Supporting Information

MEF Viability and LacZ Analysis

To assess cell viability, untreated and treated MEFs were viewed and images were acquired using a Zeiss microscope (Axiovert 200). For lacZ stained cells MEFs were viewed and images were acquired under a Nikon microscope (Eclipse TE300). Over 100 cells and at least three screen shots were counted at each time point.

Western Analysis

Total proteins were extracted using Lysis Buffer (20 mM Tris-HCl pH 7.5, 150 mM NaCl, 1 mM EDTA, 1 mM EGTA, 1% Triton, 2.5 mM sodium pyrophosphate, 1 mM beta-glycerophosphate) following cell rinsing in cold PBS. For Western analysis, 20 ug of total proteins were separated in 7.5% Tris-HCl gels (Bio-Rad), transferred to a Protran membrane (Schleicher and Schuell) using an electroblot in 1xTBST (10 mM Tris-Cl, pH 8.0; 150 mM NaCl, 0.05% Tween 20) at 250 mA for 3 hr. Blocking was carried out using 2% Blocking agent (GE/Amersham) for 1 hr at RT, followed by 3 short washes in 1xTBST, then antibody incubations, anti-Dicer (Abcam, 1:500 dilution) and GAPDH (Santa Cruz Biotech; 1:2,000 dilution), were carried out at 4^{o} C for 16 hours in 2% Block solution. Secondary anti-rabbit IgG-HRP conjugate (Sigma; 1:10,000 dilution) was incubated for 1 hr at RT in 2% Blocking solution. Washes were carried out in 1xTBST for 15 min (3 times). SuperSignal West Femto Maximum Sensitivity Substrate (Pierce) was used for chemiluminescence. As a positive control recombinant Dicer (Stratagene) was also analyzed.

LacZ Staining

Cells were washed in cold PBS+2mM $MgCl_2$, fixed in 0.2% Glutaraldehyde (Sigma; diluted in PBS+2mM $MgCl_2$) for 10 minutes on ice, then washed 3 times in PBS and rinsed with Rinse Buffer (0.1M Na phosphate, 0.1% NaDeoxycholate, 2mM $MgCl_2$, 0.2% NP-40). X-gal staining (5mM $K_3Fe(CN)_6$, 5mM $K_4Fe(CN)_6$, 1 mg/ml Xgal, in Rinse Buffer) was added for 3 hr at 37^{o}C before microscopy.

Apoptosis Assay

Cells were harvested, washed twice in cold PBS and resuspended at a concentration of 1 x 10^6 cells/ml in Binding Buffer (10mM HEPES, 140 mM NaCl, 2.5 mM $CaCl_2$, pH7.4). Annexin V-FITC (Sigma), which detects annexin V bound to apoptotic cells, and propidium iodide (2 ug/ml), which labels cellular DNA in necrotic cells, were added and incubated for 10 min at room temperature in the dark. Samples were then immediately analyzed by flow cytometer (Becton Dickinson, FACScan). Staining with both Dyes allowed differentiation among early apoptotic cells (annexin V positive, PI negative), necrotic cells (annexin V positive, PI positive), and viable cells (annexin V negative, PI negative).

Northern Analysis and Probes

Probes were generated by incubating a total of 20 ul of 20 uM oligo, 2 ul Polynucleotide Kinase (PNK; New England Biolabs), 2 ul 10x PNK Buffer (New England Biolabs) and ^{32}P gamma-ATP (6000Ci/mmol) for 1 hr at 37^{o} C. Non incorporated nucleotides were

removed using MicroSpin G-25 Columns (GE/Amersham). Sequences of oligonucleotide probes used were as follows: miR21-5'-tcaacatcagtctgataagcta; miR22-5'-acagttcttcaactggcagctt; miR23b-5'-ggtaatccctggcaatgtgat; miR34a-5'-aacaaccagctaagacactgcca; miR92-5'-acaggccgggacaagtgcaata; miR191-5'-agctgcttttgggattccgttg; miR199a2-5'-gaacaggtagtctgaacactggg; miR200b-5'-catcattaccaggcagtatta; U6-5'-ttgcgtgtcatccttgcgcagg.

MEF miRNA Microarray Preparation, Hybridization and Scanning

MicroRNA microarrays were printed using a Cartesian PixSys 5500 Arrayer on Epoxy slides (Corning) using Ambion's miRvana amine-modified DNA oligonucleotide probe set (version 1564V1). Probes were printed at 50 uM in Printing Buffer (0.25M Sodium Phosphate buffer pH8.5, 250 uM Sorkosyl) in quadruplicate. 30 ug of total RNA was separated in 15% TBE-UREA gels. The 15 to 25 nt gel region, identified using siRNA Marker (New England Biolabs) and Ethidium Bromide staining, was excised and RNA was extracted by overnight incubation at 4^{o}C in 1M NaCl followed by ethanol precipitation. Labeling of small RNA was carried out using the miRvana miRNA labeling kit (Ambion) and Cy3/5 (GE/Amersham). 10 pmol of each labeled Dye was added onto an array in 1x Hybridization Buffer (Ambion), covered by a LifterSlip (Erie Scientific), and hybridization was carried out in Corning Hybridization chambers II for 16 hr in a water bath set to 42^{o} C. Washes were performed at room temperature according to manufacturer's protocol and solutions (Ambion; salt and detergent reagents). Arrays were spun down at 500 g for 5 min and scanned immediately using an Axon Scanner GenePix 4000.

MEF miRNA Microarray Analysis

The raw GenePix (.gpr) data were imported into R (www.r-project.org) for subsequent analysis. The median background of each array was estimated by analyzing the distribution of intensities obtained for negative controls (spotted RNA for which no complementary RNA was spiked-in). The median intensity (of 4 duplicated spots) for each miRNA and array was compared to the array-background to identify miRNAs with intensities more than 2 standard deviations above the median background. To estimate changes in miRNA expression levels we compared the experiment/reference RNA (cy3/cy5; reference RNA for all arrays was size selected HeLa cell line RNA) from the control arrays with the two MEF CDKO+OHT samples across all miRNAs. Spiked-in control RNA was to verify that no systematic bias was introduced during sample processing and hybridization. This array platform probably cannot distinguish between closely related miRNA species (e.g., closely related miRNA family members) due to cross-hybridization. However, this does not affect our conclusions because all analyses using the array data were done at the level of miRNA families (defined by unique seeds) rather than individual miRNAs.

Identification of the 'Effective' siRNA Subset

For each of the 4096 possible 6mers, Refseq genes in the siRNA dataset were divided into two classes based on presence/absence of the 6mer in their annotated 3'UTR. For each 6mer, the significance of the difference in LFC distributions between these classes was tested by two-sided rank sum test. siRNAs were considered to be 'effective' if a

6mer complementary to the extended seed region (positions 1 through 8) on either the sense or antisense strand gave a P-value $<10^{-6}$ or was among the top ten 6mers having the lowest P-values. If 6mers corresponding to the sense and the antisense strands both passed these criteria, then the one with the lowest P-value was kept and UTRs having seed matches to the other strand were removed from the analysis. Of the 74 siRNAs available for analysis, 52 met these criteria and reduced to 44 unique seed regions (MAPK14-1as, -2, -3as, -4as, -5as, -6, -7, -8as, -193, -M1, -M2as, -M4as, -M5as, -M6as, -M15, -M18, IGF1R-1as, -2, -3, -4as, -5as, -6, -10as, -11as, -12, -13, MPHOSPHQ-202as, -2692, PIK3CA-2629, PIK3CB-6338as, -6340as, PLK1-1319as, -772as, PRKCE-1295, SOD1-SNPp13as, -SNPp15as, -SNPp18as, -SNPp19as, -SNPp2as, -SNPp8as, -SNPp9as, -1582as, VHL-2651as, and -2652 where 'as' indicates the strand antisense to the targeted mRNA). Data from these 44 siRNAs was pooled for analysis. For the analyses shown in Fig. 1, the subset of 33 of these sequences that began with non-U bases was used.

Identification of 'Strongly Detected' and 'Responsive' MEF miRNAs

For the analysis shown in Fig. 3A, miRNAs with hybridization intensities above a threshold of 2 standard deviations above the median background level in seven or more of the eight miRNA microarrays were considered to be 'strongly detected'. Those below this threshold on all eight microarrays were considered 'not detected'. For the analysis shown in Fig. 3C, 'responsive' miRNAs were defined as follows. From the set of conserved miRNAs (seed region m1-m8 common to a miRNA in both mouse and human miRBase 8.2 (microrna.sanger.ac.uk)), we compared the LFC CDFs for a set of

mRNAs containing an extended seed match to the miRNA with the set of all mRNAs that lacked a seed match to the miRNA. This generated a list of 31 miRNAs (listed below) where seed match containing mRNAs were significantly upregulated relative to the non-seed match containing mRNAs ($P < 0.001$ by two-sided rank sum test). For this analysis, the sets of mRNAs containing an extended seed match were selected so as to include equal numbers of conserved and non-conserved seed matches, so as to avoid introducing any biases related to conservation. This was accomplished by sampling from the (invariably larger) set of mRNAs containing non-conserved seed matches a subset of the same size as the conserved mRNA set. Such sampling was performed at least 10 times and median P-values used. Responsive miRNAs: let-7d, let-7g, miR-9*, miR-15b, miR-19b, miR-26a, miR-30a-5p, miR-101a, miR-106a, miR-106b, miR-130a, miR-135a, miR-142-5p, miR-154, miR-155, miR-181a, miR-182, miR-186, miR-200b, miR-214, miR-291a-3p, miR-291b-3p, miR-302b*, miR-302c*, miR-320, miR-367, miR-381, miR-410, miR-424, miR-448, miR-495.

