Grippa et al., http://www.jcb.org/cgi/content/full/jcb.201502070/DC1

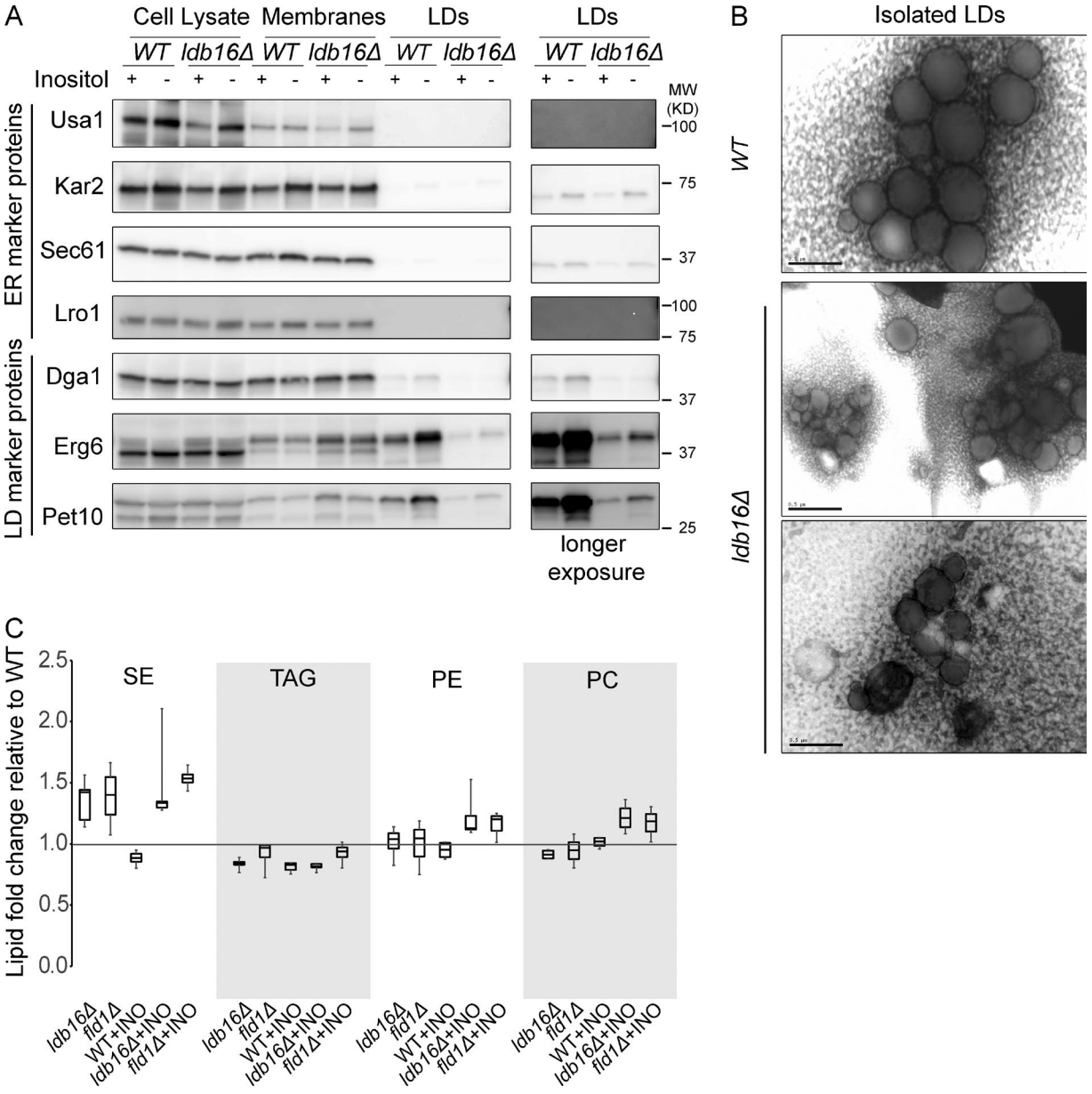


Figure S1. **Characterization of LDs isolated from *wt*, *fld1Δ*, and *ldb16Δ* cells.** (A) Whole-cell lysates, membrane, or LD fractions of cells with the indicated genotype were analyzed by SDS-PAGE followed by Western blotting with antibodies against the indicated ER or LD proteins. (B) Negative-stain electron micrographs of LDs isolated from cells with the indicated genotype. Bars, 500 nm. (C) Lipid composition of cells with the indicated genotype and grown in SC or SC supplemented with 75 µM inositol (+INO). Lipid extracts from *wt* cells grown in SC media were used as reference. The result of at least three independent experiments is shown in the graph; whiskers represent the maximum and minimum values.

