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Supplemental information

USP32-regulated LAMTOR1 ubiquitination impacts mTORC1 activation and autophagy induction

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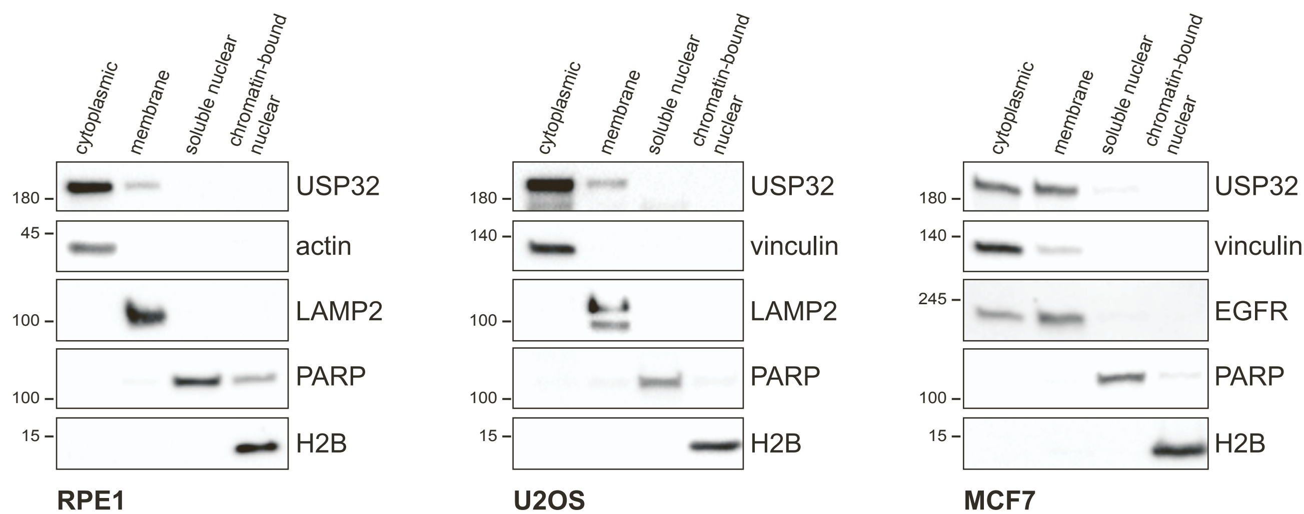
Figure S1

A



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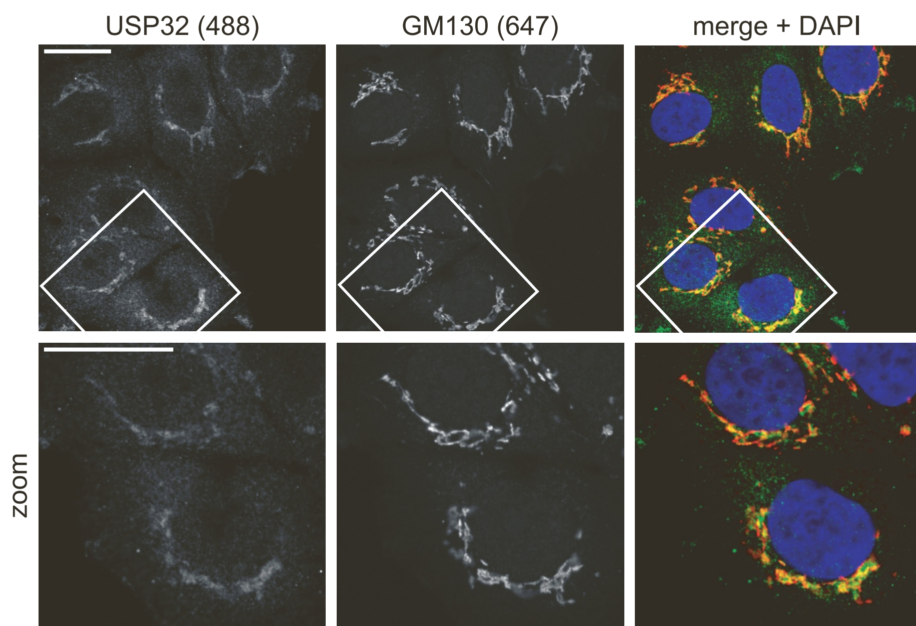
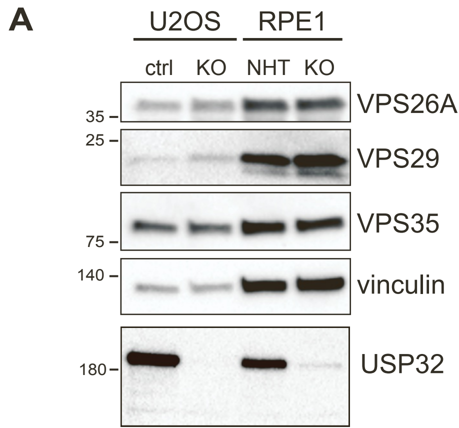