Controlling for Seed Match Type, Expression, Conservation and CG Content

Analyses of miRNA effects on mRNA levels were corrected for the effects of potentially confounding variables not under investigation. In the conservation analyses (Figs. 1C,E,G and Fig. 3C), for each mRNA in the conserved set we sampled at random and without replacement an mRNA from the non-conserved set that had the same number and type of seed matches, and roughly the same (within 10%) hybridization intensity value and fraction of conserved 7mers in its 3'UTR. Details of these controls are shown in Fig. S2. Variables that did not differ significantly between the sets (UTR length and

CG content shown in Fig. S2C) were not explicitly controlled. This same policy was applied to all analyses (e.g. mRNA expression levels across different seed match type mRNA sets). A similar approach was used to control for overall 3'UTR CG content in the nucleotide composition analyses (Fig. 5). Analyses of the effects of UTR length on targeting found either no difference (miR-1) or moderately increased downregulation for mRNAs with shorter UTRs (miR-124) (not shown). No significant effect of 3' UTR CG content was observed in the miRNA transfection data. For the analyses of seed match count shown in Fig. 4, there was not sufficient data to permit analysis of target downregulation as a function of seed match count for each seed match type separately. For each of the plots shown in Fig. 4, the proportion of seed match types for different seed match counts remained fairly constant.

No increased mRNA repression was associated with conserved versus non-conserved siRNA seed matches, when controlling for seed match type, expression and overall UTR conservation (Fig. 1G). However, slightly increased downregulation was associated with conserved siRNA seed matches when the control for overall UTR conservation was relaxed; this affect appears to be a consequence the increased local conservation that is associated with seed match conservation (not shown).

Calculation of signal:noise

Signal: noise ratios were calculated as in Lewis et al. (2005), but considering conservation only across human, mouse, rat, and dog genomes (HMRD) using cohorts of control oligonucleotides matched for both count and exact CG content. Ratios were pooled for the set of conserved human miRNAs used for target prediction by (Lewis et

al., 2005) after removal of miRNAs with common m2-m8 seed regions but different m9 nucleotides and pairs of miRNAs in the same super-family.

Orthologous 3' UTRs for zebrafish and *Tetraodon* were collected as described in Methods. Using an approach similar to Lewis et al. (2005), the number of occurrences of each 7mer was enumerated in each zebrafish 3' UTR and 7mers which also occurred in the corresponding *Tetraodon* UTR were recorded as conserved. In cases of multiple occurrences of the same 7mer in a zebrafish UTR and fewer occurrences in the *Tetraodon* UTR, only the common counts were recorded as conserved. For both the miR-430 A1 7mer and M8 7mer, sets of control 7mers with roughly equal total occurrences (within 10 counts) were collected and the mean fraction conserved for control and miR-430 7mers calculated. The ratio of miR-430 7mer to control 7mer fraction conserved is reported as the signal:noise.

Repression for non-conserved 8mers versus conserved 7mers

In the Lim miRNA transfection data, we observed stronger downregulation associated with non-conserved 8mer seed matches, especially those with a t9W, than for conserved 7mer seed matches (Fig. S8A), using conservation criteria identical to those used by the TargetScanS algorithm. This observation suggests that presence of a non-conserved 8mer seed match is at least as reliable a predictor of miRNA targeting – given co-expression with the corresponding miRNA – as is a TargetScanS prediction based on 7mer conservation (Lewis et al., 2005). Consistently, in the MZdicer knockout

system, presence of a non-conserved 8mer seed match was associated with stronger repression than for 7mer seed matches conserved to other fish (Fig. S8B).

TargetRank Scoring

TargetRank scores the seed matches in a UTR relative to a given siRNA or miRNA, and then calculates an overall score for the mRNA as a whole by summing the scores for all seed matches present in the 3' UTR. The score for each seed match, m, is calculated according to $S(m) = S_{SeedMatchType}(m) + R_{5'conservation}(m) + R_{3'AU}(m)$, where $S_{SeedMatchType}(m)$ is the mean nLFC for the seed match type represented by m, and $R_{5'conservation}(m)$ and $R_{3'AU}(m)$ represent the residual contribution to nLFC associated with the level of sequence conservation immediately 5' of the seed match and the AU content immediately 3' of the seed match, respectively. For t1A 7mer and 6mer seed matches, the $S_{SeedMatchType}(m)$ value is determined as in Fig. 1F. For M8 7mer and 8mer seed matches, the t9W effect is also incorporated by assigning $S_{SeedMatchType}(m)$ dependent on the seed match type and t9 base, as in Fig. 5A. $R_{3'AU}(m)$ is determined by first assigning the seed match to one of three bins based on the %AU content in the 50 bases immediately 3' of the seed match (as in Fig. 6D). $R_{3'AU}(m)$ is then set equal to the mean nLFC for this bin in the training set of siRNA data, less the average mean nLFC across the 3 bins. For example, if the mean nLFC values of the 3 bins are 0.10, 0.12, and 0.17 (average: 0.13), then the residual values for the three bins would be -0.03, -0.01 and 0.04, respectively. Unlike in Fig. 6D, binned mRNA sets were only controlled for seed match type and t9 composition, which are variables already accounted for in the first term of the model (parameters used: bin 1: 3’ AU < 53%, mean nLFC = 0.083, bin 2: 3’ AU between 53%

and 66%, mean nLFC = 0.126; bin 3: 3' AU > 66%, mean nLFC = 0.182). $R_{5'conservation}(m)$ is determined by assigning the seed match to one of three bins based on the %conservation in the 50 bases 5' of the seed match (as in Fig. 6C), and then calculating a residual score for this bin as described for $R_{3'AU}(m)$. Unlike in Fig. 6C, binned mRNA sets are controlled only for seed match type, t9 composition, and %AU in the 50 bp 5' of the seed match (parameters used: bin 1: 5' conservation < 33%, mean nLFC = 0.085, bin 2: 5' conservation between 33% and 56%, mean nLFC = 0.135; bin 3: 5' conservation > 56%, mean nLFC = 0.163). For a 3' UTR containing n seed matches $m_1, m_2, \ldots$, the TargetRank score is calculated simply as the sum $S(UTR) = \sum_{k=1}^{n} S(m_k)$, using the log-additivity of seed matches derived from Fig. 4. For Figs. 7A and 7C, a random subset of 8 siRNAs were held out from the Jackson/Schwarz datasets and parameters were estimated based the remaining 36 siRNA transfections. The same parameters were used for Figs. 7B and 7D.

Supplemental References

Jackson, A. L., Bartz, S. R., Schelter, J., Kobayashi, S. V., Burchard, J., Mao, M., Li, B., Cavet, G., and Linsley, P. S. (2003). Expression profiling reveals off-target gene regulation by RNAi. Nat Biotechnol *21*, 635-637.

Lewis, B. P., Burge, C. B., and Bartel, D. P. (2005). Conserved seed pairing, often flanked by adenosines, indicates that thousands of human genes are microRNA targets. Cell *120*, 15-20.

Legends to Supplemental Figures

Fig. S1. Seed match type effects on mRNA repression for conserved and non-conserved seed matches. (A) Cumulative distribution functions (CDFs) of LFCs for mRNAs containing the indicated non-conserved miR-124 seed match types. Plots are based on mRNAs containing exactly one non-conserved miR-124 seed match of each seed type (and no conserved seed matches). Set sizes are shown in parentheses. (B) LFC CDFs for mRNAs containing a single conserved miR-124 seed match (and no non-conserved seed matches). (C) LFC CDFs for mRNAs containing a single non-conserved miR-1 seed match. (D) LFC CDFs for mRNAs containing a single conserved miR-1 seed match.

Fig. S2. Effects of seed match conservation on mRNA repression following miRNA transfection for controlled and uncontrolled datasets. (A) CDF of LFCs for mRNAs containing conserved (red) or non-conserved (blue) extended seed matches to miR-124, or no seed matches (gray). mRNAs with conserved seed matches may also contain non-conserved seed matches, though the non-conserved class is strict. Set sizes are shown in parentheses. (B) mRNAs from the non-conserved set were sampled without replacement to generate a set having the same extended seed match count and distribution across 3'UTR conservation and expression as the conserved set. 3'UTR conservation is measured as the fraction of all 7mers (not just miRNA seed matches) in the 3'UTR that are perfectly conserved in human, mouse, rat and dog aligned genomes. Expression is measured as $\log_2$ of the hybridization intensity in mock transfected cells. 3'UTR conservation and expression CDFs are shown for the sampled set having the

median rank sum statistic ($P \geq 0.05$). (C) $\log_2$ 3' UTR length and fraction CG content are shown for these same sets. Although these variables were not explicitly controlled, there is no significant difference between the sets ($P \geq 0.05$). (D) CDFs of LFCs for the controlled sets (same as in Figure 1C).

Fig. S3. Genomic organization of MEF CDKO mouse

Schematic representation of the genomic organization of the MEFcdko mouse and of Dicer inactivation. (A) MEFcdko mice were generated from mice bearing three unique genomic regions: (i) CAG-ERT promoter with the Prx1-Cre allele, inducible by Orthohydroxy Tamoxifen addition (4-OHT); (ii) R26 Promoter with a LacZ gene downstream of a floxed stop codon; (iii) Dicer1 gene with a floxed exon 24. (B) The CAG-ERT promoter is activated upon addition of 4-OHT to the medium, driving expression of Cre protein. (C) The floxed stop codon upstream of the LacZ gene and (D) Dicer1 exon 24 is excised, producing beta-galactosidase and a non-functional Dicer allele.

Fig. S4. Kinetics of Dicer knockout monitored by LacZ staining

(A) MEFwt and MEFcdko cells in the absence and presence of 0.5 uM OHT were stained daily to monitor LacZ expression. At 24 hour intervals the numbers of stained (blue) and unstained cells were counted. Images are presented for MEFwt and MEFcdko in the presence of OHT. (B) The percent of stained/blue cells counted on each day is plotted (1D to 4D). The mean and standard deviation of the mean of three replicates are shown.

Fig. S5. Knockout of Dicer does not induce apoptosis in MEFs.

