Supplementary Material:

DNA extraction protocol:

We used the MoBio Power Soil DNA Extraction Kit protocol, with vortexing plus bead beating, as recommended by the manufacturer. Also, we added 15µL of Proteinasa K to each sample, incubated at -20°C for 10 minutes, and then at 60°C for 3 minutes. These two incubations were repeated twice before using the bead-beater, as recommended by the manufacturer.

Sequence coverage considerations:

To test whether our conclusions are robust to the observed sequence coverage, we performed procrustes analysis as follows (see Muegge et al Science 2011 for further details on Procrustes analysis). We calculated beta diversity rarefying at a lower level of 500 sequences per sample. The principal coordinates matrix so obtained was then transformed using rotations, scaling, and transformations to minimize the distances between the corresponding points in this and the original matrix (i.e. the principal coordinate matrix obtained at 1,500 seqs/sample). A thousand random permutations of the matrix were performed to obtain Monte Carlo p-values, resulting in p=0.000 and M^2=0.078 (unweighted UniFrac) and p=0.000 and M^2=0.117 (weighted UniFrac). These results demonstrate that even at lower coverage the observed patterns are essentially the same as the ones obtained at 1,500 sequences per sample, and thus higher coverage would not modify the conclusions here presented. It might however occur that with much deeper sequencing, the richness (number of species) would not change (if all species are there in very low abundance), but only the evenness. Since diversity is a composite of the two factors, richness and evenness, we can say based on our results that there was a decrease in diversity during strict lactation.

Table S1: Proportions of bacterial taxa in the feces of adult mice.

Phylum	Percentage (%)	Taxa	Percentage (%)	StDev
Firmicutes	52.34	Lachnospiraceae	34.7	15.42
		Lactobacillaceae (*Lactobacillus*)	5.09	4.59
		Clostridiales	3.67	2.03
		Ruminococcaceae	3.47	1.55
		Lachnospiraceae (*Bryantella*)	1.8	1.18
		Lachnospiraceae Incertae Sedis	0.88	0.49
		Bacillales	0.84	1.92
		Staphylococcaceae (*Staphylococcus*)	0.78	1.63
		Clostridia	0.39	0.79
		Staphylococcaceae (*Jeotgalicoccus*)	0.39	0.97
		Unknown	0.24	0.28
		Ruminococcaceae Incertae Sedis	0.06	0.09
		Streptococcaceae (*Streptococcus*)	0.01	0.03
Bacteroidetes	42.09	Unknown	24.07	9.31
		Prevotellaceae	13.7	6.91
		Bacteroides	3.5	4.44
		Rikenellaceae (*Alistipes*)	0.54	0.46
		Bacteroidales	0.17	0.16
		Prevotellaceae (*Prevotella*)	0.05	0.09
		Rikenellaceae (*Rikenella*)	0.05	0.09
Unknown	4.82		4.82	1.82
Proteobacteria	0.68	Unknown	0.307	0.72
		Helicobacteraceae (*Helicobacter*)	0.22	0.21
		Deltaproteobacteria	0.09	0.13
		Enterobacteriaceae	0.05	0.08
TM7	0.18	genera_incertae_sedis	0.18	0.25
Tenericutes	0.12	Anaeroplasmataceae (*Anaeroplasma*)	0.12	0.14
Actinobacteria	0.04	Corynebacteriaceae (*Corynebacterium*)	0.03	0.04
		Coriobacteriaceae	0.01	0.03
Deferribacteres	0.01	Deferribacteraceae (*Mucispirillum*)	0.01	0.03

Unknown= Short reads with no assigned taxonomy by RDP below phylum level.

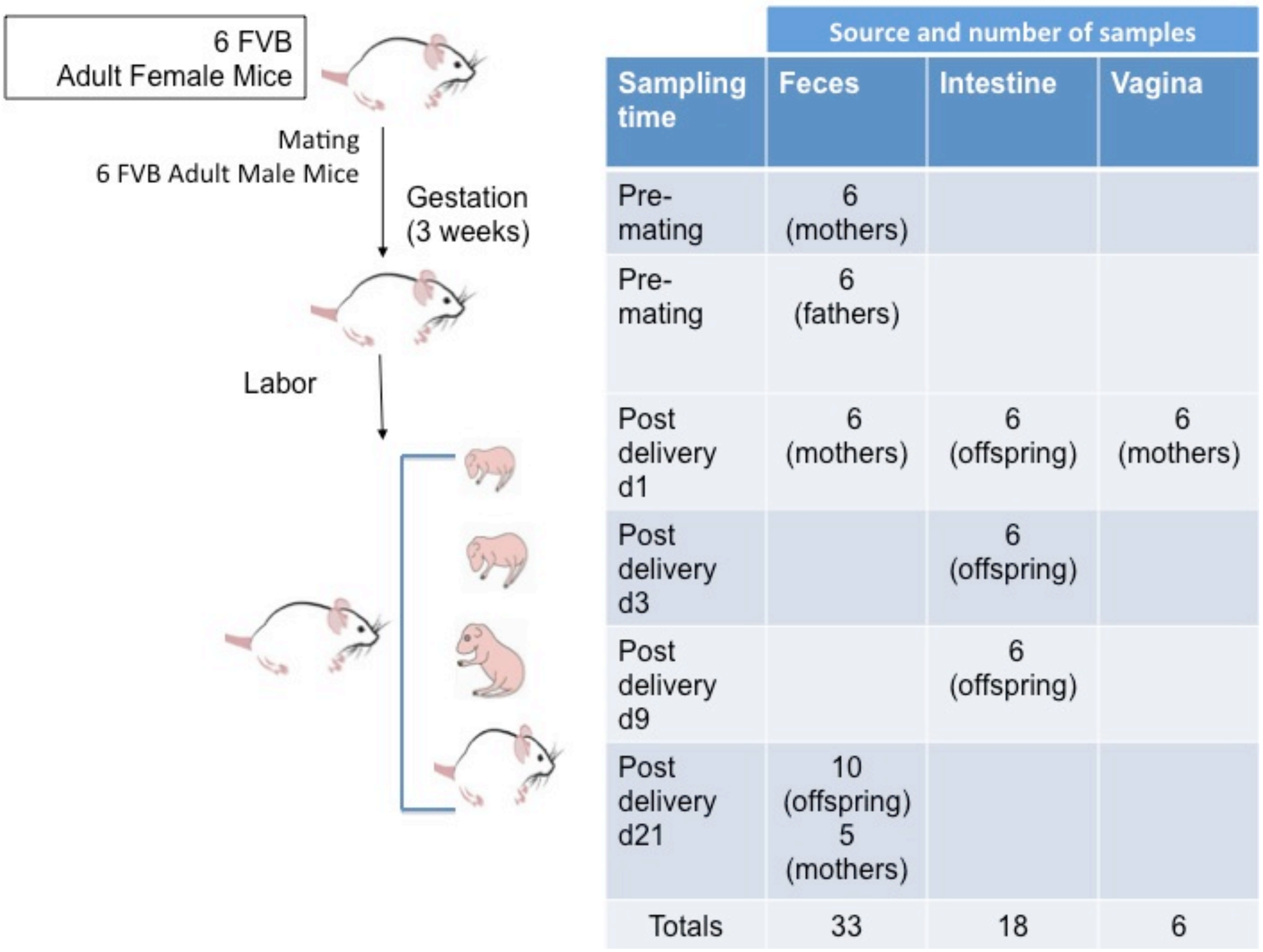
Table S2. Percentage of taxa in the mother's vagina and feces and in the offspring intestine at day 1, 3 and 9, and 21 of age.

