# Supplemental Information

## ARNAX is an ideal adjuvant for COVID-19 vaccines to enhance antigen-specific $CD4^+$ and $CD8^+$ T cell responses and neutralizing antibody induction

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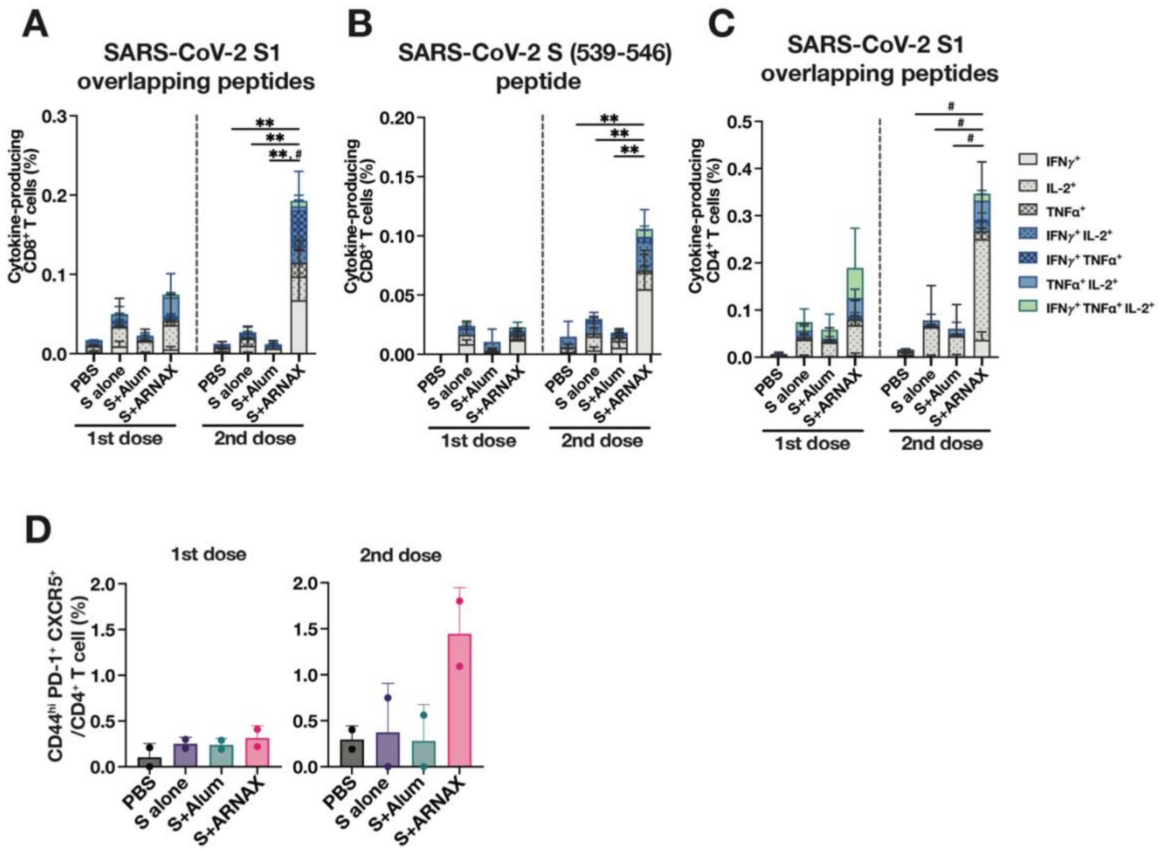


