

# Supplementary Materials for

## **Mechanism of a COVID-19 nanoparticle vaccine candidate that elicits a broadly neutralizing antibody response to SARS-CoV-2 variants**

Yi-Nan Zhang<sup>1</sup>, Jennifer Paynter<sup>1</sup>, Tatiana Fourfouris<sup>1</sup>, Cindy Sou<sup>1</sup>, Timothy Ngo<sup>1</sup>,

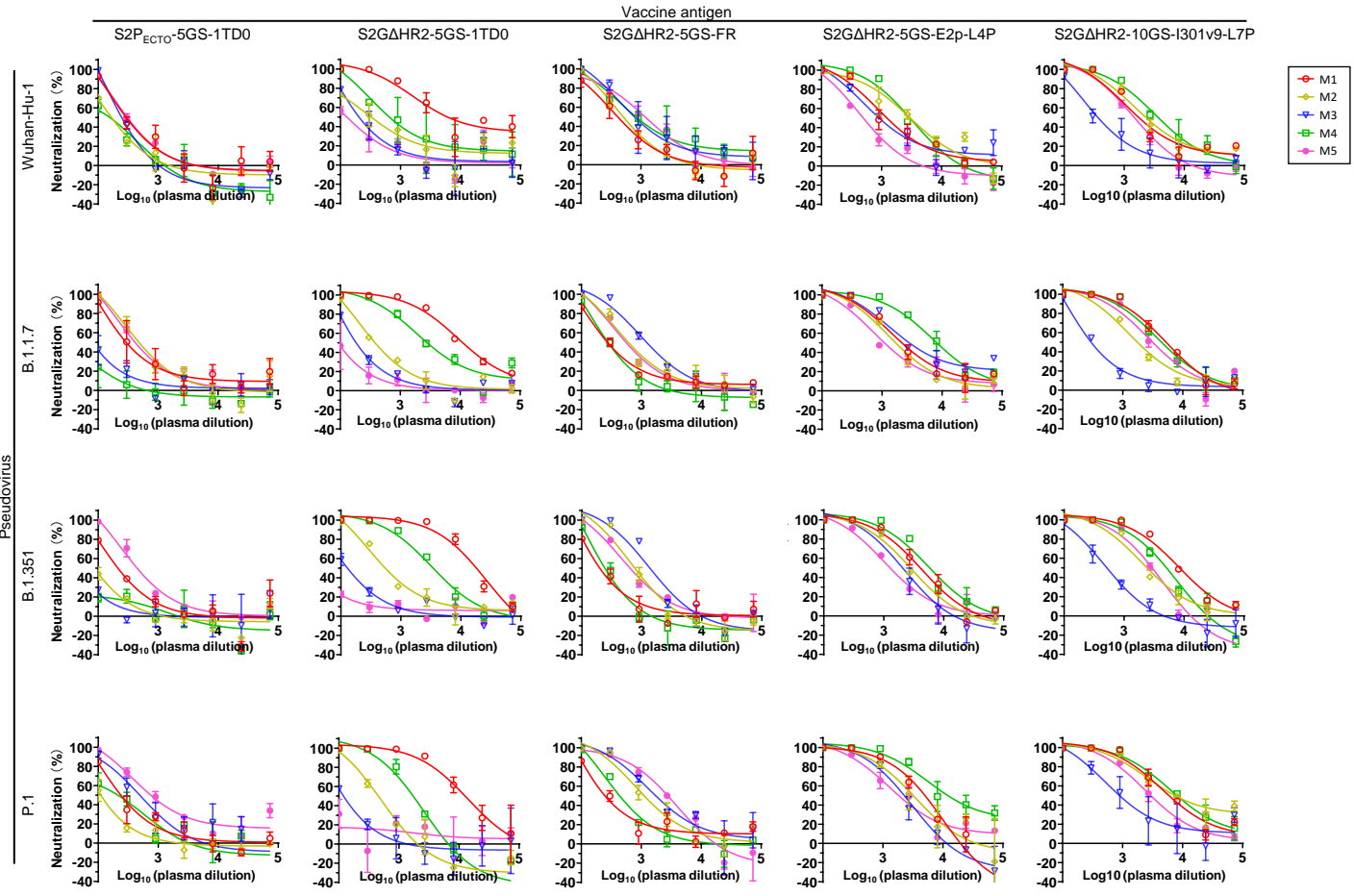
Linling He<sup>1</sup>, and Jiang Zhu<sup>1, 2, \*</sup>

Correspondence to: JZ ([jiang@scripps.edu](mailto:jiang@scripps.edu));

**This PDF file includes:**

Figs. S1 to S6

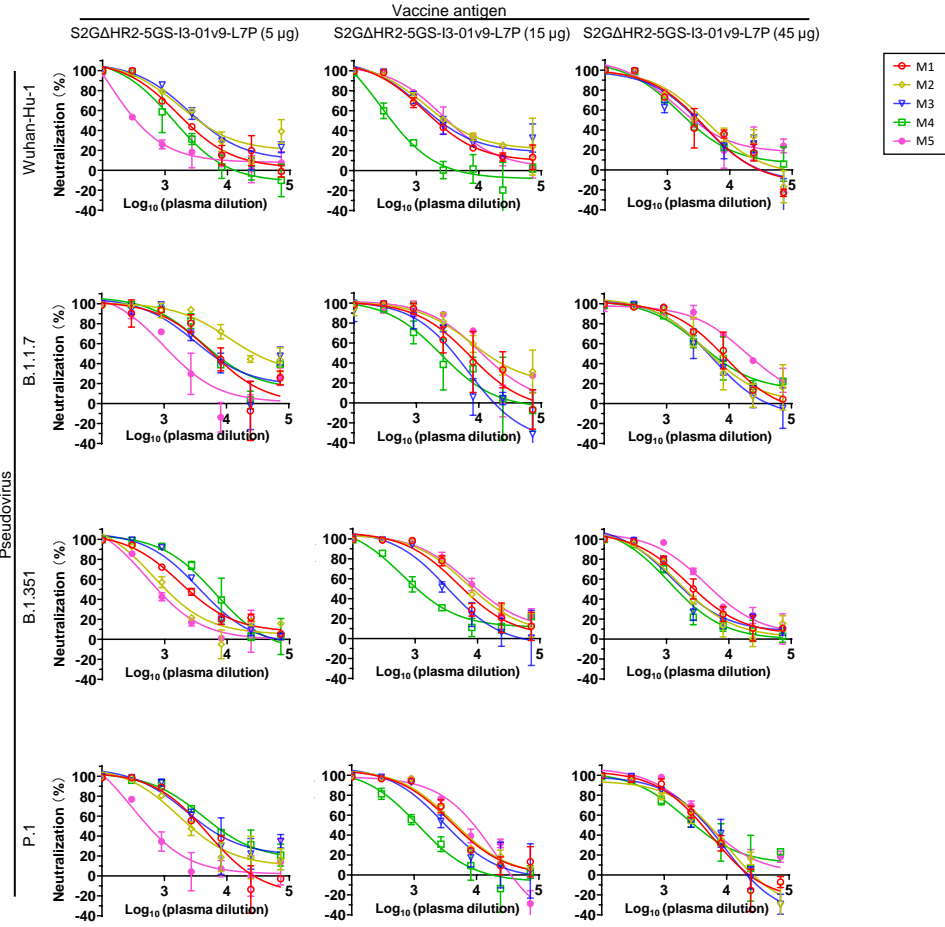
**A** Mouse plasma neutralization against SARS-CoV-2 strains Wuhan-Hu-1, B.1.1.7, B.1.351, and P.1



**B** Mouse plasma neutralization ID<sub>50</sub> titers

Vaccine antigen	Wuhan-Hu-1					B.1.1.7					B.1.351					P.1				
	M1	M2	M3	M4	M5	M1	M2	M3	M4	M5	M1	M2	M3	M4	M5	M1	M2	M3	M4	M5
S2P <sub>ECTO</sub> -5GS-1TD0 (50 µg, i.p.)	316	150	233	123	326	377	541	<100	<100	487	223	<100	<100	<100	506	274	<100	465	186	967
S2ΔHR2-5GS-1TD0 (50 µg, i.p.)	7719	370	227	911	131	11868	444	202	3252	<100	17560	654	123	3550	<100	12563	387	101	1467	<100
S2ΔHR2-5GS-FR (50 µg, i.p.)	425	544	962	1135	1154	326	700	1327	293	629	215	764	1353	236	695	316	1244	1978	488	2355
S2ΔHR2-5GS-E2p-L4P (50 µg, i.p.)	1442	3020	1107	2849	501	2458	1846	3473	8633	1331	4018	2757	2002	5971	1375	3650	3085	1966	14185	2104
S2ΔHR2-10GS-I301v9-L7P (50 µg, i.p.)	1992	2648	428	3910	1470	5313	1953	355	4715	3337	8641	2602	506	4336	2480	6461	9520	1070	8834	3180

