Supporting Online Material for

Redirection of silencing targets by adenosine-to-inosine editing of miRNAs

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Materials and Methods

Mice

All procedures involving mice were approved by the Institutional Animal Care and Use Committee of The Wistar Institute and performed in accordance to the US National Institutes of Health Guidelines.

RNA preparation

Human total RNA was obtained from Clontech (Palo Alto, CA) and Ambion (Austin, TX). A set of human RNAs and genomic DNA extracted from the brain of a single individual was obtained from BioChain Institute (Hayward, CA). Mouse total RNA was extracted from dissected tissues using TRIZOL reagent according to the manufacturer's instructions (Invitrogen, Carlsbad, CA). Small RNA (<200 nt) was extracted from 50 μg of total RNA using mirVana^{TM} miRNA Isolation Kit (Ambion).

Determination of pri-miR-376 cluster RNA editing sites

First-strand cDNA was synthesized using 1 μg of total RNA extracted from human or mouse tissues and miR-376-specific RT primers. The resultant cDNA was then amplified by PCR using PCR primers. RT-PCR products were directly sequenced. The editing frequency was determined as the % ratio of the "G" peak over the sum of "G" and "A" peaks of the sequencing chromatogram. Two independent measurements were done, which gave identical values. We confirmed that editing frequency determined by this method was not significantly different from that determined by sequencing of ~50 individual cDNA clones. Furthermore, we compared a set of "individual" human or mouse genomic DNA and pri-miR-376 RNA (cDNA) sequences and excluded the SNP possibility at any of the miR-376 RNA editing sites.

RT-PCR primers used for amplification of various pri-miR-376 RNAs

Primer sequences for RT-PCR of pri-miRNAs are listed. RT was conducted by using PriRV, then PCR was conducted by using PriFW and PriRV. Sizes of PCR products are shown in parentheses.

Pri-hs-miR-B1 (358 bp)
PriFW; 5'-TCTCCTGACCTTGTGATCTGCC-3'

PriRV; 5′-GCCCCTAATTGGTTTCTTCCG-3′

Pri-hs-miR-B2 (287 bp)
PriFW; 5′-GCAAAGTGCTCACCTGCTTCT-3′
PriRV; 5′-TCCCAGGACAGACCTTGCTAA-3′

Pri-hs-miR-368 (287 bp)
PriFW; 5′-TTAGCAAGGTCTGTCCTGGGA-3′
PriRV; 5′-ATTGTCTCTTCCCTGATGGTGG-3′

Pri-hs-miR-376a2 (280 bp)
PriFW; 5′-TGGGCTCCGTCGTCATTTT-3′
PriRV; 5′-CCATCTTTCCACTTACCCTGGA-3′

Pri-hs-miR-654 (231 bp)
PriFW; 5′-ATCAAATGCTGCCTTGGGATC-3′
PriRV; 5′-GCAACCGTTTTCAGTCCCGTA-3′

Pri-hs-miR-376b (267 bp)
PriFW; 5′-ACAGGCTGTGGTGTGTTGTCAC-3′
PriRV; 5′-GGCCTACCATCTTTGCACCTAC-3′

Pri-hs-miR-376a1 (282 bp)
PriFW; 5′-TGTGGGTCTCGTGGTGCCA-3′
PriRV; 5′-TGTGTCTGTCCGTCCTGTAC-3′

Pri-mm-miR-376c (305 bp)
PriFW; 5′-ACAGCCAGGTTGTTCCTGGAA-3′
PriRV; 5′-ACATGCTCTGTGGACCACTGAC-3′

Pri-mm-miR-376b (292 bp)
PriFW; 5′-AGGCGCCTTTCAACATTCTG-3′
PriRV; 5′-CCACGCCCCACTCATTATTACA-3′

Pri-mm-miR-376a (236 bp)
PriFW; 5′-TCTGATATACCGCCTCTTTGGG-3′
PriRV; 5′-CGCTTTCTTCGGTGAAGATGTC-3′

Pri-mm-miR-300 (316 bp)
PriFW; 5′-GGGCATGTGAATCATCTGGAA-3′
PriRV; 5′-TAGGGTCAGACGAAGAAGGAGC-3′

Characterization of mature miR-376a RNAs

Preparation of a cDNA library enriched in small RNA (<200 nt) was described previously (*1*). Briefly, 1.5 µg of small RNA was polyadenylated using the Poly(A) Tailing Kit (Ambion). A 5' adaptor (5'-CGACUGGAGCACGAGGACACUGACAUGGACUGAAGGAGUAGAAA-3') synthesized by Dharmacon (Lafayette, CO) was ligated to poly(A)-tailed RNA using T4 RNA ligase followed by RT using an RT primer: 5'-ATTCTAGAGGCCGAGGCGGCCGACATG-d$(T)_{30}$ (A, G, or C) (A, G, C, or, T)-3'. The resultant cDNA was then amplified by using PCR primers matFW, corresponding to the 5'

adaptor sequence, and a set of miR-376 isoform-specific primers to examine the 5' end sequences including editing sites. For examining the 3' end sequences, PCR was done by using PCR primers matDW, corresponding to the 3' linker sequence, and a set of miR-376 isoform-specific primers. RT-PCR products were subcloned, and more than 50 individual cDNA isolates representing each miR-376 cluster member were sequenced. Editing frequency of mature miRNAs was estimated as the % ratio of the cDNA clones containing the A→G change over the total cDNA clones examined.

