Table S1–PedsCom *rpoB* gene primers and probes. Related to STAR Methods section.

Isolate	Primer	Sequence (5' to 3')	5' Dye	3' Quencher
Anaerostipes sp. PC18	Forward Primer	TGGAACTGGCGGATAAGA	NA	NA
	Reverse Primer	GCCAGGTTTACGGTCATATT	NA	NA
	Probe	TGC CGC TGT TCA GAG TGT GCT GAT	VIC	MGBNFQ
Clostridium intestinale PC17	Forward Primer	GGTAGTTCGCAGCTTTCTC	NA	NA
	Reverse Primer	CTTCAAACCCAGCTCTTTCT	NA	NA
	Probe	TCA GCT TTA GGA CCT GGA GGA CTT TCA	JUN	QSY
Kosakonia cowanii PC08	Forward Primer	GAAGACCTGAACGTCGATAAC	NA	NA
	Reverse Primer	GCTGACACCAGAAGAGAAAC	NA	NA
	Probe	AAA CAC CGT TTG GTA CGC GCA GAG	6FAM	MGBNFQ
Enterococcus faecalis PC15	Forward Primer	CTCGTTCAATCGGACCTTAC	NA	NA
	Reverse Primer	TTCCAGTGCCCAAACTTC	NA	NA
	Probe	ACG CAA CAA CCG TTG GGT GGT AAA	JUN	QSY
Lactobacillus johnsonii PC38	Forward Primer	ACAACCACTTGGTGGTAAAG	NA	NA
	Reverse Primer	GTGTGTAAGCAGCACCATAA	NA	NA
	Probe	TTG GTG GTC AGC GTT TCG GTG AAA	6FAM	MGBNFQ
Lactobacillus murinus PC39	Forward Primer	TGGTGCTTGGTTGGAATAC	NA	NA
	Reverse Primer	CCCAAAGCACGGATCAAT	NA	NA
	Probe	TCG ACC GCA CAC GTA AGA TCC CAT	VIC	MGBNFQ
Parabacteroides distasonis PC19	Forward Primer	TGAGTAACCCTCGGTAAGG	NA	NA
	Reverse Primer	GCGTAAGACCAACCAAAGTA	NA	NA
	Probe	TCA TCG CGG ACA ACG TGT GAA AGC	JUN	QSY
Staphylococcus sciuri PC04	Forward Primer	ACGTGCTCAACTCTACCT	NA	NA
	Reverse Primer	ACCCAGAAGCACCATTTG	NA	NA
	Probe	ACG TGC AGT TAC GTG TTC CAT ACC AGT	VIC	MGBNFQ
Staphylococcus xylosus PC20	Forward Primer	GTAGTGACAAGAGGCGAAAT	NA	NA
	Reverse Primer	TCACGGCATCCTCATAGT	NA	NA
	Probe	TGT TGG TTT CAT GAC TTG GGA CGG T	6FAM	MGBNFQ

Supplemental Figure 1. Low complexity of the pre-weaning microbial community enables the PedsCom consortium to recapitulate early-life microbial abundance and function, related to Figure 1

A. Alpha diversity of the intestinal microbiomes from 14-day-old donor mice (N = 4 samples per tissue site). **B.** Percentage of the total amplicon sequence variants (ASVs) present in each tissue that were recovered from bacterial culture plates. **C.** Median relative abundance of remaining non-PedsCom donor ASVs in the fecal microbiomes of 10-week-old adult SPF NOD mice (N = 19). **D.** Species-level distribution of the non-PedsCom ASVs present in the donor mice small intestine, cecum and large intestine samples. Median relative abundance of the top 20 species shown. **E.** Assessment of the functional potential of non-PedsCom taxa in donor mice samples. Mean relative abundance of KEGG orthologs (KOs) absent in the PedsCom consortium collapsed into KEGG superpathways. **F.** Significantly different abundances of non-PedsCom ASVs present in the donor mice in pre (≤14 days-old) and post-weaning (>14 days-old) fecal microbiomes of SPF NOD mice. Differential abundances determined by ANCOM analysis with FDR corrected p-values.

Supplemental Figure 2. Community dynamics of PedsCom consortium is restricted during weaning, related to Figure 2

A. Weighted UniFrac PCoA beta-diversity of small intestinal microbiota of PedsCom (N: D14 = 3, D21 = 5, D28 = 2) and CMCom (N: D14 = 5, D21 = 8, D28 = 2) mice during ontogeny. **B.** Weighted UniFrac PCoA beta-diversity of cecal microbiota of PedsCom (N: D14 = 6, D21 = 5, D28 = 4) and CMCom (N: D14 = 5, D21 = 7, D28 = 6) mice during ontogeny. **C.** Species and family level 16S rRNA gene relative abundance of microbiota from PedsCom and CMCom mice during ontogeny in the small intestine. Two representative litters for CMCom per timepoint, one litter represented in PedsCom. **D.** Species and family level 16S rRNA gene relative abundance of microbiota from PedsCom and CMCom mice during ontogeny in the cecum. Two representative litters for communities per timepoint. **E.** Representative relative abundance of adult PedsCom and CMCom intestinal microbiota at species and family level.

