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Supplemental information

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Supplemental Information.

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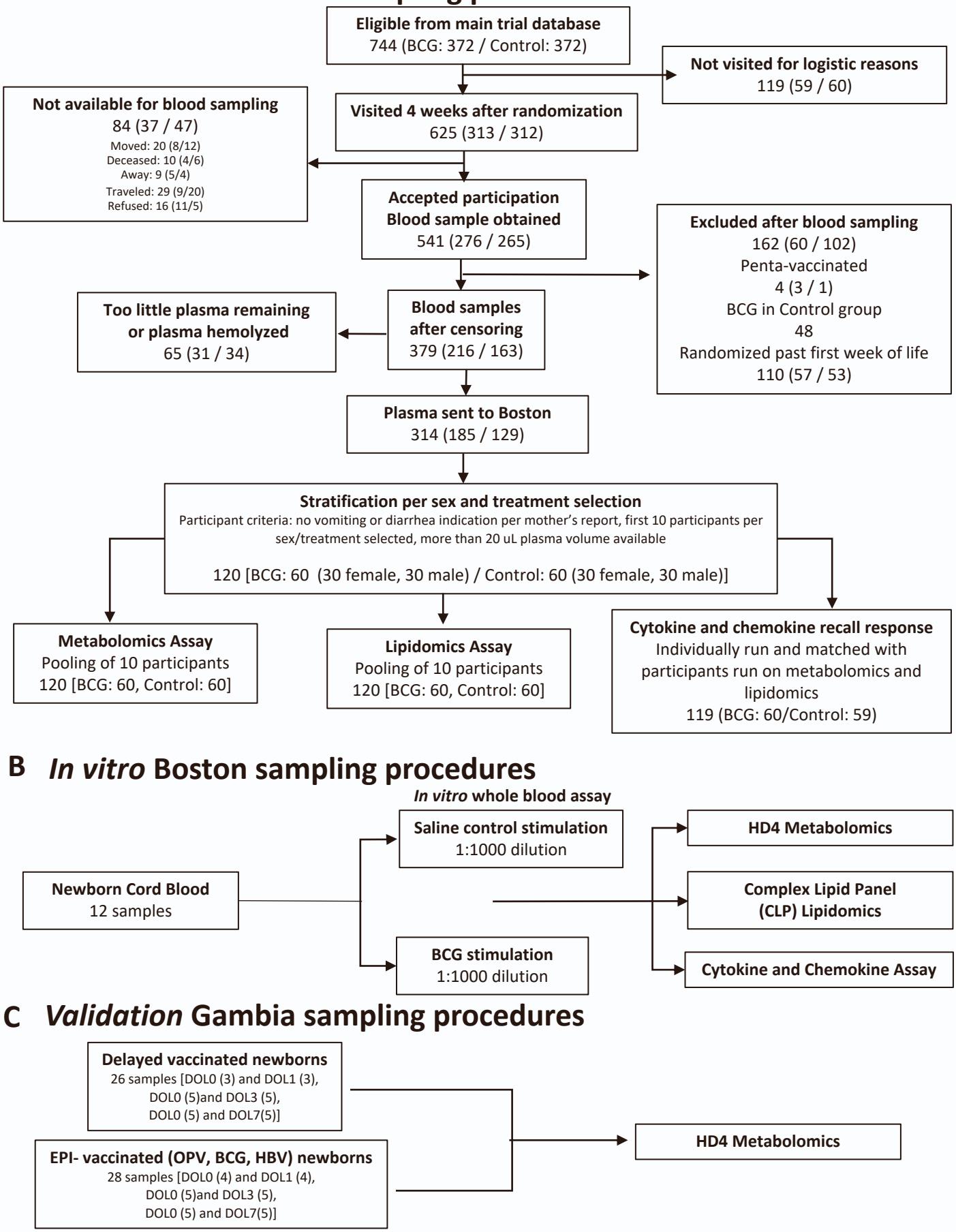

Figure S1. Flow chart of individuals with plasma samples analyzed in the study. Related to Figure 1.

