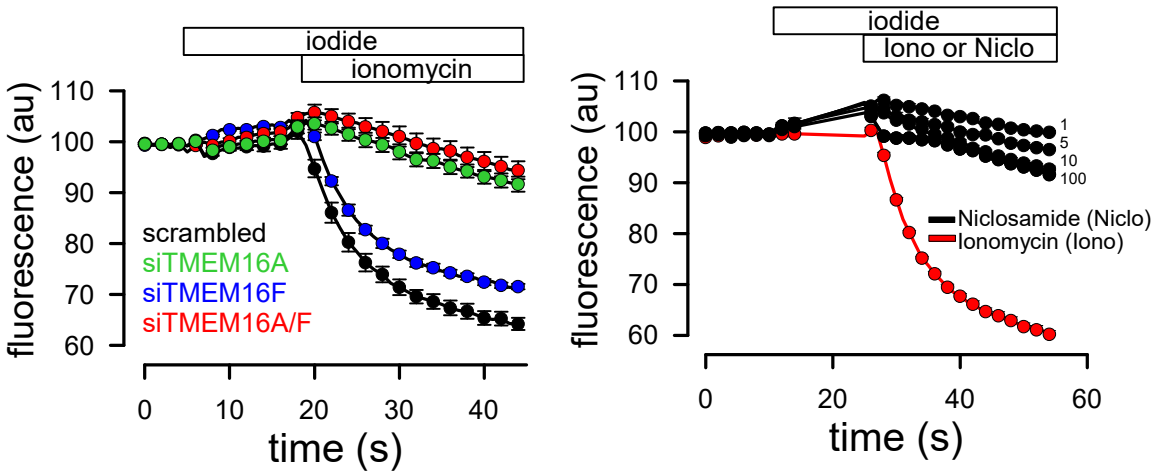
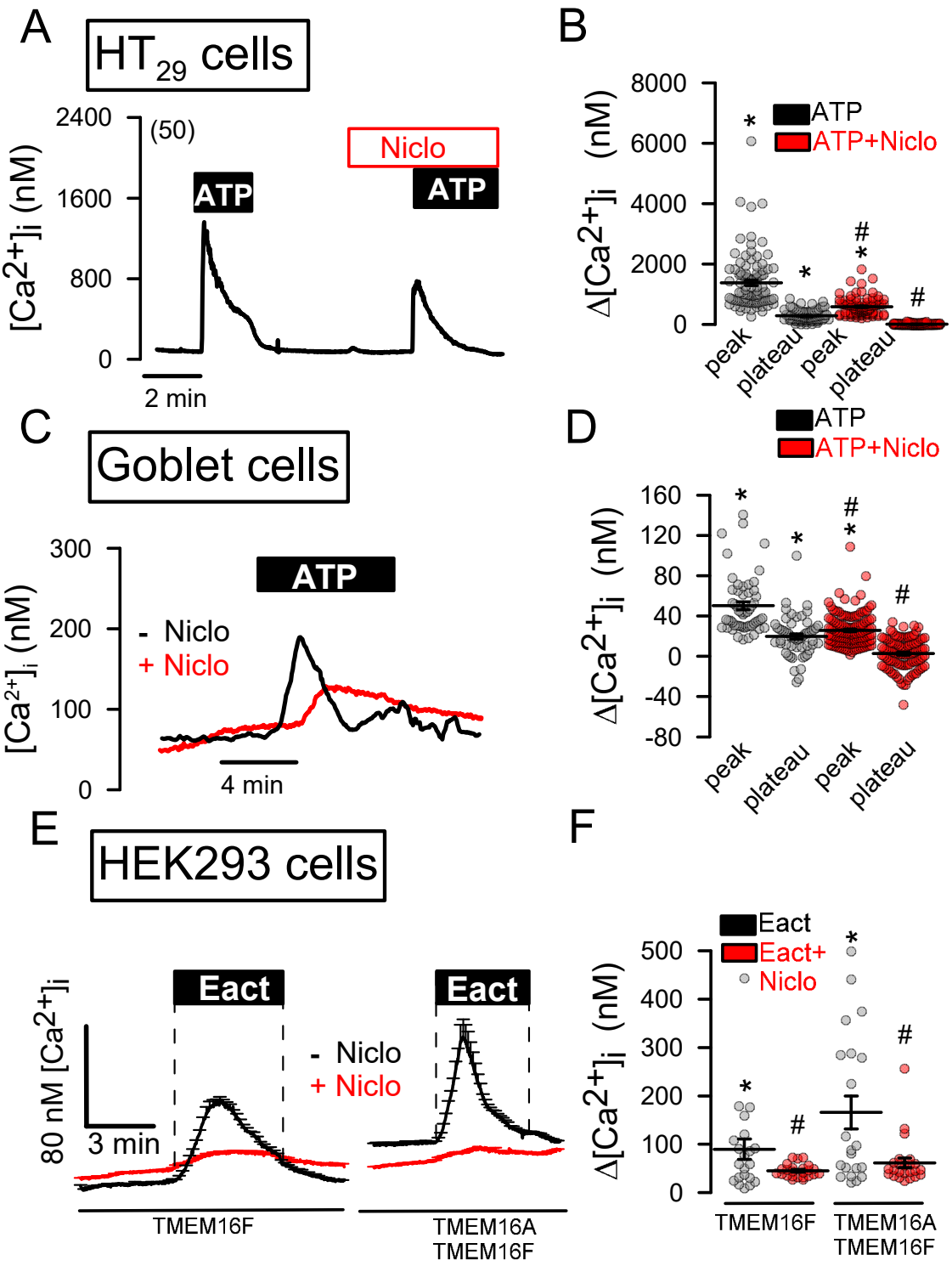
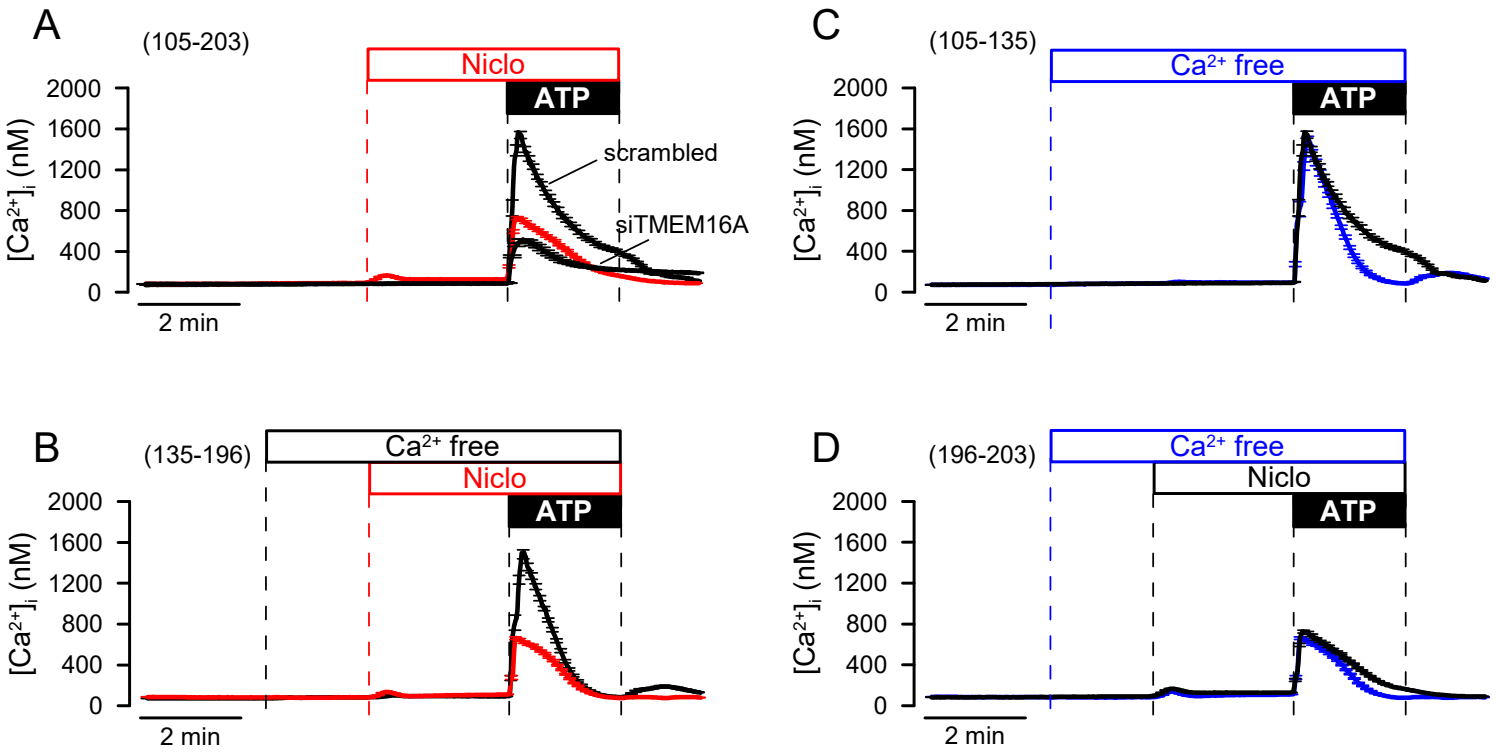


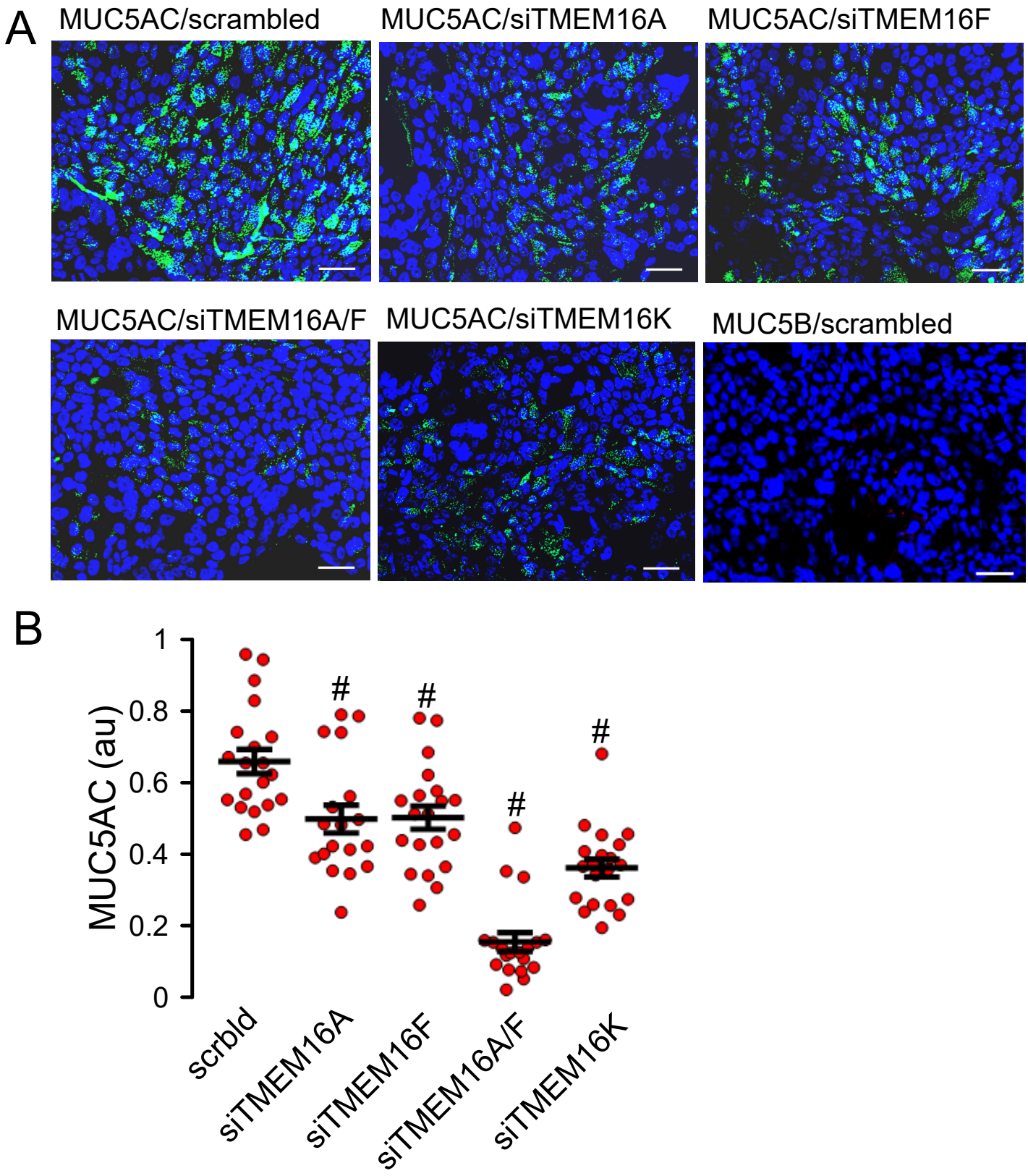
Supplemental Figure 1: *Role of TMEM16A and TMEM16F for ionomycin-induced quenching in HT_{29} cells.* YFP quenching by 20 mM extracellular iodide upon stimulation with ionomycin (1 µM). A) siTMEM16A and siTMEM16F inhibited ionomycin-induced quenching. B) Niclosamide itself did not exert any significant effect on YFP quenching, when applied at different concentrations (µM). Mean ± SEM. n=6 repeats for each condition.



