

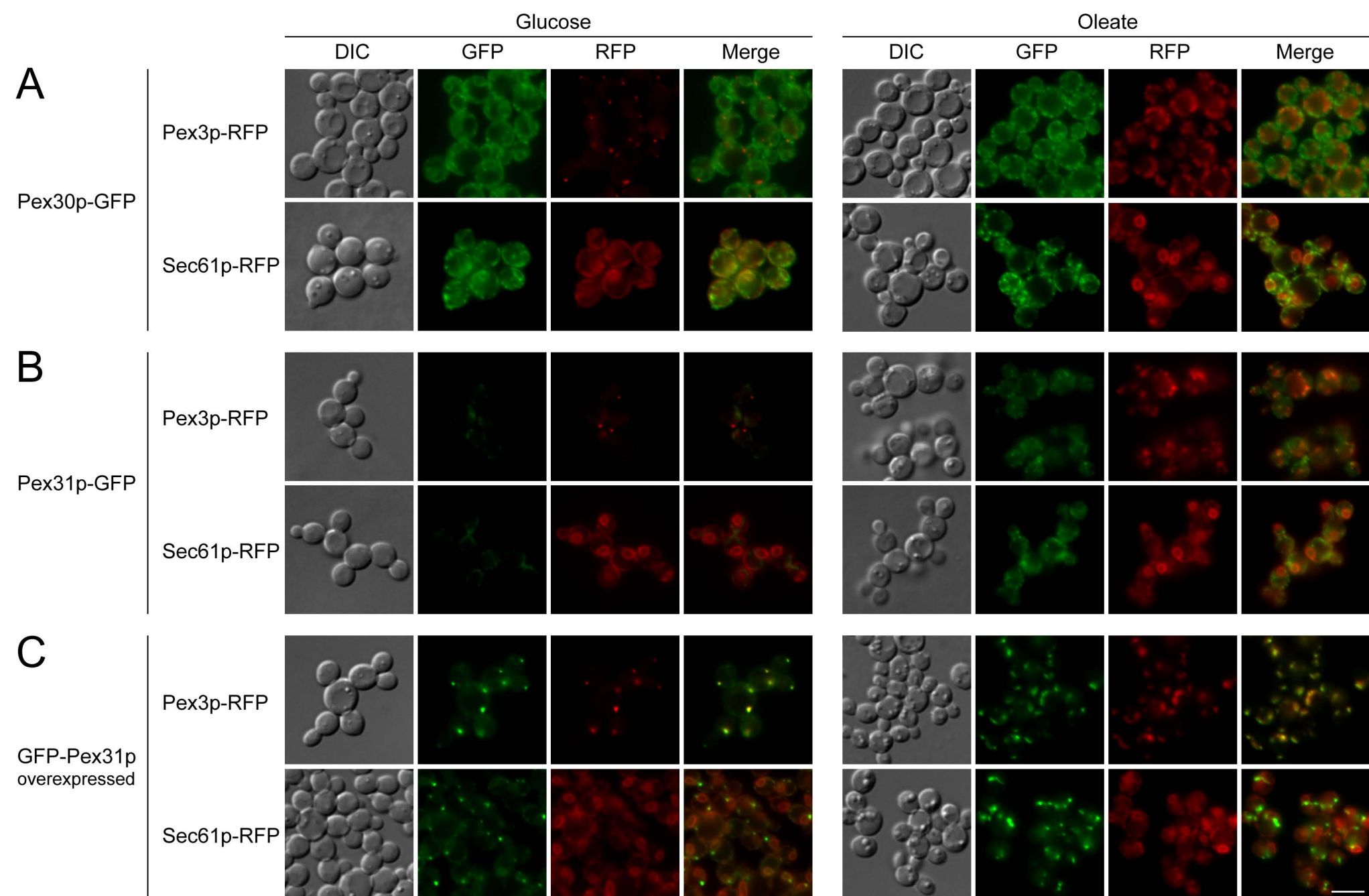
Supplementary Table I. *P. pastoris* strains used in supplementary data

