

## Supplementary Materials

### Fetal exposure to the maternal microbiota in humans and mice

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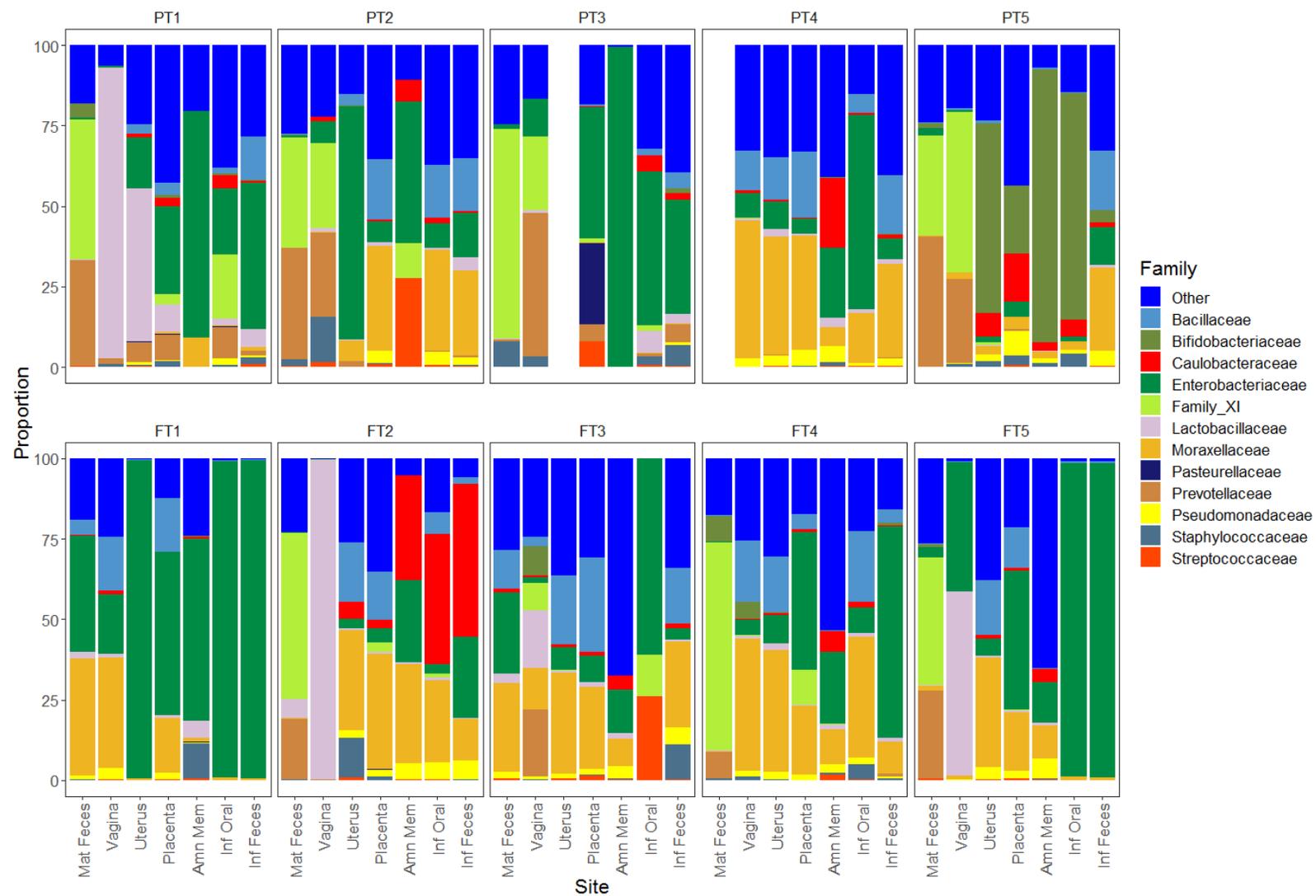
