Expanded View Figures

Figure EV1. PEX13 is required for Sindbis virophagy.

A, B Quantification of mCherry-capsid puncta per cell (A) and GFP-LC3 puncta per cell (B) for experiment shown in Fig 1B and C in HeLa/GFP-LC3 cells treated with the indicated siRNA and infected with SIN/mCherry-capsid (AO30). Results represent mean ± SEM of triplicate samples (~100 cells analyzed per sample). *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, NS = not significant; one-way ANOVA with adjustment for multiple comparisons.

C Representative images of endogenous LC3 colocalization with mCherry-capsid at 10 h after Sindbis virus (strain AO30) infection of HeLa cells treated with the indicated siRNA. Arrowheads indicate colocalization between mCherry-capsid and LC3 puncta at 10 h after Sindbis virus (strain AO30) infection of HeLa cells treated with the indicated siRNA. Scale bars, 10 μm.

D–F Quantification of colocalized LC3 and mCherry-capsid puncta normalized to the number of mCherry-capsid puncta per cell (% colocalization) (D), mCherry-capsid puncta per cell (E), and LC3 puncta per cell (F) in the experiment shown in (C). Bars are mean ± SEM of triplicate samples (~70 cells analyzed per sample). *$P < 0.05$, NS = not significant; two-tailed unpaired *t*-test.

G, H Quantification of mCherry-capsid puncta per cell (G) and GFP-LC3 puncta per cell (H) for experiment shown in Fig 1E in MEFs infected with SIN/mCherry-capsid/GFP-LC3. Results represent mean ± SEM of triplicate samples (~100 cells analyzed per sample). NS = not significant; two-tailed unpaired *t*-test.

