Rational Design of Multiclade Coronavirus Spike Immunodominant Domain Nanoparticles Elicit Broad Antibody Responses

Christian K.O. Dzuvor[1], Sydney Moak[1,2], Lindsay R. McManus[1], Abigail Thomas[1,3], Abigail E. Dzordzorme[1,4,6], Taewoo Kim[1], Jeswin Joseph[1], Valerie Foley[1,3], Laura Novik[5], Ingelise J. Gordon[5], LaSonji A. Holman[5], Lesia K. Dropulic[5], Ryan P. McNamara[1], Kizzmekia S. Corbett-Helaire[1,2,7]

[1]Department of Immunology and Infectious Diseases; Harvard T.H. Chan School of Public Health; Boston, Massachusetts, 02115; United States of America

[2]Howard Hughes Medical Institute; Chevy Chase, Maryland, 20815; United States of America

[3]Northeastern University, Boston, Massachusetts, 02115; United States of America

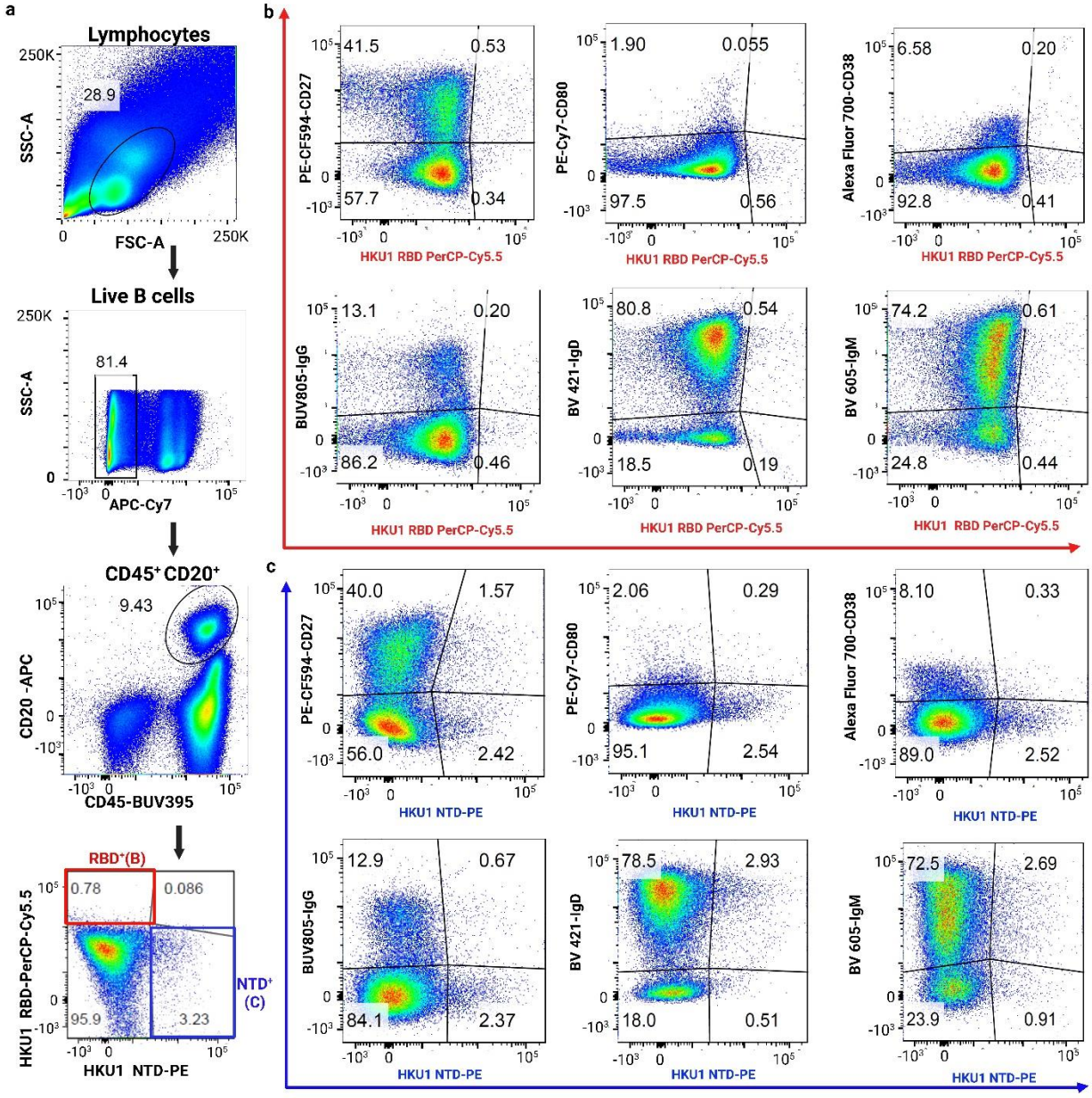
[4]Harvard-MIT Division of Health Sciences and Technology, Institute for Medical Engineering and Science, Massachusetts Institute of Technology, Cambridge, Massachusetts, 02139; United States of America