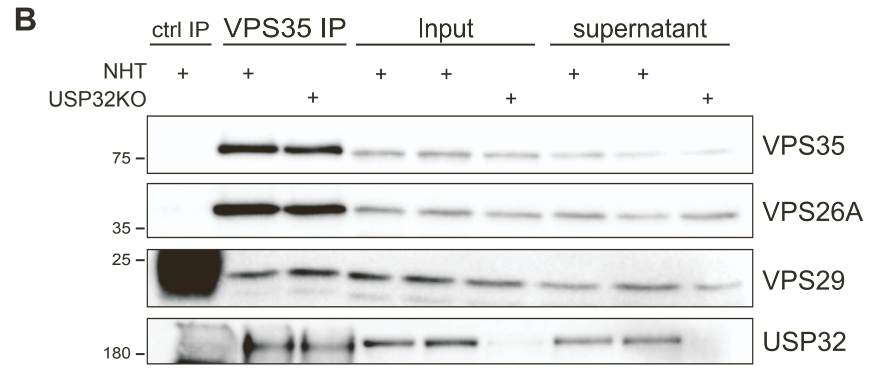
Figure S1. Cellular localization of USP32 (related to Figure 1)

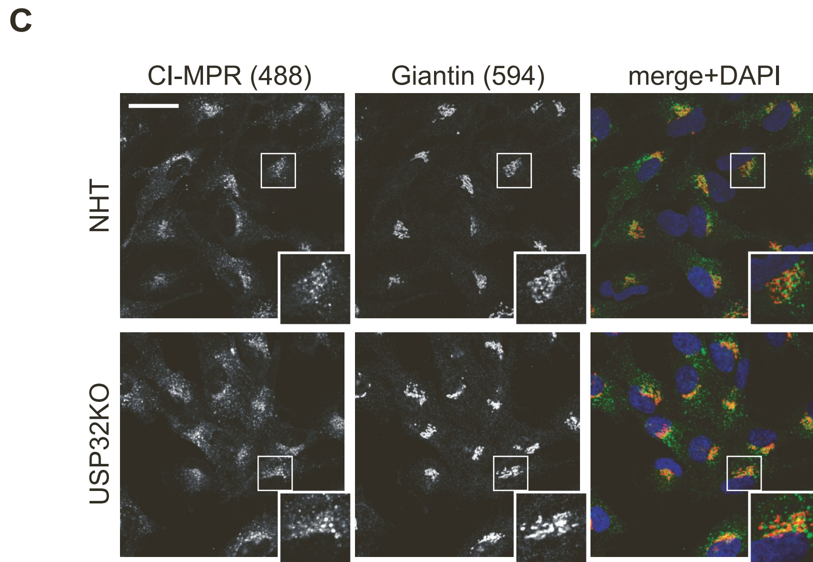
A USP32 is present in the cytoplasmic and membrane fraction in different cell lines. Subcellular fractionation of RPE1, U2OS and MCF7 cells followed by Western blotting.

B Endogenous USP32 localizes at the Golgi apparatus. MCF7 cells were co-stained for USP32 and the Golgi marker GM130. Scale bar = 20 μm.

