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Supplemental Information

Delivery Mode Affects Stability of Early Infant Gut Microbiota

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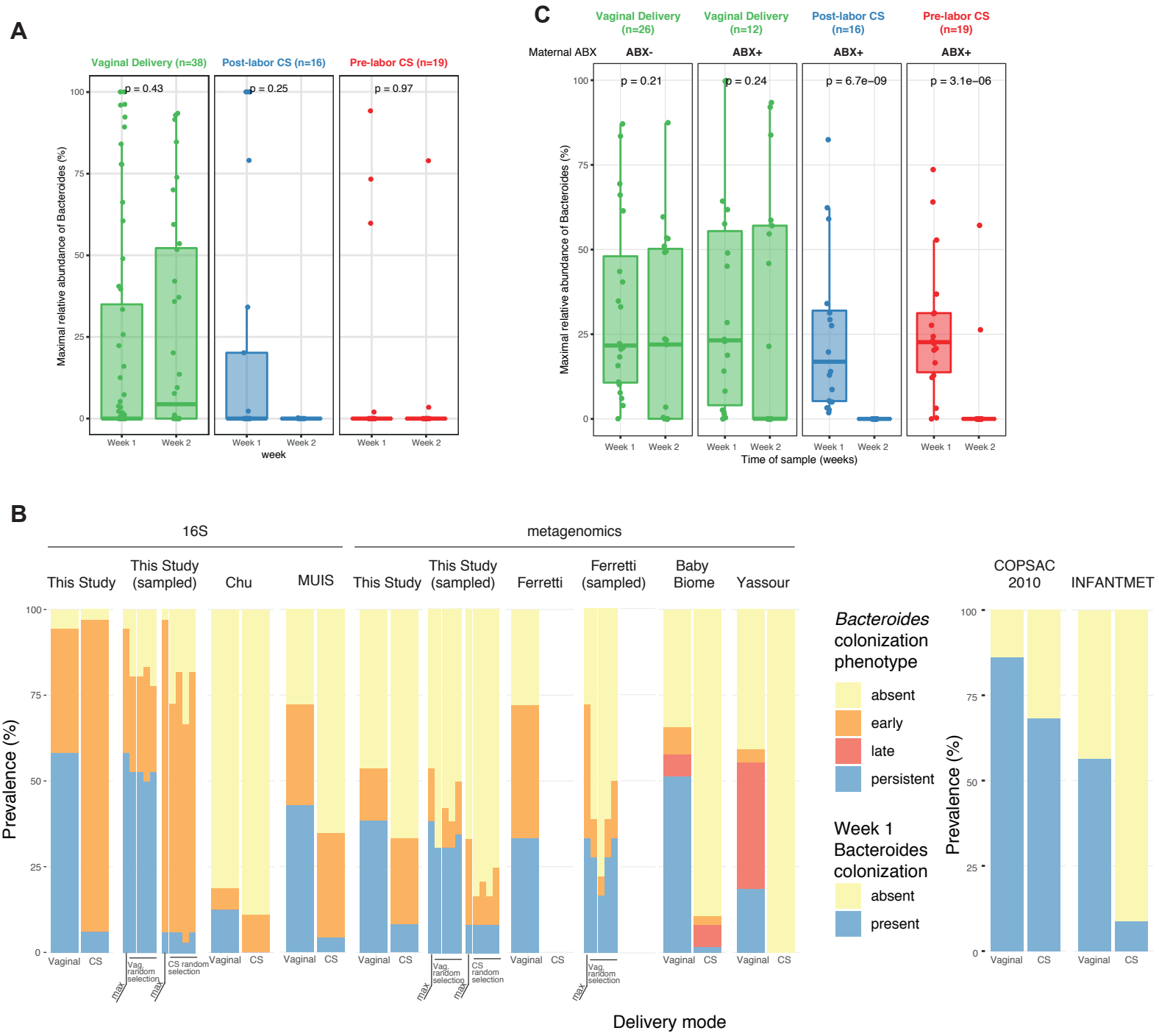


