Supplementary Figures

Fusion protein pan-sarbecovirus vaccines elicit broadly protective immune responses targeting Clade 1a, 1b, and 3 sarbecoviruses

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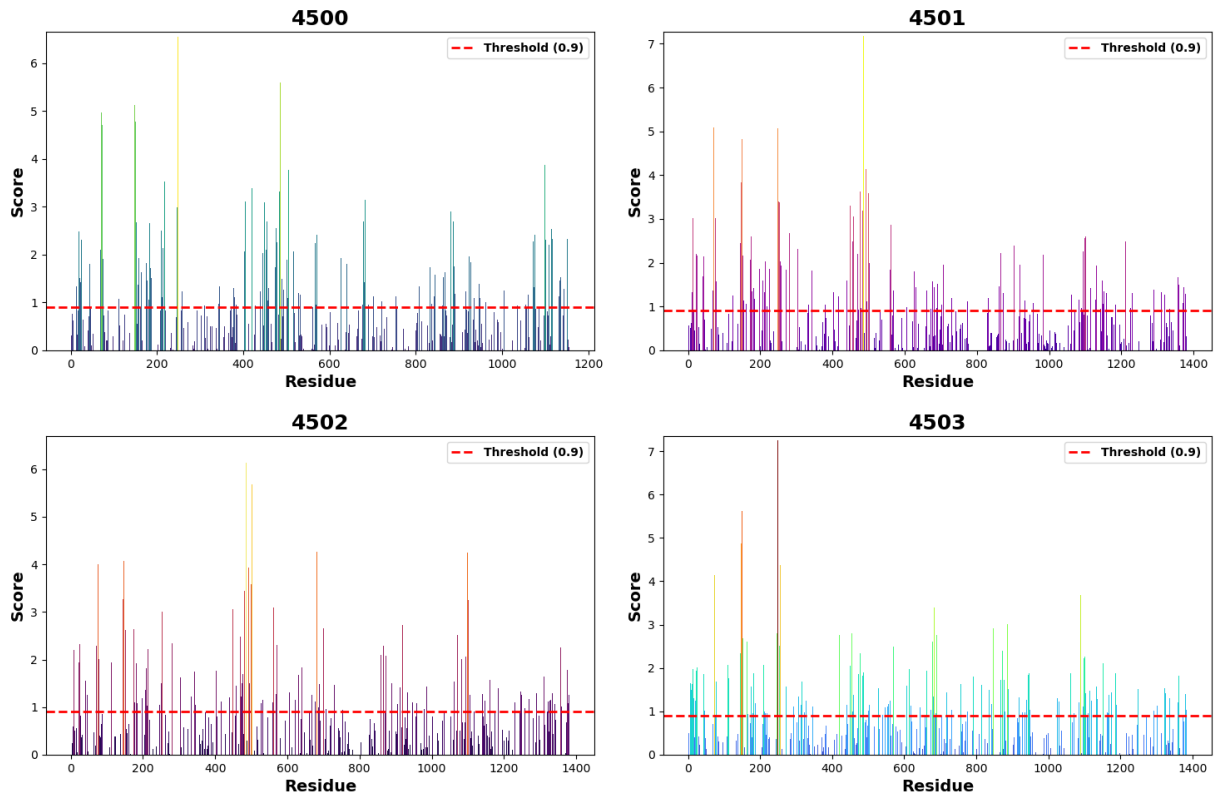
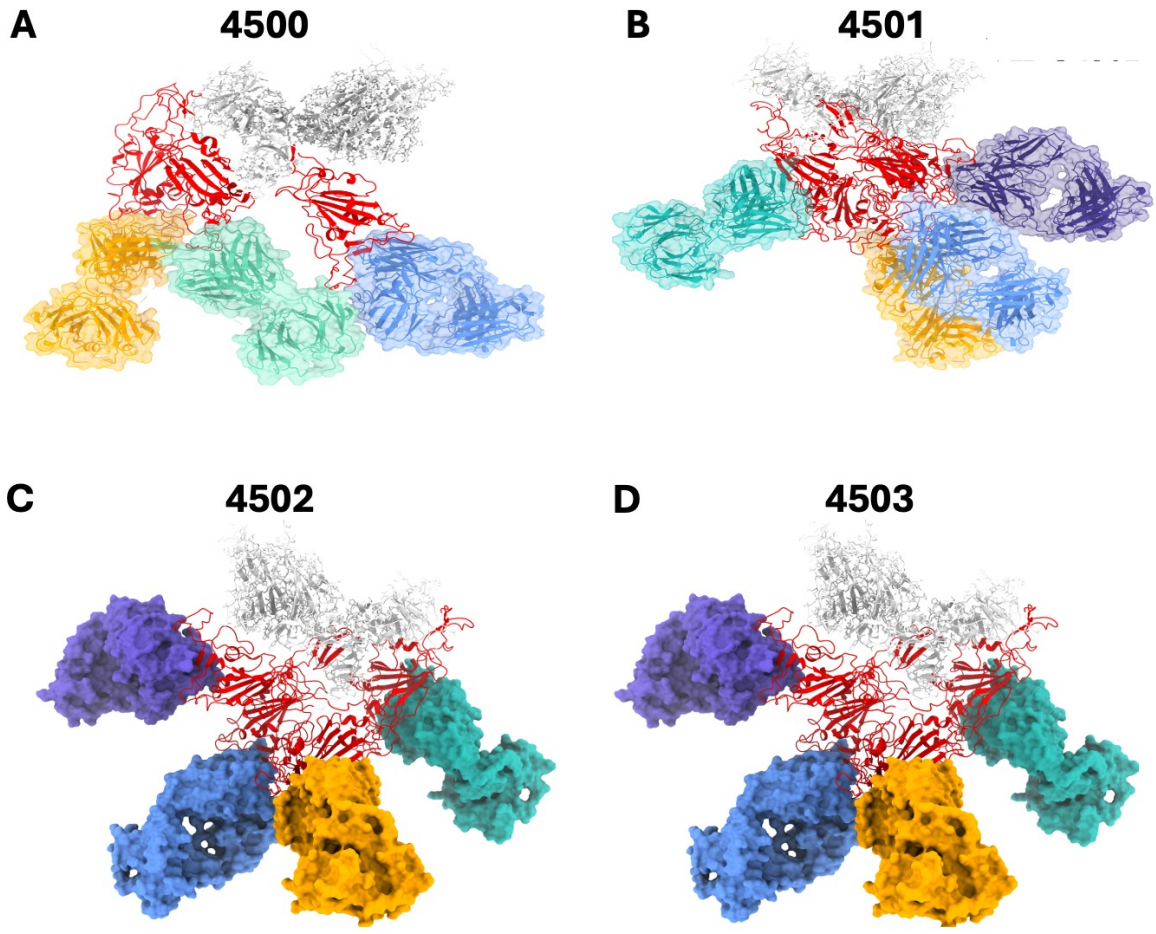
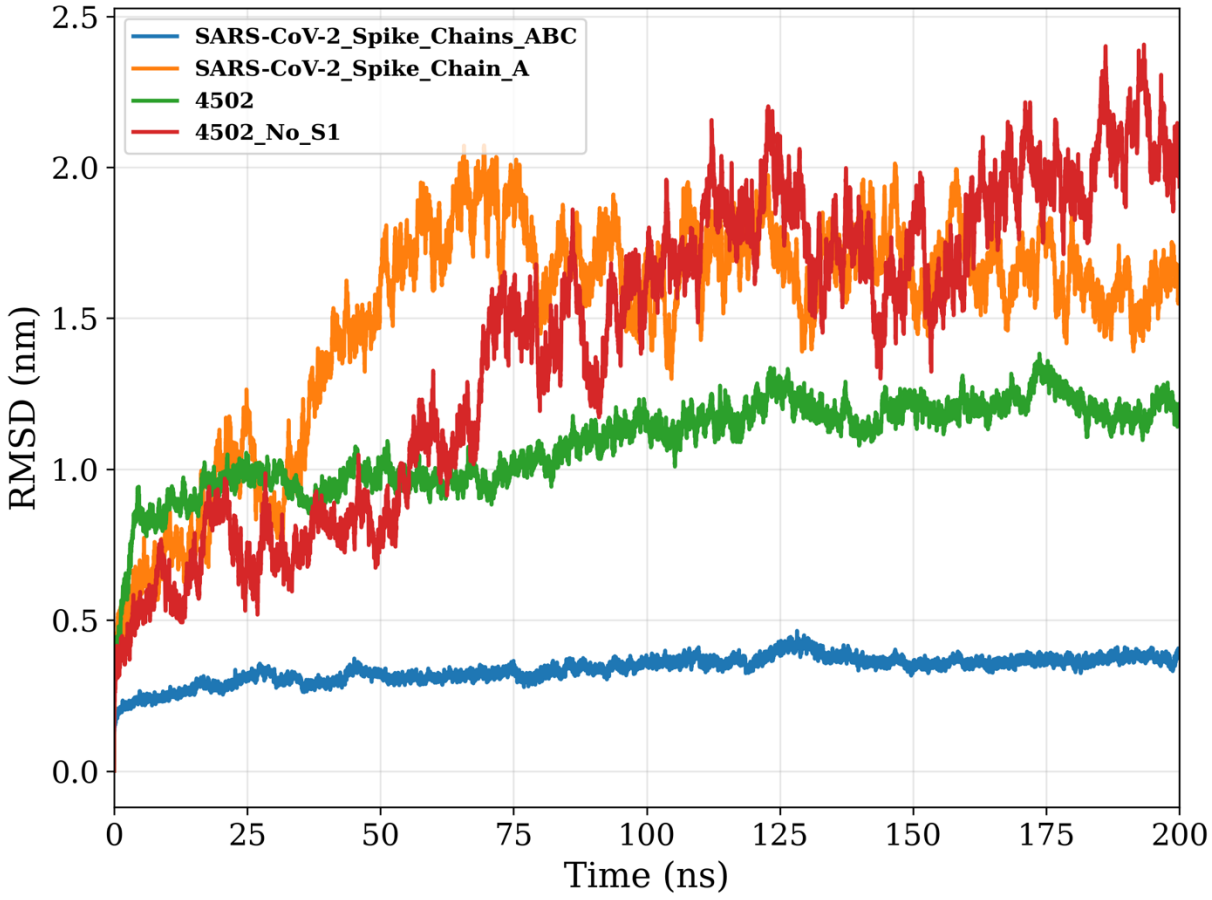


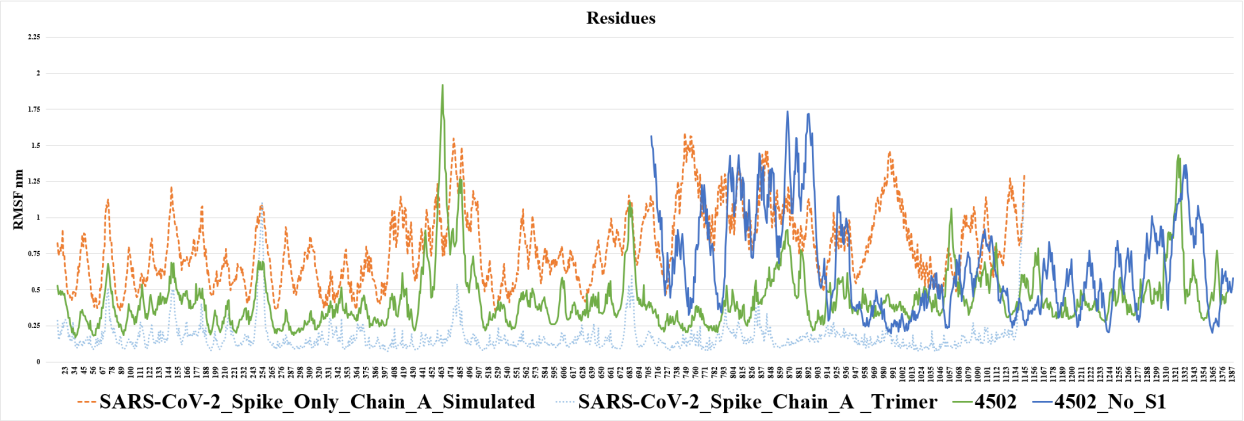
Supplementary Fig. 1. Bar graph of DiscoTope-3 predictions. The Y-axis represents the predicted likelihood scores of B-cell epitopes for all four pan-sarbecovirus vaccine candidates.



