SUPPLEMENTARY INFORMATION

Endosomal Fusion of pH-Dependent Enveloped Viruses Requires Ion Channel TRPM7

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Contents:

Reagents:

DMEM High Glucose (ThermoFisher cat# 11965-092)
Fluorobrite (ThermoFisher cat# A18967)
Optimem (ThermoFisher cat#31985)
GoScript Reverse Transcriptase (Promega Cat# A5004).
RNAimax Lipofectamine (ThermoFisher cat# 13778075)
Lipofectamine 2000 (ThermoFisher cat# 11668027)
Lipofectamine 3000 (ThermoFisher cat# L3000008)
pHrood red-succimidyl ester (ThermoFisher, cat#P36600)
siGENOME non-targeting siRNA Control Pools (horizon cat# D-001206-13)
siGENOME siRNA, human TRPM7 (horizon cat# M-005393-03)
siGENOME siRNA, human TRPM6 (horizon cat# M-005048-01-0005)
siGENOME siRNA, human TPCN1 (horizon cat# M-010710-01-0005)
siGENOME siRNA, human TPCN2 (horizon cat# M-006508-02-0005)
SensiFast SYBR no-rox kit (Bioline cat# 98020)
Optiprep (Iodixanol) (Accurate chemical and scientific corp. cat # AN1114542)
Rabbit anti-human TRPM7 (abcam cat# ab245408)
Rabbit anti-rab5 (Cell Signaling cat# CST C8B1)
Rabbit anti-GFP (Accurate chemical and scientific corp. cat# AN1114542)
Rabbit anti-EGFR (Cell Signaling cat# CST D38B1)
Rabbit anti-β-actin (Cell Signaling cat# CST D6A8)
Mouse anti-Influenza A Virus Nucleoprotein antibody (Abcam cat# 20343)
Anti-rabbit HRP secondary (JacksonImmuno cat# 111035144)
4–20% Mini-PROTEAN® TGX™ Precast Protein Gels (Biorad Cat# 4561096)
Trans-Blot™ Turbo RTA Midi 0.2 µm PVDF Transfer Kit (Biorad Cat# 1704273)
SignalBoost Immunoreaction Enhancer Kit (Millipore Sigma Cat# 407207)
SuperSignal™ West Pico PLUS Chemiluminescent Substrate (ThermoFisher cat# 34580)
Wheat Germ Agglutinin Alexa Fluor 647 (Invitrogen cat# W32466)
Janelia Fluor HaloTag Ligand 646 (Promega cat# GA1120)
FTY720 (Cayman Chemical cat# 162359560)
NS8593 (Cayman Chemical cat# 29774)
Lysosensor DND-189 (Invitrogen cat# L7535)
Epidermal Growth Factor (ThermoFisher cat# A42556)
Influenza (A/Puerto Rico/8/1934) (ATCC cat# VR-1469)
TRPM7 sgRNA (sigma-aldrich cat# HS0000409924) 5'-AAGCAACTGCTTGATTCGTGGGG-3'
Primers- IDT

Human TRPM7 F	TCGAGACGTCAAACAGGGAAA
Human TRPM7 R	TGCTGCTGGAGGTATTTCGG
Human TRPM6 F	GGATCTCTCTGCCCTGACTG
Human TRPM6 R	TTCTCTCCAGCGATCTCCAT
Human TPCN1 F	GGCTTTGAAAGGGAGCTCAAA
Human TPCN2 R	TCGTGGATGGCATAGCTGC
Human TPCN1 F	TGACCACTTACCGCAGCATC
Human TPCN2 R	ACCGTCGGTAAAGCCACATC
Human B2M F	GTCTTTCAGCAAGGACTGGTCT
Human B2M R	CTTACATGTCTCGATCCCACTTAAC

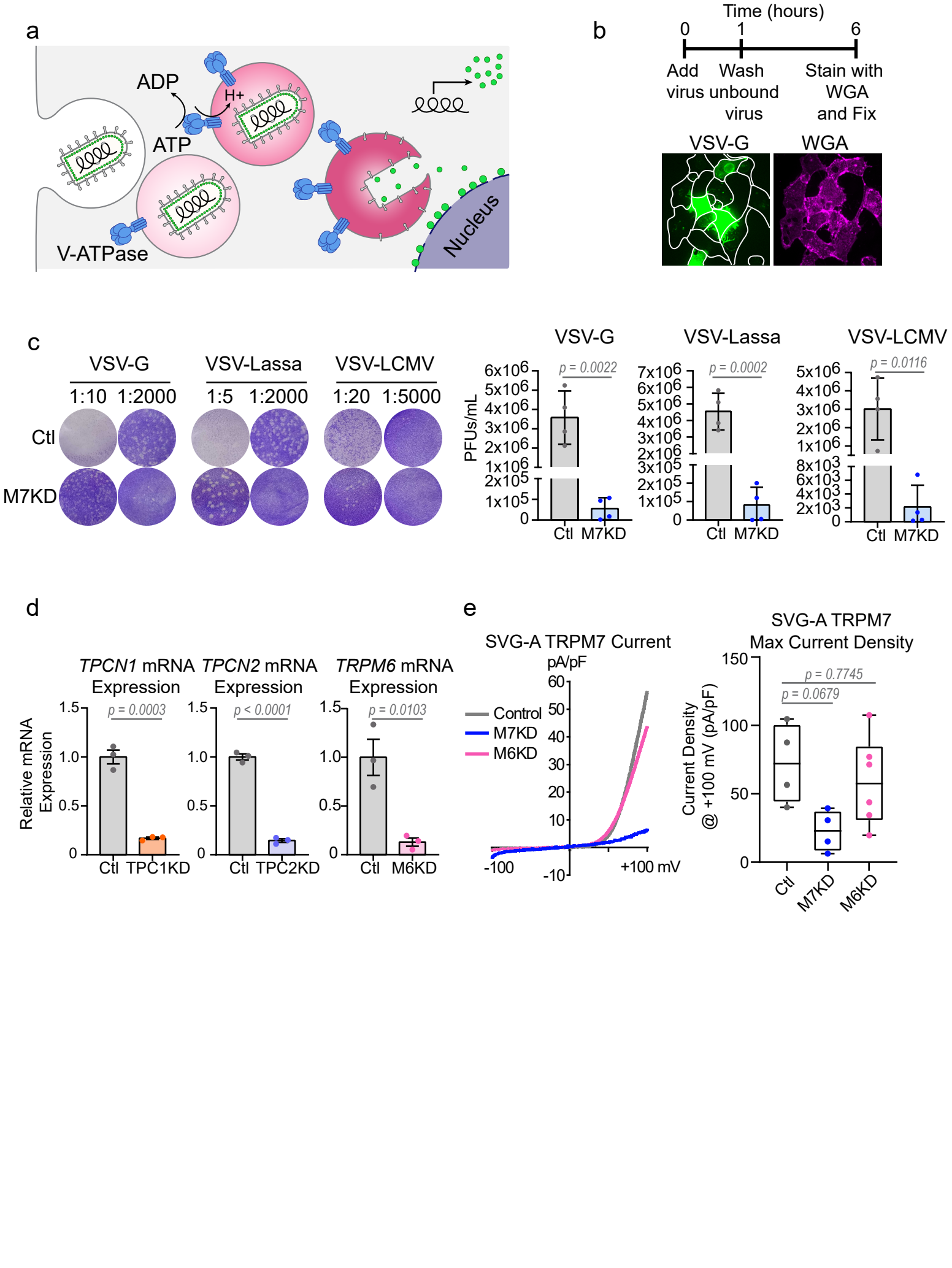
Plasmids

Glycoproteins:

