Supporting Information

Kincaid et al. 10.1073/pnas.1116107109

SI Materials and Methods

Cell Lines. HEK293 and 293T cells were obtained from American Type Culture Collection (ATCC) and maintained in DMEM supplemented with 10% (vol/vol) FBS (Cellgro). BL3.1 cells were obtained from ATCC and maintained in RPMI 1640 supplemented with 10% (vol/vol) FBS. Fetal lamb kidney-bovine leukemia virus (FLK-BLV) cells (Gift from L. Mansky, University of Minnesota, Minneapolis) were maintained in DMEM supplemented with 10% (vol/vol) FBS.

Plasmids. Cassettes encompassing the predicted miRNA genes were synthesized by Integrated DNA Technologies (IDT) in the context of the pIDT-SMART-KAN vector (sequences are listed in Table S4). The Box B mutant, pIDT-SMART-KAN-NC_001414_RNA_MUTB, was generated by PCR using phosphorylated primers (BLV_RTH_F and BLV_RTH_R) followed by DpnI digestion and self-ligation to mutate the sequence "GGG" to "AAA" at positions 6559–6561 (positions relative to refseq NC_001414). The murine herpesvirus 68 (MHV68) mir-M1-7 miRNA expression vector was made by PCR using as template a BAC containing the wild-type genome as a template (1) (kindly provided by Scott Tibbetts, University of Florida, Gainesville). The primers used for the MHV68 miRNA amplification are listed in the primer section MiR-M7-7 sense XhoI and Mir-M1-7 antisense XbaI and cloned into pIDT-SMART-KAN XhoI/XbaI. The BLV miRNA region was amplified via PCR using genomic DNA from BL3.1 cells and the BLV miRNA primers. Because of a SNP (6665 deletion) that generated an internal XhoI site, pcDNA3.1dsRluc BLV miRNAs was made by first cloning in nucleotides 6133–6620 into XhoI/XbaI site in pcDNA3.1dsRluc (2). Nucleotides 6621–6739 were then cloned into the XbaI/ApaI site. The pIDT-SMART-KAN-BLV expression vector was made by cloning nucleotides 6133–6669 into the XhoI site, which encompasses only the first four miRNA hairpins. pcDNA3.1dsRluc-776 was made by PCR amplification from SV40 776 genomic DNA using the 776 miR-Xho-F and 776 miR-Xba1-R primers and cloned into the XhoI/XbaI site. The KapB miRNA expression vector was made by subcloning the KapB 3′ UTR from pcDNA3.1dsLuc2CP-KapB expression vector (3) into the XhoI/XbaI site of pcDNA3.1dsRluc. The pcDNA3.1dsRluc-MHV68 miR-M1-7 construct was made by subcloning the insert from pIDT-SMART-KAN-MHV68 miR-M1-7. The bovine mir-29a insert was amplified from BL3.1 genomic DNA with the primers BTA_mir29a_F and BTA_mir29-a_R and cloned into the XhoI, XbaI sites of pcDNA3.1+Puro to generate pcDNA3.1-bta-mir-29a. The bovine HMG-box transcription factor 1 (HBP1) 3′UTR was amplified from BL3.1 genomic DNA using primers BTA_HPB1_3UTR_F and BTA_HPB1_3UTR_R and ligated into the XhoI, XbaI sites of pcDNA3.1dsRluc. The bovine HBP1 3′UTR seed mutant was generated by subcloning a fragment synthesized by IDT with the sequence "TG" replaced with "CC" at positions 252–253 into the EcoRV, BbvCI sites of the HBP1 3′UTR in the context of pcDNA3.1dsRluc- HBP1 3′UTR. The bovine peroxidasin homolog (PXDN) 3′UTR (ENSBTAT00000043174) was synthesized by IDT in the context of the pIDT-SMART-KAN vector and subcloned into the XhoI, XbaI sites of pcDNA3.1dsRluc. The bovine PXDN 3′UTR seed mutant was synthesized in a similar fashion with the sequence "TG" replaced with "CC" at position 193–194 by IDT in the context of the pIDT-SMART-KAN vector and subcloned into the XhoI, XbaI sites of pcDNA3.1dsRluc. The GAPDH 3′UTR was amplified from DLD1 cell genomic DNA with primers GAPDH_F and GAPDH_R and cloned into the XhoI, XbaI sites of pcDNA3.1dsRluc to generate pcDNA3.1dsRluc-GAPDH. RNA-induced silencing complex (RISC) Assay Luciferase reporters were generated with two long oligonucleotides of opposing orientations, which encoded for two complementary binding sites for the various miRNAs separated by eight nucleotides. Each oligonucleotide possesses an overlap of 20 complementary base pairs and were annealed and elongated using Taq polymerase (New England Biolabs) (98 °C 30 s, 98 °C 10 s, 55 °C 20 s, 72 °C 10 s, 72 °C 5 min). Extended target sites were then cloned into the XhoI/XbaI site pcDNA3.1dsRluc.

Pol III miRNA Prediction. The Pol III miRNA prediction algorithm was implemented as a Python script. The first step of the algorithm is to perform a search of an individual viral genome for a B Box-like promoter sequence. Next, a window of W_{AB} nucleotides (70 nt) upstream of the B Box-like sequence is searched for an A Box-like sequence. If an A Box-like sequence is found, then a window of W_{BT} nucleotides (250 nt) downstream of the B Box-like sequence is searched for a short Terminator sequence. RNA secondary-structure prediction is conducted on the sequence of the predicted gene using RNAfold (4). Predicted genes that overlap extensively with annotated viral protein coding sequences (50 or more nucleotides) are discarded and a search for secondary structural elements associated with miRNA stem-loops is performed. Each individual stem-loop is subject to a second round of secondary-structural prediction and an energy cutoff ($\Delta G \leq -22$) is applied. B Box sequences used included GTTCNANNC, GGTTSGNG, and GKWCAAGTC. A Box sequences used included TRGNNNNNNGR, TRGNNNNNGR, and TRGCTC. Terminator sequences used included TTTT and a string of four or more Ts with one substitution. The algorithm was tested on the MHV68 genomic sequence (NC_001826) and correctly identifies MHV68 encoded tRNA-like miRNAs.