[5]Vaccine Research Center; National Institutes of Allergy and Infectious Diseases; National Institutes of Health; Bethesda, Maryland, 20892; United States of America

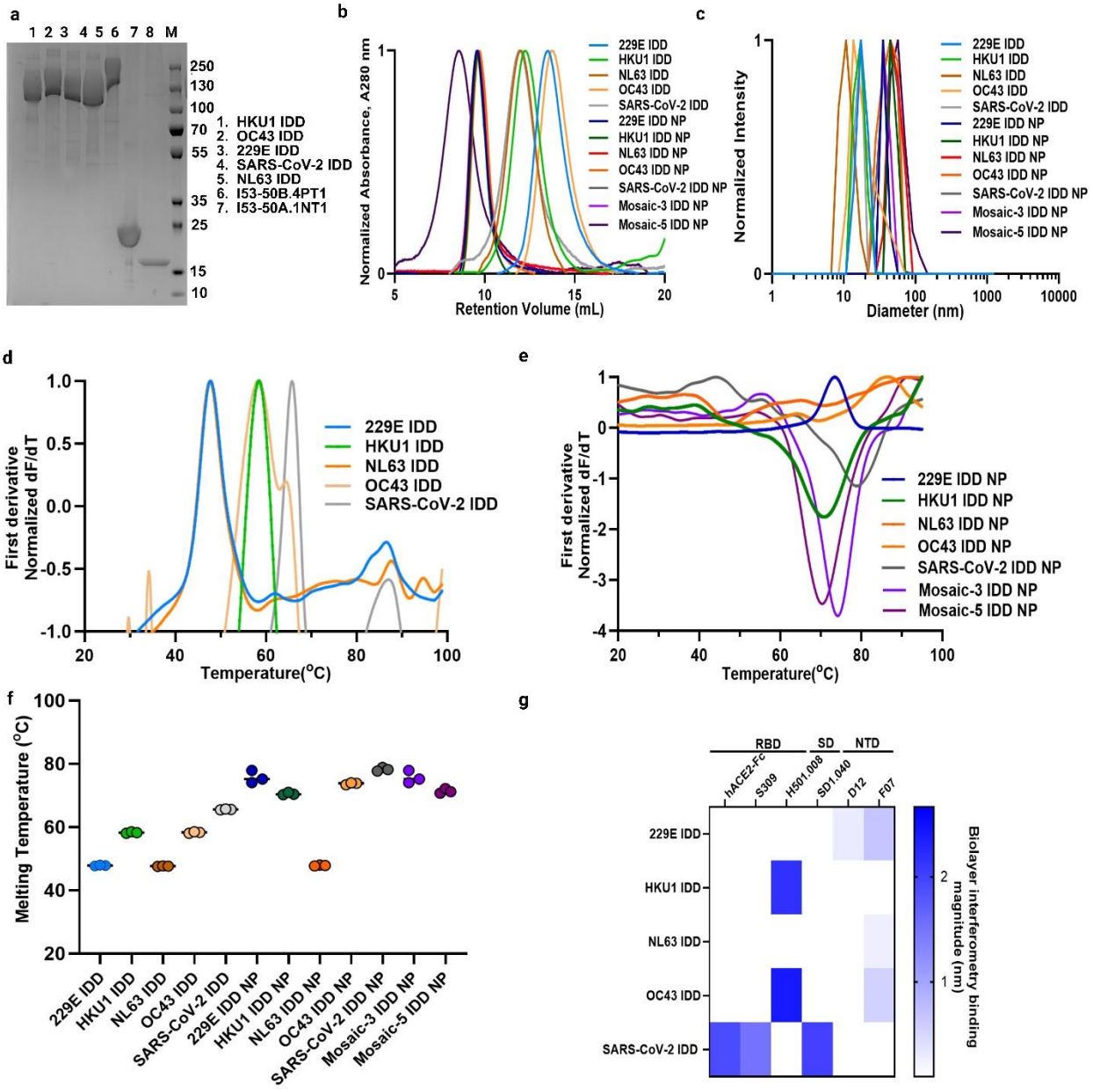
[6]Current Affiliation: Wyss Institute for Biologically Inspired Engineering, Harvard University, Boston, Massachusetts,02215; United States of America

