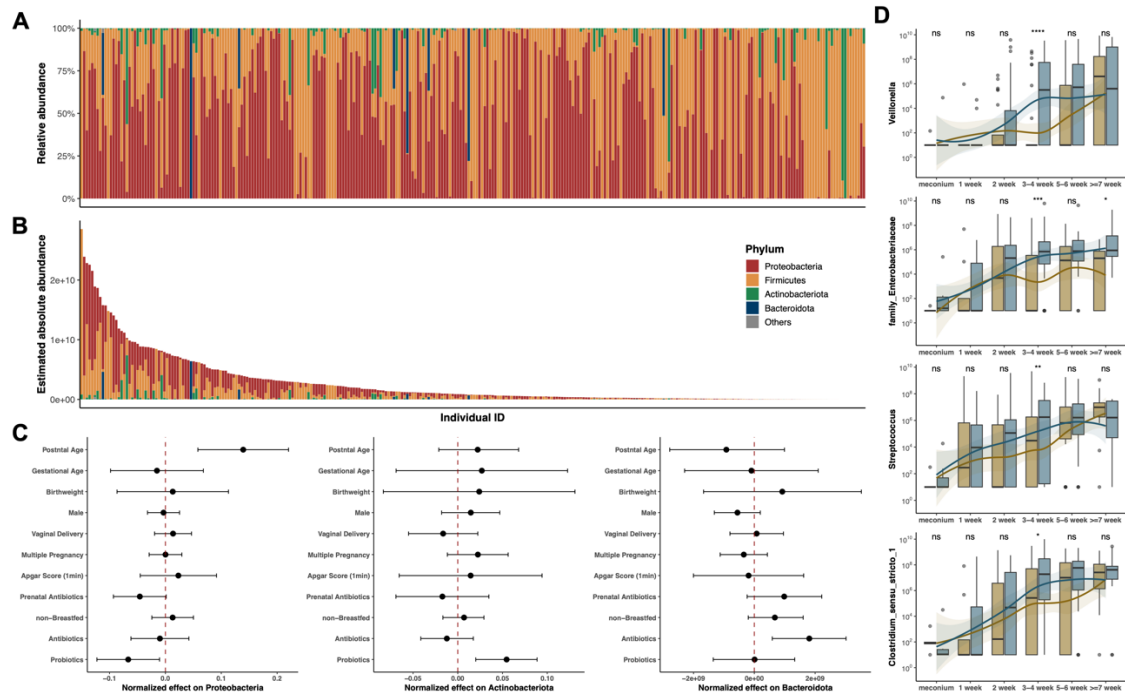


## **Supplementary materials**

### **Dynamics and Crosstalk between Gut Microbiota, Metabolome, and Fecal Calprotectin in Very Preterm Infants: Insights into Feeding Intolerance**

- Figure S1** Longitudinal change of microbiome composition and estimated bacterial abundance in very preterm infants with and without feeding intolerance. Page 2-3
- Figure S2** Correlations between the fecal calprotectin level and FC levels. Page 4
- Figure S3** Clustering of metabolome profiles and correlations between bacterial load and intensity of polyamine metabolites. Page 5

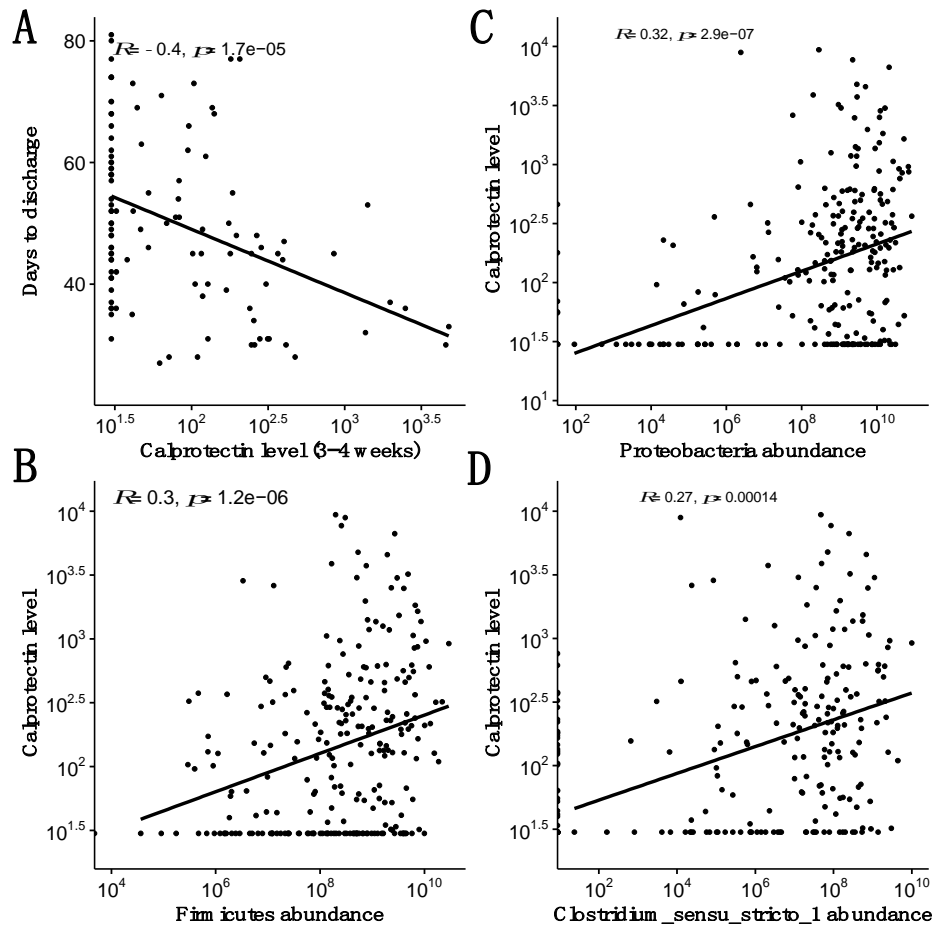
**Fig S1. Longitudinal change of microbiome composition and estimated bacterial abundance in very preterm infants with and without feeding intolerance**



