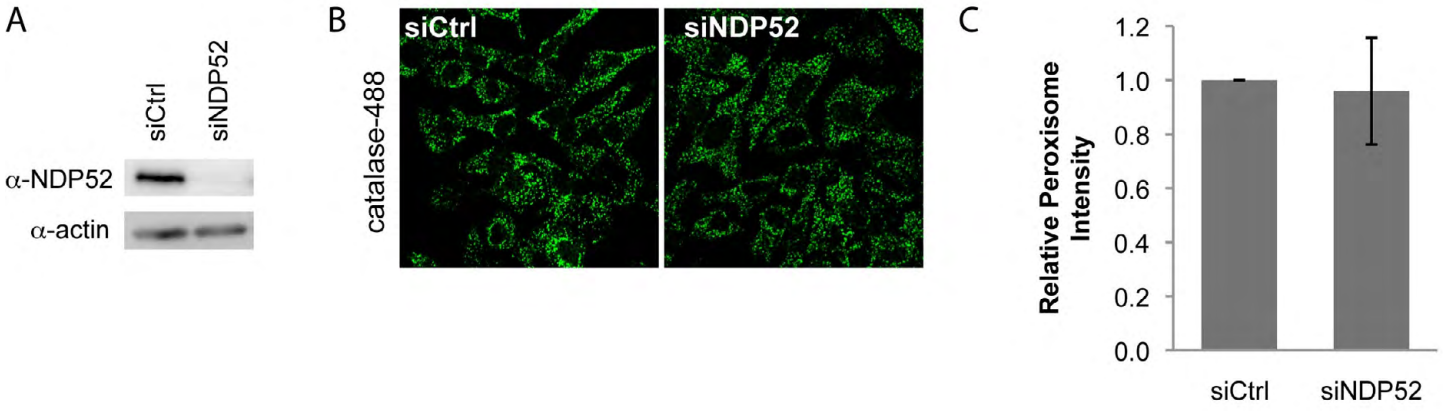


Fig. S1. NDP52 is not required for the endogenous turnover of peroxisomes. HeLa cells treated with non-targeting siRNA (siCtrl) or siRNA against NDP52. **(A)** Immunoblot analysis of cells treated with either control (siCtrl) or NDP52 siRNA (siNDP52) as indicated. The same blot was probed with antibodies against NDP52 and actin. **(B)** Representative immunofluorescence image of HeLa cells probed with anti-Catalase antibody and visualized using AlexaFluor 488 secondary antibody. **(C)** The normalized mean fluorescence intensity of catalase levels in cells treated with control or NDP52 siRNA. siRNA treated cells were fixed and immunostained for endogenous catalase. The mean catalase fluorescence intensity of at least 100 cells was measured for both control siRNA and NDP52 siRNA. The average of three independent sets of experiments normalized against the control non-targeting siRNA (siCtrl) is shown in the bar graph. Error bars represent the standard deviation.

