

Supplemental Materials

Molecular Biology of the Cell

Ding et al.

Supplementary Legends

Figure S1. Auxilin binds to Sec23 and Sec24. (A) Purified recombinant GST, GST-Sec23, GST-Sec24 and GST-Sec31 (aa 1-500) were prebound to glutathione beads and then incubated with His₆-Auxilin. After the incubation, the beads were washed and analyzed by western blot analysis using anti-His₆ antibody. (B) Ponceau S stain of the binding reactions shows equal loading of GST-Sec23, GST-Sec24 and GST-Sec 31(aa 1-500).

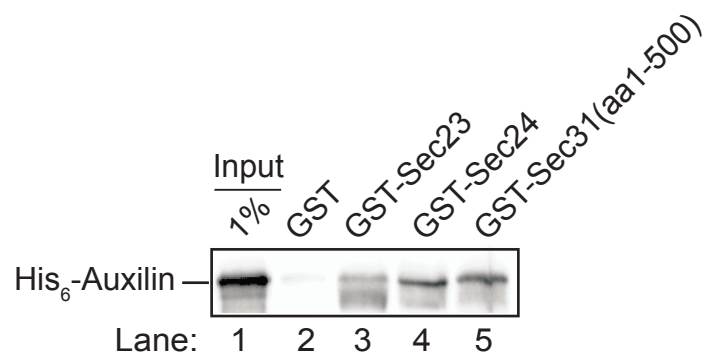
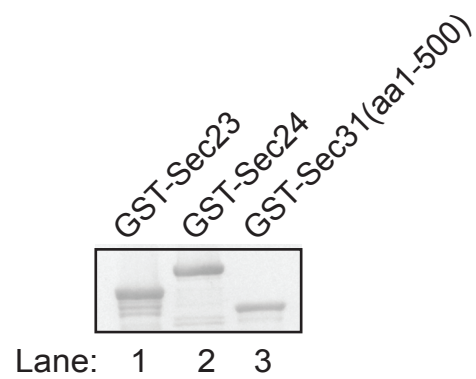
A**B**

Table S1. Yeast strains used in this study

Strain Number	Genotype	Source
SFNY998	<i>MAT a, his3Δ1, leu2Δ0, met15Δ0, ura3Δ0</i>	Ferro-Novick Lab Collection
SFNY1415	<i>MAT a, his3Δ1, leu2Δ0, met15Δ0, ura3Δ0, Ret1::COPI-TAP, HIS3</i>	Ferro-Novick Lab Collection
SFNY1741	<i>MAT a, his3Δ1, leu2Δ0, met15Δ0, ura3Δ0, Chc1::Chc1-TAP, HIS3</i>	Ferro-Novick Lab Collection
SFNY1841	<i>MAT a, ura3-52, leu2-3,112, his3Δ200</i>	Ferro-Novick Lab Collection
SFNY1842	<i>MAT α, ura3-52, leu2-3, 112, his3Δ200</i>	Ferro-Novick Lab Collection
SFNY2672	<i>MAT a, ura3-52, leu2-3,112, his3Δ200, swa2Δ::His3MX6</i>	This study
SFNY2673	<i>MAT a, ura3-52, leu2-3,112, his3Δ200, sec23-1</i>	This study
SFNY2674	<i>MAT a, ura3-52, leu2-3,112, his3Δ200, swa2Δ::His3MX6, sec23-1</i>	This study
SFNY2675	<i>MAT a, ura3-52, leu2-3,112, his3Δ200, sec24-1</i>	This study
SFNY2676	<i>MAT a, ura3-52, leu2-3,112, his3Δ200, swa2Δ::His3MX6, sec24-1</i>	This study
SFNY2677	<i>MAT a, ura3-52, leu2-3,112, his3Δ200, sec31-1</i>	This study
SFNY2678	<i>MAT a, ura3-52, leu2-3,112, his3Δ200, swa2Δ::His3MX6, sec31-1</i>	This study
SFNY2679	<i>MAT a, ura3-52, leu2-3,112, his3Δ200, sec21-1</i>	This study

SFNY2680	<i>MAT a, ura3-52, leu2-3,112, his3Δ200, swa2Δ::His3MX6, sec21-1</i>	This study
SFNY2681	<i>MAT a, ura3-52, leu2-3,112, his3Δ200, sec27-1</i>	This study
SFNY2682	<i>MAT a, ura3-52, leu2-3,112, his3Δ200, swa2Δ::His3MX6, sec27-1</i>	This study
SFNY2683	<i>MAT α, ura3-52, leu2-3, 112, his3Δ200, swa2Δ::His3MX6</i>	This study
SL1463	<i>MATα, ura3-52, leu2, trp1, his3-Δ200, scd1-v</i>	Lemmon Lab Collection
SL4827	<i>MATα, ura3-52, leu2Δ1, trp1Δ63, his3-Δ200, GAL-SWA2:TRP1</i>	From Greg Payne (GPY2598)