VSV-G – pMD2.G Addgene 12259 A gift from Didier Trono
Ebola GP Zaire strain (a gift from Judith white)
Lassa GP (a gift from Judith White)
LCMV GP (a gift from Judith White)
SARS-CoV-2 – pcDNA3.3_CoV2_D18 – Addgene 170442 A gift from David Nemazee

pCMV-lyso-pHoenix Addgene 70112 a gift from Chistian Rosenmund
Mouse TRPM7 mutant plasmids were described previously in *Desai et al, Dev Cell (2012)*

Supplementary Figure 1



Supplementary Fig. 1
Loss of TRPM7 inhibits plaque formation and validation of ion channel knockdowns

a. Diagram of VSV-chimera entry into cell. Viruses are endocytosed and exit the endosome when the pH needed for glycoprotein conformational change is achieved. M-protein from escaped viruses then coats the nucleus. Lastly new viral proteins are transcribed from the viral genome.

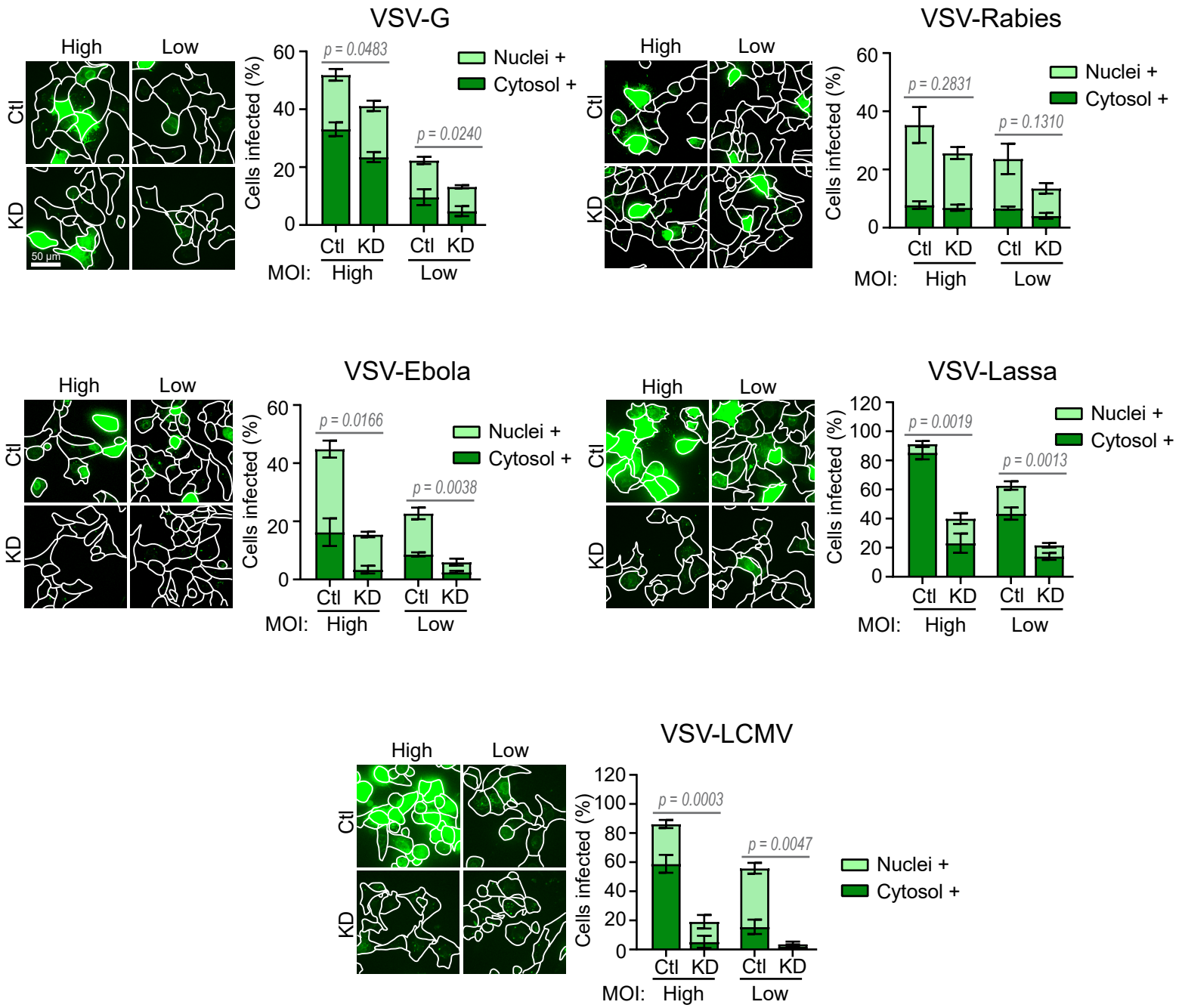
b. Top: Experimental design scheme for VSV-chimera virus infections. Virus was incubated with cells for one hour, unbound virus was washed off, and cells were incubated for a subsequent five hours. Cells were then stained with Wheat Germ Agglutinin (WGA) for 30 seconds before fixation for imaging. Bottom: Example image of VSV-G-MeGFP (left) and WGA (right) of control VSV-G-MeGFP infected SVG-A cell (from Supplementary Fig. 2, VSV-G control high MOI). White cell borders were generated by tracing the WGA signal.

c. Representative images and quantification of plaque assays of Vero cells treated with supernatant from Control (Ctl) and TRPM7 knockdown (M7KD) cells propagating VSV-G, VSV-Lassa, and VSV-LCMV. Dilution of supernatant is shown above each image. Mean and SEM shown, n = 4 independent propagations.

d. qRT-PCR of *TPCN1 (*orange, TPC1KD*), TPCN2 (*purple, TPC2KD*),* and *TRPM6 (*pink, M6KD*)* mRNA levels compared to control non-targeting siRNA (gray, Ctl) in SVG-A cells. Data points are representative of three independent transfections (n = 3). Mean and SEM shown. P-values determined by two-tailed Student's t-test.

e. TRPM7 whole-cell patch-clamp recordings shown as current-voltage (IV) relationship. The IVs of control siRNA- (gray, Ctl), *TRPM7* siRNA- (blue, M7KD), and *TRPM6* siRNA (pink, M6KD) transfected SVG-A cells are overlayed. Quantitative summary of peak current densities at +100 mV is shown on the right. P-values determined by one-way ANOVA with Tukey's multiple comparison test. n = 4 for ctl, 4 for M7KD, and 6 for M6KD.

