SUPPLEMENTAL METHODS

Prediction of alternative polyadenylation sites

The alternative polyadenylation sites shown in Figure 2 are an amalgamation of known sites annotated in the RefSeq database with very common 3'end positions of ESTs (either through analysis of EST data in the UCSC genome browser or the curated PolyA Cleavage Site & 3' UTR Database (Brockman et al. 2005)). Proposed alternative polyadenylation sites for IGF2BP1 were also derived from (Mayr and Bartel 2009).

Identification of IGF2BP1 cleavage and polyadenylation sites

To identify alternative polyadenylation sites in IGF2BP1, primers were designed 5' of predicted sites and LM-PAT assays performed. LM-PAT PCR products were run on agarose gels and products of the anticipated size were purified, cloned into the pGEM-T Easy Vector Sequence system (Promega) and sequenced by ABI 310 sequencer in the Australian Cancer Research Fund Facility at the Garvan Institute. See Supplemental Table S1 for primers.

Analsyis of HeLa cell miRNAs by next generation sequencing

Library preparation and sequencing: Total RNA was prepared from HeLa cultures using Trizol reagent. Small RNA <70nt was enriched from 50-100µg total RNA by flashPAG electrophoresis. Of the 30µl eluted, 3µl was used in the SOLiD Small RNA Expression Kit (SREK) to create the sequencing library. Briefly, after adapter ligation and reverse transcription test PCR amplifications were done to identify the minimal cycle number to visualise the libraries by agarose gel electrophoresis. Large scale library preparations (3x 100µl PCR) were enriched using MinElute columns (Qiagen) and PCR products between

110-150 nucleotide (nt) were excised from 8% Tris-Borate-EDTA polyacrylamide gels stained with SYBR Gold and eluted in 600ul of elution buffer (5mM Tris/HCl pH8, 0.5mM EDTA, 2.5M Ammonium Acetate) overnight. Gel slurry were passed through Spin-X filters (Sigma) and precipitated in two 300µl aliquots by addition of 800µl ethanol and 3µl of glycogen (5mg/ml). Pellets were washed in 75% ethanol and air-dried before being resuspended in 30µl water. Library concentrations were determined by quantitative PCR (qPCR, described in SREK manual) as well as densitometry of polyacrylamide gels (as above) loaded with serial dilutions of the HyperLadder V (Bioline). 2.8 fmol of each library were added to emulsion PCRs, and the libraries were sequenced on a SOLiD system (version 3) using SOLiD version 2 reagents. Unless stated otherwise, equipment and reagents used were from Life Technologies and used following the manufacturer's instructions.

Sequence analysis pipeline: We refer to an individual sequence read as a tag and the number of times it occurred as a count. The SOLiD Small RNA Pipeline was used to map tags to miRBase version 16. We employed this pipeline to map 18nt anchors of each tag to pre-miRNA sequences obtained from miRBase v16, allowing a maximum of three mismatches and mapping to no more than fifteen loci, before extending the alignment of the "anchor". A cumulative maximum of 6 mismatches was tolerated in aligning tags to the reference plus SOLiD adaptor. A custom-made script, termed Mismatch and Multimapping Resolver, was further applied to increase mapping specificity. Thus, tags with isolated mismatches were only accepted if the position of the mismatch coincided with a quality value score of less than 17. Tags representing multi-loci miRNAs (miRNAs of the same generic miR ID but deriving from distinct precursors with high nucleotide identity in hairpin sequence) were placed against the miRBase entry with the lowest entry number (e.g. miR-24-1 and miR-24-2; all tags were placed against miR-24-1). For all other non-uniquely mapping tags a count was placed against each matching miRBase entry. All sequence read data have been submitted to the Sequence Read Archive of NCBI with accession number SRA030449.1.

