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Supplemental information

GRAd-COV2 vaccine provides potent and durable humoral and cellular immunity to SARS-CoV-2 in randomized placebo-controlled phase 2 trial

Stefania Capone, Francesco M. Fusco, Stefano Milleri, Silvio Borrè, Sergio Carbonara, Sergio Lo Caputo, Sebastiano Leone, Giovanni Gori, Paolo Maggi, Antonio Cascio, Miriam Lichtner, Roberto Cauda, Sarah Dal Zoppo, Maria V. Cossu, Andrea Gori, Silvia Roda, Paola Confalonieri, Stefano Bonora, Gabriele Missale, Mauro Codeluppi, Ivano Mezzaroma, Serena Capici, Emanuele Pontali, Marco Libanore, Augusta Diani, Simone Lanini, Simone Battella, Alessandra M. Contino, Eva Piano Mortari, Francesco Genova, Gessica Parente, Rosella Dragonetti, Stefano Colloca, Luigi Visani, Claudio Iannacone, Rita Carsetti, Antonella Folgori, Roberto Camerini, and COVITAR study group

Table S1. Overview of adverse events in the safety population, related to Figure 2

	Vaccine single dose (n=305)	Vaccine repeated dose (n=308)	Placebo (n= 304)
Subject with at least one AE	293 (96.1%)	299 (97.1%)	239 (78.6%)
Subjects with at least one unsolicited TEAE	51 (16.7%)	42 (13.6%)	43 (14.1%)
Subjects with at least one related unsolicited TEAE	11 (3.6%)	12 (3.9%)	9 (3.0%)
Subjects who discontinued the study due to unsolicited TEAE	0	1 (0.3%)	0
Subjects with at least one SAE	2 (0.7%)	3 (1.0%)	1 (0.3%)
Subjects with at least one related SAE	0	0	0
Subjects with AESI	0	0	0
Subjects with at least one local solicited AE	253 (83.0%)	272 (88.3%)	128 (42.1%)
Subjects with at least one related local solicited TEAE	250 (82.0%)	271 (88.0%)	126 (41.4%)
Subjects who discontinued the study due to local solicited TEAE	0	0	0
Subjects with at least one systemic solicited TEAE	271 (88.9%)	280 (90.9%)	214 (70.4%)
Subjects with at least one related systemic solicited TEAE	268 (87.9%)	279 (90.6%)	210 (69.1%)
Subjects who discontinued the study due to systemic solicited TEAE	0	0	0

Notes: Treatment Emergent Adverse Events (TEAEs) are defined as AEs that started after the first dose administration and until 28 days post each dose of study intervention. Serious Adverse Events (SAEs) were recorded from the date of signature of informed consent form through the last participant contact. Solicited TEAEs were recorded for 7 days post each dose of study intervention. Solicited TEAEs recorded after 7 days post each dose are included in the summary table. All TEAEs are considered unsolicited unless categorized as solicited TEAEs. Adverse Events of Special Interest (AESIs) were recorded from day 1, post treatment, through the last participant contact. N= number of subjects, % = percentage of subjects. Percentages are calculated on the number of subjects (n) by treatment group.

Figure S1

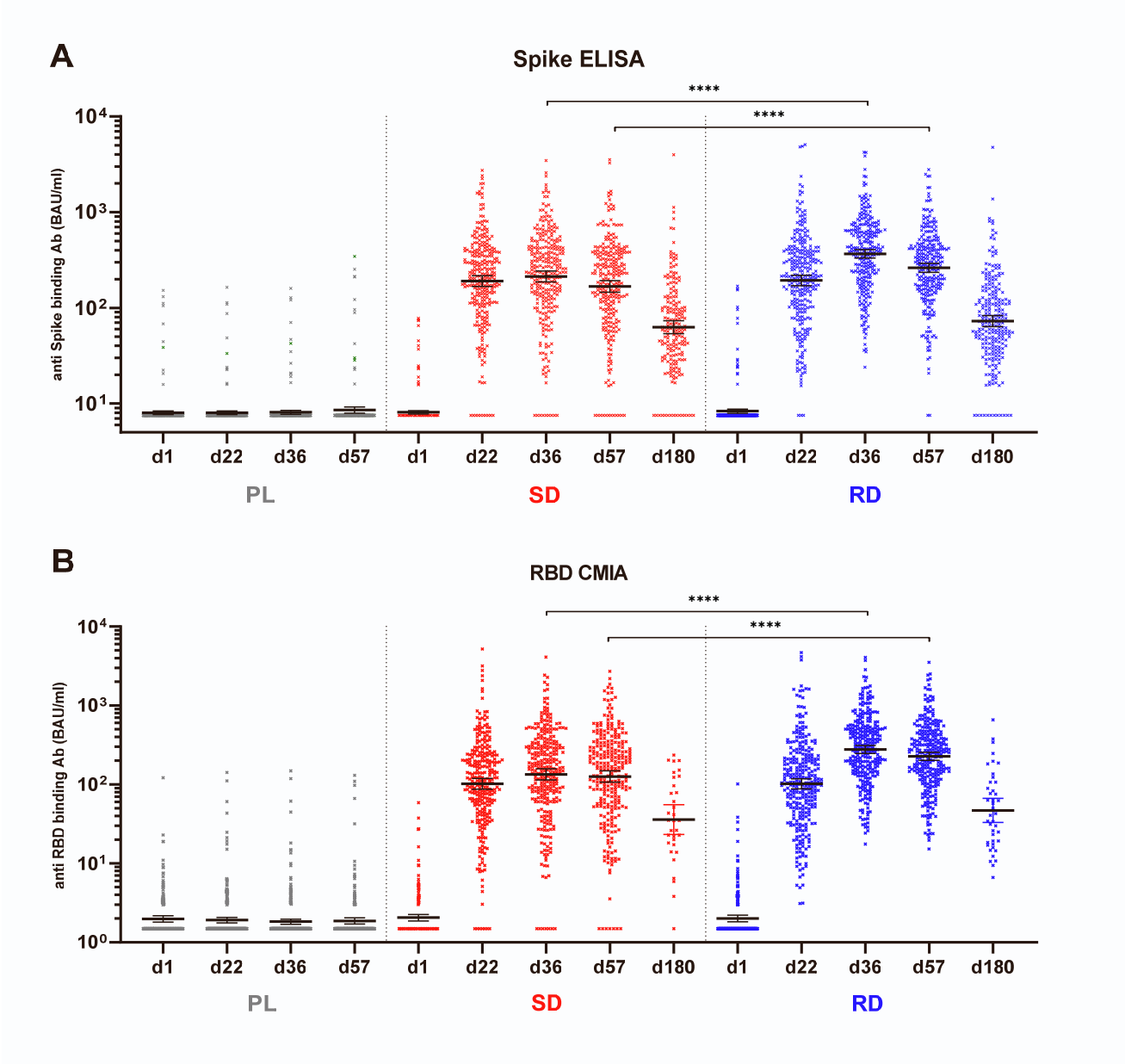
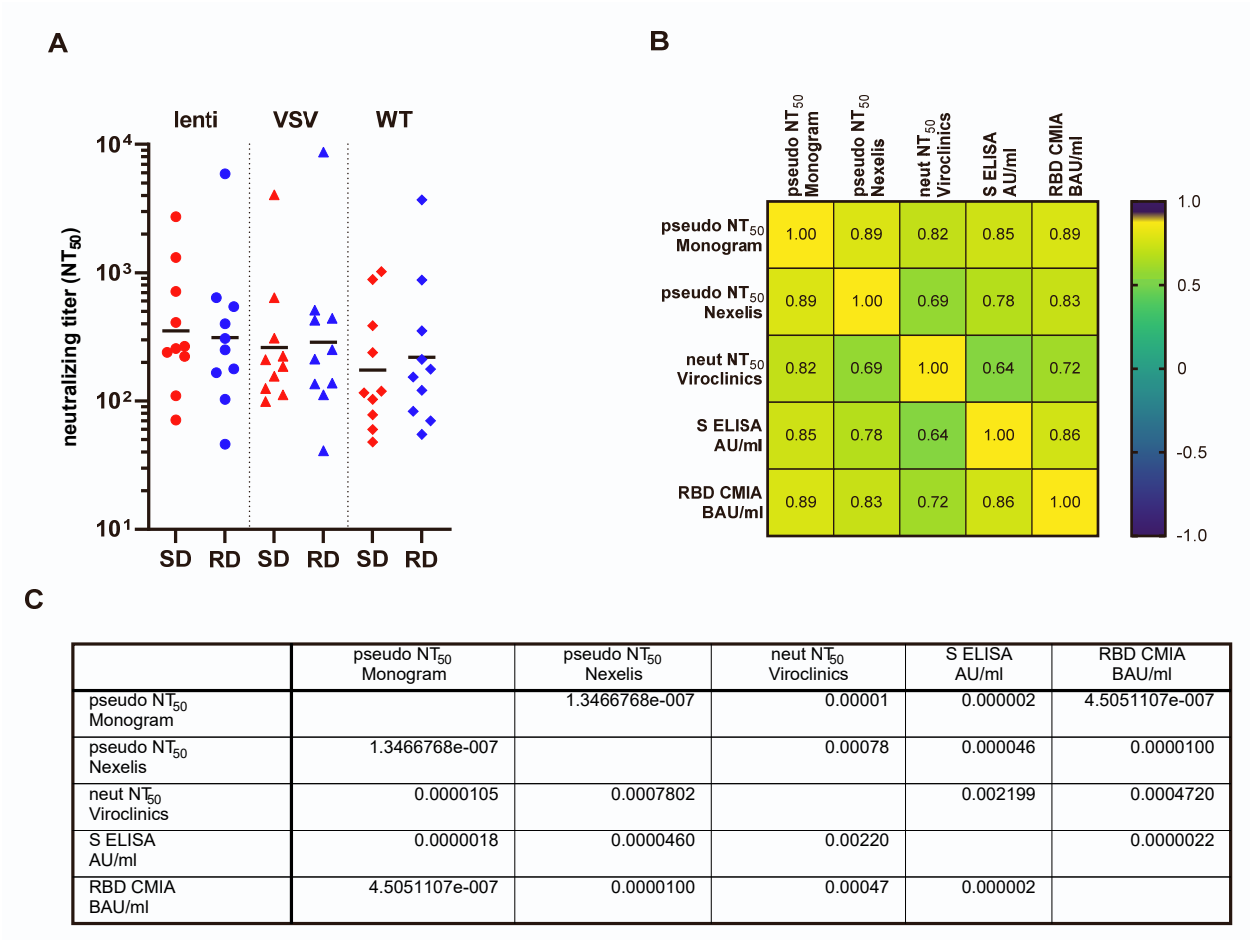