PCR primers used for cloning of mature miR-376 cluster RNAs

Primer matFW (5'-CTGACATGGACTGAAGGA-3') corresponds to the 5' adaptor sequence and matRV (5'-ATTCTAGAGGCCGAGGCGGCCGACATGT-3') to the 3' linker sequence. PCR primers used for amplification of specific mature miR-376 RNAs (indicated by green letters) are listed below. The unique nucleotide present within the PCR-extended region of each miR-376 RNA is highlighted in gray. This unique nucleotide(s) made it possible to distinguish cDNA clones derived from related miR-376 isoforms. See Fig. S2 for alignment of all pri-miR-376 RNA sequences. Editing sites are indicated by "A"s in red. We could not clone human miR-B1-3p and miR-B2-3p molecules.

hs-miR-B1-3p (matFW/matB1-3pRV; 51 bp)

```
hs-miR-B1-3p          5'-AUCACACAGAGGAAGUACCAUCAGG-3'
Primer matB1-3pRV               3'-CTCCTTCATGGTAGTCCC-5'
```

hs-miR-B2-3p (matB2-3pFW/matRV; 81 bp)

```
hs-miR-B2-3p               5'-AUCAUAGAGGAAAAUCCGCAUUU-3'
Primer matB2-3pFW        5'-AATCATRGAGGAAAATCCG-3'(R; A or G)
```

hs-miR-368-3p (matFW/mat368-3pRV; 49 bp)

```
hs-miR-368-3p         5'-AACAUAGAGGAAAUUCCACGUUU-3'
Primer mat368-3pRV           3'-CTCCTTTAAGGTGCAAAT-5'
```

hs-miR-376a2-5p (matFW/mat376a2-5pRV; 51 bp)

```
hs-miR 376a2-5p       5'-GUAGAUUUUCCUUCUAUGGUU-3'
Primer mat376a2-5pRV           3'-GGAAGATACCAATGCAC-5'
```

hs-miR-376a1-5p (matFW/mat376a1-5pRV; 51 bp)

```
hs-miR376a1-5p        5'-GGUAGAUUCUCCUUCUAUGAGU-3'
Primer mat376a1-5pRV           3'-AGGAAGATACTCATTTT-5'
```

hs-miR-376a1-3p,hs-miR-376a2-3p & hs-miR-376b-3p (matFW/mat376-3pRV; 51 bp)

```
hs-miR-376a1(or2)-3p  5'-AUCAUAGAGGAAAAUCCACGU-3'
hs-miR-376b-3p        5'-AUCAUAGAGGAAAAUCCAUGUU-3'
Primer mat376-3pRV          3'-CTCCTTTTAGGTRCAWTTTT-5'(R; A or G, W; A or T)
```

mm-miR-376a-5p (matFW/mat376a-5pRV; 51 bp)

```
mm-miR-376a-5p        5'-GGUAGAUUCUCCUUCUAUGAGU-3'
Primer mat376a-5pRV           3'-GAGGAAGATACTCATTTT-5'
```

mm-miR-376a-3p (matFW/mat376a-3pRV; 53 bp)

```
mm-miR-376a-3p        5'-AUCGUAGAGGAAAAUCCACGU-3'
Primer mat376a-3pRV         3'-CTCCTTTTAGGTGCATTTTTTT-5'
```

```
mm-miR-376b-3p (matFW/mat376b-3pRV; 49 bp)
mm-miR-376b-3p          5′-AUCAUAGAGGAACAUCCACUU-3′
Primer mat376b-3pRV           3′-CTCCTTGTAGGTGAATTT-5′

mm-miR-376c-3p (matFW/mat376c-3pRV; 49 bp)
mm-miR-376c-3p          5′-AACAUAGAGGAAAUUUCACGU-3′
Primer mat376c-3pRV           3′-CTCCTTTAAAGTGCATTT-5′
```

