Supporting Information Table 1

HLA-A*02:01 (and T134K) All Peptides SYFPEITHI	1	YLEPGPVTA
	2	YLNKIQNSL
	3	ALSNLEVKL
	4	KVAELVHFL
	5	TLNAWVKVV
	6	SLSRFSWGA
	7	FLWGPRAYA
	8	ALFDGDPHL
	9	HLIDYLVTS
	10	VLVKSPNHV
	11	ILDKKVEKV
	12	HLGNVKYLV
	13	GLHCYEQLV
	14	GLYDGMEHL
	15	RLMKQDFSV
	16	YMNGTMSQV
	17	SLLPAIVEL
	18	FLDGNELTL
	19	TLWVDPYEV
	20	ILDTGTIQL
	21	VLFSSDFRI
	22	RLNMFTPYI
	23	ILMEHIHKL
	24	QVCERIPTI
	25	HLSTAFARV
	26	RLPRIFCSC
	27	SLDQSVVEL
	28	KIFGSLAFL
	29	LLMDCSGSI
	30	MLGTHTMEV
	31	LLIENVASL
	32	MVDGTLLLL
	33	LLGATCMFV
	34	KLVANNTRL
	35	RLTRFLSRV
	36	GILGFVFTL
	37	KLLEPVLLL
	38	YLSGANLNL
	39	ILAKFLHWL
	40	RLLQETELV
	41	SLLLELEEV
	42	ALCRWGLLL
	43	FLWGPRALV
	44	ILFGHENRV
HLA-A*02:01 (and T134K) All Peptides Non-SYFPEITHI	1	ILSPHNVVT
	2	AMHYIRHRA
	3	KIFEYGFTF
	4	ALWEIQQVV
	5	FTFDNSKFV
	6	MMFDAMGAL
	7	KMVGTVQRV
	8	KLAEIFQPF
	9	VVYKEAKIK
	10	WLKEKHEEL
	11	FGKWRPVQL
	12	HLKRTILAL
	13	TMLYNKMEF
	14	IFRRDQIWF
	15	YTLNNGVAM
	16	GLAGGAATA
	17	KVRGRLLAL
	18	QLAFTYCQV
	19	WMDMWESPM
	20	ALEEGRKYV
	21	YLPEDSDIL
	22	ELADQLIHL
	23	NVWATHACV
	24	LLLGGTSEI
	25	DLYDYITRI
	26	FLYGWLFIL
	27	PLNEGIMAV
	28	FLFLYWPHY
	29	SGFGGETPV
	30	SILEYAKSI
	31	KMYEYVFKG
	32	SMFYGIFPS

	33	WFMTWQPNI
	34	SLFGAAVSL
	35	YLLLTTNGT
	36	SIFFDYMAI
	37	YQIEGAWRA
	38	FQWHEAMFL
	39	TLKPGTMSV
	40	YIITCCLFA
	41	GLYSLPHDL
	42	RQPLNIQAI
	43	SVFSRPLPL
	44	GIYGAVIPL
HLA-A*02:01 Subset EC$_{50}$ Pairs SYFPEITHI	1	HLIDYLVTS
	2	VLVKSPNHV
	3	ILDKKVEKV
	4	HLGNVKYLV
	5	RLMKQDFSV
	6	YMNGTMSQV
	7	SLLPAIVEL
	8	FLDGNELTL
	9	TLWVDPYEV
	10	RLNMFTPYI
	11	HLSTAFARV
	12	SLDQSVVEL
	13	LLIENVASL
	14	LLGATCMFV
	15	GILGFVFTL
	16	KLLEPVLLL
	17	YLSGANLNL
	18	ALCRWGLLL
	19	FLWGPRALV
	20	ILFGHENRV
	21	HLIDYLVTS
HLA-A*02:01 Subset EC$_{50}$ Pairs Non-SYFPEITHI	1	AMHYIRHRA
	2	FTFDNSKFV
	3	MMFDAMGAL
	4	KMVGTVQRV
	5	WLKEKHEEL
	6	GLAGGAATA
	7	ALEEGRKYV
	8	NVWATHACV
	9	LLLGGTSEI
	10	FLYGWLFIL

