SUPPORTING INFORMATION

Peter et al.

A pair of non-competing neutralizing human monoclonal antibodies protecting from disease in a SARS-CoV-2 infection model

SUPPLEMENTAL MATERIAL AND METHODS

Detection of SARS-CoV-2 spike binding antibodies by ELISA. 96-well microtiter plates were coated overnight at 4°C with 100ng per well of recombinant NTD (Sino Biological; Beijing, China #40591-V49H), SARS-CoV-2 Spike S2 or S1 Glycoprotein (Virion\Serion, Würzburg, Germany), RBD or an S protein stabilized in a prefusion state (both affinity-purified from the supernatant of HEK-293F cells, as described previously [1, 2]). The plates were washed and blocked with 5% skimmed milk for 1 hour at room temperature and then incubated with purified TRES antibodies or hybridoma supernatants for 1 hour. Next, the plates were washed, and goat-anti-mouse HRP (Dianova, Hamburg, Germany; #115-035-146) was added and incubated for 1hour. The plates were washed, and RLU (relative light units) were detected with the multilabel plate reader Victor X4 (Perkin Elmer, Hamburg, Germany).

FACS and ELISA-based ACE2 competition assays. For quantitative flow-based hACE2 competition assays, 10^5 SARS-CoV-2 spike-transfected HEK-293T cells were incubated with 50 µl of hACE2-Fc (250 ng/ml) composed of the ectodomain of hACE2 fused in frame to the Fc region of human IgG1 and purified as described previously [3]. Subsequently, 50 µl undiluted hybridoma supernatant or mouse sera, diluted 1:200, were added, and the cells were incubated on ice for an additional 30 minutes. Cells were washed in FACS buffer and stained on ice for 30 minutes with an Alexa647-labelled anti-human IgG-Fc antibody (Biolegend, San Diego, USA, #409320). Alexa647 mean fluorescence intensities were determined for GFP-positive HEK-293T cells with a FACS Attune Nxt and analyzed with the software Flow Logic™.

For ELISA-based hACE2 competition, 96-well microtiter plates were coated overnight at 4°C with 20ng/well recombinant RBD that was purified from the culture medium of transfected HEK-293F cells. RBD protein was generated by transient transfection of HEK-293F cells. For this, the following plasmids were transfected: The plasmid pSARS-CoV-2-RBD. It was constructed by inserting a commercially synthesized RBD fragment (AA 319-541, GenBank: QHD43416.1) with an N-terminal IgKappa signal sequence (METDTLLLWVLLLWVPGSTG) and C-terminal TwinStrep- and 3xFLAG-tags (GeneArt, ThermoFisher, Regensburg, Germany) into a pcDNA3.4(+) vector backbone. SARS-CoV-2 RBD was purified from HEK-293F supernatants via affinity chromatography with Strep-Tactin®XT 4Flow agarose (IBA, Göttingen, Germany) following the manufacturer's instructions. Eluted proteins were dialyzed against PBS in Slide A_Lyzer Dialysis Cassettes (Thermo Fisher Scientific, Waltham, USA) and stored in aliquots in 50% glycerol at -20ºC. Protein purity was assessed by SDS PAGE followed by Western blot or Coomassie staining as described previously [4]. Protein concentrations were determined by OD at 260 nm and verified by a Bradford assay (Thermo Fisher Scientific, Waltham, USA). For the ELISA plates were washed with PBS/0.05% Tween-20 and blocked with 275 µl per well of PBS with 2% BSA at room temperature (RT) for 1-3 hours. Competitional binding in the ELISA set up was achieved by applying 50 µl/well of 0.25 µg/ml human ACE2-Biotin (Acro Biosystems, Beijing, China, # A011-214) followed by 50 µl/well serially 2-fold pre-diluted TRES antibodies (2 µg/ml start concentration). This was incubated at RT for 1-2 hours. Wells were washed, 50 µl/well HRP-coupled Streptavidin (0.25 µg/ml, Merck Millipore, Darmstadt, #OR03L) were added and incubated at RT for 1-2 hours. After another washing step, HRP-bound Streptavidin was detected by adding 50 µl/well TMB substrate (BD Bioscience, Heidelberg, Germany, #555214). The reaction was stopped using 50 µl/well 0.5 M H_2SO_4 solution, and the OD at 450 nm was determined in a Spectra Max 190 (BMG Labtech, Ortenberg, Germany). EC50 values were calculated by plotting hACE2 activity against

antibody concentrations and applying a 4-parameter curve fit using GraphPad Prism 7.02 (San Diego, USA).

Determination of antibody affinity by SPR. Antibodies were captured on a Protein G Chip (Cytiva Lifesciences Protein G Chip; Marlborough, USA) to reach a response level of 500 RU. Following a kinetic titration was performed ($t_{Association}$ = 180 s, $t_{Dissociation}$ = 360 s) using a three-fold serial dilution of the S protein and spanning 5 concentrations. The chip surface was regenerated using 10 mM glycine, pH 1.5, as recommended by the manufacturer. Data were analyzed with Langmuir Kinetics and the BIAcore x100 Evaluation Software 2.1. The measurements were performed in triplicates on a Cytiva Lifesciences BIAcore X100 Plus. The S protein used was stabilized in a prefusion state as described [1]. It contained an N-terminal TPA signal peptide and a trimerization foldon followed by a His-Tag at the C-terminus. The S protein was purified from transiently transfected HEK-293F cells as described previously [2].