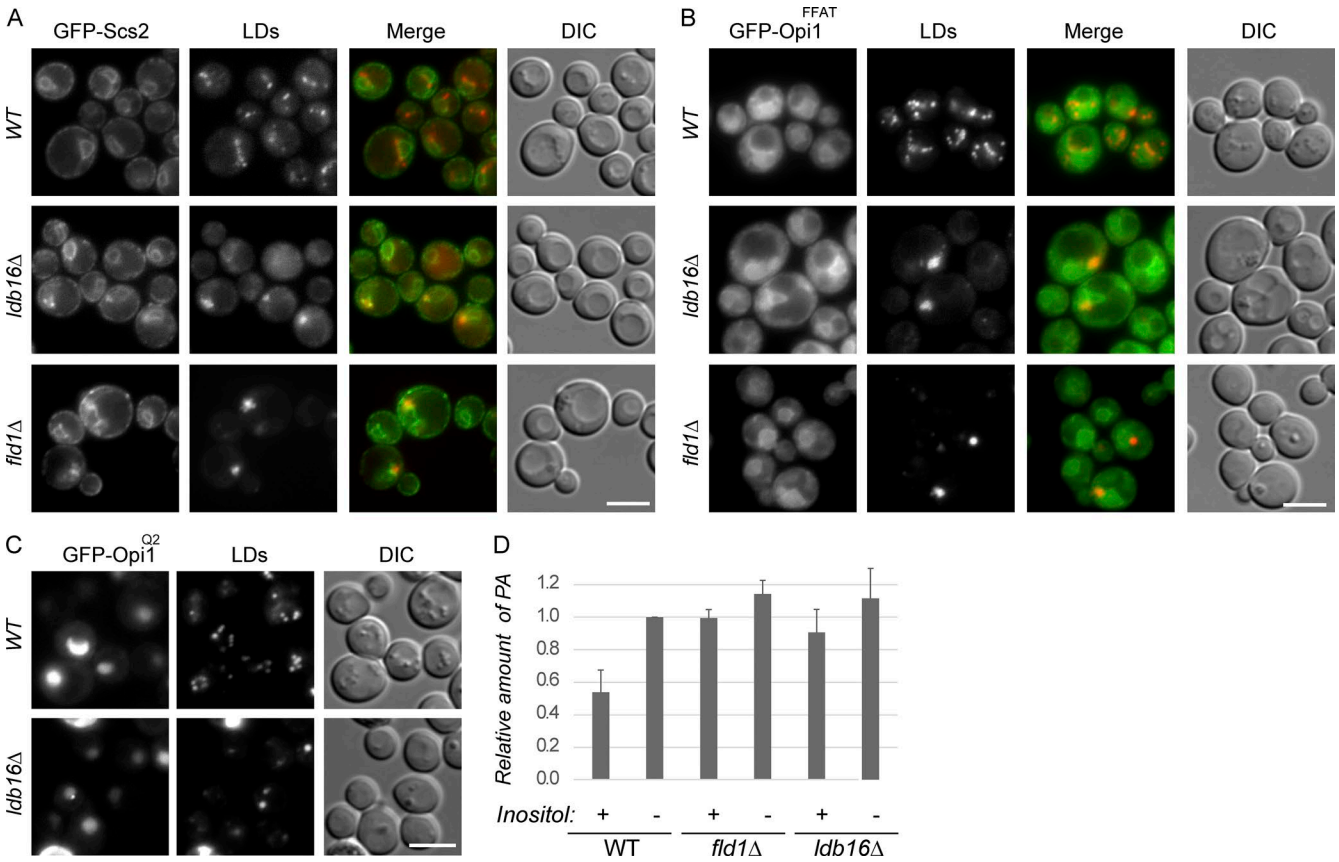


Figure S2. **Distribution of Scs2, Scs2-binding, and PA-binding domains of Opi1 and PA in *fld1Δ* and *ldb16Δ* mutants.** (A) Localization of GFP-Scs2 in *wt*, *ldb16Δ*, and *fld1Δ* cells grown in SC media to early stationary phase. LDs were stained with the neutral lipid dye MDH. Bar, 5 µm. (B) Localization of GFP-Opi1^{FFAT} in *wt*, *ldb16Δ*, and *fld1Δ* cells grown in SC media to early stationary phase. LDs were stained with the neutral lipid dye MDH. Bar, 5 µm. (C) Localization of GFP-Opi1^{Q2} in cells with the indicated genotype. Plasmid-borne GFP-Opi1^{Q2} expression was driven by the constitutive *PRC1* promoter. LDs were stained with the neutral lipid dye MDH. Bar, 5 µm. (D) Amount of PA in cells with the indicated genotype and grown in SC media or SC supplemented with inositol (75 µM). Lipids were labeled to steady state with [1-^{14}C]acetate, extracted and analyzed by thin layer chromatography. The content of PA, relative to *wt* cells grown in SC media, is presented as the mean of three independent experiments, and error bars represent SD.

