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Supplementary Information for

Utilizing TAPBPR to promote exogenous peptide loading onto cell surface MHC I molecules

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Supplementary Figures and Tables

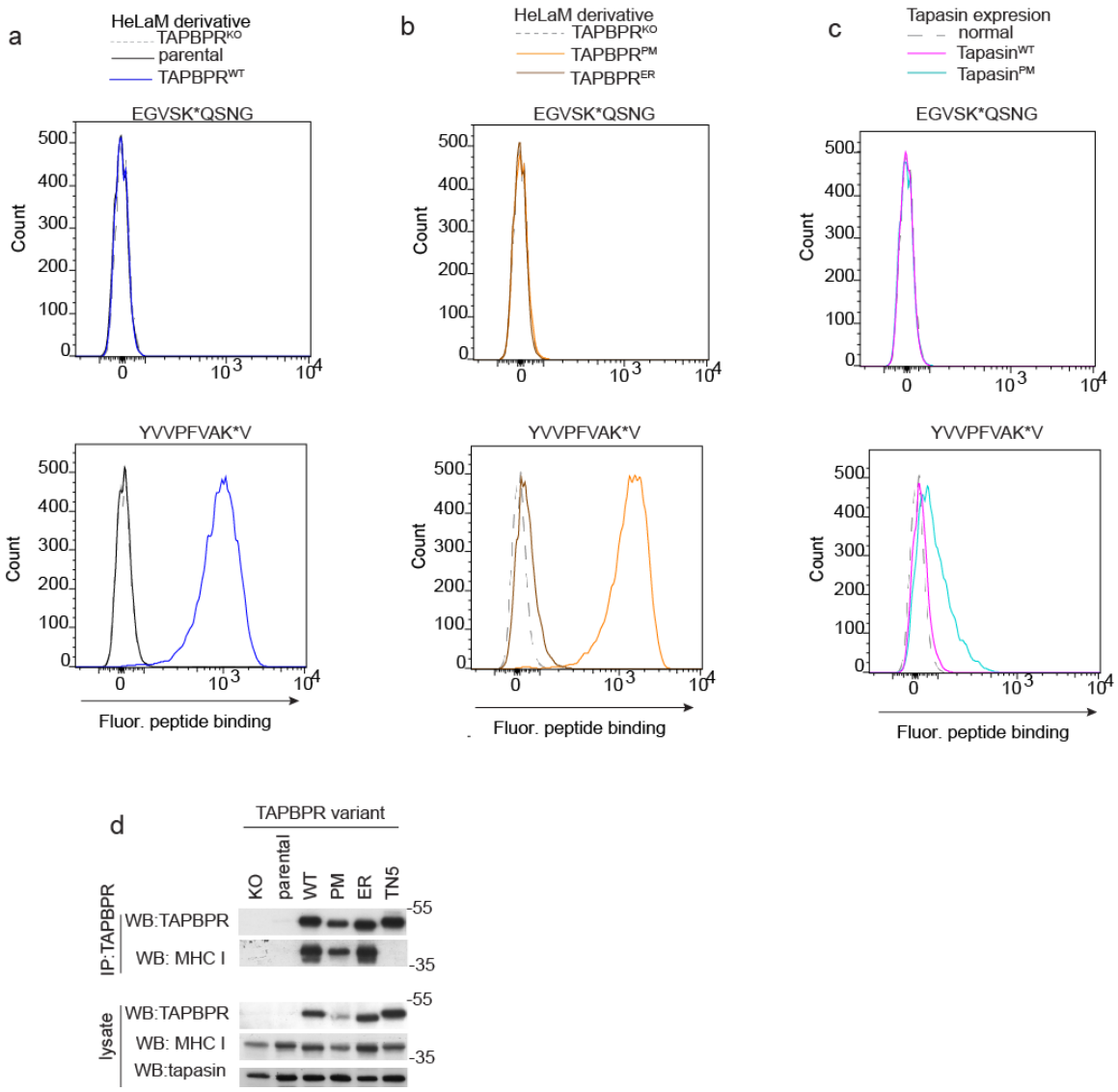

Fig. S1. Supporting data for figure 1.
Typical peptide binding observed when (**a**) HeLaM cells and HeLaM-TAPBPR^{KO} -/+ TAPBPR^{WT} transduction, (**b**) HeLaM-TAPBPR^{KO} -/+ TAPBPR^{PM} or TAPBPR^{ER} transduction or (**c**) HeLaM-TAPBPR^{KO} -/+ tapasin^{WT} or tapasin^{PM} transduction where incubated with 10 nM EGVSK*QSNG or YVVPFVAK*V for 15 min at 37^{o}C. (**d**) Classical co-immunoprecipitation of TAPBPR with MHC I from Triton X-100 lysates derived from the panel of TAPBPR expressing cell lines. TAPBPR immunoprecipitation was performed using the TAPBPR-specific mAb PeTe4, followed by Western blot analysis for TAPBPR and MHC I (using HC10). Western blotting of lysates was also performed and blotting for tapasin was included as a loading control.

