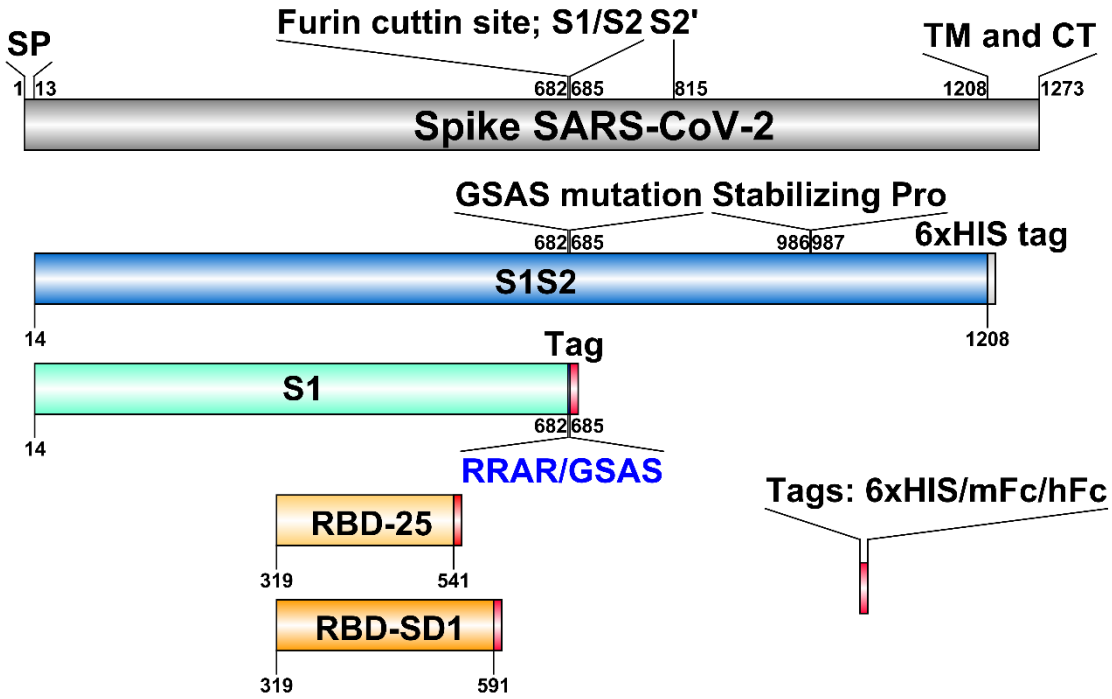
Supplementary Information

SARS-CoV-2 neutralizing human recombinant antibodies selected from pre-pandemic healthy donors binding at RBD-ACE2 interface

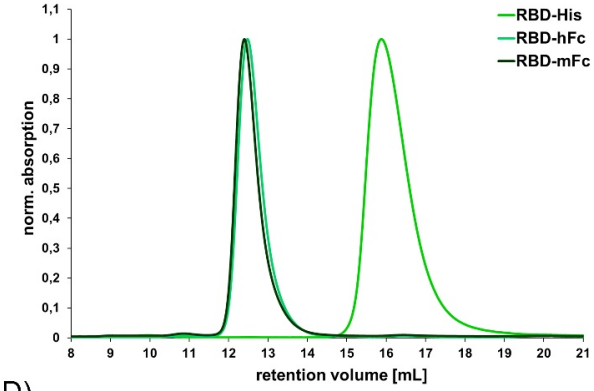
Bertoglio *et al.*

Supplementary Figure 1 Schematic overview of the expressed variants of the Spike SARS-CoV-2 protein.

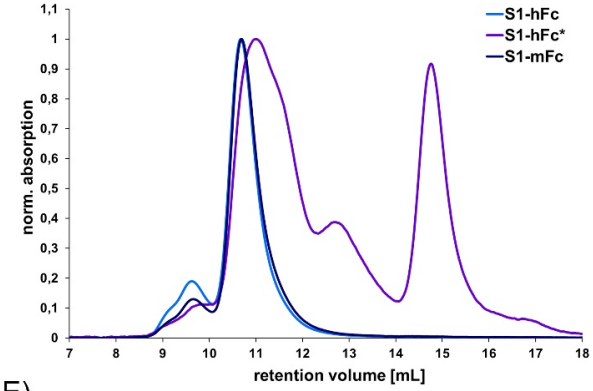


Supplementary Figure 2 SEC data of antigen produced in insect High Five cells (A-C) and mammalian Expi293F cells (D-E). The table (F) indicates the most likely conformation of the protein due to the retention volume of the peaks with the corresponding area in percentage. (*Furin site is present). Data shows a representative of two measurements.

A)



B)



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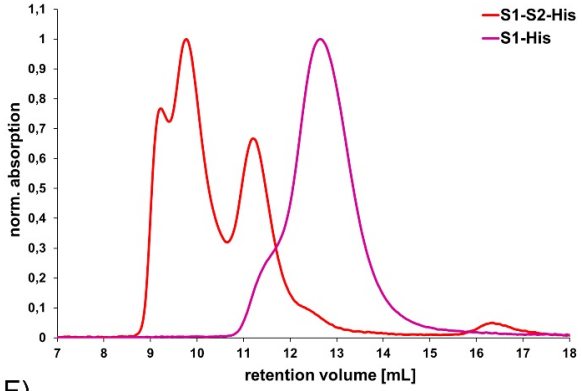
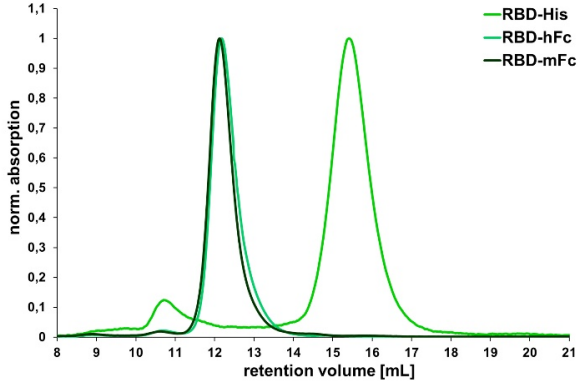
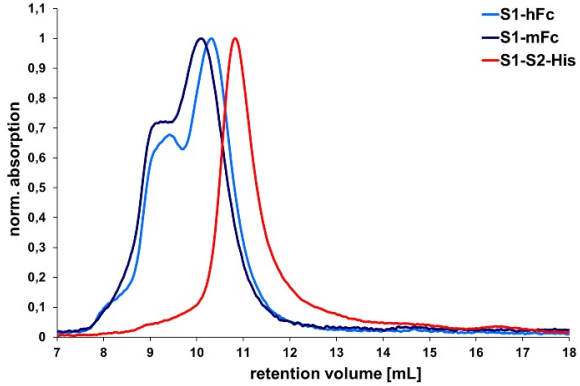


