

Figure S1

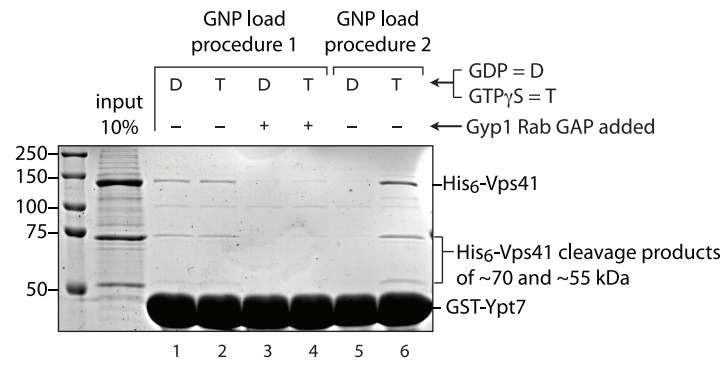
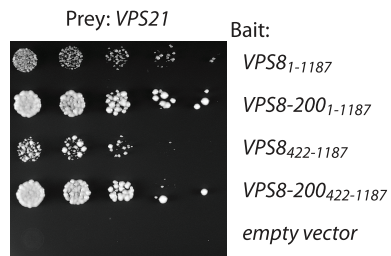
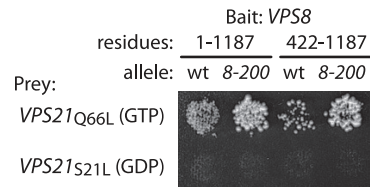


Figure S2

A



B



Supplementary Table 1. Informatic analyses: domain structures of Vps-C/HOPS and Vps-C/CORVET subunits from *Saccharomyces cerevisiae* and *Homo sapiens*

Protein	Ref/Systematic	Length	FW	DomStart	DomEnd	Method	Fold/motif	Model	ModStart	ModEnd	RawSig	AdjSig
Vps3	YDR495C	1011	117	1	140	Ginzu/msa						
				141	238	Ginzu/cutpref						
				329	416	Ginzu/msa						
				417	636	Ginzu/cutpref						
				637	853	Ginzu/cutpref						
				854	996	Ginzu/cutpref						
Vps8	VAL002W	1274	145									
				122	161	SMART	WD40				2.30E-01	0.6
				181	224	SMART	WD40				8.70E+01	-1.9
				1198	1265	SMART	RING				1.60E-02	1.8
				125	161	pfam motif scan	WD40				8.40E-01	0.1
				484	664	pfam motif scan	Clathrin				5.70E-01	0.2
				1001	1023	pfam motif scan	Vps16_C				3.00E-01	0.5
				1197	1268	pfam motif scan	PHD				2.40E-01	0.6
				1231	1265	pfam motif scan	zf-C4HC4				6.20E-02	1.2
				508	665	Ybe et al.	Clathrin (1 of 2)					
				915	1092	Ybe et al.	Clathrin (2 of 2)					
Vps11/Pep5p	YMR231W	1029	117.5									
				1	399	Manual/pdbblast	B-propellor	INR0A Aip1p ortholog	151	529	3.11E+01	31.1
				1	421	Ginzu/pdbblast	B-propellor	INR0A Aip1p ortholog	151	557	3.52E+01	35.2
				422	722	Manual/Ginzu/2djury	A-solenoid	IBK5a Srp1p karyopherin alpha	1	359	1.02E+00	1.0
				723	1187	Ginzu/pdbblast	A-solenoid	IB89a Clathrin proximal leg	25	443	2.45E+01	24.5
				1188	1274	Ginzu/ffas03		1V87a RING finger from mouse Deltex 2	5	95	2.45E+00	2.5
				882	913	SMART	coiled-coil					
				927	973	SMART	PHD					
				928	972	SMART	RING					
				373	432	pfam motif scan					3.20E-03	2.5
				406	555	pfam motif scan	pre:CLATHRIN_REPEAT				2.50E-05	4.6
				928	972	pfam motif scan	prf:ZF_RING_2				1.10E-03	3.0
				943	975	pfam motif scan	pfam_fs:PHD				1.60E-06	5.8
				928	972	pfam motif scan	pfam_fs:zf-C3HC4				5.50E-04	3.3
				927	975	pfam motif scan	pfam_ls:PHD				2.10E-04	3.7
				928	972	pfam motif scan	pfam_ls:zf-C3HC4				4.10E-04	3.4
				460	583	Ybe et al.	Clathrin					
				364	505	Ginzu/pdbblast	A-bundle	1NA0a engineered TPR A-helix array	50	105	3.52E+00	3.5