[7]Correspondance: kizzmekia_corbett@hsph.harvard.edu

Extended Data



Extended Data Fig. 1: Representative gating strategy for evaluation of HCoV-HKU1 S1 domain-specific B-cells by flow cytometry, related to Fig. 1f. (**a**) FACS analysis for identifying HCoV-HKU1 S RBD and NTD reactive B-cells (**b-c**) Representative flow cytometry plots of CD45 CD20 B-cells binding to fluorescently labelled (**b**) HCoV-HKU1 RBD probe(red) and (**c**) HCoV-HKU1 NTD probe(blue). Identification of B-cell subsets(top) and Ig isotypes (bottom) within B cell population. Numbers in each quadrant indicate percentage of gated cells.



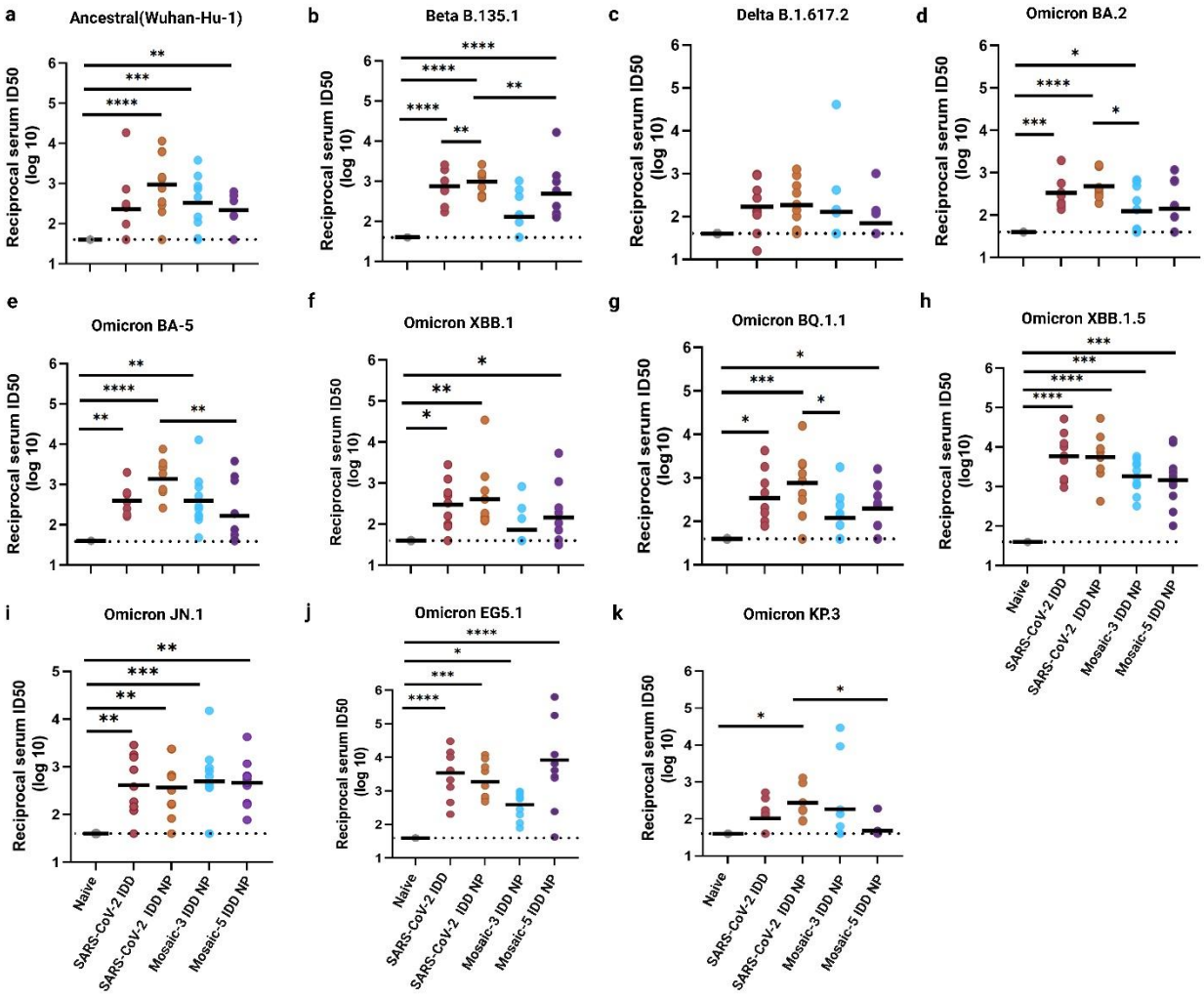
Extended Data Fig. 2: Design, production and characterization of IDD trimers and IDD NPs, related to Figs. 1h and 2. (**a**) SDS-PAGE of affinity-purified chimeric IDD trimers, I53-50A.1NT1, and I53-50B.4PT1 subunits. (**b**) Size-exclusion chromatograms of unassembled IDD trimers and assembled NPs on a Superose 6 Increase 10/300 GL column. (**c**) Dynamic light scattering (DLS) of IDD trimers and NPs. The hydrodynamic diameter and polydispersity index (PDI) of each immunogen are shown. (**d**) Representative thermal melting profiles of IDD trimers measured by conventional differential scanning fluorimetry (DSF). Data represent the average of n=3 technical replicates. (**e**) Representative thermal melting profiles of NPs measured by nano differential scanning fluorimetry (nanoDSF). Data represent the average of three technical replicates. (**f**) Thermal melting temperatures of IDD trimers and IDD NPs measured by DSF. Data represent the average of three technical replicates. (**g**) Antigenic characterization of IDD trimers by bio-layer interferometry. Antigenicity was performed by assessing binding of immunogens to receptor, hACE2-

Fc and S1 domain-specific mAbs. hACE2-Fc and S309 bind to SARS-CoV-2 RBD. H501.008 binds to HCoV-HKU1 and HCoV-OC43 RBD. SD1.040 binds to SARS-CoV-2 Subdomain, SD1. D12 and F07 bind to HCoV-229E and HCoV-NL63 NTD. Blue intensity signifies the magnitude of binding.

