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SUPPLEMENTARY INFORMATION

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A complex human gut microbiome cultured in an anaerobic intestine-on-a-chip

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Supplementary Tables

Supplementary Table 1 | Media tested for microbial diversity

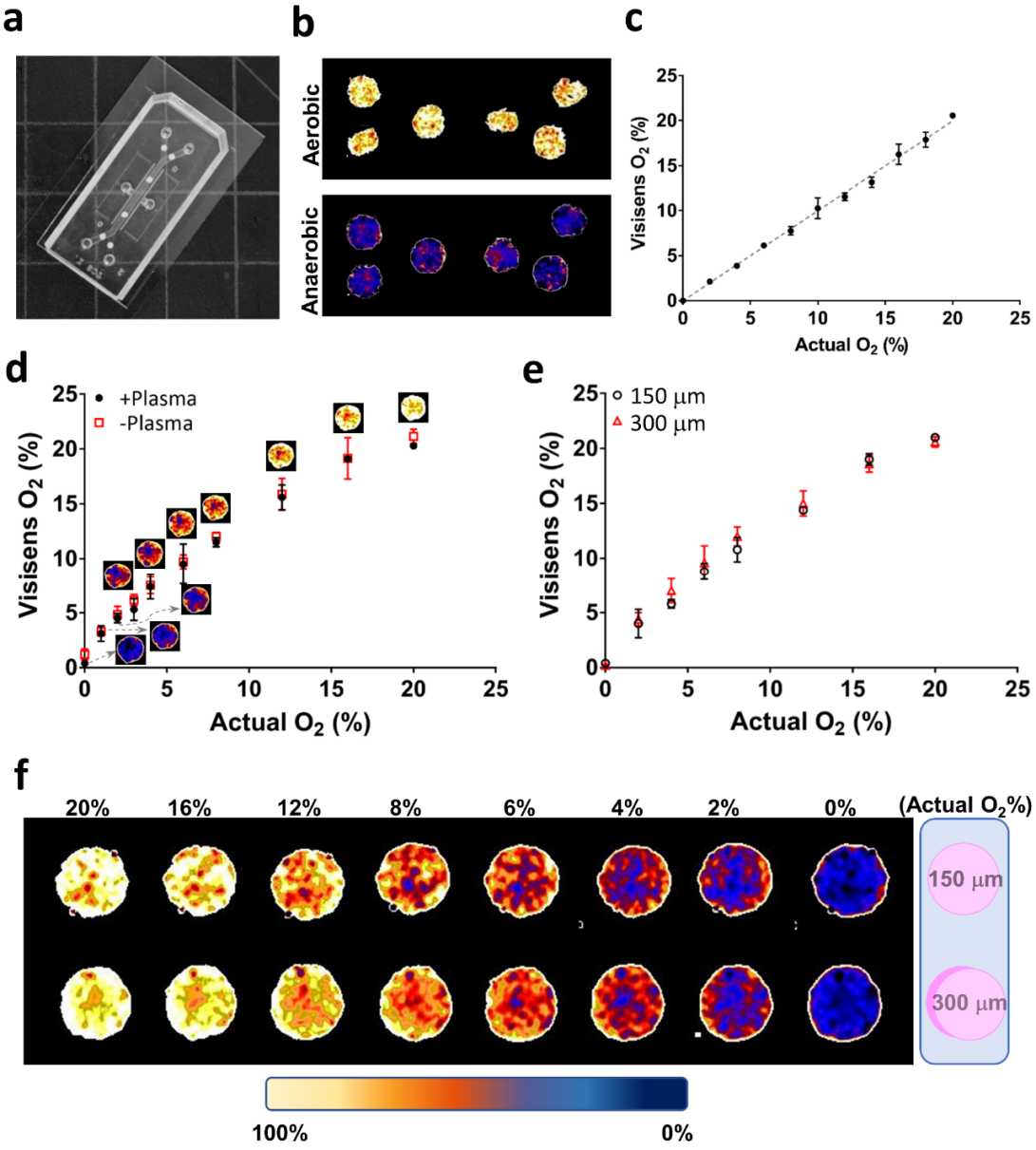
Sample number (Hmb or Mmb)	Media tested*
1	Stock
2	Brain Heart Infusion
3	Brucella
4	GMM[57]
5	Hemin 5 μg/ml
6	Hemin 5 μg/ml, Vitamin K1 0.5 μg/ml
7	Vitamin K10.5 μg/ml
8	Porcine mucin 1 mg/ml
9	Porcine mucin 1 mg/ml, Apple pectin 1 mg/ml
10	Porcine mucin 1 mg/ml, Hemin 5 μg/ml, Vitamin K1 0.5 μg/ml
11	Porcine mucin 1 mg/ml, Hemin 5 μg/ml, apple pectin 1 mg/ml Vitamin K1 0.5 μg/ml
12	Thioglycolate
13	Wilkins-Chalgren
14	YPG Yeast 20 mg/ml, NaCl 5mg/ml, K_2HPO_4 5 mg/ml, Proteose 5 mg/ml, Glucose 5 mg/ml, L-cysteine 1 mg/ml, Hemin 5 μg/ml, Vitamin K1 0.5 μg/ml

*Dissolved in DMEM 20% FBS 1% glutamax

Supplementary Table 2 | Analysis of the diversity of stool microbiome co-cultured in primary Intestine Chips.

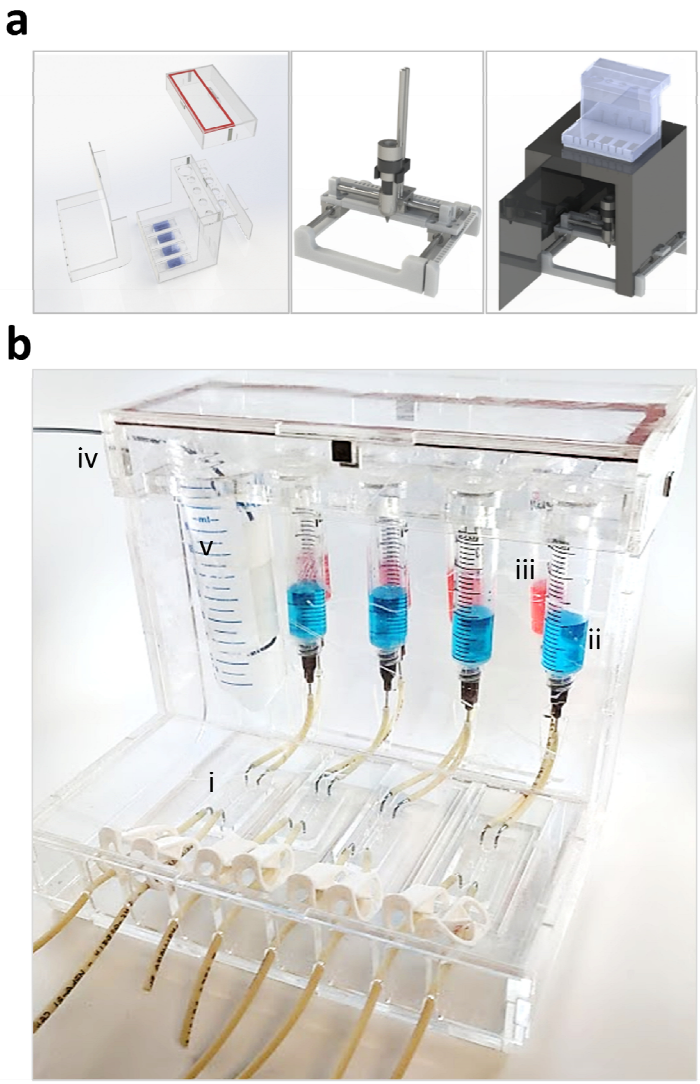
Ileum sample (day 5)	Observed richness	Shannon diversity
36 wk infant	122	0.32
36 wk infant	118	0.34
36 wk infant	121	0.31
30 wk infant	135	1.10