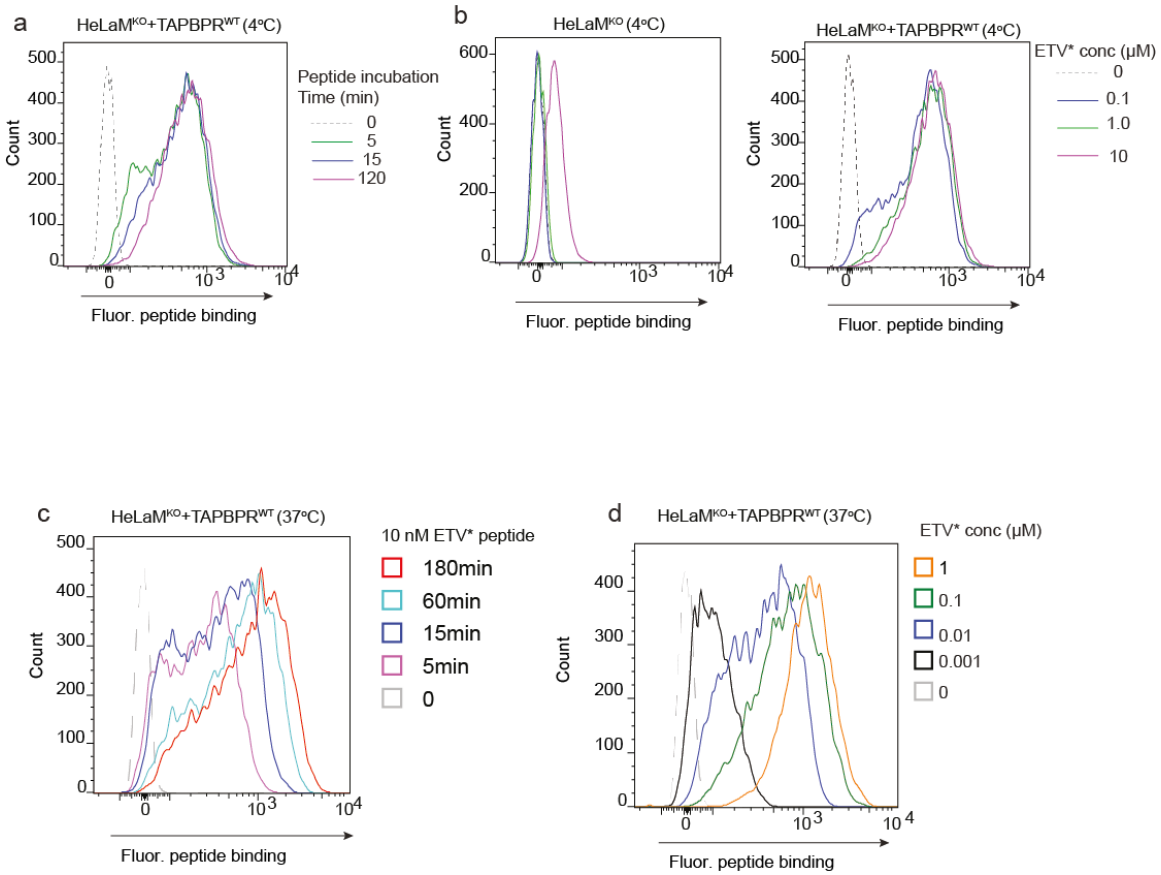

Fig. S2. **Time course and dose response data on cells expressing surface TAPBPR at 4°C and 37°C**
Binding of ETVSK*QSNV to IFN-γ treated HeLaM-TAPBPR^{KO} -/+ TAPBPR^{WT} cells at 4°C when (**a**) cells were incubated with 100 nM ETVSK*QSNV for 0-120 min or (**b**) cells were incubated with 0-10 µM ETVSK*QSNV from 60 min. Histogram displaying the typical fluorescent peptide binding observed on HeLaM-TAPBPR^{KO}+ TAPBPR^{WT} cells when (**c**) incubated with 10 nM ETVSK*QSNV from 0-180 min at 37°C and (**d**) incubated with increasing concentration of ETVSK*QSNV (0-1 µM) for 15 min at 37°C.

