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Supplemental information

mRNA vaccination of naive and COVID-19-recovered individuals elicits potent memory B cells that recognize SARS-CoV-2 variants

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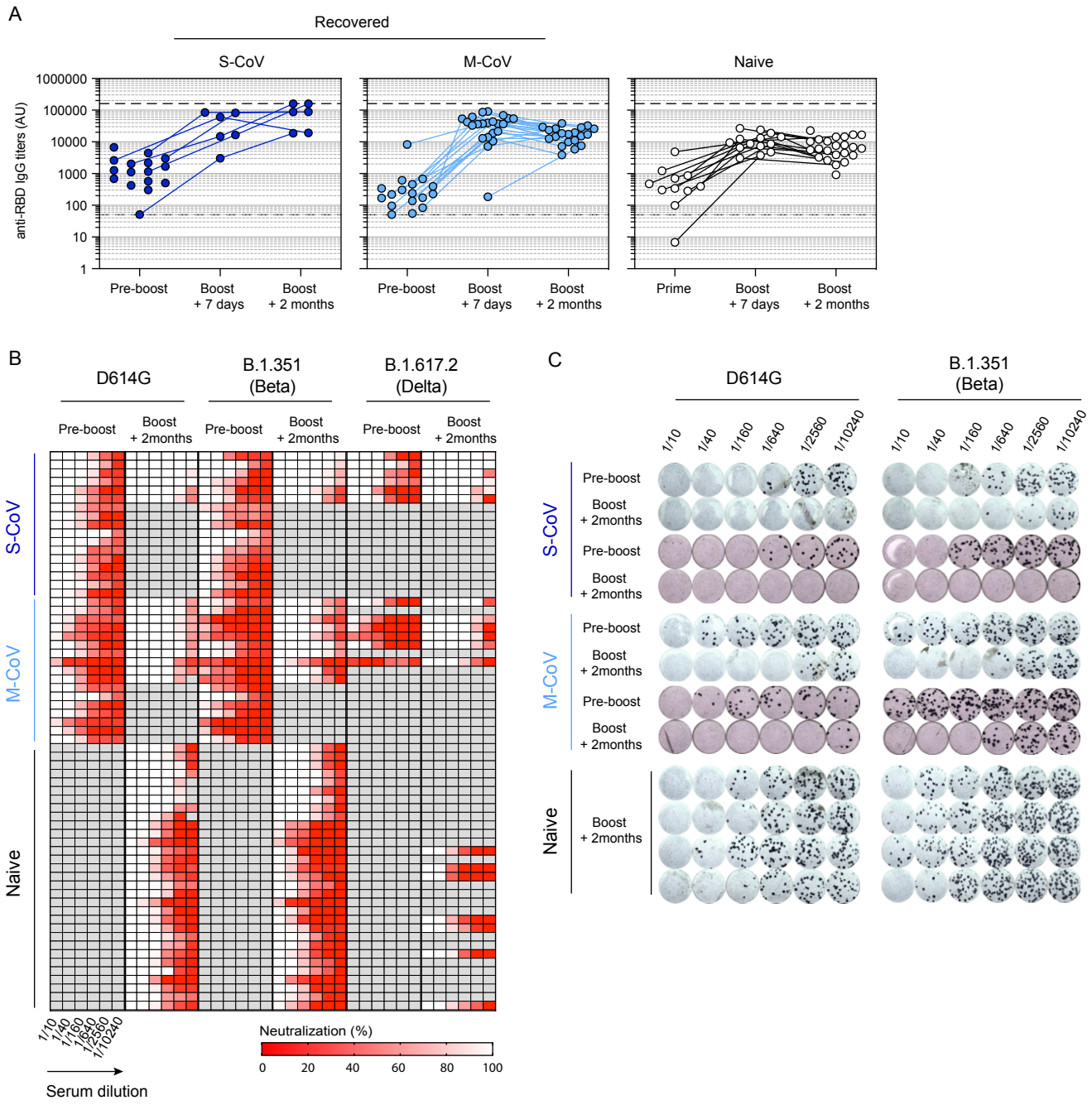

A
Recovered
S-CoV
M-CoV
Naive
anti-RBD IgG titers (AU)
1000000
100000
10000
1000
100
10
1
Pre-boost
Boost + 7 days
Boost + 2 months
Prime
B
D614G
B.1.351 (Beta)
B.1.617.2 (Delta)
Pre-boost
Boost + 2months
S-CoV
M-CoV
Naive
1/10
1/40
1/160
1/640
1/2560
1/10240
Serum dilution
Neutralization (%)
0
20
40
60
80
100
C
D614G
B.1.351 (Beta)
Pre-boost
Boost + 2months
S-CoV
M-CoV
Naive

Figure S1. mRNA vaccination boosts humoral response against WT SARS-CoV-2 and VOCs in naive and SARS-CoV-2 recovered patients. Related to Figure 1.

