Supplemental Information

Inventory

- Supplemental Figures Legends
- Supplementary Table 1- 4
- Extended Experimental Procedures.
- Supplemental References
- Supplemental Figure 1-7

Supplementary Figure Legends

Figure S1. Analysis of ADAR1 interactions with RISC member proteins. (A) Immunoblotting analysis of nuclear and cytoplasmic extracts. ADAR1p150 and ADAR1p110 are detected in both nucleus and cytoplasm. Nuclear and cytoplasmic extracts (20 μg) of HeLa and HEK293 cells were examined by immunoblotting analysis. Parental cell lines without any expression construct were used to monitor the endogenous levels of protein expression and localization relative to each other. (B) FLAG-IP purified RISC proteins and ADAR1. HEK293 FLAG-IP purifications of the cytoplasmic fraction. Various HEK293 stable cell lines expressing individual FLAG-tagged proteins were processed for their cytoplasmic extract (CE) and purified by FLAG IP. The protein complexes were eluted into five fractions with 3xFLAG-peptide (E1-E5), and examined by immunoblotting with the FLAG Ab. FT: flow through; W: wash; and E: elution. CE 20 μg, and 30 μL each of FT and W as well as 5 μl out of 1 ml each of E1-E5 were examined. (C) Mock controls for Sf9 co- and triple-infection experiments. Sf9 cells were infected with one of the three different baculovirus constructs for expression of epitope-tagged recombinant proteins: H-ADAR1, F-Dicer and HA-Ago2, or the construct for untagged TRBP. Extracts of the infected cells were made and subjected individually to each of the three affinity purification procedures used in this study, *i.e.*, TALON, FLAG, and HA column. The peak fractions were then tested by immunoblotting analysis using antibodies specific to each of the three epitope tags, *i.e.*, anti-His (HAT), anti-FLAG, and anti-HA, or anti-TRBP (untagged). The results of these control purification experiments demonstrate that each of the three tagged-proteins was captured only by the affinity purification procedure matching to its tag. For instance, F-Dicer was captured by the FLAG column but not by the TALON or HA columns. Untagged TRBP was not captured by any of the three columns. The results demonstrate that the pull-down of differentially epitope-tagged prey proteins by bait proteins shown in this study is specific. None of the pull-downs can be explained by artifactual binding of prey proteins or epitope tags to beads or to non-cognate antibody on the beads.

Figure S2. Analysis of Dicer complexes purified by two consecutive TALON and FLAG Ab affinity column chromatography. (A) Consecutive affinity chromatography is schematically shown. The F-Dicer/H-ADAR1 complexes were purified from free form Dicer and ADAR1 protein. (B) The purified F-Dicer alone and F-Dicer/H-ADAR1 complexes each containing 300 ng of Dicer were fractionated by 7% SDS-PAGE and stained with colloidal-blue. (C) The amount of Dicer, used alone or in various Dicer complexes, was adjusted to be equal. Varying amount of F-Dicer purified alone (10, 20, 30, 40 ng) and various Dicer complexes, each containing 20 ng of F-Dicer, purified via consecutive affinity chromatography, were fractionated by 7% SDS-PAGE and examined by immunoblotting analysis using the FLAG Ab. (D) The F-Dicer/H-ADAR1p110 complexes purified were fractionated by Superose 12 gel filtration column chromatography.

Figure S3. Multiple turnover Dicer cleavage assay with pre-let-7a. (A) 5' ^{32}P-labeled human pre-let-7a and Dicer cleavage reaction products are schematically presented. (B) The time course analysis of Dicer cleavage. The Dicer cleavage reaction was done at 37 °C with various concentrations of pre-let-7a and 5 nM of Dicer alone or Dicer/ADAR1 complex for various times. The reaction products were fractionated by 15% Urea-PAGE. Similar time course analyses were done also at 50, 100, and 200 nM pre-let-7a (data not shown).

Figure S4. Multiple turnover Dicer cleavage assay with pre-siRNA. (A) 5' ^{32}P-labeled 35 bp dsRNA (pre-siRNA) and Dicer cleavage reaction products are schematically presented. (B) The time course analysis of Dicer cleavage. The Dicer cleavage reaction was done at 37 °C with various concentrations of pre-siRNA and 5 nM of Dicer alone or Dicer/ADAR1 complex for various times. The reaction products were fractionated by 15% Urea-PAGE. Similar time course analyses were done also at 50, 100, and 200 nM pre-siRNA (data not shown).

Figure S5. No editing activity found with the Dicer/ADAR1 complex. (A) The two editing sites of mouse pre-miR-151 are indicated by red "A"s. The regions known to be processed into the mature miR-151-5p and miR-151-3p are highlighted in green. An individual editing site is indicated by a number with the 5' end of the mature miR-151-3p sequence counted as +1. (B) *In vitro* cleavage reaction products of ^{32}P-labeled pre-miR-151 (56 nt) by the Dicer/ADAR1p110 complex were fractionated by 15% Urea-PAGE. The cleavage assay was also done with the F-ADAR1/H-ADAR1 homodimer complex, but generated no cleaved products as expected. (C) A quantitative summary of the editing efficiency revealed by cloning and sequencing of cDNA isolates derived from the uncleaved pre-miR-151 and cleaved miR-151-3p RNAs, which were generated during the *in vitro* dicing reaction by the Dicer/ADAR1 complex.

Figure S6. Two ADAR1 functions in RNAi. ADAR1 has two separate functions, one antagonistic and another simulative, in regulation of RNAi. ADAR1 homodimer edits long dsRNA as well as certain miRNA precursors. Editing causes alteration of the dsRNA structure and makes it less accessible to Dicer cleavage, which consequently antagonizes RNAi by reducing overall production of siRNA and miRNA. In contrast, ADAR1 in the Dicer/ADAR1 heterodimer complex promotes RNAi by augmenting the Dicer cleavage activity, generating more siRNA and miRNA, and enhancing the RISC loading activity, resulting in more target RNA silencing.

Figure S7. Suppression of miRNAs results in elevated target gene expression in *ADAR1*$^{-/-}$ embryos. (A) Total and screened read counts from the E11.5 wildtype versus *ADAR1*$^{-/-}$ embryo libraries. (B) The sequence of steps and parameters used for screening of miRNA reads and RNA reads and application of the miRanda target prediction program (miRNA-target pair analysis) are shown. (C) The miRNA target prediction was performed with a selected list of miRNAs for 3'UTRs of the selected protein-coding genes, resulting in identification of 1,013 genes targeted by the miRNAs whose expression was significantly reduced in E11.5 *ADAR1*$^{-/-}$ embryos.