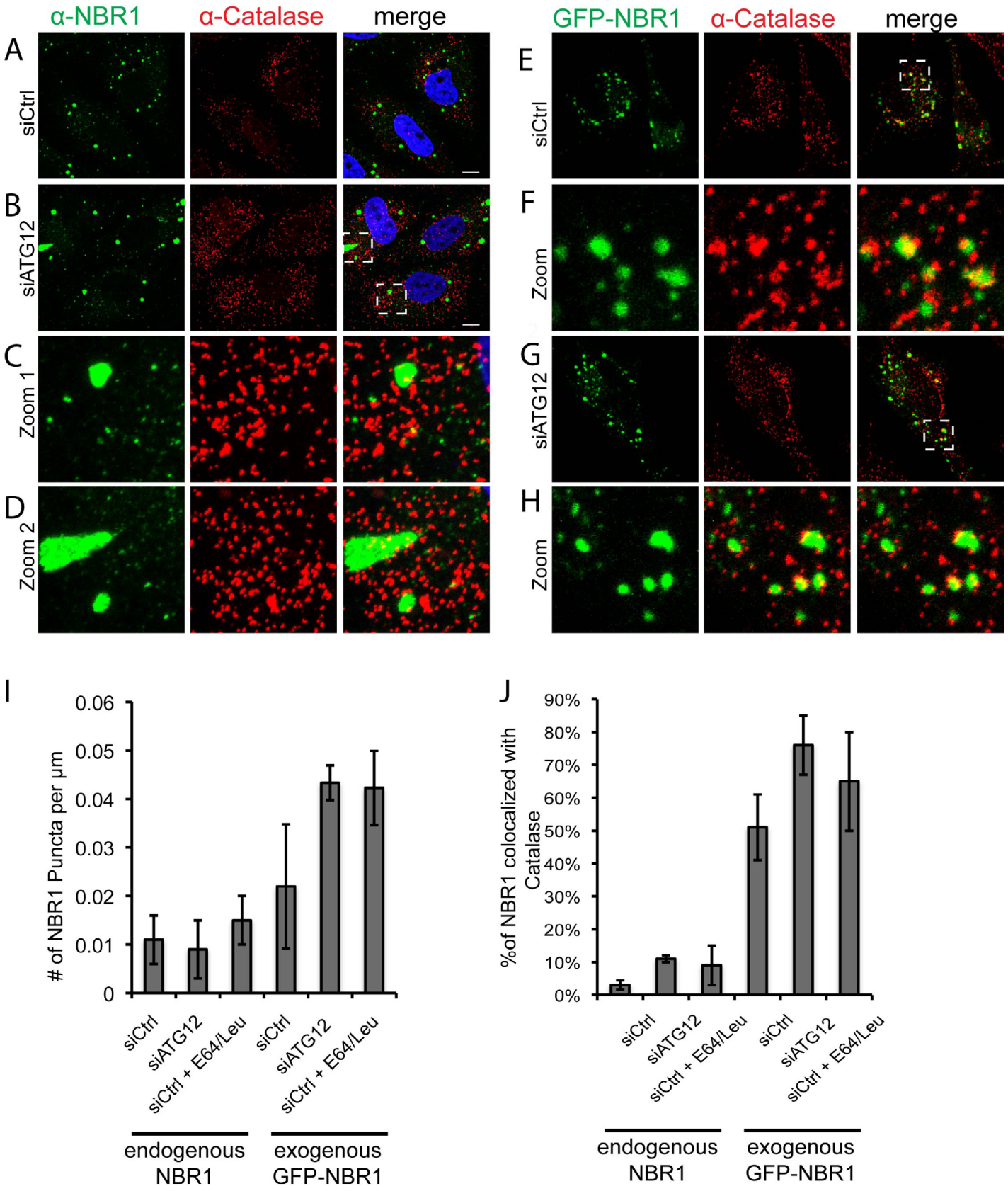


Fig. S2. NBR1/peroxisome cluster formation is dependent on NBR1 expression levels. HeLa cells treated with either non-targeting siRNA (siCtrl) **(A)**, or siRNA against ATG12 (siATG12) **(B)** were treated with lysosomal protease inhibitors E-64 and leupeptin. They were fixed and immunostained for endogenous NBR1 and catalase. **(C-D)** enlargement of the regions outlined by the box in (B). **(E-H)** HeLa cells treated with either siCtrl or siATG12 were transfected with Cherry-NBR1 and Lamp1-GFP, and treated with lysosomal protease inhibitors E-64 and leupeptin. Also shown is the magnification of the white box in the merge. **(I)** Quantification of the number of NBR1 punctate structures in cells treated with either siCtrl or siATG12. As indicated some were also treated with E64/leupeptin. Both endogenous NBR1 and exogenously expressed GFP-NBR1 quantifications are shown. **(J)** Quantification of the % of NBR1 colocalized with catalase. Scale bars, 10 µm.

