Table S1. Amino acid sequences of constructs for protein expression. scFc sequences highlighted in blue, and RBD/RBD-Stab-SpyT sequences highlighted in purple.

Construct name	Amino acid sequence
Encapsulin-DS-SpyC	MEFLKRSFAPLTEKQWQEIDNRAREIFKTQLYGRKFVDVEGGGGGHHHHHHGGGGGPYGWEYAAHP LCEVEVLSDENEVVKWGLRKSLPLIELRATFTLLWELDNLECGKPNVDLSSLEETVRKVAEFEDEVIFRG CEKSGVKGLLSFEERKIECGSTPKDLLEAIVRALSIFSKDGIEGPYTLVINTDRWINFLKEEAGHYPLEKR VEECLRGGKIITTPRIEDALVVSERGGDFKLILGQDLSIGYEDREKDAVRLFITETFTMLLKFGSGSGSVT TLSGLSGEQGPSGDMTTEEDSATHIKFSKRDEDGRELAGATMELRDSSGKTISTWISDGHVKDFYLYP GKYTFVETAAPDGYEVATAITFTVNEQGQVTVNGEATKGDAHTGSSGS
WA1-RBD-SpyT	MGWSCIILFLVATATGVHSAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVD GVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQV YTLPPSRDELTKNQVSLYCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSR WQQGNVFSCSVMHEALHNHYTQKSLSLSPGKGGGGSGGGGSGGGGSGGGGSAPELLGGPSVFLFP PKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQD WLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEW ESNGQPENNYKTTPPVLDSDGSFFLTSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGKG GSGGGGSGGLEVLFQGPAHIVMVDAYKPTKGGSGGSGGRVQPTESIVRFPNITNLCPFGEVFNATRFA SVYAWNRKRISNCVADYSVLYNSASFSTFKCYGVSPTKLNDLCFTNVYADSFVIRGDEVRQIAPGQTGK IADYNYKLPDDFTGCVIAWNSNNLDSKVGGNYNYLYRLFRKSNLKPFERDISTEIYQAGSTPCNGVEGF NCYFPLQSYGFQPTNGVGYQPYRVVVLSFELLHAPATVCGPKKSTNLVKNK
BA.5-RBD-SpyT	MGWSCIILFLVATATGVHSAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVD GVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQV YTLPPSRDELTKNQVSLYCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSR WQQGNVFSCSVMHEALHNHYTQKSLSLSPGKGGGGSGGGGSGGGGSGGGGSAPELLGGPSVFLFP PKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQD WLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEW ESNGQPENNYKTTPPVLDSDGSFFLTSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGKG GSGGGGSGGLEVLFQGPAHIVMVDAYKPTKGGSGGSGGRVQPTESIVRFPNITNLCPFDEVFNATRFA SVYAWNRKRISNCVADYSVLYNFAPFFAFKCYGVSPTKLNDLCFTNVYADSFVIRGNEVSQIAPGQTGN IADYNYKLPDDFTGCVIAWNSNKLDSKVGGNYNYRYRLFRKSNLKPFERDISTEIYQAGNKPCNGVAGV NCYFPLQSYGFRPTYGVGHQPYRVVVLSFELLHAPATVCGPKKSTNLVKNK
WA1-RBD-Stab-SpyT	MGWSCIILFLVATATGVHSAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVD GVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQV YTLPPSRDELTKNQVSLYCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSR WQQGNVFSCSVMHEALHNHYTQKSLSLSPGKGGGGSGGGGSGGGGSGGGGSAPELLGGPSVFLFP PKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQD WLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEW ESNGQPENNYKTTPPVLDSDGSFFLTSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGKG GSGGGGSGGLEVLFQGPAHIVMVDAYKPTKGGSGGSGGRVQPTESIVRFPNIMNLCPFGEVFNATRF PSVYAWNRKRISNCYYDYSVLYNSASFSTFKCYGVSPTKLNDLCFTQVFADSFVIRGDEVRQIAPGQTG KIADYNYKLPDDFTGCVIAWNSNNLDSKVGGNYNYLYRLFRKSNLKPFERDTSTEIYQAGSTPCNGVEG FNCYFPLQSYGFQPTNGVGYQPYRVVVLSFELLDAPPTVCGPKKSTNLVKNK
BA.5-RBD-Stab-SpyT	MGWSCIILFLVATATGVHSAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVD GVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQV YTLPPSRDELTKNQVSLYCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSR WQQGNVFSCSVMHEALHNHYTQKSLSLSPGKGGGGSGGGGSGGGGSGGGGSAPELLGGPSVFLFP PKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQD WLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEW ESNGQPENNYKTTPPVLDSDGSFFLTSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGKG GSGGGGSGGLEVLFQGPAHIVMVDAYKPTKGGSGGSGGRVQPTESIVRFPNIMNLCPFDEVFNATRF PSVYAWNRKRISNCYYDYSVLYNFAPFFAFKCYGVSPTKLNDLCFTQVFADSFVIRGNEVSQIAPGQTG NIADYNYKLPDDFTGCVIAWNSNKLDSKVGGNYNYRYRLFRKSNLKPFERDTSTEIYQAGNKPCNGVA GVNCYFPLQSYGFRPTYGVGHQPYRVVVLSFELLDAPPTVCGPKKSTNLVKNK

