

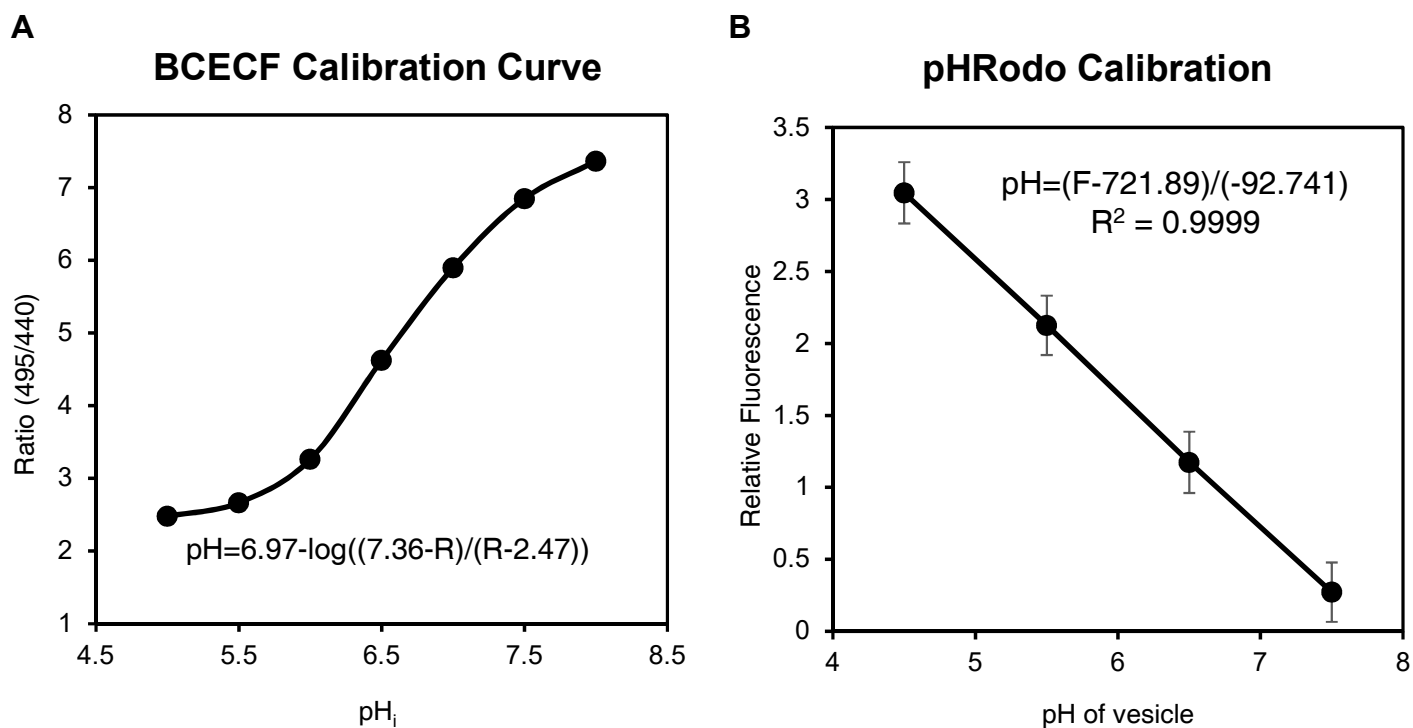
# **Modulation of lysosomal $\text{Cl}^-$ mediates migration and apoptosis through the TRPML1 as a lysosomal $\text{Cl}^-$ sensor**

**Cells**

**Dongun Lee and Jeong Hee Hong\***

Department of Health Sciences and Technology, Lee Gil Ya Cancer and Diabetes Institute, GAIHST, Gachon University, 155 Getbeolro, Yeonsu-gu, Incheon, 21999, South Korea

