The Journal of Infectious Diseases

Neonatal BCG vaccination influences cytokine responses to Toll-like receptor ligands and heterologous antigens

--Manuscript Draft--

Manuscript Number:	
Full Title:	Neonatal BCG vaccination influences cytokine responses to Toll-like receptor ligands and heterologous antigens
Short Title:	Heterologous cytokine responses after BCG
Article Type:	Major Article
Section/Category:	Bacteria
Keywords:	BCG vaccination, neonatal, cytokine, heterologous, non-specific, trained immunity
Corresponding Author:	Bridget Freyne, MbBchBAO, Royal Children's Hospital Melbourne, Victoria AUSTRALIA
Corresponding Author Secondary Information:	
Corresponding Author's Institution:	Royal Children's Hospital
Corresponding Author's Secondary Institution:	
First Author:	Bridget Freyne, MD
First Author Secondary Information:	
Order of Authors:	Bridget Freyne, MD
	Susan Donath, PhD
	Susan Germano, MSc
	Kaya Gardiner, MPHTM
	Dan Casalaz, MD
	RM Robins-Browne, PhD
	Nelly Amenyogbe
	Nicole Messina, PhD
	Mihai Netea, PhD
	Katie Flanagan, MD,PhD
	Tobias Kollmann, MD,PhD
	Nigel Curtis, MD, PhD
Order of Authors Secondary Information:	
Manuscript Region of Origin:	AUSTRALIA
Abstract:	Background (Words 192) Bacille Calmette-Guérin (BCG) vaccination is associated with a reduction in all-cause infant mortality in high mortality settings. The underlying mechanisms remain uncertain but long-term modulation of the innate immune response (trained immunity) may be involved. Methods Whole blood from 212 neonates enrolled in a randomised trial of neonatal BCG vaccination was stimulated in vitro with killed pathogens and Toll-like receptor (TLR) ligands to interrogate cytokine responses.

	Results BCG-vaccinated infants had increased production of IL-6 in unstimulated samples and decreased production of IL1-ra, IL-6, and IL-10 and the chemokines MIP-1α, MIP-1ß, MCP-1 following stimulation with peptidoglycan (TLR2) and R848 (TLR7/8). BCG-vaccinated infants also had decreased MCP-1 responses following stimulation with heterologous pathogens. Sex and maternal BCG vaccination status interacted with neonatal BCG vaccination. Conclusions Neonatal BCG vaccination influences cytokine responses to TLR ligands and heterologous pathogens. This effect is characterised by decreased anti-inflammatory cytokine and chemokine responses in the context of higher levels of IL-6 in unstimulated samples. This supports the hypothesis that BCG vaccination modulates the innate immune system. Further research is warranted to determine if there is an association between these findings and the beneficial "non-specific" effects of BCG vaccine on all-cause mortality.
Suggested Reviewers:	Kristoffer Jarlov Jensen, PhD Clinical Researcher, Serum Staten Instituit kjj@ssi.dk Dr Jensen has published a similar study focusing on the response to Toll-like receptor ligands in neonates in Guinea-Bissau.
	Hazel Dockrell, PhD Professor of Immunology, LSHTM Hazel.Dockrell@lshtm.ac.uk Prof Dockrell has published extensively on the immune response to BCG vaccination.
	Meryn Vosey, M.biostat NHR Doctoral Reserach fellow, Oxford University merryn.voysey@phc.ox.ac.uk Ms. Vosey was an author on the recent systematic review in the British Medical Journal entitled the “Immunological basis for the non-specific effects of routine neonatal immunisations.”
	Denise Faustman, MD PhD Associate Professor, Harvard Medical School DFAUSTMAN@mgh.harvard.edu Denise Faustman is the director of the immunobiology laboratory at the Massachusetts General Hospital (MGH) and an Associate Professor of Medicine at Harvard Medical School. She has published work on TNF responses following BCG vaccination.
	Eleanor Molloy, MD, PhD Professor, Trinity College Dublin molloyel@tcd.ie Professor Molloy is an expert in neonatology and has published widely on the neonatal immune response to innate ligands.

Neonatal BCG vaccination influences cytokine responses to Toll-like receptor ligands and heterologous antigens

Running title: Heterologous cytokine responses after BCG

Freyne B [1, 2], Donath S [2,3], Germano S [1], Gardiner K [1], Casalaz D [4], Robins-Browne RM [1,5], Amenyogbe N [6, 7], Messina NL [1,2], Netea MG [8,9], Flanagan KL [10,11], Kollmann T [6,7], Curtis N [1,2]

Bridget.freyne@rch.org.au

1 Infectious Diseases & Microbiology Group, Murdoch Children's Research Institute, Royal Children's Hospital Melbourne, Parkville, Australia.

2 Department of Paediatrics, The University of Melbourne, Parkville, Australia.

3 Clinical Epidemiology & Biostatistics Unit, Murdoch Children's Research Institute, Melbourne, Parkville, Australia.

4 Department of Paediatrics, Mercy Hospital for Women, Heidelberg, Australia.

5 The Department of Microbiology and Immunology, The University of Melbourne, Parkville, Australia.

6 Department of Experimental Medicine, University of British Columbia, Vancouver, Canada.

7 Division of Infectious Diseases, Department of Pediatrics, University of British Columbia, Canada.

8 Department of Internal Medicine, Radboud Institute for Molecular Life Sciences, Radboud University Medical Center, Nijmegen, The Netherlands.

[9] Radboud Center for Infectious Diseases, Radboud University Medical Center, Nijmegen, The Netherlands.

[10] School of Medicine, University of Tasmania, Launceston, Australia.

[11] Department of Immunology and Pathology, Monash University, Clayton, Australia.

Background (Words 192)

Bacille Calmette-Guérin (BCG) vaccination is associated with a reduction in all-cause infant mortality in high mortality settings. The underlying mechanisms remain uncertain but long-term modulation of the innate immune response (trained immunity) may be involved.

Methods

Whole blood from 212 neonates enrolled in a randomised trial of neonatal BCG vaccination was stimulated in vitro with killed pathogens and Toll-like receptor (TLR) ligands to interrogate cytokine responses.

Results

BCG-vaccinated infants had increased production of IL-6 in unstimulated samples and decreased production of IL1-ra, IL-6, and IL-10 and the chemokines MIP-1α, MIP-1ß, MCP-1 following stimulation with peptidoglycan (TLR2) and R848 (TLR7/8). BCG-vaccinated infants also had decreased MCP-1 responses following stimulation with heterologous pathogens. Sex and maternal BCG vaccination status interacted with neonatal BCG vaccination.

Conclusions

Neonatal BCG vaccination influences cytokine responses to TLR ligands and heterologous pathogens. This effect is characterised by decreased anti-inflammatory cytokine and chemokine responses in the context of higher levels of IL-6 in unstimulated samples. This supports the hypothesis that BCG vaccination modulates the innate immune system. Further research is warranted to determine if there is an association between these findings and the beneficial “non-specific” effects of BCG vaccine on all-cause mortality.

Key words: BCG vaccination, neonatal, cytokine, heterologous, non-specific, trained immunity.

Background (3459)

Substantial evidence supports the concept that Bacille Calmette-Guérin (BCG) vaccine reduces all-cause infant mortality in high mortality settings [1-4]. The recent WHO Special Advisory Group of Experts (SAGE) on Immunisation systematic review of the heterologous ('non-specific') effects of BCG showed that neonatal BCG vaccination is associated with a 30% reduction in all-cause mortality (RR 0.70 (95% CI 0.49-1.01) [5]. Epidemiological studies also suggest that BCG given at birth protects against the development of allergic disease [6, 7] and pneumonia [8].

Randomised controlled trials of BCG vaccination at birth report a reduction in neonatal mortality attributable to infection [1, 3]. Evidence suggests that the benefit of BCG vaccination is conferred as early as 7 days of life [4]. Clinical and demographic factors that influence vaccine responses include sex [1, 9], co-administration and timing of other neonatal vaccines or supplemental vitamins [10] and maternal BCG vaccination [11].

The need for further research into the immunological mechanisms underlying the heterologous effects of BCG has been highlighted [12]. BCG administration in animal models has shown improved survival against a wide range of pathogens including bacteria, viruses fungi and parasites [13]. Multiple pathways of innate, cellular and adaptive immunity are proposed to underpin BCG's protective effect [14]. A previous study done in West Africa showed that BCG vaccination altered neonatal cytokine responses following stimulation with Toll-like receptor (TLR) ligands [15]. Altered responses following BCG vaccination have also been reported following in vitro stimulation of peripheral blood mononuclear cells with

heterologous bacteria in adults [16]. It is proposed that this results from induction of '*trained immunity*' (immune memory in the innate immune system) through epigenetic reprogramming of monocytes [16].

Our study aimed to investigate whether BCG vaccination impacts neonatal cytokine responses to TLR ligands and clinically significant bacterial and fungal pathogens, and thus induces heterologous innate immune effects.

Methods

Participants were a subset of infants recruited from The Melbourne Infant Study: BCG for the prevention of allergy and infection (MIS BAIR), a randomised controlled trial of BCG in 1272 newborn infants with the primary outcomes of allergic sensitisation, eczema and lower respiratory tract infection (Clinical trials registration NCT01906853). Participants were randomised 1:1 to vaccination with BCG-Denmark 0.05 mL intradermally within 10 days of birth or no BCG vaccination. All participants also received a birth dose of hepatitis B vaccine as per Australian immunisation guidelines. A subset of 447 participants recruited over an 18-month period had blood sample for immunological analyses (Figure 1). Exclusion criteria for inclusion in the immunological study were: (i) suspected neonatal or maternal sepsis; (ii) infant did not receive hepatitis B vaccine prior to blood sampling; or (iii) infant received blood products in the first 7 days of life.

Written informed consent was obtained from the parents of all participants. The study was approved by the human ethics research committees of the Royal Children's Hospital (HREC 33025) and the Mercy Hospital for Women (HREC R12/28).

Participants were visited in their home 7 (±4) days after randomisation. Blood was collected via a closed loop system into sodium-heparin tubes (S-monovette, Numbrecht, Germany) pre-tested for absence of endotoxin. Blood samples were kept at room temperature, protected from direct sunlight, and transported to the laboratory and stimulated at 4 hours (± 10%; 24 minutes) after collection. Laboratory personnel were blind to the participants' BCG vaccination status.

Whole blood, diluted 1:1 with RPMI-1640 medium (GlutaMAX™ Supplement, HEPES, Gibco, Life Technologies), was added to pre-prepared stimulation assay strips (MICROLON™ 600 Greiner-One 8-well strips). The assay strips were made using a validated robotic system to maximise standardisation of stimulant volume, and were stored at –80°C until addition of blood. The final stimulant concentrations were BCG-Denmark 75 µg/mL, killed *Mycobacterium tuberculosis* 1.0 x 10^6 CFU/mL, seven heat-killed bacteria (*Escherichia coli* 1.0 x 10^6 CFU/mL, *Haemophilus influenzae* type B 1.0 x 10^6 CFU/mL, *Staphylococcus aureus* 1.0 x 10^7 CFU/mL, *Streptococcus agalactiae* 1.0 x 10^7 CFU/mL, *Streptococcus pneumoniae* serotype 15C 1.0 x 10^7 CFU/mL and *Listeria monocytogenes* 1.0 x 10^7 CFU/mL), one fungus (*Candida albicans* 1.0 x 10^6 CFU/mL) and four TLR ligands (lipopolysaccharide [LPS] 100 ng/mL, resiquimod [R848] 3.5 µg/mL, peptidoglycan [PEPG] 10 µg/mL and (S)-(2,3-bis(palmitoyloxy)-(2-RS)-propyl)-N-palmitoyl-(R)-Cys-(S)-Ser-(S)-Lys4OH,trihydrochloride [Pam3Cysk4] 1 µg/mL (all from *Invivogen*]) and RPMI. Pathogens were clinical isolates

obtained from children with invasive disease at the Royal Children's Hospital Melbourne, Parkville, Australia, and killed by heat-treatment at 70°C for 2 hours. Sterility was confirmed by the absence of growth in culture medium. Concentrations of all stimuli were optimised in preliminary experiments (data not shown). The stimulation assay was incubated at 37°C (5% CO_2: air) for 20 (± 2) hours.

Supernatants were analysed in batches using Bio-Rad human cytokine kits following the manufacturer's instructions. The mean fluorescence intensity was read for each cytokine using an xMAP Luminex 200 Analyser. Each sample was analysed in two separate kits at different dilutions to account for the anticipated dynamic range of the cytokine results indicated by preliminary experiments (data not shown): (i) sample diluted 1:100 (7-plex): IL-1β, IL-1ra, IL-6, IL-8, MCP-1 (MCAF), MIP-1α, MIP-1ß, and (ii) sample diluted 1:4 (5-plex): IFN-γ, IL-10, TNF-α, MIF and MIG.

Statistical analysis was done using Stata version 13.1. Cytokine results that were below the lower limit of detection were assigned a value of half the lowest detectable value (either the lowest standard on the curve or the lowest extrapolated value below this) multiplied by the appropriate dilution factor. There were no instances when cytokine results were above the upper limit of detection. Prior to analysis, cytokine data were log transformed.

The effect of (i) BCG vaccination, (ii) reported maternal BCG vaccination status and (iii) timing of randomisation (early: less than 48 hours after birth; late: 48 hours or more after birth) on cytokine production in response to each stimulant was investigated using logistic regression, with the log transformed value of the unstimulated (RPMI only) cytokine values

as a covariate. Results were expressed as geometric mean ratios (GMR) with 95% confidence intervals. One participant was an extreme outlier for all cytokines in unstimulated samples but this was successfully corrected for in stimulated cytokine results using the unstimulated value as a covariate; as this could not be done for the analysis of unstimulated cytokine results, this participant was excluded in this analysis. Data are presented graphically as GMR (95% CI) to illustrate the results across cytokine/stimulant pairs without emphasis on tests of statistical significance [17]. Subgroup effects (sex, mode of delivery and maternal BCG vaccination status) were investigated where there was statistical evidence of an interaction effect between BCG vaccination and the subgroup variable. In addition, to exclude the possibility of confounding by sex, mode of delivery, maternal BCG status or individual unstimulated cytokine values, the effect of BCG vaccination on cytokine expression, adjusted for these variables was investigated using multiple logistic regression. Sensitivity analyses were performed to investigate the effect of both excluding values assigned at the lower end of the standard curve and values extrapolated by the xMAP Luminex 200 Bioanalyser software.

Results

Demographic features are outlined in Table 1. There was a high degree of standardisation in the processing of samples and laboratory procedures (Supplementary Table 1). Our data exhibited the marked inter-individual variability in cytokine responses in both stimulated and unstimulated samples characteristic of cytokine data (Supplementary data, Figure 1).

In unstimulated (RPMI) samples, BCG-vaccinated infants showed increased production of IL-6, IL-1ra and IL-1ß, however the evidence that this was a real effect was weak for IL-1ra and IL-1ß (Table 2). For TLR agonist responses, chemokine and cytokine production was unchanged by BCG in response to Pam3Cysk4 and LPS, but was generally lower in BCG-vaccinated infants in response to stimulation with the TLR ligands PEPG and R848. This effect was strongest for the cytokines IL-6, IL-10 and chemokines MIP-1α, MIP-1ß, MCP-1 (Figure 2 and Supplementary data Table 2).

In response to stimulation with heterologous bacteria, the chemokines and cytokines for which production was most consistently decreased were IL-1ra, MCP-1 and MIP-1ß, particularly in response to stimulation with *L. monocytogenes*, *E. coli* and *S. pneumoniae*, and the fungus *C. albicans* (Figure 2, Supplementary Table 2).

Sex independently influenced cytokine responses in our population (data not shown). There was a significant interaction between sex and BCG vaccination for production of MIF, in response to stimulation with *L. monocytogenes, H. influenzae, S. agalactiae, M. tuberculosis* and R848 (Supplementary data Table 3). Separate analyses of males and

females showed there were generally lower MIF responses in BCG-vaccinated males and higher responses in BCG-vaccinated females. BCG-vaccinated females had higher MIF production in response to stimulation with *S. agalactiae*, whereas BCG-vaccinated males had lower MIF production in response to stimulation with the intra-cellular stimulants R848, *L. monocytogenes, M. tuberculosis* (Figure 3) compared to non BCG-vaccinated controls of the same sex.

Mode of delivery independently influenced cytokine responses in our population (data not shown), however there was minimal evidence of an interaction between BCG-vaccination and mode of delivery on cytokine responses (Supplementary data Table 3). Despite randomisation of participants to BCG vaccination, attrition from the immunological study led to differences in the distribution of sex and mode of delivery between the two groups (Table 1). A multivariate regression model including these two variables and the log transformed unstimulated cytokine yielded the same results as the primary analysis and excluded confounding as a contributor to the primary results.

Infants whose mothers had a history of BCG vaccination had increased production of IL-1ß, IL-1ra, IL6 and MIP-1α in response to Pam3Cysk4, IL6 and MIP-1α in response to *H. influenzae,* and of MIP-1ß in response to PEPG. Conversely, infants whose mothers were not BCG-vaccinated had higher production of TNF-α and IL-1ß in response to R848, and IL-10 production in response to *S. agalactia*e independent of infant BCG vaccination status. (Supplementary Table 2).

There was evidence of an interaction between maternal BCG vaccination status and infant BCG vaccination status for five cytokine/stimulant pairs (IFN-γ/*S. pneumoniae*, IL-1ß/BCG, MIP-1α/PEPG, MIP-1α/Pam3Cysk4, MIG/*H. influenzae*) (Supplementary Table 3). Separate analyses in infants of mothers who were BCG vaccinated and those whose mothers were not BCG-vaccinated showed that in the former, BCG-vaccinated infants had higher IFN-γ production in response to stimulation with *S. pneumoniae,* MIP-1α in response to PEPG and IL-10 production in response to Pam3Cysk4. Conversely for BCG-vaccinated infants whose mothers' were not BCG vaccinated MIP-1α expression was increased in response to Pam3Cysk4 (Figure 4).

Infants were categorised into those who were randomised early (less than 48 hours after birth) and late (48 hours or more after birth) (Supplementary Figure 2a and 2b). The interval between BCG vaccination and blood sampling was similar between both groups (median interval 7 days (range 0-11) vs 8 days (range 3-11)) (Supplementary Figure 2c).

Compared to those in the late BCG vaccination group, infants in the early BCG vaccination group had significantly higher cytokine and chemokine production following stimulation with all stimulants except for BCG and *M. tuberculosis* (Supplementary Table 4).

To determine how the timing of BCG vaccination influenced the results of the primary analysis, we compared cytokine production in the early BCG-vaccinated group with the non BCG-vaccinated group, and the late BCG-vaccinated group with the non BCG-vaccinated group (Supplementary Table 4). The influence of BCG vaccination on cytokine production was more marked in the late BCG-vaccinated group than in the early BCG-vaccinated group

when each was compared with the non BCG-vaccinated group. The relationship between the results of this subgroup analysis relevant to the cytokine/stimulant pairs identified as significant from our primary analysis is shown in Figure 5.

Sensitivity analyses were performed for the primary outcome, i.e. the effect of BCG vaccination on cytokine responses following in vitro stimulation, to investigate the influence of (i) the assigned values at the lower end of the standard curve and (ii) the values extrapolated by Luminex Xmap software at both the upper and lower ends of the standard curve (Supplementary Table 5). Overall, the results of the primary analysis were preserved across sensitivity analyses. Notable differences were seen only for production of IL-1ra in response to R848, MIP-1ß in response to R848, and IFN-γ in response to several stimulants, including *M. tuberculosis* (Supplementary data Table 5).

Discussion

This study adds to the growing body of immunological evidence to explain the observed heterologous effects of BCG vaccination by finding differential cytokine production between BCG-vaccinated infants and non BCG-vaccinated infants both in unstimulated samples and following in vitro stimulation with TLR ligands and heterologous pathogens.

The pattern of cytokine production observed in our study, using a well-validated system [18, 19], was consistent with previous studies in neonates. IL-10 production was high relative to IFN-γ for all stimulants [19]. IL-6 production was relatively high compared to pro-inflammatory cytokines such as TNF-α and IL-1ß [20]. In addition, there was robust

chemokine production in response to TLR ligand stimulation, particularly R848 [21]. These patterns were seen despite considerable inter-individual variability, both at in unstimulated samples and following stimulation [22].

In our study, compared to non BCG-vaccinated infants, BCG-vaccinated infants had reduced production of multiple cytokines and chemokines in response to stimulation with the TLR ligands PEPG (TLR 2/(Nod)-like receptor 1/2) and R848 (TLR 7/8). PEPG and R848 are potent activators of neonatal innate immunity when compared to Pam3CYSK43 (TLR2) and LPS (TLR4) [23] [24]. R848 induces neonatal antigen presenting cells leading to Th1 polarisation and production IL-12p70 and TNF-α in equivalent levels to adults [24]. Excess stimulation of TLR7/8 with R848 is associated with neutrophil-mediated lung injury following RSV infection and an increased incidence of viral-associated wheeze in mouse models [25, 26]. PEPG has endotoxic properties and potentiates LPS signalling, augmenting its pathogenicity in sepsis syndromes [27-29]. The observed differential production of MCP-1 following stimulation with several heterologous bacteria between BCG-vaccinated and non BCG-vaccinated infants is interesting in light of MCP-1's pivotal role in the control of mycobacterial disease. Elevated levels of MCP-1 are associated with pro-inflammatory diseases, including asthma and multiple sclerosis, and cancer progression [30].