Supplementary Table 1. E11.0 and E11.5 mouse embryo small RNAs: sequencing read statistics. Reads that were of low quality or too short (<19 nt) were removed from the total read counts provided by small RNA-seq. The adapter sequences were first removed from the filtered reads. The Bowtie program was used for aligning trimmed reads to various RNA classes and the mouse genome (mm9 assembly). Only two mismatches were permitted for alignment. The individual aligned reads were then normalized to counts per million total reads.

	E11.0		E11.5	
RNA Category	**WT Embryo**	***ADAR1*$^{-/-}$ Embryo**	**WT Embryo**	***ADAR1*$^{-/-}$ Embryo**
Original Reads	7,641,509	8,096,038	6,991,584	5,702,576
Short Reads (<19 nt)	1,282,496	1,608,821	1,190,125	1,378,965
Total Reads (≥19 nt)	6,359,013	6,487,217	5,801,459	4,323,611
Unaligned Reads	1,117,826	1,141,403	542,497	477,893
Total Aligned Reads	5,241,187	5,338,930	5,258,686	3,845,435
Normalized	1,000,000	1,000,000	1,000,000	1,000,000
miRNA	770,582	737,445	811,376	101,016
rRNA	37,551	107,704	58,987	702,020
tRNA	55,330	31,627	32,103	7,014
snoRNA	8,839	6,690	8,140	32,165
snRNA	1,278	2,446	2,327	30,962
scRNA	1,770	1,814	915	1,770
srpRNA	192	346	173	3,821
scaRNA	1	3	3	14
piRNA	40,069	27,325	30,506	26,771
7SK RNA	13	30	26	97
Repeat	22,475	18,642	12,376	13,548
Pseudo Gene	304	1,067	285	1,824
Known Transcript	37,978	48,647	26,387	68,885
mRNA	5,837	4,175	3,809	2,809
EST	5,922	4,372	3,986	3,236
Intergenic	9,252	7,006	6,124	3,087
Mitochondria chr	2,606	662	2,477	963

Extended Experimental Procedures

Mammalian Expression Plasmid Construction and Permanent Transfection of HEK293 Cells

The expression plasmid for F-ADAR1p110 was constructed in the p3xFLAG-CMV-10 vector (Sigma-Aldrich) using an ADAR1 cDNA clone BC038227 (Open Biosystems). This plasmid was co-transfected with a puromycin resistance plasmid pPUR (CloneTech) into HEK293 cells, resulting in a permanent cell line expressing F-ADAR1p110. HEK293 cell lines expressing F-Dicer, F-TRBP and F-Ago2 were described previously (Chendrimada et al., 2005).

Baculovirus Expression Constructs

Preparation of baculovirus constructs for F-ADAR1p110, H-ADAR1p150, H-ADAR1p110, and H-ADAR1p150-EAA was described previously (Cho et al., 2003; Lai et al., 1995; Valente and Nishikura, 2007). Preparation of baculovirus constructs for F-Dicer and untagged TRBP were described previously (Chendrimada et al., 2005). Additional baculovirus constructs were newly prepared for this study. F-Ago2 and HA-Ago2 were prepared by PCR cloning using a human Ago2 cDNA clone BC160104 (Open Biosystems). PCR primers used are as follows: SpeI-FLAG-Ago2FW; 5'-GACTAGTAGAATAAAAA TGAATGATTACAAGGACGACGATGACAAGTACTCGGGAGCCGGCCCC-3', SpeI-HA-Ago2FW; 5'-GACTAGTAGAATAAAAATGAATTACCCATACGATGTTCCAGATTACGCTTACTCGGGAG CCGGCCCC-3', HindIII-Ago2DW1; 5'-CCCAAGCTT**TCA**AGCAAAGTACATGGTGCGCAGAGT-3'. Restriction sites are indicated by underlining. The stop codons included for reverse primers are indicated in bold letters.

Preparation of Serial Deletion Constructs of H-ADAR1p110 and F-Dicer

Baculovirus constructs for H-ADAR1p110 and F-Dicer with C-terminal serial deletions and F-Dicer with N-terminal deletions were prepared by PCR cloning. PCR primers used are as follows: ADAR1FW; 5'-ACCCCTCCCATATGGCATTTGACAG-3', ADAR1Δ1; 5'-GCTTCTAGA**TTA**CTTC AGCTGGCACTCTGTCAGTTTCTT-3', ADAR1Δ2; 5'-GCTTCTAGA**TTA**GCTCTTCCCAGAAAA GAAGGATGTGG-3', ADAR1Δ3; 5'-GCTTCTAGA**TTA**GGTGTTCAGGTATCTCACGAGCTC-3', ADAR1Δ4; 5'-GCTTCTAGA**TTA**GCTGCCAGTGAGAGGGAGTG-3', DicerFW; 5'-CGCGGA TCC AGA ATA AAA ATG AA-3', DicerΔ1RV; 5'-ACGAAGCTT**CTA**TTGAATAGTGTCTGTCGTACC AGG-3', DicerΔ2RV; 5'-ACGAAGCTT**CTA**AACACAGTATGCTGAATCAGCGT-3', DicerΔ3RV; 5'-ACGAAGCTT**CTA**CGTGTTGATTGTGACTCGTG-3', DicerΔ4RV; 5'-ACGAAGCTT**CTA**CTCATA TGGTTTATATTTGCGTAAGATT-3'. DicerΔN1FW; 5'-TATTGCTCGAGAGAATAAAAATGAATG ATTACAAGGACGACGATGACAAGCAGTTTGAAAGCGTTGAGTGG-3', DicerΔN2FW; 5'-GACT AGTAGAATAAAAATGAATGATTACAAGGACGACGATGACAAGGAGACCAGTGTTCCAGGA AGAC-3', DicerΔN1RV; 5'-CGCATAAGCTT**TCA**GCTATTGGGAACCTGAGG-3'. Restriction sites are indicated by underlining. The stop codons included for reverse primers are indicated in bold letters.

Protein Expression, Purification, and Detection

Nuclear and cytoplasmic extracts were prepared from HEK293 cells constitutively expressing various ADAR1 or RISC member proteins by the modified Dignam method as described previously (Chendrimada et al., 2005). The complexes were then purified from ~200 mg of cytoplasmic extracts on anti-FLAG M2-monoclonal antibody (mAb)-agarose beads (Sigma). Prior to elution with the FLAG peptide, the beads were washed extensively with a buffer containing 25 mM Tris, pH7.6, 2.5 mM $MgCl_2$, 20% glycerol, 0.5 M NaCl, 1 mM β-mercaptoethanol, 0.2 mM PMSF, 0.05% NP-40. For analysis of association of recombinant proteins in the Sf9 insect cell baculovirus system, F- or H-

ADAR1 was co- or triple-infected with F-Dicer, TRBP without tagging, F-Ago2, or HA-Ago2 at an approximately equimolar ratio. The recombinant proteins were purified with an anti-FLAG M2 affinity bead and/or a TALON metal resin (BD Biosciences) as described previously (Cho et al., 2003). The interacting partner protein was determined by immunoblotting analysis. The primary antibodies used for analysis of FLAG IP products, purified recombinant proteins, and various complexes were anti-FLAG M5 mAb (Sigma), anti-HAT pAb (GenScript), anti-HA mAb (GeneScript), anti-ADAR1 mAb 15.8.6 (Cho et al., 2003), anti-ADAR2 mAb 1.3.1 (Cho et al., 2003), anti-Dicer mAb (Abcam), anti-TRBP mAb (Abnova), anti-Ago2 pAb (Upstate), and anti-Tudor-SN mAb 13.14.1 (this work). A fragment of the human Tudor-SN C-terminal region (aa698-910) was constructed in pGEX-4T-2 vector (Amersham Biosciences) as a gluthathione S-transferase (GST) fusion protein. Tudor-SN-GST fusion protein was used as antigen for preparation of mAb13.14.1 as described previously (Raitskin et al., 2001).