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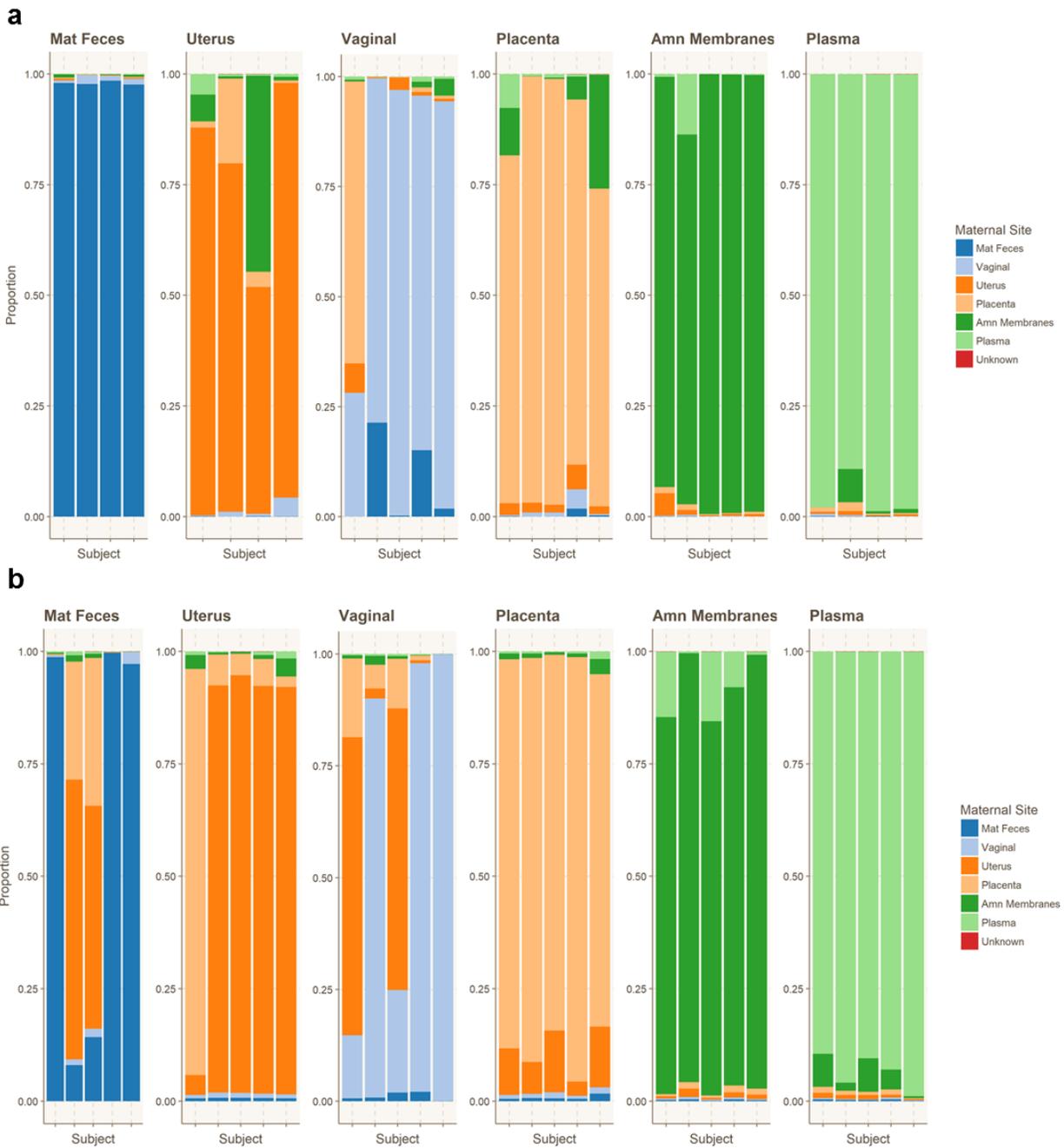
**Table S1. The proportion of amplicon sequence variants (ASVs) in infant sites that were shared with maternal body sites.**

