SUPPLEMENTARY METHODS

Viruses and cells. Virus stocks were grown in Vero (African green monkey kidney epithelial) cells. Titers of virus stocks were determined on Vero cells by a focus-forming assay (FFA). Vero

cells were maintained in Dulbecco's modified Eagle medium (DMEM) containing 5% heat-inactivated fetal bovine serum (FBS) and L-glutamine at 37°C with 5% CO_2 containing 2% fetal bovine serum (FBS), L-glutamine, and HEPES at 37ºC with 5% CO_2. HSV-1 strain NS was obtained from Dr. Harvey Friedman (University of Pennsylvania) (18). HSV-2 strain 333 was obtained from Dr. Steven Bachenheimer (UNC). Virus stock titers were quantified by focus-forming assay on Vero cells. Viral foci were detected using 1:10,000 dilution of αHSV rabbit antibody (Dako #B0114) and 1:50,000 dilution of goat αrabbit HRP conjugated antibody (Sigma #12-348), and TrueBlue peroxidase substrate (KPL). Antibody incubations were performed for at least 1 hour at room temperature. Foci were counted on a CTL Immunospot analyzer.

Mice. All experiments and husbandry were performed under the approval of the University of North Carolina at Chapel Hill Institutional Animal Care and Use Committee. Experiments used 8-12-week-old male and female mice on a C57BL/6 background, bred in-house. SKH-1 (Charles River strain #477) were received from Dr. Brian Conlon (UNC) and 10-12 week-old male and female mice were used for experiments.

HSV skin infections. One day prior to infection, mice were anesthetized by nose-cone isoflurane and depilated by plucking on the right flank unless otherwise indicated. One day later, mice were anesthetized by chamber isoflurane for infections. To perform infections, we abraded the skin of anesthetized, depilated mice with ~10 closely spaced punctures (~5mm^2 total area) using a Quintip allergy needle (Hollister Stier #8400ZA). Immediately after abrasion, we overlaid 10 µL of viral inoculum (virus + 1% FBS in PBS) and allowed the inoculum to dry while mice were anesthetized.

Viral load measurements. Viral genomes were quantified from skin that was homogenized in 500 µL of PBS and silica beads on a MagNAlyser instrument (Roche). DNA was then extracted from 200 µL of homogenate using the Qiagen DNeasy Blood & Tissue Kit (#69504). Extracted HSV-1 genomes were then quantified by TaqMan qPCR on a CFX96 Touch real-time PCR detection system (Bio-Rad) against a standard curve generated by extracting DNA from an HSV-

1 stock at 10^8 FFU/mL and serially diluting. HSV-1 genomes were detected using the following primers against the UL23 gene: F primer 5′-TTGTCTCCTTCCGTGTTTCAGTT-3′, R primer 5′-GGCTCCATACCGACGATCTG-3′, and probe 5′-FAM-CCATCTCCCGGGCAAACGTGC-MGB-NFQ-3′ (19).

Lesion area measurements. To measure HSV lesion areas, mice were anesthetized and photographed using an iPhone camera next to a ruler and an identifying card. Thereafter, images were analyzed using Image J in which pixels were converted to millimeters using the reference ruler and then lesions were outlined using the freehand tool and calculated areas within the freehand designations were reported.