Small RNA Northern Blots. Small RNA Northern blot analysis was performed as previously described (5). Specific probes are listed in Table S4.

Synthetic Gene Small RNA Sequencing. T75 flasks of subconfluent HEK293T cells were transfected with 15 μg of pIDT-SMART-KAN -NC_001414_RNA or pIDT-SMART-KAN - NC_010819_RNA using the Turbofect reagent (Fermentas). Transfections were repeated the following day. Total RNA was harvested at ∼48 h. Sixty micrograms of total RNA from each sample was pooled with additional samples for a total of 300 μg total RNA and size fractionated on a 15% (vol/vol) Urea-PAGE gel. Small RNA libraries for SOLiD sequencing were then prepared as described previously (6). Sequencing adapter sequences were trimmed from the color space reads using custom Python scripts, and any sequences with ambiguous calls or less than 18 nt in length after trimming were removed from further analysis. The preprocessed reads were then mapped to viral genomic reference sequences (NC_001414 and NC_010819). The 5′ start site counts and coverage were calculated using custom Python scripts and visualized using the gnuplot software package.

Small RNA Sequencing BL3.1 Cells. Total RNA was harvested from BL3.1 cells in log-phase growth. Then, 200 μg of total RNA was size-fractionated on a 15% (vol/vol) PAGE and used to prepare small RNA libraries for SOLiD sequencing, as previously described (6). Sequencing adapter sequences were trimmed from the color space reads using custom Python scripts, and any sequences with ambiguous calls or less than 18 nt in length after trimming were removed from further analysis. The preprocessed reads were then

mapped to the BLV reference genome (National Center for Biotechnology Information accession number NC_001414) using the SHRiMP2 software package (7). The 5′ start site counts and coverage were calculated using custom Python scripts and visualized using the gnuplot software package. Genome CDS maps were generated from the NC_001414 reference sequence and the Geneious software package (Biomatters).

Precursor miRNA Structure Prediction. Predicted secondary structures of miRNA hairpins were generated using Mfold RNA folding prediction Web server (8, 9) or the RNAfold prediction Web server (4), as indicated.

Host Target Analysis. Conservation of HBP1 and PXDN mir-29 target sites was assessed using the Targetscan Web site (10). Alignment regions flanking the predicted target sites were manually extracted from the Targetscan alignment.

Host Target Luciferase Assays. Twelve-well plates of HEK293T cells were transfected in triplicate with 5 ng *Renilla* (pcDNA3.1dsRluc) 3′ UTR reporter, 5 ng firefly reporter (pcDNA3.1dsLuc2CP), and 1 μg of miRNA expression vector (or empty expression vector) using the Turbofect reagent (Fermentas). Twenty-four hours after transfection, the cells were harvested and processed with the Dual-Glo Luciferase Assay System (Promega) according to the manufacturer's instructions. The luciferase activity was measured on a Luminoskan Ascent luminometer (Thermo Electronic). Results for the host 3′UTR reporters are presented with the relative *Renilla* to firefly luciferase levels normalized to identical reactions transfected with empty expression vectors. Student *t* test was performed to assess statistical significance of observed differences.

RNA Polymerase III Dependence. HEK293T cells were transfected with 1 μg of expression vector and, where indicated, were then treated 2 h later with a final concentration of 50 μg/mL of α-amanitin (11). Total RNA was extracted 24 h posttransfection and Northern blot analysis performed.

Box B Mutant Analysis. HEK293T cells were transfected with 1 μg of pIDT empty vector, BLV mir-B4 miRNA expression vector, or the BLV mir-B4 Box B mutant using Turbofect reagent (Fermentas) in 12-well plates. Total RNA was extracted 30 h posttransfection and Northern blot analysis was performed.

RISC Activity Assay. Five nanograms *Renilla* (pcDNA3.1dsRluc) 3′UTR reporter, 5 ng firefly reporter (pcDNA3.1dsLuc2CP), and 1 μg of BLV miRNA expression vector were cotransfected into HEK293 cells in 12-well plates using Turbofect reagent (Fermentas). Twenty-four hours after transfection, the cells were harvested and processed with the Dual-Glo Luciferase Assay System (Promega) according to the manufacturer's instructions. The luciferase activity was measured on a Luminoskan Ascent luminometer (Thermo Electronic). All reporter expression is normalized to the empty *Renilla* luciferase vector.

Drosha Knockdown. HEK293T cells in 12-well plates were treated with 20 nM of Drosha siRNA (3, 12) using Lipofectamine RNAiMAX following manufacturer's recommendations (Invitrogen). Twenty-four hours later, a second knockdown was performed followed by transfection of miRNA expression vectors using the Turbofect reagent according to the manufacturer's recommendations (Fermentas). Thirty hours later, RNA was extracted using PIG-B (13) and Northern blot analysis was performed.

Dicer Dependence. Dicer wild-type cells (DLD1) and cells hypomorphic for Dicer activity [DLD1 Dicer$^{Ex5-/-}$ cell lines (14)] were transfected with 2 μg of the BLV B4 or the positive control MHV68 M1-7 miRNA expression vectors using the Turbofect reagent according to the manufacturer's recommendations (Fermentas). Thirty-six hours posttransfection, total RNA was extracted using PIG-B (13) and Northern blot analysis was performed.

Drosha Activity Assay. HEK293 cells in 12-well plates were transfected with 5 ng *Renilla* (pcDNA3.1dsRluc) 3′UTR reporter, 5 ng firefly reporter (pcDNA3.1dsLuc2CP), and either 1 μg of pcDNA 3.1+ or 0.5 μg of Drosha and 0.5 μg of DGCR8 expression vector (12) using the Turbofect reagent. Twenty-four hours after transfection the cells were harvested and processed with the Dual-Glo Luciferase Assay System (Promega) according to the manufacturer's instructions. The luciferase activity was measured on a Luminoskan Ascent luminometer (Thermo Electronic).