Infant site	Maternal site	All <u>ASVs</u> *	
		Median (IQR) percentage of all ASVs shared with mother	Median (IQR) percentage of all ASVs shared with unrelated mothers
Oral cavity	Fecal	32.7 (14.5, 37.9)	17.2 (9.7, 33.3)
Oral cavity	Placenta	43.4 (32.0, 47.1)	20.3 (10.9, 46.4)
Oral cavity	Vaginal	26.6 (14.1, 50.0)	23.3 (10.7, 43.3)
Oral cavity	Plasma	12.6 (6.9, 13.0)	14.3 (9.9, 22.4)
Meconium	Fecal	19.4 (15.0, 34.6)	13.2 (8.7, 26.4)
Meconium	Placenta	36.9 (23.2, 41.5)	21.7 (11.3, 21.9)
Meconium	Vaginal	34.8 (16.7, 41.9)	25.6 (11.6, 38.8)
Meconium	Plasma	18.5 (12.3, 19.8)	14.1 (9.5, 19.8)

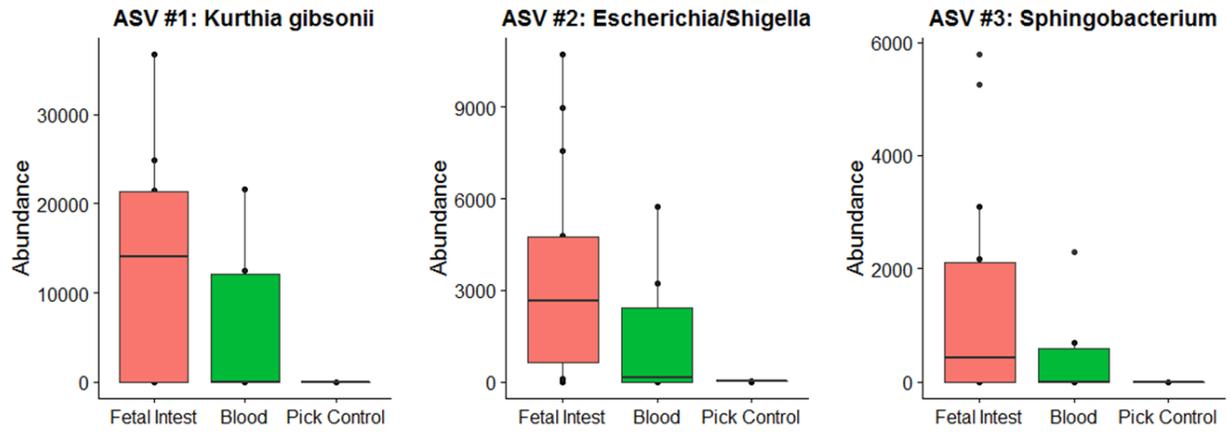
\*No filtering was performed to remove sparse ASVs before analysis.



