Supplemental Methods

Bioinformatics

To improve compression and efficiency of downstream steps, reads were first sorted using *clumpify* from the *bbtools* package v.38.96[35]. FastQC v.11.9 and MultiQC v.1.13a were used to produce read quality statistics[36,37]. Reads were deduplicated using fastp v.23.2 (parameters: -dedup, dup_calc_accuracy=6) and then filtered and trimmed with the same tool (parameters: length_required=50, n_base_limit=5, --low_complexity_filter, complexity_threshold=7, -detect_adapter_for_pe, --correction, --cut_front, --cut_tail, cut_window_size=4, cut_mean_quality=20, --overrepresentation_analysis)[38]. Reads from the phiX viral genome (sequencing internal control spike-in) were removed with bbduk v.38.96[35] (parameters: k=31 hdist=1), and reads mapping to the human (GRCh38) or mouse (GRCm39) genomes with BBMap v.38.96[35] (parameters: fast=t) were also removed.

Reads were depth normalized with BBnorm v.38.96[35] (parameters: target=100 mindepth=2 bits=16 prefilter ecc=t) and assembled per-sample with MEGAHIT[39] v.1.2.9 using the metasensitive preset. A co-assembly was also produced; reads from all samples were also concatenated and depth normalized with BBnorm v.38.96[35] (parameters: target=100 mindepth=2 bits=16 prefilter) and assembled with MEGAHIT[39] v.1.2.9 using the meta-sensitive preset.

To create coverage profiles for genomic binning, quality controlled reads from all samples were mapped to contiguous sequences (contigs) from each assembly using coverM[40] *contig* v.6.1 and the Minimap2[41] aligner v.2.24-r1122. Six different binning tools were used to cluster contigs into putative genomic bins: MetaBAT 2[42] v.2.15-8, MaxBin 2[43] v.2.2.7, CONCOCT[44] v.1.1.0, SemiBin[45] v.1.0.3, MetaDecoder[46] v.1.0.13, and VAMB[47] v.3.0.8. R scripts available at github.com/Geo-omics/GVHD_mouse_metagenomes were used to format inputs to the binners and standardize binner outputs. CheckM[48] v.1.1.3 was used to assess completeness and contamination of all bins. The taxonomy of bins was determined with GTDBtk[49] v.2.1.1 using the GTDB[50,51] release 207_v2

database. DasTool[52] v.1.1.4 was used to dereplicate and optimize bins and drep[53] v.3.3.0 was used to create a set of dereplicated bins from each assembly. Drep was additionally run on bins from all samples to determine a dereplicated set of bins representative of all samples. The abundance of these bins was determined with coverM[40] *genome* v.6.1 by mapping quality controlled reads from all samples to each bin; all community abundance information refers to these results unless otherwise indicated. A phylogenetic tree of the representative bin set was produced with GToTree[54] v.1.7.08 using the pre-built Bacteria and Archaea marker HMMs.

Prodigal[55] v.2.6.3 was used to find genes in each bin. KofamScan[56] v.1.3.0 was used to assign KEGG ortholog IDs (KO) to translated genes predicted by Prodigal. Prodigal and KofamScan were similarly used to annotate the complete metagenomic assembly from each sample. Traits including oxygen tolerance were predicted for each bin using Traitar3[57] v.3.0.1, a Python3 implementation of Traitar[58]. Additional functional annotations were determined for each bin using DRAM[59] v.1.4.6 and Bakta[60] v.1.6.1. Pathway completion in each representative bin was calculated from Bakta annotations using Pathway Tools[61] (Dec 6, 2022 build) via mpwt[62] v.0.8.3.

Community composition and functional profiles were also generated from reads.
Community composition profiles were produced with Kraken2[63] v.2.1.2 and Bracken[64] v.2.6.1 (species level) by first annotating Bacterial and Archael reads with a GTDB[51] r202 database built with Struo2[65], unannotated reads were then annotated using a RefSeq database (benlangmead.github.io/aws-indexes/k2). To reduce spurious hits, only taxa with ≥ 3 minimumhit-groups and ≥ 150 distinct minimizers were retained; the --report-minimizer-data option was used to obtain these values similar to the unique k-mer counts produced by KrakenUniq[66]; results were combined with R scripts. Functional profiles for each sample were determined with the Kraken community composition profiles and Humann3[67] v.3.0.0, with a GTDB database built with Struo2[65].

Factor analysis was conducted with MEFISTO via the MOFA2 R package v. 1.14.0[71] integrating clr-transformed taxonomic and KEGG ortholog abundance tables, and metabolite abundances normalized per sample by total peak area and centered and scaled per feature. 10 factors were calculated, and spatial and time covariates were specified to account for known spatial and temporal dependencies between the samples. Additionally, replicate sample groups were specified to better account for heterogeneity of patterns across groups. Factors were assessed for relationships with experimental variables, and feature weights were used to determine taxa, metabolites, and gene associations.

Statistical analysis

The Vegan R package[68] v.2.6-4 was used to compute diversity statistics: alpha (within-sample) diversity metrics were calculated with the *diversity* function and beta diversity (between-sample) diversity was calculated with the *vegdist* function and the Bray-Curtis diversity metric.

PERMANOVA statistics were calculated with the *adonis2* function and *metaMDS* was used to calculate nonmetric multidimensional scaling ordinations. T-tests were calculated with the *t.test* function of the R *stats* package v. 4.3.2.

Differential abundance statistics were calculated using the Aldex2 package[69,70] with either t-tests used to compare two groups, or generalized linear models (~transplant type * days after transplant * gut section) used to simultaneously evaluate additional aspects of the experimental design. In the glm, the following were used as the reference levels: syngeneic (treatment type), day 7 (days after transplant), and transverse colon (gut section).

Significance from t-tests are also displayed in several figures (*: p <= 0.05; **: p <= 0.01; ***: p <= 0.001; ****: p <= 0.0001), calculated in R via the *compare_means* function of the ggpubr package v.0.6.0.

Enriched KEGG pathways were evaluated by gene set enrichment analysis (GSEA) in the Gage R package v. 2.52.2 with rankings determined by ALDEx2 coefficients.

Intestinal Organoid Culture

In brief, small intestines were collected and flushed with ice-cold PBS, cut into ~2mm pieces and incubated in 10 mM EDTA at 4 °C for 40 minutes. Tissues were transferred to cold PBS. Crypts were mechanically separated from tissues by pipetting up and down, transferring supernatant through 70uM filter then repeating processing with new PBS five times. Purified crypts were isolated, counted then resuspended in Matrigel (Corning 356231 growth factor reduced) and culture medium (STEMCELL Technologies, Cat# 06005) (1:1) at 2-5 crypts per µl. Intestinal crypts in 50µl droplets were plated onto a flat bottom of Corning 48-well plates and allowed to solidify for 15-30 minutes in a 37°C incubator. Then, 250ul of crypt culture medium supplement with 100 U/ml penicillin and 100µg/ml streptomycin was added to each well. Wells were treated with either normal culture medium, 5uM of D-phenyllactic acid (Sigma, 376906) or culture media with HCl pH-matched to 5uM of D-phenyllactic acid. Growth and budding of organoids were assessed on day 5-6 of proliferation. Crypt culture medium was changed every two to days and maintained at 37°C in fully humidified chambers containing 5% CO2.

