Supplementary Material

Pro region engineering of nerve growth factor by deep mutational scanning enables a yeast platform for conformational epitope mapping of anti-NGF monoclonal antibodies

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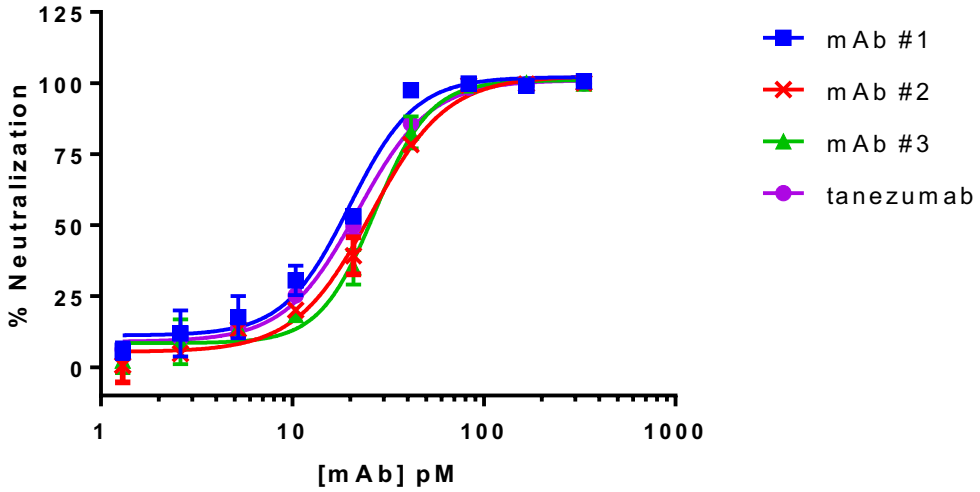
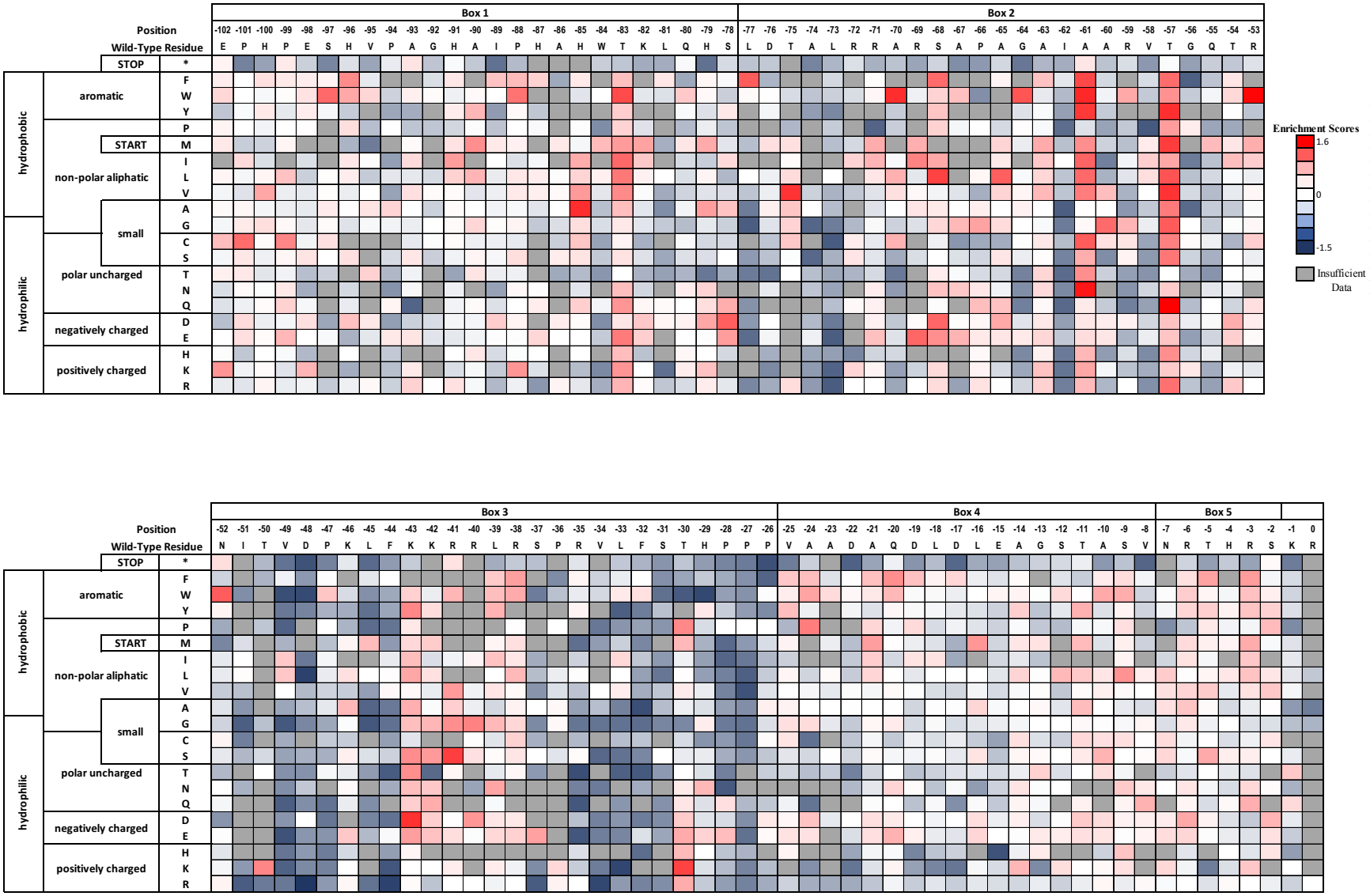
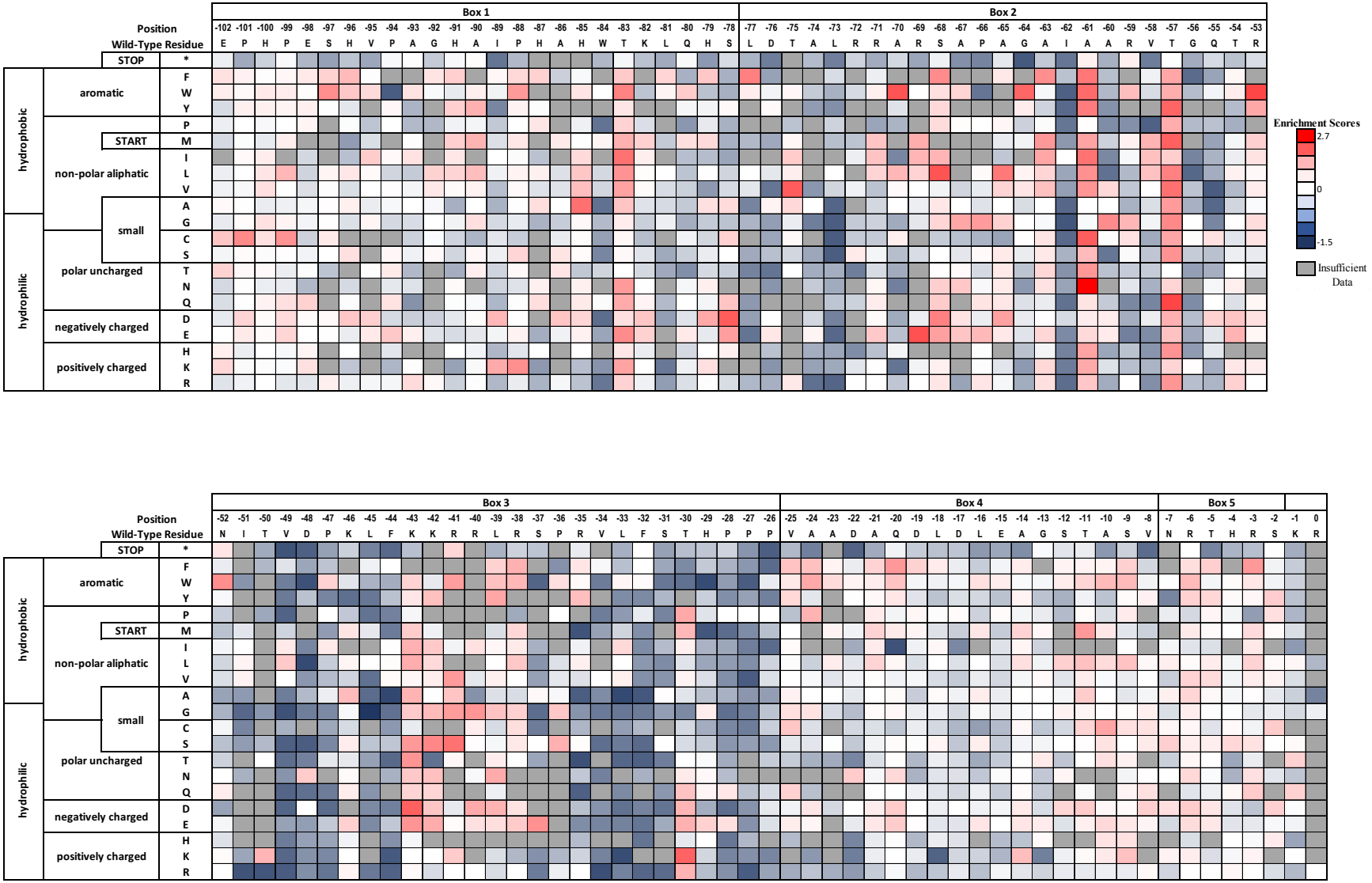