Table S2. Bacterial strains used in this study

Strain Number	Plasmid	Host strain	Sources
SFNB335	pGEX3X	BL21 (DE3)pLysS	Ferro-Novick Lab Collection
SFNB773	ORF of Aux1; inserted into the BamHI-EcoRI of pGEX4T-2	DH5 α	Ferro-Novick Lab Collection
SFNB1337	ORF of Sec13; inserted into BamHI-SalI of pGEX4T-2	BL21	Ferro-Novick Lab Collection
SFNB1346	ORF of Sec23; inserted into pGEX2T	BL21	Ferro-Novick Lab Collection
SFNB1486	ORF of Sec31(aa1-500); inserted into BamHI-SalI of pGEX4T-3	BL21	Ferro-Novick Lab Collection
SFNB1729	ORF of Aux1; inserted into XhoI-BamHI of pET15b	BL21(DE3)	Ferro-Novick Lab Collection
SFNB1781	ORF of Sec31(aa 879-1114); inserted into BamHI-NotI of pGEX4T-2	XL1 blue cells	Ferro-Novick Lab Collection
SFNB1860	ORF of Sec31(aa 501-878);	XL1 blue cells	Ferro-Novick Lab Collection
SFNB1861	ORF of Sec31(aa 1114-1274);	XL1 blue cells	Ferro-Novick Lab Collection
SFNB1906	ORF of Sec24; inserted into SmaI-XhoI of pGEX6p-1	BL-21-RIL	Ferro-Novick Lab Collection
SFNB2257	ORF of Aux1(aa 1-362); inserted into BamHI-XhoI of pGEX-6p-1	Rosetta	This study
SFNB2258	ORF of Aux1(aa 362-561); inserted into BamHI-XhoI of pGEX-6p-1	Rosetta	This study
SFNB2259	ORF of Aux1(aa 562-668); inserted into BamHI-XhoI of pGEX-6p-1	Rosetta	This study

SFNB2260	ORF of Sec31(aa 1-500); inserted into XhoI-BamHI of pET15b	Rosetta	This study
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Supplemental Table S5: WT, all mass spec hits with slice info

Slice#	Gene	Seq. Length	Protein name
3	ADE4	510	Amidophosphoribosyltransferase
5	ADE4	510	Amidophosphoribosyltransferase
6	ADE4	510	Amidophosphoribosyltransferase
7	ADE4	510	Amidophosphoribosyltransferase
8	ADE4	510	Amidophosphoribosyltransferase
9	ADE4	510	Amidophosphoribosyltransferase
6	ADH1	348	Alcohol Dehydrogenase
9	AHP1	176	Alkyl HydroPeroxide reductase
10	AHP1	176	Alkyl HydroPeroxide reductase
4	APL2	726	Beta Adaptin beta1 (AP-1 complex)
3	APL4	832	AP-1 complex Gamma
3	APL4	832	AP-1 complex Gamma
4	APL4	832	AP-1 complex Gamma
5	APM1	475	AP-1 complex medium (mu)
8	APM1	475	AP-1 complex medium (mu)
9	APS1	156	AP-1 complex small (signa)
9	ARF1	181	Arf GTPase
8	ATG27	271	Transmembrane protein involved in Autophagy
7	BMH1/BMH2	267/273	14-3-3 protein
8	BMH1/BMH2	267/273	14-3-3 protein
3	CDC19/PYK1	501	Pyruvate Kinase
5	CDC19/PYK1	501	Pyruvate Kinase
1A	CHC1	1653	Clathrin heavy chain
1	CHC1	1653	Clathrin heavy chain
2	CHC1	1653	Clathrin heavy chain
3	CHC1	1653	Clathrin heavy chain
4	CHC1	1653	Clathrin heavy chain
5	CHC1	1653	Clathrin heavy chain
6	CHC1	1653	Clathrin heavy chain
9	CHC1	1653	Clathrin Heavy Chain
10	CHC1	1653	Clathrin Heavy Chain
6	CLC1	233	Clathrin Light Chain
7	CLC1	233	Clathrin Light Chain
8	CLC1	233	Clathrin Light Chain
9	CLC1	233	Clathrin Light Chain
10	CLC1	233	Clathrin Light Chain
3	DNF2	1612	Aminophospholipid translocase (flippase)
8	EGD2	174	Alpha subunit of nascent polypeptide-associated complex
9	EMP70	667	Transmembrane 9 superfamily member 1/cell adhesion, filamentous growth, Endosome to vacuole sorting
6	ENO1/ENO2	437	Enolase
6	ENO1/ENO2	437	Enolase
7	ENO1/ENO2	437	Enolase

