

Supplemental Materials

Rapid fine conformational epitope mapping using comprehensive mutagenesis and deep sequencing

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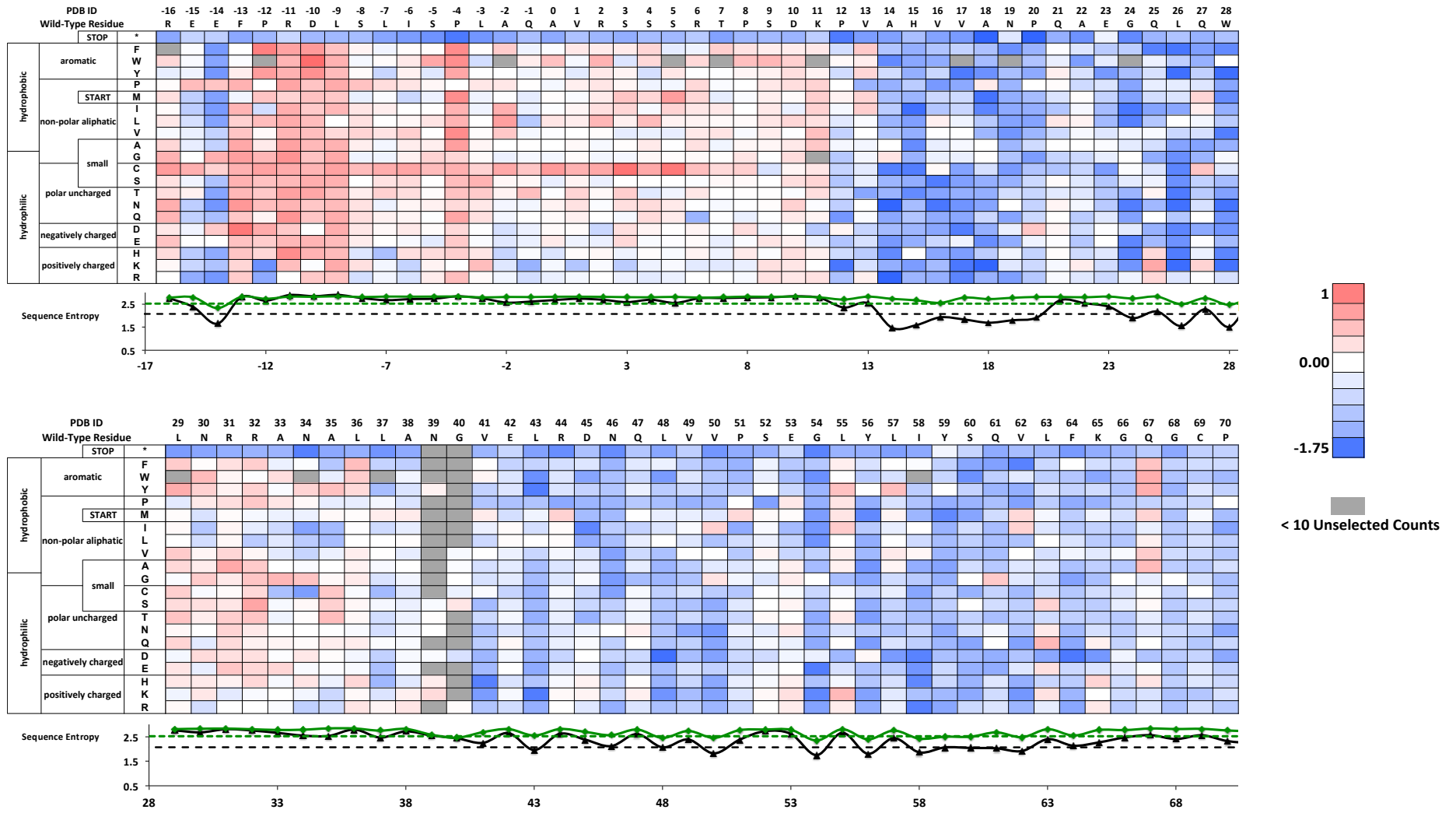
*Running title: *Rapid fine comprehensive conformational epitope mapping*

