

Mass Spectrometry and Imaging Analysis of Nanoparticle-Containing Vesicles Provide a Mechanistic Insight into Cellular Trafficking

Daniel Hofmann^{1,2}, Stefan Tenzer³, Markus B. Bannwarth^{1,4}, Claudia Messerschmidt¹, Simone-Franziska Glaser¹, Hansjörg Schild³, Katharina Landfester¹, Volker Mailänder^{1,2}*

¹ Max Planck Institute for Polymer Research, Ackermannweg 10, 55128 Mainz, Germany

² Dept. of Medicine III, Hematology, Oncology and Pneumology, University Medical Center of the Johannes Gutenberg University Mainz, Langenbeckstr. 1, 55101 Mainz, Germany

³ Dept. of Immunology, University Medical Center of the Johannes Gutenberg University Mainz, Langenbeckstr. 1, 55101 Mainz, Germany

⁴ Graduate School Materials Science in Mainz, Staudinger Weg 9, 55128 Mainz, Germany

* corresponding author: Volker Mailänder; mailaender@mpip-mainz.mpg.de

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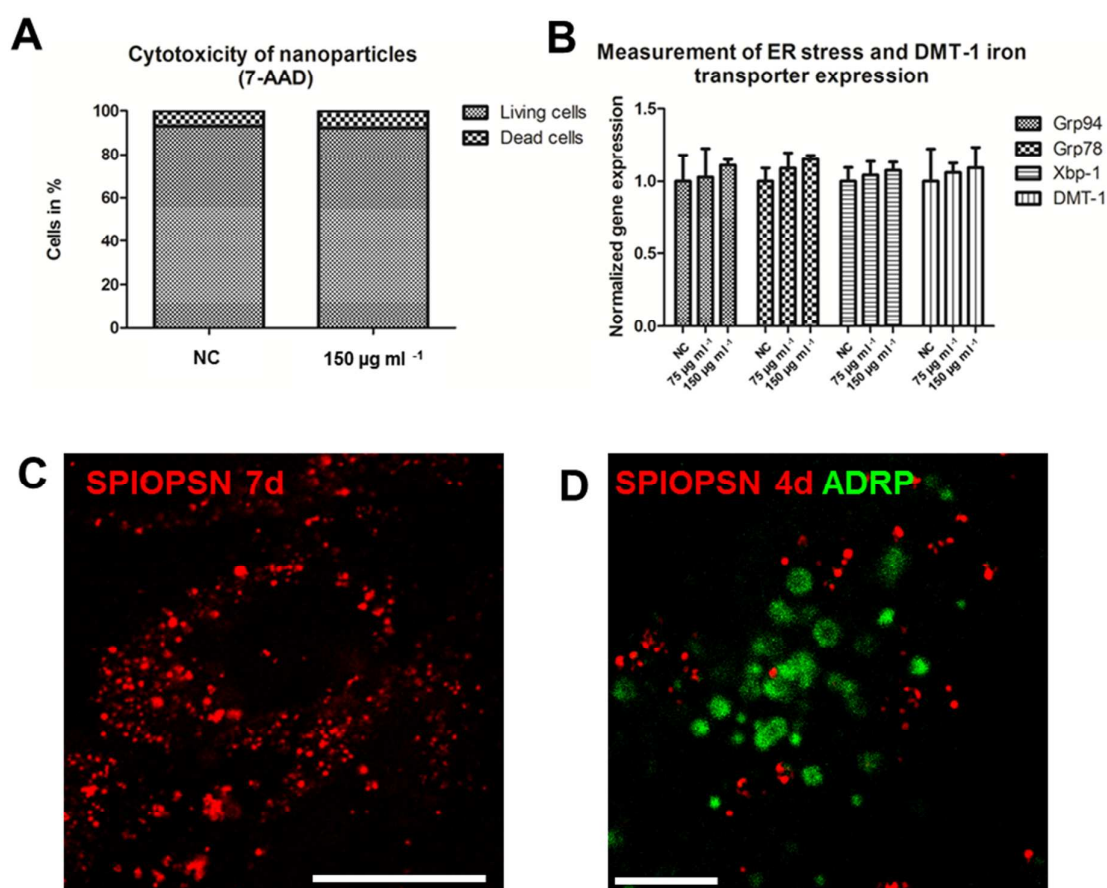
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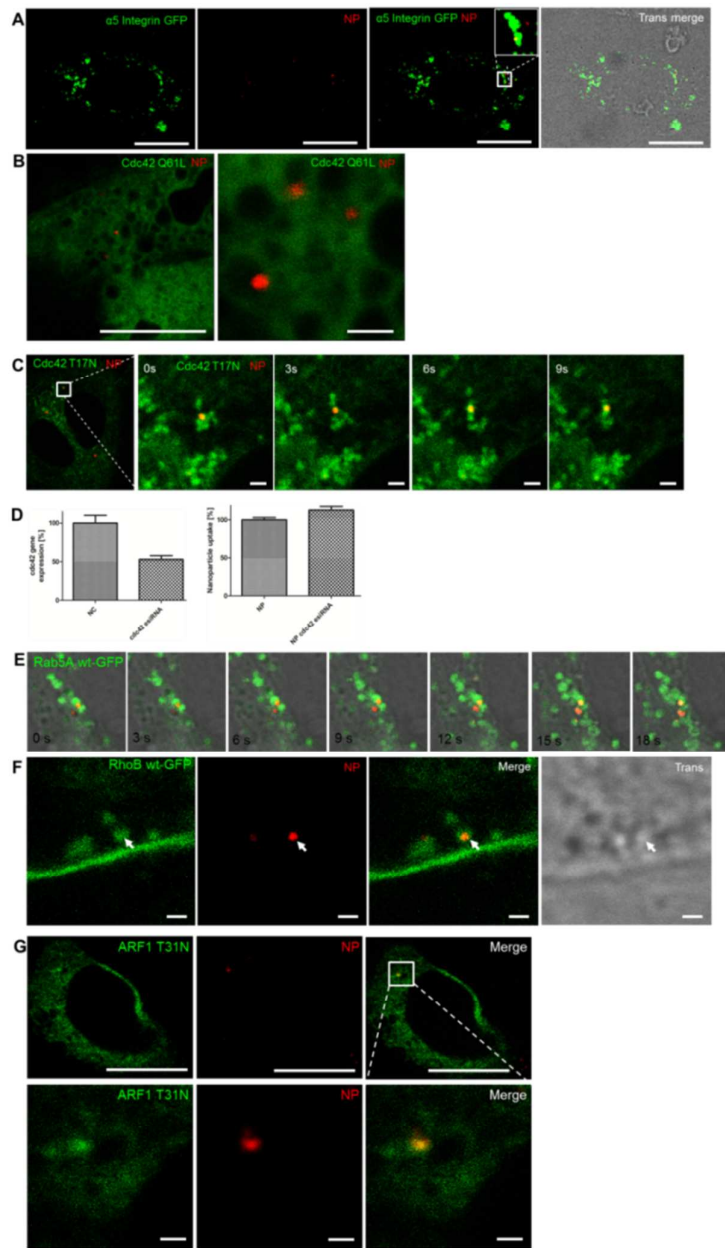
To exclude any cytotoxic effects induced by our SPIOPSN, we performed 7-AAD stainings to measure the late apoptotic/ necrotic cells after 20 h of nanoparticle exposure (**Supplementary Fig. S1**). No significant cytotoxicity was detected after 20 h and also after 7 d. This confirms the morphological investigations in cLSM and TEM lacking any signs of nanoparticle-mediated cytotoxicity. To exclude alterations in the intracellular trafficking between the ER and the endolysosomal vesicles (e.g. COP vesicles), we performed ER stress measurements. We measured the changes of Grp94, Grp78 and Xbp-1 after 20 h of nanoparticle exposure. Expression levels of DMT-1 were measured to examine the changes to examine eventually free iron in our sample. No significant changes were observed. Additionally, we performed experiments to check dye leakage of nanoparticles after long time exposure. After 4d of nanoparticle incubation, no free dye was detectable inside ADRP⁺ lipid droplets.



Supplementary Fig. S1: Cytotoxicity of nanoparticles after 20 h of exposure. (A) No cytotoxicity was determined by the measurement of 7-AAD. The number of dead cells of

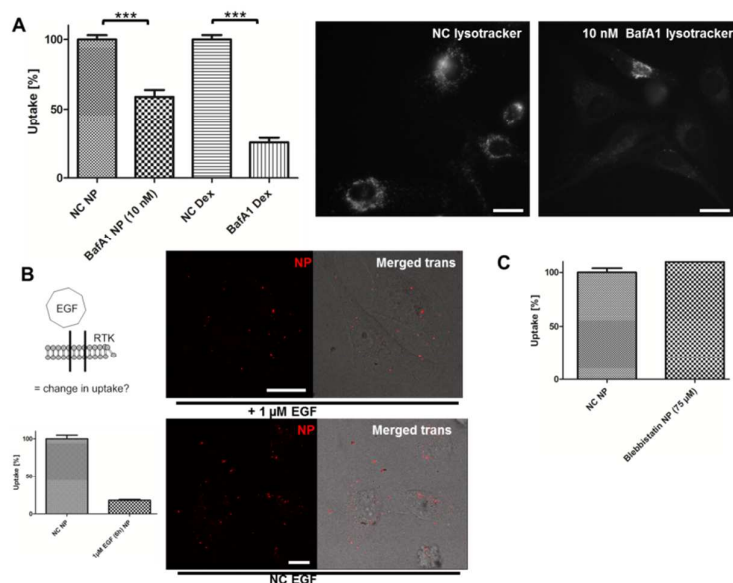
nanoparticle-treated cells is comparable to the negative control (NC). (B) Measurement of the transcriptional activity of ER stress and DMT-1 iron transporter analyzed by qPCR shows no significant changes after 20 h of nanoparticle exposure. (C) Exposure of $150\text{ }\mu\text{g ml}^{-1}$ over 7 d on HeLa cells. Scale bar: $10\text{ }\mu\text{m}$. (D) Confocal imaging of ADRP immunofluorescence staining of lipid droplets after 4d of SPIOPSN exposure. No colocalization of SPIOPSN with ADRP was observed. Scale bar: $5\text{ }\mu\text{m}$.

To confirm further proteins identified in peptide mass spectrometry by confocal imaging, we overexpressed several GFP-tagged proteins and analyzed SPIOPSN for colocalization.



Supplementary Fig. S2: Colocalization with different markers underlines the sensitivity of the peptide-mass-spectrometry. (A) $\alpha 5$ -Integrin-GFP is known to participate in macropinocytic events and was shown to rarely colocalize with SPIOPSN (scale bar = 10 μm). (B/C/E/F) cdc42 (scale bar = 10 μm /1 μm), Rab5A and RhoB (1 μm) were shown to participate in the macropinocytic machinery. (D) esiRNA knockdown of cdc42 did not affect uptake of SPIOPSN in HeLa cells. (G) ARF1 T31N rarely colocalized with SPIOPSN.

