

Supplemental material

JCB

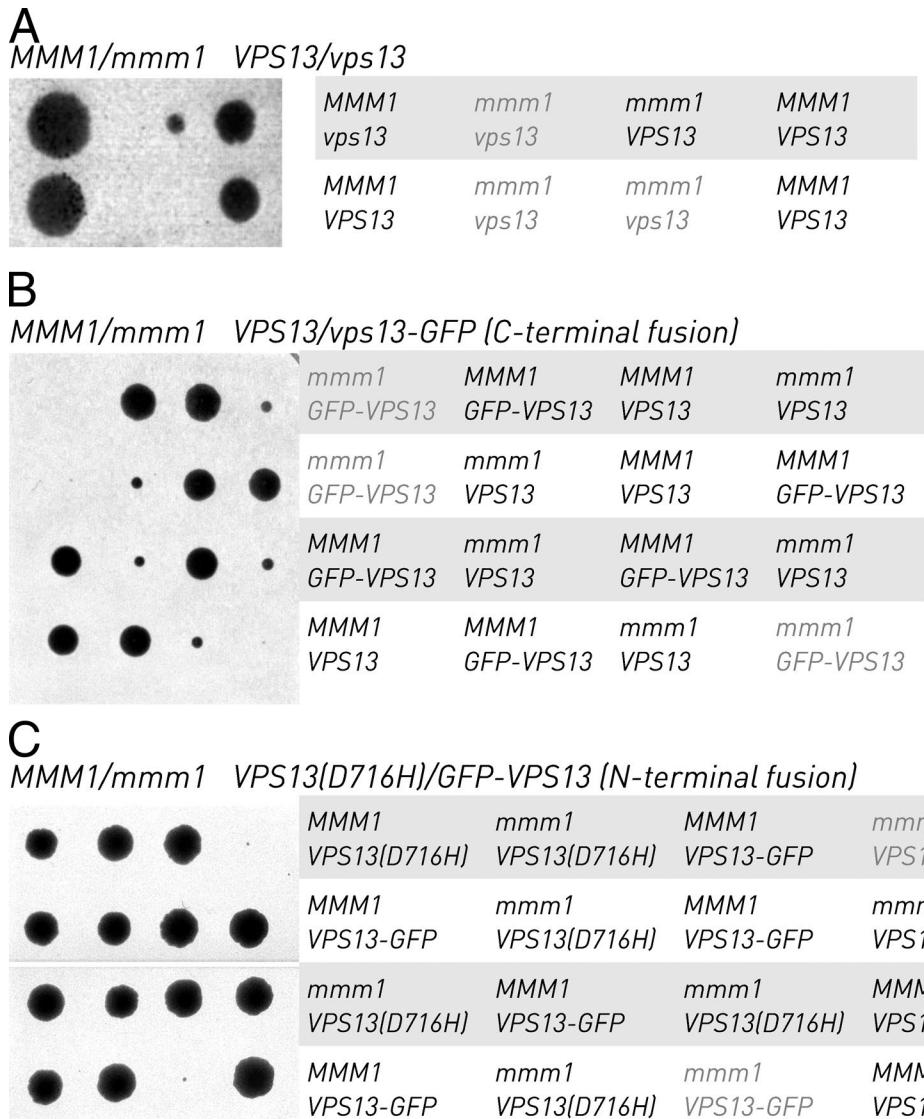
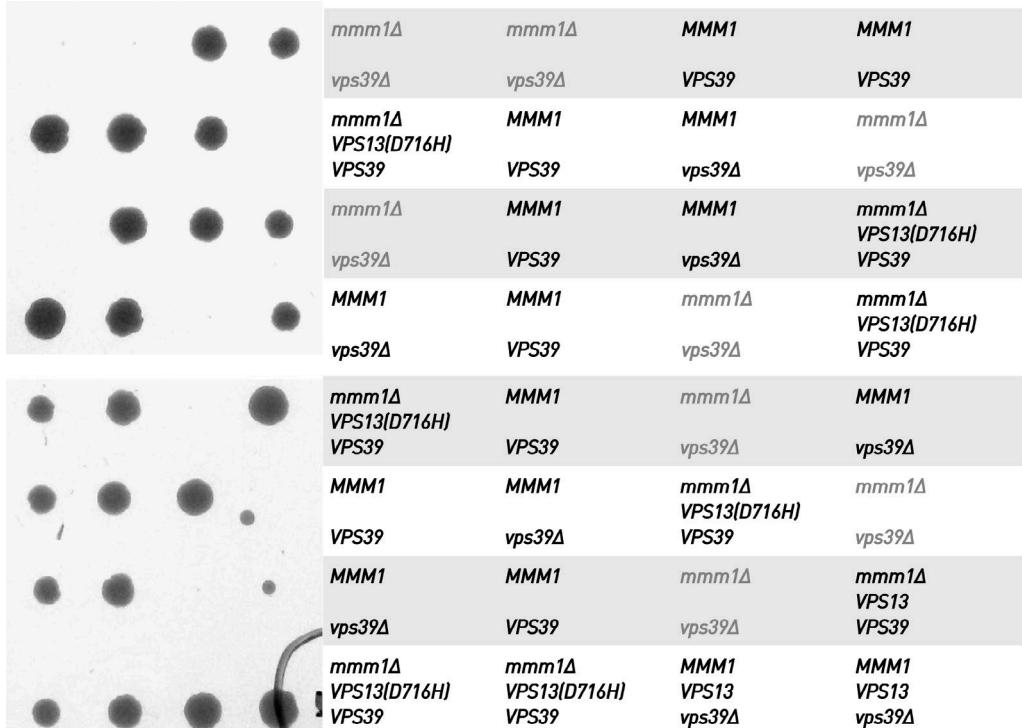
