

Description of Supplemental Video 1: Carbachol (CCH) stimulated goblet cell exocytosis in WT enteroid.

L = Lumen of crypt (Note: Contains cell debris)

BL= Basolateral side of epithelium

<u>Time (min)</u>	<u>Description</u>
3:50 - 4:15	2 min after CCH exposure (at 1:50) White arrows = Indicate two goblet cells in the epithelium
4:20	First evidence of exocytosis and granule decondensation
4:50 - 7:10	Cavitation or “cupping” into the granule cluster during exocytosis. Note brief epithelial contraction between 4:30 and 5:15.
7:15 - 10:10	Exocytosis completed at 7:15.

Description of Supplemental Video 2: Carbachol (CCH) stimulated goblet cell exocytosis in Cftr-KO enteroid.

L = Lumen of crypt

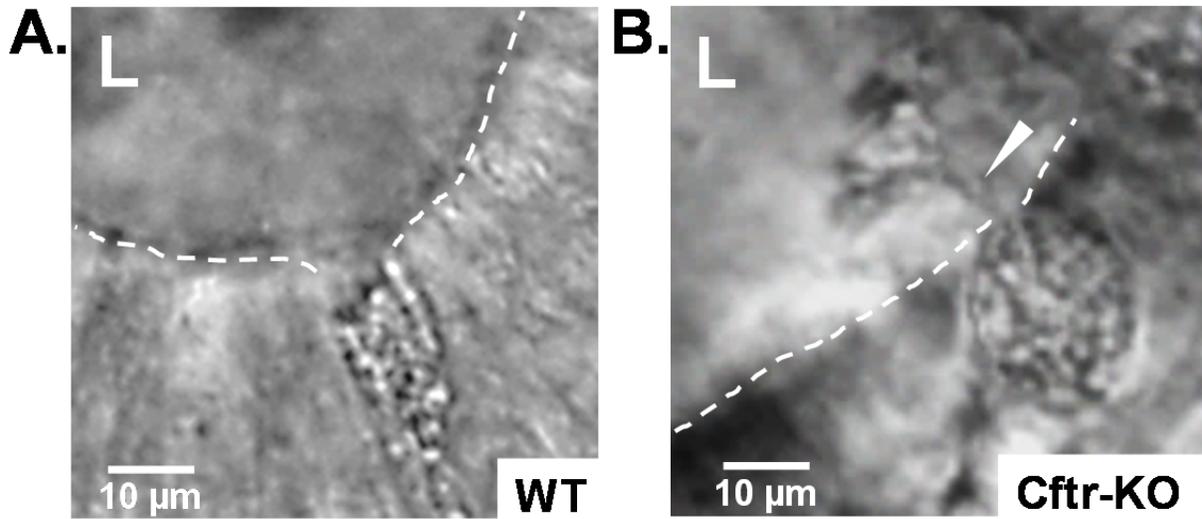
BL = Basolateral side of epithelium

<u>Time (min)</u>	<u>Description</u>
6:00 - 6:45	1.25 min after CCH exposure (at 4:45) White arrow = Goblet cell. Note enlarging size of granule cluster.
6:50 - 8:40	Black arrows = Clear amorphous material (mucus) forming bleb at basolateral surface of epithelium.
8:40 - 9:15	Yellow arrowhead = Individual granule enlarging within cluster
9:20 - 9:50	White arrowhead = Erupting exocytosis and formation of luminal mucus bleb
10:20 - 11:05	White arrowhead = Exocytosis by out of focus goblet cell and formation of second luminal mucus bleb
11:10 - 11:55	Note wave of epithelial cell distortion traveling from lumen side to basal side of epithelium.
12:00 - 13:00	Black arrow = Subsequent formation of basolateral mucus bleb.