D)



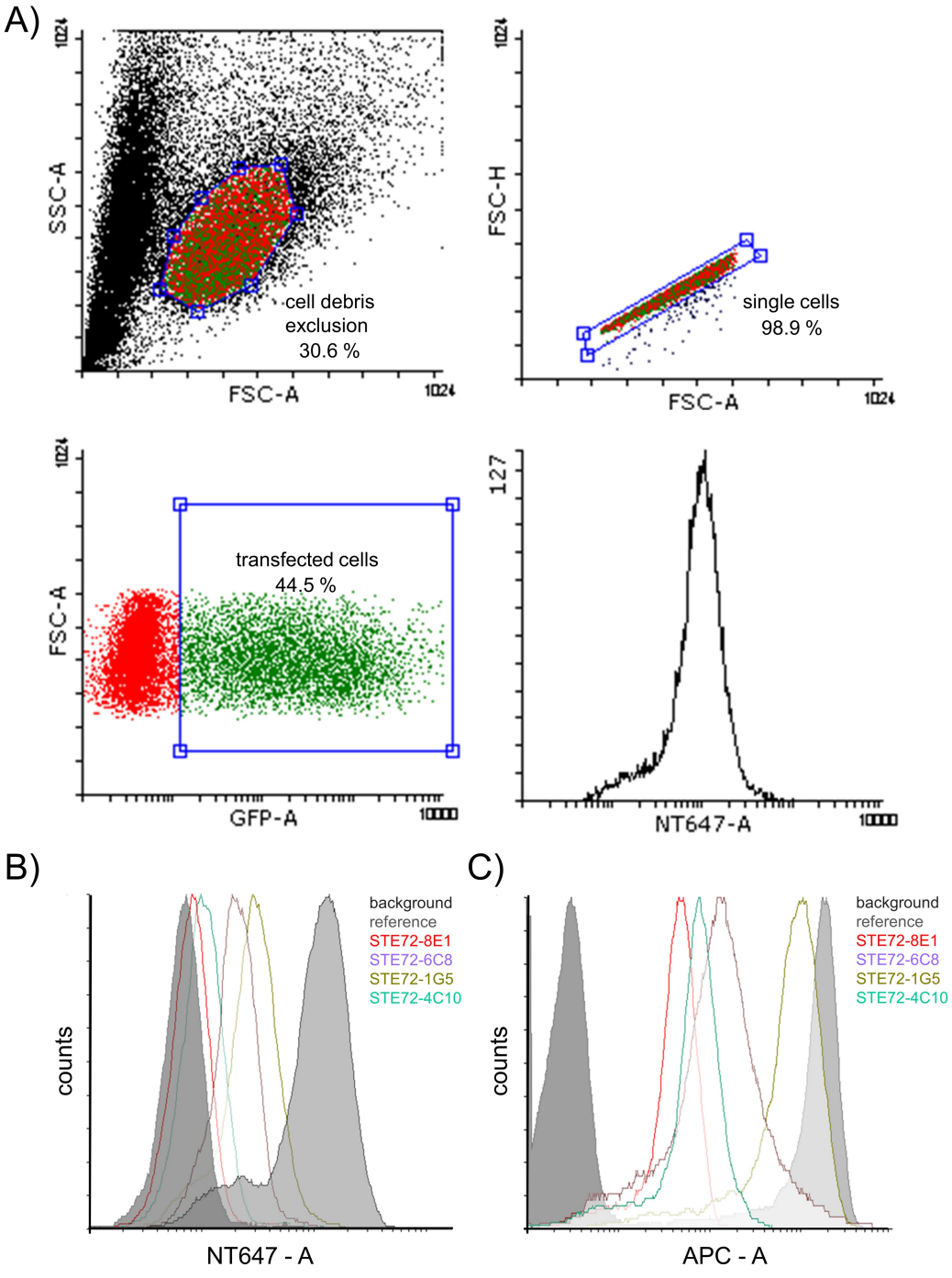
E)



F)

Antigen	Main Peak	High Five cells	Vol [mL]	Area [%]	Expi293F cells	Vol [mL]	Area [%]
RBD-His	Monomer		15.9	99		15.4	93
RBD-hFc	Dimer		12.5	100		12.2	98
RBD-mFc	Dimer		12.4	99		12.1	99
S1-His	Monomer		12.7	100		nd	nd
S1-hFc	Aggregate?		9.6	16		9.3	31
	Dimer		10.7	84		10.3	68
S1-hFc*	Dimer		10.9	58		nd	nd
	S1-hFc-hFc		12.8	9		nd	nd
	hFc Dimer		14.8	33		nd	nd
S1-mFc	Aggregate?		9.7	11		9.1	27
	Dimer		10.7	89		10.1	73
S1-S2-His	Aggregate?		9.2	23		-	-
	Trimer		9.8	50		-	-
	Monomer		11.2	27		10.8	100

Supplementary Figure 3 Flow cytometry inhibition analysis A) Gating strategy B) Analysis of spike S1-S2 trimer (50 nM in relation to monomer) binding to living cells expressing ACE2 blocked by 1500 nM antibodies and C) of RBD-mFc (10 nM in relation to monomer) binding to living cells expressing ACE2 blocked by 100 nM antibody. The antibodies STE72-1G5, STE72-4C10, STE72-8E1 and STE73-6C8 were used as example. The background control are transfected ACE2 cells and the reference are ACE2 positive cells incubated with labeled spike protein. Data shows a representative of the flow cytometry inhibition experiments.

