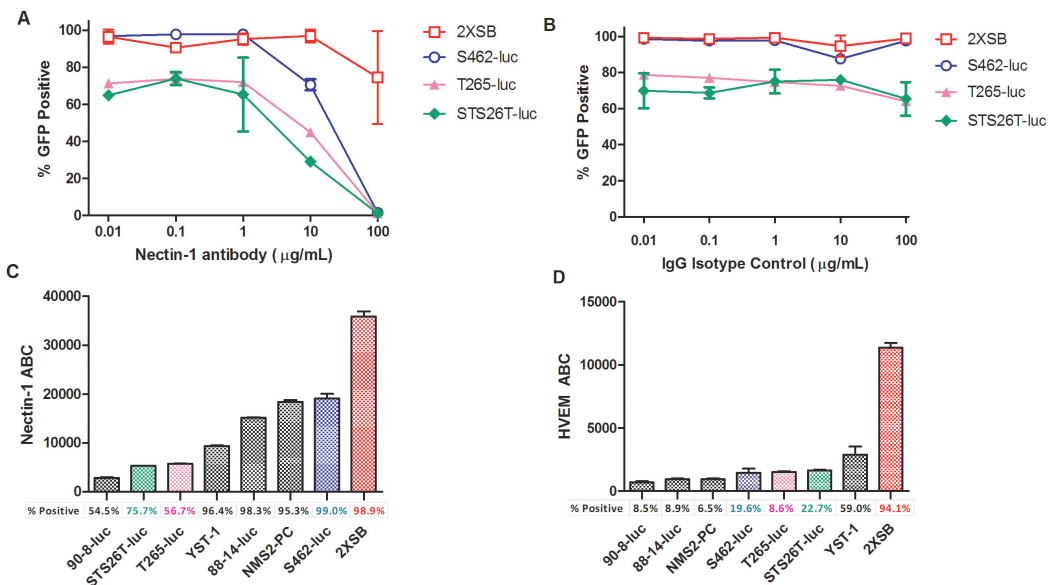
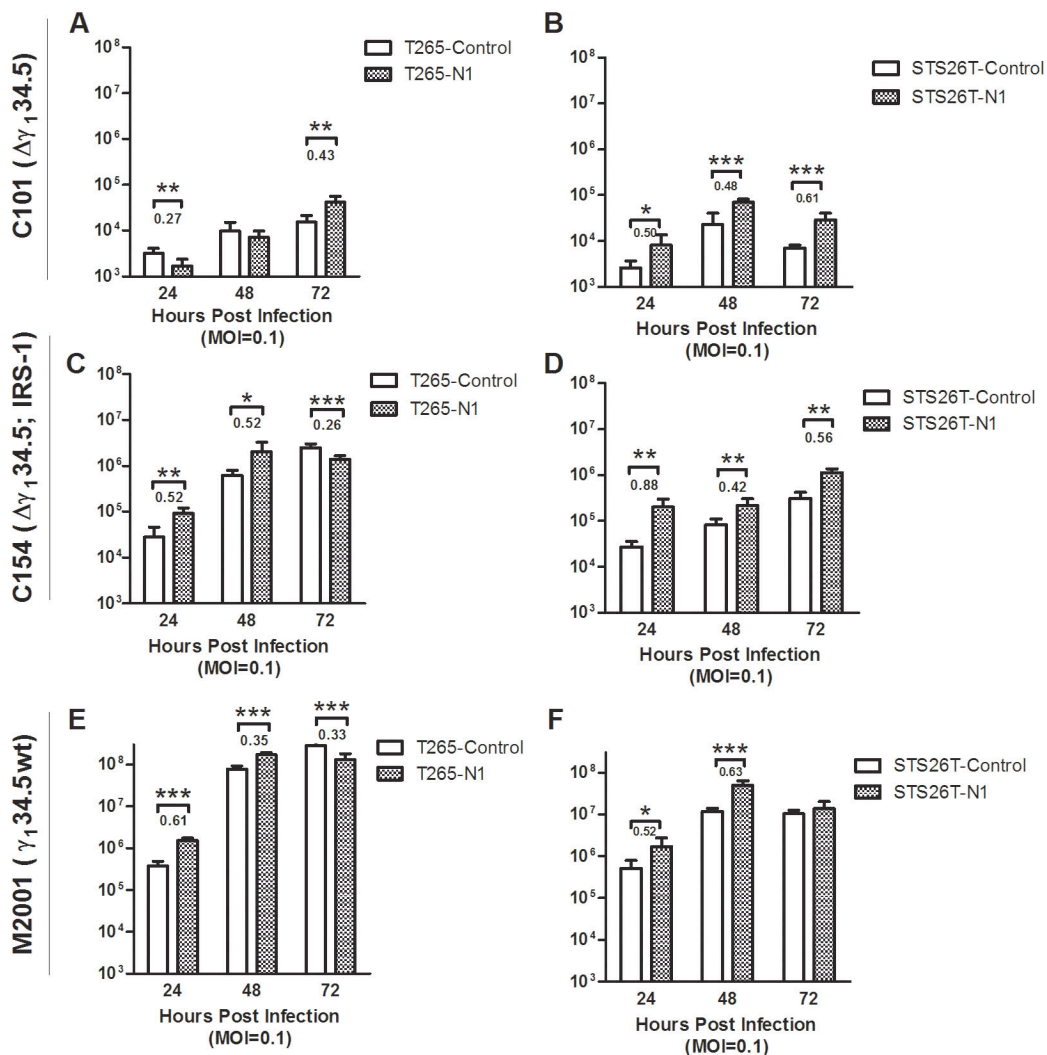


Supplemental Figure 1: Single-step viral recovery in MPNST cell lines. MPNST cell lines were subjected to single-step (MOI=10, 24 hr) infection by viruses M2001 (a), C134 (b), C101 (c), G207 (d), and R7020 (e) and the viral titers reported as the average total plaque forming units (PFU) as determined through limiting dilution plaque formation assay. Data were collected in triplicate and the standard deviation reported.



