Supporting Information

The chaperone ERp29 is required for tunneling nanotube formation by stabilizing MSec

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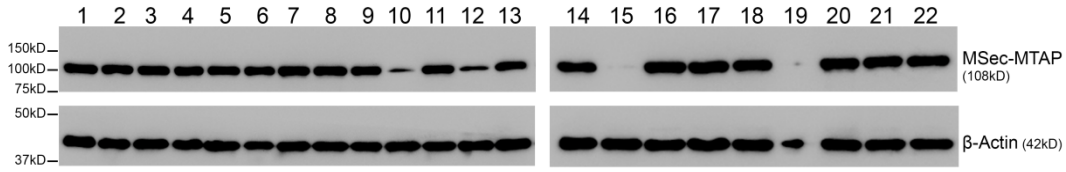
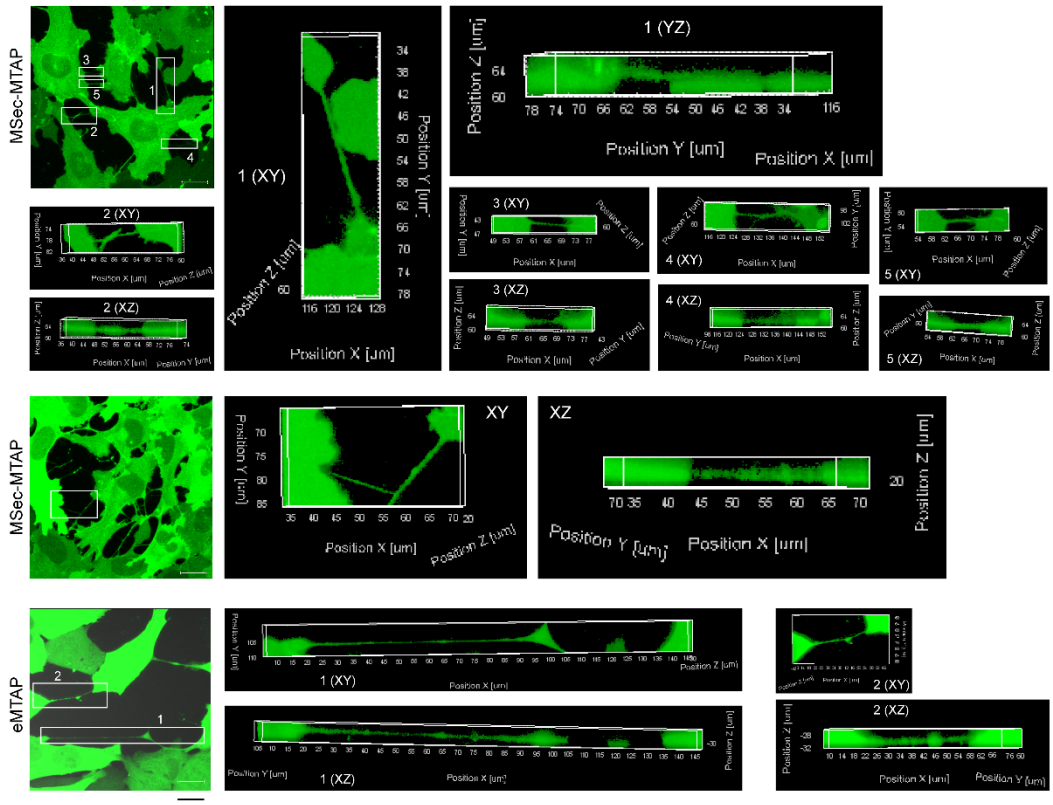
Supplementary figure S1



Supplementary figure S1. *Western blot of clonally sorted stable cell lines showing expression levels of MSec-MTAP, probed using an antibody against the Flag tag in the MTAP tag. Clone 1 (lane 1) was used for the studies in this manuscript.*