			506	925	G.inzu/3Djury	A-solenoid		1EJLI Mouse importin alpha	8	391	2.07E+00	2.1
			1	350	Manual/pdbblast	B-propeller		IGG2b G-beta			3.94E+01	39.4
			926	C	Manual/pdbblast	RING C3HC4		LJM7b Brca1BARD1 RING			4.00E+00	4.0
			1	349	Manual	B-propeller						
			350	926	Manual	A-solenoid						
			926	1029	Manual	RING						
hVPS11	Q9H270	941	107.8	860	SMART	RING					6.00E-05	4.2
			822	860	pfam motif scan	zf-C3HC4					2.10E-03	2.7
			1	72	G.inzu/pdbblast			IP22a Beta-TrCP1 WD40 domain	143	159	4.24E+01	42.4
			73	337	G.inzu/pdbblast			IP16a yeast AIp1p	55	304	1.42E+01	14.2
			338	715	G.inzu/pdbblast	A-solenoid		IB89a Clathrin proximal leg	133	449	3.11E+01	31.1
			716	941	G.inzu/pdbblast			IFBVa c-Cbl SH2-like and RING domains	235	378	5.00E+00	5.0
			716	861	G.inzu/pdbblast			IFBVa c-Cbl SH2-like and RING domains	235	378	5.00E+00	5.0
			862	941	G.inzu/3Djury			ISFFa Gamma-Aminobutyrate Aminotrans	313	381	1.02E+00	1.0
Vps16p	YPL045W	798	92.7	4	SMART	pfam:VPS16N					8.00E-216	215.1
			478	798	SMART	pfam:VPS16C					1.60E-193	192.8
			4	379	pfam motif scan	pfam_fs:Vps16_N					6.60E-218	217.2
			478	798	pfam motif scan	pfam_fs:Vps16_C					1.40E-193	192.9
			1	395	G.inzu/ffas03	B-propeller		ITYQc p40 of Arp2/3 complex	1	372	2.18E+00	2.2
			1	530	G.inzu/ffas03	B-propeller		INR0A Aip1p ortholog	74	606	2.30E+00	2.3
			396	798	G.inzu/3Djury	A-solenoid/bundle		IN1Ba bornyl diphosphate synthase	62	489	2.06E+00	2.1
			531	798	G.inzu/3Djury	A-solenoid		LIB2a Pumilio-1 PUM repeat	26	296	2.06E+00	2.1
			1	285	Manual/3Djury	B-propeller		IG0Tb Transducin beta	25	331	4.91E+00	4.9
			1	308	Manual/ffas03	B-propeller		IERJa Tup1 WD40 domain	36	392	4.95E+00	5.0
			285	798	Manual/3Djury	A-solenoid		IEE4a Srp1p karyopherin alpha			2.05E+00	2.0
			1	307	Manual	B-propeller						
			308	798	Manual	B-propeller						
hVPS16	Q9H269	839	94.6	4	SMART	pfam:Vps16_N					4.60E-270	270.0
			517	835	SMART	pfam:Vps16_N					2.10E-202	202.0
			4	420	pfam motif scan	pfam_fs:Vps16_N					3.50E-271	270.5
			517	835	pfam motif scan	pfam_fs:Vps16_C					1.60E-203	202.8

Table S4. Phenotypes resulting from HOPS/CORVET truncations

A. CPY and ALP maturation		
Genotype	CPY	ALP
Wild-type	Mature	Mature
<i>pep4Δ prb1Δ</i>	Pro [p2]	Pro-
<i>vps11^{736Δ}</i>	Pro [p2]	Pro-
<i>vps11^{926Δ} (ΔRING)</i>	Mature	Pro-
<i>vps18^{826Δ} (ΔRING)</i>	Pro [p2]	Pro-
<i>vps8^{1188Δ} (ΔRING)</i>	Mature & Pro [p2]	Mature
<i>vps3^{900Δ}</i>	Pro [p2]	Mature & Pro-
<i>vps39^{860Δ}</i>	Mature & Pro [p2]	Pro-
<i>vps39^{979Δ} (ΔRING)</i>	Mature & Pro [p2]	Mature & Pro-