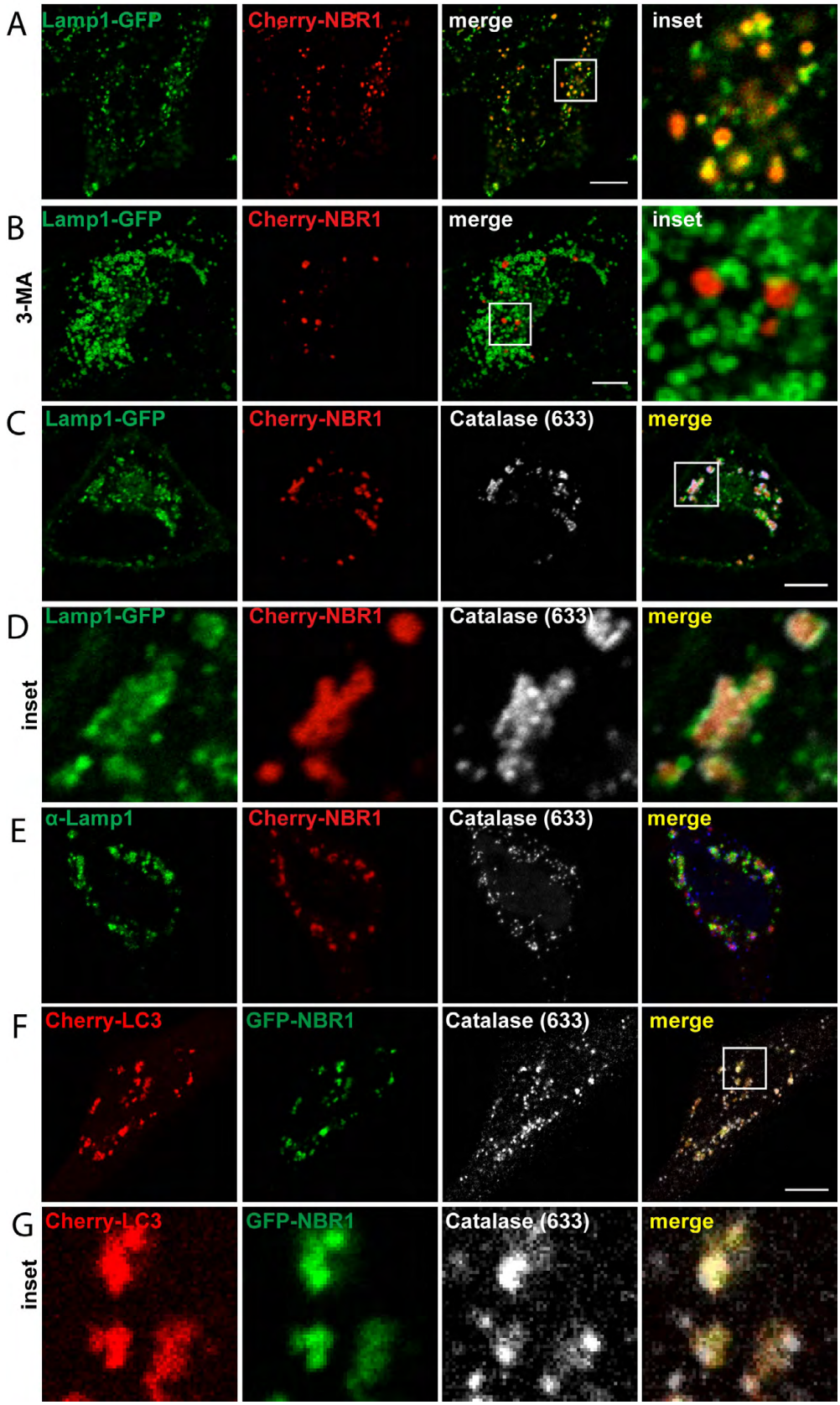
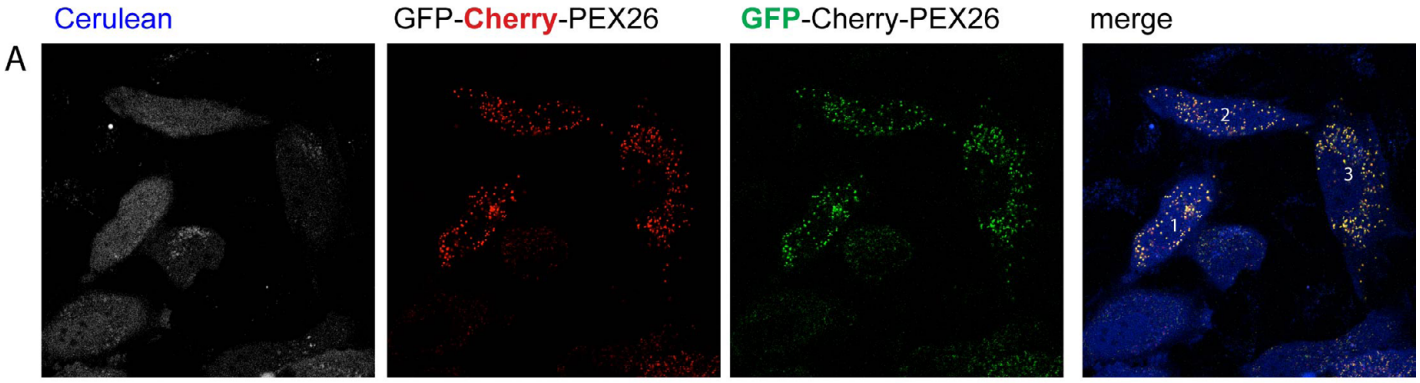
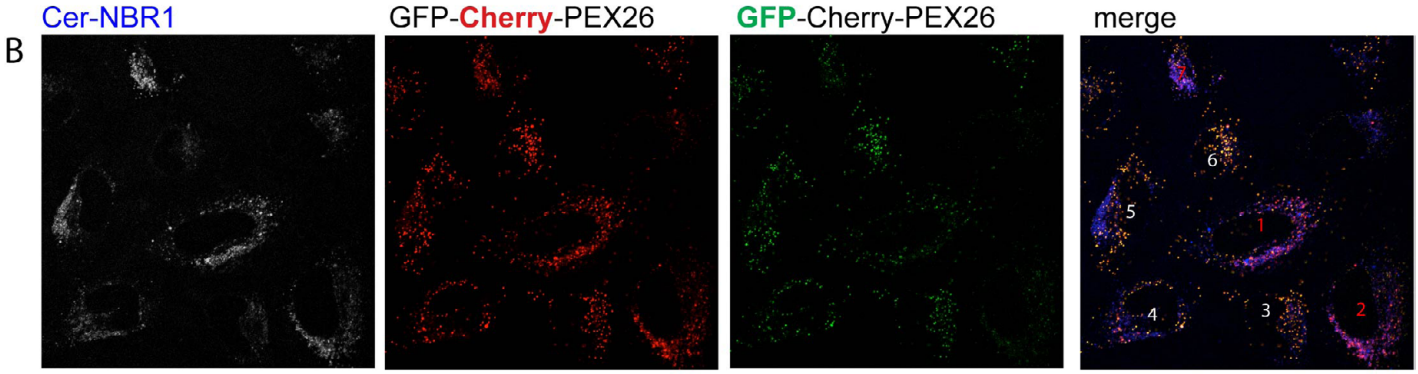