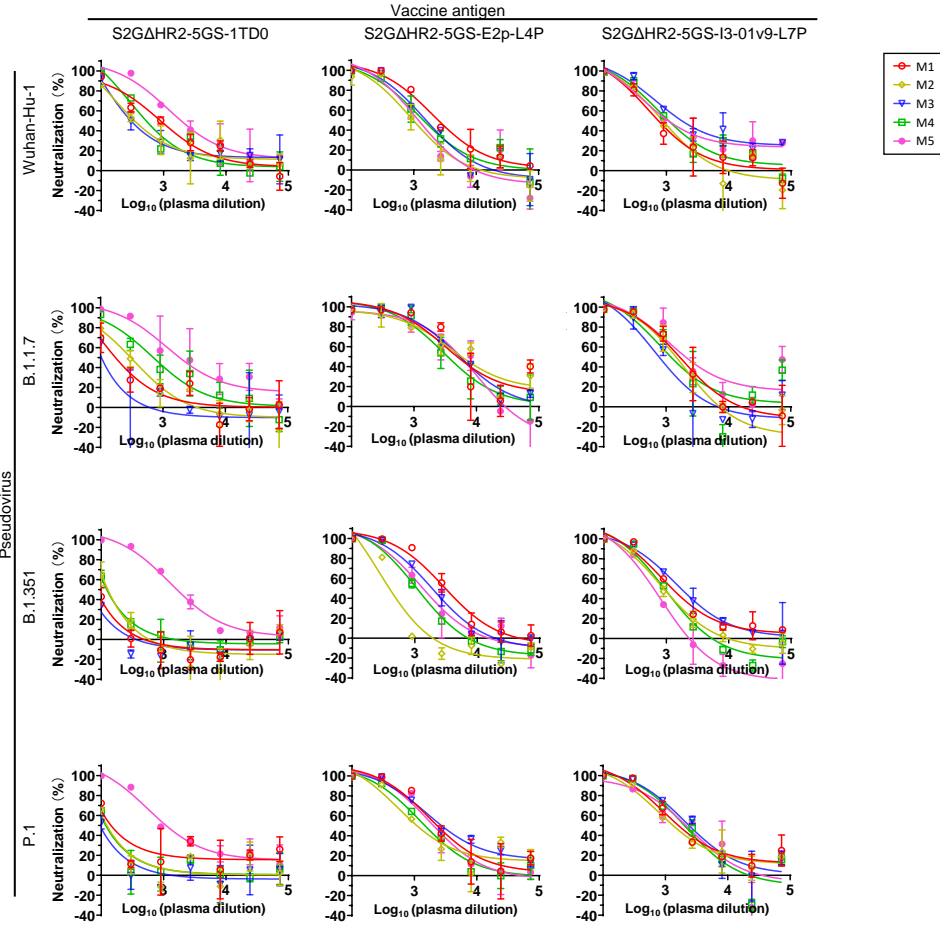
**C** Mouse plasma neutralization against SARS-CoV-2 strains Wuhan-Hu-1, B.1.1.7, B1.351, and P.1



**D** Mouse plasma neutralization ID<sub>50</sub> titers

Vaccine antigen	Wuhan-Hu-1					B.1.1.7					B.1.351					P.1				
	M1	M2	M3	M4	M5	M1	M2	M3	M4	M5	M1	M2	M3	M4	M5	M1	M2	M3	M4	M5
S2GΔHR2-10GS-I3-01v9-L7P (5 µg, i.p.)	2287	4102	4023	1364	455	6142	28391	6058	7113	1393	2624	1163	3711	5420	848	3550	2939	5351	7806	620
S2GΔHR2-10GS-I3-01v9-L7P (15 µg, i.p.)	2381	3946	2911	425	3493	6246	13941	3284	2315	11227	6220	8930	3425	1290	10607	4560	5007	3325	1143	6478
S2GΔHR2-10GS-I3-01v9-L7P (45 µg, i.p.)	2772	3753	2644	2433	3186	7277	5160	4435	6032	19255	3062	2141	2119	1538	5770	3627	3585	3469	3503	5235

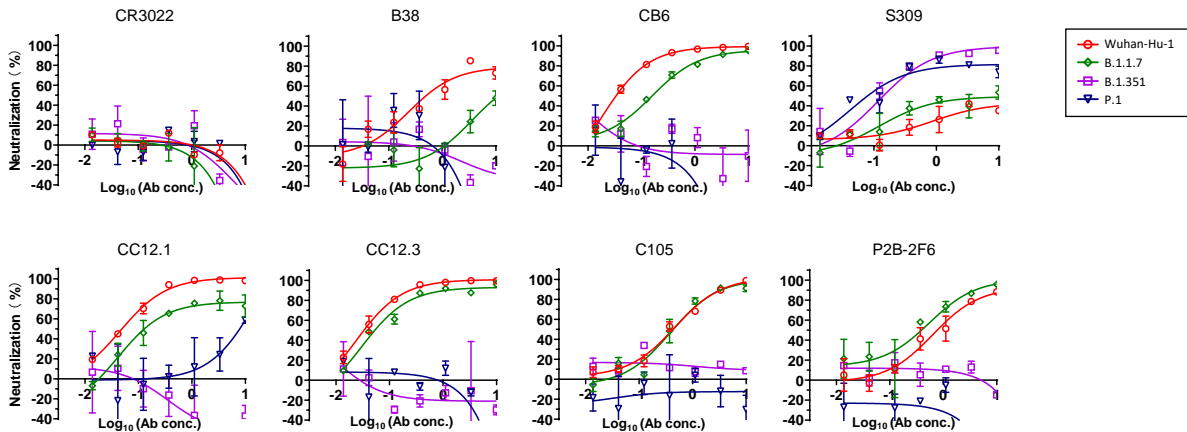
**E** Mouse plasma neutralization against SARS-CoV-2 strains Wuhan-Hu-1, B.1.1.7, B1.351, and P.1



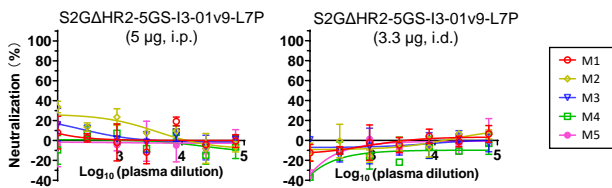
**F** Mouse plasma neutralization ID<sub>50</sub> titers

Vaccine antigen	Wuhan-Hu-1					B.1.1.7					B.1.351					P.1				
	M1	M2	M3	M4	M5	M1	M2	M3	M4	M5	M1	M2	M3	M4	M5	M1	M2	M3	M4	M5
S2GΔHR2-5GS-1TD0 (3.3 μg, i.d.)	923	435	416	640	2345	179	278	<100	721	2440	<100	<100	<100	105	1787	146	100	<100	<100	1446
S2GΔHR2-5GS-E2p-L4P (3.3 μg, i.d.)	2680	988	1586	1530	1178	5243	6549	5467	3676	4300	3149	377	1884	1048	1372	2533	1335	3524	1573	2120
S2GΔHR2-10GS-I3-01v9-L7P (3.3 μg, i.d.)	851	884	2593	1263	1504	1617	1271	776	1267	2292	1418	921	1866	891	522	1923	1616	2530	1980	1968

**G** Human monoclonal antibody neutralization against SARS-CoV-2 strains Wuhan-Hu-1, B.1.1.7, B1.351, and P.1



**H** Mouse plasma neutralization against MLV-pp



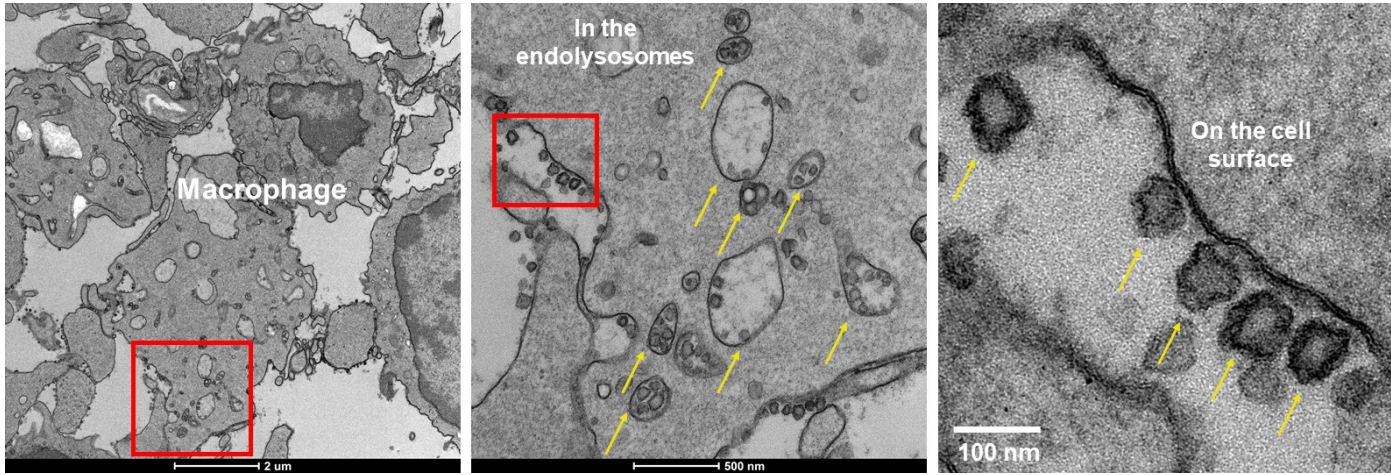
**I** Mouse plasma neutralization ID<sub>50</sub> titers

