Supplementary Material

Antibody-based soluble and membrane-bound TWEAK mimicking agonists with FcγR-independent activity

Olena Zaitseva[1,#], Annett Hoffmann[2,#], Margareta Loest[1], Mohamed A. Anany[1], Tengyu Zhang[1], Kirstin Kucka[1], Armin Wiegering[2], Christoph Otto[2] and Harald Wajant[1,*]

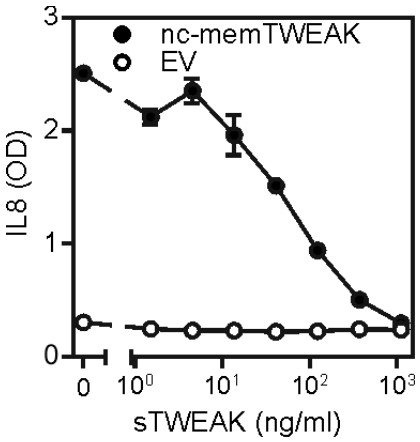
[1] University Hospital Würzburg, Division of Molecular Internal Medicine, Department of Internal Medicine II, Würzburg, Germany.

[2] University Hospital Würzburg, Department of General, Visceral, Transplant, Vascular and Pediatric Surgery, Oberdürrbacher Straße 6, 97080 Würzburg, Germany.

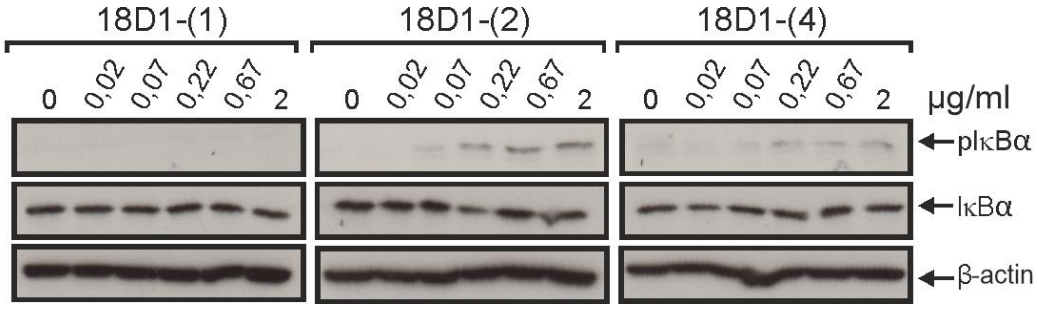
[#] These authors equally contributed to this work.

* Corresponding author: harald.wajant@mail.uni-wuerzburg.de

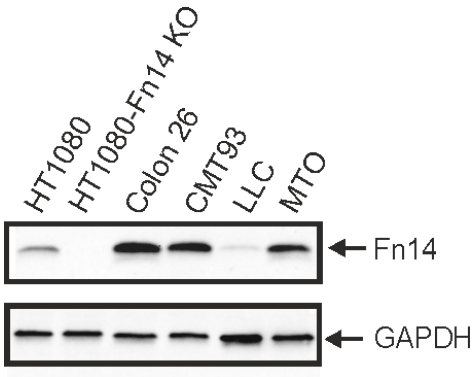
Supplementary Figures



Supplemental Figure S1. HT1080 cells, which produce IL8 in response to memTWEAK expressing cells, were mixed with HEK293 cells transiently transfected with empty vector (EV, negative control) or an expression plasmid encoding a non-cleavable form of membrane TWEAK (nc-memTWEAK). Cell mixtures were further supplemented with the indicated concentration of soluble TWEAK (sTWEAK). Next day, IL8 production was evaluated by ELISA.



Supplemental Figure S2. The tetra- and hexavalent 18D1 variants (2) and (4) but not the parental bivalent antibody (18D1-(1)) trigger IκBα phosphorylation in HT1080 cells. Cells were stimulated with the indicated concentrations of 18D1-(1), 18D1-(2) and 18D1-(4) overnight and total lysates were finally analyzed by western blotting for the indicated proteins. Please note, at early time points the initial unbalance of the coupled processes of IκBα phosphorylation, IκBα degradation and NFκB-induced IκBα resynthesis does not allow a fair comparison of dose-responses relationships at one defined time point. We therefore analyzed a later time point when these processes have reached an equilibrium resulting in IκBα phosphorylation at constant IκBα levels.



Supplemental Figure S3. Fn14 expression in murine tumor organoids (MTO) derived of tumors developing from *Apc*$^{ko/ko}$, *Kras*$^{LSL-G12D}$, *Tgfbr2*$^{ko/ko}$ and *Trp53*$^{ko/ko}$ intestinal stem cells. Total lysates of MTOs, the murine tumor cell lines Colon 26, CMT93 and Lewis lung carcinoma along with lysates of HT1080 and Fn14-deficient HT1080 cells were analyzed by western blot for the presence of Fn14, GAPDH expression was analyzed as load control.

