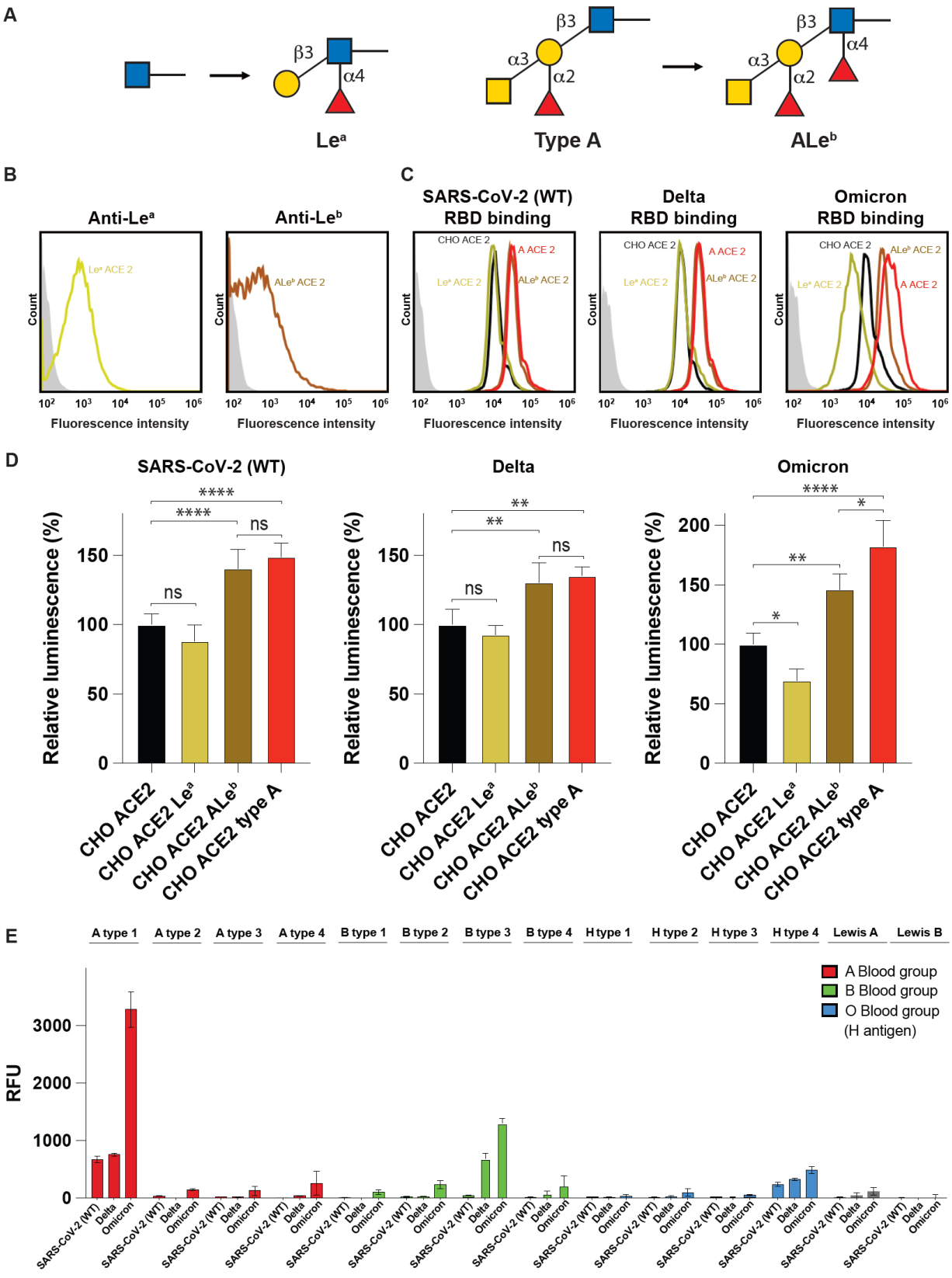
Supplemental Figure 1



Supplemental Figure 1: Distinct ABO(H) glycan configurations can impact SARS-CoV-2 interactions. (A) Schematic representation of the synthesis of Lewis a (Le^a) and blood group A Lewis b (ALe^b) antigens. (B) Flow cytometric analysis of the binding of anti-Le^a and anti-Lewis b (Le^b) antibodies to CHO cells expressing either Le^a or ALe^b as indicated. (C) Flow cytometric analysis of SARS-CoV-2 WT, Delta, and Omicron RBD binding to cells expressing either Le^a, ALe^b or blood group A antigens as indicated. (D) Infection of ACE2-expressing CHO cells expressing the Le^a, ALe^b, or blood group A antigens by SARS-CoV-2 WT, Delta, or Omicron. Results were analyzed by one-way ANOVA with Tukey's multiple comparisons test. $*P < .05$, $**P < .01$, $****P < .0001$. (E) SARS-CoV-2 WT, Delta, and Omicron RBD binding toward additional ABO(H) glycan subtypes as indicated.

Supplemental Methods

Production of SARS-CoV-2 RBD recombinant protein. SARS-CoV-2 receptor-binding domain (RBD) was cloned and purified as previously outlined[1]. In short, the SARS-CoV-2 RBD from Wuhan-Hu-1 (GenPept: QHD43416) containing natural amino-terminal signal peptide amino acids 1 (Methionine) to 14 (Glutamine) and RBD amino acids 319 (arginine) to 541 (phenylalanine) with a C-terminal 6X-His tag, was cloned into mammalian expression vector pcDNA 3.1. Plasmid DNA was prepared using the QIAGEN PlasmidPlus Maxi purification system and constructs