Intergenerational transfer of antibiotic-perturbed microbiota enhances colitis in susceptible mice

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Supplementary text for the manuscript:

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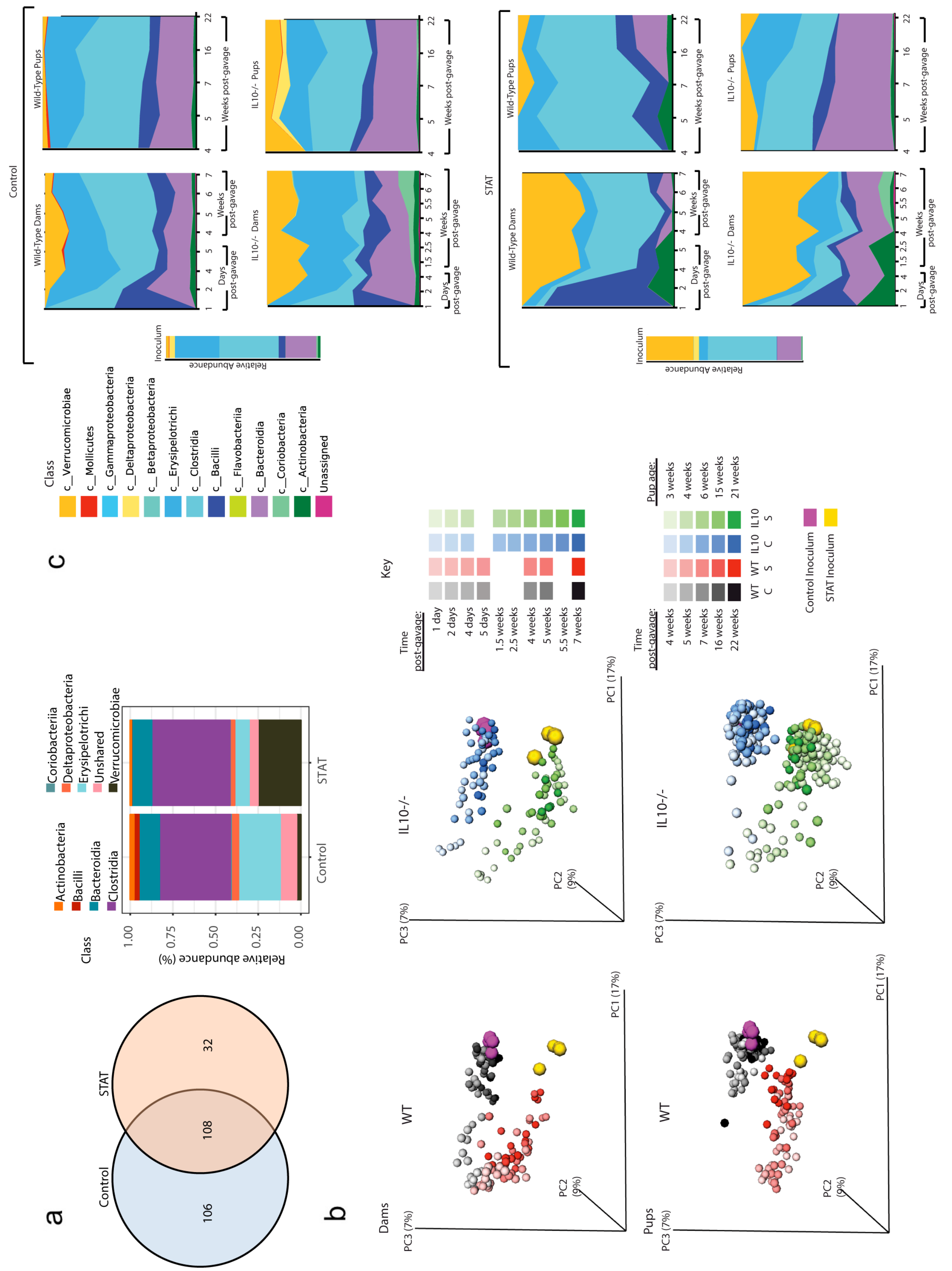
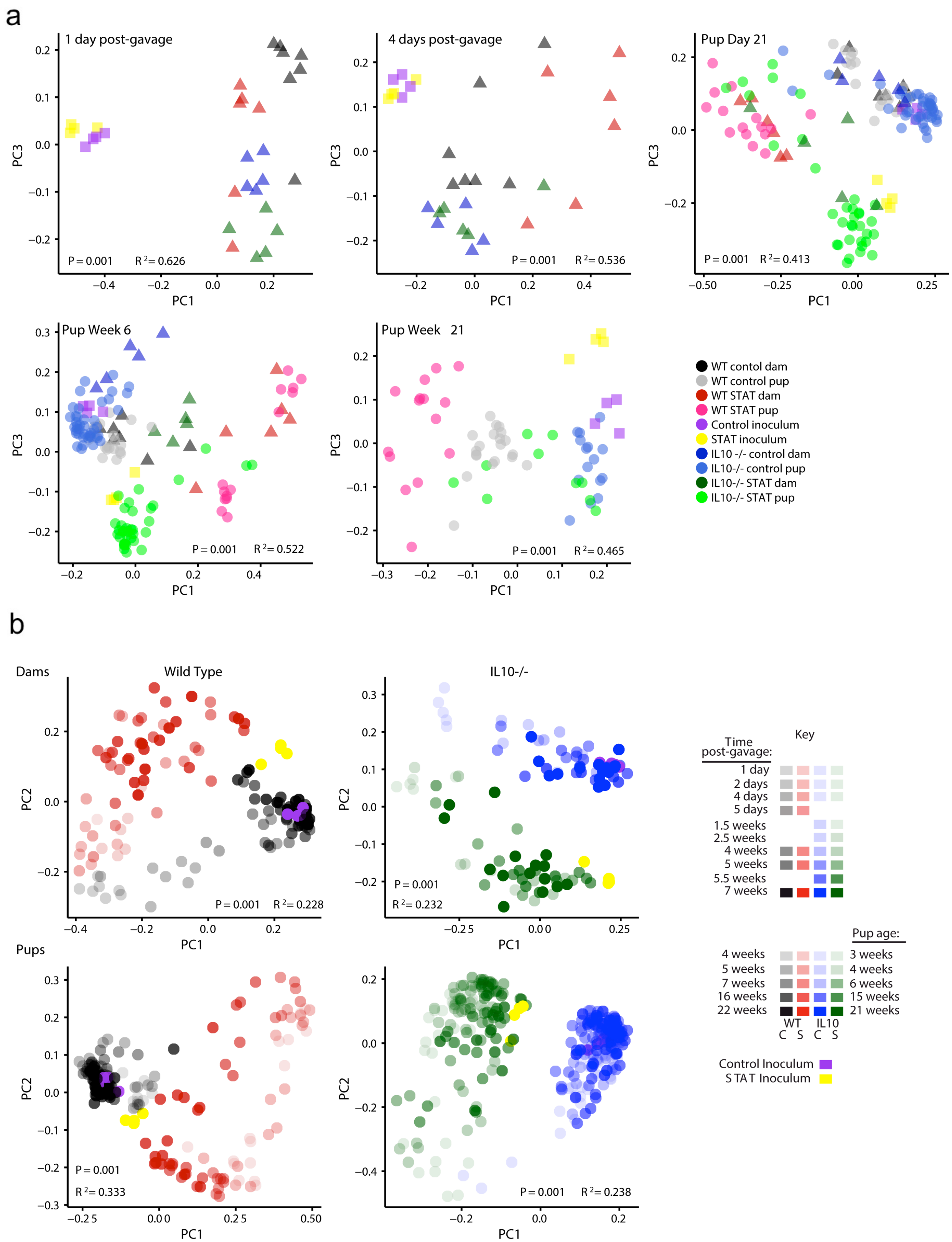
Supplementary Figures

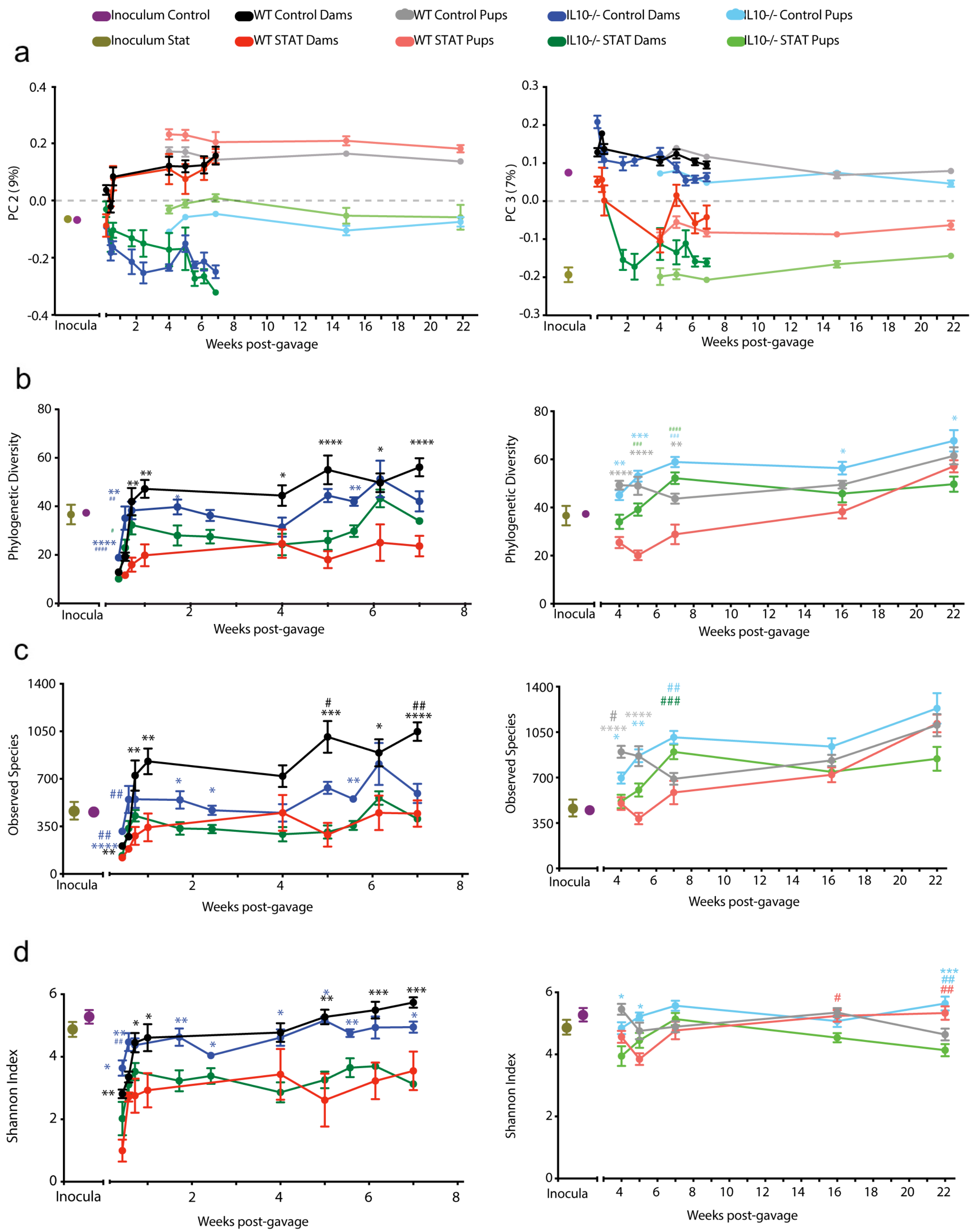


Supplementary Figure 1. Changes in microbiota and body composition over time.
(a) Analysis of inoculum samples. Left: Venn diagram of number of OTUs identified in Control, STAT, or both inocula. Right: Relative abundance of taxa identified in Control and STAT inocula. Includes analysis of 4 replicate samples of each donor material **(b)** PCoA plots showing progression of fecal bacterial communities along PC1 over time. Rotated view of PCoAs from Figure 1b. Bold, italicized times have samples from the same day for dams and pups. See Supplementary Table 1 for numbers of mice and P values from Adonis testing. **(c)** Taxa summaries showing mean relative abundance of taxa identified in each group over time; numbers of mice are as in Supplementary Table 1. Taxa are shown at the class level, as described in the color legend.



