

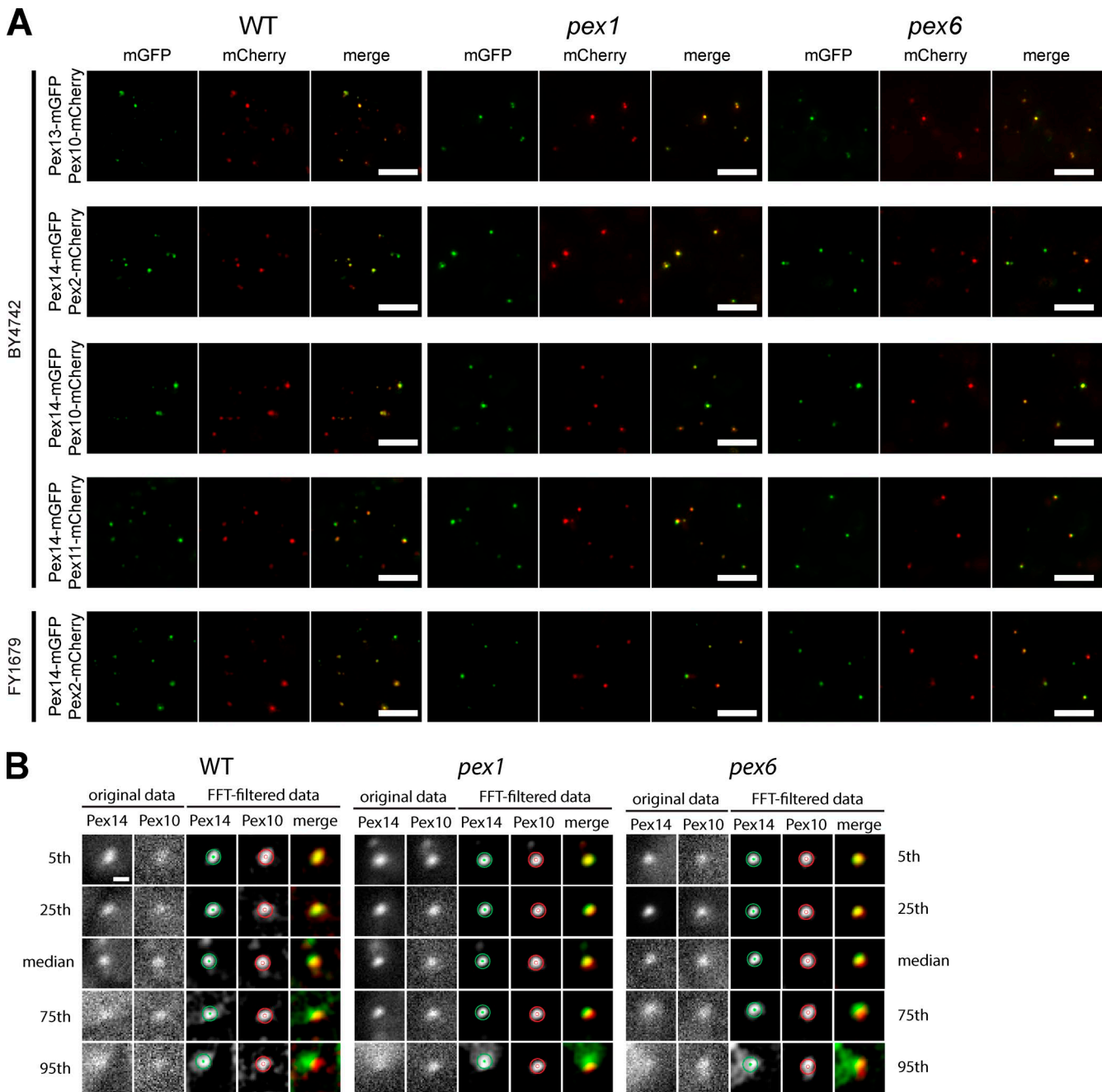
Knoops et al., <http://www.jcb.org/cgi/content/full/jcb.201506059/DC1>

Figure S1. **Docking and RING complex proteins as well as Pex11 colocalize in *pex1* and *pex6* cells.** (A) Representative FM images of WT, *pex1*, and *pex6* cells grown on glucose producing Pex13-mGFP/Pex10-mCherry, Pex14-mGFP/Pex2-mCherry, Pex14-mGFP/Pex10-mCherry, or Pex14-mGFP/Pex11-mCherry in BY4742. For the FY1679 strain, the Pex14-mGFP/Pex2-mCherry combination was analyzed. Cells were fixed in formaldehyde and embedded in agarose to prevent movement of spots and cells during imaging acquisition at the Deltavision microscope. (B) Example of the colocalization analysis of WT, *pex1*, and *pex6* cells producing Pex14-mGFP and Pex10-mCherry grown on glucose. Bars: (A) 5 μ m; (B) 10 nm.

