SUPPLEMENTARY DATA

SUPPLEMENTARY FIGURES

Figure S1. Analysis of reporter activities and mRNA levels in different human cell lines. (A) Quantification of reporter mRNA in HeLa cells transfected with RL reporter constructs. mRNA was isolated two days after transfection (60 hours post-induction with Tet). RL reporter mRNA expression was normalized to EGFP mRNA expressed from a co-transfected plasmid. Histogram shows normalized mean values of RL mRNAs relative to RL mRNA level in cells transfected with the control RL reporter (pRL-Con), which was set to 100%. Error bars (SEM) are derived from five northern blot experiments. (B) Activity of RL reporter constructs pRL-Con, pRL-Perf, and pRL-3xBulge A and B in transfected MCF-7 and A-549 cells. RL activity values are expressed relative to the activity of firefly luciferase (FL) encoded by the co-transfected pFL-Con. RL/FL values in cells transfected with the control RL reporter (pRL-Con) were set to 100%. Histogram shows normalized mean values (±SEM) of RL/FL activity from three experiments performed in duplicates. (C) Northern blot analysis of expression of RL reporters in MCF-7 and A-549 cells. mRNA was isolated two days after transfection (60 hours post-induction with Tet). RL mRNA levels (quantified from phosphorimager scans) were normalized to 28S rRNA levels, which were quantified in Image Quant (Molecular Dynamics) from .tiff images of ethidium bromide-stained agarose gels. Error bars represent a range of data from two independent northern blot experiments. Panels below the graph show images from one experiment. Abbreviations: Con, pRL-Con; Perf, pRL-Perf; 3xA, pRL-3xBulgeA; 3xB, pRL-3xBulgeB.

Figure S2. Detailed cleavage map of RL reporter constructs. 293 cells were transiently transfected with the RL reporter constructs and termini of degraded reporter transcripts were maped by 5'RACE. Perfect or bulged let-7 miRNA binding sites are indicated in a bold face. The primer binding site for the second nested PCR primer is underlined. Arrows depicts termini of individual clones mapped by the 5'RACE analysis. Numbers above arrows indicate the numbers of clones with an identical end.

Figure S3. Analysis of endogenous Ago2 protein levels in Ago-kd cells. Endogenous Ago2 levels were analyzed using a rabbit polyclonal antibody specifically recognizing Ago2 (kindly provided by Dr. Suvendra N. Bhattacharyya of our laboratory). (A) Comparison of down-regulation of transiently expressed HA-tagged Ago2 and endogenous Ago2 in different cell lines. Cells were induced with Tet for 48 hours. 293 is the parental 293T-Rex line. Bands corresponding to HA-Ago2 and endogenous Ago2 are shown in the upper panel. p63, shown in the lower panel, is an endogenous protein cross-reacting with the anti-Ago2 antibody and serves as a loading control. (B) Densitometric quantification of Ago2 expression shown in the upper part of the panel A. A raw, 12-bit scan (.tiff-file) of the exposed film was analyzed using ImageQuant software (Molecular Dynamics). Signals of Ago2 and HA-Ago2 in the 293 sample were set to 100%. (C) Levels of endogenous Ago2 and Dicer proteins in Ago2-kd and Ago3-kd cell lines. Cells were induced for 48 hours as described in Material and Methods.

Figure S4. Condition clustering analysis. (A) Hierarchical clustering using all probe sets called "Present". (B) Hierarchical clustering using all probe sets, which were found differentially expressed in at least one knock-down cell line (t-test, $p<0.05$).

Figure S5. Profiles of genes, which are significantly (t-test, $p<0.05$) up-regulated more than 1.5-fold in Ago2-kd and Dicer-kd at day 2 and day 6 (genes corresponding to the central overlap of the middle Venn diagram in the Figure 5B). Shown are data from all per gene normalized microarrays (for details of normalization, see Material and Methods). Only annotated genes are displayed. Unknown genes, ESTs, hypothetical proteins were removed from the list.

ADDITIONAL SUPPLEMENTARY FILES

Microarray Quality Data - 1 file

MIAME - 1 file

Lists of Transcripts - 1 .zip file containing:

- one .xls file (2006 Schmitter Raw Data Lists.xls). The file contains raw mean expression and fold change of all U133 plus 2.0 probes in Ago2-kd and Dicer-kd day 6. This material is supplementary for Figure 5A.
- one .xls file (2006 Schmitter Normalized Data Lists.xls). The file contains fold-change data for normalized expression of probe sets in each knockdown sample. The first sheet lists fifteen probe sets with the highest up-regulation for each knock-down.
- additional lists are available on request.

