

Supplemental information

A spike virosome vaccine induces pan-sarbecovirus antibody responses in mice

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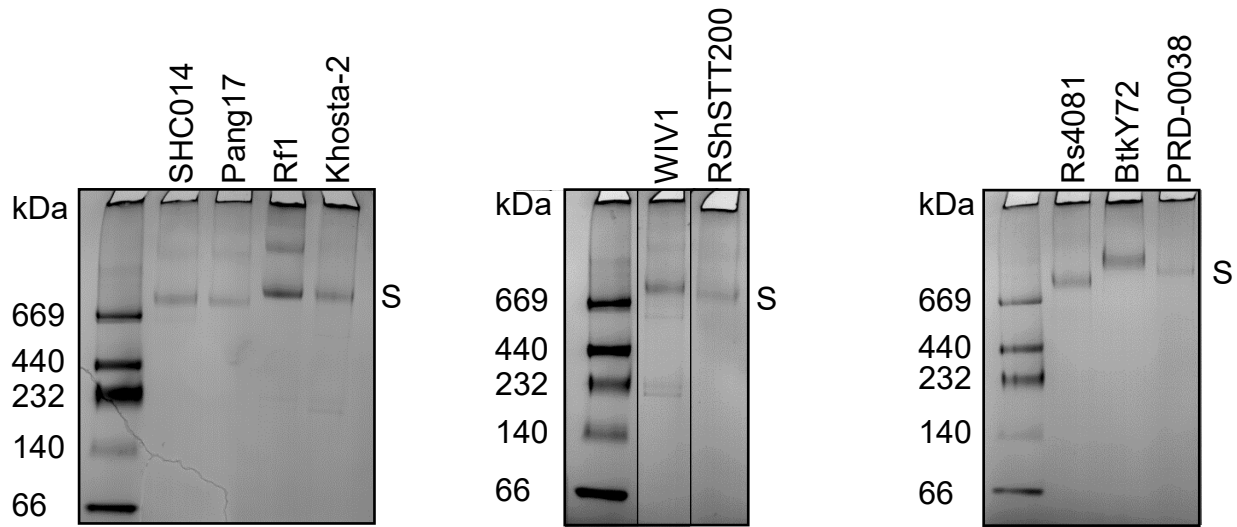
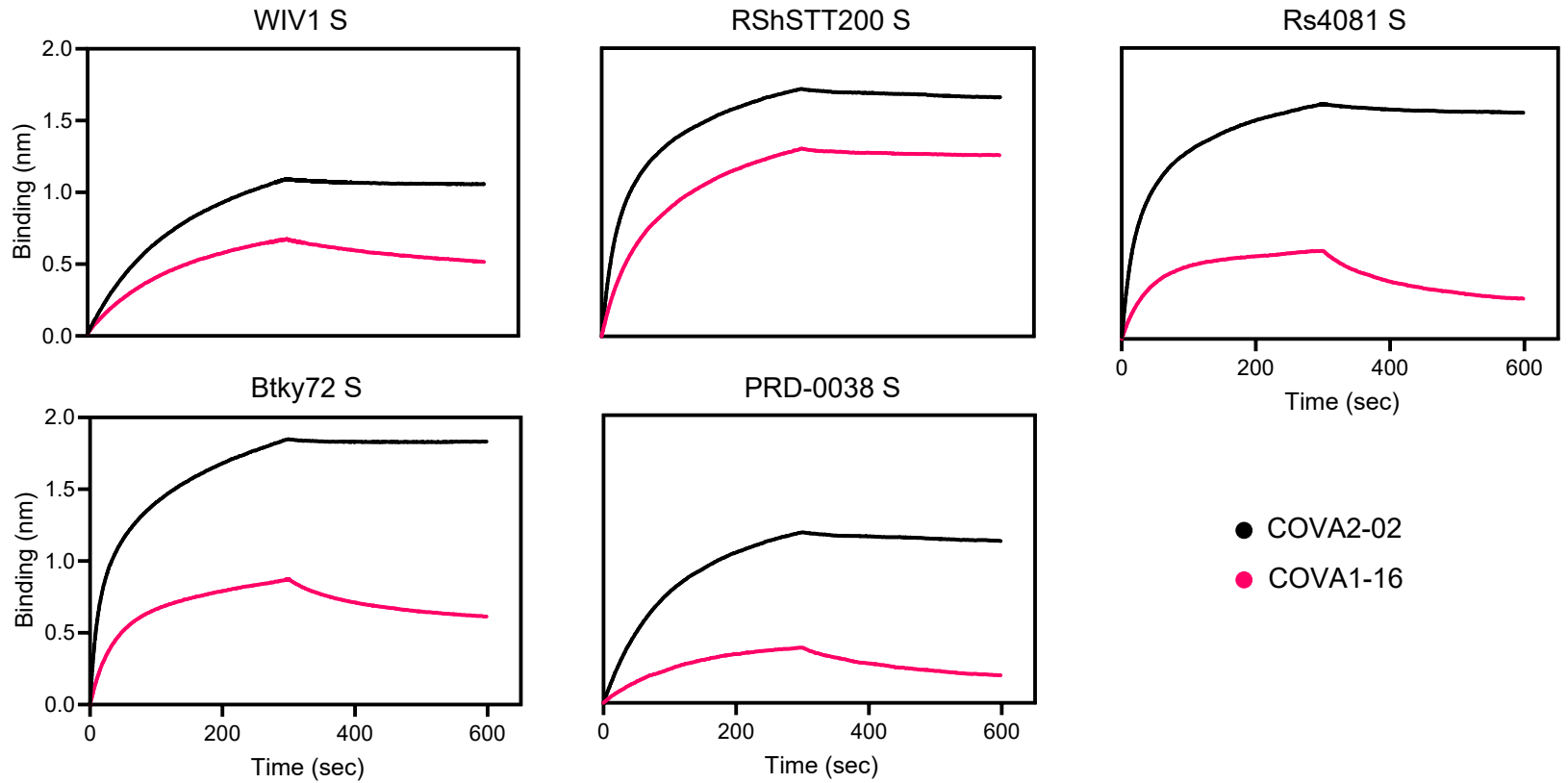
A**B**