(A) Evolution of the anti-SARS-CoV-2 RBD serum IgG titers after BNT162b2 vaccination for each patient. ELISA values are shown at pre-boost (M6 or M12) for SARS-CoV-2 recovered (S-CoV: dark blue; left panel and M-CoV: light blue, middle panel) or after the prime for naive patients (white, right panel), as well as 7 days and 2 months after the vaccine boost. **(B)** Heatmap representing the observed in vitro neutralization of D614G SARS-CoV-2 (left), B.1.351 (middle) and B.1.617.2 (right) VOCs by sera from SARS-CoV-2 recovered and naive donors at the pre-boost and boost + 2 months time points (serial dilutions: 1/10, 1/40, 1/160, 1/640, 1/2560, 1/10240). Each line represents one patient tested against SARS-CoV-2. **(C)** Representative wells for the in vitro neutralization assay of sera against D641G SARS-CoV-2 (left) and B.1.351 VOC (right). Dark blue spots represent SARS-CoV-2 infected cells.

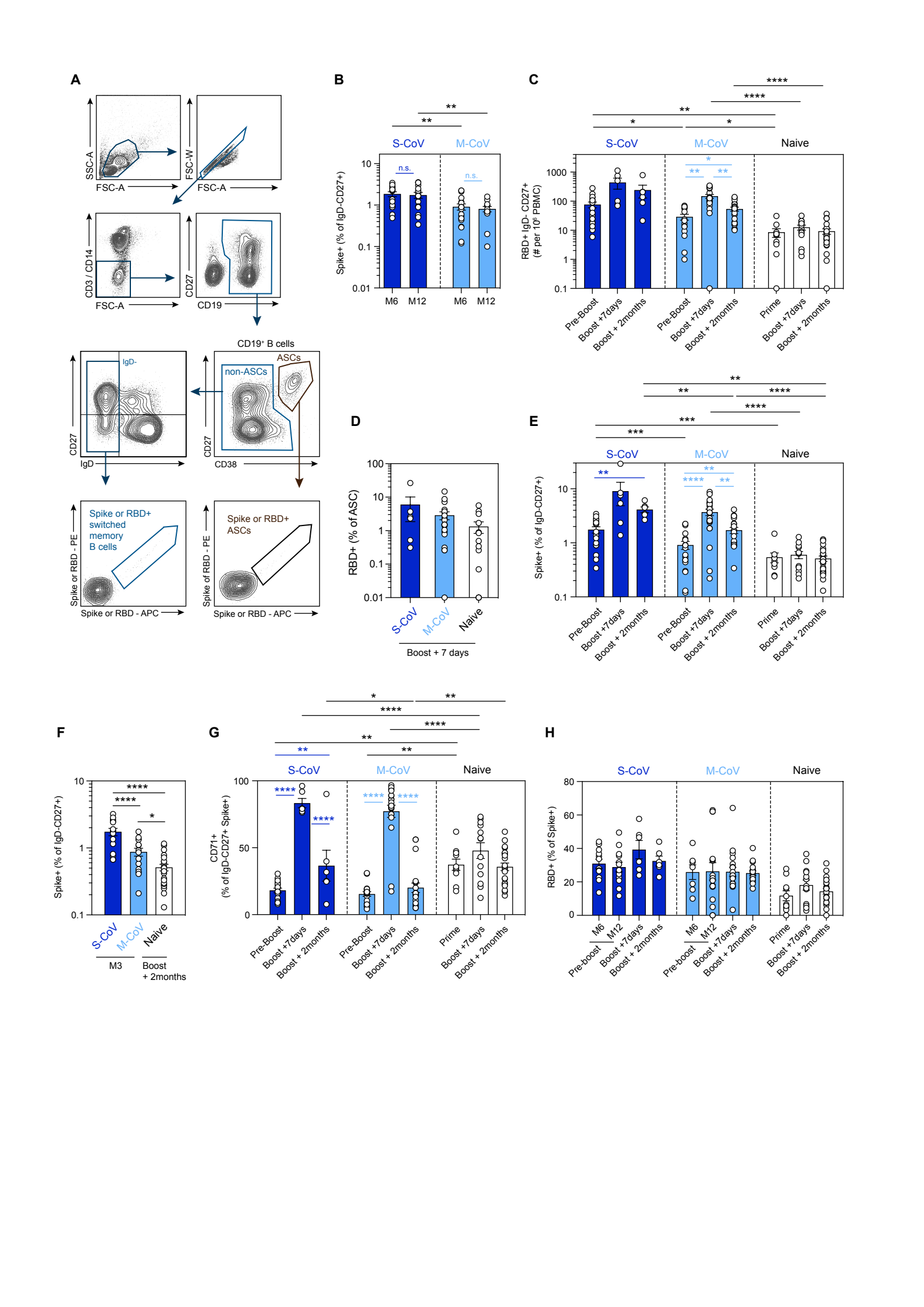

A
SSC-A
FSC-A
FSC-W
FSC-A
CD3 / CD14
FSC-A
CD27
CD19
CD19⁺ B cells
IgD-
CD27
IgD
non-ASCs
ASCs
CD27
CD38
Spike or RBD+ switched memory B cells
Spike or RBD - PE
Spike or RBD - APC
Spike or RBD+ ASCs
Spike of RBD - PE
Spike or RBD - APC
B
S-CoV
M-CoV
Spike+ (% of IgD-CD27+)
n.s.
n.s.
M6
M12
M6
M12
C
S-CoV
M-CoV
Naive
RBD+ IgD- CD27+ (# per 10⁶ PBMC)
Pre-Boost
Boost +7days
Boost + 2months
Pre-Boost
Boost +7days
Boost + 2months
Prime
Boost +7days
Boost + 2months
D
RBD+ (% of ASC)
S-CoV
M-CoV
Naive
Boost + 7 days
E
S-CoV
M-CoV
Naive
Spike+ (% of IgD-CD27+)
Pre-Boost
Boost +7days
Boost + 2months
Pre-Boost
Boost +7days
Boost + 2months
Prime
Boost +7days
Boost + 2months
F
Spike+ (% of IgD-CD27+)
S-CoV
M-CoV
Naive
M3
Boost + 2months
G
S-CoV
M-CoV
Naive
CD71+ (% of IgD-CD27+ Spike+)
Pre-Boost
Boost +7days
Boost + 2months
Pre-Boost
Boost +7days
Boost + 2months
Prime
Boost +7days
Boost + 2months
H
S-CoV
M-CoV
Naive
RBD+ (% of Spike+)
M6
M12
Pre-boost
Boost +7days
Boost + 2months
M6
M12
Pre-boost
Boost +7days
Boost + 2months
Prime
Boost +7days
Boost + 2months

Figure S2. mRNA vaccination mobilizes SARS-CoV-2 Spike-specific B cells in SARS-CoV-2 recovered and naive patients. Related to Figure 2.