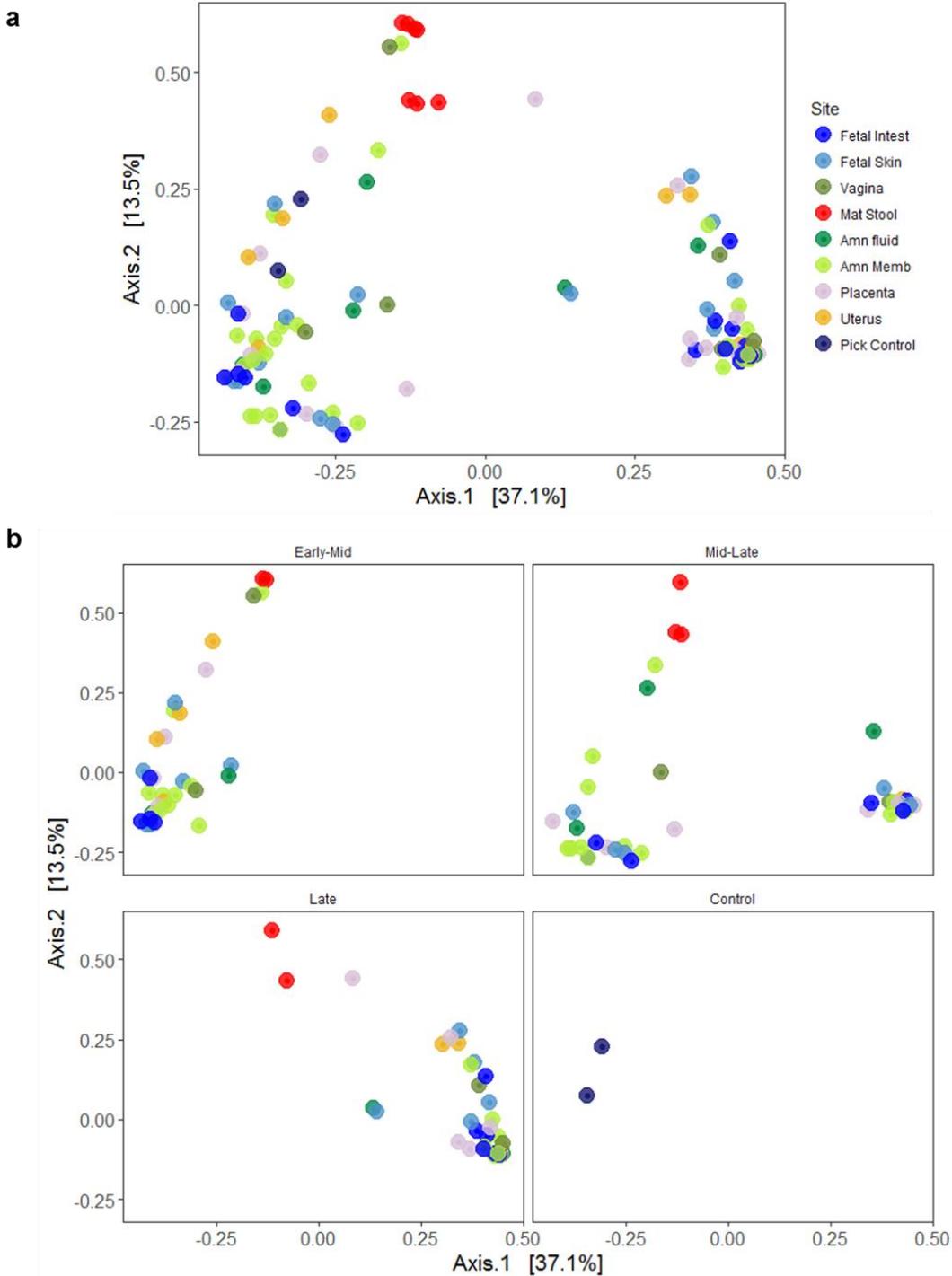
**Figure S1. Relative abundance of the top bacterial families.** Each panel represents an individual preterm (PT) or full-term (FT) mother-infant dyad.



