SUPPORTING INFORMATION

S1. Preparation of recombinant baculoviruses

Baculovirus-mediated transfection into *Spodoptera frugiperda* (Sf)9 was performed in serum-free media (HyClone SFX-Insect Cell Culture Media, Thermo Scientific) without antibiotics using Cellfectin reagent (Invitrogen) according to the manufacturer's instructions. The transfection complex was formed as follows: 3 μg of recombinant DNA (UL141 in transfer vector) + 0.05 μg of BaculoGold DNA (Invitrogen) + 20 μl of Cellfectin Reagent were filled up to 1 ml with media. As a negative control, 20 μl of Cellfectin + 1 ml media was mixed. The transfection mixture was vigorously vortexed for 30 sec and incubated at RT for 15 min in the dark. 2×10^6 healthy-dividing Sf9 cells were seeded in T-25 (25 cm^2) flasks. Culture media was removed and transfection mixture was added drop-wise. Transfection plates were then incubated at RT for 4 hours while rocking back-and-forth every 30 min in dark. After 4 hours, the transfection mixture was replaced with 5 ml fresh media containing antibiotics (mixture of 50 U ml^{-1} of penicillin and 50 μg ml^{-1} of streptomycin) and plates were incubated at 301.15 K for 5 d. For the initial screening for positive recombinant UL141 virus the dilution virus pool method was applied. Positive recombinant virus was selected and then amplified as follows. Cell supernatant containing recombinant virus was collected (1000 × g for 10 min) and used for first round of virus amplification. 500 μl of virus with a multiplicity of infection below 1 (MOI<1) was used to infect 2×10^6 cells in T-25 flask and the flask was then incubated at 301.15 K. After 5 d, the second virus amplification was performed in T-175 flask to infect 14×10^6 cells with volume of 1 ml of collected virus from the first amplification (MOI<1) in 50 ml of media and incubated for additional 5 d at 301.15 K. Virus titer was determined by end-point dilution assay (EPDA). Prior to expression, the high titer virus stock was prepared in several T-175 flasks by infection at MOI=1 of 14×10^6 cells in total 50 ml volume of media and incubated for 6 d at 301.15 K. Each flask was then directly used for infection of 2300×10^6 cells in total 1 l volume of media (MOI between 3 to 5) and incubated for 84 h at 301.15 K as a suspension culture (at 138 rpm).

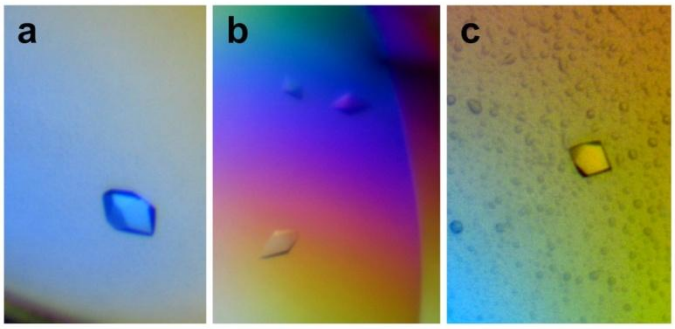

Figure S1. Crystals of HCMV UL141. Well shaped three-dimensional crystals of HCMV UL141 glycoprotein with maximal dimensions of approximately 60 × 30 × 30 μm were grown within 3-4 d at 295 K in the presence of (**a**) 0.2 M calcium acetate, 0.1 imidazole pH 8 and 10% (w/v) polyethylene glycol 8000, (**b-c**) 0.1 M bicine pH 9, 10% (w/v) polyethylene glycol 6000 by sitting drop crystallization method.

