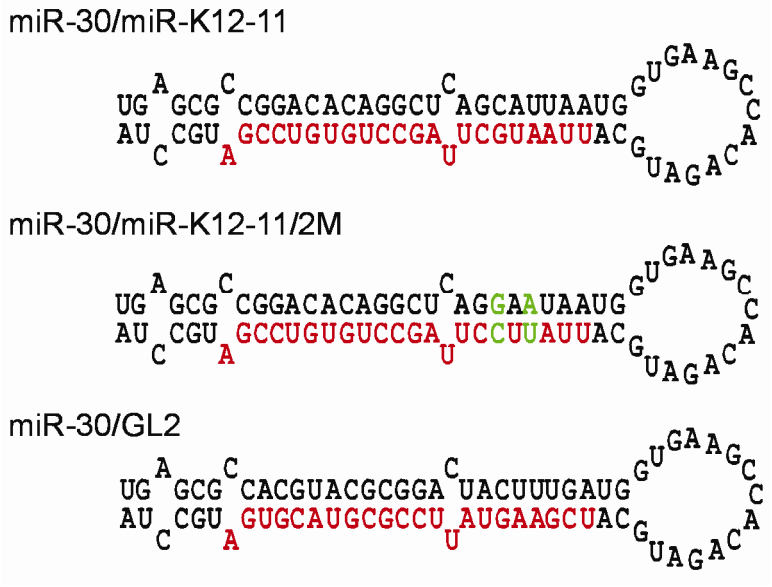
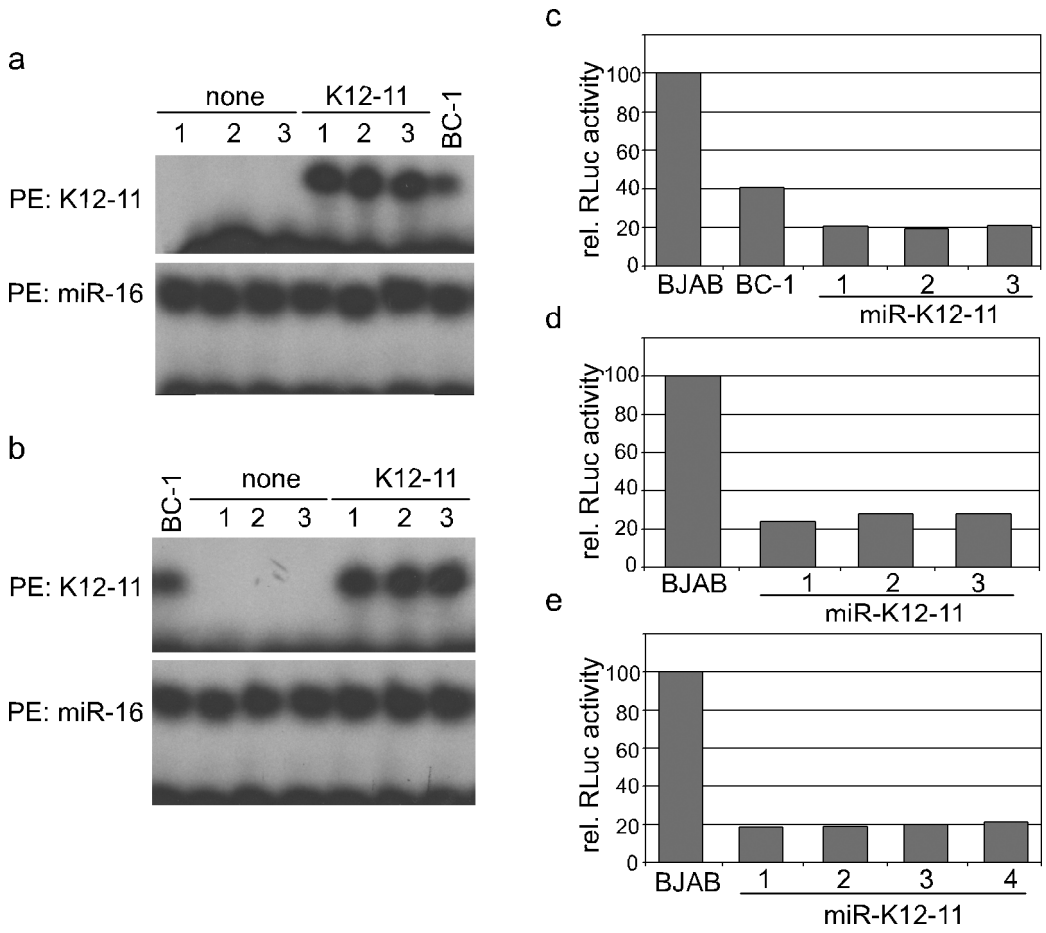


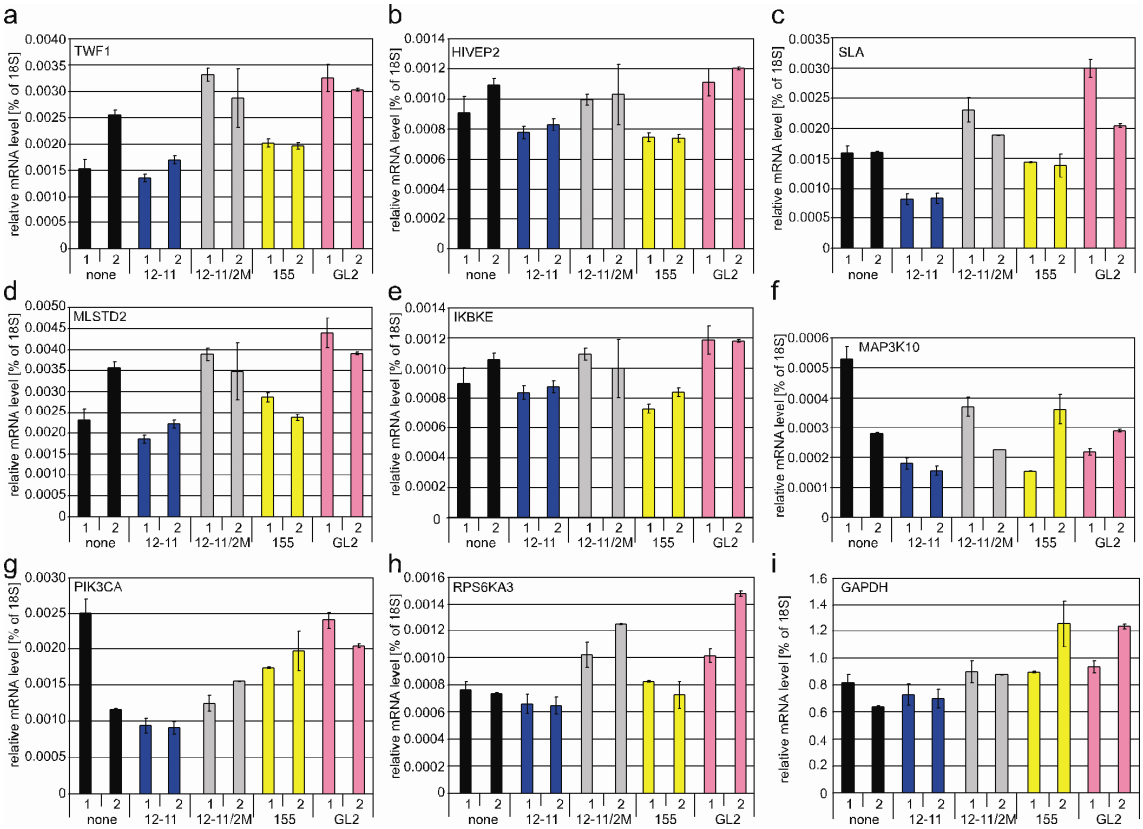
Suppl. Fig. 1

miR-30 based precursor stem-loops used for the expression of miR-K12/11, miR-K12-11/2M and miR-30/GL2. Mature miRNA sequences are highlighted in red. Bases that distinguish the miR-K12-11/2M stem-loop from the miR-K12-11 precursor are in green.



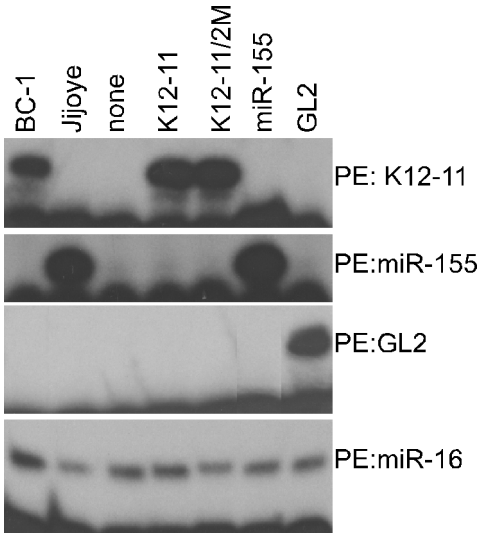
Suppl. Fig. 2

Expression of miR-K12-11 in BJAB transductants. a, b Primer extension analysis of BJAB transductants from two different experiments and latently KSHV infected BC-1 control cells for the expression of miR-K12-11 and the cellular miRNA miR-16, as indicated at left (PE). **c, d, e** Lentiviral vector based indicator assays for activity of miR-K12-11 were performed on miR-K12-11 expressing BJAB transductants from all three independent experiments included in our microarray analysis. miR-K12-11 expressing BJAB cell pools, as well as unmodified BJAB cells, were infected with a mix of Fluc control virus and Rluc indicator virus without added 3'UTR sequences or Rluc indicator virus bearing two perfectly complementary sites to miR-K12-11 in its 3'UTR. Dual luciferase assays were carried out 24 hours after infection. Rluc to Fluc ratios obtained for the RLuc vector bearing miR-K12-11 binding sites were first normalized to the values obtained from the vector lacking complementary sites. Then all values were normalized to those obtained from the negative control cell line BJAB. In all cases, levels of K12-11 indicator repression in BJAB transductants expressing miR-K12-11 were comparable to those typically observed in latently KSHV infected B cell lines (panel **c**).