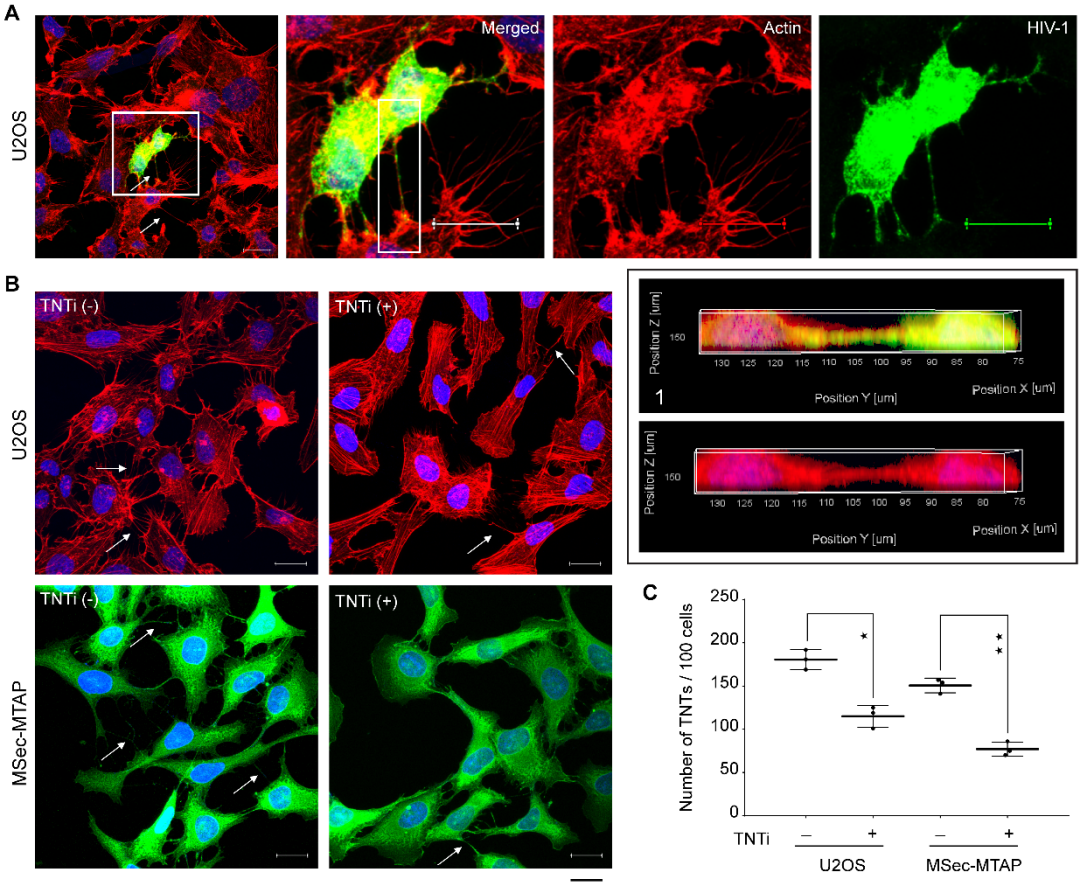
Supplementary figure S2



Supplementary figure S2. TNTs did not adhere to the substratum and "hung" between the connected cells. *Confocal snapshots taken from live cells stably expressing either MSec-MTAP or*

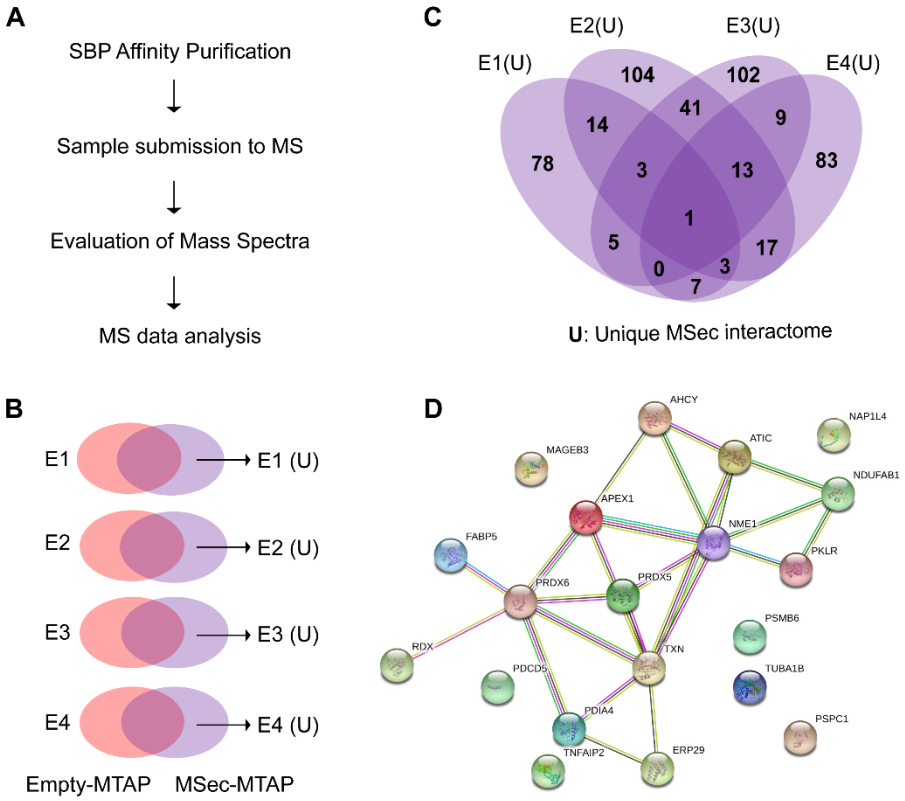
empty-MTAP as indicated (both green). TNTs are indicated by boxes. Scale bar = 20µm. X-Z/ Y-Z visualization of confocal Z-stacks of TNTs that were counted in both cell lines.

