Figure S1. Lactate dehydrogenase release after ionomycin treatment
Lactate dehydrogenase activity (Cayman Chemical) was measured in the apical and basolateral media of filter-grown MDCK cells after a 10 minute treatment with 0, 5 or 10 µM ionomycin.

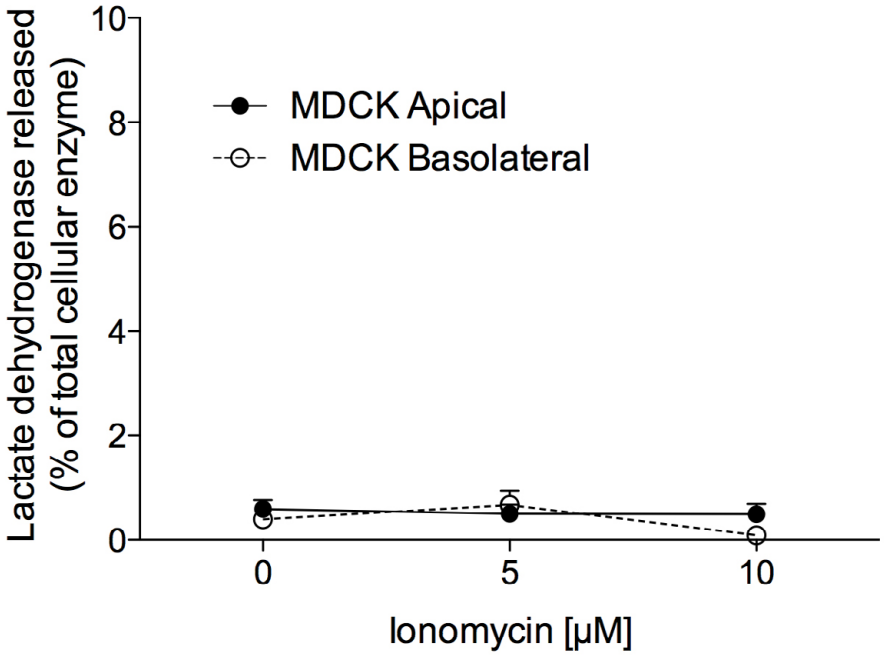
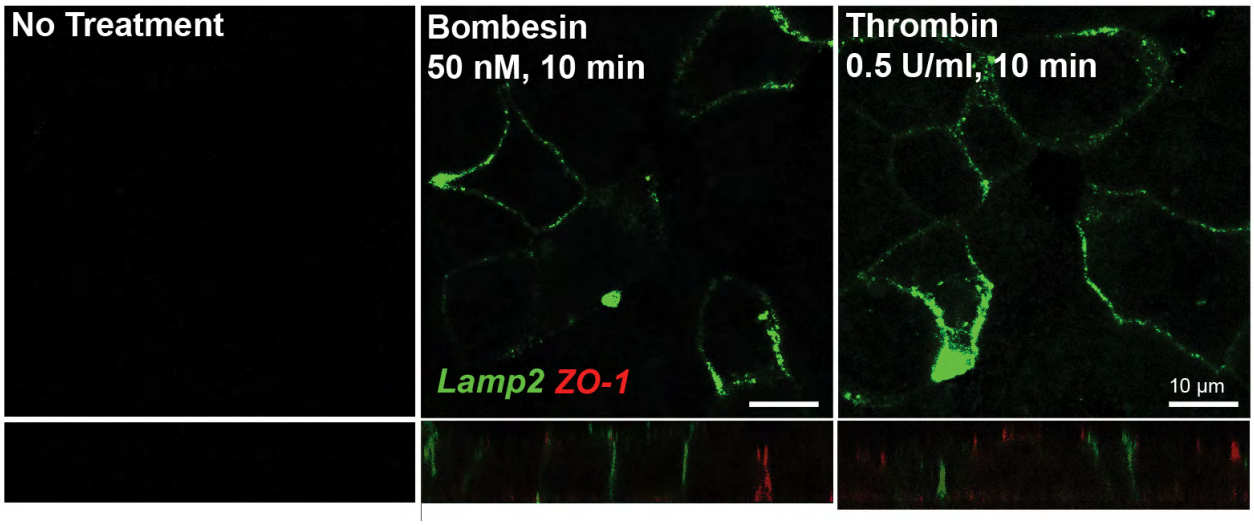


Figure S2. Lysosome exocytosis in MDCK cells induced by thrombin and bombesin.
MDCK cells were treated with either bombesin or thrombin (Sigma), labeled on ice with the LAMP-2 antibody, fixed and stained for the tight junction marker ZO-1 and the relevant secondary antibodies as described in the main text.



