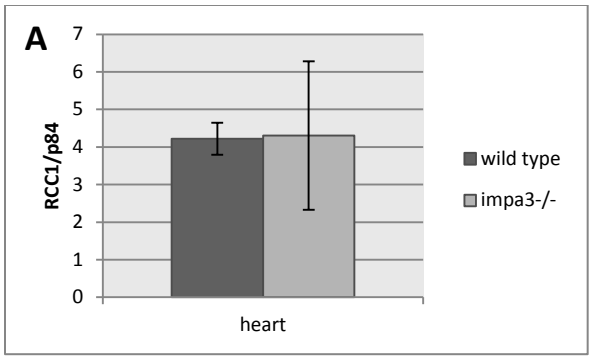


Figure 30: Nuclear RCC1 levels in different organs of importin α3-/- and wild type mice. Nuclear fractions of major organs were prepared from wild type (+/+) and importin α3 knockout (-/-) animals. P84 or pcdna served as nuclear marker proteins.



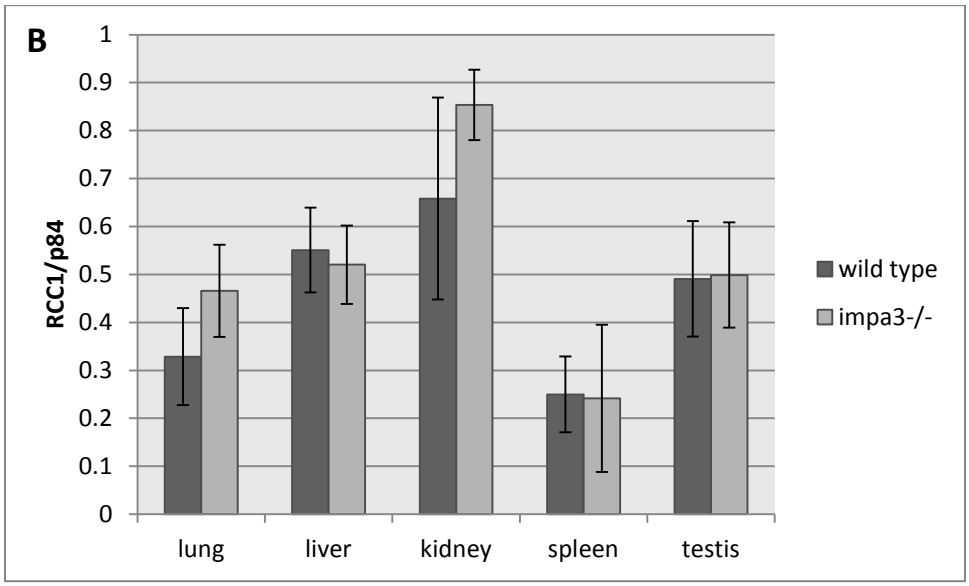


Figure 31: Signal quantification of nuclear RCC1 in different organs of importin α3-/- mice. The nuclear RCC1 Western Blot signals of hearts (A), lung, liver, kidney, spleen and testis (B) were quantified using the Odyssey software and normalized to the nuclear matrix protein p84 or pcdna signals, respectively. The diagram displays the mean values of three different samples per organ from importin α3 knockout (impa3-/-) and wild type mice. Error bar: standard deviation.

2.3. Normal IFN-γ stimulated P-STAT1 nuclear import in importin α5-/- and α7-/- MEFs

Preferred binding of STAT1 to importin α5 was shown by several different experiments [43, 45, 47-49]. Yet, importin α7 was also revealed to co-precipitate with STAT1 by another study [44]. To examine the postulated importin α dependency of STAT1 to importin α5 and α7 cytoplasmic and nuclear fractions of IFN-γ stimulated cells were analyzed by Western Blot. Beforehand, cells were stimulated with IFN-γ for different time points to find the maximum of nuclear P-STAT1

level. Activated P-STAT1 appeared in the nucleus within few minutes after IFN-γ stimulation and reached its maximum after 30 minutes. Then, nuclear P-STAT1 level did not increase any further (**Figure 32**). Therefore, cells were analyzed after 10 and 30 minutes of IFN-γ stimulation. Against expectations, P-STAT1 could be detected in nuclei of importin α5-/- MEFs (**Figure 33, A**). Western Blot signals were quantified and the mean of three different experiments was calculated. Yet, no difference between P-STAT1 nuclear/cytoplasmic ratios of importin α5-/- and wild type MEFs could be detected (**Figure 33, B**). Next, P-STAT1 nuclear import was examined in importin α7-/- MEFs. Like in importin α5-/- MEF, P-STAT1 was detectable in the nuclear fractions of importin α7-/- MEFs, as well (**Figure 34, A**). Interestingly, P-STAT1 levels were markedly increased after IFN-γ stimulation compared to wild type MEFs. This finding was reproducible but not examined in more detail. Western Blot signal quantifications revealed again no difference of P-STAT1 nuclear/cytoplasmic ratios between importin α7-/- and wild type MEFs (**Figure 34, B**).

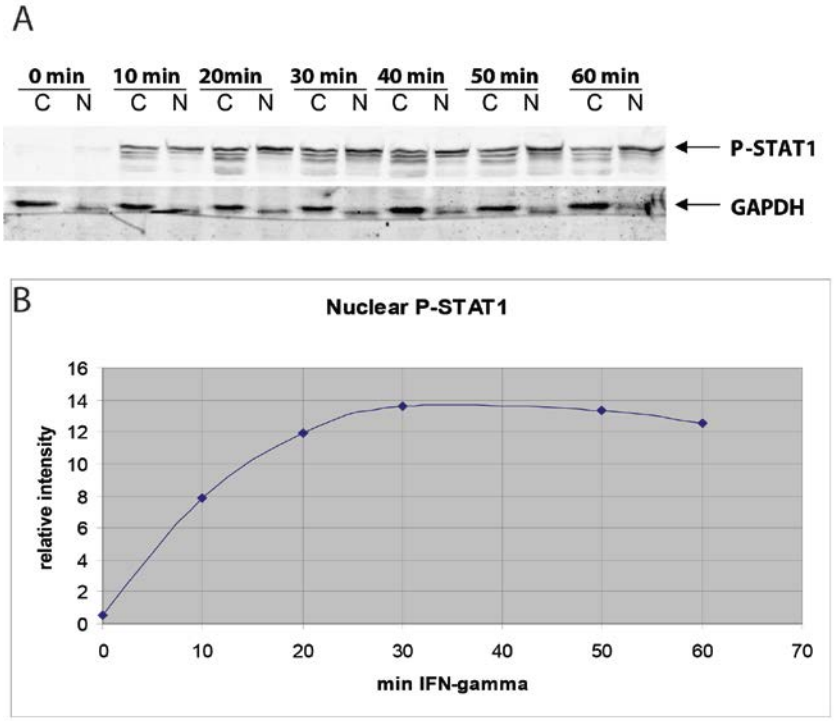


Figure 32: Time course of IFN-γ induced P-STAT1 nuclear import in MEFs. A) Western Blot of P-STAT1 levels in cytoplasmic (C) and nuclear (N) fractions of MEFs after different time points of IFN-γ stimulation. B) Quantitative time course of nuclear P-STAT1 levels. GAPDH served as cytoplasmic marker and protein loading control.

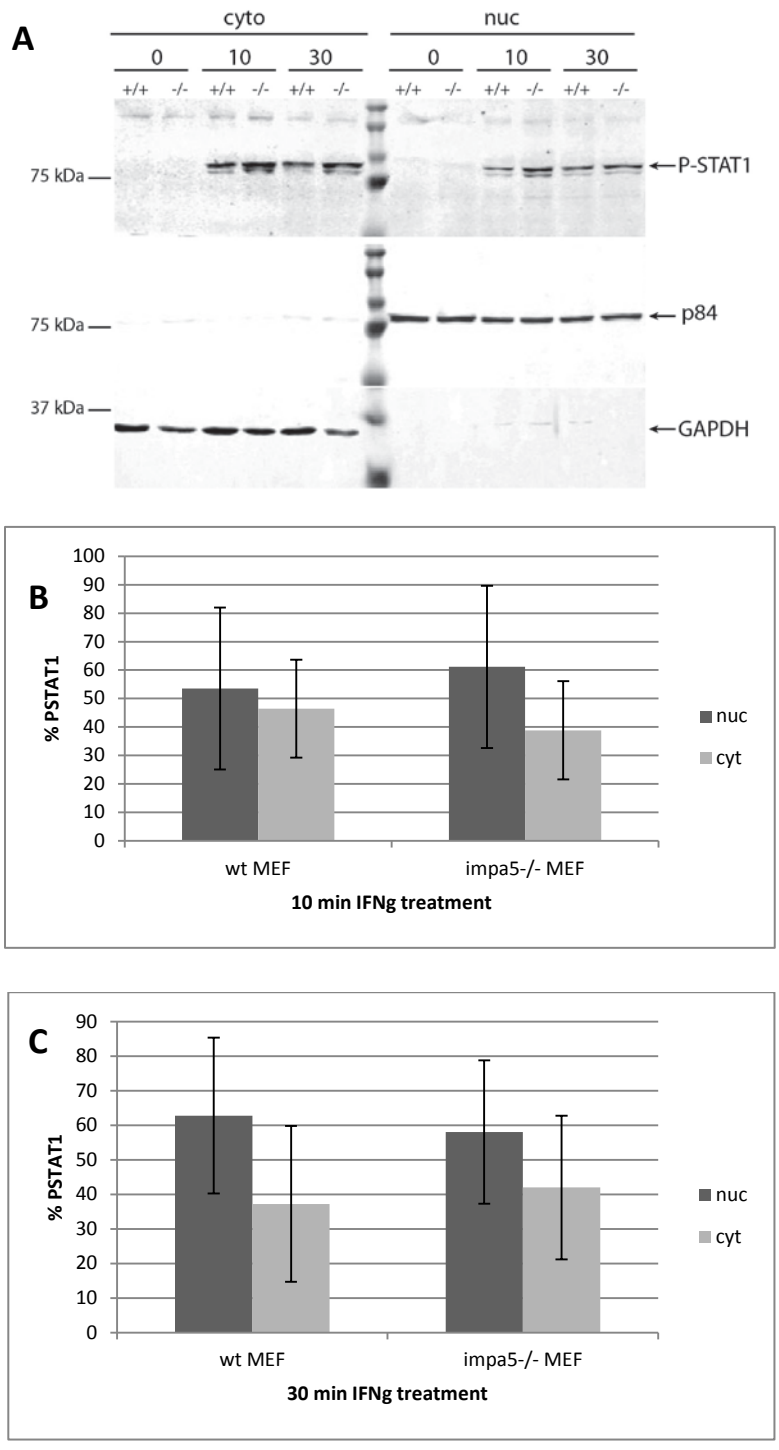


Figure 33: Cytoplasmic and nuclear P-STAT1 levels in importin α5-/- MEFs after IFN-γ stimulation. A) Representative Western Blot of P-STAT1 without (0), after 10 minutes (10) and after 30 minutes (30) of IFN-γ stimulation. B) Relative cytoplasmic and nuclear P-STAT1 levels after 10 minutes (B) and after 30 minutes (C). Cytoplasmic P-STAT1 was normalized to GAPDH and nuclear P-STAT1 was normalized to the nuclear matrix protein p84. The diagrams show percentaged P-STAT1 mean values based on overall P-STAT1 levels from cytoplasmic and nuclear fractions of three independent experiments; error bar: standard deviation.

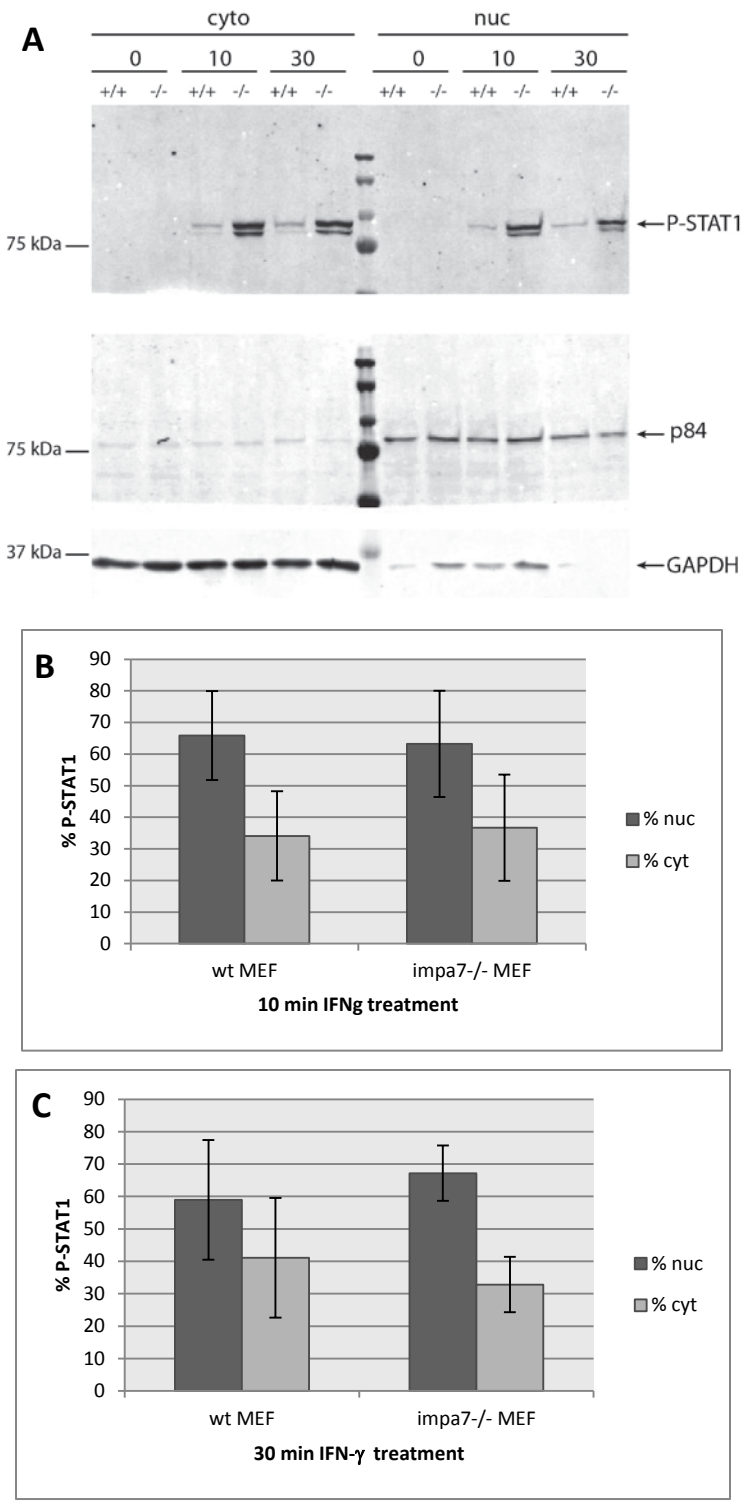


Figure 34: Cytoplasmic and nuclear P-STAT1 levels in importin α7-/- MEFs after IFN-γ stimulation. A) Representative Western Blot of P-STAT1 without (0), after 10 minutes (10) and after 30 minutes (30) of IFN-γ stimulation. B) Relative cytoplasmic and nuclear P-STAT1 levels after 10 minutes (B) and after 30 minutes (C). Cytoplasmic P-STAT1 was normalized to GAPDH and nuclear P-STAT1 was normalized to the nuclear matrix protein. The diagrams show percentaged P-STAT1 mean values based on overall P-STAT1 levels from cytoplasmic and nuclear fractions of three independent experiments; error bar: standard deviation.

2.4. Summary and Discussion Part B

Despite binding studies implicate the existence of specific importin α-cargo interactions, the *in vivo* cargo transport can behave differently. The importin α knockout MEF model serves as an excellent tool for checking nuclear cargo import according to its preferred importin α transport receptor. The studies outlined above showed in many cases that the loss of the specific α importin protein seems to be compensated by other nuclear import mechanisms. Some of these alternative transport pathways even seem to be as efficient as the one mediated by the favored importin α subtype. Recent studies described an importin α7 dependent nuclear import of the E3 ubiquitin ligase subunit Keap1 which plays a role in the Nrf2 mediated anti-oxidative stress response [56]. Decreased protein levels of importin α7 inhibited the nuclear import of Keap1 in NIH3T3 cells leading to prolonged Nrf2 signaling and subsequent upregulation of genes involved in oxidative stress response. Interestingly, in MEFs depleted of importin α7, Keap1 nuclear levels were unaltered. Hence, Nrf2-signaling was not further analyzed. Knockdown of importin α7 by RNAi led to impaired Keap1 nuclear import but not to a complete block [56]. Therefore, it is very likely that an alternative Keap1 nuclear import mechanism exists that enables Keap1 to enter the nucleus independently of importin α7, though this mechanism might be less efficient.

One of the first described importin α substrate specificities was the disturbed nuclear import of recombinant RCC1 in importin α3 depleted HeLa cells [50]. Indeed, in MEFs from importin α3 knockout mice, the nuclear RCC1 levels are significantly lower than in wild type MEFs. However, there is still 30% nuclear RCC1 left. Also in HeLa cells, nuclear import of RCC1 was not totally abolished after depletion of importin α3 by RNAi [50]. These data are consistent with a study of Nemergut and Macara who described the existence of an importin α/β independent nuclear import mechanism for RCC1 [89]. On the other hand, it has to be mentioned that the RCC1 levels may be linked to the proliferation status of a cell. Hence, RCC1 levels may be decreased in importin α3-/- MEFs since these MEFs proliferate more slowly than wildtytpe MEFs. Interestingly, in organs of importin α3 knockout mice, no significant difference in nuclear RCC1 levels was detectable compared to wild type. It cannot be excluded that there are decreased RCC1 levels in importin α3-/- tissues that were not checked here. However, it is also possible

that surviving importin α3 knockout embryos (see part C) develop normally by upregulation of the alternative RCC1 nuclear import pathway.

Moreover, importin α substrate specificities can also become visible not before certain environmental conditions appear. P-STAT1 was reported to bind importin α5 preferentially in several studies. However, nuclear import of P-STAT1 is neither abolished nor hindered in MEFs depleted of importin α5 after IFN-γ stimulation. One further study showed P-STAT1 binding to importin α7, as well. But again, despite the absence of importin α7, cytoplasmic and nuclear P-STAT1 levels were unaltered in MEFs. Therefore, it is very likely that P-STAT1 can enter the nucleus by using importin α5 or importin α7 but it is not solely dependent on the transport of one of both. In addition, an alternative STAT1 transport mechanism has been published which mediates STAT1 nuclear import via nucleolin upon differentiation of myeloid cells [90]. Hence, STAT1 seems to have more options than the classical nucleocytoplasmic transport mechanism to translocate into the nucleus. Yet, under mitogen withdrawal or low nutrient levels, this situation changes a lot. STAT1 does not need to be activated by phosphorylation to translocate into the nucleus [91]. In the recent years, it became clear that also without IFN stimulation, unphosphorylated "latent" STAT1 shuttles between the cytoplasm and the nucleus and controls genes involved in apoptosis, cell cycle arrest, and immunomodulation [92-94]. Using the here described importin α5-/- MEFs, it could be shown that at low mitogen or nutrient levels, rapamycin inhibits the serine-threonine kinase 'target of rapamycin' (mTOR) and leads to the import of latent STAT1 into the nucleus [95]. Nuclear accumulation of latent STAT1 increases expression of STAT1 and enhances cleavage of caspase-3, a marker of apoptosis. We could show that in importin α5-/- MEF, however, the rapamycin induced nuclear import of STAT1 was abolished [74]. Similarly, starvation induced increase of STAT1 mRNA levels was blocked in MEFs depleted of importin α5. Furthermore, knockdown of importin α5 by RNAi inhibited the enhancing effect of bacterial lipopolysaccharide and interferon-β induced cleavage of caspase-3. These data demonstrate that in contrast to IFN-γ stimulated nuclear import of STAT1, constitutive STAT1 nuclear translocation is dependent on importin α5 alone.

3. Characterization of importin α3 knockout mice (Part C)

3.1. Tissue specific expression pattern of importin α3

Importin α3 knockout mice were generated by conventional gene deletion strategy. The homozygous loss of both importin α3 alleles in mice was verified by PCR amplification of fragments spanning the knockout cassette integration site around exon 1 of the importin α3 gene (**Figure 35**). In order to verify the absence of importin α3, different organs were analyzed by RT-PCR (**Figure 36**) and Western Blot (**Figure 37**). Interestingly, in Western Blot experiments, importin α3 expression in wild type kidney and liver was below the detection limit although importin α3 mRNA could be detected in these tissues.

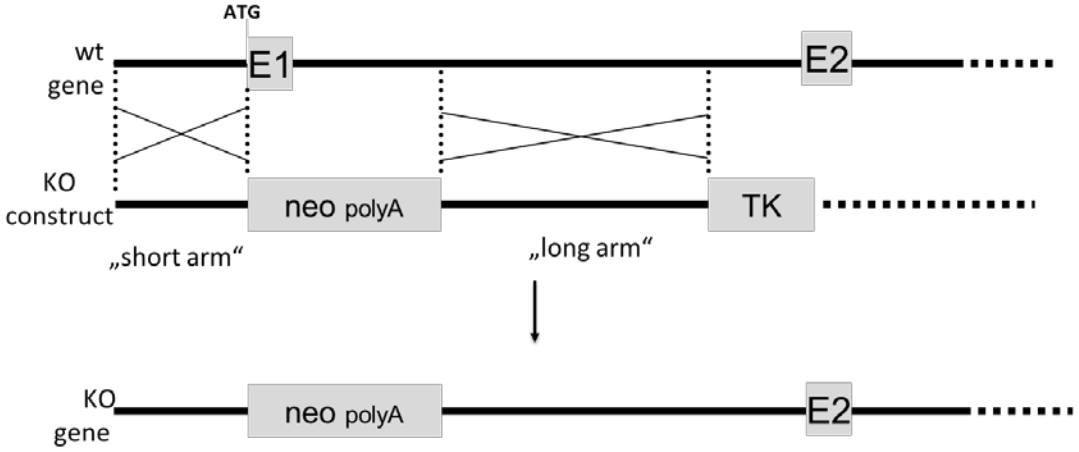


Figure 35: Importin α3 knockout construct design. The knockout (KO) construct consists of an open reading frame for neomycin (neo) resistance with a poly adenylation (polyA) transcriptional stop signal and a thymidine kinase (TK) selection cassette. The construct spans the exon 1 (E1) and exon 2 (E2) of the importin α3 gene.

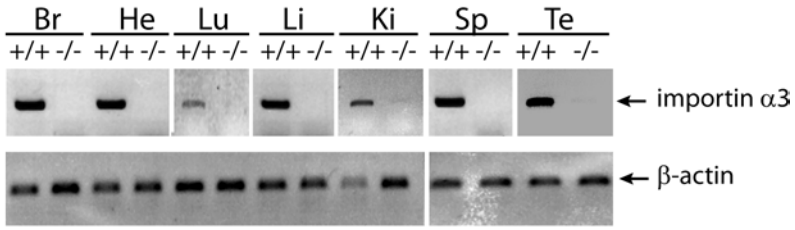


Figure 36: RT PCR of importin α3 from different organs. mRNA of wild type (+/+) and importin α3 knockout (-/-) mouse organs was prepared. Importin α3 expression was checked by reverse transcription (RT) PCR from brain (Br), heart (He), lung (Lu), liver (Li), kidney (Ki), spleen (Sp), testis (Te), and ovary (Ov). Amplification of β-actin served as cDNA loading control.

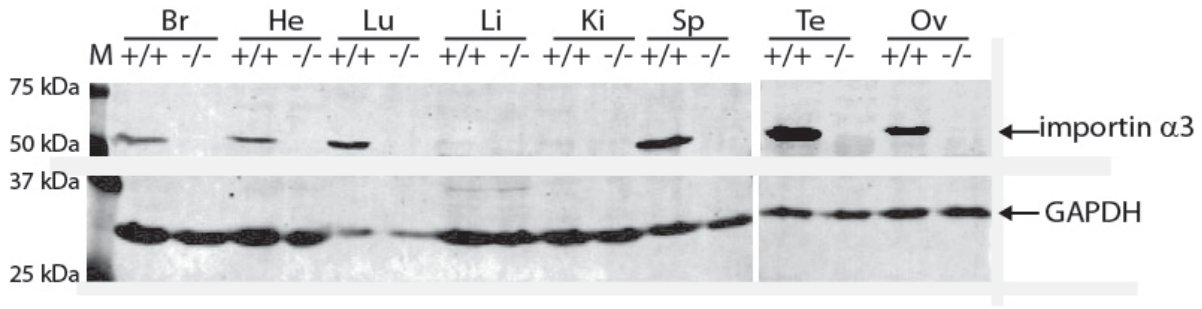


Figure 37: Verification of importin α3 knockout mice. Western Blot analysis of importin α3 protein expression in different organs of wildtype (+/+) and importin knockout (-/-) mice. Br: brain, He: heart, Lu: lung, Li: liver, Ki: kidney, Sp: spleen, Te: testis, Ov: ovary. GAPDH served as housekeeping protein.

Immunohistochemistry of cryosections, however, revealed a weak staining for importin α3 in hepatocytes of the liver (**Figure 38**) and also in tubules of the medulla of the kidney (**Figure 39, A**). Interestingly, no importin α3 staining could be detected in the cortex of the kidney (**Figure 39, B**).

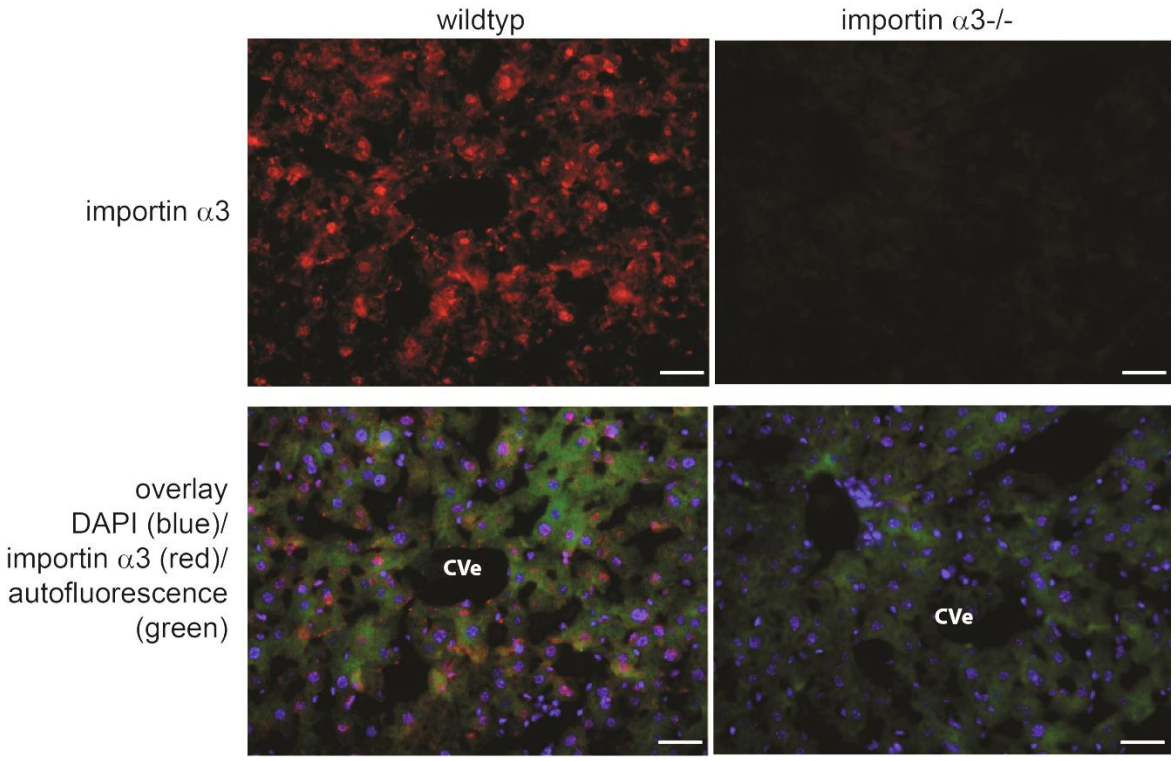


Figure 38: Immunohistochemical staining of importin α3 in liver tissue. Cryosections of wildtype liver were probed with anti-importin α3 antibody (upper panel, red). DAPI served as nuclear marker (lower panel, blue). Auto fluorescence of tissue is displayed in green. Cryosections of importin α3 KO mice served as negative control; CVe: central vein, scale bar: 100 µm.

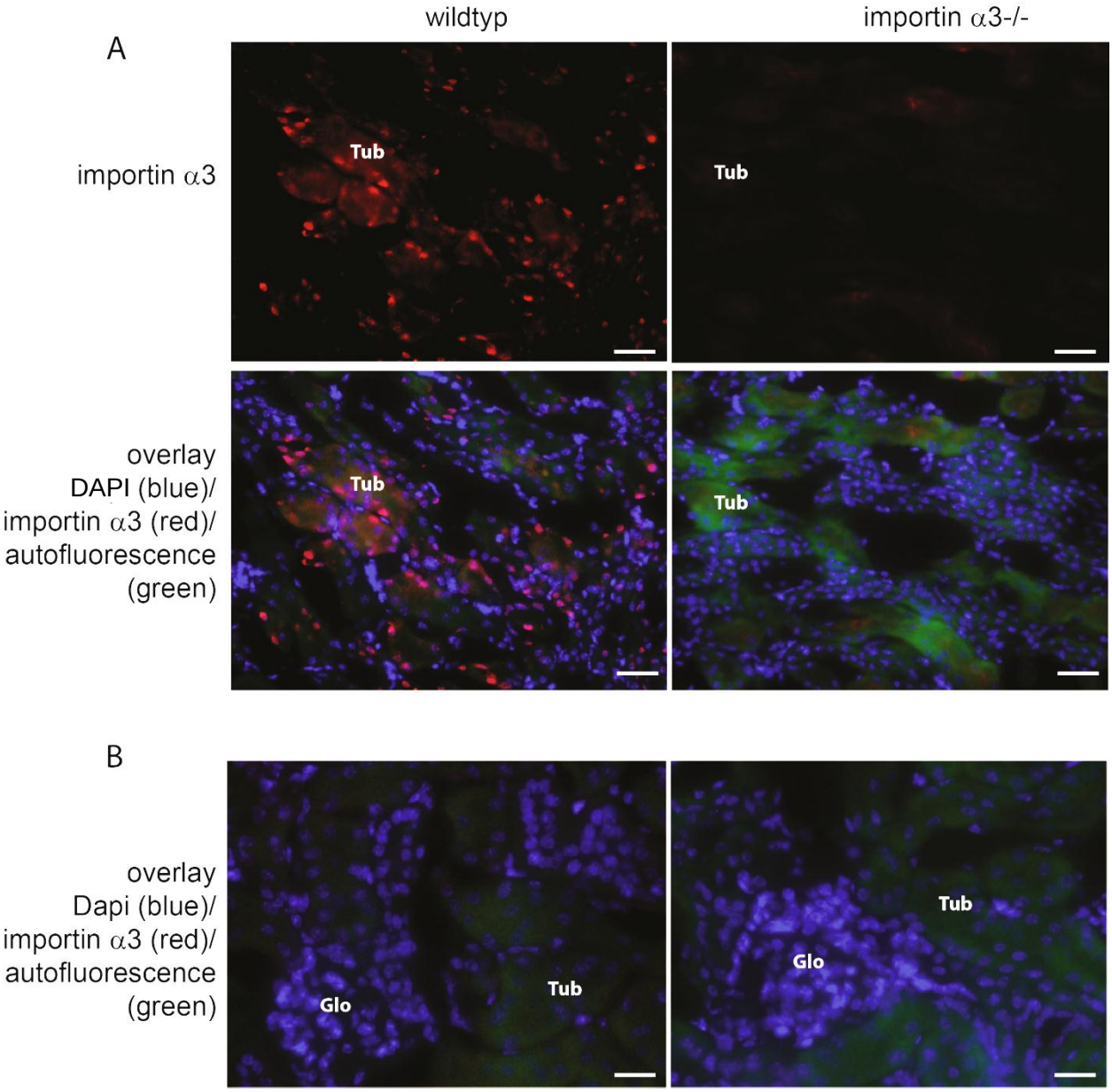


Figure 39: Immunohistochemical staining of importin α3 in kidney tissue. Cryosections of wildtype kidney were probed with anti-importin α3 antibody (A, upper panel, red). DAPI served as nuclear marker (A, lower panel, blue). Auto fluorescence of tissue is displayed in green. No importin α3 staining was detected in the cortex of wildtype kidneys (B). Cryosections of importin α3 KO mice served as negative control; Glo: glomeruli, Tub: tubule, scale bar: 100 µm.

3.2. Depletion of importin α3 led to partially embryonic lethality in mice

To gain homozygous importin α3 knockout mice, heterozygous matings were made. According to mendelian rules, 25% knockout mice are expected out of a heterozygous mating. However, mating of importin α3 heterozygous (+/-) mutant mice yield only 16% homozygous knockout offspring (**Figure 40**).

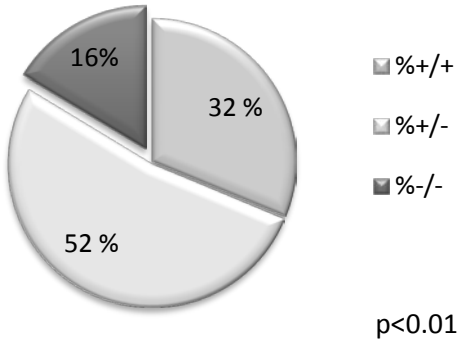


Figure 40: Analysis of importin α3 heterozygous matings according to Mendelian rules. 14 different importin α3 heterozygous (+/-) females were mated to different heterozygous males out of 34 litters, 291 pups were analyzed according to genotype.

3.3. Fertility problems of importin α3-/- mice

Only 44 % of importin α3 knockout females mated to heterozygous control males gave birth (**Table 16**) and they had a decreased litter size of 3-4 pups compared to 8-10 pups from heterozygous matings. Importin α3 knockout males showed the same fertility problems.

Table 16: Fertility analysis of importin α3-/- mice. Importin α3-/- and +/- control mice were mated to importin α3+/- partners.

female genotype	number of females	gave litter	%
+/-	28	25	89
-/-	25	11	44

male genotype	number of males	gave litter	%
+/-	16	14	88
-/-	34	14	41

3.4. Histological analysis by HE staining

To examine potential organic malformations or tissue abnormalities, different organs from adult importin α3-/- as well as wild type control mice were paraffin embedded and stained by hematoxyline and eosine (HE).

The histological examination of kidneys from importin α3 knockout females (n=2) showed misshaped glomeruli that looked smaller, unstructured and not that delimited compared to wildtype controls (**Figure 41, arrows and detailed view**). The tubuli in importin α3-/- kidneys, however, seemed to be without obvious pathological findings.

Further examination of HE stained hearts from importin α3 knockout females (n=2) revealed an elevated right ventricular wall (RVW) diameter as well as an increased right septum wall (RSW) diameter (**Figure 42**). These observations indicate an isolated right heart hypertrophy in mice depleted of importin α3. One of the main reasons for right heart hypertrophy is pulmonary dysfunction. Therefore, lung sections were examined by HE staining. In importin α3 knockout mice, the pulmonary arteries looked more contracted than in wild type lungs (**Figure 43**) (correspondence with Dr. S. Rittinghausen, Fraunhofer Fraunhofer-Institut für Toxikologie und Experimentelle Medizin, Germany). Therefore it was postulated that importin α3 knockout mice may develop a right heart hypertrophy that could be caused by pulmonary dysfunction.

All other organs seemed to be without obvious pathological findings, like for example ovary and liver (**Figure 44 and Figure 45**).

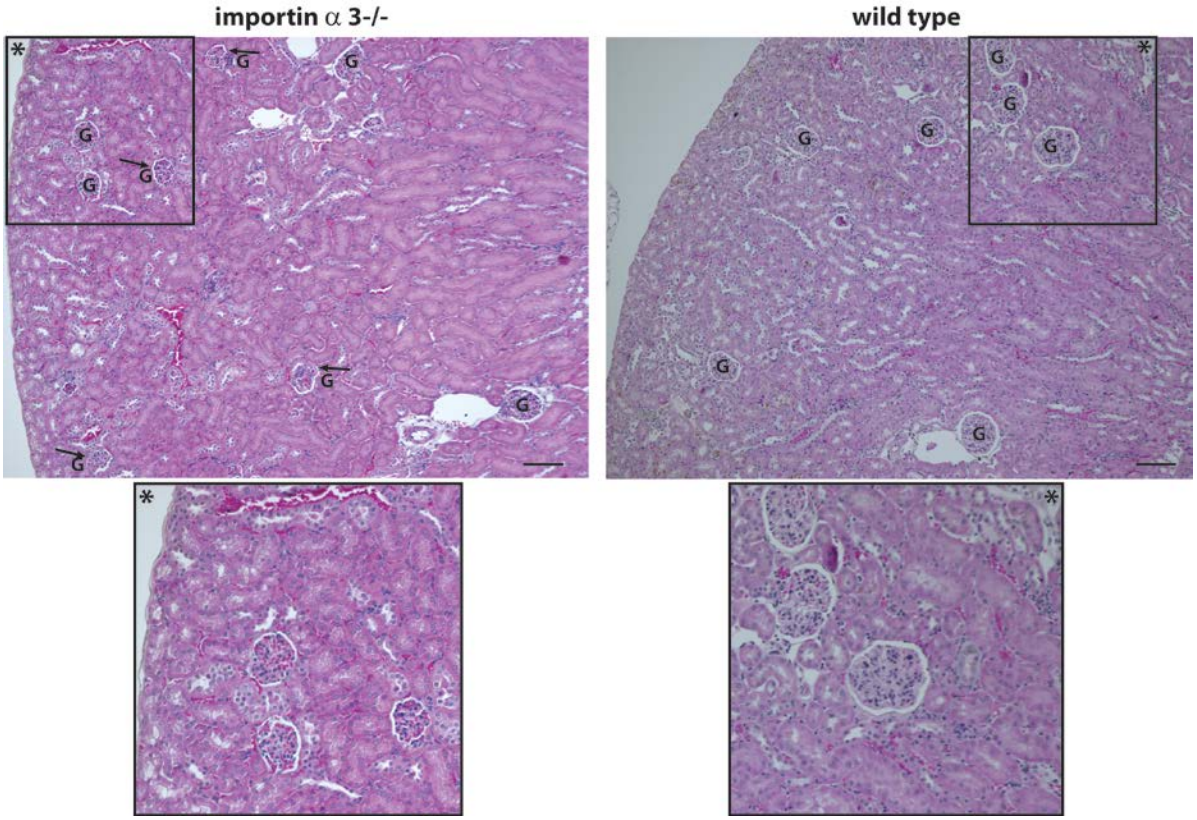


Figure 41: Histological examination of the kidney from importin α3 knockout mice. Representative pictures of the kidney from an adult female importin α3 knockout (-/-) and wild type control mouse are displayed. Tenfold magnification, G: glomerulus, arrow: wrinkled, shrunken glomerulus, scale bar: 100 µm, *: detailed view.

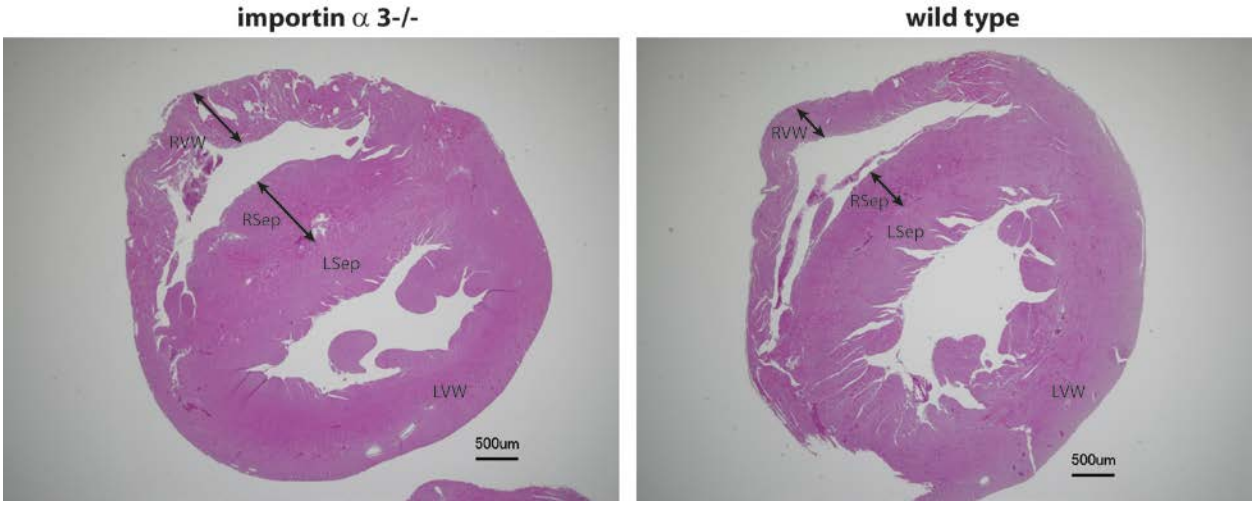


Figure 42: Histological examination of the heart from importin α3 knockout mice. Representative pictures of the heart from an adult female importin α3 knockout (-/-) and wild type control mouse are displayed. Twofold magnification, RVW: right ventricular wall, Rsep: right septum, Lsep: left septum, LVW: left ventricular wall, scale bar: 500 µm.

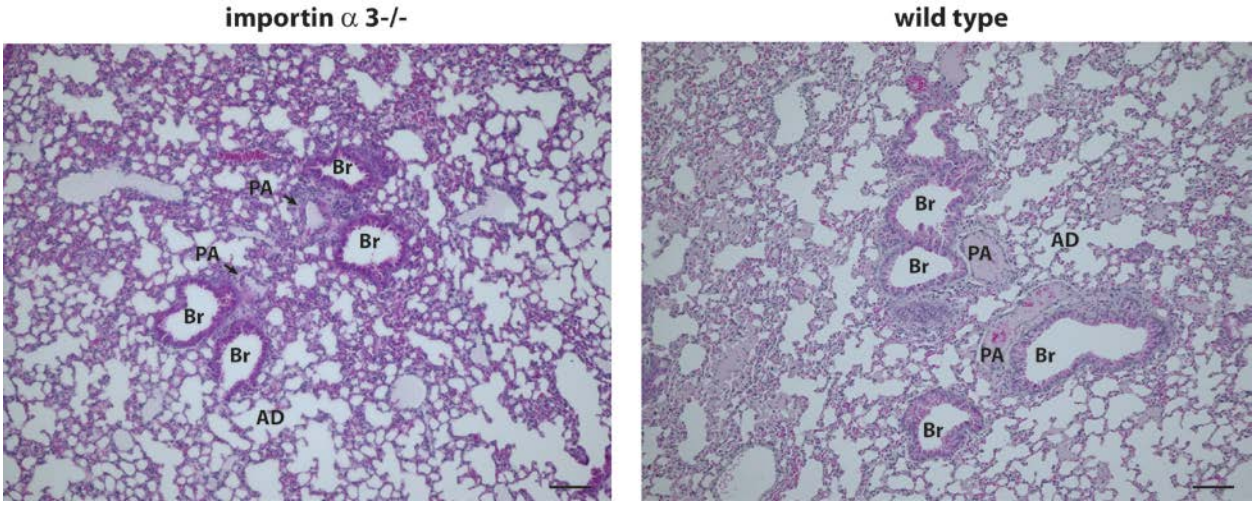


Figure 43: Histological examination of the lung from importin α3 knockout mice. Representative pictures of the lung from an adult female importin α3 knockout (-/-) and wild type control mouse are displayed. Tenfold magnification, Br: bronchiolus, AD: alveolar duct, PA: pulmonary artery, scale bar: 100 µm.

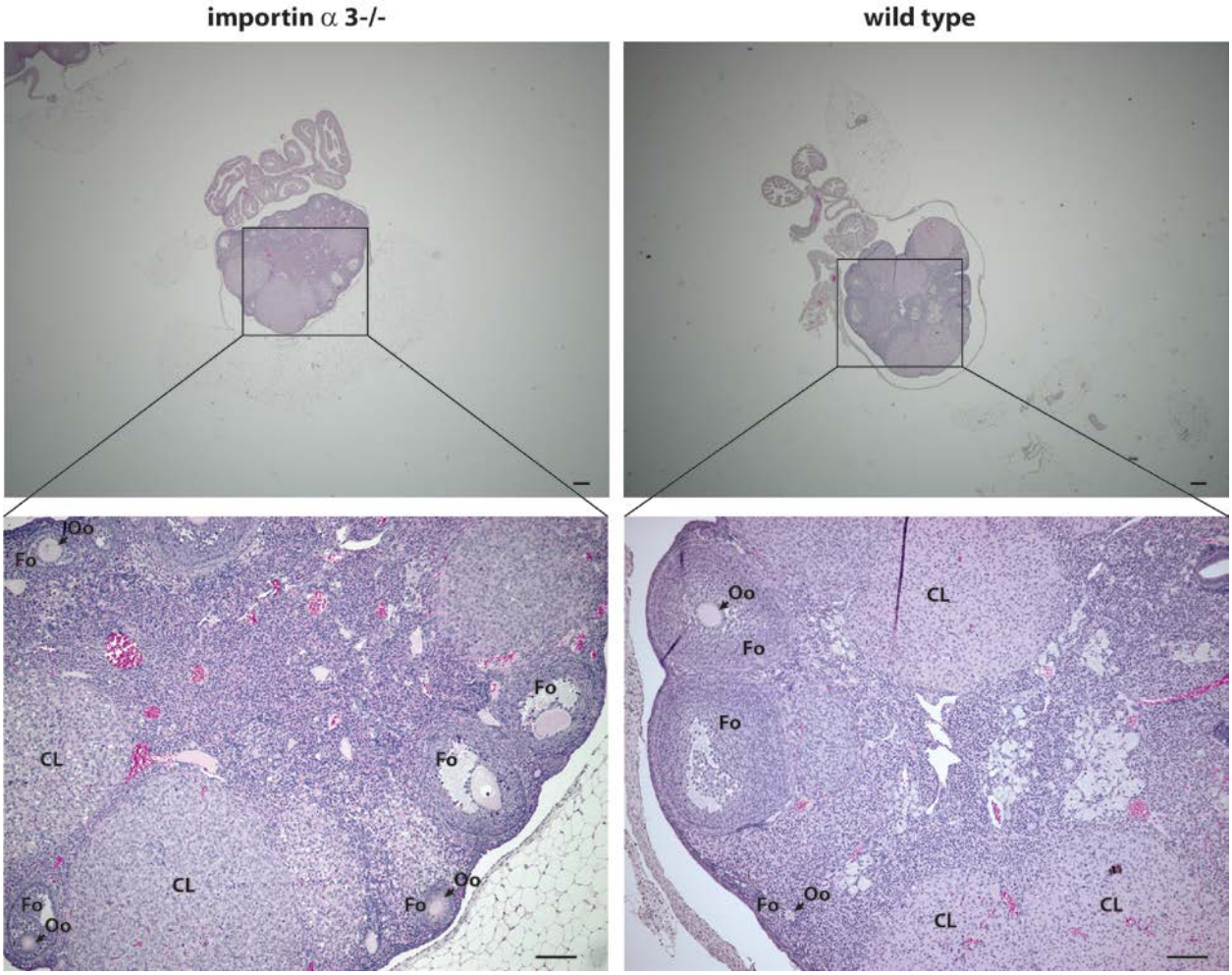


Figure 44: Histological examination of the ovary from importin α3 knockout mice. Representative pictures of the ovary from an adult female importin α3 knockout (-/-) and wild type control mouse are displayed. Upper panel: twofold magnification; overview picture, scale: 200 µm. Lower panel: tenfold magnification, scale: 100µm, Fo: follicle, Oo: oocyte, CL: corpus luteum.

