Supplementary Materials and Methods

Ethics and study samples

Human participation in this research was according to the U.S. Department of Health and Human Services and Good Clinical Practice guidelines. This included protocol approval by the Leiden University Medical Center Ethics Committee and written informed consent by all donors. Anonymous buffy coats from healthy blood bank donors were only used if donors had consented scientific use of blood products. Specifically, healthy control donor samples were *in vitro* PPD negative healthy buffy coats obtained from Sanquin blood transfusion services, The Netherlands. The group of healthy donors was supplemented with healthy Dutch donors prior to BCG vaccination, after extensive testing of their mycobacterially naïve status. These donors were subsequently BCG vaccinated and analysed at 4, 8, 12 weeks and 1 year post BCG vaccination; the BCG vaccination study was approved with protocol number P12.087 [1]. A longitudinal follow up study of individuals with latent TB infection (LTBI) was also run at LUMC and approved under protocol number P07.048 [2]. In addition, a cohort with known long-term TB latency, and age-matched uninfected controls was recruited in Norway after approval by the Regional Committees for Medical and Health Research Ethics in Norway (protocol number P027/99) [3, 4].

In addition, we obtained specific permission (protocol number B16.002) from the ethical review board at Leiden University Medical Center to use archived samples remaining after completion of previous studies, in particular contact investigations at a soccer club (original protocol number 136/97)[5], in a supermarket (original protocol number P05.053)[6-8] and amongst immigrants with a recent TB contact (original protocol number P04.114)[9, 10].

Finally, BCG vaccination studies in healthy male, volunteers were executed in Nijmegen, approved by the Arnhem-Nijmegen Medical Ethical Committee with protocol number NL50160.092.24.

Experimental design

Archived PBMCs were thawed, rested and counted. Cells were split for BCG-based MGIA as well as direct stimulation with live BCG for flowcytometry including intracellular cytokine staining. Remaining PBMCs were dispensed in Tri-zol reagens for later RNA isolation. Supernatants were collected after 4 days of co-culture in the MGIA system. Upon analysis of MGIA data as well as flowcytometry data, cytokine and chemokine measurements were initiated and functional experiments were designed.

BCG

BCG (Pasteur strain) was grown in Middlebrook 7H9 medium supplemented with 10% ADC (BD Biosciences), log phase bacteria were frozen in aliquots in glycerol (Sigma-Aldrich). The number of viable bacilli per ml was determined by plating serial dilutions of bacteria on Middlebrook 7H10 agar plates supplemented with 10% OADC (BD Biosciences) and counting of visible colonies after 3 weeks. When aliquots were thawed for infection of PBMCs they were first washed three times with PBS/Tween80 (0.05%) (Sigma-Aldrich), and were left to set for a minute to remove big clumps from the suspension.

MGIA

The EC Fp7 project Euripred optimized the protocol for human MGIA assays [11], we have utilized the optimized 'in tube' MGIA Euripred protocol in our studies.

Cryopreserved PBMCs were thawed and rested in RPMI (Gibco life sciences, ThermoFisher Scientific Inc., Bleiswijk, the Netherlands) supplemented with glutamax (Gibco) and 10% FBS (Hyclone, ThermoFisher Scientific Inc.) (=R10 medium) at a concentration of $2x10^6$ cells/ml for 2 hours in the presence of benzonase (10U/ml, Merck, Amsterdam, the Netherlands). After resting, cells were washed with R10 medium and counted with a Casy Cellcounter (Roche, Woerden, the Netherlands). $1x10^6$ PBMC's were co-cultured for 4 days in RPMI supplemented with glutamax and 10% inactivated

pooled human serum with 2.6 log CFU (+/- 0.36 SD) BCG P3 on a rotator in a 37 ^{0}C humidified CO_2 incubator in a final volume of 600 µl. All samples were run in duplicates. After 4 days, 100 µl supernatant was harvested and stored for future analysis and the remaining 500 µl per sample were transferred to a PANTA/Enrichment supplemented MGIT tube (Becton Dickinson, Erembodegem, Belgium) and placed in a Bactec 900 system (BD) until time to positivity (TTP) was reached. All tubes included in the analysis were checked visually for mycobacterial growth. Samples reaching positivity within 100 hours were considered contaminated and thus deleted as false positive, since the inoculum of 2.6 log CFU BCG is expected to reach TTP after more than 250 hours.

As a control for the BCG inoculum all experiments included a serial dilution (10^7 to 10^2) of the BCG stock for time to positivity in PANTA/Enrichment supplemented MGIT tubes and plating on Middlebrook 7H10 agar plates, supplemented with 10% OADC (BD) for CFU determination. When all dilutions showed colonies on the 7H10 plates, plates were scanned on a Canon Scanner 9000F and colonies were counted using the ImageJ software. CFU's were converted to log CFU and plotted against the TTP. Linear regression analysis was applied (GraphpadPrism software v7) and all samples were transposed and data are plotted as log CFU.

For the inhibition of mycobacterial outgrowth the same experimental setup as described above was used with the daily addition of the CXCR3 antagonist (NBI-74330, Tocris Bioscience, Bristol, United Kingdom) at a final concentration of 1 µM. After 4 days of incubation, samples were transferred to a supplemented MGIT tube and placed into the Bactec900 system.

To identify subsets responsible for the observed BCG outgrowth control, cells were separated and combined before initiation of the BCG infection experiment. Briefly, PBMCs were thawed and $CD14^+$ monocytes were enriched by magnetic bead separation using CD14 beads (Miltenyi Biotec, Leiden, The Netherlands), followed by positive selection of $CD3^+$ T-cells using CD3 beads (Milteny Biotec) on the CD14negative fraction. For each donor, PBMCs (1x10e6) were compared to isolated monocytes (150.000), and monocytes (150.000) + 0,5x10e6 T-cells or monocytes + 1x10e6 T-cells. Cells were

combined into sarstedt tubes before addition of BCG, the remainder of the procedure was identical as described above.