Supplemental Figure 3. Community similarities of PedsCom, CMCom and Oligo-MM12 mice to pre- and post-weaning SPF fecal microbiomes, related to Figure 2

A. Weighted UniFrac PCoA of large intestinal microbiota of pre- (≤14 days-old) and post-weaning (>14 days-old) vivarium 3 mice (N = 25) to PedsCom and Oligo-MM12 (N = 51). **B.** Weighted UniFrac distance comparison of pre-weaning (≤ day 14) and post-weaning (> day 14) vivarium 3 mice to all PedsCom and Oligo-MM12 timepoints. Data presented with median box and whisker plot. Mann-Whitney-Wilcoxon test ****$p<0.0001$.

Supplemental Figure 4. Lymphoid cell populations of germfree, CMCom and PedsCom mice at weaning, related to Figure 3

Percentage of $CD19^+$ B cells and γδ, αβ, $CD8^+$, $CD4^+$, Foxp3+ (Tregs), and $Foxp3^-$ $ROR\gamma^+$ (Th17) T cells in the small intestine, cecum and large intestine lamina propria of germfree, PedsCom and CMCom mice during ontogeny. N = 3 for day 21 germfree cecum, N = 3 for comparisons of day 21 PedsCom αβ, $CD8^+$, $CD4^+$, and Th17 T cells, and N ≥ 4 per other communities and timepoints. Lines represent median value for each community.

Supplemental Figure 5. Lymphoid cell populations of adult germfree, CMCom, and PedsCom mice, related to Figure 3

Percentage of CD19$^+$ B cells and γδ, αβ, CD8$^+$, CD4$^+$, Foxp3+ (Tregs), and Foxp3$^-$ RORγ$^+$ (Th17) T cells in the small intestine, cecum and large intestine lamina propria of germfree, PedsCom and CMCom 7–12-week-old adult mice. N ≥ 4 for germfree tissues, N ≥ 6 other communities and timepoints. Data presented with box and whisker plot.

Supplemental Figure 6. PedsCom consortium leads to lower cecal acetate and butyrate levels and increased systemic immunoglobulin levels, related to Figure 3-4

Acetic, propionic, isobutyric, and butyric acid concentrations in cecal contents of the indicated microbial communities at **A.** weaning (day 21) and **B.** adulthood (8-14 weeks old). N = 5 per timepoint, per group. GF = Germfree, CC = CMCom, PC = PedsCom, OMM12 = Oligo-MM12. **C.** Short-chain fatty acid concentration in cecal samples from adult CMCom (CC-ZT) and PedsCom (PC-ZT) mice sampled at ZT1500. N = 5 per group. **D.** Acetic, propionic, isobutyric, and butyric acid concentrations in cecal contents from adult CMCom and PedsCom mice sampled at ZT1500. **E.** ELISA of serum IgG1, IgG2b, IgG2c and IgE concentration in 7-12-week-old adult PedsCom and CMCom mice. N values: (Germfree = 8, CMCom = 18, PedsCom = 14). **F.** Representative gating scheme of microbial flow cytometry (mFLOW) analysis of immunoglobulin binding to fecal microbes. **G.** Acetic, propionic, isobutyric, and butyric acid concentrations in cecal contents from PedsCom co-housed mice. [Control (PC) N = 4, Co-housed (PC+CC) N = 5]. **H.** Primary bile acid concentration (median) in cecal contents from PedsCom co-housed mice (Control N = 4, Co-housed N = 5). Bile acid concentrations from SPF cecal contents included for comparison (N = 6). **I.** The median molar ratios of primary deconjugated to primary conjugated bile acids in PedsCom co-housed mice (Control N = 4, Co-housed N = 5). **J.** Percent mucosal IgA and systemic Ig binding to PedsCom co-housed fecal microbes (Control N = 4, Co-housed N = 5). **K.** Percentage of pTreg cells in the cecum, large intestine lamina propria and spleen of adult PedsCom mice supplemented with short-chain fatty acids at weaning and controls (Control N = 4, SCFA treated N = 5). Data presented with box and whisker plot. Mann-Whitney-Wilcoxon test *p<0.05, **p<0.01

