Knoops et al., http://www.jcb.org/cgi/content/full/jcb.201310148/DC1

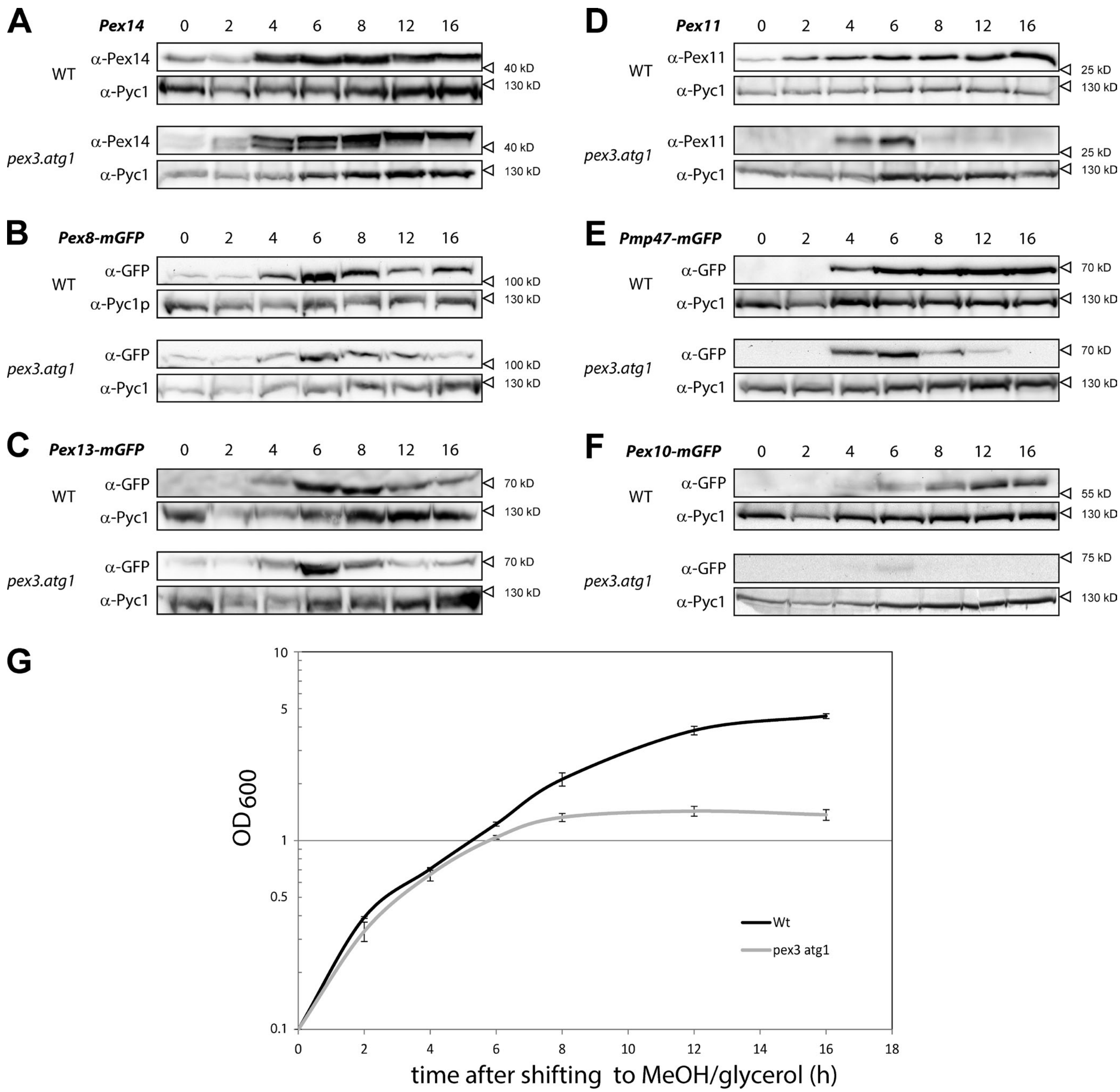


Figure S1. **Induction of PMPs in WT and *pex3 atg1* cells.** (A–F) MM-Glu grown cells were shifted to MM-M/G. Samples were taken at the indicated time points. (G) Corresponding growth curves. Error bars indicate SD of five experiments.

