

Supplementary Figures

A degradative to secretory autophagy switch mediates mitochondria clearance in the absence of the mATG8-conjugation machinery

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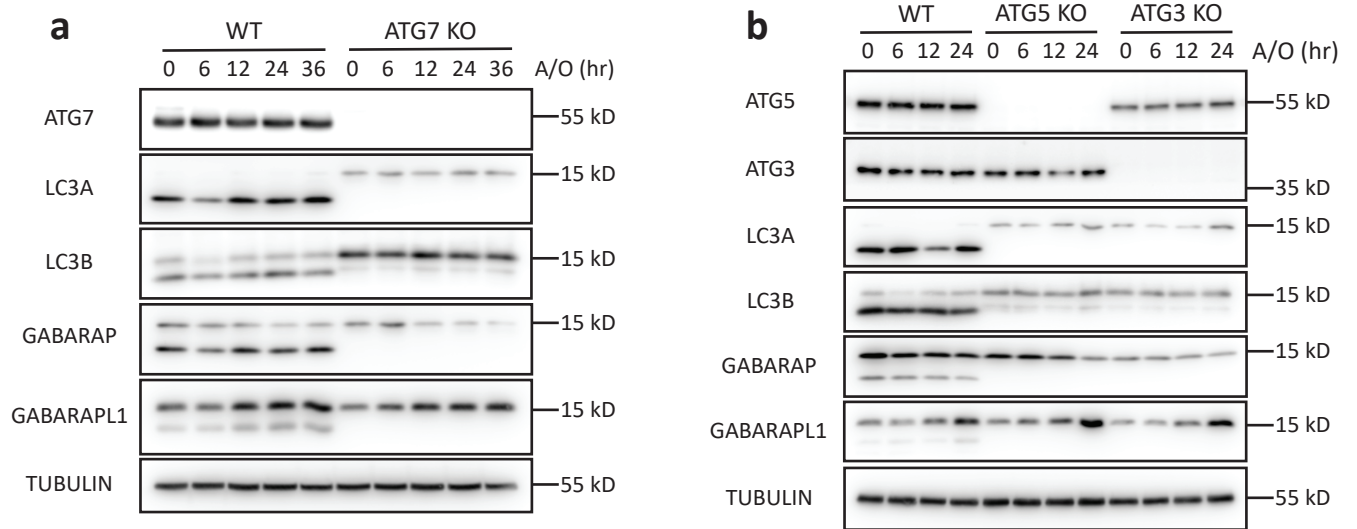
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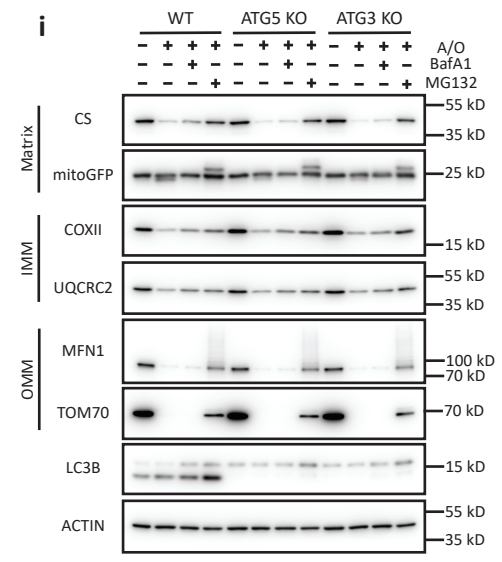