Figure S1. Spike and RBD binding IgG concentrations expressed in BAU/ml, related to Figure 3

(A) trimeric Spike binding antibody concentrations, detected by ELISA (COVID-SeroKlir, Kantaro Semi-Quantitative SARS-CoV-2 IgG Antibody Kit, R&D Systems) (B) RBD binding antibody concentrations, detected by chemiluminescent microparticle immunoassay (CMIA-Abbott SARS-CoV-2 IgG II Quant assay). For both (A) and (B), the original dataset expressed in arbitrary units/ml were converted to binding international units (BAU)/ml following manufacturers indications. Symbols for each study volunteer at each study visits are shown in grey for placebo arm (PL), in red for GRAd-COV2 single dose (SD) arm and in blue for repeated dose (RD) arm. Line and error bars indicate geometric mean (GM) and 95% confidence interval (CI). Statistical analysis is displayed only for comparison between SD and RD vaccine arms; difference between placebo and both vaccine arms was highly significant (P=<0.0001) at all post vaccination visits.

Figure S2



C

	pseudo NT_{50} Monogram	pseudo NT_{50} Nexelis	neut NT_{50} Viroclinics	S ELISA AU/ml	RBD CMIA BAU/ml
pseudo NT_{50} Monogram		1.3466768e-007	0.00001	0.000002	4.5051107e-007
pseudo NT_{50} Nexelis	1.3466768e-007		0.00078	0.000046	0.0000100
neut NT_{50} Viroclinics	0.0000105	0.0007802		0.002199	0.0004720
S ELISA AU/ml	0.0000018	0.0000460	0.00220		0.0000022
RBD CMIA BAU/ml	4.5051107e-007	0.0000100	0.00047	0.000002	

Figure S2. Correlation amongst all serology assays on d36 sera from 10 participants per vaccine arm, related to Figure 3

(A) SARS-CoV-2 50% neutralizing antibody titer (NT_{50}) measured in d36 serum from 20 volunteers (10 enrolled in SD and 10 in RD arms) by three distinct assays: PhenoSense Anti-SARS CoV-2 Neutralizing Antibody Assay based on D614G Spike pseudotyped lentivirus (Monogram Biosciences); SARS-CoV-2 pseudoparticle neutralization assay (PNA) based on VSV pseudotyped with D614 Spike (Nexelis); live Wuhan (Berlin strain) SARS-CoV-2 neutralization assay (Viroclinics). Horizontal black lines indicate geometric mean. (B) and (C) A correlation (non-parametric Spearman, two-tailed) matrix was computed for the 20 vaccinated volunteers, comparing the measured anti-SARS-CoV-2 binding and neutralizing antibody response to evaluate how the different immune parameters correlate each other. Spearman r values are shown in the heatmap (A), and the table (B) reports P values for each pair of variables.

