**S1 Text: Supplementary Materials and Methods**

**Nucleotide sequences of wild-type and miRNA deleted proviruses**

Wild-type BLV

6139-ATCAACCTTCGACCCTGCCCTTGACACCCCCGTGTTTCACGCACCCTCAGGCTGTGGTGGGGCACTGGCTTAGTGGAATAGTCAGTGTACCATCACAAGCCTCTTCTTGCTGCCAGCACCGAGTTCGAACACAGCCCTACCCTGAGCCTCTCTGAGTACATGACTGAGTGTAGCGCAGAGAGGTTGTCGCTTCTGCGTGTCACTCAGTCATTTTTTATAGCCGATTGGGGTTCGCGCCCTCCCATTGCCTGTGACACGGTTAAGACCTCTCTCACTTCTGCTTCACCATCCCCCTGCCAGCGTTGGTCTAGTGGAAAGAACTAACGCTGACGGGGGCGATTTCTTGCAGCTGTGCTAAGCGAGAGGCTCTGGTGCTGGGGATAAGATGCGGCCCCTAGCACCACAGTCTCTGCGCCTTTTGGGTTCGAATCTTCCCCATGCAGCTTCCGCTTTTTACGCCCTGTTGCACACCCTTTCTAGAGATACCTGAAAATCTCAGCTCGCACCCCAAGGAAGGTTGTGGCTCAGAGGTTAAAATAGCTCGGACCGCAACCTCCCTTTCTTTTTATTCCACCCTCGCAAGGCCCCGGGTTCTGGGCCCCCTAACGGAGGTTCAGAATTTCCTCTACTAGGGGATGCTCAGGTCCAAGTGTGCACAATATCTCTTCCAAAAGGTCCTGATGAACATCTTCCCATGTAACAAGCCCCAGCAGAGACATTCC

-6860

Junction sequence at the miRNA-deletion

6139-ATCAACCTTCGACCCTGCCCTTGACACCCCCTCTAGACCCCCTAACGGAGGTTCAGAATTTCCTCTACTAGGGGATGCTCAGGTCCAAGTGTGCACAATATCTCTTCCAAAAGGTCCTGATGAACATCTTCCCATGTAACAAGCCCCAGCAGAGACATTCC

-6860

**Construction of effector and reporter luciferase plasmids**

Effector plasmid pSUPER-miRNA was constructed by cloning the five miRNA hairpins (nucleotides 6170 to 6759 of the BLV reference genome NC\_001414.1) into BglII and HindIII sites of expression vector pSUPER (Oligoengine). pSUPER-B4 only contains the miRNA-B4 hairpin (nucleotides 6484 to 6664).

AGATCT: BglII restriction site

AAGCTT: HindIII restriction site

BLV\_B4\_BglII\_psuper

CGATCAGATCTGCAGCTGTGCTAAGCGAGA

BLV\_B4\_HindIII\_psuper

CGATCAAGCTTGAGCCACAACCTTCCTTGG

BLV\_B12345\_BglII\_psuper

CGATCAGATCTGTGTTTCACGCACCCTCAG

BLV\_B12345\_HindIII\_psuper

CGATCAAGCTTAATTCTGAACCTCCGTTAGGG

After extraction of BL3 RNA, reverse transcription and PCR amplification, a series of luciferase reporter plasmids (ANXA1, GZMA, FOS, MAP2K1, PIK3CG and PPT1) were constructed by inserting the full-length bovine cDNA sequences into psiCHECK2 (Promega). Mutations of the predicted target sequences in these reporter plasmids were introduced using the Q5 directed mutagenesis kit (NEB). Primers were the following:

Cloning of the full-length cDNAs (via AsisI and NotI restriction)

