Supplemental material

Romano et al., https://doi.org/10.1083/jcb.201901152

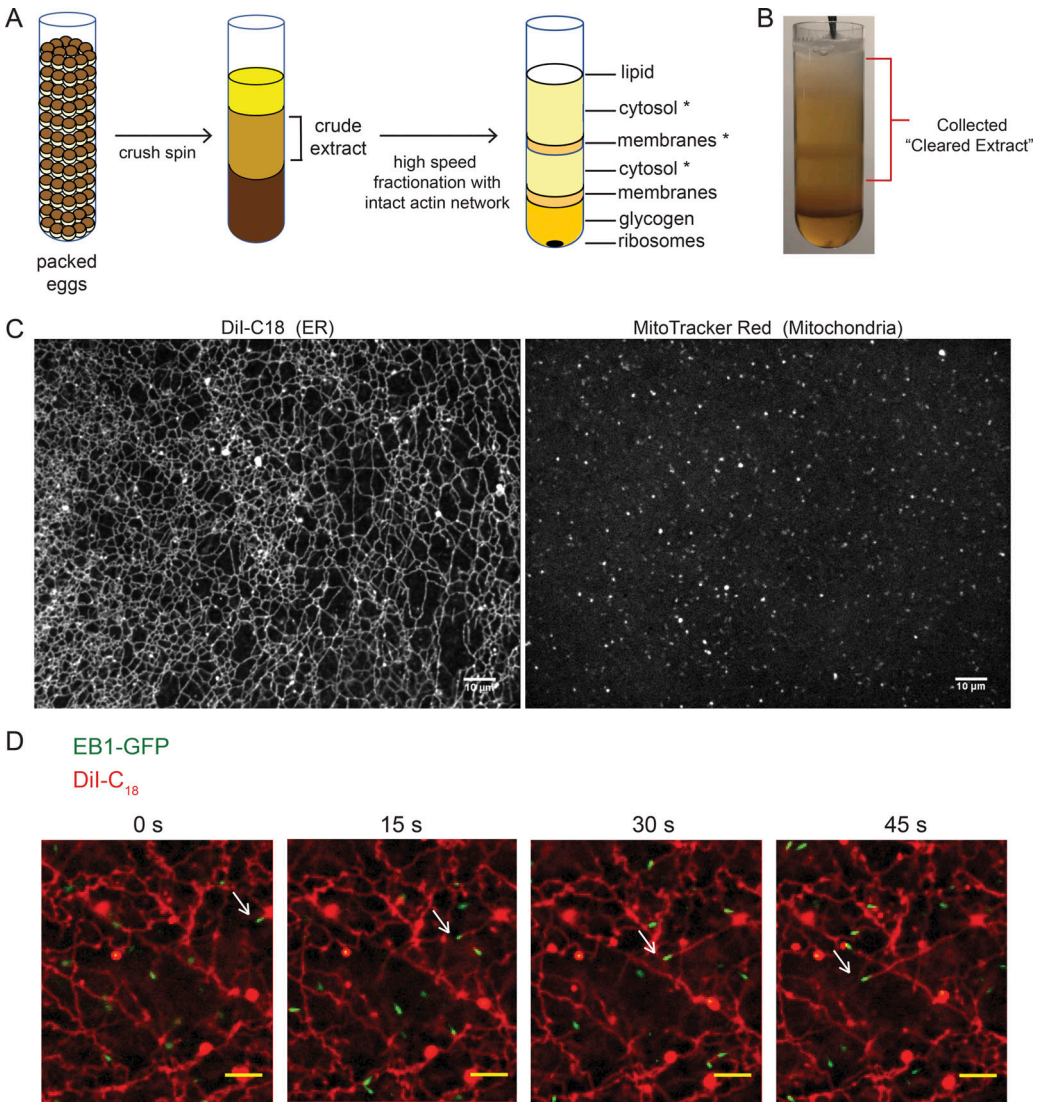


Figure S1. **The cleared *Xenopus* egg extract contains ER and mitochondria and maintains microtubule and ER dynamics. (A)** Scheme outlining the procedure for generating "cleared egg extract." The fractions indicated by a star were collected and combined. **(B)** Photograph of a tube after the last centrifugation step. The combined material is called cleared extract. **(C)** The ER was visualized by incubation of cleared extract with the hydrophobic fluorescent dye $DiIC_{18}$, dilution with fresh extract, and imaging in a spinning-disk confocal microscope. Mitochondria were visualized by incubation of the extract with 250 nM MitoTracker Red for 1 h before imaging. Bars, 10 µm. **(D)** Cleared *Xenopus* egg extract was incubated with the hydrophobic fluorescent dye $DiIC_{18}$ to stain the ER and diluted with fresh extract containing a GFP fusion of the microtubule plus-end tracking protein EB1. The sample was imaged in two fluorescence channels in a spinning-disk confocal microscope, and the images were superimposed. Shown are images at different time points. The arrows indicate the movement of a microtubule plus-end (green) together with the attached ER tubule (red). Bars, 5 µm.

