Supplemental material

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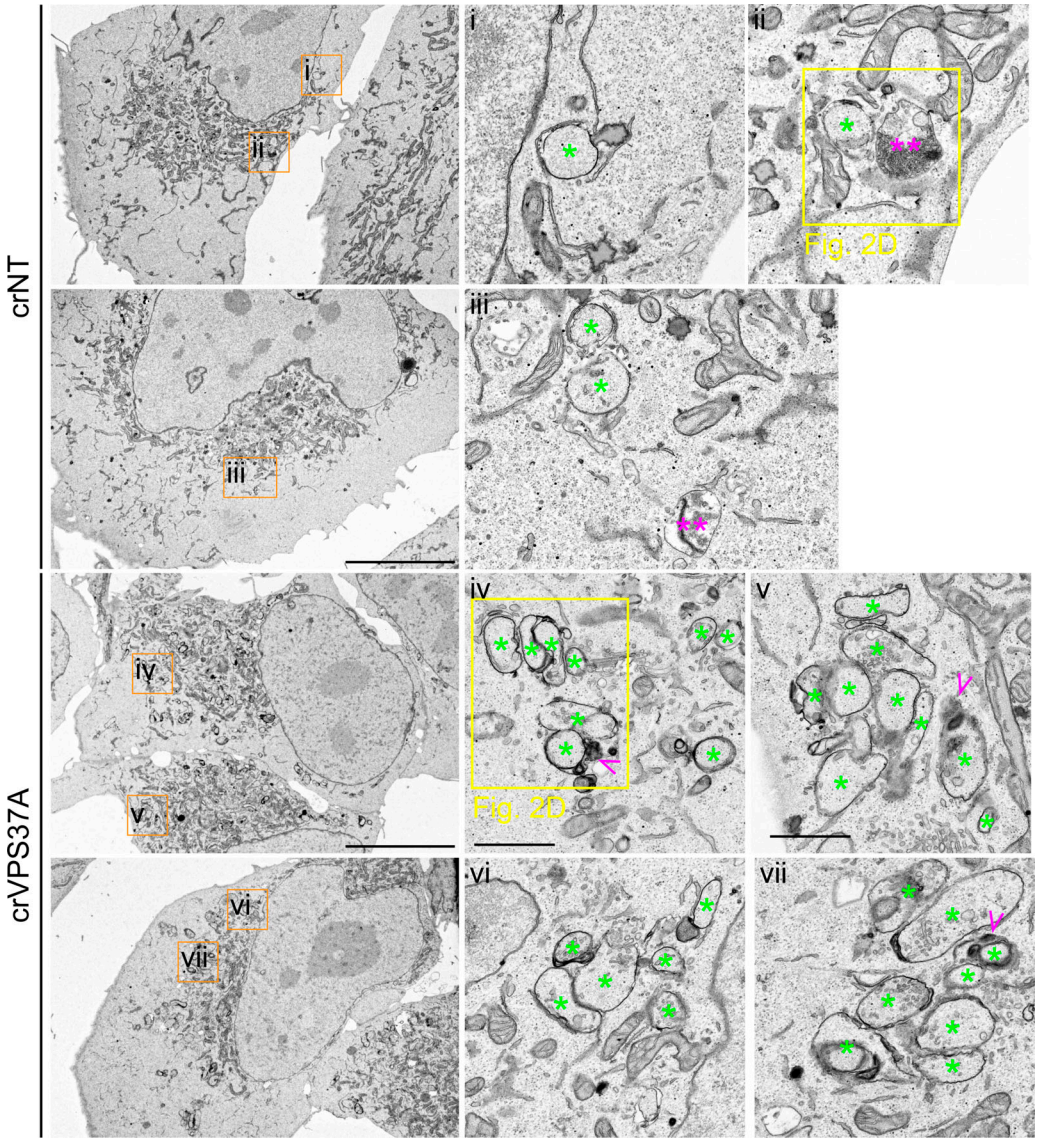

Figure S1. **VPS37A depletion accumulates immature autophagosomal membranes during starvation.** crNT and crVPS37A U-2 OS cells were starved for 3 h and subjected to electron microscopy. The samples were processed in the presence of potassium ferrocyanide. The asterisks, double asterisks, and arrowheads indicate immature autophagic structures, including phagophores, autolysosome-like structures, and lysosome contents in the intermembrane space, respectively. Magnified images in the indicated areas are shown in Fig. 2 D. The scale bars represent 10 µm in the main panels and 1 µm in the magnified images.

