Supplementary Information for "Stable, diverse, fecal-derived *in vitro* microbial communities that model the intestinal microbiota response to antibiotics"

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Supplementary Tables

Strain	Closest relative in NCBI 16S ribosomal RNA sequence database	Ciprofloxacin MIC (μg/mL)
TT1	*Enterococcus hirae* ATCC 9790	2
TT2	*Escherichia fergusonii* ATCC 35469	<0.5
TT3	*[Clostridium] symbiosum* ATCC 14940	16
TT4	*Bacteroides thetaiotaomicron* JCM 5827	16
TT5	*[Clostridium] clostridioforme* ATCC 25537	32
TT6	*Blautia producta* JCM 1471	32
TT7	*[Clostridium] scindens* strain DSM 5676	32
TT8	*Enterococcus faecium* strain DSM 20477	4
TT9	*[Clostridium] hylemonae* TN-272	32
TT10	*Enterococcus faecalis* NBRC 100480	4
TT11	*[Clostridium] hathewayi* 1313	32
TT12	*Bacteroides fragilis* NCTC 9343	4
TT13	*Flavonifractor plautii* 265	8
TT14	*Bacteroides uniformis* JCM 5828	16
TT15	*Parabacteroides distasonis* ATCC 8503	4

Table S1: Strains isolated in this study, related to Figure 5.

Medium	Formulation	Sterilization	Storage
BHI	Commercially available (BD 211061)	Autoclave 20 min	Room temperature (RT)
TYG	As described in (Whitaker et al., 2017)	0.22-µm filter	4 °C
GAM	Commercially available (HiMedia M1801)	Autoclave 20 min	RT
YCFA	As described in (Duncan et al., 2002). Vitamins and cysteine added 48 h before experiment	0.22-µm filter	4 °C

Table S2: Media used in this study, related to Figure 1.