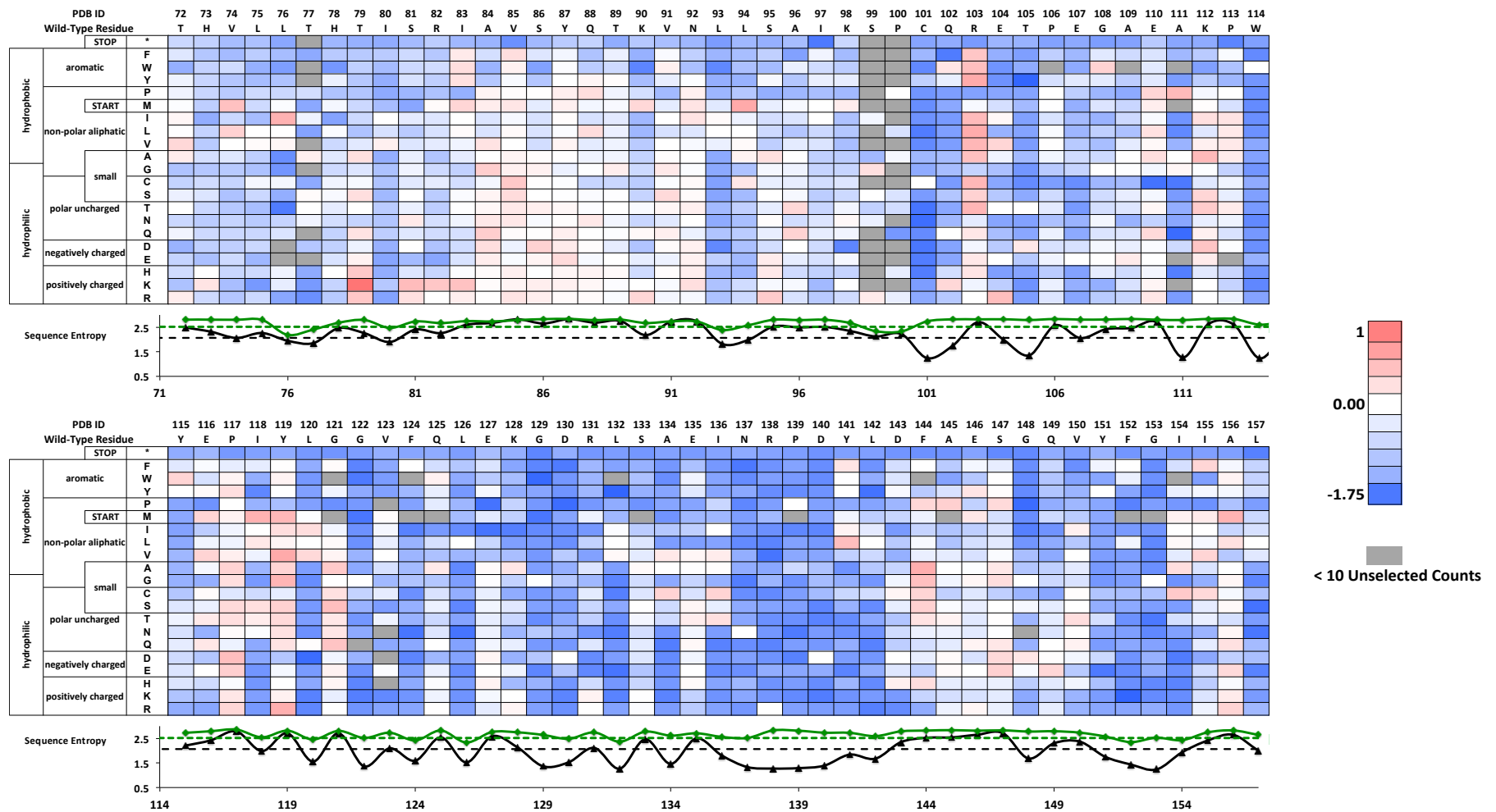
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Supporting Figures.