SUPPLEMENTARY TABLES

Supplementary Table 1

Primers and oligonucleotides used in this study.

oligonucleotides used for cloning		
pTER-Ago1_sh	sense	GATCCCGAGAAGAGGTGCTCAAGAATTCAAGAGATTCTTGAGCACCTCTTCTCTTTTTGGAAA
	antisense	AGCTTTTCCAAAAAGAGAAGAGGTGCTCAAGAATCTCTTGAATTCTTGAGCACCTCTTCTCGG
pTER-Ago2_sh2	sense	GATCCCGCAGGACAAAGATGTATTATTCAAGAGATAATACATCTTTGTCCTGCTTTTTGGAAA
	antisense	AGCTTTTCCAAAAAGCAGGACAAAGATGTATTATCTCTTGAATAATACATCTTTGTCCTGCGG
pTER-Ago3_sh	sense	GATCCCGAAATTAGCAGATTGGTAATTCAAGAGATTACCAATCTGCTAATTTCTTTTTGGAAA
	antisense	AGCTTTTCCAAAAAGAAATTAGCAGATTGGTAATCTCTTGAATTACCAATCTGCTAATTTCGG
pTER-Ago4_sh	sense	GATCCCGGCCAGAACTAATAGCAATTTCAAGAGAATTGCTATTAGTTCTGGCCTTTTTGGAAA
	antisense	AGCTTTTCCAAAAAGGCCAGAACTAATAGCAATTCTCTTGAAATTGCTATTAGTTCTGGCCGG
pTER-control_sh	sense	GATCCCATTCTCCGAACGTGTCACGTTCAAGAGACGTGACACGTTCGGAGAATTTTTTGGAAA
	antisense	GCTTTTCCAAAAAATTCTCCGAACGTGTCACGTCTCTTGAACGTGACACGTTCGGAGAATGG
pTER-Dicer_sh2	sense	GATCCCATTGGCTTCCTCCTGGTTATGTTCAAGAGACATAACCAGGAGGAAGCCAATTTTTTGGAAA
	antisense	AGCTTTTCCAAAAAATTGGCTTCCTCCTGGTTATGTCTCTTGAACATAACCAGGAGGAAGCCAATGG
pRL-3xBulgeA	sense	ACTATACAACCGTTCTACCTCAGGCCTACTATACAACCGTTCTACCTCACTCGAGACTATACAACCGTTCTACCTCA

oligonucleotides used for 5' RACE	
Adaptor	ppp-GCUGAUGGCGAUGAAUGAACACUGCGUUUGCUGGCUUUGAUGAAA
5'RACE Outer Primer	GCTGATGGCGATGAATGAACACTG
5'RACE Inner Primer	AACACTGCGTTTGCTGGCTTTGATG
5'RACE RT-primer	ATTGCAGCTTATAATGGTTAC
5'RACE RL-nested1	GCATTCTAGTTGTGGTTTGTCC
5'RACE RL-nested2	GTATCTTATCATGTCTGCTCG

Supplementary Table 2

Statistical analysis of significance of occurrence of motifs matching the seeds of miR-17/20/106, miR-30, and miR-142 (supplement for Figure 7). Statistically significant differences ($p<0.05$) are highlighted in yellow.

Ago2-kd

t-tests for panel B

	< 0.7	0.7 - 1.4	1.4 – 2	> 2
< 0.7	X	0.603200	0.000767	0.000008
0.7 - 1.4		X	0.009991	0.000295
1.4 – 2			X	0.263500
> 2				X

t-tests for panel C

	< 0.7	0.7 - 1.4	1.4 – 2	> 2
< 0.7	X	0.985200	0.005533	0.000005
0.7 - 1.4		X	0.011160	0.000017
1.4 – 2			X	0.033870
> 2				X

Dicer-kd day 2

t-tests for panel B

	< 0.7	0.7 - 1.4	1.4 – 2	> 2
< 0.7	X	0.365900	0.351700	0.007974
0.7 - 1.4		X	0.095100	0.001101
1.4 – 2			X	0.124600
> 2				X

t-tests for panel C

	< 0.7	0.7 - 1.4	1.4 – 2	> 2
< 0.7	X	0.161800	0.101600	0.001140
0.7 - 1.4		X	0.007844	0.000022
1.4 – 2			X	0.196000
> 2				X

Dicer-kd day 6

t-tests for panel B

	< 0.7	0.7 - 1.4	1.4 – 2	> 2
< 0.7	X	0.487800	0.185900	0.163900
0.7 - 1.4		X	0.076630	0.065470
1.4 – 2			X	0.983900
> 2				X

t-tests for panel C

	< 0.7	0.7 - 1.4	1.4 – 2	> 2
< 0.7	X	0.398100	0.090340	0.071840
0.7 - 1.4		X	0.026480	0.017400
1.4 – 2			X	0.858400
> 2				X