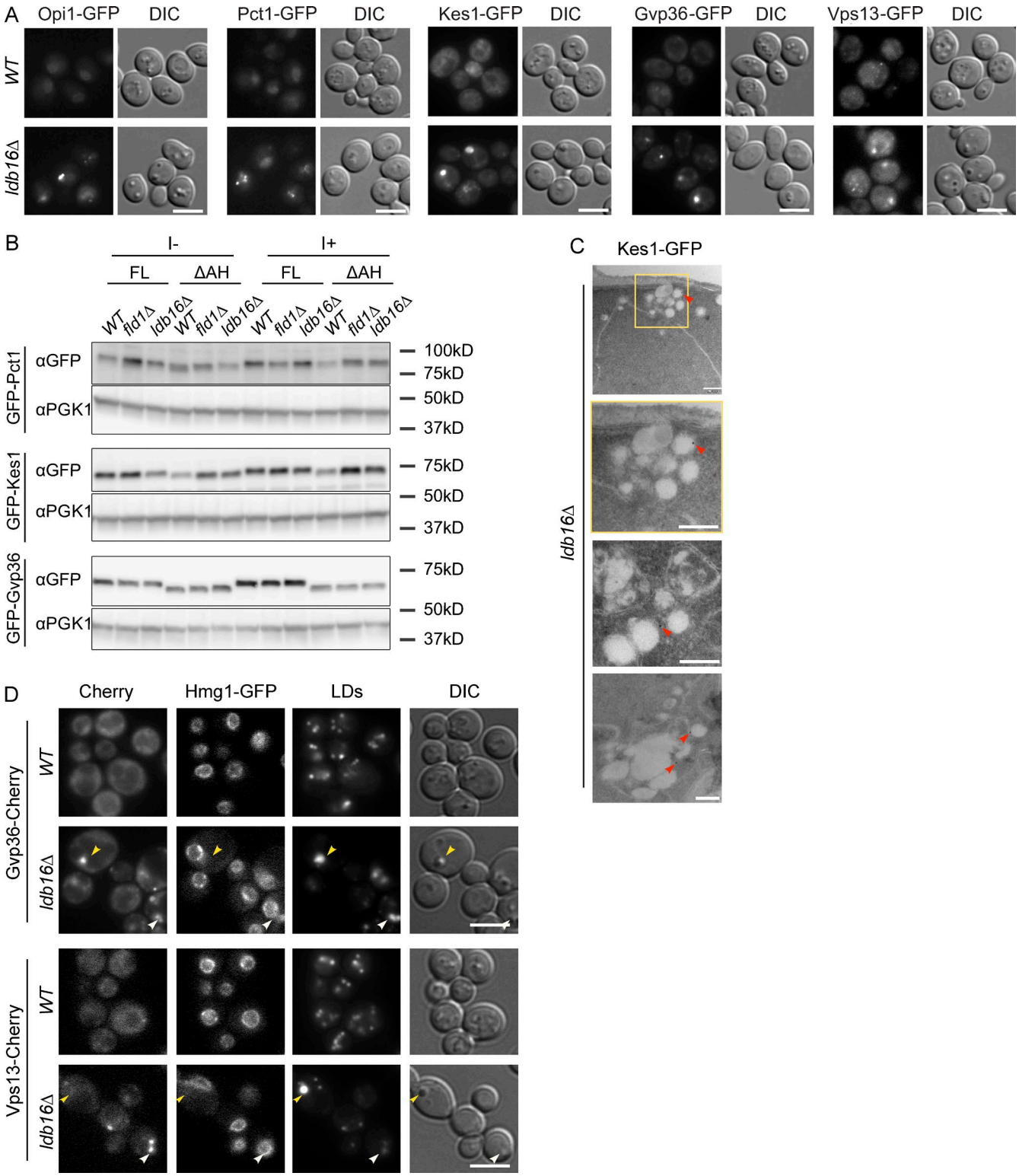


Figure S3. **AH-containing proteins are specifically relocalized in seipin complex mutants.** (A) Localization of the proteins Opi1, Pct1, Kes1, Gvp36, and Vps13 in *wt* and *Idb16Δ* cells. All proteins were expressed from their endogenous locus as C-terminal GFP fusions. Bar, 5 µm. (B) Steady-state levels of full-length Pct1, Kes1, Gvp36 (FL), and the corresponding counterparts lacking the AHs (ΔAH) in cells with the indicated genotype. Protein extracts of cells grown in the indicated media were subjected to SDS page and analyzed by Western blotting with anti-GFP antibody. Phosphoglycerate kinase 1 (Pgk1) was used as loading control and detected with anti-Pgk1 antibody. (C) Electron micrographs showing the immunolocalization of endogenously expressed Kes1-GFP in *Idb16Δ* cells, which were prepared as described in the Materials and methods section, Arrowheads point to 12-nm immunogold particles labeling specifically Kes1-GFP in association with lipid droplets aggregates. Bars, 200 nm. (D) Localization of the AH-containing proteins Gvp36 and Vps13 in *wt* and *Idb16Δ* cells grown in SC media to early stationary phase. Proteins were expressed from their endogenous locus as C-terminal mCherry fusions. Nuclear envelope is labeled by endogenously expressed Hmg1-GFP. LDs are stained with the neutral lipid dye MDH. Yellow arrowheads indicate supersized LDs; white arrowheads indicate LD aggregates. Bar, 5 µm.

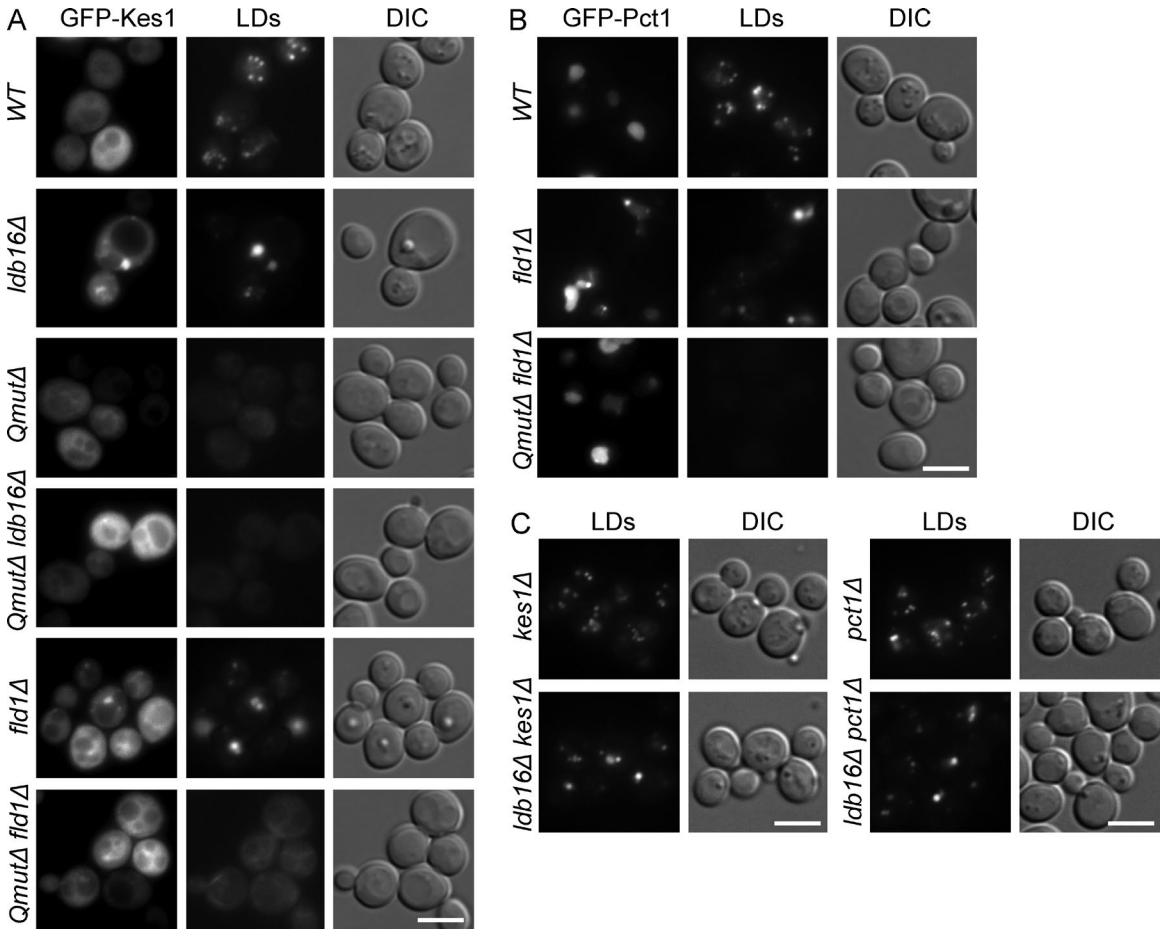
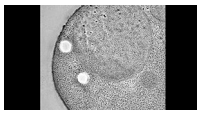
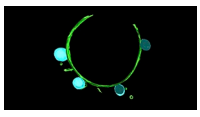


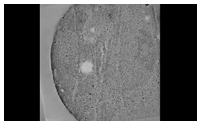
Figure S4. **LD assembly causes phospholipid-packing defects in Seipin complex mutants.** (A) Localization of GFP-Kes1 in cells with (*wt*, *ldb16Δ*, and *fld1Δ*) and without (*are1Δ are2Δ dga1Δ lro1Δ*, *are1Δ are2Δ dga1Δ lro1Δ ldb16Δ*, and *are1Δ are2Δ dga1Δ lro1Δ fld1Δ*) LDs. Cells were grown in SC media up to early stationary phase. LDs were stained with the neutral lipid dye MDH. Bar, 5 µm. (B) Localization of GFP-Pct1 in cells with the indicated genotype. LDs were stained with the neutral lipid dye MDH. Bar, 5 µm. (C) Deletion of *KES1* or *PCT1* does not affect LD morphology in presence or absence of *LDB16*. LDs of cells with the indicated genotype were stained with the neutral lipid dye MDH. Bar, 5 µm.



