Systematic identification of edited microRNAs in the human brain

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Supplementary Methods, Figures, Tables and References

Supplementary Methods

In-vitro over-expression experiments

Human ADAR (transcript variant 1), ADARB1 expression vectors or a control vector (a kind gift from Prof. Gideon Rechavi and Dr. Nurit Paz) were transiently transfected to U87 human glioblastoma cells (ATCC HTB-14). Cells were seeded in six-well plates in DMEM supplemented with 10% FBS and 1% Pen/Strep. Transfections were performed 24 hours later using Lipofectamine 2000 transfection reagent (Invitrogen, USA) according to the manufacturer's instructions. Transfection efficiencies were measured by CFP fluorescence, indicating a transfection efficiency of ~50%. Cells were harvested 24 hours later. Total RNA was extracted using the miRNeasy Mini Kit (Qiagen, USA). The final RNA concentration and purity were measured using a NanoDrop ND-1000 spectrophotometer (Thermo Scientific, USA). The elevated levels of the ADARs in the total RNA extracted were confirmed by mRNA quantitative RT–PCR. First-strand cDNA was synthesized from total RNA using a MultiScribe reverse transcriptase reaction with the High Capacity cDNA kit (Applied Biosystems, USA) and random hexamer primers (Applied Biosystems). Mixtures containing cDNA, RNase-free water, specific primers (Sigma, USA) and Power SYBR green PCR master mix (Applied Biosystems), were loaded on 96-well plates (Axygen Scientific, USA). PCR amplification was performed using ABI Prism 7900HT Sequence Detection System under the following thermal cycler conditions: 2 min at 50ºC, 10 min at 95ºC, 40 cycles of (15 sec at 95ºC and 1 min at 60ºC) and dissociation curve cycle of 15 sec at 95ºC, 15 sec at 60ºC and 15s at 95ºC. Standard curve was first created for each pair of primers to determine proper primer concentration for linear amplification. GAPDH was used as endogenous control. The primers used were:
ADAR-Fwd: tca tcc cac tat tcc aca gag a; ADAR-Rev: gct ctt ccc aga aaa gaa gga;
ADARB1-Fwd: ggt gac ctg acc gac aac tt; ADARB1-Rev: ttg gca tct tta aca tct gtg c;
GAPDH-Fwd: tgc acc acc aac tgc tta g; GAPDH-Rev: gga tgc agg gat gat gtt c;
Overall, ~100-fold and ~1000-fold increases were observed in ADAR and ADARB1, respectively (Supplementary Figure 2). We also validated that the over-expression alters the editing level of the known editing site in the coding region of CYFIP2. This was done by direct sequencing of the CYFIP2 editing site in the three conditions tested and viewing the resulting chromatograms, following the procedure described in Paz et al. (2007). As expected (Paz et al. 2007), ADARB1 over-expression increased the editing levels at this position (up to 20%), whereas in the ADAR over-expression and the control condition no editing was detected (Supplementary Figure 3). No random/nonspecific editing was found in this transcript due to ADAR overexpression, as previously described in Paz et al. (2007).

The over-expression of ADARB1 in U118 human glioblastoma cells (ATCC HTB-15) was described in Cenci et al. (2008). Briefly, EGFP-ADARB1 was stably transfected to U118 cell-line as well as EGFP alone as control (Cenci et al. 2008). The exogenous level of ADARB1 was confirmed by mRNA quantitative RT–PCR and Western blot (Supplementary Figure 4) in a similar procedure to the one described in Cenci et al. (2008). Total RNA was extracted from the U118 cells using mirVana miRNA Isolation Kit (Ambion, USA).

Sequencing of the *in-vitro* over-expression experiments

MiRNA capturing and library construction, for the U87 and U118 cell-lines samples, were conducted using Illumina's TruSeq Small RNA Sample Prep Kit following the manufacture protocol. The mature miRNA libraries from the U87 cell-line were sequenced on two lanes of Illumina HiSeq2000 instrument following the manufacture protocol. In each sequencing lane three bar-codes were used, one for each tested condition (ADAR and ADARB1 over-expression and control). The reads were filtered using Illumina's HiSeq2000 softwares. The total number of reads in both lanes after filtering was ~9.7M, ~10.4M and ~12.6M for the ADAR, ADARB1 over-expression and control, respectively. Next, the reads were aligned against the human genome, as described in the Methods section 'Aligning the reads against known miRNAs', giving ~5.3M, ~4.9M and ~6.5M reads for the ADAR, ADARB1 over-expression and control, respectively. From which ~4.1M, ~3.5M and ~5M reads were aligned to known miRNAs regions for the ADAR, ADARB1 over-expression and control, respectively.

