Filipek et al., https://doi.org/10.1083/jcb.201703061

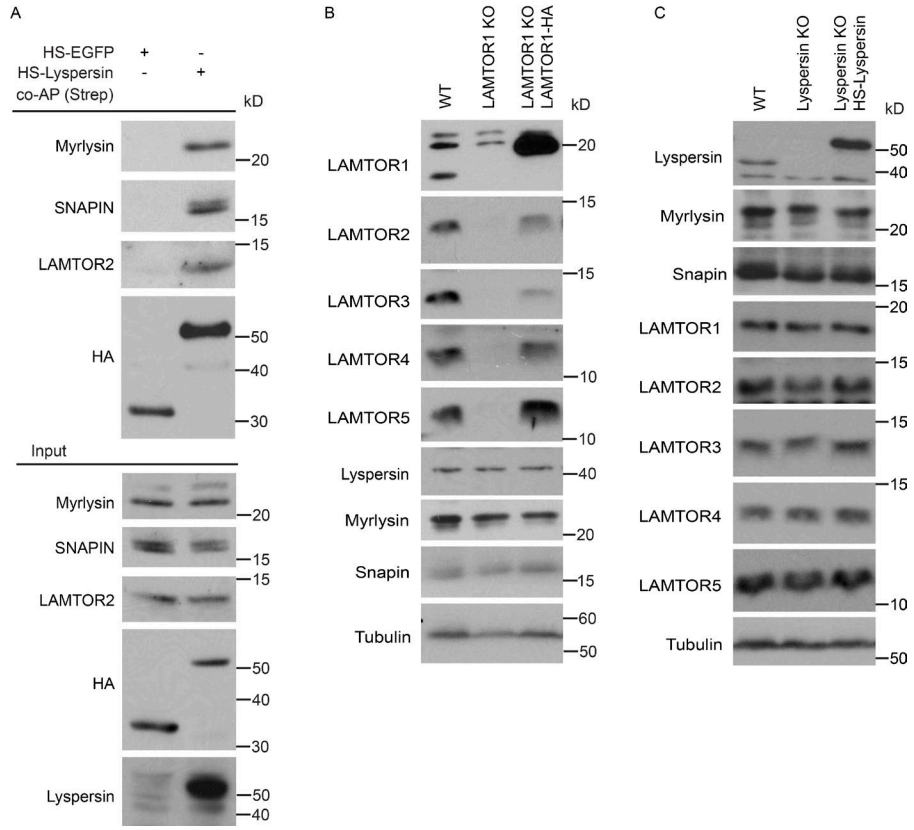


Figure S1. **Exogenously expressed HS-lyspersin incorporates into BORC and is able to interact with the LAMTOR complex; stability of LAMTOR complex and BORC upon LAMTOR1 or lyspersin deletion.** Related to Fig. 1. (A) HS-lyspersin incorporates into BORC in the presence of the endogenous lyspersin and is able to interact with LAMTOR complex. Strep-purified HS-lyspersin or HS-EGFP from transiently transfected WT HeLa cells was analyzed by immunoblotting. (B) Deletion of LAMTOR1 depletes cells from the remaining LAMTOR subunits (LAMTOR2, 3, 4, and 5), whereas BORC subunits (myrlysin, snapin) remain unaffected. The protein levels of LAMTOR2, 3, 4, and 5 are restored by reconstitution of the LAMTOR1 KO with LAMTOR1-HA. Immunoblotting analysis of lysates from WT, LAMTOR1 KO, and rescue cell lines. (C) The protein levels of BORC (myrlysin, snapin) and LAMTOR (LAMTOR1, 2, 3, 4, and 5) subunits are unaffected by lyspersin deletion. Immunoblotting analysis of lysates from WT, lyspersin KO, and rescue cell lines.