(A) Flow cytometric gating strategy for the analysis and sorting of SARS-CoV-2 S or RBD-specific MBCs or ASCs from PBMCs of SARS-CoV-2 recovered and naive donors. Lymphocytes were first gated based on morphology, before exclusion of doublets, dead cells and CD3/CD14 cells. $CD19^+$ cells were then gated and subdivided into $CD38^{int/-}$ cells (non-ASCs) and $CD27^+CD38^{hi}$ plasma cells (ASCs). $CD38^{int/-}$ B cells were further divided in four quadrants using CD27 and IgD staining. Upper left quadrant defines MBCs, lower left quadrant double-negative (DN), upper right quadrant $CD27^+IgD^+$ cells (MZB) and lower right quadrant naive B cells. SARS-Cov-2 S or RBD-specific B cells were then analyzed within the B cell population of interest using a His-tagged SARS-Cov-2 S or RBD protein further revealed by two fluorescently labeled anti-His antibodies, ASCs at this stage keeping their surface BCR expression. **(B)** Frequencies of SARS-CoV-2 S-specific cells in live $CD19^+IgD^-CD27^+CD38^{int/-}$ MBCs at 6 (M6) and 12 months (M12) post symptom onset in SARS-CoV-2 recovered patients (S-CoV; dark blue, n=17/14; M-CoV: light blue, n=16/12). **(C)** Absolute numbers of RBD-specific IgD^-CD27^+ MBCs at pre-boost, boost + 7 days and boost + 2 months time points in S-CoV (dark blue, n=17/6/6), M-CoV (light-blue, n=14/21/20) and naive (white, n=10/13/23) patients. **(D)** Frequencies of RBD-specific cells in live ASCs at boost + 7 days in SARS-CoV-2 recovered patients (S-CoV; dark blue, n=6; M-CoV: light blue, n=21) and naive (white, n=13) donors. **(E-F)** Frequencies of SARS-CoV-2 S-specific cells in live $CD19^+IgD^-CD27^+CD38^{int/-}$ MBCs at pre-boost, boost + 7 days and boost + 2 months time points in SARS-CoV-2 recovered patients (S-CoV; dark blue, n=17/6/6; M-CoV: light blue, n=14/21/20) and naive (white, n=10/13/23) (E) and at 3 months post symptoms onset in SARS-CoV-2 recovered compared to naive individuals at the boost + 2 months time point (3 months after the prime) (F). **(G)** Frequencies of SARS-CoV-2 S-specific cells displaying an activated B cell ($CD19^+CD27^+IgD^-CD71^+$) phenotype at pre-boost, boost + 7 days and boost + 2 months time points in SARS-CoV-2 recovered patients (S-CoV; dark blue, n=17/6/6; M-CoV: light blue, n=14/21/20) and naive (white, n=10/13/23) donors. Bars indicate mean ±SEM. **(H)** Proportion of Spike-specific MBCs recognizing RBD in each individual at 6 months and 12 months in SARS-CoV-2