**Fig S1.** Analysis of cytokine producing T cells

Mice were immunized with each vaccine (n = 7-10). Ten days after the first dose or 4 days after the second dose of vaccine, lymph nodes were harvested. The lymph nodes from 3-4 mice for each experiment were pooled within the vaccine group as a single sample. The pooled lymph node cells were re-stimulated with overlapping peptides (A, C) or AA 539-546 peptide of S protein (B). The results of 3 independent experiments are combined and shown in A, B, and C. Percentages of cytokine-producing $CD4^+$ and $CD8^+$ T cells (IFN-γ, IL-2, and TNF-α) were analyzed by intracellular cytokine staining using flow cytometry. Individual cytokine patterns are represented by the following: light gray for IFN-$\gamma^+$, light gray with small dots for IL-$2^+$, light gray with large dots for TNF-$\alpha^+$, light blue with small dot for IFN-$\gamma^+$ IL-$2^+$, light blue with large dot for IFN-$\gamma^+$ TNF-$\alpha^+$, light blue for TNF-$\alpha^+$ IL-$2^+$, green for IFN-$\gamma^+$ TNF-$\alpha^+$ IL-$2^+$. Each error bar represents mean ± SD. Significance is calculated for total cytokine-producing cells and multiple cytokine-producing cells (colored bars) using Two-way ANOVA with Tukey's multiple comparisons test. For total cytokine-producing cells: $^{**}P<0.01$, multiple cytokine-producing cells: $^{\#}P<0.05$. The percentage of Tfh, defined as $CD44^{hi}$, PD-$1^+$, and $CXCR5^+$, in $CD4^+$ T cells in lymph nodes was analyzed (D). The results of 2 independent experiments are combined and shown in D.

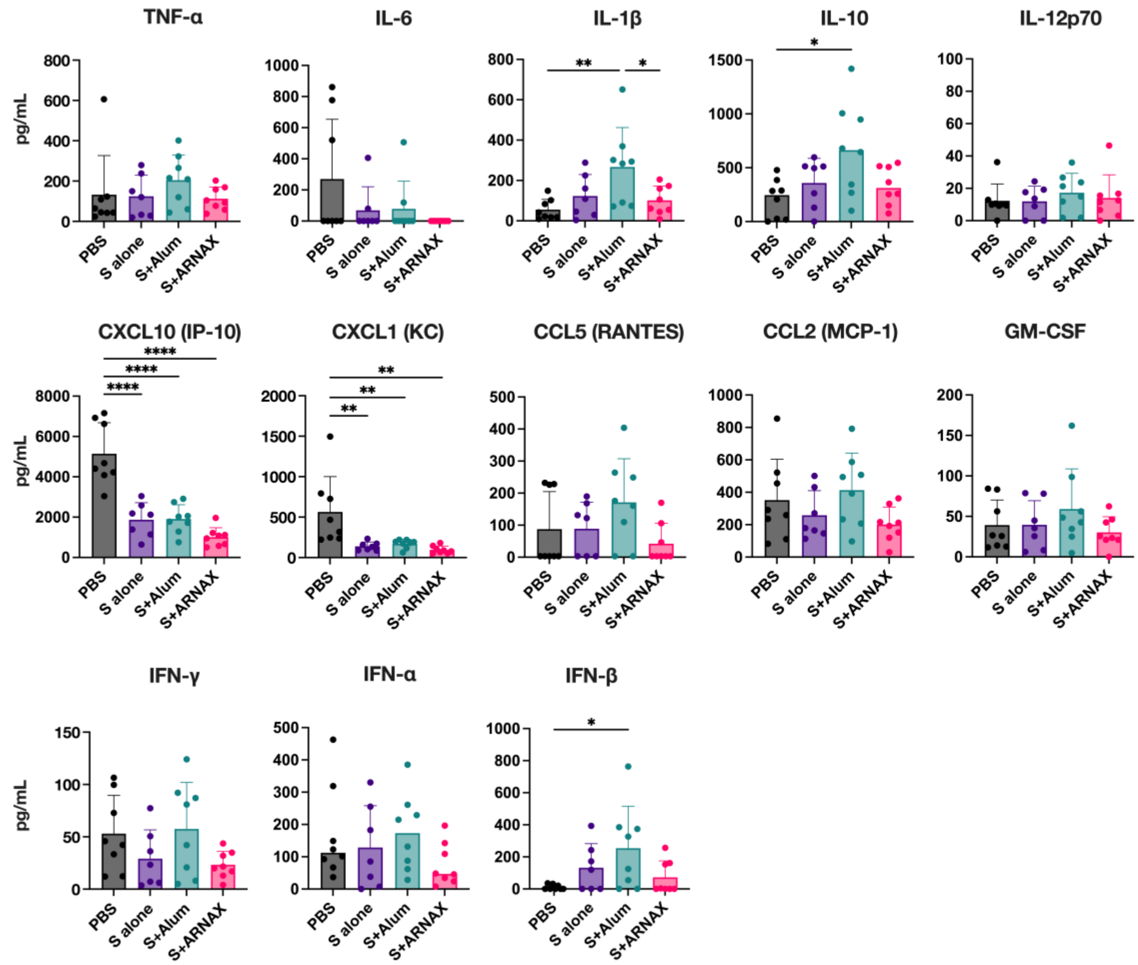


**Fig. S2.** Cytokine and chemokine levels in the sera at 3 days post-infection

Serum samples collected at 3 dpi were analyzed in a bead-based immunoassay to measure concentrations of cytokines and chemokines as indicated. Individual samples are shown as dots, horizontal lines indicate the median (n = 8). Significance was calculated using One-way ANOVA with Tukey's multiple comparisons test. *$P$<0.05, **$P$<0.01, ****$P$<0.0001.

