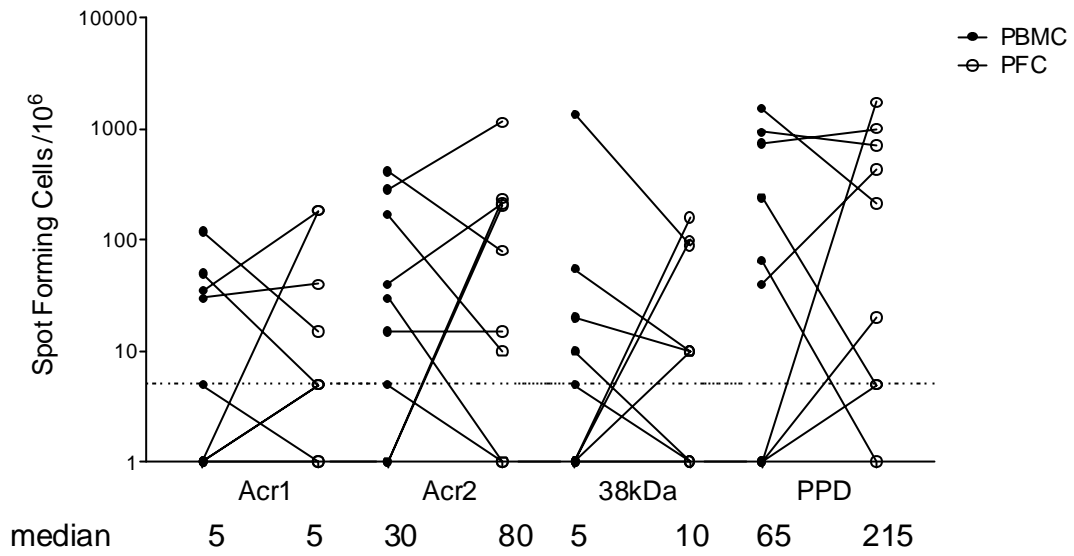
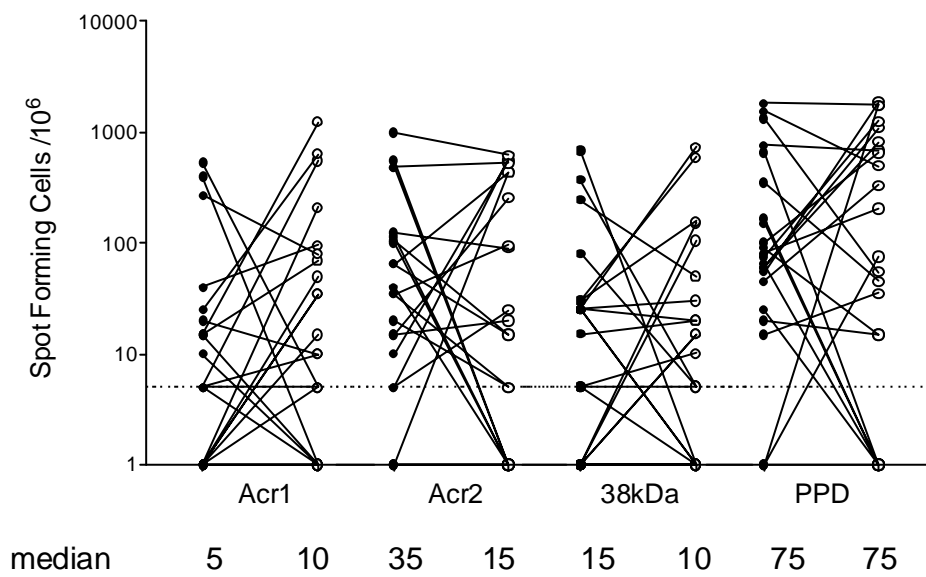