		Mean and Standard Deviation						
		Mothers			Offspring			
		Vagina	Feces		Intestine			Feces
Phylum	Family (Genus)	d1Post-delivery (N=6)	d1 Post-delivery (N=6)	d21 Post-delivery (N=5)	Day1 (N=6)	Day 3 (N=6)	Day 9 (N=6)	Day 21 (N=10)
Proteobacteria	Rhodobacteraceae (Paracoccus)	45.30 ± 6.72	0.00	0.00	0.01 ± 0.03	0.00	0.00	0.00
Proteobacteria	Sphingomonadaceae (Sphingomonas)	30.92 ± 5.23	0.00	0.00	0.02 ± 0.05	0.00	0.00	0.00
Firmicutes	Streptococcaceae (Streptococcus)	8.95 ± 5.82	0.03 ± 0.06	0.06 ± 0.13	41.25 ± 24.7	45.82 ± 36.31	13.75 ± 7.76	2.06 ± 5.87
Firmicutes	Staphylococcaceae (Staphylococcus)	3.71 ± 1.27	0.11 ± 0.13	0.05 ± 0.12	3.16 ± 3.30	3.00 ± 5.83	0.00	0.01 ± 0.02
Proteobacteria	Pasteurellaceae (Pasteurella)	2.80 ± 2.91	0.00	0.00	4.01 ± 5.74	17.24 ± 11.10	14.57 ± 14.86	0.03 ± 0.05
Firmicutes	Incertae Sedis XI (Gemella)	1.47 ± 1.07	0.00	0.00	0.00	0.65 ± 1.50	3.80 ± 3.23	0.01 ± 0.03
Firmicutes	Lachnospiraceae	0.84 ± 1.05	16.46 ± 12.45	18.57 ± 13.00	2.43 ± 2.97	0.07 ± 0.09	0.09 ± 0.20	24.22 ± 13.99
Bacteroidetes	Porphyromonadaceae (Porphyromonas)	0.71 ± 1.18	0.00	0.00	0.01 ± 0.03	0.00	0.00	0.00
Actinobacteria	Corynebacteriaceae (Corynebacterium)	0.56 ± 0.41	0.93 ± 1.42	0.00	0.73 ± 0.99	0.30 ± 0.34	0.05 ± 0.08	0.02 ± 0.07
Firmicutes	Bacillaceae 1 (Bacillus a)	0.54 ± 0.42	0.00	0.00	0.26 ± 0.28	0.01 ± 0.02	0.00	0.00
Bacteria	Unknown	0.53 ± 0.45	11.90 ± 6.71	10.79 ± 7.08	7.69 ± 7.06	0.45 ± 0.42	0.13 ± 0.15	5.63 ± 6.36
Proteobacteria	Helicobacteraceae (Helicobacter)	0.39 ± 0.74	0.25 ± 0.44	0.14 ± 0.31	0.67 ± 1.39	0.02 ± 0.03	0.03 ± 0.08	0.63 ± 0.67
Firmicutes	Lactobacillaceae (Lactobacillus)	0.33 ± 0.41	7.74 ± 6.64	22.42 ± 14.54	0.59 ± 0.65	29.34 ± 33.46	63.84 ± 19.56	18.68 ± 16.87
Proteobacteria	Moraxellaceae (Psychrobacter)	0.30 ± 0.32	0.14 ± 0.19	0.02 ± 0.05	0.40 ± 0.78	0.02 ± 0.06	0.00	0.00
Firmicutes	Carnobacteriaceae (Carnobacteriaceae 1)	0.29 ± 0.53	0.00	0.00	0.40 ± 0.50	0.48 ± 0.33	2.48 ± 3.62	0.02 ± 0.05
Firmicutes	Enterococcaceae (Enterococcus)	0.28 ± 0.25	0.00	0.00	0.03 ± 0.06	0.00	0.00	0.00
Proteobacteria	Pseudomonadaceae (Pseudomonas)	0.25 ± 0.13	0.00	0.00	0.09 ± 0.07	0.00	0.00	0.00
Firmicutes	Clostridiales	0.23 ± 0.26	4.63 ± 4.12	3.63 ± 2.55	1.74 ± 1.36	0.06 ± 0.04	0.04 ± 0.10	17.68 ± 9.03
Proteobacteria	Enterobacteriaceae	0.23 ± 0.17	0.00	0.05 ± 0.10	0.13 ± 0.18	0.06 ± 0.09	0.27 ± 0.44	0.40 ± 0.35
Firmicutes	Jeotgalicoccus	0.20 ± 0.21	0.35 ± 0.52	0.00	0.15 ± 0.12	0.01 ± 0.02	0.00	0.00
Cyanobacteria	Streptophyta	0.20 ± 0.21	0.03 ± 0.05	0.02 ± 0.04	2.78 ± 2.13	0.28 ± 0.34	0.01 ± 0.03	0.10 ± 0.08
Firmicutes	Ruminococcaceae	0.18 ± 0.19	2.74 ± 2.11	1.26 ± 0.81	0.63 ± 0.67	0.03 ± 0.03	0.01 ± 0.03	3.01 ± 2.02
Bacteroidetes	Prevotellaceae	0.17 ± 0.11	27.40 ± 16.17	7.60 ± 7.94	3.85 ± 4.10	0.20 ± 0.24	0.05 ± 0.07	0.78 ± 0.62
Proteobacteria	Xanthomonadaceae (Stenotrophomonas)	0.16 ± 0.17	0.00	0.00	0.08 ± 0.13	0.01 ± 0.02	0.00	0.00
Firmicutes	Aerococcaceae (Facklamia)	0.12 ± 0.11	0.02 ± 0.04	0.02 ± 0.05	0.23 ± 0.30	0.01 ± 0.03	0.00	0.00
Bacteroidetes	Bacteroidaceae (Bacteroides)	0.10 ± 0.15	2.99 ± 3.98	1.29 ± 2.24	0.41 ± 0.59	0.04 ± 0.09	0.00	23.17 ± 18.81
Firmicutes	Clostridiaceae 1 (Clostridium)	0.04 ± 0.08	1.26 ± 3.10	1.15 ± 2.56	0.00	0.01 ± 0.02	0.00	0.00
Firmicutes	Lachnospiraceae (Bryantella)	0.03 ± 0.05	1.31 ± 1.13	1.14 ± 1.16	0.05 ± 0.09	0.00	0.00	0.62 ± 0.73
Bacteroidetes	Flavobacteriaceae	0.03 ± 0.03	0.00	0.00	0.43 ± 0.83	0.05 ± 0.09	0.09 ± 0.10	0.00
Firmicutes	Clostridia	0.03 ± 0.05	0.15 ± 0.28	0.06 ± 0.13	0.37 ± 0.28	0.03 ± 0.08	0.09 ± 0.23	0.29 ± 0.40
Proteobacteria	Pasteurellaceae	0.03 ± 0.05	0.00	0.00	7.22 ± 11.25	0.73 ± 1.29	0.4 ± 0.55	0.00
Firmicutes	Erysipelotrichaceae (Turicibacter)	0.03 ± 0.05	16.21 ± 20.88	26.98 ± 13.71	0.34 ± 0.46	0.00	0.00	0.06 ± 0.12
Bacteroidetes	Rikenellaceae (Rikenella)	0.02 ± 0.05	0.10 ± 0.16	0.09 ± 0.16	0.04 ± 0.09	0.00	0.00	0.02 ± 0.05
Proteobacteria	Desulfovibrionaceae (Desulfovibrio)	0.02 ± 0.05	0.02 ± 0.05	0.00	0.00	0.00	0.00	0.08 ± 0.24
Firmicutes	Lachnospiraceae (Lachnospiraceae Incertae Sedis)	0.01 ± 0.03	0.82 ± 0.47	0.71 ± 0.61	0.12 ± 0.13	0.01 ± 0.02	0.00	1.07 ± 0.62
Firmicutes	Erysipelotrichaceae (Erysipelotrichaceae Incertae Sedis)	0.01 ± 0.03	0.10 ± 0.26	0.98 ± 1.70	0.00	0.00	0.00	0.33 ± 0.64
Firmicutes	Bacillales	0.01 ± 0.02	0.03 ± 0.07	0.00	0.01 ± 0.03	0.00	0.00	0.00
Firmicutes	Veillonellaceae	0.01 ± 0.02	0.00	0.00	0.33 ± 0.27	0.00	0.00	0.00
Actinobacteria	Propionibacteriaceae (Propionibacterium)	0.00	0.00	0.00	0.13 ± 0.27	0.22 ± 0.55	0.00	0.00
Actinobacteria	Coriobacteriaceae	0.00	0.15 ± 0.23	0.28 ± 0.31	0.04 ± 0.09	0.00	0.00	0.10 ± 0.24
Bacteroidetes	Bacteroidales	0.00	2.53 ± 3.02	0.34 ± 0.41	0.01 ± 0.03	0.00	0.00	0.07 ± 0.08
Bacteroidetes	Prevotellaceae (Prevotella)	0.00	0.27 ± 0.24	0.02 ± 0.05	0.00	0.00	0.00	0.04 ± 0.08
Bacteroidetes	Rikenellaceae (Alistipes)	0.00	0.34 ± 0.31	0.15 ± 0.20	0.08 ± 0.18	0.01 ± 0.02	0.00	0.01 ± 0.05
Deferribacteres	Deferribacteraceae (Mucispirillum)	0.00	0.00	0.00	0.01 ± 0.03	0.02 ± 0.05	0.00	0.18 ± 0.45
Firmicutes	Carnobacteriaceae 1 (Isobaculum)	0.00	0.00	0.00	0.43 ± 1.05	0.65 ± 1.47	0.25 ± 0.39	0.00
Firmicutes	Lachnospiraceae (Butyrivibrio)	0.00	0.00	0.00	0.42 ± 0.46	0.02 ± 0.05	0.00	0.00
Firmicutes	Erysipelotrichaceae (Coprobacillus)	0.00	0.00	0.58 ± 1.30	0.00	0.00	0.00	0.00
Firmicutes	Ruminococcaceae (Ruminococcaceae Incertae Sedis)	0.00	0.00	0.04 ± 0.05	0.00	0.00	0.00	0.08 ± 0.11
Firmicutes	Peptostreptococcaceae (Peptostreptococcaceae Incertae Sedis)	0.00	0.15 ± 0.36	1.29 ± 1.42	0.00	0.00	0.00	0.05 ± 0.07
Proteobacteria	Deltaproteobacteria	0.00	0.04 ± 0.07	0.06 ± 0.09	0.02 ± 0.04	0.00	0.00	0.42 ± 0.50
Proteobacteria	Pasteurellaceae (Mannheimia)	0.00	0.00	0.00	1.52 ± 3.03	0.04 ± 0.07	0.04 ± 0.10	0.00
TM7;TM7	genera_incertae_sedis	0.00	0.28 ± 0.38	0.22 ± 0.14	0.03 ± 0.05	0.00	0.00	0.10 ± 0.20

Table S3. Richness of intestinal bacterial OTUs identified in offspring mice during development.