Supplementary Figures

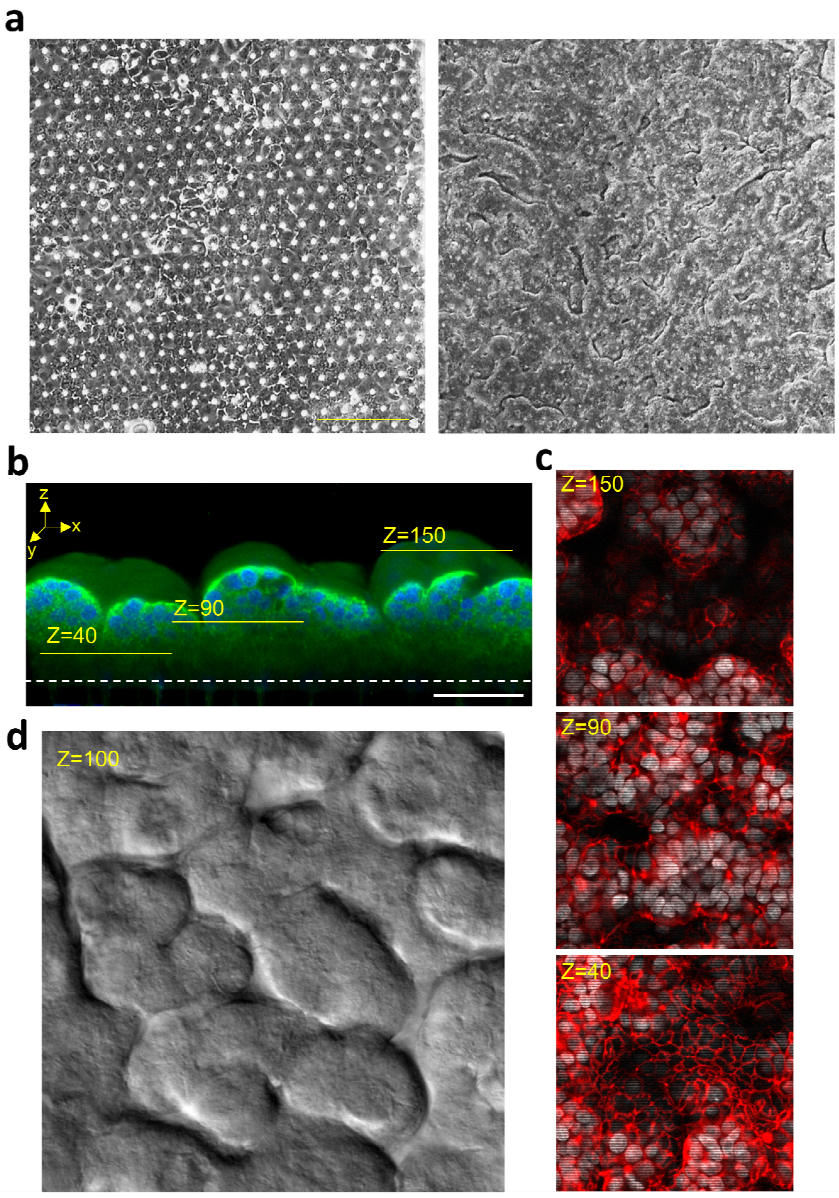


Supplementary Figure 1. (**a**) Representative optical image of an oxygen sensing Intestine Chip and (**b**) pseudocolor image of the Intestine Chip oxygen distribution under aerobic (top, yellow) and anaerobic (bottom, blue) culture conditions. (**c**) Accuracy analysis of oxygen detection by the sensor spots located in the Intestine Chip in response to defined, standard oxygen concentrations. Oxygen levels measured by the Intestine Chip sensor spots were validated by measuring levels before and after plasma treatment (**d**), which altered thickness (150 μm vs. 300 μm) of the spots (**e**). (**f**) Representative pseudocolor images of the spots when varying

