## ONLINE METHODS

**Cell lines.** Vero, Vero-SLAM [48], Vero-His [38] and BHK-T7 [49] cells were propagated in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10 % (v/v) fetal bovine serum (FBS). Vero-SLAM, Vero-His and BHK-T7 cells were also supplemented with 100 μg $ml^{-1}$ G418.

**Plasmids and mutagenesis.** Mutations were introduced into the MV H expression plasmid (pCG-H) [50] using the QuikChange Mutagenesis Kit (Stratagene, La Jolla, CA) according to the manufacturer's instructions. All cysteine substitution mutants were introduced in a vaccine-lineage H backbone (H-NSe) [51], while the hexahistidine insertions were also made in the wild type H-protein backbone (H-wt-323) [52].

**Selection of MV H dimer interface residues for cysteine substitution.** Designs were based on the MV H dimer crystal structure (PDB-code 2ZB5). First, we identified the dimer interface residues with the program GetArea [53]. Within this set of residues we then identified pairs across the dimer interface, which have their C-alpha atoms 3.0–7.5Å apart. These two filters reduced the number of potential cysteine bonded pairs to 10. Four of these residues located near the symmetry axis were predicted to bond with the same residue on the other subunit, forming a single disulfide bond, the other residues were predicted to form twin bonds, and sometimes one residue had two potential partners.

**Mammalian cell transfection and fusion assays.** Lipofectamine 2000 (Invitrogen, Carlsbad, CA) was used for all mammalian cell transfections according to the manufacturer's instructions. The standard fusion assay was performed as described [27]. Briefly, 0.8 μg each of pCG-H, the fusion protein expression plasmid, pCG-F and

peGFP (enhanced GFP expression plasmid) were transfected into Vero, Vero-SLAM or Vero-His cells ($1.5\times10^5$ cells). Fusion scores were determined 24 hours post-transfection. A fusion score of 3 denotes wild type fusion levels; 2 and 1, intermediate fusion; and 0, no fusion. In order to rescue the fusion function of cysteine substitution mutants, 24 hours post-transfection, Vero cells were treated for 20 min with 12 mM DTT in phosphate-buffered saline (PBS). Fusion was recorded 1–2 hours post DTT treatment.

**Quantitative fusion assay.** BHK-T7 cells ($1.5\times10^5$) in a 24-well plate were transfected with 0.5 µg each of pCG-H and pCG-F plasmids. A similar number of Vero, Vero-SLAM or Vero-His cells were transfected with 0.35 µg of pTM1-luc (luciferase expression plasmid under the control of T7 polymerase). Six hours post-transfection, the Vero, Vero-SLAM or Vero-His cells were washed three times with PBS and detached by Versene treatment and resuspended in DMEM (10% v/v FBS). The BHK-T7 cells were washed three times with PBS and overlaid with $1.5\times10^5$ Vero, Vero-SLAM or Vero-His cells. Six, 12- or 18-hours post-overlay, the luciferase expression levels were quantified using the Steady-Glo Luciferase Assay System (Promega, Fitchburg, WI).

**Immunoblot analysis.** For immunoblot analysis, $4\times10^5$ Vero cells in a 12-well were transfected with 1.6 µg of the relevant pCG-H expression plasmid. Twenty-four hours post-transfection the cells were washed with PBS and cytoplasmic extracts generated using 0.15 ml of lysis buffer (150 mM NaCl, 1.0% v/v NP-40, 0.5% w/v deoxycholate, 0.1% w/v sodium dodecyl sulfate [SDS], 50 mM Tris-HCl [pH 8.0]) with protease inhibitors (Complete; Roche Biochemicals, Basel, Switzerland). The lysis buffer was supplemented with 10 mM iodoacetamide for extraction of the cysteine mutant H-

proteins. The extracts were mixed with an equal volume of 2× Laemmli sample buffer and separated on a 4–15% SDS-PAGE gel in the presence or absence of β-mercaptoethanol. The proteins were transferred onto a PVDF membrane and probed with an anti-H (cytoplasmic tail) primary antibody [54] and a horseradish peroxidise conjugated goat-anti-rabbit secondary antibody (Jackson ImmunoResearch, West Grove, PA). Protein bands were revealed using the ECL Western Blotting Substrate (Pierce, Rockford, IL). β-actin expression levels were used as loading controls.

**FACS Analysis.** To quantify cells surface expression of the mutant H-proteins, $5\times10^5$ Vero cells were transfected with 4 μg of the corresponding mutant. Twenty-four hours post-transfection cells were harvested by Versene (Gibco) treatment. Cells were incubated with an anti-H ectodomain antibody (MAB8905; Chemicon), a conformation specific antibody (BH6 [37]), or an anti-His antibody (Qiagen) depending on the mutant H-protein. Primary antibodies were diluted 1:50 in FACS wash buffer (PBS, 2% v/v FCS and 0.1% w/v sodium azide) and incubated with cells for 1 hour at 4°C. Cells were washed 3× with FACS wash buffer prior to incubation with a FITC-conjugated secondary antibody (Jackson ImmunoResearch, West Grove, PA) for 1 hour at 4°C. Cells were washed 3× with FACS wash buffer and fixed with 2% (v/v) paraformaldehyde prior to FACS analysis. The H-expressing cells were gated and the mean fluorescence intensity determined for each mutant.