Table S2. Octet binding data of different immunogens with CB6 or LY-CoV1404 antibodies.

IgG	Immunogen	KD (M)	KD error	Kon (1/Ms)	Kon error	Koff (1/s)	Koff error
CB6	WA1-RBD-Stab	4.03×10^{-8}	1.35×10^{-9}	1.31×10^{5}	4.15×10^{3}	5.27×10^{-3}	5.58×10^{-5}
CB6	BA5-RBD-Stab	No binding					
CB6	WA1-RBD-Stab-EnDS-NP	$<1.0 \times 10^{-12}$	1.43×10^{-11}	2.72×10^{6}	7.70×10^{4}	$<1.0 \times 10^{-7}$	
CB6	BA.5-RBD-Stab-EnDS-NP	7.84×10^{-10}	1.19×10^{-11}	7.52×10^{5}	5.23×10^{3}	5.89×10^{-4}	7.92×10^{-6}
CB6	Mosaic-WA1-BA.5-Stab-EnDS-NP	$<1.0 \times 10^{-12}$	4.01×10^{-12}	1.99×10^{6}	1.26×10^{4}	$<1.0 \times 10^{-7}$	
LY-CoV1404	WA1-RBD-Stab	$<1.0 \times 10^{-12}$	1.63×10^{-10}	1.93×10^{5}	6.35×10^{3}	$<1.0 \times 10^{-7}$	
LY-CoV1404	BA5-RBD-Stab	$<1.0 \times 10^{-12}$	1.77×10^{-10}	2.13×10^{5}	8.90×10^{3}	$<1.0 \times 10^{-7}$	
LY-CoV1404	WA1-RBD-Stab-EnDS-NP	$<1.0 \times 10^{-12}$	1.27×10^{-11}	3.04×10^{6}	8.48×10^{4}	$<1.0 \times 10^{-7}$	
LY-CoV1404	BA.5-RBD-Stab-EnDS-NP	$<1.0 \times 10^{-12}$	9.73×10^{-12}	1.05×10^{6}	8.32×10^{3}	$<1.0 \times 10^{-7}$	
LY-CoV1404	Mosaic-WA1-BA.5-Stab-EnDS-NP	$<1.0 \times 10^{-12}$	1.43×10^{-12}	3.30×10^{6}	1.14×10^{4}	$<1.0 \times 10^{-7}$	

Table S3. Phylogenetic distance between RBDs.

