Supplemental material

Small RNA deep-sequencing identifies microRNAs and other small non-coding RNAs from human herpesvirus 6 (HHV-6B)

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Supplemental figure legends

Figure S1. 5'RACE of B1 identifies a pre-miRNA antisense to hhv6b-miR-Ro6-3

A. Representation of pre-miR-Ro6-3 (shaded) in relation to its antisense transcript, B1. Relative positions of the mature 5p and 3p forms of miR-Ro6-3 are indicated along with their respective read numbers from the Ago2 IP library in parenthesis (red). The identified cleavage site and the number of analyzed cloned is indicated with a bold arrow, it is located 3' to the expected sites (dashed arrows) if B1 was cleaved by miR-Ro6-3 within Ago2. Genomic positions are given in reference to the Z29 strain of HHV-6B. **B.** Genomic location of the antisense miRNA in the left direct repeat (DR_L) and observed sequencing reads from the Ago2IP library in reference to the Z29 strain. Nucleotides in bold match to the HST strain, underlined nucleotides do not match to either published genome; mm, mismatches to Z29. **C.** The antisense miR-Ro6-3 folds into a typical miRNA precursor, the mature miRNA is in bold; the star sequence is underlined. **D.** Northern blotting for miR-Ro6-3-5pAS, which was renamed miR-Ro6-4-3p, from total RNA (10 μg) and from RNA immunoprecipitated with Ago2 (Ago2IP), or that of a control (BrdUIP). NI, non-infected; dpi, days post infection; IP, immunoprecipitation.

Figure S2. HHV-6B pre-miRNA structures: interstrain/intervariant variation

Secondary structure predictions for HHV-6B miRNAs based on cloned sequences aligned to the Z29 strain of HHV-6B; miRNAs are highlighted in bold, cloned star sequences are underlined. The predicted precursor for hhv6b-miR-Ro6-1 is fully conserved in both HHV-6B Z29 and HST strains as well as in HHV-6A. hhv6b-miR-Ro6-2 is conserved entirely between HST and Z29 strains, but presents with 91% conservation at the sequence level with HHV-6A. This variability in sequence does not affect the predicted pre-miRNA structure; there is a final base substitution for position 22 of the mature miRNA (orange). Due to the differences at the pre-miRNA level, this miRNA from HHV-6A is represented as a separate structure. hhv6b-miR-Ro6-3 presents with the greatest variability in mature miRNA sequence, and displays both interstrain and intervariant sequence variations in the seed region of both the 3p and 5p arms for HHV-6B HST (red) and HHV-6A (orange) in relation to the HHV-6B Z29 strain.

Supplemental table legends

Table S1. Major isoforms of HHV-6B miRNAs

Sequences of the major isoforms of HHV-6B miRNAs are given along with their genomic position in respect to the left direct repeat region (DR_L). Only those sequences cloned over 1000 times and found within Ago2 are represented in the table. HHV-6B miRNA can present with 5’ and 3’ heterogeneity, as wells as present as shorter isoforms lacking a part of the 5’ region of the miRNA. Sequences are given in reference to the Z29 strain of HHV-6B; mm, mismatches are given in parentheses.

Table S2. Sequence and genomic positions of miRNA candidates

Sequences representing the major form of each candidate are presented. Viral sequences are given in reference to the Z29 strain of HHV-6B, sequences numbers are from Ago2 IP data; * candidate sequence was not identified in Ago2 IP, clone numbers derive from small RNA library conducted from total RNA.

Table S3. Bioinformatic analysis of deep-sequencing datasets

Small RNA reads obtained from libraries conducted from total RNA or from RNA immunoprecipitated with Ago2 were assessed for their candidacy as miRNAs using three web-based miRNA prediction programs: miRCat, miRDeep, and miRAnalyzer. Experimental evidence is also included for comparison. +, supportive data for the candidate miRNA; -, not-predicted as a miRNA; n/t, not tested; n/d not determinable; n/s, non-specific.

