SUPPLEMENTAL DATA

Identification of generic design principles for antibody based tumor necrosis factor (TNF) receptor 2 (TNFR2) agonists with FcγR-independent agonism

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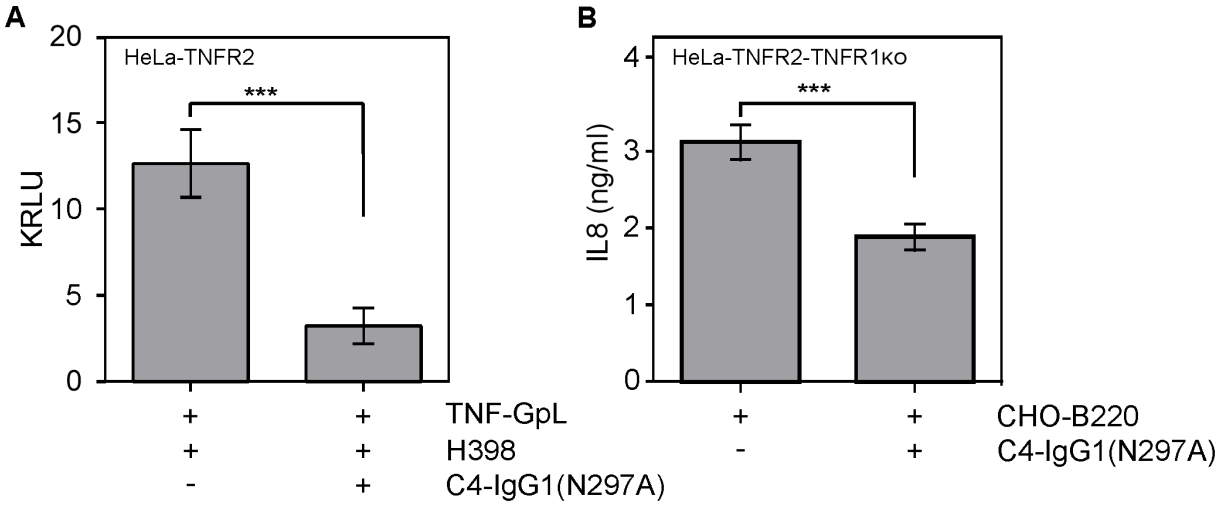
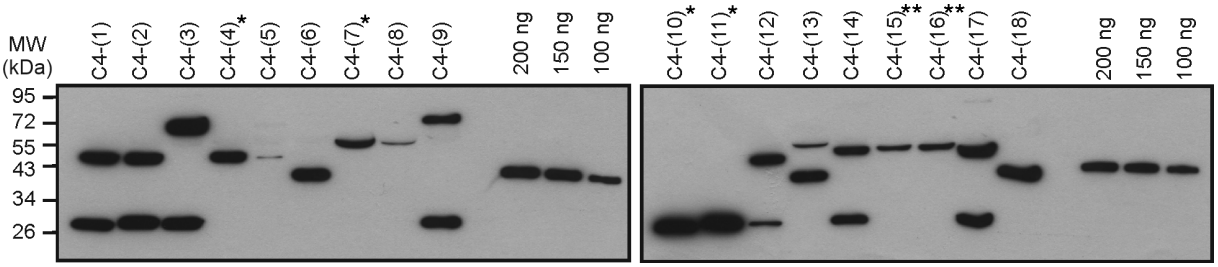


Supplemental Figure S1. **C4 is a TNFR2 antagonist.** (**A**) HeLa-TNFR2 cells were pretreated with 4 µg/ml of C4-IgG1(N297A) and were then incubated with 20 ng/ml of GpL-TNF at 37°C for 1 h. After removal of unbound GpL-TNF molecules, cell associated GpL-TNF was quantified by analysis of the cell attached luciferase activity. To prevent TNFR1 binding, cells were analyzed in the presence of 10 µg/ml of the TNFR1-blocking antibody H398. (**B**) HeLa-TNFR2-TNFR1$_{KO}$ cells were stimulated with membrane TNF-expressing CHO cells (CHO-B220) in the presence of 4 µg/ml of C4-IgG1(N297A) and next day TNFR2-mediated IL8 production was quantified by ELISA. Shown are the results of ten independent experiments in (A) and of six independent experiments in (B). ***, $p < 0.001$; repeated measures ANOVA.

