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Supplemental information

COVID-19 vaccine mRNA-1273 elicits a protective immune profile in mice that is not associated with vaccine-enhanced disease upon SARS-CoV-2 challenge

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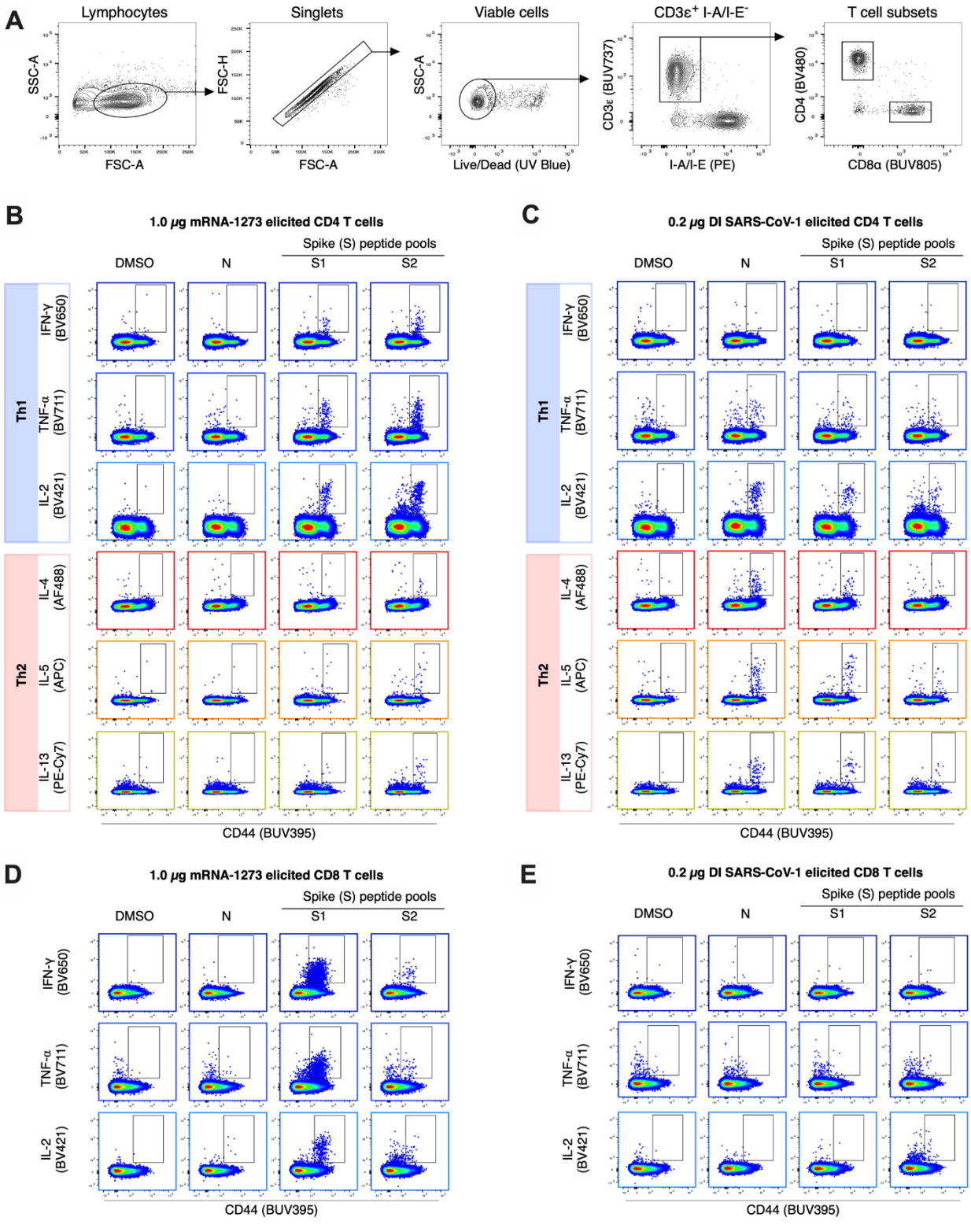
A
Lymphocytes
SSC-A
FSC-A
Singlets
FSC-H
FSC-A
Viable cells
SSC-A
Live/Dead (UV Blue)
CD3ε+ I-A/I-E-
CD3ε (BUV737)
I-A/I-E (PE)
T cell subsets
CD4 (BV480)
CD8α (BUV805)
B
1.0 μg mRNA-1273 elicited CD4 T cells
Spike (S) peptide pools
DMSO
N
S1
S2
Th1
IFN-γ (BV650)
TNF-α (BV711)
IL-2 (BV421)
Th2
IL-4 (AF488)
IL-5 (APC)
IL-13 (PE-Cy7)
CD44 (BUV395)
C
0.2 μg DI SARS-CoV-1 elicited CD4 T cells
Spike (S) peptide pools
DMSO
N
S1
S2
Th1
IFN-γ (BV650)
TNF-α (BV711)
IL-2 (BV421)
Th2
IL-4 (AF488)
IL-5 (APC)
IL-13 (PE-Cy7)
CD44 (BUV395)
D
1.0 μg mRNA-1273 elicited CD8 T cells
Spike (S) peptide pools
DMSO
N
S1
S2
IFN-γ (BV650)
TNF-α (BV711)
IL-2 (BV421)
CD44 (BUV395)
E
0.2 μg DI SARS-CoV-1 elicited CD8 T cells
Spike (S) peptide pools
DMSO
N
S1
S2
IFN-γ (BV650)
TNF-α (BV711)
IL-2 (BV421)
CD44 (BUV395)