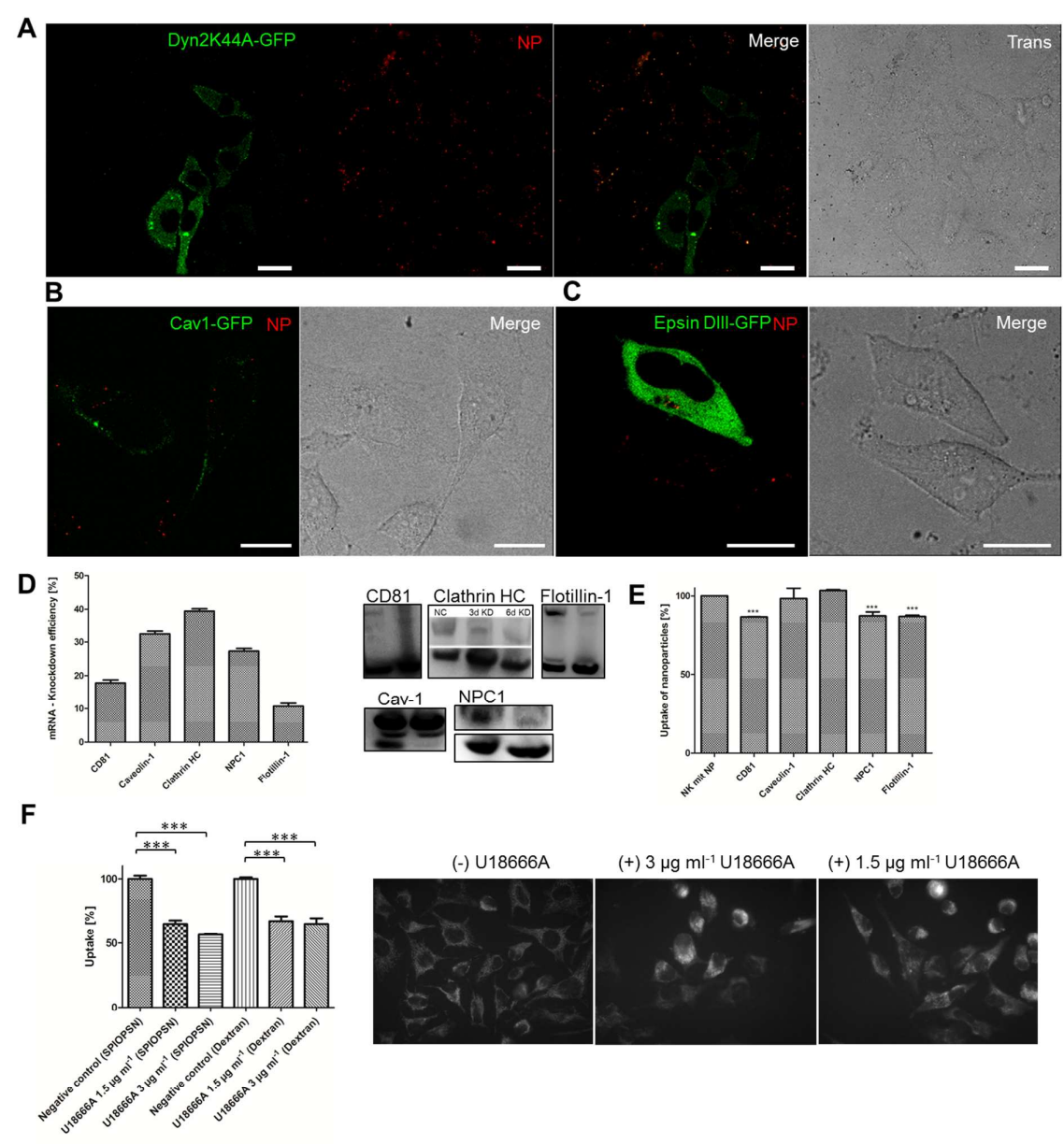
To investigate if de-acidification of the endolysosomal system influences the macropinocytic uptake of SPIOPSN, we treated HeLa cells with 10 nM Bafilomycin A1 to inhibit the v-type ATPase pumps we have identified in mass spectrometry (**Supplementary Fig. S3A**). Bafilomycin A1 treatment revealed a reduction of SPIOPSN uptake up to ~40%. AF488 Dextran (10kDa) served as a control (**Supplementary Fig. S3A**). Furthermore we were interested in the uptake behavior of SPIOPSN after receptor tyrosine kinase (RTK) stimulation by EGF. We found a massive decrease of SPIOPSN uptake after 6 h of coinubation with 1 μ M EGF. (**Supplementary Fig. S3B**). Blebbistatin is an inhibitor of non-muscular myosin II that is known to drive vesicle transport onto F-Actin filaments (**Supplementary Fig. S3C**). Uptake of SPIOPSN was not affected by myosin II inhibition.



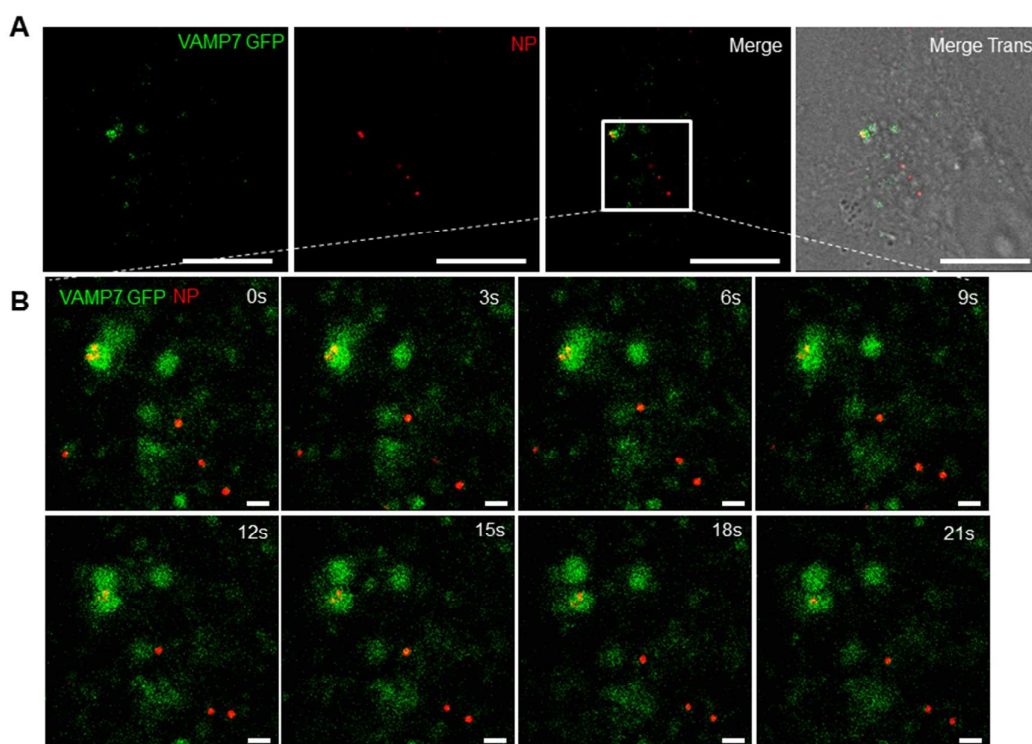
Supplementary Fig. S3: Different inhibitors/stimulators and their effect on SPIOPSN uptake.

(A) Treatment of HeLa cells with 10 nM Bafilomycin A1 (***) indicates $p < 0,001$ for SPIOPSN and Dextran) inhibited acidification of the endolysosomal system and suppressed uptake of SPIOPSN and dextran. (B) Treatment of HeLa cells with 1 μ M EGF suppressed uptake of SPIOPSN. (C) Treatment of HeLa cells with 75 μ M blebbistatin did not affect SPIOPSN uptake.

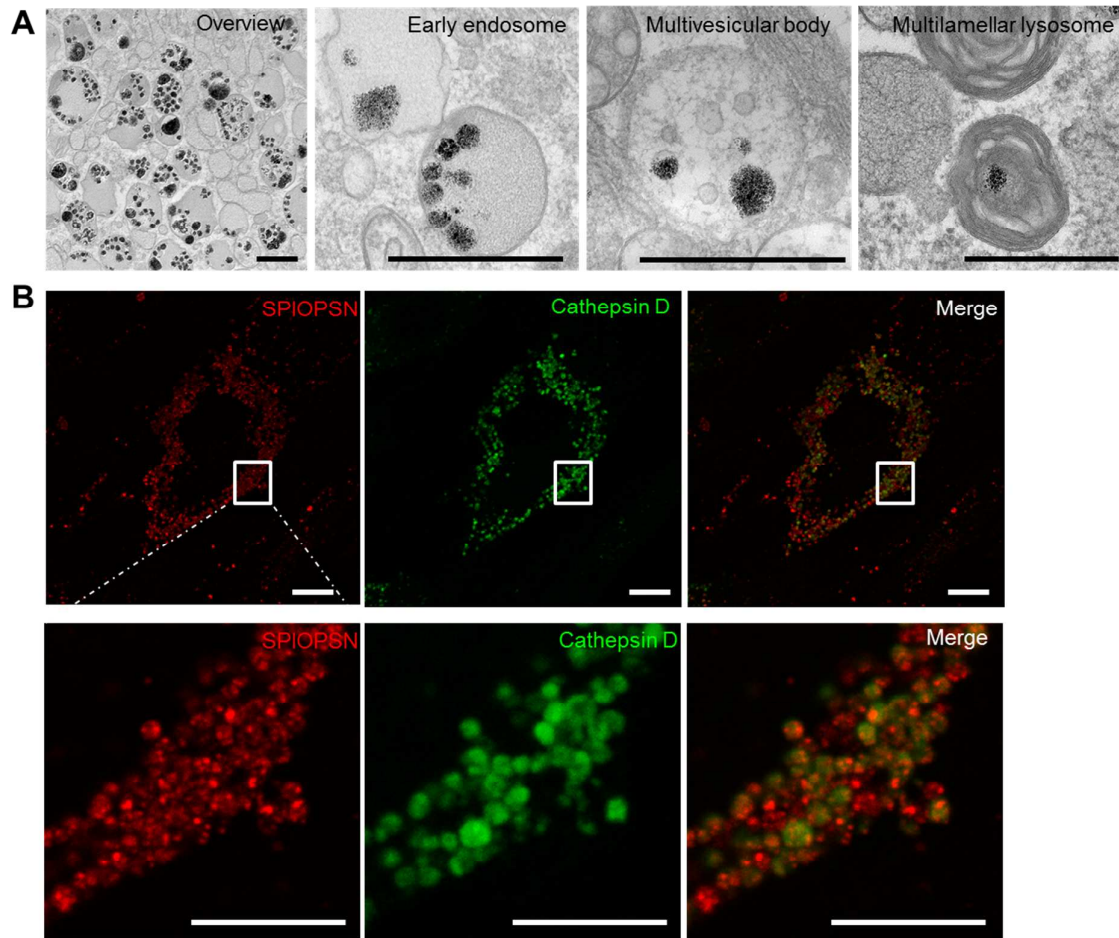
To investigate if SPIOPSN are endocytosed by a dynamin-dependent macropinocytic-like mechanism we overexpressed Dyn2 K44A-GFP. Uptake of SPIOPSN was drastically reduced. Overexpression of Caveolin-1 and Epsin DIII did not affect nanoparticle uptake. A significant decrease in SPIOPSN uptake was observed in siRNA experiments with CD81 and Flotillin-1. Deregulation of cholesterol homeostasis by U18666A suppressed SPIOPSN endocytosis (**Supplementary Fig. S4**).



