

## Supplementary Materials for **Increased weight gain by C-section: Functional significance of the primordial microbiome**

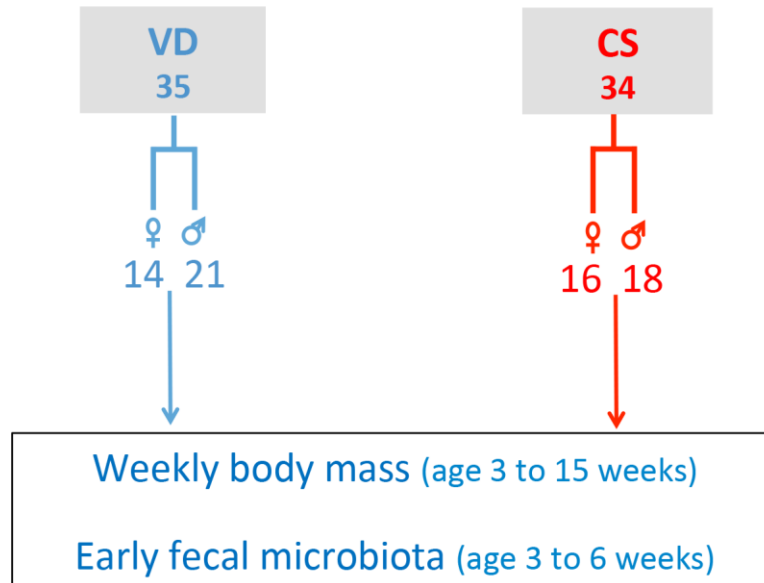
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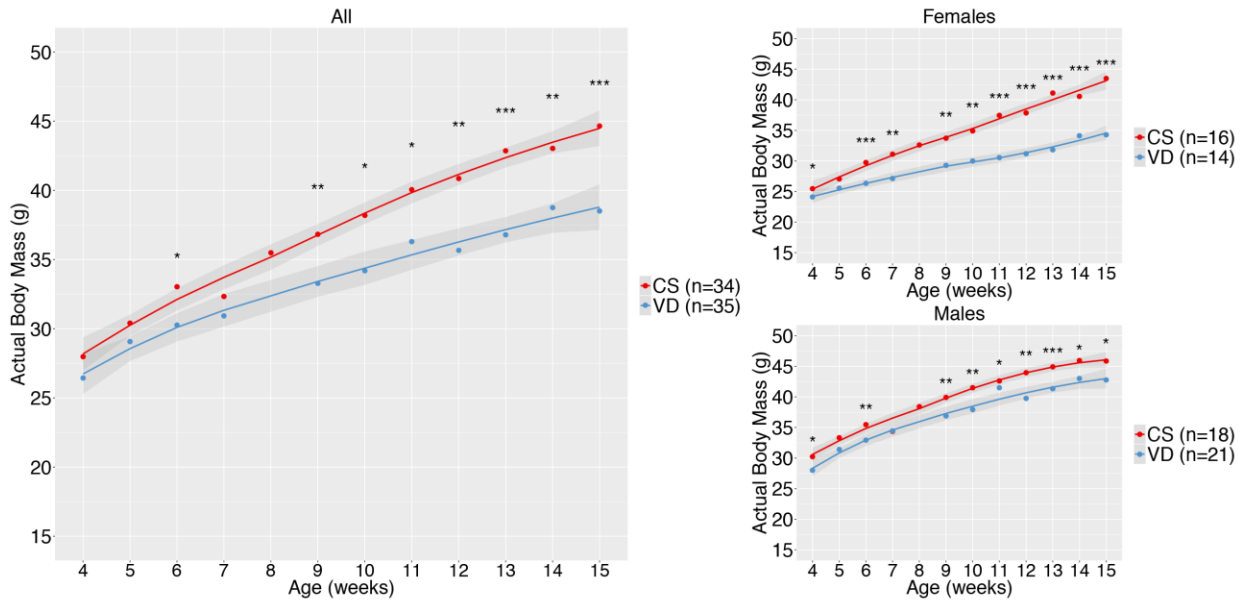
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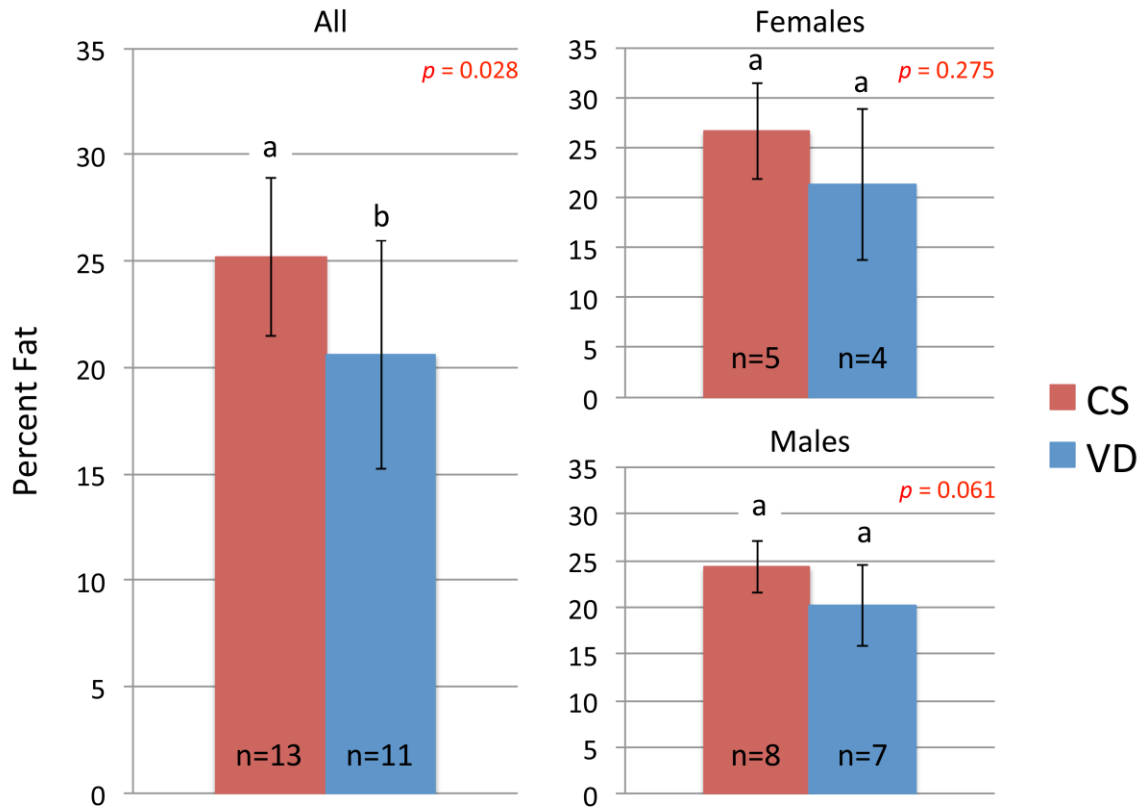
Supplementary Figures.