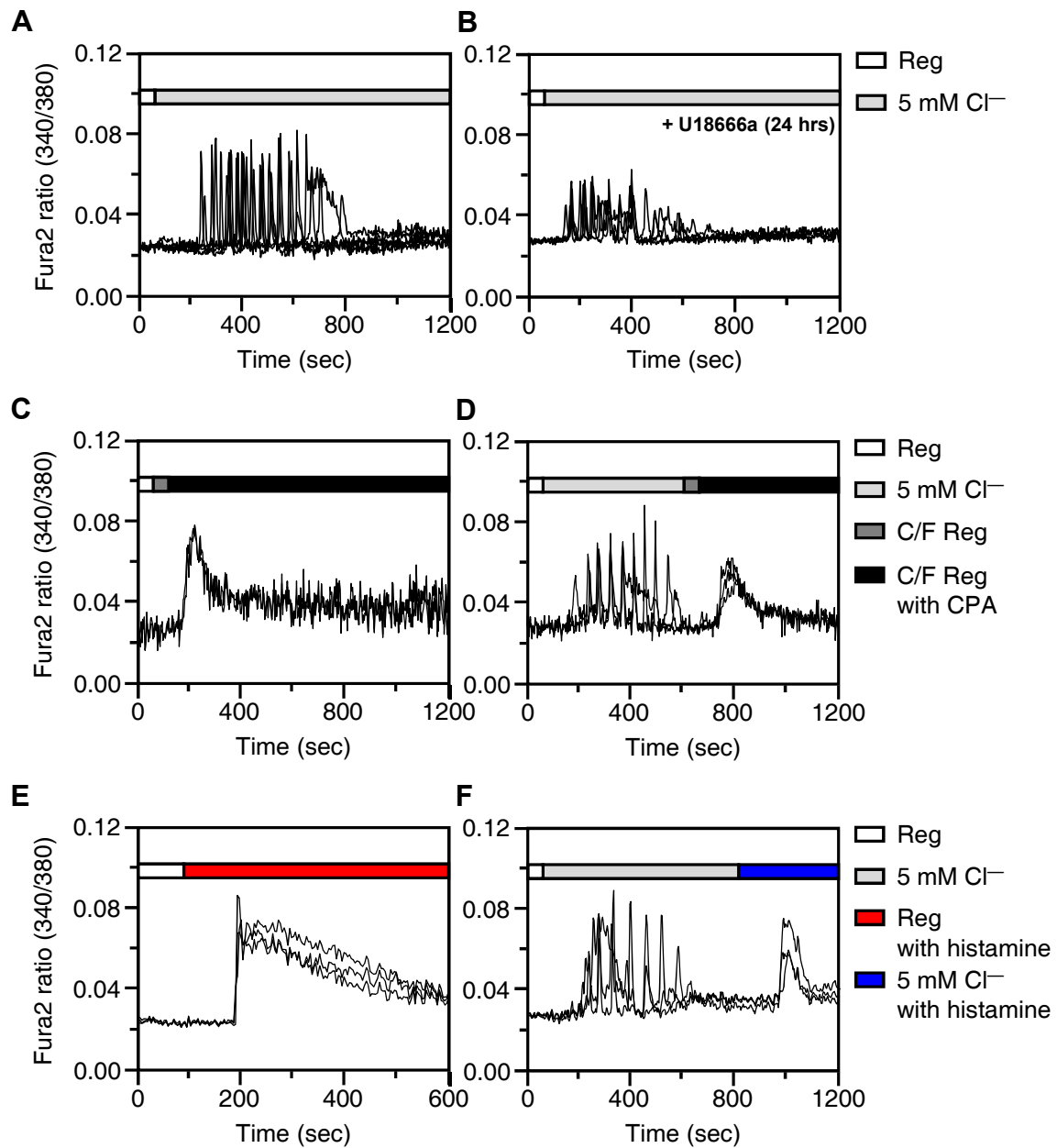
\* Correspondence to JHH ([minicleo@gachon.ac.kr](mailto:minicleo@gachon.ac.kr))



