Supplementary Information

In vivo imaging and tracking of host-microbiota interactions via metabolic labeling of gut anaerobic bacteria

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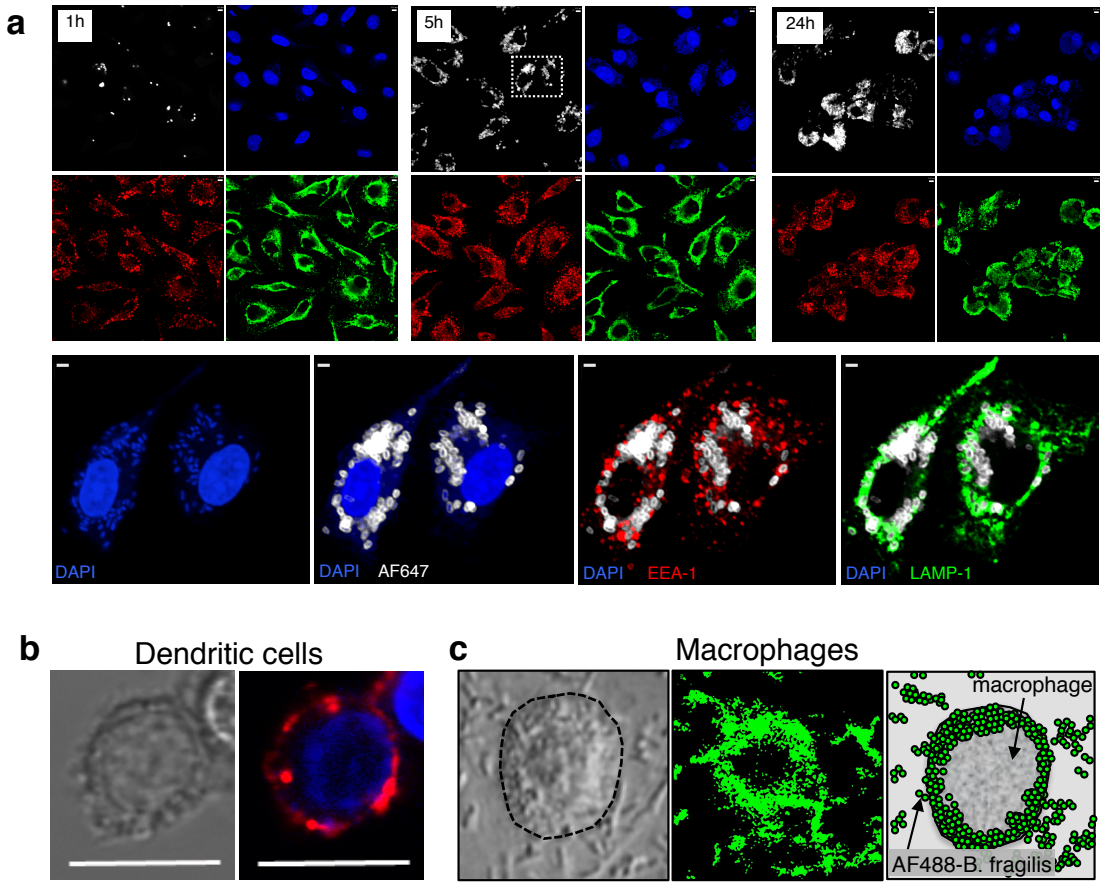
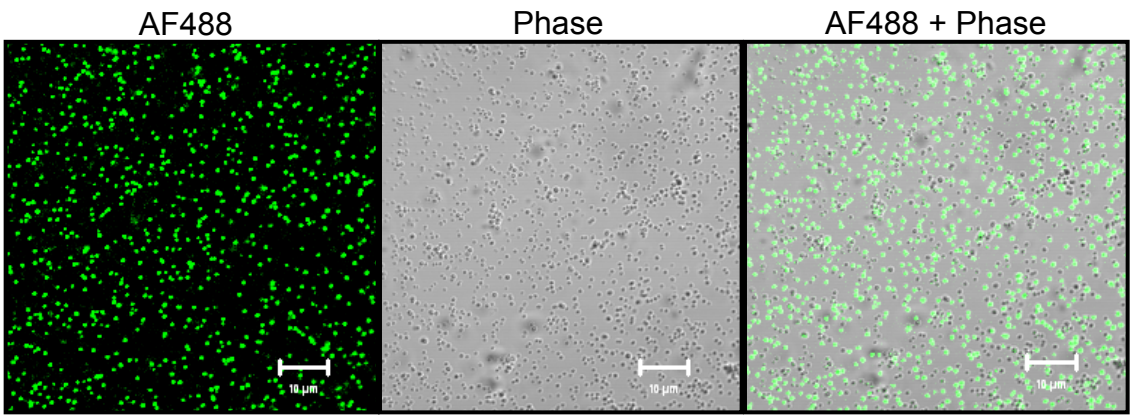
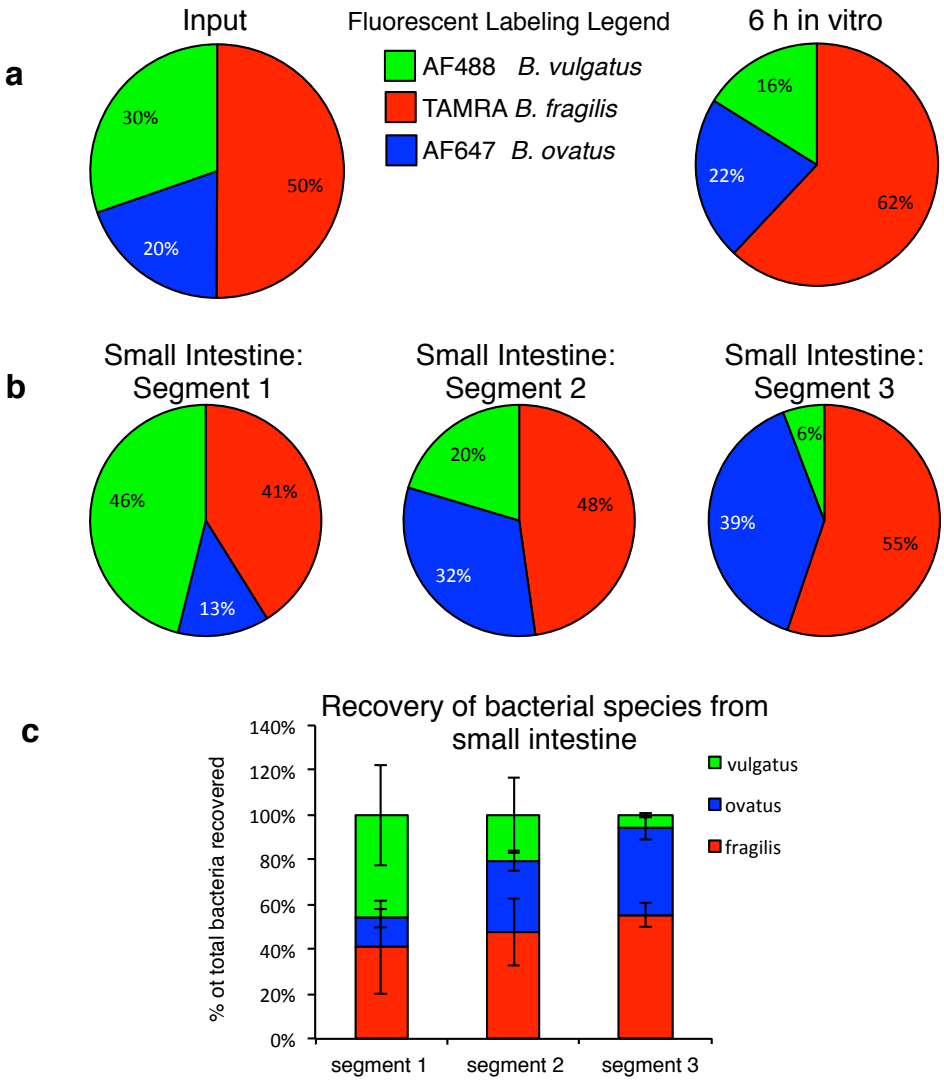
This supplement contains 3 supplementary figures, 1 supplementary table, and 4 supplementary video legends.



