Motley et al., http://www.jcb.org/cgi/content/full/jcb.201412066

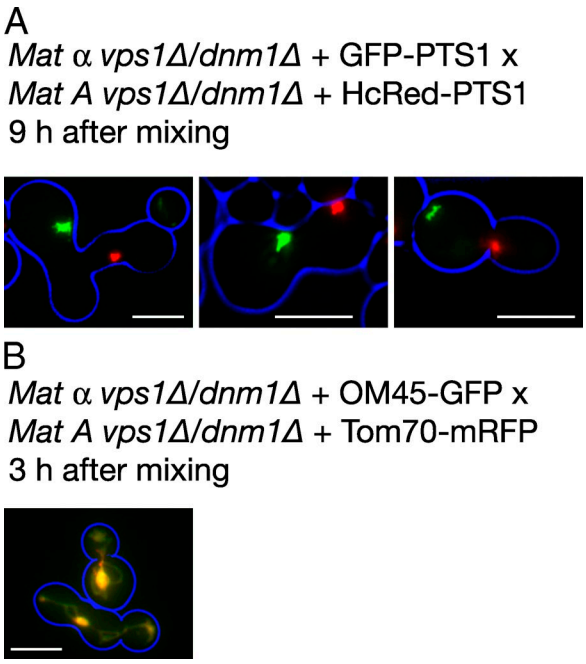


Figure S1. **Peroxisomes in *vps1Δ/dnm1Δ* cells do not fuse.** *vps1/dnm1* MatA and Matα cells transformed with galactose-controllable peroxisomal markers (GFP- or HcRed-PTS1; A) or mitochondrial markers (OM45-GFP or Tom70-mRFP; B) were grown overnight on raffinose medium and induced to express reporters for 30 min (GFP-PTS1 and OM45-GFP) or 90 min (HcRed-PTS1 and Tom70-mRFP) followed by a 1-h chase on glucose-containing medium before mating on YPD medium. Cells were restreaked on YPD after 5 h to prevent depletion of glucose. Cells were imaged 9 h (A) or 3 h (B) after mixing. Bars, 5 μm.

