SUPPLEMENTAL DATA

Insights into MHC Class I Peptide Loading from the Structure of the Tapasin-ERp57 Thiol Oxidoreductase Heterodimer

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Table S1
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Table S1. Contacts in the tapasin/ERp57 complex*							
between tapasin and ERp57				between ERp57 and tapasin			
residue/ atom in tapasin	residue/ atom in ERp57	distance (Å) in 1st complex	distance (Å) in 2nd complex	residue/ atom in ERp57	residue/ atom in tapasin	distance (Å) in 1st complex	distance (Å) in 2nd complex
Val77 Cγ1	Trp56 O	4.0	4.4	Ala54 Cβ	Asp100 Oδ1	3.9	3.6
Pro78 Cγ	Trp56 Cζ3	3.7	3.7	Trp56 O	Val77 Cγ1	4.0	4.4
Gln93 Nε2	His59 Nε2	3.4	3.4	Cζ3	Pro78 Cγ	3.7	3.7
Asn94 N	His59 Cε1	3.8	3.3	CH2	Cys95 Cβ	3.6	3.5
Oδ1	Gly99 Cα	3.7	3.9	Cz2	Pro96 O	3.6	3.4
Cβ	Pro101 N	4.4	4.0	Cζ2	Leu99 O	3.8	3.8
Cys95 Cβ	Trp56 CH2	3.6	3.5	Nε1	Asp100 Oδ1	2.9	2.6
Sγ	Cys57 Cα	3.5	3.3	Cys57 Cα	Cys95 Sγ	3.5	3.3
Sγ	Gly58 N	3.8	3.3	Gly58 N	Cys95 Sγ	3.8	3.3
Sγ	His59 Nδ1	4.1	3.8	His59 Nε2	Gln93 Nε2	3.4	3.4
O	Ser98 O	3.8	4.3	Cε1	Asn94 N	3.8	3.3
O	Gly99 Cα	3.1	3.2	Nδ1	Cys95 Sγ	4.1	3.8
N	Tyr100 O	2.8	2.7	Cε1	Tyr257 OH	3.7	3.3
Pro96 O	Trp56 Cζ2	3.6	3.4	Thr86 Cγ2	Asp100 Oδ1	4.3	4.2
C	Ser98 O	3.8	3.7	Thr89 Cγ2	Arg97 NH2	3.5	3.5
Arg97 NH2	Thr89 Cγ2	3.5	3.5	Cys92 Cβ	Arg97 NH2	3.9	3.4
NH2	Cys92 Cβ	3.9	3.4	Asn93 Nδ2	Arg97 NH1	2.5	2.8
NH1	Asn93 Nδ2	2.5	2.8	Val97 O	Arg97 NH1	3.0	2.9
NH1	Val97 O	3.0	2.9	Ser98 O	Cys95 O	3.8	4.3
N	Ser98 O	2.9	2.7		Pro96 C	3.8	3.7
Cγ	Tyr100 OH	3.2	2.7		Arg97 N	2.9	2.7
Leu99 O	Trp 56 Cζ2	3.8	3.8	Gly99 Cα	Asn94 Oδ1	3.7	3.9
Asp100 Oδ1	Ala54 Cβ	3.9	3.6		Cys95 O	3.1	3.2