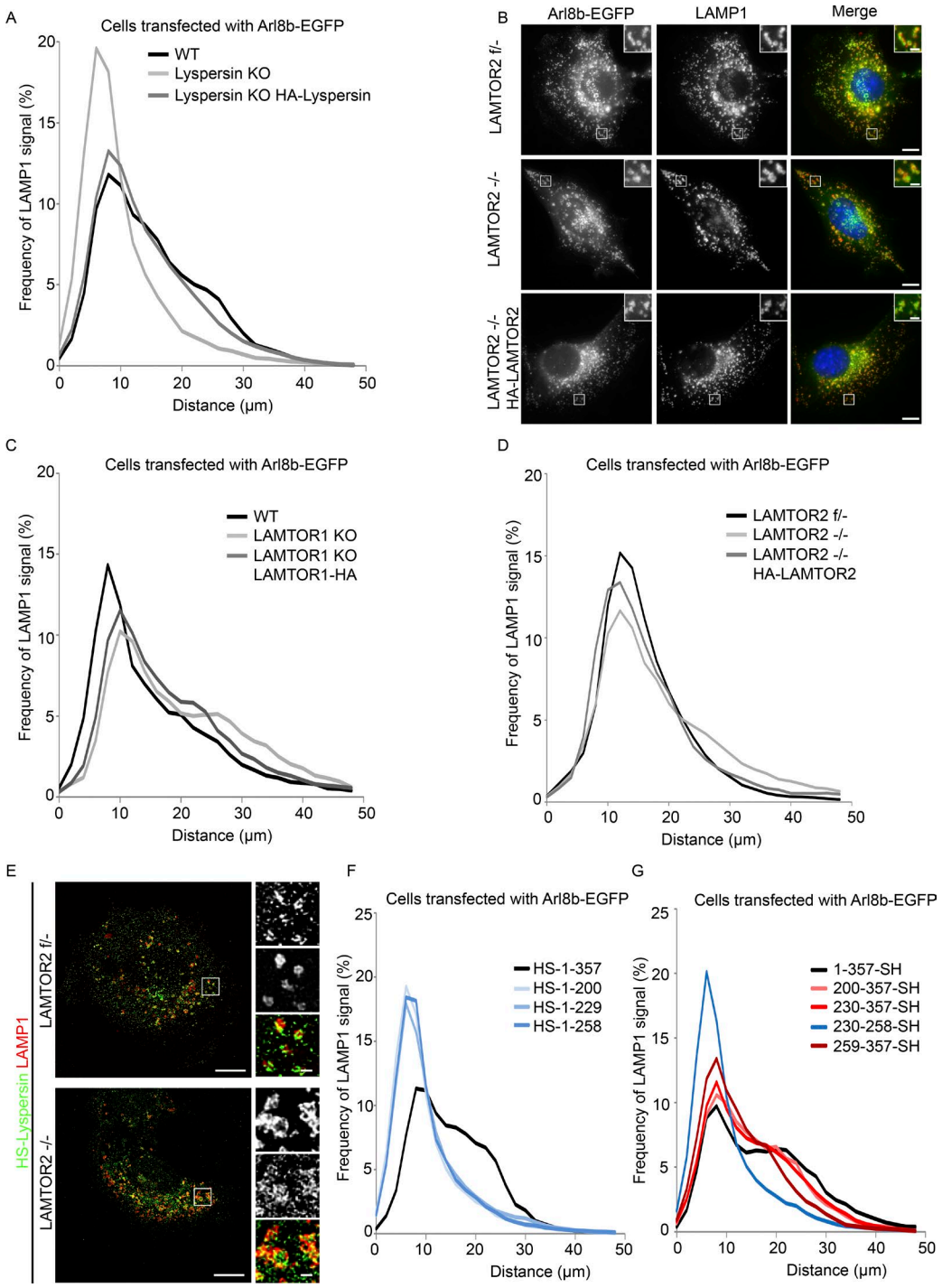


Figure S2. **Functional epistatic aligment of Arl8b, lyspersin, and the LAMTOR complex highlights the importance of the C-terminal domain of lyspersin in the regulation of lysosomal positioning.** Related to Fig. 5. (A) Lysosomes are perinuclear in lyspersin KO cells transfected with Arl8b-EGFP. Indirect IF images of HeLa WT, lyspersin KO, and rescue cell lines transfected with Arl8b-EGFP. Images were taken on an epifluorescent microscope and analyzed using ImageJ software with our macro RadialIntensityProfile (Materials and methods). Depicted is the signal intensity of LAMP1 found at a given distance from the center of the nucleus. Number of cells per genotype, $n \geq 25$. (B) LAMTOR2 deletion does not abolish Arl8b recruitment to late endosomes/lysosomes. Indirect IF images of LAMTOR2 f/−, LAMTOR2 −/−, and rescue MEFs transfected with Arl8b-EGFP. Merged and single-channel images of endogenous LAMP1 (red) and Arl8b-EGFP (green) are shown. Representative images are shown. Bars: 10 µm; (inset) 2 µm. (C) Arl8b-EGFP expression does not affect the peripheral redistribution of lysosomes found in LAMTOR1 KO cells. Cells were prepared and analyzed as in A. Number of cells per genotype, $n \geq 25$. (D) Arl8b-EGFP expression does not affect the peripheral redistribution of lysosomes found in LAMTOR2 KO MEFs. Quantification of LAMP1 signal distribution of cells from (B). Cells were prepared and analyzed as in A. Number of cells per genotype, $n \geq 25$. (E) HS-lyspersin localizes to late endosomes in a LAM TOR2-independent manner. LAMTOR2 f/− and LAMTOR2 −/− MEFs were analyzed. Shown are representative, single-plane confocal images of indirect IF of SH-tagged lyspersin (green) and endogenous endosomal marker LAMP1 (red). Representative images are shown. Bars: 10 µm; (inset) 1 µm. (F and G) Full-length lyspersin (black) and truncation mutants containing the C-domain (red) are able to restore the lysosomal distribution defect characteristic of the lyspersin KO. In contrast, truncation mutants lacking the C-domain (blue) do not rescue the perinuclear accumulation of lysosomes. Indirect IF images of lyspersin KO transfected with Arl8b-EGFP and the indicated lyspersin truncation mutants. Cells were analyzed as in A. Number of cells per condition, $n \geq 25$.