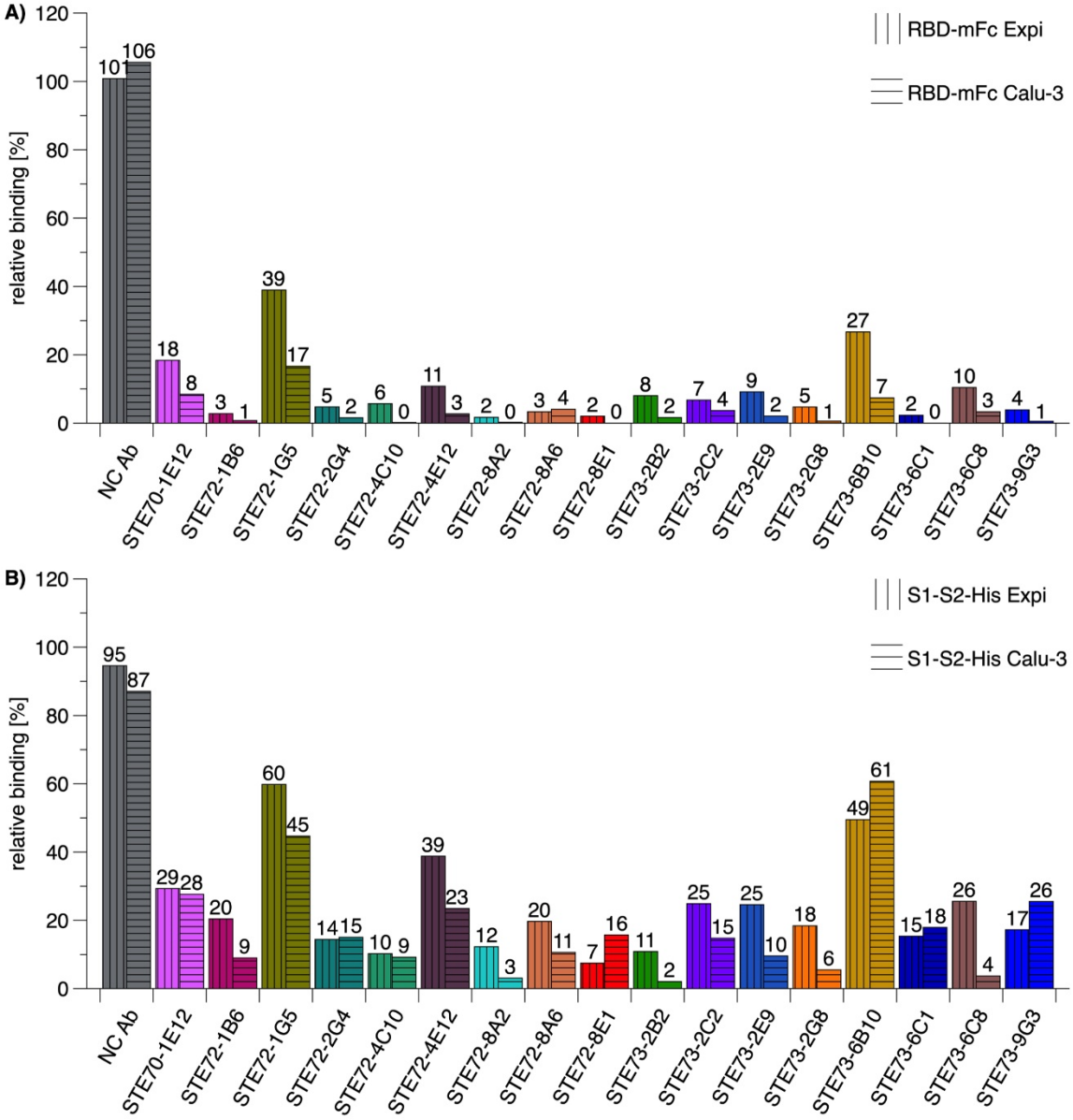


Supplementary Figure 4 VH and VL V-genes of 17 inbiting and neutralizing scFv-Fc. The CDRs are given according to the IMGT nomenclature (http://www.imgt.org/IMGTScientificChart/Numbering/IMGTIGVLsuperfamily.html) using VBASE2 (www.vbase2.org).