Figure S1. Sarbecovirus S characterization, related to figure 1 (A) Blue-Native PAGE gels of purified S proteins. (B) BLI sensorgrams of binding reactions of monoclonal Abs isolated from SARS-CoV-2 survivors to sarbecovirus S

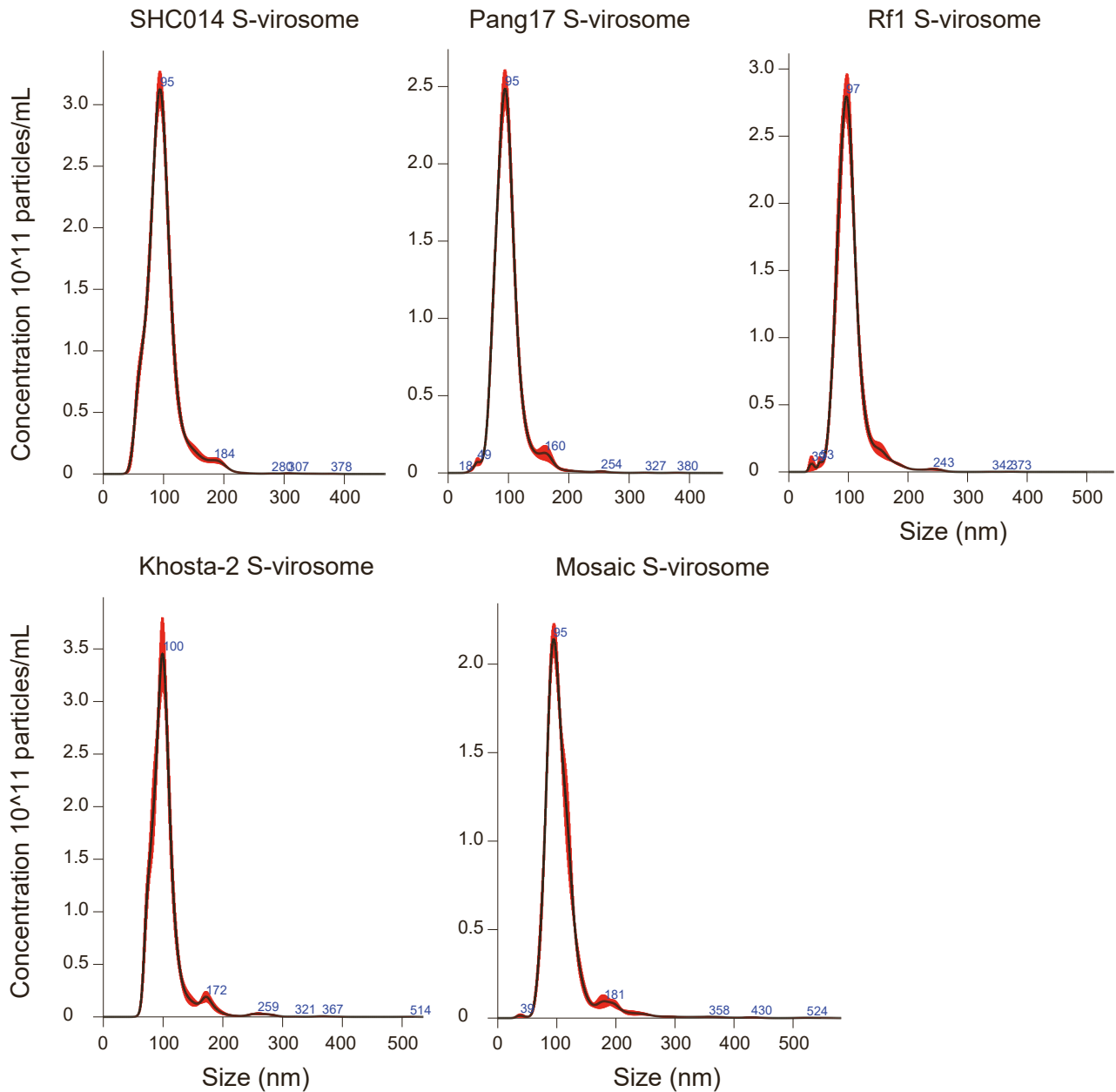


Figure S2. S-virosome size distribution, related to figure 1 Virosome size distribution as measured by nanosight.