Figure S1. *Bacteroides* relative abundance through time (week 1 vs. week 2), prevalence and persistence across different cohorts. Related to Figure 1C-D. (A) Comparison of maximal relative abundance of *Bacteroides* in week 1 vs. week 2 infant stool samples, as measured by metagenomic sequencing, within delivery mode. **(B)** A comparison with previously published data, using publicly available sequences from cohorts with samples from week 1 of life and a later time point to assign *Bacteroides* colonization phenotype (early-only, persistent, absent or late-only). In our data and the single other study with multiple week 1 time points, assignments were made based either on maximal abundance, or a randomly selected time point to demonstrate how a single sample is less likely to identify *Bacteroides* colonization in early life. The mean proportion of "early only" using maximal abundance in our vaginal deliveries, cesarean deliveries, and the Ferretti et al study (26%) was significantly different than the mean proportion of "early only" using random sampling (11%, p = 0.002). An additional comparison of week 1 *Bacteroides* detection between vaginal and C-section infants was made in two cohorts which had only that early time point, further demonstrating the range of *Bacteroides* detection in C-section delivered infants. The cohorts included a study of 81 infants from Houston, TX evaluated at day 1 and week 6 (Chu et al.[14]), a Danish cohort of 120 infants evaluated at day 1 and week 2 (MUIS et al.[12]), a cohort of 25 Italian mothers and infants with testing at birth, day 1, 3, 7 and 1 month (Ferretti et al.[18]), a cohort of 596 British infants evaluated at 4, 7 and 21 days (BabyBiome[11]), and a study of 44 Finnish infants measured at birth and 14 days (Yassour et al.[19]). We also compared week 1 Bacteroides detection in two cohorts without a follow-up sample, one that included 700 infants from Copenhagen (COPSAC2010[37]) and a cohort of 153 infants from Cork, Ireland (INFANTMET[38]). Detailed information regarding these cohorts is found in Table S3. **(C)** Comparison of maximal abundance of Bacteroides in week 1 vs. week 2 infant stool samples within vaginally delivered infants whose mothers did (N = 18) or did not (n = 20) receive antibiotics during labor, or infants delivered by pre- or post-labor C-section (all of whose mothers received antibiotics prior to delivery).