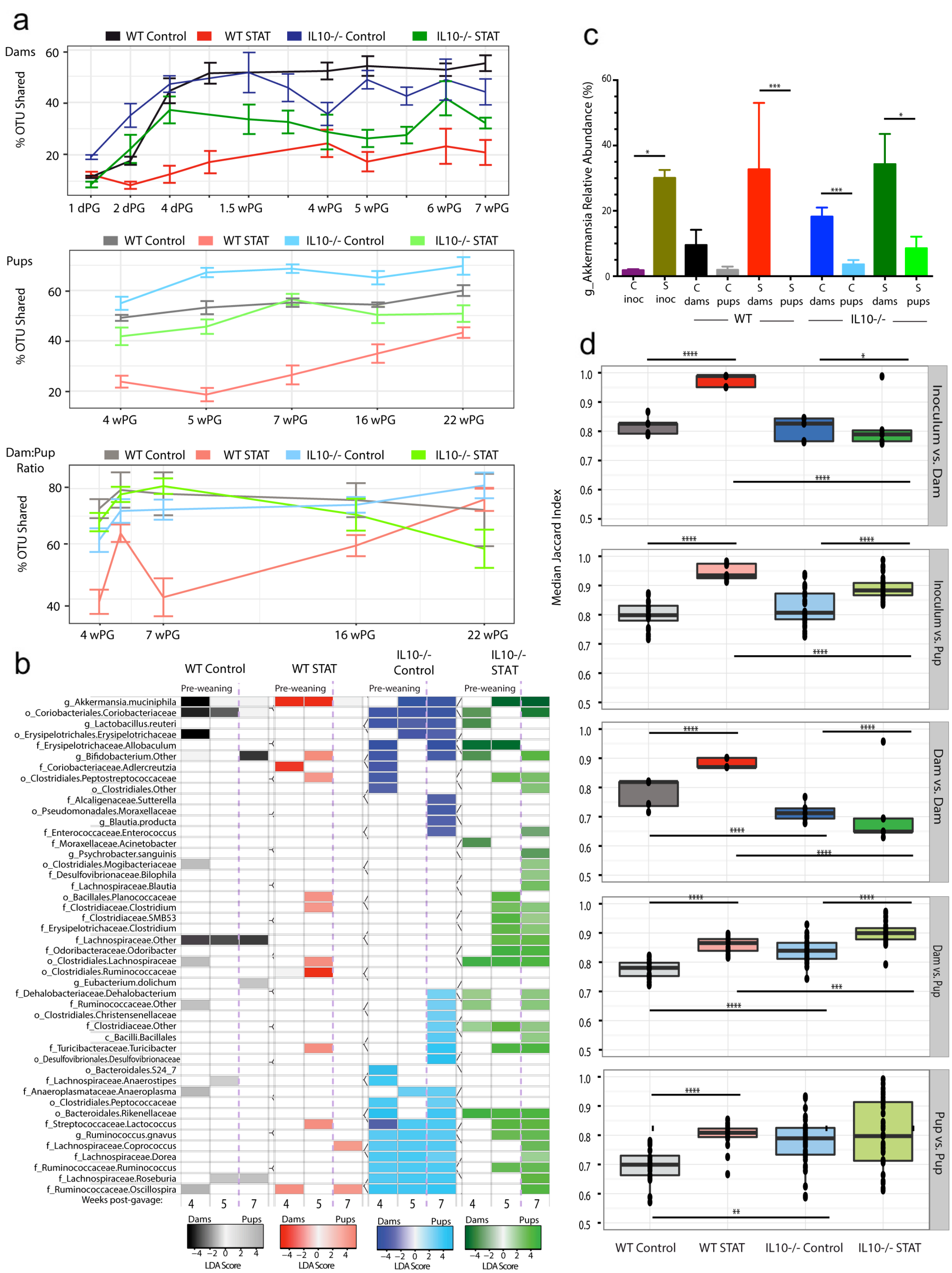
Supplementary Figure 2. Principal Coordinate analysis showing unweighted

UniFrac distances at each of 12 time-points. For each time point, Adonis testing (PERMANOVA) was performed, testing for differences across genotype/treatment groups, with p-value and R^2 shown. **(a)** Inocula (squares), dams (triangles), pups (circles). **(b)** Circles color-coded by time, and shaded by group. Numbers of mice studied for dams, pups, respectively, in each of the 4 groups are: IL10-/- Control =5, 15-42; WT Control=7, 20; IL10-/- STAT=5, 10-34; WT STAT =6, 15. Numbers for the IL10 pup groups declined after 6 weeks due to sacrifices.

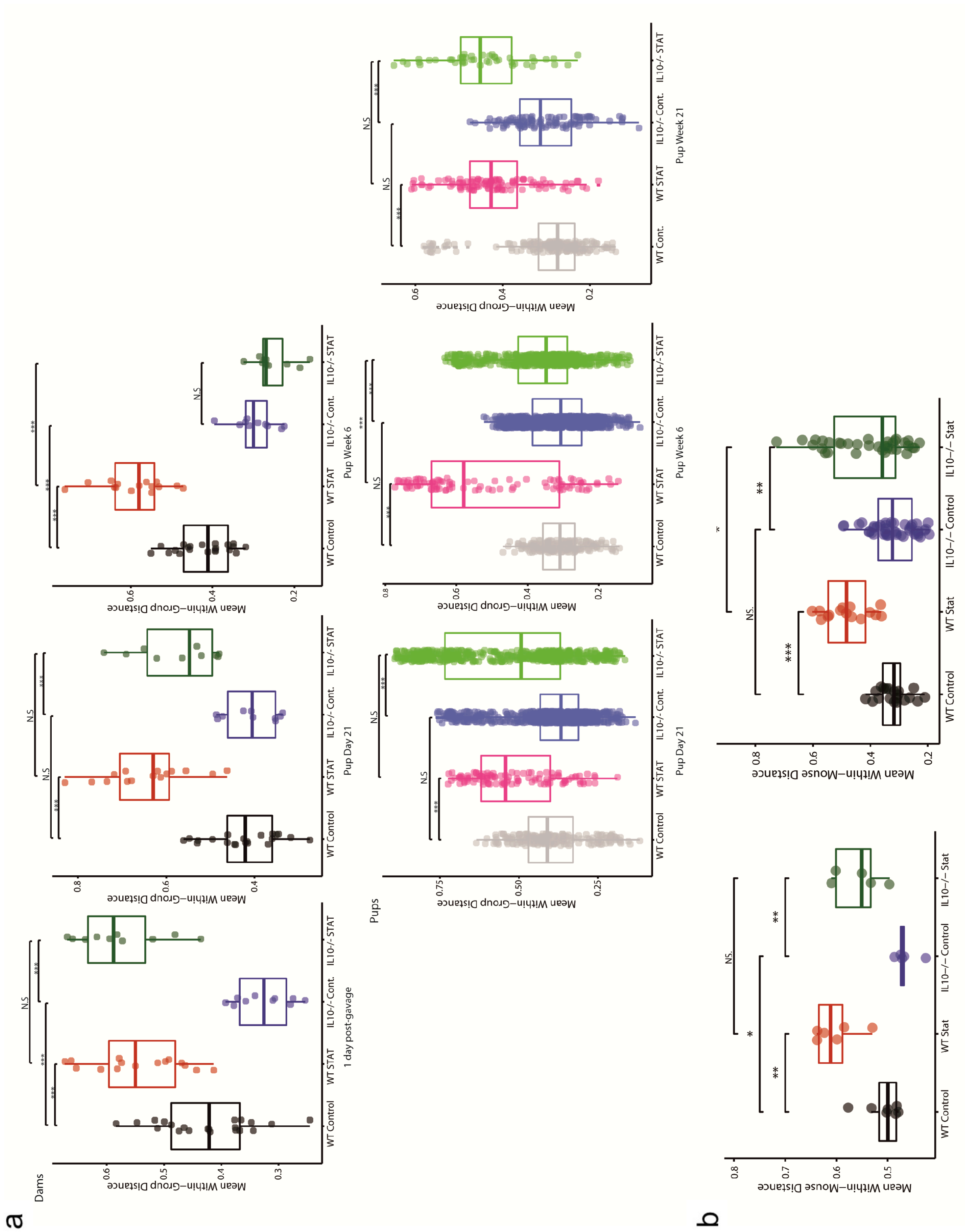


Supplementary Figure 3. Stable microbiota communities in recipient mice are determined by genotype and antibiotic influence. (a) Beta-diversity from unweighted

UniFrac principal component values over time. Numbers of mice are as in Supplementary Table 1; plots show mean +/- SEM for each group. Left, PC2: Control WT vs. Control IL10-/- and STAT WT vs. STAT IL10-/- are significantly different ($p<0.05$, one-way ANOVA with Sidak's multiple comparison test, see Supplementary Table 2) for time points after day1-post-gavage. Right, PC3: Control WT vs. STAT WT and Control IL10-/- vs. STAT IL10-/- are significantly different ($p<0.05$, one-way ANOVA with Sidak's multiple comparison test, see Supplementary Table 2) for all timepoints after 1 week post-gavage. Inocula are also significantly different ($p<0.05$, Mann-Whitney test) along PC3. **(b-d)** Alpha diversity: plots indicate mean +/- SEM. Significance indicated by color (group with higher value) and symbols: * compares Control and STAT within the same genotype, # compares the same treatment group across genotype. *$p<0.05$, **$p<0.01$, ***$p<0.001$, ****$p<0.0001$, one-way ANOVA with Sidak's multiple comparison test for all time points, except Mann-Whitney test was used when only two groups were compared, see Supplementary Table 3. **(b)** Phylogenetic Diversity in dams (left) and pups (right). **(c)** Microbial richness (observed species) in dams (left) and pups (right). **(d)** Evenness (Shannon index) over time in dams (left) and pups (right).

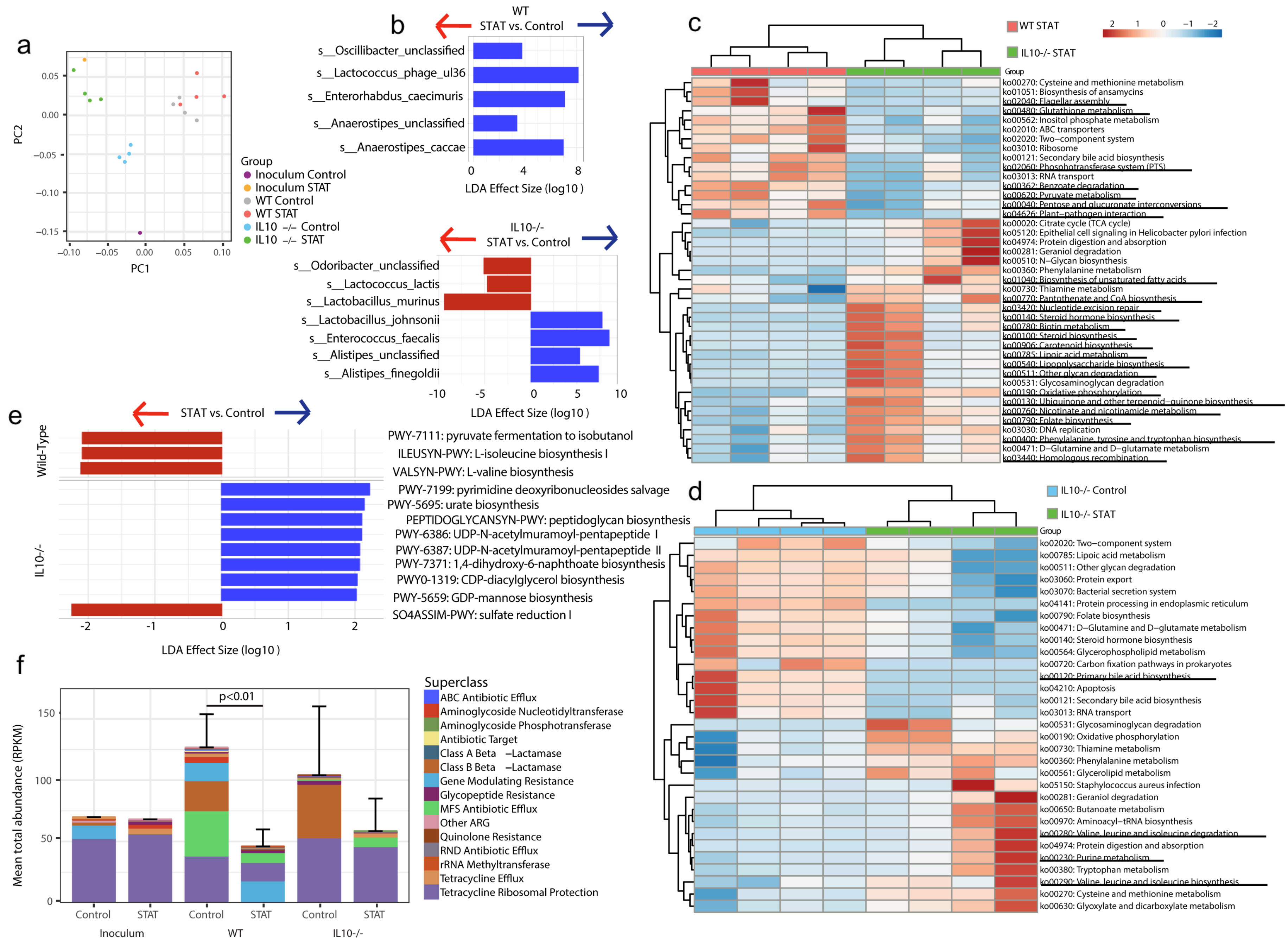


Supplementary Figure 4. Microbiota stability across transfer and generations. (a-c) Numbers of mice are as in Supplementary Table 1. **(a)** Percentage of inoculum OTUs shared by that inoculum and the mouse group that received that inoculum. Mean +/- SEM for each group of dams (top) and pups (middle) over time. Differences that were significant at individual time points include significantly greater shared OTUs in the pups and dams for the Control specimens vs STAT at all time points. IL10-/- pups also had a significantly higher proportion of shared inoculum OTUs compared to wild type. Percent OTUs shared were calculated by the number of shared OTUs between a sample and its respective inoculum, divided by the total number of OTUs found within the inoculum. The bottom graph shows sharing of the OTUs of the dams and their pups. Pups were studied from weaning (5 weeks post-gavage) through 22 weeks post-gavage. Mouse numbers are as in Supplementary Figure 2. Statistical significance was determined using the Welch's Two Sample t-test, see Supplementary Table 7 for P-values. **(b)** Linear discriminant analysis (LDA) scores based on the LEfSe analysis for time points with both dam and pup fecal samples, indicating taxa that are significantly different in abundance between dams and their pups. **(c)** Relative abundance of *Akkermansia* at 5 weeks post-gavage, mean +/- SEM. *p<0.05, ***p<0.001, Mann-Whitney U-test. See Supplementary Table 8. **(d)** Median Jaccard index values were calculated for each sample's pairwise comparison between the indicated groups (Inoculum vs. dams, dams vs. dams, etc). Boxplots indicate the median values with interquartile range. Treatment groups were compared with a set of pairwise Wilcoxon tests with Holm correction, * p < 0.05, ** p<0.01, *** p<0.001, **** p<0.0001 see Supplementary Table 9. Numbers of mice studied for dams, pups, respectively, in each of the 4 groups are: IL10-/- Control =5, 42; WT Control=7, 20; IL10-/- STAT=5, 34; WT STAT =6, 15. Each inoculum was 4 replicate samples.