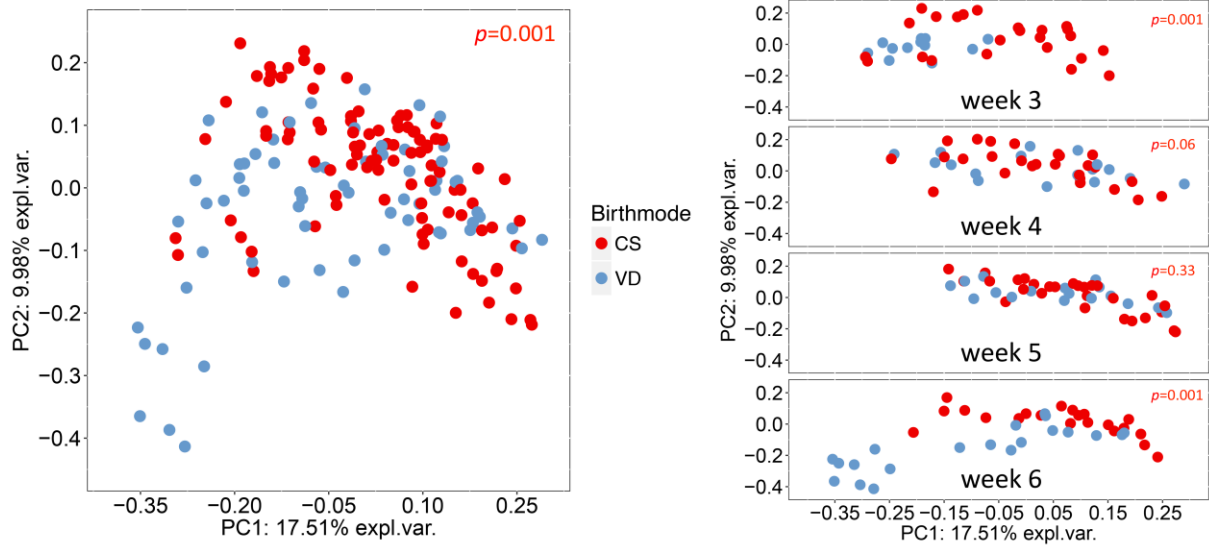
**fig. S1. Experimental design.** A total of 69 mice, 35 born vaginally from 8 families and 34 born by C-section from 5 families, not exposed to antibiotics, were followed to track their developmental body mass and gut microbiome structure.



