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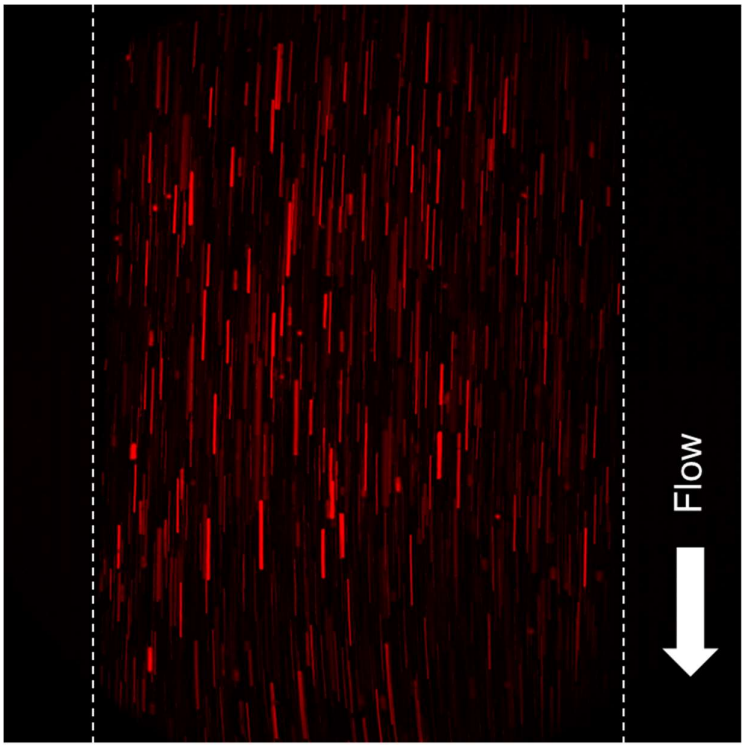

Figure S1. Max intensity projection of 1 μm fluorescent beads perfusion at 45 μl/h.

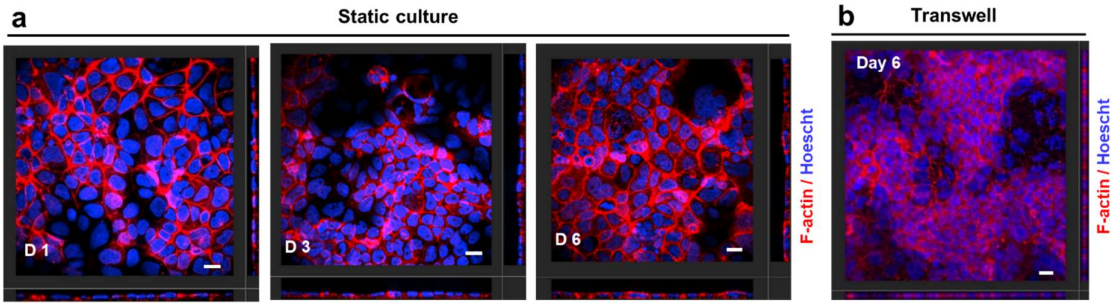

Figure S2. Caco-2 monolayer formation under the static condition.
a) Time-lapse images of Caco-2 monolayer formation in static μGut chips imaged by brightfield and confocal microscopy. b) The thin monolayer of Caco-2 formed in static Transwell® culture (Scale bar, 20 μm).