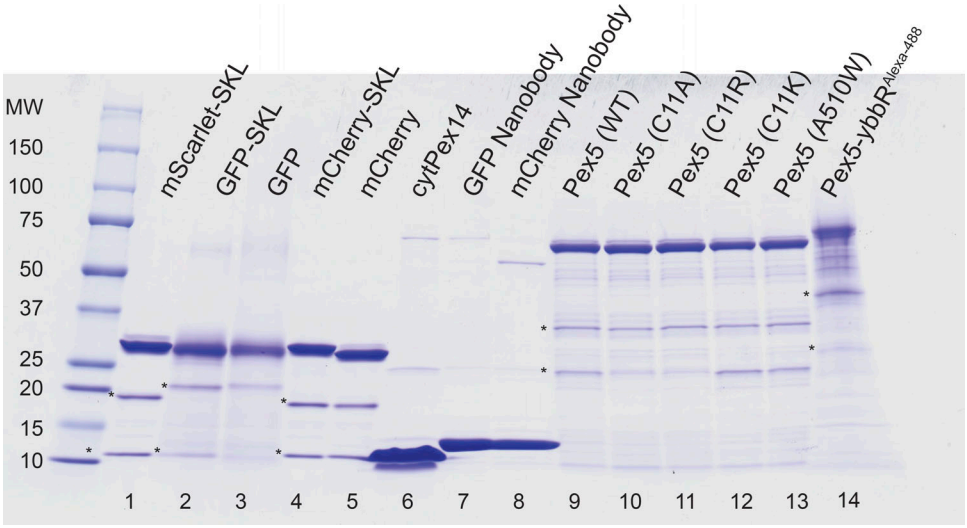


Figure S2. **SDS-PAGE of purified proteins.** The indicated purified proteins were separated in a 4–20% SDS-PAGE gel. The gel was stained with Coomassie blue. cytPex4, cytosolic fragment of Pex14. Asterisks indicate bands that likely originate from proteolysis.