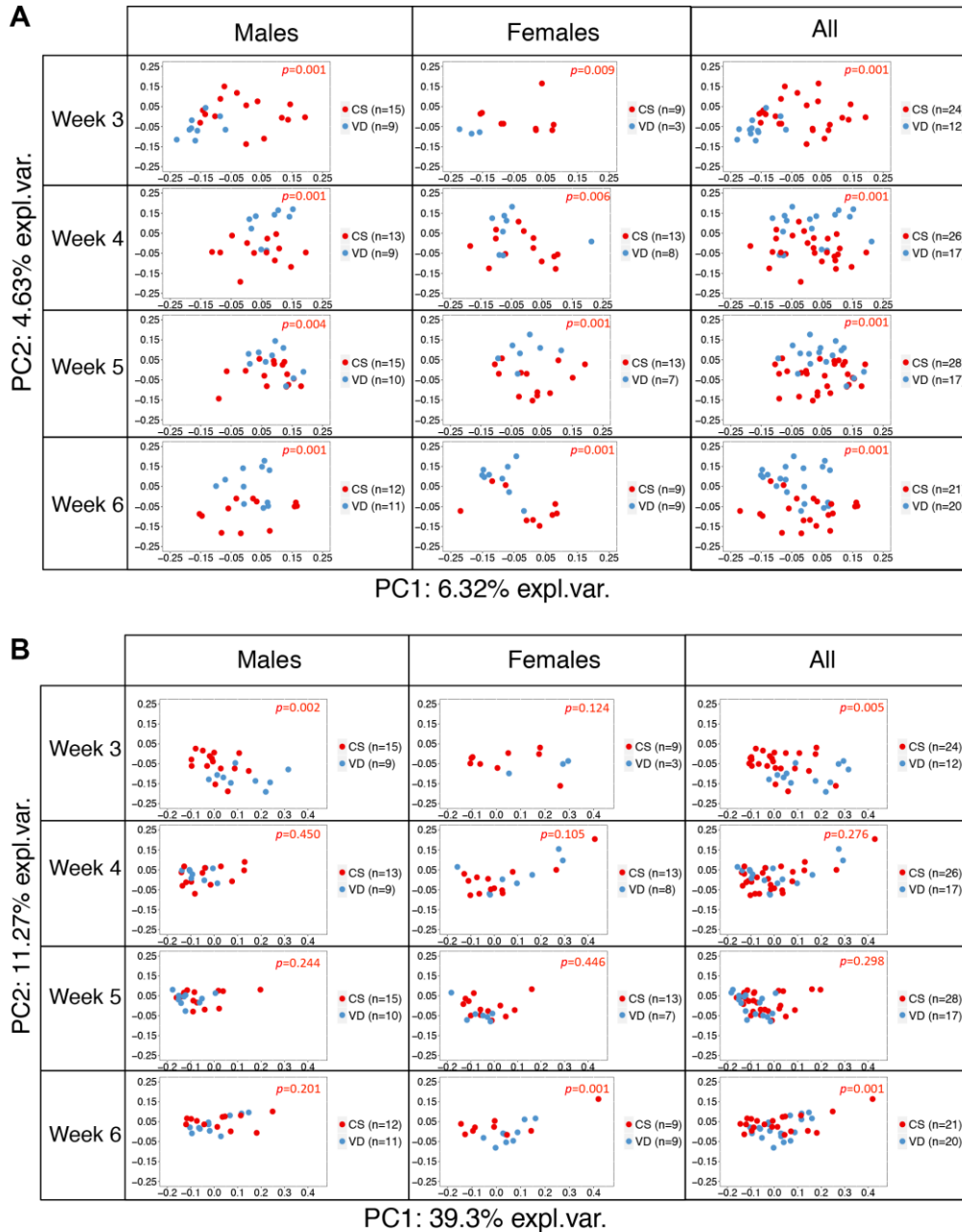
**fig. S2. Actual body mass in 69 mice discordant to birth mode during early development.** Actual body mass (g). Lines represent average, and shaded area are 95% confidence interval. Significance was determined by ANOVA test, resampling (\* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ ). C-section (CS) mice show increasingly higher body weight during development from weaning, than vaginally delivered (VD) controls. Females have a stronger weight gain phenotype.