Supplementary Figures

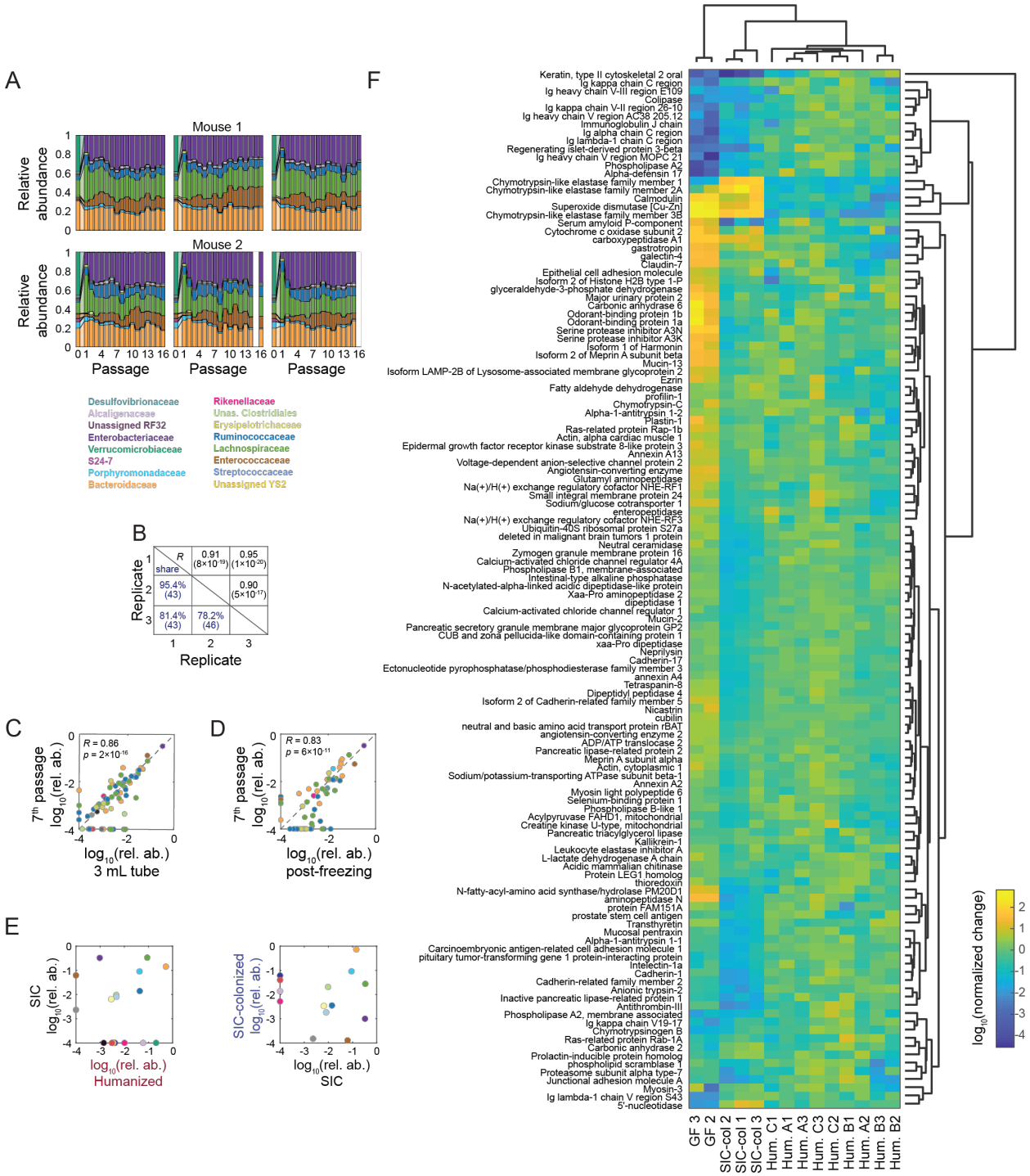

Figure S1: Robust, high-throughput cultivation of fecal-derived SICs and colonization of a germ-free mice by an SIC. Related to Figures 1 and 2.

A) *In vitro* passaging leads to stable and complex SICs. Family-level composition of three replicate SICs from two fecal samples (Pre MD mice #1 and #2) during passaging in BHI for 16 rounds *in vitro*. Passage 0 is the fecal inoculum.

B) *In vitro*-passaged SICs are highly reproducible. Summary of the reproducibility of the three replicates for the mouse 1 SICs in Fig. 1 B-D. *R* and *p* were computed using only ASVs present in both samples. Also shown is the percentage of ASVs that were present in both replicates at >0.1% abundance ("shared"), with the total number of ASVs in parentheses.

C,D) SICs can be grown in larger volumes and can be frozen and revived without affecting composition. ASV-level relative abundance values for the SIC in Fig. 1 B-D after 7 passages were compared with the same SIC grown for one passage in a larger vessel without shaking (C), and after freezing with 25% glycerol at -80 °C and reviving for one passage (D). *R* and *p* were computed using only ASVs present in both samples. ASVs with relative abundance < 10^{-4} were set to 10^{-4} for visualization.

E) Families that are overrepresented *in vitro* recede *in vivo*. Shown is a comparison of family-level relative abundance of an SIC and the humanized-mouse fecal inoculum from which it was derived (left), or ex-germ-free mice colonized with the SIC (right, SIC n=1, inoculum n=1, SIC-colonized n=3). Families with relative abundance < 10^{-4} were set to 10^{-4} for visualization. Mouse data are the same as in Fig. 2B.

F) The secreted proteome of mice colonized with SIC is similar to the proteome of humanized mice. *M. musculus* proteins present at levels 10-fold higher in mice than in SICs were normalized by their mean abundance in humanized mice. Dendrograms resulting from hierarchical clustering of normalized relative abundance of proteins in germ free (GF), SIC-colonized (SIC col.), or humanized mice (Hum.) housed in three cages (A, B, and C).