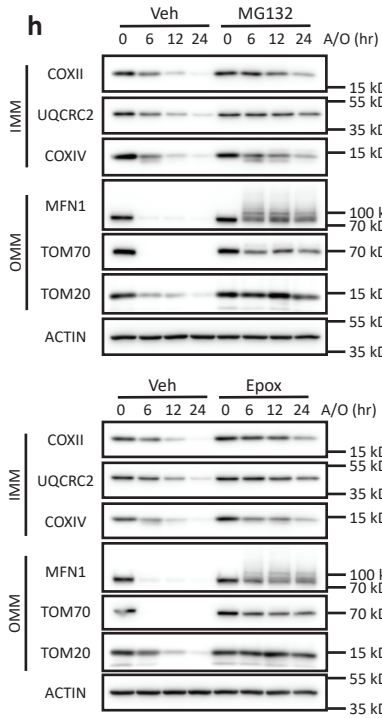
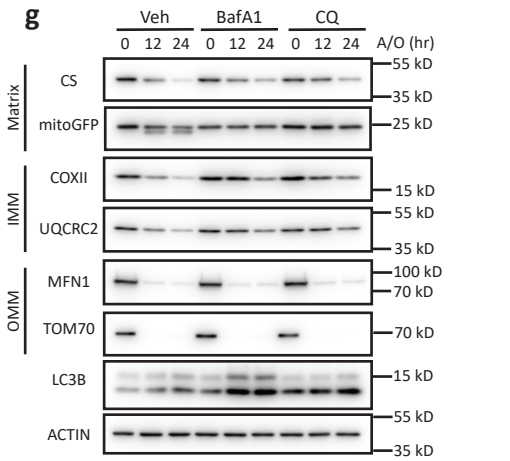
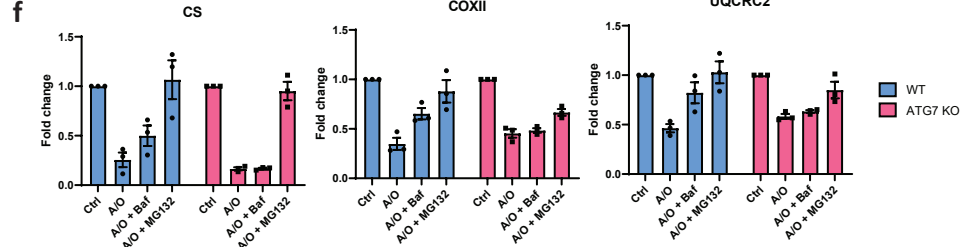
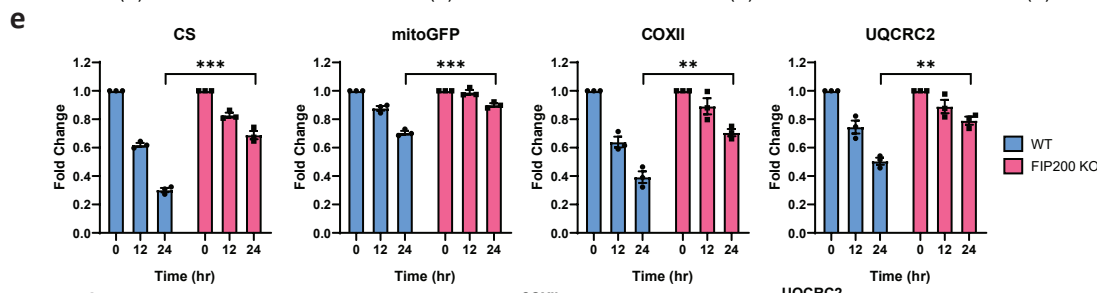
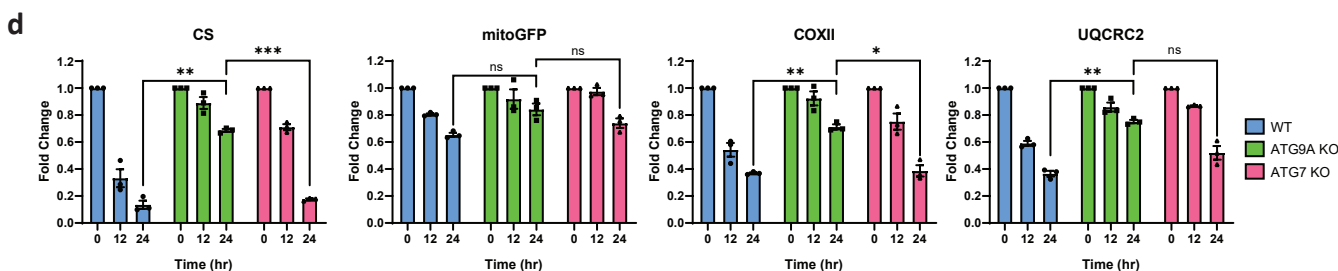
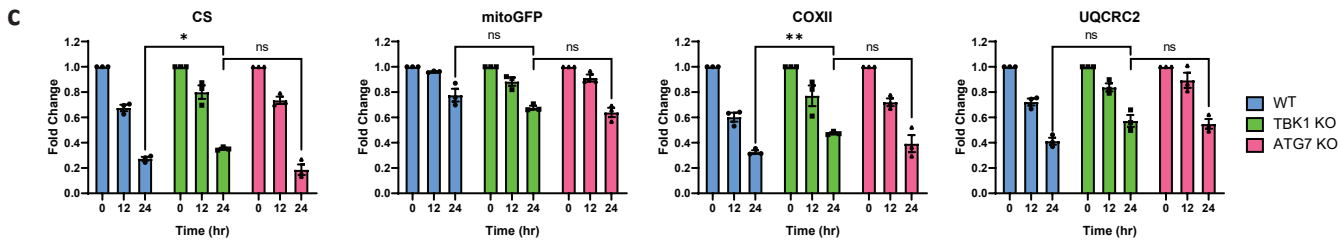
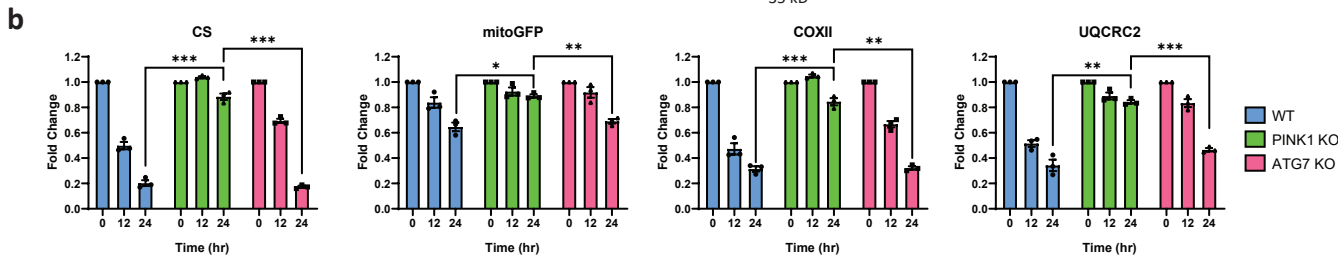
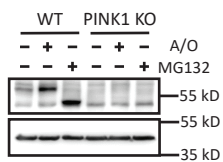
Suppl Fig 1



