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Supplementary Materials for

Multivariate mining of an alpaca immune repertoire identifies potent cross-neutralizing SARS-CoV-2 nanobodies

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The PDF file includes:

Tables S1 and S2
Figs. S1 and S2
Legend for data file S1

Other Supplementary Material for this manuscript includes the following:

Data file S1

Table S1: Nanobody amino acid sequences

Name	**AA sequence**
C11	QVQLVESGGGLVQPGGSLRLSCAASGSIVSNNAMGWYRQAPGKERELVAAITRGGITNYADSVKGRFTISRDNATNAVYLQMNTLKPEDTAVYYCNAWVPVEEVAGIARQFQEVWGQGTLVTVSS
C7	QVQLVESGGGLVQPGGSLKLSCAASGEFFRLLIMGWYRQAPGKQRELVASITPGGSTNYADSVKGRFTISRDNAKKMVYLQMNNLKPEDTALYYCNRAAVDGGGGYVPGRGDYWGQGTQVTVSS
D4	QVQLVESGGGLVQPGGSLRLSCAASGSVFIINAMGWYRQAPGKQRELVAAITSSGSTNYADSVKGRFTISRDNAKDTVYLQMNSLKPEDTAVYYCNAKGSSWYDLGGGAGDDYWGQGTQVTVSS
D9	QVQLVESGGGLVQPGGSLRLSCAASGSIFSIHGMGWYRQAPGKERELVATITSRGTTNYADSVKGRFTISRDNAKNTVYLQMNNLKPEDTAVYYCNAEFGTPPVGYDYWGQGTQVTVSS
E11	QVQLVESGGGLVQAGGSLRLSCAASGRTFSNSFVGWFRQAPGKEREFVAAISWNGVNTYYADSVKGRFTISRDNAKNTLYLQMNNLKPEDTAVYYCASGGEPLPRYYTDYASWVDYWGKGTLVTVSS
E2	QVQLVESGGGLVQPGGSLRLSCVASITMNSIVARAWYRQAPGKEREFIASITTDDITNYADSVKGRFTISRDHGKNTVYLQMNSLKPEDTAVYYCHLRTFRRAGADTIPYWGQGTHVTVSS
E4	QVQLVESGGGLVQPGGSLRLSCAASGFTLDDYAIGWFRQAPGKEREGVSFITSSDGSTYYVDSVKGRFTISRDNAKNTVYLQMNSLTPEDTAIYYCAVGPSFSYTGSTYYRSELPWDYDYWGQGTQVTVSS
F1	QVQLVESGGGLVQAGGSLRLSCAASGRTFSSYVMAWFRQAPGKEREFVATMDSSGGNRYYGDSVKDRFTISRDNAKNTVYLQMNSLKTEDTAVYYCAAYTGRMATGWGNGGWKEYDYWGQGTQVTVSS
F12	QVQLVESGGGLVQAGGSLRLSCAASGRTFSNNAMGWFRQAPGKERDFVAAISWSGDSTYYADSVKGRFTISRDNAKNTVYLQMNSLKPEDTAVYYCAAGGEPLPRYWTDYASWVDYWGKGTLVTVSS
G1	QVQLVESGGGLVQTGGSLRLSCAASGRTFSSNAMGWFRQAPGKEREFVAAINWSGGSTYYADSVKGRFTISRDNTKNTVYLQVNSLKPEDTAVYFCAAGGEPLPRYWSDYASWVDYWGKGTLVTVSS
G2	QVQLVESGGGLVQAGGSLRLSCAASGRTFSNYAMGWFRQAPGKEREFVAAISWSGGSTYYADSVKGRFTISRNNAKNTVYLQMNSLKPEDAAVYYCAAGGEGYDSDYGPPLAPDYWGQGTQVTVSS
G6	QVQLVESGGGLVQPGGSLRLSCAASGFTLDDYAIGWFRQAPGKEREGVSCIISNDGIKYYSDSVKGRFTISRDNAKNTVYLQMNSLKPEDTAVYYCAAERWGYSDCVAGYGMDYWGKGTLVTVSS

