Supplementary Data:

A universal reporter cell line for bioactivity evaluation of engineered cytokine products

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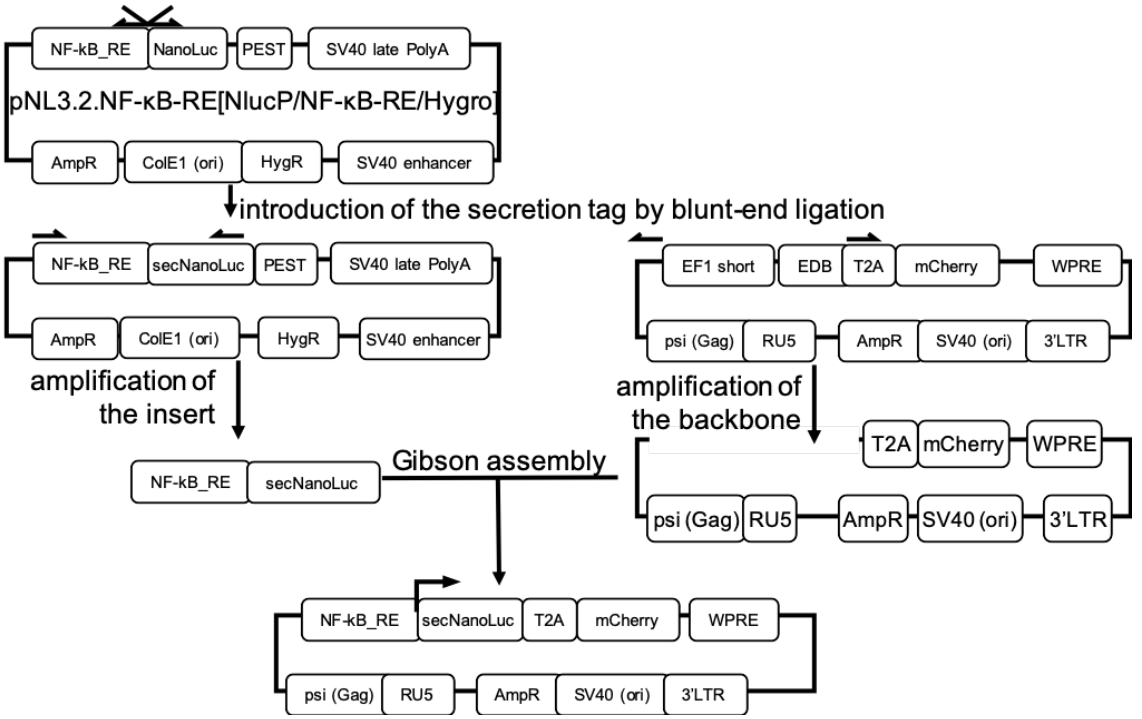


