Expanded View Figures

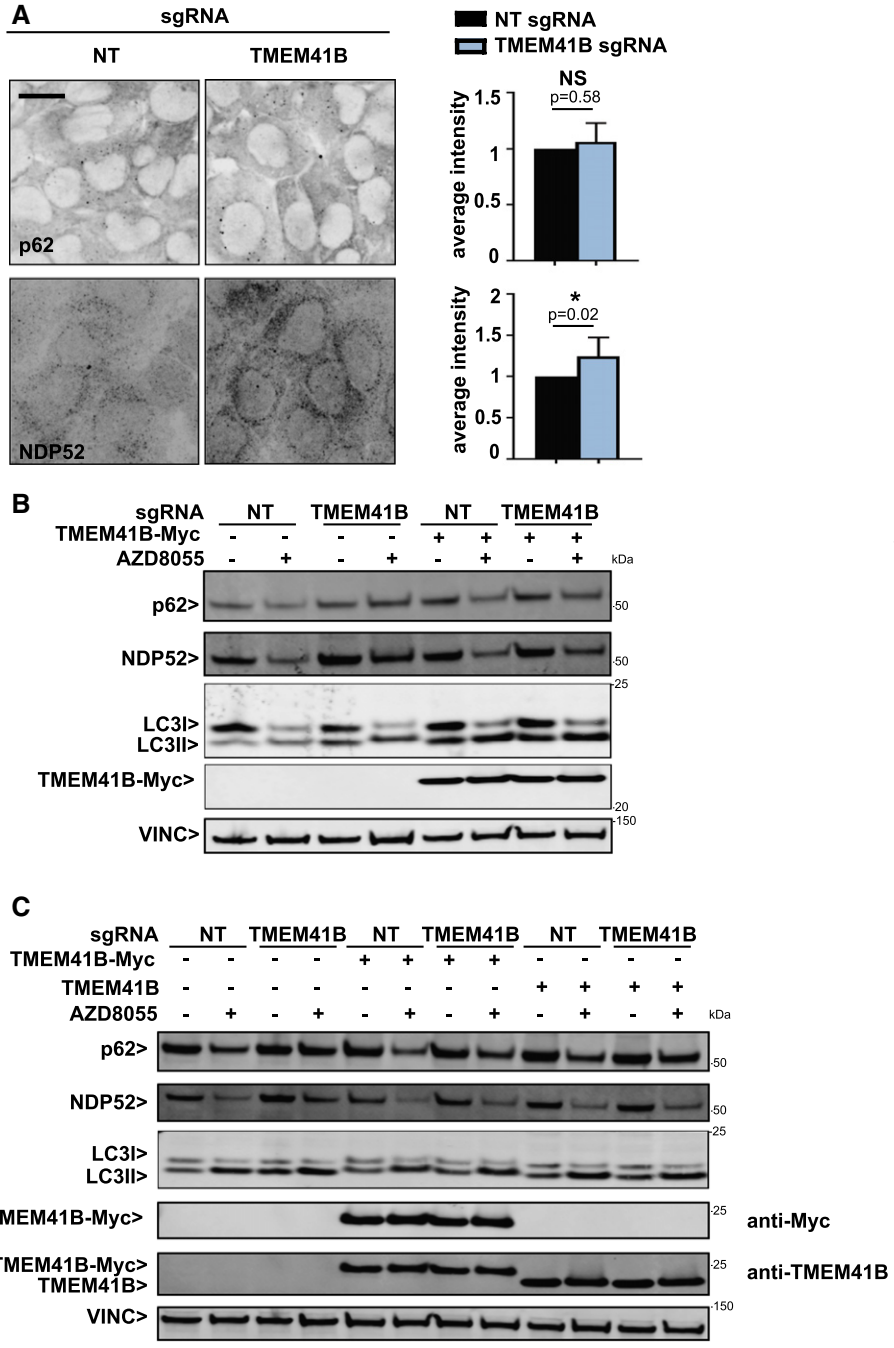


Figure EV1. Rescue of TMEM41B KO with sgRNA-resistant TMEM41B.