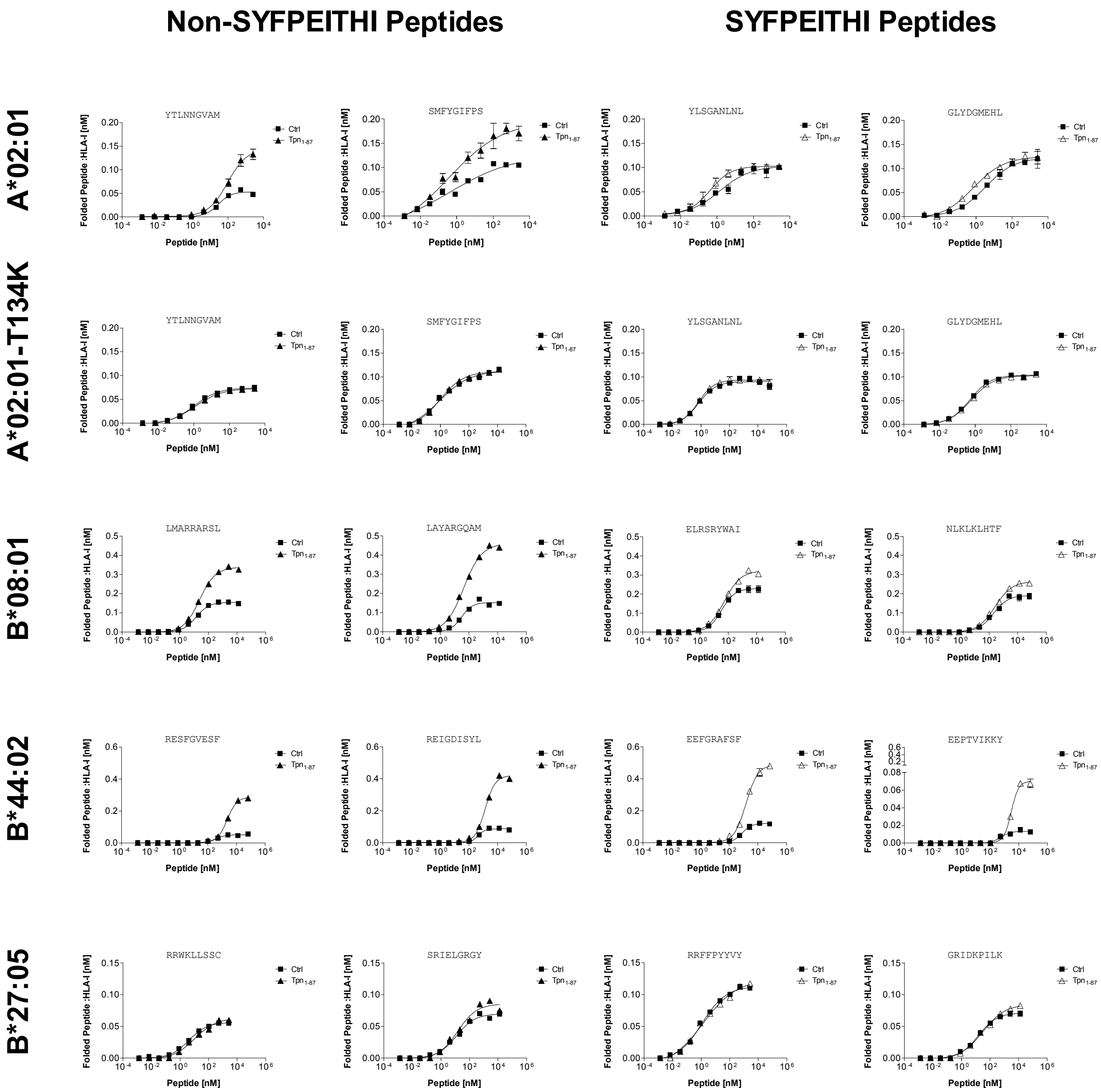
	11	PLNEGIMAV
	12	SILEYAKSI
	13	KMYEYVFKG
	14	SLFGAAVSL
	15	YLLLTTNGT
	16	SIFFDYMAI
	17	FQWHEAMFL
	18	TLKPGTMSV
	19	YIITCCLFA
	20	GLYSLPHDL
	21	GIYGAVIPL
HLA-B*08:01 All Peptides SYFPEITHI	1	DLERKVESL
	2	ELRSLYNTV
	3	EIKDTKEAL
	4	EIKDTKEAL
	5	NLKLKLHTF
	6	YLKVKGNVF
	7	ELRSRYWAI
	8	RAKFKQLL
	9	FLRGRAYGL
	10	QAKWRLQTL
	11	ELRSRYWAI
	12	LPHNHTDL
HLA-B*08:01 All Peptides Non-SYFPEITHI	1	TLRRRFAVA
	2	FPRYPLNVL
	3	FIKDRATAV
	4	QLSLRMLSL
	5	QLSLKMLSL
	6	YRRKLTNPA
	7	VPRPRFSAL
	8	FARERRLAL
	9	LAYARGQAM
	10	YGLERLAAM
	11	EAILRRFPL
	12	LARLFLYAL
	13	WLRAHPVAI
	14	LMARRARSL
	15	VLRRRRRDA
	16	RLRLLLKQM
	17	MTRRRVLSV
	18	MEQRVMATL
	19	EAKLFFQVI
	20	LAARKARAA

