**Supplemental Information**

COVID-eVax, an electroporated DNA vaccine

candidate encoding the SARS-CoV-2 RBD, elicits

protective responses in animal models

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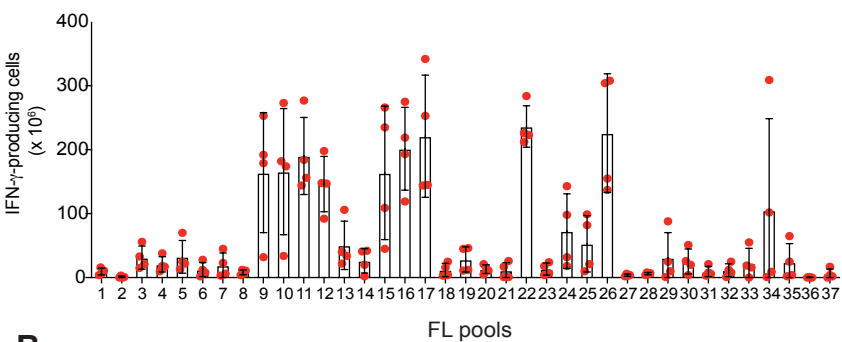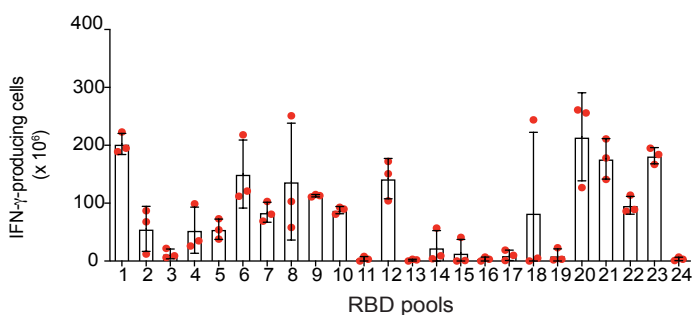
A

Electroporated mice
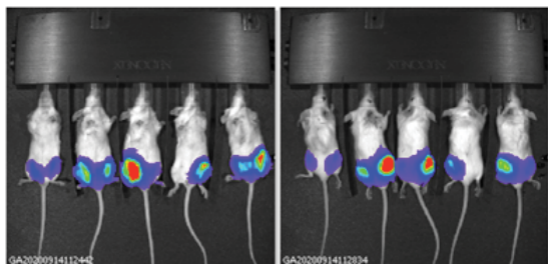
Not electroporated mice
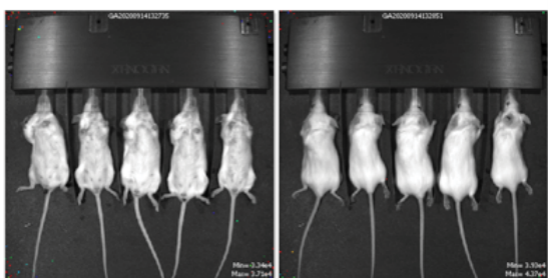
B
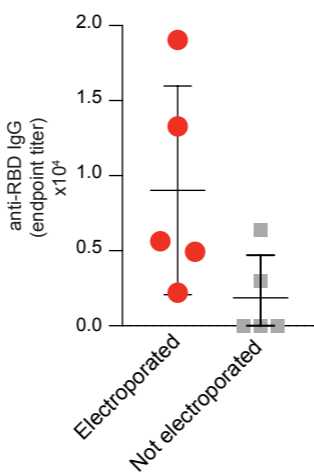
A

B

C

Peptide	Sequence	Amino Acid
69	GAAAYYVGYLQPRTF	261-275
70	YYVGYLQPRTFLLKY	265-279
71	YLQPRTFLLKYNENG	269-283
85	QPTESIVRFPNITNL	321-335
93	AWNRKRISNCVADYS	352-366
99	STFKCYGVSPTKLND	375-389
100	CYGVSPTKLNDLCFT	379-393
117	VGGNYNYLYRLFRKS	445-459
118	NYNYLYRLFRKSNLK	450-464
124	IYQAGSTPCNGVEGF	472-486
128	CYFPLQSYGFQPTNG	488-502
132	VGYQPYRVVVLSFEL	503-517
137	HAPATVCGPKKSTNL	519-533
138	TVCGPKKSTNLVKNK	523-537
139	PKKSTNLVKNKCVNF	527-541