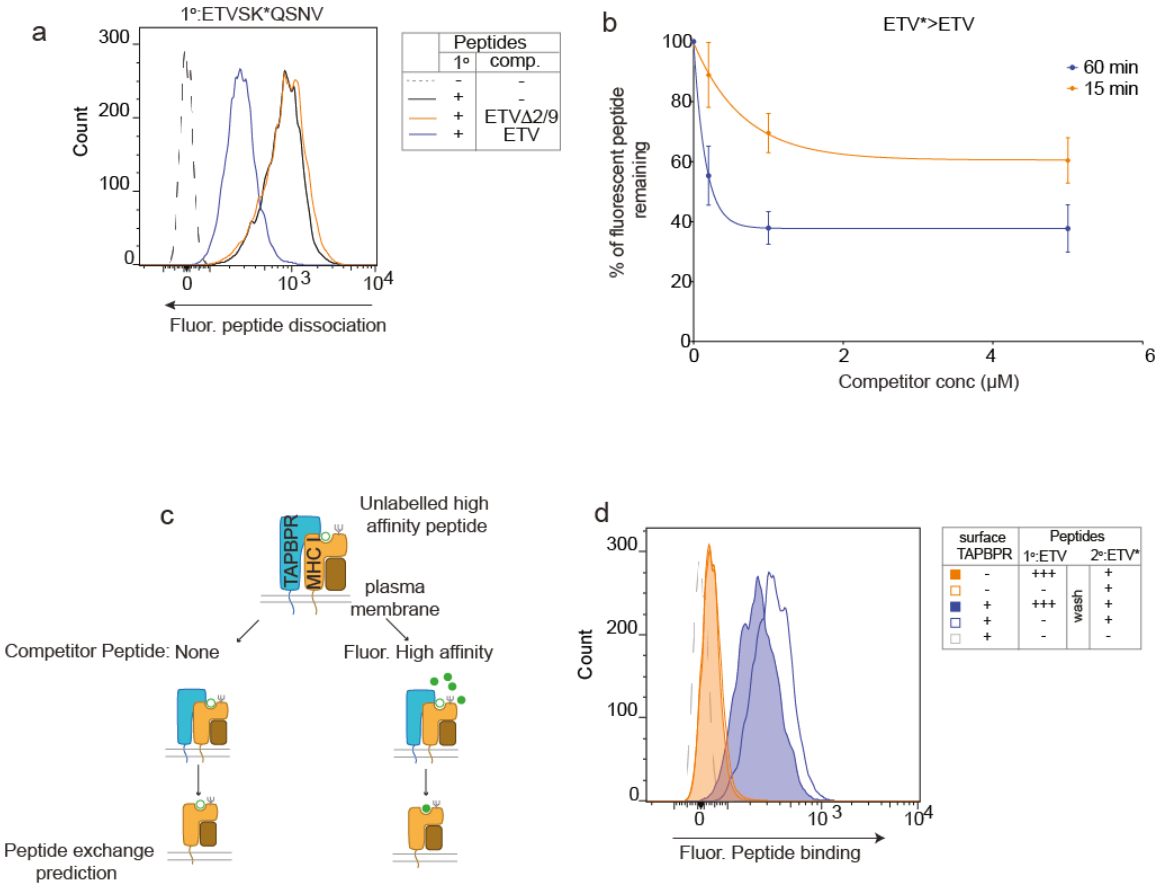

Fig. S3. TAPBPR functions as a peptide exchange catalysts at 4°C. (**a** & **b**) IFN-γ treated HeLa^{KO}TAPBPR^{WT} cells were cooled to 4°C, then incubated with 1 µM ETVSK*QSNV for 60 min at 4°C, then washed to remove unbound peptide. Dissociation of the fluorescent peptides were subsequently monitored in the absence or presence of increasing concentrations of the unlabelled competitor peptides ETVSEQSNV (ETV) or EGVSEQSNG (ETVΔ2/9) for 15 min or 60 min at 4°C. (**a**) Histograms show the typical dissociation of fluorescent peptide observed following incubation with 1 µM competitor peptide. (**b**) Line graphs show the percentage of fluorescent peptide remaining -/+ SD following treatment with increasing concentrations of unlabelled peptide from three independent experiments. (**c**) Schematic representation of the experimental work flow used to determine peptide exchange by surface TAPBPR using an alternative assay (used in **d**) in which any peptide receptive surface HLA-A*68:02 molecules were first occupied with unlabelled high affinity peptide, followed by measuring the ability of fluorescent high affinity peptide to bind to cells. (**d**) IFN-γ treated HeLaM^{KO} cell -/+ TAPBPR^{WT} cells (depicted as -/+ surface TAPBPR in key) were cooled to 4°C, then incubated without or with 5 µM unlabelled peptide ETVSEQSNV (-/+++ 1°:ETV in key) for 60 min at 4°C to occupy any peptide receptive MHC I. Cells were then washed to remove any unbound peptide, thus removing any excess unlabelled peptide from the system. Histograms show the typical fluorescent peptide binding when these cells were subsequently incubated with 1 µM fluorescent ETVSK*QSNV peptide for 60 min at 4°C.

