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Supplemental Information

Improving T Cell Receptor On-Target Specificity via Structure-Guided Design

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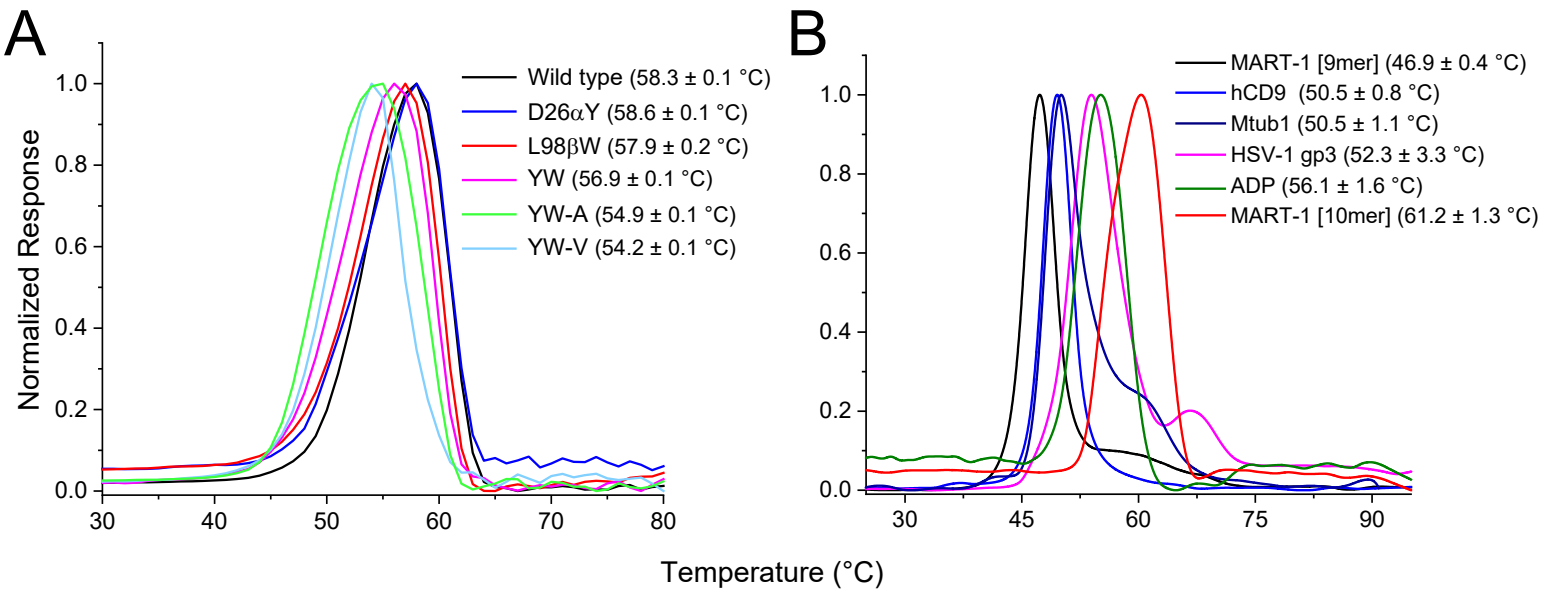

Figure S1. Thermal stabilities of TCRs (A) and peptide/HLA-A2 complexes (B). T_m values from non-linear least squares fitting and associated errors as described in ref. 69 are given in the insets.