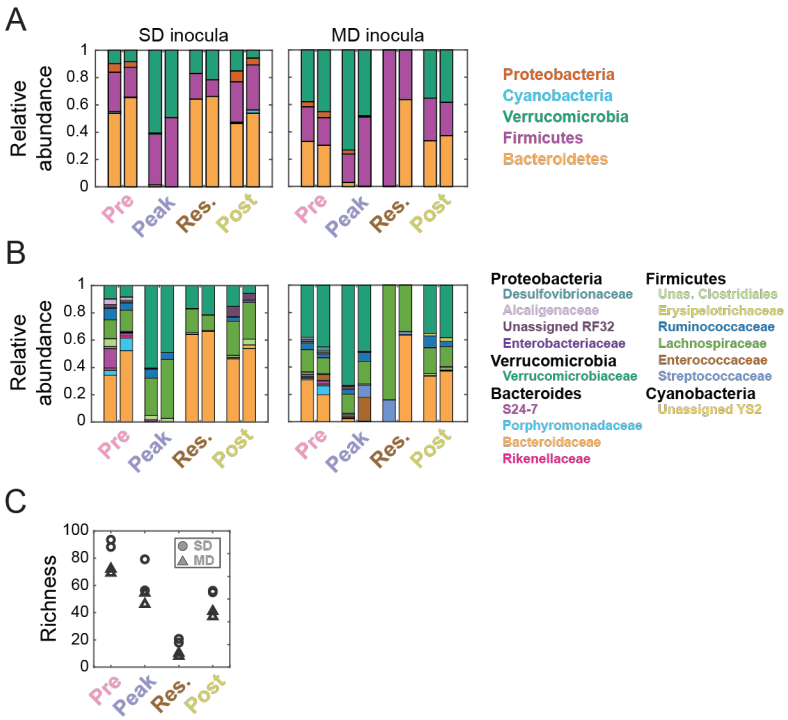

Figure S2: Diet and antibiotics have large, distinct, interacting effects on the composition of the microbiota, related to Figure 3.

A,B) Ciprofloxacin elicits large changes in microbiota composition at the phylum (A) and family (B) level *in vivo*. Each time point has two bars corresponding to the two mice in each group.

C) Diversity *in vivo* decreases during ciprofloxacin treatment and does not fully recover after treatment. Richness (number of ASVs in rarefied data) of fecal inocula.

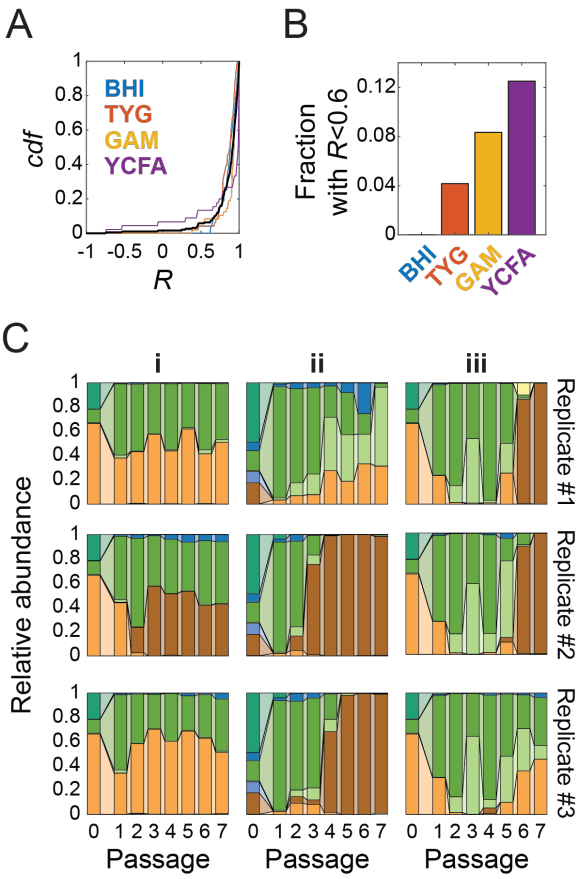

Figure S3: SICs are more reproducible in BHI than in other media, related to Figure 3.

A) Technical replicates are largely reproducible. Cumulative density function of Pearson correlation coefficient (*R*) for all pairwise comparisons between technical replicates.

B) Proportion of technical replicate pairwise correlations *R*<0.6 for the 4 growth media.

C) Non-reproducible technical replicates share similar dynamics during early passages. Family-level composition during *in vitro* passaging for 7 rounds of three SICs with low correlation coefficients after 7 passages. (i) Technical replicates of SIC originating from SD mouse #2 during pre-treatment, grown in GAM. (ii) Technical

replicates of SIC originating from MD mouse #2 during peak of treatment, grown in YCFA. (iii) Technical replicates of SIC originating from SD mouse #2 during residual treatment, grown in YCFA.

