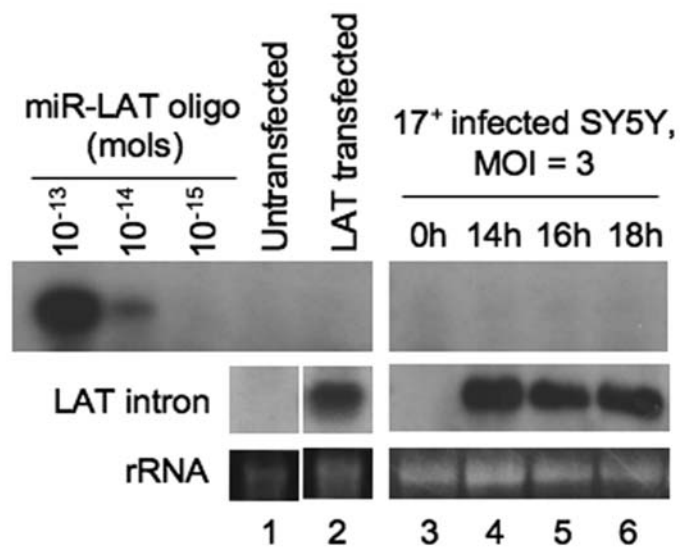
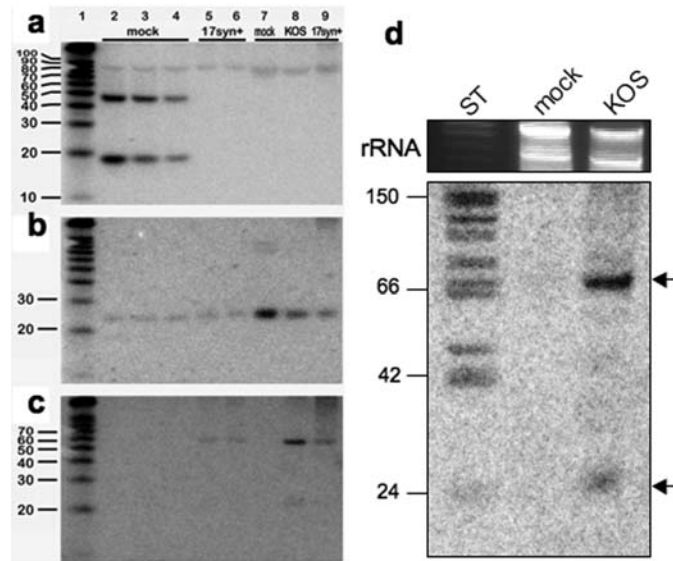


SUPPLEMENTARY INFORMATION



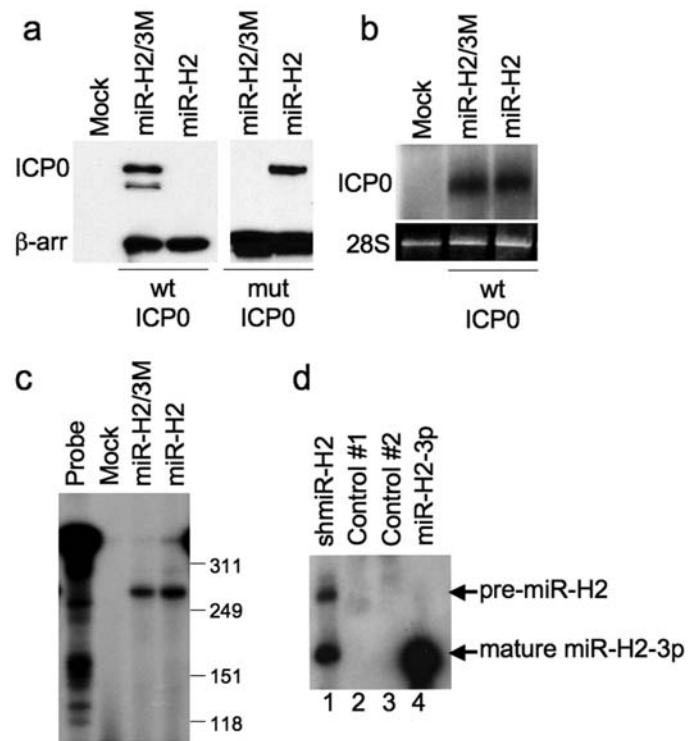
Suppl Fig. 1.**The proposed miR-LAT miRNA is not detected in HSV-1 infected cells.**

