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Supplementary Materials for

Single-component, self-assembling, protein nanoparticles presenting the receptor binding domain and stabilized spike as SARS-CoV-2 vaccine candidates

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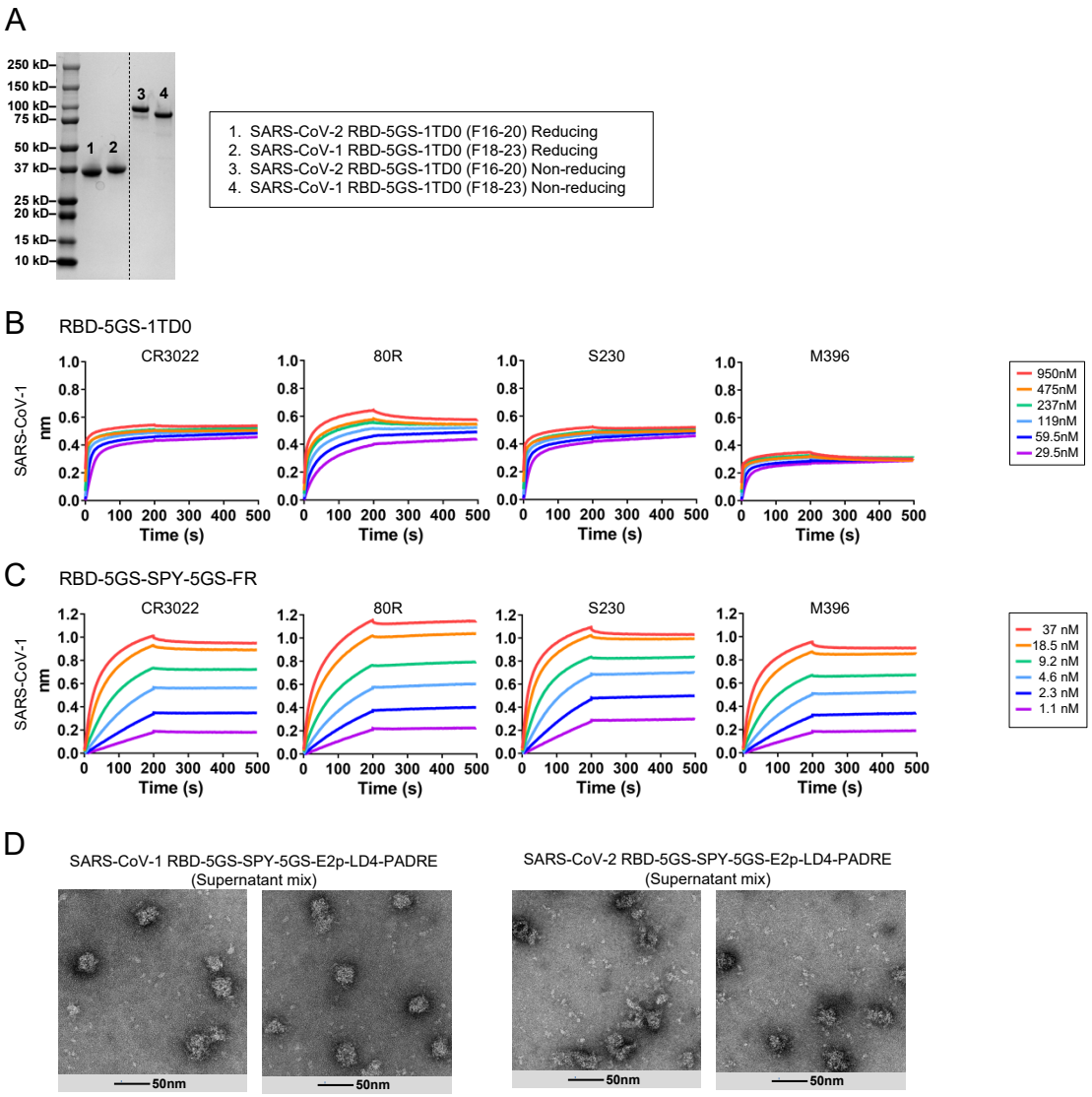


fig. S1. In-vitro characterization of SARS-CoV-1/2 RBD-based immunogens. (A) SDS-PAGE of SEC/CR3022-purified SARS-CoV-1/2 RBD-5GS-1TD0 under the reducing and non-reducing conditions. The black dashed line indicates where an unrelated protein analyzed on the same gel has been removed. **(B)** and **(C)** BLI profiles of SARS-CoV-1 RBD-5GS-1TD0 trimer and RBD-5GS-SPY-5GS-FR SApNP binding to four mAbs. Sensorgrams were obtained from an Octet RED96 instrument using a series of six concentrations (950-29.5 nM for the RBD trimer and 37-1.1 nM for the SPY-linked RBD FR SApNP, respectively, both by twofold dilutions) and AHC biosensors. **(D)** Negative-stain EM (nsEM) images of SARS-CoV-1/2 RBD-5GS-SPY-5GS-E2p-LD4-PADRE (or E2p-L4P) SApNPs. E2p-LD4-PADRE is a multilayered SApNP carrier with a locking domain (LD4) and a T-cell epitope (PADRE) fused to each E2p subunit. LD4 forms an inner layer to stabilize the NP shell from the inside and PADRE forms a cluster at the center of the SApNP.

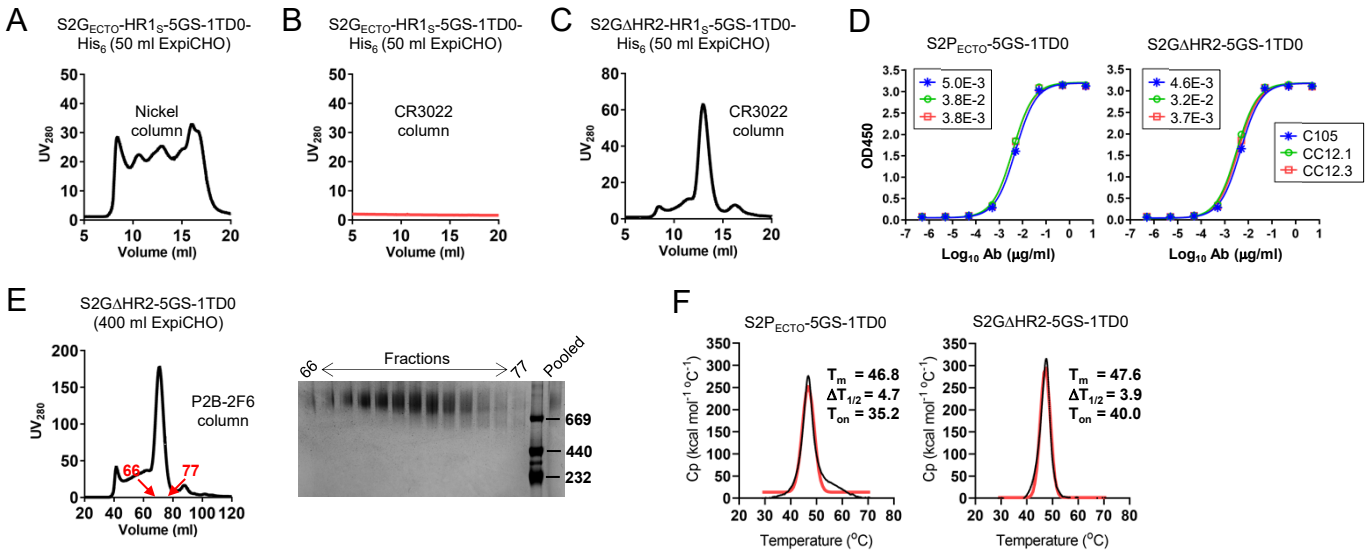


fig. S2. In-vitro characterization of SARS-CoV-2 spike antigens. (A) SEC profile of S2G$_{ECTO}$-HR1$_S$-5GS-1TD0-His$_6$ in which the SARS-CoV-2 HR1 region (L922-S943) is swapped with the equivalent SARS-CoV-1 HR1 region, thus termed "HR1$_S$". This construct was transiently expressed in 50 ml ExpiCHO cells and purified on a Nickel column. **(B)** SEC profile of S2G$_{ECTO}$-HR1$_S$-5GS-1TD0-His$_6$ following transient expression in 50 ml ExpiCHO cells and purification on a CR3022 column. **(C)** SEC profile of S2GΔHR2-HR1$_S$-5GS-1TD0-His$_6$ following transient expression in 50 ml ExpiCHO cells and purification on a CR3022 column. **(D)** ELISA curves of S2P$_{ECTO}$-5GS-1TD0 and S2GΔHR2-5GS-1TD0 binding to three newly identified potent human NAbs, C105, CC12.1, and CC12.3. **(E)** Left: SEC profile of S2GΔHR2-5GS-1TD0 obtained from a HiLoad 16/600 Superose 6 column following transient expression in 400 ml ExpiCHO cells and purification on a P2B-2F6 antibody column. The range of fractions used for the BN-PAGE analysis is indicated on the SEC profile. Right: BN-PAGE of SEC fractions (66-77 ml) and pooled fractions. **(F)** Thermostability of S2P$_{ECTO}$-5GS-1TD0 and S2GΔHR2-5GS-1TD0, with Tm, ΔT1/2, and Ton measured by differential scanning calorimetry (DSC) and labeled on the plots. The raw and Gaussian-fitted DSC data are shown in black and red lines, respectively.

