SUPPORTING INFORMATION

Quantitative affinity measurement of small molecule ligand binding to Major Histocompatibility Complex class-I related protein 1 MR1

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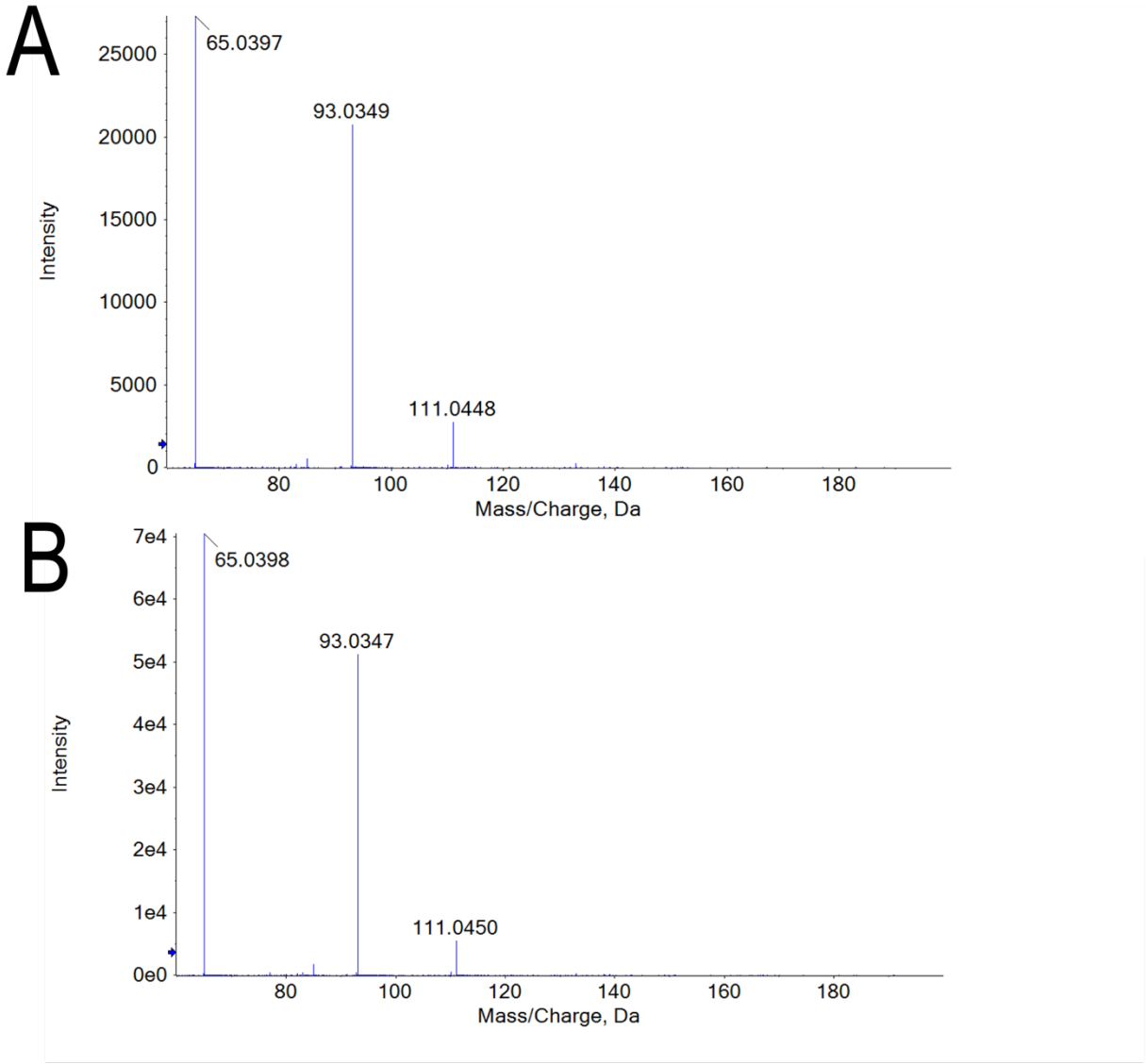

Figure S1. Mass spectrometry fragmentation analysis of ethylvanillin extracted from MAIT TCR-MR1-ethylvanillin crystals. (A) Positive mode LC-MS/MS analysis of small molecules extracted from solubilised crystals of MAIT TCR-MR1-ethylvanillin complex revealed a mass equivalent to protonated ethylvanillin (m/z 161.07), fragmenting to give ions of 65.04, 93.03 and 111.04. **(B)** Fragmentation of ethylvanillin analysed by the same workflow described in **(A)**. Spectra were visualised using PeakView® Version 2.2 (SCIEX).