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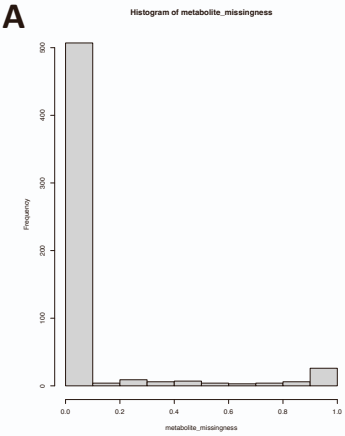
A. Low birth weight newborns in Guinea Bissau were randomized to receive BCG at birth (early BCG) or delayed by 6 weeks (delayed BCG). In an immunological study nested within the trial, capillary blood samples were collected four weeks after randomization to assess the effect of BCG on *in vitro* antigen-induced recall cytokine responses. After the primary analyses (Jensen et al., 2015), remaining plasma samples of sufficient volume were utilized for subsequent metabolomic and lipidomic assays.

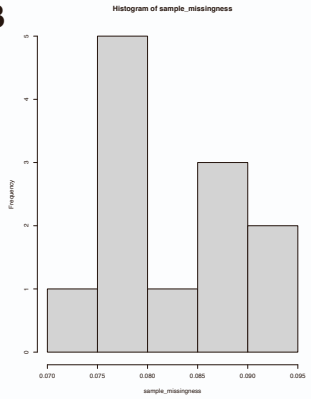
B. Human newborn cord blood samples (n= 12) were collected from healthy term newborns (≥ 37 weeks gestation) for *in vitro* stimulation with vehicle (saline) or BCG. The plasma samples were processed for metabolomics, complex lipid panel lipidomics, and multiplex cytokine/chemokine assays.

C. Newborns were recruited in The Gambia (West Africa). Each newborn provided a peripheral blood sample at the day of birth (DOL0) and subsets of newborns, each providing a second peripheral blood sample at either DOL1, 3, or 7. The newborns were assigned to either delayed-vaccinated up to 1 week (participant n= 13, total samples n=26) or EPI-vaccinated at birth (participant n=14, total samples n=28). Newborn peripheral venous blood was drawn directly into heparinized collection tubes and subjected to metabolomic assay.

A



B

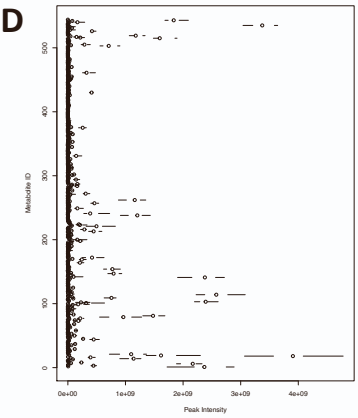


C

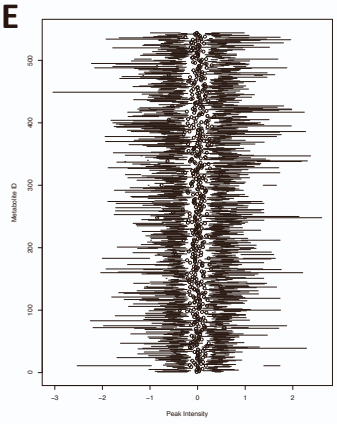
Quality control/assurance summary for *in vivo* Guinea-Bissau cohort	
# Metabolites detected	674
# Metabolites with missing values	23
# Metabolites with IQR=0	9
# Metabolites passed QC/QA	642
# Xenobiotic metabolites	98
# Metabolites included in analysis (after xenobiotics removal)	544

Guinea-Bissau *in vivo* cohort	% missing values per metabolite	% missing values per sample
Min	0	0.07292
1st Quartile	0	0.07812
Median	0	0.0816
Mean	0.08261	0.0261
3rd Quartile	0	0.08724
Max	1	0.09201

D



E



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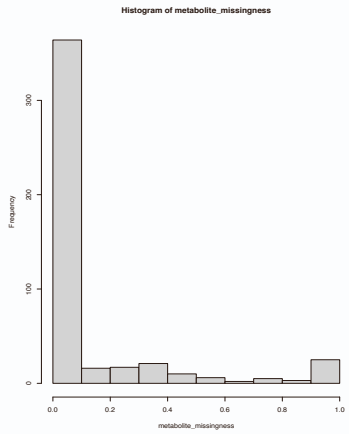
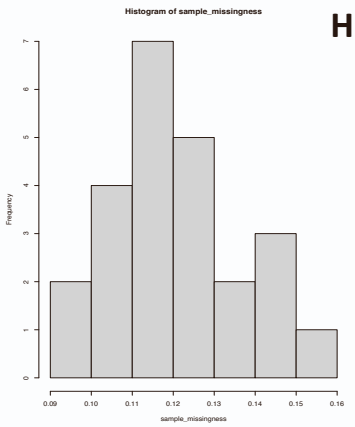