GCGATCGC: AsisI restriction site

GCGGCCGC: NotI restriction site

ANXA1\_FL\_AsisI\_fw

AGTCAGCGATCGCCCTTCAAAAATGGCAATGGT

ANXA1\_FL\_NotI\_rv

AGTCAGCGGCCGCAGGGTTTTATAGTTTGGAATAACCA

FOS\_FL\_ AsisI\_fw

AGTCAGCGATCGCGCAAGCTTAAGGAGCTGACA

FOS\_FL\_ NotI\_rv

AGTCAGCGGCCGCCAAGTTCACTTTCCACATGTCA

GZMA\_FL\_ AsisI\_fw

AGTCAGCGATCGCCTTTCTGGCAGCCACTCTCT

GZMA\_FL\_ NotI\_rv

AGTCAGCGGCCGCAAACGACAGCACTGGGTGTA

MAP2K1\_FL\_ AsisI\_fw2

AGTCAGCGATCGCGCAGCTCAGAGGGAGGAAG

MAP2K1\_FL\_ NotI\_rv

AGTCAGCGGCCGCGATTAGGCTTGTTAAACATCTGGA

PIK3CG\_FL\_ AsisI\_fw2

AGTCAGCGATCGCGTCGCCTCTGCTGCTTTATC

PIK3CG\_FL\_ NotI\_rv2

AGTCAGCGGCCGCCCTCCTCGCTCTTCCCTACT

PPT1\_FL\_ AsisI\_fw

AGTCAGCGATCGCGCGGTCACGTGATAGAGTCA

PPT1\_FL\_ NotI\_rv

AGTCAGCGGCCGCCCTTTCATCCCCTTGTTTCA

Mutagenesis of the full-length cDNA sequences (using Q5 site-directed mutagenesis)

Q5mut\_FOS\_mut\_fw

AATTAACCTGacaCTGGATATTTTCAAATTGTATC

Q5mut\_FOS\_mut\_rv

CCAATAACGAACCCAATAG

Q5mut1\_GZMA\_CDS\_fw

CCTACATGGTatTACTTGATGGGG

Q5mut1\_GZMA\_CDS\_rv

GTCTTGAATGAGGAGTCAC

Q5mut2\_GZMA\_3’UTR\_fw

TGTTAAGCGGctCTGCAATGAAC

Q5mut2\_GZMA\_3’UTR\_rv

ATAACCAGGACGTGGAAG

Q5mutsheep\_PPT1\_B4\_fw

GCAGATGGTGtgGAATGCCAATGTAATTAG

Q5mutsheep\_PPT1\_B4\_rv

TGACAGGGTGAGGCAAAG

Construction of luciferase reporters with predicted or mutated target sequences required primers:

Cloning of the predicted targets (via XhoI and NotI restriction)

GCGGCCGC: NotI restriction site

CTCGAG: XhoI restriction site

GATATC: EcoRV restriction site

Predicted target for BLV-mir-B4-3p on FOS mRNA

BLV-mir-B4-3p\_vs\_FOS\_fw

TCGAGGATATCttattggaattaACCTGGTGCTgGC

BLV-mir-B4-3p\_vs\_FOS\_rv

GGCCGCcAGCACCAGGTtaattccaataaGATATCC

Mutated predicted target

BLV-mir-B4-3p\_vs\_FOS\_mut\_fw

TCGAGGATATCttattggaattaACCTGACACTgGC

BLV-mir-B4-3p\_vs\_FOS\_mut\_rv

GGCCGCcAGTGTCAGGTtaattccaataaGATATCC

Predicted target for BLV-mir-B4-3p on GZMA mRNA (CDS)

BLV-mir-B4-3p\_vs\_GZMA(CDS)\_fw

TCGAGGATATCctcattcaAGACCCTACATGGTGCTaGC

BLV-mir-B4-3p\_vs\_GZMA(CDS)\_rv

GGCCGCtAGCACCATGTAGGGTCTtgaatgagGATATCC

Mutated predicted target

BLV-mir-B4-3p\_vs\_GZMA(CDS)\_ mut\_fw

TCGAGGATATCctcattcaAGACCCTACATGGTATTaGC

BLV-mir-B4-3p\_vs\_GZMA(CDS)\_ mut\_rv

GGCCGCtAATACCATGTAGGGTCTtgaatgagGATATCC

Predicted target for BLV-mir-B4-3p on GZMA mRNA (3’UTR)