Figure S2







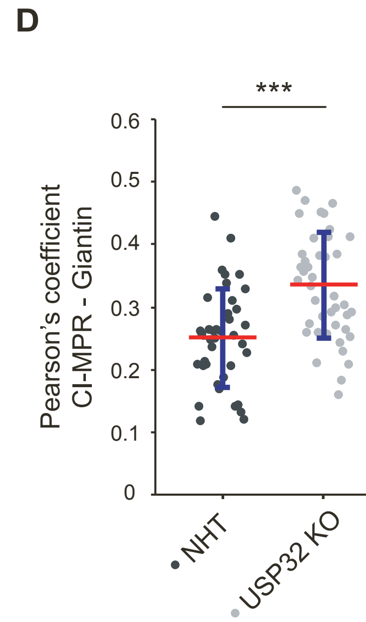


Figure S2. USP32 knockout impacts retrograde transport (related to Figure 2)

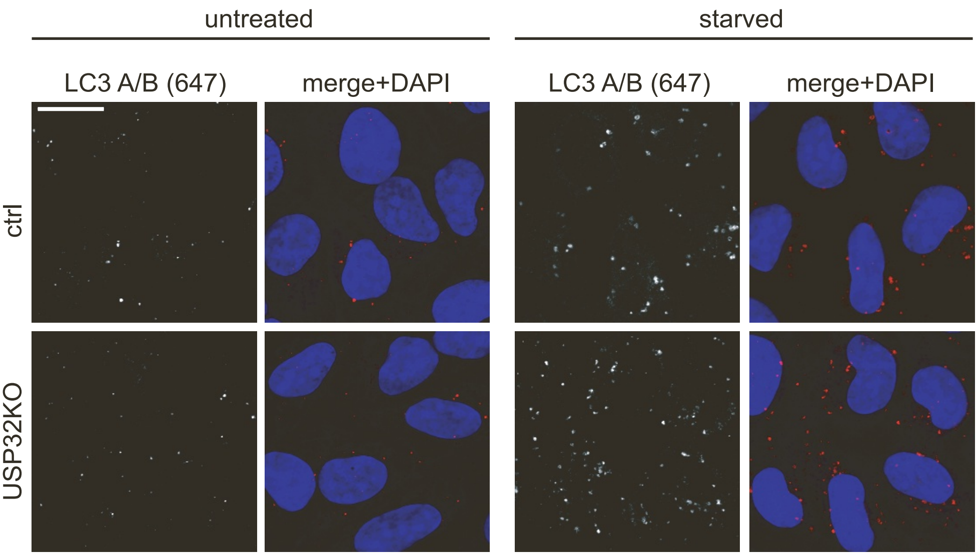
A Abundance of retromer components is stable upon loss of USP32. Lysates of U2OS and RPE1 control and USP32 KO cells were analyzed for retromer components by Western blotting.

B Composition of retromer complex does not change in USP32 KO cells. Endogenous VPS35 was immunoprecipitated from RPE1 NHT and USP32 KO cells with a specific antibody. Protein levels were detected by Western blotting. * VPS29 blot shows overexposed signal of control antibody light chain in first lane.

C CI-MPR localization at the TGN is slightly increased in USP32 KO cells. RPE1 NHT and USP32 KO cells were stained for CI-MPR and the Golgi marker Giantin. Scale bar = 15 µm.

D Quantification of CI-MPR and Giantin co-localization shown as Pearson's coefficient with indicated mean ± SD. The colocalization was quantified across ten images from two independent experiments (*** p-value < 0.001, unpaired Student's *t* test).