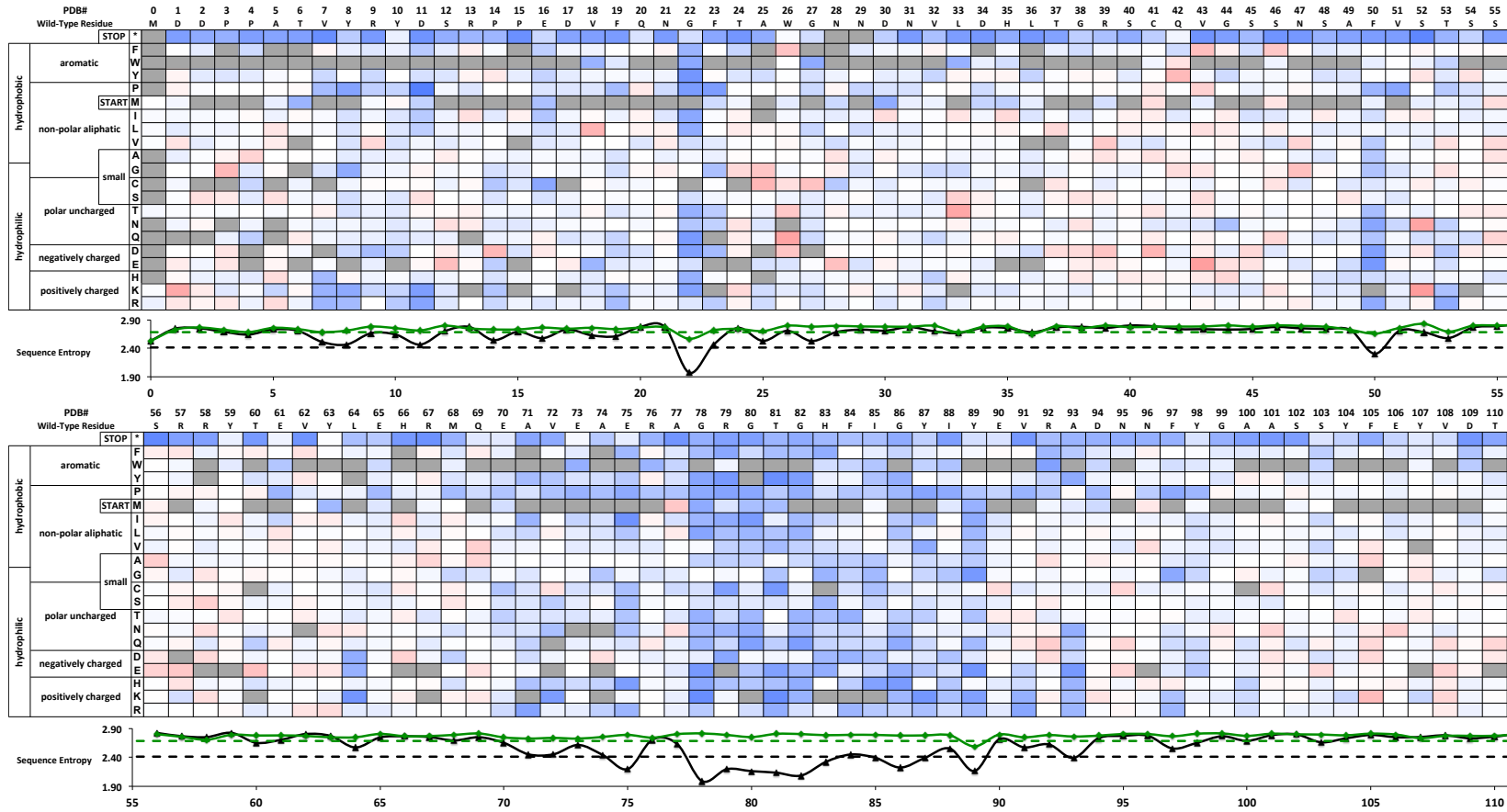
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