Figure S3

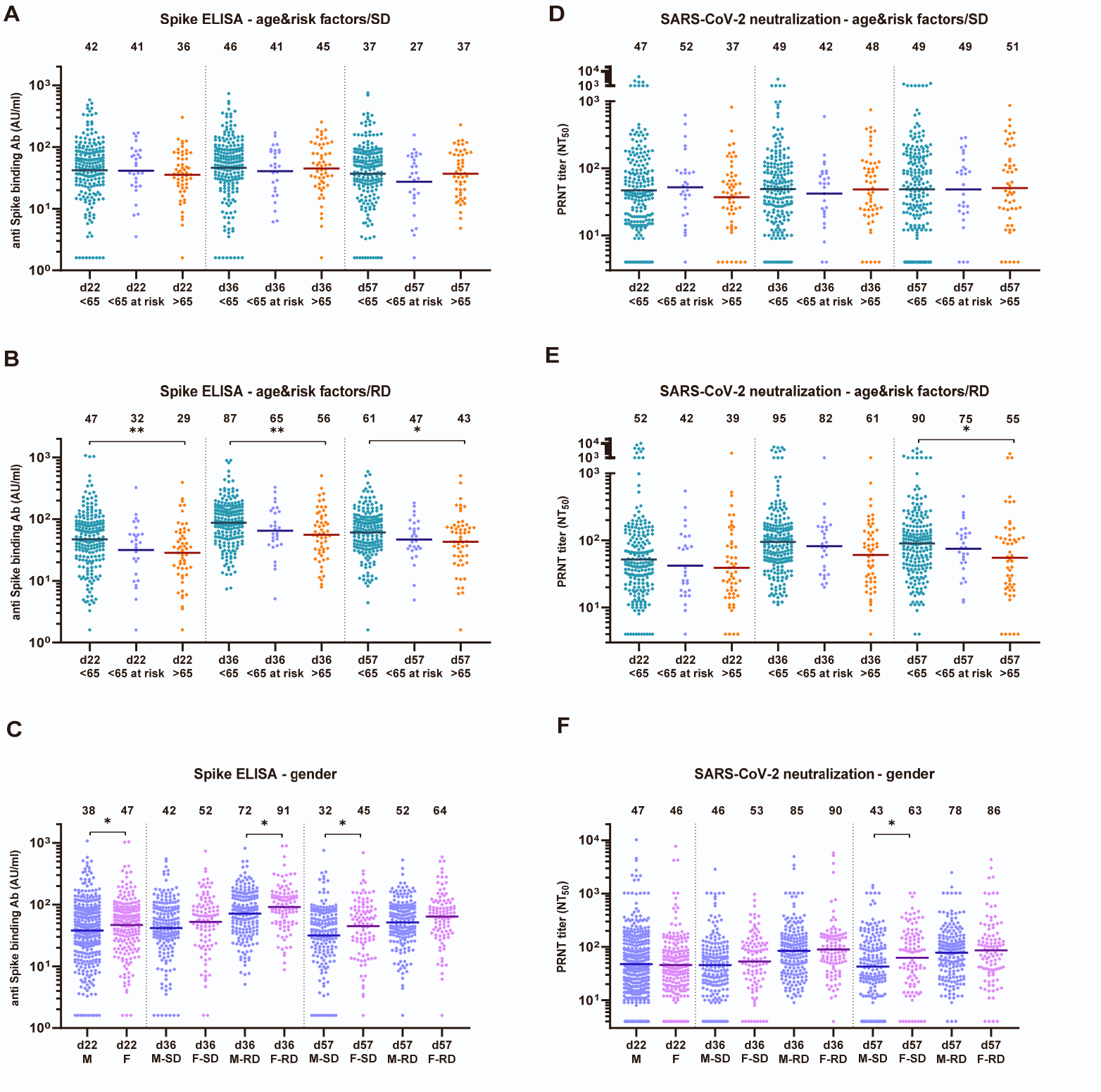

A
Spike ELISA - age&risk factors/SD
anti Spike binding Ab (AU/ml)
42 41 36 46 41 45 37 27 37
d22 <65
d22 <65 at risk
d22 >65
d36 <65
d36 <65 at risk
d36 >65
d57 <65
d57 <65 at risk
d57 >65
B
Spike ELISA - age&risk factors/RD
anti Spike binding Ab (AU/ml)
47 32 29 87 65 56 61 47 43
**
**
*
C
Spike ELISA - gender
anti Spike binding Ab (AU/ml)
38 47 42 52 72 91 32 45 52 64
*
*
*
d22 M
d22 F
d36 M-SD
d36 F-SD
d36 M-RD
d36 F-RD
d57 M-SD
d57 F-SD
d57 M-RD
d57 F-RD
D
SARS-CoV-2 neutralization - age&risk factors/SD
PRNT titer (NT50)
47 52 37 49 42 48 49 49 51
E
SARS-CoV-2 neutralization - age&risk factors/RD
PRNT titer (NT50)
52 42 39 95 82 61 90 75 55
*
F
SARS-CoV-2 neutralization - gender
PRNT titer (NT50)
47 46 46 53 85 90 43 63 78 86
*

Figure S3. Serology analysis in study subpopulations, related to Figure 3

Spike ELISA (panels A to C, expressed as AU/ml) and live SARS-CoV-2 neutralization (panels D to F, expressed as NT_{50}) dataset at visits d22, d36 and d57 are shown split by main study subpopulations. Effect of age and comorbidities on vaccine immunogenicity is shown for GRAd-COV2 SD arm (panels A and D) and for GRAd-COV2 RD arm (panels B and E), with volunteers distributed according to main per-Protocol subpopulations: age <65 years, age <65 years with comorbidities, age >65 years. Two-tailed Kruskall-Wallis test with Dunn's test correction for multiple comparisons was used to compare datasets within each study visit. Effect of gender is shown in panels C and F, with dataset split into male (M) and female (F) subjects. At visit d22, volunteers from both SD and RD arms were combined, since main statistical analysis did not detect any statistically significant difference in vaccine immunogenicity post dose 1. For d36 and d57 visits, data are shown according to study arm (SD or RD). Two tailed Mann-Whitney test was used to compare datasets within each study visit and study arm. Across all panels, horizontal lines and numbers reported above graphs indicate geometric mean. Only significant differences are displayed on the graphs.

