

SUPPLEMENTAL FIGURE LEGENDS

Supplemental Figure 1

(A) Live fluorescence images (inverted grays) of RPE-1 cells expressing either GFP-Rab45 (left), BLTP3A-mRFP (center), or both proteins together (only BLTP3A is shown) (right) as indicated. Scale bar, 5 μm . High-magnification scale bar, 2 μm .

(B) Genomic sequence of the edited BLTP3A locus (insertion of the V5 epitope) in A549 cell. Blue, small Gly-Ser linkers; green, V5 epitope sequence.

(C) AlphaFold prediction of BLTP3A. The site where the V5 epitope (V904) was inserted is indicated. The long disordered sequence and the C-terminal helix are shown in gray.

(D) Left: Fluorescence image of an RPE-1 cell expressing exogenous BLTP3B-mRFP (inverted grays) and immunolabeled with antibodies against endogenous VAMP7 (shown at right in the high magnification of the squared region in the main field). Scale bar, 5 μm . Right: zooms of different RPE-1 cells expressing exogenous BLTP3B-mRFP (magenta) and immunolabeled with antibodies (green) against endogenous VAMP4 or ATG9A. Individual channels are shown as inverted grays. Merge of channels on bottom. Scale bar, 1 μm .

(E) Live fluorescence image of RPE-1 cell expressing exogenous RFP-LRRK1^{K746G} (green), which is primarily cytosolic. Scale bar, 5 μm .

881 **(F)** Live fluorescence images (inverted grays) of RPE-1 cells expressing exogenous GFP-
882 LRRK1^{K746G} (left) and BLTP3A-mRFP (right). Scale bar, 10 μ m. High magnifications of areas of
883 overlap (orange box) or no overlap (blue box) of the fluorescence of exogenous LRRK1 (green)
884 and BLTP3A (magenta) are shown at right. Scale bar, 2 μ m.

885 **(G)** RPE-1 cell expressing BLTP3A-mRFP, GFP-LRRK1^{K746G} and mito-BFP. CLEM of a BLTP3A-
886 mRFP and GFP-LRRK1^{K746G} positive region showing abundance of small vesicles associated with
887 lysosomes in the corresponding EM image. The mito-BFP fluorescence is not shown and was
888 used for alignment. Scale bar, 2 μ m for the fluorescence and 500 nm for the EM).

889 **(H)** Western blot of lysate of RPE-1 cells expressing exogenous RFP-LRRK1^{K746G} or RFP-
890 LRRK1^{D1409A} for RFP (to detect LRRK1 fusions), Rab7, phospho-Rab7 S72, and alpha-tubulin as
891 a loading control.

892

893 **Supplemental Figure 2**

894

895 **(A)** Live fluorescence image (inverted grays) of RPE-1 cells expressing GFP-LRRK2 (left) and
896 BLTP3A-mRFP (right). Scale bar, 10 μ m. High magnifications of areas of overlap (orange box) or
897 no overlap (blue box) of the LRRK2 (green) and BLTP3A (magenta) fluorescence. Scale bar, 2
898 μ m.

899 **(B)** Live fluorescence image (inverted grays) of COS7 cells expressing GFP-LRRK2 (left) and
900 BLTP3A-mRFP (right). Scale bar, 5 μ m. High magnifications of areas of overlap (orange box) or
901 no overlap (blue box) of the LRRK2 (green) and BLTP3A (magenta) fluorescence. Scale bar, 2
902 μ m.

903 **(C)** Fluorescence images of RPE-1 cells expressing the indicated BLTP3A-mRFP construct.
904 Scale bar, 5 μ m

905 **(D)** AlphaFold3 multimer prediction of full-length MAP1LC3B (green) and a.a. 1110-1150 of
906 BLTP3A (magenta). Arrows indicate key residues of the LIR motif of BLTP3A.

907 **(E)** AlphaFold3 multimer predictions of mATG8 proteins and aa 1110-1150 of BLTP3A with and
908 without the LIR motif (Δ LIR).