FACS analysis of Annexin V was performed in MEFwt and MEFcdko in the absence and presence of 0.5 uM OHT for 1 to 4 days. Shown here are data for MEFwt and MEFcdko with and without OHT after 4 days (4D). PI Staining (dead cells) is shown on the x-axis; Annexin V-FITC staining (apoptotic cells) is shown on the y-axis. Each quadruple, clockwise from bottom left, shows: (i) unstained live cells; (ii) PI stained dead cells; (iii) PI and FITC stained dead/apoptotic cells; and (iv) FITC stained apoptotic cells, respectively. The number at the bottom right hand side in each section denotes the percentage of cells in the particular state as a fraction of the total. Percent apoptotic cells measured along a four day time course (1D to 4D) is plotted below. The day 4 experiment was repeated twice.

Fig. S6. Northern Analysis of miRNA Expression

Northern analysis is shown for four of the miRNAs represented in Fig. 2D. Background-corrected hybridization intensities were calculated for each experimental sample (MEFcdko+OHT; right lane of each gel) and for the three control samples (MEFcdko/MEFwt+OHT/MEFwt; first three lanes from the left). All bands were then normalized to U6 snRNA and the fold-change was calculated by dividing the normalized average of the control samples by the normalized experimental sample. In (A) membrane was stripped by incubating in 1% SDS solution for 10 min at 60°C and then re-hybridyzed several times.

Fig. S7. mRNA derepression following Dicer knockout in zebrafish varies with conservation status. CDFs of LCFs for mRNAs containing conserved (red) or non-conserved (blue) miR-430 extended seed matches, or no seed matches (gray). mRNAs containing conserved seed matches (see Methods) may also contain non-conserved seed matches, though the non-conserved set is strict. Set sizes are shown in parentheses.

Fig. S8. Prediction of Non-conserved miRNA Targets Containing 8mer Seed Matches. (A) Mean LFC for mRNAs containing non-conserved 7mers (blue), conserved 7mers (red), and non-conserved M8-A1 8mers (purple) to miR-1 and miR-124 following transfection of the corresponding miRNA. Dashed box indicates mean LFC for miR-1 and miR-124 W9-M8-A1 9mers. (B) Same as (A) for miR-430 following Dicer knockout in zebrafish embryos.

Fig S9. Effects of local conservation and AU content following miRNA transfection for controlled and uncontrolled datasets. (A) CDF of LFCs for equal sized sets of mRNAs containing a single siRNA extended seed match and grouped by conservation level in the 50 nt region immediately upstream of the siRNA seed match. Mean percent conservation values for the sets are as follows (most conserved (red) = 72%, moderately conserved (gray) = 44%, least conserved (green) = 11%). (B) mRNAs from the three sets were sampled without replacement such that the distributions of UTR conservation, expression level, upstream AU content and UTR AU content were not significantly different (rank sum test, $P >= 0.05$). Seed match types were also

matched across each bin (not shown). UTR conservation is measured as the number of positions in the human 3' UTR that are perfectly conserved in alignments to mouse, rat, and dog. Expression is measured as the log2 of the hybridization intensity in mock transfected cells. (C) CDFs of LFCs for the controlled sets (same data as shown in Figure 6A (upstream).

Fig. S1.

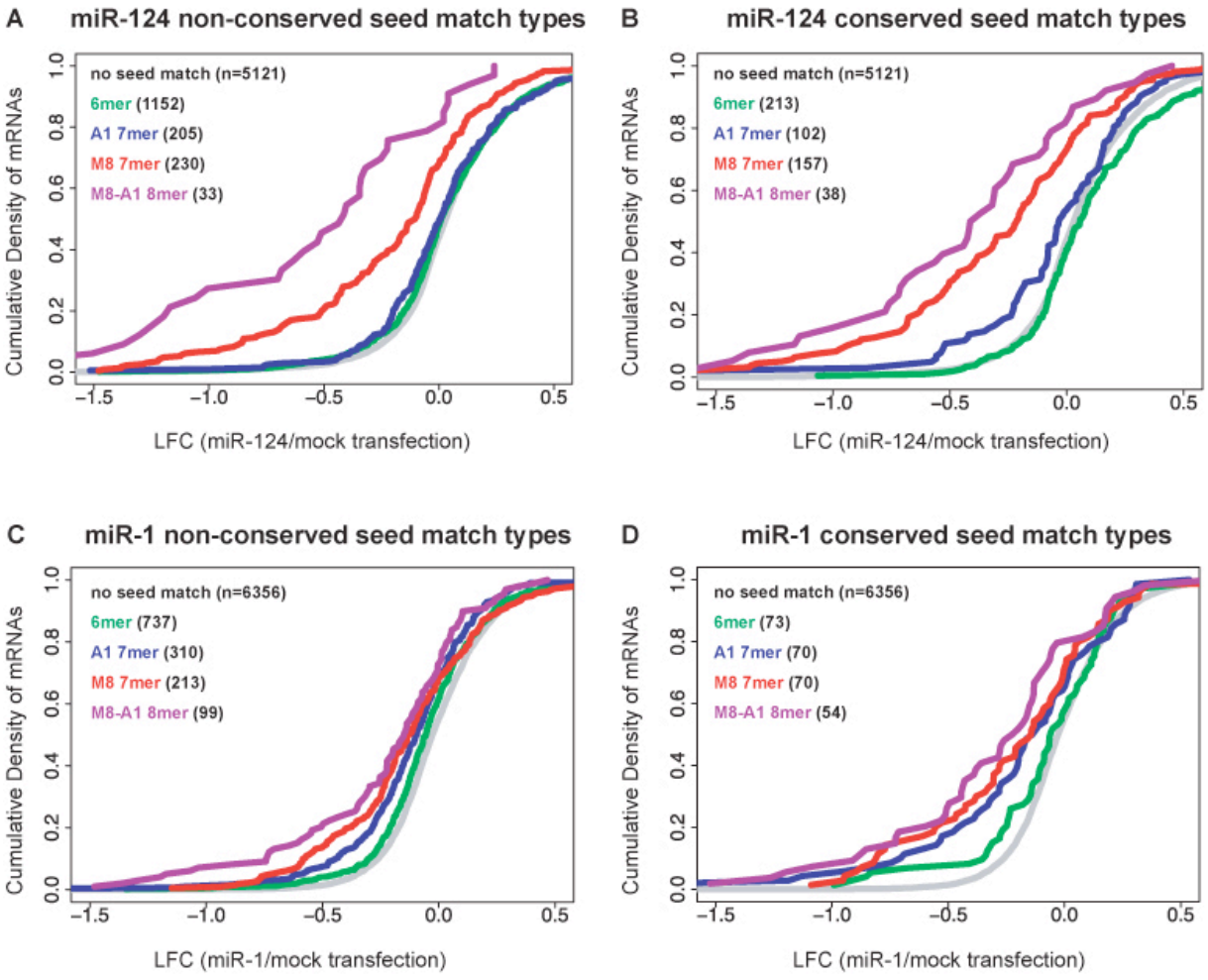
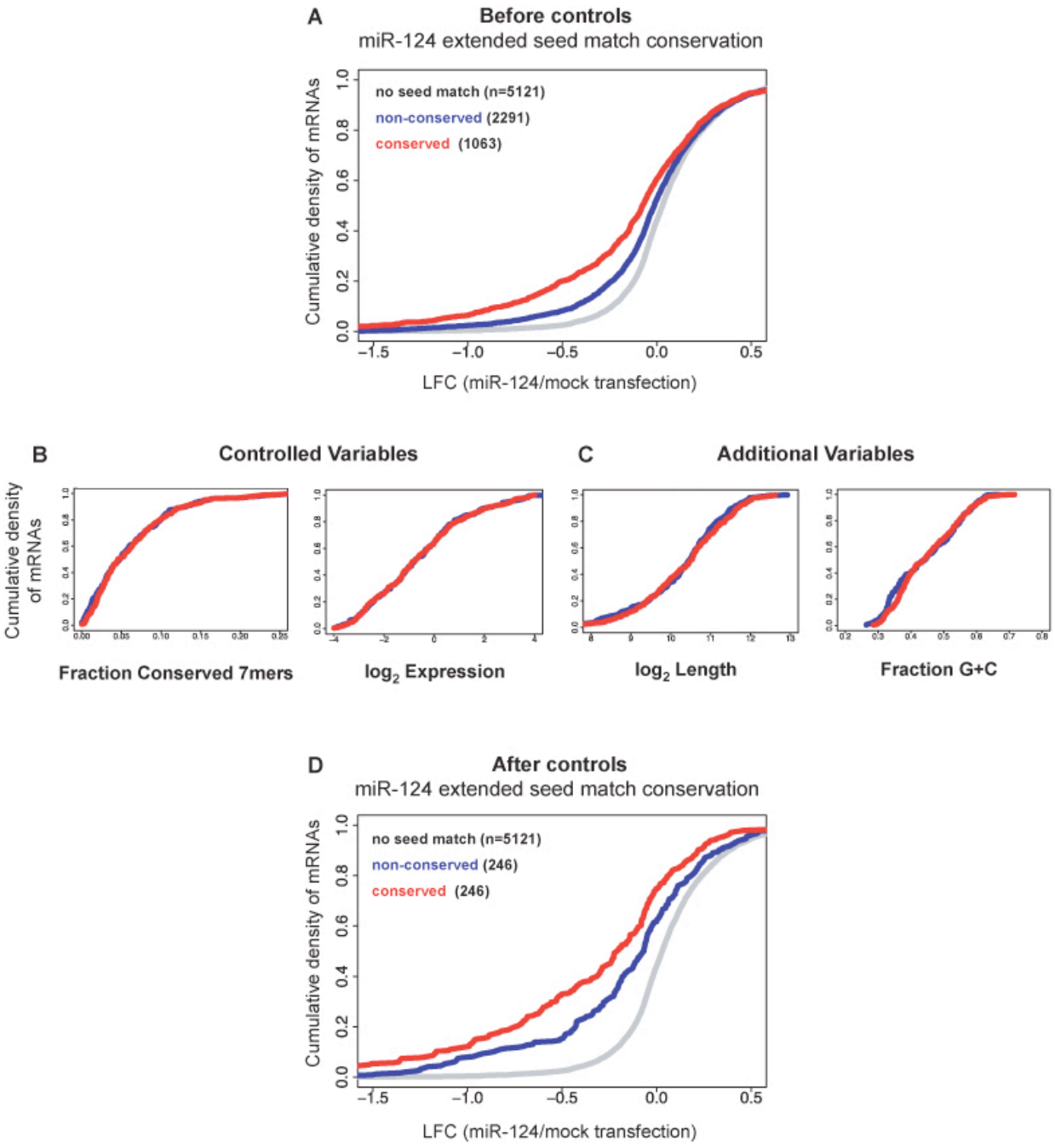

A
miR-124 non-conserved seed match types
no seed match (n=5121)
6mer (1152)
A1 7mer (205)
M8 7mer (230)
M8-A1 8mer (33)
Cumulative Density of mRNAs
LFC (miR-124/mock transfection)
B
miR-124 conserved seed match types
no seed match (n=5121)
6mer (213)
A1 7mer (102)
M8 7mer (157)
M8-A1 8mer (38)
Cumulative Density of mRNAs
LFC (miR-124/mock transfection)
C
miR-1 non-conserved seed match types
no seed match (n=6356)
6mer (737)
A1 7mer (310)
M8 7mer (213)
M8-A1 8mer (99)
Cumulative Density of mRNAs
LFC (miR-1/mock transfection)
D
miR-1 conserved seed match types
no seed match (n=6356)
6mer (73)
A1 7mer (70)
M8 7mer (70)
M8-A1 8mer (54)
Cumulative Density of mRNAs
LFC (miR-1/mock transfection)

Fig. S2.