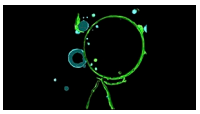
Video 1. **Morphology ER–LD contact sites in a *wt* cell shown in Fig. 7 A (left).** Late logarithmic *wt* cells were high-pressure frozen and subjected to freeze substitution. 250-nm-thick sections were analyzed by dual-axis electron tomography and tilted images aligned to generate the tomogram shown.



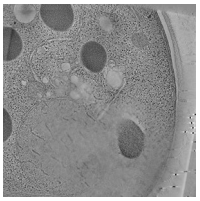
Video 2. **3D reconstruction of ER–LD contact sites of a *wt* cell shown in Video 1.** The ER is shown in green and LDs in blue.



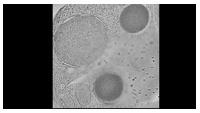
Video 3. **Morphology of a contact site between ER and a supersized LD in an *ldb16Δ* cell shown in Fig. 7 B (left).** Late logarithmic *ldb16Δ* cells were high-pressure frozen, subjected to freeze substitution and serial 250nm thick sections imaged by dual-axis electron tomography. Reconstruction was generated by aligning tomograms of serial sections.



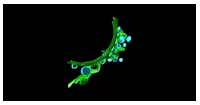
Video 4. **3D reconstruction of the ER–LD contact site in an *ldb16Δ* cell shown in Video 3.** The ER is shown in green and LDs in blue.



Video 5. **Morphology of a contact site between ER and a LD aggregate in an *ldb16Δ* cell shown in Fig. 7 C (bottom).** Late logarithmic *ldb16Δ* cells were high-pressure frozen and subjected to freeze substitution. 250-nm-thick sections were analyzed by dual-axis electron tomography and tilted images aligned to generate the tomogram shown.



Video 6. **Morphology of a contact site between ER and a LD aggregate in an *ldb16Δ* cell shown in Fig. 7 C (top).** Late logarithmic *ldb16Δ* cells were high-pressure frozen and subjected to freeze substitution. 250-nm-thick sections were analyzed by dual axis electron tomography and tilted images aligned to generate the tomogram shown.



Video 7. **3D reconstruction of the ER–LD contact site in an *ldb16Δ* cell shown in Video 6.** The ER is shown in green and LDs in blue.

Table S1. **LD-specific proteins reduced in LDs isolated from *fld1Δ* and *ldb16Δ* cells, as determined by label-free quantitative mass spectrometry**

Name		*fld1Δ*		*ldb16Δ*	
Systematic	**Standard**	**logFC**	***P***	**logFC**	***P***
YLL012W	Yeh1	−7.78649	1.13×10^{-5}	−5.05418	8.77×10^{-4}
YOL048C	Rrt8	−3.7425	1.27×10^{-4}	−3.41304	2.63×10^{-5}
YDL193W	Nus1	−3.49958	9.97×10^{-5}	−2.40426	1.02×10^{-4}
YOR246C	Yor246c	−3.25081	6.85×10^{-5}	−2.45812	1.60×10^{-5}
YGR263C	Say1	−3.24911	3.83×10^{-4}	−2.25961	2.05×10^{-4}
YMR313C	Tgl3	−3.24879	8.08×10^{-5}	−2.54358	6.01×10^{-5}
YMR148W	Osw5	−3.08516	2.28×10^{-4}	−2.58567	1.97×10^{-3}
YKL140W	Tgl1	−3.06946	2.61×10^{-4}	−2.43399	1.78×10^{-4}
YBR041W	Fat1	−3.00532	3.26×10^{-4}	−2.1922	7.36×10^{-4}
YPL206C	Pgc1	−2.9476	1.41×10^{-4}	−3.1552	8.86×10^{-5}
YIL124W	Ayr1	−2.94212	8.75×10^{-4}	−1.77792	8.77×10^{-3}
YKR046C	Pet10	−2.88843	1.59×10^{-4}	−2.14085	2.34×10^{-4}
YPR139C	Vps66	−2.88325	2.22×10^{-4}	−1.64523	2.15×10^{-2}
YHR072W	Erg7	−2.6831	1.10×10^{-3}	−3.13586	3.00×10^{-6}
YDL052C	Slc1	−2.33422	4.94×10^{-4}	−1.43428	9.79×10^{-3}
YLR100W	Erg27	−2.32554	5.69×10^{-4}	−1.90138	1.72×10^{-3}
YOR245C	Dga1	−1.98278	6.85×10^{-3}	−1.19096	5.91×10^{-2}
YKR067W	Gpt2	−1.78775	1.62×10^{-2}	−1.69292	1.24×10^{-1}
YMR246W	Faa4	−1.46686	1.10×10^{-2}	−1.74005	1.13×10^{-2}
YIL009W	Faa3	−1.44055	2.29×10^{-2}	−1.91776	1.62×10^{-2}
YMR110C	Hfd1	−1.34235	3.15×10^{-2}	−1.99597	3.29×10^{-3}
YML008C	Erg6	−1.27227	1.43×10^{-2}	−1.62012	4.79×10^{-3}
YKL094W	Yju3	−1.26406	1.66×10^{-2}	−1.23556	2.12×10^{-2}
YBR002C	Rer2	−3.43938	1.27×10^{-4}	−2.42116	$5.27E \times 10^{-3}$
YIL124W	Ayr1	−2.94212	8.75×10^{-4}	−1.77792	8.77×10^{-3}
YBR265W	Tsc10	−3.11278	9.39×10^{-5}	−2.55269	3.41×10^{-4}

