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Supplemental Material to:

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Generation of a high-fidelity antibody against nerve growth factor using library scanning mutagenesis and validation with structures of the initial and optimized Fab-antigen complexes

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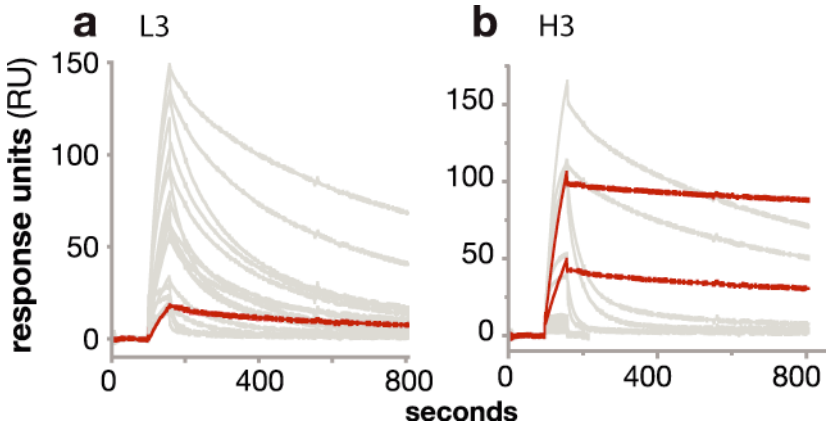


Figure S1. Raw biosensor data during LSM off-rate screening. **(a)** L3 mutant biosensor data, where red sensorgram highlights a prolonged off-rate clone **(b)** H3 mutant biosensor data, where red sensorgrams highlight prolonged off-rate clones**.**

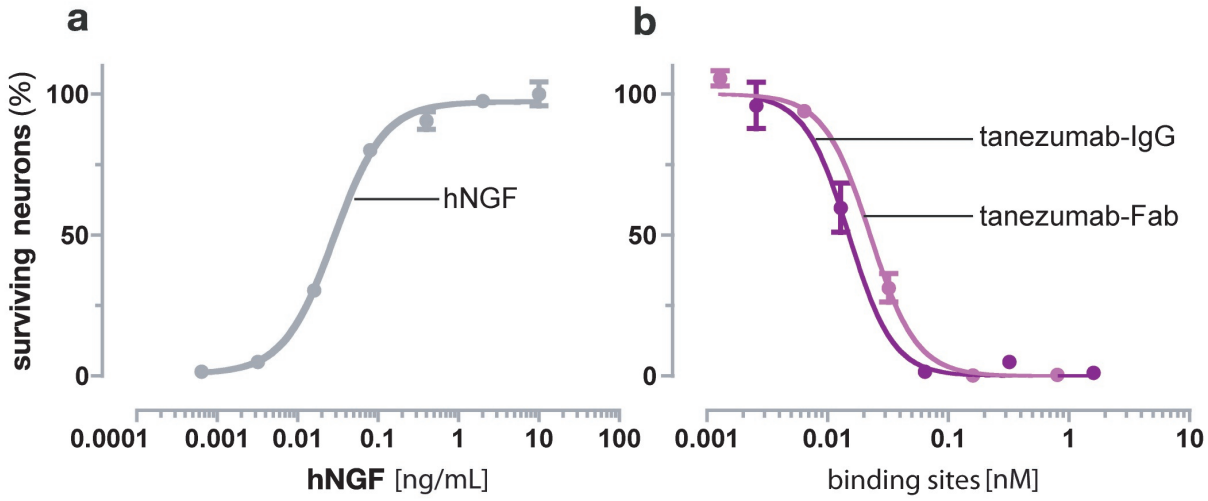


Figure S2. Neuron survival assay viability control analysis performed simultaneously with competition assay **(Figure 1e)**. **(a)** NGF-dependent survival of embryonic day 13 TG neurons were used to generate NGF dose-response curves corresponding to NGF concentration used in

competition assay. **(b)** Comparison of tanezumab as IgG and Fab to illustrate similarity in activity for bivalent IgG and monovalent Fab with NGF concentration of 15 pM.

