## Supplemental Tables

**Table S1, related to Figure 4**. $R^2$ values for correlation between *ICP0* transcripts and other transcripts in different TG at 7 dpi.

| Transcript | WT | M138 | R138 |
|---|---|---|---|
| *ICP4* | 0.54 | 0.81 | 0.61 |
| *ICP27* | 0.34 | 0.68 | 0.63 |
| *tk* | 0.30 | 0.58 | 0.52 |
| *gC* | 0.47 | 0.73 | 0.41 |
| LATs | 0.00 | 0.09 | 0.13 |

## Supplemental Experimental Procedures

### Generation of M138 and R138 BACs and viruses

The derivation of the BACs is depicted in Figure S2A. Briefly, the sequences to be mutated in one copy of *ICP0* (copy 1) were replaced with a zeocin-resistance (*Zeo*) cassette and those in the other copy (copy 2) were replaced with a kanamycin-resistance (*Kan*) cassette designed to permit engineering of mutations using two-step Red-mediated recombination (Tischer et al., 2006). After copy 2 was mutated, *Zeo* in copy 1 was replaced with the *Kan* cassette, permitting engineering of mutations into copy 1. Primers used in each step to amplify and incorporate *Zeo* and *Kan* cassettes into the HSV-1 genome are listed below. Colonies containing the desired genotypes were identified by the sizes of PCR products (Figure S2B) using primers spanning the mutation site (forward, CTGTCGCCTTACGTGAACAAGACTATCAC; reverse, CCGCGATACATCCAACACAGACAGGGAA), followed by sequencing using the same primers. The BACs resulting from each recombination step were also assessed following restriction digestion using EcoRI and HindIII (Figure S2B) followed by agarose gel electrophoresis and ethidium bromide staining. A unique restriction product pattern is seen whenever *Zeo* or *Kan* is inserted into the genome due to the existence of a HindIII restriction site in *Kan* and the addition of an EcoRI restriction site at the 5' end of *Zeo*. The integrity of the BACs was further verified by restriction digestion with two additional enzymes, EcoRV and BglII, followed by agarose gel electrophoresis and ethidium bromide staining (not shown). The BACs were transfected into Vero cells using Lipofectamine 2000 (Invitrogen) following the manufacturer's instructions to generate viruses. The sequences surrounding the mutation site in the virus stocks were confirmed by sequencing the PCR products generated by the above primers.

**Quantitative PCR and RT-PCR**

DNA and RNA purification from cells or TG samples and reverse transcription of RNA were performed using the Allprep DNA/RNA mini kit and the Quantitect RT kit (both from Qiagen), respectively, following the manufacturer's instructions except that, instead of using the provided random primers, we added to the RT reaction mixture 10 nM of each of the reverse primers to be used in later PCR reactions (listed below). Serially diluted viral DNA standards, purified as described (Pesola et al., 2005) and spiked into DNA isolated from mock-infected TG, were analyzed in parallel with TG DNA samples to quantify viral DNA. Serially diluted mock-infected TGs were used to calibrate the levels of the mouse *adipsin* gene, which serves as a cellular DNA reference. To quantify the transcripts, viral mRNA standards were combined, serially diluted and spiked into uninfected mouse brain RNA before being subjected to the same procedures as TG samples. These RNA standards were synthesized by in vitro transcription using previously developed plasmids for *ICP0*, *ICP4*, *tk* and *LAT* (Chen et al., 2002; Kramer and Coen, 1995), and pKS+ICP27 for *ICP27* and pgCup for *gC* (kindly provided by Martha Kramer). To construct plasmid pKS+ICP27, the 2461 bp BamHI-HindIII fragment from pBH27 (Rice and Knipe, 1988) was inserted into pBluescript II KS+ (Stratagene). pgCup was constructed by inserting the 3.4 kb SalI-BamHI fragment from pgCa (Mark Brockman and David Knipe, unpublished results) into pBluescript II KS+ (Stratagene). Synthesis and purification of mRNA standards and purification of mouse brain RNA were performed as described (Pesola et al., 2005). RNA standards were quantified by UV absorbance at 260 nm. Serially diluted RNA purified from 293T cells or mouse brain was used to quantify human or mouse *GAPDH* levels in 293T cells or in TG, respectively, as a cellular reference gene. PCR reactions were performed