A
Before controls
miR-124 extended seed match conservation
no seed match (n=5121)
non-conserved (2291)
conserved (1063)
Cumulative density of mRNAs
LFC (miR-124/mock transfection)
B
Controlled Variables
C
Additional Variables
Cumulative density of mRNAs
Fraction Conserved 7mers
log2 Expression
log2 Length
Fraction G+C
D
After controls
miR-124 extended seed match conservation
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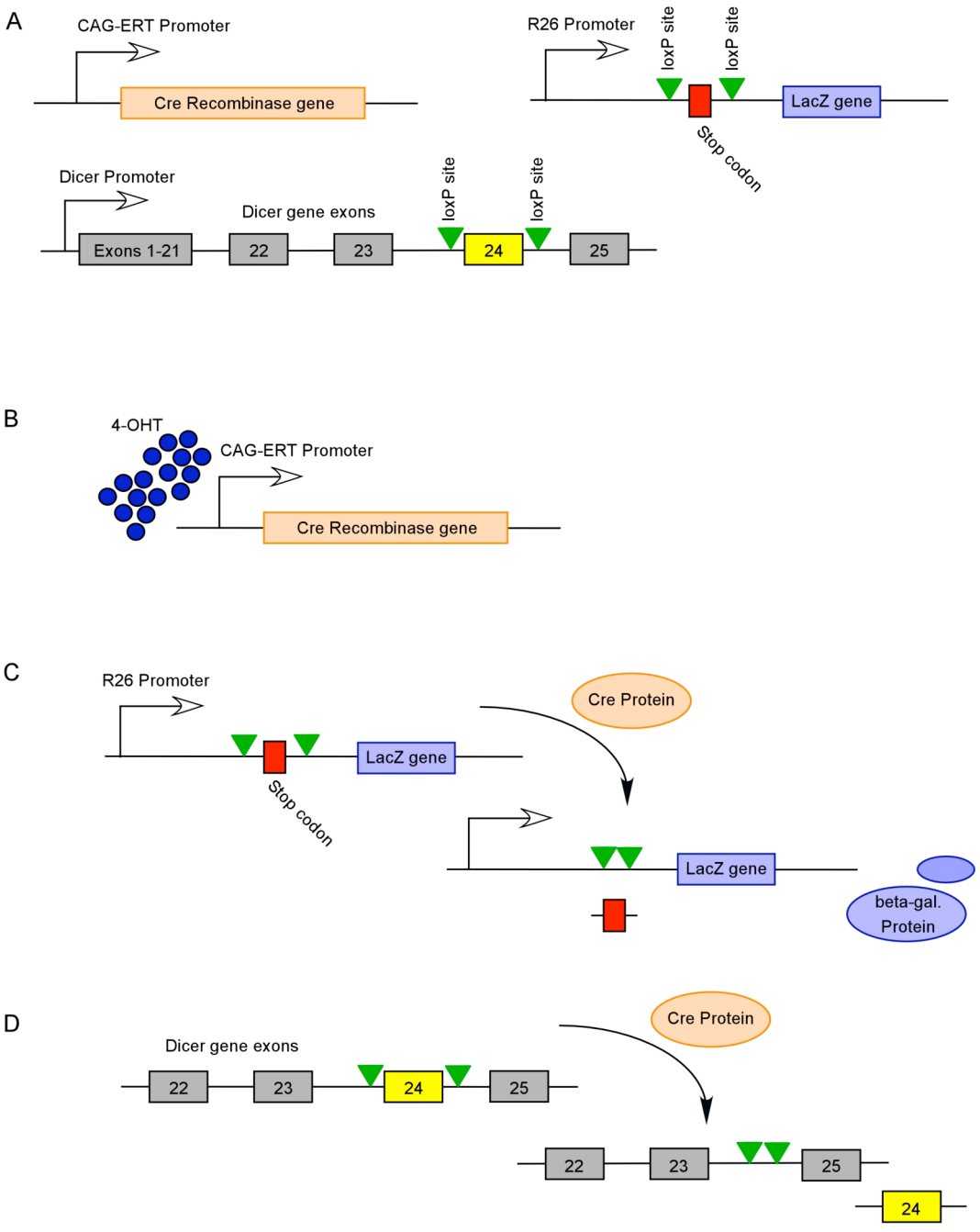
non-conserved (246)
conserved (246)
Cumulative density of mRNAs
LFC (miR-124/mock transfection)

Fig. S3.


A
CAG-ERT Promoter
Cre Recombinase gene
R26 Promoter
loxP site
loxP site
LacZ gene
Stop codon
Dicer Promoter
Dicer gene exons
loxP site
loxP site
Exons 1-21
22
23
24
25
B
4-OHT
CAG-ERT Promoter
Cre Recombinase gene
C
R26 Promoter
Cre Protein
LacZ gene
Stop codon
LacZ gene
beta-gal.
Protein
D
Dicer gene exons
Cre Protein
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25
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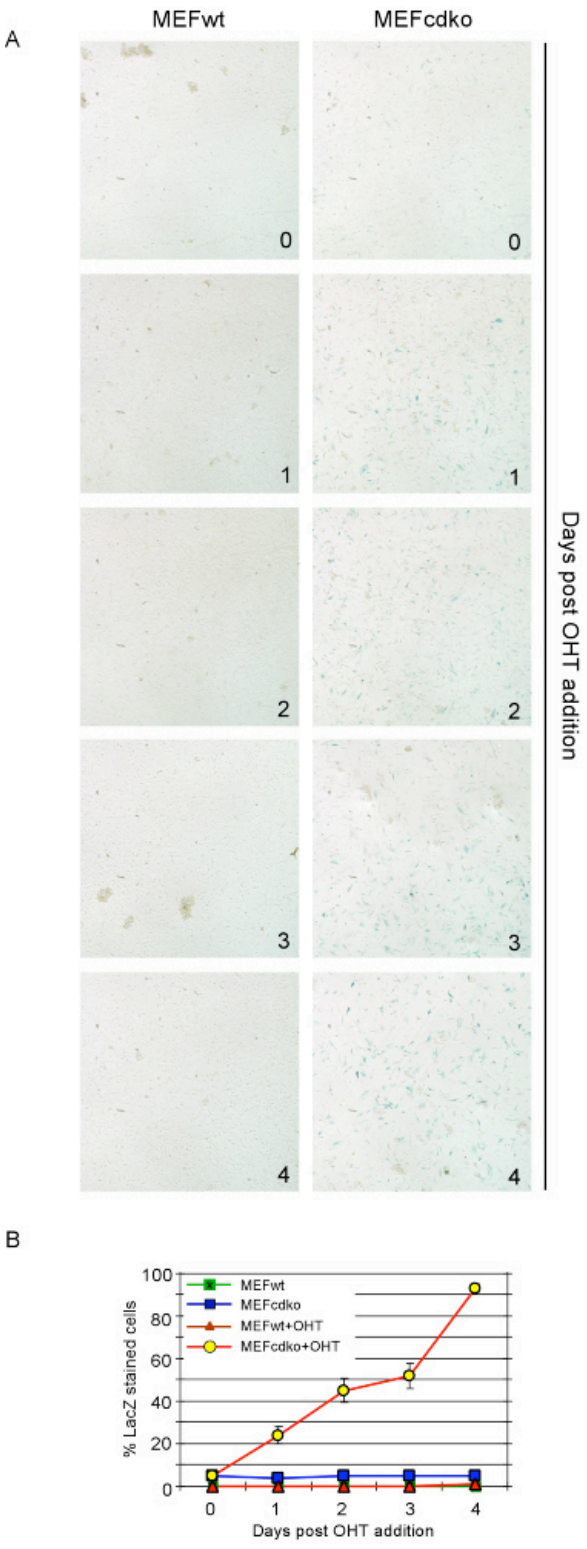
23
25
24

Fig. S4.