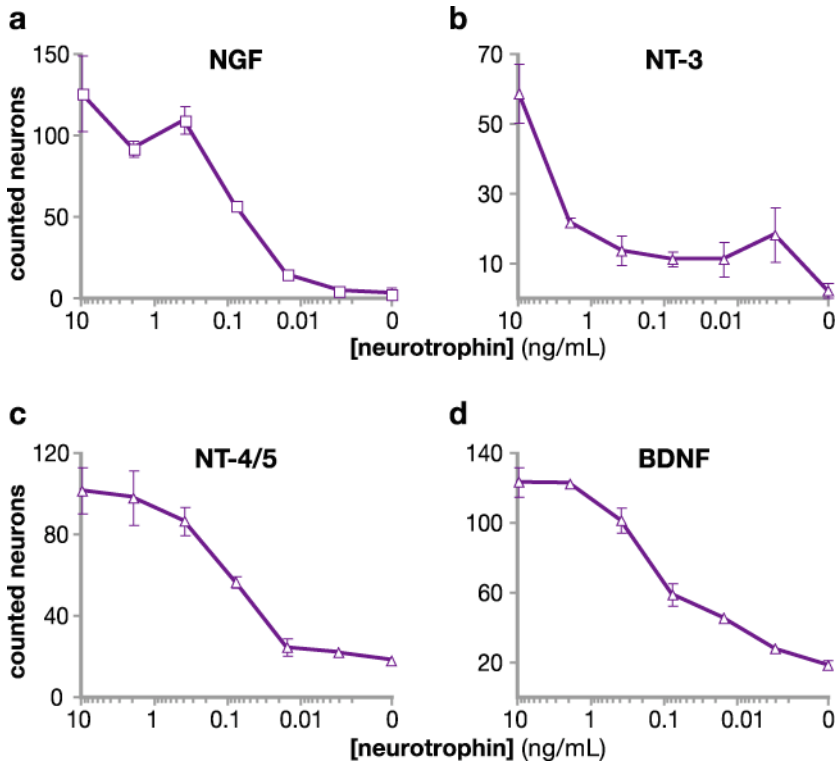


Figure S3. TG and Nodose neuron dose-response curves for each neurotrophin used with corresponding Trk-dependent survival and neurotrophin **(Figure 2a-d)**. **(a)** TG neuron survival dependent upon NGF. **(b)** TG neuron survival dependent upon NT-3. **(c)** Nodose neuron survival dependent upon NT-4/5. **(d)** Nodose neuron survival dependent upon BDNF.

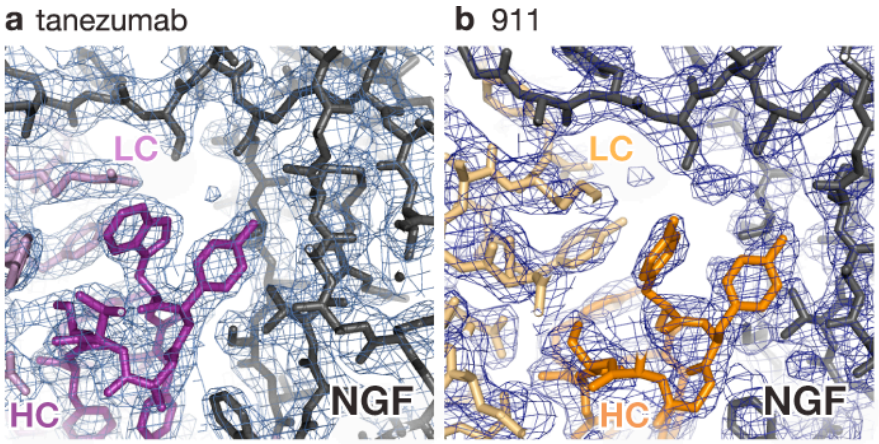


Figure S4. Model-phased electron density maps for NGF complexes calculated as 2FoFc contoured at 1σ. **(a)** HC CDR3 tanezumab (purple) and NGF (dark gray). **(b)** HC CDR3 911 (orange) and NGF (dark gray).