Vaccine antigen	MLV-pp				
	M1	M2	M3	M4	M5
S2GΔHR2-10GS-I3-01v9-L7P (5 μg, i.p.)	<100	<100	<100	<100	<100
S2GΔHR2-10GS-I3-01v9-L7P (3.3 μg, i.d.)	<100	<100	<100	<100	<100

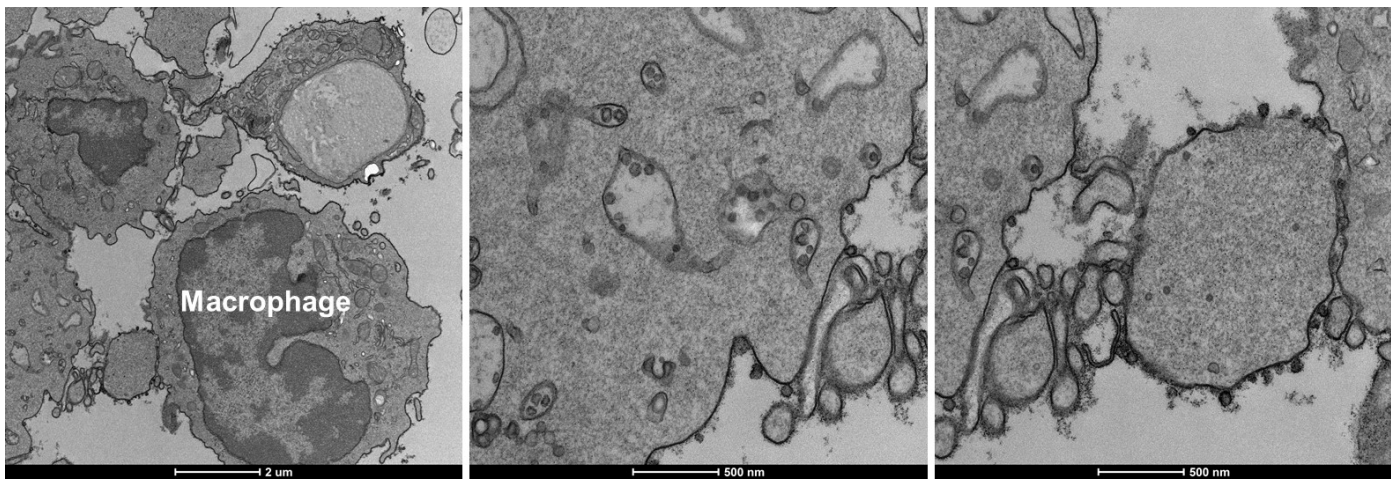
**fig. S1. Spike and spike-presenting SApNP vaccine-induced neutralizing antibody responses against SARS-CoV-2 variants of concern (VOCs).** (A) Neutralization curves of mouse plasma from 5 vaccine groups at week 5 after 2 intraperitoneal (i.p.) injections. The plasma samples were generated in the previous study (Ref 41), where mice were immunized with 50 μg of adjuvanted vaccine antigen. The five vaccines include two spikes (S2P<sub>ECTO</sub>-5GS-1TD0 and S2GΔHR2-5GS-1TD0) and three SApNPs (S2GΔHR2-5GS-FR, S2GΔHR2-5GS-E2p-LD4-PADRE (L4P), and S2GΔHR2-10GS-I3-01v9-LD7-PADRE (L7P)). (B) Summary of ID<sub>50</sub> titers measured for five SARS-CoV-2 spike-based vaccine groups in (A). (C) Neutralization curves of mouse plasma induced by the S2GΔHR2-presenting I3-01v9 SApNP vaccine at week 5 after two i.p. injections with different antigen doses (5 μg, 15 μg, and 45 μg). (D) Summary of ID<sub>50</sub> titers measured for the S2GΔHR2-presenting I3-01v9 SApNP vaccine in (C). (E) Neutralization curves of mouse plasma induced by the S2GΔHR2 spike and two S2GΔHR2-presenting SApNPs (E2p and I3-01v9) at week 5 after two intradermal (i.d.) footpad injections (0.8 μg per injection, totally 3.3 μg per mouse). (F) Summary of ID<sub>50</sub> titers measured for the three S2GΔSHR2-based vaccines against SARS-CoV-2-pps in (E). (G) Neutralization curves of human monoclonal antibodies (mAbs). In (A)-(G), SARS-CoV-2-pps that carry spikes of four strains, including the original Wuhan-Hu-1 strain and three variants, B.1.1.7, B1.351, and P.1, were tested in neutralization assays. (H) Neutralization curves of mouse plasma from two S2GΔHR2-presenting I3-01v9 SApNP vaccine groups against MLV-pps. One group was taken from (C), where mice were given 5 μg of adjuvanted antigen via i.p. injection and the other group was taken from (E), where mice were given 3.3 μg of adjuvanted antigen via i.d. injection. (I) Summary of ID<sub>50</sub> titers measured for two S2GΔHR2-presenting I3-01v9 SApNP vaccine groups against MLV-pps. In all tables, color coding indicates the level of ID<sub>50</sub> titer (white: no neutralization; green to red: low to high).



