SUPPLEMENTARY INFORMATION

Table of yeast strains (all yeast were MATa S288C-BY4741/2 background except where indicated)

Strain Name	# in MS database	Markers	Remarks	Phenotype
GFP-GEM1	939	*his3Δ1 leu2Δ0 met15Δ0 ura3Δ0* *HIS::GAL1p-GFP-GEM1*	GEM1 was endogenously tagged at its N-terminus with GFP and the promoter was replaced to the *GAL1* promoter.	Gem1 is localized to mitochondria.
GFP-GEM1 Δ*spf1*	940	*his3Δ1 leu2Δ0 met15Δ0 ura3Δ* *HIS::GAL1p-GFP-GEM1 Δspf1::Kan^r*	GEM1 was endogenously tagged at its N-terminus with GFP and the promoter was replaced to the *GAL1* promoter. on a Δ*spf1* strain from the deletion library.	Gem1 is mis-localized to the ER.
GFP-GEM1 Δ*get3*	944	*his3Δ1 leu2Δ0 met15Δ0 ura3Δ0* *HIS::GAL1p-GFP-GEM1 Δget3::URA*	GET3 was deleted from the GFP-GEM1 strain.	Gem1 is localized to mitochondria.
GFP-GEM1 Δ*spf1* Δ*get3*	945	*his3Δ1 leu2Δ0 met15Δ0 ura3Δ0* *HIS::GAL1p-GFP-GEM1 Δspf1::Kan^r Δget3::URA*	GET3 was deleted from the GFP-GEM1 Δ*spf1* Strain.	Gem1 is mis-localized to the ER.
SGA YMS 140 GFP-GEM1 MATα	140	*his3Δ1 leu2Δ0 lys2+ met15Δ0 ura3Δ0* *can1Δ::MFA1pr-LEU2 lyp1Δ cyh2* *HIS::GAL1p-GFP-GEM1*	The query strain for the SGA analysis.	Gem1 is localized to mitochondria.
Deletion library		*his3Δ1 leu2Δ0 met15Δ0 ura3Δ0*	The entire yeast knock-out collection (Giaever et al., 2002).	
DAmP library		*his3Δ1 leu2Δ0 met15Δ0 ura3Δ0*	The entire DAmP library (Breslow et al., 2008; Schuldiner et al., 2005).	
pGal-Spf1-GFP	994	*his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 can1Δ::STE2pr-spHIS5 lyp1Δ::STE3pr-LEU2 Kanr::Galpr-SPF1-GFP::Natr*	The endogenous promoter of SPF1 was replaced with an inducible one (GALp). In addition, SPF1 was C-terminally tagged with GFP.	See figure 1D.
CSY 605	1130	*KanR*	Rnt1p consensus elements were added to *ERG9*. See (abiskin & Smolke, 2011) for a detailed characterization of the strain.	Fis1 is localized to mitochondria.
CSY 692	1136	*KanR*	Rnt1p consensus elements were added to *ERG9*. See (abiskin & Smolke, 2011) for a detailed characterization of the strain.	Fis1 is localized to mitochondria.

CSY 693	1137	*KanR*	Rnt1p consensus elements were added to *ERG9*. See (abiskin & Smolke, 2011) for a detailed characterization of the strain.	Fis1 is localized to ER.
TEToff-ERG10 GFP-GEM1	1326	*his3Δ1 leu2Δ0 met15Δ0 ura3Δ0::CMV-tTA-URA3 KanR-tetO7-TATA::ERG10 His3-GFP::GEM1*	GEM1 was N terminally tagged with GFP on the background of a TEToff-ERG10 strain.	When grown in the presence of doxy for 11 hours, Gem1 is localized to the ER.
W303α		*ade2-1; can1-100; his3-11; leu2 3_112; trp1Δ2; ura3-52;*	wildtype strain	
Δ*spf1*		*ade2-1; can1-100; his3-11; leu2 3_112; trp1Δ2; ura3-52; SPF1::HIS3*	ORF of *spf1* was deleted from wildtype strain by replacement with a HIS-casette	Gem1 and Fis1 are mis-localized to ER