Figure S1, Related to Figure 2. Representative gating tree for examination of antigen-specific $CD4^+$ and $CD8^+$ T cell responses by ICS. (A) A hierarchical gating strategy was used to identify viable $CD4^+$ and $CD8^+$ T lymphocytes. (B-C) IFNγ, TNFα, IL-2, IL-4, IL-5, and IL-13 cytokine reactivity patterns from $CD4^+$ T cells elicited by (B) 1 μg mRNA-1273 and (C) 0.2 μg DI SARS CoV-1 + alum following no peptide (DMSO), N, S1, and S2 peptide pool restimulation for 6 hours at 37°C, 5% CO_2 in the presence of a protein transport inhibitor cocktail containing Brefeldin A and Monensin (Thermo product 00-4980-03). IFNγ, TNFα, and IL-2 cytokine reactivity patterns from $CD8^+$ T cells elicited by (D) 1 μg mRNA-1273 and (E) 0.2 μg DI SARS CoV-1 + alum following no peptide (DMSO), N, S1, and S2 peptide pool restimulation. Data were analyzed in FlowJo software, v10.6.2.

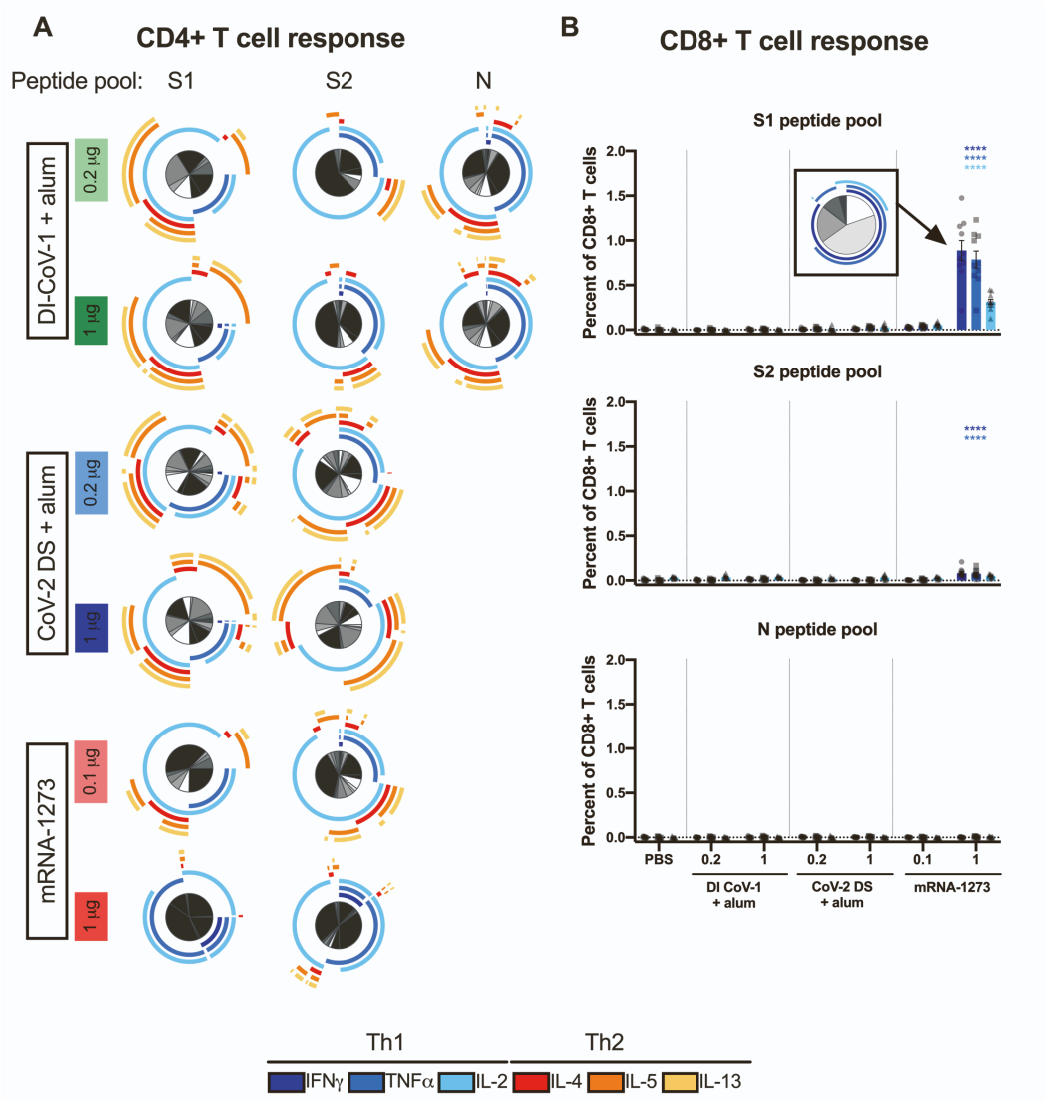

Figure S2, Related to Figure 2. Additional cytokine profiles of antigen-specific T cells from study 1. (A) Combination boolean gating analysis of cytokine co-expression patterns from CD4+ T cells isolated two weeks post-boost shown as arced pie graphs, stratified by dose and treatment group for each peptide pool (S1, S2, and N). Each slice of the pie represents the proportion of cells expressing a given cytokine expression profile, with each outer arc representing a given cytokine (Th1 cell-associated IFNγ, TNFα, IL-2 and Th2 cell-associated IL-4, IL-5, and IL-13). (B) Cytokine expression by $CD8^+$ T cells following restimulation with S1, S2, and N peptide pools. Data were analyzed by two-way ANOVA with Dunnett's multiple comparisons tests to determine expression significantly different from the naïve group for each cytokine. Significance is indicated above for each group (****$p<0.0001$). Error bars indicate the SEM.