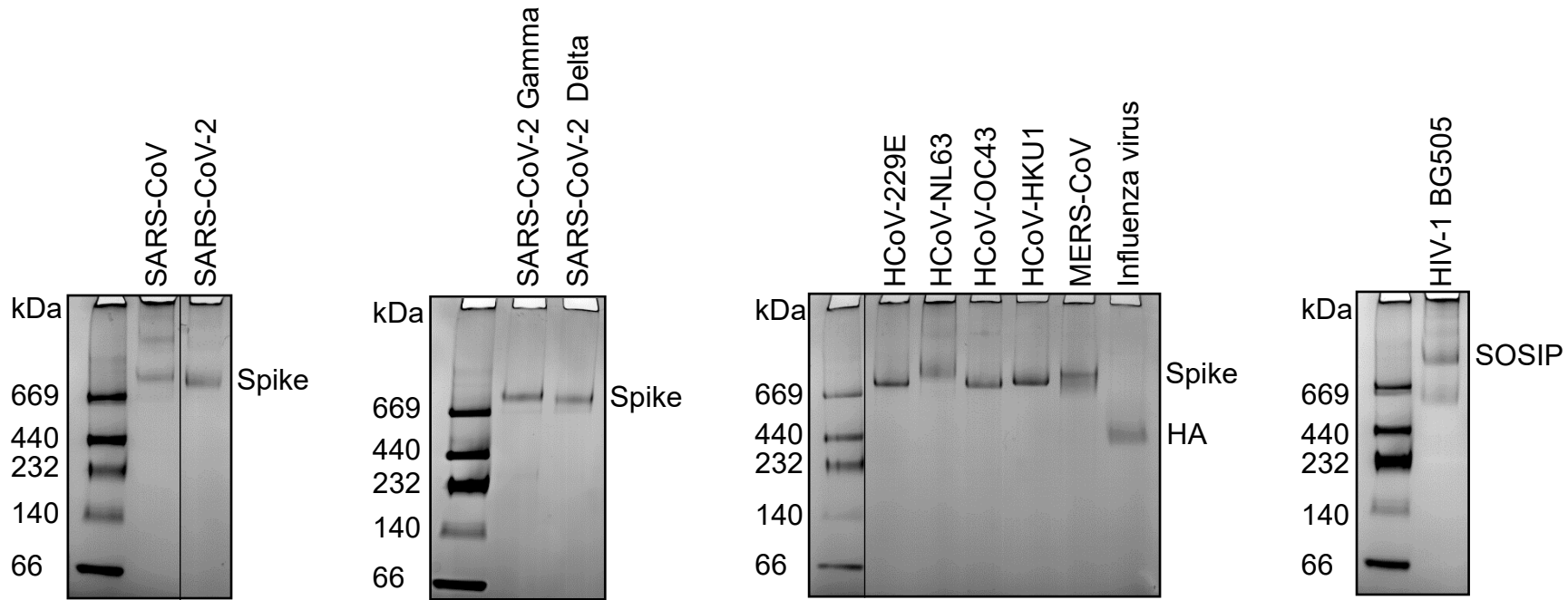


Figure S3. Luminex assay protein panel characterization, related to figure 2 Blue-Native PAGE gels of purified glycoproteins used in the custom Luminex assay.

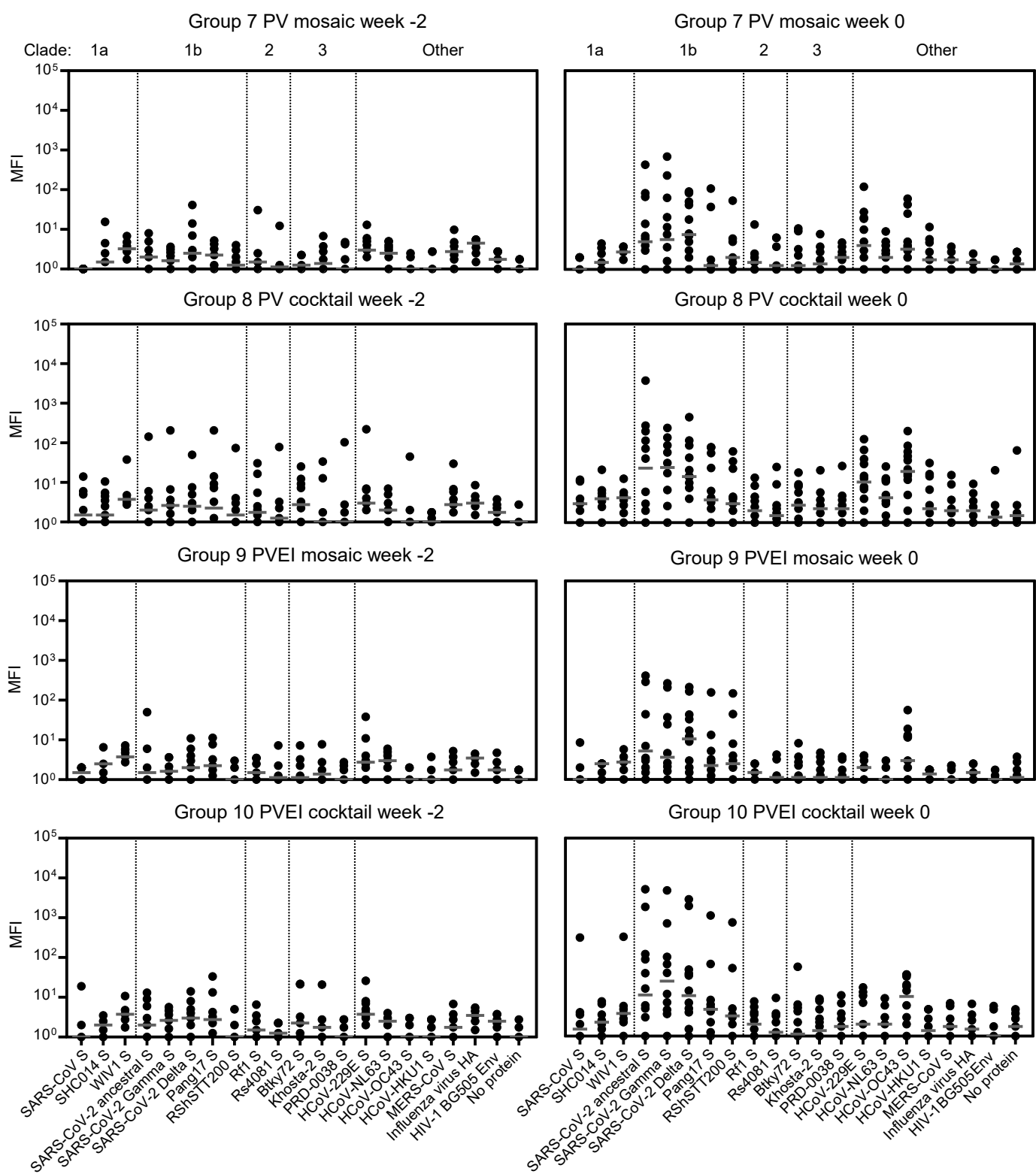


Figure S4. Ab binding responses in sera at week -2 and 0, related to figure 2 The Ab binding responses in sera from groups 7-10 at weeks -2 and 0, measured in a custom Luminex assay. The serum samples were diluted 1:1,000. The dots represent the MFI values of individual serum samples tested against each protein. The horizontal bars indicate the medians.