Microprocessor Overexpression. HEK293 cells in 12-well plates were transfected with 0.6 μg of the pcDNA3.1dsRluc-BLV miRNA, the pcDNA3.1dsRluc-MHV68 miR-M1-7, or pcDNA3.1dsRluc-SV40 miRNA and either 0.6 μg of pcDNA 3.1+ or 0.3 μg of Drosha and 0.3 μg of DGCR8 expression vectors were cotransfected into 293T cells. RNA was extracted 30 h posttransfection and Northern blot analysis was performed.

1. Adler H, Messerle M, Wagner M, Koszinowski UH (2000) Cloning and mutagenesis of the murine gammaherpesvirus 68 genome as an infectious bacterial artificial chromosome. *J Virol* 74:6964–6974.
2. Seo GJ, Fink LH, O'Hara B, Atwood WJ, Sullivan CS (2008) Evolutionarily conserved function of a viral microRNA. *J Virol* 82:9823–9828.
3. Lin YT, Sullivan CS (2011) Expanding the role of Drosha to the regulation of viral gene expression. *Proc Natl Acad Sci USA* 108:11229–11234.
4. Hofacker IL, Fontana W, Stadler PF, Bonhoeffer M, Schuster P (1994) Fast folding and comparison of RNA secondary structures. *Monatsh Chem* 125:167–188.
5. McClure LV, Lin YT, Sullivan CS (2011) Detection of viral microRNAs by Northern blot analysis. *Methods Mol Biol* 721:153–171.
6. Chen CJ, Kincaid RP, Seo GJ, Bennett MD, Sullivan CS (2011) Insights into Polyomaviridae microRNA function derived from study of the bandicoot papillomatosis carcinomatosis viruses. *J Virol* 85:4487–4500.
7. Rumble SM, et al. (2009) SHRiMP: Accurate mapping of short color-space reads. *PLOS Comput Biol* 5:e1000386.
8. Mathews DH, Sabina J, Zuker M, Turner DH (1999) Expanded sequence dependence of thermodynamic parameters improves prediction of RNA secondary structure. *J Mol Biol* 288:911–940.
9. Zuker M (2003) Mfold web server for nucleic acid folding and hybridization prediction. *Nucleic Acids Res* 31:3406–3415.
10. Lewis BP, Burge CB, Bartel DP (2005) Conserved seed pairing, often flanked by adenosines, indicates that thousands of human genes are microRNA targets. *Cell* 120: 15–20.
11. Bogerd HP, et al. (2010) A mammalian herpesvirus uses noncanonical expression and processing mechanisms to generate viral MicroRNAs. *Mol Cell* 37:135–142.
12. Han J, et al. (2004) The Drosha-DGCR8 complex in primary microRNA processing. *Genes Dev* 18:3016–3027.
13. Weber K, Bolander ME, Sarkar G (1998) PIG-B: A homemade monophasic cocktail for the extraction of RNA. *Mol Biotechnol* 9:73–77.
14. Cummins JM, et al. (2006) The colorectal microRNAome. *Proc Natl Acad Sci USA* 103: 3687–3692.

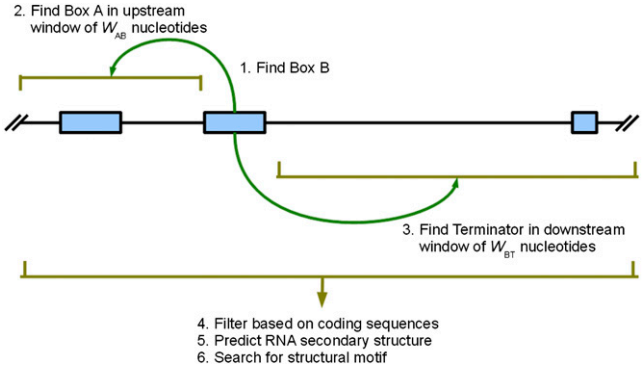

Fig. S1. Visual representation of pol III miRNA prediction algorithm. First, a search for a B Box-like sequence is performed. Once a B Box-like sequence is found, a window upstream of this position is searched for an A Box-like sequence. If an A Box-like sequence is found, then a window downstream of the B Box-like sequence is searched for a terminator like sequence. The predicted pol III gene is checked for overlap with annotated coding sequences and the secondary structural prediction is performed. Finally, the predicted secondary structure is searched for pre-miRNA like stem-loop structures.

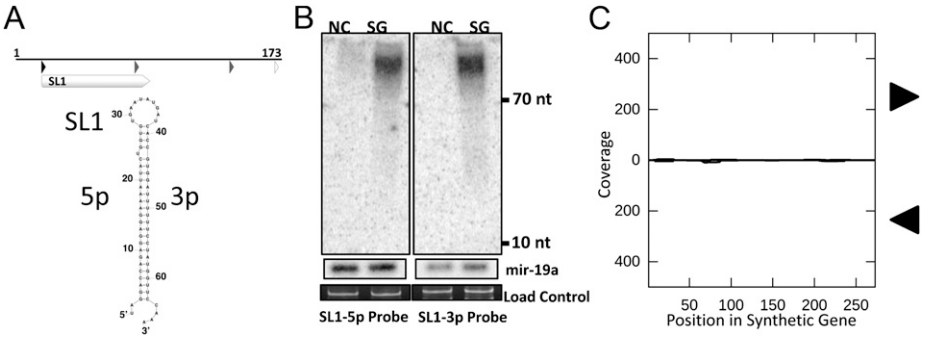

Fig. S2. A combined computational and synthetic approach fails to identify a macaque simian foamy virus-encoded miRNA. (*A*) Schematic of the organization of the predicted macaque simian foamy virus miRNA gene region. The predicted A Box-like, B Box-like, and terminator-like sequences are represented by black, gray, and white triangles, respectively, and Mfold RNA secondary structure predictions for the predicted miRNA stem-loop sequence is presented below. The genomic coordinates refer to reference sequence NC_010819. (*B*) Northern blot analysis of 293T cells transfected with the synthetic miRNA gene construct (SG lanes) or empty vector (NC lanes). Blots were stripped and reprobed for host mir-19a. The load control is ethidium bromide-stained low molecular weight RNA. (*C*) Small RNA profiling of 293T cells transfected with the synthetic miRNA construct reveals few reads mapping to the synthetic gene sequence.

