Bovine ultralong CDR-H3 derived knob paratopes elicit potent TNF-α neutralization and enable the generation of novel adalimumab-based antibody architectures with augmented features

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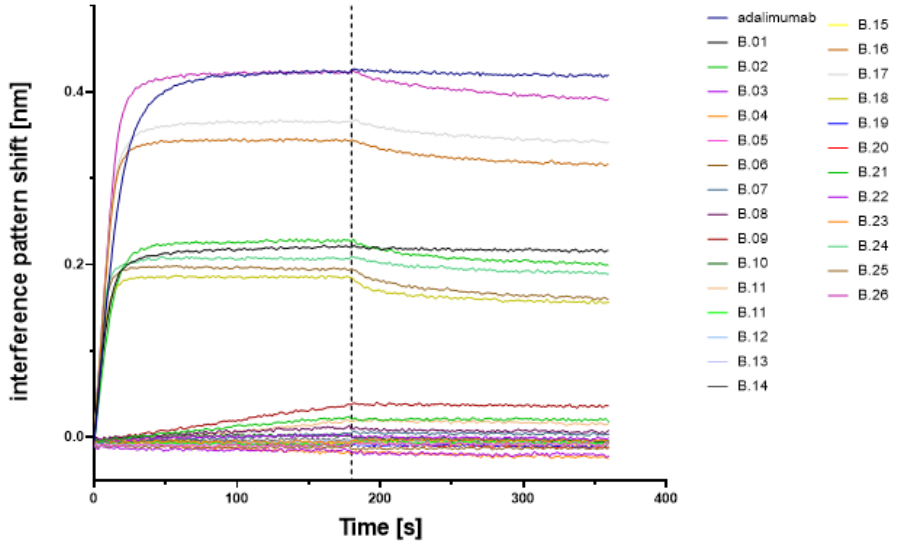
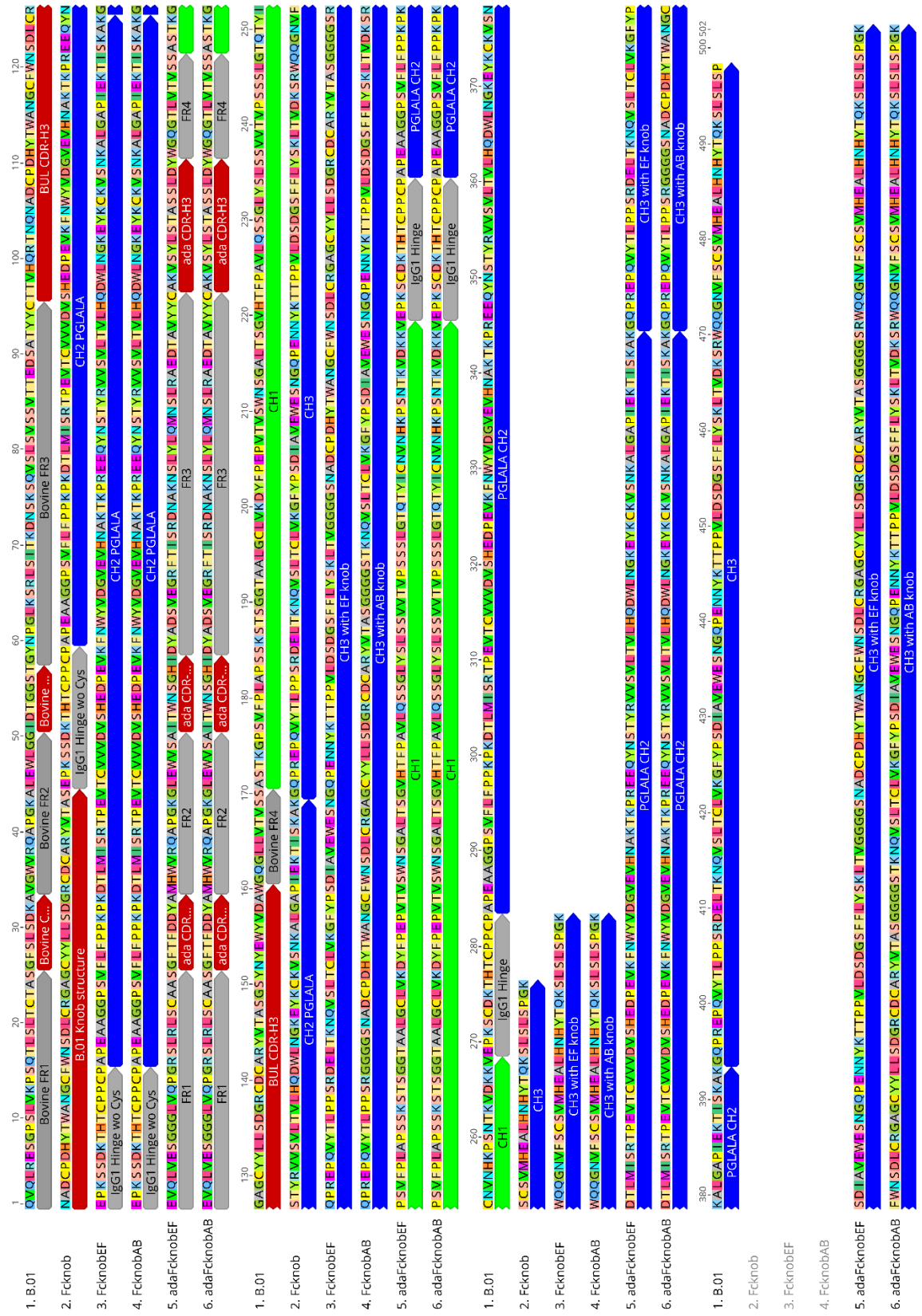
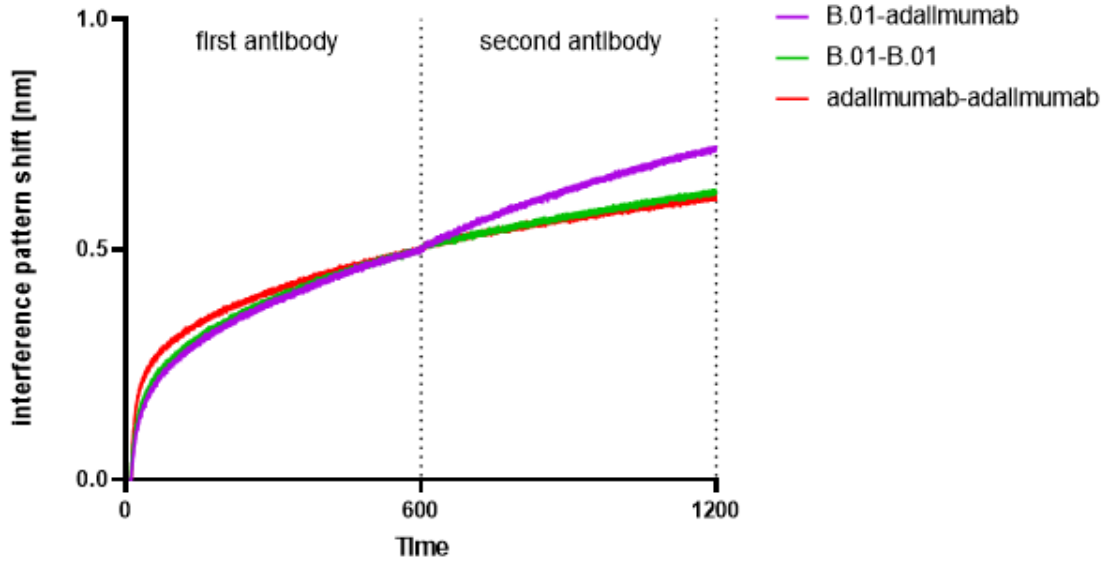
Supplementary material