Figure S3



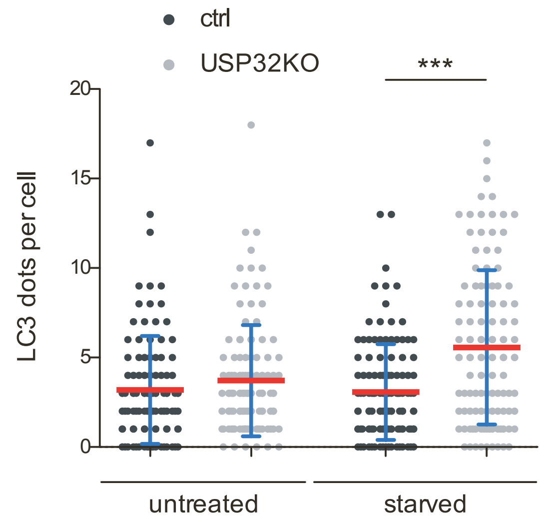


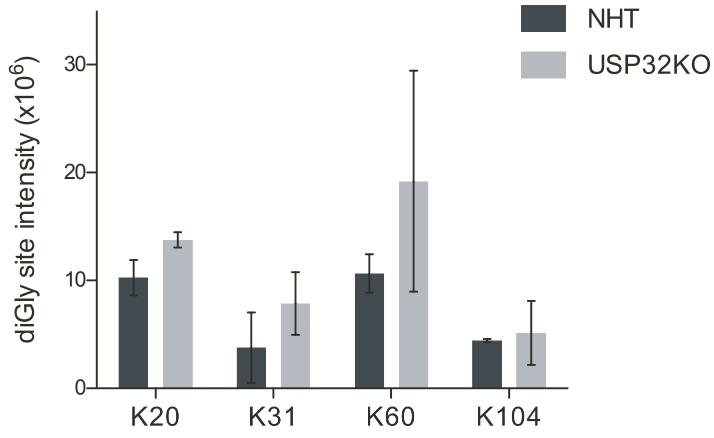
Figure S3. USP32 regulates autophagy (related to Figure 3)

A USP32 depletion impairs lysosomal degradation of autophagosomes in U2OS cells. Representative images of LC3A/B staining in untreated and 2 h amino acid-starved U2OS control (ctrl) and USP32 KO cells. Scale bar = 20 µm.

B Quantification of LC3 positive dots shown as number of dots per cell with indicated mean ± SD, *** $p<0.0001$, unpaired Student's *t* test.

Figure S4

A



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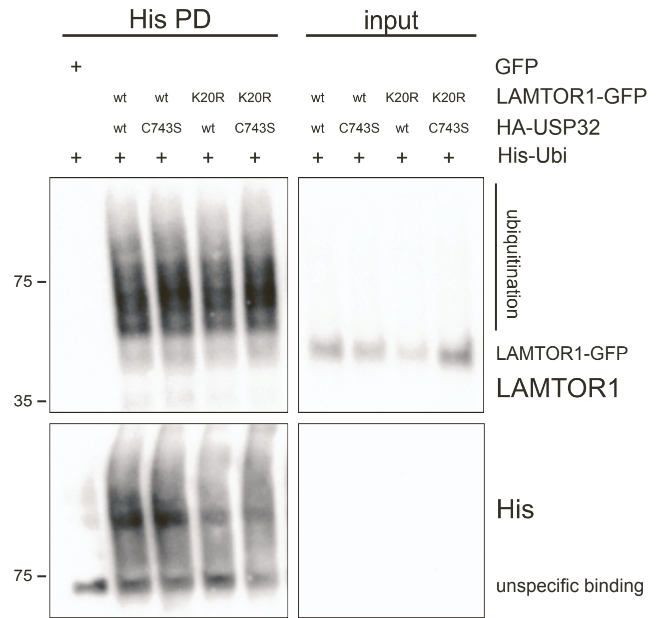