Strain	Genotype	Source
PPY12	<i>arg4, his4</i>	(Gould <i>et al.</i> , 1992)
SMY169	<i>PEX31::PEX31-HA(Zeo^R), arg4, his4</i>	This study
SMY174	<i>HIS4::P_{GAP}-GFP-PEX31, arg4</i>	This study
SMY248	<i>PEX::PEX11-HA(Zeo^R), HIS4::P_{GAP}-GFP-PEX31, arg4</i>	This study
SMY278	<i>pex11Δ::Zeo^R, arg4, his4</i>	This study
SMY371	<i>pex31Δ::Zeo^R, HIS4::P_{GAP}-PEX31-HA, arg4</i>	This study
SMY393	<i>PEX30::PEX30-GFP(ARG4), SEC61::SEC61-RFP(Hyg^R), his4</i>	This study
SMY404	<i>PEX31::PEX31-GFP(ARG4), SEC61::SEC61-RFP(Hyg^R), his4</i>	This study
SMY405	<i>PEX31::PEX31-GFP(ARG4), HIS4::PEX3-RFP</i>	This study
SMY406	<i>PEX30::PEX30-GFP(ARG4), HIS4::PEX3-RFP</i>	This study
SMY411	<i>HIS4::P_{GAP}-GFP-PEX31, SEC61::SEC61-RFP(Hyg^R), arg4</i>	This study
SMY419	<i>HIS4::P_{GAP}-GFP-PEX31, ARG4::PEX3-RFP</i>	This study

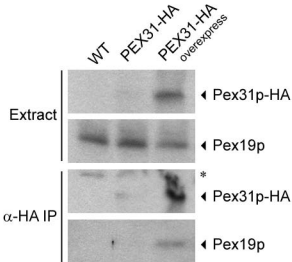
Supplementary Figure 1. **Localization of Pex30p and Pex31p in glucose and oleate medium.** (A) Pex30p-GFP expressed from a genomically tagged locus was colocalized with a peroxisome marker, Pex3p-RFP (SMY406), or an ER marker, Sec61p-RFP (SMY393), in glucose- or oleate-grown cells. (B) Pex31p-GFP expressed from its genomically tagged locus was colocalized with Pex3p-RFP (SMY405) or Sec61p-RFP (SMY404) in glucose- or oleate-grown cells. (C) Pex31p-GFP ectopically expressed from the *GAP* promoter was colocalized with Pex3p-RFP (SMY419) or Sec61p-RFP (SMY411) in glucose- or oleate-grown cells. DIC, differential interference contrast. Scale bar, 5 μ m.

Supplementary Figure 2. **Pex31p interacts with Pex19p.** Wild-type (WT, PPY12), genomically tagged *PEX31-HA* (SMY169) and ectopically overexpressed *PEX31-HA* (SMY371) cells were induced in oleate medium and analyzed by immunoprecipitation (IP) with antibody against HA. Pex31p-HA and Pex19p were visualized by western blotting. Asterisk, IgG light chain.