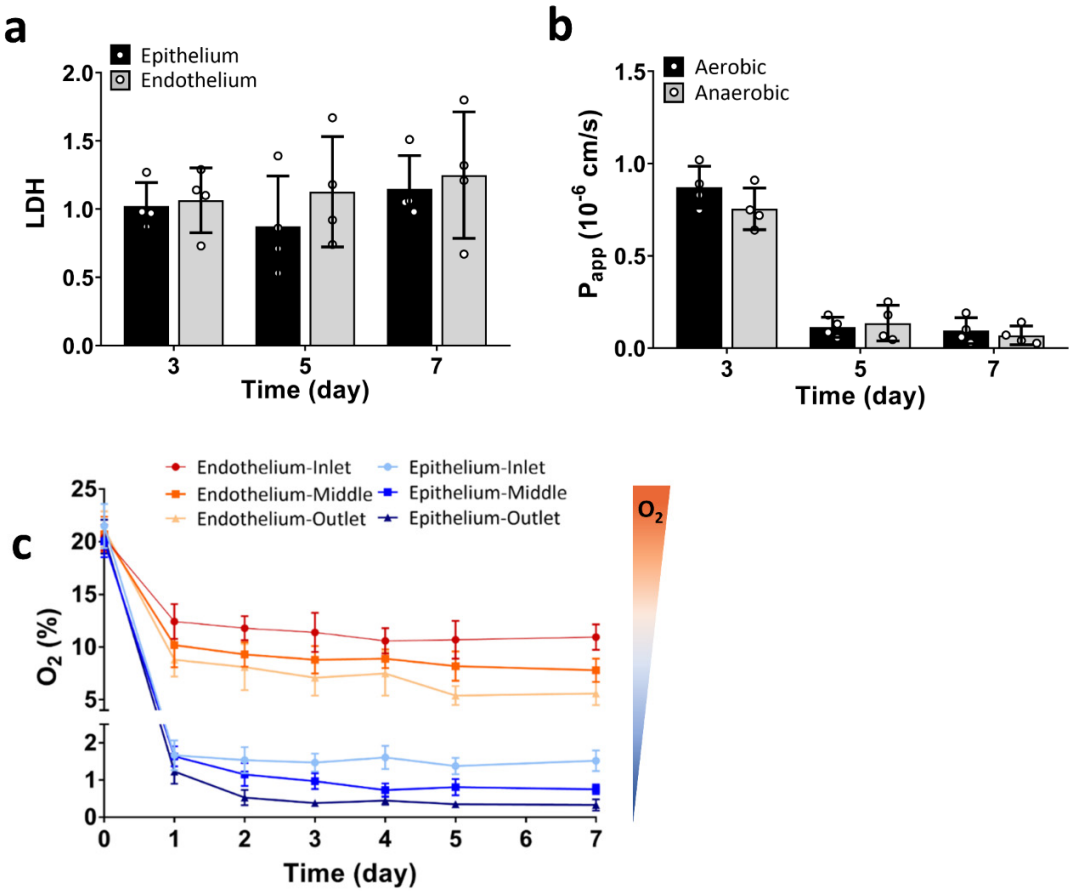
oxygen concentrations from 20% to 0% oxygen, or aerobic (yellow) to anaerobic (blue) conditions.



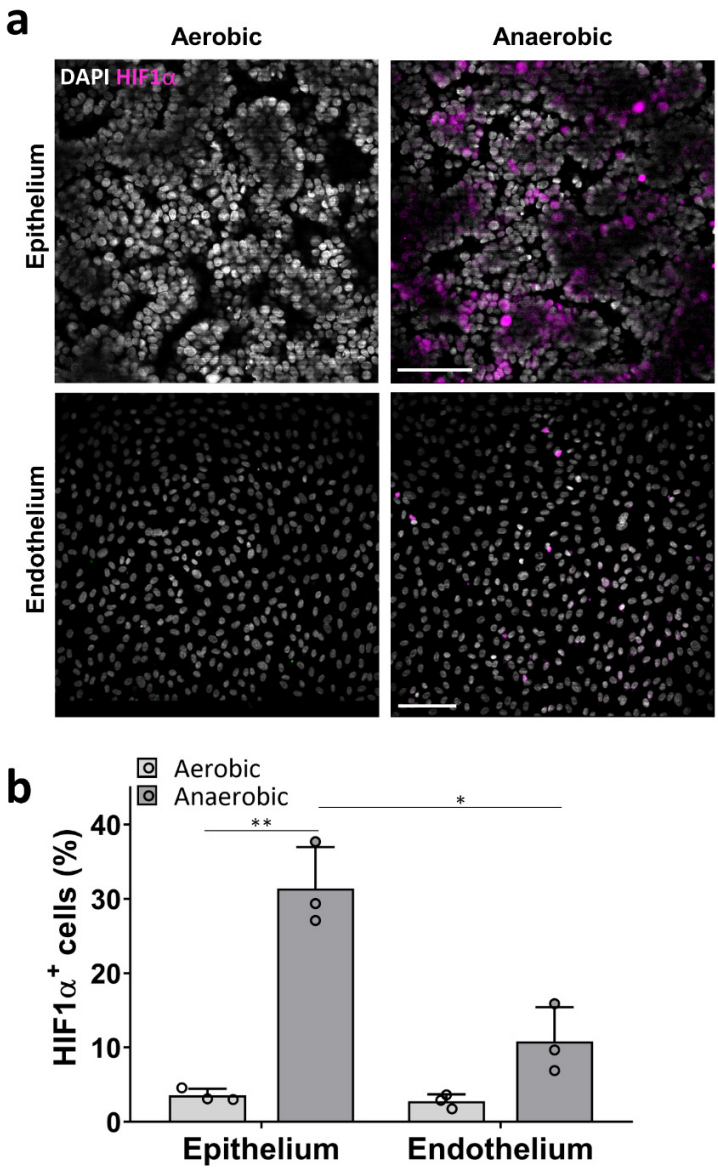
Supplementary Figure 2. (**a**) Exploded views of the anaerobic chamber (left); linear positioning system for indexed motions of the camera to any sensor spot along the chip or between the chips (middle); and rendering of hypoxic farm on imaging stand for monitoring of sensors without removing chips from anaerobic chamber (right). (**b**) Photograph of an anaerobic chamber while in use. Chips (i) are placed in the hypoxic region of the chamber and medium is flowed through epithelial channels (ii, blue) and exposed low to oxygen levels. The medium reservoirs for the vascular channels are placed outside the anaerobic chamber (iii, red) are exposed to room air and thereby, maintained under normoxia conditions. The chamber is purged with N_2 flow (iv) through a bubbler (v).