### Supplementary Figure S1

#### The pH calibration curves of BCECF and pHRodo

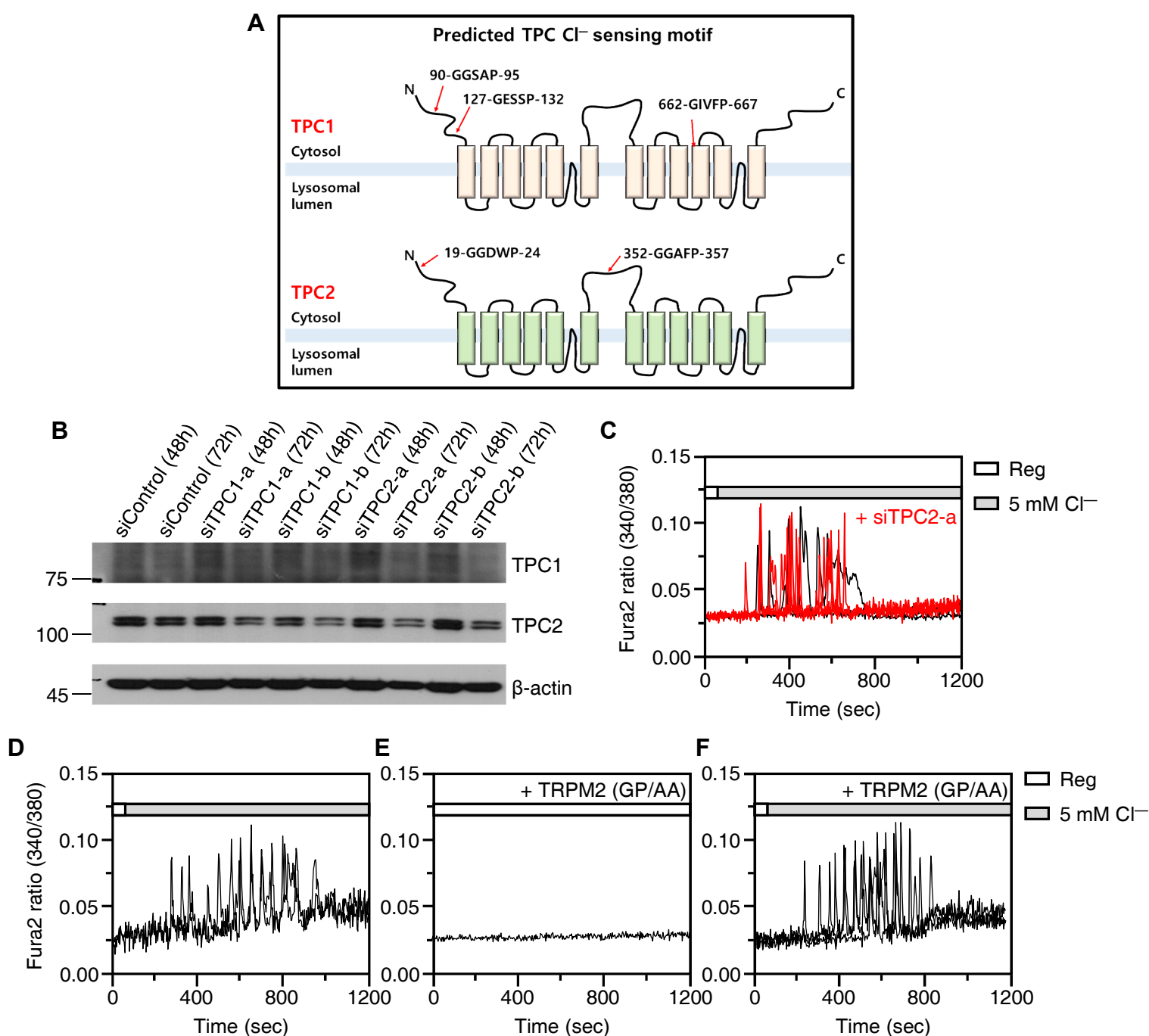
**(A)** The pH calibration curves for BCECF-loaded H1975 cells at pH 5.5, 6.0, 6.5, 7.0, 7.5, 8.0, and 8.5. The regression curve was  $\text{pH} = 6.97 - \log((7.36 - R)/(R - 2.47))$ , where R is the ratio of BCECF fluorescence. **(B)** The pH calibration curve for pHRodo-loaded H1975 cells at pH 4.5, 5.5, 6.5, and 7.5. Regression curves are  $\text{pH} = (F - 721.89)/(-92.741)$ , F: intensity of pHRodo fluorescence.