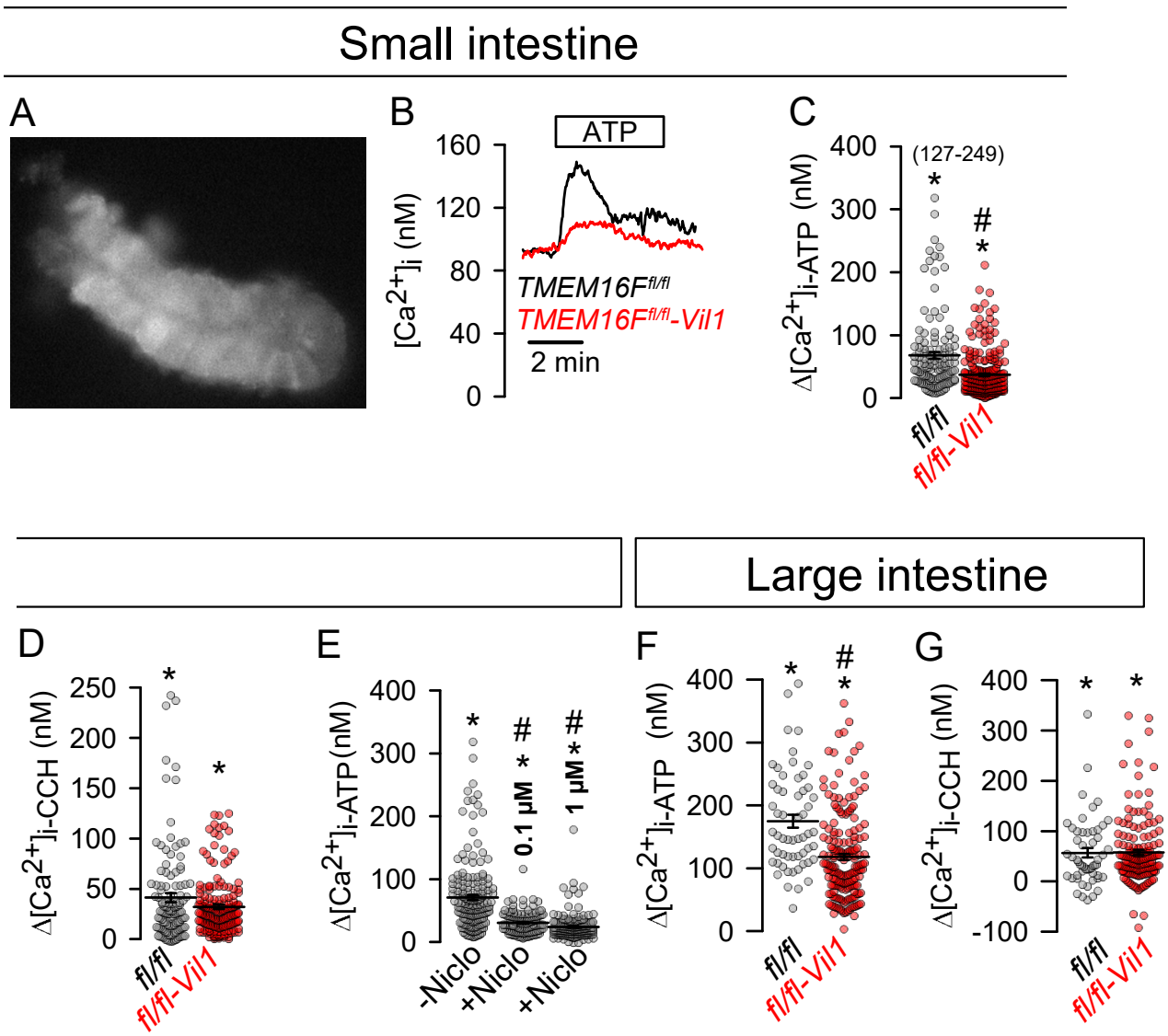
Supplemental Figure 2: *Inhibition of Ca^{2+} signaling by niclosamide in different cell types.* A,B) HT_{29} cells were loaded with the Ca^{2+}-sensitive dye Fura2 (5 µM/30 min). 10 µM ATP was applied to stimulate purinergic receptors, which increased intracellular Ca^{2+} and activated TMEM16A. Activation of TMEM16A was inhibited in the presence of niclosamide (Niclo, 5 µM), which also inhibited ATP-induced Ca^{2+}-release from the endoplasmic reticulum (peak) and blocked store operated Ca^{2+} influx (plateau) (n=50 cells). C) ATP-induced Ca^{2+} increase in goblet cells of freshly isolated colonic crypts. ATP (100 µM) was applied in the absence (black curve) or presence (red curve) of Niclo (100 nM). D) Summary of ATP-induced Ca^{2+} changes in goblet cells, indicating inhibition of peak and plateau Ca^{2+} increase by Niclo (n=55-132 cells). E,F) Ca^{2+} increase in HEK293 cells expressing endogenous TMEM16F only, or overexpressing TMEM16A together with endogenous TMEM16F. Ca^{2+}-rise by the activator of TMEM16A, Eact (50 µM), was inhibited by Niclo (1 µM; n=21-24 cells). Mean ± SEM; *significant increase ($p<0.05$; paired t-test). #significant inhibition by Niclo ($p<0.05$; unpaired t-test).



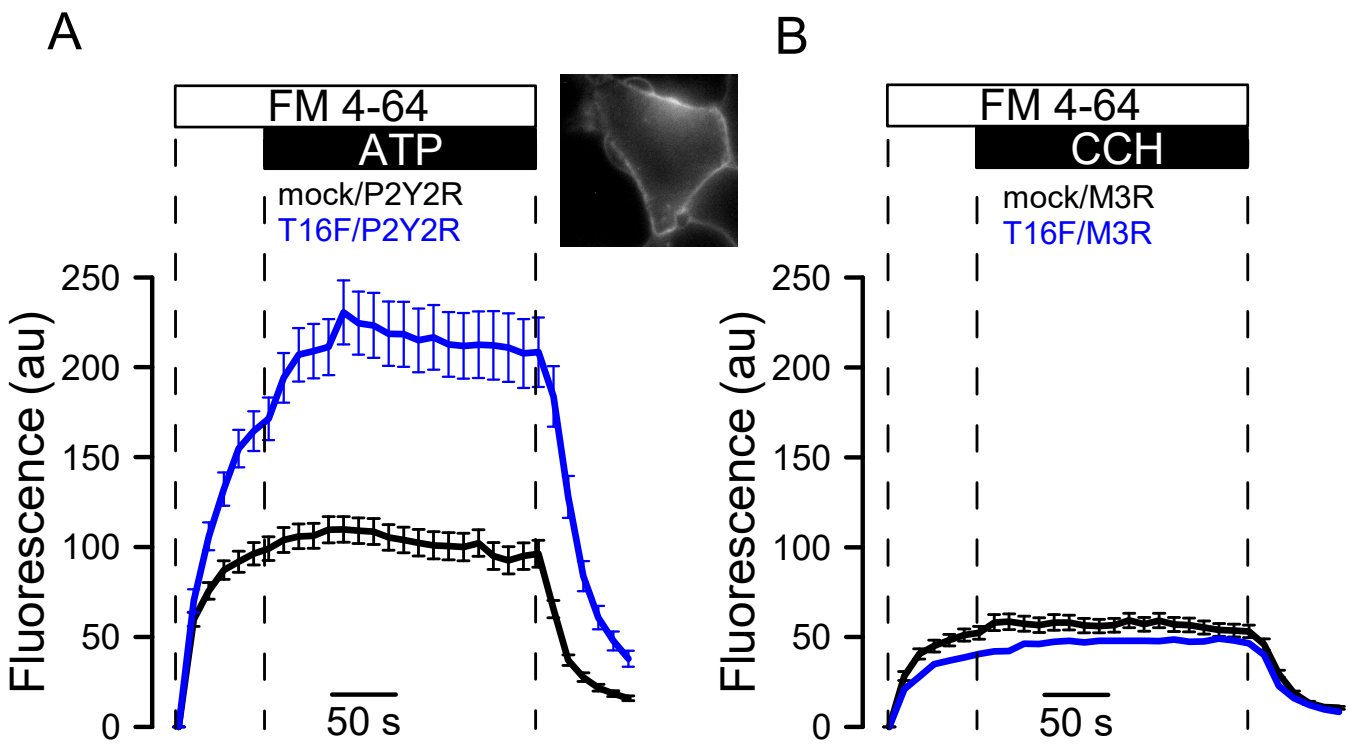