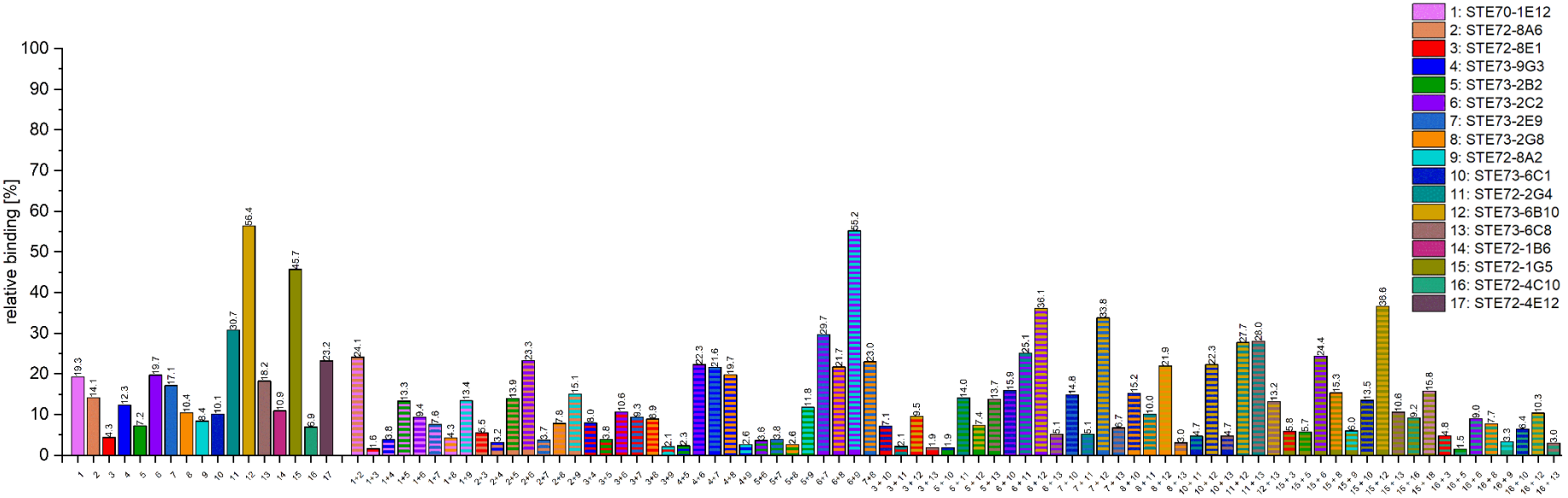
Antibody name	VH amino acid sequence CDR1, *CDR2*, CDR3	VL amino acid sequence CDR1, *CDR2*, CDR3
STE70-1E12	QMQLVQSGAEVKKPGASVKVSCKAS**GYTFTGYY**MHWVRQAP GQGLEWMGR***INPNSGGT***NYAQKFQGRVTMTRDTSTSTAYME LSRLRSDDTAVYYC**VRDGYNFNNWFDP**WGQGTLVTVSS	NFMLTQPHSVSGSPGETITISCTGS**GGSIASNY**VQWYRQRP GSAPTIVIY***EDT***QRPSGVPDRFSGSIDSSSNSASLTISGLK TEDEADYYC**QSSESTNHVM**FGGGSKLTVL
STE72-1B6	QVKLVQSGGGLVQPGGSLRLSCAAS**GFTFSSYA**MSWVRQAP GKGLEWVSG***ISGSGGST***YYADSVKGRFTISRDNSKNTLYLQ VNSLRAEDTAVYYC**AKAPYGDFRGLWYFDY**WGQGTLVTVSS	DIVMTQSPFSVSASVGDRVTISCRAS**QDIRSW**LAWYQQKPG KAPQLLIY***DAS***SLQSGVPSRFSGSGSGTDFTLTISSLQPED FATYYC**QQADSVPLT**FGGGTKVEIQ
STE72-1G5	QVQLVQSGAEVKKPGASVKVSCKAS**GGTFSSYA**ISWVRQAP GQGLEWMGG***IIPIFGTA***NYAQKFQGRVTITADESTSTAYME LSSLRSEDTAVYYC**AVGGVQLWLTY**WGQGTLVTVSS	ETTLTQSPGALSLSPGERATLSCRVS**QSLSSNS**LAWYQQKP GQAPRLLIY***DTS***IRATGIPDRFSGSGSGTDFTLTISRLEPE DFAVYYC**HKYGSWPRT**FGQGTKVEV
STE72-4C10	EVQLQESGGGVVQPGRSLRLSCAAS**GFTFSSYA**MHWVRQAP GKGLEWVAV***ISYDGSNK***YYADSVKGRFTISRDNSKNTLYLQ MNSLRAEDTAVYYC**AREYSSSWYGLGAFDI**WGQGTMVTVSS	DIRLTQSPSSLSASVGDRVTITCRAS**QNIHYY**LNWYQQKPG KAPSLLVY***GAS***SLQSGVPSRFSGSGSGTEYTLTISSLQPED FATYFC**QQSFKSPAT**FGQGTKVDIK
STE72-4E12	QVQLVQSGAEVKKPGASVKVSCKAS**GYTFTSYY**MHWVRQAP GQGLEWMGI***INPSGGST***SYAQKFQGRVTMTRDTSTSTVYME LSSLRSEDTAVYYC**ARDLSGGLDY**WGQGTLVTVSS	ETTLTQSPATLSVSPGERVTLACRAG**QTISSK**LAWYRQKPG QAPSLLIY***DAS***TRATGVPARFSGSGSGTEFTLTISSLQSED FAVYYC**QQYSDWPPT**FGQGTKVEIK
STE72-8A2	QVQLVQSGAEVKKPGASVKVSCKAS**GYTFTSYG**ISWVRQAP GQGLEWMGW***ISAYNGNT***NYAQKLQGRVTMTTDTSTSTAYME LRSLRSDDTAVYYC**ARFFYDSSGYSTDY**WGQGTLVTVSS	DIRLTQSPSSLSASVGDRVTITCQAS**QDISNY**LNWYQQKPG KAPKLLIY***DAS***NLETGVPSRFSGSGSGTDFTFTISSLQPED IATYYC**QQYDNLPRT**FGGGTKVEIK
STE72-8A6	QVQLVQSGAEVKKPGASVKVSCKAS**GYTFTSYG**ISWVRQAP GQGLEWMGW***ISAYNGNT***NYAQKLQGRVTMTTDTSTSTAYME LRSLRSDDTAVYYC**ARFFYDSSGYSTDY**WGQGTLVTVSS	DIVMTQSPSTLSASVGDRVTISCRAS**QNIGTW**LAWYQQKPG KAPKLLIS***KAS***SLESGVPSRFSGSGSGTDFTLTISSLQPDD FATYYC**QQYDSYLFT**FGQGTKLEIR
STE72-8E1	QVQLQEWGPGLVQPSETLSLTCTVS**GGSISSGGYY**WSWIRQ HPGKGLEWIGR***IYPSGST***NYNPSLKSRVTMSVDTSENQFSL KLSSVTAADTAVYYC**ARGGPKRSGSPFDV**WGQGTMVTVSS	EVVMTQSPSTLSASVGDRVTITCRAS**QTFGPW**LAWYQQKPG QAPKLLIY***MAS***ELESGVPSRFSGSGSGTEFTLSISSLQPDD FATYYC**QQYNTFSPWT**FGQGTKVDIK
STE72-2G4	QVQLVQSGAEVKKPGASVKVSCKAS**GYTFTSYG**ISWVRQAP GQGLEWMGW***ISAYNGNT***NYAQKLQGRVTMTTDTSTSTAYME LRSLRSDDTAVYYC**ARFFYDSSGYSTDY**WGQGTLVTVSS	ETTLTQSPAFMSVTPGDRAILSCRAS**QSVDSH**LAWYHQKPG QPPRLLIY***GAS***TRATGIPARFSGSRSGTEFTLTISGLQSED LGVYYC**QQYASWPPGYN**FGQGTKLEIK