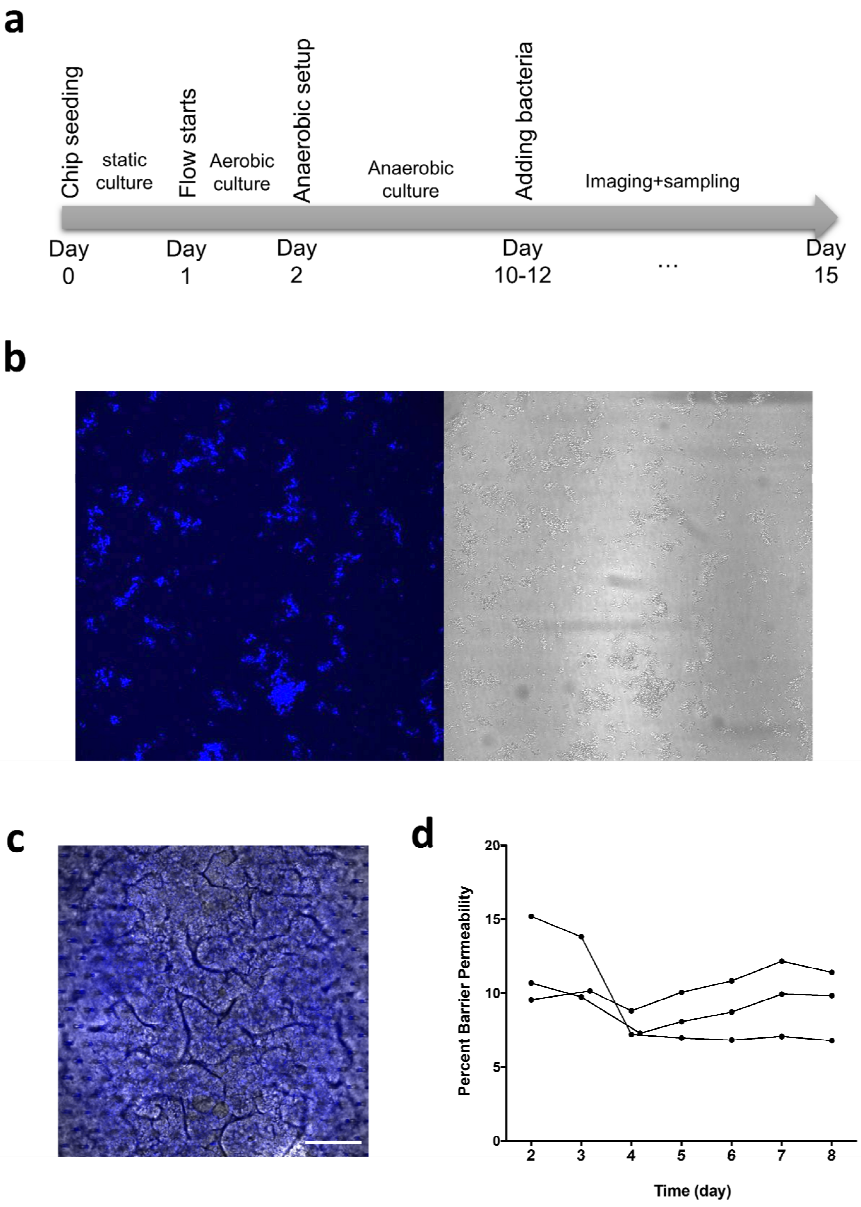
Supplementary Figure 3. (**a**) Phase contrast images of Caco2 epithelium at Day 1 (left) and 7 (right) of culture in the Intestine Chip. (**b**) Representative vertical cross-sectional, confocal micrographic views through the intestinal epithelium-membrane interface of the Intestine Chip at day 7 of culture, when immunostained for villin (green) and nuclei with DAPI (blue) (white dashed lines indicated upper surface of the porous matrix-coated membrane; bar, 100 μm). (**c**) Microscopic views showing the villus morphology of the human Caco-2 intestinal epithelium cultured for 7 days in the Intestine Chip when viewed from above by immunofluorescence staining for the tight junction protein, ZO-1, at different heights or by (**d**) DIC imaging at z=100 μm. (bar, 100 μm).



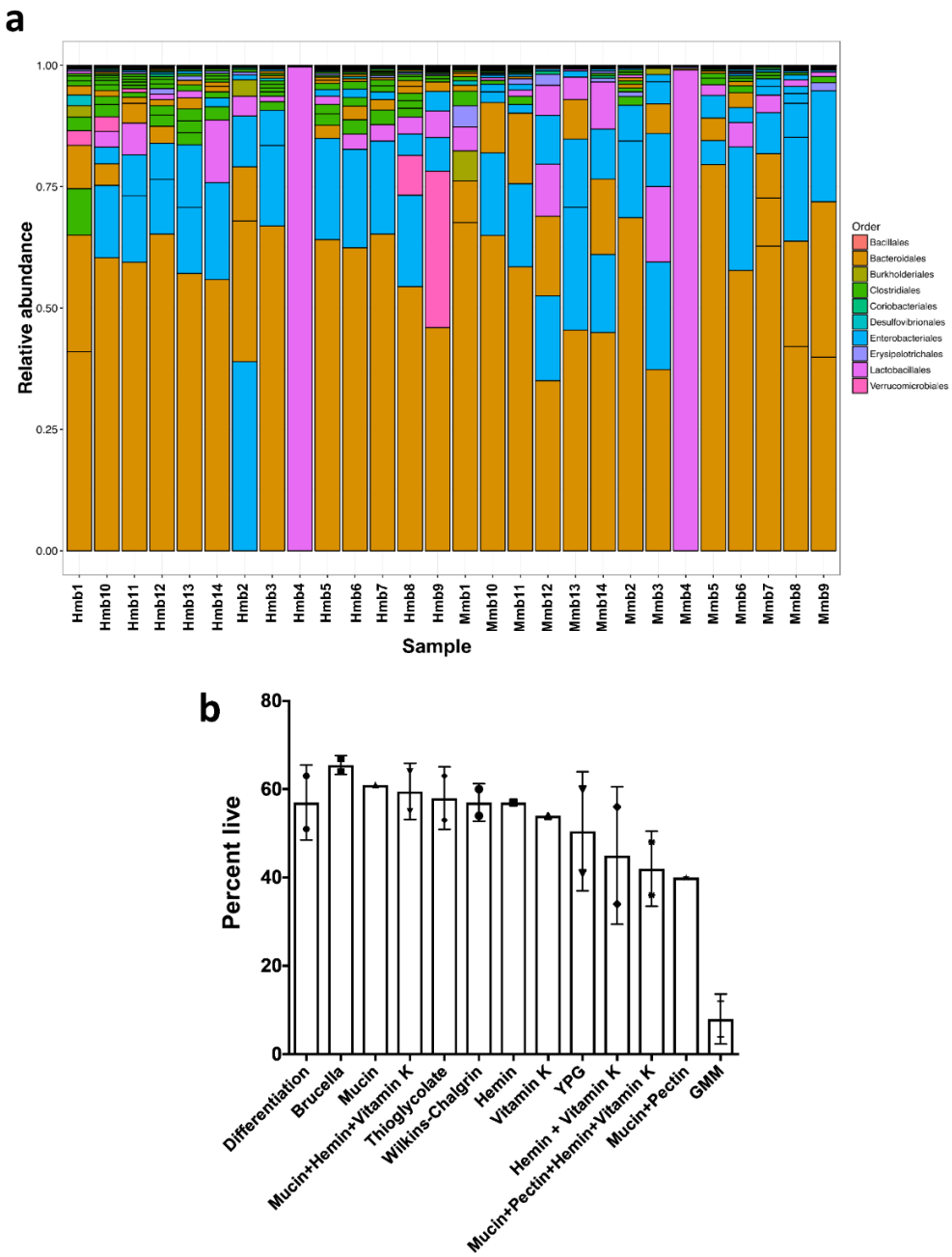
Supplementary Figure 4. (**a**) Graph showing effects of anaerobic culture on viability of intestinal epithelium and vascular endothelium, as assessed by quantifying LDH released from cells (data are presented as fold change in LDH levels relative to the aerobic control chips; n=4). (**b**) Changes in apparent permeability (P_{app}) measured by quantitating cascade blue transport across the tissue-tissue interface within the Intestine Chips cultured aerobically or anaerobically (n=4). (**c**) Radial and longitudinal oxygen profiles within anaerobically-cultured Intestine Chips during 7 days of culture. Schematic colors indicate average oxygen concentration in more aerobic (orange) versus anaerobic (blue) regions of both the epithelial and endothelial channels.