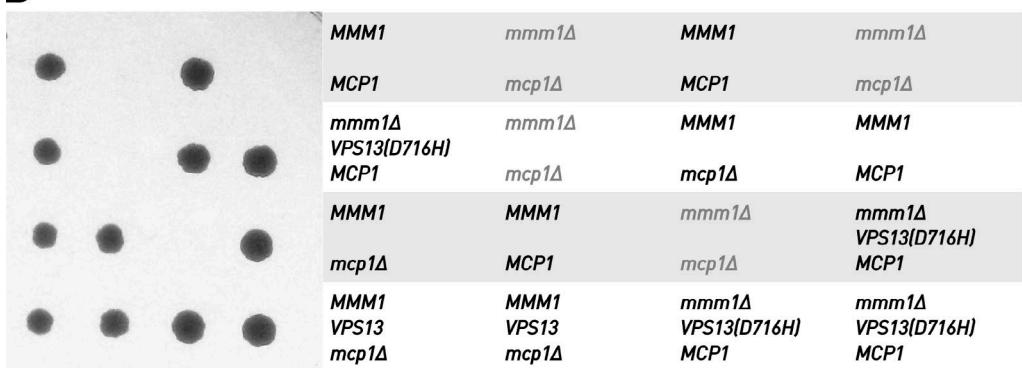
Lang et al., <http://www.jcb.org/cgi/content/full/jcb.201502105/DC1>