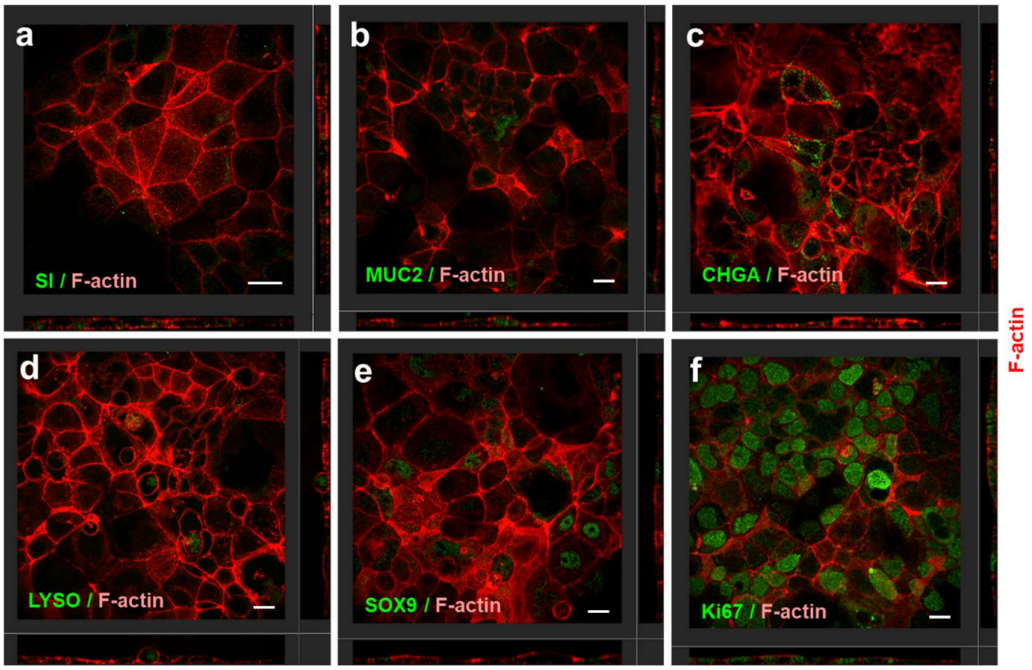

Figure S3. Immunofluorescence confocal images of key intestinal markers in Caco-2 monolayers cultured in static µGut chips. a) enterocytes (SI), b) goblet cells (MUC2), c) chromogranin A (CHGA), d) lysozyme (LYSO), e) stem cells (SOX9), f) proliferating cells (Ki67) (Scale bar, 20 µm).

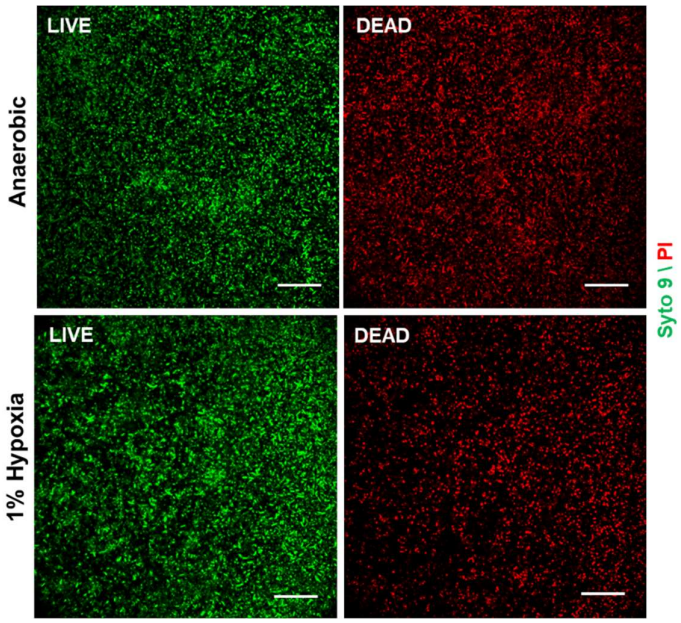

Figure S4. Viability of *B. fragilis* (NTBF) after 24 h incubation under anaerobic and 1% hypoxic conditions (Scale bar, 200 µm).

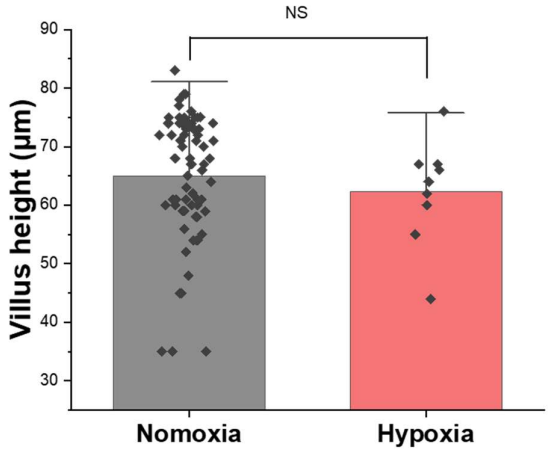

Figure S5. Comparison of villus heights of the µGuts after 24 h incubation under normoxic and 1% hypoxic conditions.