Figure S4

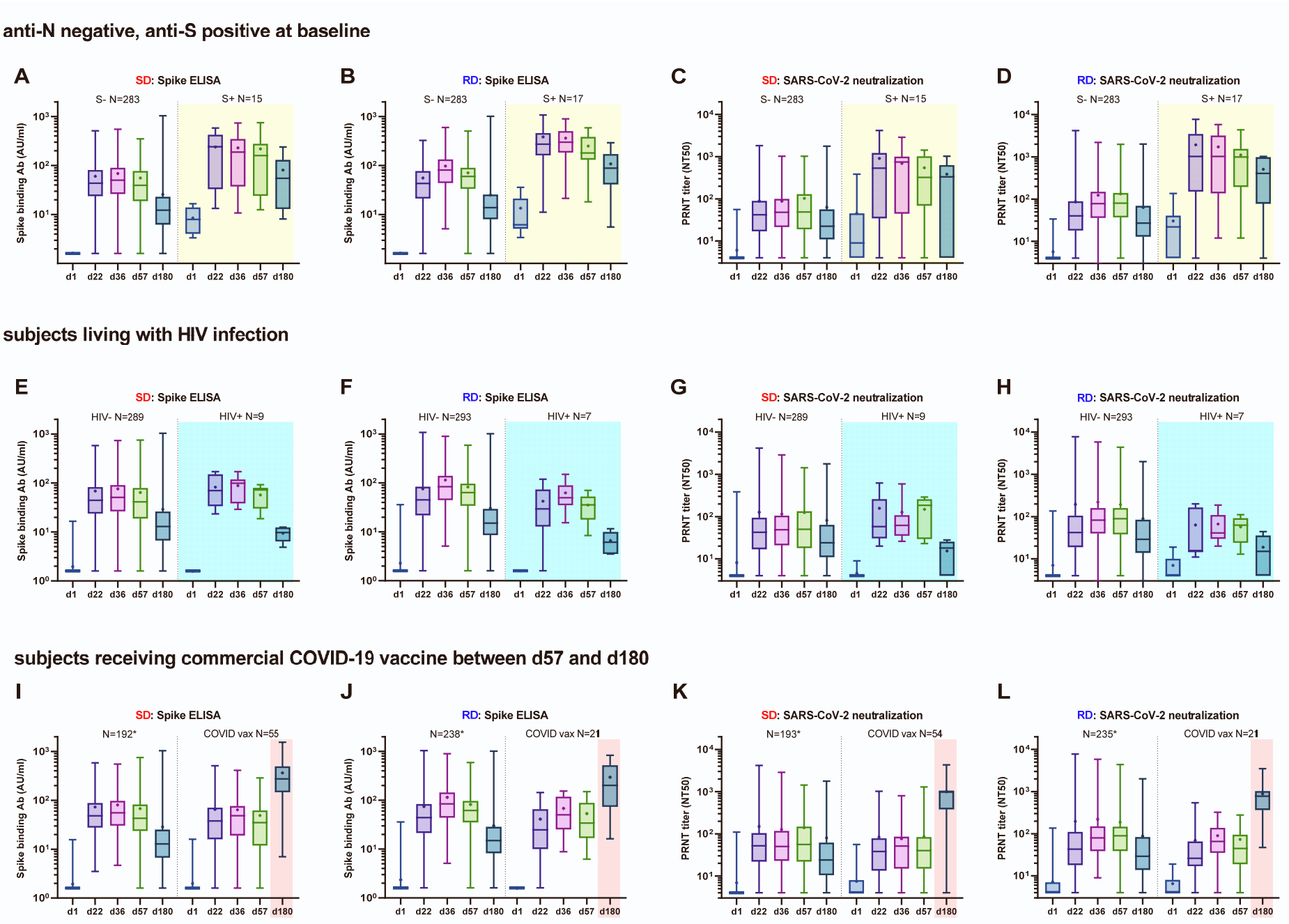

anti-N negative, anti-S positive at baseline
A
SD: Spike ELISA
S- N=283
S+ N=15
Spike binding Ab (AU/ml)
B
RD: Spike ELISA
S- N=283
S+ N=17
Spike binding Ab (AU/ml)
C
SD: SARS-CoV-2 neutralization
S- N=283
S+ N=15
PRNT titer (NT50)
D
RD: SARS-CoV-2 neutralization
S- N=283
S+ N=17
PRNT titer (NT50)
d1 d22 d36 d57 d180 d1 d22 d36 d57 d180
subjects living with HIV infection
E
SD: Spike ELISA
HIV- N=289
HIV+ N=9
Spike binding Ab (AU/ml)
F
RD: Spike ELISA
HIV- N=293
HIV+ N=7
Spike binding Ab (AU/ml)
G
SD: SARS-CoV-2 neutralization
HIV- N=289
HIV+ N=9
PRNT titer (NT50)
H
RD: SARS-CoV-2 neutralization
HIV- N=293
HIV+ N=7
PRNT titer (NT50)
d1 d22 d36 d57 d180 d1 d22 d36 d57 d180
subjects receiving commercial COVID-19 vaccine between d57 and d180
I
SD: Spike ELISA
N=192*
COVID vax N=55
Spike binding Ab (AU/ml)
J
RD: Spike ELISA
N=238*
COVID vax N=21
Spike binding Ab (AU/ml)
K
SD: SARS-CoV-2 neutralization
N=193*
COVID vax N=54
PRNT titer (NT50)
L
RD: SARS-CoV-2 neutralization
N=235*
COVID vax N=21
PRNT titer (NT50)
d1 d22 d36 d57 d180 d1 d22 d36 d57 d180

Figure S4. Binding and neutralizing antibodies in baseline Spike seropositive, HIV infected and COVID-19 commercial vaccines recipients, related to Figure 3

Spike ELISA (panels A-B-E-F-I-J, expressed as AU/ml) and live SARS-CoV-2 neutralization (panels C-D-G-H-K-L, expressed as NT_{50}) dataset at all study visits for the two GRAd-COV2 vaccine study arms (SD and RD) are shown split by population category of interest, for a descriptive analysis of vaccine immunogenicity in: subjects seronegative to SARS-CoV-2 N but seropositive (by ELISA) to S at study entry (panels A to D, yellow shaded area); subjects living with HIV infection (panels E to H, light blue shaded area); subjects that received COVID-19 approved vaccines between d57 and d180 (panels I to L, pink shaded area). Data are shown as box and whiskers plots, with whiskers extending from minimum to maximum value and mean indicated by a dot. Numerosity (N) of each subgroup at baseline is indicated for reference. For panels I to L, the asterisk on numerosity indicates that only subjects with available data at d180 visit were included.