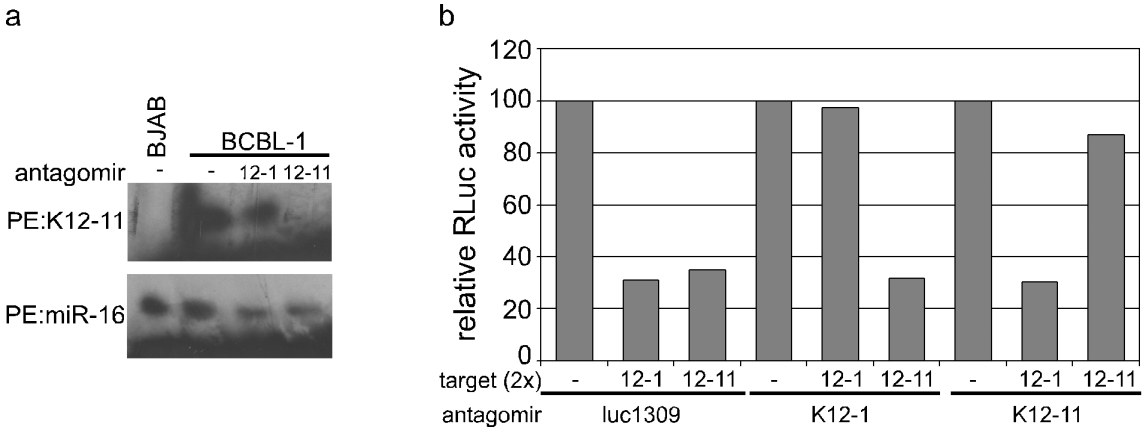
Suppl. Fig. 3

q-RT-PCR analysis of candidate target mRNAs of miR-K12-11 and miR-155. q-RT-PCR analysis of total RNA derived from two independent replicates of BJAB transductants, expressing no miRNA, miR-K12-11, miR-K12-11/2M, miR-155 or miR-30/GL2, for several candidate mRNA targets of miR-K12-11 and miR155. Relative RNA abundance is shown as a percentage of the level of 18S rRNA and error bars are derived from quadruplicate 18S replicates performed for of each sample. mRNAs tested included: TWF1 (**a**), HIVEP2 (**b**), SLA (**c**), MLSTD2 (**d**), IKBKE (**e**), MAP3K10 (**f**), PIK3CA (**g**), RPS6KA3 (**h**), and, as a negative control, GAPDH (**i**). HIVEP2, SLA, MLSTD2, IKBKE, MAP3K10 and RPS6KA3 are predicted targets of miR-155. Of these, HIVEP2 and SLA were also candidate targets of miR-K12-11 based on our microarray results (Suppl. Table 1).



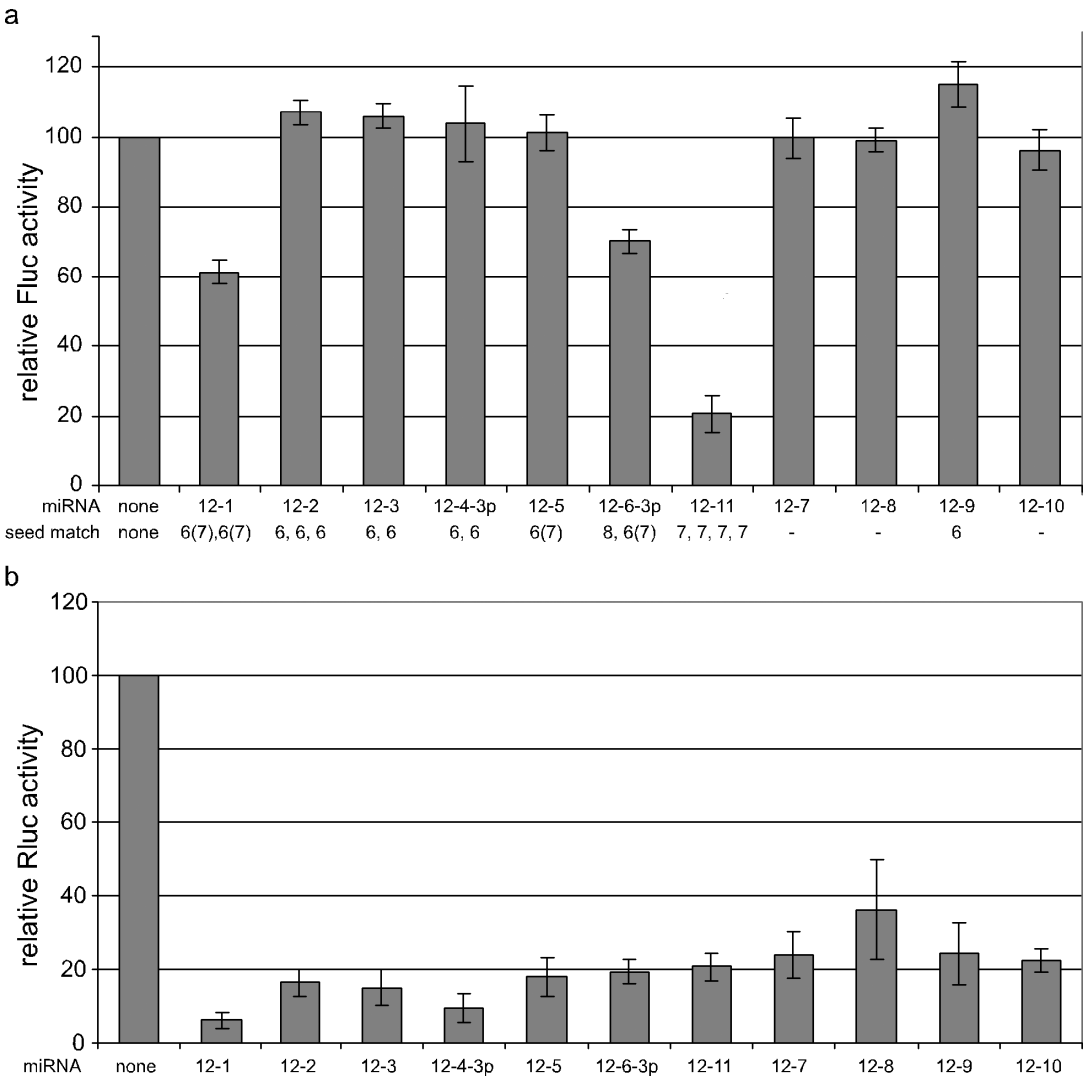
Suppl. Fig. 4

Expression of miR-K12-11 and miR-155 in THP-1 transductants. Primer extension analysis of THP-1 transductants, expressing no miRNA, miR-K12-11, miR-K12-11/2M, miR-155 or miR-30/GL2, as well as of the KSHV infected B-cell line BC-1 and the transformed B-cell line Jijoye, using primers specific for miR-K12-11, miR-155, miR-30/GL2 or the cellular miRNA miR-16 as indicated on the right (PE).