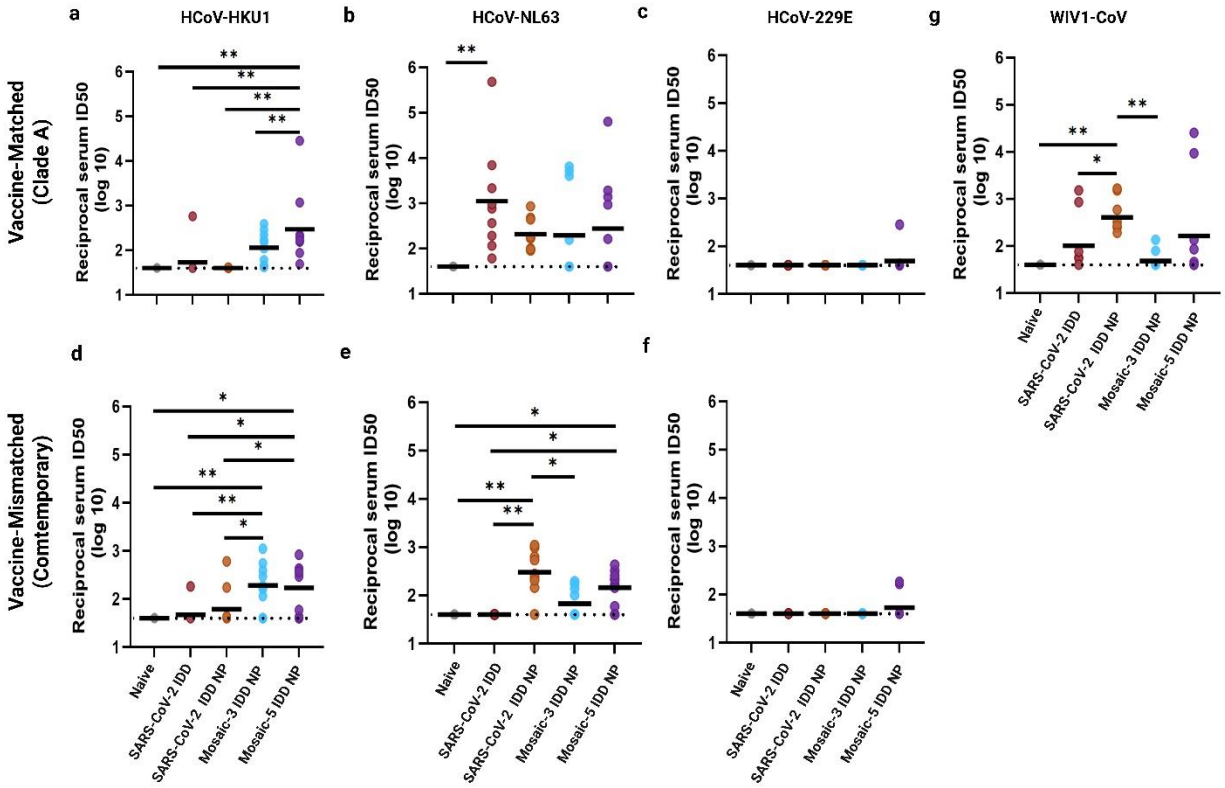
Convalescent Sera Subject ID		Coated Immunogens				
		BSA	SARS-CoV-2 IDD	SARS-CoV-2 IDD NP	Mosaic-3 IDD NP	Mosaic-5 IDD NP
Pre-pandemic Era	A	2	2	2	3.6	4.6
	B	2	2	2	3.9	4.2
	C	2	2	2	4.7	4.5
	D	2	2	2	4.5	4.8
COVID-19 Era	E	2	2.5	3.8	3.5	4.2