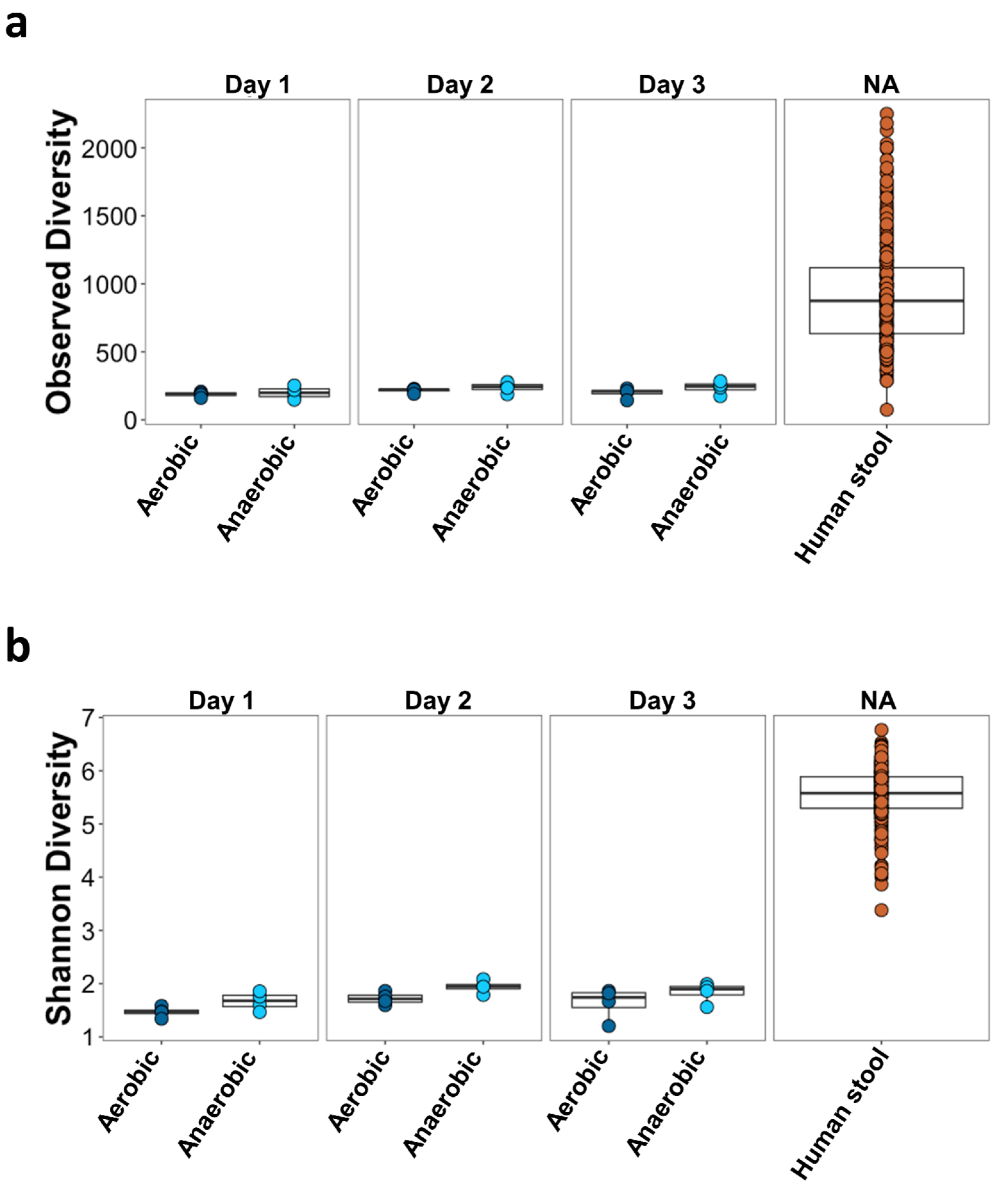
Supplementary Figure 5. (**a**) Representative images (from the middle of the chip) of immunofluorescence staining for nuclei with DAPI (white) and HIF1-α (magenta) in human intestinal epithelial cells and endothelial cells cultured aerobically and anaerobically (bar, 100 μm). (**b**) Graph showing the quantification of the percentage of epithelial and endothelial cells that expressed HIF1-α (HIF1-α^+ cells) after exposure to the conditions shown in **a** (n=3; *, P<0.05; **, P<0.01).



Supplementary Figure 6. (**a**) Diagram of the experimental timeline used for Intestine Chip co-culture studies. (**b**) Fluorescenc microscopic images of *B. fragilis* bacteria labeled with HADA (blue; left) and corresponding brightfield image (right) before they were added to the chips. (**c**) Representative immunofluorescence micrograph with simultaneous phase contrast imaging of a co-culture of HADA-labeled *Bacteroides fragilis* (blue) in the Intestine Chip showing the bacteria distributed over the top surface of the intestinal villi (viewed from above; bar, 50 μm). (**d**) Barrier permeability of Caco2 Intestine Chips cultured with *B. fragilis* under anaerobic conditions measured over time, represented by the percent of Lucifer yellow translocated from the apical channel. This panel represents data from 3 independent chip experiments normalized relative to control bacteria-free chip cultures.

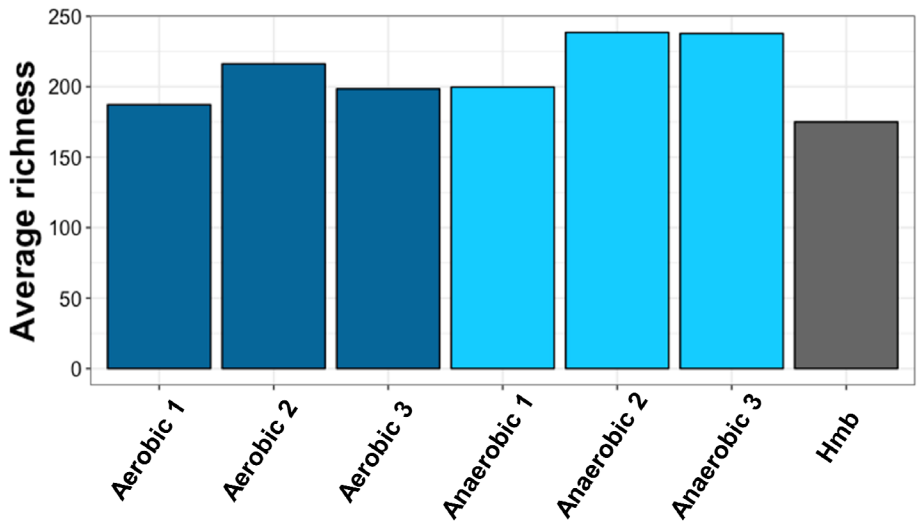


Supplementary Figure 7. (**a**) Relative abundance of identified OTUs of Hmb stock cultured in 13 different medium conditions, summarized to Phylum level. (**b**) Caco-2 viability in 13 different types of medium used for defining optimized growth of the human gut microbiome (see **Supplementary Table 1** for the composition of the various culture media tested).