HLA-B*44:02 All Peptides SYFPEITHI	1	EEFGRAFSF
	2	EEPTVIKKY
	3	EENLLDFVRF
	4	EEKRGSLHVW
	5	KEFEDDIINW
HLA-B*44:02 All Peptides Non-SYFPEITHI	1	RERIRYFHY
	2	AEHFENQVL
	3	HEGDIVPLF
	4	IEAGDEVFF
	5	REWGWRIPF
	6	LEHGLYPQL
	7	AEALLADGL
	8	REMGIVDLL
	9	REIGDISYL
	10	AELGAFFSI
	11	YEGDLRVTF
	12	QEGAMHTAL
	13	SETQGTEKL
	14	REMHHLVEF
	15	AEIESATLF
	16	AESICSYWL
	17	AETESATLF
	18	KEAVNHFHL
	19	REGGGAVRL
	20	REAGMAATL
	21	IENIDFASL
	22	CELSSHGDL
	23	WEMRAGREI
	24	TEMYIMYAM
	25	GEGPGINPI
	26	RESIVCYFM
HLA-B*27:05 All Peptides SYFPEITHI	1	ARLFGIRAK
	2	GRIDKPILK
	3	GRNSFEVRV
	4	IRHNKDRKV
	5	RRFFPYYVY
HLA-B*27:05 All Peptides Non-SYFPEITHI	1	NRRFVNVVP
	2	RRVFHGVAK
	3	SRLTYQWHK
	4	RRFGGTVIR
	5	RQWAQDLTL
	6	RRTAAGIMK
	7	RRWCFDGPR
	8	KRWGFRSGV
	9	RRLHRLLLM
	10	RRWKLLSSC
	11	SREGMFLPK
	12	RREGGGAVR
	13	KRLGDVISV
	14	KRYKNRVAS
	15	FRQCTGRPK
	16	RRRVLSVVV
	17	SRIELGRGY
	18	WRMGYRTHN
	19	GRVTVSTKR

Supporting Information Table 2

A0201/T134K	0.0770	( )
A0201/B0801	0.0253	(*)
A0201/B4402	<0.0001	(***)
A0201/B2705	0.0576	( )
T134K/B0801	<0.0001	(***)
T134K/B4402	<0.0001	(***)
T134K/B2705	<0.0001	(***)
B0801/B4402	0.0499	(*)
B0801/B2705	0.7539	( )
B4402/B2705	0.0260	(*)

