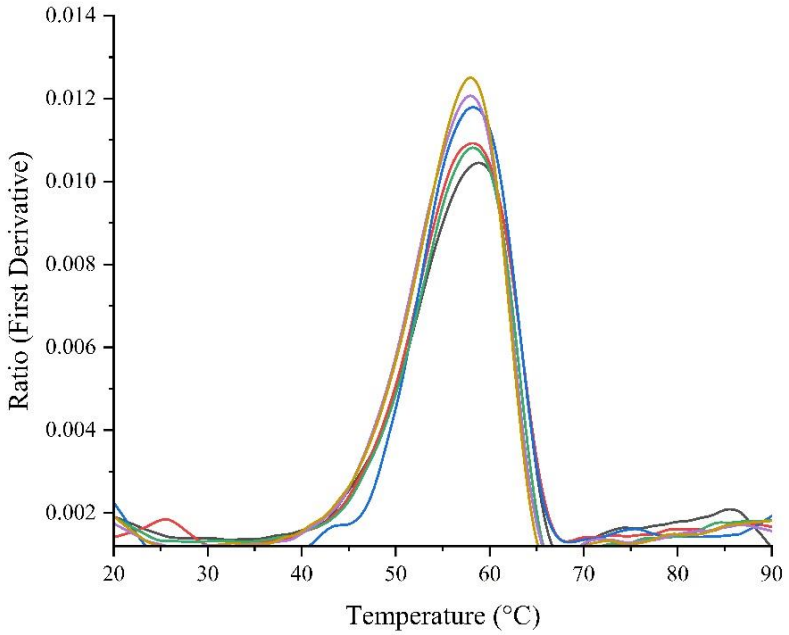
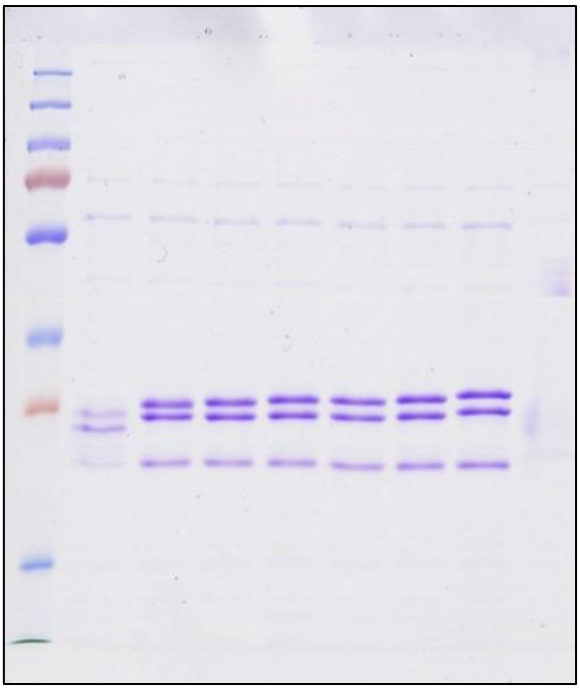
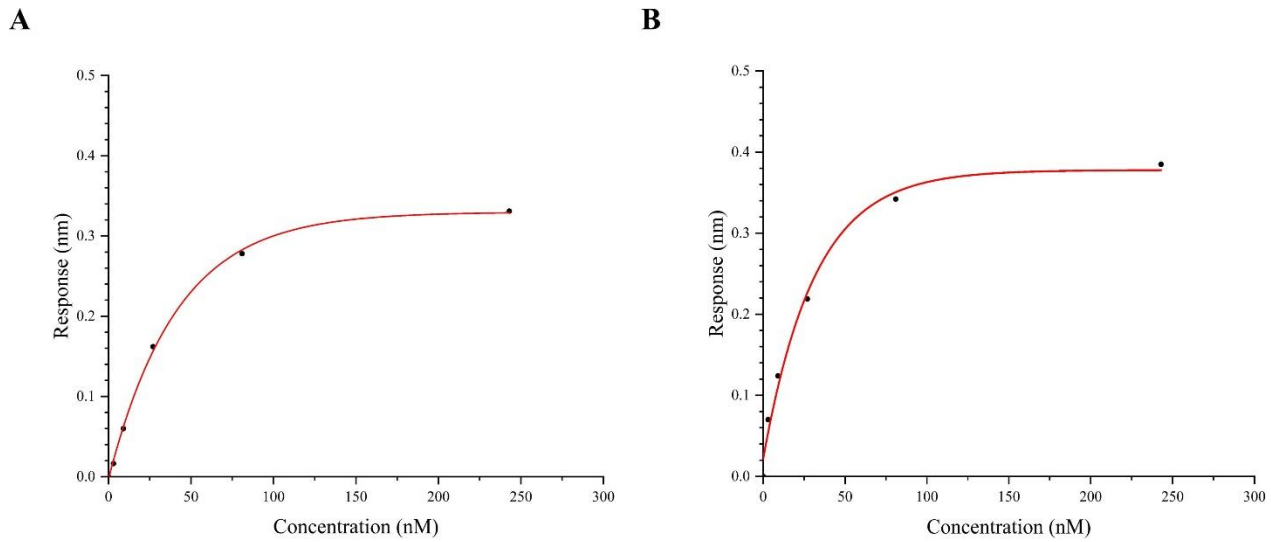
Supporting information



