

Expanded View Figures

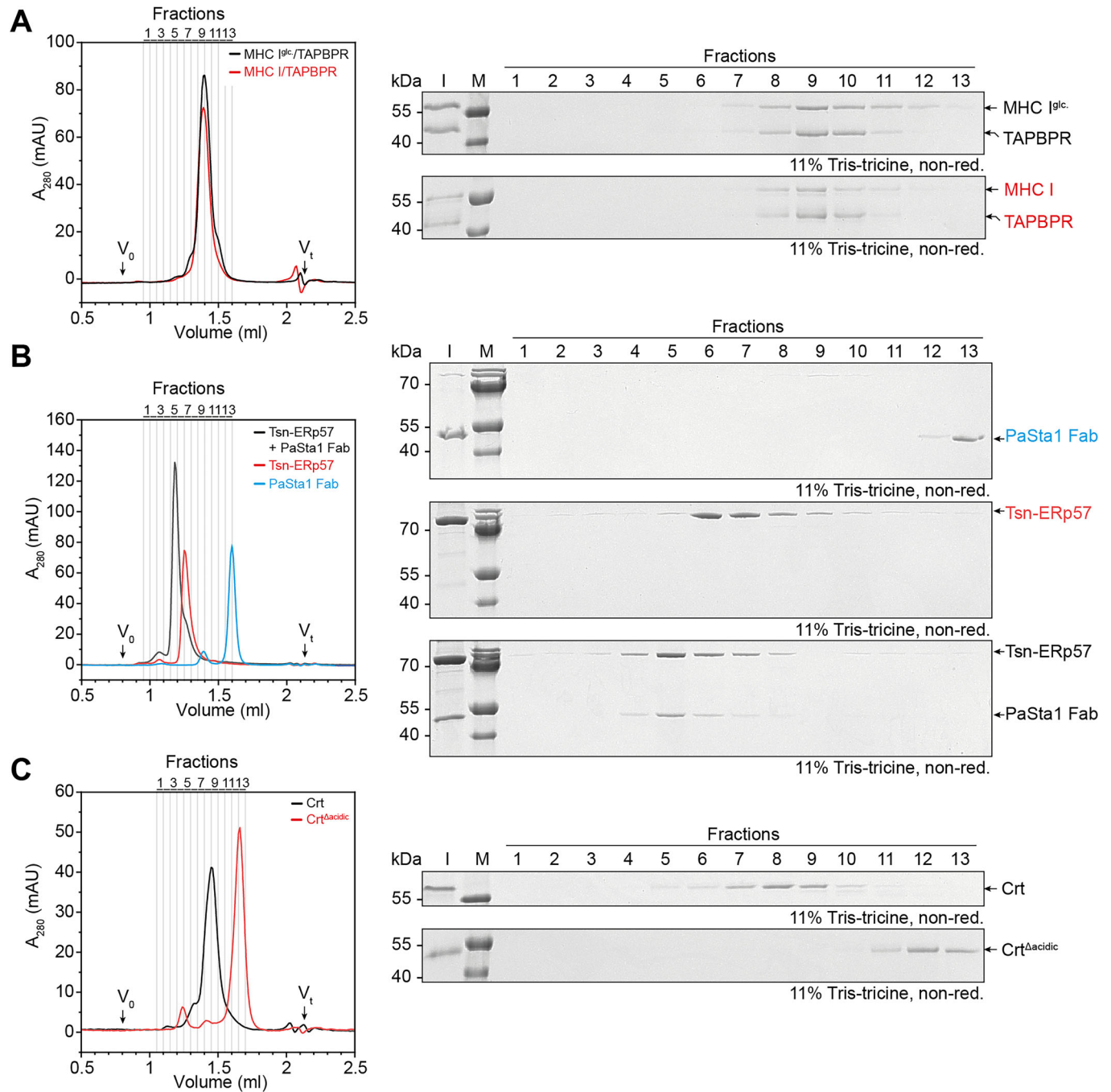