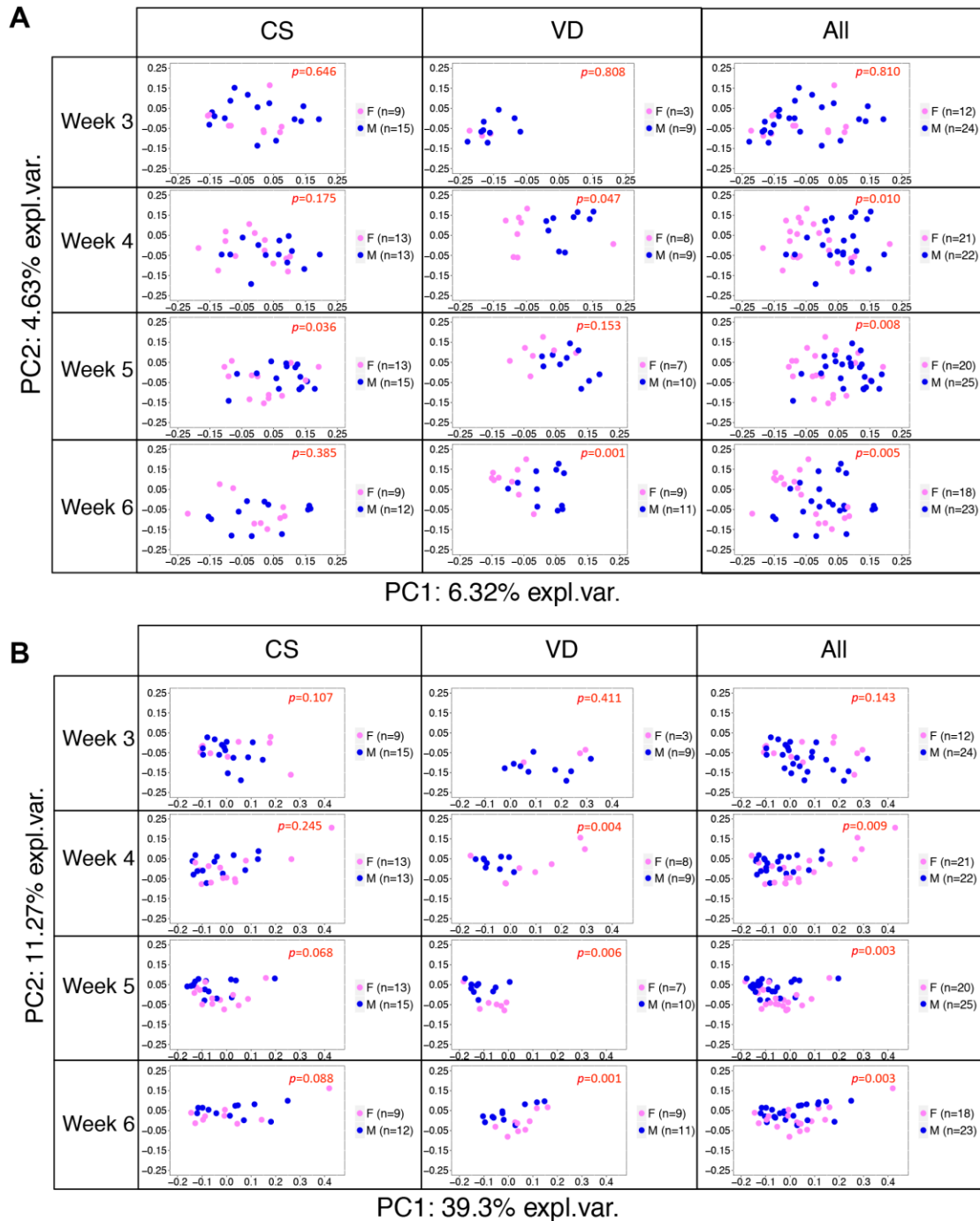
**fig. S3. Percent fat in 24 mice discordant to birth mode at 15 weeks of age.** Body fat percentage was determined by DEXA. Significance was determined by two-sample T-test  $p$ -value  $< 0.05$ . C-section mice have a higher percentage of fat at week 15 than vaginally delivered controls, although differences were not statistically significant within each gender.