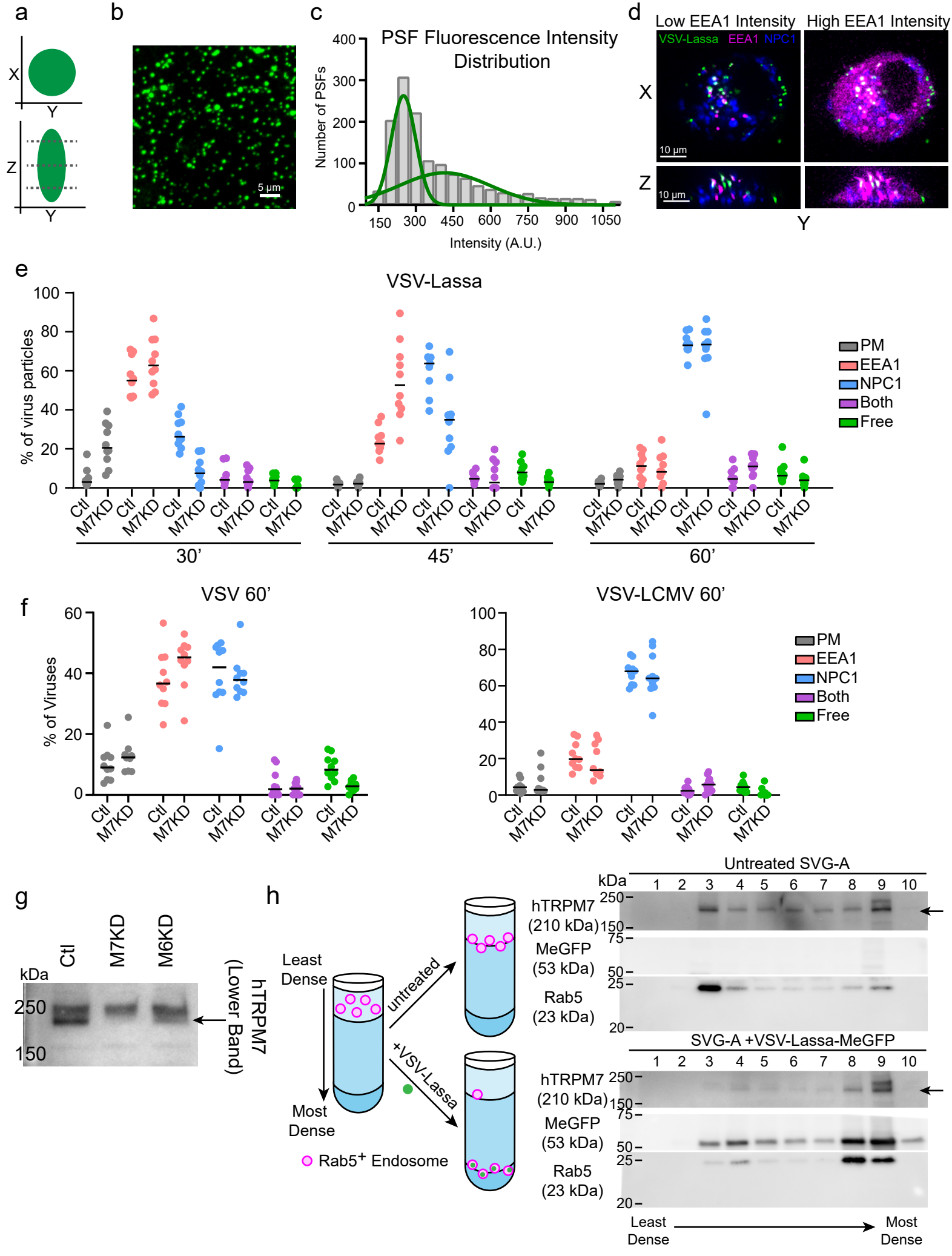
Supplementary Figure 2



Supplementary Fig. 2
Loss of TRPM7 inhibits VSV-chimeras at various MOIs

Maximum projections *(left panels)* and quantification *(right panels)* of viral infections of various VSV-chimeras. Control (Ctl) and TRPM7 Knockdown (KD) SVG-A cells were treated with a high and low multiplicity of infection (MOI) of VSV-G, VSV-Rabies, VSV-Ebola, VSV-Lassa, and VSV-LCMV. Proportion of nuclei+ (light green) and cytosol+ (dark green) are shown. Corresponding representative images are 20 μm maximum intensity projections. Experiments were performed blinded to cell genotype and virus MOI. Each experiment is plotted as average percent infected across at least five fields of view, n = 3 independent experiments. Mean and SEM are shown.

Supplementary Figure 3



Supplementary Fig. 3
Viruses are endocytosed to TRPM7-containing endosomes

a. Diagram of MeGFP virus point spread function (PSF) in x, y, and z dimensions. Because each viral particle is below the resolution of light, they appear as PSFs.

b. Maximum projection of viral particles expressing MeGFP plated on glass. Each punctum is one or more viruses.

c. Histogram of the intensity of each eGFP point spread function from five fields of view. Plotted are the gaussian fits to determine the average intensity of one and two viruses at a single point spread function.

d. Example image of VSV-Lassa-MeGFP treated cell. As EEA1 is a soluble protein that is recruited to early endosomes, increasing the intensity of EEA1 allows for visualization of the cell border and plasma membrane-bound viral particles for localization analysis. VSV-Lassa-MeGFP (green), EEA1 (magenta), and NPC1 (blue) are shown.