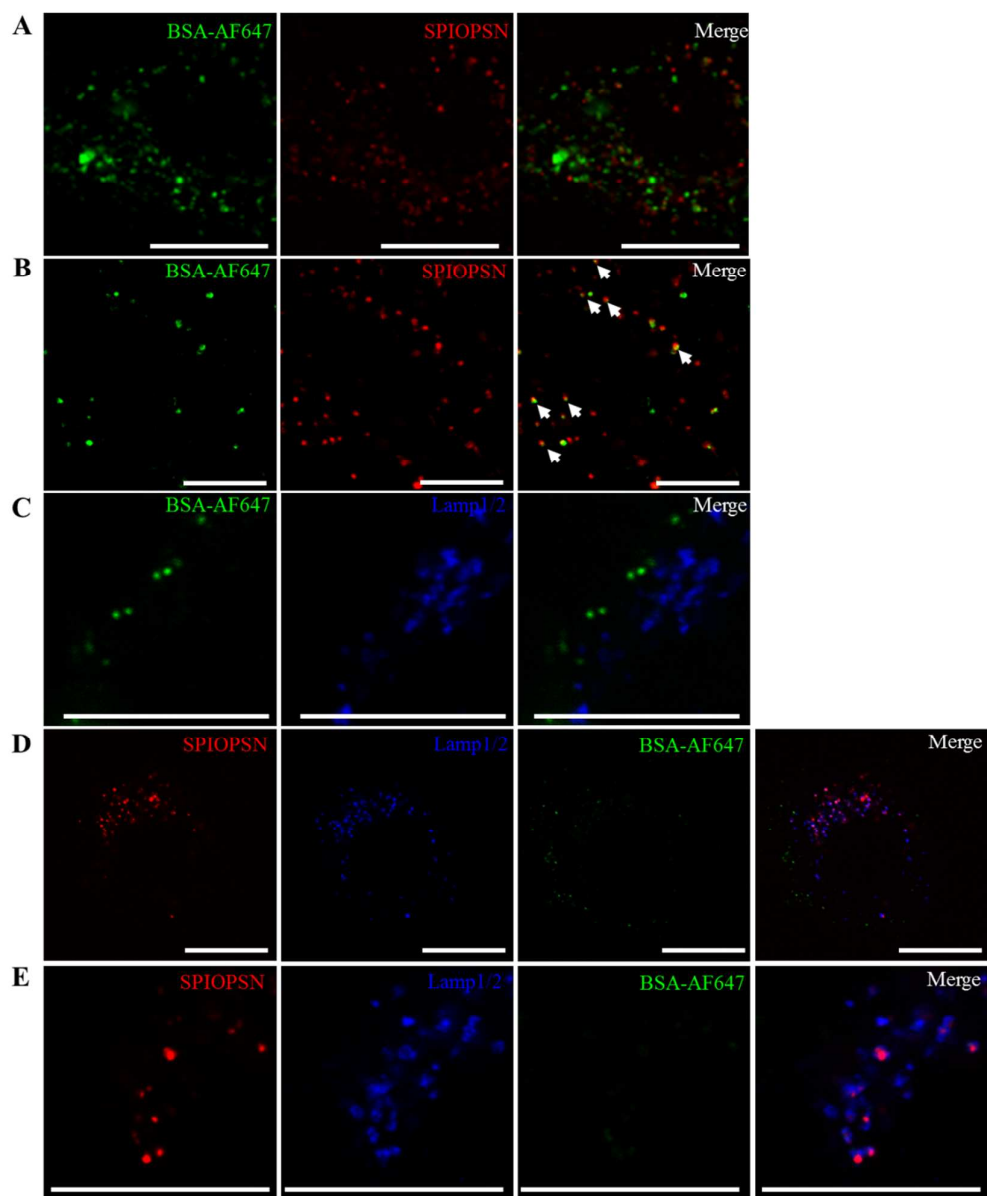
Supplementary Fig. S4: Overexpression and knockdown of different endocytosis-associated proteins. (A) Overexpression of Dyn2 K44A significantly suppressed SPIOPSN uptake (6 h SPIOPSN exposure). (B/C) Caveolin-1 as well as Epsin DIII-GFP overexpression did not significantly alter SPIOPSN uptake after 6h of exposure. (D/E) siRNA mediated knockdown of different proteins lead to a significant decrease of uptake in CD81 and Flotillin-1 silenced cells. (D) qPCR and Western blotting confirmed high rate of silencing. (F) U18666A treatment of HeLa cells inhibited SPIOPSN and dextran uptake (***) indicates $p < 0.001$). Cholesterol accumulates in endolysosomal structures (0.5 mg ml^{-1} Filipin).



Supplementary Fig. S5: Nanoparticles traffic inside late endosomal/lysosomal VAMP7⁺ vesicles. (A) Overview of VAMP7-GFP positive cell. (B) 21s of vesicle tracking of an Vamp7 positive organelle transporting three nanoparticles inside (scale bar magnification in **B** = $1 \mu\text{M}$).



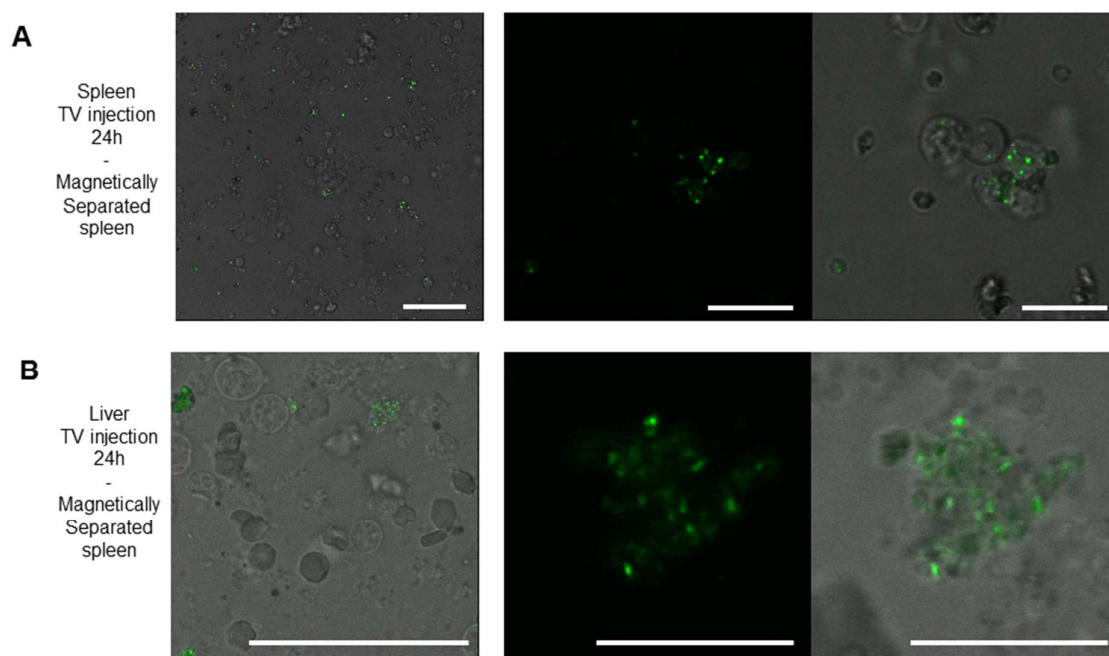
Supplementary Fig. S6: Intracellular trafficking of SPIOPSN in human mesenchymal stem cells. (A) Morphological characterization of SPIOPSN containing vesicles in hMSCs. SPIOPSN are taken up by hMSCs in a higher content than HeLa cells (overview). SPIOPSN traffic from early endosomes *via* multivesicular bodies to the multilamellar lysosome (scale bar = 1 μ m). (B) After 20 h of SPIOPSN exposure, nanoparticles are stored in Cathepsin D vesicles. Multiple fluorescent signals per vesicle are visible showing the high nanoparticle load of hMSCs (bar: cLSM: 10 μ M, bar: TEM: 1 μ M).



Supplementary Fig S7: The protein corona is cointernalized with nanoparticles and then degraded inside the lysosome. (A) HeLa cells were coexposed to $150 \mu\text{g ml}^{-1}$ SPIOPSN and 0.5 mg ml^{-1} BSA-AF647 for 8 h. Almost no colocalization was observed. (B) $150 \mu\text{g ml}^{-1}$ SPIOPSN were preincubated in 0.5 mg ml^{-1} BSA-AF647 for 60 min and then added for 6 h to HeLa cells. A large number of colocalization spots were observed (white arrows). (C) HeLa cells were incubated with BSA-AF647 for 20 h. BSA-AF647 was not detectable in Lamp1/2⁺ lysosomes. (D) Cells were exposed to BSA-AF647-preincubated SPIOPSN ($150 \mu\text{g ml}^{-1}$) for 20 h. No BSA-AF647 was present onto SPIOPSN. Minor amounts of excess BSA-AF647

accumulated inside Lamp1/Lamp2⁺ cells after 20 h of incubation. (E) Magnification of Lamp1/2⁺ lysosomes containing SPIOPSN. Less to no BSA-AF647 was detected inside lysosomes that harbored BSA-AF647-precoated SPIOPSN.

To study the *in vivo* distribution and the morphological appearance of SPIOPSN we tail-vein-injected nanoparticles into NSG mice. 20 h later we prepared liver, spleen, kidney and peripheral blood from the mice, magnetically separated the singlet cells and quantitatively analyzed their distribution. In all mice, no magnetic cells were found in peripheral blood and kidney (data not shown). Massive amounts of nanoparticles in endolysosomal structures were found in liver and spleen cells (**Supplementary Fig. S7**). This reveals that the synthesized SPIOPSN are suitable for magnetic cell separation and show similar vesicular structures as in the *in vitro* experiments.



Supplementary Fig. S8: Analysis of *in vivo* distribution of SPIOPSN after 20h (TV injection). After isolation of the organs, tissue was separated by a cell strainer and single cells were magnetically separated by MACS columns. (A) Spleen (B) Liver. Most of the nanoparticles were found in endolysosomal structures (right scale bar 10 μ m).

Lable free quantitative mass spectrometry: DATA

Supplementary Table S1: DAVID ontology analysis of proteins that were enriched > 2-fold in the magnetic fraction compared to the non-magnetic fraction.