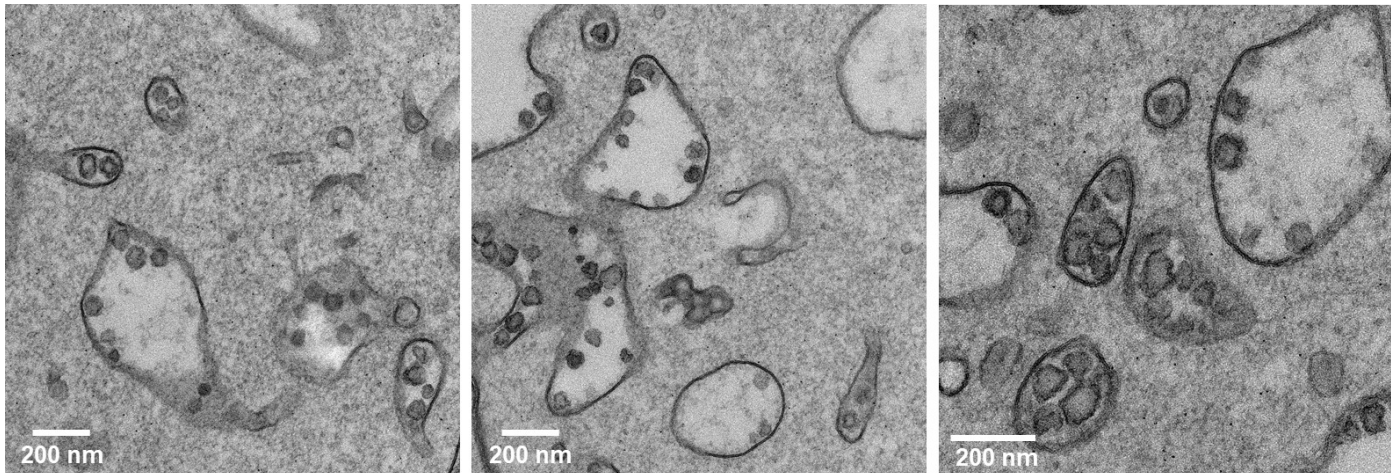
A



B



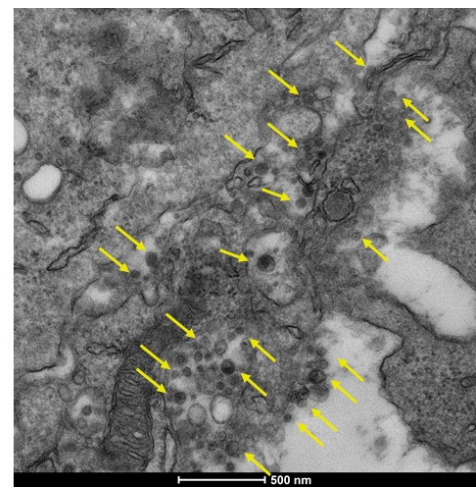
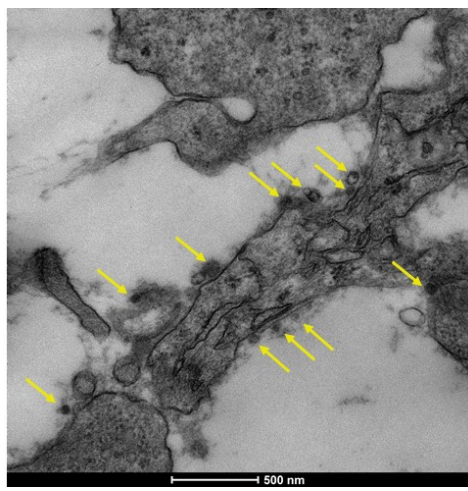
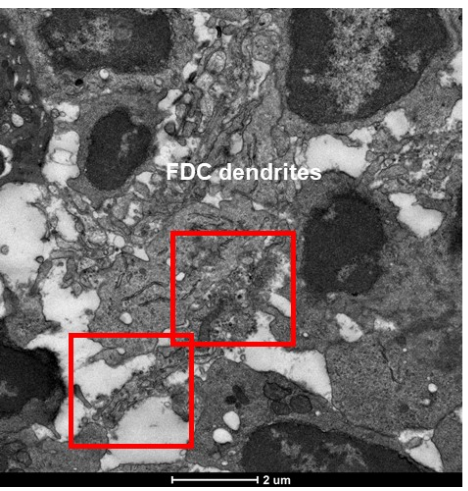
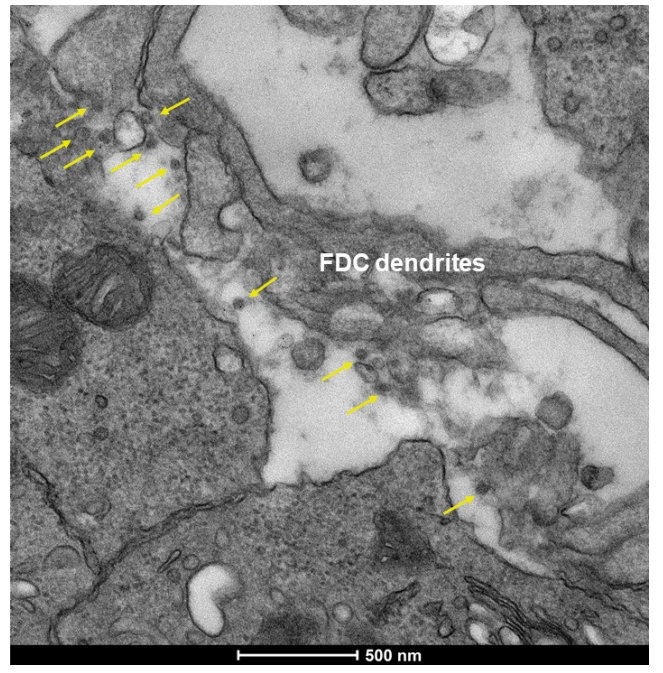
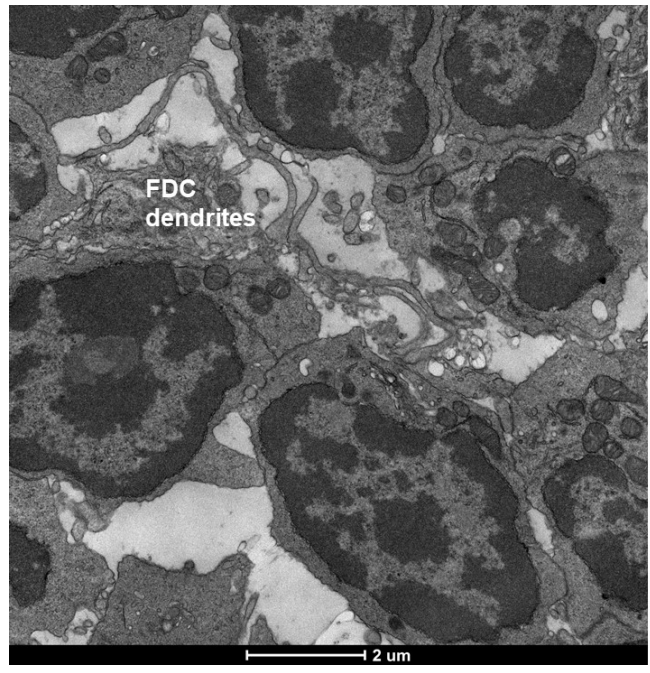
C



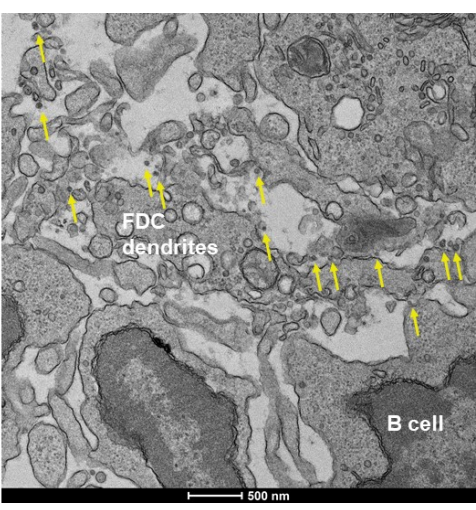
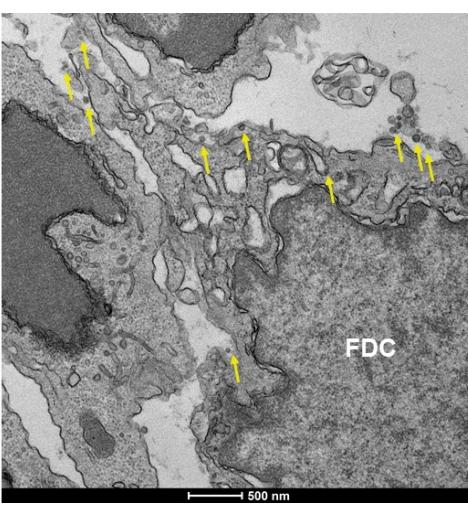
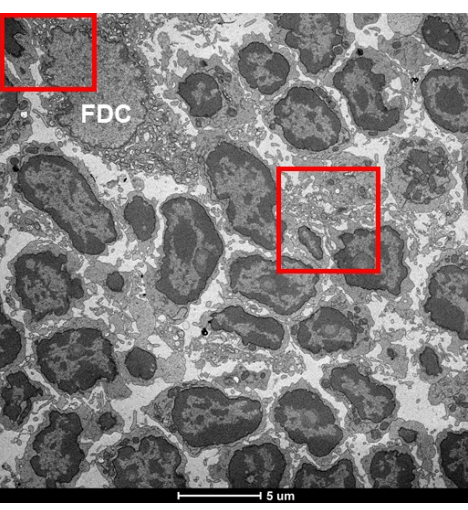
**fig. S2. SARS-CoV-2 spike-presenting I3-01v9 SApNP interaction with macrophages in a lymph node.** (A) S2G $\Delta$ HR2-presenting I3-01v9 SApNPs are sequestered by macrophages in the medullary sinus zone of a lymph node after a single-dose injection (50  $\mu$ g). S2G $\Delta$ HR2-presenting I3-01v9 SApNPs (B) aligned on macrophage surface or (C) sequestered inside the endolysosomes of a macrophage. S2G $\Delta$ HR2-presenting I3-01v9 SApNPs are pointed by yellow arrows.