RT-qPCR and qPCR

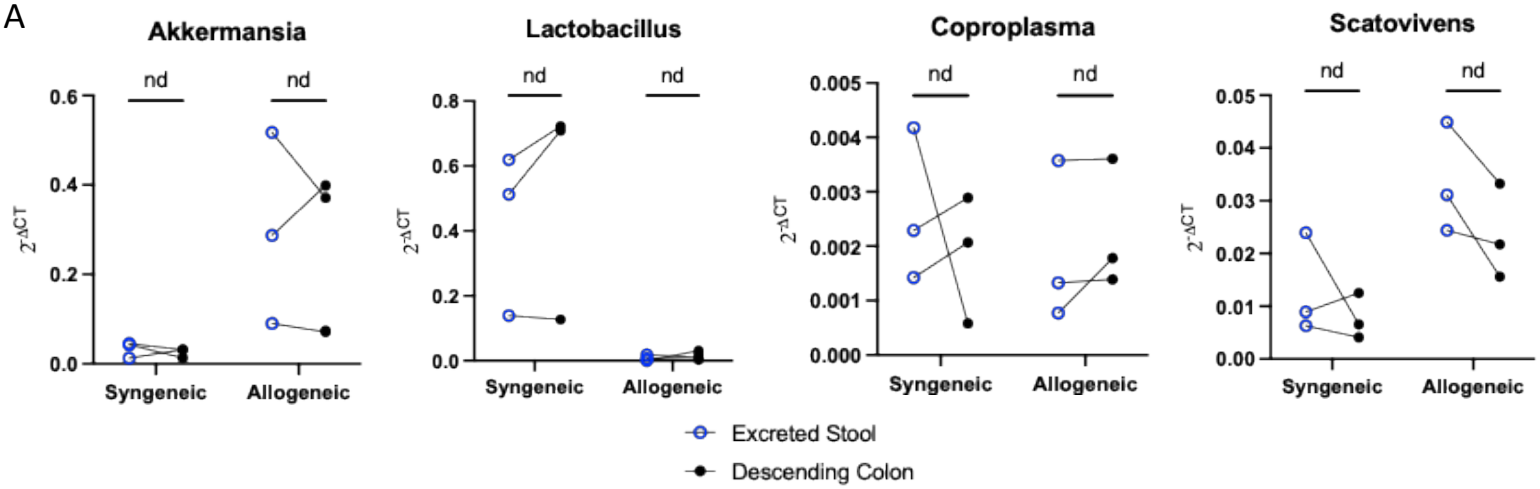
Spleens and descending colons were excised from mice 7 days post-transplant. For analysis of excreted stool, stool pellets were collected from mice and snap frozen. Stool from descending colon was collected from descending colon segment and snap frozen. Spleen and descending colon tissue (without stool) was snap frozen. DNA was isolated from stool using the QIAamp PowerFecal Pro DNA Kit (Qiagen, # 51804) and RNA was isolated from spleen and descending colon tissue using RNeasy Plus Mini Kit (Qiagen, 74134). qPCR step was performed with iTaq Universal SYBR® Green One-Step Kit (Bio-Rad, # 1725151). Primers for qPCR analysis of select taxa were built using the DECIPHER R package v. 3.0.0[72,73] and 16S rRNA gene sequences from the Genome Taxonomy Database r207. Primers are listed in table below. The relative abundance bacteria was calculated using the $2^{-\Delta Ct}$ method[74,75], where the ΔCt is calculated with the following formula: ΔCt = Ct (taxa) – Ct (16S rRNA V6-V8).

Name	Sequence
16S rRNA V6-V8	F: 5’-CAACGCGAAGAACCTTACC-3’ R: 5’-GACGGGCGGTGAGTACA-3’
Akkermansia	F: 5'-GGCGGGTTCCTGGACATT-3' R: 5'-ACTTCATCCCAGTTACCAGTCTCA-3'
Lactobacillus	F: 5'-CGGATTTATTGGGCGTAAAGCGAG-3' R: 5'-TAGCTGCAGCACTGAGAGG-3'
Coproplasma	F: 5'-AAGGTCTTCGGATTGTAAAGTTCTTT-3' R: 5'-CGCCCAGTCATTCCGGATAAC-3'
Scatovivens	F: 5'-GGAGGAAACTCTGACGCAGTG-3' R: 5'-CGAGGGATTTCACAACCAACTT-3'
IFNg	F : 5’-CAGCAACAGCAAGGCGAAAAAGG-3’ R : 5’-TTTCCGCTTCCTGAGGCTGGAT-3’
IL-17a	F : 5’-TTTAACTCCCTTGGCGCAAAA-3’

	R : 5’-CTTTCCCTCCGCATTGACAC-3’
IL-1b	F : 5’- AAGAGCTTCAGGCAGGCAGTATCA-3’ R : 5’-TGCAGCTGTCTAGGAACGTCA-3’
PPIA	F : 5’-CCCTGGCACATGAATCCTGG-3’ R : 5’-GAGCTGTTTGCAGACAAAGTTC-3’

