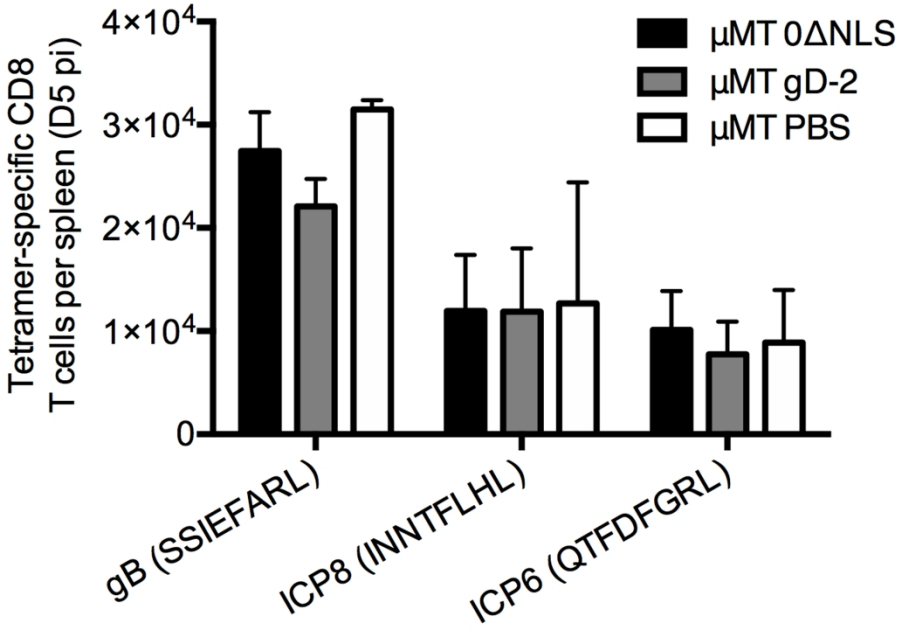
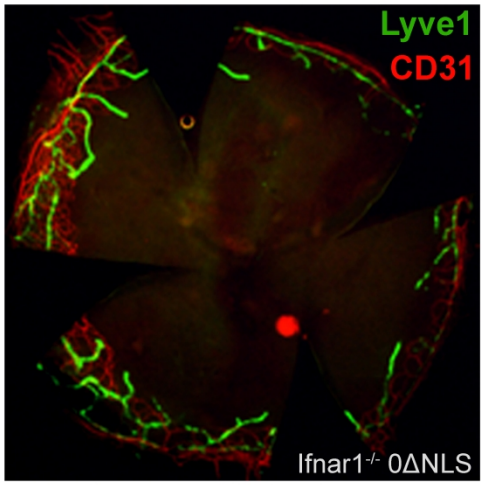
Supplementary Figure 1 (Related to Figure 4)

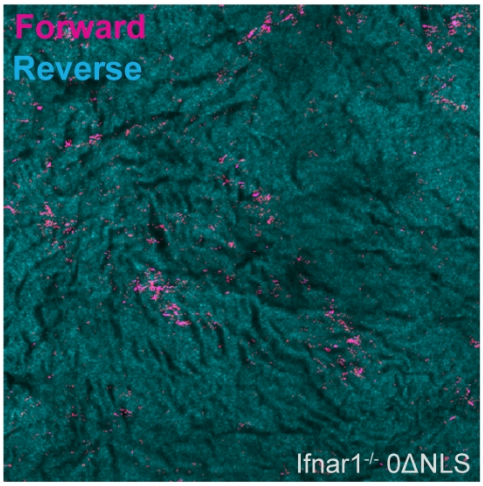


Supplementary Figure 2 (Related to Figure 7)

0ΔNLS-vaccinated Ifnar1$^{-/-}$ cornea, day 30 post-infection



Neovascularization



Fibrillar collagen SHG signal

Supplementary Materials:

List of Supplementary Materials

- Supplementary Methods
- Supplementary Figure Legends:
    - Fig. S1. (Related to Figure 4): The HSV-1 0ΔNLS vaccine does not appreciably expand the HSV-specific CD8 T cell repertoire in B cell-deficient μMT mice.
    - Fig. S2. (Related to Figure 7): Ifnar1$^{-/-}$ mice vaccinated with HSV-1 0ΔNLS do not exhibit corneal neovascularization or pathologic collagen remodeling following ocular HSV-1 challenge.

