Supplementary Experimental Procedures

Immunoprecipitation and western blots

NIH3T3 cells (2 x 10^6) were co-transfected with FLAG-m153-pcDNA (1μg) and HA-m153-pcDNA(1μg) (Amaxa Nucleofector, solution R, program U30) and seeded in 10 cm plates. For infection experiments 70-80% confluent NIH3T3 cells seeded in 10 cm plates were infected with MCMV-GFP at an m.o.i. of two. After overnight incubation for transfections and 24h incubation for infections, the cells were washed twice in ice-cold PBS and lysed in the plates with 1ml of 1% NP40, 50 mM Tris-HCl pH8, 300 mM NaCl, 5 mM EDTA, 1x protease inhibitor cocktail (Pierce), 10mM iodoacetamide, 0.02 % sodium azide. The cells were removed from the plate with a cell scraper and incubated for 15 min. on ice. All subsequent steps were performed on ice or at 4 °C. The lysate was centrifuged for 15 min. at 13,000 rpm, and supernatants were precleared by incubating with 50 μl of anti-mouse IgG beads (eBioscience) for 30 min. For transfected cells the precleared cell lysates were divided into three aliquots, and either 5 μg of FLAG M2 antibody (Sigma), anti-HA (Sigma) or mAb153.16 were incubated with the lysates for 1 h. For infection experiments 5 μg of mAb153.16 were incubated with infected and uninfected cell lysates for 1 h. Then 50 μl of anti-mouse IgG beads were added to the lysates and incubated for 1h with rotation. The beads were washed 5x with lysis buffer containing 0.1 % NP40. Reducing SDS-PAGE sample buffer (Pierce) was added to the beads and the samples heated to 100°C for ten minutes prior to electrophoresis on an 8-20 % gradient Tris-Glycine SDS-PAGE gel (Invitrogen). The proteins were transferred to polyvinylidene fluoride (PVDF) membranes using standard protocols. The membrane was blocked overnight with 5 % non-fat dried milk in 0.1 % Tween 20/PBS. The proteins were detected by incubation with either anti-FLAG M2, anti-HA (Sigma) or anti-m153 rabbit serum for 1h at room temperature. After washing in 0.1 % Tween 20/PBS the membranes were incubated with secondary antibody for 1h at room temperature (anti-mouse or anti-rabbit Trublot-eBioscience), and washed again. The blots were developed with the Supersignal West Dura Extended Duration substrate (Pierce) and exposed to Biomax XAR film (Kodak).

To determine whether m153 is glycosylated, immunoprecipitated samples on beads were incubated with 1000 U PNGaseF (New England Biolabs) in PBS at 37 °C overnight before processing for SDS-PAGE and western blot analysis as described above.

Mass spectrometry

For mass spectrometry infusion experiments, samples were diluted with ultrapure water (LC-MS-Chromasolv grade, Sigma-Aldrich), heated to 65 °C for 5 min and desalted twice in ultrapure water using Centri•Sep™ desalting spin columns according to the manufacturers procedures (Princeton Separation). After further enzymatic or chemical treatment with 0.1 μg trypsin (Promega), 0.5 mU PNGaseF (QA-Bio), 2 μg tris-(carboxyethyl)-phosphine hydrochloride (TCEP, Sigma-Aldrich) or 2 μg dithioerythritol (DTT, Sigma-Aldrich) and incubation at 37 °C for 1 – 48 hours, the respective samples were diluted 1:1 in spraying buffer (40 % Isopropanol, 40 % Acetonitrile, 20 % Ultrapure Water and 0.2 % formic acid, all Sigma-Aldrich). Infusion nano-electrospray was performed using the TriVersa™-NanoMate chip technology (Advion Biosystems) coupled to an LTQ-FT™ ICR mass spectrometer (Thermo Electron).

5 μL solution pick-up (ca. 2-15 pmol/μL) was sufficient for ca. 45 min analysis time (1.5 kV, 0.3 bar pressure assistance). MS and MS^n fragmentation data were recorded applying collision-induced dissociation (CID, 35 % energy), electron capture dissociation (ECD, 5 x e^5 energy, 10 ms delay, 70 ms duration) or infrared multi photon dissociation (IRMPD, 90 % energy, 10 ms delay, 90 ms duration) of indicative precursor ions. MS spectra recorded were the average of three or more FT-MS scans (max. ion injection time 3000 ms, resolution 100,000 to 500,000, ion accumulation values according to the manufacturer's standard values). MS^n spectra were recorded as the average of three FT-MS scans (5 Da window for precursor ion selection).

