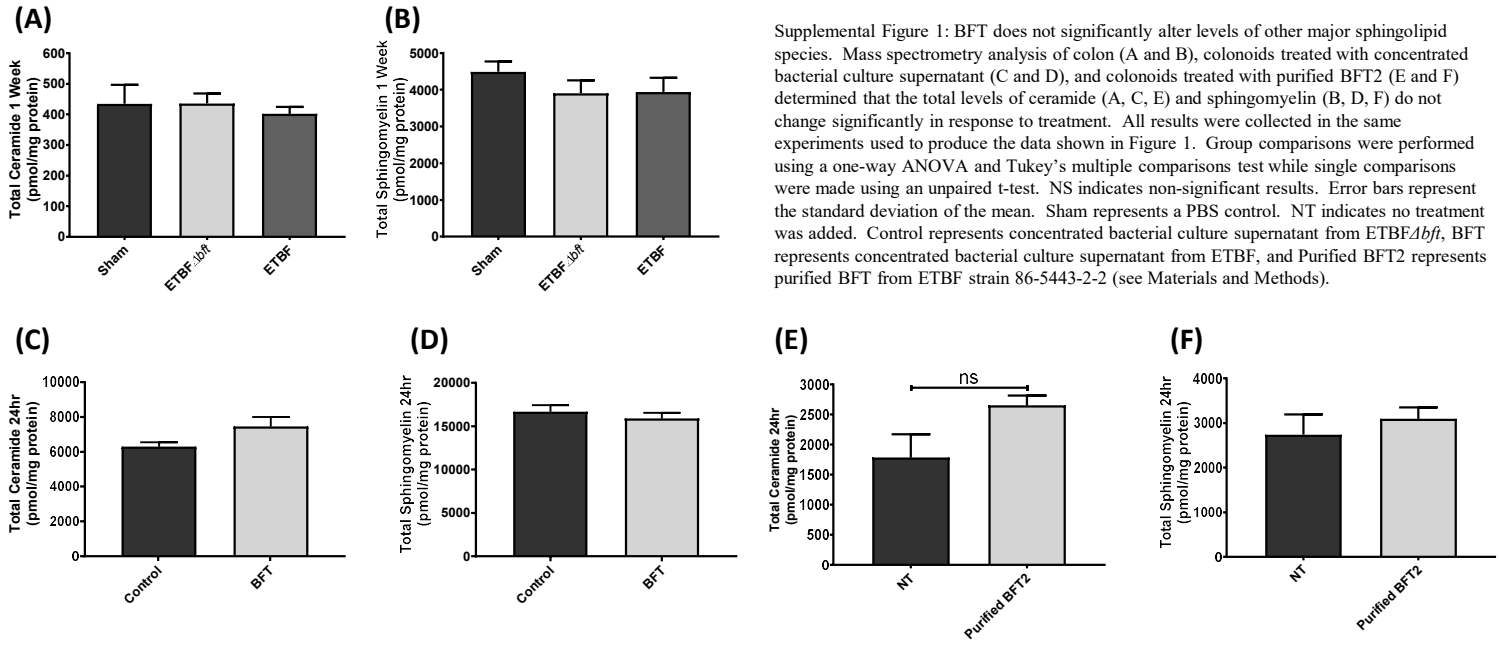
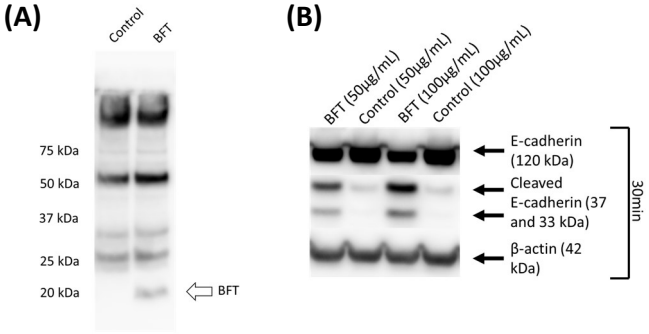