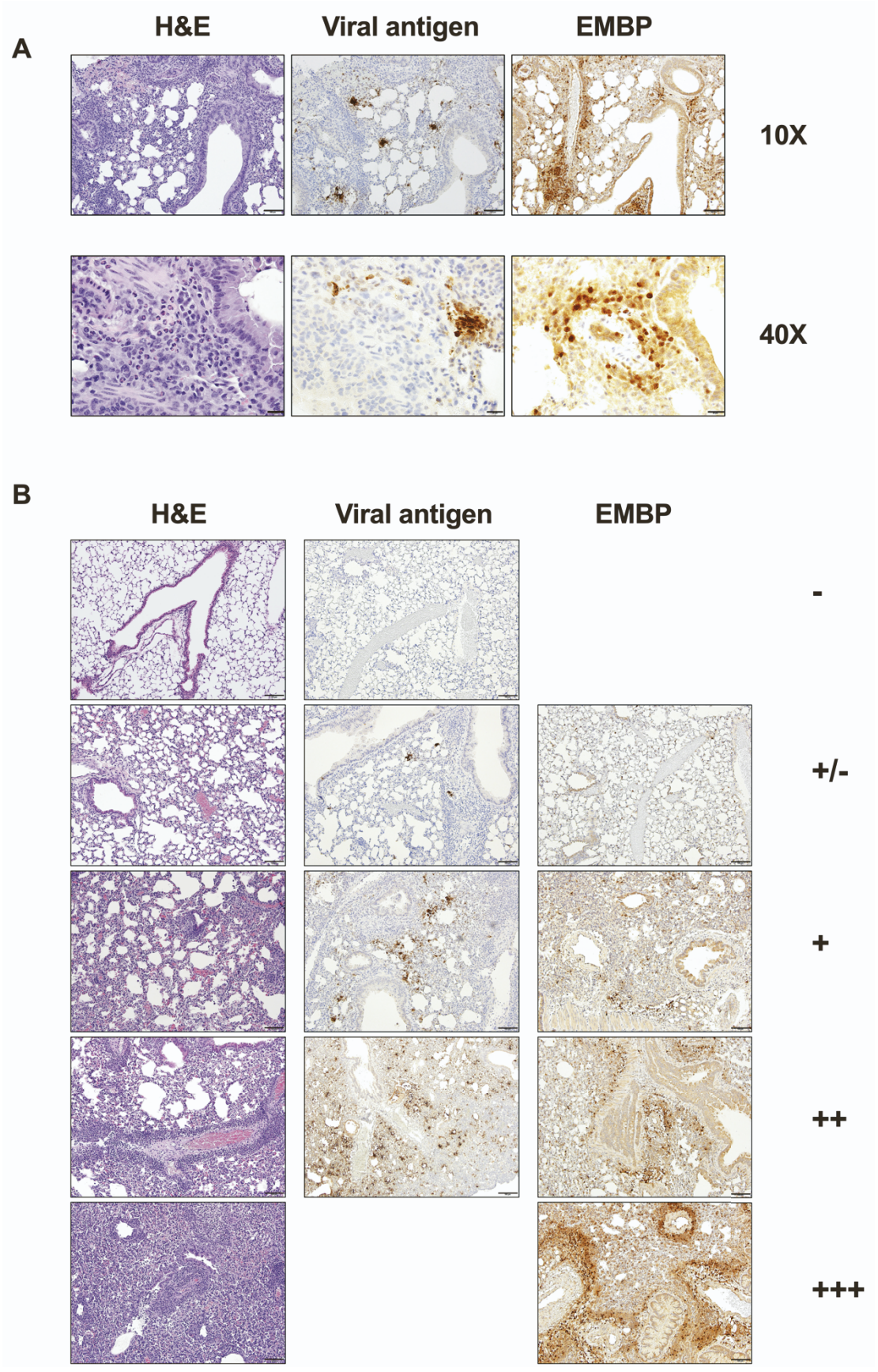
A
H&E
Viral antigen
EMBP
10X
40X
B
H&E
Viral antigen
EMBP
-
+/-
+
++
+++

Figure S3, Related to Figure 4 and Figure S5F. Histopathological assessment of lungs four days post-challenge. A) Representative photomicrographs (10X; 40X) from similar regions of the lung tissue highlight the general features and composition of the inflammatory cell infiltrates (H&E), distribution of SARS-CoV-2 virus antigen, and presence of eosinophils (EMBP). Scale bars represent 100 µm (10X) and 20 µm (40X). B) Photomicrographs (10X) demonstrating scoring for H&E, viral antigen, and EMBP. For H&E, - indicates no evidence of significant inflammation, +/- indicates mild expansion and hypercellularity of alveolar capillaries (AC), + indicates more noticeable expansion or thickening of AC, ++ indicates prominent expansion of AC with perivascular (PV) inflammation, +++ contains regions of consolidation (focal or extensive) and PV cellular infiltrates. For SARS-CoV-2 antigen (VAg), - indicates no evidence of virus antigen, +/- indicates occasional areas of VAg deposition, + indicates moderate-abundant deposition of VAg throughout the lung, and ++ indicates abundant VAg present throughout tissue. For EMBP, +/- indicates within normal limits to minimal increase, + inciates minimal to mild increase (scattered), ++ indicates mild to moderate increase (interstitium and loose perivascular), and +++ indicates moderate to abundant increase (interstitium and dense perivascular). Scale bars represent 100 µm.