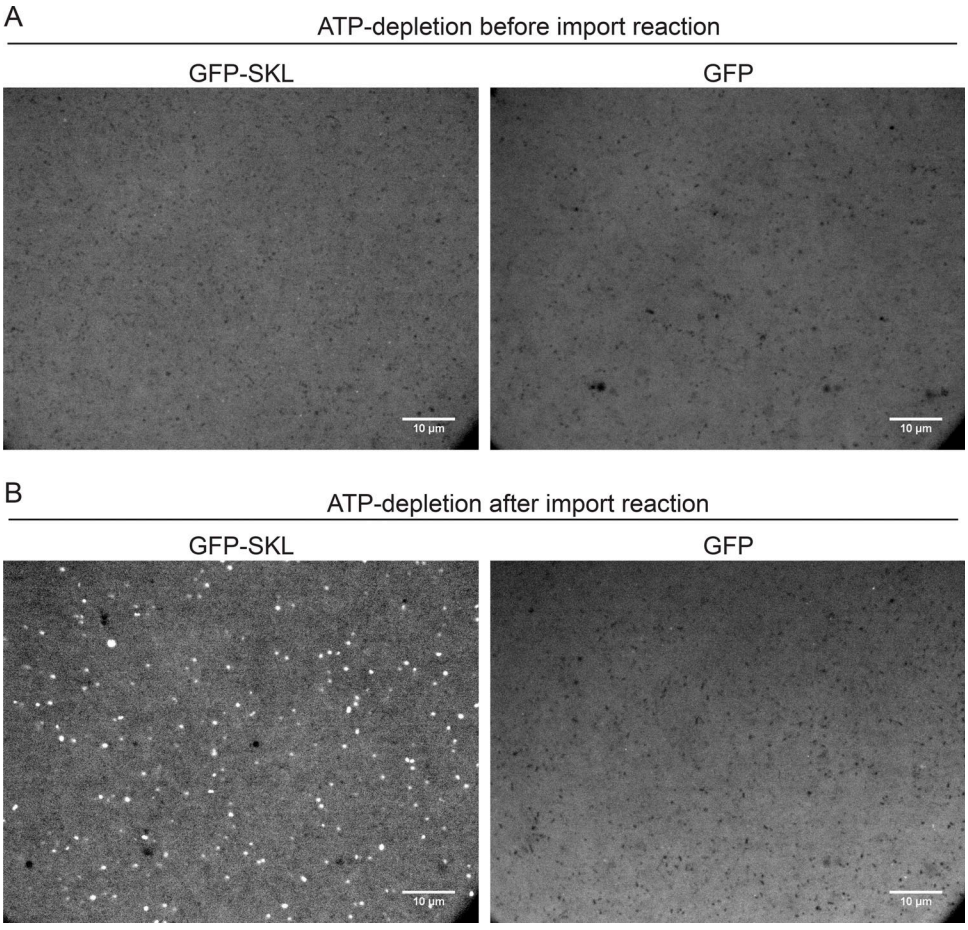


Figure S3. **ATP depletion blocks peroxisome import. (A)** Crude extract was treated with 20 µM rotenone, 20 µM antimycin, and 4 mM 2-deoxyglucose for 60 min, and then 0.4 µM GFP-SKL was added for 90 min, before imaging in a confocal microscope. The experiment was also performed with GFP lacking SKL (right panel). **(B)** As in A, but ATP depletion was done after 90 min of import reaction. Bars, 10 µm.

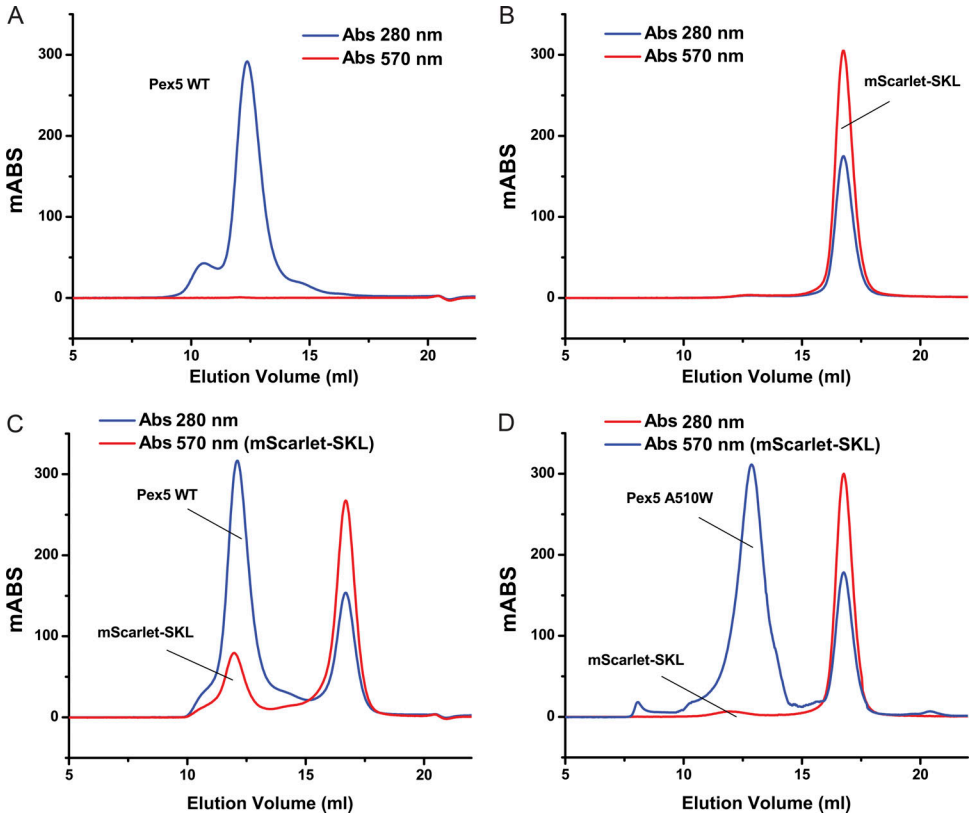


Figure S4. **Interaction of WT and mutant Pex5 with mScarlet-SKL. (A)** WT Pex5 (70 µM) was subjected to gel filtration on a Superdex 200 10/300 column in 30 mM Hepes, pH 7.8, and 150 mM KCl. The absorbance at 280 nm (Pex5) and at 570 nm was monitored. **(B)** mScarlet-SKL (70 µM) was subjected to gel filtration and its elution followed by absorbance at 280 and 570 nm. **(C)** WT Pex5 and mScarlet-SKL (both at 70 µM) were preincubated for 20 min at room temperature and then subjected to gel filtration. The absorbance at 280 and 570 nm was monitored. **(D)** As in C, but WT Pex5 was replaced with the Pex5 A510W mutant, defective in SKL binding.