Fig. S3. NBR1/peroxisome clusters are localized to lysosomes. (A) HeLa cells transfected with Cherry-NBR1 and Lamp1-GFP, and treated with lysosomal protease inhibitors E-64 and leupeptin. Also shown is the magnification of the white box in the merge. **(B)** Same as (A) except the cells were also treated with 3-MA. **(C)** HeLa cells were transfected with Cherry-NBR1 and Lamp1-GFP, and treated with lysosomal inhibitors. The cells were fixed and immunostained for endogenous catalase. **(D)** Magnification of an area in (C) indicated by the white box. **(E)** HeLa cells expressing Cherry-NBR1 and treated as in (A) were fixed and probed for endogeneous NBR1 and catalase. **(F)** HeLa cells expressing Cherry-LC3 and GFP-NBR1 were fixed and immunostained for endogenous catalase. **(G)** Magnification of the area in (E) indicated by the white box. Scale bars, 10 μm.





C

	Total Pixel with RED	Total Pixel RED ONLY(where red is 3X>green)	% of Red only Pixel	Pexophagy induced cell (>20%)
Cer Cell 1	822	0	0.0%	-
Cer Cell 2	809	0	0.0%	-
Cer Cell 3	530	0	0.0%	-
NBR1 Cell 1	2628	1487	56.6%	✓
NBR1 Cell 2	5137	3540	68.9%	✓
NBR1 Cell 3	2155	41	1.9%	-
NBR1 Cell 4	2155	345	16.0%	-
NBR1 Cell 5	3313	87	2.6%	-
NBR1 Cell 6	2417	6	0.2%	-
NBR1 Cell 7	2579	1986	77.0%	✓

Fig. S4. RG-Lysosome assay for Pexophagy using GFP-Cherry-PEX26. Representative example of cells transfected with GFP-Cherry-PEX26$_{TM}$ and Cerulean **(A)** or Cerulean-NBR1 **(B)**, and treated with lysosomal inhibitors Leupeptin and E-64. The presence of red-only peroxisomes indicates pexophagy. In the merge images Cerulean constructs are shown in blue. **(C)** Example of the quantification of pexophagy, as discussed in Materials and Methods. The cells are numbers in the merge images in (A) and (B). Cells with more than 20% of Red only pixels compare to the total red pixels are considered to have increased pexophagy (indicated with a 'check-mark')

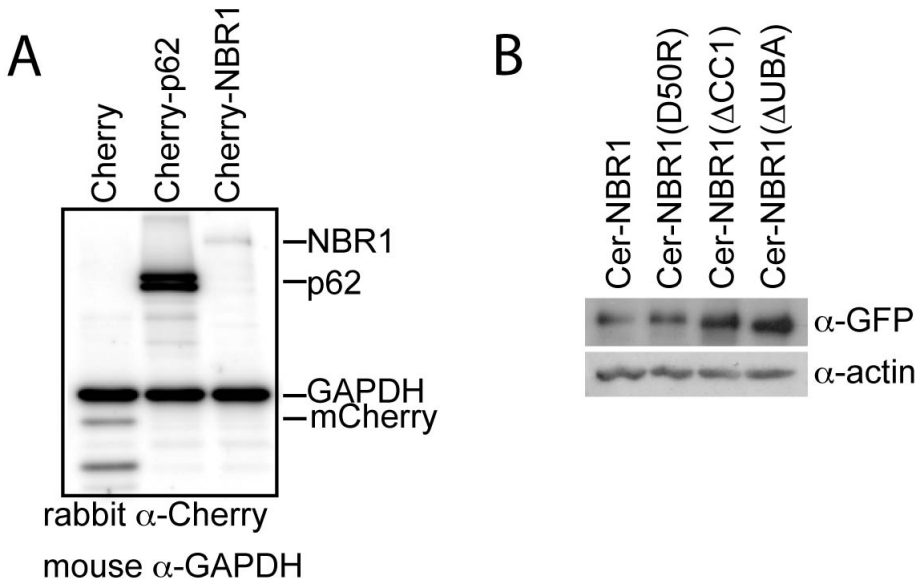
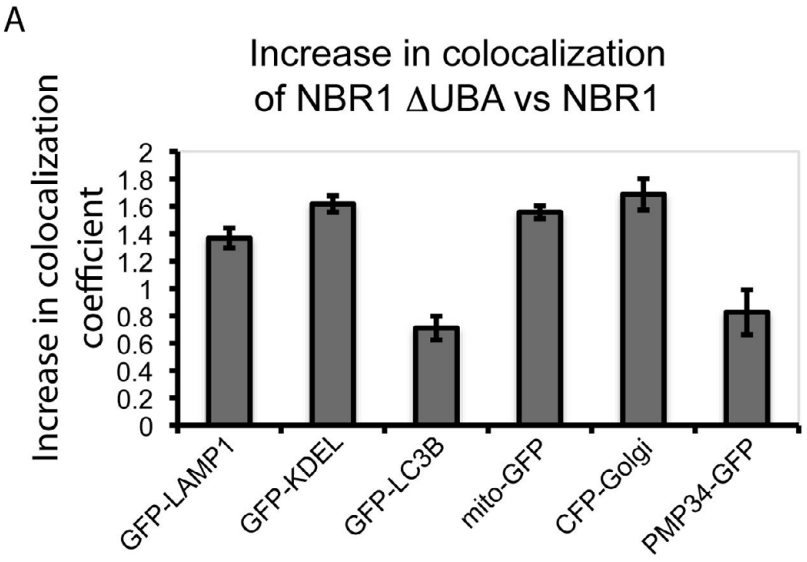