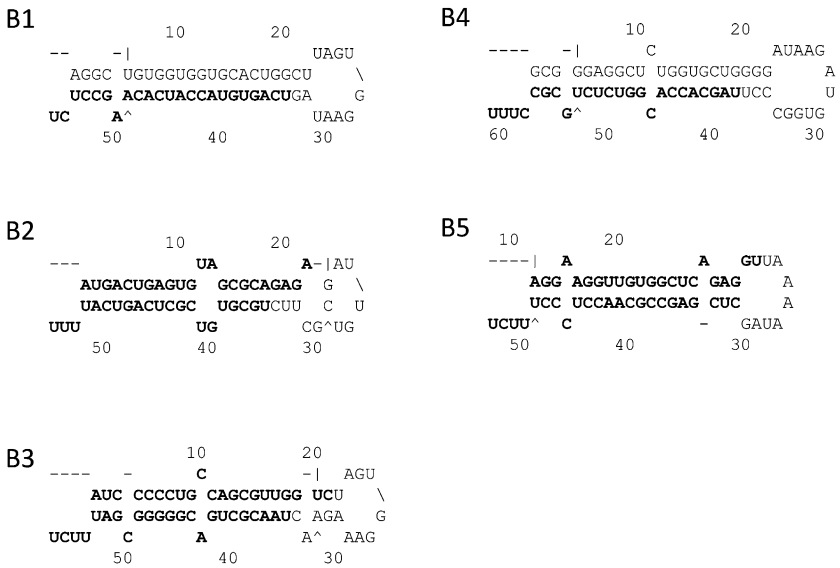

Fig. S3. Predicted RNA secondary structures of BLV-encoded pre-miRNAs. Predictions were generated using the Mfold software (9). The most commonly sequenced mature miRNA products from the BL3.1 cell line are in bold.

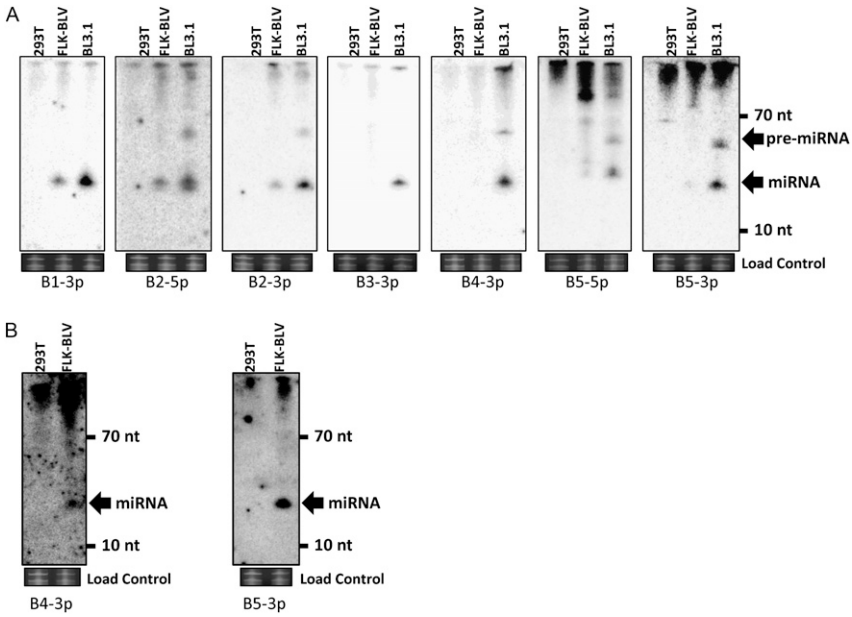

Fig. S4. Virus-derived small RNAs are detectable in both BL3.1 and FLK-BLV cell lines. (*A*) Northern blot analysis of RNA harvested from HEK293T, FLK-BLV, and BL3.1 cell lines. The load control is ethidium bromide-stained low molecular weight RNA. (*B*) Northern blot analysis of control 293T and FLK-BLV RNA probed with oligonucleotides designed to be perfectly complementary to the BLV913 miRNA sequences. The load control is ethidium bromide-stained low molecular weight RNA.

A

	BLV-mir-B1-3p	BLV-mir-B2-5p	BLV-mir-B2-3p	BLV-mir-B3-5p
Consensus:	TCAGTGTACCATCACAAGCCTCT	ATGACTGAGTGTAGCGCAGAGA	TGCGTGTCRCTCAGTCATTTT	ATCCCCCTGCCAGCGTTGGTC
BL3.1	TCAGTGTACCATCACAAGCCTCT	ATGACTGAGTGTAGCGCAGAGA	TGCGTGTCGCTCAGTCATTTT	ATCCCCCTGCCAGCGTTGGTC
BLV913	TCAGTGTACCATCACAAGCCTCT	ATGACTGAGTGTAGCGCAGAGA	TGCGTGTCGCTCAGTCATTTT	ATCCCCCTGCCAGCGTTGGTC
BLVCG	TCAGTGTACCATCACAAGCCTCT	ATGACTGAGTGTAGCGCAGAGA	TGCGTGTCGCTCAGTCATTTT	ATCCCCCTGCCAGCGTTGGTC
AF257515	TCAGTGTACCATCACAAGCCTCT	ATGACTGAGTGTAGCGCAGAGA	TGCGTGTCGCTCAGTCATTTT	ATCCCCCTGCCAGCGTTGGTC
FJ914764	TCAGTGTACCATCACAAGCCTCT	ATGACTGAGTGTAGCGCAGAGA	TGCGTGTCGCTCAGTCATTTT	ATCCCCCTGCCAGCGTTGGTC
NC_001414	TCAGTGTACCATCACAAGCCTCT	ATGACTGAGTGTAGCGCAGAGA	TGCGTGTCACTCAGTCATTTT	ATCCCCCTGCCAGCGTTGGTC
BLVGPE	TCAGTGTACCATCACAAGCCTCT	ATGACTGAGTGTAGCGCAGAGA	TGCGTGTCACTCAGTCATTTT	ATCCCCCTGCCAGCGTTGGTC

