Supplemental Information

SARS-CoV-2 mRNA Vaccines Foster Potent Antigen-Specific Germinal Center Responses Associated with Neutralizing Antibody Generation

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Figure S1

A. CD38 vs IgD for GC B cells in Inginal LN on Day 7.

B. PCs in INGUINAL LNs on Day 7.

C. GC B cells in INGUINAL LN on Day 21.

D. GC B cells in POPLITEAL LNs on Day 7.

E. PCs in POPLITEAL LNs on Day 7.
Figure S2

A

Full S+ GC B Cells Popliteal LN

B

Full S+ GC B Cells Inguinal LN

C

RBD+ GC B Cells Popliteal LN

D

Full S+ GC B Cells Popliteal LN

E

GC B Cells Immunogens 20 μg

F

RBD+ GC B Cells Immunogens 20 μg

G

Full S+ GC B Cells Inguinal LN

H

GC B Cells Inguinal LN

I

RBD+ GC B Cells Inguinal LN

J

Lymphocytes Inguinal LN

K

GC B Cells Inguinal LN

L

RBD+ GC B Cells Inguinal LN
Figure S3

A. FULL S+ MBC PRECURSORS

B. FULL S+ IgG1 MBCs

C. FULL S+ IgG1 MBCs

D. FULL S+ IgG2a/2b MBCs

E. FULL S+ IgG2a/2b MBCs

F. RBD+ IgM MBCs

G. RBD+ IgM MBCs

H. FULL S+ IgM MBCs

I. FULL S+ IgM MBCs
Figure S5

A  

Day 7 Tfh (PD-1+CXCR5+)

B  

Day 7 Tfh (PD-1+CXCR5+)

C  

Day 21 Tfh (Bcl-6+CXCR5+)

D  

Day 21 Tfh (Bcl-6+CXCR5+)

E  

nAbs MEC (log) day 14

F  

nAbs MEC (log) day 14
Figure S7

A  

Tfh cells (Bcl-6+CXCR5+)

- % of activated CD4+
- # cells x 10^5

Luc mRNA
RBD mRNA
rRBD-AddaVax

B  

# GC B cells x 10^5

nAb IC_{50}

rRBD mRNA
rRBD-AddaVax

r = 0.244
p = 0.399
Table S1. Flow cytometry panel for Tfh detection. Related to Figures 5 and 6.

| Antibody/Conjugation | Conjugation | Dilution Factor | Clone  |
|----------------------|-------------|----------------|--------|
| CD16/CD32            | Purified    | 1:1000         | 2.4G2  |
| Fixable Viability Dye| eFluor780   | 1:2000         | n/a    |
| B220                 | BV650       | 1:400          | RA3-6B2|
| CXCR5                | Biotin      | 1:50           | SPRCL5 |
| Streptavidin         | BV421       | 1:500          | n/a    |
| CD4                  | PerCP-Cy5.5 | 1:200          | RM4-5  |
| PD-1                 | PE-Cy7      | 1:200          | RMP1-30|
| CD44                 | BV605       | 1:400          | IM7    |
| CD62L                | BUV395      | 1:400          | MEL-14 |
| Bcl6                 | AF647       | 1:200          | K112-91|
| ICOS                 | AF488       | 1:400          | C398.4A|

Table S2. Flow cytometry panel for identification of total and antigen specific GC B cells, and antigen specific MBC precursors in LNs. Related to Figures 1, 2 and 3.

| Antibody, protein or reagent | Conjugation | Dilution Factor | Clone  |
|------------------------------|-------------|----------------|--------|
| CD16/CD32                    | Purified    | 1:1000         | 2.4G2  |
| Streptavidin                 | AF488       | 1:500          | n/a    |
| Fixable Viability Dye        | eFluor780   | 1:2000         | n/a    |
| CD19                         | BV605       | 1:800          | 6D5    |
| FAS                          | BV510       | 1:800          | Jo2    |
| IgD                          | PE-Cy7      | 1:400          | 11-26c |
| GL7                          | PerCP-Cy5.5 | 1:400          | GL7    |
| CD3                          | APC-Fire750 | 1:400          | 17A2   |
| Ter-119                      | APC-Fire750 | 1:400          | Ter119 |
| CD138                        | BV650       | 1:400          | 281-2  |
| CXCR4                        | Biotin      | 1:200          | 2B11   |
| CD86                         | BV421       | 1:200          | GL1    |
| CCR6                         | BV786       | 1:400          | 29-2L17|
| Recombinant RBD or full S    | AF647       | 1:3200         | n/a    |
| Recombinant RBD or full S    | PE          | 1:1500         | n/a    |