Cloning and sequencing of Ig V exon sequences. According to the manufacturer's instructions, RNA was isolated from hybridoma cell lines with the RNAeasy Mini Kit (Qiagen, Aarhus, Denmark). 500 ng total RNA was used with the Template Switching RT Enzyme Mix (NEB, Ipswich, USA) to generate 5' RACE cDNA. Briefly, 1 µM of dT_{40} VN reverse primer and 1mM dNTP Mix were incubated for 5 minutes at 70°C together with 500 ng RNA in a 6µl reaction. The RT Enzyme mix and buffer were added to the reaction together with a Template Switch Oligo containing 3' riboguanosines (rGrGrG; for primer sequences, see Supplemental Table 1). The reverse transcription/template-switching reaction was incubated for 90 minutes at 42°C and inactivated at 85°C for 5 min. The template cDNA was then PCR amplified with 5' primers annealing to the template switch oligo and 3' primers specific for the respective Ig constant region of γ, κ and λ and for $V_H/\kappa/\lambda$ with Q5 Hot Start polymerase (NEB, Ipswich, USA). PCR products were run on agarose gels, and 500-700 bp bands were excised and purified (QIAquick Gel Extraction Kit, Qiagen, Aarhus, Denmark). The purified PCR products were then sequenced with gene-specific primers or cloned with the CloneJET PCR Cloning Kit (Thermo Fisher Scientific, Waltham, USA) and introduced into competent DH5α E. coli cells. For each PCR product, at least four clones were sequenced to determine a consensus sequence. The consensus sequences were analyzed using VDJsolver [5] and IMGT/V-Quest [6].

Construction of human IgG expression vectors. The cloning strategy was adapted from Tiller et al. [7]. The vectors AbVec-hIgG1 [GenBank: LT615368.1] and AbVec-hIgKappa [GenBank: LT615369.1] were linearized by AgeI/SalI and AgeI/BsiWI digestion, respectively, separated by agarose gel electrophoresis and purified with the QIAquick Gel Extraction Kit (Qiagen, Aarhus, Denmark). Double-stranded DNA fragments (gBlocks) covering the respective mature V_H or V_L sequence, additional amino acids (VHS) to complete the leader sequence and 23bp overlap sequences at the 5' and 3' end were synthesized (IDT, San Jose, USA). The linearized vector and the synthesized fragment in a 1:2 molar ratio were assembled with the NEBuilder HiFi DNA Assembly Kit (NEB, Ipswich, USA) at 50°C for 30 min. Competent DH5α E. coli were transformed with 2 µl of the assembly mix and plated on selective agar plates. The plasmid sequences were confirmed by Sanger sequencing (Macrogen Europe, Amsterdam, The Netherlands).

Purification of antibodies from culture supernatants. Murine antibodies were purified from serum-free cell supernatants from hybridoma via Protein-G affinity chromatography. According to the manufacturer, humanized antibodies were produced by transfecting HEK-293 F (Thermo Fisher Scientific, Waltham, USA) with the respective plasmids with the FreeStyle™ 293 Expression System's instructions. 4 days post-transfection, proteins were affinity-purified.

Hybridoma cells were grown in serum-free CD hybridoma medium (ThermoFisher Scientific, Waltham, USA) supplemented with 4 mM L-Glutamine to a cell density of about 1-1,5 million cells/ml for one week. In both cases, filtered (0.2 µm) and degassed supernatants were adjusted to pH=7 and loaded onto a High-Trap Prot G column (GE Healthcare, Chicago, Illinois, USA) using an Äkta system (GE Healthcare, Chicago, Illinois, USA). Antibodies were eluted with a pH gradient ranging from pH 5.0-2.7 using 0.1 M trisodium citrate or 0.1 M glycine-HCL. 1000µl fractions were collected in tubes containing 175 µl Tris-HCl, pH=9 and dialyzed in dialysis cassettes from Thermo Fisher (Slide A_Lyzer Dialysis Cassette Cat. Nr. 87730) against PBS. Protein purity was assessed by SDS PAGE followed by Coomassie staining and Western blot analyses as described previously [4]. Protein concentration was determined by Bradford assay (Pierce, Rockford, USA).

Virus propagation. The SARS-CoV-2 strain MUC-IMB-1 (GISAID EPI ISL 406862 Germany/BavPat1/2020) was isolated from an early outbreak cluster in Bavaria [8, 9] and was propagated by infection of Vero-E6 cells (DSMZ, Braunschweig, Germany) in DMEM (Gibco, ThermoFisher Scientific, Waltham, USA) supplemented with 10% heat-inactivated fetal calf serum (FCS, Capricorn Scientific GmbH, Ebsdorfergrund, Germany), 1% penicillin/streptomycin (Gibco, ThermoFisher Scientific, Waltham, USA) and 2 mM L-glutamine (Gibco, ThermoFisher Scientific, Waltham, USA). A second virus isolate (hCoV-19/Germany/ER1/2020; CoV-ER1) was obtained from a COVID-19 patient in Erlangen, passaged twice on Vero-E6 cells in OptiPRO™ (ThermoFisher Scientific, Waltham, USA) and sequenced (GISAID: EPI_ISL_610249). Supernatants of passaged viruses were harvested and filtered through a 0.45 µm cellulose acetate membrane filter. Aliquots of the supernatant were stored at -80°C until further use. The SARS-CoV-2 Alpha (B.1.1.7) (GISAID EPI_ISL_755639) and Beta (B.1.351) (Gene Bank Accession Number MW822592.1) [10] variants of concern were obtained from a patient in München or Frankfurt, respectively. Following, they were propagated as described previously for the CoV-ER1 variant.

The infectious titers were determined by limiting dilution in 96-well plates. Three days after infection, cell culture supernatants were removed from the wells, and cells were washed with PBS and fixed with 4% paraformaldehyde in PBS for 20 min. Following permeabilization for 15 minutes with 0.5% TritonX in PBS and a blocking step with 5% skimmed milk diluted in PBS for 1 hour, they were stained with protein G purified sera from a convalescent patient diluted 1:100 in PBS containing 2% skimmed milk. After 1 hour, the cells were washed, and a goat anti-human IgG FITC (Jackson ImmunoResearch, West Grove, USA #109-096-088) antibody was applied. After 1 hour and a washing step, positive wells were identified by a CTL-ELISPOT reader (Immunospot; CTL Europe GmbH, Bonn, Germany). The signal was analyzed with the ImmunoSpot® fluoro-X™ suite (Cellular Technology Limited, Cleveland, USA) software, and TCID50s were calculated as described previously [11].