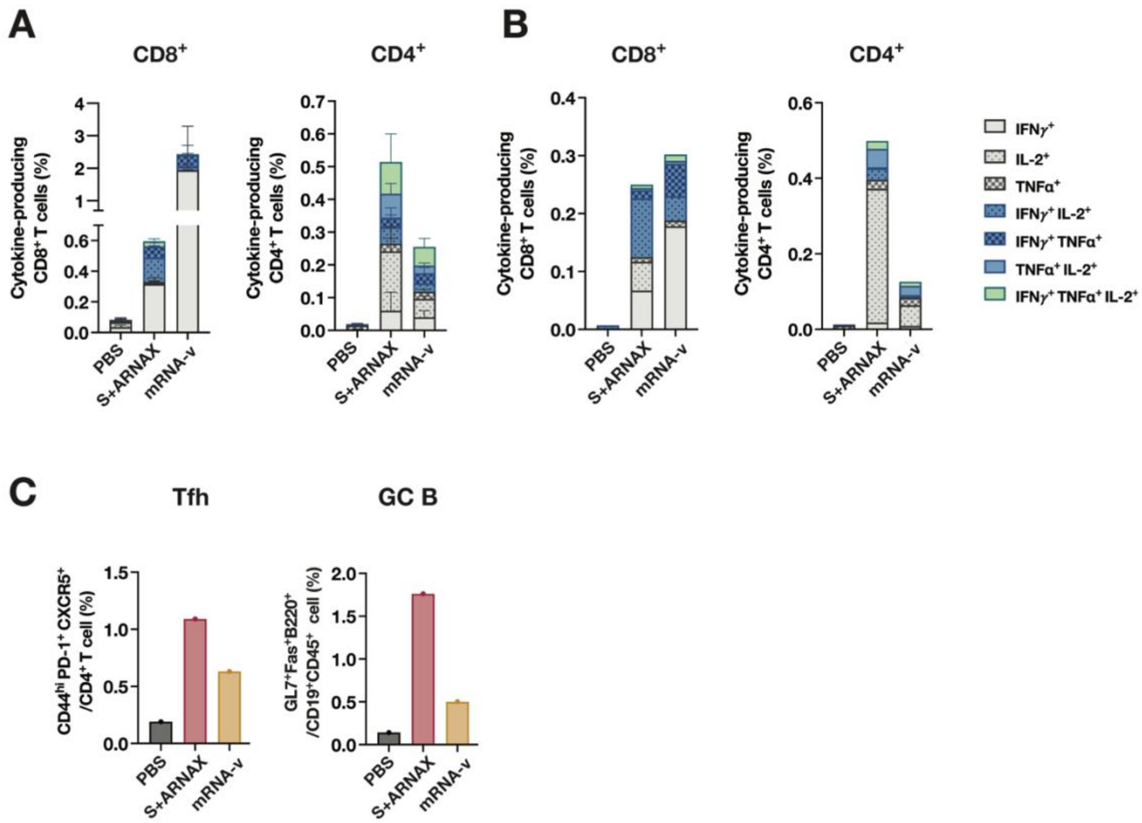


**Fig S3.** Comparison of T cell responses between S plus ARNAX and mRNA vaccines

Mice were immunized with each vaccine (n = 3). Four days after the second dose of vaccine, spleen and lymph nodes were harvested. The lymph nodes cells from 3-4 mice for each experiment were pooled within the vaccine group as a single sample. The splenocytes (A) and pooled lymph node cells (B) were re-stimulated with overlapping peptides of S protein. Percentages of cytokine-producing $CD4^+$ and $CD8^+$ T cells (IFN-γ, IL-2, and TNF-α) were analyzed by intracellular cytokine staining using flow cytometry. Individual cytokine patterns are represented by the following: light gray for IFN-$\gamma^+$, light gray with small dots for IL-$2^+$, light gray with large dots for TNF-$\alpha^+$, light blue with small dot for IFN-$\gamma^+$ IL-$2^+$, light blue with large dot for IFN-$\gamma^+$ TNF-$\alpha^+$, light