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Supplemental Material to:

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The development of gut microbiota in critically ill extremely low birth weight infants assessed with 16S rRNA gene based sequencing

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DRELL ET AL. 2014 SUPPLEMENTARY FILE S2

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Aim of the experiment:

To confirm the lack of genera *Lactobacillus, Bacteroides* and *Bifidobacterium* in gut microbiota of ELBW infants and rule out primer 27F bias towards these genera additional qPCR and PCR reactions were carried out. qPCR was carried out with genus *Bifidobacterium* and *Bacteroides* specific primers [S1] and PCR was carried out with universal 16S rRNA gene specific primers used in current study [S2] on DNA extracted from 11 *Lactobacillus* type strains.

Materials:

For qPCR experiment, DNA from 44 samples collected from 20 preterm infants participating in current study was randomly chosen. 11 samples were collected at the age of one week, 16 at the age of one month and 17 at the age of two months. Additionally, 20 vaginally born neonates (GA> 36 weeks) were included to this analysis as a comparison group with 20 samples collected at the age of one week, 20 at the age of one month and 20 at the age of three months (n=60 samples). For PCR experiment, DNA from 11 *Lactobacillus* type strains was used.

Methods:

Real-Time PCR was carried out with ABI/PRISM 7500 Sequence Detector Systems platform (Applied Biosystems, USA). Each reaction was performed in triplicate with a volume of 25µl. The reaction mixture contained 12.5 µl of 1X SYBR® Green PCR Master Mix (Applied Biosystems, USA), 0.5 µM of each primer and 2 µl of separated DNA. PCR program contained 2 minutes of 50°C, 10 minutes of 95°C and 35 cycles of 95°C for 15s, 60°C for 1 minute. Melt curve analysis was performed from 60°C to 95°C with increments of 0.5^{o}C per 10s.

For construction of standard calibration curve, DNA extracted from *Bacteroides fragilis* NCTC 8560 and *Bifidobacterium longum* DSM 14583 type strains was used. DNA was quantified with Qubit quantitation platform using Quant-iT™ Broad Range assay (2- 1000 ng) and High Sensitivity assay (0.2- 100 ng) kits (Invitrogen, USA). Further calculations to 16S rRNA gene copy number per gram of faeces (wet weight) were based on the equation 1pg=978Mb [S3], genome size of used type strains and their known 16S rRNA gene copy number [S4, S5]. To describe the relative changes in bacterial counts measured with Real-Time PCR, log10 values of 16S rRNA gene copy number per gram of faeces were calculated for each studied bacterial taxon.

PCR reaction was carried out in total volume of 20 µl from which 3 µl was template DNA, 10 µl Phusion High-Fidelity Master Mix (Fermentas) and primer concentration was 0.35 µM. Cycling paramters contained 10 min at 98 °C, followed by 5 cycles of 30 s at 95 °C, 30 s at 47 °C and 60 s at 72 °C, then 30 cycles of 30 s at 95°C, 30 s at 71 °C and 60 s at 72 °C with a final extension at 72 °C for 10 min. PCR results were visualized on 1% agarose gel with gel-electrophoresis.

Results:

qPCR confirmed that the gut microbiota of ELBW infants participating in current study is lacking of bifidobacteria and *Bacteroides* in all analyzed time points. PCR confirmed that 16S rRNA specific primers used in current study are not biased towards lactobacilli. The results of qPCR are visualized in figure S2-1 and PCR in figure S2-2.