Target prediction program

There are two major categories of mammalian miRNA-target interactions: (1) 5'-dominant and (2) 3'-compensatory (*2*). For a detailed description of these categories, we refer the reader to further publications (*2-5*). Most existing computational approaches focus primarily on predicting 5'-dominant interactions, though a very recent work from the Cohen lab makes a substantial effort to define rules for the prediction of 3'-compensatory interactions (*6*). These rules, however, have not been applied to the human genome and also have not yet been packaged into a publicly usable program. Therefore, we implemented an in-house algorithm that predicts both 5'-dominant and 3'-compensatory interactions. For 5'-dominant interactions we searched the human 3' UTR dataset for any 7-mer which has perfect base pairing to the miRNA seed sequence (nucleotides 1-7 or 2-8 starting from the miRNA 5'-end). For 3'-compensatory interactions we first searched the human 3' UTR dataset for any k-mer, where $7 \leq k \geq 8$, whose perfect base pairing to the miRNA seed sequence is interrupted by either: (a) a single G:U wobble when k=7, or (b) a single G:U wobble, a nucleotide bulge, or mismatch when k=8. We also searched for any k-mer, where $4 \leq k \geq 6$, which has a perfect base pairing to a portion of the seed sequence starting at either position 1 or 2 from the miRNA 5'-end. We call any such k-mer a *seed match*. We then used RNAhybrid to hybridize the non-seed sequence of the miRNA to the region immediately upstream of the seed match in order to compute the minimum free energy (MFE) hybridization of the non-seed. We considered the region (upstream of seed match + seed match) as a putative target site only if the MFE of the non-seed passes a specified threshold. The value of this threshold depends on the value of k for the corresponding k-mer/seed match. Thresholds were chosen as described previously by Stark et al. (*6*) Additionally, for 3'-compensatory target sites, we also searched for any 22-mer which binds to at least 80% of the entire mature miRNA, but allows G:U wobbles – although no more than one in the first consecutive four base pairings from the 5'-end of the miRNA. For conservation filtering, we defined human/mouse target site conservation as follows: (1) if k=7 or k=8, then no nucleotide differences allowed between the human and mouse seed match region, except for the special case where a single nucleotide difference introduces a G:U wobble base pair, or (2) if $4 \leq k \geq 6$, then no nucleotide differences allowed between the human and mouse seed match region and at least 80% overall target sequence identity.

Applied to the entire human genome, the program predicted 2,250 target genes for the unedited-version and 2,621 target genes for the edited-version of miR-376a-5p. We then searched for target genes with at least one target site conserved among different species (Table S2), which reduced the predictions to 327 for the unedited version and 301 for the edited version (Fig. S3A). Finally, we further filtered the predictions to retain those that have multiple target sites (at least one site conserved) yielding 78 target genes for unedited miR-376a-5p and 82 for edited miR-376a-5p (listed in Table S3 and Fig. S3A). We randomly selected three target genes from each of these two final prediction sets that have two binding sites within 1 kb for experimental verification.

Luciferase reporters

PCR products containing miR-376a-5p binding sites were amplified from human cDNAs pooled from brain, thymus, lung, spleen and liver, and then inserted into the multiple cloning sites in the luciferase reporter vector (pMIR-REPORT^{TM} Luciferase; Ambion) (Fig. S3C). Primer sequences and potential binding sites are listed below.

PCR primers used for amplification of miR376a1-5p unedited-version targets
Sequences inserted into the luciferase reporter vectors are shown. Two predicted binding sites are boxed. Primer sequences with linker sequences (*bold*) are underlined.

>SFRS11 (arginine/serine-rich splicing factor 11) 546 bp
CATCCACTAGTTGCACTAACCTTTTTTGGTGGCTAATTAGGGTTTAAATACAGAAACAAGATTTCAAAT
AAAACTGTCTTTGGCAGTGAGTAAATAGCATATTTTGAAGTAGAGTTGTATACTTTTTCATAAGATGTT
TGGGAATTTTTTTCCTGAAGTAATAATTTATTCCACATCTACATCAGTGAAAGCTATCTACCTATCCTG
AGTCTATCTTAAAGGAAAAAAAGAAAAAAACCTTATCTCTTGCCCTTATTTTGAATTTTCCACTCTTTC
ATTAATTTGTTTTAAGCTCCGTGTTGGAAAAAAGGGGTAGTGCATTTTAAATTGACCTTCATACGCTTT
TAAAATAAGACAAATCTACTTGATAATGTACCTTTATTTGATCTCAAGTTGTATAAAACCAATAAATTT
GTGTTACTGCAGTAGTAATCTTATGCACACGGTGATTTCATGTTATATATGCAAAGTAGGCAACTGTTT
TCTTAGTTACAGAAGTTTCAAGCTTCACTTTTGTGCAGTAGAAACAAAAGTAGGCTACAGTCTGTGCCA
TGTTG**ACGCGTGGATG**

>SLC16A1 (solute carrier family 16-A1 or monocarboxylic acid transporter 1 MCT 1) 981 bp
CATCCACTAGTTGAGCAGTTCATGACCCAGGATATCTGAAAATATTCTACTGGCCTGTAATCTACCAGT
GGTGCTCAATGCAAATAGTAGACATTTGTGTGGAAATCATACCAGTTGTTCATTGATGGGATTTTTGTT
TGACTCCTTACCAATAGCCTGAATTTGAGGAGGGAATGATTGGTAGCAAAGGATGGGGGAAAGAAGTAG
GTTCTGTTTTGTTTTGTTTTAATCTTAGCTTTTAATAGTGTCATAAAGATTATAATATGTGCCTTAAGT
TTTAGTCTTTAGAACTCTAGAGAGCCTTAACTTCTTAAACCATTTTTGCTGAATTCATCTATTTCGAGT
GTTGTGTTAAAAGGAAAAATAACAACTAACTTGTTTGAGGCAAATCTAAAATTTAAAATTAATCTTGCT
TCATTGTTACATGTAATATATTTCAGACATTTTCACTGGAAGATTTATGAACAGAAATATTGGTTGAAA
GTTAGAGATTTTACAAAATGCTGACAAAAATATTTTCCTAGCATCAGTAGATTTCTGGCATATGTTTCT
GCTAGCTATATATTTAGGAAATTCAAAGCATAAAACTTTGGCAACATCTTGGCTGTTCTAGACACAGTG
TACTTGTCAACCCCTCTCAGGTACCTTTTCTTGGGATGCTTATTAGAAGCCAAGTAAAGTGCTTAAGGT
TTGTTTTCATTAAATTAGCTATTTCTGCTCCCCTGTTCAAAGATGCATTTTGAGTGTTTATAGATCACT
GCCCTTTTTGAAATCACCTGGTATTATTTTTCTTACTGGAAAAGTTAGTATTAAAATCTACAGAACTAC
ATATTTGTGCCTCCTTGGTAAATACAACACATCTAATTAAATGTAGACAGATATTTCAAACATCAGCTG
AATTCACTTAAGTTTTTCCAAAACCTCAGTTAAACTGTGAAGCTATTGGAATTTTTTTTTCCTGGAATT
TTTCCCCTTTGATTCACAGTGGTCCC**AAGCTTGGATG**