Cell-cell fusion assay. A cell-cell fusion assay was performed with HEK-293T cells transiently transfected with the SARS-CoV-2-S DNA and Vero-E6 cells constitutively expressing ACE2. For this, 10^7 HEK-293T cells were transiently transfected with SARS-CoV-2-S DNA and a blue fluorescent protein (BFP) expression plasmid by standard PEI transfection. The following day 10^7 Vero E6 cells were stained with CellTraceTM CFSE (Thermo Fisher Scientific, Waltham, USA) according to the manufacturer's recommendations and plated into a flat bottom 96 well-plate. 48 hours after transfection, HEK-293T cells were detached by washing and incubated with the diluted antibodies prior to addition to the CFSE labeled Vero-E6 cells at a ratio of 1:1. The cells were co-incubated for 45minutes and then trypsinized, washed with PBS and fixed with 2% PFA for 20 minutes at RT. The cells were analyzed on a FACS Attune Nxt, and the

percentage of CFSE and BFP double-positive cells was determined using FlowJoTM (Treestar, Ashland, USA) software. The percent reduction of the percentage of double-positive cells by the TRES antibodies was calculated by dividing the percentage of double-positive cells for each antibody dilution by the percentage of double-positive cells in the absence of the antibody. The IC50 was calculated by application of a 4-parameter curve fit using GraphPad Prism 7.02.

Detection of TRES and TREShu antibody binding against Spike variants. HEK-293T cells were transfected with plasmids encoding HA-tagged S proteins of the D614G variant of the B.1 strain, the Alpha(B.1.1.7) variant or the Beta (B.1.351) variant. 48 hours after transfection, the cells were incubated for 30 minutes with of TREShu antibodies at a concentration of 1000 ng/ml in FACS buffer, stained with a secondary antibody directed against human Fc (Biolegend, San Diego, USA, #409320), fixed for 20 minutes with 2% PFA in PBS and then permeabilized with saponin (0.5% in FACS Buffer) for 10 minutes. After that, the C-terminal HA-Tag was detected with an anti-HA FITC labeled antibody (Sigma Aldrich, Taufkirchen, Germany, #7411) diluted in FACS buffer with 0,5% saponin. After flow cytometry, the binding indices were determined as follows: Binding index = (% TREShu positive cells X MFI of TREShu positive cells)/(% HA-positive cells X MFI of HA positive cells)X100.

Generation of escape mutants. $5x10^6$ Vero-E6 cells were seeded on the day before infection in T175 flasks. TRES6 and TRES328 antibodies were incubated for one hour at a 200 ng/ml concentration with $2x10^6$ TCID50 of the CoV2-ER1 virus. Subsequently, the medium in the cell culture flasks was exchanged for 20 ml of fresh medium, and the virus/antibody mix was added to the cell cultures. Control cultures were inoculated with $2x10^6$ TCID50 of the CoV2-ER1 virus in the absence of any antibody. The cells were checked daily for infection. If infection occurred, the C_T value was assessed by real-time PCR. In case of a breakthrough infection, 100 µl of the breakthrough viral supernatant were incubated with the antibody from the previous round at twice its concentration and added onto new Vero-E6 cells. This procedure was repeated five times. The TCID50 of the passaged virus stocks were measured, and the neutralization against both TRES antibodies was assessed. Additionally, the viral RNA was isolated from cell culture supernatant with a PureLink RNA Mini Kit (Thermo Fisher Scientific, Waltham, USA). The viral RNA was then sequenced for the identification of the escape mutations. Briefly, commercial reagents were used for unique dual indexed amplicon library generation according to the Artic protocol (NEBNext® ARTIC SARS-CoV-2 Library Prep Kit (Illumina®), which were sequenced with MiSeq® Reagent Kit v2 (500 Cycles) on the Illumina MiSeq instrument and data analyzed with CLC Genomic Workbench 21 (Qiagen, Aarhus, Denmark). Median coverage was obtained for SARS-CoV-2^{P5} 1914, for SARS-CoV-$2^{TRES328}$ 1326 and for SARS-CoV-2^{TRES6} 1436. The Data is available on GISAID under the following accession numbers: EPI_ISL_2718549, EPI_ISL_2718548 and EPI_ISL_2718547.

Structural modeling. The structure of the TRES328 in complex with the NTD was modeled based on the crystal structure of the spike protein bound to antibody 4A8 (PDB: 7C2L [12]). TRES328 and 4A8 exhibit a sequence identity of 85% and 94% for the heavy and light chain, respectively. Modeling of the wildtype was done with Modeller 9.23 [13], and the replacement of the sequence stretch L241-Y248 by a single phenylalanine was modeled with ModLoop [14]. Structure presentations were generated with Chimera 1.15 [15].