A
MEFwt
MEFcdko
0
0
1
1
2
2
3
3
4
4
Days post OHT addition
B
% LacZ stained cells
100
80
60
40
20
0
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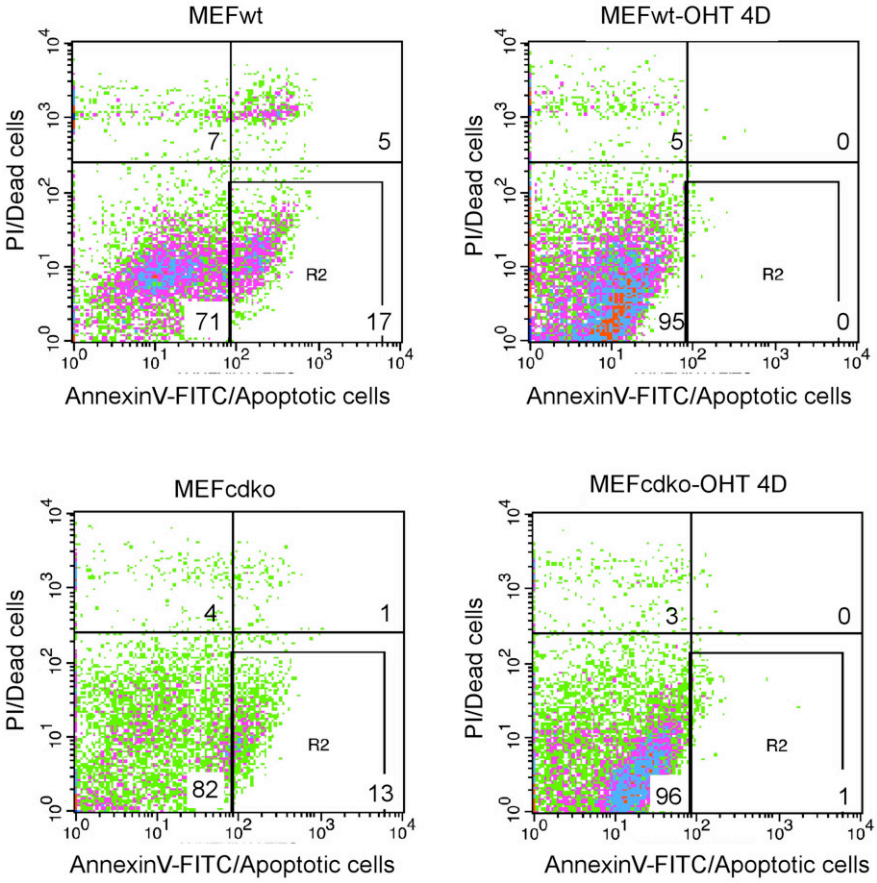
MEFcdko
MEFwt+OHT
MEFcdko+OHT
0
1
2
3
4
Days post OHT addition

Fig. S5.


MEFwt
MEFwt-OHT 4D
MEFcdko
MEFcdko-OHT 4D
PI/Dead cells
AnnexinV-FITC/Apoptotic cells
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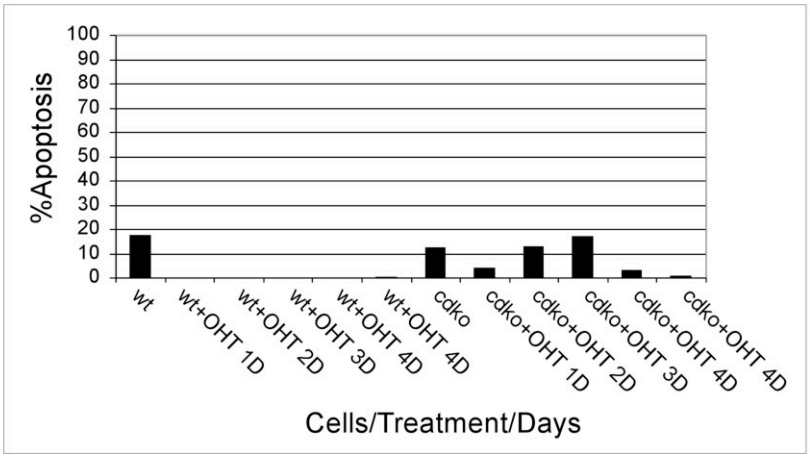
5
71
17
5
0
95
0
4
1
82
13
3
0
96
1


%Apoptosis
100
90
80
70
60
50
40
30
20
10
0
wt
wt+OHT 1D
wt+OHT 2D
wt+OHT 3D
wt+OHT 4D
wt+OHT 4D
cdko
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cdko+OHT 4D
cdko+OHT 4D
Cells/Treatment/Days

Fig. S6.

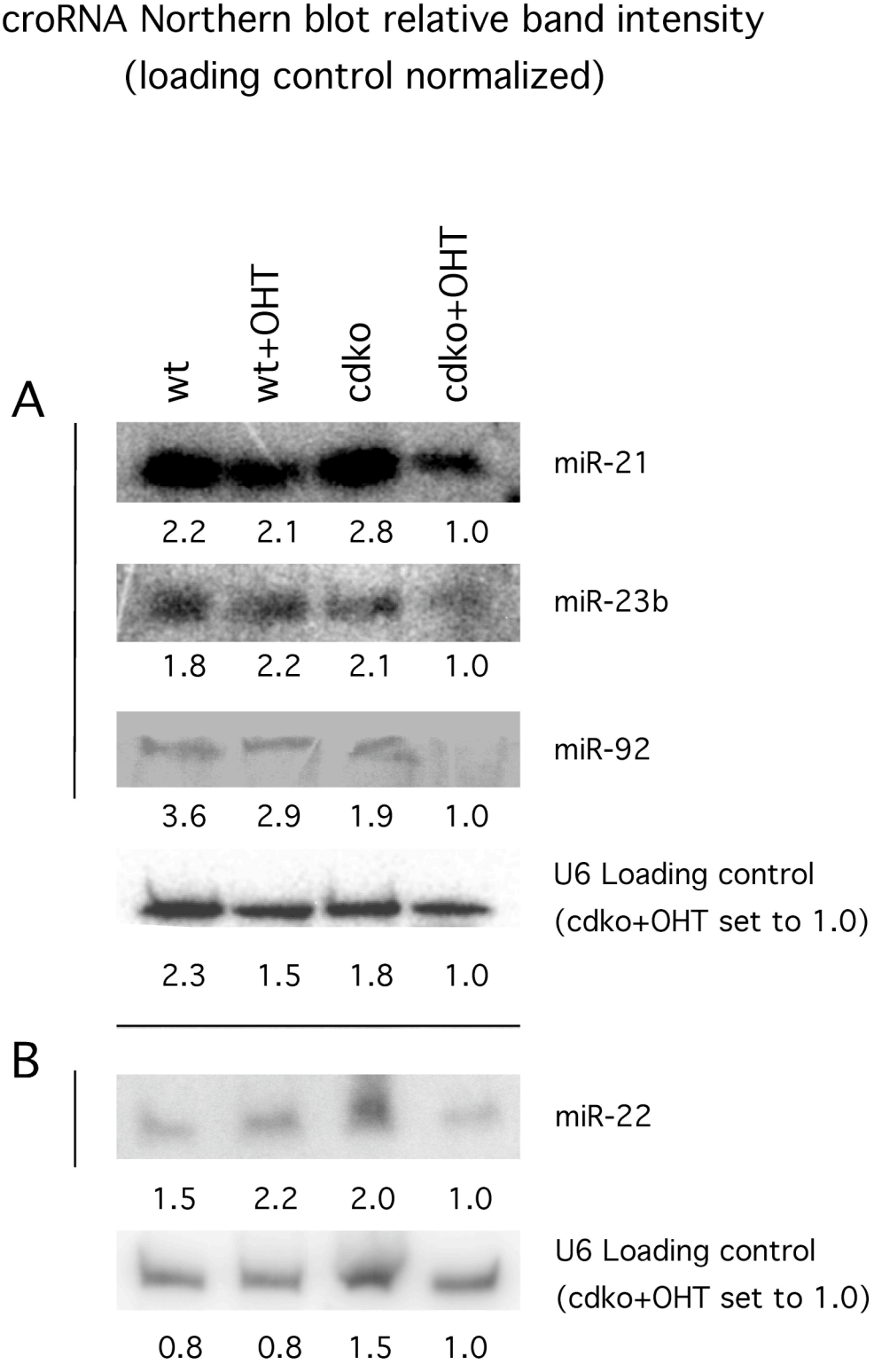
MicroRNA Northern blot relative band intensity
(loading control normalized)
A
wt
wt+OHT
cdko
cdko+OHT
miR-21
2.2 2.1 2.8 1.0
miR-23b
1.8 2.2 2.1 1.0
miR-92
3.6 2.9 1.9 1.0
U6 Loading control
(cdko+OHT set to 1.0)
2.3 1.5 1.8 1.0
B
miR-22
1.5 2.2 2.0 1.0
U6 Loading control
(cdko+OHT set to 1.0)
0.8 0.8 1.5 1.0

Fig. S7.

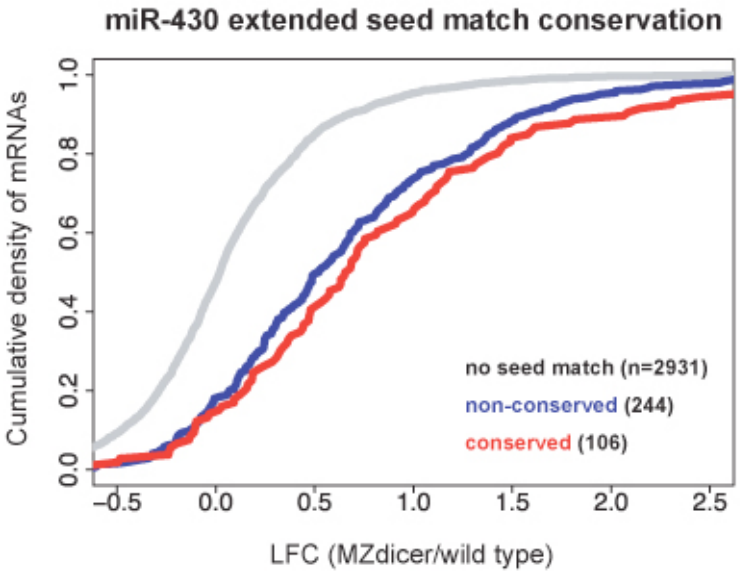
miR-430 extended seed match conservation
Cumulative density of mRNAs
0.0
0.2
0.4
0.6
0.8
1.0
−0.5
0.0
0.5
1.0
1.5
2.0
2.5
LFC (MZdicer/wild type)
no seed match (n=2931)
non-conserved (244)
conserved (106)

Fig. S8.