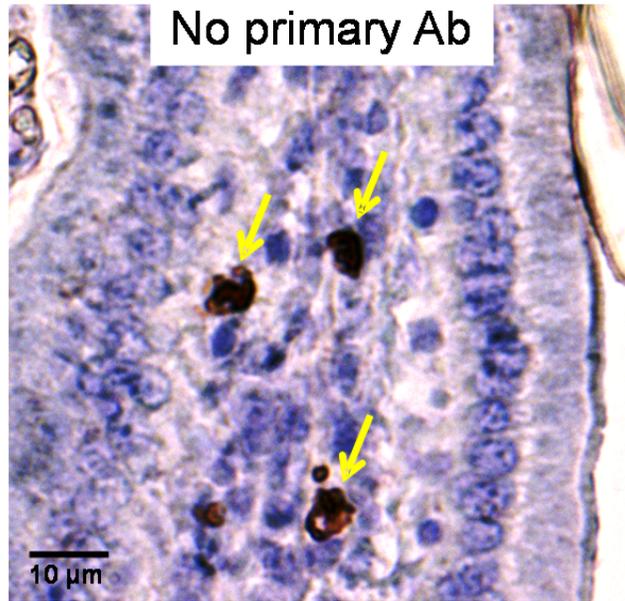
Description of Supplemental Video 3: Carbachol (CCH) stimulated goblet cell exocytosis in Cfr-KO enteroid during luminal superfusion with 115-mM HCO₃⁻ KBR.

L = Lumen of crypt

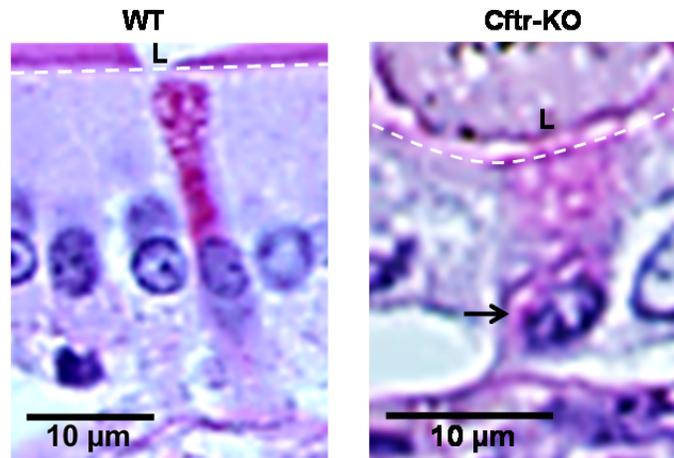
<u>Time (min)</u>	<u>Description</u>
7:00 - 7:22	2.87 min after CCH exposure (at 4:08) Normal exocytosis and decondensation of mucin granules. Note: Granule cluster is already enlarged from luminal superfusion with 115mM HCO ₃ ⁻ KBR and exposure to CCH.
7:23 - 7:31	Note movement of basal aspect of granule cluster toward luminal membrane during continuing exocytosis.
7:32 - 7:37	White arrowhead = Formation of clear mucus bleb on luminal side of granule cluster during exocytosis.
7:38 - 8:54	Continuing exocytosis of granule cluster.
8:55 - 9:07	White arrowhead = Formation of clear mucus bleb on luminal side of granule cluster during exocytosis.