using SYBR green PCR master mix (Applied Biosystems) on an Applied Biosystems StepOnePlus real-time PCR system following the manufacturer's instructions. Optimized PCR reaction conditions including primer sequences are provided below. Standard curves were linear in all cases (with $R^2$ values over 0.99) and the values reported for samples were all within the linear ranges, except for those below the detection limits , (i.e., the lowest points of the linear ranges of standard curves). The *ICP0*, *ICP27*, *tk* and *gC* assays' detection limits were 100 copies/TG, and ICP4 assay's was 1000 copies/TG.

**Luciferase assays**

Reporter vectors were generated using the renilla luciferase (RLuc) vector pNL-SIN-CMV-RLuc, as described (Gottwein et al., 2006). Briefly, two tandem fully complementary synthetic binding sites for miR-138 were inserted using ClaI and XbaI sites present in the 3'UTR of RLuc. ICP0 3'UTR RLuc was generated by PCR amplification of infected cell genomic DNA to yield a 200-bp fragment derived from the HSV-1 ICP0 3'UTR, extending from 120,463 to 120,673 bases (KOS GenBank: JQ673480.1) in the viral genome, which fully encompasses both predicted miR-138 target sites as well as flanking sequences. This DNA fragment was cloned into the ClaI and XbaI sites present in pNL-SIN-CMV-RLuc. RLuc expression constructs (50 ng) were co-transfected with 50 ng of the internal control firefly (FLuc) expression vector pGL3 (Promega) into a 24-well plate seeded with miR-138- transduced 293 cells, and RLuc and FLuc luciferase activities determined at 24 h post-transfection using a dual luciferase assay kit (Promega).

**Intracranial inoculation.**

Male CD-1 mice (Charles River Laboratories) were infected at 4.5 weeks and housed in the Center for Comparative Medicine and Research at The Geisel School of Medicine at Dartmouth. Mice were anesthetized with 4% isoflurane and injected intracranially with 5000 PFU of HSV-1 M138 or R138 in a volume of 10 µl Phosphate Buffered Saline (PBS) using a Hamilton syringe with a 26-gauge needle. Mice were weighed daily and mortality figures were generated from mice that were euthanized after ≥ 25% loss of body weight from day 0 of the experiment. Mice were housed, treated, and euthanized in accordance with all Federal and University policies.

**Lists of primer sequences and PCR conditions**

Primers used to construct plasmids.

| Purpose | Forward primer | Reverse primer |
|---|---|---|
| For PCR to insert miR-138 and flanking sequences | GTCAGGATCCGGGATGGGGAA GTTCACAAAG | GTCAGAATTCTGGAGCATTT GTGTTGGGGG |
| Mutagenesis to make ICP0-M138 plasmid (1st round) | TCTGGCCGCGCCCACTACAGC TGACAAT | ATTGTCAGCTGTAGTGGGCG CGGCCAGA |
| Mutagenesis to make ICP0-M138 plasmid (2nd round) | AAGACACGGGCACCACACAG CTGAGGG | CCCTCAGCTGTGTGGTGCCCG TGTCTT |

Primers used to construct M138 and R138 BACs.