Offspring Age (days)	Number of animals	OTUs (Average$\pm$SD)	Change in mean number of OTUs from previous time-point	% Change
1	6	$66.5 \pm 52.6^{a,b}$	–	–
3	6	24.2 ± 10.8^{a}	-43	-64.2
9	6	$8.2 \pm 5.7^{b,c}$	-16	-66.7
21	10	53.7 ± 18.4^{c}	+46	+675.0

Superscript letters indicate values that are statistically different: ^{a}day 1 vs. day 3 (*p value* 0.047); ^{b}day 1 vs. day 9 (*p value* 0.005); ^{c}day 9 vs. day 21 (*p value* 0.014).



	Source and number of samples		
Sampling time	Feces	Intestine	Vagina
Pre-mating	6 (mothers)		
Pre-mating	6 (fathers)		
Post delivery d1	6 (mothers)	6 (offspring)	6 (mothers)
Post delivery d3		6 (offspring)	
Post delivery d9		6 (offspring)	
Post delivery d21	10 (offspring) 5 (mothers)		
Totals	33	18	6

Figure S1. Experimental design. Six adult female FVB mice were sampled for feces, mated with males, and sampled after delivery (vagina and feces) and at 21 days (feces). The intestinal contents of the offspring (5 animals per mother) were sampled at ages 1, 3 and 9 days, and feces at 21 days.

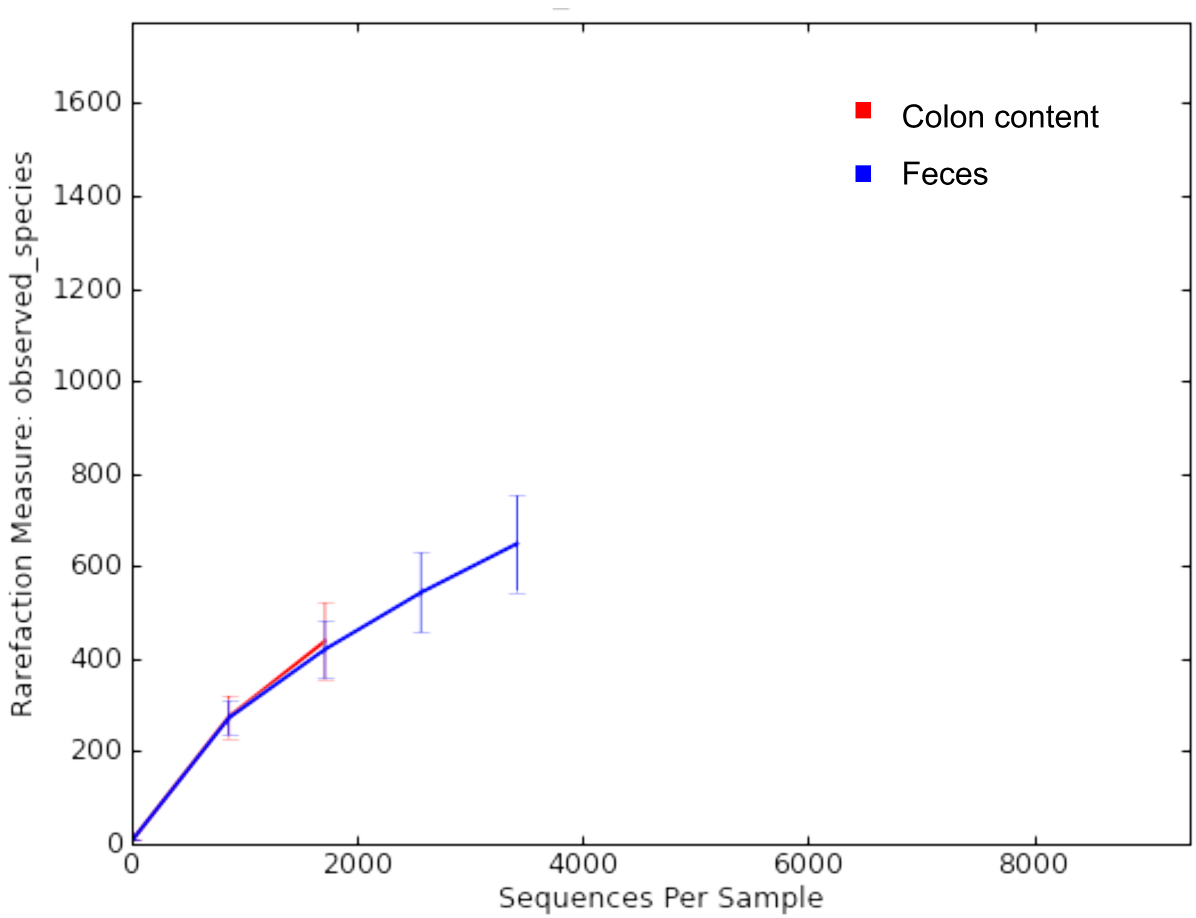


Figure S2. Rarefaction curve of observed species from mice colon content (red) and feces (blue).

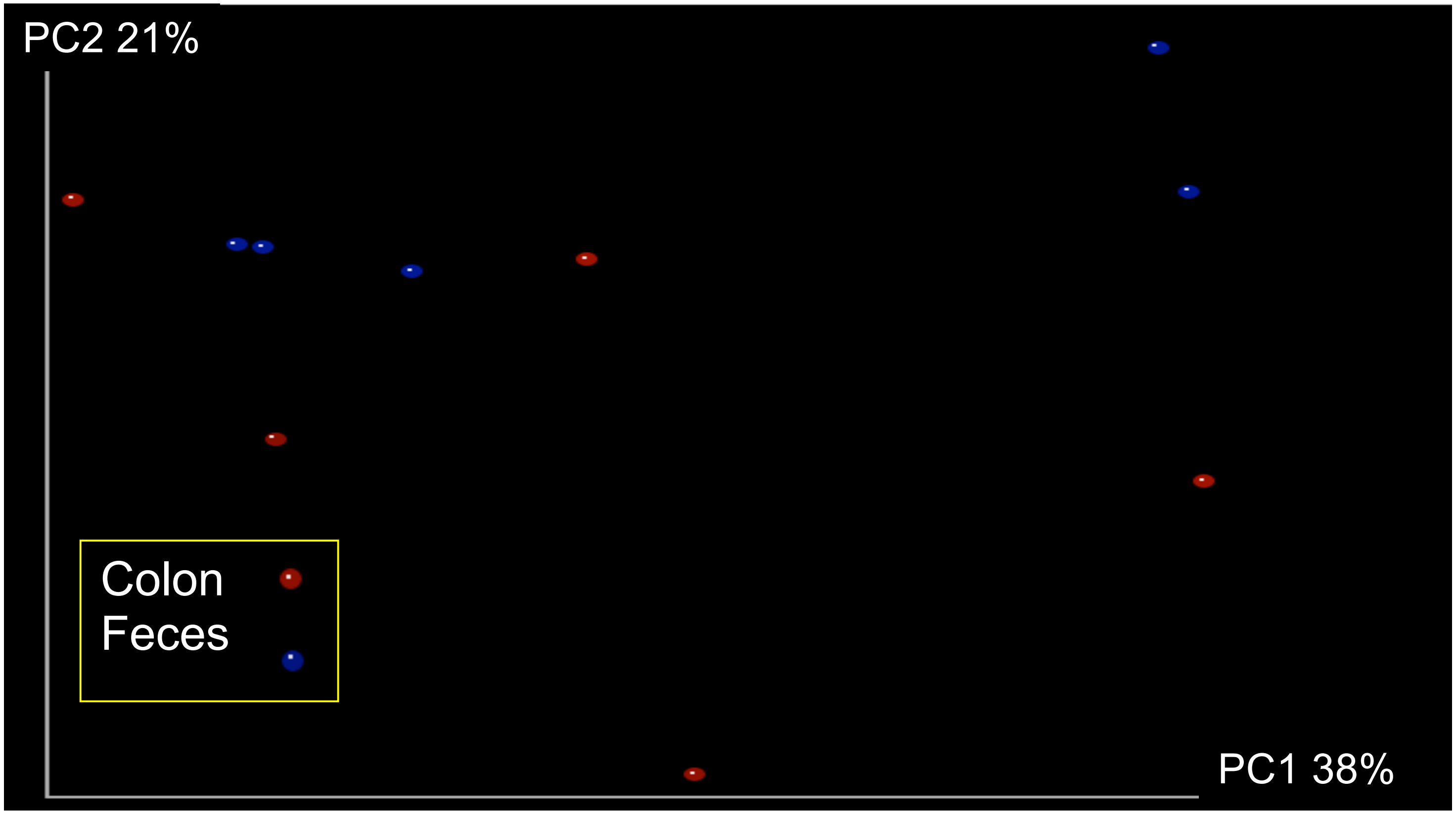


Figure S3. PCoA of bacterial communities from colon content (red) and feces (blue) from 5 adult female mice.