B. CPY missorting	
Genotype	CPY-invertase secretion
Wild-type	None
<i>pep4Δ prb1Δ</i>	None
<i>vps3Δ</i>	Strong
<i>vps3^{900Δ}</i>	Strong
<i>vps11Δ</i>	Strong
<i>vps11^{1-735Δ}</i>	Strong
<i>vps11^{926Δ} (ΔRING)</i>	Weak

C. Complementation of <i>vps11^{735Δ}</i> sorting defects by <i>vps11⁷³⁶⁻¹⁰²⁹</i>			
Genotype	CPY	ALP	GFP-CPS
Wild-type	Mature	Mature	Vacuole lumen
<i>vps11Δ</i>	Pro [p2]	Pro-form	N/A
<i>vps11^{735Δ}</i>	Pro [p2]	Pro-form	N/A
<i>vps11⁷³⁶⁻¹⁰²⁹</i>	Pro [p2]	Pro-form	N/A
<i>vps11^{735Δ} + vps11⁷³⁶⁻¹⁰²⁹</i>	Mature	Mature	Vacuole lumen

Table S5 Strains used in this study

Strain	Genotype	Source
PJ694a	<i>MATa trp1-901, leu2-3,112, ura3-52, his3-200 gal4Δ, gal80Δ, LYS2::GAL1-HIS3, GAL2-ADE2, met2::GAL7, -lacZ</i>	James <i>et al.</i> , 1996
PJ694α	<i>MATa trp1-901, leu2-3,112, ura3-52, his3-200 gal4Δ, gal80Δ, LYS2::GAL1-HIS3, GAL2-ADE2, met2::GAL7, -lacZ</i>	James <i>et al.</i> , 1996
BY4742	<i>MATa his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0</i>	ATCC
AMY942	BY4742; <i>vps39Δ::KanMX4</i>	This study
AMY885	BY4742; <i>vps39-860Δ::URA3</i>	This study
AMY886	BY4742; <i>vps39-979Δ::URA3</i>	This study
AMY1106	BY4742; <i>vps11Δ::KanMX4</i>	This study
AMY1107	BY4742; <i>vps11-350Δ::KanMX4</i>	This study
AMY1108	BY4742; <i>vps11-445Δ::KanMX4</i>	This study
AMY1109	BY4742; <i>vps11-505Δ::KanMX4</i>	This study
AMY1199	BY4742; <i>vps11-735Δ::KanMX4</i>	This study
AMY1198	BY4742; <i>vps11-926Δ::KanMX4</i>	This study
BJ8987	<i>MATa vps8-200 ura3-52 trp1 leu2Δ1 his3Δ200</i>	Jones <i>et al.</i> , 1998
BJ4490	<i>MATa pep5 (vps11Δ445)::TRP1 vps8-200 leu2Δ1 trp1Δ101</i>	Jones <i>et al.</i> , 1998
BJ6919	<i>MATa pep5 (vpsΔ445)::TRP1 leu2Δ1 trp1 ade6</i>	Jones <i>et al.</i> , 1998
BJ8866	<i>MATa leu2Δ1 ura3-52 trp1</i>	Jones <i>et al.</i> , 1998
AMY1113	<i>MATa vps8-200 ura3-52 trp1 leu2Δ1 his3Δ200 vps11-445Δ::KanMX4</i>	This study
SEY6210	<i>MATa leu2-3,112 ura3-52 his3-Δ200 trp1-Δ901 lys2-801 suc2-Δ9</i>	Robinson <i>et al.</i> , 1988
BHY10	<i>MATa leu2-3,112 ura3-52 his3-Δ200 trp1-Δ901 lys2-801 suc2-Δ9 CPY-invertase::LEU2</i>	Horazdovsky <i>et al.</i> , 1994
DNY356	<i>MATa leu2-3,112 ura3-52 his3-Δ200 trp1-Δ901 lys2-801 suc2-Δ9 CPY-invertase::LEU2 vps11Δ::KanMX4</i>	This study
DNY357	<i>MATa leu2-3,112 ura3-52 his3-Δ200 trp1-Δ901 lys2-801 suc2-Δ9 CPY-invertase::LEU2 vps11-926Δ::KanMX4</i>	This study
DNY358	<i>MATa leu2-3,112 ura3-52 his3-Δ200 trp1-Δ901 lys2-801 suc2-Δ9 CPY-invertase::LEU2 vps11-735Δ::KanMX4</i>	This study