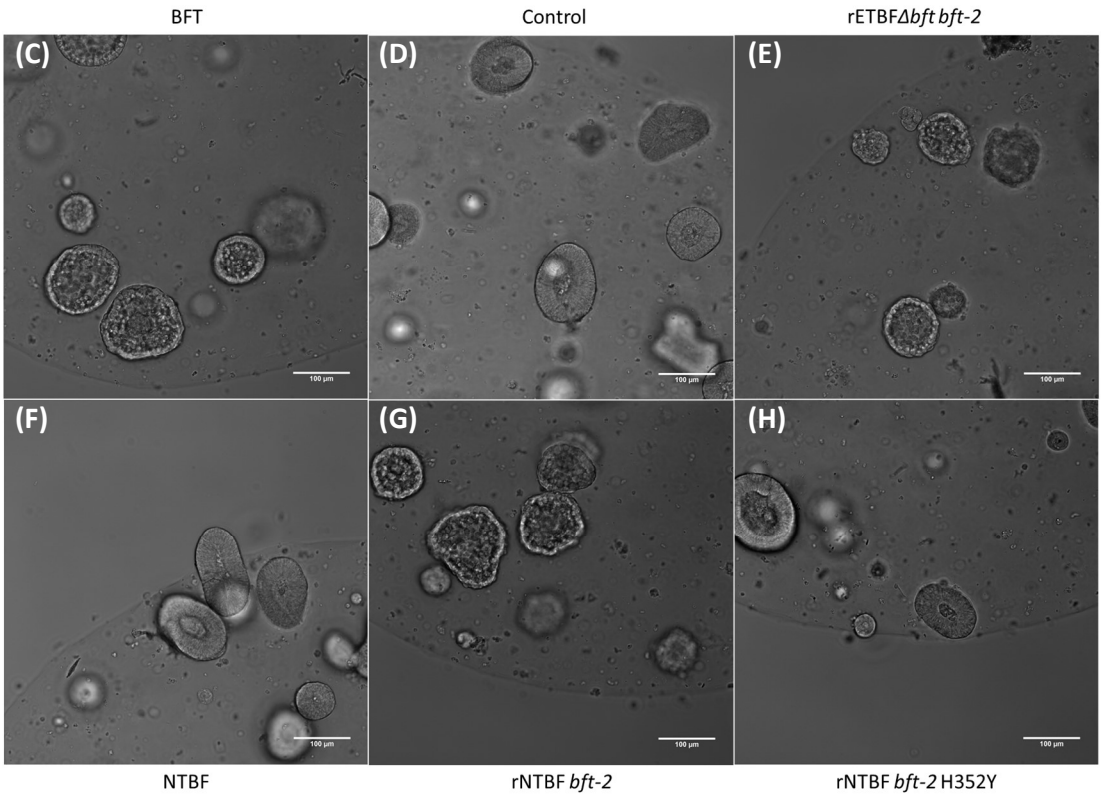
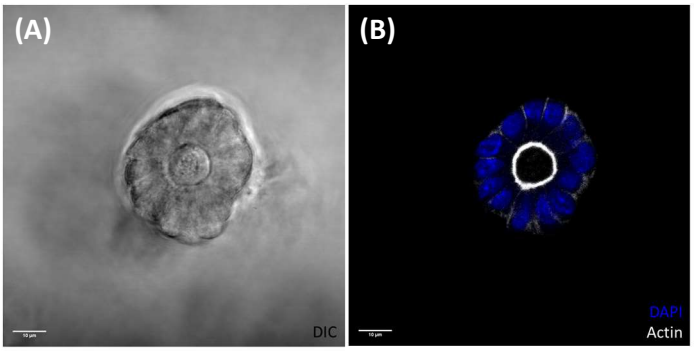