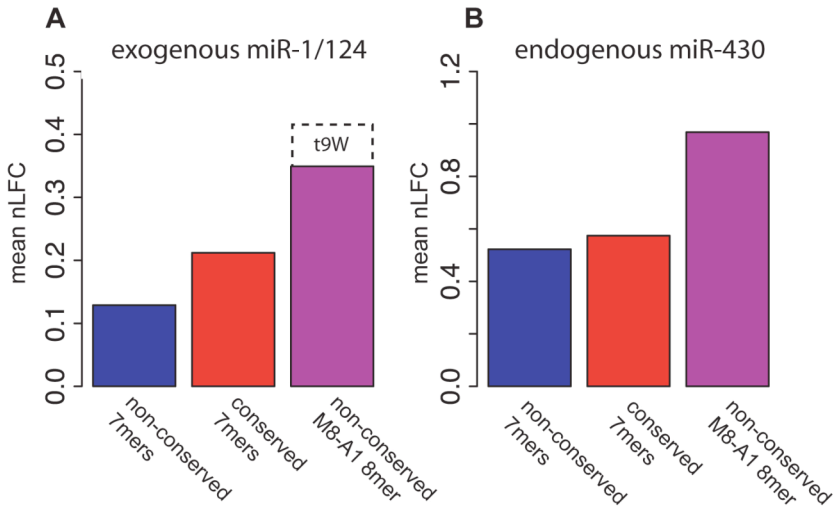
A
exogenous miR-1/124
mean nLFC
0.0
0.1
0.2
0.3
0.4
0.5
t9W
non-conserved 7mers
conserved 7mers
non-conserved M8-A1 8mer
B
endogenous miR-430
mean nLFC
0.0
0.4
0.8
1.2
non-conserved 7mers
conserved 7mers
non-conserved M8-A1 8mer

Fig. S9.

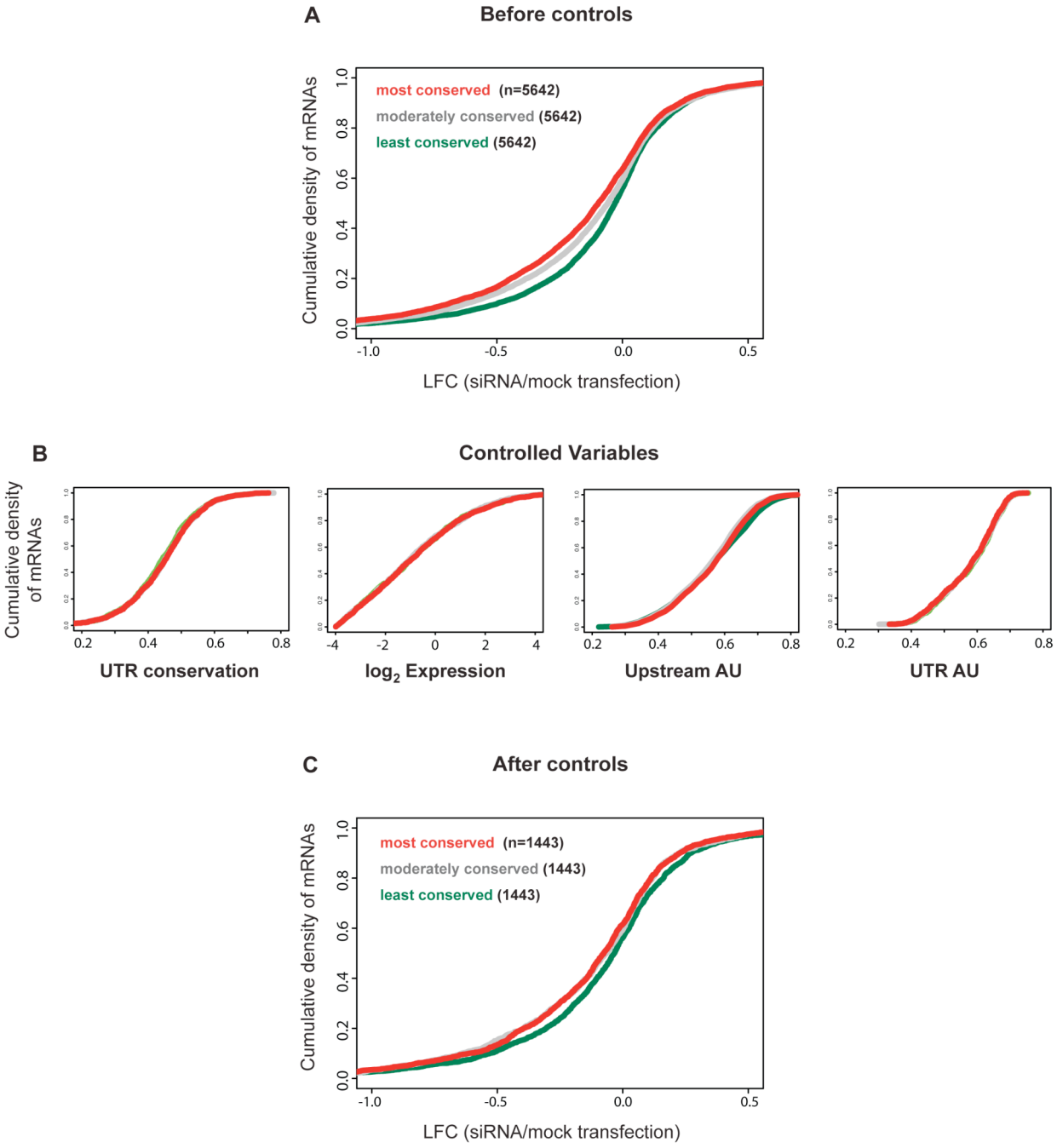
A
Before controls
most conserved (n=5642)
moderately conserved (5642)
least conserved (5642)
Cumulative density of mRNAs
LFC (siRNA/mock transfection)
B
Controlled Variables
Cumulative density of mRNAs
UTR conservation
log2 Expression
Upstream AU
UTR AU
C
After controls
most conserved (n=1443)
moderately conserved (1443)
least conserved (1443)
Cumulative density of mRNAs
LFC (siRNA/mock transfection)

Table S1. Statistics related to Fig. 1.

Figure 1B data – miR-124 seed match types

seed type	no. of mRNAs	mean LFC	mean nLFC	frac. DR	Wilcoxon Rank Sum Test (2 sided) P-values			
					no seed match	M2-7 6mer	A1 7mer	M8 7mer
no seed match	5121	0.05	0.00	0.025	-			
M2-7 6mer	1373	0.04	0.01	0.036	0.354	-		
A1 7mer	317	0	0.05	0.054	0.003	0.023	-	
M8 7mer	396	-0.25	0.25	0.250	$<10^{-43}$	$<10^{-33}$	$<10^{-13}$	-
M8-A1 8mer	77	-0.51	0.56	0.454	$<10^{-23}$	$<10^{-21}$	$<10^{-15}$	$<10^{-4}$

Figure 1C data – miR-124 extended seed match conservation

con. type	no. of mRNAs	mean LFC	mean nLFC	Wilcoxon Rank Sum Test (2 sided) P-values	
				no seed match	non-conserved
no seed match	5121	0.05	0.00	-	
non-conserved	246	-0.15	0.20	$<10^{-12}$	-
conserved	246	-0.34	0.39	$<10^{-34}$	$<10^{-3}$

Figure 1D data – miR-1 seed match types

seed type	no. of mRNAs	mean LFC	mean nLFC	frac. DR	Wilcoxon Rank Sum Test (2 sided) P-values			
					no seed match	M2-7 6mer	A1 7mer	M8 7mer
no seed match	6356	-0.01	0.00	0.025	-			
M2-7	813	-0.05	0.04	0.052	$<10^{-4}$	-		
A1 7mer	400	-0.13	0.12	0.123	$<10^{-13}$	$<10^{-5}$	-	
M8 7mer	286	-0.15	0.14	0.196	$<10^{-13}$	$<10^{-5}$	0.202	-
M8-A1 8mer	170	-0.25	0.24	0.276	$<10^{-15}$	$<10^{-8}$	0.002	0.055

Figure 1E data – miR-1 extended seed match conservation

con. type	no. of mRNAs	mean LFC	mean nLFC	Wilcoxon Rank Sum Test (2 sided) P-values	
				no seed match	non-conserved
no seed match	6356	-0.01	0.00	-	
non-conserved	178	-0.17	0.16	$<10^{-8}$	-
conserved	178	-0.29	0.28	$<10^{-20}$	0.007

Figure 1F data – non-m1U siRNA seed match types

seed type	no. of mRNAs	mean LFC	mean nLFC	frac. DR	Wilcoxon Rank Sum Test (2 sided) P-values					
					no seed	M2-7 6mer	M1 7mer	A1 7mer	M8 7mer	M8-M1 8mer
no seed match	155998	0.02	0.00	0.025	-					
M2-7	8102	-0.02	0.04	0.055	$<10^{-24}$	-				
M1 7mer	4296	-0.01	0.03	0.054	$<10^{-14}$	0.915	-			
A1 7mer	4673	-0.08	0.10	0.098	$<10^{-87}$	$<10^{-20}$	$<10^{-15}$	-		
M8 7mer	3350	-0.10	0.12	0.108	$<10^{-90}$	$<10^{-28}$	$<10^{-22}$	0.021	-	
M8-M1 8mer	1780	-0.10	0.12	0.117	$<10^{-54}$	$<10^{-20}$	$<10^{-19}$	0.014	0.561	-
M8-A1 8mer	1875	-0.21	0.23	0.186	$<10^{-149}$	$<10^{-79}$	$<10^{-67}$	$<10^{-29}$	$<10^{-19}$	$<10^{-13}$

Figure 1G data – siRNA extended seed match conservation

con. type	no. of mRNAs	mean LFC	mean nLFC	Wilcoxon Rank Sum Test (2 sided) P-values	
				no seed match	non-conserved
no seed match	187980	0.02	0.00	-	
non-conserved	1643	-0.11	0.13	$<10^{-52}$	-
conserved	1643	-0.13	0.15	$<10^{-65}$	0.198

DR = down-regulated

Table S2. Statistics related to Fig. S1.

