Supplementary Information

Crystal structure of the conserved herpesvirus fusion regulator complex gH–gL.

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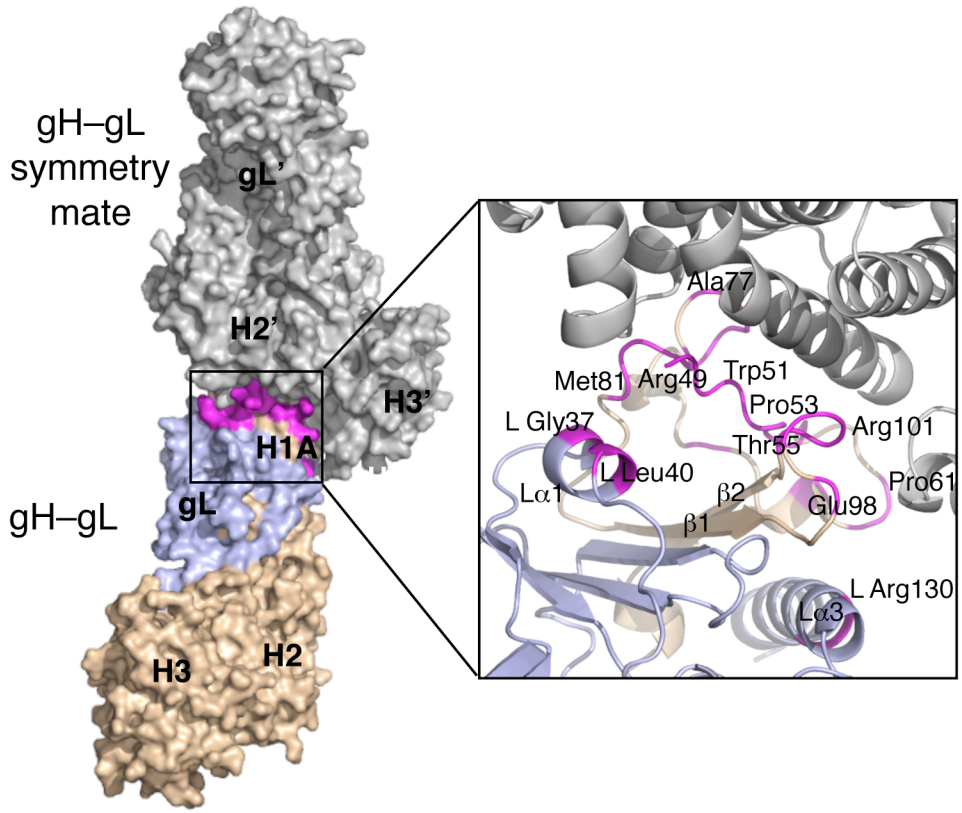
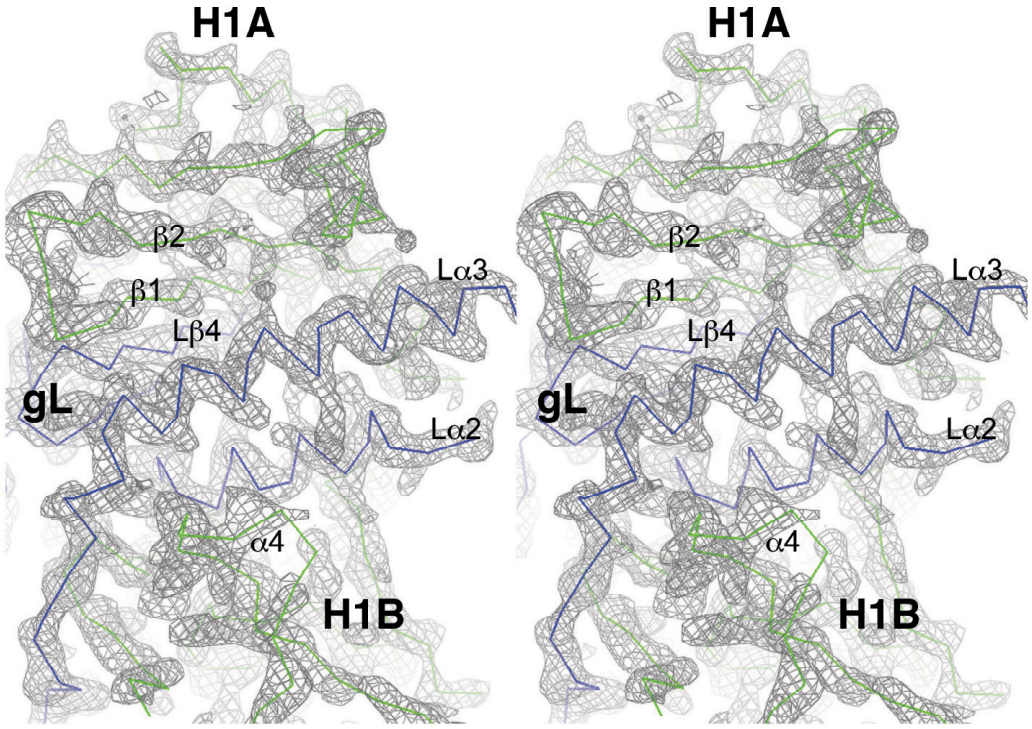
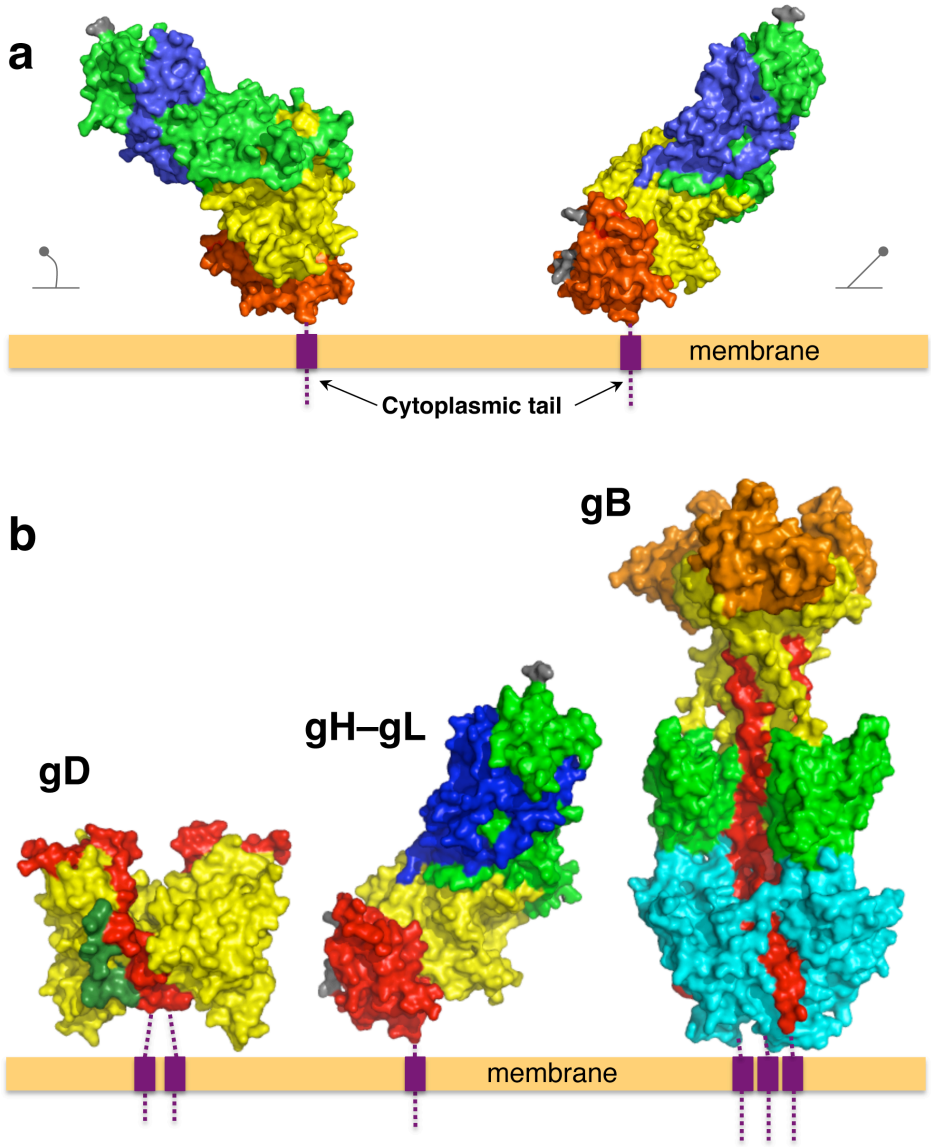