Size Exclusion Column Chromatography

The purified protein complexes were fractionated in a buffer containing 25 mM Tris, pH7.6, 0.5 M NaCl, 10% glycerol, 0.1% NP-40, 1 mM DTT, and 0.2 mM PMSF using Superose 6 or Superose 12 gel filtration column and a Fast Protein Liquid Chromatography (FPLC) system (GE Healthcare). The molecular weight of various complexes was ascertained by comparison to known molecular mass standards (Sigma): blue dextran 2000 (void), thyroglobulin (669 kDa), ferritin (440 kDa), catalase (232 kDa), aldolase (158 kDa), conalbumin (75 kDa), carbonic anhydrase (29 kDa), and chymotrypsinogen A (25 kDa). The peak position of each protein standard was determined by the optical absorption at 280 nm. The peak position for the RISC member proteins and ADAR1 was confirmed by immunoblotting analysis.

Determination of pre-miR-151 and miR-151 RNA Editing Sites

Editing of pre-miR-151 and/or miR-151 RNAs derived from the *in vitro* Dicer cleavage assay was analyzed as described previously (Kawahara et al., 2007). Dicer cleavage was assayed *in vitro* using 25 nM of pre-miR-151 RNA and F-Dicer/H-ADAR1p110 complex (5 nM Dicer equivalent) incubated at 37 °C for 90 min. Cleaved miR-151 and uncleaved pre-miR-151 RNAs were gel-purified. Editing frequency of pre-miR-151 at the -1 and/or +3 sites and of miR-151-3p at the +3 site was determined by sequencing of ~100 individual cDNA clones as described previously (Kawahara et al., 2007).

MiRNA Quantification by Real-Time PCR

Quantification of select miRNAs was done by qRT-PCR using total RNA samples extracted from wildtype and *ADAR1*$^{-/-}$ mouse embryos, miScript Reverse Transcription Kit, miScript SYBR Green PCR Kit, and miScript Primer assay systems (QIAGEN). Briefly, 1 µg of total RNA was used for reverse transcription, and the resultant cDNA equivalent to 2.5 ng of total RNA was used for qPCR. As a spike control, 2×10^{10} copies of a chemically synthesized RNA which has an exogenous sequence, 5'-AGCAGAGGAUUGACGACUACAG-3', was added to the RT reaction mixture, resulting in 5×10^{7} copies of the spike cDNA in each quantitative PCR reaction. PCR of the spike RNA was performed by using a custom ordered miScript Primer assay (QIAGEN). Quantitative PCR was done in a 20 µl reaction using 7900HT Fast Real-Time PCR System (Applied Biosystems), and each experiment was conducted in quadruplicate. After standardization into copy number by the spiked RNA, the relative miRNA expression level was presented as the expression level relative to that of the wildtype embryo at E11.0.

RNA Interference Knockdown of ADAR1, TRBP, and PACT

The human ADAR1 targeting siRNA (5'-CCGCCAUCAUUAUGAAAAAAG-3') was designed using siExplorer (Katoh and Suzuki, 2007). For human TRBP and PACT knockdown, Silencer select validated siRNAs were purchased from Life technologies (siTRBP: s13791, siPACT: s16336, Negative Control: 4390843). HeLa cells were transfected with siRNAs (5 nM) using the reverse-transfection protocol as instructed in the Lipofectamine RNAiMAX (Life technologies). Total RNA was extracted from the cells using the miRNeasy kit (QIAGEN) three days after transfection. The knockdown efficiency was confirmed by immunoblotting analysis. Quantification of select miRNAs was done by qRT-PCR with total RNA samples extracted from HeLa cells using the miRNeasy kit (QIAGEN). Primers for each human miRNA and small RNA fragments were purchased from QIAGEN (miScript Primer assay).

Immunoblotting Analysis of Mouse Embryos

Whole mouse embryos collected live at various embryonic stages (E9.0-12.0) were lysed using a homogenizer in RIPA buffer (50 mM Tris, pH 8.0, 150 mM NaCl, 1.0% NP-40, 0.5% sodium deoxycholate, and 0.1% SDS) containing a protease inhibitor cocktail (Roche Diagnostics) on ice. Insoluble material was removed by centrifugation at 15,000 g at 4°C for 15 min, and total protein concentrations were determined by the BCA assay (Thermo Scientific). For immunoblotting analysis, 12 μg of the extracted protein was fractionated by 4-15% SDS-PAGE. Proteins were transferred to Immobilon™-P nylon membrane (Millipore). Blots were blocked with SuperBlock Blocking buffer (Thermo Scientific) and hybridized overnight at 4°C with anti-ADAR1 (Cho et al., 2003), anti-Dicer (Abcam), anti-TRBP (Abcam), anti-PACT (Abcam), or anti-β-actin (Abcam) antibodies. For analysis of miRNA target gene protein levels, 6 μg of the extracted protein and anti-Dicer (Abcam), anti-c-FOS (Cell Signaling Technology), anti-FOXP1 (Cell Signaling Technology), and anti-IRX5 (Novus Biological) antibodies were used. Membranes were washed 4 times with a buffer containing 137 mM NaCl, 2.7 mM KCl, 10 mM sodium phosphate dibasic, 1.8 mM potassium phosphate monobasic, 0.1% Tween 20, pH 7.4 (PBST) on SNAP i.d. (Millipore), and incubated with anti-Mouse IgG or anti-Rabbit IgG secondary antibodies (Jackson ImmunoResearch Laboratories) for 10 min at room temperature. Finally the membranes were washed 4 times with PBST buffer, and developed with ECL (GE Healthcare). After detection of a specific protein, the membranes were stripped using Restore PLUS Western Blot Stripping Buffer (Thermo Scientific) for 15 min at room temperature, then blocking and antibody incubation were repeated.