Supporting Information Figure 1

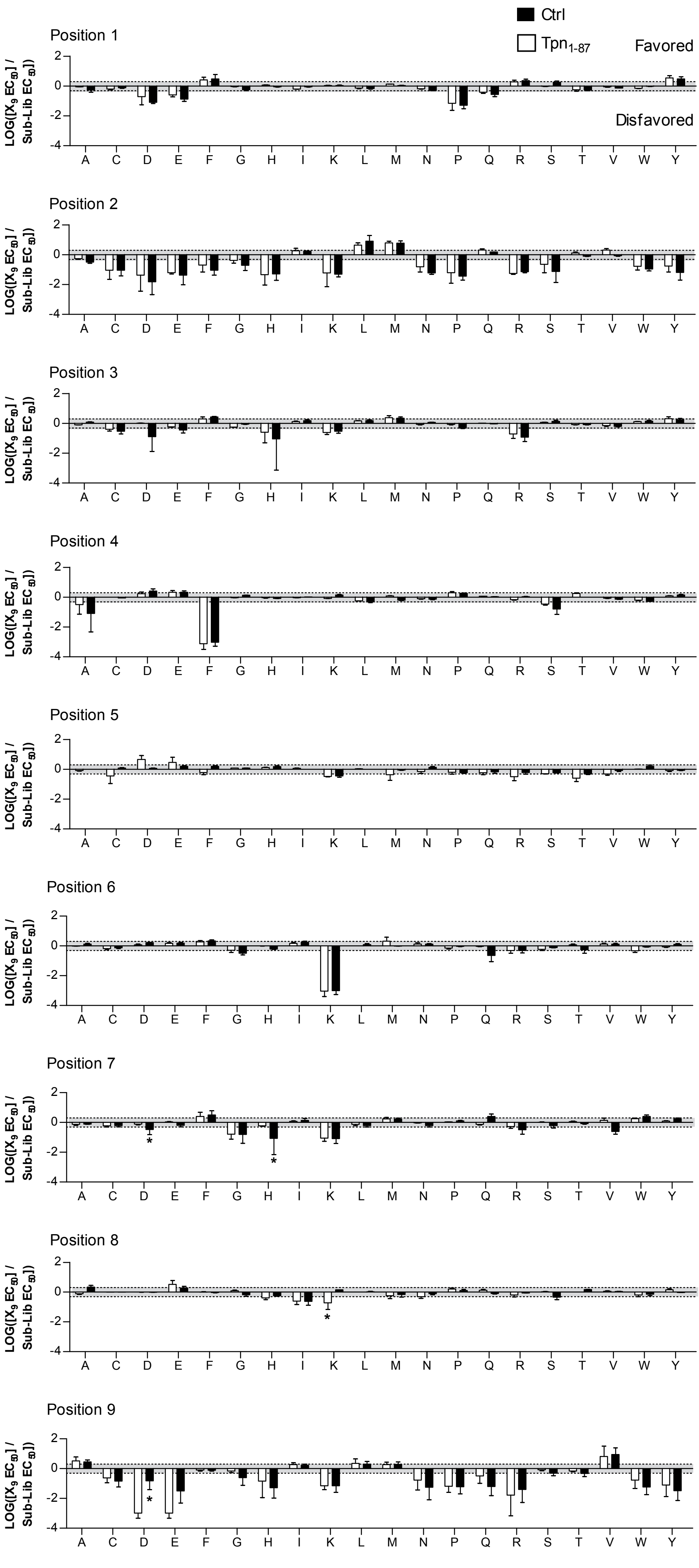


Supporting Information Figure 1

$Tpn_{1\text{-}87}$ differentially facilitates folding of peptide-HLA-I complexes.

Fixed concentrations of β_2m and HLA-I heavy chains were mixed with titrated concentrations of peptide in the presence or absence of $Tpn_{1\text{-}87}$. The mixtures were incubated at 18 ˚C for 48 hrs, and folded peptide-HLA-I complexes were detected by the W6/32 monoclonal antibody in a homogenous assay (Harndahl et al, 2009).