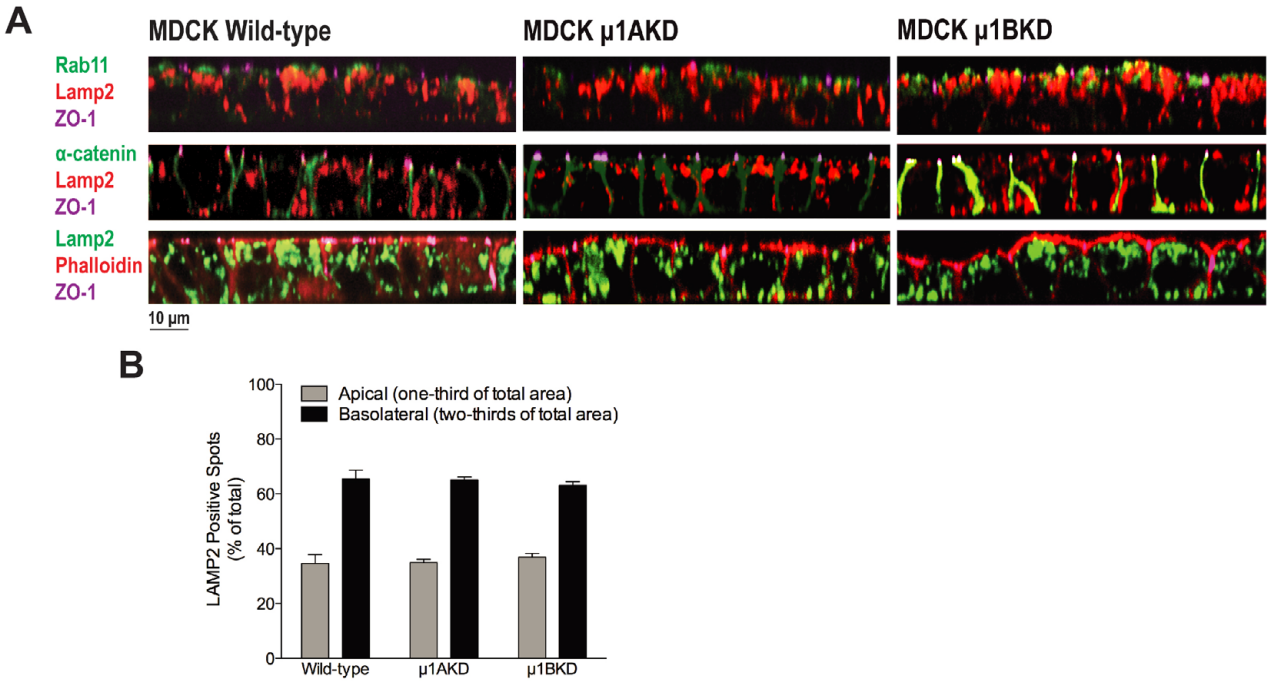


Figure S3. Distribution of lysosomes in MDCK cells.
A, Confocal x-z sections of LAMP2 staining in wild-type and µ1BKD MDCK cells with apical (rab11) and basolateral (α-catenin) markers or with phalloidin, which stains both the apical and basolateral membranes. In the top two rows, the apical recycling endosome marker rab11 and the basolateral α-catenin are in green, LAMP2 is in red and the tight junction protein ZO-1 in purple. In the bottom row, LAMP2 is in green and phalloidin is in red. Antibodies used were: rabbit polyclonal to rab11 (Invitrogen, 1:500), mouse monoclonal to LAMP2 (Abd Serotec, 1:1000), rabbit anti-α-catenin (Sigma, 1:1000) and rat anti-ZO-1. Using the Andor Revolution XD spinning disk confocal microscope, Z-stacks were acquired for 6 different fields, approximately 150 cells/field, with fields that showed as little variance in the height of the cells in Z as possible. B, Quantification of LAMP2 fluorescence above (apical) and below (basolateral) the tight junction (ZO-1) in wild-type, µ1AKD and µ1BKD MDCK cells. Imaris software was used to identify LAMP2 positive vesicles (Spots function) and the apical and basolateral surfaces were demarcated by using the Surfaces function to identify the band of ZO-1 running along the apical border of each cell. The number of LAMP2 positive spots occurring above and below ZO-1 were quantified.