Endpoint Titer (Log10)	4 – 5	3 – 4	2.5 – 3	2

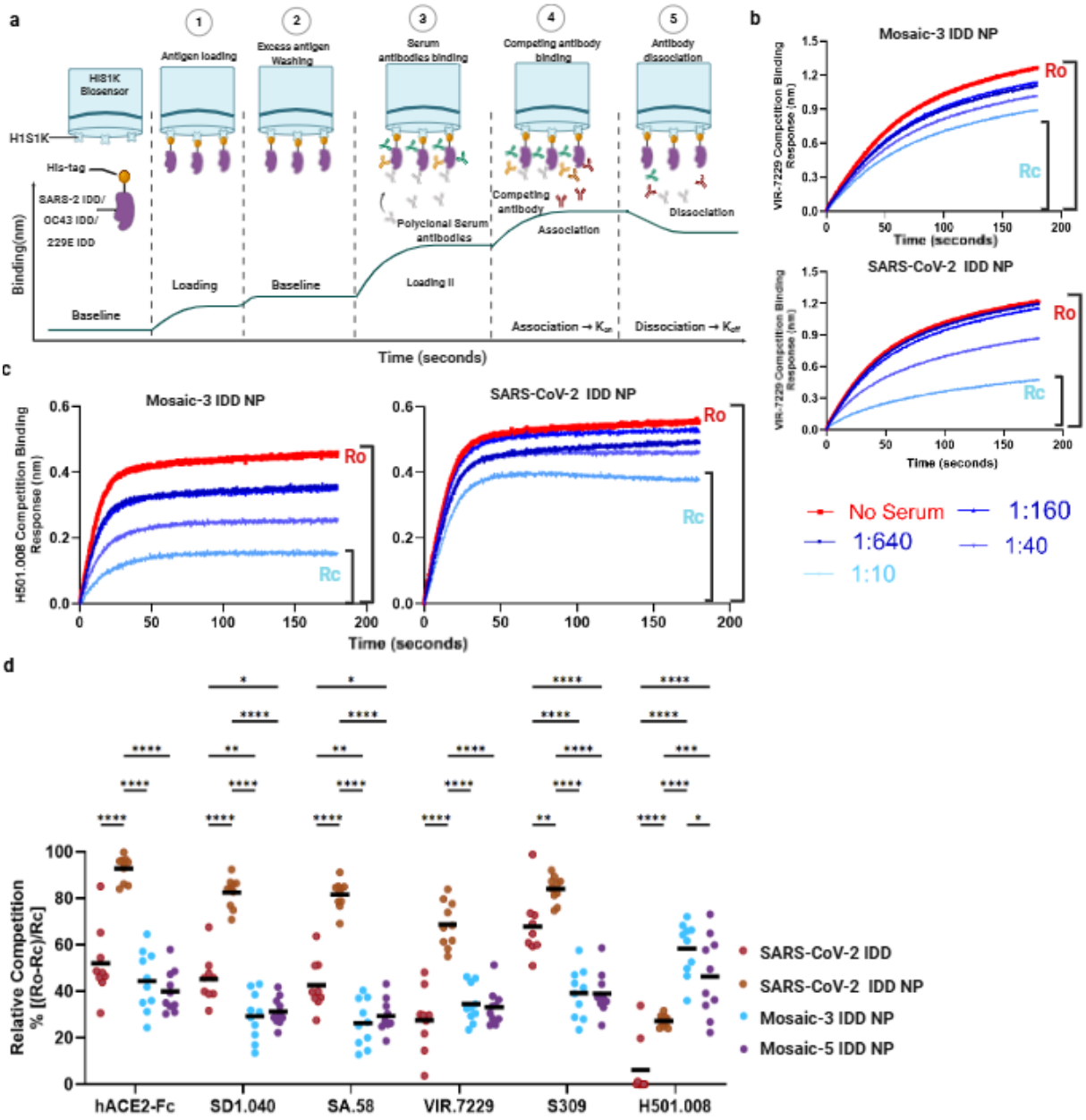
Extended Data Fig. 3: Human convalescent sera reactivity to IDD trimers. Sera from convalescent donors with pre-existing immunity acquired by infection in pre-pandemic and COVID-19 pandemic era were tested for IgG binding to IDD immunogens. Data are presented as Logarithmic(Log10) mean endpoint titer and indicated with colors. Dark red = 4-5, red = 3-4, yellow = 2.5-3, and uncolored = 2. BSA (negative control), SARS-CoV-2 IDD trimers and IDD NPs were used to coat ELISA plates, followed by incubation with serially diluted sera (starting at 1:100) and detection with type-specific secondary antibodies. Samples were run in triplicate and performed twice.