Strain	WA1	BA.5	WA1/BA.5
WA1	0	0.0802	0
BA.5	0.0802	0	0
Delta	0.0091	0.0704	0.0091
BA.2	0.0753	0.0137	0.0137
BA.2.86	0.1256	0.0607	0.0607
BQ.1.1	0.0951	0.0137	0.0137
XBB.1.5	0.1052	0.0416	0.0416
EG.5.1	0.1102	0.0464	0.0464
FL.1.5.1	0.1102	0.0511	0.0511
SARS-1	0.3197	0.3652	0.3197
WIV1	0.2886	0.352	0.2886
Pangolin_GX P2V	0.1465	0.2008	0.1465

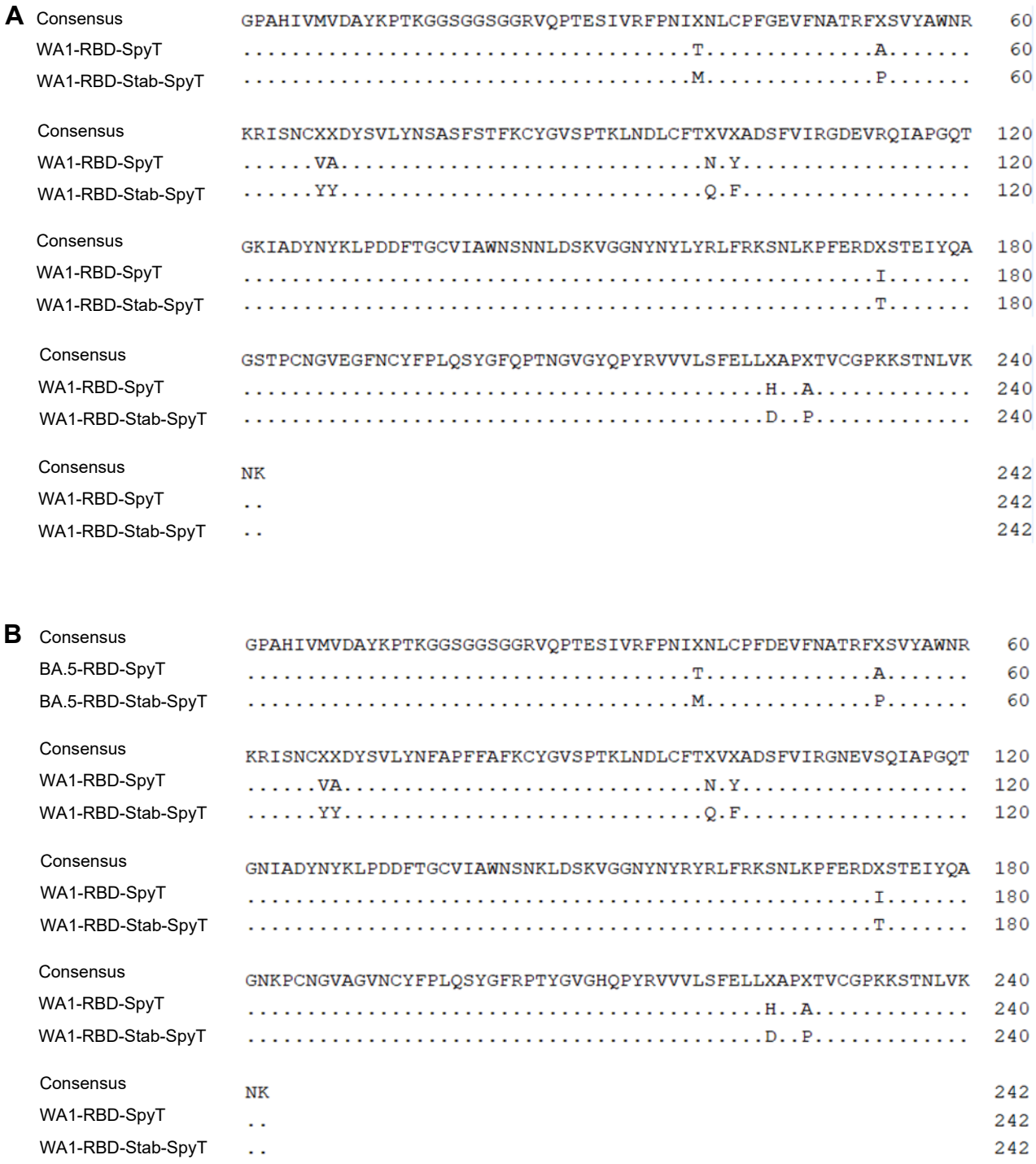

Figure S1. Amino acid sequence alignment of different RBD constructs. (A) WA1-RBD-SpyT and WA1-Stab-RBD-SpyT protein alignment. (B) BA.5-RBD-SpyT and BA.5-Stab-RBD-SpyT protein alignment.

