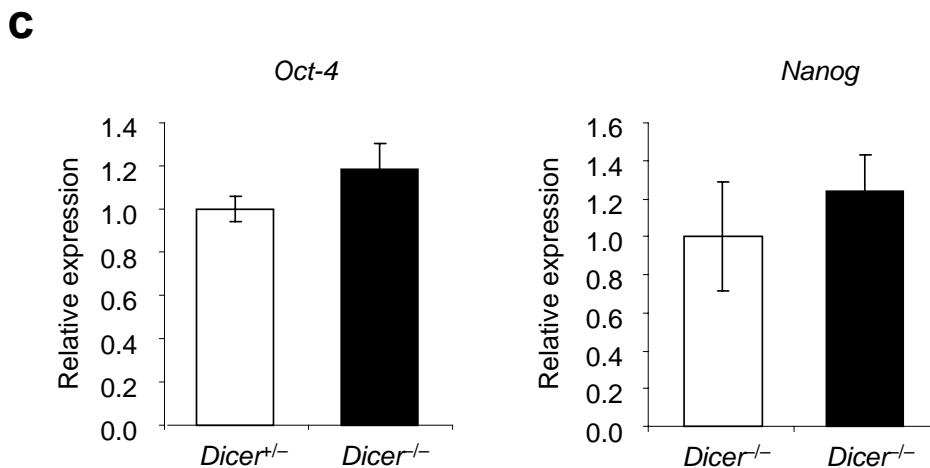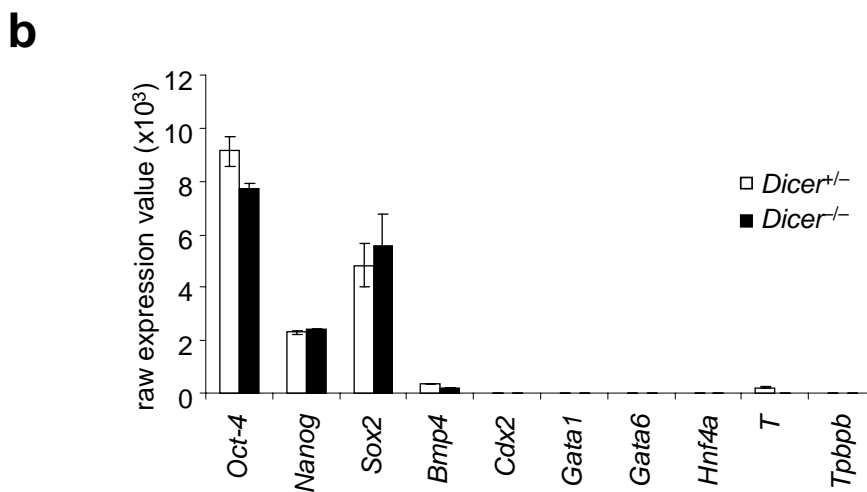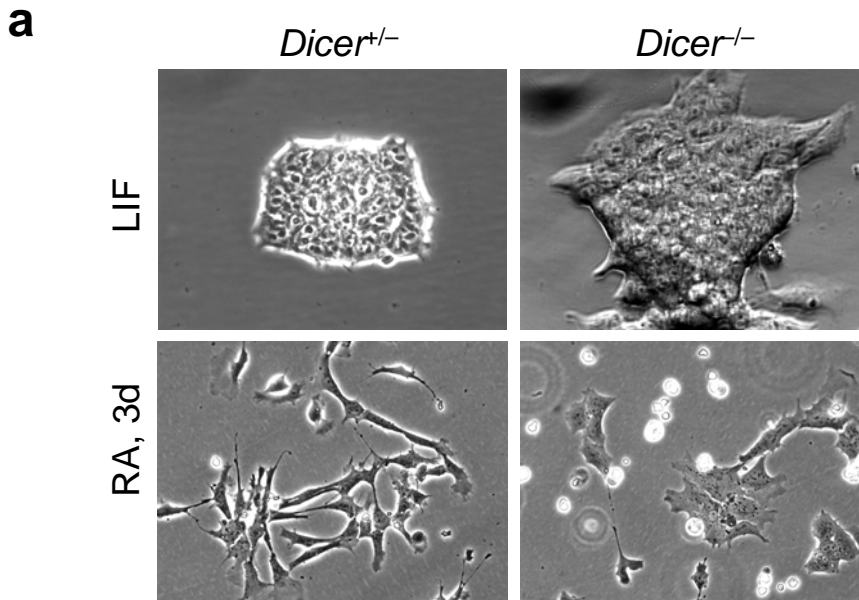# MicroRNAs control *de novo* DNA methylation through regulation of transcriptional repressors in mouse embryonic stem cells

Lasse Sinkkonen, Tabea Hugenschmidt, Philipp Berninger, Dimos Gaidatzis, Fabio Mohn, Caroline G. Artus-Revel, Mihaela Zavolan, Petr Svoboda, and Witold Filipowicz

Sinkkonen et al. Supplementary Figure 1

a

b

c

Supplementary Figure 1. Characterization of the differentiation status of *Dicer*^{+/−} and *Dicer*^{−/−} ES cells.

(a) Light microscopy images of undifferentiated cells (cells grown in the presence of LIF) and cells subjected to the differentiation treatment (grown for 3 days in the presence of 100 nM RA, in the absence of LIF; RA, 3 d). (b) Levels of mRNAs encoding core pluripotency and differentiation markers. Microarray analysis was performed with RNA isolated from undifferentiated *Dicer*^{+/−} and *Dicer*^{−/−} ES cells. Raw data were calculated as described in Materials and Methods. Median raw values (± s.d.) for each gene were taken from probe sets with the strongest hybridization signal. Other probe sets for the same genes also did not show significant differences in expression levels between *Dicer*^{+/−} and *Dicer*^{−/−} cells. Displayed differentiation markers were either used in a previous analysis of *Dicer*^{−/−} ES cells [*T* (brachyury), *Hnf4a*, *Gata1*, *Bmp4*][7] or were culled from published articles (*Tpbpb*, *Cdx2*, *Gata6*)[8,9]. These markers are indicative of the presence of cells of trophectodermal (*Tpbpb*, *Cdx2*), extraembryonic endodermal (*Gata6*), embryonic mesodermal (brachyury, *Bmp4*, *Gata1*, *Gata6*), and embryonic endodermal (*Hnf4a*, *Gata6*) lineages. It is not known why *Dicer*^{+/−} cells show a low microarray hybridization signal of brachyury. Possibly, a small fraction of cells spontaneously initiates differentiation. However, other mesodermal markers such as *Gata1* and *Gata6* remained absent. Detectable microarray hybridization signal for *Bmp4* has been previously reported for undifferentiated ES cells (GEO database, and[10]). (c) RT-qPCR analysis of *Oct-4* and *Nanog* mRNA levels confirms results of microarray analysis. Values, normalized to *Gapdh* expression, represent means (± s.e.m.) of at least 3 independent experiments.

a

1444292_at

200 bp

miR-295   miR-294   miR-293   miR-292   miR-291a   miR-290

Direction of transcription

5p

3p

```
              10        20        30        40        50        60        70        80        90
mir-290  -CUCAUCUUGCGGUACUCAAACUAUGCGGGCACUUUUUUUUUUCUUU-----AAAAGUGCCGCCUAGUUUUAAGCCCCGCCGGUUGAG--  83
mir-291  -CCUAUGUAGCGGCCAUCAAAGUGGAGCGCCUCUCUUGAGCCUGAAUG----AGAAAGUGCUUCC--ACUUUGUGUGCCACUGCAUGGG--  82
mir-292  ---CAGCCUGUCAUACUCAAACUGGGGG--CUCUCUUGGAUUUUCAUCGGAAGAAAAGUGCCGCCAGGUUUUGAGUGUCACCGGUUG----  82
mir-293  -UUCAAUCUGUGUCAAACUGUGUG--ACAUUUUGUUCUUUGU------AAGAAGUGCCGCAGAGUUUGUAGUGUUGCCGAUUGAG--  80
mir-294  UUCCAUAUAGCCAUACUCAAAGUGGAGCGCCUAAUCUAAGCUUUUAAGU--GGAAAAGUGCUUCC--CUUUUGUGUGUCAUGUGGAG-  84
mir-295  --------GGUCGGACUCAAAUGUGGGGCACACUUUCUGGACUUGGUACAU--AGAAAAGUGCUAC-UACUUUUGAGUCUCUCC--------  69
```

b

c

d

Supplementary Figure 2. Characterization of expression of the miR-290 cluster.

(a) Structure of the miR-290 locus and sequences of the miR-290 cluster miRNAs. The upper scheme depicts structure of the cluster. Shown is a 4 kb fragment from the chromosome 7 genomic contig NT_039413.7 (from 217,800 to 221,800) with relative positions of individual miRNAs and orientation of the pri-miRNA transcript. The orange line represents a region covered by oligonucleotide probes from the Affymetrix probe set 1444292_at, which detects pri-miRNA of the miR-290 cluster. Below the map is a ClustalW alignment of pre-miRNAs, which was downloaded from the miRbase[1] and edited using Genedoc alignment editor (http://www.psc.edu/biomed/genedoc). Blue lines represent mature miRNA sequences from the ascending (5p) strand and red lines from the descending (3p) strand of the pre-miRNA hairpin. Pre-miRNAs of the miR-290 cluster yield mature miRNAs primarily from descending (3p) hairpin strands. According to Landgraf et al.[6], the 5p strands also produce mature miRNAs but they represent only 13% of miRNAs generated from the cluster. Note that despite being expressed, not all mature miRNA sequences depicted here can be found in the current version of the miBase (9.2).
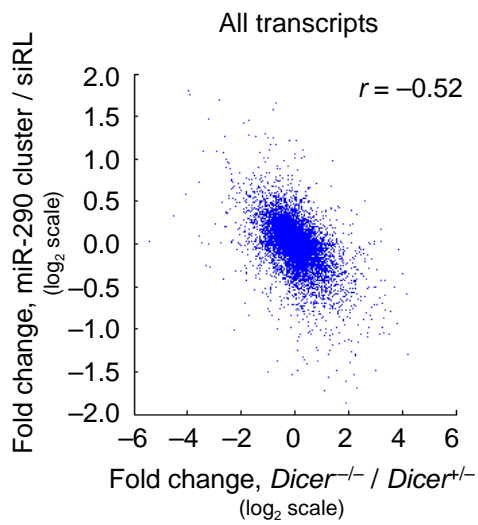
(b) Quantification of the miR-290 cluster primary transcript, followed using the microarray probe 1444292_at, indicates that the cluster is expressed zygotically. The region of the primary transcript covered by the 1444292_at probe set is indicated in panel a. The analysis was performed using previously published microarray data[11]. Raw data were calculated as described previously[12]. Median raw values ($\pm$ s.d.; n = 4) are shown.