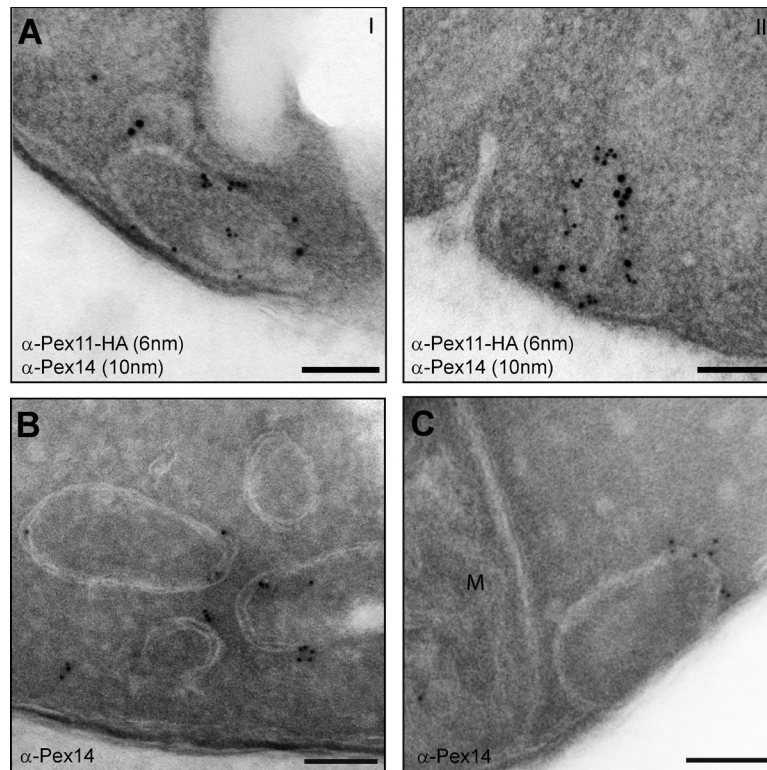


Figure S2. **Peroxisomal ghosts in glucose-grown *pex1* cells and in cells of the *pex1 atg1* and *pex6 pex11* double-deletion strains.** (A) Double immunolabeling experiment using α -HA and α -Pex14 antibodies on glucose-grown *pex1* cells producing Pex11-HA. α -Pex14 labeling of oleic acid induced *pex1 atg1* (B) or *pex6 pex11* cells (C) showing the presence of ghosts labeled with Pex14. Bars, 100 nm.

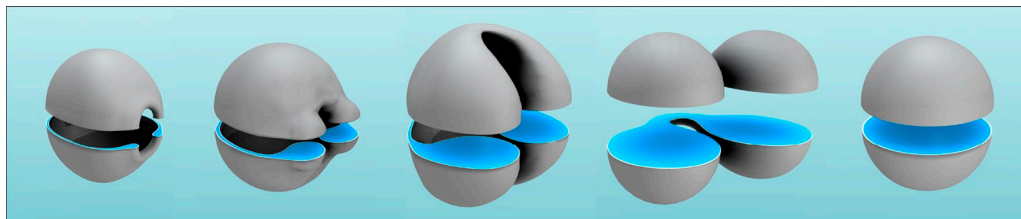


Figure S3. **Hypothetical model of peroxisome reintroduction in *pex1* or *pex6* cells.** In the absence of Pex1, peroxisomal membrane ghosts are present that contain all PMPs, but lack matrix proteins. A single ghost consists of one continuous membrane and represents a flattened membrane vesicle, which is folded into a spherical structure with a single opening toward the cytosol. In cross sections, the structure is seen as a double-membrane ring, occasionally displaying the opening. Upon reintroduction of Pex1, the space between the two membranes is filled by matrix proteins. Expansion of this space is paralleled with the formation of spherical extensions near the hole of the structure, which develop into peroxisomes, most likely by fission from the original structure.

