Supplementary Table 1. Thermostability analyses of calreticulin constructs from SYPRO Orange binding assays.

Indicated mCRT constructs (left column) were incubated with the fluorophore SYPRO Orange and subjected to a thermal stability analysis using a realtime PCR machine. Proteins (16 μM) were incubated alone (Protein), with 48 μM G1M3 (Protein + G1M3), or with 3 mM ATP (Protein + ATP). Each condition was analyzed in triplicate within an experiment. For each experiment, mean % maximum fluorescence values from triplicate wells were plotted as a function of temperature, and T_m values were estimated as the temperatures at which 50% of the maximum fluorescence was observed. T_m values shown are the averaged T_m values from n independent experiments for each condition, SEM represents the standard error of the estimated mean T_m values.

Constructs	Protein			Protein + G1M3			Protein + ATP		
	T_m	SEM	n	T_m	SEM	n	T_m	SEM	n
mCRT(WT)	47.96 ± 0.21		5	51.08 ± 0.18		5	46.24 ± 0.02		2
mCRT(ΔC)	44.29 ± 0.44		3	46.91 ± 0.17		3	---		
mCRT(ΔP)	47.63 ± 0.07		3	51.00 ± 0.27		3	45.49 ± 0.13		2
mCRT(Y92A)	45.90 ± 0.31		2	46.23 ± 0.28		2	---		
mCRT(W244A)	46.59 ± 0.46		2	50.74 ± 0.03		2	---		
mCRT(Y92AW244A)	42.96 ± 0.55		2	43.82 ± 0.01		2	---		
mCRT(W302A)	44.82 ± 0.55		3	45.67 ± 0.55		3	---		

Supplementary Figure Legends

Supplementary Fig 1. Structural model for calreticulin and depiction of truncation mutants and glycan-binding site. (A) A model for the calreticulin structure was obtained from Modbase (Pieper et al., 2004), and the PyMOL Molecular Graphics System (http://www.pymol.org) was used to render the structures. Calreticulin residues 1-32 are depicted in green. The C-terminal helix is depicted in light blue, and the core calreticulin structure in shown in orange. The P-domain is depicted in dark blue. The C-terminal acidic domain is not included in the model. **(B)** Putative glycan-binding residues of calreticulin are highlighted in purple, and the region of the calreticulin model corresponding to the glycan binding site is boxed. Mutagenesis studies (Gopalakrishnapai et al., 2006; Kapoor et al., 2003) have implicated highlighted residues (purple) in glycan binding.

Supplementary Fig 2. Calreticulin is required for the stabilization of the MHC class I – tapasin interaction within the peptide loading complex. The indicated cell lines were lysed in a digitonin lysis buffer. A rabbit anti-calreticulin antibody was used for the immunoprecipitation; lysates and samples were immunoblotted with goat anti-calreticulin, hamster anti-tapasin, or rabbit anti-ERp57 and anti-MHC class I antibodies. No antibody (No Ab) controls were performed by incubating indicated lysates with beads, and Buffer (Buf) indicates signals obtained with antibody alone (without lysates). NS indicates non-specific bands.

Supplementary Fig 3. Relative aggregation suppression activities of full-length and C-terminal truncation mutants of mCRT under various conditions. (A-D) Representative SDS-PAGE gels of aggregation assays with indicated mCRT constructs and OVA as a control.

Ovalbumin or mCRT at indicated concentrations were incubated with 4 μM LLO in the presence of 0.2-0.5 mM $CaCl_2$ or 5 mM EDTA at 37 ºC for 1 hour. Following incubations, aggregated proteins were separated from soluble proteins by centrifugation, and proteins present in supernatants (S) and pellets (P) were resolved by SDS-PAGE and visualized by staining with coomassie blue dye. **(E and F)** Band intensities of LLO in the pellet after incubation in the presence of mCRT or OVA were quantified, and are represented as a percentage relative to LLO observed in the pellet in the absence of mCRT or OVA. For each condition, data represent results from 3 or more experiments, except under conditions of 16 μM $mCRT_{1\text{-}362}$, for which data represent quantifications from 2 experiments.