Flowcytometric analysis

$1x10^6$ rested PBMC's were incubated with medium only or with $1x10^6$ CFU BCG for 6 hours after which Brefeldin A (3 µg/ml, Sigma-Aldrich, Zwijndrecht, The Netherlands) was added and samples were incubated for another 16 hours. After incubation cells were washed with PBS/0.1% BSA (Pharmacy LUMC, The Netherlands)/ (Sigma-Aldrich) and stained with Vivid live-dead stain (Invitrogen, ThermoFisher Scientific Inc.) for 10 minutes at 4 ^{0}C followed by staining with surface markers CD4-APC-H7 (clone RPA-T4), CD8-AlexaFluor700 (clone RPA-T8), CD3-Brilliant Violet510 (clone UCHT-1), CD45RA-PE-Cy5 (clone MEM-56) and CCR7-Brilliant Violet605 (Clone 3D12) (all BD Biosciences), CD19 PE-TexasRed (clone HIB19) and CD14-Qdot655 (clone 61D3) (both Ebiosciences, ThermoFisher Scientific Inc.) for 30 minutes at 4 ^{0}C. Cells were washed, fixed with fixation buffer A (fix/perm reagents Nordic MuBio, ITK, Uithoorn, The Netherlands) for 15 minutes at RT. After fixation cells were washed again and stained for the cytokines and chemokines IFN-γ-PerCP-Cy5.5 (clone 4S.B3) (Biolegend), IL-2-FITC (clone 5.344.111), TNF-α-PE-Cy7 (clone Mab11), IL-13-APC (clone JES10-5A2) and CXCL10-PE (clone 6D4/D6/G2) (all BD Biosciences) in permeabilization buffer B. After incubation for 30 minutes at room temperature (RT), cells were washed and resuspended in 1% paraformaldehyde and measured within 24 hours after staining on a LSRFortessa (BD Biosciences) using Diva software v6.2. Analysis was done using Flowjo v9.7.3 (Treestar Inc., Ashland, OR, USA) and a minimum of $7.5x10^5$ PBMC's were analysed.

For further analysis of the monocyte population also CD163-PerCP-C5.5 (clone GHI/61),CCR2-APC (clone 48607), CCR5-APC-H7 (clone 2D7/CCR5) and CX3CR1-Pacific Blue (clone 2A9-1) (all BD Biosciences) were used as surface markers but staining was performed at 37°C to prevent temperature based changes in, and thereby low detection of, the chemokine receptors.

Real-time Quantitative RT-PCR

For all PBMC samples that had cells left after the initial MGIA and flowcytometry experiments were initiated, remaining cells were directly transferred to Tri-zol reagent (Qiagen, Venlo, the Netherlands). Total RNA was extracted using direct-zol kits, including an on-column DNA digestion, according to instructions of the manufacturer (Zymo-research, Baseclear, Leiden, The Netherlands). Total RNA was reverse transcribed using oligo(dT) 12-18 primers (Thermofisher Scientific, Bleiswijk, The Netherlands) and superscript IV enzymes (Thermofisher).

Real-time quantitative RT-PCR was performed with Taqman technology and TaqMan universal PCR master mix (Thermofisher) using the following primers and probes were used: CXCR3-A: FAM probe, 5-TGAGTGACCACCAAGTGCTAAATGACGC-3; forward 5-ACCCAGCAGCCAGAGCACC-3; reverse 5-TCATAGGAAGAGCTGAAGTTCTCCA-3. CXCR3-B: FAM probe, 5-CCCGTTCCCGCCCTCACAGG-3; forward 5-TGCCAGGCCTTTACACAGC-3; reverse 5-TCGGCGTCATTTAGCACTTG-3 based on [12]. GAPDH was used for normalization using VIC labelled probes in both PCR reactions (Thermofisher). Taqman PCR was run on a Quantstudio 6 Flex (Applied Biosystems) with QuantStudio Real Time PCR v1.1 software and data expressed as the difference in Ct values between GAPDH and CXCR3A/B. Undetectable signals for CXCR3A were set at a Ct of 40 to allow calculation of the ΔCt value. Ratios were determined by dividing the ΔCt of CXCR3A by that of CXCR3B.

BCG-induction of trained immunity

To assess induction of trained immunity by BCG vaccination, 30 healthy male subjects (age: 19-37 years) were randomly assigned to receive either BCG (SSI, Denmark) or placebo (the diluent used to dissolve BCG) in a double-blind fashion. Blood was drawn before BCG/placebo and 4 weeks later. PBMCs were isolated by density centrifugation of Ficoll-Paque (GE healthcare, UK) . Cells were washed twice in PBS and resuspended in RPMI culture medium (Roswell Park Memorial Institute

medium; Invitrogen, CA, USA) supplemented with 50 μ g/mL gentamicin, 2mM Glutamax (Gibco), and 1mM pyruvate (Gibco). 5x10^6/mL cells in 100 μ L/well were added to 96 well plates, and cells were incubated for 24h with RPMI, sonicated *Mycobacterium tuberculosis* H37Rv (5 μ g/mL), heat-killed *Candida albicans* (1x10^6/mL, strain UC820) or *Staphylococcus aureus* (1x10^6/mL clinical isolate). Supernatants were collected after 24h and stored at -20^{o}C until further analysis. The study was approved by the Arnhem-Nijmegen Medical Ethical Committee, NL50160.092.24.