Supporting Information Figure 2

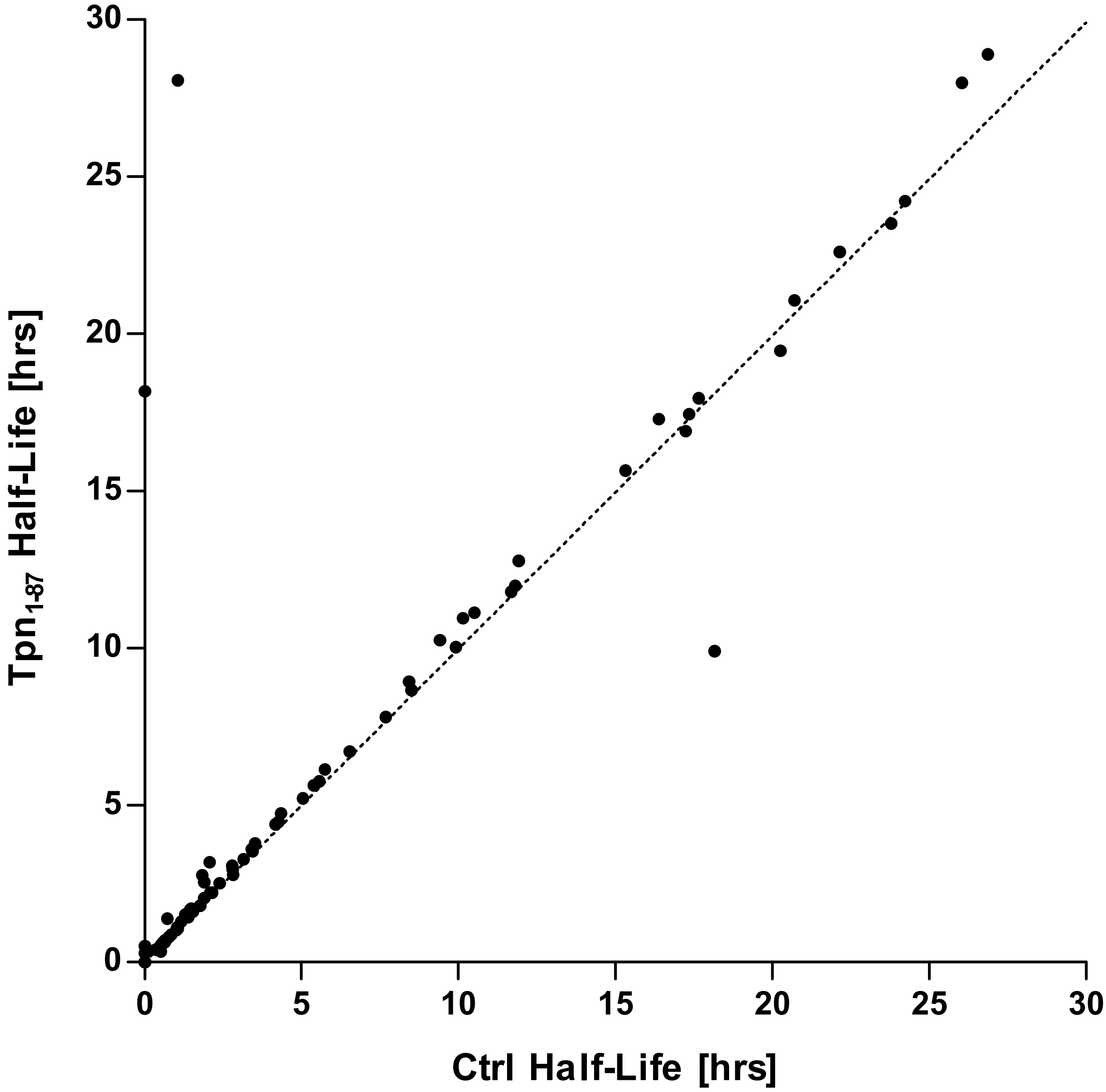


Supporting Information Figure 2

*$Tpn_{1\text{-}87}$ does not grossly alter the peptide binding specificity of HLA-A*02:01; a complete amino acid substitution scan across the entire peptide.*

Fixed concentrations of β_2-microglobulin and HLA-A*02:01 heavy chain were mixed with titrated concentrations of each 9-mer peptide sub-library in the presence or absence of $Tpn_{1\text{-}87}$. The mixture was incubated at 18 °C for 48 hrs, and subsequently folded peptide-HLA-A*02:01 complexes were measured in a biochemical assay. Each sub-library has one amino acid fixed in a position in the 9-mer peptide, and thus for a 9-mer peptide library there are 180 different sub-libraries and a library containing random amino acids at all positions (X_9). Relative binding (RB) values were calculated as the ratio between the affinity for the X_9 library and the affinity of each sub-library. The RB values were normalized to the total sum of 20 for each position in the peptide, and the log values of the normalized RB values are shown along the y-axis. The amino acid substitutions are shown along the x-axis. Each experiment was done four times, and the average and standard deviations are shown in the graph. Significant differences between RB values in the presence and absence of $Tpn_{1\text{-}87}$ were determined by unpaired t tests, and are marked with an asterisk. Only differences between RB values outside the grey area are marked, since only these are relevant for the peptide binding specificity.

