

Supplementary file 1

Additional experimental data (Supplementary files 1A, 1B, and 1C)

Supplementary file 1A

Table with results of 70 peptides screened in shark pMHC-I refolding assay

<i>Name</i>	<i>Sequence</i>	<i>Source sequence</i>	<i>%Random^a</i>	<i>Reference</i>	<i>Stability with Gici-UAA^b</i>
pep1 (Gag-LF9) ^c	LSGQREAF	Gag [Equine infectious anemia virus]	0.026	1	-
pep2 (Pol-EW9)	ESMGGQTPW	pol protein [Equine infectious anemia virus]	0.035	1	-
pep3 (Gag-DY8)	DTLEEKMY	gag [Equine infectious anemia virus]	0.014	1	-
pep4 (Gag-VL9)	VTVQGSQKL	gag [Equine infectious anemia virus]	0.035	1	-
pep5 (Env-GF9)	GSFPGCRPF	envelope protein [Equine infectious anemia virus]	0.033	1	-
pep6 (Rev-EW9)	EVLQERLEW	REV protein [Equine infectious anemia virus]	0.015	1	-
pep7 (Env-NF9)	NTPDSIAQF	envelope polyprotein [Equine infectious anemia virus]	0.026	1	-
pep8 (Env-IY9)	IAASATMSY	envelope polyprotein [Equine infectious anemia virus]	0.040	1	-
pep9 (Env-FM9)	FSYETNRSM	envelope protein [Equine infectious anemia virus]	0.077	1	-
Pep10 (Gag-CF9)	CTSEEMNAF	gag protein [Equine infectious anemia virus]	0.046	1	-
pep11 (Gag-ML9)	MTARFIRGL	gag protein [Equine infectious anemia virus]	0.072	1	+
pep12	WALSLVDLF	gag protein [Equine infectious anemia virus]	0.042		-
pep13	ITKNYEATY	pol polyprotein [Equine infectious anemia virus]	0.021		-
pep14	FLSFASLFL	nucleoprotein [Zaire ebolavirus]	0.124		-
pep15	YQVNNLEEI	nucleoprotein [Zaire ebolavirus]	0.051		-
pep16	AMNEENRFV	nucleoprotein [Zaire ebolavirus]	0.050		-
pep17	YTMQDALFL	polymerase [Zaire ebolavirus]	0.174		-
pep18	RTSFLLWVI	surface glycoprotein [Zaire ebolavirus]	0.051		-
pep19	YQVNNRNFV	artificial	0.049		-
pep20	AMNEELEEI	artificial	0.051		-
pep21	YRMKRAETY	polyprotein [Foot-and-mouth disease virus - type O]	0.033		-
pep22	RRQHTDVSF	polyprotein [Foot-and-mouth disease virus - type O]	0.014		-