Supp Fig 1

A



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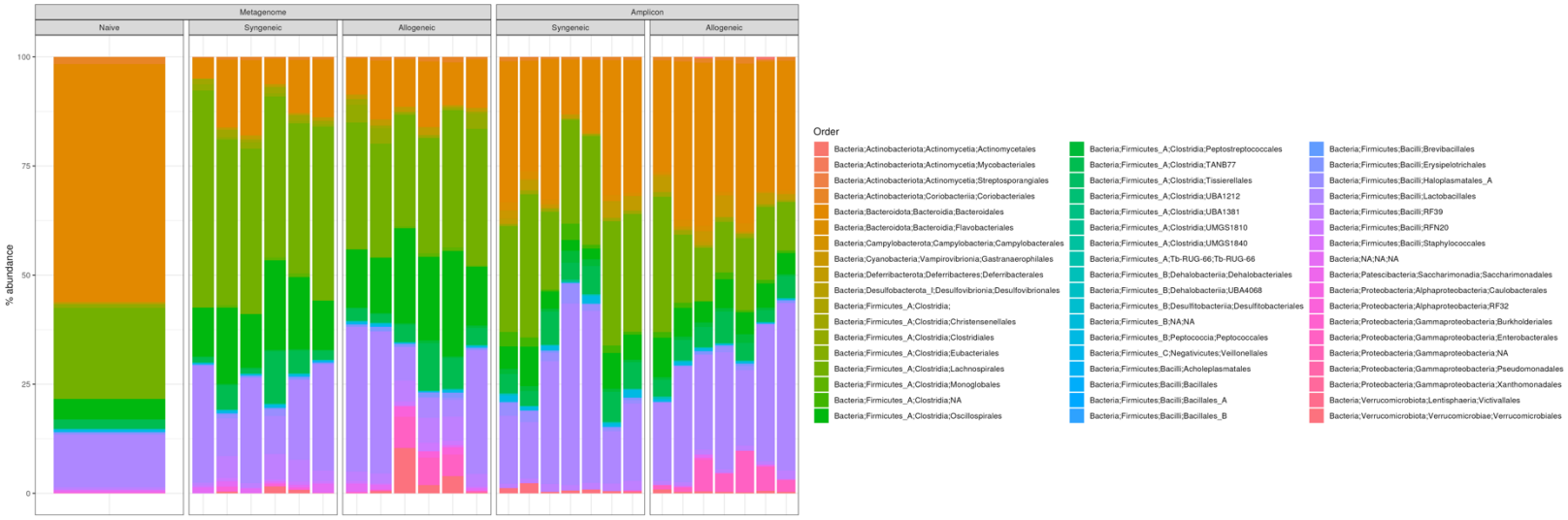

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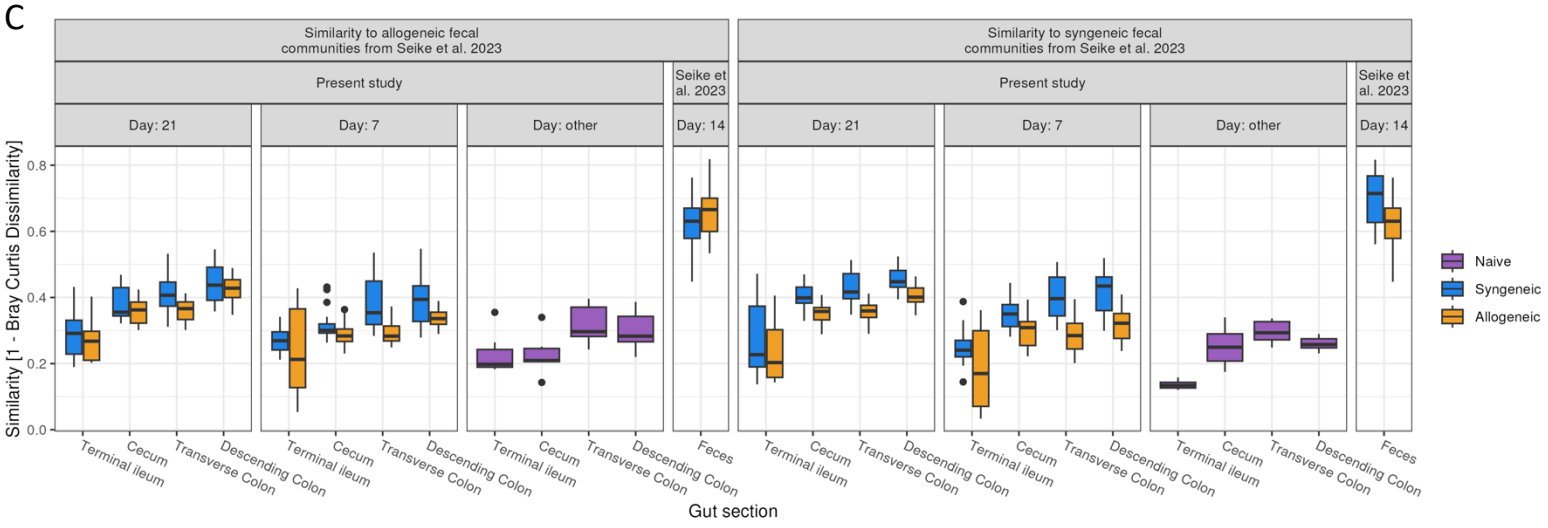

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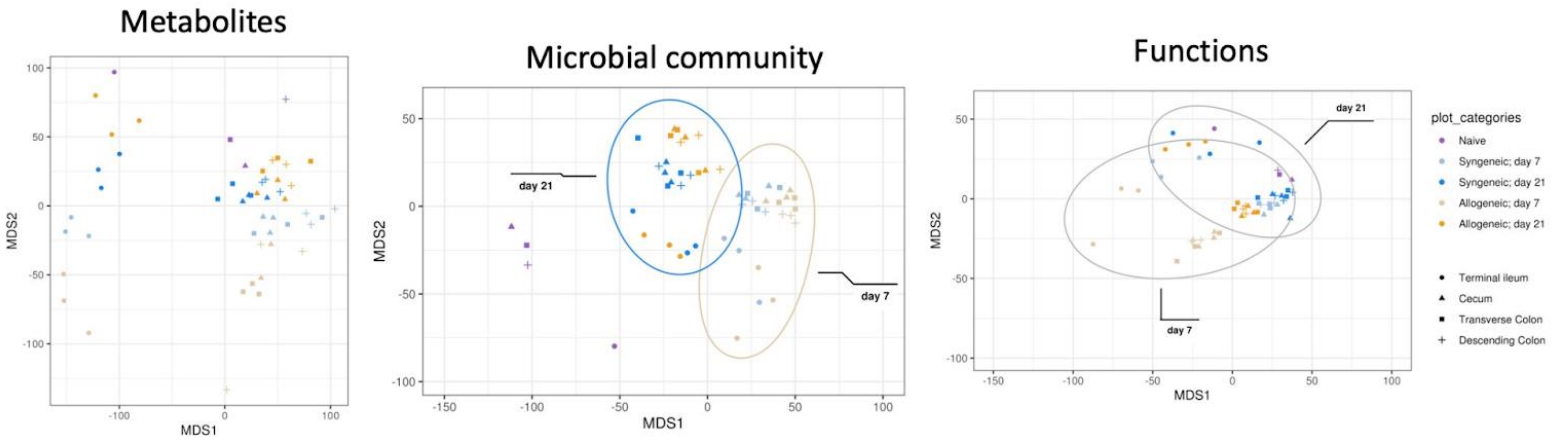


Variable	R^2	F	Pr(>F)
gut_section	0.26329	6.823	0.001
days_after_transplant	0.10303	4.005	0.001
transplant_type	0.04391	3.414	0.001
days_after_transplant: transplant_type	0.03671	2.854	0.003
Residual	0.55306		
Total	1.00000		

Variable	R^2	F	Pr(>F)
gut_section	0.25745	11.2864	0.001
transplant_type	0.16477	10.8352	0.001
days_after_transplant	0.18782	24.7019	0.001
transplant_type:days_after_transplant	0.05540	7.2862	0.001
Residual	0.33456		
Total	1.00000		