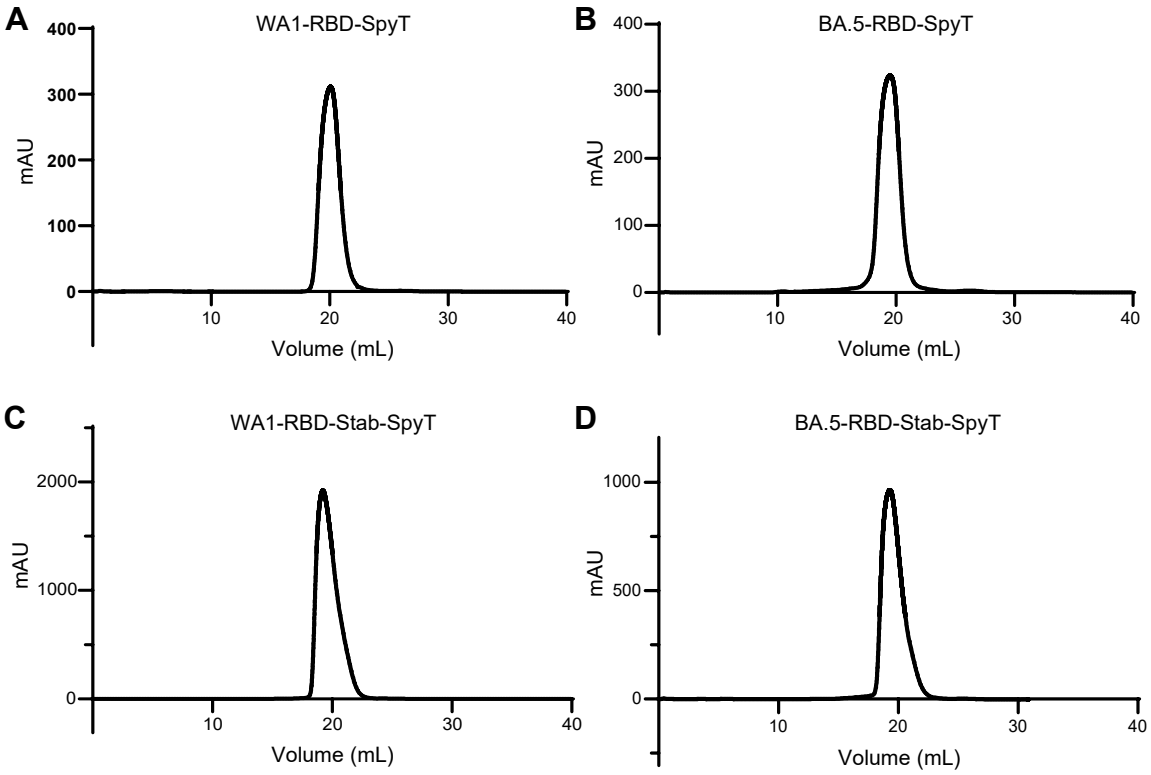

Figure S2. High purity RBD-SpyT proteins produced for nanoparticle preparation. SEC profiles of WA1-RBD-SpyT (A), BA.5-RBD-SpyT (B), WA1-RBD-Stab-SpyT (C), and BA.5-RBD-Stab-SpyT (D).