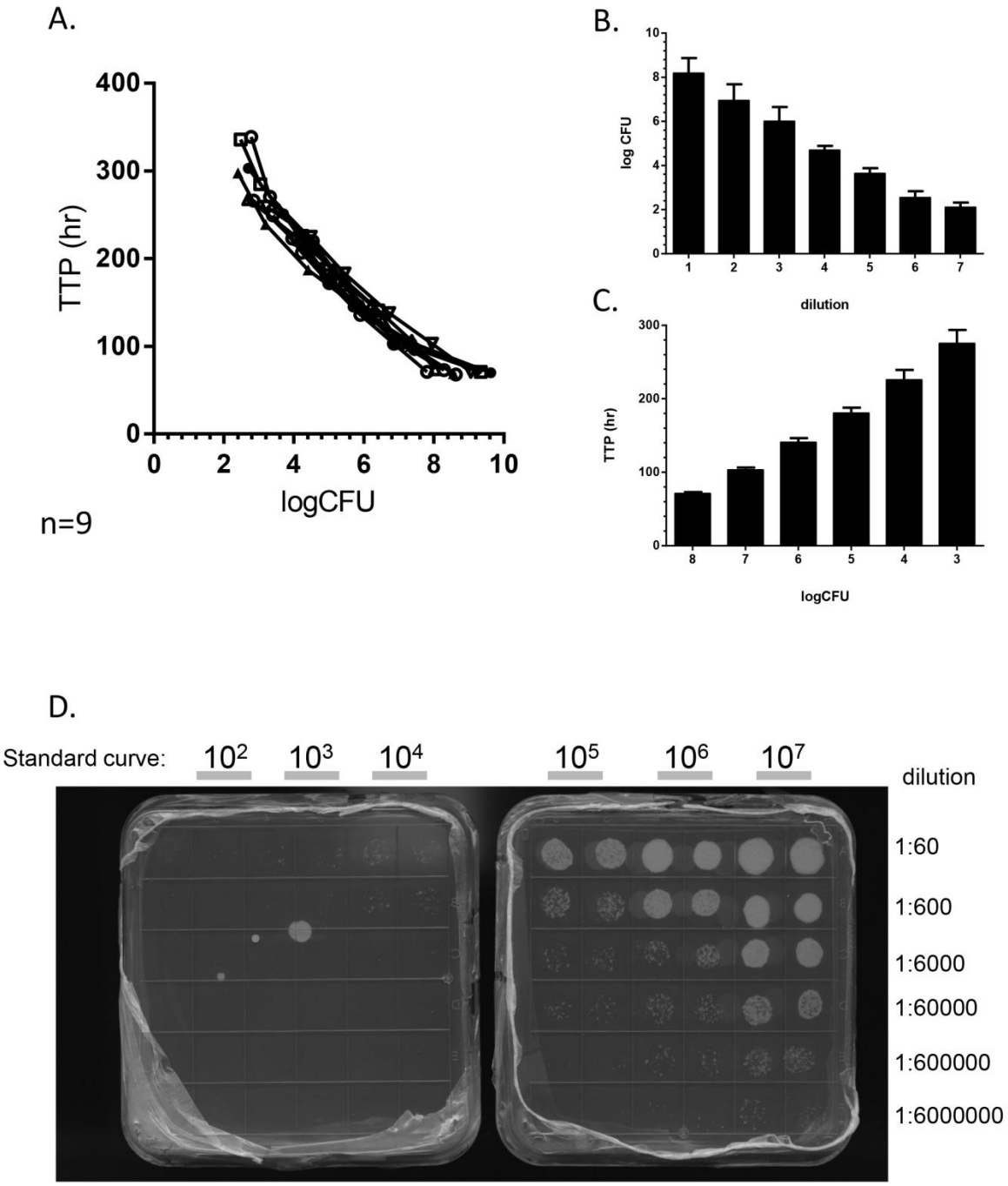
Luminex®

A 40-plex chemo-cytokine Luminex® assay was performed according to manufacturer's instructions (Bio-Rad, Veenendaal, The Netherlands) on BCG culture supernatants and on supernatants of the trained immunity experiment.

Statistical analysis

All data were analysed assuming a non-Gaussian distribution and therefore non-parametric testing was applied. All legends contain detailed information on the statistical tests applied and the number of individuals included for analysis. Generally, when comparing groups Kruskal-Wallis or Wilcoxon-paired rank tests were applied depending on the number of groups in the equation. For the analysis of longitudinal data an ANOVA with Friedman test was applied. Correlations were based on linear regression modelling. All analyses were performed using Graph Pad prism version 7.00, Graph Pad Software Inc.

Supplementary Figure 1



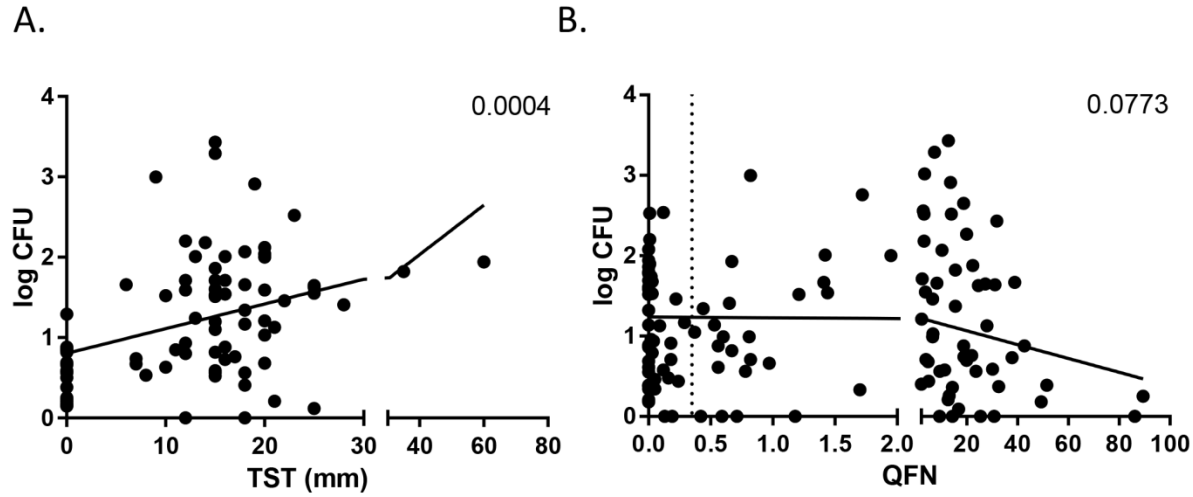
Supplementary Figure 1: **Reproducibility of standard curves in MGIT system using frozen standardized batch of BCG**

In all MGIA assays BCG was used from a single frozen stock. In each individual assay one vial was thawed using serial washes with Tween80 and diluted to generate standard curves. Dilutions were

added to BACTEC MGIT tubes and incubated in the BACTEC machine until positive (TTP). Simultaneously, all dilutions were plated on 7H10 and colony forming units were counted after 14-21 days.

A. Relation between CFU and TTP for the 9 standard curves used in this study.
B. CFU of the different dilutions of the standard curve; plotted are the mean + SD CFU for each condition out of 9 experiments.
C. Mean + SD CFU out of 9 experiments vs. TTP for all dilutions of the standard curve.
D. Scan of CFU plate of MGIA standard curve, diluted 10-fold. All samples are spotted in duplicates. CFU are determined by counting spots in highest countable dilution using high-resolution scanned images and ImageJ software.