Supplementary Figure S1. Thermal stability analysis of Fab_H3 variants with varying linker lengths at the interface of the variable domains and C_H3 domains using NanoDSF. A single co-operative unfolding peak at the same melting temperature (Tm) for each of the variants suggests no influence of the linker length on the thermal stability of the Fab_H3 format. GS-G_2: Black, GS-G_4: Red, GS-G_6: Blue, GS-G_8: Green, GS-$(G_4S)_2$: Purple, GS-$(G_4S)_3$: Yellow.

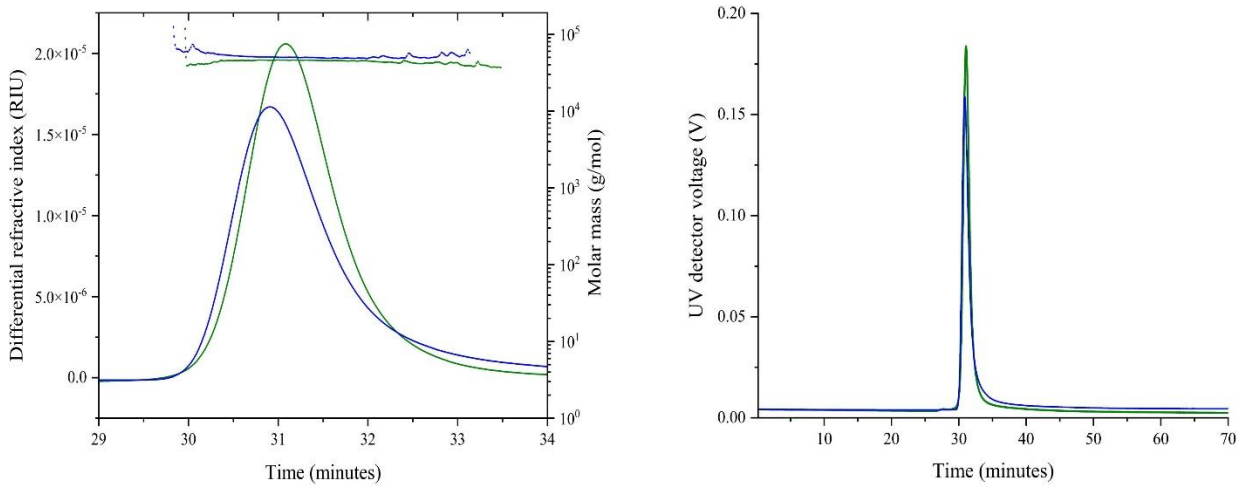


Supplementary Figure S2. Uncropped original scan of the SDS-PAGE gel image from Figure 2 in the main article.