Supporting Information Figure 3

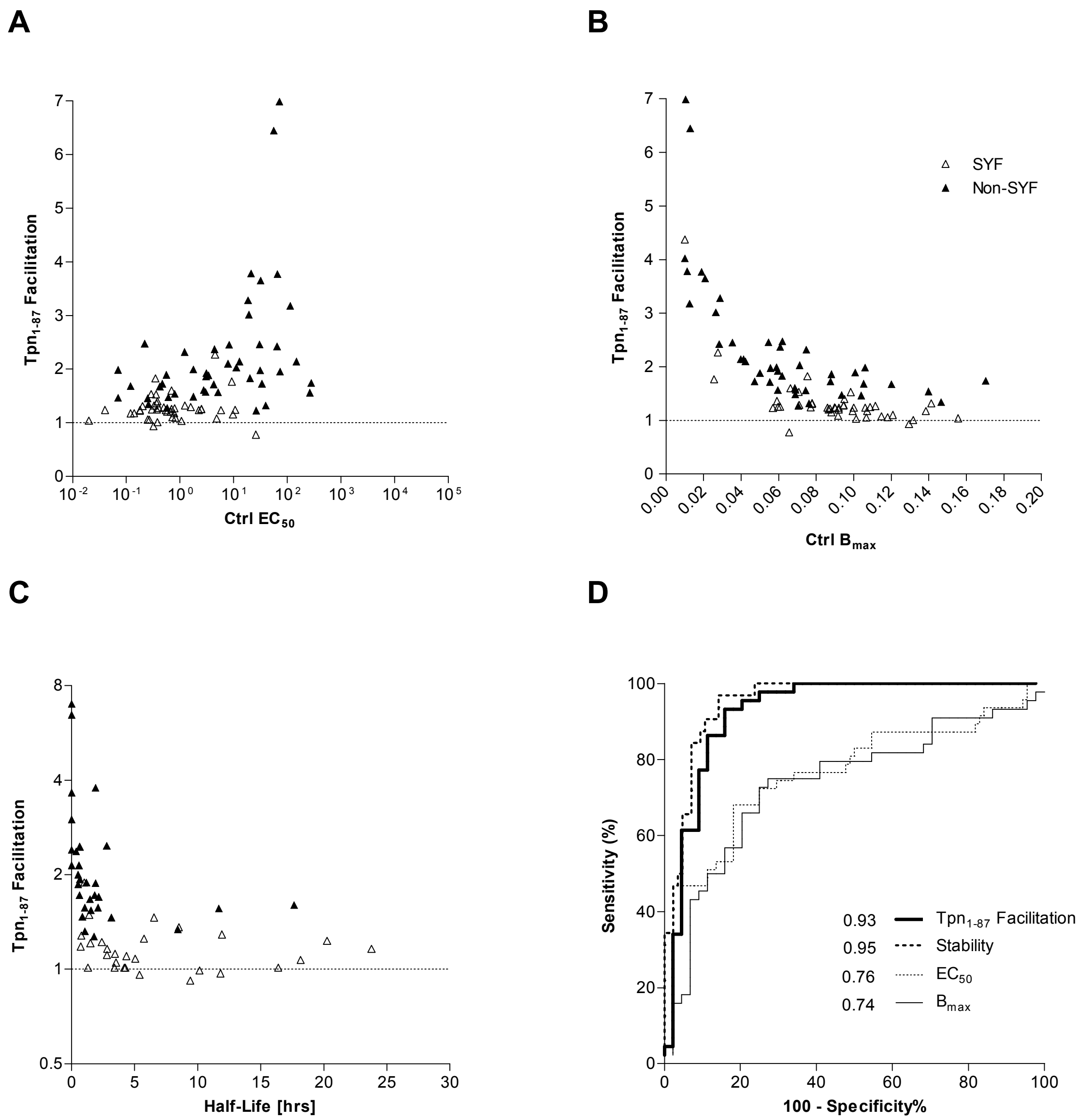


Supporting Information Figure 3

*Tpn_{1-87} does not alter the stability of the peptide-HLA-A*02:01 complex*.

Peptide and HLA-A*02:01 heavy chain in the absence (Ctrl) or presence (Tpn_{1-87}) of Tpn_{1-87} were incubated with ^{125}I labeled β_2-microglobulin. After 18 hrs of incubation at 18 ˚C, an excess amount of cold β_2-microglobulin was added, and the reaction was incubated at 37 ˚C, and read at regular intervals. The stabilities of the peptide-HLA-I complexes were calculated as half-lifes from the read-out using one-phase dissociation kinetics.