8	ENO2	437	Enolase 2
4	ENT5	411	Golgi Epsin
5	ENT5	411	Golgi Epsin
4	FAA1	700	Long Chain fatty acyl-CoA synthase
1A	FAS1	2051	Beta subunit of fatty acid synthetase
1A	FAS2	1887	Alpha subunit of fatty acid synthetase
6	FBA1	359	Fructose 1,6-bisphosphate aldolase
6	FCY2	533	Purine-cytosine permease
1A	GSC2	1895	1,3-beta-D-glucan synthase
1	FKS1	1876	1,3-beta-D-glucan synthase
2	FKS1	1876	1,3-beta-D-glucan synthase
3	FKS1	1876	1,3-beta-D-glucan synthase
3	FKS1/GSC2	1876/1895	1,3-beta-D-glucan synthase
2	GAS1	559	1,3-beta-glucanosyltransferase
3	GAS1	559	Glycolipid anchored protein
9	GEF1	779	Cl-channel-lateGolgi/Endo
8	GOS1	223	T-SNARE invovled in ER to Golgi Transport
8	GPM1	247	Phophoglycerate mutase 1
6	HXT3	567	Low-affinity glucose transporter
1A	HXT6/HXT7	570	High-affinity hexose transporter
6	HXT6/HXT7	570	High-affinity hexose transporter
7	IPP1	287	Inorganic pyrophosphatase
3	MDR1/GYP2/MIC1	950	GAP for Rabs Ypt6, Ypt31, Sec4
5	NAP1	417	Nucleosome assembly protein
9	NCE102	173	Non-classicle export protein 2; Eisosome organization, PM protein
5	PDC1	563	Pyruvate Decarboxylase
1A	PDR5	1511	Multi drug resistance transporter
1	PDR5	1511	Multi drug resistance transporter
2	PDR5	1511	Multi drug resistance transporter
3	PDR5	1522	Multi drug resistance transporter
6	PEP4	405	Vacuolar aspartyl protease (proteinase A)
6	PEP8/VPS26	379	Retromer subunit
3	PFK2	959	phosphofructokinase 2
6	PGK1	416	Phosphoglycerate Kinase
6	PGK1	416	Phosphoglycerate Kinase
7	PGK1	416	Phosphoglycerate Kinase
7	PGK1	416	Phosphoglycerate Kinase
8	PGK1	416	Phosphoglycerate Kinase
3	PMA1	918	PM ATPase 1
3	PMA1	918	PM ATPase 1
3	PMA1	918	PM ATPase 1
9	PST2	198	Flavodoxin-like fold (paralog RFS1)
6	RAS2	322	GTP binding protein, Ras-like protein 2
8	RFS1	210	Flavodoxin-like fold (like Pst2 YCp4) PM punctate Loc.

8	RHO1	209	Rho GTPase Rho1
9	RPL11A/RPL11B	174	60S ribosomal protein L11
9	RPL12A/RPL12B	165	60S ribosomal protein L12
9	RPL18	152	S.pombe 60S ribosomal protein L18
9	RPL25	142	60S ribosomal protein L25
10	RPL25	142	60S ribosomal protein L25
9	RPL28	149	60S ribosomal protein L28
10	RPL30	105	60S ribosomal protein L30
7	RPL5	297	60S ribosomal subunit L5
8	RPL7A/RPL7B	244	60S ribosomal protein L7
8	RPL9A	191	60S ribosomal protein L9
10	RPP1B	106	60S acidic ribosomal protein P1-beta
10	RPP2B	110	60S acidic ribosomal protein P2-beta
9	RPS13	151	40S ribosomal protein S13
9	RPS13	151	40S ribosomal protein S13
10	RPS14A/RPS14B	137/138	40S ribosomal protein S14
9	RPS16A/RPS16B	143	40S ribosomal protein S16
10	RPS16A/RPS16B	143	40S ribosomal protein S16
9	RPS18A/RPS18B	146	40S ribosomal protein S18
10	RPS24A/RSP24B	135	40S ribosomal protein S24
8	RPS3	240	40S Ribosomal Protein S3
8	RPS3	240	40S ribosomal protein S3
9	RPS3	240	40S Ribosomal Protein S3
10	RPS30A/RPS30B	63	40S ribosomal protein S30
8	RPS4A/RPS4B	261	40S Ribosomal Protein S4
8	RPS5	225	40S ribosomal protein S5
8	RPS5	225	40S ribosomal protein S5
9	RPS5	225	40S ribosomal protein S5
10	RPS5	225	40S ribosomal protein S5
7	SEC17	292	Alpha SNAP/vesicle fusion
8	SEC17	292	Alpha SNAP/vesicle fusion
8	SEC4	215	RAB GTPase Sec4 (Golgi to PM)
8	SFT2	215	Tetra-spanin membrane protein of late Golgi, retrograde transport from endosome to Golgi
9	SFT2	215	Tetra-spanin membrane protein of late Golgi, retrograde transport from endosome to Golgi
6	SHM2	469	Serine hydroxymethyltransferase, cytosolic
3	SKN1	771/720	Protein involved in sphingolipid synth
9	SNC2	115	v-snare for transport from Golgi to PM (SNC1 homologue)
10	SNC2	115	v-snare for transport from Golgi to PM (SNC1 homologue)
6	SNX4	423	Sorting nexin-4
8	SNX4	423	Sorting nexin-4
4	SSA1	649	HSC chaperone SSA family
4	SSB1	613	HSC chaperone SSB family
4	SSB1	613	HSC chaperone SSB family
6	SSB1/SSB2	613	HSC chaperone SSB family