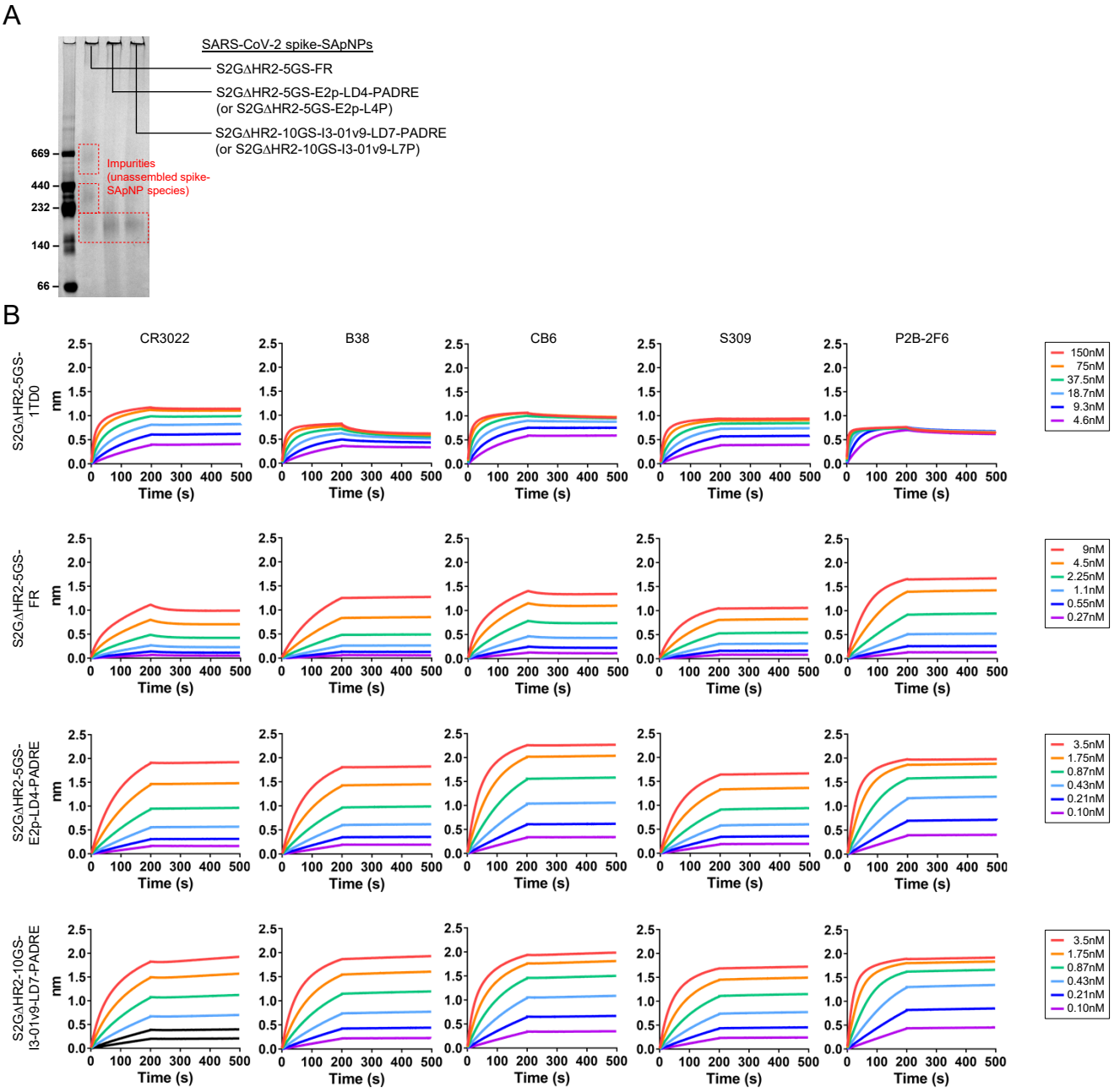
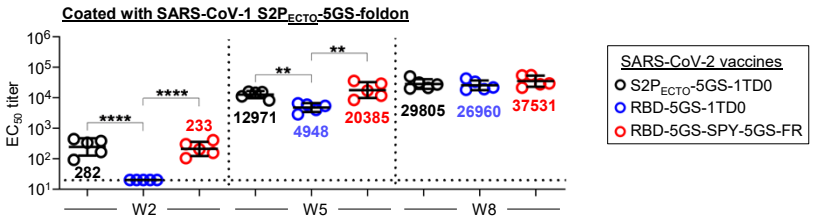


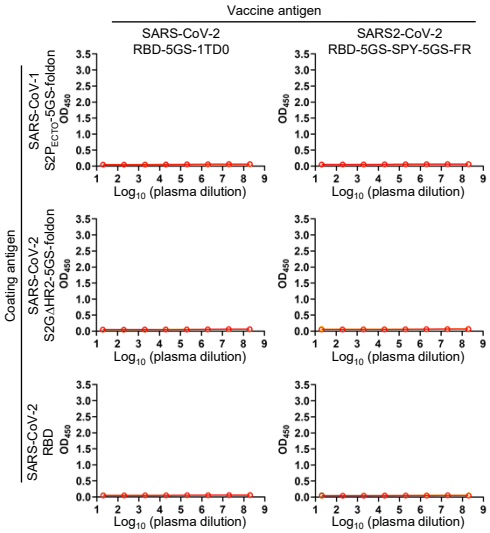
fig. S3. In-vitro characterization of SARS-CoV-2 S2GΔHR2 SApNPs. (A) BN-PAGE of CR3022-purified S2GΔHR2-presenting SApNPs. Due to the large size and molecular mass, NPs would be in the well on the top of the gel, whereas the unassembled species would be seen on the gel. **(B)** BLI profiles of SARS-CoV-2 S2GΔHR2-presenting SApNPs binding to five mAbs. The SARS-CoV-2 S2GΔHR2-5GS-1TD0 trimer was included for comparison. Sensorgrams were obtained from an Octet RED96 instrument using a series of six concentrations (150-4.6nM for the S2GΔHR2 trimer, 9-0.27nM for the FR NP, and 3.5-0.1nM for the E2p and I3-01v9 NPs, respectively, all by twofold dilutions) and AHQ biosensors.

fig. S4

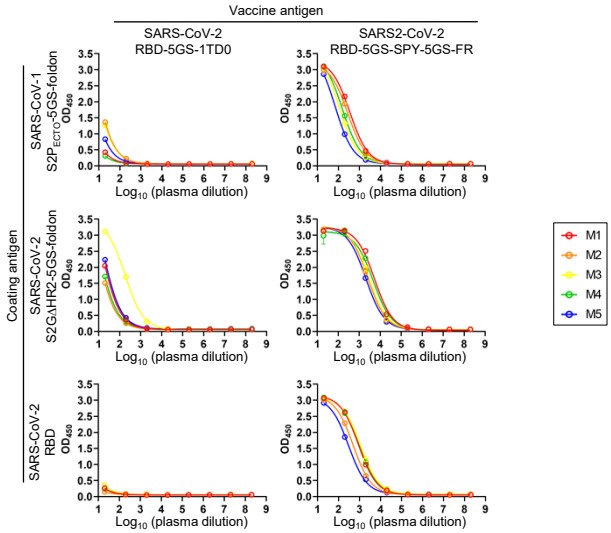
A **SARS-CoV-2 RBD/RBD-SApNP vaccine-induced binding antibody response**



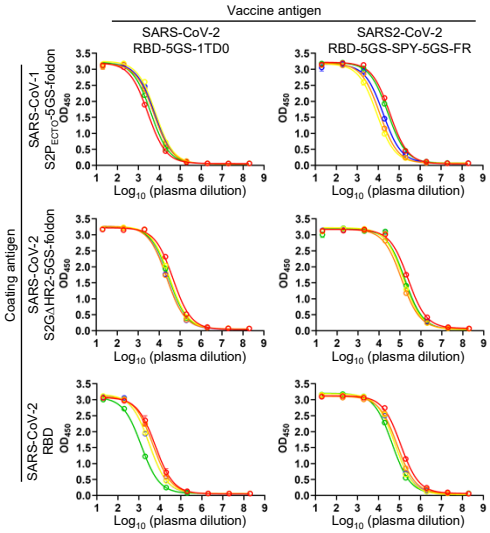
B **Mouse plasma ELISA at the 1st time point (Pre)**



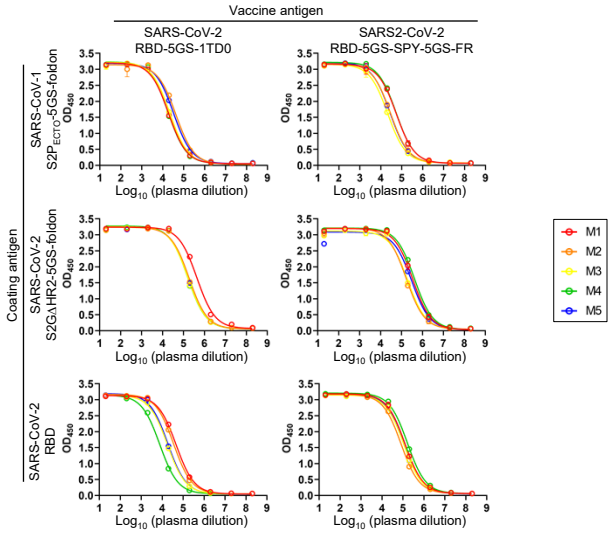
Mouse plasma ELISA at the 2nd time point (w2)



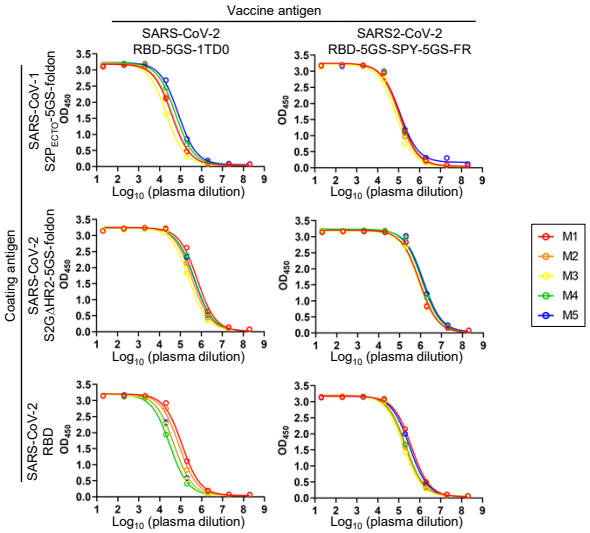
Mouse plasma ELISA at the 3rd time point (w5)



Mouse plasma ELISA at the 4th time point (w8)



Mouse plasma ELISA at the 5th time point (w11)



C **Mouse plasma ELISA EC$_{50}$ titers**

SARS-CoV-1 S2P$_{ECTO}$-5GS-foldon

SARS-CoV-2 vaccine antigen	w2					w5					w8					w11				
	M1	M2	M3	M4	M5	M1	M2	M3	M4	M5	M1	M2	M3	M4	M5	M1	M2	M3	M4	M5
RBD-5GS-1TD0	<20	<20	<20	<20	<20	2849	5426	6544	4016	5908	18964	42380	21799	18353	33308	37799	38906	21152	57386	77425
RBD-5GS-SPY-5GS-FR	402	299	155	205	105	35702	11730	8478	28869	17148	55019	29258	21429	54316	27631	116637	100384	75563	114456	117834

Coating antigen

SARS-CoV-2 S2GΔHR2-5GS-foldon

SARS-CoV-2 vaccine antigen	w2					w5					w8					w11				
	M1	M2	M3	M4	M5	M1	M2	M3	M4	M5	M1	M2	M3	M4	M5	M1	M2	M3	M4	M5
RBD-5GS-1TD0	33	<20	237	23	41	45821	22822	31754	28895	23447	437779	186022	163880	159780	177684	640237	365517	258393	495393	458394
RBD-5GS-SPY-5GS-FR	5719	2893	3239	4472	2280	245016	119447	145979	172388	146375	343504	169804	194569	415264	305726	893358	866269	801461	1181201	1352045