BLV-mir-B4-3p\_vs\_GZMA(3’UTR)\_fw

TCGAGGATATCTAGGTTGCTTCCACGTCCTGGTTATTGTTAAGCGGTGCTGCGC

BLV-mir-B4-3p\_vs\_GZMA(3’UTR)\_rv

GGCCGCGCAGCACCGCTTAACAATAACCAGGACGTGGAAGCAACCTAGATATCC

Mutated predicted target

BLV-mir-B4-3p\_vs\_GZMA(3’UTR)\_mut\_fw

TCGAGGATATCTAGGTTGCTTCCACGTCCTGGTTATTGTTAAGCGGCTCTGCGC

BLV-mir-B4-3p\_vs\_GZMA(3’UTR)\_mut\_rv

GGCCGCGCAGAGCCGCTTAACAATAACCAGGACGTGGAAGCAACCTAGATATCC

Predicted target for BLV-mir-B4-3p on PPT1 mRNA

BLV-mir-B4-3p\_vs\_PPT1\_fw

TCGAGGATATCaccctgtCAGCAGATGGTGCTgGC

BLV-mir-B4-3p\_vs\_PPT1\_rv

GGCCGCcAGCACCATCTGCTGacagggtGATATCC

Mutated predicted target

BLV-mir-B4-3p\_vs\_PPT1\_sheep\_fw

TCGAGGATATCATCCTATCAGCAGATGGTGTGGGC

BLV-mir-B4-3p\_vs\_PPT1\_sheep\_rv

GGCCGCCCACACCATCTGCTGATAGGATGATATCC

Perfect target for BLV-mir-B4-3p

BLV-mir-B4-3p\_perfect\_target\_Fw

TCGAGGATATCAAAGGCGCAGAGACCGTGGTGCTAGC

BLV-mir-B4-3p\_perfect\_target\_Rev

GGCCGCTAGCACCACGGTCTCTGCGCCTTTGATATCC

**Primers for quantification of cellular mRNAs by RT-qPCR**

ANXA1\_RTQPCR\_fw

GGGCCTTGGGACTGATGAAG

ANXA1\_RTQPCR\_rv

CTCCAGATGTGTCTGAGGCG

FOS\_RTQPCR\_fw2

CATGGGTTCTCCCGTCAATG

FOS\_RTQPCR\_rv2

TGTTGGGATGAAGTTGGCACT

GZMA\_RTQPCR\_Fw

ATGGTGCTACTTGATGGGGG

GZMA\_RTQPCR\_Rv

GAGTGGGCCCCAAGAATGAT

MAP2K1\_RTQPR\_Fw

AGAGTTGGGAGCGCCTTTC

MAP2K1\_RTQPR\_Rv

TTCCGCTGCTGTTCATCGAG

PIK3CG\_RTQPCR\_fw

CTGCGCCAAGACATGCTTAT

PIK3CG\_RTQPCR\_rv

GTTCGTGCTGGTGGTTTCCT

PPT1\_RTQPCR\_fw

CTGGTGATCTGGCATGGGATG

PPT1\_RTQPCR\_rv

GCTGTTCTCCACATCCTCTCTG

**Depletion of IgM+ B cells from PBMCs**

PBMCs from BLV WT or ∆miRNA inoculated sheep were depleted from B cells using anti-IgM monoclonal IgG1 mouse antibody (clone 1H4) in association with immunomagnetic beads coated with rat anti-mouse IgG1 and MACS columns according to the manufacturer instructions (Miltenyi Biotec, Bergisch-Gladbach, Germany). Purity (less than 5% of IgM+ in the depleted fraction, more than 95% of IgM+ in the positive fraction) was evaluated by FACS analysis. GZMA mRNA levels were determined as described previsously.