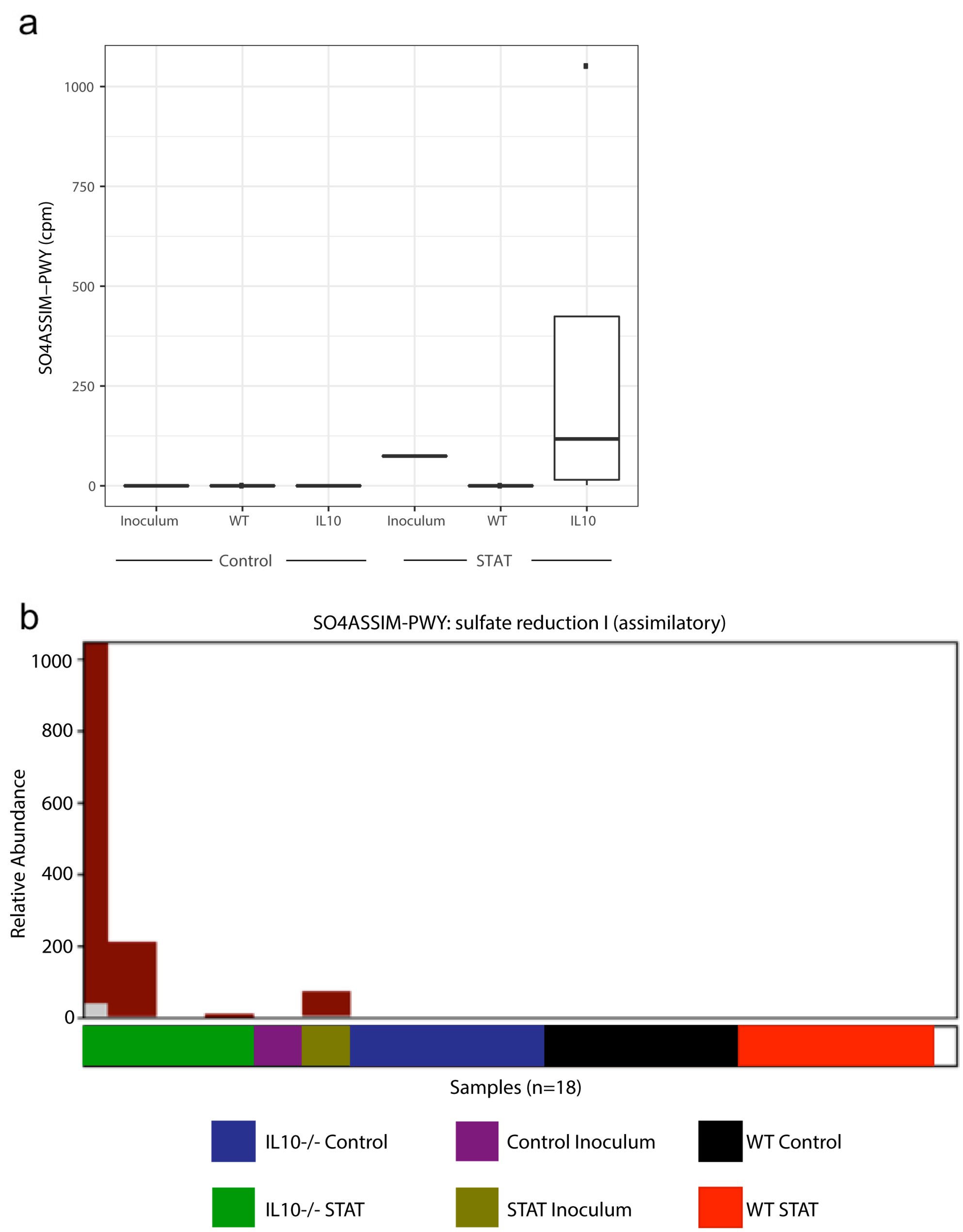


Supplementary Figure 5. Intra-litter and intra-mouse variation over time. (a)

Intragroup distances in community composition in dams and their pups according to genotype and inoculum over time. NS, not significant; p-values <0.05 are shown. **(b)** Mean within-mouse distances (variation), based on unweighted UniFrac analysis. In each comparison, there was significantly more variation in mice receiving the antibiotic-perturbed inoculum than in control (t-tests, with FDR-correction.* $p < 0.05$; ** $p < 0.01$; ***$p<0.001$; NS, not significant). IL-10-/- dams receiving the control inoculum have lower within-mouse variability than wild-type (WT) mice receiving the control inoculum, but this does not hold true for pups. IL10-/- pups receiving the STAT inoculum have lower within-mouse variability than wild-type (WT) mice receiving the STAT inoculum, but this does not hold true for dams. Numbers of mice are as detailed in Supplemental Table 1. See Supplementary Table 10 for detailed statistics.

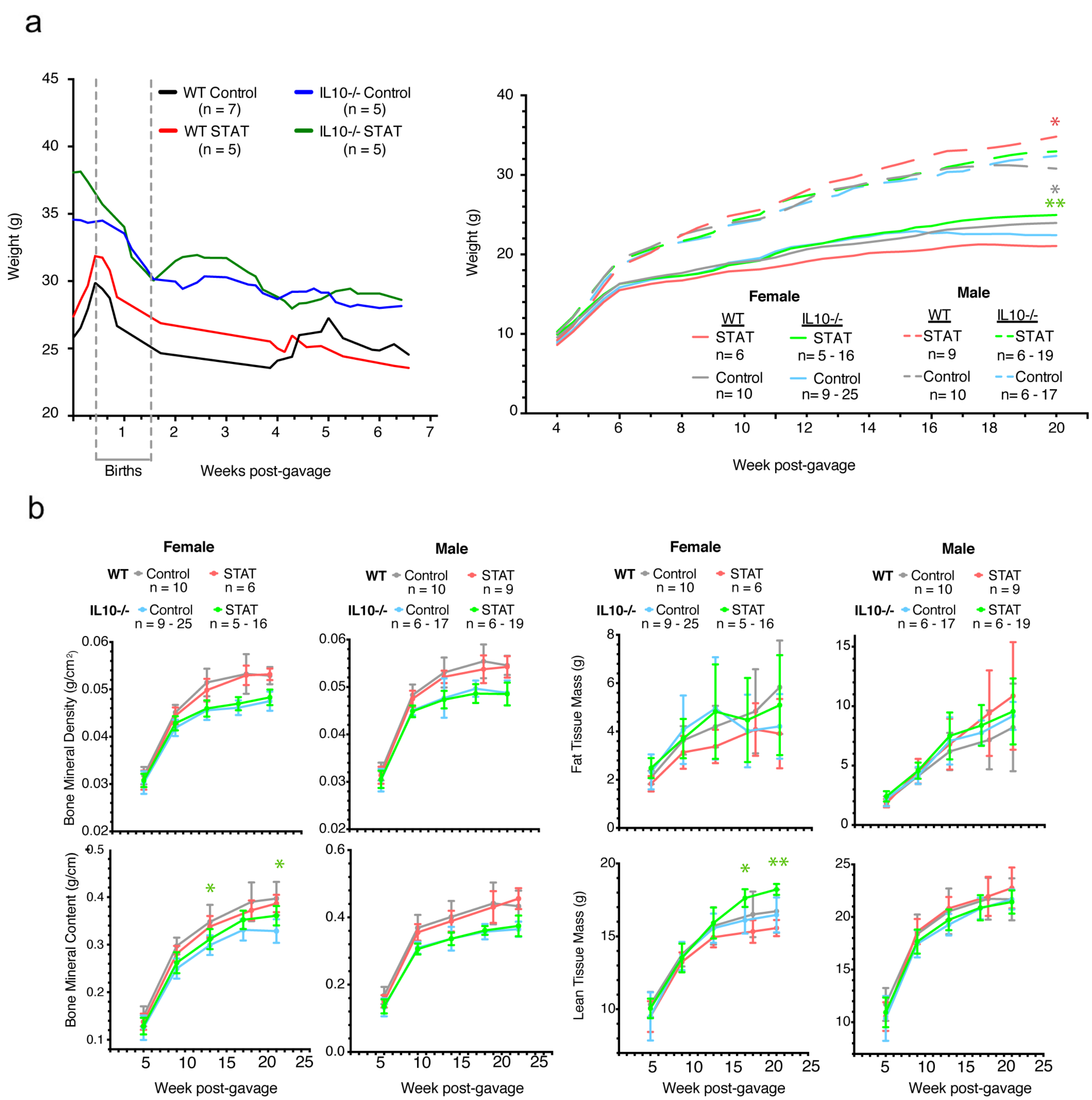

a
PC2
PC1
Group
Inoculum Control
Inoculum STAT
WT Control
WT STAT
IL10 –/– Control
IL10 –/– STAT
b
WT
STAT vs. Control
s__Oscillibacter_unclassified
s__Lactococcus_phage_ul36
s__Enterorhabdus_caecimuris
s__Anaerostipes_unclassified
s__Anaerostipes_caccae
LDA Effect Size (log10)
IL10-/-
STAT vs. Control
s__Odoribacter_unclassified
s__Lactococcus_lactis
s__Lactobacillus_murinus
s__Lactobacillus_johnsonii
s__Enterococcus_faecalis
s__Alistipes_unclassified
s__Alistipes_finegoldii
LDA Effect Size (log10)
c
WT STAT
IL10-/- STAT
Group
ko00270: Cysteine and methionine metabolism
ko01051: Biosynthesis of ansamycins
ko02040: Flagellar assembly
ko00480: Glutathione metabolism
ko00562: Inositol phosphate metabolism
ko02010: ABC transporters
ko02020: Two−component system
ko03010: Ribosome
ko00121: Secondary bile acid biosynthesis
ko02060: Phosphotransferase system (PTS)
ko03013: RNA transport
ko00362: Benzoate degradation
ko00620: Pyruvate metabolism
ko00040: Pentose and glucuronate interconversions
ko04626: Plant−pathogen interaction
ko00020: Citrate cycle (TCA cycle)
ko05120: Epithelial cell signaling in Helicobacter pylori infection
ko04974: Protein digestion and absorption
ko00281: Geraniol degradation
ko00510: N−Glycan biosynthesis
ko00360: Phenylalanine metabolism
ko01040: Biosynthesis of unsaturated fatty acids
ko00730: Thiamine metabolism
ko00770: Pantothenate and CoA biosynthesis
ko03420: Nucleotide excision repair
ko00140: Steroid hormone biosynthesis
ko00780: Biotin metabolism
ko00100: Steroid biosynthesis
ko00906: Carotenoid biosynthesis
ko00785: Lipoic acid metabolism
ko00540: Lipopolysaccharide biosynthesis
ko00511: Other glycan degradation
ko00531: Glycosaminoglycan degradation
ko00190: Oxidative phosphorylation
ko00130: Ubiquinone and other terpenoid−quinone biosynthesis
ko00760: Nicotinate and nicotinamide metabolism
ko00790: Folate biosynthesis
ko03030: DNA replication
ko00400: Phenylalanine, tyrosine and tryptophan biosynthesis
ko00471: D−Glutamine and D−glutamate metabolism
ko03440: Homologous recombination
d
IL10-/- Control
IL10-/- STAT
Group
ko02020: Two−component system
ko00785: Lipoic acid metabolism
ko00511: Other glycan degradation
ko03060: Protein export
ko03070: Bacterial secretion system
ko04141: Protein processing in endoplasmic reticulum
ko00790: Folate biosynthesis
ko00471: D−Glutamine and D−glutamate metabolism
ko00140: Steroid hormone biosynthesis
ko00564: Glycerophospholipid metabolism
ko00720: Carbon fixation pathways in prokaryotes
ko00120: Primary bile acid biosynthesis
ko04210: Apoptosis
ko00121: Secondary bile acid biosynthesis
ko03013: RNA transport
ko00531: Glycosaminoglycan degradation
ko00190: Oxidative phosphorylation
ko00730: Thiamine metabolism
ko00360: Phenylalanine metabolism
ko00561: Glycerolipid metabolism
ko05150: Staphylococcus aureus infection
ko00281: Geraniol degradation
ko00650: Butanoate metabolism
ko00970: Aminoacyl−tRNA biosynthesis
ko00280: Valine, leucine and isoleucine degradation
ko04974: Protein digestion and absorption
ko00230: Purine metabolism
ko00380: Tryptophan metabolism
ko00290: Valine, leucine and isoleucine biosynthesis
ko00270: Cysteine and methionine metabolism
ko00630: Glyoxylate and dicarboxylate metabolism
e
STAT vs. Control
Wild-Type
IL10-/-
PWY-7111: pyruvate fermentation to isobutanol
ILEUSYN-PWY: L-isoleucine biosynthesis I
VALSYN-PWY: L-valine biosynthesis
PWY-7199: pyrimidine deoxyribonucleosides salvage
PWY-5695: urate biosynthesis
PEPTIDOGLYCANSYN-PWY: peptidoglycan biosynthesis
PWY-6386: UDP-N-acetylmuramoyl-pentapeptide I
PWY-6387: UDP-N-acetylmuramoyl-pentapeptide II
PWY-7371: 1,4-dihydroxy-6-naphthoate biosynthesis
PWY0-1319: CDP-diacylglycerol biosynthesis
PWY-5659: GDP-mannose biosynthesis
SO4ASSIM-PWY: sulfate reduction I
LDA Effect Size (log10)
f
Mean total abundance (RPKM)
p<0.01
Control
STAT
Inoculum
WT
IL10-/-
Superclass
ABC Antibiotic Efflux
Aminoglycoside Nucleotidyltransferase
Aminoglycoside Phosphotransferase
Antibiotic Target
Class A Beta −Lactamase
Class B Beta −Lactamase
Gene Modulating Resistance
Glycopeptide Resistance
MFS Antibiotic Efflux
Other ARG
Quinolone Resistance
RND Antibiotic Efflux
rRNA Methyltransferase
Tetracycline Efflux
Tetracycline Ribosomal Protection