Supplemental tables

Table S1. Major isoforms of HHV-6B miRNAs

hhv6b-miR	Reads	Length	Sequence	Strand	Start	End (mm)
miR-Ro6-1	21403	22	AAGCAGUCCCGAUCUAUGCGAC	-	7590	7611(0)
miR-Ro6-1	1281	21	AGCAGUCCCGAUCUAUGCGAC	-	7590	7610(0)
miR-Ro6-2	24242	22	UUACAGUUUCCGCUGCCGCUGU	-	3754	3775(0)
miR-Ro6-2	14090	21	UUACAGUUUCCGCUGCCGCUG	-	3755	3775(0)
miR-Ro6-2	5029	22	UUACAGUUUCCGCUGCCGCUGA	-	3754	3775(1)
miR-Ro6-2	4513	16	UUUCCGCUGCCGCUGU	-	3754	3769(0)
miR-Ro6-3-3p	31540	21	AGACCGAGCGGGCUCCAUCCG	-	3097	3117(0)
miR-Ro6-3-3p	11489	20	AGACCGAGCGGGCUCCAUCC	-	3098	3117(0)
miR-Ro6-3-3p	2862	17	CGAGCGGGCUCCAUCCG	-	3097	3113(0)
miR-Ro6-3-3p	2096	18	CCGAGCGGGCUCCAUCCG	-	3097	3114(0)
miR-Ro6-3-3p	1655	22	AGACCGAGCGGGCUCCAUCCGA	-	3096	3117(1)
miR-Ro6-3-3p	1090	22	AGACCGAGCGGGCUCCAUCCGC	-	3096	3117(0)
miR-Ro6-3-3p	1004	20	GACCGAGCGGGCUCCAUCCG	-	3097	3116(0)
miR-Ro6-3-5p	10293	21	GACGGAGUCCGCUCGGUAUCG	-	3128	3148(1)
miR-Ro6-3-5p	2237	20	GACGGAGUCCGCUCGGUAUC	-	3129	3148(1)
miR-Ro6-3-5p	1977	22	ACGGAGUCCGCUCGGUAUCGCG	-	3126	3147(1)
miR-Ro6-3-5p	1109	22	GACGGAGUCCGCUCGGUAUCGC	-	3127	3148(1)

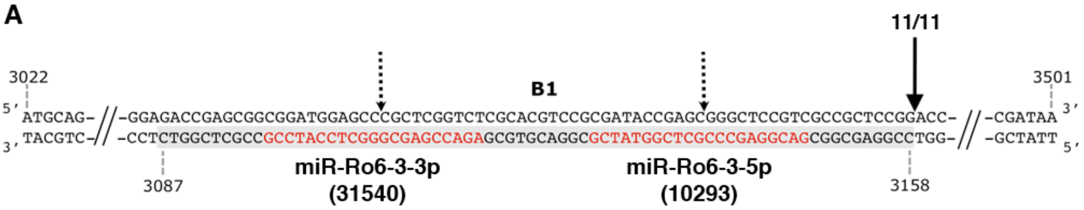
Table S2. Sequence and genomic positions of miRNA candidates

Candidate	Reads	Size	Sequence	Strand	Start	End (mm)
2	98	23	UUCGGGAUGUGCGGAGAAAGGGA	-	2083	2105(0)
5 *	3	24	UGGAUGUGUGGAAGCGUCAACGGC	-	27024	27047(0)
6	80	23	GCUGGCGCCGACCCUGCCGCGGC	+	4318	4340(0)
7	1214	22	GGUGAGAGAGCUCCGAGCGGGA	-	1392	1413(0)
8	1639	22	UGUUUUUGUGAUCGAAUCCACA	-	68744	68765(0)