Table of primers

Primer Name	**Description**	**Sequence**	**Product size (bps)**	**Template**
spf1-wtchk-f	Forward primer for check for *SPF1* deletion.	AGAGCACGCTGTTGCGCCAT	290	strain genome
spf1-wtchk-r	Reverse primer to check for *SPF1* deletion.	AATTGCGCTCTCCTCGGCGG	290	strain genome
get3-wtchk-f	Forward primer to check for *GET3* deletion.	ACGCCAGAAAGGTGACAGGCA	300	strain genome
get3-wtchk-r	Reverse primer to check for *GET3* deletion.	TGTGGCCAGTTGGAGCAGTGT	300	strain genome
KO-YDL100C-Trp-5	Forward primer to delete *GET3*' ORF.	ATGGATTTAACCGTGGAACCTAATTTGCACTCTTTAATTACACAGGAAACAGCTATGACC	1600	Plasmid: pKL-URA
KO-YDL100C-Trp-3	Reverse primer to delete *GET3*' ORF.	CTATTCCTTATCTTCTAACTCATAAATGACTTTGCCATCAGTTGTAAAACGACGGCCAGT	1600	Plasmid: pKL-URA
Gem1-NGFP-F	Forward primer for adding N-terminal GFP tag based as well as swapping promoters to GAL1p for *Gem1*.	AAATAGCGGACTTCTAAATACTAATGTGTTGAACAACACAGAATTCGAGCTCGTTTAAAC	3000	pFA6-HIS-GAL1p-GFP

Gem1-NGFP-R	Reverse primer for adding N-terminal GFP tag based as well as swapping promoters to GAL1p for *Gem1*	CTTCATCACCGCAAATAA CTACCCGAATCGTTTCTT TAGTTTTGTATAGTTCAT CCATGC	3000	pFA6a-GFP(S65T)-HIS3MX6
R5-Rev	Forward primer to check N-terminal GFP fusion into *Gem1*.	GCATGGATGAACTATACA AA	611	strain genome
Gem1-NGFPchk-R	Reverse primer to check N-terminal GFP fusion into *GEM1*	TTCACCTACCATGGCATC AA	611	strain genome
5-SPF1-KO	Forward Primer to delete *Spf1* ORF in W303α	GAC ATA GTT GAC ATA TCA GAC CTA CAG AAA CAT AGG AAT CGG TAA CGT ACG CTG CAG GTC GAC	1590	pFA6a-HIS3MX6
3-SPF1-KO	Reverse Primer to delete *Spf1* ORF in W303α	ATA TAA GTA TAT AAA TAC AAA AAG GGG TAC TAC ATA AAA GAT TTA ATC GAT GAA TTC GAG CTC G	1590	pFA6a-HIS3MX6
5-SPF1-KO-K	Forward Primer to check for deletion of *Spf1* in W303α; negative control	CCC TGC ATC TTG CGC TGC C	927	strain genome
3-SPF1-KO-K	Reverse Primer to check for deletion of *Spf1* in W303α	CCC GAA GCT ATT ATA ATT TTC GTA TAC	927	strain genome
HisC	Forward Primer to check for deletion of *Spf1* in W303α; positive control	CTT GGT TTC ATT TGT AAT ACG CT	400	strain genome
SPF1-mut1-G1459A-F	Forward Primer to introduce the first mutation that creates the ATPase dead-form of *SPF1*	GAATTGATGTTTGTTGTT TCAATAAAACTGGTACTT TGACCG	~	pSM2-SPF1-HA
SPF1-mut1-G1459A-R	Reverse Primer to introduce the first mutation that creates the ATPase dead-form of *SPF1*	CGGTCAAAGTACCAGTTT TATTGAAACAACAAACAT CAATTC	~	pSM2-SPF1-HA
SPF1-mut2-G2443A-F	Forward Primer to introduce the second mutation that creates the ATPase dead-form of *SPF1*	GGGATATCAAACTTTAAT GTGCAGTGATGGTACTAA TGATGTTGGTGCTT	~	pSM2-SPF1-HA
SPF1-mut2-G2443A-R	Reverse Primer to introduce the second mutation that creates the ATPase dead-form of *SPF1*	AAGCACCAACATCATTAG TACCATCACTGCACATTA AAGTTTGATATCCC	~	pSM2-SPF1-HA