A



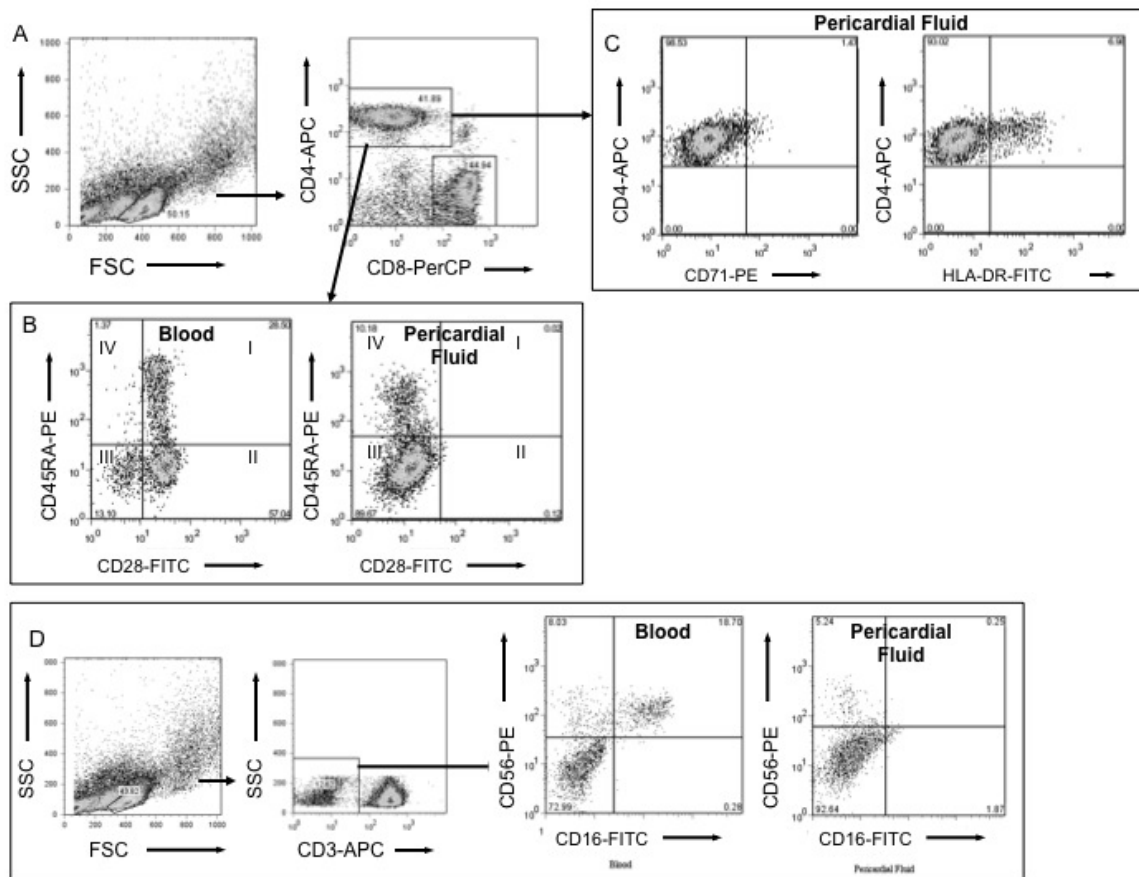
B



Supporting information, Figure 1

Heterogenous IFN- γ ELISpot responses in blood (PBMC) and disease site (PFC)

