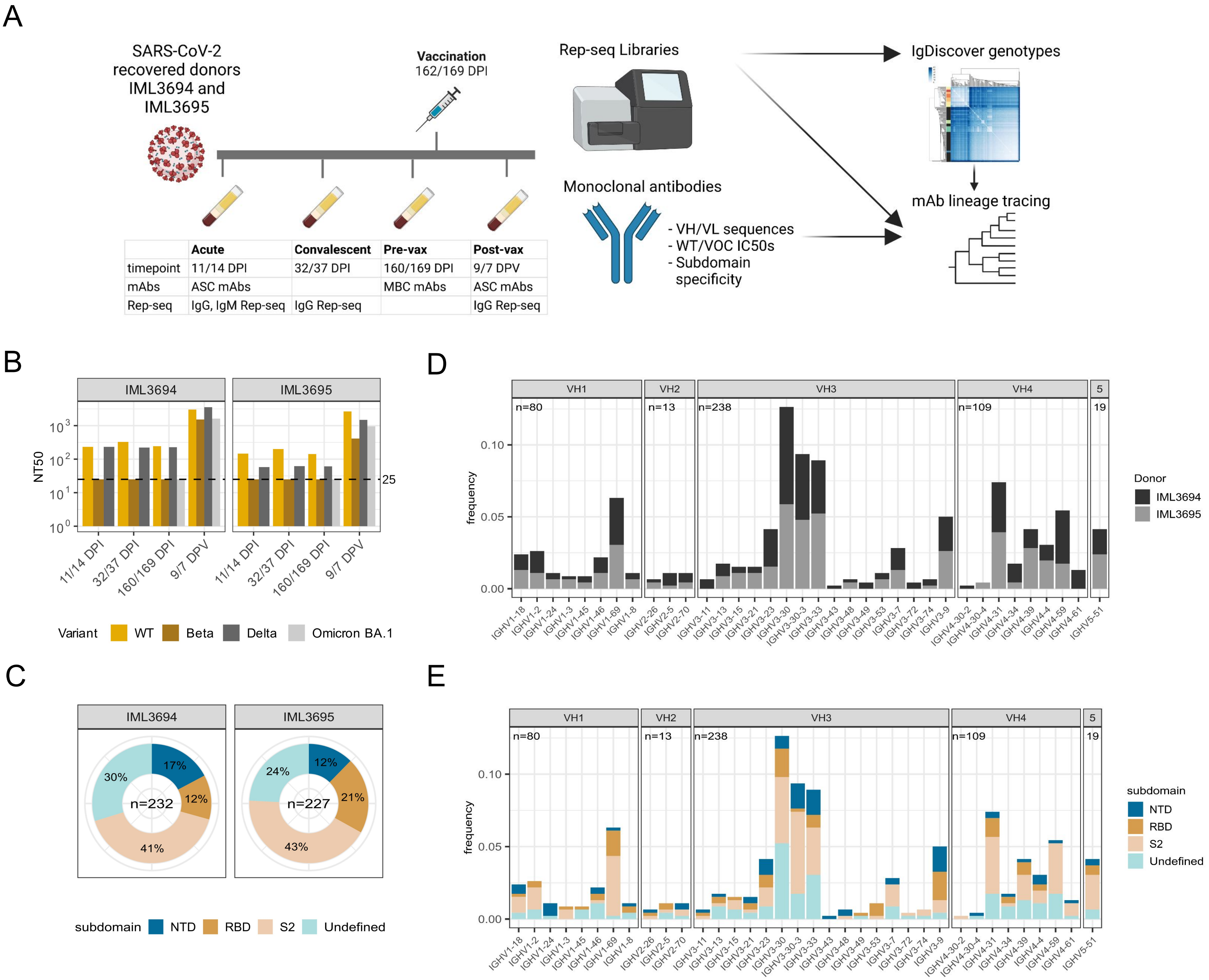


	Acute	Convalescent	Pre-vax	Post-vax
timepoint	11/14 DPI	32/37 DPI	160/169 DPI	9/7 DPV
mAbs	ASC mAbs		MBC mAbs	ASC mAbs
Rep-seq	IgG, IgM Rep-seq	IgG Rep-seq		IgG Rep-seq

FIG. 1 | Study design, serum neutralizing activity and properties of spike-binding monoclonal antibodies (mAbs). **(A)** Schematic of study design with time points for infection and vaccination, mAb isolation, and samples for NGS. **(B)** IML3694 and IML3695's serum neutralizing antibody values against ancestral SARS-CoV-2 Wuhan (WT) and variants of concern (VOCs). **(C)** Pie chart of mAb subdomain specificities in the two donors. **(D)** mAb IGHV allele frequencies colored by donor. **(E)** mAb IGHV allele frequencies colored by subdomain specificity.