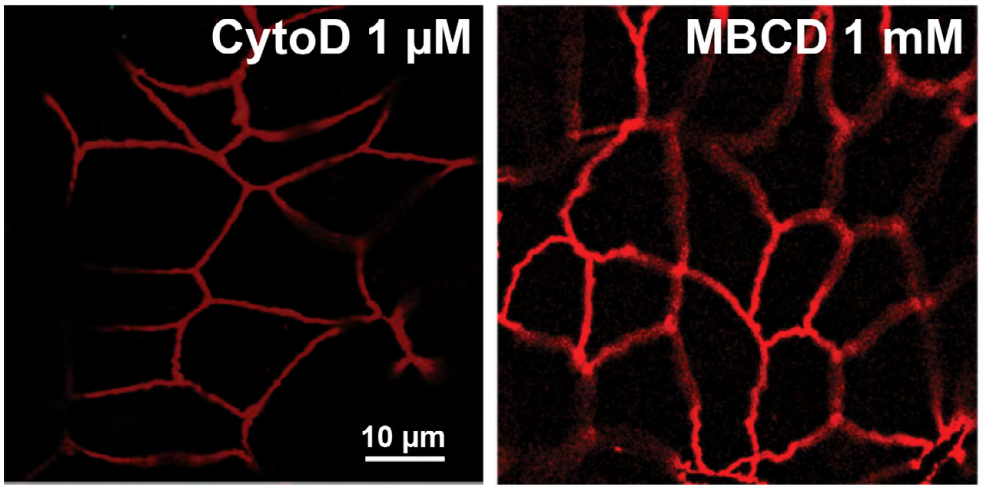


Figure S4. Drug treatments and lysosome exocytosis
Surface LAMP2 staining (green) in MDCK cells treated with the actin depolymerizing drug cytochalasin D (CytoD, 1 µM, for 1 h) or with methyl-beta-cyclodextrin (MBCD, 1 mM for 1 h) shows that these treatments alone did not induce lysosome exocytosis. ZO-1 is in red.

Figure S5. Actin and microtubule disrupting drugs

Polarized MDCK cells were treated with cytochalasin D (1 μM, 1 h) or with nocodazole (10 μM, 1 h) to disrupt actin and microtubules, respectively. Cells were fixed and stained with phalloidin to visualize the actin cytoskeleton and with antibodies to tubulin (Sigma) for microtubules, LAMP2 and syntaxin 4. Cells were imaged on the Andor Revolution XD spinning disk confocal microscope.