Figure S1A data – non-conserved miR-124 seed match types			
seed type	**no. of mRNAs**	**mean LFC**	**mean nLFC**
no seed match	5121	0.05	0.00
M2-7	1152	0.03	0.02
A1 7mer	205	0.03	0.02
M8 7mer	230	-0.21	0.26
M8-A1 8mer	33	-0.55	0.60
Figure S1B data – conserved miR-124 seed match types			
seed type	**no. of mRNAs**	**mean LFC**	**mean nLFC**
no seed match	5121	0.05	0.00
M2-7	213	0.1	-0.05
A1 7mer	102	-0.05	0.10
M8 7mer	157	-0.3	0.35
M8-A1 8mer	38	-0.44	0.49
Figure S1C data – non-conserved miR-1 seed match types			
seed type	**no. of mRNAs**	**mean LFC**	**mean nLFC**
no seed match	6356	-0.01	0.00
M2-7	737	-0.05	0.04
A1 7mer	310	-0.12	0.11
M8 7mer	213	-0.13	0.12
M8-A1 8mer	99	-0.23	0.22
Figure S1D data – conserved miR-1 seed match types			
seed type	**no. of mRNAs**	**mean LFC**	**mean nLFC**
no seed match	6356	-0.01	0.00
M2-7	73	-0.06	0.05
A1 7mer	70	-0.19	0.18
M8 7mer	70	-0.19	0.18
M8-A1 8mer	54	-0.3	0.29

Table S3. Downregulation of mRNA and Protein Levels in miR-1 and miR-124 Transfection Data

refseq ID	miRNA transfected	nLFC (Lim et al, 2005)	luciferase reporter LFC (Farh et al, 2005)
NM_170735	miR-1	0.39	0.99
NM_024652	miR-1	0.30	0.74
NM_031453	miR-1	0.34	0.37
NM_182692	miR-1	0.77	0.26
NM_181358	miR-1	0.28	0.13
NM_014325	miR-1	0.84	0.58
NM_000402	miR-1	1.14	1.77
NM_012395	miR-1	0.98	1.23
NM_015318	miR-1	0.99	0.87
NM_181358	miR-124	0.40	1.27
NM_139168	miR-124	0.24	0.21
NM_020639	miR-124	0.28	0.99
NM_014397	miR-124	1.07	1.31

Table S4. miRNAs with detectable expression in MEFs[1]

let-7a	miR-137	miR-195	miR-300
let-7b	miR-138	miR-196a	miR-301
let-7c	miR-140	miR-198	miR-302c
let-7d	miR-141	miR-199a*	miR-30a
let-7e	miR-142	miR-19a	miR-31
let-7f	miR-143	miR-200a	miR-320
let-7f	miR-144	miR-200b	miR-330
let-7g	miR-145	miR-202	miR-335
let-7i	miR-146	miR-203	miR-338
miR-101	miR-147	miR-206	miR-34c
miR-103	miR-153	miR-208	miR-361
miR-106a	miR-155	miR-214	miR-367
miR-106b	miR-15a	miR-215	miR-372
miR-107	miR-16	miR-217	miR-374
miR-10a	miR-17	miR-21	miR-376b
miR-122a	miR-181a	miR-221	miR-381
miR-124a	miR-182	miR-223	miR-382
miR-125a	miR-183	miR-22	miR-384
miR-125b	miR-184	miR-23b	miR-410
miR-126	miR-185	miR-24	miR-422a
miR-1	miR-18	miR-26a	miR-7
miR-130a	miR-190	miR-27a	miR-9
miR-130b	miR-191	miR-292	miR-93
miR-134	miR-192	miR-297	miR-98
miR-136	miR-194	miR-32	

[1]A set of 99 miRNAs were detected by microarray, defined as having median microarray intensity (of the four duplicate probes) greater than two standard deviations above background in at least 7 out of 8 samples, for those miRvana probes targeting miRNAs found in mouse (according to miRBase) that had at least five mRNA targets expressed in MEFs. The 99 different miRNAs represent 80 unique seeds.

Table S5. mRNAs with Significant Expression Change Following Dicer Knockout[2]

Refseq ID	score(d)	Fold Change	Gene Symbol
NM_028523	10.77	12.63	Dcbld2
NM_130861	4.64	8.28	Slco1a5
NM_011348	9.84	7.60	Sema3e
NM_011213	4.21	6.05	Ptprf
NM_007399	9.02	5.97	Adam10
XM_484932	3.99	5.97	NA
NM_009252	4.67	5.79	Serpina3n
NM_015762	4.30	5.02	Txnrd1
NM_029575	4.42	4.76	Tgfbr2
NM_009364	5.19	4.69	Tfpi2
NM_145390	4.45	4.69	Tnpo2
NM_013737	5.99	4.61	Pla2g7
XM_130125	6.53	4.59	NA
NM_020275	4.57	4.36	Tnfrsf10b
NM_008402	8.56	4.23	Itgav
NM_009684	8.71	4.03	Apaf1
NM_026735	4.44	3.97	Mobkl1a
NM_011052	4.46	3.82	Pdcd6ip
NM_010442	4.30	3.82	Hmox1
XM_194424	4.71	3.78	NA
NM_011198	4.65	3.70	Ptgs2
NM_011452	7.73	3.64	Serpinb9b
NM_028527	7.04	3.63	1700047I17Rik
NM_011563	4.21	3.59	Prdx2
NM_172891	4.54	3.58	Styk1
NM_030155	4.22	3.57	Sdccag3
NM_028744	6.48	3.50	Pi4k2b
NM_019819	7.84	3.48	Dusp14
NM_029438	4.63	3.43	Smurf1
NM_001004143	4.13	3.43	Usp22
NM_029000	4.15	3.37	Gvin1
NM_145413	4.17	3.35	C530043G21Rik
NM_019547	4.49	3.28	Rnpc1
XM_484088	4.34	3.25	NA
NM_011502	5.19	3.18	Stx3
NM_023785	3.73	3.16	Cxcl7
NM_153584	5.87	3.11	BC031353
NM_015806	4.84	3.11	Mapk6
NM_015760	4.91	3.09	Nox4
NM_175201	9.12	3.08	Rnf38
NM_172967	4.07	3.06	4930503L19Rik
NM_011179	4.86	3.06	Psap
NM_172507	3.72	3.03	Sh3bgrl2
NM_172787	8.84	2.96	L3mbtl3
NM_013601	6.61	2.94	Msx2
NM_026177	3.90	2.93	1200011I18Rik

NM_011267	4.71	2.93	Rgs16
NM_010913	4.47	2.91	Nfya
NM_010786	8.54	2.89	Mdm2
NM_008924	6.28	2.87	Prkar2a
XM_140740	3.93	2.83	NA
NM_017368,NM_198683	5.35	2.81	Cugbp1
NM_013609	4.40	2.80	Ngfb
NM_011951	4.59	2.79	Mapk14
NM_009648	5.80	2.78	Akap1
NM_021451	4.13	2.73	Pmaip1
NM_172513	4.68	2.70	BC049806
NM_007406	4.62	2.69	Adcy7
XM_358611,XM_359418	4.82	2.66	NA
NM_153103	3.81	2.65	Kif1c
NM_020012	3.84	2.64	Rnf14
NM_024269	5.97	2.64	Arl2bp
NM_029352	4.06	2.60	Dusp9
NM_010345	3.79	2.60	Grb10
NM_011026	5.17	2.59	P2rx4
NM_011595	7.10	2.59	Timp3
NM_025673	3.72	2.58	Golph3
NM_010923,NM_180960	3.94	2.58	Nnat
NM_009443	3.86	2.54	Tgoln1
NM_013862	4.04	2.54	Rabgap1l
NM_008338	3.76	2.54	Ifngr2
NM_053153	4.58	2.53	Klra18
NM_028932	4.46	2.52	Eaf1
NM_007690	5.80	2.51	Chd1
NM_172734	4.39	2.50	Stk38l
NM_176845	5.51	2.48	Ddhd1
NM_009831	5.12	2.47	Ccng1
NM_008442	4.90	2.46	Kif2a
NM_007453	5.35	2.45	Prdx6
XM_135842	3.92	2.41	NA
NM_178615	4.87	2.39	Rgmb
NM_007952	4.86	2.37	Pdia3
NM_030721	5.04	2.36	Acox3
NM_207239	7.63	2.34	Gtf3c1
NM_019661	7.22	2.33	0610042I15Rik
NM_019927	4.11	2.32	Arih1
XM_489703	6.15	2.30	NA
NM_026195	7.31	2.29	Atic
NM_026662	5.50	2.29	Prps2
XM_622555	6.39	2.28	NA
NM_029777	6.54	2.28	4930418P06Rik
NM_028243	3.87	2.28	Prcp
NM_028651	5.49	2.27	4930403J22Rik
NM_178907	5.63	2.26	Mapkapk3
NM_010324	4.56	2.25	Got1

NM_144543	3.94	2.25	Thy28
NM_011018	4.27	2.24	Sqstm1
NM_013882	4.34	2.24	Gtse1
NM_009516	5.10	2.22	Wee1
NM_011699	4.17	2.21	Lin7c
NM_026424	3.70	2.21	1500041J02Rik
NM_011299	6.41	2.21	Rps6ka2
NM_031256	3.95	2.20	Plekha3
NM_139154	4.35	2.19	Rab40c
NM_138681	13.21	2.19	Bcas3
NM_133349	4.69	2.18	Zfand2a
NM_019930	3.97	2.17	Ranbp9
NM_134013	4.87	2.17	Psme4
NM_007836	4.18	2.17	Gadd45a
NM_024226,NM_194052 ,NM_194053	4.98	2.17	Rtn4
NM_007614	3.68	2.16	Ctnnb1
NM_030690	3.77	2.16	Rai14
NM_172699	5.65	2.16	Foxj3
NM_026563	4.48	2.15	Sdccag3
NM_134133	5.07	2.14	2010002N04Rik
NM_023066	4.72	2.14	Asph
NM_010718	4.47	2.13	Limk2
NM_009798	4.28	2.12	Capzb
NM_008928	4.34	2.12	Map2k3
NM_030015	6.72	2.11	Peli1
NM_172863	5.29	2.11	Zfp697
NM_007922	8.75	2.10	Elk1
XM_128959	4.28	2.10	NA
NM_030246	4.42	2.10	Wdr21
NM_025762	3.69	2.08	4933434E20Rik
NM_008465	5.69	2.08	Kpna1
NM_008576	5.24	2.05	Abcc1
NM_019432	5.00	2.04	Tmem37
XM_127105	4.21	2.03	NA
NM_025951	4.70	2.03	Pi4k2b
NM_175245	3.79	2.02	2410129H14Rik
NM_177613	3.81	2.01	Cdc34
NM_019403	4.11	2.01	Rnf5
NM_010249	4.25	2.00	Gabpb1
NM_025716	5.60	2.00	4633402N23Rik
NM_026418	-2.86	-2.00	Rgs10
NM_008538	-2.79	-2.00	Marcks
NM_020276	-3.34	-2.00	Nelf
NM_175098	-3.29	-2.01	6330407D12Rik
NM_016778	-4.49	-2.01	Bok
NM_020026	-10.07	-2.01	B3galt3
NM_009685	-2.92	-2.02	Apbb1
NM_019869	-2.96	-2.03	Rbm14