(c) The miR-290 cluster primary transcript is strongly down-regulated in $Dicer^{-/-}$ ES cells (two-tailed t-test: p = 0.0053). Expression of the primary trasncript was followed using the microarray probe 1444292_at as described in panel a. The mean expression va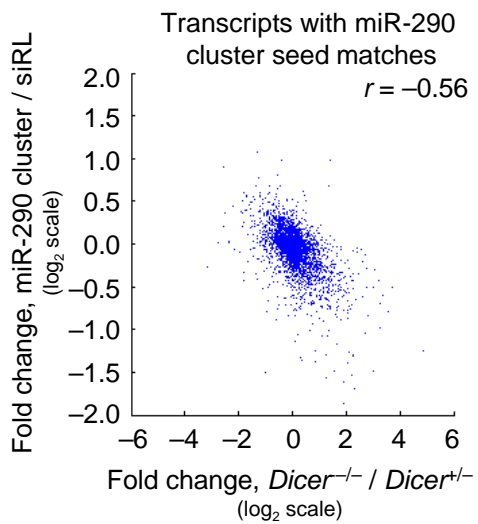lue ($\pm$ s.d.; n = 3) in $Dicer^{+/-}$ cells was set to one. (d) Levels of the mir-290 cluster miRNAs are down-regulated in $Dicer^{-/-}$ cells. Total RNA extracted from either $Dicer^{+/-}$ or $Dicer^{-/-}$ cells was analyzed on miRCURY^{TM} LNA arrays, following the manufacturer's protocol (www.exiqon.com). Values calculated for $Dicer^{+/-}$ cells were set as one. The figure shows expression levels of only 3p miRNAs of miR-290 cluster, which were reliably identified as present in the analyzed RNA samples. The 3p miRNAs represent 87% of all miRNAs generated from the cluster[6]. miR-290-3p is not shown since the Exiqon array does not contain a probe for its detection.

a

All transcripts

$r = -0.52$

Fold change, miR-290 cluster / siRL (log$_2$ scale)

Fold change, *Dicer$^{-/-}$* / *Dicer$^{+/-}$* (log$_2$ scale)

b

Transcripts with miR-290 cluster seed matches

$r = -0.56$

Fold change, miR-290 cluster / siRL (log$_2$ scale)

Fold change, *Dicer$^{-/-}$* / *Dicer$^{+/-}$* (log$_2$ scale)

c

Transcripts without miR-290 cluster seed matches

$r = -0.50$

Fold change, miR-290 cluster / siRL (log$_2$ scale)

Fold change, *Dicer$^{-/-}$* / *Dicer$^{+/-}$* (log$_2$ scale)
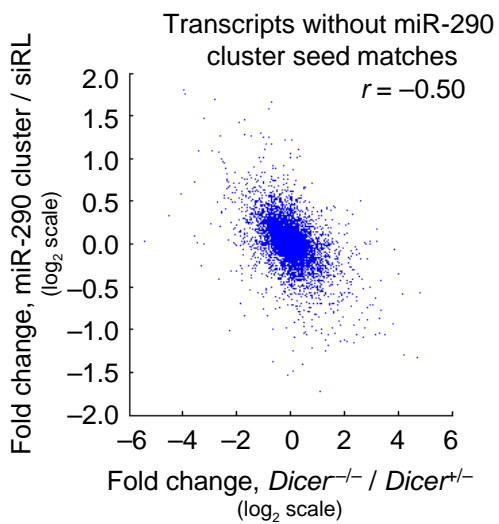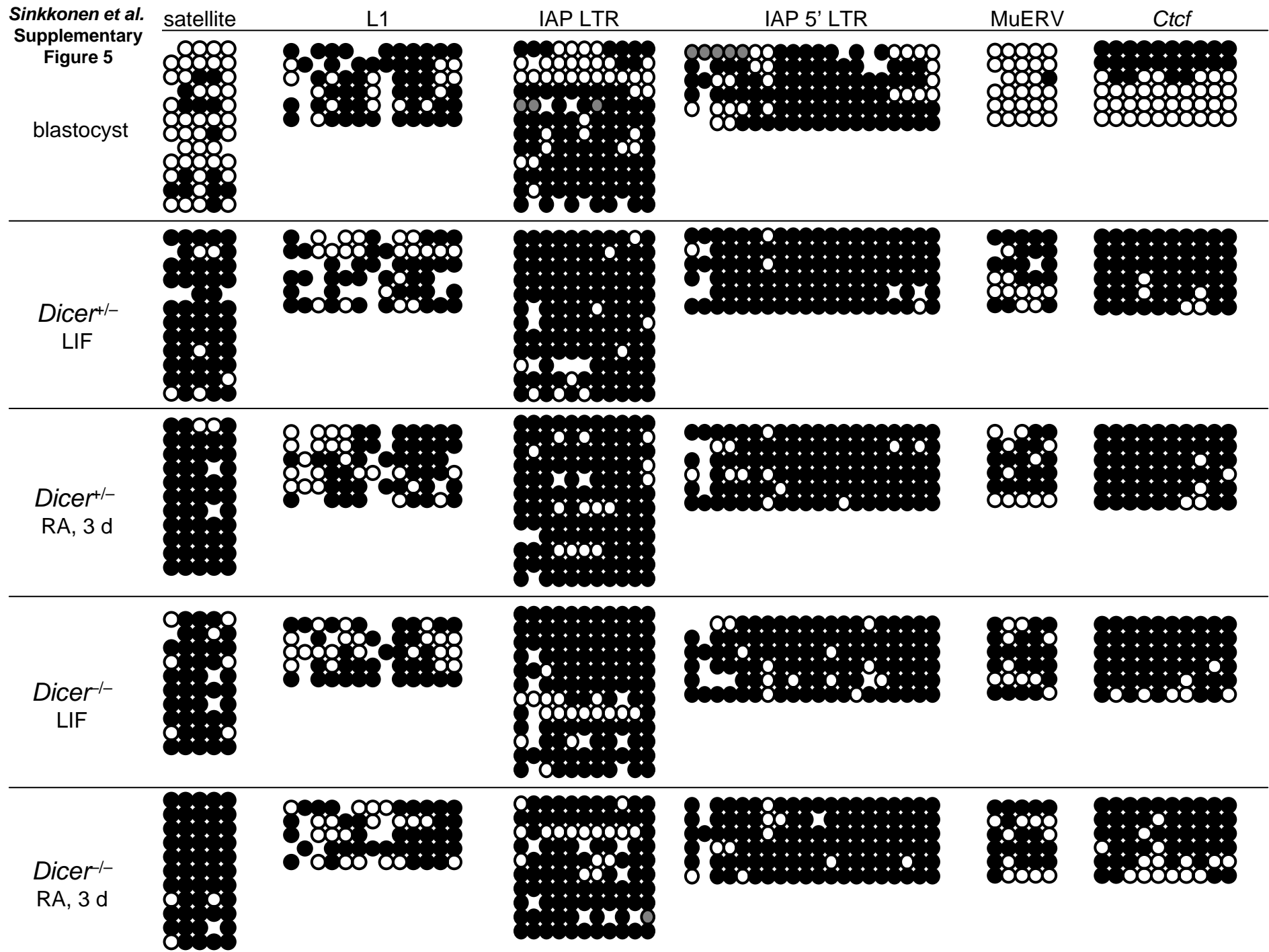
Supplementary Figure 3. Relationship between the expression changes in the *Dicer*$^{-/-}$ vs. *Dicer*$^{+/-}$ (x-axis) and miR-290s-transfected vs siRL-transfected *Dicer*$^{-/-}$ ES cells (y-axis). Each dot corresponds to a single transcript, and the panels represent: (a) all transcripts; (b) transcripts with at least one 7-mer match to one of the 1-8 positions of the miRNAs in the 290 cluster in their 3′-UTRs; and (c) transcripts with no 7-mer match to any of the miRNAs of the 290 cluster in their 3′-UTRs. The correlation coefficients are indicated in each panel.

**Sinkkonen et al. Supplementary Figure 4**
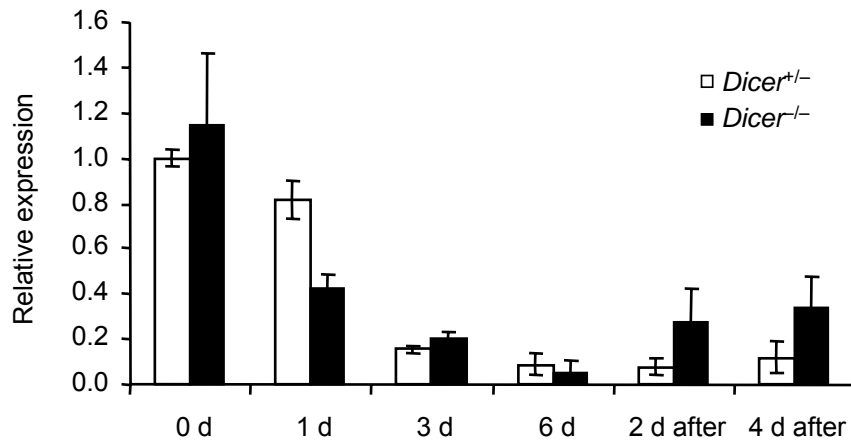
Supplementary Figure 4. RT-qPCR analysis of *Nanog* expression during the RA-induced differentiation for 0, 1, 3, or 6 days (0 d, 1 d, 3 d, 6 d), and after returning the cells to the LIF-containing medium devoid of RA for up to 4 additional days (2 d after, 4 d after). Values, normalized to GAPDH expression, represent means (± s.e.m.) of at least 3 independent experiments. Expression in control *Dicer*$^{+/-}$ cells at 0d time point was set as 1.