## Supplementary Figure S2

### The effect of low Cl<sup>-</sup>-mediated Ca<sup>2+</sup> signaling on ER Ca<sup>2+</sup> release

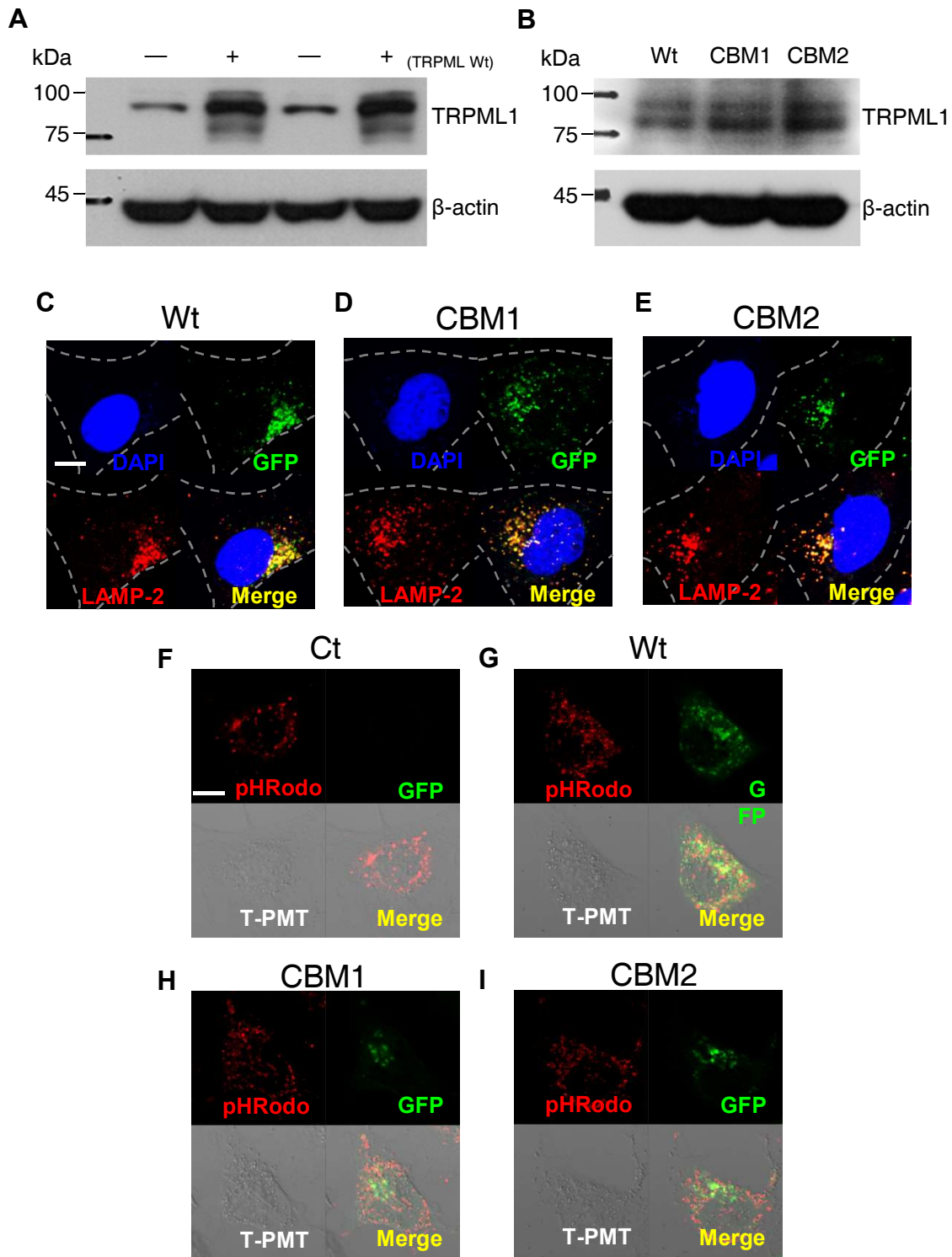
(A, B) Changes in [Ca<sup>2+</sup>]<sub>i</sub> in H1975 cells with 5 mM Cl<sup>-</sup>-induced Ca<sup>2+</sup> signaling in the absence of U18666a (4 μg/mL, 24 hrs) or in the presence of U18666a. (C, D) Changes in [Ca<sup>2+</sup>]<sub>i</sub> in H1975 cells in CPA treatment (C) and 5 mM Cl<sup>-</sup>-induced Ca<sup>2+</sup> signaling with subsequent treatment of CPA (D). Ca<sup>2+</sup> free concentration is defined as C/F. (E, F) Changes in [Ca<sup>2+</sup>]<sub>i</sub> in H1975 cells in histamine treatment (E) and 5 mM Cl<sup>-</sup>-induced Ca<sup>2+</sup> signaling with subsequent treatment of histamine (F).