Table of plasmids

Plasmid Name	# in MS database	Description	Reference
pKL-URA	70	Plasmid for knocking out genes using homologous recombination. Has the K. Lactis URA gene.	(Kitada et al., 1995)
pYM-N23: Nat::GAL1p	274	A derivative of the pYM homologous recombination plasmids family. Used to replace the native promoter with the GAL1 promoter. Integration confers nourseothricin (NAT) resistance.	(Janke et al., 2004)
C/A URA Cherry-FIS1	122	Cen/Ars plasmid expressing mCherry-FIs1. (URA+)	(Schuldiner et al., 2008)
C/A URA Cherry-SBH2	124	Cen/Ars plasmid expressing mCherry–Sbh2. (URA+).	(Schuldiner et al., 2008)
GFP-SCS2	144	Cen/Ars plasmid expressing GFP- Scs2 from a Pho5 promoter. (URA+)	(Loewen and Levine, 2005)
GFP-SED5	113	2μ plasmid driving expression of GFP-Sed5. (LEU+)	(Schuldiner et al., 2008)
pFA6-HIS-GAL1p-GFP	132	A plasmid for making N terminal GFP fusion proteins by PCR together with exchanging the endogenous promoter with the *GAL1* promoter.	(Longtine et al., 1998)
pBS35-mCherry-hygromycin	234	A plasmid for making C terminal mCherry fusion proteins by PCR mediated homologous recombination. Confers resistance to Hygromycin.	http://depts.washington.edu/yeastrc/pages/pBS35.html
pCRZ1-p	84	2μ plasmid for expressing the constitutively activated form of the Crz1 transcription factor (URA+).	(Edlind et al., 2002).
MTS-mCherry	167	Cen/Ars plasmid expressing Mitochondria localized cherry.	kind gift from Jodi Nunnari
BFGIII-GET3-OE	105	Plasmid for overexpressing GET3 with 3 N-terminal HA-Tags under control of ADH1 promotor in yeast	kind gift from Blanche Schwappach
pSM2-SPF1-HA	287	Cen/Ars plasmid expressing *SPF1* gene. This plasmid rescues the mislocalization of mCherry-Fis1 to the ER back to mitochondria on the background of Δ*spf1* cells.	
pSM2-SPF1-HA mut1 G1459A	381	The first mutation introduced to SPF1 on plasmid 287 is G1459A. This mutation changes the first letter of codon # 487 from GAT (Asp) to AAT (Asn). In Suzuki et al, 2001, this mutation was shown to cancel the rescue of a spf1 plasmid in respect to SMKT resistance phenotype. This plasmid fails to rescue the mislocalization of mCherry-Fis1 to the ER on the	

		background of Δ*spf1* cells.	
pSM2-SPF1-HA mut2 G2443A	383	The second mutation introduced to SPF1 on plasmid 287 is G2443A. This mutation changes the first letter of codon # 815 from GGT (Gly) to AGT (Ser). In Suzuki et al, 1999, this mutation was suggested to be involved in ATP binding and was shown to eliminate the SMKT resistance of yeast, which implies a loss of function of Spf1. This plasmid fails to rescue the mislocalization of mCherry-Fis1 to the ER on the background of Δ*spf1* cells.	