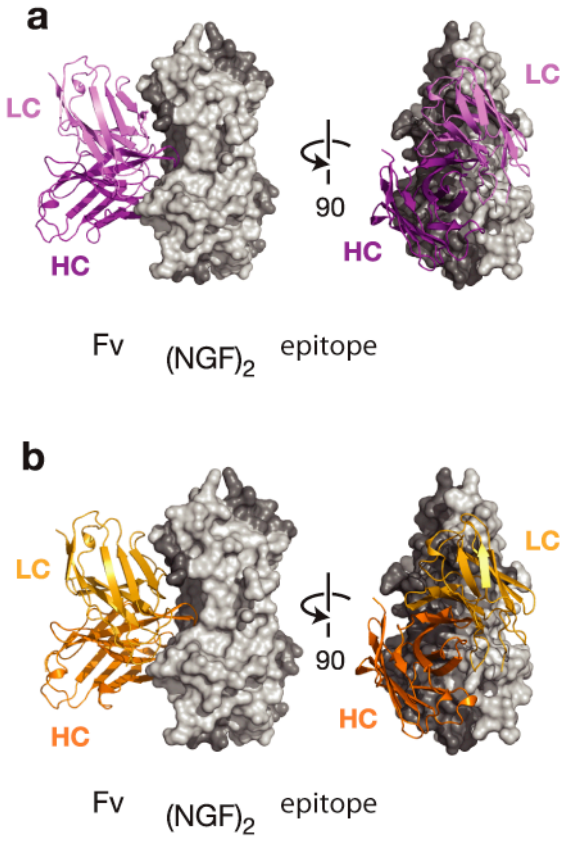


Figure S5. Comparison of Fv domains from tanezumab and 911 on $(NGF)_2$. Epitope overlap viewed through ribbon diagram representation of tanezumab **(a)** (purple) and 911 **(b)** (orange) as Fv domains on surface diagram of $(NGF)_2$(gray).