Supplementary Tables

Supplemental table SI. AA sequences of heavy and light chain variants of Fn14-specific antibody constructs. 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.

No.	Plasmid name	Sequence
1	18D1-HC(N297A)	MNFGFSLIFLVLVLKGVQCEVKLVPRQLDYKDDDDKEF*EVQLVESGGGLVQPG GSLRLSCAASGFTFSNYWMSWVRQAPGKGLEWVSGINPGGTSTYYADSVKGRF TISRDNAKNTLYLQMNSLKSEDTAVYYCAKHLGNWGEYNYWGQGTQVTVSS*GS SSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVH TFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCD KTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVK FNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKA LPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVE WESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALH NHYTQKSLSLSPGK
2	18D1-HC(N297A)-scFv:18D1	MNFGFSLIFLVLVLKGVQCEVKLVPRQLDYKDDDDKEF*EVQLVESGGGLVQPG GSLRLSCAASGFTFSNYWMSWVRQAPGKGLEWVSGINPGGTSTYYADSVKGRF TISRDNAKNTLYLQMNSLKSEDTAVYYCAKHLGNWGEYNYWGQGTQVTVSS*GS SSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVH TFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCD KTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVK FNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKA LPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVE WESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALH NHYTQKSLSLSPGKLE*EVQLVESGGGLVQPGGSLRLSCAASGFTFSNYWMSWV RQAPGKGLEWVSGINPGGTSTYYADSVKGRFTISRDNAKNTLYLQMNSLKSED TAVYYCAKHLGNWGEYNYWGQGTQVTVSS*RS*STKGPKLEEGEFSEA*QL*QSALT QPPSVSGSPGKTVTISCAGTGGDVGYRNSVSWYQQLPGMAPKLLIYDVDKRAS GITDRFSGSKSGDTASLTISGVQSEDEADYYCASQRSGIAAVFGGGTHLTVLG*
3	18D1-LC	MNFGFSLIFLVLVLKGVQCEVKLVPRQLDYKDDDDKEF*QSALTQPPSVSGSPG KTVTISCAGTGGDVGYRNSVSWYQQLPGMAPKLLIYDVDKRASGITDRFSGSK SGDTASLTISGVQSEDEADYYCASQRSGIAAVFGGGTHLTVLG*GSEIKRTVAA PSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTE QDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC
4	18D1-LC-scFv:18D1	MNFGFSLIFLVLVLKGVQCEVKLVPRQLDYKDDDDKEF*QSALTQPPSVSGSPG KTVTISCAGTGGDVGYRNSVSWYQQLPGMAPKLLIYDVDKRASGITDRFSGSK SGDTASLTISGVQSEDEADYYCASQRSGIAAVFGGGTHLTVLG*GSEIKRTVAA PSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTE QDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGECLE*EV QLVESGGGLVQPGGSLRLSCAASGFTFSNYWMSWVRQAPGKGLEWVSGINPGG TSTYYADSVKGRFTISRDNAKNTLYLQMNSLKSEDTAVYYCAKHLGNWGEYNY WGQGTQVTVSS*RS*STKGPKLEEGEFSEA*QL*QSALTQPPSVSGSPGKTVTISCA GTGGDVGYRNSVSWYQQLPGMAPKLLIYDVDKRASGITDRFSGSKSGDTASLT ISGVQSEDEADYYCASQRSGIAAVFGGGTHLTVLG*
5	scFv:18D1-CL	MNFGFSLIFLVLVLKGVQCEVKLVPRQLDYKDDDDKEF*EVQLVESGGGLVQPG GSLRLSCAASGFTFSNYWMSWVRQAPGKGLEWVSGINPGGTSTYYADSVKGRF TISRDNAKNTLYLQMNSLKSEDTAVYYCAKHLGNWGEYNYWGQGTQVTVSS*RS STKGPKLEEGEFSEA*QL*QSALTQPPSVSGSPGKTVTISCAGTGGDVGYRNSVS WYQQLPGMAPKLLIYDVDKRASGITDRFSGSKSGDTASLTISGVQSEDEADYY CASQRSGIAAVFGGGTHLTVLG*GSEIKRTVAAPSVFIFPPSDEQLKSGTASVV CLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADY EKHKVYACEVTHQGLSSPVTKSFNRGEC