Supplementary figure S3



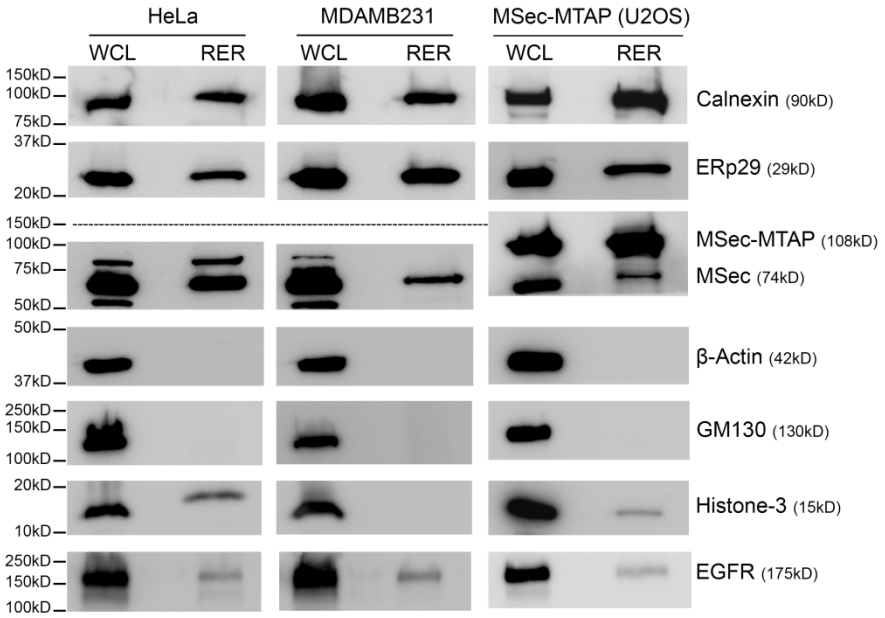
Supplementary figure S3. Functional and structural validation of TNTs. ***A)*** *Transfer of HIV-1 from the infected (green) towards the uninfected cell through a representative TNT (box). Y-z projection (right middle) clearly showing the TNT "hanging" between the two cell bodies with the HIV-1 signal (green) inside.* ***B)*** *Confocal microscopy images of cells co-stained with AlexaFluor™594 conjugated phalloidin (red, to visualize F-actin) and DAPI (blue, to visualize the nucleus) and cells stably expressing MSec-MTAP (green) after 24 hours of TNT inhibitor (TNTi) treatment. TNTs are indicated by arrows.* ***C)*** *Quantification of the number of TNTs per 100 cells from the confocal images. Data represent mean ± SD based on three independent experiments, 100 cells counted per experiment. (Paired t test, two-tailed; *P<0.05;**P<0.01).*

Supplementary figure S4



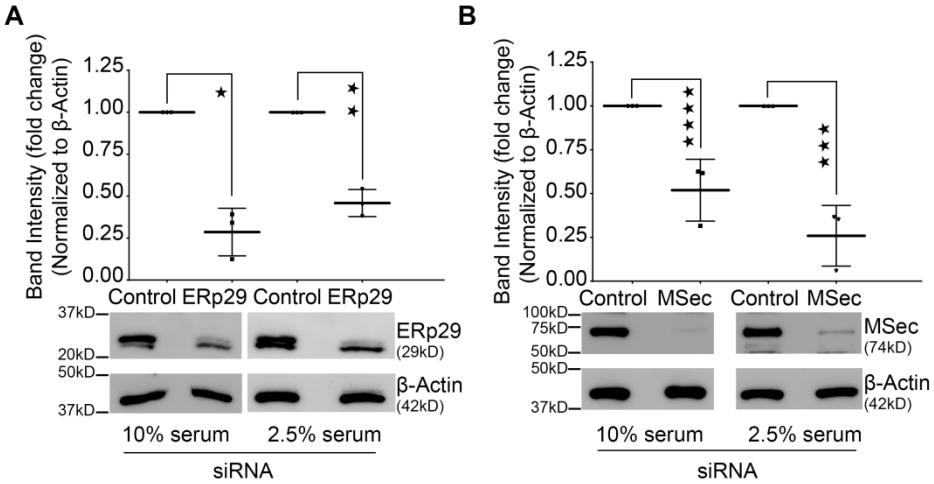
Supplementary figure S4. Identification and analysis of the MSec interactome. ***A)*** *Schematic workflow for the identification of the MSec interactome. SBP affinity purification of MSec-MTAP and empty-MTAP. Mass spectra were evaluated using protein database search program (Mascot).* ***B)*** *The empty-MTAP interactome from each experiment was subtracted from its cognate MSec-MTAP interactome to obtain the respective "unique" MSec-MTAP interactomes (E1U through E4U).* ***C)*** *The unique MSec interactomes were overlapped using the Venny 2.1 software.* ***D)*** *Analysis of protein-protein interaction networks from at least three experiments of MSec interactome by STRING database.*

Supplementary figure S5



Supplementary figure S5. MSec is associated with the ER. *Western blots of the whole cell lysate and rough endoplasmic reticulum fractions from various cell lines [HeLa cells, MDAMB231 cells and U2OS (MSec-MTAP) cells respectively] probed for the respective proteins as indicated. ER positive marker – Calnexin, ERp29, cytosolic marker - β-actin, Golgi complex marker - GM130, nuclear marker Histone-3 and cell membrane marker - EGFR.*