Figure S5

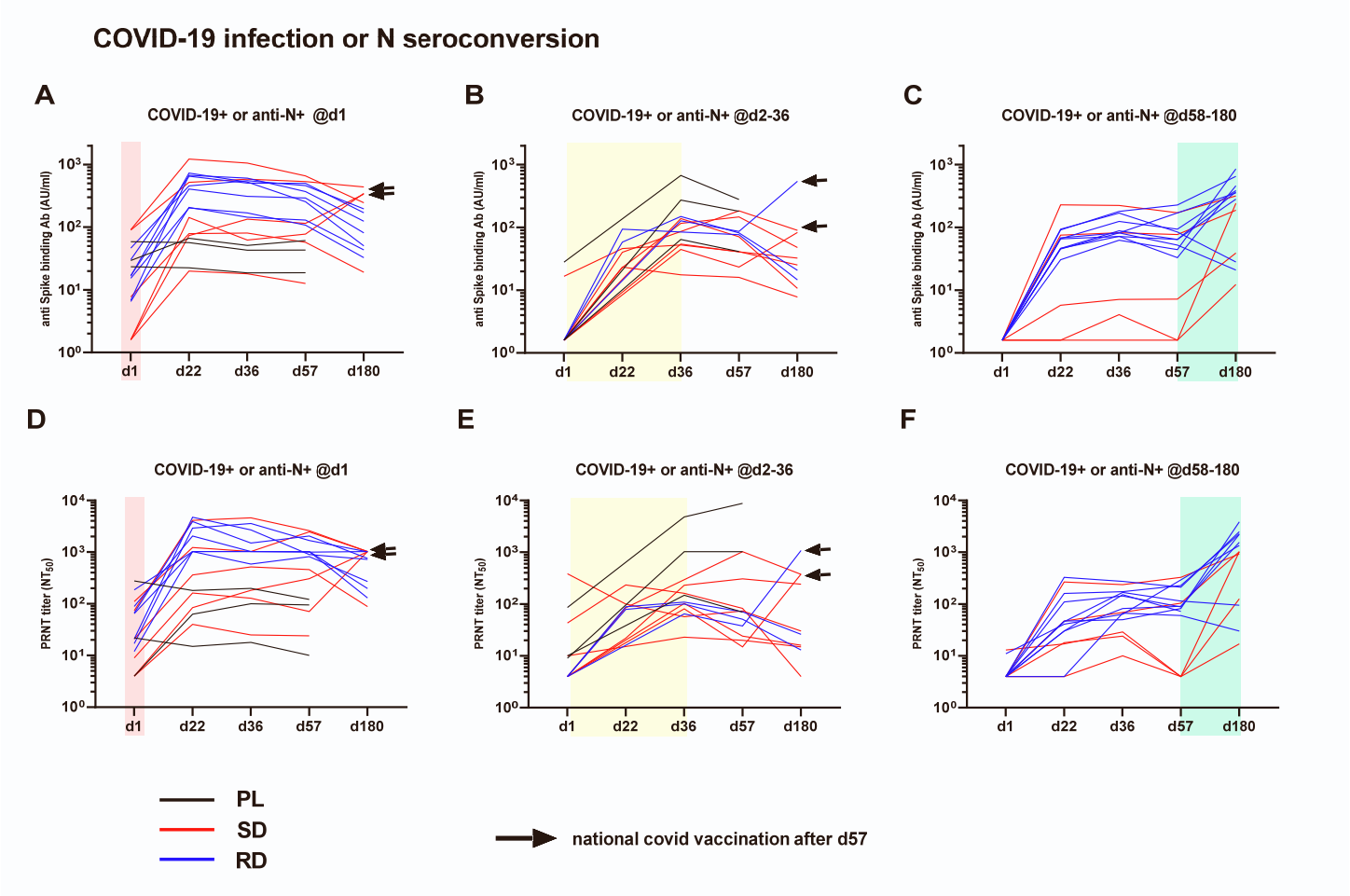
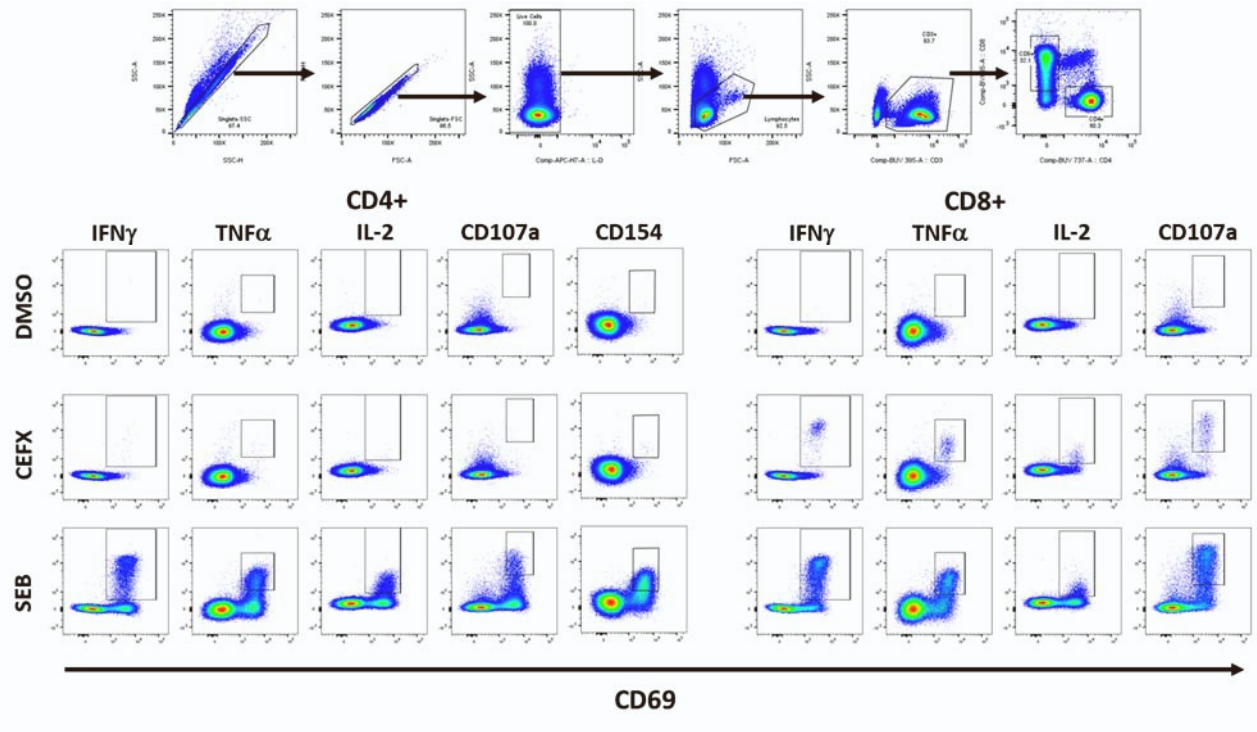


Figure S5. Binding and neutralizing antibodies in subjects with intercurrent SARS-CoV-2 exposure or infection, related to Figure 3

Kinetics of Spike binding antibodies (ELISA, panels A to C, expressed as AU/ml) and SARS-CoV-2 neutralizing antibodies (panels D to F, expressed as NT_{50}) in individual volunteers at all study visits are shown for three distinct categories: subjects found seropositive to nucleocapsid (N) antigen at study entry (panels A and D pink shaded area); subjects seroconverting to N without symptoms or with documented SARS-CoV-2 infection either during vaccination phase (between d2 and d36, panels B and E, yellow shaded area) or between d57 and d180 (panels C and F, green shaded area). The line colors indicate study arm in which the subject is enrolled: black=placebo; red=SD; blue= RD. black arrow indicates volunteers that received COVID-19 approved vaccines between d57 and d180.

Figure S6

A: Intracellular staining gating strategy



B: Intracellular staining representative CD4 and CD8 responses to spike peptide pools in one volunteer

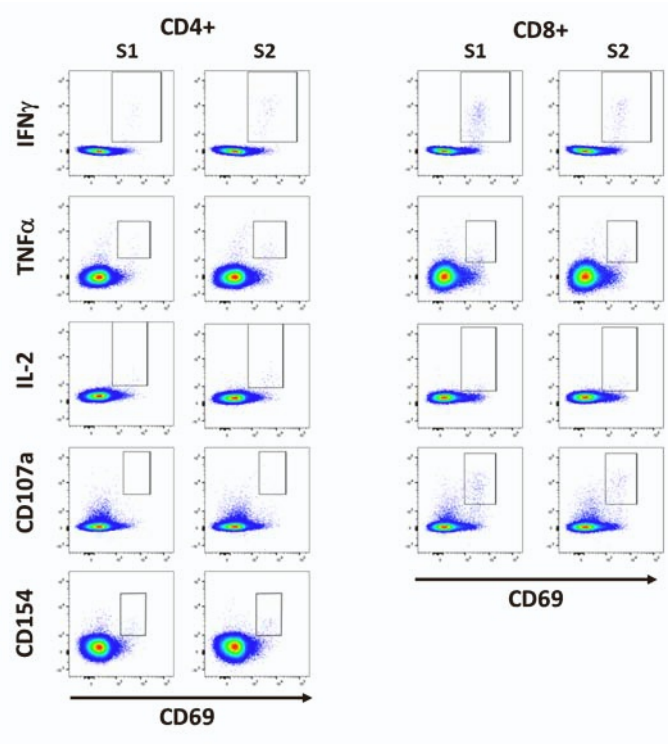
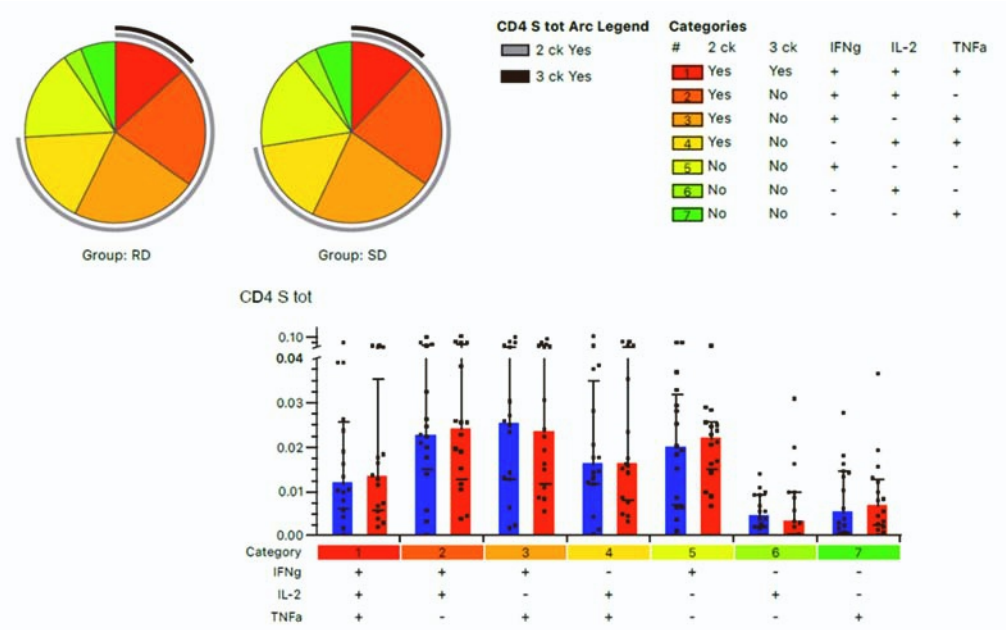