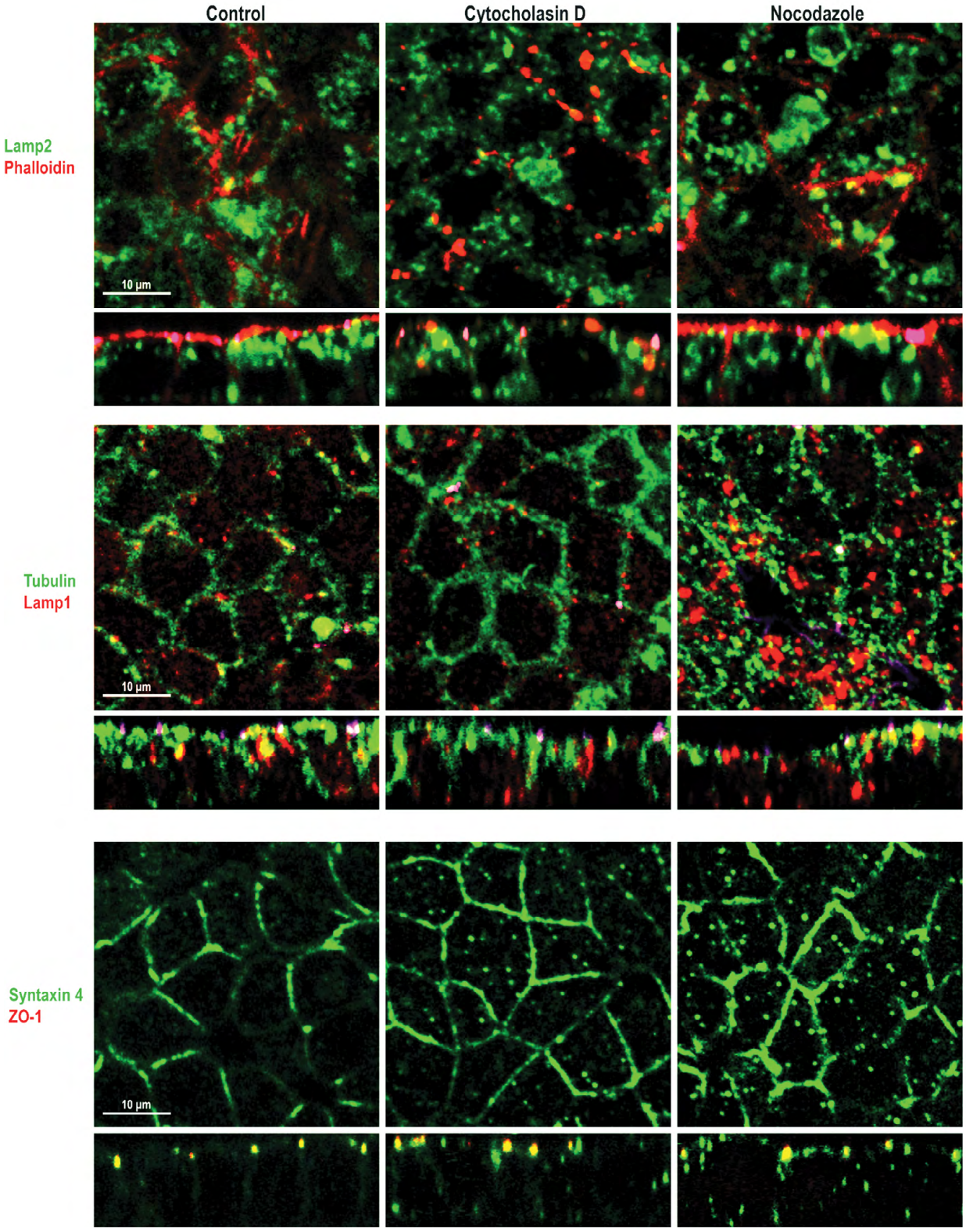
