

Supplementary Materials for

***Bifidobacterium infantis* associates with T cell immunity in human infants and is sufficient to enhance antigen-specific T cells in mice**

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The PDF file includes:

Legends for figs. S1 to S3
Legends for tables S1 to S11
Figs. S1 to S3

Other Supplementary Material for this manuscript includes the following:

Tables S1 to S11

Figure S1: Flow cytometry gating strategy for whole blood assay to measure BCG response in human infants. Related to Figure 1.

Figure S2: High versus low responder infant gut microbiota differentially impact splenic immune development in recipient gnotobiotic neonatal mice. Germ-free pups were gavaged with fecal slurry from a high responder infant (HR), low responder infant (LR) or PBS alone. Immune analyses was performed in the spleen. **(A)** Experimental schema of fecal microbiota transplantation (FMT) on to neonatal germ-free mice. **(B&C)** Body weights and spleen cellularity. **(D)** Representative flow plot showing gating strategy to determine frequencies of neutrophils (Ly6G+), Ly6Clow and Ly6Chigh monocytes in HR versus LR gavaged animals. **(E-G)** Frequencies of Ly6Chigh, Ly6Clow monocytes and Ly6G+ neutrophils. **(I)** Representative flow plot of gating for effector memory, central memory and naive CD4+ T cells **(I-K)** Proportions of naive, effector memory and central memory CD4+ T cells. **(L-M)** Gating of total CD4 memory cells and proportions of total CD4 memory cells (CD4+CD44high). Data are shown from two experiments with each experiment utilizing stool sets from different HR and LR infants. Mean values \pm SEM are plotted (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$).

Figure S3: Propidium Monoazide (PMA) qPCR experiments in SPF and GF mice orally gavaged with live or heat-killed bacteria. **(A)** Ct values of DNA extracted from stool with and without PMA treatment obtained from germ-free mice gavaged with *B. infantis*, heat-killed *B. infantis* or PBS. Analysis done at day 14 of life using *B. infantis* specific qPCR. **(B)** Ct values of DNA extracted from stool with and without PMA treatment obtained from germ-free mice gavaged with *B. thetaiotaomicron* or PBS. Analysis done at day 14 of life using *B. thetaiotaomicron* specific qPCR. **(C)** Ct values of DNA extracted from stool with and without PMA treatment obtained from SPF mice gavaged with *B. infantis* or PBS. Analysis done 6 hours after oral gavage using *B. infantis* specific qPCR. **(D)** Ct values of DNA extracted from stool with and without PMA treatment obtained from SPF mice gavaged with *B. thetaiotaomicron* or PBS. Analysis done 6 hours after oral gavage using *B. thetaiotaomicron* specific qPCR. **(E)** Ct values of DNA extracted from stool with and without PMA treatment in SPF mice gavaged with *B. infantis* or PBS. Analysis done 72 hours after oral gavage using *B. infantis* specific qPCR. **(F)** Ct values of DNA extracted from stool with and without PMA treatment obtained from SPF mice gavaged with *B. thetaiotaomicron* or PBS. Analysis done 72 hours after oral gavage using *B. thetaiotaomicron* specific qPCR.

Table S1: Mother and Infant characteristics. Related to Figure 1.

Table S2: Differentially abundant taxa in stool of high or low vaccine responders early in life. Related to Figure 2

Table S3: Differentially abundant metabolites between *Bifidobacterium infantis* and *Bacteroides thetaiotaomicron* gavaged mice. Related to Figure 6

Table S4: Differentially abundant metabolites between *Bifidobacterium infantis* and PBS gavaged mice. Related to Figure 6

Table S5: Differentially abundant metabolites between *Bifidobacterium infantis* and heat-killed *Bifidobacterium infantis* gavaged mice. Related to Figure 6

Table S6: PLS-DA analysis of metabolites across all groups ordered by variable importance projection score. Related to Figure 6

Table S7: Metabolic pathways enriched in *Bifidobacterium infantis* gavaged mice. Related to Figure 6

