Supplemental Figures

Figure S1, related to Figure 2

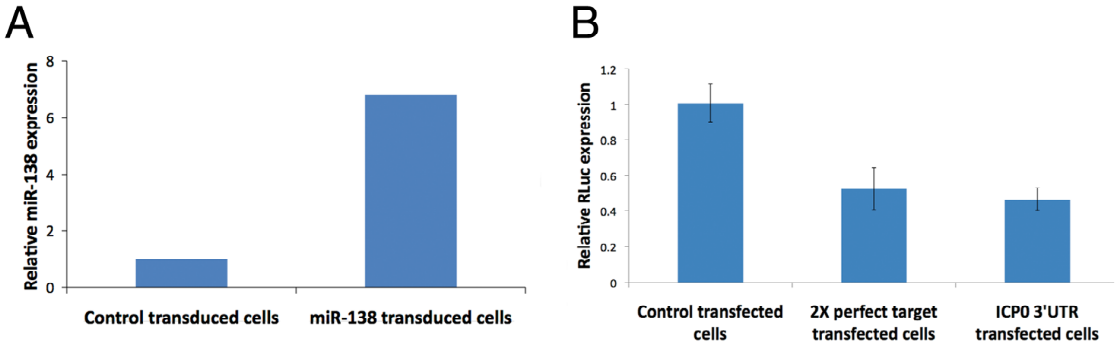


Figure S2, related to Figure 3

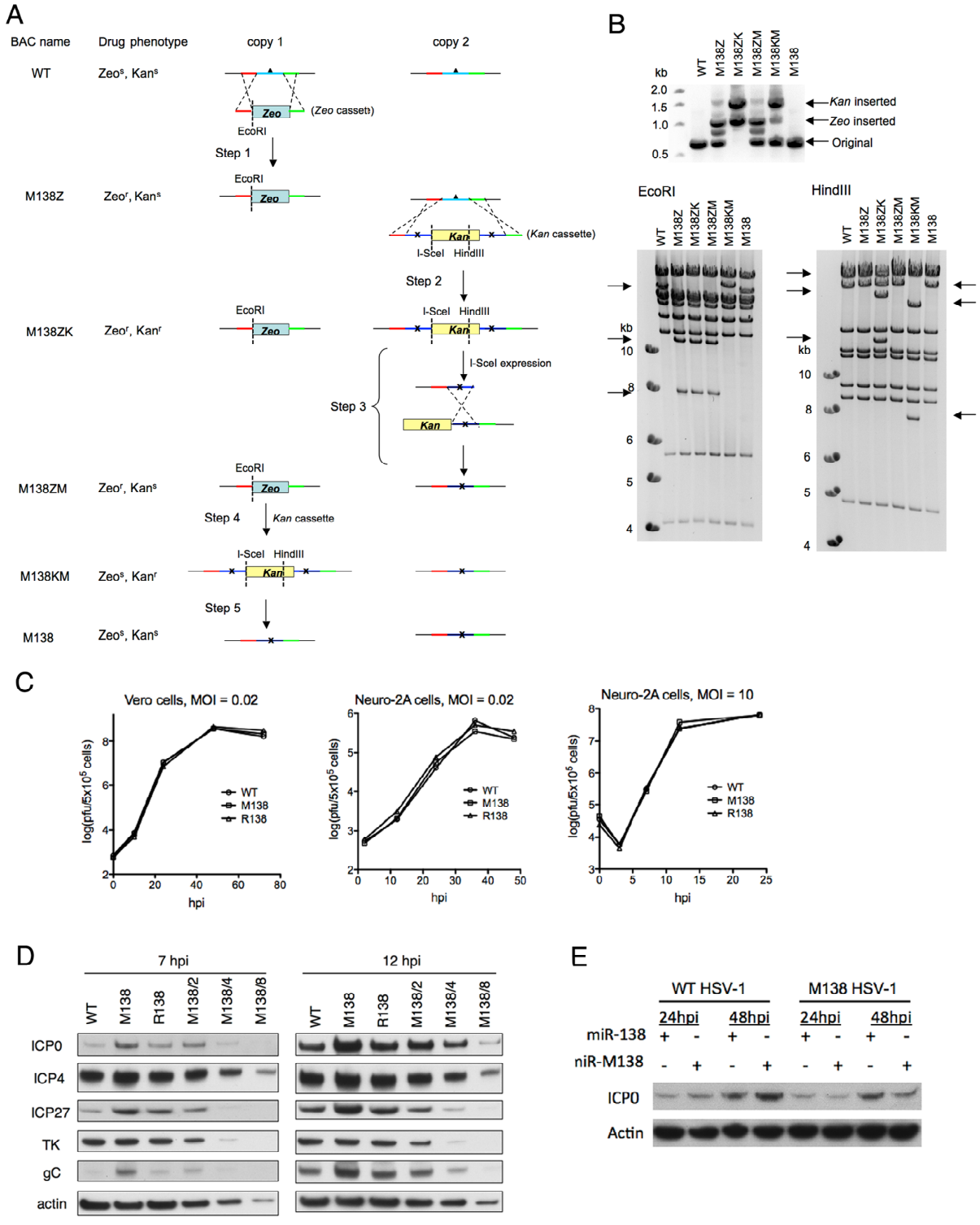


Figure S3, related to Figure 4

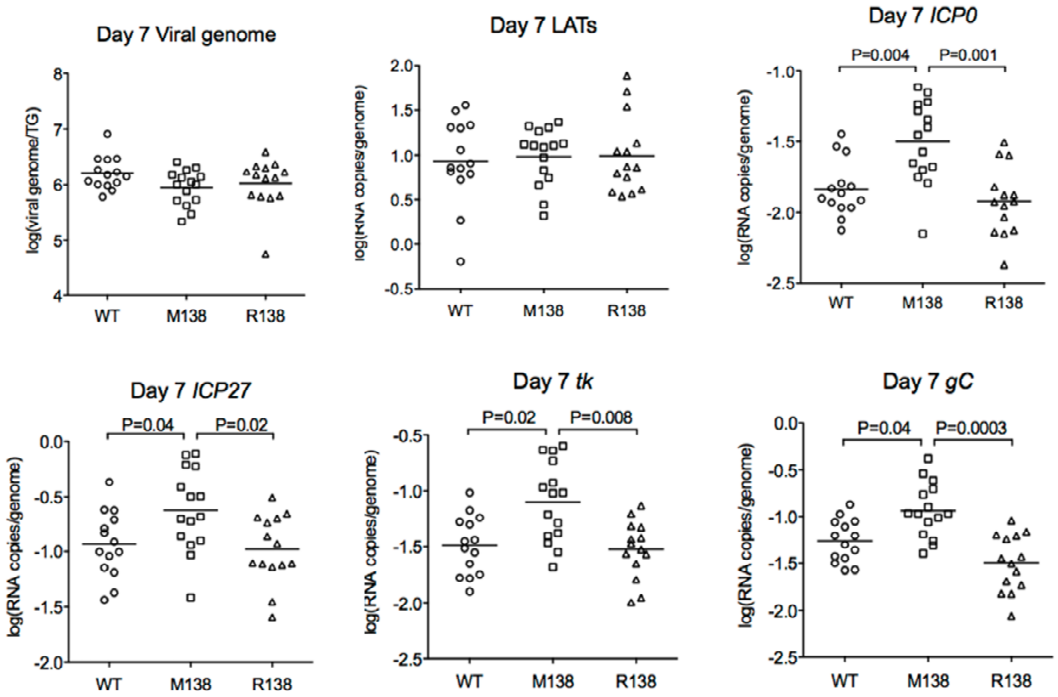
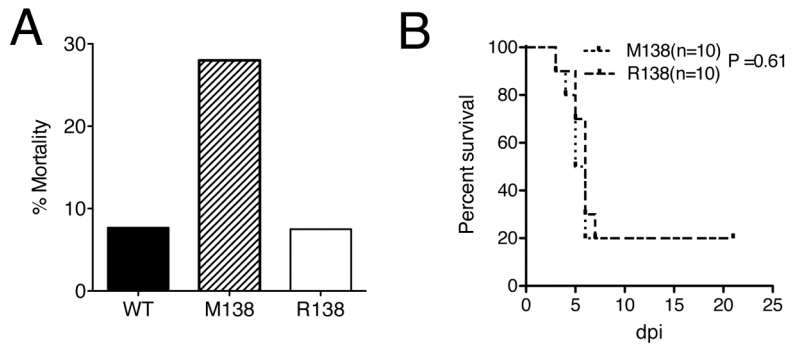


Figure S4, related to Figure 5



Supplemental Figure Legends

Figure S1. Characterization of control and miR-138 transduced 293 cells. (A) Levels of miR-138 expression relative to U6 RNA expression in cells transduced with a control vector and those with a miR-138 expression vector as determined by qRT-PCR, with the relative level in control vector transduced cells set to 1. (B) Repression of rluc expression in miR-138 transduced cells. The indicated rluc expression plasmids were transfected into 293 cells that had been transduced with miR-138 expression vector. Luciferase activities at 24 h post-tranfection are shown.