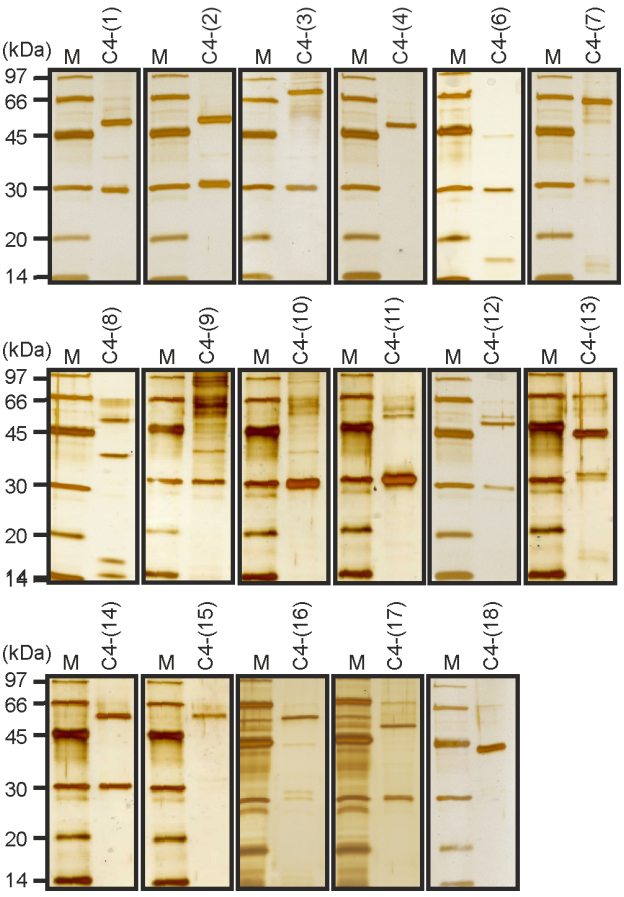
A



* constructs with two chains of similar size

** constructs composed of only one type of chain

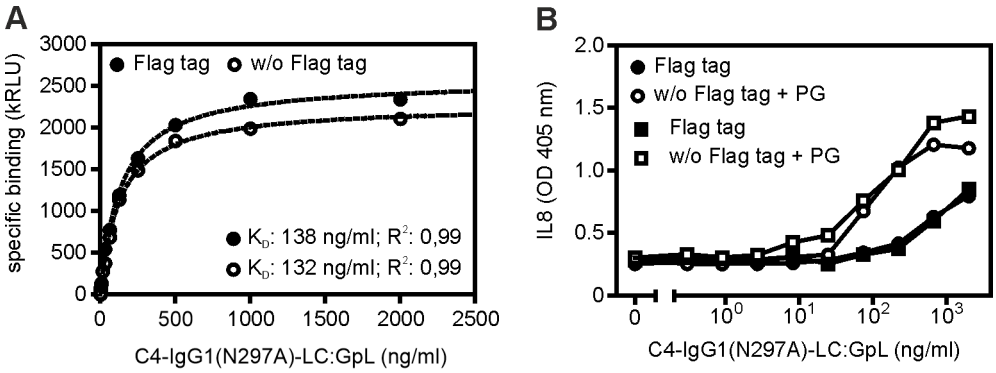
B



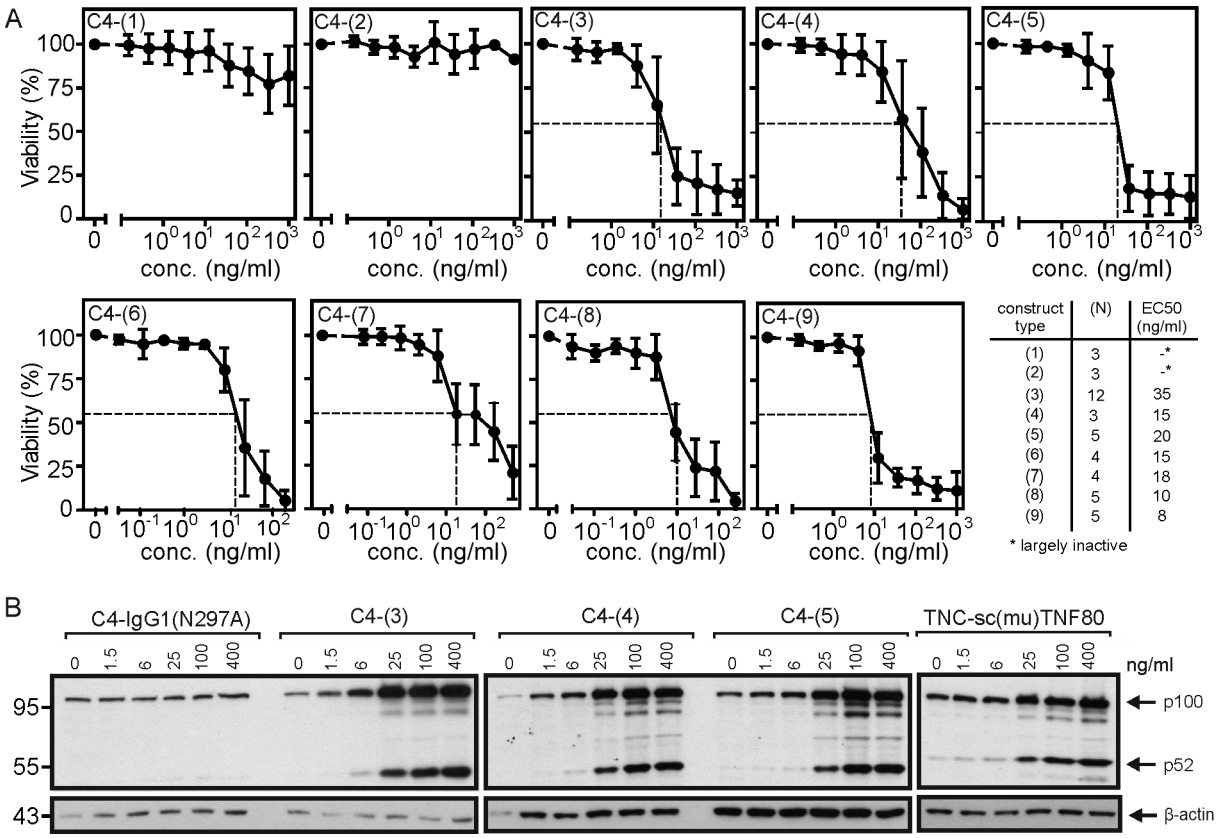
C

Construct	**Concentration (ng/ml)**	
	Individual experiments	Average
C4-(1)	50 / 50 / 40	47
C4-(2)	50 / 50 / 40	47
C4-(3)	60 / 60 / 60	60
C4-(4)	20 / 5 / 10	12
C4-(5)	~2 / < 2 / <2	< 2
C4-(6)	20 / 10 / 20	17
C4-(7)	15 / ~ 2 / 20	12
C4-(8)	~2 / 5 / 5	4
C4-(9)	40 / 50 / 40	43
C4-(10)	40 / 30 / 30	37
C4-(11)	40 / 30 / 30	33
C4-(12)	30 / 15 / 30	25
C4-(13)	30 / 20 / 30	27
C4-(14)	40 / 40 / 40	40
C4-(15)	10 / 10 / 10	10
C4-(16)	10 / 10 / 10	10
C4-(17)	50 / 30 / 40	40
C4-(18)	30 / 20 /20	23

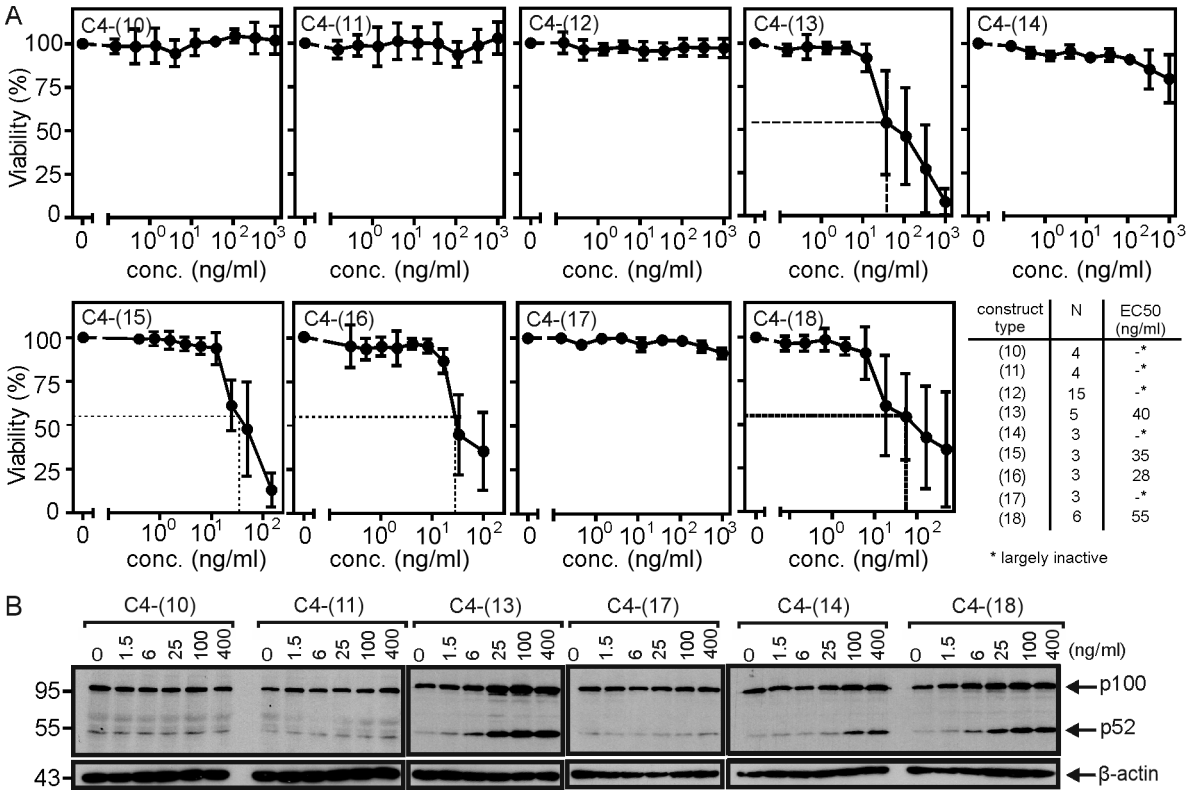
Supplemental Figure S2. **Transient expression of C4-derived variants C4-(1) to C4-(18).** (**A**) Cell culture supernatants of HEK293 cells transiently producing the indicated C4 antibody variants along with the indicated amounts of a Flag-tagged standard protein were analyzed by western blotting using the anti-Flag mAb M2 and HRP-labeled rabbit anti-mouse (#P0260, Dako). One of three independent production campaigns is shown. (**B**) Flag-tagged proteins contained in the supernatants from A were affinity purified on Flag agarose and analyzed by SDS-PAGE. Proteins in the gel were visualized by silver staining. Due to its low productivity C4-(5) was not purified. (**C**) Productivity of transiently produced C4 variants. Supernatants were subjected to western blotting as in A and the concentrations of the various C4 variants were estimated by comparison with the Flag-tagged standard.