Mouse Embryo RNA Preparation

Total RNA was extracted from whole mouse embryos collected live at various embryonic stages using TRIZOL (Invitrogen). Integrity of RNA samples was examined using the 2100 Bioanalyzer (Agilent Technologies) to confirm that the RNA Integrity Number value is greater than 8.

Small RNA-seq and RNA-seq Deep Sequencing

Preparation of small RNA libraries for deep sequencing was done using 5 μg of whole embryo or HeLa cell total RNA and Illumina Small RNA Sample Preparation Kit (v1.0 kit) and included steps for isolation of 19-24 nt small RNAs by gel purification, ligation of Illumina 5' and 3' adaptors, PCR amplification, and gel purification of amplified libraries. Preparation of RNA-seq samples was carried out using 6 μg of whole embryo total RNA and Illumina mRNA Sample Preparation Kit, according to the Illumina protocols. Briefly, this includes steps for oligo dT mRNA purification, fragmentation, cDNA synthesis, end repair, ligation of adaptors, PCR enrichment, and purification of 225-325 bp amplified DNA fragments. Sequencing for both small RNA-seq and RNA-seq was done with the GAII Illumina Genome Analyzer at a read length of 36 base pairs.

Bioinformatics Analysis of Mouse Small RNA-seq Data

We followed a multi-step approach to align and annotate small RNA reads as follows: 1) removal of adaptor nucleotides; 2) alignment to miRNA locus; 3) alignment to other non-coding RNA; 4) alignment to mitochondrial genome; 5) alignment to mouse genome. Alignment was performed using Bowtie 0.10.0 program (Langmead et al., 2009) allowing a maximum of 2 mismatches. All alignment was taken without restriction on multiple mapping of reads.

1) Adapter removal: The 3'adapter sequences (5'-TCGTATGCCGTCTTCTGCTTG-3') were first removed from the sequences. Trimmed reads of length ≥ 19 nucleotides were subjected to further analysis.

2) Alignment to miRNA: The chromosomal coordinate of miRNAs was downloaded from miRBase database (ftp://mirbase.org/ftp.shtml) (Kozomara and Griffiths-Jones, 2011). We used 36 bp sequences, both upstream and downstream of the mature miRNA sequences in the genome database, to ascertain the overlap.

3) Alignment to other non-coding RNAs: The unaligned reads from previous step were then aligned to other non-coding RNAs in the following order; ribosomal-RNAs (rRNA), transfer-RNAs (tRNA), small nucleolar RNAs (snoRNA), small nuclear RNAs (snRNA), small cajal body-specific RNA (scaRNA), and piwi-RNAs (piRNA). The complete ribosomal RNA sequence was downloaded from NCBI and Ensembl database (Flicek et al., 2012). The tRNA genes used as reference for alignment was obtained from tRNAscan-SE prediction available at UCSC genome browser and also the prediction at GtRNAdb and fnRNAdb databases (Chan and Lowe, 2009; Dreszer et al., 2012; Kin et al., 2007; Lowe and Eddy, 1997). The reference snoRNA sequences were obtained from RNAdb, snoRNABase and fnRNAdb databases (Xie et al., 2007). The reference snRNAs were downloaded from Ensembl and fnRNAdb databases. The scaRNAs were downloaded from snoRNdb database. The piRNA sequences were obtained from fnRNAdb database and those published in (Lau et al., 2006).

4) Alignment to mitochondrial genome: The unaligned reads from previous step were aligned to the mitochondrial genome of mouse. The mitochondrial genome was downloaded from UCSC genome browser.

5) Alignment to genome: The unaligned reads from the previous step were finally aligned to the mouse genome (mm9 assembly), downloaded from UCSC genome browser.

Bioinformatics Analysis of HeLa Small RNA-seq Data

Similar to analysis of mouse small RNA-seq we followed a multi-step approach to align and annotate small RNA reads as follows: 1) removal of adaptor nucleotides; 2) Removal of siRNA sequences; 3) alignment to miRNA locus; 4) alignment to other non-coding RNA; 5) alignment to mitochondrial genome; 6) alignment to human genome. Alignment was performed using Bowtie 0.10.0 program allowing a maximum of 2 mismatches. All alignment was taken without restriction on multiple mapping of reads.

1) Adapter removal: The 3'adapter sequences (5'-TGGAATTCTCGGGTGCCAAGGAACTCCAG TCAC-3') were first removed from the sequences. Trimmed reads of length ≥ 19 nucleotides were subjected to further analysis.

2) Removal of siRNA sequences: The reads containing siRNA sequences used were removed. For siLuc sample, reads with 5'-CGTACGCGGAATACTTCGAAG-3' and 5'-TCGAAGTATTCCGCGT ACGAT-3' sequences were removed. For siADAR1 sample, reads with 5'-CCGCCATCATTATGAA AAAAG-3' and 5'-TTTTTCATAATGATGGCGGAT-3' sequences were filtered out.

3) Alignment to miRNA: The chromosomal coordinate of miRNAs was downloaded from miRBase database (ftp://mirbase.org/ftp.shtml).

4) Alignment to other non-coding RNAs: The unaligned reads from previous step were then aligned to other non-coding RNAs in the following order; ribosomal-RNAs (rRNA), transfer-RNAs (tRNA), small nucleolar RNAs (snoRNA) and small nuclear RNAs (snRNA). The rRNA, tRNA, snRNA, and snoRNA genomic coordinates were downloaded from fnRNAdb databases (Chan and Lowe, 2009; Dreszer et al., 2012; Kin et al., 2007; Lowe and Eddy, 1997).

5) Alignment to mitochondrial genome: The unaligned reads from previous step were aligned to the mitochondrial genome of human. The mitochondrial genome was downloaded from UCSC genome browser.

6) Alignment to genome: The unaligned reads from the previous step were finally aligned to the human genome (hg19 assembly), downloaded from UCSC genome browser.

Bioinformatics Analysis of RNA-seq Data

We followed a three step procedure to align the mRNA-seq data. The steps include: 1) filtering; 2) alignment to a non-redundant list of mRNAs; 3) alignment to the mouse genome. Alignment was performed using the Bowtie program permitting upto 2 mismatches.

1) Filtering: The reads aligning to ribosomal RNA, mitochondrial genome of mouse and adapter like sequences were removed from further analysis.

2) Alignment to transcriptome: The filtered mRNA-seq data was aligned to a non-redundant list of mRNAs generated by combining multiple gene models: RefSeq, UCSC, Ensembl, Vega and MGI databases.

3) Alignment to genome: The unaligned reads from the previous step were aligned to the mouse genome (mm9 assembly). Multiple mapping was not allowed while aligning directly to genomic DNA.

Finally, the cufflinks 0.9.3 program (Roberts et al., 2011; Trapnell et al., 2010) was applied to the mRNA-seq aligned reads for estimating gene and mRNA expression levels.