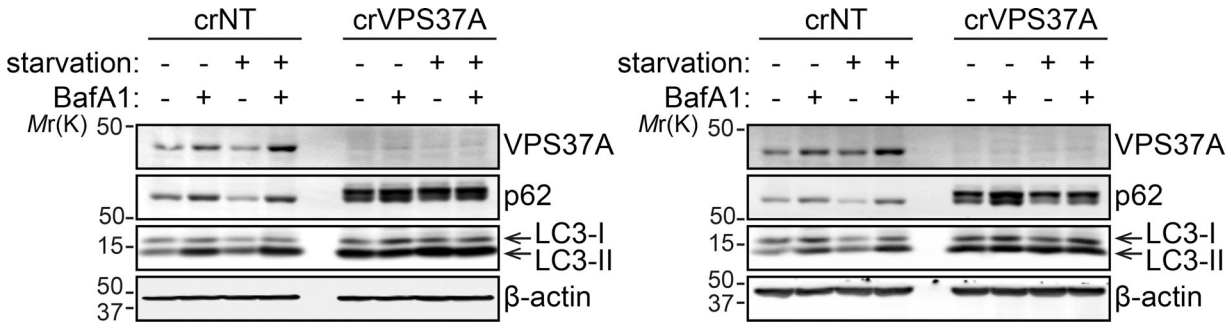

Figure S2. **Lysosomal inhibition increases the level of VPS37A during starvation.** Additional immunoblots used for quantification analyses in Fig. 4, D and E, are shown.