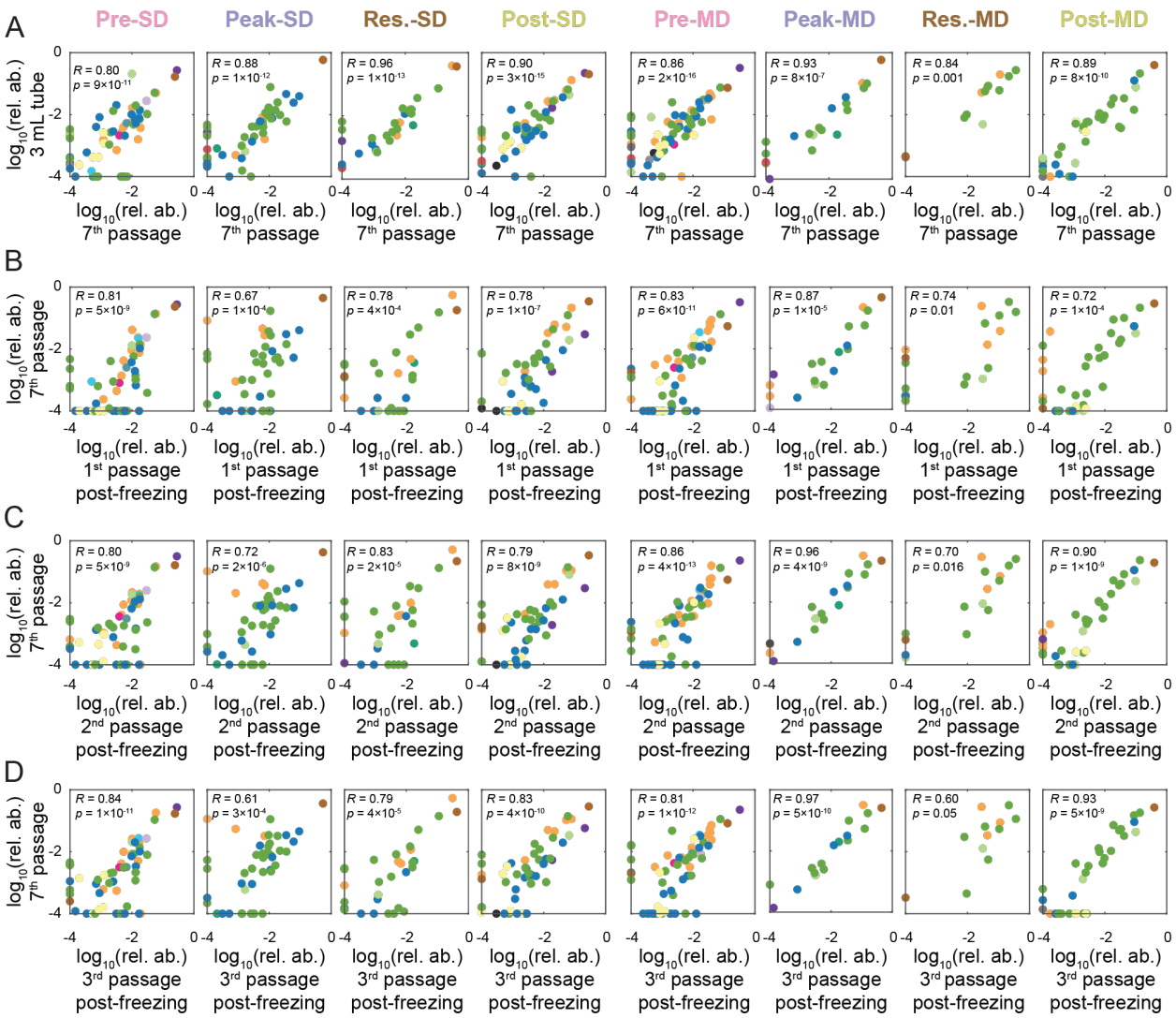

Figure S4: SICs maintain composition after growth in larger volumes without shaking and during revival after freezing, related to Figure 3.

A-D) Correlation plots of $\log_{10}$(relative abundance) at the ASV level for 8 SICs grown in BHI after 7 passages against the same SIC grown for one passage in a larger volume (3 mL) without shaking (A), and after freezing and reviving for one (B), two (C), or three (D) passages. Pearson coefficients (R) and their p-values were computed using

only data points present in both samples. ASVs with relative abundance $<10^{-4}$ were set to 10^{-4} for visualization purposes.