A H4 Cas9 TMEM41B KO and NT control cells were probed for p62 and NDP52 by immunostaining and imaged with an automated CV7000 confocal microscope. Scale bar: 20 μm. Staining intensity was quantified with ImageJ and depicted as mean ± SD (n = 4 independent experiments) with paired t-test values.

B HeLa Cas9 cells were infected with sgRNAs targeting TMEM41B or NT control alongside lentiviruses expressing sgRNA-resistant Myc-tagged TMEM41B, treated with 500 nM AZD8055 or vehicle control for 24 h, and analyzed by immunoblotting 7 days post-infection.

C H4 Cas9 cells were infected with sgRNAs targeting TMEM41B or NT control alongside lentiviruses expressing sgRNA-resistant untagged or Myc-tagged TMEM41B, treated with 500 nM AZD8055 or vehicle control for 24 h, and analyzed by immunoblotting 7 days post-infection.

Source data are available online for this figure.

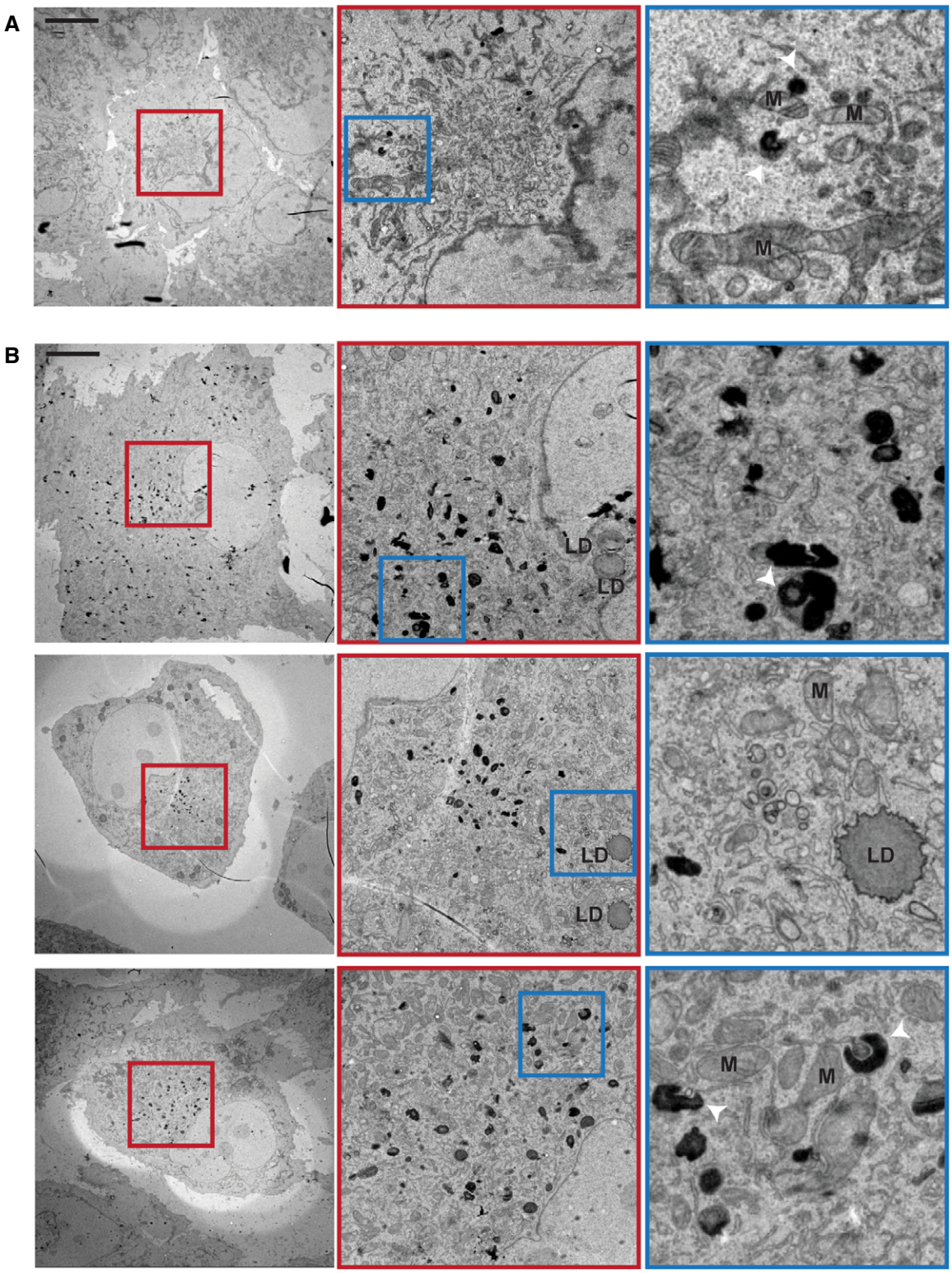


Figure EV2. Ultrastructural analysis of TMEM41B KO cells.

A, B H4 Cas9 (A) and H4 Cas9 TMEM41B KO clone 6 (B) cells were analyzed by transmission electron microscopy. Representative images are shown at the level of the entire cell, the juxtanuclear space as well as an inlet thereof. Mitochondria (M), lipid droplets (LD), and electron-dense structures (white arrowheads) are indicated. Scale bar: 10 μm.