Table S2: Nanobody CDR sequences (Chothia)

Name	**CDR1**	**CDR2**	**CDR3**
C11	GSIVSNN	TRGGI	WVPVEEVAGIARQFQEV
C7	GEFFRLL	TPGGS	AAVDGGGGYVPGRGDY
D4	GSVFIIN	TSSGS	KGSSWYDLGGGAGDDY
D9	GSIFSIH	TSRGT	EFGTPPVGYDY
E11	GRTFSNS	SWNGVN	GGEPLPRYYTDYASWVDY
E2	ITMNSIV	TTDDI	RTFRRAGADTIPY
E4	GFTLDDY	TSSDGS	GPSFSYTGSTYYRSELPWDYDY
F1	GRTFSSY	DSSGGN	YTGRMATGWGNGGWKEYDY
F12	GRTFSNN	SWSGDS	GGEPLPRYWTDYASWVDY
G1	GRTFSSN	NWSGGS	GGEPLPRYWSDYASWVDY
G2	GRTFSNY	SWSGGS	GGEGYDSDYGPPLAPDY
G6	GFTLDDY	ISNDGI	ERWGYSDCVAGYGMDY

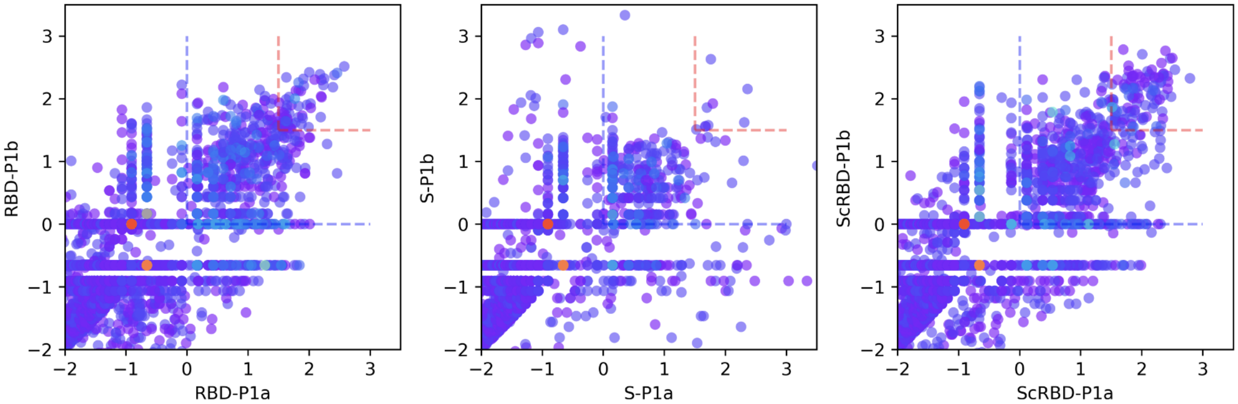

Figure S1. Within-panning consistency estimation. We compare two “versions” of each nanobody variant that differ by degenerate bases introduced with the cloning primer when establishing the baseline library. RBD and ScRBD panning shows consistency in enrichment, but spike panning is less consistent.