>TTK (threonine and tyrosine kinase or monopolar spindle type1, MPS1) 445 bp
CATCCACTAGTCCAAATGGCCAAGGGAACCACTGAAGAAATGAAATATGTTCTGGGCCAACTTGTTGGT
CTGAATTCTCCTAACTCCATTTTGAAAGCTGCTAAAACTTTATATGAACACTATAGTGGTGGTGAAAGT
CATAATTCTTCATCCTCCAAGACTTTTGAAAAAAAAAGGGGAAAAAAATGATTTGCAGTTATTCGTAAT
GTCAaATACCACCTATAAAATATATTGGACTGTTATACTCTTGAATCCCTGTGGAAATCTACATTTGAA
GACAACATCACTCTGAAGTGTTATCAGCAAAAAAAATTCAGTAGATTATCTTTAAAAGAAAACTGTAAA
AATAGCAACCACTTATGGtACTGTATATATTGTAGACTTGTTTTCTCTGTTTTATGCTCTTGTGTAATC
TACTTGACATCATTTTACTCTTGGAATAGTGGGTGGATAGCA**AAGCTTGGATG**

PCR primers used for amplification of miR376a-5p edited-version targets

>PRPS1 (phosphoribosyl pyrophosphate synthetase 1) 718 bp
CATCCACTAGTGCCATCAGGAGAACTCACAATGGAGAATCCGTTTCTTACCTATTCAGCCATGTCCCTT
TATAATAGAGTAACTTCTGAGGCTTTTTGAGAATAAAATCCACCCCACCCTTGTTTCCCCTTGGTATTT
GATGACAAATTCAGCAGAAGACCCGGCTTGCTCCAGTGTAGCTTTCTACATCCCACATCAGGTATATTA
GAGCTTATCCGAACTGGGGAAAGACGGATTGAGATTAACTGCTGGGACCTCCTACCTGCATTATCTCAT
TCTGGCTTCCTTGATAATTCTGTGGGCCTTGCAGCTTTAACTATAGCTCAGCTGCTGCAAGATTTCAGA
CTTTTGAGGATGTTGTGTGAGGGTGTTTGACTGTGACTGGGGAAGCTCAGACTACTTTGTATGTGAATG
CTTCAGGGTTTTCTTTGTTGAGAACAACTAGCAACAAAGGCAACCCATGTGTGACCAGTTCTCCCCAAG
GTCTATGCTAAATTATAGCAAGAGCCCTGGGCAACCCCAAACCTAGTCCTGGTAGCTGAGCACCCTGTA
AGGCAGGAGCAGGCAGCTCAGCTTGAGCAGACATTGGGTGGGGGGTGGGGGGTGGTTGAGGGGGGAGGC
AGCACAGTGCAGCAAATGTTTCTTGGGAGGAAGAAGCCTGATCCATCACCATCTGCTTGACTATGTAGC
TTGGATTCTCCTTTGTACCTATCCCTTTCGATTTGGCTT**AAGCTTGGATG**

>ZNF513 (zinc finger protein 513) 230 bp
CATCCACTAGTAGAAGCCACCTTTTTTCTCCCCCGCTGGCCAGGGGCTCCACACAGACTAACGTAGGCAC
TATAAGGACCAGCCCAACCCCATGGGCGGGGGGGCCCATATGGACCAGGGGACCTTGCCTTGACTGAGG
CACTTCACGAGCTCAGTGAGAAGGGCCCTGTATTCACCTCCACTGCCCCCAGGGGCTGTGGACAAACCG
GCTGGGGGACTGCCCAGCCTCCCACCTGTTTATT**AAGCTTGGATG**