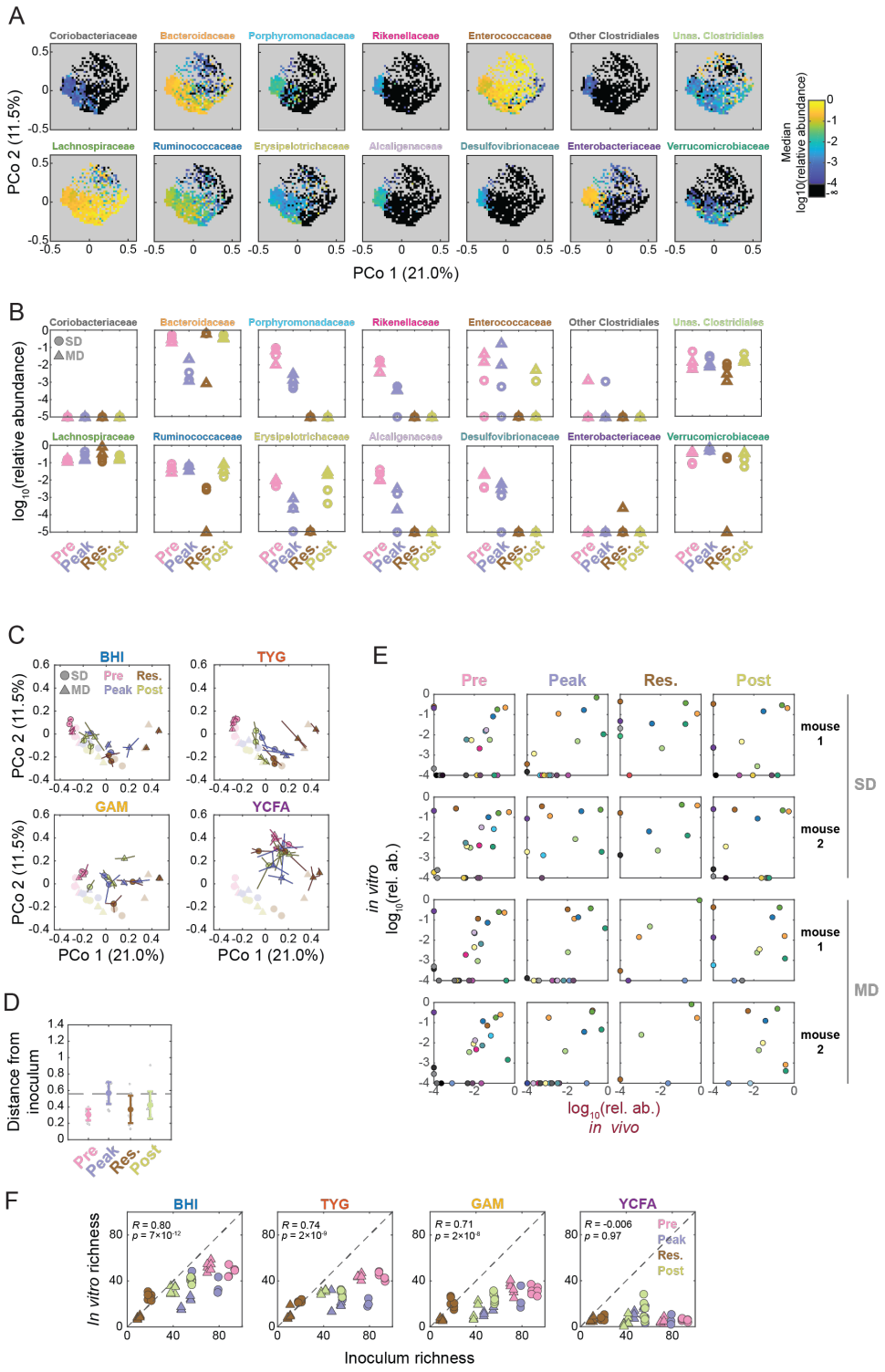

Figure S5: Certain families generally co-occur in SICs, related to Figure 3.

A) Median $\log_{10}$(relative abundance) of families across all samples (n=1728) binned by first two principal components. Only families present in >10% of the samples are shown.

B) Log_{10}(relative abundance) of families in (A) in each of the inocula. Families with relative abundance $<10^{-4}$ were set to 10^{-4} for visualization purposes.

C) Medium and inoculum determine the final composition of passaged SICs. The 7th passage of all 192 SICs in a PCoA of SIC composition using unweighted Unifrac distance computed on all *in vivo* and *in vitro* samples at the ASV level. Samples are separated by media, with colors and shapes representing the timepoint during ciprofloxacin treatment and diet, respectively, in the mice from which the inocula were taken. Symbols are the centroid of three replicates, with lines connecting the replicates to the centroid. Original fecal inocula are plotted in light colors. BHI data is the same as in Fig. 2C.