STE73-2B2	QVQLVQSGAEVKKPGASVKVSCKAS**GYTFTGYY**MHWVRQAP GQGLEWMGW***INPNSGGT***NYAQKFQGRVTITRDTSASTAYME LSSLRSEDTAVYYC**ARVSGWYFGAFDI**WGQGTMVTVSS	LPVLTQPHSLSESPGKTVNISCTGS**SGSIASNY**VQWYQQRP GSAPTTVIY***EDS***HRPSGVPDRFSGSIDSSSNSASLSISGLK TEDEADYYC**QSYDSSNNQWV**FGGGTKLTVL
STE73-2C2	QVQLVESGGGLVQPGGSLRLSCAAS**GITVSSNY**MSWVRQAP GKGLEWVSV***IYSGGST***FYADSVKGRFTISRDNSKNTLYLQM NSLRAEDTGVYYC**ARGHDNLDY**WGQGTLVTVSS	LPVLTQPHSVSESPGKTVIISCTGS**SDSIASNY**VQWYQQRP GSAPTTVIY***EDN***KRPSGVPDRFSGSTDSSSNSASLTISGLK TEDEAEYYC**QSYDSNNHWV**FGGGTKLTVL
STE73-2E9	QVQLVQSGAEVKKPGASVKVSCKAS**GYTFTSYG**ISWVRQAP GQGLEWMGW***ISAYNGNT***NYAQKLQGRVTMTTDTSTSTAYME LRSLRSDDTAVYYC**ARGKFDY**WGQGTLVTVSS	QSVLTQPPSVSEAPSQRVTISCSGS**SSNIGNNA**VNWYQQLP GKAPKLLIY***YDD***VLPSGVSDRFSGSKSGTSASLAISGLQSG DEADYYC**AAWDDSLSGVV**FGGGTKLTVL
STE73-2G8	QVQLVQSGGGLVKPGGSLRLSCAAS**GITVSSNY**MSWVRQAP GKGLEWVSI***LYSGGTT***FYTDSVKGRFTISRDNSKNTLHLQM NSLRAEDTAVYYC**ARWSGTYYDY**WGQGTLVTVSS	SSELTQDPAVSVALGQTVRITCQGD**SLRSYY**ASWYQQKPGQ APVLVIY***GKN***NRPSGIPDRFSGSSSGNTASLTITGAQAEDE ADYYC**NSRDSTNDPVL**FGAGTKLTVL
STE73-6B10	QVQLVQSGAEVKKPGASVKVSCKAS**GYTFTSYG**ISWVRQAP GQGLEWMGW***INPNSGGT***NYAQKFQGRVTMTRDTSISTAYME LSRLRSDDTAVYYC**ARDRLRYGDSGSYYYYGMDV**WGQGTTV TVSS	QSALTQPRSVSGSPGQSVTISCTGT**SSDVGGYNY**VSWYQQH PGKAPKLLIS***EVT***RRPSGVPDRFSGSKSGNTASLTVSGLQT EDEADYYC**GSYAGSNTWV**FGGGTKLTVL
STE73-6C1	QVQLVQSGGGVVQPGRSLRLSCAAS**GFTFSSYA**MHWVRQAP GKGLEWVAV***ISYDGSNK***YYADSVKGRFTISRDNSKNTLYLQ MNSLRAEDTAVYYC**ARSYVGGMDV**WGQGTTVTVSS	QLVLTQSPSVSGAPGQSVTISCTGT**DSNIGAGYN**VHWYQQL PGAAPKLIIS***YTD***NRPSGVPDRFSGSKSGTSASLAITGLQA EDEADYYC**QSSDRTLVGSL**FGTGTKVTVL
STE73-6C8	QVQLQQSGAEVKKPGSSVKVSCKAS**GGTFSSYA**ISWVRQAP GQGLEWMGR***IIPILGIA***NYAQKFQGRVTITADKSTSTAYME LSSLRSEDTAVYYC**ARSIAALNWFDP**WGQGTLVTVSS	NFMLTQPHSVSESPGKTVTIPCTGS**RGSIANNY**VQWYRQRP GRAPTTVIF***EDN***QRPSGVPDRFSGSIDRSSNSASLTISGLE TEDEADYYC**QSYDGSNVI**FGGGTKLTVL
STE73-9G3	QVQLQESGGGLVQPGGSLRLSCAAS**GFTFSSYA**MSWVRQAP GKGLEWVSA***ISGSGGST***YYADSVKGRFTISRDNSKNTLYLQ MNSLRAEDTAVYYC**ARDLVLGSGSSND**WGRGTLVTVSS	QPVLTQPPSVSGAPGQSVTISCTGT**DSNIGAGYN**VHWYQQL PGAAPKLIIS***YTD***NRPSGVPDRFSGSKSGTSASLAITGLQA EDEADYYC**QSSDRTLVGSL**FGTGTKVTVL