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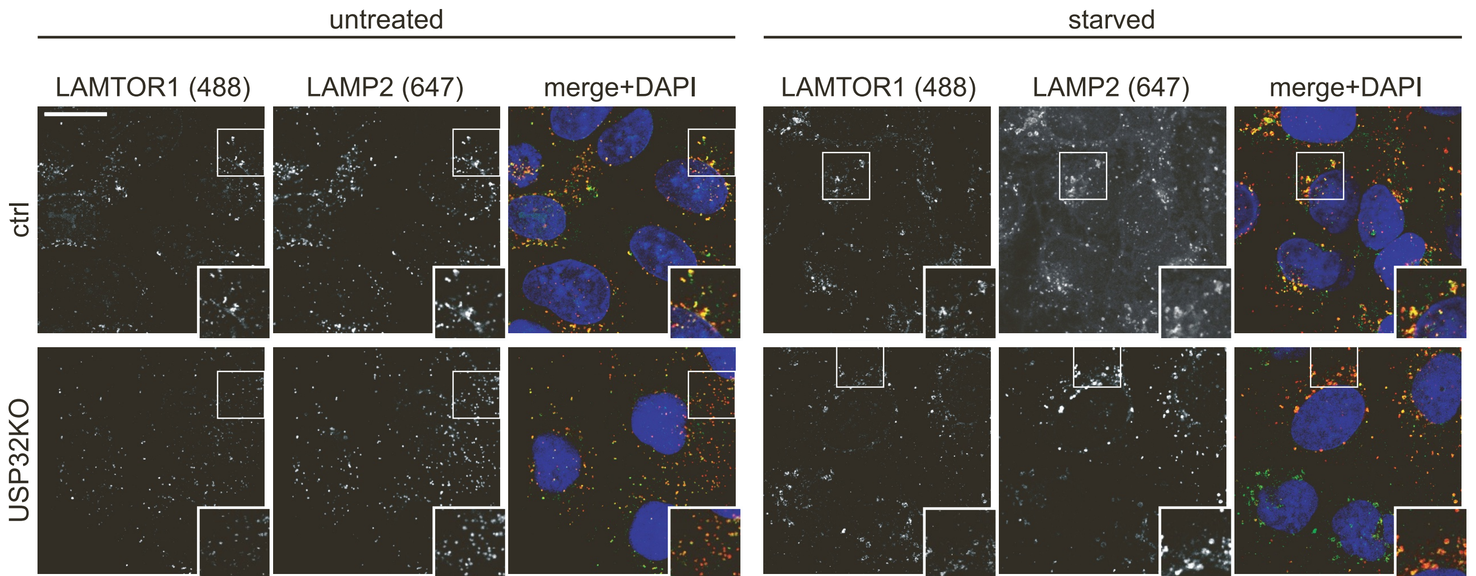

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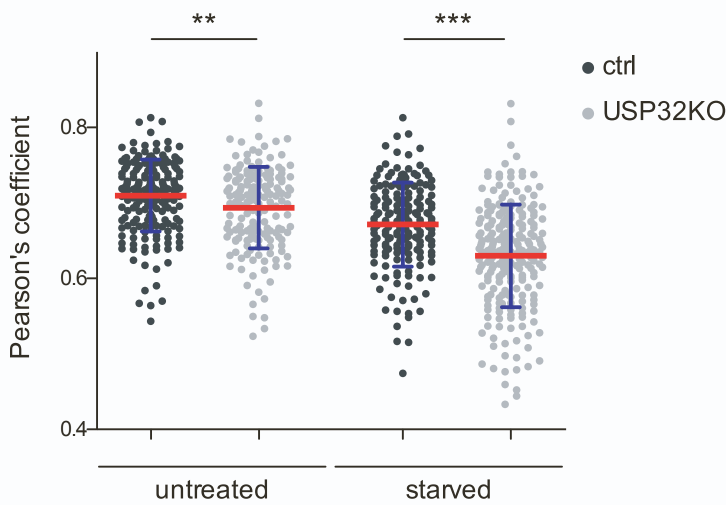

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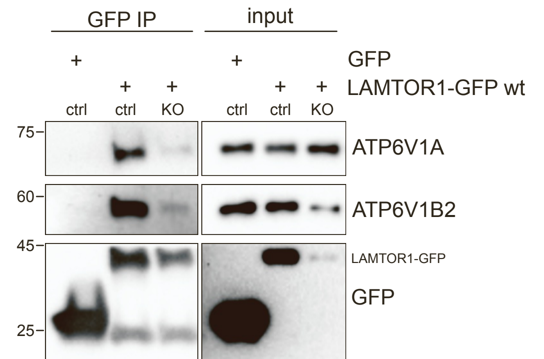


Figure S4. LAMTOR1 ubiquitination regulates its interaction with v-ATPase (related to Figure 4)

A Intensity of ubiquitination sites as identified by MaxQuant, shown as mean +/- SD.