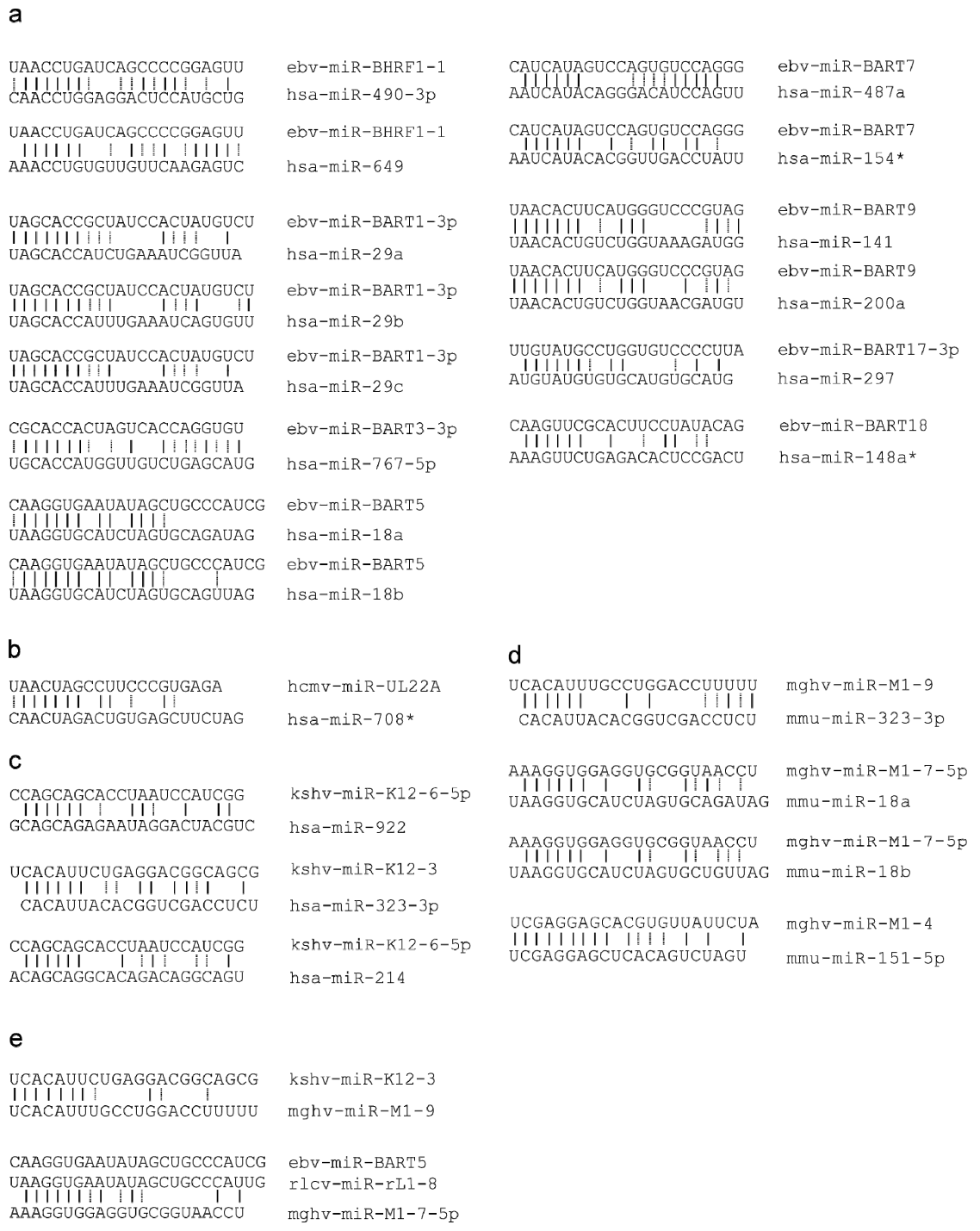
Suppl. Fig. 5

Specific inhibition of miR-K12-11 in latently KSHV infected BCBL-1 cells using antagomirs. **a**. BCBL-1 cells were serum starved for 25 hours in the presence of 1 μM antagomir against miR-K12-1 or miR-K12-11. Total RNA was analyzed by primer extension (PE) using primers specific for miR-K12-11 or the cellular miRNA miR-16. RNA prepared from untreated BJAB or BCBL-1 cells served as negative and positive controls for miR-K12-11 expression. **b**. BCBL-1 cells were incubated in serum free medium containing 1 μM antagomir antisense to miR-K12-1 or miR-K12-11, or to a luc-specific miRNA (luc-1309), for 4 hours. FCS was added to a final concentration of 10% and, after one hour, cells were infected with mixtures of **pNL-SIN-CMV-Fluc** control virus and **pNL-SIN-CMV-RLuc** indicator viruses carrying no additional sequences, or 2 perfect matches for miR-K12-1 or miR-K12-11 3' to the Rluc ORF. In parallel, virus mixtures were also used to infect KSHV-negative BJAB cells. 24 hours after infection, dual luciferase assays were carried out. RLuc to Fluc ratios were first normalized to those obtained from antagomir-luc-1309 treated cells and then normalized to values obtained for KSHV negative BJAB cells. Treatment of BCBL-1 cells with antagomir- luc-1309 did not alter indicator expression compared to untreated cells (data not shown).



Suppl. Fig. 6

BACH1 is a target of multiple KSHV miRNAs. **a**. 293T cells were cotransfected with indicator vectors carrying no additional sequences, or the BACH1 3'UTR inserted 3' to the Fluc ORF. The cells were also co-transfected with an internal RLuc control vector and either unmodified **pNL-SIN-CMV-AcGFP** or **pNL-SIN-CMV-AcGFP** expressing individual KSHV miRNAs, as indicated. Dual luciferase assays were carried out 48 hours after transfection. Data normalization was carried out as described for Fig. 3. Potential seed matches (starting at nt 2 of the miRNA) to the BACH1 3'UTR are listed from 5' to 3'. Numbers in brackets indicate the extent of seed base pairing if G:U wobble base-pairs are allowed 3' to nt 7 of the miRNA. **b.** To control for miRNA expression, **pNL-SIN-CMV-AcGFP** or **pNL-SIN-CMV-AcGFP** expressing individual KSHV miRNAs were also co-transfected with indicator vectors carrying no additional sequences, or two perfect targets sites for individual miRNAs, inserted 3' to the RLuc ORF, as well as an internal Fluc-based control vector. Data normalization was carried out as described for Fig. 3, except that Rluc vectors served as indicator vectors and the Fluc vector served as internal control.



Suppl. Fig.7

Potential cellular orthologs of other viral miRNAs. Potential cellular orthologs of miRNAs encoded by the human pathogenic herpesviruses Epstein-Barr virus (EBV) (**a**), human cytomegalovirus (**b**), and KSHV (**c**), and of murine herpesvirus (MHV) 68 (**d**). Notably, two MHV68 miRNAs also have potential viral orthologs in KSHV and EBV, as well as in rhesus lymphocryptovirus (rLCV) (**d**). In each case, alignments of potential orthologs are given. Dashed

lines indicate that both orthologs could bind to the same nucleotide if G:U wobble base pairs are allowed. G:U wobble pairs pairing was considered only outside the miRNA seed region (nt 2-7).