Table S3. Flow cytometry panel for identification of antigen specific MBCs. Related to Figure 3.

| Antibody          | Conjugation | Dilution Factor | Clone  |
|-------------------|-------------|----------------|--------|
| CD16/CD32 Purified| Purified    | 1:1000         | 2.4G2  |
| Fixable Viability | eFluor780   | 1:2000         | n/a    |
| CD19              | BV605       | 1:800          | 6D5    |
| FAS               | BV510       | 1:800          | Jo2    |
| CD3               | APC-Fire750 | 1:400          | 17A2   |
| Ter-119           | APC-Fire750 | 1:400          | Ter119 |
| B220              | BV700       | 1:400          | RA3-6B2|
| CD38              | PE-Cy7      | 1:400          | 90     |
| IgG1              | eFluor450   | 1:400          | A85-1  |
| Antibody/Conjugation                  | Conjugation | Dilution Factor | Clone    |
|--------------------------------------|-------------|----------------|----------|
| IgG2a/2b                             | BB700       | 1:400          | R2-40    |
| IgM                                  | FITC        | 1:400          | Polyclonal |
| IgD                                  | BV650       | 1:400          | 11-26c   |
| Recombinant RBD or Full Spike        | AF647       | 1:3200         | n/a      |
| Recombinant RBD or Full Spike PE     | PE          | 1:1500         | n/a      |

Table S4. Flow cytometry panel for ICS experiments. Related to Figures 5 and 6.

| Antibody | Conjugation       | Dilution Factor | Clone     |
|----------|-------------------|-----------------|-----------|
| CD16/CD32| Purified          | 1:1000          | 2.4G2     |
| Fixable Viability Dye                | eFluor780      | 1:2000          | n/a       |
| CD4     | PerCP-Cy5.5       | 1:200           | RM4-5     |
| PD-1    | PE-Cy7            | 1:200           | RMP1-30   |
| CD44    | BV605             | 1:400           | IM7       |
| CXCR5   | BV421             | 1:50            | L138D7    |
| B220    | AF700             | 1:400           | RA3-6B2   |
| IL-4    | AF647             | 1:100           | 11B11     |
| IFN-γ   | BV650             | 1:100           | XMG1.2    |
| IL-21R FC Chimera                     | n/a             | 1:20           | n/a       |
| Anti-human IgG Fc                      | PE              | 1:50           | n/a       |

Table S5. Panel for confocal microscopy. Related to Figure 1.
SUPPLEMENTAL FIGURE TITLES AND LEGENDS

Figure S1. SARS-CoV-2 vaccines induce comparable B cell responses in both draining inguinal and popliteal LNs. Related to Figure 1. Mice were immunized into the gastrocnemius muscle (i.m.) with 30 µg of Luc, full S ∆ furin or RBD mRNA or 10 µg recombinant RBD protein adjuvanted with AddaVax (rRBD-AddaVax). LNs were analyzed 7 or 21 days later. (A) Representative analysis of plasma cells (PCs): cells are gated on, live, and dump CD19+ cells from inguinal LNs at day 7. (B) Frequency (left) and absolute numbers (right) of PCs in inguinal LNs at day 7. (C) Absolute numbers of GC B cells in inguinal LNs at day 21 (D) Frequency (left) and absolute numbers (right) of GC B cells in popliteal LNs at day 7. (E) Frequency (left) and absolute numbers (right) of PCs in popliteal LNs at day 7. Data in (B) and (E) were analyzed as detailed in A. Data in (D) and (E) were analyzed as detailed in Figure 1A. In (A-B) and (D-E) n = 9 mice per group were analyzed. Data were combined from three independent experiments. In (C) n = 6 mice per group were analyzed. Data were combined from two independent experiments. Data are shown as mean ± SEM and each point represents an individual mouse. One-way-ANOVA with Bonferroni correction or unpaired two-tailed Mann-Whitney U tests was conducted according to the distribution of the data. * p ≤ 0.05, ** p ≤ 0.01, *** p ≤ 0.001, **** p ≤ 0.0001.