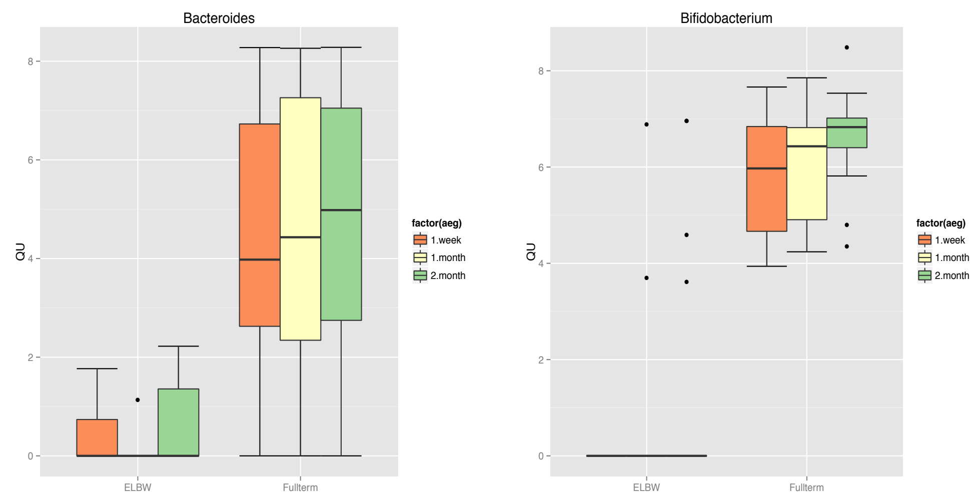

Figure S2-1. The log10 values of 16S rRNA gene copy number per gram of faeces (QU- quantitative unit) of *Bacteroides* and *Bifidobacterium* at three different sampling points in the gut microbiota of fullterm and preterm (ELBW) infants.

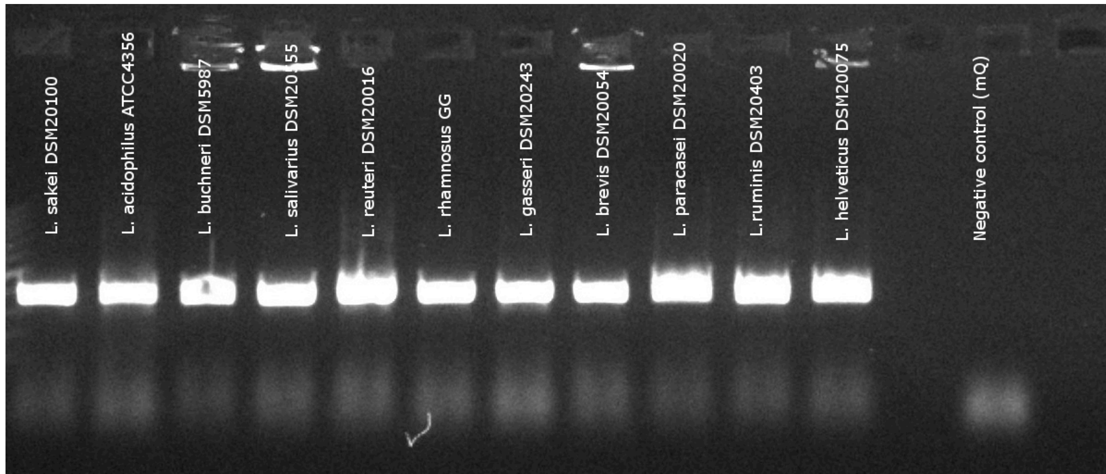

Figure S2-2. PCR with 16S rRNA specific primers carried out on DNA extracted from 11 *Lactobacillus* type strains.

References:

S1. Bartosch S, Fite A, Macfarlane G.T, McMurdo M.E.T. Characterization of bacterial communities in feces from healthy elderly volunteers and hospitalized elderly patients by using real-time PCR and effects of antibiotic treatment on the fecal microbiota. Appl Environ Microbiol; 2004; 70: 3575-3581.