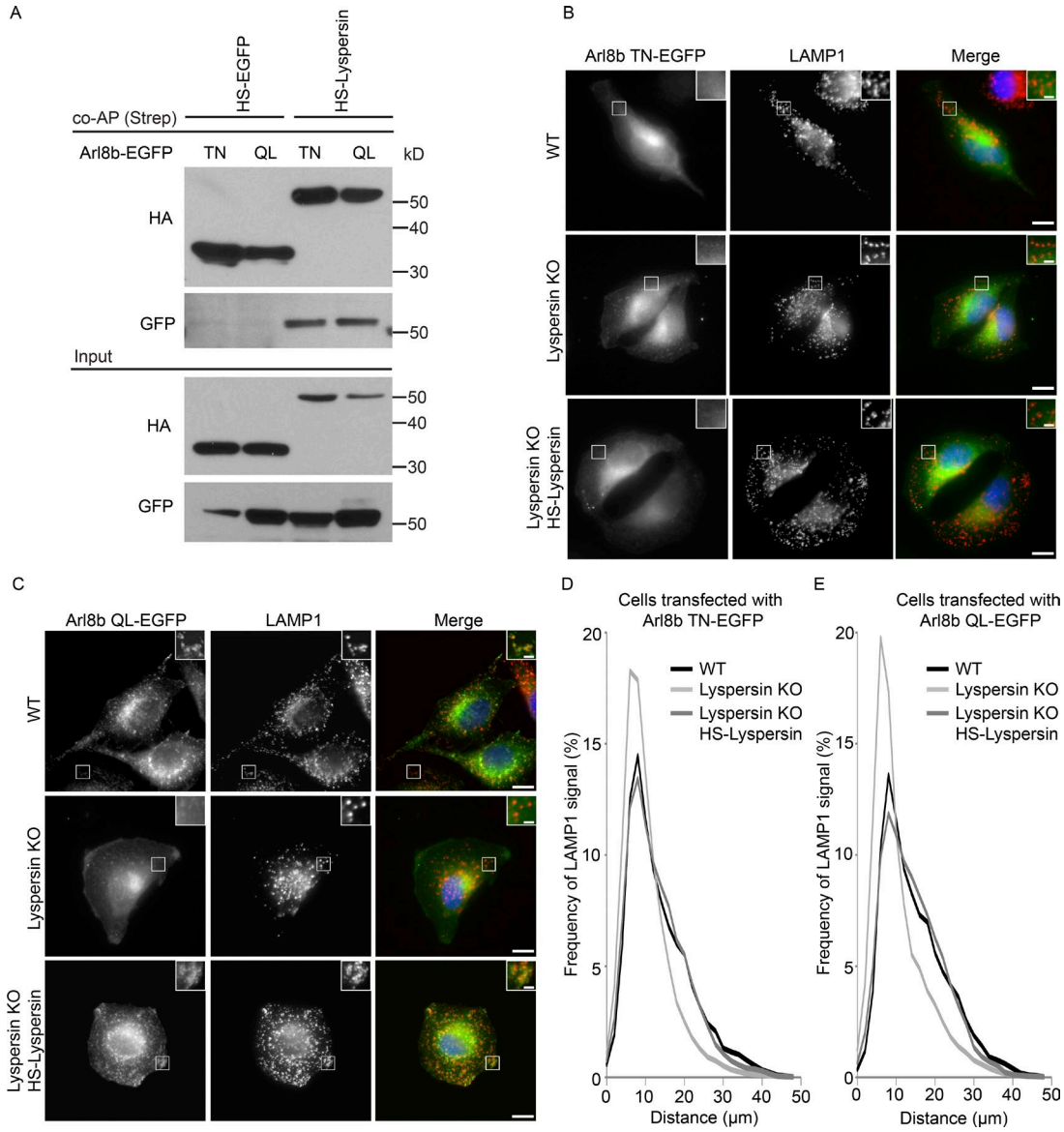


Figure S3. **Lyspersin interacts with Arl8b independent of its GDP/GTP load.** Related to Fig. 5. (A) Lyspersin (BORC) interacts with GDP- and GTP-bound Arl8b. HeLa cells stably expressing HS-lyspersin or HS-EGFP were transiently transfected with Arl8b-EGFP mutants that are found in either the GDP-bound (TN) or GTP-bound (QL) form. Shown are immunoblots of input and eluates from the Strep-purified lysates. (B) The deletion of lyspersin has no impact on the diffused distribution of Arl8b TN-EGFP (GDP-bound mutant). Control, lyspersin-depleted, and rescue cells were transfected with Arl8b TN-EGFP. Images depict Arl8b TN-EGFP (green) and indirect IF of endogenous LAMP1 (red). Representative images are shown. Bars: 10 µm; (inset) 2 µm. (C) Lyspersin is required for Arl8b QL-EGFP recruitment to LAMP1-positive endosomes. Control, lyspersin-depleted, and rescue cells were transfected with Arl8b QL-EGFP. Images show Arl8b QL-EGFP (green) and indirect IF of endogenous LAMP1 (red). Bars: 10 µm; (inset) 2 µm. (D) Lysosomes cluster in the perinuclear region in lyspersin KO cells transfected with Arl8b TN-EGFP. Cells were prepared as in B. Images were taken on an epifluorescent microscope and analyzed using ImageJ software and our macro RadialIntensityProfile (Materials and methods). Depicted is the signal intensity of LAMP1 found at a given distance from the center of the nucleus. Number of cells per genotype, $n \geq 25$. (E) Lysosomes cluster in the perinuclear region in lyspersin KO cells transfected with Arl8b QL-EGFP. Cells were prepared as in C and analyzed as described in D.