### Supplementary Figure S3

**The modulation of TPC expression and TRPM2 GXXXP motif have no effect on low Cl<sup>-</sup>-induced Ca<sup>2+</sup> signaling**

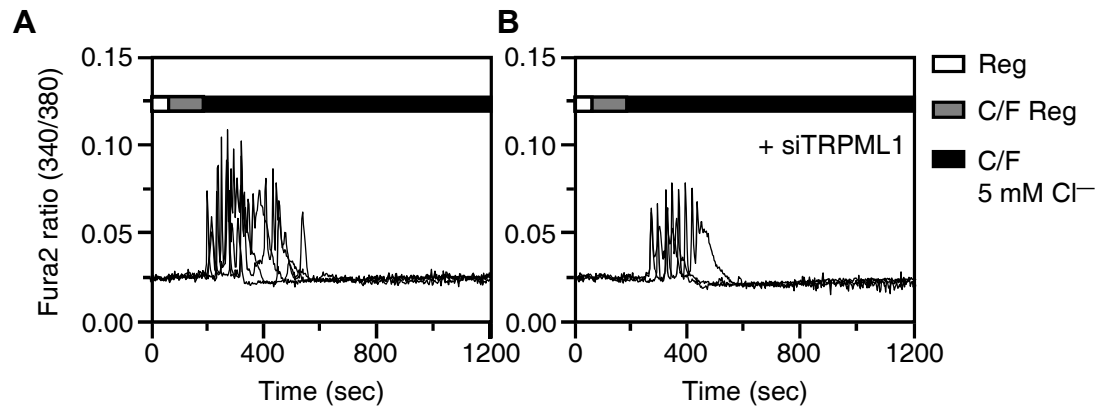
**(A)** Schematic illustration of TPCs structure marked with GXXXP motifs. **(B)** Western blotting analysis of TPC1 and TPC2 in siTPC1- or siTPC2-transfected H1975 cells. **(C)** Changes in [Ca<sup>2+</sup>]<sub>i</sub> in siTPC2-a-transfected H1975 cells with 5 mM Cl<sup>-</sup> solution. **(D-F)** Changes in [Ca<sup>2+</sup>]<sub>i</sub> in H1975 cells with 5 mM Cl<sup>-</sup> solution (D), Reg with TRPM2-GP/AA (E), 5 mM Cl<sup>-</sup> with TRPM2-GP/AA (F).