10	STE18	110	Trimeric G protein gamma subunit
4	STE7	515	Ser Thre Prot Kinase/MAP Kinase Kinase
6	SUR7	302	Plasma membrane protein, Eisosome component
7	SUR7	302	Plasma membrane protein, Eisosome component
8	SUR7	302	Plasma membrane protein, Eisosome component
4	TCB2	1178	ER protein: ER-PM tethering
6	TDH1/TDH2/TDH3	332	glyceraldehyde-3-phosphate dehydrogenase
7	TDH2	332	glyceraldehyde-3-phosphate dehydrogenase 2
7	TDH3	332	glyceraldehyde-3-phosphate dehydrogenase 3
8	TDH3	332	glyceraldehyde-3-phosphate dehydrogenase 3
8	TDH3	332	glyceraldehyde-3-phosphate dehydrogenase 3
9	TDH3	332	glyceraldehyde-3-phosphate dehydrogenase 3
6	TEF1/TEF2	458	Translation elongation factor 1-alpha
8	TEF1/TEF2	458	Translation elongation factor 1-alpha
8	TPI1	248	Triosephosphate isomerase
10	TRS31	238	Core subunit of TRAPP complex I-III (GEFs for Ypt1)
9	TSA1	196	Thioredoxin peroxidase
4	Virus Protein L-A	1505	Viral poly protein Virus L-A
3	Virus Protein L-BC	1512	Viral poly protein Virus L-BC
4	Virus Protein L-BC	1512	Viral poly protein Virus L-BC
5	Virus Protein L-BC	1512	Viral poly protein Virus L-BC
6	Virus Protein L-BC	1512	Viral poly protein Virus L-BC
7	Virus Protein L-BC	1512	Viral poly protein Virus L-BC
8	Virus Protein L-BC	1512	Viral poly protein Virus L-BC
9	Virus Protein L-BC	1512	Viral poly protein Virus L-BC
5	Vma2	517	Subunit B of V1 peripheral domain of vacuolar ATPase
6	VMA6	345	V-type proton ATPase subunit d
1A	VPS10/PEP1	1579	Sorting receptor for Vac Hydrolases
2	VPS10/PEP1	1579	CPY sorting receptor
3	VPS10/PEP1	1579	CPY sorting receptor
3	Vps35	944	Retromer subunit
8	VTI1	217	v-Snare, interacts w/Sed5+Pep12
3	YEF3	1044	Translation elongation factor EF-3A
8	YIP4	235	Trans Membrane Protein of Late Golgi that bnds YPTs
7	YIP5	310	Transmembrane protein of late Golgi that binds YPTs
9	YMR295C	197	Unknown function. Localization to cell periphery and bud
8	YPT1	206	RAB GTPase Ypt1
9	ZEO1	113	Zeocin resistance protein; Cell integrity pathway

Supplemental Table S6: Auxilin depletion, all MS hits with slice info

Slice#	Gene	Seq. Length	Protein name
1	ACC1	2233	Acetyl-CoA carboxylase
3	ADE3	946	C1-tetrahydrofolate synthase
3	ADE4	510	Amidophosphoribosyltransferase
4	ADE4	510	Amidophosphoribosyltransferase
5	ADE4	510	Amidophosphoribosyltransferase
6	ADE4	510	Amidophosphoribosyltransferase
7	ADE4	510	Amidophosphoribosyltransferase
8	ADE4	510	Amidophosphoribosyltransferase
9	ADE4	510	Amidophosphoribosyltransferase
10	ADE4	510	Amidophosphoribosyltransferase
6	ADH1	348	Alcohol Dehydrogenase
9	AHP1	177	Thiol-specific peroxiredoxin
3	APL4	832	AP-1 complex Gamma
4	APL4	832	AP-1 complex Gamma
5	APM1	475	AP-1 complex medium (mu)
5	APM1	475	AP-1 complex medium (mu)
6	APM1	475	AP-1 complex medium (mu)
6	APM1	475	AP-1 complex medium (mu)
9	APS1	156	AP-1 adaptor
10	APS1	156	AP-1 adaptor
7	ASC1	319	G-protein beta subunit/guanine dissociation inhibitor for Gpa2p
8	ATG27	271	Transmembrane protein involved in Autophagy
7	BMH1/BMH2	267/273	14-3-3 protein
5	CDC19	501	Pyruvate Kinase
9	CDC19	501	Pyruvate Kinase
10	CDC19	501	Pyruvate Kinase
5	CDC19/PYK1	501	Pyruvate Kinase
3	CDC48	835	AAA ATPase
6	CDC48	835	AAA ATPase
1	CHC1	1653	Clathrin Heavy Chain
2	CHC1	1653	Clathrin Heavy Chain
3	CHC1	1653	Clathrin Heavy Chain
4	CHC1	1653	Clathrin Heavy Chain
5	CHC1	1653	Clathrin Heavy Chain
6	CHC1	1653	Clathrin Heavy Chain
7	CHC1	1653	Clathrin Heavy Chain
3	CLC1	233	Clathrin Light Chain
6	CLC1	233	Clathrin Light Chain
7	CLC1	233	Clathrin Light Chain
8	CLC1	233	Clathrin Light Chain
9	CLC1	233	Clathrin Light Chain
10	CLC1	233	Clathrin Light Chain
3	CLU1/TIF1	1277	EIF3-TL initiation factor
4	CPS1	576	Vacuolar carboxypeptidase S
5	CPS1	576	Vacuolar carboxypeptidase S
1	DRS2	1355	TGN aminophospholipid translocase (flippase)