Supporting Information Figure 4



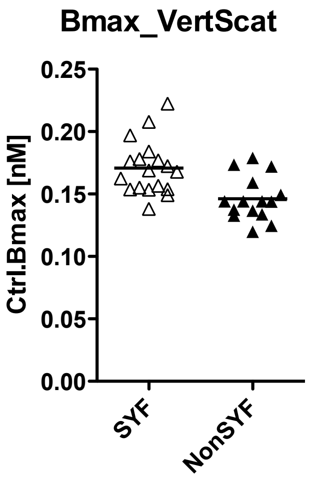
Supporting Information Figure 4

*$Tpn_{1\text{-}87}$ facilitates folding of peptide-HLA-A*02:01 complexes, and the facilitation inversely correlates with the intrinsic stability of the peptide-HLA-A*02:01 complex.*

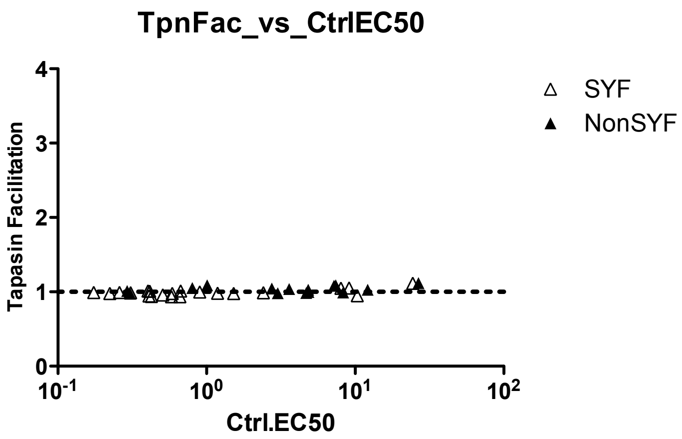
The $Tpn_{1\text{-}87}$ facilitation was analyzed using the same set of peptides tested on HLA-A*02:01 in Fig. 1. (**A**) The $Tpn_{1\text{-}87}$ facilitation was plotted against Ctrl EC_{50}, the affinity measured in the absence of $Tpn_{1\text{-}87}$. (**B**) The $Tpn_{1\text{-}87}$ facilitation was plotted against the Ctrl B_{max}, the saturation plateau measured in the absence of $Tpn_{1\text{-}87}$. (**C**) The $Tpn_{1\text{-}87}$ facilitation was plotted against the intrinsic stability of the peptide-HLA-A*02:01 complex. (**D**) A ROC analysis was used on all four parameters (EC_{50}, B_{max}, stability and $Tpn_{1\text{-}87}$ facilitation) to determine which parameter best correlates with the folding facilitation. To determine whether significant differences exist between the areas under the ROC curves a jack-knife analysis was performed on the ROC areas. A student's t test was used to determine statistical significant differences between the parameters tested.

Supporting Information Figure 5

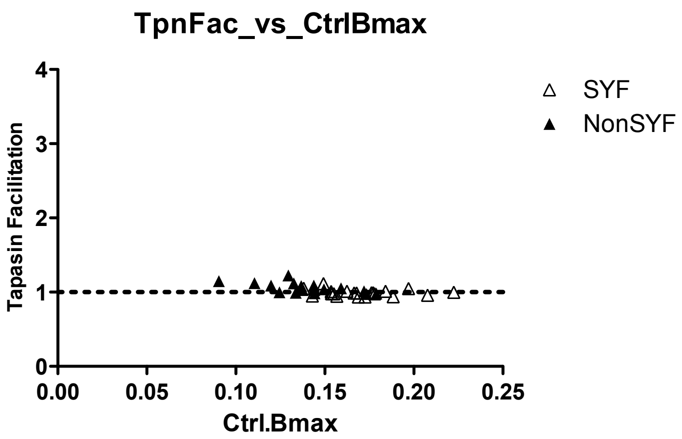
A



B



C



Supporting Information Figure 5

*$Tpn_{1\text{-}87}$ does not facilitate folding of HLA-A*02:01-T134K.*

Twenty-one SYFPEITHI and 21 non-SYFPEITHI peptides were paired, based upon affinity to HLA-A*02:01. Fixed concentrations of β_2m and HLA-A*02:01-T134K HC were mixed with various concentrations of peptide in the presence or absence of $Tpn_{1\text{-}87}$. (**A**) The B_{max} values for the SYFPEITHI and non-SYFPEITHI peptides in the absence of $Tpn_{1\text{-}87}$ were plotted in a vertical scatter diagram. (**B**) The peptide affinities (EC_{50}) to the HLA-I molecules were calculated as the peptide concentration required to reach the half-saturation point on the sigmoidal dose-response curve. The $Tpn_{1\text{-}87}$ facilitation was plotted against EC_{50}. *(*(**C**) The $Tpn_{1\text{-}87}$ facilitation was plotted against the saturation plateau, B_{max}.