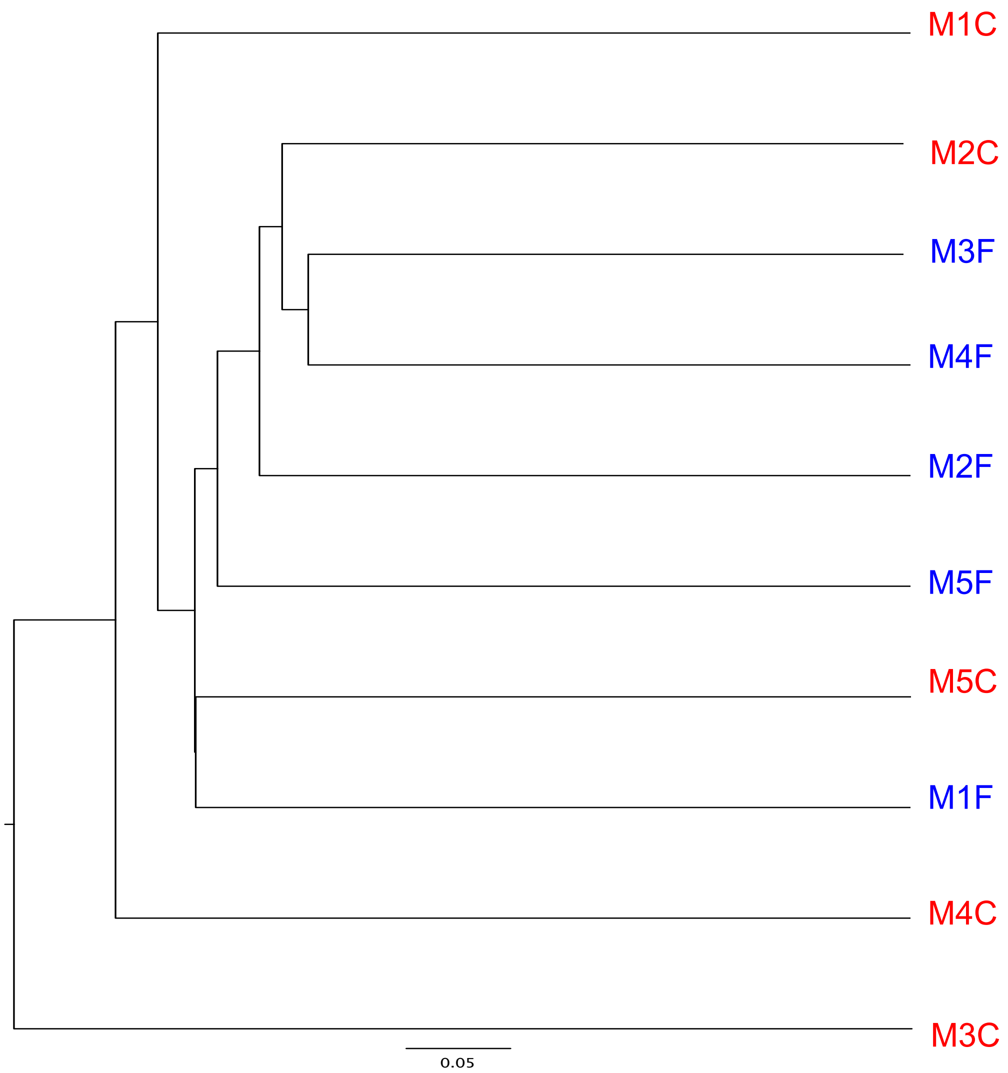


Figure S4. Unweighted Pair Group Method with Arithmetic Mean (UPGMA) cluster of bacterial communities from colon contents of female adult mice (red) and feces (blue).

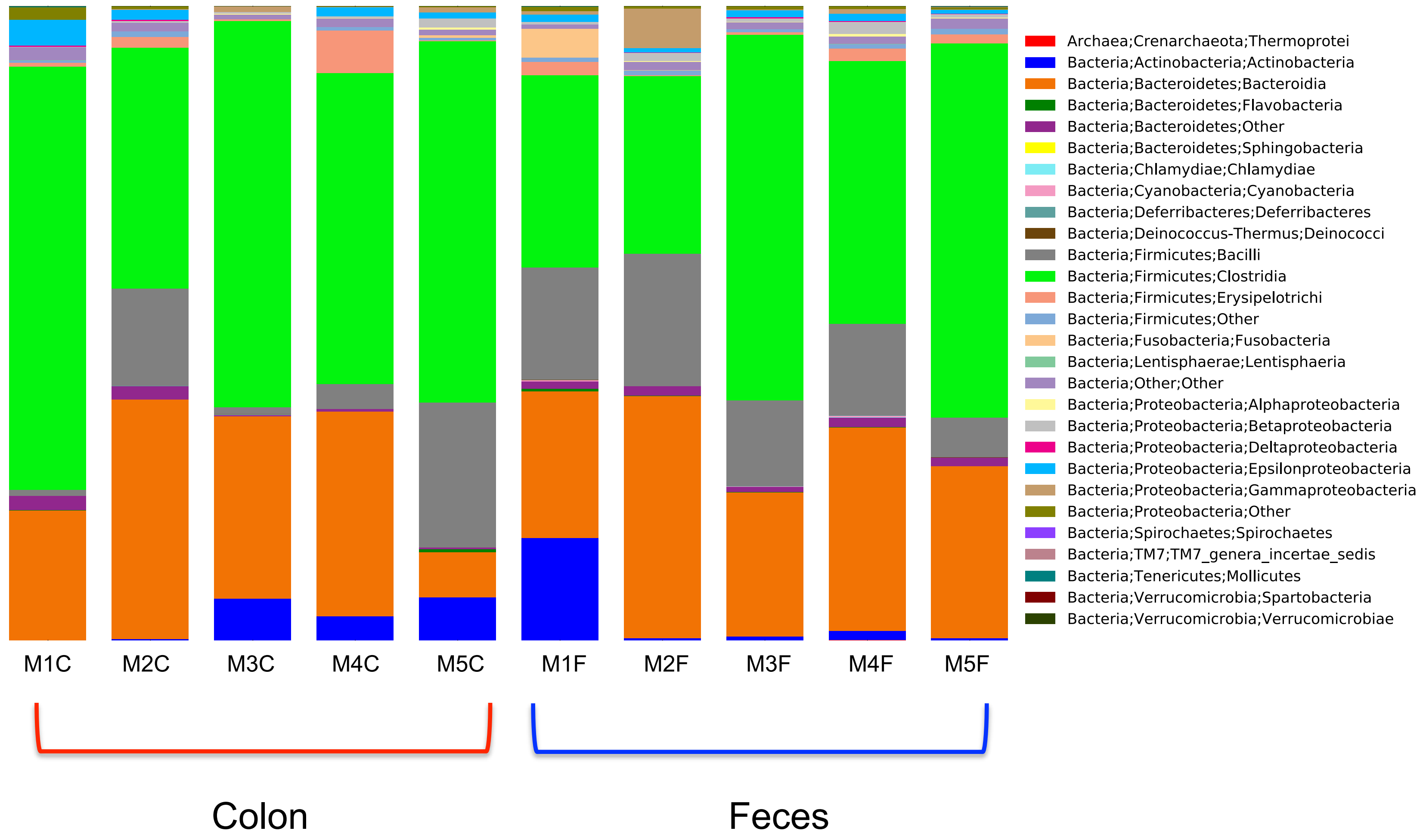


Figure S5. Proportions of colon and feces bacterial taxa from female adult mice.