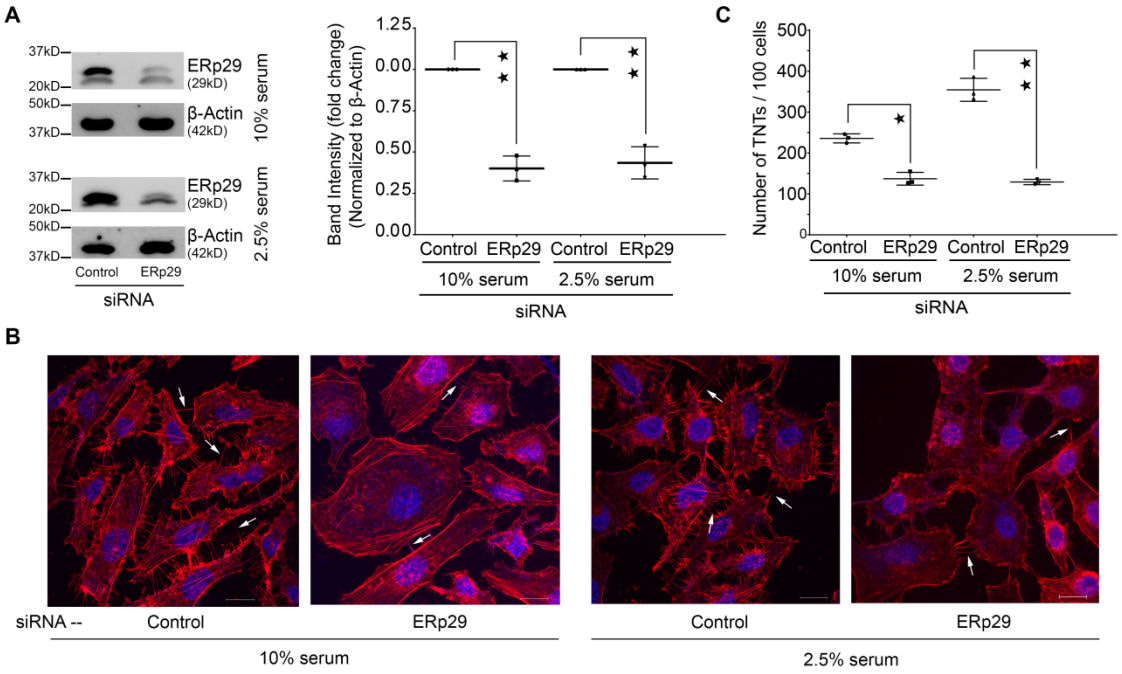
Supplementary figure S6



Supplementary figure S6. Western blot analysis of ERp29 and MSec depletion using sequence-specific siRNA treatment in U2OS cells. A) *U2OS cells were transfected with ERp29 and control (luciferase) sequence-specific siRNAs respectively for 48 hours and the efficiency of knock down analyzed by immunoblotting with anti-ERp29, anti-MSec and anti-β-actin (loading control) antibodies respectively. The fold change in expression of ERp29 or MSec protein normalized to*

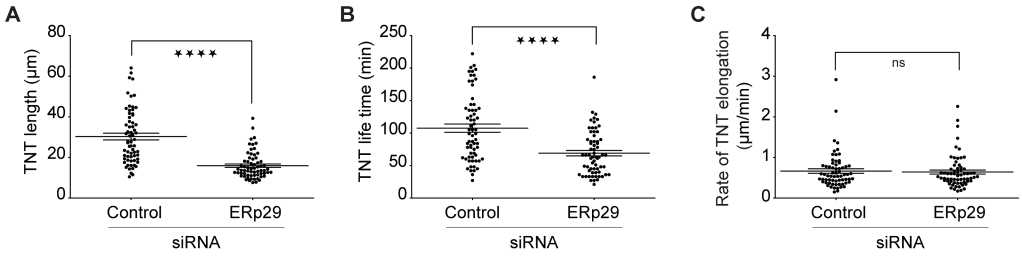
*respective β-actin levels was quantified from three independent experiments. (Paired t test, two-tailed, *P<0.05; **P<0.01; ***P<0.001; ****P<0.0001).*

Supplementary figure S7



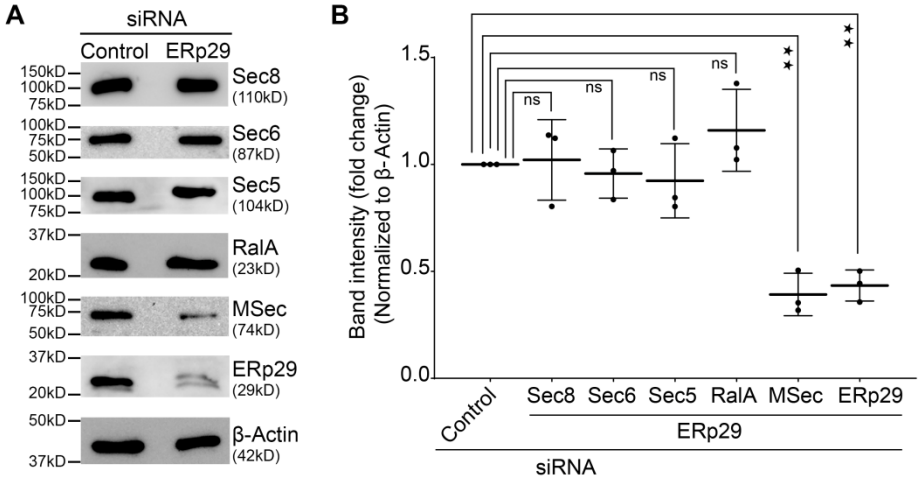
Supplementary figure S7. ERp29 is required for TNT formation in HeLa cells. ***A)*** *HeLa cells were transfected with ERp29 and control (luciferase) sequence-specific siRNAs respectively for 48 hours and the efficiency of knock down analyzed by immunoblotting with anti-ERp29 and anti-β-actin (loading control) antibodies respectively. The fold change in expression of ERp29 protein normalized to respective β-actin levels was quantified from three independent experiments.* ***B)*** *Confocal microscopy images of cells co-stained with AlexaFluor™594 conjugated phalloidin (red, to visualize F-actin) and DAPI (blue, to visualize the nucleus) after 48 hours of the indicated siRNA transfection. Arrows indicate TNTs connecting neighboring cells. Scale bar, 20µm.* ***C)*** *Quantification of the number of TNTs per 100 cells from the confocal images in both normal (10% serum) and stress (2.5% serum) conditions respectively. Data (A and C) represent mean ± SD based on three independent experiments, 100 cells counted per experiment. (Paired t test, two-tailed, *P<0.05; **P<0.01).*