6	scFv:18D1-$CH_{1,2,3}$(N297A)	MNFGFSLIFLVLVLKGVQCEVKLVPRQLDYKDDDDKEF*EVQLVESGGGLVQPG* *GSLRLSCAASGFTFSNYWMSWVRQAPGKGLEWVSGINPGGTSTYYADSVKGRF* *TISRDNAKNTLYLQMNSLKSEDTAVYYCAKHLGNWGEYNYWGQGTQVTVSS*RS *STKGPKLEEGEFSEAQLQSALTQPPSVSGSPGKTVTISCAGTGGDVGYRNSVS* *WYQQLPGMAPKLLIYDVDKRASGITDRFSGSKSGDTASLTISGVQSEDEADYY* *CASQRSGIAAVFGGGTHLTVLG*GSSSASTKGPSVFPLAPSSKSTSGGTAALGC LVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQT YICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDT LMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYASTYRVV SVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRD ELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSK LTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
7	scFv:18D1-CL-scFv:18D1	MNFGFSLIFLVLVLKGVQCEVKLVPRQLDYKDDDDKEF*EVQLVESGGGLVQPG* *GSLRLSCAASGFTFSNYWMSWVRQAPGKGLEWVSGINPGGTSTYYADSVKGRF* *TISRDNAKNTLYLQMNSLKSEDTAVYYCAKHLGNWGEYNYWGQGTQVTVSS*RS *STKGPKLEEGEFSEAQLQSALTQPPSVSGSPGKTVTISCAGTGGDVGYRNSVS* *WYQQLPGMAPKLLIYDVDKRASGITDRFSGSKSGDTASLTISGVQSEDEADYY* *CASQRSGIAAVFGGGTHLTVLG*GSEIKRTVAAPSVFIFPPSDEQLKSGTASVV CLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADY EKHKVYACEVTHQGLSSPVTKSFNRGEC*LEEVQLVESGGGLVQPGGSLRLSCA* *ASGFTFSNYWMSWVRQAPGKGLEWVSGINPGGTSTYYADSVKGRFTISRDNAK* *NTLYLQMNSLKSEDTAVYYCAKHLGNWGEYNYWGQGTQVTVSS*RS*STKGPKLE* *EGEFSEAQLQSALTQPPSVSGSPGKTVTISCAGTGGDVGYRNSVSWYQQLPGM* *APKLLIYDVDKRASGITDRFSGSKSGDTASLTISGVQSEDEADYYCASQRSGI* *AAVFGGGTHLTVLG*
8	scFv:18D1-$CH_{1,2,3}$(N297A)-scFv:18D1	MNFGFSLIFLVLVLKGVQCEVKLVPRQLDYKDDDDKEF*EVQLVESGGGLVQPG* *GSLRLSCAASGFTFSNYWMSWVRQAPGKGLEWVSGINPGGTSTYYADSVKGRF* *TISRDNAKNTLYLQMNSLKSEDTAVYYCAKHLGNWGEYNYWGQGTQVTVSS*RS *STKGPKLEEGEFSEAQLQSALTQPPSVSGSPGKTVTISCAGTGGDVGYRNSVS* *WYQQLPGMAPKLLIYDVDKRASGITDRFSGSKSGDTASLTISGVQSEDEADYY* *CASQRSGIAAVFGGGTHLTVLG*GSSSASTKGPSVFPLAPSSKSTSGGTAALGC LVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQT YICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDT LMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYASTYRVV SVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRD ELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSK LTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGKLE*EVQLVESGGGLVQ* *PGGSLRLSCAASGFTFSNYWMSWVRQAPGKGLEWVSGINPGGTSTYYADSVKG* *RFTISRDNAKNTLYLQMNSLKSEDTAVYYCAKHLGNWGEYNYWGQGTQVTVSS* RS*STKGPKLEEGEFSEAQLQSALTQPPSVSGSPGKTVTISCAGTGGDVGYRNS* *VSWYQQLPGMAPKLLIYDVDKRASGITDRFSGSKSGDTASLTISGVQSEDEAD* *YYCASQRSGIAAVFGGGTHLTVLG*
9	PDL192-HC(N297A)	MNFGFSLIFLVLVLKGVQCEVKLVPRQLDYKDDDDKEF*EVQLVESGGGLVQPG* *GSLRLSCAASGFTFSSYWMSWVRQAPGKGLEWVAEIRLKSDNYATHYAESVKG* *RFTISRDDSKNSLYLQMNSLRAEDTAVYYCTGYYADAMDYWGQGTLVTVSS*GS SSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVH TFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCD KTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVK FNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKA LPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVE WESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALH NHYTQKSLSLSPGK
10	PDL192-HC(N297A)-scFv:PDL192	MNFGFSLIFLVLVLKGVQCEVKLVPRQLDYKDDDDKEF*EVQLVESGGGLVQPG* *GSLRLSCAASGFTFSSYWMSWVRQAPGKGLEWVAEIRLKSDNYATHYAESVKG* *RFTISRDDSKNSLYLQMNSLRAEDTAVYYCTGYYADAMDYWGQGTLVTVSS*GS SSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVH TFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCD KTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVK FNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKA LPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVE WESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALH NHYTQKSLSLSPGKEF*EVQLVESGGGLVQPGGSLRLSCAASGFTFSSYWMSWV* *RQAPGKGLEWVAEIRLKSDNYATHYAESVKGRFTISRDDSKNSLYLQMNSLRA* *EDTAVYYCTGYYADAMDYWGQGTLVTVSS*RS*STKGPKLEEGEFSEAQLDIQMT*