SUPPLEMENTAL FIGURES

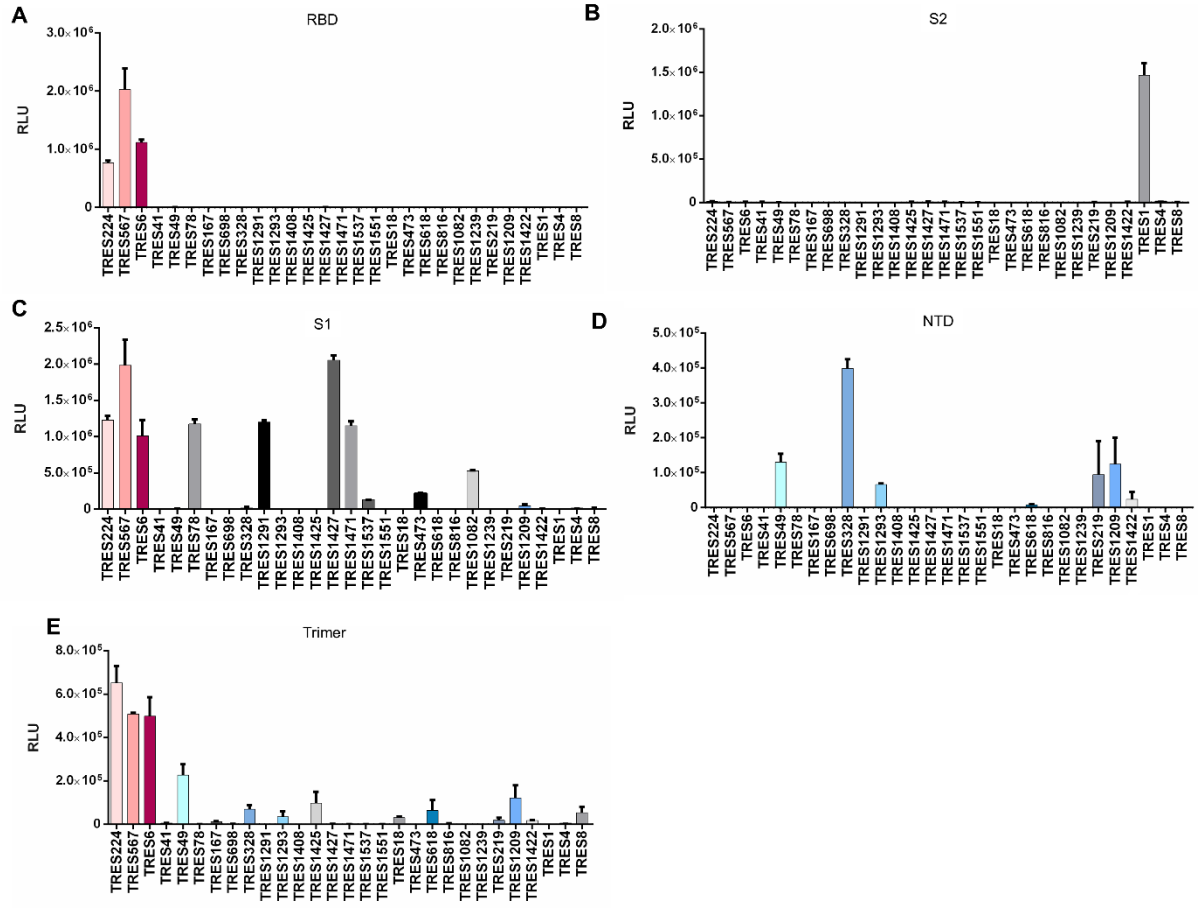

Figure S1. Binding pattern of hybridoma supernatants and purified antibodies. ELISA plates were coated with recombinant RBD (**A**), S2 (**B**), S1 (**C**), NTD (**D**), or SARS-CoV-2 spike protein stabilized in a prefusion state affinity-purified from the medium of transfected HEK-293F cells (**E**) and incubated with TRES hybridoma culture medium. Bound antibodies were detected with HRP-labeled anti-mouse IgG antibodies and the RLUs measured. Mean and standard deviations of triplicates of one experiment are shown.