Supplementary fig S8



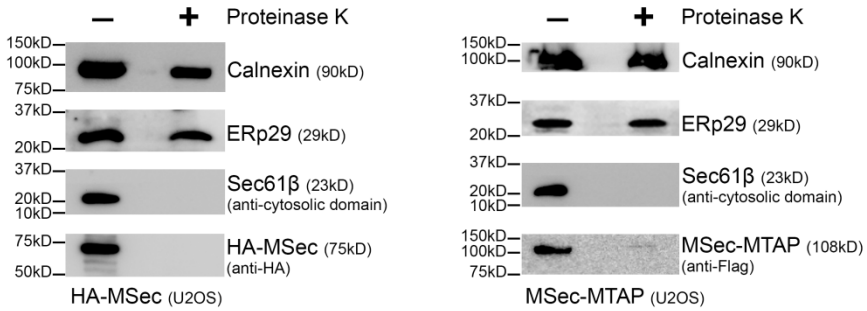
Supplementary figure S8. Analysis of TNT length, lifetime and rate of elongation. *EMTAP U2OS cells were imaged live by confocal, time-lapse microscopy. A) Measurement of the maximal length acquired by TNTs. B) Measurement of the total lifetime of TNTs (from initiation to breakage/ cell collapse). C) Measurement of the rates of TNT elongation (from initiation to reaching maximal length). A total of 65 TNTs was counted for control and ERp29 depletion across 3 independent experiments. Data represents mean ± SEM based on three independent experiments, 65 TNTs counted in total for each condition. (Paired t test, two-tailed, ****P<0.0001).*

Supplementary figure S9



Supplementary figure S9. ERp29 did not stabilize Exocyst complex and RalA. A*) siRNA-mediated depletion of ERp29 in U2OS cells using sequence-specific siRNAs. Control = anti-luciferase siRNA. Efficiency of knock down was analyzed by immunoblotting with anti-Sec8, anti-Sec6, anti-Sec5, anti-RalA, anti-MSec, anti-ERp29 and anti-β-actin (loading control) antibodies respectively.* ***B****) Fold change in protein expression was quantified through densitometry from the immunoblots of three independent experiments.*

Supplementary figure S10



Supplementary figure S10. *Limited proteolysis with proteinase-K (protease protection assay) reveals that stably expressed MSec (HA-MSec and MSec-MTAP) is associated with the ER towards its cytosolic site. Markers used – Calnexin (ER lumen) and Sec61β-cytosolic side (ER surface).*

Supplementary Movies 1 and 2. *Time lapse confocal images showing the transport of mitochondria (red, stained with Mitotracker red) from one cell to another through TNTs (green).*

Supplementary tables

Supplementary table S1. *Mouse MSec cDNA cloned into (pcDNA4-TO-Hygromycin-mVenus-MAP; Addgene #44099) in between HindIII and BamH1 sites.*