A

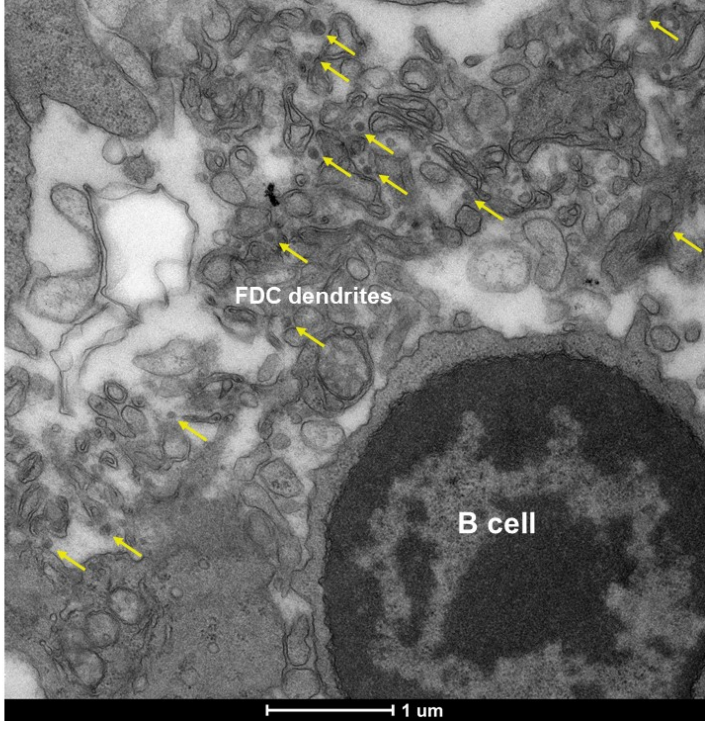
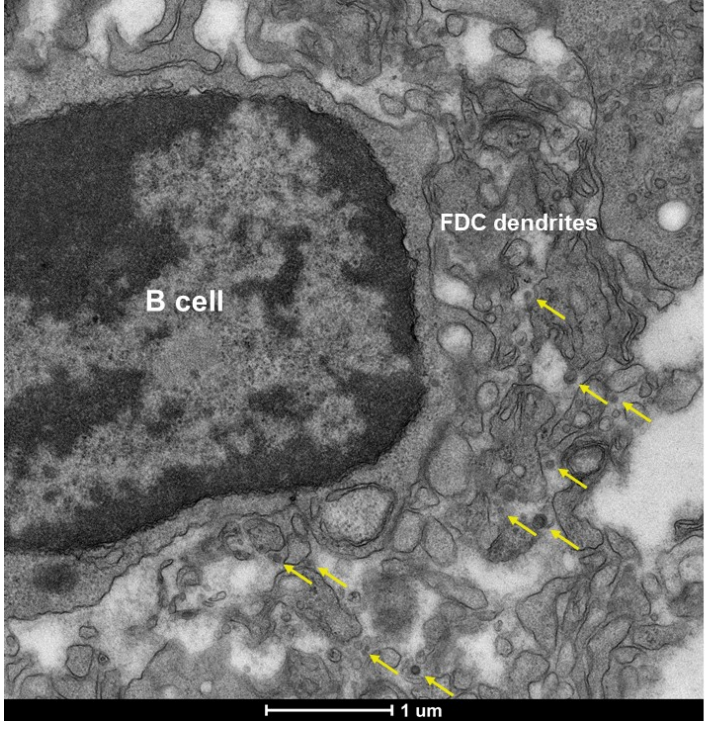
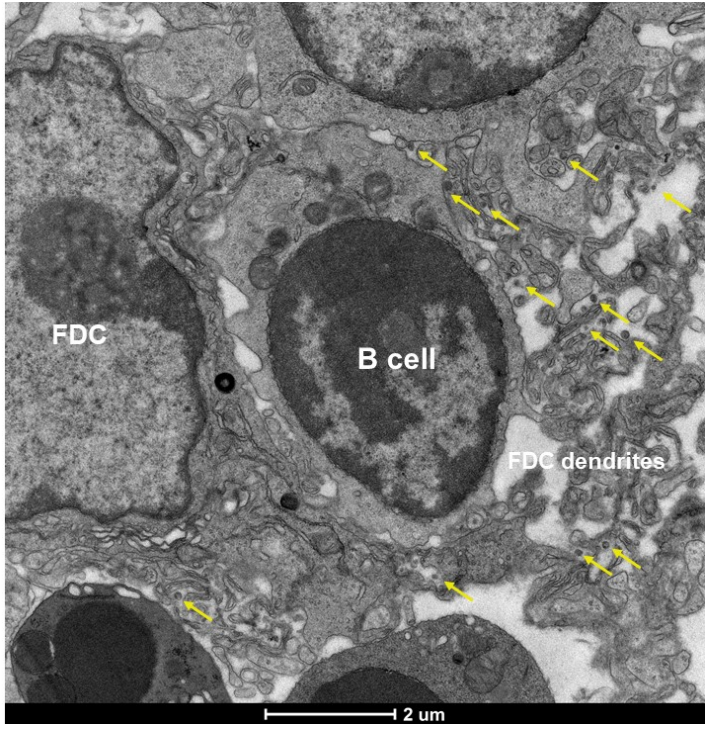
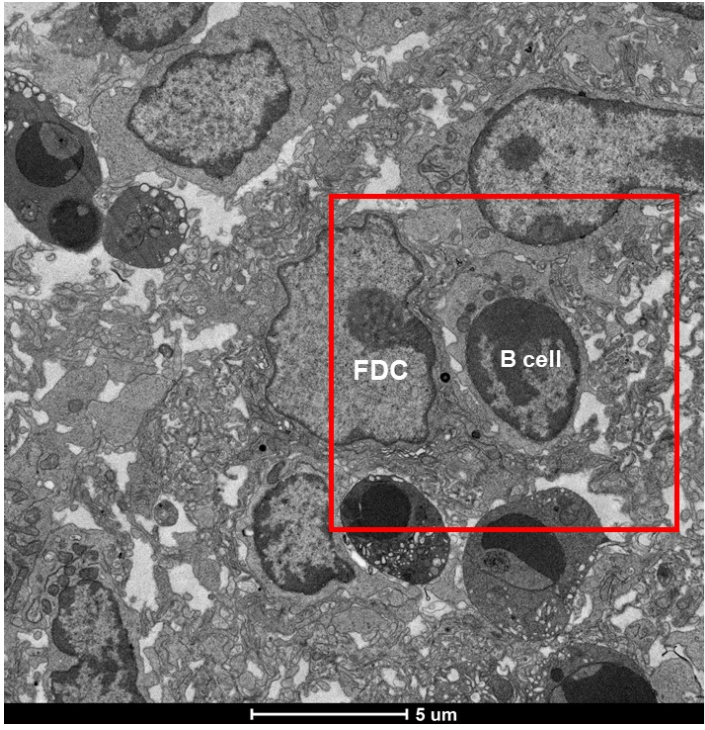


