# Supplementary materials

## Supplementary method

**Sample collection.** We enrolled 19 healthy pregnant women who vaginally delivered healthy newborns at full term. For each mother, we sampled the fecal samples, gut microbiota, and breast milk. Each infant was sampled for feces from paper diapers at 42 days postpartum. All infants were exclusively breastfed. Before milk sample collection, the maternal gland was sterilized with 75% alcohol, and the first drops were discarded to minimize contamination [1]. Spot infant fecal samples were collected from paper diapers on a super clean bench. Maternal fecal samples were stored in a plastic box. Maternal and infant fecal and breastmilk specimens were stored in plastic containers at 4°C in home refrigerators until they were brought to the study clinic no more than 24 h after collection. At the study clinic, samples were frozen at -80°C until analysis.

For each type of sample, sIgA coated microbiota was enriched by streptavidin magnetic particles. All samples were characterized by 16S rRNA gene amplicon sequencing of the V3-V4 region.

**Enrichment of sIgA-coated bacteria through magnetic-activated cell sorting.** Breast milk samples (10 mL) were divided into two parts, one (5 mL) for direct DNA extraction, and the other for enriching sIgA-coated bacteria. Nanoscale aminated magnetic beads and streptomycin affinity nanomagnetic beads were prepared using methods previously developed in our lab [2]. Thereafter, 5 mL of maternal breastmilk was centrifuged at 10,000 ×g for 10 min [3], and the precipitate was resuspended in phosphate-buffered saline (PBS). Either 5% goat serum (40 μL), biotin-labelled rabbit anti-human IgA (20 μL), or streptavidin-labelled nanomagnetic beads (250 μL) were added to the bacterial suspension with PBS, which was subsequently placed in an ice bath for 20 min. Nanomagnetic beads were adsorbed with a magnet, and the supernatant was discarded. Finally, the magnetic bead and bacterial cell combination were washed several times with PBS until the supernatant was clean and the enriched bacteria were resuspended in PBS. Aliquots (100 μL) were added to gut microbiota medium [4], and anaerobically cultured for 48 h at 37°C.

Infant and maternal faecal samples corresponding to maternal breastmilk samples were weighed and dissolved in peptone buffer to prepare a 20% faecal bacterial suspension. Half of the sample was stored with 30% glycerine for DNA extraction, and half was supplemented with 0.5% Tween 20, vortexed, and centrifuged at 1,000 ×g for 10 min. Thereafter, the supernatant was removed by washing the precipitate with

peptone buffer three or four times. Breastmilk samples were prepared in the same way; however, peptone was used instead of PBS.

All steps were carried out in an anaerobic glove cabinet (DROID Instruments and Equipment Co., Ltd., Shanghai, China).

**DNA extraction.** Bacterial genomic DNA was extracted using a reaped bead-beating method using an Ezup Column Bacteria Genomic DNA Purification Kit (Sangon Biotech, Shanghai, China) with a minor modification as previously reported [5]. Briefly, cell lysis was achieved by bead beating with zirconium beads (0.1 g, 0.7 mm: 0.3 g, 0.15 mm) on an oscillator at 6,000 rpm with two circulations (30 s per circulation; Precellys 24, Bertin Technologies, Montigny-le-Bretonneux, France) in the presence of 4% (w/v) sodium dodecyl sulphate, 500 mM NaCl, and 50 mM ethylenediaminetetraacetic acid. Ammonium acetate was used to precipitate and remove impurities, and sodium dodecyl sulphate in addition to isopropanol precipitation was used for nucleic acid recovery. RNA and proteins were removed or degraded using RNase and Proteinase K, respectively, followed using an Ezup Column Bacteria Genomic DNA Purification Kit. Genomic DNA concentration was determined using a Nanodrop 1000 spectrophotometer (Thermo Fisher Scientific, Wilmington, DE, USA), and DNA integrity was determined by electrophoresis on 1% agarose gels. DNA concentration for all samples was standardised to 10 ng/μL.