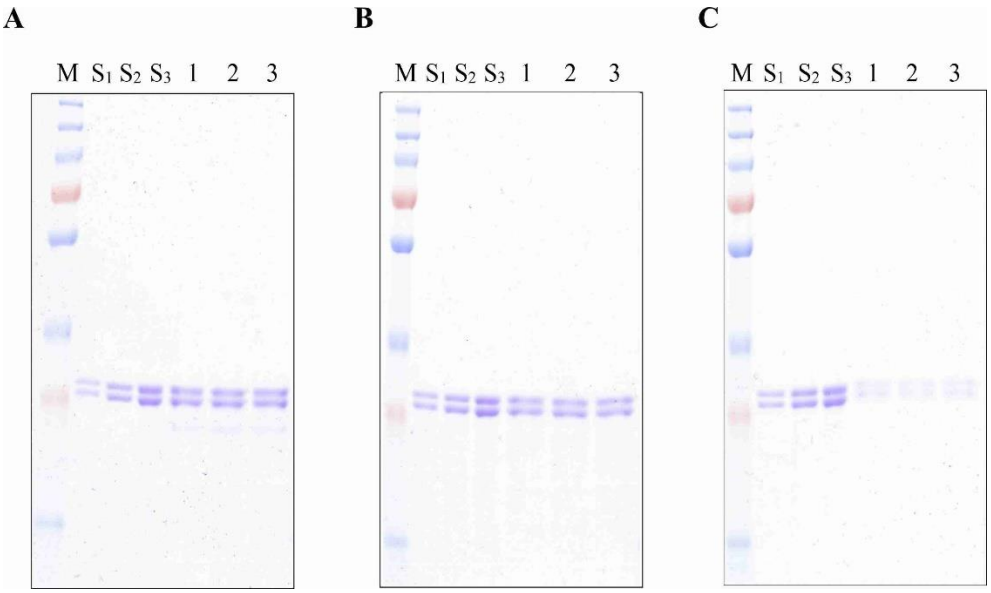


Supplementary Figure S3. Biolayer interferometry-derived steady state analysis of the binding response (nm) between **A.** REGN10987 Fab (R-square: 0.99), **B.** REGN10987-based Fab_H3 (R-square: 0.98) against SARS-CoV-2 Receptor Binding Domain (RBD) as a function of protein concentration (nM).

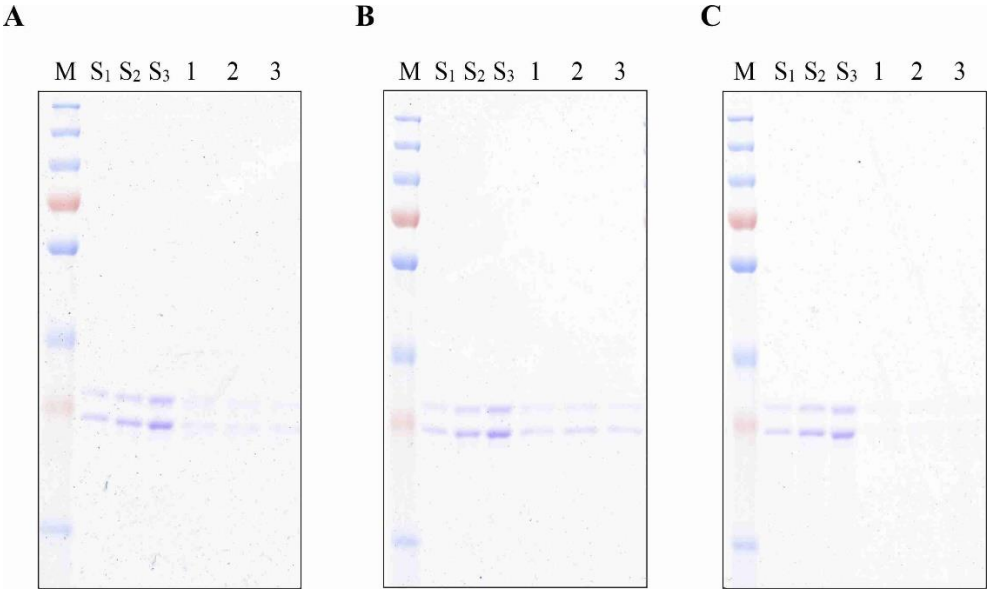
A **B**



Supplementary Figure S4. SEC-MALS analysis of the purified REGN10987-Fab (Green) and Fab_H3 (Blue). **A.** Differential refractive index (RIU) and molar mass (g/mol) against time (minutes). Polydispersity values of 1.001 and 1.002 for REGN10987-Fab and Fab_H3 respectively and a uniform molar mass distribution across the peak indicate a monodisperse species. **B.** UV signal overlay of protein separation through the SEC column against time (minutes) showing a single elution peak for the dimeric proteins and the absence of any degradants or aggregates.