The modified neonatal cytokine profile induced by BCG vaccination in our study characterised by reduced anti-inflammatory cytokine (IL-10, IL-1ra) and IL-6 responses following stimulation with heterologous bacteria suggests a pro-inflammatory bias in the BCG-vaccinated infants in the context of bacterial challenge. A concomitant down-regulation in production of several chemokines, including MCP-1, could reduce leukocyte

recruitment and associated tissue damage during infection. Overall, these effects might result in a less severe sepsis response that might contribute to the reduction in mortality reported in BCG-vaccinated infants in high mortality settings.

Jensen et al also investigated the immunological non-specific effects of BCG by measuring cytokine responses in whole blood [15]. In low-birth weight infants in Guinea-Bissau, compared to non BCG-vaccinated infants, BCG-vaccinated infants had increased IL-1ß and TNF-α production in unstimulated samples. Consistent with this, we found increased IL-6 and IL-1ra (but not TNF-α) in unstimulated samples. Also consistent, we found no effect of BCG vaccination on cytokine production following stimulation with LPS. In Jensen et al's study, BCG-vaccinated infants had increased IL-1ß, IL-6 and TNF-α production in response to stimulation with Pam3Cysk4, whereas in our study BCG vaccination had not effect on cytokine responses to Pam3Cysk4. Several factors that influence cytokine production differed between our study and that of Jensen et al. These include ethnicity, geographic locations [31], age [32] (infants were sampled at a mean of 30 days of age in Guinea-Bissau in contrast to a mean of 7 days in our study), maternal HIV status [33], weight, gestational age, underlying health, hepatitis B vaccination status, maternal BCG vaccination status, blood sampling method and anticoagulant. Jensen et al's conclusion that BCG vaccination results in a stronger pro-inflammatory profile is consistent with our findings, albeit through different mechanisms.

Kleinnijenhuis et al also found a pro-inflammatory response to heterologous bacteria induced by BCG vaccination. In this study, in adults, increased IFN-γ, TNF-α and IL-1β production in PBMC was observed following stimulation with heat-killed *M. tuberculosis,*

S. aureus and *C. albicans* [16]. More recently, a similar study in infants born in the United Kingdom showed increased IL-6 production in PBMC stimulated with C. albicans, S. aureus and Pam3Cysk4 [34]. The authors' attributed these changes to epigenetic reprogramming which resulted in 'trained immunity' or innate immune memory

Our finding that maternal BCG vaccination influenced the production of cytokines in infants is interesting. Latent maternal TB infection is associated with increased production of pro-inflammatory cytokines in response to mycobacterial stimulants in BCG-vaccinated infants [35]. Moreover, in a recently reported trial from Denmark, BCG-vaccinated infants had decreased infectious illnesses during the first three months of life if their mother had a history of BCG vaccination [36]. Two possible explanations might account for the potentiating effect of maternal BCG vaccination. Firstly, transplacentally-acquired maternal antibodies could modify the response to BCG in the infant. Secondly, trans-generational epigenetic changes in immune responses could be involved; such a phenomenon has been reported in lower organisms, but has not yet been studied in mammals[37, 38]. Understanding how maternal and infant mycobacterial exposures influence the neonatal response to BCG warrants further investigation.

It is well recognised that vaccine responses are affected by sex [39]. Although BCG vaccination alone did not affect the production of MIF in our study, there was a significant interaction between BCG vaccination and sex for MIF production in response to several heterologous antigens. MIF is produced by macrophages, lymphocytes and pituitary cells both constitutively and in response to bacterial infection or stress [40]. MIF has been implicated in severe sepsis and malaria and the development of chronic inflammatory

diseases, including dementia and atherosclerosis [41, 42]. The high levels of MIF at baseline in the infants in our study are in line with previous findings [43]. Given its pivotal role in the control of sepsis, the sex-differential effect of BCG vaccination on MIF production observed in our study warrants further investigation.

The effect of timing of administration of BCG on the vaccine response is complex. In our study, early BCG vaccination was associated with increased production of multiple cytokines across most stimulants when compared to late BCG vaccination. Furthermore, the effect of timing of BCG vaccination on the results of our primary analysis suggests these effects become more pronounced with older age. Interpretation of these results is difficult as the analysis is potentially confounded in our study by the older age of infants at blood sampling.

The strengths of our study include our ability to randomise term infants because BCG is not routinely given in Australia. Another strength was the highly standardised and controlled protocols used for blood collection, handling of samples and laboratory assays [44]. Interpretation of the results of multiplex cytokine analysis need to take into consideration the effect of assigned values outside the limit of detection of the assay and values which have been extrapolated by the bioanalyser software. There is no agreed method for standardisation or reporting of these parameters and methods described in the literature are highly variable. The findings in our study were preserved across sensitivity analyses designed to examine the effect of these assigned variables.

A potential limitation is that infants in our study were co-administered hepatitis B vaccine as part of the Australian immunisation schedule. BCG has been associated with effects on

hepatitis B antibody production in studies in the Gambia [45] and Australia [46], but not in South Africa [47]. The effects of hepatitis B vaccines on innate immunity are not well studied [48] and there have been no studies investigating the potential heterologous effects of hepatitis B vaccines or its interaction with BCG.

A further limitation of our study is the inability to perform traditional sample size calculations. Marked variability in both baseline and stimulated levels of cytokines necessitate large sample sizes. This is particularly relevant in the interpretation of subset analyses. To avoid spurious results of statistical significance in the context of multiple comparisons in subgroups of data, we used interaction analyses to explore the effect of sex, mode of delivery and maternal BCG status on our primary outcome. To aid interpretation and avoid undue emphasis on tests of statistical significance or conversely discarding results that were not significant due to type 2 error, we presented all our analyses with confidence intervals [49].

Based on our data, future studies on the innate immune response to BCG should focus on interrogation of chemokine-producing cells and pathways activated by R848 and PEPG, including TLR 7/8, TLR2, RIG and NOD. Furthermore, studies in high mortality settings would be required to confirm any association between patterns of innate immune modulation in vitro and clinical outcomes following infection..

In conclusion, our study provides evidence of differential cytokine responses to heterologous antigens between BCG vaccinated and non BCG-vaccinated term infants. The responses can be broadly described as inhibition of anti-inflammatory cytokine production,

as well as down-regulation of chemokine responses. Both sex and maternal BCG vaccination status interacted with BCG vaccination. This study supports the concept that BCG modifies the neonatal innate immune response.

Table & Figure legends

Table 1: Baseline characteristics of study participants.

Table 2: Geometric mean ratio (95% CI) for unstimulated cytokines (RPMI only). Univariate analysis was for the effect of BCG vaccination.

Figure 1: Flow sheet of eligible participants and relevant exclusions. Table shows number of individual stimulations done for each antigen.

Figure 2: The effect of BCG vaccination on cytokine expression. Significant results $p<0.05$ are pale grey. GMR > 1.0 indicates cytokine levels were higher in BCG vaccinated subjects. PEPG - Peptidoglycan (TLR2), Pam3Cysk4 - (S)-(2,3-bis(palmitoyloxy)-(2-RS)-propyl)-N-palmitoyl-(R)- Cys-(S)-Ser-(S)-Lys4OH, trihydrochloride, (TLR1/2) LPS - Lipopolysaccharide (TLR4), R848 - remisiqumod (TLR7/8).

Figure 3: Interaction and subgroup analysis for the effect of sex on BCG vaccination. MIF production (GMR 95%CI) for cytokine/stimulant pairs where a significant interaction between sex and BCG vaccination was seen are shown for the whole population, in girls alone and in boys alone. Pam3Cysk4 - (S)-(2,3-bis(palmitoyloxy)-(2-RS)-propyl)-N-palmitoyl-(R)- Cys-(S)-Ser-(S)-Lys4OH, trihydrochloride, (TLR1/2), R848 - remisiqumod (TLR7/8).

Figure 4: Interaction and subgroup analysis for the effect of maternal BCG vaccination status on neonatal BCG vaccination. Cytokine responses (GMR 95%CI) for cytokine/stimulant pairs where a significant interaction between maternal BCG vaccination status and neonatal BCG vaccination was seen are shown for the whole population and then in each subgroup. Pam3Cysk4 - (S)-(2,3-bis(palmitoyloxy)-(2-RS)-propyl)-N-palmitoyl-(R)- Cys-(S)-Ser-(S)-Lys4OH, trihydrochloride, (TLR1/2), R848 - remisiqumod (TLR7/8

Figure 5: Effect of the timing of BCG on the overall effect of BCG shown by the primary analysis. Cytokine responses (GMR 95%CI) for cytokine/stimulant pairs where a significant effect of BCG vaccination was seen are shown for the whole population and Peptidoglycan (TLR2).

References

1. Aaby P, Roth A, Ravn H, et al. Randomized trial of BCG vaccination at birth to low-birth-weight children: beneficial nonspecific effects in the neonatal period? J Infect Dis **2011**; 204:245-52.

2. Shann F. The nonspecific effects of vaccines and the expanded program on immunization. J Infect Dis **2011**; 204:182-4.

3. Biering-Sorensen S, Aaby P, Napirna BM, et al. Small randomized trial among low-birth-weight children receiving bacillus Calmette-Guerin vaccination at first health center contact. Pediatr Infect Dis J **2012**; 31:306-8.

4. Kristensen I, Aaby P, Jensen H. Routine vaccinations and child survival: follow up study in Guinea-Bissau, West Africa. BMJ **2000**; 321:1435-8.

5. Higgins JP, Soares-Weiser K, Lopez-Lopez JA, et al. Association of BCG, DTP, and measles containing vaccines with childhood mortality: systematic review. BMJ **2016**; 355:i5170.

6. Arnoldussen DL, Linehan M, Sheikh A. BCG vaccination and allergy: a systematic review and meta-analysis. J Allergy Clin Immunol **2011**; 127:246-53, 53 e1-21.

7. Freyne B, Curtis N. Does neonatal BCG vaccination prevent allergic disease in later life? Arch Dis Child **2014**; 99:182-4.

8. Hollm-Delgado MG, Stuart EA, Black RE. Acute Lower Respiratory Infection Among Bacille Calmette-Guerin (BCG)-Vaccinated Children. Pediatrics **2014**; 133:e73-81.

9. Aaby P, Jensen H, Rodrigues A, et al. Divergent female-male mortality ratios associated with different routine vaccinations among female-male twin pairs. Int J Epidemiol **2004**; 33:367-73.

10. Shann F. The non-specific effects of vaccines. [Review]. Arch Dis Child **2010**; 95:662-7.

11. Mawa PA, Webb EL, Filali-Mouhim A, et al. Maternal BCG scar is associated with increased infant proinflammatory immune responses. Vaccine **2017**; 35:273-82.

12. Kandasamy R, Voysey M, McQuaid F, et al. Non-specific immunological effects of selected routine childhood immunisations: systematic review. BMJ **2016**; 355:i5225.

13. Freyne B, Marchant A, Curtis N. BCG-associated heterologous immunity, a historical perspective: intervention studies in animal models of infectious diseases. Trans R Soc Trop Med Hyg **2015**; 109:52-61.

14. Freyne B, Marchant A, Curtis N. BCG-associated heterologous immunity, a historical perspective: experimental models and immunological mechanisms. Trans R Soc Trop Med Hyg **2015**; 109:46-51.

15. Jensen KJ, Larsen N, Biering-Sorensen S, et al. Heterologous immunological effects of early BCG vaccination in low-birth-weight infants in Guinea-Bissau: a randomized-controlled trial. J Infect Dis **2015**; 211:956-67.

16. Kleinnijenhuis J, Quintin J, Preijers F, et al. Bacille Calmette-Guerin induces NOD2-dependent nonspecific protection from reinfection via epigenetic reprogramming of monocytes. Proc Natl Acad Sci U S A **2012**; 109:17537-42.

17. Wasserstein RL, Lazar NA.

18. Kollmann TR, Crabtree J, Rein-Weston A, et al. Neonatal innate TLR-mediated responses are distinct from those of adults. J Immunol **2009**; 183:7150-60.

19. Kollmann TR, Levy O, Montgomery RR, Goriely S. Innate immune function by Toll-like receptors: distinct responses in newborns and the elderly. Immunity **2012**; 37:771-83.

20. Levy O. Innate immunity of the newborn: basic mechanisms and clinical correlates. Nat Rev Immunol **2007**; 7:379-90.

21. van Haren SD, Ganapathi L, Bergelson I, et al. In vitro cytokine induction by TLR-activating vaccine adjuvants in human blood varies by age and adjuvant. Cytokine **2016**; 83:99-109.

22. Li Y, Oosting M, Deelen P, et al. Inter-individual variability and genetic influences on cytokine responses to bacteria and fungi. Nat Med **2016**; 22:952-60.

23. Levy O, Suter EE, Miller RL, Wessels MR. Unique efficacy of Toll-like receptor 8 agonists in activating human neonatal antigen-presenting cells. Blood **2006**; 108:1284-90.

24. Levy O, Zarember KA, Roy RM, Cywes C, Godowski PJ, Wessels MR. Selective impairment of TLR-mediated innate immunity in human newborns: neonatal blood plasma reduces monocyte TNF-alpha induction by bacterial lipopeptides, lipopolysaccharide, and imiquimod, but preserves the response to R-848. J Immunol **2004**; 173:4627-34.

25. Johnson TR, Rao S, Seder RA, Chen M, Graham BS. TLR9 agonist, but not TLR7/8, functions as an adjuvant to diminish FI-RSV vaccine-enhanced disease, while either agonist used as therapy during primary RSV infection increases disease severity. Vaccine **2009**; 27:3045-52.

26. Tang FS, Van Ly D, Spann K, et al. Differential neutrophil activation in viral infections: Enhanced TLR-7/8-mediated CXCL8 release in asthma. Respirology **2016**; 21:172-9.

27. Hadley JS, Wang JE, Foster SJ, Thiemermann C, Hinds CJ. Peptidoglycan of Staphylococcus aureus upregulates monocyte expression of CD14, Toll-like receptor 2 (TLR2), and TLR4 in human blood: possible implications for priming of lipopolysaccharide signaling. Infect Immun **2005**; 73:7613-9.

28. Wang JE, Dahle MK, McDonald M, Foster SJ, Aasen AO, Thiemermann C. Peptidoglycan and lipoteichoic acid in gram-positive bacterial sepsis: receptors, signal transduction, biological effects, and synergism. Shock **2003**; 20:402-14.

29. Wang JE, Jorgensen PF, Ellingsen EA, et al. Peptidoglycan primes for LPS-induced release of proinflammatory cytokines in whole human blood. Shock **2001**; 16:178-82.

30. Deshmane SL, Kremlev S, Amini S, Sawaya BE. Monocyte chemoattractant protein-1 (MCP-1): an overview. J Interferon Cytokine Res **2009**; 29:313-26.

31. Smolen KK, Ruck CE, Fortuno ES, 3rd, et al. Pattern recognition receptor-mediated cytokine response in infants across 4 continents. J Allergy Clin Immunol **2014**; 133:818-26 e4.

32. Levy O, Wynn JL. A prime time for trained immunity: innate immune memory in newborns and infants. Neonatology **2014**; 105:136-41.

33. Reikie BA, Adams RC, Leligdowicz A, et al. Altered innate immune development in HIV-exposed uninfected infants. J Acquir Immune Defic Syndr **2014**; 66:245-55.

34. Smith SG, Kleinnijenhuis J, Netea MG, Dockrell HM. Whole Blood Profiling of Bacillus Calmette-Guerin-Induced Trained Innate Immunity in Infants Identifies Epidermal Growth Factor, IL-6, Platelet-Derived Growth Factor-AB/BB, and Natural Killer Cell Activation. Front Immunol **2017**; 8:644.

35. Mawa PA, Nkurunungi G, Egesa M, et al. The impact of maternal infection with Mycobacterium tuberculosis on the infant response to bacille Calmette-Guerin immunization. Philos Trans R Soc Lond B Biol Sci **2015**; 370.

36. Stensballe LG, Sorup S, Aaby P, et al. BCG vaccination at birth and early childhood hospitalisation: a randomised clinical multicentre trial. Arch Dis Child **2017**; 102:224-31.

37. Beemelmanns A, Roth O. Grandparental immune priming in the pipefish Syngnathus typhle. BMC Evol Biol **2017**; 17:44.

38. Green TJ, Helbig K, Speck P, Raftos DA. Primed for success: Oyster parents treated with poly(I:C) produce offspring with enhanced protection against Ostreid herpesvirus type I infection. Mol Immunol **2016**; 78:113-20.

39. Flanagan KL, Klein SL, Skakkebaek NE, et al. Sex differences in the vaccine-specific and non-targeted effects of vaccines. Vaccine **2011**; 29:2349-54.

40. Calandra T. Macrophage migration inhibitory factor and host innate immune responses to microbes. Scand J Infect Dis **2003**; 35:573-6.

41. Clark I, Awburn M. Migration inhibitory factor in the cerebral and systemic endothelium in sepsis and malaria. Crit Care Med **2002**; 30:S263-7.

42. Renner P, Roger T, Calandra T. Macrophage migration inhibitory factor: gene polymorphisms and susceptibility to inflammatory diseases. Clin Infect Dis **2005**; 41 Suppl 7:S513-9.

43. Roger T, Schneider A, Weier M, et al. High expression levels of macrophage migration inhibitory factor sustain the innate immune responses of neonates. Proc Natl Acad Sci U S A **2016**; 113:E997-1005.

44. Blimkie D, Fortuno ES, 3rd, Yan H, et al. Variables to be controlled in the assessment of blood innate immune responses to Toll-like receptor stimulation. J Immunol Methods **2011**; 366:89-99.

45. Ota MO, Vekemans J, Schlegel-Haueter SE, et al. Influence of Mycobacterium bovis bacillus Calmette-Guerin on antibody and cytokine responses to human neonatal vaccination. J Immunol **2002**; 168:919-25.

46. Ritz N, Mui M, Balloch A, Curtis N. Non-specific effect of Bacille Calmette-Guerin vaccine on the immune response to routine immunisations. Vaccine **2013**; 31:3098-103.

47. Hesseling AC, Blakney AK, Jones CE, et al. Delayed BCG immunization does not alter antibody responses to EPI vaccines in HIV-exposed and -unexposed South African infants. Vaccine **2016**; 34:3702-9.

48. Said ZN, Abdelwahab KS. Induced immunity against hepatitis B virus. World J Hepatol **2015**; 7:1660-70.

49. Halsey LG, Curran-Everett D, Vowler SL, Drummond GB. The fickle P value generates irreproducible results. Nat Methods **2015**; 12:179-85.

Footnote page

The authors of this manuscript have no conflicts of interest to disclose.

Funding for this study was provided by the Australian National Health and Medical Research Council Grant No: GNT1051228 and a Murdoch Children's Research Institute, Infection and Immunity Theme grant 2014.

BF was supported by a The University of Melbourne (Human Rights Scholarship) and a European Society of Paediatric Infectious Diseases Fellowship (2015-2017).

MGN was supported by an ERC Consolidator Grant (#310372) and a Spinoza Grant of the Netherlands Organization for Scientific Research.

Pilot data from this study was presented in poster format at the World Society for Paediatric Infectious Diseases meeting in Rio De Janeiro, October 2015. The final results were presented in oral format at the European Society for Paediatric Infectious Diseases meeting, Madrid May 2017.