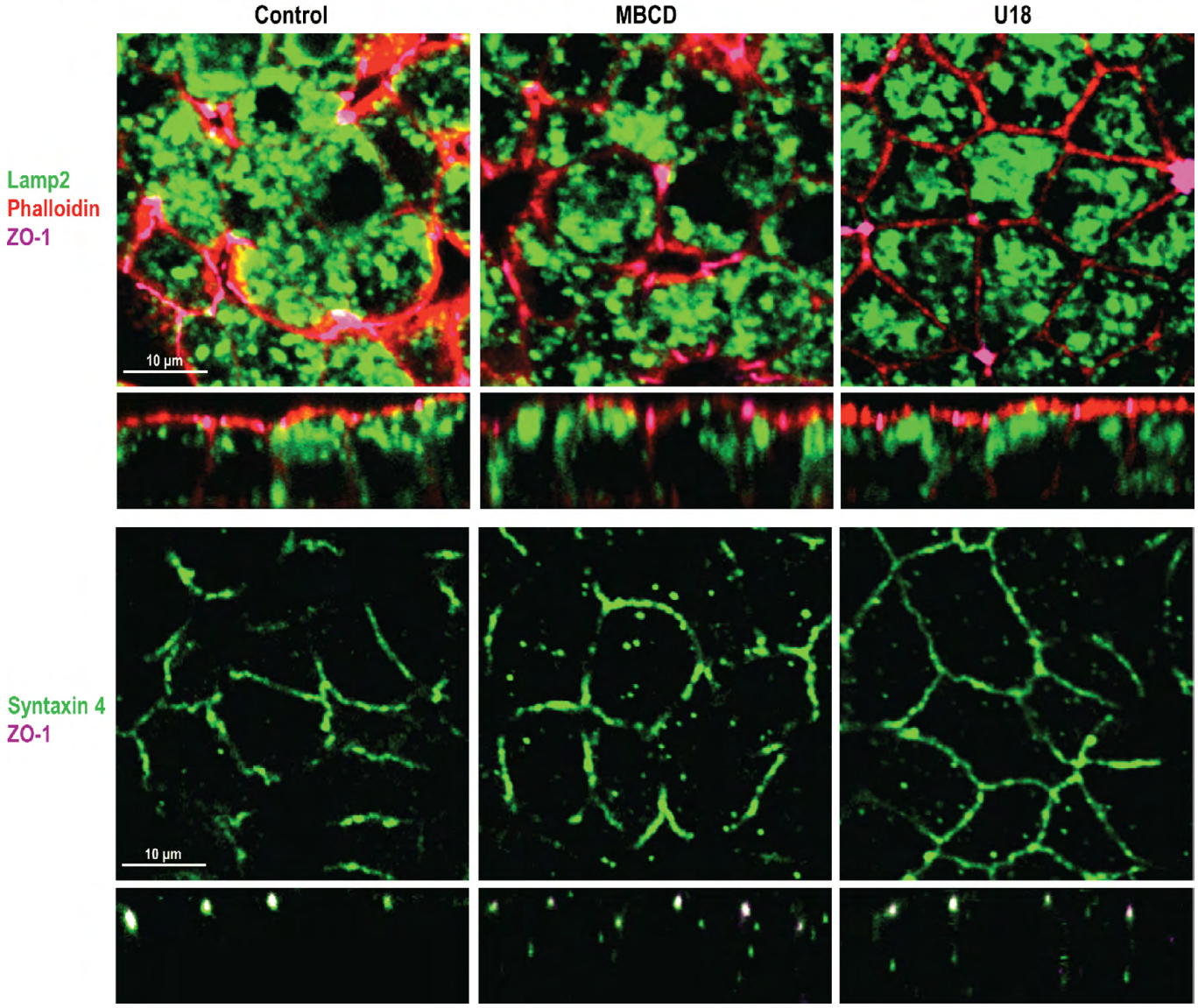