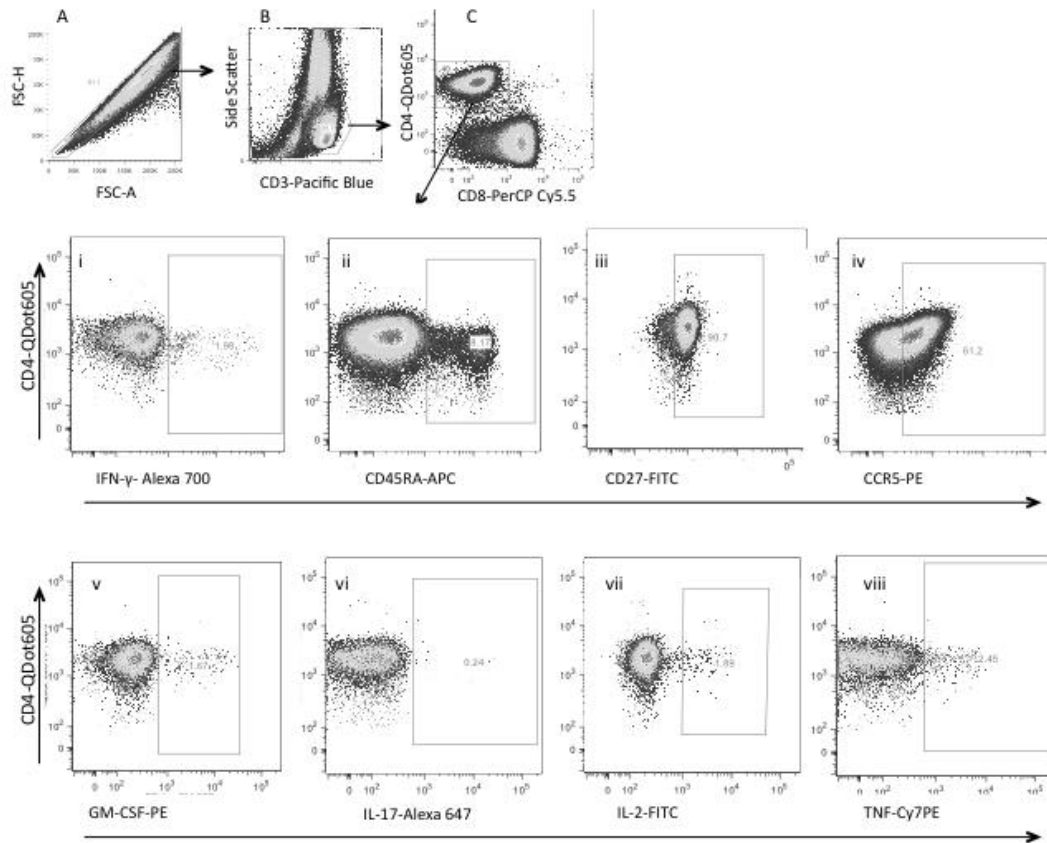
Each spot represents the number of IFN- γ Spot Forming Cells / 10^6 cells in response to Acr1, Acr2, 38kDa and PPD in HIV-1 uninfected (A) and HIV-1 infected (B) pericardial TB patients.



Supporting information, Figure 2

Representative scatter dot plots illustrating the gating strategy in four colour flow cytometry.