B





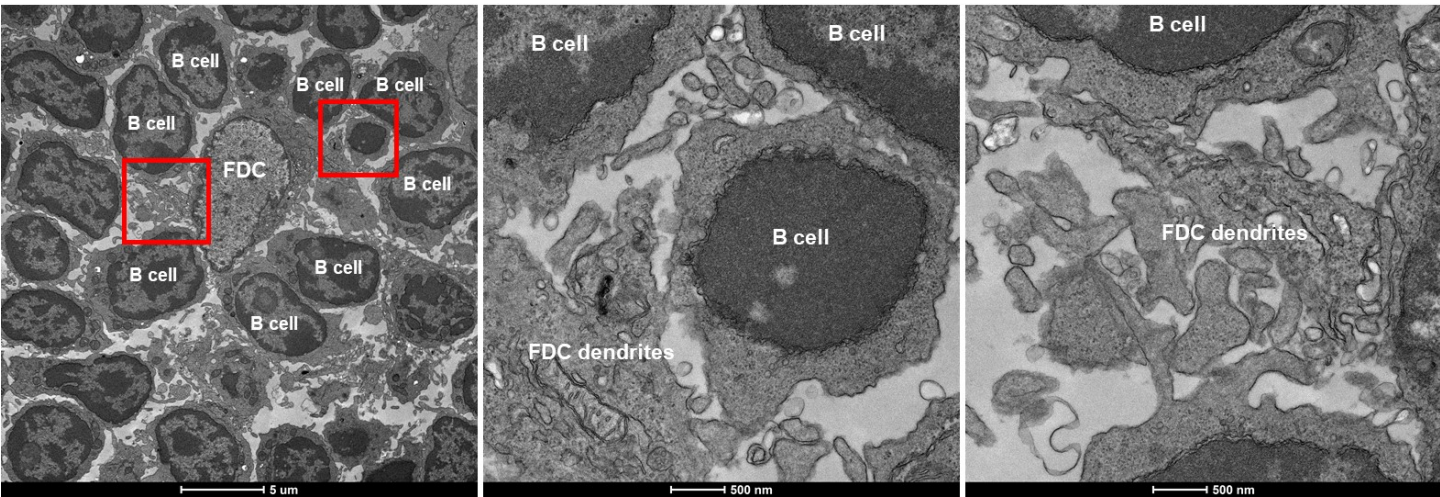
C





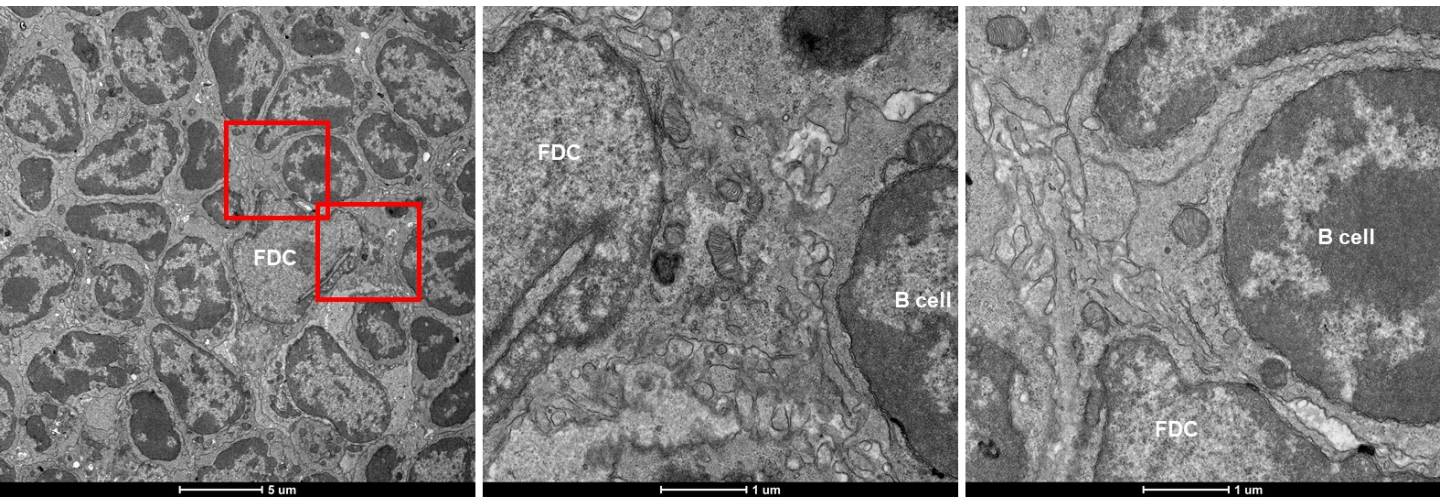
D

I3-01 nanoparticles injected at 2h



E

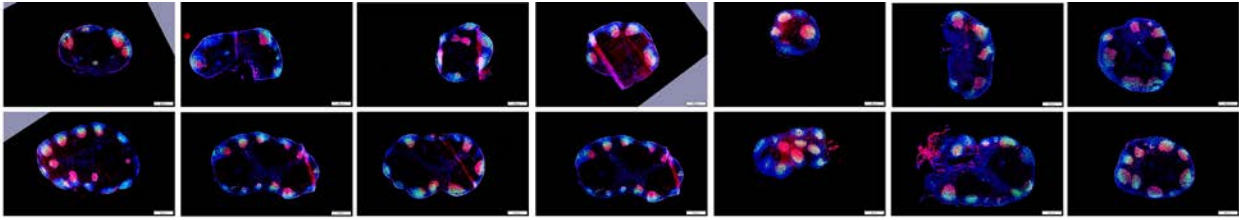
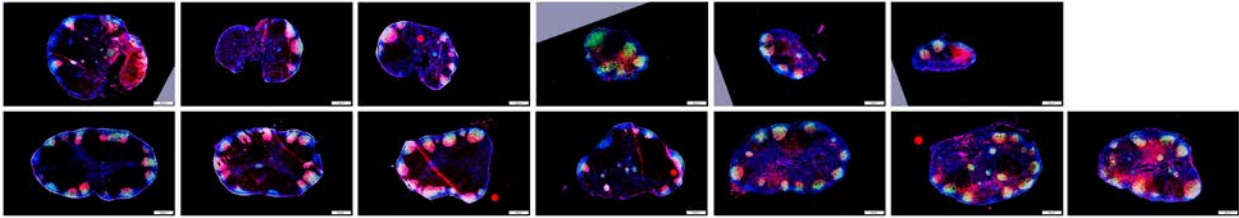
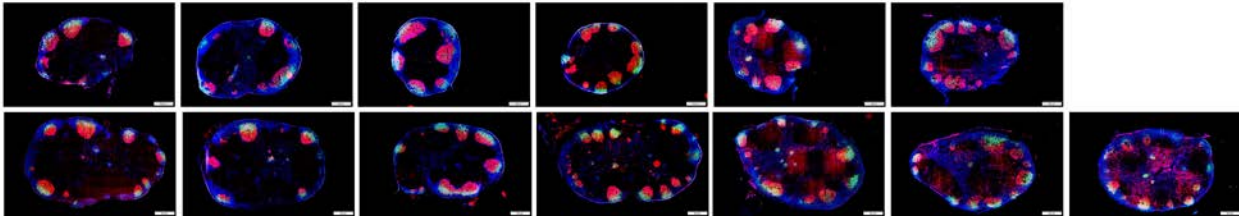
Lymph node from naïve mouse



**fig. S3. TEM images of SARS-CoV-2 spike-presenting I3-01v9 SApNP interaction with FDCs in a lymph node.** S2G $\Delta$ HR2-presenting I3-01v9 SApNPs are aligned on FDC dendrites (A) at 12 h after a single-dose injection (50  $\mu$ g), (B) at 48 h after a single-dose injection (50  $\mu$ g), and (C) at 12 h after the boost injection (50  $\mu$ g). S2G $\Delta$ HR2-presenting I3-01v9 SApNPs are pointed by yellow arrows. (D) S2G $\Delta$ HR2-presenting I3-01v9 SApNPs were barely observed at 2 h after a single-dose injection (50  $\mu$ g). (E) Lymph node from a naïve, unimmunized mouse.

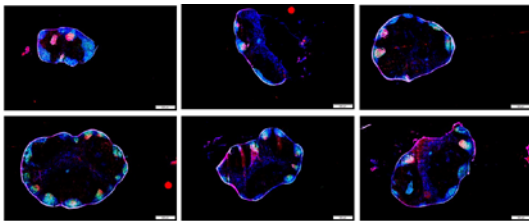
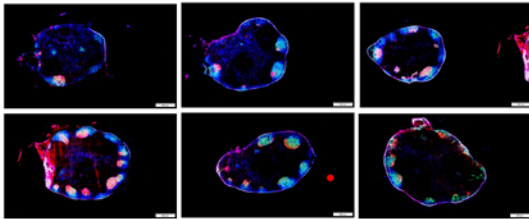
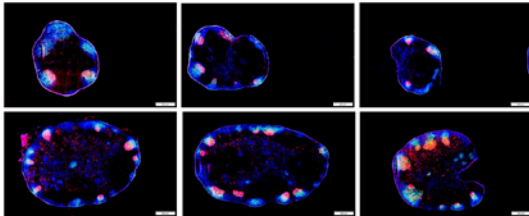
A

Single-dose – 2 w

S2G $\Delta$ HR2  
spikeE2p  
SApNPI3-01v9  
SApNP

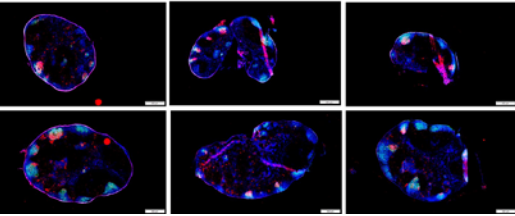
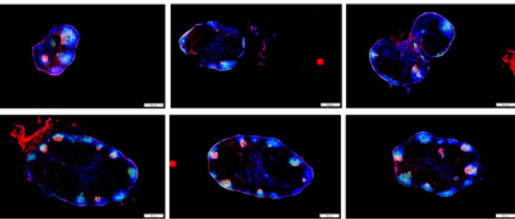
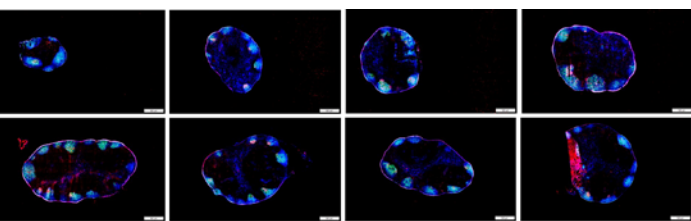
B

