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Supporting Information

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Structural basis for ineffective T-cell responses to MHC anchor residue-improved "heteroclitic" peptides

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Running title: Altered anchoring of peptide to MHC changes TCR binding

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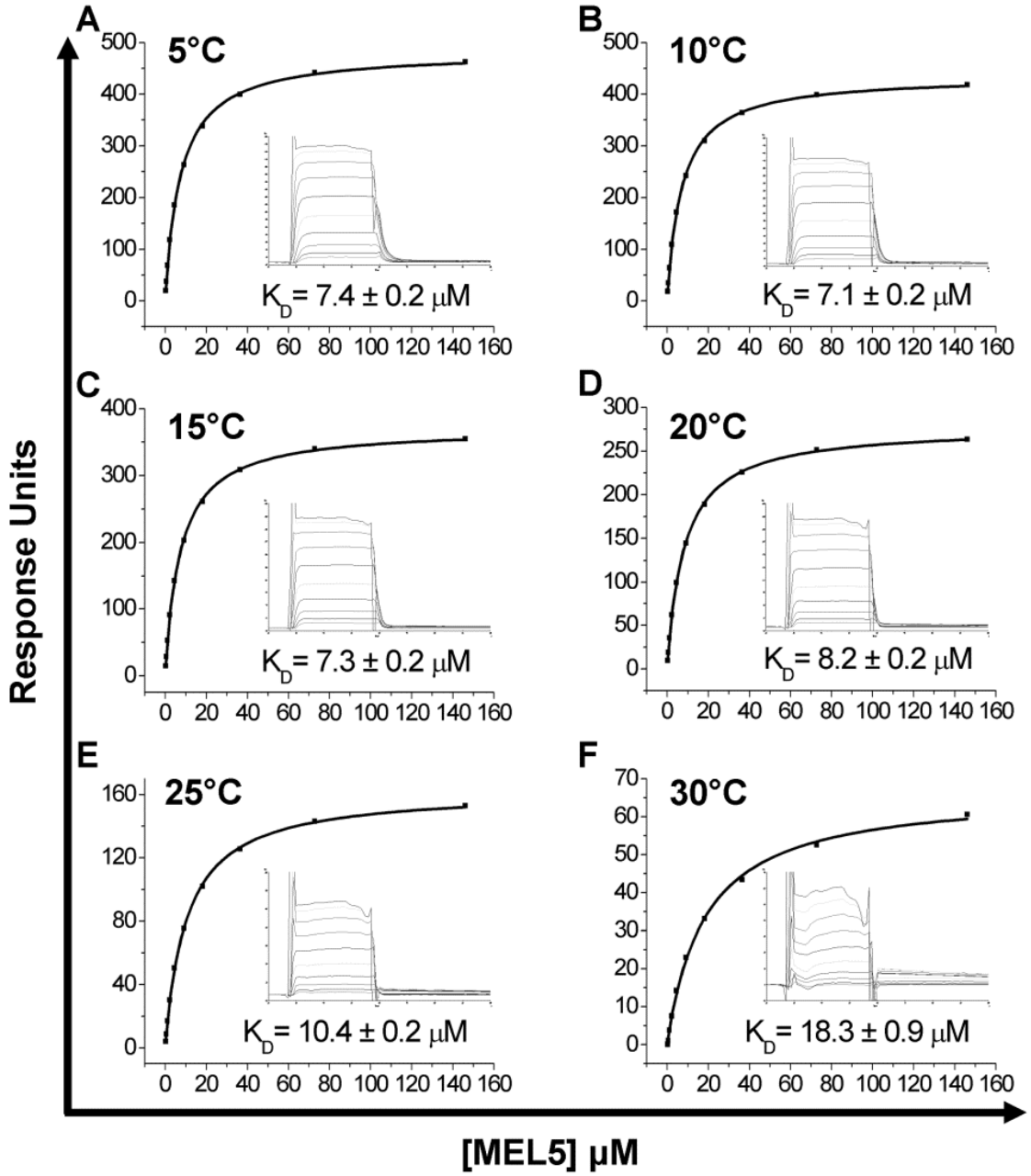
Non-standard abbreviations: pMHC, peptide-major histocompatibility complex; RU, response unit; SPR, surface plasmon resonance.

Supplementary Table S1: MEL5-A2-EAA. 3.4Å cut-off for H-bonds, 3.4Å cut-off for salt bridges and 4Å cut-off for vdW.