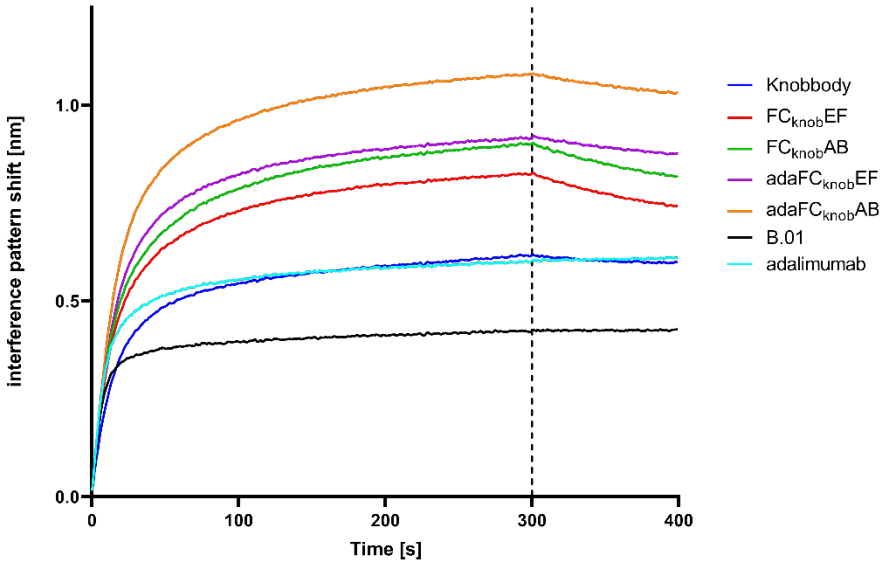
Supplementary Figure 1. Panel of bovine x human chimeric anti-TNF-α antibodies exhibit divers binding behavior in qualitative target engagement assays. Initial binding assessment of cattle-derived ultralong CDR-H3 antibodies as well as adalimumab against (rh) TNF-α antigen as determined by BLI. Sensograms show antigen association at 100 nM, respectively, and subsequent dissociation phase in kinetics buffer after sample immobilization on anti-human Fc biosensors.