Supplementary Figure S5. Uncropped SDS-PAGE gel images used for densitometry analysis of IMAC-purified REGN10987-based Fab_H3 (GS-G_4 linker) in triplicates (1,2,3) using three different concentrations of purified Fab_H3 (S1: 0.15 mg/mL, S2: 0.225 mg/mL, S3: 0.3 mg/mL) **A.** *E. coli* BL21 (DE3) in rich media, **B.** *E. coli* MG1655 in rich media, **C.** *E. coli* BL21 (DE3) in chemically defined minimal media. (M: Protein Marker)



Supplementary Figure S6. Uncropped SDS-PAGE gel images used for densitometry analysis of IMAC-purified REGN10987 Fab in triplicates (1,2,3) using three different concentrations of purified REGN10987 Fab (S1: 0.15 mg/mL, S2: 0.225 mg/mL, S3: 0.3 mg/mL) **A.** *E. coli* BL21 (DE3) in rich media, **B.** *E. coli* MG1655 in rich media, **C.** *E. coli* BL21 (DE3) in chemically defined minimal media. (M: Protein Marker)

Supplementary Table S1. Amino acid sequences of the constructs used in the study

Protein name	Sequence
REGN10987 wild-type Fab	**Light chain:** QSALTQPASVSGSPGQSITISCTGTSSDVGGYNYVSWYQQHPGKAPKLMIYDVSKRPSGV SNRFSGSKSGNTASLTISGLQSEDEADYYCNSLTSISTWVFGGGTKLTVLGQPKAAPSVTL FPPSSEELQANKATLVCLISDFYPGAVTVAWKADSSPVKAGVETTTPSKQSNNKYAASSY LSLTPEQWKSHRSYSCQVTHEGSTVEKTVAPTECS **Heavy chain:** QVQLVESGGGVVQPGRSLRLSCAASGFTFSNYAMYWVRQAPGKGLEWVAVISYDGSNK YYADSVKGRFTISRDNSKNTLYLQMNSLRTEDTAVYYCASGSDYGDYLLVYWGQGTLV TVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAV LQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDGSHHHHHH
Fab_H3 GS-G_2 linker	**Light chain:** QSALTQPASVSGSPGQSITISCTGTSSDVGGYNYVSWYQQHPGKAPKLMIYDVSKRPSGV SNRFSGSKSGNTASLTISGLQSEDEADYYCNSLTSISTWVFGGGTKLTVLGGSGGQPREPQ VYTLPPSRKEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLKSDGSFFLY SKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG **Heavy chain:** QVQLVESGGGVVQPGRSLRLSCAASGFTFSNYAMYWVRQAPGKGLEWVAVISYDGSNK YYADSVKGRFTISRDNSKNTLYLQMNSLRTEDTAVYYCASGSDYGDYLLVYWGQGTLV TVSSGSGGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYDT TPPVLDSDGSFFLYSDLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGGGSHHH HHH
Fab_H3 GS-G_4 linker	**Light chain:** QSALTQPASVSGSPGQSITISCTGTSSDVGGYNYVSWYQQHPGKAPKLMIYDVSKRPSGV SNRFSGSKSGNTASLTISGLQSEDEADYYCNSLTSISTWVFGGGTKLTVLGGSGGGGQPR EPQVYTLPPSRKEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLKSDGSF FLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG **Heavy chain:** QVQLVESGGGVVQPGRSLRLSCAASGFTFSNYAMYWVRQAPGKGLEWVAVISYDGSNK YYADSVKGRFTISRDNSKNTLYLQMNSLRTEDTAVYYCASGSDYGDYLLVYWGQGTLV TVSSGSGGGGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENN YDTTPPVLDSDGSFFLYSDLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGGGS HHHHHH