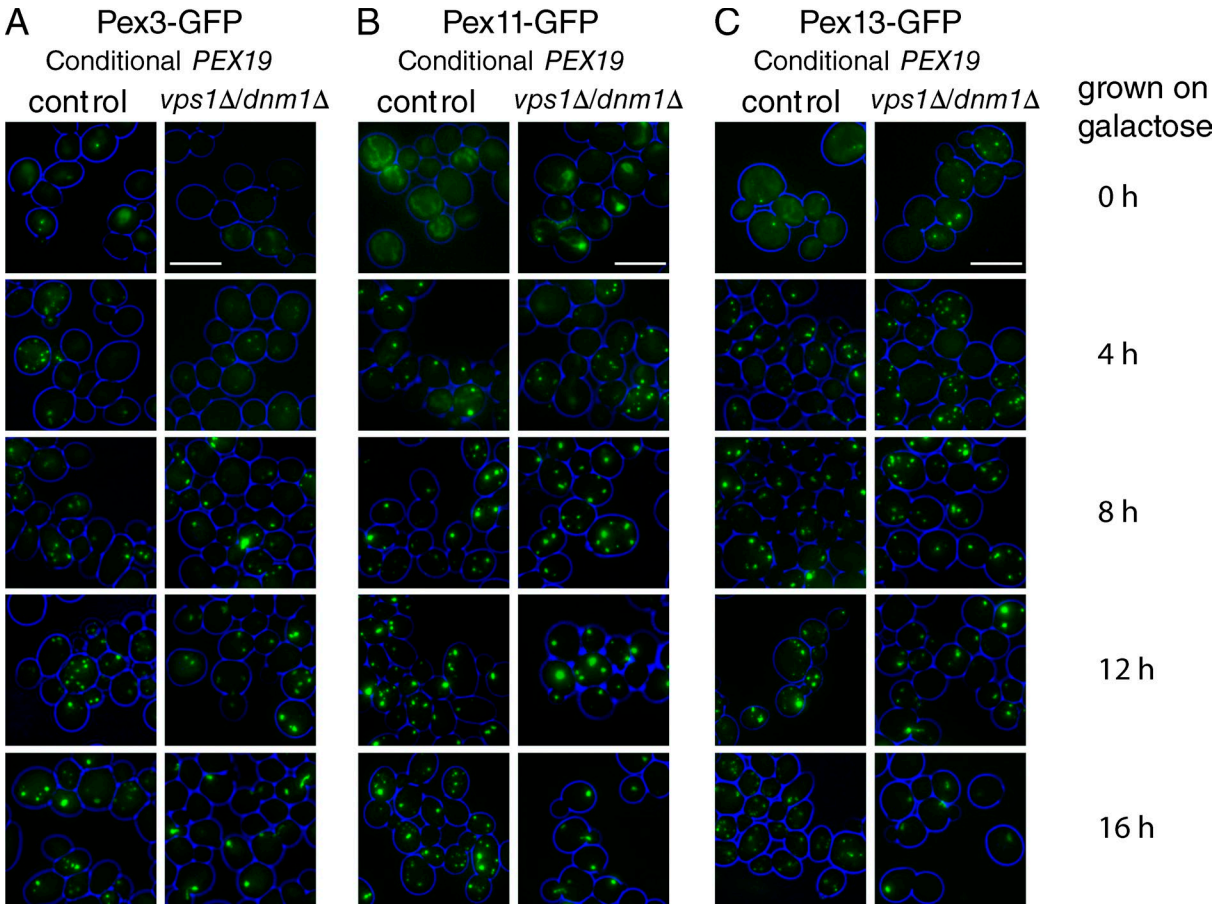


Figure S2. **De novo–formed peroxisomes grow into elongated peroxisomes in DRP-deficient cells.** (A–C) Galactose-controllable *PEX19* strains in WT and *vps1/dnm1* background transformed with Pex3-, Pex11-, or Pex13-GFP (expressed from endogenous promoters on plasmids) were grown on raffinose (top) or galactose medium for the times indicated. Note the localization of Pex11-GFP to a tubular network in control cells and how this network collapses in the *vps1/dnm1* cells. That this structure represents mitochondria is confirmed in Fig. 4 C. Bars, 5 µm.