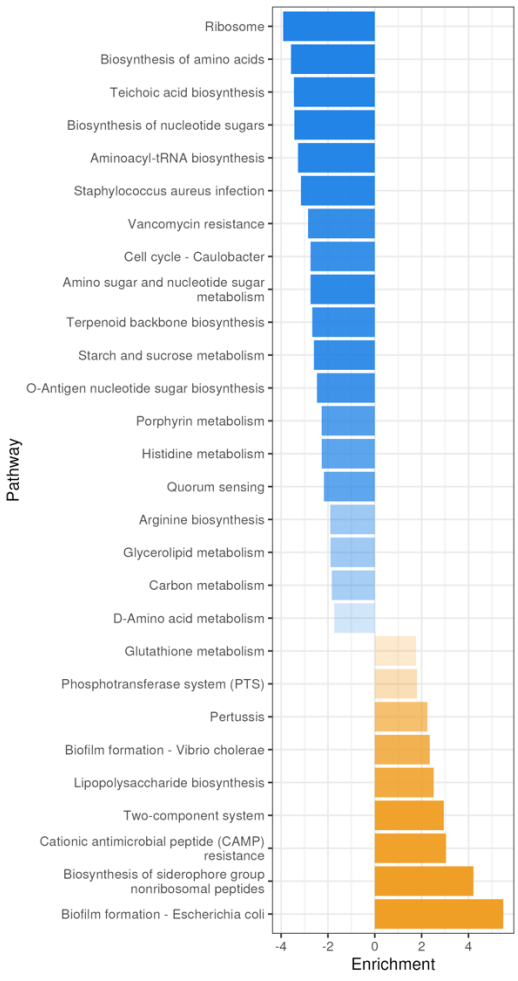
Variable	R^2	F	Pr(>F)
gut_section	0.31603	15.8687	0.001
days_after_transplant	0.16940	12.7590	0.001
transplant_type	0.18087	27.2453	0.001
days_after_transplant: transplant_type	0.04161	6.2683	0.001
Residual	0.29209		
Total	1.00000		

Supplemental Figure 1.

qPCR was performed on excreted stool or stool from descending colon from animals 7 days post-transplant (A). Plots show no significant differences in Akkermansia, Lactobacillus, Coproplasma or Scatovivens levels in between excreted stool and descending colon (A). Community compositions were compared in between metagenomic sequencing of gut samples from this study, with 16S rRNA gene sequencing of stool samples from the same GVHD model system but different harvest timepoint (14 days) published in Seike et al. (2023).(B) Similarity between communities was quantified and plotted (C). We note that the community composition was determined with different sequencing methods in the present study [shotgun metagenomics] and Seike et al. (2023) [16S rRNA gene amplicons], which limits direct comparison and likely explains much of the observed difference. Furthermore, these samples were harvested at different timepoints post-transplant: 21 days and 14 days for shotgun metagenomics and 16s rRNA sequencing respectively (C). NMDS plots based on Bray-Curtis dissimilarity (D; top) and PERMANOVA analysis (D; bottom) of metabolite, microbial community, and KEGG orthologs (function) show which factors are associated with between-sample (β) diversity (D). Gut section explained the most variance for all three feature types in the PERMANOVA analyses (D; bottom), with the second-most variance attributable to days after transplant for metabolites and functions, and transplant type for the microbial community. Experimental factors explained less of the variance in metabolites (45%) than microbial community composition (67%) or functions (71%).

Supp Fig 2

A


Day 7
Ribosome
Biosynthesis of amino acids
Teichoic acid biosynthesis
Biosynthesis of nucleotide sugars
Aminoacyl-tRNA biosynthesis
Staphylococcus aureus infection
Vancomycin resistance
Cell cycle - Caulobacter
Amino sugar and nucleotide sugar metabolism
Terpenoid backbone biosynthesis
Starch and sucrose metabolism
O-Antigen nucleotide sugar biosynthesis
Porphyrin metabolism
Histidine metabolism
Quorum sensing
Arginine biosynthesis
Glycerolipid metabolism
Carbon metabolism
D-Amino acid metabolism
Glutathione metabolism
Phosphotransferase system (PTS)
Pertussis
Biofilm formation - Vibrio cholerae
Lipopolysaccharide biosynthesis
Two-component system
Cationic antimicrobial peptide (CAMP) resistance
Biosynthesis of siderophore group nonribosomal peptides
Biofilm formation - Escherichia coli
Pathway
-4
-2
0
2
4
Enrichment