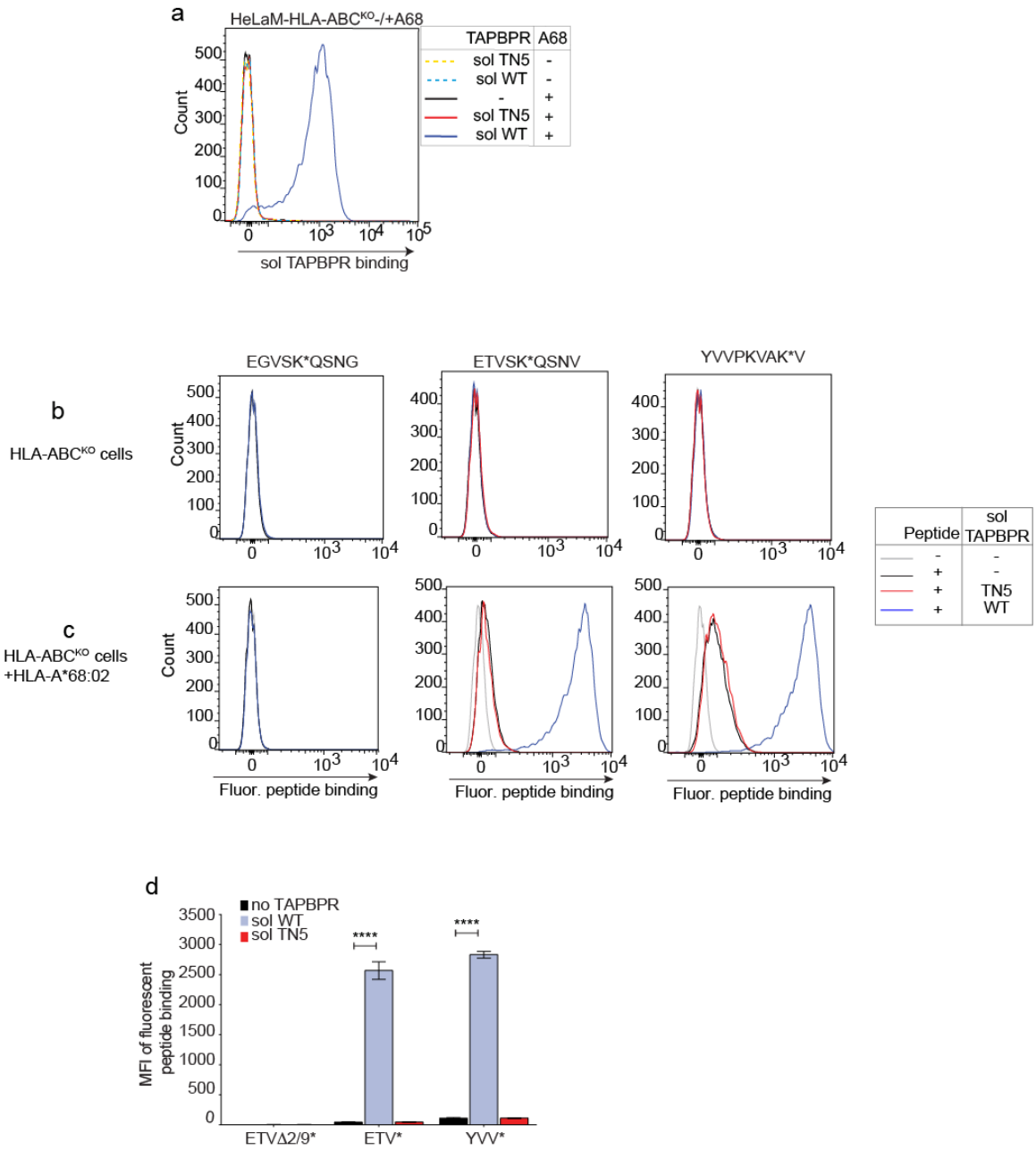

Fig. S4. The binding of specific peptides to cells is dependent on HLA-A*68:02 expression
IFN-γ treated HeLaM-HLA-ABC^{KO} -/+ reconstitution with HLA-A*68:02 were incubated -/+ 100 nM soluble TAPBPR^{WT} or TAPBPR^{TN5} for 15 min at 37ºC, followed by (**a**) detection of surface bound TAPBPR using PeTe-4, or (**b&c**) incubation -/+ 10 nM EGVSK*QSNG (ETVΔ2/9), ETVSK*QSNV (ETV*) or YVVPFVAK*V (YVV*) for 15 min at 37ºC. (**d**) Bar charts shows the MFI of fluorescent peptide binding -/+ SD from three independent experiments.