SARS-CoV-2 RBD

SARS-CoV-2 vaccine antigen	w2					w5					w8					w11				
	M1	M2	M3	M4	M5	M1	M2	M3	M4	M5	M1	M2	M3	M4	M5	M1	M2	M3	M4	M5
RBD-5GS-1TD0	<20	<20	<20	<20	<20	6061	4770	3127	1293	3140	46119	35578	17646	7671	18814	116486	82504	45221	30347	47738
RBD-5GS-SPY-5GS-FR	913	499	1092	978	292	115261	78765	61890	45215	61522	125683	81629	110472	165129	110446	402781	200112	165137	217751	328643

fig. S4. SARS-CoV-2 RBD/RBD-SApNP vaccine-induced binding antibody responses. (A) EC$_{50}$ titers derived from the ELISA binding of mouse plasma from two SARS-CoV-2 RBD-based vaccine groups (RBD-5GS-1TD0 and RBD-5GS-SPY-5GS-FR) to the coating antigen (SARS-CoV-1 S2P$_{ECTO}$-5GS-foldon) are plotted, with average EC$_{50}$ values labeled on the plots. The S2P spike vaccine group (S2P$_{ECTO}$-5GS-1TD0) is included here for comparison with RBD-based vaccine groups, with the detailed ELISA data shown in fig. S5. The *P*-values were determined by an unpaired *t* test in GraphPad Prism 8.4.3 with (*) indicating the level of statistical significance (*: 0.01<P≤0.05; **: 0.001<P≤0.01; ***: 0.0001<P≤0.001; ****: P≤0.0001). **(B)** ELISA binding curves of mouse plasma from two SARS-CoV-2 RBD-based vaccine groups to three coating antigens, SARS-CoV-1 S2P$_{ECTO}$-5GS-foldon, SARS-CoV-2 S2GΔHR2-5GS-foldon, and SARS-CoV-2 RBD. **(C)** Summary of EC$_{50}$ titers measured for two SARS-CoV-2 RBD-based vaccine groups against three coating antigens. Color coding indicates the level of EC$_{50}$ titer (white: no binding; green to red: low to high). The EC$_{50}$ values were calculated in GraphPad Prism 8.4.3. Of note, the EC$_{50}$ values at w2 were derived by setting the lower/upper constraints of OD$_{450}$ at 0.0/3.2 to achieve greater accuracy.

fig. S5

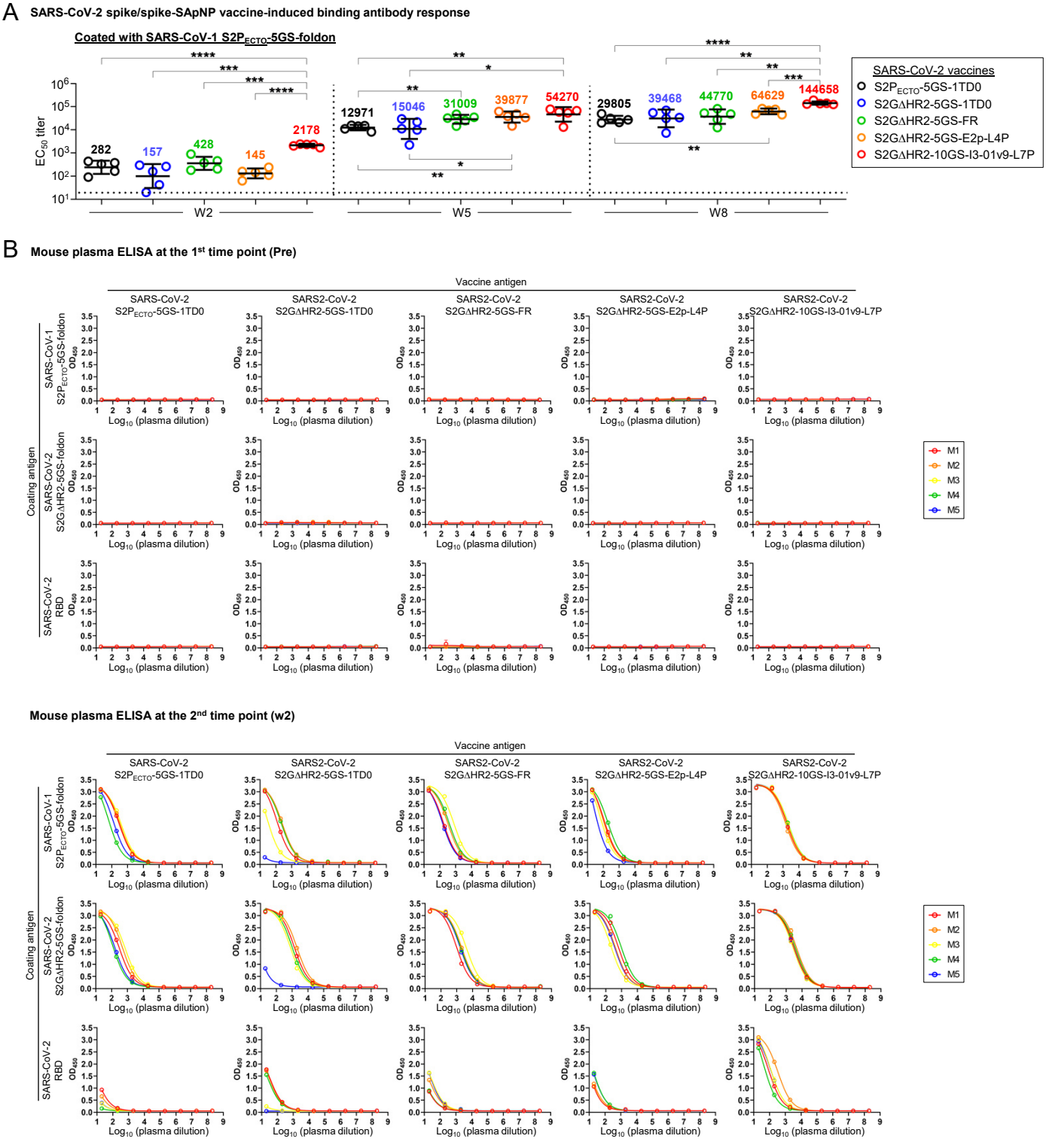
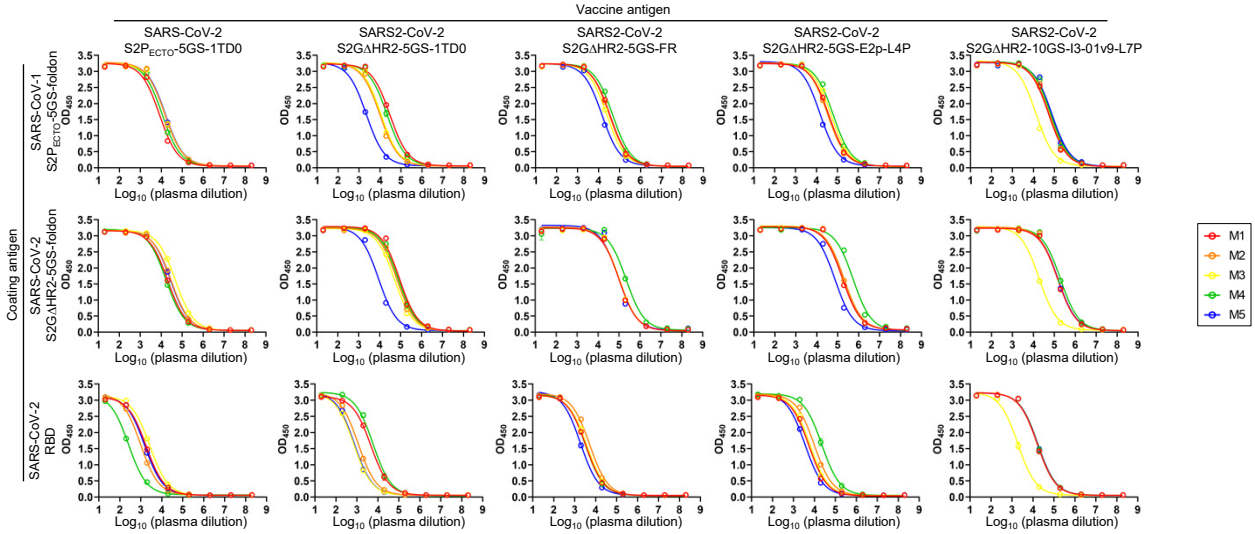

A
SARS-CoV-2 spike/spike-SApNP vaccine-induced binding antibody response
Coated with SARS-CoV-1 S2P$_{ECTO}$-5GS-foldon
EC$_{50}$ titer
282
157
428
145
2178
12971
15046
31009
39877
54270
29805
39468
44770
64629
144658
W2
W5
W8
SARS-CoV-2 vaccines
S2P$_{ECTO}$-5GS-1TD0
S2GΔHR2-5GS-1TD0
S2GΔHR2-5GS-FR
S2GΔHR2-5GS-E2p-L4P
S2GΔHR2-10GS-I3-01v9-L7P
B
Mouse plasma ELISA at the 1st time point (Pre)
Mouse plasma ELISA at the 2nd time point (w2)
Vaccine antigen
SARS-CoV-2 S2P$_{ECTO}$-5GS-1TD0
SARS2-CoV-2 S2GΔHR2-5GS-1TD0
SARS2-CoV-2 S2GΔHR2-5GS-FR
SARS2-CoV-2 S2GΔHR2-5GS-E2p-L4P
SARS2-CoV-2 S2GΔHR2-10GS-I3-01v9-L7P
Coating antigen
SARS-CoV-1 S2P$_{ECTO}$-5GS-foldon
SARS-CoV-2 S2GΔHR2-5GS-foldon
SARS-CoV-2 RBD
OD$_{450}$
Log$_{10}$ (plasma dilution)
M1
M2
M3
M4
M5

fig. S5

Mouse plasma ELISA at the 3rd time point (w5)



Mouse plasma ELISA at the 4th time point (w8)