Supplementary Figures

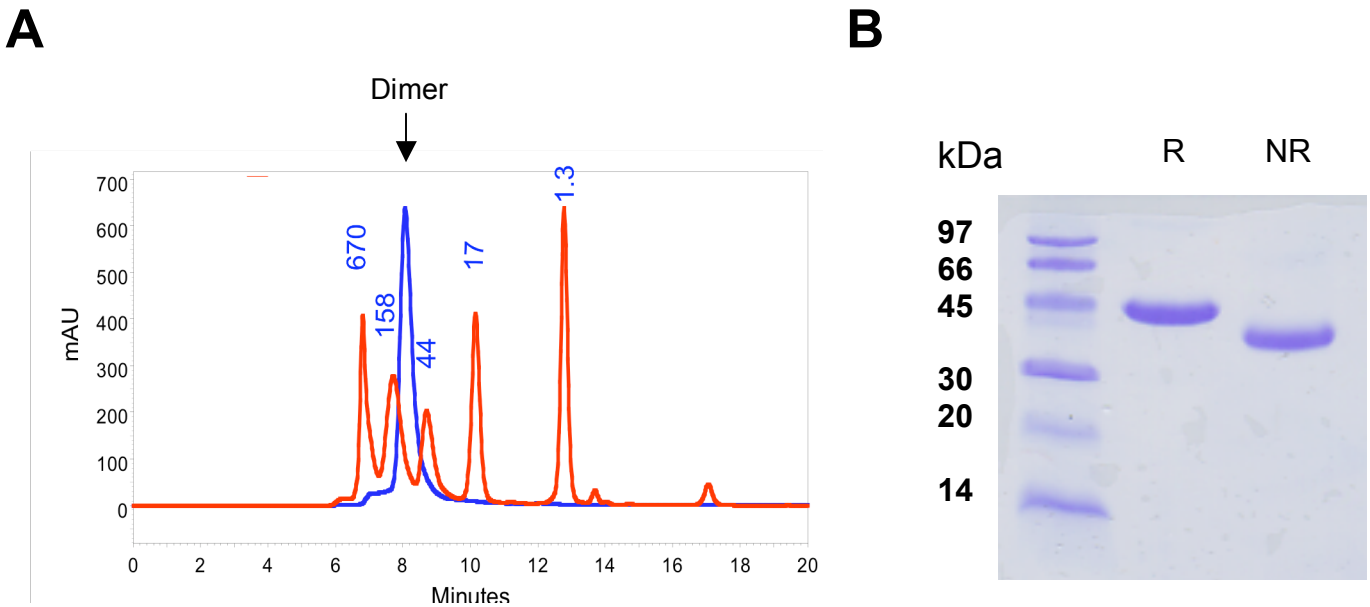

Figure S1: m153 is a non-covalent dimer when expressed in S2 insect cells. *A.* Size exclusion chromatography analysis of insect cell expressed m153 (extracellular domain – 1-314). The calculated molecular weight based on the column retention time is 85 kDa, whereas the molecular weight predicted from the amino acid sequence is 37.4 kDa for a monomer. *B.* SDS-PAGE of recombinant m153 expressed in S2 insect cells under reducing (R) and non-reducing (NR) conditions indicates that m153 is a non-covalent dimer. The molecular weight of the secreted, engineered m153 expressed in insect cells, analyzed in SDS-PAGE is accounted for by 37.4 kDa from the polypeptide and about 6 kD from the core carbohydrates. In contrast, the full length virus-encoded or transfected m153 chains migrate at about 80 kDa in SDS-PAGE and 45 kDa following deglycosylation (Fig. 1B), consistent with a length of 405 amino acids and more complex glycosylation.