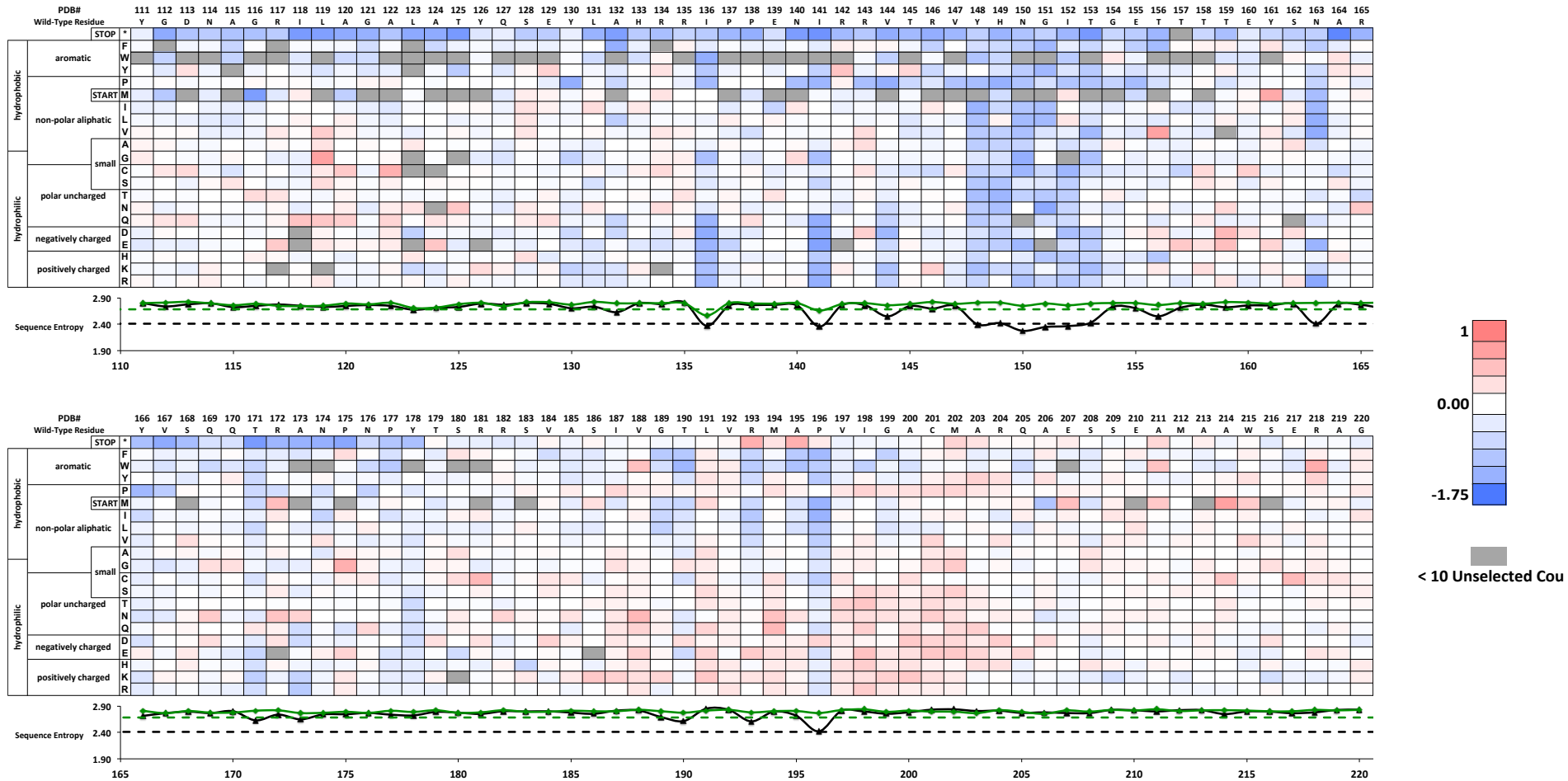