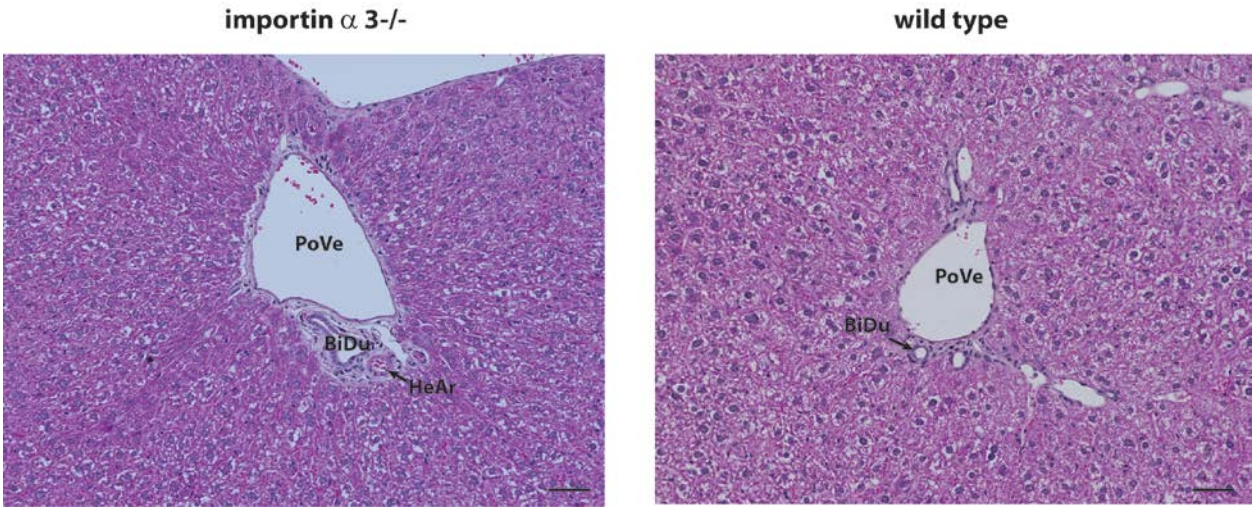
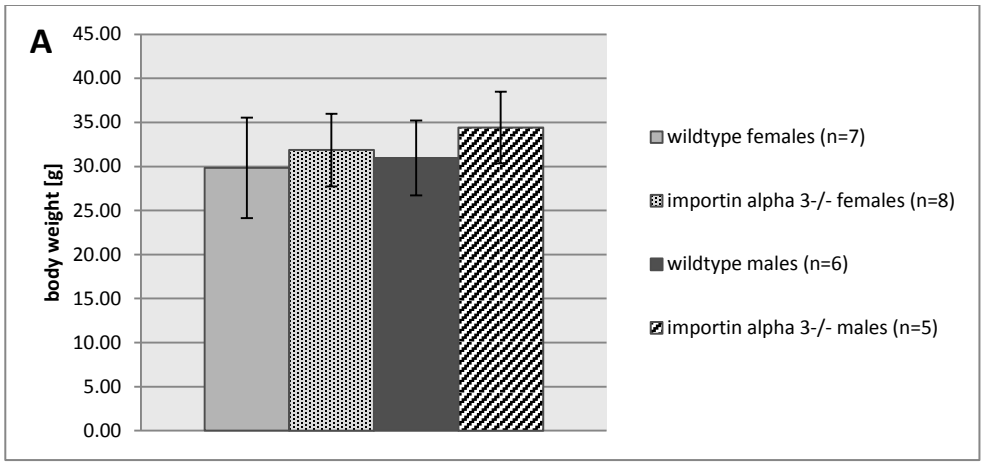
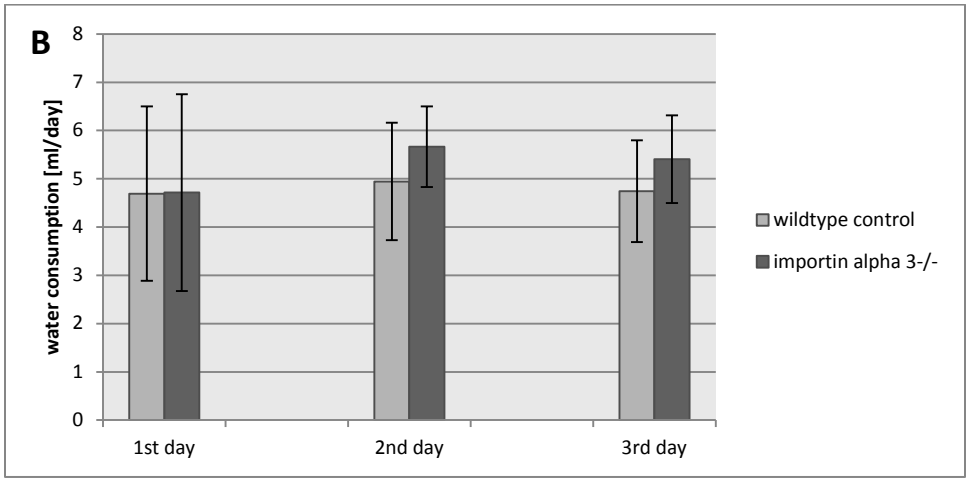


Figure 45: Histological examination of the liver from importin α3 knockout mice. Representative pictures of the liver from an adult female importin α3 knockout (-/-) and wild type control mouse are displayed. Tenfold magnification, PoVe: portal vein, BiDu: bile duct, HeAr: hepatic artery, scale bar: 100 µm.

3.5. Metabolic analysis

Due to the misshaped glomeruli found in kidneys of importin α3 knockout females, mice were examined in metabolic cages. Neither female nor male importin α3-/- mice differed significantly from their corresponding wild type controls regarding body weight (**Figure 46, A**). Furthermore, no significant differences were observed between importin α3-/- mice and wild type controls concerning water consumption and urine production (**Figure 46, B and C**).





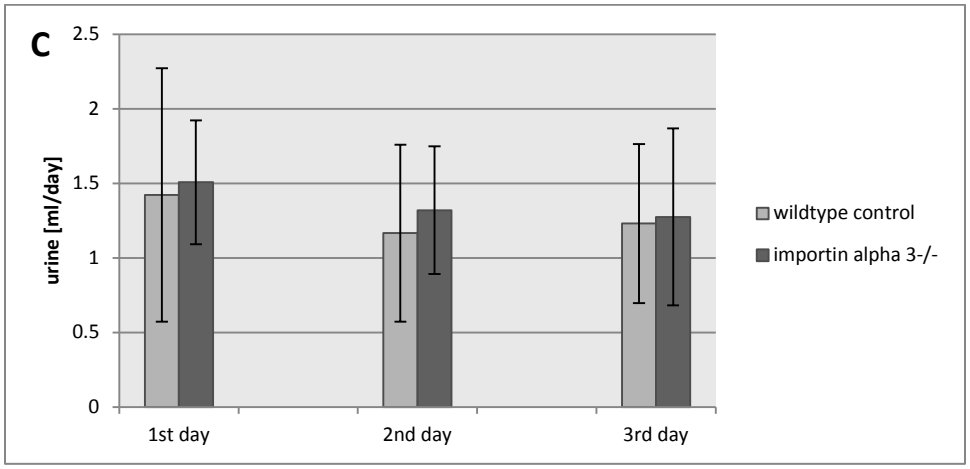


Figure 46: Metabolic analysis of importin α3-/- mice. A) Body weight of adult (>22 weeks old) mice. B) Water consumption per day on three following days in a metabolic cage (n=13/group). C) Urine production per day on three following days in a metabolic cage (n=13/group). Mean values and standard deviations are displayed.

3.6. Echocardiographic analysis

Based on the elevated right ventricular wall diameter found by histological analysis, adult (> 22 weeks) importin α3-/- mice were examined by echocardiography. However, echocardiographic measurements at diastole revealed only a slight tendency of increased right heart dimensions in importin α3 knockout males (**Figure 47, A**). In females, the right ventricular wall diameter did not differ between importin α3-/- and wild type controls (**Figure 47, B**).

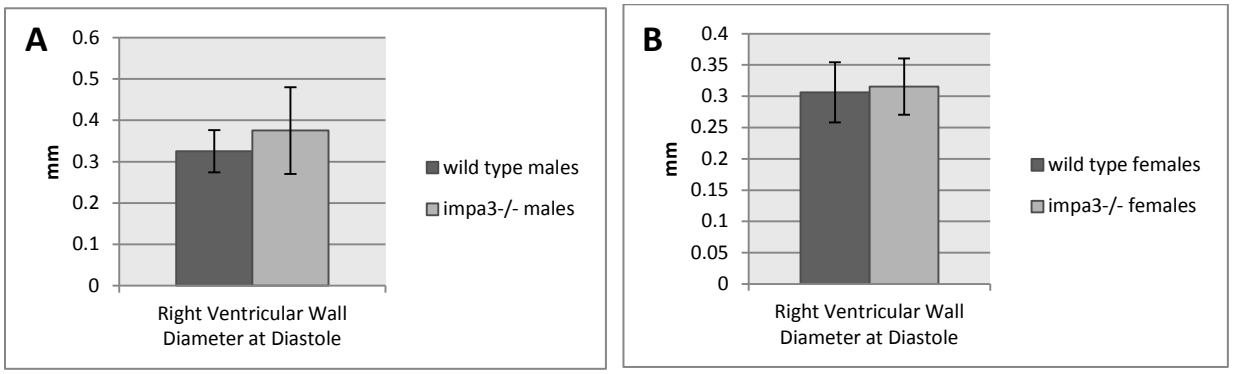


Figure 47: Right ventricular wall diameters of importin α3-/- mice. The right ventricular wall diameter was measured during diastole by echocardiography in wild type and importin α3-/- (impa3-/-) males (A; n=10-11) and females (B; n=13-15). Mean right ventricular wall diameters and corresponding standard deviations are displayed in the diagram.

Nevertheless, echocardiographic measurements of the interventricular septum at diastole revealed a significantly increased septal thickness in importin α3-/- males ($p<0.01$) by 15% (**Figure 48**, **A**). The same tendency was observed in importin α3-/- females (**Figure 48, B**). In addition, the left ventricular posterior wall dimensions were significantly ($p<0.01$) increased by 11-17% in both, males and females depleted of importin α3 (**Figure 48**, **C and D**). Furthermore, importin α3 knockout mice showed an up to 15% increased left ventricular mass (not significant) (**Figure 48**, **E and F**). Likewise, functional heart parameters were changed in importin α3-/- mice. The fractional shortening was significantly decreased by 20% in importin α3-/- males ($p<0.05$) and by 17% in importin α3-/- females ($p<0.01$) (**Figure 49, A and B**). In addition, the ejection fraction was decreased by 17% in importin α3-/- males (not significant) and by 13% in importin α3-/- females ($p<0.01$) (**Figure 49, C and D**). Furthermore, importin

α3-/- mice showed a significant decline in stroke volume by 17% in males ($p<0.05$) and by 19% in females ($p<0.001$) (**Figure 49, E and F**). Likewise, the cardiac output was significantly decreased by 20% in importin α3-/- males ($p<0.05$) and by 17% in females ($p<0.01$) (**Figure 49, G and H**).

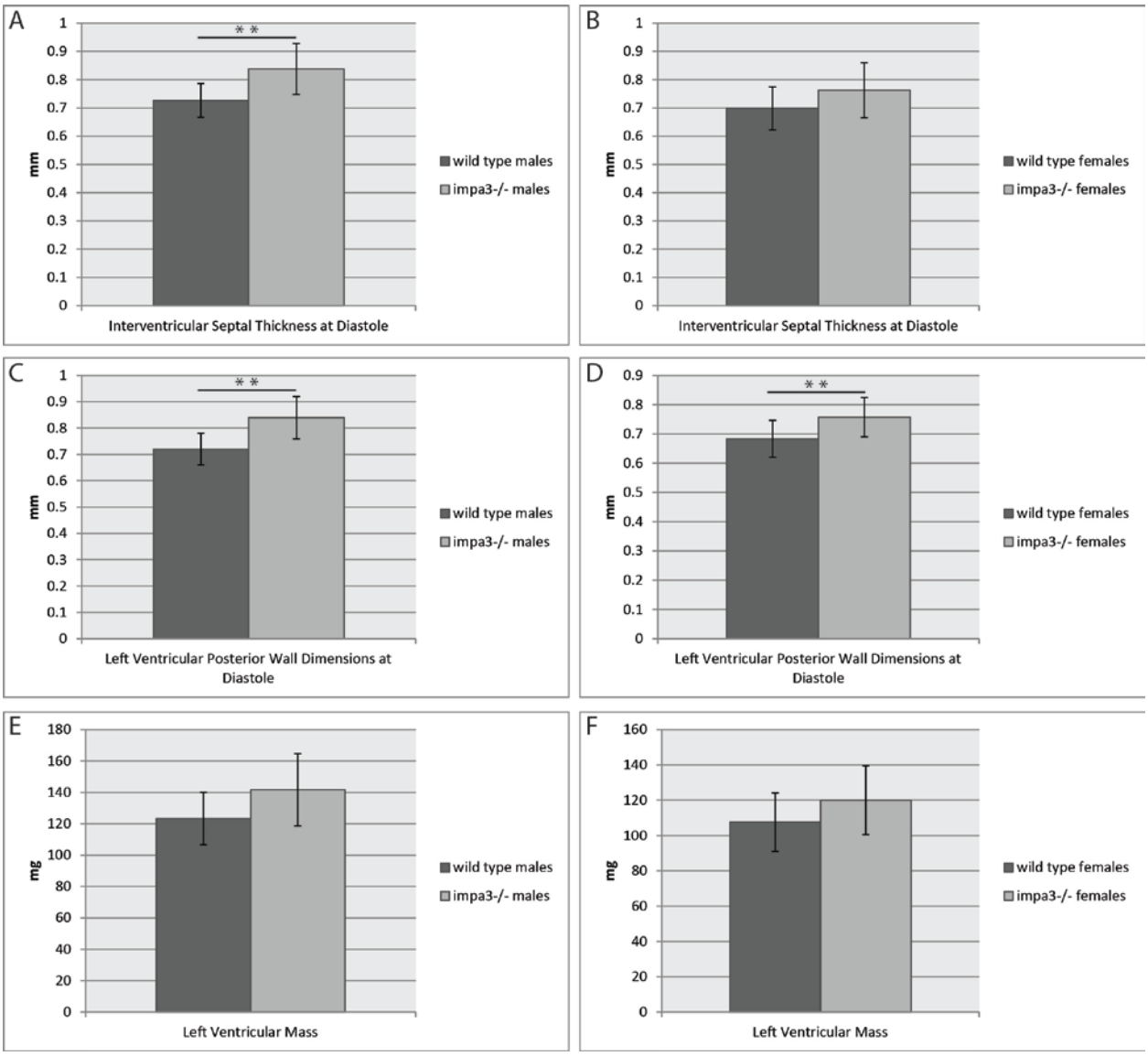


Figure 48: Echocardiographic parameters of importin α3-/- mice I. Importin α3-/- (impa3-/-) and wild type mice were analyzed by echocardiography in order to determine the interventricular septal thickness at diastole of males (A) and females (B), the left ventricular wall diameter of males (C) and females (D) as well as the left ventricular mass of males (E) and females (F). The diagrams display the mean values of 10-11 males and 13-15 females. Error bar: standard deviations, $p<0.01$ (**).

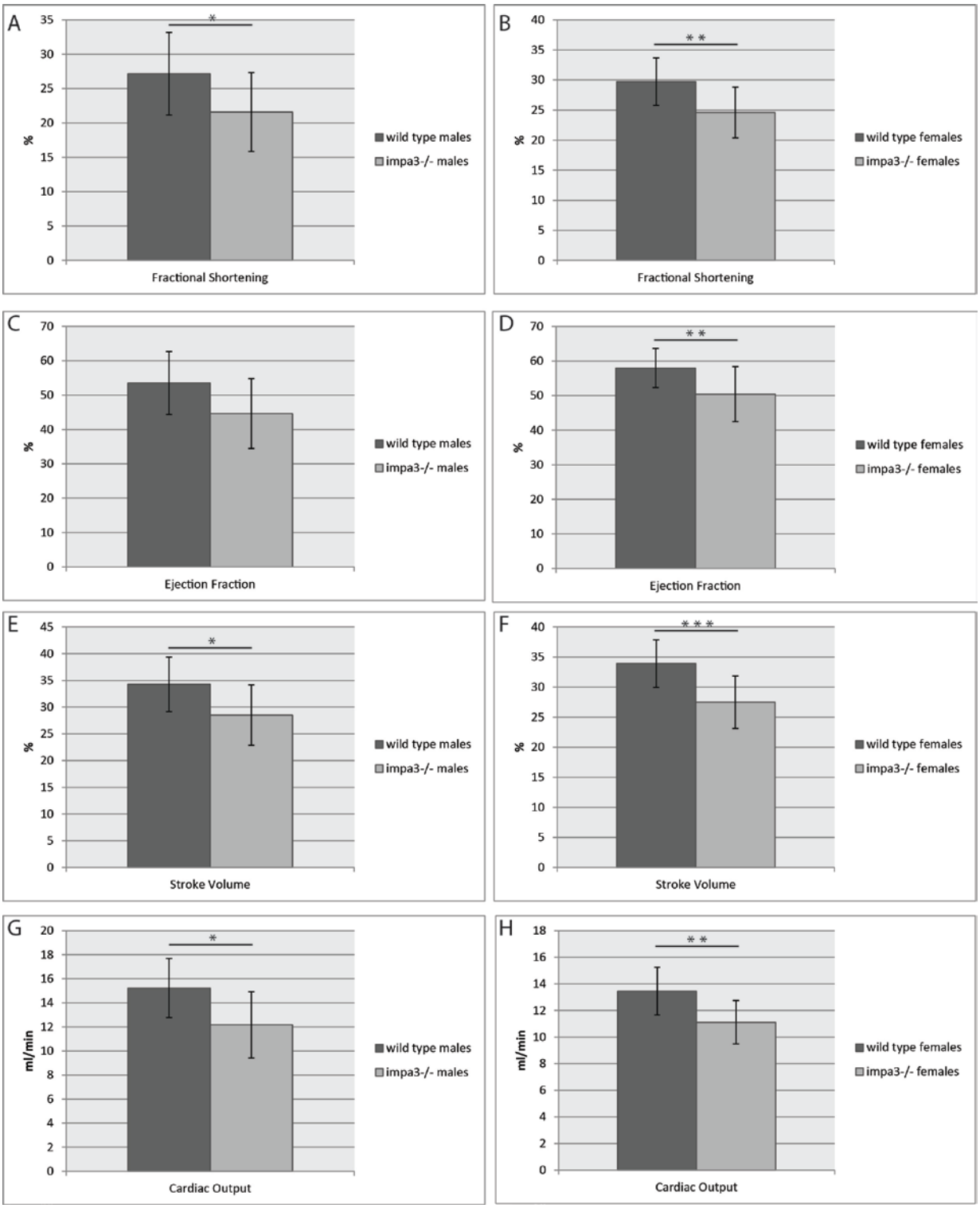


Figure 49: Echocardiographic parameters of importin α3-/- mice II. Importin α3-/- (impa3-/-) and wild type mice were analyzed by echocardiography in order to determine the fractional shortening of males (A) and females (B), the ejection fraction of males (C) and females (D) as well as the stroke volume of males (E) and females (F) and the cardiac output of males (G) and females (H). The diagrams display the mean values of 10-11 males and 13-15 females. Error bar: standard deviations, $p<0.05$ (*), $p<0.01$ (**), $p<0.001$ (***).

3.7. Respiratory frequency analysis

In order to find out whether importin α3 knockout mice may suffer from right heart hypertrophy caused by pulmonary dysfunction, mice were checked for signs of hypoxia. Therefore, the respiratory frequency was examined in adult mice by a two chambers plethysmograph. In importin α3-/- mice, the respiratory frequency was significantly upregulated by 40% compared to wild type controls (**Figure 50**, p= 0.0004).

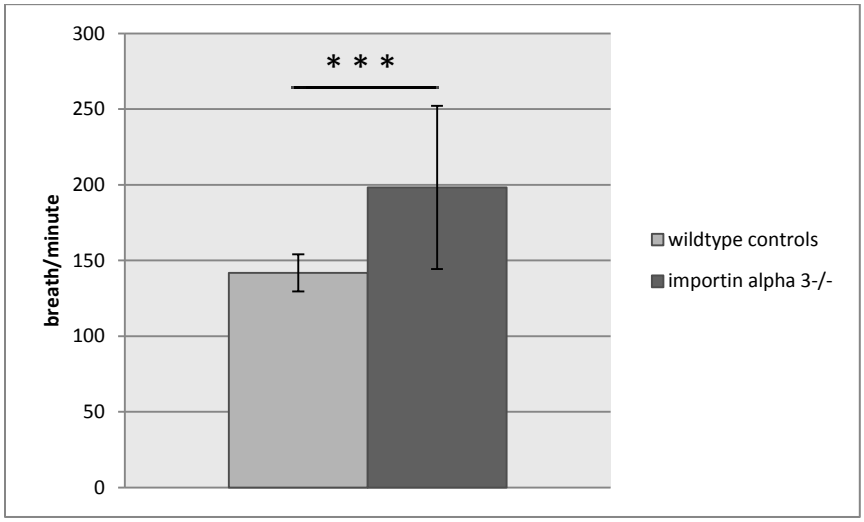


Figure 50: Respiratory frequency of importin α3-/- mice. The diagram displays the mean values of 17 importin α3 -/- and 16 wild type controls as well as corresponding standard deviations; $p < 0.001$.

3.8. Blood cell count

Importin α3 knockout mice showed an increased breathing frequency that may be caused by hypoxia. A lack of oxygen can be the result of a reduced red blood cell number or a low hemoglobin concentration. Therefore, the blood cell composition of importin α3-/- and wild type control mice was analyzed by a blood cell counter. However, none of the parameters differed significantly between importin α3-/- and wild type controls (**Figure 51** - **Figure 53**).

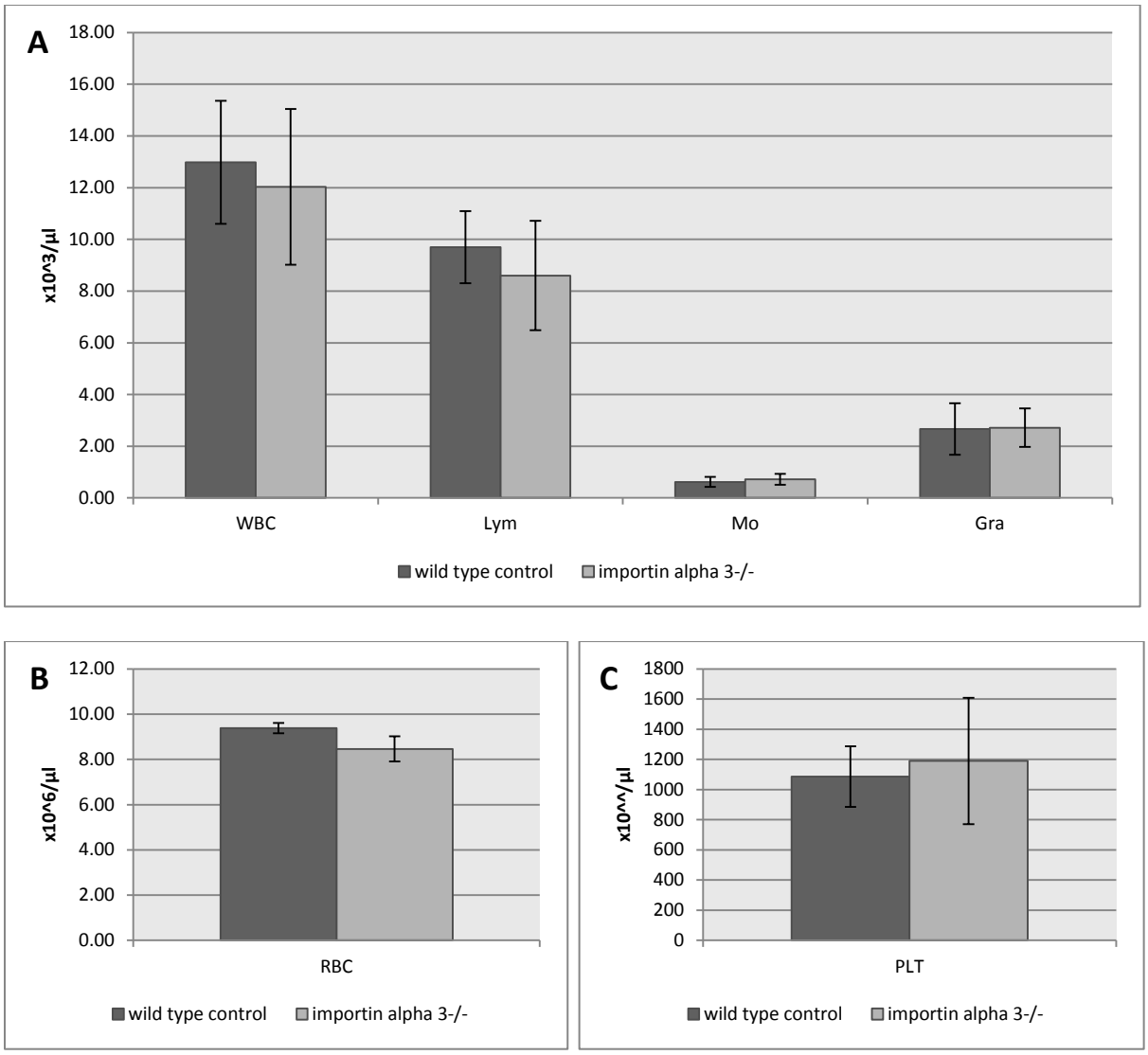
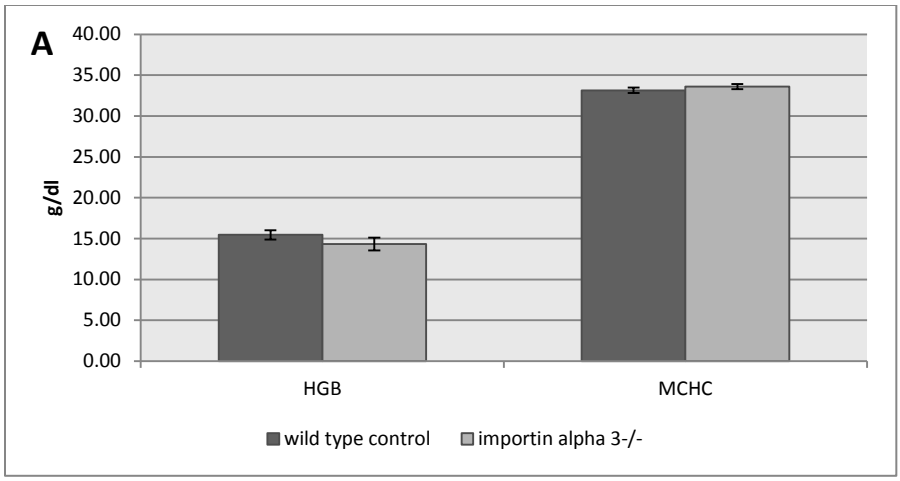
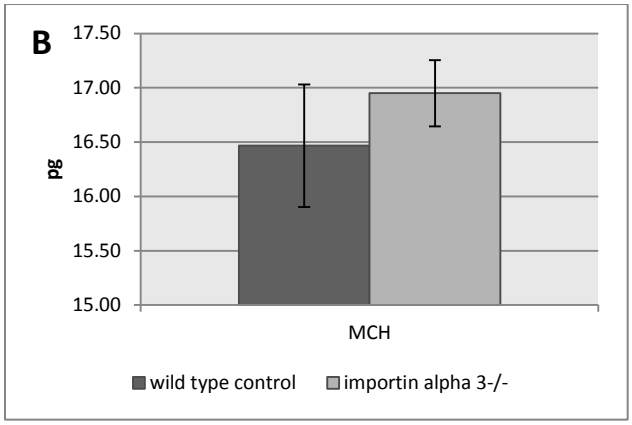
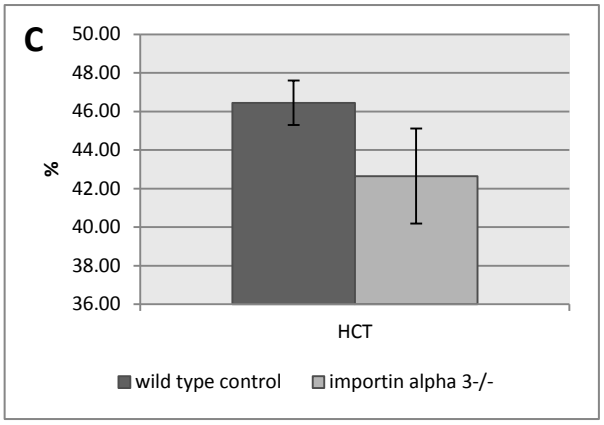


Figure 51: Blood cell count of importin α3-/- mice (I). Blood parameters of importin α3-/- and wild type control mice (n=6/group) were analyzed and mean values are displayed in the diagrams. A) WBC: white blood cells; Lym: lymphocytes; Mo: monocytes; Gra: granulocytes. B) RBC: red blood cells. C) PLT: platelets; error bar: standard deviation.







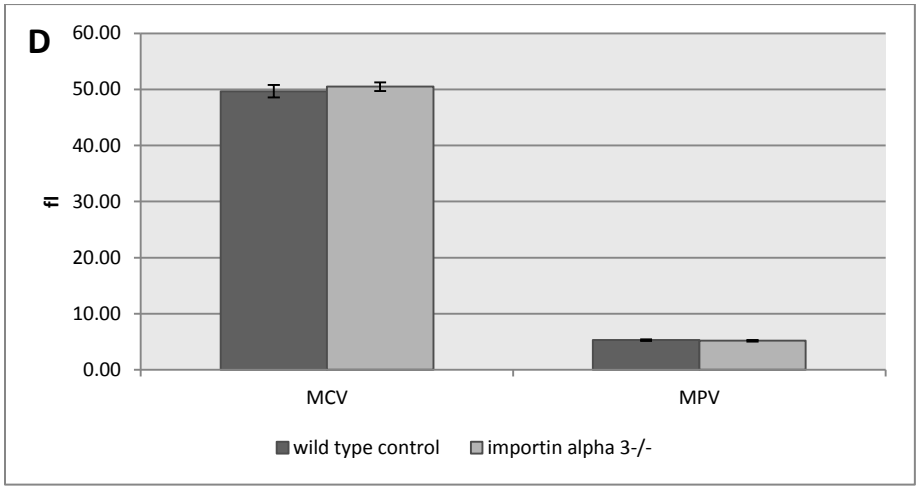


Figure 52: Blood cell count of importin α3-/- mice (II). Blood parameters of importin α3-/- and wild type control mice (n=6/group) were analyzed and mean values are displayed in the diagrams. A) HGB: hemoglobin; MCHC: Mean corpuscular hemoglobin concentration. B) MCH: Mean corpuscular hemoglobin. C) HCT: hematocrit. D) MCV: mean cell volume; MPV: mean platelet volume; error bar: standard deviation.

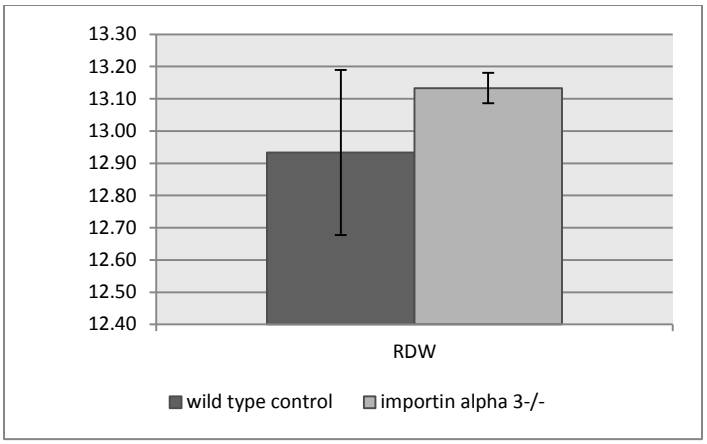


Figure 53: Blood cell count of importin α3-/- mice (III). Blood parameters of importin α3-/- and wild type control mice (n=6/group) were analyzed and mean values are displayed in the diagrams. RDW: red cell distribution width; error bar: standard deviation.

3.9. Blood gas analysis

Another reason for the increased respiratory frequency of importin α3 knockout mice may be an increased carbon dioxide level in the blood. To test this hypothesis, arterial blood was taken from mice and blood pH, oxygen (pO2), carbon dioxide (pCO_2) as well as oxygen saturation (sO2) and hydrocarbonate (HCO3) were determined by a blood gas analyzer at the Helios Clinic Berlin Buch, Germany.

The oxygen content, oxygen saturation and hydrogen carbonate blood level of importin α3-/- mice did not differ from wild type mice (**Figure 54, C-E**). However, the blood pH of importin α3 knockout mice is decreased by trend and the amount of carbon dioxide is significantly increased by 12 % (p= 0.0258) (**Figure 54, A and B**).

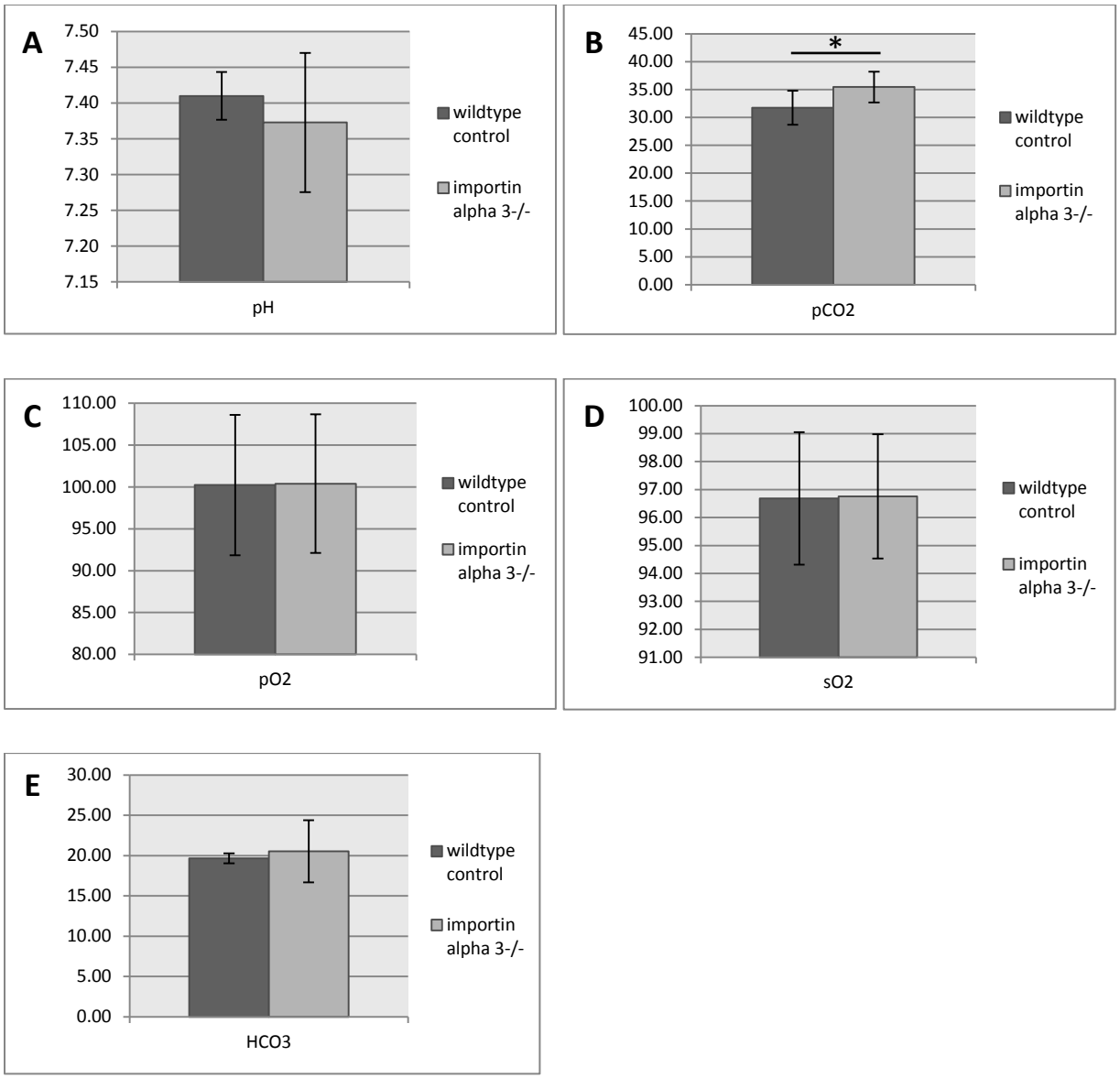


Figure 54: Blood gas analysis of importin α3-/- mice. Blood was taken from importin α3-/- (n=11) and wild type control mice (n=6) in order to determine the pH (A), partial carbon dioxide (pCO_2, B), partial oxygen (pO2, C) level as well as the oxygen saturation (sO2, D) and the hydrogen carbonate (HCO3) level. The diagram shows mean values. Error bar: standard deviation; $p<0.05$ (*).

3.10. Blood pressure and heart rate measurements

To further characterize the cardiovascular status of the importin α3 knockout mice, the basal blood pressure and heart rate were examined under physiological conditions. For this purpose, a catheter was implanted into the femoral artery of adult importin α3 -/- and wild type control mice.

The mean arterial pressure (MAP) did not differ significantly between importin α3-/- and wild type mice; neither in males nor in females (**Figure 55**). In importin α3-/- males, heart rates were slightly upregulated by trend however the differences was not significant (p=0.059) (**Figure 56, A**).

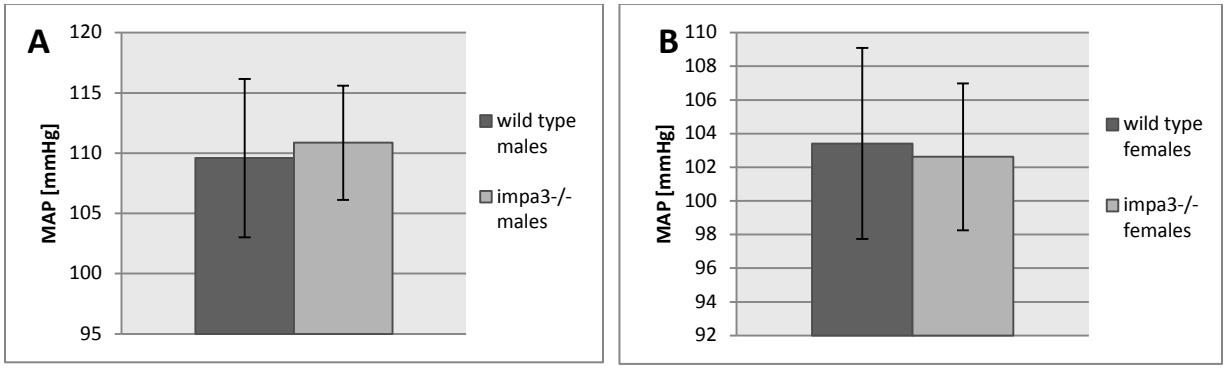


Figure 55: Basal blood pressure of importin α3-/- mice. The mean arterial blood pressure (MAP) of importin α3 knockout (impa3-/-) and wild type control males (A; n=8) and females (B; n=9-10) are depicted. Error bar: standard deviation, $p > 0.05$.

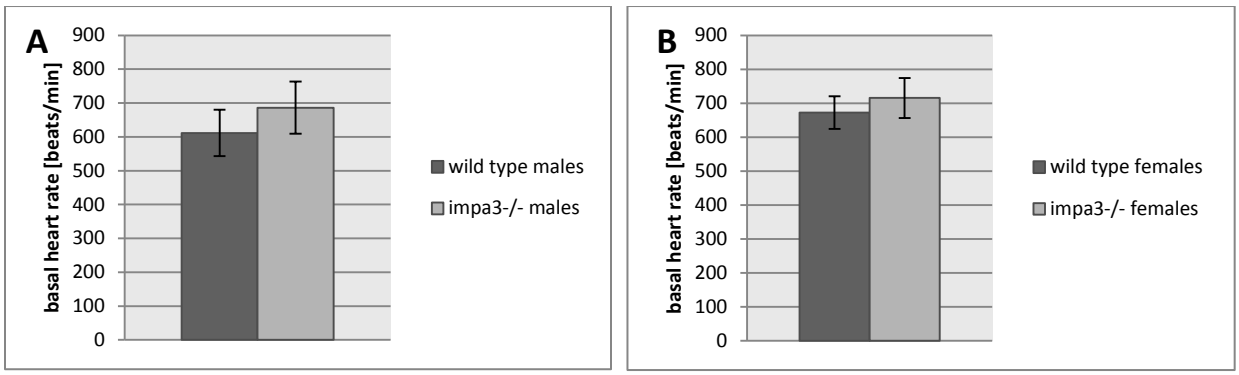


Figure 56: Basal heart rate analysis of importin α3-/- mice. The mean value of basal heart rates (HR) of importin α3 knockout (impa3-/-) and wild type control males (A; n=8) and females (B; n=9-10) are depicted. Error bar: standard deviation, $p > 0.05$.

3.11. Analysis of vasoconstriction response upon Angiotensin II treatment

Angiotensin II (Ang II) administration leads to vasoconstriction and is a model of induced hypertension in mice. Since importin α3-/- mice showed signs of impaired left ventricular function under basal conditions, Ang II administration was expected to worsen the physiological status of these mice. To test this hypothesis, Ang II was injected into adult importin α3

knockout and wild type mice. Subsequently, the blood pressure and heart rates of mice were measured for 180 seconds. In contrast to wild type control mice, the blood pressure response of importin α3-/- males and females was significantly decreased ($p<0.05$, **Figure 57**). Consistent with that, the heart rate response upon Ang II treatment was significantly ($p<0.001$) lower in importin α3-/- males and the same tendency was observed in females depleted of importin α3 (**Figure 58**).

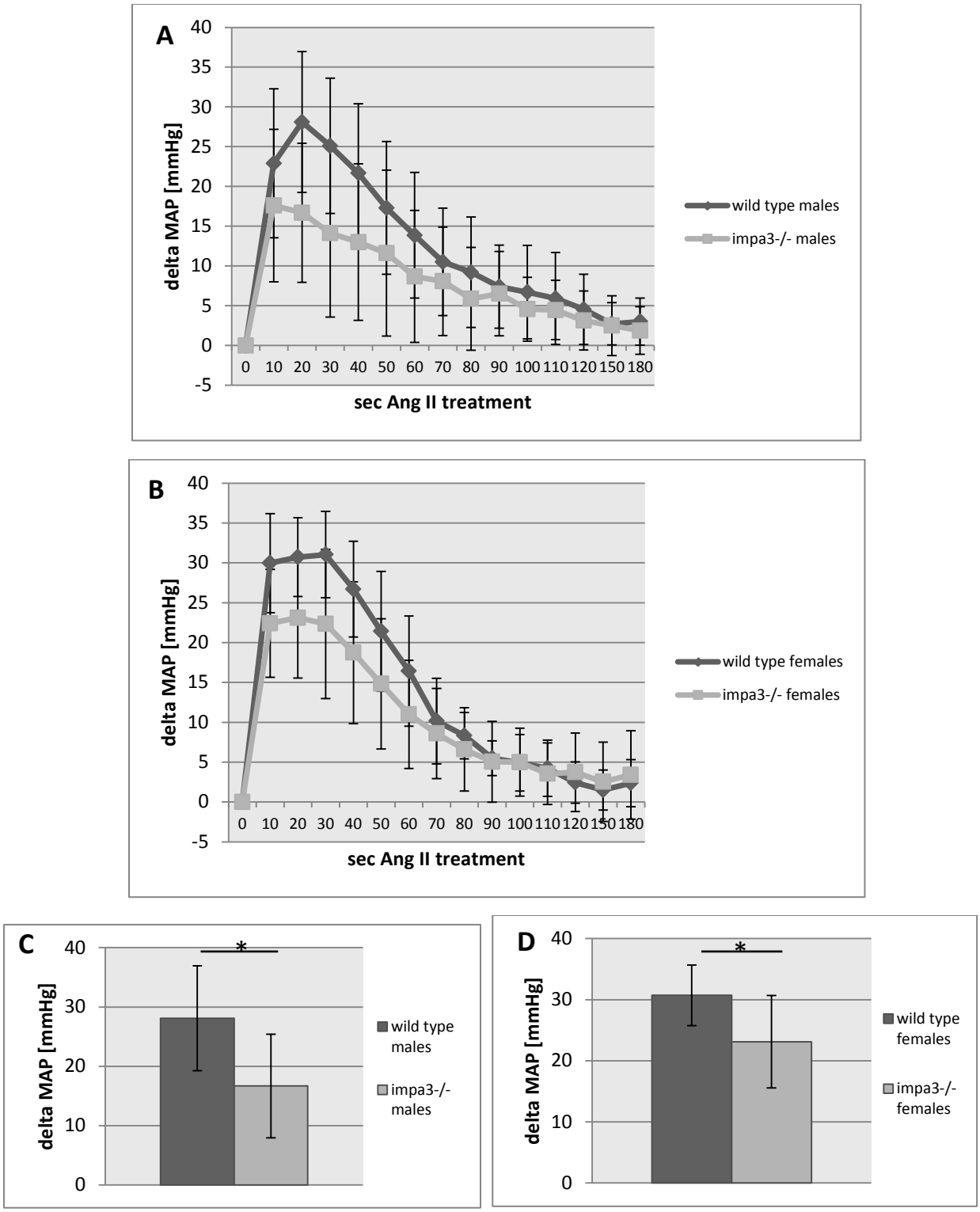


Figure 57: Blood pressure response after angiotensin II treatment of importin α3-/- mice. Time course of vasoconstriction response after Ang II injection into importin α3-/- (impa3-/-) and wild type males (A; n=8) or females (B; n=9-10). The delta mean arterial blood pressure (MAP) of impa3-/- and wild type mice are depicted for males (C) and females (D) during the first 20 seconds of Ang II response. Error bar: standard deviation, $p<0.05$ (*).

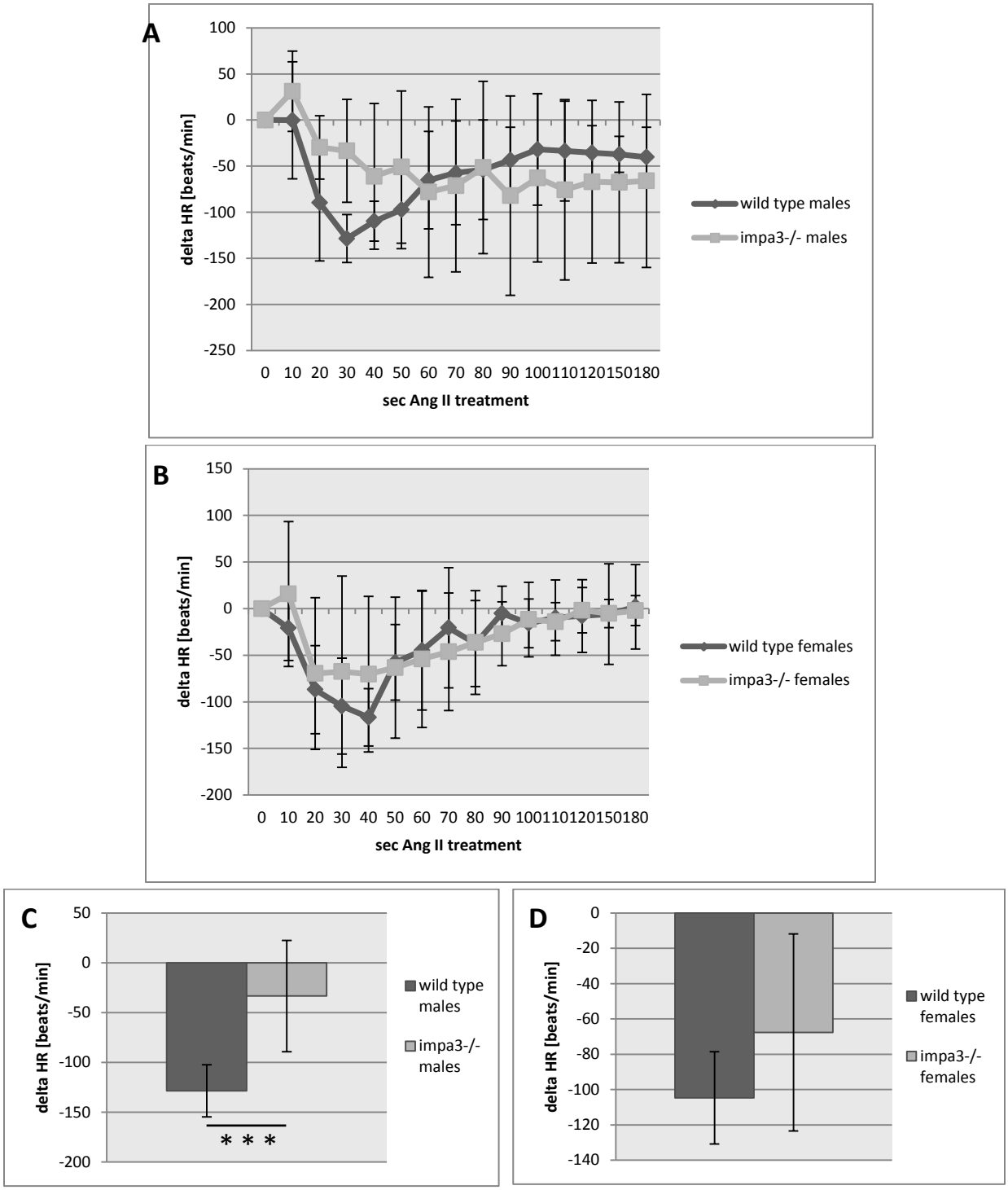


Figure 58: Heart rate response after angiotensin II treatment of importin α3-/- mice. Time course of vasoconstriction response after Ang II injection into importin α3-/- (impa3-/-) and wild type males (A; n=8; p≤0.001) or females (B; n=9-10). The delta mean heart rates (HR) of impa3-/- and wild type mice are depicted for males (C) and females (D) during the first 20 seconds of Ang II response. Error bar: standard deviation, p<0.001 (***).

3.12. Summary and Discussion Part C

The transport receptor importin α3 belongs to the second subfamily of importin α proteins. The Western Blot analysis of major murine organs revealed almost ubiquitous expression of importin α3 except in liver and kidney in which the importin α3 protein was below the detection limit. These data are in accordance with the analysis of importin α3 expression in human tissues [20]. Since importin α3 expression can be detected in liver and kidney on mRNA level it is supposed to be expressed in either low levels or by a limited number of specialized cells in these organs. Indeed, immunohistochemistry confirmed a low expression of importin α3 in hepatocytes of the liver as well as in tubules of the kidney.

3.12.1. Depletion of importin α3 caused embryonic death and fertility problems in mice

During the generation of homozygous importin α3-/- mice it became obvious that these mice are not born in the expected Mendelian ratios. Roughly one third of homozygous importin α3 knockout mice die before birth. The exact time point of developmental arrest is yet unknown. The elucidation of the stage, in which the embryos stop to develop, will help to propose biological processes, importin α3 is involved in. Therefore, the examination of embryos from homozygous importin α3 knockout females mated to heterozygous importin α3 knockout males at different days of development is planned.

Interestingly, homozygous importin α3 knockout mice showed a reduced fertility compared to heterozygous controls. Only 44% of animals produced offspring at all, regardless of sex. Moreover, importin α3-/- females displayed a decreased litter size compared to importin α3 heterozygous females. Ovaries of importin α3-/- females looked normal in initial histological examinations. Different stages of follicles could be found in importin α3-/- ovaries indicating the absence of a general follicle maturation dysfunction. Furthermore, importin α3-/- females can ovulate since several corpora lutea were present. Nevertheless, importin α3 knockout ovaries need further investigation since the number of analyzed females was very small and only mice in late adulthood were examined. In younger females, the number of follicles will be much higher helping to analyze different stages of follicle maturation in more detail. Moreover, ovaries analyzed by HE staining came from importin α3-/- females that gave offspring. Hence, it

is not clear how ovaries from unfertile importin α3-/- females look like. For upcoming histological analyses, females will be mated beforehand in order to clarify their fertility status and ovaries from fertile as well as unfertile importin α3-/- females will be compared.