Figure 1

Donor	mAb ID	VH gene	JH gene	HCDR3	% VH SHM nt	VL gene	JL gene	LCDR3	% VL SHM nt	WT IC50 (µg/ml)	Beta IC50 (µg/ml)	Delta IC50 (µg/ml)	Omicron BA.1 IC50 (µg/ml)	Omicron BA.2.75 IC50 (µg/ml)	Omicron BA.5 IC50 (µg/ml)
IML3694	ADI-67481	IGHV3-53*02	IGHJ4*02	ARDLDVMGGFDY	2	IGKV1-9*01	IGKJ1*01	QELNSYPRT	2.1	0.0589	>1	0.1227	N.D.	N.D.	N.D.
IML3694	ADI-67485	IGHV1-69*01	IGHJ2*01	ATGVRYCDTTSCRASYFDF	4.7	IGKV3-20*01	IGKJ2*01	QQYGSSPLT	1.7	0.0148	N.D.	0.2704	4.211	N.D.	N.D.
IML3694	ADI-67649	IGHV4-4*02	IGHJ4*02	ARDIRQDDCSTTRCPEY	5.1	IGKV3-11*01	IGKJ3*01	QQRSNWPPT	1.7	0.012	>1	0.0302	N.D.	N.D.	N.D.
IML3694	ADI-67652	IGHV1-2*02	IGHJ6*03	ARDRNWAIFGVESDV	5.7	IGLV2-23*02	IGLJ3*02	CSYADSSAWV	4.1	0.1698	>1	0.302	N.D.	N.D.	N.D.
IML3694	ADI-67657	IGHV3-23*01	IGHJ4*02	AKDRLRTSSLQPMPFFDY	3.7	IGKV4-1*01	IGKJ1*01	QQYYSAPYT	1.7	0.0007	N.D.	>1	2.139	N.D.	N.D.
IML3694	ADI-67679	IGHV3-53*02	IGHJ4*02	ARDLAGPGLFDH	5.8	IGKV1-33*01	IGKJ5*01	QQYDNVPSIT	3.2	0.0584	>1	0.0599	0.1204	N.D.	N.D.
IML3694	ADI-67680	IGHV3-33*01	IGHJ2*01	ARDGTIAVAGTFDRFFDL	3.4	IGKV1-33*01	IGKJ3*01	QQYHILPFT	3.9	0.1108	>1	0.2379	N.D.	N.D.	N.D.
IML3694	ADI-67722	IGHV4-34*01	IGHJ6*03	ARGVQIPEYCSMNNCPVSDHHYFYMDV	13.3	IGKV3-20*01	IGKJ1*01	QQYGGSRPWT	4.5	0.0282	0.2552	0.0122	N.D.	N.D.	N.D.
IML3694	ADI-67726	IGHV1-8*01	IGHJ6*03	ATKRVQVPRRYYYYMDL	3.1	IGKV2-28*01	IGKJ4*01	MQSLQTPLT	2	0.0258	N.D.	0.0235	N.D.	N.D.	N.D.
IML3694	ADI-67744	IGHV3-9*01	IGHJ6*02	AKGKAAGHSYYYGMDV	3.4	IGKV1-39*01	IGKJ1*01	QQSYVTPWT	3.9	0.0154	0.0272	0.0102	0.01366	0.007	>1
IML3694	ADI-67748	IGHV4-31*03	IGHJ4*02	ARSPVIYGTNSGFDY	5.7	IGKV5-2*01	IGKJ1*01	LQHDNFPYT	1.1	0.0082	0.0322	0.0431	4.405	N.D.	N.D.
IML3694	ADI-67757	IGHV1-8*01	IGHJ6*02	ARGNYFDGDGYVDY	4.1	IGKV3-15*01	IGKJ4*01	QQYNNWPLT	3.2	0.0216	N.D.	0.0302	N.D.	N.D.	N.D.
IML3694	ADI-67831	IGHV3-49*03	IGHJ4*02	TLTVTNRYYFHS	2.4	IGKV3-15*01	IGKJ2*03	QQYNNWFS	0.7	0.009	>1	0.8322	N.D.	N.D.	N.D.
IML3695	ADI-67119	IGHV3-53*02	IGHJ6*02	ARDSVRGGMDV	5.8	IGLV3-9*01	IGLJ2*01	QVWDTTSVI	8.6	0.065	0.6351	0.0808	N.D.	N.D.	N.D.
IML3695	ADI-67127	IGHV3-9*01	IGHJ3*02	AKDDYPSSWYEHHPQRWAFDI	3.7	IGLV1-51*01	IGLJ3*02	GTWDSSLSVV	0.3	0.0541	>1	>1	N.D.	N.D.	N.D.
