

Figure S1. VPS13-GFP under the transcriptional control of ADH1, TEF1 or GPD promoter complements transport defects and improves actin cytoskeleton organization in vps13 Λ . A. Secretion of CPY. Yeast cultures were spotted on nitrocellulose membrane plated on SD-leu medium, incubated for 14-16 h and washed off. The level of secreted CPY was estimated using anti-CPY antibody. B. Sensitivity of yeast strains to canavanine. Serial dilutions of wild type and vps13 Λ strains bearing indicated plasmids were spotted on synthetic minimal medium supplemented with (3 µg ml⁻¹) or without canavanine. C. Graphic representation of actin cytoskeleton organization in vps13 Λ cells. The vps13 Λ cells expressing P_{TEF1}-VPS13-GFP or bearing empty vector was grown to log-phase, fixed, stained using labeled phalloidine and observed by fluorescence microscopy. Wild-type cells bearing empty vector were used as a control. At least 100 cells were observed and the percentage of cells with well-polarized and non-polarized actin cytoskeleton is indicated.

><u>PF10351</u> **Apt1 domain**: Golgi-body localisation protein domain. This domain is found in fungi, metazoans and plants. These include FMP27 and "maize" protein APT1 and *Arabidopsis* homologues SABRE and KIP. APT1 is required for pollen tube growth. It is a Golgi-localised protein and appears to regulate vesicular trafficking. SABRE and KIP are APT1 homologues and they are involved in the elongation of root cortex cells and pollen tubes respectively.

Probab=99.68 E-value=5.9e-15 Score=141.23 Aligned cols=222 Identities=14% Similarity=0.176 Sum probs=0.0

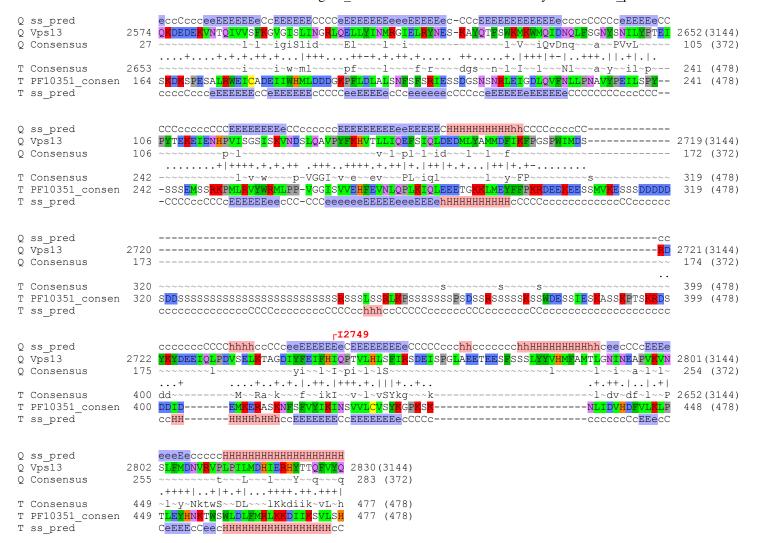


Figure S2. Prediction of the presence of APT1 domain in yeast Vps13 protein. Analysis was performed using HHpred package (Söding et al., 2005; https://toolkit.tuebingen.mpg.de/hhpred) with standard parameters and PfamA (https://toolkit.tuebingen.mpg.de/hhpred) with standard parameters and PfamA (https://ftp.ebi.ac.uk/pub/databases/Pfam/) ver. 04-Jul-16 as a template HMM database. *Upper panel*, graphical representation of predicted domain structure. *Lower panel*, an alignment with secondary structure prediction of Vps13 region L2548-R2919 to a consensus sequence for the APT1 domain (PF10351). Additionally, the position of isoleucine 2749 in Vps13 is indicated. Abbreviations: Q, query sequence; T, template sequence; ss_pred, secondary structure as predicted by PSIPRED (upper case letters: high probability, lower case letters: low probability; C, coil; E, β-sheet; H, helix). Symbols used in consensus line: "=", very bad match (column score below -1.5); "-", bad match (score between -1.5 and -0.5); ".", neutral match (score between -0.5 and +0.5); "+",good match (score between +0.5 and +1.5); "|", very good match (score above +1.5).

Söding J, Biegert A, Lupas AN (2005) The HHpred interactive server for protein homology detection and structure prediction. *Nucleic Acids Res* 33 (suppl 2), W244-W248.

Table S1. Mutant <i>vps13-I2749R</i> is defective in sporulation						
Genotype	Independent diploid	Transformant	Cells	Tetrads	% of tetrads	
vps13∆/vps13∆ [—]	I	1	259	0	0	
		2	306	0	0	
		3	156	0	0	
	II	1	321	0	0	
		2	546	0	0	
		3	384	0	0	
vps13∆/vps13∆ [VPS13]	I	1	320	144	45	
		2	256	70	27	
		3	166	43	26	
	II	1	347	147	42	
		2	359	151	42	
		3	335	129	39	
vps13∆/vps13∆ [vps13-I2749R]	I	1	281	0	0	
		2	198	0	0	
		3	215	0	0	
	II	1	373	0	0	
		2	576	0	0	
		3	457	0	0	

Diploid strain from the cross of JK197-7B ($MATa\ vps13\Delta$:: $URA3\ ura3-52\ his3\Delta200\ met15\Delta0\ leu2,3-112$) with JK197-8C ($MAT\alpha\ vps13\Delta$:: $URA3\ ura3-52\ lys2-801\ trp1-1\ leu2,3-112$) was obtained twice. Each diploid was transformed with empty vector [—] or plasmids bearing VPS13 or vps13-12749R allele. Three independent transformants were sporulated for one week at 28° C. Full tetrads were counted twice for each culture.

Table S2. Mutation *vps13-I2749R* is synthetically lethal with mutation *mmm1∆*

Strain	Transformant	Full tetrads
	1	3 PD
$vps13\Delta/VPS13 mmm1\Delta/MMM1$ [—]	2	1 PD
	3	3 PD
	1	4 PD + 3 TT
$vps13\Delta/VPS13 mmm1\Delta/MMM1 [VPS13]$	2	3 PD + 2 TT
	3	3 PD
	1	1 PD
$vps13\Delta/VPS13 mmm1\Delta/MMM1 [vps13-I2749R]$	2	6 PD
	3	5 PD

Diploid strain was obtained by crossing WR11 ($MAT\alpha$ LAS17-7Ala-mRFP::HIS3 ura3-52 leu2,3-112 lys2-801 $his3\Delta200$ trp1-1 $vps13\Delta$::URA3) with BY4741 $mmm1\Delta$ (MATa $ura3\Delta0$ $leu2\Delta0$ $his3\Delta1$ $met15\Delta0$ $mmm1\Delta$::KanMX). The diploid was transformed with empty vector [—] or plasmid bearing wild-type VPS13 or mutated vps13-I2749R allele. Three independent transformants of each type were sporulated. Full tetrads were dissected and growth of spore clones was analyzed on SC-ura and YPD+G418 media to follow $vps13\Delta$::URA3 and $mmm1\Delta$::KanMX presence. PD, parental ditype; TT, tetratype.