D) SICs derived from feces at the peak of treatment *in vivo* are most distinct from the composition of their inoculum suggesting low viability of detected strains. Weighted Unifrac distance of the 7th passage in BHI of each SIC to their fecal inoculum. Colored circles, mean distance for each medium; individual SICs in gray. Error bars, standard deviations; n=12. Dashed line, mean distance between fecal samples.

E) *In vitro* passaging in BHI can produce an SIC that retain abundant families in the fecal inoculum. Mean family-level relative abundances (from mean of passages 4-7)

for three SIC triplicates compared with the fecal inoculum from which it originated. Families with relative abundance $<10^{-4}$ were set to 10^{-4} for visualization purposes.

F) SIC yield scales with diversity. Mean OD of SICs after 20 h of growth in BHI passages 3-7 increases with increasing richness (number of ASVs in rarefied data) in passage 7. *R*, Pearson correlation coefficient; n=48. BHI data is the same as in Fig. 2E.

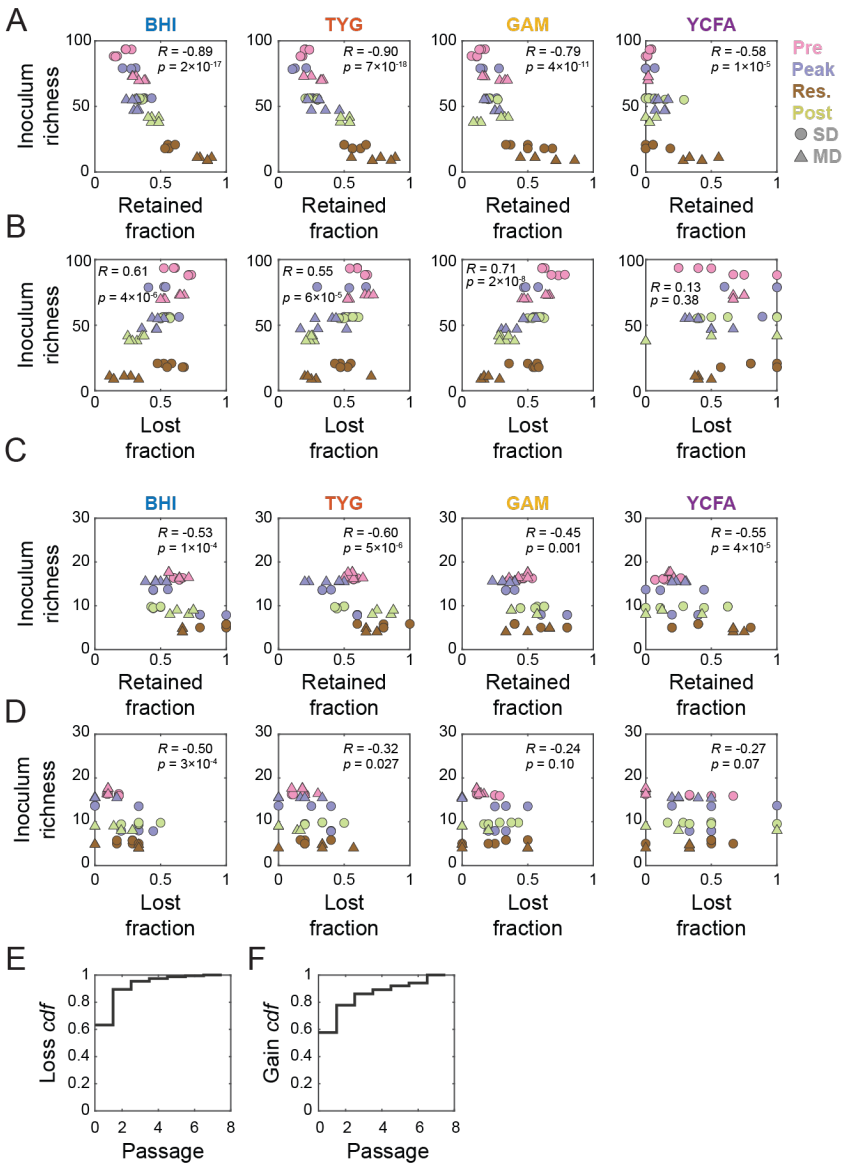

Figure S6: Taxa loss and emergence are correlated with inoculum diversity, related to Figure 3.