Moreover, importin α3-/- males will be examined in more detail, as well. Histological analyses of testis tissue as well as the analysis of sperm morphology and numbers are planned.

Besides importin α7 and importin α3 also importin α2 (Kpna7) has been recently reported to be involved in embryonic development and fertility [25, 27]. Depletion of importin α2 in bovine embryos by RNAi significantly reduced the proportion of embryos developing to the 8- to 16-cell stage as well as the proportion of embryos developing to the blastocyst stage [27]. In mice, a loss-of-function mutation of importin α2 induced sex imbalance that led to a reduction of female offspring [25]. Moreover, the female reproductive ability was markedly reduced whereas males were not affected. The litter size of importin α2 mutant females was significantly decreased since many embryos died after embryonic day 8.5. Furthermore, pre-implantation embryos developed more slowly and significantly fewer embryos reached the blastocyst stage. Parthenogenetic activation of embryos showed that the cell cycle was ahead of schedule in importin α2 mutated embryos compared to embryos from the wild type group. In addition, the expression of chromatin modification associated genes was altered and H3K37 trimethylation was reduced on maternal chromatin in importin α2 mutant embryos. Moreover, importin α5 (Kpna1) has been postulated to play a role in the female reproductive organs of mammals. The importin α5 deficient mice (generated in the lab of Prof. M. Bader) are born in the expected Mendelian ratios and most of their organs do not show any obvious histological abnormalities [88]. However, ovaries of importin α5-/- females (generated in the lab of Prof. Y. Yoneda) appear underdeveloped, which is most probably caused by a decrease in progesterone receptor mRNA levels and subsequent low serum progesterone found in these mice [96]. Although importin α5 mutant females could become pregnant, litter size was low and many pups were stillborn. In addition, some females appeared to have problems in pup delivery.

Hence, embryonic development and fertility dysfunctions seem to be common problems caused by the depletion of individual importin α subtypes.

3.12.2. *Importin α3-/- mice show signs of right ventricular hypertrophy*

Elevated right ventricular walls and an increased right septum found in initial histological examinations of the heart indicated an isolated right ventricular hypertrophy (RVH) in importin α3 knockout mice. In the first instance, echocardiographic analyses did not seem to verify these findings since the right ventricular wall of importin α3-/-mice was not significantly increased. However, due to its position in the body and its smaller wall diameter compared to the left ventricle, the right ventricle is not easily analyzed by echocardiography. Furthermore, the left and the right septum wall cannot be distinguished in echocardiographic analyses. Hence, the whole septum seems to be enlarged in importin α3 knockout mice. Although, elevated left ventricular wall diameters were found in hearts of importin α3 knockout mice there was no corresponding change in functional echocardiographic parameters (ejection fraction and cardiac output) which would have been expected to be rather increased in LVH. Thus, reasons for the apparent LVH remain unclear. In conclusion, the echocardiographic analysis strengthens the hypothesis of importin α3-/- mice suffering from RVH potentially caused by pulmonary dysfunction.

One of the common causes for RVH is a pulmonary dysfunction (PD). Interestingly, several data indicate a pathological disturbance of the lung function in importin α3-/- mice that would support this hypothesis. First histological lung examinations indicated more contracted pulmonary arteries that might lead to pulmonary hypertension in importin α3-/- mice (personal communication; Dr. S. Rittinghausen, Fraunhofer Fraunhofer-Institut für Toxikologie und Experimentelle Medizin, Germany). Moreover, importin α3-/- mice have an increased respiratory rate which possibly attends a PD. The blood cell count did not show any signs of hypoxia caused by anemia or changes in hemoglobin parameters in importin α3 knockout mice. However, the blood gas analysis (BGA) revealed an elevated CO_2 level in the blood of importin α3-/-mice indicating a disturbed blood gas exchange that may lead to a compensatory upregulation of the respiration. On the other hand, no changes in O_2, pH and bicarbonate levels were detected. Yet, the BGA results may not be that reliable. The blood gases have been analyzed using a clinical device calibrated for human blood. It is possible that this device was not suitable to analyze blood from mice. Furthermore, samples were transported

approximately 10-15 minutes cooled by a thermal pack. Although the blood values are meant to be stable in the capillary for up to 30 minutes at 0-4°C according to manufacturer's instructions it cannot be ruled out that the time of analysis was not in the optimal range.

There are further signs indicating a worsening of the pulmonary circulation and subsequent decrease in cardiac preload. The disturbed blood pressure and associated heart rate response of importin α3-/-mice on ANG II treatment fit to this hypothesis. When the preload is low, the contractility of the left ventricle decreases. Functional heart parameters like fractional shortening, cardiac output, ejection fraction and stroke volume were significantly decreased in importin α3-/- mice. Due to that, importin α3-/-mice might not be able to properly increase their blood pressure. In addition, for the maintenance of a normal blood pressure, blood vessels might be a priori contracted in importin α3-/-mice. Therefore, Ang II cannot lead to further vasoconstriction. To test this hypothesis, it is planned to treat the importin α3-/-mice with nitric oxide (NO) donors and examine their ability of vasodilatation.

In upcoming experiments, the lung function of importin α3-/- mice will be further examined. In order to determine the velocity of blood flow coming from the lung, the pulmonary artery is planned to be analyzed by echocardiography. Moreover, the respiratory volume of importin α3-/- mice will be checked by plethysmography. In addition to that, the blood vessels of importin α3 knockout lungs will be examined in more detail. On the one hand, pulmonary vascular remodeling will be checked by smooth muscle α-actin staining and on the other hand, the lung tissue will be examined according to molecular markers for hypoxia like Hif1-α and its target genes like VEGF.

3.12.3. Importin α3-/- mice have misshaped glomeruli

First histological examinations revealed misshaped glomeruli in importin α3 KO kidneys that indicate a possible glomerulopathy. However, first of all, functional parameters need to be checked in order to verify a real nephropathy. For this purpose, creatinine and urea levels will be determined in the serum of importin α3-/- mice.

Interestingly, initial immunohistological stainings showed importin α3 expression only in the medulla of the kidney suggesting a role of importin α3 in maintaining the homeostasis of

solutes and water. Some years ago, it has been shown that importin α paralogs are differentially expressed in normal rat kidneys [97]. Whereas importin α5 and α7 are found in tubuli and glomeruli, importin α1 has only been detected in the tubuli of kidneys. Moreover, in diabetic rats, importin α7 was markedly up-regulated. In addition, glucose exposure led to increased expression of importin α7 in different cultured cells whereas the up-regulation of other importin α isoforms was less consistent. The authors therefore postulated that enhanced nuclear transport in glomerular and tubular cells may participate in increased gene expression and nephrosclerosis in diabetes. Although water consumption and urine production were normal in importin α3 knockout mice, a possible diabetic nephropathy cannot be ruled out since other associated parameters like blood glucose levels have not been analyzed, yet.

3.12.4. Conclusion and Future perspectives

Taken together, this study provides first insights into the physiological roles of importin α3 in mammals. The initial characterization of importin α3 KO mice clearly demonstrated specific functions of importin α3 in embryonic development and fertility in mice. Furthermore, importin α3 seems to be involved in the maintenance of normal lung function, thereby influencing the heart physiology. Moreover, there is first evidence, importin α3 playing a role in the kidney, impacting the glomerular shape. The next steps will be to further characterize these physiological roles; moreover, future experiments will deal with the underlying molecular mechanisms.

4. MATERIAL AND METHODS

4.1. MATERIALS

4.1.1. Chemicals

Chemical	Company
2-Mercaptoethanol 99%	SIGMA
Acetic acid	Roth
Acetone	Roth
Acrylamide Ultra-Pure	ICN
Ammoniumperoxodisulfate (APS)	Sigma-Aldrich
Ampicillin trihydrate	Serva
Aqua-Rothi phenole	Roth
Bacto Agar	Becton
Bacto Tryptophan	Becton
Bacto Yeast Extract	Becton
Bovine Serum Albumin	Sigma-Aldrich
Bradford Reagent	Sigma-Aldrich
Brom Phenol Blue	Sigma-Aldrich
Calcium chloride dihydrate	Sigma-Aldrich
complete protease inhibitor cocktail Mini	Roche
complete protease inhibitor cocktail Mini EDTA-free	Roche
Coomassie Brilliant blue R 250	Ferak Berlin
Dextrose	Sigma-Aldrich
Dodecylsulfate (SDS)	Serva
DTT (1,4-Dithiothreitol)	Biomol
EDTA	Serva
EGTA	Roth
Ethidium Bromide aqueous solution 1%	Serva
Formaldehyde (37%)	Roth
Glutamine	Ferak Berlin
Glycerol	Roth
HEPES	Roth
Hyaluronidase	Merck
Hydrochloric acid (37%)	Roth
Isopropyl Alcohol	Roth
Kanamycin sulfate	Sigma-Aldrich
L-Glutathione 98%	Sigma-Aldrich
L-Phenylalanine	Sigma-Aldrich
Magnesium acetate tetrahydrat	Merck
Magnesium chloride hexahydrat	Roth
Methanol	Roth

Nonidet P40	USB
Paraformaldehyde	Merck
Phenylmethylsulfonylfluorid (PMSF)	Sigma-Aldrich
Phosphate Buffered Saline	Sigma-Aldrich
Phosphoric Acid ACS Reagent (85.5%)	Sigma-Aldrich
Ponceau S solution	Sigma-Aldrich
Potassium Acetate	Merck
Potassium chloride	Merck
Potassium dihydrogenphosphate	Merck
Potassium hydroxide	Fluka
Proteinase K	Merck
Ribonuclease A (bovine pancreas)	Serva
Roti-Phenol	Roth
Rotiphorese Gel 30 (37; 5:1) (Acrylamide- and Bisacrylamide)	Roth
SDS (Sodium dodecylsulfate)	Serva
Sepharose	Sigma-Aldrich
Sodium acide	Sigma-Aldrich
Sodium carbonate	Roth
Sodium chloride	Roth
Sodium desoxycholate	Merck
Sodium dihydrogenphosphate	Merck
Sodium hydrogencarbonate	Merck
Sucrose	Merck
TEMED	Roth
Triton X-100	Sigma-Aldrich
TRIZMA Base	Sigma-Aldrich
TRIzol Reagent	Invitrogen
Tween 20	Sigma-Aldrich
Xylene	Roth

4.1.2. Kits and Enzymes

Component	**Company**
µMax anti-His Co-IP Kit	Miltenyi
DNaseI	Roche
IVTT Kit	Promega
JetStar Plasmid Purification MAXI Kit 2.0	Genomed
M-MLV Reverse Transcriptase	Promega
Precision Blue Protein™ Standard All Blue	BioRad Laboratories
QIAquick® Gel Extraction Kit	Qiagen
Restriction enzymes	New England Biolabs
RNaseA	Boehringer Mannheim

T4-DNA-Ligase	Promega
TaqDNA Polymerase	Invitrogen
Vectashield DAPI Mounting Medium	Vector Laboratories
Wizard® SV Gel and PCR Clean-up System	Promega

4.1.3. Antibodies

Antibody	**Dilution**	**Company**
Rabbit anti-C-myc	1:500	Sigma-Aldrich
Mouse anti-Brg1	WB: 1:2000, ICC:1:80	Novus Biologicals
Rabbit anti-Brg1	WB: 1:200, ICC: 1:50	Santa Cruz
Rabbit anti-importin α1, -3, -4, -5, -7	1:1000	[20]
Rat anti-importin α1	1:1000	Sigma-Aldrich
Goat anti-importin α3	1:500	Everest Biotech
Goat anti-importin α4	1:1000	Everest Biotech
Rabbit anti-P-STAT1	1:000	Cell Signaling
Mouse anti-KEAP1	1:500	R&D Systems
Mouse anti-RCC1	1:200	Medical & Biological
Diverse IRDye coupled antibodies for the Odyssey System	1:7000 - 1:15000	LiCor, Bad Homburg
Donkey anti-rabbit Cy3	1:100 - 1:500	Jackson Immuno
Donkey anti-mouse Cy3	1:100 - 1:500	Jackson Immuno
Donkey anti-goat Alexa 594	1:100 - 1:500	Invitrogen
Donkey anti-rat Cy3	1:100 - 1:500	Jackson Immuno

4.1.4. Plasmids

- pcDNA3.1 myc-HIS (Invitrogen)
- pGEM®-Teasy (Promega)
- pJW10 (modified pQE60 [63])

4.1.5. Primer

primer name	**primer sequence**
Impa1_BamH_for	5’-TT AAA GGA TCC ATG TCC ACG AAC-3’
Impa1_BamH_rev	5’-TT AAA GGA TCC ATAGAAGTTAAA-3’

Impa2_BamH_for	5’- AAA GGA TCC ATGGCTACCTCAAAGG-3’
Impa2_BamH_rev	5’- TTG GAT CCA CAC ACT CTC AGC C-3’
Impa3_BamH_for	5’- AA GGA TCC AAA ATGGCGGACAAC -3’
Impa3_BamH_rev	5’- TTG GAT CCA TAA AAC TGG AAC CC-3’
Impa4_BamH_for	5’- AAA GGA TCC ATGGCCGAGAACC -3’
Impa4_BamH_rev	5’- TTG GAT CCA TAG AAA TTA AAT TC-3’
Impa5_BamH_for	5’- AAA GGA TCC ATGTCCACACCAG -3’
Impa5_BamH_rev	5’- TTG GAT CCA TAA AGC TGG AAA CC-3’
Impa7_BamH_for	5’-TT AAA GGA TCC ATG GCA AGC CCA GAA GGA C -3’
Impa7_Bgl_rev	5’- TTA AAA GAT CTA TAT AGC TGG AAG CCC TCC AT -3’
Impa3for1	5’- GCA CAT CAC CGT AGT CAG TCC -3’
Impa3rev1	5’- CTC CGG AGT GCT TAG CCA ATC -3’
Impa3neofor	5'- CCC TCA GTG TGA ATT ACT TCC -3'
NeoL1	5’- GTT GTG CCC AGT CAT AGC CGA ATA GCC -3’
Impa4In23for	5’-CAA AAG CAG TGG GGC TAA AC-3’
Impa4In67reva	5’-ACG AGT TTG GCC TAC AGC AC-3’
Impa4Neorev	5’-GCC CAG TCA TAG CCG AAT AG-3’
Impa5WT5	5’- TAG CTT GTT TTG GGC TGG TG -3’
Impa53	5’- GGG TAA AGT TCT AGG CTA GC -3’
BG3	5’- AGG CGA TTA AGT TGG GTA ACG -3’
Impa7Int3	5’- GAC AAA CAT GCA GGC AAA TCA C -3’
Impa7_9_for	5’- ATG AAC CAG TAT GCC CAT CAA AT -3’
Bgeo5	5’ -GAC GTC TCG TTG CTGCAT AAA C- 3’
Bgeo3	5’- CAG CAG CAG CAG ACC ATT TTC AA- 3’

4.1.6. Equipment and Expendable Material

Equipment	**Company**
Animal food	Ssniff
Bacteria shaker Certomat®H	B.Braun
Balance 440-43N	Kern & Sohn GmbH
Binocular MZFLIII	Leica
Cell culture dishes	TPP®
Cooling centrifuge Megafuge 1.0R	Heraeus Instuments GmbH
Cooling centrifuge Sigma 3K12	Sigma
Cooling centrifuge Sorvall®PC5C Plus	Kendro
Cryo 1°C Freezing Container	Nalgene®Nunc
Cryo tubes Cryo.S	Greiner bio-one

Electroporator 2510	Eppendorf
Falcon tubes	TPP®
Fast Prep benchtop homogenizer	MP Biomedicals
Fluorescence Microscope Biorevo	Keyence
GenePulse® Cuvetts	BioRad Laboratories Richmond
Incubator	Heraeus Instruments GmbH
Incubator Cell Culture C60	Labotect
Incubator Cell Culture Heracell	Heraeus Instruments GmbH
Infinite Microplate Reader M200	Tecan
Keyence Microscope	Biorevo
LabTec Chamber Slides	Nunc
Magnetic stirrer MR3001	Heidolph
Microscope CKX31	Olympus Deutschland GmbH
Microwave 8020	Privileg
Mikrotom SM2500	Leica Microsystems GmbH
Odyssey Imaging System	LiCor
One-way pipettes Cellstar® 1, 2, 5, 10, 25 ml	Greiner bio-one
Pasteur pipettes	Roth
PCR tubes	Biozym Scientific GmbH
pH meter pH level 1	WTW
Pipettes	Gilson
power supply PowerPac™HC	BioRad Laboratories
PVDF membranes	Amersham Bioscience
Refrigerated Condesation Trap RT100	Savant Instruments
Roller Mixer SRT1	Snijders
Save-Lock tubes	Eppendorf
SDS-PAGE gel electrophoresis chamber	BioRad Laboratories
Special accuracy weighing machine	Sartorius Research
SpeedVac SVC100	Savant Instruments
Sterile laminar flow work bench Laminair®HB2448	Heraeus Instruments GmbH
Sterile laminar flow work bench safe 2020	Thermo-Scientific
Swiveling platform Polymax 1040	Heidolph Instruments
Table top centrifuge 5415D	Eppendorf
Table top centrifuge Biofuge pico	Heraeus Instruments GmbH
Table top centrifuge Labofuge 400e	Heraeus Instruments GmbH
Tank blot	BioRad Laboratories
Test tube rotator	Snijders
Thermo cycler PTC-200	BioRad Laboratories
Thermo mixer 5436	Eppendorf
Trans illuminator MultiImage™Light Cabinet	A Innotech Corporation
Ultra-Turrax T25 basic	IKA® Labortechnik
Ultrasound device Sonoplus	Bandelin electronic
Vacuum pump Vacusafe comfort	IBS Integra Bioscience

Vertical gel electrophoresis chamber	Biometra
Vortexer Genic 2	Bender & Hobein AG
Water bath	GFL
Whatman paper	Whatman® International

4.2. METHODS

4.2.1.Cell culture

4.2.1.1. Generation and cultivation of murine embryonic fibroblast cell lines

Wildtype and importin α knockout (-/-) murine embryonic fibroblasts (MEFs) were prepared from murine embryos harvested from pregnant females on embryonic day 13.5. To obtain immortalized cell lines, primary MEF were passaged at least 25 times according to an adaption of the 3T3 protocol [98]. MEF and NIH3T3 cells were maintained in Dulbecco's modified Eagle's medium (Gibco) supplemented with 10% fetal bovine serum and 1% penicillin/streptomycin.

4.2.1.2. Stimulation of cells

IFN-γ-stimulation: For the analysis of activated STAT1, MEF were stimulated with 20ng/ml murine IFN-γ (Thermo Scientific, 53113 Bonn, Germany) and harvested after 10 or 30 minutes.

LMB-treatment: To analyze the nuclear levels of KEAP1 in MEFs, nuclear protein export was blocked by 50nM leptomycin B (SIGMA) treatment for two hours.

4.2.1.3. Transfection of cells

One day before transfection, cells were seeded in DMEM (GIBCO, Darmstadt, Germany), supplemented with 10% FBS, without antibiotics onto a 10 cm dish such that they would be 80-90% confluent at the time of transfection. On the next day, cells were transfected with 15-24 µg importin α expression constructs using 45-60 µl lipofectamine LTX and PLUS reagent (Invitrogen, Darmstadt, Germany) according to manufacturer's instructions.

4.2.1.4. Isotope labeling by amino acids in cell culture (SILAC)

In order to label the proteome of wildtype and importin α7-/- MEFs with heavy and light amino acids, respectively, cells were cultured in SILAC medium composed of DMEM high glucose (4.5 g/l) supplemented with 10% dialyzed FCS (Sigma-Aldrich, Munich, Germany), 1% Penicillin /Streptomycin (GIBCO, Darmstadt, Germany), L-glutamine (4 mM) as well as light (arginine-0; 28 ug/ml and lysine-0; 48 ug/ml) or heavy amino acids (arginine-10; 28 ug/ml and lysine-8; 48 ug/ml) (Sigma-Aldrich, Munich, Germany). The SILAC medium was a gift of the working group of Prof. Dr. M Selbach. Cells were cultured 2-3 weeks while they were splitted at least two-times per week 1:4.

4.2.2.Molecular biology

4.2.2.1. Cloning of importin expression constructs

For the generation of murine importin α1, α2, α3 and α5 expression constructs, the open reading frames of importin α isoforms were amplified by PCR from murine NIH3T3 cells (for primer sequences, see Materials). The open reading frames of murine importin α4 and importin α7 were amplified from vector templates (importin α4-pGEX was a gift from C. Kaether, Leibnitz Institut für Altersforschung, Jena, Germany; importin α7-YFP mammalian expression vector was purchased from RZPD German Resource Center for Genome Research). For importin α7 gene cloning, cDNA was modified to create 5'-BamHI and 3'-Bgl II restriction sites for further cloning into target vectors. All other cDNAs were modified by PCR to create BamHI restriction sites in the 5'- and 3'- flanking regions. The PCR product was first subcloned into the pGEM-T-easy vector and positive clones were detected by blue-white screening.

For mammalian expression, importin α cDNA was inserted into pcDNA3.1(-) leading to 3'-mycHis tagged fusion proteins. For bacterial expression, cDNAs of murine importin α7 and importin α2 were inserted into a modified pQE60 vector with 3'-GST-tag [47, 99]. The human importin β1-pQE60 clone was generated as described elsewhere [21].

All DNA manipulations were performed according to standard protocols. All primers used in this study were purchased from BioTez GmbH (Berlin-Buch, Germany).

4.2.3.Protein expression and analysis

4.2.3.1. Production of GST- and His-fusion proteins in E. coli cells

Importin α2-GST, α7-GST, GST and importin β-His expression vectors were inserted into *E.coli* BL21 (DE3) by electric transformation. Transformed bacteria were cultured at 17°C over night in the presence of 0.05 mM IPTG. GST fusion proteins were applied to a GST-Trap 4B column and eluted with 15 mM reduced glutathione. Importin β-His was applied onto a Ni^{2+}-charged His-trap Crude FF column and eluted by 250 mM imidazole. Further purification of recombinant proteins was done using a gel filtration 26/60 Superdex 200 prep grade column. Recombinant human importin α proteins were generated as described previously [40].

4.2.3.2. Binding studies of in vitro transcribed and translated (IVTT) proteins

The here described binding studies were performed by Dr. R. Depping in the Institute of Biology at the University of Lübeck or by the author. For *in vitro* binding studies of Brg1 to maternally expressed importin α-GST proteins, the vector containing the open reading frame of Brg1 (smarca4) was purchased from imaGenes GmbH, Berlin, Germany. Full length cDNA was transcribed and translated *in vitro* in the presence of [^{35}S]-methionine (TNT Quick Coupled Transcription/Translation Systems, Promega, USA) according to manufacturer's instructions. [^{35}S]-labeled methionine was obtained from Hartmann Analytic (Braunschweig, Germany). GST-importin pull-down assays were carried out with purified GST as a negative control. 100 µl beads were preequilibrated in IP-buffer (20 mM Hepes pH 7.5, 100 mM KOAC, 0.5 mM EGTA, 5 mM MgOAc, 250mM sucrose, 4°C), mixed with 30 µg GST protein or 30 µg GST-fusion and incubated at 4°C over night. Next day, 10 µl of the Brg1-IVTT reaction batch were allowed to bind to the immobilized fusion proteins. After washing three times with IP-buffer, the sepharose beads were dissolved in 60 µl Laemmli buffer. Proteins were separated by SDS-PAGE (10%) and visualized by Coomassie Brilliant Blue staining. To detect the [^{35}S]-labeled proteins, the dried gels were autoradiographed (16-24 h). Binding was visualized by analyzing the data with the PCBAS 2.09 g software (Raytest Isotopenmessgeräte GmbH, Germany) [100].

4.2.3.3. Protein preparation from tissues

Murine organs were homogenized in 100-1000 µl of cold 1x cell lysis buffer (Cell Signaling, Frankfurt, Germany) supplemented with 1x protease inhibitor cocktail (Roche, Grenzach-Wyhlen, Germany), 3mM DTT and 0.4 mM PMSF by using a FastPrep-24 device (MP Biomedicals, Eschwege, Germany). After short sonication and centrifugation of the lysate, soluble proteins were collected from the supernatant. Protein concentration was determined by Bradford assay [101].

4.2.3.4. Nuclear-cytoplasmic fractionation of cells and organs

Cells were washed and harvested in PBS by scrapping. The cell pellet was resuspended in hypotonic buffer A (for composition of buffers, see **Table 17**) with 2% NP40. Cells were allowed to lyse for 10 minutes on ice by additional mixing. After centrifugation for 15 minutes at 2,500x g, the cytoplasmic supernatant was collected and the pellet was resuspended in hypotonic buffer B. Afterwards, the homogenate was mixed with hypertonic buffer C and incubated for 45 minutes at 4°C with occasional mixing. Finally, the protein lysate was centrifuged at 20,000x g for 45 minutes at 4°C and the nuclear fraction supernatant was collected.

Organs were homogenized in ice cold PBS by using a FastPrep machine (2x 40 sec). Cells were pelleted by centrifugation for 3 minutes at 2600 rpm in a table top centrifuge at 4°C. Afterwards, cytoplasmic and nuclear fractions were prepared as described for monolayer cells.

Table 17: Buffer composition for nuclear and cytoplasmic fractionation of cells.

hypotonic buffer A	MgCl2 1.5mM, KCl 10mM, Tris pH 7.9 10mM
hypotonic buffer B	Glycerol 20%, MgCl2 1.5mM, KCl 10mM, Tris pH 7.9 20mM
hypertonic buffer C	Glycerol 20%, MgCl2 1.5mM, KCl 1.2M, Tris pH 7.9 20mM

4.2.3.5. SDS PAGE and Western Blot

In order to examine proteins by SDS-PAGE and Western Blot, protein solutions were mixed with 1x Laemmli buffer (Carl Roth, Karlsruhe, Germany) and heated for 5 minutes at 95°C. All

Western Blot assays were done using 10% SDS gels. 10 µl per lane of cytoplasmic or nuclear extracts were loaded for the analysis of importin α substrate specificities as well as for the examination of importin α-mycHis fusion proteins expressed in NIH3T3 cells. Western Blot analysis of tissue extracts were performed with 40 µg of protein per lane whereas expression of importin α isoforms in pre-implantation embryos was done using approximately 70-80 oocytes or zygotes per lane, respectively. After electrophoretic separation, proteins were transferred to a PVDF membrane which was subsequently blocked with Odyssey blocking solution (LiCor, Bad Homburg, Germany) for 1-2 hours at room temperature. Next, protein membranes were incubated with primary antibody (for antibody details, see 4.1.3) at 4°C over night. The following day, membranes were washed in TBST for 30 minutes and incubated with appropriate secondary antibodies which were coupled to an infrared dye (LiCor, Bad Homburg, Germany) for 1 hour at room temperature. Finally, protein detection was performed using the Odyssey Infrared Scanner (LiCor, Bad Homburg, Germany).

4.2.3.6. Co-immunoprecipitation of importin α binding partners

In order to identify importin α binding partners from NIH3T3 cells transiently transfected with importin α-mycHis expression constructs, a confluent 10cm cell culture dish was harvested after two days of transfection. The co-immunoprecipitation assay was performed using the µMACS anti-His Epitope Tag Protein Isolation kit from Miltenyi according to manufacturer's instructions. Before loading the cell extract to the columns, anti-His magnetic beads were rinsed two times with 2mg/ml BSA blocking solution.

4.2.3.7. GST pull-down of importin α binding partners

For the identification of importin α binding partners from murine tissues, organs were homogenized in 1x lysis buffer (Cell Signaling) (**Table 18**) supplemented with protease inhibitor cocktail without EDTA (Roche) using a FastPrep-24 machine (MP). 30 µg of GST-fusion proteins were coupled to 100 µl glutathione sepharose (Qiagen) overnight at 4°C. Next day, beads were washed two times with IP-buffer (20 mM Hepes, 150 mM NaCl, 150 mM K-acetate, 5 mM Mg-

acetate, 0.5 mM EGTA, 250 mM Sucrose, pH 7.5) and blocked with 2mg/ml BSA in IP-buffer. Tissue lysates were pre-cleared by incubation with empty beads for 1 hour at 4°C on a rotating wheel. Next, GST-fusion protein coupled beads were mixed with pre-cleared lysate supplemented with 45 µg importin β-His and incubated overnight at 4°C. Finally, beads were washed and bound proteins were eluted by adding hot 1x Laemmli buffer.

Table 18: Details of tissue preparation.

organ	nr. of organ/sample reaction	lysis buffer [ml]
testis	3	1
rat ovary	3	1
mouse ovary	15	0.5

4.2.4. Mass spectrometry and analysis of result lists

The mass spectrometry analysis of importin α binding partners from GST pull-down and co-immunoprecipitation experiments was done in the Core facility of the Max Delbrück Center by Dr. Gunnar Dittmar and his team. The eluted proteins from the GST pull-down and co-immunoprecipitation experiments were separated on a 10% SDS-gel. After staining with Coomassie Blue the gel lane was cut in 12 slices. Proteins in each of the slices were converted to peptides by in-gel digestion with trypsin [102]. The recovered peptides were separated on an in house packed 15 cm reverse-phase column (3 µm beads, Reprosil, Dr. Maisch HPLC GmbH, Ammerbuch-Entringen, Germany) using a 10-50% acetonitrile linear gradient on an easy-nLC system (Proxeon, Dreieich, Germany). The separated peptides were directly applied to an LTQ-OrbiTrap mass spectrometer (Thermo scientific, Dreieich, Germany). The recorded spectra were analyzed using the MaxQuant software package [103].

Identified proteins were further analyzed according to gene ontology terms using the website of the ToppGene Suite (http://toppgene.cchmc.org/).

The mass spectrometry analysis of the SILAC experiment for the identification of proteins decreased in importin α7-/- MEF nuclei was done by Dr. Matthias Sury from the work group of Prof. Matthias Selbach at the Max Delbrück Center.

Result list overlays and comparisons were performed using a software algorithm generated by Dipl.-Ing. Dennis Wieruch (Fraunhofer HHI - Heinrich Hertz Institute, 10587 Berlin, Germany).

The comparison of two unsorted lists containing case insensitive protein names is done by a function called "protein_compare". The function uses text files as input. Each text file contains a list of several proteins, where different proteins are separated by a new line and alternative names of one protein are separated by a chosen separator, e.g. ";". The two lists are compared by matching each protein name of the one list with the other list. Furthermore, multiple alternative names of each protein are taken into account, so that missing alternative names are not causing missing matches. I.e. just one alternative name of the first list has to match an alternative name of the second list to count as a match. Following, all matching proteins are stored in an independent file, where different proteins are separated by a new line.

4.2.5.Stainings of murine cells and tissues

4.2.5.1. Tissue fixation, embedding and sectioning

To prepare the paraffin sections, tissues were freshly isolated and fixed for 48h in 4% PFA at 4°C. Then the specimens were washed twice with PBS and dehydrated in a methanol bath continuously (each 1h in 25%, 50%, 75% and twice in 100% methanol in PBS). This was followed by 1h incubation in 100% ethanol and 1h in toluene to dehydrate the preparations completely. For paraffin embedding, the biopsies were immersed in paraffin at 60°C. Two days after the biopsy was placed in fresh paraffin. To cut, the tissue was placed in a metal mold, aligned, layered with fresh paraffin and the grid was covered. After the paraffin has hardened the tissue was cut slowly either at 4°C or on the microtome (Microm HM360) in 5 microns slices. These sections were placed in 45°C bath to get smoothed and placed on glass slides.

4.2.5.2. HE-staining of tissue sections

The hematoxylin and eosin (HE) staining was based on the recommendations of the manufacturer's instructions. This staining method colors nuclei of cells in blue and the counterstaining with alcoholic solutions of eosin Y, colors other structures in red, pink or orange. Before staining, the paraffin sections were deparaffinized in 3x3 min xylene baths, followed by descending ethanol series (3x3 min 100%, 1x3 min 95%, 1x3 min 80%, 1x5 min dH2O rehydrated) following the manufacturer's protocol.

Next, slides were stained with hematoxylin for 3 minutes and subsequently rinsed in deionized water. Hematoxylin staining was allowed to develop for 15 minutes in normal tap water then the slides were dipped for 8-12 times in acid ethanol to destain. Next, the tissue sections were rinsed two times with tap water for one minute and one time with deionized water for two minutes before they were stained with eosin (1x30 sec, 3x5 min 95% ethanol, 3x5 min 100% ethanol, 3x15 min Xylol). Finally, slides were mounted with Eukit mounting medium and allowed to dry overnight.

4.2.5.3. Immunocytochemical stainings of MEFs

In order to analyze the cellular localization of importin α7 binding partners, wildtype and importin α7-/- MEFs were seeded on a 24 well plate. When cells reached 70-90% confluence they were washed two times with PBS and fixed with 4% PFA for 10 minutes. After two times of washing, cells were permeabilized with 0.1% Triton-PBS for 10 minutes and subsequently blocked by incubation in 5% normal donkey serum-PBS solution for 30 minutes at room temperature. Next, cells were labeled with appropriate antibodies at 4°C overnight (for antibody details see 4.1.3). The following day, cells were washed two times with PBS and incubated with an appropriate Alexa647 coupled secondary antibody (Invitrogen, Darmstadt, Germany) (1:500 in 0.1% Triton-PBS) for 1 hour at room temperature. After final washing, cells were mounted with mounting medium supplemented with DAPI nuclear staining reagent (Vector). Analyses of immunocytochemical staining were done using a Keyence microscope (Biorevo).

4.2.6.Physiological Examinations

4.2.6.1. Respiratory frequency measurement

The respiratory frequency measurement was performed by Prof. Dr. Mihail Todiras using a two chamber plethysmograph (EMKA Technologies, Paris, France). The plethysmograph is formed from two parts which come together to form two independent airtight chambers: the animal and reference chambers. The animal chamber has a pneumotachograph, a port for connecting a differential pressure transducer. The mouse was placed whole body in the animal chamber

wherein it can freely move and its ventilator function was followed for one hour (**Figure 59**). The upper chamber of the installation is connected to a constant flow pump to ensure proper and continuous flow of fresh air (200 ml/min).

Figure 59: Mouse sitting in a plethysmograph.

4.2.6.2. Blood pressure measurements

Blood pressure measurements were done by Prof. Dr. Mihail Todiras. To examine the blood pressure directly by an arterial catheter, a modified cannula (MICRO-RENETHANE 0.25 X 0.12) was inserted into the abdominal aorta through the left femoral artery under intraperitoneal ketamine (100 mg/kg) and xylazine (10 mg/kg) anesthesia. Another catheter was placed in the femoral vein for drug infusion. The catheters were tunneled subcutaneously, exteriorized at the animal's neck and sutured between the scapulae. Postoperative analgesia was provided by adding a 2% Xylocain gel in the operated area at the end of surgery. During the entire time, animals were directly and continuously observed and monitored in a warm environment until they fully recovered from anesthesia.

Since this procedure does not influence the behavior of the mice after implantation, the systolic and diastolic blood pressure can be measured under physiological conditions. After 48 hours, blood pressure and heart rate were recorded with a transducer (MLT 1050 model) connected to a computer system for data acquisition and analysis (PowerLab, ADInstruments) in freely moving animals. After 90 minutes of basal blood pressure recording angiotensin II (100 ng / kg)

was injected into femoral vein by Hamilton syringe to examine the vasoconstriction response. Blood pressure and heart rate were recorded for 1 hour. At the end of the experiment, the catheters were taken out of the animals and mice were allowed to recover for at least 10 days.

4.2.6.3. Metabolic analysis

Metabolic analysis was performed by Ilona Kamer. In order to analyze the amount of drinking volume and urine production, mice were examined by using a metabolic cage. For this purpose, each mouse was put into a single cage and the water consumption and urine production were determined for 3 days.

4.2.6.4. Blood cell count

Mice were anesthetized by isoflurane and a short piece of tail was cut to obtain a drop of blood for the analysis. Blood cell parameters were subsequently measured by using a blood cell counter (Heska Vet ABC Animal Blood Analyzer, ABBOTT LABS).

4.2.6.5. Blood gas analysis

Approximately 50 µl blood was taken from an arterial catheter implanted beforehand in the mice. The blood samples were directly filled in a capillary (Clinitubes, 55µl, Radiometer.) and cooled at 4°C. Oxygen, carbon dioxide, oxygen saturation, blood pH and hydrogen carbonate were measured by using a blood gas analyzer (Radiometer ABL800 Flex, Helios Clinic Berlin, Germany) at the Helios Clinic Berlin, Germany with the help of Mrs. Katja Bretting.

4.2.6.6. Echocardiography

The transthoracic echocardiographic examinations in mice were performed at the MDC with the help of technical assistant Martin Taube. Before conducting the echo measurements, mice were anesthetized by isoflurane inhalation; the chest hair was removed with a topical depilatory agent and applied with ultrasound transmission gel to both the transducer and the contact area on the mouse. The measurements were made with a Visual Sonics Vevo 770 and a 45-MHz ultrasound linear probe especially suitable for the investigation of small animals. Two-dimensional images were recorded in parasternal long and short-axis projections with guided

M-mode recordings at the midventricular level in both views. LV wall thickness, interventricular septum (IVS) and posterior wall (PW) thickness, and internal dimensions at diastole and systole (LVIDd and LVIDs, respectively) were measured. LV fractional shortening [(LVIDd – LVIDs)/LVIDd] x 100 (%), LV Ejection fraction [(end-diastolic^3 - end-systolic^3) / end-diastolic^3] x 100 (%), and LV mass [1.05(IVS thickness + LVIDd + PW thickness)^3 – LVIDd^3] were calculated from the M-mode measurements.

4.2.7. Statistics

The generated data of this study was statistically analyzed by using the program Microsoft Excel. Results were displayed as mean values ± standard deviation (SD) if not mentioned differently. Statistics were done using the Student's t-Test and data were accepted as significant at a p-values of $p<0.05$.

5. LITERATURE

1. Mazzanti M, Bustamante JO, Oberleithner H: **Electrical dimension of the nuclear envelope**. *Physiol Rev* 2001, **81**(1):1-19.
2. Sorokin AV, Kim ER, Ovchinnikov LP: **Nucleocytoplasmic transport of proteins**. *Biochemistry* 2007, **72**(13):1439-1457.
3. Fried H, Kutay U: **Nucleocytoplasmic transport: taking an inventory**. *Cell Mol Life Sci* 2003, **60**(8):1659-1688.
4. Dargemont C, Schmidt-Zachmann MS, Kuhn LC: **Direct interaction of nucleoporin p62 with mRNA during its export from the nucleus**. *J Cell Sci* 1995, **108**(Pt 1):257-263.
5. Whitehurst AW, Wilsbacher JL, You Y, Luby-Phelps K, Moore MS, Cobb MH: **ERK2 enters the nucleus by a carrier-independent mechanism**. *Proc Natl Acad Sci U S A* 2002, **99**(11):7496-7501.
6. Chook YM, Blobel G: **Karyopherins and nuclear import**. *Curr Opin Struct Biol* 2001, **11**(6):703-715.
7. Kose S, Furuta M, Imamoto N: **Hikeshi, a nuclear import carrier for Hsp70s, protects cells from heat shock-induced nuclear damage**. *Cell* 2012, **149**(3):578-589.
8. Lange A, Mills RE, Lange CJ, Stewart M, Devine SE, Corbett AH: **Classical nuclear localization signals: definition, function, and interaction with importin alpha**. *J Biol Chem* 2007, **282**(8):5101-5105.
9. Goldfarb DS, Corbett AH, Mason DA, Harreman MT, Adam SA: **Importin alpha: a multipurpose nuclear-transport receptor**. *Trends Cell Biol* 2004, **14**(9):505-514.
10. Nardozzi J, Wenta N, Yasuhara N, Vinkemeier U, Cingolani G: **Molecular basis for the recognition of phosphorylated STAT1 by importin alpha5**. *J Mol Biol* 2010, **402**(1):83-100.
11. Rout MP, Aitchison JD, Magnasco MO, Chait BT: **Virtual gating and nuclear transport: the hole picture**. *Trends Cell Biol* 2003, **13**(12):622-628.
12. Lindsay ME, Plafker K, Smith AE, Clurman BE, Macara IG: **Npap60/Nup50 is a tri-stable switch that stimulates importin-alpha:beta-mediated nuclear protein import**. *Cell* 2002, **110**(3):349-360.
13. Matsuura Y, Stewart M: **Nup50/Npap60 function in nuclear protein import complex disassembly and importin recycling**. *Embo J* 2005, **24**(21):3681-3689.
14. Gilchrist D, Mykytka B, Rexach M: **Accelerating the rate of disassembly of karyopherin.cargo complexes**. *J Biol Chem* 2002, **277**(20):18161-18172.
15. Ogawa Y, Miyamoto Y, Asally M, Oka M, Yasuda Y, Yoneda Y: **Two isoforms of Npap60 (Nup50) differentially regulate nuclear protein import**. *Mol Biol Cell* 2010, **21**(4):630-638.
16. Kose S, Imamoto N, Tachibana T, Yoshida M, Yoneda Y: **beta-subunit of nuclear pore-targeting complex (importin-beta) can be exported from the nucleus in a Ran-independent manner**. *J Biol Chem* 1999, **274**(7):3946-3952.
17. Faustino RS, Nelson TJ, Terzic A, Perez-Terzic C: **Nuclear transport: target for therapy**. *Clin Pharmacol Ther* 2007, **81**(6):880-886.
18. Miyamoto Y, Boag PR, Hime GR, Loveland KL: **Regulated nucleocytoplasmic transport during gametogenesis**. *Biochim Biophys Acta* 2012, **6**:616-630.
19. Cortes P, Ye ZS, Baltimore D: **RAG-1 interacts with the repeated amino acid motif of the human homologue of the yeast protein SRP1**. *Proc Natl Acad Sci U S A* 1994, **91**(16):7633-7637.
20. Kohler M, Ansieau S, Prehn S, Leutz A, Haller H, Hartmann E: **Cloning of two novel human importin-alpha subunits and analysis of the expression pattern of the importin-alpha protein family**. *FEBS Lett* 1997, **417**(1):104-108.

21. Kohler M, Speck C, Christiansen M, Bischoff FR, Prehn S, Haller H, Gorlich D, Hartmann E: **Evidence for distinct substrate specificities of importin alpha family members in nuclear protein import**. *Mol Cell Biol* 1999, **19**(11):7782-7791.
22. Nachury MV, Ryder UW, Lamond AI, Weis K: **Cloning and characterization of hSRP1 gamma, a tissue-specific nuclear transport factor**. *Proc Natl Acad Sci U S A* 1998, **95**(2):582-587.
23. Seki T, Tada S, Katada T, Enomoto T: **Cloning of a cDNA encoding a novel importin-alpha homologue, Qip1: discrimination of Qip1 and Rch1 from hSrp1 by their ability to interact with DNA helicase Q1/RecQL**. *Biochem Biophys Res Commun* 1997, **234**(1):48-53.
24. Weis K, Mattaj IW, Lamond AI: **Identification of hSRP1 alpha as a functional receptor for nuclear localization sequences**. *Science* 1995, **268**(5213):1049-1053.
25. Hu J, Wang F, Yuan Y, Zhu X, Wang Y, Zhang Y, Kou Z, Wang S, Gao S: **Novel importin-alpha family member Kpna7 is required for normal fertility and fecundity in the mouse**. *J Biol Chem* 2010, **285**(43):33113-33122.
26. Kelley JB, Talley AM, Spencer A, Gioeli D, Paschal BM: **Karyopherin alpha7 (KPNA7), a divergent member of the importin alpha family of nuclear import receptors**. *BMC Cell Biol* 2010, **11**:63.
27. Tejomurtula J, Lee KB, Tripurani SK, Smith GW, Yao J: **Role of importin alpha8, a new member of the importin alpha family of nuclear transport proteins, in early embryonic development in cattle**. *Biol Reprod* 2009, **81**(2):333-342.
28. Hogarth CA, Calanni S, Jans DA, Loveland KL: **Importin alpha mRNAs have distinct expression profiles during spermatogenesis**. *Dev Dyn* 2006, **235**(1):253-262.
29. Geles KG, Adam SA: **Germline and developmental roles of the nuclear transport factor importin alpha3 in C. elegans**. *Development* 2001, **128**(10):1817-1830.
30. Mason DA, Fleming RJ, Goldfarb DS: **Drosophila melanogaster importin alpha1 and alpha3 can replace importin alpha2 during spermatogenesis but not oogenesis**. *Genetics* 2002, **161**(1):157-170.
31. Adam SA, Sengupta K, Goldman RD: **Regulation of nuclear lamin polymerization by importin alpha**. *J Biol Chem* 2008, **283**(13):8462-8468.
32. Hachet V, Kocher T, Wilm M, Mattaj IW: **Importin alpha associates with membranes and participates in nuclear envelope assembly in vitro**. *Embo J* 2004, **23**(7):1526-1535.
33. Askjaer P, Galy V, Hannak E, Mattaj IW: **Ran GTPase cycle and importins alpha and beta are essential for spindle formation and nuclear envelope assembly in living Caenorhabditis elegans embryos**. *Mol Biol Cell* 2002, **13**(12):4355-4370.
34. Harel A, Forbes DJ: **Importin beta: conducting a much larger cellular symphony**. *Mol Cell* 2004, **16**(3):319-330.
35. Jakel S, Mingot JM, Schwarzmaier P, Hartmann E, Gorlich D: **Importins fulfil a dual function as nuclear import receptors and cytoplasmic chaperones for exposed basic domains**. *EMBO J* 2002, **21**(3):377-386.
36. Hanz S, Perlson E, Willis D, Zheng JQ, Massarwa R, Huerta JJ, Koltzenburg M, Kohler M, van-Minnen J, Twiss JL *et al*: **Axoplasmic importins enable retrograde injury signaling in lesioned nerve**. *Neuron* 2003, **40**(6):1095-1104.
37. Fujimura K, Suzuki T, Yasuda Y, Murata M, Katahira J, Yoneda Y: **Identification of importin alpha1 as a novel constituent of RNA stress granules**. *Biochim Biophys Acta* 2010, **1803**(7):865-871.
38. Yasuda Y, Miyamoto Y, Yamashiro T, Asally M, Masui A, Wong C, Loveland KL, Yoneda Y: **Nuclear retention of importin alpha coordinates cell fate through changes in gene expression**. *Embo J* 2011, **31**(1):83-94.

39. Rother F, Shmidt T, Popova E, Krivokharchenko A, Hugel S, Vilianovich L, Ridders M, Tenner K, Alenina N, Kohler M *et al*: **Importin alpha7 is essential for zygotic genome activation and early mouse development**. *PLoS One* 2011, **6**(3):e18310.
40. Depping R, Steinhoff A, Schindler SG, Friedrich B, Fagerlund R, Metzen E, Hartmann E, Kohler M: **Nuclear translocation of hypoxia-inducible factors (HIFs): involvement of the classical importin alpha/beta pathway**. *Biochim Biophys Acta* 2008, **1783**(3):394-404.
41. Fagerlund R, Melen K, Cao X, Julkunen I: **NF-kappaB p52, RelB and c-Rel are transported into the nucleus via a subset of importin alpha molecules**. *Cell Signal* 2008, **20**(8):1442-1451.
42. Kohler M, Gorlich D, Hartmann E, Franke J: **Adenoviral E1A protein nuclear import is preferentially mediated by importin alpha3 in vitro**. *Virology* 2001, **289**(2):186-191.
43. Ushijima R, Sakaguchi N, Kano A, Maruyama A, Miyamoto Y, Sekimoto T, Yoneda Y, Ogino K, Tachibana T: **Extracellular signal-dependent nuclear import of STAT3 is mediated by various importin alphas**. *Biochem Biophys Res Commun* 2005, **330**(3):880-886.
44. Ma J, Cao X: **Regulation of Stat3 nuclear import by importin alpha5 and importin alpha7 via two different functional sequence elements**. *Cell Signal* 2006, **18**(8):1117-1126.
45. Sekimoto T, Imamoto N, Nakajima K, Hirano T, Yoneda Y: **Extracellular signal-dependent nuclear import of Stat1 is mediated by nuclear pore-targeting complex formation with NPI-1, but not Rch1**. *EMBO J* 1997, **16**(23):7067-7077.
46. Welch K, Franke J, Kohler M, Macara IG: **RanBP3 contains an unusual nuclear localization signal that is imported preferentially by importin-alpha3**. *Mol Cell Biol* 1999, **19**(12):8400-8411.
47. Melen K, Fagerlund R, Franke J, Kohler M, Kinnunen L, Julkunen I: **Importin alpha nuclear localization signal binding sites for STAT1, STAT2, and influenza A virus nucleoprotein**. *J Biol Chem* 2003, **278**(30):28193-28200.
48. Fagerlund R, Melen K, Kinnunen L, Julkunen I: **Arginine/lysine-rich nuclear localization signals mediate interactions between dimeric STATs and importin alpha 5**. *J Biol Chem* 2002, **277**(33):30072-30078.
49. Liu L, McBride KM, Reich NC: **STAT3 nuclear import is independent of tyrosine phosphorylation and mediated by importin-alpha3**. *Proc Natl Acad Sci U S A* 2005, **102**(23):8150-8155.
50. Quensel C, Friedrich B, Sommer T, Hartmann E, Kohler M: **In vivo analysis of importin alpha proteins reveals cellular proliferation inhibition and substrate specificity**. *Mol Cell Biol* 2004, **24**(23):10246-10255.
51. Bamba C, Bobinnec Y, Fukuda M, Nishida E: **The GTPase Ran regulates chromosome positioning and nuclear envelope assembly in vivo**. *Curr Biol* 2002, **12**(6):503-507.
52. Bischoff FR, Ponstingl H: **Catalysis of guanine nucleotide exchange on Ran by the mitotic regulator RCC1**. *Nature* 1991, **354**(6348):80-82.
53. Izaurralde E, Kutay U, von Kobbe C, Mattaj IW, Gorlich D: **The asymmetric distribution of the constituents of the Ran system is essential for transport into and out of the nucleus**. *EMBO J* 1997, **16**(21):6535-6547.
54. Hetzer M, Gruss OJ, Mattaj IW: **The Ran GTPase as a marker of chromosome position in spindle formation and nuclear envelope assembly**. *Nat Cell Biol* 2002, **4**(7):E177-184.
55. Quimby BB, Dasso M: **The small GTPase Ran: interpreting the signs**. *Curr Opin Cell Biol* 2003, **15**(3):338-344.
56. Sun Z, Wu T, Zhao F, Lau A, Birch CM, Zhang DD: **KPNA6 (Importin {alpha}7)-mediated nuclear import of Keap1 represses the Nrf2-dependent antioxidant response**. *Mol Cell Biol* 2011, **31**(9):1800-1811.
57. Alam J, Stewart D, Touchard C, Boinapally S, Choi AM, Cook JL: **Nrf2, a Cap'n'Collar transcription factor, regulates induction of the heme oxygenase-1 gene**. *J Biol Chem* 1999, **274**(37):26071-26078.