Oδ1	Trp56 Nε1	2.9	2.6	Try100 O	Cys95 N	2.8	2.7
Oδ1	Thr86 Cγ2	4.3	4.2	OH	Arg97 Cγ	3.2	2.7
Oδ2	Tyr100 OH	2.8	2.5		Asp100 Oδ2	2.8	2.5
Leu198 Cδ1	Trp405 O	3.9	3.7	Pro101 N	Asn94 Cβ	4.4	4.0
Ala199 O	Trp405 Cδ1 Trp405 Cγ	4.1	 4.1	Lys366 Nζ	Gln207 Nε2	2.8	3.3
Ala 200 Cβ	Trp405 Cδ2 Trp405 Cε3	3.6	 3.3	Pro404 O	Ala215 O	4.4	4.1
Pro202 Cβ	Trp405 CH2	4.3	4.4		Val216 Cγ1	3.6	3.4
Asn205 Nδ2	Val477 O	3.2	2.7	Trp405 O	Leu198 Cδ1	3.9	3.7
Oδ1	Phe450 Cζ	3.9	3.9	Cδ1 Cγ	Ala199 O	4.1	 4.1
Gln207 Nε2	Lys366 Nζ	2.8	3.3	Cδ2 Cε3	Ala200 Cβ	3.6	 3.3
Nε2	Thr437 O	4.3	4.5	CH2	Pro202 Cβ	4.3	4.4
Met208 Cγ	Trp405 Nε1	4.4	4.4	Nε1	Met208 Cγ	4.4	4.4
Ala215 O	Pro404 O	4.4	4.1	O	Val 216 Cα	3.2	3.2
Val216 Cγ1	Pro404 O	3.6	3.4		Ala217 N	2.9	2.8
Cα	Trp405 O	3.2	3.2	Cys406 C	Val216 Cβ	4.1	4.1
Cβ	Cys406 C	4.1	4.1		Ala217 O	3.9	3.7
Cγ2	Gly407 Cα	3.6	3.7	Gly407 Cα	Val216 Cγ2	3.6	3.7
Cγ2	Lys410 Cδ	4.0	3.9	N	Ala217 O	2.9	2.6
Ala217 N	Trp405 O	2.9	2.8	C	Phe218 Cε1	3.6	3.3
O	Cys406 C	3.9	3.7	O	Trp237 Cζ3	4.0	4.1
O	Gly407 N	2.9	2.6	His408 Cε1	Phe218 Cδ1	3.6	3.5
Phe218 Cε1	Gly407 C	3.6	3.3	Nε2	NAG O6	3.4	4.1
Cδ1	His408 Cε1	3.6	3.5	Lys410 Cδ	Val216 Cγ2	4.0	3.9
Cε1	Asn411 Oδ1	3.7	3.2	Nζ	Trp237 Cζ2	3.5	3.0
Trp237 Cζ3	Gly407 O	4.0	4.1		Pro239 Cβ	4.1	4.2
Cζ2	Lys410 Nζ	3.5	3.0	Asn411 Oδ1	Phe218 Cε1	3.7	3.2
CH2	Asn411 Nδ2	3.1	3.2	Nδ2	Trp237 CH2	3.1	3.2
Pro239 Cβ	Lys410 Nζ	4.1	4.2	Thr437 O	Gln207 Nε2	4.3	4.5
Tyr257 OH	His59 Ce1	3.7	3.3	Val 447 O	Ans205 Nδ2	3.2	2.7
NAG O6	His408 Nε2	3.4	4.1	Phe450 Cζ	Asn205 Oδ1	3.9	3.9
*Distances were calculated for both tapasin/ERp57 complexes in the $P2_1$ crystal form. Interactions ≤ 4.5 Å present in both of the complexes are listed.							