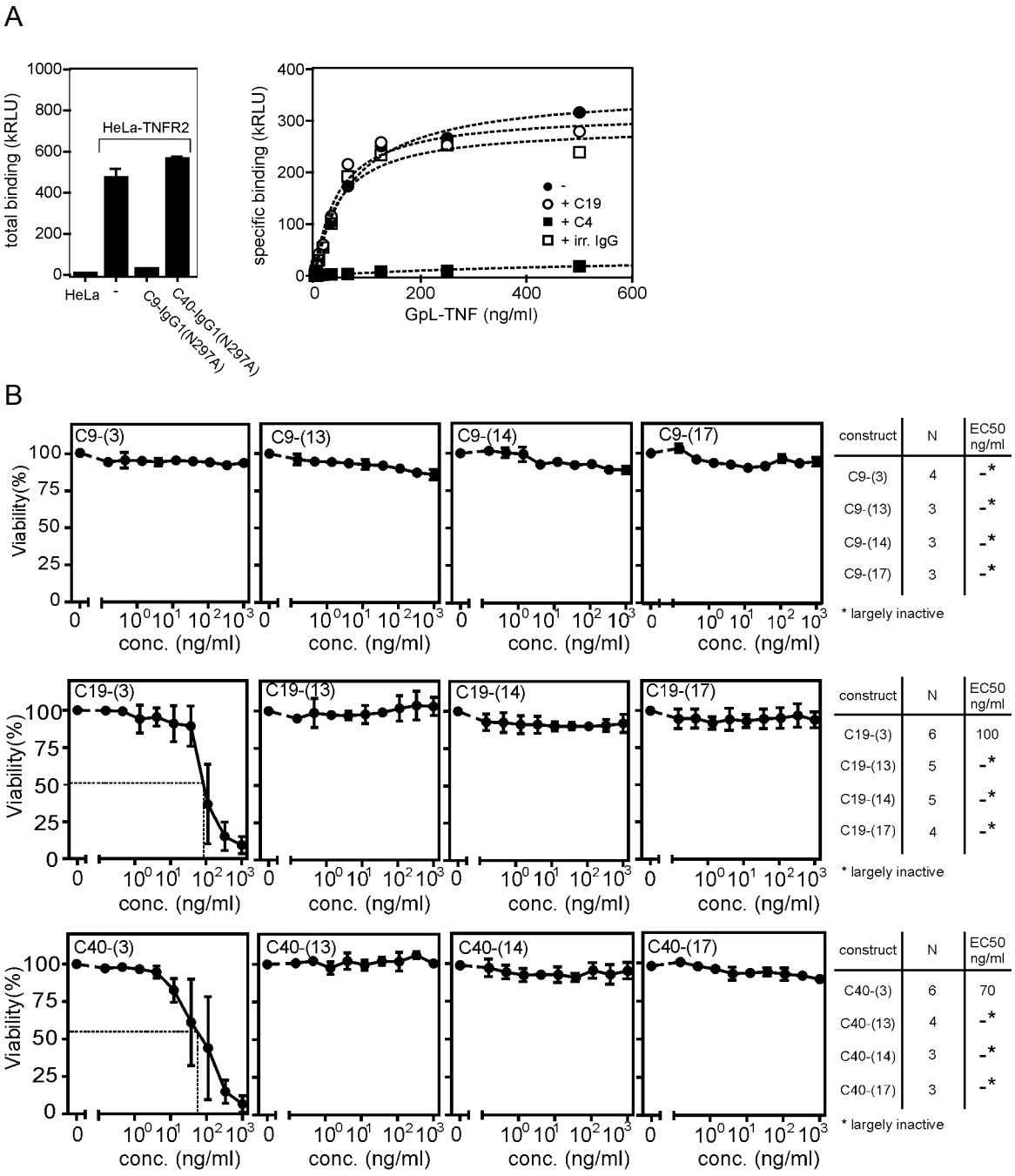
Supplemental Figure S3. **N-terminal Flag tagging of C4 does not affect its interaction with TNFR2.** (**A**) HT1080 and TNFR2-expressing HT1080 transfectants were seeded in a black 96 well tissue culture plate. The next day, wells containing the 2 cell types were pairwise incubated (1 h, 37°C) with increasing concentrations of C4-IgG1(N297A)-LC:GpL with and without N-terminal Flag tags. After removal of unbound protein molecules, cell-associated luciferase activity was measured as described in the bindung studies method paragraph. Specific binding values for the two C4-(IgG1(N297A)-LC:GpL variants were calculated by subtracting the non-specific binding values derived of the HT1080 cells from the total binding values derived from the TNFR2 transfectants. K_D values were obtained using the "nonlinear regression to a one-site specific binding curve" function of the GraphPad Prism5 software. Shown is one representative experiment of 3. (**B**) HT1080 cells and HT1080-TNFR2 transfectants were stimulated in triplicates with increasing concentrations of the indicated C4 variant in the presence and absence of 1 µg/ml protein G (PG) as „crosslinker". Next day, supernatants were investigated for the presence of IL8 by ELISA.



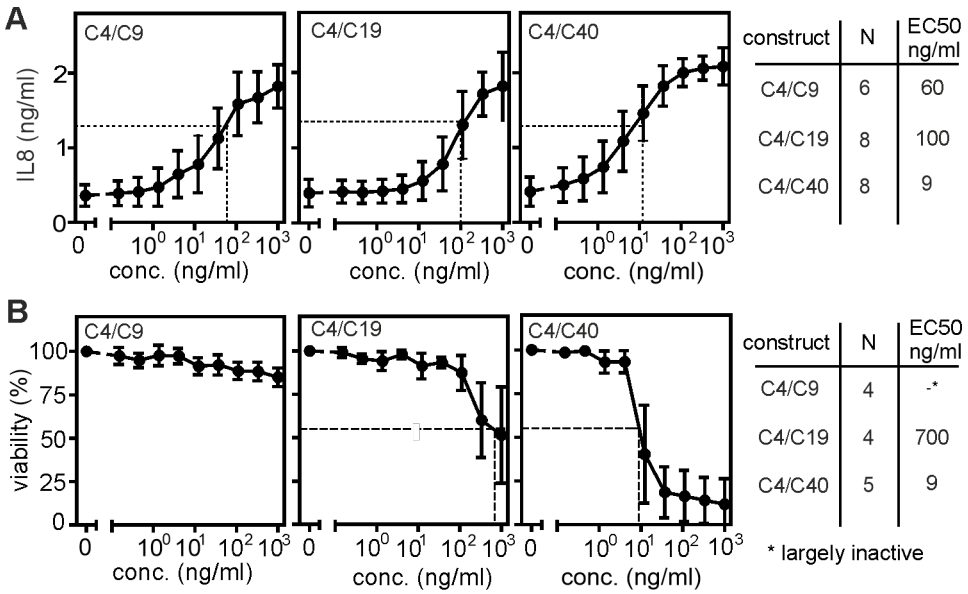
Supplemental Figure S4. **Ability of C4 antibody variants with N- and C-terminal TNFR2 binding sites to induce Kym-1 cell death and to trigger p100 processing.** (**A**) Kym-1 cells were treated with the various C4 constructs and the next day cell viability was determined by crystal violet staining. Maximal cell death induction by TNC-sc(mu)TNF80 is indicated by a dotted line. Shown are averaged data of 3–12 independent experiments. The number of experiments for each construct and their EC50 values are listed in the table. (**B**) Kym-1 cells were again stimulated overnight with the indicated C4 variants. To prevent cell death induction 20 μM ZVAD was added. Total cell lysates were analyzed by western blotting with respect to p100 processing .



Supplemental Figure S5. **Ability of C4 antibody variants with unidirectional oriented N-terminal TNFR2 binding sites to induce Kym-1 cell death and to trigger p100 processing.** (**A**) Kym-1 cells were challenged with the C4 constructs and cell viability was determined the next day by crystal violet staining. The dotted lines show the EC50 of each antibody depending on the maximal cell death induction by TNC-sc(mu)TNF80. (**B**) Total lysates of Kym-1 cells stimulated overnight with the indicated C4 constructs were analyzed by western blotting.



Supplemental Figure S6. **Intrinsic agonism of anti-TNFR2-antibody variants of format (3), (13), (14) and (17) to induce cell death**. (**A**) Effect of anti-TNFR2 antibodies C9, C19 and C40 on ligand binding. Left panel: HeLa-TNFR2 cells were pretreated with C9-IgG1(N297A) (5 µg/ml) or C40-IgG1(N297A) (3 µg/ml) and were then incubated with 5 ng/ml of GpL-TNF at 37°C for 1 h. After removal of unbound GpL-TNF molecules, cell associated GpL-TNF was quantified by analysis of the cell attached luciferase activity. Parental HeLa cells, not expressing TNFR2, served as control for non-specific binding. Right panel: HT1080-TNFR2 cells were seeded in a black 96 well tissue culture plate. The next day, cells were preincubated with 5 µg/ml of C4-IgG1(N297A), C19-IgG1(N297A) or an irrelevant human IgG1. Cells were incubated for 1 h at 37°C) with increasing concentrations of GpL-TNF and after removal of unbound protein molecules, cell-associated luciferase activity was measured. (**B**) Kym-1 cells were stimulated with the indicated anti-TNFR2 variants and the next day cell viability was determined by crystal violet staining.

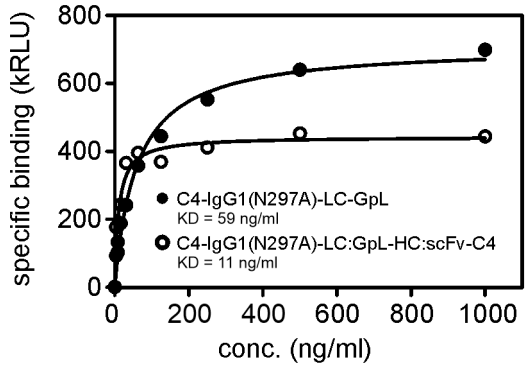


construct	N	EC50 ng/ml
C4/C9	6	60
C4/C19	8	100
C4/C40	8	9

construct	N	EC50 ng/ml
C4/C9	4	-*
C4/C19	4	700
C4/C40	5	9

Supplemental Figure S7. **Intrinsic agonism of biparatopic anti-TNFR2 variants of format (3) and (13).** (**A**) HT1080-TNFR2 cells were stimulated with the indicated anti-TNFR2 antibody variants overnight and IL8 production was quantified by ELISA. The half maximal TNFR2 response level induced by TNC-sc(mu)TNF80 is indicated by a dotted line. Averaged data of 6 – 8 independent experiments are shown. (**B**) Kym-1 cells were stimulated with the indicated anti-TNFR2 variants and analyzed for cell death induction by crystal violet staining.