A

IFN-γ-producing cells (x 10⁶)

1500
1000
500

DMSO POOL S1 POOL S2 (repeated)

0.1µg x 1 leg
0.1µg x 2 legs
0.5µg x 1 leg
0.5µg x 2 legs
1µg x 1 leg
1µg x 2 legs
5µg x 1 leg
5µg x 2 legs
10µg x 1 leg
10µg x 2 legs

B

IFN-γ-producing cells (x 10⁶)

1500
1000
500
0

RBD total dose (µg)

0.01 0.1 1 10 100

C

CD3⁺ CD4⁺ (%)

5
4
3
2
1
0

IFNγ⁺ TNFα⁺ IL4⁺ IL5⁺ IL17A⁺

CD3⁺ CD8⁺ (%)

5
4
3
2
1
0

IFNγ⁺ TNFα⁺ IL4⁺ IL5⁺ IL17A⁺

▼ male
▲ female
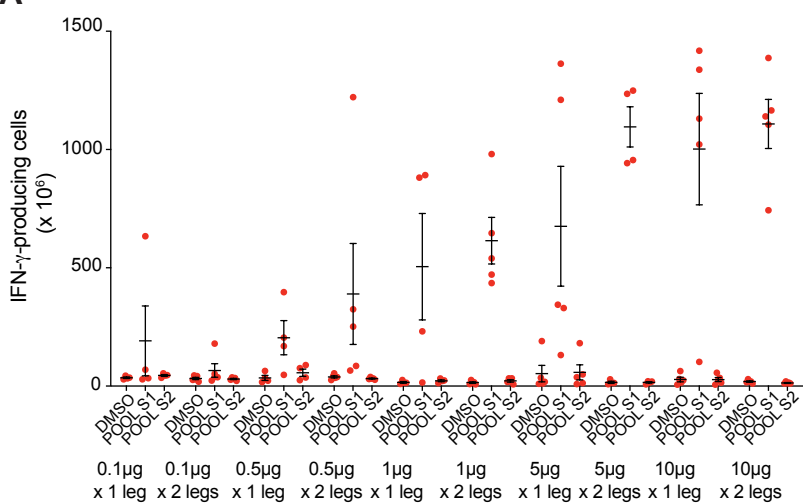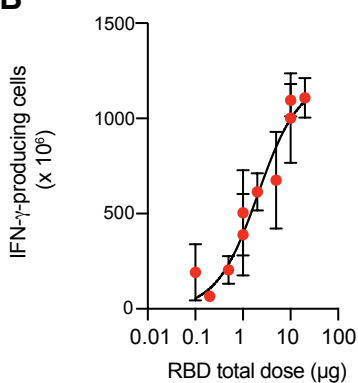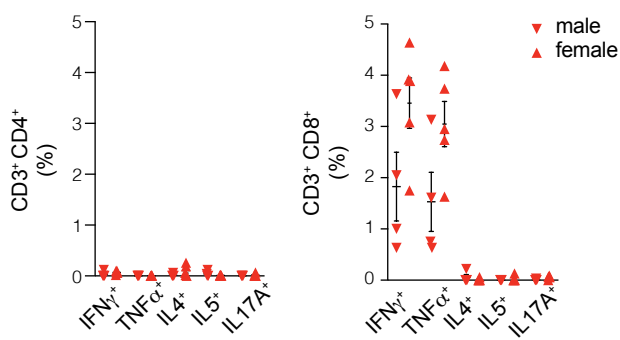
A

