Sargent et al., http://www.jcb.org/cgi/content/full/jcb.201511034/DC1

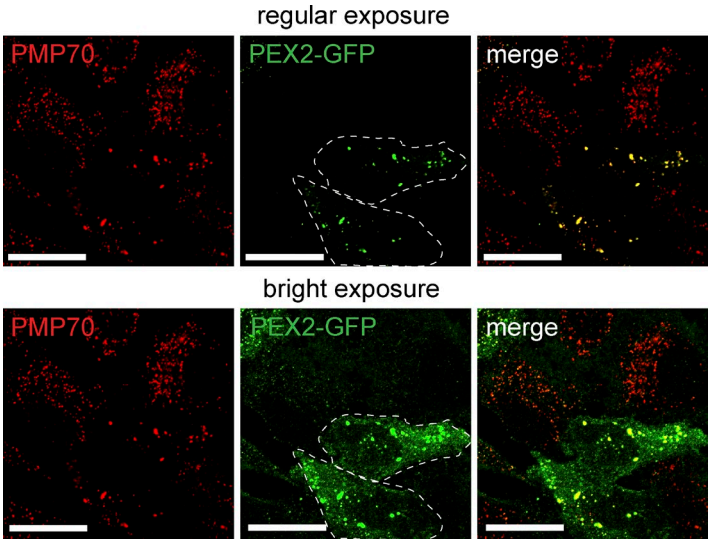

Figure S1. **PEX2-GFP–expressing cells contain fewer peroxisomes.** HeLa cells were transfected with PEX2-GFP. 48 h later, the cells were fixed and stained for the peroxisomal membrane protein PMP70. Bottom panels are the same as top panels except for the GFP panel, which has been brightened to show outline of the cells to indicate the decrease in peroxisomes is not caused changes in cell morphology. Bars, 20 µm. White lines represent the boundary of the cell.

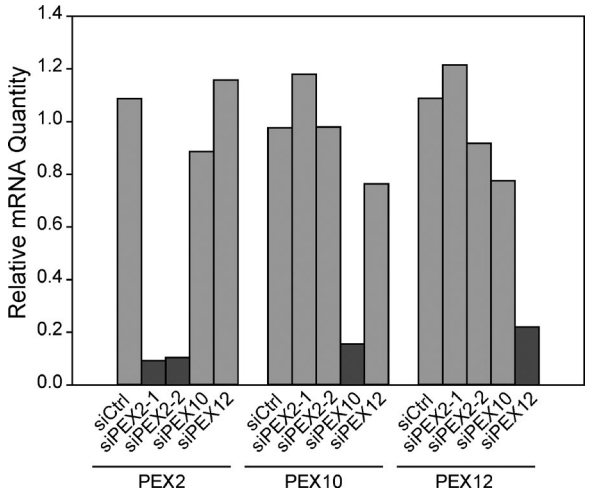

Figure S2. **Quantitative PCR verification of siRNA knockdown.** HeLa cells were mock transfected, transfected with nontargeting siRNA (siCtrl), or transfected with siRNA against PEX2, PEX10, or PEX12 for 72 h. A cDNA library was created from the cell lysates, and the relative quantities of PEX2, PEX10, and PEX12 transcripts were determined by quantitative PCR. Light gray bars denote off-target effects and dark gray bars denote expected knockdowns.