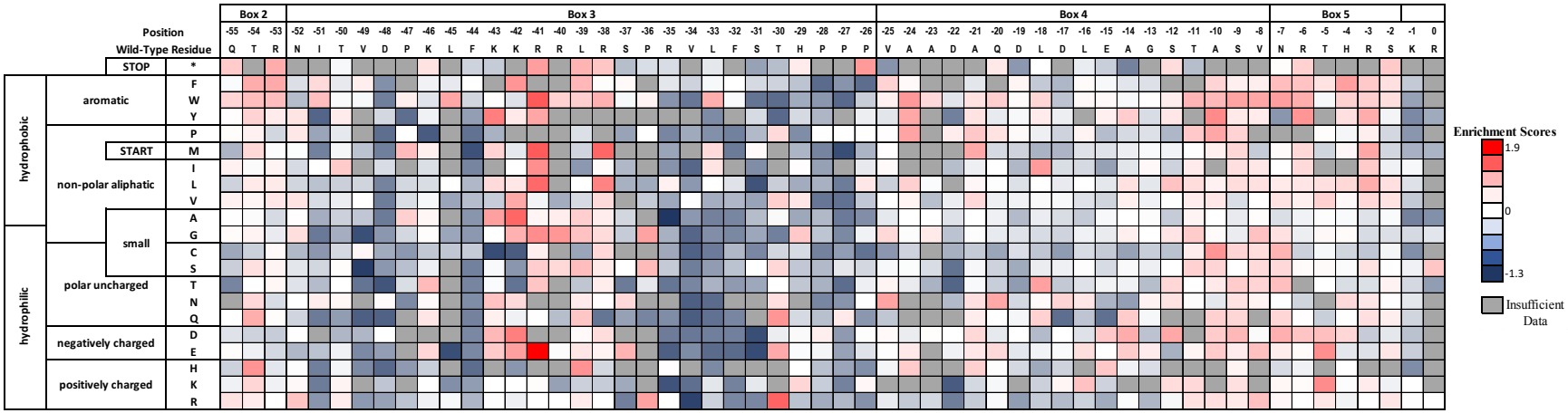
Supplementary Figure S1. Effects of anti-NGF mAbs on canine β-NGF Induced Proliferation of TF-1 Cells (representative curves). (error bars, standard deviation, n=2)



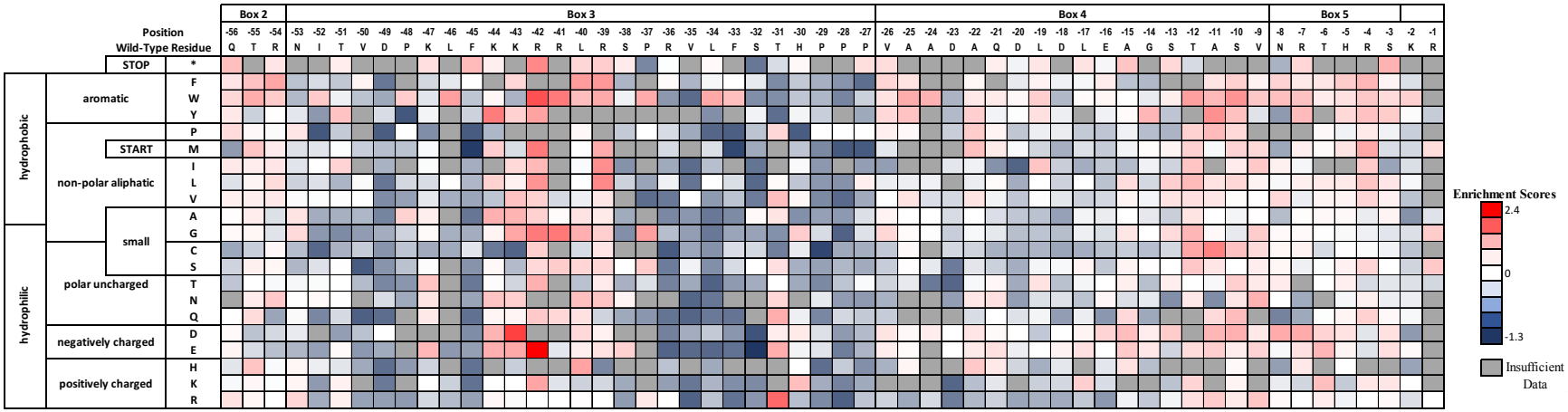
Supplementary Figure S2. Per-position heatmap of enrichment scores for pro-cNGF mutants after 1 sort with tanezumab.