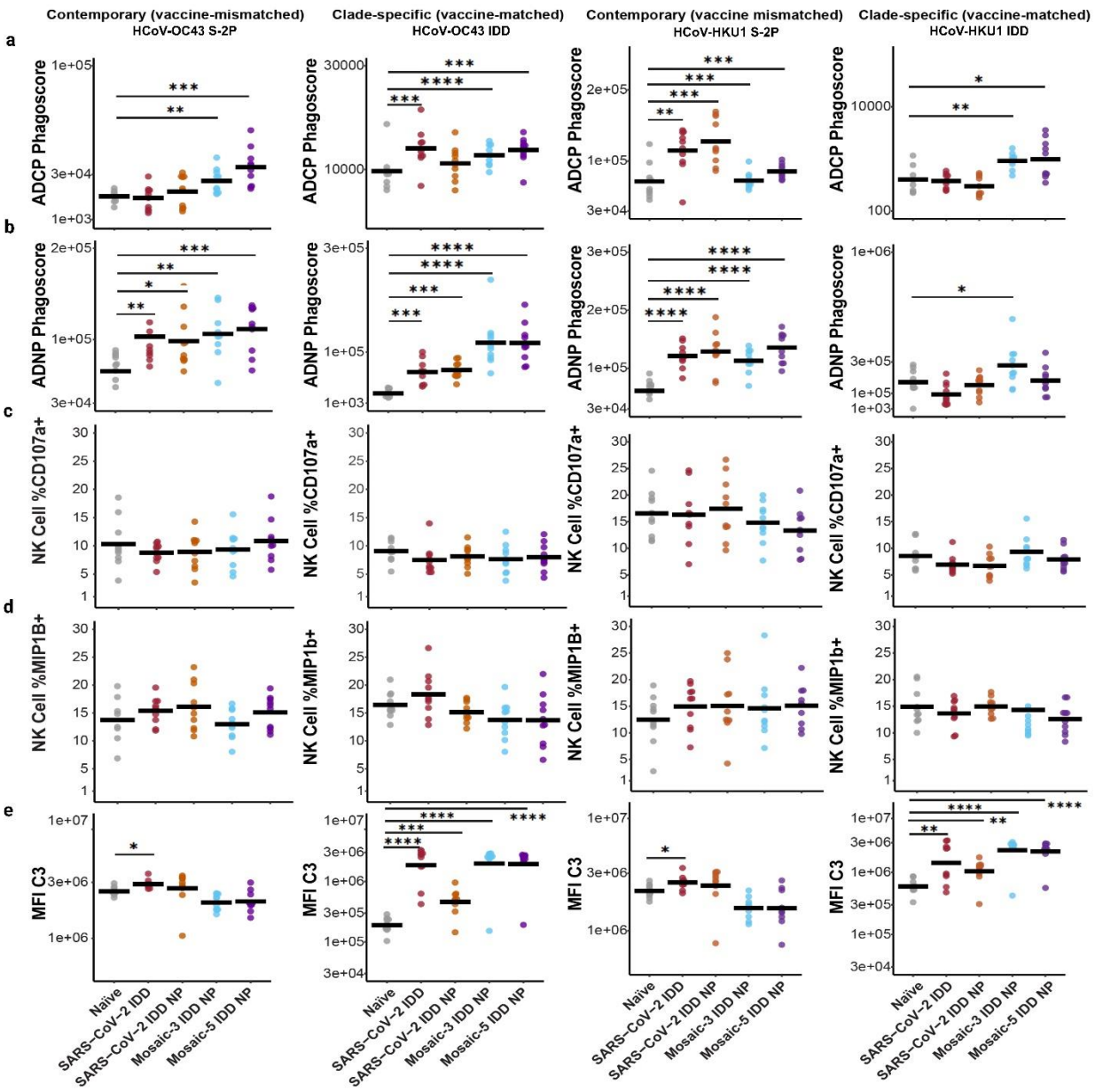
Extended Data Fig. 4: Neutralizing antibody responses in immunized mice against SARS-CoV-2 pseudotyped viruses related to Fig. 4b, left. Sera collected 2-weeks post immunization were evaluated for neutralization against the following pseudotyped SARS-CoV-2 strains: ancestral (Wuhan-Hu-1), Beta, Delta, Omicron BA.2, BA.5,XBB.1,BQ1.1, XBB 1.5,JN.1, EG5.1, and KP.3. Horizontal dashed lines represents the assay LOD. Bars indicates geometric mean ID_{50} for ten mice per group. Each dot represents an individual mouse. Two–way ANOVA with Tukey's post-hoc test was used to compare geometric mean ID_{50} titers. Statistical significance is displayed as follows: $*p < 0.05$; $**p < 0.01$; $***p < 0.001$; $****p < 0.0001$.



