Figure S1

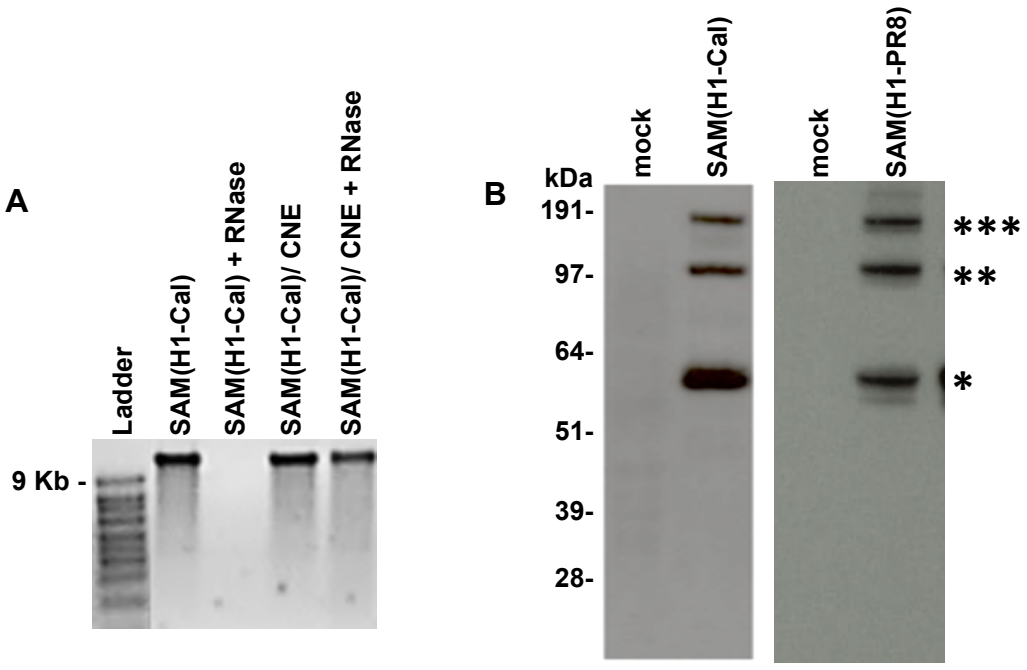

FIG. S1. Characterization of SAM(H1) replicon and formulation with CNE. **(A)** Denaturing agarose gel electrophoresis showing the protection from RNase digestion of SAM(H1-Cal) replicon naked or complexed to CNE. **(B)** HA protein expression in BHK cells transfected with SAM(H1-Cal), SAM(H1-PR8) or mock-treated. Cell lysates were analyzed by non-reducing SDS-PAGE followed by Western Blot analyses with HA-strain specific polyclonal sera (anti influenza A/Puerto Rico/8/1934 (H1N1), 03/242, NIBSC; anti influenza A/ California/7/2009 (H1N1), 11/110, NIBSC). HA *monomer; **dimer; ***trimer.