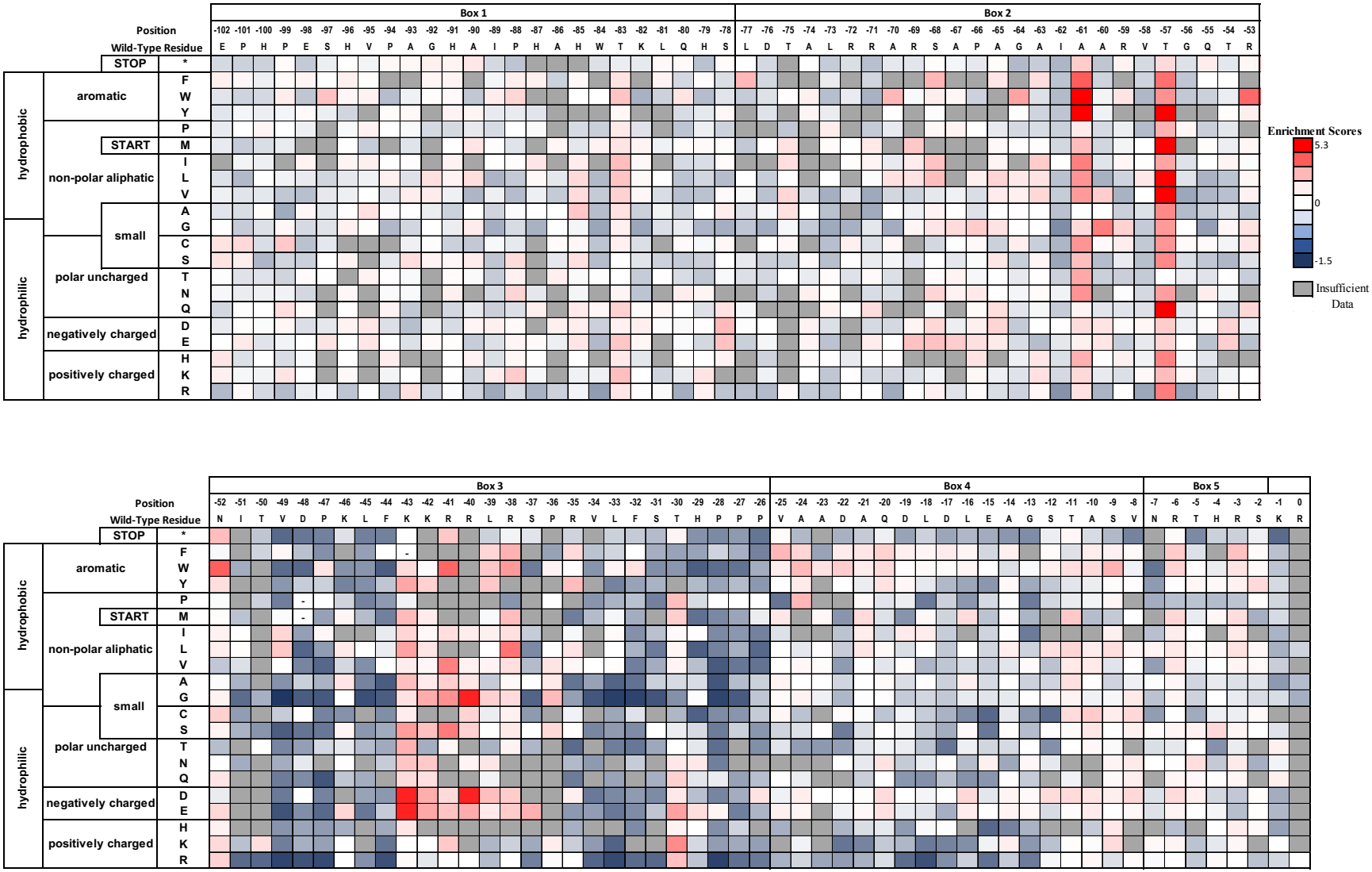
Supplementary Figure S3. Per-position heatmap of enrichment scores for pro-cNGF mutants after 1 sort with mAb #1.