IML3695	ADI-67135	IGHV3-9*01	IGHJ4*02	AKGGEYSSRWYLRESYFDY	5.1	IGKV2D-29*01	IGKJ4*01	MQSIQVPLT	1.7	0.0025	N.D.	>1	N.D.	N.D.	N.D.
IML3695	ADI-67138	IGHV4-39*01	IGHJ4*02	AVGGVRSLEWLLQFDY	6.1	IGLV2-8*01	IGLJ1*01	SSYAGSSSLV	2.7	0.0065	0.001	0.003	0.002276	0.003	>1
IML3695	ADI-67139	IGHV4-31*03	IGHJ4*02	ARDSDYGEYFDS	3.3	IGKV3-11*01	IGKJ5*01	QQRYNWPPIT	1.7	0.2125	>1	>1	N.D.	N.D.	N.D.
IML3695	ADI-67140	IGHV3-53*02	IGHJ3*02	AREAYAFDI	5.5	IGKV1-9*01	IGKJ4*01	QQLNSHPPA	2.1	0.012	>1	0.015	N.D.	N.D.	N.D.
IML3695	ADI-67183	IGHV4-39*01	IGHJ4*02	AVGGVRFFEWLLQFDY	3.7	IGLV2-8*01	IGLJ2*01	SSYAGSSSLI	3.7	0.0029	0.0027	0.0008	0.003034	0.005	>1
IML3695	ADI-67189	IGHV3-30*03	IGHJ4*02	ARDYGDYAAFDS	4.4	IGKV4-1*01	IGKJ4*01	QQYYSTPLT	1.3	0.2776	>1	>1	N.D.	N.D.	N.D.
IML3695	ADI-67218	IGHV4-34*01	IGHJ3*02	ARWDLLYPRDAFDI	3.1	IGLV2-11*01	IGLJ2*01	CSYAGSYVV	2.1	0.0657	>1	0.057	N.D.	N.D.	N.D.
IML3695	ADI-67852	IGHV3-9*01	IGHJ4*02	AKDIQFRDRDCTNGVCSVGGFDY	5.7	IGKV3-15*01	IGKJ2*01	QQYNKWPPRT	1.7	0.2345	>1	>1	1.103	N.D.	N.D.
IML3695	ADI-67857	IGHV4-39*01	IGHJ4*02	AVGGVRFLEWLLQFDY	2.4	IGLV2-8*01	IGLJ1*01	SSYAGSSNLV	1.7	0.0027	0.0727	0.0159	0.01917	0.089	>1
IML3695	ADI-67935	IGHV4-31*03	IGHJ4*02	ARGPYASGSFDS	5	IGKV3-20*01	IGKJ1*01	HHYGSSGDT	2.1	0.048	N.D.	0.039	N.D.	N.D.	N.D.
IML3695	ADI-67971	IGHV3-9*01	IGHJ3*02	AKLGGAN.D.YDFRSGPTAFDI	4.4	IGLV1-44*01	IGLJ2*01	ATWDDSLNGVVV	2	0.1363	0.3985	>1	0.1333	N.D.	N.D.
IML3695	ADI-67977	IGHV3-9*01	IGHJ6*02	VKDMSVGDRSVEYYGMDV	2	IGLV3-21*04	IGLJ2*01	QVWDSSSENVV	2.1	0.0759	0.2904	0.4427	0.02833	N.D.	N.D.
IML3695	ADI-67981	IGHV1-69*01	IGHJ3*02	ARDGRHNYDSTGYYHN.D.FDI	5.7	IGKV1-39*01	IGKJ1*01	QQSYSSRT	3.2	0.0094	>1	0.0117	0.04438	N.D.	N.D.
IML3695	ADI-67983	IGHV3-9*01	IGHJ4*02	AKGKWPSSPSFLTDY	5	IGKV1-27*01	IGKJ4*01	QKYNSVPLT	3.5	0.0031	>1	0.0049	N.D.	N.D.	N.D.
IML3695	ADI-67994	IGHV3-23*01	IGHJ6*02	ARDLGGYSYGLNFFYAMDV	3.8	IGLV2-14*03	IGLJ2*01	SSYTSSSTFVL	2	0.1967	0.3379	0.227	0.3084	N.D.	N.D.
IML3695	ADI-67999	IGHV1-69*01	IGHJ4*02	ARERGYSGYGASLYFDY	5.1	IGLV1-40*01	IGLJ1*01	QSYDSSLSGAV	1.7	0.5019	0.6414	>1	0.1381	N.D.	N.D.