Figure S1. Synthetic genetic interactions of *VPS13*. (A–C) Diploids of the indicated genotype were sporulated and tetrad dissected. The relevant genotypes of the resulting spores are indicated. Note that the greyed genotypes have been inferred from the expected Mendelian segregation of the genes. In both A and B, *mmm1Δ* strains bearing either the N- or C-terminal fusion alleles managed to grow microcolonies. However, these could not be propagated further.

A *MMM1/mmm1Δ::KanMX VPS13/VPS13(D716H) Vps39/vps39Δ::HIS3*



B *MMM1/mmm1Δ VPS13/VPS13(D716H) MCP1/mcp1Δ*



C *MMM1/mmm1Δ::KanMX VPS13/VPS13(D716H) MCP2/mcp2Δ::HIS3*

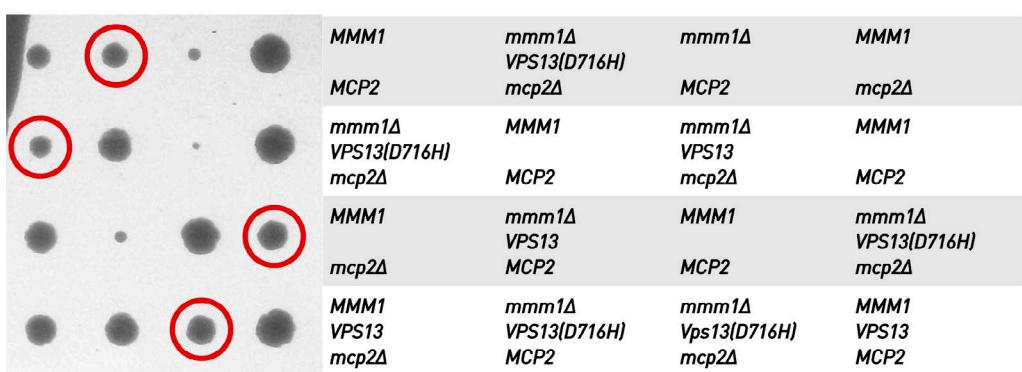


Figure S2. Synthetic genetic interactions of *MMM1* with *MCP1*, *MCP2*, and *VPS39*. Diploids of the indicated genotype were sporulated and tetrad dissected. The relevant genotypes of the resulting spores are indicated. The greyed genotypes have been inferred from the expected Mendelian segregation of the genes. The genotype at the *Vps13* locus has been determined for selected spores only. In C, the red circles highlight *mmm1Δ mcp2Δ VPS13(D716H)* spores. No *mmm1Δ mcp1Δ VPS13(D716H)* or *mmm1Δ vps39Δ VPS13(D716H)* could be retrieved, indicating that the suppressor allele does not alleviate the synthetic lethality of *mmm1Δ* with either *mcp1Δ* or *vps39Δ* strains.