Fig. S5. Expression levels of Cer-NBR1 and mutants. (A) Immunoblot of lysates of HeLa cells transiently transfected with Cherry, Cherry-p62, Cherry-NBR1 probed with Rabbit anti-Cherry and mouse anti-GAPDH. **(B)** Cer-NBR1 and its various mutants were analyzed by SDS-PAGE and immunoblotted with anti-GFP antibody and anti-actin.



B

p-values of the difference between NBR1 and NBR1-ΔUBA with various organelle markers

GFP-Lamp1 (lysosomes)	0.018
GFP-KDEL (ER)	2.91E-07
GFP-LC3B (autophagosome)	1.43E-08
GFP-mito (mitochondria)	6.25E-10
CFP-Golgi (Golgi Apparatus)	0.046
PMP34-GFP * (peroxisomes)	0.16

* Not significantly different (p>0.05)

Fig. S6. The increase in colocalization of NBR1 JΔUBA with various organelles relative to wild type NBR1. HeLa cells were co-transfected with either wild type NBR1 or NBR1 JΔUBA with various organelle markers as shown. **(A)** The fraction of NBR1 or NBR1 JΔUBA colocalized with the specific organelle markers was determined using Mander's colocalization coefficient. The change in colocalization of NBR1 JΔUBA versus wild type NBR1 is shown. The change in colocalization is the ratio of the colocalization coefficient of NBR1 JΔUBA over that of the wild type. **(B)** the p-value of the difference in the colocalization coefficient between NBR1 ΔUBA and NBR1. Box shaded in blue indicates no significant difference.