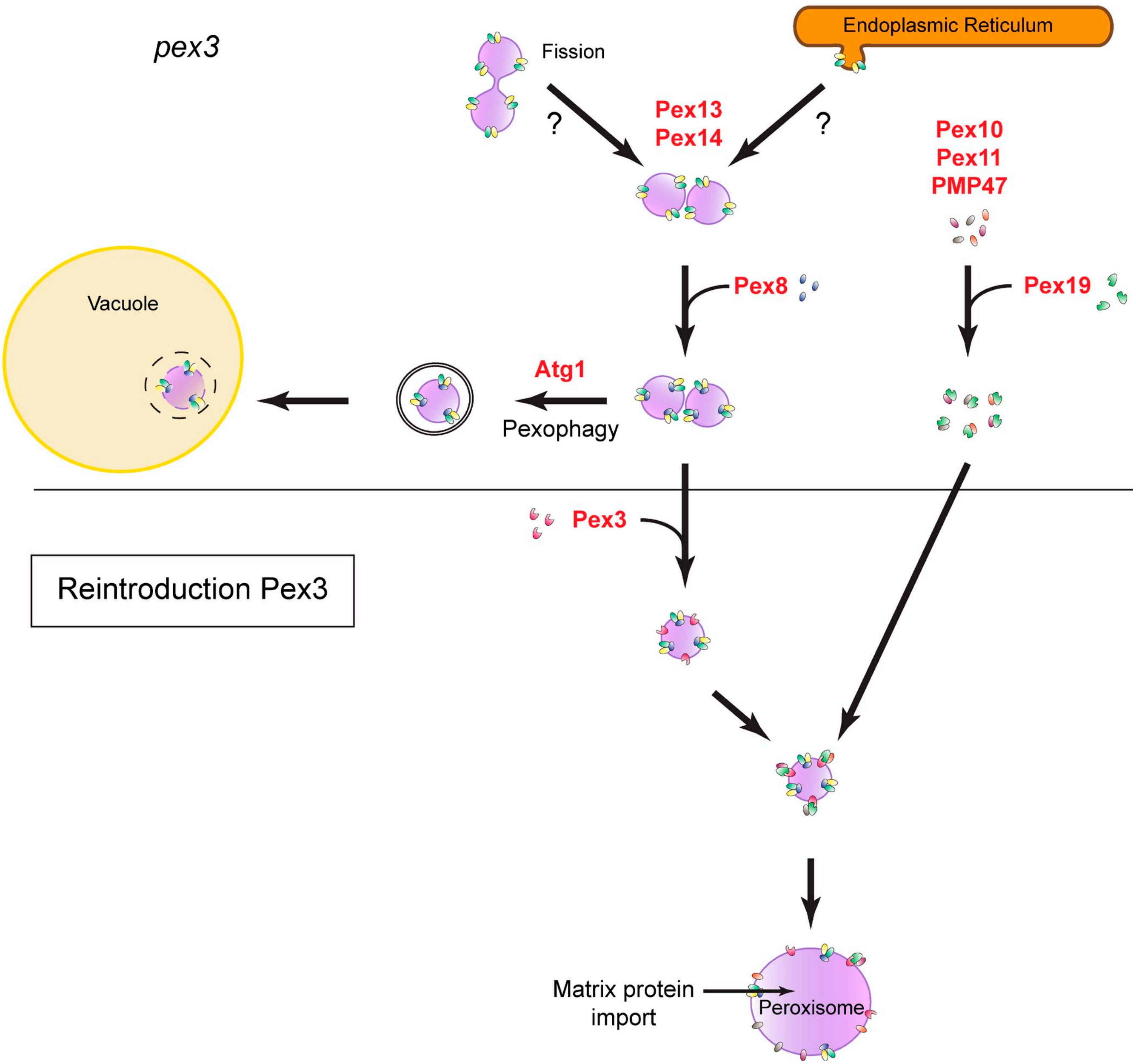


Figure S2. **Model.** In *pex3* cells, Pex13/Pex14-containing structures exist that import Pex8. These structures may arise by proliferation of a preexisting structure or form from the ER. They are constitutively degraded by autophagy, unless *ATG1* is deleted. Pex10, Pex11, and PMP47 are instable, because they are not inserted into these structures. Reintroduced Pex3 is sorted to the structures, followed by insertion of the other PMPs by the Pex3–Pex19 machinery and import of matrix proteins.