A



B

Exp. no	affinity (ng/ml)	
	C4-	C4-...-C4
1	59	11
2	49	2
3	45	24
4	13	9
5	70	22
average	47	14

Supplemental Figure S8. **Apparent affinity of GpL variants of C4-IgG1(N297A) and C4-IgG1(N297A)-HC:scFvC4.** HeLa cells and HeLa-TNFR2 cells were cultivated overnight in 24-well plates (half-plate Hela cells and half-plate HeLa-TNFR2 cells transfectants). The two HeLa variants were pairwise incubated with the indicated concentrations of C4-IgG1(N297A)-LC:GpL and C4-(3)-LC:GpL for one hour at 37 °C. After removal of unbound proteins, nonspecific (HeLa) and total (HeLa-TNFR2) cell-associated binding of the two GpL fusion proteins was quantified by measuring GpL activity. Unspecific binding values were subtracted from the corresponding total binding values to calculate specific binding values which were fitted by non-linear regression to a single binding site interaction plot with the GraphPad Prism5 software. One representative experiment is shown in (A). Affinities from 5 independent experiments were listed in the table shown in (B).

Supplemental Table S1: Biochemical and functional properties of published anti-TNFR2 antibodies

Antibody / isotype	Effect on TNF binding	FcγR-independent activation/inhibition of TNFR2*	Effect of crosslinking or FcγR-binding		Ref. ****
			In vitro	In vivo	
Ty101 anti-mTNFR2 ratIgG1	Blocking	Inhibits TNF-induced Treg proliferation	Not verified	Not verified	1)
UTR1 anti-hTNFR2 mIgG1	Blocking	Inhibits TNF-induced GM-CSF production	Agonistic after crosslinking	Not verified	2,3)
TR75-54.7 anti-mTNFR2 hamster IgG	Blocking	Inhibits TNF-induced CT6 proliferation	Agonistic after crosslinking binds mFcγRII and FcγRIII	Inhibits CT26 tumors	4,5)
An3025 anti-hTNFR2 rabbit mAbY hIgG1	Blocking	Inhibits TNF-induced TNFR2 activation	ADCC	Inhibits MC38 in hTNFR2ki-mice ADCC required	6) WO2022/122005A1 Adlai Northy
Y9 anti-mTNFR2 mIgG2a	Blocking despite binding CRD1	Not TNFR2 stimulating	Agonistic when plate-bound ADCC	Inhibit tumor FcγR-binding required	7)
Ab1 and Ab2 ABV2c anti-hTNFR2 hIgG1	Blocking	Not investigated	T-cell costimulation when plate-bound, dito NFκB reporter	Not verified	7) WO2020/061210A1 Merrimack
M861 mIgG1	Presumably Blocking	Inhibits TNF-induced proliferation of IL2-treated T-cells	Not verified	Inhibits CT26 tumors in combination with CpG ODN, FcγR relevance not checked	8)
MR2-1 anti-hTNFR2 mIgG1	-	Weak IL8 induction	Potentiated IL8 induction in presence of FcγIIb	Not verified	9)
F10, A05 anti-mTNFR2 B02, …, H10 A09, …, H03 anti-hTNFR2 Hu/mu IgG2a and IgG1	Blocking (e.g. F10) Non-blocking (e.g. A05)	Non-blocking enhance and blocking antibodies inhibit NK cytokine release, problem synergism with endogenous TNF	Not verified	FcγR-dependent anti-tumor activity	WO2020/089473A2 WO2020/089474A1 Bioinvent
BI-1808 anti-hTNFR2	Blocking	Not investigated	Not verified	FcγR-dependent Treg depletion	10)
HFB200301 anti-hTNFR2 hIgG1	Non-blocking	Low activation of CD8 and NK cells but strongly enhanced by sTNF	Not verified	Anti-tumor activity	11) WO2021/141907A1 HifiBio
SIM0235 anti-hTNFR2	Blocking	Not investigated	ADCC, ADCP	Not verified	WO2021/023098A1**

hIgG1					Simcere
SBT-001 – SBT-004 anti-hTNFR2 various IgGs	Blocking	Inhibits TNF-induced Treg proliferation	Not verified	Not verified	US10988543 B2 OPI-VI
TNFRAB1, TNFRAB2	-	Inhibits TNF-induced Treg proliferation	No effect on TNFR2 inhibition	Not verified	12) AU2017/263 833A1 General Hospital Corporation
E4 anti-hTNFR2 IgG1	Non-blocking	Neutral on TNF-induced Treg proliferation	ADCC-dependent Treg depletion***	Not verified	WO2018/21 3064A1 NIH
C4, C15, C16, C17, K21, C27, C40 covering (CRD1, CRD2, CRD3, CRD4) anti-hTNFR2 hIgG1, for C4 also hIgG2, hIgG3, hIgG4	Blocking (C4) and non-blocking (C40); this ms.	No activity per se, C4 inhibits ligand-induced IL8 (this ms.)	FcγR-dependent, IL8 induction	Not verified	9)
55F6, 25-71, 25-81 and many more anti-hTNFR2 hIgG1	> 30 blocking 10 non-blocking	Only 55F6 poor NFκB reporter (< 0.2 ng/ml TNF)	ADCC	Reduced growth of $TNFR2^{high}$ Colo205 cells (xenograft model)	WO2021/05 5253A2 Apexigen
30.083, 30.091 anti-hTNFR2 hIgG1	Not tested	Neutral on TNF-induced NFκB reporter	Not verified	Not tested	WO2022/00 3693A1 Biolojic
R2-1…R2-6, anti-hTNFR2 hIgG1	R2-1 weakly all other Abs strongly Blocking	Blocking of TNF-induced NFκB reporter	NFκB reporter activation in presence of FcγR cells	Inhibits MC38 tumors in TNFR2ki (mIgG1, mIgG2a) reduced activity with mIgG1D265A	WO2022/14 7222A1 Novarock
Hu32-C, hu3-E, anti-hTNFR2	Blocking	Not verified	T-cell costimulation when plate-bound ADCC	No verified	WO2021/24 9542A1 Nanjing leads biolabs

* Use of TNFR2 responding cells w/o FcγR expression or use of FcγR-defective IgG variants

** Patent describes several antibodies with general similar activities, concrete identity of SIM0235 is not evident

*** Demonstrated by use of defucosylated IgG1 (FUT8-deficient CHO produced)

**** S1 table references:

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Supplemental Table S2. AA sequence of light and heavy chain proteins used in the study. Leader, underlined; Linker sequences, bold; Flag tag, underlined + grey background; variable domains, italic; constant IgG1 domains, grey background; TNC trimerization domain, italic + underlined + grey background.

Plasmid No.	Encoded peptide	AA sequence (FASTA format)
1	Leader-Flag-C4-LC-scFvC4	MNFGFSLIFLVLVLKGVQCEVKLVPR**QL**DYKDDDDK**EL***DIVMTQ SHKFMSTSVGDRVSITCKASQDVDTAVAWYQQKPGQSPKLLIYW ASTRHTGVPDRFTGSGSGTDYTLTISSVQAEDLARYYCQQYYSV PPTFGGGTKL***GS***EIKRTVAAPSVFIFPPSDEQLKSGTASVVCLL NNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLT

		LSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC**LE***QVQLLQSG PELVKPGASVKLSCKASGYSFTSYDINWVKQRPGQGLEWVGWIY PRDGDTKYNEKFKGKAILTVDTSSNTAYMNLHSLTSEDSAVYFC ARLTGPYWYFDVWGTGTTVTVSS***RS*****STKGPKLEEGEFSEA*****QL***DI VMTQSHKFMSTSVGDRVSITCKASQDVDTAVAWYQQKPGQSPKL LIYWASTRHTGVPDRFTGSGSGTDYTLTISSVQAEDLARYYCQQ YYSVPPTFGGGTKLEIK*
2	Leader-Flag-C4-Fab1	MNFGFSLIFLVLVLKGVQCEVKLVPR**QL**DYKDDDDK**EL***QVQLLQ SGPELVKPGASVKLSCKASGYSFTSYDINWVKQRPGQGLEWVGW IYPRDGDTKYNEKFKGKAILTVDTSSNTAYMNLHSLTSEDSAVY FCARLTGPYWYFDVWGTGTTVTVSS***GS***SSASTKGPSVFPLAPSS KSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQS SGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSC DKTHT
3	Leader-C4-Fab2	MNFGFSLIFLVLVLKGVQCEVKLVPR**QL***QVQLLQSGPELVKPGA SVKLSCKASGYSFTSYDINWVKQRPGQGLEWVGWIYPRDGDTKY NEKFKGKAILTVDTSSNTAYMNLHSLTSEDSAVYFCARLTGPYW YFDVWGTGTTVTVSS***GS***SSASTKGPSVFPLAPSSKSTSGGTAAL GCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVV TVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCP A
4)	Leader-Flag-C4-LC	MNFGFSLIFLVLVLKGVQCEVKLVPR**QL**DYKDDDDK**EL***DIVMTQ SHKFMSTSVGDRVSITCKASQDVDTAVAWYQQKPGQSPKLLIYW ASTRHTGVPDRFTGSGSGTDYTLTISSVQAEDLARYYCQQYYSV PPTFGGGTKL***GS***EIKRTVAAPSVFIFPPSDEQLKSGTASVVCLL NNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLT LSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC
5	Leader-Flag-C4-IgG1(N297A)-scFvC4	MNFGFSLIFLVLVLKGVQCEVKLVPR**QL**DYKDDDDK**EL***QVQLLQ SGPELVKPGASVKLSCKASGYSFTSYDINWVKQRPGQGLEWVGW IYPRDGDTKYNEKFKGKAILTVDTSSNTAYMNLHSLTSEDSAVY FCARLTGPYWYFDVWGTGTTVTVSS***GS***SSASTKGPSVFPLAPSS KSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQS SGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSC DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVV DVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTV LHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLP PSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPP VLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKS LSLSPGK**EFLE***QVQLLQSGPELVKPGASVKLSCKASGYSFTSYD INWVKQRPGQGLEWVGWIYPRDGDTKYNEKFKGKAILTVDTSSN TAYMNLHSLTSEDSAVYFCARLTGPYWYFDVWGTGTTVTVSS***RS*** ***STKGPKLEEGEFSEA*****QL***DIVMTQSHKFMSTSVGDRVSITCKASQ DVDTAVAWYQQKPGQSPKLLIYWASTRHTGVPDRFTGSGSGTDY TLTISSVQAEDLARYYCQQYYSVPPTFGGGTKLEIK*
6	Leader-Flag-scFvC4-CL	MNFGFSLIFLVLVLKGVQCEVKLVPR**QL**DYKDDDDK**EF***QVQLLQ SGPELVKPGASVKLSCKASGYSFTSYDINWVKQRPGQGLEWVGW IYPRDGDTKYNEKFKGKAILTVDTSSNTAYMNLHSLTSEDSAVY FCARLTGPYWYFDVWGTGTTVTVSS***RS*****STKGPKLEEGEFSEA*****QL*** *DIVMTQSHKFMSTSVGDRVSITCKASQDVDTAVAWYQQKPGQSP KLLIYWASTRHTGVPDRFTGSGSGTDYTLTISSVQAEDLARYYC QQYYSVPPTFGGGTKLEIK***GS***EIKRTVAAPSVFIFPPSDEQLKS GTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDS TYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC
7	Leader-Flag-scFvC4-CH1/CH2/CH3(N297A)-scFvC4	MNFGFSLIFLVLVLKGVQCEVKLVPR**QL**DYKDDDDK**EF***QVQLLQ SGPELVKPGASVKLSCKASGYSFTSYDINWVKQRPGQGLEWVGW IYPRDGDTKYNEKFKGKAILTVDTSSNTAYMNLHSLTSEDSAVY FCARLTGPYWYFDVWGTGTTVTVSS***RS*****STKGPKLEEGEFSEA*****QL*** *DIVMTQSHKFMSTSVGDRVSITCKASQDVDTAVAWYQQKPGQSP KLLIYWASTRHTGVPDRFTGSGSGTDYTLTISSVQAEDLARYYC QQYYSVPPTFGGGTKLEIK***GS***SSASTKGPSVFPLAPSSKSTSGG TAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSL SSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTC

		PPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHED PEVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWL NGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDEL TKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDG SFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG K**LE***QVQLLQSGPELVKPGASVKLSCKASGYSFTSYDINWVKQRP* *GQGLEWVGWIYPRDGDTKYNEKFKGKAILTVDTSSNTAYMNLHS* *LTSEDSAVYFCARLTGPYWYFDVWGTGTTVTVSS***RS**STKGPKLE EGEFSEA**QL***DIVMTQSHKFMSTSVGDRVSITCKASQDVDTAVAW* *YQQKPGQSPKLLIYWASTRHTGVPDRFTGSGSGTDYTLTISSVQ* *AEDLARYYCQQYYSVPPTFGGGTKLEIK*
8	Leader-Flag-scFvC4-CL-scFvC4	MNFGFSLIFLVLVLKGVQCEVKLVPR**QL**DYKDDDDK**EF***QVQLLQ* *SGPELVKPGASVKLSCKASGYSFTSYDINWVKQRPGQGLEWVGW* *IYPRDGDTKYNEKFKGKAILTVDTSSNTAYMNLHSLTSEDSAVY* *FCARLTGPYWYFDVWGTGTTVTVSS***RS*STKGPKLEEGEFSEA**QL** *DIVMTQSHKFMSTSVGDRVSITCKASQDVDTAVAWYQQKPGQSP* *KLLIYWASTRHTGVPDRFTGSGSGTDYTLTISSVQAEDLARYYC* *QQYYSVPPTFGGGTKLEIK***GS**EIKRTVAAPSVFIFPPSDEQLKS GTASVVCLLNNFYPREAKVQWKVDXALQSGNSQESVTEQDSKDS TYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC**L** **E***QVQLLQSGPELVKPGASVKLSCKASGYSFTSYDINWVKQRPGQ* *GLEWVGWIYPRDGDTKYNEKFKGKAILTVDTSSNTAYMNLHSLT* *SEDSAVYFCARLTGPYWYFDVWGTGTTVTVSS***RS*STKGPKLEEG** ***EFSEA*****QL***DIVMTQSHKFMSTSVGDRVSITCKASQDVDTAVAWYQ* *QKPGQSPKLLIYWASTRHTGVPDRFTGSGSGTDYTLTISSVQAE* *DLARYYCQQYYSVPPTFGGGTKLEIK*
9	Leader-Flag-scFvC4-CH1/CH2/CH3(N297A)	MNFGFSLIFLVLVLKGVQCEVKLVPR**QL**DYKDDDDK**EF***QVQLLQ* *SGPELVKPGASVKLSCKASGYSFTSYDINWVKQRPGQGLEWVGW* *IYPRDGDTKYNEKFKGKAILTVDTSSNTAYMNLHSLTSEDSAVY* *FCARLTGPYWYFDVWGTGTTVTVSS***RS*STKGPKLEEGEFSEA**QL** *DIVMTQSHKFMSTSVGDRVSITCKASQDVDTAVAWYQQKPGQSP* *KLLIYWASTRHTGVPDRFTGSGSGTDYTLTISSVQAEDLARYYC* *QQYYSVPPTFGGGTKLEIK***GS**SSASTKGPSVFPLAPSSKSTSGG TAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSL SSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTC PPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHED PEVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWL NGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDEL TKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDG SFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG K
10	Leader-Flag-C4-IgG1(N297A)-TNCscFvC4	MNFGFSLIFLVLVLKGVQCEVKLVPR**QL**DYKDDDDK**EL***QVQLLQ* *SGPELVKPGASVKLSCKASGYSFTSYDINWVKQRPGQGLEWVGW* *IYPRDGDTKYNEKFKGKAILTVDTSSNTAYMNLHSLTSEDSAVY* *FCARLTGPYWYFDVWGTGTTVTVSS***GS**SSASTKGPSVFPLAPSS KSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQS SGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSC DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVV DVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTV LHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLP PSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPP VLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKS LSLSPGK**EFLE***DIACGCAAAPDIKDLLSRLEELEGLVSSLREQG* *TGGGS***VE***QVQLLQSGPELVKPGASVKLSCKASGYSFTSYDINWV* *KQRPGQGLEWVGWIYPRDGDTKYNEKFKGKAILTVDTSSNTAYM* *NLHSLTSEDSAVYFCARLTGPYWYFDVWGTGTTVTVSS***RS*STKG** ***PKLEEGEFSEA*****QL***DIVMTQSHKFMSTSVGDRVSITCKASQDVDT* *AVAWYQQKPGQSPKLLIYWASTRHTGVPDRFTGSGSGTDYTLTI* *SSVQAEDLARYYCQQYYSVPPTFGGGTKLEIK*
11	Leader-Flag-C4-IgG1(N297A)-TNC	MNFGFSLIFLVLVLKGVQCEVKLVPR**QL**DYKDDDDK**EL***QVQLLQ* *SGPELVKPGASVKLSCKASGYSFTSYDINWVKQRPGQGLEWVGW* *IYPRDGDTKYNEKFKGKAILTVDTSSNTAYMNLHSLTSEDSAVY*