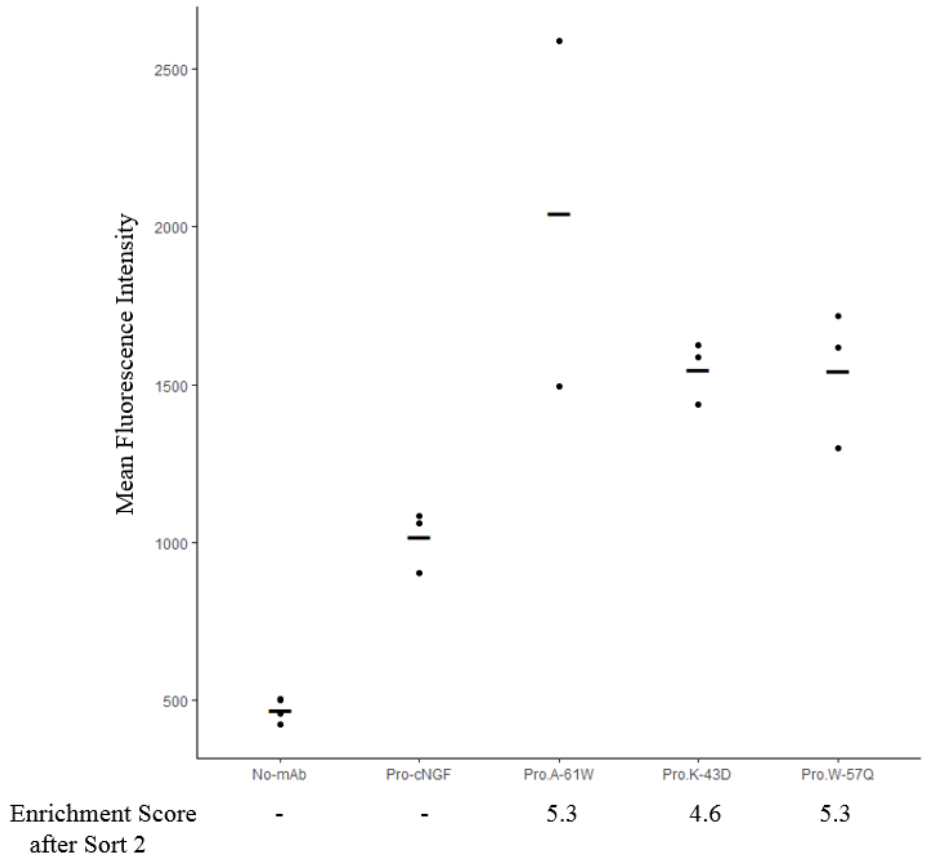
Supplementary Figure S4. Per-position heatmap of enrichment scores for proΔ1,2-cNGF mutants after 1 sort with tanezumab.



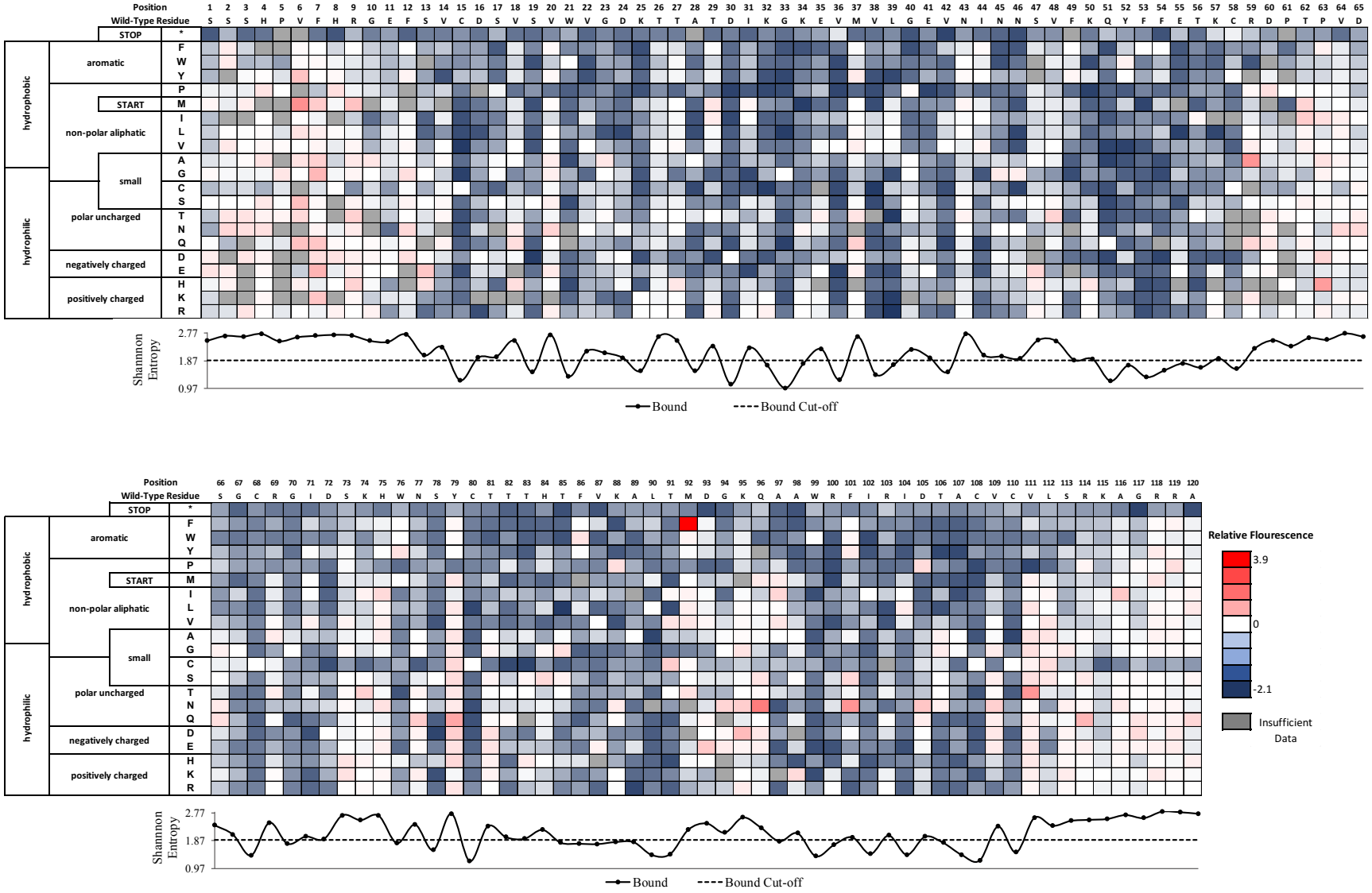
Supplementary Figure S5. Per-position heatmap of enrichment scores for proΔ1,2-cNGF mutants after 1 sort with mAb #1.