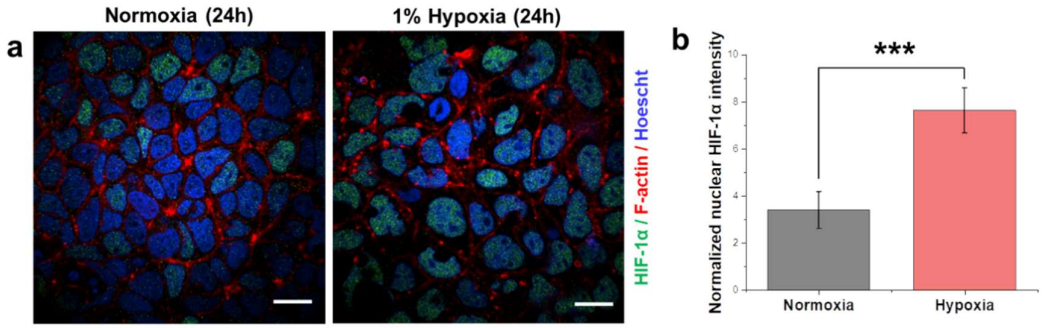

Figure S6. Comparison of HIF-1α expression in the µGut after 24 h incubation under normoxia and 1% hypoxia. a) Immunofluorescence confocal images of HIF-1α expression in the µGut and b) normalized HIF-1α densities (***$p < 0.001$) (Scale bar, 20 µm).

Table S1. In various model systems, ETBF and BFT-induced morphological changes of the intestinal epithelial cells.

Type of changes	***In vivo* model**	**BFT-treated cells**
Morphology	Extensive detachment and rounding of surface epithelial cells, as well as shortened villi in ETBF-colonized and BFT-treated animal tissue[1]	Altered cell morphology (cell rounding, loss of cell height, apical drift of cell nuclei) in monolayers of T84 cell or HT29/C1 cell[2]
ZO-1 redistribution	No reported studies	ZO-1 redistribution in BFT treated HT29/C1 cell line[3]

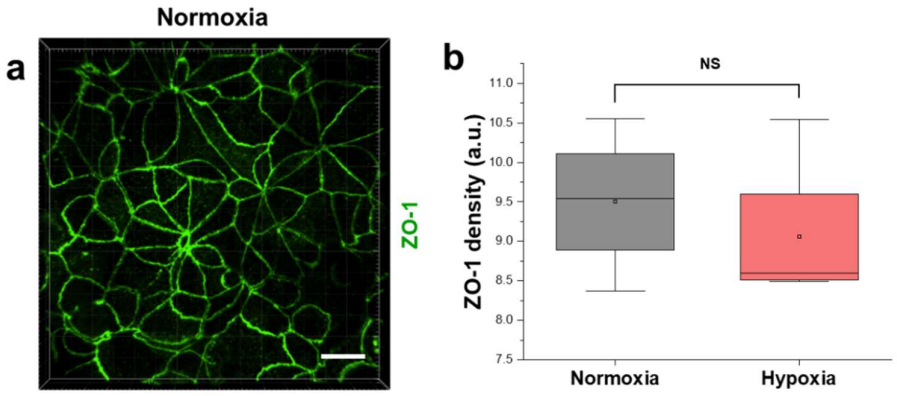

Figure S7. ZO-1 redistribution of µGuts cultured under normoxia and 1% hypoxia. a) An immunofluorescence confocal microscopy image of ZO-1 redistribution in µGut cultured under normoxic conditions (NS→ p>0.05). b) Comparison of ZO-1 densities of µGuts cultured under different oxygen conditions.