recovered and prime in naïve, and its evolution 7 days and 2 months after the boost. Bars indicate mean ±SEM. (B) two-way ANOVA with multiple comparisons of all groups means; (C and E-G) Repeated measures mixed effects model analysis with two sets of multiple comparisons (between donor groups inside each time-point (black lines) and between time points for each donor group (colored lines)) and (D) ordinary one-way ANOVA were performed (Benjamini, Krieger and Yekutieli FDR correction was used for all multiple comparisons). Only significant comparisons are highlighted in panels (C-F). (****P<0.0001, *** P<0.001, **P < 0.01, *P < 0.05)

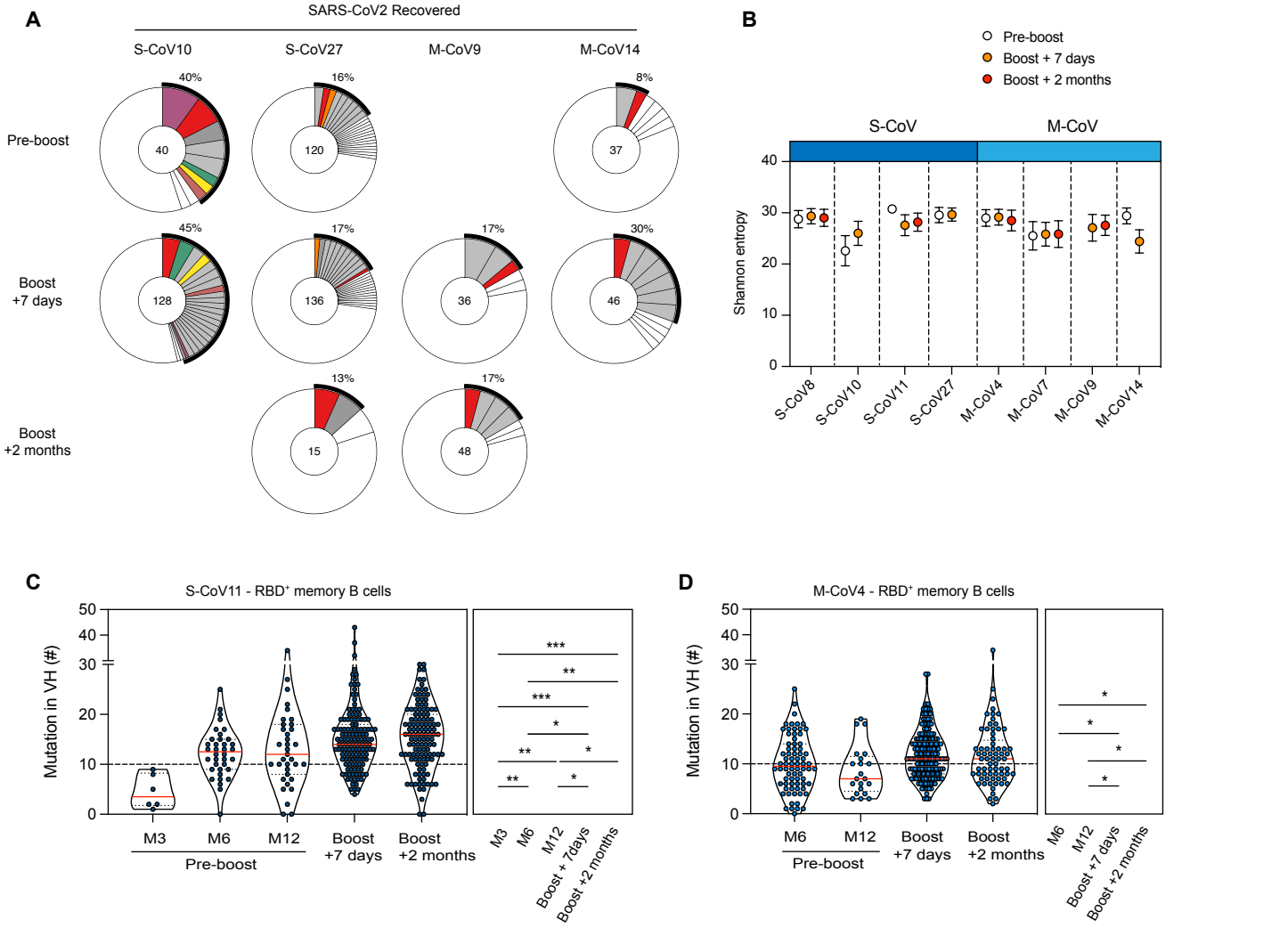
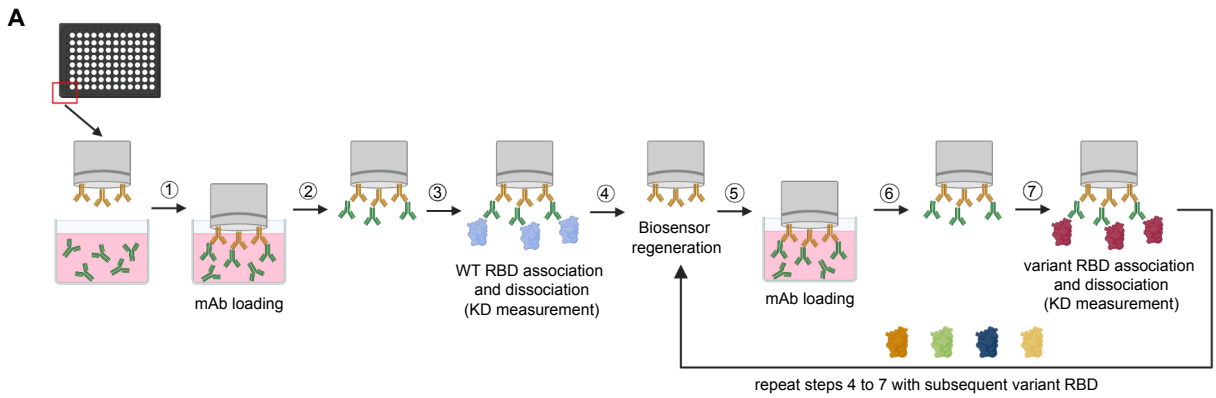
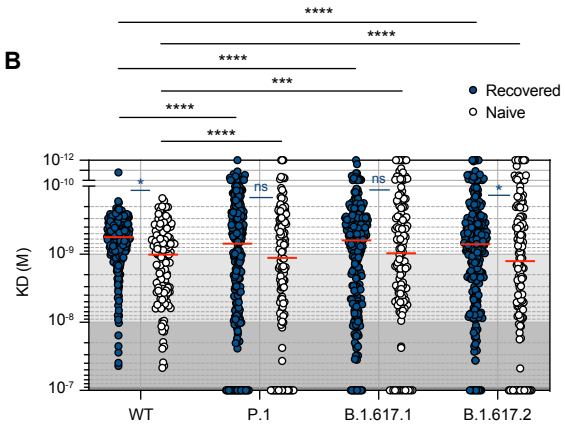