Supplementary references

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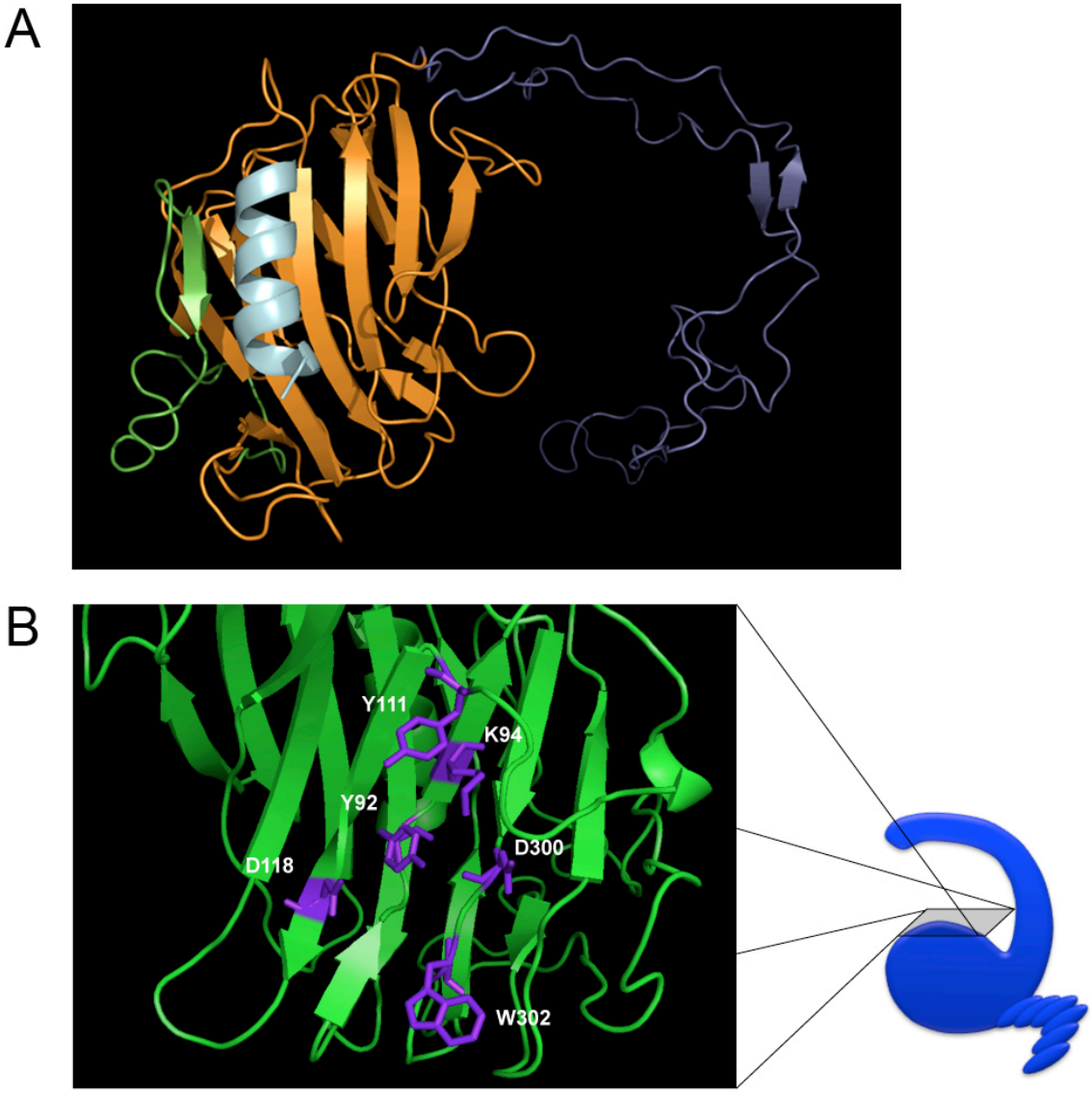
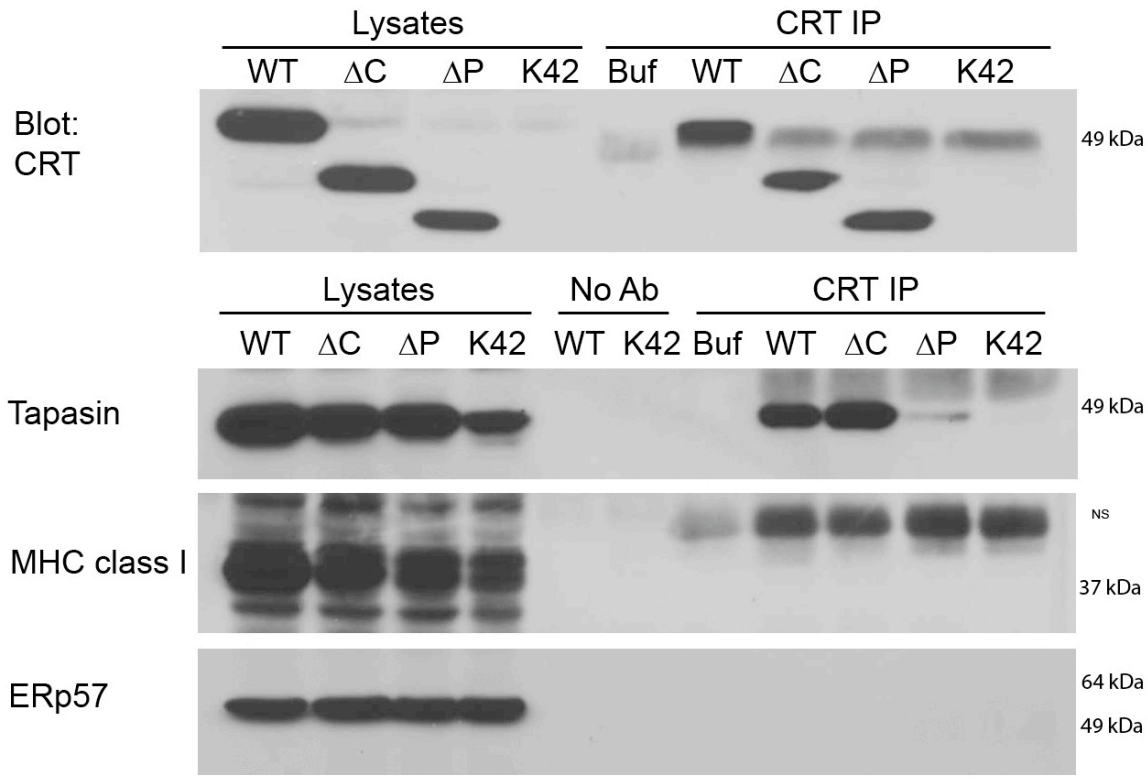
Supplementary Figure 1



Supplementary Figure 2



Supplementary Figure 3