909

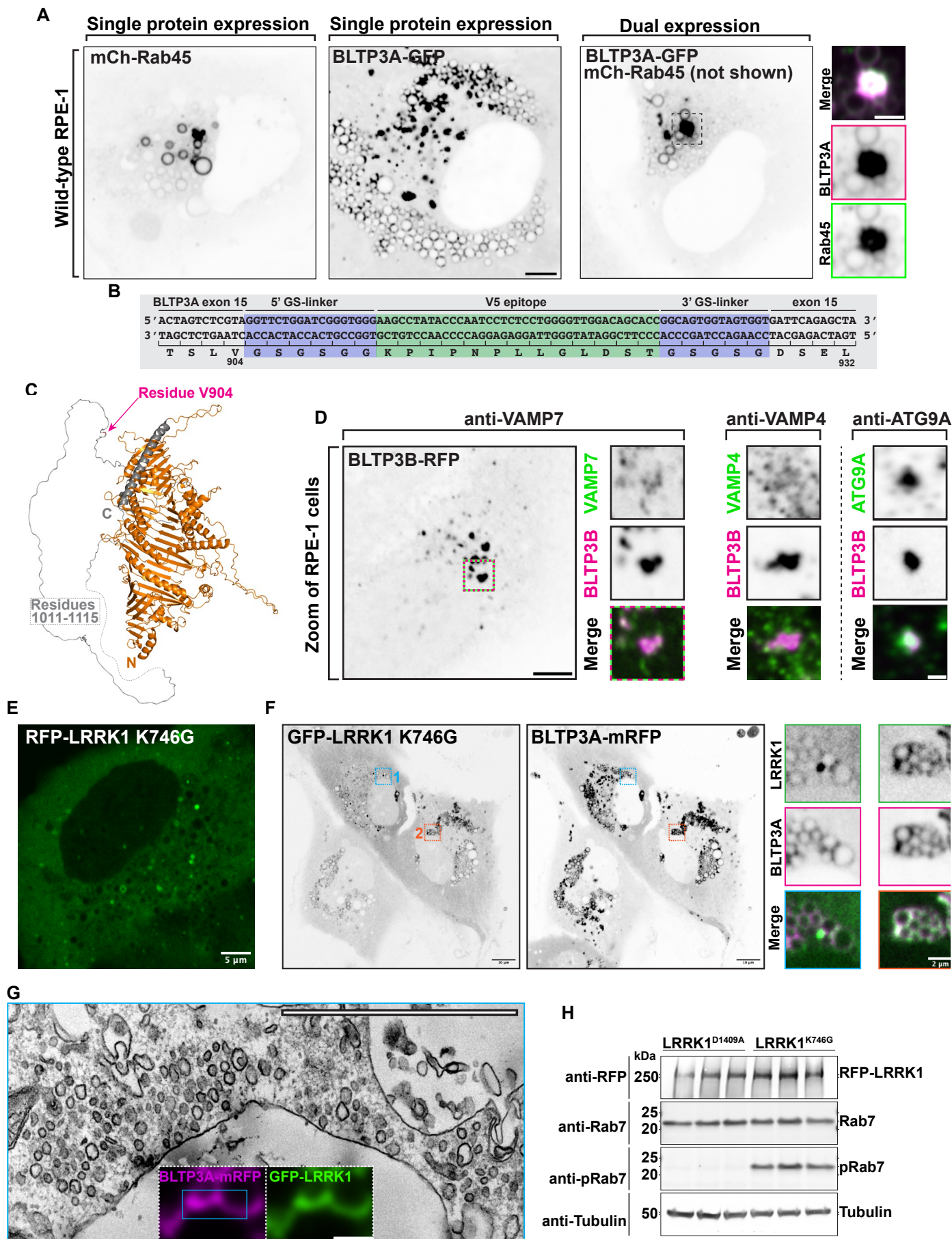
910 **Supplemental Figure 3**

911

912 Time-series of live fluorescence images (inverted grays) of BLTP3A-mRFP and GFP-LC3B before
913 and after addition of GPN. Arrowheads point to lysosomes where BLTP3A and LC3B decorate
914 the entire profile upon addition of GPN. Time, seconds. Scale bar, 5 μ m.