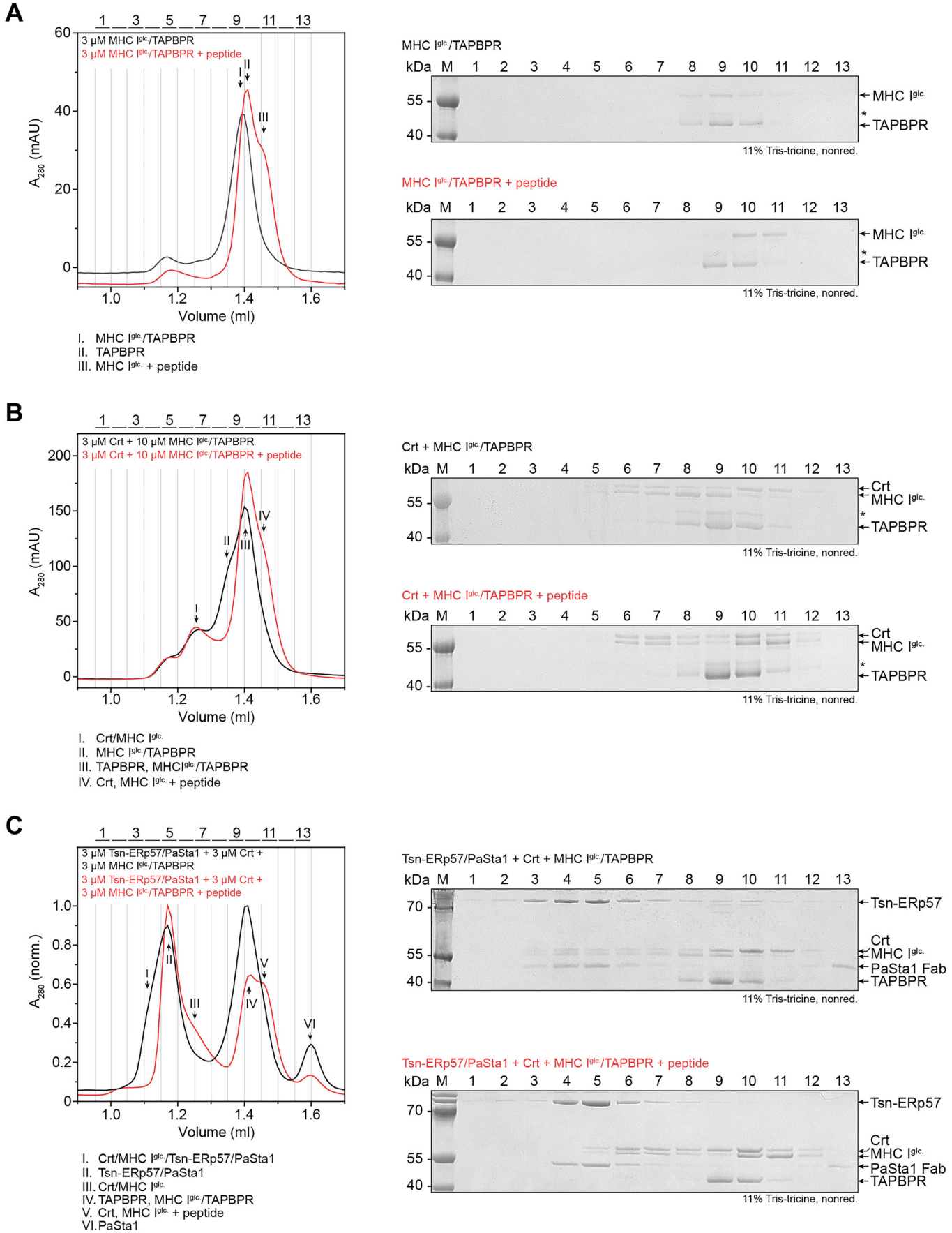