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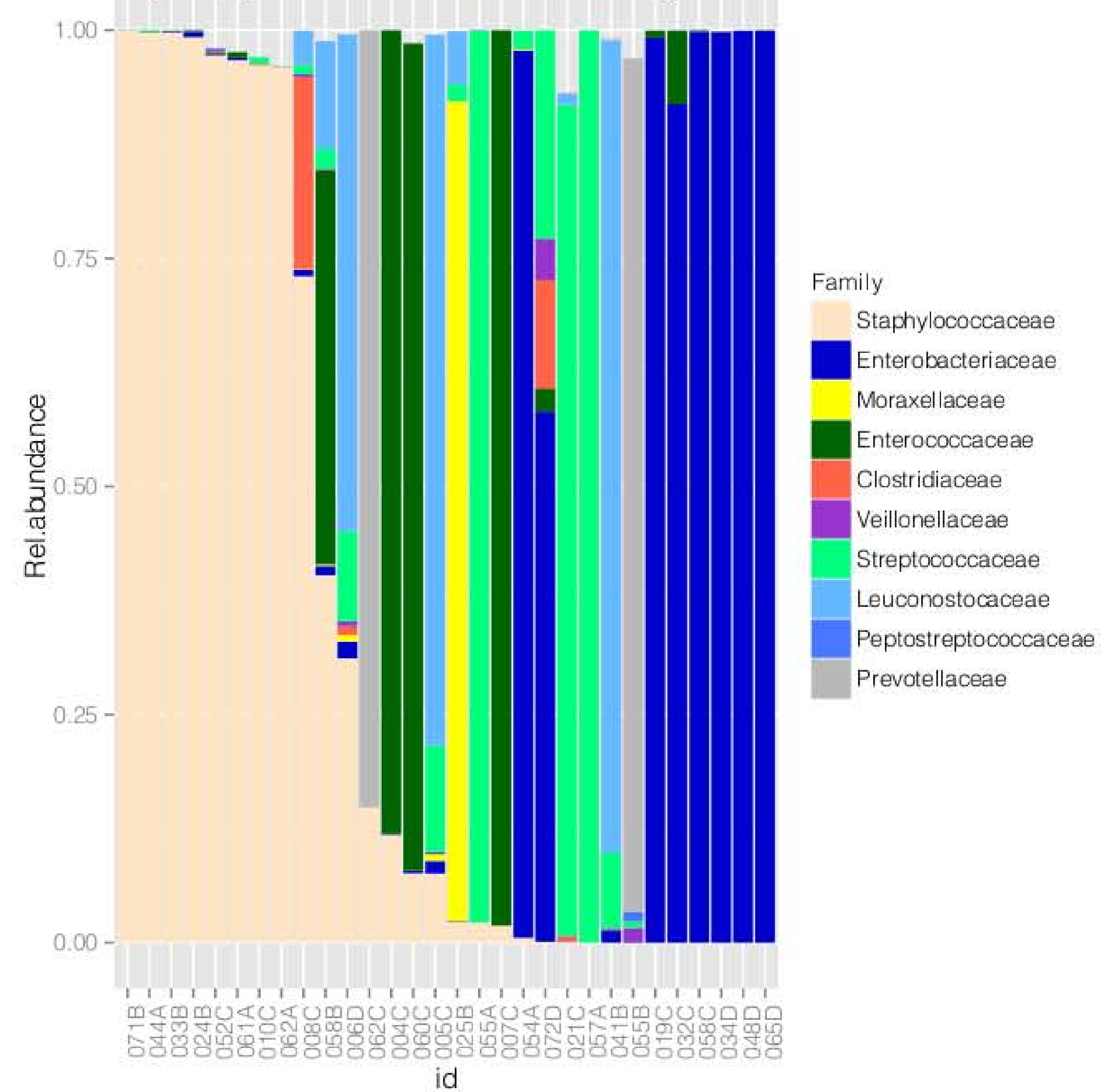
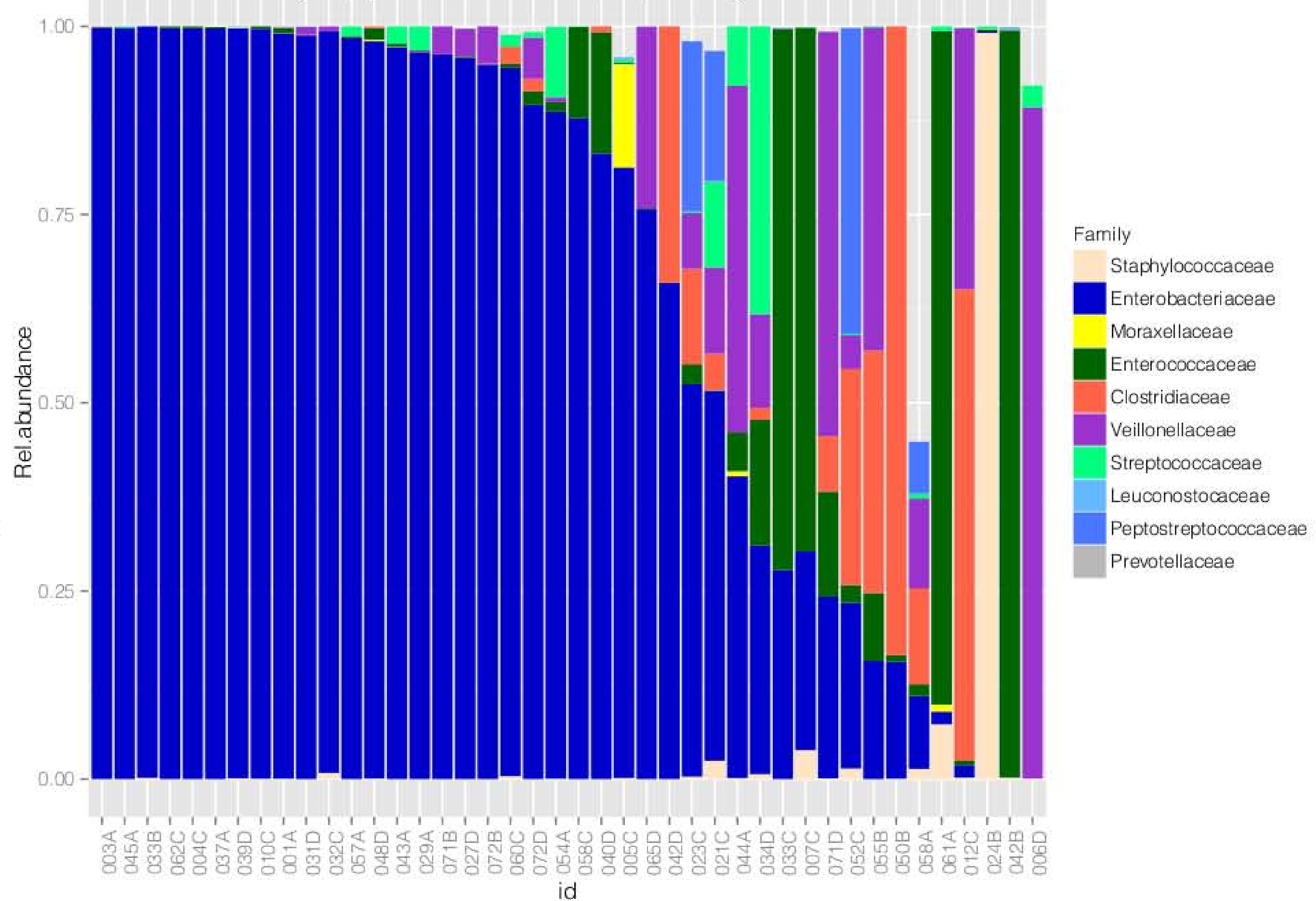
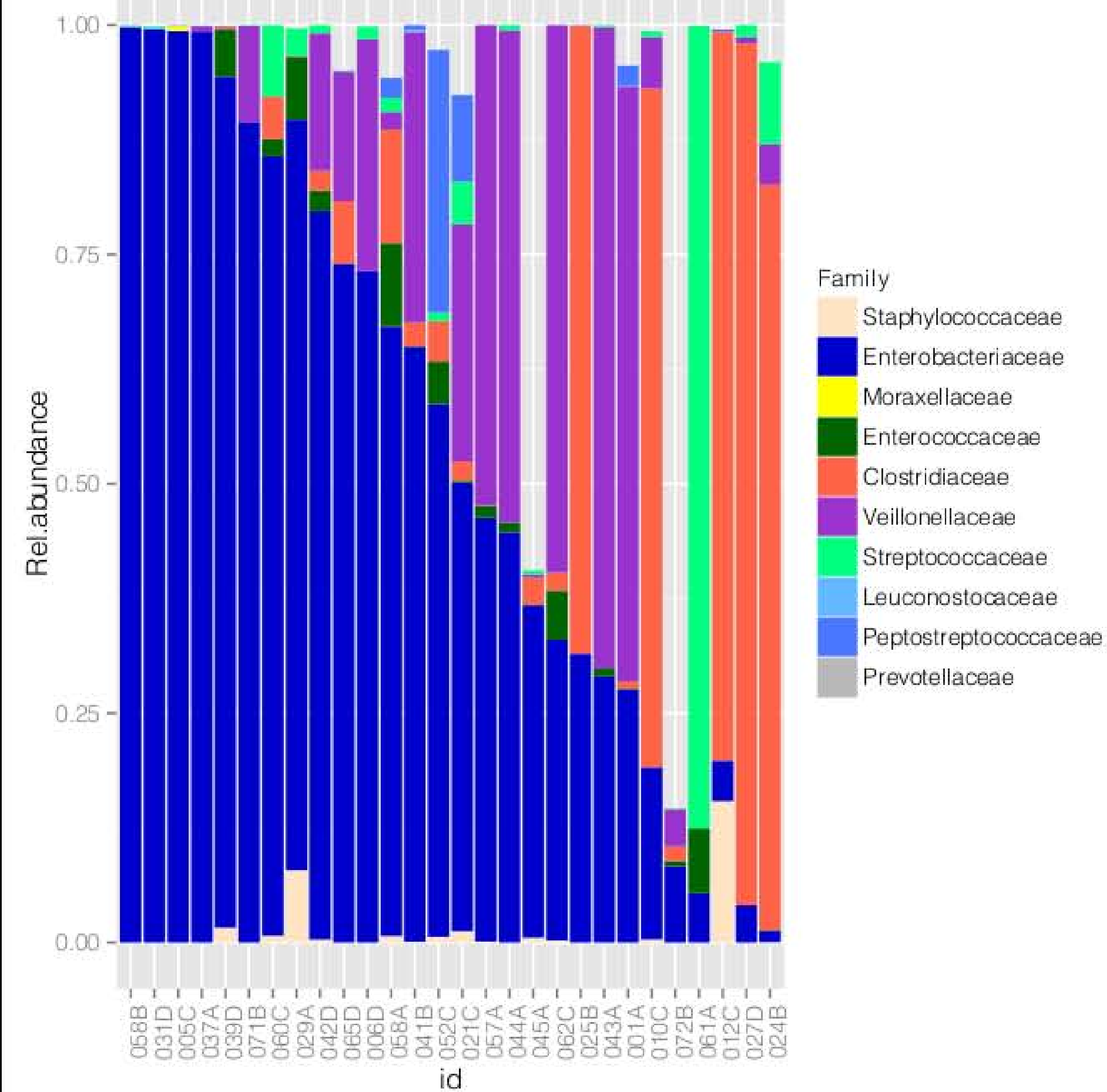
S3. Dolezel J, Bartos J, Voglmayr H, Greilhuber J. Nuclear DNA content and genome size of trout and human. Cytometry; 2003; 51A: 127-128.

S4. Kuwahara T, Yamashita A, Hirakawa H, et al. Genomic analysis of Bacteroides fragilis reveals extensive DNA inversions regulating cell surface adaption. PNAS; 2004; 101: 14919-14924.

S5. Schell M.A, Karmirantzou M, Snel B, et al. The genome sequence of Bifidobacterium longum reflects its adaptation to the human gastrointestinal tract. PNAS; 2002; 99: 14422-14427.







SUPPLEMENTARY FIGURE S1. Distribution of 10 most abundant families in individual samples.