FIG. 2 | A summary of all neutralizing mAbs with an IC_{50} below 0.4 mg/ml against any of the SARS-CoV-2 VOCs.

Figure 2

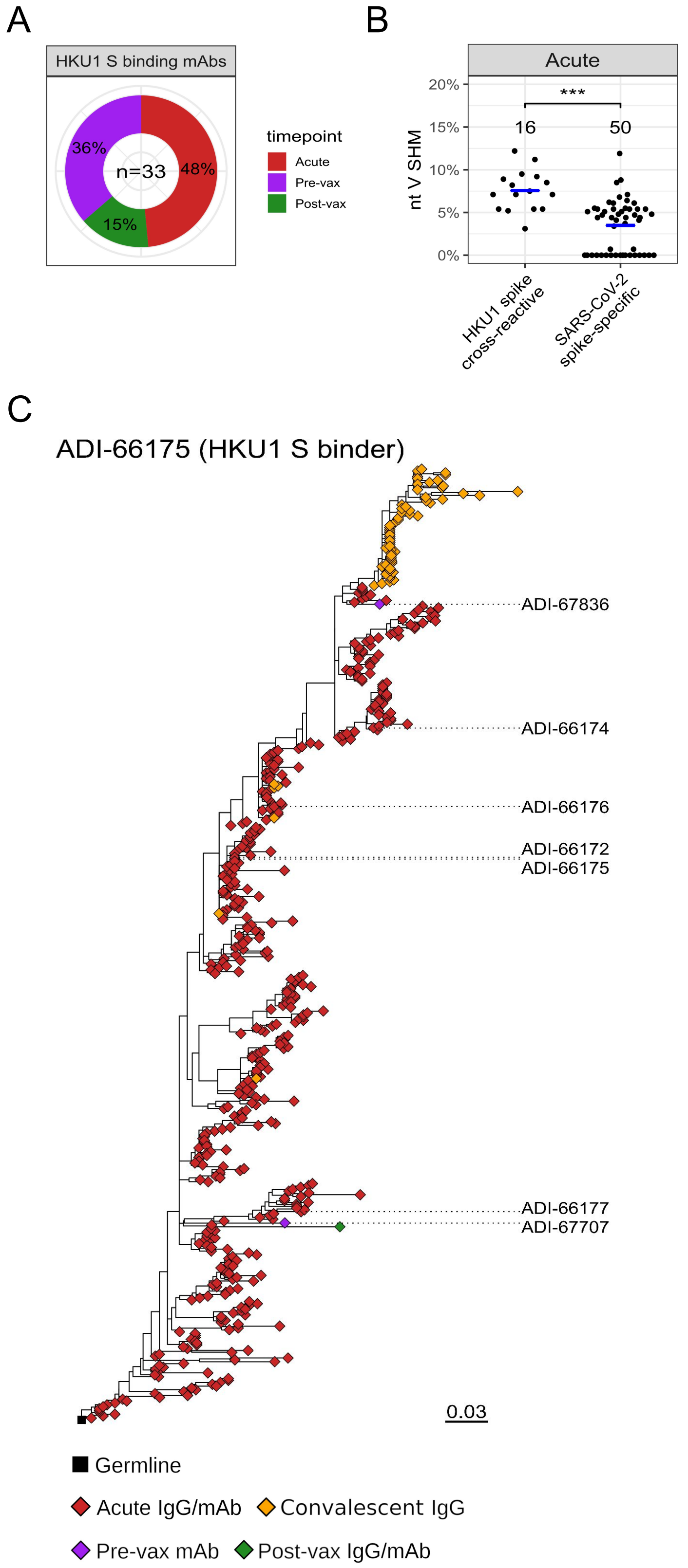


FIG. 3 | Isolation time point and evolution of HCoV-HKU1 S cross-reactive mAbs. **(A)** Pie chart of timepoints in which HCoV-HKU1 S-binding mAbs were isolated. **(B)** Dot plot of % nucleotide IGHV SHM for the HCoV-HKU1 S cross-reactive and SARS-CoV-2 S-specific mAbs isolated at the acute timepoint. Mann-Whitney U test used for comparison. **(C)** Maximum likelihood phylogenetic trees of HCoV-HKU1 S-binding traced lineage ADI-66175. The germline sequence was obtained from the IgBLAST generated “germline_alignment” column of the sequence with the minimum IGHV SHM in the lineage.

Figure 3

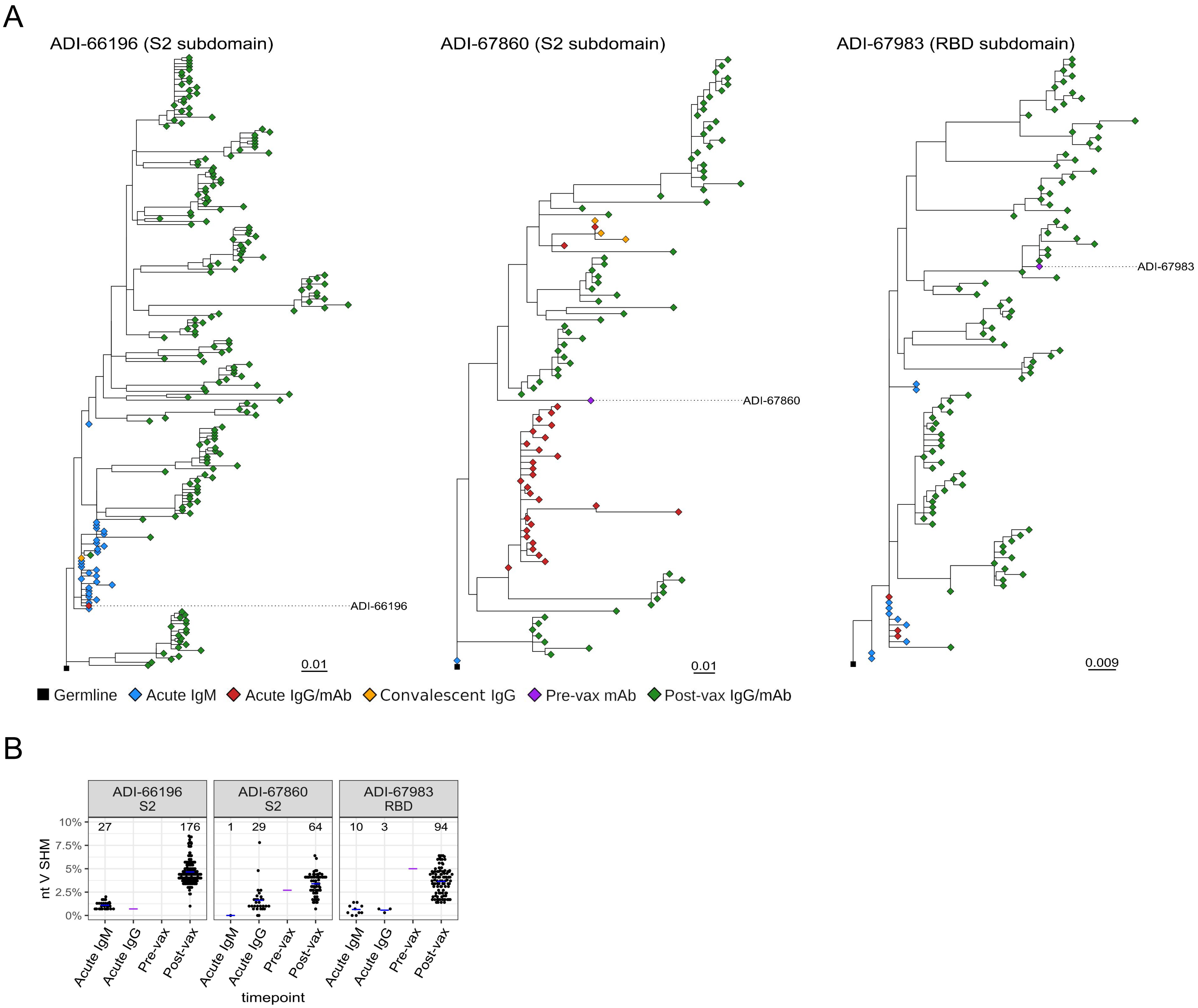


FIG. 4 | Evolution of SARS-CoV-2 S-specific antibody lineages over the sampling time points. **(A)** Maximum likelihood phylogenetic trees of traced lineages containing IgM Rep-seq sequences: ADI-66196, ADI-67860, and ADI-67983. Germlines sequences were obtained from the IgBLAST generated "germline_alignment" column of the sequence with the smallest IGHV SHM in the lineage. **(B)** Dot plot of % nucleotide IGHV SHM for the ADI-66196, ADI-67860, and ADI-67983 lineage sequences. Purple crossbars represent mAb values, while the blue crossbars represent average values of traced variants for each timepoint.