Supplemental Figures

Figure S1 Superpositions of tapasin-ERp57 complexes show flexibility between domains in both tapasin and ERp57. There were two unique tapasinERp57 complexes in one crystal form (spacegroup $P2_1$, shown in yellow and blue) and one in the second crystal form ($P2_12_12_1$, shown in orange). **(A)** A superposition of the three complexes aligned using residues in the tapasin N-terminal domain shows flexibility between the tapasin N- and C-terminal domains. **(B)** Another view of the same three complexes, related to (A) by a 90 º rotation about a vertical axis. While the tapasin-ERp57 interfaces are similar in all three complexes, there is flexibility between the four thioredoxin-like domains of ERp57. **(C)** Superpositions of ERp57 from the three different complexes, aligned using domain *b* show that there is flexibility between the ERp57 domains. **(D)** Superpositions of domains *bb'* from the tapasin-ERp57 complex structures and from structures of the ERp57 bb' domain alone (light, medium, and dark green; PDB ID 2H8L, see Kozlov et al., 2006).

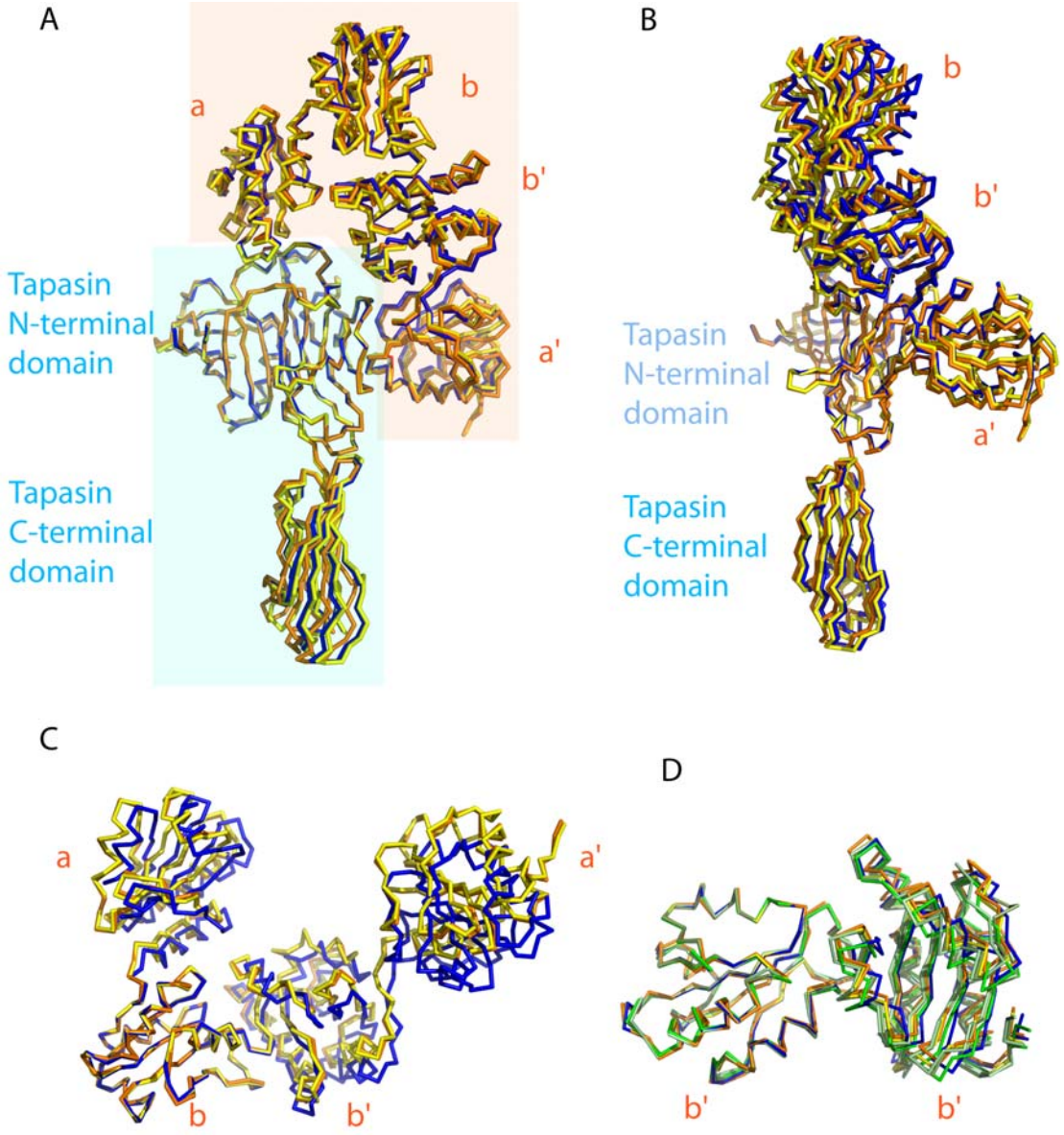


Figure S2 All tapasin mutants form the conjugate with ERp57. Pasta1 immunoprecipitation was performed using extracts of BV-infected Sf21 cells co-expressing the indicated tapasin mutants with C60A ERp57 followed by non-reducing SDS-PAGE and (**A)** Coomassie staining (~2 million cells/lane), (**B)** immunoblotting for the tapasin His_6 tag (~200,000 cells/lane using PentaHis Ab from Qiagen), (**C**) immunoblotting for ERp57 (~200,000 cells/lane using mAb MaP.ERp57). Extract preparation, immunoprecipitations, and immunoblotting were performed as described in the Methods. The predominant species expressed in insect cells was the tapasin-ERp57 conjugate (MW ~ 100 kDa) and a small percentage was isolated as free tapasin (~43 kDa).