	BLV-mir-B3-3p	BLV-mir-B4-3p	BLV-mir-B5-5p	BLV-mir-B5-3p
Consensus:	TAACGCTGACGGGGGCGATTTCT	TAGCACCAYVGTCTCTGCGCCTTT	AGGARGGTTGTGGCTCAGAGGT	CTCGRRCCGCAACCTCCCTTTCT
BL3.1	TAACGCTGACGGGGGCGATTTCT	TAGCACCACGGTCTCTGCGCCTTT	AGGAAGGTTGTGGCTCAGAGGT	CTCGGACCGCAACCTCCCTTTCT
BLV913	TAACGCTGACGGGGGCGATTTCT	TAGCACCATAGTCTCTGCGCCTTT	AGGAGGGTTGTGGCTCAGAGGT	CTCGAGCCGCAACCTCCCTTTCT
BLVCG	TAACGCTGACGGGGGCGATTTCT	TAGCACCACAGTCTCTGCGCCTTT	AGGAAGGTTGTGGCTCAGAGGT	CTCGAGCCGCAACCTCCCTTTCT
AF257515	TAACGCTGACGGGGGCGATTTCT	TAGCACCATAGTCTCTGCGCCTTT	AGGAAGGTTGTGGCTCAGAGGT	CTCGAGCCGCAACCTCCCTTTCT
FJ914764	TAACGCTGACGGGGGCGATTTCT	TAGCACCATAGTCTCTGCGCCTTT	AGGAAGGTTGTGGCTCAGAGGT	CTCGAGCCGCAACCTCCCTTTCT
NC_001414	TAACGCTGACGGGGGCGATTTCT	TAGCACCACAGTCTCTGCGCCTTT	AGGAAGGTTGTGGCTCAGAGGT	CTCGGACCGCAACCTCCCTTTCT
BLVGPE	TAACGCTGACGGGGGCGATTTCT	TAGCACCACCGTCTCTGCGCCTTT	AGGAAGGTTGTGGCTCAGAGGT	CTCGAGCCGCAACCTCCCTTTCT

B

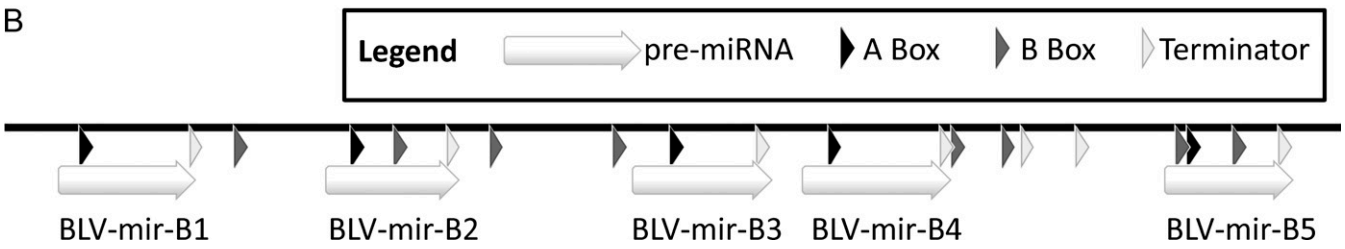

Fig. S5. BLV encoded miRNAs and predicted promoter elements are conserved across isolates. (*A*) Alignments of the most commonly sequenced mature miRNAs. Highlighted regions indicate the miRNA seed regions. Underlined sequences in consensus indicate 100% identity across isolates. (*B*) Schematic of conserved promoter-like elements across alignment of BLV isolates generated by individual promoter element searches.