Figure 4

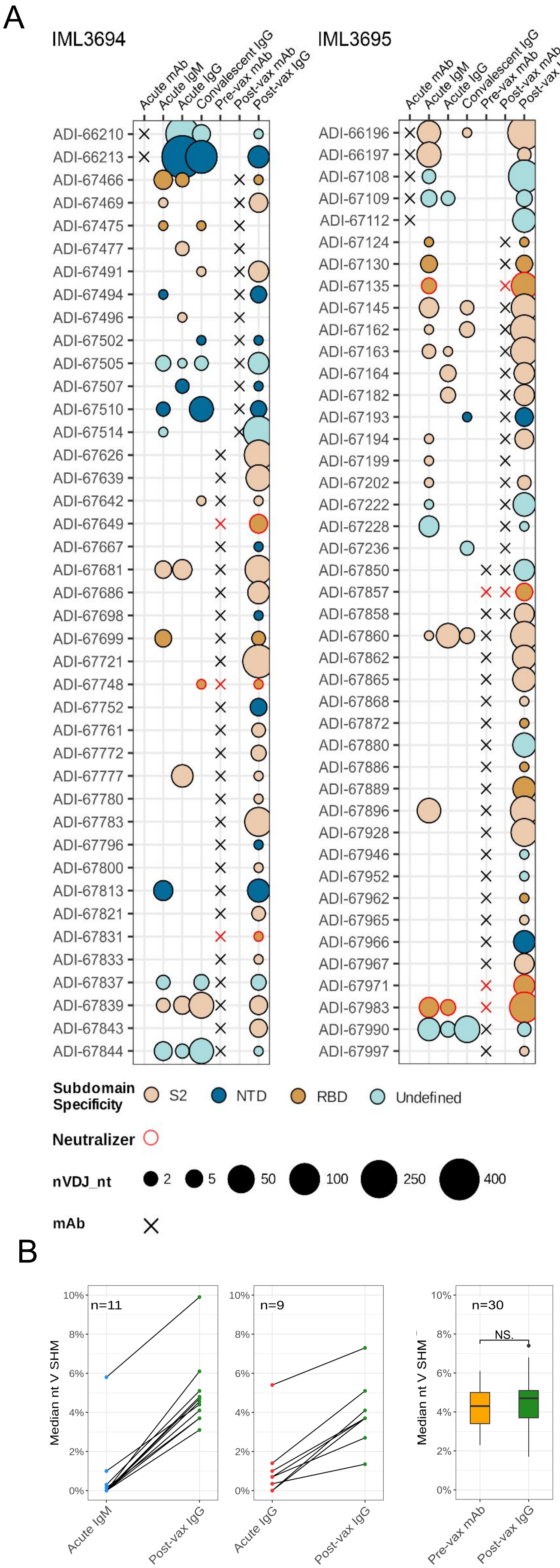
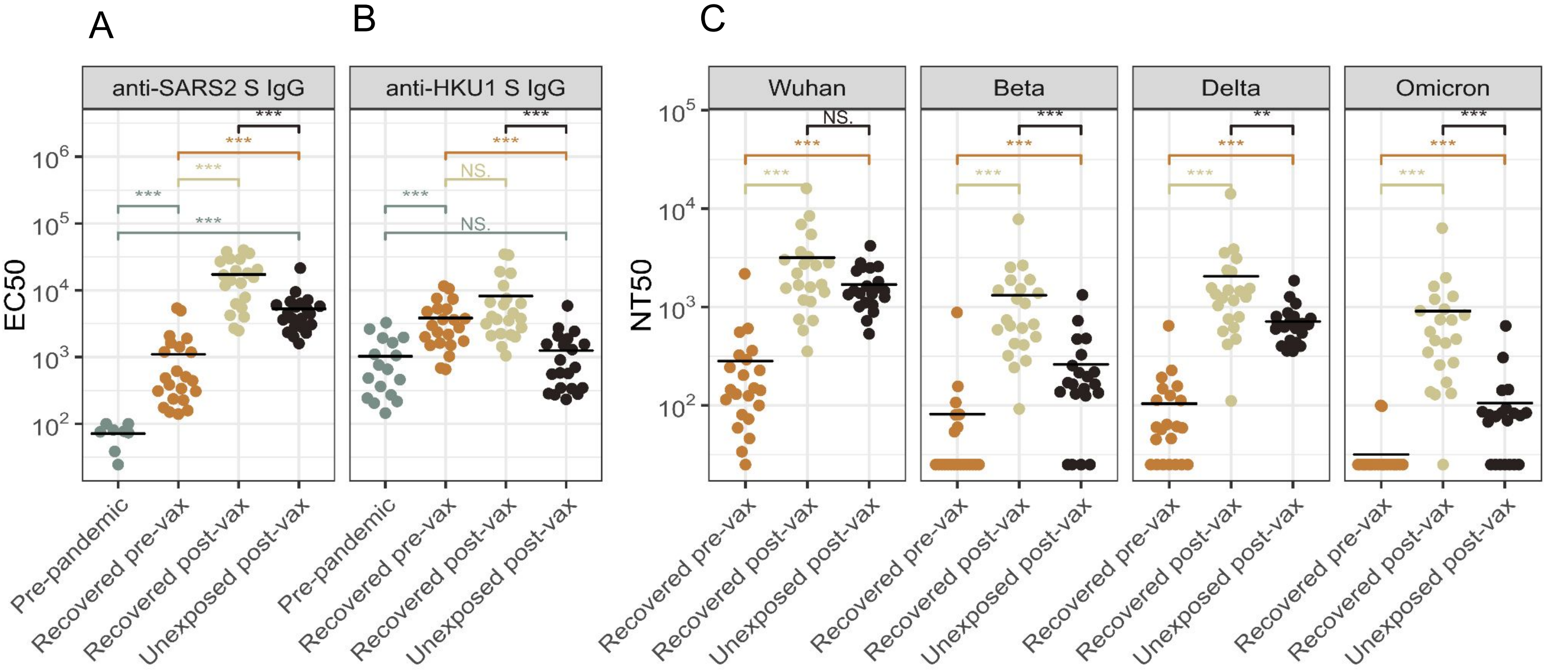