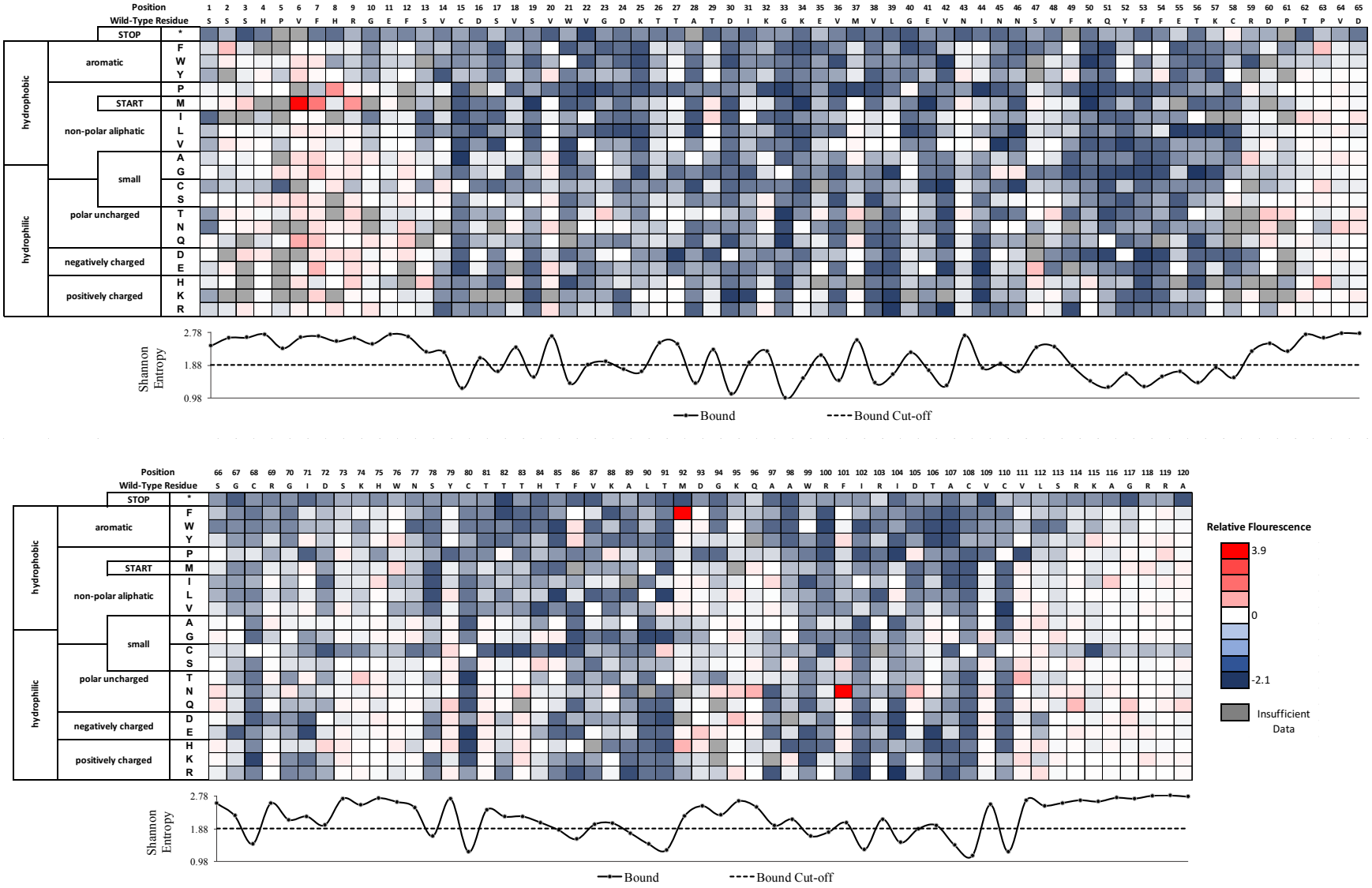
Supplementary Figure S6. Per-position heatmap of enrichment scores for pro-cNGF mutants after 2 sorts with mAb #1.



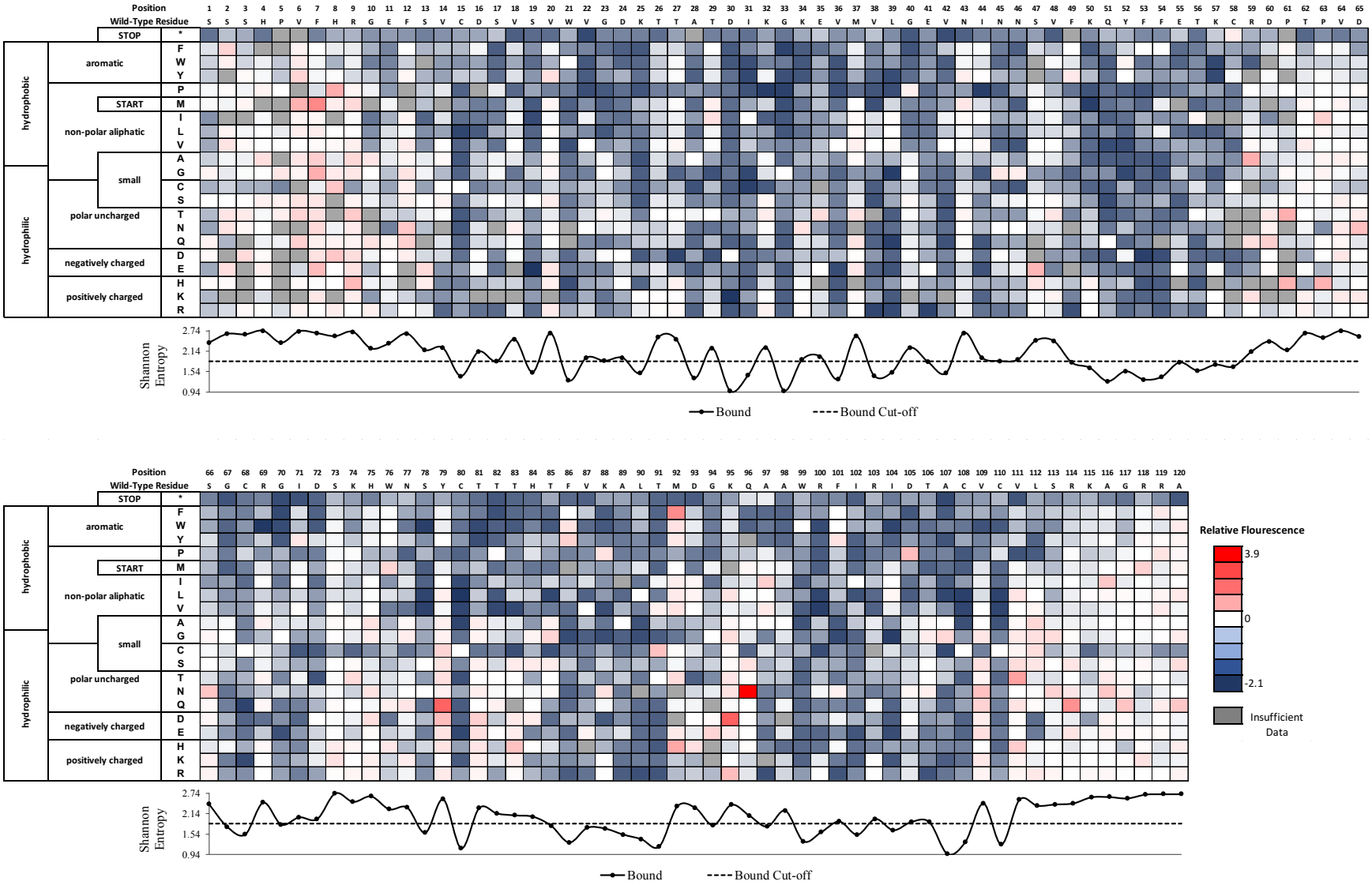
Supplementary Figure S7. Mean fluorescence intensities for individual point mutants compared with wild-type pro-cNGF.