Table S2. **Peripheral membrane proteins increased in LDs isolated from *fld1Δ* and *ldb16Δ* cells, as determined by label-free quantitative mass spectrometry and confirmed by fluorescence microscopy**

Name		*fld1Δ*		*ldb16Δ*	
Systematic	**Standard**	**logFC**	***P***	**logFC**	***P***
YHL020C	Opi1	5.489566	1.46×10^{-4}	4.20386	6.32×10^{-6}
YPR097W	Ypr097w	3.716108	1.17×10^{-4}	2.281816	6.38×10^{-4}
YLL040C	Vps13	2.827194	6.16×10^{-4}	2.056947	2.57×10^{-3}
YLR380W	Csr1	2.803219	4.00×10^{-4}	1.653667	1.12×10^{-2}
YGR202C	Pct1	2.473526	8.19×10^{-4}	2.180898	3.08×10^{-4}
YIL041W	Gvp36	2.371785	9.15×10^{-3}	2.775928	8.10×10^{-5}
YPL145C	Kes1	1.918121	5.72×10^{-3}	1.21771	6.65×10^{-2}

Table S3. **Quantification of anti-GFP immunogold particles in LDs from cells expressing the indicated GFP proteins or untagged control**

LDs	Profiles observed	Profiles with IG	Number of IG particles
Pct1-GFP			
Cytoplasmic LDs	84	7	9
Intranuclear LDs	72	12	20
Kes1-GFP			
Cytoplasmic LDs	95	21	31
Intranuclear LDs	39	0	0
Untagged			
Cytoplasmic LDs	96	1	1
Intranuclear LDs	47	0	0

IG, immunogold.

Table S4. **Quantification of anti-GFP immunogold particles in *wt* cells expressing Ldb16-GFP and untagged control cells**

Cells	ER–LD contact sites[a]	ER	Nucleus	Vacuole	Mitochondria
Untagged	1	9	127	6	40
Ldb16-GFP	10	5	109	10	31

For each strain, the quantification was performed from 100 micrographs imaged at 26,500×. Numbers correspond to the immunogold particles associated with the indicated organelles. The number of ER contact sites observed in the Ldb16-GFP strain and in the untagged control was 130 and 126, respectively.
[a]ER–LD contact sites are defined as the region within 30-nm radius of the point where ER and LDs appear in contact.

