

## SUPPLEMENTARY DATA

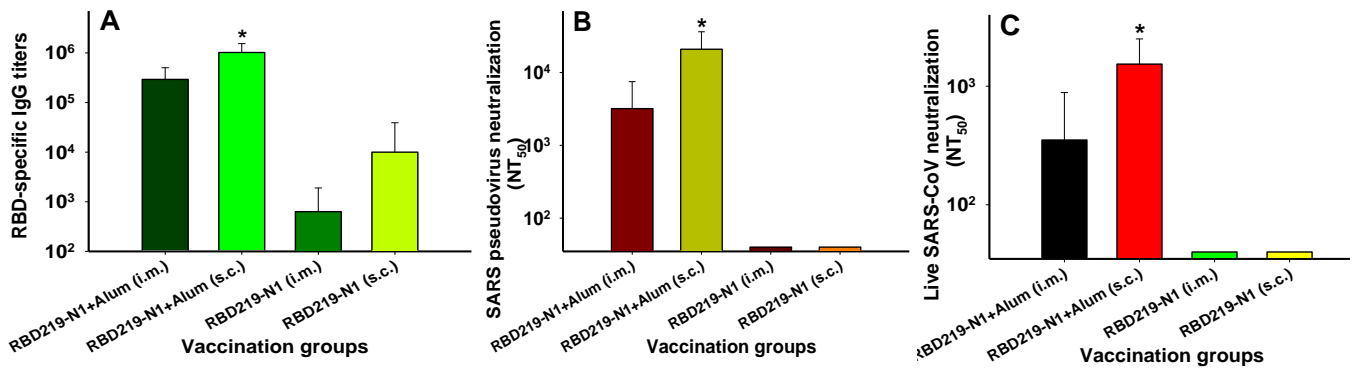
**Supplementary Table 1.** Immunogenicity and efficacy results for adjuvant screening. n/s: not significant, M: moderate, S: severe. ND: Not detected. N/A: not available

Vaccination Groups	NT <sub>100</sub> (log <sub>2</sub> )	NT <sub>50</sub> (Log <sub>2</sub> )	infectious virus/gram (dpi)	Infiltration grades (dpi)	EOS# (dpi)	Specific IgG titers (x 10 <sup>3</sup> ) (ELISA)	IgG1/IgG2a ratios
RBD219-N1/Alhydrogel	1,280 (10)	--*	ND** (3)	1 (3)	n/s (3)	512	4
	640 (9)	1,280 (10)	ND (3)			512	8
	1,280 (10)	2,560 (11)	ND (6)	1 (6)	n/s (6)	128	4
	640 (9)	1,280 (10)	ND (6)			256	8
RBD219-N1/MF59-like adjuvant	N/A	20	7.5 x 10 <sup>4</sup> (3)	3 (3)	S	128	64
	80	--	5 x 10 <sup>4</sup> (3)	3 (6)	S	256	1,024
	N/A	< 20	ND (6)			512	64
	N/A	< 20	Dead (6)			512	256
RBD219-N1 alone	N/A	< 20	5 x 10 <sup>6</sup> (3)	2 (3)	M	0.1	--
	N/A	< 20	7.5 x 10 <sup>6</sup> (3)	--	--	ND	--
	N/A	< 20	Dead (4)			0.4	--
	N/A	< 20	Dead (5)			ND	--

**Supplementary Table 2.** Immunogenicity and efficacy results for Alhydrogel dose-ranging study.

\* Sacrifice; \*\* Not applicable; # Not detected; § Death due to over anesthetization

Groups	Animal ID#	NT <sub>100</sub>	NT <sub>50</sub>	ELISA titers RBD-specific IgG antibody	RBD-specific IgG1/IgG2a antibody ratio	Infectious virus/g (dpi)	Eos-infiltration grade
RBD219-N1/ Alhydrogel (RBD: 20, 10, 10 µg; Alhydrogel: 500, 250, 250 µg)	1	> 1,280	NA**	102,400	32	ND# (3)	1
	2	640	NA	102,400	16	Death (OA)§	NA
	3	640	1,280	102,400	16	ND (3)	1
	4	160	320	51,200	8	ND (6)	1
	5	320	640	51,200	16	ND (6)	1
	6	> 1,280	NA	51,200	32	Death (OA)	NA
RBD219-N1/ Alhydrogel (RBD: 20, 10, 10 µg; Alhydrogel: 160, 80, 80 µg)	7	< 10	NA	6,400	64	1 x 10 <sup>6</sup> (3)	2
	8	< 10	NA	1,600	32	2.5 x 10 <sup>6</sup> (3)	2
	9	< 10	NA	200	NA	2.5 x 10 <sup>6</sup> (3)	2
	10	< 10	NA	25,600	256	Dead (6)	NA
	11	< 10	NA	25,600	64	5 x 10 <sup>4</sup> (6)	2
	12	40	80	25,600	64	ND (6)	2
RBD219-N1/ Alhydrogel (RBD: 10, 10, 10 µg; Alhydrogel: 80, 80, 80 µg)	13	< 10	NA	6,400	128	1 x 10 <sup>5</sup> (3)	2
	14	< 10	NA	400	NA	Dead (3)	NA
	15	160	320	25,600	64	1 x 10 <sup>4</sup> (3)	2
	16	160	NA	51,200	64	ND (6)	2
	17	10	20	25,600	4	ND (6)	2
	18	< 10	NA	800	32	2.5 x 10 <sup>4</sup> (6)	2
TRIS- Alhydrogel (Alhydrogel: 160, 160, 160 µg)	19	< 10	NA	< 100	NA	Dead (3)	NA
	20	< 10	NA	< 100	NA	Dead (3)	NA
	21	< 10	NA	< 100	NA	1 x 10 <sup>7</sup> (3)	1
	22	< 10	NA	< 100	NA	5 x 10 <sup>5</sup> (3)	1
	23	< 10	NA	< 100	NA	2.5 x 10 <sup>4</sup> (6)	1
	24	< 10	NA	< 100	NA	1 x 10 <sup>4</sup> (6)	1
SARS-S/Alum (S: 3, 3, 3 µg; Alum: pre-formulated)	25	640	NA	51,200	32	ND (3)	2+
	26	320	NA	25,600	16	ND (3)	2+
	27	320	NA	51,200	32	ND (3)	2+
	28	320	NA	51,200	32	ND (6)	2+
	29	320	NA	51,200	32	ND (6)	2+
	30	Sac*	NA	NA	NA	NA	NA



**Supplementary Figure 1. Optimization of immunization routes.** Mice were immunized with RBD219-N1 formulated with or without Alhydrogel® (1:25 ratio) subcutaneously (s.c.) or intramuscularly (i.m.), three times, at 3-week intervals. Sera were collected 10 days after the last immunization and tested for IgG antibody responses and for neutralizing antibodies against SARS pseudovirus and live SARS-CoV infections. (A) Detection of IgG antibody response by ELISA in mouse sera. Neutralization antibody titers against SARS pseudovirus (B) and live SARS-CoV (C) in mouse sera. [Alhydrogel® abbreviated as Alum.]. Pseudovirus was prepared as previously described in Chen et al., 2014 [20].