Corresponding author details:

Prof Nigel Curtis, Department of Paediatrics, The University of Melbourne, The Royal Children's Hospital Melbourne, 50 Flemington Road, Parkville 3052, Australia.

nigel.curtis@rch.org.au

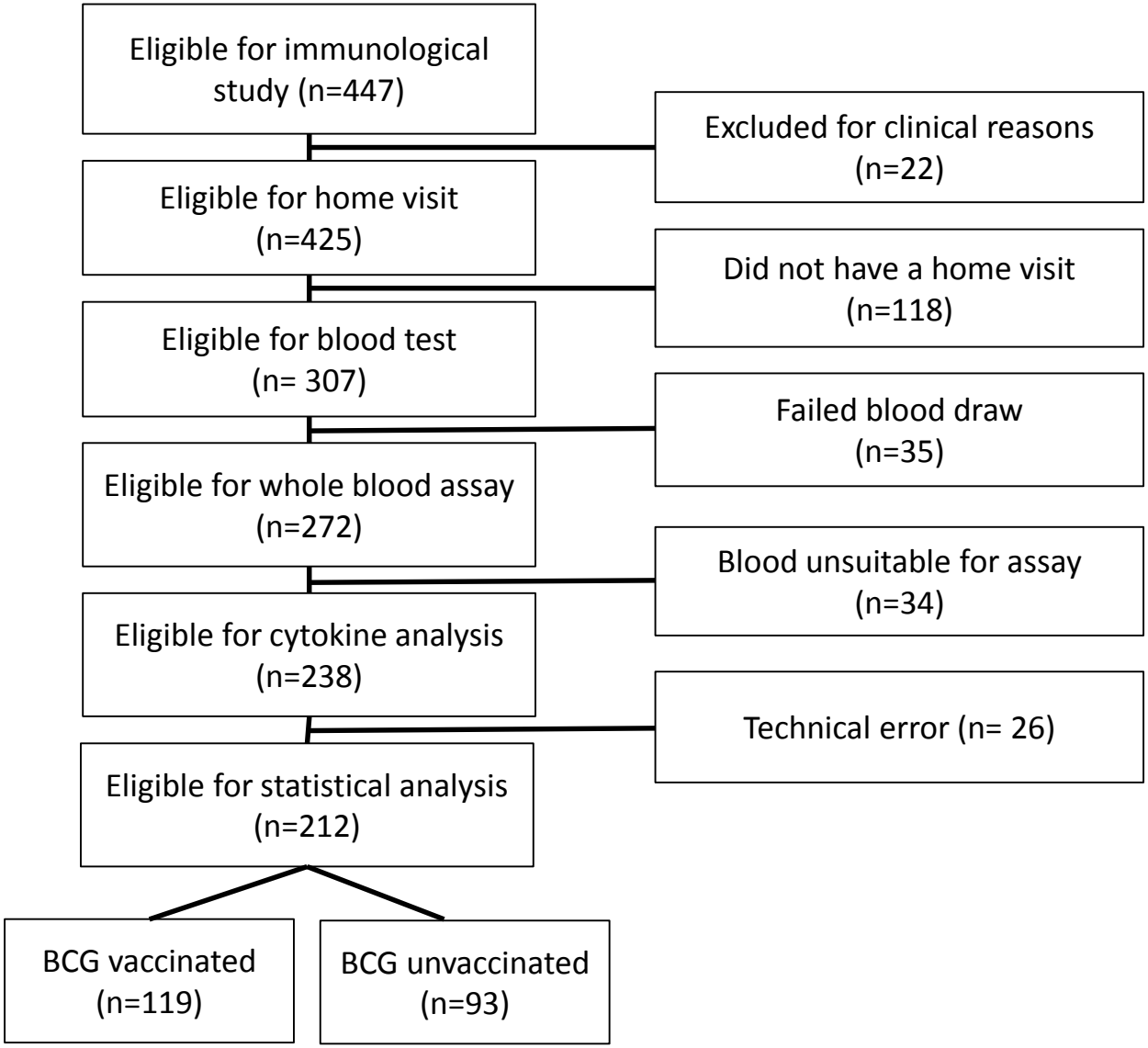
Table 1

		BCG-vaccinated n (%)	Not BCG-vaccinated n (%)	Total
All		119 (56.1)	93 (43.9)	212 (100)
Sex	Male	58 (48.7)	43 (46.2)	101 (47.6)
	Female	61 (51.3)	50 (53.8)	111 (52.3)
Gestational age (weeks)	Mean (SD)	39.3 (1.5)	39.2 (1.3)	39.3 (0.1)
Birth weight (grams)	Mean (SD)	3388 (487.3)	3333 (492.7)	3364 (489.3)
Mode of delivery	Vaginal delivery	93 (40.9)	62 (66.7)	126 (59.5)
	Caesarean section	55 (46.3)	31 (33.3)	86 (40.5)
Last trimester vaccination*	Yes	59 (49.6)	43 (46.2)	102 (48.1)
	No	58 (48.7)	49 (52.8)	107 (50.5)
	Not reported	2 (1.7)	1 (1.1)	3 (1.4)
Feeding	Breast feeding	82(69.2)	67 (71.7)	149 (70.2)
	Formula feeding	3 (2.4)	7 (7.6)	10 (4.8)
	Combination	29 (24.2)	18 (19.6)	47 (22.2)
	Not reported	5 (4.2)	1 (1.1)	6 (2.8)

Infant vitamin D supplementation	Yes	64 (53.4)	48 (51.8)	112 (52.8)
	No	55 (46.6)	45 (48.2)	100 (47.2)
Day of BCG vaccination	Mean (SD)	2.1 (1.9)	-	-
	Median (IQR)	2 (0-6)		
Interval from randomisation to blood (days)	Mean (SD)	6.9 (2.2)	-	-
Timing of hepatitis B vaccination	Hepatitis B alone	58 (48.7)	84 (90.3)	142 (67.1)
	Before BCG		-	
	Same day as BCG	37 (31.2)	-	37 (17.4)
	After BCG	24 (20.1)	-	24 (11.3)
	Not reported	0	9 (9.7)	9 (4.2)
Maternal BCG vaccinations status	Yes	24 (20.2)	31 (33.3)	55 (25.9)
	No	90 (75.6)	54 (58.1)	144 (67.9)
	Not reported	5 (4.2)	8 (8.6)	13 (6.2)

* Boostrix (DTPa) or influenza vaccine in the last trimester, in line with local recommendations

Figure 1



No of participants for each stimulant	
Nil	212
BCG	129
Heat-killed pathogens	
M. tuberculosis	98
S. agalactiae	113
S. aureus	98
S. pneumoniae	74
E. coli	157
H. influenzae	141
L. monocytogenes	69
C. albicans	86
TLR agonists	
LPS	147
PEPG	147
Pam3CYSK4	128
R848	150

Table 2

Cytokine	GMR (95% CI)	p-value
IFN-γ	0.98 (0.8-1.2)	0.85
TNF-α	1.0 (0.9-1.2)	0.80
IL-1β	1.6 (0.8-3.0)	0.18
IL-6	**2.0 (1.1-3.7)**	**0.03**
MIF	1.0 (0.9-1.2)	0.90
MIG	0.9 (0.8-1.1)	0.42
MCP-1	1.2 (0.8-1.6)	0.41
IL-8	1.0 (0.4-2.1))	0.94
MIP-1α	0.8 (0.5-1.4)	0.5
MIP-1β	1.0 (0.7-1.4)	1.00
IL-1ra	1.9 (0.9-3.8)	0.08
IL-10	1.0 (1.0-1.1	0.55

Figure 2

Click here to download Figure
FreyneB_HeterologouscytokineresponsespostBCG_Fig2.tiff

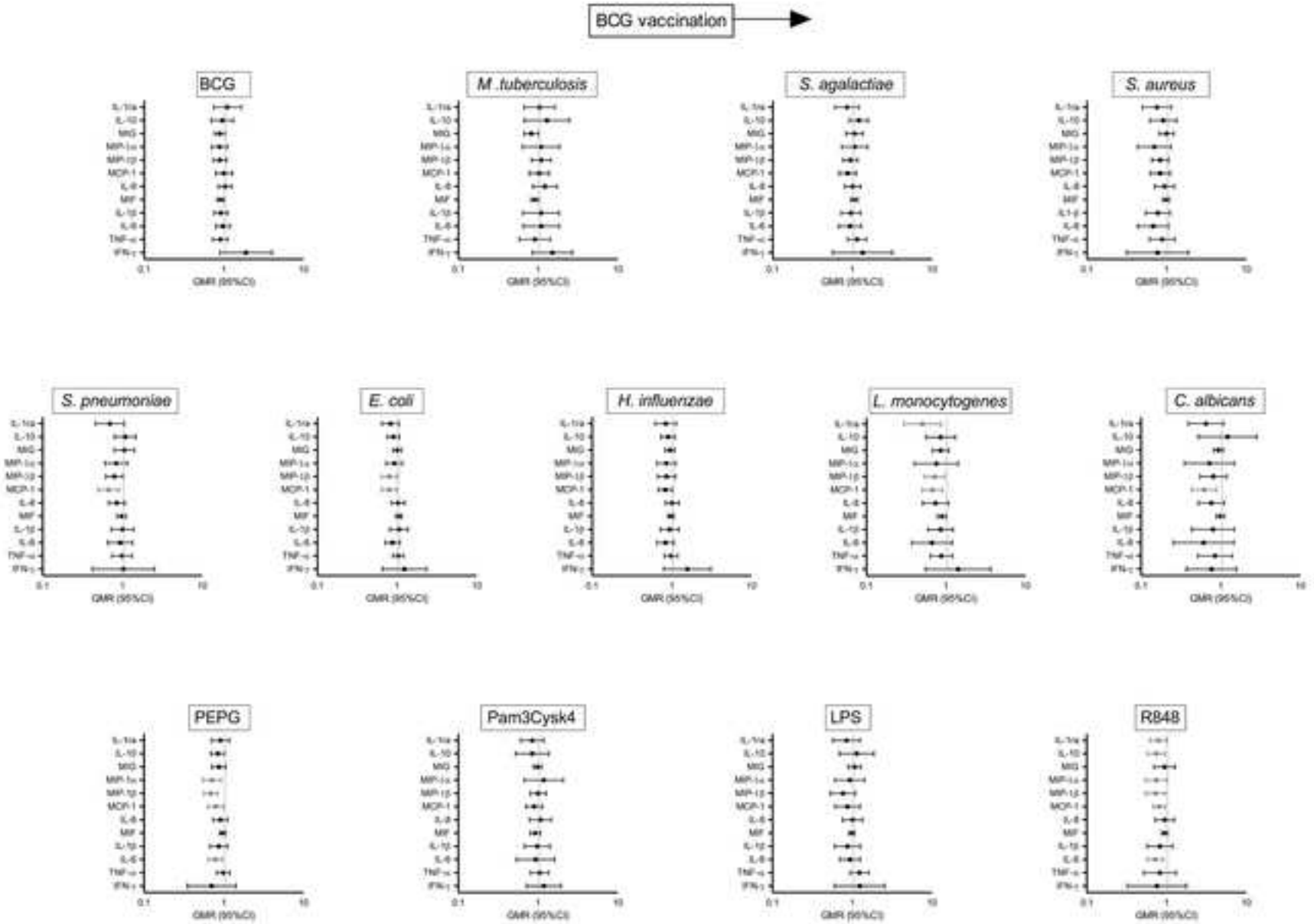


Figure 3

Click here to download Figure
FreyneB_HeterologouscytokineresponsespostBCG_Fig 3.tiff

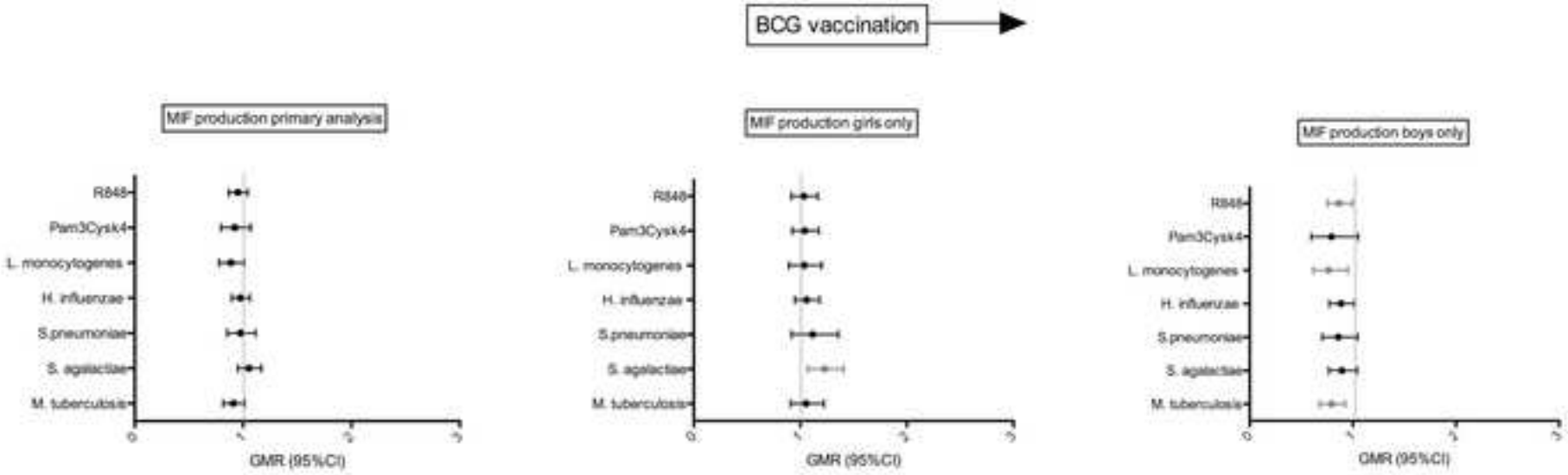


Figure 4

Click here to download Figure
FreyneB_HeterologouscytokineresponsespostBCG_Fig4.tiff

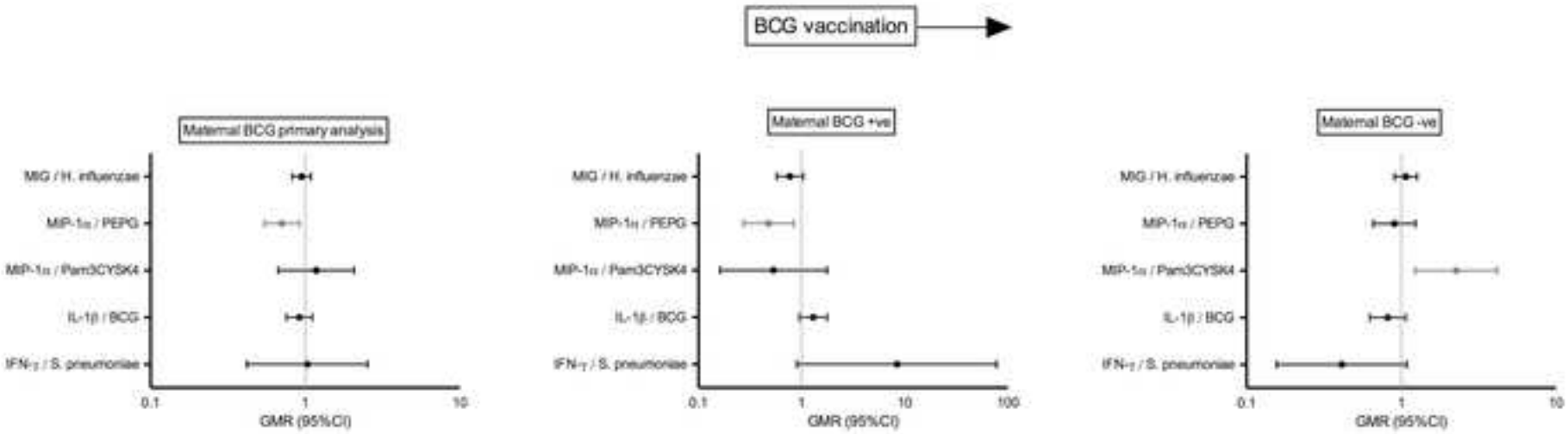
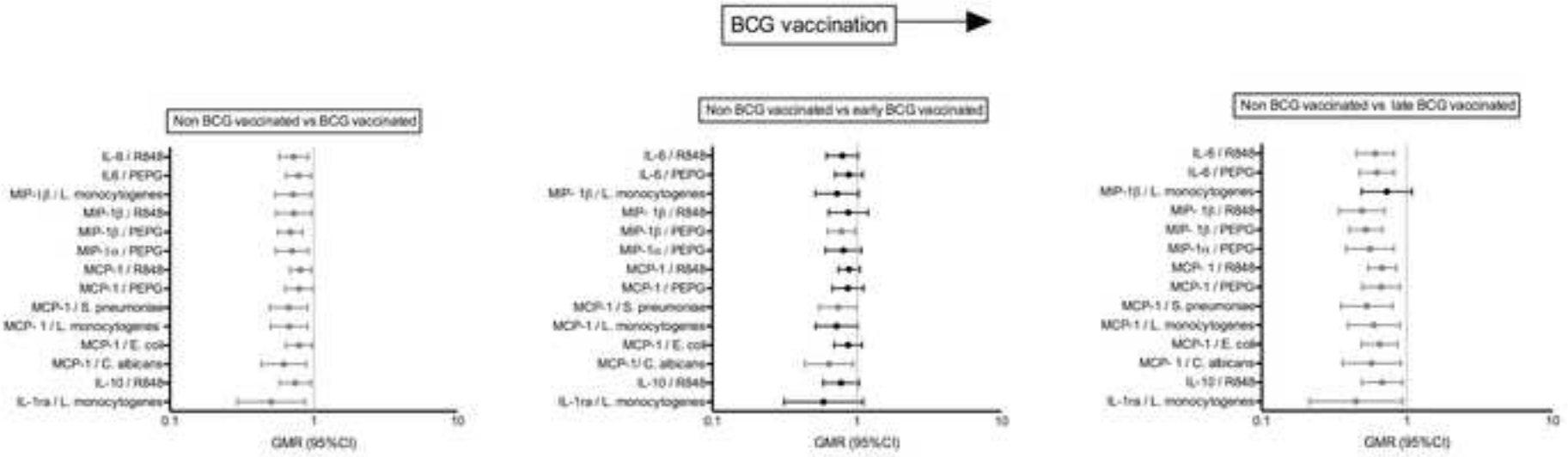


Figure 5

Click here to download Figure FreyneB_HeterologousresponsespostBCG_Fig5.tiff



The effect of neonatal BCG vaccination on cytokine responses to heterologous antigens in a randomised controlled trial

Supplementary Data

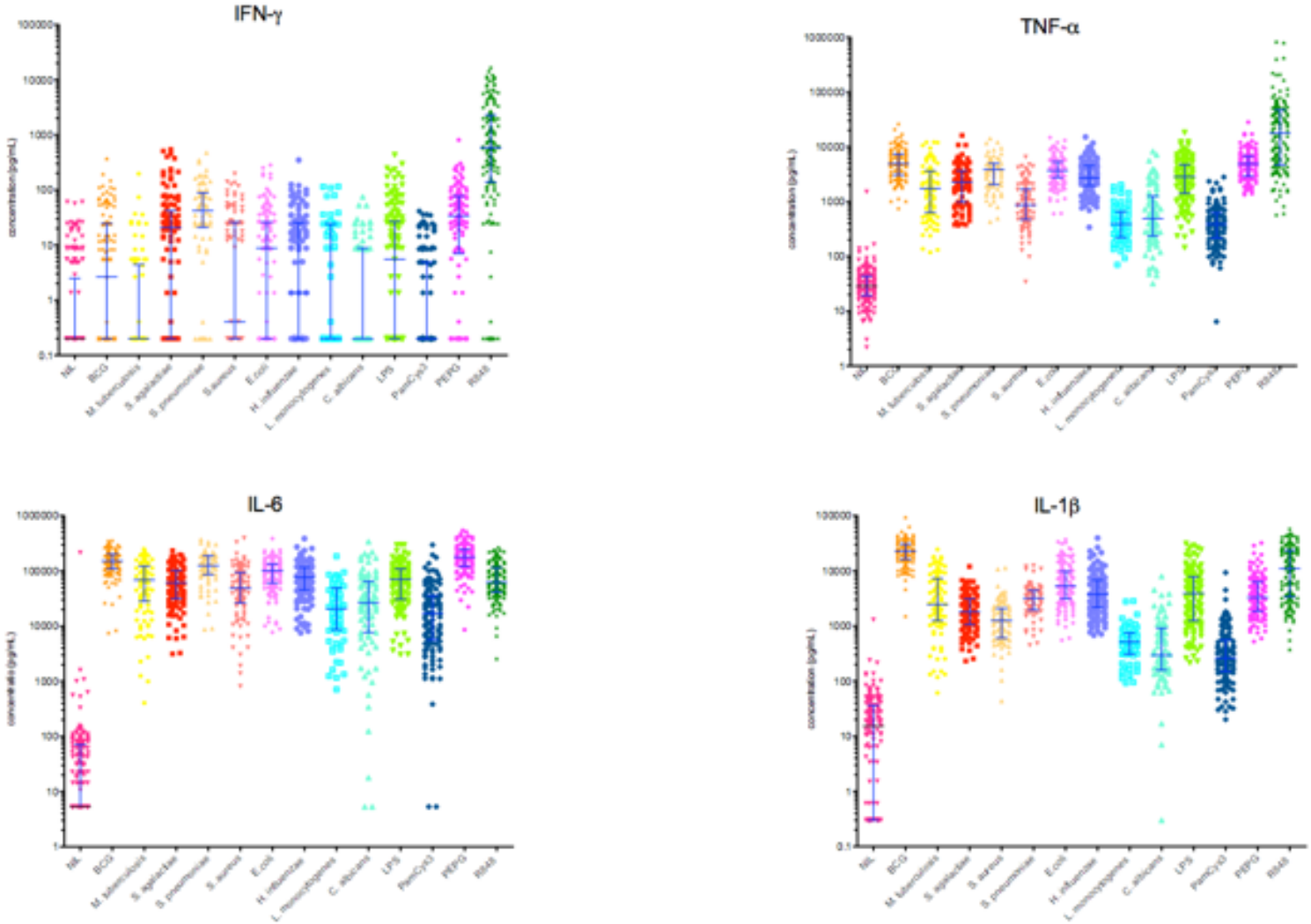


Fig 1, Panel A: Levels of pro-inflammatory cytokines (IFNγ, TNFα, IL-6, IL1β) induced by stimulation with 9 killed pathogens, 4 TLR agonists and RPMI. Error bar represent median and IQR. Nil - RPMI, BCG - Bacille Calmette Guerin, LPS - Liopolysaccarhide, PamCys3- (S)-(2,3-bis(palmitoyloxy)-(2-RS)-propyl)-N-palmitoyl-(R)-Cys-(S)-Ser-(S)-Lys4OH,trihydrochloride, PEPG - peptidoglycan, R848 - resiquimod.