Supplementary Figure 6. Early-life pup metagenome analysis. All panels represent whole genome shotgun sequencing analysis of samples from the two inocula and fecal samples from four 3-week-old mice from each of the four groups. **(a)** Principal Coordinates Analysis ordination of the Bray-Cutis Presence/Absence metric based on metagenomic analysis. MetaCyc pathway abundances generated using HUMAnN2. Significance was determined by PERMANOVA, P =0.001 WT vs. IL10-/- (all), P =0.12 Control vs STAT (WT), P =0.04 Control vs STAT (IL10-/-), P =0.04 Control vs Control, P =0.03 STAT vs STAT. **(b)** Taxa abundances were determined using Metaphlan2 and differences compared using LEfSe (P <0.05; LDA>2). Bars represent the Linear Discriminate Analysis (LDA) Effect Size, between Control and STAT pups. Colors correspond to the taxa increased in STAT pups (red), or increased in the Control pups (blue). **(c-d)** KEGG pathway abundances for each sample were calculated using *HUMAnN2* from shotgun metagenomic data. Significance was determined using the LEfSe algorithm (P <0.05, LDA >2). Intensity signifies the scaled row z-score of a pathway across all samples. **(c)** Differential KEGG pathways in WT and IL10-/- STAT pups. Pathways that are in bold and underlined had the same expression pattern in the same direction in WT and IL10-/- Control pups. **(d)** Differential KEGG pathways in IL10-/- Control and STAT pups. Pathways in bold and underlined had the same expression pattern in the same direction in WT Control and STAT pups. **(e)** MetaCyc metabolic pathways differing significantly between Control and STAT in WT and IL10-/- pups, using LEfSe. The density of the pathways increased in Control pups are in blue, while pathways increased in STAT pups are in red. Function pathway abundances were generated using the microbial metagenomics function tool, HUMAnN2. (P<0.05; LDA >2) **(f)** Using ShortBRED, the metagenomic data from the samples were queried for antibiotic resistance (AR) gene markers. Abundance is shown as Reads per Kilobase per Million mapped reads (RPKM). Differences between pairs were tested for significance by a two-sided t-test. None of the groups was significantly different from their respective inoculum. Mean ± SD abundance within groups for each AR gene Superclass.



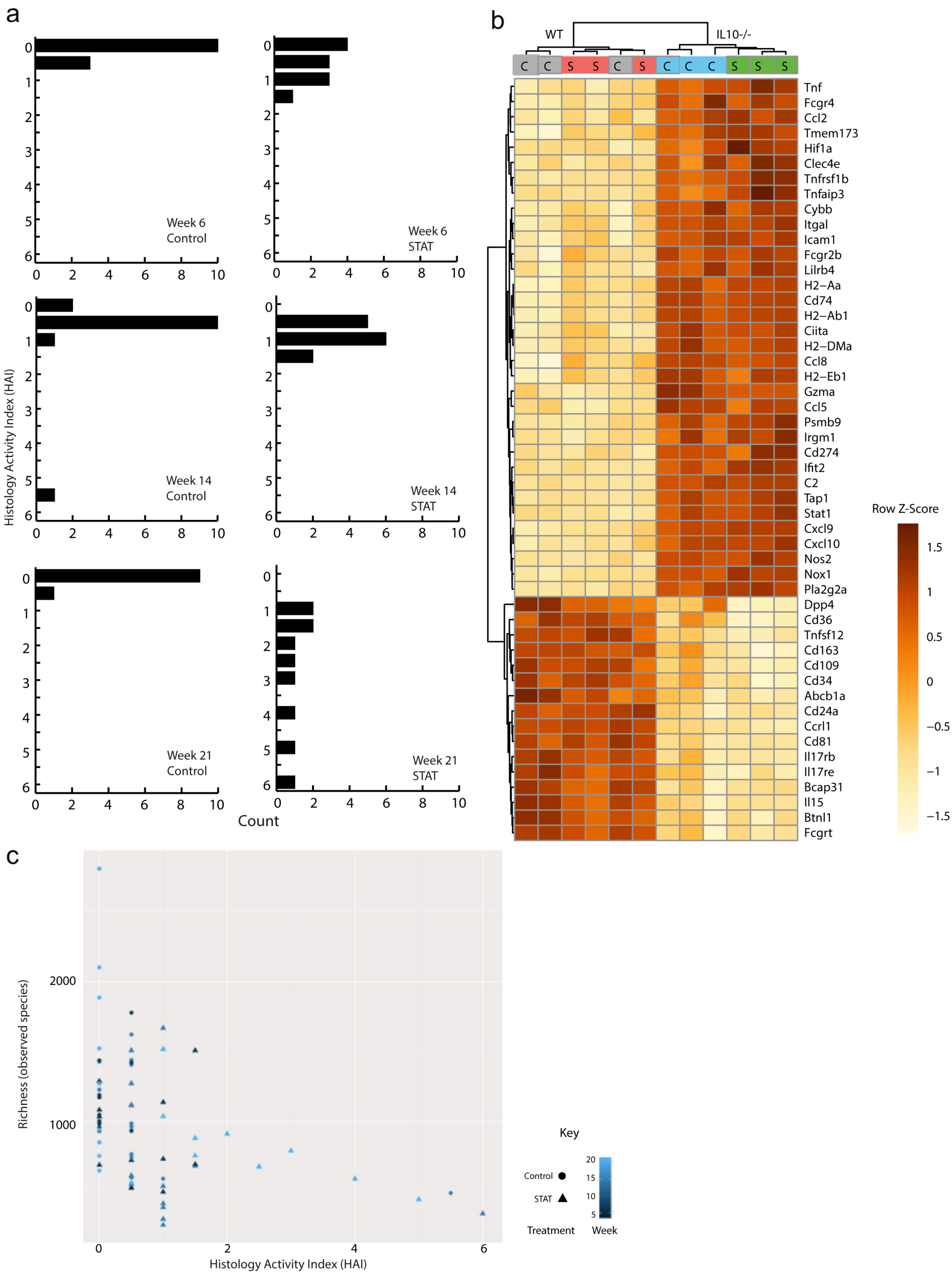
Supplementary Figure 7. Metabolic pathway analysis. (a) Abundance of the SO4SSIM metabolic pathway in metagenomic libraries from the two inocula, and from fecal contents from pups (n=4) at three weeks. Data are normalized using the total sum-scaling method of copies per million (cpm). Boxplots show median with interquartile range. The Sulfate Reduction I (SO4SSIM-PWY) Metacyc pathway significance differences in bacterial pathway abundances are shown in **Supplementary Figure 6e**.

(b) Relative abundance of taxa contributing the SO4ASSIM-pathway: sulfate reduction I, according to source of metagenomic samples. The only classifiable taxon identified by HUMANn2 as contributing genes to this pathway was *Akkermansia mucinophila* (shown in brown) in the STAT inoculum and in three of the four IL10-/- STAT pups at 3 weeks of age.

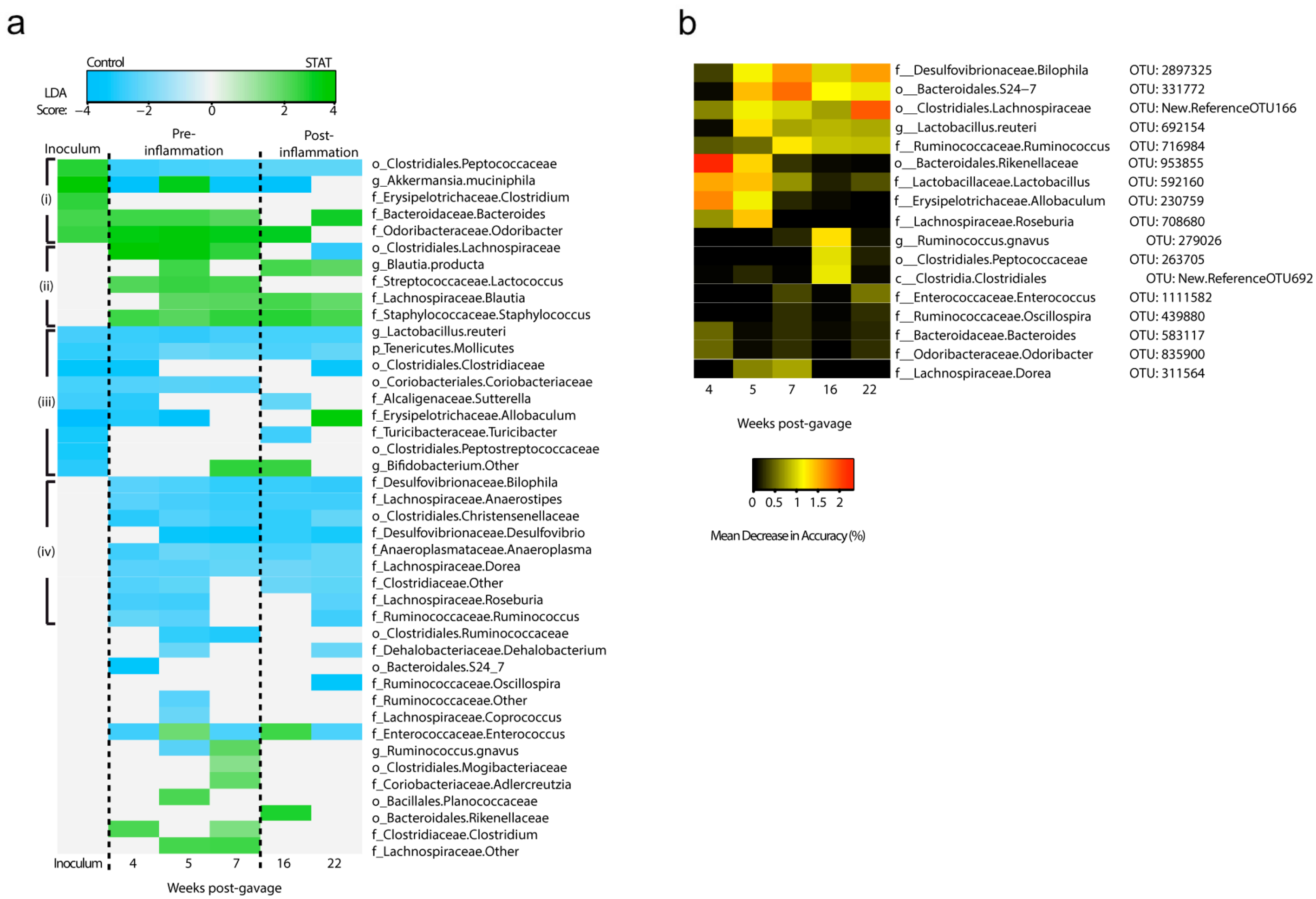


Supplementary Figure 8. Weight and body composition of dams and pups over time. (a) Scale weight over time. Group data were smoothed to the second order (3-neighbor method). Dams (left) all gave birth during the window indicated by gray dashed lines. Pups (right) show males and females of all groups. Indicated significance is between IL10-/- Control females and IL10-/- STAT females. Exact mouse numbers for

IL10-/- pups at each time point are listed in Supplementary Table 1. **(b)** DEXA results showing body composition of pups over time. Top row: bone mineral density (g/cm^2) (left), fat tissue mass (g) (right); bottom row: bone mineral content (g/cm) (left) (indicated significance is between IL10-/- Control females and IL10-/- STAT females), lean tissue mass (g) (right) (indicated significance is between IL10-/- Control females and IL10-/- STAT females). Mean +/- SEM for each group. **(a-b)** Mann-Whitney test * p< 0.05; ** p< 0.01, see Supplementary Table 11.