		*FCARLTGPYWYFDVWGTGTTVTVSS***GS**SSASTKGPSVFPLAPSS KSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQS SGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSC DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVV DVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTV LHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLP PSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPP VLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKS LSLSPGK**EFLE***DIACGCAAAPDIKDLLSRLEELEGLVSSLREQG TG*
12	Leader-Flag-scFvC4-TNC-Fc(DANA)	MNFGFSLIFLVLVLKGVQCEVKLVPR**QL**DYKDDDDK**EF***QVQLLQ SGPELVKPGASVKLSCKASGYSFTSYDINWVKQRPGQGLEWVGW IYPRDGDTKYNEKFKGKAILTVDTSSNTAYMNLHSLTSEDSAVY FCARLTGPYWYFDVWGTGTTVTVSS**RS*STKGPKLEEGEFSEA*QL** *DIVMTQSHKFMSTSVGDRVSITCKASQDVDTAVAWYQQKPGQSP KLLIYWASTRHTGVPDRFTGSGSGTDYTLTISSVQAEDLARYYC QQYYSVPPTFGGGTKLEIK**GS***DIACGCAAAPDIKDLLSRLEELE GLVSSLREQGTG***EF**KTHTCPPCPAPELLGGPSVFLFPPKPKDTL MISRTPEVTCVVVAVSHEDPEVKFNWYVDGVEVHNAKTKPREEQ YASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKA KGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWES NGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSV MHEALHNHYTQKSLSLSPGK**LE**
13	Leader-Flag-scFvC4-Fc(DANA)-TNC	MNFGFSLIFLVLVLKGVQCEVKLVPR**QL**DYKDDDDK**EF**QVQLLQ SGPELVKPGASVKLSCKASGYSFTSYDINWVKQRPGQGLEWVGW IYPRDGDTKYNEKFKGKAILTVDTSSNTAYMNLHSLTSEDSAVY FCARLTGPYWYFDVWGTGTTVTVSS**RS*STKGPKLEEGEFSEA*QL** *DIVMTQSHKFMSTSVGDRVSITCKASQDVDTAVAWYQQKPGQSP KLLIYWASTRHTGVPDRFTGSGSGTDYTLTISSVQAEDLARYYC QQYYSVPPTFGGGTKLEIK**GS**KTHTCPPCPAPELLGGPSVFLFP PKPKDTLMISRTPEVTCVVVAVSHEDPEVKFNWYVDGVEVHNAK TKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPI EKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSD IAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQG NVFSCSVMHEALHNHYTQKSLSLSPGK**LE***DIACGCAAAPDIKDL LSRLEELEGLVSSLREQGTG*
14	Leader-Flag-C4-IgG1(N297A-RGY)	MNFGFSLIFLVLVLKGVQCEVKLVPR**QL**DYKDDDDK**EL***QVQLLQ SGPELVKPGASVKLSCKASGYSFTSYDINWVKQRPGQGLEWVGW IYPRDGDTKYNEKFKGKAILTVDTSSNTAYMNLHSLTSEDSAVY FCARLTGPYWYFDVWGTGTTVTVSS**GS**SSASTKGPSVFPLAPSS KSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQS SGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSC DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVV DVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTV LHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPRRPQVYTLP PSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPP VLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHGALHNHYTQKY LSLSPGK
15	Leader-Flag-scFvC4-CH1/CH2/CH3(N297A)-TNC	MNFGFSLIFLVLVLKGVQCEVKLVPR**QL**DYKDDDDK**EF***QVQLLQ SGPELVKPGASVKLSCKASGYSFTSYDINWVKQRPGQGLEWVGW IYPRDGDTKYNEKFKGKAILTVDTSSNTAYMNLHSLTSEDSAVY FCARLTGPYWYFDVWGTGTTVTVSS**RS*STKGPKLEEGEFSEA*QL** *DIVMTQSHKFMSTSVGDRVSITCKASQDVDTAVAWYQQKPGQSP KLLIYWASTRHTGVPDRFTGSGSGTDYTLTISSVQAEDLARYYC QQYYSVPPTFGGGTKLEIK**GS**SSASTKGPSVFPLAPSSKSTSGG TAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSL SSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTC PPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHED PEVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWL NGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDEL TKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDG SFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG

		K**LE***DIACGCAAAPDIKDLLSRLEELEGLVSSLREQGTG*
16	Leader-Flag-C9-CL	MNFGFSLIFLVLVLKGVQCEVKLVPR**QL**DYKDDDDK**EL***QIVLTQ SPAIMSASPGEKVTITCSASSSVSYMHWFQQKPGTSPKLWIYST SNLASGVPARFSGSGSGTSYSLTISRMEAEDAATYYCQQRSSYP PTFGGGTKLEIK***GS***EIKRTVAAPSVFIFPPSDEQLKSGTASVVC LLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSST LTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC
17	Leader-Flag-C9-IgG1(N297A)	MNFGFSLIFLVLVLKGVQCEVKLVPR**QL**DYKDDDDK**EF***QIQLVQ SGPELKKPGETVKISCKASGYTFTTAGMQWVQKMPGKGFKWIGW INTHSGEPKYAEDFKGRFAFSLETSASTAYLQISNLKNEDTATY FCARWDGTGYWGQGTTLTVSS**RS**SSASTKGPSVFPLAPSSKSTS GGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLY SLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTH TCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSH EDPEVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQD WLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRD ELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDS DGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLS PGK
18	Leader-Flag-C9-IgG1(N297A)-scFvC9	MNFGFSLIFLVLVLKGVQCEVKLVPR**QL**DYKDDDDK**EF***QIQLVQ SGPELKKPGETVKISCKASGYTFTTAGMQWVQKMPGKGFKWIGW INTHSGEPKYAEDFKGRFAFSLETSASTAYLQISNLKNEDTATY FCARWDGTGYWGQGTTLTVSS**RS**SSASTKGPSVFPLAPSSKSTS GGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLY SLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTH TCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSH EDPEVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQD WLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRD ELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDS DGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLS PGK**LE***QIQLVQSGPELKKPGETVKISCKASGYTFTTAGMQWVQK MPGKGFKWIGWINTHSGEPKYAEDFKGRFAFSLETSASTAYLQI SNLKNEDTATYFCARWDGTGYWGQGTTLTVSS***RS STKGPKLEEG EFSEA*****QL***QIVLTQSPAIMSASPGEKVTITCSASSSVSYMHWFQQ KPGTSPKLWIYSTSNLASGVPARFSGSGSGTSYSLTISRMEAED AATYYCQQRSSYPPTFGGGTKLEIK*
19	Leader-Flag-scFvC9-CL	MNFGFSLIFLVLVLKGVQCEVKLVPR**QL**DYKDDDDK**EF***QIQLVQ SGPELKKPGETVKISCKASGYTFTTAGMQWVQKMPGKGFKWIGW INTHSGEPKYAEDFKGRFAFSLETSASTAYLQISNLKNEDTATY FCARWDGTGYWGQGTTLTVSS**RS*****STKGPKLEEGEFSEA*****QL***QIVL TQSPAIMSASPGEKVTITCSASSSVSYMHWFQQKPGTSPKLWIY STSNLASGVPARFSGSGSGTSYSLTISRMEAEDAATYYCQQRSS YPPTFGGGTKLEIK***GS***EIKRTVAAPSVFIFPPSDEQLKSGTASV VCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLS STLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC
20	Leader-Flag-scFvC9-CH1/CH2/CH3(N297A)	MNFGFSLIFLVLVLKGVQCEVKLVPR**QL**DYKDDDDK**EF***QIQLVQ SGPELKKPGETVKISCKASGYTFTTAGMQWVQKMPGKGFKWIGW INTHSGEPKYAEDFKGRFAFSLETSASTAYLQISNLKNEDTATY FCARWDGTGYWGQGTTLTVSS**RS*****STKGPKLEEGEFSEA*****QL***QIVL TQSPAIMSASPGEKVTITCSASSSVSYMHWFQQKPGTSPKLWIY STSNLASGVPARFSGSGSGTSYSLTISRMEAEDAATYYCQQRSS YPPTFGGGTKLEIK***GS***SSASTKGPSVFPLAPSSKSTSGGTAALG CLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVT VPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPA PELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKF NWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEY KCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQV SLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLY SKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
21	Leader-Flag-C9-IgG1(N297A)-TNC	MNFGFSLIFLVLVLKGVQCEVKLVPR**QL**DYKDDDDK**EF***QIQLVQ SGPELKKPGETVKISCKASGYTFTTAGMQWVQKMPGKGFKWIGW INTHSGEPKYAEDFKGRFAFSLETSASTAYLQISNLKNEDTATY