Supplementary Figure 1. TNF-Inflix_scFv Conformational Epitope Determination.

Heatmap of fitness metric of bound vs. unselected population for all possible single non-synonymous mutations in the coding sequence for extracellular TNF. Sequence entropy for the unselected/display population (green) and unselected/bound population (black) is plotted below with their respective cut-offs (dashed lines).

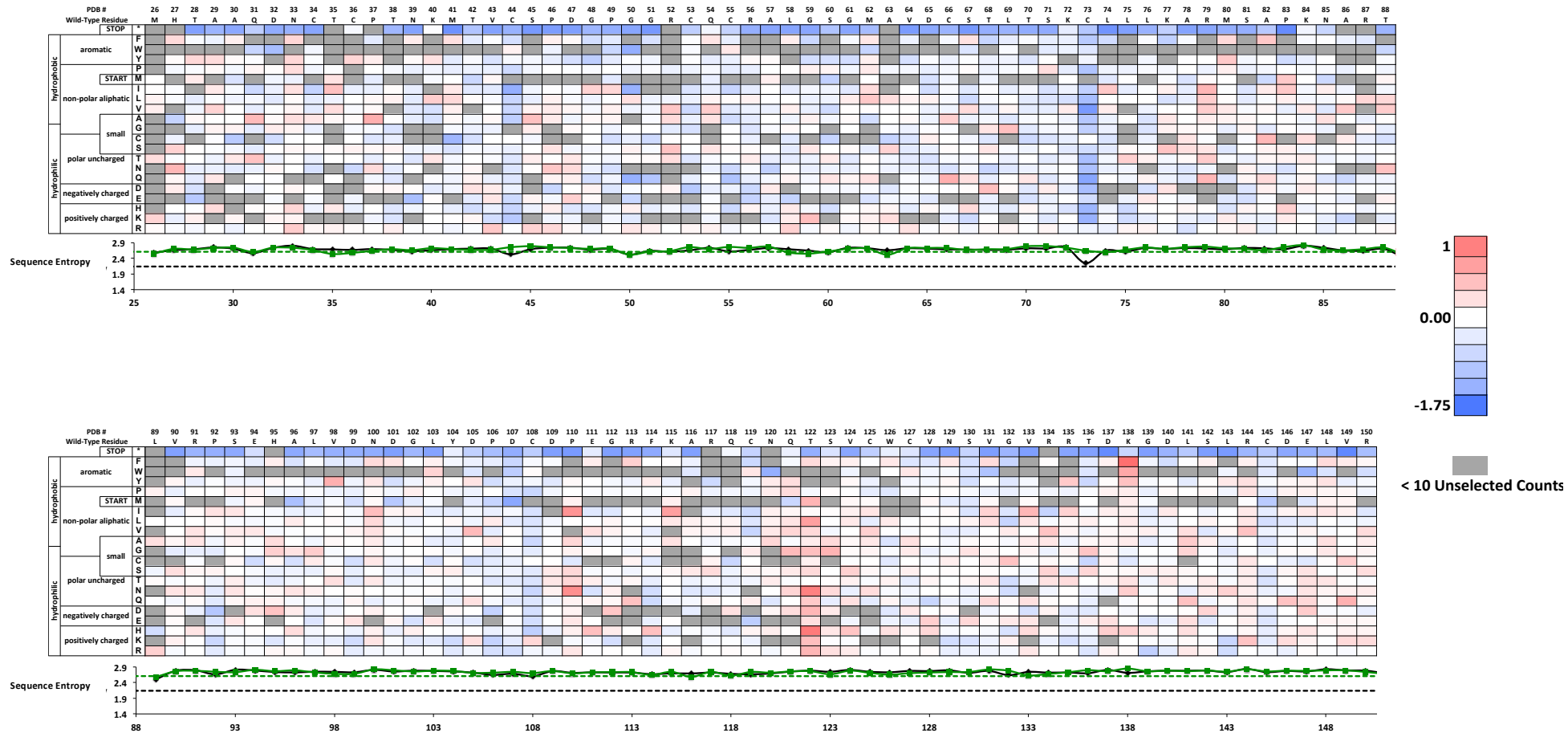


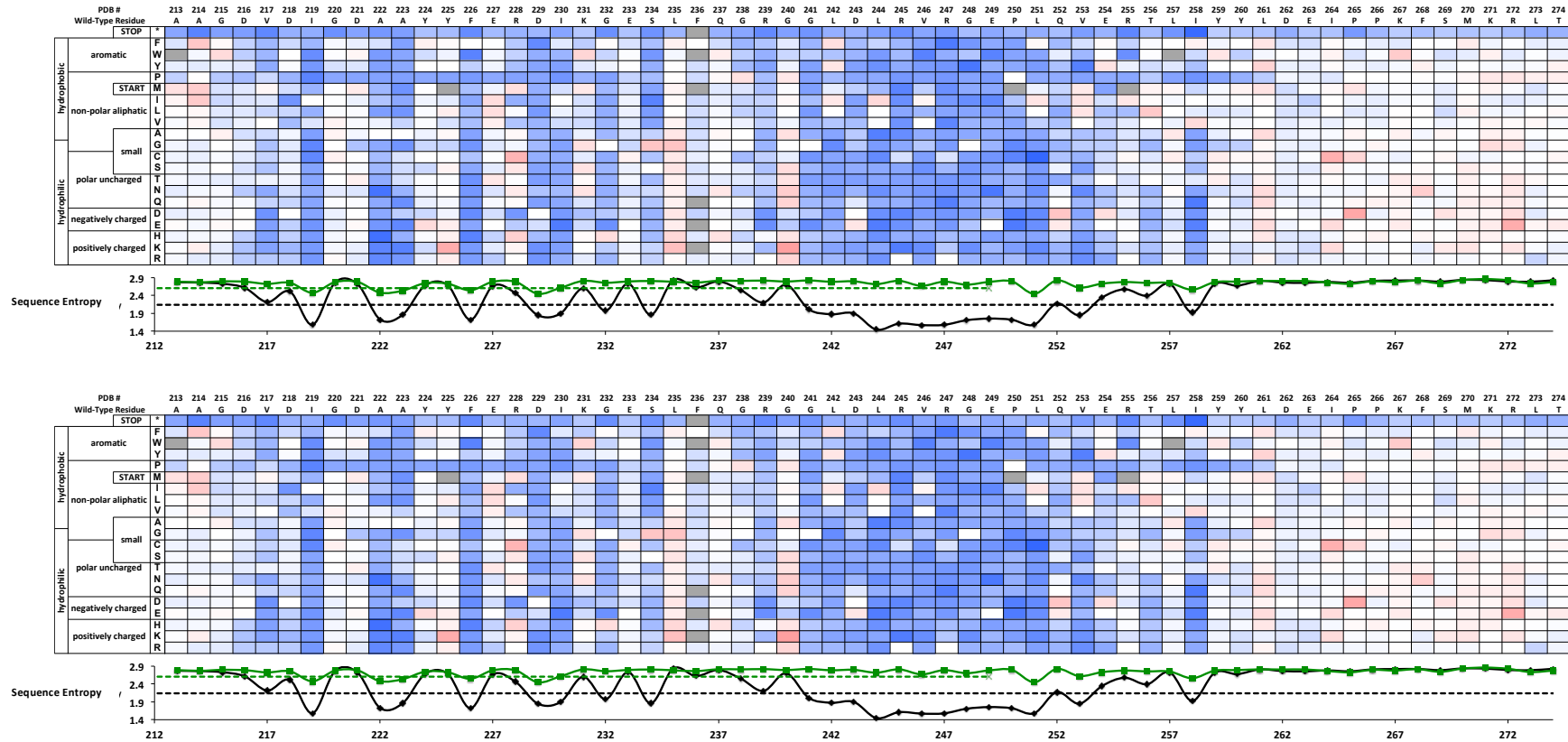


Supplementary Figure 2. PTx-S1-220-hu1B7 Conformational Epitope Determination.

Heatmap of fitness metric of bound vs. unselected population for all possible single non-synonymous mutations in the coding sequence for Asp1-Gly220 of PTx-S1. Sequence entropy for the unselected/display population (green) and unselected/bound population (black) is plotted below with their respective cut-offs (dashed lines).

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Supplementary Figure 3. TROP2-m7e6 Conformational Epitope Determination.