Figure S2. Immunization with SARS-CoV-2 mRNA vaccines elicit full S-specific GC B cell responses. Related to Figure 2. For panels (A-D) and (G-L) mice were i.m. immunized with SARS-CoV-2 mRNA vaccines, Luc mRNA control or rRBD-AddaVax as previously described. For panels (E-F) mice were immunized i.m. with 20 µg of RBD mRNA or 20 µg of rRBD-AddaVax. (A-F) Data were analyzed at day 7. (G-L) GC B cells from draining inguinal LNs were measured 7, 14, 21 or 28 days post immunization. (A) Representative contour plots showing full S-specific GC B cells in the different study groups, defined as live, dump, CD19+ FAS+ GL7+ full S-PE+ full S-AF647+ cells. (B-F) Frequency (left) and absolute numbers (right) of: (B) full S-specific GC B cells in inguinal LNs (as defined in A); (C) RBD-specific GC B cells in popliteal LNs, as defined in Figure 2A. (D) Full S-specific GC B cells in popliteal LNs (as explained in A); (E) GC B cells in inguinal LNs (as defined in Figure 1A); (F) RBD-specific GC B cells in inguinal LNs (As defined in Figure 2A). (G-L) Kinetics of: (G) absolute numbers of full S-specific GC B cells in inguinal LNs; (H) GC B cell frequencies in inguinal LNs; (I) frequency of RBD-specific
GC B cells in inguinal LNs, (J) total lymphocyte numbers in inguinal LNs; (K) absolute numbers of GC B cells in inguinal LNs of rRBD-AddaVax immunized mice; (L) absolute numbers of RBD-specific GC B cells in inguinal LNs of rRBD-AddaVax immunized mice. For kinetic plots, day 0 represents the average of 18 naive mice. For (K-L), red dotted line represents the mean of RBD mRNA vaccinated mice at designated timepoints.

In (A-C), n = 8 mice per group were analyzed for RBD mRNA group, and n = 9 mice per group for all other groups were analyzed. Data are combined from three independent experiments. In (D) n = 9 mice per group were analyzed. Data are combined from three independent experiments. In (E) and (F), n = 8 mice per group were analyzed. Data are combined from two independent experiments. In (B-F), each point represents an individual mouse. In (G) and (I), the same number of mice per group were analyzed as in (A-C) for days 7 and 14. n = 10 mice per group were analyzed and data are combined from two independent experiments at day 28. In (H) and (J), the same number of mice per group were analyzed as in (D) for days 7 and 14. n = 10 mice per group were analyzed and data are combined from two independent experiments at day 28. In (K) and (L), n = 9 mice per group were analyzed for days 7 and 14. n = 6 mice per group were analyzed for day 21. For kinetic plots (G-J), statistics were calculated versus Luc mRNA group. Data are graphed as mean ± SEM. One-way-ANOVA with Bonferroni correction or unpaired two-tailed Mann-Whitney U tests was conducted according to the distribution of the data. * p ≤ 0.05, ** p ≤ 0.01, *** p ≤ 0.001, **** p ≤ 0.0001

Figure S3. SARS-CoV-2 mRNA vaccines promote the generation of full S-specific MBC precursors and bona fide MBCs. Related to Figure 3. Mice were i.m. immunized with SARS-CoV-2 mRNA vaccines, Luc mRNA control or rRBD-AddaVax as previously described. (A) Inguinal LNs were analyzed 7 days post immunization. Frequency (left) and absolute numbers (right) of full S-specific MBC precursors. (B-I) Spleens were analyzed 60 days post immunization. (B) Representative contour plots of full S-specific IgG1+ MBCs, pre-gated on singlets, live, dump−, and CD19+B220−IgD−FAS−CD38−IgG1+ cells. (C) Frequency (left) and absolute numbers (right) of full S-specific IgG1+ MBCs as explained in (B). (D) Representative flow cytometry analysis of full S-specific IgG2a/2b+ MBCs, pre-gated on singlets, live, dump−, and CD19+B220−IgD−FAS−CD38−IgG2a/2b+ cells. (E) Frequency (left) and absolute numbers (right) of full S-specific IgG2a/2b+ MBCs as explained in (D). (F, H) Representative contour plots of: (F) RBD-specific
In (A-I), n = 8 mice per group were analyzed for RBD mRNA group, and n = 9 mice per group for all other groups were analyzed. Data are combined from three independent experiments. In (A), (C), (E), (G) and (I) data are shown as mean ± SEM and each point represents an individual mouse. One-way-ANOVA with Bonferroni correction or unpaired two-tailed Mann-Whitney U tests was conducted according to the distribution of the data. * p ≤ 0.05, ** p ≤ 0.01, *** p ≤ 0.001, **** p ≤ 0.0001.

