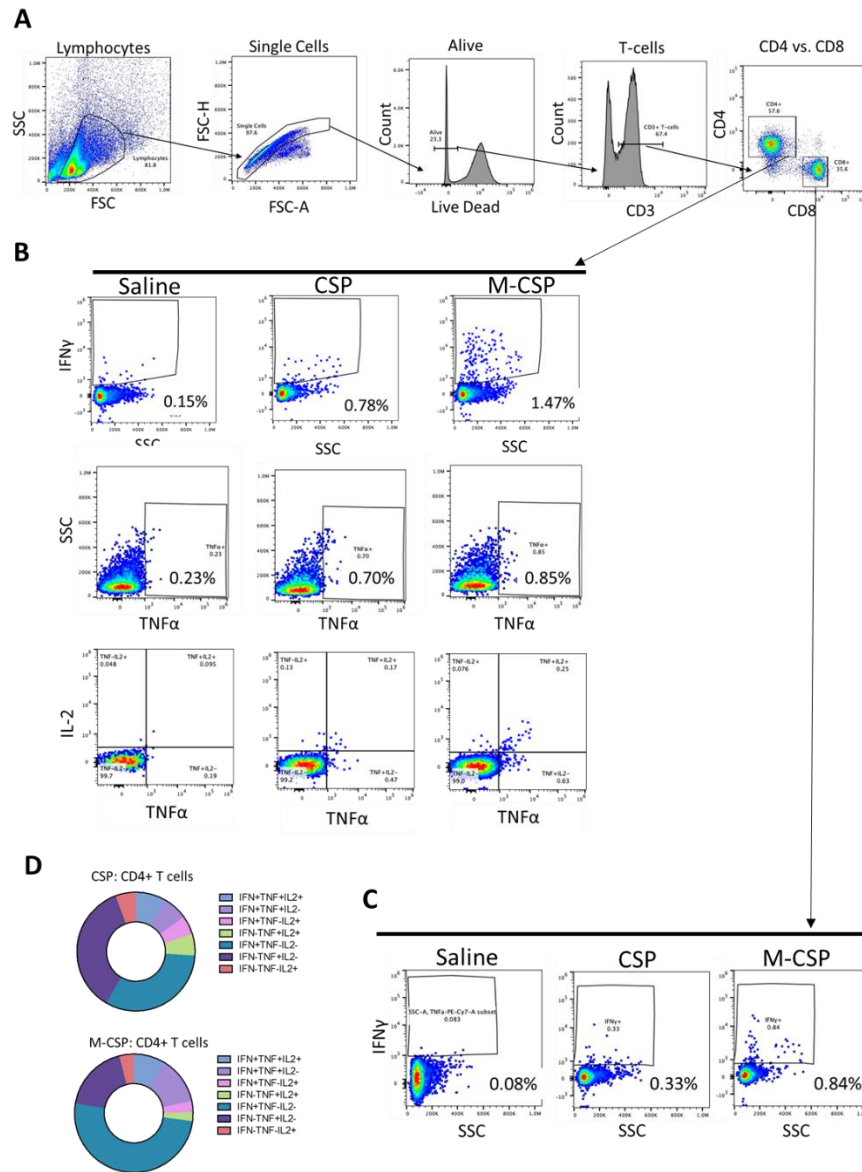


Supplementary Figure 1: Characterization and expression of CSP and MIP3 α -CSP

mRNA. (A) CSP and MIP3 α -CSP LNP formulations were run on a 2.5% formaldehyde gel. Signals of the expected sizes were obtained for both CSP and MIP3 α -CSP formulations. (B) Western blot analysis of CSP and MIP3 α -CSP transfected HEK293T cells are shown, displaying both culture supernatant and pelleted cells detected with anti-myc antibody. Signals of expected sizes of both constructs were found in pelleted but not supernatant fractions. (C) IFAs of CSP, MIP3 α -CSP and non-transfected controls probed with anti-myc antibody are shown. Punctate intracellular expression of both CSP and MIP3 α -CSP proteins is seen with transfected HEK293T cells of both groups. (D) Example images of fluorescence intensity in mice from the naïve mice, CSP vaccinated, and MIP3 α -CSP vaccinated groups are shown.