Figure S6. ICS gating strategy and example of Spike specific responses in a representative subject, related to Figure 4

Representative plots for A) gating strategy of CD4+ and CD8+ T cells populations (upper plots) and analysed functions in negative control (DMSO, top lane) and positive controls (CEFX, central lane, SEB, bottom lane). B) representative plots for Spike-specific CD4+ (left) and CD8+ (right) T cell responses against S1 (first and third columns) and S2 (second and fourth columns) Spike peptide pools.

Figure S7

A: **CD4**



B: **CD8**

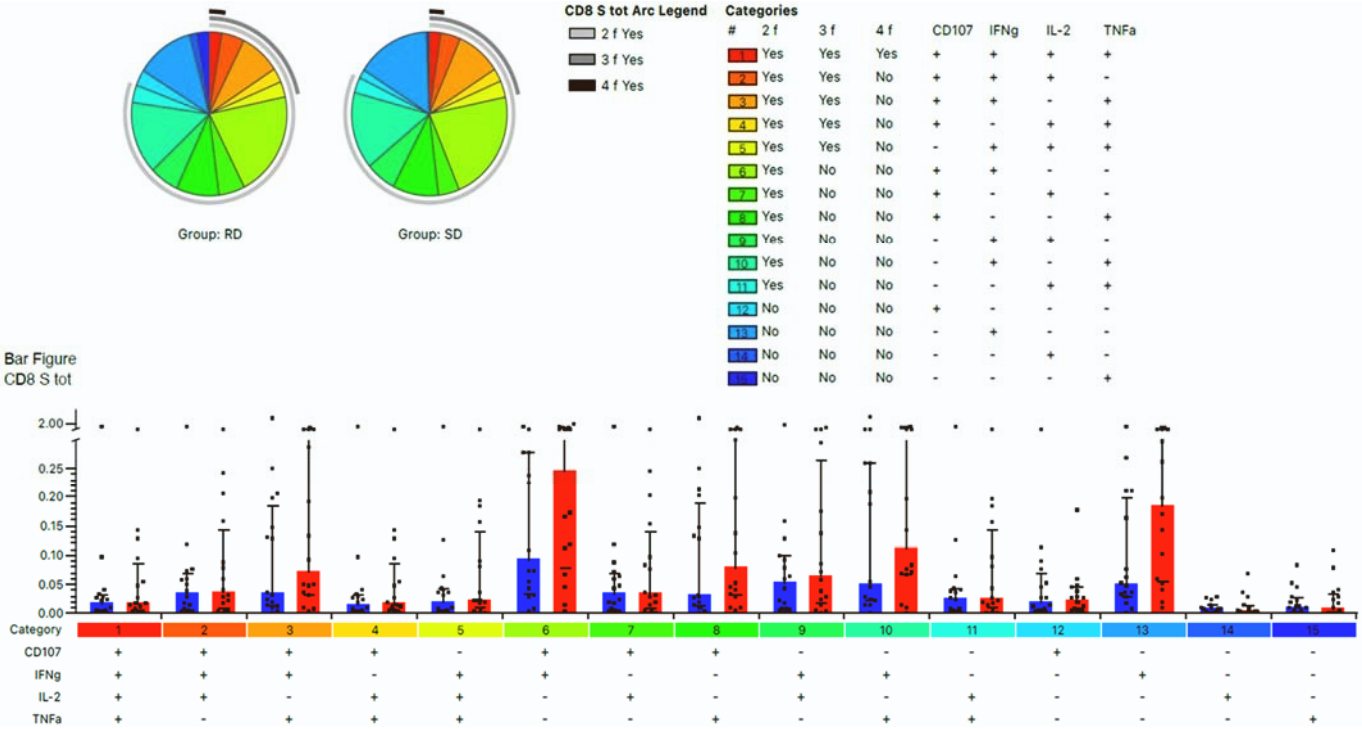


Figure S7: Polyfunctionality analysis, related to Figure 4
Co-expression of 1 to 3 (IL-2, TNFα and IFNγ) and 1 to 4 functions (CD107a, IL-2, TNFα and IFNγ) was analyzed for CD4 (A) and CD8 (B) Spike-specific T cells, respectively. Pie charts (base: median) indicates relative abundance of each population (pie slices) and co-expression (arcs) while bar graphs (set at median with whiskers representing IQR) indicate their absolute percentages and distribution in RD group (blue bars) and SD group (red bars).

Figure S8

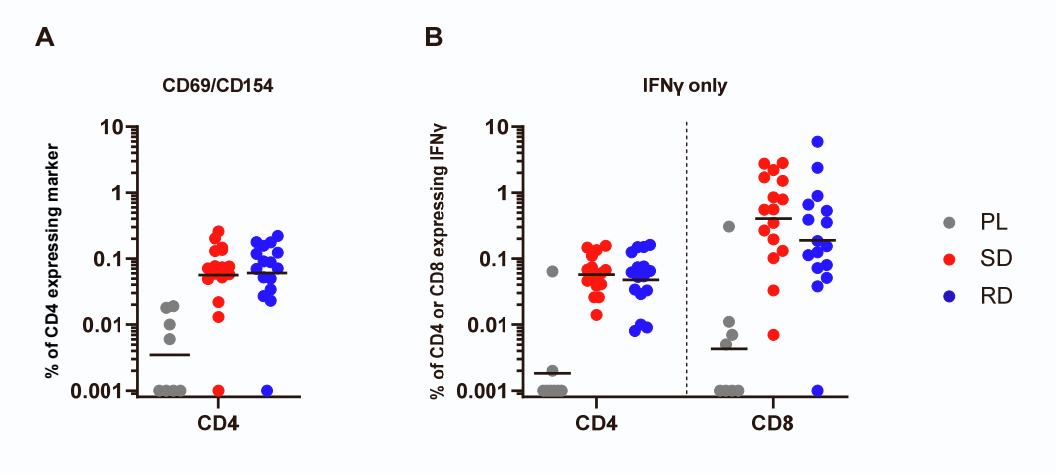


Figure S8. Additional ICS data, related to Figure 5
Dot plots represents the percentages of Spike-specific CD4 T cells co-expressing CD69 and CD154 activation markers (A) and the percentages of Spike-specific CD4 and CD8 expressing IFNγ (B) in the Placebo (grey dots), RD (blue dots) and SD (red dots) groups.