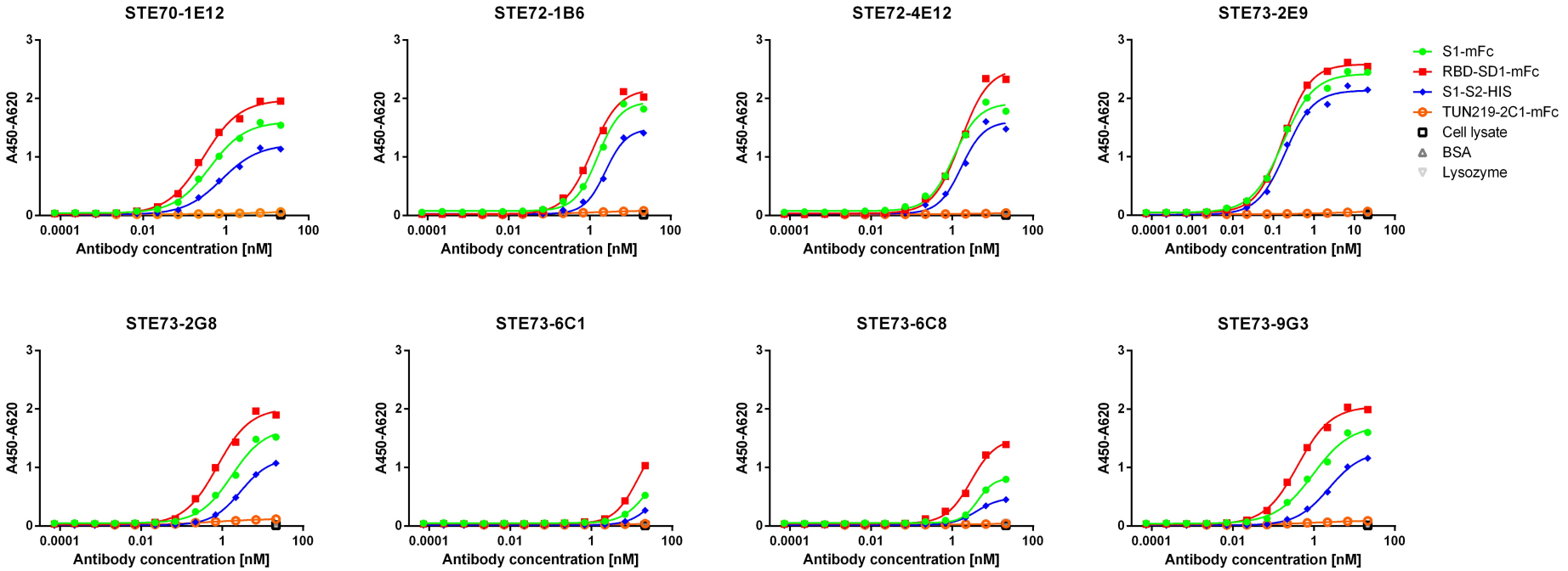
Supplementary Figure 5 Flow cytometry inhibition analysis A) Comparison of the inhibition of spike protein and RBD by flow cytometry on ACE2 positive Expi293F cells using 1000 nM scFv-Fc and 50 nM spike protein, respectively RBD (20:1 ratio). B) Inhibition of RBD binding in comparison of Expi293F transiently expressing ACE2 and Calu-3 cells. C) Inhibition of spike S1-S2 binding in comparison of Expi293F transiently expressing ACE2 and Calu-3. As negative control, the antibody SH1351-C1 was used. Data shows single measurements.



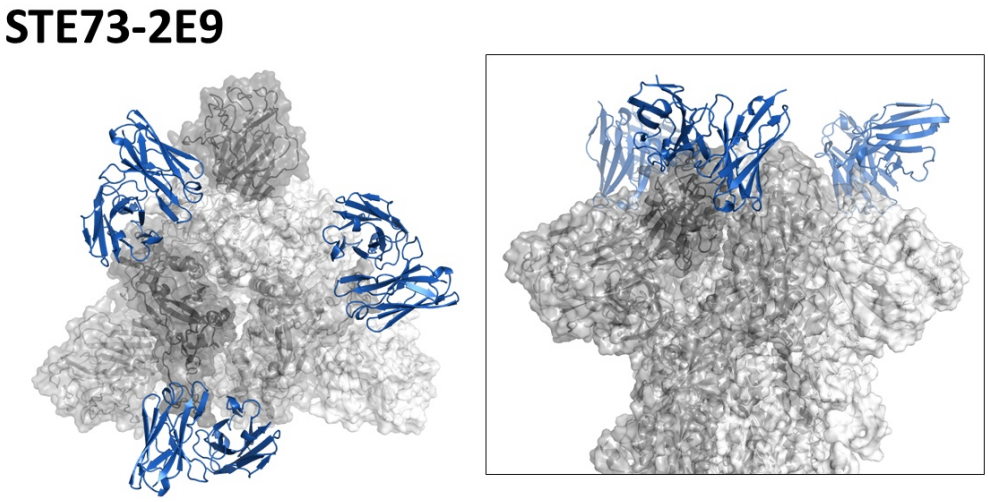
Supplementary Figure 6 Inhibition of SARS-CoV-2 spike protein binding to cells by antibody combinations. Flow cytometry analysis to determine the inhibition efficacy of antibody combinations on ACE2 positive cells and 50 nM S1-S2 and 1500 nM as a single antibody, respectively 750 nM of each antibody in a combination. Data shows single measurements.