Similarly, the mature miRNA libraries from the U118 cell-line were sequenced on two lanes of Illumina HiSeq2000 instrument following the manufacture protocol. In each sequencing lane two bar-codes were used, one for each tested condition (ADARB1 over-expression and control). The reads were filtered using Illumina's HiSeq2000 softwares. The total number of reads in both lanes after filtering was ~8M and ~3.9M for the ADARB1 over-expression and control, respectively. Next, the reads were aligned against the human genome, as described in the Methods section 'Aligning the reads against known miRNAs', giving ~4.8M and ~2.4M reads for the ADARB1 over-expression and control, respectively. From which ~4M and ~2M reads were aligned to known miRNAs regions for the ADARB1 over-expression and control, respectively.

Analyzing the *in-vitro* over-expression data

The sequencing error rate estimation was performed as described in the Methods section 'Estimating the sequencing error rate'. The total mismatch rates (summing all the 12 types of mismatches) for the different positions were 0.03-0.13%, 0.03-0.08% and 0.03-0.09%, for the ADAR, ADARB1 over-expression and control conditions in the U87 cell-line, respectively. In the U118 cell-lines the total mismatch rates for the different positions were 0.02-0.12% and 0.02-0.08%, for the ADARB1 over-expression and control conditions, respectively. These numbers are compatible with the expected estimation from the phred score (<0.1%). Importantly, in all of the examined conditions no single mismatch type had rates higher than 0.1% at any position.

Potential editing sites were first identified separately for each examined condition in the U87 cell-line (ADAR, ADARB1 over-expression and control) as described in the Methods section 'Identifying editing sites in miRNAs'. 1, 7 and 1 statistically significant A-to-G modifications were detected in the ADAR, ADARB1 over-expression and control, respectively, suggesting that ADARB1 had more impact on the editing levels of the miRNAs (Supplementary Table 5). Alternatively, the over-expression level of ADAR might have been insufficient to promote miRNA editing by ADAR in these tissue samples. Each one of the A-to-G modifications in the ADAR or

ADARB1 over-expression conditions was also tested against the control condition and the other over-expression condition in order to see if the modifications are specific to the respective sample compared with other tested conditions. Indeed, the specificity of all these events was found to be statistically significant (P-value<0.05, binomial cumulative distribution), except miR-589 which appears to be edited in the control condition as well (Supplementary Table 5). Excluding miR-589, 5 A-to-G modifications were detected in miRNAs that were also expressed in the pooled brain sample. Importantly, 4 of them were also detected as edited in the pooled brain sample, confirming our methodology (Supplementary Table 5).

Potential editing sites were also identified in each of the examined condition in the U118 cell-line (ADARB1 over-expression and control). 19 statistically significant A-to-G modifications were detected in the ADARB1 over-expression whereas no statistically significant modifications A-to-G were detected in the control (Supplementary Table 5). As before, each one of the A-to-G modifications in the ADARB1 over-expression conditions was also tested against the control condition and found to be statistically significant (P-value<0.05, binomial cumulative distribution). Even though two different cell-lines were used (U87 and U118) with two different transfection procedures (transient versus stable), all the editing sites detected in the U87 over-expression experiment were also detected in the U118 over-expression experiment, showing that the detection procedure is robust (Supplementary Table 5).

Overall, 13 miRNAs that were detected as edited in the pooled brain sample were expressed in the U87 and U118 cell-lines, 7 of which were indeed confirmed in the cell-lines. The *in-vitro* over-expression data also enabled the detection of 13 more editing sites (Supplementary Table 5). All of them seem to be ADARB1 targets (Supplementary Table 5). Two of them were also detected in the frontal lobe samples (see below and Table 1).