**16S rRNA gene sequencing.** Microbial profiles were analysed by 16S rRNA sequencing at GENEWIZ, Inc. (Suzhou, China). To maximise the effective length of the MiSeq 250PE and 300PE sequencing reads, a region of approximately 469 bp encompassing the V3 and V4 hypervariable regions of the 16S rRNA gene was targeted for sequencing. The PCR primers used to amplify V3 and V4 hypervariable regions were as follows: forward 5'- CCT ACG GRR BGC ASC AGK VRV GAA T -3' and reverse 5'- GGA CTA CNV GGG TWT CTA ATC C -3'. In addition, an indexed linker was added to the end of the 16S rRNA PCR product for next-generation sequencing (Illumina, San Diego, CA, USA). First-round PCR products were used as templates for a second round of PCR amplicon enrichment (94°C for 3 min, followed by 24 cycles at 94°C for 5 s, 57°C for 90 s, and 72°C for 10 s, and a final extension at 72°C for 5 min). PCR reactions were performed in triplicate using a 25 μL mixture containing 2.5 μL of TransStart Buffer, 2 μL of dNTPs, 1 μL of each primer, and 20 ng of template DNA. DNA library concentration was validated using a Qubit 3.0 Fluorometer. The libraries were quantified to 10 nM, and subsequently multiplexed and loaded on an Illumina MiSeq instrument according to the manufacturer's instructions (Illumina, San Diego,

CA, USA). Sequencing was performed using PE250/300 paired-end; image analysis and base calling were performed using the MiSeq Control Software embedded in the MiSeq instrument.

**Reference**


1. Albesharat R, Ehrmann MA, Korakli M, Yazaji S, Vogel RF. Phenotypic and genotypic analyses of lactic acid bacteria in local fermented food, breast milk and faeces of mothers and their babies. *Syst Appl Microbiol* 2011; **34**: 148–155.
2. Sun J, Qi C, Zhu H, Zhou Q, Xiao H, Le G, et al. IgA-Targeted *Lactobacillus jensenii* Modulated Gut Barrier and Microbiota in High-Fat Diet-Fed Mice. *Front Microbiol* 2019; **10**: 1179.
3. Khodayar-Pardo P, Mira-Pascual L, Collado MC, Martínez-Costa C. Impact of lactation stage, gestational age and mode of delivery on breast milk microbiota. *J Perinatol* 2014; **34**: 599–605.
4. Goodman AL, Kallstrom G, Faith JJ, Reyes A, Moore A, Dantas G, et al. Extensive personal human gut microbiota culture collections characterized and manipulated in gnotobiotic mice. *Proc Natl Acad Sci U S A* 2011; **108**: 6252–6257.
5. Ding M, Qi C, Yang Z, Jiang S, Bi Y, Lai J, et al. Geographical location specific composition of cultured microbiota and *Lactobacillus occurrence* in human breast milk in China. *Food Funct* 2019; **10**: 554–564.

**Supplementary Table**

**Table S1.** Selected anthropometric and descriptive variables of 19 lactating women participating in this study[1]

| Item | Range |
|---|---|
| Age, y | 30 ± 6 |
| Height, cm | 162 ± 6 |
| Pre-pregnancy weight, kg | 54 ± 8 |
| Prenatal weight, kg | 70 ± 7 |
| Maternal weight gain, kg | 17 ± 8 |
| Birth weight, kg | 3.3 ± 0.3 |
| Parity, n | 1.3 ± 0.4 |
| Female infants, % | 39 |

Values indicate the mean ± SD or unit of measure as indicated; a total of 19 women and their infants were studied.