Figure S2. Construction and characterization of mutant and rescued viruses. (A) Generation of the M138 mutant. Five steps were involved. The name of the BAC and its antibiotic resistance or sensitivity (Drug) phenotypes are listed in the left two columns. Zeo^r, zeocin resistant; Kan^r, kanamycin resistant; Zeo^s, zeocin sensitive; Kan^s, kanamycin sensitive. The two columns to the right show the changes in sequence to be mutated (mutation site) in the two copies of the *ICP0* locus. Restriction sites are indicated with vertical dashed lines. Horizontal colored lines symbolize identical sequences, whereas diagonal dashed lines indicate single homologous recombination events. Cyan line with a triangle in the middle, original sequence (in this case WT); blue lines with an X, new sequence (in this case containing the M138 mutations). Step 1, the *Zeo* cassette (light blue box) is PCR-amplified to add approximately 40 bp of the HSV-1 sequences flanking each side of the mutation site (red and green). Recombination replaces the original site in copy 1 with the *Zeo* cassette. Step 2, the *kan* cassette (yellow box) is PCR-amplified to add approximately 40 bp of the HSV-1 sequences flanking each side of the mutation site (red and green), and 57 bp of overlapping modified HSV-1 sequences including changes in both miR-138 target sites 1 and 2 (blue with an X) (Figure 1B). Recombination replaces the original sequence in copy 2 with the *Kan* cassette. Step 3, two-step Red-mediated recombination

results in replacement of copy 2 with the modified HSV-1 sequences containing the M138 mutations, resulting in Kan^s and Zeo^r colonies identified using replica plating. Step 4, the *Zeo* cassette in copy 1 is replaced with the *Kan* cassette used in step 2. Step 5, the *Kan* cassette in copy 1 is replaced with the modified HSV-1 sequences containing the M138 mutations, as in step 3. The same procedures were used for generation of the R138 BAC from the M138 BAC except that WT sequences were used to replace sequences containing the M138 mutations. (B) Analysis of BACs from each step of the generation of the M138 mutant. In all 3 gels, the left-most lane is a DNA ladder with sizes indicated in kilobasepairs (kb), and BAC names are labeled on top of the gels. Top gel, PCR products from each BAC using primers spanning the mutation site (insertion of *Kan* or *Zeo* or replacement with M138 sequences) were analyzed using a 1 % agarose gel, and stained with ethidium bromide. The arrows indicate positions of bands corresponding to no insertion (Original) or insertion of *Kan* or *Zeo*. Bottom left gel, The indicated BAC genomes were digested with EcoRI and analyzed using a 0.75% agarose gel, stained with ethidium bromide. Insertion of a *Zeo* cassette (0.4 kb and containing an EcoRI site in copy 1 converted an 18.2 kb band to a 11.1 kb and a 7.5 kb band in M138Z, M138ZK and M138ZM as indicated by arrows. Insertion of a *Kan* cassette (about 1 kb) in copy 1 converted the 18.2 kb band to one with lower mobility in M138KM. Bottom right gel, The indicated BAC genomes were digested with HindIII, and analyzed using a 0.75% agarose gel stained with ethidium bromide. Insertion of a *Kan* cassette (~1 kb, containing a HindIII site) in copy 2 resulted in the conversion of a 34.6 kb band in M138Z to a 22.6 kb and a 13.0 kb band in M138ZK as indicated by arrows pointing from the left. However, since the original 34.6 kb band overlaps with another band of a similar size (only ~0.6 kb different), its disappearance is not readily discernible. Insertion of a *Kan* cassette in copy 1 resulted in conversion of a 26.6 kb band

in M138ZM to a 20.1 kb and a 7.5 kb band in M138KM as indicated by arrows pointing from the right. The same patterns of PCR and digestion products were detected during the generation of the R138 BAC from the M138 BAC (not shown). (C) M138 virus exhibits no defect for replication in cell culture. In each plot of viral replication kinetics, the cells and MOIs used are indicated at the top. Each point represents the geometric mean titer measured in triplicate. (D) Magnitudes of effects on viral gene expression. The experiment in Figure 3C was repeated for the 7 and 12 h time points, and analyzed using SDS-PAGE and Western blotting using antibodies against the proteins indicated to the left alongside a dilution series prepared from cells infected with M138. Two fold-dilution is indicated as M138/2, etc. (E) Transfected miR-138 reduces ICP0 expression in HSV-1 infected 293T cells. 293T cells were transfected with mimics of miR-138 or miR-M138 8 hours prior to infection with WT or M138 viruses as indicated at the top of the figure. At the times indicated after infection, cells were lysed and ICP0 levels (upper panels) and actin levels as loading controls (lower panel) were analyzed by Western blot.

Figure S3. Replicate experiment measuring viral genome and transcript levels 7 days after infection of mice with WT, M138, or R138. The experiment was performed and the data are presented in exactly the same way as the experiment in Figure 4C.

Figure S4. Effects on mortality following different routes of inoculation. (A) Mortality of infected mice following corneal inoculation in the experiment shown in Fig. 5A. Vertical bars represent percentages of mice that died before 32 dpi. The viruses are indicated at the bottom. (B). Survival curves for mice infected intracranially by the viruses indicated. Total numbers of mice used and the P value by Log-rank test are shown for each virus. The survival curve (plotted according to criteria described in Supplementary Experimental Procedures) represents combined data from two independent experiments.