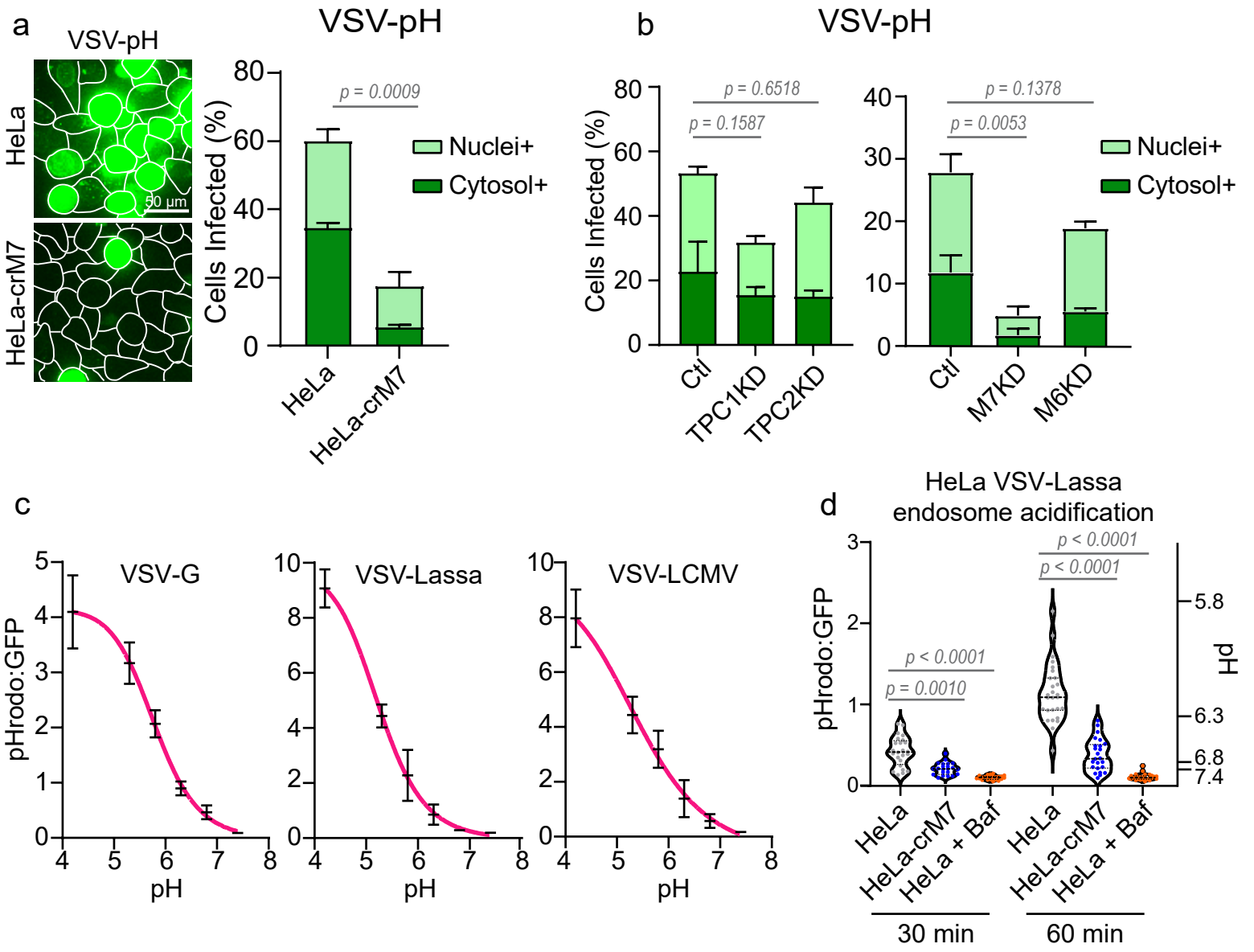
e. Individual data points used in Fig. 3c-3d. Percentage of viruses localizing to the plasma membrane (gray, PM), early endosome (red, EEA1), late endosome (blue, NPC1), $EEA1^+/NPC1^+$ (purple, Both) or free in the cytosol (green, Free). Each dot represents the percentage of viruses localizing to a specific compartment per cell. Control (Ctl) cells are shown on the left and TRPM7 knockdown (M7KD) on the right.

f. Individual data points used for VSV-G (Left) and VSV-LCMV (Right) colocalizations for figures 2e and 2f. Average percentage of viruses localizing to PM (gray), EEA1 (red), NPC1 (blue), $EEA1^+/NPC1^+$ (purple) compartments, or free (green) at 60 minutes post-infection in control and M7KD SVG-A cells.

g. Western blot of membrane fractions from Control (ctl), TRPM7 (M7KD), and TRPM6 (M6KD) siRNA-treated SVG-A cells. Arrow indicates human TRPM7 (210 kDa). A non-specific band that is not affected by either knockdown is seen at a slightly higher molecular weight. Blot is representative of two independent preparations.

h. Isosmotic iodixanol density gradient fractionation of intracellular membranes of SVG-A cells. (Left) Illustration of density gradient separation of intracellular compartments. (Right) Immunoblots of untreated cells are shown on top, while cells incubated with VSV-Lassa for one hour are shown underneath. TRPM7, M-eGFP, and early endosome marker Rab5 are blotted. Fraction 1 is the least dense, Fraction 10 the densest.

Supplementary Figure 4



Supplementary Fig. 4
TRPM7 regulates viral endosomes acidification needed for low-pH dependent viruses

a. Representative images (Left) and quantification (Right) of HeLa and HeLa-crM7 cells infected with VSV-pH. Percent of nuclei+ cells are shown in light green and cytosol+ cells in dark green. P-values determined by two-tailed Student's t-test of the average total cells infected. Mean and SEM shown, n = 3 independent experiments.

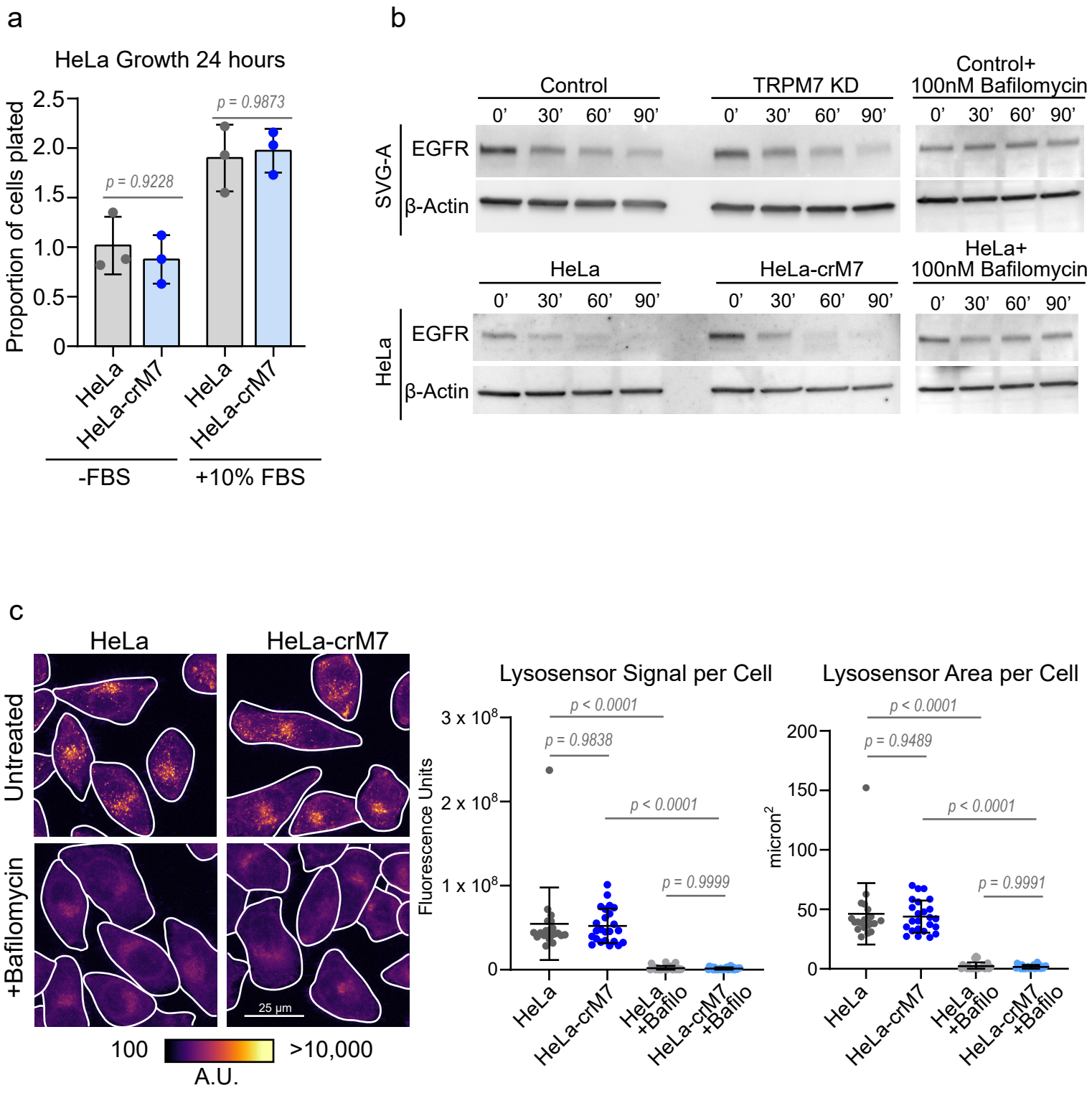
b. Percent of cells infected with VSV-pH infection in Control (Ctl), TPC1 (TPC1KD), and TPC2 (TPC2KD) as well as TRPM7 (M7KD) and TRPM6 (M6KD) knockdown SVG-A cells. Percent of nuclei+ cells are shown in light green and cytosol+ cells in dark green. P-values determined by one-way ANOVA of total cells infected with Tukey's multiple comparison test. Mean and SEM shown, n = 3 independent experiments.