Heatmap of fitness metric of bound vs. unselected population for all possible single non-synonymous mutations in the coding sequence for TROP2Ex. Sequence entropy for the unselected/display population (green) and unselected/bound population (black) is plotted below with their respective cut-offs (dashed lines).

Supplementary Table 1. Sorting Statistics

	Tile Length (AA)	Minimum Transformants for 99.9% Coverage	Sort Labeling Conditions (nM)	Events Collected for Binding Population	Percent Sorted (Display)	Percent Sorted (Binding)
TNF-Infliximab Tile 1	60	26,880	32	200,641	46.0%	6.9%
TNF-Infliximab Tile 2	60	26,880	32	200,437	43.0%	5.1%
TNF-Infliximab Tile 3	57	25,536	32	206,251	31.9%	6.5%
PTxS1-hu1B7 Tile 1	72	32,256	3	400,000	43.0%	7.7 %
PTxS1-hu1B7 Tile 2	74	33,152	3	400,000	45.0%	6.1%
cdPTxS1-hu1B7 Tile 3	73	32,704	3	400,000	46.2%	6.4%
Trop2-m7E6 Tile 1	82	36,736	22	400,000	19.8%	6.6%
Trop2-m7E6 Tile 2	83	37,184	22	400,000	24.7%	6.7%
Trop2-m7E6 Tile 3	83	37,184	22	400,000	16.9%	6.6%