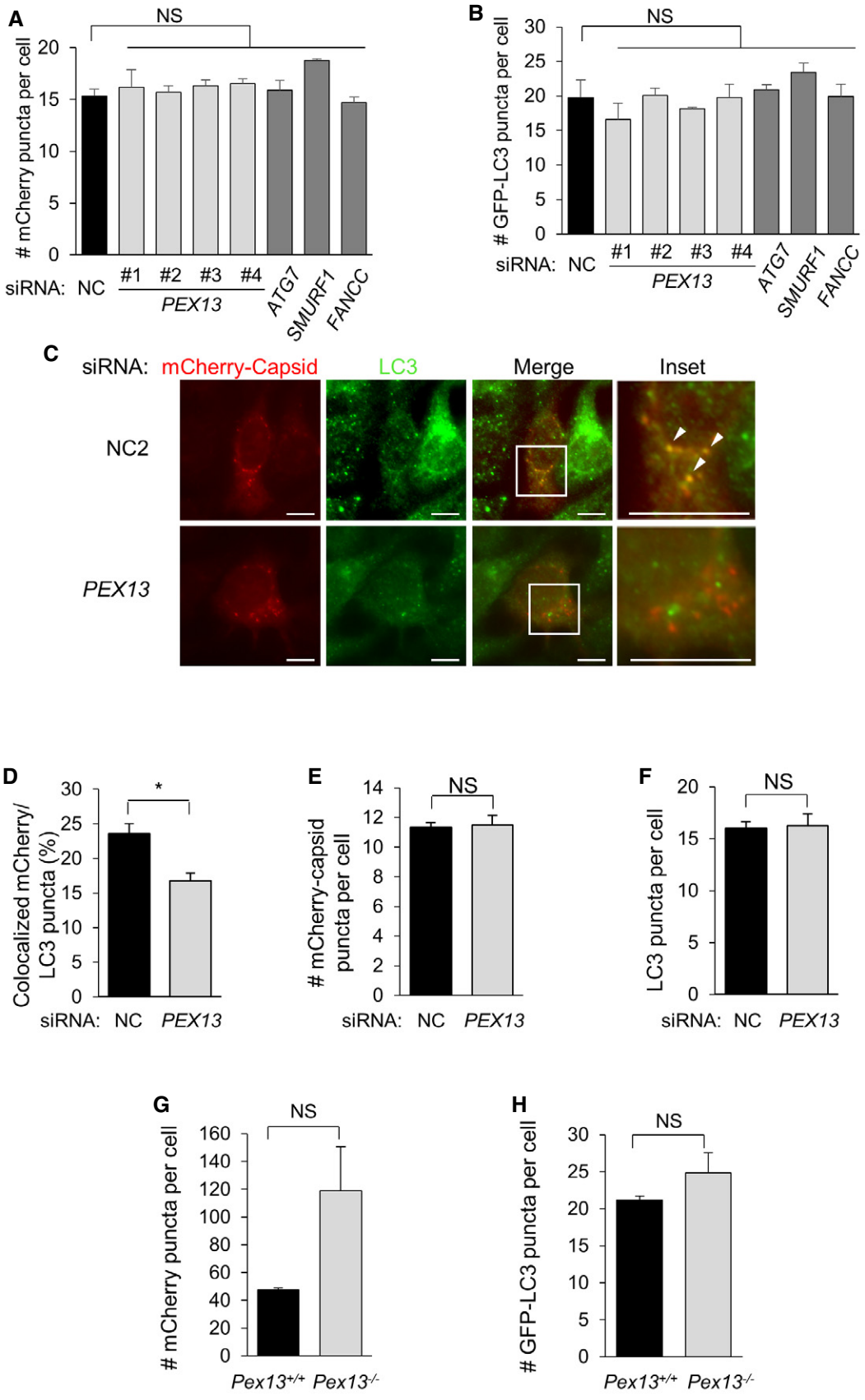

Figure EV1.

Figure EV2. PEX13 colocalization with Parkin during mitophagy and analysis of mitochondrial morphology and metabolism in *Pex13*$^{-/-}$ MEFs.

A Western blot detection of PEX13 and ATG7 in HeLa/Parkin cells transfected with the indicated siRNA. Asterisk denotes nonspecific band.

B Representative images of Parkin colocalization with TOMM20 in HeLa/Parkin cells treated with indicated siRNA after treatment with DMSO or 10 μM CCCP for 4 h. Scale bars, 20 μm.

C Transmission electron microscopic analysis of MEFs in control conditions or following CCCP-induced mitochondrial damage. Shown are representative images of mitochondrial morphology in MEFs of the indicated genotype 24 h after treatment with 30 μM CCCP or DMSO vehicle control. Black arrowhead denotes autolysosome containing damaged mitochondria; white arrowheads denote mitochondria with abnormal cristae morphology. Scale bars, 500 nm.

D Representative images of TOMM20 staining in MEFs of the indicated genotype with DMSO or 30 μM CCCP for 24 h. Insets show reticular mitochondrial morphology during basal state and fragmented damaged mitochondria after CCCP treatment. White lines indicate cell borders. Scale bars, 20 μm.

E, F Mitochondrial metabolism assay in primary MEFs of the indicated genotype. Mitochondrial oxygen consumption rate normalized to total protein level (E) and extracellular acidification rate (ECAR) adjusted to total protein level (F) are shown. Plot shows the average and SEM of 5 samples.

Source data are available online for this figure.