were sequence verified. Recombinant protein was produced using the Expi293 Expression System (Thermo Fisher Scientific) according to the manufacturer's instruction. Supernatants from transfected cells were harvested on day 5 post-transfection and protein was purified using Ni Sepharose excel affinity resin (Sigma) according to manufacturer guidelines. Protein was concentrated and buffer exchanged to phosphate-buffered saline (PBS) using Amicon Ultra-15 10 kDa cutoff Centrifugal Filter Units (Sigma). The protein was filter-sterilized (0.2 μm), aliquoted and stored at -80 °C prior to use. SARS-CoV-2 Omicron RBD (B.1.1.529) was obtained from Sino Biological (Catalog: 40592-V08H121). SARS-CoV-2 Delta RBD (B.1.617.2) was obtained from Sino Biological (Catalog: 40592-V08H90). SARS-CoV-2 Beta RBD (B.1.351) was obtained from Sino Biological (Catalog: 40592-V08H85). SARS-CoV RBD was obtained from Sino Biological (Catalog: 40150-V08B2-100). MERS RBD was obtained from Sino Biological (Catalog: 40071-V08B1-100). Common cold strain HKU Spike protein was obtained from Sino Biological (Catalog: 40606-V08B-100). Common cold strain OC43 Spike protein was obtained from Sino Biological (Catalog: 40607-V08B-100).

Production of human recombinant galectins. Expression plasmids encoding Human Gal-1, Gal-3, Gal-3C, Gal-4, Gal-4N, Gal-4C, Gal-7, Gal-9, Gal-9N and Gal-9C were expressed as outlined previously[1-8]. Briefly, transformed bacteria were cultured in LB broth containing 100 μg/ml