The sequence and structural motifs of the observed modifications that were found in the U87 and U118 samples, but not in the human brain sample or in the frontal lobe samples, were examined (Supplementary Tables 2 and 4). In line with the expected properties of true A-to-I editing sites (Kleinberger and Eisenberg 2010), we find enrichment of C or U in the nucleotide opposing the editing site (10 out of 11 sites), and depletion of G in the upstream nucleotide position (1 out of 11 sites). However, over-representation of G in the downstream nucleotide (4/11) and enrichment of U in the upstream nucleotide position (2/11) were not detected.

Human frontal lobe miRNA sequencing and analyzing

Normal frontal white matter samples, obtained from pediatric patients undergoing focal brain resection for head injury (e.g. brain contusion), were used. The two frontal lobe samples (A and B) were obtained from 3 years old boy and 3 months old girl, respectively. Brain samples were collected during neurosurgery of the patients at the Policlinico Gemelli in Rome. The study was approved by the local ethical committee.

Total RNA was extracted from the two frontal lobe samples using mirVana miRNA Isolation Kit (Ambion, USA). The mature miRNA libraries from the two frontal lobe samples were sequenced on two lanes of Illumina HiSeq2000 instrument following

the manufacture protocol. In each sequencing lane two bar-codes were used, one for each frontal lobe sample. The reads were filtered using Illumina's HiSeq2000 softwares. The total number of reads in both lanes after filtering was ~40M and ~18M for sample A and sample B, respectively. Next, the reads were aligned against the human genome, as described in the Methods section 'Aligning the reads against known miRNAs', giving ~5.5M and ~3.4M reads for sample A and sample B, respectively. From which ~3.1M and ~1.6M reads were aligned to known miRNAs regions for sample A and sample B, respectively.

The total mismatch rates for the different positions along the miRNA sequence were 0.02-0.24% and 0.03-0.19%, for sample A and sample B, respectively. These numbers are slightly higher compared to the expected estimation from the phred score (<0.1%). Importantly, in the two samples no single mismatch type had rates higher than 0.2% at any position. Therefore, the sequencing error rate was set to 0.2% in the analysis of these two samples. Potential editing sites were identified separately for each sample as described in the Methods section 'Identifying editing sites in miRNAs'.

14 statistically significant A-to-G modification sites were identified in these two samples (Table 1 and Supplementary Table 5). Importantly, 9 out of the 14 were also identified in the pooled human brain tissue (Table 1).

Two non A-to-G modifications sites were detected in the U87 *in-vitro* over-expression data, in let-7a-1 mature position 9 and miR-100 mature position 13, these modifications were U-to-G and C-to-U, respectively. One of these sites, in let-7a-1, appeared in all the tested conditions and in the second site, in miR-100, traces of the modification can be found in all the tested conditions. Two more non A-to-G modifications sites were detected in the U118 cell-line control data, in miR-148a and miR-148b, these two modifications were C-to-U in mature position 17. One of these sites, in miR-148a, appeared also in the ADARB1 over-expression and in the second site, in miR-148b, traces of the modification can also be found in the ADARB1 over-expression. The non A-to-G modifications sites might be somatic mutations that occurred in a small fraction of the cell-line sample, or might be connected to the cancerous origin of the cell-line, as they were observed in NGS of miRNAs from human melanoma (Stark et al. 2010). Four C-to-U modifications were observed in each of the frontal lobe samples, two of them appearing in both samples. The nature of these is yet unclear.

Analyzing publicly available mouse brain miRNAs sequencing data

Five datasets were downloaded from the Sequence Read Archive (SRA): SRR346417, SRR346423, SRR038744, SRR038741 and SRR345196, which contain mature miRNA NGS reads from mouse cerebellum, cortex (first sample), cortex (second sample), hippocampus (first sample) and hippocampus (second sample), respectively. The reads were filtered as described in the Methods section 'Human brain miRNA Sequencing'. The total number of reads after filtering was ~19M, ~19M, ~6M, ~3.9M and ~3.8M for the cerebellum, cortex (first sample), cortex (second sample), hippocampus (first sample) and hippocampus (second sample), respectively. Next, the reads were aligned against the mouse genome, as described in the Methods section 'Aligning the reads against known miRNAs', giving ~8.3M, ~8.2M, ~2.2M, ~1.4M and ~0.75M reads for the cerebellum, cortex (first sample), cortex (second sample),

hippocampus (first sample) and hippocampus (second sample), respectively. From which ~7.8M, ~7.6M, ~1.8M, ~1.2M and ~0.35M reads were aligned to known miRNAs regions for the cerebellum, cortex (first sample), cortex (second sample), hippocampus (first sample) and hippocampus (second sample), respectively.