NF-kB_RE-minimalPromoter-hIL6-secretionSignal-Nanoluc-T2A-mCherry

GGGAATTTCCGGGGACTTTCCGGGAATTTCCGGGGACTTTCCGGGAATTTCC-AGATCTGG
CCTCGGCGGCCAAGCTTAGACACT-AGAGGGTATATAATGGAAGCTCGACTTCCAG-CTTG
GCAATCCGGTACTGTTGGTAAAGCCACC-ATGAACTCCTTCTCCACAAGCGCCTTCGGTCC
AGTTGCCTTCTCCCTGGGCCTGCTCCTGGTGTTGCCTGCTGCCTTCCCTGCCCCA-GTCTT
CACACTCGAAGATTTCGTTGGGGACTGGCGACAGACAGCCGGCTACAACCTGGACCAAGTC
CTTGAACAGGGAGGTGTGTCCAGTTTGTTTCAGAATCTCGGGGTGTCCGTAACTCCGATCC
AAAGGATTGTCCTGAGCGGTGAAAATGGGCTGAAGATCGACATCCATGTCATCATCCCGTA
TGAAGGTCTGAGCGGCGACCAAATGGGCCAGATCGAAAAAATTTTTAAGGTGGTGTACCCT
GTGGATGATCATCACTTTAAGGTGATCCTGCACTATGGCACACTGGTAATCGACGGGGTTA
CGCCGAACATGATCGACTATTTCGGACGGCCGTATGAAGGCATCGCCGTGTTCGACGGCAA
AAAGATCACTGTAACAGGGACCCTGTGGAACGGCAACAAAATTATCGACGAGCGCCTGATC
AACCCCGACGGCTCCCTGCTGTTCCGAGTAACCATCAACGGAGTGACCGGCTGGCGGCTGT
GCGAACGCATTCTGGCG-GAGGGCAGAGGAAGTCTTCTAACATGCGGTGACGTGGAGGAGA
ATCCCGGCCCT-ATGGTGAGCAAGGGCGAGGAGGATAACATGGCCATCATCAAGGAGTTCA
TGCGCTTCAAGGTGCACATGGAGGGCTCCGTGAACGGCCACGAGTTCGAGATCGAGGGCGA
GGGCGAGGGCCGCCCCTACGAGGGCACCCAGACCGCCAAGCTGAAGGTGACCAAGGGTGGC
CCCCTGCCCTTCGCCTGGGACATCCTGTCCCCTCAGTTCATGTACGGCTCCAAGGCCTACG
TGAAGCACCCCGCCGACATCCCCGACTACTTGAAGCTGTCCTTCCCCGAGGGCTTCAAGTG
GGAGCGCGTGATGAACTTCGAGGACGGCGGCGTGGTGACCGTGACCCAGGACTCCTCCCTG
CAGGACGGCGAGTTCATCTACAAGGTGAAGCTGCGCGGCACCAACTTCCCCTCCGACGGCC
CCGTAATGCAGAAGAAGACCATGGGCTGGGAGGCCTCCTCCGAGCGGATGTACCCCGAGGA
CGGCGCCCTGAAGGGCGAGATCAAGCAGAGGCTGAAGCTGAAGGACGGCGGCCACTACGAC
GCTGAGGTCAAGACCACCTACAAGGCCAAGAAGCCCGTGCAGCTGCCCGGCGCCTACAACG
TCAACATCAAGTTGGACATCACCTCCCACAACGAGGACTACACCATCGTGGAACAGTACGA
ACGCGCCGAGGGCCGCCACTCCACCGGCGGCATGGACGAGCTGTACAAGTAA

Supplementary Figure S1: cloning strategy and sequence of the reporter vector a) outline of the cloning strategy b) sequence of the reporter vector, primer binding sites are underlined

a **F8(dDb)-4-1BBL**: F8_VH-*linker*-F8_VL-*linker*-4-1BBL-*linker*-4-1BBL-*linker*-4-1BBL
EVQLLESGGGLVQPGGSLRLSCAASGFTFSLFTMSWVRQAPGKGLEWVSAISGSGGSTYYADSVK
GRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAKSTHLYLFDYWGQGTLVTVSS-*GGSGG*-EIVLTQS
PGTLSLSPGERATLSCRASQSVSMPFLAWYQQKPGQAPRLLIYGASSRATGIPDRFSGSGSGTDFTL
TISRLEPEDFAVYYCQQMRGRPPTFGQGTKVEIK-*SSSSGSSSSGSSSSG*-ATTQQGSPVFAKLLAKN
QASLCNTTLNWHSQDGAGSSYLSQGLRYEEDKKELVVDSPGLYYVFLELKLSPTFTNTGHKVQGW
VSLVLQAKPQVDDFDNLALTVELFPCSMENKLVDRSWSQLLLLKAGHRLSVGLRAYLHGAQDAYR
DWELSYPNTTSFGLFLVKPDNPWE-*G*-ATTQQGSPVFAKLLAKNQASLCNTTLNWHSQDGAGSSY
LSQGLRYEEDKKELVVDSPGLYYVFLELKLSPTFTNTGHKVQGWVSLVLQAKPQVDDFDNLALTVE
LFPCSMENKLVDRSWSQLLLLKAGHRLSVGLRAYLHGAQDAYRDWELSYPNTTSFGLFLVKPDNP
WE-*G*-ATTQQGSPVFAKLLAKNQASLCNTTLNWHSQDGAGSSYLSQGLRYEEDKKELVVDSPGLY
YVFLELKLSPTFTNTGHKVQGWVSLVLQAKPQVDDFDNLALTVELFPCSMENKLVDRSWSQLLLLK
AGHRLSVGLRAYLHGAQDAYRDWELSYPNTTSFGLFLVKPDNPWE