Table S1. ***H. polymorpha* strains used in this study**

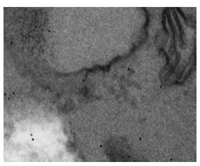
Strains	Characteristics	Reference
WT	NCYC 495 *leu1.1*	Saraya et al., 2012
atg1	*ATG1* deletion strain, *leu 1.1*	Komduur et al., 2003
pex3	*PEX3* deletion strain, *ura3*	Baerends et al., 1996
pex19	*PEX19* deletion strain, *ura3*	Otzen et al., 2004
pex10	*PEX10* deletion strain	Tan et al., 1995
pex3 atg1	*PEX3 ATG1* double deletion strain	This study
pex19 atg1	*PEX19 ATG1* double deletion strain	This study
pex3 atg1.PEX14-mCherry	*pex3 atg1* with pSEM01	This study
pex3 atg1.PEX14-mCherry.*PEX8*-mGFP	*pex3 atg1* with pSEM01 and pMCE4	This study
pex3 atg1.PEX14-mCherry.*PEX10*-mGFP	*pex3 atg1* with pSEM01and pMCE5	This study
pex3 atg1.PEX14-mCherry.*PEX11*-mGFP	*pex3 atg1* with pSEM01 and pSEM02	This study
pex3 atg1.PEX14-mCherry.*PEX13*-mGFP	*pex3 atg1* with pSEM01 and pSEM03	This study
pex3 atg1.PEX14-mCherry.*PMP47*-mGFP	*pex3 atg1* with pSEM01 and pMCE7	This study
pex3 atg1.PEX13-mGFP	*pex3 atg1* with pSEM03	This study
pex3 atg1.PEX14-mCherry-BiP$_{N30}$-eGFP-HDEL	*pex3 atg1* with pSEM01 and pRSA017	This study
pex3 atg1.PEX14-mCherry.*PEX10*-mGFP – P$_{AOX}$ *PEX19*	*pex3 atg1* with pSEM01, pMCE05 and pSEM05	This study
pex3 atg1.PEX14-mCherry.*PMP47*-mGFP – P$_{AOX}$ *PEX19*	*pex3 atg1* with pSEM01, pMCE07 and pSEM05	This study
pex3 atg1.PEX14-mGFP	*pex3 atg1* with pSNA12	This study
pex3 atg1 pex25	*pex3 atg1* with pRSA018	This study
pex3 atg1 pex25.PEX14-mGFP	*pex3 atg1 pex25* with pSNA12	This study
pex3 atg1.PEX14-mCherry – P$_{AMO}$ *PEX3*-eGFP	*pex3 atg1* with pSEM01 and pHIPZ5-P$_{AMO}$ *PEX3*-eGFP	This study
pex3 atg1.PEX14-mCherry.PEX10-mGFP–P$_{AMO}$ PEX3	*pex3 atg1* with pSEM01, pMCE5 and pSEM04	This study
pex3 atg1.PEX14-mCherry.*PMP47*-mGFP–P$_{AMO}$ PEX3	*pex3 atg1* with pSEM01, pMCE7 and pSEM04	This study
pex3 atg1.PEX14-mCherry – P$_{AMO}$ *PEX3*-eGFP – P$_{TEF1}$ eGFP-SKL	*pex3 atg1* with pSEM01, pSEM04 and pAKW27	This study
pex10. PEX8-mGFP.*PEX14*-mCherry	*pex10* with pSEM01 and pMCE4	This study
WT. *PEX13*-mGFP	WT with pSEM03	This study
WT. DsRed-SKL.*PEX8*-mGFP	WT. DsRed-SKL with pMCE4	Cepińska et al., 2011
WT. DsRed-SKL.*PEX10*-mGFP	WT. DsRed-SKL with pMCE5	Cepińska et al., 2011
WT. DsRed-SKL.*PMP47*-mGFP	WT. DsRed-SKL with pMCE7	Cepińska et al., 2011
WT. PEX10-mGFP.*PEX14*-mCherry	WT with pSEM01 and pMCE7	This study
WT. *PEX11*-mGFP.*PEX14*-mCherry	WT with pSEM01 and pMCE3	This study
WT. DsRed-SKL.*PEX13*-mGFP.	WT. DsRed-SKL with pSEM03	This study