Fig. S1

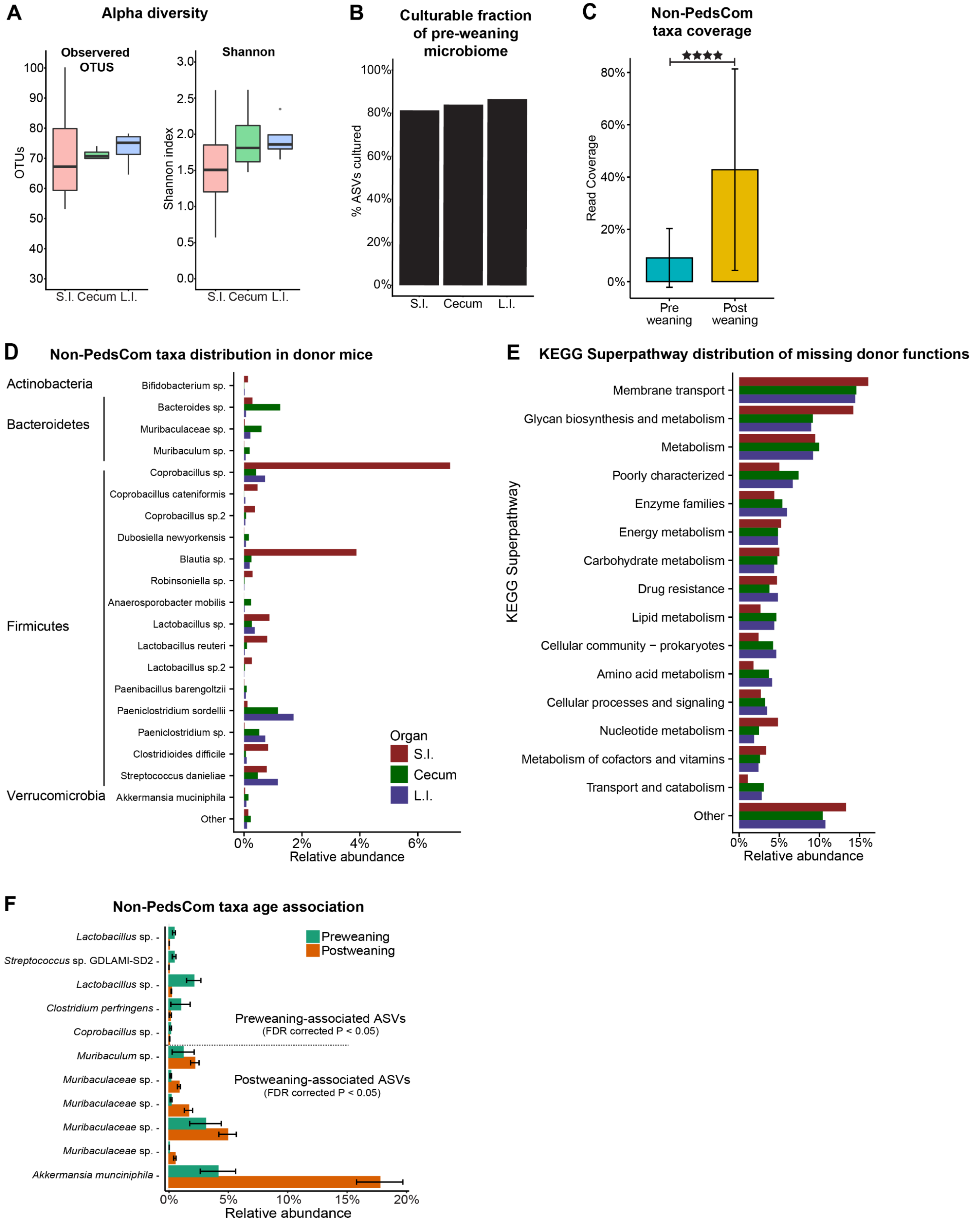

A
Alpha diversity
Observered OTUS
OTUs
100
90
80
70
60
50
40
30
S.I. Cecum L.I.
Shannon
Shannon index
3.0
2.5
2.0
1.5
1.0
0.5
0.0
S.I. Cecum L.I.
B
Culturable fraction of pre-weaning microbiome
% ASVs cultured
100%
80%
60%
40%
20%
0%
S.I.
Cecum
L.I.
C
Non-PedsCom taxa coverage
★★★★
Read Coverage
80%
60%
40%
20%
0%
Pre weaning
Post weaning
D
Non-PedsCom taxa distribution in donor mice
Actinobacteria
Bacteroidetes
Firmicutes
Verrucomicrobia
Bifidobacterium sp.
Bacteroides sp.
Muribaculaceae sp.
Muribaculum sp.
Coprobacillus sp.
Coprobacillus cateniformis
Coprobacillus sp.2
Dubosiella newyorkensis
Blautia sp.
Robinsoniella sp.
Anaerosporobacter mobilis
Lactobacillus sp.
Lactobacillus reuteri
Lactobacillus sp.2
Paenibacillus barengoltzii
Paeniclostridium sordellii
Paeniclostridium sp.
Clostridioides difficile
Streptococcus danieliae
Akkermansia muciniphila
Other
0%
2%
4%
6%
Relative abundance
Organ
S.I.
Cecum
L.I.
E
KEGG Superpathway distribution of missing donor functions
KEGG Superpathway
Membrane transport
Glycan biosynthesis and metabolism
Metabolism
Poorly characterized
Enzyme families
Energy metabolism
Carbohydrate metabolism
Drug resistance
Lipid metabolism
Cellular community − prokaryotes
Amino acid metabolism
Cellular processes and signaling
Nucleotide metabolism
Metabolism of cofactors and vitamins
Transport and catabolism
Other
0%
5%
10%
15%
Relative abundance
F
Non-PedsCom taxa age association
Preweaning
Postweaning
Lactobacillus sp.
Streptococcus sp. GDLAMI-SD2
Lactobacillus sp.
Clostridium perfringens
Coprobacillus sp.
Muribaculum sp.
Muribaculaceae sp.
Muribaculaceae sp.
Muribaculaceae sp.
Muribaculaceae sp.
Akkermansia munciniphila
Preweaning-associated ASVs
(FDR corrected P < 0.05)
Postweaning-associated ASVs
(FDR corrected P < 0.05)
0%
5%
10%
15%
20%
Relative abundance