b **F8(scFv)-4-1BBL**: F8_VH-*linker*-F8_VL-*linker*-4-1BBL
EVQLLESGGGLVQPGGSLRLSCAASGFTFSLFTMSWVRQAPGKGLEWVSAISGSGGSTYYADSVK
GRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAKSTHLYLFDYWGQGTLVTVSS-*GGGGSGGGGSG*
GGG-EIVLTQSPGTLSLSPGERATLSCRASQSVSMPFLAWYQQKPGQAPRLLIYGASSRATGIPDRF
SGSGSGTDFTLTISRLEPEDFAVYYCQQMRGRPPTFGQGTKVEIK-*SSSSGSSSSGSSSS*-GATTQQG
SPVFAKLLAKNQASLCNTTLNWHSQDGAGSSYLSQGLRYEEDKKELVVDSPGLYYVFLELKLSPTFT
NTGHKVQGWVSLVLQAKPQVDDFDNLALTVELFPCSMENKLVDRSWSQLLLLKAGHRLSVGLRA
LHGAQDAYRDWELSYPNTTSFGLFLVKPDNPWE

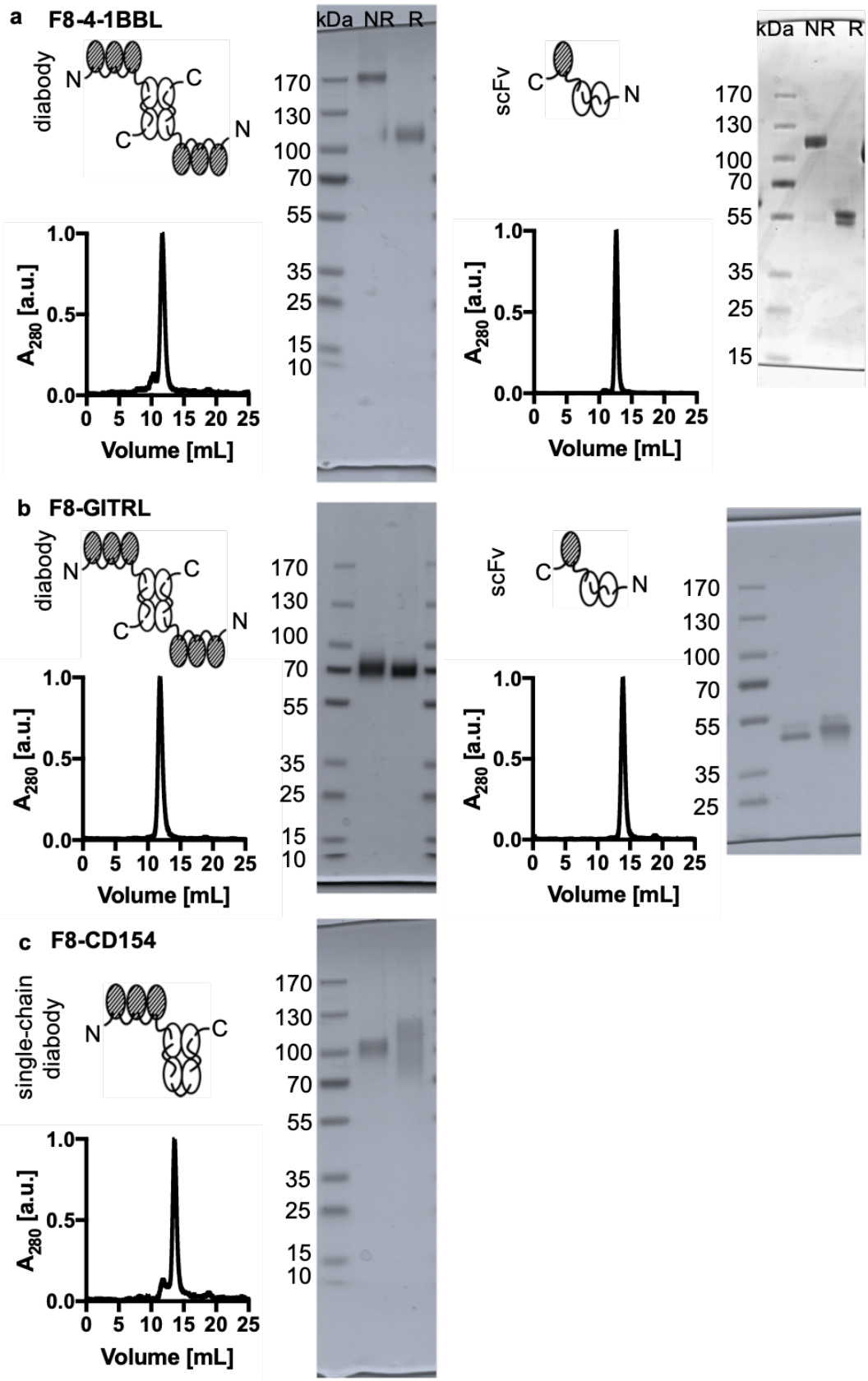
c **F8(dDb)-GITRL**: F8_VH-*linker*-F8_VL-*linker*-GITRL-*linker*-GITRL-*linker*-GITRL
EVQLLESGGGLVQPGGSLRLSCAASGFTFSLFTMSWVRQAPGKGLEWVSAISGSGGSTYYADSVK
GRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAKSTHLYLFDYWGQGTLVTVSS-*GGSGG*-EIVLTQS
PGTLSLSPGERATLSCRASQSVSMPFLAWYQQKPGQAPRLLIYGASSRATGIPDRFSGSGSGTDFTL
TISRLEPEDFAVYYCQQMRGRPPTFGQGTKVEIK-*SSSSGSSSSGSSSSG*-PTAIESCMVKFELSSSKW
HMTSPKPHCVNTTSDGKLKILQSGTYLIYGQVIPVDKKYIKDNAPFVVQIYKKNDVLQTLMNDFQIL
PIGGVYELHAGDNIYLKFNSKDHIQKNNTYWGIILMPDLP-*GGGSGGG*-PTAIESCMVKFELSSSKW
HMTSPKPHCVNTTSDGKLKILQSGTYLIYGQVIPVDKKYIKDNAPFVVQIYKKNDVLQTLMNDFQIL
PIGGVYELHAGDNIYLKFNSKDHIQKNNTYWGIILMPDLP-*GGGSGGG*-PTAIESCMVKFELSSSKW
HMTSPKPHCVNTTSDGKLKILQSGTYLIYGQVIPVDKKYIKDNAPFVVQIYKKNDVLQTLMNDFQIL
PIGGVYELHAGDNIYLKFNSKDHIQKNNTYWGIILMPDLP