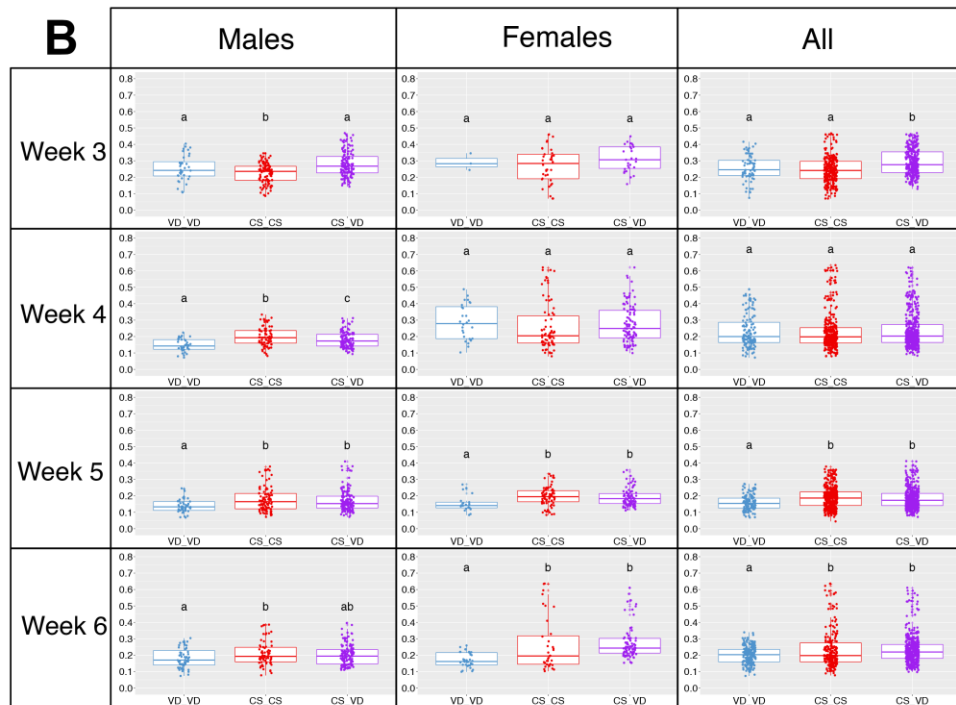
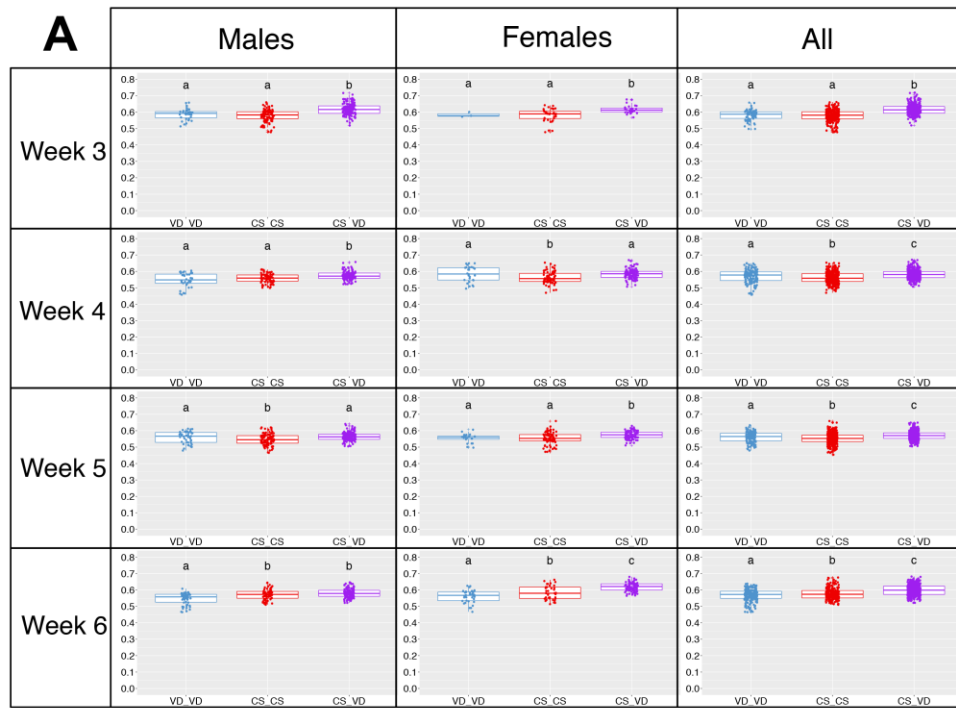
**fig. S4. PCoA of Bray-Curtis distances between fecal communities from weeks 3 to 6 in mice born vaginally or by C-section.** Fecal microbial communities at all time points by delivery mode (left panel) showed segregation that was birth mode dependent. Fecal microbial communities at each time point by delivery mode (right panels) showed segregation by birth mode at all time points except weeks 4 and 5. Significance was determined pairwise by an ANOSIM test with resampling 999 times.



**fig. S5. Fecal  $\beta$  diversity in males and females discordant to birth mode at different ages. (A) Unweighted, and (B) Weighted UniFrac distances- Principal Coordinate Analysis (PCoA). Significance was determined by ANOSIM test, resampling 999 times. Gut microbiotas segregate by birth mode more markedly in unweighted than in weighted UniFrac distances, indicating that relative proportions diminish the dissimilarities.**

