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Supplemental Information

BCG Vaccination Induces Long-Term Functional Reprogramming of Human Neutrophils

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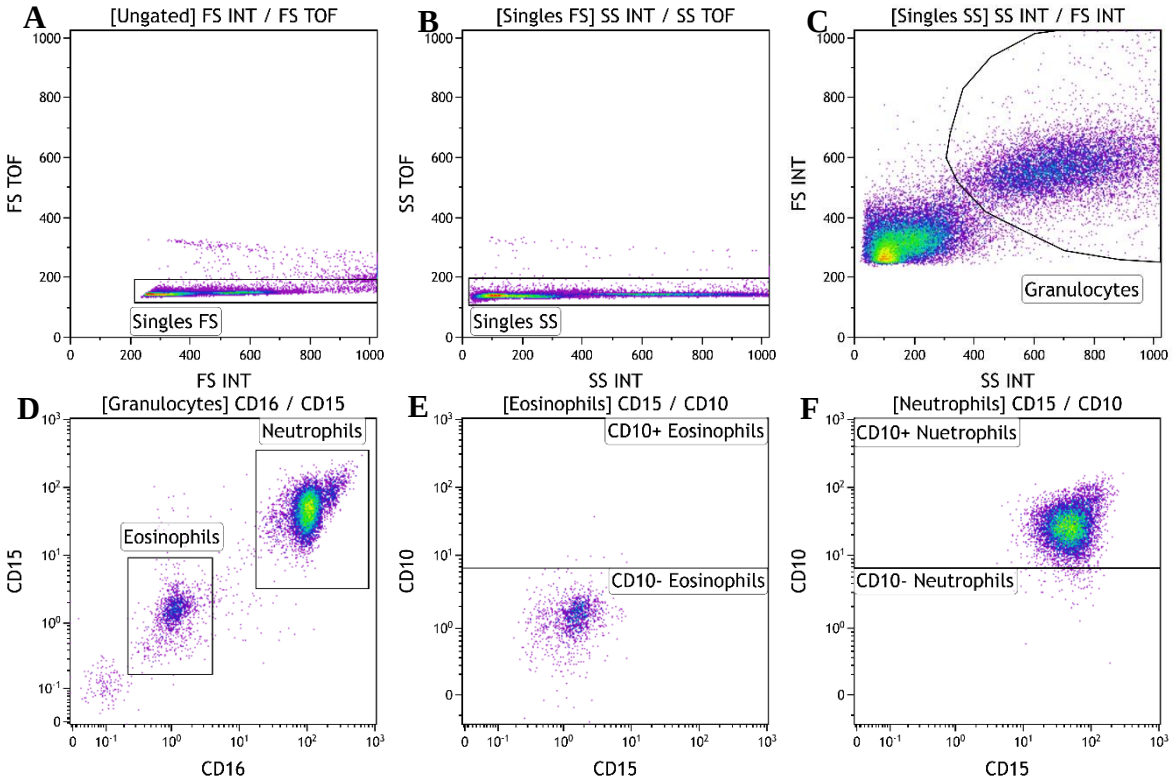


Figure S1, related to Figure 1 – Gating strategy. Total leukocytes were used to characterize the granulocyte population in the circulation of healthy individuals vaccinated with BCG. (A and B) Duplets were excluded and, (C) granulocytes were identified based on their forward and side scatter. (D) Neutrophils were identified by their high expression of CD15 and CD16 and eosinophils by their low expression of these two markers. (E) Eosinophils were used as an internal gating control to identify CD10+ and CD10- cells. (F) The gate for CD10+ and CD10- neutrophils was linked to the eosinophil gate.