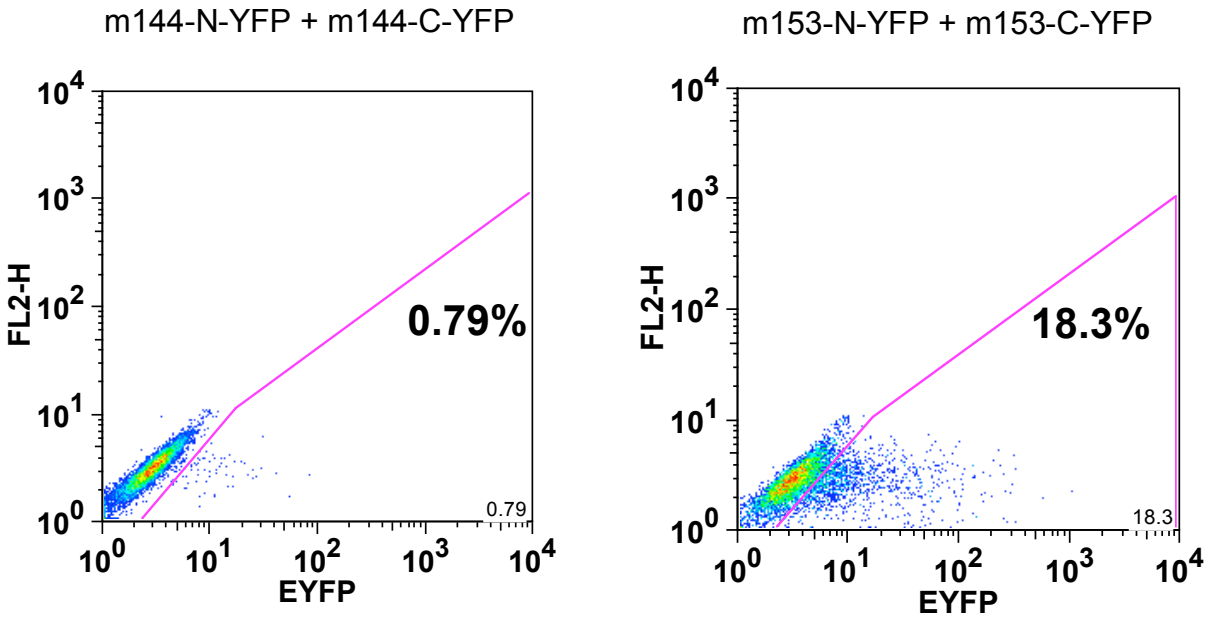

Figure S2: FACS quantification of YFP fluorescence complementation. NIH3T3 cells were transfected with m153-N-YFP+m153-C-YFP or m144-N-YFP+m144-C-YFP and following confocal microscopy the YFP-fluorescent cells were counted by FACS. YFP fluorescence was visible in 18% of m153 transfected cells but only in 0.8% of m144 transfected cells, indicating that m153 forms dimers in NIH3T3 cells.

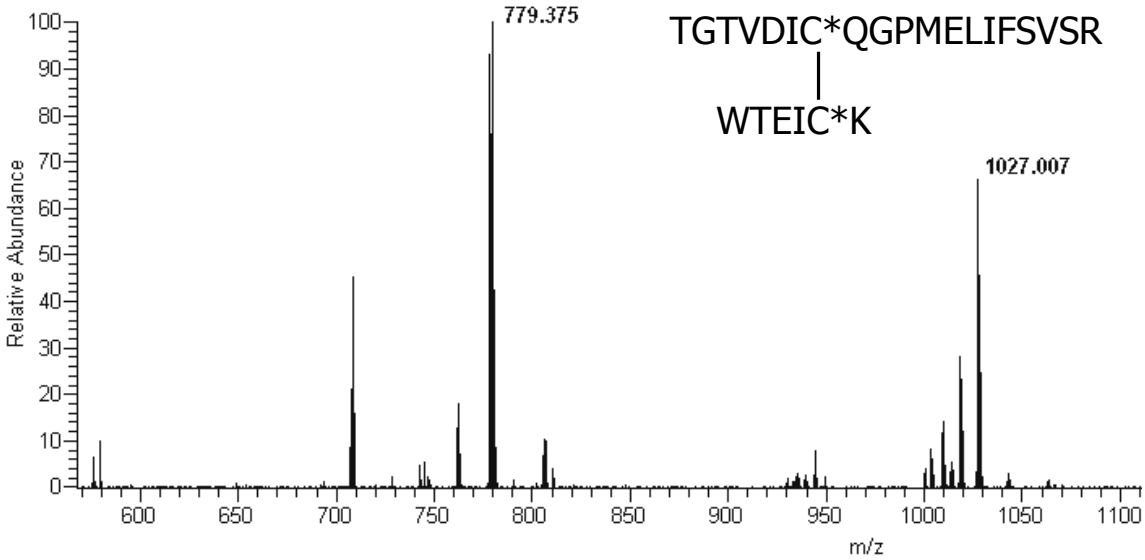

Figure S3: Mass spectrum of a disulfide-linked tryptic peptide confirms the C16-C171 disulfide bond. FT-MS/MS spectrum after electron capture dissociation (ECD) fragmentation of the quadruply charged m/z=708.097. ECD induced a fragmentation of the disulfide bond yielding the singly charged fragment m/z=779.375 and the doubly charged fragment m/z=1027.007, corresponding to the tryptic peptides TGTVDICQGPMELIFSVSR and WTEICK, respectively. ECD also resulted in further peptide fragmentation revealing the sequence tag FSVS.

