Author Contributions

Roberta Poceviciute (RP)

1. **Idea generation.** Conceived the project with RFI. Conceived the idea of imaging bacteria in the empty intestinal segments of malnourished mice gavaged with bacteria. Conceived the idea of imaging bacteria after 1 h fast. Wrote or contributed to writing grant proposals to fund the project.
2. **Preliminary experiments.** Performed preliminary 16S rRNA gene amplicon sequencing (in collaboration with OMP), 16S rRNA gene copy quantification by dPCR, and imaging (in collaboration with OMP). Supervised preliminary machine-learning based image analysis (performed by HT). Used preliminary data to refine the project.
3. **Method development (HCR v3.0 probe design and validation, hydrogel chemistry optimization).** Starting with the 52 bp region that overlaps with EUB338 binding site (selected by Molecular Technologies), designed the universal degenerate HCR v3.0 probe set. Validated HCR v3.0 probes. Screened different hydrogel chemistries for optimal transport properties.
4. **Data acquisition.** Set up all animal experiments (tail cup experiment was set up in collaboration with SB). Collected and preserved samples for imaging. Collected samples for host gene expression analysis by RTqPCR and 16S rRNA gene analysis by dPCR and sequencing with SRB. Extracted RNA for RTqPCR in collaboration with AER. Acquired all imaging data.
5. **Data analysis.** Analyzed RT-qPCR and dPCR data, processed 16S rRNA gene amplicon sequencing, and imaging data. Conceived image segmentation and filtering pipeline.
6. **Figure generation.** Created all figures in the main text and the SI.
7. **Outline writing.** Conceived and wrote outlines.
8. **Manuscript writing.** Wrote and edited the manuscript.

Said R. Bogatyrev

1. **Data acquisition.** Set up tail cup experiment with RP. Collected samples for host gene expression analysis by RTqPCR and 16S rRNA gene analysis by dPCR and sequencing with RP.

Anna E. Romano

1. **Data acquisition.** Extracted RNA with RP and quantified host gene expression by RTqPCR (Supplementary Fig. 1, b and c). Extracted DNA and quantified 16S rRNA gene copy load (Fig. 2, f and g). Prepared 16S rRNA gene amplicon library for sequencing (Fig. 2a).

Amanda H. Dilmore

1. **Data acquisition.** Extracted DNA for 16S rRNA gene amplicon sequencing and 16S rRNA gene copy quantification (Fig. 2, a-e). Quantified 16S rRNA gene copies by dPCR (Fig. 2, b-e, and Supplementary Fig. 2).

Octavio Mondragón-Palomino

1. **Preliminary experiments.** Performed preliminary 16S rRNA gene amplicon sequencing (in collaboration with RP) and 3D imaging of tissue samples from the small intestines of mice in the environmental enteropathy model (in collaboration with RP). Contributed practical and technical guidance on spectral confocal imaging of clarified tissues.

Heli Takko

1. **Preliminary experiments.** Performed preliminary machine-learning based image analysis (supervised by RP).

Ojas Pradhan

1. **Data acquisition**. Managed histopathology data acquisition (Supplementary Fig. 25)

Rustem F. Ismagilov

1. **Idea generation.** Conceived the project with RP.
2. **Data acquisition.** Provided feedback on experimental design.
3. **Funding.** Secured funding for the project.
4. **Data analysis.** Provided feedback on data analysis.
5. **Outline writing.** Provided feedback on outlines written by RP.
6. **Manuscript writing.** Edited the manuscript written by RP.