d **F8(scFv)-GITRL**: F8_VH-*linker*-F8_VL-*linker*-GITRL
EVQLLESGGGLVQPGGSLRLSCAASGFTFSLFTMSWVRQAPGKGLEWVSAISGSGGSTYYADSVK
GRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAKSTHLYLFDYWGQGTLVTVSS-*GGGGSGGGGSG*
GGG-EIVLTQSPGTLSLSPGERATLSCRASQSVSMPFLAWYQQKPGQAPRLLIYGASSRATGIPDRF
SGSGSGTDFTLTISRLEPEDFAVYYCQQMRGRPPTFGQGTKVEIK-*SSSSGSSSSGSSSS*-PTAIESCM
VKFELSSSKWHMTSPKPHCVNTTSDGKLKILQSGTYLIYGQVIPVDKKYIKDNAPFVVQIYKKNDVL
QTLMNDFQILPIGGVYELHAGDNIYLKFNSKDHIQKNNTYWGIILMPDLP

e **F8(scDb)-CD154**: F8_VH-*linker*-F8_VL- *linker*- F8_VH-*linker*-F8_VL-*linker*-CD154-*linker*-
CD154-*linker*-CD154
EVQLLESGGGLVQPGGSLRLSCAASGFTFSLFTMSWVRQAPGKGLEWVSAISGSGGSTYYADSVK
GRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAKSTHLYLFDYWGQGTLVTVSS-*GGSGG*-EIVLTQS
PGTLSLSPGERATLSCRASQSVSMPFLAWYQQKPGQAPRLLIYGASSRATGIPDRFSGSGSGTDFTL
TISRLEPEDFAVYYCQQMRGRPPTFGQGTKVEIK-*GGGGSGGGGSGGGGS*-EVQLLESGGGLVQP
GGSLRLSCAASGFTFSLFTMSWVRQAPGKGLEWVSAISGSGGSTYYADSVKGRFTISRDNSKNTLY