58. McMahon M, Itoh K, Yamamoto M, Chanas SA, Henderson CJ, McLellan LI, Wolf CR, Cavin C, Hayes JD: **The Cap'n'Collar basic leucine zipper transcription factor Nrf2 (NF-E2 p45-related factor 2) controls both constitutive and inducible expression of intestinal detoxification and glutathione biosynthetic enzymes**. *Cancer Res* 2001, **61**(8):3299-3307.
59. Cullinan SB, Gordan JD, Jin J, Harper JW, Diehl JA: **The Keap1-BTB protein is an adaptor that bridges Nrf2 to a Cul3-based E3 ligase: oxidative stress sensing by a Cul3-Keap1 ligase**. *Mol Cell Biol* 2004, **24**(19):8477-8486.
60. Zhang Y, Gordon GB: **A strategy for cancer prevention: stimulation of the Nrf2-ARE signaling pathway**. *Mol Cancer Ther* 2004, **3**(7):885-893.
61. Sun Z, Zhang S, Chan JY, Zhang DD: **Keap1 controls postinduction repression of the Nrf2-mediated antioxidant response by escorting nuclear export of Nrf2**. *Mol Cell Biol* 2007, **27**(18):6334-6349.
62. Velichkova M, Hasson T: **Keap1 regulates the oxidation-sensitive shuttling of Nrf2 into and out of the nucleus via a Crm1-dependent nuclear export mechanism**. *Mol Cell Biol* 2005, **25**(11):4501-4513.
63. Friedrich B, Quensel C, Sommer T, Hartmann E, Kohler M: **Nuclear localization signal and protein context both mediate importin alpha specificity of nuclear import substrates**. *Mol Cell Biol* 2006, **26**(23):8697-8709.
64. Zhu W, Smith JW, Huang CM: **Mass spectrometry-based label-free quantitative proteomics**. *J Biomed Biotechnol* 2010, **840518**:10.
65. Chen J, Bardes EE, Aronow BJ, Jegga AG: **ToppGene Suite for gene list enrichment analysis and candidate gene prioritization**. *Nucleic Acids Res* 2009, **37**(Web Server issue):W305-311.
66. Matsubae M, Kurihara T, Tachibana T, Imamoto N, Yoneda Y: **Characterization of the nuclear transport of a novel leucine-rich acidic nuclear protein-like protein**. *FEBS Lett* 2000, **468**(2-3):171-175.
67. Wang X, Tang J, Xing L, Shi G, Ruan H, Gu X, Liu Z, Wu X, Gao X, Xu Y: **Interaction of MAGED1 with nuclear receptors affects circadian clock function**. *Embo J* 2010, **29**(8):1389-1400.
68. Kim JH, Yang CK, Heo K, Roeder RG, An W, Stallcup MR: **CCAR1, a key regulator of mediator complex recruitment to nuclear receptor transcription complexes**. *Mol Cell* 2008, **31**(4):510-519.
69. Bultman SJ, Gebuhr TC, Pan H, Svoboda P, Schultz RM, Magnuson T: **Maternal BRG1 regulates zygotic genome activation in the mouse**. *Genes Dev* 2006, **20**(13):1744-1754.
70. Riddick G, Macara IG: **The adapter importin-alpha provides flexible control of nuclear import at the expense of efficiency**. *Mol Syst Biol* 2007, **3**:118.
71. Gabriel G, Klingel K, Otte A, Thiele S, Hudjetz B, Arman-Kalcek G, Sauter M, Shmidt T, Rother F, Baumgarte S *et al*: **Differential use of importin-alpha isoforms governs cell tropism and host adaptation of influenza virus**. *Nat Commun* 2011, **2**:156.
72. King MC, Lusk CP, Blobel G: **Karyopherin-mediated import of integral inner nuclear membrane proteins**. *Nature* 2006, **442**(7106):1003-1007.
73. Lusk CP, Blobel G, King MC: **Highway to the inner nuclear membrane: rules for the road**. *Nat Rev Mol Cell Biol* 2007, **8**(5):414-420.
74. Fielhaber JA, Tan J, Joung KB, Attias O, Huegel S, Bader M, Roux PP, Kristof AS: **Regulation of Karyopherin-alpha1 and nuclear import by mTOR**. *J Biol Chem* 2012.
75. Bailis JM, Luche DD, Hunter T, Forsburg SL: **Minichromosome maintenance proteins interact with checkpoint and recombination proteins to promote s-phase genome stability**. *Mol Cell Biol* 2008, **28**(5):1724-1738.

76. Chuang CH, Wallace MD, Abratte C, Southard T, Schimenti JC: **Incremental genetic perturbations to MCM2-7 expression and subcellular distribution reveal exquisite sensitivity of mice to DNA replication stress**. *PLoS Genet* 2010, **6**(9).
77. Hall JA, Georgel PT: **CHD proteins: a diverse family with strong ties**. *Biochem Cell Biol* 2007, **85**(4):463-476.
78. Kim JK, Huh SO, Choi H, Lee KS, Shin D, Lee C, Nam JS, Kim H, Chung H, Lee HW *et al*: **Srg3, a mouse homolog of yeast SWI3, is essential for early embryogenesis and involved in brain development**. *Mol Cell Biol* 2001, **21**(22):7787-7795.
79. Stoller JZ, Huang L, Tan CC, Huang F, Zhou DD, Yang J, Gelb BD, Epstein JA: **Ash2l interacts with Tbx1 and is required during early embryogenesis**. *Exp Biol Med* 2010, **235**(5):569-576.
80. Matilla A, Radrizzani M: **The Anp32 family of proteins containing leucine-rich repeats**. *Cerebellum* 2005, **4**(1):7-18.
81. Kular RK, Gogliotti RG, Opal P: **Cpd-1 null mice display a subtle neurological phenotype**. *PLoS One* 2010, **5**(9).
82. Murawska M, Brehm A: **CHD chromatin remodelers and the transcription cycle**. *Transcription* 2011, **2**(6):244-253.
83. Sillibourne JE, Delaval B, Redick S, Sinha M, Doxsey SJ: **Chromatin remodeling proteins interact with pericentrin to regulate centrosome integrity**. *Mol Biol Cell* 2007, **18**(9):3667-3680.
84. Cooper MT, Conant AW, Kennison JA: **Molecular genetic analysis of Chd3 and polytene chromosome region 76B-D in Drosophila melanogaster**. *Genetics* 2010, **185**(3):811-822.
85. von Zelewsky T, Palladino F, Brunschwig K, Tobler H, Hajnal A, Muller F: **The C. elegans Mi-2 chromatin-remodelling proteins function in vulval cell fate determination**. *Development* 2000, **127**(24):5277-5284.
86. Dolgner SJ: **Chromatin Remodeling in Mice**. *University of Tennessee Honors Thesis Projects* 2005.
87. Frey SH, Funnell MG, Gerry VE, Gazzaniga MS: **A dissociation between the representation of tool-use skills and hand dominance: insights from left- and right-handed callosotomy patients**. *J Cogn Neurosci* 2005, **17**(2):262-272.
88. Shmidt T, Hampich F, Ridders M, Schultrich S, Hans VH, Tenner K, Vilianovich L, Qadri F, Alenina N, Hartmann E *et al*: **Normal brain development in importin-alpha5 deficient-mice**: Nat Cell Biol. 2007 Dec;9(12):1337-8; author reply 1339.
89. Nemergut ME, Macara IG: **Nuclear import of the ran exchange factor, RCC1, is mediated by at least two distinct mechanisms**. *J Cell Biol* 2000, **149**(4):835-850.
90. Jerke U, Tkachuk S, Kiyan J, Stepanova V, Kusch A, Hinz M, Dietz R, Haller H, Fuhrman B, Dumler I: **Stat1 nuclear translocation by nucleolin upon monocyte differentiation**. *PLoS One* 2009, **4**(12):e8302.
91. Meyer T, Gavenis K, Vinkemeier U: **Cell type-specific and tyrosine phosphorylation-independent nuclear presence of STAT1 and STAT3**. *Exp Cell Res* 2002, **272**(1):45-55.
92. Kumar A, Commane M, Flickinger TW, Horvath CM, Stark GR: **Defective TNF-alpha-induced apoptosis in STAT1-null cells due to low constitutive levels of caspases**. *Science* 1997, **278**(5343):1630-1632.
93. Stephanou A, Latchman DS: **STAT-1: a novel regulator of apoptosis**. *Int J Exp Pathol* 2003, **84**(6):239-244.
94. Meyer T, Begitt A, Lodige I, van Rossum M, Vinkemeier U: **Constitutive and IFN-gamma-induced nuclear import of STAT1 proceed through independent pathways**. *EMBO J* 2002, **21**(3):344-354.

95. Fielhaber JA, Han YS, Tan J, Xing S, Biggs CM, Joung KB, Kristof AS: **Inactivation of mammalian target of rapamycin increases STAT1 nuclear content and transcriptional activity in alpha4- and protein phosphatase 2A-dependent fashion**. *J Biol Chem* 2009, **284**(36):24341-24353.
96. Moriyama T, Nagai M, Oka M, Ikawa M, Okabe M, Yoneda Y: **Targeted disruption of one of the importin alpha family members leads to female functional incompetence in delivery**. *FEBS J* 2011, **278**(9):1561-1572.
97. Kohler M, Buchwalow IB, Alexander G, Christiansen M, Shagdarsuren E, Samoilova V, Hartmann E, Mervaala EM, Haller H: **Increased importin alpha protein expression in diabetic nephropathy**. *Kidney Int* 2001, **60**(6):2263-2273.
98. Todaro GJ, Green H: **Quantitative studies of the growth of mouse embryo cells in culture and their development into established lines**. *J Cell Biol* 1963, **17**:299-313.
99. Gorlich D, Vogel F, Mills AD, Hartmann E, Laskey RA: **Distinct functions for the two importin subunits in nuclear protein import**. *Nature* 1995, **377**(6546):246-248.
100. Depping R, Schindler SG, Jacobi C, Kirschner KM, Scholz H: **Nuclear Transport of Wilms' Tumour Protein Wt1 Involves Importins alpha and beta**. *Cell Physiol Biochem* 2012, **29**(1-2):223-232.
101. Bradford MM: **A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding**. *Anal Biochem* 1976, **72**:248-254.
102. Shevchenko A, Tomas H, Havlis J, Olsen JV, Mann M: **In-gel digestion for mass spectrometric characterization of proteins and proteomes**. *Nat Protoc* 2006, **1**(6):2856-2860.
103. Cox J, Mann M: **MaxQuant enables high peptide identification rates, individualized p.p.b.-range mass accuracies and proteome-wide protein quantification**. *Nat Biotechnol* 2008, **26**(12):1367-1372.

6. ACKNOWLEDGEMENT

An dieser Stelle möchte ich mich bei allen bedanken, die diese Arbeit ermöglicht und mich während dieser Zeit unterstützt haben.

Mein besonderer Dank gilt *Prof. Michael Bader*, welcher sich stets Zeit nahm und seine positive Einstellung behielt, wenn ich in sein Büro stürmte und für mich mal wieder alles aussichtslos erschien. Neben der wissenschaftlichen Betreuung möchte ich mich auch für die sozialen Aktivitäten bedanken, die von Prof. Michael Bader ermöglicht wurden und dieser Arbeitsgruppe ein besonders angenehmes Arbeitsklima verleihen. Vor allem sind hier die jährlichen Paddeltouren zu erwähnen, welche mir immer einen guten Ausgleich zu all den doch oft frustrierenden Forschungserlebnissen boten und mir stets in Erinnerung bleiben werden.

Meinem Doktorvater *Prof. Enno Hartmann* möchte ich dafür danken, dass er mir die Arbeit in der Gruppe von Prof. Michael Bader überhaupt erst ermöglicht hat. Des Weiteren bedanke ich mich für konstruktive Diskussionen, auch wenn unsere Gruppenbesprechungen in Berlin stets die 3-Stundenmarke geknackt haben und danach allen der Kopf rauchte. Besonders dankbar bin ich dafür, dass Prof. Enno Hartmann ein hoher wissenschaftlicher Standard wichtig war, an dem ich mich immer orientieren konnte.

Insbesondere gilt mein Dank *Franziska Rother, Tanja Shmidt und Katja Tenner*, denen die mühselige Aufgabe zuteilwurde, mich in die Tiefen des Importin α-Projekts einzuführen. Vielen Dank, dass ihr mir so geduldig und kompetent zur Seite standet! Ich hätte gerne mehr von euch gehabt!

Darüber hinaus möchte ich mich bei *Dr. Reinhard Depping und Prof. Ryan Cabot* -nebst experimenteller Hilfe - für gute Ratschläge und inspirierende Gespräche bedanken. Dank euch fühle ich mich als Forscher nun nicht mehr ganz so wie im Irrenhaus.

Vielen Dank auch an *alle Kollegen*, die mir mit den Jahren sehr ans Herz gewachsen und zu guten Freunden geworden sind!

Des Weiteren möchte ich mich bei all den Personen bedanken, die mir mit fachlicher Hilfe im Labor zur Seite standen und ohne die diese Arbeit gar nicht zu Stande gekommen wäre:

Sabine Grüger und Manfred Ströhmann danke ich für all die Arbeiten im Tierhaus, vor denen ich mich gedrückt habe.

Vielen Dank an unsere Embryologen *Elena Popova, Larissa Vilianovich und Alexander Krivokharchenko*, welche mit ihren Wissen und Fähigkeiten einen großen Beitrag zur Generierung und Analyse der Importin α KO Mäuse geleistet haben.

Ebenso möchte ich *Ilona Kamer, Michail Todiras, Arnd Heuser und Martin Taube* für die Durchführung der physiologischen Tierexperimente danken.

Bei *Matthias Sury* möchte ich mich für die Hilfe und Planung beim SILAC Experiment bedanken. Außerdem vielen Dank an *Anja Schütz* und ihr Team bei der *Protein Sample Production Facility* für die freundliche Aufnahme in ihren Labors und Hilfe bei der Proteinaufreinigung.

Des Weiteren möchte ich mich bei allen technischen Assistenten der Arbeitsgruppe Bader für ihre Unterstützung bedanken. Mein besonderer Dank gilt hierbei *Andrea Müller, Tanja Schalow und Madeleine Skorna*, welche vermutlich Millionen von Genotypisieruns-PCRs durchgeführt haben.

Schließlich gilt mein besonderer Dank meiner *Familie und meinen Freunden*, welche mir immer mit aufbauenden Worten den Rücken stärkten. Besonders wichtig in dieser Zeit war für

mich die Unterstützung meines Mannes, *Michèl*, der meinen Launen stets mit Optimismus begegnete und nicht weggelaufen ist.

7. APPENDIX

7.1. Tables

see enclosed CD-ROM

7.2. List of abbreviations

Abbreviation	Meaning
-/-	knockout
µl	microliter
A	Ampere
bp	base pair
BSA	bovine serum albumin
C-	carboxyl-
C57BL/6	inbred mouse line
cDNA	complementary DNA
Co-IP	co-immunoprecipitation
DAPI	4',6-Diamidino-2-phenylindol
dl	deciliter
DMEM	Dulbecco's modified eagle's medium
DMSO	Dimethylsulfoxid
DNA	deoxyribonucleic acid
DNase	desoxyribonuclease
dNTP	Desoxyribonucleotidtriphosphat
DTT	1,4-Dithiothreitol
EDTA	Ethylendiamintetraacetic acid
EGTA	Ethylenglycoltetraacetic acid
FBS	Fetal bovine serum
FCS	Fetal calf serum
fl	femtoliter
for	forward
g	gram
GAPDH	Glycerinaldehyd-3-Phosphat-Dehydrogenase
GST	Glutathione S-transferase
h	hours
HE	Hematoxylin and Eosin
HE	Hematoxylin
His	polyhistidine tag
ICC	Immunocytochemistry
IFN-γ	interferon gamma
IHC	Immunohistochemistry

impa	importin α
IP	Immunoprecipitation
kDa	kilo Dalton
kg	kilogram
KO	knockout
l	liter
LMB	leptomycin B
LVH	left ventricular hypertrophy
M	mol/l
m	meter
MEM	minimum essential medium
mg	milligram
min	minutes
ml	milliliter
mM	millimolar
mRNA	messenger RNA
myc	EQKLISEEDL-Epitope
N-	amino-
Ni	nickel
NLS	nuclear localization signal
nt	nucleotide
ORF	open reading frame
PAGE	Polyacrylamide gel electrophoresis
PBS	phosphate buffered saline
PCR	polymerase chain reaction
PFA	Paraformaldehyde
PMSF	Phenylmethylsulfonylfluoride
rev	reverse
RNA	ribonucleic acid
RNase	ribonuclease
rpm	rotation per minute
RT	Reverse transcriptase
RT	room temperature
SD	standard deviation
SDS	Sodiumdodecylsulfate
sec	seconds
SEM	standard error of measurement
TAE	Tris-acetate-EDTA buffer
Taq	Thermus aquaticus
TBE	Tris-Borat-EDTA buffer
TBS	tris buffered saline
TE	Tris-EDTA buffer
TEMED	N,N,N',N'-Tetramethylenethylendiamide
Tris	Tris-(hydroxymethyl)-aminomethan
tRNA	Transfer RNA

u	unit
UV	ultraviolet
V	Volt
WB	Western Blot
wt	wild type

7.3. Data Tables

Table 19: Importin α7 binding partners identified by GST pull-down from ovary lysate.

1-757: proteins bound to importin α7 and not to the GST-control. 758-807 top 5 % of importin α7 binding partners found to bind also to the GST-control. Proteins were ranked according to their fold change.

	Gene name	Uniprot	Protein name
1	Ppl	Q9R269	Periplakin
2	Pfkb	P12382	6-phosphofructokinase, liver type
3	A2lp	Q7TQH0-2	Ataxin-2-like protein
4	Mapkap1	Q8BKH7-1	Mitogen-activated protein kinase 2-associated protein 1
5	Tor1b	Q9ER41	Torsin family 1 member B
6	Clim1	O70400	C-terminal LIM domain protein 1
7	1190002N15Rik	Q3USZ8	UPF0672 protein C3orf58 homolog
8	Ints4	Q8CIM8-1	Integrator complex subunit 4
9	Heatr3	Q8BQM4	HEAT repeat-containing protein 3
10	mKIAA1227	E9Q444	MKIAA1227 protein
11	Fap48	Q8BZM1	FK506-binding protein-associated protein
12	Inf2	Q0GNC1-1	Inverted formin-2
13	Plec	E9QK43	Putative uncharacterized protein
14	Abp1	Q62418-1	Actin-binding protein 1
15	Glt25d1	Q8K297	Glycosyltransferase 25 family member 1
16	Cept1	Q8BGS7-2	Choline/ethanolaminephosphotransferase 1
17	Cyp2f2	P33267	CYPIIF2
18	Dusp14	Q9JLY7	Dual specificity protein phosphatase 14
19	Fam61a	Q8K2F8	Protein FAM61A
20	Epb4.1l1	A2AUK4	Erythrocyte protein band 4.1-like 1
21	Amshlp	Q76N33-1	AMSH family protein
22	Srpr	Q9DBG7	Docking protein α
23	Hectd1	Q69ZR2	MKIAA1131 protein
24	Fmr1	P35922-8	Fragile X mental retardation protein 1 homolog
25	Bcr	E9PZL3	Breakpoint cluster region protein
26	Kiaa4077	Q8VDS4	Cyclin-dependent kinase inhibitor 2B-related protein
27	Syne2	Q6ZWQ0-3	Nesprin-2
28	Ptprs	B0V2N1-1	PTPNU-3
29	Gtrgeo22	Q99MS8	p32
30	Cycap	Q07797	Cyp-C-associated protein
31	Mfn2	B2KFT7	Mitofusin 2
32	Irf2bp2	E9Q1P8	Putative uncharacterized protein
33	Cklfsf6	Q9CZ69	Chemokine-like factor superfamily member 6
34	Chd6	A3KFM7	Chromodomain helicase DNA binding protein 6

35	Cog1	Q9Z160	Component of oligomeric Golgi complex 1
36	Hrb2	Q3UIG9	Putative uncharacterized protein
37	Got2	P05202	Aspartate aminotransferase, mitochondrial
38	Ints7	Q7TQK1-1	Integrator complex subunit 7
39	Lrp	P18052-2	LCA-related phosphatase
40	Tapbp	Q3TCU5	Putative uncharacterized protein
41	Thoc2	B1AZI6	THO complex subunit 2
42	Papd5	Q68ED3	PAP-associated domain-containing protein 5
43	Kiaa0664	Q5SW19-1	Protein KIAA0664
44	Env	E3T928	Retrovirus-related Env polyprotein from Fv-4 locus
45	RP23-351G20.1-003	B1AVD2	X-prolyl aminopeptidase (Aminopeptidase P) 2, membrane-bound
46	Kiaa0245	Q8BGK6-1	Solute carrier family 7 member 6
47	Fthfsdc1	Q3V3R1	Formyltetrahydrofolate synthetase
48	Lyar	Q08288	Cell growth-regulating nucleolar protein
49	Sbds	P70122	Protein 22A3
50	Ari	Q9Z1K5	Protein ariadne-1 homolog
51	Idh3g	P70404	Isocitrate dehydrogenase [NAD] subunit γ, mitochondrial
52	Kiaa0700	Q62141-4	Histone deacetylase complex subunit Sin3b
53	Kri1	Q8VDQ9	Protein KRI1 homolog
54	Nid2	O88322	Entactin-2
55	Fbxl19	Q6PB97-1	F-box and leucine-rich repeat protein 19
56	Exosc8	Q9D753	Exosome complex exonuclease RRP43
57	Vps53	Q8CCB4-1	Vacuolar protein sorting-associated protein 53 homolog
58	Arht1	Q8BG51-4	Mitochondrial Rho GTPase 1
59	Bb1	Q8CHK3	Bladder and breast carcinoma-overexpressed gene 1 protein
60	Arhgap21	B2RWX1	Arhgap21 protein
61	AA536749	Q5SWZ5	Myosin phosphatase Rho interacting protein
62	E330017A01Rik	Q8BU47	Putative uncharacterized protein
63	Gmds	Q8K0C9	GDP-D-mannose dehydratase
64	Tmem165	P52875	TPA-regulated locus protein
65	Fact140	Q920B9	Chromatin-specific transcription elongation factor 140 kDa subunit
66	Exoc3	Q6KAR6	Exocyst complex component 3
67	Ccnh	Q3UUW5	Putative uncharacterized protein
68	Ipo8	Q7TMY7-2	Importin-8
69	Sedl	Q9CQP2	Sedlin
70	Safb	D3YXK2	Putative uncharacterized protein Safb
71	Chac	Q5H8C4-1	Chorea-acanthocytosis protein homolog
72	Sip1	Q9R0G7	Smad-interacting protein 1

73	Rcn2	Q8BP92	Reticulocalbin-2
74	Cul5	E9PV12	Cullin-5
75	Copz2	Q9JHH9	Coatomer subunit zeta-2
76	Ctc1	Q5SUQ9-2	A-accessory factor of 132 kDa
77	Gtf2f1	Q8R5B7	General transcription factor IIF, polypeptide 1
78	Cdh1	P09803	ARC-1
79	Fbp27	Q91Z67	Formin-binding protein 2
80	Nalp4f	Q4G0D0	Nlrp4f protein
81	Atp6n1b	P15920-1	Immune suppressor factor J6B7
82	Fam49b	Q921M7	Protein FAM49B
83	Sidt2	Q8CIF6-2	SID1 transmembrane family member 2
84	Bcas2	Q9D287	Breast carcinoma-amplified sequence 2 homolog
85	Apoj	Q06890	Apolipoprotein J
86	Nol14	Q8R3N1	Nucleolar complex protein 14
87	Bre1a	Q5DTM8-1	E3 ubiquitin-protein ligase BRE1A
88	Fn1	B9EHT6	Fn1 protein
89	Anx7	Q07076	Annexin A7
90	Epc2	Q8C0I4	Enhancer of polycomb homolog 2
91	Erg1	P52019	Squalene epoxidase
92	Stx7	O70439	Syntaxin-7
93	Plat	P11214	Tissue-type plasminogen activator
94	Gmppb	Q8BTZ7	GDP-mannose pyrophosphorylase B
95	Ddx51	Q6P9R1	ATP-dependent RNA helicase DDX51
96	Ube4b	Q9ES00	Ubiquitin conjugation factor E4 B
97	Prim1	P20664	DNA primase 49 kDa subunit
98	Heatr2	B2RY24	Heatr2 protein
99	D5Wsu178e	Q80TA1	Ethanolaminephosphotransferase 1
100	Poldip2	Q91VA6	Polymerase delta-interacting protein 2
101	Alg5	Q9DB25	Asparagine-linked glycosylation protein 5 homolog
102	Pck2	Q8BH04	Phosphoenolpyruvate carboxykinase [GTP], mitochondrial
103	Ctbp2	P56546-2	C-terminal-binding protein 2
104	mKIAA4216	Q5DTH1	MKIAA4216 protein
105	Haf	Q9Z315	Hypoxia-associated factor
106	Heph	Q9Z0Z4-2	Hephaestin
107	Paf65a	Q8R2K4-1	PCAF-associated factor 65-α
108	Galnt2	Q6PB93-1	Polypeptide GalNAc transferase 2
109	Abi1	Q8CBW3-1	Abelson interactor 1
110	Anx11	P97384	Annexin A11

111	Psp1	Q8R326-1	Paraspeckle component 1
112	Cpsf1	Q9EPU4	Cleavage and polyadenylation specificity factor 160 kDa subunit
113	Arpc3	D3Z2F8	Putative uncharacterized protein Arpc3
114	Msk1	Q8C050-1	90 kDa ribosomal protein S6 kinase 5
115	Itga6	A2AU04	Integrin α 6
116	Kiaa1076	Q8CFT2-1	Histone-lysine N-methyltransferase SETD1B
117	Dis3	Q9CSH3	Exosome complex exonuclease RRP44
118	Atp13a1	B2RS54	ATPase type 13A1
119	Acatn	Q99J27	Acetyl-coenzyme A transporter 1
120	Vgll4	Q80V24	Transcription cofactor vestigial-like protein 4
121	Dctn5	Q9QZB9	Dynactin subunit 5
122	Anapc8	Q8BGZ4-1	Anaphase-promoting complex subunit 8
123	Frap	Q9JLN9-1	FK506-binding protein 12-rapamycin complex-associated protein 1
124	Sf3a2	Q7TN25	Sf3a2 protein
125	Exo70	O35250-1	Exocyst complex component 7
126	Foxk1	P42128	Forkhead box protein K1
127	Frmd5	A2AR56	FERM domain containing 5
128	Nme3	Q9WV85	DR-nm23
129	Ighg	Q569X1	Ighg protein
130	Pdk3	Q922H2	[Pyruvate dehydrogenase [lipoamide]] kinase isozyme 3, mitochondrial
131	Ptpn14	Q62130	Protein-tyrosine phosphatase PTP36
132	Ubce7	P68037	UbcM4
133	Btbd14b	Q7TSZ8	BTB/POZ domain-containing protein 14B
134	Polr2a	P08775	DNA-directed RNA polymerase II subunit A
135	Nub1	P54729	NEDD8 ultimate buster 1
136	Dhx30	Q99PU8-3	DEAH box protein 30
137	Prim2	P33610	DNA primase 58 kDa subunit
138	Kiaa0776	Q8CCJ3-3	E3 UFM1-protein ligase 1
139	Gosr2	O35166	27 kDa Golgi SNARE protein
140	Epb4.1l5	Q8BGS1-3	Band 4.1-like protein 5
141	Cal1l	P08207	Calpactin I light chain
142	Tlp46	Q91W90	Endoplasmic reticulum resident protein 46
143	Fbxo30	Q8BJL1	F-box only protein 30
144	Ints3	Q7TPD0-1	Integrator complex subunit 3
145	Adnp2	Q8CHC8	ADNP homeobox protein 2
146	Ablim2	E9Q4K0	Actin-binding LIM protein 2
147	Bckdha	P50136	2-oxoisovalerate dehydrogenase subunit α, mitochondrial
148	Palm2	Q8BR92	Paralemmin-2
149	Gtf2h4	O70422	Basic transcription factor 2 52 kDa subunit

150	Lamb-1	P02469	Laminin B1 chain
151	Aif1	O70200	Allograft inflammatory factor 1
152	Cbp	Q61990-2	A-CP2
153	Kiaa0103	Q9CRD2	Tetratricopeptide repeat protein 35
154	Rpl7l1	Q9D8M4	60S ribosomal protein L7-like 1
155	Eed	Q921E6-2	Polycomb protein EED
156	Lsm4	Q9CY46	LSM4 homolog, U6 small nuclear RNA associated (S. cerevisiae), isoform CRA_b
157	Jagn1	Q5XKN4	Protein jagunal homolog 1
158	Apoo	Q9DCZ4	Apolipoprotein O
159	Tppp3	Q9CRB6	Tubulin polymerization-promoting protein family member 3
160	D11Ertd619e	Q9D0R4	ATP-dependent 61 kDa nucleolar RNA helicase
161	Lmo7	A0T1J8	LIM domain only 7
162	Sbf1	B2RXX4	Sbf1 protein
163	Crl1	Q9JI13-1	Charged amino acid-rich leucine zipper 1
164	Esr	P19785	ER-α
165	Paf65b	Q91WQ5	PCAF-associated factor 65 β
166	Gmppa	Q922H4	GDP-mannose pyrophosphorylase A
167	Cappb1	P47757-1	CapZ β
168	Polr2e	Q80UW8	DNA-directed RNA polymerase II subunit E
169	Cclp1	O35711-4	Coiled-coil-like protein 1
170	RP23-161B9.4-001	A2AN37	Uridine kinase
171	Hk2	O08528	Hexokinase type II
172	Ugp2	Q91ZJ5-1	UDP-glucose pyrophosphorylase
173	Aat2	Q8BGT5	Alanine aminotransferase 2
174	Fug1	P46061	Ran GTPase-activating protein 1
175	Mrpl9	Q3UK02	Putative uncharacterized protein
176	Kras	P32883-2	c-Ki-ras
177	Nrm	Q8VC65	Nuclear envelope membrane protein
178	Ptpn1	P35821	Protein-tyrosine phosphatase 1B
179	Cdk16	Q04735-1	Cell division protein kinase 16
180	Rod1	A2ANH2	ROD1 regulator of differentiation 1 (S. pombe)
181	Eif4e	P63073	eIF-4F 25 kDa subunit
182	Fbp11	Q9R1C7-1	Formin-binding protein 11
183	Ctps2	P70303-1	CTP synthase 2
184	Greb1	E0CYB3	Protein GREB1
185	mCG_11651	A4QPD6	Phospholipase A2, activating protein
186	Agfg1	Q8K2K6-4	Arf-GAP domain and FG repeats-containing protein 1
187	Ccdc128	Q3TDD9-2	Coiled-coil domain-containing protein 128

188	Atx2	O70305-1	Ataxin-2
189	Cyb5r1	Q9DB73-1	NAD(P)H:quinone oxidoreductase type 3 polypeptide A2
190	Cald1	Q3TUS1	Putative uncharacterized protein
191	Krtcap2	Q5RL79	Keratinocyte-associated protein 2
192	Ptp14	Q64512	Protein tyrosine phosphatase DPZPTP
193	Datf1	Q8C9B9-1	Death-associated transcription factor 1
194	Csde1	Q80TP8	MKIAA0885 protein
195	Col1a1	P11087-1	A-1 type I collagen
196	Kiaa1561	Q8CGB3-3	Nuclear membrane-binding protein
197	Htatsf1	Q8BGC0-1	HIV Tat-specific factor 1 homolog
198	Ghitm	Q91VC9	Growth hormone-inducible transmembrane protein
199	Akap12	Q9WTQ5-1	A-kinase anchor protein 12
200	Vps29	Q9QZ88-2	Vacuolar protein sorting-associated protein 29
201	Snrpe	P62305	Sm protein E
202	Abcd2	Q61285	Adrenoleukodystrophy-related protein
203	Nek7	Q9ES74	Never in mitosis A-related kinase 7
204	Dhc1b	Q45VK7-2	Cytoplasmic dynein 2 heavy chain
205	Uba5	Q8VE47	Ubiquitin-activating enzyme E1 domain-containing protein 1
206	Bcl7b	Q921K9-1	B-cell CLL/lymphoma 7 protein family member B
207	Cds2	Q99L43	CDP-DAG synthase 2
208	Klc2	Q91YS4	Kinesin light chain 2
209	Rnasep2	O88796	Ribonuclease P protein subunit p30
210	Ints1	Q6P4S8	Integrator complex subunit 1
211	Calr	P14211	Calregulin
212	Cfh	P06909	Complement factor H
213	Cwf19l1	Q8CI33-2	CWF19-like protein 1
214	Psmd9	Q9CR00	26S proteasome non-ATPase regulatory subunit 9
215	RP23-22A15.9-001	A2AQ19	Rtf1 Paf1/RNA polymerase II complex component homolog (S. cerevisiae)
216	Mat1	P51949	CDK7/cyclin-H assembly factor
217	Atrx	A2ADH4	A thalassemia/mental retardation syndrome X-linked homolog (Human)
218	Kiaa4006	Q61151	PP2A B subunit isoform B56-epsilon
219	D12Wsu95e	Q8CFE3	Protein CoREST
220	Lrrc41	Q8K1C9	Leucine-rich repeat-containing protein 41
221	Igf2r	Q07113	300 kDa mannose 6-phosphate receptor
222	J207	Q91ZN5-1	Adenosine 3-phospho 5-phosphosulfate transporter 1
223	Myo9b	Q9QY06-2	Myosin-IXb
224	Ino1	Q9JHU9	Inositol-3-phosphate synthase 1
225	mKIAA1435	E9Q4P1	MKIAA1435 protein

226	Ndufs7	Q9DC70	Complex I-20kD
227	Kiaa1449	Q8BH57-1	USP1-associated factor 1
228	Vps16	Q920Q4-1	Vacuolar protein sorting-associated protein 16 homolog
229	Kiaa1721	Q9ESJ0	Exportin-4
230	Cops2	P61202-2	Alien homolog
231	Leng8	D3YWS8	Leukocyte receptor cluster (LRC) member 8, isoform CRA_a
232	Kiaa1311	E9QM85	Peri-implantation stem cell protein 1
233	Inppl1	Q6P549	AblSH3-binding protein
234	Cul2	Q9D4H8-1	Cullin-2
235	Tenc1	Q8CGB6-3	C1 domain-containing phosphatase and tensin homolog
236	Kiaa1471	Q6PB44-1	Tyrosine-protein phosphatase non-receptor type 23
237	Gphn	A0JNY3	Gphn protein
238	Exosc2	Q8VBV3	Exosome complex exonuclease RRP4
239	Gcp2	Q921G8	Γ-tubulin complex component 2
240	Bcl7a	Q9CXE2-1	B-cell CLL/lymphoma 7 protein family member A
241	Spty2d1	Q68FG3	Protein SPT2 homolog
242	Stx5	Q8K1E0-1	Syntaxin-5
243	mCG_17890	O08804	NK13
244	Mcm6	P97311	DNA replication licensing factor MCM6
245	Pkcd	P28867-2	nPKC-delta
246	Egfr	Q01279	Epidermal growth factor receptor
247	Vps26b	Q8C0E2-1	Vacuolar protein sorting-associated protein 26B
248	Aptx	Q7TQC5-1	Aprataxin
249	Cd9	P40240	CD9 antigen
250	Dnttip1	Q99LB0	Deoxynucleotidyltransferase terminal-interacting protein 1
251	Vps26	P40336-2	H<β>58 protein
252	Plac8	Q9JI48	Onzin
253	Dapk3	O54784	DAP-like kinase
254	Kpna2	P52293	Importin α P1
255	Abhd6	Q8R2Y0-1	2-arachidonoylglycerol hydrolase
256	2210010C04Rik	Q9CPN9	Putative uncharacterized protein
257	Polr3c	Q9D483-1	DNA-directed RNA polymerase III subunit C
258	Bst2	Q8R2Q8	Bone marrow stromal antigen 2
259	mCG_19062	Q9JMD0	Putative uncharacterized protein
260	Dbi1	Q6PCM2-1	DBI-1
261	Sec24d	Q6NXL1	Sec24 related gene family, member D (S. cerevisiae)
262	Imp9	Q91YE6	Importin-9
263	Epi64	P58802	EBP50-PDX interactor of 64 kDa
264	Cdk5	P49615	Cell division protein kinase 5

265	H1f3	P43277	H1 VAR.4
266	Spc22	Q9D365	Microsomal signal peptidase 22/23 kDa subunit
267	6330549D23Rik	Q8R3P6-1	UPF0464 protein C15orf44 homolog
268	Nvl	Q9DBY8	Nuclear valosin-containing protein-like
269	Mrpl14	Q9D1I6	39S ribosomal protein L14, mitochondrial
270	Bgn	P28653	Biglycan
271	Krev-1	P62835	Ras-related protein Krev-1
272	Dtx3l	Q3UIR3-1	E3 ubiquitin-protein ligase DTX3L
273	Zbp89	Q61624	B enolase repressor factor 1
274	Zc3hav1	Q3UPF5-1	Zinc finger CCCH-type antiviral protein 1
275	Dctn2	Q99KJ8	50 kDa dynein-associated polypeptide
276	Lig1	P37913	DNA ligase 1
277	Dhapat	P98192	Acyl-CoA:dihydroxyacetonephosphateacyltransferase
278	Nup37	Q9CWU9	Nucleoporin Nup37
279	Prkcn	Q5FWX6	Protein kinase D3
280	Kiaa0255	Q8BH24	Transmembrane 9 superfamily member 4
281	mCG_129048	A6X954	Mitochondrial ribosomal protein L39
282	Etl4	A2AQ25-1	Enhancer trap locus 4
283	Fbxo24	D3YUE2	Putative uncharacterized protein Fbxo24
284	Top1	Q04750	DNA topoisomerase 1
285	Ank3	Q4U205	Ankyrin-3
286	Arl2	Q9D0J4	ADP-ribosylation factor-like protein 2
287	Snag1	Q3U1H7	Putative uncharacterized protein
288	Madh2	Q62432-1	Mad-related protein 2
289	Baf47	Q9Z0H3-2	BRG1-associated factor 47
290	Chd2	E9PZM4	Putative uncharacterized protein
291	1200014J11Rik	Q8BZR9-1	Uncharacterized protein C17orf85 homolog
292	Col6a4	A2AX52	Collagen α-4(VI) chain
293	Rp2	Q9EPK2-2	Protein XRP2
294	Cd26	P28843	Dipeptidyl peptidase 4
295	Ring1	O35730-1	E3 ubiquitin-protein ligase RING1
296	Mp44	Q8R4R6	35 kDa nucleoporin
297	Lnpep	Q8C129	Leucyl-cystinyl aminopeptidase
298	H2-Ke6	P50171-2	17-β-hydroxysteroid dehydrogenase 8
299	Apmap	Q9D7N9	Adipocyte plasma membrane-associated protein
300	Add2	Q9QYB8-1	Add97
301	Capn2	O08529	80 kDa M-calpain subunit
302	Wapal	B9EIG8	Wings apart-like homolog (Drosophila)
303	Dom3z	O70348	Dom-3 homolog Z

304	Tceb1	P83940	Elongin 15 kDa subunit
305	Mbp-1	Q61878	Bone marrow proteoglycan
306	Gba	P17439	Acid β-glucosidase
307	Ylpm1	D3YWX2	Putative uncharacterized protein Ylpm1
308	Marcksl1	P28667	Brain protein F52
309	Tbc1d15	Q9CXF4	GTPase-activating protein RAB7
310	Ap1m1	P35585	Adaptor protein complex AP-1 mu-1 subunit
311	Fech	P22315	Ferrochelatase, mitochondrial
312	Smn	P63163	Sm protein D
313	Lrch4	Q921G6	Leucine-rich repeat and calponin homology domain-containing protein 4
314	Kiaa1064	Q6ZPZ3-1	Zinc finger CCCH domain-containing protein 4
315	Foxl2	O88470	Forkhead box protein L2
316	Elp1	Q7TT37	Elongator complex protein 1
317	Mcam	Q8R2Y2-1	Cell surface glycoprotein MUC18
318	Actr5	Q80US4-1	Actin-related protein 5
319	Dimt1	Q9D0D4	18S rRNA dimethylase
320	Qk	Q9QYS9-7	Protein quaking
321	Tnik	B2RY17	Tnik protein
322	Pold1	P52431	DNA polymerase delta catalytic subunit
323	Kiaa0349	Q6WKZ8-1	E3 ubiquitin-protein ligase UBR2
324	Cpsf3l	Q9CWS4	Cleavage and polyadenylation-specific factor 3-like protein
325	Arhgef7	Q9ES28-1	B-Pix
326	Dynlt1	P51807	Activator of G-protein signaling 2
327	Hp1bp3	Q3TEA8-1	Heterochromatin protein 1-binding protein 3
328	Fmo2	Q8K2I3	Dimethylaniline monooxygenase [N-oxide-forming] 2
329	Ppil2	E9Q0H9	Putative uncharacterized protein
330	Ppp2r5a	Q3ULN8	Putative uncharacterized protein
331	Armc10	Q9D0L7-1	Armadillo repeat-containing protein 10
332	Adam10	B8JJJ0	A disintegrin and metallopeptidase domain 10
333	Arf6	P62331	ADP-ribosylation factor 6
334	Mrps7	Q80X85	28S ribosomal protein S7, mitochondrial
335	Kiaa1927	Q922B9-3	Ki-ras-induced actin-interacting protein
336	Ssr1	E9Q9A7	Translocon-associated protein α, muscle specific isoform
337	Lss	Q8BLN5	2,3-epoxysqualene--lanosterol cyclase
338	Hmgb2l1	Q3U0Q0	Putative uncharacterized protein
339	Gstk1	Q9DCM2	Glutathione S-transferase kappa 1
340	Wdr61	Q9ERF3	Meiotic recombination REC14 protein homolog
341	Rheb	Q921J2	GTP-binding protein Rheb
342	Gtf3c5	Q8R2T8-1	General transcription factor 3C polypeptide 5

343	Ddx18	Q8K363	ATP-dependent RNA helicase DDX18
344	Kiaa0839	Q8BMG7-1	Rab3 GTPase-activating protein 150 kDa subunit
345	Grg6	Q9WVB3	Groucho-related protein 6
346	Mapkapk2	P49138	MAP kinase-activated protein kinase 2
347	Mrpl37	Q921S7	39S ribosomal protein L37, mitochondrial
348	Snap47	Q8R570-1	Synaptosomal-associated 47 kDa protein
349	RP23-291K6.1-001	B1ATW4	Slit homolog 3 (Drosophila)
350	Scyl1	Q9EQC5	105 kDa kinase-like protein
351	Tomm70a	Q8BNI6	Putative uncharacterized protein
352	Gm8394	D3YX79	Putative uncharacterized protein Gm8394
353	Kiaa0564	Q8CC88-1	Uncharacterized protein KIAA0564 homolog
354	Aacs	Q9D2R0	Acetoacetyl-CoA synthetase
355	Pccb	A0PJE6	Pccb protein
356	Polr2b	Q8CFI7	DNA-directed RNA polymerase II 140 kDa polypeptide
357	Ankrd47	Q9Z1P7	Ankyrin repeat domain-containing protein 47
358	Pcf11	A5HLW0	PCF11
359	Morf4l2	E9PZV9	Putative uncharacterized protein
360	Col4a2	P08122	Canstatin
361	Ndufa12	Q7TMF3	Complex I-B17.2
362	Lbr	Q3U9G9	Integral nuclear envelope inner membrane protein
363	Ercc2	O08811	CXPD
364	Decr2	Q9WV68	2,4-dienoyl-CoA reductase 2
365	Kiaa1113	Q99PP7-2	E3 ubiquitin-protein ligase TRIM33
366	Akr1c6	P70694	17-β-HSD 5
367	Mrps36	Q9CQX8	28S ribosomal protein S36, mitochondrial
368	H47	Q9BCZ4	Minor histocompatibility antigen H47
369	Dhrs7b	Q99J47-1	Dehydrogenase/reductase SDR family member 7B
370	Last	Q9D032-1	Lck-associated signal transducer
371	Atp6v1h	Q8BVE3	Vacuolar proton pump subunit H
372	Lpp3	Q99JY8	Lipid phosphate phosphohydrolase 3
373	Ccnt1	Q9QWV9	Cyclin-T1
374	Emp2	O88662	Epithelial membrane protein 2
375	Pitpnb	Q8JZZ5	Pitpnb protein
376	Thumpd3	P97770-1	GtROSA26asSor
377	Arhg	P84096	Rho-related GTP-binding protein RhoG
378	Espn	Q9ET47-6	Ectoplasmic specialization protein
379	Dtnb	O70585-1	B-dystrobrevin
380	Myo9a	D3Z3A8	Putative uncharacterized protein Myo9a

381	Arfgap2	Q99K28-2	ADP-ribosylation factor GTPase-activating protein 2
382	Sfrs2	E9Q975	Putative uncharacterized protein
383	Park7	Q99LX0	Parkinson disease protein 7 homolog
384	Kiaa0745	E9PUW7	Exportin-7
385	Slc14a1	Q8VHL0	Solute carrier family 14 member 1
386	Ddx24	Q9ESV0	ATP-dependent RNA helicase DDX24
387	Rhox8	Q4TU85	Reproductive homeobox on X chromosome 8
388	mCG_67985	Q9CQB4	MCG67985
389	Wdr6	Q99ME2	WD repeat-containing protein 6
390	Fam76b	Q80XP8-1	Protein FAM76B
391	MNCb-5414	Q9JJA4	Ribosome biogenesis protein WDR12
392	RP23-188H3.2-004	B1AQZ0	Septin 8
393	Eif2s2	Q99L45	Eukaryotic translation initiation factor 2 subunit 2
394	Carmil	Q6EDY6-2	CARMIL homolog
395	Afg3l2	Q8JZQ2	AFG3-like protein 2
396	Msk2	Q9Z2B9	90 kDa ribosomal protein S6 kinase 4
397	mCG_16488	Q8K1M3	cAMP-dependent protein kinase type II-α regulatory chain
398	Sap130	Q8BIH0-1	130 kDa Sin3-associated polypeptide
399	Ifi205b	Q08619	Interferon-activable protein 205-B
400	Kifc1	E9PUA5	Kifc1 protein
401	mCG_120108	D3Z0K6	MCG120108, isoform CRA_a
402	Arhgef1	Q61210-5	Lbcs second cousin
403	Mapk3	D3Z3G6	Putative uncharacterized protein Mapk3
404	Rras2	P62071	Ras-related protein R-Ras2
405	Ankfy1	B1ATS3	Ankyrin repeat and FYVE domain containing 1
406	Cald1	Q8VD79	H-caldesmon
407	Ints5	Q8CHT3	Integrator complex subunit 5
408	Nfs1	Q9Z1J3-1	Cysteine desulfurase, mitochondrial
409	Kiaa1709	Q8K224	N-acetyltransferase 10
410	Carp1	Q8CH18-1	Cell cycle and apoptosis regulatory protein 1
411	Usp14	Q9JMA1	Deubiquitinating enzyme 14
412	Elovl5	Q8BHI7	Elongation of very long chain fatty acids protein 5
413	Psme3	P61290	11S regulator complex subunit γ
414	Cobll1	Q3UMF0-2	Cobl-related protein 1
415	Fam10a1	Q99L47	Hsc70-interacting protein
416	Dnm	P39053-1	Dynamin-1
417	Ik	Q3TJY5	Putative uncharacterized protein
418	Ap19	P61967	Adapter-related protein complex 1 sigma-1A subunit

419	AI314180	A2ALV7	Novel protein containing 10 HEAT domains
420	Whip	Q91XU0-1	ATPase WRNIP1
421	Gtpbp	O08582	GTP-binding protein 1
422	Prkcsh	O08795-2	80K-H protein
423	Cdk7	Q03147	39 kDa protein kinase
424	Tpp2	Q64514-1	Tripeptidyl aminopeptidase
425	Smbp	Q9ET30	Transmembrane 9 superfamily member 3
426	Dkc1	Q9ESX5	Dyskerin
427	Smu1	Q3UKJ7-1	Smu-1 suppressor of mec-8 and unc-52 protein homolog
428	Pds5a	E9Q656	Putative uncharacterized protein
429	Tada1	Q99LM9	Transcriptional adapter 1
430	Pxdn	B2RX13	Peroxidasin homolog (Drosophila)
431	Pdlim3	O70209	Actinin-associated LIM protein
432	Acp1	Q561M1	Acp1 protein
433	Dusp3	B1AQF4	Dual specificity phosphatase 3 (Vaccinia virus phosphatase VH1-related)
434	Imp4a	Q8VI75	Importin-4
435	Pola	P33609	DNA polymerase α catalytic subunit
436	Galk2	Q68FH4	Galactokinase 2
437	Ercc3	P49135	Basic transcription factor 2 89 kDa subunit
438	Drg	P32233	Developmentally-regulated GTP-binding protein 1
439	Scfd1	Q8BRF7-1	Sec1 family domain-containing protein 1
440	Nelfa	Q8BG30	Negative elongation factor A
441	Gapex5	Q6PAR5-4	GAPex-5
442	Pes	Q9EQ61	Pescadillo homolog
443	Gas2l1	Q8JZP9-1	GAS2-like protein 1
444	Taf2g	Q8VI33	RNA polymerase II TBP-associated factor subunit G
445	Cdc73	Q8JZM7	Cell division cycle protein 73 homolog
446	Arf1gap	Q9EPJ9-1	ADP-ribosylation factor 1 GTPase-activating protein
447	Bat2l	A2AN33	Novel protein (5830434P21Rik)
448	E130309D02Rik	Q8BGA7	Uncharacterized protein C7orf26 homolog
449	Abt1	Q9QYL7	Activator of basal transcription 1
450	Ahcyl1	Q80SW1	IP3R-binding protein released with inositol 1,4,5-trisphosphate
451	Iars2	Q8BIJ6	Isoleucine--tRNA ligase
452	Aco2	Q99KI0	Aconitate hydratase, mitochondrial
453	Elp3	Q9CZX0-2	Elongator complex protein 3
454	Dsp	E9Q557	Putative uncharacterized protein
455	Csnk2a2	O54833	Casein kinase II subunit α
456	Kiaa0090	Q8C7X2-1	Uncharacterized protein KIAA0090
457	Cdc21	P49717	CDC21 homolog