G

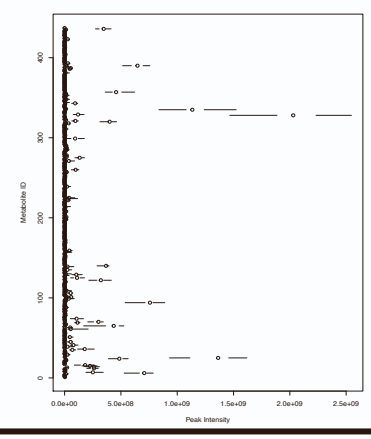


H

Quality control/assurance summary for Boston *in vitro*	
# Metabolites detected	568
# Metabolites with missing values	17
# Metabolites with IQR=0	15
# Metabolites passed QC/QA	536
# Xenobiotic metabolites	99
# Metabolites included in analysis (after xenobiotics removal)	437

Boston *in vitro* cohort	% missing values per metabolite	% missing values per sample
Min	0	0.09382
1st Quartile	0	0.1194
Median	0	0.1194
Mean	0.11976	0.11976
3rd Quartile	0.08333	0.1274
Max	1	0.15139

I



J

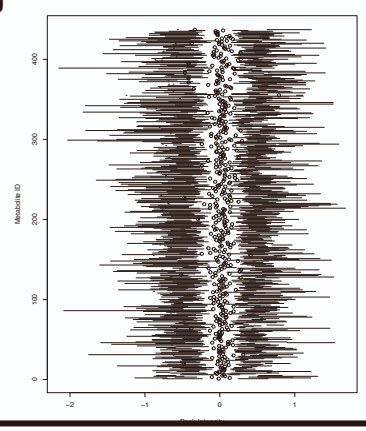


Figure S2: Summary of quality control and assurance method for metabolomics Guinea-Bissau (*in vivo*, upper panel) and Boston (*in vitro*, lower panel) datasets. Related to Figure 2.

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A and F. Histogram of the proportion of missing values for each metabolite

B and G. Histogram of the proportion of missing values for each sample

C and H. Quality control and assurance metrics summary table.

D and I. Pre-processing distribution before scaling and normalization.

E and J. Post-processing distribution after scaling and normalization.

