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Supplementary Materials for

A lymph node–targeted Amphiphile vaccine induces potent cellular and humoral immunity to SARS-CoV-2

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This PDF file includes:

Figs. S1 to S5

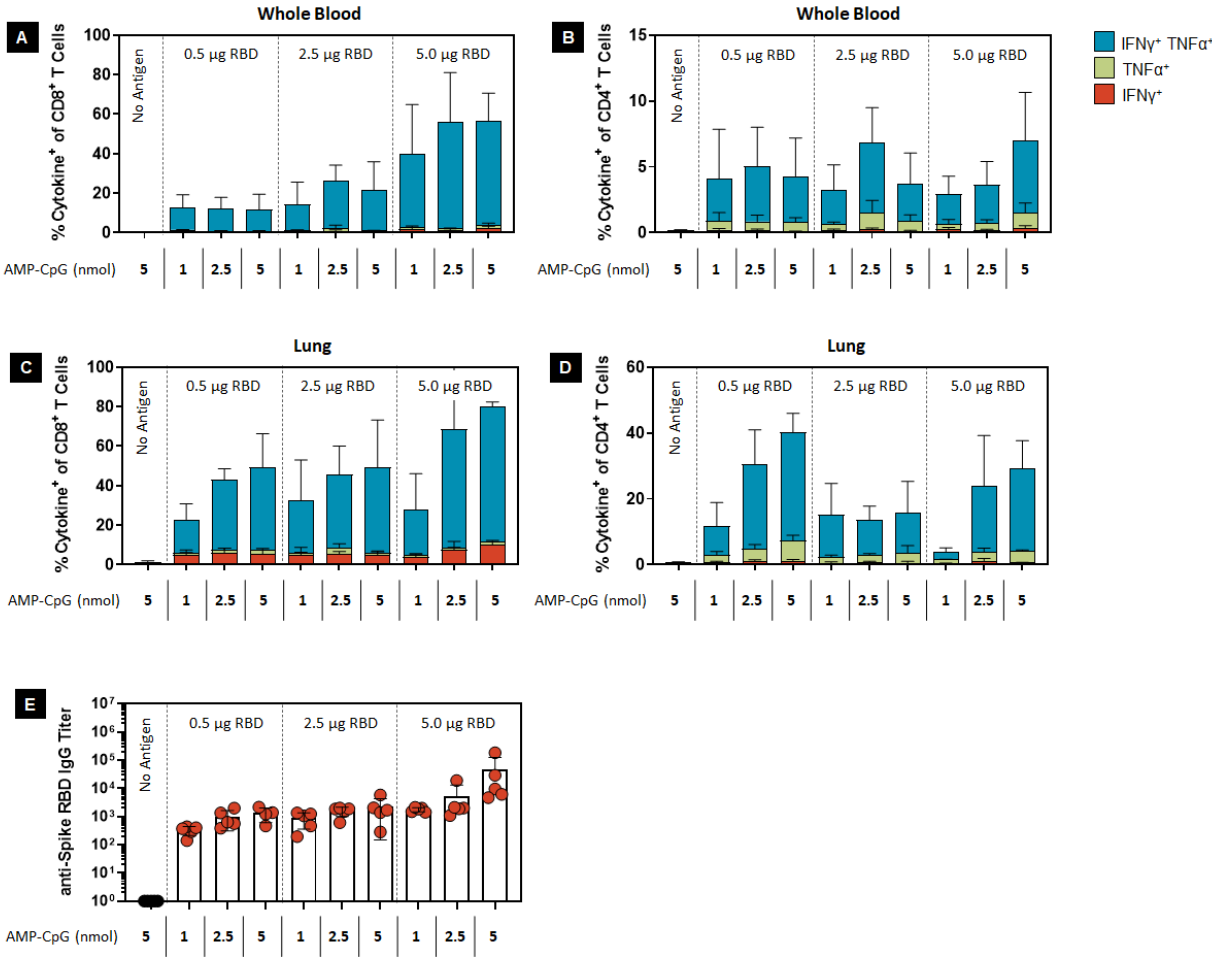

Figure S1: Two-dose vaccination with AMP-CpG-7909 elicits potent Spike RBD-specific cellular immunity in blood and lung, humoral immunity in blood. C57Bl/6 mice (n = 5 per group) were immunized on day 0 and 14 with 0.5, 1.0, or 5.0 µg Spike RBD protein admixed with 1.0, 2.5, or 5.0 nmol AMP-CpG, and T cell and IgG responses analyzed on day 21. **(A-B)** Peripheral blood cells or **(C-D)** cells collected from perfused lungs were restimulated with overlapping Spike RBD peptides and assayed by flow cytometry for intracellular cytokine production to detect antigen-specific T cell responses. Shown are frequencies of IFNγ, TNFα, and double-positive T cells among **(A, C)** $CD8^+$ and **(B, D)** $CD4^+$ T cells. Humoral responses specific to Spike RBD were assessed in serum from immunized animals by ELISA. Shown are **(E)** endpoint titers for IgG on day 35. n = 5 mice per group. Values depicted are mean ± standard deviation.

