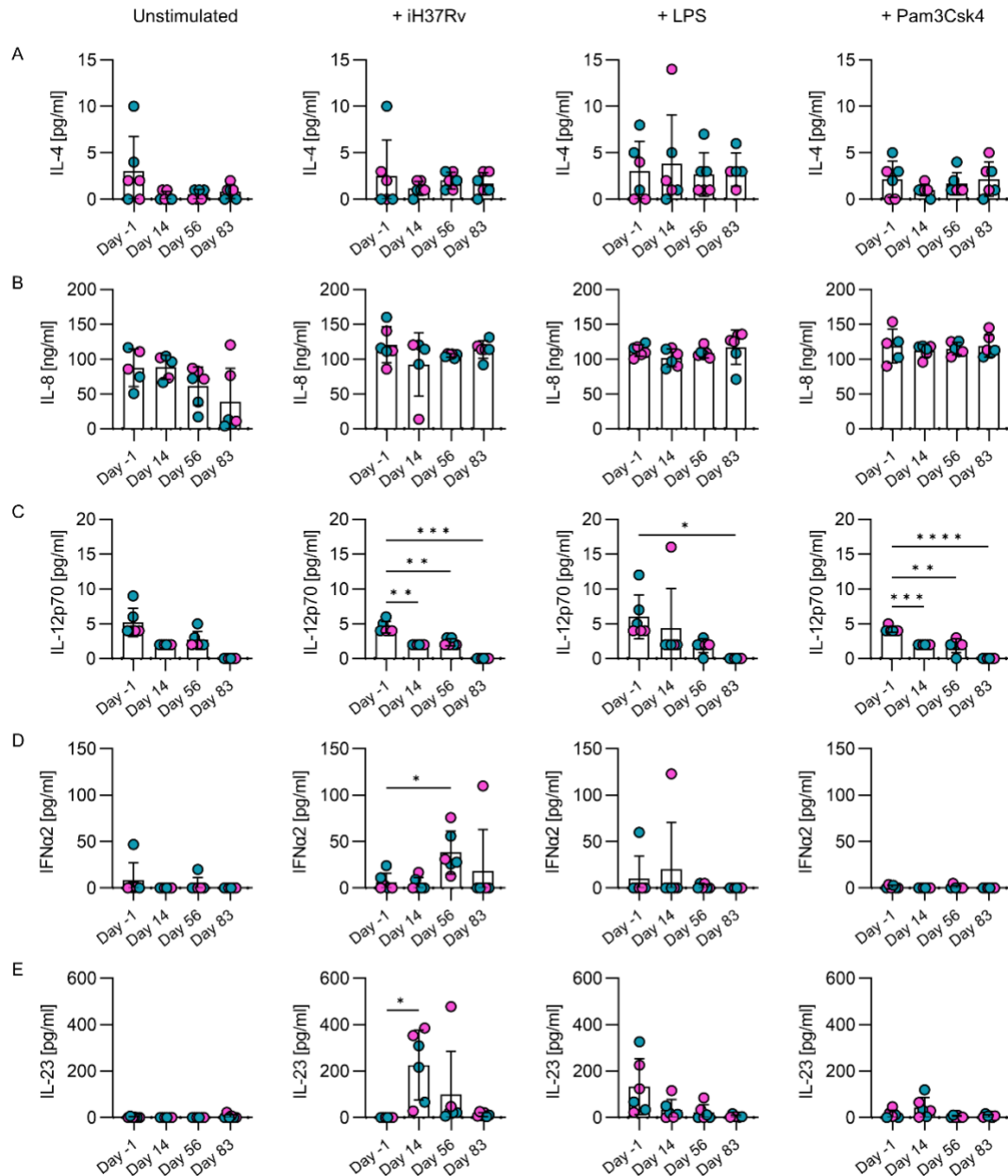


Supplemental Figure 1: Expression of HLA-DR and co-stimulatory molecules on classical, intermediate and non-classical monocytes. Monocytes were isolated from the PBMC of healthy donors on day -1 (pre-vaccine), day 14, day 56 and day 83 after vaccination using a hyperosmotic percoll gradient. Cells were Fc blocked and stained with fluorochrome-conjugated antibodies specific for CD14, and CD16. Classical monocytes were identified as CD14⁺ CD16⁻, intermediate monocytes were identified as CD14⁺ CD16⁺, and non-classical monocytes were identified as CD14^{lo} CD16⁺ with the gating strategy shown (A). The cell surface expression of the antigen presentation marker HLA-DR (B) and the T cell co-stimulatory molecules CD40 (C), CD80 (D) and CD86 (E) on all three monocyte subsets was assessed ex vivo by flow cytometry. Each dot represents an individual donor (n=10) with blue dots denoting male donors and pink dots denoting female donors. Data is graphed as the mean value \pm SD. Statistically significant differences between the groups were determined by a repeated measures two-way ANOVA using Dunnett's multiple comparisons test; ****P<0.0001, ***P<0.001, **P<0.01, *P<0.05.



Supplemental Figure 2: Cytokine production from monocytes pre and post vaccination.

Monocytes were enriched from the PBMC of healthy donors on the day before (day -1) and day 14, 56 and 83 after vaccination using a hyperosmotic percoll gradient. Monocytes were further purified using plastic adherence and were routinely over 90% pure. Monocytes were left to rest overnight and stimulated ex vivo with medium (unstimulated), irradiated *M. tuberculosis* (iH37Rv; 10 μ g/ml), LPS (10 ng/ml), or Pam3Csk4 (10 μ g/ml) for 24 hours. The concentrations of IL-4 (A), IL-8 (B), IL-12p70 (C), IL-23 (D), and IFN α 2 (E) was assessed using multiplex ELISA on cell supernatants with (A, C-E) showing [pg/ml] and (B) showing [ng/ml]. Each dot represents an individual donor (n=6) with blue dots denoting male donors and pink dots denoting female donors. Data is graphed as the mean value \pm SD. Statistically significant differences between the groups were determined by a repeated measures one-way ANOVA using Dunnett's multiple comparisons test; ****P<0.0001, ***P<0.001, **P<0.01, *P<0.05.