injection site: 2 days from EP + IM injection

500µm
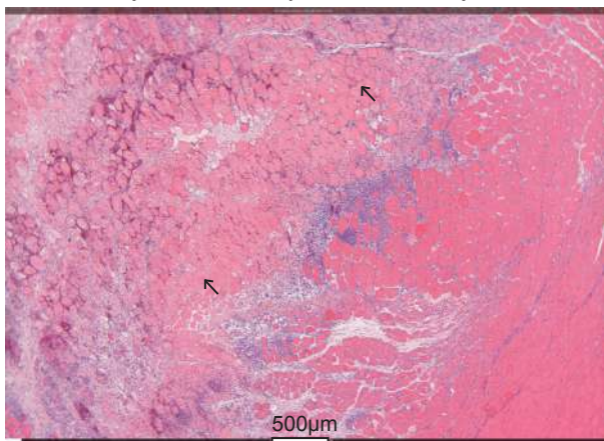
B

injection site: 4 weeks from EP + IM injection

500µm

A

BALB/c

d0
↓
RBD

d2
analysis

Untreated	-
No EP	X
EP	X

B Sera

RBD [ng/ml]

C BAL

RBD [ng/ml]

D

PBS | RBD

Specific IgG/IgM αSars-CoV-2 RBD
mg/ml

1:50 1:100 1:200 1:400 1:800 1:1600 1:3200 1:6400

E

K18-hACE2

d-2
↓

d0
↓
I.N

d2
IVIS
analysis

| PBS | X |
| RBD | X |

F

Photon/sec
(x10⁴)

*
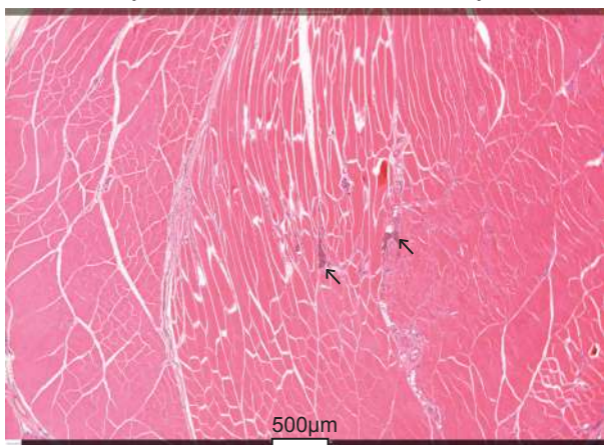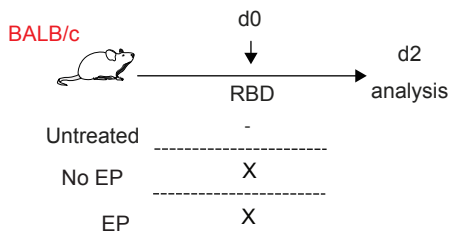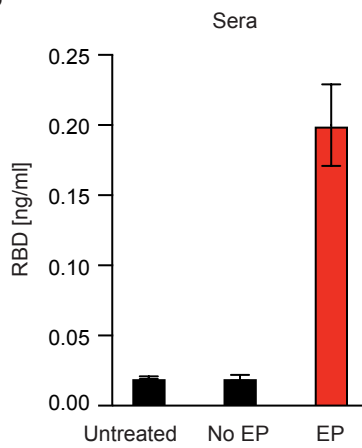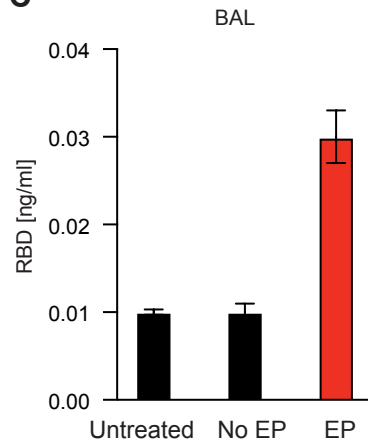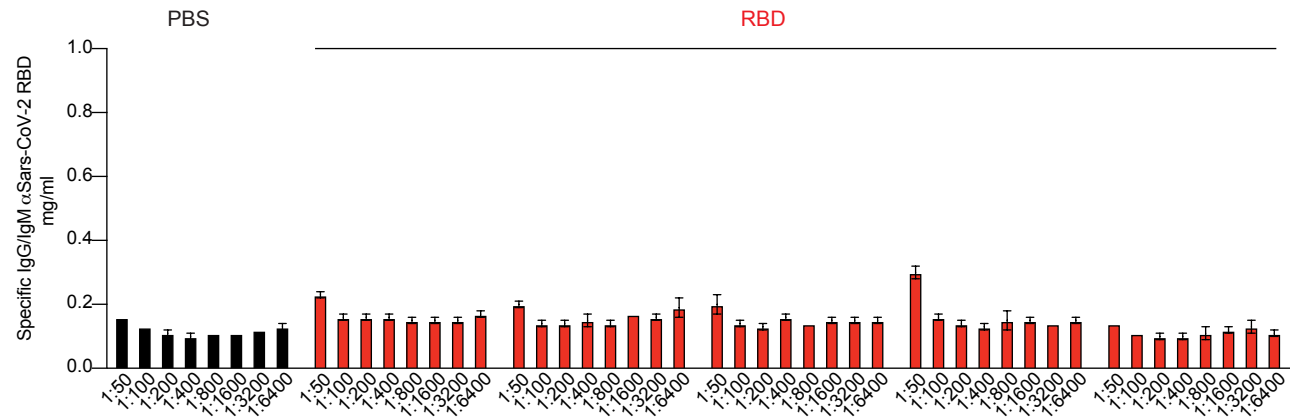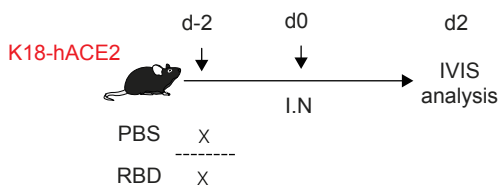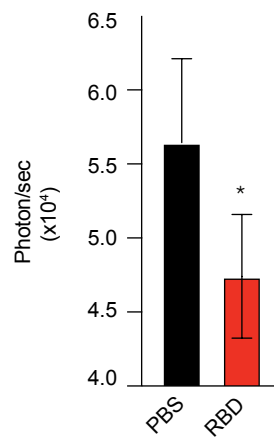
Supplementary Figure Legends