3	DRS2	1355	TGN aminophospholipid translocase (flippase)
3	ECM33	429	GPI-anchored protein of unknown function
9	EMP24	203	Member of the p24 family involved in ER to Golgi transport
6	EMP47	445	Integral membrane component of ER-derived COPII vesicles
3	ENO1	437	Enolase
5	ENO2	437	Enolase
6	ENO2	437	Enolase
8	ERP1	219	Member of the p24 family involved in ER to Golgi transport
9	ERP1	219	Member of the p24 family involved in ER to Golgi transport
10	ERP1	219	Member of the p24 family involved in ER to Golgi transport
10	ERP1	219	Member of the p24 family involved in ER to Golgi transport
8	ERP2	215	Member of the p24 family involved in ER to Golgi transport
10	Erv14	138	COPII-coated vesicle protein
8	ERV25	211	Member of the p24 family involved in ER to Golgi transport
9	ERV25	211	Member of the p24 family involved in ER to Golgi transport
1	FAS1	2051	Beta subunit of fatty acid synthetase
5	FAS2	1887	Alpha subunit of fatty acid synthetase
10	FAS2	1887	Alpha subunit of fatty acid synthetase
6	FBA1	359	Fructose 1,6-bisphosphate aldolase
10	FET3	636	Ferro-O2-oxidoreductase, Iron uptake, TM protein
1	FKS1	1876	1,3-beta-D-glucan synthase
3	FKS1	1876	1,3-beta-D-glucan synthase
3	FKS1	1876	1,3-beta-D-glucan synthase
3	FKS1/GSC2	1876/1895	1,3-beta-D-glucan synthase
10	FKS1/GSC2	1876/1895	1,3-beta-D-glucan synthase
1	GSC2	1895	1,3-beta-D-glucan synthase
3	GSC2	1895	1,3-beta-D-glucan synthase
3	GSC2	1895	1,3-beta-D-glucan synthase
3	GAS1	559	Glycolipid anchored protein
3	GAS1	559	Glycolipid anchored protein
7	GCN3	305	Alpha subunit of translation initiation factor eIF2B
9	GEF1	779	Cl-channel-lateGolgi/Endo
6	GPM1	247	Phophoglycerate mutase 1
4	GSY2	705	glycogen synthase
1	HXT6	570	High-affinity hexose transporter
3	HXT6	570	High-affinity hexose transporter
3	HXT6	570	High-affinity hexose transporter
6	HXT6	570	High-affinity hexose transporter
6	HXT7	570	High-affinity hexose transporter
3	KEX1	729	Transmembrane carboxypeptidase of TGN
5	KRE2/MNT1	442	Alpha1,2-mannosyltransferase of the Golgi
6	LSP1	341	Eisosome core component
3	MDR1/GYP2/MIC1	950	GAP for Rabs Ypt6, Ypt31, Sec4
3	PFK1?	987	phosphofructokinase 2
3	PFK2	959	phosphofructokinase 2
3	PFK2	959	phosphofructokinase 2
4	PFK2	959	phosphofructokinase 2
6	PFK2	959	phosphofructokinase 2
6	PFK2	959	phosphofructokinase 2