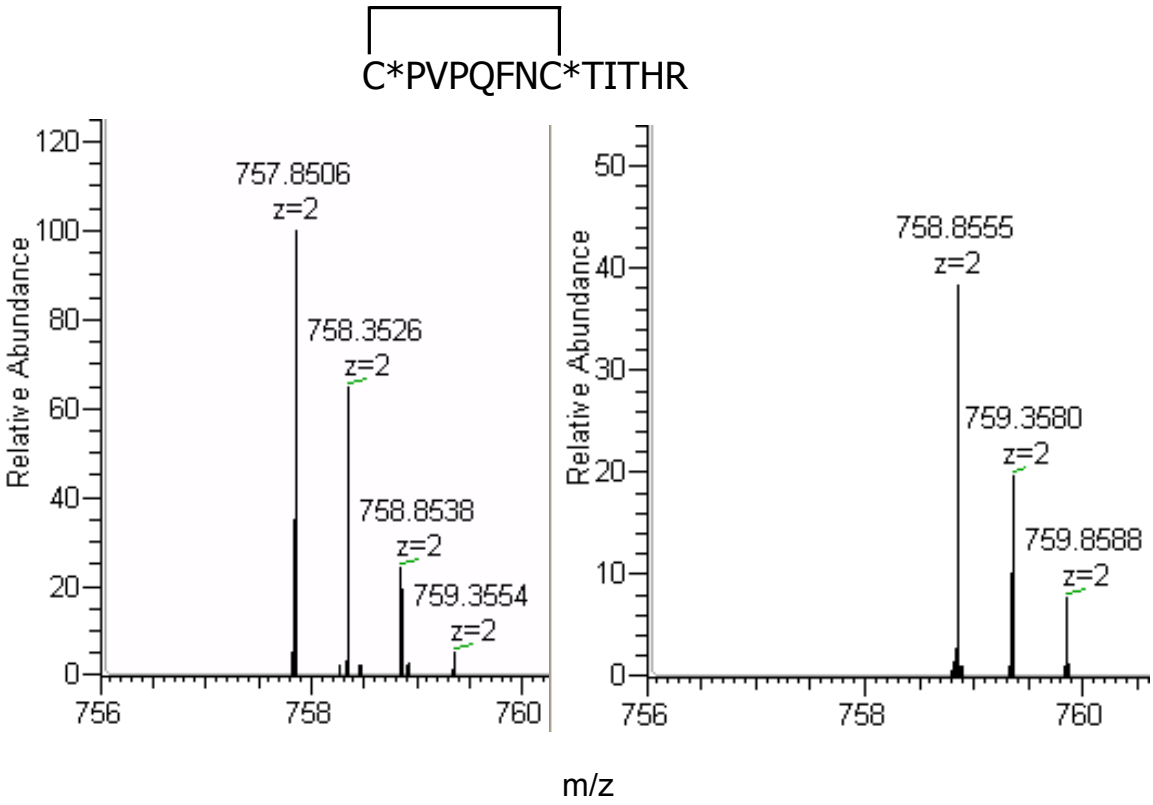

Figure S4: Mass spectra of C*PVPQFNC*TITHR peptide before and after reduction with TCEP confirmed the C101-C108 disulfide bond. Mass shift of 1.005 at the doubly charged peptide m/z=757.851 indicated addition of two protons after reduction of internal Cys-Cys bond in tryptic peptide CPVPQFNCTITHR.

FT-MS experiments for determination of Cys-Cys bonds present in MCMV-m153

Desalted and PNGaseF treated MCMV-m153 was tryptic digested and nano electrosprayed. FT-MS/MS spectra were recorded after ECD fragmentation of indicative peptides to selectively reduce Cys-Cys bonds between two tryptic peptides. Alternatively TCEP was added to the solution to obtain mass shift experiments of FT-MS survey scans for identification of e.g. internal Cys-Cys bonds in one and the same tryptic peptide. Figure S3 shows exemplary the selective Cys-Cys reduction using ECD fragmentation of the quadruply charged m/z=708.097 (tryptic peptides TGTVDIC*QGPMELIFSVSR and WTEIC*K), whereas Figure S4 shows the mass shift of 1.005 at the doubly charged m/z= 757.851 caused by TCEP induced reduction of the internal Cys-Cys bond of tryptic peptide C*PVPQFNC*TITHR. All cysteines present in MCMV-m153 are engaged in bonds with C16 bound to C171, C101 to C108 and C203 bound to C255.