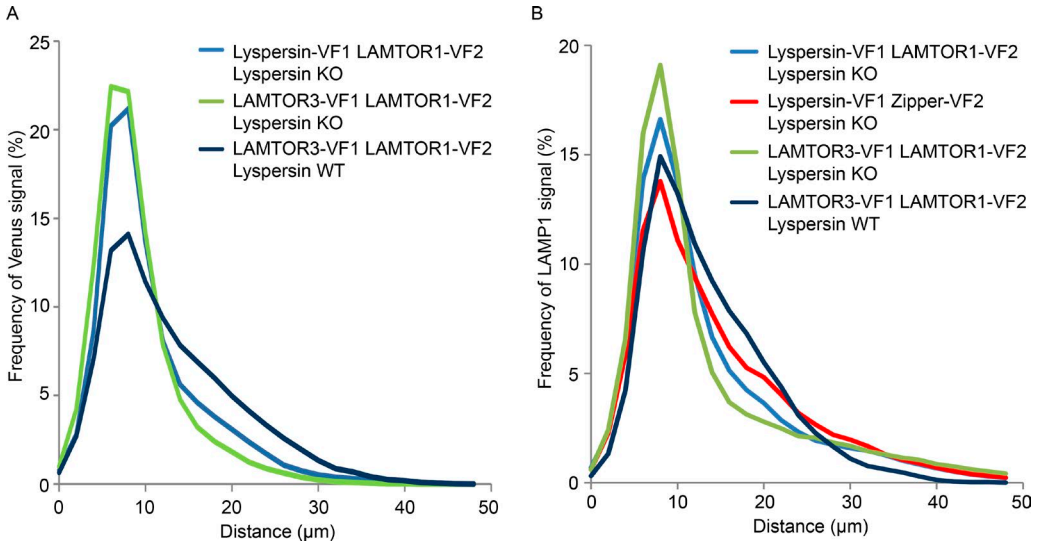


Figure S4. **Quantification of Venus and LAMP1 signal in the PCA assay.** Related to Fig. 6. (A) Vesicles positive for lyspersin-LAMTOR1 or LAMTOR3-LAMTOR1 interactions accumulate in the perinuclear region of lyspersin KO cells. In WT cells, the lysosomal Venus signal from LAMTOR3-LAMTOR1 interaction is redistributed toward the cell periphery. Cells were stained for nucleus (Hoechst). Images were taken on an epifluorescent microscope and analyzed using ImageJ software with our macro RadialIntensityProfile (Materials and methods). Depicted is the signal intensity of Venus found at a given distance from the center of nucleus. Number of cells per genotype, $n \geq 