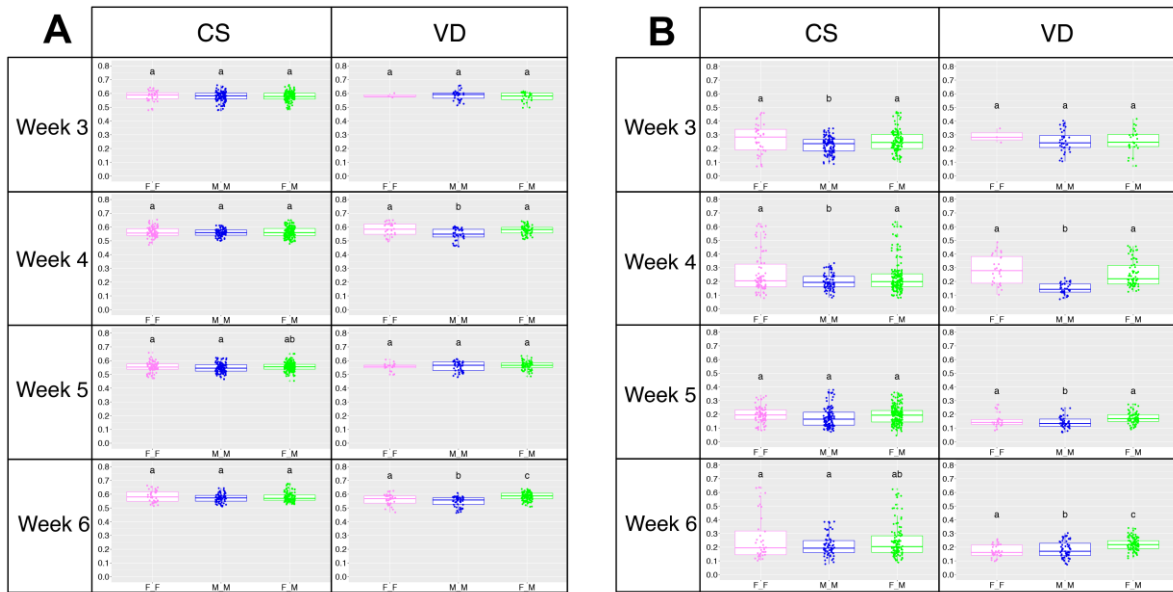


**fig. S6. Fecal  $\beta$  diversity in mice delivered by C-section or vaginally born controls by gender, at different ages. (A) Unweighted, and (B) Weighted UniFrac distances-PCoA. Significance was determined by ANOSIM test, resampling 999 times. Only VD not CS fecal communities, segregate by gender by week 6 (fig. S4). Gut microbiota gender segregation is more marked in weighted than in unweighted UniFrac distances, indicating dissimilarities are in relative proportions rather than in composition of bacteria.**

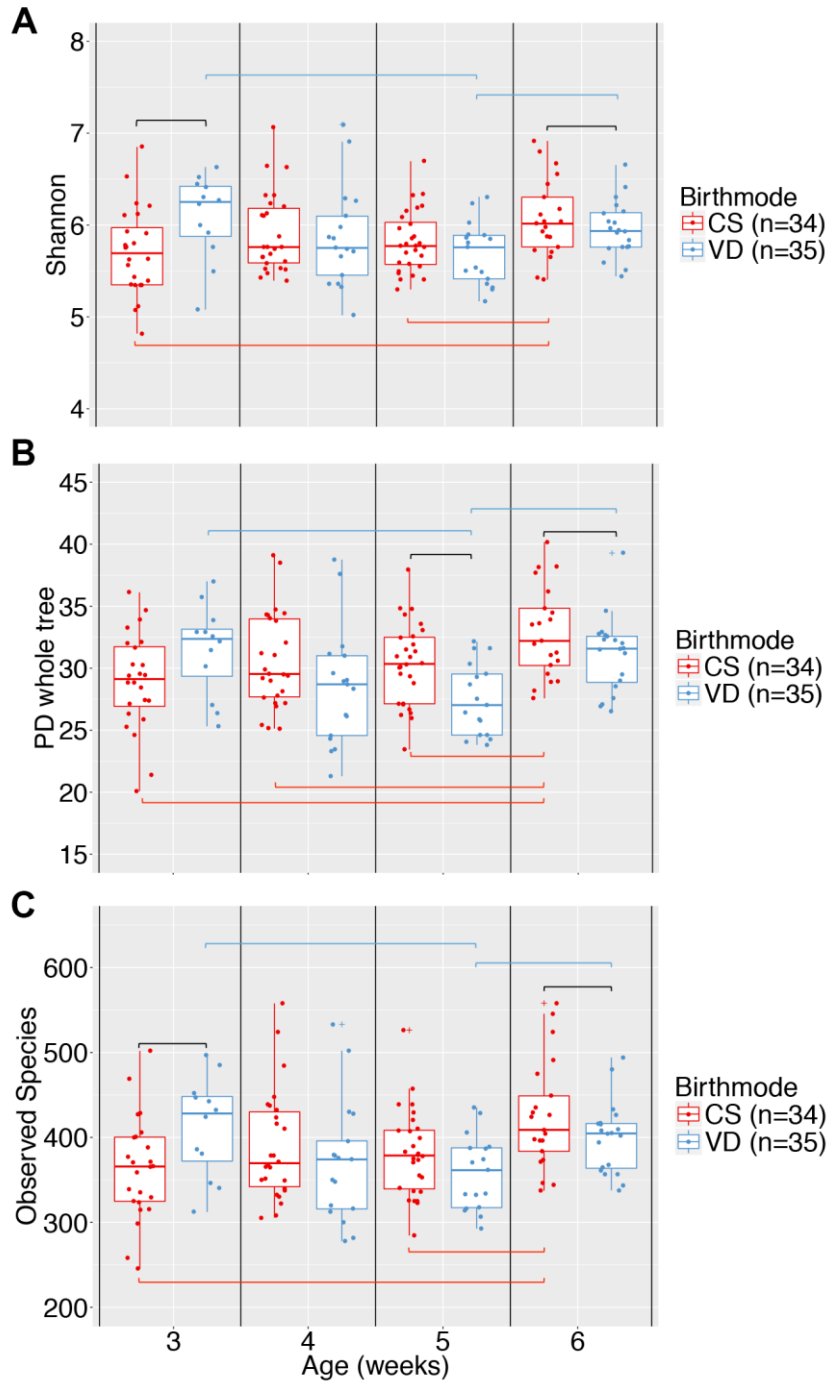


**fig. S7. Fecal intra- and inter-birth-mode-group UniFrac community distances in male and female mice at different ages. (A) Unweighted, and (B) Weighted UniFrac distances. Significance was determined by ANOVA test, resampling 999 times. At week 6, intergroup distances are bigger in females than in males.**