Fig. S2

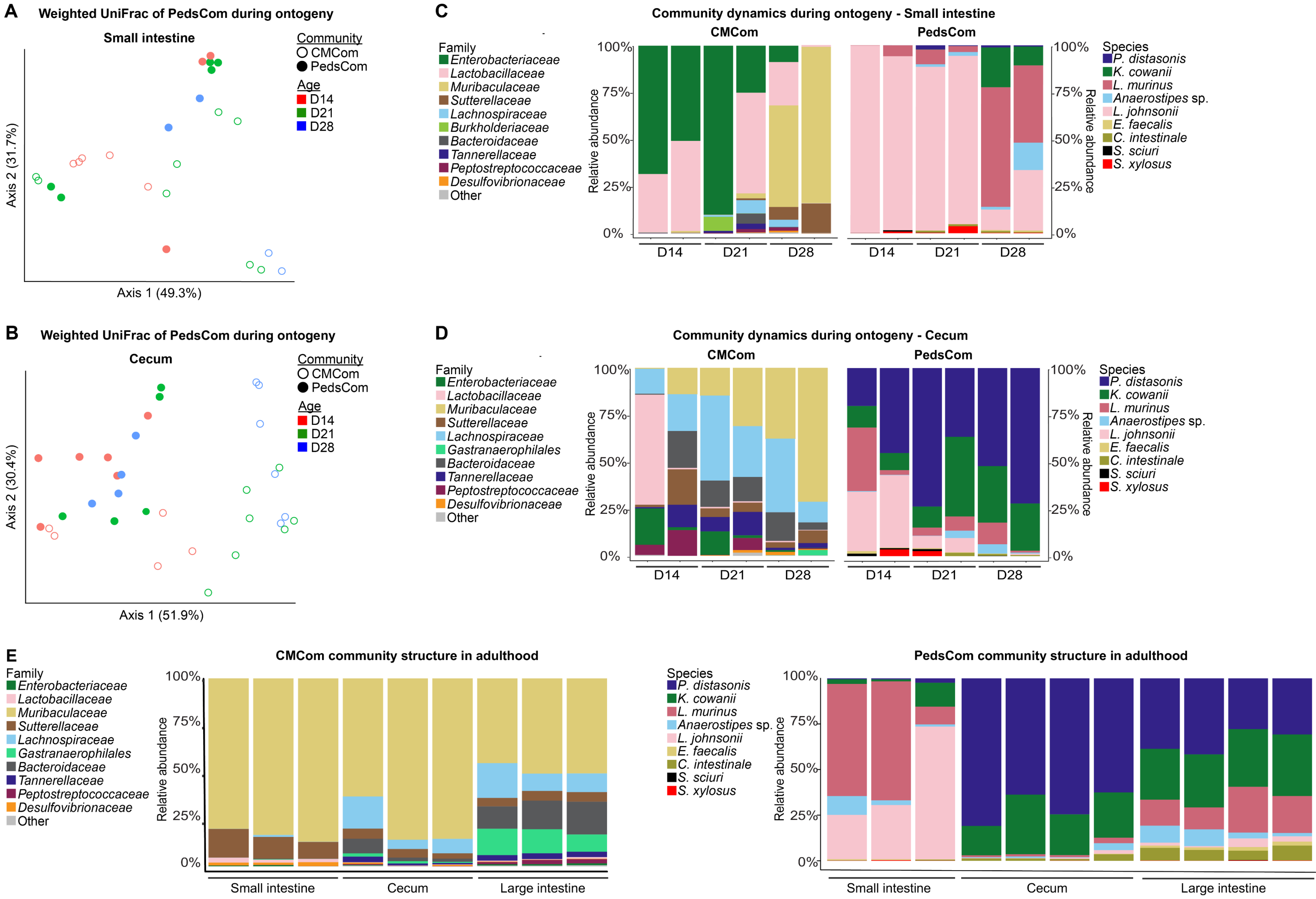
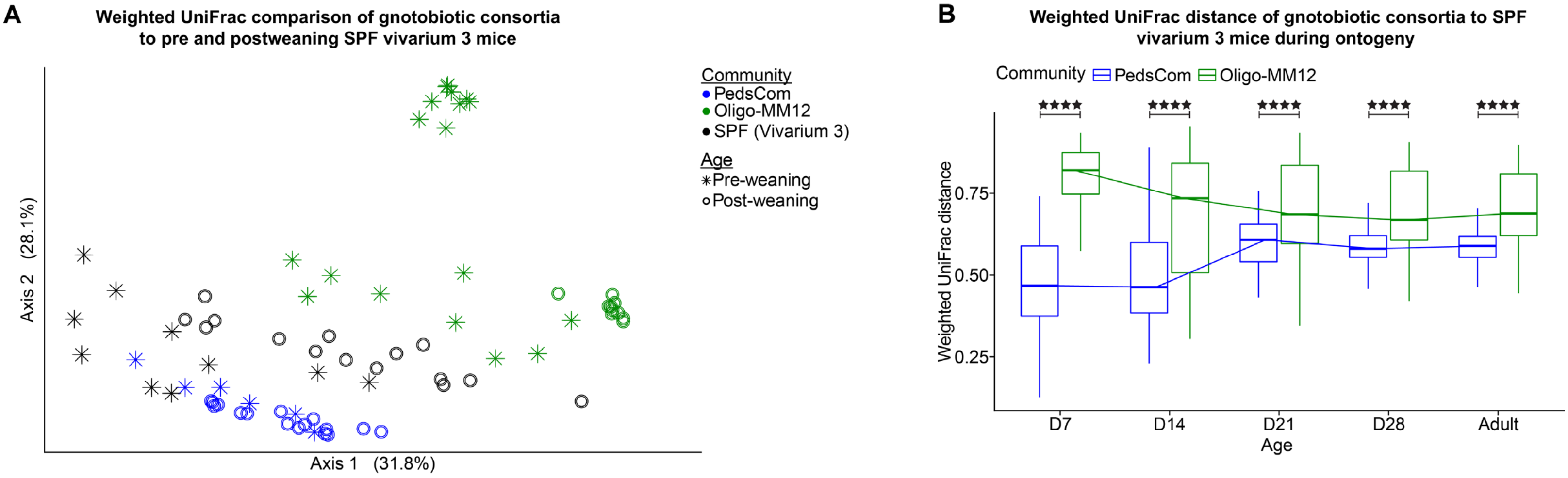