AAGCTTCCATGTCTGAGGCGTCCTCTGAGGACCTGATGCCATCCCCGGAGGCTCCCGATGGGGAGGAGA
GTCTGCGAAGAAGAAGGAGAAGAAGTCCAAAGGACTGGCCAACATGTTCAGTGTCTTCACCAAAGGGAAA
AAGAAAAAAAAAGGACCAGCCCAGATTATCAGATCTGGAAGTGCAGCCCAAGCCCAGGCCCGAGTTAGATG
GTCCACTGCCCACAGTGGAGGAGCTCAAGGAGGCCCTGGAGCACGGGCGGCTGGAGGTGGCCTGGCAGGT
GCTGGCGTTGGAGAGGCAGCTCGAGGCTGCGGCAGCGGCGGGTGGCATGAGCAACGAGGAGCTGGTGTGG
CGGCAGAGCAAGGTGGAGGCTCTGTACGTGCTGCTTTGCGACCAGGTGCTCGGGGTACTGCGGCGGCCGC
TGGAGGCGGCGCCCGAGCGGCTGAGCCAGGCGCTAGCCGTGGTGTCGCAGGAGGAGCTCGAGGACCGGCG
GGCGTCCGGGGGACCCTTGGCGGCGGCACTGGAGGCCACGCGCCCGCGGCGATGGCTGCAGCGGTGGAGG
GGCGTCGTGGCGGAGGTGGCAGCGGAACGTCTGGACGCGCAGCCGGCCACAGCGCCCGAGGGCCGCTCGG
AGGCGGAGAGCAGGTTCCTGCACATGGGTCGCACCATGAAGGAGGACCTGGAGGTGGTGGTGGAGAGGCT
GAAGCCGCTGTTCCCCGACGAGTTCAACGTCGTGCGCACCTACGCCGAAAGCTACCACTACCACTTCGCC
AGCCACCTGTGCGCCTTGGCGCAGTTCGAGTTGTGTGAAAGGGATACCTACTTGCTGCTGCTCTGGGTGC
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CTATTTTTGGACCTGAAGCCGCTGTTTAAGAAGTTCACACAGACCCGTTGGGCAAATCCAGTTGAGACCC
TGGAGGAAATCATCACTACTGTGAGCAGCAGCCTGCCTGAATTCTCAGAGCTGCAGGACTGTTTCCGGGA
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ATCCACTAGTCCAGTGTGGTGGAATTCTGCAGATATCCAGCACAGTGGCGGCCGCGGCAGCAGCatggtg
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gcagaacaccccccatcggcgacggccccgtgctgctgcccgacaaccactacctgagctaccagtccaag
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ctctcggcatggacgagctgtacaagtaaTCTAGA

Supplementary table S2. *MSec interactome appearing in at least three experiments.*

S.No	Protein Name	Accession #	Database from which it is derived	# of distinct peptides assigned for each protein				% coverage of each protein assigned			
				E1	E2	E3	E4	E1	E2	E3	E4
1	Endoplasmic reticulum resident protein 29	ERP29_HUMAN	SwissProt 57.15	9 (4)	4 (1)	5 (2)	11 (2)	22	18	19	19
2	DNA-(apurinic or apyrimidinic site) lyase	APEX1_HUMAN	SwissProt 57.15	4 (1)	5 (2)	4 (2)	-	11	16	16	-
3	Peroxiredoxin-6	PRDX6_HUMAN	SwissProt 57.15	2 (1)	2 (2)	6 (5)	-	16	6	15	-
4	Paraspeckle component 1	PSPC1_HUMAN	SwissProt 57.15	3(1)	2 (1)	2 (1)	-	11	4	5	-
5	Adenosyl homocysteinase	SAHH_HUMAN	SwissProt 57.15	4 (2)	5 (3)	-	8 (3)	4	8	-	13
6	Radixin	RADI_HUMAN	SwissProt 57.15	6 (3)	9 (2)	-	5 (3)	12	11	-	5
7	Nucleosome assembly protein 1-like 4	NP1L4_HUMAN	SwissProt 57.15	3 (1)	4 (1)	-	3 (1)	8	11	-	8
8	Tubulin alpha-1B chain	TBA1B_HUMAN	SwissProt 57.15	-	24 (19)	35 (23)	61 (43)	-	34	47	47
9	Pyruvate kinase isozymes R/L	KPYR_HUMAN	SwissProt 57.15	-	5 (3)	5 (1)	4 (2)	-	3	3	4
10	Splicing factor, arginine/serine- rich 2B	SFR2B_HUMAN	SwissProt 57.15	-	2 (1)	1 (1)	6 (2)	-	8	2	8
11	Peroxiredoxin-5, mitochondrial	PRDX5_HUMAN	SwissProt 57.15	-	3 (1)	4 (2)	3 (1)	-	26	24	8
12	Proteasome subunit beta type-6	PSB6_HUMAN	SwissProt 57.15	-	1 (1)	1 (1)	2 (2)	-	4	4	4
13	Programmed cell death protein 5	PDCD5_HUMAN	SwissProt 57.15	-	3 (2)	4 (2)	3 (1)	-	17	28	17
14	Fatty acid-binding protein, epidermal	FABP5_HUMAN	SwissProt 57.15	-	2 (1)	3 (1)	3 (1)	-	6	17	14
15	Bifunctional purine biosynthesis protein PURH	PUR9_HUMAN	SwissProt 57.15	-	3 (1)	3 (1)	2 (1)	-	7	9	2
16	Acyl carrier protein, mitochondrial	ACPM_HUMAN	SwissProt 57.15	-	2 (1)	3 (2)	4 (1)	-	8	14	13
17	Melanoma-associated antigen B3	MAGB3_HUMAN	SwissProt 57.15	-	2 (1)	3 (0)	7 (3)	-	4	4	13
18	Nucleoside diphosphate kinase A	NDKA_HUMAN	SwissProt 57.15	-	2 (1)	3 (1)	12 (6)	-	17	21	46
19	Protein disulfide-isomerase A4	PDIA4_HUMAN	SwissProt 57.15	-	3 (0)	4 (2)	1 (0)	-	3	5	1
20	Thioredoxin	THIO_HUMAN	SwissProt 57.15	-	1 (1)	4 (1)	5 (1)	-	12	10	31

Note: Numbers in parantheses refer to the number of distinct peptides with significant matches.
Supplementary table S3. *PANTHER Gene Ontology (GO) terms assigned to the MSec interactome.*