***In Silico* MiRNA Target Prediction**

The miRNA target prediction was performed with a selected list of miRNAs for 3'UTRs of selected protein-coding genes (Figure S10). miRNA clones were selected based on the three criteria: 1) miRNA clone length $\geq$ 21 nucleotides and $\leq$ 24 nucleotides; 2) miRNA clone expression $\geq$ 500 copies either in E11.5 wildtype or *ADAR1*$^{-/-}$ embryos; 3) fold change (wildtype/ *ADAR1*$^{-/-}$) of clone expression $\geq$ 4. The gene 3'UTR regions were selected based on three conditions: 1) gene expression $\geq$ 10 FPKM in E11.5 wildtype and/or *ADAR1*$^{-/-}$ embryos; 3) mRNA expression $\geq$ 5 FPKM in E11.5 wildtype and/or *ADAR1*$^{-/-}$ embryos; 4) gene and mRNA expression fold change (*ADAR1*$^{-/-}$/wildtype) $\geq$ 2. The miRNA target prediction was performed using miRanda program (John et al., 2004) using default settings.

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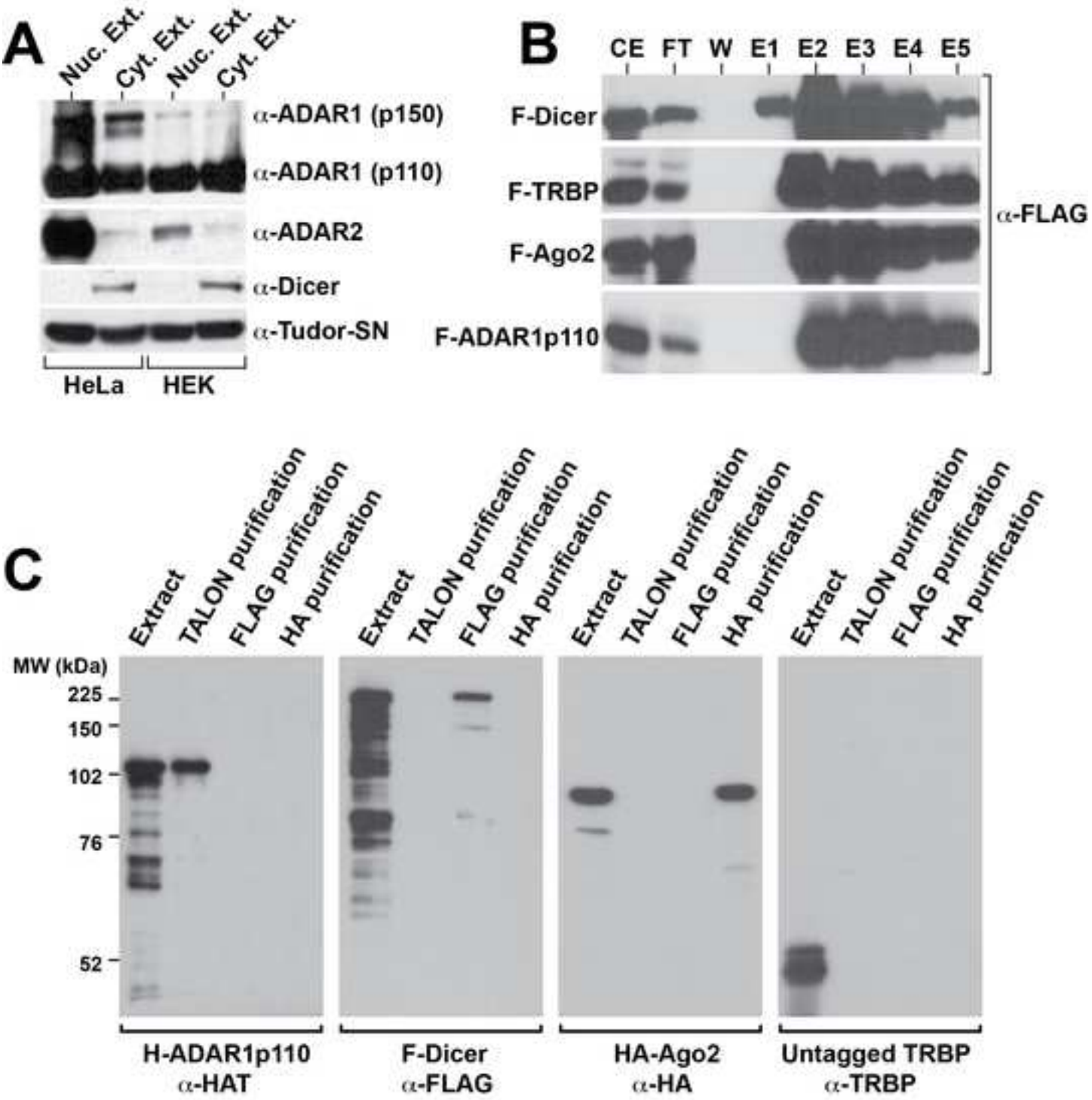
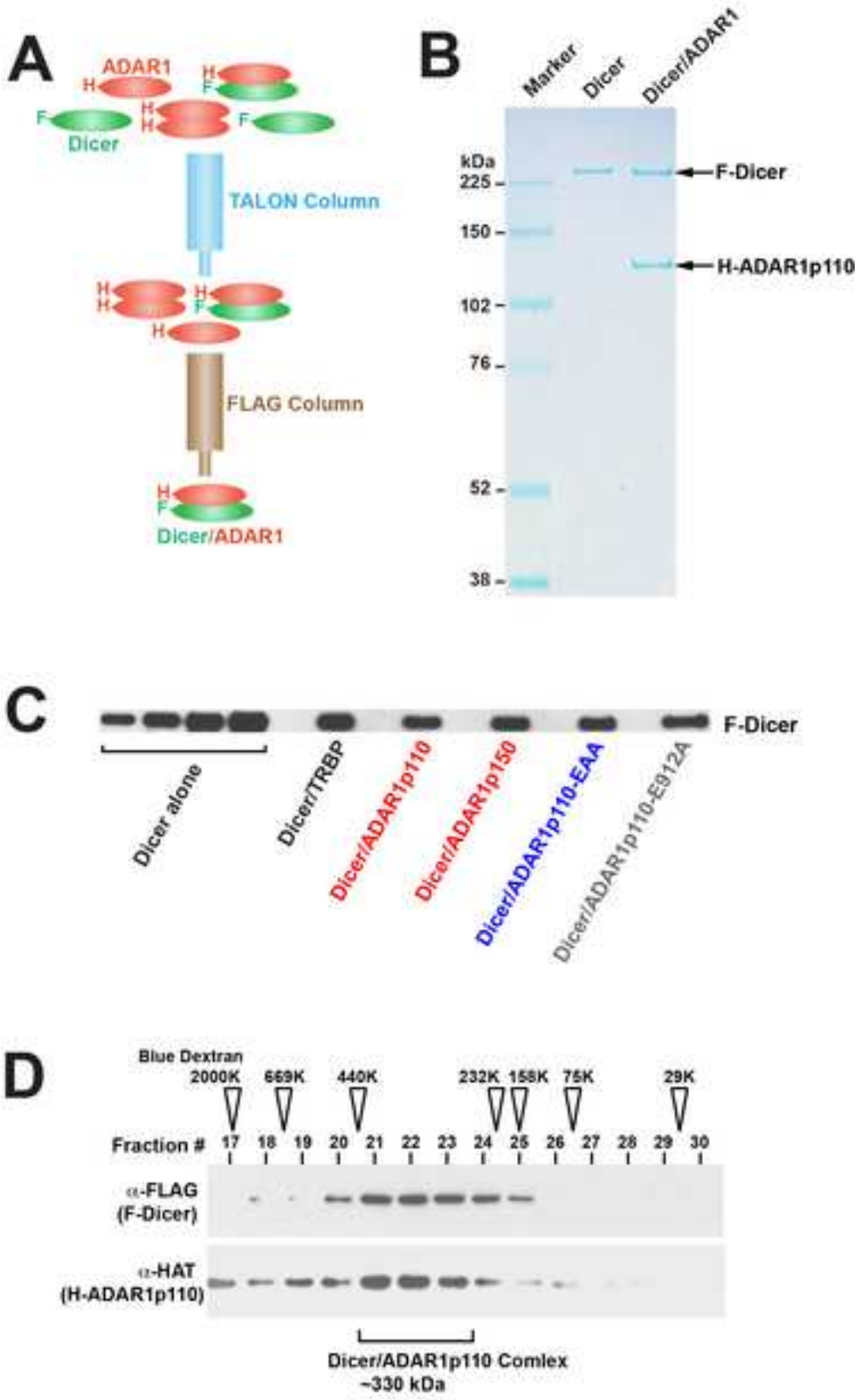