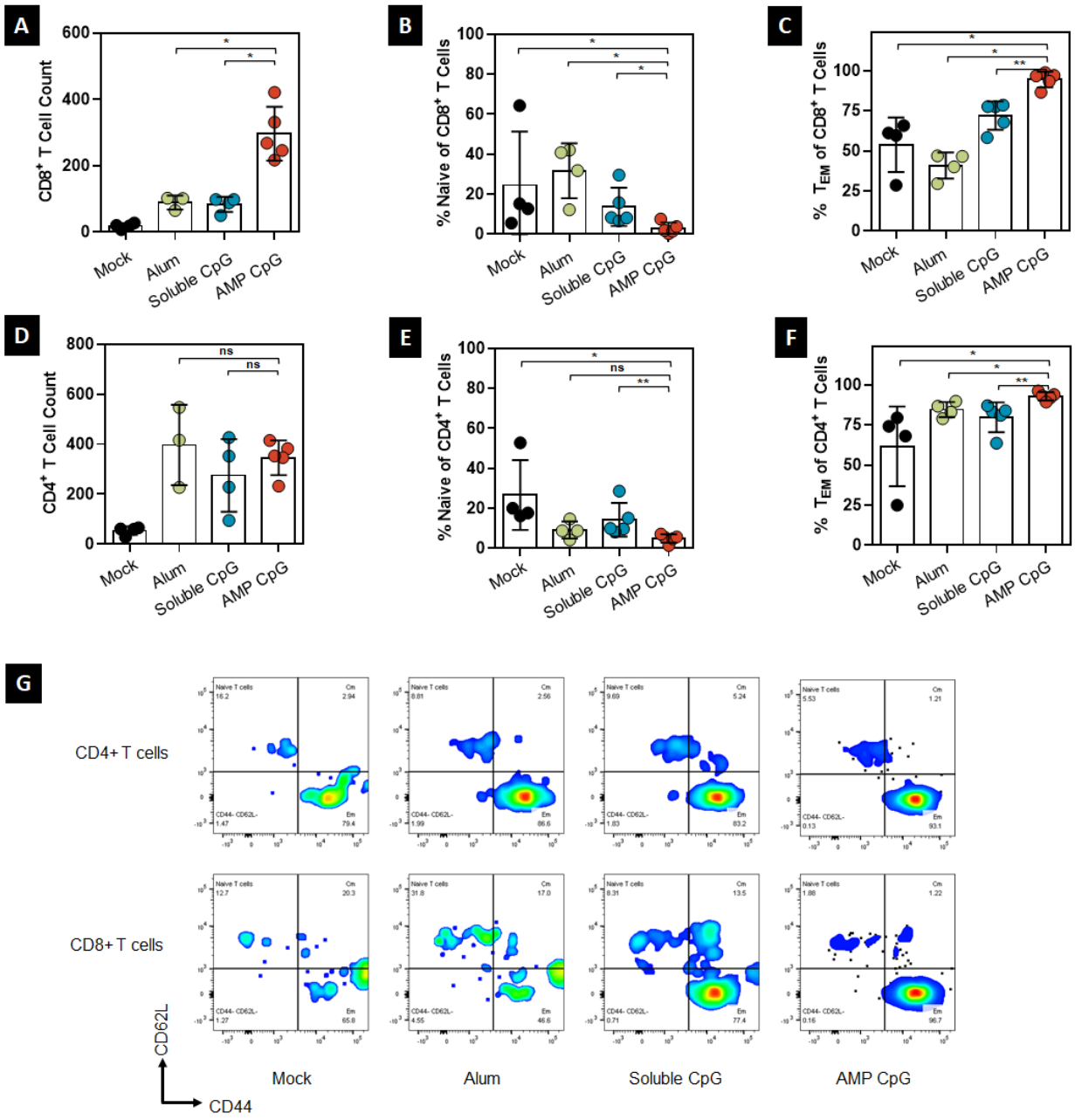

Figure S2: Vaccination with AMP-CpG elicits enhanced Spike RBD-specific T cell responses in BAL. C57Bl/6 mice (n = 10 per group) were immunized on day 0, 14, and 28 with 10 µg Spike RBD protein admixed with 100 µg Alum, 1 nmol soluble-, or AMP-CpG, and T cell responses analyzed on day 35. Cells collected from bronchoalveolar lavage (BAL) were analyzed for T cell phenotype by flow cytometry. Shown are total count (mean ± standard deviation) of **(A)** CD8+ T cells and frequencies of **(B)** naïve (CD44-CD62L+), and **(C)** effector memory (T_{EM}; CD44+ CD62L+) T cells among CD8+ T cells. Corresponding analyses are shown for **(D)** $CD4^+$ T cells, **(E)** naïve (CD44-CD62L+), and **(F)** effector memory (TEM; CD44+ CD62L+) T cells among $CD4^+$ T cells. n = 10 mice per group. **(G)** Example FACS plots for $CD4^+$ and $CD8^+$ T cells expressing CD44 and CD62L. Values depicted are mean ± standard deviation. * P < 0.05; ** P < 0.01; *** P < 0.001; **** P < 0.0001 by two-sided Mann-Whitney test applied to T cell frequencies.