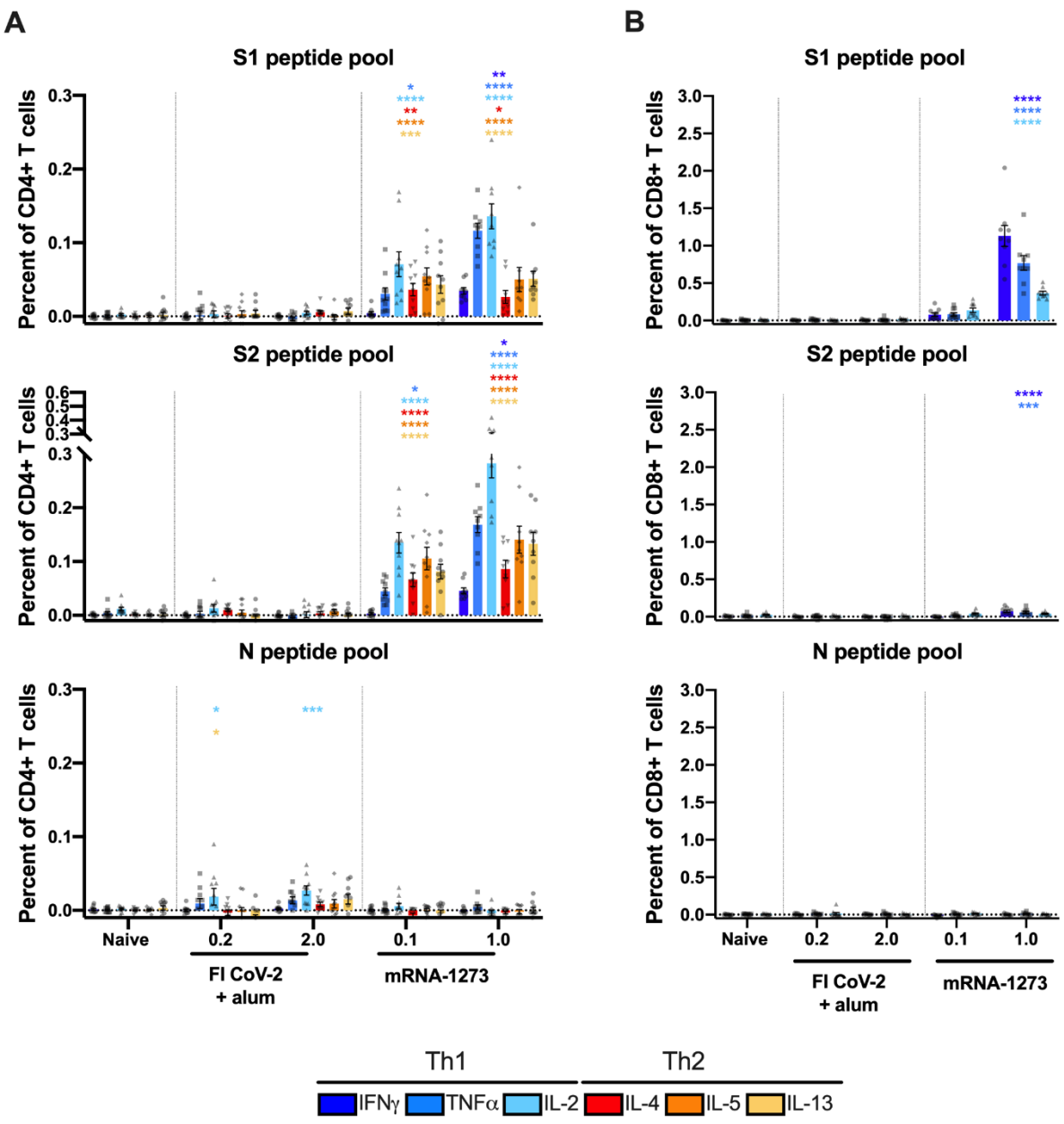

Figure S4, Related to Figure 6. T cell reactivity to spike and nucleocapsid peptide pools from study 2. (A) $CD4^+$ T cell and (B) $CD8^+$ T cell responses to S1, S2, and N peptide pools after background (no peptides, DMSO only) subtraction. Data were analyzed by two-way ANOVA with Dunnett's multiple comparisons tests to determine expression significantly different from the naïve group for each cytokine. Significance is indicated above for each group (*p<0.05, **p<0.01, ***p<0.001, ****p<0.0001). Error bars indicate the SEM.