| Mutation | Step | Forward primer* | Reverse primer |
|---|---|---|---|
| From WT to M138 | Amplify and incorporate *Zeo* cassette | GGGGGTCGGGCGCTGGGTGGTCTCTGGCC GCGCCCACTACA**G**C**T**G**A**CAATCCGTGTCG GGGAGGTG*GAATTC*tgttgacaattaatcatcggcat | AATCCCCTGAGTTTTTTTTTATTAGGGCCA ACACAAAAGACCC**T**C**A**G**C**TGTGTGGTGC CCGTGTCTTTCACTTTtcagtcctgctcctcggcca |
| | Amplify and incorporate *Kan* cassette | GGGGTCGGGCGCTGGGTGGTCTCTGGCCG CGCCCACTACA**G**C**T**G**A**CAATCCGTGTCGG GGAGGTGGAAAGTGAAAGACACGGGCAC CACACA**G**C**T**G**A**tagggataacagggtaatcgattt | TCCCCTGAGTTTTTTTTTATTAGGGCCAAC ACAAAAGACCC**T**C**A**G**C**TGTGTGGTGCCC GTGTCTTTCACTTTCCACCTCCCCGACAC GGATTG**T**C**A**G**C**gccagtgttacaaccaattaacc |
| From M138 to R138 | Amplify and incorporate *Zeo* cassette | GGGGGTCGGGCGCTGGGTGGTCTCTGGCC GCGCCCACTACA**C**C**A**G**C**CAATCCGTGTCG GGGAGGTG*GAATTC*tgttgacaattaatcatcggcat | AATCCCCTGAGTTTTTTTTTATTAGGGCCA ACACAAAAGACCC**G**C**T**G**G**TGTGTGGTGC CCGTGTCTTTCACTTTtcagtcctgctcctcggcca |
| | Amplify and incorporate *Kan* cassette | GGGGTCGGGCGCTGGGTGGTCTCTGGCCG CGCCCACTACA**C**C**A**G**C**CAATCCGTGTCGG GGAGGTGGAAAGTGAAAGACACGGGCAC CACACA**C**C**A**G**C**tagggataacagggtaatcgattt | TCCCCTGAGTTTTTTTTTATTAGGGCCAAC ACAAAAGACCC**G**C**T**G**G**TGTGTGGTGCCC GTGTCTTTCACTTTCCACCTCCCCGACAC GGATTG**G**C**T**G**G**gccagtgttacaaccaattaacc |

*In lower case are the sequences overlapping *Zeo* or *Kan* gene. In italics are the EcoRI restriction sites. In bold letters are the mutated bases.

Optimized PCR reaction conditions for qPCR and qRT-PCR.

| Assay | | Forward primer | Reverse primer | Forward (nM) | Reverse (nM) | Annealing temperature (C) |
|---|---|---|---|---|---|---|
| mRNA | ICP0 | AGCGAGTACCCGCCGGCCTG | CAGGTCTCGGTCGCAGGGAAAC | 200 | 100 | 72 |
| | ICP4 | TCGAGAGTCCGTAGGTGAC | TTGTTCTCCGACGCCATC | 500 | 500 | 65 |
| | ICP27 | GTGTGCAGCCGTGTTCCAA | AGCGACCGGGCCCGAATC | 300 | 300 | 60 |
| | Tk | ACCCGCTTAACAGCGTCAACA | CCAAAGAGGTGCGGGAGTTT | 100 | 200 | 58 |
| | gC | GCCCATTTCGTACGACTACA | GGTGCTCTAGAACGGGAATC | 300 | 300 | 60 |
| | Mouse GAPDH | GAAGGTCGGTGTGAACGGATT | GCCTTGACTGTGCCGTTGAA | 300 | 300 | 60 |
| | Human GAPDH | GAAGGTCGGAGTCAACGGATT | GCCTTGACGGTGCCATGGAA | | | |
| | LAT | CAGACAGCAAAAATCCCCTGAGT | GGGACGAGGGAAAACAATAAGGG | 300 | 300 | 60 |
| DNA | Viral genome (tk) | Same as tk | Same as tk | 300 | 300 | 60 |
| | Cellular DNA (adipsin) | TCCGGCAGCCCTCTAGT | TAGGATGACACTCGGGTATAGAC | 300 | 300 | 60 |

## Supplemental References

Gottwein, E., Cai, X., and Cullen, B.R. (2006). A novel assay for viral microRNA function identifies a single nucleotide polymorphism that affects Drosha processing. J Virol *80*, 5321-5326.

Rice, S.A., and Knipe, D.M. (1988). Gene-specific transactivation by herpes simplex virus type 1 alpha protein ICP27. J Virol *62*, 3814-3823.

Tischer, B.K., von Einem, J., Kaufer, B., and Osterrieder, N. (2006). Two-step red-mediated recombination for versatile high-efficiency markerless DNA manipulation in Escherichia coli. Biotechniques *40*, 191-197.