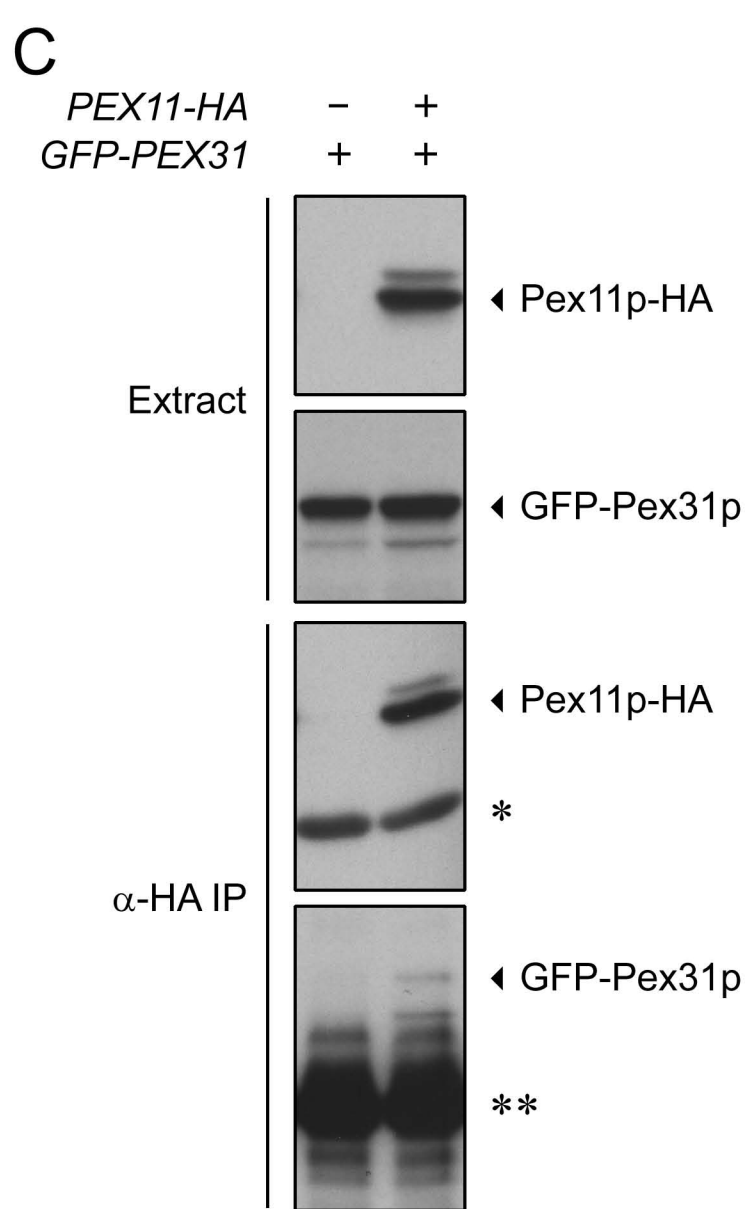
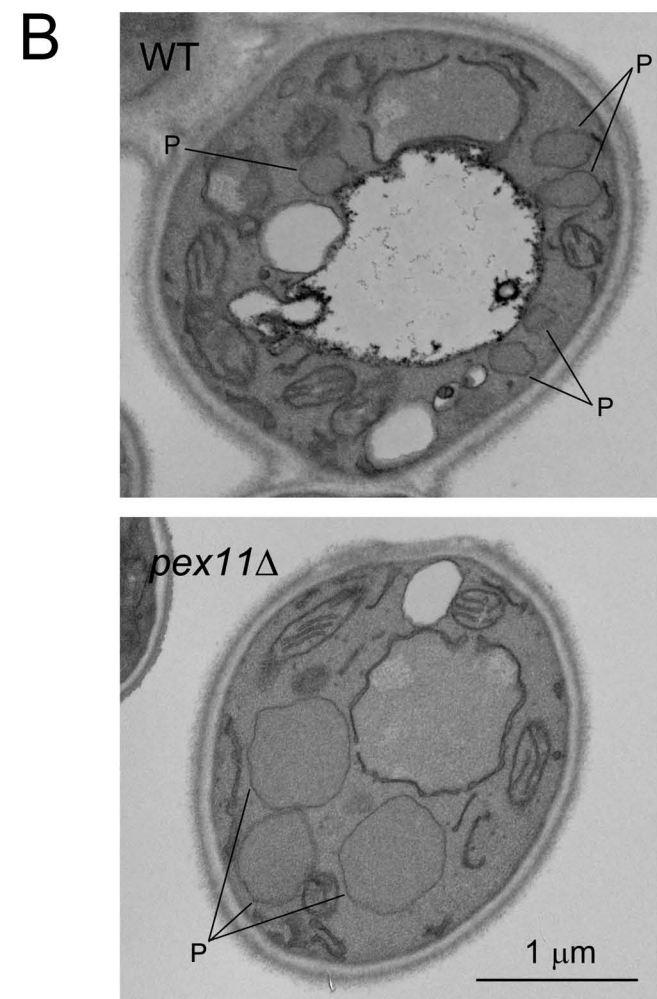
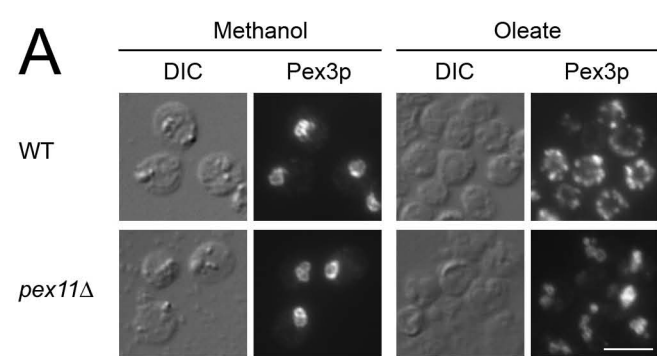
Supplementary Figure 3. **Pex11p is required for normal oleate-induced peroxisomes and interacts with Pex31p.** (A) Wild-type (WT, PPY12) and *pex11 Δ* (SMY278) cells were induced in methanol or oleate medium overnight for peroxisome proliferation and analyzed by indirect immunofluorescence after labeling with anti-Pex3p antibody and Alexa Fluor 488-conjugated secondary antibody. DIC, differential interference contrast. Scale bar, 5 μ m. (B) Wild-type and *pex11 Δ* cells were induced in oleate medium overnight and analyzed by EM at a magnification of 17,000. P, peroxisome. (C) *GFP-PEX31* was expressed ectopically in WT cells (SMY174) or in cells whose endogenous *PEX11* was tagged with HA (SMY248). Cells were induced in oleate medium overnight and analyzed by immunoprecipitation (IP) with antibody against HA. Pex30p-GFP, Pex31p-GFP and Pex11p-HA were visualized by western blotting. Asterisk, IgG light chain; double asterisk, IgG heavy chain.



Supplementary Figure 1



Supplementary Figure 2



Supplementary Figure 3