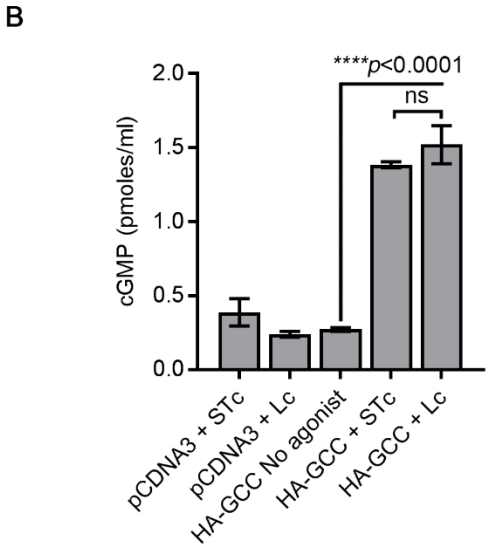
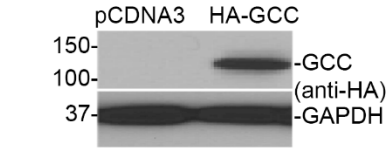
Supplementary figure legends

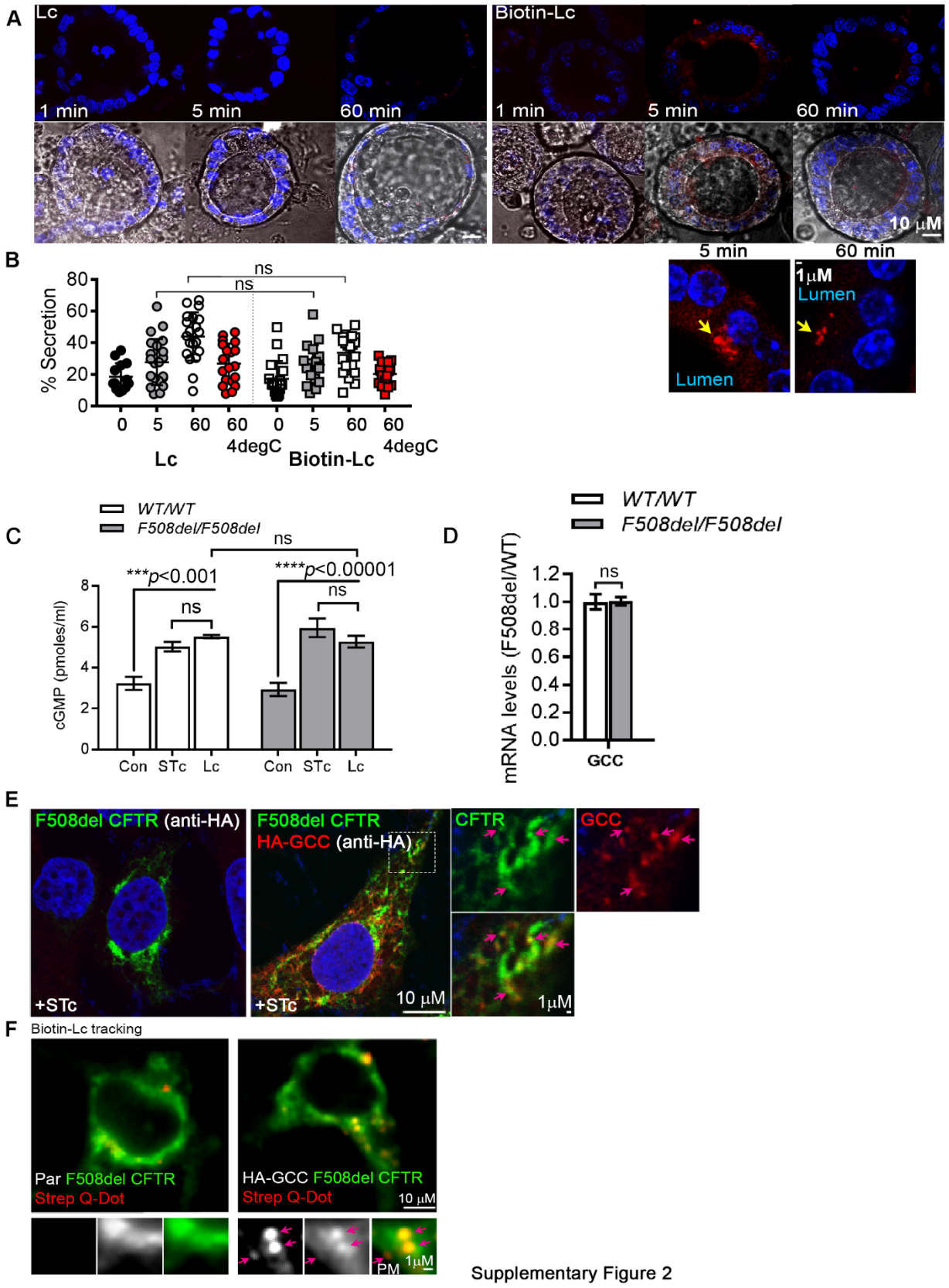




Supplementary Figure 1

Supplementary Figure 1

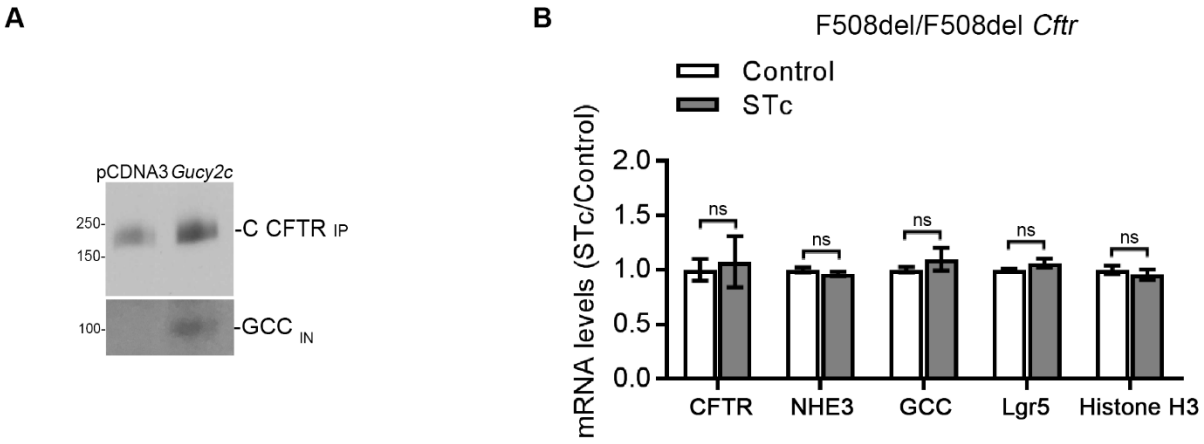
(A) An amino acid sequence comparison between heat-stable enterotoxin A2, STcore (STc), and linaclotide (Lc). (B) Left panel, cGMP-specific ELISA in whole-cell lysate obtained from HEK 293 cells expressing empty vector (pCDNA3) or pCDNA3-HA-GCC treated with ±STc or Lc (500 nM, 30 min at 37ºC). (C) Western blot data depict HA-GCC expression in HEK 293 cells used for ELISA shown in the left panel. GCC was probed using anti-HA rabbit polyclonal antibody, and GAPDH was used as a loading control. Data is ± SEM calculated from n=4 wells per condition. *P*-value was determined using one way ANOVA with Bonferroni's test for multiple comparisons, ns=nonsignificant.



Supplementary Figure 2

Supplementary Figure 2

(A) Confocal imaging to track biotinylated-linaclotide (2 μM) movement added to the media; basolateral side of the intestinal spheres at the time points 0, 5, and 60 min. Biotin-linaclotide could be identified adhering to the basolateral side and intracellular of the organoids at time point of 5 min. At the end of the 60-min incubation, the peptide was primarily identified on the luminal surface of the spheres. A set of organoids was kept at 4ºC during the assay as a negative control, and biotin-linaclotide could not be located, suggesting the process to be an active event (data not shown). (B) Dot plot shows quantitation of fluid secretion in n=18 to 32 enterospheres per condition described in panel A. Data is ± SEM. *P*-value was determined using one way ANOVA with Bonferroni's test for multiple comparisons, ns=nonsignificant. (C) Bar graph represents quantitation of cGMP