blue for TNF-$\alpha^+$ IL-$2^+$, green for IFN-$\gamma^+$ TNF-$\alpha^+$ IL-$2^+$. Each error bar represents mean ± SD for splenocytes. The percentage of Tfh (defined as $CD44^{hi}$, PD-$1^+$, and $CXCR5^+$) in $CD4^+$ T cells and GCB (defined as $GL7^+$, $Fas^+$, $B220^+$) cells in $CD19^+$ $CD45^+$ B cells in lymph nodes was analyzed (C). Results are representative of at least two independent experiments.

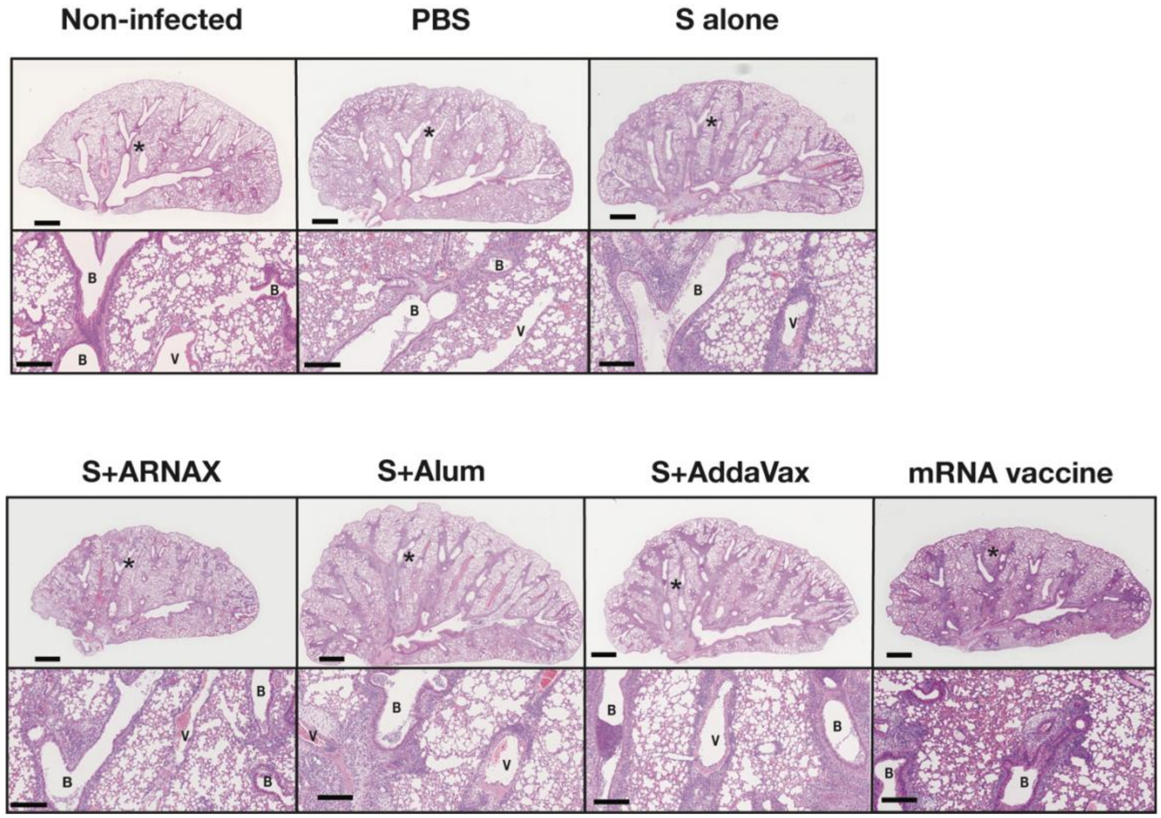


**Fig S4. Histopathology of lung section at 3 dpi**

Mice were challenged with $1 \times 10^5$ PFU of SARS-CoV-2 mouse-adapted strain 2 weeks after the second dose. The lung tissues were harvested at 3 dpi and stained with H&E. Representative pictures of each group (n = 5) were shown. The asterisk of upper panel is indicated indicates an enlarged area (bottom panel). The scale bars indicated 1mm (upper panel) and 250 µm. Bronchiole and vessel are marked as B and V respectively.