Sinkkonen et al. Supplementary Figure 5

	satellite	L1	IAP LTR	IAP 5' LTR	MuERV	*Ctcf*
blastocyst						
Dicer$^{+/-}$ LIF						
Dicer$^{+/-}$ RA, 3 d						
Dicer$^{-/-}$ LIF						
Dicer$^{-/-}$ RA, 3 d						

Supplementary Figure 5. Maintenance DNA methylation is not affected in *Dicer*^{−/−} ES cells. Bisulfite analysis shows the same methylation pattern in undifferentiated and differentiated *Dicer*^{−/−} and *Dicer*^{+/−} ES cells for typical targets of DNA methylation: repetitive DNA and a body of a gene[13]. DNA methylation of tandemly arrayed repeat sequences (centromeric satellite), interspersed LTR retrotransposon sequences (IAP and MuERV-L), interspersed non-LTR transposon sequences (L1), and a single copy gene sequence (CTCF, exon 9) were analyzed. In the case of IAP, also its 5′ LTR sequence, which serves as a promoter for the retrotransposon, was analyzed. The exon 9 sequence of the CTCF gene is hypermethylated in numerous tissues[14]. The ES cell DNA samples used to obtain the data were the same as those described in Fig. 6. Blastocyst DNA samples served as a control providing the DNA methylation status prior to establishment of ES cells. The blastocyst DNA was obtained from a pool of 30 blastocysts from uteri of C57BL/6 female mice. An equivalent of three blastocysts was used for each PCR reaction. Black dots represent methylated and white dots non-methylated CpGs. Sites for which the methylation status was not certain are in grey. Average percentages of the methylated CpG sites for ES cell samples range from 89 to 97 % for satellite repeat, 62 to 70 % for L1, 84 to 92 % for IAP LTR, 90 to 97 % for IAP 5'LTR, 62 to 67 % for MuERV, and 75 to 92 % for *Ctcf*.
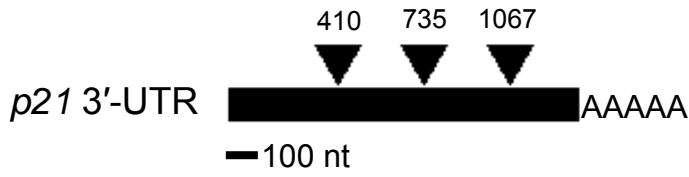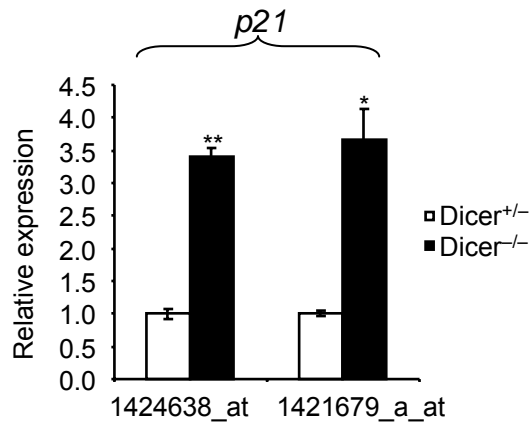
Sinkkonen et al. Supplementary Figure 6
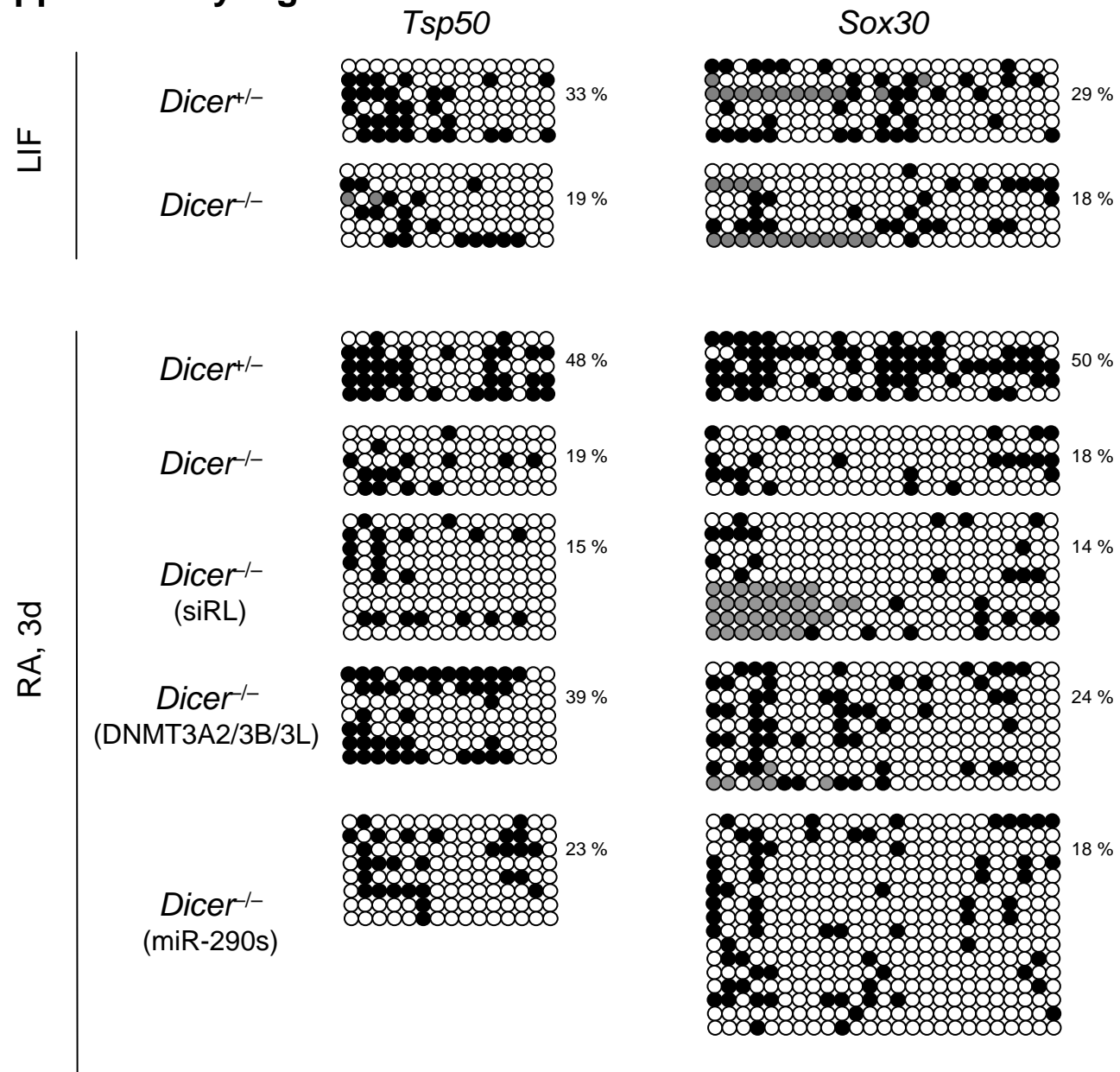
Supplementary Figure 6. Analysis of *de novo* DNA methylation of promoters of testis-specific genes *Tsp50* and *Sox30*, which are transcriptionally silent in undifferentiated ES cells and acquire DNA methylation during differentiation in the presence of RA. The promoter regions, upstream of the *Tsp50* and *Sox30* transcription start sites, were analyzed in *Dicer*$^{+/-}$ ES cells, *Dicer*$^{-/-}$ ES cells, *Dicer*$^{-/-}$ ES cells co-transfected with plasmids expressing EGFP-DNMT3a2, EGFP-DNMT3b, and EGFP-DNMT3L, *Dicer*$^{-/-}$ ES cells transfected with miR-290 cluster miRNA mimics, and *Dicer*$^{-/-}$ ES cells transfected with siRL. Cells were cultured in presence of LIF and without RA (LIF) or differentiated for 3 days with RA in the absence of LIF (RA, 3 d). Each row of dots represents CpGs in one sequenced clone. Black dots represent methylated and white dots non-methylated CpGs. Sites for which the methylation status was not certain are in grey. Average percentages of the methylated CpG sites are indicated.

a

p21 3′-UTR

410 735 1067

AAAAA

100 nt

b

p21

Relative expression

4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0

□ Dicer⁺ᐟ⁻
■ Dicer⁻ᐟ⁻

1424638_at   1421679_a_at

c

p21

Relative expression

1.2
1.0
0.8
0.6
0.4
0.2
0.0

□ siRL (24 h)
■ miR-290s (24 h)