Fab_H3 GS-G_6 linker	**Light chain:** QSALTQPASVSGSPGQSITISCTGTSSDVGGYNYVSWYQQHPGKAPKLMIYDVSKRPSGV SNRFSGSKSGNTASLTISGLQSEDEADYYCNSLTSISTWVFGGGTKLTVLGGSGGGGGGQ PREPQVYTLPPSRKEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLKSDG SFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG **Heavy chain:** QVQLVESGGGVVQPGRSLRLSCAASGFTFSNYAMYWVRQAPGKGLEWVAVISYDGSNK YYADSVKGRFTISRDNSKNTLYLQMNSLRTEDTAVYYCASGSDYGDYLLVYWGQGTLV TVSSGSGGGGGGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPE NNYDTTPPVLDSDGSFFLYSDLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGG GSHHHHHH
Fab_H3 GS-G_8 linker	**Light chain:** QSALTQPASVSGSPGQSITISCTGTSSDVGGYNYVSWYQQHPGKAPKLMIYDVSKRPSGV SNRFSGSKSGNTASLTISGLQSEDEADYYCNSLTSISTWVFGGGTKLTVLGGSGGGGGGG GQPREPQVYTLPPSRKEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLKS DGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG **Heavy chain:** QVQLVESGGGVVQPGRSLRLSCAASGFTFSNYAMYWVRQAPGKGLEWVAVISYDGSNK YYADSVKGRFTISRDNSKNTLYLQMNSLRTEDTAVYYCASGSDYGDYLLVYWGQGTLV TVSSGSGGGGGGGGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQ PENNYDTTPPVLDSDGSFFLYSDLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSP GGGSHHHHHH
Fab_H3 GS-$(G_4S)_2$ linker	**Light chain:** QSALTQPASVSGSPGQSITISCTGTSSDVGGYNYVSWYQQHPGKAPKLMIYDVSKRPSGV SNRFSGSKSGNTASLTISGLQSEDEADYYCNSLTSISTWVFGGGTKLTVLGGSGGGGSGG GGSQPREPQVYTLPPSRKEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVL KSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG **Heavy chain:** QVQLVESGGGVVQPGRSLRLSCAASGFTFSNYAMYWVRQAPGKGLEWVAVISYDGSNK YYADSVKGRFTISRDNSKNTLYLQMNSLRTEDTAVYYCASGSDYGDYLLVYWGQGTLV TVSSGSGGGGSGGGGSQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESN GQPENNYDTTPPVLDSDGSFFLYSDLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSL SPGGGSHHHHHH

Fab_H3 GS-$(G_4S)_3$ linker	**Light chain:** QSALTQPASVSGSPGQSITISCTGTSSDVGGYNYVSWYQQHPGKAPKLMIYDVSKRPSGV SNRFSGSKSGNTASLTISGLQSEDEADYYCNSLTSISTWVFGGGTKLTVLGGSGGGGSGG GGSGGGGSQPREPQVYTLPPSRKEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYK TTPPVLKSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG **Heavy chain:** QVQLVESGGGVVQPGRSLRLSCAASGFTFSNYAMYWVRQAPGKGLEWVAVISYDGSNK YYADSVKGRFTISRDNSKNTLYLQMNSLRTEDTAVYYCASGSDYGDYLLVYWGQGTLV TVSSGSGGGGSGGGGSGGGGSQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVE WESNGQPENNYDTTPPVLDSDGSFFLYSDLTVDKSRWQQGNVFSCSVMHEALHNHYTQ KSLSLSPGGGSHHHHHH

Supplementary Table S2. Details of plasmid vectors used in the study

Construct details		**Plasmid name**	**Reference**
Gene 1	**Gene 2 (Polycistronic)**		
REGN10987 Fab Light chain	Heavy chain, C-terminal His6	pAAT50	[39]
REGN10987 (V_L)- GSG_2 - IgG_1 C_H3 (E356K,D399K)	REGN10987 (V_H)- GSG_2 - IgG_1 C_H3 (K392D,K409D), C-terminal His6	pAAT202	This study
REGN10987 (V_L)- GSG_4 - IgG_1 C_H3 (E356K,D399K)	REGN10987 (V_H)- GSG_4 - IgG_1 C_H3 (K392D,K409D), C-terminal His6	pAAT203	This study
REGN10987 (V_L)- GSG_6 - IgG_1 C_H3 (E356K,D399K)	REGN10987 (V_H)- GSG_6 - IgG_1 C_H3 (K392D,K409D), C-terminal His6	pAAT204	This study
REGN10987 (V_L)- GSG_8 - IgG_1 C_H3 (E356K,D399K)	REGN10987 (V_H)- GSG_8 - IgG_1 C_H3 (K392D,K409D), C-terminal His6	pAAT205	This study
REGN10987 (V_L)- $GS(G_4S)_2$ - IgG_1 C_H3 (E356K,D399K)	REGN10987 (V_H)- $GS(G_4S)_2$ - IgG_1 C_H3 (K392D,K409D), C-terminal His6	pAAT206	This study
REGN10987 (V_L)- $GS(G_4S)_3$ - IgG_1 C_H3 (E356K,D399K)	REGN10987 (V_H)- $GS(G_4S)_3$ - IgG_1 C_H3 (K392D,K409D), C-terminal His6	pAAT207	This study
CyDisCo components		pMJS205	[26]