Supplementary Table 1

feature	symbol	description	score	fold change	6mer	7mer	Manual matching: K12-11	Manual matching: mir-155	Refseqs used for manual matching	GeneID
H200013300	BIRC4BP	XIAP ASSOCIATED FACTOR-1	8.06	2.97	0	0	7(8)mer; 8mer, 6mer	7mer,7mer,6mer	NM_017523, NM_199139	54739
H200010428	ATP6V1G1	VACUOLAR ATP SYNTHASE SUBUNIT G 1 (EC 3.6.3.14) (V-ATPASE G SUBUNIT 1) (VACUOLAR PROTON PUMP G SUBUNIT 1) (V-ATPASE 13 KDA SUBUNIT 1) (VACUOLAR ATP SYNTHASE SUBUNIT M16)	5.90	1.75	Y	0	6(7)mer, 6mer;	6(8)mer, 6mer	NM_004888	9550
H200006467	HMGCS1	HYDROXYMETHYLGLUTARYL-COA SYNTHASE, CYTOPLASMIC (EC 4.1.3.5) (HMG-COA SYNTHASE) (3-HYDROXY-3-METHYLGLUTARYL COENZYME A SYNTHASE)	5.42	1.41	0	0	6(8)mer, 7(8)mer;	6(7)mer, 7mer	NM_002130	3157
H200004399	RFK	NA	4.82	1.84	Y	0	6mer, 6mer, 6mer;	6mer, 6mer, 6mer	NM_018339	55312
H300014345	SLC33A1	ACETYL-COENZYME A TRANSPORTER	4.63	1.22	0	0	9mer	7mer	NM_004733	9197
H200006159	GALT	GALACTOSE-1-PHOSPHATE URIDYLYLTRANSFERASE (EC 2.7.7.10)	4.61	1.37	0	0	7mer	8mer	NM_000155	2592
H200003338	SAMHD1	SAM DOMAIN AND HD DOMAIN-CONTAINING PROTEIN 1 (DENDRITIC CELL-DERIVED IFNG-INDUCED PROTEIN) (DCIP) (MONOCYTE PROTEIN 5) (MOP-5)	4.42	1.36	Y	Y	8mer	7mer	NM_015474	25939
H300014965	GALT	GALACTOSE-1-PHOSPHATE URIDYLYLTRANSFERASE (EC 2.7.7.10)	4.39	1.42	Y	Y	7mer	8mer	NM_000155	2592
H200016945	PITPNM2	PYK2 N-TERMINAL DOMAIN-INTERACTING RECEPTOR 3; KIAA1457 PROTEIN; LIKELY ORTHOLOG OF MOUSE RETINAL DEGENERATION B2 HOMOLOG (DROSOPHILA) (RDGB2)	4.29	1.86	0	0	6(8)mer	6(7)mer	NM_020845	57605
H200016495	CDC42EP3	CDC42 EFFECTOR PROTEIN 3	4.08	1.58	Y	0	6mer	6mer	NM_006449	10602
H300011771	RXRA	RETINOIC ACID RECEPTOR RXR-ALPHA	3.95	1.67	0	0	7mer	8mer	NM_002957	6256
H300019089	FAM70A	NA	3.91	1.91	Y	0	6mer, 6mer	6mer, 6mer	NM_017938	55026
H300018534	DCAMKL1	SERINE/THREONINE-PROTEIN KINASE DCAMKL1 (EC 2.7.1.-) (DOUBLECORTIN- LIKE AND CAM KINASE-LIKE 1)	3.87	2.94	Y	0	6(9)mer	6(7)mer	NM_004734	9201
H300004028	TAF12	TRANSCRIPTION INITIATION FACTOR TFIID 20/15 KDA SUBUNITS (TAFII- 20/TAFII-15) (TAFII20/TAFII15)	3.54	1.30	Y	Y	8mer	7mer	NM_005644	6883
H300014063	MYB	MYB PROTO-ONCOGENE PROTEIN (C-MYB)	3.45	1.60	0	0	8mer, 8mer	7mer, 7mer	NM_005375	4602
H300000963	ARHGAP21	RHO-GTPASE ACTIVATING PROTEIN 10	3.44	1.48	Y	0	6mer	6mer	NM_020824	57584
H200013912	MADH6	MOTHERS AGAINST DECAPENTAPLEGIC HOMOLOG 6 (SMAD 6) (MOTHERS AGAINST DPP HOMOLOG 6) (SMAD6) (HSMAD6)	3.43	1.42	Y	0	6mer	6mer	NM_005585	4091
H300018782	GBA2	BILE ACID BETA-GLUCOSIDASE	3.38	1.15	Y	Y	7mer	7mer	NM_020944	57704
H200002675	NA	NA *(RNF213)*	3.34	1.35	0	0	7mer, 6mer	8mer, 6mer	NM_020914	57674
H300021965	PLEKHA5	PHOSPHOINOSITOL 3-PHOSPHATE-BINDING PROTEIN-2	3.15	1.40	Y	Y	NA	NA	NM_019012	54477
H300018100	SGK	SERINE/THREONINE-PROTEIN KINASE SGK (EC 2.7.1.-) (SERUM/GLUCOCORTICOID-REGULATED KINASE)	3.14	1.52	Y	0	6(9)mer	6(7)mer	NM_005627	6446
H200004042	ARRDC2	NA	3.13	1.60	Y	Y	7mer, 8mer	7mer, 7mer	NM_001025604, NM_015683	27106
H200005746	IKIP	NA	3.12	1.70	Y	Y	8mer, 7mer	7mer, 8mer	NM_201613 (no match); NM_201612 (no match); NM_153687	121457
H300013702	PICALM	PHOSPHATIDYLINOSITOL-BINDING CLATHRIN ASSEMBLY PROTEIN (CLATHRIN ASSEMBLY LYMPHOID MYELOID LEUKEMIA PROTEIN)	3.08	1.94	Y	Y	6mer, 8mer, 7mer	6mer, 7mer, 7mer	NM_001008660	8301
H200004464	MYO1D	MYOSIN ID (FRAGMENT)	3.08	1.51	0	0	8mer	7mer	NM_015194	4642
H200017259	GPSM2	MOSAIC PROTEIN LGN	3.07	1.23	Y	0	6(8)mer	6(7)mer	NM_013296	29899
H300021697	RNF31	NA	3.07	1.31	Y	0	NA	NA	NM_017999	55072
H300016207	NA	CASEIN KINASE I, GAMMA 2 ISOFORM (EC 2.7.1.-) (CKI-GAMMA 2)	3.02	1.23	Y	Y	7(8)mer	7mer	NM_001319	1455
H200000305	E2F3	TRANSCRIPTION FACTOR E2F3 (E2F-3)	3.02	1.43	0	0	6mer	6mer	NM_001949	1871
H200014356	SGPL1	SPHINGOSINE-1-PHOSPHATE LYASE 1; SPHINGOSINE-1-PHOSPHATE LYASE	3.01	1.32	0	0	6mer, 6mer	6mer, 6mer	NM_003901	8879
H300020598	SLA	SRC-LIKE-ADAPTOR; SRC-LIKE-ADAPTER	3.01	1.48	Y	Y	6mer, 8mer	6mer, 7mer	NM_006748	6503
H300020688	RNF166	NA	3.01	1.20	Y	0	6(7)mer	6(9)mer	NM_178841	115992
H200001254	TRIP13	THYROID RECEPTOR INTERACTING PROTEIN 13 (TRIP-13) (FRAGMENT)	2.98	1.31	0	0	7mer	8mer	NM_004237	9319
H200012266	C9orf116	NA	2.92	1.32	Y	0	6mer	6mer	NM_144654	138162
H200013833	USP8	UBIQUITIN CARBOXYL-TERMINAL HYDROLASE 8 (EC 3.1.2.15) (UBIQUITIN THIOLESTERASE 8) (UBIQUITIN-SPECIFIC PROCESSING PROTEASE 8) (DEUBIQUITINATING ENZYME 8)	2.90	1.28	Y	Y	11mer	7mer	NM_005154	9101
H200000117	BMI1	POLYCOMB COMPLEX PROTEIN BMI-1	2.87	1.22	0	0	6(7)mer	6(8)mer	NM_005180	648

Supplementary Table 1

feature	symbol	description	score	fold change	6mer	7mer	Manual matching: K12-11	Manual matching: mir-155	Refseqs used for manual matching	GeneID
H200008539	CSNK1G2	CASEIN KINASE I, GAMMA 2 ISOFORM (EC 2.7.1.-) (CKI-GAMMA 2)	2.86	1.18	Y	Y	7(8)mer	7mer	NM_001319	1455
H300021410	MRGPRF	MAS-RELATED G PROTEIN-COUPLED RECEPTOR MRGF (MAS-RELATED GENE F PROTEIN)	2.84	1.84	Y	0	6(8)mer	6(7)mer	NM_145015	219928
H200010674	C10orf35	NA	2.84	1.69	Y	0	6mer	6mer	NM_145306	219738
H200014876	CARD6	CASPASE RECRUITMENT DOMAIN PROTEIN 6	2.83	1.53	Y	0	6(7)mer	6(9)mer	NM_032587	84674
H200006385	PTDSS1	PHOSPHATIDYLSERINE SYNTHASE I (SERINE-EXCHANGE ENZYME I) (EC 2.7.8.-)	2.80	1.21	0	0	6(8)mer	6(7)mer	NM_014754	9791
H200011553	POLE3	DNA POLYMERASE EPSILON P17 SUBUNIT (DNA POLYMERASE EPSILON SUBUNIT 3) (CHROMATIN ACCESSIBILITY COMPLEX 17) (HUCHRAC17) (CHRAC-17)	2.79	1.27	Y	Y	8mer	7mer	NM_017443	54107
H200006024	WEE1	WEE1-LIKE PROTEIN KINASE (EC 2.7.1.112) (WEE1HU)	2.79	1.29	0	0	7mer	8mer	NM_003390	7465
H200007162	PIK3CA	PHOSPHATIDYLINOSITOL 3-KINASE CATALYTIC SUBUNIT, ALPHA ISOFORM (EC 2.7.1.137) (PI3-KINASE P110 SUBUNIT ALPHA) (PTDINS-3-KINASE P110) (PI3K)	2.78	1.49	Y	Y	7mer	7mer	NM_006218	5290
H300004316	NA	NA *(FBXO33)*	2.76	1.16	0	0	8mer	7mer	NM_203301	254170
H300015968	SEC13	SEC13-RELATED PROTEIN	2.75	1.32	0	0	6(8)mer	6(7)mer	NM_183352	6396
H200007079	TLE4	TRANSDUCIN-LIKE ENHANCER PROTEIN 4	2.70	1.54	Y	Y	9(10)	7	NM_007005	7091
H300018409	NA	NA	2.66	1.26	0	0	6mer	6mer	NM_014702	9729
H300008606	AGTRAP	ANGIOTENSIN II RECEPTOR-ASSOCIATED PROTEIN; ANGIOTENSIN II, TYPE I RECEPTOR-ASSOCIATED PROTEIN	2.66	1.42	Y	Y	7mer	7mer	NM_001040197, NM_001040196, NM_020350, NM_001040194, NM_001040195	57085
H300011741	ERMAP	ERYTHROBLAST MEMBRANE-ASSOCIATED PROTEIN; ERYTHROID MEMBRANE-ASSOCIATED PROTEIN	2.66	1.36	0	0	6mer	6mer	NM_001017922, NM_018538	114625
H300021317	MUC20	NA	2.64	1.35	0	0	6mer	6mer	NM_152673	200958
H300021038	AMOTL1	ANGIOMOTIN LIKE 1; SIMILAR TO LEMAN COILED-COIL PROTEIN; JUNCTION-ENRICHED AND ASSOCIATED PROTEIN	2.63	1.87	Y	0	6mer, 6(8mer)	6mer, 6(7mer)	NM_130847	154810
H300019328	RIPK5	HDCMD38P	2.60	1.24	Y	0	6mer	6mer	NM_199462, NM_015375	25778
H300000330	HSPA5	78 KDA GLUCOSE-REGULATED PROTEIN PRECURSOR (GRP 78) (IMMUNOGLOBULIN HEAVY CHAIN BINDING PROTEIN) (BIP) (ENDOPLASMIC RETICULUM LUMENAL CA2+ BINDING PROTEIN GRP78)	2.57	1.33	Y	0	6mer	6mer	NM_005347	3309
H300015718	NA	LIPASE PROTEIN	2.57	1.62	0	0	7(8mer)	7mer	NM_020676	57406
H200002530	RNF123	NA	2.57	1.14	0	0	7(9)mer	7mer	NM_022064	63891
H200014831	NA	SERINE/THREONINE-PROTEIN KINASE PRP4 HOMOLOG (EC 2.7.1.37) (PRP4 PRE-MRNA PROCESSING FACTOR 4 HOMOLOG) (PRP4 KINASE)	2.56	1.40	Y	Y	7mer	7mer	NM_003913	8899
H200012814	MGC13057	NA	2.56	1.38	1	0	6(9)mer	6(7)mer	NM_032321	84281
H200006964	TWF1	PTK9 PROTEIN TYROSINE KINASE 9; PROTEIN TYROSINE KINASE 9	2.54	1.47	Y	Y	7mer, 6mer, 6mer	8mer, 6mer, 6mer	NM_002822	5756
H200004075	SCAMP1	SECRETORY CARRIER-ASSOCIATED MEMBRANE PROTEIN 1	2.50	1.34	0	0	6(7)mer, 6mer, 7mer, 6mer	6(8)mer, 6mer, 8mer, 6mer	NM_004866	9522
H200015852	PLEKHB2	NA	2.49	1.38	0	0	8mer	7mer	NM_017958	55041
H200003548	FOS	PROTO-ONCOGENE PROTEIN C-FOS (CELLULAR ONCOGENE FOS) (G0/G1 SWITCH REGULATORY PROTEIN 7)	2.49	1.80	Y	Y	6mer, 7mer	6mer, 8mer	NM_005252	2353
H300019461	C9orf78	HEPATOCELLULAR CARCINOMA-ASSOCIATED ANTIGEN 59; HYPOTHETICAL PROTEIN	2.49	1.14	0	0	7(9)mer	7mer	NM_016520	51759
H200001984	DPY19L1	NA	2.49	1.34	0	0	6(7)mer, 7mer, 8mer	6(8)mer, 9mer, 7mer	NM_015283	23333
H200005991	HIVEP2	HUMAN IMMUNODEFICIENCY VIRUS TYPE I ENHANCER-BINDING PROTEIN 2 (HIV-EP2)	2.29	1.36	0	0	6mer, 8mer	6mer, 7mer	NM_006734	3097