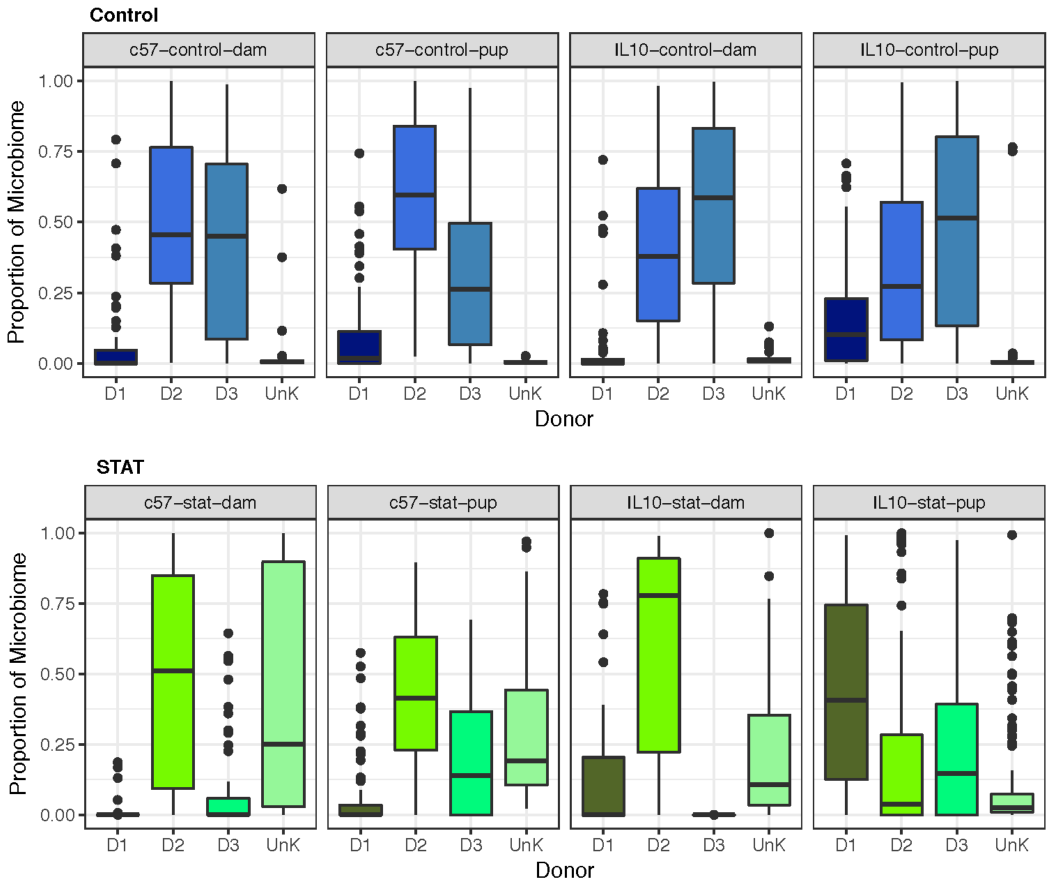

a
Histology Activity Index (HAI)
Week 6
Control
Week 6
STAT
Week 14
Control
Week 14
STAT
Week 21
Control
Week 21
STAT
Count
b
WT
IL10-/-
C C S S C S C C C S S S
Tnf
Fcgr4
Ccl2
Tmem173
Hif1a
Clec4e
Tnfrsf1b
Tnfaip3
Cybb
Itgal
Icam1
Fcgr2b
Lilrb4
H2−Aa
Cd74
H2−Ab1
Ciita
H2−DMa
Ccl8
H2−Eb1
Gzma
Ccl5
Psmb9
Irgm1
Cd274
Ifit2
C2
Tap1
Stat1
Cxcl9
Cxcl10
Nos2
Nox1
Pla2g2a
Dpp4
Cd36
Tnfsf12
Cd163
Cd109
Cd34
Abcb1a
Cd24a
Ccrl1
Cd81
Il17rb
Il17re
Bcap31
Il15
Btnl1
Fcgrt
Row Z-Score
1.5
1
0.5
0
−0.5
−1
−1.5
c
Richness (observed species)
2000
1000
Histology Activity Index (HAI)
Key
Control
STAT
Treatment
Week
20
15
10
5

Supplementary Figure 9. Distribution of histology activity indices in IL10-/- pups and differential gene expression in mouse pup colon. (a) Histology activity indices at weeks 6, 14, and 21 in the IL10-/- pups shown by inoculum status. Using these data, a Proportional Odds model was used to fit a common-slopes cumulative model, which is a parallel lines regression model based on the cumulative probabilities of the response categories rather than on their individual probabilities. Based on this model, STAT effects can be represented as the Odds Ratio (OR) of moving to the next level in the disease score of STAT versus Control. This OR was calculated to be 20.5 (CI 6.5 – 64.1). **(b)** The 50 most differential genes (all with FDR-corrected p value <0.05, see Supplementary Table 12.) expressed in the colon of the WT and IL10-/- pups according to inoculum status (n = 3 in each of the 4 groups) at week 21, measured by the Nanostring nCounter Mouse Immunology Panel v1. **(c)** Microbial richness, based on observed number of species versus colonic histology score. Scores were obtained at 6, 14, and 21 weeks; a total of 71 mice were included in the analysis; 6 animals with missing data were excluded. The intervals around the ORs represent 95% confidence limits. Figure represents a single experiment. For **(a)** and **(c)**, Control n = 13, 14, 10 and STAT n = 11, 13, 10 at weeks 6, 14, and 21 respectively.



Supplementary Figure 10. Differential taxa associated with enhanced pathology in IL10-/- STAT mice. (a) Heatmap summary of LEfSe results showing taxa that significantly differentiate Control microbiota and STAT microbiota in IL10-/- pups. Numbers of mice are as in Supplementary Table 1. The leftmost column compares communities in the inocula while the next five columns compare communities in the IL10-/- pups at different time points. Green indicates taxa that are more abundant in STAT while blue indicates taxa that are more abundant in Control, according to the LDA key shown. **(b)** Random Forest modeling results showing the features that are most predictive of week 21 histology activity index (HAI) at each experimental time point in IL10-/- pups. This model was built from 10 samples in each group (HAI 1+ and HAI <1) at each time point except for 16 weeks post-gavage where n=10 and 8, respectively and

22 weeks post-gavage where n=9 and 10, respectively. The model error was 0.050 at 4 weeks post-gavage, 0.000 at 5 weeks post-gavage, 7 weeks post-gavage, and 16 weeks post-gavage (making the error ratio infinite for these time points), and 0.030 at 22 weeks post-gavage. Baseline error was 0.5 at 4 weeks post-gavage giving an error ratio of 10.0, 0.5 at 5 weeks post-gavage and 7 weeks post-gavage, 0.444 at 16 weeks post-gavage, and 0.474 at 22 weeks post-gavage with an error ratio of 15.8.



Supplementary Figure 11. Source Tracking of the donor microbiota into recipient dams and pups. Each inoculum was comprised of cecal contents from three donor mice. Using the Source Tracker algorithm with machine learning[64], we could assign ancestry of each genus in the recipient groups of dams and pups to a single donor mouse (D1-D3), with a variable extent of non-resolution (unknown, UnK). The imputed sources are shown for the introduced taxa from the Control inoculum (**top panel**) and STAT inoculum (**bottom panel**). Boxplots depict the first quartile, median, and third quartile +/- 1.5 times the interquartile range. Outliers are depicted as black dots. Top panel: n= 100 WT control dams, 70 WT control pups, 50 IL10-/- control dams, 180 IL10-/- control pups. Bottom panel: n= 59 WT STAT dams, 74 WT STAT pups, 50 IL10-/- STAT dams, 135 IL10-/- STAT pups.

Supplementary Tables

Supplementary Table 1. Adonis P values of group pairs across time (β-diversity)

All comparisons with the original inoculum

Group 1	Group 2	Time	n	R^2	Padj
Control Inoculum	STAT Inoculum	0	8	0.61	0.032
Control Inoculum	WT Control dams	1 day post-gavage	11	0.68	0.004
	IL10-/- Control dams		9	0.75	0.008
	WT STAT dams		10	0.53	0.010
	IL10-/- STAT dams		9	0.57	0.011
STAT Inoculum	WT Control dams		11	0.70	0.005
	IL10-/- Control dams		9	0.80	0.010
	WT STAT dams		10	0.54	0.012
	IL10-/- STAT dams		9	0.59	0.010
Control Inoculum	WT Control dams	2 days post-gavage	11	0.62	0.008
	IL10-/- Control dams		9	0.55	0.008
	WT STAT dams		10	0.52	0.005
	IL10-/- STAT dams		9	0.54	0.009
STAT Inoculum	WT Control dams		11	0.68	0.005
	IL10-/- Control dams		9	0.66	0.014
	WT STAT dams		10	0.53	0.008
	IL10-/- STAT dams		9	0.50	0.008
Control Inoculum	WT Control dams	4 days post-gavage	11	0.37	0.003
	IL10-/- Control dams		9	0.51	0.007
	WT STAT dams		10	0.48	0.007
	IL10-/- STAT dams		9	0.50	0.012
STAT Inoculum	WT Control dams		11	0.49	0.007
	IL10-/- Control dams		9	0.65	0.014
	WT STAT dams		10	0.47	0.008
	IL10-/- STAT dams		9	0.40	0.009
Control Inoculum	WT Control dams	4 weeks post-gavage	11	0.36	0.008
	WT Control pups		24	0.25	0.002
	IL10-/- Control dams		9	0.51	0.014
	IL10-/- Control pups		46	0.10	0.004
	WT STAT dams		10	0.42	0.010
	WT STAT pups		19	0.38	0.002
	IL10-/- STAT dams		9	0.45	0.008
	IL10-/- STAT pups		38	0.12	0.003
STAT Inoculum	WT Control dams		11	0.48	0.004
	WT Control pups		24	0.34	0.002
	IL10-/- Control dams		9	0.63	0.014
	IL10-/- Control pups		46	0.16	0.002
	WT STAT dams		10	0.39	0.006
	WT STAT pups		19	0.38	0.002
	IL10-/- STAT dams		9	0.42	0.023
	IL10-/- STAT pups		38	0.09	0.014
Control Inoculum	WT Control dams	5 weeks post-gavage	11	0.39	0.003
	WT Control pups		25	0.28	0.002
	IL10-/- Control dams		9	0.45	0.005
	IL10-/- Control pups		46	0.11	0.002
	WT STAT dams		9	0.47	0.009
	WT STAT pups		19	0.44	0.002
	IL10-/- STAT dams		9	0.45	0.008
	IL10-/- STAT pups		39	0.14	0.002
STAT Inoculum	WT Control dams		11	0.54	0.005
	WT Control pups		25	0.41	0.002
	IL10-/- Control dams		9	0.60	0.014
	IL10-/- Control pups		46	0.23	0.002
	WT STAT dams		9	0.48	0.006
	WT STAT pups		19	0.44	0.003
	IL10-/- STAT dams		9	0.41	0.014
	IL10-/- STAT pups		39	0.11	0.007
Control Inoculum	WT Control dams	7 weeks post-gavage	11	0.37	0.008
	WT Control pups		24	0.33	0.002
	IL10-/- Control dams		9	0.56	0.008
	IL10-/- Control pups		46	0.10	0.002
	WT STAT dams		10	0.49	0.007
	WT STAT pups		18	0.35	0.002
	IL10-/- STAT dams		9	0.77	0.007
	IL10-/- STAT pups		38	0.20	0.002
STAT Inoculum	WT Control dams		11	0.49	0.004
	WT Control pups		24	0.46	0.002
	IL10-/- Control dams		9	0.67	0.014
	IL10-/- Control pups		46	0.20	0.002
	WT STAT dams		10	0.49	0.008
	WT STAT pups		18	0.34	0.003
	IL10-/- STAT dams		9	0.72	0.014
	IL10-/- STAT pups		38	0.15	0.002
Control Inoculum	WT Control pups	16 weeks post-gavage	23	0.39	0.002
	IL10-/- Control pups		31	0.13	0.002
	WT STAT pups		19	0.32	0.002
	IL10-/- STAT pups		26	0.18	0.004
STAT Inoculum	WT Control pups		23	0.48	0.002
	IL10-/- Control pups		31	0.27	0.002
	WT STAT pups		19	0.31	0.002
	IL10-/- STAT pups		26	0.14	0.003
Control Inoculum	WT Control pups	22 weeks post-gavage	24	0.33	0.002
	IL10-/- Control pups		19	0.23	0.003
	WT STAT pups		19	0.35	0.002
	IL10-/- STAT pups		14	0.31	0.004
STAT Inoculum	WT Control pups		24	0.43	0.002
	IL10-/- Control pups		19	0.39	0.002
	WT STAT pups		19	0.34	0.003
	IL10-/- STAT pups		25	0.29	0.002