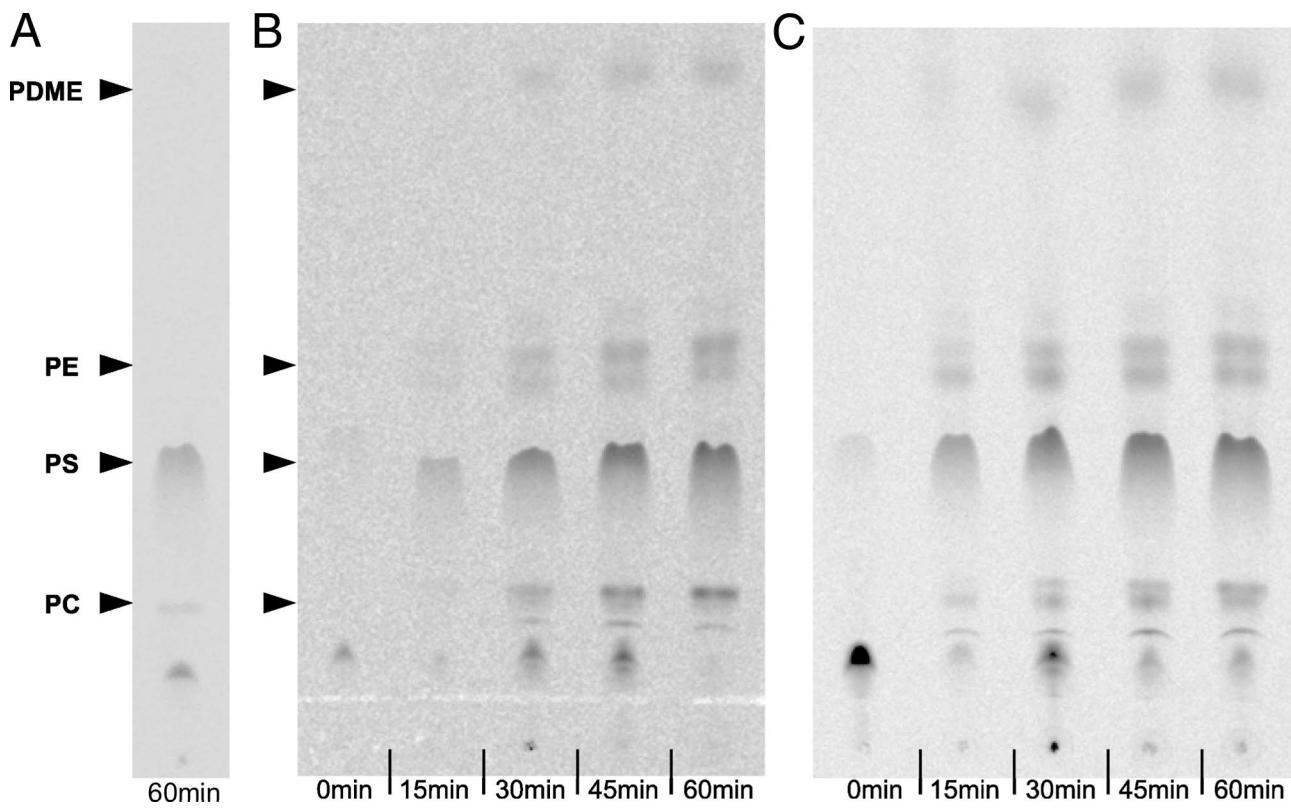


Figure S3. Phospholipid analysis of *Vps13* mutant strains using thin layer chromatography (TLC). In the absence of the phosphatidylserine (PS) decarboxylase Psd2, PS conversion to phosphatidylethanolamine (PE) is exclusively catalyzed by the mitochondrial PS decarboxylase Psd1. Since PS is generated in the ER, PS-to-PE conversion can be used as a proxy for ER-mitochondria PS exchange. The strains *psd1Δ::KanMX psd2Δ::KanMX* (A), *mmm1-1* (thermo-sensitive) *psd2Δ::KanMX* (B), and *mmm1-1 vps13Δ::HIS3 psd2Δ::KanMX* (C) were grown to OD 0.6 at 25°C, then switched to 37°C while keeping the cells in exponential growth for 12 h. This step was followed by an incubation with a final concentration of 2 μ Ci/ml [14 C]Serine. 10 OD₆₀₀ of cells were sampled at the indicated time (60 min for A) and subjected to lipid extraction according to Folch et al. (1957). TLC silica plates (#1.11845.0001; Merck) prepared with 1.8% (wt/vol) boric acid in 100% ethanol were used in a mobile-phase of chloroform/ethanol/water/triethylamine (30:35:7:35, vol/vol) to separate phospholipid components. Lipids were then detected by autoradiography. PC, phosphatidylcholine; PDME, phosphatidyl-dimethylethanolamine.