Peptide	TCR	H-bonds (≤3.4Å)	vdW (3.2Å–4Å)
Glu1^{Oε2}	αGly29^{O}	1	3
Glu1^{Oε2}	αGln31^{Nε2}	1 salt bridge	1
Ala2^{O}	αGln31^{Nε2}	1	
Ala3	αGln31		1
Gly4^{N}	αGln31^{Oε1}	1	6
Gly4	βLeu98		3
Ile5	αGln31		2
Ile5	αSer32		1
Ile5	αAsn92		1
Ile5	βLeu98		2
Ile5	βGly99		3
Gly6^{N}	βLeu98^{O}	1	2
Ile7	βGly97		2
Ile7^{O}	βLeu98^{N}	1	9
Leu8	βGly99		2
Thr9	βThr96		1
MHC	TCR	H-bonds (≤3.4Å)	vdW (3.2Å–4Å)
Gly62	αAla94		3
Arg65$^{Nε/NH2}$	αAla94^{O}	2	6
Arg65	αLys96		3
Arg65	βTyr49		6
Arg65^{NH1}	βGlu59$^{Oε1/Oε2}$	2 salt bridge	2
Lys66	αGln31		1
Lys66	βLeu98		1
Lys68	βTyr49		2
Ala69	βLeu98		4
His70	βLeu98		1
Gln72	βVal51		2
Thr73	βGly97		2
Val76	βAsn30		4
His151	αTyr51		1
Glu154	αTyr51		3
Gln155	αTyr51		5
Gln155	βGly99		2
Gln155^{Oε1}	βThr100^{N}	1	5
Ala158	αTyr51		2
Tyr159	αGln31		3
Thr163	αGln31		3
Glu166^{Oε2}	αArg28^{NH2}	1 salt bridge	2
Trp167	αArg28		1
Trp167	αGly29		1

Supplementary Table 2: MEL5-A2-ELA (1). 3.4Å cut-off for H-bonds, 3.4Å cut-off for salt bridges and 4Å cut-off for vdW.

Peptide	TCR	H-bonds (≤3.4Å)	vdW (3.2Å–4Å)
Glu1	αGly29		1
Glu1	αGln31		1
Ala3	αGln31		1
Ala3	βLeu98		1
$Gly4^{N}$	$αGln31^{Oε1}$	1	5
$Gly4^{O}$	$αAsn92^{Nδ2}$	1	
Gly4	βLeu98		3
Ile5	αGln31		1
Ile5	αSer32		1
Ile5	αTyr51		1
Ile5	αAsn92		1
Ile5	βLeu98		2
Ile5	βGly99		6
Gly6	βGly99		2
$Ile7^{O}$	$βLeu98^{N}$	1	4
Ile7	βGly99		1
Leu8	βGly99		2
Thr9	βThr96		1
MHC	TCR	H-bonds (≤3.4Å)	vdW (3.2Å–4Å)
Gly62	αAla94		2
$Arg65^{Nε}$	$αAla94^{O}$	1	5
Arg65	αLys96		2
$Arg65^{NH1}$	$βGlu59^{Oε1}$	1 salt bridge	1
Lys66	αGln31		1
Lys68	βVal51		1
Gln72	βVal51		3
Thr73	βGly97		2
Val76	βAsn30		3
Val76	βThr96		1
Glu154	αTyr51		3
Gln155	αTyr51		5
Gln155	βGly99		2
$Gln155^{Oε1}$	$βThr100^{N}$	1	7
Ala158	αTyr51		2
Tyr159	αGln31		4
Thr163	αGln31		4
$Glu166^{Oε2}$	$αArg28^{NH2}$	1 salt bridge	2
Trp167	αGly29		3



Supplementary Figure S1. Thermodynamic analysis of the MEL5 interaction with A2-EAA. Thermodynamic analysis of the MEL5-A2-EAA interaction was performed by SPR. Ten serial dilutions of MEL5 were measured in triplicate at **(A)** 5°C, **(B)** 10°C, **(C)** 15°C, **(D)** 20°C, **(E)** 25°C and **(F)** 30°C; representative data from one of these experiments is plotted. The equilibrium binding constant (K_D) values were calculated using a nonlinear curve fit (Langmuir binding equation AB = (B x AB_{max})/(K_D + B)); mean plus SD values are shown. These data were used to fit thermodynamic parameters shown in **Figure 1.**