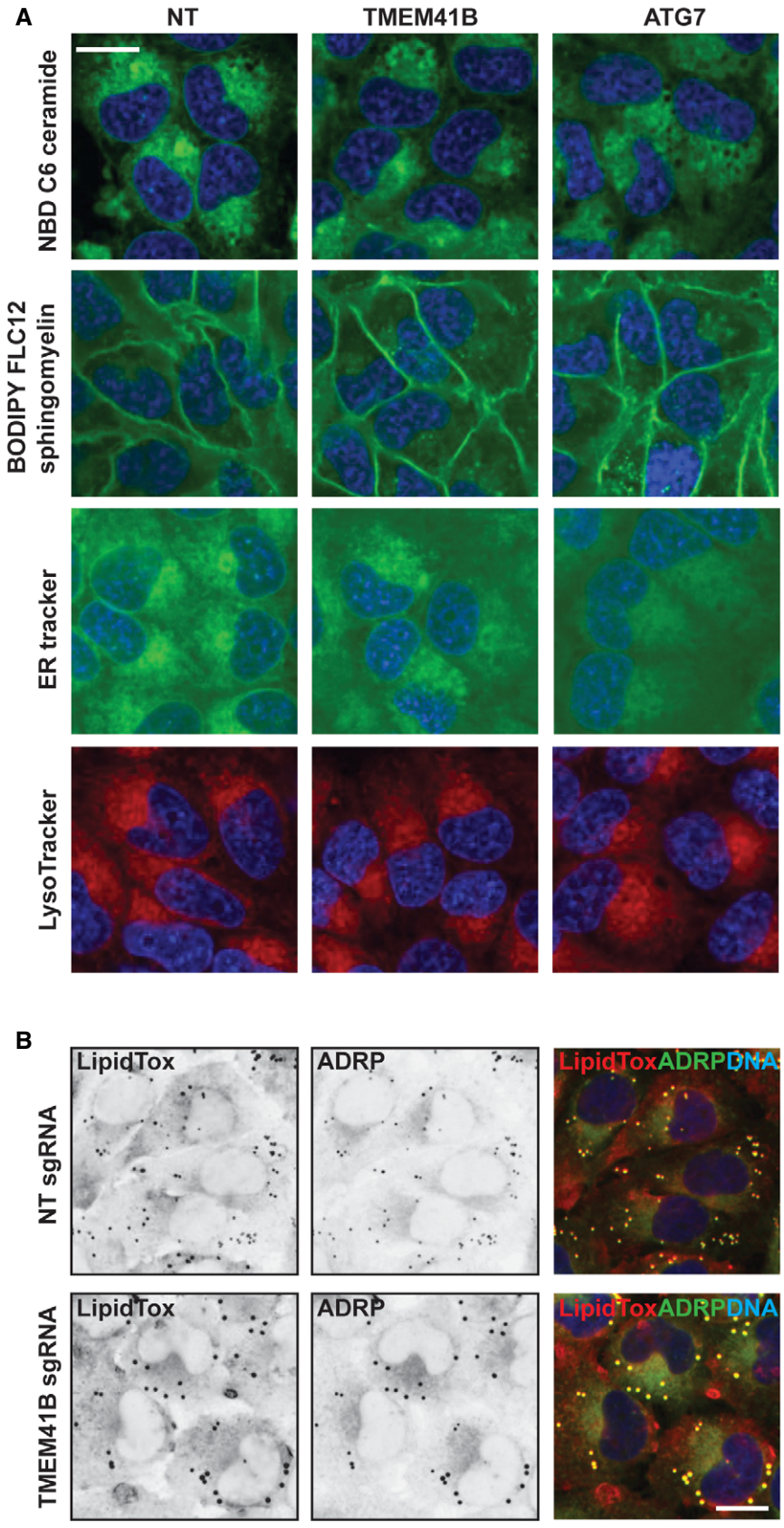


Figure EV3. Analysis of additional lipid and organelle markers.

A H4 Cas9 TMEM41B KO, ATG7 KO, and NT control cells were stained with NBD C6 ceramide, BODIPY FL C12 sphingomyelin, ER-Tracker, or LysoTracker probes. After 2 h incubation at 37°C, cells were imaged live with an automated CV7000 confocal microscope using a 60× objective. Representative images are shown, and scale bar represents 20 μm.

B H4 Cas9 TMEM41B KO and NT control cells were stained with ADRP antibodies and HCS LipidTox Deep Red Neutral Lipid Stain and imaged with an automated CV7000 confocal microscope. Representative images are shown, and scale bar represents 20 μm.