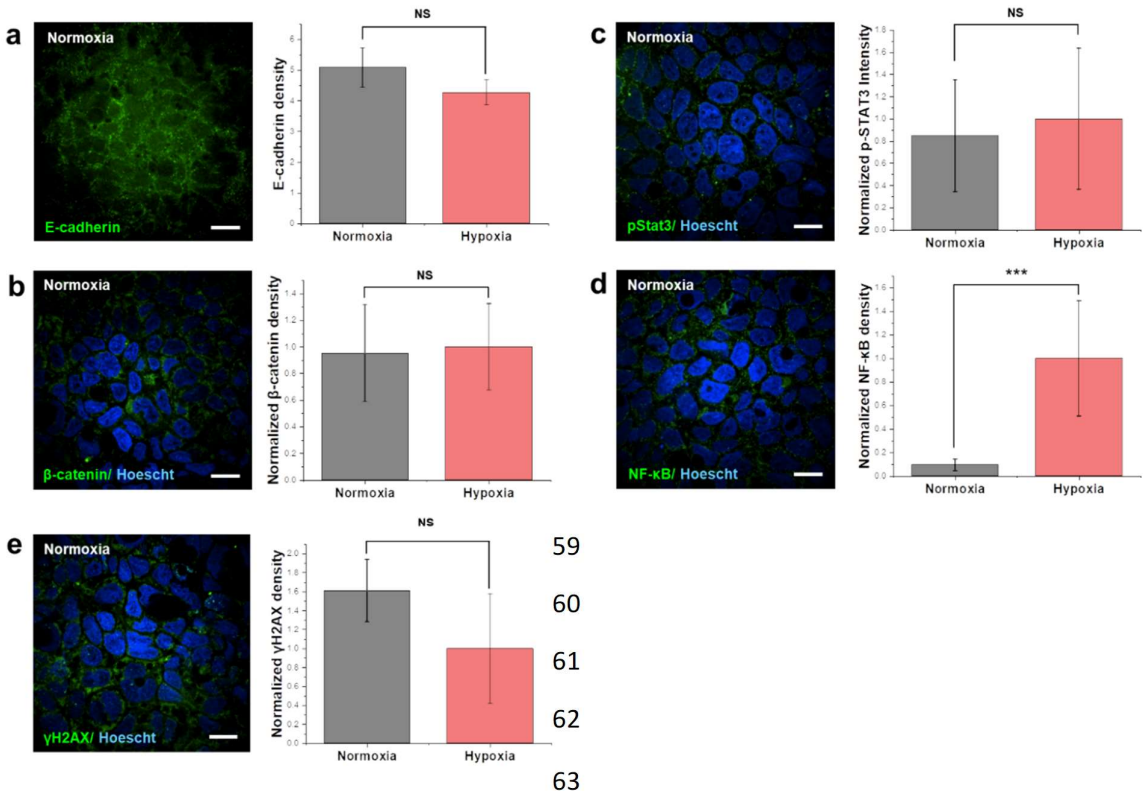

Figure S8. Immunofluorescence confocal images and quantitative analysis of various ETBF-induced signaling molecules in µGuts after 24 h incubation under normoxia. The density of signaling molecules was compared to that under 1% hypoxia. a) E-cadherin b) β-Catenin c) p-STAT3 d) NF-κB e) γH2AX (***p < 0.001, NS→ p>0.05) (Scale bar, 20 µm).

Table S2. ETBF-indued carcinogenic signaling pathways reported in *in vivo* models and BFT toxin-treated cells.

Signaling pathway	In vivo model	BFT toxin-treated cell models
E-cadherin cleavage	18~ 24 h, after inoculation in ETBF-colonized mice[4]	1 h after BFT treated HT29/C1 cell line[5]
β-catenin nuclear translocation	No reports of *in vivo* models	a. 3~6 h after BFT treatment in HT29/C1 cells[6] b. 12 h in HCT116 and CCD841CoN cells[7]
Pstat3 nuclear translocation	16~24 h after inoculation in ETBF colonized wild-type C57BL/6 and Min mice[8]	a. No detection *in vitro* model b. No detection in BFT-treated HT29/C1 cells up to 72 h and polarized T84 cells within 24 h[8b]
NF-κB p65 nuclear translocation	Day 7 of post-infection in ETBF colonized C57BL/6 mice[8a]	6 h after BFT stimulation in HT29/C1 cells[8a, 9]
DNA damage	Day 4 of inoculation in ETBF colonized AOM mice[10]	Within 6 h after BFT treatment in T84 cells[11]

Movie S1: Visualizing perfusion in the µGut channel using 1µm beads.

Movie S2: Caco-2 µGut growth under static and perfusion conditions.

Movie S3: Formation of 3D villus-like structure in µGut.