All comparisons between dams-dams or dams-pups

Group 1	Group 2	Time	n	R^2	Padj
WT Control dams	IL10-/- Control dams	1 day post-gavage	12	0.38	0.002
	WT STAT dams		13	0.27	0.002
	IL10-/- STAT dams		12	0.27	0.003
IL10-/- Control dams	IL10-/- STAT dams		10	0.34	0.010
	WT STAT dams		11	0.37	0.002
WT STAT dams	IL10-/- STAT dams		11	0.17	0.021
WT Control dams	IL10-/- Control dams	2 days post-gavage	12	0.31	0.003
	WT STAT dams		13	0.29	0.004
	IL10-/- STAT dams		12	0.35	0.003
IL10-/- Control dams	IL10-/- STAT dams		10	0.32	0.007
	WT STAT dams		11	0.40	0.007
WT STAT dams	IL10-/- STAT dams		11	0.24	0.021
WT Control dams	IL10-/- Control dams	4 days post-gavage	12	0.25	0.003
	WT STAT dams		13	0.31	0.003
	IL10-/- STAT dams		12	0.33	0.003
IL10-/- Control dams	IL10-/- STAT dams		10	0.34	0.007
	WT STAT dams		11	0.40	0.003
WT STAT dams	IL10-/- STAT dams		11	0.28	0.010
WT Control dams	WT Control pups	4 weeks post-gavage	27	0.05	0.150
	IL10-/- Control dams		12	0.40	0.005
	IL10-/- Control pups		49	0.13	0.002
	WT STAT dams		13	0.30	0.003
	WT STAT pups		22	0.34	0.002
	IL10-/- STAT dams		12	0.36	0.003
	IL10-/- STAT pups		41	0.14	0.002
IL10-/- Control dams	IL10-/- Control pups		47	0.11	0.003
	WT Control pups		25	0.34	0.002
	WT STAT dams		11	0.34	0.002
	WT STAT pups		20	0.38	0.002
	IL10-/- STAT dams		10	0.25	0.007
	IL10-/- STAT pups		39	0.14	0.002
WT STAT dams	WT STAT pups		21	0.09	0.034
	IL10-/- Control pups		48	0.23	0.002
	WT Control pups		26	0.28	0.002
	IL10-/- STAT dams		11	0.18	0.026
	IL10-/- STAT pups		40	0.09	0.003
IL10-/- STAT dams	IL10-/- STAT pups		39	0.06	0.045
	WT STAT pups		20	0.28	0.002
	IL10-/- Control pups		47	0.15	0.002
	WT Control pups		25	0.32	0.002
WT Control dams	WT Control pups	5 weeks post-gavage	28	0.05	0.195
	IL10-/- Control dams		12	0.32	0.003
	IL10-/- Control pups		49	0.16	0.002
	WT STAT dams		12	0.35	0.002
	WT STAT pups		22	0.39	0.002
	IL10-/- STAT dams		12	0.39	0.003
	IL10-/- STAT pups		42	0.17	0.002
IL10-/- Control dams	IL10-/- Control pups		47	0.09	0.002
	WT Control pups		26	0.28	0.002
	WT STAT dams		10	0.38	0.007
	WT STAT pups		20	0.41	0.002
	IL10-/- STAT dams		10	0.31	0.012
	IL10-/- STAT pups		40	0.13	0.002
WT STAT dams	WT STAT pups		20	0.15	0.003
	IL10-/- Control pups		47	0.33	0.002
	WT Control pups		26	0.32	0.002
	IL10-/- STAT dams		10	0.23	0.029
	IL10-/- STAT pups		40	0.16	0.002
IL10-/- STAT dams	IL10-/- STAT pups		40	0.07	0.023
	WT STAT pups		20	0.31	0.002
	IL10-/- Control pups		47	0.26	0.002
	WT Control pups		26	0.36	0.002
WT Control dams	WT Control pups	7 weeks post-gavage	27	0.07	0.021
	IL10-/- Control dams		12	0.49	0.004
	IL10-/- Control pups		49	0.15	0.002
	WT STAT dams		26	0.33	0.002
	WT STAT pups		34	0.27	0.002
	IL10-/- STAT dams		12	0.62	0.003
	IL10-/- STAT pups		41	0.21	0.002
IL10-/- Control dams	IL10-/- Control pups		47	0.20	0.002
	WT Control pups		25	0.48	0.002
	WT STAT dams		11	0.46	0.003
	WT STAT pups		19	0.38	0.002
	IL10-/- STAT dams		10	0.57	0.013
	IL10-/- STAT pups		39	0.27	0.002
WT STAT dams	WT STAT pups		20	0.08	0.174
	IL10-/- Control pups		48	0.35	0.002
	WT Control pups		26	0.41	0.002
	IL10-/- STAT dams		11	0.48	0.003
	IL10-/- STAT pups		40	0.28	0.002
IL10-/- STAT dams	IL10-/- STAT pups		39	0.26	0.002
	WT STAT pups		19	0.40	0.002
	IL10-/- Control pups		47	0.36	0.002
	WT Control pups		25	0.60	0.002

All comparisons between pup groups

Group 1	Group 2	Time	n	R^2	Padj
WT Control pups	WT STAT pups	4 weeks post-gavage	35	0.38	0.002
	IL10-/- Control pups		62	0.24	0.002
	IL10-/- STAT pups		54	0.24	0.002
IL10-/- Control pups	IL10-/- STAT pups		76	0.23	0.002
	WT STAT pups		57	0.41	0.002
WT STAT pups	IL10-/- STAT pups		49	0.23	0.002
WT Control pups	WT STAT pups	5 weeks post-gavage	36	0.45	0.002
	IL10-/- Control pups		63	0.29	0.002
	IL10-/- STAT pups		56	0.29	0.002
IL10-/- Control pups	IL10-/- STAT pups		77	0.29	0.002
	WT STAT pups		57	0.53	0.002
WT STAT pups	IL10-/- STAT pups		50	0.30	0.002
WT Control pups	WT STAT pups	7 weeks post-gavage	34	0.42	0.002
	IL10-/- Control pups		62	0.25	0.002
	IL10-/- STAT pups		54	0.35	0.002
IL10-/- Control pups	IL10-/- STAT pups		76	0.30	0.002
	WT STAT pups		56	0.43	0.002
WT STAT pups	IL10-/- STAT pups		48	0.33	0.002
WT Control pups	WT STAT pups	16 weeks post-gavage	34	0.32	0.002
	IL10-/- Control pups		46	0.34	0.002
	IL10-/- STAT pups		41	0.30	0.002
IL10-/- Control pups	IL10-/- STAT pups		49	0.25	0.002
	WT STAT pups		42	0.41	0.002
WT STAT pups	IL10-/- STAT pups		37	0.23	0.002
WT Control pups	WT STAT pups	22 weeks post-gavage	35	0.28	0.002
	IL10-/- Control pups		35	0.30	0.002
	IL10-/- STAT pups		30	0.33	0.002
IL10-/- Control pups	IL10-/- STAT pups		25	0.27	0.002
	WT STAT pups		30	0.39	0.002
WT STAT pups	IL10-/- STAT pups		25	0.25	0.002

Supplementary Table 2. Statistics for single unweighted UniFrac component distances

		PC3	PC2	PC1	
Time post-gavage	Comparison	P Value	P Value	P Value	Statistical test
0	Inoc Ctl vs Inoc STAT	0.0286	0.8857	0.2	t-test, Mann-whitney two tailed
1 day	WT Ctl dams vs WT STAT dams	0.0015	0.0084	0.0069	one way anova, sidak's multiple comparison test
	WT Ctl dams vs IL Ctl dams	0.0019	0.0097	< 0.0001	
	WT STAT dams vs IL STAT dams	> 0.9999	0.4809	0.0722	
	IL Ctl dams vs IL STAT dams	< 0.0001	0.4532	0.0007	
2 days	WT Ctl dams vs WT STAT dams	0.0064	0.9024	0.029	
	WT Ctl dams vs IL Ctl dams	0.427	0.0016	0.0006	
	WT STAT dams vs IL STAT dams	> 0.9999	0.0044	0.0084	
	IL Ctl dams vs IL STAT dams	0.295	0.8113	0.0075	
4 days	WT Ctl dams vs WT STAT dams	0.0148	0.9998	< 0.0001	
	WT Ctl dams vs IL Ctl dams	0.9419	0.0001	0.9925	
	WT STAT dams vs IL STAT dams	> 0.9999	0.0049	0.0007	
	IL Ctl dams vs IL STAT dams	0.1079	0.6679	0.4319	
5 days	WT Ctl dams vs WT STAT dams	> 0.9999	0.8357	0.0012	t-test, Mann-whitney two tailed
1.5 weeks	IL Ctl dams vs IL STAT dams	0.0079	0.3095	0.0952	t-test, Mann-whitney two tailed
2.5 weeks	IL Ctl dams vs IL STAT dams	0.0079	0.1508	0.0952	t-test, Mann-whitney two tailed
4 weeks	WT Ctl dams vs WT STAT dams	< 0.0001	> 0.9999	< 0.0001	one way anova, sidak's multiple comparison test
	WT Ctl dams vs IL Ctl dams	0.9998	< 0.0001	0.3029	
	WT STAT dams vs IL STAT dams	> 0.9999	< 0.0001	0.9276	
	IL Ctl dams vs IL STAT dams	< 0.0001	0.8426	0.8838	
	WT Ctl pups vs WT STAT pups	< 0.0001	0.1331	< 0.0001	
	WT Ctl pups vs IL Ctl pups	0.689	< 0.0001	0.9883	
	WT STAT pups vs IL STAT pups	0.0002	< 0.0001	< 0.0001	
	IL Ctl pups vs IL STAT pups	< 0.0001	< 0.0001	< 0.0001	
5 weeks	WT Ctl dams vs WT STAT dams	0.0034	0.9496	< 0.0001	
	WT Ctl dams vs IL Ctl dams	0.8912	< 0.0001	> 0.9999	
	WT STAT dams vs IL STAT dams	< 0.0001	< 0.0001	0.1499	
	IL Ctl dams vs IL STAT dams	< 0.0001	> 0.9999	0.0041	
	WT Ctl pups vs WT STAT pups	< 0.0001	0.1566	< 0.0001	
	WT Ctl pups vs IL Ctl pups	0.0002	< 0.0001	0.0177	
	WT STAT pups vs IL STAT pups	< 0.0001	< 0.0001	< 0.0001	
	IL Ctl pups vs IL STAT pups	< 0.0001	0.0564	< 0.0001	
5.5 weeks	IL Ctl dams vs IL STAT dams	0.0159	0.2222	0.0317	t-test, Mann-whitney two tailed
6 weeks	WT Ctl dams vs WT STAT dams	< 0.0001	0.9958	0.0001	one way anova, sidak's multiple comparison test
	WT Ctl dams vs IL Ctl dams	0.1511	< 0.0001	0.9261	
	WT STAT dams vs IL STAT dams	0.0012	< 0.0001	0.0125	
	IL Ctl dams vs IL STAT dams	< 0.0001	0.7424	0.8119	
7 weeks	WT Ctl dams vs WT STAT dams	< 0.0001	> 0.9999	< 0.0001	one way anova, sidak's multiple comparison test
	WT Ctl dams vs IL Ctl dams	0.7571	< 0.0001	0.8859	
	WT STAT dams vs IL STAT dams	< 0.0001	< 0.0001	0.0213	
	IL Ctl dams vs IL STAT dams	< 0.0001	0.6838	0.0568	
	WT Ctl pups vs WT STAT pups	< 0.0001	0.1586	< 0.0001	one way anova, sidak's multiple comparison test
	WT Ctl pups vs IL Ctl pups	< 0.0001	< 0.0001	0.2681	
	WT STAT pups vs IL STAT pups	< 0.0001	< 0.0001	< 0.0001	
	IL Ctl pups vs IL STAT pups	< 0.0001	0.0143	< 0.0001	
16 weeks	WT Ctl pups vs WT STAT pups	< 0.0001	0.4983	< 0.0001	one way anova, sidak's multiple comparison test
	WT Ctl pups vs IL Ctl pups	0.9571	< 0.0001	0.6817	
	WT STAT pups vs IL STAT pups	< 0.0001	< 0.0001	< 0.0001	
	IL Ctl pups vs IL STAT pups	< 0.0001	0.1823	< 0.0001	
22 weeks	WT Ctl pups vs WT STAT pups	< 0.0001	0.2551	< 0.0001	one way anova, sidak's multiple comparison test
	WT Ctl pups vs IL Ctl pups	0.0354	< 0.0001	0.3551	
	WT STAT pups vs IL STAT pups	< 0.0001	< 0.0001	0.1304	
	IL Ctl pups vs IL STAT pups	< 0.0001	0.969	< 0.0001	