Supplementary Figure 1. ***Electroporation increases the level of gene expression upon DNA immunization.*** **(A)** BALB/c mice ($n = 5$) were either injected i.m. with 1 mg of a plasmid expressing firefly luciferase followed by electroporation (upper panel, electroporated mice) or not (lower panel, non-electroporated mice). Forty-eight hours later, optical imaging was carried out using an IVIS 200 system. Ventral and dorsal images were taken. **(B)** BALB/c mice were injected with 5 mg of RBD vaccine, with or without electroporation. 14 days later mice were bled, and anti-RBD IgG endpoint titers were measured by ELISA.

Supplementary Figure 2. ***T cell epitope mapping in RBD vaccinated mice.*** **(A-B)** IFNγ^+ T cell response measured by ELISpot assay on splenocytes collected from FL or RBD-vaccinated BALB/c mice, following stimulation with matrix mapping FL or RBD peptide pools. **(C)** Schematic representation of the SARS-CoV-2 Spike protein and identification of immunodominant peptides in BALB/c mice.

Supplementary Figure 3. ***RBD-specific immune response in RBD vaccinated C57BL/6 mouse model.*** **(A)** IFN-γ^+ T cell response measured by ELISpot assay on splenocytes collected at day 38 from C57BL/6 mice vaccinated with increasing doses of RBD vaccine (from 0.1 to 20 μg, administered at one or two sites) and restimulated with Spike peptide pools S1 and S2. **(B)** Non-linear fitting curve of the dose-response against

RBD pool S1 peptides, measured by means of ELISpot assay performed on splenocytes from RBD-vaccinated C57BL/6 mice. **(C)** T cell characterization by intracellular staining on PBMCs collected from males and females vaccinated mice (administered dose: 5μg / leg).