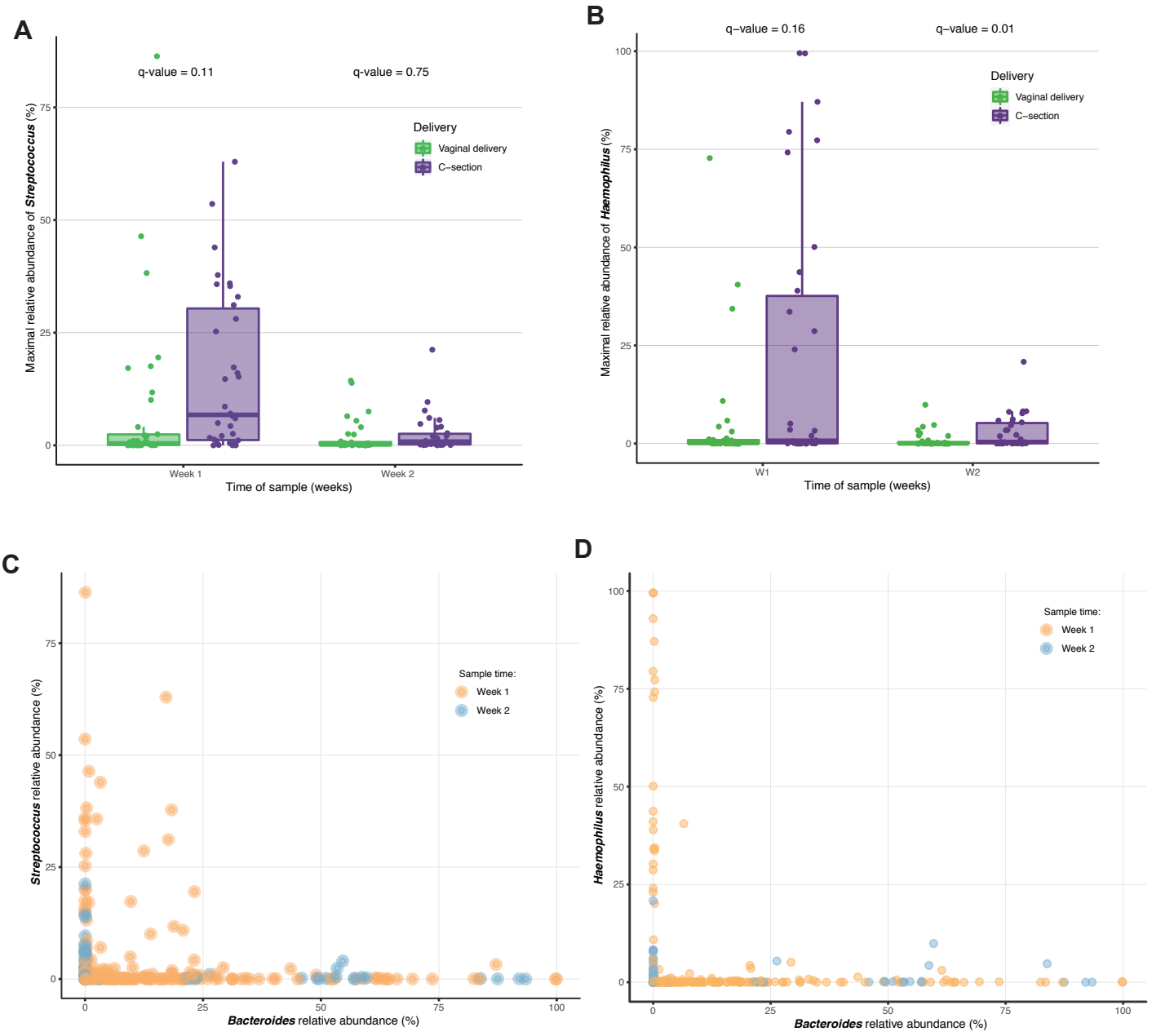
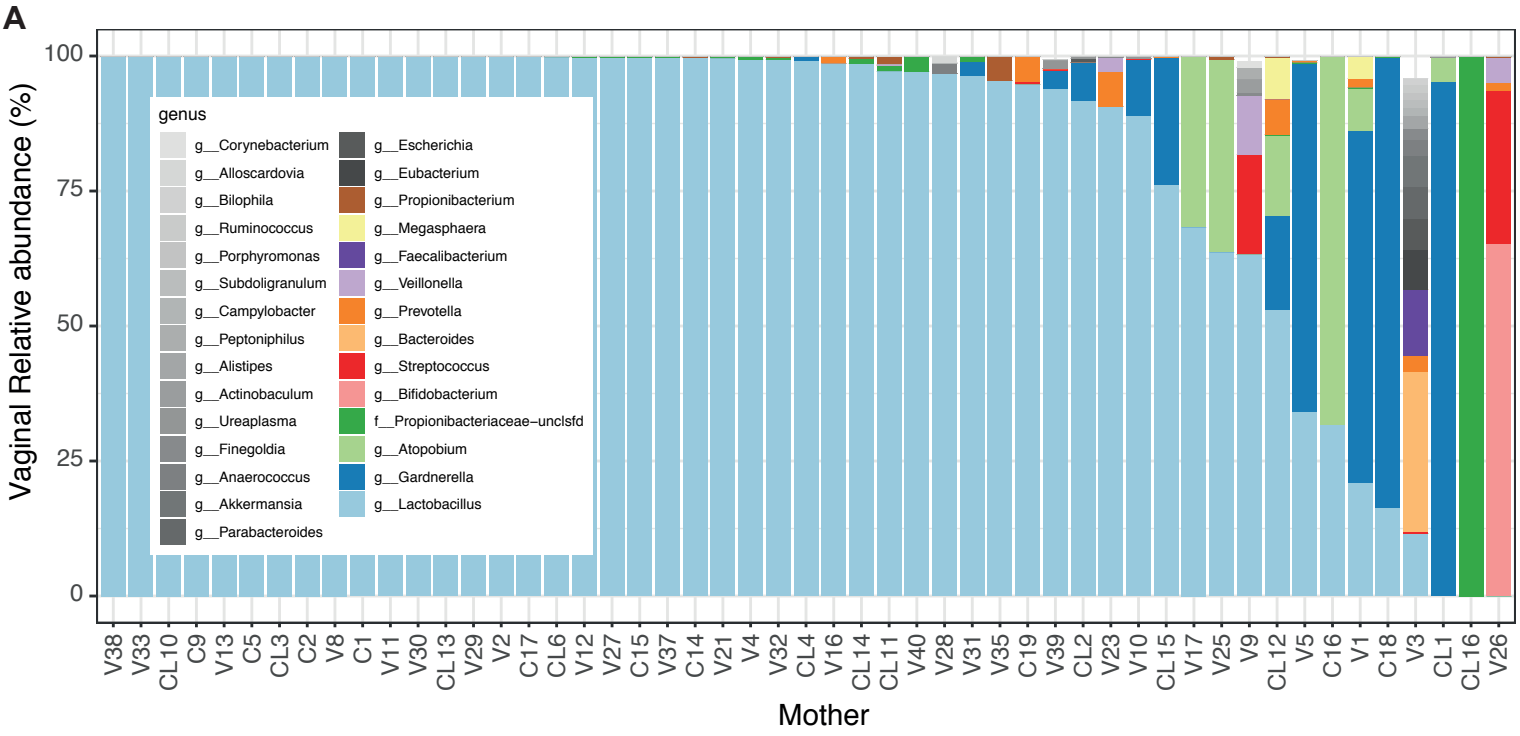