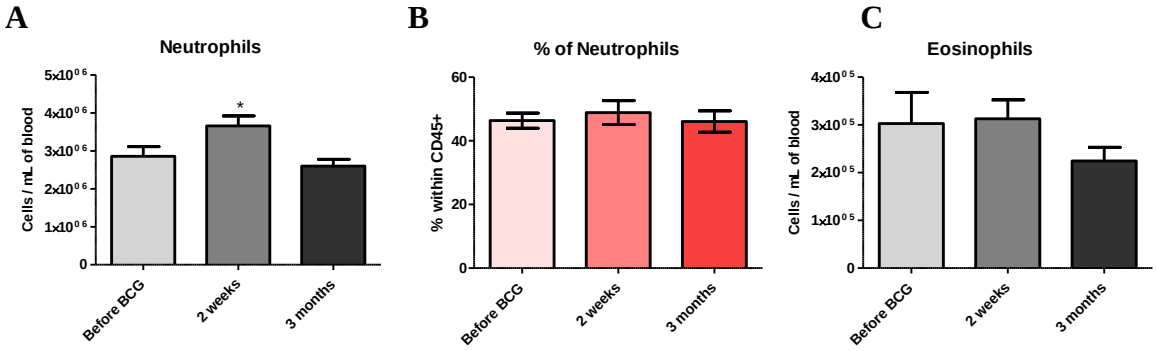


Figure S2, related to Figure 1 – Neutrophil and eosinophil counts in whole blood before and after vaccination. (A) Absolute neutrophil counts in whole blood before, 2 weeks and 3 months after BCG vaccination. (B) Neutrophil percentage was calculated related to the amount of total CD45+ cells. Data is presented as mean ± SEM, n=25, Wilcoxon signed-rank test. (C) Absolute eosinophil counts in whole blood before, 2 weeks and 3 months after BCG vaccination (mean ± SEM, n=25, Wilcoxon signed-rank test).