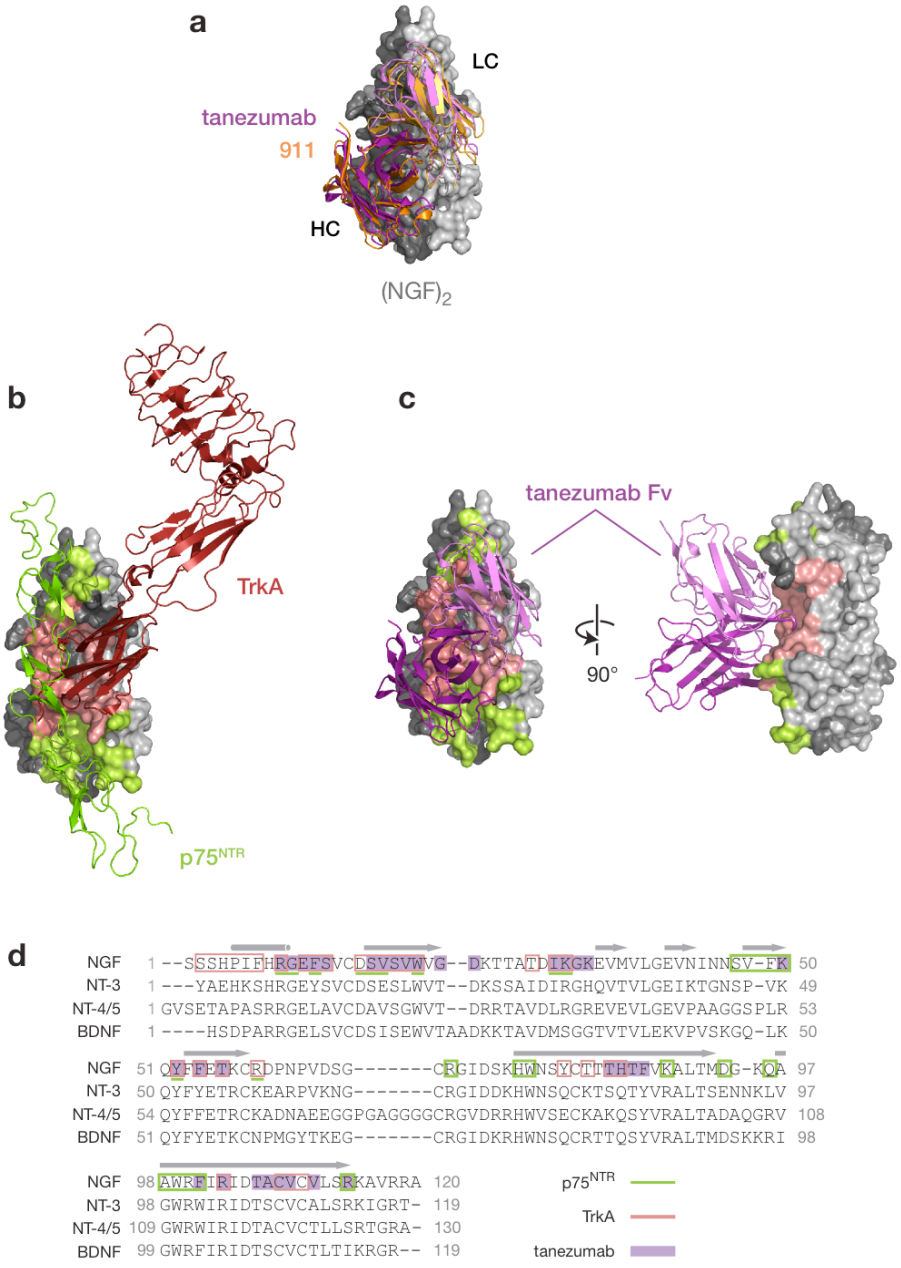


Figure S6. Molecular analysis of different complexes visualized after superposing on $(NGF)_2$ monomer V. **(a)** Epitope overlap viewed through ribbon diagram representation of tanezumab (purple) and 911 (orange) as Fv domains on surface diagram of $(NGF)_2$. **(b)** $p75^{NTR}$ (green/green), TrkA (red/pink) receptors illustrated as ribbon diagrams over the respective interation surface on the NGF-dimer. **(c)** tanezumab Fv domain (purple) as ribbon diagrams over

receptor binding surfaces (colored corresponding to receptor binding sites only) for one interface on $(NGF)_2$. **(d)** Neurotrophin sequence alignment with receptor binding sites and tanezumab epitope highlighted. NGF secondary structure elements in α-helix-cylinder and β-sheet arrow schematic are illustrated above the primary amino acid sequence for NGF given in signal amino acid letter code.