**Proliferation of BL3 and BL3-miRNA cells in vitro**

BL3 and BL3-miRNA were labelled with CFSE according to the manufacturer instructions (eBioscience). Briefly, cells were labelled in PBS containing 1M CFSE at 37°C during 10 minutes and washed with ice-cold RPMI medium. Cells were washed with RPMI medium and cultivated in RPMI 10%FCS with antibiotics. CFSE labelling was monitored every day by FACS analysis (BD FACS Calibur).

**Apoptosis of BL3 and BL3-miRNA cells in vitro**

Apoptosis of BL3 and BL3-miRNA cells was estimated using the AnnexinV-PE apoptosis detection kit I from BD biosciences according to the manufacturer instructions. Cells were analyzed by FACS (BD FACS Calibur) and percentage of AnnexinV+ 7AAD+ cells was measured.

**Expression of BLV mRNAs and BLV proteins in vitro**

HEK 293T were transfected with plasmids pBLV-WT or pBLV-∆miRNA using lipofectamine 2000 (Life Technologies) and cultivated during 48 hours in RPMI medium supplemented with L-glutamine, antibiotics and 10% FCS. Total RNA was extracted from cells using miRNeasy kit (Qiagen). Co-purified genomic DNA was removed using Turbo DNAse kit (Life Technologies). cDNAs were produced using Reverse Transcriptase Superscript III (Life Technologies) and random hexamers following manufacturer protocol. The following primers were used: HPRT (5’-GGTCAAGAAGCATAAACCAAAG-3’ and 5’-AAGGGCATATCCCACAACAAAC-3’), Tax/Rex (5’-GCGTTTGCTGAAAGCCTTCAA-3’ and 5’-GGGCAGGCATGTAGAGAGTG-3’), Gag/genomic (5’-TCCCTTTCTCATCACGTTCC-3’ and 5’-GTGGGGGTGAATGGTGTAAC-3’) and Env (5’-CTATCCGGCAGCGGTCAG-3’ and 5’-GAGGAGAGTAAGAGTGAGACTTACCC-3’). Three dilutions of cDNA were amplified by real-time quantitative PCR in a Roche light cycler using MESA green master mix (Eurogentec). The thermal protocol used started by a 95°C 5 min denaturation step; then 45 cycles as follow (95°C 15 sec, 60°C 20 sec, 72°C 40 sec) and terminated with a melting curve. PCR efficacies were calculated for each sample using the three dilutions. Relative BLV transcript expressions were calculated from HRPT expression using delta-delta Ct method. To determine the proportion of cells expressing the p24 viral protein, cells were fixed in 4% paraformaldehyde (Sigma Aldrich) and permeabilized using PBS-0.5% Triton X-100 (Sigma Aldrich). Intracellular detection of p24 was performed by sequential incubation with 4'G9 monoclonal antibody and a rat anti-mouse IgG1 Alexa488 conjugate (Life Technologies) for 30 min at 4 °C. Ten thousand events per sample were collected by flow cytometry (Becton Dickinson FACScalibur) and analyzed with the Cellquest software. To estimate the overall quantities of viral proteins p24 (CA), gp51 (SU) and p34 (Tax), cells were lyzed in RIPA buffer supplemented with the Complete protease inhibitor (Roche) during 30 min on ice. Protein concentration was measured by the Pierce BCA protein assay kit. Proteins (25μg) were resolved by SDS-PAGE and analyzed by Western blotting using standard procedures. Actin, p24, gp51 and Tax were detected with polyclonal rabbit anti-actin (Sigma Aldrich), anti-p24 4’G9, anti-SU BLV2 (VMRD) and anti-Tax 5A5 monoclonal antibodies, respectively. After incubation with HRP-conjugated anti-IgG secondary antibodies (Dako), immunoblots were revealed with the Pierce ECL western blotting substrate and a GE ImageQuant LAS imager.