>SNX19 (sorting nexin 19) 567 bp
CATCCACTAGTTGCCATCCTCCCTGAGATTGTTTGCCAGAATCCTGGCCCTGTGCACACATGTGCTTAA
TCCACCAGCACACAAGCTCTAGATAGAACAGGAAAAAAACTGTGGCCAGATTTCCTTGGAGGAGAAAAC
AACCTTCTCAGCTCTCTGTTCCTACCCGGAAACTAAAAATTCATTCAGGGCCATTCTCAAAAACCAGAC
TCCTGGGTCTAATCTAGTTTCCCAACAGTTCCAGAAGAAGAAGGAGGGAAGAAAATTTTTCAAGGAACT
TCTTGCTCATGTGTTGCTGTTTCCCAACACATCAGCCTTATGGAAGATGTCATGAGGCTGCTTGCCTGG
GAGGCTACACTTTCCTTTCCAGAAGACTTCAAAGCTGACTGCCAAAGCTTCTGGTAAATCCACCCCACC
CTTCACACCCTTTCCTGAAAGGCATTTATACCACCCATTTTATTTATTGGCTTTGCTCCCAACCCCCTG
CCCTAGAGCAATTGAGTTTTTGAATTTTTGACCCTCTCTCTGTCTTCAGCAAAACTGCATGACAAGCTG
CATATTCAGCCTCAGTTTTTCCAAGG**ACGCGTGGATG**

Transfections
Both unedited miR376a-5p (adenosine at +4 site) and "edited" miR376a-5p (A→G at +4 site) duplexes were synthesized at Ambion (Pre-miR™ miRNA). Edited miR376a-5p (A→I at +4 site) duplex was synthesized at Dharmacon (MiRIDIAN™ microRNA Mimic). Mismatches were introduced into duplexes to help promote activation of the sense strand. A negative control miRNA cel-miR-67 (miRIDIAN™ microRNA Mimic Negative Control) was also obtained from Dharmacon. All transfections were carried out in triplicate as described previously (*7*). Briefly, HeLa cells were pre-plated in 24-well tissue culture plates. Two hundred ng of luciferase reporter plasmid and 200 ng of control vector pMIR-REPORT™ β–gal Plasmid (Ambion) were diluted into 50 μl Opti-MEM (Invitrogen) with or without 10 pmol of either unedited or edited miR376a-5p duplex, followed by addition of 3 μl of lipofectamine 2000 (Invitrogen). After incubation according to the manufacturer's instructions, 100 μl transfection mixture was added to the HeLa cells in 500 μl of the growth medium. No expression of endogenous mature miR-376 RNAs was detected in HeLa cells. The luciferase activity was measured at 48 hours after transfection, using the Luciferase Assay System (Promega, Madison,

WI), together with the β-galactosidase activity by reading the absorbance at 415nm in a plate reader with β-Galactosidase Enzyme Assay System (Promega).

Protein Extraction

Cerebral cortices and livers of 3 wild-type and 3 *ADAR2 null* mice (12 week old) were minced using a pair of scissors in a buffer containing 0.25 M Tris (pH 7.8), 2 mM $MgCl_2$, 1 mM DTT, 1X complete protease inhibitor mixture (Roche Diagnostics, Indianapolis, IN), and 0.5 mM phenylmethylsulfonyl fluoride (PMSF), and kept on ice for 30 min. After sonication followed by centrifugation at 14,000 rpm for 15 min, the supernatant was transferred to new tubes and diluted with an equal volume of a buffer containing 0.1 M Tris (pH 7.0), 0.2 M NaCl, 2 mM $MgCl_2$, 40% glycerol, 1 mM DTT, 1X complete protease inhibitor mixture, and 0.5 mM PMSF. After the measurement of the protein concentration, the samples were kept at –80°C until use.

Western Immunoblot Analysis

Cerebral cortex or liver protein extracts of 12 week old wild-type or *ADAR2 null* mice (100 μg) were fractionated on an SDS-7% (or 15%) polyacrylamide gel and transferred to Immobilon™-P nylon membrane (Millipore, Bedford, MA) as described previously (*8*). Blots were blocked in a buffer containing phosphate-buffered saline (PBS), 0.1% Tween-20, and 5% nonfat dry milk. For analysis, we used the following antibodies: anti-TTK (Mps1) polyclonal antibody (Abcam, Cambridge, MA); anti-PRPS1 polyclonal antibody (Novus Biologicals, Littleton, CO); anti-Actin monoclonal antibody (Sigma, St. Louis, MO). The antibodies were diluted (anti-TTK, 1 : 2500; anti-PRPS1 and anti-Actin, 1 : 500), and incubated with the membrane for 1 hr at room temperature. Each protein band was detected by HRP-conjugated secondary antibodies using ECL Western blotting detection reagents (GE Healthcare, Piscataway, NJ). Both TTK and PRPS1 expression level were normalized by that of β-actin.

Uric Acid measurement

Cerebral cortices (0.3 g) and livers (1.0 g) of 6 wild-type and 6 *ADAR2 null* mice (12 weeks old) were minced using a pair of scissors in 3 ml and 10 ml of PBS buffer (pH 7.0), respectively, after removal of blood by perfusion. After sonication followed by centrifugation at 14,000 rpm for 15 min, the supernatants were then filtered through 0.2-μm syringe filters (Corning, Corning, NY) and stored at -80°C until use as previously described (*9*). Uric acid was measured with an Amplex Red-based assay kit (Invitrogen) using 50 μl of cerebral cortex extract and 5 μl of liver extract in duplicate.