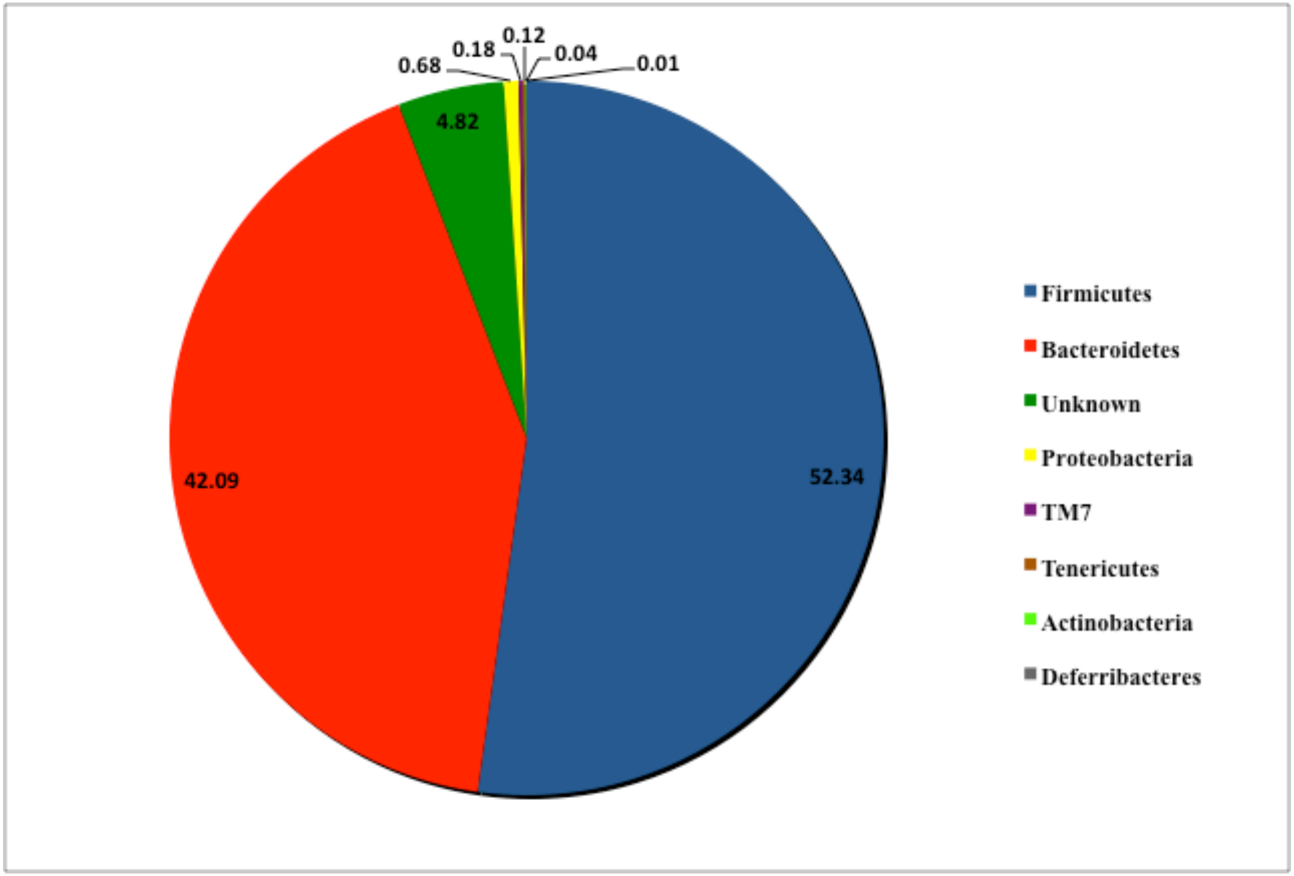


Figure S6. Distribution of 7 fecal bacterial phyla found in 6 adult female mice aged 21 days, Unknown represents short reads with no assigned taxonomy by RDP, below the level of Bacteria.

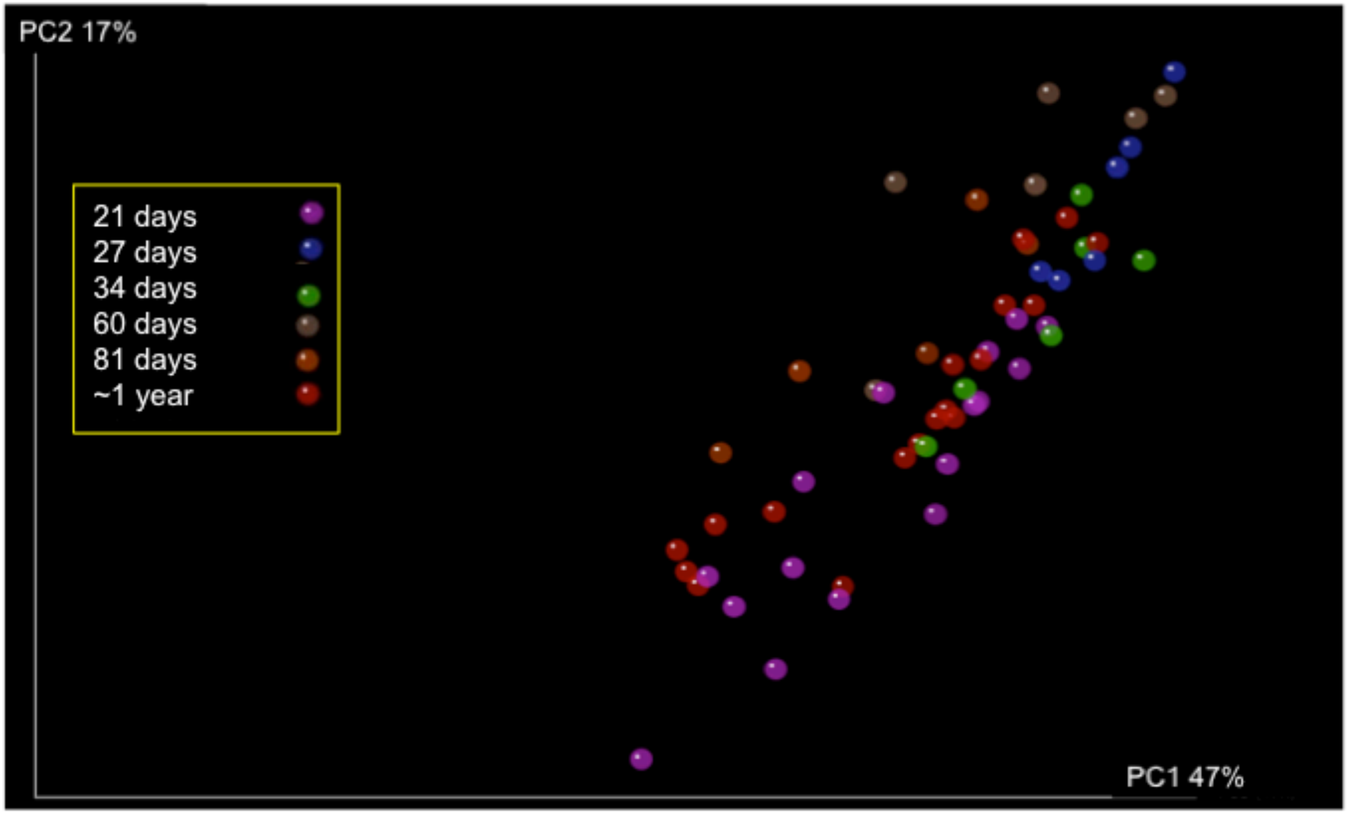


Figure S7. PCoA of the fecal bacterial communities of adult mice as they age (21, 27 34, 6, 81 days and 1 year).

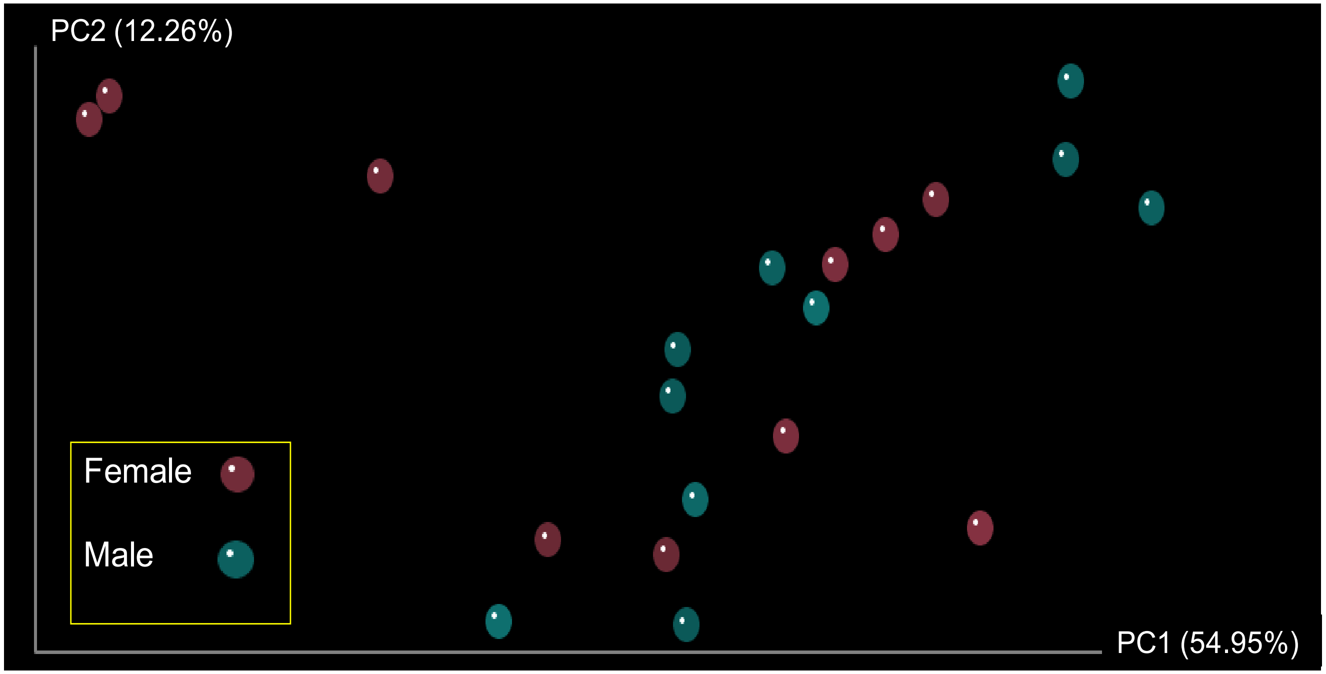


Figure S8. PCoA of fecal bacterial communities of adult, 21-28 day old females and males.