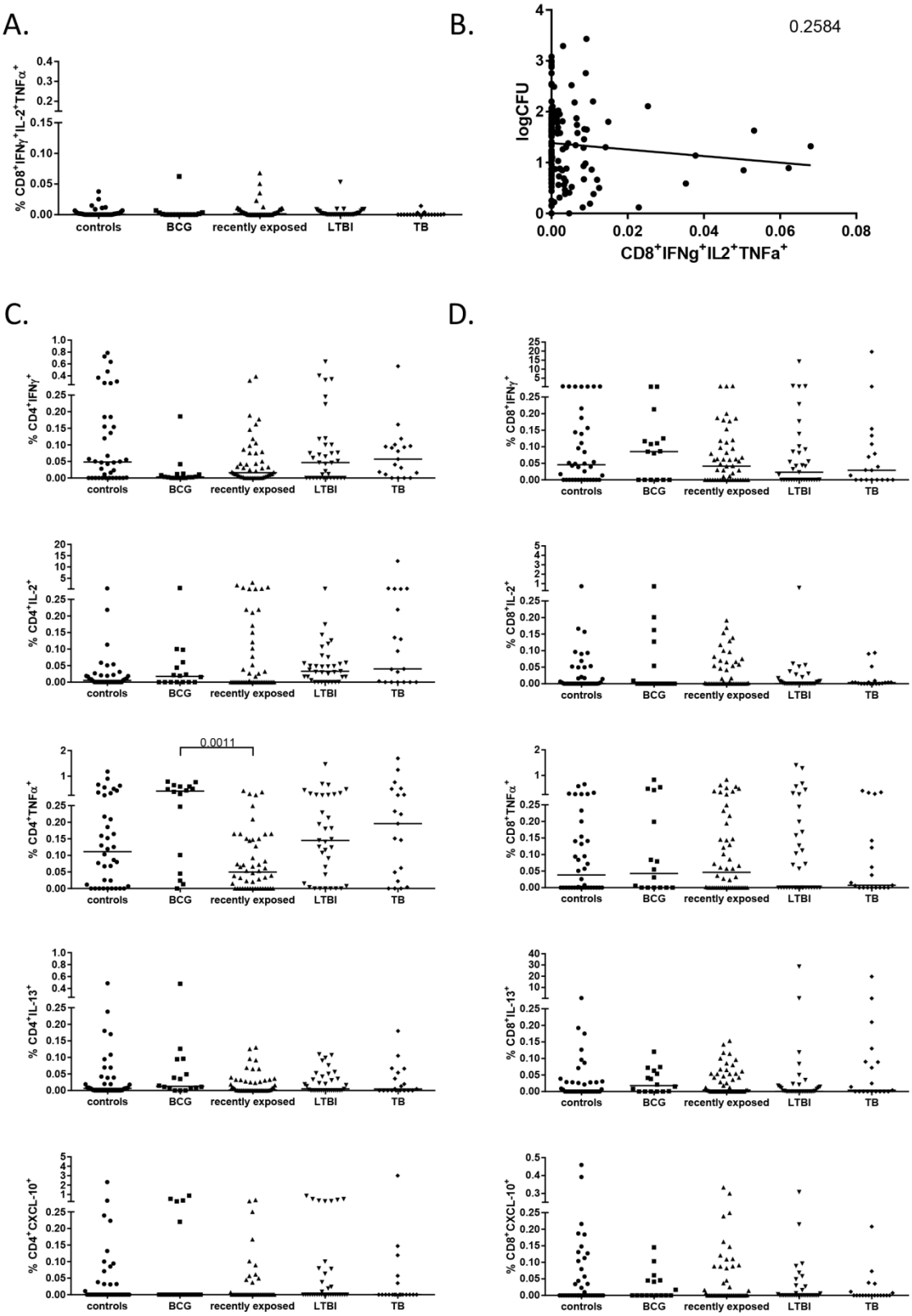
Supplementary Figure 2

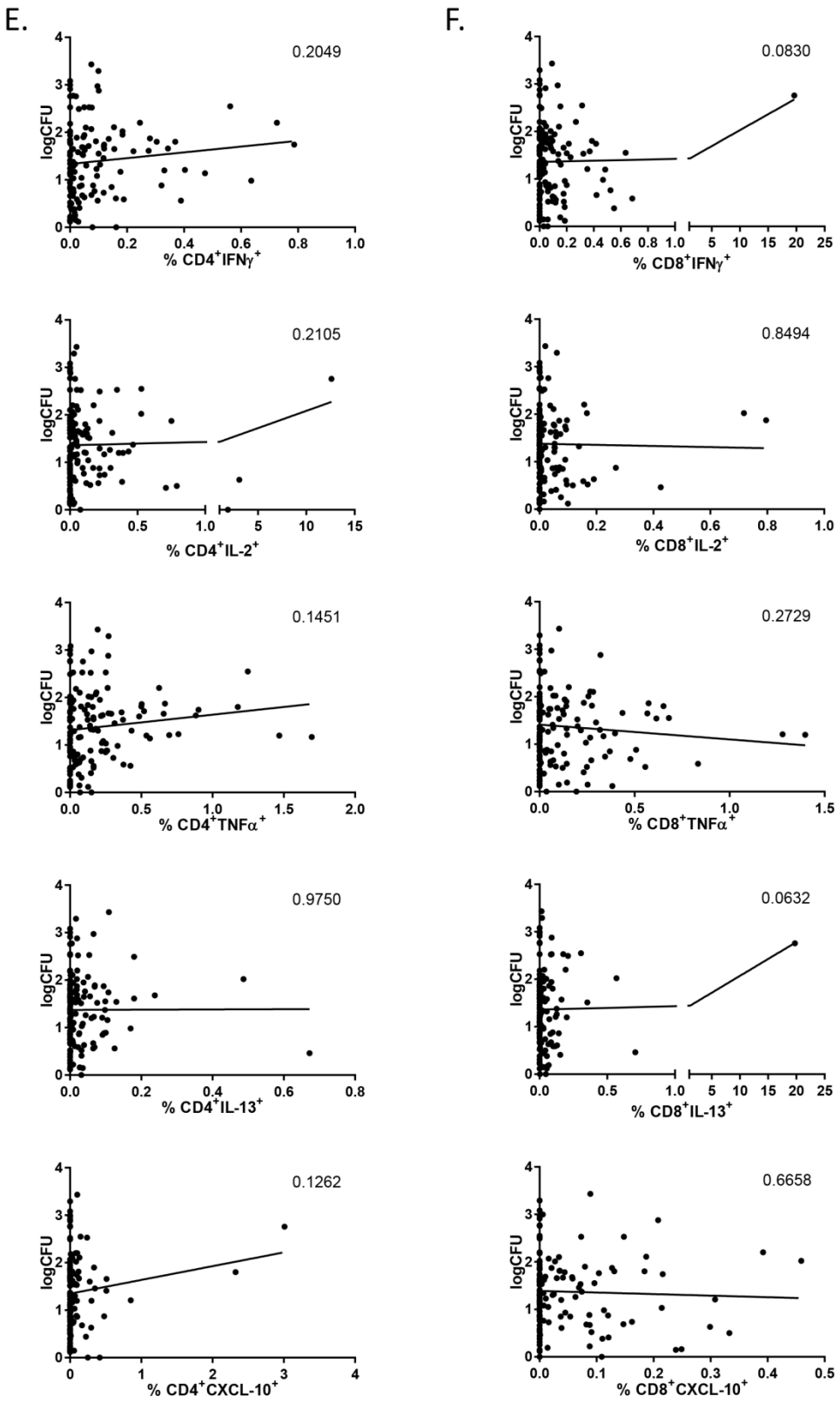


Supplementary Figure 2: **Capacity to control BCG outgrowth correlates with TST but not QFN test results**

Combining all results from all cohorts, mycobacterial growth is indicated in log CFU on the Y-axis and TST in mm (panel A, n=81) or QFN IFN-γ production in IU/ml (panel B, n=125) on the X-axis. Associations were determined using linear regression modelling.