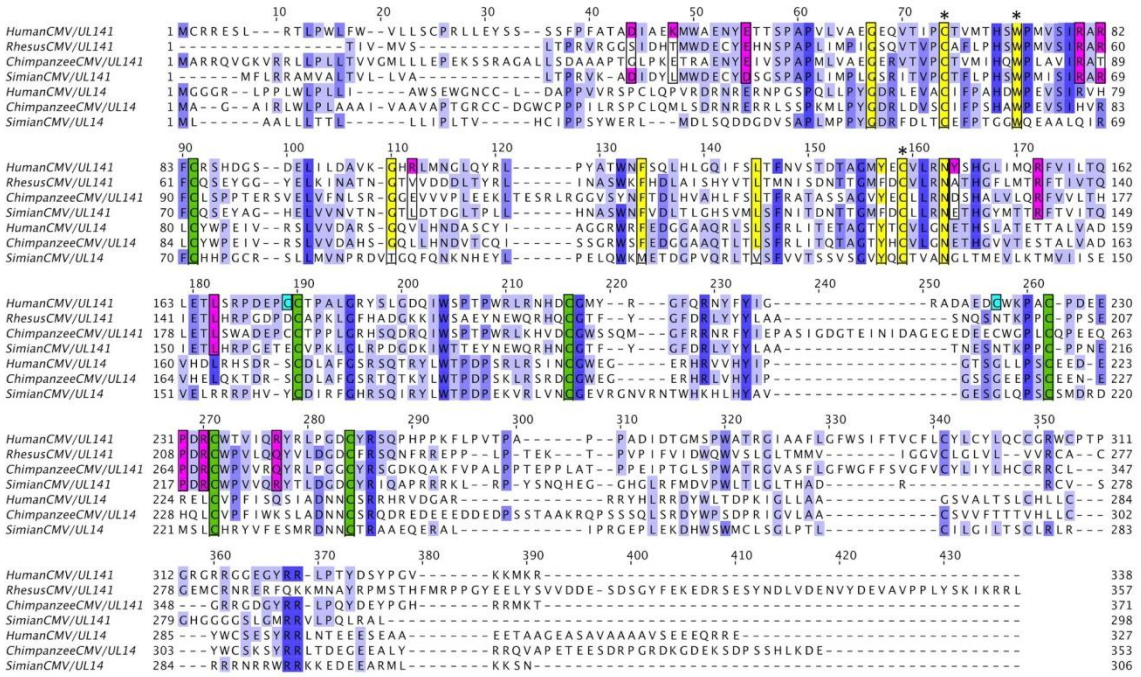

Figure S2. Sequence alignment of cytomegalovirus UL141 and UL14. Sequence alignment of Human CMV UL141, its orthologs in Chimpanzee, Simian and Rhesus CMV compared to related UL14 genes. Residues that are conserved in all the viral sequences are shown in dark blue (lower degree of conservation shown in light blue). Residues that are also conserved within most Ig domains are depicted in yellow, where two conserved cysteine residues (asterixed) forming disulfide bond packed over the conserved tryptophan residue (asterixed). All conserved cysteines in viral sequences are also highlighted (green), while additional two cysteines are highlighted in HCMV UL141 sequence only (cyan). Residues that are responsible for death receptor (TRAIL-R2) binding and show degree of conservation in viral sequences are highlighted in magenta.