Figure S6. Methyl-beta-cyclodextrin and U18666A treatments

Polarized MDCK cells were treated with methyl-beta-cyclodextrin (MBCD, 1 mM, 1 h) to deplete membrane cholesterol or with U18666A (U18, 1 μM, 16 h) to induce lysosomal cholesterol storage. Cells were fixed and stained with phalloidin to visualize the actin cytoskeleton and with antibodies to LAMP2 and syntaxin 4. Cells were imaged on the Andor Revolution XD spinning disk confocal microscope.



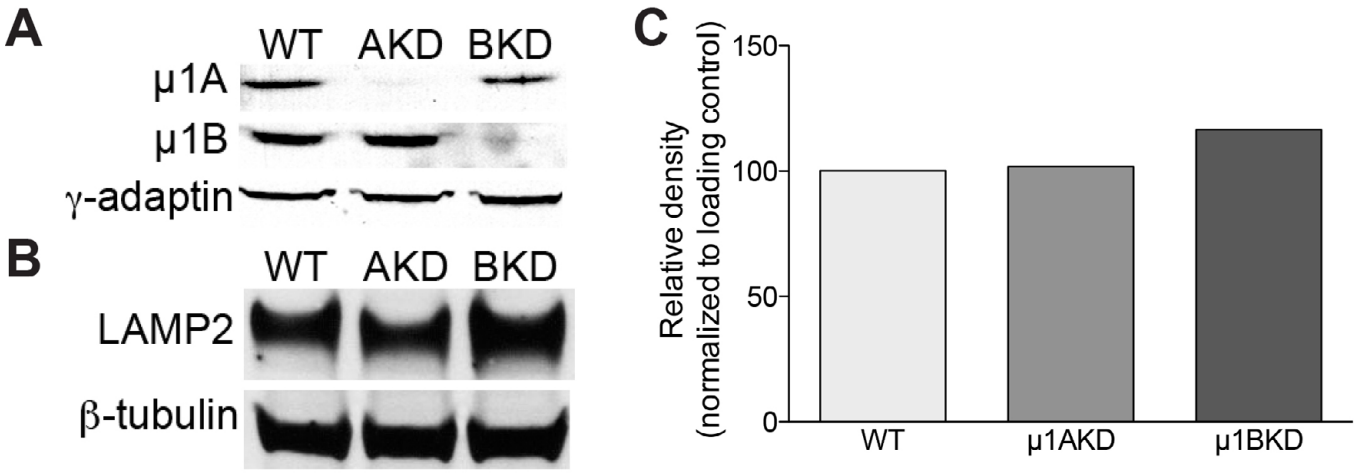


Figure S7. μ1A and μ1B expression in wild-type, μ1AKD and μ1BKD MDCK cells
A, μ1A, μ1B and γ-adaptin levels in wild-type (WT), μ1AKD (AKD) and μ1BKD (BKD) cell lysates were determined by immunoblotting with specific μ1A and μ1B antibodies (Gravotta et al, 2012).
B, Representative immunoblot showing total LAMP2 levels in wild-type, μ1AKD and μ1BKD cell lysates were determined by immunoblotting with anti-LAMP2 (ABD Serotec). **C**, Quantitation of LAMP2 bands normalized to loading control, mean of two independent experiments.

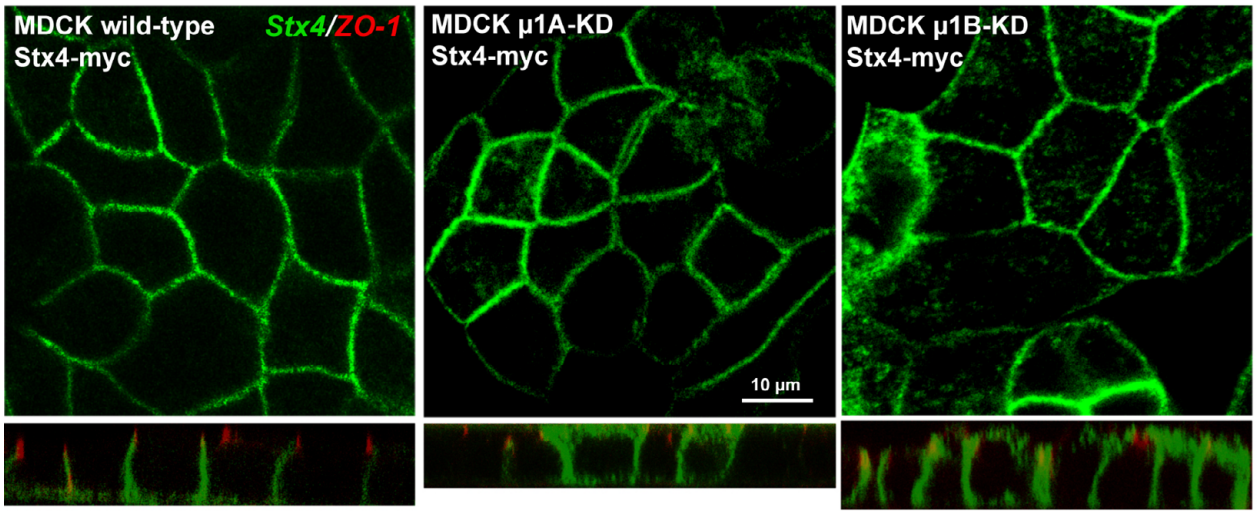


Figure S8. Localization of exogenously expressed syntaxin 4-myc in MDCK cells.
Wild-type, μ1AKD and μ1BKD cells were transfected with syntaxin 4-myc and stable cell lines were generated using zeocin. Polarized monolayers were fixed, permeabilized and stained with anti-syntaxin 4 (green) and ZO-1 (red).

Table S1. Trans-epithelial resistance (TER) of polarized MDCK cells cultured on Transwell filters before and immediately after treatment with ionomycin.

	MDCK TER ($\Omega.cm^2$)	
Ionomycin, 10 min	Before	After
0 μM	114 ± 11.5	113.7 ± 7.8
5 μM	106.7 ± 4.93	109.7 ± 8.74
10 μM	111.7 ± 11.55	106.0 ± 5.29

TER was measured using the EVOM-2 epithelial voltohmmeter (World Precision Instruments). Mean ± SD, n = 3 for each condition.