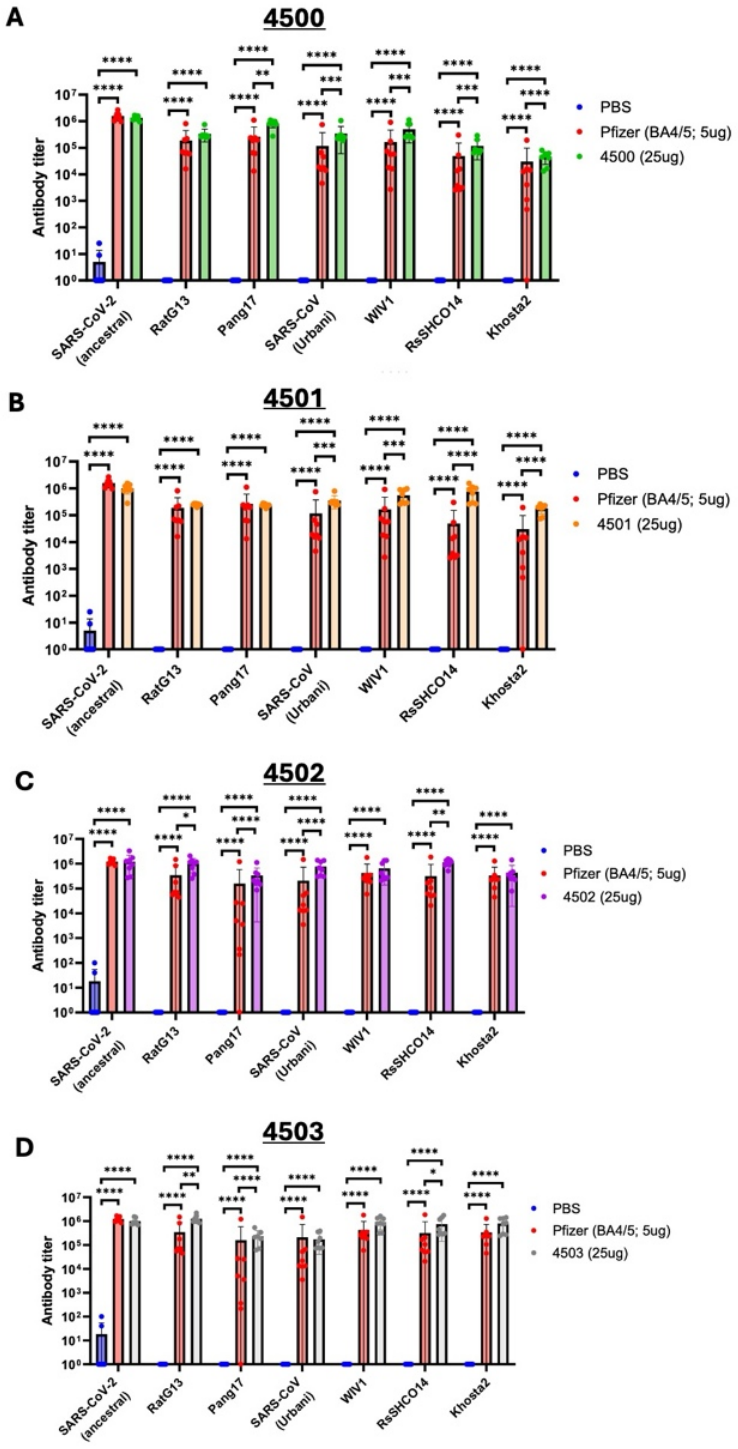
Supplementary Fig. 2. Docking results for pan-sarbecovirus vaccine candidates to the neutralizing CT-P59 antibody. Docking predictions of CT-P59 with 4500 (**A**), 4501 (**B**), 4502 (**C**), and 4503 (**D**). The CT-P59 antibody is shown in surface and colored in blue, green, orange and dark blue for the sarbecovirus RBDs of all vaccine candidates. Each panel illustrates the interaction between the RBD of the candidates (shown in red for all RBDs) and the CT-P59 antibody, revealing how the RBD engage key residues in the binding regions. The antibody's heavy and light chains are shown in surface representation, while each candidate includes multiple spike protein domains, only in red, the white colour shows the N and C-terminal of the SARS-CoV-2 Ancestral S1. These models demonstrate the stable and accessible binding orientations achieved across all four constructs. All RBDs are coloured in red.



Supplementary Fig. 3. Backbone RMSD of the native SARS-CoV-2 spike trimer, its monomer, VIDO4502 and its version with no S1 during 200 ns of MDS. The RMSD plots comparing the structural stability across the full spike trimer (blue), the monomeric spike chain A (orange), designed constructs, VIDO4502 (green) and its variant that lacks the S1 region (VIDO4502_No_S1, red). The ancestral Wuhan trimer remained highly stable throughout the simulation with a very low fluctuation at around 0.25 nm, opposite to the monomer and VIDO4502_No_S1, which have higher fluctuation. VIDO4502 reached equilibrium after ~50ns and remained stable during the course of the simulation.

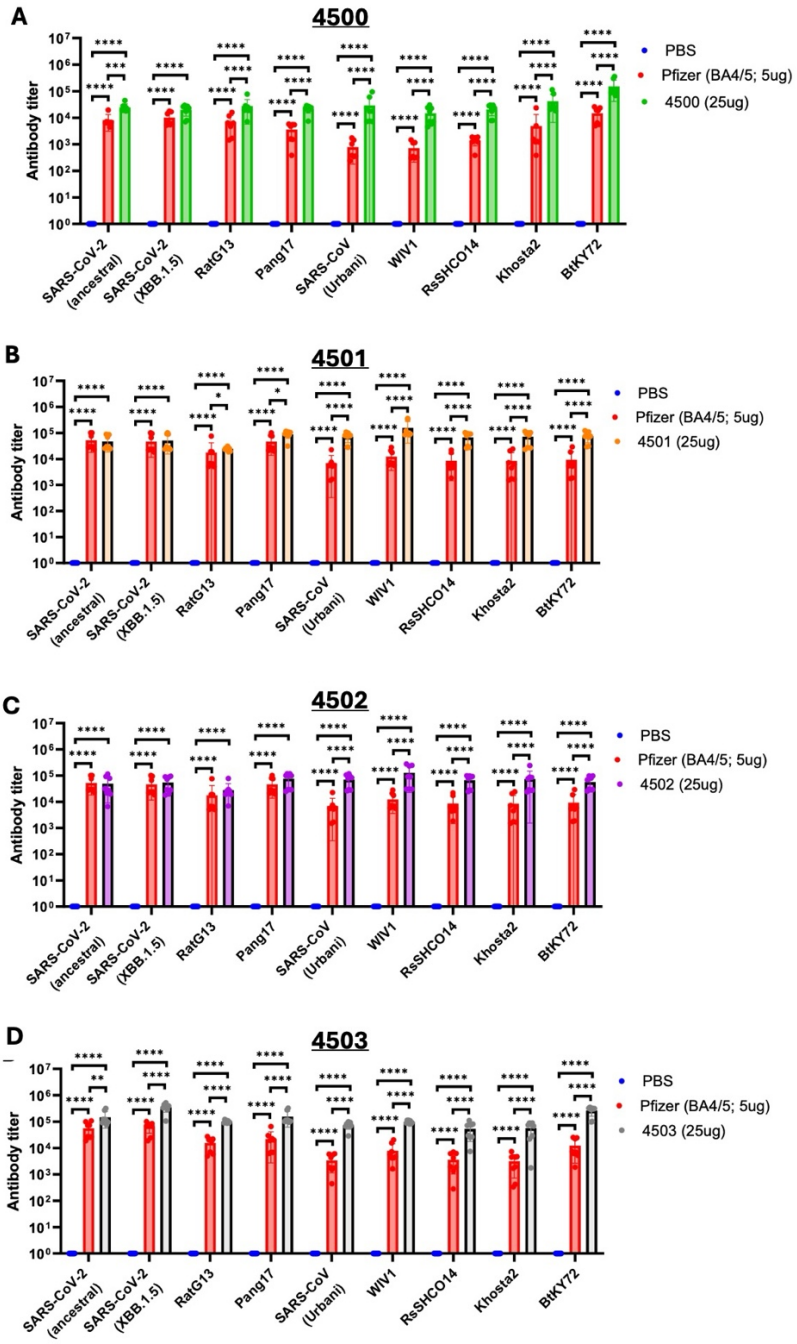


Supplementary Fig. 4. Residue-wise RMSF profiles for 4502 and three simulated proteins as control. SARS-CoV-2_Spike_Chain_A, SARS-CoV-2_Spike_Chains_ABC, 4502, 4502_No_S1 are colored in orange, dashed blue, green and deep blue, respectively.



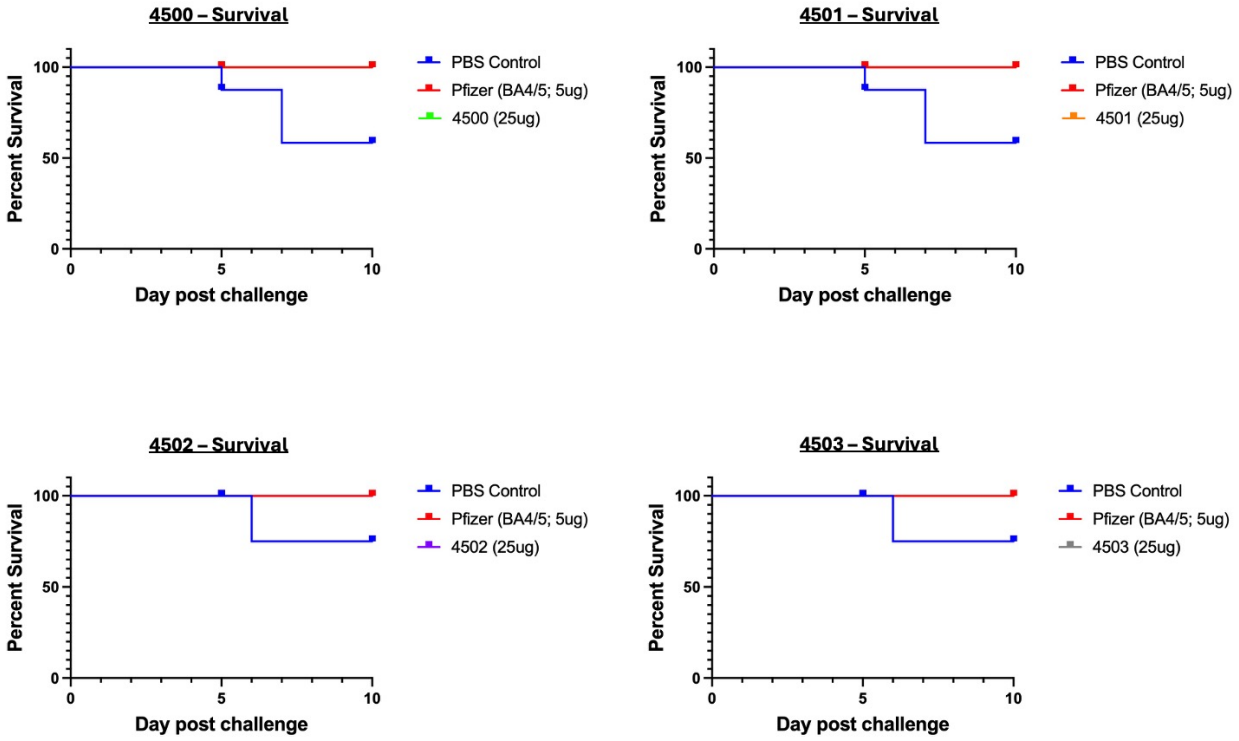
Supplementary Fig. 5. Multivalent protein subunit pan-sarbecovirus vaccine candidates induce broadly-reactive antibodies against sarbecovirus antigens after immunization in mice. Plasma isolated from immunized BALB/c mice post immunization were evaluated for broad-binding capabilities using ELISAs. Antibodies were evaluated for binding abilities toward a panel of sarbecovirus spike protein RBD (Ancestral SARS-CoV-2, WIV1, Khosta2,