SUPPLEMENTAL FIGURE LEGENDS

SUPPLEMENTAL FIGURE S1. Normalisation of qRT-PCR data from polysome gradient fractions to accurately reflect differential sedimentation of target mRNAs after let-7 inhibition. After ultracentrifugation each gradient is divided into 24 fractions and 100ng of total yeast RNA is spiked into each fraction. RNA is purified from all fractions (see Methods) and combined to make 12 fractions in total. qRT-PCR is performed on yeast genes CMD1 and/or RPS3, human GAPDH and then target mRNAs of interest. Two steps of normalisation were performed on each gradient. Firstly, each fraction was divided by the spike-in to provide mRNA abundance from an equal portion of each fraction. After multiple experiments using this normalisation it was determined that the distribution of GAPDH mRNA in polysome profiles was largely invariant between anti-miR treatments (miR-499, let-7 and miR-34a). This property of GAPDH mRNA allowed further normalisation of polysome gradient data to more accurately reflect the difference between polysome profiles in control and miRNA inhibited samples. For example, for each fraction target mRNA abundance in let-7 anti-miR samples is normalised to the deviation of GAPDH mRNA between miR-499 and let-7 (target let-7 x GAPDH miR-499/GAPDH let-7). Examples of normalisation using GAPDH are shown.

SUPPLEMENTAL FIGURE S2. Effect of puromycin treatment on the sedimentation of R-luc-3xb mRNA after let-7 inhibition. Duplicate plates of HeLa cells were transfected with R-luc-3xb and anti-miRs to let-7 anti-miR (or control) as for Fig. 1 except that one duplicate of cells was additionally incubated with puromycin before harvest (see Methods). (A) Distribution of Rluc-3xB mRNA in untreated cells +/- let-7 anti-miR, with A_{254} nm absorbance profile below. (B) Distribution of R-luc-3xb mRNA in puromycin treated cells

+/- let-7 anti-miR. A_{254} nm absorbance profile below is not on the same scale as in A due to measurement of the large monosome peak requiring reduction of the sensitivity of the UV absorbance detector.

SUPPLEMENTAL FIGURE S3. The RDH10 3'UTR is unaffected by let-7 inhibition in HeLa cells. Plasmids with either the RDH10 or GAPDH 3'UTR engineered behind the Firefly luciferase ORF (from Switchgear Genomics), as well as the Renilla luciferase reporter R-luc-3xb (see Fig. 1), were individually transfected into HeLa cells with either let-7 anti-miRs or control anti-miR against miR-499. To normalise for transfection efficiencies pTK-Rluc and pGL3-Fluc were co-transfected, respectively (reporter luciferase activity was normalised as follows: Reporter/normalisation control). Luciferase activity was measured after 48 hours (as previously described in (Beilharz et al. 2009)). The fold increase in luciferase activity (let-7 anti-miR/control anti-miR) is shown with standard error and is an average of triplicate experiments for GAPDH and RDH10 reporters. Illustrative data for one experiment is shown for R-luc-3xb (see also (Beilharz et al. 2009)).

SUPPLEMENTAL FIGURE S4. Identification of alternative polyadenylation sites of IGF2BP1 used in HeLa cells. To verify alternative polyadenylation sites primers were designed to sites upstream of predicted sites and PAT products generated and sequenced (see Supplemental Methods). Above is a schematic of the let-7 binding sites (purple lines) and predicted and annotated alternative polyadenylation sites (blue triangles) in the 3'UTR of IGF2BP1. Underneath are 3'UTR sequences surrounding the sequenced alternative polyadenylation sites used in HeLa cells with the cleavage site indicated, number of clones supporting use of this site in HeLa cells, and likely polyadenylation signals upstream of the cleavage site.

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SUPPLEMENTAL TABLE S1. Primer information.