c. Calibration curves of pHrodo:GFP ratio to pH. pHrodo-tagged viruses were plated on glass coverslips in pH 7.4, 6.8, 6.3, 5.8, 5.3, 4.2 NTE buffer and five fields of view imaged to determine single particle pHrodo:GFP ratios. Calibrations for VSV-G, VSV-Lassa, and VSV-LCMV are shown. Curves were fitted with four-parameter logistic equations.

d. HeLa (gray) and HeLa-crM7 (blue) cells were infected with VSV-Lassa tagged with pHrodo dye. Cells were incubated with virus at 37°C for 20 minutes, washed, and then imaged at 30-, 45-, and 60-minutes post-infection. Cells were imaged at 37°C with 5% CO_2. HeLa cells were also pretreated with 50 nM Bafilomycin (orange) prior to infection and throughout the infection protocol to inhibit endosomal acidification. 25^{th} and 75^{th} percentiles are indicated by thin dotted lines and median by thick dotted lines. P-values determined by two-way ANOVA with Sidak's multiple comparisons test. For Hela n = 24, 27; HeLa-crM7 n = 22, 24; Baf n = 23, 27 at 30 and 60 minutes respectively

e. Comparison of pHrodo:eGFP ratios to pH measurement calibrations used for Fig. 3e. Y-axis was calibrated with curves determined in Supplementary Fig. 4c.

Supplementary Figure 5



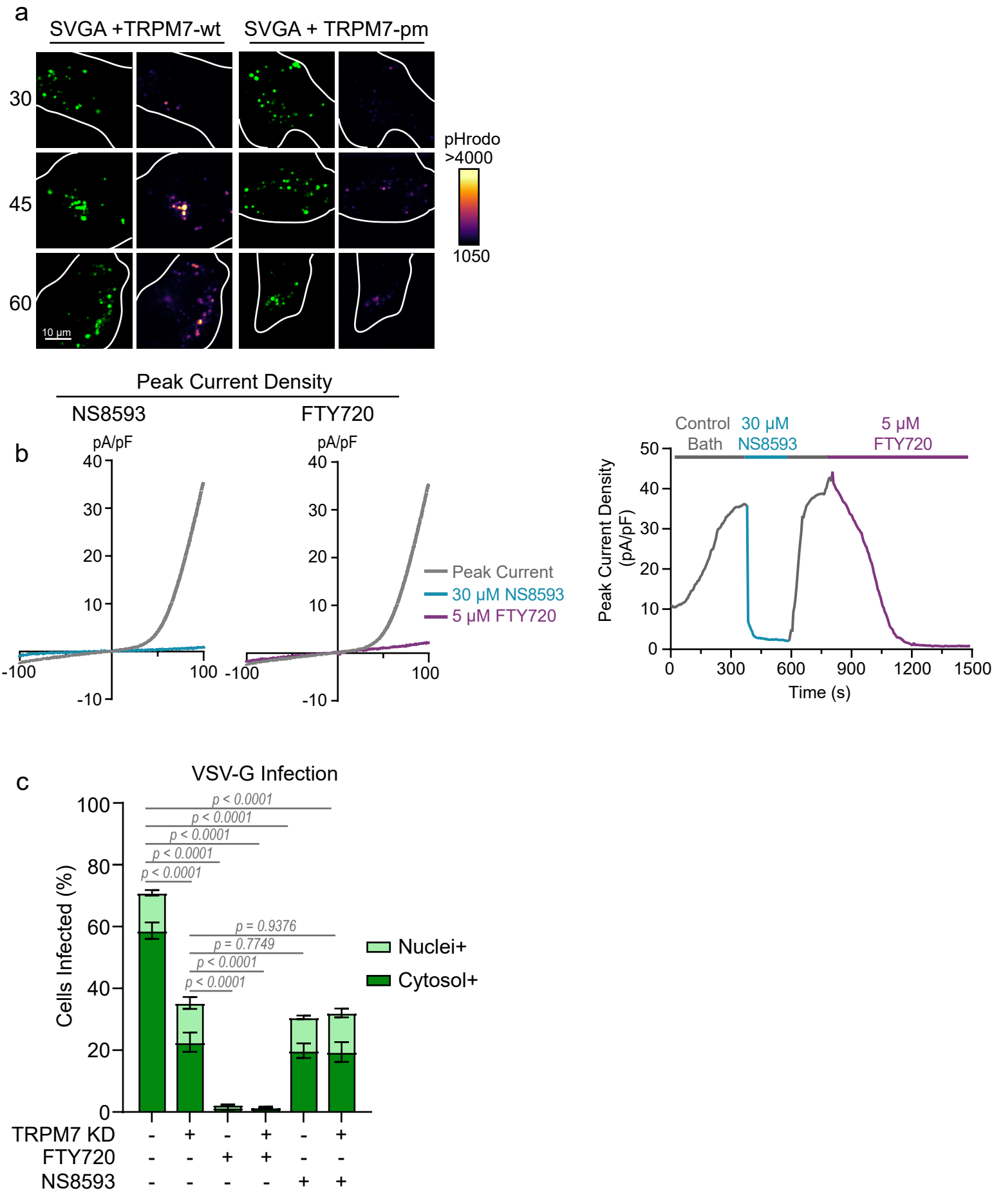
Supplementary Fig. 5
Non-viral endosomal processes are not affected by loss of TRPM7

a. Survival and growth of HeLa (gray) and HeLa-crM7 (blue) cells over 24 hours in DMEM with or without serum. P-values determined by two-tailed Student's t-test. n = 3 independent samples

b. Western blot of EGFR in SVG-A (Top) and HeLa (Bottom) cell lysates following stimulation with 50 ng/mL of human EGF. Cells were serum starved for 2.5 hours then incubated for 0, 30, 60, or 90 minutes with EGF prior to lysis. β-actin is shown as a loading control. Blots are representative of three independent replicates.

c. (Left) Maximum projection images of Lysosensor DND-189 dye fluorescence in HeLa and HeLa-crM7 cells. Top images represent cells loaded with dye. Bottom images show fluorescence after pretreatment with 50 nM Bafilomycin for 30 minutes and throughout the one hour dye incubation. Pseudocolor scale indicates fluorescence intensity. (Right) Quantification of overall Lysosensor fluorescence per cell in HeLa and HeLa-crM7 cells with or without the presence of 50 nM Bafilomycin. n = 21, 24 for HeLa and HeLa-crM7 untreated respectively; n = 24, 26 for HeLa and HeLa-crM7 with Bafilomycin respectively.