Using splint-ligation, miR-LAT was not detected in SY5Y cells infected with HSV-1 strain 17syn+, (lanes 4 to 6) or in 293T cells transfected with pcDNA3/LAT (lane 2). In contrast, the LAT intron, and a synthetic RNA identical to miR-LAT, were readily detected, in the latter case at a level equivalent to ~300 copies per cell.



Suppl Fig. 2.**Northern analysis detects miR-H1 and miR-H6, but not miR-LAT, in HSV-1**

infected cells. HSV-1 or mock-infected SY5Y cells were harvested at 16 hr P.I., the time reported by Gupta et al.¹⁹ Short-enriched RNA was isolated using the mirVana™ miRNA Isolation Kit. ³²P-end-labeled probes had specific activities of $>1 \times 10^8$ cpm/pmol. Synthetic miR-LAT and pre-miR-LAT mimic (3, 1.5, and 0.75×10^{10} molecules, lanes 2, 3, 4) were combined with cellular RNA. **a.** Probed with a miR-LAT probe (5'-GGGCCCGGGCCGGCCGCCACG-3'). **b.** Same blot as in panel **a**, stripped and re-probed for cellular let-7a. **c.** Parallel blot re-probed with miR-H1 after having been probed with the miR-LAT probe, then stripped. **d.** Northern blot for HSV-1 miR-H6 in infected Vero cells. Arrows indicate pre-miR-H6 and mature miR-H6. rRNA served as a loading control. Numbers indicate size markers.



Suppl Fig. 3.**miR-H2-3p inhibits ICP0 protein expression but does not affect ICP0 mRNA**

levels. a. 293T cells were co-transfected with a β -arrestin expression plasmid and a wildtype or mutant ICP0 expression construct (Fig. 3a), as well as either miR-H2 or miR-H2/3M synthetic pre-miRNA duplex intermediates. ICP0 and β -arrestin expression were assayed by Western blot. **b.** Northern analysis of ICP0 mRNA levels in samples shown in panel A that were transfected with wildtype ICP0. 28S rRNA was used as a loading control. **c.** RPA for ICP0 mRNA levels, and potential miR-H2-3p-induced mRNA cleavage products. miRNA cleavage of the ICP0 mRNA would result in the generation of 120 nt and 164 nt probe fragments, while full-length ICP0 mRNA should rescue a 284 nt probe fragment. **d.** Northern analysis of 293T cells transfected with pSuper-based expression plasmids designed to express an shRNA that mimics pre-miR-H2 (lane 1) or two irrelevant shRNAs (lanes 2 and 3). Synthetic mature miR-H2 was used as a positive control (lane 4).

Supplementary Table 1
Short RNA sequence analysis

	<u>293/LAT</u>		<u>TG/HSV-1</u>	
Total reads	225,439		254,651	
Num. of unique sequences	23,683		28,152	
Total num. of sequences 18-24nts	192,864	86%	235,114	92%
Num. of unique sequences 18-24nts	11,398		15,315	
<hr/>				
Total num. of sequences ≥ 2 hits	185,204	82%	224,729	88%
Num. of unique sequences ≥ 2 hits	3,738		4,930	
<hr/>				
Total num. of cellular miRNAs ≥ 2 hits	144,955	64%	204,867	80%
Num. of HSV-1 miRNAs ≥ 2 hits	619	0.3%	144	0.1%
<hr/>				
Single hit HSV-1 sequences	67		27	
Alternative variants of miR-H2 to miR-H6	32		20	
Other short RNAs of HSV-1 origin	35		7	
<hr/>				
Total num. of HSV-1 miRNAs	651	0.3%	164	0.1%

Suppl Table 1

Short RNA sequence analysis. Overall summary of 454 sequencing data of 18-24 bp cDNAs obtained from pcDNA3/LAT-transfected 293T cells and mouse TG latently infected with HSV-1. The central section summarizes the characteristics of cDNA sequences obtained two or more times, while the lower section describes HSV-1 sequences obtained only once in this analysis.