Table S1. Data Collection and Refinement for Fab911/NGF & Fab-tanezumab/NGF

	Fab-911/NGF	**Fab-tanezumab/NGF**
Data collection	SSRL 11-1	ALS 5.0.2
space group	P1	$C222_1$
cell parameters (Å,˚)	***a***=57.75, ***b***=69.93, ***c***=83.74 α=104.34, β=94.13, γ=110.42	***a***=64.70, ***b***=92.71, ***c***=252.73
V_M (Å^3/Dalton)	3.6	4.4
Resolution (Å)	50–2.5 (2.59–2.50)	50–2.5 (2.59–2.50)
Rsym[a,b]	0.067 (0.274)	0.062 (0.270)
Number of observations	95822	170755
Unique reflections	38098	25872
Completeness (%)[b]	96.0 (96.9)	95.2 (71.9)
I/σI[b]	13 (2.6)	24 (4.4)
Refinement		
Resolution (Å)	50 – 2.5	50 – 2.5
Number of reflections	37866	24244
Final R[c], R_{FREE}	0.208, 0.258	0.207, 0.265
Number of residues	1063	536
Number solvent molecules	98	37
Number of atoms[d]	8298 (3)	4178 (10)
Mean B-factor (Å^2)	32	43
Rmsd bonds (Å)	0.008	0.011
Rmsd angles (˚)	1.1	1.4
Rmsd bonded Bs (Å^2)	2.2/2.3	3.1/3.2
Number of TLS groups	10	5
Ramachandran analysis (%)	90.3/9.0/0.3/0.4	87.7/11.5/0.4/0.4

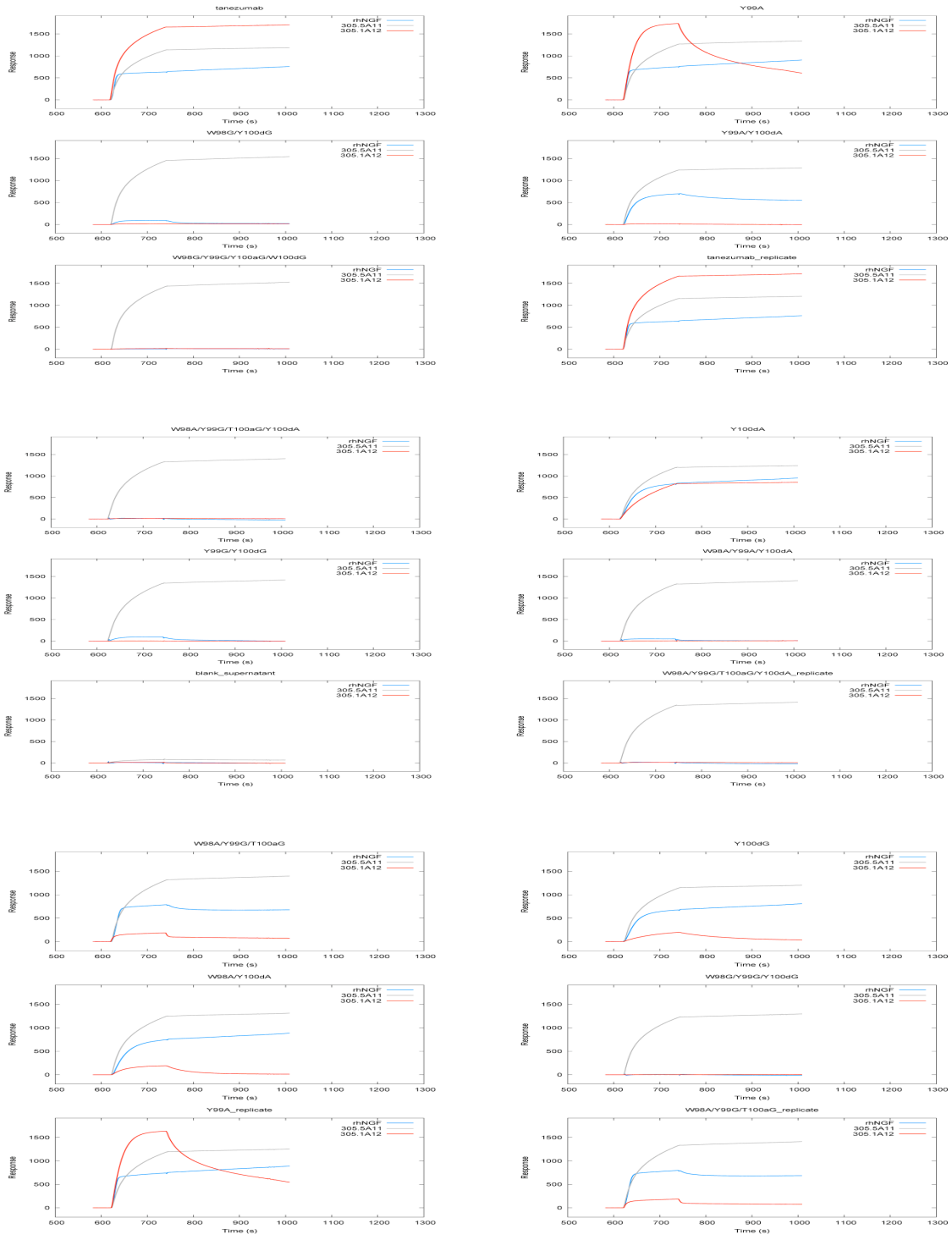
[a] $R_{sym} = \Sigma | |I| - |\langle I \rangle| | / \Sigma |\langle I \rangle|$, where I is the intensity of a single observation and <I> the average intensity for symmetry equivalent observations.