		QSPSSLSASVGDRVTITCRASQSVSTSSYSYMHWYQQKPGKAPKLLIKYASNL ESGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQHSWEIPYTFGGGTKVEIK
11	PDL192-LC	MNFGFSLIFLVLVLKGVQCEVKLVPRQLDYKDDDDKEF*DIQMTQSPSSLSASV GDRVTITCRASQSVSTSSYSYMHWYQQKPGKAPKLLIKYASNLESGVPSRFSG SGSGTDFTLTISSLQPEDFATYYCQHSWEIPYTFGGGTKV*EIKRTVAAPSVFI FPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKD STYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC
12	PDL192-LC-scFv:PDL192	MNFGFSLIFLVLVLKGVQCEVKLVPRQLDYKDDDDKEF*DIQMTQSPSSLSASV GDRVTITCRASQSVSTSSYSYMHWYQQKPGKAPKLLIKYASNLESGVPSRFSG SGSGTDFTLTISSLQPEDFATYYCQHSWEIPYTFGGGTKV*EIKRTVAAPSVFI FPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKD STYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGECEF*EVQLVES GGGLVQPGGSLRLSCAASGFTFSSYWMSWVRQAPGKGLEWVAEIRLKSDNYAT HYAESVKGRFTISRDDSKNSLYLQMNSLRAEDTAVYYCTGYYADAMDYWGQGT LVTVSS*RS*STKGPKLEEGEFSEA*QL*DIQMTQSPSSLSASVGDRVTITCRASQS VSTSSYSYMHWYQQKPGKAPKLLIKYASNLESGVPSRFSGSGSGTDFTLTISS LQPEDFATYYCQHSWEIPYTFGGGTKVEIK*
13	scFv:PDL192-CL	MNFGFSLIFLVLVLKGVQCEVKLVPRQLDYKDDDDKEF*EVQLVESGGGLVQPG GSLRLSCAASGFTFSSYWMSWVRQAPGKGLEWVAEIRLKSDNYATHYAESVKG RFTISRDDSKNSLYLQMNSLRAEDTAVYYCTGYYADAMDYWGQGTLVTVSS*RS *STKGPKLEEGEFSEA*QL*DIQMTQSPSSLSASVGDRVTITCRASQSVSTSSYSY MHWYQQKPGKAPKLLIKYASNLESGVPSRFSGSGSGTDFTLTISSLQPEDFAT YYCQHSWEIPYTFGGGTKVEIK*GSEIKRTVAAPSVFIFPPSDEQLKSGTASVV CLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADY EKHKVYACEVTHQGLSSPVTKSFNRGEC
14	scFv:PDL192-$CH_{1,2,3}$(N297A)	MNFGFSLIFLVLVLKGVQCEVKLVPRQLDYKDDDDKEF*EVQLVESGGGLVQPG GSLRLSCAASGFTFSSYWMSWVRQAPGKGLEWVAEIRLKSDNYATHYAESVKG RFTISRDDSKNSLYLQMNSLRAEDTAVYYCTGYYADAMDYWGQGTLVTVSS*RS *STKGPKLEEGEFSEA*QL*DIQMTQSPSSLSASVGDRVTITCRASQSVSTSSYSY MHWYQQKPGKAPKLLIKYASNLESGVPSRFSGSGSGTDFTLTISSLQPEDFAT YYCQHSWEIPYTFGGGTKVEIK*GSSSASTKGPSVFPLAPSSKSTSGGTAALGC LVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQT YICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDT LMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYASTYRVV SVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRD ELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSK LTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

Supplemental table SII. Heavy and light chain composition of Fn14-specific antibody variants.

Antibody variant	**Plasmid no. from supplemental table I**
18D1-(1)	1+3
18D1-(2)	2+3
18D1-(3)	1+4
18D1-(4)	2+4
18D1-(5)	6+5
18D1-(6)	8+5
18D1-(7)	6+7
18D1-(8)	8+7
PDL192-(1)	9+11
PDL192-(2)	10+11
PDL192-(3)	9+12
PDL192-(4)	10+12
PDL192-(5)	14+13