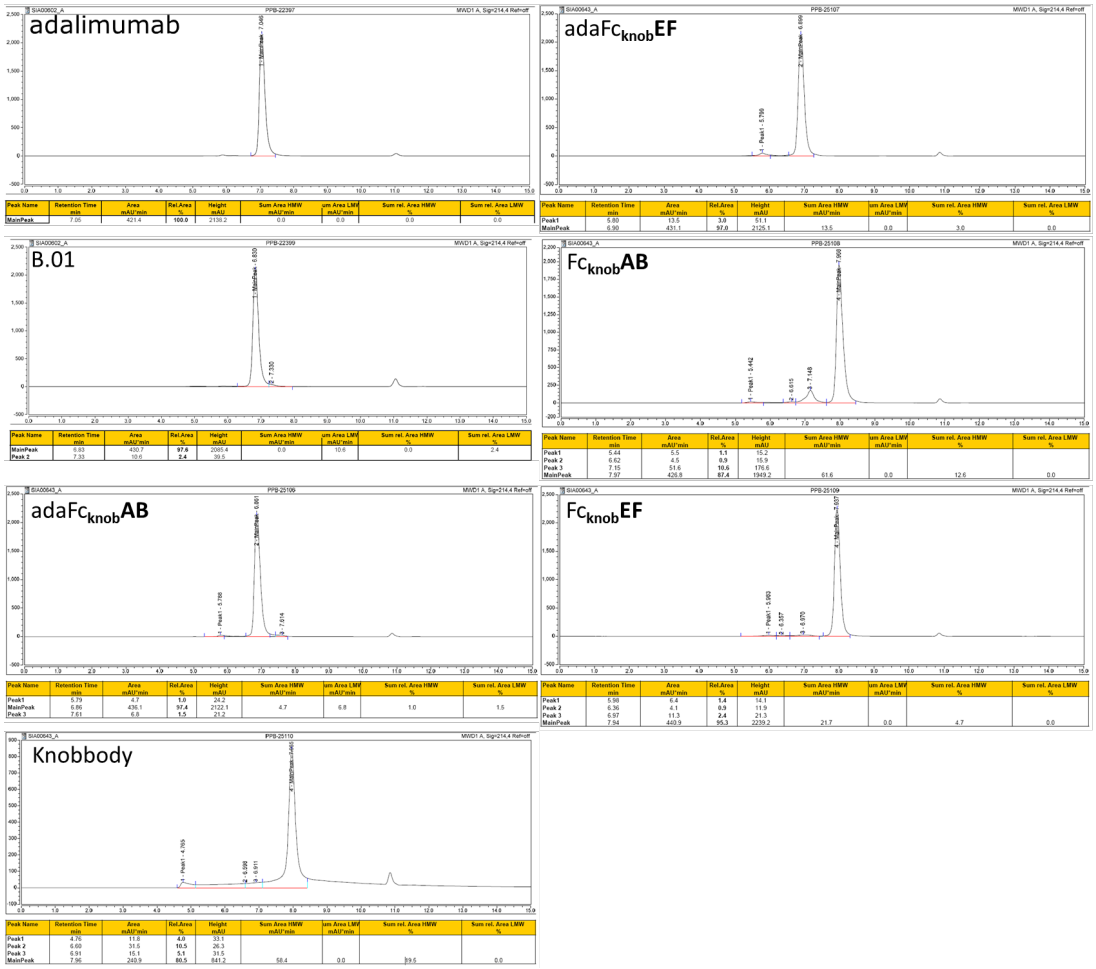
Supplementary Figure 2. Sequence list of generated constructs. Amino acid sequences of TNF-α targeting bovine x human chimeric antibody clone B.01 and engineered derivates Knobbody, $Fc_{knob}EF$, $Fc_{knob}AB$, $adaFc_{knob}EF$ $adaFc_{knob}AB$. Sequence list and annotations generated with Geneious Prime® 2023.1.2 software using standard coloring scheme highlighting every amino acid with distinct color.