458	Pon3	Q62087	Serum paraoxonase/lactonase 3
459	Nampt	Q99KQ4	Nicotinamide phosphoribosyltransferase
460	Maged2	Q3TFU3	Putative uncharacterized protein
461	Ubap2l	Q80X50-5	Ubiquitin-associated protein 2-like
462	Col18a1	D3Z5C2	Putative uncharacterized protein Col18a1
463	Clpx	E9QLZ8	ATP-dependent Clp protease ATP-binding subunit clpX-like, mitochondrial
464	Fen1	E9PYV9	Putative uncharacterized protein
465	Nsdhl	Q9R1J0	Sterol-4-α-carboxylate 3-dehydrogenase, decarboxylating
466	Scnm1	Q8K136-1	Sodium channel modifier 1
467	Stxbp3	Q60770-1	Mammalian homolog of Unc-18c
468	X99384	B2RQ13	X99384 protein
469	Cdgsh2	Q9CQB5	CDGSH iron sulfur domain-containing protein 2
470	0610010K14Rik	D3Z687	Putative uncharacterized protein 0610010K14Rik
471	Cdk9	Q99J95-1	Cell division protein kinase 9
472	Pgrmc	O55022	Membrane-associated progesterone receptor component 1
473	Met	Q8CH25-1	Modulator of estrogen-induced transcription
474	Hspa12b	Q9CZJ2	Heat shock 70 kDa protein 12B
475	D1Ucla4	Q8N7N5-1	DDB1- and CUL4-associated factor 8
476	Ddit1	P48316	DNA damage-inducible transcript 1 protein
477		Q8R3C0	UPF0557 protein C10orf119 homolog
478	mCG_15097	Q8R2K3	Single-stranded DNA binding protein 1
479	Csnk1d	Q3USK2	Casein kinase 1, delta, isoform CRA_b
480	Dync1i2	D6Q0F5	Dynein cytoplasmic 1 intermediate chain 2
481	H2-Ke4	Q31125	Histidine-rich membrane protein Ke4
482	Sa2	O35638	Cohesin subunit SA-2
483	Bptf	A2A654	Bromodomain PHD finger transcription factor
484	Abcf1	Q6P542	ATP-binding cassette sub-family F member 1
485	Kctd14	Q5EER8	Potassium channel tetramerisation domain containing 14
486	Grcc3f	Q91V01	1-acylglycerophosphocholine O-acyltransferase
487	mCG_21756	Q6ZQG1	MCG21756, isoform CRA_a
488	Rap1gds1	E9Q912	Putative uncharacterized protein
489	Glud	P26443	Glutamate dehydrogenase 1, mitochondrial
490	Kiaa1978	Q9CW46-1	Protein raver-1
491	Kiaa0099	Q80U78-1	Pumilio homolog 1
492	Plod2	Q9R0B9	Lysyl hydroxylase 2
493	mKIAA0118	Q6A0C7	MKIAA0118 protein
494	Ssr3	Q9DCF9-1	Signal sequence receptor subunit γ
495	Aclp	Q640N1-1	Adipocyte enhancer-binding protein 1

496	Cdc47	Q61881	CDC47 homolog
497	Gipc	Q9Z0G0	GAIP C-terminus-interacting protein
498	Git1	Q68FF6	ARF GTPase-activating protein GIT1
499	Hsd17b7	Q8C5N9	Putative uncharacterized protein
500	Ebp	P70245	3-β-hydroxysteroid-Delta(8),Delta(7)-isomerase
501	Pth2	Q8R2Y8	Peptidyl-tRNA hydrolase 2, mitochondrial
502	D13Wsu123e	Q1HFZ0-1	Myc-induced SUN domain-containing protein
503	Gtl1-13	Q9Z0W3-1	160 kDa nucleoporin
504	Fkbp38	O35465-2	38 kDa FK506-binding protein
505	Scamp2	Q9ERN0	Secretory carrier-associated membrane protein 2
506	Eif3i	Q9QZD9	eIF3 p36
507	Aos1	Q9R1T2-1	SUMO-activating enzyme subunit 1
508	Phldb1	D3Z0X5	Putative uncharacterized protein Phldb1
509	Faf2	Q3TDN2-1	FAS-associated factor 2
510	Apg1	P48722-1	Heat shock 70 kDa protein 4L
511	Cyp1b1	Q64429	CYPIB1
512	Slit2	E9PZ51	Slit homolog 2 protein
513	Letm1	Q9Z2I0	LETM1 and EF-hand domain-containing protein 1, mitochondrial
514	P2pr	P97868-1	p53-associated cellular protein of testis
515	Tsg101	Q61187	ESCRT-I complex subunit TSG101
516	F	Q99JR1	Sideroflexin-1
517	Itpr2	Q9Z329-3	Inositol 1,4,5-trisphosphate receptor type 2
518	Cbx3	P23198	Chromobox protein homolog 3
519	Prelp	Q9JK53	Prolargin
520	Exosc10	B1ARY9	Exosome component 10
521	Msy1	P62960	CCAAT-binding transcription factor I subunit A
522	Col3a1	P08121	Collagen α-1(III) chain
523	Kiaa1741	P58871	182 kDa tankyrase-1-binding protein
524	Srf	Q9JM73	Serum response factor
525	Numa1	E9Q7G0	Putative uncharacterized protein
526	Sec62	Q8BU14	Translocation protein 1
527	Acad9	Q3ULL9	Putative uncharacterized protein
528	Sr140	Q6NV83-2	140 kDa Ser/Arg-rich domain protein
529	Prkaa1	Q5EG47	5-AMP-activated protein kinase catalytic subunit α-1
530	Wdr74	Q8VCG3	WD repeat-containing protein 74
531	Arht2	Q8JZN7	Mitochondrial Rho GTPase 2
532	Cape	Q8CG48	Chromosome-associated protein E
533	Aimp1	P31230	Aminoacyl tRNA synthase complex-interacting multifunctional protein 1
534	Rbbp5	Q8BX09-1	Retinoblastoma-binding protein 5

535	Fam164a	Q8BJH1-1	Protein FAM164A
536	Igh	P01806	Ig heavy chain V region 441
537	Hnrnpr	Q3U8W9	Putative uncharacterized protein
538	AL022818	Q3TMP1	General transcription factor IIIC, polypeptide 3
539	Brcc3	P46737-1	BRCA1/BRCA2-containing complex subunit 3
540	Nhp2l1	E9PWL3	Fertilization antigen 1
541	Fiz1	Q9WTJ4	Flt3-interacting zinc finger protein 1
542	Pbx1	P41778-1	Homeobox protein PBX1
543	AI597080	Q3V203	Putative uncharacterized protein
544	Thumpd2	Q9CZB3	THUMP domain-containing protein 2
545	Skiv2l2	Q9CZU3	ATP-dependent helicase SKIV2L2
546	Ilf3	Q9Z1X4-3	Interleukin enhancer-binding factor 3
547	Dus3l	Q91XI1-1	tRNA-dihydrouridine synthase 3-like
548	Abra1	Q8BPZ8-1	BRCA1-A complex subunit Abraxas
549	MNCb-2643	Q9CYH6	Ribosome biogenesis regulatory protein homolog
550	Igh	Q58E56	Igh protein
551	Bxdc1	Q9JJ80	Brix domain-containing protein 1
552	Baf45c	P58269-1	BRG1-associated factor 45C
553	Kiaa1789	Q3V0C1-1	Zinc finger matrin-type protein 1
554	Eif3h	Q91WK2	eIF3 p40 subunit
555	Ctps	P70698	CTP synthase 1
556	Dinb1	Q9QUG2-1	DINB protein
557	Erf3a	E9QK49	Eukaryotic peptide chain release factor GTP-binding subunit ERF3A
558	Ube2d3	P61079	Ubiquitin carrier protein D3
559	Nol1	Q922K7	Nucleolar protein 1
560	Mgst3	Q9CPU4	Microsomal glutathione S-transferase 3
561	mCG_17426	A2ASQ2	Prostaglandin E synthase 2
562	Srbd1	Q497V5-2	S1 RNA-binding domain-containing protein 1
563	Tpr	E9PZZ3	Tpr protein
564	Gnai1	B2RSH2	Guanine nucleotide binding protein (G protein), α inhibiting 1
565	Fam82a	Q8BSE0	Protein FAM82A1
566	Chdh	Q8BJ64	Choline dehydrogenase, mitochondrial
567	Slc25a35	Q5SWT3	Solute carrier family 25 member 35
568	Hnrnpa0	Q9CX86	Putative uncharacterized protein
569	Hells	Q60848-1	Lymphocyte-specific helicase
570	D3Wsu161e	Q05CL8-1	La ribonucleoprotein domain family member 7
571	Aralar1	Q8BH59	Calcium-binding mitochondrial carrier protein Aralar1
572	Loh11cr2a	Q99KC8	Loss of heterozygosity 11 chromosomal region 2 gene A protein homolog
573	Bre	Q8K3W0-	Brain and reproductive organ-expressed protein

		1	
574	Cd47	Q61735-2	Integrin-associated protein
575	Kars	Q99MN1	Lysine--tRNA ligase
576	Kif13b	E9Q4K7	Motor domain of KIF13B
577	Dhx16	Q80TX4	MKIAA0577 protein
578	Trrap	E9PWT1	Tra1 homolog
579	Cyp2s1	Q9DBX6	CYPIIS1
580	Ddx19	Q61655	ATP-dependent RNA helicase DDX19A
581	Tm9sf2	E9Q0K1	Putative uncharacterized protein
582	Dcaf7	P61963	DDB1- and CUL4-associated factor 7
583	Tbl2	Q8CFY0	Putative uncharacterized protein
584	Dance	Q9WVH9	Developmental arteries and neural crest EGF-like protein
585	Ythdc1	E9PW44	Putative uncharacterized protein
586	Brms1l	Q3U1T3-1	Breast cancer metastasis-suppressor 1-like protein
587	Baf180	Q8BSQ9-1	BRG1-associated factor 180
588	Fam103a1	Q9CQY2	Protein FAM103A1
589	B2m	P01887	B-2-microglobulin
590	Ascc3l1	Q69ZZ3	MKIAA0788 protein
591		F2Z452	
592	Yes	Q04736	p61-Yes
593	Tu52	B2M0S2	Clk2-Scamp3 protein
594	Adrm1	Q9JKV1	110 kDa cell membrane glycoprotein
595	Hr21	Q61550	Double-strand-break repair protein rad21 homolog
596	Xpot	Q9CRT8	Exportin(tRNA)
597	Dbr1	Q923B1	Lariat debranching enzyme
598	Bsg	P18572-1	Basic immunoglobulin superfamily
599	Dlg1	Q811D0-3	Disks large homolog 1
600	Rps27l	Q6ZWY3	40S ribosomal protein S27-like
601	Hausp	E9PXY8	Deubiquitinating enzyme 7
602	Aldh3b1	Q80VQ0	Aldehyde dehydrogenase 7
603	Tif1	Q64127-1	Transcription intermediary factor 1-α
604	Kiaa0425	A2A791-1	Zinc finger MYM-type protein 4
605	Atbp	Q5HZI1-1	Angiotensin-II type 2 receptor-interacting protein
606	Baz1b	Q9Z277-1	Bromodomain adjacent to zinc finger domain protein 1B
607	Garp45	Q9JL35	High mobility group nucleosome-binding domain-containing protein 5
608	Dscaml1	Q8R4B4	Down syndrome cell adhesion molecule-like protein
609	Ddx52	Q8K301	ATP-dependent RNA helicase ROK1-like
610	Tmem109	Q3UBX0	Mitsugumin-23
611	C130057N11Rik	A2AU63	HnRNP-associated with lethal yellow

612	Mll3	Q8BRH4-1	Histone-lysine N-methyltransferase MLL3
613	Snrpd3	P62320	Small nuclear ribonucleoprotein Sm D3
614	Fau	P62862	40S ribosomal protein S30
615	H2-D1	P01899	H-2 class I histocompatibility antigen, D-B α chain
616	Rrp1b	Q91YK2	Ribosomal RNA processing protein 1 homolog B
617	Aip5	Q8R5J9	Addicsin
618	Lancl2	Q9JJK2	LanC-like protein 2
619	Baf60c	Q6P9Z1	60 kDa BRG-1/Brm-associated factor subunit C
620	Ash2l	Q91X20	ASH2-like protein
621	Sf3a1	Q8K4Z5	SF3a120
622	Kiaa0326	Q6A085	Zinc finger protein 629
623	Wdr18	Q4VBE8	WD repeat-containing protein 18
624	Nab2	Q3TBL2	Putative uncharacterized protein
625	Chd3	B1AR17	Chromodomain helicase DNA binding protein 3
626	Ppil1	Q9D0W5	Peptidyl-prolyl cis-trans isomerase-like 1
627	Kiaa3002	Q80T69	Round spermatid basic protein 1
628	Metap1	Q8BP48	Methionine aminopeptidase 1
629	Fatp	Q60714	Long-chain fatty acid transport protein 1
630	Nup88	Q8CEC0-1	88 kDa nucleoporin
631	Apc	Q61315-1	Adenomatous polyposis coli protein
632	Iws1	Q8C1D8-1	IWS1-like protein
633	Cdyl	Q9WTK2-2	Chromodomain Y-like protein
634	BC031601	E9Q5F9	MKIAA1732 protein
635	Parp3	Q3UGL7	Putative uncharacterized protein
636	Baf60a	Q61466-1	60 kDa BRG-1/Brm-associated factor subunit A
637	Dlc1	P63168	8 kDa dynein light chain
638	Smc1a	A0JLM6	Smc1a protein
639		Q9D735	Uncharacterized protein C19orf43 homolog
640	Gtf3c1	Q8K284-1	General transcription factor 3C polypeptide 1
641	Dlc2	Q9D0M5	8 kDa dynein light chain b
642	Col14a1	Q80X19-1	Collagen α-1(XIV) chain
643	Ctr9	Q62018-1	RNA polymerase-associated protein CTR9 homolog
644	Gnl3	Q8CI11-1	Guanine nucleotide-binding protein-like 3
645	Syb3	P63024	Cellubrevin
646	Kiaa0152	Q6ZQI3	Malectin
647	Gpam	Q61586	Glycerol-3-phosphate acyltransferase 1, mitochondrial
648	Hnrnpl	E9Q8W8	Putative uncharacterized protein
649	Ssr4	Q9D6F7	Putative uncharacterized protein
650	Ledgf	Q99JF8-1	Lens epithelium-derived growth factor

651	Rbmxrt	Q91VM5	Putative uncharacterized protein
652	Maged1	Q9QYH6	Dlxin-1
653	Bcdin3	Q8K3A9	7SK snRNA methylphosphate capping enzyme
654	D14Ertd231e	Q8K114-1	Integrator complex subunit 9
655	Sap155	Q99NB9	Pre-mRNA-splicing factor SF3b 155 kDa subunit
656	Ftsjd2	Q9DBC3	FtsJ methyltransferase domain-containing protein 2
657	Hnrpq	Q7TMK9-1	Glycine- and tyrosine-rich RNA-binding protein
658	Cdk2ap2	Q9CPY4	Cyclin-dependent kinase 2-associated protein 2
659	Atp5e	P56382	ATP synthase subunit epsilon, mitochondrial
660	Aifm2	Q8BUE4-1	Apoptosis-inducing factor 2
661	Cwc15	Q9JHS9	Embryonic development factor 1
662	Kpna4	Q3UGH8	Putative uncharacterized protein
663	Mnar	Q9DBD5	Modulator of non-genomic activity of estrogen receptor
664	Baf155	P97496-1	BRG1-associated factor 155
665	Acadm	P45952	Medium-chain specific acyl-CoA dehydrogenase, mitochondrial
666	Rtn3	Q9ES97-1	Reticulon-3
667	Kiaa4072	Q8C181-4	Muscleblind-like protein 2
668	Cdw5	Q8BFQ4	WD repeat-containing protein 82
669	Drb1	Q8BHN5	Developmentally-regulated RNA-binding protein 1
670	Dpf2	D3Z5N6	Putative uncharacterized protein Dpf2
671	Nipbl	Q6KCD5-1	Delangin homolog
672	Aaas	P58742	Adracalin
673		Q9DAM7	UPF0444 transmembrane protein C12orf23 homolog
674	Bcl7c	O08664	B-cell chronic lymphocytic leukemia/lymphoma 7C protein
675	Ddx27	Q921N6-1	DEAD box protein 27
676	4933424B01Rik	Q8QZV7	Cell cycle regulator Mat89Bb homolog
677	Srp54	P14576-1	Signal recognition particle 54 kDa protein
678	Sar1a	Q3TXJ4	Putative uncharacterized protein
679	Ftsj3	Q9DBE9	Protein ftsJ homolog 3
680	Ncln	Q8VCM8	Nicalin
681	Gpkow	Q56A08	G patch domain and KOW motifs-containing protein
682	Hnrnpul1	Q8VDM6-1	Heterogeneous nuclear ribonucleoprotein U-like protein 1
683	Nup121	Q8K3Z9	Nuclear envelope pore membrane protein POM 121
684	mKIAA0035	E9Q5C9	MKIAA0035 protein
685	Frg1	P97376	FSHD region gene 1 protein
686	Ints10	Q8K2A7	Integrator complex subunit 10
687	Sf1	Q64213-2	CW17
688	Carf	Q8BI72	CDKN2A-interacting protein

689	Pcif1	P59114	Phosphorylated CTD-interacting factor 1
690	Phax	Q9JJT9	Phosphorylated adapter RNA export protein
691	Rps26	P62855	40S ribosomal protein S26
692	H2afv	Q3THW5	H2A.F/Z
693	Smarca2	F2Z4A9	Smarca2 protein
694	Smarca1	Q6PGB8-2	ATP-dependent helicase SMARCA1
695	Paf53	Q8K202-1	DNA-directed RNA polymerase I subunit E
696	Mta3	A4FTZ3	Mta3 protein
697	Cry1	P97784	Cryptochrome-1
698	Mbd2	E9QMV9	Methyl-CpG-binding domain protein 2
699	Nnp1	P56183-1	Novel nuclear protein 1
700	Apip	Q9WVQ5	APAF1-interacting protein
701	Abtap	Q3V1V3	ABT1-associated protein
702	Hnrpll	Q921F4-1	Heterogeneous nuclear ribonucleoprotein L-like
703	Tex10	Q3URQ0	Testis-expressed sequence 10 protein
704	Anp32	O35381	Acidic leucine-rich nuclear phosphoprotein 32 family member A
705	Mbd3	Q9Z2D8-1	Methyl-CpG-binding domain protein 3
706	Arid1b	E9Q4N7	Putative uncharacterized protein
707	Cbp20	Q9CQ49	20 kDa nuclear cap-binding protein
708	AF013969	E9Q6J5	Antigen containing epitope to monoclonal antibody MMS-85/12
709	D1Ertd578e	Q8BIG4	F-box only protein 28
710	Ptpn2	Q06180-1	MPTP
711	Jak1	B1ASP2	Tyrosine-protein kinase
712	Hnrnpul2	Q00PI9	Heterogeneous nuclear ribonucleoprotein U-like protein 2
713	Cdc5l	Q6A068	Cdc5-like protein
714	Myo1c	Q9WTI7-2	Myosin I β
715	Arid1a	A2BH40-1	AT-rich interactive domain-containing protein 1A
716	Bing4	Q9Z0H1	WD repeat-containing protein 46
717	Baf57	O54941	BRG1-associated factor 57
718	Nol5a	Q9D6Z1	Nucleolar protein 56
719	Crept	Q9CSU0-1	Cell cycle-related and expression-elevated protein in tumor
720	Gatad2a	E9QMN5	GATA zinc finger domain-containing protein 2A
721	Sdad1	Q80UZ2-1	Protein SDA1 homolog
722	Nomo1	Q6GQT9	Nodal modulator 1
723	Nol5	Q6DFW4	MSSP
724	Sin3a	E9QLW4	Histone deacetylase complex subunit Sin3a
725	Baf47	Q9Z0H3-1	BRG1-associated factor 47
726	Kifc1	Q9QWT9-1	Kinesin-like protein KIFC1
727	Abh5	Q3TSG4	Alkylated DNA repair protein alkB homolog 5

728	Baf60b	Q99JR8-1	60 kDa BRG-1/Brm-associated factor subunit B
729	Dcps	Q9DAR7	DCS-1
730	Sdcbp	O08992	Scaffold protein Pbp1
731	Cggbp1	Q8BHG9	20 kDa CGG-binding protein
732	Tcof1	Q05CS0	Tcof1 protein
733	Hdac1	O09106	Histone deacetylase 1
734	Aprin	Q4VA53-3	Androgen-induced proliferation inhibitor
735	Adnp	Q9Z103	Activity-dependent neuroprotective protein
736	Anp32e	P97822-1	Acidic leucine-rich nuclear phosphoprotein 32 family member E
737	Myh7b	A2AQP0	Myosin cardiac muscle β chain
738	Edd	Q80TP3	E3 ubiquitin-protein ligase UBR5
739	Kiaa1113	Q99PP7-1	E3 ubiquitin-protein ligase TRIM33
740	Cap1a	O55236	GTP--RNA guanylyltransferase
741	Rsl1d1	Q3TAJ5	Putative uncharacterized protein
742	Ctnnbl1	Q9CWL8	B-catenin-like protein 1
743	Kiaa0162	Q62383	Transcription elongation factor SPT6
744	Parn	Q8VDG3	Poly(A)-specific ribonuclease PARN
745	Syne1	Q6ZWR6-4	Enaptin
746	Tceb3	Q8CB77	Elongin 110 kDa subunit
747	Ddx47	Q9CWX9	DEAD box protein 47
748	Sbno1	A0JLU6	Sbno1 protein
749	Calnb	P48453-1	Calmodulin-dependent calcineurin A subunit β isoform
750	Baf190a	Q3TKT4-2	ATP-dependent helicase SMARCA4
751	Gatad2b	Q8VHR5-1	GATA zinc finger domain-containing protein 2B
752	Cbp80	Q3UYV9	80 kDa nuclear cap-binding protein
753	AV249152	Q8C456-1	Homoloc-13
754	Acta2	P62737	Actin, aortic smooth muscle
755	Ddx55	Q6ZPL9	ATP-dependent RNA helicase DDX55
756	Taf2s	Q8CGF7-1	Formin-binding protein 28
757	Kiaa0398	Q9D0L8-1	mRNA (guanine-N(7)-)-methyltransferase
758	Chd4	E9QAS5	MKIAA4075 protein
759	Hnrnpl	Q8R081	Heterogeneous nuclear ribonucleoprotein L
760	Dhm1	Q9DBR1-1	5-3 exoribonuclease 2
761	Anp32b	Q9EST5-2	Acidic leucine-rich nuclear phosphoprotein 32 family member B
762	Mta1	Q8K4B0	Metastasis-associated protein MTA1
763	Mybbp1a	Q7TPV4	Myb-binding protein 1A
764	Ars2	Q99MR6-1	Arsenite-resistance protein 2
765	Baf170	Q6PDG5-1	BRG1-associated factor 170

766	Mta1l1	Q9R190	Metastasis-associated 1-like 1
767		Q8VDP4	Protein KIAA1967 homolog
768	Kpna5	O35345	Importin α-S2
769	Mcm3	P25206	DNA polymerase α holoenzyme-associated protein P1
770	Chd8	Q09XV5-1	ATP-dependent helicase CHD8
771	Hnrnpu	Q8VEK3	Heterogeneous nuclear ribonucleoprotein U
772	Smarca5	Q91ZW3	Sucrose nonfermenting protein 2 homolog
773	Ddx21	Q3TVJ3	DEAD (Asp-Glu-Ala-Asp) box polypeptide 21
774	Ase1	Q76KJ5	A34.5
775	mCG_14998	Q52KC3	Minichromosome maintenance deficient 5, cell division cycle 46 (S. cerevisiae)
776	Bap135	Q9ESZ8-1	Bruton tyrosine kinase-associated protein 135
777	Hdac2	P70288	Histone deacetylase 2
778	Npap60	Q9JIH2	50 kDa nucleoporin
779	Lmn1	P48678-1	Lamin-A/C
780	Rbap48	Q60972	Chromatin assembly factor 1 subunit C
781	Skiip	Q9CSN1	Nuclear protein SkiP
782	Kiaa0156	Q9JLI8-1	Squamous cell carcinoma antigen recognized by T-cells 3
783	Msh6	Q61061	Mus musculus probable G/T mismatch binding protein
784	Fbl	P35550	Nucleolar protein 1
785	Rbap46	Q60973	Histone acetyltransferase type B subunit 2
786	Nup153	E9Q3G8	Putative uncharacterized protein
787	Actl6	Q9Z2N8	53 kDa BRG1-associated factor A
788	Hnrnpc	Q9Z204-2	Heterogeneous nuclear ribonucleoproteins C1/C2
789	Krip1	Q62318-1	KRAB-A-interacting protein
790	Caper	Q8VH51-2	Coactivator of activating protein 1 and estrogen receptors
791	Tfdp2	Q3TY79	Putative uncharacterized protein
792	Lmnb1	P14733	Lamin-B1
793	Tpm3	P21107-2	Γ-tropomyosin
794	Rpl27	P61358	60S ribosomal protein L27
795	cbp37	O88380	Cbp37
796	Cspg6	E9Q762	Putative uncharacterized protein
797	Ssb	P32067	La autoantigen homolog
798	Ddx15	O35286	DEAH box protein 15
799	Sbx	Q02053	Ubiquitin-activating enzyme E1
800	Fkbp5	Q64378	51 kDa FK506-binding protein
801	Prp19	Q99KP6-1	Nuclear matrix protein 200
802	Fubp1	Q0P6B2	Far upstream element (FUSE) binding protein 1
803	Csnk1a1	E9PWB2	Putative uncharacterized protein
804	Nfib	P97863-1	CCAAT-box-binding transcription factor

805	Cbp	Q61990-1	A-CP2
806	Ruvbl2	Q9WTM5	p47 protein
807	Eif3d	O70194	eIF3 p66

Table 20: Importin α7 binding partner identified by co-immunoprecipitation from fibroblast cells.

1-267: proteins bound to importin α7 and not to the mycHis-control. 268-299: top 5 % of importin α7 binding partners found to bind also to the mycHis-control peptide. Proteins were ranked according to their fold change.

	Gene name	Uniprot	Protein name
1	Celf2	Q9Z0H4-6	Bruno-like protein 3
2	H1fx	Q80ZM5	H1 histone family, member X
3	Dtd1	Q9DD18-1	D-tyrosyl-tRNA(Tyr) deacylase 1
4	Ccdc55	Q5NCR9	Coiled-coil domain-containing protein 55
5	Zfp593	Q9DB42	Zinc finger protein 593
6	Kiaa4103	Q924W5-1	Structural maintenance of chromosomes protein 6
7	Dhx33	Q80VY9	DEAH box protein 33
8	Fbp21	Q61048	Formin-binding protein 21
9	Dnajc9	Q91WN1	DnaJ homolog subfamily C member 9
10	RP23-158L6.2-002	Q9D3L3	Synaptosomal-associated protein
11	Cacybp	Q9CXW3	Calcyclin-binding protein
12	mCG_53108	Q9D3U4	MCG53108
13	Samd4	Q8CBY1-1	Protein Smaug homolog 1
14	Armc8	Q9DBR3-1	Armadillo repeat-containing protein 8
15	Lsm3	P62311	U6 snRNA-associated Sm-like protein LSm3
16	D10Wsu102e	Q9CX66	Uncharacterized protein C12orf45 homolog
17	Hox-1.3	P09021	Homeobox protein Hox-1.3
18	Clk	P22518-1	CDC-like kinase 1
19	Fam32a	Q9CR80	Protein FAM32A
20	C130039O16Rik	E9Q2I4	Putative uncharacterized protein
21	Gtf2a1	Q99PM3	General transcription factor IIA subunit 1
22	Abcf2	Q99LE6	ATP-binding cassette sub-family F member 2
23	Qk	Q9QYS9-7	Protein quaking
24	Med11	Q9D8C6	Mediator complex subunit 11
25	Crsp3	Q80YQ2-2	Cofactor required for Sp1 transcriptional activation subunit 3
26	Hnrnpl	Q8R081	Heterogeneous nuclear ribonucleoprotein L
27	Chd5	A2A8L1	Chromodomain helicase DNA binding protein 5
28	Rpl39	P62892	60S ribosomal protein L39
29	Kiaa1113	Q99PP7-1	E3 ubiquitin-protein ligase TRIM33
30	Xpc	P51612	DNA repair protein complementing XP-C cells homolog
31	Wdr18	Q4VBE8	WD repeat-containing protein 18
32	Ppp1cc	P63087-2	Protein phosphatase 1C catalytic subunit
33	Arg1	Q61176	Arginase-1
34	Hnrnpc	Q9Z204-4	Heterogeneous nuclear ribonucleoproteins C1/C2
35	Rrp7a	Q9D1C9	Gastric cancer antigen Zg14 homolog
36	Efr3a	E9QLX7	Protein EFR3 homolog A
37	Ccnk	Q3U3M5	Putative uncharacterized protein
38	Sf3b10	Q923D4	Pre-mRNA-splicing factor SF3b 10 kDa subunit
39	Zfhx4	E9Q5A7	Zfhx4 protein
40	Hnrnpk	P61979-3	Heterogeneous nuclear ribonucleoprotein K

41	Rbm	E9Q2F9	RNA-binding motif protein 1
42	Ythdc2	B2RR83	Probable ATP-dependent RNA helicase YTHDC2
43		Q8VDP4	Protein KIAA1967 homolog
44	Atxn2l	Q3TGG2	Putative uncharacterized protein
45	Myof	B9EK95	Myof protein
46	Cdkn2aipnl	Q9D211	CDKN2A-interacting protein N-terminal-like protein
47	Recql4	E9QMJ2	Recql4 protein
48	Lsm11	Q8BUV6	U7 snRNA-associated Sm-like protein LSm11
49	Prph	P15331-2	Peripherin
50	Rpa12	Q791N7	DNA-directed RNA polymerase I subunit RPA12
51	Pabpc4	A3KFU5	Poly A binding protein, cytoplasmic 4
52	Tgase3	Q08189	Protein-glutamine γ-glutamyltransferase E
53	Ppih	Q9D868-1	Peptidyl-prolyl cis-trans isomerase H
54	Top2b	Q64511	DNA topoisomerase 2-β
55	Tpx2	A2APB8	Targeting protein for Xklp2
56	Srp14	P16254	Signal recognition particle 14 kDa protein
57	Gtf2h3	Q8VD76	Basic transcription factor 2 34 kDa subunit
58	Ell	O08856	Eleven-nineteen lysine-rich leukemia protein
59	Cbp	Q61990-2	A-CP2
60	MNCb-1192	Q9D7Z3-1	Nucleolar protein 7
61	Myo1c	Q9WTI7-3	Myosin I β
62	Evi3	E9PWM6	Ecotropic viral integration site 3 protein
63	mCG_49644	D3Z4Z0	MCG49644
64	P4ha1	Q60715-1	Procollagen-proline,2-oxoglutarate-4-dioxygenase subunit α-1
65	Pnpla7	A2AJ88-1	Patatin-like phospholipase domain-containing protein 7
66	Nfx1	B1AY10-1	Nuclear transcription factor, X box-binding protein 1
67	Dhx16	Q80TX4	MKIAA0577 protein
68	Kiaa1582	Q3UHC0	Trinucleotide repeat-containing gene 6C protein
69	D3Ertd300e	Q7TT00-2	p38-interacting protein
70	Kiaa0007	Q6ZQL4	WD repeat-containing protein 43
71	Ash2l	Q91X20	ASH2-like protein
72	Dinb1	Q9QUG2-1	DINB protein
73	Ctcf	Q61164	11-zinc finger protein
74	Cbfb	Q08024-2	Core-binding factor subunit β
75	Zcchc10	Q9CX48	Zinc finger CCHC domain-containing protein 10
76	Baf60c	Q6P9Z1	60 kDa BRG-1/Brm-associated factor subunit C
77	Prnp	P04925	Major prion protein
78	Polr1d	Q8R1M3	Polymerase (RNA) I polypeptide D
79	Npm3	Q9CPP0	Nucleoplasmin-3
80	Kiaa1839	Q3UZ01-1	RNA-binding motif protein 40
81	Tex10	Q3URQ0	Testis-expressed sequence 10 protein
82	Grsf1	Q8C5Q4	G-rich sequence factor 1
83	Dsg1b	Q7TSF1	Desmoglein-1-β
84	Mars	E9QB02	Methionine--tRNA ligase
85	Garg16	Q64282	Glucocorticoid-attenuated response gene 16 protein
86	Sec61b	E9PW43	Protein transport protein Sec61 subunit β
87	Eef1b	O70251	Elongation factor 1-β
88	Hdac2	E9PXS3	Putative uncharacterized protein

89	mCG_120108	D3Z0K6	MCG120108, isoform CRA_a
90	Tu52	B2M0S2	Clk2-Scamp3 protein
91	Wdr36	Q3TAQ9	Putative uncharacterized protein
92	Drim	Q5XG71	Down-regulated in metastasis protein
93	Paf65b	Q91WQ5	PCAF-associated factor 65 β
94	Nol6	Q8R5K4-1	Nucleolar protein 6
95	Wdr26	E9PX83	WD repeat-containing protein 26
96	Nol9	Q3TZX8-1	Nucleolar protein 9
97	Ott	Q62013	Ott protein
98	Anx2	P07356	Annexin A2
99	Rmi1	Q9D4G9	RecQ-mediated genome instability protein 1
100	D14Ucla2	Q99LB2	Dehydrogenase/reductase SDR family member 4
101	G7b	O35900	Protein G7b
102	Kiaa1034	Q8VI24-1	DNA-binding protein SATB2
103	Mnar	Q9DBD5	Modulator of non-genomic activity of estrogen receptor
104	Med6	Q921D4	Mediator complex subunit 6
105	Metap1	Q8BP48	Methionine aminopeptidase 1
106	Ranbp2	E9QM01	Putative uncharacterized protein
107	Nvl	Q9DBY8	Nuclear valosin-containing protein-like
108	Tcfap4	Q8BWA4	Putative uncharacterized protein
109	Edd1	Q3TSI5	Putative uncharacterized protein
110	mCG_21074	Q8K357	Procr protein
111	Dsp	E9Q557	Putative uncharacterized protein
112	Ktn1	E9QJY4	Kinectin
113	Mkrn2	Q9ERV1	Probable E3 ubiquitin-protein ligase makorin-2
114	Glut1	P17809	Glucose transporter type 1, erythrocyte/brain
115	Dek	Q7TNV0	Protein DEK
116	mCG_67924	Q8BYM4	Peter pan homolog (Drosophila), isoform CRA_c
117	Pno1	Q9CPS7	RNA-binding protein PNO1
118	Supt7l	Q9CZV5	SPTF-associated factor 65 γ
119	Gm1878	E9QMB1	A-NAC, muscle-specific form
120	D4Cole1e	Q9EPA7	Nicotinamide mononucleotide adenylyltransferase 1
121	Paip1	Q8VE62	Polyadenylate-binding protein-interacting protein 1
122	Coil	E9Q284	Putative uncharacterized protein
123	Baf60a	Q61466-1	60 kDa BRG-1/Brm-associated factor subunit A
124	Supt3h	Q8BU36	Putative uncharacterized protein
125	Crsp7	Q7TN02	Cofactor required for Sp1 transcriptional activation subunit 7
126	Dars	Q922B2	Aspartate--tRNA ligase
127	Kiaa0425	A2A791-1	Zinc finger MYM-type protein 4
128	Prp31	Q8CCF0-1	Pre-mRNA-processing factor 31
129	Aimp1	P31230	Aminoacyl tRNA synthase complex-interacting multifunctional protein 1
130	Csnk1d	Q9DC28-1	Casein kinase I isoform delta
131	Kiaa1265	Q6P5F6	Solute carrier family 39 member 10
132	Cul1	Q9WTX6	Cullin-1
133	Ars2	Q99MR6-1	Arsenite-resistance protein 2
134	Tbp	P29037	TATA sequence-binding protein
135	Bend3	Q6PAL0	BEN domain-containing protein 3
136	Hmga1l4	Q99PT3	High mobility group AT-hook 1-like 4

137	Zfp384	E9Q1A5	Zfp384 protein
138	Smc1a	A0JLM6	Smc1a protein
139	Gas1	Q01721	Growth arrest-specific protein 1
140	Aff4	Q9ESC8	AF4/FMR2 family member 4
141	Gtf2h4	O70422	Basic transcription factor 2 52 kDa subunit
142	Mgcracgap	Q9WVM1	Male germ cell RacGap
143	Anp32e	P97822-1	Acidic leucine-rich nuclear phosphoprotein 32 family member E
144	Rps19	Q5M9P3	Rps19 protein
145	Myo1b	P46735-1	MIH-L
146	Dpf2	D3Z5N6	Putative uncharacterized protein Dpf2
147	Thrap3	Q569Z6	Thyroid hormone receptor-associated protein 3
148	Cpsf4	E0CXT7	Cleavage and polyadenylation specific factor 4 isoform 2
149	Tada1	Q99LM9	Transcriptional adapter 1
150	Sp3	O70494-1	Transcription factor Sp3
151	Myo5a	B8JK04	Myosin Va
152	Dlx2	P40764	Homeobox protein DLX-2
153		E9PV77	
154	Myh9	Q8VDD5	Cellular myosin heavy chain, type A
155	Sec13	Q9D1M0	Protein SEC13 homolog
156	Bat6	Q9Z1Q9	Protein G7a
157	Bclaf1	Q8K019-1	Bcl-2-associated transcription factor 1
158	Polr2h	Q923G2	DNA-directed RNA polymerase II subunit H
159	Cspg6	E9Q762	Putative uncharacterized protein
160	Atf7	Q3TZR9	Activating transcription factor 7, isoform CRA_a
161	Dhx8	A2A4P0	ATP-dependent RNA helicase DHX8
162	Ctnnbl1	Q9CWL8	B-catenin-like protein 1
163	Fam103a1	Q9CQY2	Protein FAM103A1
164	D19Bwg1357e	Q8BKS9	Pumilio domain-containing protein KIAA0020
165	Polr2e	Q80UW8	DNA-directed RNA polymerase II subunit E
166	Brcc3	P46737-1	BRCA1/BRCA2-containing complex subunit 3
167	Ddx2a	P60843	ATP-dependent RNA helicase eIF4A-1
168	Thoc3	Q8VE80	THO complex subunit 3
169	Ifi204	E9QLE0	Interferon-activable protein 204
170	Kif23	E9Q5G3	Kif23 protein
171	Nfic	P70255-1	CCAAT-box-binding transcription factor
172	Eif5b	Q05D44	Eukaryotic translation initiation factor 5B
173	Rbbp5	Q8BX09-1	Retinoblastoma-binding protein 5
174	Rap80	Q5U5Q9-1	BRCA1-A complex subunit RAP80
175	Hcfc1	Q61191	C1 factor
176	Gnb1	P62874	Guanine nucleotide-binding protein G(I)/G(S)/G(T) subunit β-1
177	Khsrp	E9QKH3	KH type splicing regulatory protein KSRP
178	G22p1	P23475	ATP-dependent DNA helicase 2 subunit 1
179	Maged1	Q9QYH6	Dlxin-1
180	Gapd	P16858	Glyceraldehyde-3-phosphate dehydrogenase
181	Abh5	Q3TSG4	Alkylated DNA repair protein alkB homolog 5
182	Ran	P62827	GTPase Ran
183	Kifc1	Q9QWT9-1	Kinesin-like protein KIFC1
184	Folr1	Q03514	Gag protein

185	Ppig	A2AR02	Cyclophilin G
186	mKIAA0035	E9Q5C9	MKIAA0035 protein
187	Aprin	Q4VA53-3	Androgen-induced proliferation inhibitor
188	Kiaa1341	Q8C854-4	Myelin expression factor 2
189	Bp75	O88665	75 kDa bromodomain protein
190	Cyp33	Q9QZH3	Cyclophilin E
191	Cwc15	Q9JHS9	Embryonic development factor 1
192	Vwc2	Q8C8N3	Brain-specific chordin-like protein
193	Ino80d	Q66JY2-1	INO80 complex subunit D
194	Baf155	P97496-1	BRG1-associated factor 155
195		Q8R3C0	UPF0557 protein C10orf119 homolog
196	Klhl7	Q8BUL5	Kelch-like protein 7
197	Hrmt1l2	Q9JIF0-1	Protein arginine N-methyltransferase 1
198	Rplp1	P47955	60S acidic ribosomal protein P1
199	Chd3	B1AR17	Chromodomain helicase DNA binding protein 3
200	Ptrf	O54724	Cavin-1
201	RP23-18D18.4-001	B1AZM2	Novel protein similar to ovary testis transcribed (Ott)
202	Adprp	P11103-1	NAD(+) ADP-ribosyltransferase 1
203	Trrap	E9PWT1	Tra1 homolog
204	Rpl3l	E9PWZ3	Rpl3l protein
205	Arid1b	E9Q4N7	Putative uncharacterized protein
206	Rps17	P63276	40S ribosomal protein S17
207	Arid2	E9Q7E2	MCG141061
208	BC010250	E9PYH6	Setd1a protein
209	Spty2d1	Q68FG3	Protein SPT2 homolog
210	Mcm3	P25206	DNA polymerase α holoenzyme-associated protein P1
211	Mbd2	E9QMV9	Methyl-CpG-binding domain protein 2
212	Abra1	Q8BPZ8-1	BRCA1-A complex subunit Abraxas
213	Lmnb2	P21619-1	Lamin-B2
214	Ylpm1	D3YWX2	Putative uncharacterized protein Ylpm1
215	Myh10	Q3UH59	Myosin, heavy polypeptide 10, non-muscle
216	Cbll1	Q9JIY2-1	Casitas B-lineage lymphoma-transforming sequence-like protein 1
217	Alg4	Q6NS46	Apoptosis-linked gene 4 protein
218	Nkrf	A2A3V7	NF-kappaB repressing factor
219	Paf65a	Q8R2K4-1	PCAF-associated factor 65-α
220	Serbp1	Q3UMP4	Putative uncharacterized protein
221	Emp2	O88662	Epithelial membrane protein 2
222	Bptf	A2A654	Bromodomain PHD finger transcription factor
223	Ppil1	Q9D0W5	Peptidyl-prolyl cis-trans isomerase-like 1
224	mCG_21006	Q3UMX7	DNA-directed RNA polymerase
225	Farsa	Q8C0C7	Phenylalanine--tRNA ligase α chain
226	D3Ertd250e	Q9D478-1	Outer dense fiber protein 2-like
227	Rnu3ip2	Q91WM3	RRP9 homolog
228	H1f3	P43277	H1 VAR.4
229	Lrrc59	Q922Q8	Leucine-rich repeat-containing protein 59
230	Smarca5	Q91ZW3	Sucrose nonfermenting protein 2 homolog
231	Fbll1	Q80WS3	rRNA/tRNA 2-O-methyltransferase fibrillarin-like protein 1
232	Mta3	Q924K8-1	Metastasis-associated protein MTA3

233	Nipbl	Q6KCD5-1	Delangin homolog
234	Smy	P13675	Y-linked testis-specific protein 1
235	Baf47	Q9Z0H3-1	BRG1-associated factor 47
236	Anp32b	Q9EST5-2	Acidic leucine-rich nuclear phosphoprotein 32 family member B
237	Myo1c	Q9WTI7-1	Myosin I β
238	Paf53	Q8K202-1	DNA-directed RNA polymerase I subunit E
239	Ase1	Q76KJ5	A34.5
240	Baf180	Q8BSQ9-1	BRG1-associated factor 180
241	Baf60b	Q99JR8-1	60 kDa BRG-1/Brm-associated factor subunit B
242	Trmt112	Q9DCG9	TRM112-like protein
243	Rpl37	Q9D823	60S ribosomal protein L37
244	Arid1a	A2BH40-1	AT-rich interactive domain-containing protein 1A
245	Hist3h2a	Q8BFU2	Histone H2A type 3
246	Baf57	O54941	BRG1-associated factor 57
247	Nup121	Q8K3Z9	Nuclear envelope pore membrane protein POM 121
248	Adnp	Q9Z103	Activity-dependent neuroprotective protein
249	Polr1a	O35134	DNA-directed RNA polymerase I largest subunit
250	mCG_5485	Q3UI38	Putative uncharacterized protein
251	Carf	Q8BI72	CDKN2A-interacting protein
252	Mocs3	A2BDX3	Adenylyltransferase and sulfurtransferase MOCS3
253	Mbd3	Q9Z2D8-1	Methyl-CpG-binding domain protein 3
254	Nup153	E9Q3G8	Putative uncharacterized protein
255	Hdac2	P70288	Histone deacetylase 2
256	AF013969	E9Q6J5	Antigen containing epitope to monoclonal antibody MMS-85/12
257	Rbap48	Q60972	Chromatin assembly factor 1 subunit C
258	Taf2s	Q8CGF7-1	Formin-binding protein 28
259	Gatad2a	E9QMN5	GATA zinc finger domain-containing protein 2A
260	Mta1	Q8K4B0	Metastasis-associated protein MTA1
261	Kifc1	E9PUA5	Kifc1 protein
262	Gatad2b	Q8VHR5-1	GATA zinc finger domain-containing protein 2B
263	Edd	Q80TP3	E3 ubiquitin-protein ligase UBR5
264	Kiaa0398	Q9D0L8-1	mRNA (guanine-N(7)-)-methyltransferase
265	Mta1l1	Q9R190	Metastasis-associated 1-like 1
266	mCG_13583	Q6P1F4	Nucleoporin 50
267	Kpna5	O35345	Importin α-S2
268	Lmnb1	P14733	Lamin-B1
269	Chd4	E9QAS5	MKIAA4075 protein
270	Rbap46	Q60973	Histone acetyltransferase type B subunit 2
271	Lmn1	P48678-1	Lamin-A/C
272	Baf170	Q6PDG5-1	BRG1-associated factor 170
273	Hdac1	O09106	Histone deacetylase 1
274	Dhm1	Q9DBR1-1	5-3 exoribonuclease 2
275	Baf190a	Q3TKT4-2	ATP-dependent helicase SMARCA4
276	Nhp2l1	E9PWL3	Fertilization antigen 1
277	Nol5a	Q9D6Z1	Nucleolar protein 56
278	Fbl	P35550	Nucleolar protein 1
279	Impnb	P70168	Importin subunit β-1
280	Fbxo11	Q7TPD1-1	F-box only protein 11

281	Kpna1	Q60960	Importin α-S1
282	Nol5	Q6DFW4	MSSP
283	Lyar	Q08288	Cell growth-regulating nucleolar protein
284	mCG_14998	Q52KC3	Minichromosome maintenance deficient 5, cell division cycle 46 (S. cerevisiae)
285	Ddx21	Q3TVJ3	DEAD (Asp-Glu-Ala-Asp) box polypeptide 21
286	H3.2	P84228	Histone H3.2
287	Gnl3	Q8CI11-1	Guanine nucleotide-binding protein-like 3
288	Zc3h13	B9EHN9	Zinc finger CCCH type containing 13
289	Pcif1	Q6ZWS8	HIB homolog 1
290	Carp1	Q8CH18-1	Cell cycle and apoptosis regulatory protein 1
291	Snrpd1	P62315	Small nuclear ribonucleoprotein Sm D1
292	Ino80c	Q8BHA0	INO80 complex subunit C
293	Eif2s3x	Q9Z0N1	Eukaryotic translation initiation factor 2 subunit 3, X-linked
294	Crfg	Q99ME9	Chronic renal failure gene protein
295	Hnrnpu	Q8VEK3	Heterogeneous nuclear ribonucleoprotein U
296	Eprs	B9EIU1	Glutamyl-prolyl-tRNA synthetase
297	Ahnak	A0JLR7	Ahnak protein
298	Rps27l	Q6ZWY3	40S ribosomal protein S27-like
299	Cbp80	Q3UYV9	80 kDa nuclear cap-binding protein

Table 21: Importin α2 binding partner identified by co-immunoprecipitation from fibroblast cells.

1-111: proteins bound to importin α2 and not to the mycHis-control. 112-137: top 5 % of importin α2 binding partners found to bind also to the mycHis-control peptide. Proteins were ranked according to their fold change.