Gene name	Primer sequences	Ref Seq	Amplicon
GAPDH	For: GACATCAAGAAGGTGGTGAA Rev: TGTCATACCAGGAAATGAGC	NM_002046.3	871-1048
TMEM2	For: GGGACAAATGGCTTGCTAATA Rev: AGAGTACCCGGCTTGGTGA	NM_013390.2	2296-2429
TMEM2 Long 3'UTR	For: CCCCCGACGCATAAAGTGTA Rev: GCCCCTAGAAGAGGAAAGTGAAA	NM_013390.2	5980-6091
IGF2BP1	For: AGGGCCGAGCAGGAAATA Rev: GAAGCAGCCCCAGTAACG	NM_006546.3	1346-1516
IGF2BP1 Long 3'UTR	For: ACCACCCGATTTTGATTGTA Rev: CACGGGAAAGAAAACTGGAG	NM_006546.3	8558-8692
HMGA2	For: CAGAAGCCACTGGAGAAAAAC Rev: TGCTGCTGAGGTAGAAATCG	NM_003483.4	1014-1175
HMGA2 Long 3'UTR	For: GAATCGCTTGCTTGTTGAA Rev: GAGGCTGTTATGTTTATTGTGC	NM_003483.4	3989-4063
NRAS	For: TTTGCCAACAAGGACAGTT Rev: CTGAGTCCCATCATCACT	NM_002524.3	611-791
KRAS	For: CACAAAACAGGCTCAGGA Rev: CTTTTTACCATCTTTGCTCA	NM_004985.3	559-833
KRAS Long 3'UTR	For: CCACACCCCCACAGAGC Rev: CAGCGCAACCAAATGATG	NM_004985.3	4851-5124
CCND1	For: ACGCGCAGACCTTCGTT Rev: AGCGTGTGAGGCGGTAGTA	NM_053056.2	751-903
SPRYD4	For: AGATGGCGCTGCTTTTTG Rev: CTGCTGTGGGCGGTTTT	NM_207344.3	74-200
SLC25A24	For: CCCCTTTGGACCGTCTGA Rev: AGCGAGCGGATACCTCCTT	NM_013386.3	852-963
SLC25A24 Long 3'UTR	For: AGGCATTTTCTTTATTTGAACCTA Rev: AAAGTAGAATCCCAGGCTTATCAT	NM_013386.3	2983-3146
MYC	For: TTCTGTGGAAAAGAGGCAGGC Rev: TCACGCAGGGCAAAAAAGC	NM_002467.4	1365-1706
GPR56	For: ACACGGCCGCTCACAAT Rev: GGCCTTCTGGGGATGCT	NM_001145771.1	946-1037
GPR56 isoform 1	For: GCCCAGACAAGCTCAGACT Rev: GGGAGGTGGAGGTTACGA	NM_001145770.1	35-207
GPR56 isoform 2	For: GTGGCCCAGCTTCAAAGT Rev: GAACGTGCGTGGCAGAT	NM_001145774.1	219-334
RDH10	For: CAAAGAGAAAGGTGAAGTTCGTCG Rev: ACTCCGGCAGTACTGAACAATC	NM_172037.3	788-880
Rluc-3xB	For: CAAAGAGAAAGGTGAAGTTCGTCG Rev: GCATTGGAAAAGAATCCTGGGT	AF025843.2	1685-1861
CMD1 *S.cerevisiae*	For: TGGCTCTGATGTCTCGTCAA Rev: CAAAGCAGCGAATTGTTGAA	NM_001178457.1	209-432
RPS3 *S.cerevisiae*	For: CTCCAACCAAGACCGAAGTT Rev: ATCTGACGACACCGTAAGCA	NM_001183016.1	125-373
IGF2BP1 LM-PAT primers	2416: CCACCAAGAGGGTGGATCA 3779: CAAGGTATGAAGTTACCTCAG 8769: GATGGATGAACGAACACTCC	NM_006546.3	2351-2369 3706-3779 8657-8676
GAPDH LM-PAT	For: GGACCACCAGCCCCAGCAAG Universal and TVN primers see (Beilharz,2009)	NM_002046.3	1118-1137
Ubiquitin	For: ATTTGGGTCGCGGTTCTTG Rev: TGCCTTGACATTCTCGATGGT	NM_021009.5	410-542
RPL13a	For: CCTGGAGGAGAAGAGGAAAGAGA Rev: TTGAGGACCTCTGTGTATTTGTCAA	NM_012423.2	487-612