A
SARS-CoV2 Recovered
S-CoV10
S-CoV27
M-CoV9
M-CoV14
Pre-boost
Boost +7 days
Boost +2 months
40%
16%
8%
45%
17%
17%
30%
13%
17%
40
120
37
128
136
36
46
15
48
B
Pre-boost
Boost + 7 days
Boost + 2 months
S-CoV
M-CoV
Shannon entropy
S-CoV8
S-CoV10
S-CoV11
S-CoV27
M-CoV4
M-CoV7
M-CoV9
M-CoV14
C
S-CoV11 - RBD+ memory B cells
Mutation in VH (#)
M3
M6
M12
Boost +7 days
Boost +2 months
Pre-boost
Boost + 7days
Boost +2 months
D
M-CoV4 - RBD+ memory B cells
Mutation in VH (#)
M6
M12
Boost +7 days
Boost +2 months
Pre-boost

Figure S3. mRNA vaccination elicits a diverse RBD-specific memory B cell repertoire with increased mutational load in SARS-CoV-2 recovered patients. Related to Figure 3.

(A) Pie charts representing the clonal distribution of RBD-specific MBCs sorted from 2 S-CoV and 2 M-CoV at pre-boost, boost + 7 days and boost + 2 months time points. Colored slices indicate an expanded MBC clone (2 or more sequences at a given time-point) found at several time points in the same patient (persistent), grey slices indicate an expanded MBC clone found at a single time-point and white slices indicate persistent unique sequences. Outer black semi circular line indicates the proportion of sequences belonging to expanded clones at a given time point. Total number of sequences is indicated in the middle of the pie. **(B)** Shannon entropy index at indicated time points for the 4 S-CoV and the 4 M-CoV patients from Figure 3B and S3A. Estimated diversity ±SD. **(C-D)** Violin plots showing the longitudinal evolution of the number of mutations in V_H genes of sorted RBD^+ MBCs in one S-CoV (C) and one M-CoV donor (D). Red lines indicate median. Ordinary one-way ANOVA with multiple comparisons (Benjamini, Krieger and Yekutieli FDR correction) (*** $P<0.001$, **$P < 0.01$, *$P < 0.05$).