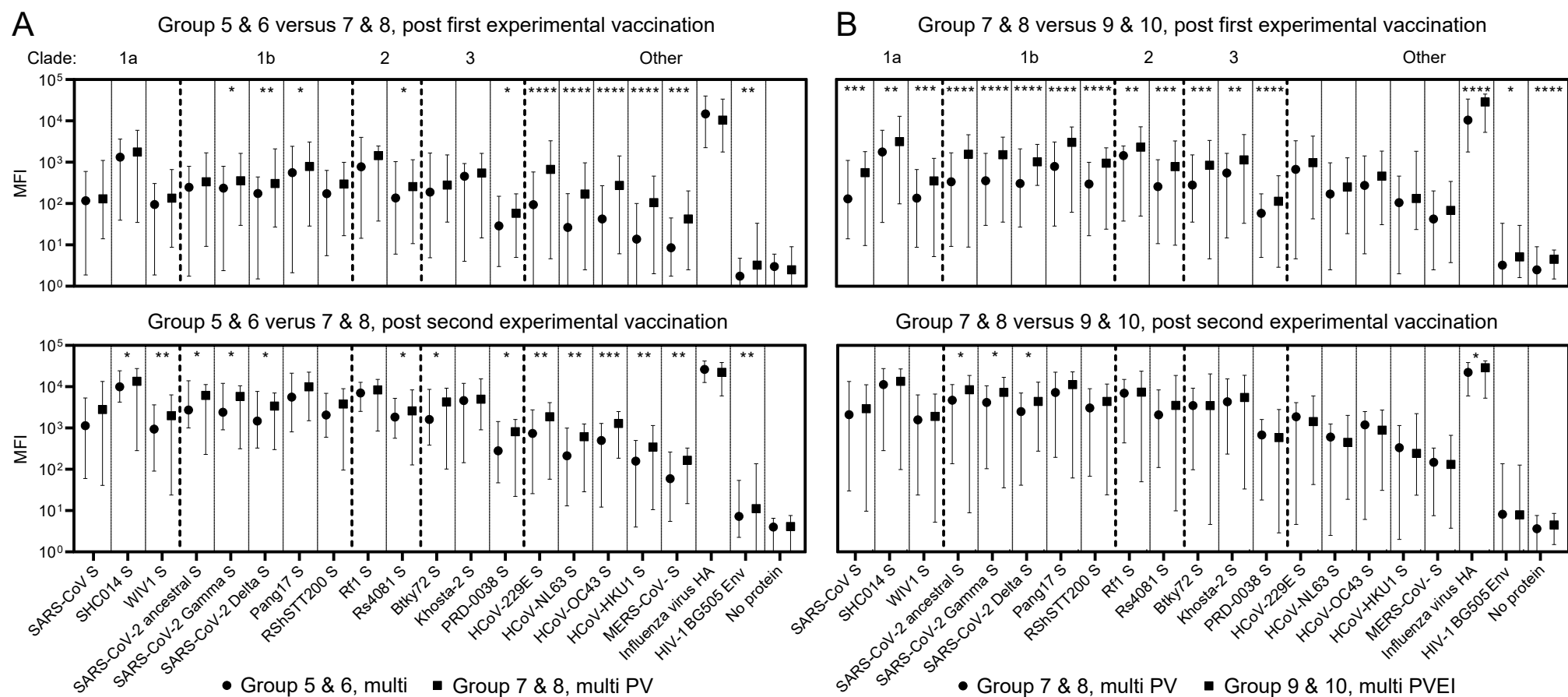


Figure S5. Ab binding responses in sera, comparing different vaccination protocols, related to figure 2 (A) Comparison of mice that received the multivalent vaccines to mice that received the multivalent vaccines and were pre-vaccinated. The Ab binding responses post the first experimental vaccination (week 6) (upper), and the Ab binding responses post the second experimental vaccination (week 8) (lower) (B) Comparison of mice that received the multivalent vaccines and were pre-vaccinated to mice that received the multivalent vaccines and were pre-vaccinated, with extended vaccination intervals. The Ab binding responses post the first experimental vaccination (week 6) (upper), and the Ab binding responses in mice post the second experimental vaccination (lower). (A,B) The Ab binding responses were measured in a custom Luminex assay. The sera were diluted 1:10,000. The dots represent the median MFI values. The whiskers indicate the range of MFIs measured in all serum samples. The MFI values between groups were compared using the Mann-Whitney U test (*, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$; ****, $p < 0.001$).