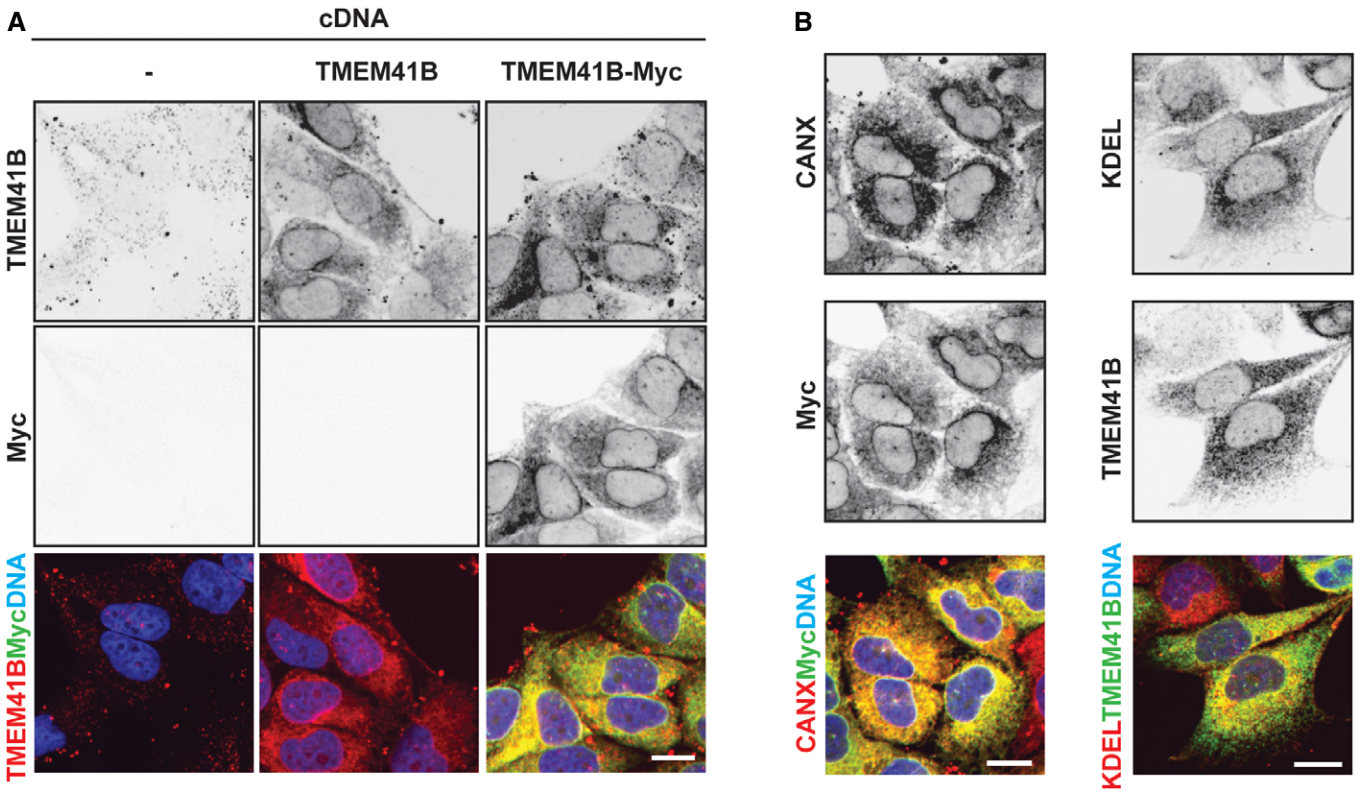


Figure EV4. Localization of untagged and Myc-tagged TMEM41B.

A H4 Cas9 cells were transduced with untagged or Myc-tagged TMEM41B expression constructs, and stable cell populations were selected by antibiotics treatment. Cells were plated into a 96-well plate, fixed, stained with antibodies directed against Myc epitope or TMEM41B, and imaged with an automated CV7000 confocal microscope. Note that