Supporting Information

Glibenclamide reduces primary human monocyte functions against tuberculosis infection by enhancing M2 polarization

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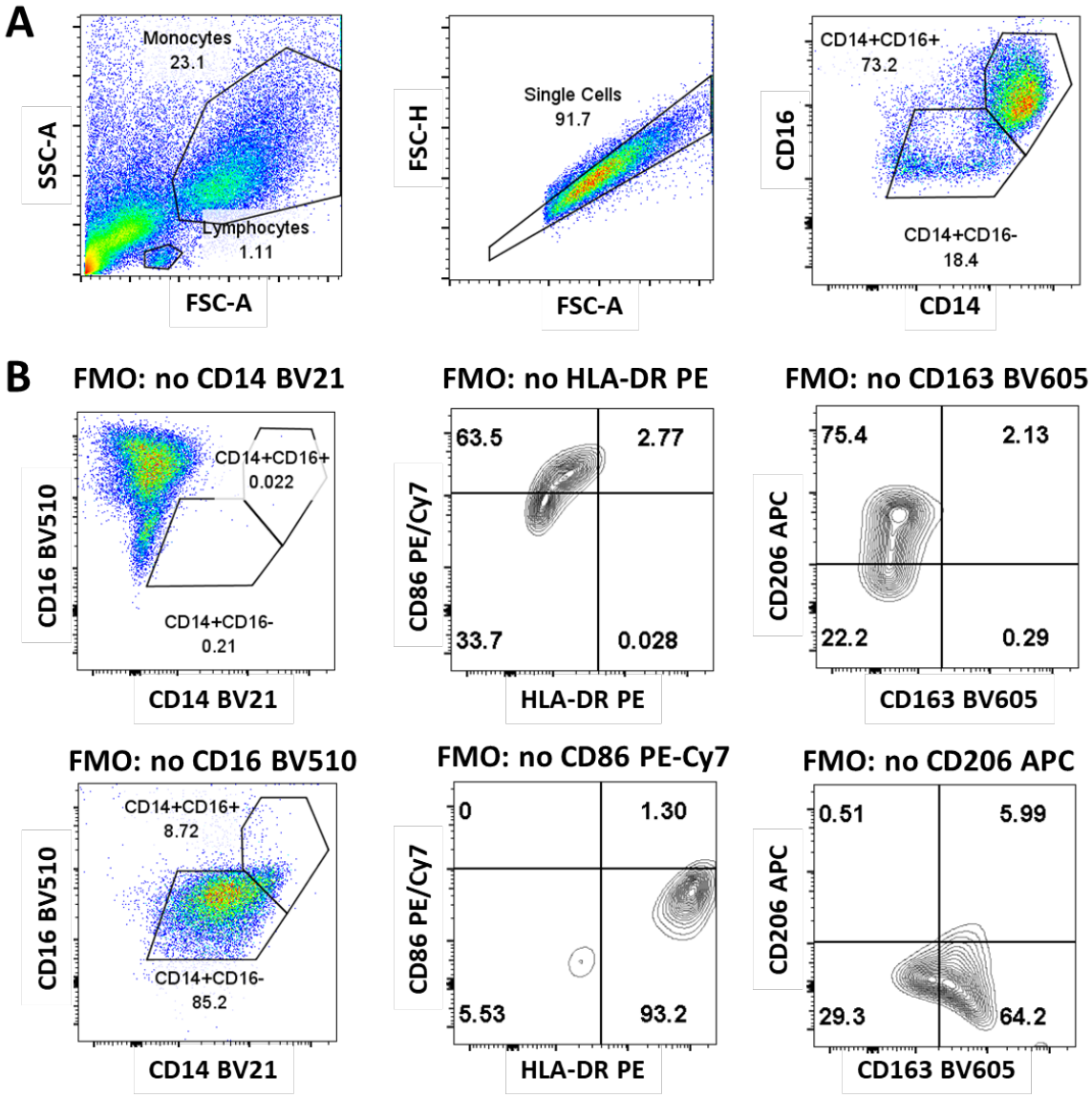


Figure S1: Gating strategy and Fluorescence minus one (FMO) controls.

(A) Total monocyte population from UK healthy individual was gated and then detected as $CD14^{+}CD16^{-}$ and $CD14^{hi}CD16^{+}$ populations. Each population was further analyzed for M1, HLA-$DR^{+}CD86^{+}$ or M2, $CD163^{+}CD206^{+}$ expression. (B) 5% contour plots with outliers show how a FMO control (left panel) was used to identify stained cells in the fully stained sample (Fig 1A). There were CD14 BV21, CD16 BV510, HLA-DR PE, CD86 PE-Cy7, CD163 BV605 and CD206 APC FMO.

