ONLINE METHODS

Expression and purification of recombinant HSV-2 gHΔ48/gL. The construct for expression of soluble HSV-2 gHΔ48/gL complex was generated by site-directed mutagenesis using construct pTC605[45] as a template. The resulting construct (pLF761) encodes gH, residues 48-803, followed by a C-terminal His_6 tag, and full-length gL in the pFastBacDUAL vector. Transmembrane region and the cytoplasmic tail of gH, residues 804-838, are replaced with a His_6 tag. Both proteins retain their native signal sequences. Sf9 cells in spinner flasks were infected with the recombinant baculovirus expressing HSV-2 gHΔ48/gL (viral passage P3) at 10 ml per 2×10^9 cells. Culture supernatant was separated from cells 72 h post-infection by centrifugation followed by filtration. HSV-2 gHΔ48/gL was purified from the supernatant by Ni affinity chromatography (GE Healthcare) and gel-filtration chromatography using Superdex S200 column (GE Healthcare). For crystallization, gHΔ48/gL complex was concentrated to 1 mg ml^{-1} using Amicon Ultra-4 (MW cutoff 50 kDa) (Millipore).

Crystallization and data collection. Crystals of gHΔ48/gL were grown by vapor diffusion at room temperature in hanging drops with 1 μl protein and 1 μl reservoir solution (20% (w/v) PEG 4000, 0.1 M Na·citrate, pH 4.5). Tetragonal crystals appeared after 4-5 days and grew to their final size in over two weeks. For data collection, crystals were transferred stepwise to 20% xylitol (w/v), 20% PEG 4000 (w/v), 0.1 M Na·citrate, pH 4.5, 0.1 M NaCl, and plunged into liquid N_2. A native data set was collected at the wavelength of 0.9795 Å at 100 K at beamline 24ID at the Advanced Photon Source and processed to 3.0 Å resolution using HKL2000[46] (Table 1). Crystals took space group $P4_{(1/3)}2_12$ with a=b=88.2 Å, c=333.4 Å, $\alpha=\beta=\gamma=90°$. To obtain experimental phases, selenomethionine-substituted (SeMet) gHΔ48/gL was expressed using

methionine-deficient insect-cell media ESF921 (Expression Systems, CA, USA) supplemented with L-selenomethionine. 71% SeMet incorporation was confirmed by mass spectrometry. SeMet gHΔ48/gL was crystallized under conditions similar to those for the native gHΔ48/gL, 22.5% PEG 3350 (w/v) and 0.1 M Na·citrate, pH 4.5. A single-wavelength anomalous dispersion dataset was collected at the peak wavelength of 0.9789 Å at 100 K at X25 beamline at the National Synchrotron Radiation Source and processed to 3.29 Å resolution using HKL2000[46] (Table 1).

Structure determination. Eight out of 9 Se sites were found using direct methods as implemented in HKL2MAP in the ShelX suite[47] and refined using SHARP[48]. At this point, the space group ambiguity was resolved in favor of $P4_12_12$. The residual maps revealed the location of an additional site. All sites were then refined in SHARP[48]. After phase improvement by density modification, the experimental electron-density map (Supplementary Fig. 1) allowed tracing and sequence assignment for over 80% of the ordered polypeptide chain, using Coot[49]. Prior to refinement, 10% of the data were set aside for cross-validation. Data from 39.23 to 3.29 Å resolution were used in refinement, which included simulated annealing, group atomic displacement parameter refinement, and TLS refinement, all as implemented in Phenix[50].

Next, test set flags were transferred to the native dataset; additionally, 10% of the native data between 3.29 and 3.0 Å were set aside for cross-validation. The model was then rigid-body refined against the native data to 3.0 Å resolution using Phenix[50]. After several rounds of refinement, rebuilding, and addition of solvent molecules, the current R is 17.0% and the Rfree is 24.3% (Table 1). The final model contains residues Arg49 to Pro797 of gH, except for three disordered loops Gly116 to Pro136, Thr328 to Asp331, and Arg720 to Arg724; residues Thr24 to

Asn203 of gL, except for two disordered loops, Phe112 to Ala114 and Leu166 and Pro196; and 52 solvent molecules. Sequence Pro197 to Asn203 was tentatively assigned to a short, 7-amino-acid-long stretch of residues, but due to poor side-chain density, its precise sequence is unclear. Three asparagines in gH, Asn73, Asn670, and Asn784, are glycosylated, and four N-acetyl-glucosamine moieties are visible in the structure. According to Molprobity[51], 94.8% of residues lie in the most favored and 5.1%, in the additionally allowed regions of the Ramachandran plot. Pro80 is the only outlier.

Structure analysis. Sequence alignments were made using ClustalW[52] and ALSCRIPT[53] or Multalign[54] and ESPRIPT[55]. Interfaces were analyzed using PISA[56] and SurfRace[25]. All structure figures were made in Pymol (http://www.pymol.org).

Bi-molecular fluorescence complementation (BiMC) and immunofluorescence. C10 cells (mouse melanoma cells expressing nectin-1) were transfected with plasmids encoding gL (pPEP101)[57,58], EYFP-tagged gB (gBc, pCW803) and gH (gHn, pBR770) as described previously[15]. 20 h post transfection, cells were incubated with 250 µg ml^{-1} of soluble gD306 to trigger fusion. For blocking of BiMC, cells were exposed to 100 µg ml^{-1} of an appropriate anti-gH–gL monoclonal antibody (mAb) for 1 h at 37°C before adding soluble gD306. Cells were then incubated with gD306 for 1 h at 37°C. For immunofluorescence or EYFP fluorescence, cells were processed as described earlier[15] and labeled with a mixture of anti-gB mAbs, SS55 and A22. Samples were examined by confocal microscopy with a Nikon TE-300 inverted microscope coupled to a Perkin Elmer imaging system. A two-line argon krypton laser (488/514 and 568/647 nm) was used to excite the fluorescence of AlexaFluor 594 (590/617) and EYFP

(515/528). For testing expression of truncated gL mutant, C10 cells were transfected with plasmids for wild-type or mutant gL in the absence or presence of gH, and expression detected with anti-gH–gL mAb CHL2.

Fluorescence-activated cell sorting (FACS). Surface expression of the truncated gL mutant in C10 cells was detected using FACS with either the anti-gH–gL polyclonal antibody R176 or the mAb CHL2.

Luciferase fusion assay. The luciferase reporter assay[57,58] was used with some modifications[15]. Effector CHO-K1 cells (Chinese hamster ovary cells) were transfected with plasmids encoding HSV-1 gB (pPEP98[57,58]), gH, gD (pPEP99[57,58]), the T7 RNA polymerase (pCAGT7), plus either wild-type gL, truncated gL, or no gL. CHO-K1 cells transfected with plasmids encoding HVEM (pSC386[59]) and luciferase under control of the T7 promoter (pT7EMCLuc[57,58]) were used as receptor-bearing target cells. Target cells were co-cultivated with the effector cells for 20 h at 37°C. Luciferase production was assayed by luminometry using a BioTek plate reader.