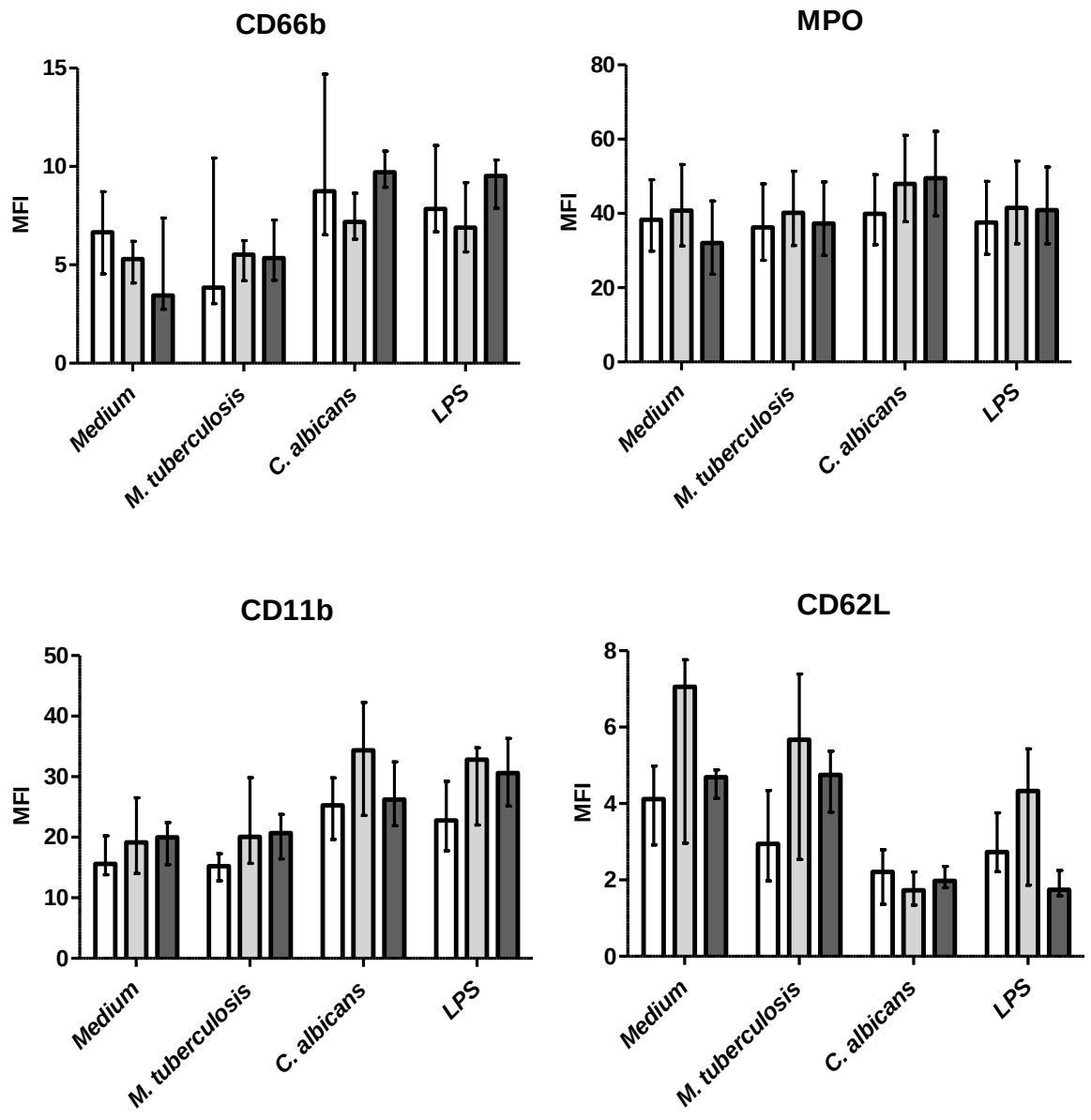


Figure S3, related to Figure 2 – Median fluorescence intensity of each activation marker before and after vaccination. The expression of these four markers was assessed by flow cytometry after 17 hr of culture before BCG vaccination (white bars), after 2 weeks (light gray bars), and after 3 months (dark gray bars). Bars depict median, error bars depict IQR.