Figure S2. Analysis of the relative abundance of *Streptococcus* and *Haemophilus* exemplifying the link with *Bacteroides*. Related to Figure 1B. Maximal relative abundance of *Streptococcus* **(A)** and *Haemophilus* **(B)** measured by 16S rRNA sequencing compared between vaginally delivered and C-section delivered infants at week 1 or week 2. Q-values calculated by linear regression, accounting for the feeding of each infant (using MaAsLin2, see Methods). Relative abundance of **(C)** *Streptococcus* vs. *Bacteroides* or **(D)** *Haemophilus* vs. *Bacteroides* in week 1 and week 2 infant stool samples.



FigureS3. Vaginal microbiome composition of mothers and their detected species transmission to infants. Related to Figure 1B. (A) Genus-level microbiome composition of maternal vaginal swabs collected prior to delivery, using metagenomic sequencing. We included the top 29 vaginal taxa across all samples. **(B)** A comparison of shared species between maternal vaginal samples and infant stool samples in 25 dyads (as in Figure 2D, only for the vaginal samples). Bold numbers indicate shared species, in parentheses is noted whether this was a vaginal delivery (V) or C-section after labor (CL). Only one species (*B. breve*) in one family (V26) had enough sequencing coverage in each sample to actually test for strains.

a

	Absent (N = 3)	*Early-only* (N = 43)	*Persistent* (N = 23)	P value*
Delivery Mode				
Vaginal	2 (67%)	13 (30%)	21 (91%)	
Pre-labor C-section	1 (33%)	15 (35%)	2 (9%)	< 0.001
Post-labor C-section	0	15 (35%)	0	
Maternal race: White	3 (100%)	35 (81%)	17 (74%)	0.67
BMI	27 ± 6	27 ± 5	27 ± 7	0.93
Chorioamnionitis	0	6 (14%)	4 (17%)	0.71
Maternal antibiotics	2 (67%)	38 (88%)	10 (43%)	0.001
Meconium stained amniotic fluid	0	9 (21%)	7 (30%)	0.43
Birthweight	4243 ± 605	3484 ± 479	3592 ± 372	0.35
Infant antibiotics	0	7 (16%)	3 (13%)	0.68
Formula in hospital	2 (67%)	16 (37%)	6 (26%)	0.33
Exclusive breastfeeding at week 2	2 (67%)	23 (53%)	14 (61%)	0.79
Duration of 2nd stage (median, IQR)	3.75 (n = 1)	2.4 (0.75, 4.03)	0.5 (0.32, 0.9)	0.04
Duration rupture of membranes (median, IQR)	3.7 (0, 13.45)	2.5 (0, 10.37)	3.5 (0.8, 15.5)	0.29