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

Plasmid	Characteristics	Reference
pSEM01	pHIPN plasmid containing C-terminal part of *PEX14* fused to mCherry; nat^R; amp^R	This study
pSEM02	pHIPZ plasmid containing C-terminal part of *PEX11* fused to mGFP; zeo^R; amp^R	This study
pSEM03	pHIPZ plasmid containing C-terminal part of *PEX13* fused to mGFP; zeo^R; amp^R	This study
pSEM04	pHIPH5 plasmid containing *PEX3* under control of P$_{AMO}$, Hph^{R,} ; amp^R	This study
pSEM05	pHIPH4 plasmid containing *PEX19* under control of P$_{AOX}$, Hph^{R,} ; amp^R	This study
pSEM188	pBS plasmid containing *PEX19* deletion cassette; *LEU2*; amp^R	This study
pHIPZ5-PEX3-eGFP	pHIPZ5 plasmid containing *PEX3*-eGFP under control of P$_{AMO}$, zeo^R; amp^R	Nagotu et al., 2008
pRSA017	pHIPZ4 plasmid containing BiP$_{N30}$-eGFP-HDEL under control of P$_{AOX}$; zeo^R; amp^R	Saraya et al., 2011
pHIPH4	pHIPH plasmid containing hygromycine B marker, amp^R	Saraya et al., 2011
pHIPZ7	pHIPZ plasmid containing TEF1 promoter, zeo ^{R}; amp^R	Baerends et al., 1997
pFEM35	pHIPX7 plasmid containing eGFP with C-terminal PTS1 under control of P$_{AOX}$; Leu2; kan^R	Krikken et al., 2009
pHIPZ-mGFP fusinator	pHIPZ plasmid containing mGFP and *AMO* terminator; zeo^R; amp^R	Saraya et al., 2010
pMCE02	pHIPN plasmid containing mCherry; nat^R; amp^R	Cepińska et al., 2011
pSNA12	pHIPZ plasmid containing C-terminal part of *PEX14* fused to mGFP; zeo^R; amp^R	Cepińska et al., 2011
pMCE3	pHIPN pPlasmid containing C-terminal part of *PEX11* fused to mCherry; nat^R; amp^R	Cepińska et al., 2011
pMCE4	pHIPZ plasmid containing C-terminal part of *PEX8* fused to mGFP; zeo^R; amp^R	Cepińska et al., 2011
pRSA018	pDEST-R4-R3 containing *PEX25* deletion cassette, nat^R; amp^R	Saraya et al., 2011
pMCE5	pHIPZ plasmid containing C-terminal part of *PEX10* fused to GFP; zeo^R; amp^R	Cepińska et al., 2011
pMCE7	pHIPZ plasmid containing C-terminal part of *PMP47* fused to GFP; zeo^R; amp^R	Cepińska et al., 2011
pAKW27	pHIPZ plasmid containing eGFP-SKL under control of P$_{TEF1}$; zeo^R; amp^R	This study
pHOR30b	pBS plasmid containing *PEX19* deletion cassette; *URA3*; amp^R	Otzen et al., 2004
pBS-CaLeu2	pBS plasmid containing *C. albicans LEU2* gene; amp^R	Otzen et al., 2004

Table S3. **Primers used in this study**

Primer	Sequence	Reference
Pex25-F	5′-CTGGATGGAGGCTTCATCTC-3′	Saraya et al., 2011
Pex25-R	5′-GGAGCTGCTGTGCTTGTATG-3′	Saraya et al., 2011
H5-F	5′-GTGCTGCAAGGCGATTAAGT-3′	This study
H5-R	5′-AGAGCTCGAGGTTAAGCATCGAAATTAGAGTAGAC-3′	This study
Pex13-F	5′-AAAAGCTTATGACTACACCACGTCCAAAGCC-3′	This study
Pex13-R	5′-AAAGATCTGATCAATAGCTTTTGATCTTTCTTGAAC-3′	This study
Pex14-F	5′-CCCAAGCTTCGTTGCAGGAAGTCGACGAA-3′	This study
Pex14-R	5′-AGATCTTCCGGCATTCAGCTGCCACGCCG-3′	This study
Pex19-F	5′-AAAAGCTTATGAGCGAGAAAAAGTCC-3′	This study
Pex19-R	5′-ATCTAGACTATGTTTGTTTGCAAGTGTCTTCC-3′	This study



Video 1. **Electron tomography of *pex3 atg1* cells.** Two perpendicular tilt series (−70° to 70°) were acquired of 150-nm sections of $KMnO_4$-fixed cells with a transmission EM microscope (Tecnai 12; FEI) and reconstructed into a single tomographic volume by IMOD. Organelle masks were segmented manually in Amira followed by nonlinear anisotropic diffusion filtering, thresholding, and 3D surface rendering, using an identical color scheme as in Fig. 1 E.