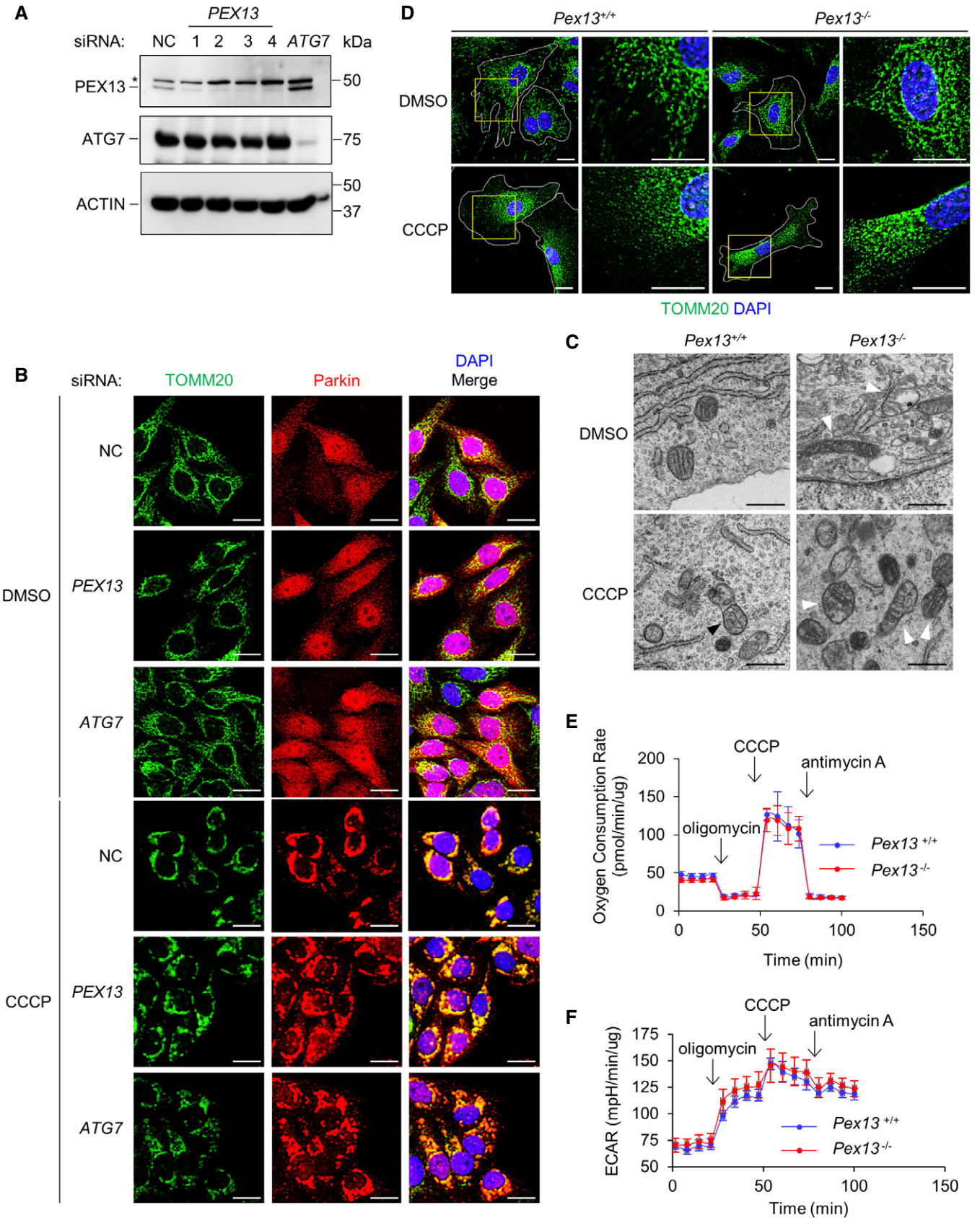
A
PEX13
siRNA: NC 1 2 3 4 ATG7 kDa
PEX13 *
ATG7
ACTIN
50
75
50
37
D
Pex13+/+
Pex13-/-
DMSO
CCCP
TOMM20 DAPI
B
siRNA:
TOMM20
Parkin
DAPI
Merge
NC
PEX13
ATG7
DMSO
CCCP
C
Pex13+/+
Pex13-/-
DMSO
CCCP
E
Oxygen Consumption Rate (pmol/min/ug)
CCCP
antimycin A
oligomycin
Pex13 +/+
Pex13 -/-
Time (min)
F
ECAR (mpH/min/ug)
CCCP
antimycin A
oligomycin
Pex13 +/+
Pex13 -/-
Time (min)

Figure EV2.