Supplementary Figure 6



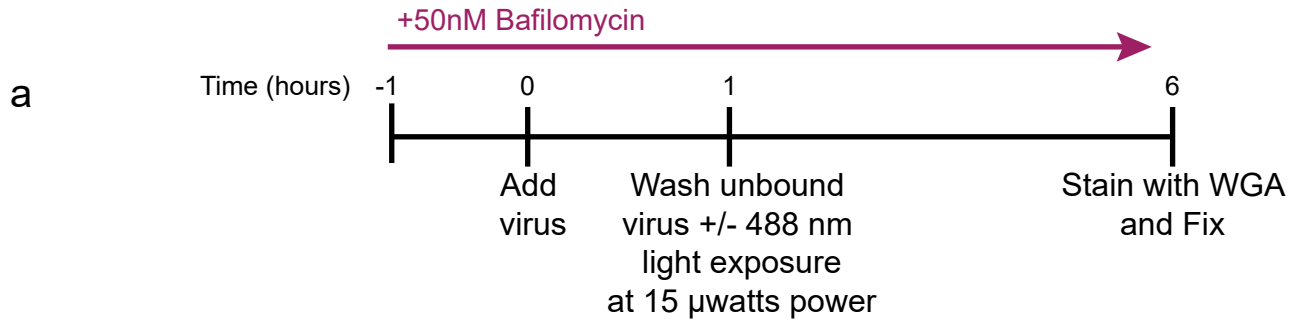
Supplementary Fig. 6
Inhibition of TRPM7 ion channel activity dictates phenotype induced by loss of TRPM7

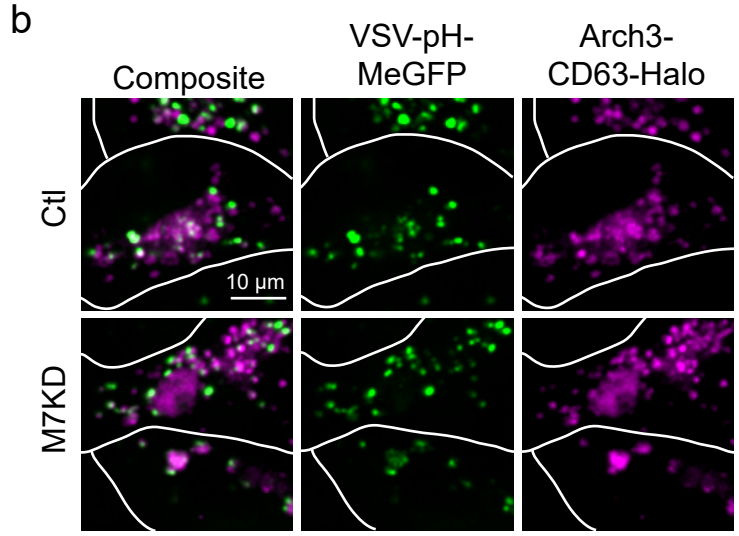
a. Representative images of pHrodo tagged VSV-pH infected SVG-A cells expressing TRPM7 variants TRPM7-wt, and TRPM7-pm. Viral MeGFP is shown in green and pHrodo signal is shown pseudo-colored on an intensity spectrum ranging from black/purple (low) to yellow (high). Images are maximum z-projections (1.08 μm in depth). Images are representative of at least 25 cells imaged.

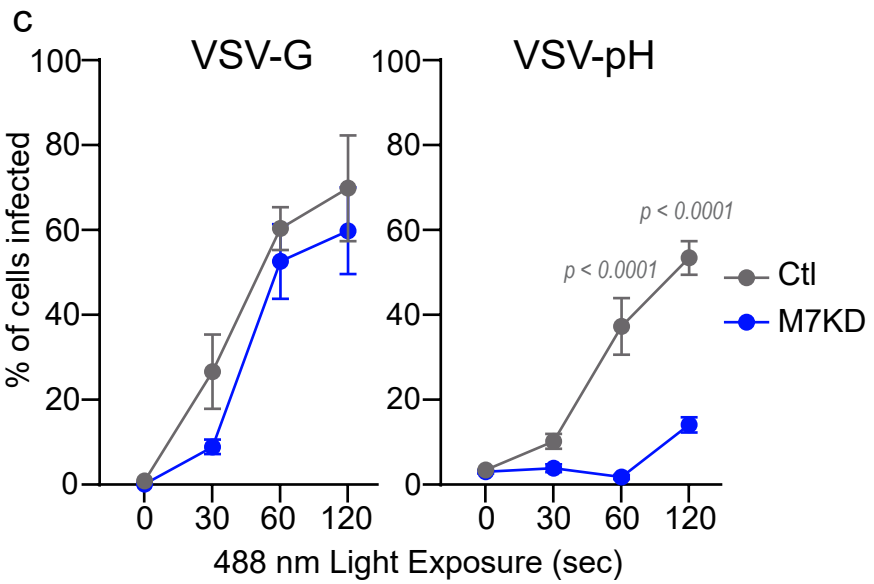
b. (Top) Whole-cell patch-clamp recordings showing the inhibition of I_{TRPM7} by 30 μM NS8593 (teal) and 5 μM FTY720 (purple) in SVG-A cells. (Bottom) Peak current density recorded from an SVG-A treated with 30 μM NS8593, washed with bath solution, and incubated with 5 μM FTY720. Currents are representative of three cellular replicates.