**Supplementary Figure S4**

#### The effect of TRPML1 clones on LAMP2 location and vesicle pH

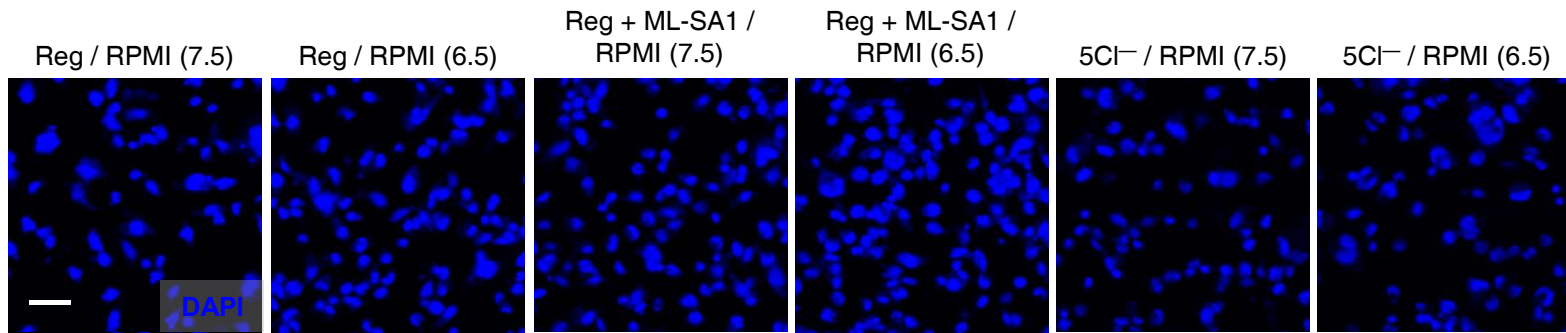
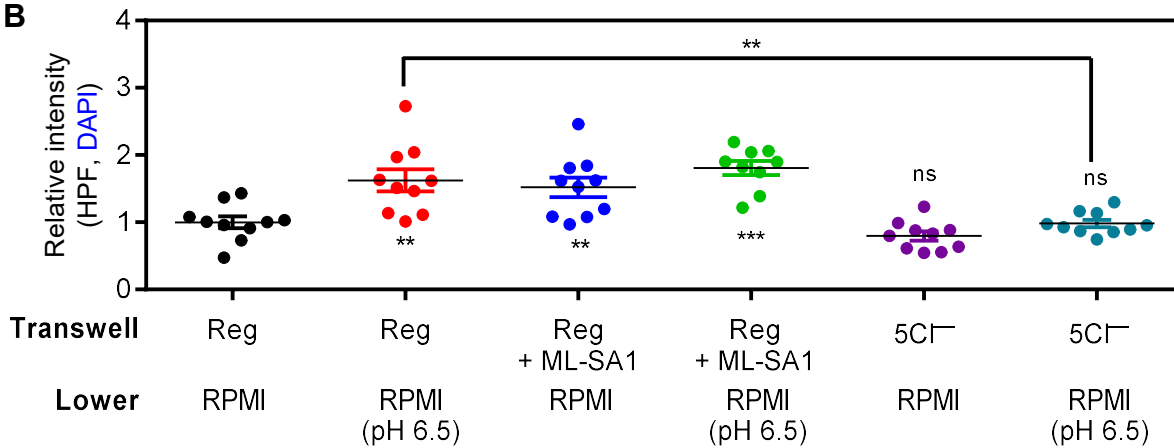
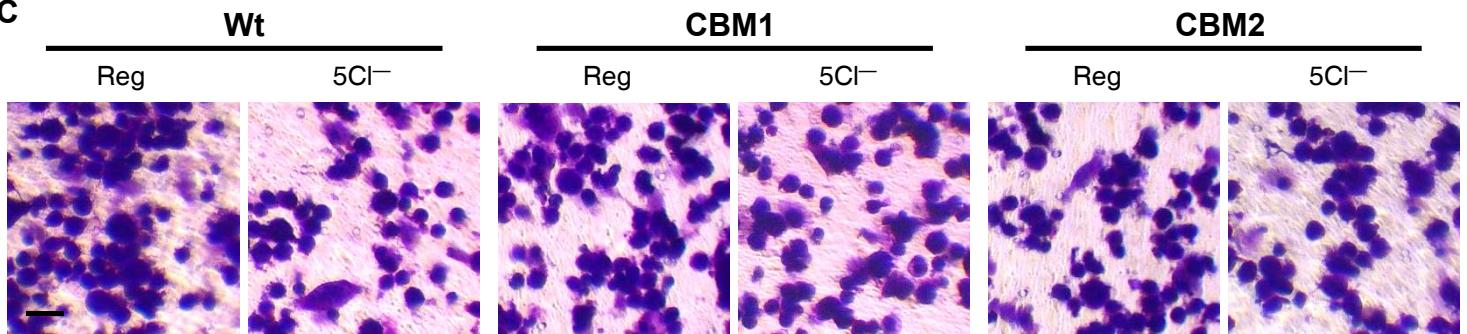
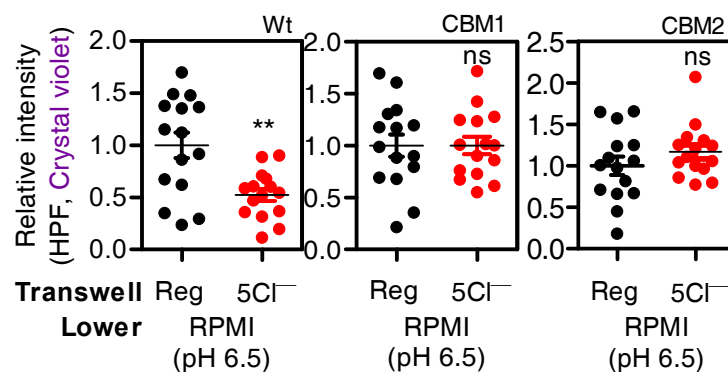
**(A)** Western blotting analysis of TRPML1 protein in H.T cells transfected with TRPML1 Wt. **(B)** Western blotting analysis of TRPML1 protein in H1975 cells transfected with TRPML1 Wt, CBM1, and CBM2. **(C-E)** Confocal images of immunofluorescence staining of LAMP-2 (red) and DAPI (blue) which transfected with GFP-tagged TRPML1 Wt, CBM1, and CBM2 (green). The scale (white) represents 10  $\mu$ m. **(F-I)** Confocal images of immunofluorescence staining of pHRedo (red) in control (F) and cells transfected with GFP-tagged TRPML1 Wt (G), CBM1 (H), or CBM2 (I). The scale bar (white) is 10  $\mu$ m.