The sequencing error rate estimation was performed as described in the Methods section 'Estimating the sequencing error rate'. Potential editing sites were identified separately for each sample as described in the Methods section 'Identifying editing sites in miRNAs' (with the sole exception that a Bonferroni corrected P-value=0.05 was used instead of Benjamini-Hochberg false-discovery rate of 5%). As expected, almost all of the modifications detected (98.8%) were A-to-G (Supplementary Figure 5). Overall, 32 statistically significant A-to-G modifications were detected (Supplementary Tables 7-8). Note that the number of detected editing sites increases with the total number of reads in the sample (Supplementary Table 7, the samples are arranged by the total number of reads).

The sequence and structural motifs of the observed modifications in the mouse brain samples are in line with the expected properties of true A-to-I editing sites (Figure 2, Supplementary Tables 2 and 9, Kleinberger and Eisenberg 2010). We find enrichment of U and depletion of G in the upstream nucleotide position (14 U and 1 G out of 32 sites). The nucleotide opposing the editing site is typically C or U (30/32), and G is over-represented in the downstream nucleotide (20/32).

The detected sites include most of the previously discovered editing sites: 10 out of 16 editing sites reported in Chiang et al. (2010), as well as 16 novel editing sites (Supplementary Table 7). 10 of the sites identified appear also in the results obtained for human brain and brain-originated human cell-lines, demonstrating that many of the editing sites in miRNAs are conserved (Table 2).

Four non A-to-G modifications sites were detected in the mouse brain samples: miR-34a mature position 15 (cerebellum and cortex), miR-409 5p mature position 9 (cerebellum), miR-411 mature position 9 (cerebellum and cortex) and let-7a-1 mature position 9 (hippocampus and cortex), all these modifications were C-to-U except the latter which was U-to-G. One of these sites, in let-7a-1, also appeared in the human U87 cell-line dataset. Again, all the non A-to-G sites taken together consists only a small fraction from the total number of modifications detected (Supplementary Figure 5).

Functional analysis of the edited miRNAs

From the list of 18 miRNAs that were edited in their binding sites (Table 1), 6 had statistically significant (Benjamini-Hochberg corrected P-value<0.05) over-representation of molecular functions in their mRNA targets. In miR-381 the over-represented molecular functions were: 'Long-term potentiation' and 'Insulin signaling pathway' (KEGG pathways; 7 and 10 genes, respectively), 'Homophilic cell adhesion' (GO Biological process, 11 genes) and 'Cytosol' (GO Cellular component, 35 genes). In miR-376a-1 'Transcription regulator activity' (GO Molecular function, 29 genes) was detected. In miR-589 'Neuron projection' and 'Cell projection' (GO Cellular component; 18 and 23 genes, respectively) were detected. In miR-497 'Regulation of

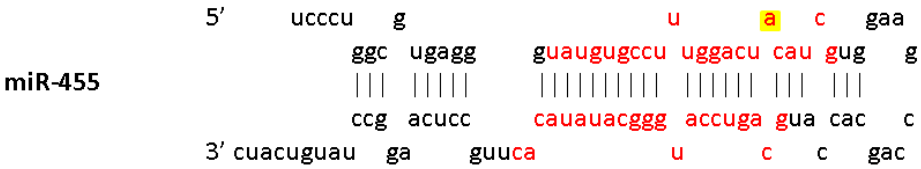
transforming growth factor beta receptor signaling pathway' (GO Biological process, 8 genes), 'Regulation of actin cytoskeleton' and 'Phosphatidylinositol signaling system' (KEGG pathways; 16 and 7 genes, respectively) were detected. In miR-376c 'Regulation of transcription' (GO Biological process, 45 genes), 'Lamellipodium' (GO Cellular component, 6 genes) and 'Transcription factor binding' (GO Molecular function, 16 genes) were detected. Finally, in miR-411 'Ion transport' (GO Biological process, 10 genes) was detected.