Supplementary Fig. 1 Characterization of mATG8-conjugation system knockouts.

a Validation of ATG7 knockout in HeLa stably expressing mCherry-Parkin. **b** Validation of ATG5 and ATG3 knockout in HeLa stably expressing mCherry-Parkin.

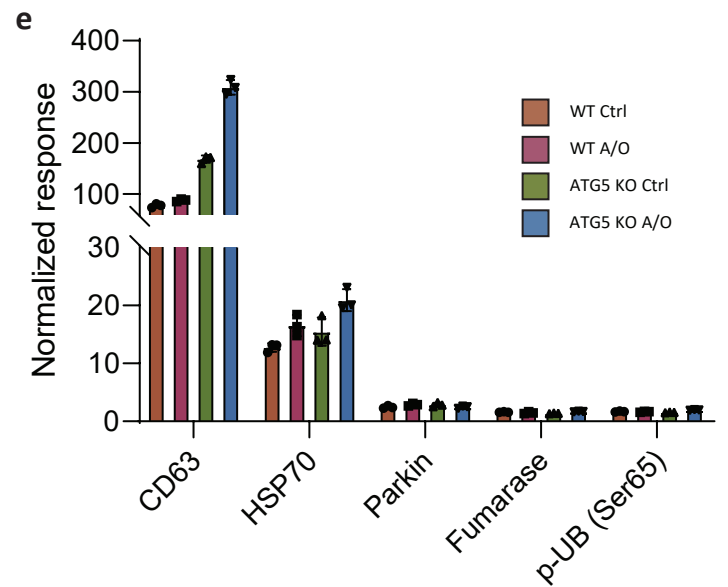
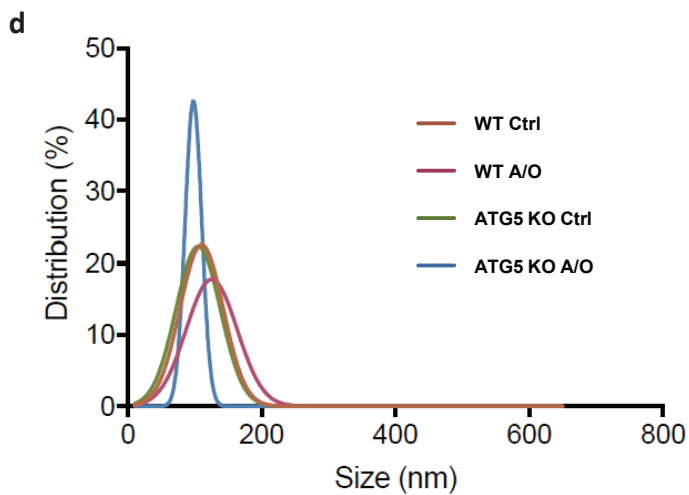