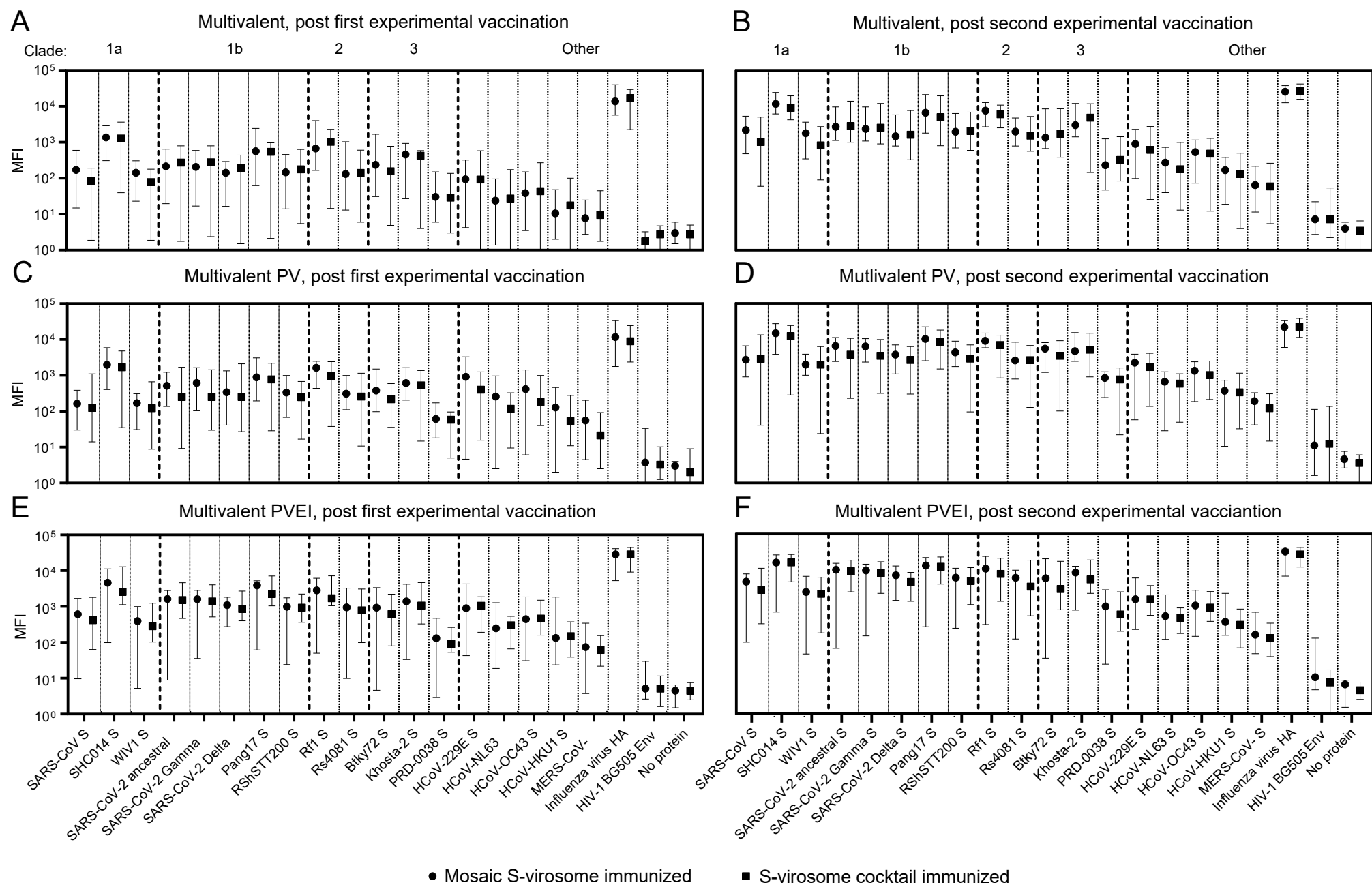


Figure S6. Ab binding responses in sera, comparing mosaic S-virosome and S-virosome cocktail vaccinations, related to figure 2 (A) Comparison of Ab binding responses in mosaic S-virosome versus cocktail S-virosome vaccinated mice post the first experimental vaccination (week 6). (B) Comparison of Ab binding responses in mosaic S-virosome versus cocktail S-virosome vaccinated mice post the second experimental vaccination (week 8). (C) Comparison of mosaic S-virosome versus cocktail S-virosome vaccinated mice, mice received the CoV-pre-vaccination post the first experimental vaccination (week 6). (D) Comparison of mosaic S-virosome versus cocktail S-virosome vaccinated mice, mice received the CoV-pre-vaccination post the second experimental vaccination (week 8). (E) Comparison of mosaic S-virosome versus cocktail S-virosome vaccinated mice, mice received the CoV-pre-vaccination, and were vaccinated with extended vaccination intervals post the first experimental vaccination (week 6). (F) Comparison of mosaic S-virosome versus cocktail S-virosome vaccinated mice, mice received the CoV-pre-vaccination, and were vaccinated with extended vaccination intervals post the second experimental vaccination (week 8). (A-E) The Ab binding responses in sera measured in a custom Luminex assay. The sera were diluted 1:10,000. The dots represent the median MFI values. The whiskers indicate the range of MFIs measured in all serum samples. The MFIs between groups were compared using the Mann-Whitney U test.