Suppl. Table 1

Candidate target genes based on microarray analysis of miR-K12-11 expressing BJAB transductants. Genes that were significantly downregulated in miR-K12-11 expressing BJAB cells and contained at least a 6nt seed match (nt 2-7 of the miRNA) to miR-K12-11 in their 3'UTR were considered to be candidate targets for miR-K11 regulation and are listed. The "feature" column refers to the oligo-ID in the Human Genome Oligo Set V3 (Operon); T score cut-offs were as follows: T ≥ 3.883 (~ p≤ 0.001), T ≥ 2.861 (~ p≤ 0.01) and T ≥2.093 (~ p ≤ 0.05). Fold changes indicate downregulation of the gene in comparison to BJAB transductants expressing only AcGFP. " 6mer" and "7mer" columns indicate whether at least one 6mer or 7mer 3'UTR match was detected using our computational pipeline (0: no match, Y: at least one match.) We also manually checked the top 325 downregulated genes (T ≥ 2.49) for the presence and extent of 3'UTR seed matches to miR-K12-11 and miR-155 (columns denoted "manual matching"). Seed matches (starting at nt 2 of the miRNA) are listed from 5' to 3'. Numbers in brackets indicate the extent of seed base pairing if G:U wobble base-pairs are allowed 3' to nt 7 of the miRNA. Refseqs used for manual matching are shown in an extra column. HIVEP2 (T score = 2.29) was included because it is a predicted target of miR-155. Other candidate targets with T scores between 2.49 and 2.1 are not shown.

Supplementary Table 2

GENE SYMBOL	GENE_TITLE	RANK	RANK METRIC SCORE	RUNNING ES
UBE2W	ubiquitin-conjugating enzyme E2W (putative)	57	0.71	0.00
CSNK1G2	casein kinase 1, gamma 2	78	0.66	0.02
TLE4	transducin-like enhancer of split 4 (E(sp1) homolog, Drosophila)	96	0.63	0.05
WEE1	WEE1 homolog (S. pombe)	98	0.63	0.08
PICALM	phosphatidylinositol binding clathrin assembly protein	122	0.59	0.10
RNF123	ring finger protein 123	130	0.58	0.13
MYB	v-myb myeloblastosis viral oncogene homolog (avian)	152	0.56	0.15
MYO1D	myosin ID	201	0.52	0.17
KBTBD2	kelch repeat and BTB (POZ) domain containing 2	204	0.52	0.19
HIVEP2	human immunodeficiency virus type I enhancer binding protein 2	222	0.51	0.21
RAB11FIP2	RAB11 family interacting protein 2 (class I)	337	0.46	0.23
SGK3	serum/glucocorticoid regulated kinase family, member 3	342	0.46	0.24
NDFIP1	Nedd4 family interacting protein 1	348	0.46	0.26
H3F3A	H3 histone, family 3A	360	0.45	0.28
SOX11	SRY (sex determining region Y)-box 11	381	0.45	0.30
ARID2	AT rich interactive domain 2 (ARID, RFX-like)	502	0.40	0.31
ACTA1	actin, alpha 1, skeletal muscle	506	0.40	0.33
PKIA	protein kinase (cAMP-dependent, catalytic) inhibitor alpha	568	0.39	0.34
CARHSP1	calcium regulated heat stable protein 1, 24kDa	594	0.38	0.35
SLA	Src-like-adaptor	620	0.38	0.37
CUTL1	cut-like 1, CCAAT displacement protein (Drosophila)	637	0.38	0.38
STRN3	striatin, calmodulin binding protein 3	676	0.37	0.40
RPS6KA3	ribosomal protein S6 kinase, 90kDa, polypeptide 3	768	0.35	0.40
KRAS	v-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog	779	0.35	0.42
SKI	v-ski sarcoma viral oncogene homolog (avian)	982	0.32	0.42
BOC	Boc homolog (mouse)	1037	0.31	0.43
TRIM2	tripartite motif-containing 2	1136	0.30	0.43
SPI1	spleen focus forming virus (SFFV) proviral integration oncogene spi1	1167	0.30	0.44
MAP3K10	mitogen-activated protein kinase kinase kinase 10	1257	0.28	0.45
N4BP1	-	1400	0.27	0.45
HIF1A	hypoxia-inducible factor 1, alpha subunit (basic helix-loop-helix transcription factor)	1488	0.26	0.45
ATP2B1	ATPase, Ca++ transporting, plasma membrane 1	1509	0.26	0.46
KIAA1411	KIAA1411	1628	0.25	0.46
ZNF238	zinc finger protein 238	1656	0.24	0.47
ZNF652	zinc finger protein 652	1724	0.24	0.48
PSIP1	PC4 and SFRS1 interacting protein 1	2138	0.21	0.45
RREB1	ras responsive element binding protein 1	2151	0.21	0.46
DNAJB7	DnaJ (Hsp40) homolog, subfamily B, member 7	2377	0.19	0.45
CDC73	cell division cycle 73, Paf1/RNA polymerase II complex component, homolog (S. cerevisiae)	2380	0.19	0.46
CEBPB	CCAAT/enhancer binding protein (C/EBP), beta	2423	0.19	0.46
YWHAZ	tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, zeta polypeptide	2527	0.18	0.46
INPP5D	inositol polyphosphate-5-phosphatase, 145kDa	2554	0.18	0.47
MAP3K7IP2	mitogen-activated protein kinase kinase kinase 7 interacting protein 2	2595	0.18	0.47
MAP3K14	mitogen-activated protein kinase kinase kinase 14	2614	0.18	0.48
BACH1	BTB and CNC homology 1, basic leucine zipper transcription factor 1	2689	0.17	0.48
PSKH1	protein serine kinase H1	2775	0.17	0.48

Suppl. Table 2

List of genes that contribute to the enrichment of predicted targets for miR-155 in miR-K12-11 downregulated genes detected using Gene Set Enrichment Analysis (GSEA). Also given are the rank of each gene in the rank ordered gene list derived from comparison of BJAB transductants expressing miR-K12-11 to BJAB transductants expressing only AcGFP, rank metric scores and running enrichment scores for the GSEA analysis of miR-155 predicted targets.