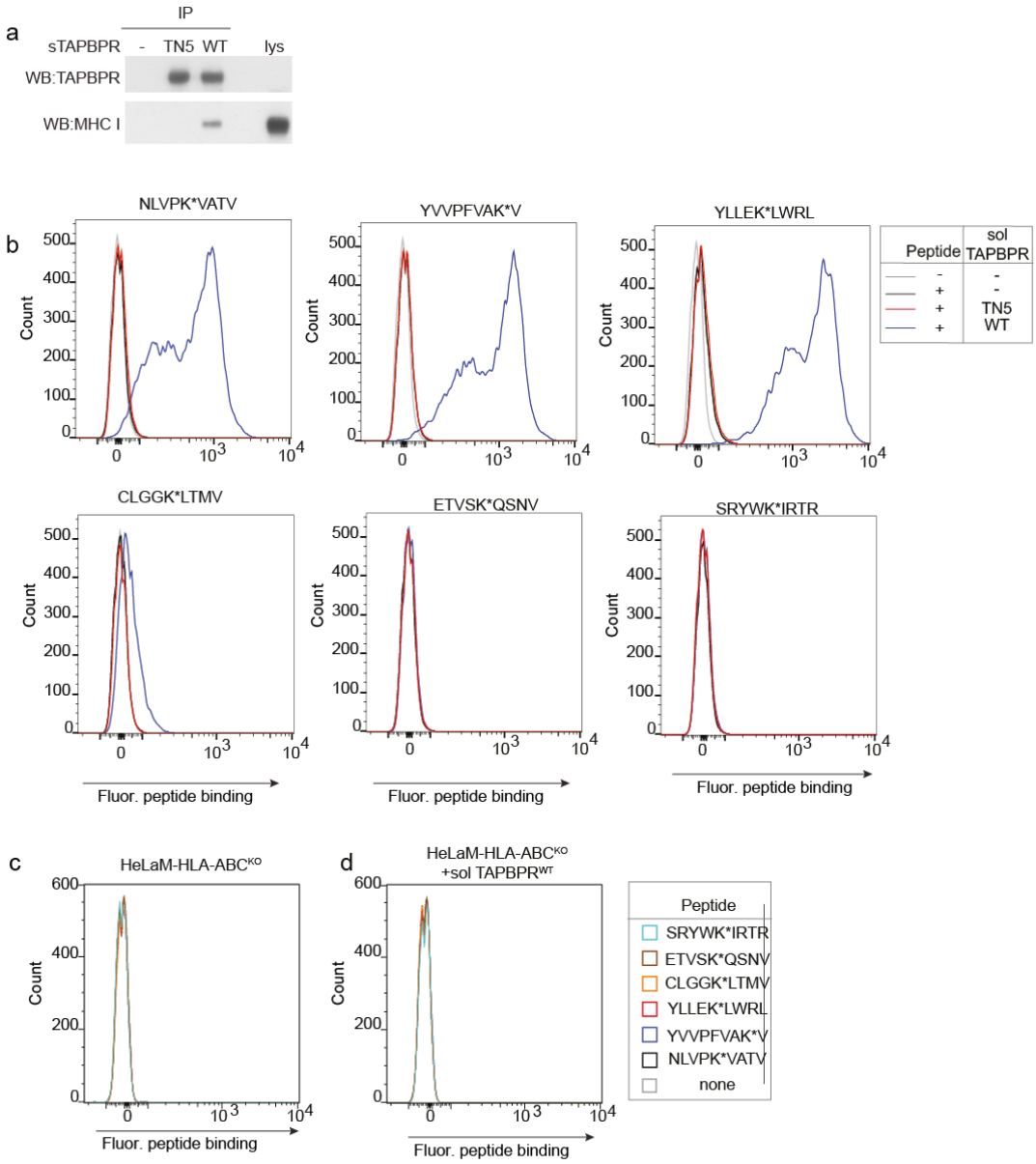

Fig. S5. The binding of specific peptides to cells is dependent on HLA-A*02:01

(**a**) TAPBPR pulldowns on precleared lysates from IFNγ treated HeLaM-HLA-ABC^{KO} cells expressing HLA-A2 incubated -/+ 5 µg soluble TAPBPR^{TN5} or TAPBPR^{WT}. Pulldowns were performed using the TAPBPR specific mAb PeTe4 and protein A sepharose beads. Western blot analysis was performed for TAPBPR-his and MHC I. (**b**) IFN-γ treated HeLaM-HLA-ABC^{KO} expressing HLA-A*02:01 were incubated -/+ 1 µM soluble TAPBPR^{WT} or TAPBPR^{TN5} for 15 min at 37^{o}C, followed by incubation -/+ 10 nM NLVPK*VATV, YVVPFVAK*V, YLLEK*LWRL, CLGGK*LTMV, ETVSK*QSNV or SRYWK*IRTR for 60 min. The histograms shown are representative of the typical results used to generate bar graph in Fig. 3G. (**c**) HeLaM-HLA-ABC^{KO} cells and (**d**) HeLaM-HLA-ABC^{KO} cells incubated with 1µM soluble TAPBPR^{WT} were incubated with 10 nM NLVPK*VATV, YVVPFVAK*V, YLLEK*LWRL, CLGGK*LTMV, ETVSK*QSNV or SRYWK*IRTR for 60 min. No peptide binding was observed to these cell lines that lacked HLA expression.