18 Cluster(s)

Annotation Cluster 1	Enrichment Score: 42.07	Count	P_Value	Benjamini
GOTERM_CC_FAT	envelope	145	3.6E-45	5.1E-43
GOTERM_CC_FAT	organelle envelope	144	1.2E-44	1.4E-42
GOTERM_CC_FAT	mitochondrial envelope	115	7.1E-43	6.5E-41
GOTERM_CC_FAT	mitochondrial membrane	111	1.6E-42	1.2E-40
GOTERM_CC_FAT	mitochondrial inner membrane	96	5.3E-41	3.7E-39
GOTERM_CC_FAT	organelle inner membrane	99	1.5E-40	9.3E-39
Annotation Cluster 2	Enrichment Score: 29.76	Count	P_Value	Benjamini
GOTERM_CC_FAT	lysosome	69	1.3E-30	7.4E-29
GOTERM_CC_FAT	lytic vacuole	69	1.3E-30	7.4E-29
GOTERM_CC_FAT	vacuole	75	2.9E-30	1.5E-28
Annotation Cluster 3	Enrichment Score: 16.73	Count	P_Value	Benjamini
GOTERM_CC_FAT	vesicle	105	3.8E-18	1.5E-16
GOTERM_CC_FAT	membrane-bounded vesicle	93	2.5E-17	9.1E-16
GOTERM_CC_FAT	cytoplasmic membrane-bounded vesicle	91	2.9E-17	1.0E-15
GOTERM_CC_FAT	cytoplasmic vesicle	100	4.2E-17	1.4E-15
Annotation Cluster 4	Enrichment Score: 9.24	Count	P_Value	Benjamini
GOTERM_CC_FAT	membrane fraction	99	2.9E-10	6.2E-9
GOTERM_CC_FAT	insoluble fraction	101	4.7E-10	9.6E-9
GOTERM_CC_FAT	cell fraction	120	1.4E-9	2.6E-8
Annotation Cluster 5	Enrichment Score: 7.22	Count	P_Value	Benjamini
GOTERM_CC_FAT	outer membrane	26	1.2E-8	2.2E-7
GOTERM_CC_FAT	organelle outer membrane	24	1.2E-7	1.9E-6
GOTERM_CC_FAT	mitochondrial outer membrane	22	1.5E-7	2.4E-6

Annotation Cluster 6	Enrichment Score: 5.93	Count	P_Value	Benjamini
GOTERM_CC_FAT	respiratory chain complex I	14	1.2E-6	1.6E-5
GOTERM_CC_FAT	mitochondrial respiratory chain complex I	14	1.2E-6	1.6E-5
GOTERM_CC_FAT	NADH dehydrogenase complex	14	1.2E-6	1.6E-5
Annotation Cluster 7	Enrichment Score: 5.61	Count	P_Value	Benjamini
GOTERM_CC_FAT	membrane-enclosed lumen	171	2.6E-7	3.8E-6
GOTERM_CC_FAT	organelle lumen	163	3.2E-6	4.0E-5
GOTERM_CC_FAT	intracellular organelle lumen	156	1.8E-5	1.9E-4
Annotation Cluster 8	Enrichment Score: 3.33	Count	P_Value	Benjamini
GOTERM_CC_FAT	actomyosin	9	2.0E-4	1.6E-3
GOTERM_CC_FAT	stress fiber	8	5.5E-4	3.9E-3
GOTERM_CC_FAT	actin filament bundle	8	9.4E-4	6.4E-3
Annotation Cluster 9	Enrichment Score: 2.51	Count	P_Value	Benjamini
GOTERM_CC_FAT	cell-substrate junction	17	1.9E-3	1.2E-2
GOTERM_CC_FAT	cell-substrate adherens junction	16	2.9E-3	1.7E-2
GOTERM_CC_FAT	focal adhesion	15	5.2E-3	2.8E-2
Annotation Cluster 10	Enrichment Score: 2.29	Count	P_Value	Benjamini
GOTERM_CC_FAT	clathrin adaptor complex	8	1.9E-3	1.2E-2
GOTERM_CC_FAT	AP-type membrane coat adaptor complex	8	2.3E-3	1.4E-2
GOTERM_CC_FAT	clathrin coat	9	2.8E-3	1.6E-2
GOTERM_CC_FAT	endocytic vesicle membrane	6	5.6E-2	2.0E-1
Annotation Cluster 11	Enrichment Score: 2.15	Count	P_Value	Benjamini
GOTERM_CC_FAT	AP-2 adaptor complex	4	2.3E-3	1.4E-2
GOTERM_CC_FAT	clathrin coat of coated pit	5	2.5E-3	1.5E-2
GOTERM_CC_FAT	clathrin-coated endocytic vesicle	5	3.8E-3	2.1E-2
GOTERM_CC_FAT	clathrin coat of endocytic vesicle	4	4.5E-3	2.4E-2
GOTERM_CC_FAT	clathrin-coated endocytic vesicle membrane	4	1.1E-2	5.5E-2
GOTERM_CC_FAT	clathrin vesicle coat	4	1.2E-1	3.5E-1

Annotation Cluster 12	Enrichment Score: 2.1	Count	P_Value	Benjamini
GOTERM_CC_FAT	microbody part	9	5.2E-3	2.8E-2
GOTERM_CC_FAT	peroxisomal part	9	5.2E-3	2.8E-2
GOTERM_CC_FAT	microbody membrane	7	1.2E-2	5.9E-2
GOTERM_CC_FAT	peroxisomal membrane	7	1.2E-2	5.9E-2
Annotation Cluster 13	Enrichment Score: 2.09	Count	P_Value	Benjamini
GOTERM_CC_FAT	protein-lipid complex	8	5.8E-3	3.0E-2
GOTERM_CC_FAT	plasma lipoprotein particle	8	5.8E-3	3.0E-2
GOTERM_CC_FAT	triglyceride-rich lipoprotein particle	6	7.2E-3	3.7E-2
GOTERM_CC_FAT	very-low-density lipoprotein particle	6	7.2E-3	3.7E-2
GOTERM_CC_FAT	high-density lipoprotein particle	6	1.9E-2	8.6E-2
Annotation Cluster 14	Enrichment Score: 1.58	Count	P_Value	Benjamini
GOTERM_CC_FAT	tricarboxylic acid cycle enzyme complex	4	7.4E-3	3.8E-2
GOTERM_CC_FAT	dihydrolipoyl dehydrogenase complex	3	3.6E-2	1.4E-1
GOTERM_CC_FAT	mitochondrial alpha-ketoglutarate dehydrogenase complex	3	3.6E-2	1.4E-1
GOTERM_CC_FAT	mitochondrial tricarboxylic acid cycle enzyme complex	3	5.1E-2	1.9E-1
Annotation Cluster 15	Enrichment Score: 1.38	Count	P_Value	Benjamini
GOTERM_CC_FAT	COPI vesicle coat	4	2.9E-2	1.2E-1
GOTERM_CC_FAT	COPI coated vesicle membrane	4	3.7E-2	1.4E-1
GOTERM_CC_FAT	COPI-coated vesicle	4	6.6E-2	2.3E-1
Annotation Cluster 16	Enrichment Score: 0.97	Count	P_Value	Benjamini
GOTERM_CC_FAT	vesicle lumen	7	7.0E-2	2.3E-1
GOTERM_CC_FAT	platelet alpha granule lumen	6	1.2E-1	3.5E-1
GOTERM_CC_FAT	cytoplasmic membrane-bounded vesicle lumen	6	1.5E-1	4.1E-1
Annotation Cluster 17	Enrichment Score: 0.58	Count	P_Value	Benjamini
GOTERM_CC_FAT	contractile fiber part	11	1.8E-1	4.8E-1
GOTERM_CC_FAT	contractile fiber	11	2.4E-1	5.7E-1

GOTERM_CC_FAT	myofibril	9	4.1E-1	7.6E-1
Annotation Cluster 18	Enrichment Score: 0.44	Count	P_Value	Benjamini
GOTERM_CC_FAT	spectrin	3	1.1E-1	3.4E-1
GOTERM_CC_FAT	cortical actin cytoskeleton	3	5.4E-1	8.5E-1
GOTERM_CC_FAT	cortical cytoskeleton	3	8.3E-1	9.8E-1

Supplementary Table S2: Reconstruction of SPIOPSN trafficking is based on the GOTERM: *Lysosome*.

Uniprot_ID	Protein name
ARL8A_HUMAN	ADP-ribosylation factor-like 8A
ARL8B_HUMAN	ADP-ribosylation factor-like 8B
VATG1_HUMAN	ATPase, H ⁺ transporting, lysosomal 13kDa, V1 subunit G1
CD63_HUMAN	CD63 molecule
SAP3_HUMAN	GM2 ganglioside activator
NAGAB_HUMAN	N-acetylgalactosaminidase, alpha-
ANAG_HUMAN	N-acetylglucosaminidase, alpha-
ASAHI_HUMAN	N-acylsphingosine amidohydrolase (acid ceramidase) 1
SPHM_HUMAN	N-sulfoglucosamine sulfohydrolase
NPC1_HUMAN	Niemann-Pick disease, type C1
NPC2_HUMAN	Niemann-Pick disease, type C2
RAB14_HUMAN	RAB14, member RAS oncogene family
RB27A_HUMAN	RAB27A, member RAS oncogene family
RAB7A_HUMAN	RAB7A, member RAS oncogene family
RAB9A_HUMAN	RAB9A, member RAS oncogene family
PPAL_HUMAN	acid phosphatase 2, lysosomal
AP3M1_HUMAN	adaptor-related protein complex 3, mu 1 subunit
ARSA_HUMAN	arylsulfatase A

ARSB_HUMAN	arylsulfatase B
CATA_HUMAN	catalase
PPGB_HUMAN	cathepsin A
CATB_HUMAN	cathepsin B
CATC_HUMAN	cathepsin C
CATD_HUMAN	cathepsin D
CATL1_HUMAN	cathepsin L1
CATZ_HUMAN	cathepsin Z
CLN3_HUMAN	ceroid-lipofuscinosis, neuronal 3
CLN5_HUMAN	ceroid-lipofuscinosis, neuronal 5
CLCN7_HUMAN	chloride channel 7
NCUG1_HUMAN	chromosome 1 open reading frame 85
DNS2A_HUMAN	deoxyribonuclease II, lysosomal
DPP2_HUMAN	dipeptidyl-peptidase 7
EPDR1_HUMAN	ependymin related protein 1 (zebrafish)
GALNS_HUMAN	galactosamine (N-acetyl)-6-sulfate sulfatase
AGAL_HUMAN	galactosidase, alpha
BGAL_HUMAN	galactosidase, beta 1
GGH_HUMAN	gamma-glutamyl hydrolase (conjugase, folylpolyglutamyglutamyl hydrolase)
GNS_HUMAN	glucosamine (N-acetyl)-6-sulfatase
LYAG_HUMAN	glucosidase, alpha; acid
GLCM_HUMAN	glucosidase, beta; acid (includes glucosylceramidase)
BGLR_HUMAN	glucuronidase, beta
HEXA_HUMAN	hexosaminidase A (alpha polypeptide)
HEXB_HUMAN	hexosaminidase B (beta polypeptide)
MPRI_HUMAN	insulin-like growth factor 2 receptor
GILT_HUMAN	interferon, gamma-inducible protein 30

LGMN_HUMAN	legumain
LICH_HUMAN	lipase A, lysosomal acid, cholesterol esterase
LAMP1_HUMAN	lysosomal-associated membrane protein 1
LAMP2_HUMAN	lysosomal-associated membrane protein 2
MPRD_HUMAN	mannose-6-phosphate receptor (cation dependent)
MA2B1_HUMAN	mannosidase, alpha, class 2B, member 1
MANBA_HUMAN	mannosidase, beta A, lysosomal
MCLN1_HUMAN	mucolipin 1
NICA_HUMAN	nicastrin
PPT1_HUMAN	palmitoyl-protein thioesterase 1
PPT2_HUMAN	palmitoyl-protein thioesterase 2
PAG15_HUMAN	phospholipase A2, group XV
PCYOX_HUMAN	prenylcysteine oxidase 1
PCP_HUMAN	prolylcarboxypeptidase (angiotensinase C)
SAP_HUMAN	prosaposin
SCR2_HUMAN	scavenger receptor class B, member 2
NEUR1_HUMAN	sialidase 1 (lysosomal sialidase)
S15A4_HUMAN	solute carrier family 15, member 4
ASM_HUMAN	sphingomyelin phosphodiesterase 1, acid lysosomal
STX7_HUMAN	syntaxin 7
STXB2_HUMAN	syntaxin binding protein 2
TM192_HUMAN	transmembrane protein 192
TPP1_HUMAN	tripeptidyl peptidase I
VAMP7_HUMAN	vesicle-associated membrane protein 7