pep23	RTLPTSFNY	polyprotein [Foot-and-mouth disease virus - type O]	0.022		-
pep24	SKFTFSIPY	polyprotein [Foot-and-mouth disease virus - type A]	0.012		-
pep25	RELYQLTLF	polyprotein [Foot-and-mouth disease virus - type O]	0.008		-
pep26	RFGTHFAQY	polyprotein [Foot-and-mouth disease virus - type A]	0.021		-
pep27	RQHTDVSFI	polyprotein [Foot-and-mouth disease virus - type O]	0.027		-
pep28	AQYRNVWDV	polyprotein [Foot-and-mouth disease virus - type A]	0.040		-
pep29	MLADTGLEI	polyprotein [Foot-and-mouth disease virus - type A]	0.122		-
pep30	YMQYQNSM	polyprotein [Foot-and-mouth disease virus - type A]	0.159		-
pep31	SIWITDETV	glycoprotein [Carp sprivivirus]	0.035		-
pep32	KRKINNQEK	glycoprotein [Carp sprivivirus]	0.006		-
pep33	MKKSRRPAT	glycoprotein [Carp sprivivirus]	0.004		-
pep34	CVACTYLMK	glycoprotein [Carp sprivivirus]	0.012		-
pep35	YLNHLITNM	glycoprotein [Viral hemorrhagic septicemia virus]	0.089		-
pep36	CMTVAVDEV	glycoprotein [Infectious hematopoietic necrosis virus]	0.055		-
pep37	SLHWSFWPT	glycoprotein [Infectious hematopoietic necrosis virus]	0.038		-
pep38	TMGPASVSF	polyprotein [Infectious pancreatic necrosis virus]	0.032		-
pep39 (SVCV-FAN9)	FANFCLMMI	L protein [Carp sprivivirus]	0.082	2	+
pep40	FA A FCLMMI	Mutant pep39	/	2	-
pep41	FANFC A MMI	Mutant pep39	/	2	+
pep42	FANFCLM M A	Mutant pep39	/	2	-
pep43	F TNFCLMMI	Mutant pep39	/	2	+
pep44	GRRTRIAY	nucleoprotein [Influenza A virus]	0.010		-
pep45	RREVHTYY	polymerase PA [Influenza A virus]	0.011		-
pep46	VRNGTYDY	hemagglutinin, partial [Influenza A virus]	0.010		-
pep47	TSADQQLY	hemagglutinin [Influenza A virus]	0.038		-
pep48	GTFDLGGLY	polymerase PA [Influenza A virus]	0.022		-
pep49	WSQDPTMLY	PB2 [Influenza A virus]	0.036		-
pep50	GTEKLITY	PB2 [Influenza A virus]	0.020		-
Pep51	ASQGTKRSY	nucleoprotein [Influenza A virus]	0.012	3	-
pep52	MLNRFIRGL	artificial	0.081		+
pep53	MTARFIRMI	artificial	0.068		++
pep54	MTARCLMMI	artificial	0.095		+
pep55	MTNRFIRGL	artificial	0.064		++
pep56	FANFFIRGL	artificial	0.070		++
pep57	FANFF R RGL	mutant pep56	0.087		++
pep58	A ANFFIRGL	mutant pep56	/		++
pep59	FA A FFIRGL	mutant pep56	/		+

pep60	FANAFIRGL	mutant pep56	/		++
pep61	FANFAIRGL	mutant pep56	/		+
pep62	FANFFARGL	mutant pep56	/		+
pep63	FANFFIAGL	mutant pep56	/		++
pep64	FANFFIRAL	mutant pep56	/		++
pep65	FANFFIRGA	mutant pep56	/		+
pep66	AANFFIRGA	mutant pep56	/		+
pep67 (IE8)	IDWFDGKE	FUS proto-onc gene	0.001	4	-
pep68 (MVM9)	MVMELIRMI	nucleoprotein [Influenza A virus]	0.082	5	++
pep69	RTSDMRTEI	nucleoprotein [Influenza A virus]	0.062		++
pep70	IAYERMCNI	nucleocapsid protein [Influenza A virus]	0.089		++

^a % Random is a base value for predicting the binding affinities of peptides with the NetMHCpan 2.8 server (<http://www.cbs.dtu.dk/services/NetMHCpan/>): Rank threshold for strongly binding peptides, 0.100; rank threshold for weakly binding peptides, 1.000.

^b -, peptide did not detectably form complexes with Gici-UAA*01 and Gici- β ₂-m; +, peptide formed complexes with Gici-UAA*01 and Gici- β ₂-m as revealed by size exclusion chromatography (gel filtration) but could not tolerate anion-exchange chromatography; ++, peptide bound strongly and the pMHC-I complex could tolerate anion-exchange chromatography;

^c The peptide name used in the article listed in the Reference column is in parentheses. Some of the tested peptides were originally synthesized for use in other study projects, whereas others were newly ordered.

References

1. Yao S, Liu J, Qi J, Chen R, Zhang N, Liu Y, Wang J, Wu Y, Gao GF, Xia C. 2016. Structural Illumination of Equine MHC Class I Molecules Highlights Unconventional Epitope Presentation Manner That Is Evolved in Equine Leukocyte Antigen Alleles. *J Immunol* 196:1943-1954.
2. Chen Z, Zhang N, Qi J, Chen R, Dijkstra JM, Li X, Wang Z, Wang J, Wu Y, Xia C. 2017. The Structure of the MHC Class I Molecule of Bony Fishes Provides Insights into the Conserved Nature of the Antigen-Presenting System. *J Immunol* 199:3668-3678.
3. Nakada R, Hirano H, Matsuura Y. 2015. Structure of importin-alpha bound to a non-classical nuclear localization signal of the influenza A virus nucleoprotein. *Sci Rep* 5:15055.
4. Zhang J, Chen Y, Qi J, Gao F, Liu Y, Liu J, Zhou X, Kaufman J, Xia C, Gao GF. 2012. Narrow groove and restricted anchors of MHC class I molecule BF2*0401 plus peptide transporter restriction can explain disease susceptibility of B4 chickens. *J Immunol* 189:4478-4487.
5. Wu Y, Wang J, Fan S, Chen R, Liu Y, Zhang J, Yuan H, Liang R, Zhang N, Xia C. 2017. Structural Definition of Duck Major Histocompatibility Complex Class I Molecules That Might Explain Efficient Cytotoxic T Lymphocyte Immunity to Influenza A Virus. *J Virol* 91:e02511-16.