SUPPLEMENTAL TABLE S2. miRNA binding sites in the 3'UTRs of let-7 targets.

mRNA target	miRNA[1]	Tag count[2]	Evidence[3]	3'UTR miRNA binding site[4]
IGF2BP1	Let-7 family	107807	Experimental (Mayr and Bartel 2009)	1632, 1651, 4269, 4923, 5568
	miR-17-5p family	83484	Predicted	4050
KRAS	Let-7 family	107807	Experimental (Chin et al. 2008)	2624
	miR-19a/b	56690	Predicted	3578
	miR-27a/b	36647	Predicted	225,4458
	miR-30 family	68038	Predicted	4242
MYC	Let-7 family	107807	Experimental (Kong et al. 2008)	141
	miR-34a	12076	Experimental (Kong et al. 2008)	139
	miR-145	19827	Experimental (Chen et al. 2010b)	147
	miR-24	255003	Experimental (Lal et al. 2009)	255,447
TMEM2	Let-7 family	107807	Experimental (Selbach et al. 2008)	1469[#]
	miR23a/b	238978	Predicted	34
	miR-26a/b	17883	Predicted	382
HMGA2	Let-7 family	107807	Experimental (Lee and Dutta 2007)	21,1107,1256,1668,2521,2541
	miR-15 family	26384	Predicted	1302
	miR-17-5p family	83484	Predicted	1214
	miR-26a/b	17883	Predicted	1867
CCND1	Let-7 family	107807	Experimental (Schultz et al. 2008)	3311, 3970[#]
	miR-193b	40633	Experimental (Chen et al. 2010a)	1448
	miR-34a	12076	Experimental (Jiang et al. 2009)	2076
	miR-17-5p family	83484	Predicted	583,1014
SPRYD4	Let-7 family	107807	Experimental (Selbach et al. 2008)	1128[#]
	miR-25 family	59353	Predicted	121
NRAS	Let-7 family	107807	Experimental (Johnson et al. 2005)	187[#]
GPR56	Let-7 family	107807	Experimental (Selbach et al. 2008)	570
SLC25A24	Let-7 family	107807	Experimental (Selbach et al. 2008)	3003[#]
RDH10	Let-7 family	107807	Experimental (Selbach et al. 2008)	1331[#]

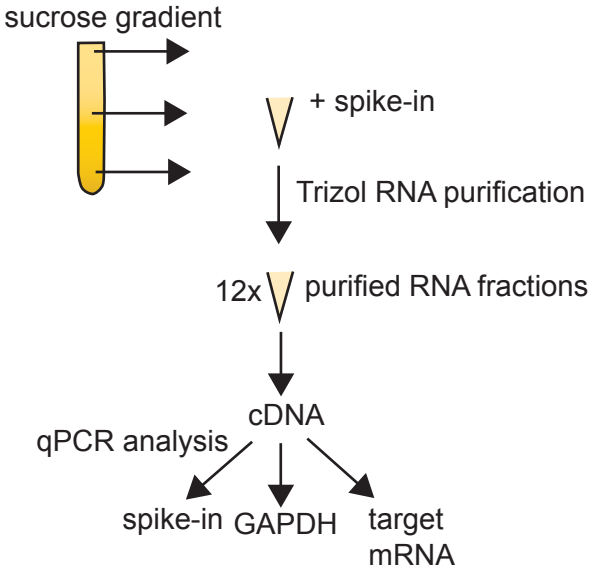
[1] miRNA families defined by Targetscan 5.1

[2] Expression level of miRNAs given as tag counts in a HeLa cell small RNA deep sequencing dataset of 2,297,595 tags that mapped to known miRNAs (see Supplemental Methods). Only miRNAs with a >11,000 tag count (~10% of let-7 expression) were considered.

[3] Predictions were performed using Targetscan 5.1 with a $P_{CT} \geq 0.5$ and a context score < -0.13 (Grimson et al. 2007).