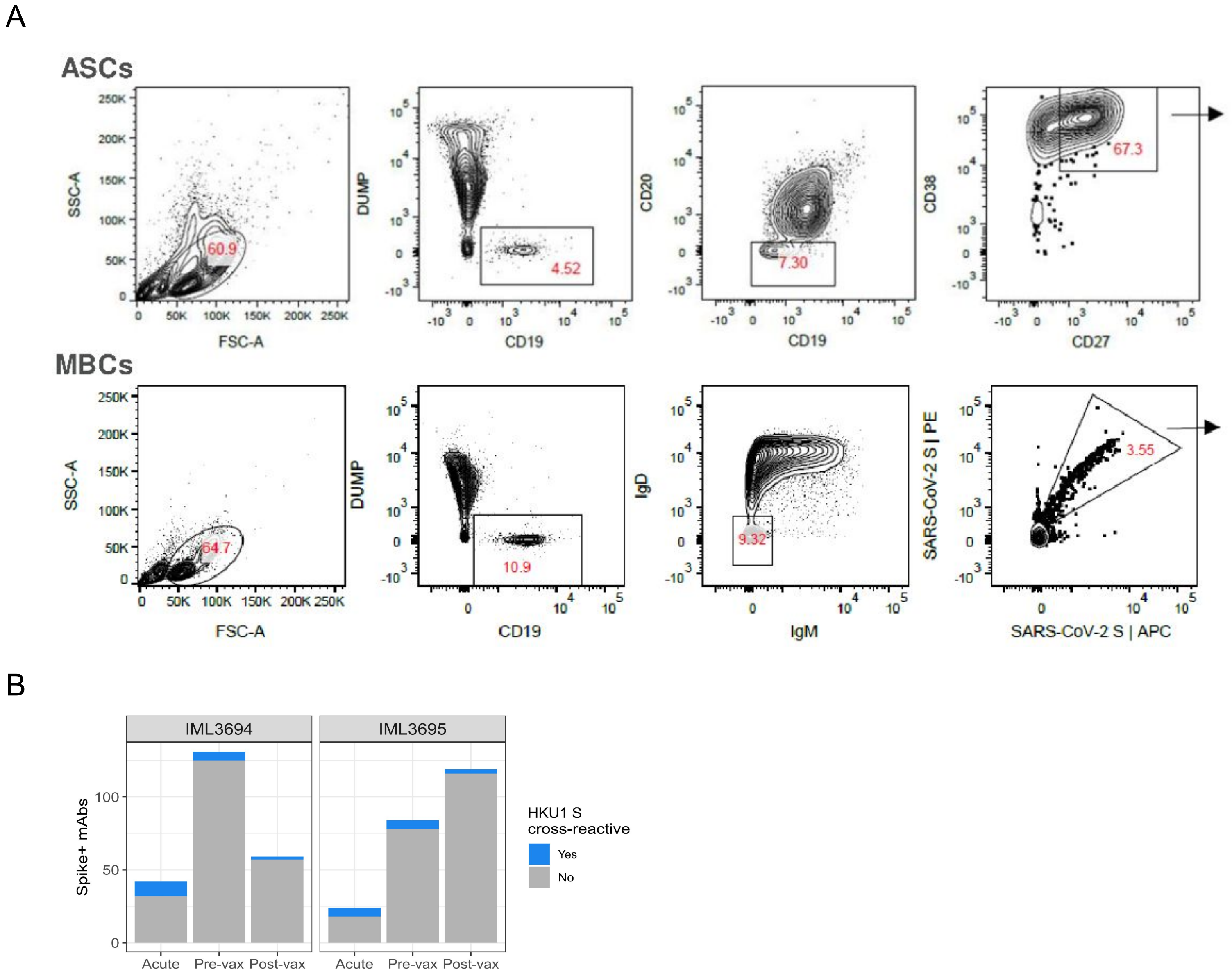
FIG. 5 | IgM and IgG repertoire sequencing and lineage tracing of SARS-CoV-2 S-specific antibody lineages at the longitudinal time points. **(A)** Bubble plots of SARS-CoV-2 S-specific mAb lineages found before and after vaccination in IML3694 and IML3695. The bubble sizes correspond to post-FAD and chimera cleaned data. **(B)** Line plots of median % nucleotide IGHV SHM for lineages found in acute IgM/IgG and post-vax IgG Rep-seq data. **(C)** Boxplot of median % nucleotide IGHV SHM for lineages containing pre-vax timepoint mAbs and post-vax IgG Rep-seq. Wilcoxon signed-rank test used for comparison.