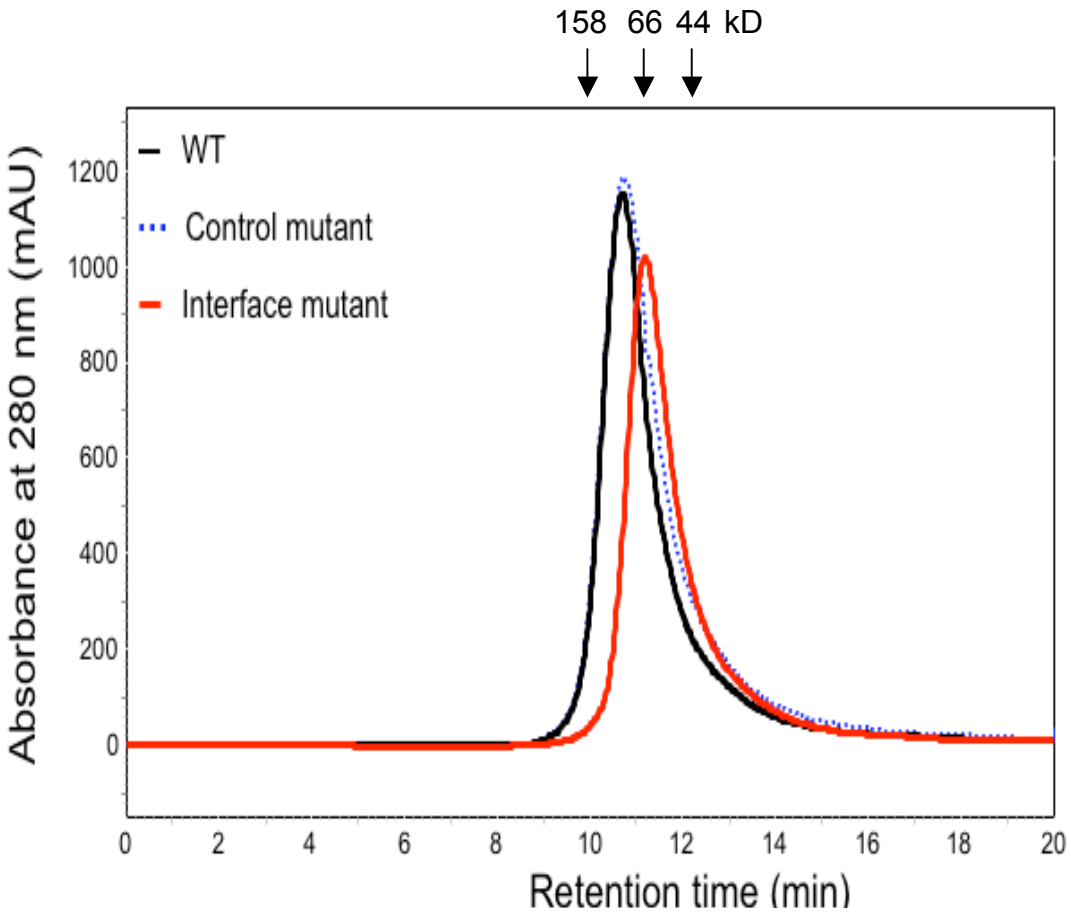

Figure S5: Mutations at the interface destabilize the m153 dimer. Size exclusion chromatography analysis of wild type (black), quadruple interface mutant (T128A, S131A, R225A, S241A - red) and control mutant (D194A - dashed blue) m153 purified from SF9 insect cells. The quadruple interface mutant migrates at a slower rate through the column, indicating a smaller size compared to wild type m153.

Table S1: Hinge angle between the α1α2 and α3 domains of viral and MHC-Ia and MHC-Ib molecules.

Protein	PDB code	Angle (degrees)	Associated subunits
m153	2O5N	76	m153
m157	2NYK	81	None
m144	1U58	99	β2m
H-2K^b	2VAA	71	β2m, peptide
CD1d	1ZHN	75	β2m, lipid
T22	1C16	72	β2m
ZAG	1TW7	90	β2m
MICA	1B3J	116	β2m

Hinge angles were calculated with the HINGE program as described in Experimental Procedures.