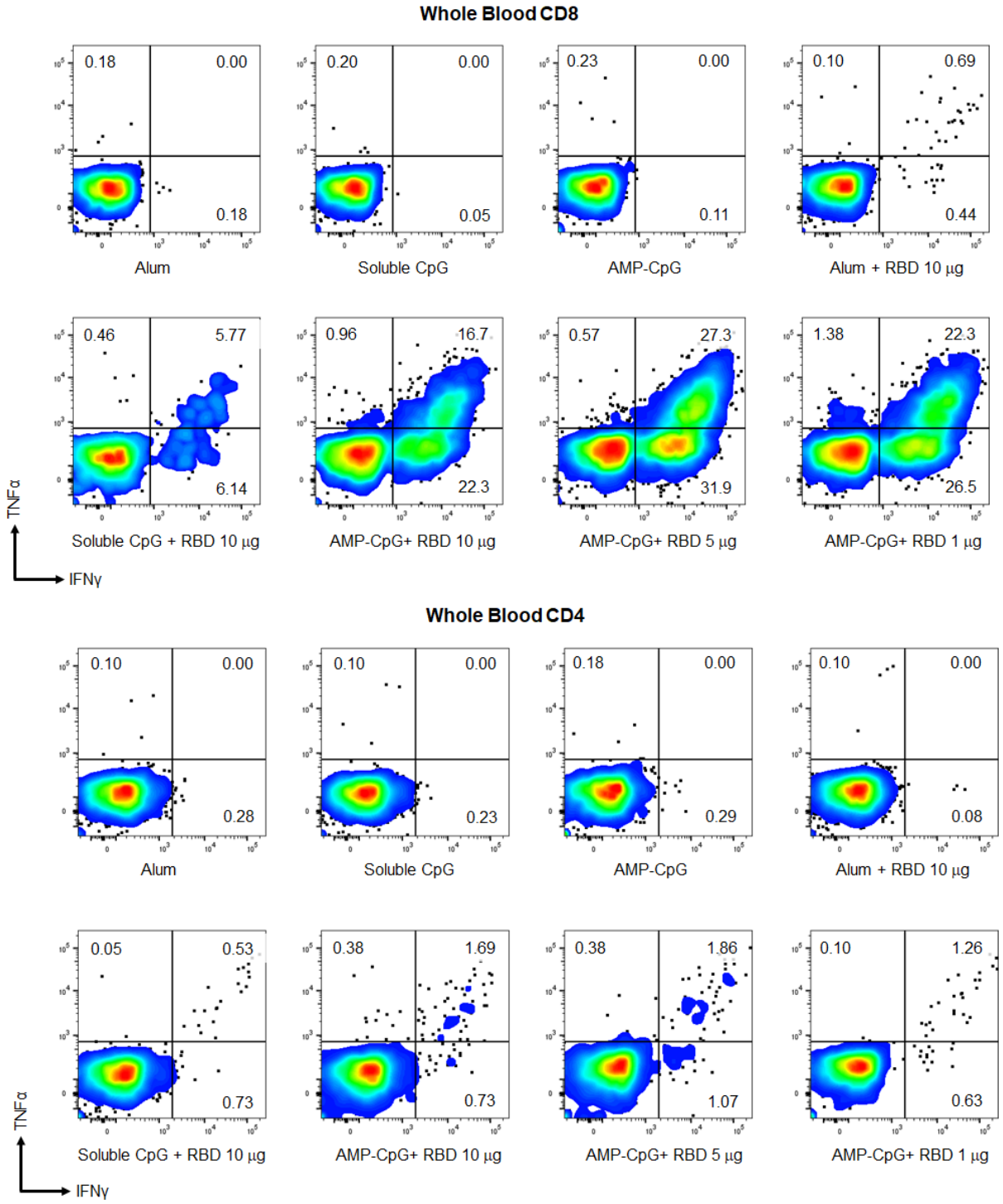

Figure S3: Example FACS plots for whole blood ICS in Figure 5. C57Bl/6 mice (n = 10 per group) were immunized on day 0, 14, and 28 with 10 µg Spike RBD protein admixed with 100 µg Alum or 1 nmol soluble-CpG. Comparator animals were dosed with 1, 5, or 10 µg Spike RBD admixed with 1 nmol AMP-CpG. Control animals were dosed with adjuvant alone. T cell responses were analyzed on day 35. Cells collected from peripheral blood were restimulated with overlapping Spike RBD peptides and assayed for intracellular cytokine production to detect antigen-specific T cell responses. Shown are example FACS plots for CD4+ and CD8+ T cells expressing IFNγ and TNFα.