	Gene name	Uniprot	Protein name
1	Get4	Q9D1H7-1	UPF0363 protein C7orf20 homolog
2	Prr11	Q8BHE0	Proline-rich protein 11
3	mCG_6742	A2AWN8	YTH domain family 1
4	Akirin2	B1AXD8	Akirin-2
5	ENSMUSG00000074697	Q7TQ39	Gag protein
6	mCG_17339	Q9WV02	Heterogeneous nuclear ribonucleoprotein G
7	Stc2	O88452	Stanniocalcin-2
8	Wdr3	Q8BHB4	WD repeat-containing protein 3
9	Gna11	P21278	Guanine nucleotide-binding protein subunit α-11
10	Immt	Q8CAQ8-3	Mitochondrial inner membrane protein
11	Nle1	B1ARD5	Notchless homolog 1 (Drosophila)
12	Aimp2	E9QP67	Aminoacyl tRNA synthase complex-interacting multifunctional protein 2
13	Uch37	Q9WUP7-1	Ubiquitin carboxyl-terminal hydrolase isozyme L5
14		D3YY62	Putative uncharacterized protein ENSMUSP00000083721
15	Ltv1	Q6NSQ7	Protein LTV1 homolog
16	Kif3c	O35066	Kinesin-like protein KIF3C
17	Farsa	Q8C0C7	Phenylalanine--tRNA ligase α chain
18	Myh14	B3F3T1	Nonmuscle myosin II-C2
19	Tim50	Q9D880	Mitochondrial import inner membrane translocase subunit TIM50
20	Gm929	Q3UPE3-1	RecQ-mediated genome instability protein 2
21	Mtch2	Q791V5	Mitochondrial carrier homolog 2
22	Dimt1	Q9D0D4	18S rRNA dimethylase
23	Nap1l1	E9PW66	Brain protein DN38
24	Mgcracgap	Q9WVM1	Male germ cell RacGap
25	Sfxn3	Q91V61-1	Sideroflexin-3
26	Igf2bp2	A6X8Z3	Insulin-like growth factor 2 mRNA binding protein 2
27	Dnaja2	Q9QYJ0	DnaJ homolog subfamily A member 2
28	Iqgap3	B9EKT6	IQ motif containing GTPase activating protein 3
29	Dpm1	O70152	Dolichol-phosphate mannose synthase
30	Shmt2	Q3TFD0	Serine hydroxymethyltransferase
31	Cttn	Q3UGC2	Cortactin, isoform CRA_e
32	Simp	Q3TDQ1	B6dom1 antigen
33	Ehmt1	Q5DW34-1	GLP/Eu-HMTase1
34	Myl6	Q60605-2	17 kDa myosin light chain
35	Fxr1	Q61584-4	Fragile X mental retardation syndrome-related protein 1
36	Rpn1	Q91YQ5	Dolichyl-diphosphooligosaccharide--protein glycosyltransferase 67 kDa subunit
37	Chchd3	Q9CRB9	Coiled-coil-helix-coiled-coil-helix domain-containing protein 3, mitochondrial
38	Abcf2	Q99LE6	ATP-binding cassette sub-family F member 2
39	Ddx2a	P60843	ATP-dependent RNA helicase eIF4A-1
40	Ring1	O35730-1	E3 ubiquitin-protein ligase RING1

41	Rps19	Q5M9P3	Rps19 protein
42	Dhrs7b	Q99J47-1	Dehydrogenase/reductase SDR family member 7B
43	Coro1c	Q9WUM4	Coronin-1C
44	Hcngp	Q02614	SAP30-binding protein
45	Aif	Q9Z0X1	Apoptosis-inducing factor 1, mitochondrial
46	Rrp1b	Q91YK2	Ribosomal RNA processing protein 1 homolog B
47	Myo1c	Q9WTI7-2	Myosin I β
48	Ddx52	Q8K301	ATP-dependent RNA helicase ROK1-like
49	Arc20	P59999	Actin-related protein 2/3 complex subunit 4
50	Mta1l1	Q9R190	Metastasis-associated 1-like 1
51	Prp31	Q8CCF0-1	Pre-mRNA-processing factor 31
52	Gja1	E9Q0S1	Putative uncharacterized protein
53	Sec61a	P61620	Protein transport protein Sec61 subunit α isoform 1
54	Smt3c	P63166	Small ubiquitin-related modifier 1
55	Myo9b	Q9QY06-2	Myosin-IXb
56	Abcd3	E9QNT7	Putative uncharacterized protein
57	Mct1	P53986	Monocarboxylate transporter 1
58	Scd2	P13011	Acyl-CoA desaturase 2
59	Anx2	P07356	Annexin A2
60	Alms1	Q8K4E0-2	Alstrom syndrome protein 1 homolog
61	Actr2	P61161	Actin-like protein 2
62	H2a.x	P27661	Histone H2A.x
63	Kiaa1341	Q8C854-4	Myelin expression factor 2
64	Hig1	Q8R472	HIG1 domain family, member 1A, isoform CRA_a
65	Pk3	P52480-1	Pyruvate kinase isozymes M1/M2
66	Hist3h2a	Q8BFU2	Histone H2A type 3
67	Myo1g	Q5SUA5	Myosin-Ig
68	Copg	Q9QZE5	Coatomer subunit γ
69	Cfl1	P18760	Cofilin, non-muscle isoform
70	Metap2	O08663	Initiation factor 2-associated 67 kDa glycoprotein
71	Uqcrq	Q9CQ69	Complex III subunit 8
72		A2IXM7	Anti-CD30 moab Ki-4 scFv
73	Gstp1	P19157	Glutathione S-transferase P 1
74	Tubb6	Q922F4	Tubulin β-6 chain
75	Ahnak2	E9PYB0	Putative uncharacterized protein
76	Tmco1	Q3V4A0	Putative uncharacterized protein
77	Gapd	P16858	Glyceraldehyde-3-phosphate dehydrogenase
78	Kdelr2	Q9CQM2	ER lumen protein retaining receptor 2
79	Msh2	P43247	DNA mismatch repair protein Msh2
80	Arpc1b	Q3TBA2	Putative uncharacterized protein
81	Hypk	Q9CR41-1	Huntingtin yeast partner K
82	Ran	P62827	GTPase Ran
83	Rangap1	Q3UZD8	Putative uncharacterized protein
84	Hkdc1	Q91W97	Hexokinase domain-containing protein 1
85	Cdipt	Q8VDP6	CDP-diacylglycerol--inositol 3-phosphatidyltransferase
86	Drim	Q5XG71	Down-regulated in metastasis protein
87	Lrrc59	Q922Q8	Leucine-rich repeat-containing protein 59
88	Uba52	E9QNP0	60S ribosomal protein L40

89	Rps17	P63276	40S ribosomal protein S17
90	Folr1	Q03514	Gag protein
91	Gclm	O09172	Γ-ECS regulatory subunit
92	Ndufa4	Q62425	Complex I-MLRQ
93		E9QPE7	
94	Gm4883	D3YVT7	Putative uncharacterized protein Gm4883
95	Gstm2	P15626	Glutathione S-transferase Mu 2
96	Ranbp2	E9QM01	Putative uncharacterized protein
97	Sh2b1	Q91ZM2-1	Pro-rich, PH and SH2 domain-containing signaling mediator
98	Actg	P63260	Actin, cytoplasmic 2
99	Myo5a	B8JK04	Myosin Va
100	Kifc1	E9PUA5	Kifc1 protein
101	Rplp1	P47955	60S acidic ribosomal protein P1
102	Myo1b	P46735-1	MIH-L
103	Rpl37	Q9D823	60S ribosomal protein L37
104	Myo1d	Q5SYD0-1	Myosin-Id
105	H1f3	P43277	H1 VAR.4
106	Gstm1	P10649	Glutathione S-transferase GT8.7
107	Myh10	Q3UH59	Myosin, heavy polypeptide 10, non-muscle
108	EG225058	Q3UTE1	Putative uncharacterized protein
109	Myo1c	Q9WTI7-1	Myosin I β
110	Myh9	Q8VDD5	Cellular myosin heavy chain, type A
111	AW146299	C0LLJ1	Kpna2-like protein transcript variant 2
112	Hsd17b12	O70503-1	17-β-hydroxysteroid dehydrogenase 12
113	Lbr	Q3U9G9	Integral nuclear envelope inner membrane protein
114	Far1	Q922J9-4	Fatty acyl-CoA reductase 1
115	Gpsn2	Q9CY27	Synaptic glycoprotein SC2
116	Ckap4	Q8BMK4	63 kDa membrane protein
117	Impnb	P70168	Importin subunit β-1
118	Atp5c1	Q91VR2	ATP synthase subunit γ, mitochondrial
119	Kiaa0093	P46935	E3 ubiquitin-protein ligase NEDD4
120	Mybbp1a	Q7TPV4	Myb-binding protein 1A
121	Actg1	E9Q607	Γ actin-like protein
122	Cd44	P15379-14	CD44 antigen
123	Rps27l	Q6ZWY3	40S ribosomal protein S27-like
124	Slc25a3	Q8VEM8	Phosphate carrier protein, mitochondrial
125	Atad3	Q925I1-1	AAA-ATPase TOB3
126	Myo1e	E9Q634	Putative uncharacterized protein
127	Immt	Q8CAQ8-2	Mitochondrial inner membrane protein
128	Iqgap1	Q9JKF1	Ras GTPase-activating-like protein IQGAP1
129	Thoc2	B1AZI6	THO complex subunit 2
130	Atp5a1	Q03265	ATP synthase subunit α, mitochondrial
131	Ant2	P51881	Adenine nucleotide translocator 2
132	Apg2	Q61316	Heat shock 70 kDa protein 4
133	Rpl35	Q6ZWV7	60S ribosomal protein L35
134	Thoc6	Q5U4D9	THO complex subunit 6 homolog
135	Rps24	P62849-1	40S ribosomal protein S24
136	Tubb5	P99024	Tubulin β-5 chain

137	Kpna5	O35345	Importin α-S2

Table 22: Importin α3 binding partner identified by co-immunoprecipitation from fibroblast cells.

1-425: proteins bound to importin α3 and not to the mycHis-control. 426-434: top 5 % of importin α3 binding partners found to bind also to the mycHis-control peptide. Proteins were ranked according to their fold change.

	Gene name	Uniprot	Protein name
1	Mrt4	Q9D0I8	mRNA turnover protein 4 homolog
2	Lsm11	Q8BUV6	U7 snRNA-associated Sm-like protein LSm11
3	Fam65a	Q68FE6	Protein FAM65A
4	Cappa1	P47753	CapZ α-1
5	Dal1	Q9WV92-8	4.1B
6	Srebf2	Q3U1N2-1	Processed sterol regulatory element-binding protein 2
7	Diap1	E9PV41	Diap1 protein
8	Ldc4	Q9ESX4-1	Nucleolar protein of 40 kDa
9	Inrf2	Q9Z2X8	Cytosolic inhibitor of Nrf2
10	Golgb1	B2RWW2	Golgb1 protein
11	Naa15	Q80UM3	N-α-acetyltransferase 15, NatA auxiliary subunit
12	Jbp1	Q8CIG8	Histone-arginine N-methyltransferase PRMT5
13	Hk2	O08528	Hexokinase type II
14	Uqcrq	Q9CQ69	Complex III subunit 8
15	mKIAA0118	Q6A0C7	MKIAA0118 protein
16	Metap2	O08663	Initiation factor 2-associated 67 kDa glycoprotein
17	Recql4	E9QMJ2	Recql4 protein
18	Nol14	Q8R3N1	Nucleolar complex protein 14
19	Afg3l1	Q920A7-1	AFG3-like protein 1
20	Ehd1	Q9WVK4	EH domain-containing protein 1
21	Sec63	E9QKG1	SEC63
22	Arhgap18	Q8K0Q5	Rho GTPase-activating protein 18
23	Pilt	Q9DCD5	Protein incorporated later into tight junctions
24	Lars	Q6ZPT2	MKIAA1352 protein
25	Mrpl44	Q9CY73-1	39S ribosomal protein L44, mitochondrial
26	Zfhx4	E9Q5A7	Zfhx4 protein
27	Cav	P49817-2	Caveolin-1
28	Kif5b	Q5BL10	Kinesin family member 5B
29	Usmg5	Q78IK2	Up-regulated during skeletal muscle growth protein 5
30	Dhx8	A2A4P0	ATP-dependent RNA helicase DHX8
31	G2an	Q8BHN3-2	A-glucosidase 2
32	Xpc	P51612	DNA repair protein complementing XP-C cells homolog
33	Gc1	Q9D6M3	Glutamate/H(+) symporter 1
34	Flot2	Q3UEG9	Putative uncharacterized protein
35	Zfp62	Q8C827-3	Zinc finger protein 62
36	Bend3	Q6PAL0	BEN domain-containing protein 3
37	Hectd1	Q69ZR2	MKIAA1131 protein
38	Aars2	Q14CH7	Alanine--tRNA ligase
39	Crop	Q5SUF2-2	Cisplatin resistance-associated-overexpressed protein
40	Snap47	Q8R570-1	Synaptosomal-associated 47 kDa protein
41	Hcfc1	Q61191	C1 factor
42	Smarca5	Q91ZW3	Sucrose nonfermenting protein 2 homolog

43	Alb	P07724	Serum albumin
44	Kiaa1582	Q3UHC0	Trinucleotide repeat-containing gene 6C protein
45	Tuba4	P68368	A-tubulin 4
46	Rin1	Q921Q7	Ras and Rab interactor 1
47	Zfp385	Q9QY68	Hematopoietic zinc finger protein
48	Ipo13	Q8K0C1-2	Importin-13
49	Abcb10	Q9JI39	ABC-mitochondrial erythroid protein
50	Hltf	Q6PCN7	Helicase-like transcription factor
51	MNCb-5414	Q9JJA4	Ribosome biogenesis protein WDR12
52	Sun2	Q8BJS4-1	Protein unc-84 homolog B
53	Emd	O08579	Emerin
54	Mrps9	Q14AZ3	Mitochondrial ribosomal protein S9
55	Cars	Q9ER72-1	Cysteine--tRNA ligase
56	Atp12a	Q32MR8	ATPase, H+/K+ transporting, nongastric, α polypeptide
57	Afg3l2	Q8JZQ2	AFG3-like protein 2
58	Fryl	Q80UX7	Fryl protein
59	RP23-216M21.1-001	A2AD18	Ubiquitin carboxyl-terminal hydrolase
60	Inf2	Q0GNC1-1	Inverted formin-2
61	H2afy	Q9QZQ8-1	Core histone macro-H2A.1
62	Eps15	P42567-1	Epidermal growth factor receptor substrate 15
63	Kif3c	O35066	Kinesin-like protein KIF3C
64	Obsl1	D3YYU8	Putative uncharacterized protein Obsl1
65	Utp23	Q9CX11	rRNA-processing protein UTP23 homolog
66	Crmp2	O08553	Dihydropyrimidinase-related protein 2
67	Kiaa1881	E9QN72	Adipocyte protein S3-12
68	Arhgap23	Q69ZH9-1	Rho GTPase-activating protein 23
69	Ccbl2	Q71RI9-1	Cysteine-S-conjugate β-lyase 2
70	Patl1	Q3TC46	PAT1-like protein 1
71	Ano10	Q8BH79-4	Anoctamin-10
72	Hspg2	Q05793	Basement membrane-specific heparan sulfate proteoglycan core protein
73	Eset	O88974-7	ERG-associated protein with SET domain
74	Fastkd5	Q7TMV3	FAST kinase domain-containing protein 5
75	Vps16	Q920Q4-1	Vacuolar protein sorting-associated protein 16 homolog
76	Coro1c	Q9WUM4	Coronin-1C
77	C2f	O35130	Nucleolar protein EMG1 homolog
78	Dvl2	Q60838	DSH homolog 2
79	Ylpm1	D3YWX2	Putative uncharacterized protein Ylpm1
80	Fhod1	Q6P9Q4	FH1/FH2 domain-containing protein 1
81	Nle1	B1ARD5	Notchless homolog 1 (Drosophila)
82	Fasn	P19096	[Acyl-carrier-protein] S-acetyltransferase
83	Dnajc9	Q91WN1	DnaJ homolog subfamily C member 9
84	Copg	Q9QZE5	Coatomer subunit γ
85	Smt3h1	Q9Z172-1	Small ubiquitin-related modifier 3
86	Des	P31001	Desmin
87	D14Ertd231e	Q8K114-1	Integrator complex subunit 9
88	Bag3	A6H663	BCL2-associated athanogene 3

89	Ubap2l	Q80X50-2	Ubiquitin-associated protein 2-like
90	Actg	P63260	Actin, cytoplasmic 2
91	scFv B8E5	Q6KB05	ScFv B8E5 protein
92	Myl6b	Q8CI43	Myosin light chain 6B
93	Arsdr1	Q9QYF1	Androgen-regulated short-chain dehydrogenase/reductase 1
94	Ki-67	E9PVX6	Ki-67 protein
95	Nfx1	B1AY10-1	Nuclear transcription factor, X box-binding protein 1
96	Efha1	Q8CD10	EF-hand domain-containing family member A1
97	Phgdh	Q61753	A10
98	Ask2	Q9WTR2	Apoptosis signal-regulating kinase 2
99	Ndufa9	Q9DC69	Complex I-39kD
100	Blvra	Q9CY64	Biliverdin reductase A
101	Nrd1	A2A9Q2	Nardilysin, N-arginine dibasic convertase, NRD convertase 1
102	Nek3	Q9R0A5	Never in mitosis A-related kinase 3
103	Galk	Q9R0N0	Galactokinase
104	Actn3	O88990	A-actinin skeletal muscle isoform 3
105	Uso1	Q9Z1Z0-1	General vesicular transport factor p115
106	Acsvl4	Q91VE0	Long-chain fatty acid transport protein 4
107	Jmjd2a	A2A8L8	Jumonji domain containing 2A
108	Ncoa4	Q3UI10	Putative uncharacterized protein
109	Cyfip1	Q7TMB8-1	Cytoplasmic FMR1-interacting protein 1
110	Myo1b	P46735-1	MIH-L
111	Exosc10	B1ARY9	Exosome component 10
112	RP23-92C4.1-001	B1AVZ0	Novel protein
113		E9Q264	
114	Fbxo30	Q8BJL1	F-box only protein 30
115	Rpl37a	P61514	60S ribosomal protein L37a
116	Gbf1	A0JLN9	Gbf1 protein
117	A2mr	Q91ZX7	A-2-macroglobulin receptor
118	Bat2l	Q7TPM1-1	HLA-B-associated transcript 2-like 1
119	Kiaa0564	Q8CC88-1	Uncharacterized protein KIAA0564 homolog
120	Spag5	Q7TME2	Mastrin
121	Grlf1	Q91YM2	Glucocorticoid receptor DNA-binding factor 1
122	Eif4g1	E9PVC6	Eif4g1 protein
123	Kiaa0256	Q6A098	Selenocysteine insertion sequence-binding protein 2-like
124	Srcap	E9Q9V7	Putative uncharacterized protein
125	Sdcbp	O08992	Scaffold protein Pbp1
126	Dlx2	P40764	Homeobox protein DLX-2
127		E9PZ11	
128	Dtd1	Q9DD18-1	D-tyrosyl-tRNA(Tyr) deacylase 1
129	Cpeb4	Q7TN98-1	Cytoplasmic polyadenylation element-binding protein 4
130	H2afv	Q3THW5	H2A.F/Z
131	Nr3c1	E9PYV1	Glucocorticoid receptor
132	Scfd2	Q8BTY8-1	Neuronal Sec1
133	Rrp8	E9PVA2	Putative uncharacterized protein
134	Tens1	Q5SSZ5-1	Tensin-3
135	Aimp2	E9QP67	Aminoacyl tRNA synthase complex-interacting multifunctional protein 2
136		A2AG58-1	Uncharacterized protein CXorf23 homolog

137	Ttf1	Q62187	RNA polymerase I termination factor
138	Tpx2	A2APB8	Targeting protein for Xklp2
139	Hbp	O88811-1	Hrs-binding protein
140	Nup153	E9Q3G8	Putative uncharacterized protein
141	Tjp1	B9EHJ3	Tjp1 protein
142	Baf60a	Q61466-1	60 kDa BRG-1/Brm-associated factor subunit A
143	mCG_17339	Q9WV02	Heterogeneous nuclear ribonucleoprotein G
144	AA536749	Q5SWZ5	Myosin phosphatase Rho interacting protein
145	Ips1	Q8VCF0	CARD adapter inducing interferon β
146	Atg2b	Q80XK6-2	Autophagy-related protein 2 homolog B
147	Zfp384	E9Q1A5	Zfp384 protein
148	Fxr1	Q61584-4	Fragile X mental retardation syndrome-related protein 1
149	Gm1040	E9QPB9	Nucleolar MIF4G domain-containing protein 1
150	Il1rl1	P14719-1	Interleukin-1 receptor-like 1
151	Ptrf	O54724	Cavin-1
152	Fhl3	A6H6N4	Four and a half LIM domains 3
153	Hmgcs1	Q8JZK9	3-hydroxy-3-methylglutaryl coenzyme A synthase
154	ORF18	Q9QY73	Thymic dendritic cell-derived factor 1
155		Q80XC6	UPF0614 protein C14orf102 homolog
156	Acads	Q07417	Butyryl-CoA dehydrogenase
157	Smy	P13675	Y-linked testis-specific protein 1
158	Cbll1	Q9JIY2-1	Casitas B-lineage lymphoma-transforming sequence-like protein 1
159	Iars	Q8BU30	Isoleucine--tRNA ligase
160	Nsa2	Q9CR47	L-name-related protein 42
161	Fgf7	P36363	Fibroblast growth factor 7
162	Farsa	Q8C0C7	Phenylalanine--tRNA ligase α chain
163	Rslcan12	E9Q1L8	Regulator of sex-limitation candidate 12
164	Kiaa0999	Q6P4S6-1	Salt-inducible kinase 3
165	Cdw5	Q8BFQ4	WD repeat-containing protein 82
166	Canx	P35564	Calnexin
167	Uch37	Q9WUP7-1	Ubiquitin carboxyl-terminal hydrolase isozyme L5
168	Rbm4	Q8C7Q4-1	Lark homolog
169	Imp4a	Q8VI75	Importin-4
170	Kiaa2005	Q69Z37	Sterile α motif domain-containing protein 9-like
171	Mmp14	P53690	Matrix metalloproteinase-14
172	Abpl	Q8VHX6-1	ABP-280-like protein
173	Man2a1	B9EHS6	Mannosidase 2, α1
174	Snt2b2	Q61235	59 kDa dystrophin-associated protein A1 basic component 2
175	Map1a	Q9QYR6-2	MAP1 light chain LC2
176	Trip13	Q3UA06	Thyroid hormone receptor interactor 13
177	Utp15	Q8C7V3	Src-associated protein SAW
178	Spty2d1	Q68FG3	Protein SPT2 homolog
179	Otub1	Q7TQI3	Deubiquitinating enzyme OTUB1
180	Ctnnbl1	Q9CWL8	B-catenin-like protein 1
181	Hypk	Q9CR41-1	Huntingtin yeast partner K
182	Wdr62	Q3U3T8-1	WD repeat-containing protein 62
183	Col5a1	O88207	Collagen α-1(V) chain
184	Kif5c	P28738	Kinesin heavy chain isoform 5C

185	Igf2bp2	A6X8Z3	Insulin-like growth factor 2 mRNA binding protein 2
186	Myo1c	Q9WTI7-1	Myosin I β
187	Cycap	Q07797	Cyp-C-associated protein
188	Oasl2	Q9Z2F2	54 kDa 2-5-oligoadenylate synthase-like protein 2
189	Kif3	P28741	Kinesin-like protein KIF3A
190	Gm9026	D3YX54	MCG6146
191	Abp1	Q62418-1	Actin-binding protein 1
192	Tbl2	Q8CFY0	Putative uncharacterized protein
193	Frmd4	Q8BIE6-2	FERM domain-containing protein 4A
194	Whip	Q91XU0-1	ATPase WRNIP1
195	Acsl4	Q5D071	Acyl-CoA synthetase long-chain family member 4
196	Mapkap1	Q8BKH7-1	Mitogen-activated protein kinase 2-associated protein 1
197	Kiaa1040	Q80TL7-1	Protein MON2 homolog
198	Mre11	Q61216-1	Double-strand break repair protein MRE11A
199	Myh14	B3F3T1	Nonmuscle myosin II-C2
200	Capl	P07091	Metastasin
201	Lrrc59	Q922Q8	Leucine-rich repeat-containing protein 59
202	Adnp	Q9Z103	Activity-dependent neuroprotective protein
203	Nte	Q3TRM4-4	Neuropathy target esterase
204	Prr11	Q8BHE0	Proline-rich protein 11
205	Hist1h2ak	Q8CGP7	Histone H2A type 1-K
206	D11Ertd619e	Q9D0R4	ATP-dependent 61 kDa nucleolar RNA helicase
207	Fmnl2	A2APV2-3	Formin-like protein 2
208	Angptl2	Q8BM09	Angiopoietin-like 2
209	Tarsl2	Q8BLY2	Probable threonyl-tRNA synthetase 2, cytoplasmic
210	Isg20l2	Q3U1G5	Interferon-stimulated 20 kDa exonuclease-like 2
211	Mcm6	P97311	DNA replication licensing factor MCM6
212	Paf53	Q8K202-1	DNA-directed RNA polymerase I subunit E
213	Spcs3	Q6ZWQ7	Putative uncharacterized protein
214	Kiaa0991	Q80UG5-3	Septin-9
215	Dsn	Q9R0P5	Actin-depolymerizing factor
216	Eif2s3y	Q9Z0N2	Eukaryotic translation initiation factor 2 subunit 3, Y-linked
217	Ubc12	P61082	NEDD8 carrier protein
218	Asc1p100	Q91WR3-3	Activating signal cointegrator 1 complex subunit 2
219	Iag2	Q9CQY5-3	Implantation-associated protein
220	Sybl1	P70280	Synaptobrevin-like protein 1
221	Dcs	Q8R013	C1-tetrahydrofolate synthase
222	D14Wsu89e	Q8BIK4-2	Cdc42 guanine nucleotide exchange factor zizimin-1
223	D14Ertd453e	Q8BX90	Fibronectin type-III domain-containing protein 3A
224	Sco2	Q8VCL2	Protein SCO2 homolog, mitochondrial
225	9030624J02Rik	D3YW19	Putative uncharacterized protein 9030624J02Rik
226	Rars2	Q3U186	Arginine--tRNA ligase
227	Etl1	Q04692-1	ATP-dependent helicase SMARCAD1
228	Cspg6	E9Q762	Putative uncharacterized protein
229	Gbp	P16045	14 kDa lectin
230	Htf9c	E9QA27	Putative uncharacterized protein

231	Mrps24	Q9CQV5	28S ribosomal protein S24, mitochondrial
232	Kiaa0147	Q80U72-3	Protein LAP4
233	Ap1gbp1	Q5SV85-1	AP1 subunit γ-binding protein 1
234	Gna-rs1	P36916-2	GTP-binding protein MMR1
235	Kiaa0913	Q3UHH1-1	Zinc finger SWIM domain-containing protein KIAA0913
236	Metap1	Q8BP48	Methionine aminopeptidase 1
237	Lrp130	Q6PB66	130 kDa leucine-rich protein
238	Kiaa0156	Q9JLI8-1	Squamous cell carcinoma antigen recognized by T-cells 3
239	mCG_5513	Q148U2	NIMA (Never in mitosis gene a)-related expressed kinase 9
240	Kiaa0090	Q8C7X2-1	Uncharacterized protein KIAA0090
241	Hint	P70349	Adenosine 5-monophosphoramidase
242	Akt1	A4FUQ9	Akt1 protein
243	Prpsap2	Q8R574	41 kDa phosphoribosypyrophosphate synthetase-associated protein
244	Emilin1	Q99K41	Elastin microfibril interface-located protein 1
245	Cand2	Q6ZQ73	Cullin-associated and neddylation-dissociated protein 2
246	Nol6	Q8R5K4-1	Nucleolar protein 6
247	Mat2a	Q3THS6	Methionine adenosyltransferase 2
248	Ngd	Q9DB96	EIF4E-binding protein
249	Atp2a2	O55143-1	Calcium pump 2
250	Cacy	P14069	5B10
251	Zfp593	Q9DB42	Zinc finger protein 593
252	Ncor1	Q60974-1	Nuclear receptor corepressor 1
253	Myh11	O08638-1	Myosin heavy chain 11
254	Tpm2	P58774-2	B-tropomyosin
255	Bat6	Q9Z1Q9	Protein G7a
256	Prkar1a	Q3TYK4	Putative uncharacterized protein
257	Vwc2	Q8C8N3	Brain-specific chordin-like protein
258	Serpinb6a	E9QP98	Putative uncharacterized protein
259	Ift140	B2RSU5	Intraflagellar transport 140 homolog (Chlamydomonas)
260	Hmga1l4	Q99PT3	High mobility group AT-hook 1-like 4
261	Psmd11	Q8BG32	26S proteasome non-ATPase regulatory subunit 11
262	Der1	Q99J56	Degradation in endoplasmic reticulum protein 1
263	Etf1	Q8BWY3	Eukaryotic peptide chain release factor subunit 1
264	Grb10	Q60760-1	GRB10 adapter protein
265	Ars2	Q99MR6-1	Arsenite-resistance protein 2
266	Ccdc61	Q3UJV1	Coiled-coil domain-containing protein 61
267	Rab5ep	O35551-1	Rab GTPase-binding effector protein 1
268	Sh2b1	Q91ZM2-1	Pro-rich, PH and SH2 domain-containing signaling mediator
269	Baf60c	Q6P9Z1	60 kDa BRG-1/Brm-associated factor subunit C
270	mCG_2696	Q7TNL5	Protein phosphatase 2, regulatory subunit B (B56), delta isoform, isoform CRA_a
271	Glut1	P17809	Glucose transporter type 1, erythrocyte/brain
272	Dlg7	Q8K4R9-1	Discs large homolog 7
273	Ric8	Q3TIR3	Protein Ric-8A
274	Acadsb	Q9DBL1	2-methyl branched chain acyl-CoA dehydrogenase
275	Ugcgl2	E9QAH9	Ugcgl2 protein
276	Gapr1	Q9CYL5	Glioma pathogenesis-related protein 2
277	Herc2	Q4U2R1-2	HECT domain and RCC1-like domain-containing protein 2
278	Cyp11a	Q9QZ82	Cholesterol desmolase

279	Ankrd25	Q8BX02-1	Ankyrin repeat domain-containing protein 25
280	Zfp668	Q8K2R5	Zinc finger protein 668
281	Myh6	Q02566	Myosin heavy chain 6
282	Pop4	Q9CR08	Ribonuclease P protein subunit p29
283	Rnasep2	O88796	Ribonuclease P protein subunit p30
284	Ndufs3	Q9DCT2	Complex I-30kD
285	cbp37	O88380	Cbp37
286	Cdk4	P30285	Cell division protein kinase 4
287	Brms1l	Q3U1T3-1	Breast cancer metastasis-suppressor 1-like protein
288	Solo	Q3UPH7-1	Protein SOLO
289	Cdc37	Q61081	Hsp90 chaperone protein kinase-targeting subunit
290	Ccdc131	B2RT41	Zinc finger, C3H1-type containing
291	Copg2	Q9QXK3-1	Coatomer subunit γ-2
292	Adrm1	Q9JKV1	110 kDa cell membrane glycoprotein
293	Gcat	O88986	2-amino-3-ketobutyrate coenzyme A ligase, mitochondrial
294	Calm	Q7M6Y3-1	Clathrin assembly lymphoid myeloid leukemia
295	Pkp1	P97350	Plakophilin-1
296	Itm1	Q8BMP9	Putative uncharacterized protein
297	Odr4	Q4PJX1-1	Protein odr-4 homolog
298	Mrpl16	Q99N93	39S ribosomal protein L16, mitochondrial
299	Vat1	Q62465	Synaptic vesicle membrane protein VAT-1 homolog
300	MNCb-3848	Q9DCU6	39S ribosomal protein L4, mitochondrial
301	Nipbl	Q6KCD5-1	Delangin homolog
302	Rplp1	P47955	60S acidic ribosomal protein P1
303	RP23-396N8.6-001	Q8BPK2	Putative uncharacterized protein
304	H1f1	P43275	H1 VAR.3
305	Fam195b	Q3UGS4	Protein FAM195B
306	Lrrc40	Q9CRC8	Leucine-rich repeat-containing protein 40
307	Capri	Q6PFQ7-1	Calcium-promoted Ras inactivator
308	Celf2	Q9Z0H4-6	Bruno-like protein 3
309	Cdc47	Q61881	CDC47 homolog
310	Phb	P67778	B-cell receptor-associated protein 32
311	Polr2b	Q8CFI7	DNA-directed RNA polymerase II 140 kDa polypeptide
312	Mrps6	P58064	28S ribosomal protein S6, mitochondrial
313	Garg16	Q64282	Glucocorticoid-attenuated response gene 16 protein
314	Pgd	Q9DCD0	6-phosphogluconate dehydrogenase, decarboxylating
315	Dennd2b	Q924W7-1	DENN domain-containing protein 2B
316	RP23-291K6.1-001	B1ATW4	Slit homolog 3 (Drosophila)
317	Trrap	E9PWT1	Tra1 homolog
318	Twistnb	Q78WZ7	DNA-directed RNA polymerase I subunit RPA43
319	Kiaa1151	Q8CH77-1	Neuron navigator 1
320	Abra1	Q8BPZ8-1	BRCA1-A complex subunit Abraxas
321	Pdha1	P35486	PDHE1-A type I
322	Kbtbd2	Q6ZPP6	MKIAA1489 protein
323	H2-L	P01897	H-2 class I histocompatibility antigen, L-D α chain
324	Tbl1	Q9QXE7	F-box-like/WD repeat-containing protein TBL1X

325	Iqcb1	Q8BP00-1	IQ calmodulin-binding motif-containing protein 1
326	Kiaa1341	Q8C854-4	Myelin expression factor 2
327	Wdr1	Q80ZI9	WD repeat domain 1
328	Kiaa2010	E9Q481	Serine/threonine-protein phosphatase 4 regulatory subunit 3A
329	Hdac2	E9PXS3	Putative uncharacterized protein
330	mKIAA0177	E9PYK3	MKIAA0177 protein
331	Ran	P62827	GTPase Ran
332	Kifc1	Q9QWT9-1	Kinesin-like protein KIFC1
333	Lrrfip1	Q8BLV4	Putative uncharacterized protein
334	Iqgap3	B9EKT6	IQ motif containing GTPase activating protein 3
335	Kpnb2	Q8BFY9-1	Importin β-2
336	Copa	E9Q075	Coatomer protein complex subunit α, isoform CRA_b
337	Ighmbp2	D3Z5P5	Putative uncharacterized protein Ighmbp2
338	Atl3	Q91YH5-1	Atlastin-3
339	Anapc5	Q8BTZ4-1	Anaphase-promoting complex subunit 5
340	Lrrk2	E9QNJ2	Putative uncharacterized protein
341	Bp75	O88665	75 kDa bromodomain protein
342	Ccdc93	Q7TQK5	Coiled-coil domain-containing protein 93
343	Eno2	P17183	2-phospho-D-glycerate hydro-lyase
344	Kiaa1043	Q80XJ3	Tetratricopeptide repeat protein 28
345	Sec24b	Q3UDH4	Putative uncharacterized protein
346	Aif	Q9Z0X1	Apoptosis-inducing factor 1, mitochondrial
347	Eps8	Q3TM41	Putative uncharacterized protein
348	Oxct	Q9D0K2	3-oxoacid-CoA transferase 1
349	Rere	A2A7T4	Arginine glutamic acid dipeptide (RE) repeats
350	Ruk	Q8R550-1	Regulator of ubiquitous kinase
351	H1f3	P43277	H1 VAR.4
352	Ddx49	Q4FZF3	DEAD box protein 49
353	Epb4.1l2	B6ZHD0	Erythrocyte protein band 4.1-like 2
354	Tmem57	Q7TQE6	Brain-specific adaptor protein C61
355	Kiaa0430	Q8BJ34-5	Limkain-b1
356	Fads2	Q9Z0R9	Delta(6) fatty acid desaturase
357	Crsp8	Q9DB40-1	Cofactor required for Sp1 transcriptional activation subunit 8
358	Nop16	D3Z7M5	Putative uncharacterized protein Nop16
359	Carmil	Q6EDY6-2	CARMIL homolog
360	Maged1	Q9QYH6	Dlxin-1
361	Fen1	E9PYV9	Putative uncharacterized protein
362	Ppil2	Q9D787	Cyclophilin-60
363	Nup88	Q8CEC0-1	88 kDa nucleoporin
364	Hspbp	Q99P31	Heat shock protein-binding protein 1
365	Arhgef17	Q80U35-1	Rho guanine nucleotide exchange factor 17
366	Cdc42bpa	B2RXX8	Cdc42bpa protein
367	Tada1	Q99LM9	Transcriptional adapter 1
368	Eif2b1	Q99LC8	eIF-2B GDP-GTP exchange factor subunit α
369	Mess1	Q9JI10-1	Mammalian STE20-like protein kinase 2
370	Dip2	Q8BWT5	Disco-interacting protein 2 homolog A
371	Camsap1	A2AHC3-1	Calmodulin-regulated spectrin-associated protein 1
372	Klhl7	Q8BUL5	Kelch-like protein 7

373	Kiaa1230	Q8C8U0-3	Liprin-β-1
374	Prbp	P97473	Protamine-1 RNA-binding protein
375	Mrps21	P58059	28S ribosomal protein S21, mitochondrial
376	Lcb1	O35704	Long chain base biosynthesis protein 1
377	Tpi	E0CXH5	Triosephosphate isomerase
378	Emp2	O88662	Epithelial membrane protein 2
379	Supt3h	Q8BU36	Putative uncharacterized protein
380	Mrps35	Q8BJZ4	28S ribosomal protein S35, mitochondrial
381	Acadl	P51174	Long-chain specific acyl-CoA dehydrogenase, mitochondrial
382	Rabex5	Q9JM13	Rab5 GDP/GTP exchange factor
383	Pip4k2b	Q80XI4	1-phosphatidylinositol-5-phosphate 4-kinase 2-β
384	mCG_19062	Q9JMD0	Putative uncharacterized protein
385	Atp1a1	Q8VDN2	Sodium pump subunit α-1
386	Gls	D3Z7P3	Putative uncharacterized protein Gls
387	Apaf1	O88879-1	Apoptotic protease-activating factor 1
388	Tbl3	Q8C4J7	Transducin β-like protein 3
389	Parf	Q5U3K5	Putative GTP-binding protein Parf
390	Dncli2	Q6PDL0	Cytoplasmic dynein 1 light intermediate chain 2
391	Eno3	P21550	2-phospho-D-glycerate hydro-lyase
392	1110002N22 Rik	Q5SSK3	UPF0629 protein C17orf42 homolog
393	mCG_6742	A2AWN8	YTH domain family 1
394	Svil	Q8K4L3	Archvillin
395	Npm3	Q9CPP0	Nucleoplasmin-3
396	Plec1	Q9QXS1-3	Plectin-1
397	Lgals3	P16110	35 kDa lectin
398	Ifrd2	Q7TSB3	IRFD2
399	Surf6	P70279	Surfeit locus protein 6
400	Alkbh6	Q8K2U2	Alkylated DNA repair protein alkB homolog 6
401	Cttn	Q3UGC2	Cortactin, isoform CRA_e
402	Gaa	P70699	Acid maltase
403	Acot7	Q91V12-1	Acyl-CoA thioesterase 7
404	Eck	Q03145	Ephrin type-A receptor 2
405	Stat6	P70383	STAT6
406	Efemp1	Q8BPB5	EGF-containing fibulin-like extracellular matrix protein 1
407	Mrpl47	Q8K2Y7	39S ribosomal protein L47, mitochondrial
408	Hba	P01942	A-globin
409	Mrnp41	Q8C570	mRNA export factor
410	Spata5l1	Q3TER3	Putative uncharacterized protein
411	Sbx	Q02053	Ubiquitin-activating enzyme E1
412	Fhad1	A6PWD2-1	Forkhead-associated domain-containing protein 1
413	Akap8l	Q9R0L7	A-kinase anchor protein 8-like
414	Ard1	Q3V4D5	N-acetyltransferase ARD1 homolog
415	Fsc2	P48774	Fibrous sheath component 2
416	Ahdc1	Q6PAL7	AT-hook DNA-binding motif-containing protein 1
417	Dpy19l1	A6X919-1	Dpy-19-like protein 1
418	Ifi204	E9QLE0	Interferon-activable protein 204
419	Scyl1	Q9EQC5	105 kDa kinase-like protein
420	Vps33a	Q9D2N9	Vacuolar protein sorting-associated protein 33A

421	Uba52	E9QNP0	60S ribosomal protein L40
422	Ecm29	Q6PDI5-1	Proteasome-associated protein ECM29 homolog
423	Aldh18a1	Q3TWN8	Putative uncharacterized protein
424	Kiaa0440	Q8C0T5-1	Signal-induced proliferation-associated 1-like protein 1
425	Spnb3	O35411	B III spectrin
426	Rbm14/Rbm 4 fusion	B0LM42	Transcriptional coactivator CoAZ
427	Clk3	O35492	CDC-like kinase 3
428	Fhl2	O70433	Four and a half LIM domains protein 2
429	Taf4b	Q8C0S6	Putative uncharacterized protein
430	Kiaa1741	P58871	182 kDa tankyrase-1-binding protein
431	Zfp36l2	B9EIF6	Zinc finger protein 36, C3H type-like 2
432	Ppil4	Q9CXG3	Cyclophilin-like protein PPIL4
433	Crsp6	Q8VCD5	Cofactor required for Sp1 transcriptional activation subunit 6
434	RP23-307E15.7-001	A2AT37	UPF2 regulator of nonsense transcripts homolog (Yeast)

Table 23: Preferentially importin α7 binding partners from fibroblast cells.

Importin α7 binding partners identified from fibroblast cells by co-immunoprecipitation were ranked according to their fold change and the top 5 % of importin α7 binding partners found to bind also to the GST-control were included as well. Importin α2 and – α3 binding partners identified by co-immunoprecipitation were arranged in the same way and excluded from the list of importin α7 binding partners. Proteins are ranked by name

	Gene.Names	Protein Descriptions	Uniprot
1		Protein KIAA1967 homolog	Q8VDP4
2		Histone H3	E9PV77
3		Mini-chromosome maintenance complex-binding protein	Q8R3C0
4		14 kDa protein	
5	Abh5	Probable α-ketoglutarate-dependent dioxygenase ABH5	Q3TSG4
6	Abra1	Isoform 1 of BRCA1-A complex subunit Abraxas	Q8BPZ8-1
7	Adnp	activity-dependent neuroprotector homeobox protein	Q9Z103
8	Adprp	Isoform 1 of Poly [ADP-ribose] polymerase 1	P11103-1
9	AF013969	biorientation of chromosomes in cell division 1-like	E9Q6J5
10	Ahnak	AHNAK nucleoprotein isoform 1	A0JLR7
11	Aimp1	aminoacyl tRNA synthase complex-interacting multifunctional protein 1	P31230
12	Alg4	Protein RRP5 homolog	Q6NS46
13	Anp32b	Isoform 2 of Acidic leucine-rich nuclear phosphoprotein 32 family member B	Q9EST5-2
14	Anp32e	Isoform 1 of Acidic leucine-rich nuclear phosphoprotein 32 family member E	P97822-1
15	Aprin	Uncharacterized protein	Q4VA53-3
16	Arg1	Arginase-1	Q61176
17	Arid1a	Uncharacterized protein	A2BH40-1
18	Arid1b	AT rich interactive domain 1B	E9Q4N7
19	Arid2	AT rich interactive domain 2	E9Q7E2
20	Armc8	Isoform 1 of Armadillo repeat-containing protein 8	Q9DBR3-1
21	Ase1	DNA-directed RNA polymerase I subunit RPA34	Q76KJ5
22	Ash2l	Set1/Ash2 histone methyltransferase complex subunit ASH2	Q91X20
23	Atf7	Activating transcription factor 7, isoform CRA_a	Q3TZR9
24	Atxn2l	Putative uncharacterized protein	Q3TGG2
25	Baf155	Isoform 1 of SWI/SNF complex subunit SMARCC1	P97496-1
26	Baf170	Isoform 1 of SWI/SNF complex subunit SMARCC2	Q6PDG5-1
27	Baf180	protein polybromo-1	Q8BSQ9-1
28	Baf190a	transcription activator BRG1 isoform 1	Q3TKT4-2
29	Baf47	Isoform A of SWI/SNF-related matrix-associated actin-dependent regulator of chromatin subfamily B member 1	Q9Z0H3-1
30	Baf57	SWI/SNF-related matrix-associated actin-dependent regulator chromatin subfamily E member 1	O54941
31	Baf60a	Isoform 2 of SWI/SNF-related matrix-associated actin-dependent regulator of chromatin subfamily D member 1	Q61466-1
32	Baf60b	Isoform 1 of SWI/SNF-related matrix-associated actin-dependent regulator of chromatin subfamily D member 2	Q99JR8-1
33	Baf60c	SWI/SNF-related matrix-associated actin-dependent regulator of chromatin subfamily D member 3	Q6P9Z1

34	Bat6	Valyl-tRNA synthetase	Q9Z1Q9
35	BC010250	SET domain containing 1A	E9PYH6
36	Bclaf1	Isoform 1 of Bcl-2-associated transcription factor 1	Q8K019-1
37	Bend3	BEN domain-containing protein 3	Q6PAL0
38	Bp75	Bromodomain-containing protein 7	O88665
39	Bptf	Bromodomain PHD finger transcription factor	A2A654
40	Brcc3	Isoform 1 of Lys-63-specific deubiquitinase BRCC36	P46737-1
41	C130039O16 Rik	hypothetical protein LOC238317	E9Q2I4
42	Cacybp	Calcyclin-binding protein	Q9CXW3
43	Carf	CDKN2A-interacting protein	Q8BI72
44	Cbfb	Isoform 2 of Core-binding factor subunit β	Q08024-2
45	Cbll1	Isoform 1 of E3 ubiquitin-protein ligase Hakai	Q9JIY2-1
46	Cbp	Isoform 2 of Poly(rC)-binding protein 2	Q61990-2
47	Ccdc55	Coiled-coil domain-containing protein 55	Q5NCR9
48	Ccnk	cyclin-K	Q3U3M5
49	Cdkn2aipnl	CDKN2AIP N-terminal-like protein	Q9D211
50	Celf2	Isoform 6 of CUGBP Elav-like family member 2	Q9Z0H4-6
51	Chd3	Chromodomain helicase DNA binding protein 3	B1AR17
52	Chd4	Uncharacterized protein	E9QAS5
53	Chd5	chromodomain helicase DNA binding protein 5 isoform1	A2A8L1
54	Clk	Isoform Long of Dual specificity protein kinase CLK1	P22518-1
55	Coil	coilin	E9Q284
56	Cpsf4	Uncharacterized protein	E0CXT7
57	Crfg	Nucleolar GTP-binding protein 1	Q99ME9
58	Crsp3	mediator of RNA polymerase II transcription subunit 23 isoform 1	Q80YQ2-2
59	Csnk1d	Isoform 1 of Casein kinase I isoform delta	Q9DC28-1
60	Cspg6	Uncharacterized protein	E9Q762
61	Ctcf	Transcriptional repressor CTCF	Q61164
62	Ctnnbl1	B-catenin-like protein 1	Q9CWL8
63	Cul1	Cullin-1	Q9WTX6
64	Cwc15	Protein CWC15 homolog	Q9JHS9
65	Cyp33	Peptidyl-prolyl cis-trans isomerase E	Q9QZH3
66	D10Wsu102e	Uncharacterized protein C12orf45 homolog	Q9CX66
67	D14Ucla2	Dehydrogenase/reductase SDR family member 4	Q99LB2
68	D19Bwg1357 e	D19Bwg1357e protein	Q8BKS9
69	D3Ertd250e	Isoform 1 of Outer dense fiber protein 2-like	Q9D478-1
70	D3Ertd300e	Isoform 2 of Protein FAM48A	Q7TT00-2
71	D4Cole1e	Nicotinamide mononucleotide adenylyltransferase 1	Q9EPA7
72	Dars	Aspartyl-tRNA synthetase, cytoplasmic	Q922B2
73	Ddx21	nucleolar RNA helicase 2	Q3TVJ3
74	Dek	Protein DEK	Q7TNV0
75	Dhm1	Isoform 1 of 5-3 exoribonuclease 2	Q9DBR1-1
76	Dhx16	DEAH (Asp-Glu-Ala-His) box polypeptide 16	Q80TX4
77	Dhx33	Putative ATP-dependent RNA helicase DHX33	Q80VY9
78	Dhx8	ATP-dependent RNA helicase DHX8	A2A4P0
79	Dinb1	Isoform 1 of DNA polymerase kappa	Q9QUG2-1
80	Dnajc9	DnaJ homolog subfamily C member 9	Q91WN1

81	Dpf2	Uncharacterized protein	D3Z5N6
82	Dsg1b	Desmoglein-1-β	Q7TSF1
83	Dsp	desmoplakin	E9Q557
84	Dtd1	Isoform 1 of D-tyrosyl-tRNA(Tyr) deacylase 1	Q9DD18-1
85	Edd	E3 ubiquitin-protein ligase UBR5 isoform 1	Q80TP3
86	Edd1	E3 ubiquitin-protein ligase UBR5 isoform 2	Q3TSI5
87	Eef1b	Elongation factor 1-β	O70251
88	Efr3a	Uncharacterized protein	E9QLX7
89	Eif2s3x	Eukaryotic translation initiation factor 2 subunit 3, X-linked	Q9Z0N1
90	Eif5b	Eukaryotic translation initiation factor 5B	Q05D44
91	Ell	RNA polymerase II elongation factor ELL	O08856
92	Emp2	Epithelial membrane protein 2	O88662
93	Eprs	bifunctional aminoacyl-tRNA synthetase	B9EIU1
94	Evi3	Uncharacterized protein	E9PWM6
95	Fam103a1	Protein FAM103A1	Q9CQY2
96	Fam32a	13 kDa protein	Q9CR80
97	Farsa	Phenylalanyl-tRNA synthetase α chain	Q8C0C7
98	Fbl	rRNA 2-O-methyltransferase fibrillarin	P35550
99	Fbll1	rRNA/tRNA 2-O-methyltransferase fibrillarin-like protein 1	Q80WS3
100	Fbp21	WW domain-binding protein 4	Q61048
101	Fbxo11	Uncharacterized protein	Q7TPD1-1
102	G22p1	X-ray repair cross-complementing protein 6	P23475
103	G7b	U6 snRNA-associated Sm-like protein LSm2	O35900
104	Garg16	Interferon-induced protein with tetratricopeptide repeats 1	Q64282
105	Gas1	growth arrest-specific protein 1	Q01721
106	Gatad2a	transcriptional repressor p66 α isoform a	E9QMN5
107	Gatad2b	Isoform 1 of Transcriptional repressor p66-β	Q8VHR5-1
108	Glut1	Solute carrier family 2, facilitated glucose transporter member 1	P17809
109	Gm1878	nascent polypeptide-associated complex subunit α isoform a	E9QMB1
110	Gnb1	Guanine nucleotide-binding protein G(I)/G(S)/G(T) subunit β-1	P62874
111	Gnl3	Isoform 1 of Guanine nucleotide-binding protein-like 3	Q8CI11-1
112	Gtf2a1	Transcription initiation factor IIA subunit 1	Q99PM3
113	Gtf2h3	General transcription factor IIH subunit 3	Q8VD76
114	Gtf2h4	General transcription factor IIH subunit 4	O70422
115	H1fx	H1 histone family, member X	Q80ZM5
116	Hcfc1	Host cell factor C1	Q61191
117	Hdac1	Histone deacetylase 1	O09106
118	Hdac2	Histone deacetylase 2	P70288
119	Hdac2	Uncharacterized protein	E9PXS3
120	Hist3h2a	Histone H2A type 3	Q8BFU2
121	Hmga1l4	INO80 complex subunit B	Q99PT3
122	Hnrnpc	Isoform 4 of Heterogeneous nuclear ribonucleoproteins C1/C2	Q9Z204-4
123	Hnrnpk	Uncharacterized protein	P61979-3
124	Hnrnpl	Heterogeneous nuclear ribonucleoprotein L	Q8R081
125	Hnrnpu	Heterogeneous nuclear ribonucleoprotein U	Q8VEK3
126	Hox-1.3	Homeobox protein Hox-A5	P09021
127	Hrmt1l2	Isoform 1 of Protein arginine N-methyltransferase 1	Q9JIF0-1
128	Ifi204	Uncharacterized protein	E9QLE0