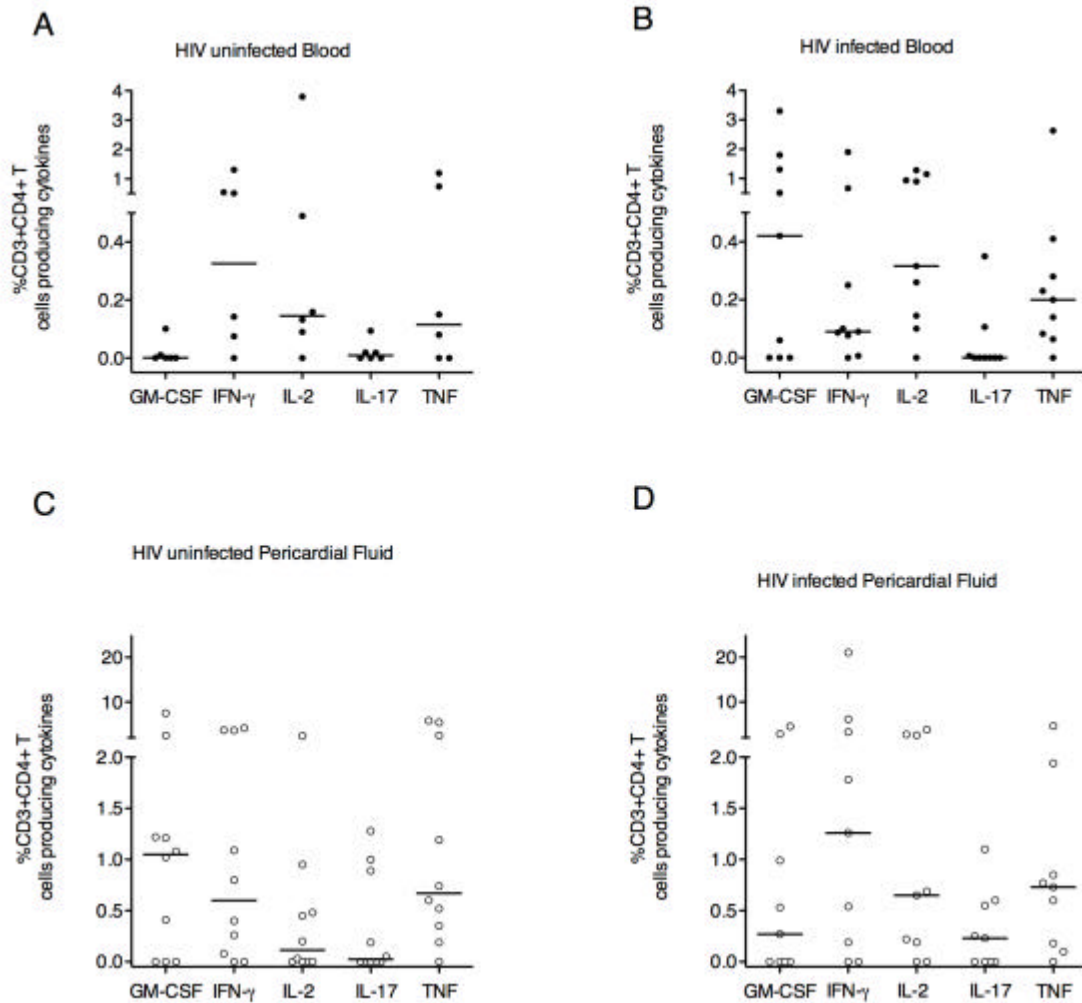
- A. Selection of CD4 and CD8 positive cells: a scatter plot using Side Scatter (SSC) and Forward Scatter (FSC) was applied to select for lymphocytes. These were further divided into CD4 and CD8 positive cells by gating on CD4-PE and CD8-PerCP fluorochromes.
- B. CD4 positive cells were further analysed for their surface expression of CD45RA-PE and CD28-FITC as follows (I) CD4⁺CD28⁺CD45RA⁺, (II) CD4⁺CD28⁺CD45RA⁻, (III) CD4⁺CD28⁻CD45RA⁻, (IV) CD4⁺CD28⁻CD45RA⁺ cells
- C. CD4 positive cells were also analysed for their surface expression of CD71-PE and HLA-DR-FITC.
- D. Representative example of gating on NK cells: CD3-APC negative cells were selected from the lymphocytes, and analysed as CD16-FITC⁺CD56-PE⁺ NK cells.



Supporting information, Figure 3

Representative scatter dot plots illustrating the gating strategy in eight colour flow cytometry.