Figure EV3. Disease-associated PEX13 mutant protein colocalization with peroxisomes and effects on mitochondrial morphology, and structured illumination microscopic analyses of mitochondria in PEX13 patient mutant fibroblasts.

A Representative images of wild-type and mutant PEX13 colocalization with PMP70 in HeLa/Parkin cells transduced with lentivirus containing the indicated PEX13-MYC-DDK cDNA. Scale bars, 20 μm.

B Representative images of TOMM20 morphology 16 h after DMSO vehicle treatment in HeLa/Parkin cells transfected with *PEX13* siRNA and indicated *PEX13* siRNA-resistant plasmids. Scale bars, 20 μm.

C Representative structured illumination microscopy (SIM) 3D projections of wild-type and PEX13 W313G mutant primary human fibroblasts treated with MitoTracker CMX rosamine (CMXROS, 50 nM, 30 min). White arrowheads indicate mitochondrial fragments. Scale bar, 5 μm.

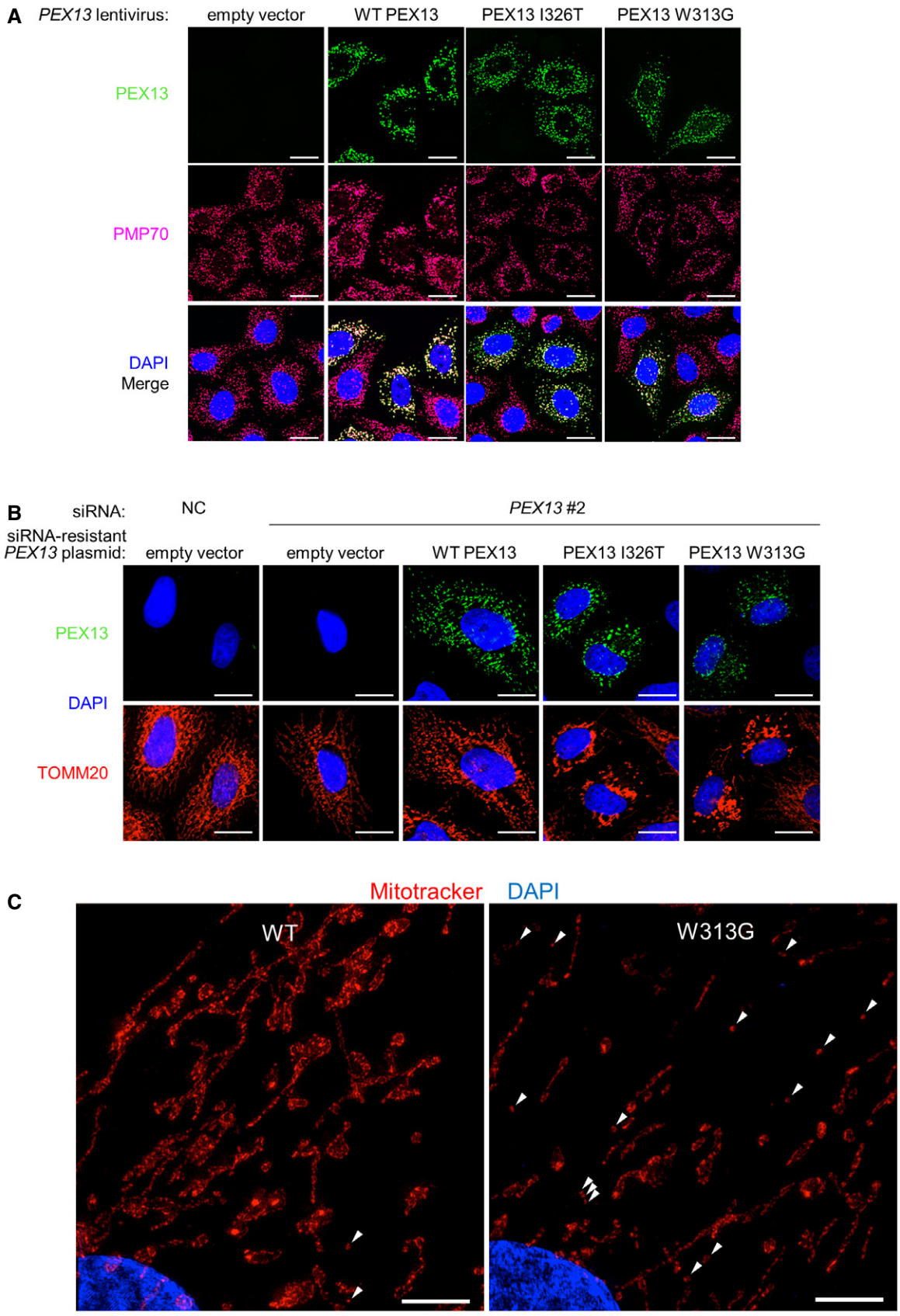

Figure EV3.

Figure EV4. PEX3 is required for mitophagy.

A Quantitative real-time PCR of *PEX3* mRNA detection in HeLa/Parkin cells transfected with indicated siRNA. Results represent mean ± SEM of triplicate samples. Similar results were observed in three independent experiments. ***$P < 0.001$; one-way ANOVA with adjustment for multiple comparisons.

B Representative images of Parkin colocalization with TOMM20 in HeLa/Parkin cells treated with indicated siRNA after treatment with DMSO or 10 μM CCCP for 4 h. Scale bars 20 μm.