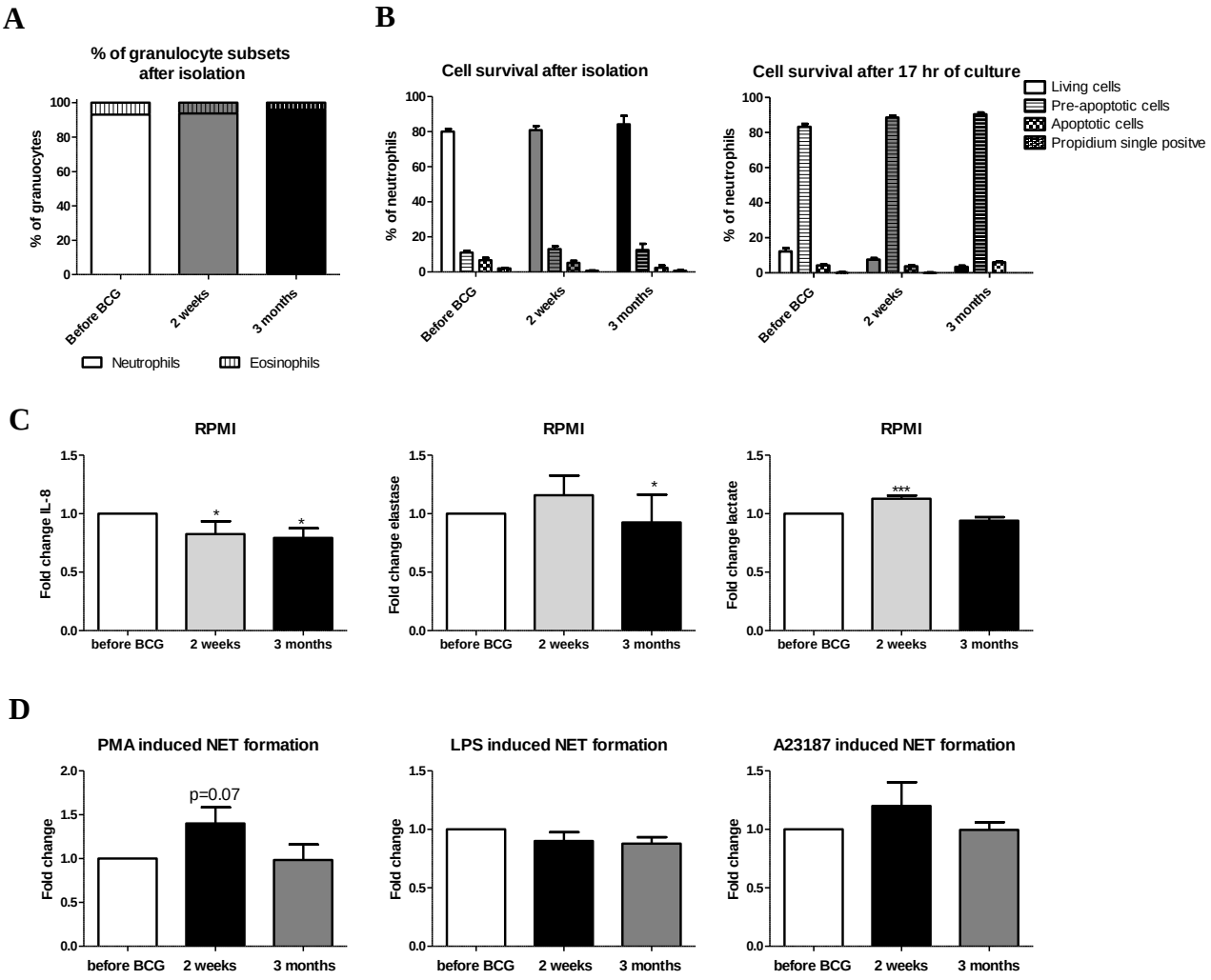


Figure S4, related to Figures 2 and 3 – Cell purity, survival and antimicrobial function. (A) Percentage of neutrophils after isolation from whole blood before and after vaccination. (B) Percentages of live, pre-apoptotic, apoptotic and dead neutrophils after isolation and 17 hr of culture at all 3 timepoints determined by annexin V and PI staining (n=25). (C) Fold change (compared to levels before BCG) in the production of IL-8, elastase and lactate upon incubation with RPMI medium (control) (mean ± SEM, Wilcoxon signed-rank test). (D) Fold change (compared to before BCG) in NET formation upon stimulation with PMA, LPS or A23187, Wilcoxon signed-rank test.