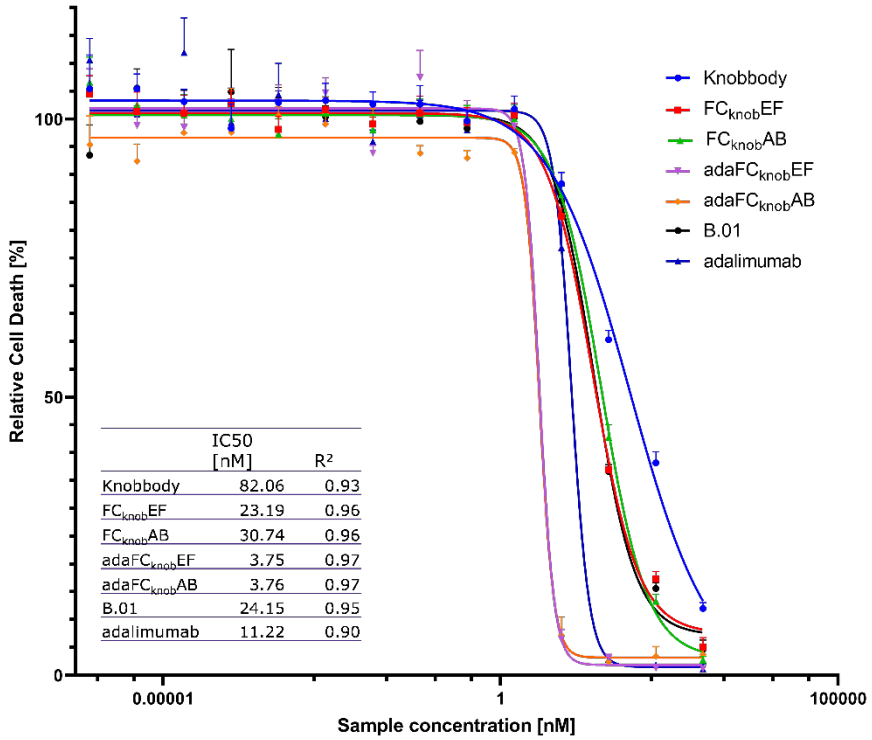
Supplementary Figure 3. Competition assay reveals non-identical but overlapping epitopes or steric hindrance of bovine x human chimeric antibody clone B.01 and adalimumab. Competitive binding assay for (rh) TNF-α of cattle-derived clone B.01 and adalimumab as determined by BLI. Sensograms show consecutive association of B.01 and adalimumab (magenta) in comparison with individual samples B.01 (green) and adalimumab (red) for 600 s, respectively, following prior TNF-α capture.



Supplementary Figure 4. Qualitative target engagement assays unveil superior TNF-α binding capacities for cattle-derived knob Fc engrafted variants. BLI-based binding evaluation of bovine x human chimeric antibody B.01 and engineered derivates Knobbody, Fc$_{knob}$EF, Fc$_{knob}$AB, adaFc$_{knob}$EF, adaFc$_{knob}$AB as well as adalimumab. Graphs indicate association of 100 nM (rh) TNF-α antigen, respectively, followed by dissociation in kinetics buffer after sample immobilization on anti-human Fc biosensors.



Supplementary Figure 5. Analytical SEC profiles of adalimumab, anti-TNF-α bovine x human antibody B.01 and B.01 derived Knobbody, Fc$_{knob}$EF, Fc$_{knob}$AB, adaFc$_{knob}$EF, adaFc$_{knob}$AB variants.



Supplementary Figure 6. Bovine antibody constructs neutralize the cytotoxic capacities of TNF-α towards L929 cells. Evaluation of the inhibitory capacities of bovine x human chimeric antibody B.01 and adalimumab as well as engineered derivates in an L929 cytotoxicity assay using (rh) TNF-α. Resulting IC_{50} values are given. A concentration of 10 nM was used for (rh) TNF-α. Dose response curves ±SEM were calculated using a non-linear regression variable slope four parameter fit from at least three independent experiments.

Supplementary Table 1. SEC purities and expression yields of anti-TNF-α bovine x human chimeric antibody panel derived from yeast surface display selection campaign.

ID	Purity [%]	Yield [mg/L]
B.01	97.5	96.7
B.02	76.2	30.5
B.03	90.2	20.1
B.04	95.4	22.3
B.05	54.2	38.8
B.06	89.9	30.2
B.07	96.1	30.2
B.08	61	34.9
B.09	73	16.2
B.10	72.4	30.7
B.11	98.7	95.0
B.12	97.2	17.4
B.13	68	26.4
B.14	68.9	28.7
B.15	96.1	59.0
B.16	98.2	66.3
B.17	98.1	55.6
B.18	98.8	40.4
B.19	91.4	33.1
B.20	98.3	39.3
B.21	94.7	34.2
B.22	98.8	41.1
B.23	98	48.8
B.24	100	33.5
B.25	95.8	83.5
B.26	98.5	69.8

Purities were determined by analytical size exclusion chromatography post protein A purification. Expression yields were determined after protein A purification.