Supplementary Table S3: GOTERM: *Vesicle*.**Not considered in GOTERM analysis; manually inserted.*

ADA10_HUMAN	ADAM metallopeptidase domain 10
VA0D1_HUMAN	ATPase, H ⁺ transporting, lysosomal 38kDa, V0 subunit d1
VATC1_HUMAN	ATPase, H ⁺ transporting, lysosomal 42kDa, V1 subunit C1
VATB2_HUMAN	ATPase, H ⁺ transporting, lysosomal 56/58kDa, V1 subunit B2
VPP1_HUMAN	ATPase, H ⁺ transporting, lysosomal V0 subunit a1
AT1A1_HUMAN	ATPase, Na ⁺ /K ⁺ transporting, alpha 1 polypeptide
AT1B3_HUMAN	ATPase, Na ⁺ /K ⁺ transporting, beta 3 polypeptide
CD9_HUMAN	CD9 molecule
DNJC5_HUMAN	DnaJ (Hsp40) homolog, subfamily C, member 5
EHD1_HUMAN	EH-domain containing 1
RAI3_HUMAN	G protein-coupled receptor, family C, group 5, member A
GIPC1_HUMAN	GIPC PDZ domain containing family, member 1
GNAS2_HUMAN	GNAS complex locus
RB11A_HUMAN	RAB11A, member RAS oncogene family
RB11B_HUMAN	RAB11B, member RAS oncogene family
RAB14_HUMAN	RAB14, member RAS oncogene family
RAB21_HUMAN	RAB21, member RAS oncogene family
RB27A_HUMAN	RAB27A, member RAS oncogene family
RAB2A_HUMAN	RAB2A, member RAS oncogene family
RAB3A_HUMAN	RAB3A, member RAS oncogene family
RAB3D_HUMAN	RAB3D, member RAS oncogene family
RAB5A_HUMAN	RAB5A, member RAS oncogene family
RAB5C_HUMAN	RAB5C, member RAS oncogene family
RAB6B_HUMAN	RAB6B, member RAS oncogene family
RAB7A_HUMAN	RAB7A, member RAS oncogene family

SH3B4_HUMAN	SH3-domain binding protein 4
SC23A_HUMAN	Sec23 homolog A (S. cerevisiae)
VAPA_HUMAN	VAMP (vesicle-associated membrane protein)-associated protein A, 33kDa
YKT6_HUMAN	YKT6 v-SNARE homolog (S. cerevisiae)
DP13A_HUMAN	adaptor protein, phosphotyrosine interaction, PH domain and leucine zipper containing 1
AP1B1_HUMAN	adaptor-related protein complex 1, beta 1 subunit
AP2A1_HUMAN	adaptor-related protein complex 2, alpha 1 subunit
AP2A2_HUMAN	adaptor-related protein complex 2, alpha 2 subunit
AP2B1_HUMAN	adaptor-related protein complex 2, beta 1 subunit
AP2M1_HUMAN	adaptor-related protein complex 2, mu 1 subunit
AP2S1_HUMAN	adaptor-related protein complex 2, sigma 1 subunit
AMPN_HUMAN	alanyl (membrane) aminopeptidase
A4_HUMAN	amyloid beta (A4) precursor protein
APOA1_HUMAN	apolipoprotein A-I
ARSA_HUMAN	arylsulfatase A
BASI_HUMAN	basigin (Ok blood group)
CATB_HUMAN	cathepsin B
CATD_HUMAN	cathepsin D
CAV1_HUMAN	caveolin 1, caveolae protein, 22kDa
CAV2_HUMAN	caveolin 2
CLN3_HUMAN	ceroid-lipofuscinosis, neuronal 3
CLIC4_HUMAN	chloride intracellular channel 4
CLUS_HUMAN	clusterin
COPB_HUMAN	coatamer protein complex, subunit beta 1
COPB2_HUMAN	coatamer protein complex, subunit beta 2 (beta prime)
COPE_HUMAN	coatamer protein complex, subunit epsilon
COPZ1_HUMAN	coatamer protein complex, subunit zeta 1

DLDH_HUMAN	dihydrolipoamide dehydrogenase
DPP2_HUMAN	dipeptidyl-peptidase 7
DAB2_HUMAN	disabled homolog 2, mitogen-responsive phosphoprotein (Drosophila)
ECE1_HUMAN	endothelin converting enzyme 1
EGFR_HUMAN	epidermal growth factor receptor (erythroblastic leukemia viral (v-erb-b) oncogene homolog, avian)
FIBA_HUMAN	fibrinogen alpha chain
FIBB_HUMAN	fibrinogen beta chain
FIBG_HUMAN	fibrinogen gamma chain
FLOT1_HUMAN	flotillin 1
GGH_HUMAN	gamma-glutamyl hydrolase (conjugase, folylpolygammaglutamyl hydrolase)
GANAB_HUMAN	glucosidase, alpha; neutral AB
GPNMB_HUMAN	glycoprotein (transmembrane) nmb
GNAI3_HUMAN	guanine nucleotide binding protein (G protein), alpha inhibiting activity polypeptide 3
CH60_HUMAN	heat shock 60kDa protein 1 (chaperonin) pseudogene 5;
HEXB_HUMAN	hexosaminidase B (beta polypeptide)
MPRI_HUMAN	insulin-like growth factor 2 receptor
ITA1_HUMAN	integrin, alpha 1
ITB1_HUMAN	integrin, beta 1
LAMP1_HUMAN	lysosomal-associated membrane protein 1
LAMP2_HUMAN	lysosomal-associated membrane protein 2
MYOF_HUMAN	myoferlin
MYH11_HUMAN	myosin, heavy chain 11, smooth muscle
NICA_HUMAN	nicastatin
PPT1_HUMAN	palmitoyl-protein thioesterase 1
STOM_HUMAN	phosphatidylethanolamine binding protein 1
P4K2A_HUMAN	phosphatidylinositol 4-kinase type 2 alpha
PICAL_HUMAN	phosphatidylinositol binding clathrin assembly protein

PA24A_HUMAN	phospholipase A2, group IVA (cytosolic, calcium-dependent)
PLD1_HUMAN	phospholipase D1, phosphatidylcholine-specific
PTN1_HUMAN	protein tyrosine phosphatase, non-receptor type 1
RPN1_HUMAN	ribophorin I
SCFD1_HUMAN	sec1 family domain containing 1
NEUR1_HUMAN	sialidase 1 (lysosomal sialidase)
RAB35_HUMAN	similar to hCG1778032; RAB35, member RAS oncogene family
SATT_HUMAN	solute carrier family 1 (glutamate/neutral amino acid transporter), member 4
AAAT_HUMAN	solute carrier family 1 (neutral amino acid transporter), member 5
GTR1_HUMAN	solute carrier family 2 (facilitated glucose transporter), member 1
SPG21_HUMAN	spastic paraplegia 21 (autosomal recessive, Mast syndrome)
ASM_HUMAN	sphingomyelin phosphodiesterase 1, acid lysosomal
STOM_HUMAN	stomatin
SYPL1_HUMAN	synaptophysin-like 1
SDCB1_HUMAN	syndecan binding protein (syntenin)
STX12_HUMAN	syntaxin 12
STXB1_HUMAN	syntaxin binding protein 1
STXB2_HUMAN	syntaxin binding protein 2
STXB3_HUMAN	syntaxin binding protein 3
SNTB2_HUMAN	syntrophin, beta 2 (dystrophin-associated protein A1, 59kDa, basic component 2)
TSP1_HUMAN	thrombospondin 1
TFR1_HUMAN	transferrin receptor (p90, CD71)
TMED2_HUMAN	transmembrane emp24 domain trafficking protein 2
TMEDA_HUMAN	transmembrane emp24-like trafficking protein 10 (yeast)
TPP1_HUMAN	tripeptidyl peptidase I
VAMP7_HUMAN	vesicle-associated membrane protein 7
1B55_HUMAN*	HLA class I histocompatibility antigen, B-55 alpha chain

1C06_HUMAN*	HLA class I histocompatibility antigen, Cw-6 alpha chain
ARF1_HUMAN*	ADP-ribosylation factor 1
ARF4_HUMAN*	ADP-ribosylation factor 4
COPG1_HUMAN*	Coatomer subunit gamma-1
LTOR1_HUMAN*	Ragulator complex protein LAMTOR1
LTOR2_HUMAN*	Ragulator complex protein LAMTOR2
LTOR3_HUMAN*	Ragulator complex protein LAMTOR3
RAB1A_HUMAN*	Ras-related protein Rab-1A
RAP1A_HUMAN*	Ras-related protein Rap-1A
RAP1B_HUMAN*	Ras-related protein Rap-1b

Supplementary Table S4: GOTERM: *Membrane fraction.*

5NTD_HUMAN	5'-nucleotidase, ecto (CD73)
ARF6_HUMAN	ADP-ribosylation factor 6
MRP1_HUMAN	ATP-binding cassette, sub-family C (CFTR/MRP), member 1
AT2A1_HUMAN	ATPase, Ca ⁺⁺ transporting, cardiac muscle, fast twitch 1
VATF_HUMAN	ATPase, H ⁺ transporting, lysosomal 14kDa, V1 subunit F
VA0D1_HUMAN	ATPase, H ⁺ transporting, lysosomal 38kDa, V0 subunit d1
VATB1_HUMAN	ATPase, H ⁺ transporting, lysosomal 56/58kDa, V1 subunit B1
AT1A1_HUMAN	ATPase, Na ⁺ /K ⁺ transporting, alpha 1 polypeptide
AT1A2_HUMAN	ATPase, Na ⁺ /K ⁺ transporting, alpha 2 (+) polypeptide
CD59_HUMAN	CD59 molecule, complement regulatory protein
EHD2_HUMAN	EH-domain containing 2
ERO1A_HUMAN	ERO1-like (S. cerevisiae)
GIPC1_HUMAN	GIPC PDZ domain containing family, member 1

GNAS2_HUMAN	GNAS complex locus
L1CAM_HUMAN	L1 cell adhesion molecule
SNAG_HUMAN	N-ethylmaleimide-sensitive factor attachment protein, gamma
NCPR_HUMAN	P450 (cytochrome) oxidoreductase
RAB14_HUMAN	RAB14, member RAS oncogene family
RAB3A_HUMAN	RAB3A, member RAS oncogene family
RAB5A_HUMAN	RAB5A, member RAS oncogene family
USO1_HUMAN	USO1 homolog, vesicle docking protein (yeast)
VAPA_HUMAN	VAMP (vesicle-associated membrane protein)-associated protein A, 33kDa
PPAL_HUMAN	acid phosphatase 2, lysosomal
ACSL4_HUMAN	acyl-CoA synthetase long-chain family member 4
DP13A_HUMAN	adaptor protein, phosphotyrosine interaction, PH domain and leucine zipper containing 1
A4_HUMAN	amyloid beta (A4) precursor protein
AIP_HUMAN	aryl hydrocarbon receptor interacting protein
NCEH1_HUMAN	arylacetamide deacetylase-like 1
ARSA_HUMAN	arylsulfatase A
CPT1A_HUMAN	carnitine palmitoyltransferase 1A (liver)
COMT_HUMAN	catechol-O-methyltransferase
CTNB1_HUMAN	catenin (cadherin-associated protein), beta 1, 88kDa
CTND1_HUMAN	catenin (cadherin-associated protein), delta 1
CAV1_HUMAN	caveolin 1, caveolae protein, 22kDa
CAV2_HUMAN	caveolin 2
CLN3_HUMAN	ceroid-lipofuscinosis, neuronal 3