C Representative images of Parkin-mediated clearance of TOMM20 in HeLa/Parkin cells transfected with indicated siRNA and after treatment with DMSO or 10 μM CCCP for 16 h. Scale bars, 20 μm.

D Quantification of TOMM20 clearance in the experiment shown in (C). Results represent mean ± SEM of triplicate samples (~100 cells analyzed per sample). Similar results were observed in more than three independent experiments. *$P < 0.05$, *** $P < 0.001$, NS = not significant; one-way ANOVA with adjustment for multiple comparisons.

E Representative images of Parkin-mediated clearance of mtDNA after 8 h of treatment with 2.5 μM oligomycin and 250 nM antimycin A (OA) or DMSO vehicle control in HeLa/HA-Parkin cells treated with indicated siRNA. si*PEX3* oligo #1 is shown; similar results were observed with three other si*PEX3* oligos. Nuclear DNA staining was masked using DAPI. Scale bars, 20 μm.

F Quantification of mtDNA clearance in the experiment shown in (E). Results represent box plots of ~300 cells analyzed per sample. Whiskers represent 5–95% range and the horizontal lines of the boxes indicate the 25^{th}, 50^{th}, and 75^{th} percentiles of the dataset. Each outlier is represented by a dot. Similar results were observed in three independent experiments. ***$P < 0.001$; Kruskal–Wallis *H*-test.