Supplementary Figure 8. (**a**) Observed alpha diversity (richness) and (**b**) Shannon diversity between the Intestine-Chip samples on either condition and the human microbiome stool sample (from HMP).

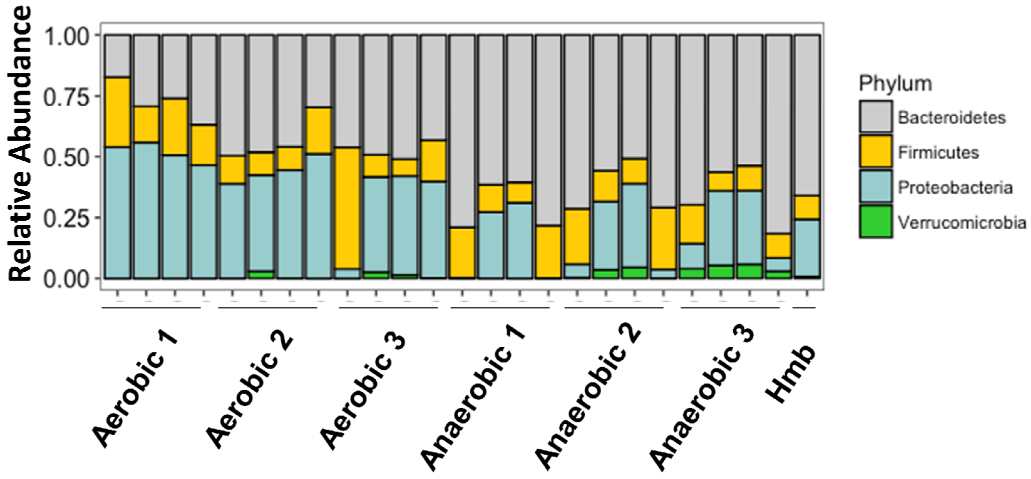
a



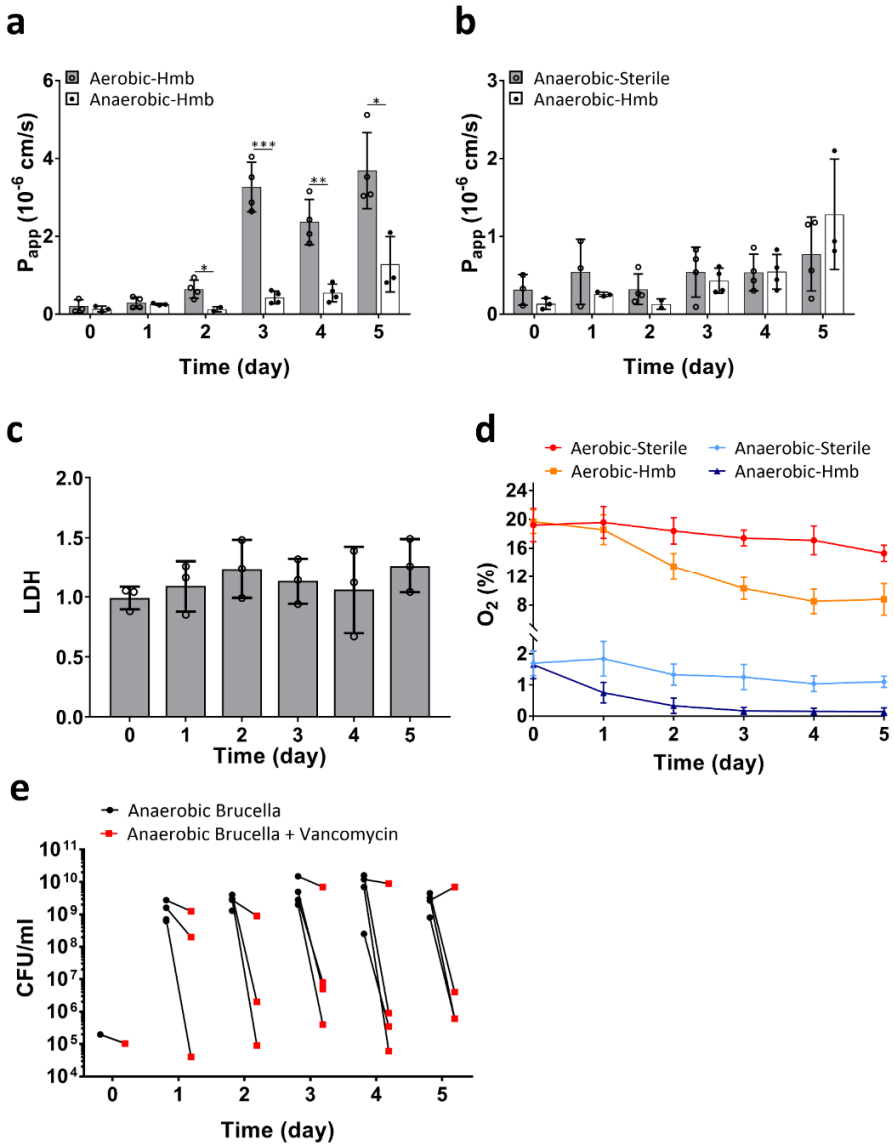
b

Sample	Mean	SD
Starting culture	175	NA
Aerobic, day 1	187	19.7
Aerobic, day 2	216	17.1
Aerobic, day 3	198	37.7
Anaerobic, day 1	200	45.9
Anaerobic, day 2	238	38
Anaerobic, day 3	238	46.7