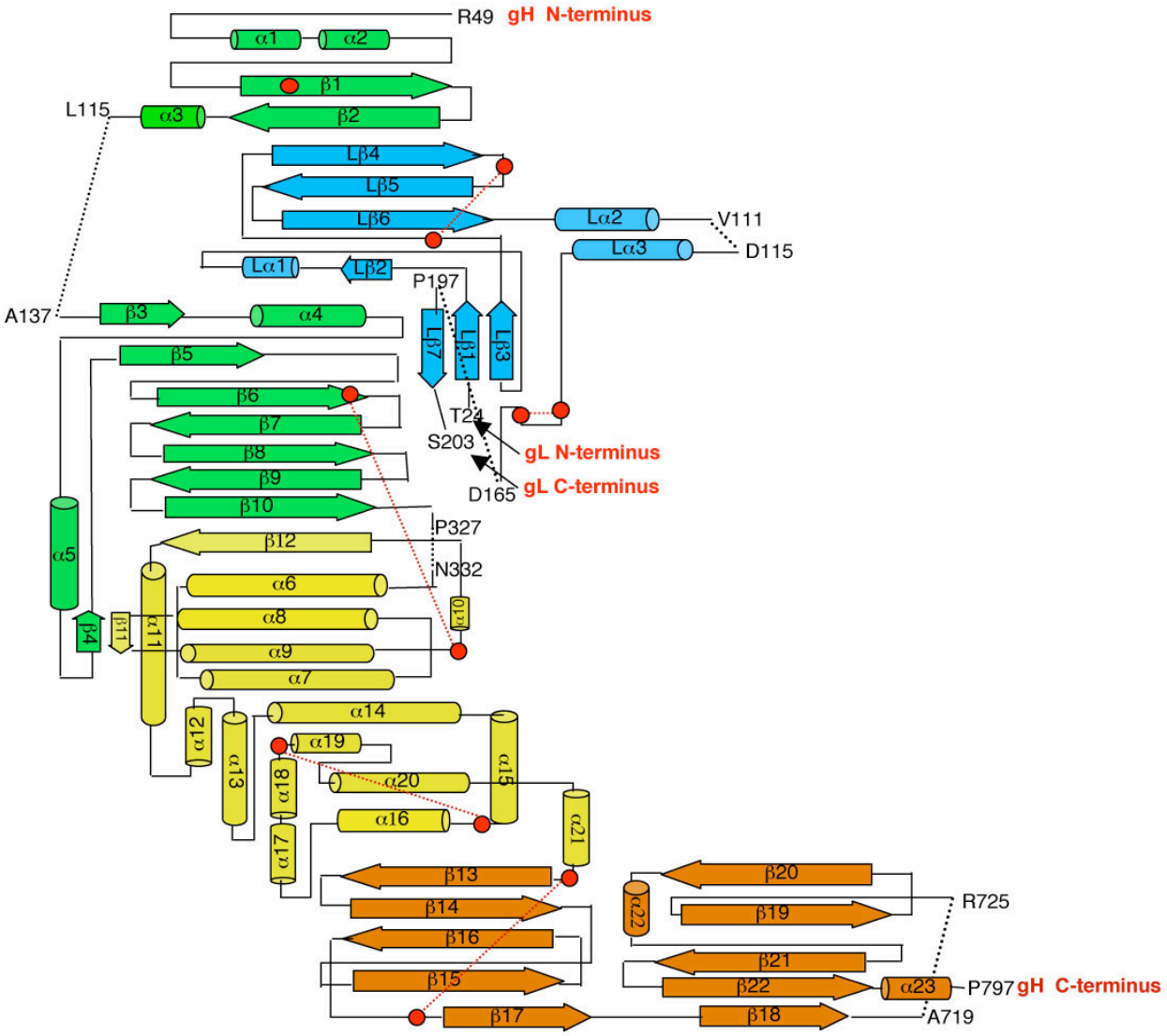
Supplementary Fig. 1. The N terminus of gH participates in crystal contacts. One gH–gL complex (beige and light blue) is shown in surface representation with domains labeled as in Fig. 1. 26 gH and 5 gL residues (magenta) make crystal contacts with a neighboring gH–gL symmetry mate (grey). View is rotated 90º counterclockwise along the vertical axis with respect to Fig. 1c. The inset shows a close-up view of crystal contacts at the interface, with molecules shown in ribbon representation. Selected residues making crystal contacts are labeled. If residues His19 to Thr47 were present, they would interfere with these crystal contacts, consistent with the fact that the full-length soluble gH–gL complex does not form crystals. Also, residues Tyr26 to Cys44 of HSV-1 gL are not accessible to antibodies when gL is in complex with gH[1]. However, in the structure, which lacks first 47 residues of gH, these gL residues are exposed, with residues Gly37, Leu40, and Arg41 involved in crystal contacts. Thus, the missing N-terminus could be located in the vicinity of gH residues Arg49 to Pro61 and gL residues Gly37, Leu40, and Arg41.