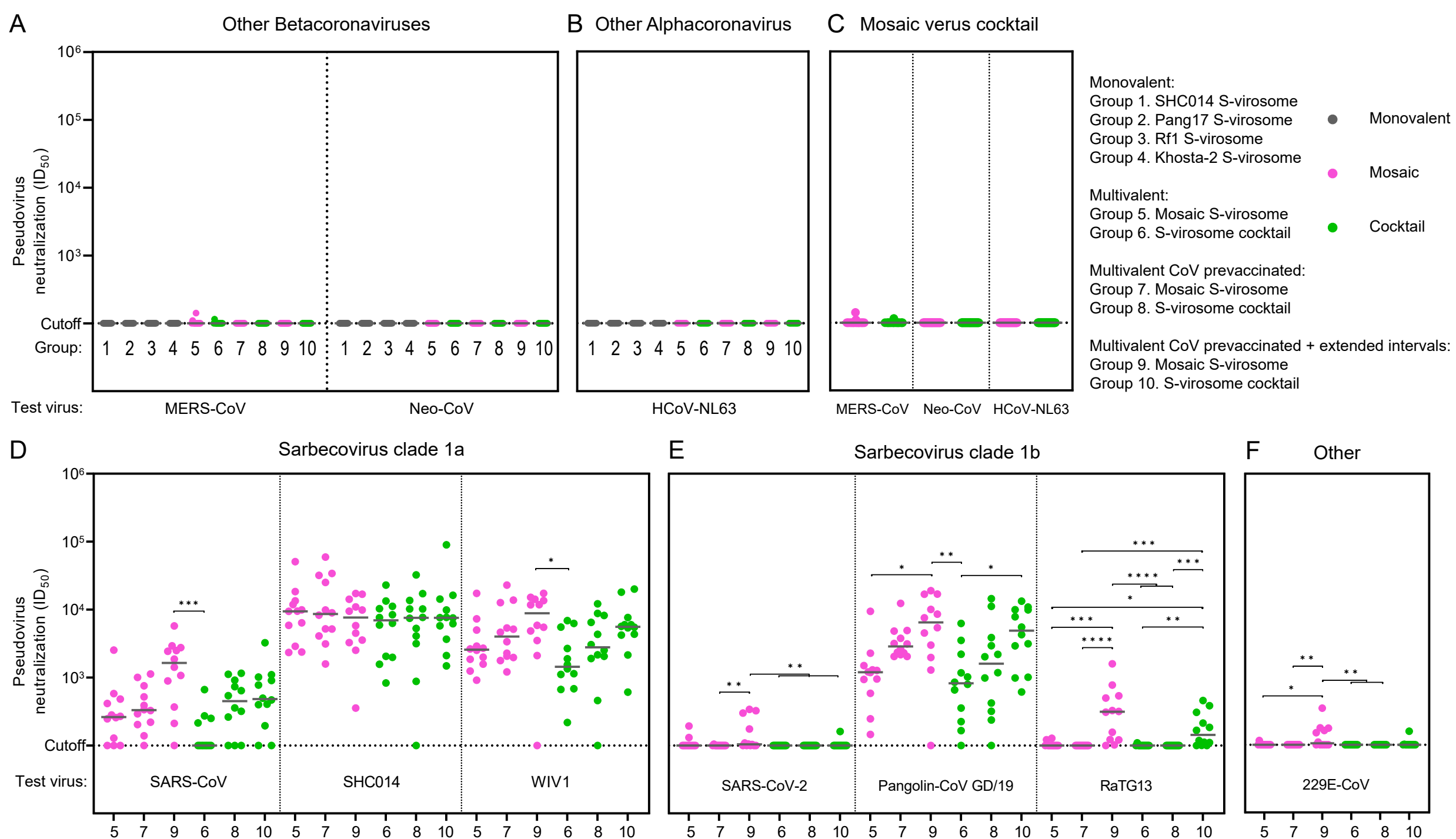


Figure S7. Pseudovirus neutralization at final bleed, related to figure 3 (A) Pseudovirus neutralization of betacoronaviruses MERS-CoV and Neo-CoV. (B) Pseudovirus neutralization of alphacoronavirus hCoV-NL63. (C) Comparison of pseudovirus neutralization by mosaic S-virosome immunized animals (groups 5, 7 and 9) to S-virosome cocktail immunized animals (groups 6, 8 and 10). (D) Pseudovirus neutralization of clade 1a sarbecoviruses by animals in groups 5-10. (E) Pseudovirus neutralization of clade 1b sarbecoviruses by animals in groups 5-10. (F) Pseudovirus neutralization of hCoV-229E by animals in groups 5-10. (A-F) The dots represent ID₅₀ titers of individual serum samples against each virus. The horizontal bars indicate the median. The titers between groups were compared using the Kruskal-Wallis test, and Dunn's post-test (*, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$; ****, $p < 0.001$).