Supplementary Table 3

Target	Name	Protein function	matches K12-11	matches miR-155	predicted target?	GeneID
AGTRAP	angiotensin II receptor-associated protein	regulates angiotensin II type I receptor signaling	7mer	7mer		57085
BACH1	BTB and CNC homology 1, basic leucine zipper transcription factor 1	transcription factor of the cap'n'collar type of basic region leucine zipper (CNC/bZIP) family. Functions in heterodimers with small Maf familiy members as a repressor of the Maf recognition element (MARE).	7mer, 7mer, 7mer, 7mer	7mer, 9mer, 9mer, 8mer	P, T	571
BIRC4BP	XIAP associated factor-1	BIRC4BP antagonizes the anticaspase activity of XIAP	7(8)mer; 8mer, 6mer	7mer,7mer,6mer		54739
FOS	v-fos FBJ murine osteosarcoma viral oncogene homolog	leucine zipper protein of the FOS family. FOS family members dimerize with JUN family proteins to form the transcription factor AP-1. FOS proteins have been implicated in cell cycle regulation, differentiation, transformation and apoptosis.	6mer, 7mer	6mer, 8mer		2353
HIVEP2	human immunodeficiency virus type I enhancer binding protein 2	transcription factor that binds specific DNA sequences, including the kappa-B motif.	6mer, 8mer	6mer, 7mer	P, T	3097
IKBKE	inhibitor of kappa light polypeptide gene enhancer in B-cells, kinase epsilon	kinase in the toll like receptor signaling pathway	8mer, 7mer	7mer, 7mer	P, T	9641
MAP3K10	mitogen-activated protein kinase kinase kinase 10	serine/threonine kinase that functions in the JNK pathway	7(8)mer	7mer	P	4294
MLSTD2	male sterility domain containing 2	fatty acyl CoA reductase	7mer, 6mer, 7mer, 6mer, 8mer	7mer, 6mer, 8mer, 6mer, 7mer	P, T	84188
PIK3CA	phosphoinositide-3-kinase, catalytic, alpha polypeptide	catalytic subunit of phosphatidylinositol 3-kinase	7mer	7mer		5290
TWF1	twinfilin, actin-binding protein, homolog 1 (Drosophila)	actin monomer-binding protein	7mer, 6mer, 6mer	8mer, 6mer, 6mer		5756
RFK	riboflavin kinase	riboflavin kinase	6mer, 6mer, 6mer;	6mer, 6mer, 6mer		55312
RPS6KA3	ribosomal protein S6 kinase, 90kDa, polypeptide 3	member of the RSK (ribosomal S6 kinase) family of serine/threonine kinases. Substrates include members of the mitogen-activated kinase (MAPK) signalling pathway. Implicated in cell growth and differentiation.	7mer, 6(8)mer	8mer, 6(7)mer	T	6197
SAMHD1	SAM domain and HD domain 1	unknown	8mer	7mer		25939
SLA	Src-like-adaptor	regulation of signal transduction. Has been implicated in B cell development and function.	6mer, 8mer	6mer, 7mer	P, T	6503

Suppl. Table 3

List of shared targets for miR-K12-11 and miR-155 validated in this study. Shown are the names of the targets, a brief summary of known functions of each gene product, the extent of seed base pairing to miR-K12-11 and miR-155 (as in Suppl Table 1) and whether the mRNA is a predicted target for miR-155 based on PicTar (P) or TargetScan 4.0 (T). BACH1, IKBKE, MAP3K10, MLSTD2 and RPS3KA3 were included because they are predicted targets for miR-155. The remaining genes were included based on our microarray analysis of miR-K12-11 expressing BJAB cells. Of these, HIVEP2 and SLA are also predicted targets for miR-155.

Supplementary Methods

MiRNA expression cassettes. MiRNA expression cassettes were placed into the 3'UTR of the *AcGFP* gene in the context of the **pcDNA3** based vector **pcDNA3/AcGFP**. The AcGFP coding region was PCR amplified using primer pair 129/131. The resulting PCR product was digested with NotI, blunt-ended using Klenow enzyme and then cut with HindIII. **pcDNA3** was cut with EcoRI, blunt-ended using Klenow enzyme, cut with HindIII and ligated to the PCR product.

Since miR-K12-11 expression cassettes derived from KSHV genomic sequences did not yield high levels of miR-K12-11 after transduction (data not shown), the sequence of mature KSHV miR-K12-11 was embedded into a fragment of the pri-miRNA gene for miR-30. This strategy has been previously validated and is now widely employed for the expression of small RNAs[22,27]. First, 5' and 3'flanking regions of miR-30 were amplified from BJAB genomic DNA using primer pairs 76/77 (5'-flanking region) and 78/79 (3'-flanking region) and cut with MfeI and XhoI or XhoI and BamHI, respectively. Both fragments were ligated into the EcoRI and BamHI sites of a shuttle vector, **pLNCX2M**. **pLNCX2M** is a modified version of the MLV based retroviral vector **pLNCX2** (BD Biosciences) and contains EcoRI and BamHI sites upstream of a CMV promoter driven Neo cassette. This arrangement of the miR-30 flanking regions was originally described by Silva et al. and results in unique XhoI and EcoRI (introduced with primer 78) sites that allow the insertion of miRNA precursor hairpin sequences[27]. The precursor hairpins for miR-K12-11 and miR30/GL2 were designed to express the miRNA from the 3'arm of the pre-miRNA hairpin. The passenger strand was adjusted to contain one bulge (at nt 9 from the 5' end of the mature miRNA sequence). The stem-loop sequences used for expression of miR-K12-11 and miR-30/GL2 are shown in Suppl. Fig. 1. Two primers covering each miRNA stem-loop coding sequence (miR-K12-11: primer pair 117/118; miR-30/GL2 primer pair: 119/120) were designed to overlap over the central 31 nt.

Extension of the annealed primers using PFU polymerase yielded the entire extended pre-miRNA stem-loop coding sequence. The resulting fragment was cut with XhoI and EcoRI and inserted between the miR-30 flanking regions. The same strategy was used to clone expression cassettes for miR-K12-2 (primers 103/104), miR-K12-4-3p (primers 107/108), miR-K12-5 (primers 109/110), miR-K12-6-3p (primers 125/126) and miR-K12-7 (primers 127/128).

miR-30 based miRNA expression cassettes were amplified from the **pLNCX2M** shuttle vectors described above using primers 140/141. A ~300 bp fragment of the *BIC* gene (corresponding to nt 150-449 of the RNA; accession number AF402776) was cloned from BJAB cDNA using primers 485/486. Expression cassettes for miR-K12-1 (primers 198/199), miR-K12-3 (primers 200/201), miR-K12-8 (primers 204/205), miR-K12-9 (primers 206/207), and miR-K12-10 (primers 208/209) were PCR-amplified from the KSHV genome using BC-1 genomic DNA. 5' primers contained an NruI site and 3' primers contained XbaI and EcoRV sites. PCR products were cut with NruI and XbaI and inserted into the EcoRV and XbaI sites of **pcDNA3/AcGFP**, thus regenerating an EcoRV site upstream of the XbaI site to facilitate the insertion of further miRNA expression cassettes (see below).

The miR-K12-11/2M expression cassette was generated using overlap PCR. Using the miR30/K12-11 expression cassette described above as template, two overlapping PCR products were generated using primer pairs 140/390R and 141/390F and used as templates in a second round of PCR with primer pair 140/141. The resulting expression cassette (miR30K11/2M) was inserted into **pcDNA3/AcGFP** as described above.

In THP-1 cells, tandem miRNA expression cassettes were used in order to allow higher expression levels of the miRNA at lower AcGFP expression levels. The miRNA in question was amplified from the corresponding **pcDNA3/AcGFP** construct using primers

140/141, cut with NruI and XbaI and cloned into the EcoRV and XbaI sites of the appropriate **pcDNA3/AcGFP** vectors containing one cassette miRNA expression cassette.

To clone **pNL-SIN-CMV-AcGFP** and derivatives, the **pcDNA3/AcGFP** vector and its derivatives containing one or more miRNA expression cassettes were cut with XbaI, blunt-ended using Klenow enzyme, and then cut with MluI, yielding a fragment containing part of the CMV promoter, the AcGFP coding region and the miRNA expression cassette(s). This fragment was used to replace the corresponding fragment of **pNL-SIN-CMV-BLR**[31], which was cut with XhoI, blunt-ended with Klenow enzyme and then cut with MluI.

pL/SV40 RL and FL indicator vectors. Indicator and control vectors to test candidate cellular 3'UTRs were based on the self-inactivating lentiviral vector **pLL3.7**[32]. First, a polylinker was inserted between the ApaI and EcoRI sites of **pLL3.7** (primers 238/39), introducing the unique restriction sites ApaI, BamHI, XhoI, XbaI, NotI, EcoRI. The resulting vector (**pL**) retained only the 5'promoter sequences and regulatory sequences, as well as the WRE element and the 3'SIN LTR.