Supplementary Figures Legends

Krumpe *et al*, Supplementary Figure 1

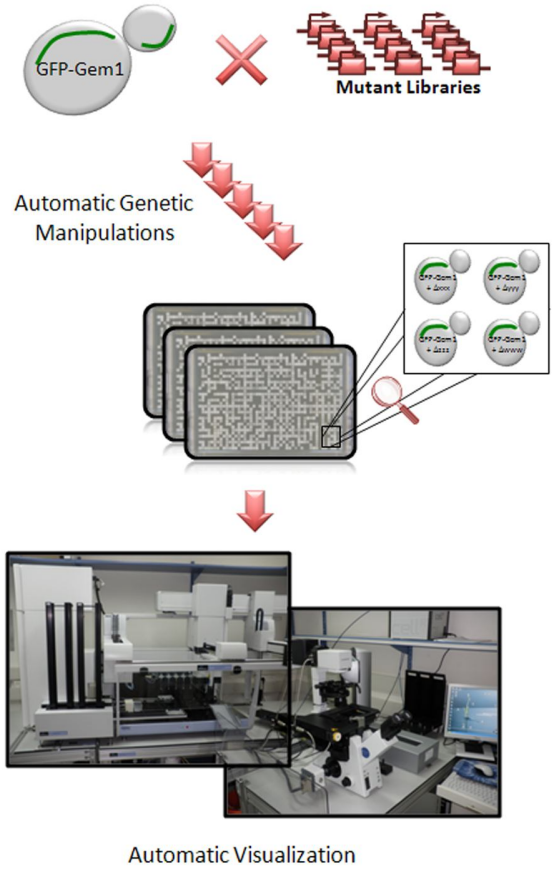

Figure 1: SGA methodology. GFP-Gem1 as a marker for mitochondrial TA protein insertion as it localizes correctly to mitochondrial membranes. To visualize GFP-Gem1 on the background of mutations in all yeast genes we mated a strain expressing GFP-Gem1 into a library of all mutations. Following selection of diploids and sporulation we could select only the haploid cells expressing both the marker and the mutation.

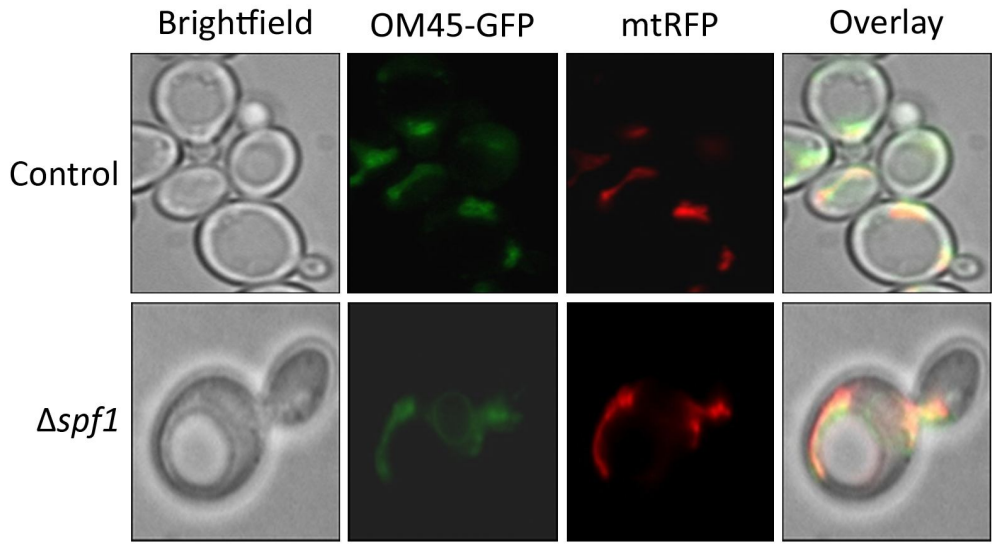

Figure 2: Om45 is partially mislocalized to the ER in *Δspf1* cells. Plasmids encoding GFP-Om45 and MTS-mCherry were transformed into control and *Δspf1* cells. Transformed cells were grown at 30°C to mid-log phase and analyzed by fluorescence microscopy.