B

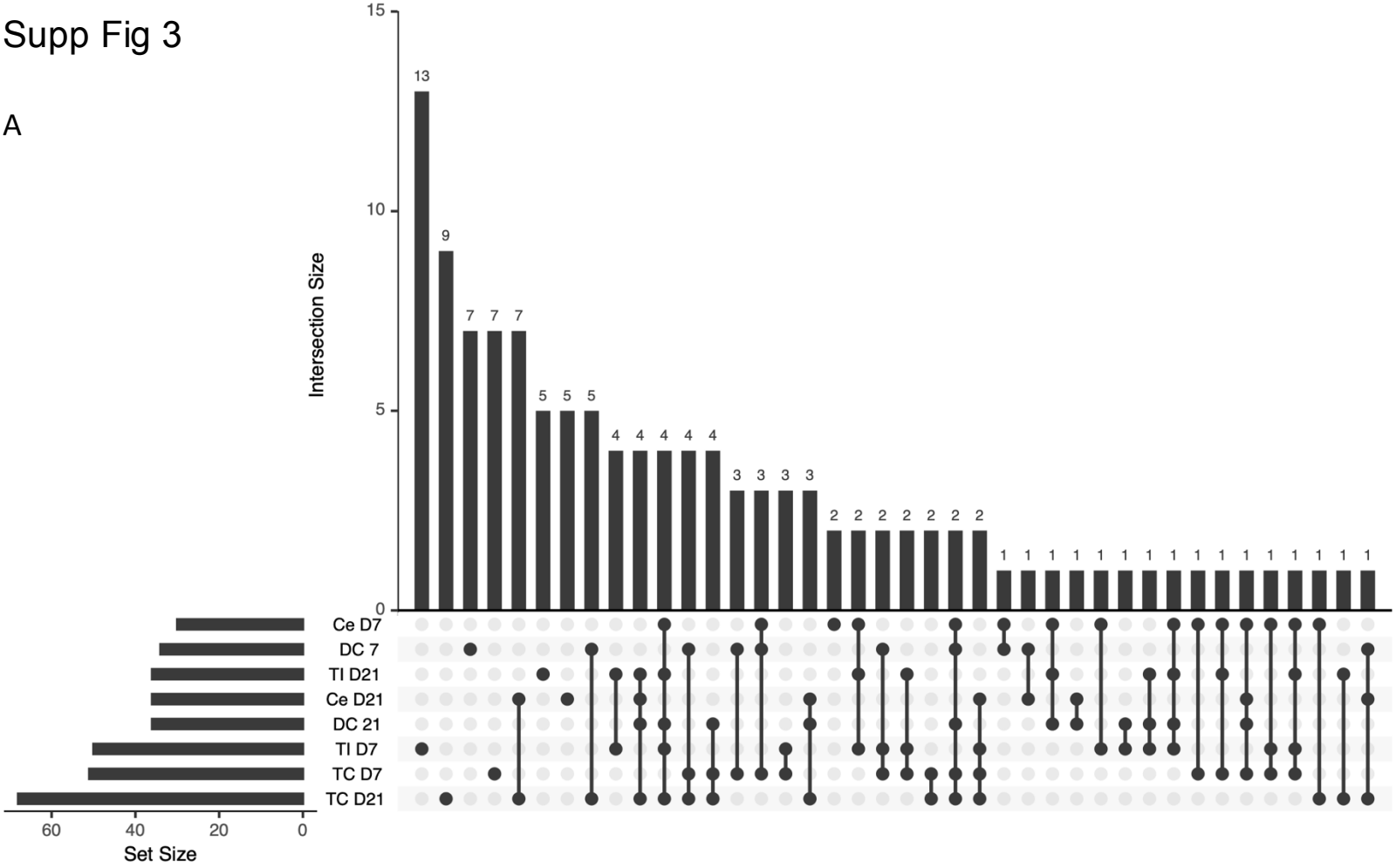

Day 21
Biosynthesis of amino acids
Biosynthesis of cofactors
Biosynthesis of nucleotide sugars
Histidine metabolism
Microbial metabolism in diverse environments
Pentose and glucuronate interconversions
Arginine biosynthesis
One carbon pool by folate
Lysosome
Quorum sensing
Porphyrin metabolism
Degradation of aromatic compounds
O-Antigen nucleotide sugar biosynthesis
Glycine, serine and threonine metabolism
Vancomycin resistance
Cysteine and methionine metabolism
Carbon metabolism
Ribosome
Amino sugar and nucleotide sugar metabolism
Aminoacyl-tRNA biosynthesis
D-Amino acid metabolism
Aminobenzoate degradation
Biosynthesis of siderophore group nonribosomal peptides
Pertussis
Cationic antimicrobial peptide (CAMP) resistance
Two-component system
Biofilm formation - Escherichia coli
Pathway
-4
-2
0
2
4
Enrichment
Transplant type
Syngeneic
Allogeneic
p.val
0.01
0.02
0.03
0.04

Supplemental Figure 2.

Gene set enrichment analysis (GSEA) was used to evaluate enriched KEGG pathways (B) in syngeneic and allogeneic transplant samples 7 (A) and 21 (B) days post-transplant for all samples.

Supp Fig 3

A



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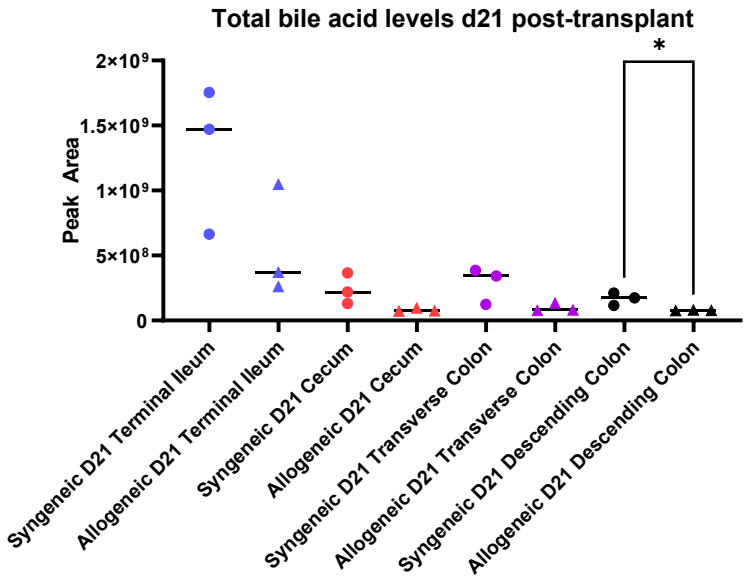

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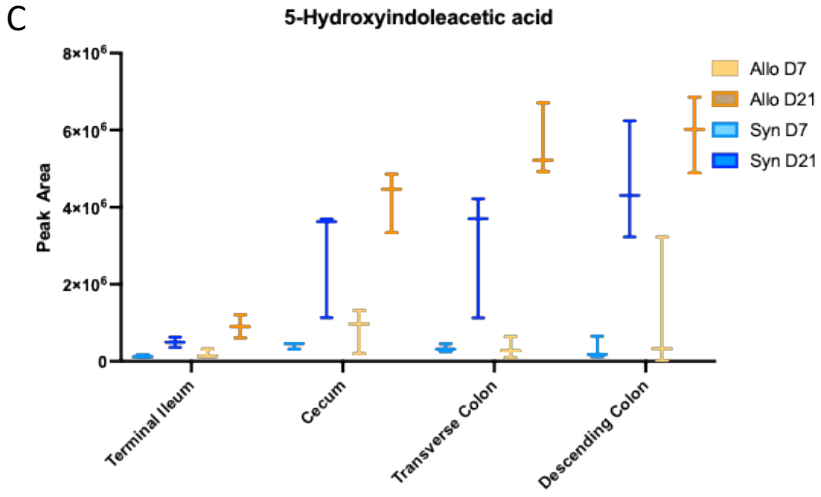

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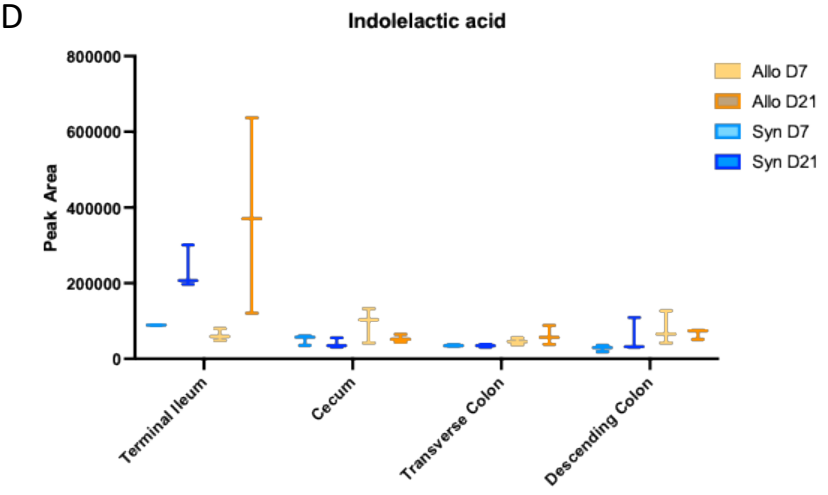