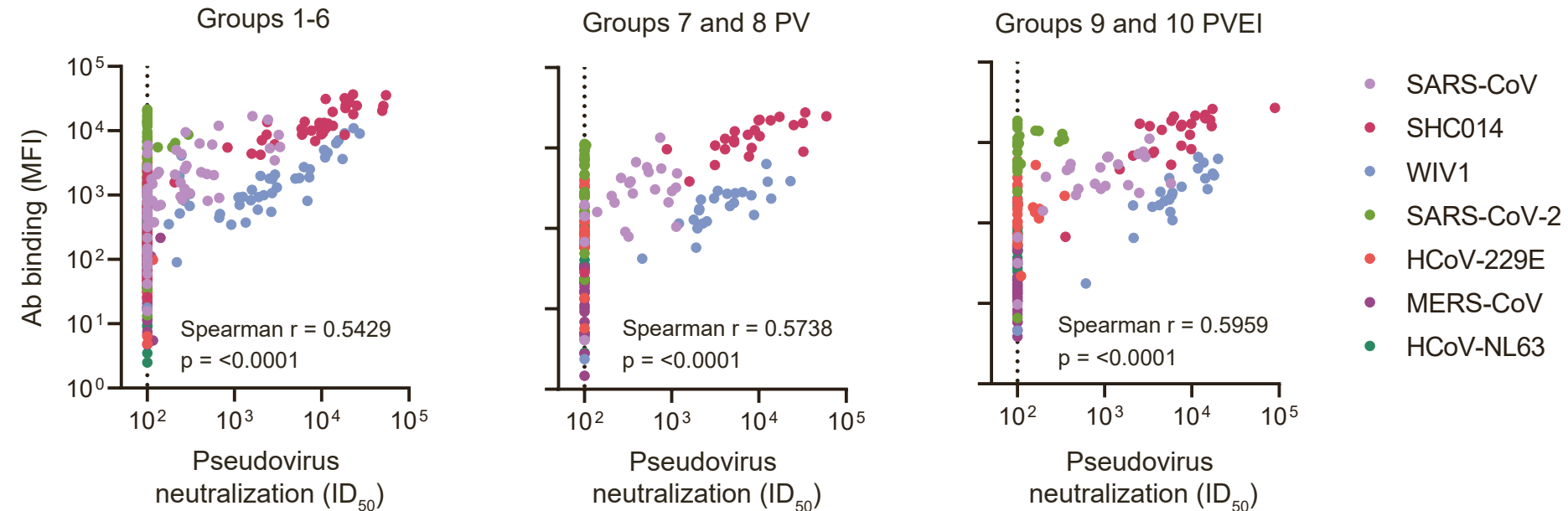


Figure S8. Correlation between pseudovirus neutralization and antibody binding, related to figures 2 and 3 Correlation between pseudovirus neutralization and antibody binding in groups 1-6 (right), 7 and 8 (middle), and 9 and 10 (left).