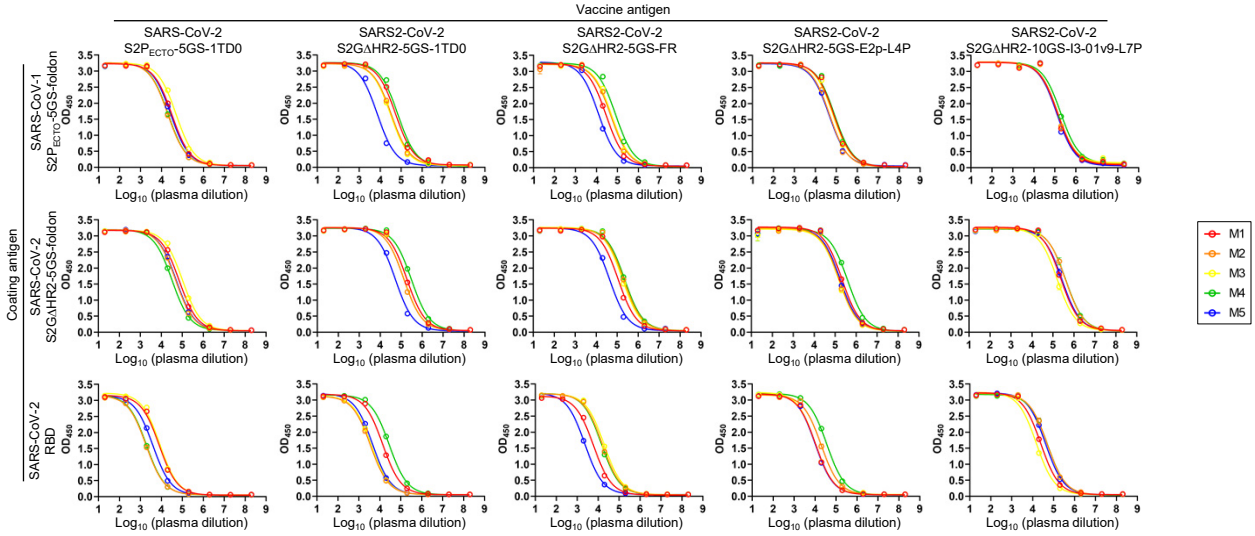
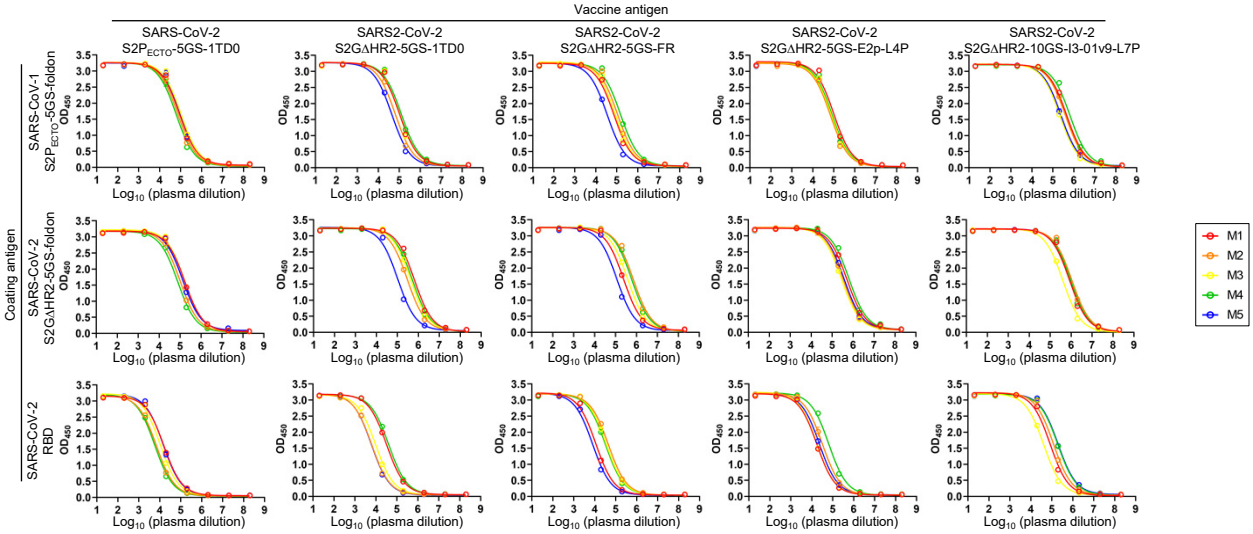


fig. S5

Mouse plasma ELISA at the 5th time point (w11)



C **Mouse plasma ELISA EC_{50} titers**

Coating antigen: SARS-CoV-1 S2P$_{ECTO}$-5GS-foldon

SARS-CoV-2 vaccine antigen	w2					w5					w8					w11				
	M1	M2	M3	M4	M5	M1	M2	M3	M4	M5	M1	M2	M3	M4	M5	M1	M2	M3	M4	M5
S2P$_{ECTO}$-5GS-1TD0	337	378	438	92	166	8229	14598	15271	11029	15730	31346	19923	49495	20899	27362	87963	74675	105473	61814	98729
S2GDHR2-5GS-1TD0	160	300	43	264	<20	29889	9934	11211	21972	2225	54736	33977	31597	69701	7331	104856	65140	102307	125912	44213
S2GDHR2-5GS-FR	207	379	907	463	182	35236	32621	25025	47534	14631	28314	48030	53004	81962	12542	73512	110204	85157	152546	35108
S2GDHR2-5GS-E2p-L4P	161	145	111	244	64	38631	34068	49277	62620	14788	76610	48384	69459	83674	45019	101670	68765	75147	83008	78758
S2GDHR2-10GS-I3-01v9-L7P	2047	1697	2368	2434	2342	53863	59267	13164	67367	77688	136410	139928	130590	190016	126344	461522	400892	212809	662353	240540

Coating antigen: SARS-CoV-2 S2GΔHR2-5GS-foldon

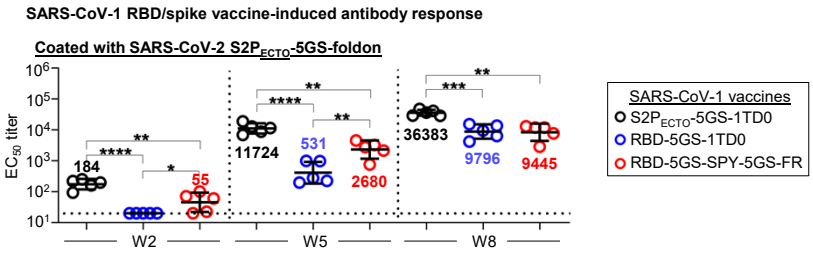
SARS-CoV-2 vaccine antigen	w2					w5					w8					w11				
	M1	M2	M3	M4	M5	M1	M2	M3	M4	M5	M1	M2	M3	M4	M5	M1	M2	M3	M4	M5
S2P$_{ECTO}$-5GS-1TD0	342	564	786	152	186	20525	30418	47919	16989	28307	71336	48544	104033	32473	51430	168104	101372	164947	77599	136078
S2GDHR2-5GS-1TD0	1623	2329	915	1190	<20	88704	66897	51587	77015	8882	188541	133257	137492	308124	53005	648885	297906	427211	524920	103434
S2GDHR2-5GS-FR	1168	2365	4609	2102	1925	100424	99021	105640	217243	94779	112191	207821	177619	227282	43471	240083	710983	381026	592260	116127
S2GDHR2-5GS-E2p-L4P	748	481	268	1132	435	168389	197689	167303	549503	74194	209083	153974	150854	389845	173525	500160	324937	276279	631496	369062
S2GDHR2-10GS-I3-01v9-L7P	3695	5177	3135	4338	3353	147140	146498	19018	197789	152653	239618	402217	162393	412328	272905	843216	1100695	403762	949208	944347

Coating antigen: SARS-CoV-2 RBD

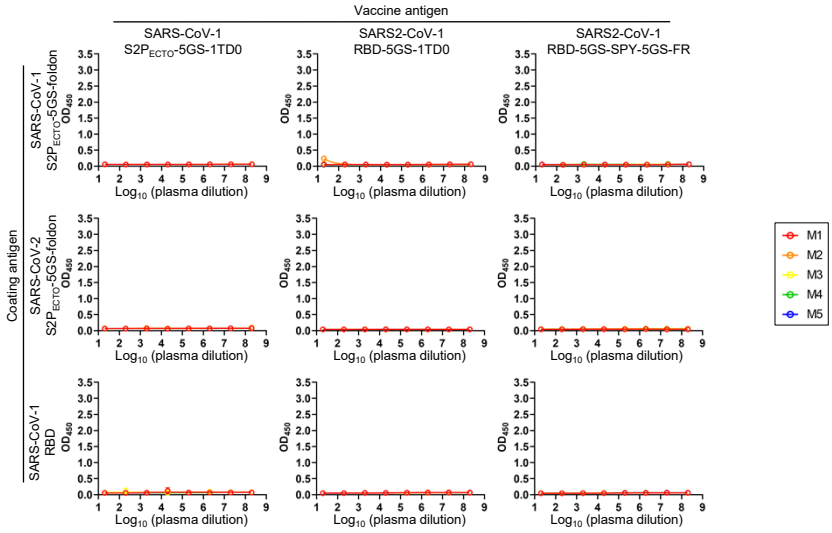
SARS-CoV-2 vaccine antigen	w2					w5					w8					w11				
	M1	M2	M3	M4	M5	M1	M2	M3	M4	M5	M1	M2	M3	M4	M5	M1	M2	M3	M4	M5
S2P$_{ECTO}$-5GS-1TD0	<20	<20	<20	<20	<20	1801	980	2693	260	1533	7881	1861	8448	1952	3948	15828	6935	10668	5826	14807
S2GDHR2-5GS-1TD0	26	24	<20	<20	<20	4693	1161	703	5881	751	13835	3578	3906	28190	4493	32268	6328	9751	40928	6118
S2GDHR2-5GS-FR	<20	<20	22	<20	21	3340	5205	3799	3229	1900	5996	15596	18918	14222	2293	11360	44589	35885	32606	7947
S2GDHR2-5GS-E2p-L4P	<20	<20	<20	21	<20	5425	9801	6186	22466	3554	10682	19414	10367	36238	10091	17641	32324	17662	64941	23122
S2GDHR2-10GS-I3-01v9-L7P	112	384	159	73	151	16265	16054	1920	16797	17368	24415	49820	15026	51700	41325	84065	120318	40866	194224	199672

fig. S5. SARS-CoV-2 spike/spike-SApNP vaccine-induced binding antibody responses. (A) EC_{50} titers derived from the ELISA binding of mouse plasma from five SARS-CoV-2 spike-based vaccine groups (S2P$_{ECTO}$-5GS-1TD0, S2GΔHR2-5GS-1TD0, S2GΔHR2-5GS-FR, S2GΔHR2-5GS-E2p-L4P, and S2GΔHR2-10GS-I3-01v9-L7P) to the coating antigen (SARS-CoV-1 S2P-5GS-foldon) are plotted, with average EC_{50} values labeled on the plots. The S2P spike vaccine group (S2P$_{ECTO}$-5GS-1TD0) is included here for comparison with S2GΔHR2 spike-based vaccine groups. The *P*-values were determined by an unpaired *t* test in GraphPad Prism 8.4.3 with (*) indicating the level of statistical significance (*: 0.01<P≤0.05; **: 0.001<P≤0.01; ***: 0.0001<P≤0.001; ****: P≤0.0001). **(B)** ELISA binding curves of mouse plasma from five SARS-CoV-2 spike-based vaccine groups to three coating antigens, SARS-CoV-1 S2P$_{ECTO}$-5GS-foldon, SARS-CoV-2 S2GΔHR2-5GS-foldon, and SARS-CoV-2 RBD. **(C)** Summary of EC_{50} titers measured for five SARS-CoV-2 spike-based vaccine groups against three coating antigens. Color coding indicates the level of EC_{50} titer (white: no binding; green to red: low to high). The EC_{50} values were calculated in GraphPad Prism 8.4.3. Of note, the EC_{50} values at w2 were derived by setting the lower/upper constraints of OD_{450} at 0.0/3.2 to achieve greater accuracy.