Table S5. **Yeast strains used in this study**

Strain	Genotype
BY4741	*Mat a ura3Δ0 his3Δ1 leu2Δ0 met15Δ0*
BY4742	*Mat α ura3Δ0 his3Δ1 leu2Δ0 lys2Δ0*
yPC3975	*Mat a ura3Δ0 his3Δ1 leu2Δ0 met15Δ0 fld1::KAN*
yPC4002	*Mat a ura3Δ0 his3Δ1 leu2Δ0 met15Δ0 fld1::HYGB*
yPC4246	*Mat a ura3Δ0 his3Δ1 leu2Δ0 met15Δ0 ldb16::KAN*
yPC4307	*Mat a ura3Δ0 his3Δ1 leu2Δ0 met15Δ0* OSW5-GFP-KAN
yPC4397	*Mat a ura3Δ0 his3Δ1 leu2Δ0 met15Δ0* OSW5-GFP-KAN *ldb16::HYGB*
yPC5777	*Mat a ura3Δ0 his3Δ1 leu2Δ0 met15Δ0* PET10-GFP-HIS3
yPC5837	*Mat ? ura3Δ0 his3Δ1 leu2Δ0* PET10-GFP-HIS3 *ldb16::HYGB*
yPC5778	*Mat a ura3Δ0 his3Δ1 leu2Δ0 met15Δ0* TGL1-GFP-HIS3
yPC5840	*Mat ? ura3Δ0 his3Δ1 leu2Δ0* TGL1-GFP-HIS3 *ldb16::HYGB*
yPC5776	*Mat a ura3Δ0 his3Δ1 leu2Δ0 met15Δ0* YEH1-GFP-HIS3
yPC6438	*Mat a ura3Δ0 his3Δ1 leu2Δ0 met15Δ0* YEH1-GFP-HIS3 *ldb16::HYGB*
yPC5266	*Mat a ura3Δ0 his3Δ1 leu2Δ0 met15Δ0* OPI1-GFP-HIS3
yPC5583	*Mat ? ura3Δ0 his3Δ1 leu2Δ0* OPI1-GFP-HIS3 *ldb16::HYGB*
yPC5558	*Mat a ura3Δ0 his3Δ1 leu2Δ0 met15Δ0* PCT1-GFP-HIS3
yPC5577	*Mat ? ura3Δ0 his3Δ1 leu2Δ0* PCT1-GFP-HIS3 *ldb16::HYGB*
yPC7303	*Mat a ura3Δ0 his3Δ1 leu2Δ0 met15Δ0* PCT1-GFP-HIS3 *opi3::KAN*
yPC5559	*Mat a ura3Δ0 his3Δ1 leu2Δ0 met15Δ0* KES1-GFP-HIS3
yPC7811	*Mat a ura3Δ0 his3Δ1 leu2Δ0 met15Δ0* KES1-GFP-HIS3 *ldb16::HYGB*
yPC5581	*Mat a ura3Δ0 his3Δ1 leu2Δ0 met15Δ0* KES1-GFP-HIS3 *fld1::NAT*
yPC5554	*Mat a ura3Δ0 his3Δ1 leu2Δ0 met15Δ0* GVP36-GFP-HIS3
yPC5563	*Mat a ura3Δ0 his3Δ1 leu2Δ0 met15Δ0* GVP36-GFP-HIS3 *ldb16::HYGB*
yPC7300	*Mat a ura3Δ0 his3Δ1 leu2Δ0 met15Δ0* GVP36-GFP-HIS3 *opi3::KAN*
yPC5923	*Mat a ura3Δ0 his3Δ1 leu2Δ0 met15Δ0* VPS13-GFP-HIS3
yPC5985	*Mat a ura3Δ0 his3Δ1 leu2Δ0 met15Δ0* VPS13-GFP-HIS3 *ldb16::HYGB*
yPC6450	*Mat a ura3Δ0 his3Δ1 leu2Δ0 met15Δ0* OPI1-mCHERRY-URA2 HMG1-GFP-HIS3
yPC6453	*Mat ? ura3Δ0 his3Δ1 leu2Δ0* OPI1-mCHERRY-URA2 HMG1-GFP-HIS3 *ldb16::HYGB*
yPC7989	*Mat a ura3Δ0 his3Δ1 leu2Δ0 met15Δ0* PCT1-mCHERRY-URA3 HMG1-GFP-HIS3
yPC7425	*Mat a ura3Δ0 his3Δ1 leu2Δ0 met15Δ0* PCT1-mCHERRY-URA3 HMG1-GFP-HIS3 *ldb16::HYGB*
yPC7453	*Mat a ura3Δ0 his3Δ1 leu2Δ0 met15Δ0* KES1-mCHERRY-URA3 HMG1-GFP-HIS3
yPC7455	*Mat ? ura3Δ0 his3Δ1 leu2Δ0* KES1-mCHERRY-URA3 HMG1-GFP-HIS3 *ldb16::HYGB*
yPC8124	*Mat ? ura3Δ0 his3Δ1 leu2Δ0* PCT1-mCHERRY-URA3 KES1-GFP-HIS3
yPC8125	*Mat ? ura3Δ0 his3Δ1 leu2Δ0* PCT1-mCHERRY-URA3 KES1-GFP-HIS3 *fld1::NAT*
yPC8129	*Mat ? ura3Δ0 his3Δ1 leu2Δ0* PCT1-mCHERRY-URA3 KES1-GFP-HIS3 *ldb16::HYGB*
yPC5266	*Mat a ura3Δ0 his3Δ1 leu2Δ0 met15Δ0* OPI1-GFP-HIS3
yPC5585	*Mat a ura3Δ0 his3Δ1 leu2Δ0 met15Δ0* OPI1-GFP-HIS3 *fld1::NAT*
yPC5583	*Mat a ura3Δ0 his3Δ1 leu2Δ0 met15Δ0* OPI1-GFP-HIS3 *ldb16::HYGB*
yPC5950	*Mat ? ura3Δ0 his3Δ1 leu2Δ0* OPI1-GFP-HIS3 *scs2::KAN*
yPC5949	*Mat ? ura3Δ0 his3Δ1 leu2Δ0* OPI1-GFP-HIS3 *scs2::KAN fld1::NAT*
yPC5952	*Mat ? ura3Δ0 his3Δ1 leu2Δ0* OPI1-GFP-HIS3 *scs2::KAN ldb16::HYGB*
yPC6260	*Mat a leu2-3,122 ura3-52 his3D200 trp-D900 lys2-8-1 suc2D9* <PHO5p-GFP-Opi1^{FFAT} >
yPC6264	*Mat a leu2-3,122 ura3-52 his3D200 trp-D900 lys2-8-1 suc2D9 fld1::NAT* <PHO5p-GFP-Opi1^{FFAT} >
yPC6340	*Mat a leu2-3,122 ura3-52 his3D200 trp-D900 lys2-8-1 suc2D9 ldb16::HYGB* <PHO5p-GFP-Opi1^{FFAT} >
yPC6037	*Mat a ura3Δ0 his3Δ1 leu2Δ0 met15Δ0* <TEFp-GFP-Spo20$^{51–91}$,2micron,URA>
yPC6038	*Mat a ura3Δ0 his3Δ1 leu2Δ0 met15Δ0 fld1::HYGB* <TEFp-GFP-Spo20$^{51–91}$,2micron,URA>
yPC6058	*Mat a ura3Δ0 his3Δ1 leu2Δ0 met15Δ0 ldb16::KANR* <TEFp-GFP-Spo20$^{51–91}$,2micron,URA>
yPC6039	*Mat a ura3Δ0 his3Δ1 leu2Δ0 met15Δ0 opi3::KANR* <TEFp-GFP-Spo20$^{51–91}$,2micron,URA>
yPC7647	*Mat a ura3Δ0 his3Δ1 leu2Δ0 met15Δ0* < Flag-GFP-KES1, CEN, URA>
yPC7648	*Mat a ura3Δ0 his3Δ1 leu2Δ0 met15Δ0 ldb16::KAN* < Flag-GFP-KES1, CEN, URA>
yPC7669	*Mat a ura3Δ0 his3Δ1 leu2Δ0 met15Δ0 fld1::HYGB* < Flag-GFP-KES1, CEN, URA>
yPC7888	*Mat a ura3Δ0 his3Δ1 leu2Δ0 met15Δ0* < Flag-GFP-KES1 $^{Δ2-29}$, CEN, URA>
yPC7882	*Mat a ura3Δ0 his3Δ1 leu2Δ0 met15Δ0 ldb16::KAN* < Flag-GFP-KES1 $^{Δ2-29}$, CEN, URA>
yPC7885	*Mat a ura3Δ0 his3Δ1 leu2Δ0 met15Δ0 fld1::HYGB* < Flag-GFP-KES1 $^{Δ2-29}$, CEN, URA>
yPC7645	*Mat a ura3Δ0 his3Δ1 leu2Δ0 met15Δ0* < Flag-GFP-PCT1, CEN, URA>
yPC7646	*Mat a ura3Δ0 his3Δ1 leu2Δ0 met15Δ0 ldb16::KAN* < Flag-GFP-PCT1, CEN, URA>
yPC7668	*Mat a ura3Δ0 his3Δ1 leu2Δ0 met15Δ0 fld1::HYGB* < Flag-GFP-PCT1, CEN, URA>
yPC7881	*Mat a ura3Δ0 his3Δ1 leu2Δ0 met15Δ0* < Flag-GFP-PCT1 $^{Δ261-282}$, CEN, URA>
yPC7884	*Mat a ura3Δ0 his3Δ1 leu2Δ0 met15Δ0 ldb16::KAN* < Flag-GFP-PCT11 $^{Δ261-282}$, CEN, URA>
yPC788?	*Mat a ura3Δ0 his3Δ1 leu2Δ0 met15Δ0 fld1::HYGB* < Flag-GFP-PCT11 $^{Δ261-282}$, CEN, URA>
yPC8237	*Mat a ura3Δ0 his3Δ1 leu2Δ0 met15Δ0* < GVP36-GFP, CEN, URA>