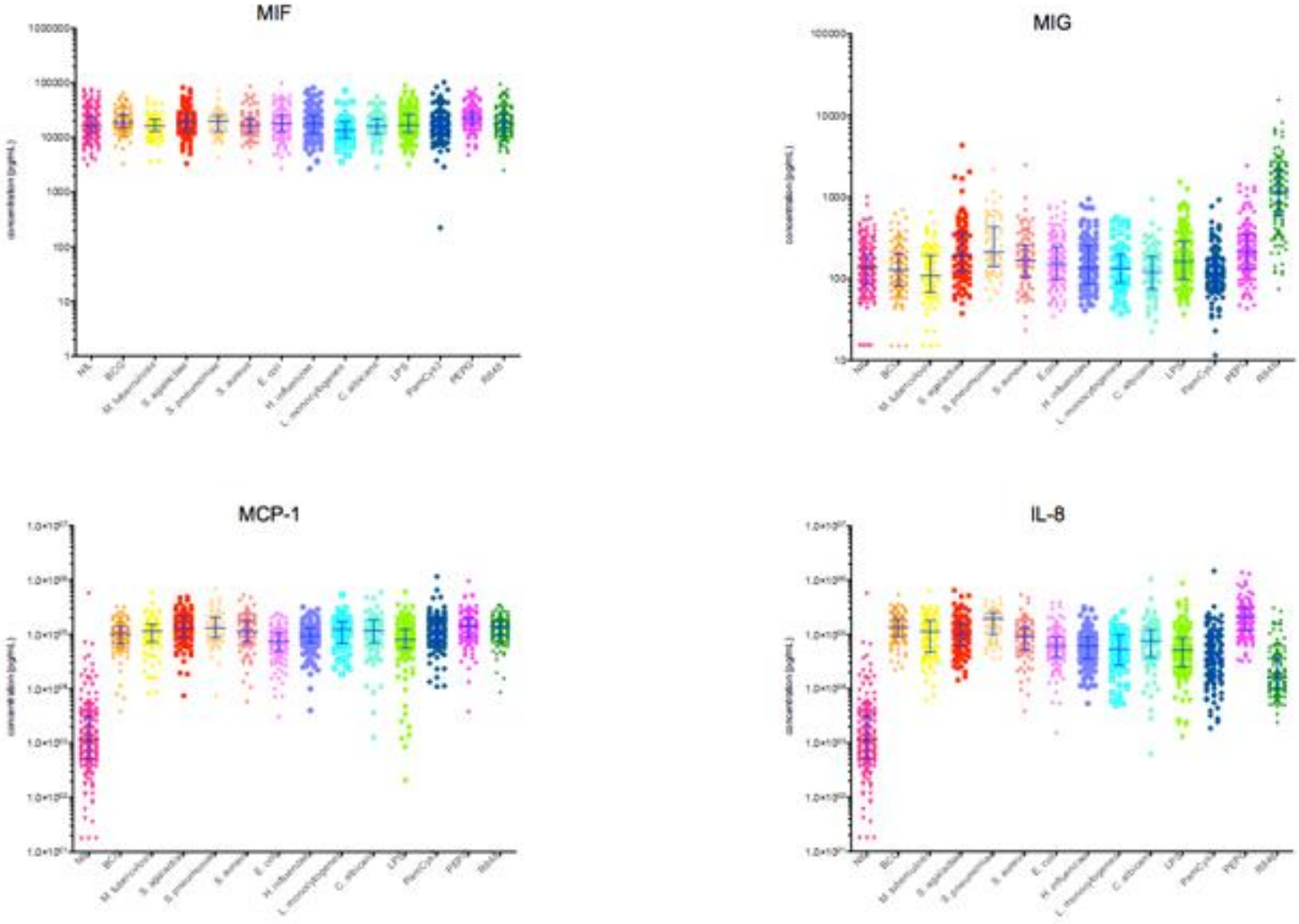


Fig 1, Panel B: Levels of chemokines (MIF, MIG, MCP-1, IL-8) induced by stimulation with 9 killed pathogens, 4 TLR agonists and RPMI. Error bar represent median and IQR. Nil - RPMI, BCG - Bacille Calmette Guerin, LPS - Liopolysaccarhide, PamCys3-(S)-(2,3-bis(palmitoyloxy)-(2-RS)-propyl)-N-palmitoyl-(R)-Cys-(S)-Ser-(S)-Lys4OH,trihydrochloride, PEPG - peptidoglycan, R848 - resiquimod.

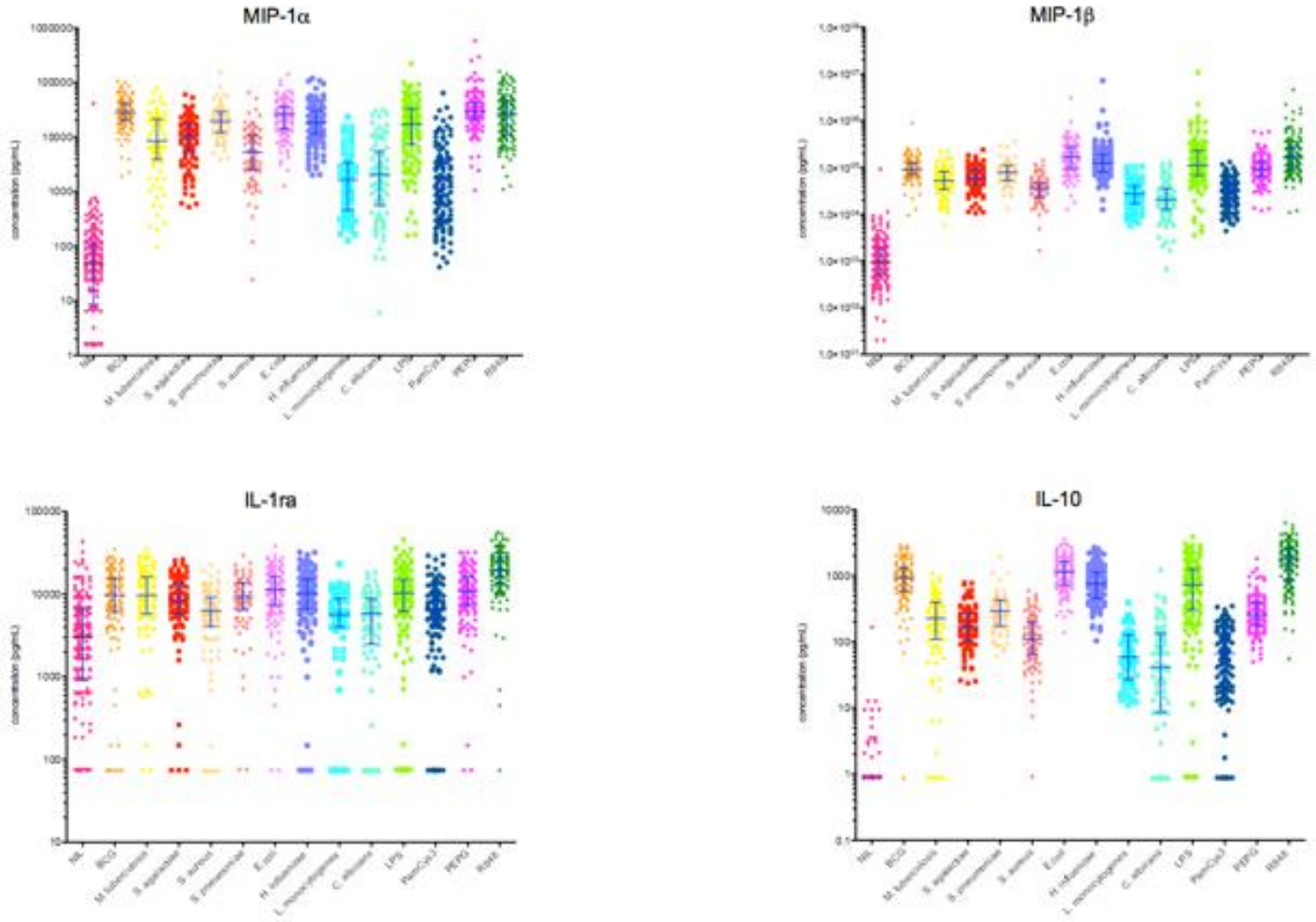


Fig 1, Panel C: Levels of chemokines (MIP-1α, MIP-1β) and anti-inflammatory cytokines IL-1ra and IL10 induced by stimulation with 9 killed pathogens, 4 TLR agonists and RPMI. Error bar represent median and IQR. Nil - RPMI, BCG - Bacille Calmette Guerin, LPS - Liopolysaccarhide, PamCys3- (S)-(2,3-bis(palmitoyloxy)-(2-RS)-propyl)-N-palmitoyl-(R)-Cys-(S)-Ser-(S)-Lys4OH,trihydrochloride, PEPG - peptidoglycan, R848 - resiquimod.

		BCG-vaccinated	Not BCG-vaccinated	Total
All	n (%)	119 (56.1)	93 (43.9)	212
Age at blood collection (days)	Mean (SD)	9.1 (2.9)	8.6 (2.5)	8.9 (2.8)
Volume of blood (mL)	Mean (SD)	2.8 (1.6)	2.9 (1.6)	2.9 (1.6)
Time between blood collection and stimulation (hours)	Mean (SD)	4.0 (0.3)	4.1 (0.3)	4.0 (0.3)
Within 4 hours ± 24 minutes	n (%)	74 (62.2)	69 (74.2)	143 (67.5)
Incubation duration (hours)	Mean (SD)	20.5 (0.8)	20.3 (0.8)	20.4 (0.8)
Within 20 ± 2 hours	n (%)	115 (96.6)	92 (99.2)	207 (97.6)

Supplementary Table 1: Standardisation of blood collection, handling and laboratory methods.

Cytokine	Stim	Total (n)	BCG GMR	BCG 95%CI LCI	BCG 95%CI UCI	BCG p value	Maternal BCG GMR	Maternal BCG 95%CI LCI	Maternal BCG 95%CI UCI	Maternal BCG p value
MIP-1β	BCG	129	0.9	0.7	1.1	0.23	1.1	0.9	1.4	0.42
	MTBK	98	1.1	0.8	1.4	0.55	1.2	0.9	1.6	0.28
	LPS	147	0.8	0.5	1.1	0.14	0.9	0.6	1.4	0.73
	PEPG	147	0.7	0.6	0.8	0.00	1.2	1.0	1.6	0.05
	Pam3	128	1.0	0.8	1.3	0.96	1.2	0.9	1.6	0.16
	R848	150	0.7	0.5	1.0	0.03	0.9	0.7	1.3	0.58
	SA	98	0.8	0.7	1.1	0.14	1.2	0.9	1.5	0.25
	SP	74	0.8	0.6	1.0	0.07	1.3	1.0	1.7	0.06
	GBS	113	0.9	0.8	1.2	0.57	1.0	0.8	1.3	0.98
	EC	157	0.8	0.6	1.0	0.05	1.1	0.8	1.4	0.66
	HI	141	0.9	0.7	1.1	0.25	1.1	0.9	1.5	0.30
	LM	69	0.7	0.5	1.0	0.03	1.0	0.7	1.4	0.95
	CA	86	0.8	0.5	1.2	0.28	1.1	0.7	1.7	0.68
MCP-1	BCG	129	1.0	0.8	1.3	0.98	1.0	0.8	1.4	0.77
	MTBK	98	1.0	0.8	1.4	0.82	1.0	0.7	1.4	0.95
	LPS	147	0.9	0.6	1.3	0.45	1.3	0.8	1.9	0.27
	PEPG	147	0.8	0.6	1.0	0.04	1.2	0.9	1.6	0.14
	Pam3	128	0.9	0.7	1.1	0.36	1.2	0.9	1.5	0.30
	R848	150	0.8	0.7	1.0	0.01	1.1	0.9	1.4	0.29
	SA	98	0.8	0.6	1.1	0.20	1.1	0.8	1.6	0.47
	SP	74	0.7	0.5	0.9	0.01	1.2	0.8	1.6	0.37
	GBS	113	0.9	0.7	1.1	0.28	1.0	0.7	1.3	0.91
	EC	157	0.8	0.6	1.0	0.03	1.1	0.8	1.4	0.52
	HI	141	0.8	0.7	1.0	0.07	1.1	0.8	1.3	0.63
	LM	69	0.7	0.5	0.9	0.01	1.0	0.7	1.3	0.80
	CA	86	0.6	0.4	0.9	0.01	1.3	0.8	1.9	0.28
IL-8	BCG	129	1.0	0.9	1.3	0.70	1.1	0.9	1.4	0.38
	MTBK	98	1.2	0.9	1.7	0.27	1.2	0.8	1.8	0.26
	LPS	147	1.0	0.8	1.3	0.94	1.1	0.8	1.5	0.52
	PEPG	147	0.9	0.7	1.1	0.37	1.1	0.9	1.4	0.40
	Pam3	128	1.1	0.8	1.5	0.68	1.3	0.9	1.8	0.19
	R848	150	0.9	0.7	1.2	0.70	0.9	0.7	1.2	0.58
	SA	98	0.9	0.7	1.3	0.67	1.0	0.8	1.4	0.77
	SP	74	0.8	0.7	1.1	0.17	1.0	0.8	1.3	0.87
	GBS	113	1.0	0.8	1.3	0.95	1.0	0.7	1.2	0.79
	EC	157	1.0	0.8	1.2	0.81	1.2	0.9	1.4	0.16
	HI	141	1.0	0.8	1.2	0.92	1.1	0.9	1.4	0.30
	LM	69	0.7	0.5	1.1	0.11	0.9	0.6	1.4	0.61
	CA	86	0.8	0.5	1.1	0.15	1.2	0.8	1.9	0.37
MIF	BCG	129	0.9	0.8	1.0	0.06	1.0	0.9	1.1	0.79
	MTBK	98	0.9	0.8	1.0	0.09	1.0	0.9	1.2	0.67
	LPS	147	1.0	0.9	1.1	0.81	1.0	0.9	1.1	0.44
	PEPG	147	1.0	0.9	1.1	0.42	1.0	0.9	1.1	0.98
	Pam3	128	0.9	0.8	1.1	0.29	1.0	0.8	1.1	0.62
	R848	150	1.0	0.9	1.0	0.28	1.0	0.9	1.1	0.81
	SA	98	1.0	0.9	1.1	0.76	1.0	0.9	1.1	0.62
	SP	74	1.0	0.9	1.1	0.72	1.0	0.9	1.2	0.69
	GBS	113	1.1	0.9	1.2	0.32	1.0	0.9	1.1	0.97
	EC	157	1.1	1.0	1.1	0.25	1.0	0.9	1.1	0.64
	HI	141	1.0	0.9	1.1	0.56	1.0	0.9	1.1	0.44
	LM	69	0.9	0.8	1.0	0.07	1.0	0.9	1.2	0.61
	CA	86	1.0	0.9	1.1	0.88	1.0	0.9	1.1	0.76

Supplementary data Table 2 p1.

Cytokine	Stim	Total (n)	BCG				Maternal BCG			
			GMR	95%CI		p value	GMR	95%CI		p value
				LCI	UCI			LCI	UCI	
IL-1ra	BCG	129	1.1	0.7	1.7	0.62	1.0	0.7	1.6	0.95
	MTBK	98	1.0	0.7	1.6	0.85	1.3	0.8	2.0	0.34
	LPS	147	0.8	0.6	1.3	0.40	1.3	0.8	2.0	0.32
	PEPG	147	0.9	0.7	1.2	0.46	1.0	0.7	1.3	0.98
	Pam3	128	0.8	0.6	1.2	0.32	1.5	1.0	2.2	0.03
	R848	150	0.8	0.6	1.0	0.06	1.3	1.0	1.7	0.08
	SA	98	0.8	0.5	1.2	0.19	1.5	0.9	2.4	0.09
	SP	74	0.7	0.5	1.1	0.09	1.3	0.8	2.1	0.26
	GBS	113	0.9	0.6	1.2	0.37	1.1	0.8	1.7	0.49
	EC	157	0.8	0.6	1.1	0.13	1.1	0.8	1.4	0.59
	HI	141	0.8	0.6	1.1	0.25	1.2	0.9	1.7	0.23
	LM	69	0.5	0.3	0.9	0.01	1.5	0.8	2.8	0.18
	CA	86	0.7	0.4	1.1	0.10	1.3	0.7	2.2	0.43
IL-10	BCG	129	1.0	0.7	1.3	0.77	1.0	0.7	1.4	0.81
	MTBK	98	1.3	0.7	2.5	0.44	0.9	0.5	1.8	0.84
	LPS	147	1.1	0.7	1.9	0.62	0.6	0.3	1.0	0.06
	PEPG	147	0.8	0.7	1.0	0.09	0.9	0.7	1.1	0.30
	Pam3	128	0.8	0.5	1.4	0.49	1.1	0.6	1.8	0.81
	R848	150	0.7	0.6	1.0	0.02	0.8	0.6	1.0	0.08
	SA	98	0.9	0.6	1.3	0.61	1.0	0.6	1.5	0.88
	SP	74	1.1	0.8	1.5	0.62	0.9	0.6	1.2	0.38
	GBS	113	1.2	0.9	1.6	0.18	0.7	0.6	1.0	0.05
	EC	157	0.9	0.7	1.1	0.23	0.9	0.7	1.1	0.22
	HI	141	0.9	0.7	1.1	0.31	0.9	0.7	1.1	0.22
	LM	69	0.9	0.6	1.3	0.47	0.8	0.5	1.3	0.45
	CA	86	1.2	0.5	2.9	0.64	1.0	0.4	2.7	0.93
MIG	BCG	129	0.9	0.8	1.0	0.14	1.1	1.0	1.4	0.11
	MTBK	98	0.8	0.7	1.0	0.06	1.0	0.8	1.3	0.80
	LPS	147	1.1	0.9	1.3	0.50	0.9	0.8	1.2	0.60
	PEPG	147	0.9	0.7	1.1	0.17	1.1	0.9	1.4	0.49
	Pam3	128	1.0	0.9	1.1	0.81	0.9	0.8	1.1	0.23
	R848	150	0.9	0.7	1.3	0.70	1.1	0.8	1.5	0.65
	SA	98	1.0	0.8	1.2	0.99	1.0	0.8	1.2	0.85
	SP	74	1.1	0.8	1.4	0.71	1.1	0.8	1.5	0.64
	GBS	113	1.1	0.8	1.4	0.63	1.0	0.8	1.3	0.90
	EC	157	1.0	0.9	1.1	0.94	1.0	0.9	1.2	0.67
	HI	141	0.9	0.8	1.1	0.45	1.1	0.9	1.2	0.48
	LM	69	0.9	0.7	1.1	0.19	1.1	0.8	1.4	0.63
	CA	86	0.9	0.8	1.1	0.34	1.0	0.9	1.2	0.92
MIP-1α	BCG	129	0.9	0.7	1.1	0.28	1.1	0.8	1.4	0.69
	MTBK	98	1.1	0.6	1.9	0.76	1.2	0.6	2.2	0.60
	LPS	147	0.9	0.6	1.4	0.75	1.0	0.6	1.6	0.99
	PEPG	147	0.7	0.5	0.9	0.01	1.3	1.0	1.8	0.06
	Pam3	128	1.2	0.7	2.1	0.57	2.3	1.3	4.3	0.01
	R848	150	0.7	0.5	1.0	0.06	0.9	0.6	1.3	0.64
	SA	98	0.7	0.4	1.1	0.15	1.7	1.0	2.8	0.07
	SP	74	0.8	0.6	1.2	0.27	1.3	0.9	1.8	0.17
	GBS	113	1.1	0.7	1.6	0.73	1.4	0.9	2.0	0.15
	EC	157	0.9	0.7	1.2	0.50	1.2	0.9	1.5	0.24
	HI	141	0.9	0.6	1.1	0.25	1.4	1.0	1.8	0.04
	LM	69	0.8	0.4	1.4	0.38	1.1	0.5	2.1	0.89
	CA	86	0.7	0.3	1.5	0.38	1.2	0.5	2.8	0.60

Supplementary data Table 2 p2.