Table S8: Metabolic pathways enriched in *Bacteroides thetaiotaomicron* gavaged mice. Related to Figure 6

Table S9: Differentially expressed genes in colon between *Bifidobacterium infantis* and *Bacteroides thetaiotaomicron* gavaged mice at day 14 of life. Related to Figure 7

Table S10: Differentially expressed genes in colon between *Bifidobacterium infantis* and PBS gavaged mice at day 14 of life. Related to Figure 7

Table S11: Gene Set Enrichment Analysis (GSEA) of enriched pathways between *Bifidobacterium infantis* and *Bacteroides thetaiotaomicron* gavaged mice. Related to Figure 7

Figure S1

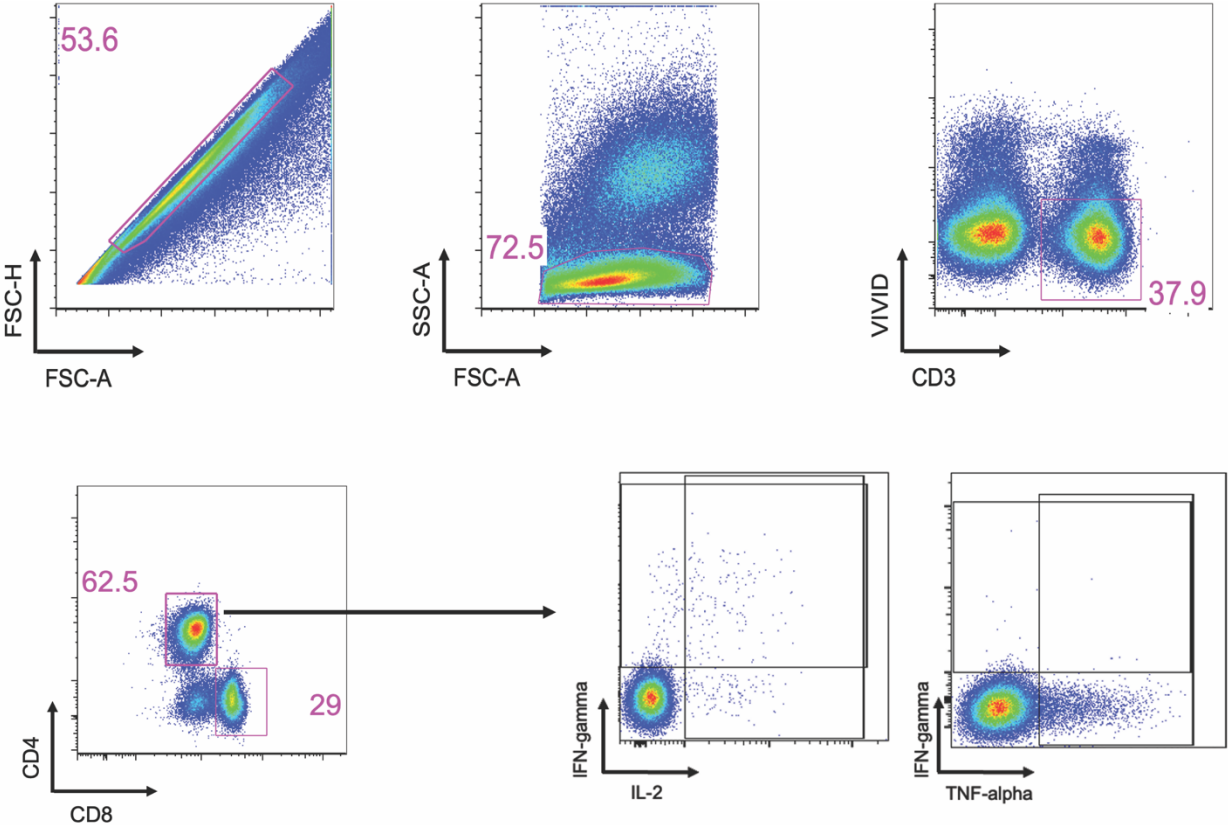


Figure S2

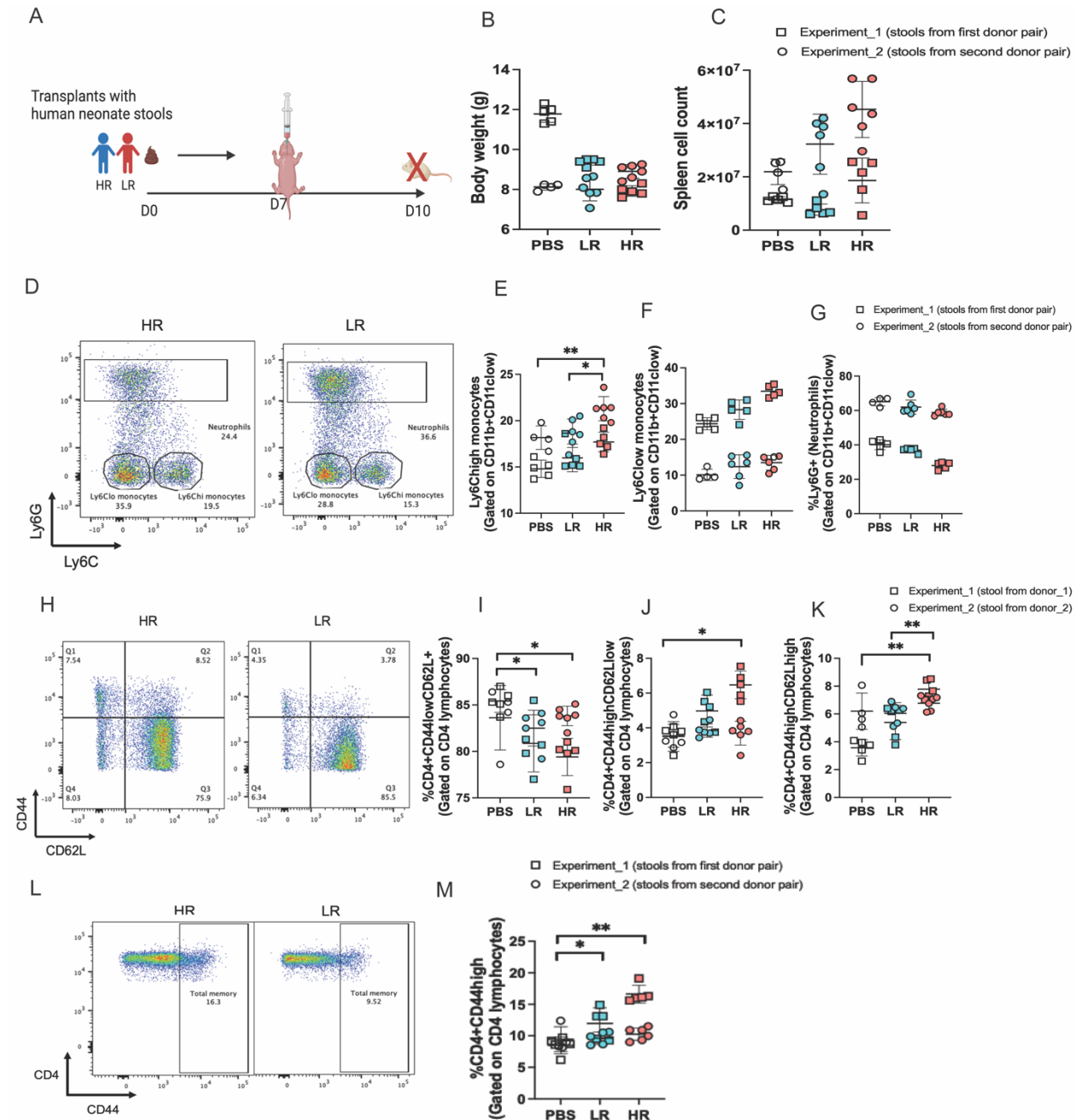
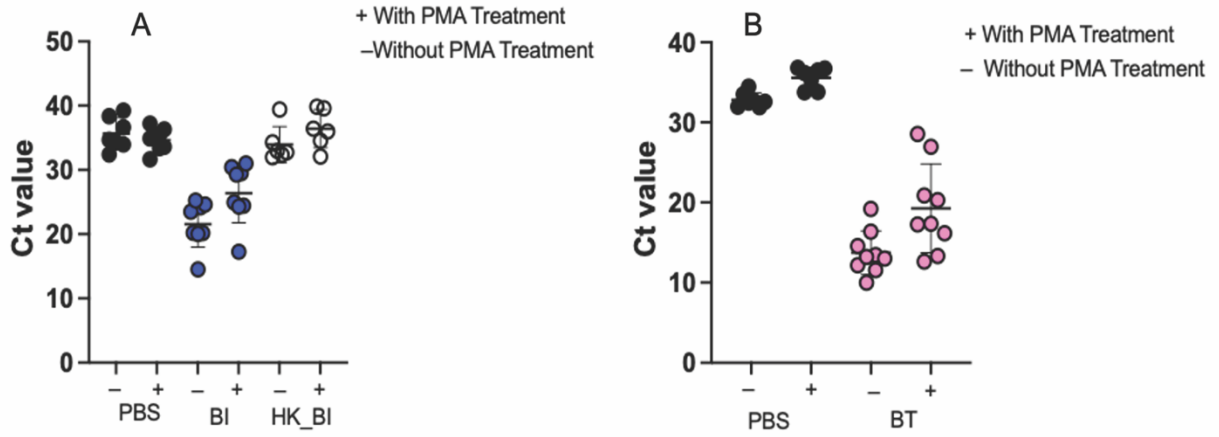
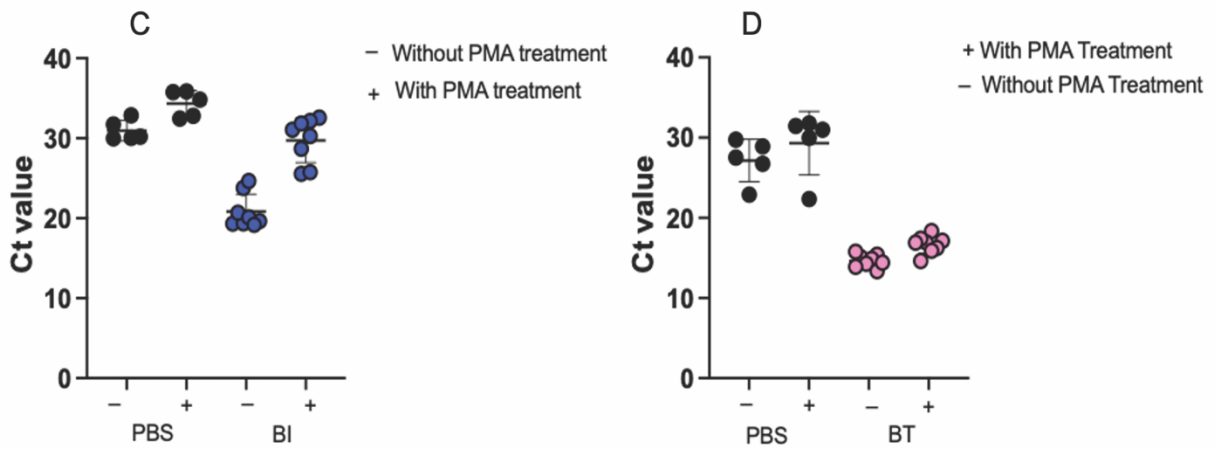


Figure S3

Gnotobiotic mice, day 14 post gavage



SPF mice, 6 hours post gavage



SPF mice, 72 hours post gavage

