Figure S6

A

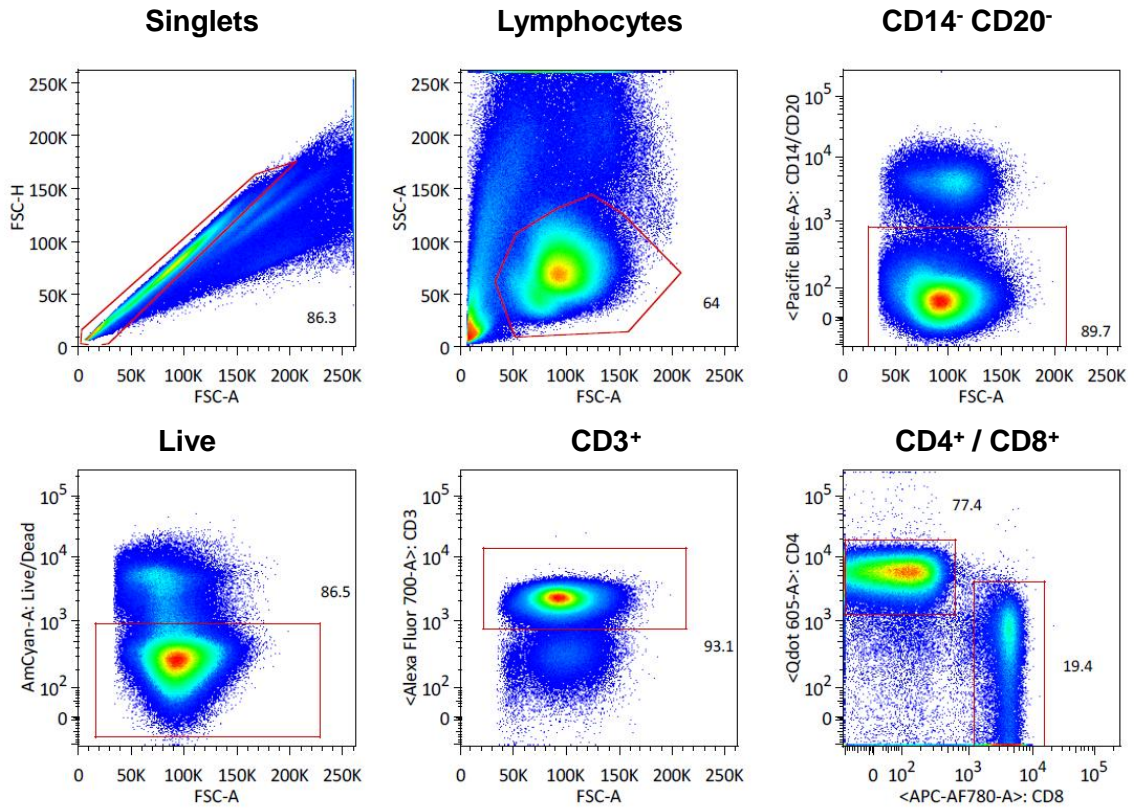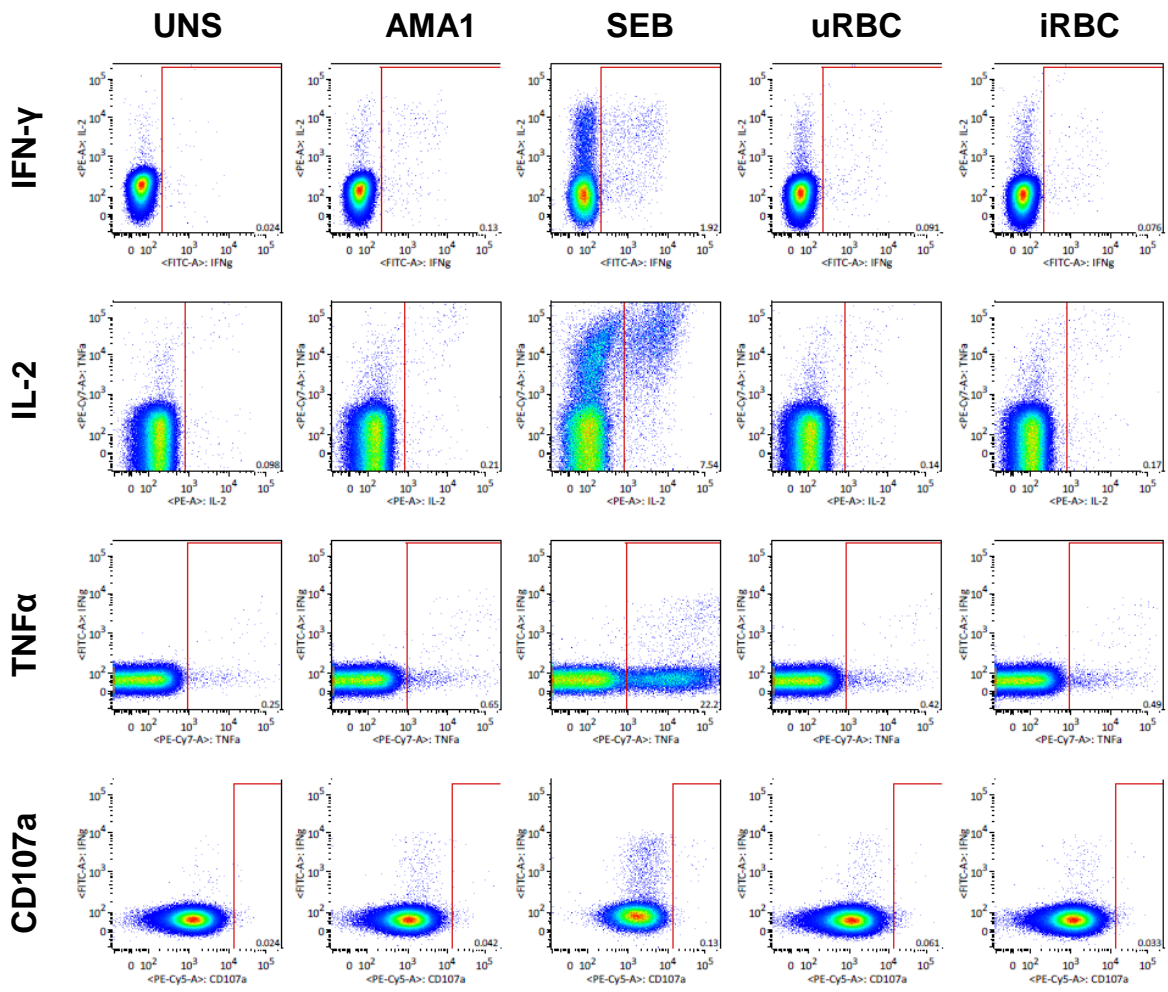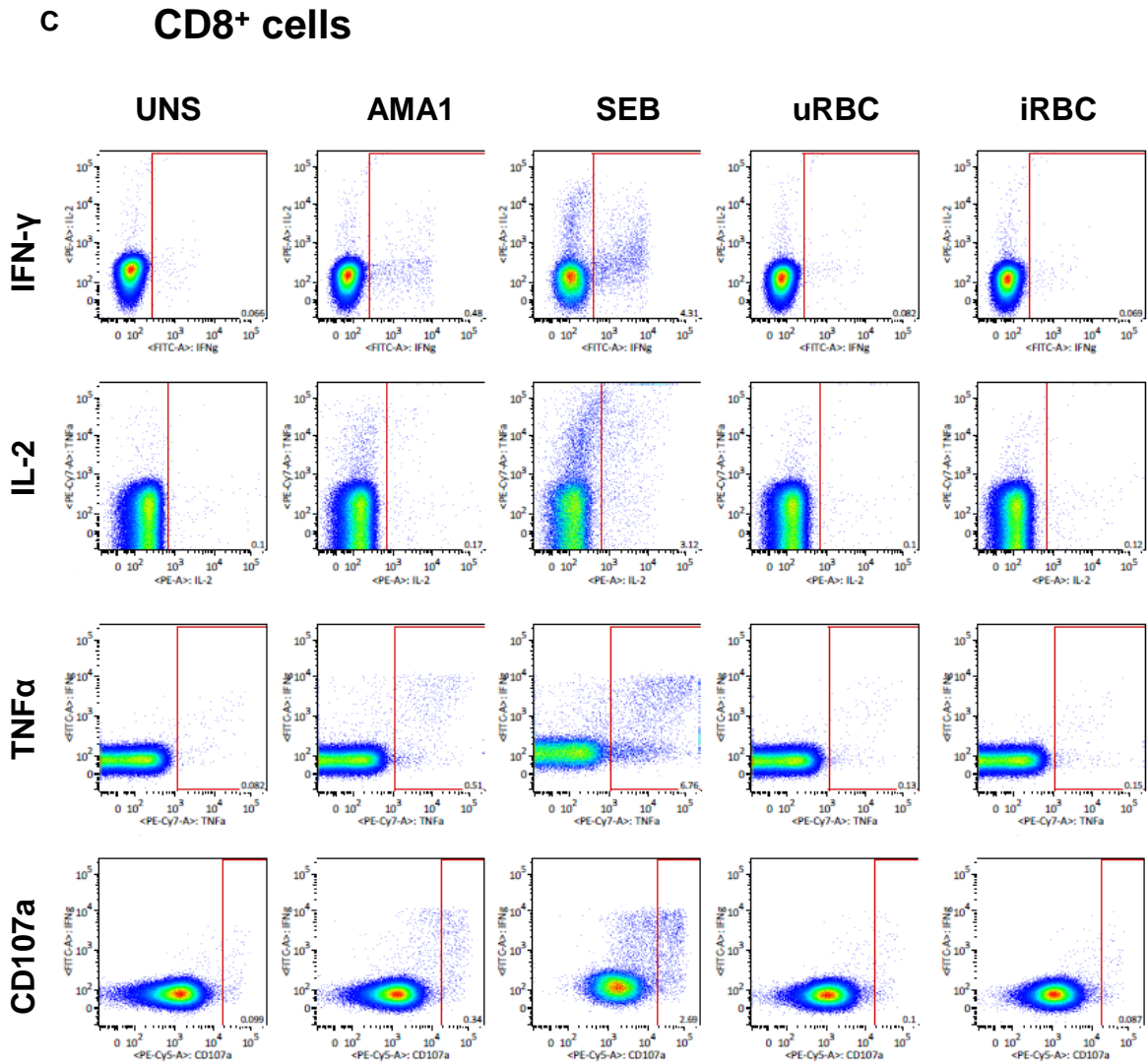**B** **CD4⁺ cells**

Figure S6. Gating strategy for analysis of AMA1-specific T cell responses. Representative flow cytometry plots are shown for the analysis of AMA1-specific T cell responses from volunteers immunized with ChAd63-MVA AMA1. **(A)** Initial gating used (from top left to bottom right) forward scatter area (FSC-A) versus forward scatter height (FSC-H) to remove doublet events and select singlet cells; then following this small lymphocytes were gated using FSC-A versus side scatter area (SSC-A); then live CD14$^-$ CD20$^-$ CD3$^+$ cells were selected; then CD4 versus CD8 was used to select the total CD4$^+$ CD8$^-$ cell population and vice versa for the CD8$^+$ CD4$^-$ population. Cytokine (IFN-γ, IL-2 and TNFα) and CD107a gating using bivariate plots is shown for **(B)** CD4$^+$ cells and **(C)** CD8$^+$ cells. **(B)** Representative plots for un-stimulated (UNS), AMA1 peptide stimulated (AMA1), SEB, uRBC and iRBC

stimulated samples are shown. IFN-γ (top row), IL-2 (second row), TNFα (third row) and CD107a (bottom row) for the CD8$^-$ CD4$^+$ T cell population were analyzed using bivariate plots. Percentages refer to the % of CD8$^-$ CD4$^+$ cells that express the specific cytokine or marker. Background responses in UNS or uRBC control cells were subtracted from the AMA1 and iRBC response respectively during the analysis. **(C)** Same analysis as in **(B)**, except for the CD4$^-$ CD8$^+$ T cell population.