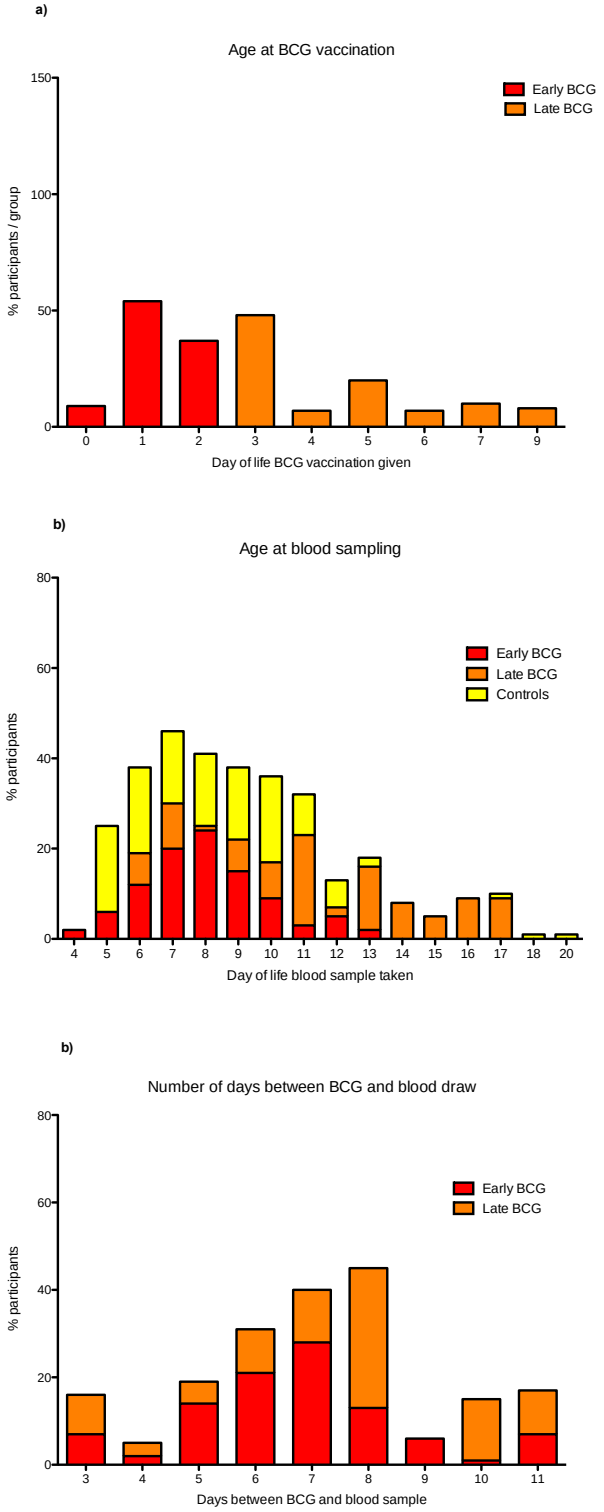
Cytokine	Stim	Total (n)	BCG				Maternal BCG			
			GMR	95%CI		p value	GMR	95%CI		p value
				LCI	UCI			LCI	UCI	
IL-1β	BCG	129	0.9	0.8	1.1	0.38	1.0	0.8	1.2	0.89
	MTBK	98	1.1	0.6	1.8	0.76	1.1	0.6	2.0	0.75
	LPS	147	0.9	0.6	1.3	0.46	0.7	0.5	1.1	0.11
	PEPG	147	0.9	0.7	1.1	0.27	1.1	0.8	1.4	0.58
	Pam3	128	1.0	0.7	1.4	0.91	1.5	1.0	2.2	0.04
	R848	150	0.8	0.6	1.2	0.30	0.6	0.4	1.0	0.03
	SA	98	0.8	0.5	1.1	0.16	1.4	0.9	2.0	0.12
	SP	74	1.0	0.7	1.4	0.98	1.1	0.8	1.5	0.68
	GBS	113	1.0	0.7	1.3	0.75	1.1	0.8	1.5	0.58
	EC	157	1.1	0.8	1.4	0.68	1.2	0.9	1.6	0.27
	HI	141	0.9	0.7	1.2	0.63	1.2	0.9	1.7	0.13
	LM	69	0.9	0.6	1.2	0.40	0.9	0.6	1.3	0.43
	CA	86	0.8	0.4	1.5	0.48	1.2	0.6	2.4	0.60
IL-6	BCG	129	1.0	0.8	1.2	0.77	1.0	0.8	1.3	0.87
	MTBK	98	1.1	0.6	1.8	0.74	1.1	0.6	2.0	0.70
	LPS	147	0.9	0.7	1.3	0.66	1.0	0.7	1.4	0.96
	PEPG	147	0.8	0.6	1.0	0.02	1.2	0.9	1.5	0.17
	Pam3	128	0.9	0.5	1.6	0.80	2.2	1.2	3.9	0.01
	R848	150	0.7	0.6	0.9	0.01	0.9	0.7	1.1	0.26
	SA	98	0.7	0.4	1.1	0.09	1.5	0.9	2.4	0.13
	SP	74	0.9	0.7	1.3	0.73	1.2	0.8	1.8	0.35
	GBS	113	0.9	0.7	1.3	0.69	1.2	0.8	1.6	0.39
	EC	157	0.9	0.7	1.1	0.22	1.2	1.0	1.5	0.10
	HI	141	0.8	0.6	1.1	0.14	1.3	1.0	1.8	0.03
	LM	69	0.7	0.4	1.2	0.17	1.2	0.6	2.3	0.59
	CA	86	0.6	0.3	1.5	0.26	1.8	0.7	4.7	0.25
TNF-α	BCG	129	0.9	0.7	1.1	0.35	1.0	0.8	1.3	0.83
	MTBK	98	0.9	0.6	1.4	0.67	1.4	0.9	2.3	0.18
	LPS	147	1.2	0.9	1.6	0.14	0.8	0.6	1.1	0.19
	PEPG	147	1.0	0.8	1.2	0.89	1.2	1.0	1.5	0.10
	Pam3	128	1.0	0.8	1.4	0.74	0.9	0.7	1.2	0.43
	R848	150	0.8	0.5	1.3	0.42	0.5	0.3	0.9	0.01
	SA	98	0.9	0.6	1.3	0.49	1.2	0.8	1.7	0.47
	SP	74	1.0	0.7	1.3	0.94	1.1	0.8	1.5	0.68
	GBS	113	1.1	0.9	1.5	0.34	1.0	0.8	1.4	0.78
	EC	157	1.0	0.9	1.2	0.71	1.0	0.8	1.2	0.71
	HI	141	1.0	0.8	1.2	0.83	1.1	0.9	1.4	0.33
	LM	69	0.9	0.6	1.2	0.41	1.0	0.7	1.4	0.98
	CA	86	0.8	0.5	1.4	0.52	0.9	0.5	1.6	0.69
IFN-γ	BCG	129	1.9	0.9	4.0	0.10	1.3	0.6	3.1	0.49
	MTBK	98	1.5	0.8	2.7	0.16	1.3	0.6	2.5	0.49
	LPS	147	1.2	0.6	2.6	0.56	0.7	0.3	1.6	0.37
	PEPG	147	0.7	0.3	1.4	0.33	0.9	0.4	2.0	0.84
	Pam3	128	1.2	0.7	2.0	0.51	1.0	0.6	1.8	0.96
	R848	150	0.8	0.3	1.8	0.52	0.7	0.3	1.7	0.41
	SA	98	0.8	0.3	1.9	0.56	1.5	0.5	3.9	0.45
	SP	74	1.0	0.4	2.5	0.95	0.6	0.2	1.7	0.38
	GBS	113	1.3	0.6	3.2	0.50	1.8	0.7	4.5	0.21
	EC	157	1.2	0.7	2.3	0.51	0.9	0.5	1.8	0.80
	HI	141	1.6	0.8	3.1	0.19	0.9	0.4	1.8	0.69
	LM	69	1.4	0.5	3.7	0.47	0.6	0.2	1.7	0.33
	CA	86	0.8	0.4	1.6	0.47	0.7	0.3	1.5	0.34

Supplementary data Table 2 p3.

Cytokine	Stimulant	Total (n)	Sex				MOD				Maternal BCG			
			GMR	95%CI LCI	95%CI UCI	p value	GMR	95%CI LCI	95%CI UCI	p value	GMR	95%CI LCI	95%CI UCI	p value
IL-1ra	BCG	129	0.8	0.4	1.9	0.65	0.9	0.4	2.1	0.88	0.9	0.4	2.3	0.83
	MTBK	98	0.8	0.3	1.8	0.54	0.8	0.3	2.1	0.68	0.6	0.2	1.6	0.27
	LPS	147	0.7	0.3	1.6	0.45	1.9	0.8	4.3	0.13	0.7	0.3	1.9	0.53
	PEPG	147	0.7	0.4	1.2	0.17	1.3	0.7	2.2	0.41	0.8	0.5	1.6	0.60
	Pam3	128	0.8	0.4	1.7	0.63	1.7	0.8	3.4	0.14	0.6	0.3	1.3	0.18
	R848	150	1.1	0.7	1.8	0.74	0.9	0.5	1.5	0.69	1.0	0.6	1.8	0.91
	SA	98	1.0	0.4	2.4	0.97	2.2	0.9	5.2	0.08	0.6	0.2	1.6	0.29
	SP	74	1.0	0.4	2.3	0.97	1.5	0.7	3.6	0.32	0.5	0.2	1.4	0.18
	GBS	113	1.1	0.6	2.3	0.74	1.1	0.5	2.3	0.82	0.6	0.3	1.4	0.25
	EC	157	1.0	0.6	1.6	0.99	0.9	0.5	1.6	0.77	0.9	0.5	1.5	0.60
	HI	141	0.7	0.4	1.3	0.27	1.2	0.7	2.3	0.52	0.6	0.3	1.1	0.10
	LM	69	1.0	0.3	3.1	0.99	1.8	0.6	5.5	0.30	0.6	0.2	2.2	0.47
	CA	86	1.5	0.5	4.3	0.43	1.8	0.6	5.0	0.29	0.9	0.3	2.9	0.82
IL-10	BCG	129	1.8	0.9	3.4	0.08	1.1	0.6	2.1	0.77	0.9	0.4	1.8	0.72
	MTBK	98	0.9	0.2	3.2	0.82	1.6	0.4	6.4	0.47	0.6	0.2	2.5	0.49
	LPS	147	1.1	0.4	3.1	0.80	2.6	0.9	7.4	0.07	1.5	0.5	4.8	0.49
	PEPG	147	1.3	0.9	1.9	0.25	1.4	0.9	2.1	0.14	0.9	0.6	1.4	0.64
	Pam3	128	1.0	0.4	2.7	0.98	2.0	0.7	5.6	0.18	0.4	0.1	1.0	0.06
	R848	150	1.0	0.6	1.6	0.86	1.2	0.7	2.0	0.49	1.4	0.8	2.4	0.28
	SA	98	2.0	0.9	4.2	0.09	1.1	0.5	2.4	0.87	1.3	0.5	3.2	0.57
	SP	74	1.5	0.8	2.9	0.17	1.1	0.6	2.2	0.73	1.4	0.7	2.9	0.37
	GBS	113	1.0	0.6	1.7	0.90	1.5	0.8	2.6	0.17	1.6	0.9	2.9	0.12
	EC	157	1.1	0.7	1.5	0.76	1.2	0.8	1.7	0.35	1.2	0.8	1.8	0.39
	HI	141	1.0	0.6	1.4	0.83	1.1	0.7	1.8	0.56	1.2	0.7	1.8	0.53
	LM	69	1.5	0.6	3.6	0.35	1.6	0.6	3.9	0.31	1.8	0.7	5.0	0.23
	CA	86	1.3	0.2	6.9	0.79	4.9	0.8	28.8	0.08	2.4	0.3	18.0	0.40
MIG	BCG	129	1.0	0.8	1.4	0.77	1.0	0.7	1.3	0.87	1.1	0.8	1.5	0.64
	MTBK	98	1.1	0.7	1.7	0.65	0.9	0.6	1.3	0.47	0.9	0.6	1.5	0.77
	LPS	147	1.0	0.7	1.5	0.88	1.2	0.8	1.8	0.29	1.0	0.6	1.5	0.82
	PEPG	147	0.8	0.5	1.2	0.30	0.8	0.6	1.3	0.41	1.2	0.7	1.9	0.50
	Pam3	128	0.8	0.6	1.0	0.08	0.7	0.6	1.0	0.02	0.9	0.7	1.1	0.29
	R848	150	0.7	0.4	1.2	0.18	1.0	0.5	1.9	0.94	1.1	0.6	2.3	0.71
	SA	98	1.5	1.0	2.3	0.06	1.3	0.9	2.0	0.20	1.2	0.7	1.9	0.50
	SP	74	1.1	0.6	2.1	0.64	0.8	0.4	1.5	0.45	1.3	0.7	2.6	0.45
	GBS	113	0.9	0.6	1.5	0.74	1.2	0.7	2.0	0.52	0.6	0.4	1.0	0.07
	EC	157	0.8	0.6	1.0	0.10	0.9	0.7	1.1	0.22	0.8	0.6	1.1	0.16
	HI	141	0.8	0.6	1.1	0.22	0.9	0.7	1.2	0.41	0.7	0.5	1.0	0.04
	LM	69	1.2	0.8	2.0	0.40	1.0	0.6	1.7	0.93	1.2	0.7	2.1	0.53
	CA	86	0.9	0.7	1.2	0.45	1.1	0.8	1.5	0.46	1.0	0.7	1.4	0.84
MIP-1α	BCG	129	1.2	0.8	1.9	0.42	1.1	0.7	1.7	0.79	0.7	0.4	1.1	0.11
	MTBK	98	0.6	0.2	1.6	0.28	1.4	0.5	4.3	0.55	0.5	0.1	1.9	0.32
	LPS	147	1.1	0.5	2.6	0.85	1.7	0.7	4.2	0.23	0.8	0.3	2.1	0.59
	PEPG	147	0.8	0.5	1.4	0.48	1.1	0.7	2.0	0.63	0.5	0.3	0.9	0.02
	Pam3	128	0.4	0.1	1.2	0.10	1.0	0.3	3.4	0.95	0.2	0.1	0.7	0.01
	R848	150	1.0	0.5	1.8	0.91	1.3	0.7	2.4	0.45	0.7	0.4	1.4	0.34
	SA	98	1.1	0.4	2.8	0.84	1.4	0.5	3.7	0.55	0.7	0.2	2.2	0.60
	SP	74	0.8	0.4	1.6	0.55	1.4	0.7	2.7	0.33	0.8	0.4	1.7	0.50
	GBS	113	0.8	0.4	1.6	0.50	1.5	0.7	3.4	0.28	0.7	0.3	1.5	0.34
	EC	157	0.9	0.6	1.5	0.74	1.2	0.7	2.0	0.41	0.8	0.5	1.3	0.33
	HI	141	0.8	0.4	1.3	0.31	1.1	0.6	1.9	0.86	0.6	0.3	1.1	0.12
	LM	69	1.5	0.4	5.3	0.56	2.1	0.6	7.7	0.27	0.5	0.1	2.4	0.42
	CA	86	1.1	0.3	4.3	0.89	2.4	0.5	10.9	0.24	0.9	0.2	5.2	0.94
MIP-1β	BCG	129	1.2	0.8	1.7	0.48	1.1	0.7	1.7	0.59	0.9	0.6	1.4	0.53
	MTBK	98	0.8	0.5	1.4	0.46	1.4	0.8	2.6	0.22	0.7	0.3	1.3	0.21
	LPS	147	1.1	0.6	2.4	0.71	2.2	1.0	4.6	0.04	1.2	0.6	2.7	0.59
	PEPG	147	0.9	0.6	1.4	0.79	1.5	1.0	2.3	0.05	0.7	0.5	1.1	0.13
	Pam3	128	0.8	0.5	1.3	0.40	1.3	0.8	2.1	0.31	0.6	0.4	1.0	0.07
	R848	150	1.0	0.6	1.9	0.88	1.3	0.7	2.4	0.34	1.0	0.5	1.9	0.98
	SA	98	1.2	0.7	1.9	0.52	1.2	0.7	1.9	0.58	0.8	0.5	1.3	0.37
	SP	74	1.1	0.7	1.9	0.61	1.5	0.9	2.5	0.12	0.8	0.4	1.4	0.41
	GBS	113	1.2	0.8	1.8	0.42	1.4	0.9	2.1	0.15	0.8	0.5	1.3	0.33
	EC	157	1.0	0.6	1.6	0.98	1.7	1.0	2.7	0.04	1.1	0.6	1.8	0.75
	HI	141	1.1	0.6	1.8	0.82	1.3	0.8	2.3	0.28	0.9	0.5	1.5	0.62
	LM	69	1.5	0.8	2.6	0.21	1.6	0.9	2.9	0.11	0.7	0.4	1.4	0.29
	CA	86	1.3	0.6	2.8	0.46	1.8	0.8	4.0	0.14	1.0	0.4	2.4	0.93
MCP-1	BCG	129	1.3	0.8	2.1	0.33	1.0	0.6	1.6	0.99	0.8	0.5	1.4	0.44
	MTBK	98	1.1	0.6	1.9	0.80	1.1	0.6	2.0	0.80	1.0	0.5	2.0	0.97
	LPS	147	0.9	0.4	1.8	0.69	1.8	0.8	3.7	0.14	1.3	0.6	3.2	0.50
	PEPG	147	0.7	0.5	1.2	0.19	1.1	0.7	1.7	0.75	0.8	0.5	1.4	0.41
	Pam3	128	0.8	0.5	1.3	0.39	1.1	0.6	1.8	0.78	0.8	0.5	1.4	0.39
	R848	150	1.1	0.8	1.5	0.69	1.1	0.8	1.5	0.66	1.1	0.8	1.7	0.57
	SA	98	1.2	0.7	2.2	0.46	1.1	0.6	1.9	0.85	0.8	0.4	1.6	0.58
	SP	74	1.4	0.8	2.5	0.29	1.1	0.6	2.0	0.81	1.5	0.8	3.1	0.22
	GBS	113	1.1	0.7	1.9	0.61	1.2	0.7	2.0	0.51	0.8	0.5	1.4	0.49
	EC	157	0.8	0.5	1.3	0.42	0.9	0.6	1.5	0.75	1.1	0.7	1.8	0.71
	HI	141	0.8	0.5	1.1	0.18	1.2	0.8	1.8	0.44	1.0	0.6	1.7	0.86
	LM	69	1.2	0.7	2.2	0.52	1.2	0.6	2.1	0.62	1.3	0.7	2.6	0.42
	CA	86	1.5	0.7	2.9	0.29	1.4	0.7	2.9	0.39	1.7	0.7	4.0	0.22