Movie S4: NTBF adhesion and proliferation in µGut.

Movie S5: Adhesion and proliferation of ETBF8 in µGut.

Movie S6: Adhesion and proliferation of ETBF9 in µGut.

Movie S7: ETBF biofilm formation in the µGut.

Movie S8: *Lactobacillus rhamnosus* GG (LGG) enriched µGut (LGG- µGut).

Reference

[1] a) C. L. Sears, L. L. Myers, A. Lazenby, R. L. Van Tassell, *Clin. Infect. Dis.* **1995**, *20 Suppl 2*, S142; b) R. J. Obiso, Jr., D. M. Lyerly, R. L. Van Tassell, T. D. Wilkins, *Infect. Immun.* **1995**, *63* (10), 3820.

[2] F. G. Chambers, S. S. Koshy, R. F. Saidi, D. P. Clark, R. D. Moore, C. L. Sears, *Infect. Immun.* **1997**, *65* (9), 3561.

[3] S. Wu, K. C. Lim, J. Huang, R. F. Saidi, C. L. Sears, *Proc. Natl. Acad. Sci. U. S. A.* **1998**, *95* (25), 14979.

[4] K. J. Rhee, S. Wu, X. Wu, D. L. Huso, B. Karim, A. A. Franco, S. Rabizadeh, J. E. Golub, L. E. Mathews, J. Shin, R. B. Sartor, D. Golenbock, A. R. Hamad, C. M. Gan, F. Housseau, C. L. Sears, *Infect. Immun.* **2009**, *77* (4), 1708.

[5] S. Wu, K. J. Rhee, M. Zhang, A. Franco, C. L. Sears, *J. Cell. Sci.* **2007**, *120*, 1944.

[6] S. Wu, P. J. Morin, D. Maouyo, C. L. Sears, *Gastroenterology* **2003**, *124* (2), 392.

[7] J. I. Jeon, S. H. Ko, J. M. Kim, V. J. Torres, *Infect. Immun.* **2019**, *87* (11).

[8] a) L. Chung, E. T. Orberg, A. L. Geis, J. L. Chan, K. Fu, C. E. DeStefano Shields, C. M. Dejea, P. Fathi, J. Chen, B. B. Finard, A. J. Tam, F. McAllister, H. Fan, X. Wu, S. Ganguly, A. Lebid, P. Metz, S. W. Van Meerbeke, D. L. Huso, E. C. Wick, D. M. Pardoll, F. Wan, S. Wu, C. L. Sears, F. Housseau, *Cell Host Microbe* **2018**, *23* (3), 421; b) E. C. Wick, S. Rabizadeh, E. Albesiano, X. Wu, S. Wu, J. Chan, K. J. Rhee, G. Ortega, D. L. Huso, D. Pardoll, F. Housseau, C. L. Sears, *Inflamm. Bowel. Dis.* **2014**, *20* (5), 821; c) S. Wu, K. J. Rhee, E. Albesiano, S. Rabizadeh, X. Wu, H. R. Yen, D. L. Huso, F. L. Brancati, E. Wick, F. McAllister, F. Housseau, D. M. Pardoll, C. L. Sears, *Nat. Med.* **2009**, *15* (9), 1016.

[9] J. M. Kim, S. J. Cho, Y. K. Oh, H. Y. Jung, Y. J. Kim, N. Kim, *Clin. Exp. Immunol.* **2002**, *130* (1), 59.

[10] C. M. Dejea, P. Fathi, J. M. Craig, A. Boleij, R. Taddese, A. L. Geis, X. Wu, C. E. DeStefano Shields, E. M. Hechenbleikner, D. L. Huso, R. A. Anders, F. M. Giardiello, E. C. Wick, H. Wang, S. Wu, D. M. Pardoll, F. Housseau, C. L. Sears, *Science* **2018**, *359* (6375), 592.

[11] A. C. Goodwin, C. E. D. Shields, S. Wu, D. L. Huso, X. Wu, T. R. Murray-Stewart, A. Hacker-Prietz, S. Rabizadeh, P. M. Woster, C. L. Sears, R. A. Casero, *Proc. Natl. Acad. Sci. U. S. A.* **2011**, *108* (37), 15354.