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.

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	1 10 20 30 40 50 60 70 80 90 100 110 120
1. B.01	ÓVOLRESGPÉLUKPSOTI SITTOTASGESLÉDKAVGWROÁPGKALEWLGEIDTGGSTGYŃPGLKSRUSITÍKDNSKSQUSÚSVSSVI TEDŠATYYCT TVHÖRTNONADCPDHYTWANGCEWNSDLCR Rovina EPi Bruina C Rovina EPi Bruina C Rovina EPi Bruina EPi Bruina EPi Bruina EPi Bruina EPi Bruina EPi Bruina
2. Fcknob	
20120-1-1	B.01 Knob structure IgG1 Hinge wo Cys
3. FcknobEF	EPKSSDKTHTCPPCPAPEAAGGPSWFLEPPKPKDTLMISRTPEWTCVVVDVSHEDPEWKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKWSNKALGAPTEKTTISKAKG IgG1 Hinge wo Cys
4. FcknobAB	EPKS SDK THTCPPCPAPEAAGGPSWFILFPPK PKDTLMI SR TPEWTCWVVDVSHEDPEVKFNWYVDGVEVHNAK TKPREEQYNSTYRVV SWLIVLHQDWLNGKEYKCKWSNKALIGAPI IEKTI SKAKG LigG1 Hinge wo Cys
5. adaFcknobEF	ENQLVESGGGLVOPGRSLRLSCASGFTFDDYAMHWWRQAPGKGLEWVSALTWNSGHIDYADSNEGRFTLSRDNAKNSLYLQMNSLRAEDTAVYYCAKUSYLSTASSLDYWGQGTLVTMSSASTKG FR1 ada CDR
6. adaFcknobAB	E WOL VE SGGGL WOP GRSLI'R LSCASGETEDDYAMHWVRQAPGKGL EWVSALTWNSGH I DYADSVEGRETLI SRDNAKNSLYLQMNSLI RAEDTAVYYCAKVSYL STASSLDYWGQGTL VTVSSASTKG FR1 ada CDR
1. B.01	130 140 140 150 150 150 170 170 180 270 20 270 270 250 240 250 240 250 240 250 240 250 240 250 250 240 250 250 250 250 250 250 250 250 250 25
2. Fcknob	STYRUVSVLITVLHODWINGKEYKCKWSNKALIGAPLIEKTIJSKAKGOPREPOVYTUPPSRDELITKNOVSLITCLWKGFYPSDLIAVEWESNGOPENNYKTTPPVLDSDGSFFLYSKLITVDKSRWQOGNVF
3. FcknobEF	OP REPONYTUPPSRDELTKNOVSUTCUNKGFYPSDIANEWESNGOPENNYKTTPPVLDSDGSFFUYSKUTVGGGGSNADCPDHYTWANGCFWNSDUCRGAGCYYLUSDGRCDCARYNTASGGGGSR CH3 with EF knob
4. FcknobAB	QPREPQVYTLPPSRGGGGSNADCPDHYTWANGCFWNSDLCRGAGCYYLLSDGRCDCARYWTASGGGGSTKNQWSLTCLWKGFYPSDIIAWEWESNGQPENNYKTTPPVLDSDGSFFLYSKLITWDKSR CH3 with AB knob
5. adaFcknobEF	PSWFPLAPSSKSTSGGTAALGCLWKDYFPEPMTWSWNSGALTSGWHTFPAVLQSSGLYSLSSVUTWPSSSLGTQTYICNNNHKPSNTKWDKKWEPKSCDKTHTCPPCPAPEAAGGPSWFLFPPKPK CHI
6. adaFcknobAB	PSWFPLLAPSSKSTSGGTAALGCLUKDYFPEPNTNSWNSGALTSGVHTFPAVLQSSGLYSLUSSVUTVPSSSLGTQTYICNNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPEAAGGEVFLEPPKPK CH1 CH1
1. B.01	260 270 280 290 300 300 300 300 370 270 280 370 270 270 270 270 270 270 270 270 270 2
2. Fcknob	SC SVMH A LH NHY T QK S L S L S P G K CH3
3. FcknobEF	WOOGNUF S C SUMMEA LENNHYTOK S IL S I S P G K CH3 with EF knob MOODENWE S C SUMMEA ALENNEVTOK S IL S D C K
4. FONTOURIO	
	PGLALA CH2
6. adaFcknobAB	
1. B.01	30 30 40 40 40 40 40 40 50 50 50 50 50 50 50 50 50 50 50 50 50
2. Fcknob	
3. FcknobEF	
4. FcknobAB	
5. adaFcknobEF	SD I AVEWE SNG QPENNYKTTPPVLD SDG SFFLYSKLTVGGGG SNADCPDHYTWANGC FWN SDLCRGAGCYYLL SDGRCDCARYVTA SGGGG SRWQ QBNVFSC SWMHEALHNHYTQK SLL SPGK CH3 with FF knob
6. adaFcknobAB	FWNSDLCRGAGCYYLLSDGRCDCARYWTASGGGGSTKNQVSLTCLWKGFYPSDIAVEWESNGPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLLSPGK CH3 with AB knob

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 IC₅₀ 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.

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

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.

B.2698.569.8Purities were determined by analytical size exclusion chromatography post protein A
purification. Expression yields were determined after protein A purification.