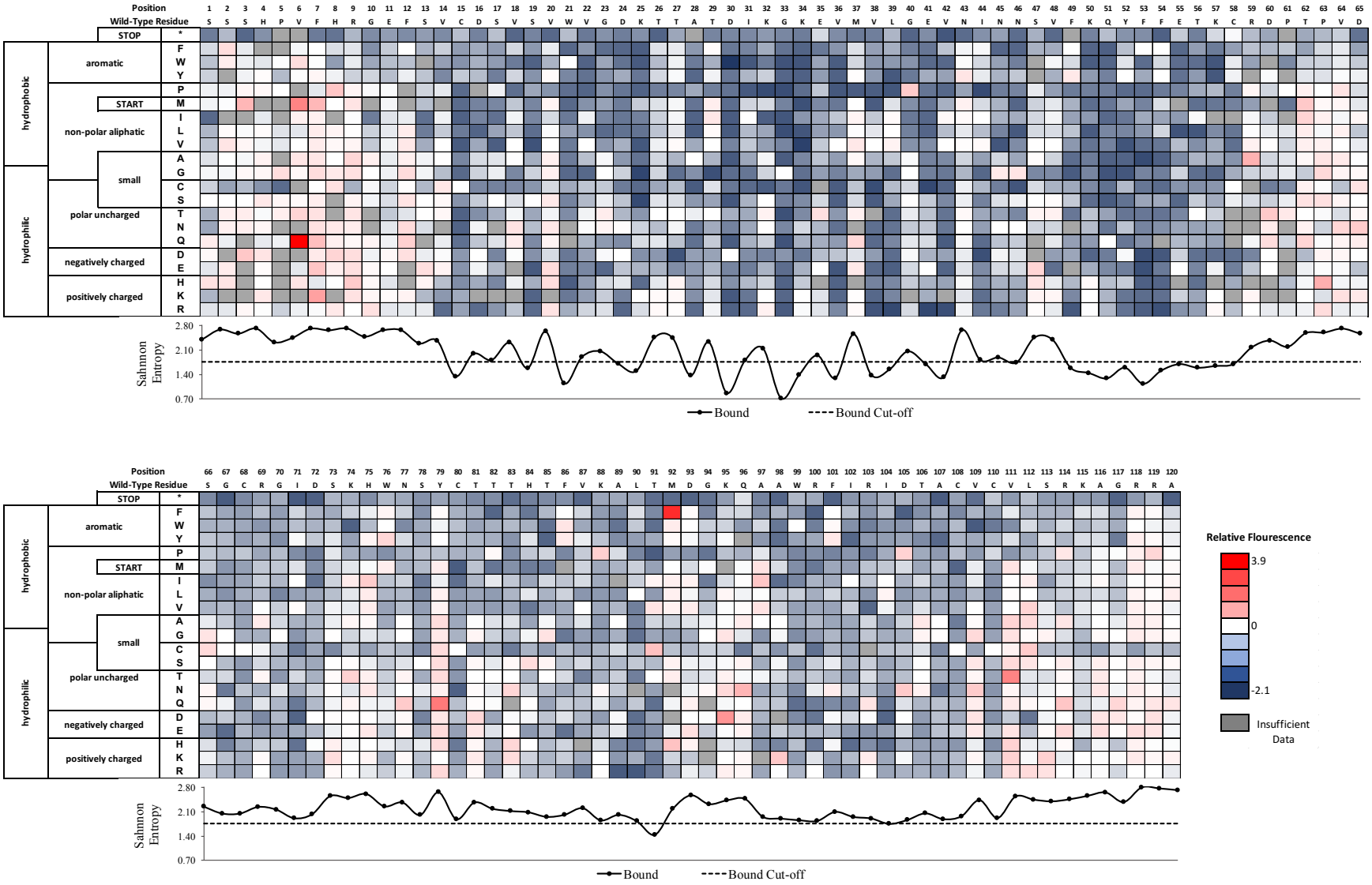
Supplementary Figure S8. Determination of conformational epitope for cNGF_tanezumab. Fitness metric heatmap of the top 7% bound population vs the unselected population. Shannon entropy is plotted below with its respectively cut-off (dashed line).



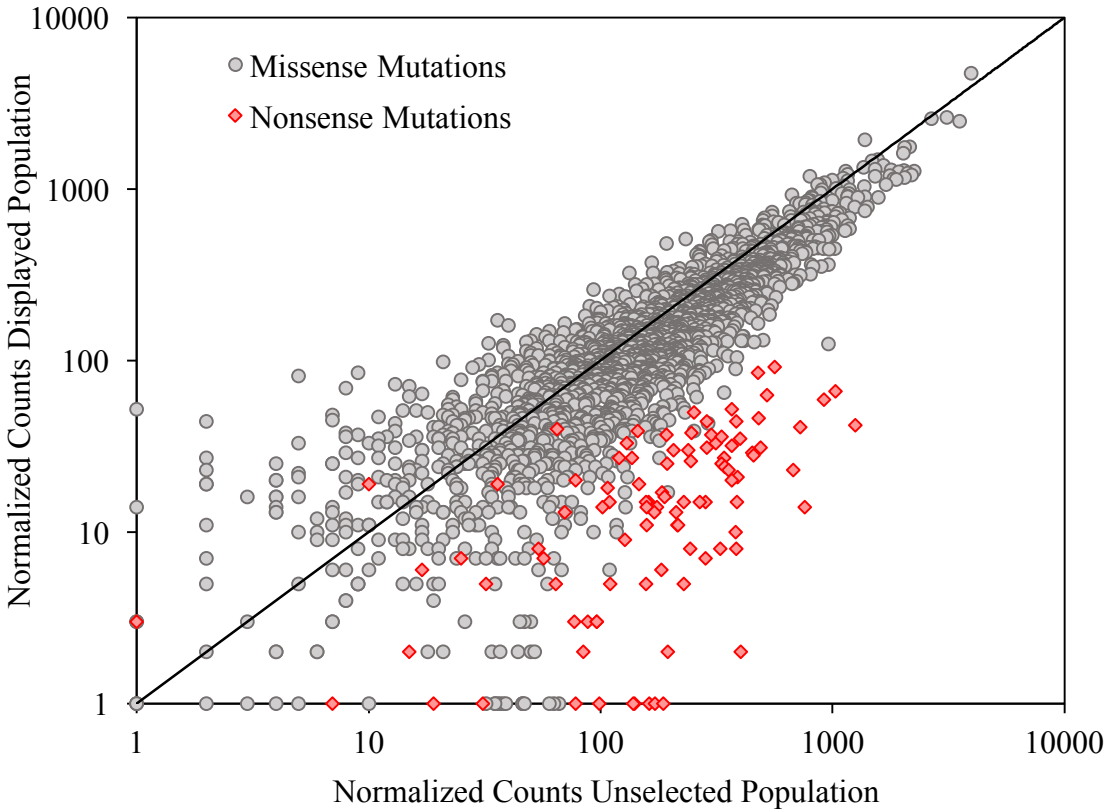
Supplementary Figure S9. Determination of conformational epitope for cNGF_mAb #1 Fitness metric heatmap of the top 7% bound population vs the unselected population. Shannon entropy is plotted below with its respectively cut-off (dashed line).