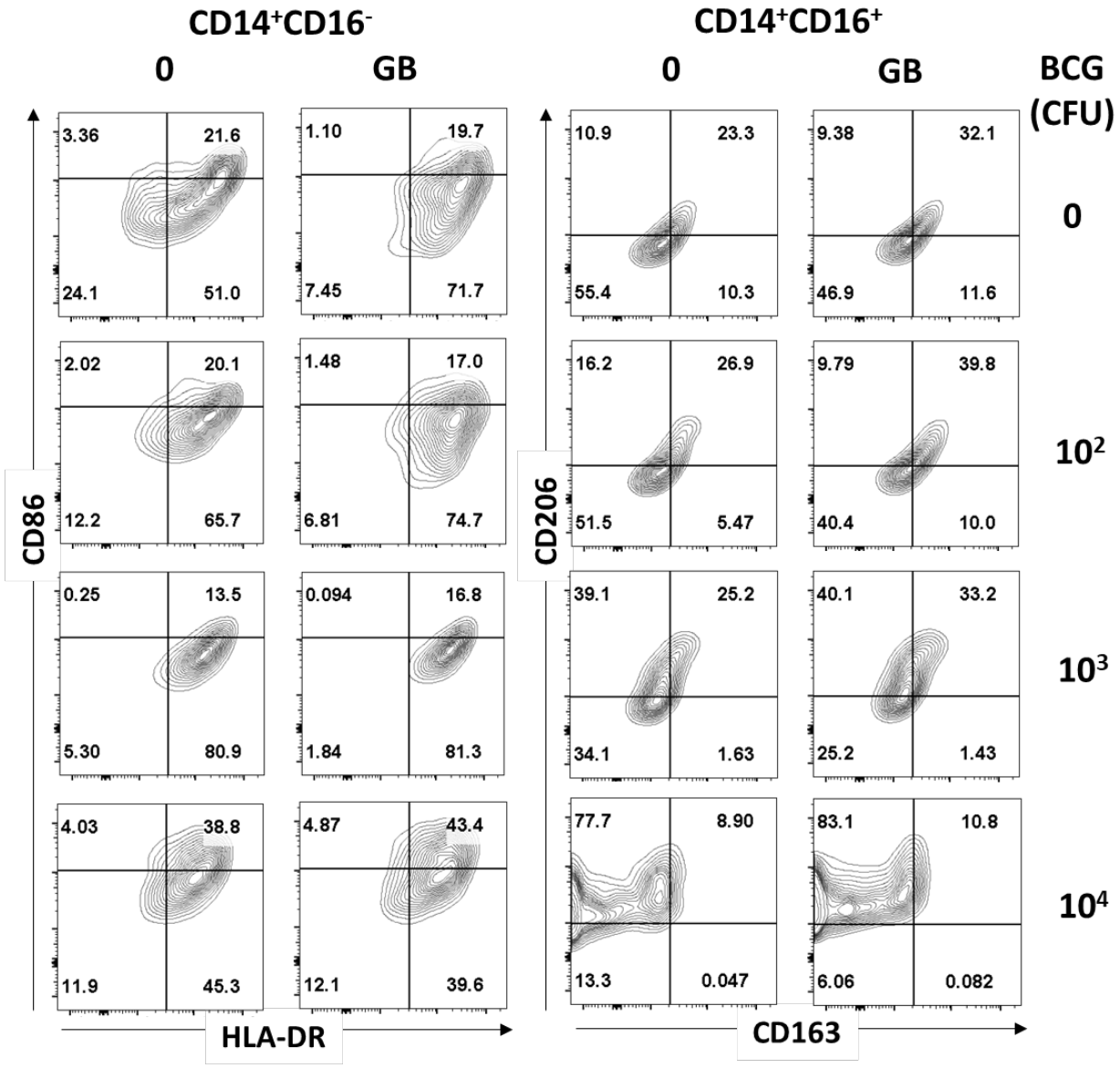


Figure S2: M1 and M2 marker expression in response to varied concentrations of BCG. Monocytes from UK healthy individuals (n=10) were treated with glibenclamide (GB, 25 μM) or vehicle (0) for 30 min., incubated with 10^2, 10^3 or 10^4 CFU of BCG or RPMI medium (Med) for 96 h and then analysed for M1 (HLA-DR$^+$CD86$^{+)}$ or M2 (CD163$^+$CD206$^{+)}$ expression by flow cytometry.