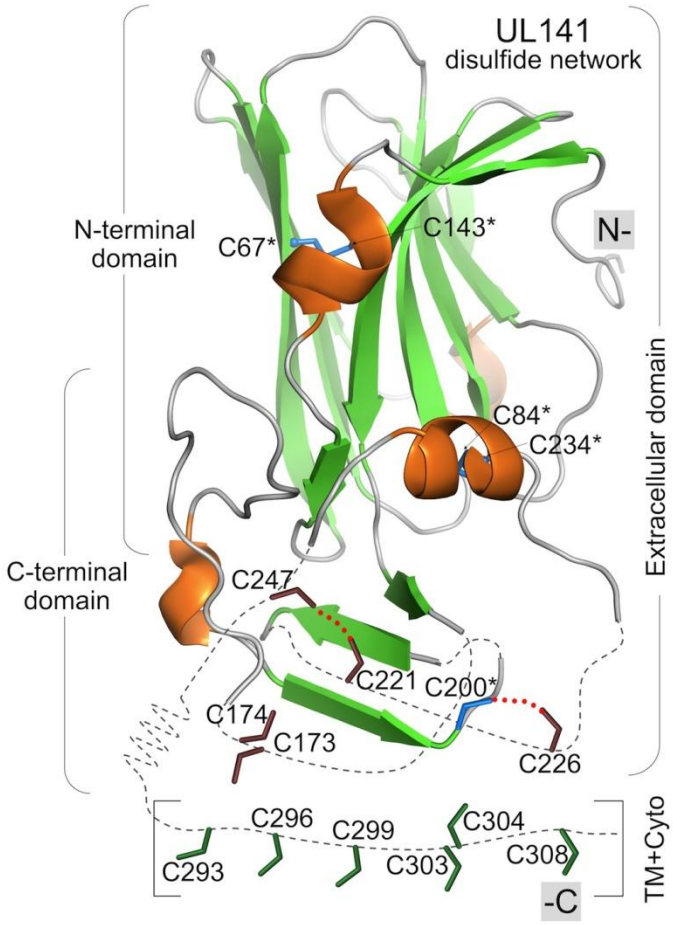

Figure S3. Predicted disulfide bond network on UL141. Structure of the HCMV UL141 monomer in ribbon representation with α-helices colored orange and β-sheets shown in green. Four well-defined cysteines forming two disulfide bridges C67-C143 and C84-C234 and one free cysteine C200 are asterixed and shown as blue sticks. Additional five cysteines within the C-terminal appendix domain of UL141 (C173, C174, C221, C226 and C247) are located in disordered regions (dashed line) and approximate position is indicated as brown sticks. The red dotted lines indicate predicted disulfide linkages between C200-C226 and C221-C247. Of note, the transmembrane and cytoplasmic domains of UL141 contain 6 cysteines (C293, C296, C299 and C303, C304, C308; respectively), whose functions are currently unknown.

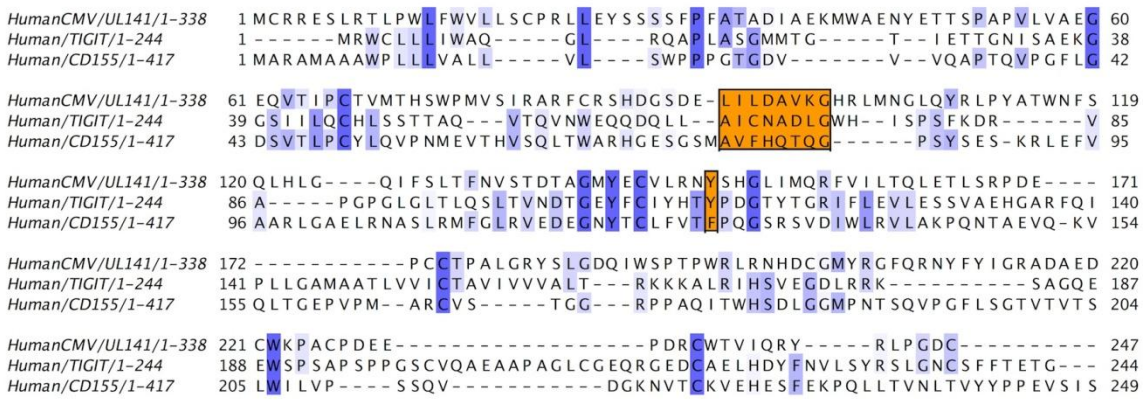

Figure S4. Sequence alignment of cytomegalovirus UL141, human CD155 and TIGIT. Sequence alignment of Human CMV UL141, Human CD155 (poliovirus receptor) and TIGIT. Residues that are conserved in all the sequences are shown in dark blue (lower degree of conservation shown in light blue). Newly identified hydrophobic 'lock' motif '(L/A)X_6G' and 'key' motif '(Y/F)' that are potentially responsible for CD155 binding are highlighted in orange.