Supplementary Figure S10. Determination of conformational epitope for cNGF_mAb #2. Fitness metric heatmap of the top 7% bound population vs the unselected population. Shannon entropy is plotted below with its respectively cut-off (dashed line).



Supplementary Figure S11. Determination of conformational epitope for cNGF_mAb #3. Fitness metric heatmap of the top 7% bound population vs the unselected population. Shannon entropy is plotted below with its respectively cut-off (dashed line).



Supplementary Figure S12. Correlation between counts in the displayed population relative to the counts in the unselected population for cNGF.

Supplementary Table S1. FACS collection statistics for Pro-cNGF and ProΔ1,2-cNGF library screening experiments.

	Library Size (aa's)	Sort Round 1		Sort Round 2	
		tanezumab	mAb #1	tanezumab	mAb #1
Pro-cNGF_tanezumab library 1	51	2.9%	2.4%	3.1%	2.7%
Pro-cNGF_tanezumab library 2	52	3.3%	3.1%	2.8%	2.8%
ProΔ1,2-cNGF_tanezumab	56	3.3%	3.6%	-	-

Supplementary Table S2. Summary of Average Dissociation Constant, K_D values using Surface Plasmon Resonance (SPR) for human pro-NGF and canine NGF and Yeast Surface Display (YSD) for pro.v4-cNGF, and sorting conditions for library screening using pro.v4-cNGF. The K_D values from SPR were obtained using the 1:1 biding model. Error bars represent 1 standard deviation of the regression. One tail t-test assuming unequal variances was used to calculate p-values for Hill coefficients. ($n \geq 3$)

	Surface Plasmon Resonance Data						Yeast Surface Display Data				
mAb	Human Pro-NGF		Human NGF		Canine NGF		Average K_D values with Hill coefficient, H=1 [pM]	Labeling Concentrations for Screening Libraries [pM]	Average K_D values varying Hill coefficient, H [pM]	Hill coefficient values	p- values for Hill coefficient
	Average K_D values [pM]	Chi2	Average K_D values [pM]	Chi2	Average K_D values [pM]	Chi2					
tanezumab	1610	0.01	15.9	1.26	19	0.77	801 ± 164	400.6	1319 ± 200	0.66 ± 0.05	0.0033
mAb #1	286	0.22	0.243	2.32	0.118	1.92	209 ± 65	104.6	189 ± 36	0.70 ± 0.18	0.0103
mAb #2	48	0.05	0.308	1.47	0.074	1.29	307 ± 173	153.6	461 ± 300	0.62 ± 0.09	0.0003
mAb #3	2500	0.07	1.24	5.36	0.179	4.92	143 ± 44	71.6	325 ± 328	0.58 ± 0.19	0.0106

Supplementary Table S3. FACS collection statistics for cNGF libraries.

mAb	Amount of collected cells	cNGF	
		Library 1 (90 aa's)	Library 2 (90 aa's)
tanezumab	250,000	8.01%	6.10%
mAb #1	250,000	7.36%	6.33%
mAb #2	250,000	7.28%	6.60%
mAb #3	250,000	6.59%	8.48%

Supplementary Table S4. Primers set for deep sequencing. L1: library 1, L2: library 2, Blue: Illumina Universal Sequence, **NNNNNN**: Indexing Barcode, and Green: Illumina Adapter

Name	Sequence
Inner Primers	
Pro-cNGF_L1_FWD	5'- GTTCAGAGTTCTACAGTCCGACGATCGATGACGACAAGCATATG -3'
Pro-cNGF_L1_REV	5'- CCTTGGCACCCGAGAATTCCAAACTTTGGATCAACTGTGAT -3'
Pro-cNGF_L2_FWD	5'- GTTCAGAGTTCTACAGTCCGACGATC TTACAGGTCAAACTAGAAAC-3'
Pro-cNGF_L2_REV	5'- CCTTGGCACCCGAGAATTCCAAACTGGATGAGATGAAGA -3'
ProΔ1,2-cNGF_FWD	5'- GTTCAGAGTTCTACAGTCCGACGATCGATGACGACAAGCATATG-3'
ProΔ1,2-cNGF_REV	5'- CCTTGGCACCCGAGAATTCCAAACTGGATGAGATGAAGA -3'
cNGF_L1_FWD	5'- GTTCAGAGTTCTACAGTCCGACGATCAACTGGATGAGATGAAGA -3'
cNGF_L1_REV	5'- CCTTGGCACCCGAGAATTCCAATCAACTGGAGTTGG -3'
cNGF_L2_FWD	5'- GTTCAGAGTTCTACAGTCCGACGATCCTTTTTTGAAACAAAATGTAGAGAT -3'
cNGF_L2_REV	5'- CCTTGGCACCCGAGAATTCCAGCCTCCTCCACC -3'
Foward Outer Primer	
Illumina_FWD	5'- AATGATACGGCGACCACCGAGATCTACACGTTCAGAGTTCTACAGTCCGACGATC - 3'
Reverse Outer Primers	
Pro-cNGF_L1_Unsel	5'- CAAGCAGAAGACGGCATACGAGAT**TGACAT**GTGACTGGAGTTCCTTGGCACCCGAGAATTCCA - 3'
Pro-cNGF_L1_Display	5'- CAAGCAGAAGACGGCATACGAGAT**GGACGG**GTGACTGGAGTTCCTTGGCACCCGAGAATTCCA - 3'
Pro-cNGF_L1_tanezumab	5'- CAAGCAGAAGACGGCATACGAGAT**CTCTAC**GTGACTGGAGTTCCTTGGCACCCGAGAATTCCA - 3'