Supplementary Figure 3



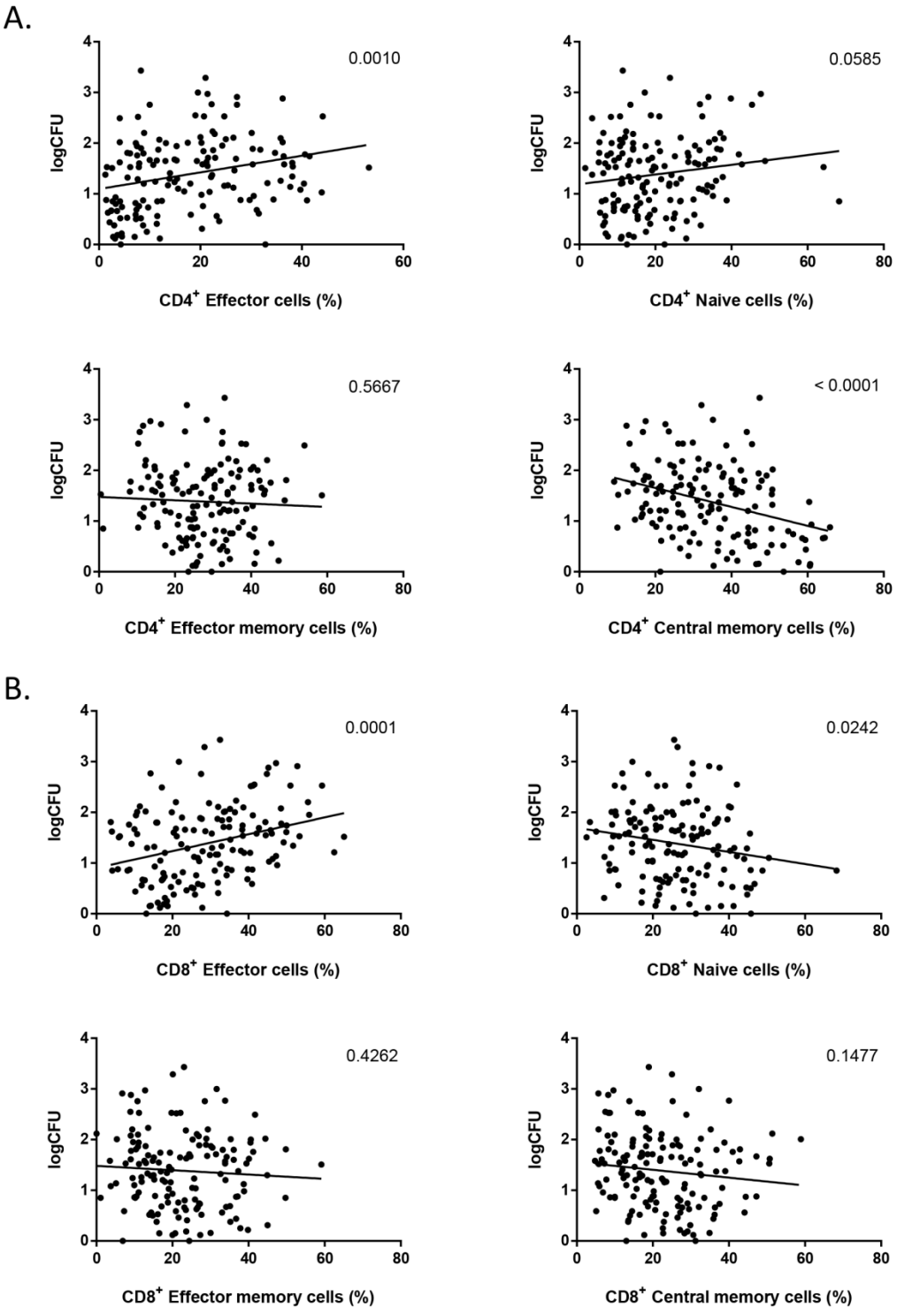


Supplementary Figure 3: **CD4 or CD8 T-cell cytokine production do not correlate with capacity to control mycobacterial outgrowth**

PBMCs were infected with live BCG, rotated for 4 days and samples incubated in MGIT tubes in the BACTEC machine. Antibody marker staining for FACS analysis was performed after 16 hours of BCG stimulation in the presence of BFA. Associations were determined using linear regression modelling. Controls n=38, BCG n=16, exposed n=50, LTBI n=35, TB n=19.

A. $CD8^+$ multifunctional T-cells (IFN-γ^+ TNF-α^+ IL-2^+) are hardly detectable in clinical groups.
B. The frequency of $CD8^+$ multifunctional T-cells does not correlate with the capacity to control mycobacterial outgrowth (n=142).
C. $CD4^+$ T-cells producing single cytokines (IFN-γ, IL-2, TNF-α, IL-13 or IL-10) are not different across the clinical groups, only $CD4^+$ T-cell TNF-α production is significantly higher in BCG vaccinated donors compared to recently exposed individuals.
D. $CD8^+$ T-cells producing single cytokines (IFN-γ, IL-2, TNF-α, IL-13 or IL-10) are not different across the clinical groups.
E. $CD4^+$ T-cells producing single cytokines (IFN-γ, IL-2, TNF-α, IL-13 or IL-10) do not correlate with the capacity to control mycobacterial outgrowth (n=144).
F. $CD8^+$ T-cells producing single cytokines (IFN-γ, IL-2, TNF-α, IL-13 or IL-10) do not correlate with the capacity to control mycobacterial outgrowth (n=144).

Supplementary Figure 4

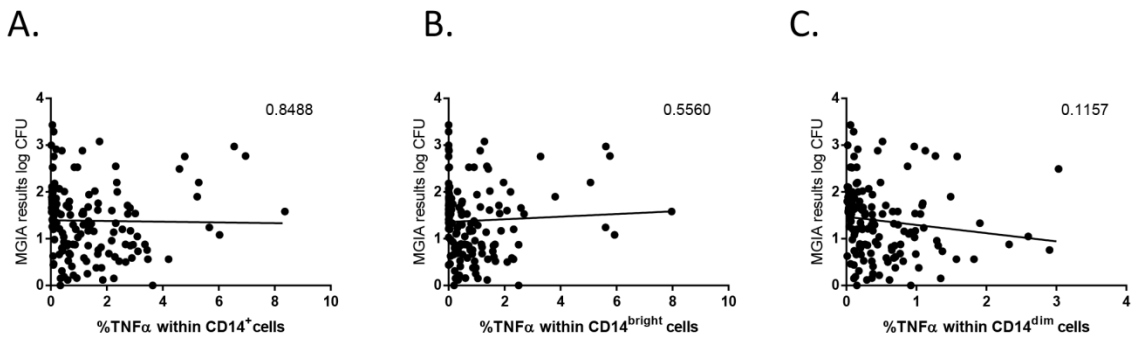


Supplementary Figure 4: **Central memory and effector T-cell populations correlate with capacity to control mycobacterial outgrowth**

PBMCs were infected with live BCG, rotated for 4 days and incubated in MGIT tubes. FACS staining was performed after 16 hours of BCG stimulation. Associations were determined using linear regression modelling (n=148).