## Supplementary Figure

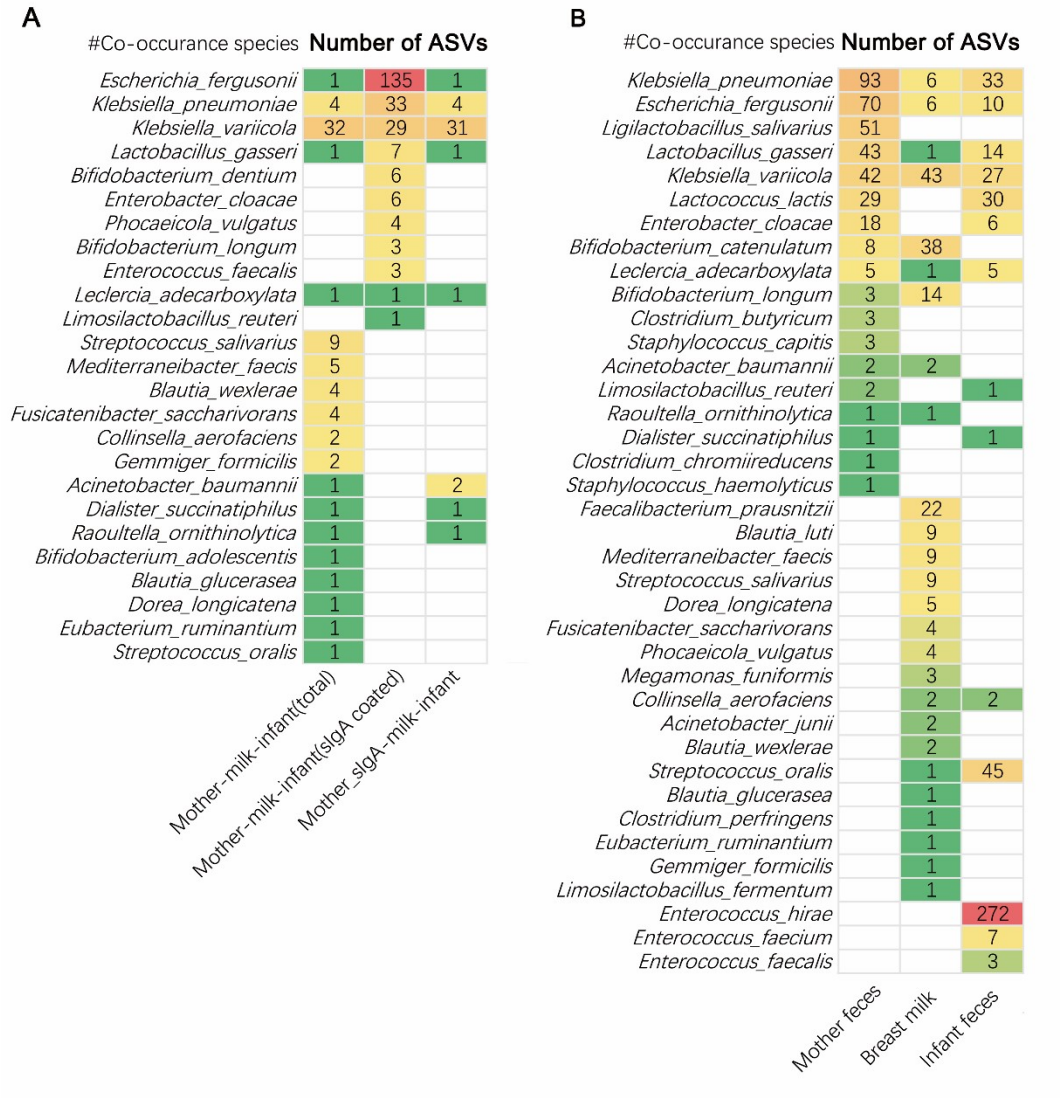


Figure S1.
The number of co-occurring sub-dominant (0.01%) ASVs and corresponding species for total microbiota (A) and secretory immunoglobulin A (sIgA) coated microbiota (B).