1424638_at   1421679_a_at

d

□ LIF
▨ LIF + 25 nM rapamycin
▨ RA, 3 d
■ RA, 3 d + 25 nM rapamycin

Relative expression

1,8
1,6
1,4
1,2
1,0
0,8
0,6
0,4
0,2
0

Dnmt3a2

Relative expression

1,2
1,0
0,8
0,6
0,4
0,2
0

Dnmt3b1/6

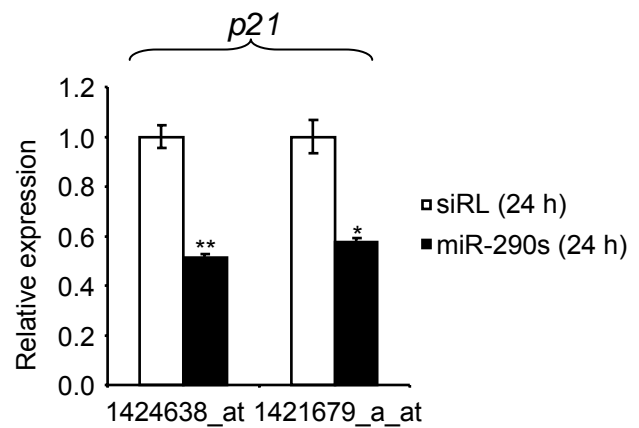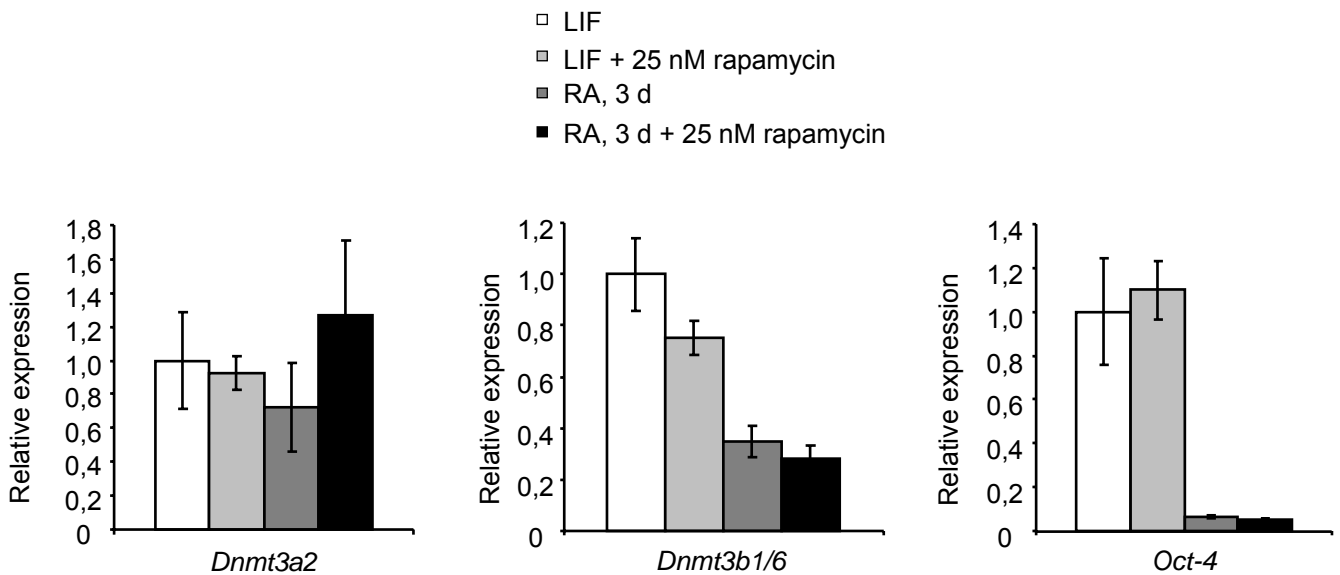Relative expression

1,4
1,2
1,0
0,8
0,6
0,4
0,2
0

Oct-4

Supplementary Figure 7. (a,b,c) *p21* is up-regulated in *Dicer*$^{-/-}$ ES cells and down-regulated in response to transfection of miR-290s. (a) Schematic representation of localization of predicted binding sites for AAGUGC-seed containing miRNAs in 3′-UTR of *p21* mRNA are marked with black triangles. (b) The level of *p21* mRNA is up-regulated in *Dicer*$^{-/-}$ cells. RNA isolated from *Dicer*$^{+/-}$ and *Dicer*$^{-/-}$ cells was analyzed by Affymetrix microarrays. The probe sets detecting expression of *p21* mRNA are indicated. Mean expression value (± s.d.; n = 3) in *Dicer*$^{+/-}$ cells was set to 1. The two-tailed t-test p-values were 0.0021 and 0.0120. (c) Transfection of miR-290s to *Dicer*$^{-/-}$ ES cells down-regulates the level of *p21* mRNA. Cells were transfected with either a mixture of the miR-290 cluster miRNAs or siRL used as a control. RNA was isolated 24 h after transfection. Mean expression value (± s.d; n = 3) in siRL transfected cells was set to 1. The two-tailed t-test p-values were 0.0012 and 0.0070. (d) Expression of *Dnmt3a2* and *Dnmt3b* is independent of the growth rate of the *Dicer*$^{+/-}$ ES cells. *Dicer*$^{+/-}$ ES cells were cultured with or without 25 nM rapamycin and differentiated for three days with RA in the absence of LIF (RA, 3 d). mRNA levels of *Dnmt3a2*, *Dnmt3b* and *Oct-4* were analyzed by RT-qPCR. Expression (± s.d.; n = 3) was normalized to that of *Gapdh* and is shown relative to corresponding samples cultured in the presence of LIF without rapamycin, whose expression values were set to 1.

Sinkkonen et al. **SUPPLEMENTARY TABLES**

Supplementary Table 1. Motifs most significantly enriched in 3′-UTRs of mRNAs up-regulated in *Dicer*$^{-/-}$ ES cells or down-regulated in *Dicer*$^{-/-}$ ES cells transfected with miRNAs of the miR-290 cluster.

Enrichments of the 7-mers were analyzed as described in Materials and Methods. Shown are the motifs most significantly (posterior probability > 0.99) enriched in 3′-UTRs of mRNAs up-regulated in *Dicer*$^{-/-}$ ES cells or down-regulated in *Dicer*$^{-/-}$ ES cells transfected with miRNAs of the miR-290 family. For each motif, the sequence of the motif, posterior probability of the enrichment, occurrence of the motif among up- or down-regulated 3′-UTRs, and the enrichment of the motif are shown. For motifs that are complementary to sequences within miRNAs, the names and sequences of the matching miRNAs are also shown. Sequences complementary to the enriched motif are in capitals. A number of top scoring 7-mer motifs enriched in 3′-UTRs of mRNAs down-regulated upon transfection of miR-290 cluster miRNAs contain seven or six U residues. The significance of these motifs and their enrichment is unknown.

Supplementary Table 1 - Significantly enriched motifs (posterior probability > 0.99)

Motif	Posterior probability	Occurrence	Enrichment	Position	miRNA	Sequence of miRNA
Motifs enriched in transcripts upregulated in Dicer -/- cells						
GCACUUU	0.9999999999	489	1.615			
				(2-8)	mmu-miR-17-5p	cAAAGUGCuuacagugcagguagu
				(1-7)	mmu-miR-291a-3p	AAAGUGCuuccacuuugugugcc
				(2-8)	mmu-miR-93	cAAAGUGCuguucgugcagguag
				(2-8)	mmu-miR-20b	cAAAGUGCucauagugcaggua
				(1-7)	mmu-miR-291b-3p	AAAGUGCauccauuuuguuuguc
				(2-8)	mmu-miR-106b	uAAAGUGCugacagugcagau
				(2-8)	mmu-miR-106a	cAAAGUGCuaacagugcaggua
				(2-8)	mmu-miR-20a	uAAAGUGCuuauagugcagguag
				(1-7)	mmu-miR-294-3p	AAAGUGCuucccuuuugugugu
				(1-7)	mmu-miR-295-3p	AAAGUGCuacuacuuuugagucu
AGCACUU	0.9999999994	545	1.495			
				(3-9)	mmu-miR-17-5p	caAAGUGCUuacagugcagguagu
				(1-7)	mmu-miR-302b	AAGUGCUuccauguuuuaguag
				(2-8)	mmu-miR-291a-3p	aAAGUGCUuccacuuugugugcc
				(3-9)	mmu-miR-93	caAAGUGCUguucgugcagguag
				(3-9)	mmu-miR-20b	caAAGUGCUcauagugcaggua
				(1-7)	mmu-miR-302d	AAGUGCUuccauguuugagugu
				(2-8)	mmu-miR-302c	cAAGUGCUuccauguuucagugg
				(3-9)	mmu-miR-106b	uaAAGUGCUgacagugcagau
				(3-9)	mmu-miR-106a	caAAGUGCUaacagugcaggua
				(3-9)	mmu-miR-20a	uaAAGUGCUuauagugcagguag
				(2-8)	mmu-miR-294-3p	aAAGUGCUucccuuuugugugu
				(2-8)	mmu-miR-295-3p	aAAGUGCUacuacuuuugagucu
				(2-8)	mmu-miR-302	uAAGUGCUuccauguuuuggga
GCACUUA	0.9999281990	255	1.614			
				(2-8)	mmu-miR-467*	gUAAGUGCcugcauguauaug
				(1-7)	mmu-miR-302	UAAGUGCuuccauguuuuggga
UGCACUU	0.9988487421	415	1.402			
				(2-8)	mmu-miR-291b-3p	aAAGUGCAuccauuuuguuuguc
AAGCACU	0.9967070829	406	1.392			

				Position	miRNA	Sequence of miRNA
				(4-10)	mmu-miR-17-5p	caaAGUGCUUacagugcagguagu
				(2-8)	mmu-miR-302b	aAGUGCUUccauguuuuaguag
				(3-9)	mmu-miR-291a-3p	aaAGUGCUUccacuuugugugcc
				(11-17)	mmu-miR-471	uacguaguauAGUGCUUuucaca
				(2-8)	mmu-miR-302d	aAGUGCUUccauguuugagugu
				(3-9)	mmu-miR-302c	caAGUGCUUccauguuucagugg
				(4-10)	mmu-miR-20a	uaaAGUGCUUauagugcagguag
				(3-9)	mmu-miR-294-3p	aaAGUGCUUcccuuuugugugu
				(3-9)	mmu-miR-302	uaAGUGCUUccauguuuugguga

Motifs enriched in transcripts downregulated in Dicer -/- cells transfected with miR-290 cluster