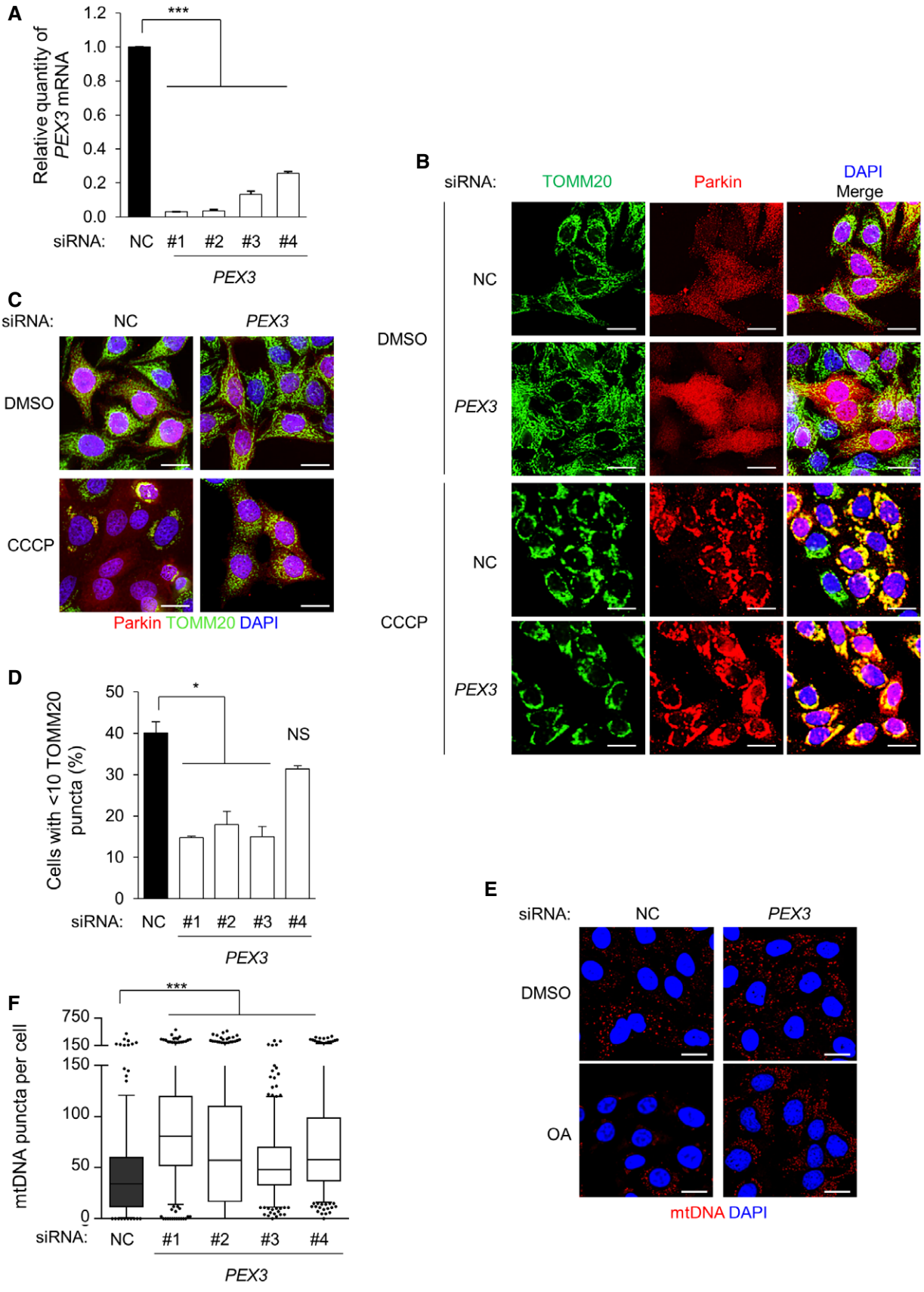

Figure EV4.