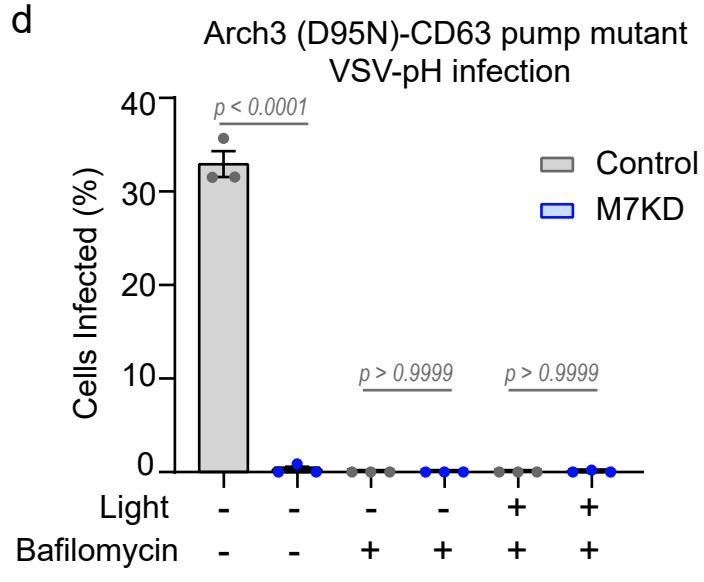
c. Percentage of cells infected with VSV-G in in SVG-A cells with or without TRPM7 knockdown (TRPM7 KD) in the presence or absence of 5 μM FTY720 or 30 μM NS8593. Nuclei+ cells are in light green while cytosol+ cells are in dark green. P-values are determined by one-way ANOVA with Tukey's multiple comparison test of total percent of cells infected. Mean and SEM are shown, n = 3 independent experiments.

Supplementary Figure 7









Supplementary Fig. 7
Viruses are endocytosed and escape in a light dependent manner in Arch3-CD63 expressing cells

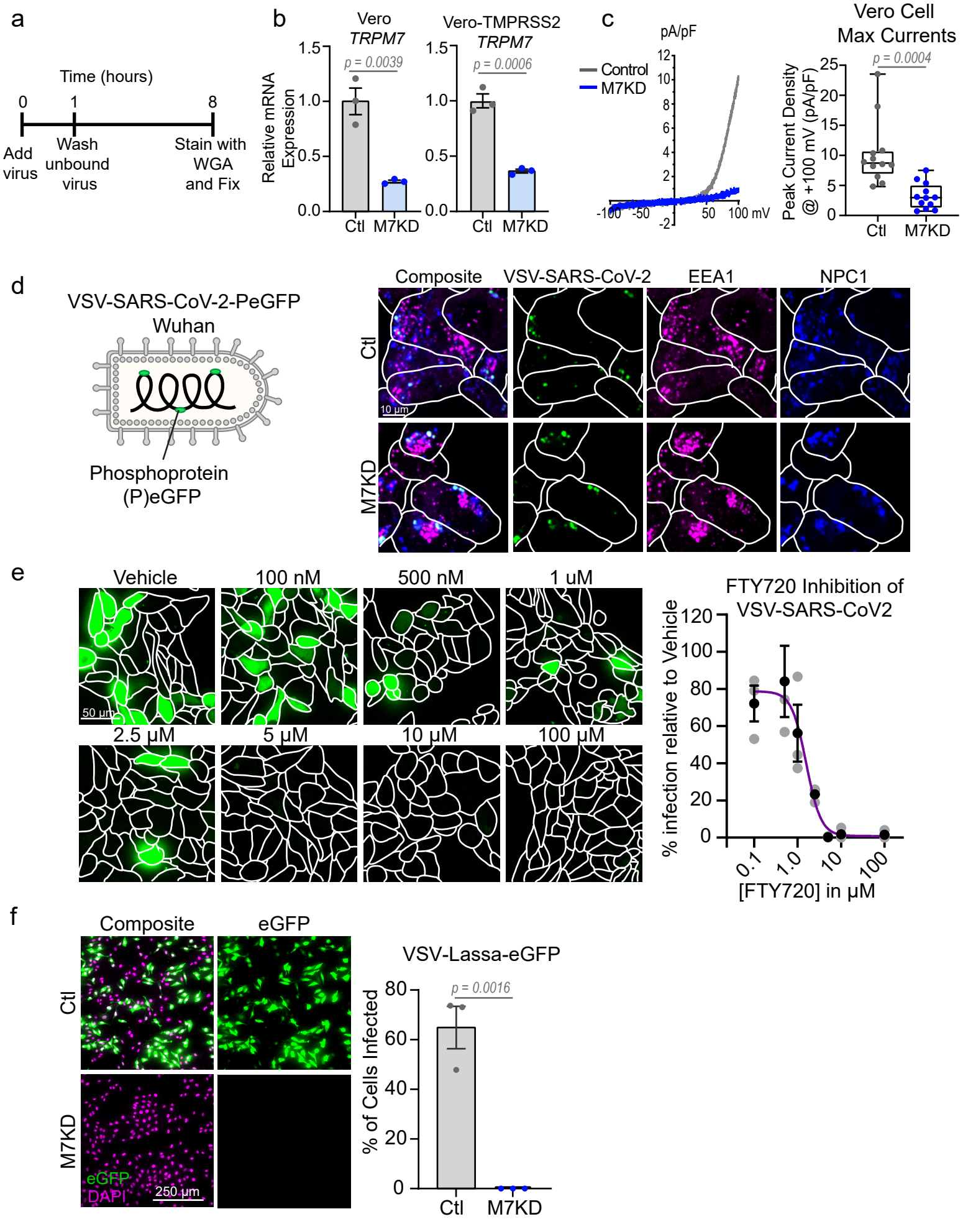
a. Diagram of the experimental design of Arch3-CD63 infections with or without Bafilomycin and 488 nm light exposure. SVG-A cells were transfected with Control (Ctl) or TRPM7 (M7KD) siRNA. Appropriate cells were pretreated with 50 nM Bafilomycin for one hour prior to infection and throughout the experiment. Cells were incubated with virus for one hour, washed, and then incubated at 37°C and 5% CO_2 for 5 hours. At the time of washing, cells that required light activation were exposed to 15 µW of 488 nm laser for one minute and returned to incubate.

b. Representative images of Control (Ctl) and TRPM7 Knockdown (M7KD) SVG-A cells expressing Arch3-CD63-Halo visualized with JFX647 (magenta) and infected with VSV-pH-MeGFP (green) in the presence of 50 nM Bafilomycin. Cells were pretreated with 50 nM Bafilomycin, incubated with virus, washed, and imaged in the presence of Bafilomycin. Representative of eight fields of view.

c. Calibration curve of VSV-G and VSV-pH infection in Control (Ctl, gray) and TRPM7 knockdown (M7KD, blue) SVG-A cells expressing CD63-Arch3. Cells were treated with 50 nM Bafilomycin throughout the experiment. Cells were exposed to 15 µWatt 488 nm laser for 0-, 30-, 60-, or 120-seconds after 1 hour of incubation with the virus. Infection readouts were determined at 6 hours post-infection. P-values determined by two-way ANOVA with Sidak multiple comparisons test. Mean and SEM are shown. n = 3 independent experiments.

d. Quantification of percent VSV-pH infected Control (Ctl, gray) and TRPM7 knockdown (M7KD, blue) SVG-A cells expressing a mutated Arch3 (Arch3(D95N)-CD63-Halo). Arch3(D95N)-CD63-Halo is incapable of pumping H^+ into the endosomal lumen. Cells were untreated or exposed to 50 nM Bafilomycin +/- Light exposure (15 µWatt 488 nm, 60s). P-values determined by one-way ANOVA with Tukey's multiple comparison analysis of triplicate replicates. Mean and SEM are shown. n = 3 independent experiments.