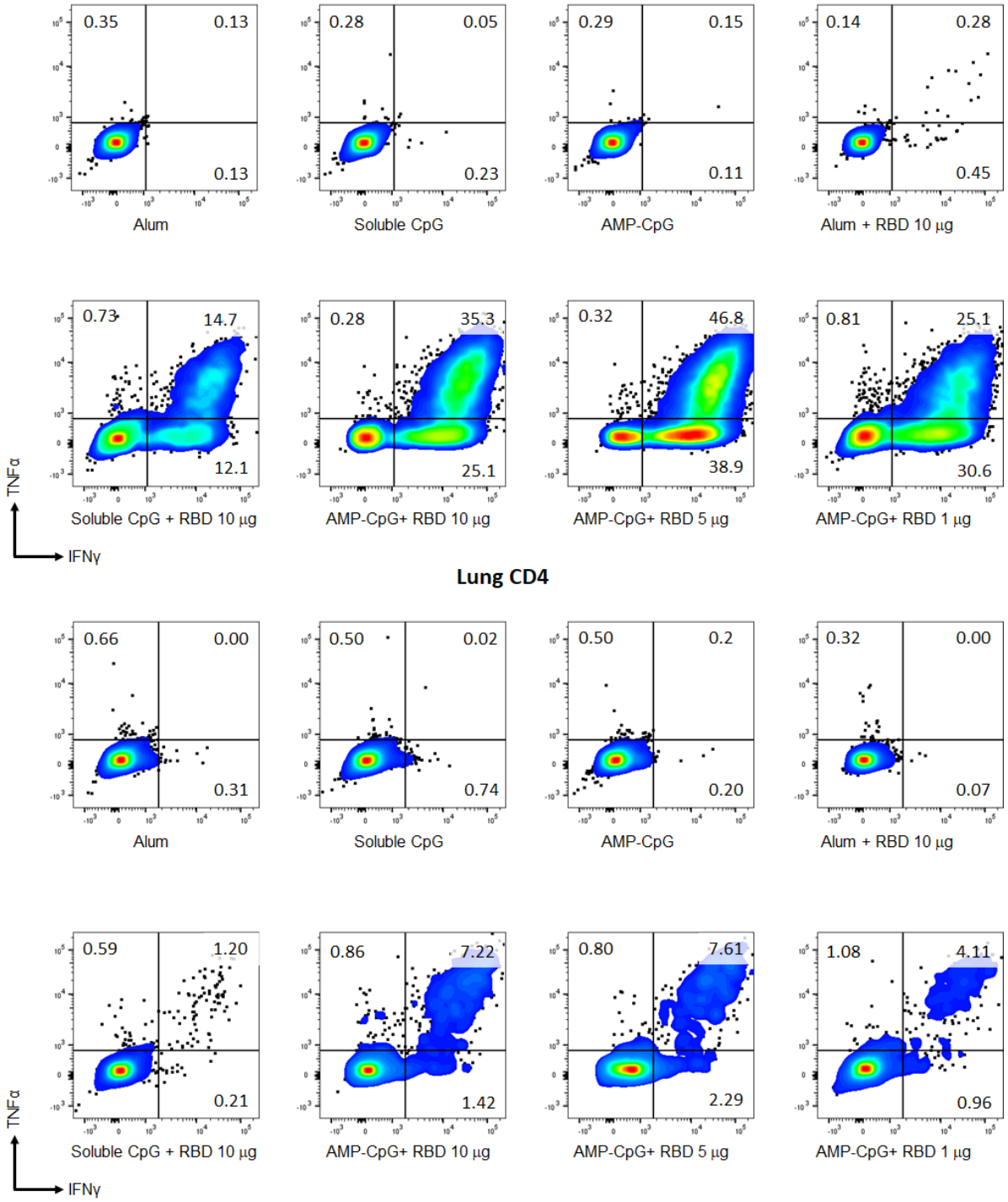

Figure S4: Example FACS plots for lung ICS in Figure 5. C57Bl/6 mice (n = 10 per group) were immunized on day 0, 14, and 28 with 10 µg Spike RBD protein admixed with 100 µg Alum or 1 nmol soluble-CpG. Comparator animals were dosed with 1, 5, or 10 µg Spike RBD admixed with 1 nmol AMP-CpG. Control animals were dosed with adjuvant alone. T cell responses were analyzed on day 35. Cells collected from perfused lung tissue were restimulated with overlapping Spike RBD peptides and assayed for intracellular cytokine production to detect antigen-specific T cell responses. Shown are example FACS plots for $CD4^+$ and $CD8^+$ T cells expressing IFNγ and TNFα.