A
Weighted UniFrac of PedsCom during ontogeny
Small intestine
Community
○ CMCom
● PedsCom
Age
D14
D21
D28
Axis 2 (31.7%)
Axis 1 (49.3%)
B
Weighted UniFrac of PedsCom during ontogeny
Cecum
Community
○ CMCom
● PedsCom
Age
D14
D21
D28
Axis 2 (30.4%)
Axis 1 (51.9%)
C
Community dynamics during ontogeny - Small intestine
CMCom
PedsCom
Family
Enterobacteriaceae
Lactobacillaceae
Muribaculaceae
Sutterellaceae
Lachnospiraceae
Burkholderiaceae
Bacteroidaceae
Tannerellaceae
Peptostreptococcaceae
Desulfovibrionaceae
Other
Relative abundance
100%
75%
50%
25%
0%
D14
D21
D28
Species
P. distasonis
K. cowanii
L. murinus
Anaerostipes sp.
L. johnsonii
E. faecalis
C. intestinale
S. sciuri
S. xylosus
D
Community dynamics during ontogeny - Cecum
CMCom
PedsCom
Family
Enterobacteriaceae
Lactobacillaceae
Muribaculaceae
Sutterellaceae
Lachnospiraceae
Gastranaerophilales
Bacteroidaceae
Tannerellaceae
Peptostreptococcaceae
Desulfovibrionaceae
Other
Relative abundance
100%
75%
50%
25%
0%
D14
D21
D28
Species
P. distasonis
K. cowanii
L. murinus
Anaerostipes sp.
L. johnsonii
E. faecalis
C. intestinale
S. sciuri
S. xylosus
E
CMCom community structure in adulthood
Family
Enterobacteriaceae
Lactobacillaceae
Muribaculaceae
Sutterellaceae
Lachnospiraceae
Gastranaerophilales
Bacteroidaceae
Tannerellaceae
Peptostreptococcaceae
Desulfovibrionaceae
Other
Relative abundance
100%
75%
50%
25%
0%
Small intestine
Cecum
Large intestine
PedsCom community structure in adulthood
Species
P. distasonis
K. cowanii
L. murinus
Anaerostipes sp.
L. johnsonii
E. faecalis
C. intestinale
S. sciuri
S. xylosus
Relative abundance
100%
75%
50%
25%
0%
Small intestine
Cecum
Large intestine

Fig. S3



A Weighted UniFrac comparison of gnotobiotic consortia to pre and postweaning SPF vivarium 3 mice

B Weighted UniFrac distance of gnotobiotic consortia to SPF vivarium 3 mice during ontogeny