ampicillin with agitation (250 rpm) at 37 °C. When bacteria were grown to the mid-log phase, protein expression was induced by addition of isopropyl 1-thio-β-D-galactopyranoside (IPTG, final concentration 1.5 mM). After 20 h induction at 16 °C, 6L cultured bacteria were pelleted and harvested by centrifugation and then resuspended in 60 mL bacterial lysis buffer (PBS pH7.4 with 14 mM β-mercaptoethanol (β-ME), 60 μL ribonuclease A, 60 μL DNase I, 60 μL lysozyme, and 2 protease inhibitor cocktail tablets, Thermo Fisher Scientific, A32965). Lysozyme (MilliporeSigma, L6876) dissolved in sterile water to a stock concentration of 100 mg/mL. Ribonuclease A (MilliporeSigma, R4875) dissolved in sterile water to a stock concentration of 10 mg/mL. DNase I (MilliporeSigma, 11284932001) dissolved in sterile water to a stock concentration of 10 mg/mL. The suspension was passed through a cell disruptor, and the lysate was centrifuged at 20,000 × *g* at 4 °C for 1h and repeated twice. Supernatant was applied to lactosyl-sepharose affinity chromatography column. For elution, the elution buffer (PBS pH7.4 with 14 mM β-ME and 100 mM lactose) was added. The desired fractions were pooled and stained with Coomassie blue on SDS-PAGE gel to test purity. The purified protein was aliquoted and stored at -80 °C prior to use. Galectins were labeled with Alexa Fluor™ 647 NHS Ester (succinimidyl ester) by incubating 2 mg/mL galectin with 1 mg Alexa Fluor™ 647 for 1h at room temperature and avoid from light as outlined previously[9,10]. Unconjugated Alexa Fluor™ 647 and free lactose were separated using a PD-10 gel filtration column (GE Healthcare). Labeled galectins were purified again by lactosyl-sepharose column to remove possible inactive protein that may have formed during labeling process. Bound galectin was eluted with 100 mM lactose in PBS plus β-ME and then β-ME and lactose were removed from galectins using a PD-10 gel filtration column prior to the use of galectins for experiments. While β-ME is not required for some galectins, this approach was used to provide a consistent protocol for all galectin purification[11].

Mammalian glycan array analysis. Glycans were prepared as previously outlined[12], followed by printing onto NHS-activated glass slides in 300 mM phosphate, pH 8.5 containing 0.005% Tween-20, glycan was printed in a replicate of 6 at a concentration of 10 μM. SARS-CoV-2 RBD and variants were incubated with the microarray as described previously[1,13]. In short, the protein was diluted with PBS containing 0.05% Tween 20 and 1% BSA and incubated with the glycan microarray for 1h at room temperature in a dark humid chamber. Slides were washed by successive immersion in PBS containing 0.05% Tween 20 (4x) followed by PBS (4x) and then H_2O (4x) followed by incubation with 5 μg/ml anti-His antibody (Anti-His-tag mAb-Alexa Fluor® 647 from MBL Code No. D291-A64). Slides were washed by successive immersion in PBS containing 0.05% Tween 20 (4x) followed by PBS (4x) and then H_2O (4x). An image of bound fluorescence was then obtained using a microarray scanner (GenePix 4000 B, Molecular devices). Integrated spot intensities were acquired using Imagene software (GenePix Pro 7)[14,15].

Production of SARS-CoV-2 pseudovirus and infection assay. HIV-based pseudoviral particles were produced using expression plasmids with codon-optimized SARS-CoV-2 Spike (Wuh-1, Delta, and Omicron), encoding a 21 amino acid intracellular domain truncation mutant of each variant protein. For pseudovirus production, HEK293T cells were seeded in DMEM supplemented with 10% FBS for 24 hours prior to transfection. Cells were co-transfected with the appropriate entry receptor plasmid and Luciferase-IRES-ZsGreen (NR-52516) backbone plasmid along with HDM-Hgpm2 (NR=52517), pRC-CMV-Rev1b (NR-52519), and HDM-tat1b (NR-52518) accessory plasmids[16]. 48 hours after transfection, supernatants containing SARS-CoV-2 pseudotyped viral particles were harvested and clarified by centrifugation. Pseudoviral stocks were then aliquoted and stored at -80 °C for use in infectivity assays. Bright-Glo™ Luciferase Assay System (Promega, E2610) was used to determine the pseudovirus infection on the blood group A or O CHO cells. The luciferase reagent was incubated for 2 min in the dark before

measuring luminescence with no attenuation and a 1 second integration time using ELISA reader (Molecular Devices, SpectraMax M3).