Similar analysis was performed for the edited miRNAs detected in the mouse brain samples (Supplementary Table 10). Again, the overlap between the putative mRNA targets before and after the editing was only ~3% on average (TargetScanMouse 5.2 Custom was used). From the list of 19 miRNAs that were edited in their binding sites, 5 had statistically significant (Benjamini-Hochberg corrected P-value<0.05) over-representation of molecular functions in their mRNA targets (that were created by the editing events). In miR-212 'Plasma membrane', 'Synapse' and 'Cell junction' (GO Cellular component; 29, 9 and 9 genes, respectively) were detected. In miR-376c 'DNA binding' (GO Molecular function, 23 genes) was detected. In miR-381 'Transcription factor activity' (GO Molecular function, 28 genes), 'Regulation of transcription' (GO Biological process, 46 genes), 'Neurotrophin signaling pathway' (KEGG pathways, 8 genes) and 'Adherens junction' (KEGG pathways, 6 genes) were detected. In miR-497 'Glioma', 'Regulation of actin cytoskeleton', 'Chronic myeloid leukemia', 'B cell receptor signaling pathway' and 'Pancreatic cancer' (KEGG pathways; 7, 11, 6, 6 and 6 genes, respectively) were detected. In miR-1251 'Homophilic cell adhesion' (GO Biological process, 13 genes), 'Cell-cell adhesion' (GO Biological process, 15 genes), 'Insoluble fraction' (GO Cellular component, 18 genes), 'Membrane fraction' (GO Cellular component, 17 genes) and 'Calcium ion binding' (GO Molecular function, 17 genes) were detected.

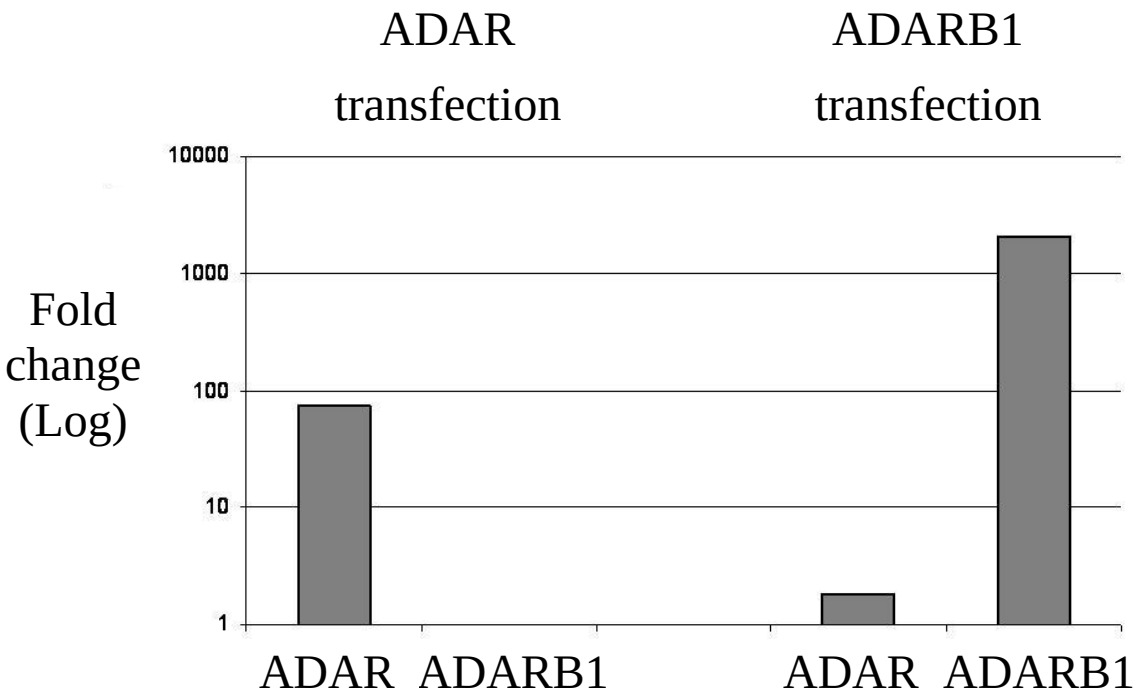
This analysis was also performed for the A-to-G modifications sites detected in the *in-vitro* ADAR over-expression experiments but not in the human pooled brain sample or in the human frontal lobe (Supplementary Table 4). Only two miRNAs change in their binding site. The overlap between the putative mRNA targets before and after the editing was ~2% on average. Statistically significant over-representation of molecular functions in their mRNA targets (that were created by the editing events) was not detected.