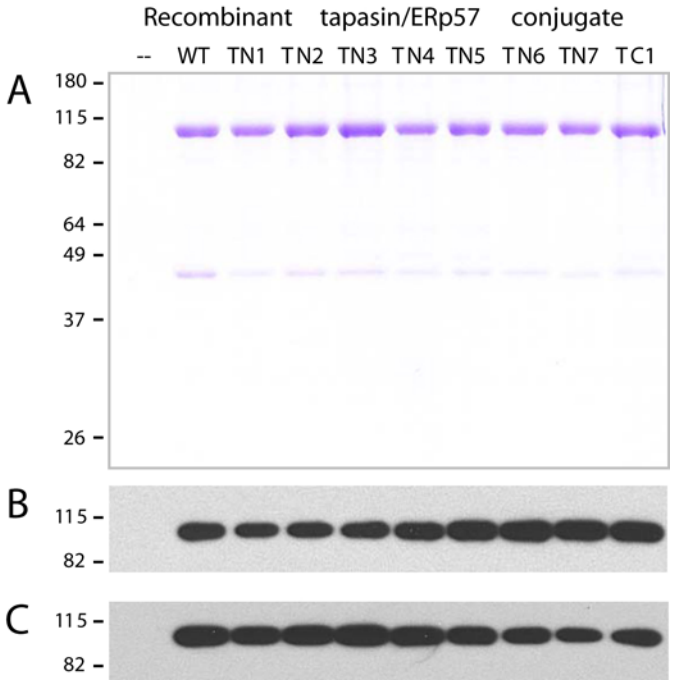
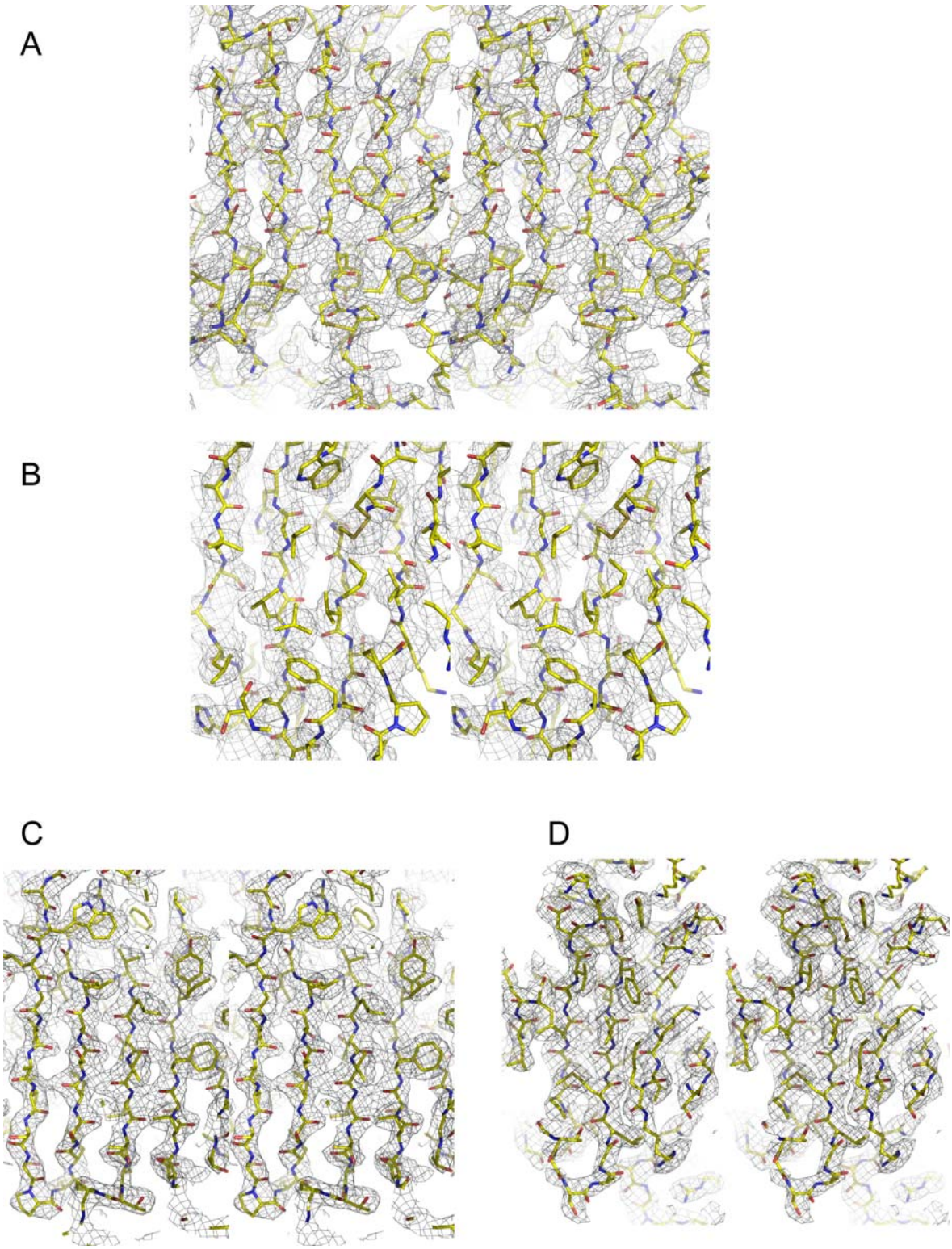