Ota *et al.*
Supplementary Figure 1



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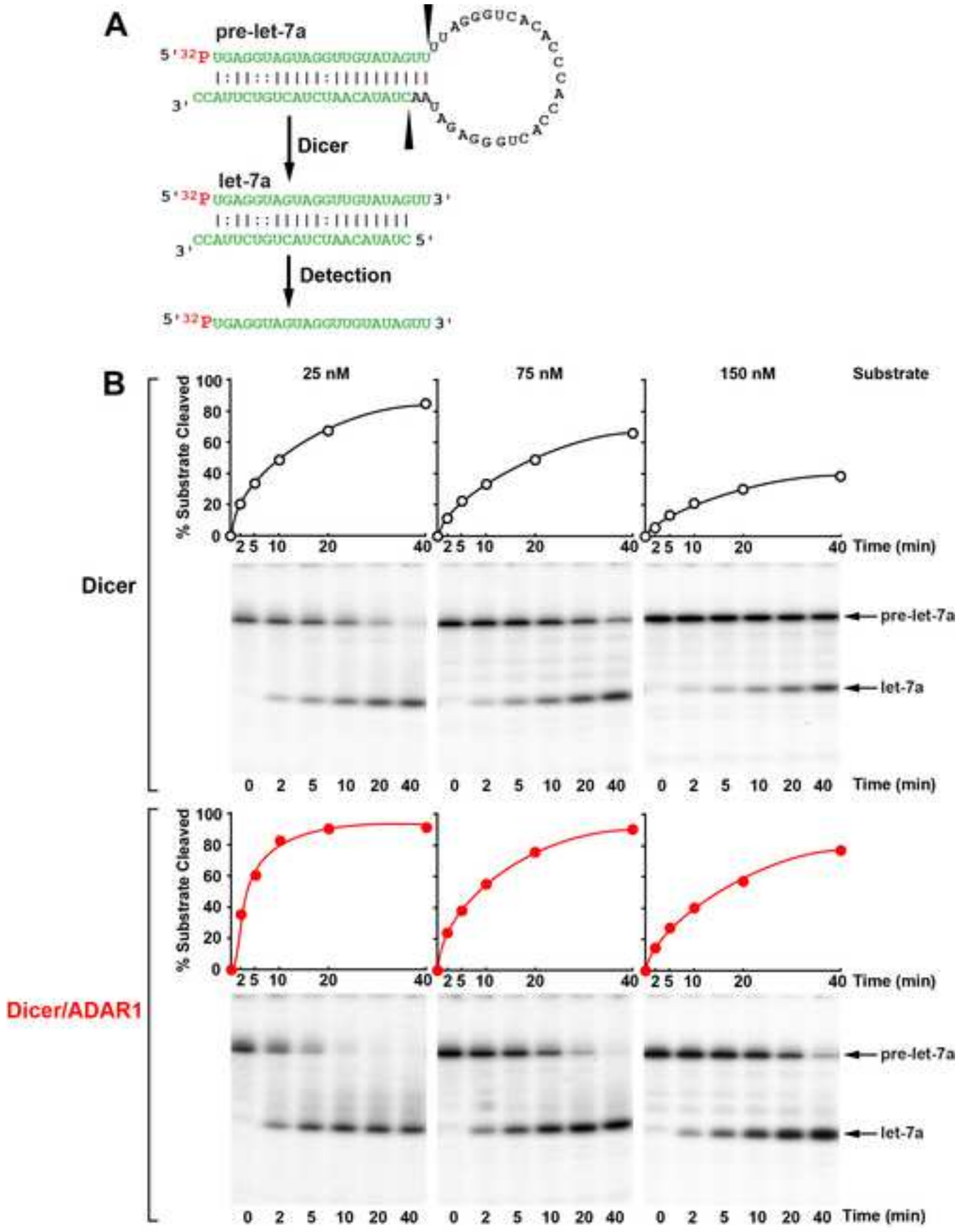
Supplementary Figure 2