NM_021605	-2.80	-2.03	Nek7
XM_622635	-3.62	-2.05	NA
NM_009878	-6.10	-2.05	Cdkn2d
NM_175130	-3.29	-2.06	Trpm4
NM_026530	-8.58	-2.06	E130307M08Rik
XM_143175	-2.92	-2.08	NA
NM_016765	-6.30	-2.08	Ddah2
NM_027309	-4.16	-2.08	Lysmd2
NM_172546	-2.97	-2.09	Cnksr3
NM_026447,NM_198931	-8.46	-2.09	Ppm1m
NM_027878	-3.94	-2.11	1200002N14Rik
NM_172711	-4.94	-2.11	AA407526
NM_011838	-3.29	-2.12	Lynx1
NM_009746	-2.84	-2.12	Bcl7c
NM_009166	-2.82	-2.12	Sorbs1
NM_025656	-2.94	-2.12	Sip1
NM_145524	-3.70	-2.14	BC004636
NM_152813	-3.00	-2.14	Plcd3
NM_030004	-4.13	-2.16	Cryl1
NM_001003946	-3.02	-2.18	Als2cr13
NM_207269	-2.93	-2.18	D330050I23Rik
NM_144862	-4.02	-2.19	Lims2
NM_011146	-3.49	-2.20	Pparg
NM_026298	-3.23	-2.23	4930553F24Rik
NM_009968	-3.31	-2.23	Cryz
NM_026122	-4.88	-2.24	Hmgn3
NM_013496	-2.91	-2.25	Crabp1
NM_017373	-3.71	-2.25	Nfil3
NM_175074	-4.84	-2.26	Hmgn3
NM_026024	-2.88	-2.27	Ube2t
NM_001024225	-4.46	-2.28	Defcr24
NM_007852	-4.46	-2.28	Defcr6
NM_029624	-2.99	-2.29	2400010G15Rik
NM_007760	-2.92	-2.29	Crat
NM_009004	-2.83	-2.34	Kif20a
NM_009672	-5.35	-2.34	Anp32a
NM_013543	-4.16	-2.35	H2-Ke6
NM_173752	-4.26	-2.35	1110067D22Rik
NM_025658	-4.15	-2.37	Ms4a4d
NM_009822	-2.85	-2.38	Cbfa2t1h
NM_133990	-3.53	-2.40	Il13ra1
XM_133813	-5.01	-2.40	NA
NM_016762	-4.39	-2.44	Matn2
NM_016764	-4.88	-2.44	Prdx4
NM_025522	-2.94	-2.45	Dhrs7
NM_010216	-3.22	-2.47	Figf
NM_178660	-2.81	-2.48	Rbms3
NM_010744	-4.28	-2.48	Tmed1
NM_133859	-3.40	-2.48	Olfml3

NM_175205	-2.80	-2.49	4632419I22Rik
NM_183254	-3.09	-2.49	1700025K23Rik
NM_029413	-3.07	-2.50	Morc4
NM_199195	-3.54	-2.50	Bckdhb
NM_134163	-2.99	-2.51	Mbnl3
NM_146162	-3.02	-2.53	BC025600
NM_010194	-3.24	-2.54	Fes
XM_134902	-3.02	-2.54	NA
NM_173011	-3.65	-2.56	Idh2
NM_148928	-2.91	-2.57	Gtf3c5
XM_619217	-2.96	-2.58	NA
NM_146040	-2.78	-2.60	Cdca7l
NM_178884	-2.95	-2.61	AW822216
NM_173426	-2.77	-2.66	1700012H17Rik
NM_009155	-3.73	-2.67	Sepp1
NM_021342	-3.11	-2.68	Kcne4
NM_010726	-3.99	-2.71	Phyh
NM_009472	-3.16	-2.76	Unc5c
XM_194370	-2.91	-2.76	NA
NM_028915	-2.86	-2.79	Lrrcc1
NM_026303	-2.80	-2.81	4930562C03Rik
XM_620727	-2.80	-2.81	NA
NM_010826,NM_194464	-2.85	-2.90	Mrvi1
NM_146249	-3.59	-2.91	BC031441
NM_026772	-5.04	-2.92	Cdc42ep2
XM_355247	-3.06	-2.99	NA
XM_283635	-3.22	-3.00	NA
NM_013665	-3.03	-3.04	Shox2
NM_016873	-4.56	-3.05	Wisp2
NM_144794	-4.38	-3.05	Tmem63a
NM_008046	-3.58	-3.07	Fst
NM_134147	-3.45	-3.10	D930010J01Rik
NM_011129	-3.15	-3.10	4-Sep
NM_177135	-3.20	-3.21	D830030K20Rik
XM_489067	-7.18	-3.28	NA
NM_028724	-2.91	-3.31	Rin2
NM_138315	-3.80	-3.34	Mical1
NM_007630	-3.14	-3.41	Ccnb2
NM_012006	-3.23	-3.43	Cte1
NM_001012335,NM_001012336,NM_010784	-3.14	-3.52	Mdk
NM_009776	-4.62	-3.57	Serping1
NM_026125	-2.91	-3.61	1110035L05Rik
NM_010931	-2.92	-3.74	Uhrf1
NM_027954	-4.92	-3.88	Syce2
NM_026514	-3.61	-3.93	Cdc42ep3
NM_010226	-4.13	-3.98	Fkhl18
NR_001592	-3.48	-4.01	NA
NM_009141	-3.06	-4.04	Cxcl5

XM_358515	-4.71	-4.05	NA
NM_001004359,NM_001005385,NM_026081	-2.91	-4.07	Gprasp1
XM_354836	-3.09	-4.10	NA
XM_130991	-3.21	-4.14	NA
NM_026928	-5.71	-4.31	1810014F10Rik
NM_007825	-4.31	-4.42	Cyp7b1
NM_172604	-2.99	-4.48	Scara3
NM_008452	-3.10	-4.51	Klf2
NM_008987	-3.20	-4.74	Ptx3
NM_016847	-2.76	-5.17	Avpr1a
NM_198161	-3.74	-5.23	Bhlhb9
XM_181304	-3.02	-6.86	NA
NM_148948	-2.76	-8.23	Dicer1
NM_138304	-3.38	-12.70	Calml4

[2]Statistically significant differences in mRNA expression levels following Dicer knockout in MEFs were identified using Significance Analysis of Microarrays (SAM) with a False Discovery Rate cutoff of 2%, and then requiring a fold change of at least two (up or down). Refseq transcript identifiers, d-statistics (from SAM), fold change and gene symbols are listed for each significant mRNA and ordered according to fold change. In cases where multiple Refseq transcripts from the same gene were not distinguishable by the probes on the Mouse 430 2.0 array, all Refseq ids are listed. This list includes 135 mRNAs whose expression increased following Dicer knockout and 119 mRNAs whose expression decreased (including Dicer1).

Table S6. Statistics related to Fig. 3.

Figure 3B data – zebrafish miR-430 seed match types								
seed type	**no. of mRNAs**	**mean LFC**	**mean nLFC**	**fraction up-regulated**	**Wilcoxon Rank Sum Test (2 sided) P-values**			
					no seed match	**6mer**	**A1 7mer**	**M8-A1 8mer**
no seed match	2931	0.07	0.00	0.025	-			
6mer	269	0.45	0.38	0.082	$<10^{-25}$	-		
A1 7mer	71	0.65	0.58	0.155	$<10^{-14}$	0.017	-	
M8 7mer	170	0.59	0.52	0.159	$<10^{-20}$	0.070	0.364	-
M8-A1 8mer	23	1.04	0.97	0.348	$<10^{-7}$	0.001	0.066	0.020

Figure 3C data – MEF extended seed match conservation					
conservation type	**no. of mRNAs**	**mean LFC**	**mean nLFC**	**Wilcoxon Rank Sum Test (2 sided) P-values**	
				no seed match	**non-conserved**
no seed match	556	-0.24	0.00	-	
non-conserved	761	-0.08	0.16	$<10^{-6}$	-
conserved	761	-0.01	0.23	$<10^{-10}$	0.030

Table S7. Statistics related to Fig. 7.

Figure 7A data –ranked siRNA 7mer targets (test set)					
mRNA subset	**no. of mRNAs**	**mean LFC**	**mean nLFC**	**Fraction down-regulated**	**Wilcoxon Rank Sum Test (2 sided) P-values**
					Lowest ranking 20%
top 20%	638	-0.24	0.26	0.20	-
bottom 20%	638	-0.05	0.07	0.05	0.0
Figure 7B data – ranked miR-155 non-conserved 7mer targets					
mRNA subset	**no. of mRNAs**	**mean LFC**	**mean nLFC**	**Fraction up-regulated**	**Wilcoxon Rank Sum Test (2 sided) P-values**
					Lowest ranking 20%
top 20%	97	0.09	0.09	0.09	-
bottom 20%	97	0.01	0.01	0.04	$<10^{-2}$