Table S1. Pseudovirus neutralization ID50 titers, related to figures 3 and S7

Pseudovirus sera neutralization ID50s		Pseudovirus Panel										
Virosome vaccine	Immunization protocol	Micro ID	SARS-CoV	SHC014	WV1	SARS-CoV-2 D614G	Pangolin-CoV GD/19	RaTG13 T403R	MERS-CoV	Neo CoV T510F	HCoV-NL63 I507L	HCoV-229E-CoV
SHC014 S	Standard	gr1-1	281	10620	3953	<100	1111	<100	<100	<100	<100	<100
		gr1-2	491	28215	2156	<100	<100	<100	<100	<100	<100	<100
		gr1-3	655	18840	14588	<100	<100	<100	<100	<100	<100	<100
		gr1-4	101	18742	245	<100	<100	<100	<100	<100	<100	<100
		gr1-5	398	54455	10588	<100	338	<100	<100	<100	<100	<100
		gr1-6	2425	105332	18373	<100	3258	<100	<100	<100	<100	<100
		gr1-7	176	22998	27169	<100	2605	969	<100	<100	<100	<100
		gr1-8	1605	18076	23238	<100	1960	<100	<100	<100	<100	<100
		gr1-9	3218	11077	15611	261	3755	338	<100	<100	<100	<100
		gr1-10	3335	20861	11642	<100	1745	<100	<100	<100	<100	<100
		gr1-11	2879	45278	7215	209	789	218	<100	<100	<100	<100
		gr1-12	554	18283	10912	<100	1114	<100	<100	<100	<100	<100
Pang17 S	Standard	gr2-13	268	<100	268	<100	9318	929	<100	<100	<100	<100
		gr2-14	<100	<100	<100	<100	12896	<100	<100	<100	<100	<100
		gr2-15	<100	<100	<100	<100	2887	<100	<100	<100	<100	<100
		gr2-16	136	<100	<100	<100	8284	492	<100	<100	<100	<100
		gr2-17	119	<100	<100	<100	3231	<100	<100	<100	<100	<100
		gr2-18	267	<100	<100	<100	15153	<100	<100	<100	<100	<100
		gr2-19	114	<100	306	<100	2233	<100	<100	<100	<100	<100
		gr2-20	115	306	306	<100	6631	<100	<100	<100	<100	<100
		gr2-21	306	<100	<100	<100	3322	<100	<100	<100	<100	<100
		gr2-22	111	<100	233	<100	5979	<100	<100	<100	<100	<100
		gr2-23	<100	<100	<100	<100	1391	<100	<100	<100	<100	<100
		gr2-24	207	<100	<100	<100	2719	<100	<100	<100	<100	<100
Rf-1 S	Standard	gr3-25	<100	<100	<100	<100	<100	<100	<100	<100	<100	<100
		gr3-26	<100	<100	<100	<100	589	<100	<100	<100	<100	<100
		gr3-27	<100	<100	<100	<100	<100	<100	<100	<100	<100	<100
		gr3-28	<100	<100	<100	<100	<100	<100	<100	<100	<100	<100
		gr3-29	<100	<100	<100	<100	<100	<100	<100	<100	<100	<100
		gr3-30	<100	<100	<100	<100	<100	<100	<100	<100	<100	<100
		gr3-31	<100	<100	<100	<100	<100	<100	<100	<100	<100	<100
		gr3-32	<100	<100	<100	<100	<100	<100	<100	<100	<100	<100
		gr3-33	<100	<100	<100	<100	<100	<100	<100	<100	<100	<100
		gr3-34	<100	<100	<100	<100	436	186	<100	<100	<100	<100
		gr3-35	<100	<100	<100	<100	<100	<100	<100	<100	<100	<100
		gr3-36	<100	<100	<100	<100	<100	<100	<100	<100	<100	<100
Khosta-2 S	Standard	gr4-37	<100	<100	<100	<100	<100	<100	<100	<100	<100	<100
		gr4-38	<100	<100	<100	<100	<100	<100	<100	<100	<100	<100
		gr4-39	<100	<100	<100	<100	<100	<100	<100	<100	<100	<100
		gr4-40	<100	<100	<100	<100	<100	<100	<100	<100	<100	<100
		gr4-41	<100	<100	<100	<100	<100	<100	<100	<100	<100	<100
		gr4-42	<100	<100	<100	<100	<100	<100	<100	<100	<100	<100
		gr4-43	<100	<100	<100	<100	<100	<100	<100	<100	<100	<100
		gr4-44	<100	<100	<100	<100	<100	<100	<100	<100	<100	<100
		gr4-45	<100	<100	<100	<100	<100	<100	<100	<100	<100	<100
		gr4-46	<100	<100	<100	<100	<100	<100	<100	<100	<100	<100
		gr4-47	<100	<100	<100	<100	<100	<100	<100	<100	<100	<100
		gr4-48	<100	<100	<100	<100	<100	<100	<100	<100	<100	<100
Mosaic	Standard	gr5-49	578	5852	2703	<100	939	<100	<100	<100	<100	<100
		gr5-50	2535	11852	17345	<100	9426	127	110	<100	<100	<100
		gr5-51	297	2339	1853	<100	1210	<100	<100	<100	<100	<100
		gr5-52	414	2367	2323	381	1497	<100	<100	<100	<100	<100
		gr5-53	<100	18386	915	<100	586	<100	<100	<100	<100	<100
		gr5-54	246	50790	1988	193	2277	<100	142	<100	<100	<100
		gr5-55	481	2886	1245	<100	1273	121	1245	<100	<100	<100
		gr5-56	268	13425	2897	<100	950	<100	<100	<100	<100	<100
		gr5-57	128	9998	1578	<100	247	<100	<100	<100	<100	<100
		gr5-58	286	9414	4995	<100	146	<100	<100	<100	<100	<100
		gr5-59	<100	6378	2629	<100	1298	<100	<100	<100	<100	<100
		gr5-60	<100	9449	2522	<100	1184	<100	<100	<100	<100	116
Cocktail	Standard	gr6-61	<100	11173	1882	<100	2197	110	<100	<100	<100	<100
		gr6-62	214	13373	6258	<100	6252	<100	<100	<100	<100	<100
		gr6-63	<100	6287	1111	<100	1018	<100	<100	<100	<100	<100
		gr6-64	<100	1982	1137	<100	258	<100	<100	<100	<100	<100
		gr6-65	<100	1954	638	<100	322	<100	<100	<100	<100	<100
		gr6-66	<100	22820	683	<100	355	<100	<100	<100	<100	<100
		gr6-67	665	10329	1342	<100	165	<100	<100	<100	<100	<100
		gr6-68	<100	7632	6869	<100	1175	<100	<100	<100	<100	<100
		gr6-69	251	8413	666	<100	657	<100	<100	<100	<100	<100
		gr6-70	<100	2057	5521	<100	3514	<100	<100	<100	<100	<100
		gr6-71	<100	629	678	<100	852	<100	<100	<100	<100	<100
		gr6-72	272	5935	1540	<100	794	<100	<100	<100	<100	<100
Mosaic	Pre-vaccination	gr7-73	337	8996	13788	<100	3178	<100	<100	<100	<100	<100
		gr7-74	293	1586	1209	<100	3783	<100	<100	<100	<100	<100
		gr7-75	519	9889	12630	<100	2305	<100	<100	<100	<100	<100
		gr7-76	389	5174	3372	<100	4827	<100	<100	<100	<100	<100
		gr7-77	<100	9498	2486	<100	2045	<100	<100	<100	<100	<100
		gr7-78	38	3256	2275	<100	2173	<100	<100	<100	<100	<100
		gr7-79	757	25057	22954	194	12345	<100	<100	<100	<100	<100
		gr7-80	1003	58364	5361	<100	2605	<100	<100	<100	<100	<100
		gr7-81	326	31817	4634	<100	3124	<100	<100	<100	<100	<100
		gr7-82	220	3126	1779	<100	2346	<100	<100	<100	<100	<100
		gr7-83	295	5193	5149	<100	4823	<100	<100	<100	<100	<100
		gr7-84	1129	4093	1972	<100	2047	<100	<100	<100	<100	<100
Cocktail	Pre-vaccination	gr8-85	1153	32400	3050	<100	2946	<100	<100	<100	<100	<100
		gr8-86	642	3148	2163	<100	2189	<100	<100	<100	<100	<100
		gr8-87	<100	5275	2518	<100	318	<100	<100	<100	<100	<100
		gr8-88	916	4096	8767	<100	14467	<100	<100	<100	<100	<100
		gr8-89	<100	876	458	<100	237	<100	<100	<100	<100	<100
		gr8-90	1118	13626	6467	<100	3878	<100	<100	<100	<100	<100
		gr8-91	359	7692	2057	<100	1177	<100	<100	<100	<100	<100
		gr8-92	<100	<100	<100	<100	<100	<100	<100	<100	<100	<100
		gr8-93	318	10206	1901	<100	986	<100	<100	<100	<100	<100
		gr8-94	262	10215	8174	<100	2018	<100	<100	<100	<100	<100
		gr8-95	541	7452	4330	<100	421	<100	<100	<100	<100	<100
		Mosaic	Pre-vaccination with extended protocol	gr9-96	734	17145	12177	<100	11161	<100	<100	<100
gr9-97	5735			9538	15117	325	16932	1597	<100	<100	<100	<100
gr9-98	893			14221	5873	<100	1971	102	<100	<100	<100	175
gr9-99	2930			16848	17346	105	16792	538	<100	<100	<100	150
gr9-100	368			9481	2107	103	7567	773	<100	<100	<100	347
gr9-101	2493			3558	5499	109	4516	327	<100	<100	<100	175
gr9-102	<100			356	<100	<100	<100	<100	<100	<100	<100	116
gr9-103	1892			3059	4538	<100	1290	325	<100	<100	<100	<100
gr9-104	2429			2525	11769	<100	8682	310	<100	<100	<100	<100
gr9-105	1072			17207	11824	299	5399	316	<100	<100	<100	162
gr9-106	2760			5805	13599	339	10037	500	<100	<100	<100	<100
gr9-107	1417			10937	14162	175	1899					