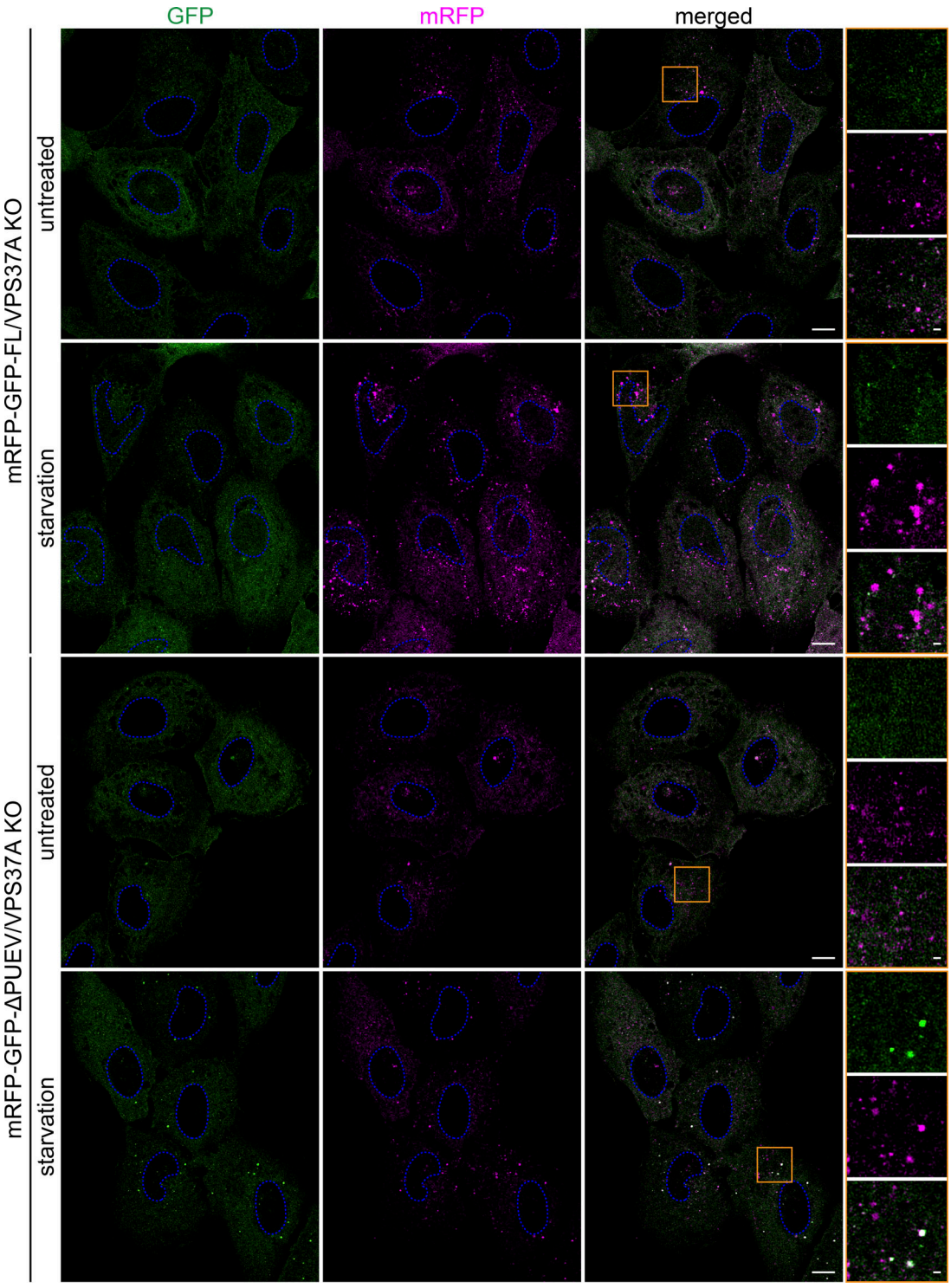

Figure S3. **VPS37A FL undergoes autophagic degradation.** VPS37A KO U-2 OS cells stably expressing mRFP-GFP-VPS37A FL or ΔPUEV were starved for 3 h and subjected to confocal microscopy. Representative images from three independent experiments are shown. Magnified images in the boxed areas are shown in the right panels. The scale bars represent 10 µm in the main panels and 1 µm in the magnified images.