Fig. S4

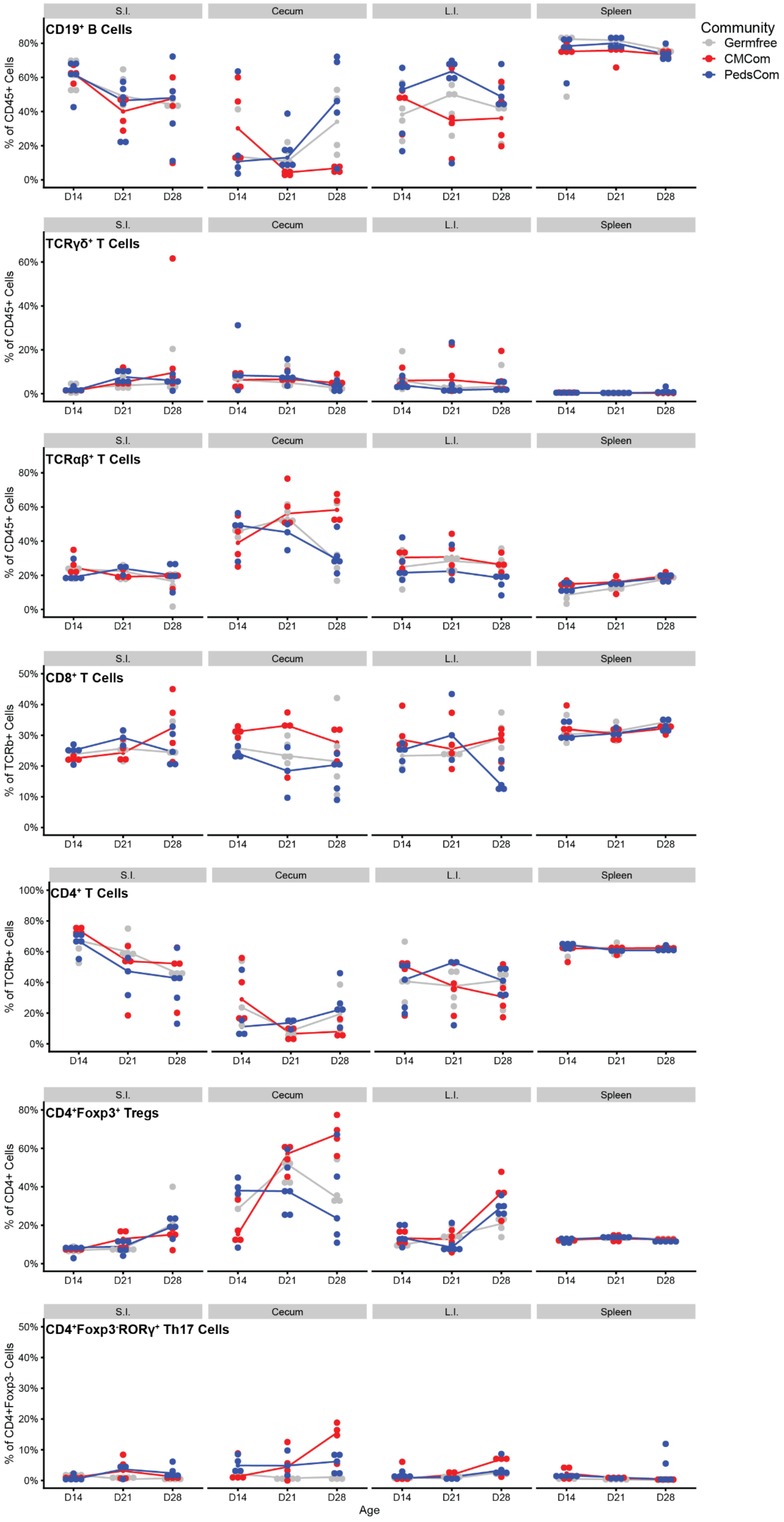