Supporting Information Table 3

SYFPEITHI peptides			
Batch Number	Sequence	Pos 2	C-term
4179	YLEPGPVTA	L	A
4183	YLNKIQNSL	L	L
4184	ALSNLEVKL	L	L
4185	KVAELVHFL	V	L
4186	TLNAWVKVV	L	V
4187	SLSRFSWGA	L	A
4189	FLWGPRAYA	L	A
4190	ALFDGDPHL	L	L
4193	HLIDYLVTS	L	S
4194	VLVKSPNHV	L	V
4196	ILDKKVEKV	L	V
4197	HLGNVKYLV	L	V
4198	GLHCYEQLV	L	V
4199	GLYDGMEHL	L	L
4200	RLMKQDFSV	L	V
4201	YMNGTMSQV	M	V
4202	SLLPAIVEL	L	L
4203	FLDGNELTL	L	L
4204	TLWVDPYEV	L	V
4205	ILDTGTIQL	L	L
4207	VLFSSDFRI	L	I
4211	RLNMFTPYI	L	I
4213	ILMEHIHKL	L	L
4216	QVCERIPTI	V	I
4217	HLSTAFARV	L	V
4220	RLPRIFCSC	L	C
4221	SLDQSVVEL	L	L
4223	KIFGSLAFL	I	L
4224	LLMDCSGSI	L	I
4226	MLGTHTMEV	L	V
4227	LLIENVASL	L	L
4228	MVDGTLLLL	V	L
4229	LLGATCMFV	L	V
4231	KLVANNTRL	L	L
4232	RLTRFLSRV	L	V
4233	GILGFVFTL	I	L
4234	KLLEPVLLL	L	L
4235	YLSGANLNL	L	L
4239	ILAKFLHWL	L	L
4244	RLLQETELV	L	V
4245	SLLLELEEV	L	V
4251	ALCRWGLLL	L	L
4253	FLWGPRALV	L	V
4254	ILFGHENRV	L	V
Suboptimal anchors		5	5
		44	44
		11%	**11%**

Non-SYFPEITHI peptides			
Batch Number	Sequence	Pos 2	C-term
6411	ILSPHNVVT	L	T
6436	AMHYIRHRA	M	A
6438	KIFEYGFTF	I	F
6945	ALWEIQQVV	L	V
8711	FTFDNSKFV	T	V
8712	MMFDAMGAL	M	L
8718	KMVGTVQRV	M	V
9585	KLAEIFQPF	L	F
9929	VVYKEAKIK	V	K
10042	WLKEKHEEL	L	L
10051	FGKWRPVQL	G	L
10055	HLKRTILAL	L	L
10179	TMLYNKMEF	M	F
10570	IFRRDQIWF	F	F
10621	YTLNNGVAM	T	M
10851	GLAGGAATA	L	A
10886	KVRGRLLAL	V	L
11076	QLAFTYCQV	L	V
11083	WMDMWESPM	M	M
11091	ALEEGRKYV	L	V
11250	YLPEDSDIL	L	L
11503	ELADQLIHL	L	L
11514	NVWATHACV	V	V
11689	LLLGGTSEI	L	I
11866	DLYDYITRI	L	I
13533	FLYGWLFIL	L	L
14600	PLNEGIMAV	L	V
16321	RSLYNTVAVL	S	L
16556	SGFGGETPV	G	V
16682	SILEYAKSI	I	I
16683	KMYEYVFKG	M	G
16782	RSLYNTIATL	S	L
16784	RSLFNTVAVL	S	L
17803	SLFGAAVSL	L	L
17917	YLLLTTNGT	L	T
17920	SIFFDYMAI	I	I
18458	YQIEGAWRA	Q	A
18475	FQWHEAMFL	Q	L
18928	TLKPGTMSV	L	V
18949	YIITCCLFA	I	A
20123	GLYSLPHDL	L	L
20126	RQPLNIQAI	Q	I
20139	SVFSRPLPL	V	L
20142	GIYGAVIPL	I	L
20154	FLFLYWPHY	L	Y
20163	SMFYGIFPS	M	S
20165	WFMTWQPNI	F	I
Suboptimal anchors		21	16
		47	47
		45%	**34%**