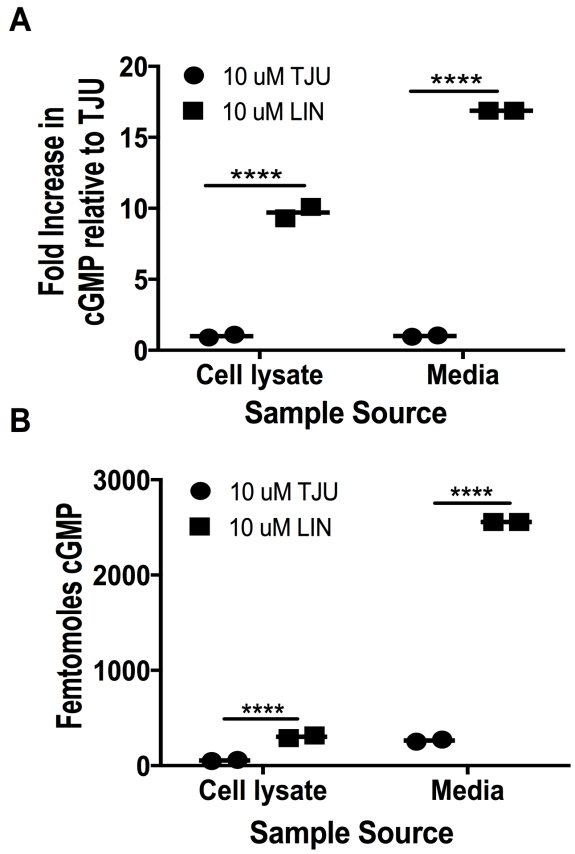
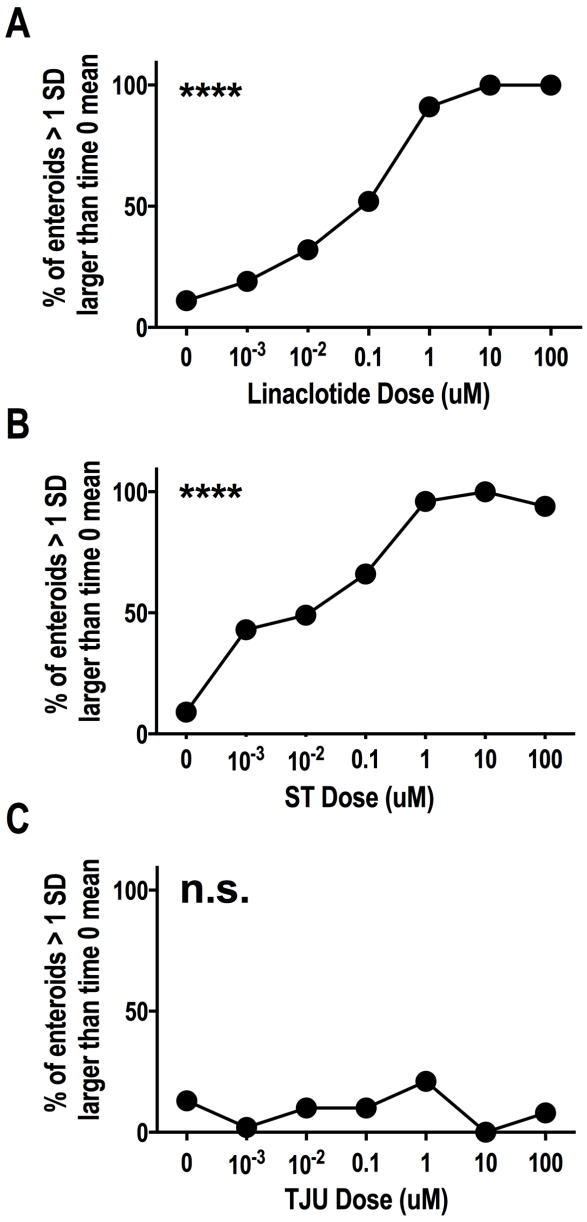
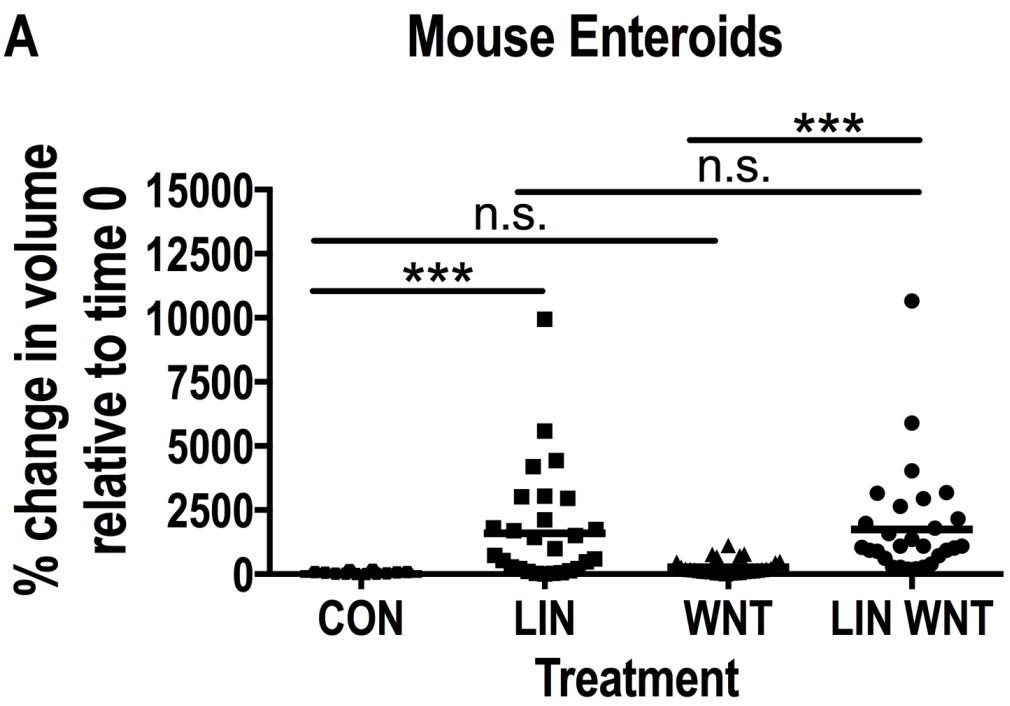
Supplementary Figures