RsSHC014, Pang17, Urbani, and RatG13) using EISAs for animals immunized with 4500 (A), 4501 (B), 4502 (C), and 4503 (D). Results are depicted as histograms where group results are compared to antibody levels from the control PBS group for statistical evaluation. Statistical analysis was conducted by Log transforming the raw and analyzing differences among groups using a Kruskal Wallis (One-way ANOVA) test with Dunn's multiple comparisons. A p value of ≤ 0.05 was considered statistically significant with $p<0.05$:*; $p<0.01$:**; $p<0.001$:***; $p<0.0001$:****.



Supplementary Fig. 6. Multivalent protein subunit pan-sarbecovirus vaccine candidate immunizations in Syrian hamsters elicit cross-binding antibodies against RBDs from various sarbecoviruses. Plasma isolated from Syrian hamsters post immunization in challenge studies were evaluated for broad-binding capabilities using ELISAs. Plasma was collected on Day 48 post immunization and boost with 25 ug of multivalent protein subunit pan-sarbecovirus vaccine candidates. Antibodies were evaluated for binding abilities toward a panel of sarbecovirus spike protein RBD (Ancestral SARS-CoV-2, WIV1, Khosta2, RsSHC014, Pang17, Urbani, and RatG13) using EISAs for animals immunized with 4500 (**A**), 4501 (**B**), 4502 (**C**), and 4503 (**D**). Results are depicted as histograms where group results are compared to antibody

levels from the control PBS group for statistical evaluation. Statistical analysis was conducted by Log transforming the raw and analyzing differences among groups using a Kruskal Wallis (One-way ANOVA) test with Dunn's multiple comparisons. A p value of ≤ 0.05 was considered statistically significant with $p<0.05$:*; $p<0.01$:**; $p<0.001$:***; $p<0.0001$:****.



Supplementary Fig. 7. Survival following SARS-CoV challenge in hamsters immunized with pan-sarbecovirus vaccine candidates, Pfizer COVID-19 vaccine, or PBS. Immunized Syrian hamsters were prime and boosted on Day 0 and 28, respectively, and then intranasally challenged on Day 48 with SARS-CoV Tor2 strain (6.24×10^4 $TCID_{50}$). Following challenge, hamsters that met criteria for humane intervention points were euthanized according to approved protocols. Four hamsters per group had planned removals for tissue collection necropsies on Day 5 and then four per group on Day 10. Survival was calculated in Prism according to the experimental design and calculated out of 8 hamsters until Day 5 and out of 4 hamsters for end day on Day 10.