129	Ino80d	INO80 complex subunit D isoform 1	Q66JY2-1
130	Khsrp	far upstream element-binding protein 2	E9QKH3
131	Kiaa0007	WD repeat-containing protein 43	Q6ZQL4
132	Kiaa0398	Isoform 1 of mRNA cap guanine-N7 methyltransferase	Q9D0L8-1
133	Kiaa0425	Isoform 1 of Zinc finger MYM-type protein 4	A2A791-1
134	Kiaa1034	Isoform 1 of DNA-binding protein SATB2	Q8VI24-1
135	Kiaa1113	Isoform A of E3 ubiquitin-protein ligase TRIM33	Q99PP7-1
136	Kiaa1265	Zinc transporter ZIP10	Q6P5F6
137	Kiaa1582	trinucleotide repeat-containing gene 6C protein	Q3UHC0
138	Kiaa1839	Isoform 1 of RNA-binding protein 40	Q3UZ01-1
139	Kiaa4103	Isoform 1 of Structural maintenance of chromosomes protein 6	Q924W5-1
140	Kif23	kinesin family member 23	E9Q5G3
141	Klhl7	Kelch-like protein 7	Q8BUL5
143	Kpna1	Importin subunit α-1	Q60960
144	Kpna5	Importin subunit α-7	O35345
145	Ktn1	Uncharacterized protein	E9QJY4
146	Lmn1	Isoform A of Prelamin-A/C	P48678-1
147	Lmnb1	Lamin-B1	P14733
148	Lmnb2	Isoform B2 of Lamin-B2	P21619-1
149	Lsm11	U7 snRNA-associated Sm-like protein LSm11	Q8BUV6
150	Lsm3	U6 snRNA-associated Sm-like protein LSm3	P62311
151	Lyar	Cell growth-regulating nucleolar protein	Q08288
152	Maged1	Melanoma-associated antigen D1	Q9QYH6
153	Mars	methionyl-tRNA synthetase, cytoplasmic isoform 1	E9QB02
154	Mbd2	methyl-CpG-binding domain protein 2	E9QMV9
155	Mbd3	Isoform 1 of Methyl-CpG-binding domain protein 3	Q9Z2D8-1
156	mCG_13583	Nucleoporin 50	Q6P1F4
157	mCG_14998	DNA replication licensing factor MCM5	Q52KC3
158	mCG_21006	DNA-directed RNA polymerase I subunit RPA2	Q3UMX7
159	mCG_21074	Protein C receptor, endothelial, isoform CRA_b	Q8K357
160	mCG_49644	transcriptional adaptor 2B	D3Z4Z0
161	mCG_53108	RNA binding motif 31, Y-linked	Q9D3U4
162	mCG_5485	Transcription elongation factor B (SIII), polypeptide 3	Q3UI38
163	mCG_67924	suppressor of SWI4 1 homolog	Q8BYM4
164	Mcm3	DNA replication licensing factor MCM3	P25206
165	Med11	Mediator of RNA polymerase II transcription subunit 11	Q9D8C6
166	Med6	Mediator of RNA polymerase II transcription subunit 6	Q921D4
167	Metap1	Methionine aminopeptidase 1	Q8BP48
168	Mgcracgap	Rac GTPase-activating protein 1	Q9WVM1
169	mKIAA0035	nucleolar and coiled-body phosphoprotein 1 isoform C	E9Q5C9
170	Mkrn2	Probable E3 ubiquitin-protein ligase makorin-2	Q9ERV1
171	Mnar	Proline-, glutamic acid- and leucine-rich protein 1	Q9DBD5
172	MNCb-1192	Isoform 1 of Nucleolar protein 7	Q9D7Z3-1
173	Mocs3	Adenylyltransferase and sulfurtransferase MOCS3	A2BDX3
174	Mta1	Metastasis-associated protein MTA1	Q8K4B0
175	Mta1l1	Metastasis-associated protein MTA2	Q9R190
176	Mta3	Isoform 1 of Metastasis-associated protein MTA3	Q924K8-1
177	Myo1c	Isoform 3 of Myosin-Ic	Q9WTI7-3

178	Myof	Myof protein	B9EK95
179	Nfic	Isoform 1 of Nuclear factor 1 C-type	P70255-1
180	Nfx1	Isoform 1 of Transcriptional repressor NF-X1	B1AY10-1
181	Nhp2l1	Uncharacterized protein	E9PWL3
182	Nipbl	Isoform 1 of Nipped-B-like protein	Q6KCD5-1
183	Nkrf	NF-kappa-B-repressing factor	A2A3V7
184	Nol5	Nucleolar protein 58	Q6DFW4
185	Nol5a	Nucleolar protein 56	Q9D6Z1
186	Nol6	Isoform 1 of Nucleolar protein 6	Q8R5K4-1
187	Nol9	Isoform 1 of Polynucleotide 5-hydroxyl-kinase NOL9	Q3TZX8-1
188	Npm3	Nucleoplasmin-3	Q9CPP0
189	Nup121	Nuclear envelope pore membrane protein POM 121	Q8K3Z9
190	Nvl	Nuclear valosin-containing protein-like	Q9DBY8
191	Ott	Ott protein (Fragment)	Q62013
192	P4ha1	Putative uncharacterized protein	Q60715-1
193	Pabpc4	Poly A binding protein, cytoplasmic 4	A3KFU5
194	Paf53	Isoform 1 of DNA-directed RNA polymerase I subunit RPA49	Q8K202-1
195	Paf65a	TAF6-like RNA polymerase II p300/CBP-associated factor-associated factor 65 kDa subunit 6L isoform 1	Q8R2K4-1
196	Paf65b	TAF5-like RNA polymerase II p300/CBP-associated factor-associated factor 65 kDa subunit 5L	Q91WQ5
197	Paip1	Protein	Q8VE62
198	Pcif1	Speckle-type POZ protein	Q6ZWS8
199	Pno1	RNA-binding protein PNO1	Q9CPS7
200	Pnpla7	Isoform 1 of Patatin-like phospholipase domain-containing protein 7	A2AJ88-1
201	Polr1a	DNA-directed RNA polymerase I subunit RPA1	O35134
202	Polr1d	Polymerase (RNA) I polypeptide D	Q8R1M3
203	Polr2e	DNA-directed RNA polymerases I, II, and III subunit RPABC1	Q80UW8
204	Polr2h	DNA-directed RNA polymerases I, II, and III subunit RPABC3	Q923G2
205	Ppig	Peptidyl-prolyl cis-trans isomerase G	A2AR02
206	Ppih	peptidyl-prolyl cis-trans isomerase H-like	Q9D868-1
207	Ppil1	Peptidyl-prolyl cis-trans isomerase-like 1	Q9D0W5
208	Ppp1cc	Isoform Γ-2 of Serine/threonine-protein phosphatase PP1-γ catalytic subunit	P63087-2
209	Prnp	Major prion protein	P04925
210	Prph	Isoform 3u of Peripherin	P15331-2
211	Ptrf	Polymerase I and transcript release factor	O54724
212	Qk	Isoform 7 of Protein quaking	Q9QYS9-7
213	Rap80	Isoform 1 of BRCA1-A complex subunit RAP80	Q5U5Q9-1
214	Rbap46	Histone-binding protein RBBP7	Q60973
215	Rbap48	Uncharacterized protein	Q60972
216	Rbbp5	Isoform 1 of Retinoblastoma-binding protein 5	Q8BX09-1
217	Rbm	Uncharacterized protein	E9Q2F9
218	Recql4	Uncharacterized protein	E9QMJ2
219	Rmi1	RecQ-mediated genome instability protein 1	Q9D4G9
220	Rnu3ip2	U3 small nucleolar RNA-interacting protein 2	Q91WM3
221	RP23-158L6.2-002	synaptosomal-associated protein 23 isoform a	Q9D3L3
222	RP23-	Novel protein similar to ovary testis transcribed	B1AZM2

	18D18.4-001		
223	Rpa12	DNA-directed RNA polymerase I subunit RPA12	Q791N7
224	Rpl39	60S ribosomal protein L39-like	P62892
225	Rpl3l	ribosomal protein L3-like isoform 1	E9PWZ3
226	Rrp7a	Ribosomal RNA-processing protein 7 homolog A	Q9D1C9
227	Samd4	Isoform 1 of Protein Smaug homolog 1	Q8CBY1-1
228	Sec13	Protein SEC13 homolog	Q9D1M0
229	Sec61b	Uncharacterized protein	E9PW43
230	Serbp1	plasminogen activator inhibitor 1 RNA-binding protein isoform 2	Q3UMP4
231	Sf3b10	Splicing factor 3B subunit 5	Q923D4
232	Smarca5	SWI/SNF-related matrix-associated actin-dependent regulator of chromatin subfamily A member 5	Q91ZW3
233	Smc1a	structural maintenance of chromosomes protein 1A	A0JLM6
234	Smy	y-linked testis-specific protein 1-like	P13675
235	Snrpd1	Small nuclear ribonucleoprotein Sm D1	P62315
236	Sp3	Isoform 1 of Transcription factor Sp3	O70494-1
237	Spty2d1	Protein SPT2 homolog	Q68FG3
238	Srp14	Signal recognition particle 14 kDa protein	P16254
239	Supt3h	Putative uncharacterized protein	Q8BU36
240	Supt7l	STAGA complex 65 subunit γ	Q9CZV5
241	Tada1	Transcriptional adapter 1	Q99LM9
242	Taf2s	Isoform 1 of Transcription elongation regulator 1	Q8CGF7-1
243	Tbp	TATA-box-binding protein	P29037
244	Tcfap4	Transcription factor AP4	Q8BWA4
245	Tex10	Testis-expressed sequence 10 protein	Q3URQ0
246	Tgase3	Protein-glutamine γ-glutamyltransferase E	Q08189
247	Thoc3	THO complex subunit 3	Q8VE80
248	Thrap3	Thyroid hormone receptor-associated protein 3	Q569Z6
249	Top2b	DNA topoisomerase 2-β	Q64511
250	Trrap	Uncharacterized protein	E9PWT1
251	Tu52	Clk2-Scamp3 protein	B2M0S2
252	Vwc2	Brorin	Q8C8N3
253	Wdr18	WD repeat-containing protein 18	Q4VBE8
254	Wdr26	WD repeat-containing protein 26	E9PX83
255	Xpc	DNA repair protein complementing XP-C cells homolog	P51612
256	Ylpm1	YLP motif-containing protein 1	D3YWX2
257	Ythdc2	Probable ATP-dependent RNA helicase YTHDC2	B2RR83
258	Zc3h13	Zinc finger CCCH type containing 13	B9EHN9
259	Zcchc10	Zinc finger CCHC domain-containing protein 10	Q9CX48
260	Zfhx4	zinc finger homeobox protein 4	E9Q5A7
261	Zfp384	Zfp384 protein	E9Q1A5
262	Zfp593	Zinc finger protein 593	Q9DB42

Table 24: Potential importin α3 dependent cargoes associated with abnormal muscle fiber morphology.

536 proteins were analyzed using the ToppFun analysis software from the ToppGene Suite according enrichment of associated mouse phenotypes. Genes were ranked by name.

	Entrez Gene ID	Gene Symbol	Gene Name
1	10939	AFG3L2	AFG3 ATPase family gene 3-like 2 (S. cerevisiae)
2	7204	TRIO	triple functional domain (PTPRF interacting)
3	488	ATP2A2	ATPase, Ca++ transporting, cardiac muscle, slow twitch 2
4	8087	FXR1	fragile X mental retardation, autosomal homolog 1
5	1674	DES	desmin
6	4629	MYH11	myosin, heavy chain 11, smooth muscle
7	4624	MYH6	myosin, heavy chain 6, cardiac muscle, α
8	9531	BAG3	BCL2-associated athanogene 3
9	3066	HDAC2	histone deacetylase 2
10	858	CAV2	caveolin 2
11	57410	SCYL1	SCY1-like 1 (S. cerevisiae)
12	207	AKT1	v-akt murine thymoma viral oncogene homolog 1
13	9611	NCOR1	nuclear receptor corepressor 1
14	2010	EMD	emerin
15	3508	IGHMBP2	immunoglobulin mu binding protein 2
16	2548	GAA	glucosidase, α; acid
17	2318	FLNC	filamin C, γ
18	5339	PLEC	plectin
19	3339	HSPG2	heparan sulfate proteoglycan 2
20	4323	MMP14	matrix metallopeptidase 14 (membrane-inserted)
21	9131	AIFM1	apoptosis-inducing factor, mitochondrion-associated, 1

Table 25: Nuclear proteins less abundant in importin α7-/- MEF nuclei.

Nuclear proteins from wild type (wt) and importin α7-/- (impa7-/-) MEFs were quantitatively examined by SILAC and subsequent mass spectrometry analysis. Identified proteins were ranked according to their nuclear fold change wt/impa7-/- based on peptide intensity. Proteins displayed have at least a fold change of 1.5. With increasing values the protein abundance in importin α7-/- MEF nuclei decreases.

	Majority protein IDs	Gene names	Protein names	fold change wt/impa7-/-
1	Q3USR5	Fbxo2	F-box only protein 2	19.329
2	A2A5T5	Ptgis	Prostacyclin synthase	9.5111
3	Q8BH97	Rcn3	Reticulocalbin-3	9.2692
4	P37804	Tagln	Transgelin	8.4302
5	P97299	Sfrp2	Secreted frizzled-related protein 2	7.8375
6	Q9R1Z8	Sorbs3	Vinexin	7.7692
7	B2RSH3	Cnn1	Calponin-1	7.4879
8	P18872-2	Gnao1	Guanine nucleotide-binding protein G(o) subunit α	6.3906
9	Q6GU68	Islr	Immunoglobulin superfamily containing leucine-rich repeat protein	6.0789
10	Q9CZX0-2	Elp3	Elongator complex protein 3	6.0091
11	P97314	Csrp2	Cysteine and glycine-rich protein 2	5.9924
12	Q60847	Col12a1	Collagen α-1(XII) chain	5.9407
13	Q8BY78	Eya4	Eyes absent homolog 4	5.7879
14	P62737	Acta2	Actin, aortic smooth muscle	5.7523
15	E9PYF4	Lmo7		5.5858
16	A2AEY2	Fhl1	Four and a half LIM domains protein 1	5.5822
17	Q02788	Col6a2	Collagen α-2(VI) chain	5.3543
18	Q61391	Mme	Neprilysin	5.0762
19	Q549C9	Trp53	Cellular tumor antigen p53	5.0016
20	E9PWQ3	Col6a3		4.9912
21	O35646	Capn6	Calpain-6	4.9852
22	A9C491	Cul7	Cullin-7	4.8771
23	F8VPU2	Farp1		4.8764
24	Q04857	Col6a1	Collagen α-1(VI) chain	4.8535
25	Q9ET54-6	Palld	Palladin	4.7539
26	E9Q8G0	Col6a3		4.6753
27	Q9D385	Arl2bp	ADP-ribosylation factor-like protein 2-binding protein	4.6577
28	Q8BKG3	Ptk7	Inactive tyrosine-protein kinase 7	4.5594
29	Q8CFE2		UPF0609 protein C4orf27 homolog	4.4701
30	P10404		MLV-related proviral Env polyprotein	4.3113
31	A6H6K1	Aspn	Asporin	4.2973

32	Q8R326	Pspc1	Paraspeckle component 1	4.2935
33	Q925B0	Pawr	PRKC apoptosis WT1 regulator protein	4.1594
34	O08553	Dpysl2	Dihydropyrimidinase-related protein 2	4.1546
35	Q8VCN5	Cth	Cystathionine γ-lyase	4.1323
36	G5E892	Cspg4	Chondroitin sulfate proteoglycan 4	4.0875
37	Q5SNZ0	Ccdc88a	Girdin	4.0854
38	E9QAH9	Uggt2		4.0467
39	A2AQN4	Acss2	Acetyl-coenzyme A synthetase, cytoplasmic	4.0172
40	B2RTE5	Ccdc102a	Coiled-coil domain-containing protein 102A	3.977
41	Q8R2Y2	Mcam	Cell surface glycoprotein MUC18	3.9592
42	P68033	Actc1	Actin, α cardiac muscle 1	3.789
43	P20152	Vim	Vimentin	3.7693
44	F6PZC9	Sorbs1	Sorbin and SH3 domain-containing protein 1	3.7429
45	Q8R3X6	Gpc6	Glypican-6	3.7162
46	Q3TXU5	Dhps	Deoxyhypusine synthase	3.708
47	O70306	Tbx15	T-box transcription factor TBX15	3.699
48	Q9WTR5	Cdh13	Cadherin-13	3.6716
49	Q9Z0P5	Twf2	Twinfilin-2	3.6334
50	Q8R3B1	Plcd1	1-phosphatidylinositol 4,5-bisphosphate phosphodiesterase delta-1	3.5457
51	Q64364	Cdkn2a	Cyclin-dependent kinase inhibitor 2A, isoform 3	3.504
52	P30681	Hmgb2	High mobility group protein B2	3.4684
53	Q7TPW1	Nexn	Nexilin	3.4459
54	Q9WV34-2	Mpp2	MAGUK p55 subfamily member 2	3.427
55	E9PUM4	Tln2	Talin-2	3.421
56	E9PUW7	Xpo7	Exportin-7	3.401
57	F6W687	Hmgn2	Non-histone chromosomal protein HMG-17	3.3805
58	Q91W92	Cdc42ep1	Cdc42 effector protein 1	3.368
59	A2A5V4	Sh3bp1	SH3 domain-binding protein 1	3.3607
60	Q542S9	Sntb2	B-2-syntrophin	3.3576
61	E9PUJ6	Mycbp2	Probable E3 ubiquitin-protein ligase MYCBP2	3.3422
62	Q8BIA4	Fbxw8	F-box/WD repeat-containing protein 8	3.3419
63	Q3TYK3	Nfix	Nuclear factor 1	3.3185
64	P59108	Cpne2	Copine-2	3.3172
65	Q7TSZ8	Nacc1	Nucleus accumbens-associated protein 1	3.3081
66	Q8CG70	Leprel2	Prolyl 3-hydroxylase 3	3.2742
67	Q8CHX7	Rftn2	Raftlin-2	3.2646
68	P61971	Nutf2	Nuclear transport factor 2	3.2044
69	Q3UJF5	Top3b	DNA topoisomerase	3.1918
70	Q9JLM8	Dclk1	Serine/threonine-protein kinase DCLK1	3.1731
71	E9QM38	Slc12a2	Solute carrier family 12 member 2	3.1647

72	A2APZ7	Prrx2	Paired mesoderm homeobox protein 2	3.1511
73	Q5DTG2	Col3a1	Collagen α-1(III) chain	3.1377
74	Q8BMC4		Pumilio domain-containing protein C14orf21 homolog	3.1292
75	A2AWQ5	Pygb	Phosphorylase	3.129
76	Q9JK38	Gnpnat1	Glucosamine 6-phosphate N-acetyltransferase	3.1076
77	Q9D1G2	Pmvk	Phosphomevalonate kinase	3.1036
78	B2RY04	Dock5	Dedicator of cytokinesis protein 5	3.0875
79	P10287	Cdh3	Cadherin-3	3.0363
80	P48722	Hspa4l	Heat shock 70 kDa protein 4L	3.0129
81	E9Q9C1	Ablim1		3.0012
82	A2A7Z0	Plod1	Procollagen-lysine,2-oxoglutarate 5-dioxygenase 1	2.9888
83	O88322	Nid2	Nidogen-2	2.9758
84	Q545R3	Ndrg1	Protein NDRG1	2.9588
85	Q8C7E7	Stbd1	Starch-binding domain-containing protein 1	2.9529
86	Q64727	Vcl	Vinculin	2.9425
87	Q8VDI9	Alg9	A-1,2-mannosyltransferase ALG9	2.9372
88	Q3U1G5	Isg20l2	Interferon-stimulated 20 kDa exonuclease-like 2	2.9099
89	G3UWA6	Slc3a2	4F2 cell-surface antigen heavy chain	2.8861
90	Q5FWI3	Tmem2	Transmembrane protein 2	2.8781
91	F8WIH8	Bcat1	Branched-chain-amino-acid aminotransferase	2.8071
92	P58771-2	Tpm1	Tropomyosin α-1 chain	2.7766
93	Q8VDP3	Mical1	Protein-methionine sulfoxide oxidase MICAL1	2.7696
94	Q99L04	Dhrs1	Dehydrogenase/reductase SDR family member 1	2.7582
95	Q6P9P6	Kif11	Kinesin-like protein KIF11	2.7381
96	P58242	Smpdl3b	Acid sphingomyelinase-like phosphodiesterase 3b	2.7364
97	Q3UH59	Myh10	Myosin-10	2.7356
98	Q8C167	Prepl	Prolyl endopeptidase-like	2.7131
99	Q3V1M1	Igsf10	Immunoglobulin superfamily member 10	2.7074
100	Q9CQ18	Rnaseh2c	Ribonuclease H2 subunit C	2.6991
101	A2AJA8	Phpt1	14 kDa phosphohistidine phosphatase	2.6953
102	P97863	Nfib	Nuclear factor 1 B-type	2.6786
103	F8WGQ4	Fermt2	Fermitin family homolog 2	2.6537
104	Q3TX08	Trmt1	tRNA (guanine(26)-N(2))-dimethyltransferase	2.6297
105	E0CYH7	Filip1l	Filamin A-interacting protein 1-like	2.6219
106	Q9CSH3	Dis3	Exosome complex exonuclease RRP44	2.6142
107	Q91WG4	Elp2	Elongator complex protein 2	2.5929
108	Q8R2N2	Cirh1a	Cirhin	2.5666
109	Q6WVG3	Kctd12	BTB/POZ domain-containing protein KCTD12	2.563
110	F8WI17	Sh3d19	SH3 domain-containing protein 19	2.5536
111	O08915	Aip	AH receptor-interacting protein	2.5518
112	O35144	Terf2	Telomeric repeat-binding factor 2	2.5509

113	P58774	Tpm2	Tropomyosin β chain	2.5344
114	Q8K4R9	Dlgap5	Disks large-associated protein 5	2.5266
115	Q571J7	Ppp2r2a	Serine/threonine-protein phosphatase 2A 55 kDa regulatory subunit B α isoform	2.5158
116	Q01721	Gas1	Growth arrest-specific protein 1	2.5146
117	Q8VD75	Hip1	Huntingtin-interacting protein 1	2.5095
118	Q6P9Q4	Fhod1	FH1/FH2 domain-containing protein 1	2.5011
119	D3Z6Q3	Foxp1	Forkhead box protein P1	2.4955
120	O54784	Dapk3	Death-associated protein kinase 3	2.482
121	Q9JKB1	Uchl3	Ubiquitin carboxyl-terminal hydrolase isozyme L3	2.4717
122	G5E8A2	Fam65a	Protein FAM65A	2.4702
123	Q80ZV0	Rnaseh2b	Ribonuclease H2 subunit B	2.4671
124	Q3U0K8	Ogfod1	2-oxoglutarate and iron-dependent oxygenase domain-containing protein 1	2.4522
125	E9QP49	Ehbp1l1	EH domain-binding protein 1-like protein 1	2.4483
126	B7ZMT1	Cdk19	Cyclin-dependent kinase 19	2.4441
127	Q543H0	Srm	Spermidine synthase	2.4374
128	O54998	Fkbp7	Peptidyl-prolyl cis-trans isomerase FKBP7	2.4365
129	Q0KL02-4	Trio	Triple functional domain protein	2.4358
130	E9QLZ9	Enah	Protein enabled homolog	2.4313
131	P97352	S100a13	Protein S100-A13	2.3959
132	Q9ESD7-2	Dysf	Dysferlin	2.3941
133	Q8CG71	Leprel1	Prolyl 3-hydroxylase 2	2.3928
134	Q8K0C9	Gmds	GDP-mannose 4,6 dehydratase	2.3921
135	P81069	Gabpb2	GA-binding protein subunit β-2	2.3911
136	Q7TT37	Ikbkap	Elongator complex protein 1	2.3897
137	Q922X9	Prmt7	Protein arginine N-methyltransferase 7	2.3811
138	Q9WVA4	Tagln2	Transgelin-2	2.3722
139	P68181-4	Prkacb	cAMP-dependent protein kinase catalytic subunit β	2.366
140	D3YYU8	Obsl1		2.3467
141	Q61292	Lamb2	Laminin subunit β-2	2.3411
142	Q8R0K9	E2f4	Transcription factor E2F4	2.334
143	G3UW90	Trps1	Zinc finger transcription factor Trps1	2.3318
144	E9PYW4	Rnf168	E3 ubiquitin-protein ligase RNF168	2.3316
145	Q6NZN0	Rbm26	RNA-binding protein 26	2.3271
146	Q2PZL6	Fat4	Protocadherin Fat 4	2.3267
147	B2RS36	Acd	Adrenocortical dysplasia protein	2.3217
148	P19157	Gstp1	Glutathione S-transferase P 1	2.3157
149	Q91Z31-2	Ptbp2	Polypyrimidine tract-binding protein 2	2.3094
150	Q8K297	Glt25d1	Procollagen galactosyltransferase 1	2.3054
151	Q6P5D8	Smchd1	Structural maintenance of chromosomes flexible hinge domain-containing protein 1	2.2978

152	B3RH23	Lmna	Prelamin-A/C	2.2962
153	Q8BX90	Fndc3a	Fibronectin type-III domain-containing protein 3A	2.2948
154	A2ASB6	Ncaph	Condensin complex subunit 2	2.2941
155	Q80UE5	Epb4.1l2		2.2871
156	Q8BK67	Rcc2	Protein RCC2	2.2793
157	Q05816	Fabp5	Fatty acid-binding protein, epidermal	2.2739
158	Q3UFV9	Dnajc3	DnaJ homolog subfamily C member 3	2.273
159	Q9EP52	Twsg1	Twisted gastrulation protein homolog 1	2.2668
160	P56564	Slc1a3	Excitatory amino acid transporter 1	2.2645
161	E9Q450	Tpm1		2.253
162	Q9DCC4	Pycrl	Pyrroline-5-carboxylate reductase 3	2.2472
163	F8WIT2	Anxa6	Annexin	2.2463
164	Q9R1X4	Timeless	Protein timeless homolog	2.2463
165	E9QPB3	Wdhd1	WD repeat and HMG-box DNA-binding protein 1	2.2463
166	Q8BGW1	Fto	A-ketoglutarate-dependent dioxygenase FTO	2.2434
167	E9QL65	Cog3	Conserved oligomeric Golgi complex subunit 3	2.2364
168	Q99LD8	Ddah2	N(G),N(G)-dimethylarginine dimethylaminohydrolase 2	2.2211
169	P70268-2	Pkn1	Serine/threonine-protein kinase N1	2.2163
170	G5E8Y3	Gm5590	Protein FAM32A	2.2051
171	F6TFN2	Lmo7		2.202
172	Q6NXL1	Sec24d		2.2006
173	G3X8Y8	Tlr2	Toll-like receptor	2.1937
174	P54726	Rad23a	UV excision repair protein RAD23 homolog A	2.1899
175	P54763-2	Ephb2	Ephrin type-B receptor 2	2.1858
176	A2AJP1	Rxra	Retinoic acid receptor RXR-α	2.1751
177	Q4FJN9	Fdps	Farnesyl pyrophosphate synthase	2.1709
178	Q5SX75	P4ha2	Prolyl 4-hydroxylase subunit α-2	2.163
179	O35954	Pitpnm1	Membrane-associated phosphatidylinositol transfer protein 1	2.1577
180	Q6PCM2	Ints6	Integrator complex subunit 6	2.1538
181	F8VQ95	Tacc1	Transforming acidic coiled-coil-containing protein 1	2.1527
182	Q8VDP4		DBIRD complex subunit KIAA1967 homolog	2.1526
183	Q8BI72	Cdkn2aip	CDKN2A-interacting protein	2.1525
184	P63028	Tpt1	Translationally-controlled tumor protein	2.1496
185	E9PWG6	Ncapg		2.145
186	Q9WV92-6	Epb41l3	Band 4.1-like protein 3	2.1432
187	Q3U335	Stk24	Serine/threonine-protein kinase 24	2.1424
188	E9Q6R7	Utrn		2.1274
189	H9H9T1	Fam107b		2.1102
190	P53798	Fdft1	Squalene synthase	2.1058
191	Q9ESJ0	Xpo4	Exportin-4	2.1042
192	Q52KC3	Mcm5	DNA replication licensing factor MCM5	2.1035

193	E9QPT2	Ncapd2	Condensin complex subunit 1	2.0993
194	G5E8R4	Ppp6r3	Serine/threonine-protein phosphatase 6 regulatory subunit 3	2.0987
195	F6Q641	Sipa1		2.0985
196	D3YVY9	Dscc1	Sister chromatid cohesion protein DCC1	2.0977
197	Q8JZK9	Hmgcs1	Hydroxymethylglutaryl-CoA synthase, cytoplasmic	2.0962
198	Q08775	Runx2	Runt-related transcription factor 2	2.0899
199	B1AVE8	Sh3kbp1	SH3 domain-containing kinase-binding protein 1	2.0878
200	Q9JHC9-4	Elf2	ETS-related transcription factor Elf-2	2.0875
201	D3Z3N4	Hnrnph3		2.0845
202	P97346	Nxn	Nucleoredoxin	2.0839
203	E9PV22	Lrrc47	Leucine-rich repeat-containing protein 47	2.0774
204	Q3TE40	Rpa2	Replication protein A 32 kDa subunit	2.0753
205	Q9EPC1	Parva	A-parvin	2.0699
206	P10605	Ctsb	Cathepsin B	2.0632
207	Q3U962	Col5a2	Collagen α-2(V) chain	2.0627
208	B2RRH9	Gmps	GMP synthase [glutamine-hydrolyzing]	2.0605
209	Q6IRU2	Tpm4	Tropomyosin α-4 chain	2.0581
210	A2ADA4	Cpsf3l	Integrator complex subunit 11	2.0576
211	Q8K2B0	Leprel4	Synaptonemal complex protein SC65	2.0555
212	Q61210-5	Arhgef1	Rho guanine nucleotide exchange factor 1	2.0524
213	Q5SWN2	Rpa1	Replication protein A 70 kDa DNA-binding subunit	2.0524
214	P80318	Cct3	T-complex protein 1 subunit γ	2.0513
215	Q9CQ71	Rpa3	Replication protein A 14 kDa subunit	2.0495
216	Q9D0D5	Gtf2e1	General transcription factor IIE subunit 1	2.0447
217	Q8BIW1	Prune	Protein prune homolog	2.0442
218	P11983	Tcp1	T-complex protein 1 subunit α	2.031
219	Q99NF7	Ppm1b	Protein phosphatase 1B	2.0309
220	P47857	Pfkm	6-phosphofructokinase, muscle type	2.029
221	Q7TMF2	Eri1	3-5 exoribonuclease 1	2.026
222	Q59IW6	Abi3bp		2.0258
223	Q8BKC5	Ipo5	Importin-5	2.0244
224	Q9CX34	Sugt1	Suppressor of G2 allele of SKP1 homolog	2.0219
225	Q8K212	Pacs1	Phosphofurin acidic cluster sorting protein 1	2.0197
226	P80313	Cct7	T-complex protein 1 subunit eta	2.0175
227	B1AWV1	Palm2	Paralemmin-2	2.0165
228	P97313	Prkdc	DNA-dependent protein kinase catalytic subunit	2.0128
229	P80316	Cct5	T-complex protein 1 subunit epsilon	2.0124
230	E9PVY0	Cdc42bpa	Serine/threonine-protein kinase MRCK α	2.0109
231	P54227	Stmn1	Stathmin	2.0084
232	P80317	Cct6a	T-complex protein 1 subunit zeta	2.0069
233	P51807	Dynlt1	Dynein light chain Tctex-type 1	2.0038

234	Q8CHG7	Rapgef2	Rap guanine nucleotide exchange factor 2	1.9998
235	Q91VH2	Snx9	Sorting nexin-9	1.9995
236	Q9D0R2	Tars	Threonine--tRNA ligase, cytoplasmic	1.997
237	F8WHB0	Efr3a	Protein EFR3 homolog A	1.9963
238	P29595	Nedd8	NEDD8	1.9956
239	G5E896	Edc4	Enhancer of mRNA-decapping protein 4	1.995
240	A6PW84	Lepre1	Prolyl 3-hydroxylase 1	1.9946
241	P25206	Mcm3	DNA replication licensing factor MCM3	1.9935
242	Q8CG47	Smc4	Structural maintenance of chromosomes protein 4	1.9912
243	Q91WN1	Dnajc9	DnaJ homolog subfamily C member 9	1.9879
244	P70677	Casp3	Caspase-3	1.9872
245	Q60715	P4ha1	Prolyl 4-hydroxylase subunit α-1	1.9871
246	P80315	Cct4	T-complex protein 1 subunit delta	1.9779
247	F8WI30	Snx7	Sorting nexin-7	1.9775
248	Q61553	Fscn1	Fascin	1.9756
249	P28352	Apex1	DNA-(apurinic or apyrimidinic site) lyase	1.9746
250	P42932	Cct8	T-complex protein 1 subunit theta	1.9745
251	P36916	Gnl1	Guanine nucleotide-binding protein-like 1	1.9723
252	Q3UHU8	Gtf2i	General transcription factor II-I	1.9699
253	Q923T9	Camk2g	Calcium/calmodulin-dependent protein kinase type II subunit γ	1.9695
254	P21279	Gnaq	Guanine nucleotide-binding protein G(q) subunit α	1.9689
255	Q60875	Arhgef2	Rho guanine nucleotide exchange factor 2	1.9683
256	P80314	Cct2	T-complex protein 1 subunit β	1.9672
257	Q9Z179	Shcbp1	SHC SH2 domain-binding protein 1	1.9666
258	Q6ZQK0	Ncapd3	Condensin-2 complex subunit D3	1.9633
259	P63013-2	Prrx1	Paired mesoderm homeobox protein 1	1.958
260	Q80X98	Dhx38		1.9567
261	P39053-6	Dnm1	Dynamin-1	1.9565
262	Q5SVD0	Fam101b	Protein FAM101B	1.9539
263	P31266	Rbpj	Recombining binding protein suppressor of hairless	1.9536
264	B7ZNM7	41157	Septin-5	1.9516
265	Q3TRG2	B230120H23Rik	Mitogen-activated protein kinase kinase kinase MLT	1.949
266	Q91XI1	Dus3l	tRNA-dihydrouridine(47) synthase [NAD(P)(+)]-like	1.9481
267	Q9D832	Dnajb4	DnaJ homolog subfamily B member 4	1.948
268	P28867-2	Prkcd	Protein kinase C delta type	1.9395
269	E9PZ16	Hspg2	Basement membrane-specific heparan sulfate proteoglycan core protein	1.9369
270	Q8C788	Snx18	Sorting nexin-18	1.9302
271	Q9Z247	Fkbp9	Peptidyl-prolyl cis-trans isomerase FKBP9	1.9282
272	B2RRX2	Ppp3ca	Serine/threonine-protein phosphatase	1.9281
273	P14873	Map1b	Microtubule-associated protein 1B	1.9224

274	Q811S7	Ubp1	Upstream-binding protein 1	1.9211
275	Q61171	Prdx2	Peroxiredoxin-2	1.9179
276	Q8BGQ7	Aars	Alanine--tRNA ligase, cytoplasmic	1.9126
277	P97315	Csrp1	Cysteine and glycine-rich protein 1	1.9067
278	F6YRW4	Kdm2a	Lysine-specific demethylase 2A	1.9056
279	E9Q1T1	Camk2d	Calcium/calmodulin-dependent protein kinase type II subunit delta	1.9024
280	G5E8V9	Arfip1		1.9021
281	Q3ULF2	Uhrf1	E3 ubiquitin-protein ligase UHRF1	1.9015
282	Q3TIV6	Kars	Lysyl-tRNA synthetase	1.9008
283	Q8R3Y8	Irf2bp1	Interferon regulatory factor 2-binding protein 1	1.9004
284	Q9JK30	Orc3	Origin recognition complex subunit 3	1.8952
285	E9Q4K7	Kif13b		1.8855
286	Q8BXK8	Agap1	Arf-GAP with GTPase, ANK repeat and PH domain-containing protein 1	1.8853
287	O88572	Lrp6	Low-density lipoprotein receptor-related protein 6	1.878
288	Q6DFV1	Ncapg2	Condensin-2 complex subunit G2	1.8761
289	Q9CYD3	Crtap	Cartilage-associated protein	1.8753
290	Q01730	Rsu1	Ras suppressor protein 1	1.8738
291	Q63810	Ppp3r1	Calcineurin subunit B type 1	1.8723
292	Q3U379	Gpc1	Glypican-1	1.8707
293	Q3U4T8	Mcm7	DNA replication licensing factor MCM7	1.8707
294	E9PXY8	Usp7	Ubiquitin carboxyl-terminal hydrolase	1.8626
295	Q60848	Hells	Lymphocyte-specific helicase	1.8568
296	P54276	Msh6	DNA mismatch repair protein Msh6	1.8558
297	P11087	Col1a1	Collagen α-1(I) chain	1.8516
298	A6H644	Ppp1r12b	Protein phosphatase 1 regulatory subunit 12B	1.8508
299	Q9QWF0	Chaf1a	Chromatin assembly factor 1 subunit A	1.8506
300	P97310	Mcm2	DNA replication licensing factor MCM2	1.8506
301	Q9JK23	Psmg1	Proteasome assembly chaperone 1	1.8496
302	Q6VN19	Ranbp10	Ran-binding protein 10	1.8485
303	Q8R016	Blmh	Bleomycin hydrolase	1.8478
304	Q9QYJ0	Dnaja2	DnaJ homolog subfamily A member 2	1.8396
305	Q9DC33-2	Hmg20a	High mobility group protein 20A	1.8396
306	Q8BGK6	Slc7a6	Y+L amino acid transporter 2	1.8376
307	Q9D0N7	Chaf1b	Chromatin assembly factor 1 subunit B	1.8363
308	A2AUK5	Epb4.1l1	Band 4.1-like protein 1	1.836
309	P30412	Ppic	Peptidyl-prolyl cis-trans isomerase C	1.8353
310	Q9D7B2	Lims1	LIM and senescent cell antigen-like-containing domain protein 1	1.8302
311	Q9CRC8	Lrrc40	Leucine-rich repeat-containing protein 40	1.8294
312	A0PK78	Ccdc25	Coiled-coil domain-containing protein 25	1.8288

313	P97311	Mcm6	DNA replication licensing factor MCM6	1.8278
314	Q9WUV0	Orc5	Origin recognition complex subunit 5	1.8272
315	Q3TEA8	Hp1bp3	Heterochromatin protein 1-binding protein 3	1.8232
316	Q99LI5	Zfp281		1.8232
317	A2AJ02	Cdc26	Anaphase-promoting complex subunit CDC26	1.8224
318	O35345	Kpna6	Importin subunit α-7	1.8193
319	E9QPK4	Atr	Serine/threonine-protein kinase ATR	1.8183
320	E9QK89	Mdc1	Mediator of DNA damage checkpoint protein 1	1.8095
321	Q5SPX8	Tbc1d10a	TBC1 domain family member 10A	1.8042
322	P19324	Serpinh1	Serpin H1	1.8023
323	P07742	Rrm1	Ribonucleoside-diphosphate reductase large subunit	1.8019
324	G3X8Y4	Dhx36	Probable ATP-dependent RNA helicase DHX36	1.7997
325	Q8CIM8	Ints4	Integrator complex subunit 4	1.7945
326	Q5SYD0	Myo1d	Unconventional myosin-Id	1.7933
327	Q99LZ3	Gins4	DNA replication complex GINS protein SLD5	1.7921
328	P49717	Mcm4	DNA replication licensing factor MCM4	1.7915
329	Q9QXV3	Ing1	Inhibitor of growth protein 1	1.7903
330	P23492	Pnp	Purine nucleoside phosphorylase	1.7889
331	Q9R045	Angptl2	Angiopoietin-related protein 2	1.7863
332	Q8BIW9	Chtf18	Chromosome transmission fidelity protein 18 homolog	1.7842
333	A1A4T2	Ganab		1.7839
334	Q7TQK1	Ints7	Integrator complex subunit 7	1.782
335	Q62141	Sin3b	Paired amphipathic helix protein Sin3b	1.7784
336	Q8CGB3-3	Uaca	Uveal autoantigen with coiled-coil domains and ankyrin repeats	1.7758
337	Q8BIK4-2	Dock9	Dedicator of cytokinesis protein 9	1.7738
338	Q9ESJ4	Nckipsd	NCK-interacting protein with SH3 domain	1.7736
339	Q9CPV5	Pmf1	Polyamine-modulated factor 1	1.7717
340	F8WIL9	Elmo1	Engulfment and cell motility protein 1	1.7686
341	Q3UH60	Dip2b	Disco-interacting protein 2 homolog B	1.7681
342	Q543K5	Psat1	Phosphoserine aminotransferase	1.7604
343	Q61686	Cbx5	Chromobox protein homolog 5	1.7597
344	Q3UMJ4	Gtf2f2	General transcription factor IIF subunit 2	1.7597
345	Q8BW72	Kdm4a	Lysine-specific demethylase 4A	1.7592
346	F8WIG5	Diap3	Protein diaphanous homolog 3	1.7576
347	P13864	Dnmt1	DNA (cytosine-5)-methyltransferase 1	1.7549
348	F8VPM7	Erc1	ELKS/Rab6-interacting/CAST family member 1	1.7528
349	Q9JJK2	Lancl2	LanC-like protein 2	1.7511
350	O70400	Pdlim1	PDZ and LIM domain protein 1	1.7511
351	Q9WVM1	Racgap1	Rac GTPase-activating protein 1	1.7506
352	G3X961	Klhdc4	Kelch domain-containing protein 4	1.7491
353	Q3UK27	Nae1	NEDD8-activating enzyme E1 regulatory subunit	1.7485

354	E9Q3E2	Synpo	Synaptopodin	1.748
355	Q8CGA0	Ppm1f	Protein phosphatase 1F	1.7443
356	D3Z024	Lrrc16a	Leucine-rich repeat-containing protein 16A	1.7426
357	Q8VE70	Pdcd10	Programmed cell death protein 10	1.74
358	Q8BQM4	Heatr3	HEAT repeat-containing protein 3	1.7393
359	Q8CDJ8	Ston1	Stonin-1	1.7379
360	O55222	Ilk	Integrin-linked protein kinase	1.7377
361	Q9D735		Uncharacterized protein C19orf43 homolog	1.7358
362	Q5HZJ0	Drosha	Ribonuclease 3	1.7352
363	H3BKF6	Cux1		1.7333
364	Q6PIP5	Nudcd1	NudC domain-containing protein 1	1.7329
365	Q9D8S3	Arfgap3	ADP-ribosylation factor GTPase-activating protein 3	1.731
366	F8WH45	Lars	Leucine--tRNA ligase, cytoplasmic	1.7302
367	Q99KK2	Cmas	N-acylneuraminate cytidylyltransferase	1.7284
368	O35344	Kpna3	Importin subunit α-3	1.7273
369	Q3UYC1	Mvd	Diphosphomevalonate decarboxylase	1.7199
370	Q5FWX6	Prkd3	Serine/threonine-protein kinase D3	1.7176
371	P43247	Msh2	DNA mismatch repair protein Msh2	1.7167
372	A6X966	Cggbp1	CGG triplet repeat-binding protein 1	1.7163
373	P33611	Pola2	DNA polymerase α subunit B	1.7158
374	E9QN12	Pdgfrb	Platelet-derived growth factor receptor β	1.7156
375	Q52KE7	Ccnl1	Cyclin-L1	1.7133
376	E9QB05	Clasp2	CLIP-associating protein 2	1.7091
377	Q80ZM5	H1fx		1.7091
378	O54950	Prkag1	5-AMP-activated protein kinase subunit γ-1	1.7056
379	Q8C3P7	Mettl3	N6-adenosine-methyltransferase 70 kDa subunit	1.7028
380	Q8BHT6	B3galtl	β-1,3-glucosyltransferase	1.7026
381	Q924A2	Cic	Protein capicua homolog	1.7018
382	Q3V0C5-2	Usp48	Ubiquitin carboxyl-terminal hydrolase 48	1.7011
383	Q9ER73	Elp4	Elongator complex protein 4	1.6999
384	O08795-2	Prkcsh	Glucosidase 2 subunit β	1.6994
385	Q8BK58	Hspbap1	HSPB1-associated protein 1	1.6991
386	B1AWD5	Glipr2	Golgi-associated plant pathogenesis-related protein 1	1.6973
387	A2A3Z8	Rcn1	Reticulocalbin-1	1.6936
388	B1AR17	Chd3		1.6931
389	P70460	Vasp	Vasodilator-stimulated phosphoprotein	1.6928
390	Q91VS8	Farp2	FERM, RhoGEF and pleckstrin domain-containing protein 2	1.6896
391	Q80WE4	Kif20b	Kinesin-like protein KIF20B	1.6884
392	P17809	Slc2a1	Solute carrier family 2, facilitated glucose transporter member 1	1.6871
393	Q99LG2	Tnpo2	Transportin-2	1.6862
394	Q09143	Slc7a1	High affinity cationic amino acid transporter 1	1.684

395	E9Q664	Ube4b	Ubiquitin conjugation factor E4 B	1.6839
396	P97822	Anp32e	Acidic leucine-rich nuclear phosphoprotein 32 family member E	1.6786
397	P58774-2	Tpm2		1.6778
398	O88413	Tulp3	Tubby-related protein 3	1.6777
399	Q3U4X8	Lig1	DNA ligase	1.6769
400	Q9JHH9	Copz2	Coatomer subunit zeta-2	1.6758
401	Q4FZF3	Ddx49	Probable ATP-dependent RNA helicase DDX49	1.6757
402	P47856	Gfpt1	Glucosamine--fructose-6-phosphate aminotransferase [isomerizing] 1	1.6749
403	Q9DCA5	Brix1	Ribosome biogenesis protein BRX1 homolog	1.6714
404	G5E837	Hcfc2		1.6695
405	Q8VHM5	Hnrnpr		1.6658
406	Q91X78	Erlin1	Erlin-1	1.6637
407	A2A6Q1	Cdc42ep4	Cdc42 effector protein 4	1.6584
408	Q8BH04	Pck2	Phosphoenolpyruvate carboxykinase [GTP], mitochondrial	1.6583
409	Q78PY7	Snd1	Staphylococcal nuclease domain-containing protein 1	1.6578
410	E9QJS2	Psph	Phosphoserine phosphatase	1.6571
411	Q8R4U7	Luzp1	Leucine zipper protein 1	1.6537
412	Q8C878	Uba3	NEDD8-activating enzyme E1 catalytic subunit	1.6537
413	P51432	Plcb3	1-phosphatidylinositol 4,5-bisphosphate phosphodiesterase β-3	1.653
414	Q9EP97	Senp3	Sentrin-specific protease 3	1.6519
415	Q9R0E1	Plod3	Procollagen-lysine,2-oxoglutarate 5-dioxygenase 3	1.6516
416	O88271	Cfdp1	Craniofacial development protein 1	1.6429
417	Q9CQ60	Pgls	6-phosphogluconolactonase	1.6429
418	Q9Z0M5	Lipa	Lysosomal acid lipase/cholesteryl ester hydrolase	1.6413
419	O09110	Map2k3	Dual specificity mitogen-activated protein kinase kinase 3	1.6407
420	P40237	Cd82	CD82 antigen	1.6396
421	P10630-2	Eif4a2	Eukaryotic initiation factor 4A-II	1.6377
422	Q540H0	Naa10	N-α-acetyltransferase 10	1.6374
423	Q8BGD9	Eif4b	Eukaryotic translation initiation factor 4B	1.6371
424	Q3UG25	Nfkb2	Nuclear factor NF-kappa-B p100 subunit	1.6368
425	Q5SUQ9	Ctc1	CST complex subunit CTC1	1.6365
426	P05132	Prkaca	cAMP-dependent protein kinase catalytic subunit α	1.6363
427	P51859	Hdgf	Hepatoma-derived growth factor	1.636
428	Q9CZW4	Acsl3	Long-chain-fatty-acid--CoA ligase 3	1.6333
429	P28658	Atxn10	Ataxin-10	1.633
430	Q3TRJ7	Sh3gl1	Endophilin-A2	1.6327
431	A2AE27	Ampd2	AMP deaminase 2	1.6308
432	E9QQ56	Myo9b	Unconventional myosin-IXb	1.6302
433	Q8BLH7	Hirip3	HIRA-interacting protein 3	1.6285
434	Q64737	Gart	Trifunctional purine biosynthetic protein adenosine-3	1.6276
435	Q3THK3	Gtf2f1	General transcription factor IIF subunit 1	1.6269