Figure S3 Electron density maps contoured at 1.0 times the rms deviation of the map. (**A)** Experimental phases were combined with phases from a partial model, for ERp57, and used in modeling tapasin. A region in the tapasin N-terminal domain is shown (in stereo). (**B)** A region in the C-terminal domain of tapasin in the same map. (**C)** A 2Fo-Fc composite omit map was calculated in CNS using an annealing temperature of 3000K. A portion of the N-terminal domain of tapasin is shown (in stereo). (**D)** A portion of domain *a* in ERp57 is shown in the same omit map.



SUPPLEMENTAL EXPERIMENTAL PROCEDURES

Cloning, expression, and isolation of recombinant tapasin mutants

Mutants of soluble tapasin were generated using the QuikChange II XL Site-Directed Mutagenesis kit (Stratagene), cloned into the pFastBac Dual vector along with C60A ERp57, and confirmed by sequencing. Recombinant baculovirus was generated and amplified using Sf21 insect cells according to the manufacturer's instructions (Invitrogen). For protein production, small-scale infections were performed for the various mutants at MOI's ranging from 0.2-2 for 64-68 hr. All 8 tapasin mutants were stably expressed and conjugated to ERp57 with high efficiency (see Figure S2). Cell extracts were prepared as described (Wearsch and Cresswell, 2007) and incubated with A15m beads (BioRad) coupled to the tapasin-specific mAb PaSta1 (Dick et al., 2002) for 2 hr at a ratio of ~6 μg of recombinant protein per 25 μl of 50% bead slurry. The beads were washed 3x with 0.1% Triton X-100 in TBS (150 mM NaCl, 25 mM Tris-Cl, pH 7.4) and then 5x in TBS to remove the detergent. The advantage of this purification procedure is that PaSta1 is conformation-specific, thus ensuring that the isolated mutants were properly folded and stable. The amount of recombinant protein on the beads was quantitated by Sypro Orange (Molecular Probes) staining of SDS-PAGE gels according to the manufacturer's instructions using purified tapasin-ERp57 conjugate as a standard. The purified proteins immobilized on PaSta1 beads were used for experiments within 4 days to ensure optimal stability and activity.