Supplementary Figures

Supplementary Figure 1: An example of a novel editing site detected in mature miRNA. The pre-miRNA sequence and secondary structure of miR-455 are presented. The 5p and 3p mature sequences are marked with red and the editing site is marked with a yellow box.

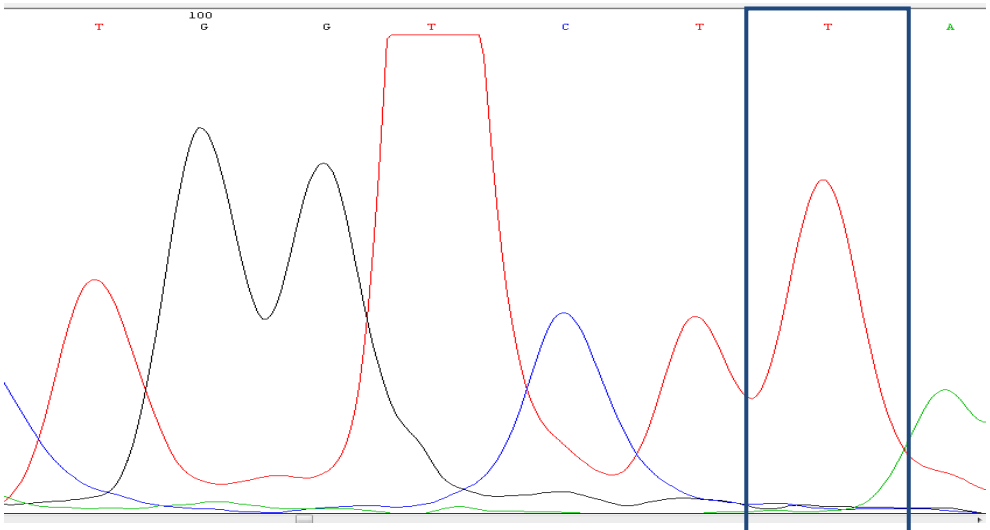


Supplementary Figure 2: mRNA quantitative RT–PCR confirms the elevated levels of the ADARs in the *in-vitro* U87 cell-line experiments.

