

Supplemental Material to

Calcium-activated chloride channel regulator 1 (CLCA1) controls mucus expansion in colon by proteolytic activity

Figure S1 and S2, page 2 and 3
Video S1 as separate file.

Video S1: Videos of bead tracking in untreated and rCLCA1 treated *Clca1*^{-/-} mucus

A: Tracking of beads in the buffer 120 μm above the mucus surface in untreated mucus at $t = 60$ min.

B: Tracking of beads in the buffer 120 μm above the mucus surface in rCLCA1 (10 μg) mucus at $t = 60$ min (30 min of treatment).

Red/Purple spheres = tracked beads in untreated and rCLCA1 treated mucus respectively, cylinders = bead tracks colored by mean speed ($\mu\text{m} / \text{second}$). The videos were recorded at 2.5 times the live speed.

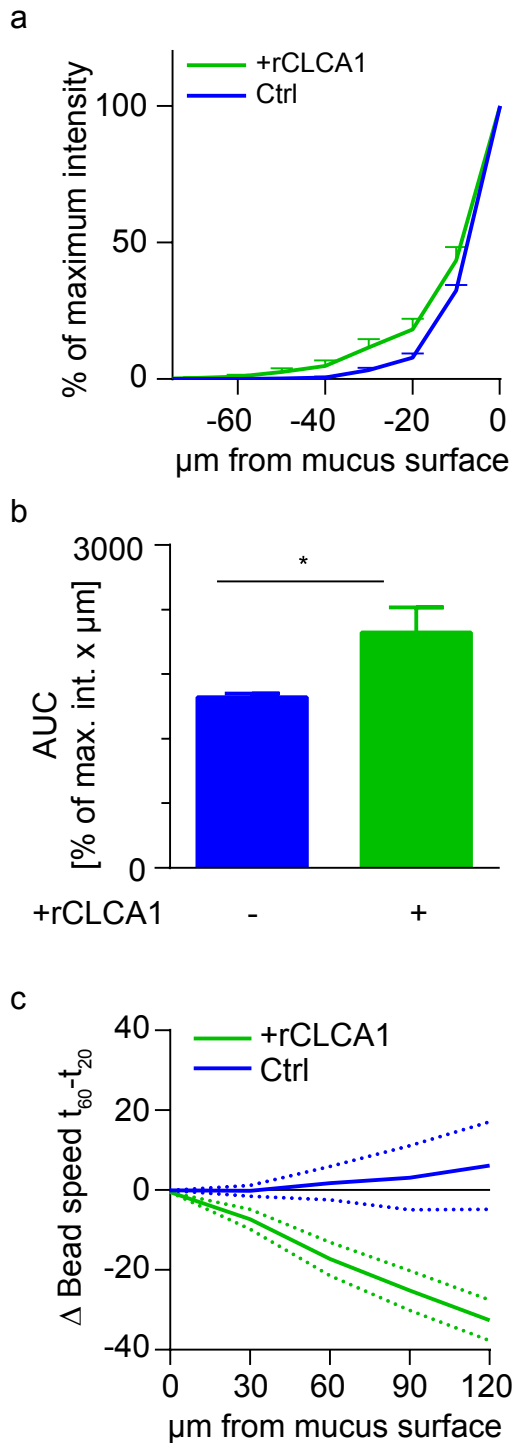


Figure S1: Effect of rCLCA1 on WT mucus penetrability and bead tracking.

(A) Distribution of beads in control or rCLCA1 (10µg) treated WT mucus. The z-position with highest bead intensity was defined as the mucus surface, and the bead penetrance in the mucus was plotted as % of maximum intensity at each z-position below the mucus surface. N=4 in each group. (B) Area under curve (AUC) analysis of bead penetrability data (A) comparing rCLCA1 treated with control mucus. C: Change in average bead speed in different z-planes above the mucus surface between $t=20$ min and $t=60$ min in control or rCLCA1 (10µg) treated WT mucus, where the treatments were applied at $t=30$ min. Data are presented as mean \pm SEM (n=4 in each group). * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$ with either two-way ANOVA with Tukey's multiple correlation test (MCT) (B) or unpaired 2-tailed t-test (E).

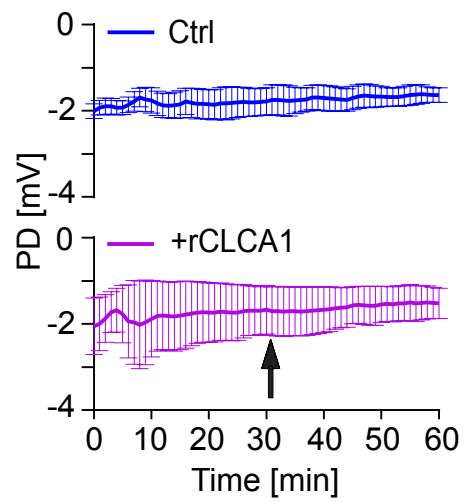


Figure S2: potential difference in human colon biopsies with rCLCA1

Transepithelial potential difference (PD) was recorded in untreated (Ctrl, blue) and rCLCA1(purple) treated human sigmoid colon biopsies. rCLCA1 was added at t= 32 min (arrow). Data is presented as mean \pm SEM (n= 4).