A-D) Correlation plots for inoculum diversity (number of ASVs in (A) and (B), and families in (C) and (D), in rarefied data) with fraction of taxa lost in (A) and (C) and emerged in (B) and (D), for all 192 passaged SICs. Pearson correlation coefficients (R) and their p-values were computed from all data points in each plot (n=48).

E,F) Most ASV loss or emergence occurred within the first three passages. Cumulative density function for the passage at which an ASV was lost (E) or emerged (F).

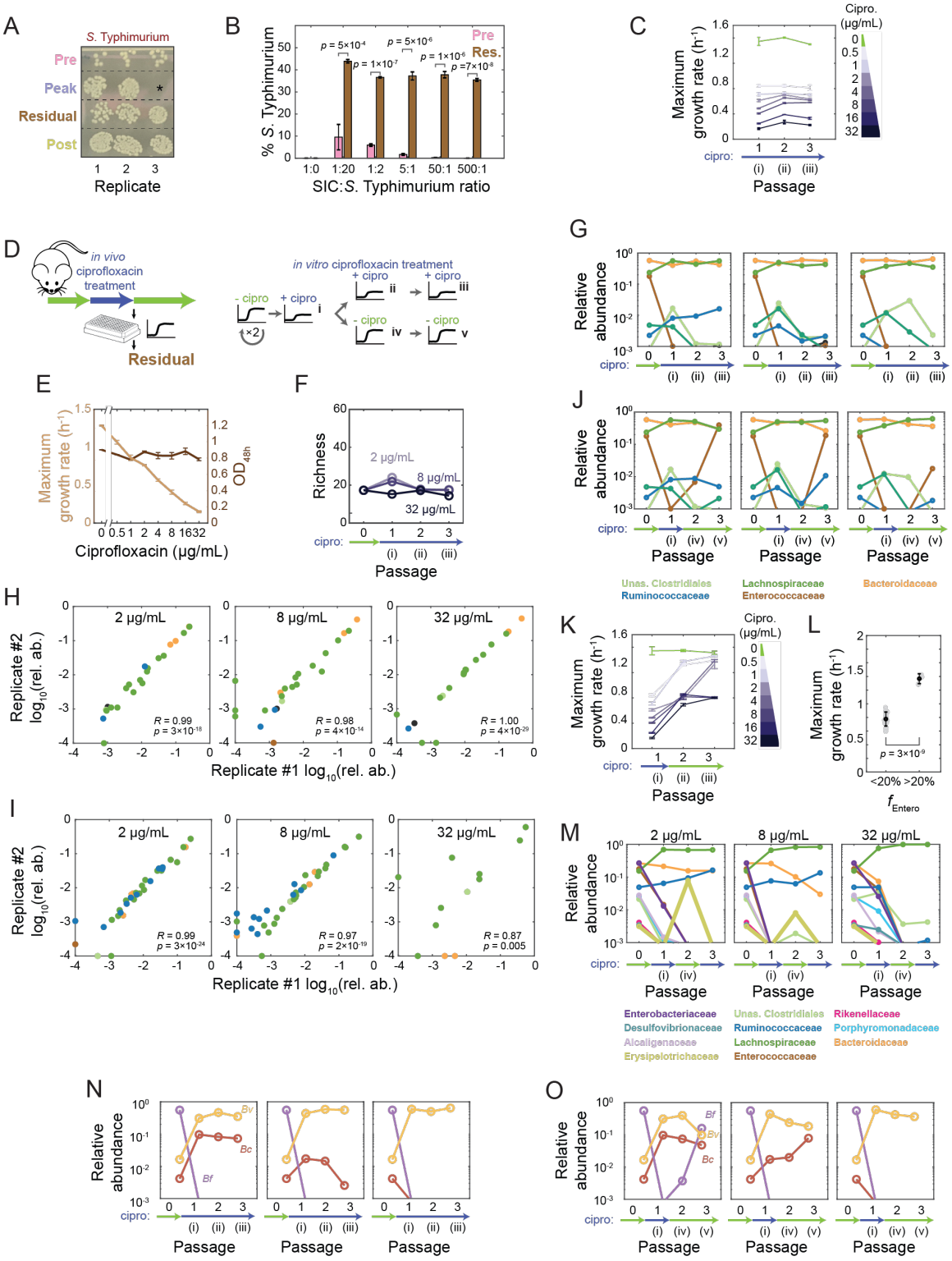

Figure S7: Effects and dynamics of antibiotic treatment on SICs, related to Figures 4-6.