Supplemental Figure 3: *Inhibition of Ca^{2+} signaling by niclosamide in the presence and absence of extracellular Ca^{2+}.* A) Niclosamide (1 µM) inhibits the agonist induced ER Ca^{2+} store release (Ca^{2+} peak) and eliminates the Ca^{2+} influx (Ca^{2+} plateau) (red tracing). Black tracing was obtained in the absence of niclosamide, before (scrambled) and after siRNA-knockout of TMEM16A (siTMEM16A). The inhibitory effect of niclosamide and siTMEM16A on ATP-induced store release were comparable. B) In Ca^{2+} free solution, ATP-induced store release is fully maintained, but the plateau is eliminated (black tracing). Niclosamide in Ca^{2+} free solution still blocks store release (red tracing). Additional effects of niclosamide on Ca^{2+} plateau cannot be detected in Ca^{2+} free buffer. C,D) Both peak and plateau are inhibited in the presence of niclosamide and in extracellular free Ca^{2+} (blue tracing was obtained in extracellular free buffer). n=105-203 cells analyzed.



Supplemental Figure 4: *Role of TMEM16A, TMEM16F and TMEM16K for mucus production in Calu3 cells.* A) IL-13 (20 ng/ml; 72 hrs) induced expression of MUC5AC but not MUC5B in Calu3 cells. siRNA for TMEM16A, TMEM16F, and TMEM16K suppressed expression of MUC5AC. B) Inhibition of MUC5AC expression by knockdown of TMEM16 proteins. (n=19-21 images analyzed for each). Mean ± SEM; #significant inhibition ($p<0.05$; ANOVA).



Supplemental Figure 5: *Inhibition of intestinal Ca^{2+} signals in the absence of TMEM16F, and by niclosamide.