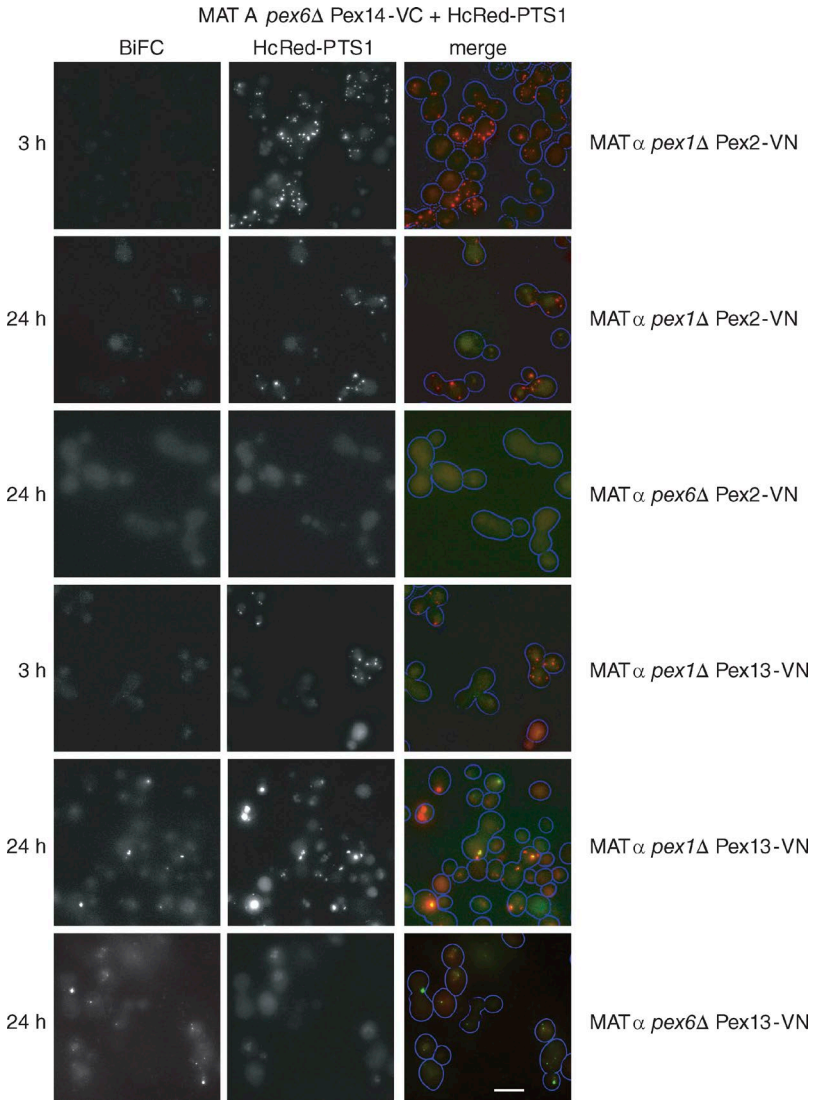


Figure S3. **PTS1 import is restored 3 h after mating *pex1* with *pex6* cells.** *Pex6* MatA cells tagged with Pex14-VC and expressing HcRed-PTS1 on a plasmid were mated with *pex1* or *pex6* Matα cells tagged with Pex2- or Pex13-VN as indicated. Cells were imaged 3 and 24 h after mixing. Similar observations were made after mating *pex1* MatA cells tagged with Pex14-VC with *pex1* or *pex6* Matα cells tagged with Pex2- or Pex13-VN. VN and VC tags were genomically integrated. Bar, 5 μm.

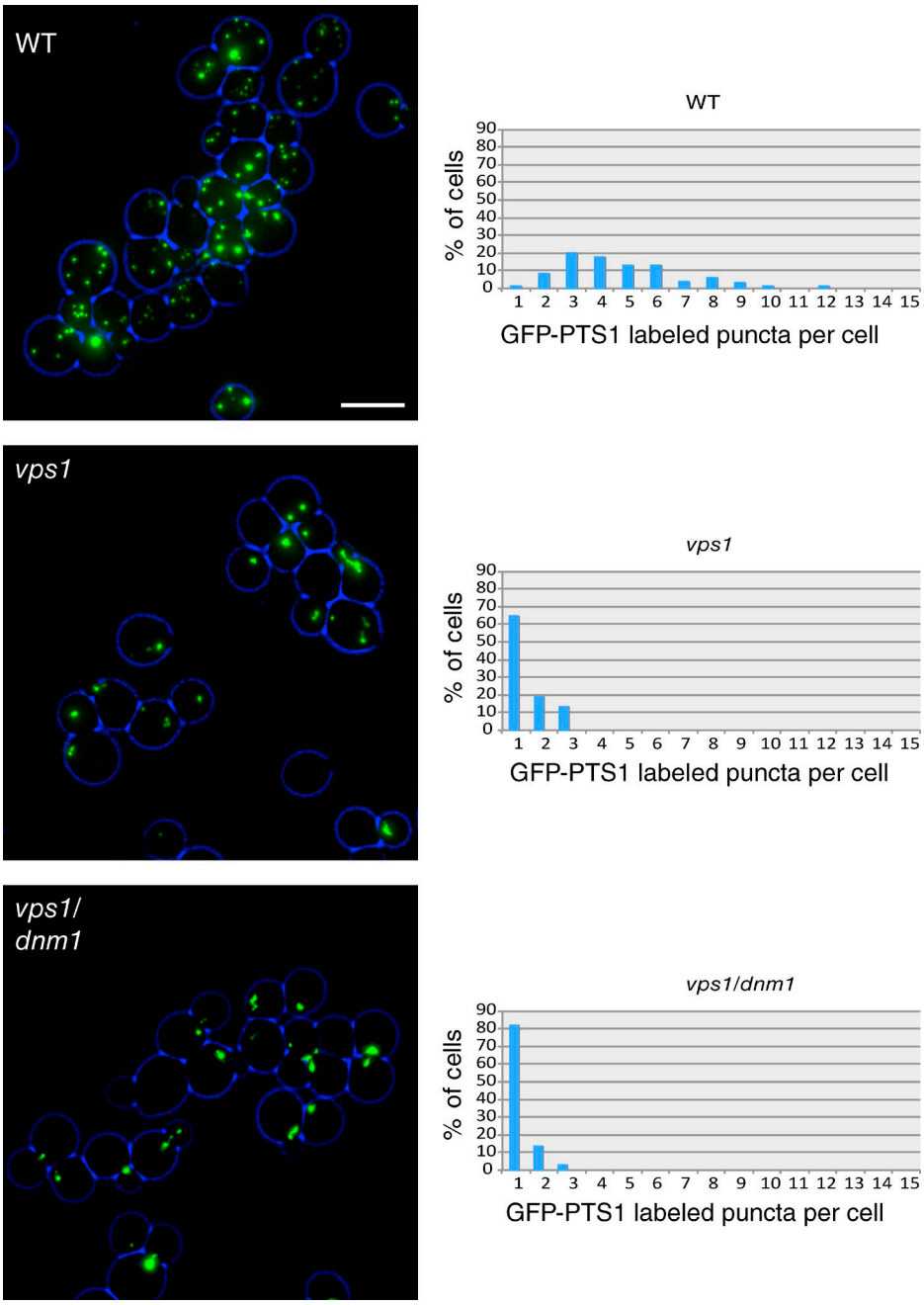
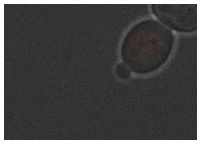
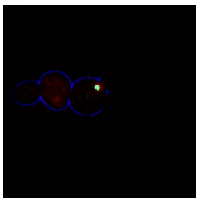