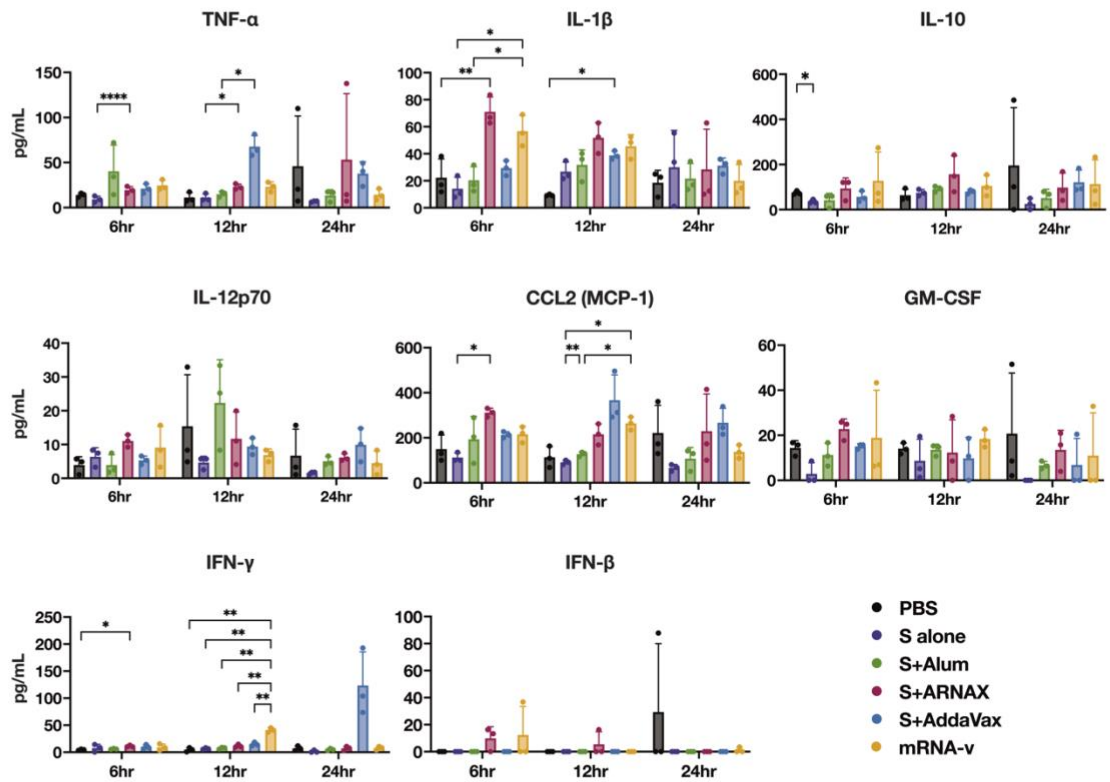


**Fig S5.** Cytokine and chemokine levels 6-24 hours after vaccination

Sera were collected from immunized mice at 6-24 hours after the first dose of vaccine, and concentrations of the indicated cytokines and chemokines were measured using a bead-based immunoassay (n = 3). Individual samples are shown as dots, horizontal lines indicate the median. Significance was calculated using One-way ANOVA with Tukey's multiple comparisons test. *$P$<0.05, **$P$<0.01, ****$P$<0.0001.

**Table S1** Cryo-EM data collection, refinement, and validation statistics

| | EMD-63071 spike protein |
| --- | --- |
| EMDB entry | EMD-63071 |
| Data collection and processing | |
| Magnification | 130,000 |
| Microscope | Krios G4 |
| Voltage (kV) | 300 |
| Detector | Gatan K3 |
| Energy filter | Gatan BioContinuum, 10 eV slit |
| Electric exposure (e-/Å) | 52.85 |
| Deforcus range (μm) | −0.5 to −3.0 |
| Pixel size (Å) | 0.67 |
| Data Processing Program | CryoSPARC (v.4.2.1) |
| Movies | 3,500 |
| Initial / Final particle images (no.) | 14,448,075 / 31,127 |
| Symmetry imposed | C1 |
| Map resolution (Å) | 3.67 |
| FSC threshold | 0.143 |