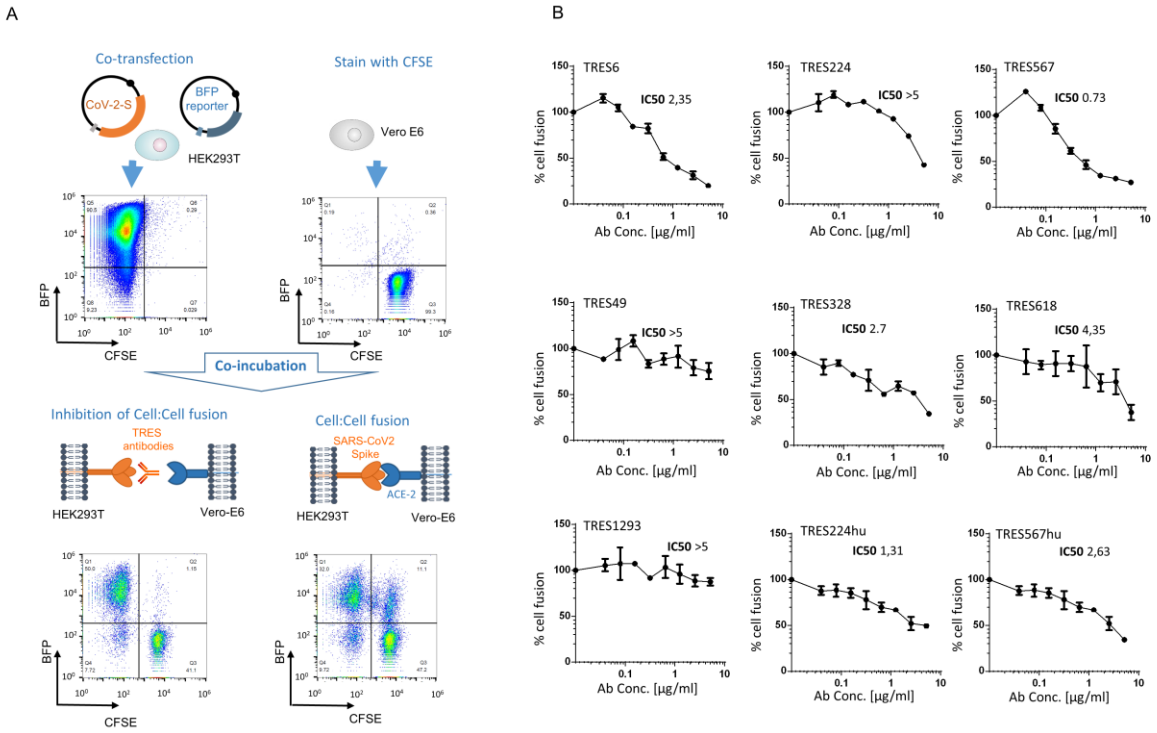

Figure S2: Cell-cell fusion inhibition by addition of spike binding antibodies (A) HEK-293T cells were transiently co-transfected with a BFP reporter plasmid and an expression vector encoding the complete spike protein. One day later, Vero-E6 cells, constitutively expressing ACE2, were stained with CFSE. 48 hours post-transfection and 24 hours post-labeling, Vero E6 and HEK-293T cells were mixed and incubated 1:1 for 45 minutes in the presence of different dilutions of spike binding antibodies. Double positive cells were identified by gating on Vero-E6 cells and the BFP+ CFSE+ cells. **(B)** The percentage of CFSE BFP double-positive cells was acquired, and the fusion inhibition was determined by dividing the percentage of double-positive cells for each antibody dilution with the percentage of double-positive cells non-incubated with antibodies. The IC50 was calculated in µg/ml by application of a 4-parameter curve fit using GraphPad Prism 7.02. One representative experiment, out of two performed in duplicates, is shown.

Sequences from TRES clones of Cluster 1

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                               CDR1                       CDR2                                             CDR3
VH-GL  QVQLVESGGGVVQPGRSLRLSCAASGFTFSSYGMHWVRQAPGKGLEWVAVIWYDGSNKYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARETVDGMDVWGKGTTVTVSS
VH567  ...V...................................................................................V........V...........Q........          MZ466572
VH6    ...V...................................................................................V........V...........Q........          MZ466574
VH224  ...V..........................G..........................Q.............................V......H.V...........Q........          MZ466570
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                               CDR1                    CDR2                                         CDR3
Vκ-GL  NIQMTQSPSAMSASVGDRVTITCRARQGISNYLAWFQQKPGKVPKHLIYAASSLQSGVPSRFSGSGSGTEFTLTISSLQPEDFATYYCLQHNSYPCSFGQGTKLEIK
Vκ567  .................S.........D.N........................L........................................YT..........          MZ466573
Vκ6    .................S.........D.N........................L........................................YT..........          MZ466575
Vκ224  .................S.........D.N........................L........................................YT..........          MZ466571
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Sequences from TRES clones of Cluster 2

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                                 CDR1                       CDR2                                                 CDR3
VH-GL   QVQLVQSGAEVKKPGASVKVSCKVSGYTLTELSMHWVRQAPGKGLEWMGGFDPEDGETIYAQKFQGRVTMTEDTSTDTAYMELSSLRSEDTAVYYCATAPAVAGPFYYYYYGMDVWGQGTTVTVSS
VH618   ...............................V.V.....................A......................................F............NF...I............. MZ466576
VH1209  ...............................V.V.......E.............A......................................F............................... MZ466578
VH1293  ...............................V.V.....................AK.............................S.......F............................... MZ466580
VH49    ...............................V.V.....................A...............................Y......F............................... MZ466582
VH328   ..H..........................V.V.V..........F.........NAA..............................Y......F..........L.................... MZ466584
VH219   ...........................S.I.V.V....................NA.....RG.R.....................KY......F............................... MZ466586
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                                    CDR1                    CDR2                                          CDR3
Vκ-GL    DIVMTQTPLSSPVTLGQPASISFRSSQSLVHSDGNTYLSWLQQRPGQPPRLLIYKVSNRFSGVPDRFSGSGAGTDFTLKISRVEAEDVGVYYCTQATQFPHSFGQGTKLEIK
Vκ618    ......................C..............................................................................T..........          MZ466577
Vκ1293   ......................C..............................................................................T..........          MZ466581
Vκ1209   ......................C............................I........C........................................T..........          MZ466579
Vκ49     ......................C...................................................E..........................T..........          MZ466583
Vκ328    ......................C...................................................E..........................T..........          MZ466585
Vκ219    ......................C...................................................E..........................T..........          MZ466587
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Figure S3: Alignments of the mature V regions of neutralizing TRES cluster 1 and cluster 2 antibodies. The virtual germline sequence is shown above the TRES sequences. The sequences in each of the indicated clusters are clonally related and descended from a common naive B cell. Sequence annotations and positions of CDR regions were determined with the abYsis software program. Amino acids are shown in the one-letter code. The positions of CDRs using the Kabat (blue underlined) and IMTG (bold blue letters) algorithms are indicated. Identical Vk sequences in cluster 2 antibodies are presented in the same color. Periods, identical amino acids; CDR, complementarity determining region. The sequences have been submitted to Genbank with the accession numbers indicated in bold at the end of each sequence.