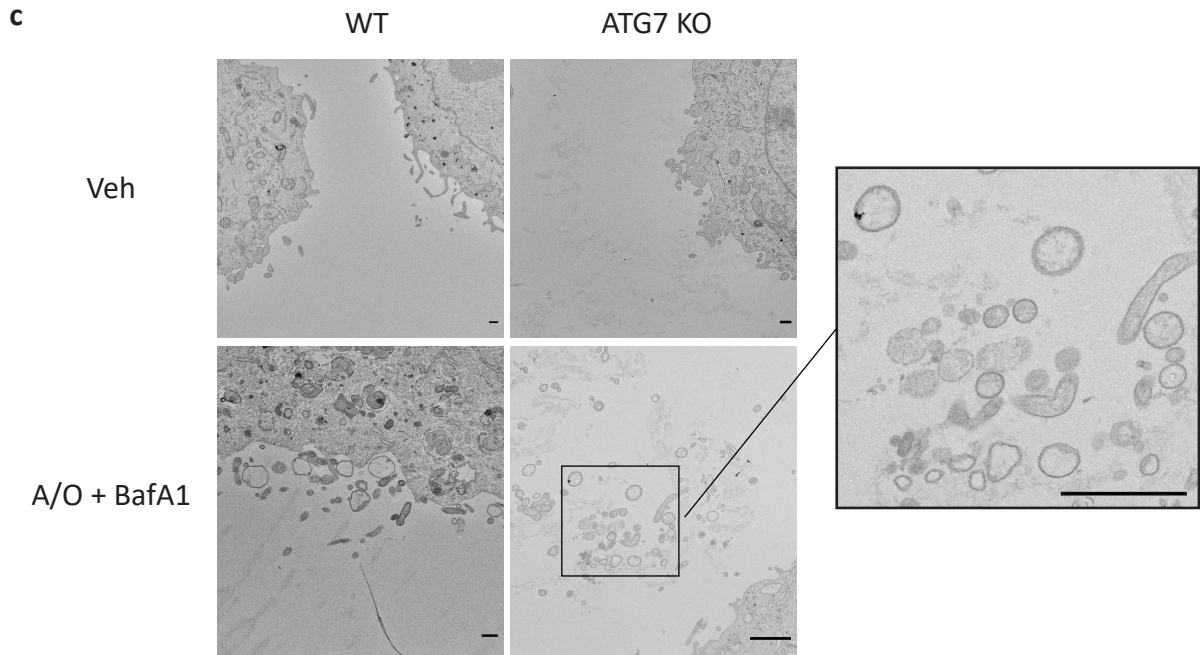
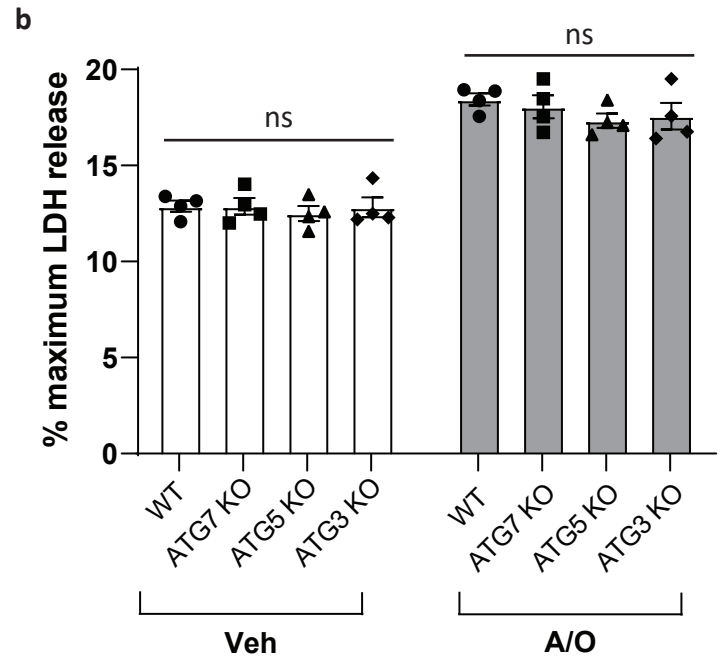
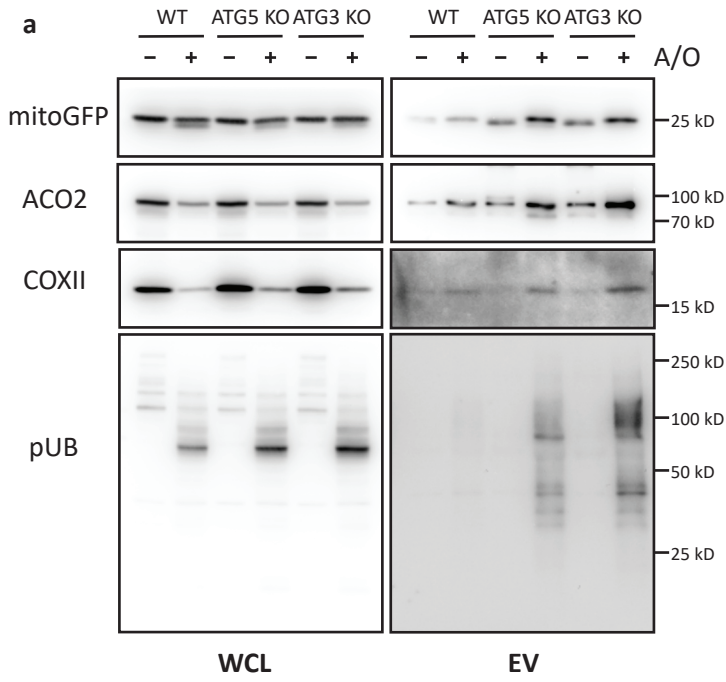
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Supplementary Fig. 2 Effect of lysosomal or proteasomal inhibition on mitochondria clearance and protein quantifications.