References

Baerends, R.J., S.W. Rasmussen, R.E. Hilbrands, M. van der Heide, K.N. Faber, P.T. Reuvekamp, J.A. Kiel, J.M. Cregg, I.J. van der Klei, and M. Veenhuis. 1996. The *Hansenula polymorpha* PER9 gene encodes a peroxisomal membrane protein essential for peroxisome assembly and integrity. *J. Biol. Chem.* 271:8887–8894. http://dx.doi.org/10.1074/jbc.271.15.8887

Baerends, R.J., F.A. Salomons, K.N. Faber, J.A. Kiel, I.J. Van der Klei, and M. Veenhuis. 1997. Deviant Pex3p levels affect normal peroxisome formation in *Hansenula polymorpha*: high steady-state levels of the protein fully abolish matrix protein import. *Yeast*. 13:1437–1448. http://dx.doi.org/10.1002/(SICI)1097-0061(199712)13:15<1437::AID-YEA192>3.0.CO;2-U

Cepińska, M.N., M. Veenhuis, I.J. van der Klei, and S. Nagotu. 2011. Peroxisome fission is associated with reorganization of specific membrane proteins. *Traffic*. 12:925–937. http://dx.doi.org/10.1111/j.1600-0854.2011.01198.x

Komduur, J.A., M. Veenhuis, and J.A. Kiel. 2003. The *Hansenula polymorpha* PDD7 gene is essential for macropexophagy and microautophagy. *FEMS Yeast Res.* 3:27–34.

Krikken, A.M., M. Veenhuis, and I.J. van der Klei. 2009. *Hansenula polymorpha* pex11 cells are affected in peroxisome retention. *FEBS J.* 276:1429–1439. http://dx.doi.org/10.1111/j.1742-4658.2009.06883.x

Nagotu, S., R. Saraya, M. Otzen, M. Veenhuis, and I.J. van der Klei. 2008. Peroxisome proliferation in *Hansenula polymorpha* requires Dnm1p which mediates fission but not *de novo* formation. *Biochim. Biophys. Acta*. 1783:760–769. http://dx.doi.org/10.1016/j.bbamcr.2007.10.018

Otzen, M., U. Perband, D. Wang, R.J.S. Baerends, W.H. Kunau, M. Veenhuis, and I.J. Van der Klei. 2004. *Hansenula polymorpha* Pex19p is essential for the formation of functional peroxisomal membranes. *J. Biol. Chem.* 279:19181–19190. http://dx.doi.org/10.1074/jbc.M314275200

Saraya, R., M.N. Cepińska, J.A. Kiel, M. Veenhuis, and I.J. van der Klei. 2010. A conserved function for Inp2 in peroxisome inheritance. *Biochim. Biophys. Acta*. 1803:617–622. http://dx.doi.org/10.1016/j.bbamcr.2010.02.001

Saraya, R., A.M. Krikken, M. Veenhuis, and I.J. van der Klei. 2011. Peroxisome reintroduction in *Hansenula polymorpha* requires Pex25 and Rho1. *J. Cell Biol.* 193:885–900. http://dx.doi.org/10.1083/jcb.201012083

Saraya, R., A.M. Krikken, J.A. Kiel, R.J. Baerends, M. Veenhuis, and I.J. van der Klei. 2012. Novel genetic tools for *Hansenula polymorpha. FEMS Yeast Res.* 12:271–278. http://dx.doi.org/10.1111/j.1567-1364.2011.00772.x

Tan, X., H.R. Waterham, M. Veenhuis, and J.M. Cregg. 1995. The *Hansenula polymorpha* PER8 gene encodes a novel peroxisomal integral membrane protein involved in proliferation. *J. Cell Biol.* 128:307–319. http://dx.doi.org/10.1083/jcb.128.3.307