Motif	Posterior probability	Occurrence	Enrichment	Position	miRNA	Sequence of miRNA
UUUUUUU	0.9999999542	2299	1.175			
GCACUUA	0.9999976429	126	2.047			
				(2-8)	mmu-miR-467*	gUAAGUGCcugcauguauaug
				(1-7)	mmu-miR-302	UAAGUGCuuccauguuuugguga
UUUGUUU	0.9999952321	931	1.265			
				(1-7)	mmu-miR-495	AAACAAAcauggugcacuucuu
UUUUGUU	0.999912871	776	1.272			
AGCACUU	0.999696131	224	1.576			
				(3-9)	mmu-miR-17-5p	caAAGUGCUuacagugcagguagu
				(1-7)	mmu-miR-302b	AAGUGCUuccauguuuuaguag
				(2-8)	mmu-miR-291a-3p	aAAGUGCUuccacuuugugugcc
				(3-9)	mmu-miR-93	caAAGUGCUguucgugcagguag
				(3-9)	mmu-miR-20b	caAAGUGCUcauagugcaggua
				(1-7)	mmu-miR-302d	AAGUGCUuccauguuugagugu
				(2-8)	mmu-miR-302c	cAAGUGCUuccauguuucagugg
				(3-9)	mmu-miR-106b	uaAAGUGCUgacagugcagau
				(3-9)	mmu-miR-106a	caAAGUGCUaacagugcaggua
				(3-9)	mmu-miR-20a	uaAAGUGCUuauagugcagguag
				(2-8)	mmu-miR-294-3p	aAAGUGCUucccuuuugugugu
				(2-8)	mmu-miR-295-3p	aAAGUGCUacuacuuuugagucu
				(2-8)	mmu-miR-302	uAAGUGCUuccauguuuugguga
GCACUUU	0.9996301511	193	1.635			
				(2-8)	mmu-miR-17-5p	cAAAGUGCuuacagugcagguagu

				(1-7)	mmu-miR-291a-3p	AAAGUGCuuccacuuugugugcc
				(2-8)	mmu-miR-93	cAAAGUGCuguucgugcagguag
				(2-8)	mmu-miR-20b	cAAAGUGCucauagugcaggua
				(1-7)	mmu-miR-291b-3p	AAAGUGCauccauuuuguuuguc
				(2-8)	mmu-miR-106b	uAAAGUGCugacagugcagau
				(2-8)	mmu-miR-106a	cAAAGUGCuaacagugcaggua
				(2-8)	mmu-miR-20a	uAAAGUGCuuauagugcagguag
				(1-7)	mmu-miR-294-3p	AAAGUGCuucccuuuugugugu
				(1-7)	mmu-miR-295-3p	AAAGUGCuacuacuuuugagucu
UUGUUUU	0.9989237718	802	1.245			
UAUUUUU	0.9983591123	691	1.264			

Supplementary Table 2. Profiling of miRNA levels in *Dicer*$^{+/-}$ and *Dicer*$^{-/-}$ ES cells using miRCURY^{TM} Exqon microarrays.

Microarray analysis was performed as described in Supplementary Materials and Methods. 190 miRNAs gave significant hybridization signals with both analyzed *Dicer*$^{+/-}$ RNA samples and thus were considered for further analysis. The miRNA expression levels in *Dicer*$^{-/-}$ ES cells were compared to the expression levels in *Dicer*$^{+/-}$ ES cells. The numbers of miRNAs down- or up-regulated, or not changing, with cut-offs of 1.2-fold, 1.5-fold and 2.0-fold are shown in the table. The miRNAs that were down-regulated more than 1.5-fold or 2.0-fold were considered as expressed in ES cells in a Dicer-dependent manner. These miRNAs are listed and their expression levels normalized to the expression levels in a control reference sample (a mixture of total RNA from 11 different tissues) are indicated to identify miRNAs expressed preferentially in ES cells.

Supplementary Table 2 - miRNA expression profiling in Dicer +/- and Dicer -/- ES cells

Changes in miRNA levels in Dicer -/- cells	Number of miRNAs	% of all detected miRNAs (n=190)
CUT-OFF 1.2-FOLD		
downregulated 1.2-fold	115	60,3
upregulated 1.2-fold	23	12,2
not changed above 1.2-fold	52	27,5
CUT-OFF 1.5-FOLD		
downregulated 1.5-fold	69	36,0
upregulated 1.5-fold	8	4,2
not changed above 1.5-fold	113	59,8
CUT-OFF 2.0-FOLD		
downregulated 2.0-fold	29	14,8
upregulated 2.0-fold	3	1,6
not changed above 2.0-fold	158	83,6

miRNAs DOWNREGULATED >2.0-fold		
Expressed > 1.5-fold higher relative to control reference mixture	Expressed between 1.5- to 0.5-fold relative to control reference mixture	Expressed < 0.5-fold relative to control reference mixture
mmu-let-7d*	mmu-miR-136	mmu-miR-101a
mmu-miR-291a-3p	mmu-miR-18	mmu-miR-103
mmu-miR-292-3p	mmu-miR-337	mmu-miR-106a
mmu-miR-293 (miR-293-3p)	mmu-miR-376a	mmu-miR-130a
mmu-mir294 (miR-294-3p)	mmu-miR-467*	mmu-miR-140*
mmu-miR-295 (miR-295-3p)	mmu-miR-541	mmu-miR-146b
		mmu-miR-16
		mmu-miR-17-5p
		mmu-miR-191
		mmu-miR-193
		mmu-miR-200a
		mmu-miR-20b
		mmu-miR-21
		mmu-miR-22
		mmu-miR-23a

| | | mmu-miR-335 |
| | | mmu-miR-98 |

Expressed > 1.5-fold higher relative to control reference mixture	**Expressed between 1.5- to 0.5-fold relative to control reference mixture**	**Expressed < 0.5-fold relative to control reference mixture**
mmu-let-7d*	mmu-miR-136	mmu-let-7i
mmu-miR-291a-3p	mmu-miR-18	mmu-miR-101a
mmu-miR-292-3p	mmu-miR-337	mmu-miR-101b
mmu-miR-293 (miR-293-3p)	mmu-miR-341	mmu-miR-103
mmu-mir294 (miR-294-3p)	mmu-miR-376a	mmu-miR-106a
mmu-miR-295 (miR-295-3p)	mmu-miR-379	mmu-miR-107
mmu-miR-697	mmu-miR-467*	mmu-miR-10b
	mmu-miR-541	mmu-miR-122a
		mmu-miR-127
		mmu-miR-128a
		mmu-miR-130a
		mmu-miR-133a*
		mmu-miR-140
		mmu-miR-140*
		mmu-miR-142-5p
		mmu-miR-143
		mmu-miR-146
		mmu-miR-146b
		mmu-miR-148a
		mmu-miR-148b
		mmu-miR-15a
		mmu-miR-15b
		mmu-miR-16
		mmu-miR-17-5p
		mmu-miR-191
		mmu-miR-193
		mmu-miR-195
		mmu-miR-19b
		mmu-miR-200a

		mmu-miR-200b
		mmu-miR-20b
		mmu-miR-21
		mmu-miR-22
		mmu-miR-222
		mmu-miR-223
		mmu-miR-23a
		mmu-miR-23b
		mmu-miR-24
		mmu-miR-26a
		mmu-miR-27a
		mmu-miR-27b
		mmu-miR-29a
		mmu-miR-29c
		mmu-miR-30a-5p
		mmu-miR-30b
		mmu-miR-30c
		mmu-miR-30e
		mmu-miR-335
		mmu-miR-338
		mmu-miR-376b
		mmu-miR-449
		mmu-miR-451
		mmu-miR-9*
		mmu-miR-98

Supplementary Table 3 - Predicted primary targets of the miR-290 cluster miRNAs

RefSeq	Gene symbol	Description	Number of GCACUU motifs
NM_025326	0610011I04Rik	Mus musculus RIKEN cDNA 0610011I04 gene	1
XM_001003634	1110060D06Rik	PREDICTED: Mus musculus RIKEN cDNA 1110060D06 gene, transcript variant 2	1
XM_978179	1810013L24Rik	PREDICTED: Mus musculus RIKEN cDNA 1810013L24 gene, transcript variant 1	2
NM_026437	1810055E12Rik	Mus musculus RIKEN cDNA 1810055E12 gene	2
NM_175381	2700081O15Rik	Mus musculus RIKEN cDNA 2700081O15 gene	4
NM_172877	4732496O08Rik	Mus musculus RIKEN cDNA 4732496O08 gene	1
NM_029037	4930444A02Rik	Mus musculus RIKEN cDNA 4930444A02 gene	2
NM_175172	4930506M07Rik	Mus musculus RIKEN cDNA 4930506M07 gene	1
NM_173764	4932414K18Rik	Mus musculus RIKEN cDNA 4932414K18 gene	2
NM_175263	5730593N15Rik	Mus musculus RIKEN cDNA 5730593N15 gene	1
NM_146091	5730596K20Rik	Mus musculus RIKEN cDNA 5730596K20 gene	1
NM_025697	6330409N04Rik	Mus musculus RIKEN cDNA 6330409N04 gene	1
XM_991839	9030420J04Rik	PREDICTED: Mus musculus RIKEN cDNA 9030420J04 gene	1
NM_153117	9530068E07Rik	Mus musculus RIKEN cDNA 9530068E07 gene	4
NM_001007577	A630018P17Rik	Mus musculus RIKEN cDNA A630018P17 gene	1
NM_177118	A830073O21Rik	Mus musculus RIKEN cDNA A830073O21 gene	2
NM_015729	Acox1	Mus musculus acyl-Coenzyme A oxidase 1, palmitoyl	1
NM_007394	Acvr1	Mus musculus activin A receptor, type 1	1
NM_007404	Adam9	Mus musculus a disintegrin and metallopeptidase domain 9 (meltrin gamma)	2
NM_007408	Adfp	Mus musculus adipose differentiation related protein	1
NM_001005605	Aebp2	Mus musculus AE binding protein 2	2
NM_001033476	AI450948	Mus musculus expressed sequence AI450948	1
NM_177907	AI593442	Mus musculus expressed sequence AI593442	1
NM_145489	AI661453	Mus musculus expressed sequence AI661453	1
NM_011785	Akt3	Mus musculus thymoma viral proto-oncogene 3	1
NM_028270	Aldh1b1	Mus musculus aldehyde dehydrogenase 1 family, member B1	1
NM_019998	Alg2	Mus musculus asparagine-linked glycosylation 2 homolog (yeast, alpha-1,3-mannosyltransfe	1
NM_009667	Ampd3	Mus musculus AMP deaminase 3	1
NM_134071	Ankrd32	Mus musculus ankyrin repeat domain 32	1
XM_001000870	Ap1g1	PREDICTED: Mus musculus adaptor protein complex AP-1, gamma 1 subunit	1
NM_007457	Ap1s1	Mus musculus adaptor protein complex AP-1, sigma 1	1
NM_009686	Apbb2	Mus musculus amyloid beta (A4) precursor protein-binding, family B, member 2	1