Figure 5



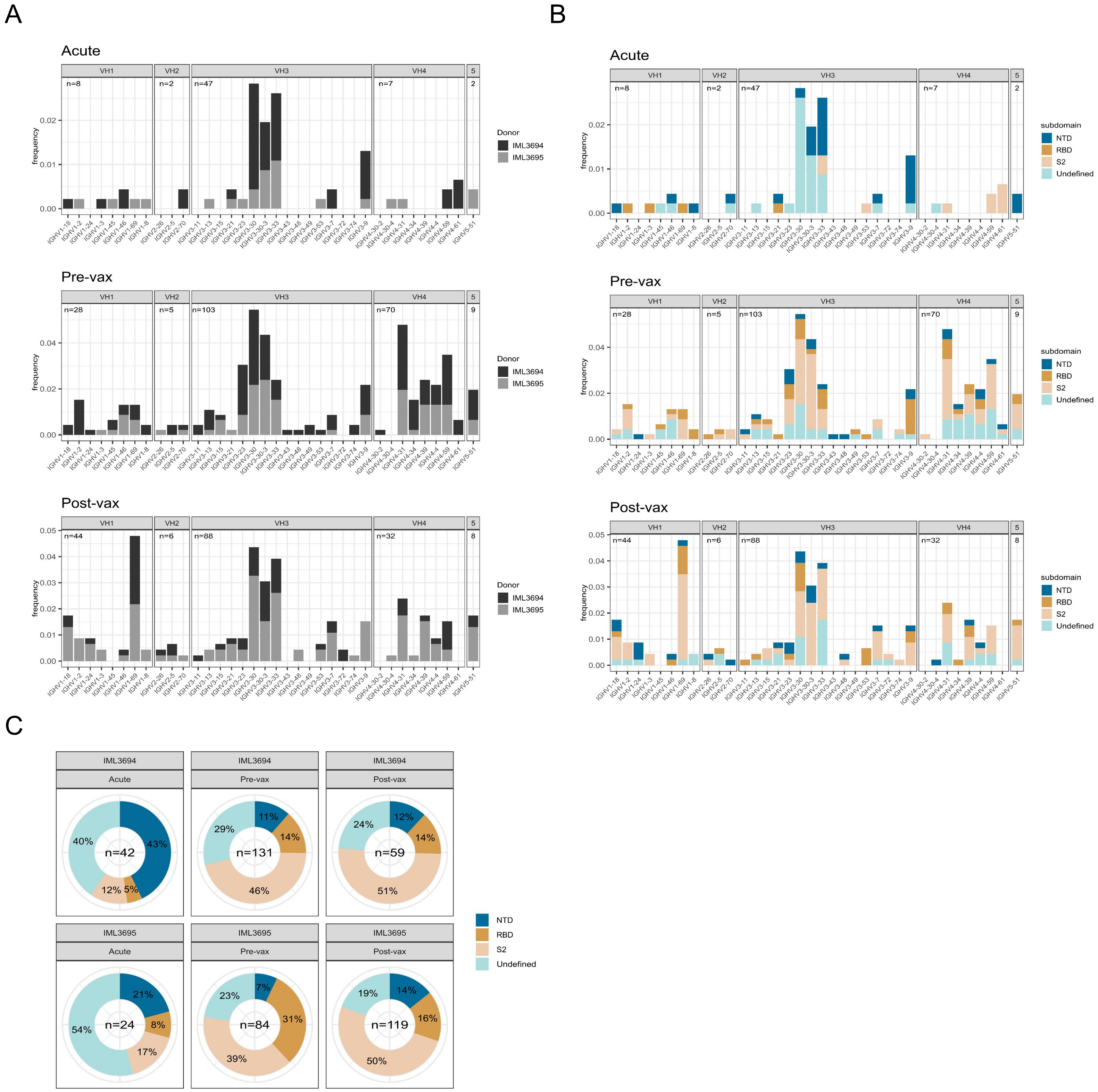
Supplementary Fig. 1 | Serum IgG titers against the SARS-CoV-2 **(A)** and HCoV-HKU1 S glycoproteins **(B)** as well as neutralizing antibody titers against SARS-CoV-2 WT and variants **(C)** in a cohort of individuals a median of 8 days (range: 7-14) days after vaccination by mRNA-1273 (n = 22) or BNT162b2 (n = 21), 22 of whom were previously infected with SARS-CoV-2 (Recovered) and given one dose and 21 who were not previously infected (Unexposed) and given two doses. The previously infected individuals were vaccinated a median of 179 days after disease onset (range: 40-213 days). For these individuals, we also included serum samples collected a median of 42 (range 0-176 days) days before the mRNA vaccination. We used Mann-Whitney U-tests to compare titers. *P < 0.05, **P < 0.01, ***P < 0.001, and ****P < 0.0001.