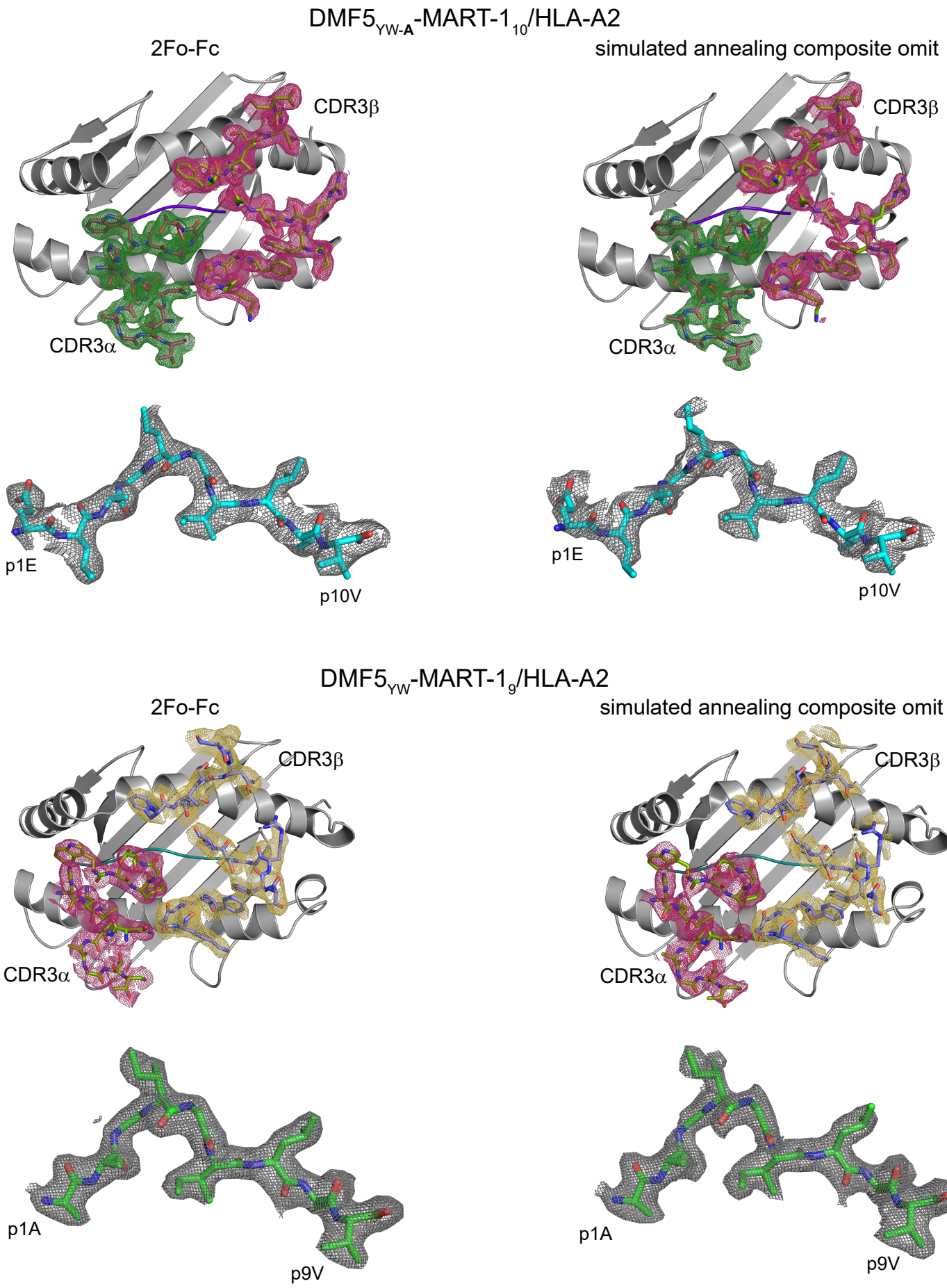

Figure S2. Electron density for the hypervariable loops and the peptides in the $DMF5_{YW-A}$ (top) and $DMF5_{YW}$ (bottom) TCR-pMHC structures. The left hand side shows 2Fo-Fc maps, whereas the right hand side shows simulated annealing composite omit maps. All maps are contoured at 1σ.

Table S1. Binding data for pMHC complexes and variant TCRs.