CYB5B_HUMAN	cytochrome b5 type B (outer mitochondrial membrane)
CKAP4_HUMAN	cytoskeleton-associated protein 4
ODO2_HUMAN	dihydrolipoamide S-succinyltransferase
OST48_HUMAN	dolichyl-diphosphooligosaccharide-protein glycosyltransferase
DPM1_HUMAN	dolichyl-phosphate mannosyltransferase polypeptide 1, catalytic subunit
EGLN_HUMAN	endoglin
ECE1_HUMAN	endothelin converting enzyme 1
HYEP_HUMAN	epoxide hydrolase 1, microsomal (xenobiotic)
FLOT1_HUMAN	flotillin 1
FLOT2_HUMAN	flotillin 2
FOLR1_HUMAN	folate receptor 1 (adult)
GNA11_HUMAN	guanine nucleotide binding protein (G protein), alpha 11 (Gq class)
GNAI2_HUMAN	guanine nucleotide binding protein (G protein), alpha inhibiting activity polypeptide 2
GNAI3_HUMAN	guanine nucleotide binding protein (G protein), alpha inhibiting activity polypeptide 3
GNAQ_HUMAN	guanine nucleotide binding protein (G protein), q polypeptide
HMOX1_HUMAN	heme oxygenase (decycling) 1
HMOX2_HUMAN	heme oxygenase (decycling) 2
IGHG2_HUMAN	immunoglobulin heavy constant gamma 2 (G2m marker)
IGHG4_HUMAN	immunoglobulin heavy constant gamma 4 (G4m marker)
MPRI_HUMAN	insulin-like growth factor 2 receptor
ITA1_HUMAN	integrin, alpha 1
ITA5_HUMAN	integrin, alpha 5 (fibronectin receptor, alpha polypeptide)
ITB1_HUMAN	integrin, beta 1 (fibronectin receptor, beta polypeptide, antigen CD29 includes MDF2,

	MSK12)
PLAK_HUMAN	junction plakoglobin
ERG7_HUMAN	lanosterol synthase (2,3-oxidosqualene-lanosterol cyclase)
LMAN1_HUMAN	lectin, mannose-binding, 1
LCAP_HUMAN	leucyl/cystinyl aminopeptidase
LIN7C_HUMAN	lin-7 homolog C (C. elegans)
LAMP1_HUMAN	lysosomal-associated membrane protein 1
LAMP2_HUMAN	lysosomal-associated membrane protein 2
1B55_HUMAN, 1C06_HUMAN	major histocompatibility complex, class I, C; major histocompatibility complex, class I, B
MET_HUMAN	met proto-oncogene (hepatocyte growth factor receptor)
ANPRC_HUMAN	natriuretic peptide receptor C/guanylate cyclase C (atrionatriuretic peptide receptor C)
RASN_HUMAN	neuroblastoma RAS viral (v-ras) oncogene homolog
NICA_HUMAN	nicastatin
PPT1_HUMAN	palmitoyl-protein thioesterase 1
PPIF_HUMAN	peptidylprolyl isomerase F
STOM_HUMAN	phosphatidylethanolamine binding protein 1
P4K2A_HUMAN	phosphatidylinositol 4-kinase type 2 alpha
PA24A_HUMAN	phospholipase A2, group IVA (cytosolic, calcium-dependent)
PLD1_HUMAN	phospholipase D1, phosphatidylcholine-specific
LIS1_HUMAN	platelet-activating factor acetylhydrolase, isoform Ib, subunit 1 (45kDa)
PGRC1_HUMAN	progesterone receptor membrane component 1
KPCA_HUMAN	protein kinase C, alpha

KAP2_HUMAN	protein kinase, cAMP-dependent, regulatory, type II, alpha
RHOB_HUMAN	ras homolog gene family, member B
SCRB1_HUMAN	scavenger receptor class B, member 1
SCRB2_HUMAN	scavenger receptor class B, member 2
SEPT2_HUMAN	septin 2
SPCS2_HUMAN	signal peptidase complex subunit 2 homolog (S. cerevisiae); signal peptidase complex subunit 2 homolog pseudogene
SPCS3_HUMAN	signal peptidase complex subunit 3 homolog (S. cerevisiae)
AAAT_HUMAN	solute carrier family 1 (neutral amino acid transporter), member 5
MOT1_HUMAN	solute carrier family 16, member 1 (monocarboxylic acid transporter 1)
GTR1_HUMAN	solute carrier family 2 (facilitated glucose transporter), member 1
SPTA2_HUMAN	spectrin, alpha, non-erythrocytic 1 (alpha-fodrin)
SNTB2_HUMAN	syntrophin, beta 2 (dystrophin-associated protein A1, 59kDa, basic component 2)
TMX1_HUMAN	thioredoxin-related transmembrane protein 1
TOM22_HUMAN	translocase of outer mitochondrial membrane 22 homolog (yeast)
TOM40_HUMAN	translocase of outer mitochondrial membrane 40 homolog (yeast)
TMED2_HUMAN	transmembrane emp24 domain trafficking protein 2
TMEDA_HUMAN	transmembrane emp24-like trafficking protein 10 (yeast)
VDAC3_HUMAN	voltage-dependent anion channel 3
FACE1_HUMAN	zinc metallopeptidase (STE24 homolog, S. cerevisiae)
ADAM9_HUMAN	ADAM metallopeptidase domain 9 (meltrin gamma)
ARL1_HUMAN	ADP-ribosylation factor-like 1
AT2B1_HUMAN	ATPase, Ca ⁺⁺ transporting, plasma membrane 1

AT2B4_HUMAN	ATPase, Ca ⁺⁺ transporting, plasma membrane 4
RENH_HUMAN	ATPase, H ⁺ transporting, lysosomal accessory protein 2
AT12A_HUMAN	ATPase, H ⁺ /K ⁺ transporting, nongastric, alpha polypeptide
AT1B1_HUMAN	ATPase, Na ⁺ /K ⁺ transporting, beta 1 polypeptide
UFO_HUMAN	AXL receptor tyrosine kinase
CD276_HUMAN	CD276 molecule
DAF_HUMAN	CD55 molecule, decay accelerating factor for complement (Cromer blood group)
LFA3_HUMAN	CD58 molecule
CD81_HUMAN	CD81 molecule
CD82_HUMAN	CD82 molecule
DD19A_HUMAN	DEAD (Asp-Glu-Ala-As) box polypeptide 19A
GBG10_HUMAN	DnaJ (Hsp40) homolog, subfamily C , member 25; guanine nucleotide binding protein (G protein), gamma 10; DNAJC25-GNG10 readthrough transcript
EPHA2_HUMAN	EPH receptor A2
EPHB2_HUMAN	EPH receptor B2
GPR56_HUMAN	G protein-coupled receptor 56
LIMA1_HUMAN	LIM domain and actin binding 1
RAB18_HUMAN	RAB18, member RAS oncogene family
RAB1B_HUMAN	RAB1B, member RAS oncogene family
RAB31_HUMAN	RAB31, member RAS oncogene family
RAP2A_HUMAN	RAP2A, member of RAS oncogene family
RAP2B_HUMAN	RAP2B, member of RAS oncogene family
RAGP1_HUMAN	Ran GTPase activating protein 1

RHEB_HUMAN	Ras homolog enriched in brain
RHG17_HUMAN	Rho GTPase activating protein 17
VPP3_HUMAN	T-cell, immune regulator 1, ATPase, H ⁺ transporting, lysosomal V0 subunit A3
THY1_HUMAN	Thy-1 cell surface antigen
ANKH_HUMAN	ankylosis, progressive homolog (mouse)
CADH2_HUMAN	cadherin 2, type 1, N-cadherin (neuronal)
CNN2_HUMAN	calponin 2
CTNA1_HUMAN	catenin (cadherin-associated protein), alpha 1, 102kDa
CTNA2_HUMAN	catenin (cadherin-associated protein), alpha 2
CDC42_HUMAN	cell division cycle 42 (GTP binding protein, 25kDa); cell division cycle 42 pseudogene 2
CERU_HUMAN	ceruloplasmin (ferroxidase)
CXAR_HUMAN	coxsackie virus and adenovirus receptor pseudogene 2; coxsackie virus and adenovirus receptor
DSG2_HUMAN	desmoglein 2
DIAP1_HUMAN	diaphanous homolog 1 (Drosophila)
DLG1_HUMAN	discs, large homolog 1 (Drosophila)
DYN2_HUMAN	dynammin 2
LAP2_HUMAN	erbB2 interacting protein
FERM2_HUMAN	fermitin family homolog 2 (Drosophila)
GBB1_HUMAN	guanine nucleotide binding protein (G protein), beta polypeptide 1
GBG12_HUMAN	guanine nucleotide binding protein (G protein), gamma 12
HGS_HUMAN	hepatocyte growth factor-regulated tyrosine kinase substrate
APOE_HUMAN	hypothetical LOC100129500; apolipoprotein E

CD166_HUMAN	hypothetical protein LOC100133690; activated leukocyte cell adhesion molecule
ITA2_HUMAN	integrin, alpha 2 (CD49B, alpha 2 subunit of VLA-2 receptor)
ITA3_HUMAN	integrin, alpha 3 (antigen CD49C, alpha 3 subunit of VLA-3 receptor)
ITA6_HUMAN	integrin, alpha 6
ITB4_HUMAN	integrin, beta 4
ITB5_HUMAN	integrin, beta 5
ILK_HUMAN	integrin-linked kinase
IL6RB_HUMAN	interleukin 6 signal transducer (gp130, oncostatin M receptor)
1A68_HUMAN	major histocompatibility complex, class I, A
1B55_HUMAN, 1C06_HUMAN	major histocompatibility complex, class I, C; major histocompatibility complex, class I, B
MPZL1_HUMAN	myelin protein zero-like 1
MYO1C_HUMAN	myosin IC
NEXN_HUMAN	nexilin (F actin binding protein)
NU155_HUMAN	nucleoporin 155kDa
PARVA_HUMAN	parvin, alpha
PARVB_HUMAN	parvin, beta
PODXL_HUMAN	podocalyxin-like
PVRL2_HUMAN	poliovirus receptor-related 2 (herpesvirus entry mediator B)
EPCR_HUMAN	protein C receptor, endothelial (EPCR)
PACN3_HUMAN	protein kinase C and casein kinase substrate in neurons 3
PTPRJ_HUMAN	protein tyrosine phosphatase, receptor type, J
RHOG_HUMAN	ras homolog gene family, member G (rho G)

RRAS_HUMAN	related RAS viral (r-ras) oncogene homolog
SCRIB_HUMAN	scribbled homolog (Drosophila)
STAM1_HUMAN	signal transducing adaptor molecule (SH3 domain and ITAM motif) 1
S12A7_HUMAN	solute carrier family 12 (potassium/chloride transporters), member 7
CTR1_HUMAN	solute carrier family 7 (cationic amino acid transporter, y ⁺ system), member 1
NHRF1_HUMAN	solute carrier family 9 (sodium/hydrogen exchanger), member 3 regulator 1
NHRF2_HUMAN	solute carrier family 9 (sodium/hydrogen exchanger), member 3 regulator 2
TRIP6_HUMAN	thyroid hormone receptor interactor 6
ZO1_HUMAN	tight junction protein 1 (zona occludens 1)
TPBG_HUMAN	trophoblast glycoprotein
RALA_HUMAN	v-ral simian leukemia viral oncogene homolog A (ras related)
VASP_HUMAN	vasodilator-stimulated phosphoprotein
ZYX_HUMAN	zyxin

Interestingly, we identified several mitochondrial proteins in the magnetic fraction that showed an enrichment factor > 2. Since we have identified a large number of membrane whorls inside TEM experiments we propose that the fusion with the autophagosomal pathway introduces several nonendolysosomal proteins into the pathway. However, less impurities were found from other compartments as seen by DAVID ontology analysis of the remaining proteins that have not been clustered in the GOTERMs of Supplementary Table S1-S5. (see

DAVID ontology analysis of remaining proteins in Supplementary Table S6).