Supplemental Figure 1



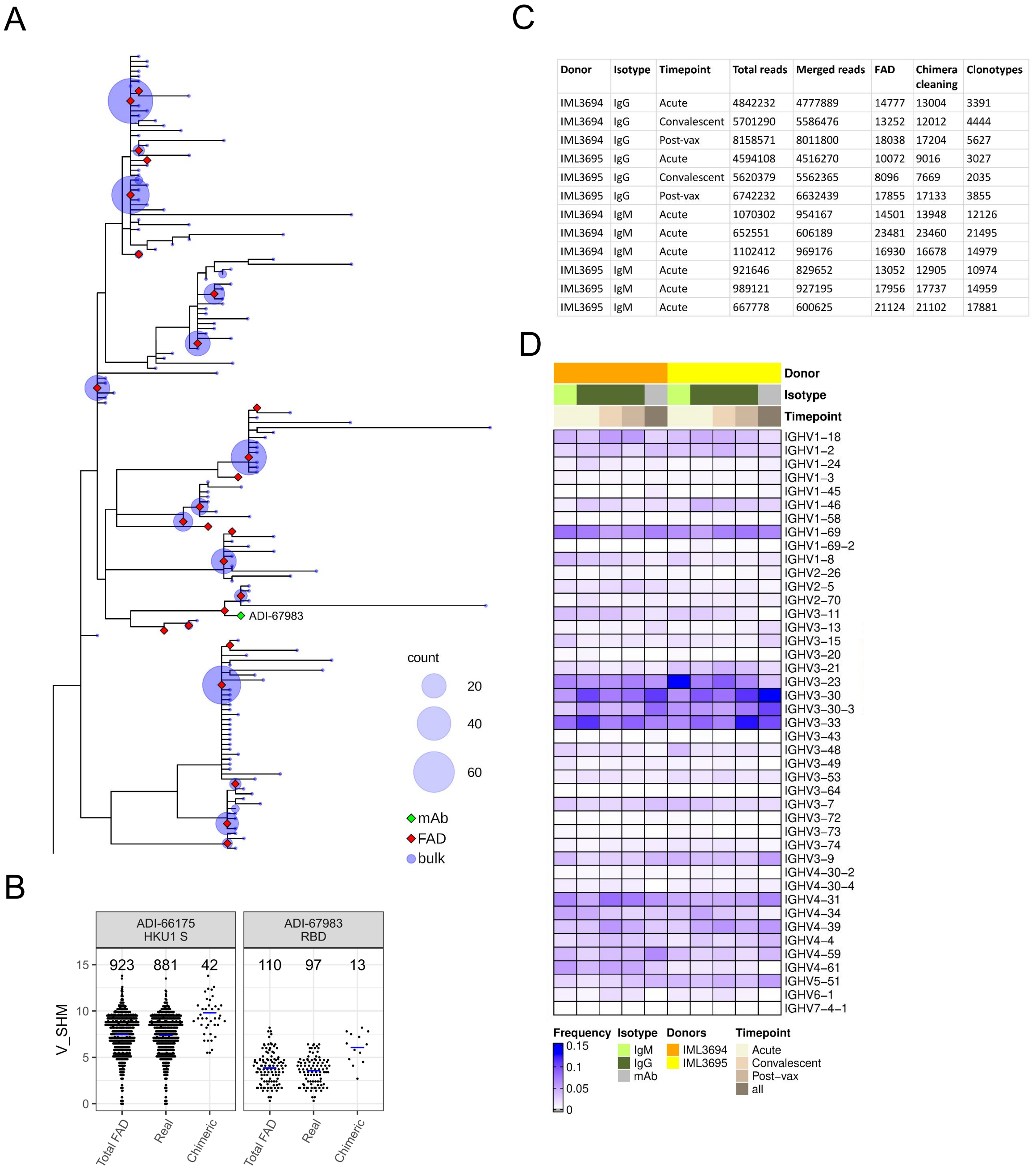
Supplementary Fig. 2 | (A) Flow cytometry plots showing the staining and gating strategy for sorting ASCs and spike-specific MBCs. **(B)** Number of HCoV-HKU1 and SARS-CoV-2 S cross-reactive mAbs versus SARS-CoV-2 S-specific mAbs isolated at the different time points.

Supplemental Figure 2



Supplementary Fig. 3 | (A) mAb IGHV allele frequencies colored by donor shown separately for the three sets of mAbs isolated at the different time points. **(B)** mAb IGHV allele frequencies colored by subdomain specificity shown separately for the three sets of mAbs isolated at the different time points. **(C)** Pie charts of mAb subdomain frequencies shown separately for the three sets of mAbs isolated at the different time points.

Supplemental Figure 3



Donor	Isotype	Timepoint	Total reads	Merged reads	FAD	Chimera cleaning	Clonotypes
IML3694	IgG	Acute	4842232	4777889	14777	13004	3391
IML3694	IgG	Convalescent	5701290	5586476	13252	12012	4444
IML3694	IgG	Post-vax	8158571	8011800	18038	17204	5627
IML3695	IgG	Acute	4594108	4516270	10072	9016	3027
IML3695	IgG	Convalescent	5620379	5562365	8096	7669	2035
IML3695	IgG	Post-vax	6742232	6632439	17855	17133	3855
IML3694	IgM	Acute	1070302	954167	14501	13948	12126
IML3694	IgM	Acute	652551	606189	23481	23460	21495
IML3694	IgM	Acute	1102412	969176	16930	16678	14979
IML3695	IgM	Acute	921646	829652	13052	12905	10974
IML3695	IgM	Acute	989121	927195	17956	17737	14959
IML3695	IgM	Acute	667778	600625	21124	21102	17881

Supplementary Fig. 4 | (A) Maximum likelihood phylogenetic tree of IgM NGS, IgG NGS, and mAb sequences belonging to the same lineage as the ADI-67983 mAb. Sequences which remained after Fast Amplicon Denoising are marked in red. The tree is rooted on an inferred germline sequence obtained from the IgBLAST generated “germline_alignment” column of the sequence with the smallest IGHV SHM in the lineage. **(B)** IGHV SHM nucleotide percentage dot plots for the ADI-66175 and ADI-67983 clones IgG Rep-seq sequences, demonstrating the difference between SHM distributions with and without chimera removal. Blue crossbars are NGS data averages. **(C)** Sequence count data at each processing step. **(D)** Heatmap of clonally collapsed IGHV gene frequencies of bulk libraries and mAbs.

Supplemental Figure 4