Supplementary Fig. 2. Electron density for the gH–gL structure. A stereo view of a representative section. Experimental selenomethionine SAD electron density map, contoured at 1.25σ is shown in grey. The model is shown as a Cα trace with gH in green and gL in blue. Secondary structure elements as well as domains are labeled.

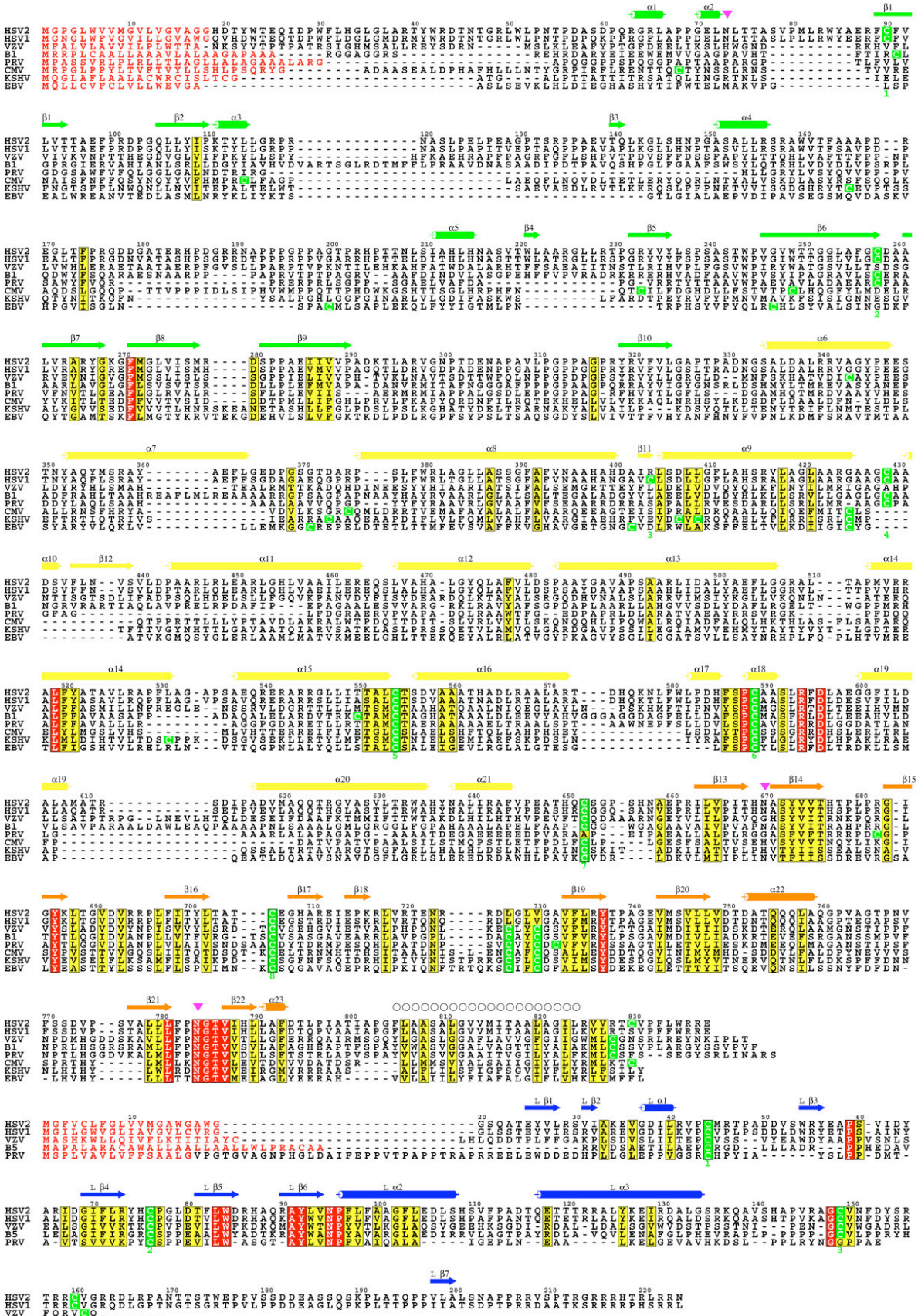


Supplementary Fig. 3. Proposed orientation of gH–gL with respect to the viral envelope or cell surface. a, Two different particle shapes detected on the virion surface[2] likely represent two different views of the same molecule. **b**, Relative sizes of HSV entry glycoproteins, HSV-2 gH–gL, HSV-1 gD (2c36) and HSV-1 gB (2gum). gB is in its presumed postfusion conformation; the prefusion conformation is unknown. Proteins are colored by domain[3,4] (Fig. 1). Transmembrane regions and cytoplasmic domains are shown schematically and not to scale with the ectodomains. Relative glycoprotein orientations may not reflect those on the viral envelope or cell surface.



Supplementary Fig. 4. Topology diagram of gH–gL. Coloring scheme is the same as in Fig. 1. β-strands are shown as arrows and α-helices as cylinders. Both are numbered numerically. All secondary structure elements are labeled. Cysteines are indicated as red circles and disulfides, as red dotted lines. Disordered regions are indicated as black dotted lines. Terminal residues as well as residues bordering disordered regions are labeled.