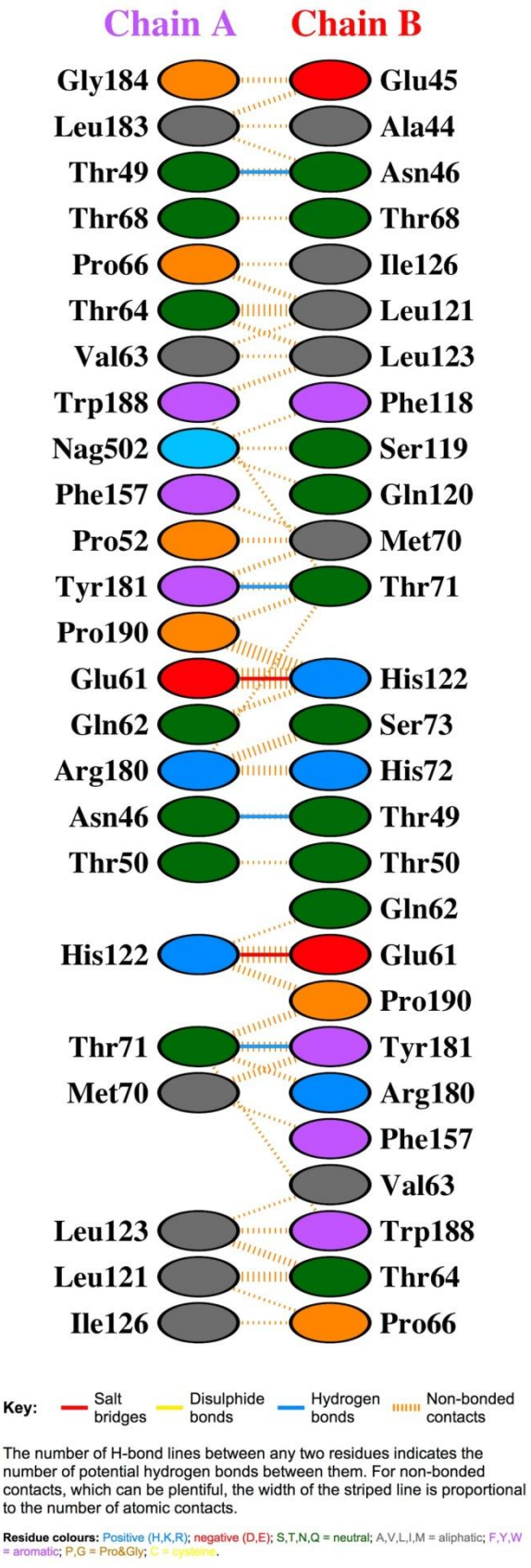

Figure S5. List of UL141 dimerization interface interactions derived from PDBsum analysis.

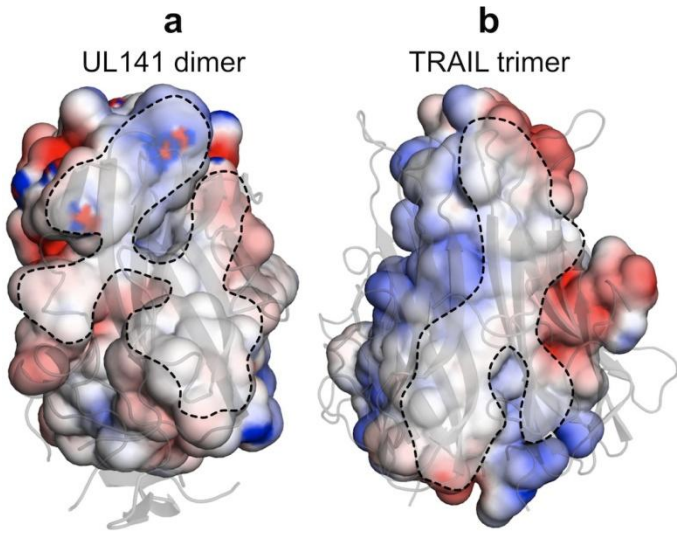

Figure S6. Electrostatic properties of dimerization (UL141) and trimerization (TRAIL) interfaces. Molecular surface representation of the dimerization (**a**, HCMV UL141 dimer) and trimerization (**b**, Human TRAIL trimer) interfaces (encircled with dashed line), colored according to electrostatic potential; the other subunits are shown by grey cartoon only. Positive potential is shown in blue, and negative potential is in red; the contouring value of the potential is show from -5 to +5 kTe^{-1}. The image was prepared using PyMolX11Hybrid (The PyMOL Molecular Graphics System, Version 1.5.0.4 Schrödinger, LLC).