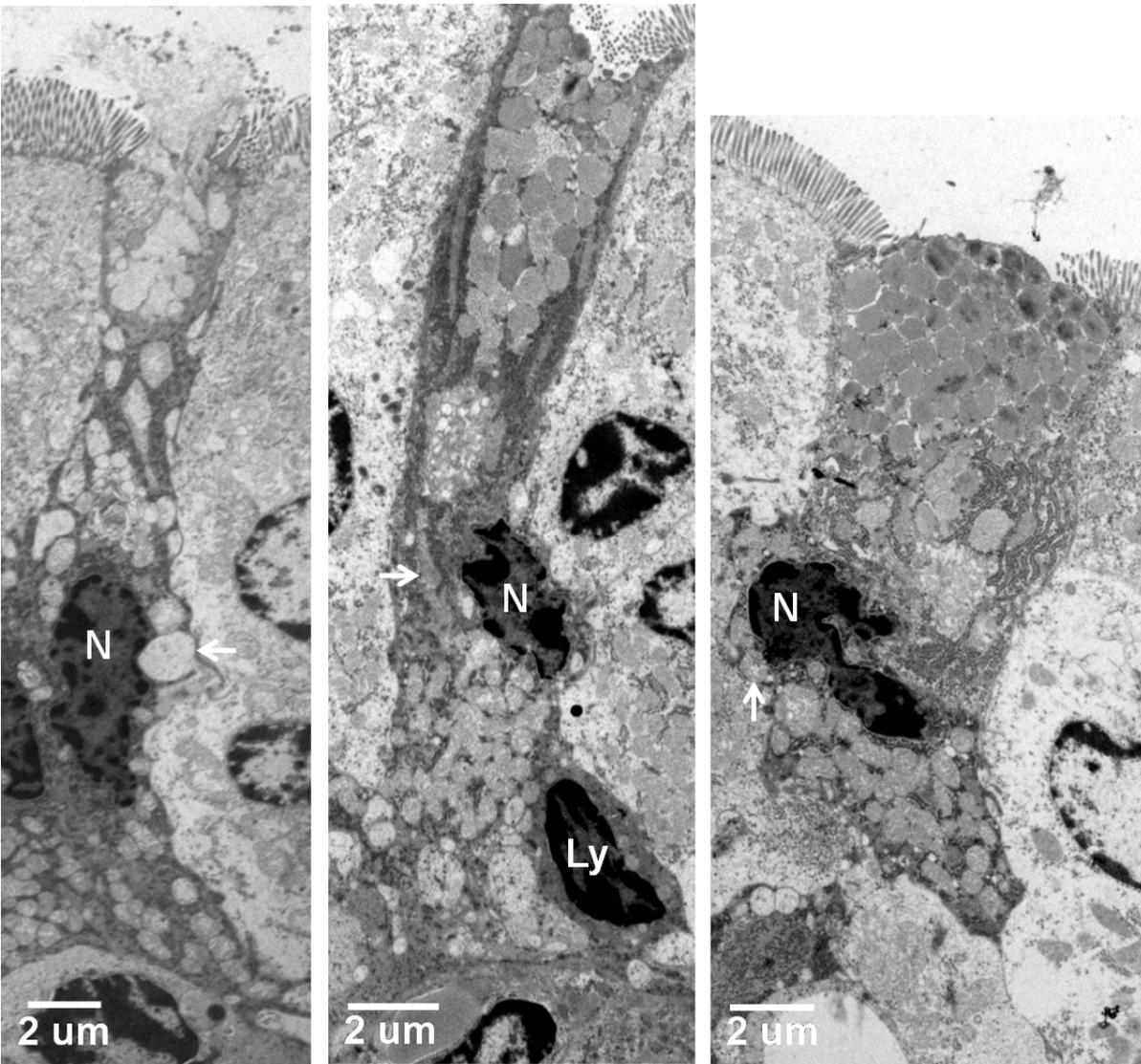
Supplemental Figure 1. Enlarged images of WT and Cftr-KO goblet cells following 100μM carbachol stimulation of exocytosis. A. WT goblet cell. Note cupping at apical aspect. B. Cftr-KO goblet cell. White arrowhead, luminal mucus bleb attached to content in granule cluster in goblet cell theca. L, crypt lumen. White dashed line, apical membrane of adjacent enterocytes.



Supplemental Figure 2: No primary Muc2 antibody control showing only incidental HRP staining of submucosal granulocytes (yellow arrows) in lamina propria.



Supplemental Figure 3. Periodic acid-Schiff (PAS) staining of WT and Cftr-KO intestine exposed to 100 μ M carbachol for 20 min. WT, note PAS-stained granule cluster at apical aspect of goblet cell. PAS staining extends from the luminal membrane to the apical aspect of the nucleus. Cftr-KO, PAS-stained granules at and below level of nucleus (black arrow). L, lumen. White dashed line, apical membrane of adjacent enterocytes.



Supplemental Figure 4. TEM images of Cftr-KO goblet cells. Left, spontaneous exocytosis in Cftr-KO goblet cell. Note luminal mucus bleb. Middle and Right, Cftr-KO goblet cells 20 min post-carbachol (100µM). Note distorted shape and location of nucleus. Right also shows apical granule compaction. N, nucleus. Ly, lymphocyte. White arrows, indicate mislocalized granules at and basal to nuclear level.