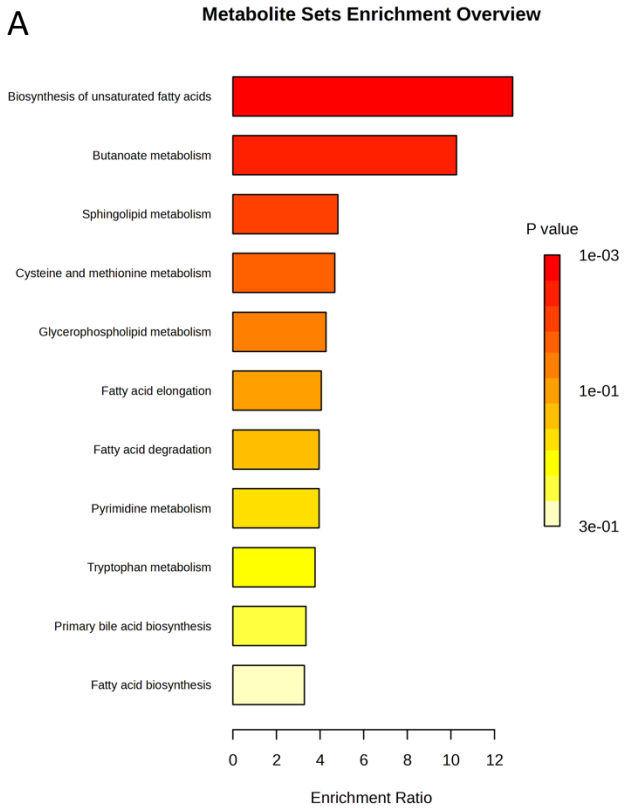
Supplemental Figure 3.

The differentially abundant metabolites were identified by comparing syngeneic and allogeneic experimental groups within each gut location and timepoint post-transplant (7 or 21 days posttransplant) using the Metaboanalyst platform (Methods). The overlap in significantly differentially abundant metabolites within each gut location and timepoint post-transplant were compared to evaluate coordinated changes (A); in this upset plot points indicate group overlap, vertical bars indicate the number of shared metabolites, and horizontal bars indicate the number of metabolites differentially abundant metabolites between syngeneic and allogeneic samples in each gut location at each timepoint (A). The peak area of bile acids observed on day 21 post- transplant is shown for each gut location and treatment condition (B). The peak area of indole metabolites 5-Hydroxyindoleacetic acid (5-HIAA) (C) and indolelactic acid (D) observed on day 7 and day 21 post-transplant is shown for each gut location and treatment condition (C-D).