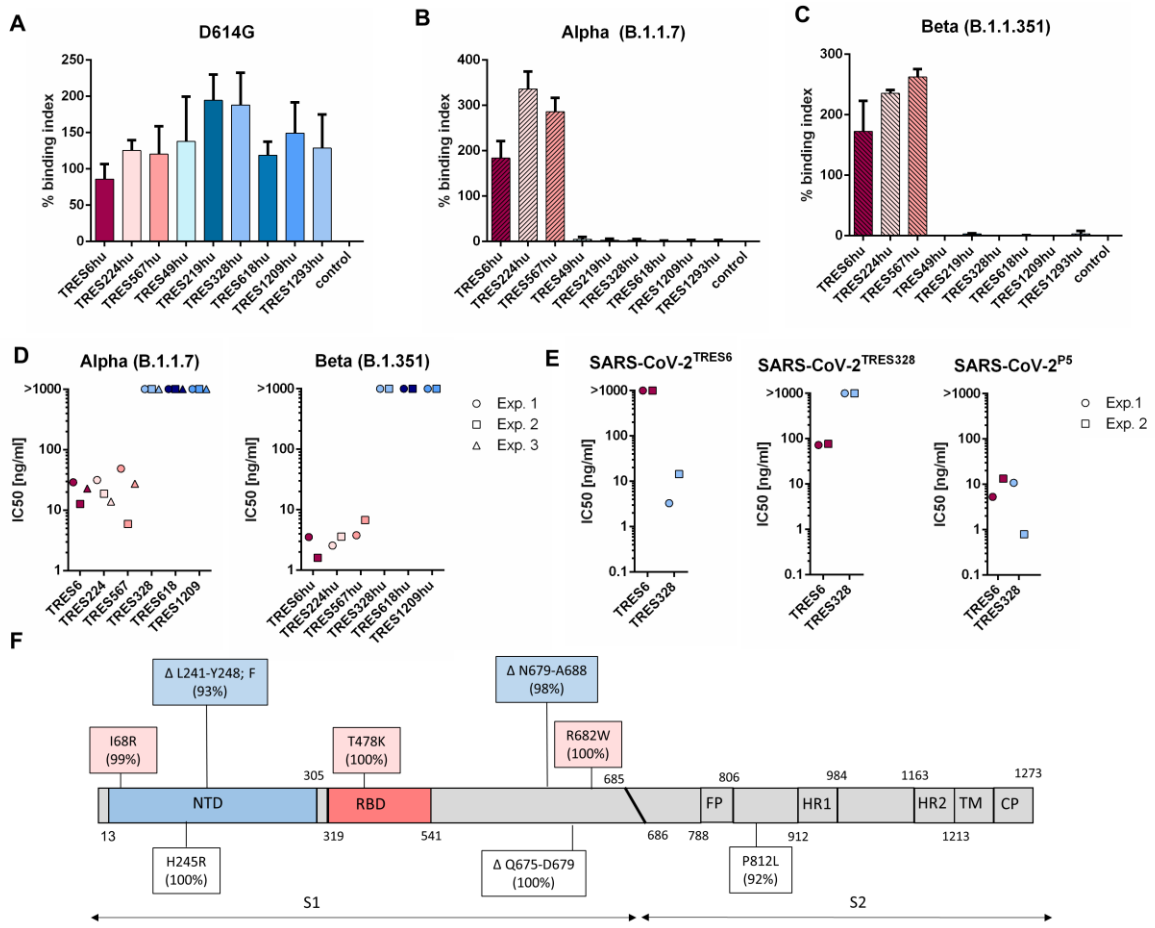

Figure S4: Breadth of neutralization by TRES antibodies and emergence of escape mutants. For the assessment of binding of TRES against the S proteins of SARS-CoV-2 variants, HEK-293T cells were transfected with plasmids encoding HA-tagged S proteins of the D614G mutant of the B.1 variant **(A)**, the Alpha (B.1.1.7) variant **(B)** or the Beta (B.1.351) variant **(C)**. Cells were subsequently incubated with 1000 ng/ml of TREShu antibodies. TREShu antibodies bound to the spike protein were detected with an anti-IgGFc antibody. Additionally, the cells were stained for intracellular HA expression. The binding indices were calculated as described in Supplemental Material and Methods. One experiment with standard deviations performed in triplicates is shown. (**D)** Neutralizing activity of hybridoma (TRES) or recombinant human TRES (TREShu) antibodies towards Alpha (B.1.1.7) or Beta (B.1.351) was determined as described in Fig. 3B. Shown are IC50s calculated from two to three experiments, each performed in triplicates. **(E)** Emergence of escape mutants in cell culture during 5 passages on Vero-E6 cells in the absence or presence of increasing TRES6 or TRES328 antibody concentrations. IC50s were determined in two independent experiments performed in triplicates as described previously in Fig. 3B. **(F)** Mutations in the S-Protein (not scaled) of the SARS-CoV-2 TRES6 (pink) and TRES328 (blue) escape mutants and the P5 variant passaged without antibody (white) as determined by whole-genome sequencing. The percentage describes the frequency of the mutations in the virus population. Exp (Experiment), NTD (N-terminal domain), RBD (receptor-binding domain), FP (fusion peptide), HR (heptat repeat), TM (transmembrane domain), CT (cytoplasmic tail).