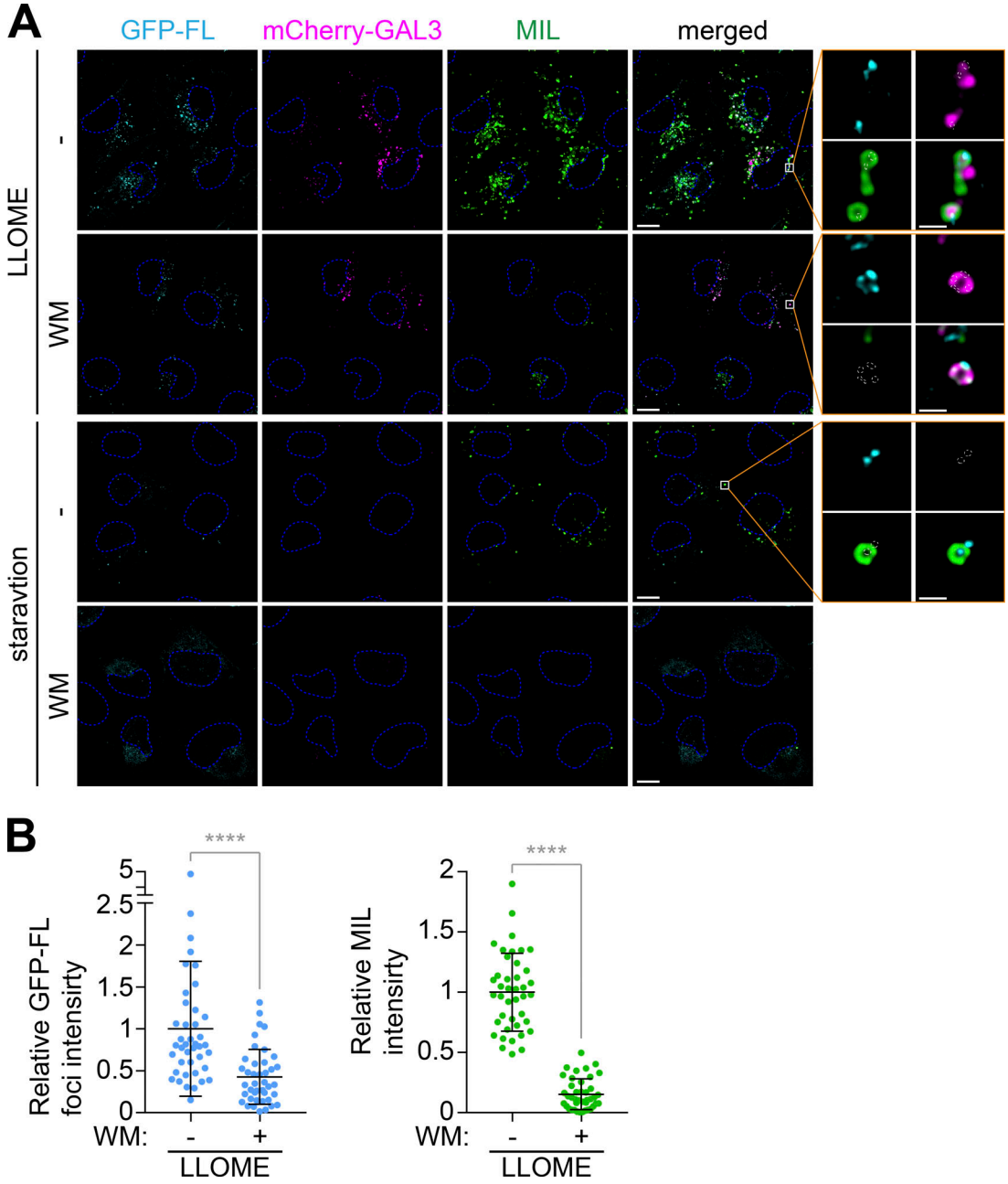

Figure S4. **VPS37A translocates to immature autophagosomal membranes and damaged lysosomal membranes upon LLOME exposure. (A)** VPS37A KO U-2 OS cells stably expressing GFP-VPS37A FL and HT-LC3 were nucleofected with mCherry-GAL3 for 16 h, preincubated with or without 10 µM WM for 10 min followed by the treatment with 1 mM LLOME for 15 min, or starved in the presence or absence of WM for 3 h. Cells were subjected to the HT-LC3 assay using AF660-conjugated MIL and subjected to confocal microscopy. Representative images from two independent experiments are shown. Magnified images in the boxed areas are shown in the right panels. The scale bars represent 10 µm in the main panels and 1 µm in the magnified images. **(B)** The fluorescence intensities of GFP-VPS37A foci (right) and MIL (left) per cell were quantified and normalized to the respective mean fluorescence intensities of LLOME-treated cells (n = 41). Statistical significance was determined by Student's t test. All values are mean ± SD. ****, P ≤ 0.0001.

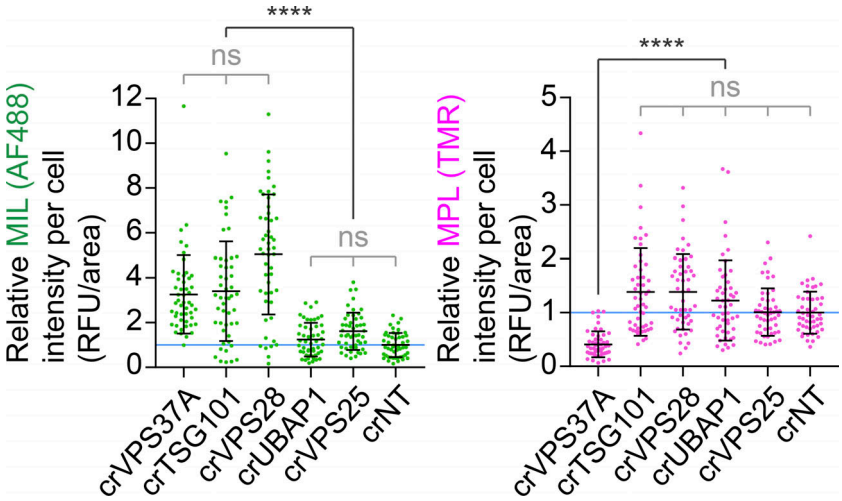

Figure S5. **Loss of the core ESCRT-I components TSG101 and VPS28 accumulates immature autophagosomal membranes.** The cytoplasmic fluorescence intensities of MIL and MPL in each cell in Fig. 8 A were quantified and normalized to the respective mean fluorescence intensities of crNT cells (n = 50). Statistical significance was determined by two-way ANOVA followed by Tukey's multiple comparison test. All values are mean ± SD. ****, $P \leq 0.0001$; ns, not significant.

Provided online are two tables in Excel. Table S1 shows data from the primary screen. Table S2 lists sgRNA sequences used for the experiments.