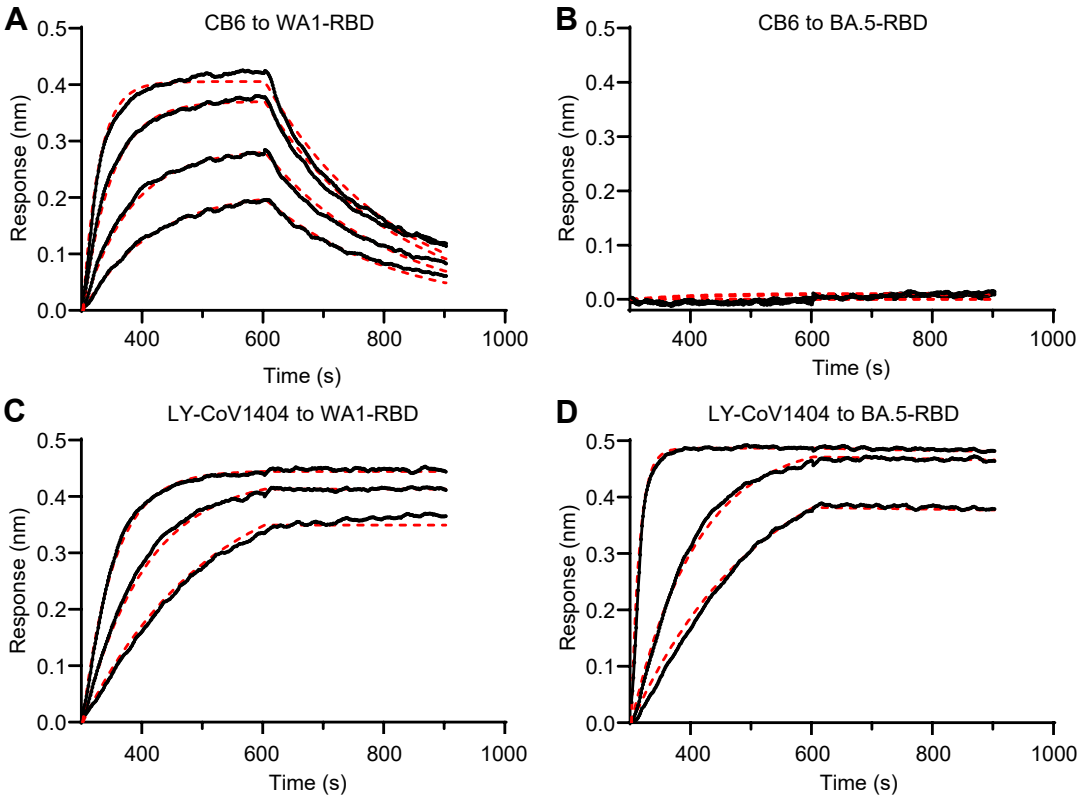

Figure S3. Antigenic analysis of different RBD proteins. (A-D) BLI measurements are shown in black with fitting provided with dotted red lines. (A) Antigenic data with CB6 antibody dipped into WA1-RBD. (B) Antigenic data with CB6 antibody dipped into BA.5-RBD. (C) Antigenic data with LY-CoV1404 antibody dipped into WA1-RBD. (D) Antigenic data with LY-CoV1404 antibody dipped into BA.5-RBD.