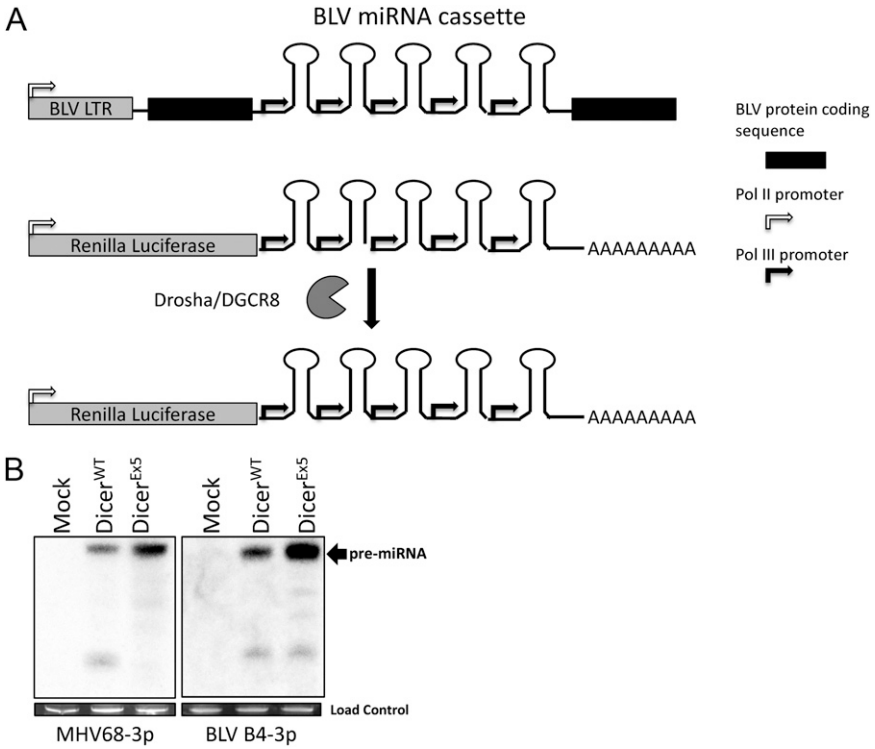

Fig. S6. Characterization of BLV-mir-B4 biogenesis. (*A*) Schematic of BLV miRNAs in the context of the viral genome. The miRNA cluster is transcribed by pol II in the production of full-length genomic RNA. Below is a schematic of the BLV miRNAs in the context of the *Renilla* luciferase reporter. The miRNA cluster is transcribed by pol II from the vector CMV promoter. The viral miRNA cluster is resistant to Drosha cleavage when transcribed in the context of the long pol II transcripts. (*B*) Northern blot analysis of MHV68-M1-7 and BLV-B4 miRNA expression vectors transfected into DLD1 cells with wild-type Dicer ($Dicer^{WT}$) or hypomorphic for Dicer activity ($Dicer^{Ex5}$) (14). The load control is ethidium bromide-stained low molecular weight RNA. As expected, reduced Dicer activity results in an accumulation of the Dicer substrate pre-miRNA for the positive control MHV68 miRNA. Accumulation of the pre-miRNA was also observed for the BLV miRNA, most consistent with its biogenesis being Dicer-dependent.

Table S1. Summary of retroviruses with predicted pol III miRNA genes

Family	Subfamily	Genus	Species	Detected by Northern blot	Detected by sequencing
Retroviridae	*Orthoretrovirinae*	Deltaretrovirus	Bovine leukemia virus	+	+
Retroviridae	*Spumaretrovirinae*	Spumavirus	Bovine foamy virus	N/A	N/A
Retroviridae	*Spumaretrovirinae*	Spumavirus	Equine foamy virus	N/A	N/A
Retroviridae	*Spumaretrovirinae*	Spumavirus	Feline foamy virus	N/A	N/A
Retroviridae	*Spumaretrovirinae*	Spumavirus	Macaque simian foamy virus	—	—
Retroviridae	*Spumaretrovirinae*	Spumavirus	Simian foamy virus	N/A	N/A

N/A refers to candidates not screened. + indicates detected in screen. – indicates inability to detect in screen.

Table S2. Most-often sequenced small RNA sequences from BL3.1 cells that map to the BLV miRNA loci

No. mapped	Start	Sequence	End	Length
26,334	6220	TCAGTGTACCATCACAAGCCTCT	6242	23
9,379	6298	ATGACTGAGTGTAGCGCAGAGA	6319	22
5,881	6460	TAACGCTGACGGGGGCGATTTCT	6482	23
5,679	6220	TCAGTGTACCATCACAAGCCTC	6241	22
3,751	6298	ATGACTGAGTGTAGCGCAGA	6317	20
3,680	6649	AAGGAAGGTTGTGGCTCAGAGGT	6670	23
3,556	6297	ACATGACTGAGTGTAGCGCAGA	6317	22
3,451	6220	TCAGTGTACCATCACAAGCCTCTT	6243	24
3,271	6333	TGCGTGTCACTCAGTCATTTT	6352	21
2,870	6220	TCAGTGTACCATCACAAGCCTCT	6241	23
2,680	6220	TCAGTGTACCATCACAAGCCT	6240	21
2,025	6679	CTCGGACCGCAACCTCCCTTTCT	6701	23
1,973	6298	ATGACTGAGTGTAGCGCAGAGAG	6319	23
1,948	6460	TAACGCTGACGGGGGCGATTTC	6481	22
1,824	6534	TAGCACCACGGTCTCTGCGCCTTT	6557	24
1,674	6298	ATGACTGAGTGTAGCGCAGA	6316	20
1,234	6220	TCAGTGTACCATCACAAGCCTCTTC	6243	25
1,200	6298	ATGACTGAGTGTAGCGCAGAGA	6318	22
1,128	6298	ATGACTGAGTGTAGCGCAGAG	6317	21
1,122	6649	AAGGAAGGTTGTGGCTCAGAGGTT	6671	24
1,081	6298	ATGACTGAGTGTAGCGCAGAG	6318	21
1,039	6220	TCAGTGTACCATCACAAGCCTCTT	6242	24
998	6298	ATGACTGAGTGTAGCGCAGAGA	6317	22
939	6333	TGCGTGTCACTCAGTCATTT	6351	20
905	6297	ACATGACTGAGTGTAGCGCAGA	6316	22
860	6220	TCAGTGTACCATCACAAGCCTCT	6240	23
845	6649	AAGGAAGGTTGTGGCTCAGAGG	6669	22
802	6220	TCAGTGTACCATCACAAGCCTCTTC	6242	25
762	6220	TCAGTGTACCATCACAAGCCTC	6240	22
724	6534	TAGCACCACGGTCTCTGCGCCTTTT	6558	25
716	6460	TAACGCTGACGGGGGCGATTTCT	6481	23
709	6649	AAGGAAGGTTGTGGCTCAGAGG	6668	22
686	6460	TAACGCTGACGGGGGCGATTT	6480	21
620	6220	TCAGTGTACCATCACAAGCCTCTT	6241	24
617	6649	AAGGAAGGTTGTGGCTCAGAGGTTA	6672	25
573	6330	TCTGCGTGTCACTCAGTCATTT	6351	22
564	6460	TAACGCTGACGGGGGCGATTTCTT	6482	24
532	6297	ACATGACTGAGTGTAGCGCAGAG	6317	23
529	6649	AAGGAAGGTTGTGGCTCAGAG	6668	21
465	6534	TAGCACCACGGTCTCTGCGCCTT	6556	23
435	6220	TCAGTGTACCATCACAAGCCTCTTCT	6243	26
414	6649	AAGGAAGGTTGTGGCTCAGAGGT	6669	23
413	6333	TGCGTGTCACTCAGTCATTTTT	6353	22
393	6649	AAGGAAGGTTGTGGCTCAGAGGTTA	6671	25
361	6680	TCGGACCGCAACCTCCCTTTCT	6701	22
351	6298	ATGACTGAGTGTAGCGCAGAGAGG	6321	24
341	6649	AAGGAAGGTTGTGGCTCAGAGGTT	6670	24
335	6220	TCAGTGTACCATCACAAGCCTCTTCT	6242	26
312	6298	ATGACTGAGTGTAGCGCAGAGAGG	6319	24
309	6679	CTCGGACCGCAACCTCCCTTTC	6700	22