Supplementary Table 2. Library Statistics

	TNF			PTxS1			Trop2		
	Tile 1	Tile 2	Tile 3	Tile 1	Tile 2	Tile 3	Tile 1	Tile 2	Tile 3
Reads passing through enrich	895912	1594215	1018624	428831	447909	1610608	401980	523168	657434
Percent of possible codon substitutions observed									
1-base substitution	99.8%	100.0%	99.6%	100.0%	100.0%	100.0%	99.6%	99.9%	100.0%
2-base substitutions	92.0%	96.0%	95.4%	94.3%	96.9%	99.0%	87.3%	96.1%	99.2%
3-base substitutions	91.0%	94.8%	91.3%	91.7%	96.7%	95.6%	81.0%	95.4%	98.4%
All substitutions	92.7%	96.1%	94.2%	94.0%	97.2%	97.8%	86.4%	96.3%	98.9%
Percent of reads with									
No nonsynonymous mutations	15.9%	44.1%	10.4%	16.0%	20.9%	28.4%	8.2%	7.3%	14.7%
One nonsynonymous mutation	62.9%	45.8%	75.6%	32.7%	36.0%	42.2%	31.0%	26.5%	56.7%
Multiple nonsynonymous mutations	21.2%	10.1%	14.0%	51.3%	43.1%	29.4%	60.8%	66.2%	28.6%
Coverage of possible single nonsynonymous mutations	93.6%	97.1%	97.2%	94.7%	96.8%	98.7%	90.2%	97.1%	99.5%

Supplementary Table 3. Primer Sequences used for deep sequencing. Each primer is listed as X_Y_Z, where X is the gene name, Y is the Tile (T1=Tile 1, etc), and F/R denotes forward or reverse reads, respectively.

Primer Name	Sequence
TNFa_T1_F	G TTCAGAGTTCTACAGTCCGACGATCTCGGCTAGCCATATG
TNFa_T1_R	CCTTGGCACCCGAGAATCCAAGCTGGTTGTCCCTCAGCTC
TNFa_T2_F	G TTCAGAGTTCTACAGTCCGACGATCACGCCCTGCTGG
TNFa_T2_R	CCTTGGCACCCGAGAATCCAGGGTCTCCCTCTGG
TNFa_T3_F	G TTCAGAGTTCTACAGTCCGACGATCGAGCGCCATCAAGA
TNFa_T3_R	CCTTGGCACCCGAGAATCCATTACATCTACACTGTTGTTATCA
pETCON_T1_F	G TTCAGAGTTCTACAGTCCGACGATCGGGTTCGGCTAGCCATATG
pETCON_TE_R	CCTTGGCACCCGAGAATCCAGCCCCCTCGAG
PTxS1_T1_R	CCTTGGCACCCGAGAATCCAAAGTGGCCGGTGCCC
PTxS1_T2_F	G TTCAGAGTTCTACAGTCCGACGATCAGGAAGCGGTCGAG
PTxS1_T2_R	CCTTGGCACCCGAGAATCCAGTGATGCCGTTGTGATA
PTxS1_T3_F	G TTCAGAGTTCTACAGTCCGACGATCGGGTAACGCGGGTC
Trop2_T1_R	CCTTGGCACCCGAGAATCCAGCGCGCCTTGAAGC
Trop2_T2_F	G TTCAGAGTTCTACAGTCCGACGATCCGATGGCCTCTACGAC
Trop2_T2_R	CCTTGGCACCCGAGAATCCACTCGTAGTGCACGGC
Trop2_T3_F	G TTCAGAGTTCTACAGTCCGACGATCCACCCAAGTTCGTG
Trop2amp_F	G CATCATATGCACACGGCCGCGCAGGACA ACTGCACGTG
Trop2amp_R	CAGGCTCGAGGGTGAGGCGCTTCATGGAGAACTTCGGGGGAATCTCG
PTxS1amp_F	G CGGTAGCGGAGGCGGAGGGTCGGCTAGCCATATGGACGATCCTCCCGC
PTxS1amp_R	CAGAAATAAGCTTTTGTTCGGATCCGCCCCCTCGAGGCCGGCGCGT