Figure S2

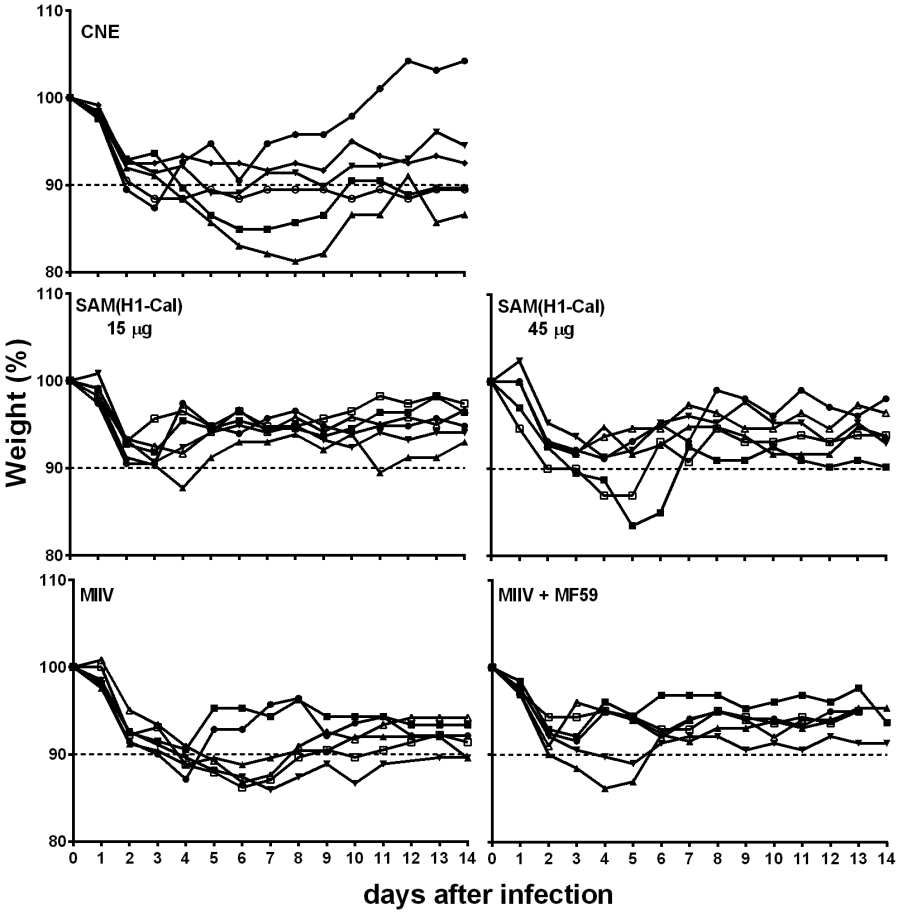

FIG. S2. Percentage of body weight loss in individual ferrets. Ferrets (n=6) were immunized i.m. on day 0 and day 56 with 15 or 45 mg SAM(H1-Cal)/CNE, 15 mg MIIV±MF59, or with CNE alone. At day 94, ferrets were infected i.n. with 10^6 pfu/ animals of pandemic influenza A/California/07/2009 (H1N1) virus. Body weights were monitored for 14 days after challenge. The curves represent the percentage of body weight change relative to the weight before challenge. The dotted line indicates the 10% drop in weight used to determine illness onset.