Creation of CHO cells and analysis with flow cytometry. CHO cells pgsB-618 (ATCC® CRL-2241™) were purchased from ATCC. PgsB-618 is a Chinese hamster ovary cell mutant deficient in galactosyltransferase I (UDP-D-galactose:xylose-1,4-D-galactosyl-transferase). Stable CHO cell line constitutively expressing human ACE2 was developed using the same protocol as described previously[16]. Flow cytometric analysis was accomplished for verifying ACE-2 expression. CHO cells were harvested and stained with anti-human ACE-2 Alexa Fluor® 647-conjugated Antibody (R&D Systems, FAB9332R-100UG) according to the manufacturer's instruction. Lentivirus packaging human β3GALT5 (Dsred), human FUT3 (ECFP), rat α1,2-fucosyltransferase (EGFP) and rat Enzyme A (BFP, blue fluorescent protein) were generated via co-transfection in HEK293T cells (ATCC, CRL-3216) with β3GALT5, FUT3, α1,2-fucosyltransferase or Enzyme A plasmids in the presence of lentiviral helper plasmids (HDM-VSVG, HDM-Hgpm2, HDM-tat1b, and pRC-CMV-Rev1b). The resulting lentivirus of β3GALT5 and α1,2-fucosyltransferase were used to infect CHO cells in the presence of 5 μg/mL polybrene to produce blood group O CHO cells. Blood group A CHO cell line was generated with three lentiviruses including β3GALT5, α1,2-fucosyltransferase and Enzyme A. Additional modification to generate ALe^b was achieved by adding FUT3 lentiviruses. The blood group Le^a CHO cell line was generated using two lentiviruses including β3GALT5 and FUT3. Based on the fluorescent markers, blood group O CHO cells were sorted by flow sorter for Dsred and EGFP double positive events. Blood group A CHO cells were sorted by flow sorter for Dsred, EGFP and BFP triple positivity. These cells were further sorted on ECFP to select for ALe^b CHO cell. Blood group Le^a CHO cells were selected by flow sorting Dsred and ECFP double positive events. Flow cytometric analysis was accomplished for verifying blood group A expression. CHO cells were harvested

and stained with mouse IgM anti-Blood Group A Antigen antibody (Abcam, ab2521) and Alexa Fluor® 647 anti-mouse IgM Antibody (Jackson, Code: 115-605-075). The SARS-COV-2 RBD was incubated with blood group A or O CHO cells, CHO cells expressing human ACE-2, followed by detection with anti-His antibody (Anti-His-tag mAb-Alexa Fluor® 647 from MBL Code No. D291-A64) and flow cytometric analysis. Flow cytometric analysis was accomplished using a FACSCanto II flow cytometer (BD Biosciences).

Flow sorting was accomplished by BD FACSAria Fusion Cell Sorter. The data were processed with FlowJo version 10.

Molecular docking analysis and visualization. Molecular docking was performed using Autodock Vina to get ligand docking poses[17]. SARS-CoV-2 RBD (PDB ID: 7DK3), Delta variant (PDB ID: 7T9K), Omicron variant (PDB ID: 7V8A), and type I blood group A antigen were converted to the pdbqt format using the MGLTools program of the AutoDockTools package. Amide bonds in the ligand were treated as non-rotatable bonds during docking, while RBD receptor atoms were all kept rigid. Each native complex was docked three times with different initial random seeds, and the top 10 scoring poses were retained each time. The top 3 docked poses with the highest negative binding free-energy (kcal/mol) were selected as the further ligand-protein binding analysis. The best-predicted poses were visualized and analyzed by PyMol version 1.7 to show ligand-protein interactions in detail.

Data sharing. All data in this manuscript will be made available upon request to the corresponding author.

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