Table S3. Most-often sequenced small RNA sequences from BL3.1 cells that map to bovine host miRNAs listed in miRBase (1)

No. mapped	Sequence	Length
36,224	UGUGCAAAUCCAUGCAAAACUGA	23
21,239	AACCCGUAGAUCCGAUCUUGUGG	23
20,644	UCCCUGAGACCCUAACUUGUGA	22
14,433	UAUUGCACUUGUCCCGGCCUGU	22
13,565	UGUGCAAAUCCAUGCAAAACUG	22
12,391	AACCCGUAGAUCCGAUCUUGUG	22
11,554	AACAUUCAACGCUGUCGGUGAGU	23
6,909	UGUGCAAAUCUAUGCAAAACUGA	23
6,588	UAGCACCAUCUGAAAUCGGUUA	22
6,376	CAACGGAAUCCCAAAAGCAGCU	22
5,738	UAGCUUAUCAGACUGAUGUUGAC	23
4,712	UGAGGUAGUAGGUUGUAUGGUU	22
4,442	AACCCGUAGAUCCGAUCUUGU	21
3,978	UAGCACCAUUUGAAAUCAGUGUU	23
3,928	CAUUGCACUUGUCUCGGUCUGA	22
3,736	AGCAGCAUUGUACAGGGCUAUGA	23
3,467	UAGCAGCACAUCAUGGUUUACA	22
3,428	CAAAGUGCUUACAGUGCAGGUAG	23
3,231	UGAGGUAGUAGGUUGUAUAGUU	22
3,196	UGUGCAAAUCCAUGCAAAACUG	22
2,774	UGUGCAAAUCCAUGCAAAACUGA	23
2,669	UGGCUCAGUUCAGCAGGAACAGG	23
2,551	UGUGCAAAUCUAUGCAAAACUG	22
2,548	UAGCACCAUUUGAAAUCGGUUA	22
2,347	UCCCUGUCCUCCAGGAGCUCA	21
2,112	AUCACAUUGCCAGGGAUUUCCA	22
2,075	CAACGGAAUCCCAAAAGCAGCUG	23
2,016	UAAAGUGCUUAUAGUGCAGGUAG	23
1,914	UUCAAGUAAUCCAGGAUAGGCU	22
1,899	UGAGGUAGUAGAUUGUAUAGUU	22
1,783	CAAAGUGCUUACAGUGCAGGUAGU	24
1,726	UCCCUGAGACCCUAACUUGUG	21
1,697	UCCCUGAGACCCUAACUUGUGAU	23
1,632	UAGCAGCACGUAAAUAUUGGCGU	23
1,604	UAGCAGCACGUAAAUAUUGGCG	22
1,568	UGUAGUGUUUCCUACUUUAUGGA	23
1,510	UGUGCAAAUCCAUGCAAAACU	21
1,506	UCCCUGUCCUCCAGGAGCUCACU	23
1,506	UGUAGUGUUUCCUACUUUAUGG	22
1,452	UGAGGUAGUAGUUUGUACAGUU	22
1,446	UAUUGCACUUGUCCCGGCCUGUU	23
1,278	UAUUGCACUUGUCCCGGCCUGUUG	24
1,243	AACAUUCAACGCUGUCGGUGAGUUU	25
1,173	UAGCUUAUCAGACUGAUGUUGACU	24
1,139	AAUGACACGAUCACUCCCGUUGA	23
1,109	CAAAGUGCUGUUCGUGCAGGUAG	23
1,107	UGUAGUGUUUCCUACUUUAUGGA	23
1,106	UAAAGUGCUUAUAGUGCAGGUAG	23
1,103	CAGUGCAAUGAUGAAAGGGCAU	22
1,059	UGAGGUAGUAGGUUGUAUGGUUU	23