Supplementary Table 2A
Identity of cellular miRNAs detected by sequence analysis

hsa-miR-19b	24406	hsa-miR-193b	3	hsa-miR-361-5p	2
hsa-let-7a	570	hsa-miR-194	63	hsa-miR-374a	4
hsa-let-7b	99	hsa-miR-195	48	hsa-miR-374b	12
hsa-let-7c	141	hsa-miR-196a	334	hsa-miR-378	4
hsa-let-7d	16	hsa-miR-196b	144	hsa-miR-421	16
hsa-let-7e	36	hsa-miR-197	63	hsa-miR-423-3p	2
hsa-let-7f	189	hsa-miR-19a	2675	hsa-miR-424	178
hsa-let-7g	109	hsa-miR-19b	4450	hsa-miR-425	25
hsa-let-7i	98	hsa-miR-200c	3	hsa-miR-454	18
hsa-miR-101	8	hsa-miR-20a	3153	hsa-miR-455-3p	27
hsa-miR-103	434	hsa-miR-20b	44	hsa-miR-484	349
hsa-miR-106a	686	hsa-miR-21	599	hsa-miR-486-3p	14
hsa-miR-106b	149	hsa-miR-210	61	hsa-miR-486-5p	129
hsa-miR-107	317	hsa-miR-218	452	hsa-miR-497	17
hsa-miR-10a	731	hsa-miR-221	16068	hsa-miR-503	143
hsa-miR-10b	237	hsa-miR-222	1717	hsa-miR-505	20
hsa-miR-124	21	hsa-miR-224	4	hsa-miR-532-3p	91
hsa-miR-125a-5p	250	hsa-miR-23a	12	hsa-miR-532-5p	38
hsa-miR-125b	216	hsa-miR-23b	21	hsa-miR-542-5p	14
hsa-miR-126	9	hsa-miR-24	51	hsa-miR-548b-3p	62
hsa-miR-127-3p	3	hsa-miR-25	7992	hsa-miR-548d-5p	2
hsa-miR-128b	82	hsa-miR-26a	67	hsa-miR-551a	3
hsa-miR-130b	562	hsa-miR-26b	100	hsa-miR-551b	83
hsa-miR-132	2	hsa-miR-27a	178	hsa-miR-574-3p	72
hsa-miR-133a	2	hsa-miR-27b	477	hsa-miR-589	3
hsa-miR-138	47	hsa-miR-28-3p	16	hsa-miR-598	41
hsa-miR-139-5p	2	hsa-miR-29a	15	hsa-miR-615-3p	34
hsa-miR-140-3p	479	hsa-miR-29b	53	hsa-miR-628-3p	2
hsa-miR-140-5p	2	hsa-miR-29c	16	hsa-miR-660	102
hsa-miR-142-3p	8	hsa-miR-301a	18	hsa-miR-675	15
hsa-miR-146b-5p	2	hsa-miR-301b	10	hsa-miR-7	21
hsa-miR-148b	6	hsa-miR-30a	79	hsa-miR-708	10
hsa-miR-149	10	hsa-miR-30b	1639	hsa-miR-744	69
hsa-miR-151-3p	23	hsa-miR-30c	764	hsa-miR-760	2
hsa-miR-151-5p	58	hsa-miR-30d	95	hsa-miR-767-5p	22
hsa-miR-152	7	hsa-miR-30e	11	hsa-miR-877	2
hsa-miR-15a	713	hsa-miR-31	107	hsa-miR-891a	20
hsa-miR-15b	6007	hsa-miR-32	18	hsa-miR-92a	5766
hsa-miR-16	14523	hsa-miR-320	6	hsa-miR-92b	109
hsa-miR-17	14777	hsa-miR-324-3p	86	hsa-miR-93	9504
hsa-miR-181a	35	hsa-miR-324-5p	34	hsa-miR-935	8
hsa-miR-181b	18	hsa-miR-328	3	hsa-miR-940	2
hsa-miR-181d	2	hsa-miR-330-3p	2	hsa-miR-941	53
hsa-miR-185	76	hsa-miR-331-3p	37	hsa-miR-98	3
hsa-miR-187	79	hsa-miR-338-3p	12	hsa-miR-99a	118
hsa-miR-188-3p	6	hsa-miR-339-5p	22	hsa-miR-99b	10361
hsa-miR-188-5p	2	hsa-miR-340	2	Total	144955
hsa-miR-18a	2069	hsa-miR-342-3p	528		
hsa-miR-18b	37	hsa-miR-346	4		
hsa-miR-191	463	hsa-miR-34a	6049		
hsa-miR-192	119	hsa-miR-361-3p	15		