Cytokine	Stimulant	Total (n)	Sex				MOD				Maternal BCG			
			GMR	95%CI LCI	95%CI UCI	p value	GMR	95%CI LCI	95%CI UCI	p value	GMR	95%CI LCI	95%CI UCI	p value
IL-8	BCG	129	1.3	0.9	1.9	0.19	1.0	0.7	1.5	0.95	1.2	0.8	1.9	0.38
	MTBK	98	0.9	0.4	1.8	0.69	0.9	0.4	1.9	0.78	0.8	0.3	1.7	0.53
	LPS	147	1.3	0.7	2.3	0.42	1.3	0.7	2.3	0.40	1.3	0.7	2.5	0.42
	PEPG	147	1.0	0.6	1.5	0.93	1.0	0.7	1.6	0.84	1.0	0.6	1.6	0.95
	Pam3	128	0.8	0.4	1.6	0.58	1.0	0.5	2.0	0.89	0.7	0.4	1.4	0.34
	R848	150	1.3	0.7	2.2	0.40	1.1	0.6	2.0	0.70	0.8	0.4	1.5	0.51
	SA	98	1.4	0.8	2.4	0.26	1.0	0.5	1.8	0.94	0.9	0.5	1.8	0.83
	SP	74	1.2	0.7	1.9	0.49	1.0	0.6	1.6	0.94	1.3	0.8	2.3	0.34
	GBS	113	1.3	0.8	2.0	0.34	1.2	0.7	1.9	0.54	0.9	0.5	1.5	0.69
	EC	157	1.0	0.7	1.5	0.98	0.8	0.6	1.3	0.39	1.0	0.7	1.6	0.90
	HI	141	0.8	0.5	1.2	0.23	1.0	0.7	1.5	0.95	0.9	0.6	1.4	0.73
	LM	69	1.4	0.6	2.9	0.42	1.1	0.5	2.3	0.87	1.0	0.4	2.4	0.97
	CA	86	1.2	0.6	2.4	0.64	1.3	0.6	2.8	0.48	1.5	0.6	3.6	0.39
MIF	BCG	129	1.0	0.8	1.2	0.97	1.0	0.8	1.2	0.69	1.0	0.8	1.3	0.79
	MTBK	98	0.7	0.6	0.9	0.01	0.9	0.7	1.1	0.36	0.9	0.7	1.2	0.56
	LPS	147	0.9	0.8	1.1	0.53	0.9	0.8	1.1	0.49	1.0	0.8	1.3	0.81
	PEPG	147	1.0	0.8	1.2	0.76	1.0	0.8	1.2	0.78	0.9	0.8	1.2	0.50
	Pam3	128	0.8	0.6	1.0	0.06	0.7	0.5	1.0	0.03	0.8	0.6	1.1	0.20
	R848	150	0.8	0.7	1.0	0.06	0.9	0.7	1.1	0.27	1.0	0.8	1.2	0.92
	SA	98	0.9	0.7	1.1	0.19	0.9	0.7	1.1	0.39	0.9	0.7	1.2	0.60
	SP	74	0.8	0.6	1.0	0.06	0.9	0.7	1.2	0.53	1.0	0.7	1.3	0.88
	GBS	113	0.7	0.6	0.9	0.00	0.9	0.7	1.1	0.21	1.0	0.8	1.3	0.98
	EC	157	0.9	0.7	1.0	0.10	0.9	0.7	1.0	0.13	1.0	0.9	1.3	0.70
	HI	141	0.8	0.7	1.0	0.04	0.9	0.8	1.1	0.45	1.0	0.8	1.2	0.80
	LM	69	0.8	0.6	1.0	0.03	0.9	0.7	1.1	0.24	1.1	0.8	1.5	0.59
	CA	86	0.8	0.6	1.0	0.10	1.0	0.8	1.3	0.98	1.0	0.8	1.4	0.95
IL-1β	BCG	129	0.9	0.6	1.3	0.51	0.9	0.6	1.4	0.64	1.6	1.0	2.4	0.04
	MTBK	98	0.4	0.2	1.3	0.12	0.9	0.3	2.6	0.79	0.6	0.2	1.9	0.35
	LPS	147	1.4	0.7	3.0	0.33	1.1	0.5	2.5	0.73	1.2	0.5	2.7	0.72
	PEPG	147	1.5	0.9	2.5	0.15	1.0	0.6	1.8	0.92	0.8	0.5	1.5	0.51
	Pam3	128	0.8	0.4	1.7	0.54	1.4	0.6	3.0	0.42	0.7	0.3	1.5	0.35
	R848	150	1.2	0.6	2.4	0.68	1.3	0.6	2.8	0.50	1.3	0.6	3.0	0.48
	SA	98	1.1	0.6	2.3	0.71	0.6	0.3	1.3	0.21	0.8	0.4	1.7	0.55
	SP	74	1.1	0.6	2.1	0.79	0.9	0.4	1.7	0.67	0.8	0.4	1.8	0.60
	GBS	113	0.8	0.5	1.5	0.52	1.0	0.6	1.9	0.92	1.0	0.5	1.8	0.97
	EC	157	1.2	0.7	1.9	0.57	1.3	0.8	2.2	0.32	1.1	0.6	2.0	0.74
	HI	141	1.0	0.6	1.7	0.91	1.1	0.6	1.9	0.80	0.9	0.5	1.6	0.72
	LM	69	1.1	0.5	2.3	0.83	1.0	0.5	2.1	0.97	0.7	0.3	1.7	0.49
	CA	86	1.2	0.3	3.9	0.81	1.1	0.3	4.0	0.91	0.8	0.2	3.3	0.71
IL6	BCG	129	1.2	0.8	1.8	0.44	0.9	0.6	1.3	0.50	1.0	0.6	1.7	0.88
	MTBK	98	0.6	0.2	1.6	0.29	0.9	0.3	2.8	0.90	0.5	0.1	1.5	0.19
	LPS	147	1.0	0.5	1.8	0.98	1.4	0.7	2.5	0.32	1.2	0.6	2.3	0.67
	PEPG	147	0.9	0.6	1.4	0.79	1.1	0.8	1.7	0.51	0.8	0.5	1.2	0.31
	Pam3	128	0.6	0.2	1.9	0.42	0.9	0.3	2.8	0.84	0.4	0.1	1.3	0.14
	R848	150	1.0	0.6	1.6	0.99	1.2	0.8	2.0	0.41	1.1	0.7	1.9	0.72
	SA	98	1.4	0.6	3.3	0.46	0.7	0.3	1.9	0.50	0.8	0.3	2.2	0.69
	SP	74	1.2	0.6	2.5	0.59	0.9	0.4	1.9	0.79	1.0	0.4	2.3	0.94
	GBS	113	1.0	0.5	1.8	0.91	1.1	0.6	2.2	0.76	0.9	0.4	1.8	0.76
	EC	157	0.9	0.6	1.4	0.64	0.9	0.6	1.5	0.75	0.8	0.5	1.3	0.36
	HI	141	0.7	0.5	1.2	0.24	1.0	0.6	1.6	0.86	0.9	0.5	1.6	0.73
	LM	69	1.5	0.5	5.0	0.47	1.2	0.3	4.0	0.79	0.9	0.2	3.7	0.92
	CA	86	1.9	0.4	10.2	0.43	1.3	0.2	8.5	0.75	1.5	0.2	12.0	0.70
TNF-α	BCG	129	1.0	0.6	1.5	0.90	1.5	1.0	2.4	0.06	1.4	0.9	2.4	0.15
	MTBK	98	0.9	0.3	2.1	0.75	2.3	0.9	5.9	0.07	0.7	0.3	2.1	0.55
	LPS	147	1.0	0.6	1.7	0.96	1.8	1.0	3.2	0.04	1.2	0.6	2.2	0.61
	PEPG	147	0.9	0.6	1.4	0.78	1.2	0.8	1.8	0.40	0.7	0.5	1.2	0.18
	Pam3	128	0.7	0.4	1.3	0.28	1.1	0.6	1.9	0.73	0.6	0.4	1.2	0.13
	R848	150	0.8	0.3	2.1	0.68	1.4	0.5	3.6	0.50	0.9	0.3	2.6	0.90
	SA	98	1.0	0.5	2.1	0.96	1.1	0.5	2.4	0.80	1.0	0.4	2.3	0.99
	SP	74	0.9	0.5	1.6	0.66	1.6	0.9	2.9	0.14	0.7	0.4	1.5	0.40
	GBS	113	0.6	0.4	1.1	0.08	1.5	0.9	2.6	0.16	0.9	0.5	1.7	0.74
	EC	157	0.9	0.6	1.2	0.52	1.3	0.9	1.8	0.14	1.1	0.8	1.6	0.53
	HI	141	0.9	0.6	1.3	0.52	1.2	0.8	1.7	0.44	0.9	0.6	1.5	0.79
	LM	69	1.0	0.5	1.9	1.00	1.3	0.7	2.5	0.46	0.6	0.3	1.2	0.15
	CA	86	0.9	0.3	2.5	0.89	2.2	0.8	6.3	0.13	0.6	0.2	2.1	0.47
IFN-γ	BCG	129	1.0	0.2	4.7	0.98	0.4	0.1	2.0	0.29	4.6	0.9	24.7	0.08
	MTBK	98	1.2	0.4	4.0	0.71	0.4	0.1	1.2	0.10	0.3	0.1	1.2	0.09
	LPS	147	1.2	0.3	5.0	0.84	2.1	0.5	9.6	0.32	2.3	0.4	11.9	0.33
	PEPG	147	1.8	0.4	7.7	0.40	1.0	0.2	4.5	0.97	2.9	0.6	14.3	0.20
	Pam3	128	1.6	0.6	4.4	0.37	0.5	0.2	1.4	0.17	0.8	0.3	2.4	0.65
	R848	150	1.4	0.2	8.0	0.70	1.3	0.2	7.8	0.76	1.6	0.2	11.1	0.62
	SA	98	2.1	0.3	13.0	0.43	4.5	0.7	28.3	0.11	5.4	0.7	40.5	0.10
	SP	74	2.9	0.5	18.2	0.24	1.3	0.2	8.7	0.78	19.5	2.6	146.3	0.00
	GBS	113	2.6	0.5	15.2	0.27	0.9	0.1	5.2	0.87	0.3	0.0	1.7	0.16
	EC	157	1.0	0.3	3.5	0.94	0.9	0.2	3.4	0.85	1.8	0.4	7.6	0.40
	HI	141	2.4	0.6	9.3	0.22	1.5	0.4	6.0	0.59	0.6	0.1	2.6	0.46
	LM	69	3.6	0.5	23.9	0.19	2.5	0.4	18.0	0.35	1.6	0.2	14.2	0.69
	CA	86	2.8	0.6	12.2	0.17	2.3	0.5	10.3	0.27	2.2	0.4	11.7	0.34