Table S1. Curve fit for ligands

Ligand	5-OP-RU	2'D-5-OP-RU	JYM72	Ac-6-FP	3'D-5-OP-RU	6-FP	3-F-SA	Ribityl-less analogue	RL-6-Me-7-OH
Best-fit values									
LogIC50	0.62	1.80	1.43	1.60	2.12	2.03	2.75	2.69	3.42
Std. Error									
LogIC50	0.05	0.05	0.04	0.13	0.07	0.03	0.03	0.04	0.02
95% CI									
LogIC50	0.53 to 0.71	1.73 to 1.89	1.36 to 1.50	1.38 to 1.80	2.01 to 2.24	1.97 to 2.09	2.70 to 2.81	2.62 to 2.76	3.38 to 3.46
Goodness of Fit									
Degrees of Freedom	51	42	42	30	33	48	51	42	51
R squared	0.97	0.98	0.97	0.90	0.97	0.99	0.99	0.98	0.99
Sum of Squares	3089	1661	1016	7248	2309	1127	857.9	1088	464.2
Sy.x	7.78	6.29	4.92	15.54	8.36	4.85	4.10	5.09	3.02
Number of points									
# of X values	111	51	48	42	42	51	168	219	285
# Y values analyzed	54	45	45	33	36	51	54	45	54

Ligand	2'D-RL-7-Me	DB28	RL-6-Me	Ethylvanillin	Vanillin	NV18.1	HMB	5-OH-DCF	DCF
Best-fit values									
LogIC50	4.05	4.29	5.05	5.51	5.51	5.72	5.89	6.05	6.42
Std. Error									
LogIC50	0.05	0.03	0.06	0.024	0.024	0.02	0.03	0.07	0.09
95% CI									
LogIC50	3.97 to 4.13	4.24 to 4.34	4.94 to 5.16	5.47 to 5.56	5.47 to 5.56	5.68 to 5.75	5.84 to 5.94	5.92 to 6.18	6.27 to 6.57
Goodness of Fit									
Degrees of Freedom	45	45	42	45	42	51	33	51	33
R squared	0.98	0.99	0.96	0.99	0.99	1.00	0.99	0.94	0.9
Sum of Squares	1606	594.8	2197	450.6	397.2	266.6	330.1	2349	3666
Sy.x	5.98	3.64	7.23	3.16	3.08	2.29	3.16	6.79	10.54
Number of points									
# of X values	291	285	282	273	270	285	261	282	261
# Y values analyzed	48	48	45	48	45	54	36	54	36

Table S2. Data collection and refinement statistics

	AF-7 TCR-MR1-ethylvanillin
Data collection	
Temperature	100K
Resolution limits (Å)	48.43-2.5 (2.55-2.5)
Space Group	C121
Cell dimensions (Å)	*a*=217.94, *b*=70.89, *c*=144.39 α=β=90° γ=104.86°
Total N°. observations	256341 (16092)
N°. unique observations	73278 (4496)
Multiplicity	3.5 (3.6)
Data completeness	99.1 (98.9)
Wilson B-factors (Å^2)	35.7
I/σ_I	9.1 (1.9)
$R_{p.i.m}$ [1] (%)	6.9 (47.5)
Refinement statistics	
R_{factor} [2] (%)	17.7
R_{free} [3] (%)	23.6
Non hydrogen atoms	
- Protein	13151
- Water	379
- Heterogen	116
Ramachandran plot (%)	
- Most favoured	97
- Allowed	2.9
B-factors (Å^2)	
- protein	46.6
- ligands	50.3
rmsd bonds (Å)	0.008
rmsd angles (°)	1.01

[1] $R_{p.i.m} = \Sigma_{hkl} [1/(N-1)]^{1/2} \Sigma_i | I_{hkl,i} - \langle I_{hkl} \rangle | / \Sigma_{hkl} \langle I_{hkl} \rangle$

[2] $R_{factor} = (\Sigma | |F_o| - |F_c| |) / (\Sigma |F_o|)$ - for all data except as indicated in footnote 3.