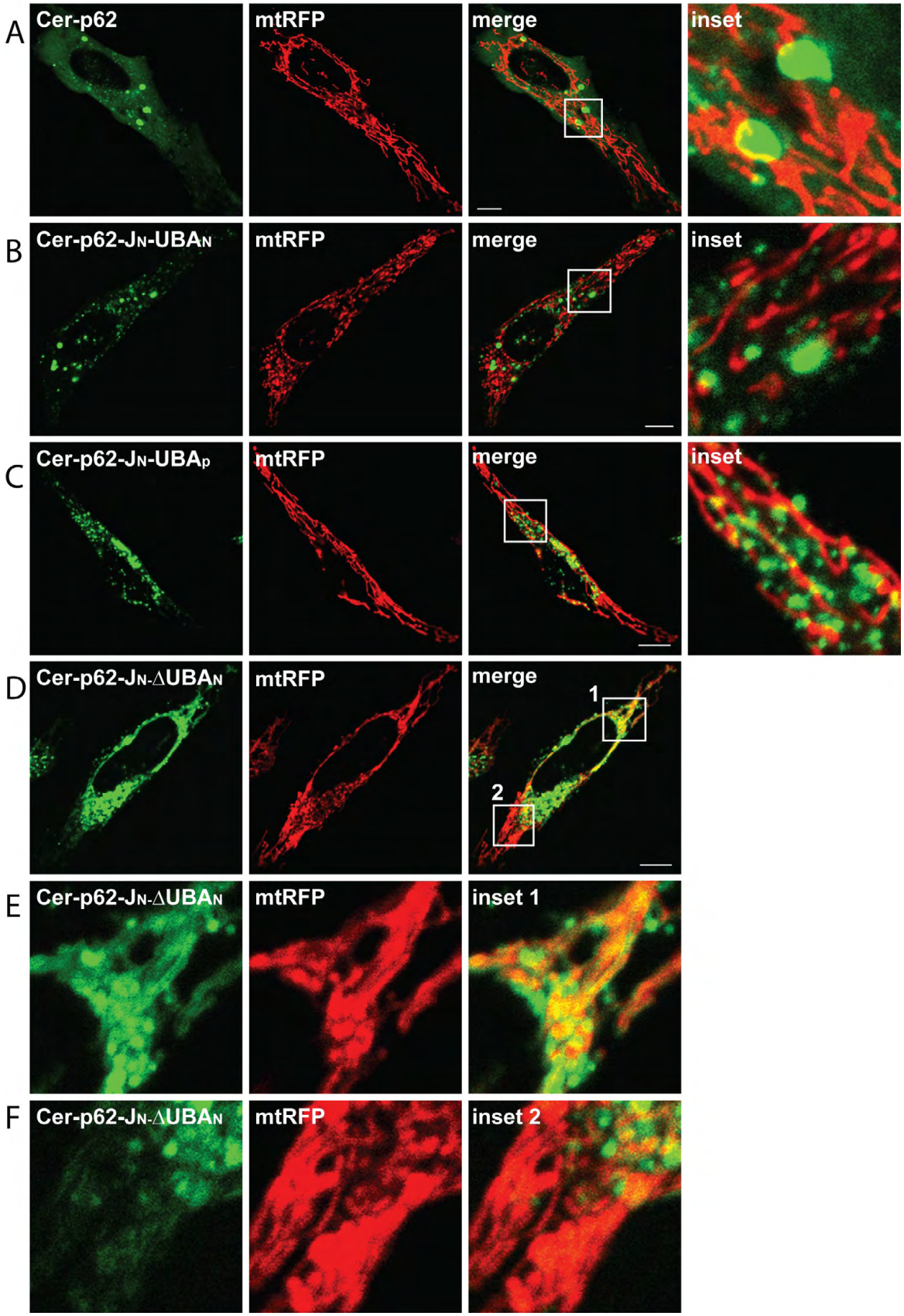


Fig. S7. HeLa cells were co-transfected with mitochondrial-targeting red fluorescent protein (mtRFP) and **(A)** Cerulean-p62, **(B)** Cer-p62-JN-UBA$_N$, **(C)** Cer-p62-J$_N$-UBA$_p$, or **(D)** Cer-p62-J$_N$-ΔUBA for 16 hours. The cells were imaged live. The white box area is magnified in the frame labeled 'inset'. Two insets were shown in the cell expressing mtRFP and Cer-p62-J$_N$-ΔUBA (D), which are shown in **(E)** inset 1 and **(F)** inset 2.