		MART-1 [9mer]	MART-1 [10mer]	hCD9	HSV-1 gp3	ADP	Mtub1	NLS	SLA	MMW
		AAGIGILTV	ELAGIGILTV	AVGIGIAVV	IAGIGILAI	VDGIGILTI	LGGLGLFFA	NLSNLGILPV	SLANIGILPV	MMWDRGLGMM
TCR	DMF5 WT [a]	37 ± 3 (-6.0)	11 ± 1 (-6.8)	NQ [d]	96 ± 6 (-5.5)	NQ	NQ	10 ± 1 (-6.8)	33 ± 2 (-6.1)	35 ± 2 (-6.1)
	DMF5 L98βW	12 ± 1 (-6.7)	5.3 ± 0.3 (-7.2)	NQ	87 ± 7 (-5.5)	179 ± 11 (-5.1)	NQ			
	DMF5 D26αY	7 ± 2 (-7.0)	1.3 ± 0.2 (-8.0)	420 ± 36 (-4.6)	8 ± 2 (-6.9)	54 ± 11 (-5.8)	148 ± 19 (-5.2)			
	$DMF5_{YW}$ [e]	1.8 ± 0.3 (-7.8)	43 ± 7 [b] (-10.0)	22 ± 2 (-5.4)	2.5 ± 0.5 (-7.2)	58 ± 6 (-4.9)	50 ± 5 (-5.0)	12 ± 2 (-6.7)	16 ± 2 (-6.5)	97.6 ± 0.1 (-9.6) [b]
	DMF5 Y50αF		24.0 ± 8.4 (-6.3)							
	DMF5 Y50αV		200 ± 35 (-5.1)							
	DMF5 Y50αA		NBD [c]							
	$DMF5_{YW\text{-}A}$ [f]		36.4 ± 7.1 (-6.1)					NQ	NDB	13 ± 1 (-6.7)
	$DMF5_{YW\text{-}V}$ [g]		16.1 ± 1.8 (-6.5)					NQ	NQ	

[a] Entries for TCRs give K_D values in micromolar (except where indicated) along with standard fitting error. Values in parenthesis are binding free energies in kcal/mol.

[b] K_D value is in nanomolar.

[c] NBD = no binding detected.

[d] NQ = binding detected, but weak and not quantified.

[e] DMF5 double mutant incorporating the D26αY and L98βW mutations. Binding data from refs. 30 and 33.

[f] DMF5 triple mutant incorporating the D26αY, L98βW, and Y50αA mutations

[g] DMF5 triple incorporating the D26αY, L98βW, and Y50αV mutations

Table S2. X-ray data collection and refinement statistics

Data Collection	DMF5$_{YW\text{-}A}$-MART-1$_{10}$/HLA-A2	DMF5$_{YW}$-MART-1$_{9}$/HLA-A2
Source	24-ID-C	22-ID
Wavelength (Å)	1.0	1.0
Resolution range (Å)	47.94 - 2.97 (3.07 - 2.97)*	40 - 2.35 (2.43 - 2.35)*
Space group	C 1 2 1	C 1 2 1
Unit cell		
a,b,c (Å)	226.49, 49.06, 92.50	226.90, 44.09, 83.22
α,β,γ (°)	90.0, 95.0, 90.0	90.0, 106.4, 90.0
Total reflections	48804 (5130)	66493 (6397)
Unique reflections	20514 (2090)	33491 (3222)
Multiplicity	2.4 (2.4)	2.0 (2.0)
Completeness (%)	94.03 (96.71)	99.55 (96.87)
Mean I/σ(I)	10.55 (1.95)	11.04 (1.77)
Wilson B-factor (Å^2)	87.02	35.35
R_{merge}	0.076 (0.745)	0.048 (0.307)
$CC_{1/2}$	0.996 (0.836)	0.998 (0.856)
Refinement		
Reflections used in refinement	20214 (2090)	33472 (3219)
Reflections used for R_{free}	1033 (107)	1660 (148)
R_{work}	0.229 (0.376)	0.204 (0.273)
R_{free}	0.286 (0.420)	0.256 (0.347)
CC_{work}	0.949 (0.821)	0.949 (0.875)
CC_{free}	0.930 (0.773)	0.928 (0.704)
Number of non-hydrogen atoms	6539	6652
RMS deviations from ideality		
Bond lengths (Å)	0.02	0.003
Angles (°)	0.53	0.89
Ramachandran statistics		
Favored (%)	95.26	98.09
Allowed (%)	4.11	1.91
Outliers (%)	0.62	0.00
Rotamer outliers (%)	0.43	0.00
Clashscore	4.16	2.00
Average B-factor (Å^2)	118.0	41.00
PDB ID	6DKP	6D78

*Statistics for the highest-resolution shells are shown in parentheses.