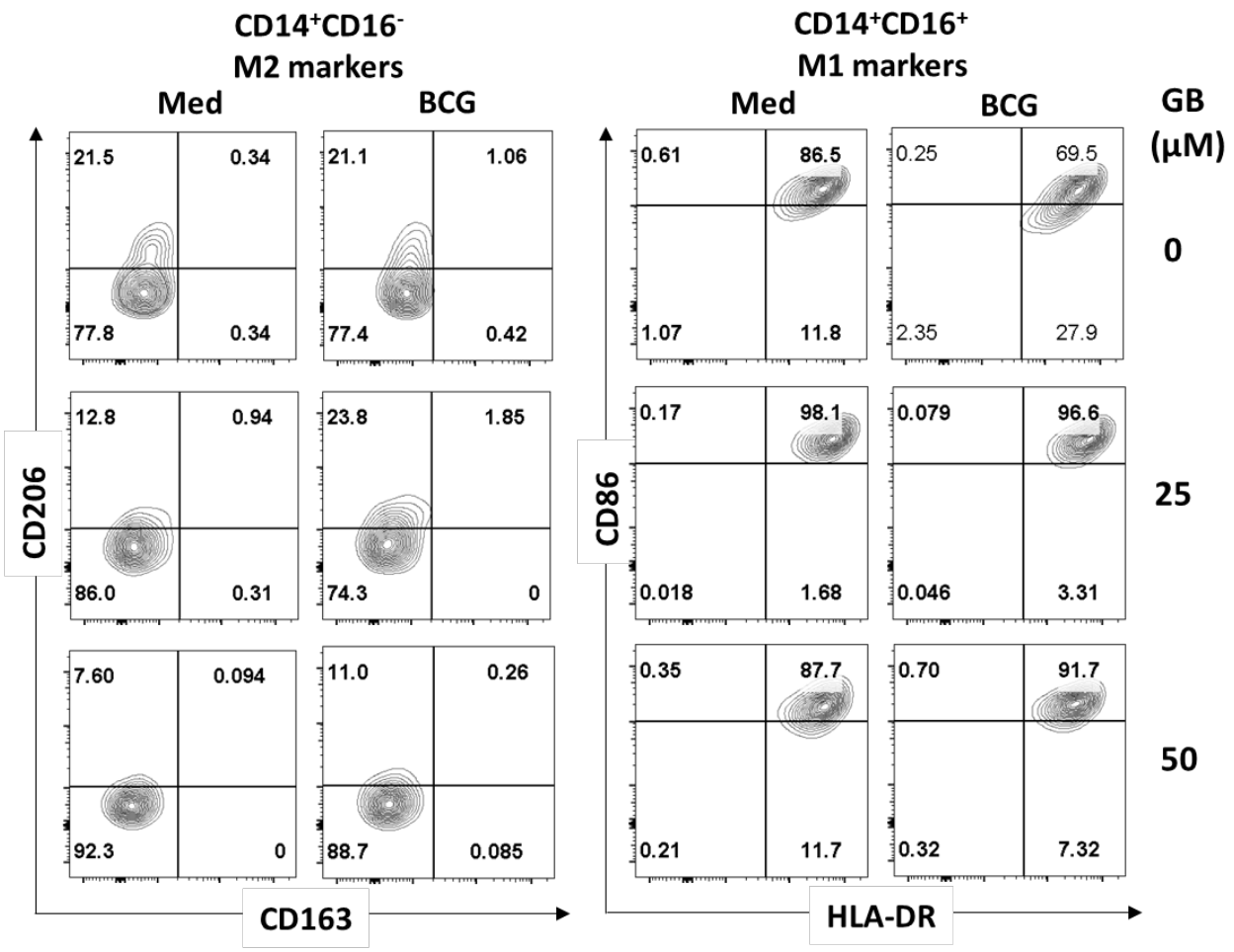


Figure S3: Effect of glibenclamide on M2 markers of $CD14^{+}CD16^{-}$ population and M1 markers of $CD14^{+}CD16^{+}$ population.

Monocytes from UK healthy individuals (n=10) were treated with glibenclamide (GB, 0, 25, 50 µM) for 30 min., incubated with 100 CFU per well of BCG or RPMI medium (Med) for 96 h and then analysed for M1 and M2 markers by flow cytometry. After exclusion of debris and doublets, monocytes were detected as $CD14^{+}CD16^{-}$ and $CD14^{+}CD16^{+}$ populations and then further analysed for M2, $CD163^{+}CD206^{+}$ and M1, $HLA\text{-}DR^{+}CD86^{+}$ expression, respectively.