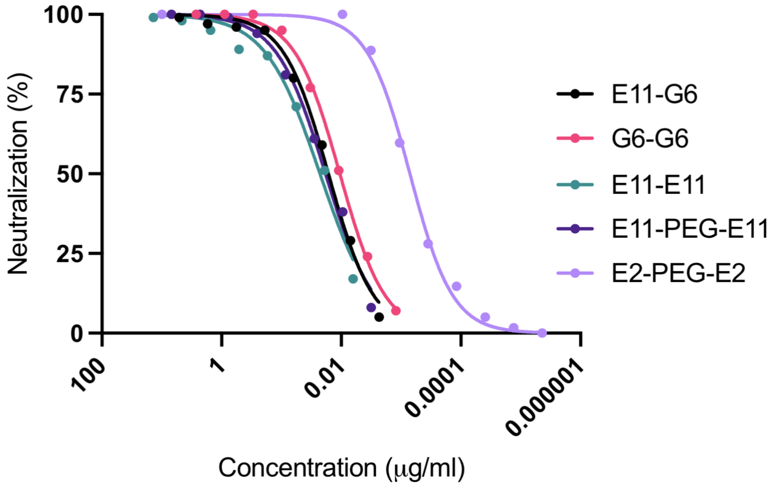

Figure S2. Ultrapotent neutralization of SARS-CoV-2 by nanobody homo- and heterodimers. Neutralization of SARS-CoV-2 spike pseudotyped lentiviruses are shown for nanobody dimers generated by Cu-free strain-promoted azide-alkyne click chemistry (SPAAC) reaction. E11 and G6 homo- and heterodimers neutralized with similarly high potency. An E2 dimer (with a short PEG11 spacer, E2-PEG-E2) was ultrapotent, with an IC50 of approximately 0.7 ng/ml (23 pM).