Figure EV1. Analysis of chaperone-MHC I complexes and their individual components by SEC.

(A) SEC profiles (left) and SDS-PAGE analyses of indicated fractions (right) for the single-chain β_2 m-HLA-A*68:02/TAPBPR complex (3 μ M, black line) or the monoglucosylated complex (3 μ M, red line). (B) SEC profiles (left) and SDS-PAGE analysis of indicated fractions (right) for PaSta1 Fab alone (3 μ M, blue line), soluble tapasin-ERp57 (3 μ M, red line), and the tapasin-ERp57/PaSta1 complex (3 μ M, black line). (C) SEC profiles (left) and SDS-PAGE analysis of indicated fractions (right) for calreticulin (Crt, 3 μ M, black line) and its deletion variant (Crt^{Δacidic}, 3 μ M, red line). M marker, I input, kDa kilodalton, A_{280} absorption at 280 nm. Data shown in panels (A–C) are representative of three independent experiments ($n = 3$). Source data are available online for this figure.



◀ **Figure EV2. Influence of high-affinity peptides on MHC I quality control and recycling complexes.**

(A) SEC profiles (left) and SDS-PAGE analysis of indicated fractions (right) for the monoglucosylated β_2 m-HLA-A*68:02/TAPBPR complex (3 μ M) in the presence (red line) or absence (black line) of peptide ETVSKQSNV (0.75 mM). (B) SEC profiles (left) and SDS-PAGE analysis (right) of mixtures of calreticulin (Crt, 3 μ M) with monoglucosylated single-chain β_2 m-HLA-A*68:02/TAPBPR complex (10 μ M) in the presence (red line) or absence (black line) of peptide ETVSKQSNV (2.5 mM). (C) SEC profiles (left) and SDS-PAGE analysis (right) of mixtures of tapasin-ERp57/PaSta1 Fab (3 μ M), monoglucosylated single-chain β_2 m-HLA-A*68:02/TAPBPR complex, and Crt with (red line) or without (black line) peptide ETVSKQSNV (0.75 mM). Note that SEC profiles and corresponding SDS-PAGE analyses shown in panels (B, C) are identical to those in Figs. 3B, C and 1C, E, respectively. Image shown in panel (B) was taken from the same gel as shown in Fig. 3C, but at a different exposure time. Roman numbers above each peak correspond to the proteins or protein complexes defined below. M marker, I input, kDa kilodalton, A_{280} absorption at 280 nm. Data shown in panels (A–C) are representative of two independent experiments ($n = 2$) with peptide and three independent experiments ($n = 3$) without peptide. Source data are available online for this figure.

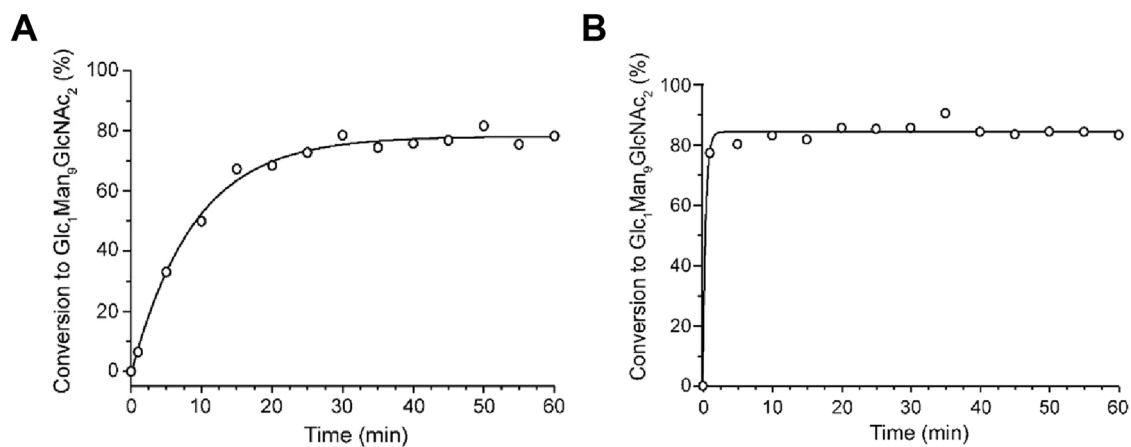


Figure EV3. Comparison of the monoglucosylation capacity of human UGGT1 and *Chaetomium thermophilum* UGGT on MHC I/TAPBPR complexes.

(A) Human UGGT1 (HsUGGT1, 1 μ M) (Sagert et al, 2023) or (B) *Chaetomium thermophilum* UGGT (CtUGGT, 1 μ M) was incubated with MHC I/TAPBPR complexes (3 μ M) in 1 $\times$ HBS buffer supplemented with UDP-glucose (UDP-Glc; 200 μ M) and CaCl₂ (5 mM) at room temperature. Conversion to the monoglucosylated Glc₁Man₉GlcNAc₂ glycan (MHC I^{glc}) was analyzed by LC-MS. Source data are available online for this figure.