Supplementary Methods: Mass Spectrometry for Identification of Viral Targets

LC-MS/MS Analysis

Trypsin digest of immunoprecipitated proteins was performed according to the filter-aided sample preparation (FASP) protocol.[78] Briefly, the eluate was buffer exchanged to 8M urea, the proteins were reduced with 10mM DTT (dithiothreitol) and then alkylated with 10mM iodoacetamide. The peptides were eluted in 10mM ammonium acetate pH 8.0, dried and resuspended in 10mM ammonium formate pH 10.0. Liquid chromatography tandem mass spectrometry was performed by coupling a nanaoAcquity UPLC (Waters Corp., Manchester, UK) to a Q-TOF SYNAPT G2S instrument (Waters Corp., Manchester, UK). Each protein digest (about 100ng of peptide) was delivered to a trap column (300 µm × 50 mm nanoAcquity UPLC NanoEase Column 5 µm BEH C18, Waters Corp, Manchester, UK) at a flow rate of 2 µl/min in 99.9% solvent A (10mM ammonium formate pH 10, in HPLC grade water). After 3 min of loading and washing, peptides were transferred to another trap column (180 µm × 20 nanoAcquity UPLC 2G-V/MTrap 5 µm Symmetry C18, Waters Corp, Manchester, UK) using a gradient from 1% to 60% solvent B (100% acetonitrile). The peptides were then eluted and separated at a flow rate of 200 nL/min using a gradient from 1% to 40% solvent B (0.1% FA in acetonitrile) for 60min on an analytical column (7.5 µm × 150 mm nanoAcquity UPLC 1.8 µm HSST3, Waters Corp, Manchester, UK). The eluent was sprayed via PicoTip Emitters (Waters Corp, Manchester, UK) at a spray voltage of 3.0 kV and a sampling cone voltage of 30 V and a source offset of 60 V. The source temperature was set to 70 °C. The cone gas flow was turned off, the nano flow gas pressure was set at 0.3 bar and the purge gas flow was set at 750 ml/h. The SYNAPT G2S instrument was operated in data-independent mode with ion mobility (HDMSe). Full scan MS and MS2 spectra (*m*/*z* 50 - 2000) were acquired in resolution mode (20,000 resolution FWHM at *m*/*z* 400). Tandem mass spectra were generated in the trapping region of the ion mobility cell by using a collisional energy ramp from 20 V (low mass, start/end)

to 35 V (high mass, start/end). A variable IMS wave velocity was used. Wave velocity was ramped from 300 m/s to 600 m/s (start to end) and the ramp was applied over the full IMS cycle. A manual release time of 500 µs was set for the mobility trapping and a trap height of 15 V with an extract height of 0 V. The pusher/ion mobility synchronization for the HDMSe method was performed using MassLynx V4.1 and DriftScope v2.4. LockSpray of Glufibrinopeptide-B (*m*/*z* 785.8427) was acquired every 60 s and lock mass correction was applied post acquisition.

Protein Identification

Raw MS data were processed by PLGS (ProteinLynx Global Server, Waters Corp., Manchester, UK) for peptide and protein identification. MS/MS spectra were searched against the Uniprot HSV-1 proteome database (release date November 2, 2017 containing 1,776 unreviewed sequences) with the following search parameters: full tryptic specificity, up to two missed cleavage sites; carbamidomethylation of cysteine residues was set as a fixed modification and N-terminal protein acetylation and methionine oxidation were set as variable modifications. Proteins reported were identified in ≥2 out of 5 samples per group.

Supplementary Figure Legends:

Figure S1 (Related to Figure 4A): The HSV-1 0ΔNLS vaccine does not appreciably expand the HSV-specific CD8 T cell repertoire in B cell-deficient μMT mice.

MHC class I tetramers were utilized to quantify the numbers of HSV-specific CD8 T cells in the spleen of vaccinated and naive B cell-deficient μMT mice at day 5 post-infection as in Fig. 4A for wild-type mice. No differences were detected between groups by one-way ANOVA for each epitope-specific tetramer (*n* = 4 vaccinated and 2 naive mice per group from 2 independent experiments). Viral proteins and epitope amino acid sequences are listed. gB, glycoprotein B; ICP, infected cell protein.

Figure S2 (Related to Figure 7): Ifnar1$^{-/-}$ mice vaccinated with HSV-1 0ΔNLS do not exhibit corneal neovascularization or pathologic collagen remodeling following ocular HSV-1 challenge.

Ifnar1$^{-/-}$ mice were vaccinated with HSV-1 0ΔNLS, challenged ocularly with 1x10^4 PFU HSV-1 McKrae, and evaluated at day 30 post infection for pathological corneal neovascularization (left) and collagen remodeling by second harmonic generation (SHG) microscopy (right). Representative images taken from three mice are shown.