A. Correlation between $CD4^+$ subsets based on the expression of CD45RA and CCR7 with the capacity to control mycobacterial outgrowth. $CD45RA^+CCR7^+$: naïve cells, $CD45RA^-CCR7^+$: central memory cells, $CD45RA^-CCR7^-$: effector memory cells, $CD45RA^+CCR7^-$: effector cells.
B. Correlation between $CD8^+$ subsets based on the expression of CD45RA and CCR7 with the capacity to control mycobacterial outgrowth. $CD45RA^+CCR7^+$ cells are naïve cells, $CD45RA^-CCR7^+$ cells are central memory cells, $CD45RA^-CCR7^-$ cells are effector memory cells and $CD45RA^+CCR7^-$ cells are effector cells.

Supplementary Figure 5

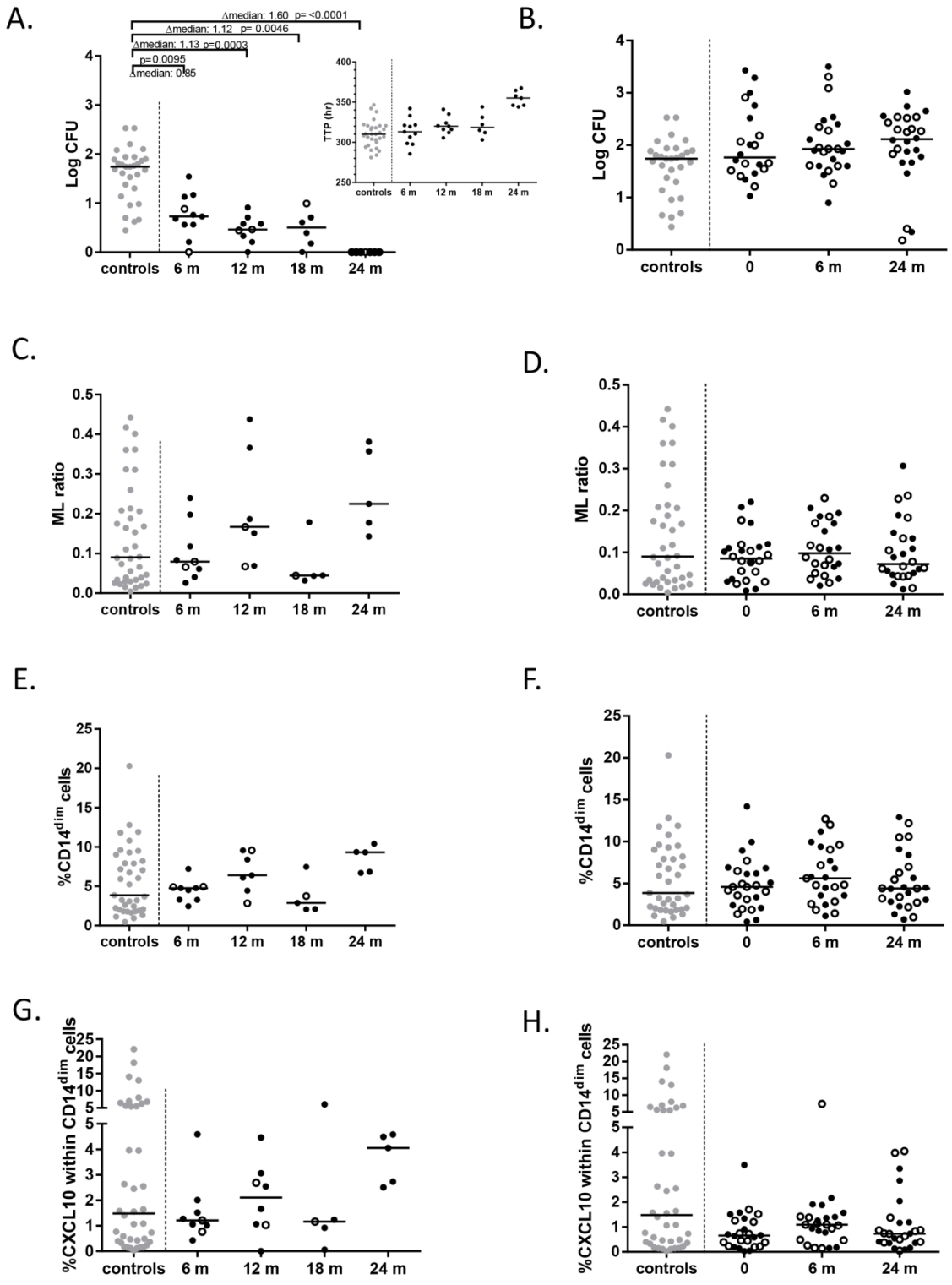


Supplementary Figure 5: **Monocyte TNF-α production does not associate with the capacity to inhibit mycobacterial control**

PBMCs were infected with live BCG, rotated for 4 days and samples incubated in MGIT tubes in the BACTEC machine. FACS staining was performed after 16 hours of negative control stimulation in the presence of BFA. Associations were determined using linear regression modelling (n=144).

A. TNF-α production in the total CD14 population does not correlate with mycobacterial outgrowth control.
B. TNF-α production in the $CD14^{bright}$ population does not correlate with mycobacterial outgrowth control.
C. TNF-α production in the $CD14^{dim}$ population does not correlate with mycobacterial outgrowth control.