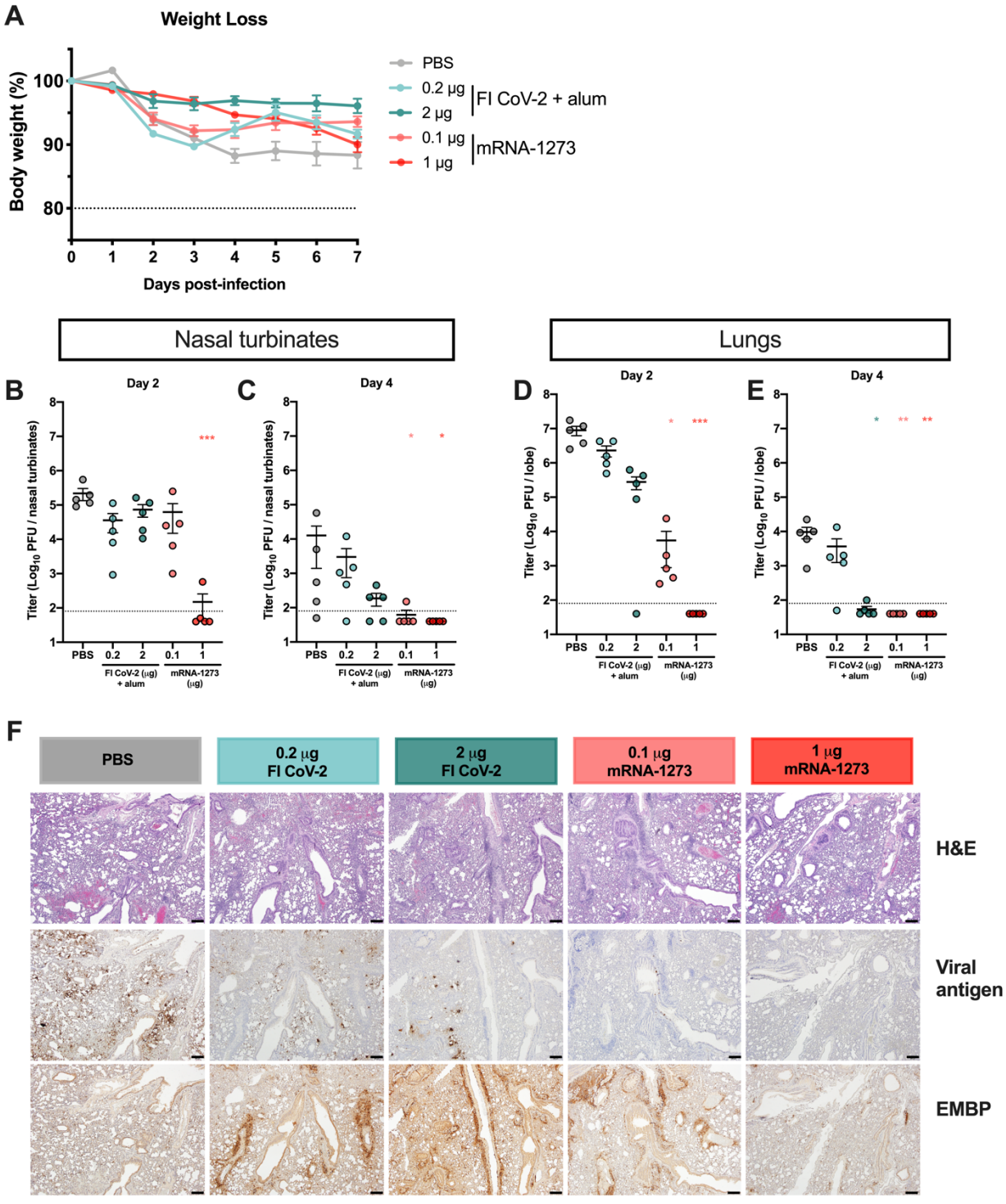
A
Weight Loss
Body weight (%)
Days post-infection
PBS
0.2 µg
2 µg
0.1 µg
1 µg
Fl CoV-2 + alum
mRNA-1273
Nasal turbinates
Lungs
B
Day 2
C
Day 4
D
Day 2
E
Day 4
Titer (Log10 PFU / nasal turbinates)
Titer (Log10 PFU / lobe)
Fl CoV-2 (µg) + alum
mRNA-1273 (µg)
F
PBS
0.2 µg Fl CoV-2
2 µg Fl CoV-2
0.1 µg mRNA-1273
1 µg mRNA-1273
H&E
Viral antigen
EMBP

Figure S5, Related to Figure 6. mRNA-1273 controls viral replication while FI CoV-2 enhances disease. (A) The percent of starting weight (day 0) was calculated for animals weighed through day 7 post-infection. N=10 mice per group, mean and SEM for each group is shown. The dotted line represents 80% of starting weight. Plaque-forming units of SARS-CoV-2 were measured from nasal turbinates on (B) day 2 and (C) day 4 post-infection, and in clarified lung supernatants obtained on (D) day 2 and (E) day 4 post-infection. The dotted line indicates the limit of detection, and samples with no detectable virus are plotted at half the limit of detection. Viral titer data were analyzed using a Kruskal-Wallis test of log-transformed data to identify groups significantly different than the PBS group (*$p<0.05$, **$p<0.01$, ***$p<0.001$). (F) Inflammation, viral antigen, and eosinophilia were assessed four days after infection using H&E staining and IHC for viral antigen, and eosinophil major basic protein (EMBP) as indicated in the methods. Micrographs are 4X and the scale bar indicated is 200 μm; a representative animal from each group is shown. Scoring for each animal and a summary for each group are presented in Table S4. See also Figure S3.