Supplementary Figure 1. Rapid phagocytic uptake of fluorescently labeled *B. fragilis* by macrophages *in vitro*. (**a**) Representative confocal images showing phagocytic uptake of AF647-labeled *B. fragilis* (white) by macrophages *in vitro* 1 h, 5 h, and 24 h after infection. Panels show separate channels for LAMP-1 (green), EEA-1 (red), AF647 (white), and DAPI (blue) staining. Scale bar for top row panels, 3 μm. Zoomed inserts (bottom row) are from 5 h (dotted box); scale bar for zoomed images, 2 μm. (**b**) Representative confocal images of splenic DCs presenting fluorescently labeled PSA. Panels show bright-field image (left) and fluorescence image (right), with TAMRA-labeled PSA (red) and Hoechst nuclear stain (blue). Scale bar, 10 μm. Data in (**a**) and (**b**) represent three independent experiments. (**c**) Time-lapse imaging of phagocytic uptake of fluorescently labeled *B. fragilis* by macrophages *in vitro*. Bone marrow–derived macrophages were grown in optical imaging chambers and allowed to adhere for 1 h before addition of fluorescently AF488-labeled *B. fragilis*. Images were recorded every minute for up to ~12 h and rendered into a time-lapse video (see **Supplementary Video 2**). Left and center panels show static images of time-lapse movies, showing macrophages in differential interference contrast (DIC) and fluorescently labeled *B. fragilis* in the AF488 channel, respectively. On the right is an illustration of a macrophage and individual bacteria as seen in the images and **Supplementary Video 2**. Time-lapse imaging data are representative of four different regions of interest (ROI).