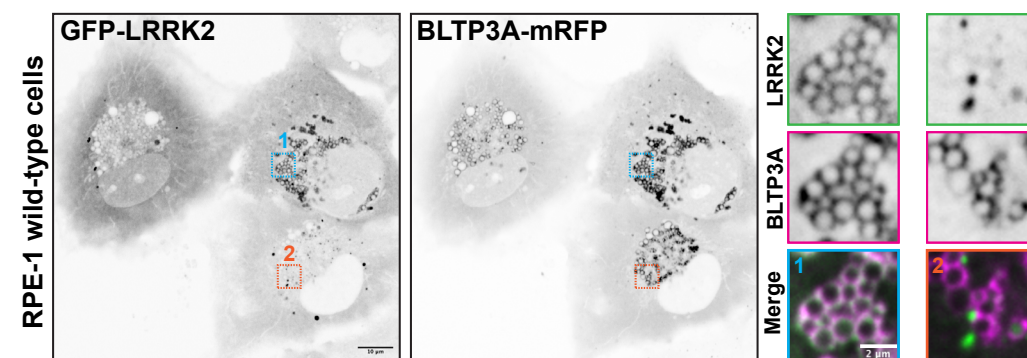
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Supplemental Figure 1

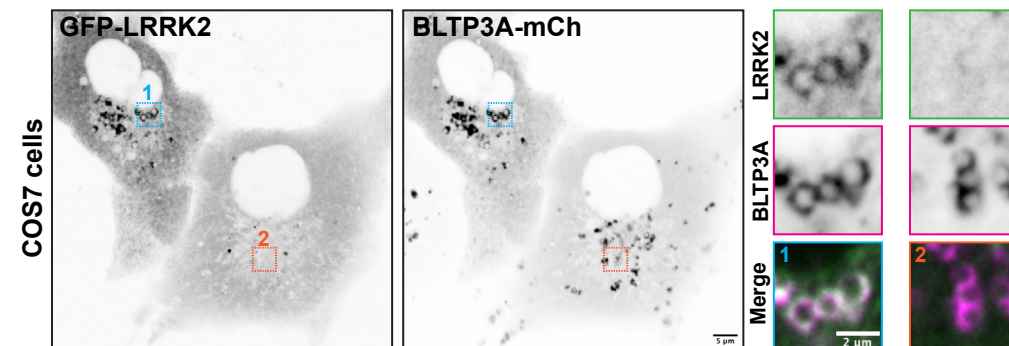


Supplemental Figure 2

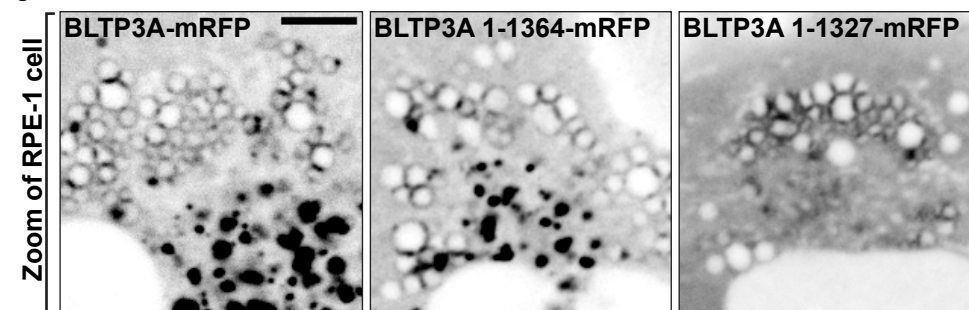
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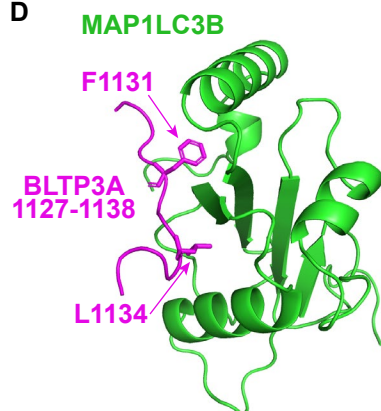
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C



D



E

AF3 predictions of BLTP3A with mAtg8 proteins

| | BLTP3A: 1110-1150 | | 1110-1150 ΔLIR | |
|-----------|-------------------|------|----------------|------|
| | ipTM | pTM | ipTM | pTM |
| MAP1LC3A | 0.66 | 0.7 | 0.31 | 0.69 |
| MAP1LC3B | 0.56 | 0.68 | 0.32 | 0.7 |
| MAP1LC3C | 0.57 | 0.62 | 0.32 | 0.62 |
| GABARAP | 0.74 | 0.76 | 0.55 | 0.74 |
| GABARAPL1 | 0.73 | 0.75 | 0.52 | 0.72 |
| GABARAPL2 | 0.72 | 0.74 | 0.53 | 0.73 |

Supplemental Figure 3