Supp Fig 4

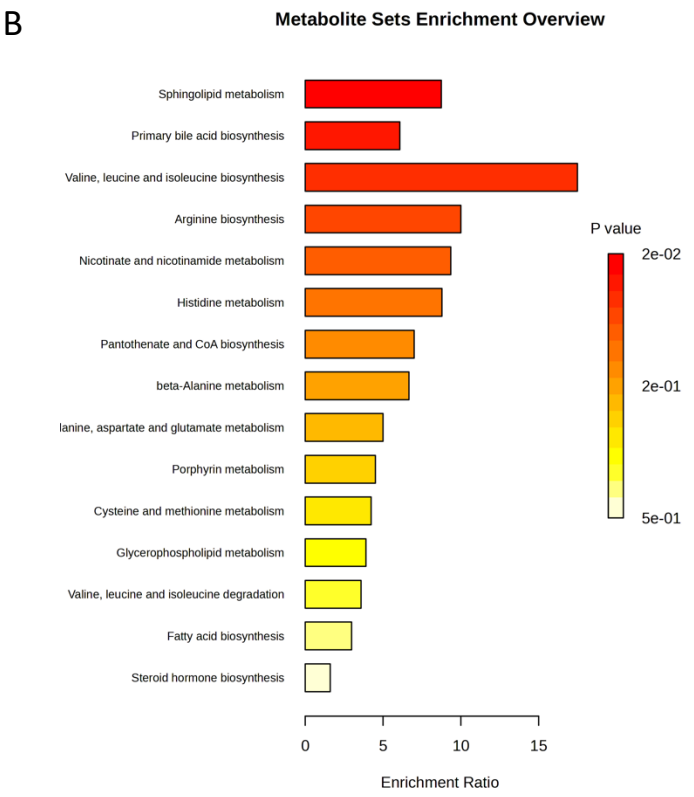
Terminal Ileum
Day 7

A



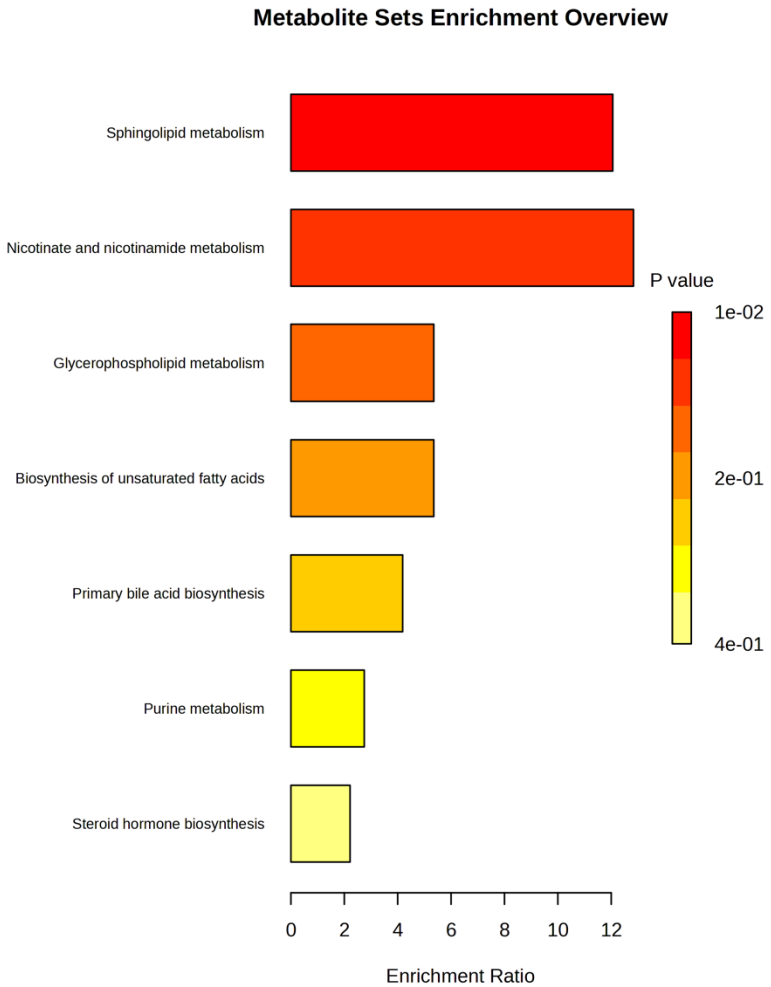
Terminal Ileum
Day 21

B



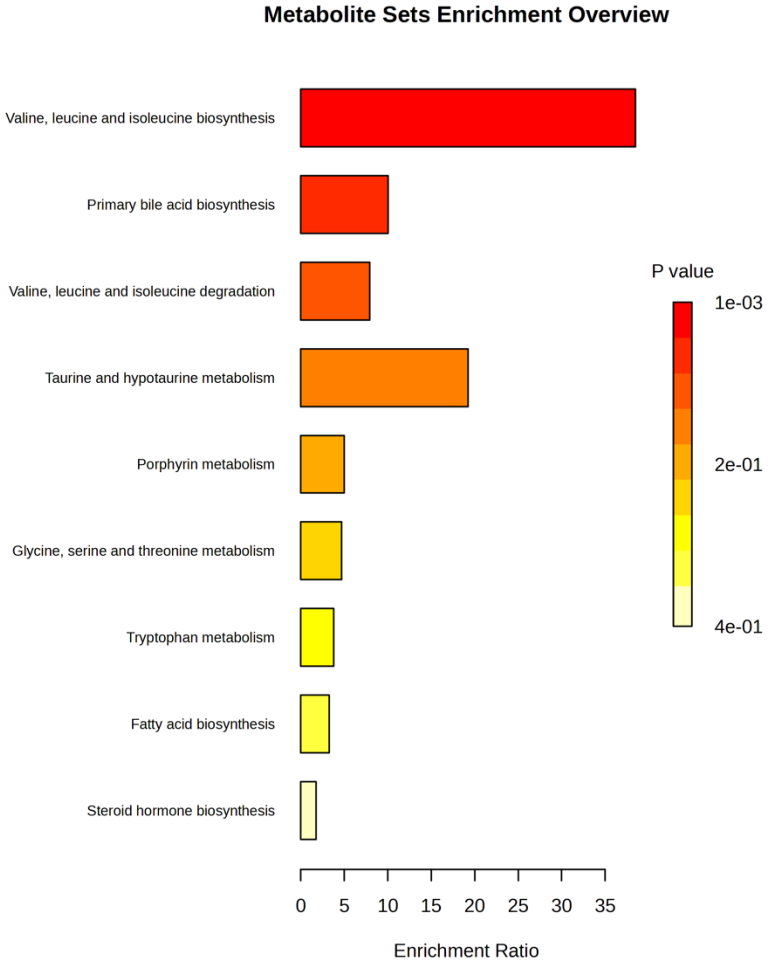
C

Cecum
Day 7



D

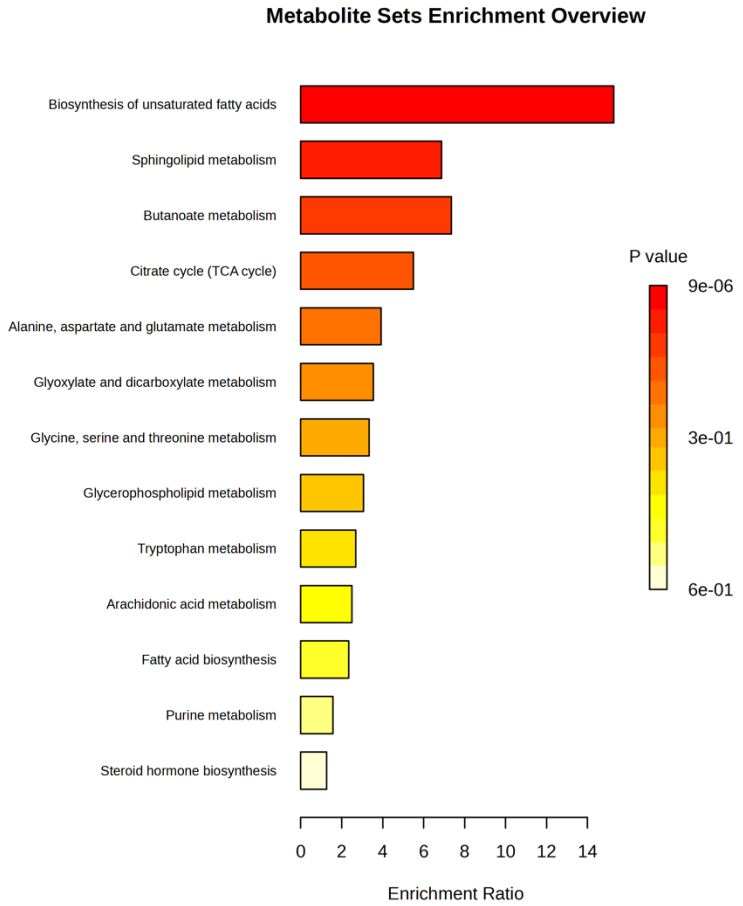
Cecum
Day 21



Supp Fig 4

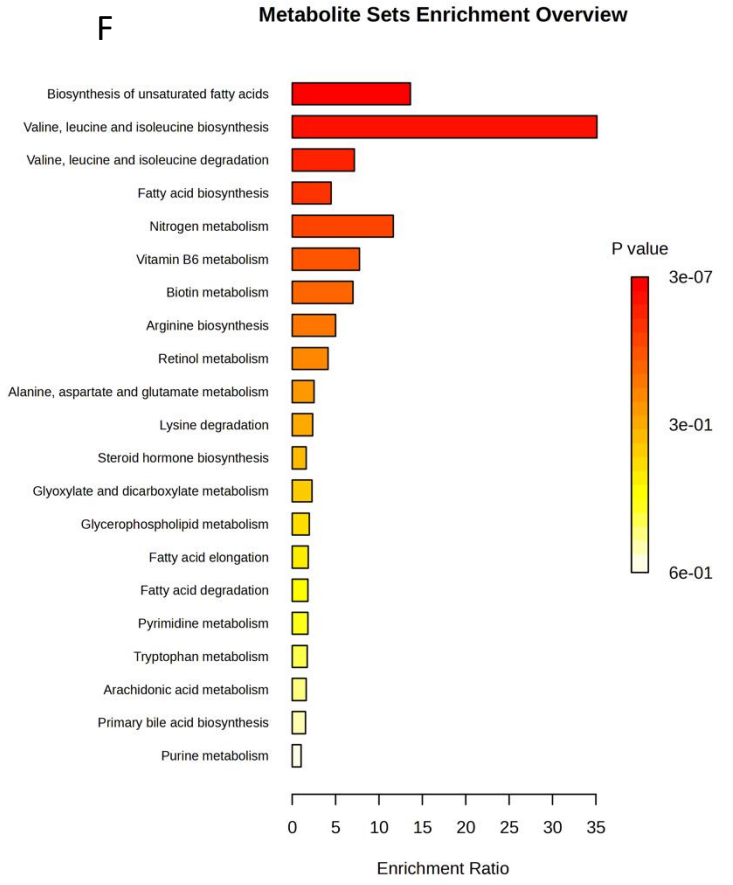
Transverse Colon
Day 7

E



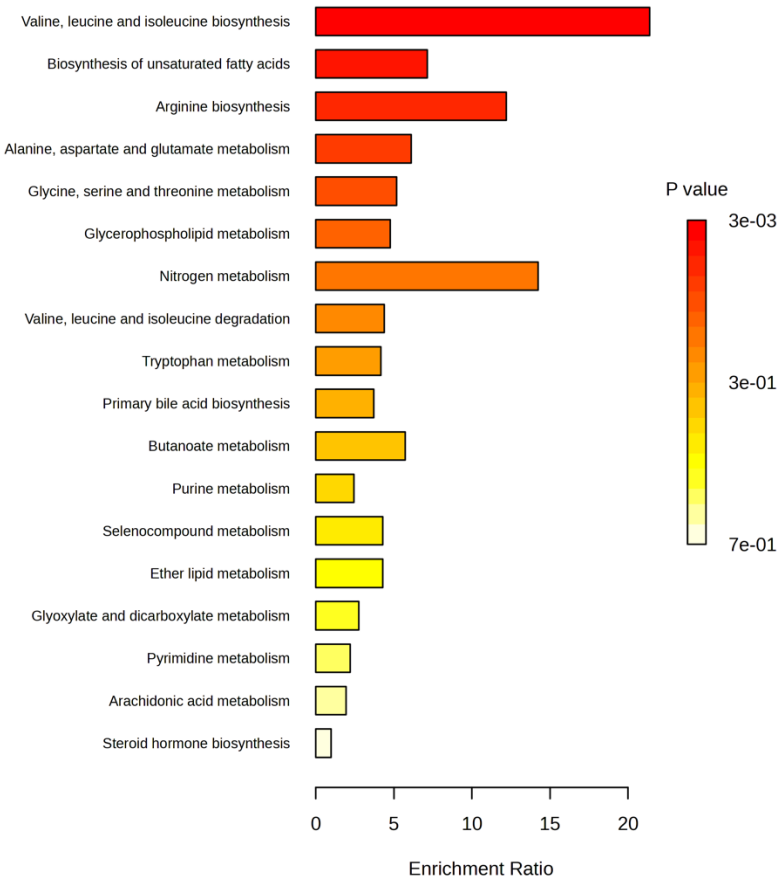
Transverse Colon
Day 21

F



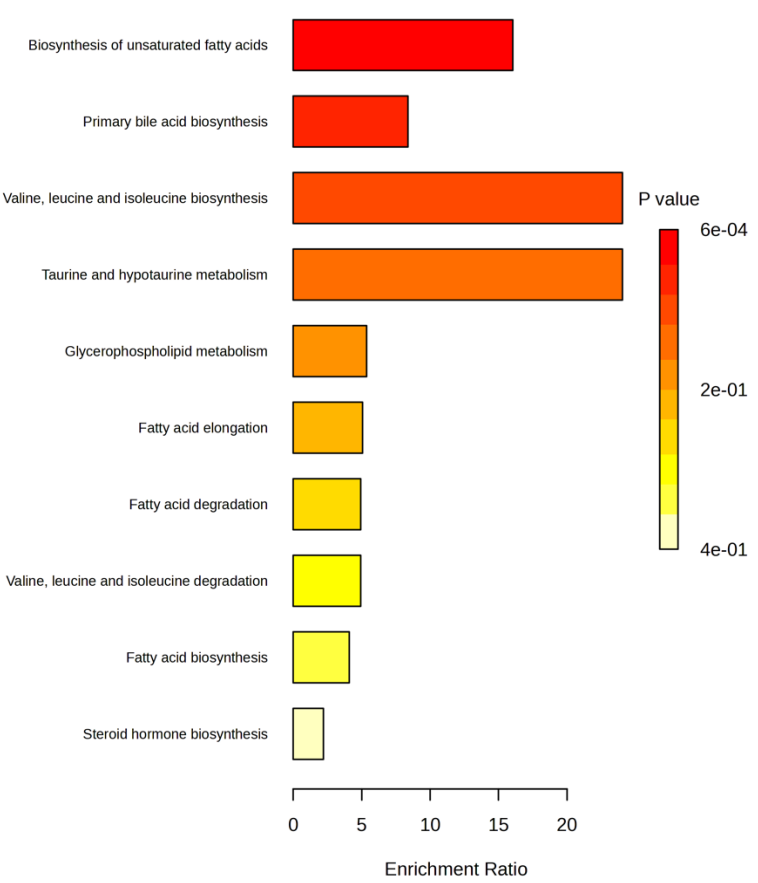
Descending Colon
Day 7

G



Descending Colon
Day 21

H

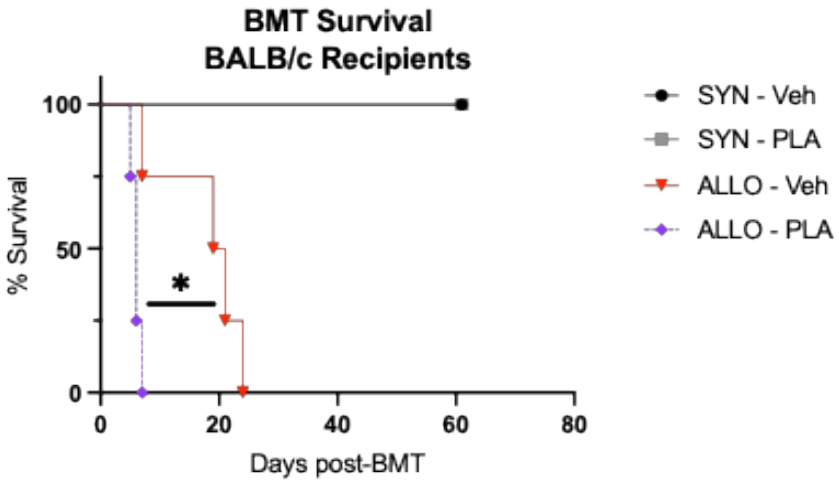


Supplemental Figure 4.

Enrichment analysis was performed using the Metaboanalyst 5.0 platform for metabolites that were significantly different between syngeneic and allogeneic samples from each gut location at each timepoint to determine which pathways are most affected (A-H).

Supp Fig 5

A



Supplemental Figure 5.
BALB/c recipients of syngeneic or allogeneic transplants received daily oral gavage of either vehicle (water) or PLA for the duration of experiment.
Allogeneic n =8, syngeneic n=6, 1 independent experiment