A
①
②
③
④
⑤
⑥
⑦
mAb loading
WT RBD association
and dissociation
(KD measurement)
Biosensor
regeneration
mAb loading
variant RBD association
and dissociation
(KD measurement)
repeat steps 4 to 7 with subsequent variant RBD


B
Recovered
Naive
KD (M)
WT
P.1
B.1.617.1
B.1.617.2
ns
****
***
*

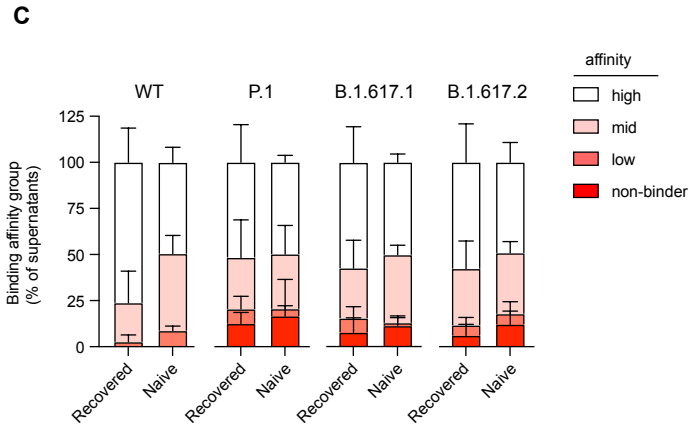

C
affinity
high
mid
low
non-binder
Binding affinity group
(% of supernatants)
WT
P.1
B.1.617.1
B.1.617.2
Recovered
Naive

Figure S4. The memory B cell pool of vaccinated individuals contains high-affinity clones against WT SARS-CoV-2 and P.1, B.1.617.1 and B.1.617.2 VOCs. Related to Figure 4.

(A) Scheme of the experimental procedure of affinity measurement from single-cell culture supernatants against SARS-CoV-2 variant RBD using bio-layer interferometry (BLI). Monoclonal antibodies in a given supernatant were captured on an anti-human Fc coated biosensor and affinity was first measured against the WT RBD. The biosensors were then regenerated, re-loaded with the same supernatant before testing affinity against another RBD variant. This procedure was repeated to test a total of five variants (B.1.1.7; B.1.351; P.1; B.1.617.1 and B.1.617.2). **(B**) KD (M) measured by BLI for 382 naturally expressed monoclonal antibodies analyzed in Figure 4 and tested here against WT, P.1, B.1.617.1 and B.1.617.2 RBD variants. Tested monoclonal antibodies were randomly selected from single-cell culture supernatants of RBD-specific MBCs isolated from SARS-CoV-2 recovered (n=251) and naive donors (n=131) and displaying WT RBD ELISA blank ratio ≥3. Background colors define high (KD $<10^{-9}$ M), mid ($10^{-9} \leq$ KD $<10^{-8}$ M) and low ($10^{-8} \leq$ KD $<10^{-7}$) affinity monoclonal antibodies. All monoclonal antibodies with no measurable affinity (KD $\geq 10^{-7}$) were considered non-binders. **(C)** Histogram showing binding affinity distribution of tested monoclonal antibodies against WT, P.1, B.1.617.1 and B.1.617.2 RBD variants, as defined in (A)**,** for SARS-CoV-2 recovered or naive donors**.** Bars indicate mean±SEM. (A) A two-way ANOVA with two sets of multiple comparisons (between tested variants inside each group (black lines) and between groups for each tested variants (colored lines) was performed (Benjamini, Krieger and Yekutieli FDR correction) (*** P<0.001; **** P<0.0001).