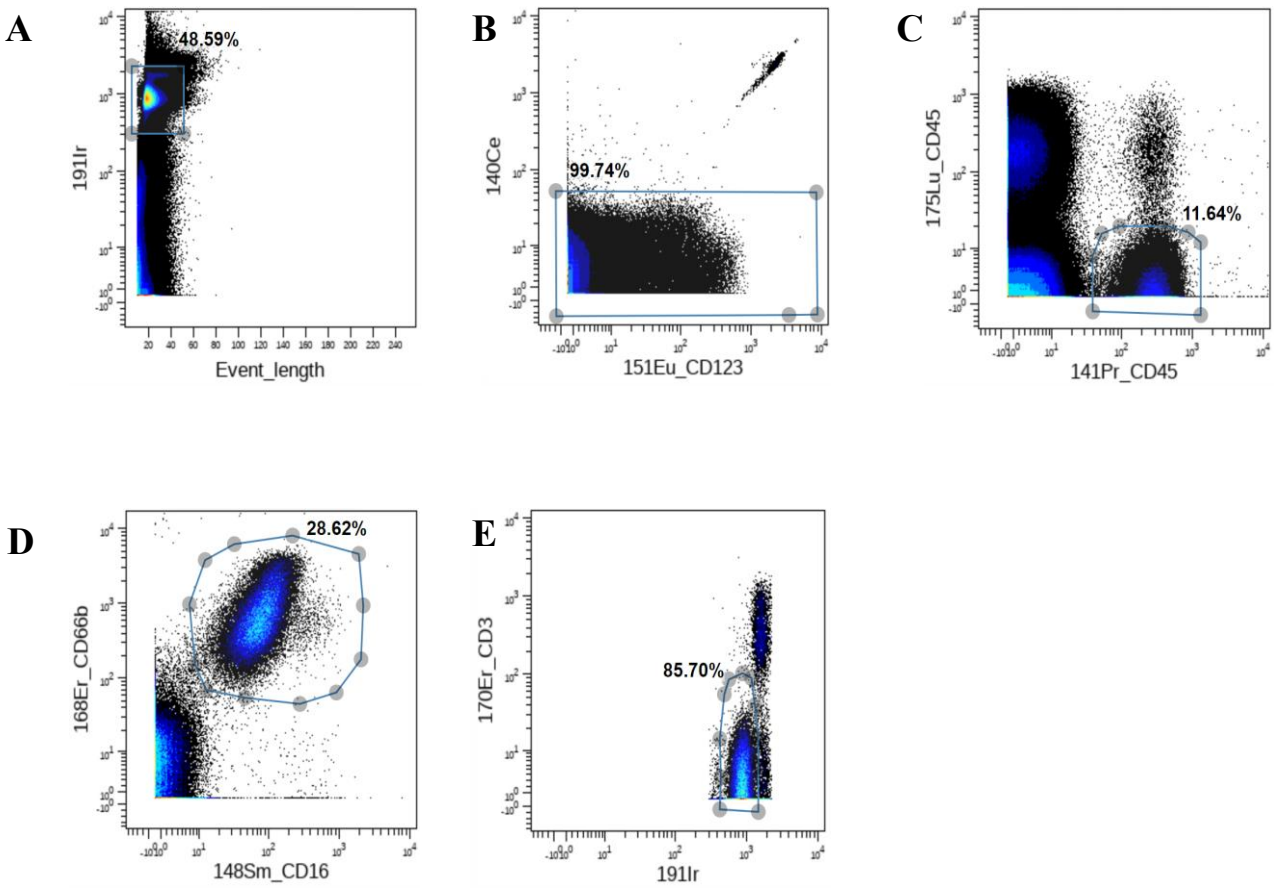


Figure S5, related to Figure 4 – Gating strategy of neutrophils from whole blood for analysis of phosphoproteins. Bivariate flow cytometry plots are shown for a representative volunteer sample. Gating was performed using Cytobank software. The final neutrophil population used for analysis was defined as follows: (A) Cells were identified and cell debris was excluded, (B) beads were excluded, (C) CD45+ barcoded cells were identified and then (D) Neutrophils were defined as the CD66b+ CD16+ population, from which (E) singlets were identified.

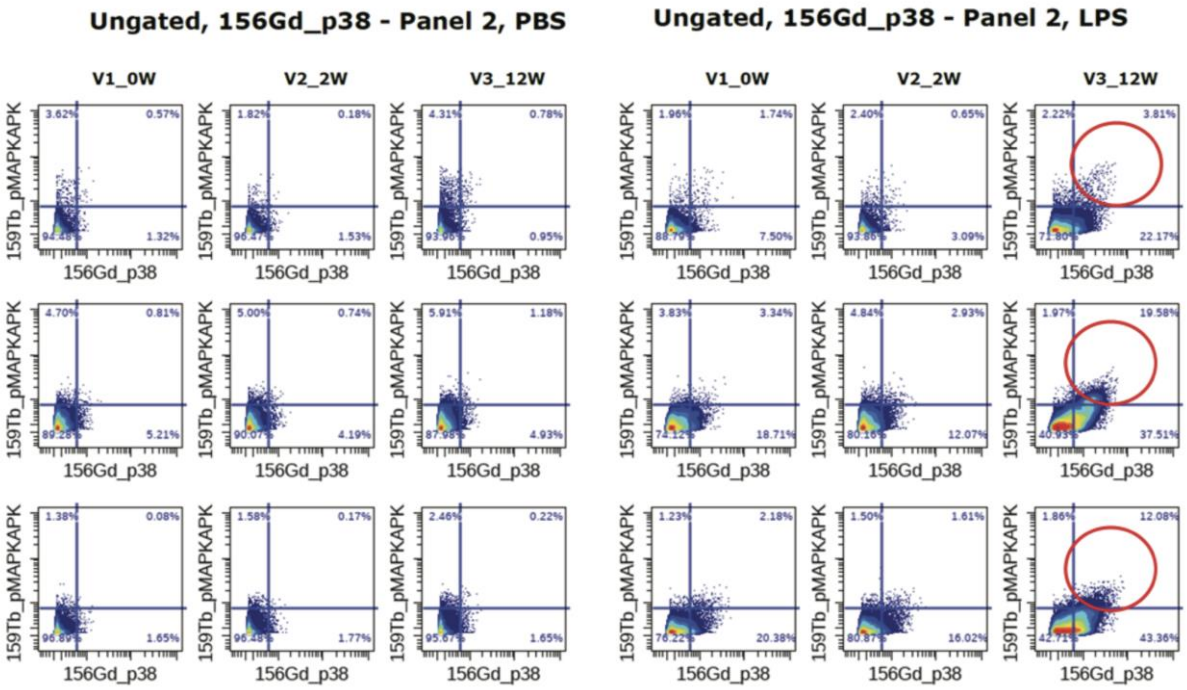


Figure S6, related to Figure 4 – Gating strategy for pMAPKAPK2 and pp38 co-expression. Representative bivariate flow cytometry plots of neutrophils for three donors (rows) at the specified timepoints either unstimulated (left, PBS) or in response to LPS stimulation (right).