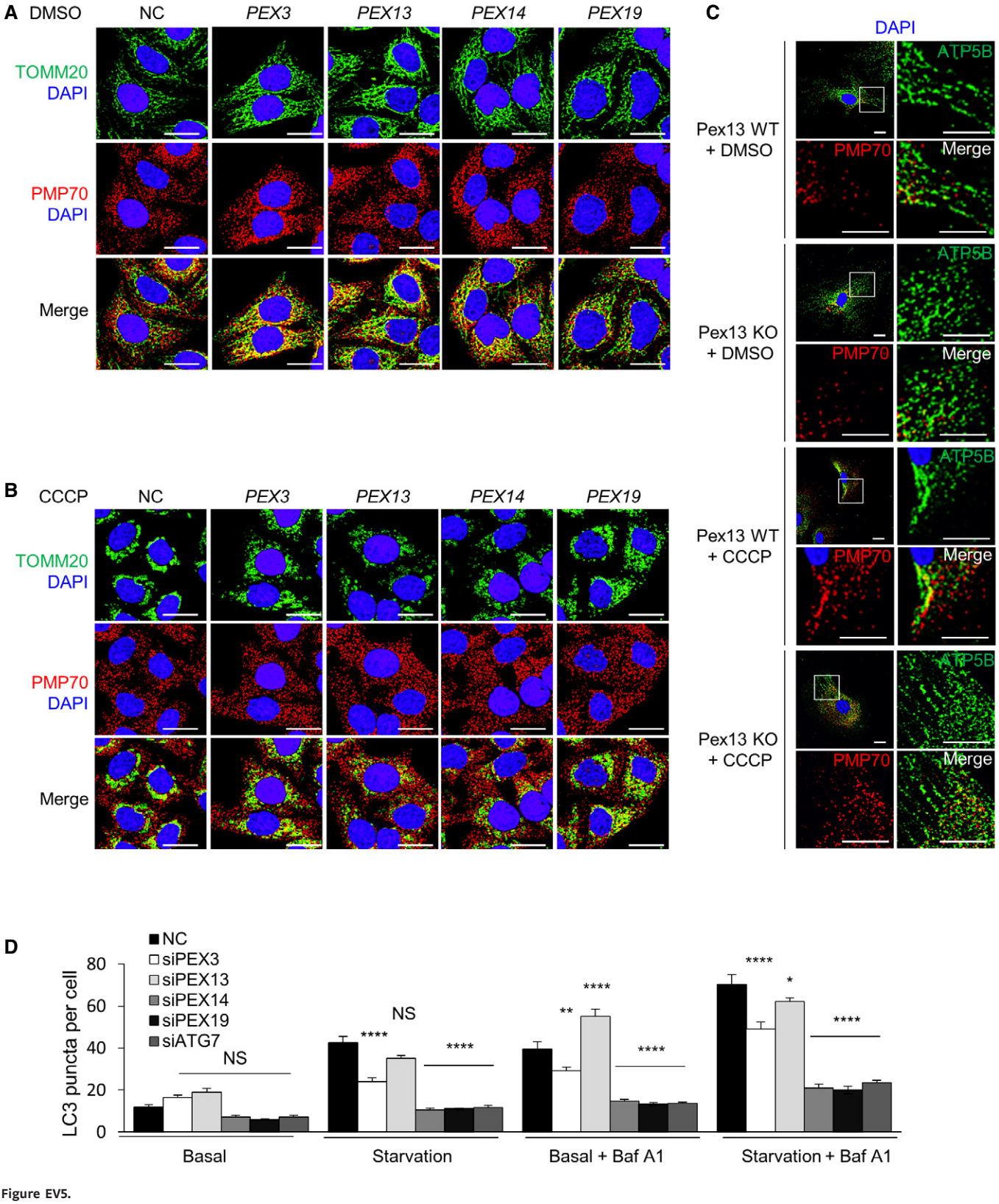

Figure EV5.

Figure EV5. PEX protein colocalization with peroxisomes and mitochondria in baseline and mitophagy conditions, and roles of different PEX proteins in general autophagy.

A, B Representative images of TOMM20 and PMP70 co-staining in HeLa/Parkin cells treated with the indicated siRNA 4 h after treatment with DMSO (A) or 10 μM CCCP (B). Scale bars, 20 μm.

C Representative images of mitochondrial inner membrane protein ATP5B and peroxisome membrane protein PMP70 co-staining in primary MEFs of the indicated genotype 24 h after treatment with DMSO or 30 μM CCCP. Scale bars, 20 μm.

D Quantification of endogenous LC3 puncta in HeLa/GFP-LC3 cells treated with 10 nM Baf A1 or DMSO vehicle and cultured in normal medium or 3 h HBSS starvation media. Results represent mean ± SEM in triplicate samples (~100 cells analyzed per sample). Similar results were observed in three independent experiments. *$P < 0.05$, **$P < 0.01$, ****$P < 0.001$, NS = not significant; one-way ANOVA with adjustment for multiple comparisons. Statistical analyses refer to the differences between *Pex* or *ATG7* siRNAs vs. NC siRNA within each treatment group.