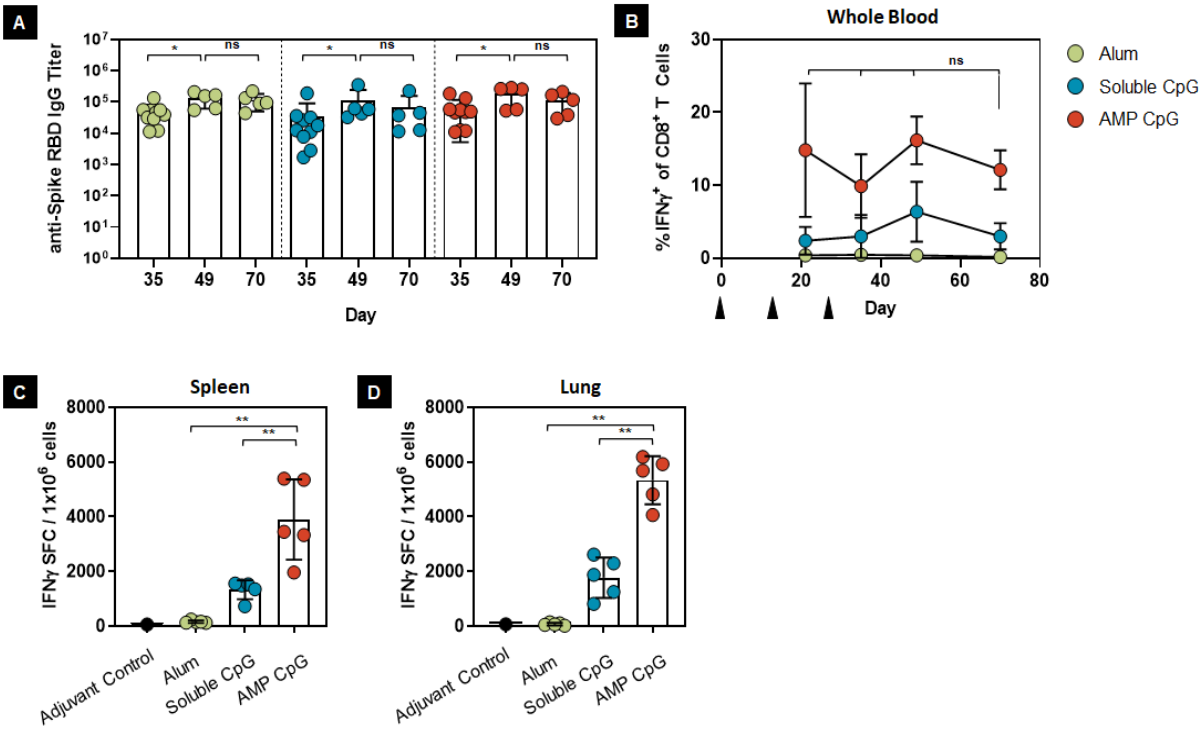

Figure S5: Vaccination with AMP-CpG in aged mice enables durable Spike RBD-specific T cells in blood, spleen, and lung tissue. 37-week-old C57Bl/6 mice (n = 5-10 per group) were immunized on day 0, 14, and 28 with 10 µg Spike RBD protein admixed with 100 µg Alum or 1 nmol soluble-, or AMP-CpG. Adjuvant control animals were dosed with AMP-CpG adjuvant alone. Humoral responses specific to Spike RBD were assessed in serum from immunized animals by ELISA on day 35, 49, and 70. Shown are **(A)** endpoint titers determined for IgG. T cell responses were analyzed on day 21, 35, 49, and 70. Cells were collected from **(B)** peripheral blood on day 21, 35, 49, and 70 and were restimulated with overlapping Spike RBD peptides and assayed for intracellular cytokine production to detect antigen-specific T cell responses. Shown are frequencies of IFNγ-positive cells among **(A)** peripheral blood $CD8^+$ T cells. **(C-D)** Cells were collected from **(C)** spleen and **(D)** lungs and were restimulated with overlapping Spike RBD peptides and assayed for IFNγ production by ELISpot assay. Shown are representative images of ELISpot and frequency of IFNγ spot forming cells (SFC) per $1x10^6$ cells. n = 5 mice per group. Values depicted are mean ± standard deviation. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; **** $P < 0.0001$ by two-sided Mann-Whitney test applied to cytokine+ T cell frequencies.