436	P39054-2	Dnm2	Dynamin-2	1.6268
437	Q6VH22	Ift172	Intraflagellar transport protein 172 homolog	1.6267
438	Q3TZR9	Atf7	Cyclic AMP-dependent transcription factor ATF-7	1.6256
439	G5E8R8	Ubxn7		1.6248
440	Q64318	Zeb1	Zinc finger E-box-binding homeobox 1	1.6235
441	Q7TPD0	Ints3	Integrator complex subunit 3	1.6215
442	Q8QZV7	Asun	Protein asunder homolog	1.6203
443	Q3TI84	Cdc16	Cell division cycle protein 16 homolog	1.6185
444	Q91ZS8	Adarb1	Double-stranded RNA-specific editase 1	1.6171
445	Q9DAW9	Cnn3	Calponin-3	1.6166
446	O35215	Ddt	D-dopachrome decarboxylase	1.6165
447	P20664	Prim1	DNA primase small subunit	1.6161
448	O35887	Calu	Calumenin	1.616
449	Q9CR16	Ppid	Peptidyl-prolyl cis-trans isomerase D	1.6146
450	Q8CGC7	Eprs	Bifunctional glutamate/proline--tRNA ligase	1.6134
451	Q9D662	Sec23b	Protein transport protein Sec23B	1.6115
452	Q8K2X3	Obfc1	CST complex subunit STN1	1.6106
453	G3X9Z0	Zwilch	Protein zwilch homolog	1.6099
454	Q9CZD3	Gars	Glycine--tRNA ligase	1.6095
455	Q9D903	Ebna1bp2	Probable rRNA-processing protein EBP2	1.6068
456	A2CEK3	Pgm2	Phosphoglucomutase-1	1.6061
457	Q09XV5	Chd8	Chromodomain-helicase-DNA-binding protein 8	1.6053
458	Q99MJ9	Ddx50	ATP-dependent RNA helicase DDX50	1.6052
459	E9Q718	Plod2	Procollagen-lysine,2-oxoglutarate 5-dioxygenase 2	1.6048
460	Q8C7V8	Ccdc134	Coiled-coil domain-containing protein 134	1.6044
461	P97770	Thumpd3	THUMP domain-containing protein 3	1.6044
462	A2ASC7	Rae1	mRNA export factor	1.6036
463	Q8BUI3	LRWD1	Leucine-rich repeat and WD repeat-containing protein 1	1.6034
464	A2AQB0	Rif1	Telomere-associated protein RIF1	1.603
465	A8C756	Thada	Thyroid adenoma-associated protein homolog	1.6009
466	Q9R1T4	41158	Septin-6	1.6004
467	P62892	Rpl39	60S ribosomal protein L39	1.6003
468	Q6P4S8	Ints1	Integrator complex subunit 1	1.5938
469	Q9Z127	Slc7a5	Large neutral amino acids transporter small subunit 1	1.5937
470	Q9D720	Nsmce1	Non-structural maintenance of chromosomes element 1 homolog	1.5931
471	B0V2V2	Cnpy3	Protein canopy homolog 3	1.5914
472	P11440	Cdk1	Cyclin-dependent kinase 1	1.5905
473	Q8BU30	Iars	Isoleucine--tRNA ligase, cytoplasmic	1.59
474	Q5SUR3	Skp1a	S-phase kinase-associated protein 1	1.5851
475	A2ADY9	Ddi2	Protein DDI1 homolog 2	1.5833
476	E9QMQ8	Ptk2	Focal adhesion kinase 1	1.5831

477	G3X8U9	Mapk8	Mitogen-activated protein kinase 8	1.5807
478	Q9Z1A1	Tfg		1.5802
479	A5GZX3	Glo1	Lactoylglutathione lyase	1.5799
480	G3X8Q1	Cabin1		1.5783
481	Q8K4X7	Agpat4	1-acyl-sn-glycerol-3-phosphate acyltransferase delta	1.5778
482	P58389	Ppp2r4	Serine/threonine-protein phosphatase 2A activator	1.5776
483	E9PYV9	Fen1	Flap endonuclease 1	1.5728
484	O35648	Cetn3	Centrin-3	1.5727
485	Q01149	Col1a2	Collagen α-2(I) chain	1.5721
486	O88574	Sap30	Histone deacetylase complex subunit SAP30	1.5717
487	Q9ERS5	Plekha2	Pleckstrin homology domain-containing family A member 2	1.571
488	F8WJE0	Samhd1	SAM domain and HD domain-containing protein 1	1.5697
489	Q4FK31	Mid1ip1	Mid1-interacting protein 1	1.5693
490	Q3UEG9	Flot2	Flotillin-2	1.5685
491	G3X8Y3	Naa15	N-α-acetyltransferase 15, NatA auxiliary subunit	1.5654
492	B2MWM9	Calr	Calreticulin	1.5627
493	Q9CZ04-2	Cops7a	COP9 signalosome complex subunit 7a	1.5621
494	E9Q1E9	Smc2	Structural maintenance of chromosomes protein	1.5597
495	Q9JKB3	Csda	DNA-binding protein A	1.5592
496	Q8R3P6		UPF0464 protein C15orf44 homolog	1.5564
497	Q9D7K2	Ten1	CST complex subunit TEN1	1.5561
498	Q9Z2I9	Sucla2	Succinyl-CoA ligase [ADP-forming] subunit β, mitochondrial	1.5526
499	A2AKT7	Xpo1	Exportin-1	1.5517
500	Q08761	Pros1	Vitamin K-dependent protein S	1.5515
501	Q3TJC2	Pafah1b3	Platelet-activating factor acetylhydrolase IB subunit γ	1.5501
502	Q9CY45	N6amt2	N(6)-adenine-specific DNA methyltransferase 2	1.5495
503	P14094	Atp1b1	Sodium/potassium-transporting ATPase subunit β-1	1.5493
504	Q8VDM6	Hnrnpul1	Heterogeneous nuclear ribonucleoprotein U-like protein 1	1.5471
505	A2AS06	Gmeb2	Glucocorticoid modulatory element-binding protein 2	1.5469
506	P48036	Anxa5	Annexin A5	1.5467
507	Q3UA06	Trip13	Pachytene checkpoint protein 2 homolog	1.544
508	P62874	Gnb1	Guanine nucleotide-binding protein G(I)/G(S)/G(T) subunit β-1	1.5407
509	D3Z7B5	C330027C09Rik	Protein CIP2A	1.5378
510	E9Q0H9	Ppil2	Peptidyl-prolyl cis-trans isomerase-like 2	1.5378
511	D3Z0X5	Phldb1	Pleckstrin homology-like domain family B member 1	1.5377
512	Q3UFB2	Znhit6	Box C/D snoRNA protein 1	1.5354
513	Q9CQK7	Rwdd1	RWD domain-containing protein 1	1.535
514	B9EIE9	Adss	Adenylosuccinate synthetase	1.5342
515	P97496	Smarcc1	SWI/SNF complex subunit SMARCC1	1.5334
516	Q9QZ73	Dcun1d1	DCN1-like protein 1	1.533
517	Q6DID7	Wls	Protein wntless homolog	1.5327

518	Q91ZJ5	Ugp2	UTP--glucose-1-phosphate uridylyltransferase	1.5293
519	Q3TDP6	Trpc4ap	Short transient receptor potential channel 4-associated protein	1.5274
520	Q8BWW9	Pkn2	Serine/threonine-protein kinase N2	1.5273
521	D3YUP1	Carm1	Histone-arginine methyltransferase CARM1	1.5265
522	P27641	Xrcc5	X-ray repair cross-complementing protein 5	1.5255
523	Q91X20	Ash2l	Set1/Ash2 histone methyltransferase complex subunit ASH2	1.525
524	D3YTT6	Osbpl3	Oxysterol-binding protein	1.5234
525	Q9EPU0	Upf1	Regulator of nonsense transcripts 1	1.5219
526	Q8BP27	Sfr1	Swi5-dependent recombination DNA repair protein 1 homolog	1.5203
527	Q99ME2	Wdr6	WD repeat-containing protein 6	1.5187
528	Q61024	Asns	Asparagine synthetase [glutamine-hydrolyzing]	1.5186
529	Q921K2	Parp1	Poly [ADP-ribose] polymerase 1	1.5174
530	Q9D902	Gtf2e2	General transcription factor IIE subunit 2	1.5135
531	Q8CAY6	Acat2	Acetyl-CoA acetyltransferase, cytosolic	1.5099
532	P61222	Abce1	ATP-binding cassette sub-family E member 1	1.5081
533	O55201	Supt5h	Transcription elongation factor SPT5	1.5056
534	Q80X50-5	Ubap2l	Ubiquitin-associated protein 2-like	1.5056
535	Q4VBG1	Ddx47	Probable ATP-dependent RNA helicase DDX47	1.5038
536	E9QNE9	Map4k3	Mitogen-activated protein kinase kinase kinase kinase 3	1.5037

Table 26: Nuclear proteins more abundant in importin α7-/- MEF nuclei.

Nuclear proteins from wild type (wt) and importin α7-/- (impa7-/-) MEFs were quantitatively examined by SILAC and subsequent mass spectrometry analysis. Identified proteins were ranked according to nuclear fold change wt/impa7-/- based on peptide intensity. Proteins displayed have at least a fold change ratio of 0.5. With decreasing values the protein abundance in importin α7-/- MEF nuclei increases.

	Majority protein IDs	Gene names	Protein names	fold change wt/impa7-/-
1	Q61599	Arhgdib	Rho GDP-dissociation inhibitor 2	0.026417
2	E9Q1I0	Gm3662		0.034381
3	Q9QZ85	Iigp1	Interferon-inducible GTPase 1	0.045876
4	Q8BW75	Maob	Amine oxidase [flavin-containing] B	0.051075
5	Q64133	Maoa	Amine oxidase [flavin-containing] A	0.052703
6	P22437	Ptgs1	Prostaglandin G/H synthase 1	0.059817
7	A2AFI6	Gm364		0.061296
8	E9QJW0	Mgst1	Microsomal glutathione S-transferase 1	0.061314
9	Q2EMV9	Parp14	Poly [ADP-ribose] polymerase 14	0.065027
10	Q3TNK3	Gpx7	Glutathione peroxidase	0.065918
11	Q8VHQ1	Serpinb9g		0.069198
12	P16460	Ass1	Argininosuccinate synthase	0.075394
13	Q6NSP9	Hmga2	High mobility group protein HMGI-C	0.075586
14	Q8VI93	Oas3		0.077916
15	Q3TBT3-2	Tmem173	Transmembrane protein 173	0.079382
16	Q6ZQM8	Ugt1a7c	UDP-glucuronosyltransferase 1-7C	0.084354
17	Q64282	Ifit1	Interferon-induced protein with tetratricopeptide repeats 1	0.084925
18	Q8VDU3	Apol9a		0.088245
19	E9Q5T9	Stat2	Signal transducer and activator of transcription 2	0.089067
20	Q8BUL5	Klhl7	Kelch-like protein 7	0.090845
21	Q4FJR9	Isg15	Ubiquitin-like protein ISG15	0.096059
22	Q07797	Lgals3bp	Galectin-3-binding protein	0.09638
23	P55097	Ctsk	Cathepsin K	0.096518
24	P06869	Plau	Urokinase-type plasminogen activator	0.0982
25	F2Z419	Oas1a	2-5-oligoadenylate synthase 1A	0.098341
26	Q9D379	Ephx1	Epoxide hydrolase 1	0.10103
27	Q60766	Irgm1	Immunity-related GTPase family M protein 1	0.10221
28	E9QMK9	9030617O03Rik	UPF0317 protein C14orf159 homolog, mitochondrial	0.10402
29	Q62293	Tgtp		0.10489
30	E9Q555	Rnf213	E3 ubiquitin-protein ligase RNF213	0.10867
31	D0QMC3	Mndal	Myeloid cell nuclear differentiation antigen-like protein	0.11295

32	D3Z2E7	AI607873		0.11325
33	Q9D1A2	Cndp2	Cytosolic non-specific dipeptidase	0.11895
34	Q9CPX4	Ftl1	Ferritin	0.12418
35	Q99P91	Gpnmb	Transmembrane glycoprotein NMB	0.12647
36	Q8CAS9	Parp9	Poly [ADP-ribose] polymerase 9	0.12659
37	Q99PA7	4930550L24Rik		0.12791
38	P20065-2	Tmsb4x		0.12889
39	Q9DAV6	Serpinb9b		0.132
40	Q3UIR3	Dtx3l	E3 ubiquitin-protein ligase DTX3L	0.13455
41	P97333	Nrp1	Neuropilin-1	0.13487
42	P97742	Cpt1a	Carnitine O-palmitoyltransferase 1, liver isoform	0.13666
43	Q9CZH7	Mxra7	Matrix-remodeling-associated protein 7	0.13911
44	Q8BMD8	Slc25a24	Calcium-binding mitochondrial carrier protein SCaMC-1	0.14294
45	Q571M4	Akr1c13	Aldo-keto reductase family 1 member C13	0.1449
46	P10493	Nid1	Nidogen-1	0.14724
47	P82343	Renbp	N-acylglucosamine 2-epimerase	0.1496
48	P00405	Mtco2	Cytochrome c oxidase subunit 2	0.15296
49	F8WJ19	Gvin1	Interferon-induced very large GTPase 1	0.15302
50	P50427	Sts	Steryl-sulfatase	0.15603
51	E9QK78	Stat1	Signal transducer and activator of transcription 1	0.15712
52	E9PX59	Samd9l	Sterile α motif domain-containing protein 9-like	0.15943
53	P03975	Iap	IgE-binding protein	0.1629
54	A2A4Q1	Ifi35	Interferon-induced 35 kDa protein homolog	0.16608
55	Q91ZE0	Tmlhe	Trimethyllysine dioxygenase, mitochondrial	0.16741
56	Q9DCE9	Igtp		0.16929
57	Q9JJX6	P2rx4	P2X purinoceptor 4	0.17287
58	Q3TMX7	Qsox2	Sulfhydryl oxidase 2	0.17677
59	Q61592	Gas6	Growth arrest-specific protein 6	0.1792
60	P51881	Slc25a5	ADP/ATP translocase 2	0.18243
61	Q8BGZ6	Gla	A-galactosidase A	0.18303
62	Q499X4	Vat1	Synaptic vesicle membrane protein VAT-1 homolog	0.18327
63	F7DBB3	Ahnak2		0.18488
64	Q8VEM8	Slc25a3	Phosphate carrier protein, mitochondrial	0.18564
65	A2AC28	Ebp	3-β-hydroxysteroid-Delta(8),Delta(7)-isomerase	0.18655
66	Q3UJU9	Fam82a2	Regulator of microtubule dynamics protein 3	0.19048
67	Q91WS0	Cisd1	CDGSH iron-sulfur domain-containing protein 1	0.19095
68	Q9CQW9	Ifitm3	Interferon-induced transmembrane protein 3	0.19108
69	Q5I0W0	Atp5f1	ATP synthase subunit b, mitochondrial	0.19235
70	E9PYB0	Ahnak2		0.19278
71	Q9DCX2	Atp5h	ATP synthase subunit d, mitochondrial	0.19535
72	Q9CPU4	Mgst3	Microsomal glutathione S-transferase 3	0.19607

73	A8Y5N3	Hsd17b11	Estradiol 17-β-dehydrogenase 11	0.19743
74	P26040	Ezr	Ezrin	0.19762
75	B2KGQ6	Gdpd1	Glycerophosphodiester phosphodiesterase domain-containing protein 1	0.19908
76	P15626	Gstm2	Glutathione S-transferase Mu 2	0.20096
77	E9PVY4	Ppt1	Palmitoyl-protein thioesterase 1	0.20109
78	Q8BIJ7	Rufy1	RUN and FYVE domain-containing protein 1	0.20119
79	Q8VCQ8	Cald1		0.20151
80	P97363	Sptlc2	Serine palmitoyltransferase 2	0.20333
81	Q3U6G1	Blvrb	Flavin reductase (NADPH)	0.20335
82	G3UWC2	Naalad2	N-acetylated-α-linked acidic dipeptidase 2	0.20444
83	B2RRE0	Akap12	A-kinase anchor protein 12	0.21079
84	Q99KN6	Htatip2	Oxidoreductase HTATIP2	0.21154
85	Q9QYR9	Acot2	Acyl-coenzyme A thioesterase 2, mitochondrial	0.21343
86	Q08509	Eps8	Epidermal growth factor receptor kinase substrate 8	0.21658
87	F7CVJ5	Ahnak2		0.21704
88	P51174	Acadl	Long-chain specific acyl-CoA dehydrogenase, mitochondrial	0.21806
89	P41731	Cd63	CD63 antigen	0.21936
90	Q3U3K9	Aup1	Ancient ubiquitous protein 1	0.22
91	Q6Q899	Ddx58	Probable ATP-dependent RNA helicase DDX58	0.22038
92	Q60953	Pml	Protein PML	0.22153
93	Q60930	Vdac2	Voltage-dependent anion-selective channel protein 2	0.22267
94	O70311	Nmt2	Glycylpeptide N-tetradecanoyltransferase 2	0.22446
95	P56395	Cyb5a	Cytochrome b5	0.22696
96	Q99JY0	Hadhb	Trifunctional enzyme subunit β, mitochondrial	0.22794
97	A2AFW7	Mtch2	Mitochondrial carrier homolog 2	0.22853
98	B1AQF4	Dusp3	Dual specificity protein phosphatase 3	0.22983
99	Q9DB20	Atp5o	ATP synthase subunit O, mitochondrial	0.23125
100	P17047-2	Lamp2	Lysosome-associated membrane glycoprotein 2	0.23161
101	E9Q7L0	Ogdhl		0.23205
102	Q6PB93	Galnt2	Polypeptide N-acetylgalactosaminyltransferase 2	0.2325
103	Q07235	Serpine2	Glia-derived nexin	0.23376
104	Q549X4	Rab27b	Ras-related protein Rab-27B	0.23483
105	P68368	Tuba4a	Tubulin α-4A chain	0.23594
106	Q8K1R3	Pnpt1	Polyribonucleotide nucleotidyltransferase 1, mitochondrial	0.23626
107	Q5SXC8	Mrps23	28S ribosomal protein S23, mitochondrial	0.23696
108	Q9DBU1	Ggta1	N-acetyllactosaminide α-1,3-galactosyltransferase	0.23892
109	O08749	Dld	Dihydrolipoyl dehydrogenase, mitochondrial	0.2393
110	Q91WJ4	Gnpda1	Glucosamine-6-phosphate isomerase 1	0.24202
111	Q3U9N4	Grn	Granulins	0.24268
112	Q1XID4	Atp6ap2	Renin receptor	0.24282
113	Q9D2G2	Dlst	Dihydrolipoyllysine-residue succinyltransferase component of 2-	0.24315

			oxoglutarate dehydrogenase complex, mitochondrial	
114	Q9QYF1	Rdh11	Retinol dehydrogenase 11	0.24518
115	P06797	Ctsl1	Cathepsin L1	0.24528
116	P56480	Atp5b	ATP synthase subunit β, mitochondrial	0.24609
117	Q9QYA2	Tomm40	Mitochondrial import receptor subunit TOM40 homolog	0.24617
118	Q8BWT1	Acaa2	3-ketoacyl-CoA thiolase, mitochondrial	0.24784
119	F8WHK2	Thbs2	Thrombospondin-2	0.25289
120	Q921T2-2	Tor1aip1	Torsin-1A-interacting protein 1	0.2543
121	F8VQ05	Fryl		0.25587
122	A2RSV8	Cox4i1	Cytochrome c oxidase subunit 4 isoform 1, mitochondrial	0.25617
123	Q03265	Atp5a1	ATP synthase subunit α, mitochondrial	0.25693
124	Q8K157	Galm	Aldose 1-epimerase	0.25841
125	Q91WK1	Spryd4	SPRY domain-containing protein 4	0.25938
126	O35114	Scarb2	Lysosome membrane protein 2	0.26055
127	Q8BMS1	Hadha	Trifunctional enzyme subunit α, mitochondrial	0.26164
128	Q69ZY2	Endod1	Endonuclease domain-containing 1 protein	0.26182
129	O35704	Sptlc1	Serine palmitoyltransferase 1	0.26225
130	O35604	Npc1	Niemann-Pick C1 protein	0.26328
131	O35405	Pld3	Phospholipase D3	0.26419
132	Q3TWN7	Atp6ap1	V-type proton ATPase subunit S1	0.26526
133	Q9CPQ3	Tomm22	Mitochondrial import receptor subunit TOM22 homolog	0.26601
134	Q9R0X4	Acot9	Acyl-coenzyme A thioesterase 9, mitochondrial	0.26739
135	Q62425	Ndufa4	NADH dehydrogenase [ubiquinone] 1 α subcomplex subunit 4	0.26753
136	E9QLA0	Immt	Mitochondrial inner membrane protein	0.26942
137	Q64435	Ugt1a6	UDP-glucuronosyltransferase 1-6	0.2716
138	E9Q296	Acox3	Acyl-coenzyme A oxidase	0.2724
139	Q8BL66	Eea1	Early endosome antigen 1	0.27285
140	D3YWD3	D730040F13Rik	Transmembrane protein 245	0.27314
141	Q9D6K8	Fundc2	FUN14 domain-containing protein 2	0.27421
142	E9PVN6	Gm20498	Synaptojanin-2-binding protein	0.27485
143	P67778	Phb	Prohibitin	0.27676
144	Q3UD06	Atp5c1	ATP synthase γ chain	0.27683
145	Q9CQE1	Nipsnap3b	Protein NipSnap homolog 3B	0.2774
146	Q62426	Cstb	Cystatin-B	0.2788
147	Q99LP6	Grpel1	GrpE protein homolog 1, mitochondrial	0.27882
148	Q8R5L1	C1qbp	Complement component 1 Q subcomponent-binding protein, mitochondrial	0.27886
149	Q05769	Ptgs2	Prostaglandin G/H synthase 2	0.28091
150	Q8VI84	Noc3l	Nucleolar complex protein 3 homolog	0.28146
151	O09131	Gsto1	Glutathione S-transferase omega-1	0.28317

152	O35129	Phb2	Prohibitin-2	0.28422
153	P0CW02	Ly6c1	Lymphocyte antigen 6C1	0.2853
154	Q80TA1	Ept1	Ethanolaminephosphotransferase 1	0.28586
155	Q9D6S2	Oasl2	54 kDa 2-5-oligoadenylate synthase-like protein 2	0.28715
156	Q8R1V4	Tmed4	Transmembrane emp24 domain-containing protein 4	0.28813
157	P97450	Atp5j	ATP synthase-coupling factor 6, mitochondrial	0.2889
158	A2RS96	Tmed5	Transmembrane emp24 domain-containing protein 5	0.29021
159	O09005	Degs1	Sphingolipid delta(4)-desaturase DES1	0.29025
160	B1AU74	Mospd2	Motile sperm domain-containing protein 2	0.29049
161	Q60932	Vdac1	Voltage-dependent anion-selective channel protein 1	0.29131
162	P52825	Cpt2	Carnitine O-palmitoyltransferase 2, mitochondrial	0.29231
163	Q8BU33	Ilvbl	Acetolactate synthase-like protein	0.29307
164	Q9DB73	Cyb5r1	NADH-cytochrome b5 reductase 1	0.29384
165	P17439	Gba	Glucosylceramidase	0.29483
166	Q8C1E7	Tmem120a	Transmembrane protein 120A	0.29492
167	Q9Z1G4	Atp6v0a1	V-type proton ATPase 116 kDa subunit a isoform 1	0.29552
168	Q8BG51-4	Rhot1	Mitochondrial Rho GTPase 1	0.29582
169	E9QN70	Lamb1	Laminin subunit β-1	0.29603
170	E9QLE0	Ifi204		0.29795
171	E9PVI2	Irgm2		0.29871
172	A2BFA6	Naglu		0.29903
173	Q08619	Ifi205b	Interferon-activable protein 205-B	0.29922
174	P25785	Timp2	Metalloproteinase inhibitor 2	0.3007
175	O88477	Igf2bp1	Insulin-like growth factor 2 mRNA-binding protein 1	0.30118
176	Q0VBA4	Rpl22l1	60S ribosomal protein L22-like 1	0.30238
177	Q9D997	Nagk	N-acetyl-D-glucosamine kinase	0.30271
178	Q8R0X7	Sgpl1	Sphingosine-1-phosphate lyase 1	0.30432
179	Q8BYY4	Ttc39b	Tetratricopeptide repeat protein 39B	0.30624
180	E9Q1S7	Wbp2	WW domain-binding protein 2	0.30633
181	P56695	Wfs1	Wolframin	0.30653
182	Q9DB77	Uqcrc2	Cytochrome b-c1 complex subunit 2, mitochondrial	0.30686
183	P97479	Myo7a	Unconventional myosin-VIIa	0.30803
184	B9EI57	Sltm	SAFB-like transcription modulator	0.30812
185	P45591	Cfl2	Cofilin-2	0.31067
186	Q99M08		Uncharacterized protein C4orf3 homolog	0.31121
187	Q9CQF9	Pcyox1	Prenylcysteine oxidase	0.31191
188	Q6A0C7	Rab21	Ras-related protein Rab-21	0.31218
189	O08715-4	Akap1	A-kinase anchor protein 1, mitochondrial	0.31395
190	Q8BYU6	Tor1aip2	Torsin-1A-interacting protein 2	0.31461
191	O54782	Man2b2	Epididymis-specific α-mannosidase	0.31709

192	Q9D1M7	Fkbp11	Peptidyl-prolyl cis-trans isomerase FKBP11	0.31857
193	Q99JP6-2	Homer3	Homer protein homolog 3	0.31887
194	O35640	Anxa8	Annexin A8	0.31904
195	Q9CZU6	Cs	Citrate synthase, mitochondrial	0.32068
196	Q9ET22	Dpp7	Dipeptidyl peptidase 2	0.32098
197	P12787	Cox5a	Cytochrome c oxidase subunit 5A, mitochondrial	0.32173
198	O89023	Tpp1	Tripeptidyl-peptidase 1	0.32227
199	Q922Q1	2-Mar	MOSC domain-containing protein 2, mitochondrial	0.32238
200	Q9DCW4	Etfb	Electron transfer flavoprotein subunit β	0.32302
201	Q8K411	Pitrm1	Presequence protease, mitochondrial	0.32468
202	Q8BMF4	Dlat	Dihydrolipoyllysine-residue acetyltransferase component of pyruvate dehydrogenase complex, mitochondrial	0.32649
203	Q99K70	Rragc	Ras-related GTP-binding protein C	0.32668
204	P70441	Slc9a3r1	Na(+)/H(+) exchange regulatory cofactor NHE-RF1	0.32728
205	P28653	Bgn	Biglycan	0.32803
206	A2AQR0	Gpd2	Glycerol-3-phosphate dehydrogenase, mitochondrial	0.3282
207	E0CZ22	Heatr7a		0.3292
208	Q91V61	Sfxn3	Sideroflexin-3	0.32956
209	Q8BIJ6	Iars2	Isoleucine--tRNA ligase, mitochondrial	0.32994
210	Q9EQH2	Erap1	Endoplasmic reticulum aminopeptidase 1	0.33006
211	Q9D0L7	Armc10	Armadillo repeat-containing protein 10	0.33056
212	Q8R3H7	Hs2st1	Heparan sulfate 2-O-sulfotransferase 1	0.33089
213	P09528	Fth1	Ferritin heavy chain	0.33234
214	Q00519	Xdh	Xanthine dehydrogenase/oxidase	0.33442
215	Q61029	Tmpo	Lamina-associated polypeptide 2, isoforms β/delta/epsilon/γ	0.33494
216	P15092	Ifi204	Interferon-activable protein 204	0.33637
217	B1B1B4	Etfa	Electron transfer flavoprotein subunit α, mitochondrial	0.33649
218	Q8VEH3	Arl8a	ADP-ribosylation factor-like protein 8A	0.33684
219	P08249	Mdh2	Malate dehydrogenase, mitochondrial	0.33756
220	G3X8R0	Reep5	Receptor expression-enhancing protein 5	0.33845
221	F8WJE2	Ube2g2	Ubiquitin-conjugating enzyme E2 G2	0.33875
222	B1AU24	Aifm1	Apoptosis-inducing factor 1, mitochondrial	0.3388
223	Q3UQ44	Iqgap2	Ras GTPase-activating-like protein IQGAP2	0.34045
224	F7AWB4	Uap1l1	UDP-N-acetylhexosamine pyrophosphorylase-like protein 1	0.34254
225	Q9CZ13	Uqcrc1	Cytochrome b-c1 complex subunit 1, mitochondrial	0.34335
226	A2ALW3	Ptgr1	Prostaglandin reductase 1	0.34478
227	E9PV45	Usp24	Ubiquitin carboxyl-terminal hydrolase	0.34539
228	Q8BYC6	Taok3	Serine/threonine-protein kinase TAO3	0.3462
229	Q8BFR4	Gns	N-acetylglucosamine-6-sulfatase	0.34624
230	Q8BH24	Tm9sf4	Transmembrane 9 superfamily member 4	0.3464
231	Q9CRD0	Ociad1	OCIA domain-containing protein 1	0.34663
232	Q00993	Axl	Tyrosine-protein kinase receptor UFO	0.34707

233	Q9D5T0	Atad1	ATPase family AAA domain-containing protein 1	0.34904
234	Q9R0H0	Acox1	Peroxisomal acyl-coenzyme A oxidase 1	0.34942
235	Q5SXR0	Ptrh2	Peptidyl-tRNA hydrolase 2, mitochondrial	0.35249
236	B1AXB8	Wdr44	WD repeat-containing protein 44	0.35318
237	Q9Z2I8	Suclg2	Succinyl-CoA ligase [GDP-forming] subunit β, mitochondrial	0.35511
238	Q9D0M3	Cyc1	Cytochrome c1, heme protein, mitochondrial	0.35522
239	B1AX58	Pls3	Plastin-3	0.35625
240	Q64FW2	Retsat	All-trans-retinol 13,14-reductase	0.36018
241	P16092-3	Fgfr1	Fibroblast growth factor receptor	0.36123
242	Q99JR1	Sfxn1	Sideroflexin-1	0.36214
243	Q8BR63	Fam177a1	Protein FAM177A1	0.36436
244	Q3TC93	Hs1bp3	HCLS1-binding protein 3	0.3663
245	P35762	Cd81	CD81 antigen	0.36741
246	O35143	Atpif1	ATPase inhibitor, mitochondrial	0.3677
247	Q07813	Bax	Apoptosis regulator BAX	0.36779
248	Q8BXZ1	Tmx3	Protein disulfide-isomerase TMX3	0.36804
249	Q91XU3	Pip4k2c	Phosphatidylinositol 5-phosphate 4-kinase type-2 γ	0.37021
250	A2AFQ2	Hsd17b10	3-hydroxyacyl-CoA dehydrogenase type-2	0.37142
251	Q9ET30	Tm9sf3	Transmembrane 9 superfamily member 3	0.37151
252	G3X8U5	Hmgcr	3-hydroxy-3-methylglutaryl-coenzyme A reductase	0.37284
253	P70280	Vamp7	Vesicle-associated membrane protein 7	0.37494
254	G5E895	Akr1b10		0.37579
255	Q03963	Eif2ak2	Interferon-induced, double-stranded RNA-activated protein kinase	0.37583
256	Q99KE1	Me2	NAD-dependent malic enzyme, mitochondrial	0.37686
257	Q76LV0	Gpx4	Glutathione peroxidase	0.37692
258	Q93092	Taldo1	Transaldolase	0.37701
259	F8WIV2	Serpinb6a	Serpin B6	0.37754
260	E9Q137	Tex264		0.37781
261	A2AFP5	Rab9	Ras-related protein Rab-9A	0.37813
262	A2AL38	Stom	Erythrocyte band 7 integral membrane protein	0.37959
263	A1L3P4	Slc9a6	Sodium/hydrogen exchanger	0.37964
264	O88736	Hsd17b7	3-keto-steroid reductase	0.37967
265	Q80X95	Rraga	Ras-related GTP-binding protein A	0.38105
266	Q9CQB4	Uqcrb	Cytochrome b-c1 complex subunit 7	0.38353
267	Q9CQW2	Arl8b	ADP-ribosylation factor-like protein 8B	0.3837
268	Q60597	Ogdh	2-oxoglutarate dehydrogenase, mitochondrial	0.38495
269	P26041	Msn	Moesin	0.38499
270	Q8VDY9	Caap1	Caspase activity and apoptosis inhibitor 1	0.38532
271	A2A4H7	Jup	Junction plakoglobin	0.38557
272	Q8VBT0	Tmx1	Thioredoxin-related transmembrane protein 1	0.38882
273	P37040	Por	NADPH--cytochrome P450 reductase	0.38888

274	Q6NZR2	Msantd2	Myb/SANT-like DNA-binding domain-containing protein 2	0.3911
275	Q9JMH6	Txnrd1	Thioredoxin reductase 1, cytoplasmic	0.39114
276	Q3TXV4	Rab31	Ras-related protein Rab-31	0.39181
277	O55022	Pgrmc1	Membrane-associated progesterone receptor component 1	0.39184
278	B2RR82	Itsn2	Intersectin-2	0.39202
279	Q9D880	Timm50	Mitochondrial import inner membrane translocase subunit TIM50	0.39293
280	P63038	Hspd1	60 kDa heat shock protein, mitochondrial	0.3943
281	Q791T5	Mtch1	Mitochondrial carrier homolog 1	0.39821
282	Q9QYE6	Golga5	Golgin subfamily A member 5	0.39862
283	Q9JKF7	Mrpl39	39S ribosomal protein L39, mitochondrial	0.39904
284	Q99KK1	Reep3	Receptor expression-enhancing protein 3	0.40002
285	A3KGQ8	Golga1	Golgin subfamily A member 1	0.40017
286	P51863	Atp6v0d1	V-type proton ATPase subunit d 1	0.40039
287	P35569	Irs1	Insulin receptor substrate 1	0.40041
288	P01887	B2m	Β-2-microglobulin	0.40076
289	Q14C51	Ptcd3	Pentatricopeptide repeat-containing protein 3, mitochondrial	0.40084
290	P59017	Bcl2l13	Bcl-2-like protein 13	0.40144
291	Q9QYB5	Add3	Γ-adducin	0.40183
292	Q99KU0	Vmp1	Vacuole membrane protein 1	0.4029
293	P20352	F3	Tissue factor	0.40322
294	Q921W7	Tes	Testin	0.40345
295	Q91YP2	Nln	Neurolysin, mitochondrial	0.40416
296	Q07417	Acads	Short-chain specific acyl-CoA dehydrogenase, mitochondrial	0.40444
297	Q80X71	Tmem106b	Transmembrane protein 106B	0.40496
298	Q9JIY5	Htra2	Serine protease HTRA2, mitochondrial	0.40497
299	Q9DCV4	Fam82b	Regulator of microtubule dynamics protein 1	0.40531
300	Q8BX10-2			0.4063
301	Q99K41	Emilin1	EMILIN-1	0.40653
302	D3YVN7	Gm9755	Elongation factor Tu	0.40696
303	Q9D1K2	Atp6v1f	V-type proton ATPase subunit F	0.40816
304	G3XA41	Gm6055	Microtubule-associated proteins 1A/1B light chain 3B	0.40856
305	Q9CQ22	Lamtor1	Ragulator complex protein LAMTOR1	0.40883
306	O55242	Sigmar1	Sigma non-opioid intracellular receptor 1	0.40995
307	G5E833	Osbpl5	Oxysterol-binding protein	0.41018
308	Q9DBS1	Tmem43	Transmembrane protein 43	0.41047
309	Q544R8	Trappc4	Trafficking protein particle complex subunit 4	0.41058
310	P62075	Timm13	Mitochondrial import inner membrane translocase subunit Tim13	0.41069
311	Q9Z0L0	Tpbg	Trophoblast glycoprotein	0.41074
312	O09117	Sypl1	Synaptophysin-like protein 1	0.41109
313	Q9DB15	Mrpl12	39S ribosomal protein L12, mitochondrial	0.41191
314	P51480	Cdkn2a	Cyclin-dependent kinase inhibitor 2A, isoforms 1/2	0.41209

315	E2JF22	Piezo1	Piezo-type mechanosensitive ion channel component 1	0.41291
316	P40630	Tfam	Transcription factor A, mitochondrial	0.41461
317	Q02819	Nucb1	Nucleobindin-1	0.41473
318	A2API8	Akap2	A-kinase anchor protein 2	0.41547
319	Q9CQV4	Fam134c	Protein FAM134C	0.41618
320	Q9CX13	Cnih4	Protein cornichon homolog 4	0.41676
321	Q80TT4	Tomm70a	Mitochondrial import receptor subunit TOM70	0.41706
322	Q80WW9	Ddrgk1	DDRGK domain-containing protein 1	0.41717
323	A3KG50	Ube2j1	Ubiquitin-conjugating enzyme E2 J1	0.41758
324	P70302	Stim1	Stromal interaction molecule 1	0.41808
325	F8WI94	Atp2a2	Sarcoplasmic/endoplasmic reticulum calcium ATPase 2	0.41959
326	Q6NS82	Fam134a	Protein FAM134A	0.41977
327	Q9CQB5	Cisd2	CDGSH iron-sulfur domain-containing protein 2	0.42018
328	Q9CQ43	Dut		0.42029
329	B9EHJ3	Tjp1	Tight junction protein ZO-1	0.42032
330	Q0PD23	Rab32	Ras-related protein Rab-32	0.42039
331	Q62087	Pon3	Serum paraoxonase/lactonase 3	0.42207
332	Q9QYC0	Add1	α-adducin	0.4221
333	Q91Y74	St3gal4	CMP-N-acetylneuraminate-β-galactosamide-α-2,3-sialyltransferase 4	0.42318
334	B1APM8	Nkap	NF-kappa-B-activating protein	0.42466
335	E9Q120	Lonp1	Lon protease homolog	0.42486
336	Q8BY89	Slc44a2	Choline transporter-like protein 2	0.42515
337	Q9WV03	Fam50a	Protein FAM50A	0.42619
338	Q64429	Cyp1b1	Cytochrome P450 1B1	0.42678
339	Q61263	Soat1	Sterol O-acyltransferase 1	0.42687
340	O08797	Serpinb9		0.42774
341	Q3TM70	Ehd4	EH domain-containing protein 4	0.42868
342	Q6R891	Ppp1r9b	Neurabin-2	0.42872
343	P48962	Slc25a4	ADP/ATP translocase 1	0.42894
344	A2ALM8	Bcap31	B-cell receptor-associated protein 31	0.42985
345	G5E8C4	Tmtc3	Transmembrane and TPR repeat-containing protein 3	0.43013
346	Q921H9	Selrc1	Sel1 repeat-containing protein 1	0.43075
347	P04627	Araf	Serine/threonine-protein kinase A-Raf	0.43146
348	O35639	Anxa3	Annexin A3	0.43193
349	Q9CPN8	Igf2bp3	Insulin-like growth factor 2 mRNA-binding protein 3	0.43214
350	O08579	Emd	Emerin	0.43223
351	Q9JHF5	Tcirg1		0.43368
352	A6ZI44	Aldoa	Fructose-bisphosphate aldolase	0.43489
353	Q8BH59	Slc25a12	Calcium-binding mitochondrial carrier protein Aralar1	0.43572
354	P11438	Lamp1	Lysosome-associated membrane glycoprotein 1	0.4367

355	P35293	Rab18	Ras-related protein Rab-18	0.43821
356	B2RV69	Tmem199	Transmembrane protein 199	0.43868
357	A2AV60	Vps18	Vacuolar protein sorting-associated protein 18 homolog	0.43886
358	Q4KL76	Hspe1	10 kDa heat shock protein, mitochondrial	0.43926
359	Q8R048	Mycbp	C-Myc-binding protein	0.4395
360	Q6A058	Armcx2	Armadillo repeat-containing X-linked protein 2	0.43994
361	P62748	Hpcal1	Hippocalcin-like protein 1	0.44052
362	O35598	Adam10	Disintegrin and metalloproteinase domain-containing protein 10	0.44205
363	P57746	Atp6v1d	V-type proton ATPase subunit D	0.44243
364	Q8CCJ3	Ufl1	E3 UFM1-protein ligase 1	0.44273
365	P38647	Hspa9	Stress-70 protein, mitochondrial	0.44369
366	Q3UVK0	Ermp1	Endoplasmic reticulum metallopeptidase 1	0.4439
367	G3UWZ0	Baz1a	Bromodomain adjacent to zinc finger domain protein 1A	0.44401
368	P47738	Aldh2	Aldehyde dehydrogenase, mitochondrial	0.44402
369	Q9D1D4	Tmed10	Transmembrane emp24 domain-containing protein 10	0.44432
370	Q9D2V8	Mfsd10	Major facilitator superfamily domain-containing protein 10	0.44573
371	Q9CWK8	Snx2	Sorting nexin-2	0.44649
372	H3BKH2	Ctage5	Cutaneous T-cell lymphoma-associated antigen 5 homolog	0.4468
373	Q569N0	Ndufab1	Acyl carrier protein	0.44681
374	Q8C266	Rab5c	Ras-related protein Rab-5C	0.44753
375	Q99N84	Mrps18b	28S ribosomal protein S18b, mitochondrial	0.44754
376	F6WIU1	Kri1	Protein KRI1 homolog	0.44762
377	H3BK65	Eps15	Epidermal growth factor receptor substrate 15	0.44769
378	Q8BML1	Mical2	Protein-methionine sulfoxide oxidase MICAL2	0.44785
379	A2A6E3	Cdk5rap3	CDK5 regulatory subunit-associated protein 3	0.44814
380	Q07113	Igf2r	Cation-independent mannose-6-phosphate receptor	0.44816
381	Q91VA7	Idh3b		0.44948
382	Q9CYR6	Pgm3	Phosphoacetylglucosamine mutase	0.45004
383	Q3UL31	Zc3h11a	Zinc finger CCCH domain-containing protein 11A	0.45048
384	Q8K273	Mmgt1	Membrane magnesium transporter 1	0.45091
385	Q7TQ95	Lnp	Protein lunapark	0.451
386	Q9QUJ7	Acsl4	Long-chain-fatty-acid--CoA ligase 4	0.45211
387	P21956	Mfge8	Lactadherin	0.45242
388	Q6GV12	Kdsr	3-ketodihydrosphingosine reductase	0.45274
389	D3Z7P3-2	Gls	Glutaminase kidney isoform, mitochondrial	0.45322
390	P35564	Canx	Calnexin	0.45369
391	Q9JIK9	Mrps34	28S ribosomal protein S34, mitochondrial	0.45401
392	Q62504	Spen	Msx2-interacting protein	0.45407
393	P70227	Itpr3	Inositol 1,4,5-trisphosphate receptor type 3	0.45456
394	A6PWS1	Tollip	Toll-interacting protein	0.45485
395	P53690	Mmp14	Matrix metalloproteinase-14	0.455

396	E9PVZ8	Golgb1		0.45523
397	Q8BLF1	Nceh1	Neutral cholesterol ester hydrolase 1	0.45554
398	Q8K2B3	Sdha	Succinate dehydrogenase [ubiquinone] flavoprotein subunit, mitochondrial	0.45724
399	Q7TMM9	Tubb2a	Tubulin β-2A chain	0.45801
400	Q91VM9	Ppa2	Inorganic pyrophosphatase 2, mitochondrial	0.46007
401	Q8BGS2	Bola2	BolA-like protein 2	0.46031
402	Q8VE99	Ccdc115	Coiled-coil domain-containing protein 115	0.46083
403	Q9DB05	Napa	A-soluble NSF attachment protein	0.46138
404	A2AA77	Sdhb	Succinate dehydrogenase [ubiquinone] iron-sulfur subunit, mitochondrial	0.46265
405	P51150	Rab7a	Ras-related protein Rab-7a	0.46278
406	P20108	Prdx3	Thioredoxin-dependent peroxide reductase, mitochondrial	0.46296
407	O88811	Stam2	Signal transducing adapter molecule 2	0.46298
408	O55126	Gbas	Protein NipSnap homolog 2	0.46323
409	G5E878	Cntn3	Contactin-3	0.46511
410	Q3TFD2	Lpcat1	Lysophosphatidylcholine acyltransferase 1	0.46664
411	Q76KJ5	Cd3eap	DNA-directed RNA polymerase I subunit RPA34	0.46679
412	P35486	Pdha1	Pyruvate dehydrogenase E1 component subunit α, somatic form, mitochondrial	0.46686
413	A2AIW8	Pmpca	Mitochondrial-processing peptidase subunit α	0.46752
414	A2A5R2	Arfgef2		0.46893
415	Q9DBG7	Srpr	Signal recognition particle receptor subunit α	0.46909
416	E9QMC5	Ptpmt1	Protein-tyrosine phosphatase mitochondrial 1	0.46937
417	Q3TX38	Vdac3	Voltage-dependent anion-selective channel protein 3	0.46956
418	Q8BXV2	Bri3bp	BRI3-binding protein	0.46991
419	P17426	Ap2a1	AP-2 complex subunit α-1	0.47062
420	B1AV37	Timm8a1	Mitochondrial import inner membrane translocase subunit Tim8 A	0.47135
421	A2RS23	Pepd	Xaa-Pro dipeptidase	0.47141
422	P29533	Vcam1	Vascular cell adhesion protein 1	0.4716
423	A2RS53	Tmed2	Transmembrane emp24 domain-containing protein 2	0.47171
424	Q4FJK0	Decr1	2,4-dienoyl-CoA reductase, mitochondrial	0.47218
425	Q8VBZ3	Clptm1	Cleft lip and palate transmembrane protein 1 homolog	0.47247
426	Q91W86	Vps11	Vacuolar protein sorting-associated protein 11 homolog	0.47387
427	F6ZJB0	Iqgap1	Ras GTPase-activating-like protein IQGAP1	0.4739
428	A2AEV6	Praf2	PRA1 family protein 2	0.47474
429	Q91VN6	Ddx41	Probable ATP-dependent RNA helicase DDX41	0.47512
430	P07091	S100a4	Protein S100-A4	0.47567
431	Q64337	Sqstm1	Sequestosome-1	0.4763
432	B1AWK6	Meis2	Homeobox protein Meis2	0.4776
433	B2RPS1	Rab5b	Ras-related protein Rab-5B	0.47762
434	P54071	Idh2	Isocitrate dehydrogenase [NADP], mitochondrial	0.4788

435	Q8R502	Lrrc8c	Leucine-rich repeat-containing protein 8C	0.48005
436	Q80TL7	Mon2	Protein MON2 homolog	0.48059
437	Q8K2C7	Os9	Protein OS-9	0.48167
438	Q9CYG7	Tomm34	Mitochondrial import receptor subunit TOM34	0.48278
439	O08997	Atox1	Copper transport protein ATOX1	0.48304
440	Q8C243	Ctsd	Cathepsin D	0.4832
441	F8WHJ5	Lpgat1	Acyl-CoA:lysophosphatidylglycerol acyltransferase 1	0.48333
442	F6VW30	Ywhaq	14-3-3 protein theta	0.48389
443	Q8BVE3	Atp6v1h	V-type proton ATPase subunit H	0.48445
444	P26443	Glud1	Glutamate dehydrogenase 1, mitochondrial	0.4845
445	A6H6H4	Tmsb10	Thymosin β-10	0.48504
446	O54962	Banf1	Barrier-to-autointegration factor	0.48579
447	G3X8X7	Vps16	Vacuolar protein sorting-associated protein 16 homolog	0.48688
448	Q5KU39	Vps41	Vacuolar protein sorting-associated protein 41 homolog	0.48723
449	Q8C0T5	Sipa1l1	Signal-induced proliferation-associated 1-like protein 1	0.48737
450	P17156	Hspa2	Heat shock-related 70 kDa protein 2	0.4884
451	Q91ZX7	Lrp1	Prolow-density lipoprotein receptor-related protein 1	0.48869
452	Q80V26	Impad1	Inositol monophosphatase 3	0.4896
453	Q3V307	Pigs	GPI transamidase component PIG-S	0.48977
454	Q9R0Q7	Ptges3	Prostaglandin E synthase 3	0.49039
455	Q6NSR8	Npepl1	Probable aminopeptidase NPEPL1	0.49091
456	E9PW66	Nap1l1	Nucleosome assembly protein 1-like 1	0.49116
457	E9Q4P1	Wdfy1		0.49131
458	B1B0W8	Hprt	Hypoxanthine-guanine phosphoribosyltransferase	0.49162
459	O08992	Sdcbp	Syntenin-1	0.49177
460	O35134	Polr1a	DNA-directed RNA polymerase I subunit RPA1	0.49195
461	Q9CXT8	Pmpcb	Mitochondrial-processing peptidase subunit β	0.49202
462	B1AUP1	Timp1	Metalloproteinase inhibitor 1	0.49209
463	Q80YQ1	Thbs1	Thrombospondin-1	0.49273
464	Q8BFZ9	Erlin2	Erlin-2	0.49274
465	Q8BKE6	Cyp20a1	Cytochrome P450 20A1	0.49423
466	Q3UBU9	Fkbp3	Peptidyl-prolyl cis-trans isomerase	0.49433
467	A2A4P4	Aarsd1	Alanyl-tRNA editing protein Aarsd1	0.49458
468	Q80YS6	Afap1	Actin filament-associated protein 1	0.49485
469	Q8VEJ4	Nle1	Notchless protein homolog 1	0.49514
470	Q91VW5	Golga4	Golgin subfamily A member 4	0.4953
471	O88630	Gosr1	Golgi SNAP receptor complex member 1	0.49543
472	Q9CWW6	Pin4	Peptidyl-prolyl cis-trans isomerase NIMA-interacting 4	0.49583
473	Q9WTP7	Ak3	GTP:AMP phosphotransferase, mitochondrial	0.49602
474	E9PYM9	Rock2	Rho-associated protein kinase 2	0.49713
475	A1ILG8	Vps13c	Vacuolar protein sorting-associated protein 13C	0.49773

476	O08528	Hk2	Hexokinase-2	0.49774
477	Q8BL63	Pigk	GPI-anchor transamidase	0.4979
478	Q9D6R2	Idh3a	Isocitrate dehydrogenase [NAD] subunit α, mitochondrial	0.49848
479	Q4VAI2	Acp1	Low molecular weight phosphotyrosine protein phosphatase	0.49864
480	Q11011	Npepps	Puromycin-sensitive aminopeptidase	0.49887
481	A2AMX4	Syap1	Synapse-associated protein 1	0.49922
482	P70206	Plxna1	Plexin-A1	0.49928
483	F6UF97	Ncstn	Nicastrin	0.50021