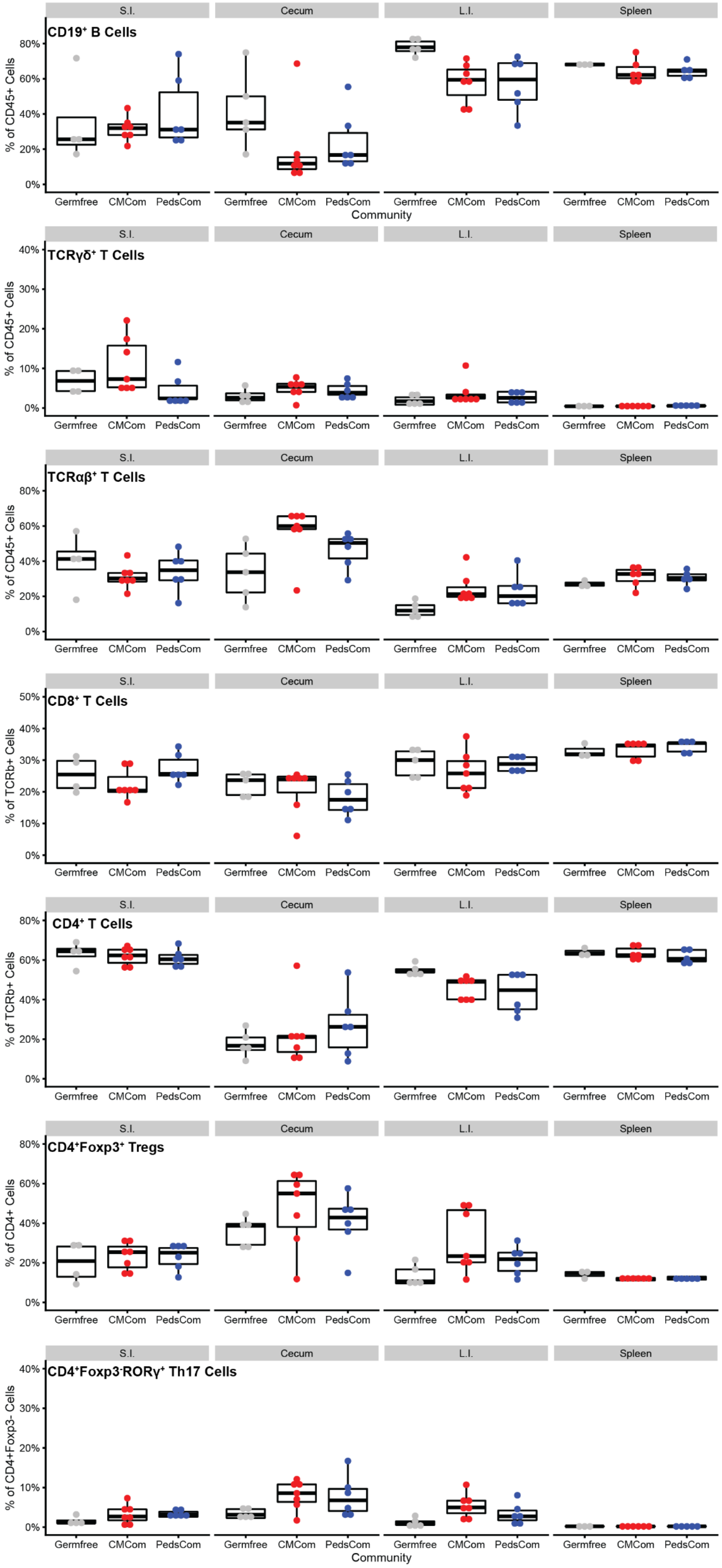
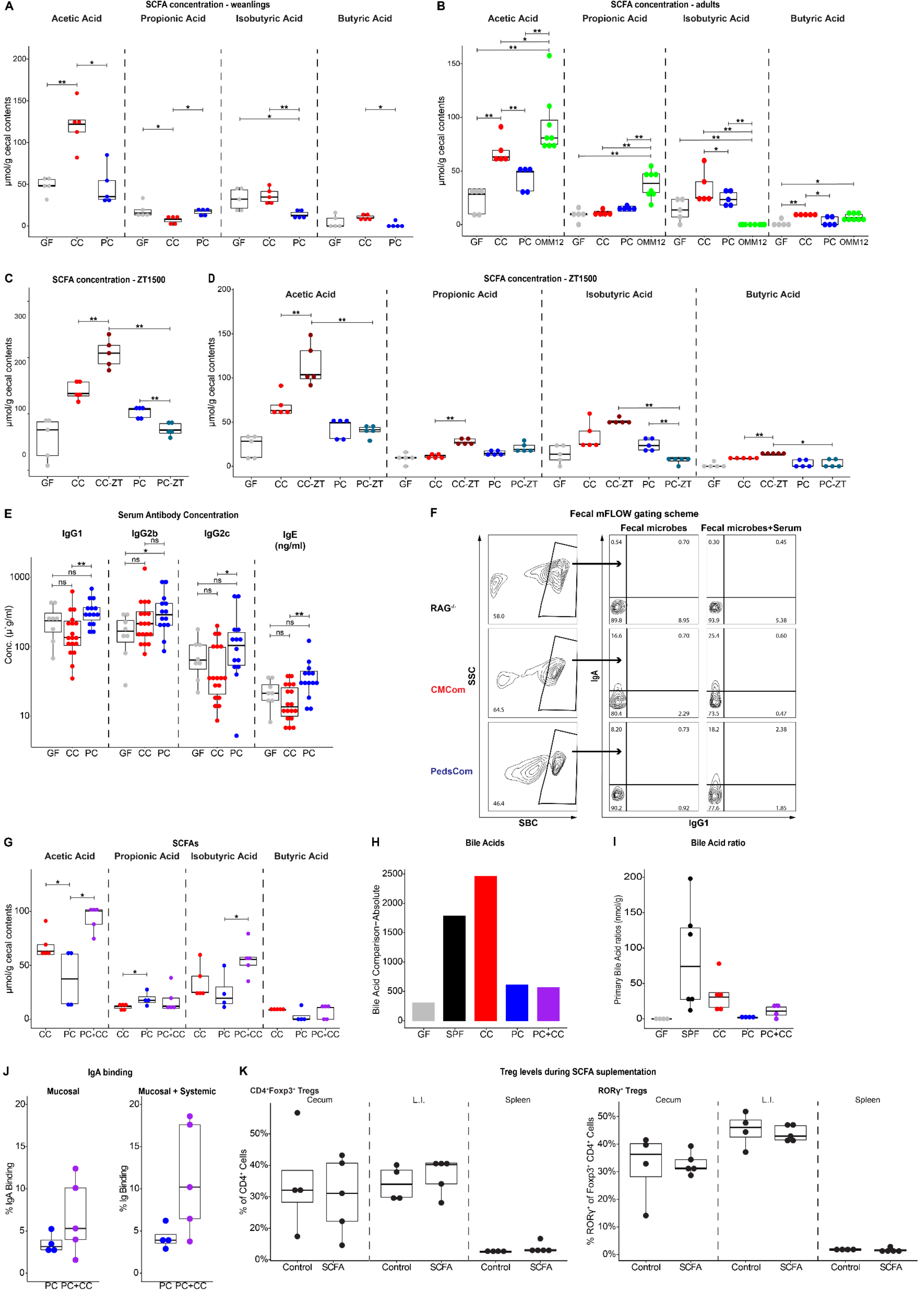