Table S1. Yeast strains used in this study

Strain	Original background	Genotype
ByK45	BY4741	MA α his3Δ1 leu2Δ0 met15Δ0 ura3Δ0
ByK46	BY4742	MA α his3Δ1 leu2Δ0 met15Δ0 ura3Δ0
ByK47	BY4743	diploid <i>MMM1/mmm1Δ::KanMX VPS13/VPS13[D716H] his3Δ1 leu2Δ0 met15Δ0 ura3Δ0</i>
ByK48	BY4741	<i>mdm34-GFP::HIS3 his3Δ1 leu2Δ0 met15Δ0 ura3Δ0</i>
ByK71	BY4742	MA α <i>mdm34-GFP::HIS3 mdm10Δ::KanMX his3Δ1 leu2Δ0 met15Δ0 ura3Δ0</i>
ByK76	BY4742	MA α <i>mdm34-GFP::His mmm1Δ::KanMX his3Δ1 leu2Δ0 met15Δ0 ura3Δ0</i>
ByK85	BY4742	<i>psd1Δ::KanMX psd2Δ::KanMX his3Δ1 leu2Δ0 met15Δ0 ura3Δ0</i>
ByK201	BY4742	MA α <i>mmm1Δ::KanMX VPS13[D716H] his3Δ1 leu2Δ0 met15Δ0 ura3Δ0</i>
ByK204	BY4741	MA α <i>mmm1Δ::KanMX his3Δ1 leu2Δ0 met15Δ0 ura3Δ0</i>
ByK226	YH8	MA α <i>mmm1-1 leu2-A1 trp1-A1 his3-A200</i>
ByK231 ^a	YH8	MA α <i>mmm1-1 Vps13Δ^GFP leu2-A1 trp1-A1 his3-A200</i>
ByK297	YH8	MA α <i>mmm1-1 vps13Δ::HIS3 psd2Δ::KanMX leu2-A1 trp1-A1 his3-A200</i>
ByK308	YH8	MA α <i>mmm1-1 psd2Δ::KanMX leu2-A1 trp1-A1 his3-A200</i>
ByK311 ^b	BY4741	<i>mmm1Δ::KANMX +pVTU100-mtDsRed his3Δ1 leu2Δ0 met15Δ0 ura3Δ0</i>
ByK312 ^b	BY4741	<i>VPS13[D716H] +pVTU-mtDsRed his3Δ1 leu2Δ0 met15Δ0 ura3Δ0</i>
ByK313 ^b	BY4741	<i>mmm1Δ::KanMX VPS13[D716H] +pVTU100-mtDsRed his3Δ1 leu2Δ0 met15Δ0 ura3Δ0</i>
ByK314 ^b	BY4741	<i>+pVTU100-mtDsRed his3Δ1 leu2Δ0 met15Δ0 ura3Δ0</i>
ByK337 ^c	BY4741	MA α <i>VPS13Δ^GFP his3Δ1 leu2Δ0 met15Δ0 ura3Δ0</i>
ByK342 ^c	BY4741	MA α <i>VPS13Δ^GFP Sec61-mCherry-HIS3 his3Δ1 leu2Δ0 met15Δ0 ura3Δ0</i>
ByK362 ^c	BY4741	<i>mmm1Δ::KanMX VPS13[D716H] Δ^GFP his3Δ1 leu2Δ0 met15Δ0 ura3Δ0</i>
ByK363	BY4741	<i>mmm1Δ::KanMX his3Δ1 leu2Δ0 met15Δ0 ura3Δ0</i>
ByK365 ^c	BY4741	<i>Vps13[D716H]Δ^GFP his3Δ1 leu2Δ0 met15Δ0 ura3Δ0</i>
ByK381 ^c	BY4741	<i>mmm1Δ::KanMX Vps13Δ^GFP his3Δ1 leu2Δ0 met15Δ0 ura3Δ0</i>
ByK382 ^c	BY4741	<i>Vps13Δ^GFP his3Δ1 leu2Δ0 met15Δ0 ura3Δ0</i>
ByK422 ^c	BY4741	MA α <i>Vps13[L1627S]Δ^GFP his3Δ1 leu2Δ0 met15Δ0 ura3Δ0</i>

^aByK231 bears a non-functional *Vps13Δ^GFP* (see Table S2, primers 7 and 8).^bByk311 to Byk314 are haploid spores of the same diploid "ByK47 pVTU-mtdsRed" strain.^cStrains with a functional *Vps13Δ^GFP* (created via primers 9 and 10; Table S2).