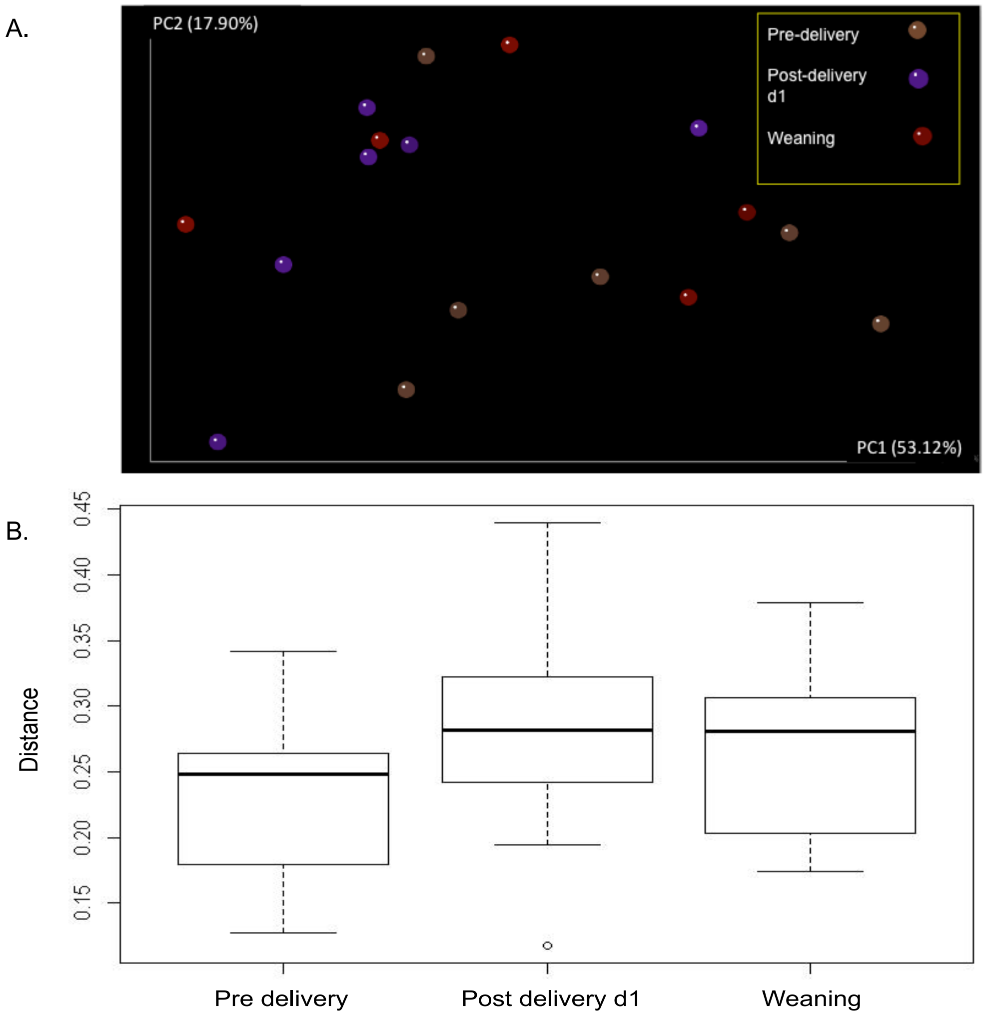


Figure S9. Fecal bacterial communities in 6 female mice. Mothers at different stages (prior to pregnancy (34 days old) andpost-delivery day 1 and 21 (weaning). A) PCoA; B) Pairwise Unifrac distances. Differences are not significant (ANOVA *P*=0.124).

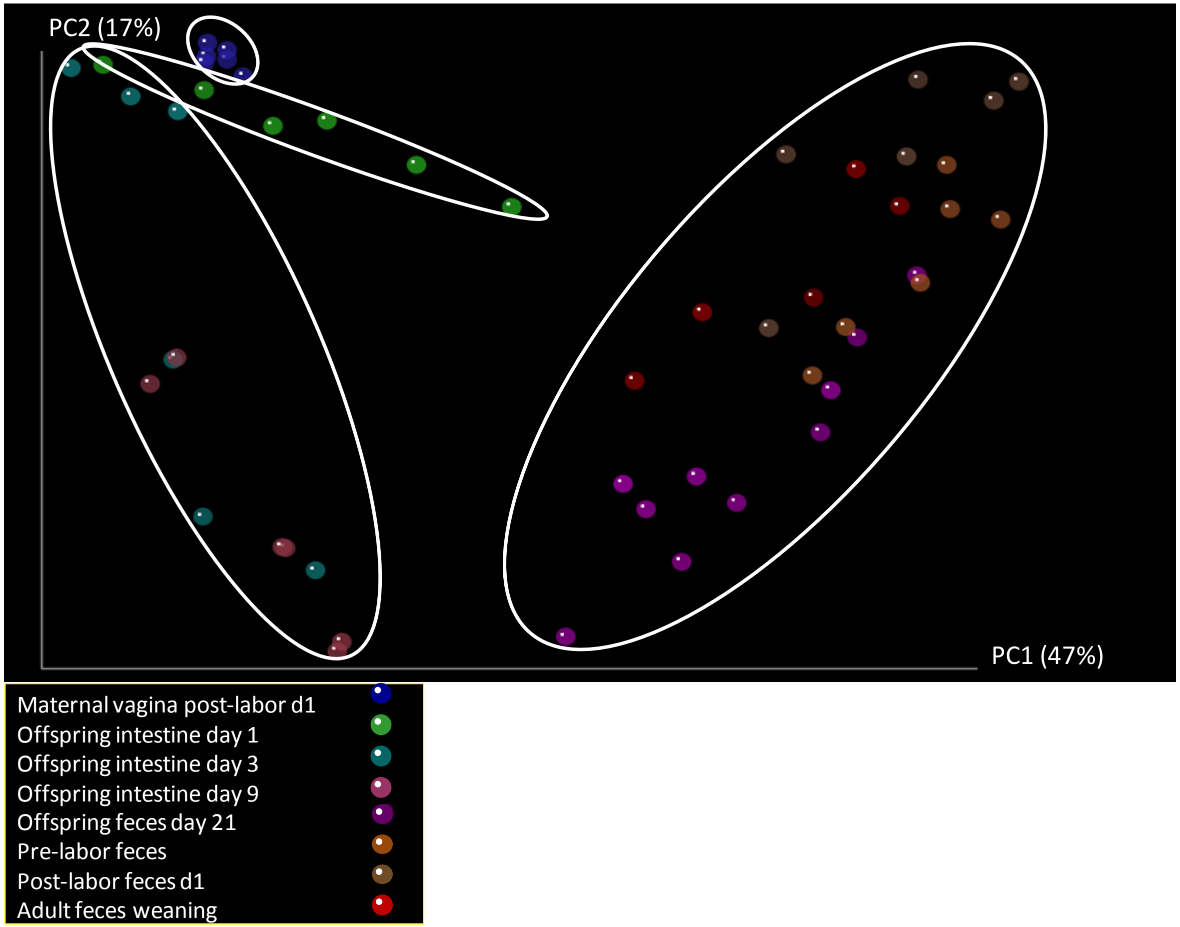


Figure S10. Principal Coordinates Analysis (PCoA) of the bacterial communities from the maternal vagina and feces and from offspring intestines.