Extended Data Fig. 5: Comparison of neutralizing antibody response against endemic pseudotyped CoVs and pandemic-threat WIV1-CoV, related to Fig. 4b. (**a-g**) Sera collected 2-weeks post-boost immunization were evaluated for neutralization of a panel of pseudotyped viruses displaying vaccine-matched (**a-c**) and mismatched (**d-f**): HCoV-HKU1, HCoV-NL63, HCoV-229E spikes and pandemic-threat WIV1-CoV (**g**). The horizontal dashed line represents the limit of detection(LOD). Bar indicates geometric mean of ID_{50} for N=10 mice per group. Each dot represents a mouse in a group. Two–way ANOVA with Tukey's post-hoc test was performed to convert ID_{50} values to log_{10} scale. The statistical significance is displayed as follows: $*p < 0.05$; $**p < 0.01$; $***p < 0.001$; $****p < 0.0001$.



Extended Data Fig. 6: Detection of antibodies and epitope mapping in immune sera using bio layer interferometry competition binding assay. (a) Experimental workflow for serum competition assay as measured by BLI. **(b, c)** Representative binding data curves for mAbs VIR-7229 and H501-008 competing with serially diluted immune sera from mice immunized with Mosaic-3 IDD NP and SARS-CoV-2 IDD NP. Ro represents the highest binding signals of VIR-7229 and H501-008 without serum. Rc represents the highest binding signals of VIR-7229 and H501.008 tested with sera from vaccinated mice. (**d**) Cross-competition and inhibition of immune sera by indicated mAbs. 1:10 sera dilution is shown. Bar indicates mean percent inhibition for N=10 mice per group. Each dot represents an individual mouse in a group. Two–way ANOVA with Tukey's post-hoc test was performed. The statistical significance is displayed as follows: $*p < 0.05$; $**p < 0.01$; $***p < 0.001$; $****p < 0.0001$.



Extended Data Fig. 7: Fc antibody-dependent functional scores (ADCP, ADNP, ADNK, ADCD) against endemic HCoV-OC43 and HCoV-HKU1. (**a-e)** Fc-mediated effector functions of the naïve and immune sera after vaccination with IDD NPs against contemporary HCoV-OC43 S-2P (left-hand column), clade-specific HCoV-OC43 IDD (left-middle column), contemporary HCoV-HKU1 S-2P (right middle column), and clade-specific HCoV-HKU1 IDD. Antibody-mediated cellular phagocytosis with monocytes (ADCP, **a**) or neutrophils (ADNP, **b**) using IDD-vaccine-induced immune and naïve sera and beads coated with indicated HCoV-HKU1 and HCoV-OC43 proteins. (**c-d)**, Antibody-dependent natural killer cell activation (ADNKA) using IDD-vaccine-induced immune and naïve sera and beads coated with indicated HCoV-OC43 and HCoV-HKU1 proteins. **c**, Percentages of NK cells expressing CD107a against the antigens by vaccine group. **d**, Percentages of NK cells expressing MIP-1β against the antigens by vaccine group. The experiment was run with PBMCs from two separate donors. (**e**) Antibody-dependent deposition of complement(ADCD) on beads coated with indicated HCoV-HKU1 and HCoV-OC43 proteins and

incubated with naïve or immune sera. In all figures, the horizontal black bar indicates the group mean value (N=10 mice per group). Each dot represents an individual mouse in a group. Two–way ANOVA with Tukey's post-hoc test was performed. The statistical significance is displayed as follows: $*p < 0.05$; $**p < 0.01$; $***p < 0.001$; $****p < 0.0001$. Source data file is provided.