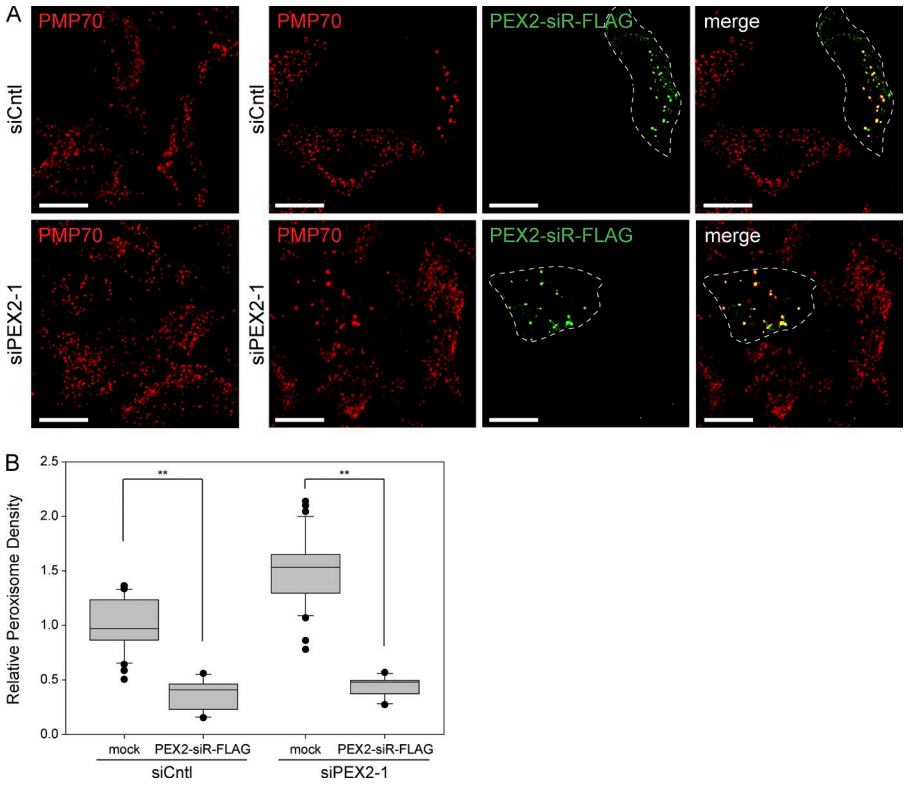

Figure S3. **siPEX2 is complemented by PEX2-siR-FLAG.** (A) HeLa cells were transfected with siRNA against PEX2 (siPEX2-1) for 72 h. 1 d before fixation, cells were complemented with a siPEX2-1–resistant PEX2-FLAG construct. Bars, 20 µm. (B) Box plot of the relative peroxisome density in 150 cells as shown in A. Boxes show the 25th, 50th, and 75th percentiles, and lines show one-way standard deviations. Points represent all cells that did not fall within one standard deviation. **, P < 0.01.