* statistical comparison made using chi square, ANOVA or Kruskal-Wallis

b

	Absent (N = 2)	*Early-only* (N = 13)	*Persistent* (N = 21)	P value
Maternal race: White	2 (100%)	11 (85%)	15 (73%)	0.63
BMI	25 ± 7	25 ± 3	28 ± 7	0.39*
Chorioamnionitis	0	3 (23%)	4 (19%)	0.74
Maternal antibiotics	1 (50%)	8 (62%)	8 (38%)	0.41
Meconium stained amniotic fluid	0	3 (23%)	7 (33%)	0.54
Birthweight	3968 ± 993	3428 ± 367	3548 ± 366	0.19*
Infant antibiotics	1	8 (73%)	8 (36%)	0.12
Formula in hospital	1 (50%)	2 (15%)	5 (24%)	0.53
Exclusive breastfeeding at week 2	2 (100%)	8 (62%)	13 (62%)	0.55
Duration of 2nd stage (median, IQR)	3.75	1.2 (0.25, 2.32)	0.48 (0.32, 0.87)	0.43*
Duration rupture of membranes (median, IQR)	8.58 (3.7, 13.45)	8.98 (3.13, 10.23)	3.5 (1.08, 15.5)	0.22*

*comparison only between Early Only vs. Persistent

Table S1. Metadata on our 75 dyads cohort. Related to all Figures. A comparison of demographic and clinical information by *Bacteroides* colonization phenotype in **(a)** all infants and **(b)** only vaginally delivered infants. *Bacteroides* colonization phenotype was characterized as absent (not detected at week 1 or 2), early-only (detected at week 1 only), late (detected at week 2 only) or persistent (detected at both week 1 and week 2). A total of 69 families had data from 16S sequencing for both week 1 and week 2 which allowed this classification. Detection was defined as a relative abundance of 0.1%. Categorical variables were compared between all three groups by chi square. For continuous variables, Early-only and Persistent groups were compared by t-test or Kruskall Wallis.

Study	Country	N (vaginal)	N (C-section)	Timing of infant stool samples	Sequencing	Reference (PMID)
Chu et al	US (Texas)	82	33	Delivery & 6 weeks (longitudinal subset)	16S V3-V5 and metagenomics (subset)	28112736
MUIS	Holland	74	46	Delivery, Day 1, Week 1, Week 2, Month 1, 2,4, 6, 9,12	16S V4	31676793
Ferretti et al	Italy	25	0	Day 1, Day 3, Day 7, 1 month, 4 months	Metagenomics	30001516
Baby Biome	United Kingdom	314	282	Day 4, Day 7, Day 21, Infancy	Metagenomics	31534227
Yassour et al	Finland	37	7	Birth, 2 weeks, 1, 2 and 3 months	Metagenomics	30001517
COPSAC	Denmark	~538*	~152*	1 week, 1 month, 1 year	16S V4	29321519
INFANTMET	Ireland	83 term, 4 preterm	70 term, 35 preterm	Week 1, Week 4, Week 8, Week 24	16S V4-V5	28095889

*Estimated from total N and the percent delivered via C-section in parent manuscript, as exact numbers are not reported

Table S3: Validation cohorts information. Related to Figure S1B. Description of the cohorts included in the validation analysis on Bacteroides persistence.