Next, fragments containing the SV40 promoter and RLuc or FLuc (*luc*+ gene) coding regions were inserted using BamHI and NheI (SV40 promoter) and NheI and XhoI restriction (*luc* genes) sites. The SV40 promoter was amplified from **pcDNA3** using PCR with primers 256 and 257. The RLuc fragment was obtained by digesting the previously described vector **pNL-SIN-CMV-RLuc** with NheI and XhoI. The *luc*+ ORF was amplified from **pGL3-CMV** using PCR primers 363 and 364. The resulting vectors were named **pL/SV40/Rluc** and **pL/SV40/GL3** and contain unique XhoI, XbaI, NotI and EcoRI sites downstream of the Luc ORFs to facilitate 3'UTR cloning. Throughout this study, **pL/SV40/Rluc** served as negative control vector and **pL/SV30/GL3** was used to

insert candidate 3'UTRs. Candidate 3'UTR sequences were amplified from BJAB cDNA or genomic DNA using the following primer pairs: 376/377 (IKBKE), 380/381 (PIK3CA), 400/401 (FOS), 425/428 (BIRC4BP), 447/448 (RFK), 449/450 (TWF1). 451/452 (SLA), 453/454 (SAMHD1), 457/458 (AGTRAP), 462/463 (RPS6KA3), 464/465 (MAP3K10), and 501/504 (BACH1). **pNL-SIN-CMV-FL** and **pNL-SIN-CMV-RL** as well as the indicator vectors bearing two sites perfectly complementary to each KSHV miRNA were described previously[17].

Generation of BJAB transductants. Lentiviral vectors were produced by transfection of 293T cells as described[31] and used to transduce ~1 x 10^6 BJAB or THP-1 cells at a cell concentration of ~0.5 x 10^6/ml . The next day, media were exchanged and 48 hours after transduction, cells were collected by centrifugation and resuspended in RPMI containing 2mM EDTA. AcGFP expressing cells were sorted (using the 488nm line of a 20mW laser) and analyzed using a BD FACSAria™ cell sorter with DiVa software (BD Biosciences, San Jose, CA). In experiment one, 60,000 BJAB cells expressing only AcGFP or AcGFP and miR-K12-11 were sorted and, after sorting, split into three biological replicates. In the case of experiments two and three, BJAB cells were transduced in three or four independent replicates, respectively, and ~40,000 cells were collected for each replicate. Cell populations of similar mean fluorescence intensities were collected for all samples. In the case of experiment three, cells were resorted on day 6 after transduction to ensure comparable AcGFP expression levels. Cytoplasmic RNA for microarray analysis was prepared using the RNeasy Mini kit (Qiagen) and harvested on day 12 (experiment 2) and day 16 (experiments 1 and 3) after transduction of BJAB cells. Indicator assays shown in Suppl. Fig. 2, were conducted on the day of RNA preparation (experiment 3) or one day before RNA preparation (experiment 1 and 2).

In the case of THP-1, cells were sorted three times to achieve similar levels of AcGFP expression levels.

RNA preparation and primer extension. For analysis of miRNA expression by primer extension, total RNA was prepared using TRIzol reagent as instructed and 10μg of RNA was used per reaction. Primer extension was carried out using the Promega Primer Extension System. Probe sequences were as follows for miR-16a: cgccaatatttacgtg; miR-K11 and miR-K11/2M: tcggacacaggctaag; miR-155: cccctatcacgattagc; miR30/GL2: tcacgtacgcggaata.

Independently, miR-K12-11 expression from the miR30 based expression cassette described above was validated using Northern analysis (not shown). Taken together, these results prove that the miRNA expressed from our miR30 based miR-K12-11 expression cassette is identical to miR-K12-11.

Spotted microarray, RNA and microarray probe preparation and hybridization. Arrays were printed at the Duke Microarray Facility using the Genomics Solutions OmniGrid 300 Arrayer. The arrays contain the Human Operon v3.0.2 arrays (Oligo Source) that possess 34,602 unique optimized 70-mers.

RNA quality was ascertained using an Agilent 2100 bioanalyzer (Agilent technologies). Cytoplasmic RNA (10 μg) from each sample and the reference (Universal Human Reference RNA, Stratagene) were hybridized to oligo (dT) primers at 65° C and then incubated at 42° C for 2 hours in the presence of reverse transcriptase, Cy5- or Cy3-dUTP and Cy5- or Cy3-dCTP, and a deoxynucleotide mix. In all cases, BJAB derived RNA samples were labeled with Cy5 and reference samples were labeled with Cy3. NaOH was used to destroy residual RNA. Sample and reference cDNA were then pooled, purified with QIAquick Purification Columns (Qiagen), mixed with hybridization buffer (50% formamide, 5 SSC, and 0.1% SDS), COT-1 DNA, and poly-deoxyadenylic acid to limit nonspecific binding, and heated to 95° C for 2 minutes. This mixture was pipetted onto a microarray slide, and hybridized overnight at 42° C on the MAUI hybridization

system (BioMicro Systems). The array was then washed at increasing stringencies and scanned on a GenePix 4000B microarray scanner (Axon Instruments). All protocols are available in greater detail on the Duke Microarray Facility Web site (http://microarray.genome.duke.edu/services/spotted-arrays/protocols). Array results were submitted to the GEO database.

Microarray normalization and analysis. All arrays were subject to background subtraction followed by loess normalization within each array and scale normalization across all arrays using the arrayMagic package in R[28]. The KNN impute package in GenePattern[29] was used to impute missing data if a probe had intensity values for at least half the samples. Otherwise the probes were excluded from analysis. Replicate probes were collapsed to one probe corresponding to the median value of all the replicates.

Probe IDs which had a t-score > 4.032 (~ p-value of ≤ 0.01) from a two-sided T-test, calculated using the Comparative Marker Selection package from GenePattern, comparing AcGFP only expressing cell populations to the unmodified BJAB cell line were considered to be activated by AcGFP and excluded from our further analysis (895 probes). At this stage, 23,330 probes remained and represent the set on which the analysis was conducted. T-scores were calculated for comparisons between cell populations expressing AcGFP only to those expressing AcGFP and miR-K12-11 using the Comparative Marker Selection package from GenePattern.

miRNA target mapping. We applied an in-house computational pipeline[30] to analyze a high-quality set of human 3'UTR sequences, based on the hg18 assembly, for the presence of sites complementary to miR-K12-11. The set of 3' UTRs were first mapped from human Refseq IDs to Human Operon v3.0.2 probe IDs using the array annotation table downloaded from the Operon website. All successfully mapped 3' UTRs were analyzed for exact 6mer and 7mer matches to the reverse complement of the miRNA seed

(positions 2 to 7 or 2 to 8 from the 5' end of the mature miR-K12-11 sequence, respectively). Probes which contained at least one seed match in any transcript isoform were regarded as hits, without any requirements regarding conservation of seed matches in related species. These sets of UTRs and seed matches were used to build a null distribution and assess the significance of miR-K12-11 target site frequency in the top list of downregulated genes.

Gene set enrichment analysis and enrichment of K11 seed matches. To identify sets of genes which showed a correlated change of expression upon introduction of K11, we performed a Gene Set Enrichment Analysis (GSEA-P)[19] on the changes observed when comparing AcGFP only expressing cell populations to those expressing both AcGFP and miR-K12-11 (signal-to-noise ratio was used as ranking metric). We obtained a list of pre-defined gene sets from the Molecular Signatures Database (http://www.broad.mit.edu/gsea/msigdb/index.jsp), specifically the c3 motif gene sets, which included gene sets of predicted targets for human miRNAs[20].

To assess whether we saw a significant enrichment of 6-or 7-mer seed matches in the 3'UTRs of genes for which we observed the most prominent changes in expression, we further performed an empirical p-value calculation as follows. We selected random samples of 150 genes from all probes contained in the set of our analysis defined above, and for each of 10,000 of such randomized gene sets, we calculated the percentage of genes which had a 6mer or 7mer seed match to miR-K12-11 in their 3'UTR. From these 10,000 samples, we obtained a null distribution, which was compared against the percentage of genes with 6mer or 7mer seed match to miR-K12-11 in the 150 most downregulated genes (ranked as above using Gene Set Enrichment Analysis). Enrichment p-values were then determined from the null distribution as the fraction of gene sets that had an equal or higher percentage of seed matches. Histograms were created using JMP 6.0 (SAS).

Real-time quantitative PCR (qRT-PCR). Total RNA from BJAB cells was prepared using TRIzol extraction and further processed with the RNeasy Mini Kit (Qiagen) including an on-column DNase digestion step (RNase-free DNase set; Qiagen). Absence of DNA contamination was proven through minus-RT controls in qRT-PCR reactions. cDNA synthesis was carried out using ABI HighCapacity cDNA reagents. Real-time qPCR was performed using the Quantace SybrGreen qPCR mix. qPCR was performed on an ABI7900HT machine. Per plate, four PCR replicates for 18S rRNA were run per sample to make normalization more robust. Specific target genes were measured as single data points per sample, error bars are derived from the variance of the four 18S rRNA replicates. Relative mRNA levels were calculated against 18S rRNA amplification. RPL13A (geneID 23521) was included into the analyses as a second verifying "house-keeper" (data not shown), which however did not yield results different from those normalized against 18S rRNA. Primer sequences are given in Suppl. Table 4. In the case of BACH1 and HIVEP2, one representative result out of two distinct primer sets per gene is shown, in the case of BIRC4BP, one representative result for three different primer sets is shown.