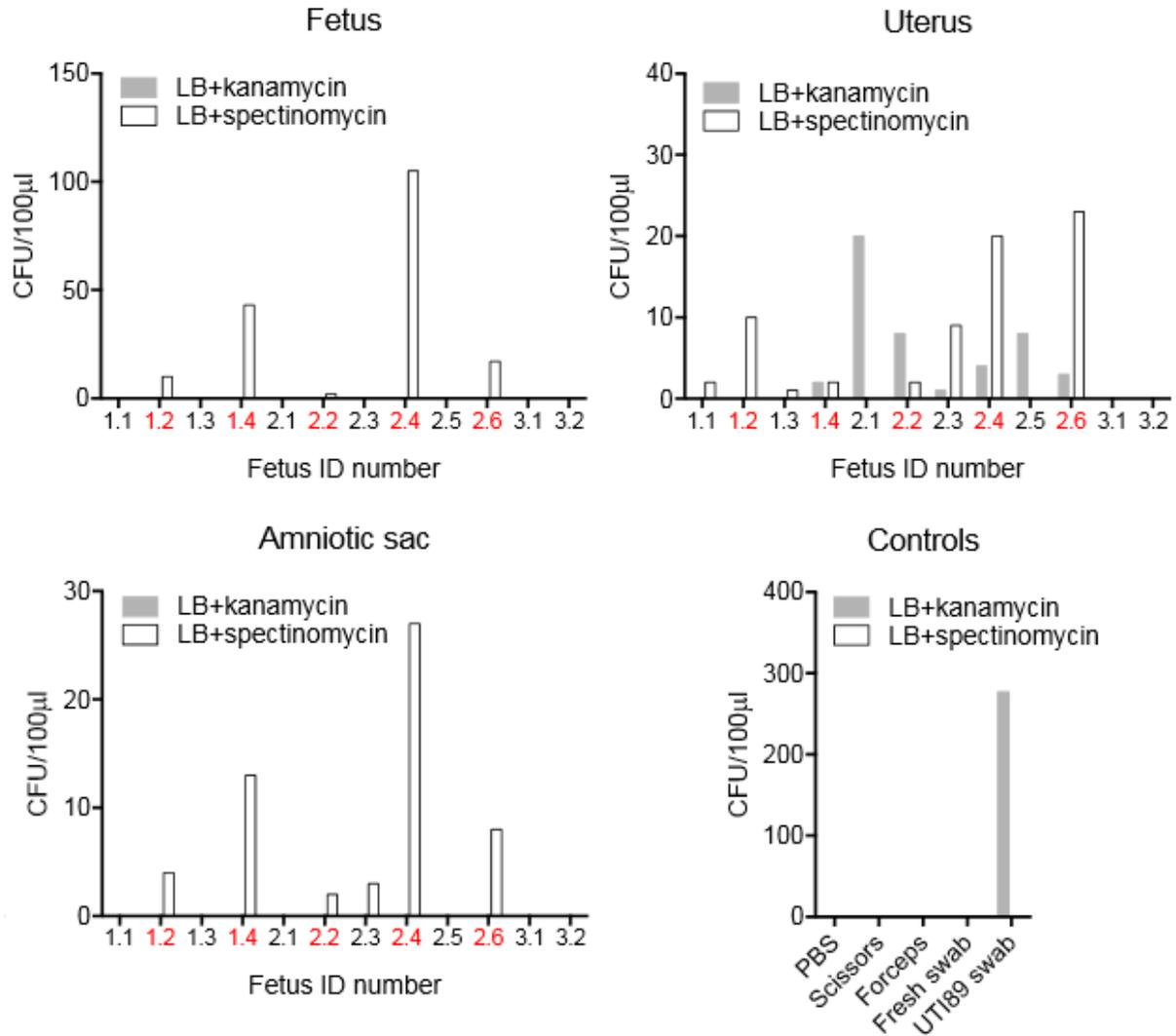
**Figure S2. Validation of bacterial source-tracking model.** To assess the ability of the source-tracking model to accurately predict the source of the microbiota, each site was input separately as a “sink” in the model. The model generally predicted the same site type as the source of microbiota for each sample for the preterm (**a**) and full term (**b**) cohorts.



**Figure S3.** Normalized abundances of the top 3 ASVs identified in the fetal intestine, compared to the abundance of these ASVs in the maternal blood and pick control samples.



**Figure S4.** Principal coordinates analysis of murine maternal and fetal samples based on Bray-Curtis distances. All samples are shown in composite (**a**), then shown divided by gestational age at the time of sampling (**b**). Early-mid refers to samples collected between E14-16; mid-late refers to samples collected between E17-18; and late refers to samples collected between E19-20 (n=2 dams per time point and  $\geq 3$  fetuses per dam).



**Figure S5. Control experiments for *E. coli* maternal-fetal cross-contamination.** In anesthetized pregnant dams (Dams 1 and 2; day E14), the exterior uterine surface was swabbed with a solution of  $1 \times 10^5$  CFU/ml of kanamycin-resistant *E. coli* (UTI89pCOMgfp). The fetal liver of every other fetus within the uterus (noted in red text) was then transmurally injected with  $5 \mu\text{l}$  of a  $1 \times 10^5$  CFU/ml solution of a spectinomycin-resistant *E. coli* ( $\Delta 6\text{H}$ ). Each fetal-placental unit was then ligated with a sterile surgical suture to allow for individual dissection of the uterus, amniotic membrane, and fetus. Dams 3 received no microbial manipulation. Cultures from the uninjected fetuses and accompanying amniotic sacs did not grow either of the bacterial strains. The kanamycin-resistant *E. coli* (UTI89) was recovered from the uterus, but not the fetal tissues. The injected spectinomycin-resistant *E. coli* ( $\Delta 6\text{H}$ ) was cultured from all tissues, indicating some spillage into the uterus. The results demonstrate that the fetal tissues were not contaminated by the maternal uterine tissue during dissection. Control cultures were obtained from the PBS solution used to prepare the tissue suspension, and the surgical instruments and swabs used in the experiment. A swab of UTI89pCOMgfp was included as a positive control.