Supplementary Table 2B
Identity of cellular miRNAs detected by sequence analysis

mmu-miR-34a	25463	mmu-miR-181a	187	mmu-miR-324-3p	4	mmu-miR-666-3p	32
mmu-let-7a	2272	mmu-miR-181b	36	mmu-miR-324-5p	127	mmu-miR-666-5p	3
mmu-let-7b	18414	mmu-miR-181c	2	mmu-miR-326	120	mmu-miR-667	46
mmu-let-7c	21409	mmu-miR-182	21	mmu-miR-328	223	mmu-miR-669b	11
mmu-let-7d	325	mmu-miR-183	14	mmu-miR-329	2	mmu-miR-672	16
mmu-let-7e	392	mmu-miR-185	267	mmu-miR-331-3p	34	mmu-miR-674	527
mmu-let-7f	1451	mmu-miR-187	55	mmu-miR-335-5p	2	mmu-miR-676	1151
mmu-let-7g	981	mmu-miR-188-3p	8	mmu-miR-337-5p	41	mmu-miR-700	22
mmu-let-7i	4623	mmu-miR-191	120	mmu-miR-338-3p	10174	mmu-miR-744	425
mmu-miR-1	11	mmu-miR-192	32	mmu-miR-338-5p	24	mmu-miR-760	2
mmu-miR-100	95	mmu-miR-194	98	mmu-miR-341	478	mmu-miR-770-3p	2
mmu-miR-101a	23	mmu-miR-195	500	mmu-miR-342-3p	38	mmu-miR-770-5p	2
mmu-miR-101b	8	mmu-miR-199a-5p	34	mmu-miR-344	5	mmu-miR-7a	28
mmu-miR-103	381	mmu-miR-199b	1381	mmu-miR-345-3p	5	mmu-miR-7b	2
mmu-miR-106a	25	mmu-miR-19b	27	mmu-miR-345-5p	32	mmu-miR-92a	21
mmu-miR-106b	2	mmu-miR-200a	16	mmu-miR-34a	10468	mmu-miR-92b	5
mmu-miR-107	72	mmu-miR-200b	27	mmu-miR-34c	15	mmu-miR-93	573
mmu-miR-124	4053	mmu-miR-200c	8	mmu-miR-350	5	mmu-miR-96	6
mmu-miR-125a-5p	256	mmu-miR-204	3	mmu-miR-351	34	mmu-miR-98	96
mmu-miR-125b-3p	21	mmu-miR-206	3	mmu-miR-370	4	mmu-miR-99a	532
mmu-miR-125b-5p	25332	mmu-miR-20b	29	mmu-miR-376a	4	mmu-miR-99b	30961
mmu-miR-126-3p	763	mmu-miR-21	5724	mmu-miR-378	2	Total	204867
mmu-miR-126-5p	9	mmu-miR-210	1200	mmu-miR-379	13		
mmu-miR-127	3407	mmu-miR-214	301	mmu-miR-383	196		
mmu-miR-130a	252	mmu-miR-217	15	mmu-miR-423-3p	2		
mmu-miR-130b	11	mmu-miR-218	14	mmu-miR-431	84		
mmu-miR-134	7	mmu-miR-221	60	mmu-miR-433	2		
mmu-miR-136	6	mmu-miR-222	37	mmu-miR-434-5p	4		
mmu-miR-138	10140	mmu-miR-23a	17	mmu-miR-449a	11		
mmu-miR-139-3p	18	mmu-miR-23b	32	mmu-miR-451	6		
mmu-miR-139-5p	64	mmu-miR-24	1223	mmu-miR-455	163		
mmu-miR-140	19	mmu-miR-25	161	mmu-miR-466b-5p	2		
mmu-miR-141	2	mmu-miR-26a	2	mmu-miR-466c-5p	6		
mmu-miR-142-3p	136	mmu-miR-26b	2	mmu-miR-466f-5p	9		
mmu-miR-142-5p	10	mmu-miR-27a	433	mmu-miR-466h	23		
mmu-miR-143	760	mmu-miR-27b	412	mmu-miR-484	57		
mmu-miR-144	14	mmu-miR-299	2	mmu-miR-485	2		
mmu-miR-146a	2	mmu-miR-29a	256	mmu-miR-486	275		
mmu-miR-146b	8	mmu-miR-29b	57	mmu-miR-497	1753		
mmu-miR-147	2	mmu-miR-29c	27	mmu-miR-503	29		
mmu-miR-148b	5	mmu-miR-300	111	mmu-miR-511	3		
mmu-miR-149	30	mmu-miR-301a	21	mmu-miR-532-3p	48		
mmu-miR-150	32	mmu-miR-30a	100	mmu-miR-532-5p	24		
mmu-miR-151-3p	4	mmu-miR-30b	458	mmu-miR-540-3p	13		
mmu-miR-151-5p	97	mmu-miR-30c	602	mmu-miR-540-5p	4		
mmu-miR-152	290	mmu-miR-30d	142	mmu-miR-542-5p	3		
mmu-miR-154	5	mmu-miR-30e	33	mmu-miR-551b	63		
mmu-miR-15a	830	mmu-miR-31	22	mmu-miR-574-3p	2656		
mmu-miR-15b	424	mmu-miR-32	4	mmu-miR-574-5p	3		
mmu-miR-16	5680	mmu-miR-322	42	mmu-miR-592	2		
mmu-miR-17	81	mmu-miR-323-3p	9	mmu-miR-665	3		