Figure S3

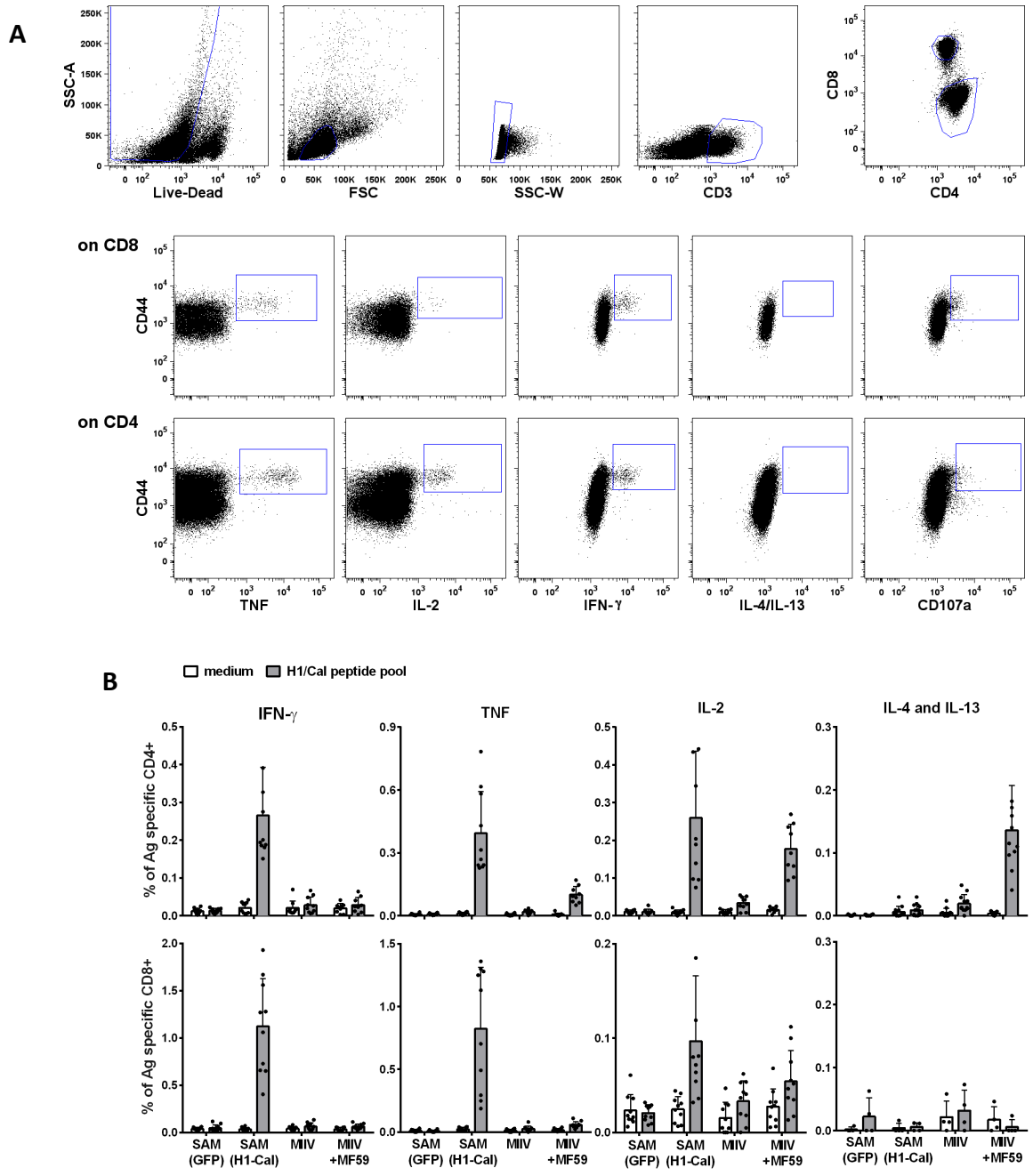

FIG. S3. (A) Gating strategy for flow cytometry analyses of CD4 and CD8 T cells. Live cells were negatively selected based on dye exclusion, and lymphocytes were further identified based on morphology. $CD3^+$ T cells were selected after discrimination of singlets, and CD4 and CD8 T cells were identified based on $CD4^+$ and $CD8^+$ expression, respectively. Cytokine+ (IFN-γ, IL-4, IL-13, IL-2 and TNF) and $CD107a^+$ cells were identified in the $CD4^+CD44^{hi}$ and $CD8^+CD44^{hi}$ T cell populations. Data shown are representative dot plots. **(B)** Frequency of cytokine positive CD4 and CD8 T cells upon in vitro stimulation with medium alone or H1/Cal peptide pool of splenocytes of mice immunized as indicated. Dots represent single mice, bars the calculated average. Data shown are the merge of three independent experiments.