NM_027144	Arhgef12	Mus musculus Rho guanine nucleotide exchange factor (GEF) 12	2
NM_001039515	Arl4a	Mus musculus ADP-ribosylation factor-like 4A	1
NM_007488	Arnt2	Mus musculus aryl hydrocarbon receptor nuclear translocator 2	1
NM_030711	Arts1	Mus musculus type 1 tumor necrosis factor receptor shedding aminopeptidase regulator	1
NM_025541	Asf1a	Mus musculus ASF1 anti-silencing function 1 homolog A (S. cerevisiae)	2
NM_138679	Ash1l	Mus musculus ash1 (absent, small, or homeotic)-like (Drosophila)	3
NM_007496	Atbf1	Mus musculus AT motif binding factor 1	2
NM_177632	BC022623	Mus musculus cDNA sequence BC022623	1
NM_153407	BC035295	Mus musculus cDNA sequence BC035295	1
XM_984947	BC053401	PREDICTED: Mus musculus cDNA sequence BC053401, transcript variant 2	1
NM_012060	Bcap31	Mus musculus B-cell receptor-associated protein 31	1
NM_080708	Bmp2k	Mus musculus BMP2 inducible kinase	1
NM_027430	Brp44	Mus musculus brain protein 44	1
NM_009790	Calm1	Mus musculus calmodulin 1	1
NM_177343	Camk1d	Mus musculus calcium/calmodulin-dependent protein kinase ID	4
XM_001000085	Camta1	PREDICTED: Mus musculus calmodulin binding transcription activator 1	1
XM_985577	Cand1	PREDICTED: Mus musculus cullin associated and neddylation disassociated 1	2
NM_007610	Casp2	Mus musculus caspase 2	4
NM_009817	Cast	Mus musculus calpastatin	1
NM_028763	Cbx6	Mus musculus chromobox homolog 6	1
NM_198164	Cdc2l6	Mus musculus cell division cycle 2-like 6 (CDK8-like)	2
NM_007669	Cdkn1a	Mus musculus cyclin-dependent kinase inhibitor 1A (P21)	3
NM_028760	Cep55	Mus musculus centrosomal protein 55	2
NM_019950	Chst5	Mus musculus carbohydrate (N-acetylglucosamine 6-O) sulfotransferase 5	1
NM_013885	Clic4	Mus musculus chloride intracellular channel 4 (mitochondrial)	2
XM_921620	Cnot6	PREDICTED: Mus musculus CCR4-NOT transcription complex, subunit 6, transcript variant	4
NM_178854	Cnot6l	Mus musculus CCR4-NOT transcription complex, subunit 6-like	2
NM_013495	Cpt1a	Mus musculus carnitine palmitoyltransferase 1a, liver	1
NM_009963	Cry2	Mus musculus cryptochrome 2 (photolyase-like)	2
NM_026444	Cs	Mus musculus citrate synthase	1
NM_173185	Csnk1g1	Mus musculus casein kinase 1, gamma 1	4
NM_177662	Ctso	Mus musculus cathepsin O	2
NM_177640	D030056L22Rik	Mus musculus RIKEN cDNA D030056L22 gene	2
XM_984040	D630040G17Rik	PREDICTED: Mus musculus RIKEN cDNA D630040G17 gene	3
NM_011873	Dazap2	Mus musculus DAZ associated protein 2	3
NM_026302	Dctn4	Mus musculus dynactin 4	4

XM_001005781	Ddef2	PREDICTED: Mus musculus development and differentiation enhancing factor 2	1
NM_001039106	Ddhd1	Mus musculus DDHD domain containing 1	3
NM_007916	Ddx19a	Mus musculus DEAD (Asp-Glu-Ala-Asp) box polypeptide 19a	1
XM_898691	Dip2a	PREDICTED: Mus musculus DIP2 disco-interacting protein 2 homolog A (Drosophila), transc	1
XM_619261	Dock5	PREDICTED: Mus musculus dedicator of cytokinesis 5, transcript variant 1	1
NM_053090	Drctnnb1a	Mus musculus down-regulated by Ctnnb1, a	1
NM_001013371	Dtx3l	Mus musculus deltex 3-like (Drosophila)	3
NM_001013380	Dync1li2	Mus musculus dynein, cytoplasmic 1 light intermediate chain 2	2
NM_173386	E330016A19Rik	Mus musculus RIKEN cDNA E330016A19 gene	3
NM_001001932	Eea1	Mus musculus early endosome antigen 1	1
NM_007915	Ei24	Mus musculus etoposide induced 2.4 mRNA	1
NM_207685	Elavl2	Mus musculus ELAV (embryonic lethal, abnormal vision, Drosophila)-like 2 (Hu antigen B)	2
NM_007936	Epha4	Mus musculus Eph receptor A4	2
NM_007961	Etv6	Mus musculus ets variant gene 6 (TEL oncogene)	2
NM_153118	Fnbp1l	Mus musculus formin binding protein 1-like	1
NM_173182	Fndc3b	Mus musculus fibronectin type III domain containing 3B	1
NM_028194	Fryl	Mus musculus furry homolog-like (Drosophila)	1
XM_980423	Furin	PREDICTED: Mus musculus furin (paired basic amino acid cleaving enzyme), transcript vari	1
NM_008056	Fzd6	Mus musculus frizzled homolog 6 (Drosophila)	1
NM_019749	Gabarap	Mus musculus gamma-aminobutyric acid receptor associated protein	1
NM_013814	Galnt1	Mus musculus UDP-N-acetyl-alpha-D-galactosamine:polypeptide N-acetylgalactosaminyltrar	1
NM_013529	Gfpt2	Mus musculus glutamine fructose-6-phosphate transaminase 2	1
NM_133236	Glcci1	Mus musculus glucocorticoid induced transcript 1	1
NM_025374	Glo1	Mus musculus glyoxalase 1	1
NM_021610	Gpa33	Mus musculus glycoprotein A33 (transmembrane)	1
NM_173747	Gpkow	Mus musculus G patch domain and KOW motifs	3
NM_019986	Habp4	Mus musculus hyaluronic acid binding protein 4	2
NM_026812	Hddc3	Mus musculus HD domain containing 3	1
NM_010437	Hivep2	Mus musculus human immunodeficiency virus type I enhancer binding protein 2	2
NM_008253	Hmgb3	Mus musculus high mobility group box 3	1
NM_008258	Hn1	Mus musculus hematological and neurological expressed sequence 1	2
NM_010470	Hp1bp3	Mus musculus heterochromatin protein 1, binding protein 3	2
XM_985333	Hs6st1	PREDICTED: Mus musculus heparan sulfate 6-O-sulfotransferase 1	2
NM_175185	Hsdl1	Mus musculus hydroxysteroid dehydrogenase like 1	2
NM_008331	Ifit1	Mus musculus interferon-induced protein with tetratricopeptide repeats 1	1
NM_019440	Iigp2	Mus musculus interferon inducible GTPase 2	1

NM_008371	Il7	Mus musculus interleukin 7	2
NM_172161	Irak2	Mus musculus interleukin-1 receptor-associated kinase 2	1
NM_008390	Irf1	Mus musculus interferon regulatory factor 1	1
XM_001002526	Irf2bp2	PREDICTED: Mus musculus interferon regulatory factor 2 binding protein 2	3
NM_008394	Isgf3g	Mus musculus interferon dependent positive acting transcription factor 3 gamma	2
NM_008402	Itgav	Mus musculus integrin alpha V	1
NM_010580	Itgb5	Mus musculus integrin beta 5	1
NM_008410	Itm2b	Mus musculus integral membrane protein 2B	1
NM_021310	Jmy	Mus musculus junction-mediating and regulatory protein	1
XM_978811	Kctd1	PREDICTED: Mus musculus potassium channel tetramerisation domain containing 1, transc	1
NM_207682	Kif1b	Mus musculus kinesin family member 1B	2
XM_994052	Klf3	PREDICTED: Mus musculus Kruppel-like factor 3 (basic)	2
NM_021284	Kras	Mus musculus v-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog	1
NM_145743	Lace1	Mus musculus lactation elevated 1	2
NM_010685	Lamp2	Mus musculus lysosomal membrane glycoprotein 2	3
NM_172153	Lcorl	Mus musculus ligand dependent nuclear receptor corepressor-like	2
NM_010094	Lefty1	Mus musculus left right determination factor 1	1
NM_177099	Lefty2	Mus musculus Left-right determination factor 2	1
NM_001029878	Lonrf2	Mus musculus LON peptidase N-terminal domain and ring finger 2	2
NM_172950	Lpin1	Mus musculus lipin 1	1
NM_080420	Lpo	Mus musculus lactoperoxidase	1
NM_172784	Lrp11	Mus musculus low density lipoprotein receptor-related protein 11	1
NM_173038	Lrrc35	Mus musculus leucine rich repeat containing 35	3
XM_984916	Lycat	PREDICTED: Mus musculus lysocardiolipin acyltransferase, transcript variant 3	1
NM_172865	Manea	Mus musculus mannosidase, endo-alpha	1
NM_027920	March8	Mus musculus membrane-associated ring finger (C3HC4) 8	3
NM_010773	Mbd2	Mus musculus methyl-CpG binding domain protein 2	1
NM_020007	Mbnl1	Mus musculus muscleblind-like 1 (Drosophila)	2
NM_175341	Mbnl2	Mus musculus muscleblind-like 2	1
NM_175088	Mdfic	Mus musculus MyoD family inhibitor domain containing	1
XM_976104	Mef2a	PREDICTED: Mus musculus myocyte enhancer factor 2A, transcript variant 3	2
XM_001002380	Mfn2	PREDICTED: Mus musculus mitofusin 2, transcript variant 4	1
XM_912670	Mgat5	PREDICTED: Mus musculus mannoside acetylglucosaminyltransferase 5, transcript variant 2	3
NM_008606	Mmp11	Mus musculus matrix metallopeptidase 11	1
NM_011985	Mmp23	Mus musculus matrix metallopeptidase 23	1
NM_008636	Mtf1	Mus musculus metal response element binding transcription factor 1	2