Figure S4. **Quantitation of peroxisome numbers in DRP-deficient cells.** WT, *vps1*, and *vps1/dnm1* cells expressing GFP-PTS1 under control of the Tpi1 promoter and Pgk1 terminator from a plasmid were grown exponentially for 16 h in 2% glucose-containing medium. GFP puncta were counted manually. At least 100 cells of each strain were counted. Bar, 5 µm.



Video 1. **Segregation of de novo–formed peroxisomes in *vps1/dnm1* cells.** Haploid *vps1/dnm1* cells with galactose-controllable Pex19 constitutively expressing HcRed-PTS1 (red) were grown in media containing galactose. Image acquisition started 4.5 h after first exposure to galactose. Images were acquired using time-lapse epifluorescence microscopy on a widefield microscope (Axio Observer). Stacks were taken every 10 min for 15 h. Video runs at one frame per second.



Video 2. **Asymmetric segregation of BiFC complexes with HcRed-PTS1 in *vps1* cells.** Diploid *vps1* cells expressing both Pex13-VN and Pex14-VC (green) and HcRed-PTS1 (red). Images were acquired using time-lapse epifluorescence microscopy on a widefield microscope (Axio Observer). Stacks were taken every 20 min for 14 h. Video runs at one frame per second.

Table S1. **Yeast strains used in this study**

Strain and genotype	Reference
BY4741 MATA *his3-1 leu2-0 met15-0 ura3-0*	EUROSCARF
BY4742 MATα *his3-1 leu2-0 lys2-0 ura3-0*	EUROSCARF
FY1679-08A MATA *ura3-52 leu2Δ1 trp1Δ63 his3Δ200 GAL2*	EUROSCARF
FY1679-06C MATα *ura3-52 leu2Δ1 trp1Δ63 his3Δ200 GAL2*	EUROSCARF
BY4742 *his3MX6-pGAL1-3HA-PEX19*	This study
BY4742 *dnm1Δ::kanMX4 vps1Δ::loxP:His3MX6-pGAL1-3HA-PEX19*	This study
BY4741 *dnm1Δ::kanMX4 vps1Δ::HIS5*	Motley et al., 2008
BY4742 *dnm1Δ::kanMX4 vps1Δ::HIS5*	Motley et al., 2008
BY4742 *pex1Δ:: kanMX4*	EUROSCARF
BY4742 *pex6Δ:: kanMX4*	EUROSCARF
BY4742 *pex1Δ:: kanMX4 atg36Δ::hphMX4*	This study
BY4742 *pex6Δ:: kanMX4 atg36Δ::hphMX4*	This study
BY4742 *atg36Δ::hphMX4*	Motley et al., 2008
BY4742 *dnm1Δ::kanMX4 vps1Δ::HIS5 pex1Δ::LEU2 atg36Δ::hphMX4*	This study
FY1679-08A *pex1Δ::hphMX4*	This study
FY1679-08A *pex6Δ::hphMX4*	This study
FY1679-06C *pex1Δ::hphMX4*	This study
FY1679-06C *pex6Δ::hphMX4*	This study
FY1679-06C *pex1Δ::hphMX4 PEX2-VN::HIS5*	This study
FY1679-06C *pex1Δ::hphMX4 PEX13-VN::HIS5*	This study
FY1679-08A *pex1Δ::hphMX4 PEX14-VC::HIS5*	This study
FY1679-06C *pex6Δ::hphMX4 PEX2-VN::HIS5*	This study
FY1679-06C *pex6Δ::hphMX4 PEX13-VN::HIS5*	This study
FY1679-08A *pex6Δ::hphMX4 PEX14-VC::HIS5*	This study
FY1679-08A *PEX14-VC::HIS5*	This study
FY1679-08A *PEX14-VC::HIS5 PEX2-VN::TRP1*	This study
FY1679-08A *PEX14-VC::HIS5 PEX13-VN::TRP1*	This study
FY1679-08A *pex6Δ::hphMX4 PEX14-VC::HIS5 PEX2-VN::TRP1*	This study
FY1679-08A *pex6Δ::hphMX4 PEX14-VC::HIS5 PEX13-VN::TRP1*	This study
FY1679-06C *PEX13-GFP::HIS5 vps1Δ::LEU2*	This study
FY1679-08A *PEX14-VC::HIS5 PEX2-VN::TRP1*	This study
FY1679-08A *pex1Δ::hphMX4 PEX14-VC::HIS5 PEX2-VN::TRP1*	This study
FY1679-08A *pex6Δ::hphMX4 PEX14-VC::HIS5 PEX2-VN::TRP1*	This study
FY1679-08A *PEX14-VC::HIS5 PEX13-VN::TRP1 vps1Δ::LEU2*	This study
FY1679-08A *PEX14-VC::HIS5 PEX2-VN::TRP1 vps1Δ::LEU2*	This study
W303-1A MATA *ura3-1::ADH1-OsTIR1-9Myc(URA3) ade2-1 his3-11 15 leu 2-3 112 trp1-1 can1-100 PEX1-3HA-IAA17::hphMX4*	Nuttall et al., 2014
BY4742 *pex1Δ::kanMX4 pex6Δ::HIS5*	This study
FY1679-06C *PEX14-VC::HIS5 TOM20-VN::TRP1*	This study
FY1679-06C *TOM70-VC::HIS5 PEX2-VN::TRP1*	This study
FY1679-06C *TOM70-VC::HIS5 TOM20-VN::TRP1*	This study
FY1679-06C *pex1Δ::hphMX4 PEX14-VC::HIS5 TOM20-VN::TRP1*	This study
FY1679-06C *pex6Δ::hphMX4 PEX14-VC::HIS5 TOM20-VN::TRP1*	This study
BY4742 *His3MX6-pGAL1-3HA-PEX19 pex1Δ::hphMX4*	This study