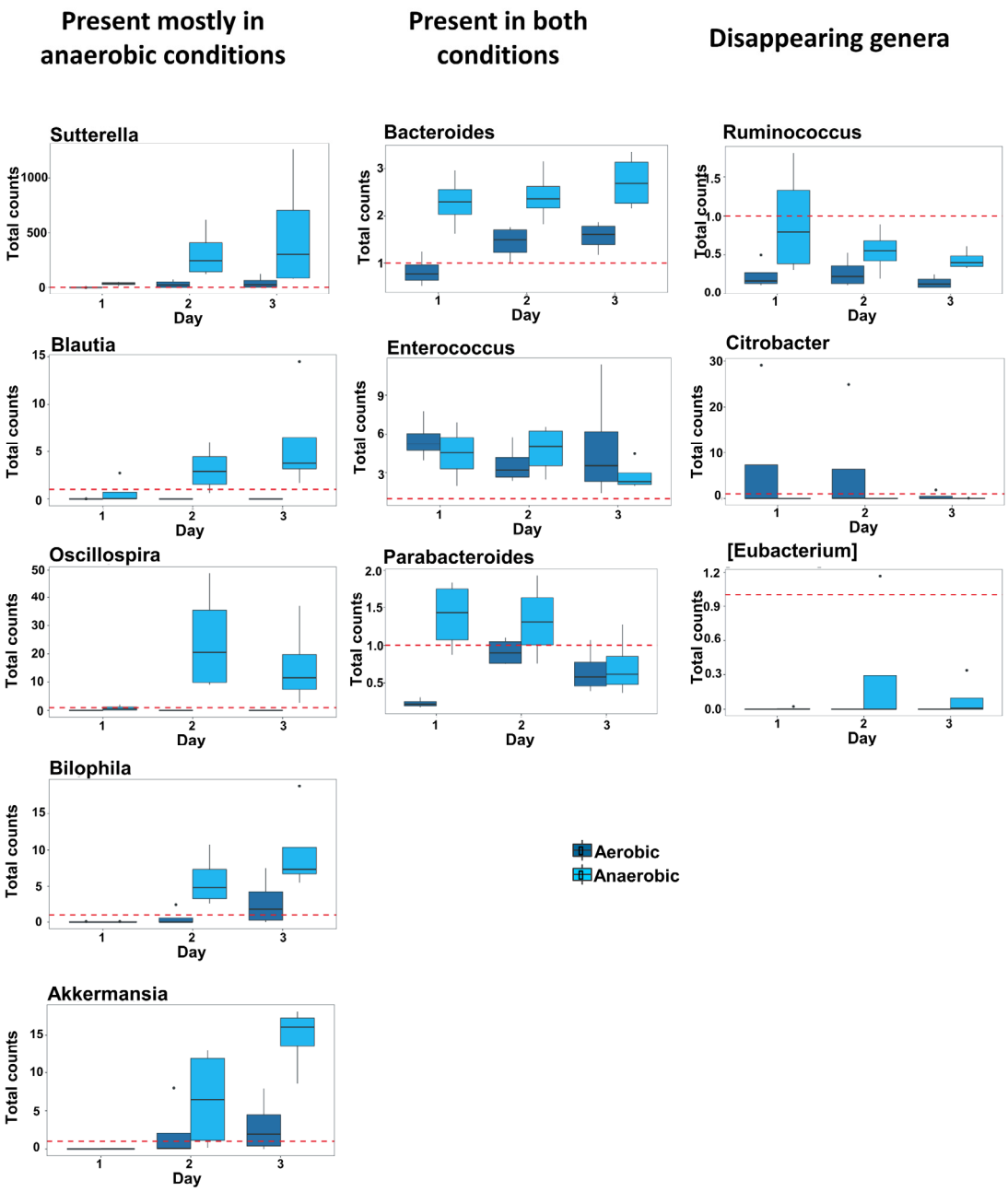
Supplementary Figure 9. (**a**) Observed alpha diversity (richness) for the aerobic or anaerobic chips, as well as the original (starter) microbiome culture. (**b**) Table shows the mean and standard deviation of this population richness metric across all groups.



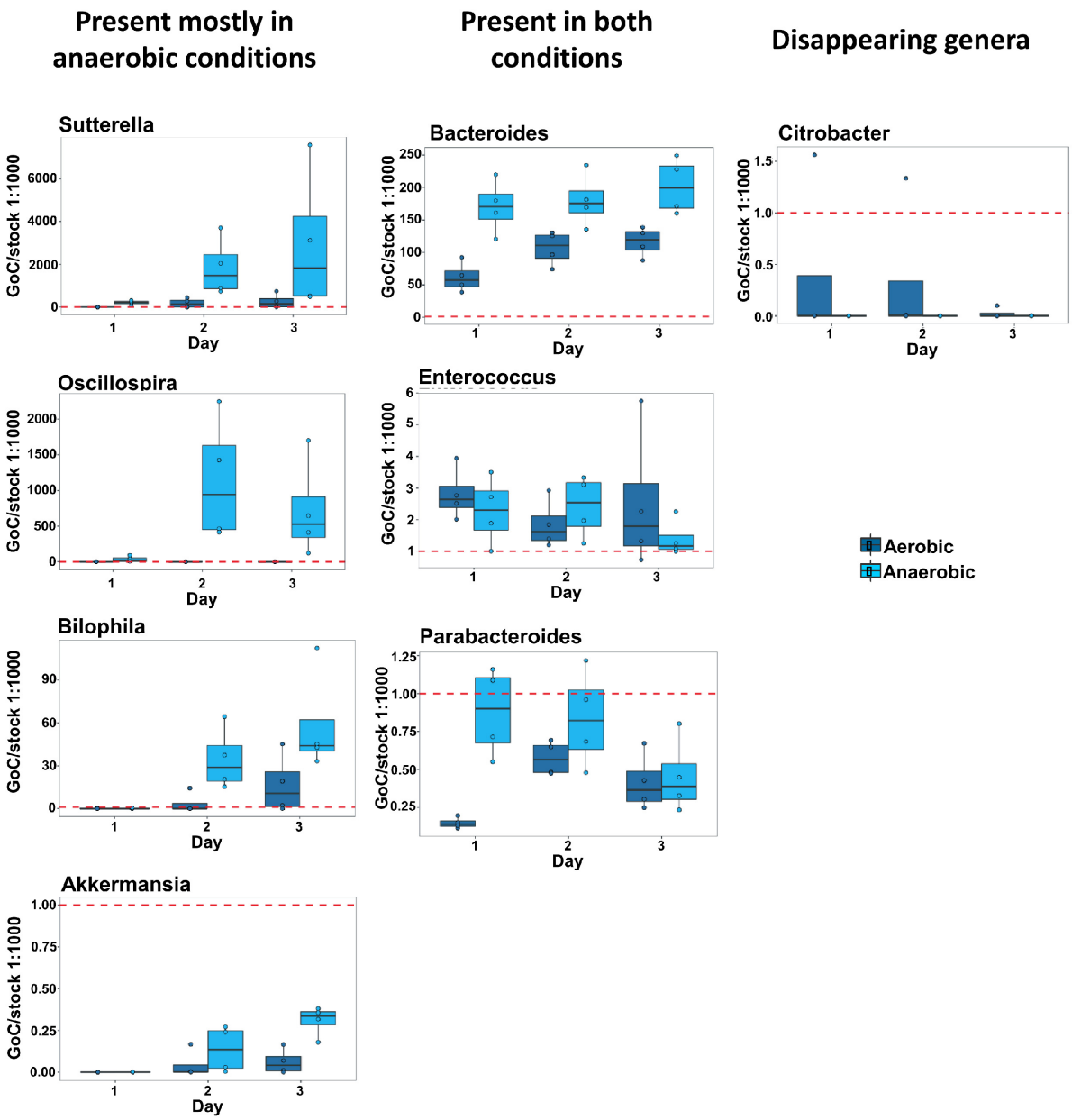
Suplementary Figure 10. Relative abundance of identified OTUs under aerobic or anaerobic conditions, summarized to Phylum level. The 4 bars presented for each of the 3 replicates cultured under aerobic or anaerobic conditions represent results at days 0, 1, 2 and 3 days of culture (left to right, respectively); Hmb stock indicates phyla abundance in the complex microbiome stock at time 0.