NM_001005864	Mtus1	Mus musculus mitochondrial tumor suppressor 1	1
NM_139063	Muted	Mus musculus muted	2
NM_008659	Myo1c	Mus musculus myosin IC	1
NM_019542	Nagk	Mus musculus N-acetylglucosamine kinase	1
XM_973478	Nck2	PREDICTED: Mus musculus non-catalytic region of tyrosine kinase adaptor protein 2, transc	1
NM_172495	Ncoa7	Mus musculus nuclear receptor coactivator 7	1
NM_008684	Neo1	Mus musculus neogenin	2
NM_008687	Nfib	Mus musculus nuclear factor I/B	1
NM_023526	Nkiras1	Mus musculus NFKB inhibitor interacting Ras-like protein 1	2
NM_009697	Nr2f2	Mus musculus nuclear receptor subfamily 2, group F, member 2	2
NM_172416	Ostm1	Mus musculus osteopetrosis associated transmembrane protein 1	1
NM_008775	Pafah1b2	Mus musculus platelet-activating factor acetylhydrolase, isoform 1b, alpha2 subunit	1
NM_011864	Papss2	Mus musculus 3'-phosphoadenosine 5'-phosphosulfate synthase 2	1
NM_028829	Paqr8	Mus musculus progestin and adipoQ receptor family member VIII	1
XM_992943	Pbx3	PREDICTED: Mus musculus pre B-cell leukemia transcription factor 3, transcript variant 6	1
XM_982935	Pcgf4	PREDICTED: Mus musculus polycomb group ring finger 4, transcript variant 4	1
NM_008786	Pcmt1	Mus musculus protein-L-isoaspartate (D-aspartate) O-methyltransferase 1	1
XM_912421	Pde3b	PREDICTED: Mus musculus phosphodiesterase 3B, cGMP-inhibited	1
XM_920266	Pdzd2	PREDICTED: Mus musculus PDZ domain containing 2, transcript variant 9	1
NM_019410	Pfn2	Mus musculus profilin 2	4
NM_171824	Pgbd5	Mus musculus piggyBac transposable element derived 5	2
XM_895539	Phip	PREDICTED: Mus musculus pleckstrin homology domain interacting protein, transcript varia	3
NM_201406	Pigs	Mus musculus phosphatidylinositol glycan anchor biosynthesis, class S	1
NM_181585	Pik3r3	Mus musculus phosphatidylinositol 3 kinase, regulatory subunit, polypeptide 3 (p55)	1
NM_001025309	Pja2	Mus musculus praja 2, RING-H2 motif containing	1
NM_018807	Plagl2	Mus musculus pleiomorphic adenoma gene-like 2	3
NM_031256	Plekha3	Mus musculus pleckstrin homology domain-containing, family A (phosphoinositide binding sp	1
NM_183034	Plekhm1	Mus musculus pleckstrin homology domain containing, family M (with RUN domain) member	2
NM_023564	Plscr3	Mus musculus phospholipid scramblase 3	1
NM_010127	Pou6f1	Mus musculus POU domain, class 6, transcription factor 1	2
NM_024209	Ppp6c	Mus musculus protein phosphatase 6, catalytic subunit	1
NM_178738	Prss35	Mus musculus protease, serine, 35	2
NM_008971	Ptk9	Mus musculus protein tyrosine kinase 9	1
NM_011877	Ptpn21	Mus musculus protein tyrosine phosphatase, non-receptor type 21	2
NM_027514	Pvr	Mus musculus poliovirus receptor	2
NM_024436	Rab22a	Mus musculus RAB22A, member RAS oncogene family	3

NM_026405	Rab32	Mus musculus RAB32, member RAS oncogene family	1
NM_144875	Rab7l1	Mus musculus RAB7, member RAS oncogene family-like 1	1
NM_001038621	Rabgap1l	Mus musculus RAB GTPase activating protein 1-like	1
XM_983626	Rapgef2	PREDICTED: Mus musculus Rap guanine nucleotide exchange factor (GEF) 2	1
NM_009826	Rb1cc1	Mus musculus RB1-inducible coiled-coil 1	2
NM_011250	Rbl2	Mus musculus retinoblastoma-like 2	3
NM_029777	Rhbdd1	Mus musculus rhomboid domain containing 1	1
NM_007484	Rhoc	Mus musculus ras homolog gene family, member C	1
NM_145491	Rhoq	Mus musculus ras homolog gene family, member Q	1
NM_023894	Rhox9	Mus musculus reproductive homeobox 9	1
NM_009068	Ripk1	Mus musculus receptor (TNFRSF)-interacting serine-threonine kinase 1	1
NM_023270	Rnf128	Mus musculus ring finger protein 128	1
NM_011277	Rnf2	Mus musculus ring finger protein 2	1
XM_903197	Rora	PREDICTED: Mus musculus RAR-related orphan receptor alpha, transcript variant 4	4
NM_009075	Rpia	Mus musculus ribose 5-phosphate isomerase A	1
NM_199476	Rrm2b	Mus musculus ribonucleotide reductase M2 B (TP53 inducible)	1
NM_030179	Rsnl2	Mus musculus restin-like 2	1
NM_030692	Sacm1l	Mus musculus SAC1 (suppressor of actin mutations 1, homolog)-like (S. cerevisiae)	1
NM_011452	Serpinb9b	Mus musculus serine (or cysteine) peptidase inhibitor, clade B, member 9b	2
NM_031179	Sf3b1	Mus musculus splicing factor 3b, subunit 1	2
XM_988661	Sh3glb1	PREDICTED: Mus musculus SH3-domain GRB2-like B1 (endophilin)	1
NM_177364	Sh3pxd2b	Mus musculus SH3 and PX domains 2B	3
NM_172966	Sh3rf2	Mus musculus SH3 domain containing ring finger 2	1
NM_134038	Slc16a6	Mus musculus solute carrier family 16 (monocarboxylic acid transporters), member 6	1
NM_025807	Slc16a9	Mus musculus solute carrier family 16 (monocarboxylic acid transporters), member 9	1
NM_172773	Slc17a5	Mus musculus solute carrier family 17 (anion/sugar transporter), member 5	2
NM_018861	Slc1a4	Mus musculus solute carrier family 1 (glutamate/neutral amino acid transporter), member 4	1
NM_021435	Slc35b4	Mus musculus solute carrier family 35, member B4	2
NM_133741	Snrk	Mus musculus SNF related kinase	1
NM_029068	Snx16	Mus musculus sorting nexin 16	1
NM_028937	Sohlh2	Mus musculus spermatogenesis and oogenesis specific basic helix-loop-helix 2	1
NM_009274	Srpk2	Mus musculus serine/arginine-rich protein specific kinase 2	3
NM_138744	Ssx2ip	Mus musculus synovial sarcoma, X breakpoint 2 interacting protein	1
NM_011374	St8sia1	Mus musculus ST8 alpha-N-acetyl-neuraminide alpha-2,8-sialyltransferase 1	1
NM_024239	Stambp	Mus musculus Stam binding protein	1
NM_133810	Stk17b	Mus musculus serine/threonine kinase 17b (apoptosis-inducing)	2

NM_134115	Stk38	Mus musculus serine/threonine kinase 38	1
NM_026343	Stx17	Mus musculus syntaxin 17	1
NM_025932	Syap1	Mus musculus synapse associated protein 1	2
XM_903544	Syde1	PREDICTED: Mus musculus synapse defective 1, Rho GTPase, homolog 1 (C. elegans)	1
XM_981719	Synj1	PREDICTED: Mus musculus synaptojanin 1	1
NM_145968	Tagap	Mus musculus T-cell activation Rho GTPase-activating protein	1
NM_198294	Tanc1	Mus musculus tetratricopeptide repeat, ankyrin repeat and coiled-coil containing 1	2
NM_023755	Tcfcp2l1	Mus musculus transcription factor CP2-like 1	2
NM_146142	Tdrd7	Mus musculus tudor domain containing 7	1
NM_009371	Tgfbr2	Mus musculus transforming growth factor, beta receptor II	3
NM_009373	Tgm2	Mus musculus transglutaminase 2, C polypeptide	1
NM_133352	Tm9sf3	Mus musculus transmembrane 9 superfamily member 3	1
XM_892747	Tmcc1	PREDICTED: Mus musculus transmembrane and coiled coil domains 1, transcript variant 2	2
NM_134020	Tmed4	Mus musculus transmembrane emp24 protein transport domain containing 4	1
NM_144792	Tmem23	Mus musculus transmembrane protein 23	1
NM_009395	Tnfaip1	Mus musculus tumor necrosis factor, alpha-induced protein 1 (endothelial)	2
XM_982893	Tnrc6a	PREDICTED: Mus musculus trinucleotide repeat containing 6a	1
NM_009277	Trim21	Mus musculus tripartite motif protein 21	2
NM_178872	Trim36	Mus musculus tripartite motif-containing 36	1
XM_987804	Trps1	PREDICTED: Mus musculus trichorhinophalangeal syndrome I (human)	1
NM_133681	Tspan1	Mus musculus tetraspanin 1	2
NM_026954	Tusc1	Mus musculus tumor suppressor candidate 1	1
NM_028339	Txndc1	Mus musculus thioredoxin domain containing 1	2
NM_153162	Txnrd3	Mus musculus thioredoxin reductase 3	1
NM_019586	Ube2j1	Mus musculus ubiquitin-conjugating enzyme E2, J1	1
NM_172300	Ube2z	Mus musculus ubiquitin-conjugating enzyme E2Z (putative)	1
NM_011670	Uchl1	Mus musculus ubiquitin carboxy-terminal hydrolase L1	2
NM_009466	Ugdh	Mus musculus UDP-glucose dehydrogenase	1
NM_177561	Usp46	Mus musculus ubiquitin specific peptidase 46	2
XM_977760	Wdr26	PREDICTED: Mus musculus WD repeat domain 26, transcript variant 7	1
NM_011701	Vim	Mus musculus vimentin	1
NM_172643	Zbtb41	Mus musculus zinc finger and BTB domain containing 41 homolog	4
NM_010731	Zbtb7a	Mus musculus zinc finger and BTB domain containing 7a	4
NM_028864	Zc3hav1	Mus musculus zinc finger CCCH type, antiviral 1	1
NM_011749	Zfp148	Mus musculus zinc finger protein 148	2
NM_175494	Zfp367	Mus musculus zinc finger protein 367	4

NM_009557	Zfp46	Mus musculus zinc finger protein 46	2
NM_133218	Zfp704	Mus musculus zinc finger protein 704	5
NM_133906	Zkscan1	Mus musculus zinc finger with KRAB and SCAN domains 1	5
NM_144516	Zmynd11	Mus musculus zinc finger, MYND domain containing 11	1
XM_893176	Znrf3	PREDICTED: Mus musculus zinc and ring finger 3, transcript variant 2	2

Supplementary Table 4. Primers used in the study.