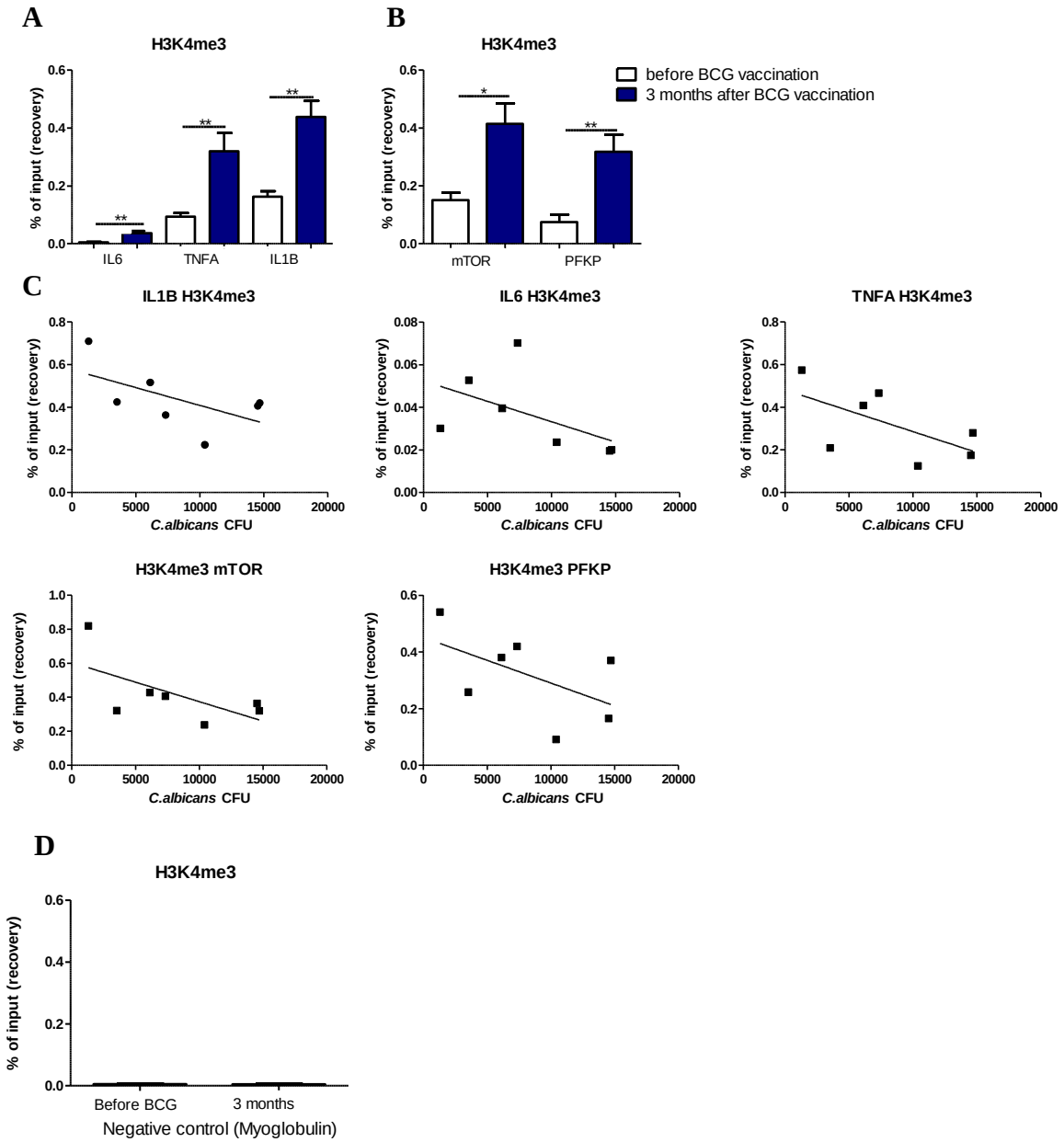
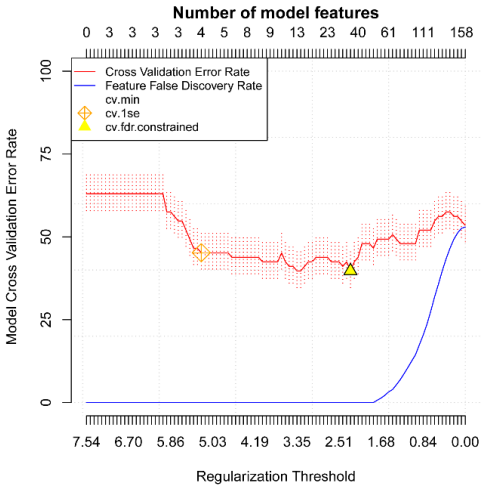


Figure S7, related to Figure 5 – Vaccination with BCG induces chromatin remodeling of neutrophils.
(A) Neutrophils were analyzed by ChIP-qPCR to determine the enrichment of H3K4me3 at the promoters of *IL6*, *TNFA*, *IL1B*, *MTOR* and *PFKP* before BCG vaccination and 3 months after BCG vaccination (mean ± SEM, n=7, *p <0.05, **p <0.01 Wilcoxon signed-rank test). (B) Correlation plots showing the relationship between levels of H3K4me3 and *C. albicans* CFU after 17 hr *ex vivo* incubation with neutrophils. (D) Negative control (myoglobulin) (n=7).