7	PFK2	959	phosphofructokinase 2
3	PGK1	417	Phosphoglycerate kinase
6	PGK1	417	Phosphoglycerate kinase
6	PGK1	417	Phosphoglycerate kinase
8	PHO84	587	High-affinity inorganic phosphate (Pi) transporter
7	PIL1	339	Eisosome core component
6	PIL1	339	Eisosome core component
9	PIL1	339	Eisosome core component
1	PMA1	918	PM ATPase
3	PMA1	918	PM ATPase
3	PMA1/2	918/947	PM ATPase
3	PMA1/2	918/947	PM ATPase
5	PMA1/PMA2	918/947	PM ATPase
6	PMA1/PMA2	918/947	PM ATPase
1	PMA2	947	PM ATPase
3	PMA2	947	PM ATPase
4	PMA2/PMA1	947/918	PM ATPase
8	RET2	546	Delta subunit of CopI Coatmer
8	RHO1	209	Rho GTPase Rho1
6	RPG1	964	eIF3alpha translation initiation subunit
9	RPL11A/B	174	ribosomal 60S subunit protein L11
10	Rpl12A/B	165	ribosomal 60S subunit protein L12
9	Rpl12A/B	165	ribosomal 60S subunit protein L12
9	RPL14	138	ribosomal 60S subunit protein L14
9	RPL14	138	ribosomal 60S subunit protein L14
10	Rpl14	138	ribosomal 60S subunit protein L14
10	Rpl14	138	ribosomal 60S subunit protein L14
9	RPL17A	184	ribosomal 60S subunit protein L17
9	RPL20	172	Ribosomal 60S subunit protein L20
10	Rpl20	172	Ribosomal 60S subunit protein L20
10	Rpl22	122	Ribosomal 60S subunit protein L22
8	RPL22A	122	Ribosomal 60S subunit protein L22
9	RPL22A	122	Ribosomal 60S subunit protein L22
10	RPL23A/B	137	Ribosomal 60S subunit protein L23
9	RPL25	142	Ribosomal 60S subunit protein L25
9	RPL25	142	Ribosomal 60S subunit protein L25
9	RPL25	142	Ribosomal 60S subunit protein L25
10	Rpl25	142	Ribosomal 60S subunit protein L25
10	Rpl25	142	Ribosomal 60S subunit protein L25
9	Rpl27	136	Ribosomal 60S subunit protein L27
10	Rpl27	136	Ribosomal 60S subunit protein L27
9	Rpl28	149	Ribosomal 60S subunit protein L28
6	RPL2A	254	Ribosomal 60S subunit protein L2
6	RPL3	387	Ribosomal 60S subunit protein L3
6	RPL3	387	Ribosomal 60S subunit protein L3
10	Rpl3	387	Ribosomal 60S subunit protein L3
10	Rpl30	105	Ribosomal 60S subunit protein L30
9	Rpl32	130	Ribosomal 60S subunit protein L32
10	RPL33A	107	Ribosomal 60S subunit protein L33

10	Rpl33B	107	Ribosomal 60S subunit protein L33
10	Rpl35	120	Ribosomal 60S subunit protein L35
10	Rpl38	78	Ribosomal 60S subunit protein L38
6	RPL4	362	Ribosomal 60S subunit protein L4
6	RPL4	362	Ribosomal 60S subunit protein L4
8	RPL4A/B	362	Ribosomal 60S subunit protein L4
6	RPL40	128	Ubiquitin-ribosomal 60S subunit protein L40A fusion protein
6	RPL40	128	Ubiquitin-ribosomal 60S subunit protein L40A fusion protein
6	RPL5	297	Ribosomal 60S subunit protein L5
7	Rpl5	297	Ribosomal 60S subunit protein L5
9	RPL5	297	Ribosomal 60S subunit protein L5
9	Rpl5	297	Ribosomal 60S subunit protein L5
7	RPL7A/B	244	Ribosomal 60S subunit protein L7
8	RPL7A/B	244	Ribosomal 60S subunit protein L7
7	RPP0	312	Ribosomal protein P0 of the ribosomal stalk
8	RPP0	312	Ribosomal protein P0 of the ribosomal stalk
8	RPP0	312	Ribosomal protein P0 of the ribosomal stalk
10	Rpp1A	106	Ribosomal Protein P1 Alpha
10	Rpp1B	106	Ribosomal Protein P1 Beta
10	Rpp2A	106	Ribosomal protein P2 alpha
10	Rpp2B	110	Ribosomal protein P2 beta
9	Rps0a&b	252	Ribosomal 40S subunit protein S0
9	RPS12	143	Ribosomal 40S subunit protein S12
10	Rps12	143	Ribosomal 40S subunit protein S12
9	Rps13	151	Ribosomal 40S subunit protein S13
10	Rps13	151	Ribosomal 40S subunit protein S13
10	Rps14	137	Ribosomal 40S subunit protein S14
9	RPS15	142	Ribosomal 40S subunit protein S15
10	RPS15	142	Ribosomal 40S subunit protein S15
10	Rps16A/B	143	Ribosomal 40S subunit protein S16
9	RPS16A/B	143	Ribosomal 40S subunit protein S16
9	Rps16A/B	143	Ribosomal 40S subunit protein S16
9	Rps17	136	Ribosomal 40S subunit protein S17
10	Rps17	136	Ribosomal 40S subunit protein S17
9	RPS19A/B	144	Ribosomal 40S subunit protein S19
9	Rps20	121	Ribosomal 40S subunit protein S20
9	RPS22	130	Ribosomal 40S subunit protein S22
10	RPS22	130	Ribosomal 40S subunit protein S22
10	RPS22	130	Ribosomal 40S subunit protein S22
10	RPS24A/B	135	Ribosomal 40S subunit protein S24
10	RPS28	67	Ribosomal 40S subunit protein S28
7	Rps3	240	Ribosomal 40S subunit protein S3
7	RPS3	240	Ribosomal 40S subunit protein S3
8	RPS3	240	Ribosomal 40S subunit protein S3
9	RPS3	240	Ribosomal 40S subunit protein S3
9	RPS3	240	Ribosomal 40S subunit protein S3
10	RPS3	240	Ribosomal 40S subunit protein S3
10	Rps31	152	Fusion protein cleaved to yield ribosomal protein S31 and ubiquitin
10	Rps31	152	Fusion protein cleaved to yield ribosomal protein S31 and ubiquitin