Figure S4

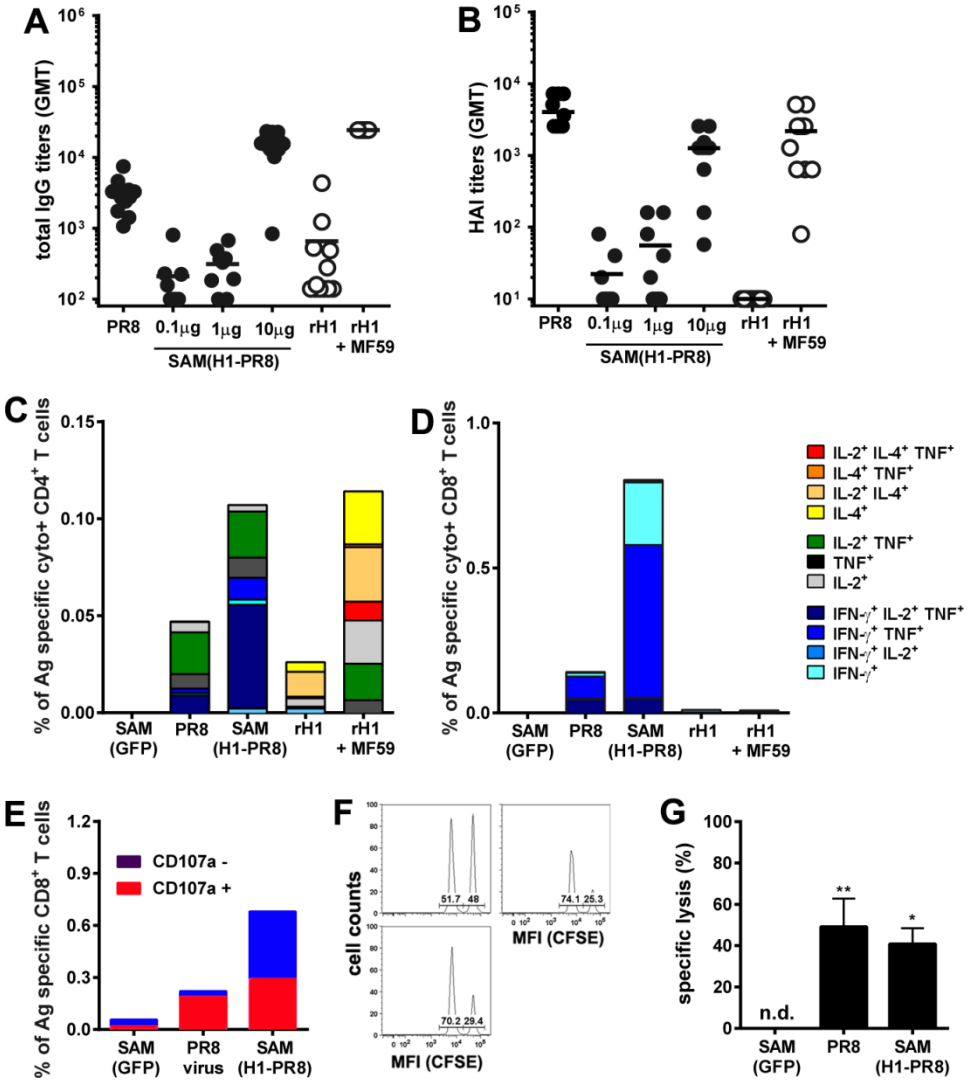

FIG. S4. Immunogenicity of SAM(H1-PR8) in mice. Mice (n=8) were immunized i.m on day 0 and 56 with 10 mg of SAM(GFP), SAM(H1-PR8) at 0.01, 0.1, 1 and 10 mg, or 1 mg of rH1(PR8) -/+ MF59. Sera and spleens were collected 2 wk after the second immunization. Sera were analyzed for H1N1-specific **(A)** total IgG titers, **(B)** and HI titers. *p<0.05; ***p<0.001 compared to rH1(PR8). **(C – E)** Splenocytes (from n=4 mice) were stimulated in vitro with a H1-PR8 peptide pool and T cells were analyzed for cytokine production and cytotoxicity by flow cytometry following the gating strategy presented in Suppl. Fig. 3. The bars represent the cumulative frequency of H1-specific (D) CD4+ T cells, and (E) CD8+ T cells expressing combinations of cytokines, as indicated in the graph. (F) CD107a expression by CD8+ T cells. **(G - H)** In vivo cytotoxicity assay. CFSE-labeled Flu-$H1_{533-541}$ or HIV-$Gag_{107-205}$ -pulsed target cells were administered i.v. to mice previously immunized with PBS or SAM(H1-PR8), or sublethally infected with PR8 virus. Splenocytes were harvested 20 h later and analyzed for the presence of CFSE+ target cells by flow cytometry, as described in the methods section. **(G)** Representative histograms showing the percentage of recovered $CFSE_{high}$ and $CFSE_{low}$ target cells. **(H)** Percentage of specific target cell lysis. **p<0.01; *p<0.05 compared to PBS