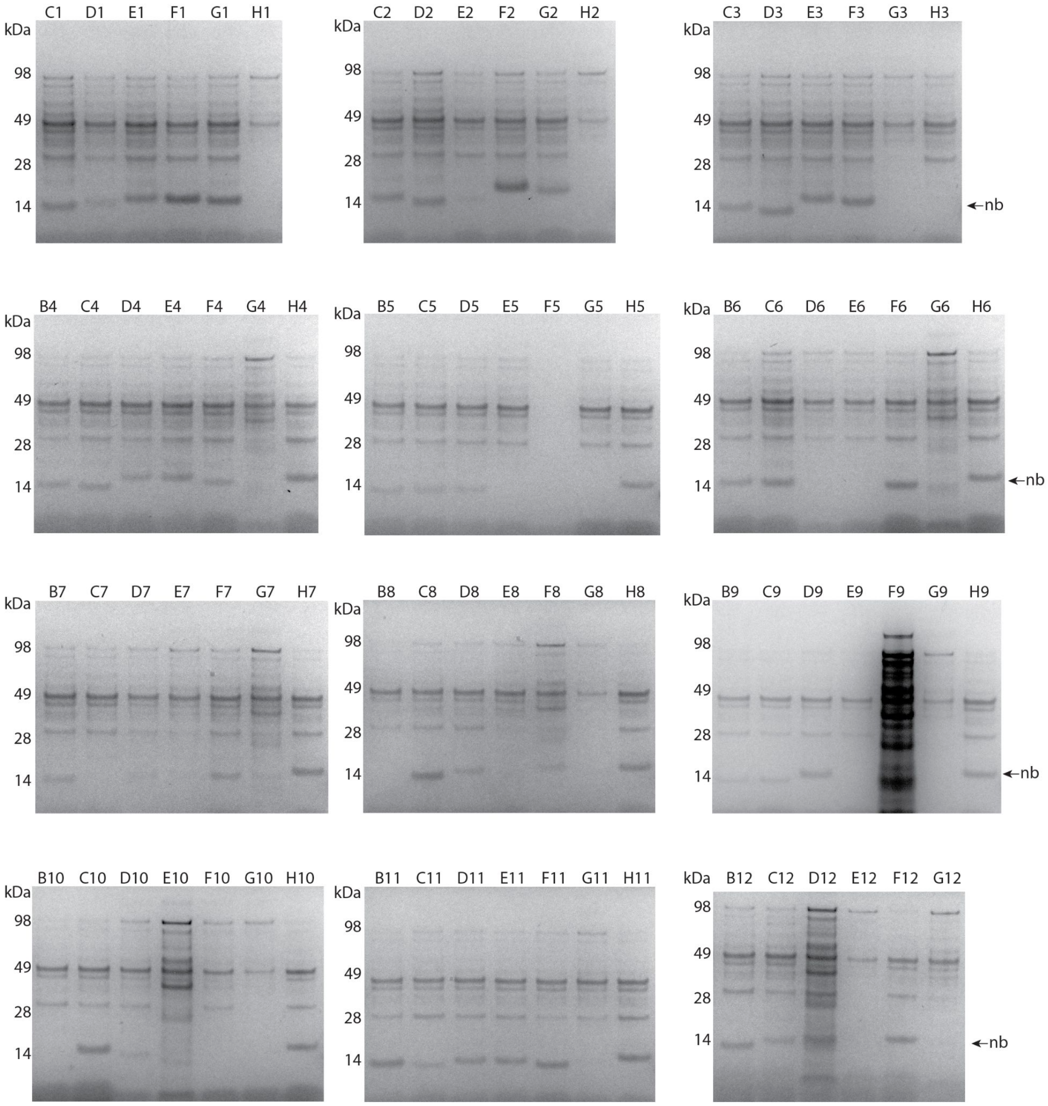

Figure S3. SDS-PAGE analysis and Coomassie staining of periplasmic extracts. Nanobodies (nb) run at 15 kDa.