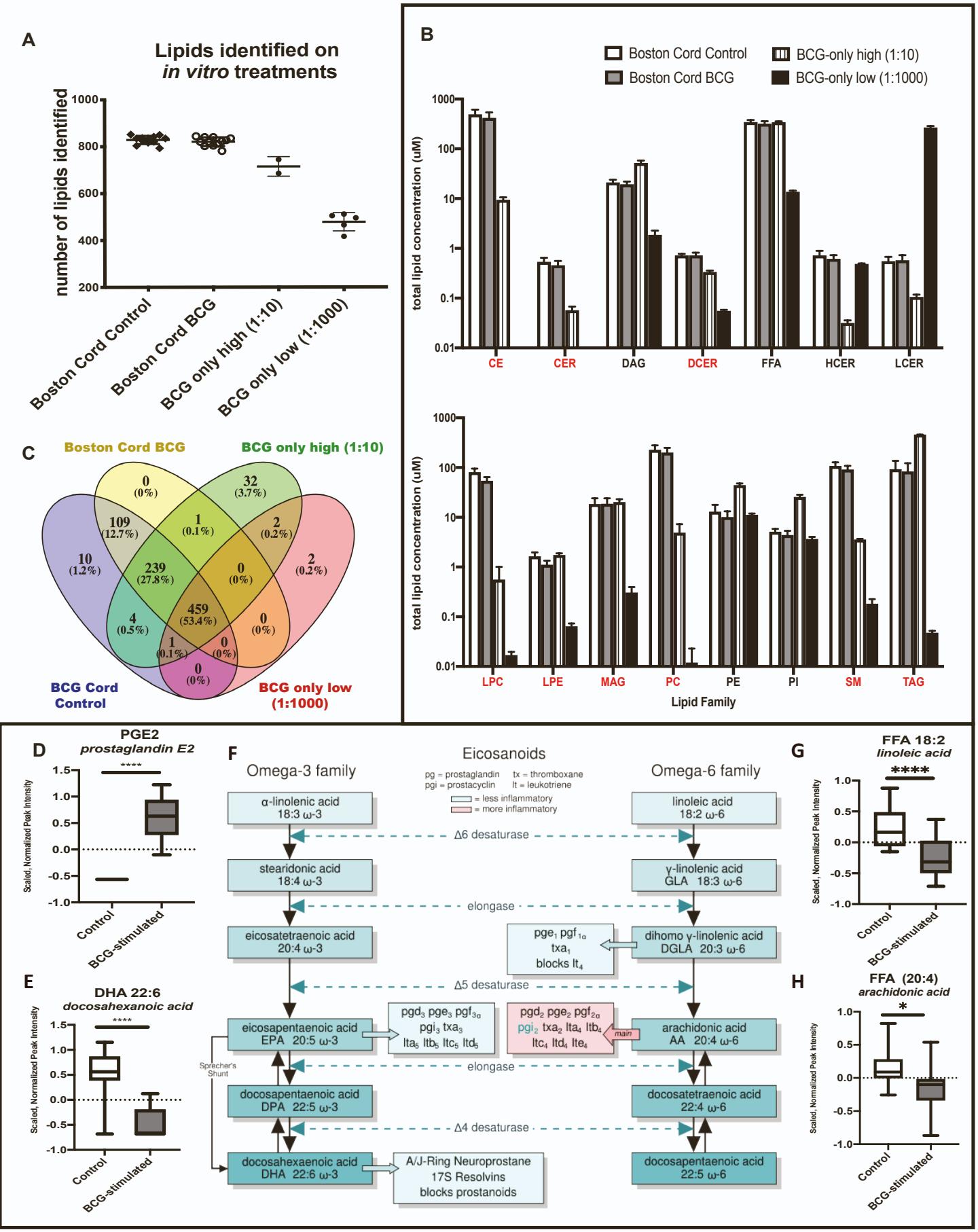

Figure S3: Addition of BCG to human newborn cord blood *in vitro* perturbs the eicosanoid lipid pathway. Related to Figure 4.

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A. Heparinized human cord blood was stimulated with saline control or BCG vaccine for 18 hr after which the extracellular medium was collected and analyzed via complex lipid panel lipidomics. BCG alone was tested at high (1:10 v/v) and low (1:1000v/v) concentrations as controls. For *in vitro* studies, the 1:1000 vol/vol ratio was used. A summary of the number of lipids quantified is illustrated. Data are presented as mean ± SEM.

B. Comparison of lipid families showed that BCG-only controls (BCG vaccine alone) have lower measured lipids in most families than cord blood stimulated with vehicle or BCG. For *in vitro* treatments, BCG low (1:1000 vol/vol) concentration was used to stimulate cord blood. Lipid families with very low or undetectable concentrations after BCG-only low or BCG cord stimulation (1:1000 vol/vol) are depicted in red.

C. The Venn diagram of detected lipids illustrates that BCG-only controls had fewer lipids detected than *in vitro* control- or BCG-stimulated cord blood samples.

D-H. BCG-induced differentially abundant lipids (DALs) included fatty acid components such as prostaglandin E_2 (PGE_2) (panel D), docosahexaenoic acid (DHA 22:6) (panel E), linoleic acid (FFA 18:2) (panel G), and arachidonic acid (FFA 20:4) (panel H) suggesting BCG modulates lipid mediators of inflammation (panel F). BCG induced a pro-inflammatory eicosanoid pathway pattern *in vitro*, decreasing anti-inflammatory DHA 22:6, FFA 18:2, and FFA 20:4 while increasing pro-inflammatory PGE_2. Statistical analyses employed repeated measures t-test for participant samples. Data are presented with box and whiskers depicting quartiles and variability outside the quartiles.