Table S2. Primers used in this study

Number	Name	Sequence
1	Sec61-mCherry_fwd	CGCCAAGGAAGGTGGTTACTAAGAACCTCGTCCAGGATTTCTGATTGATGcgatccccggtaattaa
2	Sec61-mCherry_rev	GGGGTGTGGCTAAATGC GATT TTTTTCTTGGATATTTCATTTATA Tgaa tgcagtcgtttaaac
3	Vps13_iGFP_+124_fwd	AAGATGAGGAAGGTAAAGTGAATGGGAACTGACAAATCAGGCTCGCtgaggc gacaacccttaat
4	Vps13_iGFP_+124_rev	TGAAAAGAAGAAGAGGTTTGTCTCTGATGGTACTCAGTAtgcaggc gata gggccact
5	Vps13_iGFP_+446_fwd	TGGCATGACGGAAGACTCACCCACACCAACAGCTAGCTCAAATATTGAAtgcaggc gacaacccttaat
6	Vps13_iGFP_+446_rev	GAGAGCCAGCTCCATTATTAGTAGTGCGGACTGTGGAGTTGTG Gcgccgc gata gggccact
7	Vps13_iGFP_+473_fwd	GGAGCTGGCTCTCTTGGGAAACGTAACAGAAGAAAtgcaggc gacaacccttaat
8	Vps13_iGFP_+473_rev	AAGCTCTGACGCTTCTCAGTCATTAAATCTCGTCAcggccgc gata gggccact
9	Vps13_iGFP_+499_fwd ^a	AACAGCGTCAGGAGCTTATGATGCTATTGAATTGACGAGAATGAAGATgcaggc gacaacccttaat
10	Vps13_iGFP_+499_rev ^a	GTTACACGAAGTCAACCCCTCTAGGAACCTGCAGTACAGGACCTTgcggccgc gata gggccact
11	Vps13_iGFP_+587_fwd	TTATATAAACATATCATTAGTGTCAAGAAACTCATCTAAAGATCAAtgcaggc gacaacccttaat
12	Vps13_iGFP_+587_rev	ATCCTCTCTCTCCCTCCGGTAGCGTGTGCAATGAAAGAgccgc gata gggccact
13	Vps13_iGFP_+597_fwd	TCATCTAAAGATCAATCTCAATTGACAACCACGCTACCGGGGAGGcgaggc gacaacccttaat
14	Vps13_iGFP_+597_rev	AGGTATAGAATTAGCAATCAAATCATAATAGTCTGTATTGAATgcggccgc gata gggccact
15	Vps13_iGFP_+860_fwd	CCTCTATGATGATGAGAAGAAAACAACGGCATTACGTTAATgcaggc gacaacccttaat
16	Vps13_iGFP_+860_rev	CAATTGAATTGCTCTTCATTCTTCCGAACCAGAAAGACTAgccgc gata gggccact
17	Vps13_iGFP_+1543_fwd	AAAAGCCACGAAAACAATTATACTATCCTGAAAACACAAACCAAtgcaggc gacaacccttaat
18	Vps13_iGFP_+1543_rev	ACCACCTCTGAACGCTTCAACATAGCCTGTTCAAGCTTgcggccgc gata gggccact
19	Vps13_iGFP_+1889_fwd	AACGATACAGAATTAGATTTGACGCTGGATACAGGATAAAACGtgaggc gacaacccttaat
20	Vps13_iGFP_+1889_rev	AGTGTGCTTIAAGAGCACAACCTCGTTTGTCTCAGTgcggccgc gata gggccact
21	Vps13_iGFP_+2007_fwd	ACTTCAACTGAGATTGAGCTTGGTGA CACTCAAAGATCAAATgcaggc gacaacccttaat
22	Vps13_iGFP_+2007_rev	AGATTACTTGATGTTAATAGCATATTAAAGATGGCTTgcggccgc gata gggccact
23	Vps13_iGFP_+2239_fwd	CAAAGTAGTCTCTAAATATTGACAATCCAAGATTTGCTGAAtgcaggc gacaacccttaat
24	Vps13_iGFP_+2239_rev	TTATCAAATGAAAACATTITGAAATAGTATCTTTCGTTgcggccgc gata gggccact
25	Vps13_iGFP_+233_fwd	TCACTCTGTTGTATTGAAATACAGACTCTCCGCCCTGATTCTgcaggc gacaacccttaat
26	Vps13_iGFP_+233_rev	GAAACCCCTACAAAATTTCAGGCTCTATCTGATCATCAGTgcggccgc gata gggccact
27	Vps13_iGFP_+2449_fwd	ATATTACCAAAAGAGATCTCGTAAAGTTAGTGTACCTACAAAGtgaggc gacaacccttaat
28	Vps13_iGFP_+2449_rev	GATAGTTGAAACACTACGGCTTGGGACTCGTAGACTGGTACAGTgcggccgc gata gggccact
29	Vps13_iGFP_+2562_fwd	TTATACAAATGAAAACCTGCTCGCAACTACCACGCTCTCAGTTgcaggc gacaacccttaat
30	Vps13_iGFP_+2562_rev	CTCATCCTTIGCACAAAGCCATCAGTCACTGA ACTATTACTGAgccgc gata gggccact
31	Vps13_iGFP_+2769_fwd	CATCTGCTTATACGCTCGATGAAATTAGTCCAGGATTGGCTTgcaggc gacaacccttaat
32	Vps13_iGFP_+2769_rev	ATGGACATAGTACATGATGACGAAAATGACTCCTCGGTCTTCgcggccgc gata gggccact