9	Rps4A/B	261	Ribosomal 40S subunit protein S4
6	RPS5	225	Ribosomal 40S subunit protein S5
6	RPS5	225	Ribosomal 40S subunit protein S5
7	Rps5	225	Ribosomal 40S subunit protein S5
8	RPS5	225	Ribosomal 40S subunit protein S5
9	RPS5	225	Ribosomal 40S subunit protein S5
9	RPS5	225	Ribosomal 40S subunit protein S5
10	Rps5	225	Ribosomal 40S subunit protein S5
7	RPS6	236	Ribosomal 40S subunit protein S6
10	Rps6	236	Ribosomal 40S subunit protein S6
8	RPS6A/B	236	Ribosomal 40S subunit protein S6
7	SEC17	292	Alpha SNAP/vesicle fusion
7	SEC17	292	Alpha SNAP/vesicle fusion
3	SEC23	768	Component of COPII vesicle coat; GAP for Sar1
4	SEC23	768	Component of COPII vesicle coat; GAP for Sar1
3	SEC24	926	Component of COPII vesicle coat
3	SEC24	926	Component of COPII vesicle coat
3	SEC26	973	Beta subunit of the COPI coatomer
3	SEC27	889	Beta' subunit of the COPI coatomer
3	RPN2/SEN3	945	26S proteasome subunit
7	RPN11	306	Proteasome, Metalloprotease subunit of 19S regulatory particle
5	RPN3	523	Essential non-ATPase regulatory subunit of the 26S proteasome lid
9	SFT2	215	syntxn 5-golgi to endosome(synt lw/ypt6, ric1, got1)
6	SHM2	469	seine hydroxymethyl transferase
3	SKN1	771	Protein involved in sphingolipid synth
9	SNA3	133	Membrane Prot, MVB sorting
9	SNA3	133	Membrane Prot, MVB sorting
9	SOD1	154	Superoxide dismutase
4	SSA2	639	HSC chaperone SSA family
9	SYN8	255	Endosomal t-SNARE
10	SYN8	255	Endosomal t-SNARE
6	TCP1	559	Alpha subunit of chaperonin-containing T-complex
7	TDH1/2/3	332	glyceraldehyde-3-phosphate dehydrogenase
7	TDH1/2/3	332	glyceraldehyde-3-phosphate dehydrogenase
6	TDH3	332	glyceraldehyde-3-phosphate dehydrogenase
7	TDH3	332	glyceraldehyde-3-phosphate dehydrogenase
6	TLG1	224	T-snare:late golgi
8	TLG1	224	T-snare:late golgi
9	TLG1	224	T-snare:late golgi
7	TPM1	199	tropomyosin
8	TPM2	161	tropomyosin
6	TUB2	457	Beta Tubulin
1	UBI	128	Ubiquitin
4	UBI	128	Ubiquitin
6	UBI	128	Ubiquitin
7	UBI	128	Ubiquitin
5	UGP1	499	UDP-glucose pyrophosphorylase
6	Virus Protein L-BC	1512	Viral poly protein Virus L-BC
4	Viral Polyprot	1512	Viral Poly Protein Virus L-BC