Suppl Table 2

Identity of cellular miRNAs detected by sequence analysis. Mature cellular miRNAs obtained from 454 sequencing, as identified by miRBASE. **A.** pcDNA3/LAT transfected 293T cells. hsa; human. **B.** Mouse TG latently infected with HSV-1. mmu; mouse.

Supplementary Table 3

HSV-1 miRNAs identified by 454 sequencing of cDNAs from pcDNA3/LAT transfected 293T cells

	Freq	Sequences	Nts	Start	End	Orientation	Designation
	7	TCGCACGCGCCCGGCACAGACT	22	121832	121853	+	miR-H2-5p
	2	TCGCACGCGCCCGGCACAGA	20	121832	121851		
Total	9						
	113	CCTGAGCCAGGGACGAGTGCGA	22	121871	121892	+	miR-H2-3p
	89	CCTGAGCCAGGGACGAGTGCGACT	24	121871	121894		
	27	CCTGAGCCAGGGACGAGTGCGAC	23	121871	121893		
	10	CCTGAGCCAGGGACGAGTGCG	21	121871	121891		
	4	CCTGAGCCAGGGACGAGTGCGACa	24	121871	121893		
	4	TGAGCCAGGGACGAGTGCGACTGa	24	121873	121895		
	3	TGAGCCAGGGACGAGTGCGA	20	121873	121892		
	2	CCTGAGCCAGGGACGAGTGCGAt	23	121871	121892		
	2	GAGCCAGGGACGAGTGCGACTGt	23	121874	121895		
	2	CTGAGCCAGGGACGAGTGCGA	21	121872	121892		
	2	CCTGAGCCAGGGACGAGT	18	121871	121888		
Total	258						
	3	CTGGGACTGTGCGGTTGGGA	20	125775	125794	+	miR-H3
	2	CTGGGACTGTGCGGTTGGGAC	21	125775	125795		
Total	5						
	25	GGTAGAGTTTGACAGGCAAGC	21	125882	125902	+	miR-H4-5p
	11	GGTAGAGTTTGACAGGCAAGCA	22	125882	125903		
	11	GTAGAGTTTGACAGGCAAGCA	21	125883	125903		
	4	GTAGAGTTTGACAGGCAAGC	20	125883	125902		
	2	GGTAGAGTTTGACAGGCAAGCa	23	125882	125903		
Total	53						
	219	CTTGCCCTGTCTAACTCGCTAGT	22	125927	125948	+	miR-H4-3p
	12	CTTGCCCTGTCTAACTCGCTAG	21	125927	125947		
	9	TTGCCTGTCTAACTCGCTAGT	21	125928	125948		
	6	CTTGCCCTGTCTAACTCGCTA	20	125927	125946		
	3	CTTGCCCTGTCTAACTCGCT	19	125927	125945		
	2	CTTGCCCTGTCTAACTCGCTAGTa	23	125927	125948		
	2	CTTGCCCTGTCTAACTCGCTAGTt	23	125927	125948		
	2	CTTaGCCTGTCTAACTCGCTAGT	23	125930	125948		
Total	255						
	29	GTCAGAGATCCAAACCCTCCGGt	23	126769	126790	+	miR-H5
	5	GTCAGAGATCCAAACCCTCCGGtt	24	126769	126790		
	5	TCAGAGATCCAAACCCTCCGGt	22	126770	126790		
Total	39						
TOTAL	619						