[b] In parenthesis, for the highest resolution shell.

[c] $R = \Sigma |Fo-Fc| / \Sigma |Fo|$, where Fo and Fc are observed and calculated structure factor amplitudes, respectively. R_{FREE} is calculated as R for 1015 reflections (2.6%) sequestered from refinement.

[d] In parenthesis, the number of atoms assigned less than unit occupancy.

A


tanezumab
Y99A
W98G/Y100dG
Y99A/Y100dA
W98G/Y99G/Y100aG/W100dG
tanezumab_replicate
W98A/Y99G/T100aG/Y100dA
Y100dA
Y99G/Y100dG
W98A/Y99A/Y100dA
blank_supernatant
W98A/Y99G/T100aG/Y100dA_replicate
W98A/Y99G/T100aG
Y100dG
W98A/Y100dA
W98G/Y99G/Y100dG
Y99A_replicate
W98A/Y99G/T100aG_replicate
rhNGF
305.5A11
305.1A12
Response
Time (s)

B

	Response (RU)		
Ligand	NGF	305.5A11 (Non-Blocker)	305.1A12 (Blocker)
W98A/Y99G/T100aG/Y100dA	6	1341	14
W98A/Y99G/T100aG/Y100dA (replicate)	6	1330	14
W98G/Y99G/Y100dG	7	1232	2
W98G/Y99G/Y100aG/W100dG	13	1440	16
W98A/Y99A/Y100dA	21	1326	4
W98G/Y100dG	61	1461	17
Y99G/Y100dG	63	1348	1
tanezumab	649	1140	1658
tanezumab	654	1155	1665
Y99A/Y100dA	682	1243	10
Y100dG	692	1153	177
W98A/Y99G/T100aG	742	1323	100
W98A/Y99G/T100aG (replicate)	748	1337	100
Y99A	761	1198	1374
W98A/Y100dA	766	1253	143
Y99A (replicate)	770	1274	1449
Y100dA	838	1201	822

C

H3 Mutants	NGF	305.5A11(NB)
Y99A	++	++
W98A/Y99G/T100aG	+	++
W98A/Y99G/T100aG/Y100dA	-	++
Y100dA	++	++
Y100dG	++	++
Y99A/Y100dA	++	++
Y99G/Y100dG	+	++
W98A/Y100dA	++	++
W98G/Y100dG	+	++
W98A/Y99A/Y100dA	+	++
W98G/Y99G/Y100dG	-	++
W98G/Y99G/Y100aG/W100dG	-	++

Figure S7. Single injection binding characterization by SPR of tanezumab mutants. (a) Panel for binding SPR signal by each mutant resulting from the injection of either 200 nM NGF (red), 100 nM non-blocking (NB) antibody 305.5A11 (black) or 100 nM blocking antibody 305.1A12 (blue). **(b)** Table ranking Response of each mutant. **(c)** Table summarizing binding according to mutant name.