Figure S1

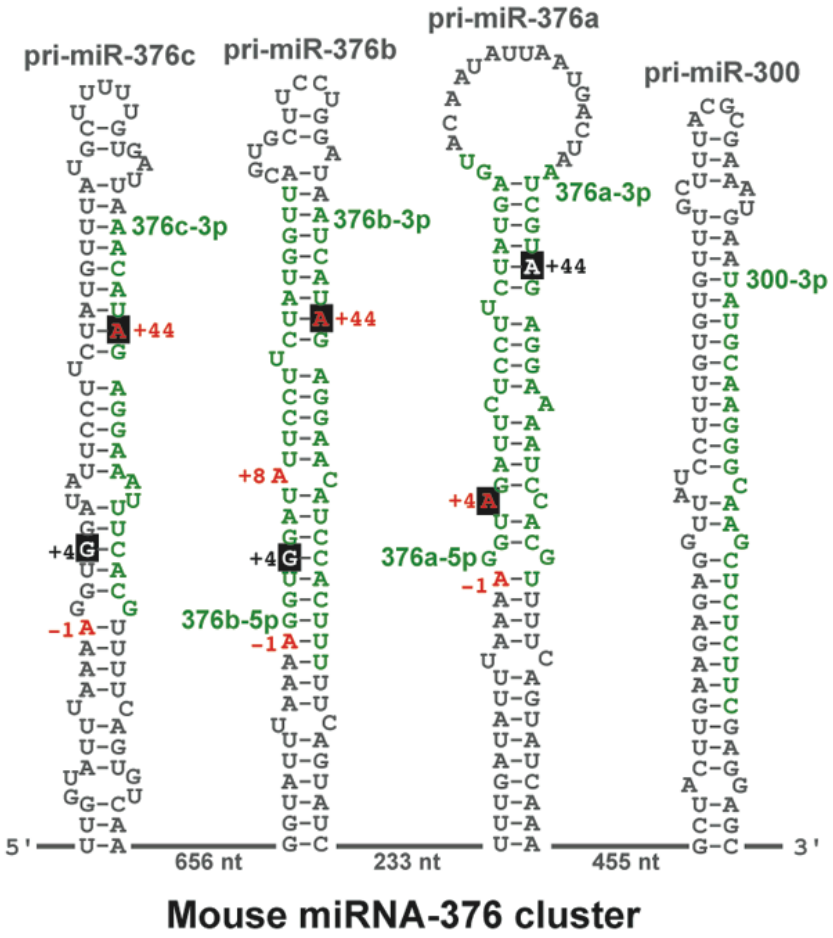

Fig. S1: A→I RNA editing of mouse pri-miR-376 RNAs. Hairpin structures of three mouse miR-376 cluster pri-miRNAs are shown. Editing sites are indicated by red “A”s. The two most highly edited adenosines (+4 and +44) are indicated by red "A"s highlighted in black. Each individual editing site is numbered, with the 5’ end of the human miR-376a1-5p sequence counted as +1. Editing sites of other miR-376 family members are numbered based on the human miR-376a1 hairpin structure. Regions known to be processed into mature miRNAs are highlighted in green. The genomically encoded guanosine at +4 is also highlighted. The genomic distance between miRNA genes is indicated by the numbers at the bottom.

Figure S2

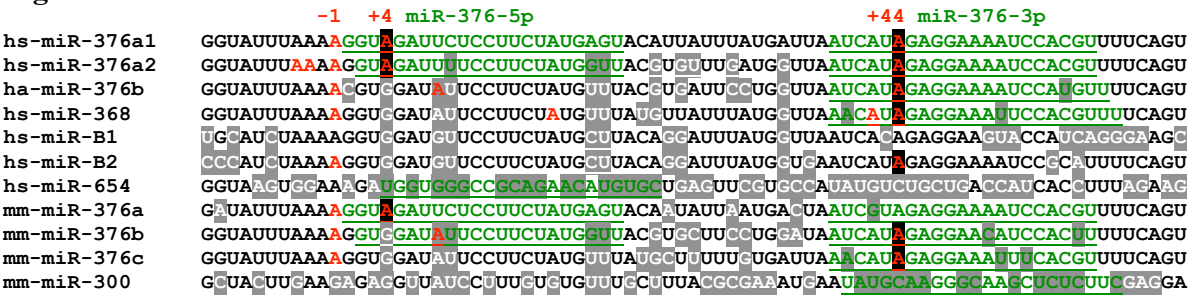

Figure S2: Alignment of pri-miR-376 cluster RNA Sequences. Sequences of all pri-miR-376 cluster RNAs as well as unrelated pri-miR-654 and pri-miR-300 sequences are aligned. Mature miRNA sequences are indicated by green letters and underlines. The sequence difference of all pri-miRNAs in comparison to the human pri-miR-376a1 sequence is highlighted in gray. These unique nucleotide differences made it possible to distinguish cDNA clones derived from related miR-376 isoforms. Editing sites are indicated by "A"s in red. Two major editing sites (+4 and +44 sites) are further highlighted in black.