A


SARS-CoV-1 RBD/spike vaccine-induced antibody response
Coated with SARS-CoV-2 S2P$_{ECTO}$-5GS-foldon
EC$_{50}$ titer
184
55
11724
531
2680
36383
9796
9445
W2
W5
W8
SARS-CoV-1 vaccines
S2P$_{ECTO}$-5GS-1TD0
RBD-5GS-1TD0
RBD-5GS-SPY-5GS-FR

B


Mouse plasma ELISA at the 1st time point (Pre)
Vaccine antigen
SARS-CoV-1 S2P$_{ECTO}$-5GS-1TD0
SARS2-CoV-1 RBD-5GS-1TD0
SARS2-CoV-1 RBD-5GS-SPY-5GS-FR
Coating antigen
SARS-CoV-1 S2P$_{ECTO}$-5GS-foldon OD$_{450}$
SARS-CoV-2 S2P$_{ECTO}$-5GS-foldon OD$_{450}$
SARS-CoV-1 RBD OD$_{450}$
Log$_{10}$ (plasma dilution)
M1
M2
M3
M4
M5

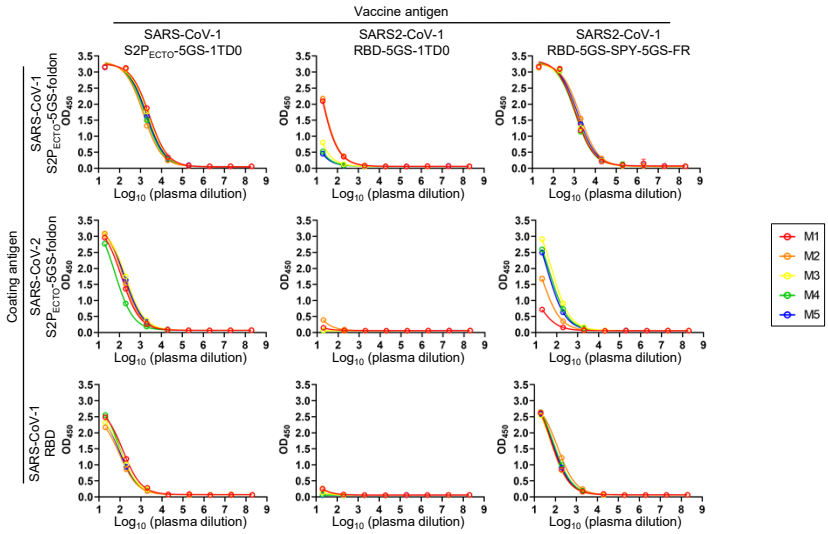
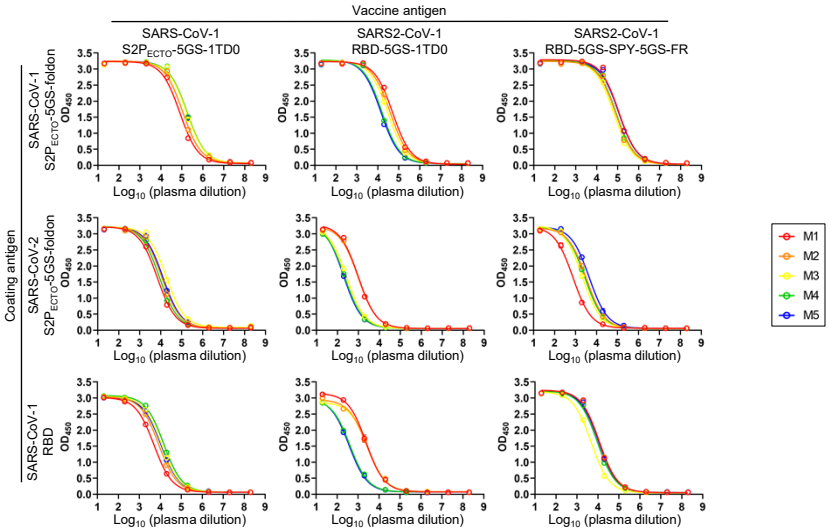
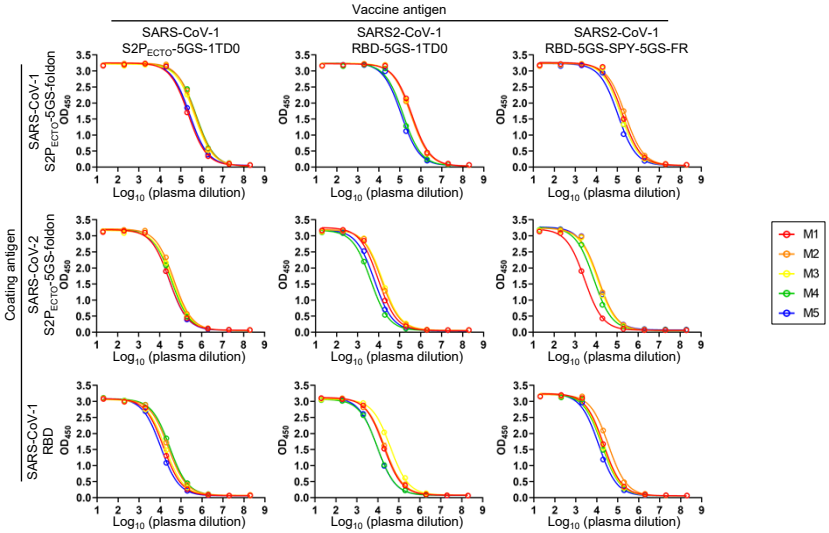

Mouse plasma ELISA at the 2nd time point (w2)
Vaccine antigen
SARS-CoV-1 S2P$_{ECTO}$-5GS-1TD0
SARS2-CoV-1 RBD-5GS-1TD0
SARS2-CoV-1 RBD-5GS-SPY-5GS-FR
Coating antigen
SARS-CoV-1 S2P$_{ECTO}$-5GS-foldon OD$_{450}$
SARS-CoV-2 S2P$_{ECTO}$-5GS-foldon OD$_{450}$
SARS-CoV-1 RBD OD$_{450}$
Log$_{10}$ (plasma dilution)
M1
M2
M3
M4
M5

fig. S6

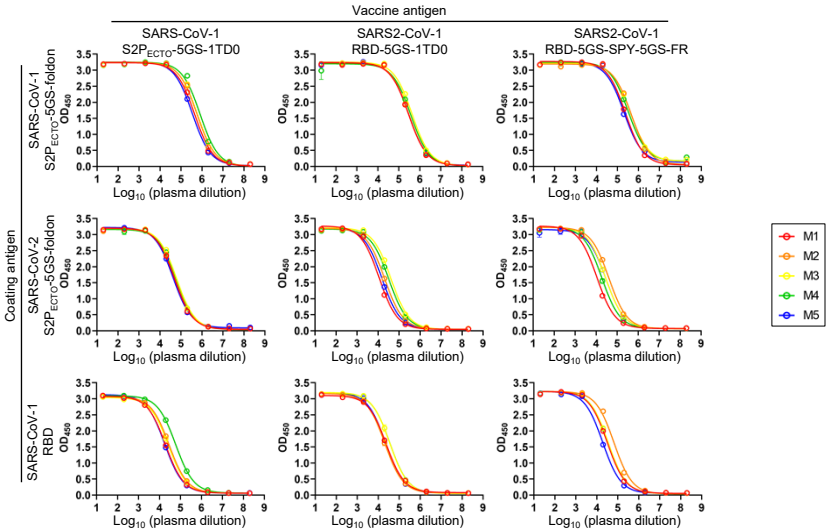
Mouse plasma ELISA at the 3rd time point (w5)



Mouse plasma ELISA at the 4th time point (w8)



Mouse plasma ELISA at the 5th time point (w11)



C **Mouse plasma ELISA EC_{50} titers**

Coating antigen: SARS-CoV-1 S2P$_{ECTO}$-5GS-foldon

SARS-CoV-1 vaccine antigen	w2					w5					w8					w11				
	M1	M2	M3	M4	M5	M1	M2	M3	M4	M5	M1	M2	M3	M4	M5	M1	M2	M3	M4	M5
S2P$_{ECTO}$-5GS-1TD0	2874	1611	2546	1924	2196	79871	111383	167209	179036	170765	226510	499160	444229	539886	264941	449939	577309	607415	818477	352607
RBD-5GS-1TD0	35	38	<20	<20	<20	51448	39666	29849	15658	13955	378373	337915	353896	144261	117614	290621	294825	425889	370917	290601
RBD-5GS-SPY-5GS-FR	1410	2047	1496	1320	1692	113510	78891	64381	83234	111601	178346	237355	151022	147743	107270	242440	454498	412469	328237	204991