Supplementary Table 3. Statistics for α–diversity measurements

		PD_whole_tree	Observed Species	Shannon	
Time post-gavage	Comparison	P Value	P Value	P Value	Statistical test
0	Inoc C vs Inoc S	0.8857	0.6857	0.3429	t-test, Mann-whitney two tailed
1 day	WT Ctl dams vs WT STAT dams	> 0.9999	0.0077	0.0019	one way anova, sidak's multiple comparison test
	WT Ctl dams vs IL10-/- Ctl dams	< 0.0001	0.0015	0.298	
	WT STAT dams vs IL10-/- STAT dams	0.1016	0.9676	0.1556	
	IL10-/- Ctl dams vs IL10-/- STAT dams	< 0.0001	< 0.0001	0.0153	
2 days	WT Ctl dams vs WT STAT dams	0.2001	0.5986	0.1691	one way anova, sidak's multiple comparison test
	WT Ctl dams vs IL10-/- Ctl dams	0.0026	0.0048	0.0081	
	WT STAT dams vs IL10-/- STAT dams	0.0451	0.2102	0.6692	
	IL10-/- Ctl dams vs IL10-/- STAT dams	0.037	0.051	0.0028	
4 days	WT Ctl dams vs WT STAT dams	0.0012	0.0031	0.0124	one way anova, sidak's multiple comparison test
	WT Ctl dams vs IL10-/- Ctl dams	0.9626	0.4813	0.9998	
	WT STAT dams vs IL10-/- STAT dams	0.0764	0.6616	0.5352	
	IL10-/- Ctl dams vs IL10-/- STAT dams	0.8545	0.8229	0.4913	
5 days	WT Ctl dams vs WT STAT dams	0.0047	0.0047	0.0326	t-test, Mann-whitney two tailed
1.5 weeks	IL10-/- Ctl dams vs IL10-/- STAT dams	0.0317	0.0317	0.0079	t-test, Mann-whitney two tailed
2.5 weeks	IL10-/- Ctl dams vs IL10-/- STAT dams	0.0556	0.0317	0.0317	t-test, Mann-whitney two tailed
4 weeks	WT Ctl dams vs WT STAT dams	0.022	0.1519	0.2108	one way anova, sidak's multiple comparison test
	WT Ctl dams vs IL10-/- Ctl dams	0.2379	0.1862	0.9989	
	WT STAT dams vs IL10-/- STAT dams	> 0.9999	0.6902	0.8979	
	IL10-/- Ctl dams vs IL10-/- STAT dams	0.8037	0.7224	0.1186	
	WT Ctl pups vs WT STAT pups	< 0.0001	0.0001	0.2106	one way anova, sidak's multiple comparison test
	WT Ctl pups vs IL10-/- Ctl pups	0.6991	0.0237	0.3457	
	WT STAT pups vs IL10-/- STAT pups	0.1564	0.9995	0.4574	
	IL10-/- Ctl pups vs IL10-/- STAT pups	0.0022	0.0155	0.0189	
5 weeks	WT Ctl dams vs WT STAT dams	< 0.0001	< 0.0001	0.0011	one way anova, sidak's multiple comparison test
	WT Ctl dams vs IL10-/- Ctl dams	0.3904	0.0287	0.9995	
	WT STAT dams vs IL10-/- STAT dams	0.7139	0.9998	0.7904	
	IL10-/- Ctl dams vs IL10-/- STAT dams	0.0609	0.0991	0.0315	
	WT Ctl pups vs WT STAT pups	< 0.0001	< 0.0001	0.0671	one way anova, sidak's multiple comparison test
	WT Ctl pups vs IL10-/- Ctl pups	0.7632	> 0.9999	0.3984	
	WT STAT pups vs IL10-/- STAT pups	0.0002	0.0797	0.2861	
	IL10-/- Ctl pups vs IL10-/- STAT pups	0.0003	0.001	0.0122	
5.5 weeks	IL10-/- Ctl dams vs IL10-/- STAT dams	0.0035	0.0079	0.0079	t-test, Mann-whitney two tailed
6 weeks	WT Ctl dams vs WT STAT dams	0.0202	0.042	0.0008	one way anova, sidak's multiple comparison test
	WT Ctl dams vs IL10-/- Ctl dams	0.9994	0.9755	0.7181	
	WT STAT dams vs IL10-/- STAT dams	0.1609	0.9485	0.859	
	IL10-/- Ctl dams vs IL10-/- STAT dams	0.8231	0.4811	0.1178	
7 weeks	WT Ctl dams vs WT STAT dams	< 0.0001	< 0.0001	0.0006	one way anova, sidak's multiple comparison test
	WT Ctl dams vs IL10-/- Ctl dams	0.0529	0.0011	0.407	
	WT STAT dams vs IL10-/- STAT dams	0.245	0.9937	0.8842	
	IL10-/- Ctl dams vs IL10-/- STAT dams	0.5077	0.3651	0.0109	
	WT Ctl pups vs WT STAT pups	0.0076	0.7981	0.9956	one way anova, sidak's multiple comparison test
	WT Ctl pups vs IL10-/- Ctl pups	0.0002	0.0009	0.0729	
	WT STAT pups vs IL10-/- STAT pups	< 0.0001	0.0072	0.7214	
	IL10-/- Ctl pups vs IL10-/- STAT pups	0.1179	0.3903	0.2698	
16 weeks	WT Ctl pups vs WT STAT pups	0.064	0.7525	0.992	one way anova, sidak's multiple comparison test
	WT Ctl pups vs IL10-/- Ctl pups	0.3065	0.6673	0.6853	
	WT STAT pups vs IL10-/- STAT pups	0.322	0.999	0.0368	
	IL10-/- Ctl pups vs IL10-/- STAT pups	0.0241	0.0993	0.0793	
22 weeks	WT Ctl pups vs WT STAT pups	0.8867	> 0.9999	0.0929	one way anova, sidak's multiple comparison test
	WT Ctl pups vs IL10-/- Ctl pups	0.6053	0.7759	0.0022	
	WT STAT pups vs IL10-/- STAT pups	0.6853	0.3637	0.0056	
	IL10-/- Ctl pups vs IL10-/- STAT pups	0.0159	0.0616	0.0001	

Supplementary Table 4. Statistics for mean consecutive pairwise Jaccard distances

	Comparison	P value
Dams	WT Ctl vs WT STAT	0.0012
	IL10-/- Ctl vs IL10-/- STAT	>0.9999
	WT Ctl vs IL10-/- Ctl	0.2733
	WT STAT vs IL10-/- STAT	>0.9999
Pups	WT Ctl vs WT STAT	0.0054
	IL10-/- Ctl vs IL10-/- STAT	0.0003
	WT Ctl vs IL10-/- Ctl	0.3609
	WT STAT vs IL10-/- STAT	0.8726

Supplementary Table 5. Statistics for colonic inflammation metrics

Test	Time	Group 1	Group 2	P value
HAI	Week 6	IL10-/- STAT	IL10-/- Control	0.2187
	Week 14			0.8767
	Week 21	WT Control	WT STAT	>0.9999
			IL10-/- Control	>0.9999
		IL10-/- STAT	IL10-/- Control	<0.0001
			WT STAT	<0.0001
Calprotectin	Week 7	WT Control	WT STAT	0.9998
			IL10-/- Control	0.0736
		IL10-/- STAT	IL10-/- Control	0.0016
			WT STAT	0.3268
	Week 14	WT Control	WT STAT	>0.9999
			IL10-/- Control	0.1342
		IL10-/- STAT	IL10-/- Control	<0.0001
			WT STAT	<0.0001
	Week 19	WT Control	WT STAT	>0.9999
			IL10-/- Control	0.9953
		IL10-/- STAT	IL10-/- Control	0.0038
			WT STAT	0.0020
Lipocalin-2	Week 7	WT Control	WT STAT	>0.9999
			IL10-/- Control	0.1988
		IL10-/- STAT	IL10-/- Control	0.3184
			WT STAT	0.0050
	Week 14	WT Control	WT STAT	>0.9999
			IL10-/- Control	0.8539
		IL10-/- STAT	IL10-/- Control	0.2316
			WT STAT	0.0429
	Week 19	WT Control	WT STAT	>0.9999
			IL10-/- Control	0.5927
		IL10-/- STAT	IL10-/- Control	0.0223
			WT STAT	0.0012
TNFa	Week 21	IL10-/- STAT	IL10-/- Control	0.7613
IFNg	Week 21	IL10-/- STAT	IL10-/- Control	0.0311

Supplementary Table 6. Statistics for differentially expressed genes in IL10-/- pup colon (Control vs STAT)

Gene	P value (FDR-corrected)
Ifit2	0.0463
Il13ra1	0.0538
Blnk	0.0705
Nox1	0.0996
Hif1a	0.0778
Lif	0.0631
Il1rn	0.0631
Irf7	0.0251
Tnfaip3	0.0536
Ccl25	0.0224
Cfd	0.0833
Dpp4	0.0108
Maf	0.0116
Il6ra	0.0705
Masp2	0.0154
Tbx21	1.22E-06
Il2	0.0022
Cxcl12	0.0108
Cd109	0.0538
Cd163	0.0011
Il18	0.0631
Ccr8	0.0173
Cd36	0.0022

Supplementary Table 7. Statistics for percent shared OTUs

Time post-gavage	Group 1	Group 2	P value (dams)	P value (pups)
1 day	Control (both genotypes)	STAT (both genotypes)	0.3458	--------
	WT (both treatments)	IL10-/- (both treatments)	0.0176	--------
2 days	Control (both genotypes)	STAT (both genotypes)	0.0037	--------
	WT (both treatments)	IL10-/- (both treatments)	0.0366	--------
4 days	Control (both genotypes)	STAT (both genotypes)	0.0661	--------
	WT (both treatments)	IL10-/- (both treatments)	0.0007	--------
4 weeks	Control (both genotypes)	STAT (both genotypes)	0.2688	0.0017
	WT (both treatments)	IL10-/- (both treatments)	0.0018	2.45E-06
5 weeks	Control (both genotypes)	STAT (both genotypes)	0.8732	2.50E-05
	WT (both treatments)	IL10-/- (both treatments)	1.71E-07	4.37E-11
6 weeks	Control (both genotypes)	STAT (both genotypes)	0.5108	--------
	WT (both treatments)	IL10-/- (both treatments)	0.0068	--------
7 weeks	Control (both genotypes)	STAT (both genotypes)	0.8567	4.08E-07
	WT (both treatments)	IL10-/- (both treatments)	1.68E-05	1.02E-06
16 weeks	Control (both genotypes)	STAT (both genotypes)	--------	0.0002
	WT (both treatments)	IL10-/- (both treatments)	--------	3.25E-06
22 weeks	Control (both genotypes)	STAT (both genotypes)	--------	0.015
	WT (both treatments)	IL10-/- (both treatments)	--------	4.64E-08

Time post-gavage	Group 1	Group 2	P value (dam:pup ratio)
4 weeks	WT Control	WT STAT	0.0002
		IL10-/- Control	0.0635
	IL10-/- STAT	IL10-/- Control	0.3164
		WT STAT	0.0002
5 weeks	WT Control	WT STAT	0.0596
		IL10-/- Control	0.3562
	IL10-/- STAT	IL10-/- Control	0.3264
		WT STAT	0.0002
7 weeks	WT Control	WT STAT	0.0084
		IL10-/- Control	0.4875
	IL10-/- STAT	IL10-/- Control	0.0635
		WT STAT	0.0077
16 weeks	WT Control	WT STAT	0.0127
		IL10-/- Control	0.6948
	IL10-/- STAT	IL10-/- Control	0.6277
		WT STAT	0.1420
22 weeks	WT Control	WT STAT	0.8028
		IL10-/- Control	0.6558
	IL10-/- STAT	IL10-/- Control	0.0045
		WT STAT	0.0088

Supplementary Table 8. Statistics for g_*Akkermansia* relative abundance

	Group	P value
Inoculum	Ctl vs STAT	0.0286
Dams vs pups	WT Ctl	0.0889
	WT STAT	0.0001
	IL10-/- Ctl	0.0005
	IL10-/- STAT	0.0160

Supplementary Table 9. Statistics for median Jaccard index

	Comparison	P value
Inoculum vs. Pup	WT Ctl vs WT STAT	3.70E-09
	IL10-/- Ctl vs IL10-/- STAT	8.70E-06
	WT Ctl vs IL10-/- Ctl	0.2
	WT STAT vs IL10-/- STAT	8.70E-06
Inoculum vs. Dam	WT Ctl vs WT STAT	1.90E-06
	IL10-/- Ctl vs IL10-/- STAT	4.70E-02
	WT Ctl vs IL10-/- Ctl	0.744
	WT STAT vs IL10-/- STAT	2.70E-06
Dam vs. Dam	WT Ctl vs WT STAT	1.90E-06
	IL10-/- Ctl vs IL10-/- STAT	4.40E-05
	WT Ctl vs IL10-/- Ctl	2.20E-05
	WT STAT vs IL10-/- STAT	6.10E-05
Dam vs. Pup	WT Ctl vs WT STAT	3.00E-08
	IL10-/- Ctl vs IL10-/- STAT	6.70E-10
	WT Ctl vs IL10-/- Ctl	4.40E-08
	WT STAT vs IL10-/- STAT	2.00E-04
Pup vs. Pup	WT Ctl vs WT STAT	2.10E-05
	IL10-/- Ctl vs IL10-/- STAT	8.53E-01
	WT Ctl vs IL10-/- Ctl	0.00031
	WT STAT vs IL10-/- STAT	9.83E-01

Supplementary Table 10. Statistics for litter and mouse variation over time

	Time	Group 1	Group 2	P value
Dam	1 day post-gavage	WT Control	WT STAT	5.23E-04
			IL10-/- Control	6.67E-04
		IL10-/- STAT	IL10-/- Control	1.11E-06
			WT STAT	0.332
	Pup day 21 (4 weeks post-gavage)	WT Control	WT STAT	6.35E-07
			IL10-/- Control	0.482
		IL10-/- STAT	IL10-/- Control	5.95E-4
			WT STAT	0.198
	Pup week 6 (7 weeks post-gavage)	WT Control	WT STAT	9.00E-7
			IL10-/- Control	1.11E-5
		IL10-/- STAT	IL10-/- Control	0.0709
			WT STAT	1.26E-11
Pup	Pup day 21	WT Control	WT STAT	1.755E-12
			IL10-/- Control	0.96
		IL10-/- STAT	IL10-/- Control	8.03E-44
			WT STAT	0.229
	Pup week 6	WT Control	WT STAT	6.79E-16
			IL10-/- Control	0.259
		IL10-/- STAT	IL10-/- Control	1.02E-17
			WT STAT	5.27E-10
	Pup week 21	WT Control	WT STAT	7.56E-21
			IL10-/- Control	0.352
		IL10-/- STAT	IL10-/- Control	1.45E-11
			WT STAT	0.213
Dam	All samples per animal	WT Control	WT STAT	5.56E-3
			IL10-/- Control	0.0413
		IL10-/- STAT	IL10-/- Control	0.0166
			WT STAT	0.143
Pup	All samples per animal	WT Control	WT STAT	2.4E-6
			IL10-/- Control	0.981
		IL10-/- STAT	IL10-/- Control	1.35E-03
			WT STAT	0.0351