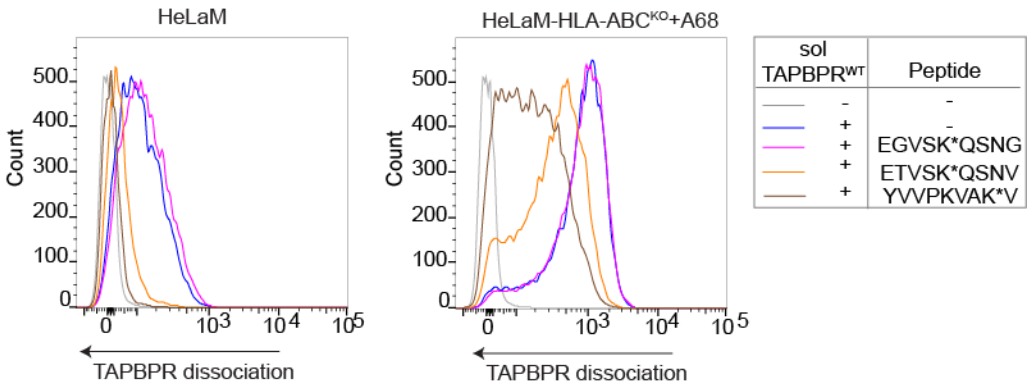

Fig. S6. Soluble TAPBPR dissociates from cells upon high affinity peptide binding

IFN-γ treated HeLaM cell and HeLa-HLA-ABC^{KO} reconstituted with HLA-A*68:02 were incubated -/+ 100 nM soluble TAPBPR^{WT} for 15 min at 37ºC, followed by incubation with -/+ 10 nM EGVSK*QSNG, ETVSK*QSNV or YVVPFVAK*V for 15 min at 37ºC. Subsequently, the amount of TAPBPR remaining on the cell surface was detected by staining with the TAPBPR specific mAb PeTe4.

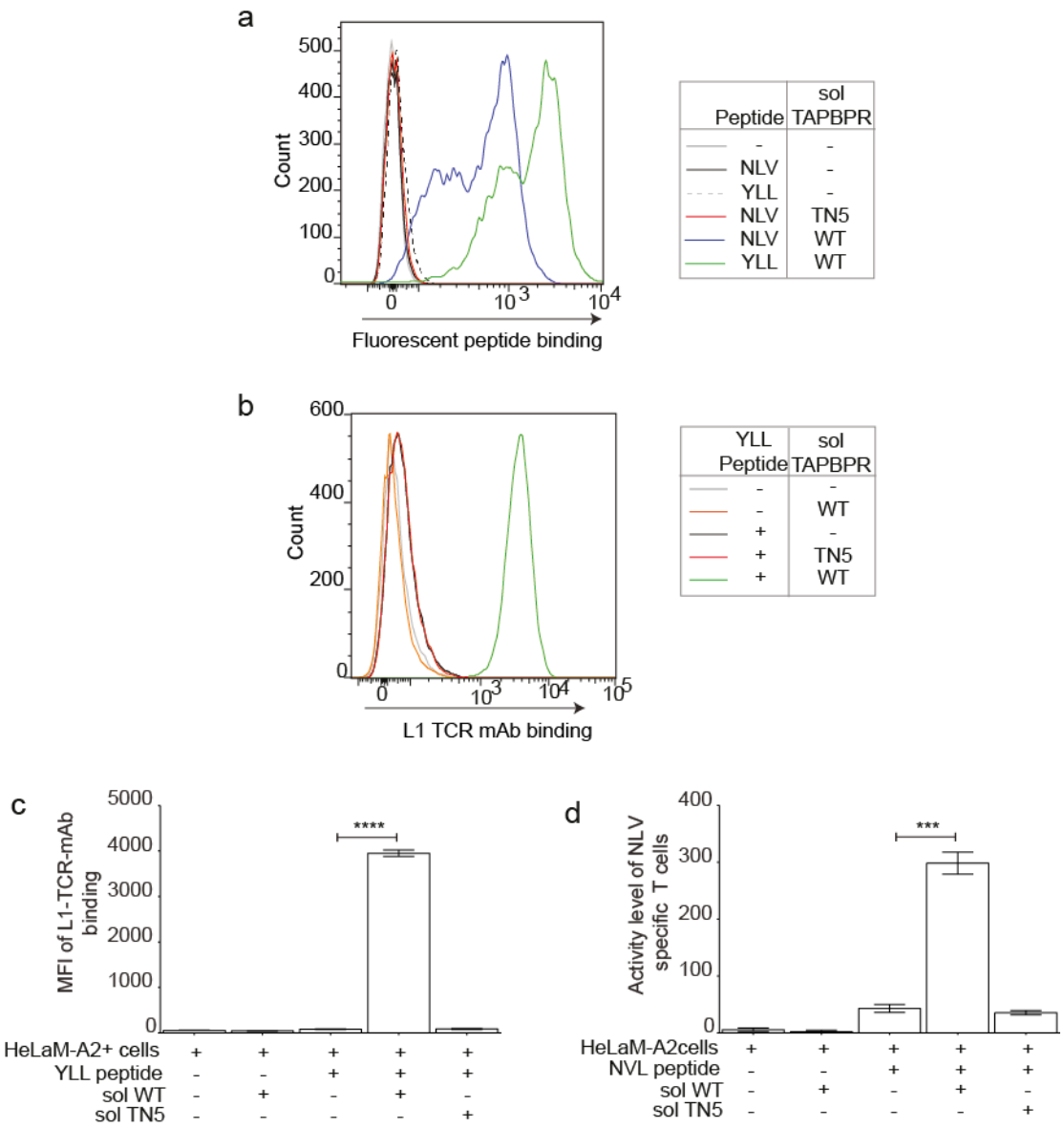

Fig. S7. Peptide loading and TCR recognition of HLA-A2 molecules expressed HeLaM cells.