Table S5. **Yeast strains used in this study** *(Continued)*

Strain	Genotype
yPC8241	*Mat a ura3Δ0 his3Δ1 leu2Δ0 met15Δ0 ldb16::KAN* < GVP36-GFP, CEN, URA>
yPC8239	*Mat a ura3Δ0 his3Δ1 leu2Δ0 met15Δ0 fld1::HYGB* < GVP36-GFP, CEN, URA>
yPC8238	*Mat a ura3Δ0 his3Δ1 leu2Δ0 met15Δ0* < GVP36 $^{\Delta 2\text{-}35}$-GFP, CEN, URA>
yPC8242	*Mat a ura3Δ0 his3Δ1 leu2Δ0 met15Δ0 ldb16::KAN* < GVP36 $^{\Delta 2\text{-}35}$-GFP, CEN, URA>
yPC8240	*Mat a ura3Δ0 his3Δ1 leu2Δ0 met15Δ0 fld1::HYGB* < GVP36 $^{\Delta 2\text{-}35}$-GFP, CEN, URA>
yPC7791	*Mat a ura3Δ0 his3Δ1 leu2Δ0 are1::KAN are2::HYGB dga1::NAT lro1::KAN* < Flag-GFP-PCT1, CEN, URA>
yPC7794	*Mat a ura3Δ0 his3Δ1 leu2Δ0 are1::KAN are2::HYGB dga1::NAT lro1::KAN ldb16::HIS* < Flag-GFP-PCT1, CEN, URA>
yPC8538	*Mat a ura3Δ0 his3Δ1 leu2Δ0 are1::KAN are2::HYGB dga1::NAT lro1::KAN fld1::HIS* < Flag-GFP-PCT1, CEN, URA>
yPC7267	*Mat a ura3Δ0 his3Δ1 leu2Δ0 are1::KAN are2::HYGB dga1::NAT lro1::KAN* <TEFp-GFP-Spo20$^{51-91}$, 2micron, URA>
yPC7268	*Mat a ura3Δ0 his3Δ1 leu2Δ0 are1::KAN are2::HYGB dga1::NAT lro1::KAN ldb16::HIS* <TEFp-GFP-Spo20$^{51-91}$, 2micron, URA>
yPC8540	*Mat a ura3Δ0 his3Δ1 leu2Δ0 are1::KAN are2::HYGB dga1::NAT lro1::KAN fld1::HIS* <TEFp-GFP-Spo20$^{51-91}$, 2micron, URA>
yPC8307	*Mat ? ura3Δ0 his3Δ1 leu2Δ0 are1::KANR are2::HYGB lro1::HIS KANR-GALp-DGA1* < Flag-GFP-KES1, CEN, URA> <ADH1p-GAL4-ER-VP16,CEN,LEU >
yPC8306	*Mat ? ura3Δ0 his3Δ1 leu2Δ0 are1::KANR are2::HYGB lro1::HIS KANR-GALp-DGA1 ldb16::NAT* < Flag-GFP-KES1, CEN, URA> <ADH1p-GAL4-ER-VP16,CEN,LEU >
yPC8305	*Mat ? ura3Δ0 his3Δ1 leu2Δ0 are1::KANR are2::HYGB lro1::HIS KANR-GALp-DGA1* < Flag-GFP-PCT1, CEN, URA> <ADH1p-GAL4-ER-VP16,CEN,LEU >
yPC8304	*Mat ? ura3Δ0 his3Δ1 leu2Δ0 are1::KANR are2::HYGB lro1::HIS KANR-GALp-DGA1 ldb16::NAT* < Flag-GFP-PCT1, CEN, URA> <ADH1p-GAL4-ER-VP16,CEN,LEU >
yPC8070	*Mat ? ura3Δ0 his3Δ1 leu2Δ0* KAN-GPD-CDS1 KES1-GFP-HIS3
yPC8073	*Mat ? ura3Δ0 his3Δ1 leu2Δ0* KAN-GPD-CDS1 KES1-GFP-HIS3 *fld1::NAT*
yPC8071	*Mat ? ura3Δ0 his3Δ1 leu2Δ0* KAN-GPD-CDS1 KES1-GFP-HIS3 *ldb16::HYGB*
yPC4092	*Mat a ura3Δ0 his3Δ1 leu2Δ0 met15Δ0 cho2::KAN*
yPC5617	*Mat ? ura3Δ0 his3Δ1 leu2Δ0 cho2::KAN ldb16::HYGB*
yPC4067	*Mat a ura3Δ0 his3Δ1 leu2Δ0 met15Δ0 opi3::KAN*
yPC5752	*Mat a ura3Δ0 his3Δ1 leu2Δ0 opi3::KAN ldb16::HYGB*
yPC7452	*Mat a ura3Δ0 his3Δ1 leu2Δ0 met15Δ0* GVP36-mCherry-URA3 HMG1-GFP-HIS3
yPC7456	*Mat ? ura3Δ0 his3Δ1 leu2Δ0* GVP36-mCherry-URA3 HMG1-GFP-HIS3 *ldb16::HYGB*
yPC7454	*Mat a ura3Δ0 his3Δ1 leu2Δ0 met15Δ0* VPS13-mCherry-URA3 HMG1-GFP-HIS3
yPC7458	*Mat ? ura3Δ0 his3Δ1 leu2Δ0* VPS13-mCherry-URA3 HMG1-GFP-HIS3 *ldb16::HYGB*
yPC7064	*Mat a ura3Δ0 his3Δ1 leu2Δ0 met15Δ0* Opi1-3HA-HIS3
yPC7065	*Mat a ura3Δ0 his3Δ1 leu2Δ0 met15Δ0* Opi1-3HA-HIS3 *fld1::HYGB*
yPC7066	*Mat a ura3Δ0 his3Δ1 leu2Δ0 met15Δ0* Opi1-3HA-HIS3 *ldb16::KAN*
yPC6258	*Mat a leu2-3,122 ura3-52 his3D200 trp-D900 lys2-8-1 suc2D9* <PHO5p-GFP-Scs2>
yPC6262	*Mat a leu2-3,122 ura3-52 his3D200 trp-D900 lys2-8-1 suc2D9 fld1::NAT* <PHO5p-GFP-Scs2>
yPC6338	*Mat a leu2-3,122 ura3-52 his3D200 trp-D900 lys2-8-1 suc2D9 ldb16::HYGB* <PHO5p-GFP-Scs2>
yPC6446	*Mat a ura3Δ0 his3Δ1 leu2Δ0 met15Δ0* <FLAG-GFP-OPI1 ^{Q2}, CEN, URA>
yPC6447	*Mat a ura3Δ0 his3Δ1 leu2Δ0 met15Δ0 ldb16::KAN*<FLAG-GFP-OPI1 ^{Q2}, CEN, URA>
yPC7792	*Mat a ura3Δ0 his3Δ1 leu2Δ0 are1::KAN are2::HYGB dga1::NAT lro1::KAN* < Flag-GFP-KES1, CEN, URA>
yPC7795	*Mat a ura3Δ0 his3Δ1 leu2Δ0 are1::KAN are2::HYGB dga1::NAT lro1::KAN ldb16::HIS* < Flag-GFP-KES1, CEN, URA>
yPC8539	*Mat a ura3Δ0 his3Δ1 leu2Δ0 are1::KAN are2::HYGB dga1::NAT lro1::KAN fld1::HIS*? < Flag-GFP-KES1, CEN, URA>
yPC7828	*Mat a ura3Δ0 his3Δ1 leu2Δ0 met15Δ0* KAN-GPD-CDS1 <Flag-GFP-PCT1, CEN, URA>
yPC7832	*Mat a ura3Δ0 his3Δ1 leu2Δ0 met15Δ0* KAN-GPD-CDS1 *ldb16::HYGB* <Flag-GFP-PCT1, CEN, URA>
yPC7834	*Mat a ura3Δ0 his3Δ1 leu2Δ0 met15Δ0* KAN-GPD-CDS1 *fld1::NAT* <Flag-GFP-PCT1, CEN, URA>
yPC5144	*Mat α ura3Δ0 his3Δ1 leu2Δ0 lys2Δ0* ELO3-mCherry-URA
yPC5199	*Mat ? ura3Δ0 his3Δ1 leu2Δ0* ELO3-mCherry-URA *fld1::NAT*
yPC5201	*Mat ? ura3Δ0 his3Δ1 leu2Δ0* ELO3-mCherry-URA *ldb16::HYGB*
yPC1573	*Mat a ura3Δ0 his3Δ1 leu2Δ0 met15Δ0* SEC63-GFP-HIS5
yPC8122	*Mat a ura3Δ0 his3Δ1 leu2Δ0 met15Δ0* SEC63-GFP-HIS5 *fld1::NAT*
yPC8121	*Mat a ura3Δ0 his3Δ1 leu2Δ0 met15Δ0* SEC63-GFP-HIS5 *ldb16::NAT*