**Figure S4. SARS-CoV-2 mRNA immunized animals exhibit elevated SARS-CoV-2-specific Ab titers and nAbs. Related to Figure 4.** Mice were i.m. immunized with SARS-CoV-2 mRNA vaccines, Luc mRNA control or rRBD-AddaVax as previously described. (A-D) Serum was collected at 14 (left), 28 (middle) and 60 (right) days post immunization. SARS-CoV-2 specific Ab titers were determined by ELISA. RBD-specific: (A) IgG; (B) IgG1; (C) IgG2a and (D) IgG2b responses are plotted. (E) Bone marrow (BM) was collected at day 60 post immunization. Quantification of full S-specific IgG+ ASC in BM was determined by ELISPOT. (F-J) Spearman correlations of: (F) RBD-specific IgG titers and nAb levels (MEC) at 60 days post immunization; (G) RBD-specific IgG+ ASC and nAb levels (MEC) at 60 days post immunization; (H) inguinal LN GC B cells (cells x 10^5) and nAb levels (MEC) at 14 days post immunization; (I) RBD-specific GC B cells (cells x 10^5) from inguinal LNs and nAb levels (MEC) at 14 days post immunization; and (J) full S-specific GC B cells (cells x 10^5) from inguinal LNs and nAb levels (MEC) at 14 days post immunization.

In (A-D) and (F-H) n = 9 mice per group were analyzed. Data are combined from three independent experiments. In (E) n = 6 mice per group were analyzed. Data are combined from two independent experiments. In (I-J) n = 8 mice per group were analyzed for RBD mRNA group, and n = 9 mice per all other groups were analyzed. Data are combined from three independent experiments. In (A-D) data is shown as geometric mean ± geometric SD. In (E) mean ± SEM are shown. In all graphs, each point represents an individual mouse. One-way-ANOVA with Bonferroni correction or unpaired two-tailed Mann-Whitney U tests was conducted according to the distribution of the data. * p ≤ 0.05, ** p ≤ 0.01, *** p ≤ 0.001, **** p ≤ 0.0001.
Figure S5. Tfh cells are increased in mRNA-vaccinated animals. Related to Figure 5. Mice were i.m. immunized with SARS-CoV-2 mRNA vaccines, Luc mRNA control or rRBD-AddaVax as previously described. Naive mice were also included as control. Tfh cells from (A-E) inguinal or pooled inguinal and (F) popliteal LNs were evaluated at different time points. (A) Representative contour plots of Tfh cell defined as CXCR5⁺PD-1⁺ cells pre-gated on singlets, live, and B220⁻CD4⁺CD44hiCD62L⁻ populations at 7 days post immunization. (B) Frequency (left) and absolute numbers (right) of Tfh cells as defined in (A). (C) Frequency (left) and absolute numbers (right) of Tfh cells defined as live, B220⁻CD4⁺CD44hiCD62L⁻CXCR5⁺Bcl-6⁺ at 21 days post immunization. (D-E) Spearman correlations of: (D) GC B cells (cells x 10⁵) and CXCR5⁺PD-1⁺ Tfh cells (cells x 10⁵) at 7 days post immunization and (E) CXCR5⁺PD-1⁺ Tfh cells (cells x 10⁵) and nAb levels at 14 days post immunization. (F) Representative gating strategy defining Tfh cells in ICS experiments upon SARS-CoV-2 peptide pool stimulation at 7 days post immunization. In (A-B) and (D-E) n = 8 mice per group were analyzed for RBD mRNA group, and n = 9 mice per group for all other groups were analyzed. Data are combined from three independent experiments. In (C) n = 6 mice per group were analyzed. Data are combined from two independent experiments. In (B-C) data is graphed as mean ± SEM. In (B-E) each point represents an individual mouse. One-way-ANOVA with Bonferroni correction or unpaired two-tailed Mann-Whitney U tests was conducted according to the distribution of the data. * p ≤ 0.05, ** p ≤ 0.01, *** p ≤ 0.001, **** p ≤ 0.0001