Single-dose – 5 w

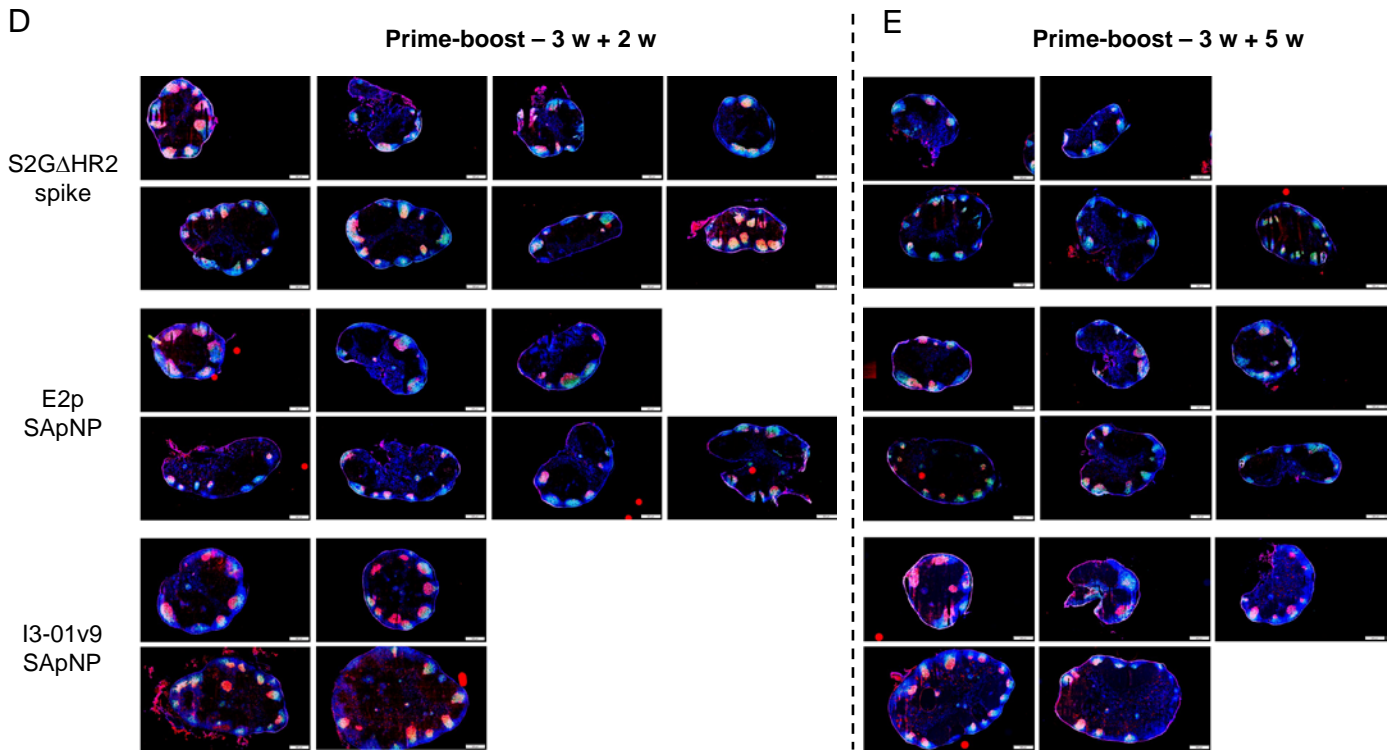
S2G $\Delta$ HR2  
spikeE2p  
SApNPI3-01v9  
SApNP

C

Single-dose – 8 w

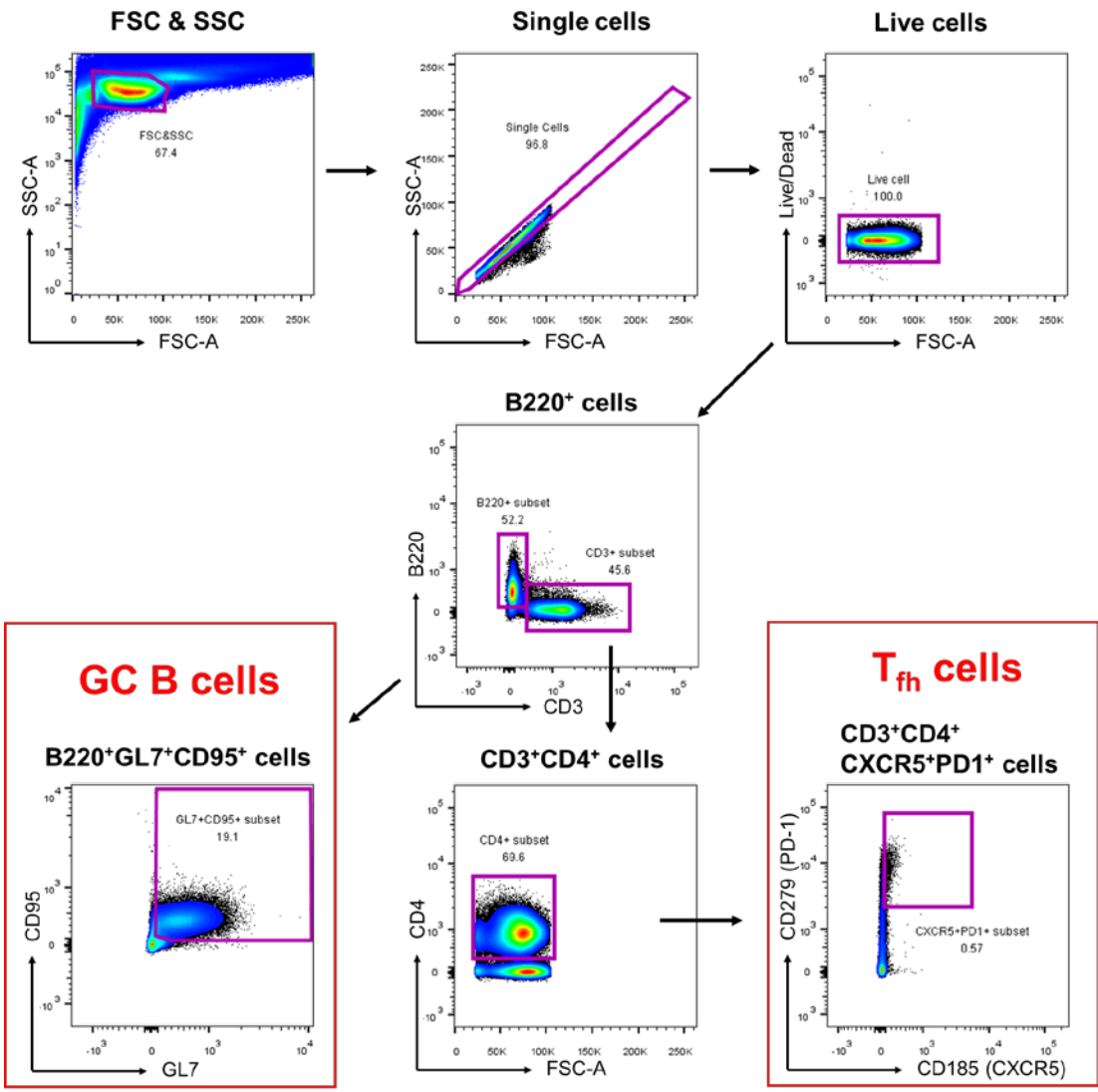






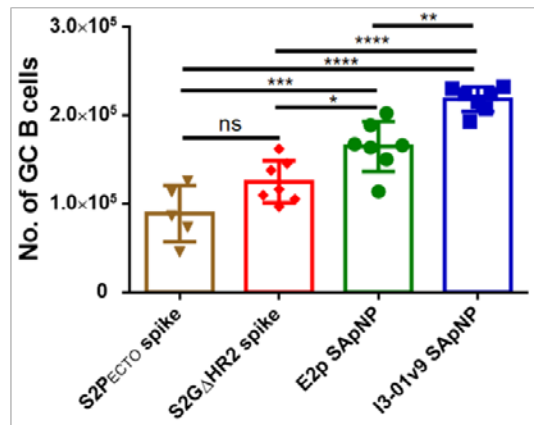
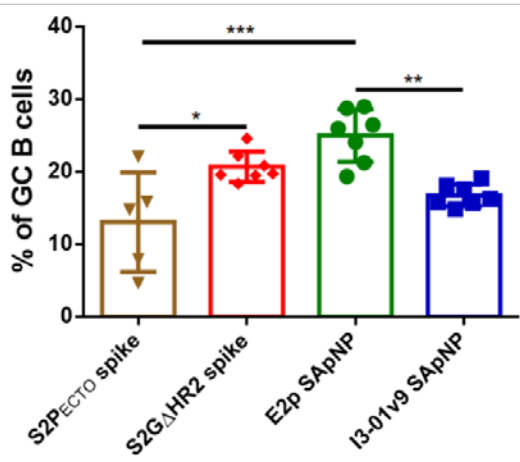
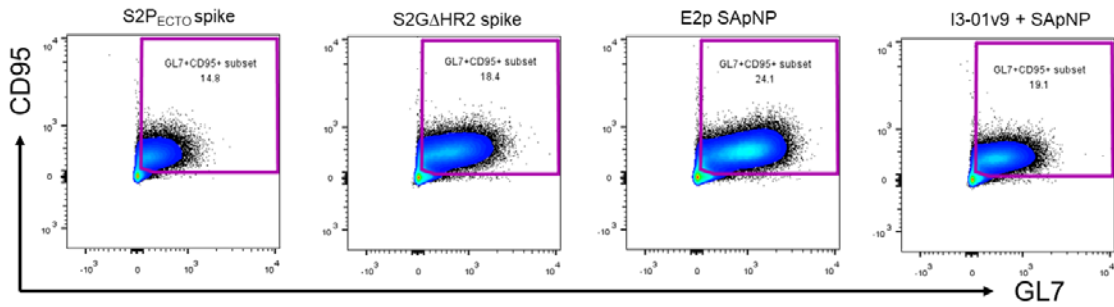
**fig. S4. Immunohistological analysis of SARS-CoV-2 spike/spike-presenting SApNP vaccine-induced GCs.** Images of germinal centers at (A) week 2, (B) week 5, and (C) week 8 after a single-dose injection of S2G $\Delta$ HR2 spike and S2G $\Delta$ HR2-presenting E2p and I3-01v9 SApNP vaccines (10  $\mu$ g per injection, 40  $\mu$ g for a mouse). Images of germinal centers at (D) week 2 and (E) week 5 after prime-boost injections.