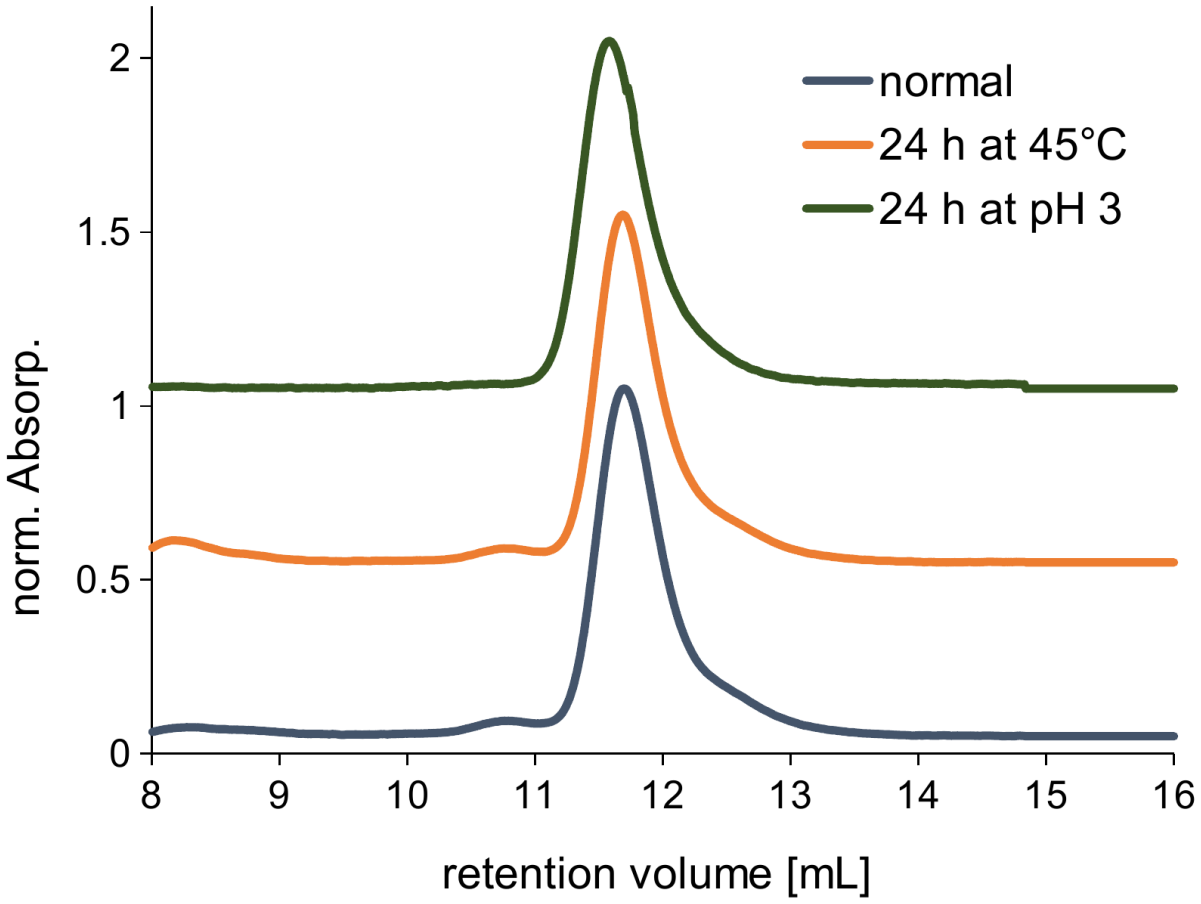
Supplementary Figure 7 Binding of the IgGs to RBD, S1 and S1-S2 in ELISA. Experiments were performed in duplicate and mean values are given.



Supplementary Figure 8 Computational models of the antibodies (colored cartoon) on the spike trimer (surface, each monomer is in a different shade of grey). Only the variable region of the antibodies is shown.



Supplementary Figure 9 SEC analysis of STE73-2E9 IgG under normal conditions (4°C, PBS, pH7.4), heat stress conditions (45°C for 24 h, PBS, pH7.4) and pH stress (4°C, 100 mM Na Acetate, pH3 for 24 h). Data shows a representative of two experiment.



Supplementary Table 1 Overview oligononucleotide primers used in this study

primer name	sequence (5' > 3')	application
MBL-NcoI-RBD 32kDa_f	attaccatggcccgggtgcagcccac	cloning of RBD-SD1 and RBD-25
RBD-SD1-Not-rev	taatgcggccgcgctgcaaggggtgatg	cloning of RBD-SD1
GiR-RBD25kDa-NotI_r	taatgcggccgcgaagttcacgcatttgttcttc	cloning of RBD-25
Nco-S1-for	cattaccatggcccagtgcgtgaacc	cloning of S1 and S1-S2
S1-Not-rev (with furin)	ataatgcggccgcgcgcgcgcgtctggg	cloning of S1 with Furin
S1-Not-rev (without furin)	tggcggccgcggacgcagagccggggctgtttgtct	cloning of S1 without Furin
S1-S2-Not-rev	gcacgcgcggccgcctgttcatacttc	cloning of S1-S2
Nco-ACE-for	gcgtgccatggcccagtccaccattgaggaac	cloning of ACE2
ACE-extracell-Not-rev	cgcacgcggccgcggaaacaggggctgg	cloning of ACE2 extracellular domain
ACE-fulllength-Not-rev	cgcacgcggccgcaaaggaggtctgaac	cloning of ACE2 fulllength
V367F-for	ctactccttcctgtacaactccgccagcttc	insertion of mutation V367F
V367F-rev	gtacaggaaggagtagtcggccacgcaattgc	insertion of mutation V367F
V483A-for	cggcgcggaaggcttcaactgctac	insertion of mutation V483A
V483A-rev	cttccgcgccgttacaaggggtgc	insertion of mutation V483A
G476S-for	ggccagcagcacccttgtaacggc	insertion of mutation G476S
G476S-rev	gctgctggcctgatagatctcggtggag	insertion of mutation G476S
N439K-for	cagcaagaacctggactccaaagtcggcgg	insertion of mutation N439K
N439K-rev	ccaggttcttgctgttccaggcaatcacacagc	insertion of mutation N439K
E484K-for	gcgtgaaaggcttcaactgctacttccc	insertion of mutation E484K
E484K-rev	gcctttcacgccgttacaaggggtg	insertion of mutation E484K
F486V-for	gaaggcgtcaactgctacttcccactgc	insertion of mutation F486V
F486V-rev	agttgacgccttccacgccgttacaagg	insertion of mutation F486V
G485R-for	gtggaacgcttcaactgctacttcccactgc	insertion of mutation G485R
G485R-rev	gaagcgttccacgccgttacaagggg	insertion of mutation G485R
OpiE2_for	cgcttatcgcgcctataaatac	sequencing of OpiE2 vector forward
IE1-rev	caaaaccccacaccaacaac	sequencing of OpiE2 vector reverse
ACE-seq-for	ccatgctaacggacccagg	internal sequencing of ACE2
S1Spike-seq-int-FBE-f	ccgaatccatcgtgcggttcc	internal sequencing of S1
S1+S2-int-S2-seq_F	gtggccagccagagcatcattgc	internal sequencing of S1-S2