1. Griffiths-Jones S (2006) miRBase: The microRNA sequence database. *Methods Mol Biol* 342:129–138.

Table S4. Oligonucleotides used in cloning and Northern blot analysis

Oligonucleotide name	Sequence
776miR-xbal-R	ATCGATCGATCTAGAGTGGCTATGGGAATTGGA
776miR-xho1-F	ATCGATCGACTCGAGTTATGTTTCAGGTTCAGGGGG
BLV B4 3p target xho1 sense	AGTCTCGAGGGCGCAGAGACTGTGGTGCTAGGGA TCGGCTAGG
BLV B4 3p target xba1 anti sense	CATTCTAGACCCTAGCACCACAGTCTCTGCGCCTAG CCGATCCC
blv miRNAs antisense xba1	TTAGGGGGCCCAGAACCCG
blv miRNAs sense xho1	AGTCTCGAGGATTACATCAACCTTCGACCCT
BLV_B1_3p_probe	AGAGGCTTGTGATGGTACACTGA
BLV_B2_3p_probe	AAAATGACTGAGCGACACGCA
BLV_B2_5p_probe	TCTCTGCGCTACACTCAGTCAT
BLV_B3_3p_probe	AGAAATCGCCCCCGTCAGCGTTA
BLV_B3_5p_probe	GACCAACGCTGGCAGGGGGAT
BLV_B4_3p_probe	AAAGGCGCAGAGACCGTGGTGCTA
BLV_B5_3p_probe	AGAAAGGGAGGTTGCGGTCCGAG
BLV_B5_5p_probe	ACCTCTGAGCCACAACCTTCCT
BLV_RTH_F	AAATTCGAATCTTCCCCATGCAGCTTCCG
BLV_RTH_R	AAAAGGCGCAGAGACTGTGGTGCTAG
BTA_HBP1_3UTR_R	ATCGTCTAGAGGGGCAACATCATGAAGTGTAATGAACTG
BTA_HPB1_3UTR_F	ATCGCTCGAGACAGGATGCTTGGCTGTTGATGGAAAG
BTA_PXDN_3UTR	GCGTCTGCGAGCCCGGAGCTTGTCCC AGCCCTTGGGAGCGAGGCCGCGTAGGCGGCGAGGCT GGGACGCGGGGGCCGCGGCTCGGGGAACCCCG GCCTGAGCGCTCGCCAGCAGACCACGGGGCAGACGC GTGCCTGACTCCATGTTAAGGCTTCTCAGGGTTCTAT TTAATTTTTTTTAATGAAAAATTGGTGCTACTATTAAA TTGCACAGTTCAATCATTTAGGCTCCTAATTGATTTCACC TAGTATCACCGTTTCTTCTCAACTGAAAGTTAGTCCC GCCGGTCCCGGCTCCAGAAGCGTGCAGAGCGGAGC AGGCGGAGGAGGGACGCAGGATTTGAAAGAGAAT CCGTCCCTGGCAAAC
FLK_B4_3p_probe	AAAGGCGCAGAGACTATGGTGCTA
FLK_B5_3p_probe	AGAAAGGGAGGTTGCGGCTCGAG
GAPDH_FWD	ATCGCTCGAGATGGCCCACATGGCCTCCAA
GAPDH_REV	ATCGTCTAGATGAGCACAGGGTACTTTATTGATGGT
MHV68 -M1-7–3p probe	AATAAAGGTGGGCGCGATATC
MiR-m-7 anti xba1	ATCTCTAGATTTGGTGGGAGGCTAGATGGT
MiR-M7-7 sense xho1	GATCTCGAGCAAAGTCTAAGTCCCTGTACAGG
NC_001414_RNA	ATTTCTTGCAGCTGTGCTAAGCGAGAGGCTCT GGTGCTGGGGATAAGATGCGGCCCCTAGCACCACAGTC TCTGCGCCTTTTGGGTTCGAATCTTCCCCATGCAGCTTCC GCTTTTTACGCCCTGTTGCACACCCTTTCTAGAGATACCTG AAAATCTCAGCTCGCACCCCAAGGAAGGTTGTGGC TCAGAGGTTAAAATAGCTCGGACCGCAACCTCCCTTTC TTTTTATTCCACCCTCGCAA
NC_001414_SL1_3p	GGCGCAGAGACTGTGGTGCTAGGGG
NC_001414_SL1_5p	TCCCCAGCACCAGAGCCTCTCGCT
NC_001414_SL2_3p	AAAGGGAGGTTGCGGTCCGAG
NC_001414_SL2_5p	CTCTGAGCCACAACCTTCCTTG
NC_010819_RNA	TGTGGCAGACAGCCACTAAATGTATAGGACCAGAGGAGGAA AATTACTGGTGTGAATATGATCACCGTGGATTTTTTCCTATG GTTCCAAACAAGCTGTCTCCTACCTGGGTGAGACATGCTGCC CCCTACTGTATCCAGAGGTTCGCAACACCATATGATCTCCAG TATTTTGCAAATGAGCTTCTACCCCCTGGTTTCAGTATAAC GACTCCTAAGGGAGTGAGTTATACCAGTGATCGAAGGCTC CATTATGGAAATGAAGGAACTCTCC
NC_010819_SL1_3p	TGGAACCATAGGAAAAAATCCACGGTG
NC_010819_SL1_5p	CACCAGTAATTTTCCTCCTCTGGTCCTA
SV40 776 3p probe	CATGGAAGACTCAGGGCATGAAACAGGCATTGATTCAC
U6_probe	CGTTCCAATTTTAGTATATGTGCTGCC
hsa-mir-19a probe	TCAGTTTTGCATAGATTTGCACA

Table S4. Cont.

Oligonucleotide name	Sequence
BTA_PXDN SM_3UTR	TCTAGAGTTTGCCAGGGACGGATTCTCTTTCAAATCCTGCGT CCCTCCTCCGCCTGCTCCGCTCTGCACGCTTCTGGAGCCGGGA CCGGCGGGACTAACTTTCAGTTGAGAAGAAACGGTGATACT AGGTGAAATCAATTAGGAGCCTAAATGATTGAACTGTGCAA TTTAATAGTAGGGCCAATTTTTCATTAAAAAAAATTAAA TAGAACCCTGAGAAGCCTTAACATGGAGTCAGGCACG CGTCTGCCCCGTGGTCTGCTGGCGAGCGCTCAGGCCGG GGTTCCCCGAGCCGCGGCCCCCGCGTCCCAGCCTCGCC GCCTACGCGGCCTCGCTCCCAAGGGCTGGGACAAGCT CCGGGCTCGCAGACGCCTCGAG
BTA_HPB1_SM Fragment	GATATCCTGTGGCCCTAAAGTACAGTAGAAAAGAGAGGAG AAGTGTATACATGTTTTATTTTAAATTGTACAAAA GGGGAATTTAAAAATATGTAACTGCTGTTTATACATT GGCTCCTTACTGCTTATTAATCTGTATTGTACACATAA TGGGGTGAAGCAGAAGCCGGGAGTTGGCCTTCCTT GAGCAACCACCACATGGCTCAGCATCTGTGCCAAA CACAGGGGCTCCTAGTCTGGCCAGTGCCAAGAGGTT GCCAGGACACAGGGCCGGTGGATGGTGCCAGTGCCAG CCTAGCCACTGGCCGCGGCTCCTGAGCATGCTGAGTT GGACACACCGGGCGGCGGCGCCCACCCTTGACTTCAGT GGAGACCCAGCCCAGGCCAAGGTAAAGTTAGTTAATA GCATTGGGATATTGTCACTGTAATGGTGCTGTTAACAGTTG ACACCATTGTATTTTTAACTTTGTGTCCTATATCTCCTCAGC