Primer pair		Primer sequences (5´-3´)	Reference
Primers for chromatin immunoprecipitation			
GAPDH	forward	TCCCCTCCCCCTATCAGTTC	
	reverse	TTGGACCCGCCTCATTTTT	
Oct-4	forward	TGGGCTGAAATACTGGGTTC	
	reverse	TTGAATGTTCGTGTGCCAAT	
Primers for real-time quantitative RT-PCR			
GAPDH	forward	CCATCACCATCTTCCAGG	
	reverse	CCTGCTTCACCACCTTCTTG	
Oct-4	forward	GGCGTTCGCTTTGGAAAGGTGTTC	
	reverse	CTCGAACCACATCCTTCTCT	
Nanog	forward	TGATTCAGAAGGGCTCAGCAC	
	reverse	GGGATAGCTGCAATGGATGC	
GCNF	forward	TGAATTGGCAGAGCTTGATCC	
	reverse	CGATCATCTGGGACGGAAAC	
Dnmt3a2	forward	AGGGGCTGCACCTGGCCTT	[1]
	reverse	TCCCCCACACCAGCTCTCC	[1]
Dnmt3b1/b6	forward	TGGGATCGAGGGCCTCAAAC	[1]
	reverse	TTCCACAGGACAAACAGCGG	[1]
Rbl2	forward	CCCGGAGCCAGGTGTACA	
	reverse	CCTCATCACTGGGCTGGAAT	
Primers for bisulfite sequencing			
Oct-3/4 [-2069]	forward	GGGAGGAATTGGGTGTGGGGAGGTT	[2]
Oct-3/4 [-1677]	reverse	AAAAATCCCCTCCTTCTACCACAT	[2]
Oct-3/4 [-1505]	forward	TGATGAAGATTATTATTAAGAGAT	[2]
Oct-3/4 [-992]	reverse	CCCCAATCCCCTCACACAAAACTT	[2]
Oct-3/4 [-212]	forward	AGGATTTTGAAGGTTGAAAATGAAGG	[2]
Oct-3/4 [-8]	reverse	TCCCTCCCCAATCCCACCCTC	[2]
Oct-3/4 [+1173]	forward	GTAATTAGTTTTAAGAATAAGGTG	[2]
Oct-3/4 [+1340]	reverse	AAATAAACTATTAATACCTTCCTA	[2]
CTCF.bis.9.fwd	forward	GTTAATTTTTAAGGATGATAGTTTTGTGATT	
CTCF.bis.9.rev	reverse	AAAACCATAACAAAAACCTAAACCTTAC	
IAP.bis.fwd	forward	TTGATAGTTGTGTTTTAAGTGGTAAATAAA	[3]
IAP.bis.rev	reverse	AAAACACCACAAACCAAAATCTTCTAC	[3]
IAP 5'.bis.fwd	forward	ATGGGTTGTAGTTAATTAGGGAGTGATA	
IAP 5'.bis.rev	reverse	CATACAATTAAATCCTTCTTAACAATCTAC	
LINE1.bis.fwd	forward	TAGGAAATTAGTTTGAATAGGTGAGAGGT	
LINE1.bis.rev	reverse	TCAAACACTATATTACTTTAACAATTCCCA	
satellite.bis.fwd	forward	ATACACACTTTAAAACATAAAATATAA	[5]
satellite.bis.rev	reverse	TTYGTTATATTTTAGGTTTTTTAGA	[5]
MuERV.bis.fwd	forward	GTTATTATGTGATTTGAATTA	[3]
MuERV.bis.rev	reverse	ACATACAAAACCATCAATAAAC	[3]

Sox30.bis.fwd	forward	AGGTGTTTTTATATTTGAGAATGATTAGAA	4
Sox30.bis.rev	reverse	ATTAAAACCCTTCCAAAACCTTAACTA	4
Tsp50.bis.fwd	forward	TAAAAATTGTTATTGAAGTTAAGTTTGG	4
Tsp50.bis.rev	reverse	CTAAACCCTTTCTCTAAATCCCTATAC	4

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mRNA microarray analysis

Undifferentiated *Dicer*$^{+/-}$ and *Dicer*$^{-/-}$ cells were grown in the presence of LIF as described in the main text. Prior to harvesting, *Dicer*$^{+/-}$ and *Dicer*$^{-/-}$ cells were grown in triplicates for more than one week. For the rescue with the miR-290s mimics, three independently cultured *Dicer*$^{-/-}$ cell samples were transfected separately with either miR-290 cluster miRNA mimics or siRL, as described in Materials and Methods (main text), and harvested 24 h later. We note that at the time of study the miR-290 annotation in miRBase[1] was for the miR-290-5p and not miR-290-3p. The miR-290-5p mimic was therefore included in the transfection together with miR-291a-3p through miR-295-3p miRNAs, which represent the main products of their respective hairpins. The complement of miR-290-5p seed was not found to be significantly enriched in any seed motif analysis, arguing that miR-290-5p does not play a major role in ES cells.

Total RNA was isolated using Absolutely RNA Miniprep Kit (Stratagene). 5 µg of total RNA from each triplicate culture was reverse transcribed with the Affymetrix cDNA synthesis kit and cRNA was produced by *in vitro* transcription (IVT) by T7 RNA polymerase, using the Affymetrix IVT kit as per manufacturer's instructions. 20 µg of biotinylated cRNA was fragmented by heating in the presence of Mg^{2+} (as per Affymetrix's instructions) and 15 µg of fragmented cRNA from each triplicate was hybridized to Mouse MOE430 v2.0 GeneChips™. All arrays yielded hybridization signals of comparable intensity and quality. BioConductor[2] Affymetrix package of the R software was used to import the CEL files from the Affymetrix Mouse Genome 430 2.0 Array. Probe set intensities were then background-corrected, adjusted for non-specific binding and quantile normalized with the GCRMA algorithm[3]. GCRMA-normalized microarray data were deposited in the GEO database (GSE7141 and GSE8503).

Data analysis

To extract a non-redundant set of transcripts for subsequent analyses of 3′-UTR sequences, probe sets with _s or _x tags, which map to multiple transcripts from different genes, were discarded. Then, the Affymetrix annotation from December 2006 was used

to obtain the corresponding reference sequence (RefSeq[4]) for each probe set. When the Affymetrix array contained probe sets for alternative RefSeq transcripts for the same gene, we only used the RefSeq transcript with the median length 3′-UTR. Through this procedure, we obtained an n-to-1 probe set to RefSeq transcript mapping. For transcripts that had multiple probe sets, we discarded those that were deficient, as indicated by their very low variance across a set of unrelated experiments performed with different cell types using the same platform (Affymetrix Mouse Genome 430 2.0). Finally, the log2 intensities of the probe sets corresponding to a given transcript were averaged to obtain a transcript level measurement. We used Limma[5] to estimate the fold change and the corresponding p-value in the three replicate experiments for each condition.

To identify those motifs whose frequency in up-regulated (in $Dicer^{-/-}$) or down-regulated (in $Dicer^{-/-}$ ES cells transfected with miRNA mimics of the miR-290 family) 3′-UTRs is significantly different relative to the frequency in the entire set of 3′-UTRs, we extracted the set of transcripts up-regulated in the Dicer-/- cells (p-value < 0.001) and computed the relative frequency of all 7-mers in the 3′-UTRs of these transcripts compared with the entire set of 3′-UTRs represented on the microarray. For each 7-mer, we then plotted the log2(number of occurrences in up-regulated 3′-UTRs) on the x-axis, and the enrichment in up-regulated 3′-UTRs compared to the entire set of 3′-UTRs on the y-axis (Fig. 1b and 1e). We then used a Bayesian model that we previously introduced for comparing miRNA frequencies between samples[6]. Briefly, we estimate the posterior probabilities of the model that assumes that the frequency of a given motif is different between two sets of transcripts (call this "different" model), and the model that assumes that the frequency is the same (call this "same" model), given the observed counts m and n of the motif among M and N total motifs in the two samples. We selected as significant those motifs that were enriched in the up-regulated or down-regulated set, respectively, with a posterior probability of the "different" model > 0.99

miRNA microarray analysis

Total RNA from two independent cultures of $Dicer^{+/-}$ cells and single culture of $Dicer^{-/-}$ cells was extracted using MirVana miRNA Isolation Kit (Ambion). 5 μg of each RNA preparation was used for miRNA miRCURY™ microarray analysis as a service by

Exiqon (Vedbäck, Denmark). As a control, a mixture of 5 μg of total RNA originating from 10 mouse tissues (Ambion) supplemented with 500 ng of total RNA from *Dicer*^{+/−} cells was labeled with Hy5 (spectrally equivalent to Cy5) and co-hybridized with either the *Dicer*^{+/−} or the *Dicer*^{−/−} RNA samples, which were labeled with Hy3 (spectrally equivalent to Cy3). The expression level of reliably detected miRNAs was calculated relative to the levels in *Dicer*^{+/−} sample as well as relative to the level in the control mixture of total RNAs (reference sample). Most miRNA probes exhibited hybridization signal also with *Dicer*^{−/−} samples, suggesting that the arrays also detect precursors of miRNAs or cross-hybridize to unrelated RNAs. The original data are available upon request.

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