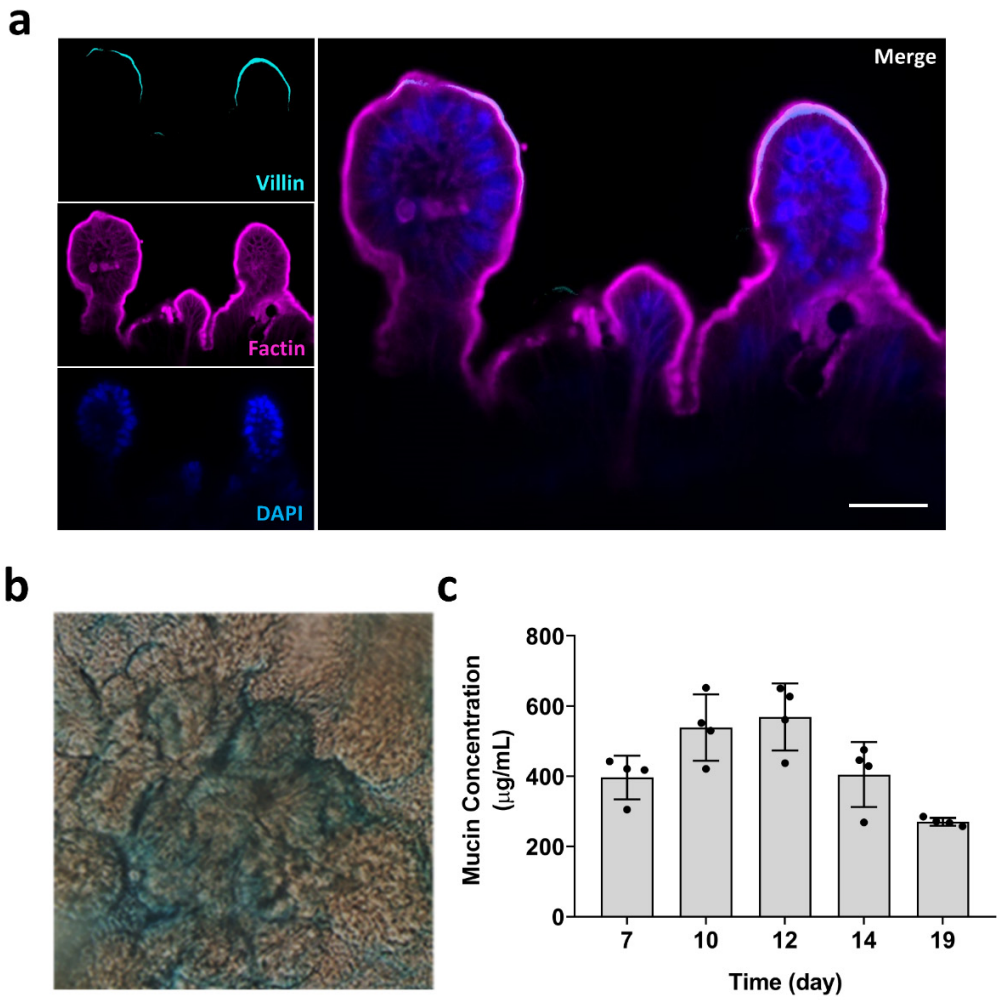
Supplementary Figure 11. Changes in apparent paracellular permeability (Papp) measured by quantifying cascade blue transport across the tissue-tissue interface within aerobic or anaerobic Intestine Chips co-cultured for 1 to 5 days with complex Hmb gut microbiome (**a**) or over a similar timecourse in anaerobic Intestine Chips cultured with or without (sterile) microbiome (**b**) (n=4; *P<0.05, **<0.01, ***P<0.001). (**c**) Graph showing effects of complex microbiome co-culture on viability of intestinal epithelium, as assessed by quantifying LDH released from cells (data are presented as fold change in LDH levels relative to the sterile control chips; n=3). (**d**) Oxygen concentration profiles measured in sterile aerobic and anaerobic Intestine Chips and similar Intestine Chips co-cultured with complex microbiome for 5 days. (**e**) CFU/ ml measured within effluent samples flushed from the epithelial microchannel anaerobic chips, as determined by plating serial dilutions on Brucella culture plates in the presence or absence of vancomycin and grown in the anaerobic chamber at 37°C.



Supplementary Figure 12. Total counts of genera detected in Caco2 Intestine Chips over 1 to 3 days of co-culture with Hmb microbiome. For comparison, the dashed red line represents the number of counts identified in the anaerobic liquid culture sample.



Supplementary Figure 13. Normalized counts of genera detected the in Intestine Chips over 1 to 3 days of co-culture with microbiome, compared to the starting human Hmb microbiome stock. The counts measured in both the aerobic and anaerobic chips are normalized to the total number of reads of that genus in the human stock. The dashed red line represents the normalized counts for the human stock. When a genus is not present in the stock, its line is set to 0 and the plot represents total counts of the genus in the chips.



Supplementary Figure 14. (**a**) Confocal fluorescence microscopic views showing the villus morphology of the primary ileal epithelium stained for villin (cyan), F-actin (magenta) and DAPI (blue) (bar, 50μm). (**b**) Phase contrast views of ileum chips stained with alcian blue. (**c**) Quantitation of alcian blue staining in cultures shown in **b**.