Supplementary Table 3



Supplementary Figure 2

Cytokine	Stimulant	Total (n)	Early (n)	Late (n)	Controls (n)	BCG				Early v Late				Early v controls				Late v controls			
						GMR	95%CI LCI	95%CI UCI	p value	GMR	95%CI LCI	95%CI UCI	p value	GMR	95%CI LCI	95%CI UCI	p value	GMR	95%CI LCI	95%CI UCI	p value
IL-1ra	BCG	129	51	19	59	1.1	0.7	1.7	0.62	0.7	0.4	1.2	0.18	1.3	0.8	1.9	0.30	0.9	0.4	1.7	0.64
	MTBK	98	37	17	44	1.0	0.7	1.6	0.85	0.7	0.4	1.3	0.26	1.3	0.8	2.0	0.28	0.8	0.4	1.7	0.57
	LPS	147	54	26	67	0.8	0.6	1.3	0.40	0.6	0.4	1.0	0.07	1.0	0.7	1.6	0.94	0.6	0.3	1.1	0.09
	PEPG	147	53	26	67	0.9	0.7	1.2	0.46	0.8	0.6	1.0	0.08	1.0	0.8	1.4	0.90	0.7	0.5	1.1	0.15
	Pam3	128	49	21	58	0.8	0.6	1.2	0.32	1.0	0.7	1.7	0.86	0.9	0.6	1.2	0.43	0.8	0.5	1.4	0.44
	R848	150	57	27	66	0.8	0.6	1.0	0.06	0.8	0.6	1.2	0.28	0.8	0.6	1.1	0.22	0.7	0.5	1.0	0.04
	SA	98	36	16	46	0.8	0.5	1.2	0.19	0.8	0.4	1.5	0.49	0.8	0.5	1.3	0.45	0.6	0.3	1.2	0.14
	SP	74	27	13	34	0.7	0.5	1.1	0.09	0.6	0.3	1.0	0.06	0.9	0.6	1.4	0.66	0.5	0.2	0.9	0.03
	GBS	113	43	19	51	0.9	0.6	1.2	0.37	0.8	0.5	1.3	0.30	1.0	0.7	1.4	0.78	0.7	0.4	1.2	0.17
	EC	157	62	28	67	0.8	0.6	1.1	0.13	0.7	0.5	1.0	0.05	0.9	0.7	1.2	0.67	0.6	0.4	0.9	0.02
	HI	141	56	22	63	0.8	0.6	1.1	0.25	0.8	0.5	1.2	0.23	0.9	0.7	1.3	0.69	0.7	0.4	1.1	0.11
	LM	69	23	12	33	0.5	0.3	0.9	0.01	0.9	0.4	2.0	0.79	0.6	0.3	1.1	0.09	0.4	0.2	0.9	0.03
	CA	86	32	15	39	0.7	0.4	1.1	0.10	1.0	0.5	2.1	0.97	0.7	0.4	1.2	0.19	0.6	0.3	1.3	0.20
IL-10	BCG	129	51	19	59	1.0	0.7	1.3	0.77	0.7	0.5	1.1	0.15	1.0	0.7	1.5	0.86	0.8	0.4	1.3	0.35
	MTBK	98	37	17	44	1.3	0.7	2.5	0.44	1.3	0.6	3.0	0.52	1.2	0.5	2.6	0.66	1.6	0.6	3.9	0.34
	LPS	147	54	26	67	1.1	0.7	1.9	0.62	0.6	0.3	1.1	0.08	1.4	0.8	2.4	0.27	0.8	0.4	1.6	0.49
	PEPG	147	53	26	67	0.8	0.7	1.0	0.09	0.7	0.5	0.9	0.00	1.0	0.8	1.2	0.68	0.7	0.5	0.9	0.00
	Pam3	128	49	21	58	0.8	0.5	1.4	0.49	1.3	0.6	2.8	0.52	0.8	0.5	1.4	0.41	1.0	0.5	1.9	0.98
	R848	150	57	27	66	0.7	0.6	1.0	0.02	0.8	0.6	1.2	0.38	0.8	0.6	1.0	0.08	0.7	0.5	0.9	0.02
	SA	98	36	16	46	0.9	0.6	1.3	0.61	0.7	0.4	1.2	0.18	1.0	0.7	1.5	0.98	0.7	0.4	1.3	0.29
	SP	74	27	13	34	1.1	0.8	1.5	0.62	0.5	0.3	0.7	0.00	1.3	1.0	1.9	0.07	0.7	0.4	1.1	0.10
	GBS	113	43	19	51	1.2	0.9	1.6	0.18	0.8	0.5	1.1	0.17	1.3	1.0	1.8	0.08	1.0	0.7	1.5	0.95
	EC	157	62	28	67	0.9	0.7	1.1	0.23	0.7	0.5	0.9	0.00	1.0	0.8	1.2	0.99	0.7	0.6	0.9	0.00
	HI	141	56	22	63	0.9	0.7	1.1	0.31	0.7	0.5	1.0	0.03	1.0	0.8	1.2	0.93	0.7	0.5	0.9	0.02
	LM	69	23	12	33	0.9	0.6	1.3	0.47	0.8	0.5	1.5	0.58	0.9	0.5	1.5	0.67	0.8	0.4	1.5	0.44
	CA	86	32	15	39	1.2	0.5	2.9	0.64	0.7	0.2	2.4	0.58	1.4	0.5	3.6	0.52	1.0	0.3	3.1	0.96
MIG	BCG	129	51	19	59	0.9	0.8	1.0	0.14	1.0	0.8	1.2	0.98	0.9	0.8	1.0	0.16	0.9	0.7	1.1	0.35
	MTBK	98	37	17	44	0.8	0.7	1.0	0.06	1.0	0.7	1.4	0.94	0.8	0.6	1.1	0.12	0.8	0.6	1.0	0.10
	LPS	147	54	26	67	1.1	0.9	1.3	0.50	0.8	0.6	1.1	0.20	1.1	0.9	1.4	0.25	0.9	0.8	1.1	0.53
	PEPG	147	53	26	67	0.9	0.7	1.1	0.17	1.1	0.8	1.4	0.51	0.8	0.7	1.1	0.14	0.9	0.7	1.2	0.54
	Pam3	128	49	21	58	1.0	0.9	1.1	0.81	1.1	0.9	1.4	0.34	1.0	0.8	1.1	0.52	1.1	0.9	1.2	0.30
	R848	150	57	27	66	0.9	0.7	1.3	0.70	0.9	0.6	1.5	0.74	1.0	0.7	1.4	0.85	0.9	0.6	1.3	0.41
	SA	98	36	16	46	1.0	0.8	1.2	0.99	1.1	0.8	1.6	0.41	1.0	0.8	1.2	0.71	1.1	0.8	1.5	0.47
	SP	74	27	13	34	1.1	0.8	1.4	0.71	0.7	0.5	1.1	0.15	1.2	0.9	1.6	0.31	0.8	0.6	1.3	0.41
	GBS	113	43	19	51	1.1	0.8	1.4	0.63	0.9	0.6	1.3	0.65	1.1	0.8	1.4	0.55	1.0	0.7	1.4	0.98
	EC	157	62	28	67	1.0	0.9	1.1	0.94	0.9	0.8	1.1	0.50	1.0	0.9	1.2	0.73	0.9	0.8	1.1	0.50
	HI	141	56	22	63	0.9	0.8	1.1	0.45	1.0	0.8	1.2	0.86	1.0	0.8	1.1	0.55	0.9	0.8	1.1	0.43
	LM	69	23	12	33	0.9	0.7	1.1	0.19	0.9	0.7	1.3	0.73	0.9	0.7	1.1	0.32	0.8	0.6	1.2	0.32
	CA	86	32	15	39	0.9	0.8	1.1	0.34	1.1	0.9	1.4	0.19	0.9	0.8	1.1	0.19	1.0	0.8	1.2	0.90
MIP-1α	BCG	129	51	19	59	0.9	0.7	1.1	0.28	0.9	0.6	1.3	0.45	0.9	0.7	1.2	0.56	0.8	0.6	1.0	0.09
	MTBK	98	37	17	44	1.1	0.6	1.9	0.76	1.0	0.5	2.2	0.93	1.1	0.6	2.0	0.77	1.1	0.5	2.3	0.81
	LPS	147	54	26	67	0.9	0.6	1.4	0.75	0.5	0.3	0.9	0.02	1.2	0.7	1.9	0.55	0.6	0.3	1.2	0.13
	PEPG	147	53	26	67	0.7	0.5	0.9	0.01	0.7	0.5	1.0	0.03	0.8	0.6	1.1	0.14	0.6	0.4	0.8	0.00
	Pam3	128	49	21	58	1.2	0.7	2.1	0.57	1.1	0.5	2.2	0.86	1.2	0.6	2.2	0.62	1.3	0.5	3.0	0.59
	R848	150	57	27	66	0.7	0.5	1.0	0.06	0.6	0.4	1.0	0.04	0.9	0.6	1.2	0.41	0.5	0.4	0.8	0.00
	SA	98	36	16	46	0.7	0.4	1.1	0.15	0.9	0.4	1.7	0.63	0.7	0.4	1.2	0.25	0.6	0.3	1.4	0.24
	SP	74	27	13	34	0.8	0.6	1.2	0.27	0.8	0.5	1.2	0.26	0.9	0.6	1.3	0.58	0.7	0.4	1.2	0.17
	GBS	113	43	19	51	1.1	0.7	1.6	0.73	0.7	0.4	1.2	0.19	1.2	0.8	1.8	0.43	0.9	0.5	1.5	0.60
	EC	157	62	28	67	0.9	0.7	1.2	0.50	0.7	0.5	1.0	0.07	1.0	0.8	1.3	0.94	0.8	0.5	1.1	0.12
	HI	141	56	22	63	0.9	0.6	1.1	0.25	0.7	0.5	1.1	0.11	0.9	0.7	1.3	0.65	0.7	0.5	1.0	0.07
	LM	69	23	12	33	0.8	0.4	1.4	0.38	1.2	0.5	3.2	0.65	0.7	0.3	1.4	0.32	0.9	0.4	2.1	0.75
	CA	86	32	15	39	0.7	0.3	1.5	0.38	0.9	0.3	2.6	0.83	0.7	0.3	1.7	0.50	0.7	0.3	1.7	0.40
MIP-1β	BCG	129	51	19	59	0.9	0.7	1.1	0.23	0.8	0.6	1.0	0.06	1.0	0.8	1.2	0.69	0.7	0.5	1.0	0.03
	MTBK	98	37	17	44	1.1	0.8	1.4	0.55	0.9	0.6	1.3	0.55	1.1	0.8	1.6	0.43	1.0	0.7	1.5	1.00
	LPS	147	54	26	67	0.8	0.5	1.1	0.14	0.6	0.4	0.8	0.01	0.9	0.6	1.4	0.71	0.5	0.3	0.9	0.01
	PEPG	147	53	26	67	0.7	0.6	0.8	0.00	0.7	0.5	0.9	0.01	0.8	0.6	1.0	0.03	0.5	0.4	0.7	0.00
	Pam3	128	49	21	58	1.0	0.8	1.3	0.96	0.9	0.6	1.2	0.45	1.0	0.8	1.4	0.75	0.9	0.7	1.3	0.65
	R848	150	57	27	66	0.7	0.5	1.0	0.03	0.6	0.4	0.9	0.01	0.9	0.6	1.2	0.39	0.5	0.3	0.7	0.00
	SA	98	36	16	46	0.8	0.7	1.1	0.14	0.8	0.6	1.1	0.11	0.9	0.7	1.2	0.40	0.7	0.5	1.0	0.08
	SP	74	27	13	34	0.8	0.6	1.0	0.07	0.8	0.5	1.1	0.20	0.9	0.6	1.1	0.26	0.7	0.5	1.0	0.03
	GBS	113	43	19	51	0.9	0.8	1.2	0.57	0.7	0.5	0.9	0.01	1.1	0.8	1.3	0.62	0.7	0.5	1.0	0.02
	EC	157	62	28	67	0.8	0.6	1.0	0.05	0.7	0.5	0.9	0.01	0.9	0.7	1.2	0.45	0.6	0.4	0.8	0.00
	HI	141	56	22	63	0.9	0.7	1.1	0.25	0.6	0.4	0.9	0.01	1.0	0.7	1.3	0.95	0.6	0.4	0.9	0.00
	LM	69	23	12	33	0.7	0.5	1.0	0.03	1.0	0.7	1.5	0.99	0.7	0.5	1.0	0.07	0.7	0.5	1.1	0.11
	CA	86	32	15	39	0.8	0.5	1.2	0.28	0.8	0.4	1.3	0.34	0.9	0.6	1.4	0.54	0.7	0.4	1.1	0.14
MCP-1	BCG	129	51	19	59	1.0	0.8	1.3	0.98	0.7	0.5	1.1	0.11	1.1	0.8	1.4	0.52	0.8	0.6	1.2	0.25
	MTBK	98	37	17	44	1.0	0.8	1.4	0.82	0.8	0.5	1.2	0.28	1.1	0.8	1.5	0.56	0.9	0.6	1.4	0.62
	LPS	147	54	26	67	0.9	0.6	1.3	0.45	0.7	0.4	1.0	0.06	1.0	0.7	1.5	1.00	0.7	0.4	1.1	0.13
	PEPG	147	53	26	67	0.8	0.6	1.0	0.04	0.8	0.5	1.1	0.12	0.9	0.7	1.1	0.25	0.7	0.5	0.9	0.01
	Pam3	128	49	21	58	0.9	0.7	1.1	0.36	0.9	0.6	1.2	0.37	0.9	0.7	1.2	0.63	0.8	0.6	1.1	0.21
	R848	150	57	27	66	0.8	0.7	1.0	0.01	0.8	0.6	1.0	0.05	0.9	0.7	1.0	0.16	0.7	0.5	0.8	0.00
	SA	98	36	16	46	0.8	0.6	1.1	0.20	0.8	0.5	1.2	0.27	0.9	0.6	1.2	0.43	0.7	0.5	1.1	0.15
	SP	74	27	13	34	0.7	0.5	0.9	0.01	0.7	0.4	1.1	0.11	0.7	0.5	1.0	0.05	0.5	0.3	0.8	0.00
	GBS	113	43	19	51	0.9	0.7	1.1	0.28	0.7	0.5	1.1	0.09	1.0	0.7	1.2	0.75	0.7	0.5	1.0	0.06
	EC	157	62	28	67	0.8	0.6	1.0	0.03	0.7	0.5	1.0	0.06	0.9	0.7	1.1	0.21	0.6	0.5	0.9	0.00
	HI	141	56	22	63	0.8	0.7	1.0	0.07	0.8	0.6	1.2	0.30	0.9	0.7	1.1	0.22	0.7	0.6	1.0	0.03
	LM	69	23	12	33	0.7	0.5	0.9	0.01	0.8	0.5	1.2	0.29	0.7	0.5	1.0	0.06	0.6	0.4	0.9	0.01
	CA	86	32	15	39	0.6	0.4	0.9	0.01	0.9	0.5	1.6	0.65	0.6	0.4	0.9	0.02	0.6	0.4	0.9	0.02
IL-8	BCG	129	51	19	59	1.0	0.9	1.3	0.70	0.9	0.7	1.2	0.44	1.1	0.9	1.3	0.53	1.0	0.7	1.3	0.73
	MTBK	98	37	17	44	1.2	0.9	1.7	0.27	1.1	0.7	1.8	0.63	1.2	0.8	1.8	0.45	1.3	0.8	2.1	0.25
	LPS	147	54	26	67	1.0	0.8	1.3	0.94	0.7	0.5	1.0	0.05	1.1	0.8	1.6	0.44	0.8	0.5	1.2	0.27
	PEPG	147	53	26	67	0.9	0.7	1.1	0.37	0.8	0.6	1.1	0.11	1.0	0.8	1.3	0.90	0.8	0.6	1.0	0.06
	Pam3	128	49	21	58	1.1	0.8	1.5	0.68	0.9	0.6	1.4	0.63	1.1	0.8	1.6	0.61	1.0	0.7	1.6	0.88
	R848	150	57	27	66	0.9	0.7	1.2	0.70	0.7	0.4	1.0	0.05	1.1	0.8	1.5	0.62	0.7	0.5	1.0	0.08
	SA	98	36	16	46	0.9	0.7	1.3	0.67	1.1	0.8	1.7	0.52	0.9	0.7	1.3	0.56	1.0	0.7	1.6	0.94
	SP	74	27	13	34	0.8	0.7	1.1	0.17	1.0	0.7	1.4	0.93	0.8	0.6	1.1	0.17	0.9	0.6	1.2	0.39
	GBS	113	43	19	51	1.0	0.8	1.3	0.95	0.7	0.5	1.0	0.05	1.1	0.9	1.4	0.39	0.8	0.6	1.1	0.14
	EC	157	62	28	67	1.0	0.8	1.2	0.81	0.9	0.7	1.1	0.26	1.1	0.9	1.3	0.58	0.9	0.7	1.2	0.61
	HI	141	56	22	63	1.0	0.8	1.2	0.92	0.9	0.7	1.2	0.41	1.0	0.8	1.3	0.70	0.9	0.7	1.2	0.65
	LM	69	23	12	33	0.7	0.5	1.1	0.11	1.0	0.6	1.7	0.92	0.7	0.5	1.2	0.19	0.8	0.5	1.2	0.27
	CA	86	32	15	39	0.8	0.5	1.1	0.15	1.2	0.7	2.0	0.55	0.7	0.5	1.1	0.13	0.8	0.5	1.4	0.50
MIF	BCG	129	51	19	59	0.9	0.8	1.0	0.06	0.9	0.8	1.1	0.34	0.9	0.8	1.0	0.18	0.9	0.7	1.0	0.04
	MTBK	98	37	17	44	0.9	0.8	1.0	0.09	0.9	0.8	1.1	0.32	0.9	0.8	1.1	0.26	0.9	0.7	1.0	0.06
	LPS	147	54	26	67	1.0	0.9	1.1	0.81	1.0	0.9	1.2	0.74	1.0	0.9	1.1	0.71	1.0	0.9	1.1	0.93
	PEPG	147	53	26	67	1.0	0.9	1.1	0.42	1.0	0.8	1.1	0.46	1.0	0.9	1.1	0.68	0.9	0.8	1.1	0.27
	Pam3	128	49	21	58	0.9	0.8	1.1	0.29	1.0	0.8	1.3	0.85	0.9	0.8	1.1	0.33	0.9	0.8	1.1	0.30
	R848	150	57	27	66	1.0	0.9	1.0	0.28	1.0	0.9	1.1	0.78	1.0	0.9	1.1	0.42	0.9	0.8	1.0	0.26
	SA	98	36	16	46	1.0	0.9	1.1	0.76	1.0	0.9	1.1	0.92	1.0	0.9	1.1	0.82	1.0	0.8	1.1	0.78
	SP	74	27	13	34	1.0	0.9	1.1	0.72	1.0	0.8	1.2	0.71	1.0	0.8	1.2	0.87	1.0	0.8	1.2	0.60
	GBS	113	43	19	51	1.1	0.9	1.2	0.32	1.0	0.8	1.1	0.70	1.1	0.9	1.2	0.32	1.0	0.9	1.2	0.66
	EC	157	62	28	67	1.1	1.0	1.1	0.25	1.0	0.9	1.2	0.75	1.0	0.9	1.2	0.37	1.1	0.9	1.2	0.29
	HI	141	56	22	63	1.0	0.9	1.1	0.56	1.1	0.9	1.2	0.40	1.0	0.9	1.1	0.37	1.0	0.9	1.1	0.77
	LM	69	23	12	33	0.9	0.8	1.0	0.07	1.1	0.9	1.3	0.22	0.9	0.7	1.0	0.04	1.0	0.8	1.1	0.57
	CA	86	32	15	39	1.0	0.9	1.1	0.88	1.0	0.8	1.2	0.81	1.0	0.9	1.1	0.98	1.0	0.8	1.1	0.72
IL-1β	BCG	129	51	19	59	0.9	0.8	1.1	0.38	0.9	0.6	1.2	0.47	0.9	0.8	1.2	0.61	0.8	0.6	1.1	0.17
	MTBK	98	37	17	44	1.1	0.6	1.8	0.76	1.1	0.5	2.3	0.88	1.1	0.6	2.1	0.69	1.0	0.5	2.2	0.89
	LPS	147	54	26	67	0.9	0.6	1.3	0.46	0.5	0.3	0.9	0.02	1.1	0.7	1.6	0.72	0.6	0.3	1.0	0.05
	PEPG	147	53	26	67	0.9	0.7	1.1	0.27	0.7	0.5	0.9	0.01	1.0	0.7	1.3	0.95	0.7	0.5	1.0	0.03
	Pam3	128	49	21	58	1.0	0.7	1.4	0.91	0.5	0.3	0.9	0.02	1.2	0.8	1.8	0.41	0.6	0.4	1.1	0.08
	R848	150	57	27	66	0.8	0.6	1.2	0.30	0.7	0.4	1.1	0.13	1.0	0.6	1.4	0.81	0.6	0.4	1.0	0.06
	SA	98	36	16	46	0.8	0.5	1.1	0.16	0.8	0.5	1.4	0.39	0.8	0.6	1.2	0.31	0.7	0.4	1.1	0.14
	SP	74	27	13	34	1.0	0.7	1.4	0.98	0.7	0.4	1.0	0.05	1.2	0.8	1.7	0.41	0.8	0.5	1.2	0.24
	GBS	113	43	19	51	1.0	0.7	1.3	0.75	0.6	0.5	0.9	0.01	1.1	0.8	1.5	0.58	0.7	0.5	1.1	0.12
	EC	157	62	28	67	1.1	0.8	1.4	0.68	0.7	0.5	1.0	0.03	1.2	0.9	1.6	0.23	0.8	0.6	1.2	0.29
	HI	141	56	22	63	0.9	0.7	1.2	0.63	0.6	0.4	0.9	0.01	1.1	0.8	1.5	0.53	0.6	0.4	0.9	0.03
	LM	69	23	12	33	0.9	0.6	1.2	0.40	0.8	0.5	1.4	0.46	0.9	0.6	1.4	0.72	0.8	0.4	1.3	0.28
	CA	86	32	15	39	0.8	0.4	1.5	0.48	0.7	0.3	2.0	0.55	0.9	0.4	1.8	0.72	0.7	0.3	1.4	0.27
IL-6	BCG	129	51	19	59	1.0	0.8	1.2	0.77	0.8	0.6	1.1	0.24	1.0	0.8	1.3	0.88	0.8	0.6	1.1	0.25
	MTBK	98	37	17	44	1.1	0.6	1.8	0.74	1.4	0.7	3.1	0.35	1.0	0.5	1.9	0.99	1.3	0.6	2.5	0.49
	LPS	147	54	26	67	0.9	0.7	1.3	0.66	0.6	0.4	0.9	0.00	1.1	0.8	1.5	0.56	0.7	0.4	1.0	0.07
	PEPG	147	53	26	67	0.8	0.6	1.0	0.02	0.7	0.5	0.9	0.01	0.9	0.7	1.1	0.25	0.6	0.5	0.8	0.00
	Pam3	128	49	21	58	0.9	0.5	1.6	0.80	0.9	0.4	1.9	0.73	1.0	0.5	1.8	0.91	0.9	0.4	2.0	0.75
	R848	150	57	27	66	0.7	0.6	0.9	0.01	0.7	0.5	1.1	0.09	0.8	0.6	1.0	0.07	0.6	0.4	0.8	0.00
	SA	98	36	16	46	0.7	0.4	1.1	0.09	0.9	0.5	1.7	0.69	0.7	0.4	1.1	0.14	0.6	0.3	1.3	0.19
	SP	74	27	13	34	0.9	0.7	1.3	0.73	0.7	0.5	1.2	0.17	1.0	0.7	1.5	0.85	0.8	0.4	1.4	0.39
	GBS	113	43	19	51	0.9	0.7	1.3	0.69	0.7	0.5	1.1	0.12	1.0	0.7	1.5	0.84	0.7	0.5	1.2	0.22
	EC	157	62	28	67	0.9	0.7	1.1	0.22	0.7	0.6	1.0	0.05	0.9	0.7	1.2	0.67	0.7	0.5	1.0	0.04
	HI	141	56	22	63	0.8	0.6	1.1	0.14	0.8	0.5	1.1	0.11	0.9	0.7	1.2	0.44	0.7	0.5	1.0	0.05
	LM	69	23	12	33	0.7	0.4	1.2	0.17	0.8	0.4	1.9	0.66	0.7	0.4	1.4	0.32	0.6	0.3	1.4	0.23
	CA	86	32	15	39	0.6	0.3	1.5	0.26	0.6	0.1	2.9	0.56	0.7	0.3	1.8	0.43	0.5	0.2	1.5	0.21
TNF-α	BCG	129	51	19	59	0.9	0.7	1.1	0.35	0.8	0.5	1.1	0.15	1.0	0.8	1.2	0.73	0.7	0.5	1.0	0.07
	MTBK	98	37	17	44	0.9	0.6	1.4	0.67	1.0	0.5	2.0	0.91	0.9	0.5	1.5	0.68	0.9	0.5	1.7	0.78
	LPS	147	54	26	67	1.2	0.9	1.6	0.14	0.7	0.5	1.0	0.03	1.4	1.0	1.9	0.04	0.9	0.6	1.4	0.71
	PEPG	147	53	26	67	1.0	0.8	1.2	0.89	0.6	0.5	0.8	0.00	1.1	0.9	1.4	0.20	0.7	0.6	1.0	0.03
	Pam3	128	49	21	58	1.0	0.8	1.4	0.74	0.9	0.6	1.3	0.51	1.1	0.8	1.5	0.57	1.0	0.7	1.3	0.78
	R848	150	57	27	66	0.8	0.5	1.3	0.42	0.7	0.4	1.4	0.30	0.9	0.5	1.6	0.77	0.7	0.3	1.2	0.18
	SA	98	36	16	46	0.9	0.6	1.3	0.49	0.9	0.5	1.6	0.79	0.9	0.6	1.3	0.54	0.8	0.5	1.5	0.54
	SP	74	27	13	34	1.0	0.7	1.3	0.94	0.8	0.5	1.2	0.30	1.1	0.8	1.5	0.67	0.8	0.5	1.3	0.44
	GBS	113	43	19	51	1.1	0.9	1.5	0.34	0.7	0.5	1.1	0.12	1.2	0.9	1.7	0.15	0.9	0.6	1.4	0.73
	EC	157	62	28	67	1.0	0.9	1.2	0.71	0.8	0.6	1.0	0.02	1.1	0.9	1.3	0.27	0.9	0.7	1.1	0.19
	HI	141	56	22	63	1.0	0.8	1.2	0.83	0.8	0.6	1.0	0.07	1.1	0.9	1.3	0.64	0.8	0.6	1.1	0.11
	LM	69	23	12	33	0.9	0.6	1.2	0.41	1.4	0.9	2.2	0.18	0.8	0.6	1.1	0.17	1.0	0.6	1.7	0.88
	CA	86	32	15	39	0.8	0.5	1.4	0.52	0.8	0.4	1.7	0.53	0.9	0.5	1.6	0.78	0.7	0.4	1.4	0.30
IFN-γ	BCG	129	51	19	59	1.9	0.9	4.0	0.10	1.1	0.3	3.9	0.83	1.9	0.8	4.2	0.12	2.0	0.6	6.1	0.23
	MTBK	98	37	17	44	1.5	0.8	2.7	0.16	1.7	0.8	3.7	0.15	1.2	0.7	2.3	0.52	2.3	0.9	5.6	0.07
	LPS	147	54	26	67	1.2	0.6	2.6	0.56	0.4	0.1	1.1	0.07	1.6	0.7	3.7	0.25	0.7	0.3	1.9	0.53
	PEPG	147	53	26	67	0.7	0.3	1.4	0.33	0.9	0.3	2.5	0.83	0.7	0.3	1.6	0.43	0.7	0.2	1.9	0.46
	Pam3	128	49	21	58	1.2	0.7	2.0	0.51	1.3	0.6	2.8	0.45	1.1	0.6	1.9	0.76	1.5	0.7	3.1	0.28
	R848	150	57	27	66	0.8	0.3	1.8	0.52	0.5	0.1	1.7	0.27	0.9	0.4	2.5	0.89	0.5	0.2	1.7	0.28
	SA	98	36	16	46	0.8	0.3	1.9	0.56	2.5	0.7	9.3	0.18	0.6	0.2	1.6	0.28	1.4	0.4	5.1	0.59
	SP	74	27	13	34	1.0	0.4	2.5	0.95	0.3	0.1	1.0	0.06	1.5	0.6	3.9	0.37	0.4	0.1	1.7	0.23
	GBS	113	43	19	51	1.3	0.6	3.2	0.50	0.6	0.2	1.9	0.37	1.6	0.6	4.1	0.34	0.9	0.3	3.5	0.92
	EC	157	62	28	67	1.2	0.7	2.3	0.51	1.3	0.6	3.3	0.50	1.1	0.5	2.3	0.76	1.6	0.7	4.0	0.29
	HI	141	56	22	63	1.6	0.8	3.1	0.19	1.3	0.5	3.6	0.64	1.5	0.7	3.1	0.32	1.8	0.7	4.9	0.22
	LM	69	23	12	33	1.4	0.5	3.7	0.47	0.6	0.2	2.4	0.49	1.7	0.6	5.1	0.34	1.1	0.3	3.9	0.93
	CA	86	32	15	39	0.8	0.4	1.6	0.47	0.5	0.2	1.4	0.19	0.9	0.4	2.1	0.88	0.5	0.2	1.4	0.18