Capital nucleotide sequences represent homology regions for homologous recombination. Lowercase sequences represent homology regions for PCR amplification.

^aPrimer-set yielding a functional Vps13-GFP.

Table S3. Plasmids used in this study

Plasmid	Name	Reference
pBK37	pVTU100-miDsRed	Westermann and Neupert, 2000
pBK64	pVTU100-miBFP	Westermann and Neupert, 2000
pBK91	pOM42	Gauss et al., 2005
pBK98	pVPS13	pSOI1-1; Brickner and Fuller, 1997
pBK106	pVPS13[D716H]	Same as pVPS13 with the <i>VPS13[D716H]</i> allele
pBK117	pVPS13[L1627S]	Same as pVPS13 with the <i>VPS13[L1627S]</i> allele
pBK177	pFA6a mCherry:His	Kind gift from E. Ceboller-Presmanes (ETH Zürich, Zürich, Switzerland)

Script S1 is an ImageJ macro used to calculate mitochondrial shape quotient. Script S2 is an ImageJ macro used to quantify Vps13 localization at the mitochondria and vacuole. Script S3 is an ImageJ macro used to measure the percentage of Vps13 at NVJs. All three are available for download in a single ZIP file.

References

- Brickner, J.H., and R.S. Fuller. 1997. SOI1 encodes a novel, conserved protein that promotes TGN-endosomal cycling of Kex2p and other membrane proteins by modulating the function of two TGN localization signals. *J. Cell Biol.* 139:23–36. <http://dx.doi.org/10.1083/jcb.139.1.23>
- Folch, J., M. Lees, and G.H. Sloane Stanley. 1957. A simple method for the isolation and purification of total lipides from animal tissues. *J. Biol. Chem.* 226:497–509.
- Gauss, R., M. Trautwein, T. Sommer, and A. Spang. 2005. New modules for the repeated internal and N-terminal epitope tagging of genes in *Saccharomyces cerevisiae*. *Yeast.* 22:1–12. <http://dx.doi.org/10.1002/yea.1187>
- Westermann, B., and W. Neupert. 2000. Mitochondria-targeted green fluorescent proteins: convenient tools for the study of organelle biogenesis in *Saccharomyces cerevisiae*. *Yeast.* 16:1421–1427.