Data S1

A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Feature False Discovery Rate
cv.min
cv.1se
cv.fdr.constrained
Model Cross Validation Error Rate
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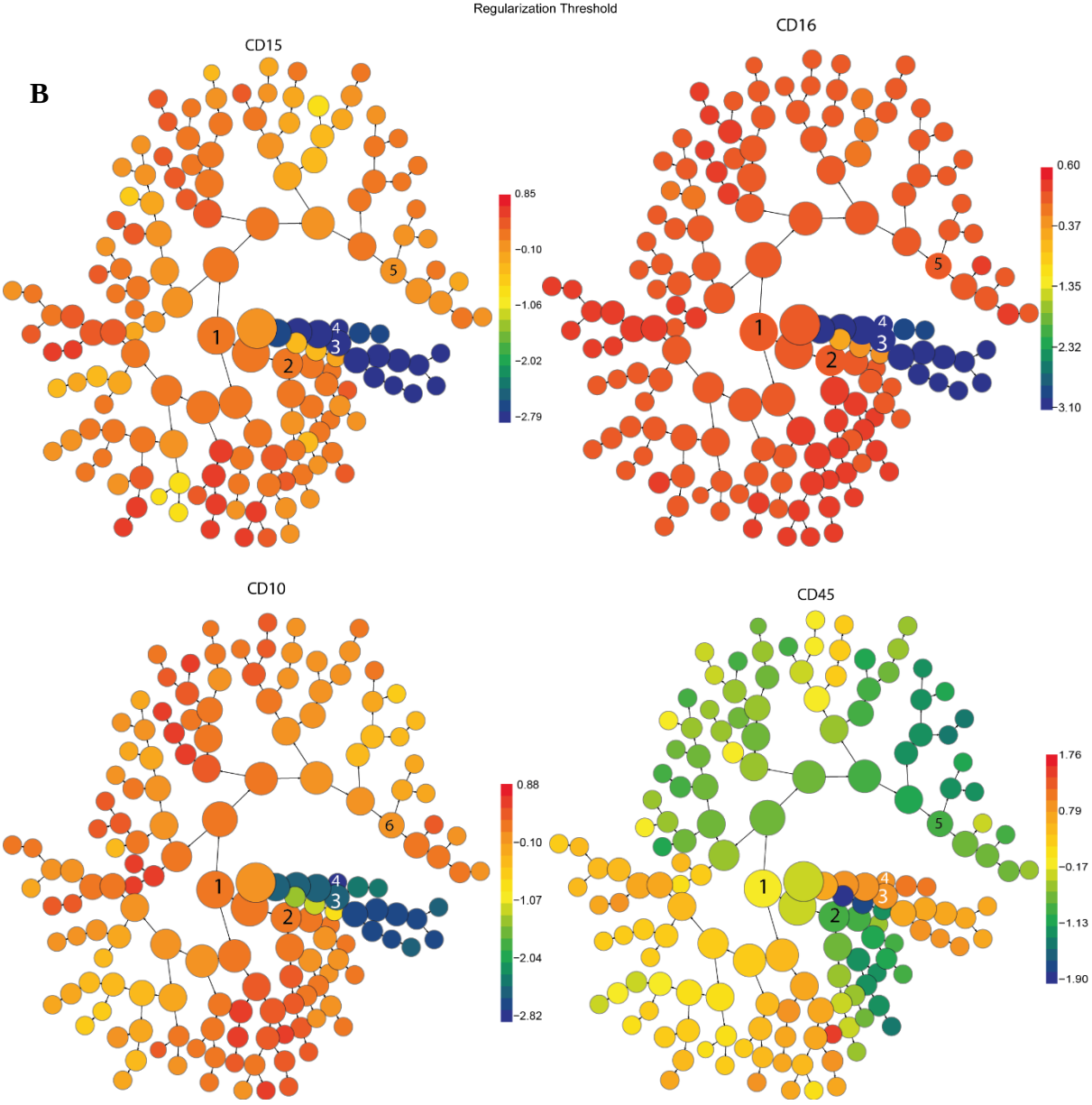
7.54 6.70 5.86 5.03 4.19 3.35 2.51 1.68 0.84 0.00
Regularization Threshold