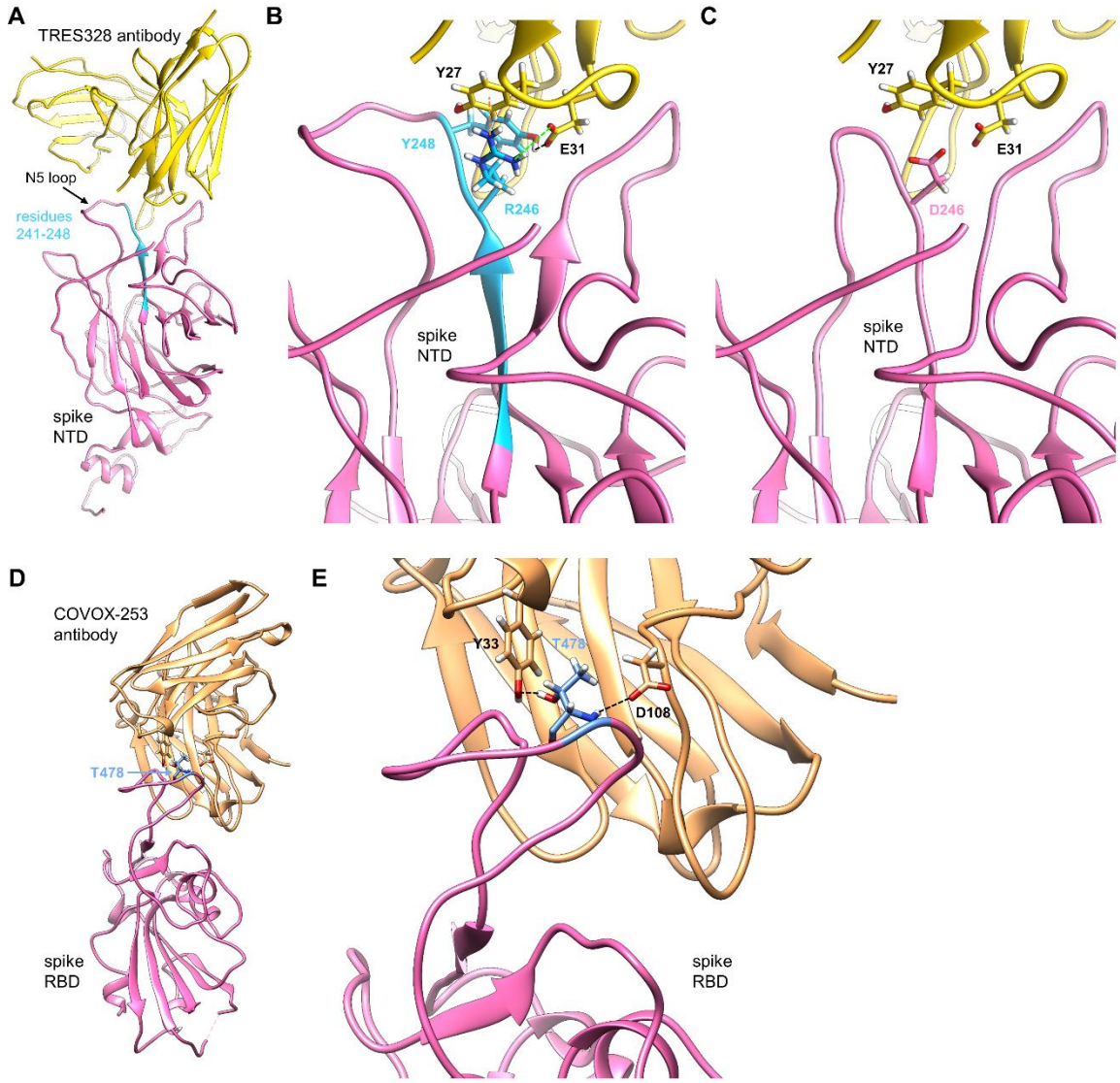

Figure S5: Structural effect of the L241-Y248 deletion on TRES328 binding and recognition of COVOX-253 antibody with the T478 epitope of the RBD domain. (A) Model of TRES328 (yellow) in complex with the NTD of wildtype spike protein (pink). Spike residues 241-248 are highlighted in blue. **(B)** Enlargement of the binding site indicating the stabilizing polar interactions in the wildtype NTD: R246 is interacting with Y27 (cation–π interaction, orange dashed line) and E31 (salt bridge, green dashed line). Y248 is forming an additional hydrogen bond with E31 (black dashed line). **(C)** The deletion of L241-Y248 shortens the N5 loop and results in a loss of the stabilizing interactions of the wildtype and electrostatic repulsion between two negatively charges residues (E31 – D246) is observed instead. **(D)** Spike protein receptor-binding domain (pink) in complex with the neutralizing COVOX-253 antibody (PDB; 7BEN [16]). T478 of the RBD is represented as blue sticks, whereas the antibody residues in close proximity to T478 (<5 Å) are represented as orange sticks. **(E)** Enlargement of the vicinity of T478 showing the hydrogen bonds with Y33 and D108 (black dotted lines)