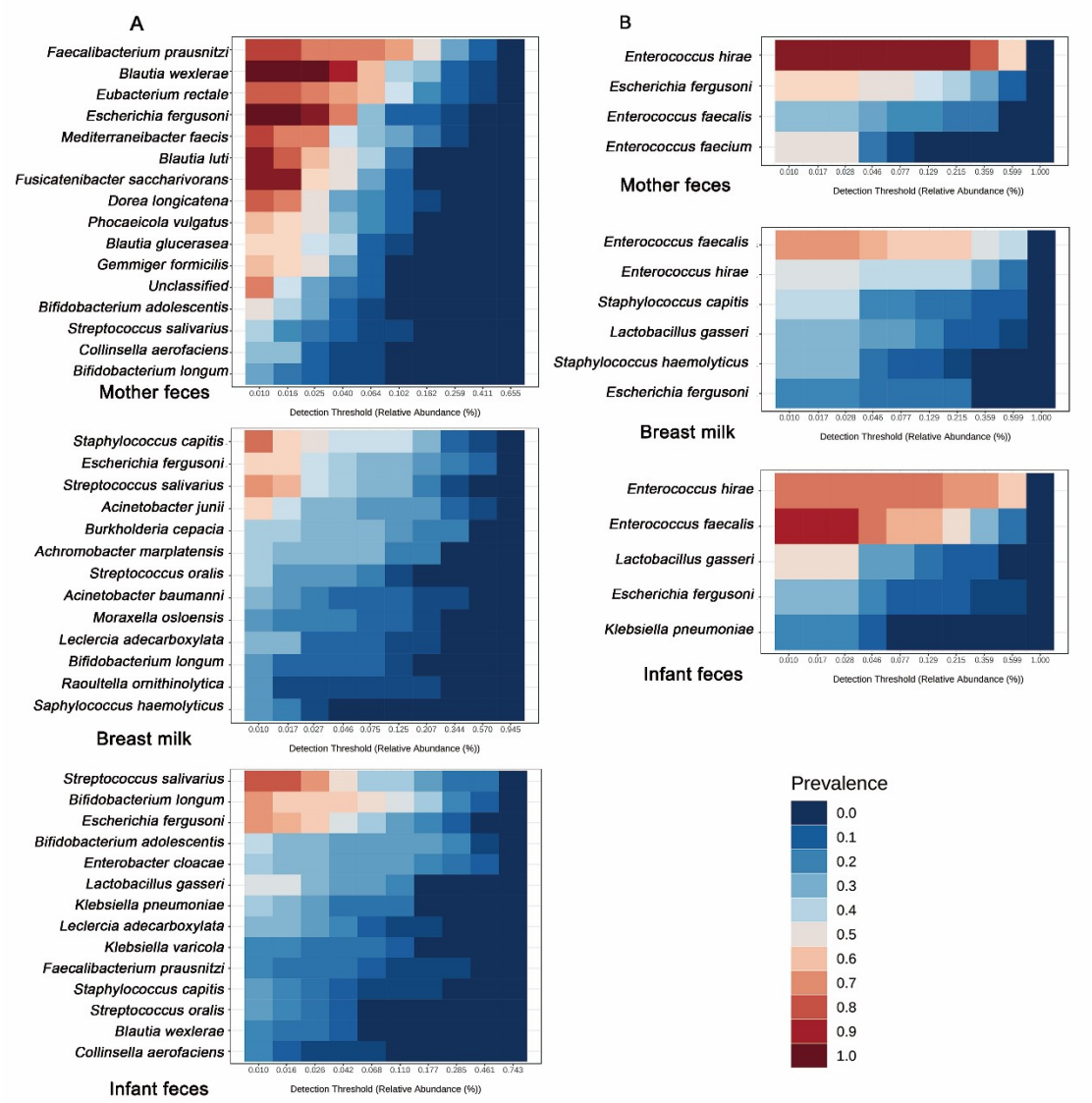


Figure S2.
Core species in microbiota of total microbiota (A) and secretory immunoglobulin A coated microbiota (B) in mother feces, breast milk and infant feces.