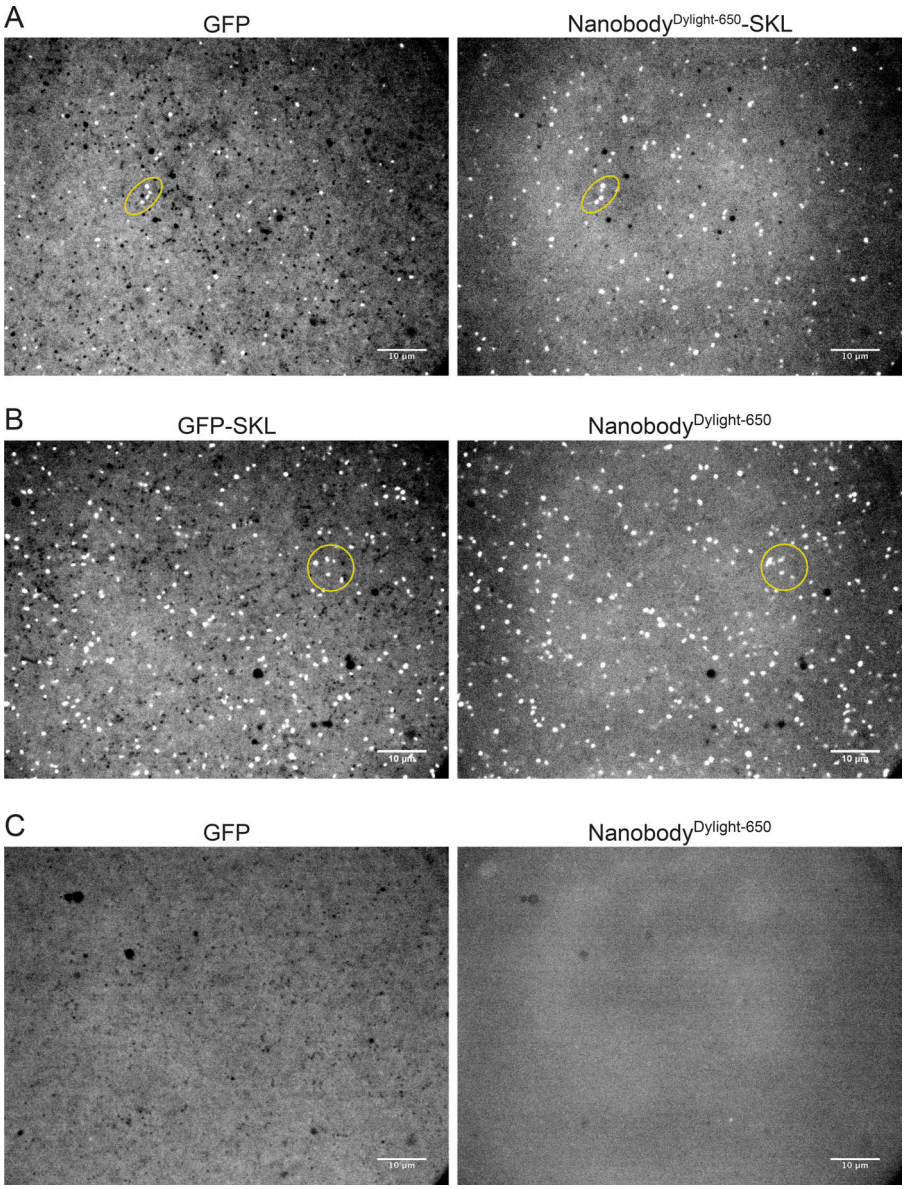
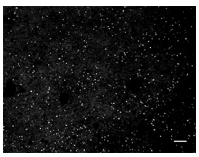


Figure S5. **Piggyback peroxisome import. (A)** A 1:1 complex between GFP lacking SKL and Dylight-650–labeled nanobody-SKL ("enhancer" nanobody [Kirchhofer et al., 2010], which does not quench GFP fluorescence) was formed and incubated at 0.4 mM with crude extract for 2 h. The sample was imaged in both the GFP and Dylight-650 fluorescence channels. **(B)** As in A, but with a complex of GFP-SKL and nanobody lacking SKL. **(C)** As in A, but with both proteins lacking SKL. These experiments were performed at least three times. Bars, 10 µm.



Video 1. **Mobility of peroxisomes.** Cleared egg extract was incubated with 0.6 µM purified mScarlet-SKL for 2 h at 18°C. The sample was visualized in real time with a spinning-disk confocal microscope. The sample was continuously illuminated, and five images were acquired per second. The video is displayed at five frames per second. Bar, 10 µm.

Reference

Kirchhofer, A., J. Helma, K. Schmidthals, C. Frauer, S. Cui, A. Karcher, M. Pellis, S. Muyldermans, C.S. Casas-Delucchi, M.C. Cardoso, et al. 2010. Modulation of protein properties in living cells using nanobodies. *Nat. Struct. Mol. Biol.* 17:133–138. https://doi.org/10.1038/nsmb.1727