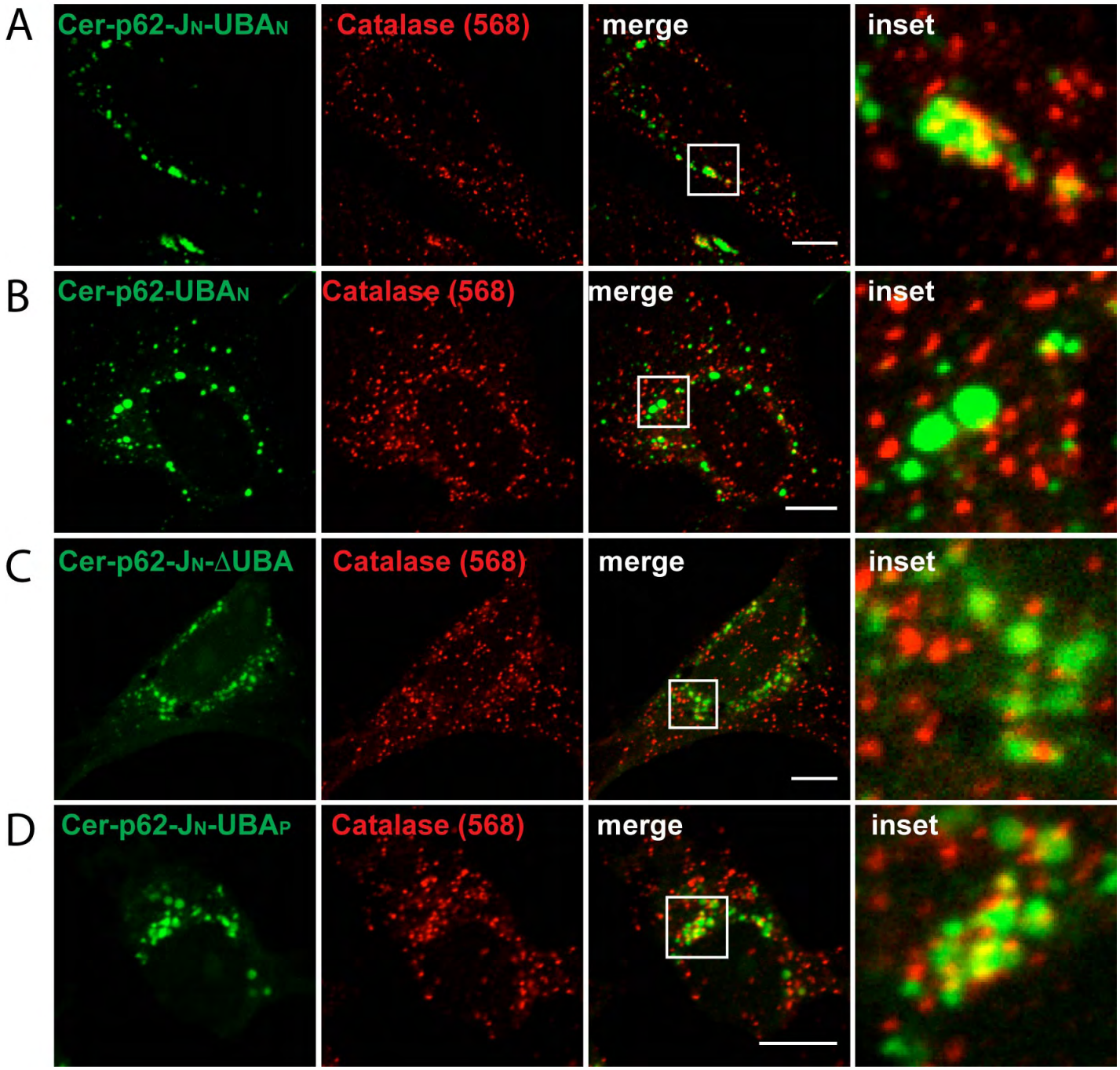


Fig. S8. Representative images of HeLa cells transfected with (**A**) p62 with the J and UBA domain of NBR1 (Cer-p62-J_N-UBA_N); (**B**) p62 with only the UBA domain of NBR1 (Cer-p62-UBA_N); (**C**) p62 with only the J domain and no UBA (Cer-p62-J-ΔUBA); or (**D**) p62 with NBR1 J domain and its own UBA domain (Cer-p62-J_N-UBA_p). Cells were fixed and immunostained for catalase. Scale bars, 10 μm.