S.I.
Cecum
L.I.
Spleen
Community
Germfree
CMCom
PedsCom
CD19⁺ B Cells
% of CD45+ Cells
80%
60%
40%
20%
0%
TCRγδ⁺ T Cells
% of CD45+ Cells
60%
40%
20%
0%
TCRαβ⁺ T Cells
% of CD45+ Cells
80%
60%
40%
20%
0%
CD8⁺ T Cells
% of TCRb+ Cells
50%
40%
30%
20%
10%
0%
CD4⁺ T Cells
% of TCRb+ Cells
100%
80%
60%
40%
20%
0%
CD4⁺Foxp3⁺ Tregs
% of CD4+ Cells
80%
60%
40%
20%
0%
CD4⁺Foxp3⁻RORγ⁺ Th17 Cells
% of CD4+Foxp3- Cells
50%
40%
30%
20%
10%
0%
D14
D21
D28
Age

Fig. S5



S.I.
Cecum
L.I.
Spleen
CD19⁺ B Cells
% of CD45+ Cells
0%
20%
40%
60%
80%
Germfree
CMCom
PedsCom
Community
TCRγδ⁺ T Cells
% of CD45+ Cells
0%
10%
20%
30%
40%
TCRαβ⁺ T Cells
% of CD45+ Cells
0%
20%
40%
60%
80%
CD8⁺ T Cells
% of TCRb+ Cells
0%
10%
20%
30%
40%
50%
CD4⁺ T Cells
% of TCRb+ Cells
0%
20%
40%
60%
80%
CD4⁺Foxp3⁺ Tregs
% of CD4+ Cells
0%
20%
40%
60%
80%
CD4⁺Foxp3⁻RORγ⁺ Th17 Cells
% of CD4+Foxp3- Cells
0%
10%
20%
30%
40%
Germfree
CMCom
PedsCom
Community

Fig. S6


A
SCFA concentration - weanlings
Acetic Acid
Propionic Acid
Isobutyric Acid
Butyric Acid
µmol/g cecal contents
0
50
100
150
200
GF CC PC
B
SCFA concentration - adults
Acetic Acid
Propionic Acid
Isobutyric Acid
Butyric Acid
µmol/g cecal contents
GF CC PC OMM12
C
SCFA concentration - ZT1500
µmol/g cecal contents
GF CC CC-ZT PC PC-ZT
D
SCFA concentration - ZT1500
Acetic Acid
Propionic Acid
Isobutyric Acid
Butyric Acid
µmol/g cecal contents
GF CC CC-ZT PC PC-ZT
E
Serum Antibody Concentration
IgG1
IgG2b
IgG2c
IgE (ng/ml)
Conc. (µ`g/ml)
10
100
1000
ns
GF CC PC
F
Fecal mFLOW gating scheme
Fecal microbes
Fecal microbes+Serum
RAG-/-
CMCom
PedsCom
SSC
SBC
IgA
IgG1
58.0
64.5
46.4
0.54
0.70
89.8
8.95
0.30
0.45
93.9
5.38
16.6
0.70
80.4
2.29
25.4
0.60
73.5
0.47
8.20
0.73
90.2
0.92
18.2
2.38
77.6
1.85
G
SCFAs
Acetic Acid
Propionic Acid
Isobutyric Acid
Butyric Acid
µmol/g cecal contents
CC PC PC+CC
H
Bile Acids
Bile Acid Comparison–Absolute
0
500
1000
1500
2000
2500
GF SPF CC PC PC+CC
I
Bile Acid ratio
Primary Bile Acid ratios (nmol/g)
0
50
100
150
200
GF SPF CC PC PC+CC
J
IgA binding
Mucosal
Mucosal + Systemic
% IgA Binding
% Ig Binding
0
5
10
15
20
PC PC+CC
K
Treg levels during SCFA suplementation
CD4+Foxp3+ Tregs
RORγ+ Tregs
Cecum
L.I.
Spleen
% of CD4+ Cells
% RORγ+ of Foxp3+ CD4+ Cells
0%
10%
20%
30%
40%
50%
Control SCFA