Table S1. Yeast strains used in this work

Name	Genotype	Reference
WT	BY4742; Mata α ; his3 Δ 1; leu2 Δ 0; lys2 Δ 0; ura3 Δ 0	Euroscarf
<i>pex1</i>	BY4742; Mata α ; his3 Δ 1; leu2 Δ 0; lys2 Δ 0; ura3 Δ 0; YKL197c::kanMX4	Euroscarf
<i>pex6</i>	BY4742; Mata α ; his3 Δ 1; leu2 Δ 0; lys2 Δ 0; ura3 Δ 0; YNL329c::kanMX4	Euroscarf
WT Pex14-mGFP	BY4741; Mata α ; his3::PEX14-mGFP; leu2 Δ 0; met15 Δ 0 ura3 Δ 0	Invitrogen
WT Pex13-mGFP	BY4741; Mata α ; his3::PEX13-mGFP; leu2 Δ 0; met15 Δ 0 ura3 Δ 0	Invitrogen
FY1679 WT	FY1679 (S288C isogenic yeast strain): Mata α ; his3 Δ 200; leu2 Δ 1; ura3-52; trp1-63; GAL2	Euroscarf
TIR1	DF5; Mata α ; his3 Δ 200; leu2-3,2-112; lys2-801; trp1-1; ura3::ADH-AfTIR1-9myc	Morawska and Ulrich, 2013
<i>pex1</i> Pex14-mGFP Sec63-mRFP	BY4742; Mata α ; his3::PEX14-mGFP; leu2 Δ 0; lys2 Δ 0; URA3::Sec63-mRFP; YKL197c::kanMX4	This study
<i>pex6</i> Pex14-mGFP Sec63-mRFP	BY4742; Mata α ; his3::PEX14-mGFP; leu2 Δ 0; lys2 Δ 0; URA3::Sec63-mRFP; YNL329c::kanMX4	This study
<i>pex1</i> Pex14-mGFP	BY4742; Mata α ; his3::PEX14-mGFP; leu2 Δ 0; lys2 Δ 0; ura3 Δ 0; YKL197c::kanMX4	This study
<i>pex6</i> Pex14-mGFP	BY4742; Mata α ; his3::PEX14-mGFP; leu2 Δ 0; lys2 Δ 0; ura3 Δ 0; YNL329c::kanMX4	This study
WT Pex10-mCherry Pex14-mGFP	BY4742; Mata α ; his3::PEX14-mGFP; leu2 Δ 0; lys2 Δ 0; ura3 Δ 0; PEX10-mCHERRY::ZeocinR	This study
<i>pex1</i> Pex10-mCherry Pex14-mGFP	BY4742; Mata α ; his3::PEX14-mGFP; leu2 Δ 0; lys2 Δ 0; ura3 Δ 0; YKL197c::kanMX4; PEX10-mCHERRY::ZeocinR	This study
<i>pex6</i> Pex10-mCherry Pex14-mGFP	BY4742; Mata α ; his3::PEX14-mGFP; leu2 Δ 0; lys2 Δ 0; ura3 Δ 0; YNL329c::kanMX4; PEX10-mCHERRY::ZeocinR	This study
WT Pex10-mCherry Pex13-mGFP	BY4742; Mata α ; his3::PEX13-mGFP; leu2 Δ 0; lys2 Δ 0; ura3 Δ 0; PEX10-mCHERRY::ZeocinR	This study
<i>pex1</i> Pex10-mCherry Pex13-mGFP	BY4742; Mata α ; his3::PEX13-mGFP; leu2 Δ 0; lys2 Δ 0; ura3 Δ 0; YKL197c::kanMX4; PEX10-mCHERRY::ZeocinR	This study
<i>pex6</i> Pex10-mCherry Pex13-mGFP	BY4742; Mata α ; his3::PEX13-mGFP; leu2 Δ 0; lys2 Δ 0; ura3 Δ 0; YNL329c::kanMX4; PEX10-mCHERRY::ZeocinR	This study
WT Pex2-mCherry Pex14-mGFP	BY4742; Mata α ; his3::PEX14-mGFP; leu2 Δ 0; lys2 Δ 0; ura3 Δ 0; PEX2-mCHERRY::ZeocinR	This study
<i>pex1</i> Pex2-mCherry Pex14-mGFP	BY4742; Mata α ; his3::PEX14-mGFP; leu2 Δ 0; lys2 Δ 0; ura3 Δ 0; YKL197c::kanMX4; PEX2-mCHERRY::ZeocinR	This study
<i>pex6</i> Pex2-mCherry Pex14-mGFP	BY4742; Mata α ; his3::PEX14-mGFP; leu2 Δ 0; lys2 Δ 0; ura3 Δ 0; YNL329c::kanMX4; PEX2-mCHERRY::ZeocinR	This study
<i>pex1.pex6</i> Pex2-mCherry Pex14-mGFP	BY4742; Mata α ; his3::PEX14-mGFP; leu2 Δ 0; lys2 Δ 0; ura3 Δ 0; YNL329c::kanMX4; YKL197c::Hygromycin; PEX2-mCHERRY::ZeocinR	This study
WT Pex11-mCherry Pex14-mGFP	BY4742; Mata α ; his3::PEX14-mGFP; leu2 Δ 0; lys2 Δ 0; ura3 Δ 0; PEX11-mCHERRY::ZeocinR	This study
<i>pex1</i> Pex11-mCherry Pex14-mGFP	BY4742; Mata α ; his3::PEX14-mGFP; leu2 Δ 0; lys2 Δ 0; ura3 Δ 0; YKL197c::kanMX4; PEX11-mCHERRY::ZeocinR	This study
<i>pex6</i> Pex11-mCherry Pex14-mGFP	BY4742; Mata α ; his3::PEX14-mGFP; leu2 Δ 0; lys2 Δ 0; ura3 Δ 0; YNL329c::kanMX4; PEX11-mCHERRY::ZeocinR	This study
<i>pex1 atg1</i> Pex10-mCherry Pex14-mGFP	BY4742; Mata α ; his3::PEX14-mGFP; leu2 Δ 0; lys2 Δ 0; ura3 Δ 0; YKL197c::kanMX4; YKL180w::NatR; PEX10-mCHERRY::ZeocinR	This study
<i>pex1</i> Pex11-HA	BY4742; Mata α ; his3 Δ 1; leu2 Δ 0; lys2 Δ 0; ura3 Δ 0; YKL197c::kanMX4; PEX11-HA::ZeocinR	This study
<i>pex1</i> Pex2-6HA	BY4742; Mata α ; his3 Δ 1; leu2 Δ 0; lys2 Δ 0; ura3 Δ 0; YKL197c::kanMX4; PEX2-6HA::Hygromycin	This study
FY1679 <i>pex1</i>	FY1679 (S288C isogenic yeast strain): Mata α ; his3 Δ 200; leu2D1; ura3-52; trp1-63; GAL2; YKL197c::kanMX4	This study
FY1679 <i>pex6</i>	FY1679 (S288C isogenic yeast strain): Mata α ; his3 Δ 200; leu2D1; ura3-52; trp1-63; GAL2; YNL329c::kanMX4	This study
FY1679 <i>pex6</i> Pex14-mGFP Sec63-mRFP	FY1679 (S288C isogenic yeast strain): Mata α ; his3::PEX14-mGFP; leu2D1; URA3::Sec63-mRFP; trp1-63; GAL2; YNL329c::kanMX4	This study
FY1679 WT Pex2-mCherry Pex14-mGFP	FY1679 (S288C isogenic yeast strain): Mata α ; his3::PEX14-mGFP; leu2D1; ura3-52; trp1-63; GAL2; PEX2-mCHERRY::ZeocinR	This study
FY1679 <i>pex1</i> Pex2-mCherry Pex14-mGFP	FY1679 (S288C isogenic yeast strain): Mata α ; his3::PEX14-mGFP; leu2D1; ura3-52; trp1-63; GAL2; YKL197c::kanMX4; PEX2-mCHERRY::ZeocinR	This study
FY1679 <i>pex6</i> Pex2-mCherry Pex14-mGFP	FY1679 (S288C isogenic yeast strain): Mata α ; his3::PEX14-mGFP; leu2D1; ura3-52; trp1-63; GAL2; YNL329c::kanMX4; PEX2-mCHERRY::ZeocinR	This study
PEX1-AID*-6HA	DF5; Mata α ; his3::PEX14-mGFP; leu2::DsRed-SKL; lys2-801; trp1-1; ura3::ADH-AfTIR1-9myc; Pex1-AID*-6HA::hygromycin	This study