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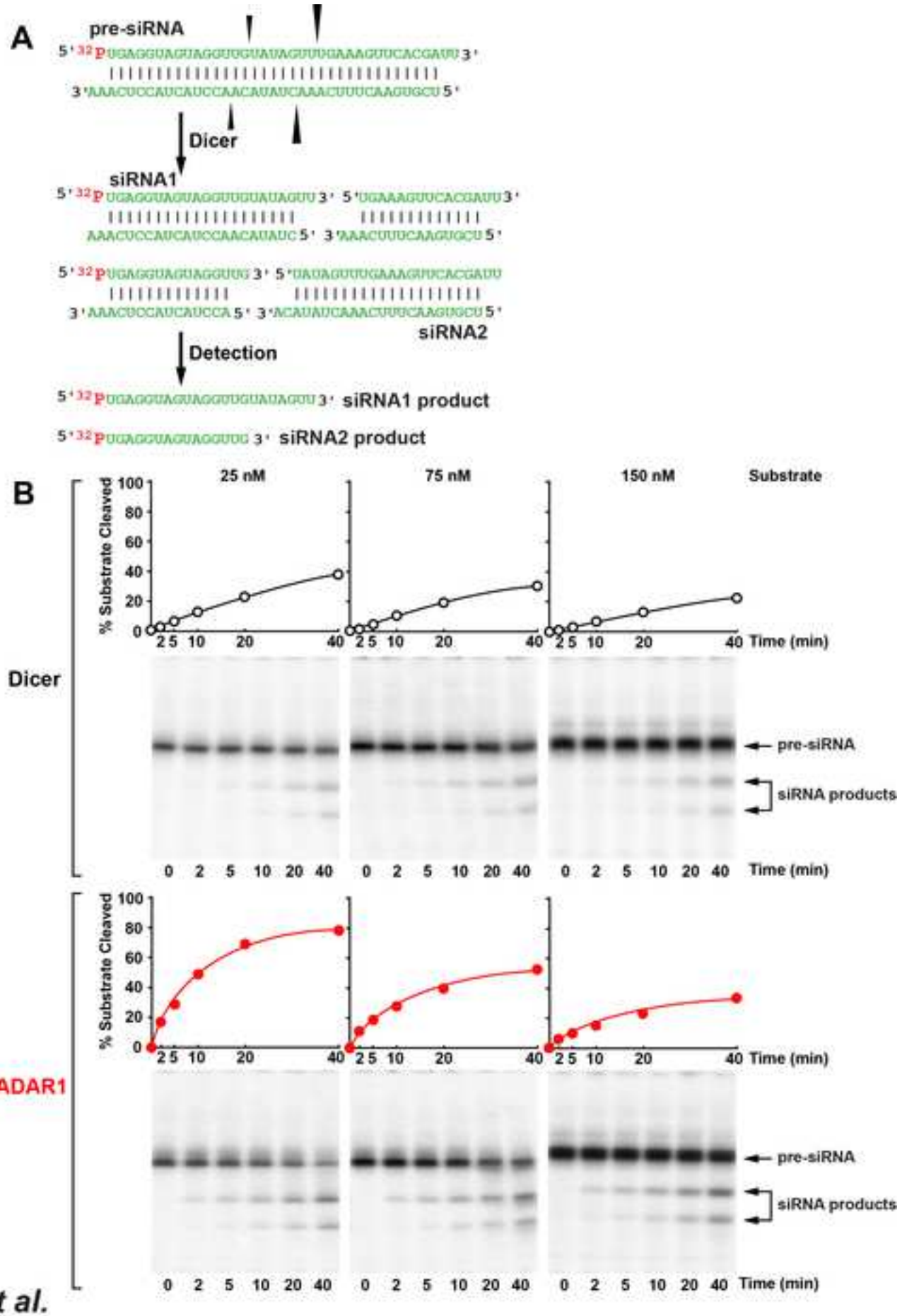
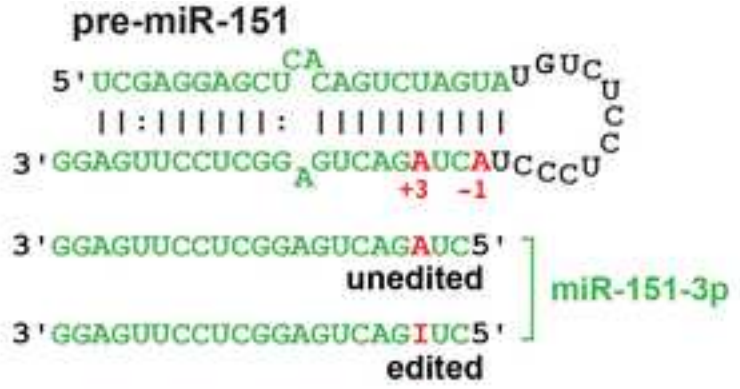
Supplementary Figure 3