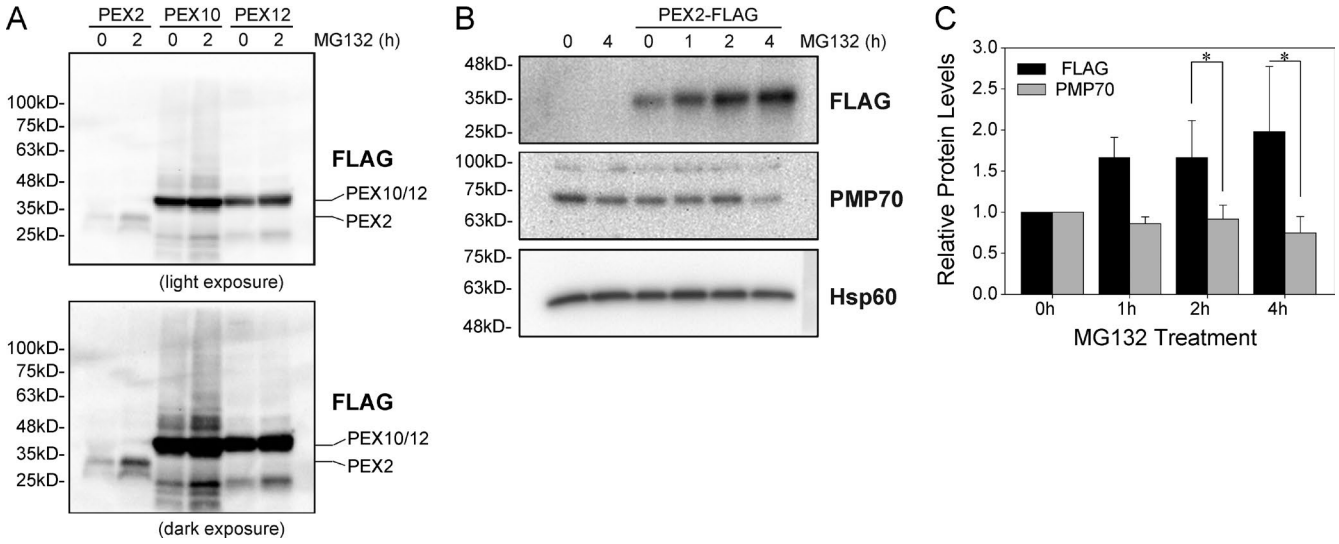

Figure S4. **PEX2 is stabilized by the proteasomal inhibitor MG132.** (A) HeLa cells were transfected with PEX2-FLAG, PEX10-FLAG, or PEX12-FLAG. 2 h before lysis, cells were treated with the proteasomal inhibitor MG132 (10 µM) or with DMSO as a vehicle control. Cell lysates were analyzed by blotting with an antibody against the FLAG epitope. (B) HeLa cells were mock transfected or were transfected with PEX2-FLAG. 24 h after transfection and 4 h before lysis, cells were treated with the proteasomal inhibitor MG132 (10 µM) or with DMSO as a vehicle control. Cell lysates were analyzed by blotting with antibodies against the FLAG epitope, PMP70, or Hsp60 as a loading control. (C) Bar graphs represent the PMP70/Hsp60 ratio normalized to 0 h loading control with means and stardard devations shown for three independent experiments. *n* = 3. *, P < 0.05.

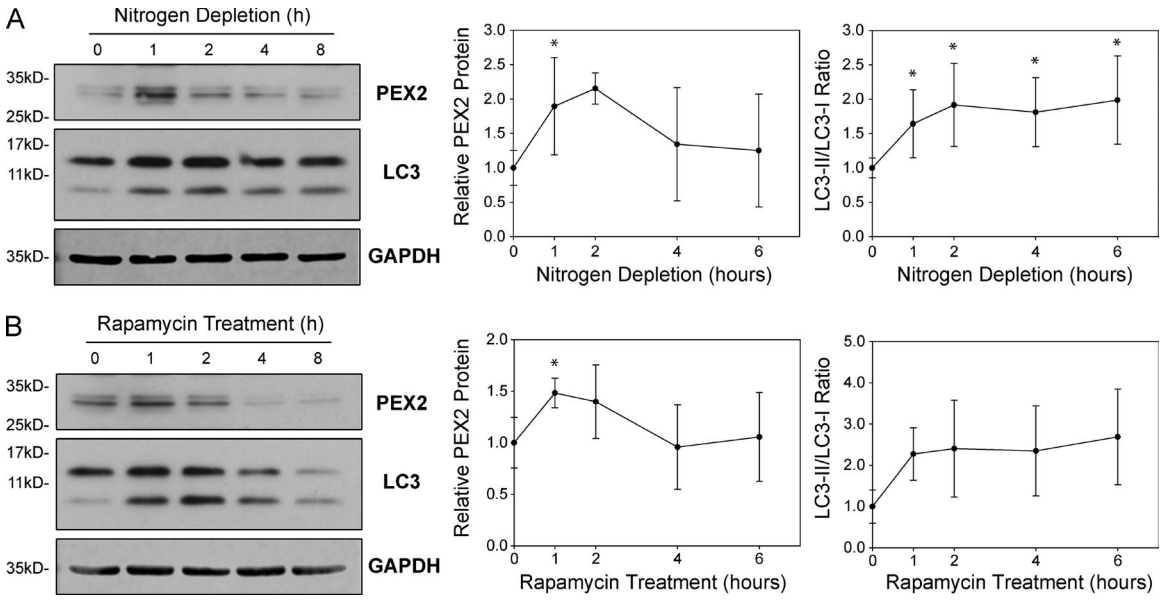

Figure S5. **PEX2 regulation occurs on a timescale similar to LC3 conversion.** (A) HeLa cells were depleted of amino acids for 0 to 6 h as indicated in HBSS and analyzed by immunoblotting for PEX2, LC3, and GAPDH. Line graphs show the PEX2/GAPDH ratio and the LC3-II/LC3-I ratio normalized to 0 h. (B) HeLa cells were treated with 5 μM rapamycin for 0 to 6 h as indicated to inhibit mTOR and analyzed by immunoblotting for PEX2, LC3, and GAPDH. Line graphs show the amount of PEX2/GAPDH ratio and the LC3-II/LC3-I ratio normalized to 0 h. Means and stardard deviations are shown. $n = 3$. *, $P < 0.05$.