A

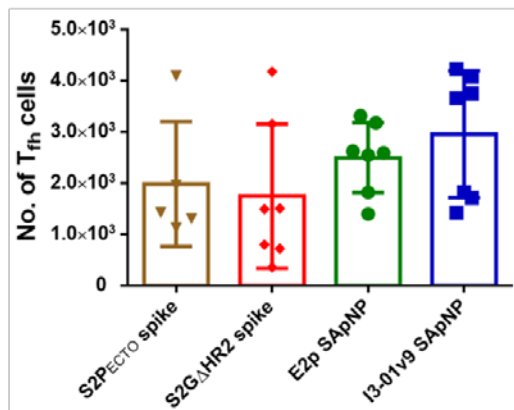
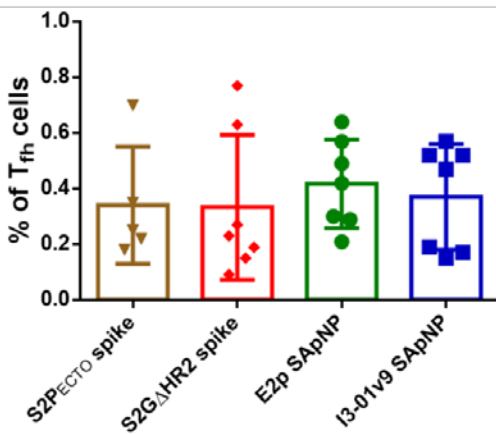
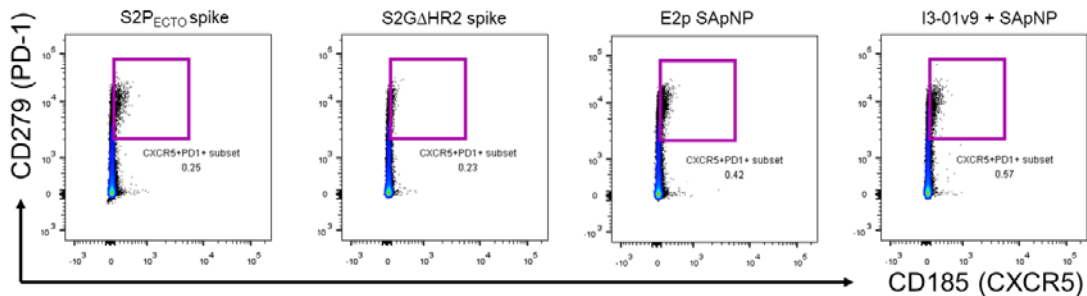




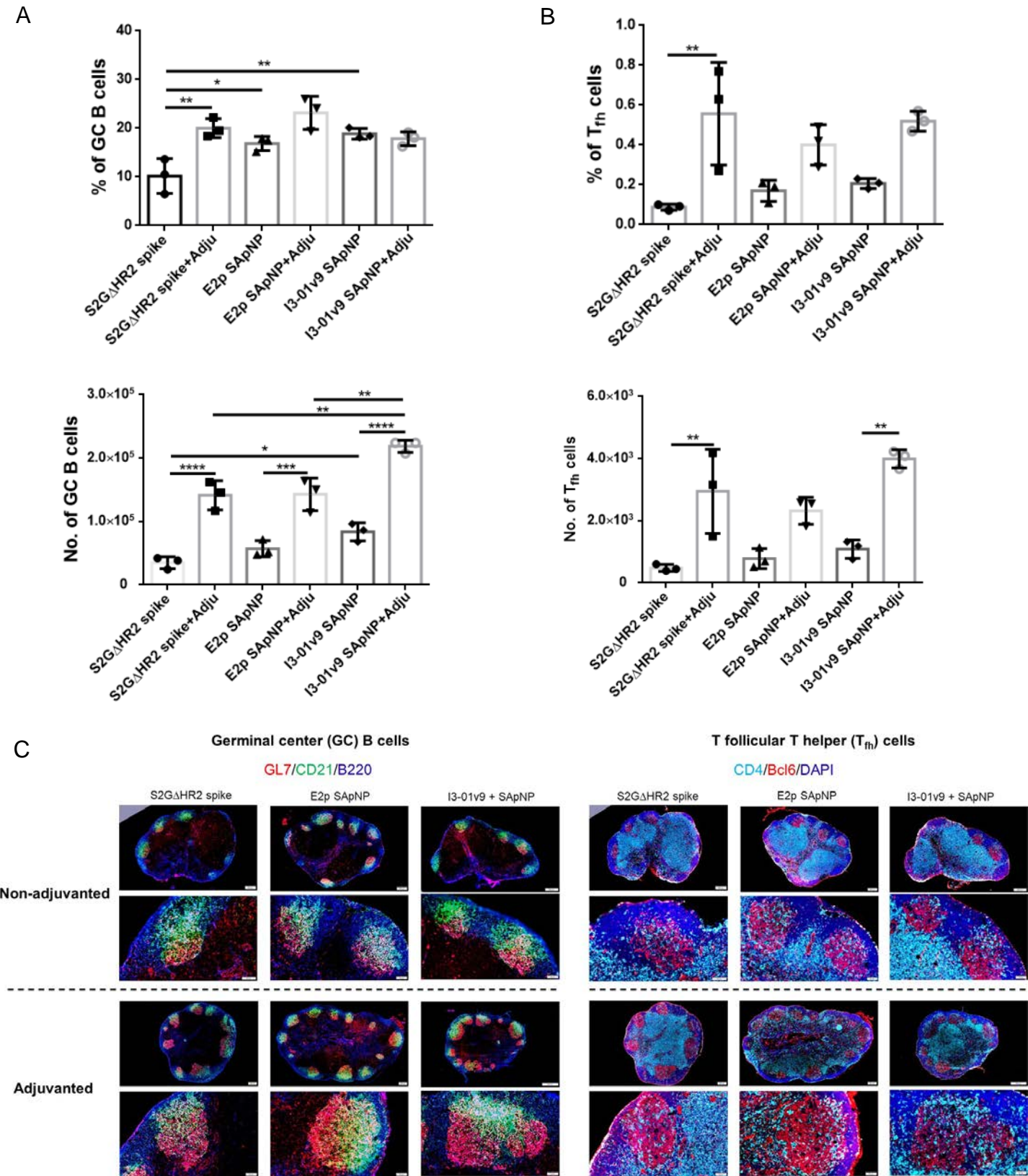
B



C



**fig. S5. Flow cytometry analysis of SARS-CoV-2 spike/spike-presenting SApNP vaccine-induced GCs.** (A) Gating strategy for analyzing germinal center reactions using flow cytometry. Quantification of germinal center reactions, including (B) GC B cells and (C) T follicular helper cells at week 2 after a single-dose injection of S2P<sub>ECTO</sub>, S2G $\Delta$ HR2, and S2G $\Delta$ HR2-presenting E2p and I3-01v9 SApNP vaccines (10  $\mu$ g per injection, 40  $\mu$ g for a mouse). Data points are presented as mean  $\pm$  SD. The  $P$  values were determined by one-way ANOVA followed by Tukey's multiple comparisons *post hoc* test for each timepoint. \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ , \*\*\*\* $p < 0.0001$ .



**fig. S6. Adjuvant effect on SARS-CoV-2 spike/spike-presenting SApNP vaccine-induced germinal centers.** Quantification of germinal center reactions, including (A) GC B cells and (B) T follicular helper cells at week 2 after a single-dose injection of S2P<sub>ECTO</sub>, S2G $\Delta$ HR2, and S2G $\Delta$ HR2-presenting E2p and I3-01v9 SApNP vaccines with/without adjuvants using flow cytometry (10  $\mu$ g per injection, 40  $\mu$ g for a mouse). (C) Immunohistology of germinal centers at week 2 after a single-dose injection with/without adjuvants. Data points are presented as mean  $\pm$  SD. The *P* values were determined by one-way ANOVA followed by Tukey's multiple comparisons *post hoc* test for each timepoint. \**p* < 0.05, \*\**p* < 0.01, \*\*\**p* < 0.001, \*\*\*\**p* < 0.0001.