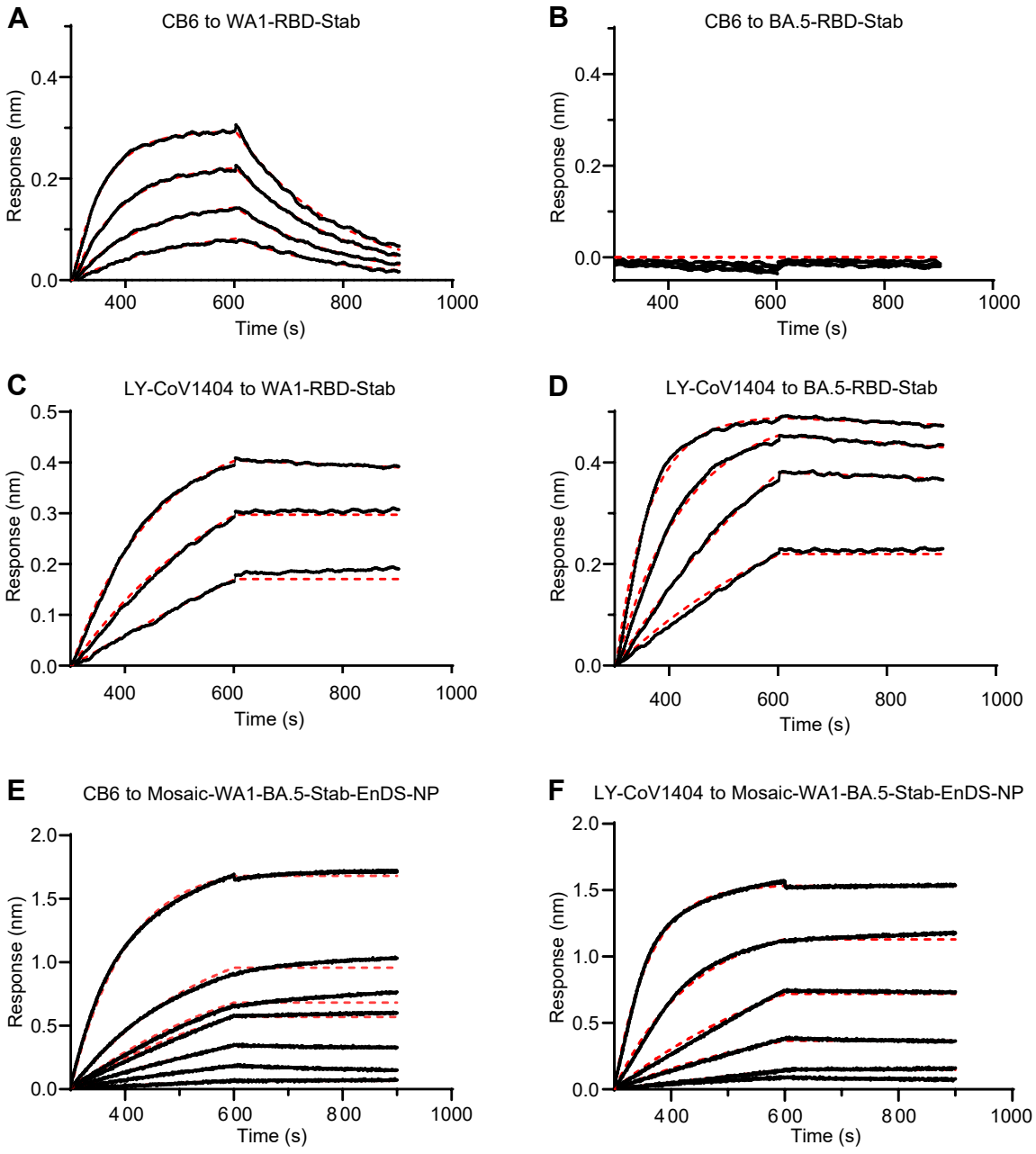

Figure S4. Antigenic analysis of different RBD proteins and Mosaic-WA1-BA.5-Stab-EnDS-NP. (A-F) BLI measurements are shown in black with fitting provided with dotted red lines. (A) Antigenic data with CB6 antibody dipped into WA1-RBD-Stab. (B) Antigenic data with CB6 antibody dipped into BA.5-RBD-Stab. (C) Antigenic data with LY-CoV1404 antibody dipped into WA1-RBD-Stab. (D) Antigenic data with LY-CoV1404 antibody dipped into BA.5-RBD-Stab. (E) Antigenic data with CB6 antibody dipped into Mosaic-WA1-BA.5-Stab-EnDS-NP. (F) Antigenic data with LY-CoV1404 antibody dipped into Mosaic-WA1-BA.5-Stab-EnDS-NP.