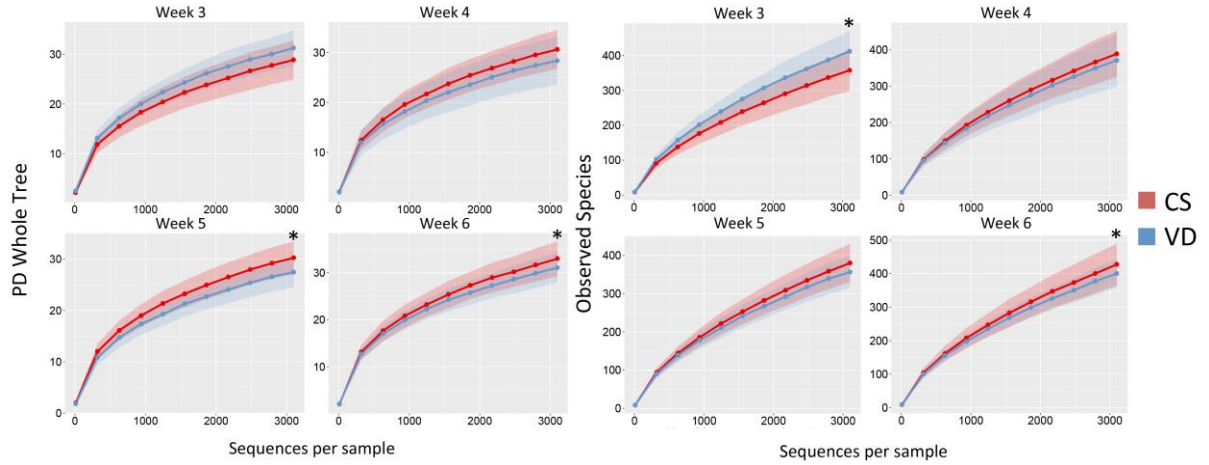


**fig. S8. Fecal intra- and inter-gender-group UniFrac community distances in C-section and vaginally born controls at different ages. (A) Unweighted (B) weighted UniFrac distances. Significance was determined by ANOVA test, resampling 999 times. Gender distances within birth groups were greater in unweighted than in weighted UniFrac. In weighted distances, intragroup distance in males are lower than in females ( $p < 0.05$ ), except in Week 5 and week 6 CS mice.**