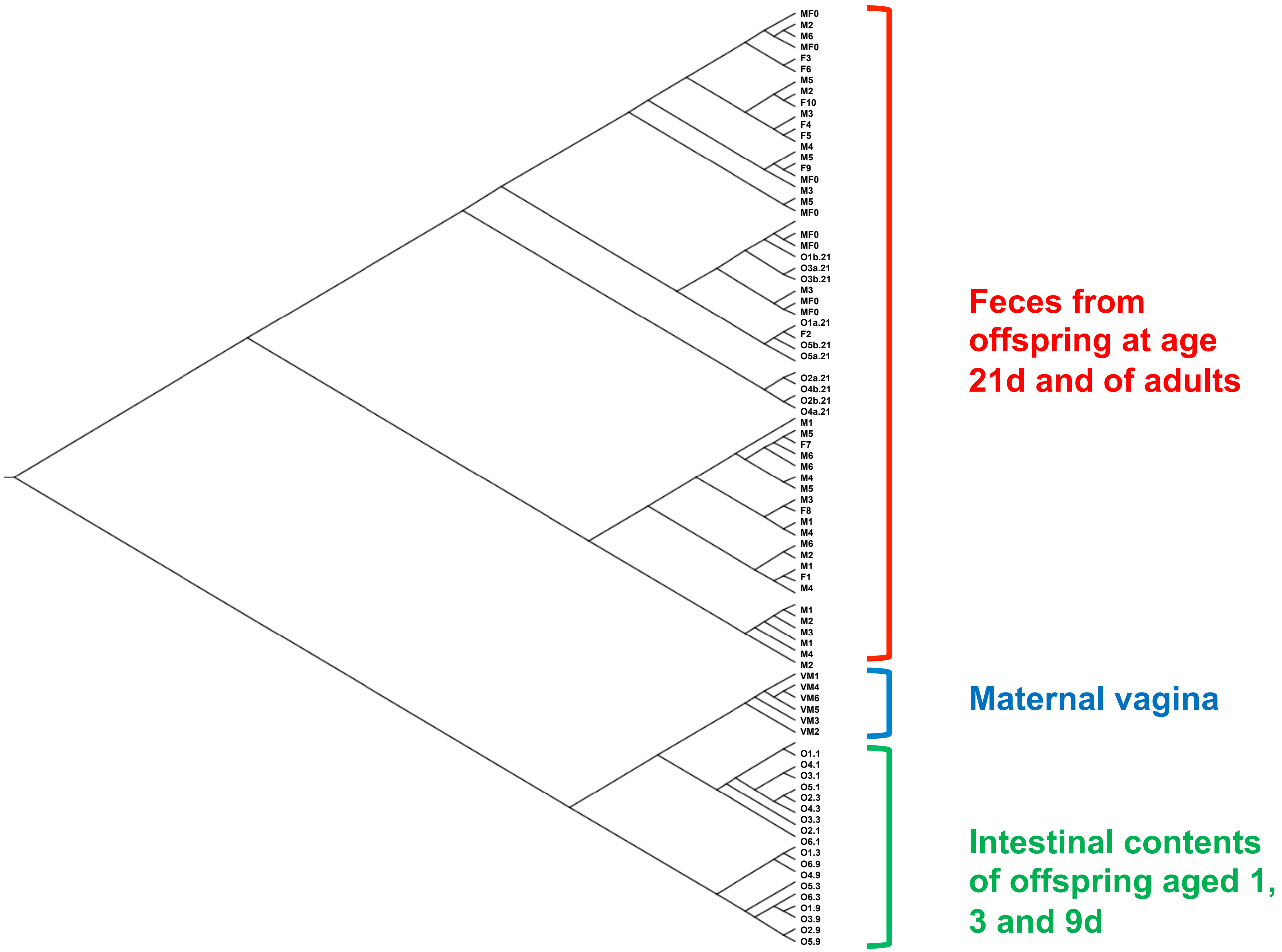


Figure S11. Unweighted Pair Group Method with Arithmetic Mean (UPGMA) cluster of bacterial communities from maternal vaginas, and maternal and offspring intestines and feces.

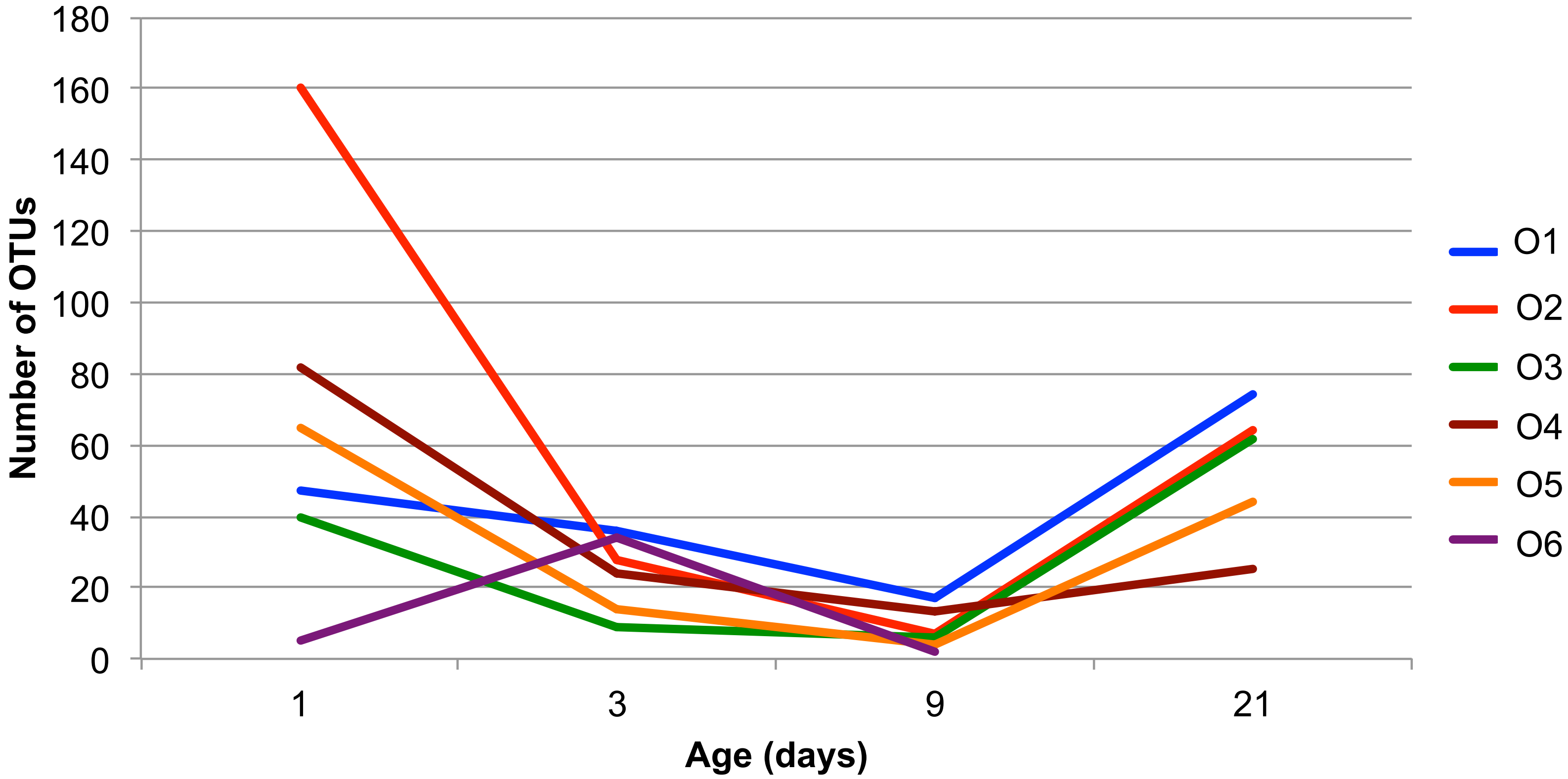


Figure S12. **Number of bacterial OTUs in each of the 6 newborn mice by age.** Six mice were sampled across the first 21 days of life.

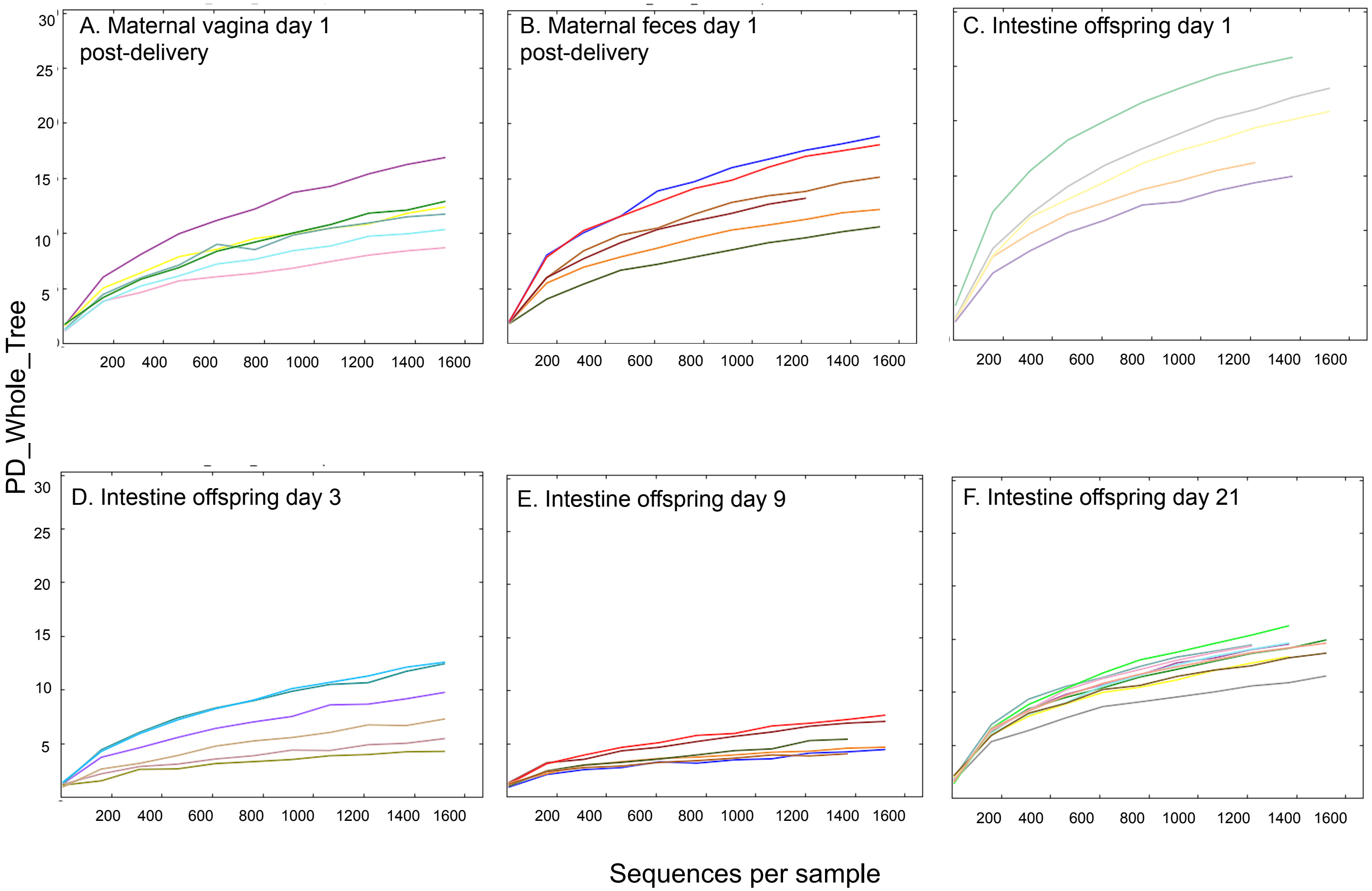


Fig S13. Rarefaction curves of Phylogenetic Diversity from mothers and offspring mice. Panel A, maternal vagina, day 1 post-delivery; Panel B, maternal feces, day 1 post-delivery; Panel C, offspring intestine, day 1 of life; Panel D, offspring intestine, day 3 of life; Panel E, offspring intestine, day 9 of life; Panel F, offspring intestine, day 21 of life.