HeLaM-HLA-ABC^{KO} cells reconstituted with HLA-A*02:01 were incubated -/+ 1 µM soluble TAPBPR^{WT} or TAPBPR^{TN5} for 15 min at 37°C followed by 60 min treatment -/+ 10 nM (**a**) NLVPK*VATV or YLLEK*LWRL or (**b**) YLLEMLWRL (YLL) followed by staining with the TCR-like mAb L1 specific for YLLEMLWRL/HLA-A2 complexes. (**c**) The MFI of L1 binding to HeLaM-HLA-ABC^{KO} cells -/+ SD from three independent experiments. (**d**) Bar graphs show T cell activity measured by IFN-γ secretion in fluorospot assays of a HLA-A2 restricted NLVPMVATV specific CD8+ T cell line when incubated with HeLaM-HLA-ABC^{KO} target cells as treated in **a** with the exception that non-fluorescent NLVPMVATV peptide at 100 pM was used. Results are from triplicate wells representative of two independent experiments. Error bars -/+ SD. Note: In **a & d** IFNγ treated cells were used. ***P ≤ 0.001, ****P≤0.0001 using unpaired two-tailed t-test.

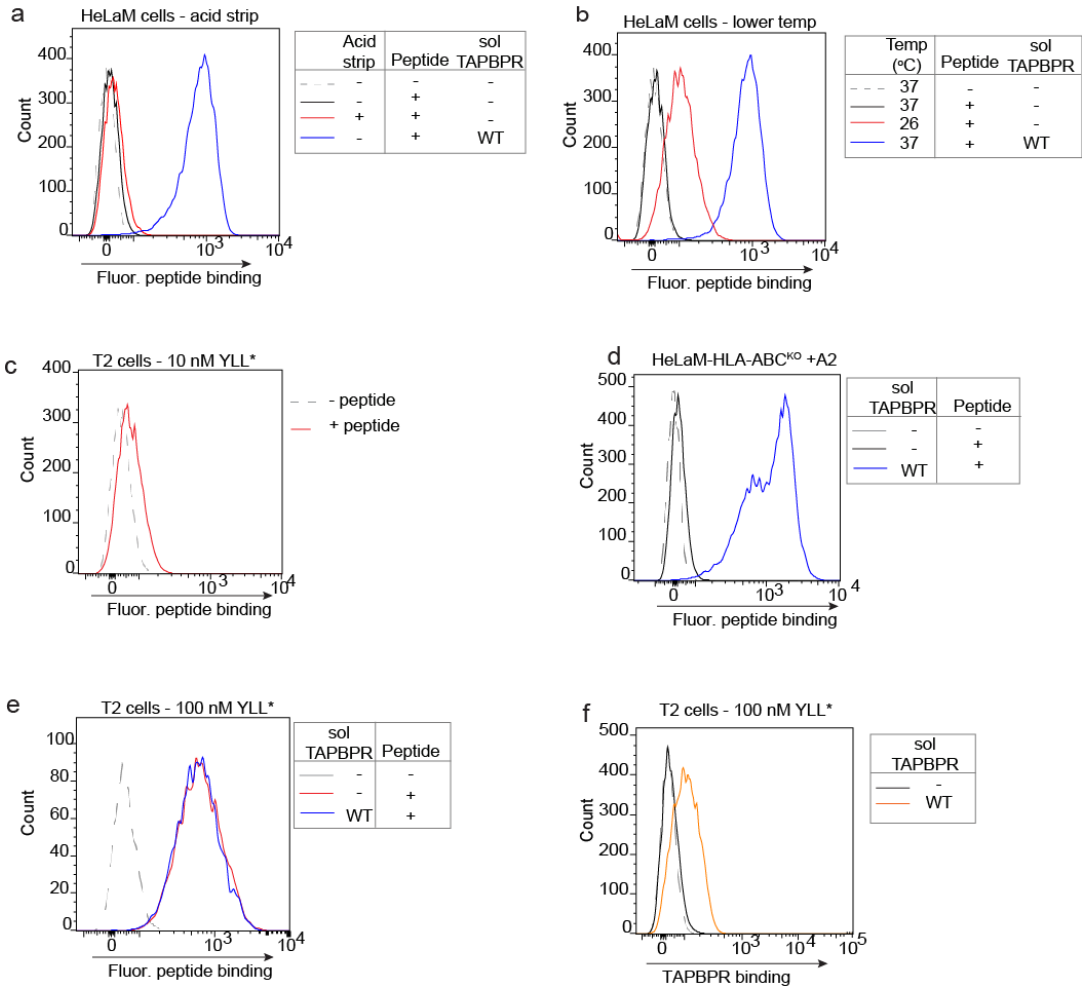

Fig. S8. Comparison of soluble TAPBPR-mediated peptide loading to classical peptide loading methods. (**a**) IFNγ treated HeLaM cells were acid stripped by incubating cells in a citric acid based stripping buffer (pH 4.5) for 5 mins followed by washing in PBS. Acid stripped cells (red line) and their non-stripped counterparts were subsequently incubated without or with 10 nM ETVSK*QSNV for 15 min at 37ºC. (**b**) IFNγ treated HeLaM cells were either incubated at 26ºC overnight (red line) or maintained at 37ºC. Cell were subsequently incubated without or with 10 nM ETVSK*QSNV for 15 min at the indicated temperature. For both **a** & **b** IFNγ treated HeLaM cells incubated with soluble TAPBPR^{WT} for 15 min followed by 10 nM ETVSK*QSNV for 15 min at 37ºC is included for comparison (blue line). In **a,** only extremely low levels of exogenous peptide binding was observed to acid stripped cells while in **b**, a notable increase in exogenous peptide binding was observed to cells upon incubation at 26ºC. However, the level of exogenous peptide binding was >8 fold higher by soluble TAPBPR compared to incubating cells at low temperature. (**c**) Histogram