Supplementary Figure 8



Supplementary Fig. 8
VSV-SARS-CoV-2 is endocytosed and escape is dependent on TRPM7 ion channel activity

a. Diagram of VSV coronavirus chimera infection protocol. Cells were incubated with virus for one hour, washed, and allowed to incubate for seven hours at 37°C and 5% CO_2 before WGA staining and fixation.

b. Quantification of Vero and Vero-TMPRSS2 knockdown efficiency by human TRPM7 siRNA. Control (Ctl, gray) siRNA is shown in gray, TRPM7 (M7KD, blue) knockdown is shown in blue. Mean and SEM shown, n = 3 independent transfections. P-values determined by two-tailed Student's t-test.

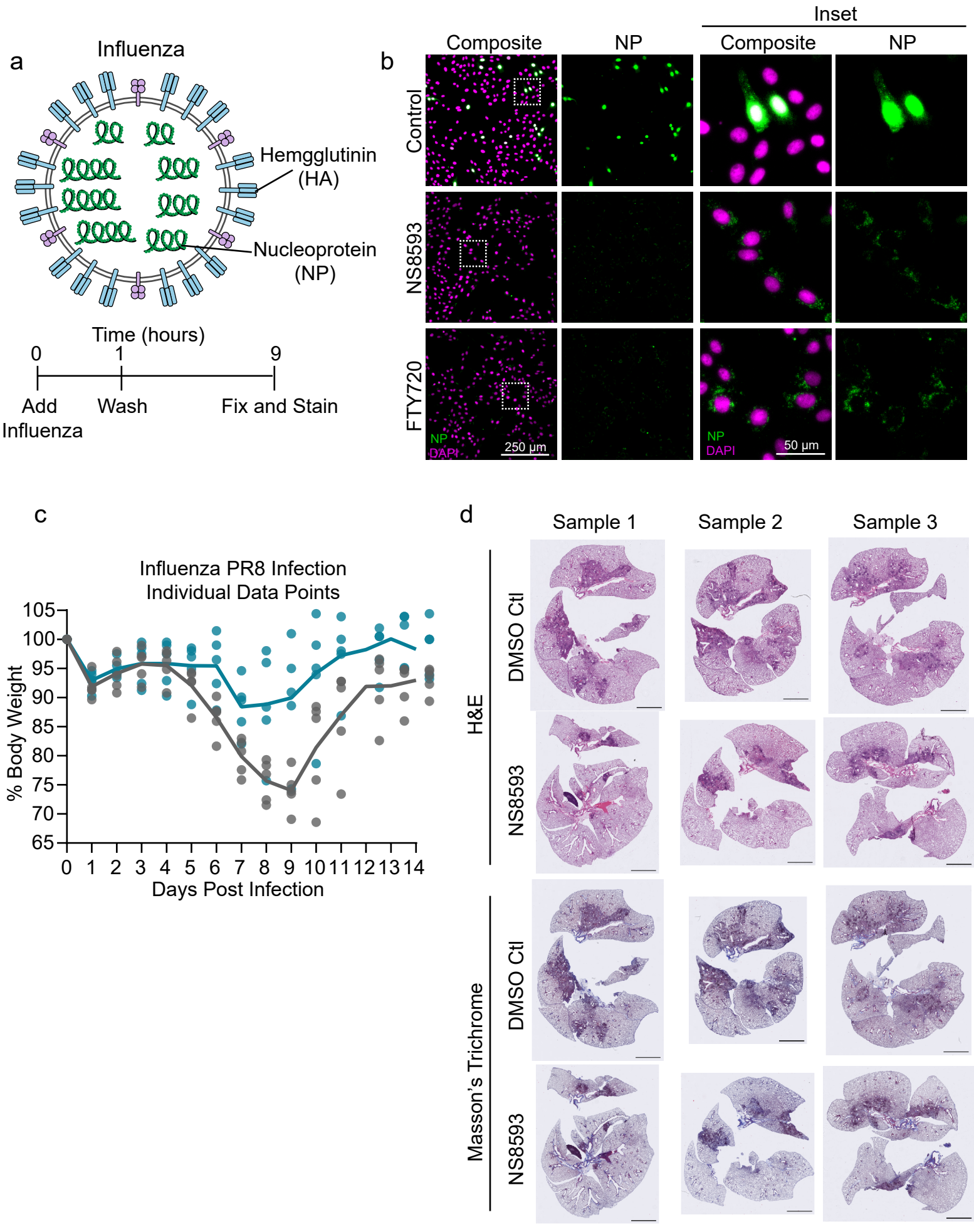
c. (Left) Whole-cell patch-clamp recordings shown as current-voltage (IV) relationship. The IVs of control siRNA- (Ctl, gray) and TRPM7 siRNA- (M7KD, blue) transfected Vero cells are overlayed. (Right) Quantitative summary of peak current densities at +100 mV is shown as a box chart (right panel). P-values determined by two-tailed Student's t-test. Median, 25^{th}, and 75^{th} quartiles are shown with maximum and minimum. n =12 cells.

d. (Left) Diagram of VSV-SARS-CoV-2-PeGFP with the P-protein tagged with eGFP. (Right) Maximum intensity projection of Control (Ctl) and TRPM7 Knockdown (M7KD) SVG-A cells overexpressing ACE2 with genetically encoded EEA1-scarlet (magenta) and NPC1-Halo-JFX647 (blue). Cells were infected with the Wuhan VSV-SARS-CoV-2-PeGFP variant. Representative of ten fields of view.

e. Representative maximum projection images and quantification of Vero cells infected with VSV-SARS-CoV-2-eGFP pseudotyped for the Wuhan variant, in the presence of various concentrations of FTY720. Individual data points are shown in gray and average percent of cells infected is shown in black and SEM is plotted. Concentration-response curve (purple) was fit with a sigmoidal 4PL curve. IC_{50} = ~1.58 μM. Mean and SEM shown, n = 3 independent experiments.

f. Control (Ctl, gray) and TRPM7 Knockdown (M7KD, blue) Vero cells treated with VSV-Lassa-eGFP, which expresses eGFP after successful infection. eGFP is shown in green and DAPI is shown in magenta. P-value determined by two-tailed Student's t-test. Mean and SEM shown, n = 3 independent experiments.

Supplementary Figure 9



Supplementary Figure 9
Loss of TRPM7 is protective from influenza infection at a cellular and tissue level

a. (Top) Schematic of Influenza highlighting nucleoprotein, the antigen used to detect viral infection by immunofluorescence, and Hemagglutinin, the envelope fusion protein of Influenza. (Bottom) Schematic for Influenza first-round infection assays. Vero cells were incubated with influenza for 1 hour, washed, and incubated again for a subsequent 8 hours before fixation and DAPI staining.

b. Non-trypsin-cleaved influenza infection in Vero cells treated with 5 μM FTY720 or 30 μM NS8593 from Fig. 7d with inset to show accumulated nucleoprotein in intracellular compartments.

c. Individual mouse weight data points used in Fig. 7e. The mean is plotted as a solid line.

d. Images of the three DMSO control and NS8593 treated murine lung samples stained with H&E or Masson's trichrome. Scale bar is 2.5 mm