Figure S5

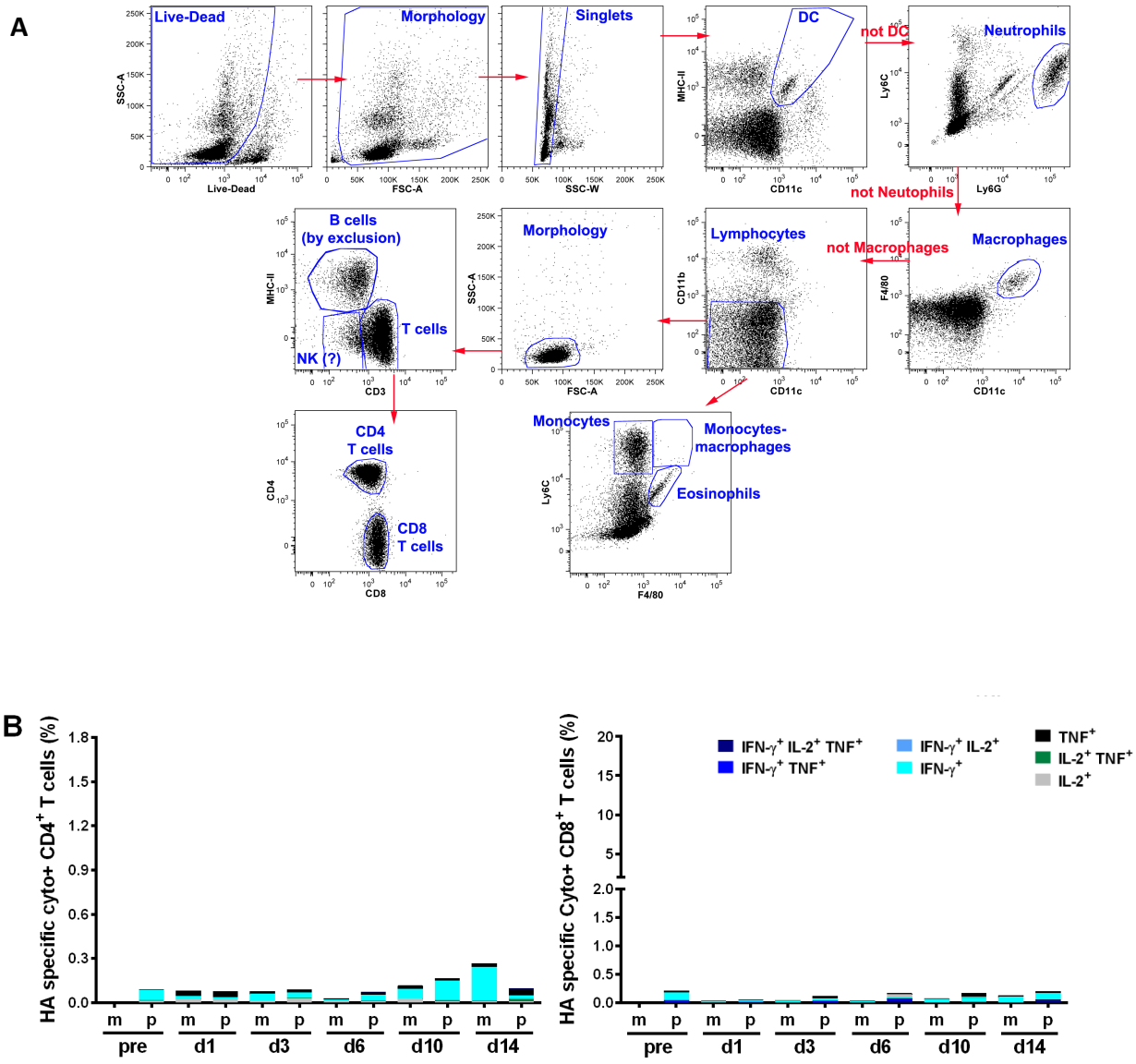

FIG. S5. Recruitment of immune cells in the lungs after challenge with A/PR/8/34 (H1N1). Mice were immunized i.m. on day 0 and 56 with saline, 10 μg of SAM(H1-Cal) or exposed to a sub-lethal dose of PR8 virus. Four weeks after the second immunization, mice were infected with 20 $TCID_{50}$ of PR8 virus. Lung cell suspensions were analyzed at different time points after infection by flow cytometry. **(A)** Leukocyte populations in lung cell suspensions were gated on live cells followed by morphology and singlet discrimination. Neutrophils were identified as $Ly6G^{hi}$ $CD11b^{hi}$; T cells as $CD3^{+}$ and $CD4^{+}$ or $CD8^{+}$; B cells as $MHCII^{hi}CD3^{-}$, DCs as $CD11c^{+}$ $MHCII^{hi}$; monocytes as $Ly6C^{hi}$ $F4/80^{-}$; monocyte-macrophages as $Ly6C^{hi}$ $F4/80^{+}$; eosinophils as $Ly6C^{int}$ $F4/80^{+}$. **(B)** Mice were exposed to a sub-lethal dose of PR8 virus. Four weeks after the second treatment, mice were infected with 20 $TCID_{50}$ of PR8 virus. Lung cell suspensions were analyzed at different time points after infection by flow cytometry. Frequencies of cytokine-producing $CD4^{+}$ and $CD8^{+}$ T cells after in vitro stimulation with H1-Cal peptide pool (p) or medium (m).