Suppl Table 3**HSV-1 miRNAs identified by 454 sequencing of cDNAs from**

pcDNA3/LAT-transfected 293T cells. Sequence start and end coordinates are as aligned with the HSV-1 strain 17 genome sequence (NC_001806). As a consequence of being located within LAT, which itself is contained within a repeat region, there are two inverted copies of each HSV-1 miRNA in the viral genome (Fig. 1a). Coordinates and orientations provided are in relation to the positively orientated LAT. Lower case letters indicate nucleotides that do not match the genome sequence and are presumably artifacts generated by the cDNA cloning process. Only HSV-1 sequences recovered at least twice are included in this table. No evidence of RNA editing of HSV-1 miRNAs was noted.

Supplementary Table 4
HSV-1 miRNAs identified by 454 sequencing of cDNAs from latently infected mouse trigeminal ganglia

Freq	Sequences	Nts	Start	End	Orientation	Designation
20	CCTGAGCCAGGGACGAGTGCGA	22	121871	121892	+	miR-H2-3p
20	GAGCCAGGGACGAGTGCGACTGt	23	121874	121895		
10	CCTGAGCCAGGGACGAGTGCGACT	24	121871	121894		
5	CCTGgGCCAGGGACGAGTGCGA	22	121871	121892		
4	TGAGCCAGGGACGAGTGCGACTGt	24	121873	121895		
3	cGAGCCAGGGACGAGTGCGACTGt	24	121874	121895		
3	GAGCCAGGGACGAGTGCGACTGc	23	121874	121895		
3	CCTGAGCCAGGGACGAGTGCGAC	23	121871	121893		
3	TGAGCCAGGGACGAGTGCGACTG	23	121873	121895		
3	TGAGCCAGGGACGAGTGCGACT	22	121873	121894		
2	CCTGAGCCAGGGACGAGTGCGACa	24	121871	121893		
2	CTGAGCCAGGGACGAGTGCGACT	23	121872	121894		
2	GAGCCAGGGACGAGTGCGACTG	22	121874	121895		
2	TGAGCCAGGGACGAGTGCGA	20	121873	121892		
2	CgGGGACGAGTGCGACTGt	19	121880	121895		
Total	84					
10	CTGGGACTGTGCGGTTGGGA	20	125775	125794	+	miR-H3
2	CaGGGACTGTGCGGTTGGGA	20	125777	125794		
2	CTGGGACTGTGCGGTTGGA	19	125775	125792		
Total	14					
25	CACTTCCCGTCCTTCCATCCC	21	118348	118328	-	miR-H6
11	CACTTCCCGTCCTTCCATCCCa	22	118348	118328		
6	CACTTCCCGTCCTTCCATCC	20	118348	118329		
2	CACTTCCCGTCCTTCCATCCCC	22	118348	118327		
2	CACTTCCCGTCCTTCCATCCct	22	118348	118328		
Total	46					
TOTAL	144					