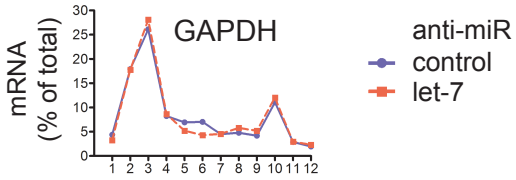
[4] Nucleotides from stop codon. [#] denotes experimentally verified targets with sites predicted by Targets scan, others are derived from references in previous column.

Supplemental Figure S1.



(1) Normalisation to spike in

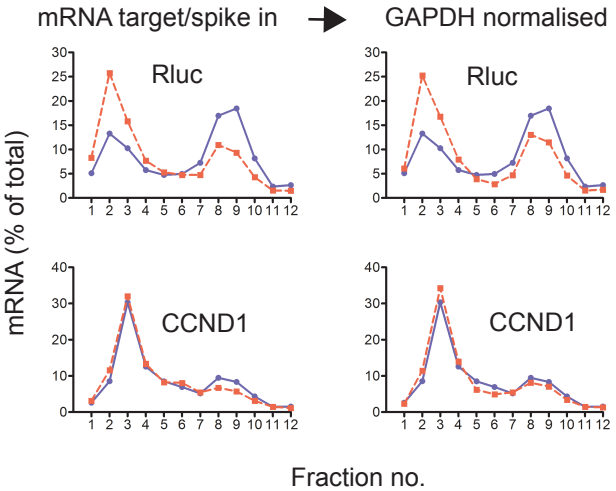
$$\frac{\text{GAPDH}}{\text{spike-in}}$$



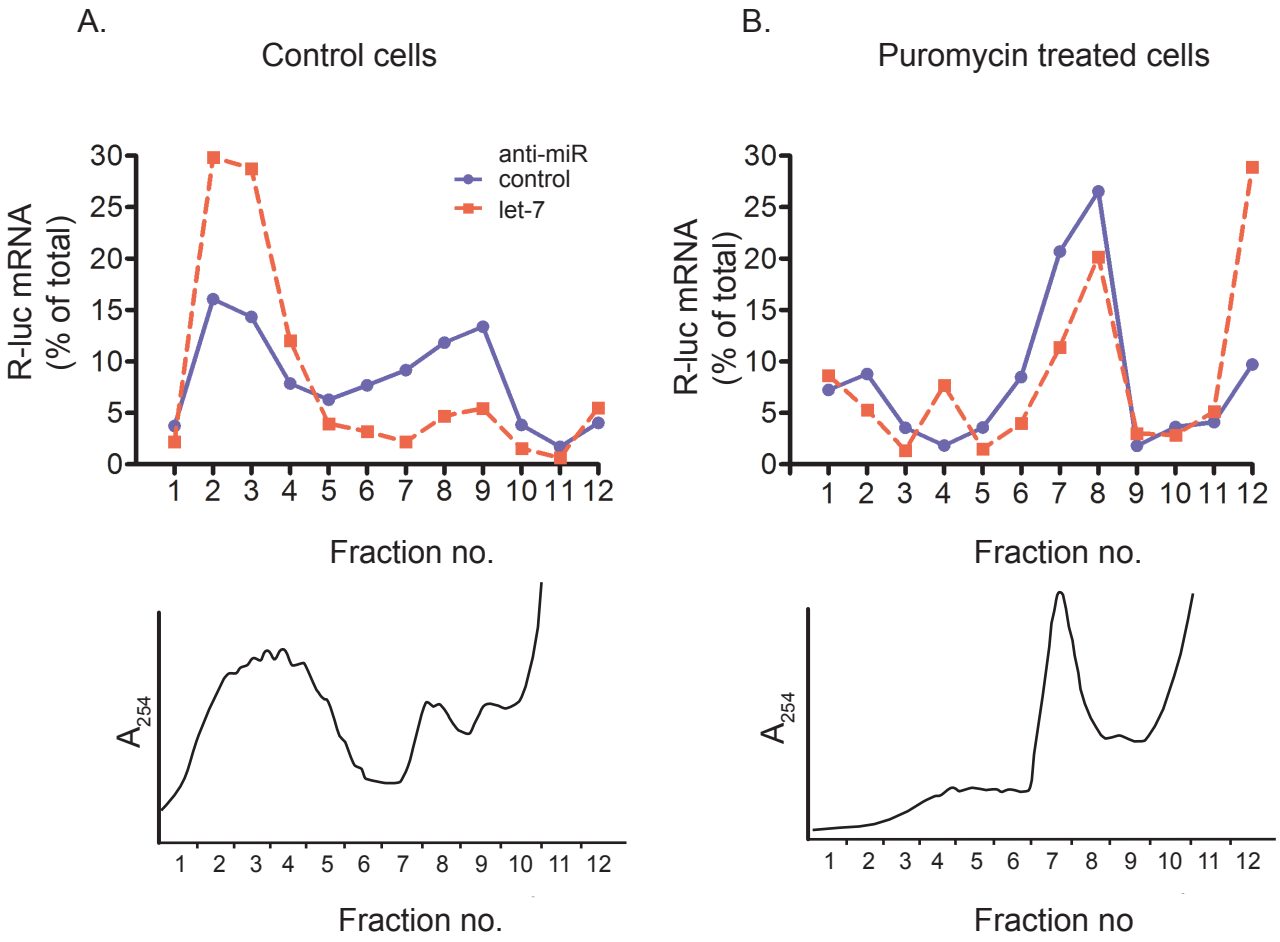
QC for similar gradient sedimentation between treatments