In vitro MHC class I binding and peptide loading assays

Both assays were performed as described (Wearsch and Cresswell, 2007) using extracts from .220.B*0801 cells (Greenwood et al., 1994) with the exception that the recombinant

tapasin-ERp57 conjugates were immobilized on PaSta1 beads rather than free in solution. Immobilization had minimal, if any, effect on the ability of recombinant tapasin-ERp57 to interact with and load MHC class I molecules (data not shown). For the analysis of the MHC class I interaction, ~25 μl of beads loaded with the various wild type and mutant tapasin conjugates were added at a final concentration of 250 nM to digitonin extracts from $5x10^6$ cells for 15 min at RT with mixing. The samples were then chilled and washed 4x with 0.1% digitonin in TBS. Samples were analyzed by SDS-PAGE and then subjected to immunoblotting with the MHC class I HC-specific Ab 3B10.7 (Lutz and Cresswell, 1987) as described (Wearsch and Cresswell, 2007). For peptide loading assays, 250 nM of immobilized conjugate was incubated with 1 μM of [^{125}I]-NP(380-387L) peptide (which is specific for HLA-B8 binding) and extracts from $5x10^6$ cells for 45 min at RT. Following centrifugation for 5 min at 10,000x*g* to pellet the PaSta1 beads, supernatants were subjected to immunoprecipitation with the W6/32 Ab (Parham et al., 1979) which is specific for MHC class I heavy chain-β_2m heterodimers. The amount of peptide bound was quantitated by gamma counting and the activity of the mutant tapasin-ERp57 conjugates was normalized to that observed with wild type tapasin.

Generation and analysis of transduced 721.220.B*4402 cell lines

Wild type and mutant tapasin were cloned into the pBMN-IRES-EGFP retroviral expression vector (Peaper and Cresswell, 2008) and all constructs were confirmed by sequencing. 721.220 cells expressing HLA-B*4402 (Peh et al., 1998) were retrovirally transduced with wild type and mutant tapasin constructs by spinfection as described (Peaper and Cresswell, 2008). For FACS analysis, 10^6 transduced cells were stained with mouse anti-HLA-A,B,C or an isotype control directly coupled to APC (Becton Dickinson Clone #G46-2.6) in PBS with 1 mM EDTA, 0.05% FCS, and 0.02% azide according to the

manufacturer's instructions. Data was collected on a FACSCalibur (Becton Dickinson) and analyzed using FlowJo software (Treestar Inc.). The data presented in Figure 4C is gated for $EGFP^+$ cells. For analysis of the PLC in cells expressing wild type and mutant tapasin molecules, $5x10^6$ cells per sample were washed in MMTS, lysed in digitonin, subjected to immunoprecipitation with the PaSta1 mAb, and analyzed by non-reducing SDS-PAGE. Following transfer to Immobilon-P (Millipore), membranes were immunoblotted as described (Peaper and Cresswell, 2008) for MHC class I heavy chain and TAP using the 3B10.7 mAb (Lutz and Cresswell, 1987) and RING4.C polyclonal Ab (Meyer et al., 1994), respectively.

Analysis of the tapasin-ERp57 conjugate using mAb PaSta2

The PaSta2 mAb was generated against soluble tapasin expressed in insect cells as part as the same mAb production used to generate PaSta1 (Dick et al., 2002). To evaluate their relative ability to bind MHC class I-associated tapasin, extracts from $5x10^6$ 220.B*0801 cells were supplemented with 100-300 nM recombinant WT tapasin-ERp57 conjugate and incubated for 15 min at RT. Samples were then immunoprecipitated with PaSta1 or PaSta2 coupled A15m beads and processed for immunoblotting against the MHC class I HC as described above. To screen the recombinant mutants for recognition by PaSta2, $\sim 4x10^6$ baculovirus-infected Sf21 cells were harvested and lysed as described (Wearsch and Cresswell, 2007). Immunoprecipitations were performed using PaSta1 or PaSta2 coupled beads and analyzed by non-reducing SDS-PAGE. The isolated recombinant proteins were detected by staining with Coomassie Blue.

Supplemental References

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