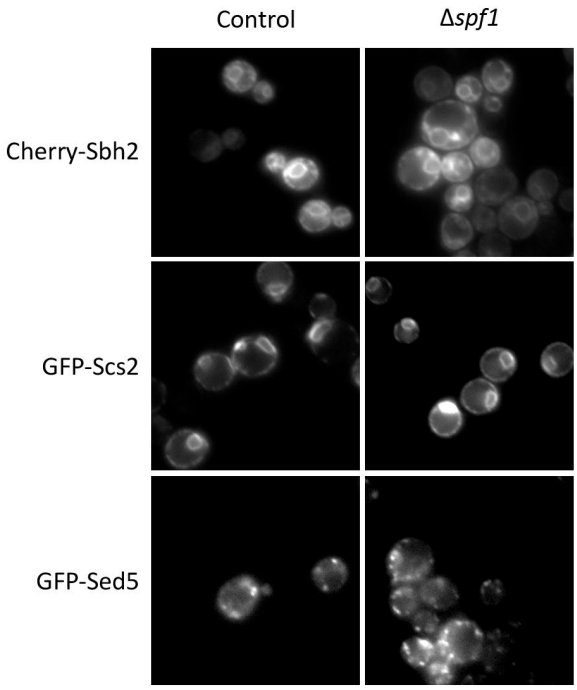

Figure 3: Secretory pathway TA proteins are not affected in *Δspf1* cells. A. Sbh2, Scs2 (ER TA proteins) and Sed5 (Golgi TA protein) are correctly localized on Δ*spf1* background (X60 lens).

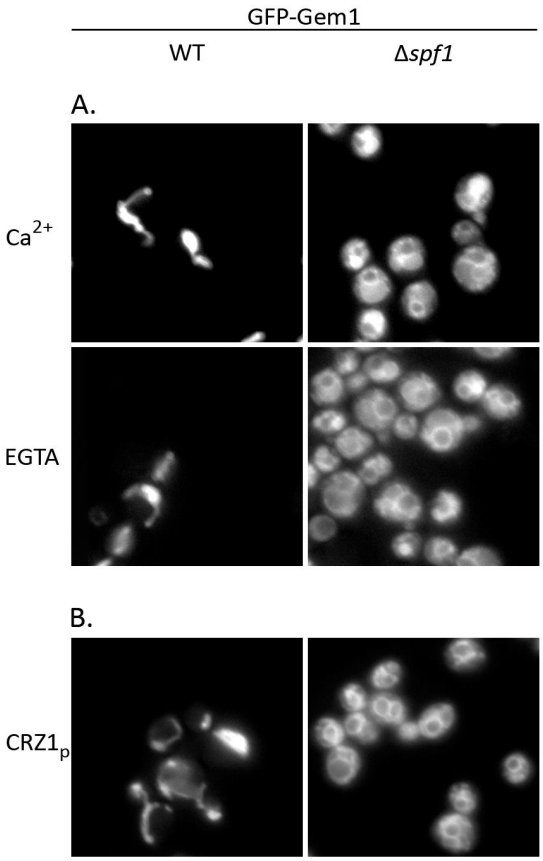

Figure 4: The mislocalization of MOM TA proteins on Δ*spf1* background cannot be explained by activation of the Calcium response:A. Neither Ca^{2+} nor EGTA affected the mislocalization of GFP-Gem1 on Δ*spf1* background nor phenocopied it in control cells. Control and Δ*spf1* cells were grown in the presence of either 4mM Ca^{2+} or 1mM EGTA and visualized using a X60 lens. **B.** Activation of the calcium response does not modulate MOM TA protein localization. A plasmid expressing a constitutively-phosphorylated Crz1 (a constitutively activated form of the calcineurin pathway transcription factor) was transformed into both control and Δ*spf1* cells and cells were visualized in mid log (X60 lens).