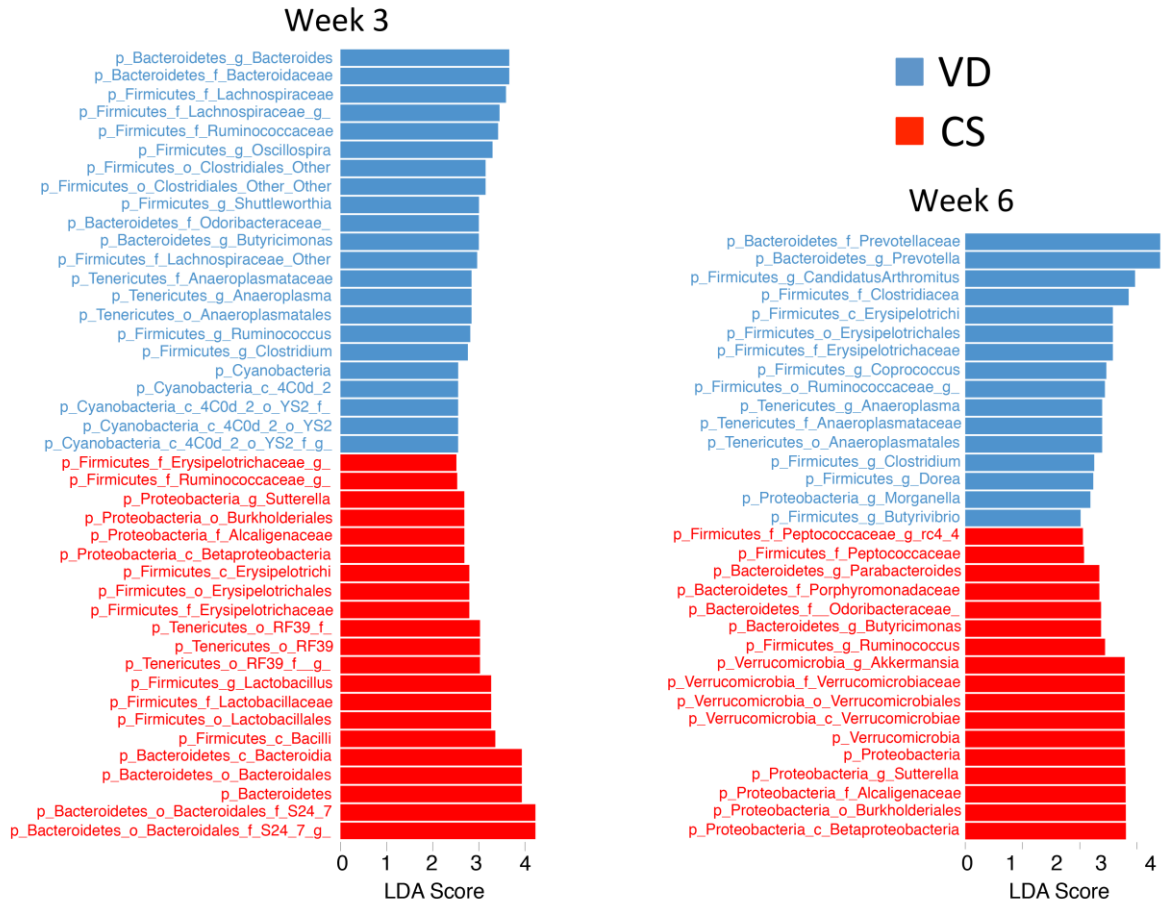


**fig. S9. Fecal  $\alpha$  diversity in mice discordant to birth mode at different ages.**

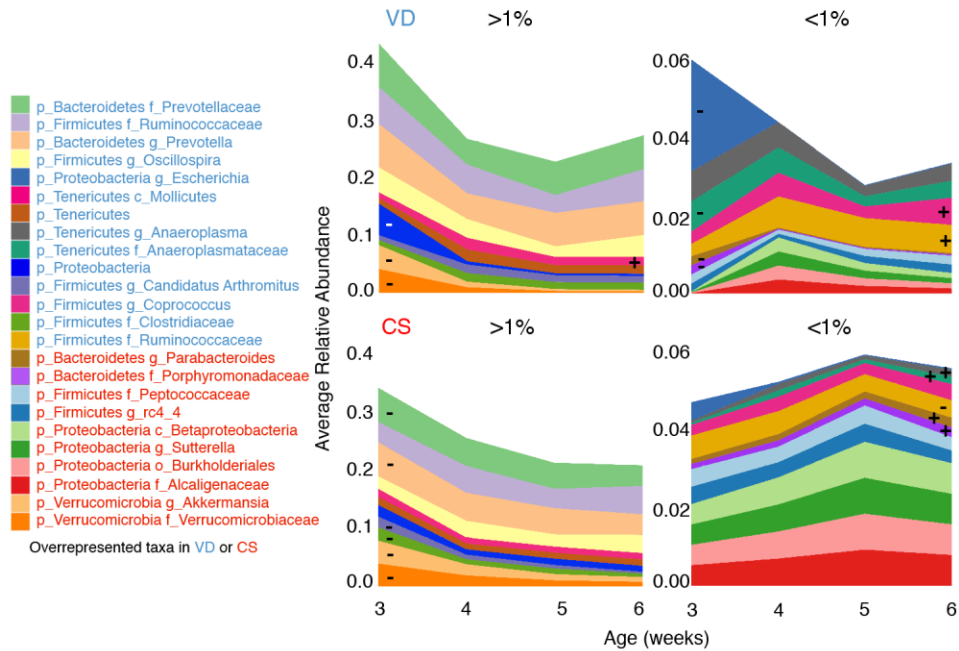
(A) Shannon, (B) Phylogenetic diversity (PD Whole tree) and (C) Observed species for ages 3 to 6 weeks. Significance was determined by ANOVA test, resampling 999 times. Feces from C-section mice have lower alpha diversity at weaning, but higher at week 6 of age, in relation to control mice.



**fig. S10. Rarefaction curves of PD whole tree and observed species  $\alpha$  diversity metrics.** Maximum rarefaction was 3,100 sequences per sample, and curves still had a slope, indicating insufficient sequencing depth to capture the whole sample 16S diversity. Brackets and asterisks indicate significant differences by delivery mode.



**fig. S11. LEfSE of communities at weaning (week 3) and young adulthood (week 6) by delivery mode.** LDA of communities at weaning (week 3, left panel) and young adulthood (week 6, right panel) by delivery mode.



**fig. S12. Average relative abundance of 24 birth mode LEfSe-discriminant taxa over 3 to 6 weeks of age.** Time-course taxa plots show decrease in dominant taxa such as *Akkermansia* and *Verrucomicrobiaceae* regardless of birth mode, with more significant decrease in taxa from C-section mice.

**table S1. Number of fecal specimens from mouse lineages differing in birth mode.**

N samples per delivery mode and time-point									
Delivery Mode	Week 3		Week 4		Week 5		Week 6		All
	F	M	F	M	F	M	F	M	
C-section Delivery	9	15	13	13	13	15	9	12	104
Vaginal Delivery	3	9	8	9	7	10	9	11	71
Total	36		43		45		41		175