3	Viral Polyprot	1512	Viral Poly Protein Virus L-BC
4	Viral Polyprot	1512	Viral Poly Protein Virus L-BC
7	Viral Polyprot	1512	Viral Poly Protein Virus L-BC
7	Viral Polyprot	1512	Viral Poly Protein Virus L-BC
5	Viral Polyprot	1512	Viral Poly Protein Virus L-BC
5	VMA2	517	Subunit B of V1 peripheral domain of vacuolar ATPase
5	VMA2	517	Subunit B of V1 peripheral domain of vacuolar ATPase
7	VMA6	345	vacuolar ATPase--Vo domain
4	VPS1	704	Dynamin
5	VPS1	704	Dynamin
7	VPS1	704	Dynamin
3	VPS1	704	Dynamin
4	VPS1	704	Dynamin
2	VPS10/PEP1	1577	CPY sorting receptor
3	VPS10/PEP1	1577	CPY sorting receptor
6	VPS10/PEP1	1577	CPY sorting receptor
9	VPS10/PEP1	1577	CPY sorting receptor
3	VPS10/PEP1	1577	CPY sorting receptor
10	VPS10/PEP1	1577	CPY sorting receptor
5	VPS17/PEP21	551	Retromer subunit
3	VPS35	944	Retromer subunit
8	VTI1	217	v-Snare, interacts w/Sed5+Pep12
9	YCL012C	134	Membrane protein, Unknown Function
3	YDJ1	409	DNA J protein
5	YDJ1	409	DNA J protein
6	YDJ1	409	DNA J protein
7	YDJ1	409	DNA J protein
8	YDJ1	409	DNA J protein
9	YDL121C	149	ER Membrane Prot, function unknown
7	YIP5	310	Transmembrane protein of late Golgi that binds YPTs
9	ZEO1	113	Zeocin resistance protein; Cell integrity pathway

Supplemental Table S7: Protein hits found only in auxilin depletion fractions

Systematic Name*	Standard Name	Seq. Length	Protein name or Description
ER membrane proteins			
YGL200C	EMP24	203	Member of the p24 family involved in ER to Golgi transport
YFL048C	EMP47	445	Integral membrane component of ER-derived COPII vesicles
YAR002C-A	ERP1	219	Member of the p24 family involved in ER to Golgi transport
YAL007C	ERP2	215	Member of the p24 family involved in ER to Golgi transport
YGL054C	Erv14	138	COPII-coated vesicle protein
YML012W	ERV25	211	Member of the p24 family involved in ER to Golgi transport
YDL121C	YDL121C	149	ER Membrane Prot, function unknown
COP subunits			
YPR181C	SEC23	768	Component of COPII vesicle coat; GAP for Sar1
YIL109C	SEC24	926	Component of COPII vesicle coat
YDR238C	SEC26	973	Beta subunit of the COPI coatmer
YGL137W	SEC27	889	Beta' subunit of the COPI coatmer
YFR051C	RET2	546	Delta subunit of COPI Coatmer
Other membrane transport			
YKR001C	VPS1	704	Dynamin
YOR132W	VPS17/PEP21	551	Retromer subunit
Other membrane proteins			
YJL172W	CPS1	576	Vacuolar carboxypeptidase S
YAL026C	DRS2	1355	TGN aminophospholipid translocase (flippase)
YBR078W	ECM33	429	GPI-anchored protein of unknown function
YMR058W	FET3	636	Ferro-O2-oxidoreductase, Iron uptake, TM protein
YGL203C	KEX1	729	Transmembrane carboxypeptidase of TGN
YDR483W	KRE2/MNT1	442	Alpha1,2-mannosyltransferase of the Golgi
YML123C	PHO84	587	High-affinity inorganic phosphate (Pi) transporter
YJL151C	SNA3	133	Membrane protein, MVB sorting
YAL014C	SYN8	255	Endosomal t-SNARE
YDR468C	TLG1	224	T-snare late golgi
YCL012C	YCL012C	134	Membrane protein, Unknown Function
Eisosome components			
YPL004C	LSP1	341	Eisosome core component
YGR086C	PIL1	339	Eisosome core component
Proteasome			
YFR004W	RPN2/SEN3	945	26S proteasome subunit
YIL075C	RPN11	306	Proteasome, Metalloprotease subunit of 19S regulatory particle
YER021W	RPN3	523	Essential non-ATPase regulatory subunit of the 26S proteasome lid
NA	UBI	128	Ubiquitin
Metabolic processes			
YNR016C	ACC1	2233	Acetyl-CoA carboxylase
YGR204W	ADE3	946	C1-tetrahydrofolate synthase
YMR116C	ASC1	319	G-protein beta subunit/guanine dissociation inhibitor for Gpa2p
YLR258W	GSY2	705	Glycogen synthase
YJR104C	SOD1	154	Superoxide dismutase
YKL035W	UGP1	499	UDP-glucose pyrophosphorylase
Chaperone/Protein folding			
YDL126C	CDC48	835	AAA ATPase
YDR212W	TCP1	559	Alpha subunit of chaperonin-containing T-complex
YNL064C	YDJ1	409	DNA J protein
Cytoskeleton			
YNL079C	TPM1	199	Tropomyosin
YIL138C	TPM2	161	Tropomyosin
YFL037W	TUB2	457	Beta Tubulin

*Notes: Proteins involved in translation were eliminated from the auxilin only hit list.