Supplementary Figure 6

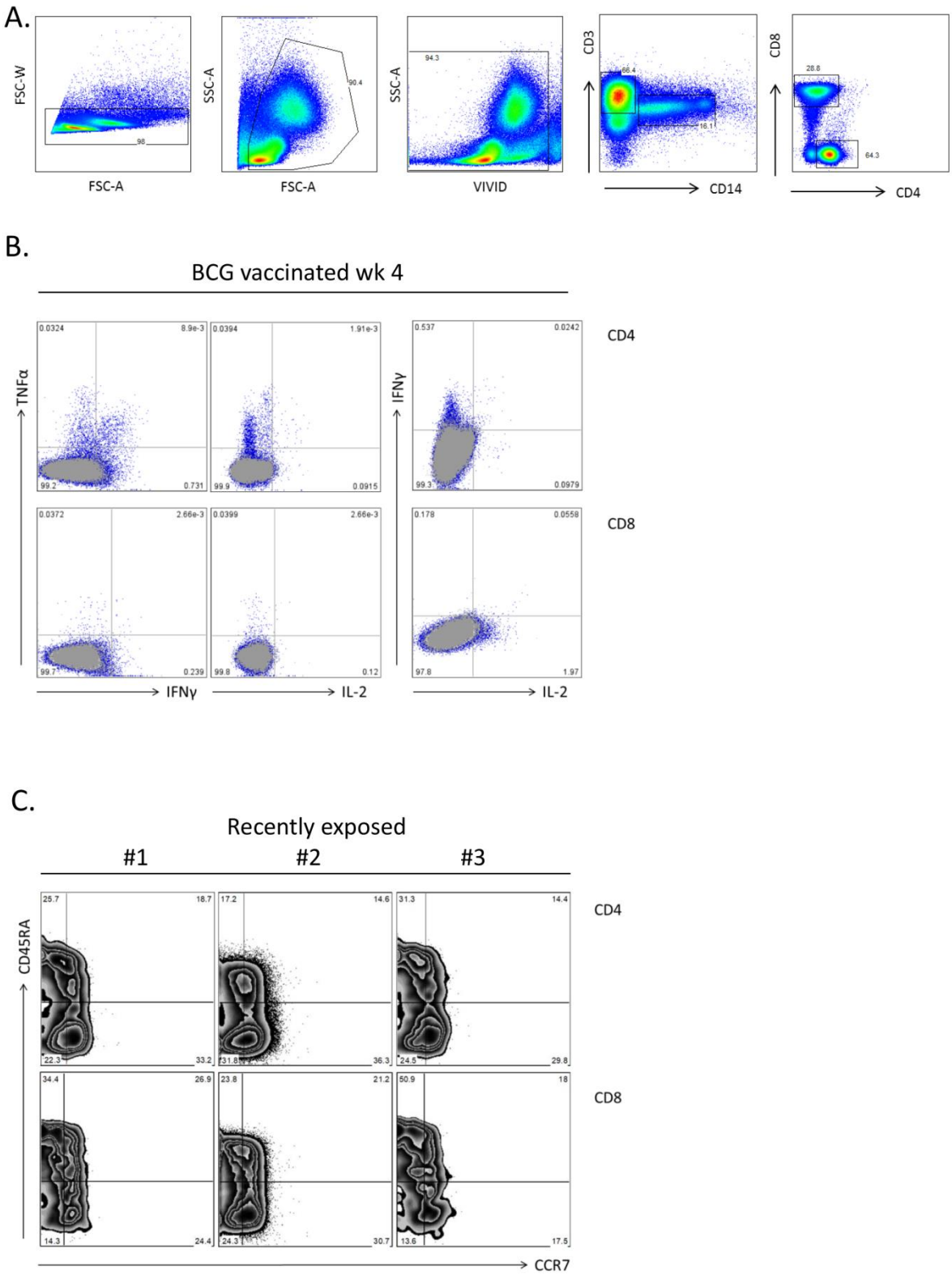


Supplementary Figure 6: **Mycobacterial growth control may increase in early stages following infection**

The Dutch LTBI cohort as well as the contact investigation in the Dutch supermarket both included longitudinal follow up. All samples from the same individual were tested in the same MGIA assay. The control population shown in this graph is the same as used in figure 1C-G and was comprised of healthy Dutch individuals.

A. The contact investigation in the supermarket also included longitudinal follow up at 3-monthly intervals. Only 2 individuals were treated prophylactically (open symbols). All groups had increased BCG outgrowth control compared to the healthy control population. (Kruskal Wallis). The insert shows the actual time to positivity (TTP) in the BACTEC culture to illustrate that although values at 12 months are very low they do not indicate sterile elimination of BCG. Controls n=30, m6 n=11, m12 n=9, m18 n=6, m24 n=7.
B. The LTBI cohort from the Netherlands had samples collected at inclusion (month 0), 6 and 24 months later. Individuals with prophylactic treatment are depicted with open symbols. Controls n=30, m0 n=22, m6 n=25, m24 n=26.
C. Monocyte lymphocyte ratios over different time points of follow up in the supermarket contact investigation. Controls n=30, m6 n=11, m12 n=9, m18 n=6, m24 n=7.
D. Monocyte lymphocyte ratios over different time points of follow up in the LTBI cohort. Controls n=30, m0 n=22, m6 n=25, m24 n=26.
E. The proportion of $CD14^{dim}$ cells at different time points of follow up in the supermarket contact investigation. Controls n=30, m6 n=11, m12 n=9, m18 n=6, m24 n=7.
F. The proportion of CXCL10 producing $CD14^{dim}$ cells at time points of follow up in the LTBI cohort. Controls n=30, m0 n=22, m6 n=25, m24 n=26.
G. The proportion of CXCL10 producing $CD14^{dim}$ cells at different time points of follow up in the supermarket contact investigation. Controls n=30, m6 n=11, m12 n=9, m18 n=6, m24 n=7.
H. The proportion of $CD14^{dim}$ cells at time points of follow up in the LTBI cohort. Controls n=30, m0 n=22, m6 n=25, m24 n=26.

Supplementary Figure 7



Supplementary Figure 7: **gating strategies**

A. Flow cytometry data were analysed using Flow Jo software Initial gating included gating on singlets using FSC-A and W gates, followed by gating on lymphocytes and live cells ($VIVID^-$). T-cells were gated as $CD3^+CD14^-$ cells, followed by CD4 and CD8 gates and myeloid cells were $CD14^+CD3^-$.

B. Cytokine production upon BCG stimulation (blue) compared to unstimulated samples (grey), for CD4 in top row and CD8 in bottom row. Boolean gating was used to analyse T-cell cytokine production capacities according to MIATA guidelines [13].