Pro-cNGF_L1_mAb #1	5'- CAAGCAGAAGACGGCATACGAGAT**GCGGAC**GTGACTGGAGTTCCTTGGCACCCGAGAATTCCA - 3'
Pro-cNGF_L2_Unsel	5'- CAAGCAGAAGACGGCATACGAGAT**TTTCAC**GTGACTGGAGTTCCTTGGCACCCGAGAATTCCA - 3'
Pro-cNGF_L2_Display	5'- CAAGCAGAAGACGGCATACGAGAT**GGCCAC**GTGACTGGAGTTCCTTGGCACCCGAGAATTCCA - 3'
Pro-cNGF_L2_tanezumab	5'- CAAGCAGAAGACGGCATACGAGAT**CGAAAC**GTGACTGGAGTTCCTTGGCACCCGAGAATTCCA - 3'
Pro-cNGF_L2_mAb #1	5'- CAAGCAGAAGACGGCATACGAGAT**CGTACG**GTGACTGGAGTTCCTTGGCACCCGAGAATTCCA - 3'
ProΔ1,2-cNGF_Unsel	5'- CAAGCAGAAGACGGCATACGAGAT**CCACTC**GTGACTGGAGTTCCTTGGCACCCGAGAATTCCA - 3'
ProΔ1,20-NGF_Display	5'- CAAGCAGAAGACGGCATACGAGAT**GCTACC**GTGACTGGAGTTCCTTGGCACCCGAGAATTCCA - 3'
ProΔ1,2-cNGF_tanezumab	5'- CAAGCAGAAGACGGCATACGAGAT**ATCAGT**GTGACTGGAGTTCCTTGGCACCCGAGAATTCCA - 3'
ProΔ1,2-cNGF_mAb #1	5'- CAAGCAGAAGACGGCATACGAGAT**GCTCAT**GTGACTGGAGTTCCTTGGCACCCGAGAATTCCA - 3'
Pro-cNGF_L1_S2_tanezumab	5'- CAAGCAGAAGACGGCATACGAGAT**AGGAAT**GTGACTGGAGTTCCTTGGCACCCGAGAATTCCA - 3'
Pro-cNGF_L1_S2_mAb #1	5'- CAAGCAGAAGACGGCATACGAGAT**CTTTTG**GTGACTGGAGTTCCTTGGCACCCGAGAATTCCA - 3'
Pro-cNGF_L2_S2_tanezumab	5'- CAAGCAGAAGACGGCATACGAGAT**TAGTTG**GTGACTGGAGTTCCTTGGCACCCGAGAATTCCA - 3'
Pro-cNGF_L2_S2_mAb #1	5'- CAAGCAGAAGACGGCATACGAGAT**CCGGTG**GTGACTGGAGTTCCTTGGCACCCGAGAATTCCA - 3'
cNGF_L1_Unsel	5'- CAAGCAGAAGACGGCATACGAGAT**CGTGAT**GTGACTGGAGTTCCTTGGCACCCGAGAATTCCA - 3'
cNGF_L1_Display	5'- CAAGCAGAAGACGGCATACGAGAT**ACATCG**GTGACTGGAGTTCCTTGGCACCCGAGAATTCCA - 3'
cNGF_L1_mAb #1	5'- CAAGCAGAAGACGGCATACGAGAT**GCCTAA**GTGACTGGAGTTCCTTGGCACCCGAGAATTCCA - 3'
cNGF_L1_mAb #2	5'- CAAGCAGAAGACGGCATACGAGAT**TGGTCA**GTGACTGGAGTTCCTTGGCACCCGAGAATTCCA - 3'
cNGF_L1_mAb #3	5'- CAAGCAGAAGACGGCATACGAGAT**CACTGT**GTGACTGGAGTTCCTTGGCACCCGAGAATTCCA - 3'
cNGF_L1_tanezumab	5'- CAAGCAGAAGACGGCATACGAGAT**ATTGGC**GTGACTGGAGTTCCTTGGCACCCGAGAATTCCA - 3'
cNGF_L2_Unsel	5'- CAAGCAGAAGACGGCATACGAGAT**CTGATC**GTGACTGGAGTTCCTTGGCACCCGAGAATTCCA - 3'
cNGF_L2_Display	5'- CAAGCAGAAGACGGCATACGAGAT**AAGCTA**GTGACTGGAGTTCCTTGGCACCCGAGAATTCCA - 3'
cGF_L2_mAb #1	5'- CAAGCAGAAGACGGCATACGAGAT**GTAGCC**GTGACTGGAGTTCCTTGGCACCCGAGAATTCCA - 3'
cNGF_L2_mAb #2	5'- CAAGCAGAAGACGGCATACGAGAT**TACAAG**GTGACTGGAGTTCCTTGGCACCCGAGAATTCCA - 3'
cNGF_L2_mAb #3	5'- CAAGCAGAAGACGGCATACGAGAT**TTGACT**GTGACTGGAGTTCCTTGGCACCCGAGAATTCCA - 3'
cNGF_L2_tanezumab	5'- CAAGCAGAAGACGGCATACGAGAT**GGAACT**GTGACTGGAGTTCCTTGGCACCCGAGAATTCCA - 3'

Supplementary Table S5. Library Statistics Results.

	Pro-cNGF		***ProΔ1,2-cNGF***	***NGF***	
	Tile 1	***Tile 2***		***Tile 1***	***Tile 2***
Percent of possible codon subsititions observed in the unselected population:					
1-base substitution	96.50%	95.10%	94.20%	98.70%	99.80%
2-base substitutions	59.50%	52.80%	53.20%	55.70%	60.00%
3-base substitutions	47.40%	43.20%	44.80%	44.50%	47.90%
Percent of unselected reads with:					
No nonsynonymous mutations:	35.20%	30.80%	28.60%	39.50%	34.50%
One nonsynonymous mutation:	56.00%	61.80%	56.60%	55.40%	57.10%
Multiple nonsynonymous mutations:	8.80%	7.40%	14.80%	5.10%	8.50%
Coverage of possible single nonsynonymous amino acid mutations:	**85.50%**	**83.20%**	**84.20%**	**92.90%**	**98.20%**