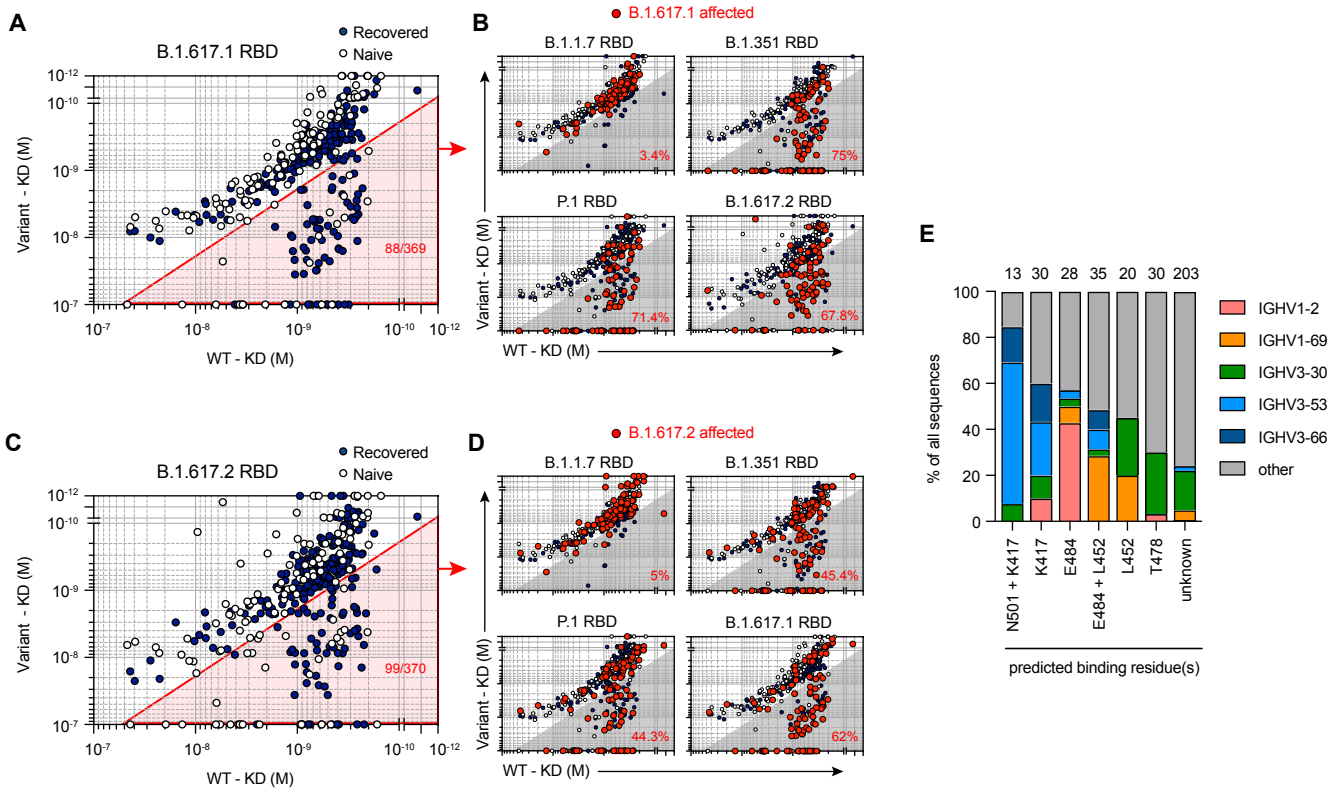

A
B.1.617.1 RBD
Recovered
Naive
Variant - KD (M)
WT - KD (M)
88/369
B
B.1.617.1 affected
B.1.1.7 RBD
B.1.351 RBD
P.1 RBD
B.1.617.2 RBD
3.4%
75%
71.4%
67.8%
C
B.1.617.2 RBD
99/370
D
B.1.617.2 affected
5%
45.4%
44.3%
62%
E
13 30 28 35 20 30 203
% of all sequences
IGHV1-2
IGHV1-69
IGHV3-30
IGHV3-53
IGHV3-66
other
N501 + K417
K417
E484
E484 + L452
L452
T478
unknown
predicted binding residue(s)

Figure S5: Variant RBD recognition profile reveals key residues recognized by memory B cells mobilized by the mRNA vaccine boost. Related to Figure 5.

(A) Dot plots representing the KDs for B.1.617.1 RBD versus WT RBD for all tested monoclonal antibodies from SARS-CoV-2 recovered (dark blue dots) and naive donors (white dots). The red shaded zone indicates B.1.617.1-affected monoclonal antibodies, defined as those whose KD for B.1.617.1 is increased by at least two-fold as compared to WT RBD. **(B)** Dot plots representing the KDs for B.1.1.7, B.1.351, P.1 and B.1.617.2 RBD versus WT RBD. B.1.617.1-affected monoclonal antibodies are highlighted as larger size red dots (corresponding to the red sector in (A)). Percentages indicate the proportion of B.1.617.1 affected monoclonal antibodies also affected by other RBD variants. **(C)** Dot plots representing the KDs for B.1.617.2 RBD versus WT RBD for all tested monoclonal antibodies from SARS-CoV-2 recovered (dark blue dots) and naive donors (white dots). The red shaded zone indicates B.1.617.2 affected monoclonal antibodies, defined as those whose KD for B.1.617.2 is increased by at least two-fold as compared to WT RBD. **(D)** Dot plots representing the KDs for B.1.1.7, B.1.351, P.1 and B.1.617.1 RBD versus WT RBD. B.1.617.2-affected monoclonal antibodies are highlighted as larger size red dots (corresponding to the red sector in (C)). Percentages indicate the proportion of B.1.617.2 affected monoclonal antibodies also affected by other RBD variants. **(D)** Proportion of IGHV1-2, IGHV1-69, IGHV3-30, IGHV3-53 and IGHV3-66 usage among all tested monoclonal antibodies with available V_H sequence and grouped based on their predicted essential binding residues, as defined by RBD variant recognition profile in BLI.