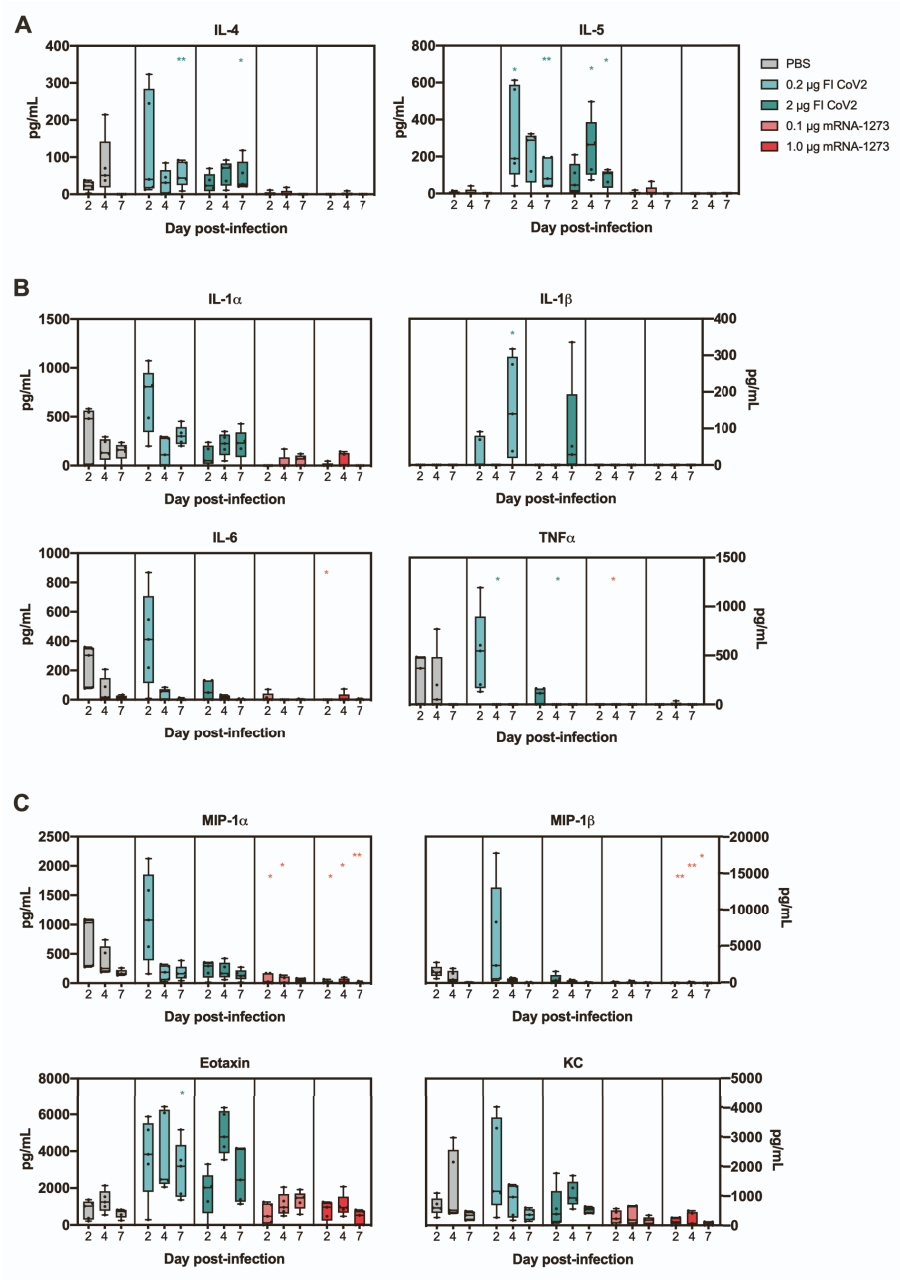

Figure S6, Related to Figure 6. Kinetic analysis of pulmonary cytokine signature in immunized mice infected with MA10 SARS-CoV-2 from study 2. Concentrations of (A) type 2 cytokines, IL-4 and IL-5, (B) inflammatory cytokines, IL-1α and IL-1β, IL-6, and TNFα, and (C) chemotactic mediators, MIP-1α, MIP-1β, Eotaxin, and KC on days 2, 4, and 7 post challenge using 10^5 PFU MA10 SARS-CoV-2. Data were analyzed using a Kruskal-Wallis test comparing all groups the PBS group at each timepoint (*p<0.05, **p<0.01, ***p<0.001)

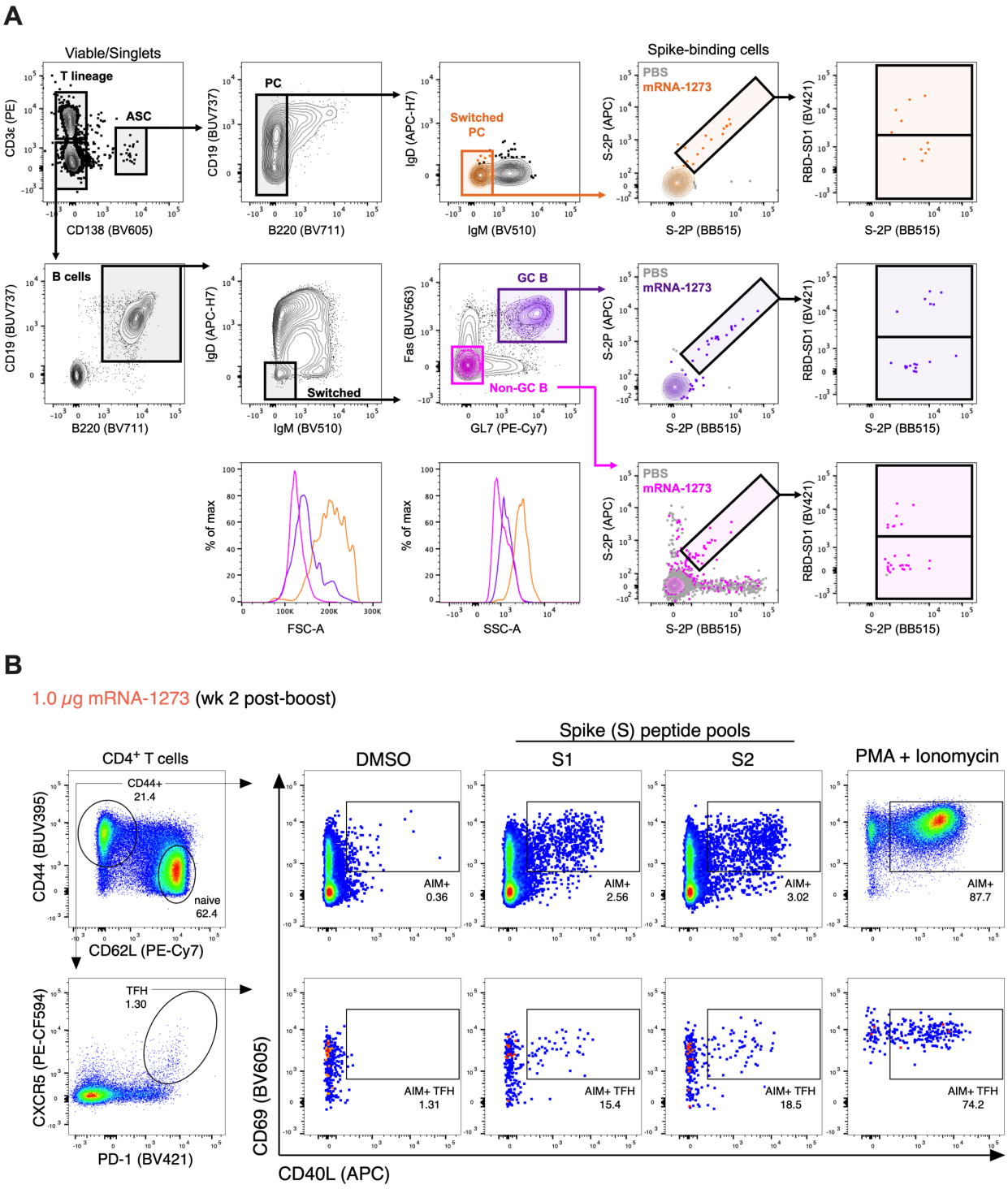
A
Viable/Singlets
T lineage
ASC
CD3ε (PE)
CD138 (BV605)
PC
CD19 (BUV737)
B220 (BV711)
Switched PC
IgD (APC-H7)
IgM (BV510)
Spike-binding cells
PBS
mRNA-1273
S-2P (APC)
S-2P (BB515)
RBD-SD1 (BV421)
B cells
Switched
GC B
Non-GC B
Fas (BUV563)
GL7 (PE-Cy7)
% of max
FSC-A
SSC-A
B
1.0 μg mRNA-1273 (wk 2 post-boost)
Spike (S) peptide pools
CD4+ T cells
DMSO
S1
S2
PMA + Ionomycin
CD44+
21.4
naive
62.4
CD44 (BUV395)
CD62L (PE-Cy7)
AIM+
0.36
2.56
3.02
87.7
TFH
1.30
CXCR5 (PE-CF594)
PD-1 (BV421)
AIM+ TFH
1.31
15.4
18.5
74.2
CD69 (BV605)
CD40L (APC)