LQMNSLRAEDTAVYYCAKSTHLYLFDYWGQGTLVTVSS-*GGSGG*-EIVLTQSPGTLSLSPGERATLS
CRASQSVSMPFLAWYQQKPGQAPRLLIYGASSRATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYC
QQMRGRPPTFGQGTKVEIK-*SSSSGSSSSGSSSS*-QRGDEDPQIAAHVVSEANSNAASVLQWAKK
GYYTMKSNLVMLENGKQLTVKREGLYYVYTQVTFCSNREPSSQRPFIVGLWLKPSSGSERILLKANT
HSSSQLCEQQSVHLGGVFELQAGASVFVNVTEASQVIHRVGFSSFGLLKL-*GGGS*-QRGDEDPQIA
AHVVSEANSNAASVLQWAKKGYYTMKSNLVMLENGKQLTVKREGLYYVYTQVTFCSNREPSSQR
PFIVGLWLKPSSGSERILLKANTHSSSQLCEQQSVHLGGVFELQAGASVFVNVTEASQVIHRVGFSS
FGLLKL-*GGGS*-QRGDEDPQIAAHVVSEANSNAASVLQWAKKGYYTMKSNLVMLENGKQLTVKR
EGLYYVYTQVTFCSNREPSSQRPFIVGLWLKPSSGSERILLKANTHSSSQLCEQQSVHLGGVFELQA
GASVFVNVTEASQVIHRVGFSSFGLLKL

Supplementary Figure S2: Sequences of the immunocytokines that were developed in this study, the linker sequences are depicted in italics



Supplementary Figure S3: The proteins were characterized by SDS PAGE (NR: non-reducing, R: reducing sample buffer) and size exclusion chromatography (Superdex 200 Increase, 10/300 GL, GE Healthcare) after purification.

	conventional assay			luciferase assay			mCherry expression		
	EC_{50}	95% CI	R^2	EC_{50}	95% CI	R^2	EC_{50}	95% CI	R^2
L19-IL2	47 pM	26 to 83 pM	0.95	15 pM	8.5 to 26 pM	0.94	21 pM	6.3 to 74 pM	0.82
L19-IL12	0.1 nM	0.1 to 0.2 nM	0.99	3.5 nM	1.9 to 6.0 nM	0.95	2.6 nM	2.0 to 3.4 nM	0.99
F8-TNF	0.7 pM	0.6 to 0.9 pM	0.98	450 pM	280 to 730 pM	0.96	540 pM	450 to 650 pM	0.99

Supplementary Table T1: EC_{50} obtained from a sigmoidal curve fit for conventional assay as well as for the readouts with the new cell lines (95% CI: 95% confidence interval)

		+ EDA						no EDA					
		luciferase activity			mCherry expression			luciferase activity			mCherry expression		
		EC_{50}	95% CI	R^2	EC_{50}	95% CI	R^2	EC_{50}	95% CI	R^2	EC_{50}	95% CI	R^2
F8-4-1BBL	diabody	1.1 nM	0.9 to 1.4 nM	0.99	1.2 nM	0.9 to 1.5 nM	0.99	1.1 nM	0.8 to 1.5 nM	0.98	1.0 nM	0.8 to 1.3 nM	0.99
	scFv	1.1 nM	0.7 to 1.8 nM	0.97	1.0 nM	0.7 to 1.6 nM	0.98	n/a	n/a	n/a	n/a	n/a	n/a
F8-GITRL	diabody	7.6 nM	6.2 to 9.3 nM	0.99	7.9 nM	7.0 to 8.9 nM	1.00	4.7 nM	3.9 to 5.7 nM	0.99	4.1 nM	3.6 to 4.6 nM	1.00
	scFv	11 nM	7.5 to 18 nM	0.97	9.6 nM	7.5 to 12 nM	0.99	56 nM	43 to 72 nM	0.99	67 nM	57 to 79 nM	0.99
F8-CD154	diabody	65 pM	45 to 9.3 pM	0.98	64 pM	49 to 85 pM	0.99	660 pM	0.4 to 1.1 nM	0.97	1.1 nM	0.4 to 2.7 nM	0.93

Supplementary Table T2: EC_{50} values obtained from a sigmoidal curve fit for the different F8-TNFSF fusion proteins (95% CI: 95% confidence interval)