		*FCARWDGTGYWGQGTTLTVSS***RS**SSASTKGPSVFPLAPSSKSTS GGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLY SLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTH TCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSH EDPEVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQD WLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRD ELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDS DGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLS PGK**LE***DIACGCAAAPDIKDLLSRLEELEGLVSSLREQGTG*
22	Leader-Flag-C19-CL	MNFGFSLIFLVLVLKGVQCEVKLVPR**QL**DYKDDDDK**EL***DIVMAQ SQKFMSVSVGDRVSVTCKASQNVGTNVAWYQQRPGQSPKALIYS ASYRYSGVPDRFTGSGSGTDFTLTISNVQSEDLAEYFCQQFDSH PLTFGAGTKLELK***GS***EIKRTVAAPSVFIFPPSDEQLKSGTASVV CLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSS TLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC
23	Leader-C19-IgG1(N297A)	MNFGFSLIFLVLVLKGVQCEVKLVPR**QL***QIQLVQSGPEVKKPGE TVKISCKASGYTFTIHGMSWVKQAPGKGLKWMGWINTYSGVPTY ANDFKGRFAFSLETSASTAYLQINNLKNEDTATYFCARDEVRRG FGFAYWGQGTLVTVSA***GS***SSASTKGPSVFPLAPSSKSTSGGTAA LGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSV VTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPC PAPELLGGPSVFLFPPXPKDTLMISRTPEVTCVVVDVSHEDPEV KFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGK EYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKN QVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFF LYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
24	Leader-Flag-C19-IgG1(N297A)-scFvC19	MNFGFSLIFLVLVLKGVQCEVKLVPR**QL**DYKDDDDK**EL***QIQLVQ SGPEVKKPGETVKISCKASGYTFTIHGMSWVKQAPGKGLKWMGW INTYSGVPTYANDFKGRFAFSLETSASTAYLQINNLKNEDTATY FCARDEVRRGFGFAYWGQGTLVTVSA***GS***SSASTKGPSVFPLAPS SKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQ SSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKS CDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVV VDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLT VLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTL PPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTP PVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQK SLSLSPGK**LE***QIQLVQSGPEVKKPGETVKISCKASGYTFTIHGM SWVKQAPGKGLKWMGWINTYSGVPTYANDFKGRFAFSLETSAST AYLQINNLKNEDTATYFCARDEVRRGFGFAYWGQGTLVTVSA***RS STKGPKLEEGEFSEA*****QL***DIVMAQSQKFMSVSVGDRVSVTCKASQ NVGTNVAWYQQRPGQSPKALIYSASYRYSGVPDRFTGSGSGTDF TLTISNVQSEDLAEYFCQQFDSHPLTFGAGTKLELK
25	Leader-Flag-scFvC19-CL	MNFGFSLIFLVLVLKGVQCEVKLVPR**QL**DYKDDDDK**EF***QIQLVQ SGPEVKKPGETVKISCKASGYTFTIHGMSWVKQAPGKGLKWMGW INTYSGVPTYANDFKGRFAFSLETSASTAYLQINNLKNEDTATY FCARDEVRRGFGFAYWGQGTLVTVSA**RS*STKGPKLEEGEFSEA*****QL***DIVMAQSQKFMSVSVGDRVSVTCKASQNVGTNVAWYQQRPGQS PKALIYSASYRYSGVPDRFTGSGSGTDFTLTISNVQSEDLAEYF CQQFDSHPLTFGAGTKLELK***GS***EIKRTVAAPSVFIFPPSDEQLK SGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKD STYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC
26	Leader-Flag-scFvC19-CH1/CH2/CH3(N297A)	MNFGFSLIFLVLVLKGVQCEVKLVPR**QL**DYKDDDDK**EF***QIQLVQ SGPEVKKPGETVKISCKASGYTFTIHGMSWVKQAPGKGLKWMGW INTYSGVPTYANDFKGRFAFSLETSASTAYLQINNLKNEDTATY FCARDEVRRGFGFAYWGQGTLVTVSA**RS*STKGPKLEEGEFSEA*****Q L***DIVMAQSQKFMSVSVGDRVSVTCKASQNVGTNVAWYQQRPGQS PKALIYSASYRYSGVPDRFTGSGSGTDFTLTISNVQSEDLAEYF CQQFDSHPLTFGAGTKLELK***GS***SSASTKGPSVFPLAPSSKSTSG GTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYS LSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHT CPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHE

		DPEVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDW LNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDE LTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSD GSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSP GK
27	Leader-Flag-C19-IgG1(N297A)-TNC	MNFGFSLIFLVLVLKGVQCEVKLVPR**QL**DYKDDDDK**EF***QIQLVQ* *SGPEVKKPGETVKISCKASGYTFTIHGMSWVKQAPGKGLKWMGW* *INTYSGVPTYANDFKGRFAFSLETSASTAYLQINNLKNEDTATY* *FCARDEVRRGFGFAYWGQGTLVTVSA***RS**SSASTKGPSVFPLAPS SKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQ SSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKS CDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVV VDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLT VLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTL PPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTP PVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQK SLSLSPGK**LE***DIACGCAAAPDIKDLLSRLEELEGLVSSLREQGT* *G*
28	Leader-Flag-C40-CL	MNFGFSLIFLVLVLKGVQCEVKLVPR**QL**DYKDDDDK**EL***DIQMTQ* *SPSSLSASLGGKVTITCKASQDINKFIAWYQHKPGKGPRLLIHY* *TSTLQPGIPSRFSGSGSGRDYSFSISNLEPEDIATYYCLQYDNL* *YTFGGGTKLEIK***GS**EIKRTVAAPSVFIFPPSDEQLKSGTASVVC LLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSST LTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC
29	Leader-Flag-C40-IgG1(N297A)	MNFGFSLIFLVLVLKGVQCEVKLVPR**QL**DYKDDDDK**EF***QVTLKE* *SGPGILQPSQTLSLTCSFSGFSLSTFGMGVGWIRQPSGKGLEWL* *AHIWWDDDKYYNPALKSRLTISKDTSENQVFLKIANVDTADTAT* *YYCVRIAGTRYFDVWGTGTTVTVSS***RS**SSASTKGPSVFPLAPSS KSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQS SGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSC DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVV DVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTV LHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLP PSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPP VLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKS LSLSPGK
30	Leader-Flag-C40-IgG1(N297A)-scFvC40	MNFGFSLIFLVLVLKGVQCEVKLVPR**QL**DYKDDDDK**EF***QVTLKE* *SGPGILQPSQTLSLTCSFSGFSLSTFGMGVGWIRQPSGKGLEWL* *AHIWWDDDKYYNPALKSRLTISKDTSENQVFLKIANVDTADTAT* *YYCVRIAGTRYFDVWGTGTTVTVSS***RS**SSASTKGPSVFPLAPSS KSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQS SGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSC DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVV DVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTV LHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLP PSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPP VLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKS LSLSPGK**LE***QVTLKESGPGILQPSQTLSLTCSFSGFSLSTFGMG* *VGWIRQPSGKGLEWLAHIWWDDDKYYNPALKSRLTISKDTSENQ* *VFLKIANVDTADTATYYCVRIAGTRYFDVWGTGTTVTVSS***RS*****ST** ***KGPKLEEGEFSEA*****QL***DIQMTQSPSSLSASLGGKVTITCKASQDI* *NKFIAWYQHKPGKGPRLLIHYTSTLQPGIPSRFSGSGSGRDYSF* *SISNLEPEDIATYYCLQYDNLYTFGGGTKLEIK*
31	Leader-Flag-scFvC40-CL	MNFGFSLIFLVLVLKGVQCEVKLVPR**QL**DYKDDDDK**EF***QVTLKE* *SGPGILQPSQTLSLTCSFSGFSLSTFGMGVGWIRQPSGKGLEWL* *AHIWWDDDKYYNPALKSRLTISKDTSENQVFLKIANVDTADTAT* *YYCVRIAGTRYFDVWGTGTTVTVSS***RS*****STKGPKLEEGEFSEA*****QL*** *DIQMTQSPSSLSASLGGKVTITCKASQDINKFIAWYQHKPGKGP* *RLLIHYTSTLQPGIPSRFSGSGSGRDYSFSISNLEPEDIATYYC* *LQYDNLYTFGGGTKLEIK***GS**EIKRTVAAPSVFIFPPSDEQLKSG TASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDST YSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