Supplemental Figure 1. Enteroids secrete cGMP into the media. Following a 24 h incubation with 10 uM linaclotide or TJU, media was collected from each well for cGMP analysis. Separately, enteroids were lysed with 1x Passive lysis buffer and then processed for cGMP analysis. Both approaches revealed that linaclotide significantly increased cGMP production compared to TJU. However, >90% of the cGMP produced by enteroids was secreted into the media. Each sample was assayed in duplicate, with the mean represented as a single data point. Each point represents an independent well of enteroids or media. Error bars represent standard deviation; for some data, error bars are too small to be depicted. ****, $p< 0.0001$.



Supplemental Figure 2. GUCY2C ligands increase the fraction of enteroids secreting in a concentration-dependent fashion. For each experiment, enteroid volume at time 0 was calculated to determine mean and standard deviation. After 120 min, 25-50 enteroids were measured at each dose to determine their volume; enteroids with a volume greater than the mean + SD were considered responders. ****, $p < 0.0001$.



Supplemental Figure 3. ST-mediated secretion occurs in the presence of WNT treatment. (A) Normalized swelling assay of *Gucy2c*$^{+/+}$ mouse enteroids that were co-treated with Wnt3a conditioned media and 10 µM linaclotide for 48 hrs.

Supplementary Video 1. TJU (control)-induced swelling of *Gucy2c*$^{+/+}$ mouse enteroids.

Supplementary Video 2. Linaclotide-induced swelling of *Gucy2c*$^{+/+}$ mouse enteroids.

Supplementary Video 3. TJU (control)-induced swelling of *Gucy2c*$^{-/-}$ mouse enteroids.

Supplementary Video 4. Linaclotide-induced swelling of *Gucy2c*$^{-/-}$ mouse enteroids.

Supplementary Video 5. 8-Br-cGMP-induced swelling of *Gucy2c*$^{-/-}$ mouse enteroids.