a WT and PINK1-KO cells were treated with A/O or MG132 for 4 hrs to accumulate full length or cleaved PINK1 respectively. **b** Quantification of mitochondrial protein changes from WT, PINK1-KO and ATG7-KO cells in Fig. 2a. **c** Quantification of mitochondrial protein changes from WT, TBK1-KO and ATG7-KO cells in Fig. 2b. **d** Quantification of mitochondrial protein changes from WT, ATG9A-KO and ATG7-KO cells in Fig. 2c. Mean of $n = 3$ independent replicates \pm SEM are shown for (**b-d**). P values are calculated by two-way ANOVA followed by Dunnett's multiple comparisons test. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, and ns denotes not significant. See source data for exact P values. **e** Quantification of mitochondrial protein changes from WT and FIP200-KO cells in Fig. 2d. Mean of $n = 3$ independent replicates \pm SEM is shown. P values are calculated by two-tailed student's T test. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, and ns denotes not significant. See source data for exact P values. **f** Quantification of mitochondrial protein changes from WT and ATG7-KO cells in Fig. 2f. Mean of $n = 3$ independent replicates \pm SEM is shown. **g** HeLa stably expressing mCherry-Parkin was treated with A/O in the absence or presence of Bafilomycin A1 (BafA1, 200 nM) or chloroquine (CQ, 100 μ M). **h** HeLa stably expressing YFP-Parkin were treated with A/O for the indicated duration. Proteasomal degradation was inhibited by MG132 (10 μ M) or epoxomicin (1 μ M). **i** WT, ATG5-KO and ATG3-KO cells stably expressing mCherry-Parkin were treated with A/O for 24 hrs, with or without Bafilomycin A1 (BafA1, 200 nM) or MG132 (10 μ M) to inhibit lysosomal or proteasome degradation respectively.