Figure S7, Related to Figure 7. Representative gating tree for identification of probe-binding B cells and their subsets following mRNA-1273 immunization and representative staining of activation-induced markers (CD69 and CD40L) by $CD4^+$ T cells from mRNA-1273 immunized mice. (A) Antibody secreting cells (ASC) were defined as $CD3\varepsilon^-CD138^+$. Mature plasma cells (PC) were distinguished from plasmablasts based on B220 expression and class-switched cells were defined as IgM^-IgD^-. B cells were negative for CD138 but positive for B220 and CD19, and naïve cells (IgM^+IgD^+) were excluded from further analysis. Germinal center (GC) B cells or non GC B cells were defined by differential expression of GL7 and CD95 (Fas). Spike-binding cells were determined using bivariate analysis of cells co-capturing S-2P probes conjugated to two distinct fluorophores. Overlays of PC (orange), GC B (purple), and non GC B (magenta) cells from mice immunized with PBS (gray) or 1 μg mRNA-1273 reveal low background staining of probe reagents. The specificity of spike-binding cells were subsequently broken down into those targeting the RBD vs other protein surfaces using a RBD-SD1 probe. Analysis of forward (FSC) and side (SSC) scatter properties confirm the larger size and internal complexity of PC and GC B cells relative to non GC B cells. (B) Antigen experienced $CD4^+$ T cells were separated from naïve cells based on CD44 and CD62L expression. Shown are responses from all $CD44^+$ $CD4^+$ T cells (top row) and Tfh cells (bottom row) two weeks post-boost from 1 μg mRNA-1273-immunized mice, restimulated for 6 hours at 37°C, 5% CO_2 with no peptides (DMSO) compared to S1 and S2 peptide pools. Responses from PMA+ionomycin stimulated cells confirmed the functional responsiveness of cells used in the assay.

Supplemental Tables

Table S1, Related to Figure 1: Disposition of animals in Study 1

Animals	Mice used	Readout N	Experimental Readouts	Time point	Main Figures	Supplemental Figures	Supplemental Tables
Mice 1-10 Not Challenged	1-10	10	Spike-binding IgG subclass	2 weeks post-boost	Figure 2A-C		
	1-10	10	T cell intracellular cytokine staining	2 weeks post-boost	Figure 2D-F	Figure S1, S2	
Mice 11-30 Challenged with 10^4 PFU of SARS-CoV-2 MA10	11-30	20	Antibody binding and neutralization	2 weeks post-boost	Figure 1B-D		
	11-15	5	Day 2 post-infection nose, lung viral titers	2 days post-infection	Figure 3B, 3D		
	11-15	5	Day 2 post-infection lung cytokines	2 days post-infection	Figure 5A-C		
	16-20	5	Day 4 post-infection nose, lung viral titers	4 days post-infection	Figure 3C, 3E		
	16-20	5	Day 4 post-infection lung cytokines	4 days post-infection	Figure 5A-C		
	16-20	5	Day 4 post-infection histopathology	4 days post-infection	Figure 4	Figure S3	Table S2
	20-25	5	Day 7 post-infection lung cytokines	7 days post-infection	Figure 5A-C		
	20-30	10	Weight loss through 7 days post-infection	Days 0-7 post-infection	Figure 3A		

Table S2, Related to Figure 4: Day 4 histopathological scoring in the lungs of mice in Study 1