Figure S6

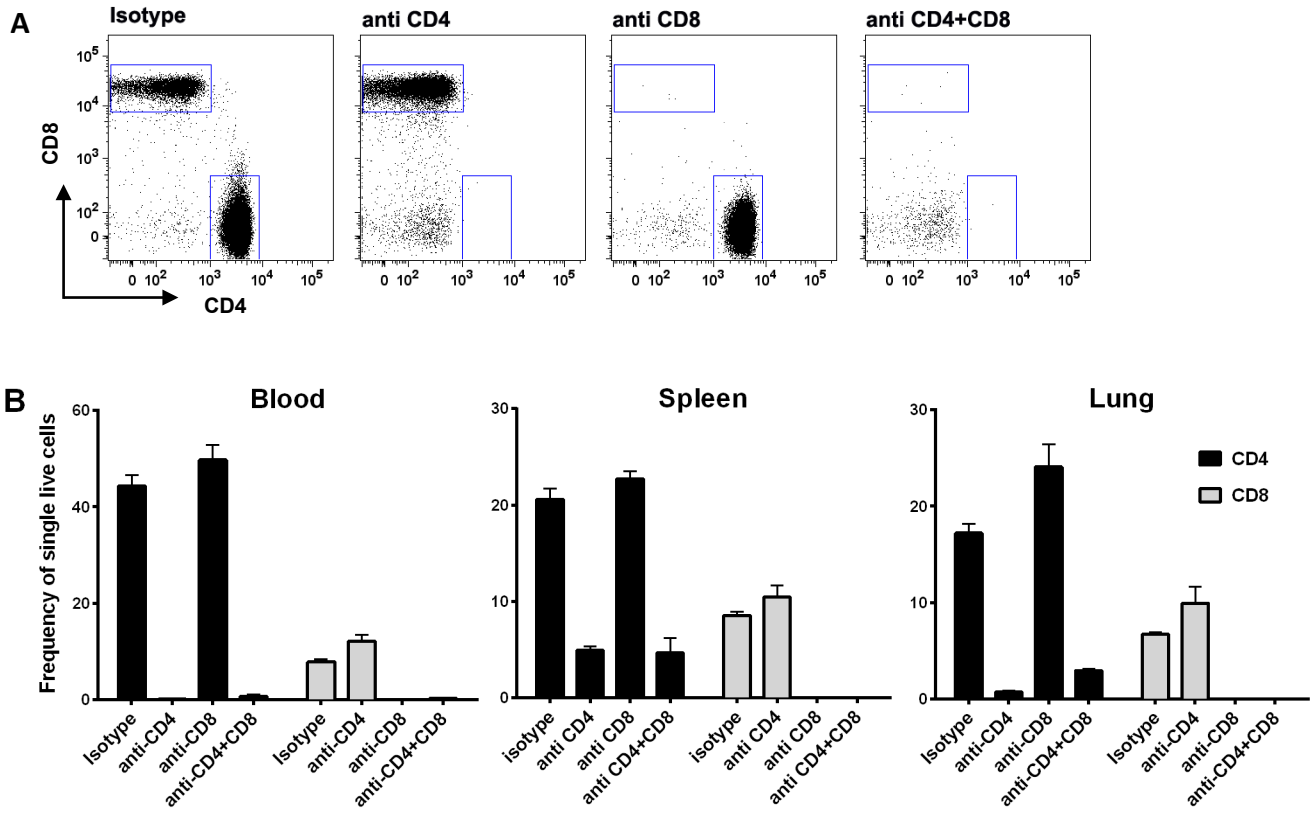

FIG. S6. Efficiency of CD4 and CD8 T-cell depletion. Mice immunized with SAM(H1-Cal) were treated with anti-CD4, anti-CD8, anti-CD4 and anti-CD8 depleting antibodies, or with an isotype control. **(A)** Dot plots representative of the frequencies of blood circulating CD4 and CD8 T cells after the different treatments. **(B)** Frequencies of CD4 and CD8 T cells after treatment in whole blood, spleens and lungs.