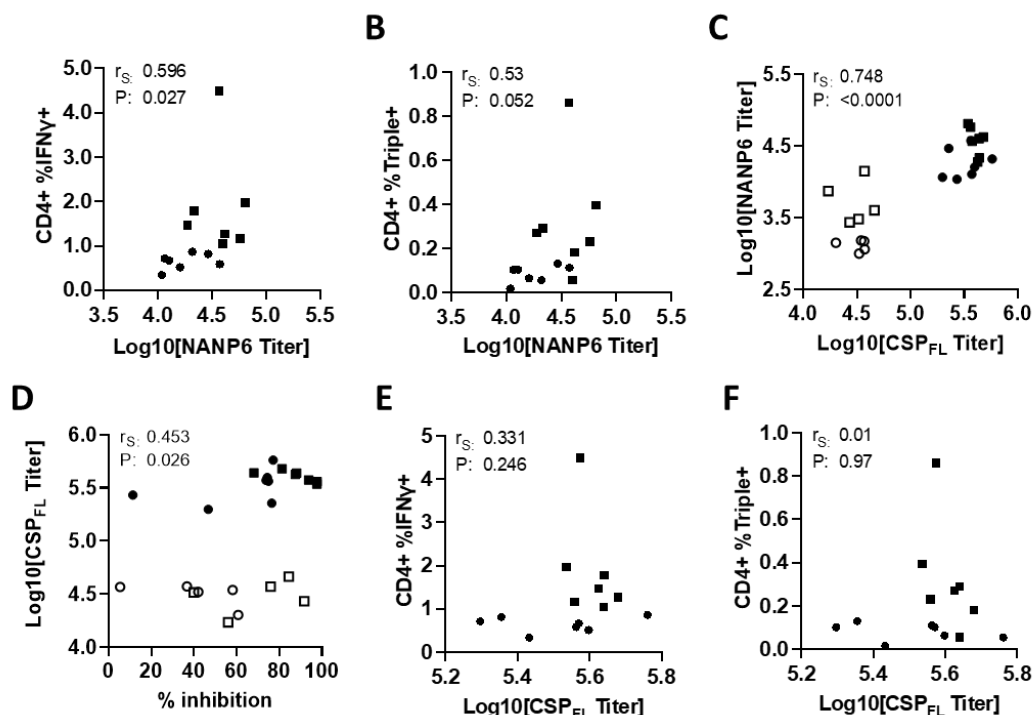


Supplementary Figure 2: Flow cytometry gating strategy. (A) All flow cytometry data passed through initial gates for lymphocytes, single cells, live cells, and CD3+ cells. CD3+ cells were then gated into two distinct groups, CD4+ and CD8+ cells. (B) CD4+ T-cell IFN γ + gates, CD4+ T-cells TNF α + gates, and polyfunctional CD4+ T-cell gates are shown as examples of gating strategy here. (C) Example image of gating of CD8+ T-cell IFN γ + cells from a single mouse. (D) Qualitative pie chart analysis of the cytokine expression patterns of all 7 mice in CSP and MIP3 α -CSP vaccination groups from second vaccination study. A

38 greater proportion of triple and double positive CD4+ T cells are seen in the MIP3 α -CSP
 39 group.

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41



42 **Supplementary Figure 3: Association between antibody titers, inhibition of liver stage**
 43 **parasitemia, and polyfunctional T cells.** (A-B) Relationship between anti-NANP6 antibody
 44 titers and IFN γ + CD4+ T cells (A) or triple positive CD4+ T cells (B). Data points represent
 45 individual mice from the dose de-escalation, delayed boost challenge study. Circles represent
 46 mice from the CSP vaccinated group, and squares represent mice from the MIP3 α -CSP
 47 vaccinated group. Spearman's rank correlation coefficients were used to compare variables.
 48 There was a significant relationship between anti-NANP6 antibody titers and IFN γ + CD4+
 49 cells (A) ($p = 0.027$, $r_s = 0.596$). Likewise, there was a strong relationship between anti-
 50 NANP6 antibody titers and triple positive CD4+ cells (B) ($p = 0.052$ and $r_s = 0.53$). (C)
 51 Relationship between log₁₀ anti-full length CSP antibody titers and log₁₀ anti-NANP6 antibody
 52 titers is shown. Unfilled and filled symbols represent single mice from the standard regimen

and dose de-escalation, delayed boosting challenge studies respectively. Circles represent mice from the CSP vaccinated group, and squares represent mice from the MIP3 α -CSP vaccinated group. Spearman's rank correlation coefficient was used to compare variables. There was a significant relationship between anti-full length CSP antibody titers and anti-NANP6 antibody titers ($p < 0.0001$, $r_s = 0.748$). (D) Relationship between log₁₀ anti-full length CSP antibody titers and percent inhibition of liver stage parasitemia is shown. Percent inhibition of liver stage parasitemia was calculated relative to naïve, sporozoite challenged mice. Unfilled and filled symbols represent single mice from the standard regimen and dose de-escalation, delayed boost challenge studies respectively. Circles represent mice from the CSP vaccinated group, and squares represent mice from the MIP3 α -CSP vaccinated group. Spearman's rank correlation coefficient was used to compare variables. There was a significant relationship between anti-full length CSP antibody titers and inhibition of liver stage parasitemia ($p = 0.026$, $r_s = 0.0453$). (E-F) Relationship between anti-full length CSP antibody titers and IFN γ + CD4+ T cells (E) or IFN γ , TNF α , and IL-2 triple positive CD4+ T cells (F). Data points represent individual mice from the dose de-escalation, delayed boost challenge study. Circles represent mice from the CSP vaccinated group, and squares represent mice from the MIP3 α -CSP vaccinated group. Spearman's rank correlation coefficients were used to compare variables. There was no significant relationship between anti-full length CSP antibody titers and IFN γ + CD4+ cells (E) ($p = 0.331$, $r_s = 0.246$) nor anti-full length CSP antibody titers and triple positive CD4+ cells (F) ($p = 0.97$ and $r_s = 0.01$).