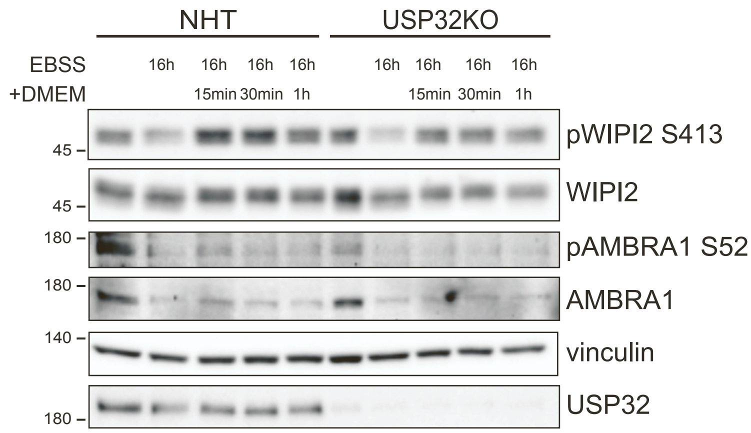
B His-tagged ubiquitin pulldown assay performed in 293 cells co-expressing His-ubiquitin and GFP-LAMTOR1 in the presence of wildtype HA-USP32 or catalytic inactive HA-USP32 (C743S).

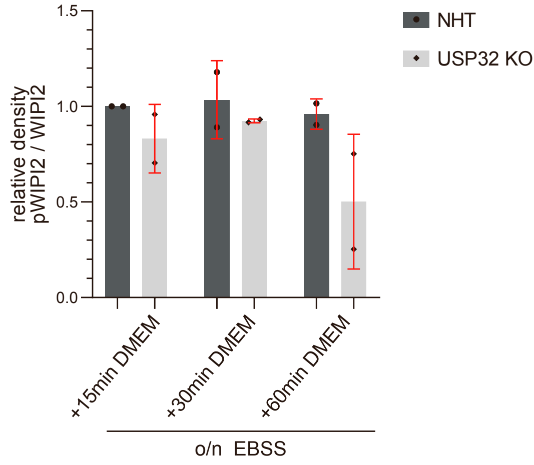
C Lysosomal LAMTOR1 localization is reduced in U2OS USP32 KO cells. Representative immunofluorescence microscopy images of LAMTOR1 and LAMP2 co-staining in untreated and 2 h amino acid-starved U2OS control (ctrl) and USP32 KO cells. Scale bar = 20 µm.

D Quantification of LAMTOR1 co-localization to LAMP2 positive structures shown as Pearson's coefficient (per cell) with indicated mean ± SD, ** $p<0.01$, *** $p<0.0001$, unpaired Student's *t* test (n=3)

E Co-immunoprecipitation of LAMTOR1-GFP and endogenous lysosomal v-ATPase subunits shown in control and USP32 KO cells.

Figure S5





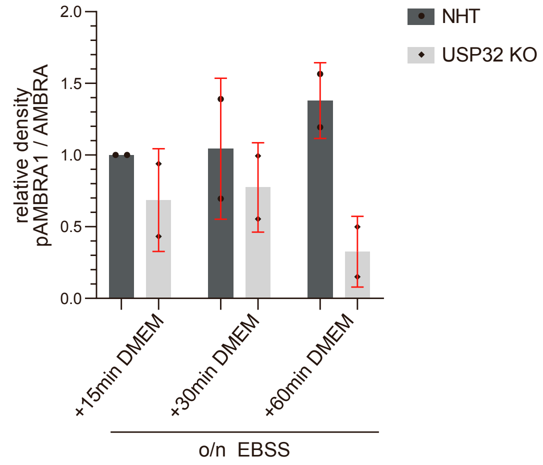


Figure S5. mTORC1 substrate phosphorylation (related to Figure 5)

A RPE1 NHT and USP32 KO cells were amino acid-starved for 16 h (EBSS medium) and subsequently cultured in full medium (DMEM) for the indicated time points. Protein levels were detected by Western blotting.

B Relative quantification of immunoblot of pWIPI2 S413 normalized to total WIPI2 level from panel A (n=2)

C Relative quantification of immunoblot of pAMBRA1 S52 normalized to total AMBRA1 level from panel A (n=2)