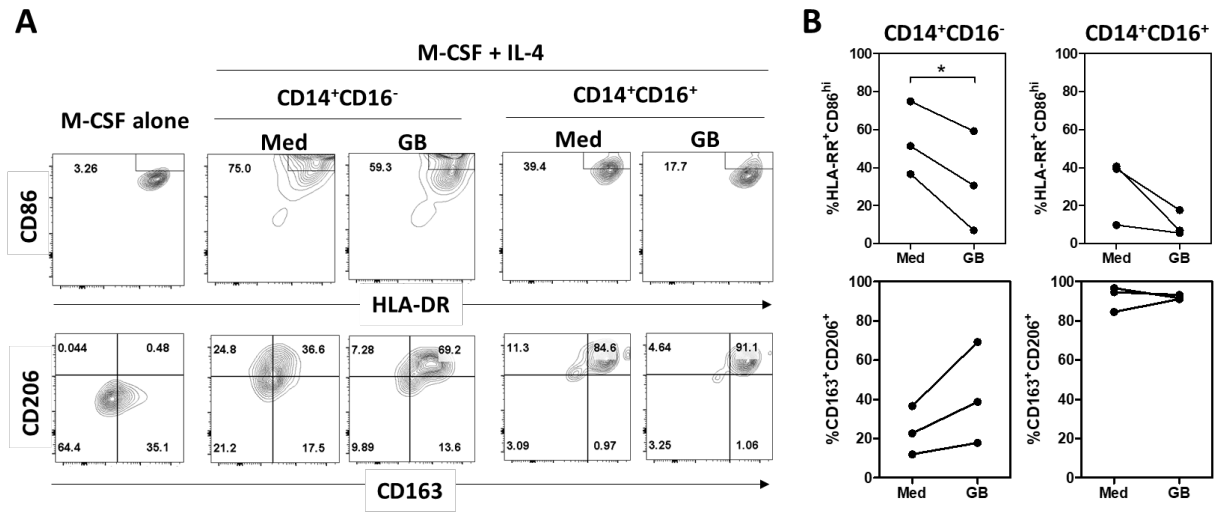
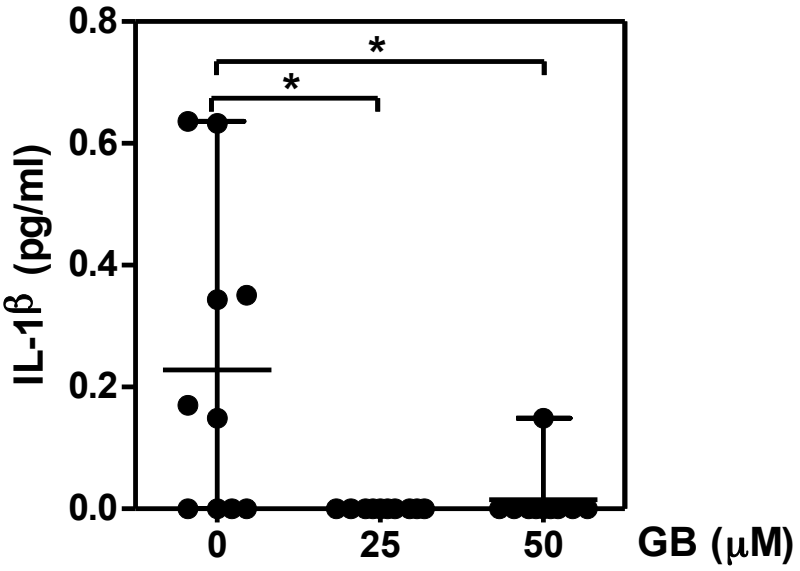


Figure S4: Glibenclamide reduces M1 markers of CD14$^+$CD16$^-$ population in M2 polarized macrophages.

(A) Monocytes from UK healthy donors (n=3) were treated with 50 ng/ml monocyte-colony stimulating factor (M-CSF) for M2 differentiation. At day 5, monocyte-derived macrophages (MDM) were stimulated for 2 days with 20 ng/ml IL-4 to obtain M2 phenotypes and then analysed M1 and M2 markers by flow cytometry. (B) The percentage of M1 or M2 positive cells gated on CD14$^+$CD16$^-$ and CD14$^+$CD16$^+$ cells are shown. Each dot represents median with interquartile range. Statistical analysis was performed using Paired *t* test to compare between with and without GB (w/o), *$P < 0.05$. No asterisk, non significant.



High sensitivity IL-1β ELISA: 0.125 - 8 ρg/ml

Figure S5: Glibenclamide reduces IL-1β from primary human monocytes in response to BCG.

Monocytes from UK healthy individuals were treated with glibenclamide (GB, 25, 50 μM) for 30 min. Drug-treated monocytes were incubated with 10^2 CFU per well of BCG (n=10) for 96 h and then the supernatants were collected for IL-1β detection. Error bars represent. Statistical analysis was performed using One Way ANOVA. Data are expressed as median with interquartile range. *P < 0.05. No asterisk, non significant.