ABC2	gtaacgtctctcagccaggtacagctgcagc	VH_f for cloning into pCSEHh1c.2 (STE73-6C8)
ABC4	gtaacgtctctcagccaggtgcaactgcagg	VH_f for cloning into pCSEHh1c.2 (STE73-9G3)
ABC5	gtaacgtctctcagccaggtgcagctggtg	VH_f for cloning into pCSEHh1c.2 (STE72-4E12)
ABC8	gtaacgtctctcagccaggtccagctggtg	VH_f for cloning into pCSEHh1c.2 (STE73-2E9, STE73-6B10, STE73-6C1)
ABC23	gtaacgtctctcggttcttctgagctgactcagg	VLλ_f for cloning into pCSL3hl.2 (STE73-2G8)
ABC28	gtaacgtctctcggtcagcttgtgctgactcaatc	VLλ_f for cloning into pCSL3hl.2 (STE73-6C1)
ABC34	tcctcgtctcagacctaggacggtgaccttgg	VLλ_r for cloning into pCSL3hl.2 (STE73-6C1, STE73-9G3)
ABC35	tcctcgtctcagacctaggacggtcagcttgg	VLλ_r for cloning into pCSL3hl.2 (STE73-2E9, STE73-2G8, STE73-6B10)
ABC40	gtaacgtctctcagccaggttcagctggtgc	VH_f for cloning into pCSEHh1c.2 (STE72-8A6, STE72-8A2)
ABC41	gtaacgtctctcggtcagcctgtgctgactcag	VLλ_f for cloning into pCSL3hl.2 (STE73-9G3)
ABC57	gtaacgtctctcggtcagtctgtgctgactcagc	VLλ_f for cloning into pCSL3hl.2 (STE73-2E9)
ABC59	gtaacgtctctcggtcagtctgccctgactcag	VLλ_f for cloning into pCSL3hl.2 (STE73-6B10)
ABC61	tcctcgtctcatagctgaggatacggtgacc	VH_r for cloning into pCSEHh1c.2 (STE70-1E12, STE72-8A6, STE72-8A2, STE72-4E12, STE72-1B6, STE73-2E9, STE73-2G8, STE73-6B10, STE73-6C1, STE73-6C8, STE73-9G3)
ABC63	gtaacgtctctcggtgaaacgacactcacgcagtc	VLk_f for cloning into pCSL3hk.2 (STE72-4E12)
ABC64	gtaacgtctctcggtgatattgtgatgacacagtctcc	VLk_f for cloning into pCSL3hk.2 (STE72-1B6)
ABC67	tcctcgtctcatacgtttgatttccaccttgg	VLk_r for cloning into pCSL3hk.2 (STE72-4E12)
ABC76	gtaacgtctctcggtgacatcgtgatgacccagtc	VLk_f for cloning into pCSL3hk.2 (STE72-8A6)
ABC107	tcctcgtctcatacgtttgatctccaccttgg	VLk_r for cloning into pCSL3hk.2 (STE72-8A2, STE72-1B6)
ABC110	tcctcgtctcatacgtttgatctccagcttgg	VLk_r for cloning into pCSL3hk.2 (STE72-8A6)
ABC113	tcctcgtctcagacctaggacggtcagtttgg	VLλ_r for cloning into pCSL3hl.2 (STE70-1E12, STE73-6C8)
GG_STE70-1E12_VH_f	gtaacgtctctcagccagatgcagctggtac	VH_f for cloning into pCSEHh1c.2 (STE70-1E12)
GG_STE70-1E12_VLl_f	gtaacgtctctcggtaattttatgctgactcagc	VLλ_f for cloning into pCSL3hl.2 (STE70-1E12)
GG_STE72-8A2_VLk_f	gtaacgtctctcggtgacatccggttgacccag	VLk_f for cloning into pCSL3hk.2 (STE72-8A2)
GG_STE73-2G8_VH_f	gtaacgtctctcagccaggtccagctggtacaatc	VH_f for cloning into pCSEHh1c.2 (STE73-2G8)
GG_STE73-6C8_VLl_f	gtaacgtctctcggtaattttatgctgactcaac	VLλ_f for cloning into pCSL3hl.2 (STE73-6C8)
GG_STE72-1B6_VH_f	gtaacgtctctcagccaggtgaagctggtacagtc	VH_f for cloning into pCSEHh1c.2 (STE72-1B6)