the Myc signal appears virtually absent in untransduced cells (−) and for untagged TMEM41B as exposure settings were kept constant for all conditions. Scale bar: 20 μm.

B H4 Cas9 cells expressing C-terminally Myc-tagged TMEM41B were probed for Myc epitope, CANX, or KDEL and imaged with an automated CV7000 confocal microscope. Scale bar: 20 μm.

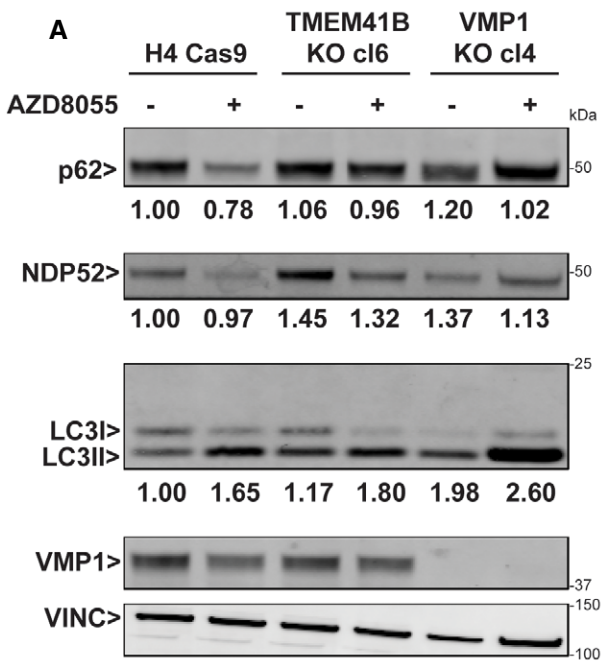
Figure EV5. Comparison of TMEM41B and VMP1 KO cells.