Figure S9

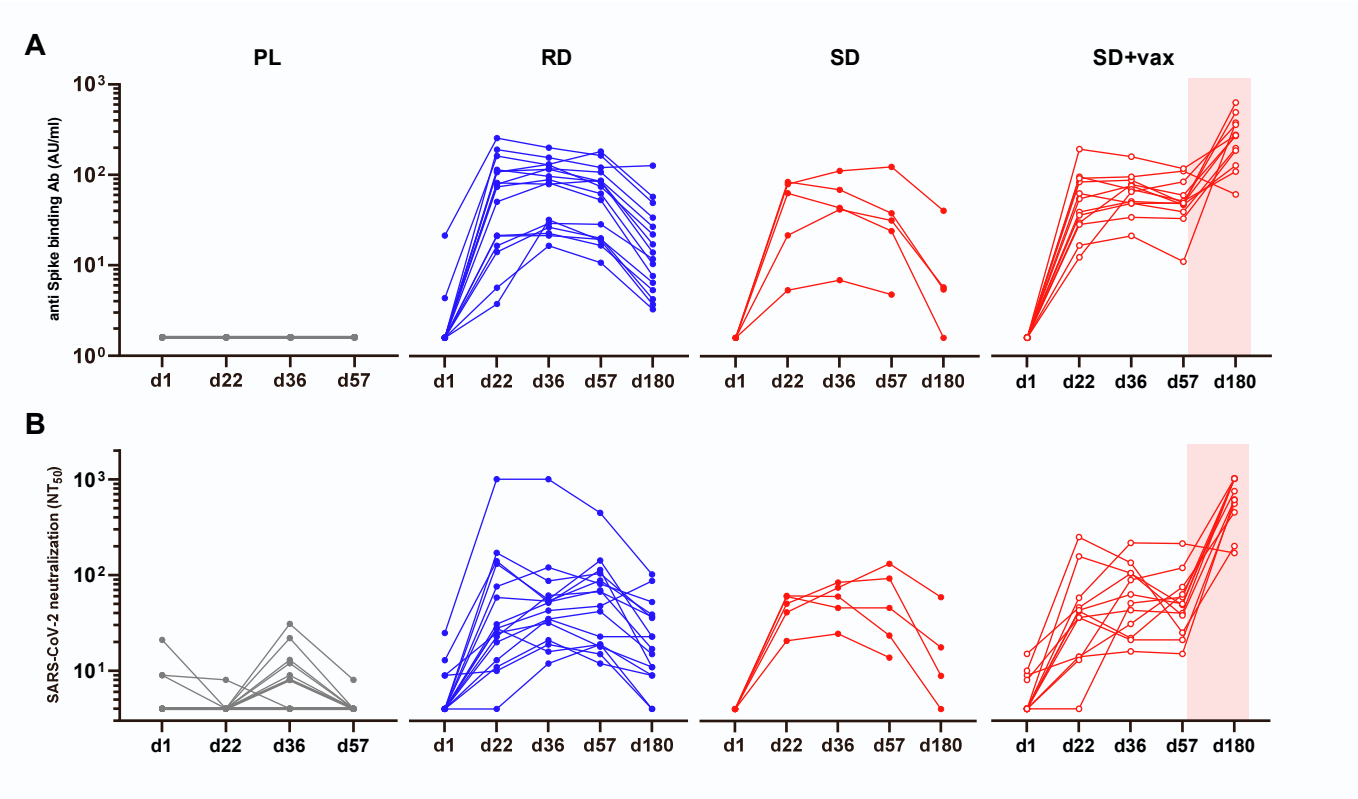


Figure S9. Serology kinetics in PBMC sub-study volunteers, related to Figure 5
Kinetic of Spike-specific binding (A) and neutralizing (B) antibodies in each single donor belonging to the PBMC sub-study, divided by Placebo (grey dots and lines), RD (blue dots and lines), SD (red dots and lines) and SD+vax (empty red dots and red lines, with pink shaded area indicating time frame of COVID-19 approved vaccine receipt) groups.