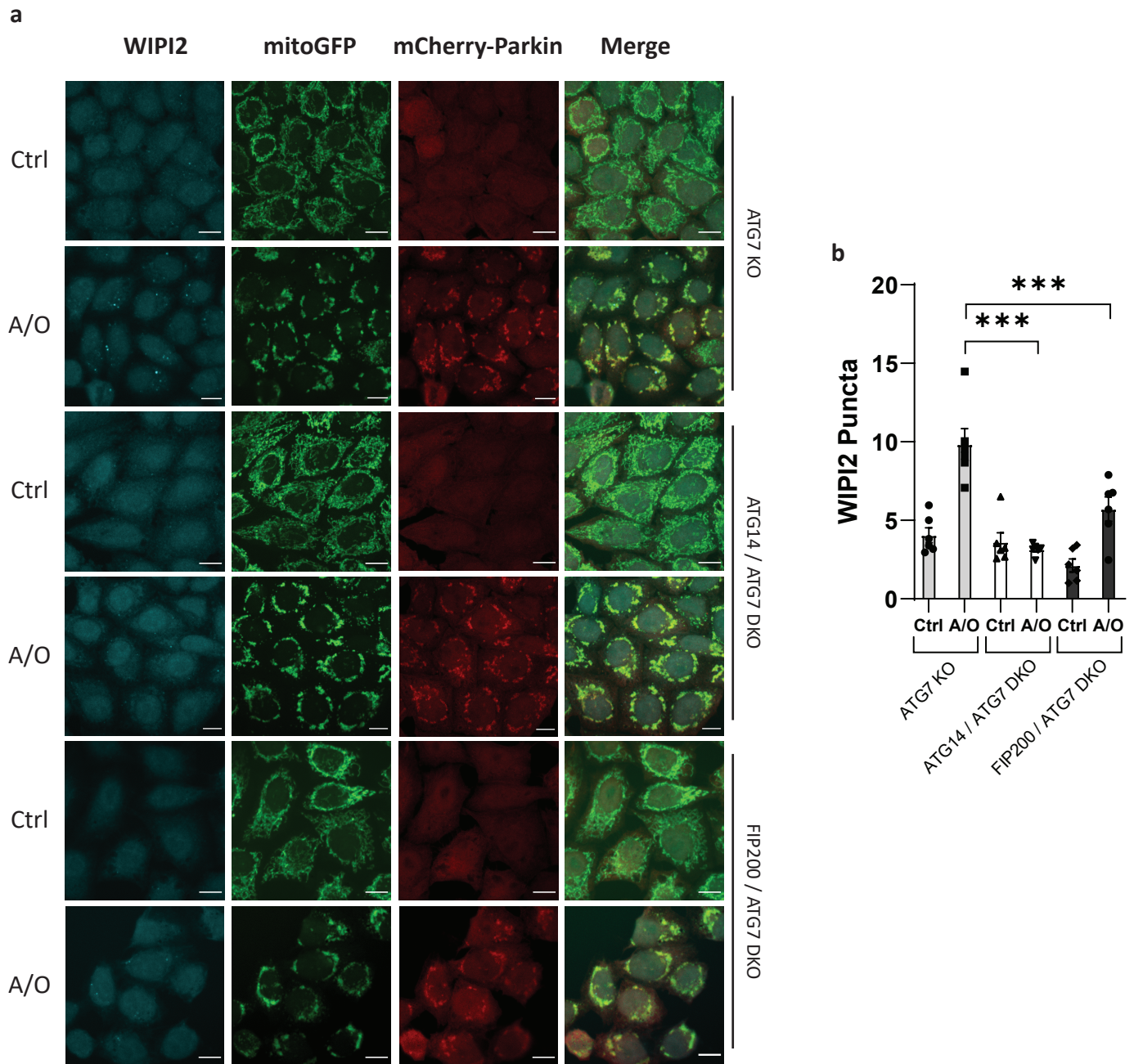
Suppl Fig 3



Supplementary Fig. 3 Characterization of secreted mitochondria in other mATG8-conjugation defective cell lines.