Supplementary Table S5: GOTERM: *Mitochondrial envelope + mitochondria*

HMGCL_HUMAN	3-hydroxymethyl-3-methylglutaryl-Coenzyme A lyase
AT5F1_HUMAN	ATP synthase, H ⁺ transporting, mitochondrial F0 complex, subunit B1
ATP5I_HUMAN	ATP synthase, H ⁺ transporting, mitochondrial F0 complex, subunit E
ATPK_HUMAN	ATP synthase, H ⁺ transporting, mitochondrial F0 complex, subunit F2
ATP5J_HUMAN	ATP synthase, H ⁺ transporting, mitochondrial F0 complex, subunit F6
ATP5L_HUMAN	ATP synthase, H ⁺ transporting, mitochondrial F0 complex, subunit G
ATP5H_HUMAN	ATP synthase, H ⁺ transporting, mitochondrial F0 complex, subunit d
ATPO_HUMAN	ATP synthase, H ⁺ transporting, mitochondrial F1 complex, O subunit
ATPA_HUMAN	ATP synthase, H ⁺ transporting, mitochondrial F1 complex, alpha subunit 1, cardiac muscle
ATPB_HUMAN	ATP synthase, H ⁺ transporting, mitochondrial F1 complex, beta polypeptide
ATPD_HUMAN	ATP synthase, H ⁺ transporting, mitochondrial F1 complex, delta subunit
ATPG_HUMAN	ATP synthase, H ⁺ transporting, mitochondrial F1 complex, gamma polypeptide 1
ABCB6_HUMAN	ATP-binding cassette, sub-family B (MDR/TAP), member 6
BAX_HUMAN	BCL2-associated X protein
BCS1_HUMAN	BCS1-like (yeast)
CISD1_HUMAN	CDGSH iron sulfur domain 1
CLPX_HUMAN	ClpX caseinolytic peptidase X homolog (E. coli)
COX2_HUMAN	Cytochrome c oxidase subunit 2
DJC11_HUMAN	DnaJ (Hsp40) homolog, subfamily C, member 11
NDUAA_HUMAN	NADH dehydrogenase (ubiquinone) 1 alpha subcomplex, 10, 42kDa
NDUAB_HUMAN	NADH dehydrogenase (ubiquinone) 1 alpha subcomplex, 11, 14.7kDa
NDUAD_HUMAN	NADH dehydrogenase (ubiquinone) 1 alpha subcomplex, 13
NDUA2_HUMAN	NADH dehydrogenase (ubiquinone) 1 alpha subcomplex, 2, 8kDa
NDUA4_HUMAN	NADH dehydrogenase (ubiquinone) 1 alpha subcomplex, 4, 9kDa
NDUA5_HUMAN	NADH dehydrogenase (ubiquinone) 1 alpha subcomplex, 5, 13kDa

NDUF3_HUMAN	NADH dehydrogenase (ubiquinone) 1 alpha subcomplex, assembly factor 3
NDUBA_HUMAN	NADH dehydrogenase (ubiquinone) 1 beta subcomplex, 10, 22kDa
NDUB3_HUMAN	NADH dehydrogenase (ubiquinone) 1 beta subcomplex, 3, 12kDa
ACPM_HUMAN	NADH dehydrogenase (ubiquinone) 1, alpha/beta subcomplex, 1, 8kDa
NDUS1_HUMAN	NADH dehydrogenase (ubiquinone) Fe-S protein 1, 75kDa (NADH-coenzyme Q reductase)
NDUS2_HUMAN	NADH dehydrogenase (ubiquinone) Fe-S protein 2, 49kDa (NADH-coenzyme Q reductase)
NDUS3_HUMAN	NADH dehydrogenase (ubiquinone) Fe-S protein 3, 30kDa (NADH-coenzyme Q reductase)
NDUS8_HUMAN	NADH dehydrogenase (ubiquinone) Fe-S protein 8, 23kDa (NADH-coenzyme Q reductase)
NDUV1_HUMAN	NADH dehydrogenase (ubiquinone) flavoprotein 1, 51kDa
NDUV2_HUMAN	NADH dehydrogenase (ubiquinone) flavoprotein 2, 24kDa
THIL_HUMAN	acetyl-Coenzyme A acetyltransferase 1
ACSL4_HUMAN	acyl-CoA synthetase long-chain family member 4
ACADV_HUMAN	acyl-Coenzyme A dehydrogenase, very long chain
KAD2_HUMAN	adenylate kinase 2
P5CS_HUMAN	aldehyde dehydrogenase 18 family, member A1
AL3A2_HUMAN	aldehyde dehydrogenase 3 family, member A2
AIFM2_HUMAN	apoptosis-inducing factor, mitochondrion-associated, 2
CPSM_HUMAN	carbamoyl-phosphate synthetase 1, mitochondrial
CPT1A_HUMAN	carnitine palmitoyltransferase 1A (liver)
CPT2_HUMAN	carnitine palmitoyltransferase 2
CATA_HUMAN	catalase
CHCH3_HUMAN	coiled-coil-helix-coiled-coil-helix domain containing 3
NB5R3_HUMAN	cytochrome b5 reductase 3
CYB5B_HUMAN	cytochrome b5 type B (outer mitochondrial membrane)
COX4I_HUMAN	cytochrome c oxidase subunit IV isoform 1
CX7A2_HUMAN	cytochrome c oxidase subunit VIIa polypeptide 2 (liver)
COX5A_HUMAN	cytochrome c oxidase subunit Va

DHRS1_HUMAN	dehydrogenase/reductase (SDR family) member 1
FIS1_HUMAN	fission 1 (mitochondrial outer membrane) homolog (S. cerevisiae)
FAHD1_HUMAN	fumarylacetoacetate hydrolase domain containing 1
GSTK1_HUMAN	glutathione S-transferase kappa 1
GPDM_HUMAN	glycerol-3-phosphate dehydrogenase 2 (mitochondrial)
GOLP3_HUMAN	golgi phosphoprotein 3 (coat-protein)
CH60_HUMAN	heat shock 60kDa protein 1
CCHL_HUMAN	holocytochrome c synthase (cytochrome c heme-lyase)
HCDH_HUMAN	hydroxyacyl-Coenzyme A dehydrogenase
ECHA_HUMAN	hydroxyacyl-Coenzyme A dehydrogenase/3-ketoacyl-Coenzyme A thiolase/enoyl-Coenzyme A hydratase (trifunctional protein), alpha subunit
ECHB_HUMAN	hydroxyacyl-Coenzyme A dehydrogenase/3-ketoacyl-Coenzyme A thiolase/enoyl-Coenzyme A hydratase (trifunctional protein), beta subunit
HCD2_HUMAN	hydroxysteroid (17-beta) dehydrogenase 10
THIM_HUMAN	hypothetical LOC648603; acetyl-Coenzyme A acyltransferase 2
IMMT_HUMAN	inner membrane protein, mitochondrial (mitofilin)
LETM1_HUMAN	leucine zipper-EF-hand containing transmembrane protein 1
MDHM_HUMAN	malate dehydrogenase 2, NAD (mitochondrial)
MTX1_HUMAN	metaxin 1
MTX2_HUMAN	metaxin 2
MTCH2_HUMAN	mitochondrial carrier homolog 2 (C. elegans)
MFF_HUMAN	mitochondrial fission factor
NEUL_HUMAN	neurolysin (metallopeptidase M3 family)
NNTM_HUMAN	nicotinamide nucleotide transhydrogenase
NIPS1_HUMAN	nipsnap homolog 1 (C. elegans)
OPA1_HUMAN	optic atrophy 1 (autosomal dominant)
ODO1_HUMAN	oxoglutarate (alpha-ketoglutarate) dehydrogenase (lipoamide)
MPPA_HUMAN	peptidase (mitochondrial processing) alpha

MPPB_HUMAN	peptidase (mitochondrial processing) beta
PPIF_HUMAN	peptidylprolyl isomerase F
STOM_HUMAN	phosphatidylethanolamine binding protein 1
PNPT1_HUMAN	polyribonucleotide nucleotidyltransferase 1
PHB_HUMAN	prohibitin
PHB2_HUMAN	prohibitin 2
PP1G_HUMAN	protein phosphatase 1, catalytic subunit, gamma isoform
PYC_HUMAN	pyruvate carboxylase
GLYM_HUMAN	serine hydroxymethyltransferase 2 (mitochondrial)
QCR7_HUMAN	similar to ubiquinol-cytochrome c reductase binding protein; ubiquinol-cytochrome c reductase binding protein pseudogene; ubiquinol-cytochrome c reductase binding protein
CMC1_HUMAN	solute carrier family 25 (mitochondrial carrier, Aralar), member 12
GHC1_HUMAN	solute carrier family 25 (mitochondrial carrier: glutamate), member 22
ADT2_HUMAN	solute carrier family 25 (mitochondrial carrier; adenine nucleotide translocator), member 5; solute carrier family 25 (mitochondrial carrier; adenine nucleotide translocator), member 5 pseudogene 8
ADT3_HUMAN	solute carrier family 25 (mitochondrial carrier; adenine nucleotide translocator), member 6
TXTP_HUMAN	solute carrier family 25 (mitochondrial carrier; citrate transporter), member 1
DIC_HUMAN	solute carrier family 25 (mitochondrial carrier; dicarboxylate transporter), member 10
M2OM_HUMAN	solute carrier family 25 (mitochondrial carrier; oxoglutarate carrier), member 11
SCMC1_HUMAN	solute carrier family 25 (mitochondrial carrier; phosphate carrier), member 24
MPCP_HUMAN	solute carrier family 25 (mitochondrial carrier; phosphate carrier), member 3
CMC2_HUMAN	solute carrier family 25, member 13 (citrin)
SPNS1_HUMAN	spinster homolog 1 (Drosophila)
STML2_HUMAN	stomatin (EPB72)-like 2
DHSA_HUMAN	succinate dehydrogenase complex, subunit A, flavoprotein (Fp)
DHSB_HUMAN	succinate dehydrogenase complex, subunit B, iron sulfur (Ip)
SYJ2B_HUMAN	synaptojanin 2 binding protein
TIM44_HUMAN	translocase of inner mitochondrial membrane 44 homolog (yeast)