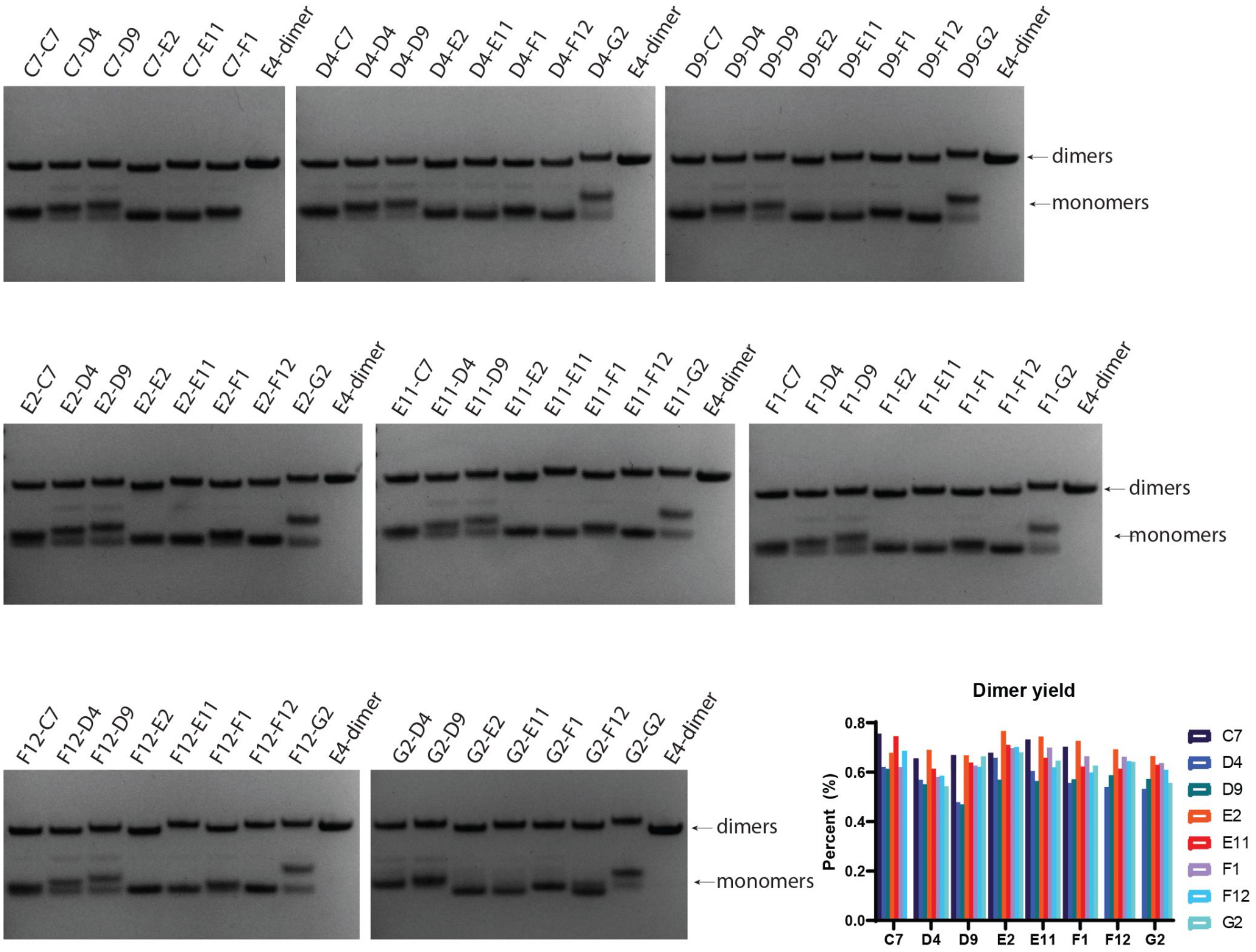

Figure S4. SDS-PAGE analysis and Coomassie staining of nanobody dimers generated to rapidly screen for potently neutralizing dimer pairs.

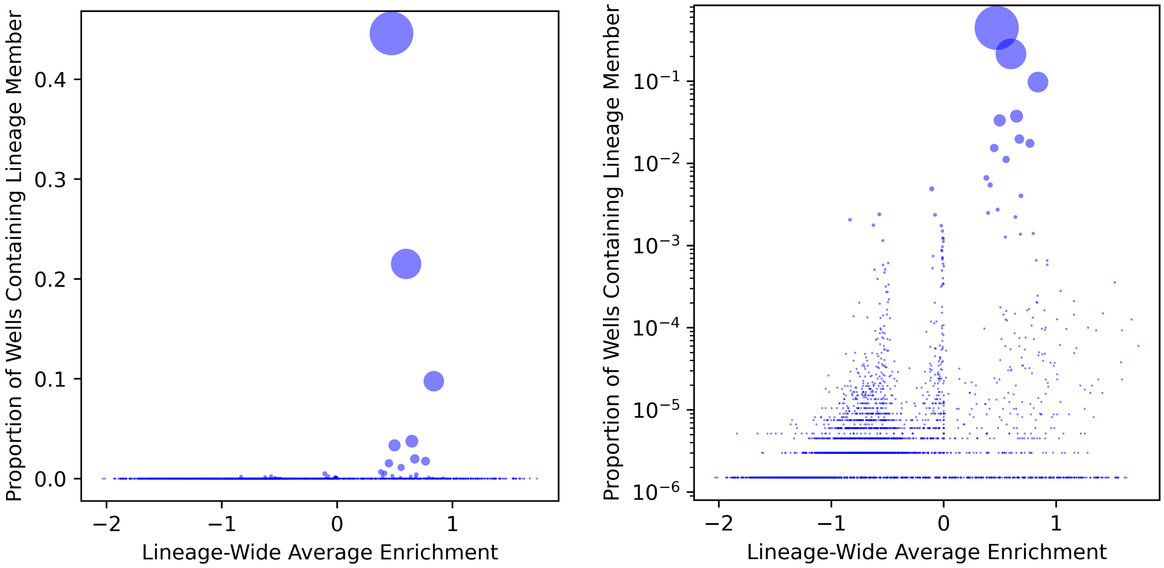

Figure S5. Lineage enrichment vs sampling probability. Each bubble represents a lineage, showing the mean enrichment for each lineage vs the probability that a single well would contain a member from that lineage, after a second round of RBD panning, estimated from NGS frequency data. The skewed size distribution of lineages, where a small number of lineages are extremely high frequency, shows that colony-based sampling approaches will not reach the lower frequency lineages. As an example, we would have had to screen nearly 500 colonies to have a >50% chance of discovering a member from the lineage of our highest affinity RBD binding nanobody (F1).

Supplementary File S1. HDX Experimental Details. Excel file containing HDX experimental conditions and results for five nanobodies.