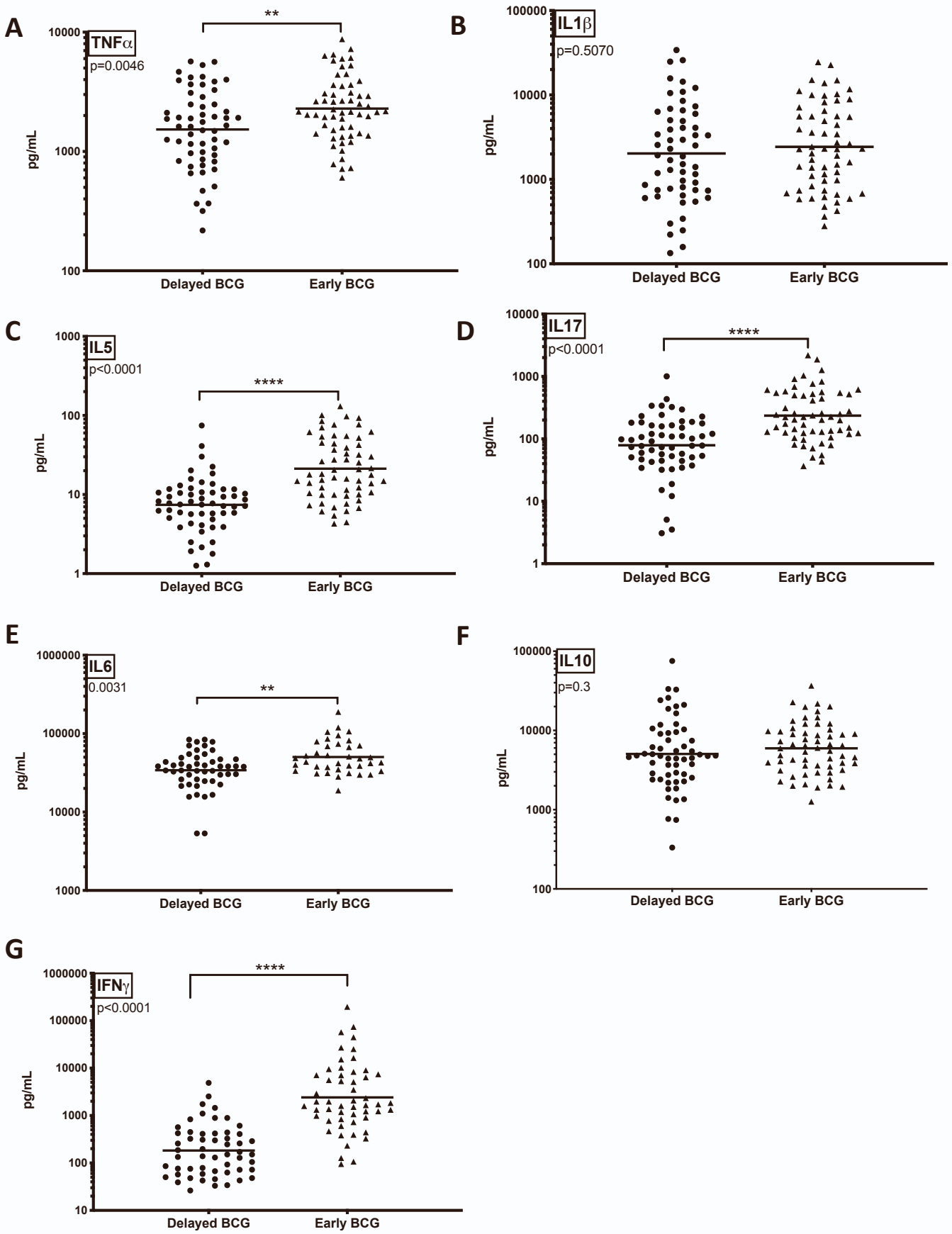

Figure S4: Early BCG group demonstrated enhanced cytokine production upon PPD re-stimulation of heparinized blood collected 4 weeks post BCG vaccination. Related to Figure 6 and Table S6.

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A-G. PPD-induced cytokines were compared in the early vs. delayed BCG groups. The early BCG group demonstrated significantly greater PPD-induced production of TNFα, IL5, IL6, IL17, and IFNγ. Groups were compared using the Wilcoxon Rank Sum Test. Data are depicted as geometric means. * $P < 0.05$: ** $P < 0.01$; *** $P < 0.001$