Supplemental Figure 1: BFT does not significantly alter levels of other major sphingolipid species. Mass spectrometry analysis of colon (A and B), colonoids treated with concentrated bacterial culture supernatant (C and D), and colonoids treated with purified BFT2 (E and F) determined that the total levels of ceramide (A, C, E) and sphingomyelin (B, D, F) do not change significantly in response to treatment. All results were collected in the same experiments used to produce the data shown in Figure 1. Group comparisons were performed using a one-way ANOVA and Tukey's multiple comparisons test while single comparisons were made using an unpaired t-test. NS indicates non-significant results. Error bars represent the standard deviation of the mean. Sham represents a PBS control. NT indicates no treatment was added. Control represents concentrated bacterial culture supernatant from ETBF*Δbft*, BFT represents concentrated bacterial culture supernatant from ETBF, and Purified BFT2 represents purified BFT from ETBF strain 86-5443-2-2 (see Materials and Methods).



Supplemental Figure 2: Concentrated bacterial culture supernatant from ETBF contains *Bacteroides fragilis* toxin and BFT is biologically active. Western blot of concentrated bacterial culture supernatants (200μg protein) using an anti-BFT antibody shows presence of toxin in concentrated bacterial culture supernatant from BFT but not control (A). Addition of BFT to HT29/C1 cells for 30 minutes leads to E-cadherin cleavage, illustrating toxin activity in our BFT preparations (B). Confocal imaging of colonoids treated for six hours with different concentrated bacterial culture supernatant preparations demonstrates that presence of toxin leads to morphology changes (C-H). Concentrated ETBF bacterial culture supernatant, containing BFT, dramatically alters colonoid morphology (C). Concentrated bacterial culture supernatant from ETBF*Δbft* does not alter colonoid morphology (D), but when a recombinant version of this strain that produces BFT2 is used (rETBF*Δbft bft-2*), morphology changes are similar to those seen with BFT (E). Concentrated bacterial culture supernatant from NTBF (NCTC 9343), which does not produce BFT, does not cause morphology changes in colonoids (F). In contrast, a recombinant NTBF strain that produces BFT2 (rNTBF *bft-2*) alters morphology, similar to BFT (G). A recombinant NTBF strain, which secretes a biologically inactive mutant of BFT2 (rNTBF *bft-2* H352Y) no longer causes morphology changes (H). Confocal images were captured using 10x magnification. Scale bar indicates a distance of 100μm.





Supplemental Figure 3: Confocal immunofluorescence of actin demonstrates the apical membrane faces the interior of the colonoid. Colonoids were extracted from the Matrigel, fixed using 2% paraformaldehyde in 100mM phosphate buffer (pH 7.4), and stained using Alexa Fluor 633 phalloidin (actin) and DAPI (nuclear stain). A representative colonoid is shown with DIC imaging (A). Confocal immunofluorescence of Alexa Fluor 633 (white) and DAPI (blue) is shown with strong actin staining at the apical ring (B). Confocal images were captured using a 63x oil-immersion lens. Scale bar indicates a distance of 10µm.

Supplemental Video Figure Legends

Supplemental Video 1: Time-lapse of colonoids treated with concentrated bacterial culture supernatant from ETBF*Δbft*. Colonoid morphology does not change throughout treatment. Images were captured using confocal microscopy every 15 minutes for 48 hours, and resulting images were assembled, at 15 frames per second, into a video. Confocal images were captured using 20x magnification.

Supplemental Video 2: Time-lapse of colonoids treated with concentrated bacterial culture supernatant from ETBF*Δbft* and ibiglustat. Addition of 5µM ibiglustat did not alter colonoid morphology throughout treatment. Images were captured using confocal microscopy every 15 minutes for 48 hours, and resulting images were assembled, at 15 frames per second, into a video. Confocal images were captured using 20x magnification.

Supplemental Video 3: Time-lapse of colonoids treated with concentrated bacterial culture supernatant from ETBF. Colonoids treated with BFT undergo dramatic swelling and bubbling by six hours before returning to normal morphology by 48 hours. Images were captured using confocal microscopy every 15 minutes for 48 hours, and resulting images were assembled, at 15 frames per second, into a video. Confocal images were captured using 20x magnification.

Supplemental Video 4: Time-lapse of colonoids treated with concentrated bacterial culture supernatant from ETBF and ibiglustat. Colonoids treated with BFT and 5µM ibiglustat burst open, spilling contents into the extracellular matrix. Images were captured using confocal microscopy every 15 minutes for 48 hours, and resulting images were assembled, at 15 frames per second, into a video. Confocal images were captured using 20x magnification.

Supplemental Video 5: Time-lapse of colonoids cultured in CBE and treated with concentrated bacterial culture supernatant from ETBF*Δbft*. Colonoids cultured in 20µM CBE do not undergo morphology changes throughout treatment. Images were captured using confocal microscopy every 20 minutes for 48 hours, and resulting images were assembled, at 15 frames per second, into a video. Confocal images were captured using 10x magnification.

Supplemental Video 6: Time-lapse of colonoids cultured in CBE and treated with concentrated bacterial culture supernatant from ETBF*Δbft* and ibiglustat. Colonoids cultured in 20µM CBE do not undergo morphology changes throughout treatment, even in the presence of 5µM ibiglustat. Images were captured using confocal microscopy every 20 minutes for 48 hours, and resulting images were assembled, at 15 frames per second, into a video. Confocal images were captured using 10x magnification.

Supplemental Video 7: Time-lapse of colonoids cultured in CBE and treated with concentrated bacterial culture supernatant from ETBF. Colonoids cultured in 20µM CBE and treated with BFT undergo dramatic swelling and bubbling and are still in the process of recovering and returning to normal morphology by 48 hours. Images were captured using confocal microscopy every 20 minutes for 48 hours, and resulting images were assembled, at 15 frames per second, into a video. Confocal images were captured using 10x magnification.

Supplemental Video 8: Time-lapse of colonoids cultured in CBE and treated with concentrated bacterial culture supernatant from ETBF and ibiglustat. Colonoids cultured in 20µM CBE and treated with BFT and ibiglustat show a normal BFT response, delayed recovery, but no colonoid bursting. Images were captured using confocal microscopy every 20 minutes for 48 hours, and resulting images were assembled, at 15 frames per second, into a video. Confocal images were captured using 10x magnification.