Figure S3

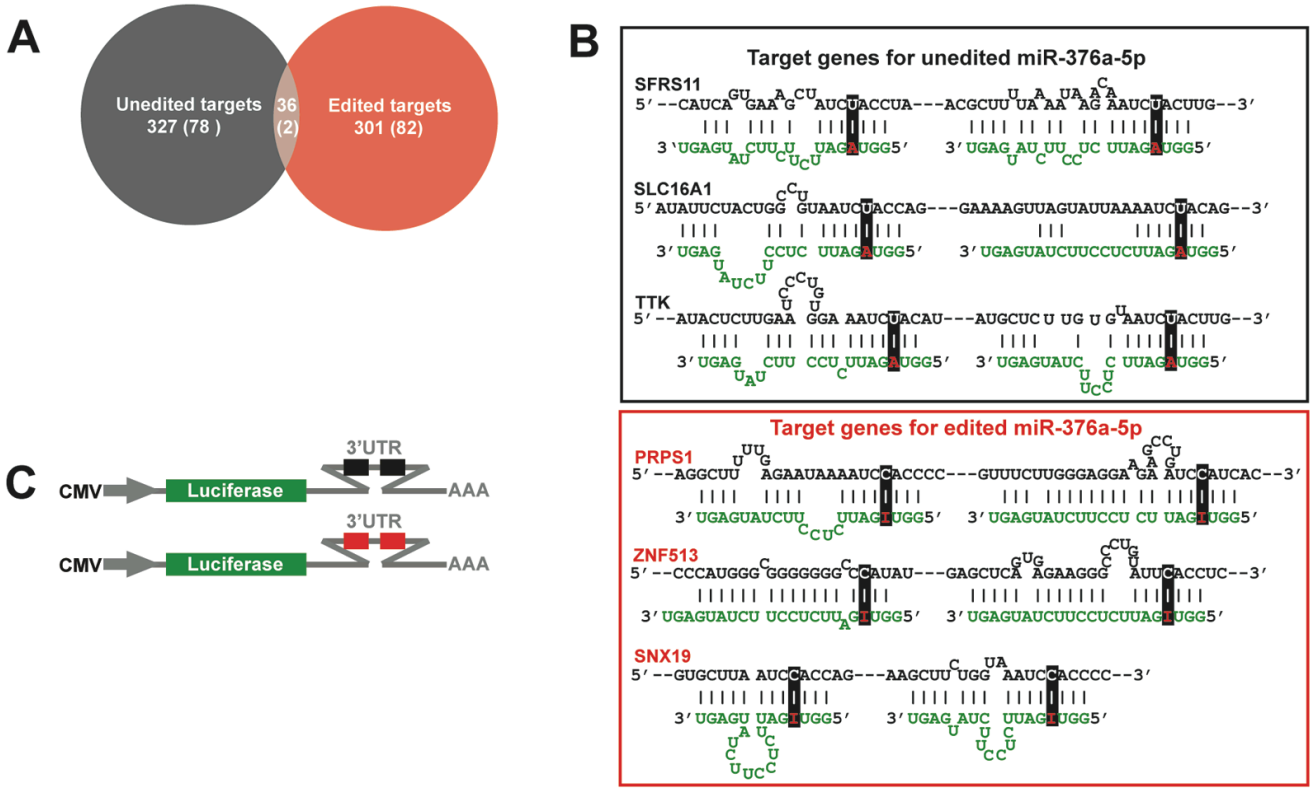

Fig. S3: Prediction of the targets for edited miR-376a1-5p. (A) *In silico* prediction of the targets of unedited and edited miR-376a-5p. The number of targets with at least one conserved site, and with multiple target sites (parentheses) is indicated. **(B)** RNA duplex formation between 3' UTR target sites and unedited or edited miR-376a-5p are diagrammed. The target location that pairs with the +4 site of unedited or edited miR-376a-5p is highlighted. **(C)** Diagram of luciferase reporter plasmids containing miR-376a-5p unedited-version (black boxes) or miR-376a-5p edited-version (red boxes) target sites in the 3' UTR.

Table S1: Editing of pri-miR-376 RNAs in human and mouse tissues

		Major sites			Minor sites				
miRNA	Tissue	site -1	site +4	site +44	site -4	site -3	site +8	site +17	site +42
hs-miR-376a1	cortex	16	46	41					
	medulla	14	41	25					
	amygdala	39	49	45					
	liver	0	0	17					
	lung	17	30	20					
hs-miR-376a2	cortex	55	90	98	8	13			
	medulla	52	97	98	9	17			
	lung	44	68	90	6	7			
hs-miR-376b	cortex	0	Genomically G	95			7		
	medulla	6	Genomically G	94			18		
	lung	6	Genomically G	55			8		
hs-miR-368	cortex	23	Genomically G	94				11	14
	medulla	28	Genomically G	96				24	12
	lung	19	Genomically G	59				0	0
hs-miR-B1	cortex	0	Genomically G	0					
	medulla	0	Genomically G	0					
	lung	0	Genomically G	0					
hs-miR-B2	cortex	0	Genomically G	13					
	medulla	7	Genomically G	29					
	lung	8	Genomically G	0					
mm-miR-376a	cortex/wild-type	24	48	0					
	cortex/*ADAR 2null*	6	9	0					
	heart/wild-type	6	12	0					
	kidney/wild-type	33	38	0					
	liver/wild-type	0	7	0					
	lung/wild-type	7	28	0					
	E11.5 embryo/wild-type	0	8	0					
	E11.5 embryo/*ADAR1 null*	0	13	0					
mm-miR-376b	cortex/wild-type	22	Genomically G	52			31		

	cortex/*ADAR 2null*	0	Genomically G	78			0		
	lung/wild-type	0	Genomically G	14			17		
	E11.5 embryo/wild-type	0	Genomically G	14			0		
	E11.5 embryo/*ADAR1 null*	0	Genomically G	0			0		
mm-miR-376c	cortex/wild-type	18	Genomically G	66					
	cortex/*ADAR 2null*	0	Genomically G	96					
	lung/wild-type	18	Genomically G	0					
	E11.5 embryo/wild-type	0	Genomically G	40					
	E11.5 embryo/*ADAR1 null*	0	Genomically G	0					