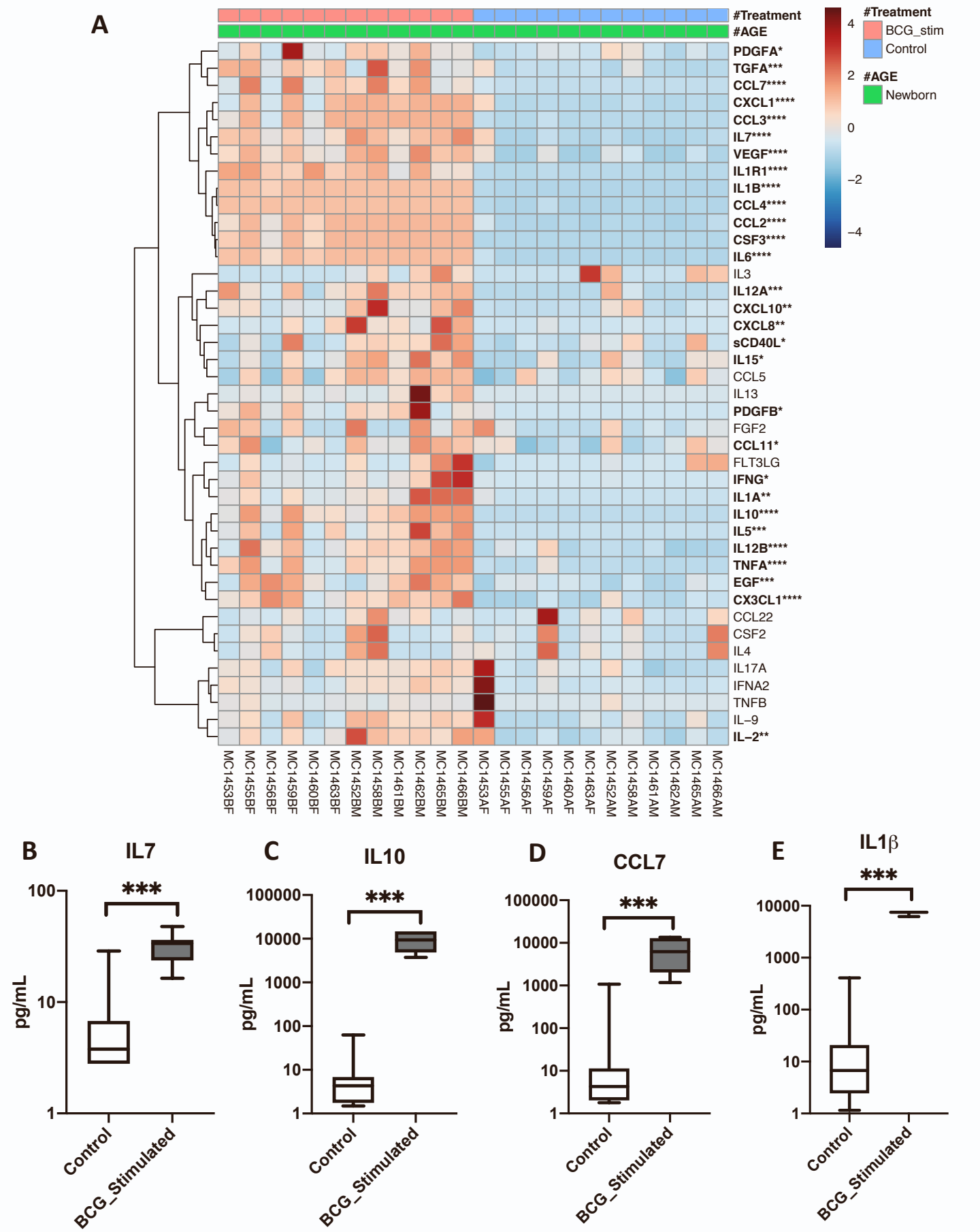

Figure S5: BCG-induced cytokine and chemokine production in Boston human newborn cord blood *in vitro*. Related to Figure 1.

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A. Heatmap depicting changes in the production of 41 selected cytokines and chemokines after BCG *in vitro* stimulation for 18 hr. Two-sample t-tests with Benjamini, Krieger and Yekutieli correction comparing BCG-stimulated vs. vehicle control * $P < 0.05$: ** $P < 0.01$; *** $P < 0.001$. Data are reported using Euclidean distance and Ward algorithm.

B-E. Selected inflammatory cytokines showed differences between vehicle control stimulated cord blood vs. BCG-stimulated cord blood. Paired student t-test used for data analysis * $P < 0.05$: ** $P < 0.01$; *** $P < 0.001$.