a

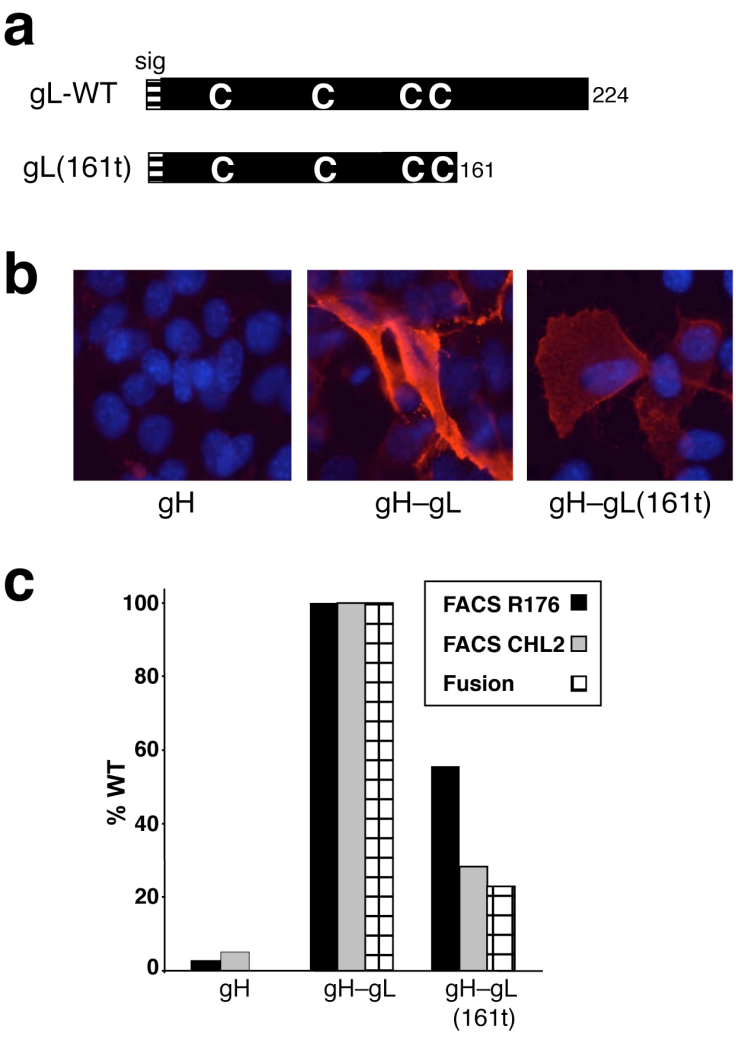

HSV2
HSV1
VZV
Bl
PRV
CMV
KSHV
EBV

b

HSV2
HSV1
VZV
Bl
PRV
CMV
KSHV
EBV

Supplementary Fig. 5. Alignment of amino acid sequences of herpesvirus gH and gL proteins. a, Alignment of secondary structure of Herpes Simplex virus 2 (HSV-2) gH with the sequences of gH proteins from several representatives of three major herpesvirus subfamilies. α-herpesviruses: HSV-2, strain HG52; Herpes Simplex virus 1 (HSV-1), strain KOS; Varicella-zoster virus (VZV); bovine herpesvirus type 1.1 (B1), strain Cooper; and Pseudorabies virus (PRV), strain Kaplan. β-herpesviruses: human cytomegalovirus (HCMV), strain AD169. γ-

herpesviruses: Kaposi's Sarcoma virus (KSHV); and Epstein-Barr virus (EBV). Residue numbering for HSV-2 is shown above its sequence. Signal sequences, absent from mature glycoproteins, are in red. Secondary structure elements are indicated above sequence alignment in colors corresponding to Fig. 1 and are labeled according to Supplementary Fig. 4. Purple triangles mark glycosylation sites. Open circles mark the predicted transmembrane region. Conserved cysteines are labeled sequentially underneath the alignment. Identical residues are boxed in red, conserved residues, in yellow, cysteines, in green. **b,** Alignment of secondary structure of HSV-2 gL with the sequences of gL proteins from several α-herpesviruses: HSV-2, strain HG52; HSV-1, strain KOS; VZV; bovine herpesvirus 5 (B5); and PRV, strain Kaplan. Residue numbering for HSV-2 is shown above its sequence. Signal sequences, absent from mature glycoproteins, are shown in red letters. Secondary structure elements are indicated above sequence alignment in blue and are labeled according to Supplementary Fig. 4. Conserved cysteines are labeled sequentially underneath the alignment. Identical residues