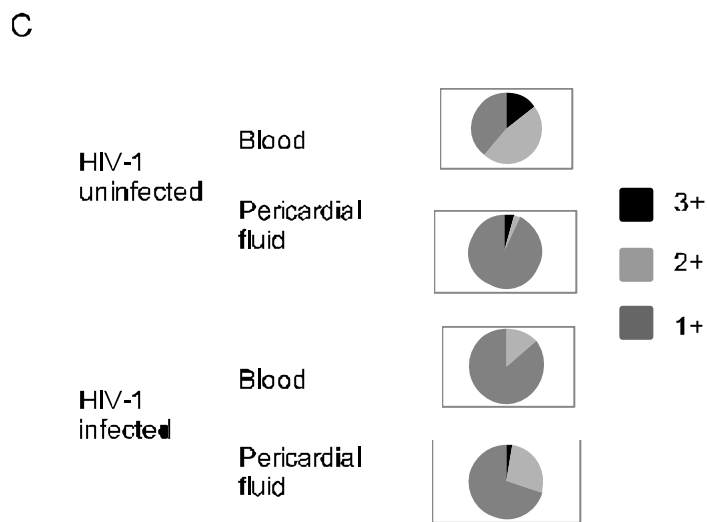
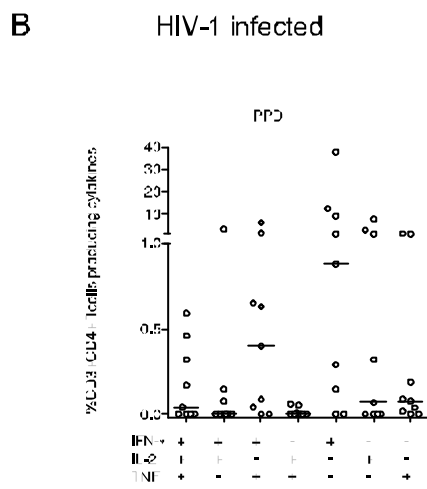
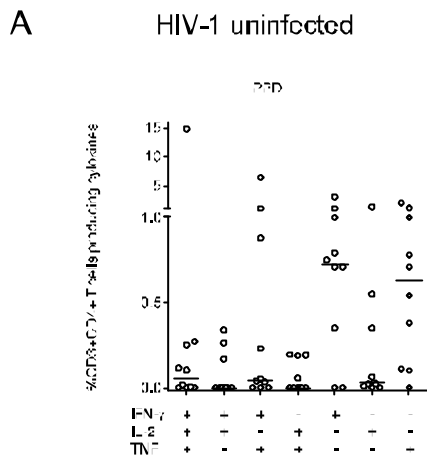
Plotting FSC-Area against FSC-Height allowed to select for singlet cells (Panel A). CD3⁺ cells were selected using SSC and CD3-PacBlue (Panel B), after which CD4⁺ and CD8⁺ cells were selected for by plotting CD4-Qdot 605 against CD8-PerCP Cy5.5 fluorochromes (Panel C). CD4⁺ or CD8⁺ cells on the Y axes were plotted against i. IFN- γ -Alexa 700, ii. CD45RA-APC, iii. CD27-FITC, iv. CCR5-PE; and v. GM-CSF-PE, vi. IL-17-Alexa 647, vii. IL-2-FITC and viii. TNF-Cy7PE on the X axes, after which Boolean gating was applied to look at combinations of the markers.



Supporting information, Figure 4

Total cytokine production in response to hkB37Rv in blood and pericardial fluid of HIV-1 infected and HIV-1 uninfected pericardial TB patients

Total cytokine production for GM-CSF, IFN- γ , IL-2, IL-17 and TNF in blood (Panel A and B) and pericardial fluid (Panel C and D) for HIV-1 uninfected (Panel A and C) and HIV-1 infected (Panel B and D) pericardial TB patients.



Supporting information, Figure 5

PPD specific polyfunctional CD3⁺CD4⁺ T cells in the blood and pericardium

CD3⁺CD4⁺ T cells expressing various combinations of IFN- γ , IL-2 and TNF in response to PPD stimulation, in the pericardial fluid of HIV-1 uninfected (panel A), and HIV-1 infected (panel B) patients. The pie-charts (Panel C) illustrate the overall proportional contribution of cytokine expressing cells to the PPD specific response in both blood and pericardial fluid. 1+: any one cytokine, 2+: any two cytokines, 3+: all three cytokines. IL-2 single positive cells were significantly increased in the blood of HIV-1 infected patients compared to HIV-1 uninfected patients (p=0.014).