TIM50_HUMAN	translocase of inner mitochondrial membrane 50 homolog (S. cerevisiae)
TOM22_HUMAN	translocase of outer mitochondrial membrane 22 homolog (yeast)
TOM40_HUMAN	translocase of outer mitochondrial membrane 40 homolog (yeast)
TOM70_HUMAN	translocase of outer mitochondrial membrane 70 homolog A (S. cerevisiae)
QCR1_HUMAN	ubiquinol-cytochrome c reductase core protein I
QCR2_HUMAN	ubiquinol-cytochrome c reductase core protein II
UCRI_HUMAN	ubiquinol-cytochrome c reductase, Rieske iron-sulfur polypeptide-like 1; ubiquinol-cytochrome c reductase, Rieske iron-sulfur polypeptide 1
USMG5_HUMAN	up-regulated during skeletal muscle growth 5 homolog (mouse)
VDAC1_HUMAN	voltage-dependent anion channel 1; similar to voltage-dependent anion channel 1
VDAC2_HUMAN	voltage-dependent anion channel 2
VDAC3_HUMAN	voltage-dependent anion channel 3
DECR_HUMAN	2,4-dienoyl CoA reductase 1, mitochondrial
3HIDH_HUMAN	3-hydroxyisobutyrate dehydrogenase
SCOT1_HUMAN	3-oxoacid CoA transferase 1
VATE1_HUMAN	ATPase, H ⁺ transporting, lysosomal 31kDa, V1 subunit E1
VATA_HUMAN	ATPase, H ⁺ transporting, lysosomal 70kDa, V1 subunit A
B2L13_HUMAN	BCL2-like 13 (apoptosis facilitator)
CX4NB_HUMAN	COX4 neighbor
CLPP_HUMAN	ClpP caseinolytic peptidase, ATP-dependent, proteolytic subunit homolog (E. coli)
DNJA3_HUMAN	DnaJ (Hsp40) homolog, subfamily A, member 3
EFGM_HUMAN	G elongation factor, mitochondrial 1
GRPE1_HUMAN	GrpE-like 1, mitochondrial (E. coli)
NFU1_HUMAN	NFU1 iron-sulfur cluster scaffold homolog (S. cerevisiae)
PTCD3_HUMAN	Pentatricopeptide repeat domain 3
RAB32_HUMAN	RAB32, member RAS oncogene family
TRAP1_HUMAN	TNF receptor-associated protein 1
EFTS_HUMAN	Ts translation elongation factor, mitochondrial

EFTU_HUMAN	Tu translation elongation factor, mitochondrial
ABHDA_HUMAN	abhydrolase domain containing 10
ACON_HUMAN	aconitase 2, mitochondrial
ACO13_HUMAN	acyl-CoA thioesterase 13
ACOT9_HUMAN	acyl-CoA thioesterase 9
ACADM_HUMAN	acyl-Coenzyme A dehydrogenase, C-4 to C-12 straight chain
ACOX1_HUMAN	acyl-Coenzyme A oxidase 1, palmitoyl
KAD3_HUMAN	adenylate kinase 3
AL1B1_HUMAN	aldehyde dehydrogenase 1 family, member B1
ALDH2_HUMAN	aldehyde dehydrogenase 2 family (mitochondrial)
ADAS_HUMAN	alkylglycerone phosphate synthase
SYDM_HUMAN	aspartyl-tRNA synthetase 2, mitochondrial
ODBB_HUMAN	branched chain keto acid dehydrogenase E1, beta polypeptide
SDHF2_HUMAN	chromosome 11 open reading frame 79
SLIRP_HUMAN	chromosome 14 open reading frame 156
CB047_HUMAN	chromosome 2 open reading frame 47
ES1_HUMAN	chromosome 21 open reading frame 33
CISY_HUMAN	citrate synthase
C1QBP_HUMAN	complement component 1, q subcomponent binding protein
RT29_HUMAN	death associated protein 3
ODP2_HUMAN	dihydrolipoamide S-acetyltransferase
ODB2_HUMAN	dihydrolipoamide branched chain transacylase E2
DPYL2_HUMAN	dihydropyrimidinase-like 2
ETFA_HUMAN	electron-transfer-flavoprotein, alpha polypeptide
ETFB_HUMAN	electron-transfer-flavoprotein, beta polypeptide
ECH1_HUMAN	enoyl Coenzyme A hydratase 1, peroxisomal
ETHE1_HUMAN	ethylmalonic encephalopathy 1

RMD3_HUMAN	family with sequence similarity 82, member A2
FUMH_HUMAN	fumarate hydratase
DHE3_HUMAN	glutamate dehydrogenase 1
GLSK_HUMAN	glutaminase
HINT2_HUMAN	histidine triad nucleotide binding protein 2
DHB4_HUMAN	hydroxysteroid (17-beta) dehydrogenase 4
HSDL2_HUMAN	hydroxysteroid dehydrogenase like 2
ISOC2_HUMAN	isochorismatase domain containing 2
IDH3A_HUMAN	isocitrate dehydrogenase 3 (NAD+) alpha
IDH3G_HUMAN	isocitrate dehydrogenase 3 (NAD+) gamma
IVD_HUMAN	isovaleryl Coenzyme A dehydrogenase
AMPL_HUMAN	leucine aminopeptidase 3
LPPRC_HUMAN	leucine-rich PPR-motif containing
SYLM_HUMAN	leucyl-tRNA synthetase 2, mitochondrial
LYPA1_HUMAN	lysophospholipase I
MCCB_HUMAN	methylcrotonoyl-Coenzyme A carboxylase 2 (beta)
C1TM_HUMAN	methylenetetrahydrofolate dehydrogenase (NADP+ dependent) 1-like
MTDC_HUMAN	methylenetetrahydrofolate dehydrogenase (NADP+ dependent) 2, methenyltetrahydrofolate cyclohydrolase
RM12_HUMAN	mitochondrial ribosomal protein L12
RM19_HUMAN	mitochondrial ribosomal protein L19
RM37_HUMAN	mitochondrial ribosomal protein L37
RM39_HUMAN	mitochondrial ribosomal protein L39
RM44_HUMAN	mitochondrial ribosomal protein L44
RM46_HUMAN	mitochondrial ribosomal protein L46
RM49_HUMAN	mitochondrial ribosomal protein L49
RT10_HUMAN	mitochondrial ribosomal protein S10
RT22_HUMAN	mitochondrial ribosomal protein S22

RT23_HUMAN	mitochondrial ribosomal protein S23
RT27_HUMAN	mitochondrial ribosomal protein S27
RT28_HUMAN	mitochondrial ribosomal protein S28
RRFM_HUMAN	mitochondrial ribosome recycling factor
MECR_HUMAN	mitochondrial trans-2-enoyl-CoA reductase
OAT_HUMAN	ornithine aminotransferase (gyrate atrophy)
PTH2_HUMAN	peptidyl-tRNA hydrolase 2
PRDX3_HUMAN	peroxiredoxin 3
PRDX4_HUMAN	peroxiredoxin 4
PCKGM_HUMAN	phosphoenolpyruvate carboxykinase 2 (mitochondrial)
PDIP2_HUMAN	polymerase (DNA-directed), delta interacting protein 2
P4HA1_HUMAN	prolyl 4-hydroxylase, alpha polypeptide I
PGES2_HUMAN	prostaglandin E synthase 2
IPYR2_HUMAN	pyrophosphatase (inorganic) 2
P5CR1_HUMAN	pyrroline-5-carboxylate reductase 1
ODPA_HUMAN	pyruvate dehydrogenase (lipoamide) alpha 1
ODPB_HUMAN	pyruvate dehydrogenase (lipoamide) beta
SFXN3_HUMAN	sideroflexin 3
PRS6B_HUMAN	similar to 26S protease regulatory subunit 6B (MIP224) (MB67-interacting protein) (TAT-binding protein 7) (TBP-7); proteasome (prosome, macropain) 26S subunit, ATPase, 4
ABCE1_HUMAN	similar to ATP-binding cassette, sub-family E, member 1; ATP-binding cassette, sub-family E (OABP), member 1
SUCB2_HUMAN	similar to sucB; succinate-CoA ligase, GDP-forming, beta subunit
SSBP_HUMAN	single-stranded DNA binding protein 1
P5CR1_HUMAN	sirtuin (silent mating type information regulation 2 homolog) 7 (S. cerevisiae)
NLTP_HUMAN	sterol carrier protein 2
SUCB1_HUMAN	succinate-CoA ligase, ADP-forming, beta subunit
SYTM_HUMAN	threonyl-tRNA synthetase 2, mitochondrial (putative)

Supplementary Table S6: DAVID ontology analysis: Other proteins identified.

Annotation Cluster 1	Enrichment Score: 3.53	Count
GOTERM_CC_FAT	cytoskeletal part	21
GOTERM_CC_FAT	cytoskeleton	26
GOTERM_CC_FAT	actin cytoskeleton	11
GOTERM_CC_FAT	non-membrane-bounded organelle	32
GOTERM_CC_FAT	intracellular non-membrane-bounded organelle	32
Annotation Cluster 2	Enrichment Score: 2.12	Count
GOTERM_CC_FAT	nuclear pore	6
GOTERM_CC_FAT	pore complex	6
GOTERM_CC_FAT	endomembrane system	16
GOTERM_CC_FAT	nuclear envelope	7
GOTERM_CC_FAT	organelle envelope	7
GOTERM_CC_FAT	envelope	7
Annotation Cluster 3	Enrichment Score: 1.81	Count
GOTERM_CC_FAT	endomembrane system	16
GOTERM_CC_FAT	endoplasmic reticulum	18
GOTERM_CC_FAT	endoplasmic reticulum membrane	7
GOTERM_CC_FAT	nuclear envelope-endoplasmic reticulum network	7
GOTERM_CC_FAT	endoplasmic reticulum part	7
GOTERM_CC_FAT	organelle membrane	9
Annotation Cluster 4	Enrichment Score: 1.77	Count
GOTERM_CC_FAT	microtubule cytoskeleton	12
GOTERM_CC_FAT	centrosome	6
GOTERM_CC_FAT	microtubule organizing center	6
Annotation Cluster 5	Enrichment Score: 1.34	Count
GOTERM_CC_FAT	keratin filament	4

GOTERM_CC_FAT	intermediate filament	5
GOTERM_CC_FAT	intermediate filament cytoskeleton	5
Annotation Cluster 6	Enrichment Score: 1.32	Count
GOTERM_CC_FAT	ribonucleoprotein complex	10
GOTERM_CC_FAT	cytosolic ribosome	4
GOTERM_CC_FAT	cytosolic part	5
GOTERM_CC_FAT	ribosomal subunit	4
GOTERM_CC_FAT	ribosome	4
Annotation Cluster 7	Enrichment Score: 1.16	Count
GOTERM_CC_FAT	dynein complex	3
GOTERM_CC_FAT	microtubule	6
GOTERM_CC_FAT	microtubule associated complex	3
Annotation Cluster 8	Enrichment Score: 0.52	Count
GOTERM_CC_FAT	kinetochore	3
GOTERM_CC_FAT	chromosome, centromeric region	3
GOTERM_CC_FAT	chromosome	5
GOTERM_CC_FAT	chromosomal part	4