Coating antigen: SARS-CoV-2 S2P$_{ECTO}$-5GS-foldon

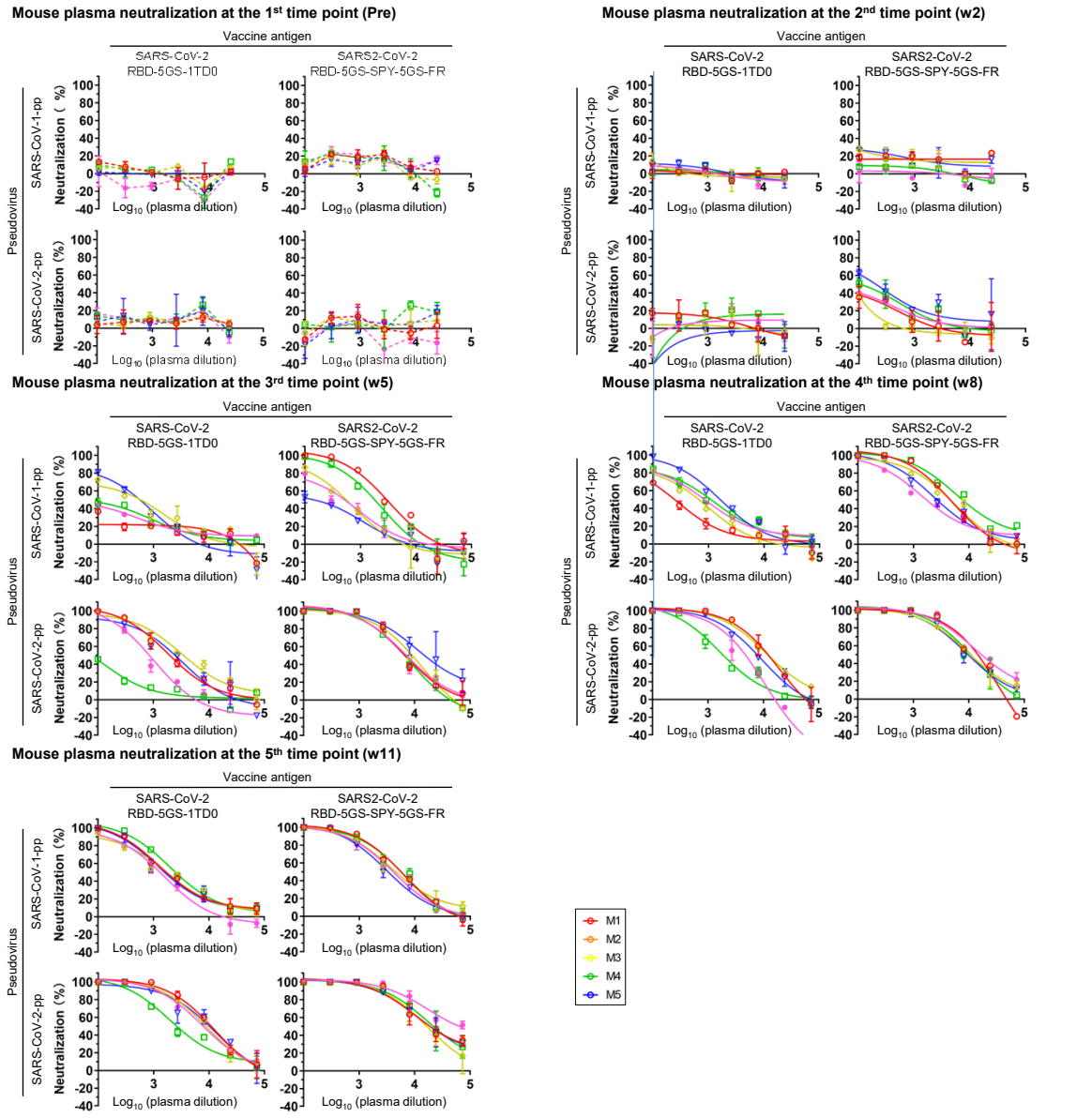
SARS-CoV-1 vaccine antigen	w2					w5					w8					w11				
	M1	M2	M3	M4	M5	M1	M2	M3	M4	M5	M1	M2	M3	M4	M5	M1	M2	M3	M4	M5
S2P$_{ECTO}$-5GS-1TD0	159	196	251	92	222	6913	11383	18692	8754	12877	28975	47458	41115	36059	28310	49277	52192	62700	60806	42133
RBD-5GS-1TD0	<20	<20	<20	<20	<20	985	975	274	224	197	9476	13677	15302	4216	6307	11223	20517	40915	32465	15295
RBD-5GS-SPY-5GS-FR	<20	22	100	70	60	751	3243	2124	2768	4513	2840	13086	11753	7693	11851	10886	40615	28253	18689	29739

Coating antigen: SARS-CoV-1 RBD

SARS-CoV-1 vaccine antigen	w2					w5					w8					w11				
	M1	M2	M3	M4	M5	M1	M2	M3	M4	M5	M1	M2	M3	M4	M5	M1	M2	M3	M4	M5
S2$_{PECTO}$-5GS-1TD0	104	55	73	89	68	4967	8355	11547	14466	10683	14673	24576	17828	28878	10869	19455	28689	26660	58793	17679
RBD-5GS-1TD0	<20	<20	<20	<20	<20	2446	2600	2827	415	371	18820	20963	39469	9503	9100	24044	20641	35559	36058	22822
RBD-5GS-SPY-5GS-FR	79	118	74	94	83	11483	11758	4772	9294	10888	21284	36104	18587	17645	12351	31380	66025	34973	34373	19241

fig. S6. SARS-CoV-1 spike/RBD/RBD-SApNP vaccine-induced binding antibody responses. (A) EC_{50} titers derived from the ELISA binding of mouse plasma from three SARS-CoV-1 vaccine groups (S2P$_{ECTO}$-5GS-1TD0, RBD-5GS-1TD0, and RBD-5GS-SPY-5GS-FR) to the coating antigen (SARS-CoV-2 S2P$_{ECTO}$-5GS-foldon) are plotted, with average EC_{50} values labeled on the plots. The S2P spike vaccine group (S2P$_{ECTO}$-5GS-1TD0) is included here for comparison with RBD-based vaccine groups. The *P*-values were determined by an unpaired *t* test in GraphPad Prism 8.4.3 with (*) indicating the level of statistical significance (*: 0.01<P≤0.05; **: 0.001<P≤0.01; ***: 0.0001<P≤0.001; ****: P≤0.0001). **(B)** ELISA binding curves of mouse plasma from three SARS-CoV-1 vaccine groups to three coating antigens, SARS-CoV-1 S2P-5GS-foldon, SARS-CoV-2 S2P$_{ECTO}$-5GS-foldon, and SARS-CoV-1 RBD. **(C)** Summary of EC_{50} titers measured for three SARS-CoV-1 vaccine groups against three coating antigens. Color coding indicates the level of EC_{50} titer (white: no binding; green to red: low to high). The EC_{50} values were calculated in GraphPad Prism 8.4.3. Of note, the EC_{50} values at w2 were derived by setting the lower/upper constraints of OD_{450} at 0.0/3.2 to achieve greater accuracy.

A



B

Mouse serum neutralization ID_{50} titers

Pseudovirus	SARS-CoV-2 vaccine antigen	W2					W5					W8					W11				
		M1	M2	M3	M4	M5	M1	M2	M3	M4	M5	M1	M2	M3	M4	M5	M1	M2	M3	M4	M5
SARS-CoV-1-pp	RBD-5GS-1TD0	<100	<100	<100	<100	<100	72	428	459	166	124	250	570	1608	1037	859	1979	1710	1816	2759	1183
SARS-CoV-1-pp	RBD-5GS-SPY-5GS-FR	<100	<100	<100	<100	<100	2870	482	189	1543	427	4456	4199	2858	8390	1941	5389	4986	3418	5401	4066
SARS-CoV-2-pp	RBD-5GS-1TD0	<100	<100	<100	<100	<100	2069	3655	2043	<100	746	10882	12545	7382	2010	3245	10639	8742	9094	3047	8031
SARS-CoV-2-pp	RBD-5GS-SPY-5GS-FR	<100	<100	220	143	<100	6923	7926	16669	6079	7400	11048	11051	10012	10613	18178	20964	16955	20948	22920	51997

fig. S7. SARS-CoV-2 RBD/RBD-SApNP vaccine-induced neutralizing antibody responses. (A) Neutralization curves of mouse plasma from two SARS-CoV-2 RBD-based vaccine groups against SARS-CoV-1/2-pps. **(B)** Summary of ID_{50} titers measured for two SARS-CoV-2 RBD-based vaccine groups against SARS-CoV-1/2-pps. Color coding indicates the level of ID_{50} titer (white: no neutralization; green to red: low to high). The ID_{50} values were calculated in GraphPad Prism 8.4.3, with the lower/upper constraints of %neutralization set at 0.0/100.0.

fig. S8

A

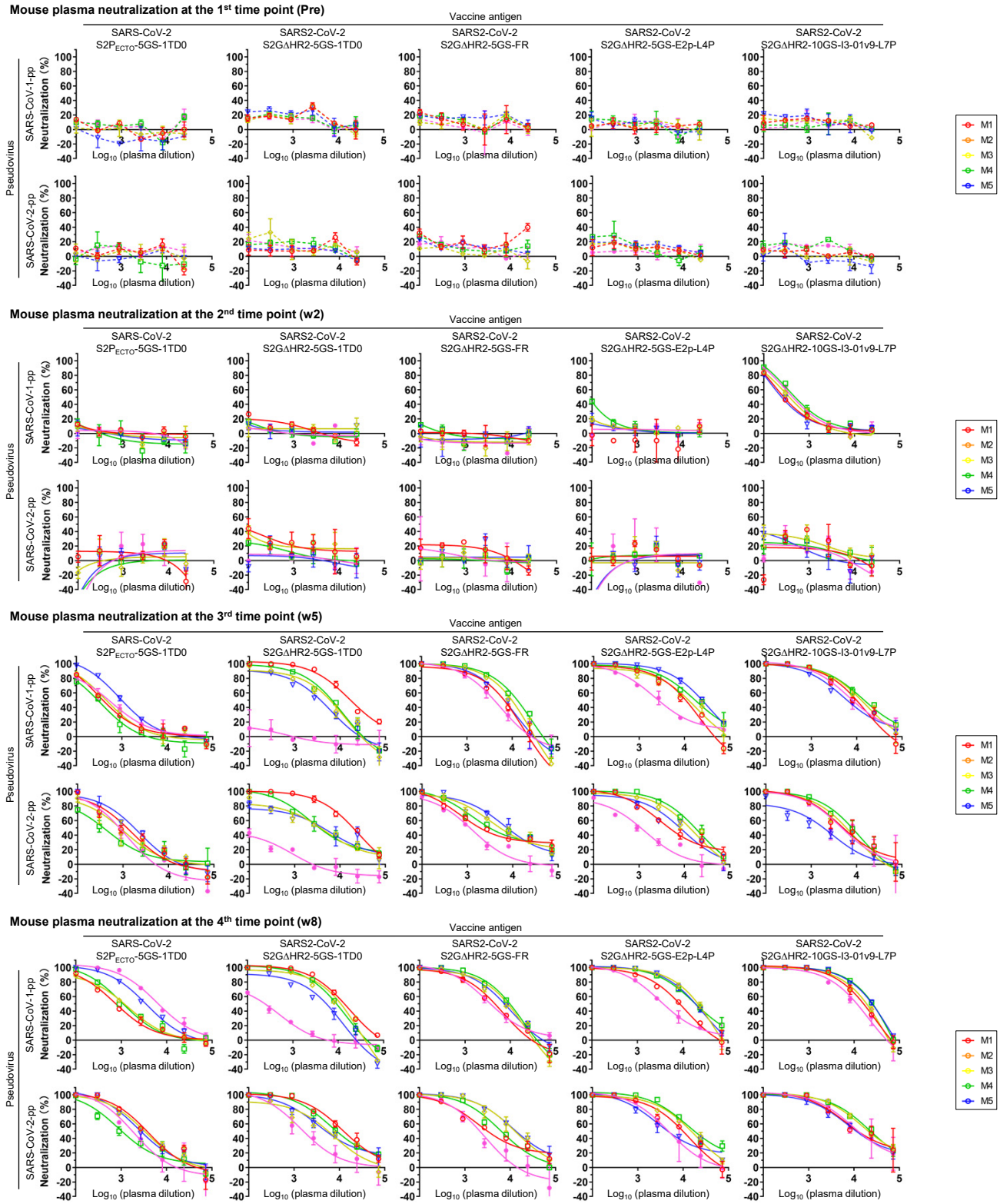
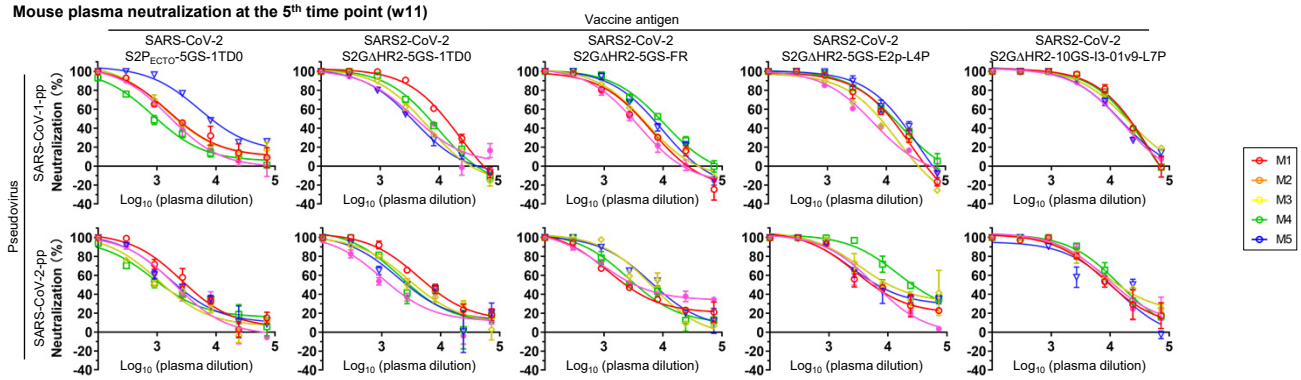