Supplementary Table 4

Cytokine	Stimulant	Dataset1					Dataset2					Dataset3				
		DS1(n)	GMR	95%CI LCI	95%CI UCI	p-value	DS2(n)	GMR	95%CI LCI	95%CI UCI	p-value	DS3(n)	GMR	95%CI LCI	95%CI UCI	p-value
IL-1ra	BCG	129	1.1	0.7	1.7	0.62	123	1.3	0.8	2.1	0.26	121	1.0	0.8	1.4	0.72
	MTBK	98	1.0	0.7	1.6	0.85	96	1.2	0.8	2.0	0.39	94	1.4	1.0	1.9	0.06
	LPS	147	0.8	0.6	1.3	0.40	138	0.9	0.6	1.5	0.75	137	0.9	0.7	1.1	0.22
	PEPG	146	0.9	0.7	1.2	0.46	145	1.0	0.7	1.3	0.79	144	0.9	0.7	1.1	0.19
	Pam3	128	0.8	0.6	1.2	0.32	121	0.9	0.6	1.4	0.73	117	1.0	0.8	1.3	0.89
	R848	150	0.8	0.6	1.0	0.06	149	0.8	0.6	1.0	0.11	149	0.8	0.6	0.9	0.01
	SA	98	0.8	0.5	1.2	0.19	92	0.8	0.5	1.4	0.47	91	0.9	0.7	1.2	0.35
	SP	74	0.7	0.5	1.1	0.09	72	0.8	0.5	1.3	0.39	72	0.8	0.6	1.1	0.15
	GBS	113	0.9	0.6	1.2	0.37	110	0.9	0.6	1.4	0.71	108	0.9	0.7	1.2	0.40
	EC	157	0.8	0.6	1.1	0.13	155	0.9	0.7	1.2	0.41	154	0.9	0.7	1.1	0.17
	HI	141	0.8	0.6	1.1	0.25	137	0.9	0.6	1.3	0.56	136	0.9	0.7	1.1	0.43
	LM	69	0.5	0.3	0.9	0.01	63	0.6	0.3	1.2	0.16	62	0.7	0.5	1.0	0.04
	CA	86	0.7	0.4	1.1	0.10	76	0.8	0.4	1.6	0.56	73	1.0	0.7	1.3	0.79
IL-10	BCG	129	1.0	0.7	1.3	0.77	128	0.9	0.7	1.3	0.72	127	0.8	0.6	1.1	0.12
	MTBK	98	1.3	0.7	2.5	0.44	93	1.3	0.7	2.5	0.44	89	0.9	0.6	1.3	0.63
	LPS	147	1.1	0.7	1.9	0.62	143	1.1	0.7	1.8	0.67	141	0.9	0.7	1.3	0.72
	PEPG	146	0.8	0.7	1.0	0.09	147	0.8	0.7	1.0	0.07	145	0.8	0.7	1.0	0.08
	Pam3	128	0.8	0.5	1.4	0.49	128	0.8	0.5	1.3	0.41	100	0.9	0.6	1.2	0.40
	R848	150	0.7	0.6	1.0	0.02	150	0.7	0.6	0.9	0.02	150	0.7	0.6	1.0	0.02
	SA	98	0.9	0.6	1.3	0.61	98	0.9	0.6	1.3	0.56	93	0.9	0.7	1.2	0.40
	SP	74	1.1	0.8	1.5	0.62	74	1.1	0.8	1.5	0.60	74	1.1	0.8	1.5	0.60
	GBS	113	1.2	0.9	1.6	0.18	113	1.2	0.9	1.6	0.24	113	1.2	0.9	1.6	0.24
	EC	157	0.9	0.7	1.1	0.23	157	0.9	0.7	1.1	0.18	157	0.9	0.7	1.1	0.26
	HI	141	0.9	0.7	1.1	0.31	141	0.9	0.7	1.1	0.24	141	0.9	0.7	1.1	0.21
	LM	69	0.9	0.6	1.3	0.47	69	0.9	0.6	1.3	0.47	57	0.8	0.5	1.2	0.24
	CA	86	1.2	0.5	2.9	0.64	71	1.2	0.5	2.7	0.73	55	1.1	0.7	1.8	0.71
MIG	BCG	129	0.9	0.8	1.0	0.14	129	0.8	0.7	1.1	0.15	126	0.8	0.6	1.0	0.04
	MTBK	98	0.8	0.7	1.0	0.06	98	0.8	0.6	1.1	0.23	95	0.9	0.6	1.1	0.25
	LPS	147	1.1	0.9	1.3	0.50	147	0.9	0.7	1.2	0.63	146	1.0	0.8	1.2	0.79
	PEPG	146	0.9	0.7	1.1	0.17	146	0.8	0.6	1.0	0.05	146	0.8	0.6	1.0	0.05
	Pam3	128	1.0	0.9	1.1	0.81	127	0.9	0.7	1.1	0.17	126	0.9	0.8	1.2	0.65
	R848	150	0.9	0.7	1.3	0.70	150	0.9	0.6	1.2	0.43	150	0.9	0.7	1.2	0.47
	SA	98	1.0	0.8	1.2	0.99	98	0.9	0.7	1.2	0.50	98	0.9	0.7	1.2	0.50
	SP	74	1.1	0.8	1.4	0.71	74	1.0	0.7	1.5	0.84	74	1.0	0.7	1.4	0.98
	GBS	113	1.1	0.8	1.4	0.63	113	1.0	0.7	1.3	0.83	113	1.0	0.7	1.3	0.83
	EC	157	1.0	0.9	1.1	0.94	157	0.9	0.7	1.1	0.18	157	0.9	0.7	1.1	0.34
	HI	141	0.9	0.8	1.1	0.45	141	0.8	0.7	1.0	0.09	141	0.8	0.7	1.0	0.10
	LM	69	0.9	0.7	1.1	0.19	69	0.8	0.6	1.2	0.31	69	0.8	0.6	1.2	0.31
	CA	86	0.9	0.8	1.1	0.34	86	0.8	0.6	1.1	0.24	85	0.9	0.7	1.1	0.31
MIP-1α	BCG	129	0.9	0.7	1.1	0.28	129	0.9	0.7	1.1	0.29	118	0.9	0.7	1.1	0.31
	MTBK	98	1.1	0.6	1.9	0.76	98	1.1	0.6	1.9	0.70	96	1.2	0.7	2.1	0.44
	LPS	147	0.9	0.6	1.4	0.75	147	1.0	0.6	1.5	0.86	135	1.1	0.7	1.7	0.65
	PEPG	146	0.7	0.5	0.9	0.01	147	0.7	0.5	0.9	0.01	124	0.8	0.6	1.0	0.06
	Pam3	128	1.2	0.7	2.1	0.57	128	1.2	0.7	2.1	0.57	127	1.2	0.7	2.2	0.44
	R848	150	0.7	0.5	1.0	0.06	150	0.8	0.6	1.1	0.13	126	0.8	0.6	1.1	0.13
	SA	98	0.7	0.4	1.1	0.15	98	0.7	0.4	1.1	0.15	96	0.7	0.4	1.0	0.05
	SP	74	0.8	0.6	1.2	0.27	74	0.8	0.6	1.2	0.28	71	0.9	0.7	1.2	0.49
	GBS	113	1.1	0.7	1.6	0.73	113	1.1	0.7	1.6	0.65	111	1.1	0.8	1.6	0.63
	EC	157	0.9	0.7	1.2	0.50	157	0.9	0.7	1.2	0.48	143	0.9	0.7	1.2	0.61
	HI	141	0.9	0.6	1.1	0.25	141	0.9	0.6	1.1	0.24	113	0.9	0.7	1.2	0.50
	LM	69	0.8	0.4	1.4	0.38	69	0.8	0.4	1.4	0.39	69	0.8	0.4	1.4	0.39
	CA	86	0.7	0.3	1.5	0.38	86	0.7	0.3	1.5	0.36	85	0.8	0.4	1.6	0.53
MIP-1β	BCG	129	0.9	0.7	1.1	0.23	129	0.9	0.7	1.1	0.23	114	0.9	0.8	1.1	0.28
	MTBK	98	1.1	0.8	1.4	0.55	98	1.1	0.8	1.4	0.54	94	1.1	0.8	1.4	0.69
	LPS	147	0.8	0.5	1.1	0.14	147	0.7	0.5	1.1	0.11	100	0.9	0.5	1.4	0.52
	PEPG	146	0.7	0.6	0.8	0.00	147	0.7	0.6	0.8	0.00	120	0.8	0.6	0.9	0.01
	Pam3	128	1.0	0.8	1.3	0.96	128	1.0	0.7	1.3	0.81	127	1.0	0.7	1.3	0.84
	R848	150	0.7	0.5	1.0	0.03	150	0.7	0.5	1.0	0.02	95	0.8	0.6	1.1	0.10
	SA	98	0.8	0.7	1.1	0.14	98	0.8	0.6	1.0	0.08	98	0.8	0.6	1.0	0.08
	SP	74	0.8	0.6	1.0	0.07	74	0.8	0.6	1.0	0.11	68	0.9	0.7	1.1	0.19
	GBS	113	0.9	0.8	1.2	0.57	113	0.9	0.7	1.1	0.42	109	0.9	0.8	1.2	0.62
	EC	157	0.8	0.6	1.0	0.05	157	0.8	0.6	1.0	0.06	93	1.0	0.7	1.3	0.72
	HI	141	0.9	0.7	1.1	0.25	141	0.8	0.6	1.1	0.21	101	0.9	0.7	1.1	0.25
	LM	69	0.7	0.5	1.0	0.03	69	0.7	0.5	1.0	0.07	69	0.7	0.5	1.0	0.07
	CA	86	0.8	0.5	1.2	0.28	86	0.8	0.5	1.2	0.19	86	0.8	0.5	1.2	0.19
MCP-1	BCG	129	1.0	0.8	1.3	0.98	129	1.0	0.8	1.3	0.96	129	1.0	0.8	1.3	0.96
	MTBK	98	1.0	0.8	1.4	0.82	98	1.1	0.8	1.4	0.67	98	1.1	0.8	1.4	0.67
	LPS	146	0.9	0.6	1.3	0.45	147	0.9	0.6	1.3	0.53	147	0.9	0.6	1.3	0.52
	PEPG	147	0.8	0.6	1.0	0.04	147	0.8	0.6	1.0	0.04	146	0.8	0.7	1.0	0.07
	Pam3	128	0.9	0.7	1.1	0.36	128	0.9	0.7	1.2	0.46	127	1.0	0.7	1.2	0.77
	R848	150	0.8	0.7	1.0	0.01	150	0.8	0.7	1.0	0.02	147	0.8	0.7	1.0	0.05
	SA	98	0.8	0.6	1.1	0.20	98	0.9	0.6	1.2	0.39	97	0.9	0.6	1.2	0.33
	SP	74	0.7	0.5	0.9	0.01	74	0.7	0.5	1.0	0.04	73	0.7	0.5	0.9	0.02
	GBS	113	0.9	0.7	1.1	0.28	113	0.9	0.7	1.1	0.35	113	0.9	0.7	1.1	0.35
	EC	157	0.8	0.6	1.0	0.03	157	0.8	0.6	1.0	0.04	157	0.8	0.7	1.0	0.06
	HI	141	0.8	0.7	1.0	0.07	141	0.8	0.7	1.0	0.08	141	0.8	0.7	1.0	0.07
	LM	69	0.7	0.5	0.9	0.01	69	0.8	0.6	1.1	0.15	69	0.8	0.6	1.1	0.15
	CA	86	0.6	0.4	0.9	0.01	86	0.7	0.4	1.0	0.06	86	0.7	0.4	1.0	0.06

Cytokine	Stimulant	Dataset1					Dataset2					Dataset3				
		DS1(n)	GMR	95%CI LCI	95%CI UCI	p-value	DS2(n)	GMR	95%CI LCI	95%CI UCI	p-value	DS3(n)	GMR	95%CI LCI	95%CI UCI	p-value
IL-8	BCG	129	1.0	0.9	1.3	0.70	129	1.0	0.9	1.3	0.70	129	1.0	0.9	1.3	0.70
	MTBK	98	1.2	0.9	1.7	0.27	98	1.2	0.9	1.8	0.24	98	1.2	0.9	1.8	0.24
	LPS	147	1.0	0.8	1.3	0.94	147	1.0	0.7	1.4	0.96	147	1.0	0.7	1.4	0.90
	PEPG	146	0.9	0.7	1.1	0.37	146	0.9	0.7	1.1	0.40	142	0.9	0.7	1.1	0.39
	Pam3	128	1.1	0.8	1.5	0.68	128	1.0	0.7	1.5	0.89	127	1.1	0.8	1.7	0.48
	R848	150	0.9	0.7	1.2	0.70	150	1.0	0.7	1.3	0.77	150	1.0	0.7	1.3	0.85
	SA	98	0.9	0.7	1.3	0.67	98	0.9	0.7	1.3	0.70	98	0.9	0.7	1.3	0.70
	SP	74	0.8	0.7	1.1	0.17	74	0.9	0.7	1.2	0.64	74	0.9	0.7	1.2	0.64
	GBS	113	1.0	0.8	1.3	0.95	113	1.0	0.8	1.3	0.95	113	1.0	0.8	1.3	0.95
	EC	157	1.0	0.8	1.2	0.81	157	1.1	0.8	1.4	0.58	157	1.1	0.8	1.4	0.56
	HI	141	1.0	0.8	1.2	0.92	141	1.0	0.8	1.3	0.99	141	1.0	0.8	1.3	0.97
	LM	69	0.7	0.5	1.1	0.11	69	0.8	0.5	1.3	0.48	69	0.8	0.5	1.3	0.48
	CA	86	0.8	0.5	1.1	0.15	86	0.8	0.5	1.2	0.24	85	0.8	0.5	1.3	0.36
MIF	BCG	129	0.9	0.8	1.0	0.06	129	0.9	0.7	1.0	0.06	129	0.9	0.7	1.0	0.06
	MTBK	98	0.9	0.8	1.0	0.09	98	0.9	0.7	1.0	0.11	98	0.9	0.7	1.0	0.11
	LPS	147	1.0	0.9	1.1	0.81	147	1.0	0.8	1.2	0.87	147	1.0	0.8	1.2	0.88
	PEPG	146	1.0	0.9	1.1	0.42	146	1.0	0.8	1.1	0.66	146	1.0	0.8	1.1	0.66
	Pam3	128	0.9	0.8	1.1	0.29	128	0.9	0.7	1.2	0.39	128	1.0	0.8	1.2	0.93
	R848	150	1.0	0.9	1.0	0.28	150	1.0	0.8	1.2	0.74	150	1.0	0.8	1.2	0.80
	SA	98	1.0	0.9	1.1	0.76	98	1.0	0.8	1.3	0.70	98	1.0	0.8	1.3	0.70
	SP	74	1.0	0.9	1.1	0.72	74	1.1	0.9	1.3	0.60	74	1.1	0.9	1.3	0.60
	GBS	113	1.1	0.9	1.2	0.32	113	1.1	0.9	1.3	0.43	113	1.1	0.9	1.3	0.43
	EC	157	1.1	1.0	1.1	0.25	157	1.1	0.9	1.3	0.39	157	1.1	0.9	1.3	0.36
	HI	141	1.0	0.9	1.1	0.56	141	1.0	0.8	1.2	0.95	141	1.0	0.8	1.2	0.94
	LM	69	0.9	0.8	1.0	0.07	69	1.0	0.8	1.3	0.94	69	1.0	0.8	1.3	0.94
	CA	86	1.0	0.9	1.1	0.88	86	1.0	0.8	1.3	0.74	86	1.0	0.8	1.3	0.74
IL-1β	BCG	129	0.9	0.8	1.1	0.38	129	0.9	0.8	1.1	0.39	128	0.9	0.8	1.1	0.34
	MTBK	98	1.1	0.6	1.8	0.76	98	1.1	0.6	1.8	0.81	97	1.1	0.6	1.9	0.75
	LPS	147	0.9	0.6	1.3	0.46	147	0.9	0.6	1.4	0.68	145	0.9	0.6	1.3	0.67
	PEPG	146	0.9	0.7	1.1	0.27	146	0.9	0.7	1.2	0.39	144	0.9	0.7	1.1	0.29
	Pam3	128	1.0	0.7	1.4	0.91	128	1.0	0.7	1.5	0.95	117	1.1	0.8	1.6	0.54
	R848	150	0.8	0.6	1.2	0.30	150	0.9	0.6	1.2	0.43	150	0.9	0.6	1.2	0.40
	SA	98	0.8	0.5	1.1	0.16	98	0.8	0.5	1.1	0.16	95	0.9	0.6	1.2	0.41
	SP	74	1.0	0.7	1.4	0.98	74	1.0	0.7	1.4	0.90	74	1.0	0.7	1.4	0.90
	GBS	113	1.0	0.7	1.3	0.75	113	1.0	0.7	1.3	0.83	109	0.9	0.7	1.3	0.69
	EC	157	1.1	0.8	1.4	0.68	157	1.1	0.9	1.4	0.44	150	1.0	0.8	1.4	0.80
	HI	141	0.9	0.7	1.2	0.63	141	1.0	0.7	1.3	0.81	136	1.0	0.7	1.3	0.74
	LM	69	0.9	0.6	1.2	0.40	69	0.9	0.6	1.3	0.45	69	0.9	0.6	1.3	0.45
	CA	86	0.8	0.4	1.5	0.48	86	0.8	0.4	1.5	0.49	81	0.8	0.5	1.3	0.42
IL-6	BCG	129	1.0	0.8	1.2	0.77	129	1.0	0.8	1.2	0.97	129	1.0	0.8	1.2	0.97
	MTBK	98	1.1	0.6	1.8	0.74	98	1.1	0.7	1.9	0.63	98	1.1	0.7	1.9	0.63
	LPS	147	0.9	0.7	1.3	0.66	147	1.0	0.7	1.3	0.97	147	1.0	0.7	1.4	0.96
	PEPG	146	0.8	0.6	1.0	0.02	146	0.8	0.7	1.0	0.04	147	0.8	0.7	1.0	0.04
	Pam3	128	0.9	0.5	1.6	0.80	126	1.0	0.6	1.8	0.96	126	1.0	0.6	1.6	0.98
	R848	150	0.7	0.6	0.9	0.01	150	0.8	0.6	1.0	0.03	150	0.8	0.6	1.0	0.05
	SA	98	0.7	0.4	1.1	0.09	98	0.7	0.4	1.1	0.12	98	0.7	0.4	1.1	0.12
	SP	74	0.9	0.7	1.3	0.73	74	1.0	0.7	1.4	0.80	74	1.0	0.7	1.4	0.80
	GBS	113	0.9	0.7	1.3	0.69	113	1.0	0.7	1.4	0.91	113	1.0	0.7	1.4	0.91
	EC	157	0.9	0.7	1.1	0.22	157	0.9	0.7	1.1	0.38	157	0.9	0.7	1.1	0.40
	HI	141	0.8	0.6	1.1	0.14	141	0.9	0.7	1.1	0.25	141	0.9	0.7	1.1	0.25
	LM	69	0.7	0.4	1.2	0.17	69	0.7	0.4	1.3	0.27	69	0.7	0.4	1.3	0.27
	CA	86	0.6	0.3	1.5	0.26	84	0.6	0.2	1.5	0.29	86	0.9	0.5	1.7	0.73
TNF-α	BCG	129	0.9	0.7	1.1	0.35	129	0.9	0.7	1.1	0.37	129	0.9	0.7	1.1	0.37
	MTBK	98	0.9	0.6	1.4	0.67	98	0.9	0.6	1.4	0.69	98	0.9	0.6	1.4	0.69
	LPS	147	1.2	0.9	1.6	0.14	147	1.2	0.9	1.6	0.31	147	1.2	0.9	1.6	0.26
	PEPG	146	1.0	0.8	1.2	0.89	146	1.0	0.8	1.2	0.64	145	1.0	0.8	1.2	0.67
	Pam3	128	1.0	0.8	1.4	0.74	127	1.0	0.7	1.3	0.89	127	1.0	0.8	1.4	0.78
	R848	150	0.8	0.5	1.3	0.42	150	0.8	0.5	1.3	0.30	120	0.9	0.5	1.4	0.52
	SA	98	0.9	0.6	1.3	0.49	98	0.8	0.6	1.2	0.37	98	0.8	0.6	1.2	0.37
	SP	74	1.0	0.7	1.3	0.94	74	1.0	0.7	1.4	0.92	74	1.0	0.7	1.4	0.92
	GBS	113	1.1	0.9	1.5	0.34	113	1.1	0.8	1.5	0.64	113	1.1	0.8	1.5	0.64
	EC	157	1.0	0.9	1.2	0.71	157	1.0	0.8	1.2	0.95	156	1.0	0.8	1.2	0.89
	HI	141	1.0	0.8	1.2	0.83	141	0.9	0.7	1.2	0.50	141	0.9	0.7	1.2	0.52
	LM	69	0.9	0.6	1.2	0.41	69	0.9	0.6	1.3	0.54	69	0.9	0.6	1.3	0.54
	CA	86	0.8	0.5	1.4	0.52	86	0.8	0.5	1.4	0.40	86	0.8	0.5	1.4	0.40
IFN-γ	BCG	129	1.9	0.9	4.0	0.10	66	1.8	0.8	4.0	0.12	26	1.0	0.5	2.0	0.99
	MTBK	98	1.5	0.8	2.7	0.16	28	1.4	0.7	2.6	0.32	7	0.1	0.0	0.7	0.02
	LPS	147	1.2	0.6	2.6	0.56	78	1.4	0.7	3.1	0.37	44	0.8	0.5	1.4	0.52
	PEPG	146	0.7	0.3	1.4	0.33	117	0.8	0.4	1.5	0.43	88	0.7	0.5	1.0	0.04
	Pam3	128	1.2	0.7	2.0	0.51	40	1.4	0.8	2.4	0.29	10	0.6	0.1	2.5	0.40
	R848	150	0.8	0.3	1.8	0.52	139	0.8	0.3	1.9	0.61	133	0.8	0.5	1.3	0.36
	SA	98	0.8	0.3	1.9	0.56	50	0.8	0.3	2.1	0.66	26	1.2	0.7	2.1	0.46
	SP	74	1.0	0.4	2.5	0.95	65	1.0	0.4	2.5	0.93	44	1.0	0.7	1.6	0.86
	GBS	113	1.3	0.6	3.2	0.50	75	1.6	0.6	3.8	0.32	46	0.5	0.3	1.0	0.04
	EC	157	1.2	0.7	2.3	0.51	92	1.4	0.7	2.9	0.29	47	0.7	0.4	1.2	0.19
	HI	141	1.6	0.8	3.1	0.19	73	1.8	0.9	3.7	0.12	32	1.0	0.5	2.0	0.91
	LM	69	1.4	0.5	3.7	0.47	32	1.6	0.6	4.3	0.38	11	0.6	0.3	1.4	0.22
	CA	86	0.8	0.4	1.6	0.47	26	0.8	0.4	1.7	0.56	5	0.2	0.1	1.2	0.06

Supplementary Table 5

Supplementary data Table 2: Univariate analysis of the effect of BCG vaccination and maternal BCG vaccination on neonatal cytokine response to toll like receptor ligands and a selection of heterologous antigens. Total (n) refers to the number of stimulations included in the analysis. GMR= Geometric Means Ratio. Staistically significant values (p<0.05) are depicted in red. GMR >1.0 indicates the effect was stronger in BCG vaccinated subjects and infants whose mothers' were BCG-vaccinated. BCG= Bacille Calmette Guerin, MTBK= Killed Mycobacterium Tuberculosis, LPS = Lipopolysaccharide, PEPG = peptidoglycan, PamCys3=(S)-(2,3-bis(palmitoyloxy)-(2-RS)-propyl)-N-palmitoyl-(R)-Cys-(S)-Ser-(S)-Lys4OH,trihydrochloride, R848= resiquimod, SA= *S. aureus*, SP= *S. pneumoniae*, GBS= Group B Streptococcus, EC= *E.coli*, HI= *H. influenzae*, LM= *L. monocytogenes*, CA= *C. albicans*.

Supplementary data Table 3: **Interaction analyses looking for a differential effect on cytokine expression following BCG vaccination between (i) boys and girls (ii)** infants born by vaginal delivery compared with infants born by Cesarean section (iii) infants whose mothers are BCG vaccinated compared with infants whose mothers **are non BCG vaccinated**. Total (n) refers to the number of stimulations included in the analysis. GMR= Geometric Means Ratio. Statistically significant values are depicted in red (p<=0.05) and those approaching significance p<=0.07 in orange). BCG= Bacille Calmette Guerin, MTBK= Killed Mycobacterium Tuberculosis, LPS = Lipopolysaccharide, PEPG = peptidoglycan, PamCys3=(S)-(2,3-bis(palmitoyloxy)-(2-RS)-propyl)-N-palmitoyl-(R)-Cys-(S)-Ser-(S)-Lys4OH,trihydrochloride, R848= resiquimod, SA= *S .aureus,* SP= *S. pneumoniae,* EC= *E. coli,* HI= *H. influenzae*, LM= *L. monocytogenes,* CA= *C. albicans,* GBS= Group B Streptococcus, GAS= Group A streptococcus.

Supplementary Figure 2: Relationship between timing of randomisation (early BCG = randomisation < 48 hours, late BCG= randomisation >48 hours) and a) age at BCG vaccination, b) age at blood sample and c) time to blood sample.

Supplementary data Table 4: **Univariate analysis of the effect of BCG vaccination based on the timing of randomisation. Early BCG = randomised < 48 hours of life, Late BCG vaccination > 48 hours of life on neonatal cytokine response to toll like receptor ligands and a selection of heterologous antigens.** BCG= Bacille Calmette Guerin, MTBK= Killed Mycobacterium Tuberculosis, LPS = Lipopolysaccharide, PEPG = peptidoglycan, PamCys3=(S)-(2,3-bis(palmitoyloxy)-(2-RS)-propyl)-N-palmitoyl-(R)-Cys-(S)-Ser-(S)-Lys4OH,trihydrochloride, R848= resiquimod, SA= *S. aureus*, SP= *S. pneumoniae*, GBS= Group B Streptococcus, EC= *E.coli*, HI= *H. influenzae*, LM= *L. monocytogenes*, CA= *C. albicans*.

Supplementary data Table 5: **Sensitivity analyses for the effect of BCG vaccination. Dataset 1= Complete dataset. Dataset 2= Complete dataset following removal of values assigned at the lower limit of detection. Dataset 3= Complete dataset following removal of values assigned at the lower limit of detection and values extrapolated by the Luminex Xmap software at both the upper and lower ends of the curve**. Total (n) refers to the number of stimulations included in the analysis. GMR= Geometric Means Ratio. Staistically significant values (p<0.05) are depicted in red, those approaching significance (p<0.07) are shown in orange to illustrate where trends are maintained. GMR >1.0 indicates the effect was stronger in BCG vaccinated subjects.