GO terms	GO ID	Number of hits
PANTHER GO-Slim molecular function (Total # genes: 19; Total # function hits: 17)		
Binding	(GO:0005488)	TBA1B, NP1L4, ACPM, PRDX6, PSPC1
Structural molecular activity	(GO:0005198)	TBAIB, RADI
Molecular function regular	(GO:0098772)	NP1L4
Catalytic activity	(GO:0003824)	PRDX5, PDIA4, PRDX6, KPYR, NDKA, PUR9, PSB6, APEX1, SAHH
PANTHER GO-Slim Biological process (Total # genes: 19; Total # process hits: 22)		
reproduction	(GO:0000003)	MAGB3
response to stimulus	(GO:0050896)	PRDX5, PRDX6, PDIA4, KPYR
developmental process	(GO:0032502)	RADI
cellular process	(GO:0009987)	NP1L4, PDIA4, RADI, MAGB3
metabolic process	(GO:0008152)	PRDX5,PRDX6, NP1L4, ACPM, KPYR, NDKA, PUR9, PSB6, APEX1,
biological regulation	(GO:0065007)	PRDX1,
Biogenesis	(GO:0071840)	TBA1B,NP1L4
PANTHER GO-Slim Cellular component (Total # genes: 19; Total # component hits: 18)		
supramolecular complex	(GO:0099080)	TBA1B
protein-containing complex	(GO:0032991)	PSB6
organelle	(GO:0043226)	ERP29, PDIA4, PSPC1, PDCD5, PSB6
cell	(GO:0005623)	PRDX5, PRDX6, PDIA4, TBA1B, KPYR, ERP29, PDCD5, RADI, PSB6, SAHH, ACPM
PANTHER Protein class (Total # genes: 19; Total # protein class hits: 13)		
membrane traffic protein	(PC00150)	ERP29
hydrolase	(PC00121)	PUR9, PSB6, SAHH
oxidoreductase	(PC00176)	PRDX6
cell adhesion molecule	(PC00069)	MAGB3
enzyme modulator	(PC00095)	NP1L4, PDCD5
transfer/carrier protein	(PC00219)	ACPM
transferase	(PC00220)	PUR9
nucleic acid binding	(PC00171)	PSPC1
cytoskeletal protein	(PC00085)	TBA1B, RADI
PANTHER pathway (Total # genes: 19; Total # pathway hits: 10)		
Pyruvate metabolism	(P02772)	KPYR
Gonadotropin-releasing hormone receptor pathway	(P06664)	TBA1B
De novo pyrimidine deoxyribonucleotide biosynthesis	(P02739)	NDKA
De novo purine biosynthesis	(P02738)	NDKA, PUR9
De novo pyrimidine ribonucleotides biosythesis	(P02740)	NDKA
Hypoxia response via HIF activation	(P00030)	THIO
Glycolysis	(P00024)	KPYR
Parkinson disease	(P00049)	PSB6
Oxidative stress response	(P00046)	THIO

Supplementary table S4. *Analysis summary of MSec interactome appearing in at least three experiments (analyzed by PANTHER)*

Analysis type	PANTHER Overrepresentation Test (Released 20181113)
Annotation Version and Release Date	Reactome version 65 Released 2018-06-12
Analyzed list	Client Text Box Input (Homo sapiens)
Reference list	Homo sapiens (all genes in database)
Test type	Binomial
Correction	Used the Bonferroni correction for multiple testing
Results	Displayed only results for Bonferroni-corrected for P < 0.05

	Reference list	Client text box input
Mapped IDs	20996 out of 20996	19 out of 19
Unmapped IDs	0	1
Multiple mapping information	0	0

Annotation Data Set	Homo sapiens (REF)	Client text box input			
	#	#	Expected	Fold enrichment	P value
PANTHER pathways (Bonferroni count: 152)					
1) De novo purine biosynthesis	29	2	0.03	76.21	4.88E-02
2) Unclassified	18402	13	16.65	0.78	0.00E+00
PANTHER GO-Slim molecular function (Bonferroni count: 462)					
1) Unclassified	11259	5	10.19	0.49	0.00E+00
PANTHER GO-Slim biological process (Bonferroni count: 1467)					
1) Unclassified	10756	6	9.73	0.62	0.00E+00
PANTHER GO-Slim cellular component (Bonferroni count: 383)					
1) Unclassified	11922	7	10.79	0.65	0.00E+00
PANTHER Protein class (Bonferroni count: 203)					
1) Unclassified	12726	7	11.52	0.61	0.00E+00
GO molecular function complete (Bonferroni count: 2783)					
1) Unclassified	3290	1	2.98	0.34	0.00E+00
GO biological process complete (Bonferroni count: 8749)					
1) Cell redox homeostasis	77	5	0.07	71.76	6.47E-05
2) Unclassified	3224	1	2.92	0.34	0.00E+00
GO cellular component complete (Bonferroni count: 1423)					
1) Extracellular exosome	2096	11	1.9	5.8	4.91E-04
2) Extracellular vesicle	2117	11	1.92	5.74	5.43E-04
3) Vesicle	3812	13	3.45	3.77	2.98E-03
4) Extracellular organelle	2119	11	1.92	5.74	5.49E-04
5) Extracellular region part	3529	12	3.19	3.76	1.12E-02
6) Extracellular space	3335	12	3.02	3.98	6.12E-03
7) Unclassified	2169	1	1.96	0.51	0.00E+00
Reactome pathways (Bonferroni count: 1974)					
1) Unclassified	10452	8	9.46	0.85	0.00E+00