Mouse plasma neutralization at the 1st time point (Pre)
Mouse plasma neutralization at the 2nd time point (w2)
Mouse plasma neutralization at the 3rd time point (w5)
Mouse plasma neutralization at the 4th time point (w8)
Vaccine antigen
SARS-CoV-2 S2P$_{ECTO}$-5GS-1TD0
SARS2-CoV-2 S2GΔHR2-5GS-1TD0
SARS2-CoV-2 S2GΔHR2-5GS-FR
SARS2-CoV-2 S2GΔHR2-5GS-E2p-L4P
SARS2-CoV-2 S2GΔHR2-10GS-I3-01v9-L7P
Pseudovirus
SARS-CoV-1-pp Neutralization (%)
SARS-CoV-2-pp Neutralization (%)
Log10 (plasma dilution)
M1
M2
M3
M4
M5

fig. S8



B **Mouse plasma neutralization ID_{50} titers**

Pseudovirus: SARS-CoV-1-pp

SARS-CoV-2 vaccine antigen	W2					W5					W8					W11				
	M1	M2	M3	M4	M5	M1	M2	M3	M4	M5	M1	M2	M3	M4	M5	M1	M2	M3	M4	M5
S2P$_{ECTO}$-5GS-1TD0	<100	<100	<100	<100	<100	405	444	1032	270	501	777	1062	3410	1121	6783	2588	2431	9374	1074	1806
S2GDHR2-5GS-1TD0	<100	<100	<100	<100	<100	17433	3960	2747	5170	<100	13124	7579	3443	7902	221	11228	4377	3271	6009	4176
S2GDHR2-5GS-FR	<100	<100	<100	<100	<100	4152	6773	5414	10007	3527	3735	5968	6443	7605	3872	3384	4103	5704	8194	2697
S2GDHR2-5GS-E2p-L4P	<100	<100	<100	<100	<100	7954	10197	23509	17140	2915	7676	18213	17917	19538	4017	9170	5651	13334	12484	4799
S2GDHR2-10GS-I3-01v9-L7P	278	330	247	486	417	8250	11666	6695	15034	8694	14174	14888	22024	21474	9393	18257	18901	14175	17014	13137

Pseudovirus: SARS-CoV-2-pp

SARS-CoV-2 vaccine antigen	w2					w5					w8					w11				
	M1	M2	M3	M4	M5	M1	M2	M3	M4	M5	M1	M2	M3	M4	M5	M1	M2	M3	M4	M5
S2P$_{ECTO}$-5GS-1TD0	<100	<100	<100	<100	<100	1050	787	1524	369	667	3515	3169	2897	1141	1683	3431	1428	2509	1494	1994
S2GDHR2-5GS-1TD0	<100	<100	<100	<100	<100	18419	3271	3238	4382	<100	12641	4656	6148	9660	1707	7026	4096	3226	3491	1686
S2GDHR2-5GS-FR	<100	<100	<100	<100	<100	3065	6659	8044	5164	1072	4156	12681	14238	6453	1827	3170	6292	7318	4051	5202
S2GDHR2-5GS-E2p-L4P	<100	<100	<100	<100	<100	5778	11650	7370	16399	977	8613	14120	5999	19054	4733	6849	12699	8468	28041	6138
S2GDHR2-10GS-I3-01v9-L7P	<100	<100	<100	<100	<100	5465	5151	1440	6831	4722	14684	22060	12830	23608	13575	10747	16081	8855	14951	12578

fig. S8. SARS-CoV-2 spike/spike-SApNP vaccine-induced neutralizing antibody responses. (A) Neutralization curves of mouse plasma from five SARS-CoV-2 spike-based vaccine groups against SARS-CoV-1/2-pps. **(B)** Summary of ID_{50} titers measured for five SARS-CoV-2 spike-based vaccine groups against SARS-CoV-1/2-pps. Color coding indicates the level of ID_{50} titer (white: no neutralization; green to red: low to high). The ID_{50} values were calculated in GraphPad Prism 8.4.3, with the lower/upper constraints of %neutralization set at 0.0/100.0.

fig. S9

A

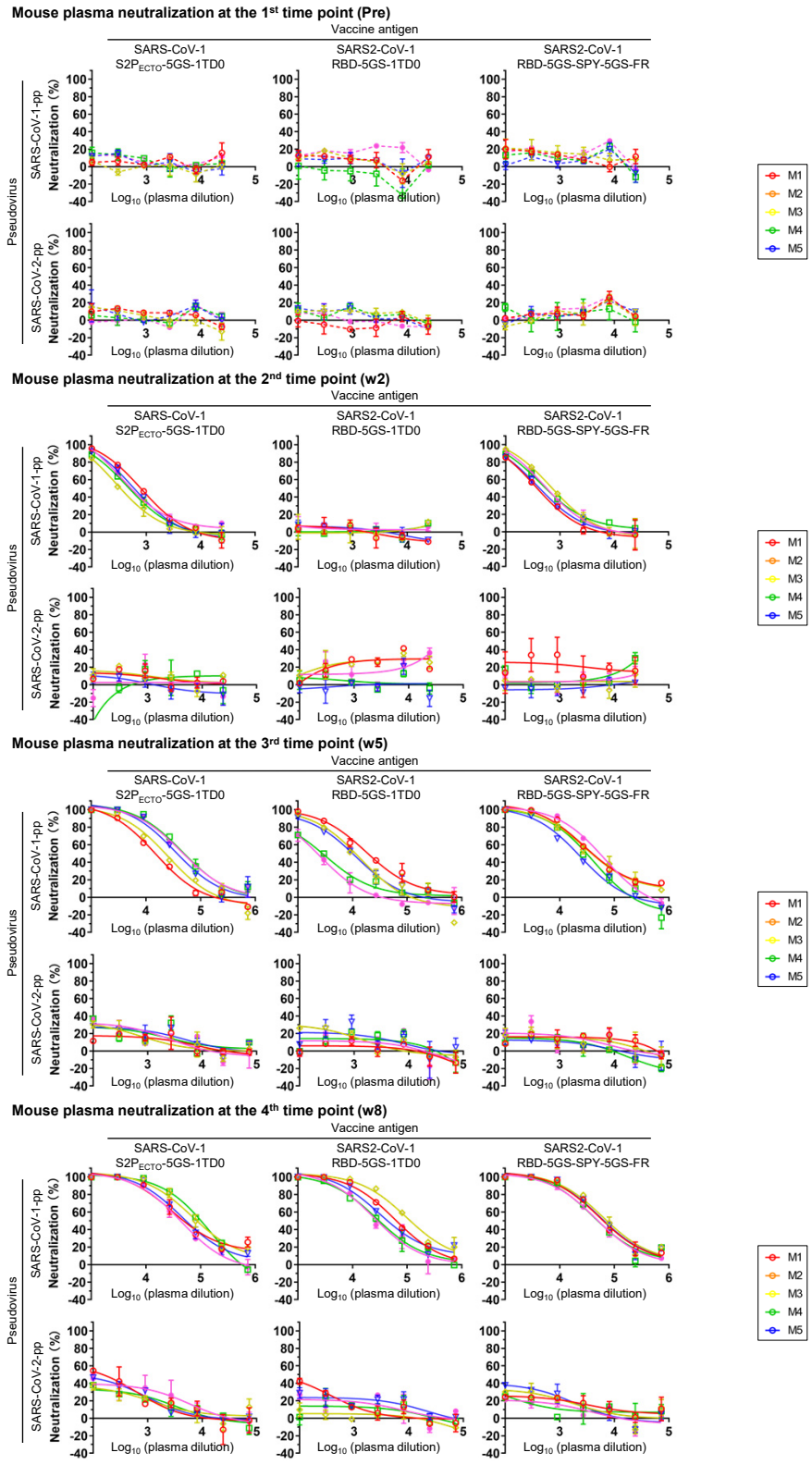
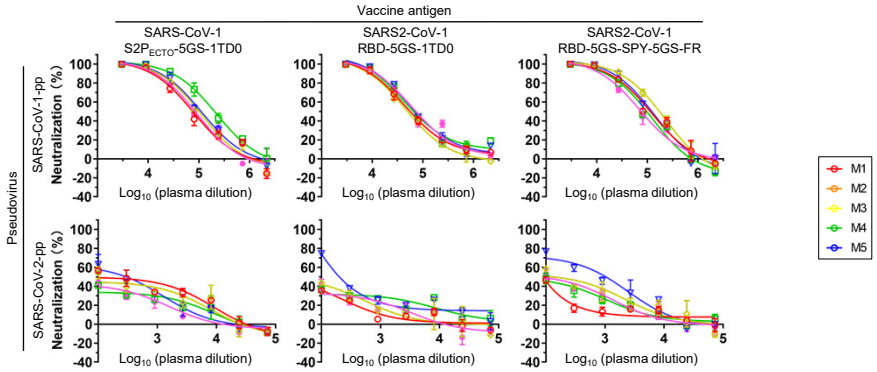