Figure S6. Features of Tfh cells induced by SARS-CoV-2 vaccines. Related to Figure 6. Balb/c (A-E) and (L-M) or C57BL/6J mice (F-K) were i.m. immunized with SARS-CoV-2 mRNA or rRBD-AddaVax as previously described and Tfh cells were evaluated at 7 days post immunization. (A) Representative gating strategy defining Tfh cells (CXCR5⁺PD-1⁺) by flow cytometry intracellular staining after PMA/ionomycin activation in inguinal LNs. (B, D) Representative flow cytometry analysis of: (B) IFN-γ⁺ or (D) IL-4⁺ Tfh cells (CXCR5⁺PD-1⁺) in pooled inguinal and popliteal LNs of the different study groups upon SARS-CoV-2 peptide stimulation. Cells were pre-gated on singlets, live, and CD4⁺B220⁻CD44lo populations. (C, E) Frequency of: (C) IFN-γ⁺ or (E) IL-4⁺ producing Tfh cells analyzed as detailed in (B) and (D), respectively. (F-G) In C57BL/6J mice, frequency of (F) IFN-γ⁺ or (G) IL-4⁺ producing Tfh cells analyzed as detailed in
(B) and (D), respectively. (H) Ratio of IL-4+ to IFN-γ+ producing Tfh cells in C57BL/6J mice. (I-K) In inguinal LNs of C57BL/6J mice, absolute numbers of: (I) GC B cells defined as live dump−CD19+ FAS+ GL7+; (J) RBD+ GC B cells defined as live dump−CD19+ FAS+ GL7+ RBD-AF647+ RBD-PE+; and (K) Tfh cells defined as live −CD4+B220+ CD44hi CD62L−CXCR5+ Bcl-6+. (L) Mean Fluorescence Intensity (MFI) of Bcl-6 on Tfh cells (CXCR5+ PD-1+) in inguinal LNs. (M) Spearman correlation of ICOS MFI and the frequency of Tfh cells (CXCR5+ Bcl-6+) in pooled inguinal and popliteal LNs.

In (A) and (L) n = 9 mice per group were analyzed. Data were combined from three independent experiments. In (B-E) n = 10 mice per group were analyzed. Data were combined from four independent experiments. In (F-K) n = 8 mice per group were analyzed. Data were combined from two independent experiments. In (M) n = 8 mice per group across two independent experiments. For (C), (E), and (F-K) data are graphed as mean ± SEM and each point represents an individual mouse. One-way-ANOVA with Bonferroni correction or unpaired two-tailed Mann-Whitney U tests was conducted according to the distribution of the data. * p ≤ 0.05, ** p ≤ 0.01, *** p ≤ 0.001, **** p ≤ 0.0001.

**Figure S7. Secondary Tfh cell differentiation following a booster immunization.** Related to Figure 7. Mice were i.m. immunized with Luc mRNA control, RBD mRNA, or rRBD-AddaVax as previously described. After 28 days, all groups received a second immunization with the same vaccine. Serum and draining inguinal LNs were analyzed 10 days following the second immunization. (A) Frequencies (left) and absolute numbers (right) of Tfh cells defined as live CD4+B220+ CD44hi CD62L− CXCR5+ Bcl-6+. (B) Spearman correlation analysis of absolute numbers of GC B cells and nAb IC50. In (A-B) n = 7 mice per group were analyzed. Data were combined from two independent experiments. Data are graphed as Mean ± SEM and each point represents an individual mouse. Unpaired two-tailed Mann-Whitney U tests was conducted according to the distribution of the data. * p ≤ 0.05, ** p ≤ 0.01, *** p ≤ 0.001, **** p ≤ 0.0001.