Table S6. **Plasmids used in this study**

Name	Insert/gene	Primers used	Vector	Source
pPC941	PHO5p-GFP-Opi1^{FFAT}, URA3	NA	pRS406	Loewen et al., 2003
pPC933	TEFp-GFP-Spo20$^{51–91}$,2μ,URA3	NA	pRS426	Nakanishi et al., 2004
pPC1131	Flag-GFP-KES1, CEN, URA3	1688 and 1689	pRS316	This study
pPC1224	Flag-GFP-KES1 $^{\Delta 2\text{-}29}$, CEN, URA3	1731	pRS316	This study
bPC1130	Flag-GFP-PCT1, CEN, URA3	1690 and 1691	pRS316	This study
pPC1226	Flag-GFP-PCT1 $^{\Delta 261\text{-}282}$, CEN, URA3	1733	pRS316	This study
pPC1129	Flag-GFP-GVP36, CEN, URA3	1686 and 1687	pRS316	This study
pPC1184	GVP36-GFP, CEN, URA3	NA	pRS316	This study
pPC1183	GVP36 $^{\Delta 2\text{-}35}$-GFP, CEN, URA3	1818	pRS416	This study
pPC924	ADH1p-GAL4-ER-VP16,CEN,LEU	NA	pRS415	This study (based on Louvion et al., 1993)
pPC938	PHO5p-GFP-Scs2	NA	pRS406	Loewen et al., 2003
pPC974	FLAG-GFP-OPI1 ^{Q2}, CEN, URA3	1339 and 1340	pRS316	This study

Table S7. **Primers used in this study**

Number	Name	Sequence (5′ to 3′)
1688	Kes1FXhoI	CATGGATGAACTATACAAACTCGAGATGTCTCAATACGCAAGCTC
1689	Kes1RBamHI	CTATAGGGCGAATTGGCTAGTGGATCCGAGCGATCTGTCTATCAATAATTA
1731	Kes1Δ(2–29)	GATGAACTATACAAACTCGAGATGCCTCCATTCATTTTATCTCCAATC
1690	Pct1FXhoI	GATGAACTATACAAACTCGAGATGGCAAACCCAACAACAGGGAAG
1691	Pct1RBamHI	GCGAATTGGCTAGTGGATCCTAATCAACTTTCTCTCCTTCAAATC
1733	Pct1Δ(261–282)	GACAGGAGCTGAACGTTTCTCACATCAATGAATTCAGGTC
1686	Gvp36FXhoI	GAACTATACAAACTCGAGATGTCGTTTAATGCCTTCGCCAG
1687	Gvp36RBamHI	GCGAATTGGCTAGTGGATCCGTATTGCGGTTGAGTAGCGTC
1818	Gvp36Δ(2–35)	CAAATCATAGTCATCAATGCAAAGAATGGTCCAGGAAC
185	Yos9R5	CTATTGTACTCGAGCGAGGCAAGCTAAACAGATC
1339	Opi1Q2FXhoI	GAACTATACAAACTCGAGGATGAGTTCTTCACCAACAAG
1340	Opi1Q2RHindIII	CACATACACGCTAAGCTTTTACTCGTCCTCGCCAGCTCCAG

References

Loewen, C.J., A. Roy, and T.P. Levine. 2003. A conserved ER targeting motif in three families of lipid binding proteins and in Opi1p binds VAP. *EMBO J.* 22:2025–2035. http://dx.doi.org/10.1093/emboj/cdg201

Louvion, J.F., B. Havaux-Copf, and D. Picard. 1993. Fusion of GAL4-VP16 to a steroid-binding domain provides a tool for gratuitous induction of galactose-responsive genes in yeast. *Gene.* 131:129–134. http://dx.doi.org/10.1016/0378-1119(93)90681-R

Nakanishi, H., P. de los Santos, and A.M. Neiman. 2004. Positive and negative regulation of a SNARE protein by control of intracellular localization. *Mol. Biol. Cell.* 15:1802–1815. http://dx.doi.org/10.1091/mbc.E03-11-0798