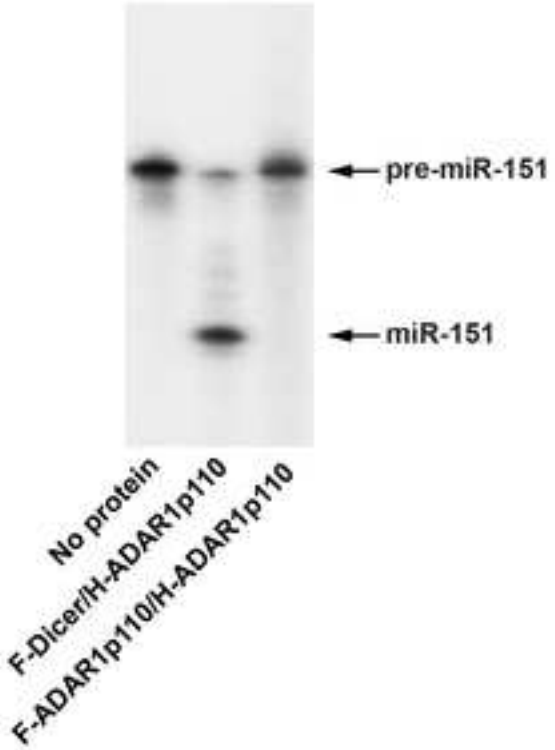


Ota *et al.*
Supplementary Figure 4

A



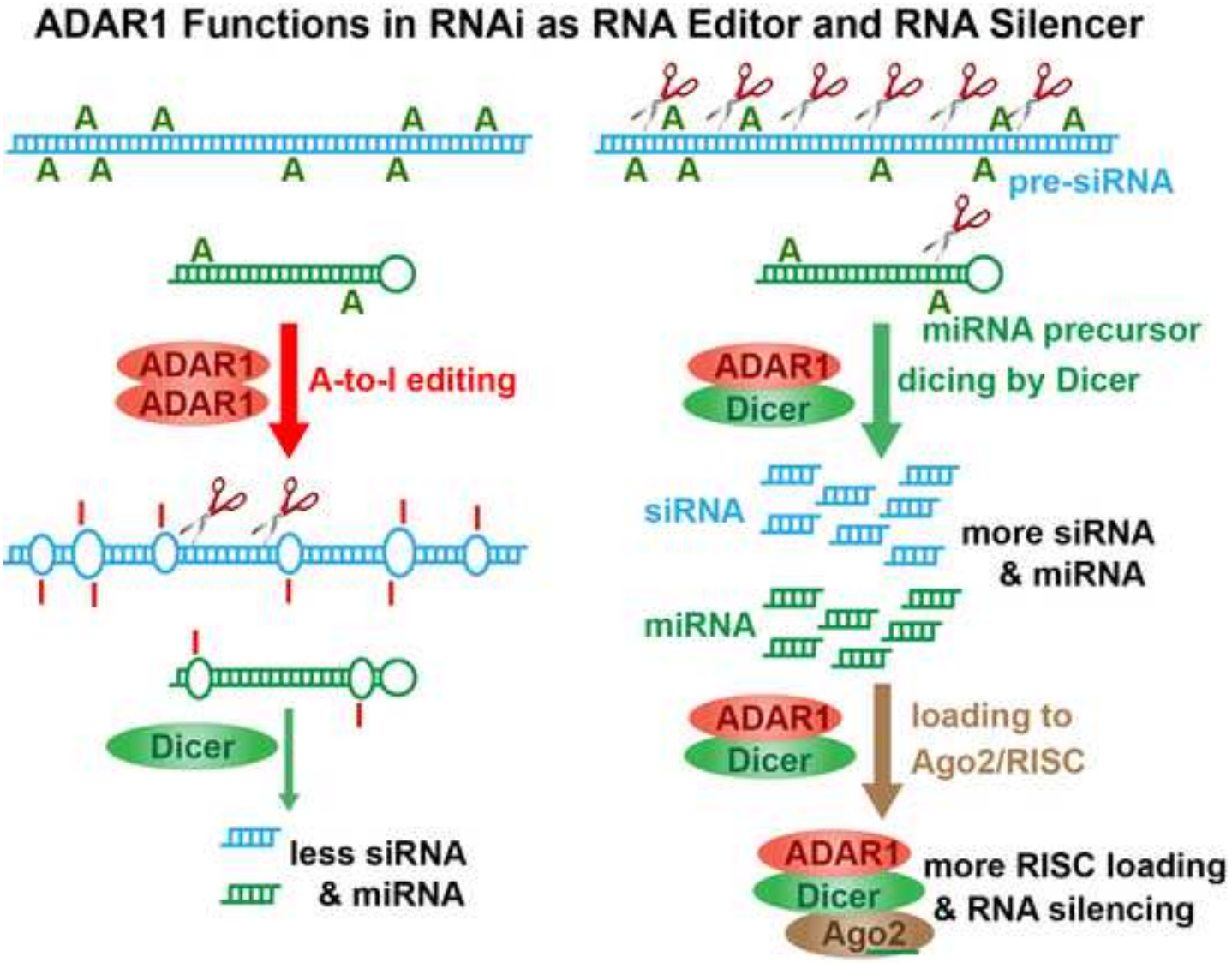
B



C

		cDNA clones analyzed	Cloning efficiency (%)
pre-miR-151	..CUACUAGAC.. (-1 +3)	101	100
	..CUACUIGAC..	0	0
	..CUICUAGAC..	0	0
	..CUICUIGAC..	0	0
miR-151-3p (5'-end)	CUAGAC..	139	100
	CUIGAC..	0	0

Ota *et al.*
Supplementary Figure 5



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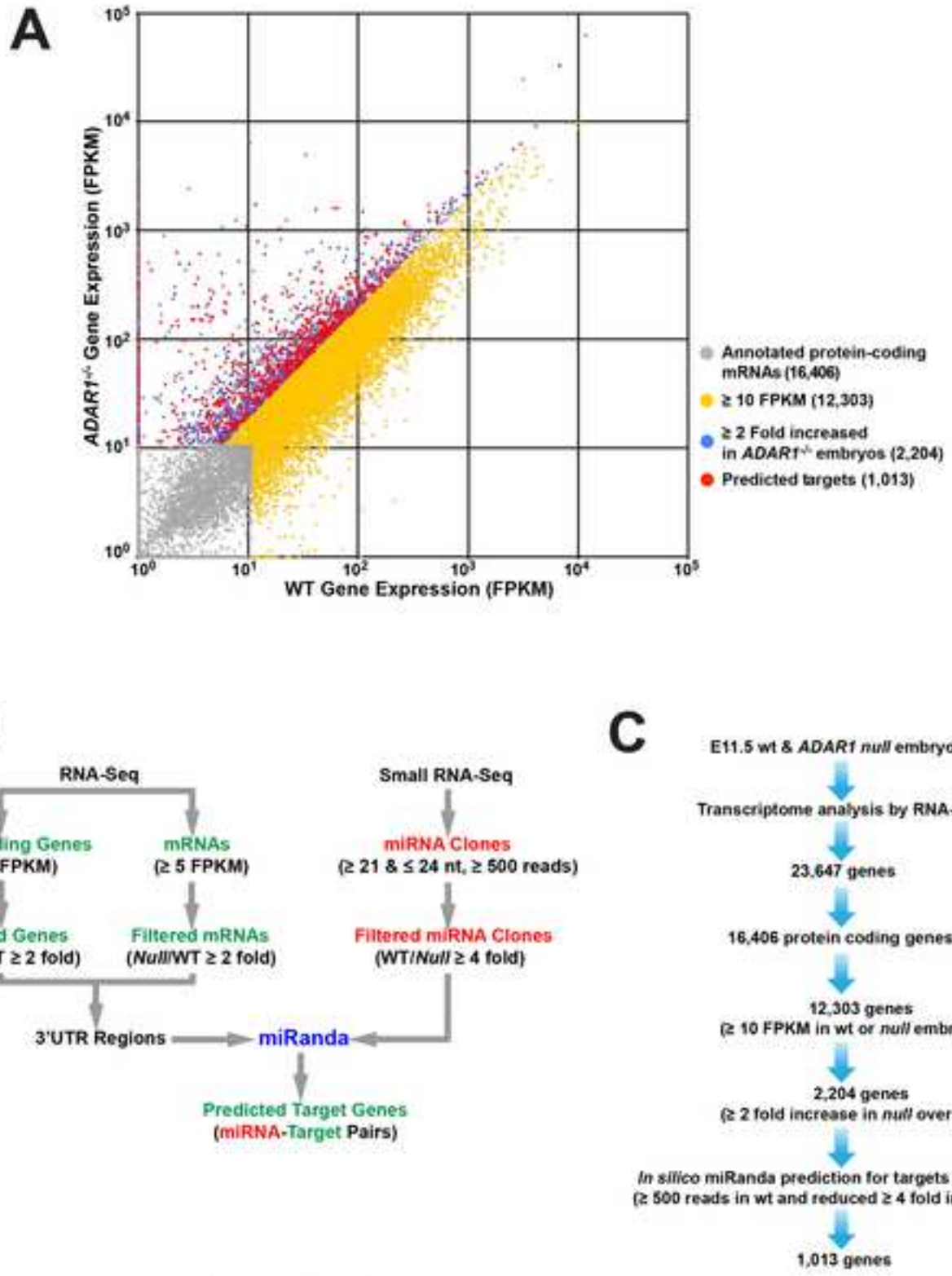
Supplementary Figure 6



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Supplementary Figure 7