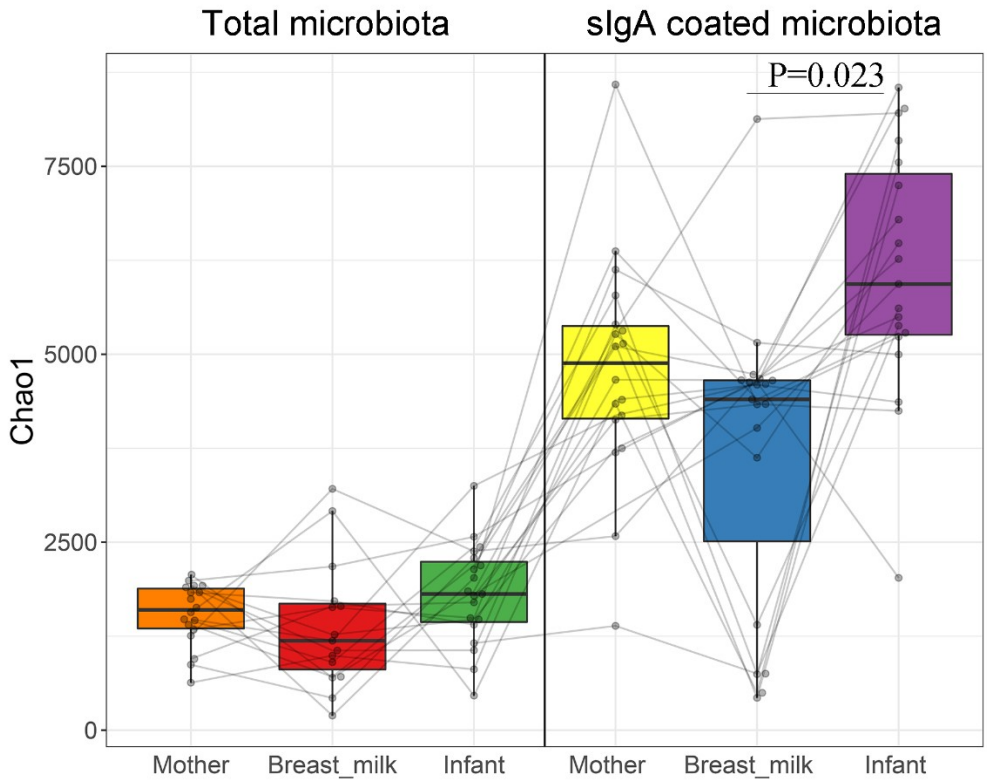


Figure S3.
Chao1 index for microbiota in maternal gut, breast milk, and infant's gut dyads of total and secretory immunoglobulin A (sIgA) coated bacteria. Wilcoxon matched-pairs signed rank test

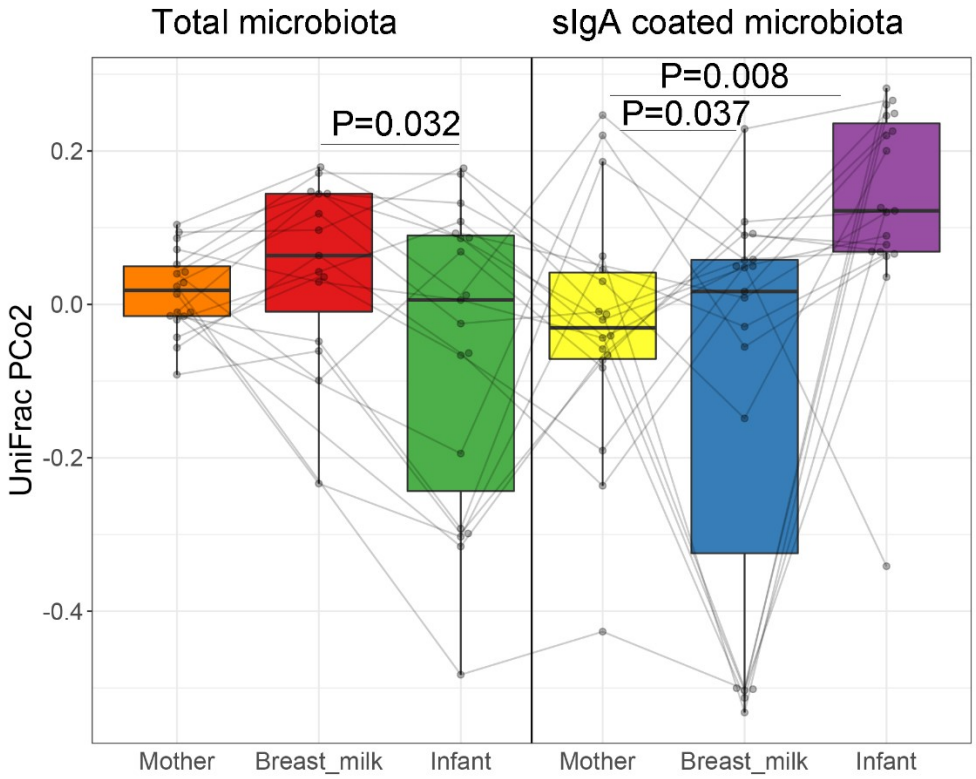


Figure S4.

The source of variability between mother and infant fecal and breast milk samples (PCo2) was showed by ordination from unweighted UniFrac. Data were analyzed by Wilcoxon matched-pairs signed rank test.