a Conditioned media from WT, ATG5-KO, ATG3-KO HeLa stably expressing mCherry-Parkin treated with A/O for 24 hr were collected. Extracellular vesicles (EVs) were isolated by differential ultracentrifugation and immunoblotted for mitochondria markers. **b** LDH release assay from WT, ATG7-KO, ATG5-KO and ATG3-KO cells treated with or without A/O for 24 hrs. Mean of $n = 4$ independent replicates \pm SEM is shown. P values are calculated by two-way ANOVA followed by Dunnett's multiple comparisons test. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, and ns denotes not significant. See source data for exact P values. **c** Representative TEM images of extracellular regions from WT or ATG7-KO HeLa stably expressing mCherry-Parkin untreated or treated with A/O + BafA1. Scale bar represents 500 nm. **d** Nanoparticle tracking analysis of EVs from WT and ATG5-KO cells untreated or treated with A/O for 24 hrs. **e** nPLEX analysis of mitochondria cargo from immunocaptured CD63+ EVs isolated from WT and ATG5-KO cells stably expressing YFP-Parkin untreated or treated with A/O for 24 hrs. Mean of $n = 3$ technical replicates \pm SD is shown.

Suppl Fig 4

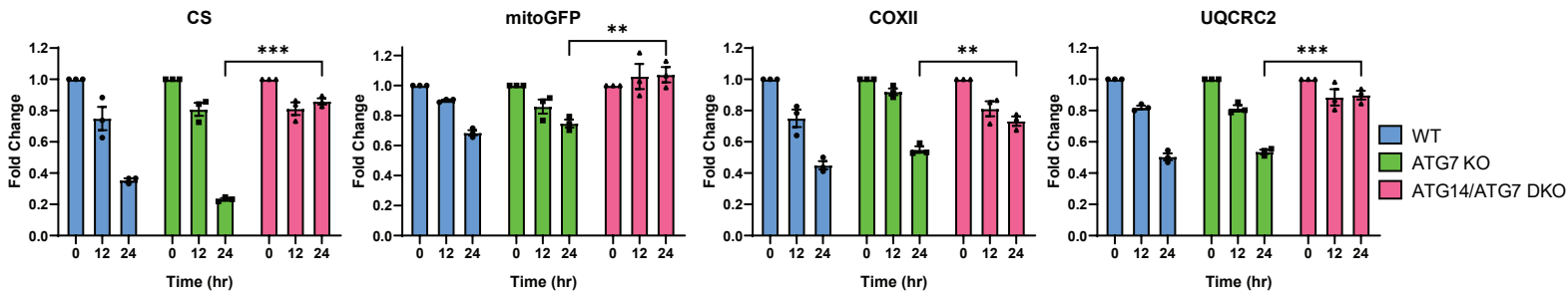


Supplementary Fig. 4 Mitophagosome formation in ATG14/ATG7 and FIP200/ATG7 DKO cells.

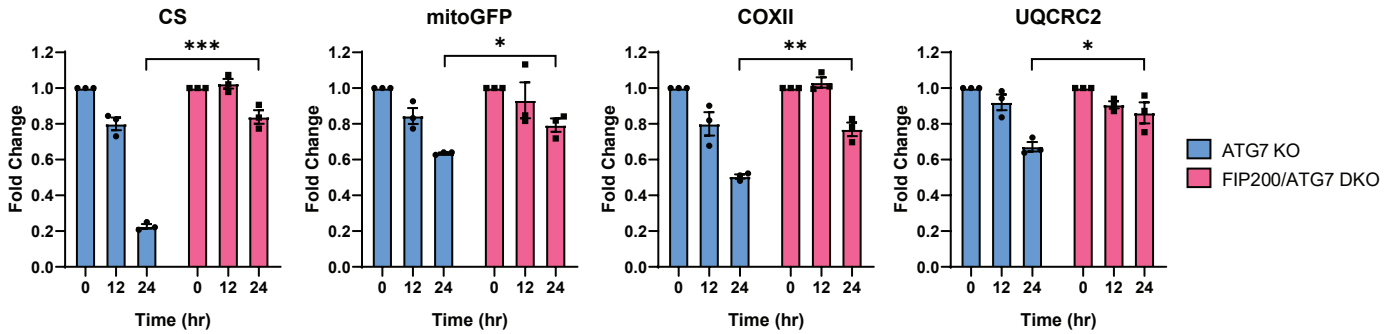
a Immunostaining of WIPI2 (cyan), mito-GFP (green) and mCherry-Parkin (red) in ATG7-KO, ATG14/ATG7 DKO and FIP200/ATG7 DKO cells treated with A/O for 2.5 hr. Scale bar = 10 μ m. **b** Quantification of WIPI2 puncta are shown. Mean of $n = 6$ fields of view \pm SEM is shown. P values are calculated by two-way ANOVA followed by Dunnett's multiple comparisons test. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, and ns denotes not significant. See source data for exact P values.