25$. (B) Lyspersin KO cells transfected with lyspersin-VF1 LAMTOR1-VF2 show an intermediate distribution of LAMP1-positive vesicles. Vesicles are more perinuclear than lyspersin KO reconstituted with lyspersin-VF1, Zipper-VF2, but more peripheral than lyspersin KO transfected with LAMTOR3-VF1, LAMTOR1-VF2. The lyspersin-VF1 LAMTOR1-VF2 results are a consequence of averaging the late endosomal distribution of perinuclear (Venus-positive) and peripheral (Venus-negative) vesicles. Cells treated as in A. Depicted is signal intensity of LAMP1 found at a given distance from the center of nucleus. Number of cells per genotype, $n \geq 25$.

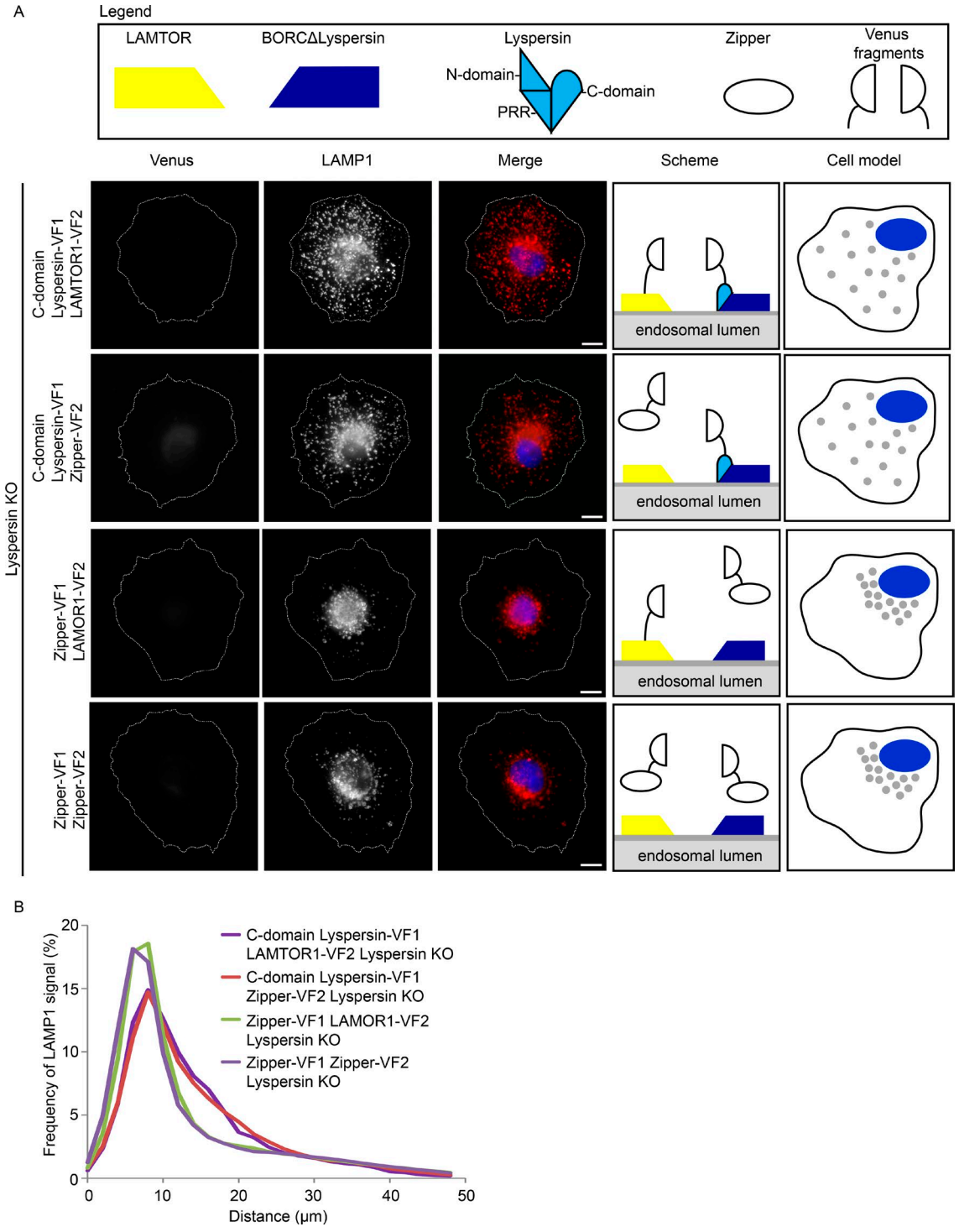
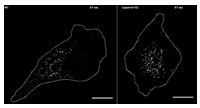