[3] 5% of data was used for the R_{free} calculation

Values in parentheses refer to the highest resolution bin.

Table S3. AF-7 MAIT TCR contacts with MR1

TCR gene	TCR residues	MR1 residues	Bond type
CDR1α	Gly28α	Glu160	VDW
CDR1α	Phe29α^{N}	Glu160^{Oε2}	HB
CDR1α	Phe29α^{O}	Asn155^{Nδ2}, Glu160^{Oε2}	HB
CDR1α	Phe29α	Gln160	VDW
CDR1α	Asn30α^{Oδ1}	Tyr152, Trp156, Glu160	VDW
CDR2α	Val50α	Leu151, Tyr152, Asn155	VDW
CDR2α	Leu51α	Leu151, Lys154, Asn155	VDW
FWα	Tyr48α	His148, Tyr152	VDW
FWα	Glu55α^{Oε1}	His148^{Nε2}	HB
FWα	Glu55α	His148	VDW
FWα	Arg66α^{Nη1}	Asn155^{Oδ1}	HB
FWα	Arg66α	Asn155, Glu159	VDW
CDR3α	Ser93α	Tyr62^{Oη}	HB
CDR3α	Ser93α	Tyr62, Glu160, Trp164	VDW
CDR3α	Asn94α^{Oδ1}	Tyr62^{Oη}	HB
CDR3α	Asn94α^{O}	Arg61^{Nε}, Arg61^{Nη2}	HB
CDR3α	Asn94α	Arg61, Tyr62, Trp164	VDW
CDR3α	Tyr95α^{O}	Arg61^{Nη2}	HB
CDR3α	Tyr95α^{OH}	Trp156^{Nε1}	HB
CDR3α	Tyr95α	Arg61, Tyr152, Trp156	VDW
CDR3α	Gln96α	Arg61	VDW
CDR2β	Ala50β	Gln64	VDW
CDR2β	Ser51β	Gly68	VDW
CDR2β	Thr54β^{Oγ1}	Arg67^{Nη1}, Gln64^{Oε1}	HB
CDR2β	Thr54β	Arg67, Gln64,	VDW
FWβ	Tyr48β^{Oη}	Arg61^{Nη1}	HB
FWβ	Tyr48β	Arg61, Gln64	VDW
FWβ	Thr55β^{O}	Gln64^{Oε1}	HB
FWβ	Thr55β	Gln64	VDW
FWβ	Asp56β	Gln64	VDW
CDR3β	Trp96β	Gly68, Met72	VDW
CDR3β	Thr97β^{O}	Trp69^{Nε1}	HB
CDR3β	Thr97β	Leu65	VDW
CDR3β	Gly98β	Trp69, Glu149, Tyr152	VDW
CDR3β	Gly99β	Glu149	VDW
CDR3β	Gly100β	Glu149, Tyr152	VDW
CDR3β	Ser101β^{Oγ}	Glu149^{N}, Glu149^{Oε1}	HB
CDR3β	Ser101β^{N}	Glu149^{Oε1}	HB
CDR3β	Ser101β	Glu149	VDW

HB: Hydrogen bond, VDW: van der Waals, Cut-off at 4 Å for VDW interactions and 3.5 Å for HB.

Table S4. Ethylvanillin contacts with MR1

Ethylvanillin	MR1 residues	Bond type
C1	Tyr7, Arg9, Trp69	VDW
C2	Ser24	VDW
C3	Tyr7, Ser24, Lys43, Leu66	VDW
C4	Tyr7, Lys43	VDW
C5	Tyr7, Lys43	VDW
C6	Tyr7, Lys43, Trp69	VDW
C7	Tyr7, Trp69	VDW
C8	Arg9, Trp69, Arg94, Ile96	VDW
C9	Trp156	VDW
O1	$Arg9^{N\varepsilon}$, $Arg9^{N\eta 2}$	HB
O1	Tyr7, Arg9, Arg94	VDW
O3	Tyr7, Trp69	VDW

HB: Hydrogen bond, VDW: van der Waals, Cut-off at 4 Å for VDW interactions and 3.5 Å for HB.