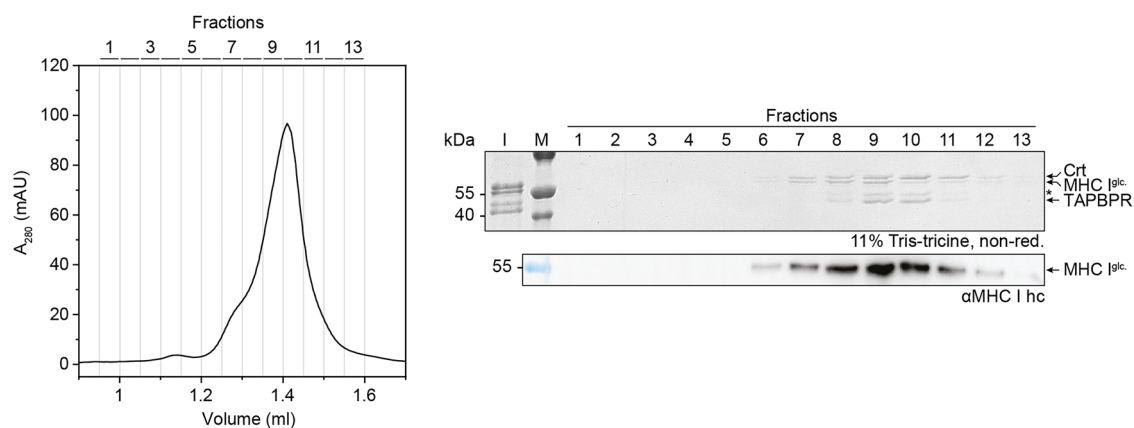


Figure EV4. Chaperone exchange of MHC I at equimolar concentrations of MHC I^{glc}/TAPBPR and calreticulin.

Size-exclusion chromatography (SEC) profiles (left) with SDS-PAGE and immunoblot analyses (right) of monoglucosylated single-chain β_2 m-HLA-A*68:02/TAPBPR complex and calreticulin (Crt) (3 μ M each). The HC10 antibody was used to detect the MHC I heavy chain (α MHC I hc). M marker, I input, kDa kilodalton. Data shown in panels are representative of three independent experiments ($n = 3$). Source data are available online for this figure.

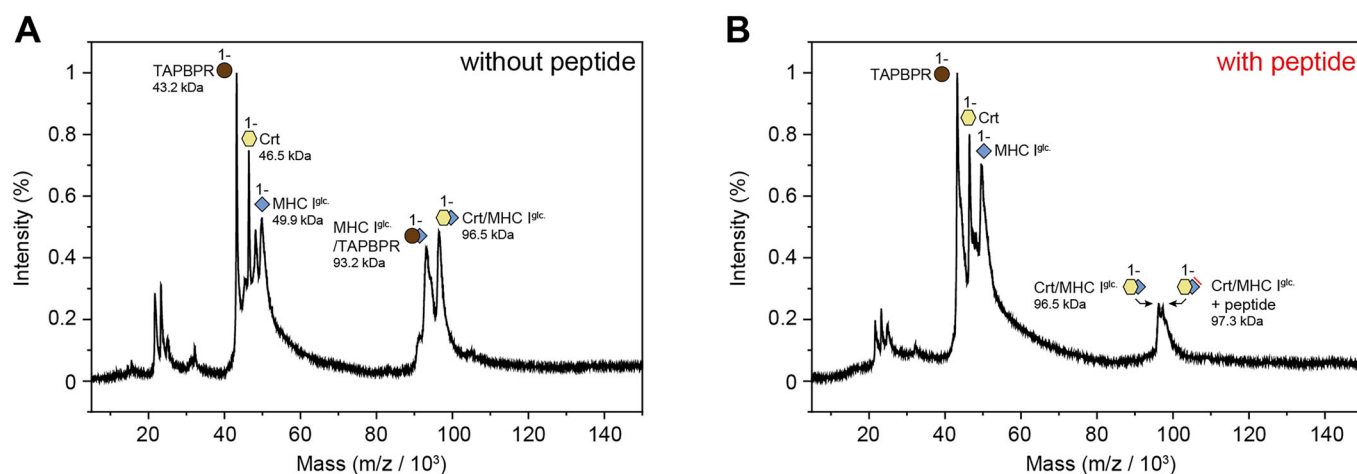


Figure EV5. High-affinity peptide induces disengagement of MHC I from TAPBPR.

(A, B) LILBID mass spectra of complexes formed upon mixture of monoglucosylated β_2m -HLA-A*68:02/TAPBPR complex (10 μ M) with calreticulin (Crt; 3 μ M) in the absence (A) or presence (B) of peptide ETVSKQSNV (2.5 mM). Charge states of the individual proteins or complexes and the corresponding observed masses are indicated. Brown circle - TAPBPR; yellow hexagon - Crt; blue square - MHC 1^{glc} . Source data are available online for this figure.