Table S2. Plasmids used in this work

Name	Genotype	Reference
pAG25	pFA6, nourseothricin ^R , amp ^R	Goldstein and McCusker, 1999
pANL31	pHIPZ-eGFP fusionator, amp ^R	Leao-Helder et al., 2003
pCDNA3.1 mCherry	Plasmid containing mCherry, amp ^R	Shaner et al., 2004
pHIPX7 GFP-SKL	pHIPX, TEFl promoter, kan ^R , LEU2	Krikken et al., 2009
pHyg-AID*-6HA	pSM409 Auxin-inducible degron, amp ^R , hph ^R	Morawska and Ulrich, 2013
pHyg-Pex1-AID*-6HA	pSM409 Pex1-Auxin-inducible degron, amp ^R , hph ^R	This study
pHyg-Pex2-6HA	pSM409 6xHA, amp ^R , hph ^R	This study
pRSA01	pHIPZ4 mCherry fusionator, amp ^R , zeo ^R	This study
pSM1960	pSM1960 Sec63-mRFP, amp ^R , URA3	Metzger et al., 2008
pPtdh3 GFP-SKL	pHIPX, TDH3 promoter, kan ^R , LEU2	This study
pPtdh3 DsRed-SKL	pHIPX, TDH3 promoter, kan ^R , LEU2	This study