levels in whole-cell lysate obtained from WT/WT *cftr* (n=6 wells) and F508del/F508del *cftr* (n=6 wells) enterospheres treated ±STc or Lc (500 nM, 60 min at 37ºC). Data is ± SEM. *P*-value was determined using one way ANOVA with Bonferroni's test for multiple comparisons, ns=nonsignificant. (D) Bar graph represents relative mRNA levels of GCC in WT/WT *cftr* (n=3 biological replicates) and F508del/F508del *cftr* (n=3 biological replicates) enterospheres. Histone H3 was used as a reference gene. Data is ± SEM. *P*-value was determined using an unpaired two-tailed Student's *t* test, ns=nonsignificant. (E) Confocal images of HEK 293 cells expressing YFP-F508del CFTR ± HA-GCC treated with STc (500 nM, 24 h) were stained with anti-HA antibody to study co-localization between CFTR and GCC (indicated by pink arrows). (F) Fluorescent images of HEK 293 cells obtained using a wide-field microscope demonstrate biotin-linaclotide (2 μM) imaged at 30 min postincubation by using streptavidin-conjugated Q dot in HEK 293 cells with no GCC (Parental or Par) or a stable expression of HA-GCC and transiently expressing GFP-tagged F508del CFTR.



Supplementary Figure 3

Supplementary Figure 3

(A) Western blot data depict increased Band C of CFTR immunoprecipitated from HEK 293 cells that overexpressed FLAG WT CFTR using protein A resin with cross-linked 24-1 anti-CFTR antibody with and without co-expression of HA-GCC. (B) Bar graph represents relative mRNA levels corresponding to CFTR, Na^+/H^+ exchanger 3 (NHE3), Lgr5, and Histone H3 in F508del/F508del *cftr* intestinal spheres ± STc. GAPDH was used as a reference gene. Error bars represent SEM calculated from n=3 independent experiments (biological replicates). Data is ± SEM. *P*-value was determined using one way ANOVA with Bonferroni's test for multiple comparisons, ns=nonsignificant.