B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−2.02
−2.79
CD16
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0.37
−1.35
−2.32
−3.10
CD10
0.88
−0.10
−1.07
−2.04
−2.82
CD45
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0.79
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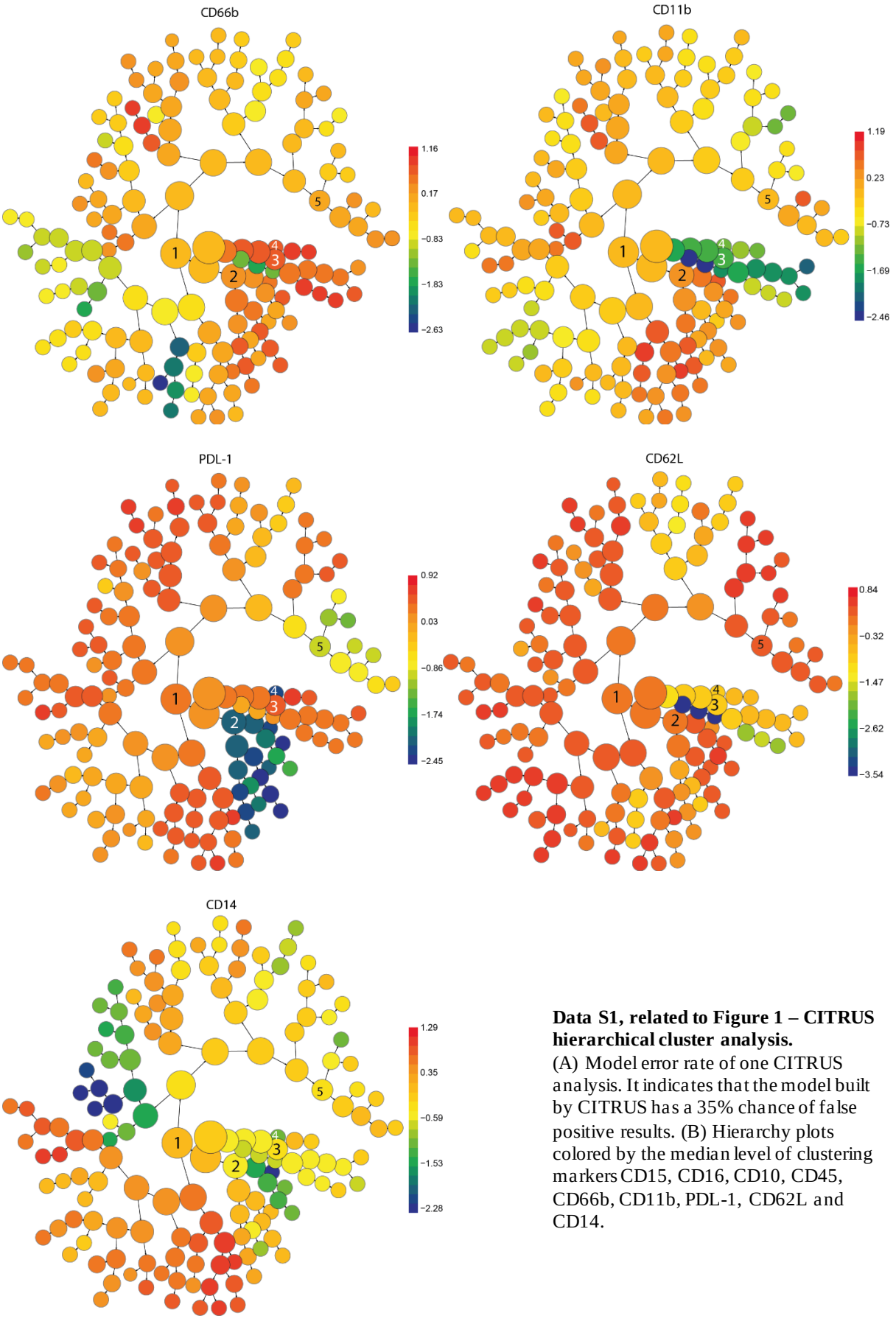
−1.13
−1.90



Data S1, related to Figure 1 – CITRUS hierarchical cluster analysis.
(A) Model error rate of one CITRUS analysis. It indicates that the model built by CITRUS has a 35% chance of false positive results. (B) Hierarchy plots colored by the median level of clustering markers CD15, CD16, CD10, CD45, CD66b, CD11b, PDL-1, CD62L and CD14.