

Supporting Table 2. Amino acids in *CfAvr4* that interact with (GlcNAc)₆ in the dimeric assembly and the effect of mutating these residues on the (GlcNAc)₆ affinity of the protein and its recognition by the Cf-4 immune receptor.

Amino acids interacting with (GlcNAc) ₆ as determined by the solution structure of the <i>CfAvr4</i> -(GlcNAc) ₆ complex		Thermodynamic binding parameters to (GlcNAc) ₆ as determined by isothermal titration calorimetry (ITC)						Protein stability and recognition by Cf-4 as determined by pure protein infiltrations and ATTAs		
Amino acid (Chain)	Interaction with GlcNAc unit in (GlcNAc) ₆ (Chain)	Introduced mutation	Protein (mM)	(GlcNAc) ₆ Ligand (mM)	K _d (μM)	n	ΔH (kJ/mol)	Recognition by Cf-4 (pure protein infiltr)	Recognition by Cf-4 (ATTAs)	Stability (Subtilisin assay)
WT- <i>CfAvr4</i>	(GlcNAc) ₆	NA	0.198	2.10	7.63 ± 1.74	1.17 ± 0.11	-40.87 ± 1.42	Y/Y ¹	Y/Y/Y ²	S3
WT- <i>CfAvr4</i> w/o tag	(GlcNAc) ₆	NA	0.333	2.05	6.73 ± 1.49	1.09 ± 0.12	-37.58 ± 3.51	-	-	-
WT- <i>CfAvr4</i> reverse titration	(GlcNAc) ₆	NA	1.03	0.33	7.16 ± 0.76	1.01 ± 0.06	-41.97 ± 1.90	NA	NA	NA
Lys49 (Chain A)	GlcNAc-1 (Chain A)	-	-	-	-	-	-	-	-	-
Cys50 (Chain A)	GlcNAc-2 (Chain A)	-	-	-	-	-	-	-	-	-
Met51 (Chain A)	GlcNAc-2 (Chain A)	M51A	0.293	1.56	9.20 ± 1.47	1.01 ± 0.08	-33.30 ± 0.87	Y/Y	Y/Y/Y	
Pro53 (Chain A)	GlcNAc-1 (Chain A)	P53A	0.300	1.52	11.34 ± 2.58	0.94 ± 0.04	-40.86 ± 2.26	Y/Y	Y/Y/Y	
Tyr67 (Chain A)	GlcNAc-2 (Chain B)	Y67F	0.201	2.08	6.86 ± 1.24	1.01 ± 0.09	-27.70 ± 1.01	Y/Y	Y/Y/Y	
Tyr67 (Chain B)	GlcNAc-4 (Chain A)									
Gln69 (Chain A)	GlcNAc-2 (Chain A)	Q69N	0.304	2.98	130.95 ± 31.47	1.01 ± 0.10	-18.84 ± 4.38	P/Y	Y/Y/Y	PU
Gln69 (Chain B)	GlcNAc-2 (Chain B) GlcNAc-3 (Chain A)									
Lys84 (Chain A)	GlcNAc-1 (Chain B)	K84A	0.598	3.11	120.67 ± 18.77	0.92 ± 0.11	-19.53 ± 1.73	Y/Y	Y/Y/Y	S
Lys84 (Chain B)	GlcNAc-3 (Chain A)									
Pro87 (Chain A)	GlcNAc-3 (Chain B)	P87A	0.298	1.04	17.94 ± 0.44	0.91 ± 0.03	-41.82 ± 1.94	Y/Y	Y/Y/Y	
Pro87 (Chain A)	GlcNAc-4 (Chain B)									
Pro87 (Chain B)	GlcNAc-5 (Chain A)									
Pro87 (Chain B)	GlcNAc-6 (Chain A)									
Trp100 (Chain A)	GlcNAc-5 (Chain A)	W100A	0.300	no binding	no binding	no binding	no binding	Y/Y	Y/Y/Y	S
Cys101 (Chain A)	GlcNAc-5 (Chain A)	-	-	-	-	-	-	-	-	-
Asp102 (Chain A)	GlcNAc-6 (Chain B)	D102A	0.295	no binding	no binding	no binding	no binding	P/Y	Y/Y/Y	PU
Tyr103 (Chain A)	GlcNAc-6 (Chain A) GlcNAc-5 (Chain B)	Y103F	0.310	2.10	12.19 ± 4.50	1.01 ± 0.07	-32.32 ± 0.50	Y/Y	Y/Y/Y	
Asn93 (Chain A/B)	None	N93A	0.240	2.43	44.70 ± 2.74	0.95 ± 0.12	-8.78 ± 0.98	N/N	Y/Y/Y	PU
Asp94 (Chain A/B)	None	D94A	0.213	2.26	37.63 ± 7.22	1.08 ± 0.07	-12.83 ± 0.75	N/N	Y/Y/Y	PU
Asn95 (Chain A/B)	None	N95A	0.206	1.10	18.85 ± 6.03	1.06 ± 0.03	-25.20 ± 0.87	Y/Y	Y/Y/Y	

¹ Infiltrations with 5 μg/ml / 10 μg/ml. Y: Yes HR; P: Partial HR; N: No HR; ² Co-infiltrations between effector and receptor of at cell density ratios of 0.5:1 / 1:1 / 2:1. Y: Yes HR; ³ S: stable; PU: partially unstable