32	Leader-Flag-scFvC40-CH1/CH2/CH3(N297A)	MNFGFSLIFLVLVLKGVQCEVKLVPR**QL**DYKDDDDK**EF***QVTLKE* *SGPGILQPSQTLSLTCSFSGFSLSTFGMGVGWIRQPSGKGLEWL* *AHIWWDDDKYYNPALKSRLTISKDTSENQVFLKIANVDTADTAT* *YYCVRIAGTRYFDVWGTGTTVTVSS***RS*****STKGPKLEEGEFSEA*****QL*** *DIQMTQSPSSLSASLGGKVTITCKASQDINKFIAWYQHKPGKGP* *RLLIHYTSTLQPGIPSRFSGSGSGRDYSFSISNLEPEDIATYYC* *LQYDNLYTFGGGTKLEIK***GS**SSASTKGPSVFPLAPSSKSTSGGT AALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLS SVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCP PCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDP EVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLN GKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELT KNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGS FFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
33	Leader-Flag-C40-IgG1(N297A)-TNC	MNFGFSLIFLVLVLKGVQCEVKLVPR**QL**DYKDDDDK**EF***QVTLKE* *SGPGILQPSQTLSLTCSFSGFSLSTFGMGVGWIRQPSGKGLEWL* *AHIWWDDDKYYNPALKSRLTISKDTSENQVFLKIANVDTADTAT* *YYCVRIAGTRYFDVWGTGTTVTVSS***RS**SSASTKGPSVFPLAPSS KSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQS SGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSC DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVV DVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTV LHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLP PSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPP VLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKS LSLSPGK**LE***DIACGCAAAPDIKDLLSRLEELEGLVSSLREQGTG*
34	Leader-Flag-C4-IgG1(N297A)-scFvC9	MNFGFSLIFLVLVLKGVQCEVKLVPR**QL**DYKDDDDK**EL***QVQLLQ* *SGPELVKPGASVKLSCKASGYSFTSYDINWVKQRPGQGLEWVGW* *IYPRDGDTKYNEKFKGKAILTVDTSSNTAYMNLHSLTSEDSAVY* *FCARLTGPYWYFDVWGTGTTVTVSS***GS**SSASTKGPSVFPLAPSS KSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQS SGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSC DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVV DVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTV LHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLP PSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPP VLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKS LSLSPGK**EFLE***QIQLVQSGPELKKPGETVKISCKASGYTFTTAG* *MQWVQKMPGKGFKWIGWINTHSGEPKYAEDFKGRFAFSLETSAS* *TAYLQISNLKNEDTATYFCARWDGTGYWGQGTTLTVSS***RS*****STKG*** ***PKLEEGEFSEA*****QL***QIVLTQSPAIMSASPGEKVTITCSASSSVSY* *MHWFQQKPGTSPKLWIYSTSNLASGVPARFSGSGSGTSYSLTIS* *RMEAEDAATYYCQQRSSYPPTFGGGTKLEIK*
35	Leader-Flag-C4-IgG1(N297A)-scFvC19	MNFGFSLIFLVLVLKGVQCEVKLVPR**QL**DYKDDDDK**EL***QVQLLQ* *SGPELVKPGASVKLSCKASGYSFTSYDINWVKQRPGQGLEWVGW* *IYPRDGDTKYNEKFKGKAILTVDTSSNTAYMNLHSLTSEDSAVY* *FCARLTGPYWYFDVWGTGTTVTVSS***GS**SSASTKGPSVFPLAPSS KSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQS SGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSC DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVV DVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTV LHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLP PSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPP VLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKS LSLSPGK**EFLE***QIQLVQSGPEVKKPGETVKISCKASGYTFTIHG* *MSWVKQAPGKGLKWMGWINTYSGVPTYANDFKGRFAFSLETSAS* *TAYLQINNLKNEDTATYFCARDEVRRGFGFAYWGQGTLVTVSA***R** ***SSTKGPKLEEGEFSEA*****QL***DIVMAQSQKFMSVSVGDRVSVTCKAS* *QNVGTNVAWYQQRPGQSPKALIYSASYRYSGVPDRFTGSGSGTD* *FTLTISNVQSEDLAEYFCQQFDSHPLTFGAGTKLELK*
36	Leader-Flag-C4-IgG1(N297A)-scFvC40	MNFGFSLIFLVLVLKGVQCEVKLVPR**QL**DYKDDDDK**EL***QVQLLQ* *SGPELVKPGASVKLSCKASGYSFTSYDINWVKQRPGQGLEWVGW*

		*IYPRDGDTKYNEKFKGKAILTVDTSSNTAYMNLHSLTSEDSAVY FCARLTGPYWYFDVWGTGTTVTVSS***GS**SSASTKGPSVFPLAPSS KSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQS SGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSC DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVV DVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTV LHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLP PSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPP VLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKS LSLSPGK**EFLE***QVTLKESGPGILQPSQTLSLTCSFSGFSLSTFG MGVGWIRQPSGKGLEWLAHIWWDDDKYYNPALKSRLTISKDTSE NQVFLKIANVDTADTATYYCVRIAGTRYFDVWGTGTTVTVSS***RS* ***STKGPKLEEGEFSEA*****QL***DIQMTQSPSSLSASLGGKVTITCKASQ DINKFIAWYQHKPGKGPRLLIHYTSTLQPGIPSRFSGSGSGRDY SFSISNLEPEDIATYYCLQYDNLYTFGGGTKLEIK*

37	Leader-C4-LC-GpL Flag less	MNFGFSLIFLVLVLKGVQCEVKLVPR**QL***DIVMTQSHKFMSTSVG DRVSITCKASQDVDTAVAWYQQKPGQSPKLLIYWASTRHTGVPD RFTGSGSGTDYTLTISSVQAEDLARYYCQQYYSVPPTFGGGTKL* **GS**EIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKV QWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHK VYACEVTHQGLSSPVTKSFNRGEC**LE**KPTENNEDFNIVAVASNF ATTDLDADRGKLPGKKLPLEVLKEMEANARKAGCTRGCLICLSH IKCTPKMKKFIPGRCHTYEGDKESAQGGIGEAIVDIPEIPGFKD LEPMEQFIAQVDLCVDCTTGCLKGLANVQCSDLLKKWLPQRCAT FASKIQGQVDKIKGAGGD
38	Leader-Flag-C4-IgG1(N297A)	MNFGFSLIFLVLVLKGVQCEVKLVPR**QL**DYKDDDDK**EF***QVQLLQ SGPELVKPGASVKLSCKASGYSFTSYDINWVKQRPGQGLEWVGW IYPRDGDTKYNEKFKGKAILTVDTSSNTAYMNLHSLTSEDSAVY FCARLTGPYWYFDVWGTGTTVTVSS***RS***SSASTKGPSVFPLAPSS KSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQS SGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSC DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVV DVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTV LHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLP PSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPP VLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKS LSLSPGK

Supplemental Table S3. Antibodies and variants thereof used in this study. For the amino acid sequences of the expression plasmid encoded proteins please see supplemental table SI.

Proteine name		Expression plasmid(s) no. in table SI	
Short	Systematic name		
C4-(1)	C4-Fab1-LC:scFvC4	1	2
C4-(2)	C4-Fab2-LC:scFvC4	1	3
C4-(3)	C4-IgG1(N297A)-HC:scFvC4	4	5
C4-(4)	C4-IgG1(N297A)-LC:scFvC4	1	38
C4-(5)	C4-IgG1(N297A)-LC:scFvC4-HC:scFvC4	1	5
C4-(6)	C4(scFv)-IgG1(N297A)-HC:scFvC4	6	7
C4-(7)	C4(scFv)-IgG1(N297A)-LC:scFvC4	8	9
C4-(8)	C4(scFv)-IgG1(N297A)-LC:scFvC4-HC:scFvC4	8	7
C4-(9)	C4-IgG1(N297A)-HC:TNC-scFvC4	4	10
C4-(10)	C4-Fab1	4	2
C4-(11)	C4-Fab2	4	3
C4-(12)	C4-IgG1(N297A)	4	38
C4-(13)	C4(scFv)-IgG1(N297A)	6	9
C4-(14)	C4-IgG1(N297A)-HC:TNC	4	11
C4-(15)	C4(scFv)-TNC-Fc(DANA)	12	
C4-(16)	C4(scFv)-Fc(DANA)-TNC	13	
C4-(17)	C4-IgG1(N297A)-HC:RGY	4	14
C4-(18)	C4(scFv)-IgG1(N297A)-HC:TNC	6	15
C9-(12)	C9-IgG1(N297A)	16	17
C9-(3)	C9-IgG1(N297A)-HC:scFvC9	16	18
C9-(13)	C9(scFv)-IgG1(N297A)	19	20
C9-(14)	C9-IgG1(N297A)-HC:TNC	16	21
C19-(12)	C19-IgG1(N297A)	22	23
C19-(3)	C19-IgG1(N297A)-HC:scFvC19	22	24
C19-(13)	C19(scFv)-IgG1(N297A)	25	26
C19-(14)	C19-IgG1(N297A)-HC:TNC	22	27
C40-(12)	C40-IgG1(N297A)	28	29
C40-(3)	C40-IgG1(N297A)-HC:scFvC40	28	30
C40-(13)	C40(scFv)-IgG1(N297A)	31	32
C40-(14)	C40-IgG1(N297A)-HC:TNC	28	33
C4/C9-(3)	C4-IgG1(N297A)-HC:scFvC9	4	34
C4/C19-(3)	C4-IgG1(N297A)-HC:scFvC19	4	35
C4/C40-(3)	C4-IgG1(N297A)-HC:scFvC40	4	36
C4-(3)-GpL	C4-IgG1(N297A)-HC:scFvC4-LC:GpL	37	5
C4-(12)-GpL	C4-IgG1(N297A)-LC:GpL	37	38