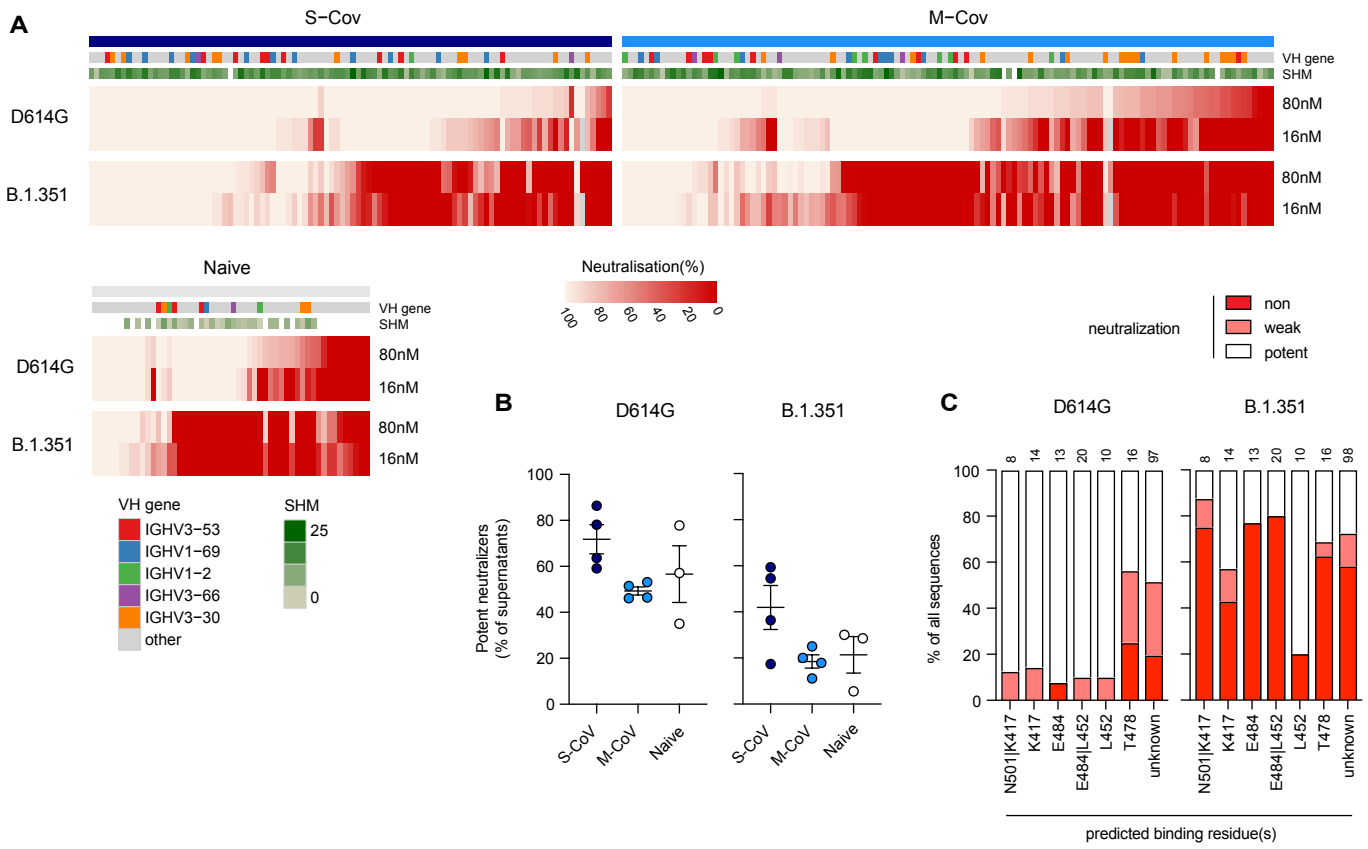

A
S−Cov
M−Cov
VH gene
SHM
D614G
B.1.351
80nM
16nM
Naive
Neutralisation(%)
100
80
60
40
20
0
neutralization
non
weak
potent
VH gene
IGHV3−53
IGHV1−69
IGHV1−2
IGHV3−66
IGHV3−30
other
SHM
25
0
B
D614G
B.1.351
Potent neutralizers
(% of supernatants)
S-CoV
M-CoV
Naive
C
% of all sequences
8
14
13
20
10
16
97
98
N501|K417
K417
E484
E484|L452
L452
T478
unknown
predicted binding residue(s)

Figure S6: A substantial proportion of memory B cells in vaccinated individuals neutralizes D614G SARS-CoV-2 and B.1.351 VOC. Related to Figure 6.

(A) Heatmap showing the in vitro neutralization of D614G SARS-CoV-2 and B.1.351 SARS-CoV-2 at 80 nM and 16 nM for all tested culture supernatants (S-CoV, n=104; M-CoV, n=123 and naive, n=52). V_H gene name and V_H gene mutation numbers (SHM) for each monoclonal antibody are represented on the top. **(B)** Percentage of potent neutralizers against SARS-CoV-2 D614G or variant B.1.351 viruses among monoclonal antibodies analyzed for each donor **(C)** Proportion of potent, weak or non-D614G or B.1.351 SARS-CoV-2 neutralizers among all tested monoclonal antibodies, grouped based on their predicted binding residues.