Figure S5. **Additional PCA assay controls.** Related to Fig. 6. (A) C-domain lyspersin-VF1 interacts with neither LAMTOR1-VF2 nor Zipper-VF2. However, this domain is sufficient to restore the transport of late endosomes toward the cell periphery in lyspersin-deficient cells. No interaction could be detected for the cotransfections of Zipper-VF1 and LAMTOR1-VF2 or Zipper-VF1 and Zipper-VF2. Neither of those two conditions reverts the perinuclear accumulation of late endosomes found in lyspersin KO cells. Lyspersin KO cells were cotransfected with the indicated proteins tagged with complementary fragments of the Venus fluorophore. Shown are images of Venus-fluorophore reconstitution (green) and indirect IF of endogenous LAMP1 (red). Dashed line indicates cell borders. Representative images are shown. Bars, 10 µm. Legend: yellow trapezoid, LAMTOR complex; dark blue trapezoid, BORCΔlyspersin; light blue shape with discrimination of individual domains (N-domain, PRR, C-domain), lyspersin; white ellipse, Zipper (negative control); white semicircles, VFs; two green semicircles together, Venus reconstitution and interaction between tagged proteins. For each transfection combination, a graphical scheme is shown that highlights the tagged proteins and their interaction status on late endosomes. The adjacent cell model depicts the presence (green) or absence of interaction (gray) and the overall position of late endosomes in the transfected cells. (B) Lyspersin KO cells transfected with combinations containing C-domain lyspersin-VF1 show LAMP1-positive vesicles with a more peripheral distribution than that observed in lyspersin KO cells expressing Zipper-VF1 LAMTOR1-VF2 or Zipper-VF1 Zipper-VF2. Cells treated as in A. Depicted is signal intensity of LAMP1 found at a given distance from the center of the nucleus. Number of cells per genotype, $n \geq 25$.



Video 1. **Lysosomal movement in WT and lyspersin KO cells.** Cells were transfected with LAMP1-mCherry and analyzed by time-lapse epifluorescent microscopy. Frames were taken for 2 min at 1-s intervals.

Provided online is Table S1 in Excel, showing the complete mass spectrometry analysis of the LAMTOR, RagA, RagC, and BORC interactomes. The supplemental text file contains the custom software, RadialIntensityProfile, which is a FIJI and ImageJ macro for analyzing the radial distribution of a fluorescent signal of interest in relation to a cell nucleus.