C. Example of gates used to identify memory T-cell populations based on CD45RA and CCR7 for $CD4^+$ and $CD8^+$ T-cells.

Supplementary Table 1

	MGIA: donors with BCG growth control							
	log CFU				log CFU + Antagonist			
	replicates			mean	replicates			mean
1	1,7	0,28		0,99	1,64	1,7		1,67
2	1,7	1,86		1,78	3,06	2,81		2,94
3	1,92	0,03		0,98	2,81	2,08		2,45
4	0,01	0,37	1,41	0,60	1,95	1,95	2,32	2,07
5	0,48	0,77		0,63	0	2,05		1,03
6	0,71	1,06		0,89	1,76	3,17		2,47
7	1,04	1,07	1,26	1,12	1,62	1,65	2,29	1,85
8	0,71	1,56		1,14	2,29	1,62		1,96
9	1,83	0,92	1,04	1,26	0,86	1,47	1,59	1,31
10	0	2,59		1,30	2,56	1,54		2,05
11	1,92	0,87		1,40	2,5	1,41		1,96

	MGIA: donors without BCG growth control							
	log CFU				log CFU + Antagonist			
	replicates			mean	replicates			mean
1	2,81	2,62		2,715	1,92	2,59		2,26
2	2,56	2,52		2,54	2,27			2,27
3	2,46	2,40		2,43	1,10			1,10
4		2,68		2,68	1,51	0,12		0,82
5	3,00	3,03		3,02	2,37	1,42		1,90
6	2,46	2,14		2,30	1,16	1,39		1,28
7	2,24	2,46		2,35	2,62	2,94		2,78
8	2,27	2,43		2,35	2,30	2,46		2,38
9	2,49	2,40		2,45	2,75	2,46		2,61
10	2,84	2,78		2,81	2,43	1,96		2,20
11	1,74	2,56		2,15	2,17			2,17
12	2,35	2,35		2,35	2,05	1,89		1,97
13	2,11	2,74	2,50	2,45		2,23		2,23
14	2,90	2,05		2,48	2,17	2,44		2,31
15	2,78	2,26		2,52	2,05	1,89	2,29	2,08
16	2,59	2,59		2,59	2,17	1,71		1,94
17	2,47	2,84	2,68	2,66	2,14	2,35		2,25
18	2,65	2,78		2,72	2,68	2,29		2,49
19	2,84	2,71		2,78	2,02	2,08		2,05
20	2,40	2,72		2,56	1,70	1,86		1,78
21	2,08	3,10		2,59		2,62		2,62
22	3,23	0,71		1,97	0,48	1,63		1,06
23	2,69	3,45		3,07	1,57	1,57		1,57
24	1,03	2,21		1,62	1,41	0,71		1,06
25	1,51	1,38		1,45	1,35	1,06		1,21
26	1,54	1,95		1,75	2,27	1,95		2,11
27	2,72	0,96		1,84		1,98		1,98

References in supplementary information

1. Boer, M. C., Prins, C., van Meijgaarden, K. E., van Dissel, J. T., Ottenhoff, T. H. and Joosten, S. A., Mycobacterium bovis BCG Vaccination Induces Divergent Proinflammatory or Regulatory T Cell Responses in Adults. *Clin.Vaccine Immunol.*, 2015. **22**: p. 778-788.
2. de Paus, R. A., van Meijgaarden, K. E., Prins, C., Kamphorst, M. H., Arend, S. M., Ottenhoff, T. H. M. and Joosten, S. A., Immunological characterization of latent tuberculosis infection in a low endemic country. *Tuberculosis* 2017. **106**: p. 62-72.
3. Commandeur, S., Lin, M. Y., van Meijgaarden, K. E., Friggen, A. H., Franken, K. L., Drijfhout, J. W., Korsvold, G. E., et al., Double- and monofunctional CD4(+) and CD8(+) T-cell responses to Mycobacterium tuberculosis DosR antigens and peptides in long-term latently infected individuals. *Eur.J.Immunol.*, 2011. **41**: p. 2925-2936.
4. Commandeur, S., van Meijgaarden, K. E., Lin, M. Y., Franken, K. L., Friggen, A. H., Drijfhout, J. W., Oftung, F., et al., Identification of human T-cell responses to Mycobacterium tuberculosis resuscitation-promoting factors in long-term latently infected individuals. *Clin Vaccine Immunol*, 2011. **18**: p. 676-683.
5. Arend, S. M., Engelhard, A. C., Groot, G., de Boer, K., Andersen, P., Ottenhoff, T. H. and van Dissel, J. T., Tuberculin skin testing compared with T-cell responses to Mycobacterium tuberculosis-specific and nonspecific antigens for detection of latent infection in persons with recent tuberculosis contact. *Clin Diagn Lab Immunol*, 2001. **8**: p. 1089-1096.
6. Arend, S. M., Thijsen, S. F., Leyten, E. M., Bouwman, J. J., Franken, W. P., Koster, B. F., Cobelens, F. G., et al., Comparison of two interferon-gamma assays and tuberculin skin test for tracing tuberculosis contacts. *Am J Respir Crit Care Med*, 2007. **175**: p. 618-627.
7. Franken, W. P., Arend, S. M., Thijsen, S. F., Bouwman, J. J., Koster, B. F., van Dissel, J. T. and Bossink, A. W., Interferon-gamma release assays during follow-up of tuberculin skin test-positive contacts. *Int J Tuberc Lung Dis*, 2008. **12**: p. 1286-1294.
8. Franken, W. P., Koster, B. F., Bossink, A. W., Thijsen, S. F., Bouwman, J. J., van Dissel, J. T. and Arend, S. M., Follow-up study of tuberculosis-exposed supermarket customers with negative tuberculin skin test results in association with positive gamma interferon release assay results. *Clin Vaccine Immunol*, 2007. **14**: p. 1239-1241.
9. Kik, S. V., Franken, W. P., Arend, S. M., Mensen, M., Cobelens, F. G., Kamphorst, M., van Dissel, J. T., et al., Interferon-gamma release assays in immigrant contacts and effect of remote exposure to Mycobacterium tuberculosis. *Int J Tuberc Lung Dis*, 2009. **13**: p. 820-828.
10. Kik, S. V., Franken, W. P., Mensen, M., Cobelens, F. G., Kamphorst, M., Arend, S. M., Erkens, C., et al., Predictive value for progression to tuberculosis by IGRA and TST in immigrant contacts. *Eur Respir J*, 2010. **35**: p. 1346-1353.
11. www.euripred.eu, E. C.-. Optimization of MGIA assay. *in preparation*, 2017.
12. Lasagni, L., Francalanci, M., Annunziato, F., Lazzeri, E., Giannini, S., Cosmi, L., Sagrinati, C., et al., An alternatively spliced variant of CXCR3 mediates the inhibition of endothelial cell growth induced by IP-10, Mig, and I-TAC, and acts as functional receptor for platelet factor 4. *J Exp Med*, 2003. **197**: p. 1537-1549.
13. Britten, C. M., Janetzki, S., Butterfield, L. H., Ferrari, G., Gouttefangeas, C., Huber, C., Kalos, M., et al., T cell assays and MIATA: the essential minimum for maximum impact. *Immunity.*, 2012. **37**: p. 1-2.