Group	H&E	SARS-CoV-2 IHC	EMBP
PBS	++	+	+/-
PBS	++	+	+/-
PBS	++	+	+
PBS	++	+	+/-
Group summary: Moderate to severe pulmonary inflammation, virus antigen detected occasionally, and eosinophils within normal limits.			
0.2 µg DI CoV-1	+++	+/-	++
0.2 µg DI CoV-1	+++	+/-	+++
0.2 µg DI CoV-1	+++	+	+++
0.2 µg DI CoV-1	+++	+/-	+++
0.2 µg DI CoV-1	+	-	+/-
Group summary: Severe pulmonary inflammation, virus antigen detected occasionally, and eosinophils moderately abundant			
1 µg DI CoV-1	+++	+/-	++
1 µg DI CoV-1	++	+/-	++
1 µg DI CoV-1	+++	+/-	++
1 µg DI CoV-1	++	+/-	+
1 µg DI CoV-1	++	+/-	++
Group summary: Moderate to severe pulmonary inflammation, virus antigen detected occasionally, and eosinophils mild to moderately increased			
0.2 µg CoV-2 DS	++	+/-	++
0.2 µg CoV-2 DS	+++	+/-	+++
0.2 µg CoV-2 DS	+++	+/-	++
Group summary: Severe pulmonary inflammation, virus antigen detected occasionally, and eosinophils range from minimally increased to moderately abundant			
1 µg CoV-2 DS	++	-	+
1 µg CoV-2 DS	+	+/-	+++
1 µg CoV-2 DS	++	+/-	+++
1 µg CoV-2 DS	+	+/-	+
1 µg CoV-2 DS	+	+/-	+
Group summary: Mild to moderate pulmonary inflammation, virus antigen detected occasionally, eosinophils range from minimally increased to moderately abundant			
0.1 µg mRNA-1273	++	+/-	+/-
0.1 µg mRNA-1273	++	+	+/-
0.1 µg mRNA-1273	+	+/-	+/-
0.1 µg mRNA-1273	+	+/-	+/-
0.1 µg mRNA-1273	++	+/-	+
Group summary: Moderately severe pulmonary inflammation, virus antigen detected occasionally, and eosinophils largely within normal limits			
1 µg mRNA-1273	+	-	+/-
1 µg mRNA-1273	+	-	+/-
1 µg mRNA-1273	+	-	+/-
1 µg mRNA-1273	+	-	+/-
1 µg mRNA-1273	+	-	+/-
Group summary: Mild to moderate pulmonary inflammation, virus antigen not detected, and eosinophils within normal limits			

Table S3, Related to Figure 6: Disposition of animals in Study 2

Animals	Mice used	Readout N	Experimental Readouts	Time point	Main Figures	Supplemental Figures	Supplemental Tables
Mice 1-10 Not Challenged	1-10	10	Total TFH and probe-binding B cell analysis	2 weeks post-boost	Figure 7A-D	Figure S7	
	1-10	10	Activation-induced marker assay – CD4+ and TFH cells	2 weeks post-boost	Figure 7E-G	Figure S8	
	1-10	10	T cell intracellular cytokine staining	2 weeks post-boost		Figures S1, S2, S4	
Mice 11-30 Challenged with 10^5 PFU of SARS-CoV-2 MA10	11-30	20	Antibody binding and neutralization	2 weeks post-boost	Figure 6B-E		
	11-15	5	Day 2 post-infection nose, lung viral titers	2 days post-infection		Figure S5B, S5D	
	11-15	5	Day 2 post-infection lung cytokines	2 days post-infection		Figure S6	
	16-20	5	Day 4 post-infection nose, lung viral titers	4 days post-infection		Figure S5C, S5E	
	16-20	5	Day 4 post-infection lung cytokines	4 days post-infection		Figure S6	
	16-20	5	Day 4 post-infection histopathology	4 days post-infection		Figure S5F, Figure S3	Table S4
	20-25	5	Day 7 post-infection lung cytokines	7 days post-infection		Figure S6	
	20-25	10	Weight loss through 7 days post-infection	Days 0-7 post-infection		Figure S5A	

Table S4, Related to Figure 6: Day 4 histopathological scoring in the lungs of mice in Study 2

Group	H&E	SARS-CoV-2 IHC	EMBP
PBS	+	++	+/-
PBS	++	++	+/-
PBS	+	++	+/-
PBS	++	++	+/-
PBS	+++	++	+/-
Group summary: Pulmonary inflammation ranged from mild/moderate to moderate/severe, virus antigen abundant, and eosinophils within normal limits to minimally elevated			
0.2 µg FI CoV-2	++	+	++
0.2 µg FI CoV-2	+++	+	++
0.2 µg FI CoV-2	++	+/-	++
0.2 µg FI CoV-2	+++	+	++
0.2 µg FI CoV-2	++	++	++
Group summary: Moderate/severe to severe pulmonary inflammation, virus antigen detected moderately to occasionally, eosinophils mild to moderately elevated			
2 µg FI CoV-2	++	+/-	++
2 µg FI CoV-2	++	+/-	++
2 µg FI CoV-2	++	-	++
2 µg FI CoV-2	+++	+/-	++
2 µg FI CoV-2	++	-	++
Group summary: Moderate to severe pulmonary inflammation, virus antigen ranged from no detection to detected occasionally, eosinophils mild to moderately elevated			
0.1 µg mRNA-1273	+	+/-	++
0.1 µg mRNA-1273	++	+/-	+
0.1 µg mRNA-1273	++	+/-	++
0.1 µg mRNA-1273	+	-	+/-
0.1 µg mRNA-1273	++	+/-	++
Group summary: Moderate to severe pulmonary inflammation, virus antigen detected occasionally, eosinophils mild to moderately elevated			
1 µg mRNA-1273	+	-	+
1 µg mRNA-1273	+	-	+/-
1 µg mRNA-1273	++	-	+
1 µg mRNA-1273	++	-	+
1 µg mRNA-1273	++	-	+/-
Group summary: Pulmonary inflammation ranged from mild/moderate to moderate/severe, virus antigen not detected, and eosinophils minimally elevated			