**(A-B)** Longitudinal change of bacterial composition **(A)** and estimated absolute abundance **(B)** in fecal samples of very preterm infants. **(C)** Effects of different clinical factors on the estimated absolute abundance of *Proteobacteria*, *Firmicutes*, *Actinobacteria*, and *Bacteroidota*. Error bars indicate 95% confidence intervals. **(D)** Longitudinal change of inferred bacterial abundance of genera *Veillonella*, *Enterobacteriaceae*, *Streptococcus*, and *Clostridium\_sensu\_stricto\_1* in infants with and without FI, respectively. Yellow and blue color for infants with and without FI, respectively. Group-wise

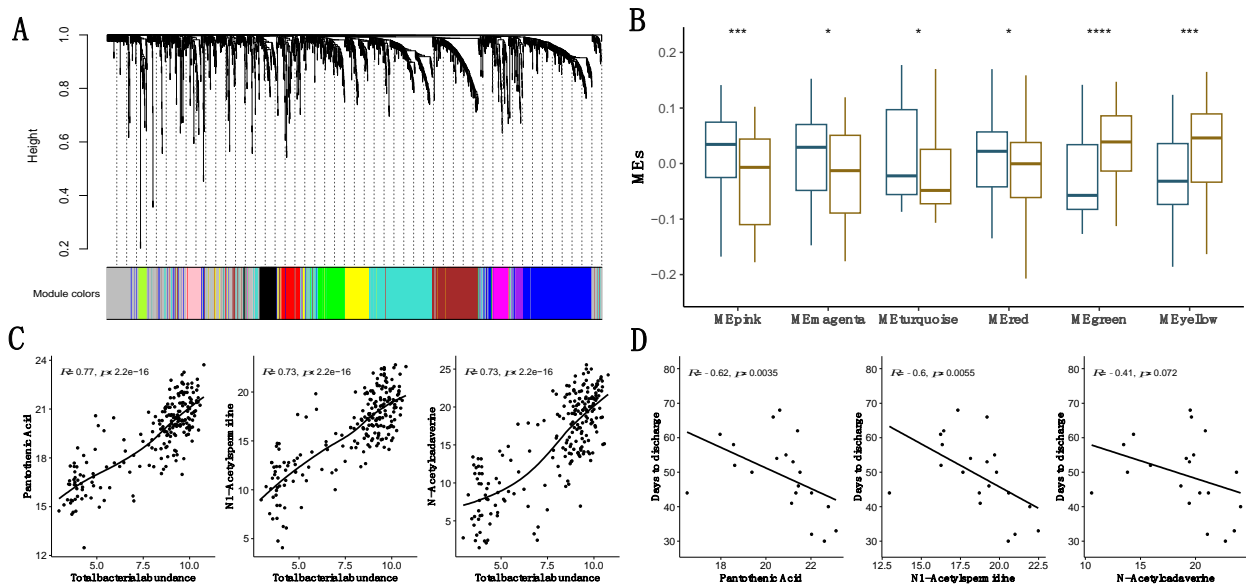
comparisons were shown using at-risk. Asterisks represent p values: ns:  $p > 0.05$ ,  $*p < 0.05$ ,  $**p < 0.01$ ,  $***p < 0.001$ ,  $****p < 0.0001$ .

**Fig S2. Correlations between the fecal calprotectin level and FC levels**



**(A)** Correlations between fecal calprotectin level at 4 weeks of age and time to discharge using Spearman's method. **(B-D)** Correlations between inferred bacterial abundances of phyla *Firmicutes* **(B)** and *Proteobacteria* **(C)** and genus *Clostridium\_sensu\_stricto\_1* **(D)** in samples collected at and large than 2 weeks of age using Spearman's method, respectively.

**Fig S3. WGCNA analyses of metabolome profiles and Spearman's correlations between total bacterial load and intensity of polyamine metabolites**



**(A)** WGCNA clustering of all metabolites with HMDB annotations. **(B)** The overall changes of different metabolite modules in infants with and without FI, respectively. Group-wise comparisons were shown using at-risk. Asterisks represent  $p$  values: ns:  $p > 0.05$ , \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ , \*\*\*\* $p < 0.0001$ . Yellow and blue color for infants with and without FI, respectively. **(C)** Correlations between fecal metabolite intensities and total bacterial abundance using Spearman's method in infants with and without FI, respectively. **(D)** Correlations between fecal metabolite intensities and days to discharge using Spearman's method in infants with and without FI, respectively.