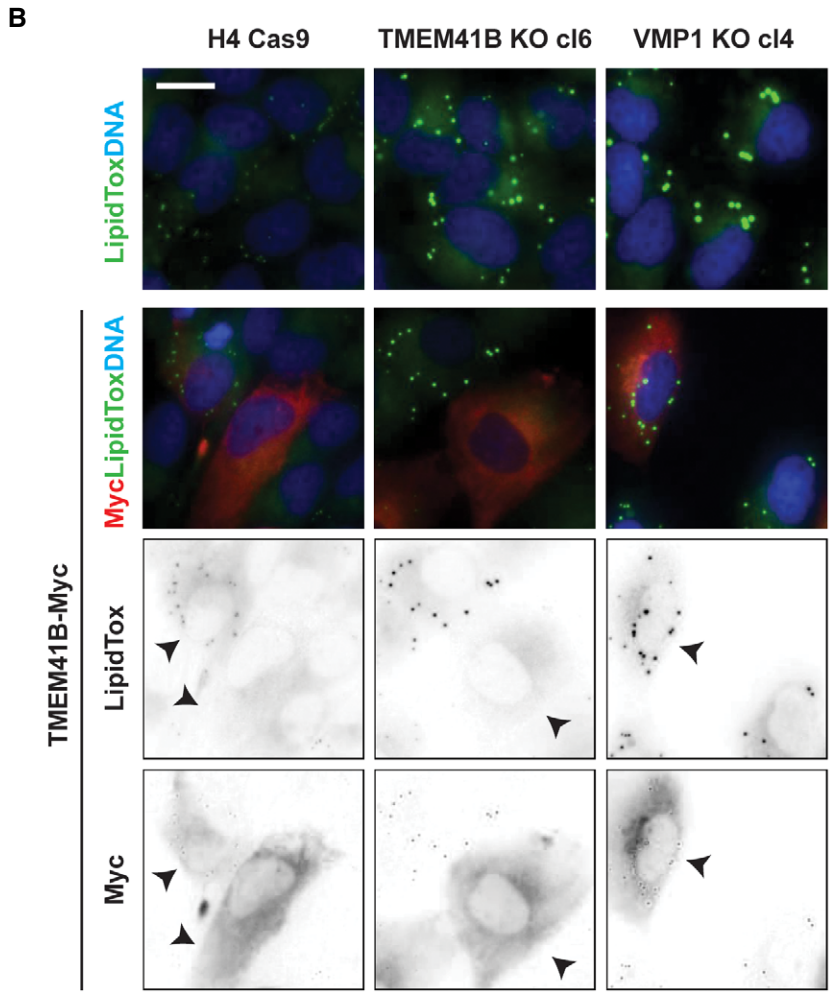
A H4 Cas9, H4 Cas9 TMEM41B KO clone 6, and H4 Cas9 VMP1 KO clone 4 cells were treated overnight with 500 nM AZD8055 or vehicle control and analyzed by immunoblot. Band intensities were quantified by ImageJ, and values from one experiment are depicted for p62, NDP52, and LC3-II:I ratio.

B H4 Cas9, H4 Cas9 TMEM41B KO clone 6, and H4 Cas9 VMP1 KO clone 4 cells were infected with TMEM41B-Myc lentivirus as indicated, fixed 72 h post-infection, and stained with HCS LipidTox Green Neutral Lipid Stain and anti-Myc antibodies. Cells were imaged on an automated Operetta microscope. Representative images are shown, and scale bar represents 20 μm. Arrowheads indicate Myc-positive cells.

C Lipid droplet size was quantified using Harmony software. For infected condition, only Myc-positive cells were included in the analysis. Data are depicted as mean ± SD (n = 3 technical replicates).

Source data are available online for this figure.





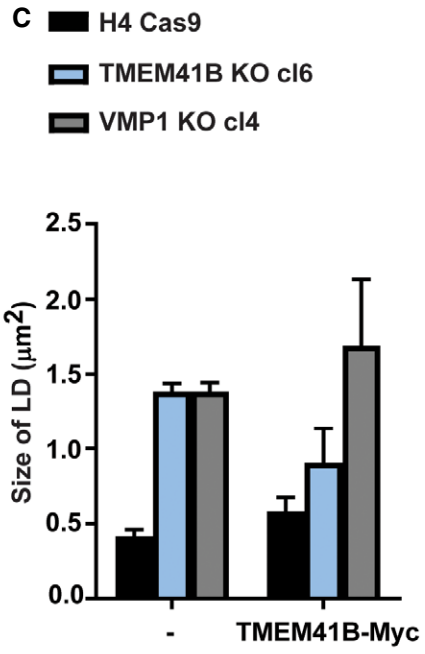


Figure EV5.