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Supplementary Figure 4. *Histopathological evaluation of electroporated tissues in rat model.* **(A)** Histological section of the left injection site in a 400 μg RBD-vaccinated rat performed two days after the third and last DNA injection (i.e. day 30). Arrows indicate the necrosis of muscle fibers, surrounded by inflammatory reaction (i.e.,

10   polymorphonuclear cells, mixed mononuclear cell infiltration, predominantly macrophages). The cavities surrounded by the necrotic carbonized muscle fibers are suggested to be related to the electroporation procedure. The lesions were mostly scored as mild to moderate and were similar in all the groups. **(B)** Histological section of the left injection site in a 400 μg RBD vaccinated rat performed 4 weeks after third and

15   last DNA injection (i.e. day 57). Arrows indicate brownish pigmented muscle fibers, probably related to a minimal chronic inflammation due to the electroporation procedure. This image demonstrates a complete recovery of the injection site lesions at this stage, in comparison to (A).

20   **Supplementary Figure 5.** *Assessment of secreted RBD in RBD vaccinated mice.* **(A)** Schematic representation of the experimental setup. BALB/c mice were vaccinated with 20 µg of RBD vaccine, with or without electroporation, and 48 hours later the

secretion of RBD protein was assessed in sera and BALs. **(B)** Measurement of secreted

RBD protein in sera from control mice and RBD vaccinated mice, with or without EP. **(C)**

Measurement of secreted RBD protein in BALs from same groups of mice as in (B). **(D)**

Measurement of anti-RBD antibodies in the sera at day 2 after RBD or PBS vaccination

5 **(E)** Schematic representation of the experimental setup. K18-hACE2 mice were

vaccinated with 20 µg of RBD vaccine and 2 days later a lentiviral vector pseudotyped

with the SARS-CoV-2 spike protein and encoding for luciferase RBD protein was

intranasally administered. Two days later the lungs of treated mice were assessed for

bioluminescence using an in vivo imaging system. **(F)** Comparison of bioluminescence

10 assessed by means of *in vivo* imaging system in control K18-hACE2 mice and K18-

hACE2 RBD vaccinated mice. * p value < 0.05

Supplementary Table Legends

Table S1. *List of immunodominant B epitopes.*

5   **Table S2**. *Scheme of RBD peptide pool matrix.*

Immunodominant B epitope list

Peptide	Sequence	Amino Acid
5	NLTTRTQLPPAYTNS	17-31
6	RTQLPPAYTNSFTRG	21-35
22	RFDNPVLPFNDGVYF	85-99
36	CEFQFCNDPFLGVYY	141-155
37	FCNDPFLGVYYHKNN	145-159
56	INLVRDLPQGFSALE	221-235
57	RDLPQGFSALEPLVD	225-239
76	AVDCALDPLSETKCT	301-315
77	ALDPLSETKCTLKSF	305-319
90	VFNATRFASVYAWNR	357-371
92	SVYAWNRKRISNCVA	365-379
120	FRKSNLKPFERDIST	477-491
121	NLKPFERDISTEIYQ	481-495

Epitope T mapping description.
RBD peptides are 132 out of the 338 peptides covering the whole Spike protein (from peptide nr.4 to peptide nr.136). In order to identify immunodominant RBD epitopes, Elispot assay was performed by stimulating splenocytes from RBD vaccinated Balb/c mice for 20h with RBD peptide pools. Pools (from 1 to 24) were distributed as a matrix (the intersection of two pools identifies one RBD peptide), with each pool comprising up to 12 RBD peptides, as shown in table…. Immunodominant RBD peptides were identified at the intersection of pools showing >50 SFCs.

1	2	3	4	5	6	7	8	9	10	11	12	
4	5	6	7	8	9	10	11	12	13	14	15	13
16	17	18	19	20	21	22	23	24	25	26	27	14
28	29	30	31	32	33	34	35	36	37	38	39	15
40	41	42	43	44	45	46	47	48	49	50	51	16
52	53	54	55	56	57	58	59	60	61	62	63	17
64	65	66	67	68	69	70	71	72	73	74	75	18
76	77	78	79	80	81	82	83	84	85	86	87	19
88	89	90	91	92	93	94	95	96	97	98	99	20
100	101	102	103	104	105	106	107	108	109	110	111	21
112	113	114	115	116	117	118	119	120	121	122	123	22
124	125	126	127	128	129	130	131	132	133	134	135	23
136												24