Table S2: Summary of miR-376a-5p target genes

# Target Genes	All genes (with conserved and/or non-conserved target sites)	At least one conserved target site among human, chimp and mouse
Unedited miR-376a-5p	**2250**	**327**
Edited miR-376a-5p	**2621**	**301**
Common to both	732	36
Common to both (with same target site)	189	17
# Target Genes with Multiple Sites (not necessarily conserved)		
Unedited miR-376a-5p	**344**	**78**
Edited miR-376a-5p	**487**	**82**
Common to both	66	2
Common to both (with same target site)	28	0

Table S3: Target genes of unedited and edited miR-376a-5p RNAs

Unedited miR-376a-5p target genes with multiple target sites (at least one conserved site)	Edited miR-376a-5p target genes with multiple target sites (at least one conserved site)
ENSG00000017427	ENSG00000071537
ENSG00000186628	ENSG00000155545
ENSG00000101892	ENSG00000065371
ENSG00000113657	ENSG00000096063
ENSG00000125266	ENSG00000103061
ENSG00000125375	ENSG00000141622
ENSG00000172260	**ENSG00000163795 (ZNF513)**
ENSG00000172057	ENSG00000110436
ENSG00000114127	ENSG00000062716
ENSG00000157617	ENSG00000111269
ENSG00000172493	ENSG00000104643
ENSG00000110680	ENSG00000110680
ENSG00000185640	ENSG00000186908
ENSG00000075391	ENSG00000163914
ENSG00000177098	ENSG00000183715
ENSG00000157020	ENSG00000158715
ENSG00000133121	ENSG00000116455
ENSG00000183715	ENSG00000177000
ENSG00000170624	ENSG00000114735
ENSG00000157680	ENSG00000138138
ENSG00000101856	ENSG00000165495
ENSG00000131697	ENSG00000197774
ENSG00000109171	ENSG00000172403
ENSG00000163743	ENSG00000149485
ENSG00000116754 (SFRS11)	ENSG00000198915
ENSG00000153814	ENSG00000154478
ENSG00000137075	ENSG00000168314
ENSG00000135913	ENSG00000176276
ENSG00000147246	ENSG00000162599
ENSG00000155380 (SLC16A1)	ENSG00000157106
ENSG00000141150	ENSG00000116133
ENSG00000139350	ENSG00000108774
ENSG00000143437	ENSG00000186642
ENSG00000112742 (TTK)	ENSG00000077279
ENSG00000138757	ENSG00000161526
ENSG00000165983	ENSG00000166170
ENSG00000144406	ENSG00000101445
ENSG00000157450	ENSG00000172115
ENSG00000130779	ENSG00000115194
ENSG00000161896	ENSG00000101337
ENSG00000067082	ENSG00000187210
ENSG00000154114	ENSG00000134369
ENSG00000083642	ENSG00000185920

ENSG00000174013	ENSG00000142619
ENSG00000188167	ENSG00000172137
ENSG00000165424	ENSG00000160714
ENSG00000070808	ENSG00000092421
ENSG00000077157	ENSG00000171604
ENSG00000169991	ENSG00000100403
ENSG00000073282	ENSG00000149256
ENSG00000143653	ENSG00000186702
ENSG00000163071	ENSG00000002834
ENSG00000087301	ENSG00000140320
ENSG00000179241	ENSG00000171152
ENSG00000134121	ENSG00000103888
ENSG00000119042	ENSG00000048991
ENSG00000141084	ENSG00000157483
ENSG00000155111	ENSG00000196090
ENSG00000101109	ENSG00000148943
ENSG00000065559	ENSG00000070366
ENSG00000170044	ENSG00000175606
ENSG00000167580	ENSG00000154102
ENSG00000169330	ENSG00000156687
ENSG00000170921	ENSG00000022355
ENSG00000044524	ENSG00000119912
ENSG00000197959	ENSG00000142149
ENSG00000153234	ENSG00000139926
ENSG00000135503	ENSG00000164088
ENSG00000116604	ENSG00000009307
ENSG00000157224	ENSG00000113721
ENSG00000102753	ENSG00000163349
ENSG00000119457	**ENSG00000147224 (PRPS1)**
ENSG00000062725	ENSG00000158604
ENSG00000115137	ENSG00000100170
ENSG00000182836	ENSG00000165886
ENSG00000125447	ENSG00000156860
ENSG00000101266	ENSG00000164742
ENSG00000015153	**ENSG00000120451 (SNX19)**
	ENSG00000181007
	ENSG00000198838
	ENSG00000168672
	ENSG00000090776

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