Figure S10

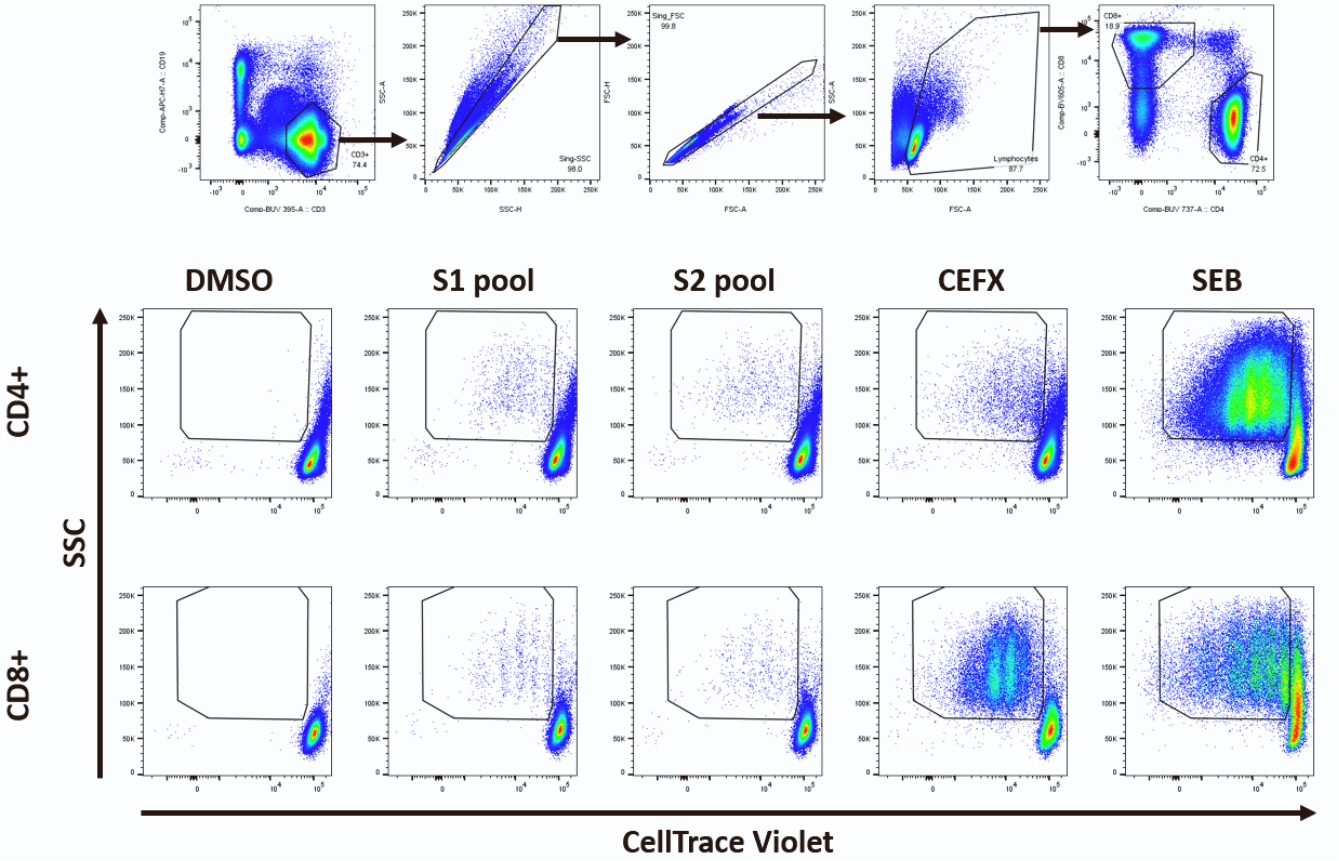


Figure S10. T cell proliferation assay gating strategy, related to Figure 5
Representative plots for gating strategy (upper lane) of T cell populations and proliferative response (i.e. dilution of CellTrace dye) of CD4 (middle lane) and CD8 (lower lane) after 5 days stimulation with DMSO (first column-negative control), S1 Spike sub-pool (second column) S2 Spike sub-pool (third column), CEFX (fourth column-peptide pool positive control) and SEB (fifth column-superantigen positive control).

Figure S11

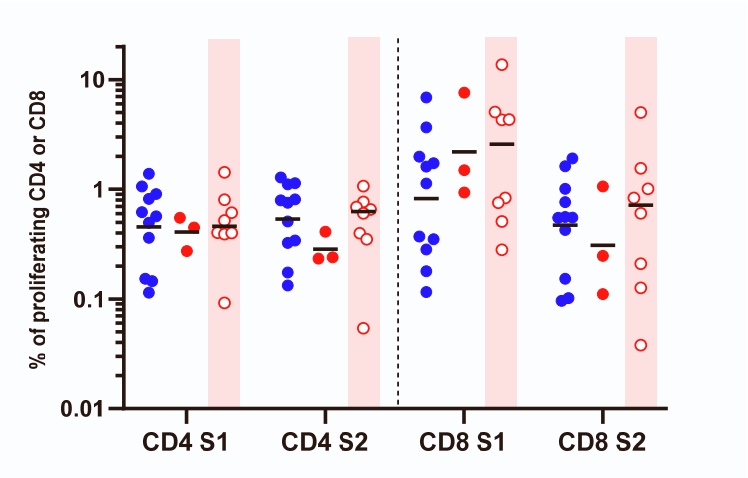


Figure S11. breadth of proliferative response at d180, related to Figure 5

Dot plot represent the percentage of proliferating CD4 (left part) and CD8 (right part) T cells stimulated with either S1 spike sub-pool (first and third groups) or S2 Spike sub-pool (second and fourth groups) in subjects representing the RD (blue dots), SD (red dots) and SD+vax (empty red dots and pink shaded area, highlighting that before d180 visit the volunteers have received a COVID-19 approved vaccine) groups.

Figure S12

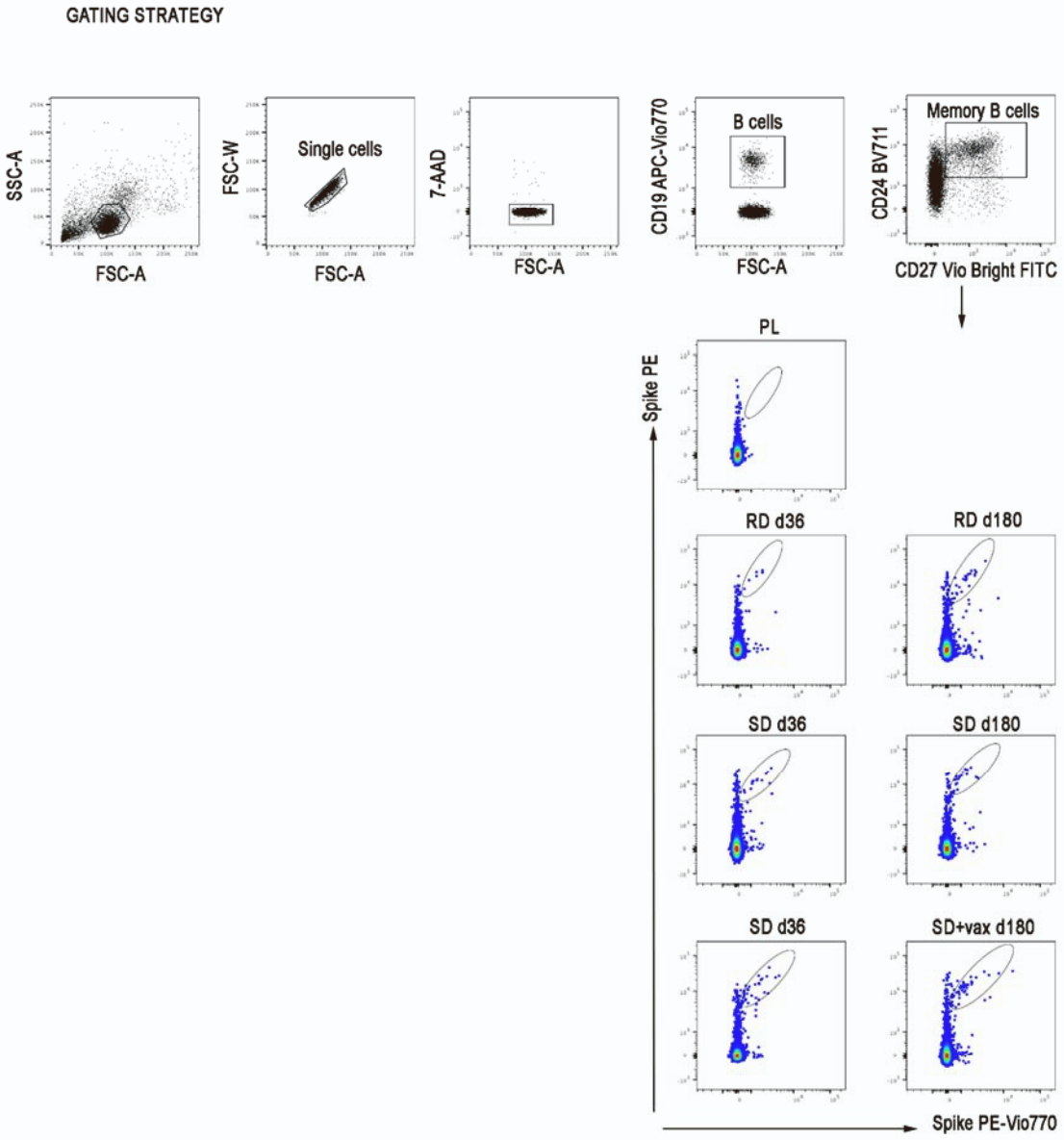


Figure S12. Spike-specific B cell staining gating strategy, related to Figure 5
Representative plots for gating strategy (upper lane) of B cell populations and Spike-specific memory B cells in Placebo (upper plot), RD (second lane plots), SD (third lane plots) and SD+vax (fourth lane plots) at day 36 (left column) and day 180 (right column).