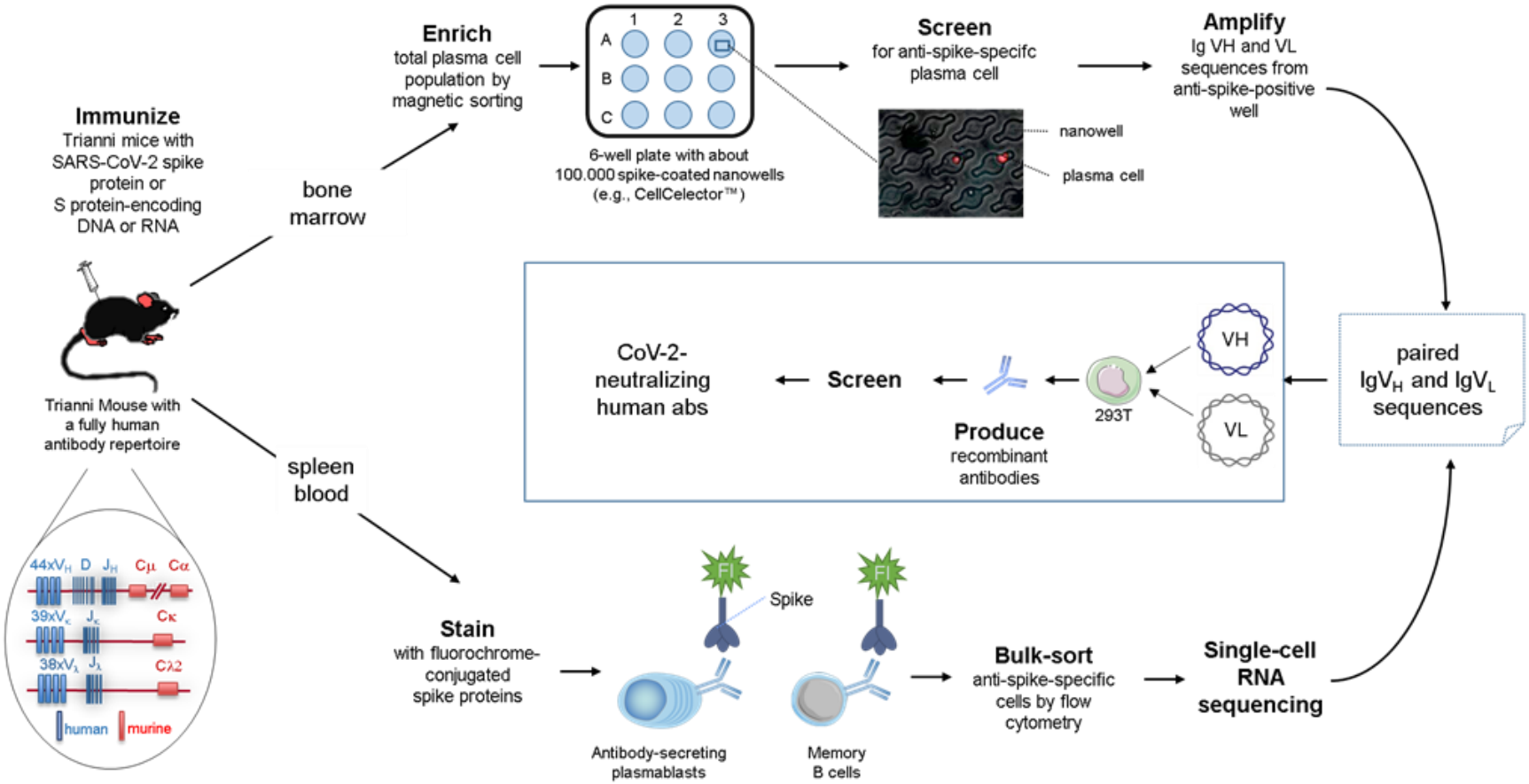

Figure S6: Isolation of CoV-2 neutralizing antibodies with the Trianni mouse platform.

Table S1: Overview: TRianni-Erlangen anti -CoV2- Spike (TRES) antibodies

Cluster	TRES antibody[1]	Binding to						ACE2 block (nM)	Trimer KD (nM)[6]	Neut IC50 (ER-1) (ng/ml)[6]	VH	VH % nt identity	VL	VL % nt identity
		S ecto-domain[2]	RBD[3]	NTD[4]	S1[5]	S2[5]	Trimer[3]							
1	6	+	+	-	+	-	+	0.75	9.5	102	V3-33*01 or V3-33*06	98,96	KV1D-17*01	97,85
	224	+	+	-	+	-	+	0.69	1.6	66	V3-33*01 or V3-33*06	97,92	KV1D-17*01	98,21
	567	+	+	-	+	-	+	1.14	3.4	64	V3-33*01 or V3-33*06	98,96	KV1D-17*01	98,57
2	49	+	-	+	-	-	+	-	6.1	34	V1-24*01	98,26	KV2D-24*01	98,64
	219	+	-	+	-	-	+	-	3.3	5	V1-24*01	96,18	KV2D-24*01	98,98
	328	+	-	+	-	-	+	-	5.8	11	V1-24*01	94,44	KV2D-24*01	98,64
	618	+	-	+	-	-	+	-	ND	18	V1-24*01	98,26	KV2D-24*01	99,66
	1209	+	-	+	-	-	+	-	7.6	11	V1-24*01	97,57	KV2D-24*01	98,64
	1293	+	-	+	-	-	+	-	ND	8	V1-24*01	97,57	KV2D-24*01	99,66
3	78	+	-	-	+	-	+	-	ND	-	V5-51*01	99,65	mLV3*01	100
	473	+	-	-	+	-	+	-	ND	-	V5-51*01	99,65	mLV3*01	99,32
	1082	+	-	-	+	-	+	-	ND	-	V5-51*01	99,65	mLV3*01	100
	1239	+	-	-	+	-	+	-	ND	-	V5-51*01	99,65	mLV3*01	100
	1291	+	-	-	+	-	+	-	ND	-	V5-51*01	99,65	mLV3*01	100
	1427	+	-	-	+	-	+	-	ND	-	V5-51*01	99,65	mLV3*01	98,98
	1471	+	-	-	+	-	+	-	ND	-	V5-51*01	99,65	mLV3*01	100
	1537	+	-	-	+	-	+	-	ND	-	V5-51*01	99,65	mLV3*01	98,98
	1	+	-	-	-	+	-	-	ND	-	V3-11*01	99,65	KV1-9*01	97,85
	167	+	-	-	-	-	-	-	ND	-	V4-59*01	99,65	KV2-28*01 or KV2D-28*01	97,96
	816	+	-	-	-	-	-	-	ND	-	V3-30*03 or V3-30*18 or V3-30-5*01	100	KV3D-7*01	99,29
	4	+	-	-	-	-	-	-	ND	-	V3-30*03 or V3-30*18 or V3-30-5*01	97,57	KV3D-7*01	100
	41	+	-	-	-	-	-	-	ND	-	V3-30*03 or V3-30*18 or V3-30-5*01	98,61	KV6D-21*02	100
	1425	+	-	-	-	-	+	-	ND	-	V3-30*03 or V3-30*18 or V3-30-5*01	97,22	KV6-21*01 or KV6D-21*01	97,85

[1]Included are all TRES antibodies with identified productive heavy and light chain sequences, [2] as determined by flow cytometry; [3] affinity-isolated form the medium of transfected HEK-293F cells, binding determined by ELISA; [4] Sino Biological, binding determined by ELISA; [5] Virion/Serion, binding determined by ELISA; [6] mean of at least two independent experiments ; ND (not determined), mL (murine lambda L chain)

Table S2: List of oligonucleotide sequences

Name	Sequence	Description
Template Switch Oligo	GCTAATCATTGCAAGCAGTGGTATC AACGCAGAGTACATrGrGrG	TSO for 5'RACE RT
TSO Primer	CATTGCAAGCAGTGGTATCAAC	PCR Forward Primer
p350_mIgG1	ATGGAGTTAGTTTGGGCAGCAGAT	Gene Specific PCR Reverse Primers
p354_mIgG2b	AGGAACCAGTTGTATCTCCACACC	
p616_mIgG2c	GAGCCAGTTGTACCTCCACACAC	
p355_mkappa	CTCCAGATGTTAACTGCTCACTGG	
p357_mlambda1	ATCTACCTTCCAGTCCACTGTCAC	
p358_mlambda2/3	ATTTGCCTTCCAGGCCACTGTCAC	
mCgamma1/2b/2c_Seq	GGCCAGTGGATAGACHGATG	mIgG PCR Sequencing Primer [17]
mkappa_Seq	CACTGGATGGTGGGAAGATGGATA	mkappa PCR Sequencing Primer [18]

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