Supplementary Figure 2. Fluorescent labeling of the aerobic pathogen *Staphylococcus aureus* by metabolic oligosaccharide engineering and bioorthogonal click-chemistry. Fluorescence confocal microscopy of *Staphylococcus aureus* fluorescently labeled with azide-modified D-GalNAz analogs and AF488-DIBO. Images depict AF488 channel (left), phase contrast (middle), and merged channels (right). Scale bar, 10 μm.



Supplementary Figure 3. Assessment of bacterial species competition in the mouse intestine following administration of three gut symbionts. (Extension of **Fig. 6b,c**). The fluorescent labels were swapped compared to **Fig. 6b,c** to rule out negative affects of the fluorescent label. Three members of the Bacteroidetes phylum—*B. vulgatus*, *B. fragilis*, and *B. ovatus*—were grown with the same azide-modified substrate (GalNAz), and labeled with AF488-DIBO, TAMRA-DIBO, and AF647-DIBO, respectively. The bacterial mixture was administered by oral gavage. (**a**) Bacteria were mixed in near-equal proportions; the frequency of each species was determined by flow cytometry and computed as input ratio (left). Bacterial mixtures were also grown *in vitro* and in parallel, and ratios measured 5 h later (right). (**b**) Flow cytometry ratios of labeled bacteria 5 h after oral delivery of the three color-coded bacteria. Lumen suspensions were harvested from the proximal (segment 1), medial (segment 2), and distal (segment 3) regions of the small intestine. Data for each intestinal region appear as individual pie charts showing the relative counts of each bacterial species recovered from the lumen, expressed as a percentage of total bacteria. (**c**) Bar graph quantifying recovery of each bacterial species in the mixture from all three small intestine segments. Data in (**a-c**) are representative of two independent experiments (n=3 mice, see **Fig. 6b**).

Table 1: *B. fragilis* strains used in this study

Strain Name	Phenotype	Polysaccharides synthesized								Description	Reference
		A	B	C	D	E	F	G	H		
Bacteroides fragilis NCTC9343	WT	+	+	+	+	+	+	+	+	clinical isolate from abdominal infection	
B. fragilis 9343ΔPSA	no PSA synthesis	-	+	+	+	+	+	+	+	deletion in an 8kb region of the PSA locus	29
B. fragilis 9343ΔmpiM44	expresses only PSA	+	-	-	-	-	-	-	-	Deletion of the Mpi recombinase; PSA and PSE promoters locked on; 5 remaining invertible PS promoters locked off; PSE and PSC synthesis inhibited by the anti-termination factor UpaZ	30

Supplementary Video Legends

Supplementary Video 1. MOE fluorescent labeling of surface glycans of *B. fragilis*. Three-dimensional rendered confocal image of an individual bacterium following MOE-BCC labeling with AF488-DIBO. Green depicts AF488-labeled *B. fragilis*; magenta denotes propidium iodide DNA stain. Video displays labeled *B. fragilis* with outer-surface labeling by AF488-DIBO and internal DNA labeling by propidium-iodide. (See main **Fig. 1j**)

Supplementary Video 2. Phagocytic uptake of *B. fragilis* by macrophages. Time-lapsed imaging of AF488-labeled *B. fragilis* (green) uptake by bone marrow–derived macrophages (DIC channel). Time-lapsed images were acquired in a single Z-plane at 1-min intervals. Video playback is at 15 frames per second. Time is displayed as hh:mm:ss. (See **Supplementary Fig. 1c**)

Supplementary Video 3. Visualization of *B. fragilis* in the distal small intestine by intravital two-photon microscopy. Video depicts AF488-labeled *B. fragilis* in the intravillous space *in vivo*. Green depicts AF488-labeled *B. fragilis*, and blue denotes Hoechst nuclear stain. Time-lapsed images were acquired in a single Z-plane at 1.3 second intervals. Video playback is at 18 frames per second. Scale bar, 10 μm. Time is displayed as hh:mm:ss. (See main **Fig. 5e**)

Supplementary Video 4. Non-invasive 3D whole-body longitudinal optical fluorescence imaging of live mice after oral administration of labeled *B. fragilis*. MOE-BCC labeling was used to label *B. fragilis* with the near-infrared dye Cy7. GF mice were imaged by IVIS at baseline (t = 0), then gavaged and imaged at 2, 6, 9, 12, 24, 72, and 96 h post administration. Video shows 3D rendered IVIS images for each time point fitted to the same scale. (See main **Fig. 5f,g**)