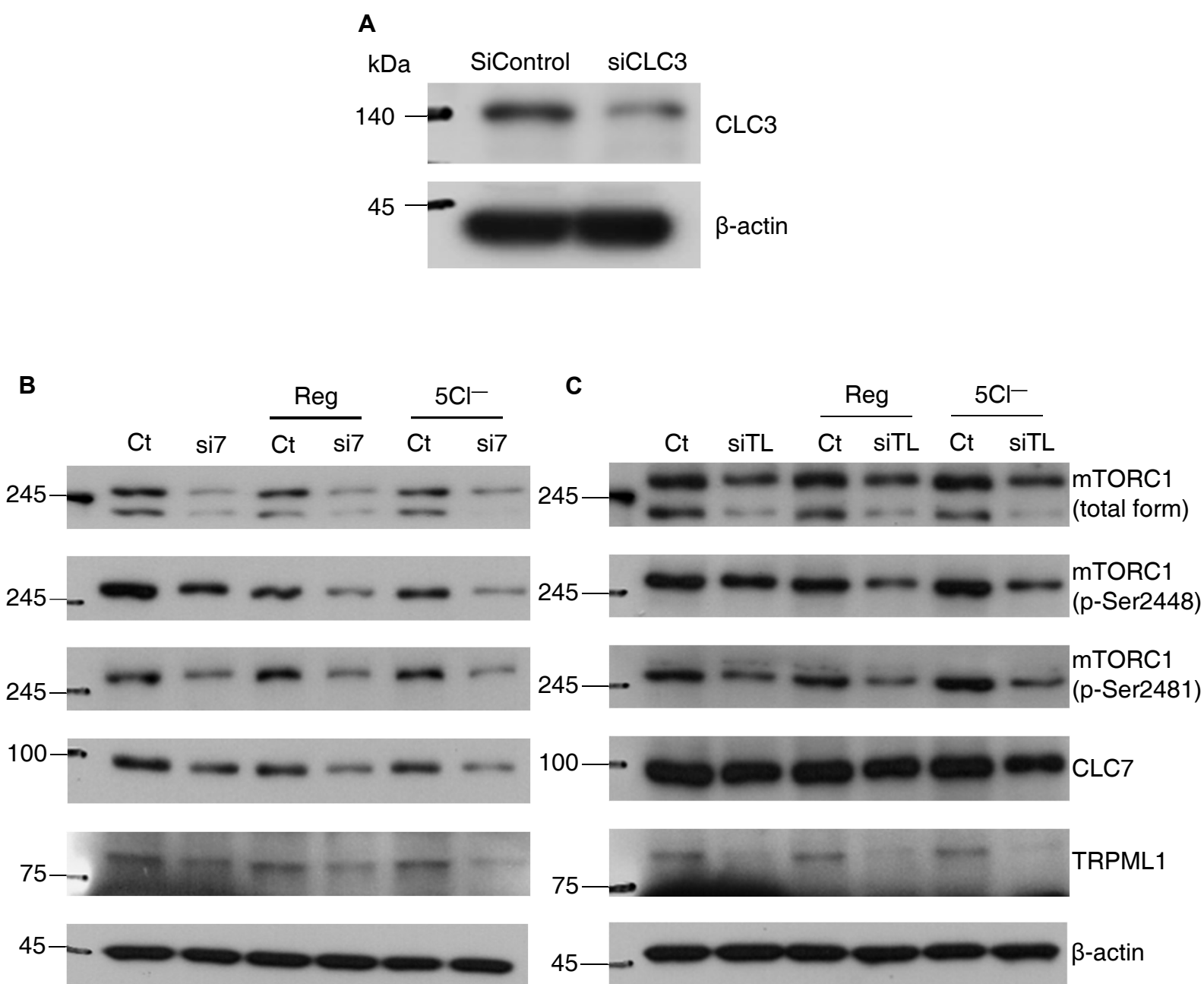
#### Supplementary Figure S5

#### The effect of siTRPML1 on C/F 5 mM Cl<sup>-</sup> solution-mediated [Ca<sup>2+</sup>]<sub>i</sub> signaling

(A, B) Changes in [Ca<sup>2+</sup>]<sub>i</sub> in H1975 cells (A) and with knock-down of TRPML1 (B).

**A****B****C****D****Supplementary Figure S6****Transwell migration assay in presence of ML-SA1 or overexpressed all types of TPRML1 clones**

**(A)** Images of transwell migration assay with DAPI (blue) which incubated for under the indicated conditions. **(B)** The dot plots are presented as means  $\pm$  SEMs of the relative intensity of DAPI (\*\*p < 0.01, ns; non-significance). **(C)** Images of transwell migration assay with crystal violet (purple) in TRPML1, CBM1, and CBM2-transfected cells with the indicated conditions. **(D)** The dot plots are presented as means  $\pm$  SEMs of the relative intensity of crystal violet (\*p < 0.05).



**Supplementary Figure S7**

**Western blotting analysis with siCLC3, siCLC7 and siTRPML1**

**(A)** Western blotting analysis of CLC3 transfected with siRNA-CLC3 (siCLC3). **(B)** Western blotting analysis of mTORC1, two types of phosphorylated mTORC1 (ser-2448 and ser-2481), CLC7 and TRPML1 transfected with siRNA-CLC7 (si7) in presence of Reg or 5 mM Cl<sup>-</sup> solution for 30 min. **(C)** Western blotting analysis of mTORC1, phosphorylated mTORC1 (ser-2448 and ser-2481), CLC7 and TRPML1 transfected with siRNA-TRPML1 (siTL) in presence of Reg or 5 mM Cl<sup>-</sup> solution for 30 min.