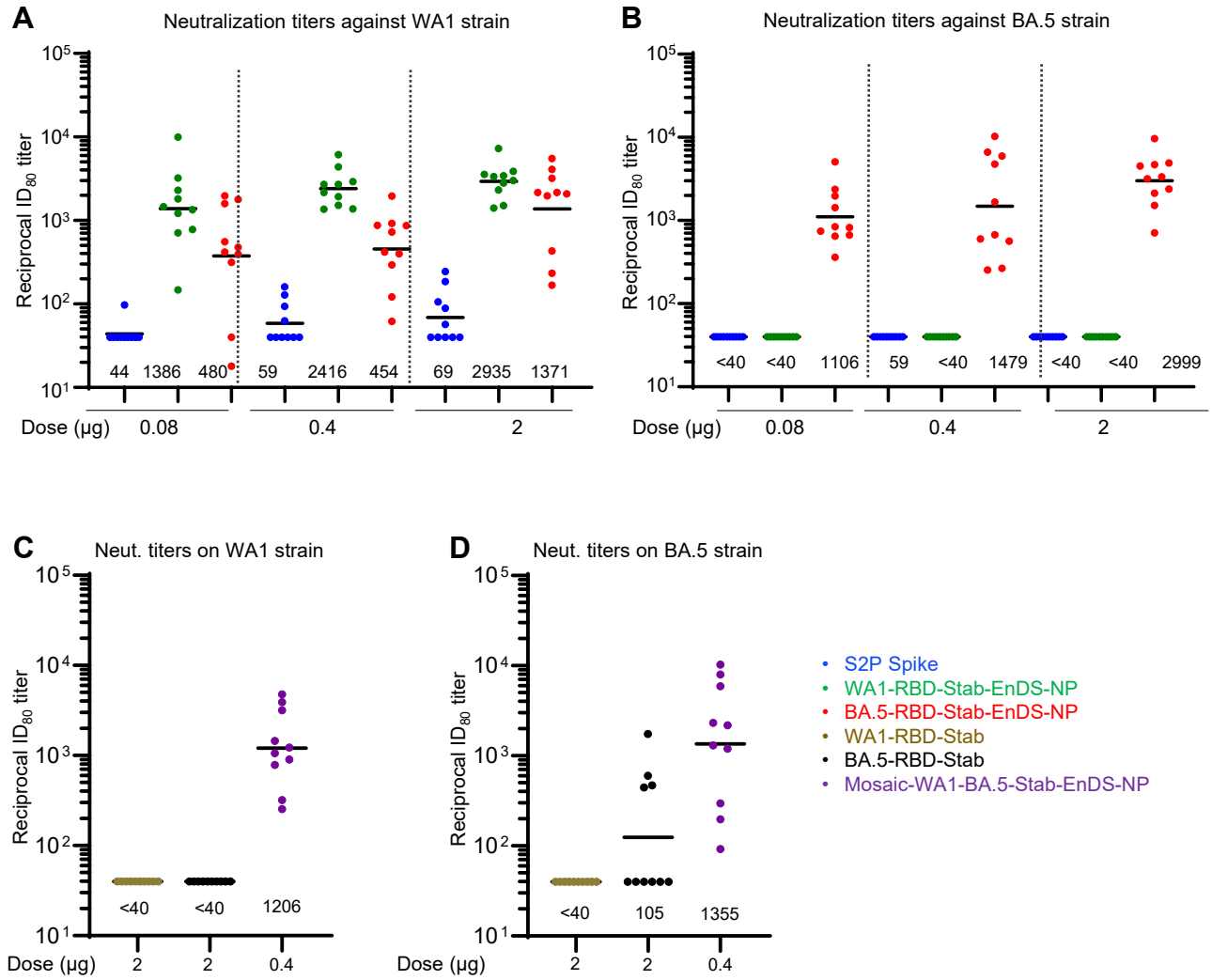

Figure S5. Immunization of stabilized RBD-EnDS nanoparticles elicited strong anti-SARS-CoV-2 pseudovirus neutralizing responses in mice. (A) Anti-SARS-CoV-2 WA1 pseudovirus neutralization ID80 titers from different immunogens. (B) Anti-SARS-CoV-2 BA.5 pseudovirus neutralization ID80 titers from different immunogens. (C) Anti-SARS-CoV-2 WA1 pseudovirus neutralization ID80 titers from different immunogens. (D) Anti-SARS-CoV-2 BA.5 pseudovirus neutralization ID80 titers from different immunogens.