Mouse plasma neutralization at the 1st time point (Pre)
Mouse plasma neutralization at the 2nd time point (w2)
Mouse plasma neutralization at the 3rd time point (w5)
Mouse plasma neutralization at the 4th time point (w8)
Vaccine antigen
SARS-CoV-1 S2P$_{ECTO}$-5GS-1TD0
SARS2-CoV-1 RBD-5GS-1TD0
SARS2-CoV-1 RBD-5GS-SPY-5GS-FR
Pseudovirus
SARS-CoV-1-pp Neutralization (%)
SARS-CoV-2-pp Neutralization (%)
Log$_{10}$ (plasma dilution)
M1
M2
M3
M4
M5

fig. S9

Mouse plasma neutralization at the 5th time point (w11)



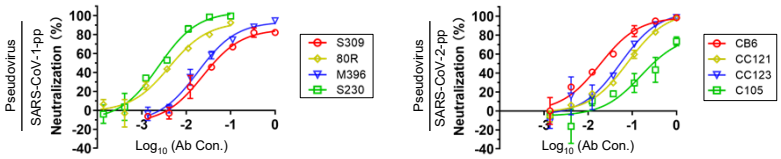
B

Mouse serum neutralization ID_{50} titers

Pseudovirus	SARS-CoV-1 vaccine antigen	w2					w5					w8					w11				
		M1	M2	M3	M4	M5	M1	M2	M3	M4	M5	M1	M2	M3	M4	M5	M1	M2	M3	M4	M5
SARS-CoV-1-pp	S2P$_{ECTO}$-5GS-1TD0	770	343	591	487	593	14583	21202	38507	51532	49913	56161	91290	57692	90786	42757	74328	94977	109706	198568	80619
	RBD-5GS-1TD0	<100	<100	<100	<100	<100	19980	10126	9368	2830	2110	65863	120732	49566	32475	30098	62357	52065	78969	74719	80296
	RBD-5GS-SPY-5GS-FR	370	719	423	588	584	45123	43109	19859	26996	50574	66993	77427	69400	53156	52677	109989	157931	117525	86514	77985

Pseudovirus	SARS-CoV-1 vaccine antigen	w2					w5					w8					w11				
		M1	M2	M3	M4	M5	M1	M2	M3	M4	M5	M1	M2	M3	M4	M5	M1	M2	M3	M4	M5
SARS-CoV-2-pp	S2P$_{ECTO}$-5GS-1TD0	<100	<100	<100	<100	<100	<100	<100	<100	<100	<100	174	<100	142	<100	140	325	216	268	112	114
	RBD-5GS-1TD0	<100	<100	<100	<100	<100	<100	<100	<100	<100	<100	<100	<100	<100	<100	<100	<100	103	272	<100	<100
	RBD-5GS-SPY-5GS-FR	<100	<100	<100	<100	<100	<100	<100	<100	<100	<100	<100	<100	<100	<100	<100	<100	240	717	150	184

C



D

Mouse serum neutralization against MLV-pp at the 4th time point (w8)

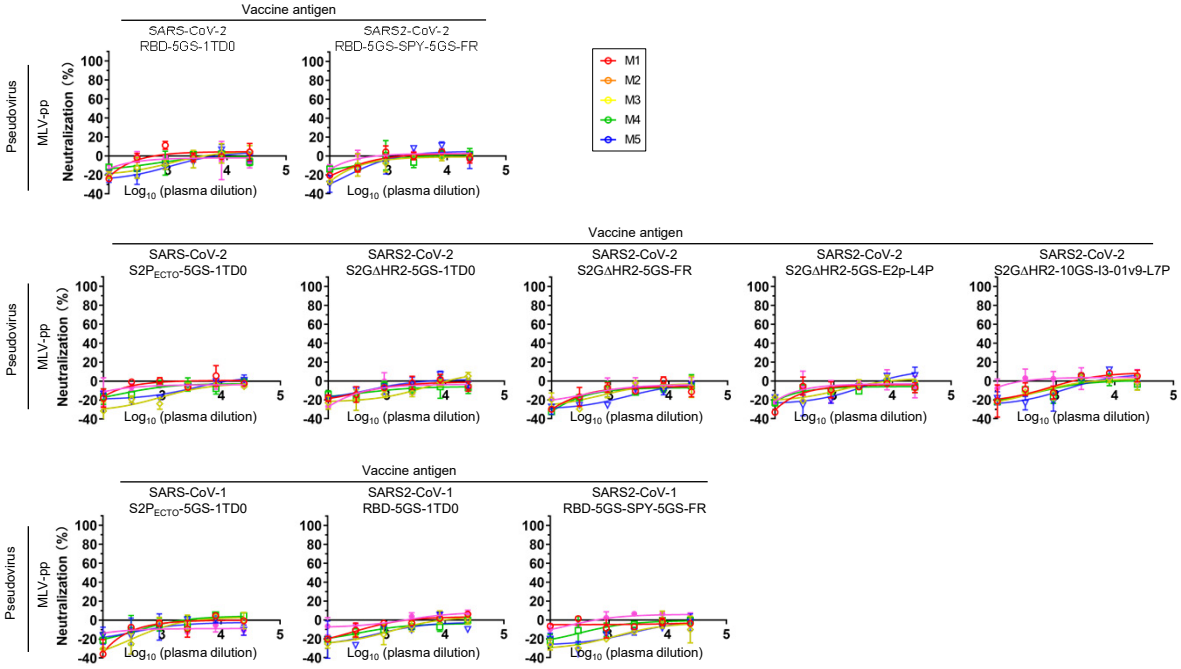


fig. S9

fig. S9. SARS-CoV-1 spike/RBD/RBD-SApNP vaccine-induced neutralizing antibody responses. (A) Neutralization curves of mouse plasma from three SARS-CoV-1 vaccine groups against two SARS-CoV-1/2-pps. **(B)** Summary of ID_{50} titers measured for three SARS-CoV-1 vaccine groups against SARS-CoV-1/2-pps. Color coding indicates the level of ID_{50} titer (white: no neutralization; green to red: low to high). The ID_{50} values were calculated in GraphPad Prism 8.4.3, with the lower/upper constraints of %neutralization set at 0.0/100.0. **(C)** Neutralization curves of four known SARS-CoV-1/2 NAbs against respective pseudoviruses. SARS-CoV-1-pp (left) and SARS-CoV-2-pp (right). **(D)** Neutralization curves of mouse plasma from all vaccine groups at w8 against MLV-pps as a negative control. No MLV-pp neutralization was observed.

Table S1

Table S1. Key *in vitro* and *in vivo* characteristics of RBD and spike-based SARS-CoV-2 vaccines [a]

	RBD-based constructs				Spike-based constructs				
	RBD-5GS-1TD0	RBD-5GS-SPY-5GS-FR	RBD-5GS-SPY-5GS-E2p-LD4-PADRE	RBD-5GS-SPY-5GS-I3-01v9-LD7-PADRE	$S2P_{ECTO}$-5GS-1TD0	S2GΔHR2-5GS-1TD0	S2GΔHR2-5GS-FR	S2GΔHR2-5GS-E2p-LD4-PADRE	S2GΔHR2-10GS-I3-01v9-LD7-PADRE
Display scaffold [b]	1TD0	Ferritin	E2p	I3-01v9	1TD0	1TD0	Ferritin	E2p	I3-01v9
Multilayered (Y/N) [c]	NA	N	Y	Y	NA	NA	N	Y	Y
PADRE included (Y/N) [d]	NA	N	Y	Y	NA	NA	N	Y	Y
Antigen (Ag) type [e]	Monomer	Monomer	Monomer	Monomer	Trimer	Trimer	Trimer	Trimer	Trimer
Number of Ags [f]	3	24	60	60	1	1	8	20	20
Display method [g]	Fusion	SPY	SPY	SPY	Fusion	Fusion	Fusion	Fusion	Fusion
Number of plasmids [h]	1	2	2	2	1	1	1	1	1
Protein yield [i]	+++++	++++	+	+++	+++	+++	+++	++	+++
Protein purity [j]	+++++	++++	+	+++	++++	+++++	+++	++	+++
ELISA binding [k]	++++	+++++	ND	ND	++++	++++	+++++	+++++	+++++
BLI binding [l]	++	+++++	ND	ND	++	++	+++	+++++	+++++
W8 binding Ab titer [m]	++++	+++++	ND	ND	++	+++	+++	++++	+++++
W8 NAb titer [n]	++	+++	ND	ND	++	+++	+++	++++	+++++
T-cell response [o]	ND	ND	ND	ND	++	ND	ND	+++	++++

[a] Summary of key vaccine attributes with "+" indicating positive outcome (+ being broadline and +++++ being optimal), "NA" not applicable, and "ND" not determined.
[b] Display scaffold – A trimerization motif (1TD0) and three self-assembling protein nanoparticles (SApNPs), ferritin, E2p, and I3-01, are used to display the RBD and spike.
[c] Multilayered (Y/N) – Whether the SApNP construct contains an inner layer of locking domains (LD) and a cluster of T-cell epitopes (PADRE). Y: yes, N: no.
[d] PADRE included (Y/N) – Whether the SApNP construct contains a pan DR epitope that activates $CD4^+$ T cells.
[e] Antigen (Ag) type – The RBD is a monomer, whereas the spike is a trimer of cleaved S1/S2 heterodimers or uncleaved S proteins.
[f] Number of Ags – Number of antigens in the properly folded and assembled vaccine immunogen.
[g] Display method – The antigen is either conjugated to SApNPs using the SpyTag/SpyCatcher (SPY) system or genetically fused to a trimerization motif or SApNP subunit.
[h] Number of plasmids – The number of expression plasmids needed to produce the vaccine immunogen in ExpiCHO cells.
[i] Protein yield – Protein yield is measured after purification on a CR3022 antibody column. Of note, individual antigens usually have higher yield than their SApNPs, and the 24-meric SApNP (ferritin) tends have greater yield than the 60-meric SApNPs, E2p and I3-01.
[j] Protein purity – Protein purity is measured by qualitatively comparing the ratio of various species (aggregate, SApNP, trimer, dimer and monomer) in the SEC profile.
[k] ELISA binding – EC_{50} values are calculated from ELISA binding to a panel of known antibodies.
[l] BLI binding – Binding signals (nm) are compared as most constructs show negligible off-rates in the Octet experiments.
[m] W8 binding Ab titer – EC_{50} titers are compared at week 8, where serum binding antibody response reaches the plateau.
[n] W8 NAb titer – ID_{50} titers are compared at week 8, where serum NAb response reaches the plateau.
[o] T-cell response – End-point T-cell (Th1, cytolytic $CD4^+$, and CD8) responses.