Supplementary table S4 (continued). *Analysis summary of MSec interactome appearing in at least three experiments (analyzed by PANTHER)*

Annotation Data Set	**Client text box input**

PANTHER pathways (Bonferroni count: 152)		
1) De novo purine biosynthesis	2	NDKA, PUR9
2) Unclassified	13	PRDX5, PRDX6, NP1L4, ACPM, PDIA4, ERP29, MAGB3, PSPC1, PDCD5, RADI,FABP5, APEX1, SAHH
PANTHER GO-Slim molecular function (Bonferroni count: 462)		
1) Unclassified	5	ERP29, MAGB3, PDCD5, FABP5, THIO
PANTHER GO-Slim biological process (Bonferroni count: 1467)		
1) Unclassified	6	ERP29, PSPC1, PDCD5, FABP5, SAHH, THIO
PANTHER GO-Slim cellular component (Bonferroni count: 383)		
1) Unclassified	7	THIO, NP1L4, NDKA, MAGB3, PUR9, FABP5, APEX1
PANTHER Protein class (Bonferroni count: 203)		
1) Unclassified	7	PRDX5, THIO, PDIA4, KPYR, NDKA, FABP5, APEX1
GO molecular function complete (Bonferroni count: 2783)		
1) Unclassified	1	MAGB3
GO biological process complete (Bonferroni count: 8749)		
1) Cell redox homeostasis	5	PRDX5, PRDX6, THIO, PDIA4, APEX1
2) Unclassified	1	MAGB3
GO cellular component complete (Bonferroni count: 1423)		
1) Extracellular exosome	11	PRDX5, PRDX6, THIO, KPYR, NDKA, PUR9, PDCD5, RADI, FABP5, PSB6, SAHH
2) Extracellular vesicle	11	PRDX5, PRDX6, THIO, KPYR, NDKA, PUR9, PDCD5, RADI, FABP5, PSB6, SAHH
3) Vesicle	13	ERP29, PDIA4, PRDX5, PRDX6, THIO, KPYR, NDKA, PUR9, PDCD5, RADI, FABP5, PSB6, SAHH
4) Extracellular organelle	11	PRDX5, PRDX6, THIO, KPYR, NDKA, PUR9, PDCD5, RADI, FABP5, PSB6, SAHH
5) Extracellular region part	12	PDIA4, PRDX5, PRDX6, THIO, KPYR, NDKA, PUR9, PDCD5, RADI, FABP5, PSB6, SAHH
6) Extracellular space	12	PDIA4, PRDX5, PRDX6, THIO, KPYR, NDKA, PUR9, PDCD5, RADI, FABP5, PSB6, SAHH
7) Unclassified	1	MAGB3
Reactome pathways (Bonferroni count: 1974)		
1) Unclassified	8	PRDX5, NP1L4, PDIA4, KPYR, ERP29, MAGB3, PSPC1, PDCD5

Supplementary table S5. *Primers used for RT-qPCR and site directed mutagenesis.*

Name	Primer sequence (5'-3')
MSec	Sense: TCAAGAGGTTCACGCACA

	Antisense: CCCTTGCTGAGTTGGATG
ERp29	Sense: CTTTGAGAACCCAGTCCC Antisense: GTTATCTTGCCCCTGCTT
18S	Sense: GAGGGACAAGTGGCGTTCA Antisense: CCGGACATCTAAGGGCATCA
(D61A)	Sense: AGTTCGTCTTGGTGAAGTTCGCCACCCAGTACCCCTACGGTGA Antisense: TCACCGTAGGGGTACTGGGTGGCGAACTTCACCAAGACGAACT
(Y64S)	Sense: TGGTGAAGTTCGACACCCAGTCCCCCTACGGTGAGAAGCAGGA Antisense: TCCTGCTTCTCACCGTAGGGGGACTGGGTGTCGAACTTCACCA
(Y66K)	Sense: AGTTCGACACCCAGTACCCCAAGGGTGAGAAGCAGGATGAGTT Antisense: AACTCATCCTGCTTCTCACCCTTGGGGTACTGGGTGTCGAACT
(Y96Q)	Sense: CAGAGGTGGGGATCTCAGATCAAGGTGACAAGCTGAACATGGA Antisense: TCCATGTTCAGCTTGTCACCTTGATCTGAGATCCCCACCTCTG
(P116D)	Sense: AGCTGGACAAAGAGAGCTACGACGTCTTCTACCTCTTCCGGGA Antisense: TCCCGGAAGAGGTAGAAGACGTCGTAGCTCTCTTTGTCCAGCT
(C157S)	Sense: TCTACCTAGGTATGCCTGGTAGCCTGCCTGTATACGACGCCCT Antisense: AGGGCGTCGTATACAGGCAGGCTACCAGGCATACCTAGGTAGA