Suppl Table 4

HSV-1 miRNAs identified through 454 sequencing of cDNAs from latently infected mouse TG. Sequence start and end coordinates are as aligned with the HSV-1 strain 17 genome sequence (NC_001806). As a consequence of being located within LAT, which itself is contained within a repeat region, there are two inverted copies of each HSV-1 miRNA in the genome (Fig. 1a). Coordinates and orientation provided are in relation to the positively orientated LAT. Lower case letters indicate nucleotides that do not match the genome sequence and are presumably cloning artifacts. Only HSV-1 sequences recovered at least twice are included in this table. No evidence of RNA editing of HSV-1 miRNAs was noted.

Supplementary Table 5A
Stem-loop RT PCR Analysis of HSV-1 microRNAs

	Log ₁₀ copies per ng short-enriched RNA							
	let-7a	miR-H1	miR-H2-3p	miR-H3	miR-H4-3p	miR-H4-5p	miR-H5	miR-H6
LAT/293T	6.7	Nt	5.2	5.1	6.6	6.0	5.8	2.3
293T control	6.7	Nt	1.9	1.2	2.3	1.8	1.9	2.3
Latently-infected ganglia	6.8	Nt	3.7	2.8	4.8	3.4	3.8	3.5
Mock-infected ganglia	6.8	Nt	1.8	1.8	3.2	1.9	2.3	1.7
HSV-1-infected Vero	6.1	5.6	4.1	2.7	3.8	3.1	3.3	5.0
Mock-infected Vero	6.1	2.0	0.7	0.1	-0.5	-0.4	1.3	1.5

Supplementary Table 5B
Oligonucleotides used for stem-loop RT PCR quantification of microRNAs

miRNA	Reagent	Length	Sequence
all	Reverse Primer	20	CCAGTGCAGGGTCCGAGGTA
let-7a	RNA Standard	22	UGAGGUAGUAGGUUGUAUAGUU
let-7a	RT-loop	50	GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACAACCTAT
let-7a	Forward Primer	22	GCCCGGTGAGGTAGTGGGTTGT
let-7a	Probe	17	(6FAM) CTGGATACGACAACCTAT(MGB)
H1	RNA Standard	21	UGGAAGGACGGGAAGUGGAAG
H1	RT-loop	50	GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACCTTCCA
H1	Forward Primer	19	CACGCATGGAAGGACGGGA
H1	Probe	18	(6-FAM) TGGATACGACCTTCCACT (MGB)
H2-3p	RNA Standard	24	CCUGAGCCAGGGACGAGUGCGACU
H2-3p	RT-loop	50	GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACAGTCGC
H2-3p	Forward Primer	22	TCATAACCTGAGCCAGGGACGA
H2-3p	Probe	18	(6-FAM) TACGACAGTCGCACT (MGB)
H3	RNA Standard	20	CUGGGACUGUGCGGUUGGGA
H3	RT-loop	50	GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACTCCCAA
H3	Forward Primer	18	GCTTGGCTGGGACTGTGC
H3	Probe	18	(6-FAM) TACGACTCCCAACCGCAC (MGB)
H4-3p	RNA Standard	22	CUUGCCUGUCUAACUCGCUAGU
H4-3p	RT-loop	50	GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACACTAGC
H4-3p	Forward Primer	20	GCCTGGCTTGCCTGTCTAAC
H4-3p	Probe	18	(6-FAM) CTGGATACGACACTAGCG (MGB)
H4-5p	RNA Standard	21	GGUAGAGUUUGACAGGCAAGC
H4-5p	RT-loop	50	GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACGCTTGC
H4-5p	Forward Primer	21	GCCCGGGGTAGAGTTTGACAG
H4-5p	Probe	17	(6-FAM) CTGGATACGACGCTTGC (MGB)
H5	RNA Standard	23	GUCAGAGAUCCAAACCCUCCGGU
H5	RT-loop	50	GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACACCGGA
H5	Forward Primer	22	GCCTGGGTCAGAGATCCAAACC
H5	Probe	18	(6-FAM) CTGGATACGACACCGGAG (MGB)
H6	RNA Standard	22	CACUUCCCGUCCUCCAUCCCC
H6	RT-loop	50	GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACGGGGAT
H6	Forward Primer	22	TCATAACACTTCCCGTCCTTCC
H6	Probe	18	(6FAM) GGATACGACGGGGATGGA (MGB)