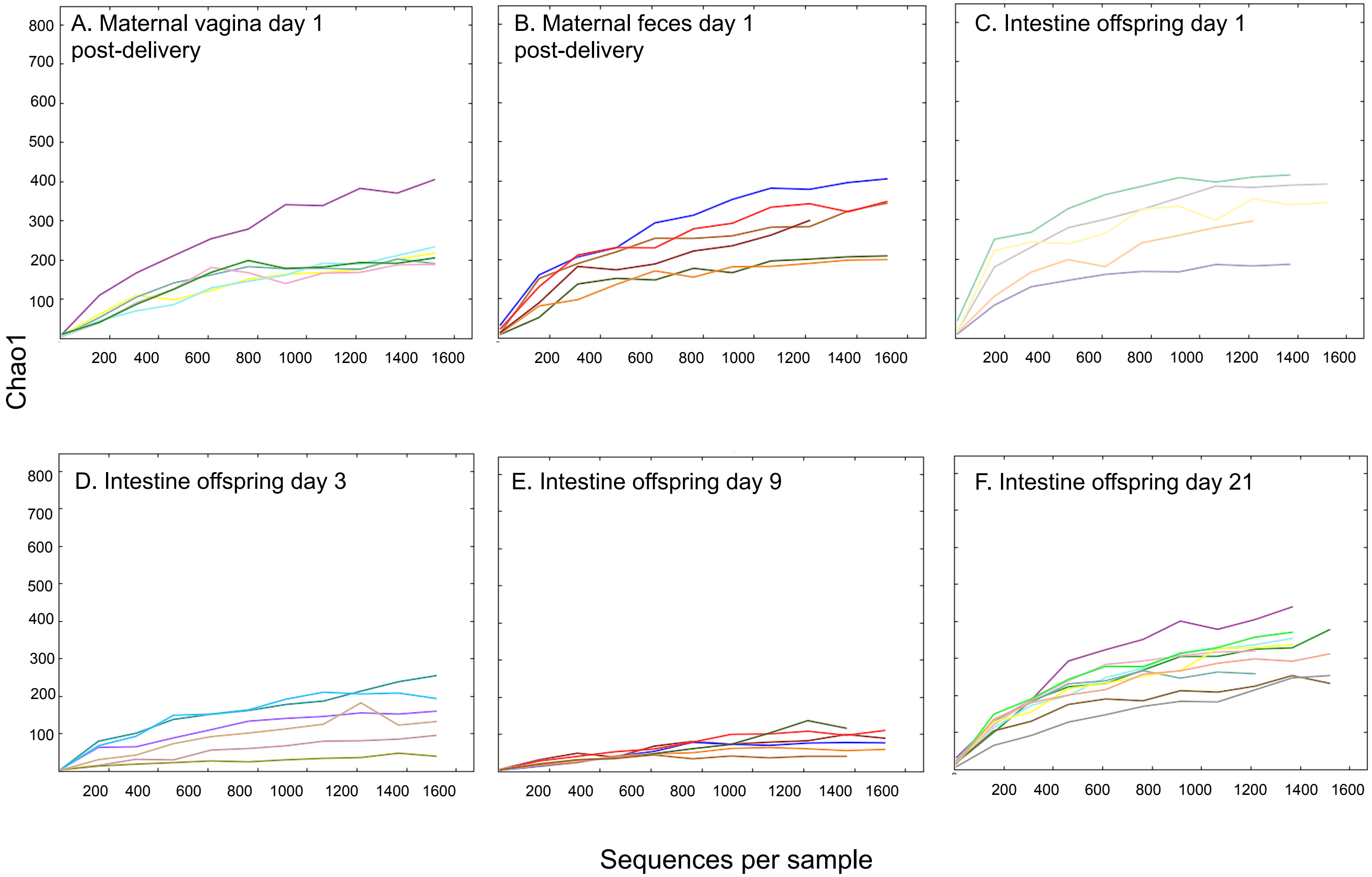


Fig S14. Rarefaction curves of Chao1 from mothers and offspring mice. Panel A, maternal vagina, day 1 post-delivery; Panel B, maternal feces, day 1 post-delivery; Panel C, offspring intestine, day 1 of life; Panel D, offspring intestine, day 3 of life; Panel E, offspring intestine, day 9 of life; Panel F, offspring intestine, day 21 of life.

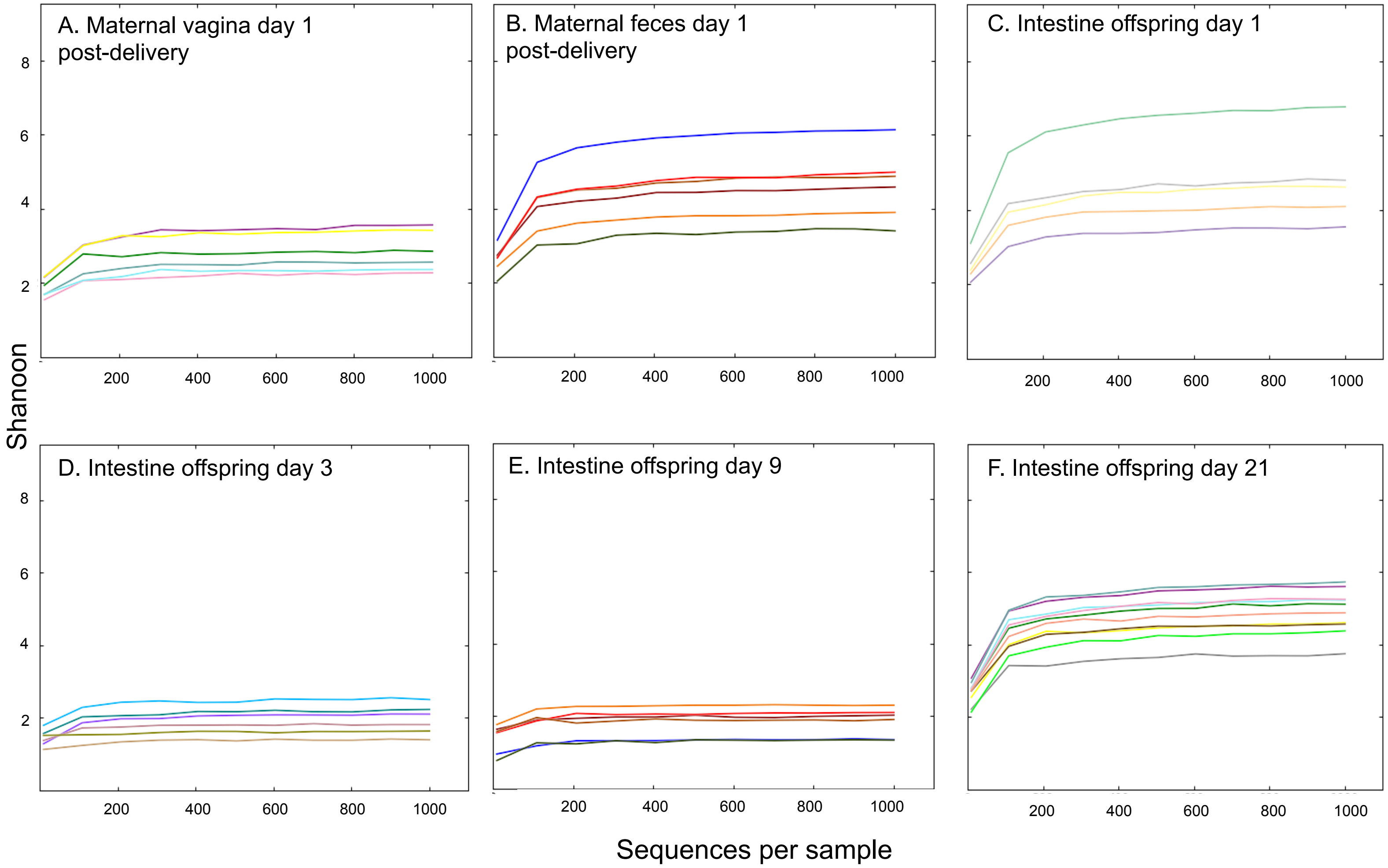


Fig S15. Rarefaction curves of Shannon index from mothers and offspring mice. Panel A, maternal vagina, day 1 post-delivery; Panel B, maternal feces, day 1 post-delivery; Panel C, offspring intestine, day 1 of life; Panel D, offspring intestine, day 3 of life; Panel E, offspring intestine, day 9 of life; Panel F, offspring intestine, day 21 of life.