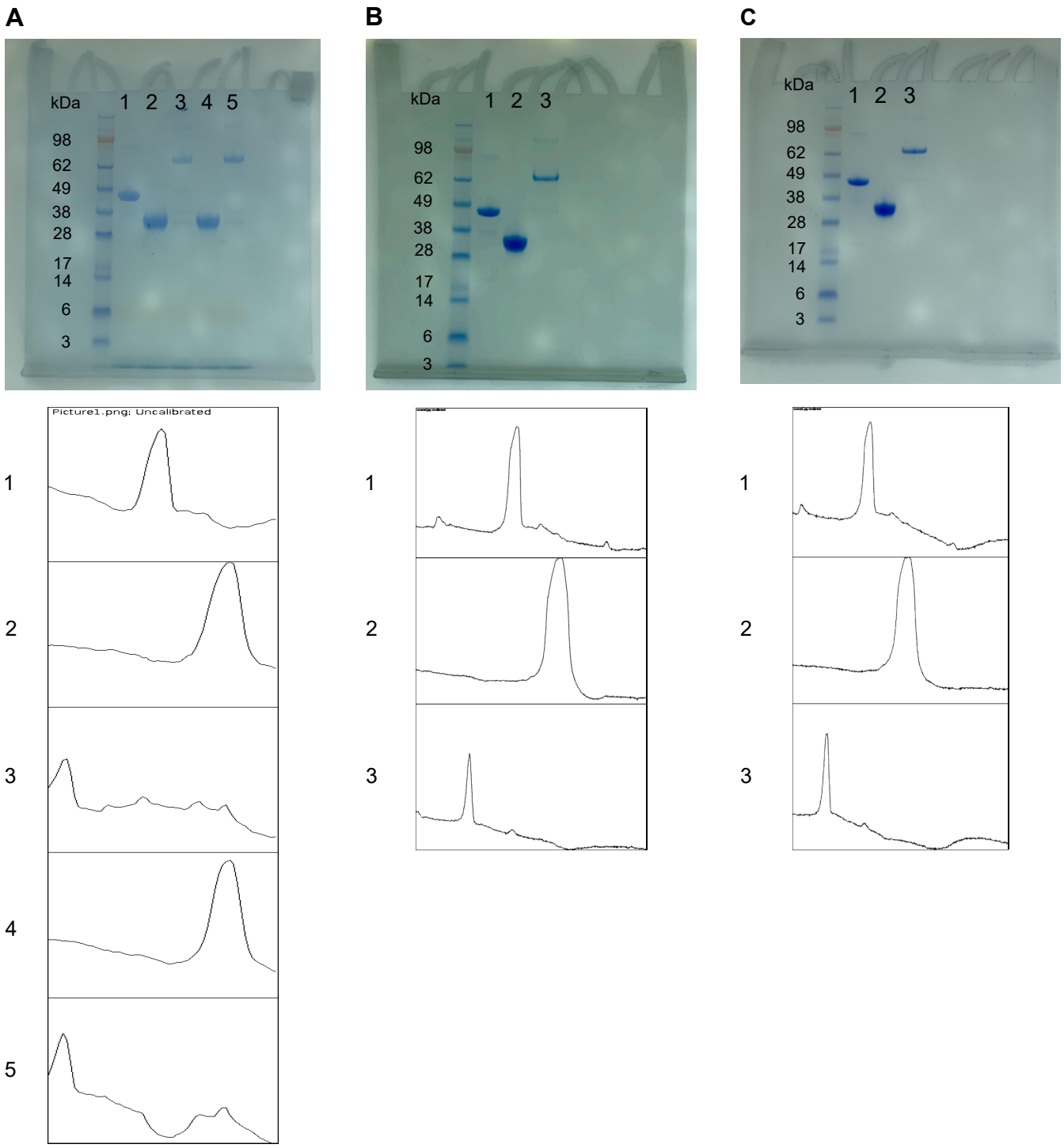

Figure S6. Uncropped SDS-PAGE images and densitometry readings of each lane. (A) Uncropped image of Figure 1C and densitometry readings of each lane. (B) Uncropped image of Figure 3E and densitometry readings of each lane. (C) Uncropped image of Figure 3F and densitometry readings of each lane. Molecular weight markers are show to the left of each gel.