Suppl Table 5

Validation of HSV-1 miRNAs by quantitative stem-loop RT-PCR. Stem-loop RT-PCR assays were designed for each HSV-1 miRNA and for cellular miRNA let-7a, and developed using synthetic miRNAs, which were used as quantification standards. **A.** Representative results, shown as log miRNA copies per ng short-enriched RNA, are shown for RNA isolated from 293T cells transfected with pcDNA3/LAT, latently-infected mouse TG, and Vero cells infected with HSV-1 at 16 hours post-infection. Each assay included reactions lacking reverse-transcriptase (RT) or the RT primer, and mixtures of synthetic miRNAs other than those being assayed, as well as RNA from non-transfected 293T or Vero cells, or mock-infected ganglia, as negative controls. Background levels, which are equivalent to the highest values obtained from these negative controls, are presented in italics. Nt, not tested. **B.** Reagents for each stem-loop RT PCR assay. The sequences of the RNA standards and the RT and PCR primers are derived from the data in Supplementary Tables 3 and 4 for HSV-1, and from Chen et al.³⁷ for let-7a. FAM, TaqMan fluorescent probe; MGB, minor groove binder.

Supplementary Table 6**A** Molecules per 30 pg total RNA = molecules/cell

	let-7a	H1	H2-3p	H3	H4-3p	H4-5p	H5	H6
293T tx	15,000	Nt	480	390	12,000	3,000	1,890	≤1
293T mock	15,000	Nt	0	0	≤1	0	0	≤1
Latent TG	18,900	Nt	15	2	189	8	19	10
TG mock	18,900	Nt	0	0	≤5	0	≤1	0
Inf Vero	3,900	1,200	38	2	19	4	6	300
Mock Vero	3,900	0	0	0	0	0	0	0

B Molecules/cell x 2×10^6 cells = molecules/TG

let-7a	H1	H2-3p	H3	H4-3p	H4-5p	H5	H6
3.2×10^{10}	-	3.2×10^7	4.0×10^6	4×10^8	1.6×10^7	4.0×10^7	2.0×10^7

C Molecules/500 LAT⁺ neurons in each TG = molecules/LAT⁺ neuron

let-7a	H1	H2	H3	H4-3p	H4-5p	H5	H6
18,900	-	63,000	8,000	800,000	32,000	80,000	40,000

Suppl Table 6**Estimation of HSV-1 miRNA expression levels in latently or productively**

infected cells. A. Estimation of miRNA molecules per cell. We found that short-enriched RNA comprised roughly 10% of total RNA. Mammalian cells contain around 30 pg of total RNA per cell, so molecules/cell equals molecules per ng of short enriched RNA multiplied by 0.003. **B.** Estimation of miRNA molecules per ganglion. To estimate the number of miRNA molecules per ganglion, we assumed a constant 2×10^6 cells per TG based on measurement of how many copies of a murine single-copy gene, adipsin, could be detected per TG using qRT-PCR (unpublished results). **C.** Estimation of miRNA molecules per latently infected ganglionic neuron. Most TG cells do not harbor latent HSV-1. In mice infected with HSV-1 strain syn17+, the number of TG neurons containing HSV-1 DNA has been shown to range from <240 to ~7200 neurons, depending upon inoculum titer and other conditions¹⁸. In mice infected with HSV-1 strain KOS, the average number of TG neurons in which LAT was detected by *in situ* hybridization (LAT+ cells) is ~500¹⁷. For the purpose of estimating HSV-1 miRNAs per latently infected cell, we therefore divided molecules per ganglion by 500 LAT+ neurons, which could be as much as a 10-fold underestimate of latently infected neurons. This calculation was not applied to cellular miRNA let-7a, which is simply the number obtained in panel **A**.