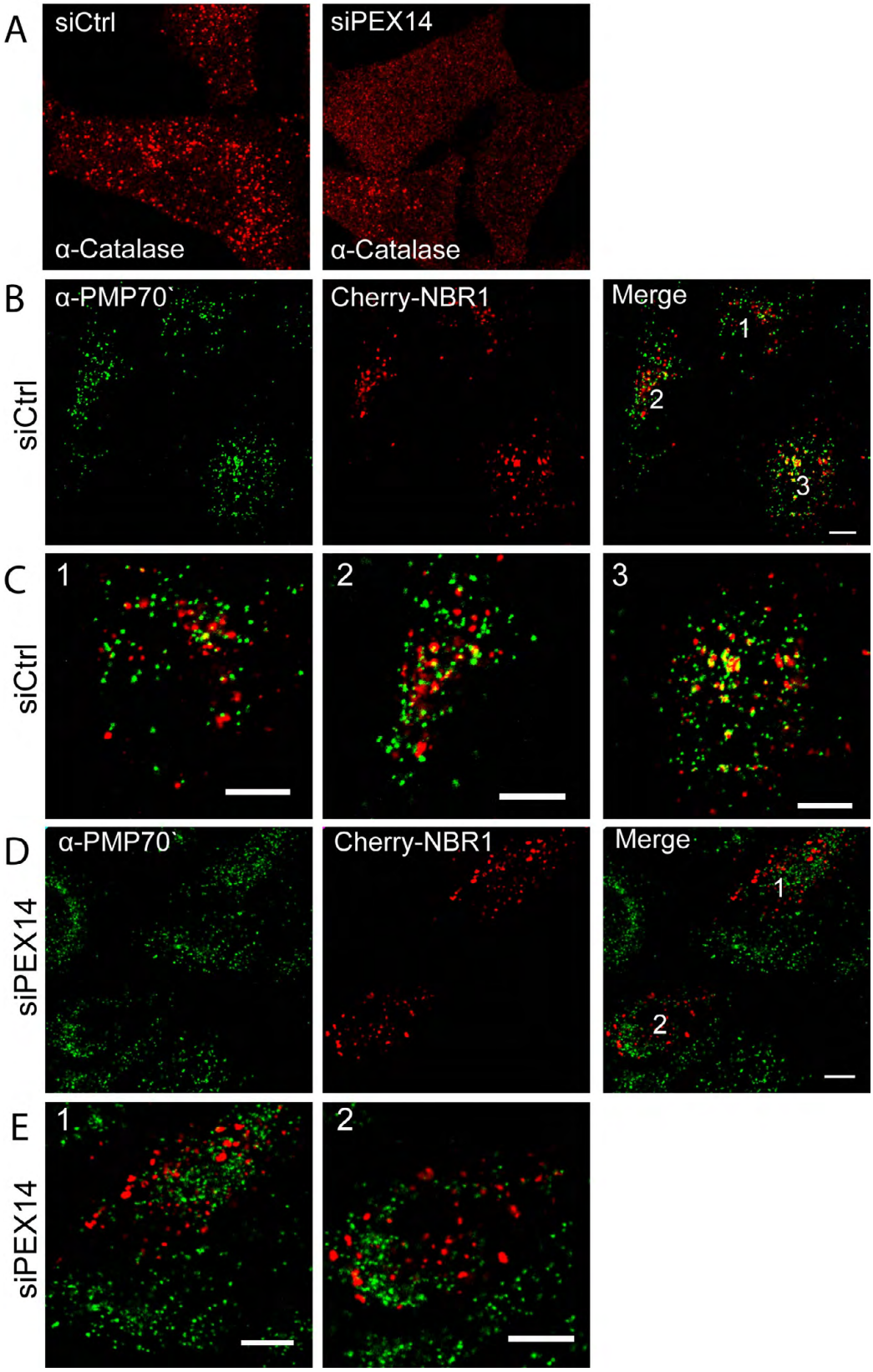
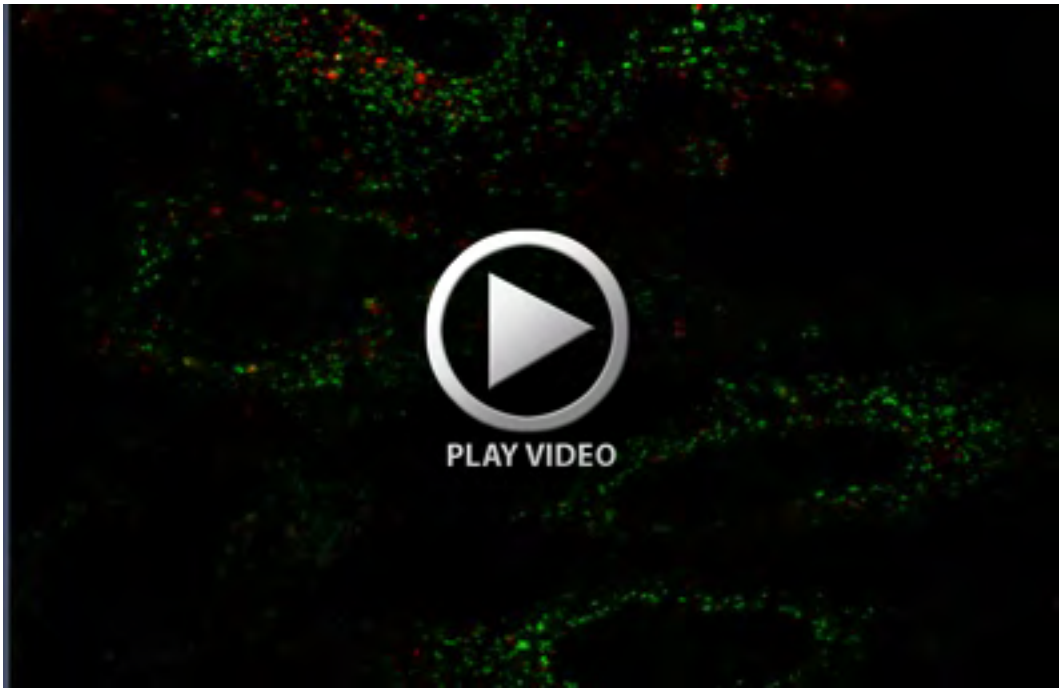


Fig. S9. Representative images of HeLa cells treated with siRNA against PEX14. **(A)** Cells treated with either control siRNA (siCtrl) or siRNA against PEX14 (siPEX14) immunostained for catalase. The siPEX14 consist of two siRNA sequences: (1) 5'-GGCAGGCAUUGCAUUUTT-3' and (2) 5'-GAACUCAAGUCCGAAAUUTT-3'. Note the increase in cytosolic localization of catalase in siPEX14 treated cells. **(B)** siCtrl treated cells expressing Cherry-NBR1 immunostained for PMP70. **(C)** Magnification of cells in (B). Cells 2 and 3 show NBR1/PMP70 clustering. **(D)** siPEX14 treated cells transiently expressing Cherry-NBR1 were immunostained for PMP70. **(E)** Magnification of cells in (D).



Movie 1. Time-lapse imaging of a HeLa cell expressing Cherry-NBR1 and PMP34-GFP. Images were acquired 16 hours after transfection at 2 hour intervals for 14 hours. Images were compiled using Zeiss Zen software.

Movie 2. Time-lapse imaging of a HeLa cell expressing Cer-NBR1 and GFP-Cherry-PEX26$_{TM}$. Images were acquired every hour for 48 hours. Images were compiled using Zeiss Zen software and Adobe Photoshop.