A,B) *In vivo* ciprofloxacin treatment makes SICs more susceptible to *S*. Typhimurium invasion. (A) Colonies of *S*. Typhimurium SL1344 after 48 h of growth with SICs spotted on LB+streptomycin in aerobic conditions after a $1{:}10^4$ dilution. These data are a biological replicate (SICs derived from mice housed in a different cage) of Fig. 4B. *: missing replicate. (B) Single-cell quantification of mCherry-tagged *S*. Typhimurium 14028s after 48 h of co-culturing with SICs derived from pre- and residual-treatment mice fecal inocula. *p*-values are from a Student's two-sided *t*-test between each pairwise comparison, n=3.

C) SICs show little adaptation to continuous ciprofloxacin treatment. Maximum growth rate decreases or remains approximately constant across three passages in ciprofloxacin.

D-J) SICs derived from pre-exposed inocula show increased resilience to ciprofloxacin treatment. (D) Experimental setup for *in vitro* antibiotic treatment of a pre-exposed SIC. An SIC passaged in BHI from residual treatment humanized mouse fecal inoculum (Res-SD) was revived after freezing and passaged twice in BHI. The SIC was passaged in ciprofloxacin three times (i,ii,iii) or in ciprofloxacin once and then twice without the drug (i,iv,v). (E) In contrast to the Pre-SD SIC (Fig. 3C), the yield of Res-SD SIC was virtually unaffected by ciprofloxacin, although the growth rate decreased with concentration. OD was measured after 48 h of growth with ciprofloxacin. Lines, means of triplicate growth curves; error bars, standard

deviations. (F) Richness (number of ASVs in rarefied data) of Pre-SD SIC remained constant during continuous ciprofloxacin treatment. Data are means of two technical replicates. (G) Bacteroidaceae and Lachnospiraceae dominated during three rounds of ciprofloxacin treatment of the Res-SD SIC. Data are the mean of family-level abundances across two technical replicates. (H) Treatment of the residual treatment humanized mouse fecal inoculum (Res-SD) SIC led to highly reproducible outcomes. Shown are comparisons of relative abundance at the ASV level between replicates after 3 passages of growth in BHI with ciprofloxacin. Pearson coefficient (*R*) and its *p*-value were computed only from data points present in both samples. ASVs with relative abundance $<10^{-4}$ were set to 10^{-4} for visualization. (I) Treatment outcome of Pre-SD SICs was less reproducible than Res-SD SICs at high concentrations. Shown are comparisons of relative abundance at the ASV level between replicates after 3 passages of growth in BHI with ciprofloxacin. Pearson coefficient (*R*) and its *p*-value were computed only from data points present in both samples. ASVs with relative abundance $<10^{-4}$ were set to 10^{-4} for visualization. (J) Enterococcaceae can recover after one round of ciprofloxacin treatment in the Res-SD SIC. Data are the mean of two replicates.

K-M) SICs derived from pre-exposed inocula show increased resilience to ciprofloxacin treatment. (K) Pre-SD SIC growth rate can recover to levels similar to values before treatment at low concentrations. Maximum growth rate was calculated across

ciprofloxacin concentrations during one round of antibiotic treatment and two rounds of recovery. Lines, mean of three technical replicates; error bars, standard deviations. (L) Growth rate recovery after transient ciprofloxacin treatment is linked to the recovery of fast-growing species. Maximum growth rate of SICs grown in BHI after one round of treatment with ciprofloxacin and two rounds without drug. SICs were classified by their summed relative abundances of Enterococcaceae and Enterobacteriaceae (f_{Entero}). Black circles are the mean maximum growth rate for each group (n=21 for $f_{\text{Entero}} < 20\%$ and n=3 for $f_{\text{Entero}} > 20\%$), error bars are standard deviations. Individual data points are plotted in gray. p-value is from a Student's two-sided t-test between the two groups. (M) Erysipelotrichaceae recovery is reversed by a second ciprofloxacin treatment. Data are the family-level mean $\log_{10}$(relative abundance) of two replicates during one round of ciprofloxacin treatment followed by one round of recovery and a second treatment.

N,O) Bacteroidaceae are remodeled in the Res-SD SIC during and after ciprofloxacin treatment, with *B. vulgatus* as the only member to generally survive. Relative abundances of *Bacteroides* species in the Res-SD SIC during continuous ciprofloxacin treatment (N) or after one round of treatment followed by two rounds of recovery (O).