Table S3. Bioinformatic analysis of deep-sequencing datasets

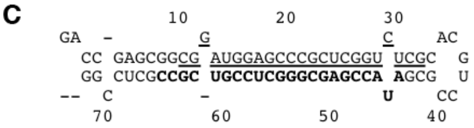
Candidate	Defined as miRNA in this study	qPCR	Northern Total RNA	NORTHERN (AGO)	miRCat	miRCat (AGO)	miRAnalyzer v2.0	miRAnalyzer v2.0 (AGO)	miRDeep v2.0	miRDeep v2.0 (AGO)
1	miR-Ro6-1	+	+	+	+	+	+	-	+	+
1*	miR-Ro6-1*	n/t	n/t	n/t	+	+	+	+	+	+
2	-	+	-	-	-	-	+	+	-	-
3-3p	miR-Ro6-3-3p	+	+	+	+	-	+	+	-	+
3-5p	miR-Ro6-3-5p	n/t	+	+	-	-	+	-	-	+
4	miR-Ro6-2	+	+	+	+	-	-	-	*	+
4*	miR-Ro6-2*	n/t	n/t	n/t	-	-	-	+	*	+
5#	-	-	-	-	-	-	+	-	-	-
6	-	n/s	+	-	-	-	-	+	+	-
7	-	n/d	-	-	-	-	-	-	-	-
8	-	n/d	-	-	-	-	-	-	-	-
-	miR-Ro6-4*	n/t	n/t	n/t	-	-	-	+	-	+
-	miR-Ro6-4	n/t	+	+	-	-	+	+	-	+

Figure S1



B

HHV-6B-miRNA	Reads	Length	Sequence	Strand	Start	End (mm)
miR-Ro6-3-5pAS	947	22	ATACCGAGCGG**A**CTCCGTCGCT	+	3130	3151 (2)
miR-Ro6-3-3pAS	20	23	CGGATGGAGCCCGCTCGGTCTCG	+	3097	3119 (0)



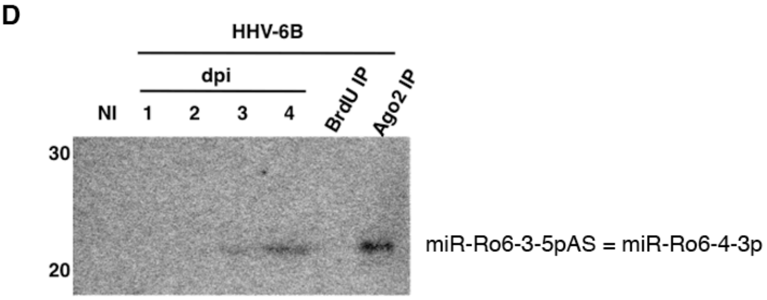


Figure S2

Candidate 1: hhv6b-miR-Ro6-1

```
          10         20          30
GCGCGCUUUCA      -      -  -       UCA
           AGCAGU CCCGAU CU AUGCGAC   \
           UCGUCG GGGCUA GG UGCGCUG   C
GAUA-------      C      C  U       UGC
             60        50        40
```

Candidate 3: hhv6b-miR-Ro6-3

```
           10         20        30
--   G       AC-              A     G
  CCG AGCGGCG   GGAGCCCGCUCGGU UCGCG A
  GGC UCGCCGC   CCUCGGGCGAGCCA AGCGU C
CU   -       CUA              G     G
 70         60        50        40
```

Candidate 4: hhv6b-miR-Ro6-2

```
             10           20        30
-       ---   C  C  C---  C  U  U     U    A
 CGGCGAC   AGC GC GC     GC GU AC GUCAC GUUC \
 GCCGCUG   UCG CG CG     UG CA UG CGGUG CAAG A
C       GUG   C  U  CCUU  A  U  C     -    A
80        70        60        50         40
```

Candidate 4: hhv**6a**-miR-Ro6-2

```
          10        20        30         40
-      CAA    C  C  C  CGUU     C  U   -  GA
 CGGCGA   CCGC GC GC GC     ACUGU GC GUU CG  \
 GCCGCU   GGCG CG CG CG     UGACA UG CGG GC  G
C      ---    U  C  U  CCUU     U  C   U  AA
   80           70        60        50
```