showing the level of fluorescent peptide binding to T2 (TAP negative) when cells were incubated -/+ 10 nM YLLEK*LWRL (HLA-A2 binding peptide). For comparison, (**d**) shows the level of fluorescent peptide binding to HeLaM-HLA-ABC^{KO} HLA-A2 expressing cells incubated -/+ 1 µM soluble TAPBPR followed by treatment -/+ 10 nM YLLEK*LWRL (HLA-A2 binding peptide). (**e**) Histogram show the level of fluorescent peptide binding to T2 cells incubated -/+ 1 µM soluble TAPBPR^{WT}, followed treatment -/+ 100 nM YLLEK*LWRL (10 fold higher than in **c**). (**f**) Histogram shows the level of TAPBPR binding to T2 cells incubated -/+ 1 µM soluble TAPBPR^{WT}. Staining with an isotype control antibody is included (grey dashed line). Although soluble TAPBPR binding was observed to T2 cells (**f**), this did not change the amount of peptide receptive HLA-A2 molecules on the surface of these cells. This supports the concept that TAPBPR promotes exogenous peptide binding to cells by making MHC I molecules peptide receptive (i.e. by first promoting dissociation of peptide from pMHC complexes), rather than facilitating peptide binding to pre-existing peptide receptive pMHC molecules.

Table S1. Primers used for cloning and chimeric protein generation

Primer name	Primer sequence
TAPBPR^{WT}-BamHI-for	5′-GCGCGGATCCAGCAGCCTCCATGGGCACACAGGAGGGC-3′3′
TAPBPR^{WT}-NotI-rev	5′-GCGCGCGGCCGCTCAGCTGGGCTGGCTTACA-3′
TAPBPR^{PM}-for	5′-GATGTTCCTGGGGCTTCAGAGACGAAGACGTGTTTGCAAATGTCC-3′
TAPBPR^{PM}-rev	5′-GGACATTTGCAAACACGTCTTCGTCTCTGAAGCCCCAGGAACATC-3′
tapasin^{WT}-BamHI-for	5′-GCGCGGATCCCGCAGCGCCATGAAGTCCCTGTCTCTGCTCC-3′
tapasin^{WT}- NotI-rev	5′-GCGCGCGGCCGCTCACTCTGCTTTCTTCTTTGAATCCTTG-3′
tapasin^{PM}-for	5'-CTTCAAGGCACTGGGCTGGCGAAGACGTGTTTGCAAATGTCC-3′
tapasin^{PM}-rev	5′-GGACATTTGCAAACACGTCTTCGCCAGCCCAGTGCCTTGAAG-3′
TAPBPR^{ER}-for	5′-CCTGGAGGTAGCAGGTCTTTCAGCCTTGGGAGTCATCTTTGC-3′
TAPBPR^{ER}-rev	5′-GTTCTCAAGGGAGGGCCCTGTTCTCCGCTCTGGTGGG-3′
CD8 tail-NotI-rev	5′-GCGCGCGGCCGCTTAGACGTATCTCGCCGAAAGGC-3′
TAPBPR-soluble-for	5′- GCGCGCTAGCCACCATGGGCACACAGGAGGGC – 3′
TAPBPR-soluble-rev	5′-GCGCGCGGCCGCTCATCAGTGATGGTGATGGTGGTGTCTCCGCTCTGGTGGGACA-3′