(2) Normalisation to GAPDH

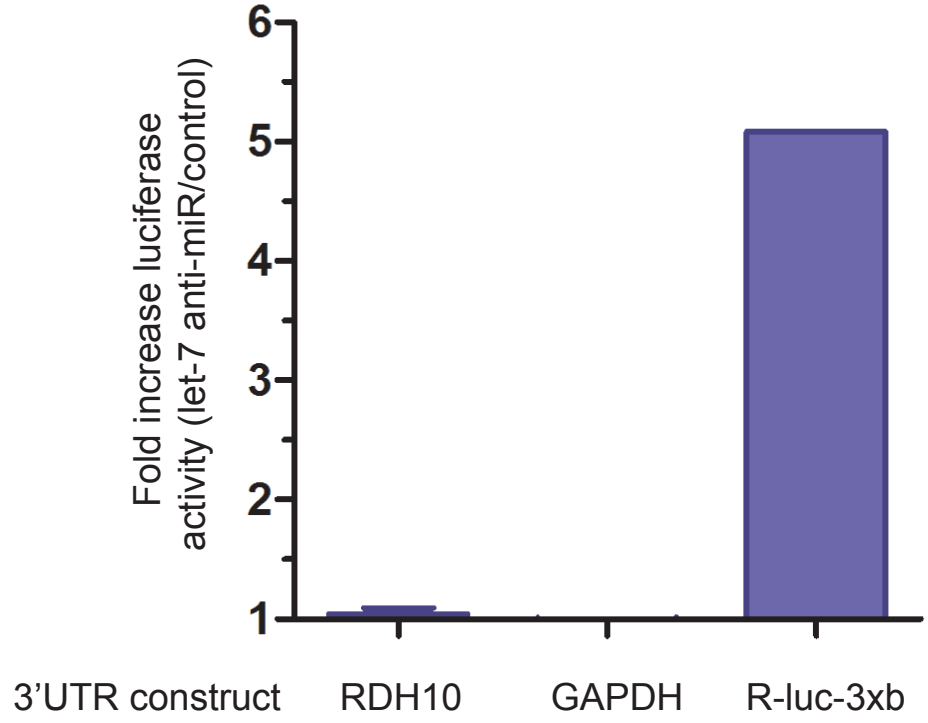
$$\frac{\text{let-7 GAPDH}}{\text{control GAPDH}} \times \text{let-7 target mRNA}$$



Supplemental Figure S2.



Supplemental Figure S3.



Supplemental Figure S4