* A) Crypt from small intestine (jejunum) loaded with the Ca^{2+} dye Fura2 (5 µM/30 min). B) ATP (100 µM) induced Ca^{2+} signals in crypt cells from *TMEM16F$^{flox/flox}$* (*fl/fl*) and *TMEM16F$^{flox/flox}$ Vil1Cre* (*fl/fl-Vil1*) littermates. C,D) Summary of ATP (100 µM) and carbachol (CCH, 100 µM) induced Ca^{2+} peaks, respectively (n=118-249 cells). E) Inhibition of ATP-induced Ca^{2+} increase by two different concentrations of niclosamide (Niclo; n=175-195 cells). F,G) Summary of ATP (100 µM) and carbachol (CCH, 100 µM) induced Ca^{2+} peaks in colonic crypt cells from *fl/fl* and *fl/fl-Vil1* littermates (n=53-183 cells). Mean ± SEM; *significant activation ($p<0.05$; paired t-test). #significant difference when compared to *fl/fl* or –niclo, respectively ($p<0.05$; unpaired t-test).



Supplemental Figure 6: *Coupling of $P2Y_2$ receptors but not muscarinic M3 receptors with TMEM16F.* A) Effect of ATP (100 µM) on plasma membrane insertion of the lipid dye FM4-64, present in the extracellular bath solution. Increase in FM4-64 fluorescence in the plasma membrane of HEK293 cells can be taken as a measure for endosomal recycling/exocytosis. Cells expressing TMEM16F in addition to $P2Y_2$ receptors, show stronger basal FM4-64 fluorescence, which is further enhanced by stimulation with ATP. Inset: FM4-64 labeled HEK293 cell. B) Effect of CCH (carbachol; 100 µM) on FM4-64 insertion and staining of the plasma membrane in HEK293 cells expressing mock/M3R or TMEM16F/M3R. Lower basal FM6-64 fluorescence and lack of muscarinic stimulation of membrane exocytosis was observed in these cells. Mean ±SEM; n = 30 cells for each experimental series.