Table S3. Primers used in this work

Name	Sequence
pKEK036	5'-ATGAGATCAGTGTCCCTGACTGGCAAATGGACAGGTCGA-3'
pKEK038	5'-CCTTGTGCAGACAACACTGTCAACCACAGGAAATCTGGTCTGCGGCAAGTGAGCAAGGGCGAGGAGGA-3'
pKEK039	5'-TATTTCCCTTCGCGTTTTACTTCTCTGCTCAGACGTCTGCGTTATTTGTGAATTGTAATACGACTCA-3'
pKEK041	5'-GTTCCGGGTAACAATCAG-3'
pKEK123	5'-CGGGCACATGGAAATCACT-3'
pKEK124	5'-TATGCTTGCCTGTGGCGTTA-3'
pKEK127	5'-CCGAAGCCTTGCTCATCTTG-3'
pKEK128	5'-GTTGCCGGCTTAGCTCTGTA-3'
pKEK131	5'-CTAAAGGAACAAAAGCTGG-3'
pKEK136	5'-GGTTATCCATACGATGTTCTGACTATGCTAGCGGTTAGGTCGACTCTAGAGGATC-3'
pKEK137	5'-CCGGTGTGTCACATCTATCCTGGTATGCAAGACATGTGAAAGCTACAGGTTATCCATACGATGTTCC-3'
pKEK138	5'-ATAAATTATAAAGAAGGGTCGAATCAAACATAAGCGGAGAATAGCCAAATCTAAAGGGAACAAAAGCTGG-3'
pKEK164	5'-CTGGTGGAAAGTGAGGACAAAG-3'
pKEK165	5'-GTACTTCGTCTCGTTTCTGCTG-3'
pKEK178	5'-CAGCTGAAGCTTGTTAAATATGCGGATTGTGAAACATTGCACG-3'
pKEK179	5'-GCACCGTCGACCATAAGGGAGAGTCGCGTACCAATGTCGA-3'
pKEK183	5'-AATATGAATATAGTATACACATATATAGAGATACAAGCGAGGAAACGGGGTGAATTGTAATACGACTCA-3'
pKEK184	5'-CCGGTGTGTCACATCTATCCTTGGTATGCAAGACATGTGAAAGCTACAGTGAGCAAGGGCGAGGAGGA-3'
pKEK184	5'-AATTATAAAGAAGGGTCGAATCAAACATAAGCGGAGAATAGCCAAATAAAGTGAATTGTAATACGACTCA-3'
pKEK202	5'-GGTCGACGGTGCAGGCTGTGTTATGTGCGCGACGAGG-3'
pKEK203	5'-GAACATCGTATGGGATTTCCCGTACACTGGTGGGCGGTCAGT-3'
pKEK229	5'-ATGTGTTGCATACCTCCAAAGAAAGCGATTATAGTAACATTAATGCATGATGTGACTGTGCGCCCG-3'
pKEK230	5'-GGGACATATATTTACAAATTTACCTATACGCTCTGAGTTGATATTACTTACGCATAGGCCACTAGTGGATCTGATATCA-3'
pTER208	5'-ACCCATATTTCAAATCTCTTTTACAACACCAGACGAGAAAATAAGAAACCAGATCTGTTTACGCTTGCCTT-3'
pTER209	5'-ATAGCAGGTCATTTGACTTAATAAGAAAACCATATTATGCATCACTTAAATTCGAGCTCGTTTTTCGACA-3'
TDH3_NotI.F	5'-GCATCAGCGCCGCCACGCTTTTTTCAGTTCGAGT-3'
TDH3_BamHI.R	5'-GCGCGGGATCCTTTGTTGTTTATGTGTGTTTAT-3'
RSA10fw	5'-GAAGATCTATGGTGAGCAAGGGCGAGGAG-3'
RSA11rev	5'-GCGTGTCGACTTACTTGTACAGCTCGTCCATGCC-3'

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