Suppl Fig 5

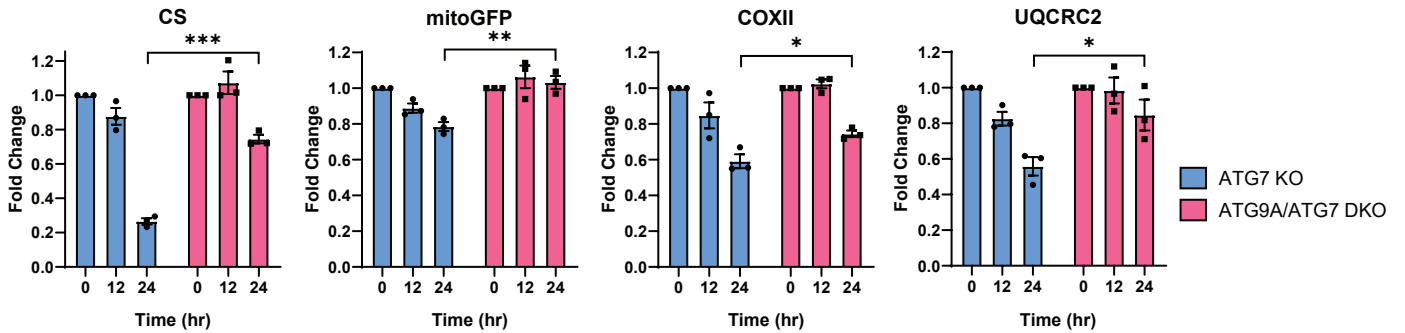
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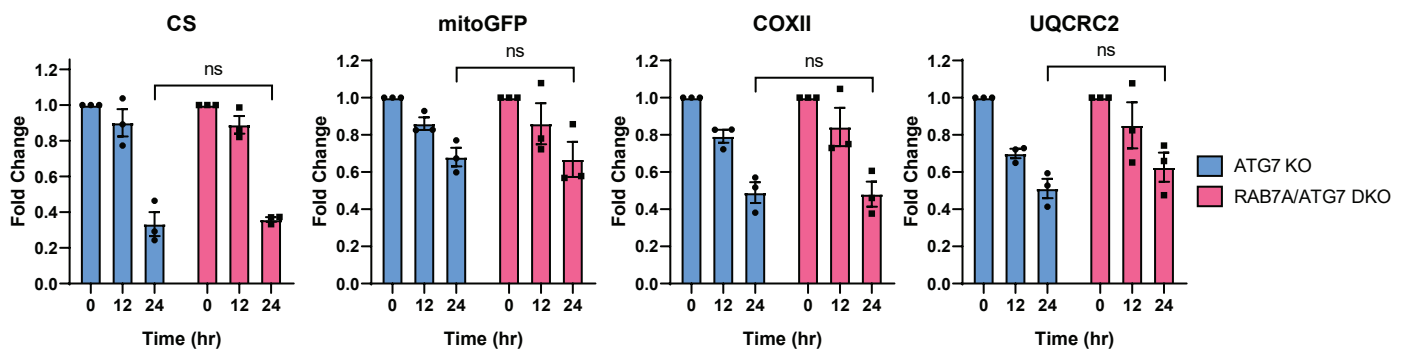
b



c



d



Supplementary Fig. 5 Quantifications of mitochondria protein changes.

a Quantification of mitochondrial protein changes in WT, ATG7-KO and ATG14/ATG7 DKO cells. **b** Quantification of mitochondrial protein changes in ATG7-KO and FIP200/ATG7 DKO cells. **c** Quantification of mitochondrial protein changes in in ATG7-KO and ATG9A/ATG7 DKO cells. **d** Quantification of mitochondrial protein changes in in ATG7-KO and RAB7A/ATG7 DKO cells. Mean of $n = 3$ independent replicates \pm SEM are shown for (a-d). P values are calculated by two-tailed student's T test. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, and ns denotes not significant. See source data for exact P values.