Indicator assays. Each well of a 24 well dish of 293T cells was co-transfected with 2.5 ng of each indicator and control retroviral vector as well as 0.4 μg of a **pNL-SIN-CMV-AcGFP** based miRNA expression construct using FuGENE6 (ROCHE). 48 hours after transfection, Dual-Luciferase Reporter Assays (Promega) were carried as instructed. Fluc to Rluc ratios were calculated and, for co-transfection with each miRNA expression constructs and empty **pNL-SIN-CMV-AcGFP**, values obtained for each Fluc construct bearing a candidate 3'UTR were normalized on those obtained from the corresponding value of the unmodified Fluc vector. These normalized values were then normalized to the values derived from co-transfections with the empty **pNL-SIN-CMV-AcGFP** vector, which were set at 100%. Error bars were calculated from three independent experiments.

Indicator assays for miRNA activity in BJAB and BCBL1 cells (Suppl. Figs. 2 and 5) were carried out using the lentiviral vectors **pNL-SIN-CMV-FL** and **pNL-SIN-CMV-RL** as described[17].

Antibodies and Western blotting. To analyze BACH1 protein levels, cell numbers of BJAB or THP-1 cell lines were counted and equal numbers were plated in 10 cm dishes. The following day, cells were collected by centrifugation, washed once with PBS and then lysed in denaturing lysis buffer (40mM Tris [pH 6.8], 2% sucrose, 1% SDS). Cell lysates were boiled immediately for 5 min at 95°C and vortexed. Protein concentration was determined using the BCA™ protein assay kit (Pierce).

In order to determine the abundance of FOS protein after serum starvation and TPA induction, cells were counted, washed twice in serum free RPMI and 0.5 x 10^6 cells/ml serum free RPMI were plated into wells of 6 well dishes. After starving the cells for 26 hours in serum free medium, the medium was aspirated (BJAB cells adhere to the culture dish during serum starvation) and cells were incubated with serum free RPMI with or without 20ng/ml TPA for 2 hours. Cells were rinsed with PBS, lysed by addition of 200µl denaturing lysis buffer/well and further processed as described above. Equal amounts of protein (~120 µg/lane for BACH1; ~15 µg/lane for β-actin and FOS) were analyzed by Western blotting. Primary goat anti-BACH1 was from Santa-Cruz Biotechnology, Inc. (C-20, sc-14700), primary rabbit anti-FOS was a gift from Dr. Tom Curran's laboratory and primary anti-β-actin was from Santa-Cruz Biotechnology, Inc. (C-4, sc-47778). Secondary anti-goat IgG HRP was from Santa-Cruz Biotechnology, Inc. (sc-2020) and secondary anti-rabbit IgG HRP was from Amersham (NA934V). Signals were developed using SuperSignal West Femto (Pierce) in the case of BACH1 or Lumi-Light (Roche) in the case of β-actin and FOS.

Antagomir treatment. Antagomirs were synthesized as described[23]. Sequences were 5'-$u_sc_s$ggacacaggcuaagcau$_su_sa_sa_s$-Chol-3' (antagomir-miR-K12-K11), 5'-$a_sc_s$cgccugaagucucugau$_su_sa_sa_s$-Chol-3' (antagomir-luc1309), and 5'-$g_sc_s$uuacacccaguuuccugu$_sa_sa_su_s$-Chol-3' (antagomir-miR-K12-1). Lower case letters represent 2'-*O*Me-modified nucleotides, subscript 's' represents a phosphorothioate linkage, and 'Chol' represents linked cholesterol.

For detection of FOS by Western blotting, BCBL-1 cells were washed with PBS and 0.5 x 10^6 cells/ml were incubated with serum free medium in the presence of 1 μM antagomir for 25 hours. FOS expression was induced by treatment with 20ng/ml TPA for 1 hour. Cells were recovered by centrifugation, washed with PBS and lysed as described above. For indicator assays, BCBL-1 cells were washed with serum-free medium and 0.5 x 10^6 cells/ml were incubated with serum-free medium in the presence of 1μM antagomir for 4 hours. FCS was added to a final concentration of 10% and, after one hour, cells were recovered by centrifugation and infected with mixtures of **pNL-SIN-CMV-Fluc** control virus and **pNL-SIN-CMV-RLuc** indicator viruses carrying no additional sequences or 2 perfect matches for miR-K12-1 or miR-K12-11 inserted 3' to the RLuc ORF. In parallel, virus mixtures were also used to infect KSHV-negative BJAB cells. Indicator assays and normalization were carried out as described previously[17].

31. Lee, M.T., Coburn, G.A., McClure, M.O. & Cullen, B.R. Inhibition of human immunodeficiency virus type 1 replication in primary macrophages by using Tat- or CCR5-specific small interfering RNAs expressed from a lentivirus vector. *J Virol* **77**, 11964-11972 (2003).

32. Rubinson, D.A. et al. A lentivirus-based system to functionally silence genes in primary mammalian cells, stem cells and transgenic mice by RNA interference. *Nat Genet* **33**, 401-406 (2003).

Suppl. Table 4. PCR primers used for q-RT-PCRs.

Symbol	Gene ID	F Oligo Sequence	R Oligo Sequence
MAP3K10	4294	CCTCAAGTCCATCAACATCCTG	TTTTGGAGAAGAGGGAGAGACG
RPS6KA3	6197	TGTCAAGCACGTACTGGAAGGT	GTTAAAGTTGGGGGTCTGTTGC
FOS	2353	AAAGGAGAATCCGAAGGGAAAG	TGAGCTGCCAGGATGAACTCTA
SLA	6503	TCCAACTATTGCCTGGGATCTT	ATGATCCAATGTTTCGTGATGC
IKBKE	9641	ACTCAGTGCTGAGCTTGGTGTC	TTCAGGGCTCACTGTCATTCAT
SAMHD1	25939	TCGTCCGAATCATTGATACACC	CCAGTGCGTGAACTAGACATCC
RFK	55312	TCCAAGGCAAATCTAAATGGGT	GGCCCAAAGCATCTGTAATCTT
AGTRAP	57085	CGTAGTGCCTACCAGACGATTG	TCTTAGGTCCAAGAGACGGAGG
MLSTD2	84188	AGTGGATGGATGATGGCCTAGT	CAATCCATCCTGGAAAAGGTTC
TWF1	5756	GCATCCAAGCAAGTGAAGATGT	TCCAACAGGGGTAAAACAAAGG
BACH1	571	ATTCATGCTTCTGTTCAGCCAA	GGCACTGAGAAGCAGGATCTTT
HIVEP2	3097	TTTTCCCTGAGGGTCCTAATGA	GCAATGTGGGTGAAGGATGTAA
18S	100008588	CGGCTTAATTTGACTCAACACG	TTAGCATGCCAGAGTCTCGTTC

Suppl. Table 5. Sequences of primers used in this study.

Number	Primer sequence (5' to 3')
76	gcacgcaattgggatcctgtttgaatgaggcttcag
77	ggttgctcgagccttctgttgggttaacctg
78	gaaggctcgagcaacaagaattcaaggggctactttaggagc
79	gcacgggatccaaagtgatttaatttatacc
103	gaaggctcgagaaggtatattgctgttgacagtgagcgaagatcgacccggcctacagtttggtgaagccacagatgc
104	cttccgaattccgaggcagtaggcacagatcgacccggactacagtttgcatctgtggcttcaccaaactgtaggccgg
107	gaaggctcgagaaggtatattgctgttgacagtgagcgccagctaggcctccgtattctatggtgaagccacagatgc
108	cttccgaattccgaggcagtaggcatcagctaggcctcagtattctatgcatctgtggcttcaccatagaatacggagg
109	gaaggctcgagaaggtatattgctgttgacagtgagcgacggcaagttccaagcatcttatggtgaagccacagatgc
110	cttccgaattccgaggcagtaggcaccggcaagttccaggcatcctatgcatctgtggcttcaccataagatgcttgga
117	gaaggctcgagaaggtatattgctgttgacagtgagcgccggacacaggctcagcattaatggtgaagccacagatgc
118	cttccgaattccgaggcagtaggcatcggacacaggctaagcattaatgcatctgtggcttcaccattaatgctgagcc
119	gaaggctcgagaaggtatattgctgttgacagtgagcgccacgtacgcggactactttgatggtgaagccacagatgc
120	cttccgaattccgaggcagtaggcatcacgtacgcggaatacttcgatgcatctgtggcttcaccatcaaagtagtccg
125	gaaggctcgagaaggtatattgctgttgacagtgagcgatcaacagcccgacaaccattatggtgaagccacagatgc
126	cttccgaattccgaggcagtaggcactcaacagcccgaaaaccatcatgcatctgtggcttcaccataatggttgtcg
127	gaaggctcgagaaggtatattgctgttgacagtgagcgaagcgccagcaacctgggattatggtgaagccacagatgc
128	cttccgaattccgaggcagtaggcagagcgccagcaacatgggatcatgcatctgtggcttcaccataatcccaggttg
129	gcgcgaagcttcgccaccatggtgagcaagg
131	gcgcggcggccgctcacttgtacagctcatcc
140	gcgcgtcgcgatgtttgaatgaggcttcag
141	gcgcgtctagagcgacgatatcaaagtgatttaatttatacc
198	gcgcgtcgcgagggaggaaggatgtgggg
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