are highlighted in red, conserved residues, in yellow, cysteines, in green. **C,** Sequence alignment of gH proteins from HSV-2, strains HG52 and 333, and HSV-1, strains KOS and 17, and sequence alignment of gL proteins from HSV-2, strain HG52, and HSV-1, strains KOS and 17. Identical residues are boxed in red.



Supplementary Fig. 6. The C-terminal truncation of HSV-2 gL decreases the surface expression and with it, the cell-cell fusion activity of gH–gL. a, Schematic diagram showing wild-type (WT) HSV-2 gL and the C-terminal truncation mutant gL(161t). The signal sequence (sig, hatched box) and the cysteine residues (denoted as "C") are shown. **b,** Cell surface expression of HSV-2 gH–gL (red), as seen by immunofluorescence using MAb CHL2. Cell nuclei were stained with DAPI (blue). **c,** Cell surface expression and cell-cell fusion activity of the gH–gL(161t) complex, expressed as percent of WT control. Surface expression was assayed using fluorescence-activated cell sorting (FACS) and either the anti-gH–gL pAb R176 (black column) or the mAb CHL2 (gray column). Cell-cell fusion activity was assayed quantitatively using luciferase assay (checkered column).

Supplementary Table 1. **gH–gL MAbs used in BiMC experiments.**

Mab	BiMC*	Neutralization†	epitope†
none	+++		
52S	+++	yes	discontinuous
53S	+++	no	discontinuous
MP6	+++	no	19-276
MP7	+++	no	19-276
MP8	+++	no	19-276
CHL2	+++	no	discontinuous
H12	+++	no	475-648
LP11	-/+	yes	discontinuous

*BiMC stands for bi-molecular fluorescence complementation. +++ indicates little to no change in the fluorescence compared to no antibody control. -/+ indicates a signal barely above the background.

†References for the MAbs: 52S, 53S[5], H12, MP series[1], CHL2[6], LP11[7-9].

Supplementary references

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