Supplementary file 1B

Table with X-ray diffraction data collection and refinement statistics of Gici- β_2 -m and pGici-UAA*01

	Gici- β_2 -m	pGici-UAA*01 (aka shark pUAA)
Data collection		
Space group	<i>P</i> 3 ₂ 21	<i>P</i> 6 ₄
Cell dimensions		
a,b,c (Å)	88.23, 88.23, 67.15	125.86, 125.86, 132.47
α,β,γ (°)	90.00, 90.00, 120.00	90.00, 90.00, 120.00
Resolution (Å)	50.00-2.30 (2.38-2.30) ^a	50.00-2.30 (2.36-2.30) ^a
<i>R</i> _{merge} (%) ^b	8.2 (20.3)	11.0 (57.1)
<i>I</i> / σ <i>I</i>	33.733 (14.608)	21.185 (4.267)
Completeness (%)	94.0 (100.0)	100.00 (100.00)
Redundancy	11.9 (12.2)	4.7 (4.8)
refinement		
Resolution (Å)	33.21-2.30	50.00-2.30
Number of reflections	12603	49982
<i>R</i> factor (%) ^c	22.75	23.29
<i>R</i> _{free} (%)	25.33	26.20
Number of atoms		
Protein	1544	6032
Ligand/ion	0	0
Water	127	160
Total	1671	6192
<i>B</i> -factors		
Protein	42.5	63.2
Ligand/ion	0	0
Water	42.5	39.5
Total	42.5	62.6
r.m.s.d.		
Bond lengths (Å)	0.006	0.005
Bond angles (°)	0.988	0.963
Most favored (%)	97.78	95.59
Allowed region (%)	0.56	0.14
Disallowed (%)	1.66	4.27

^a Values in parentheses are for highest-resolution shell.

^b $R_{\text{merge}} = \frac{\sum_{\text{hkl}} \sum_i |I_i(\text{hkl}) - \langle I(\text{hkl}) \rangle|}{\sum_{\text{hkl}} \sum_i I_i(\text{hkl})}$, where $I_i(\text{hkl})$ is the observed intensity and $\langle I(\text{hkl}) \rangle$ is the average intensity from multiple measurements.

^c $R \text{ factor} = \frac{\sum_{\text{hkl}} \sum_i |F_{\text{obs}} - k| |F_{\text{calc}}|}{\sum_{\text{hkl}} \sum_i |F_{\text{obs}}|}$, where R_{free} is calculated for a randomly chosen 5% of reflections and R_{work} is calculated for the remaining 95% of reflections used for structure refinement.

Supplementary file 1C

Table with RMSD values of pMHC-I total structures or domains when superimposed on shark pUAA

		total*	$\alpha 1\alpha 2$ -domain	$\beta 2$ -m	$\alpha 3$ -domain
pMHC-I	Shark UAA (6LUP)				
	Carp UAA (5Y91)	1.057 (1843 atoms)	0.716 (963 atoms)	0.630 (543 atoms)	0.979 (409 atoms)
	Frog UAA (6A2B)	1.347 (1947 atoms)	0.763 (943 atoms)	0.790 (462 atoms)	0.955 (411 atoms)
	Chicken BF2*0401 (4E0R)	0.996 (1665 atoms)	0.741 (933 atoms)	0.504 (463 atoms)	0.732 (393 atoms)
	Human HLA-A2 (3PWN)	1.072 (1692 atoms)	0.685 (900 atoms)	0.486 (508 atoms)	1.230 (357 atoms)

RMSD values were calculated by PyMOL software using the “super” function. *“Total” refers to full-size structures while neglecting the peptide ligands.