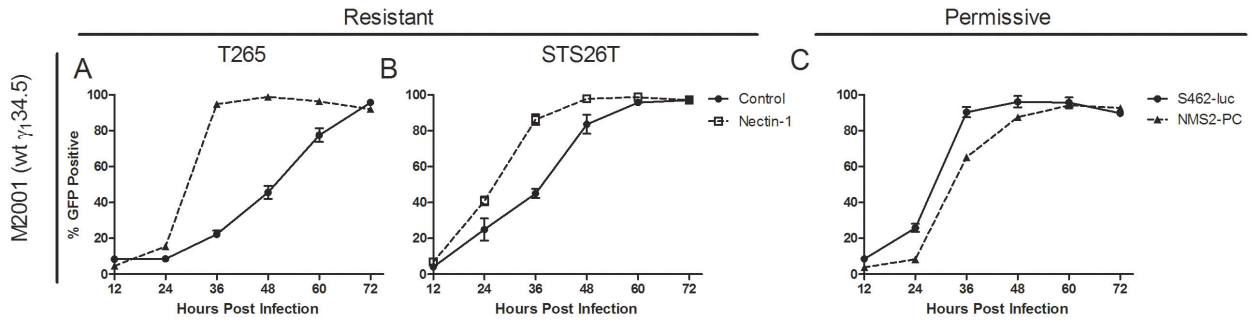
Supplemental Figure 2: Nectin-1 dependence of productive HSV-1 infection in resistant MPNST cell lines. MPNST cells were seeded  $3 \times 10^4$  cells per well in 96 well plates and allowed to adhere overnight. Nectin-1 antibody (clone R1.302, Biolegend) (A) or mouse IgG1 isotype control (Cell Signaling) (B) was diluted in growth media and incubated with cells at 4°C for 30

minutes. Cells were then infected with GFP expressing wt HSV-1 M2001 at an MOI of 5 and incubated at 37 °C for 20 hrs (S26T-luc, T265-luc, and S462-luc) or 12 hrs (2XSB). The earlier timepoint of analysis for 2XSB was necessary due to the rapid infection and deterioration of these cells at longer timepoints. Cells were trypsinized and resuspended in FACSs buffer and analyzed by flow cytometry for GFP expression. The % GFP positive is calculated as the percentage of viable cells which have a GFP intensity greater than an uninfected control. Measurements were taken in duplicate and the standard deviation reported. Nectin-1 (C) and HVEM (D) expression was quantified by flow cytometry after incubation with PE-conjugated mouse monoclonal antibody (Biolegend) with subsequent quantification using antibody quantification beads (Bangs Labs). The percentage of cells staining above the isotype control is also reported. Receptor quantification was performed in triplicate with the standard deviation reported.

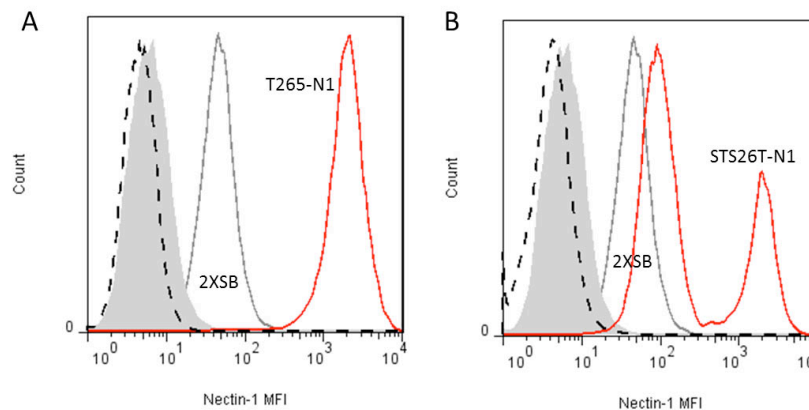


Supplemental Figure 3: Impact of increased nectin-1 expression on multi-step viral replication. Multi-step (MOI=0.1) replication assays using C101 (A,B), C154 (C,D), and M2001 (E,F)

viruses were performed in S26T-luc and T265-luc transduced with nectin-1 or control lentivirus. Samples were collected at the indicated timepoints following infection and titered using standard limiting dilution plaque formation assays. Data were collected in triplicate and the standard deviation reported. Comparison was made between nectin-1 overexpressing cells and control by 2-tailed Student's t-test. Significance was set at  $P < 0.05$ . For cells with significant changes in titer, the logarithm of the increase is reported below the significance marking.



Supplemental Figure 4: Impact of increased nectin-1 expression on wild-type HSV-1 spread in vitro. Resistant cell lines T265-luc (A) and STS26T-luc (B) and their nectin-1 transduced variants as well as permissive cell lines S462-luc and NMS2-PC (C) were infected in a multi-step assay (MOI=0.1) with GFP expressing wild-type HSV-1 M2001 and monitored over time for viral infection as evident by expression of viral GFP monitored by flow cytometry.



Supplemental Figure 5: Comparison of nectin-1 expression in 2XSB to nectin-1 overexpressing cell lines T265-N1 (A) and STS26T-N1 (B). Cell lines were analyzed by flow cytometry as described in the submitted manuscript. Dashed black or solid red lines respectively represent unlabeled or nectin-1 labeled resistant cell lines T265-N1 or STS26T-N1. Resistant cell lines in each graph are shown to express nectin-1 at an intensity greater than the highest natural nectin-1 expressing cell line 2XSB (gray line). Unlabeled 2XSB is also shown (shaded gray).