Table S2. **Oligonucleotides used in this study**

Protein	Sequence
PEX2F	5′-GATGCCTGTGGATCCTCTGGGAGACTGACCGCCTCACCAGTGTACGGTCGACGGATCCCCGGGTTAATT-3′
PEX2R	5′-TGAATATAGTATACACATATATAGAGATACAAGCGAGGGAACGGGGCCCTGCATAGGCCACTAGTGGATC-3′
PEX13F	5′-GACGGAAGAAAATTGAGCATGTTGATGATGAAACGCGTACACACGGTCGACGGATCCCCGGGTTAATT-3′
PEX13R	5′-TAGATTTTACTATATATATATGCGAATATATGTGTGCAAATATTGATGCAGCATAGGCCACTAGTGGATC-3′
Pex14F	5′-AGTGTCCCTGACTGGCAAAATGGACAGGTCGAAGACTCCATCCCAGGTCGACGGATCCCCGGGTTAATT-3′
Pex14R	5′-GTTACAATTACAATTTCCGTTAAAAAACTAATTACTTACATAGAATTGCGGCATAGGCCACTAGTGGATC-3′
TOM20F	5′-GCAAGGCCGAATCTGATGCGGTTGCTGAAGCTAACGATATCGATGACGGTCGACGGATCCCCGGGTTAATT-3′
TOM20R	5′-CAGAGTAAAAGAAACAAAAACGGAGAAAAAAAGCAAGCAAAATGTTACTCGCATAGGCCACTAGTGGATC-3′
TOM70F	5′-TAAAAAGATTCAAGAAACTTTAGCTAAATTACGCGAACAGGGTTTAATGGGTCGACGGATCCCCGGGTTAATT-3′
TOM70R	5′-TGTTACTTAGTTTTTGTCTTCTCCTAAAAGTTTTTAAGTTTATGTTTACTGTGCATAGGCCACTAGTGGATC-3′

References

Motley, A.M., G.P. Ward, and E.H. Hettema. 2008. Dnm1p-dependent peroxisome fission requires Caf4p, Mdv1p and Fis1p. *J. Cell Sci.* 121:1633–1640. http://dx.doi.org/10.1242/jcs.026344

Nuttall, J.M., A.M. Motley, and E.H. Hettema. 2014. Deficiency of the exportomer components Pex1, Pex6, and Pex15 causes enhanced pexophagy in *Saccharomyces cerevisiae*. *Autophagy.* 10:835–845. http://dx.doi.org/10.4161/auto.28259