Supplementary Table 11. Statistics for weight and body composition over time

Weeks post-gavage	Comparison	Sex	P value (weight)	P value (BMD)	P value (BMC)	P value (FTM)	P value (LTM)
5	WT Control vs WT STAT	Male	0.4344	0.4618	0.1823	0.2428	0.2110
		Female	0.2428	0.3676	0.2635	0.2198	0.1471
	IL10-/- Control vs IL10-/- STAT	Male	0.3418	0.8367	0.9747	0.3664	0.4661
		Female	0.2291	0.7081	0.7283	0.5838	0.3034
9	WT Control vs WT STAT	Male	0.5890	0.7344	0.4967	0.2775	0.6038
		Female	0.1101	0.3001	0.0727	0.2198	0.2635
	IL10-/- Control vs IL10-/- STAT	Male	0.5547	0.7436	0.5316	0.1495	0.6833
		Female	0.5834	0.1149	0.1725	0.5409	0.8508
13	WT Control vs WT STAT	Male	0.4587	0.8577	0.5490	0.4967	0.4967
		Female	0.0931	0.3312	0.5887	0.1320	0.3939
	IL10-/- Control vs IL10-/- STAT	Male	0.7364	0.3687	0.1295	0.7648	0.2648
		Female	0.9740	0.8691	0.0482	0.8508	0.4239
17/18	WT Control vs WT STAT	Male	0.1564	0.2871	0.7197	0.1333	0.7197
		Female	0.1471	0.8971	0.3132	0.5622	0.1471
	IL10-/- Control vs IL10-/- STAT	Male	0.4589	0.4848	0.9372	0.5887	1.0000
		Female	0.0629	0.6993	0.0829	0.7972	0.0120
21	WT Control vs WT STAT	Male	0.0205	0.6461	0.3154	0.2428	0.2428
		Female	0.0328	0.8540	0.6354	0.0559	0.0879
	IL10-/- Control vs IL10-/- STAT	Male	0.8182	0.9372	0.8182	1.0000	0.8182
		Female	0.0070	0.6882	0.0190	0.6993	0.0070

Supplementary Table 12. Statistics for differentially expressed genes in pup colon (WT vs IL10-/-)

Gene	P value (FDR-corrected)
Tnf	5.31E-19
Fcgr4	1.28E-16
Ccl2	1.36E-16
Tmem173	3.44E-15
Hif1a	4.04E-17
Clec4e	7.22E-09
Tnfrsf1b	4.05E-20
Tnfaip3	8.53E-14
Cybb	2.45E-19
Itgal	7.63E-23
Icam1	2.28E-14
Fcgr2b	2.67E-15
Lilrb4	2.79E-15
H2-Aa	7.63E-29
Cd74	2.74E-63
H2-Ab1	2.92E-51
Ciita	1.90E-28
H2-DMa	1.07E-27
Ccl8	1.09E-19
H2-Eb1	2.56E-15
Gzma	2.10E-17
Ccl5	6.33E-14
Psmb9	2.84E-23
Irgm1	7.49E-14
Cd274	9.42E-13

Gene	P value (FDR-corrected)
Ifit2	1.87E-44
C2	2.39E-29
Tap1	1.70E-40
Stat1	5.08E-28
Cxcl9	2.21E-63
Cxcl10	9.37E-27
Nos2	5.35E-43
Nox1	4.54E-90
Pla2g2a	3.88E-44
Dpp4	4.00E-13
Cd36	2.39E-17
Tnfsf12	5.92E-13
Cd163	6.61E-21
Cd109	9.78E-25
Cd34	2.00E-12
Abcb1a	4.37E-15
Cd24a	2.00E-15
Ccrl1	1.42E-34
Cd81	1.20E-13
Il17rb	4.02E-16
Il17re	2.70E-15
Bcap31	1.71E-12
Il15	8.08E-18
Btnl1	4.17E-13
Fcgrt	5.08E-13

Supplementary Notes

OTU Transfer

An advantage of pooling donor samples to create a single inoculum for each treatment group is that we could track which bacteria from each original donor mouse colonized both genotypes of mice. Using SourceTracking[64], we provide evidence that all three donor mice contributed genera to the communities colonizing each group of pups and dams with only a single exception in which only two donors were identified. (**Supplementary Figure 11**). In general, for the Control inoculum, the source for nearly all genera could be identified, whereas for the STAT inoculum, there was somewhat less identification by source donor, reflecting in part the extensive intragroup variation in the recipients of the STAT inoculum (**Supplementary Figure 11**).

We hypothesized that there would be a bottleneck with the transfer of the inoculum to the new hosts, and the figures showing a-diversity (**Supplementary Figure 3b-d**) clearly indicate that for the dams. This is shown as a loss of richness (PD and Observed Species) and of evenness (Shannon Index). All of the data are constrained by a finite sequencing depth; with loss of evenness, dominant taxa are crowding out the less abundant ones. Over time, evenness is restored and the previously less abundant organisms apparently bloom and are more detectable. In particular, in the dams that received the STAT inocula, there is dominance by a single taxon *Akkermansia* (Verrucomicrobiae) with a mean abundance of 34.5 ± 34.1%. Interestingly, in 3 of 4 comparisons, *Akkermansia* was significantly more dominant in the dams than in the pups (**Supplementary Figure 4c**).

Metagenomic Pathways

We identified a single pathway that was significantly enriched in the STAT-recipient pups in relation to the Control-recipients in the IL10-/- background (**Supplementary Figure 7**). This pathway also was significantly enriched in the IL10-/- STAT mice compared to the WT STAT mice, further indicating that this pathway is associated with the mice with the most severe disease. Upon inspection, this pathway was found to only have increased abundance in the IL10-/- STAT mice and in the STAT inoculum, suggesting it may have originated in the donor material that the dams received. The pathway is SO4ASSIM-PWY or Sulfate Reduction I (**Supplementary Figure 7, panel a**), which is involved in the reduction of sulfate into hydrogen sulfide. The literature indicates that H_2S can cause colonic dysfunction.[30] We also found that *Akkermansia muciniphila* was the only taxon significantly contributing to the Sulfate Reduction pathway in our model (**panel b**). In genomic studies, *Akkermansia* has been shown to contain the glycosulfatases needed to contribute to the pathway.[31] This finding is consistent with the taxonomic studies, based on 16S analyses, that show the high abundance of Verrucomicrobiae in the inoculum (**Supplementary Figure 1a**), and in the STAT compared to Control dams and pups, especially in the IL10-/- background (**Supplementary Figure 4c**). In an analysis of the taxa associated with the IL10-/- recipient pups, *Akkermansia* again was significantly increased in the STAT pups in the inoculum and at week 5 post-gavage. This work is consistent with a recent publication identifying H_2S production as an important pathway in both early-onset CD in children and in an experiment mouse model.[65] Finally, in another experimental model in IL10-/- mice, another H_2S-producing organism, *Bilophila wadsworthia,* was implicated in colitis pathogenesis.[66] In our experiments, *B. wadsworthia* was inversely related to disease, suggesting that it is the H_2S production, rather than the specific taxon, that is the critical factor.

Microbiota Transfer into IL10-/- Mice

From the LEfSe analyses, we can compare the representation of taxa from each inoculum across the two genotypes (WT and IL10-/-). Although this shows differences in relative abundances, it provides clues about the taxonomic differences. Analysis of the β-diversity of the dam and pup communities after inoculation shows mouse genotype-related differences along PC2, and treatment-related differences along PC3 (**Supplementary Figures 2, 3**). For both dams and pups, receipt of the antibiotic-perturbed microbiota resulted in significantly higher intra-group variability compared to controls for most time points, except for IL10-/- dams at the pup week 6 time point, as examined with t-tests (fdr-corrected) (**Supplementary Figure 5, panel a**). However, the intra-group variation among the IL10-/- mice was not greater than for the WT mice, regardless of treatment. We also tested within-animal differences (**panel b**). For both dams and pups, receipt of the antibiotic-perturbed inoculum resulted in significantly higher within-mouse variability. There were no consistent significant differences of within-mouse variability between WT and IL10-/- mice, regardless of treatment.

Microbiota Richness and Tissue Injury

Next, we asked whether in this experimental model, the α-diversity of the bacterial community correlated with, or could be used as a predictor of, tissue injury in the IL10-/- mice that develop colitis spontaneously. We did so because there is a growing literature that patients with IBD have lower α-diversity than in healthy controls.[24,50] Based on a proportional odds model correcting for time and treatment, we estimated the relationship between microbiota composition and histology. The microbial richness (observed species) for samples obtained at the same time as histology had an inverse relationship with the histology score (**Supplementary Figure 9c**). An Odds Ratio (OR) of 18.0 (CI 1.4-228.7) was found for a 10-fold decrease in the observed number of species. Thus, there was an 18-fold greater chance of moving to a higher score in histology when a sample had a 10 times lower number of observed species. This study shows a significant inverse relationship of α-diversity with histological score, which is both consistent with the human data, and provides a model for future studies.

Supplementary Discussion

Microbial Succession

Studies of the early life development of the microbiota indicate that the dominant microbiota in the early days of life, during obligate lactation, are derived from the vaginal microbiota.[67] Subsequently, there is a dramatic loss in bacterial diversity resulting from lactation, followed by a shift in dominance to microbiota resembling that of the maternal gut. This nonlinear succession pattern follows the introduction of solid food coinciding with an increased diversity of anaerobes. Further reports have shown a succession of the microbiota in human children that parallels these phenomena.[44,68,69]

In our study, from the specimens from the WT mice, whose mothers received the Control microbiota, we observed a specific succession of decreased abundance of Firmicutes, specifically Clostridia and Bacilli, corresponding to an increase in Erysipelotrichi (**Supplementary Figure 1c**). This succession has been observed in the literature, such as a study on early microbiota perturbation in which the unperturbed microbiota of control pups showed a dramatic increase in Erysipelotrichaceae coinciding with a decrease in Clostridiaceae and Lactobacillus starting at 8 weeks of age extending through 30 weeks of ages.[2] The time window of these experiments align well with the succession observed in this current study. Decreasing Firmicutes and constant Bacteroidetes ratios among control pups was also observed in a recent study of murine peripartum antibiotic exposure.[40]

Antimicrobial Resistance

An increasing body of work is providing evidence that the microbiota of laboratory-raised mice is abnormal compared to wild free-ranging mice,[70–72] probably due to the constraints of chow diets, trace antibiotics in the diet over generations, chlorinated water, and high dose antibiotic treatments over the generations. As such, it is not surprising that antibiotic resistance genes are present in the microbiota of the mice that inherited the 'normal' microbiota. The low dose antibiotics, by reducing bacterial richness, may have led to loss of resistance genes that were unrelated to the penicillin selection. The metagenomic analysis provides evidence for this since the major differential AR superclasses are independent of beta-lactamases, but rather involve tetracycline resistance. An alternate but related hypothesis is that in the context of one antibiotic selection, carriage of unrelated AR genes confers fitness cost to its host and is selected against, which may be particularly important in clonal species. A third observation is that when the antibiotic selection ends (as in the STAT inoculum), resistance genes have a fitness cost that is not counterbalanced by positive selection, and thus strains in the STAT inoculum with these genes are outcompeted.[73–75]

Supplementary References

64. Knights, D. *et al.* Bayesian community-wide culture-independent microbial source tracking. *Nat Meth* **8,** 761–763 (2011).

65. Mottawea, W. *et al.* Altered intestinal microbiota–host mitochondria crosstalk in new onset Crohn's disease. *Nat. Commun.* **7,** 13419 (2016).

66. Devkota, S. *et al.* Dietary-fat-induced taurocholic acid promotes pathobiont expansion and colitis in Il10-/- mice. *Nature* **487,** 104–108 (2012).

67. Pantoja-Feliciano, I. G. *et al.* Biphasic assembly of the murine intestinal microbiota during early development. *ISME J.* **7,** 1112–1115 (2013).

68. Bäckhed, F. *et al.* Dynamics and stabilization of the human gut microbiome during the first year of life. *Cell Host Microbe* **17,** 690–703 (2015).

69. Yassour, M. *et al.* Natural history of the infant gut microbiome and impact of antibiotic treatment on bacterial strain diversity and stability. *Sci. Transl. Med.* **8,** 343ra81 LP-343ra81 (2016).

70. Andersson, D. I. & Hughes, D. Microbiological effects of sublethal levels of antibiotics. *Nat Rev Micro* **12,** 465–478 (2014).

71. Weldon, L. *et al.* The gut microbiota of wild mice. *PLoS One* **10,** 1–15 (2015).

72. Kreisinger, J., Čížková, D., Vohánka, J. & Piálek, J. Gastrointestinal microbiota of wild and inbred individuals of two house mouse subspecies assessed using high-throughput parallel pyrosequencing. *Mol. Ecol.* **23,** 5048–5060 (2014).

73. Bolotin, E. & Hershberg, R. Gene loss dominates as a source of genetic variation within clonal pathogenic bacterial species. *Genome Biol. Evol.* **7,** 2173–2187 (2015).

74. Sandegren, L. & Andersson, D. I. Bacterial gene amplification: implications for the evolution of antibiotic resistance. *Nat Rev Micro* **7,** 578–588 (2009).

75. Andersson, D. I. & Hughes, D. Persistence of antibiotic resistance in bacterial populations. *FEMS Microbiol. Rev.* **35,** 901–911 (2011).