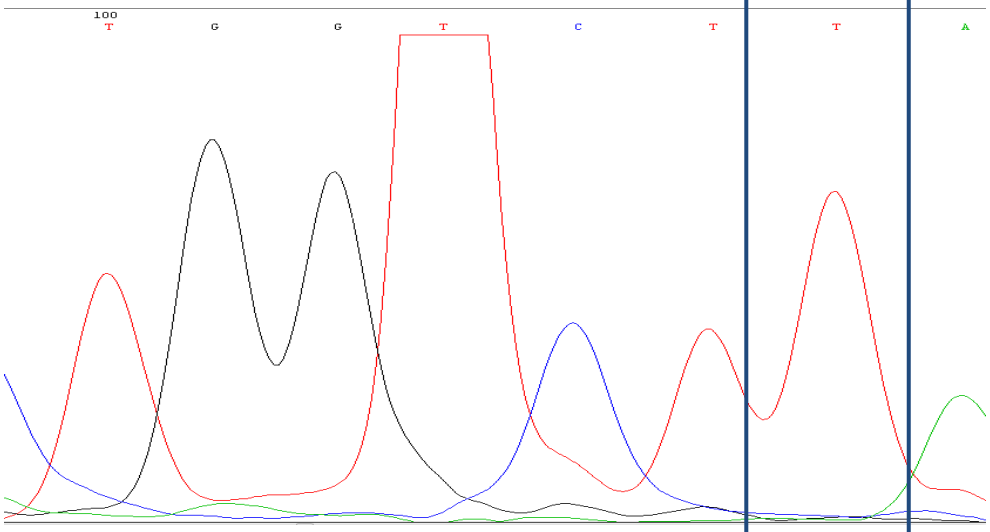


Supplementary Figure 3: Direct sequencing of a known ADARB1 editing site in CYFIP2 in U87 transfected cells. **(A)** Control condition. **(B)** ADAR over-expression. **(C)** ADARB1 over-expression. The editing position is marked with blue box.

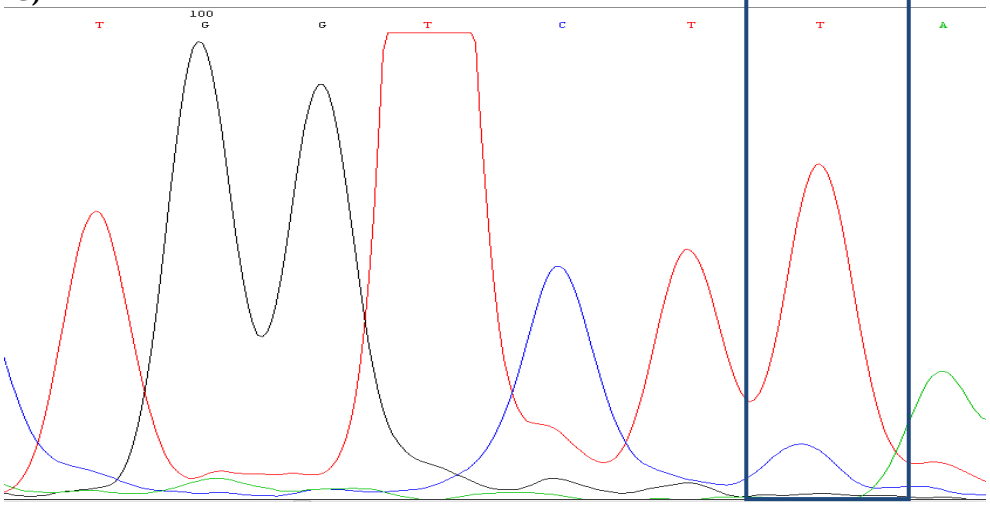
A)



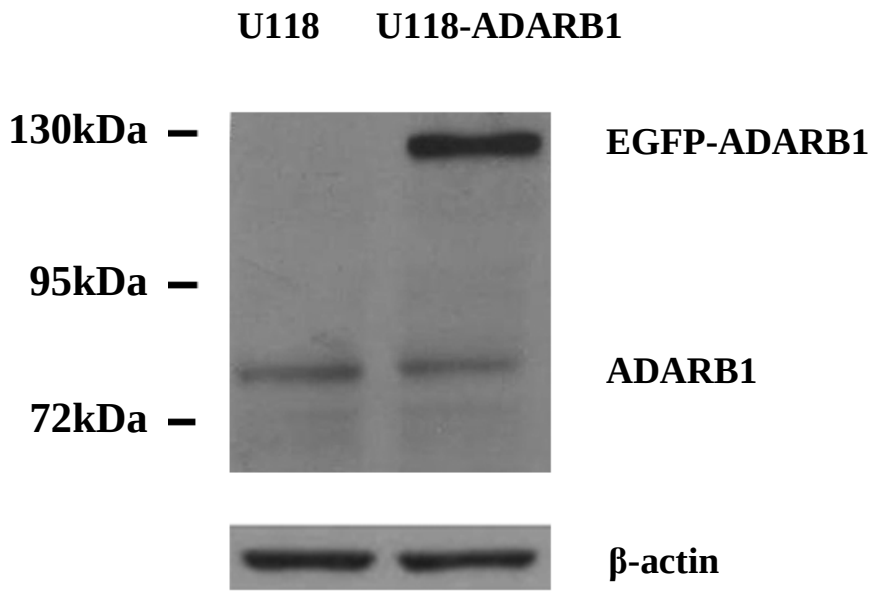
B)



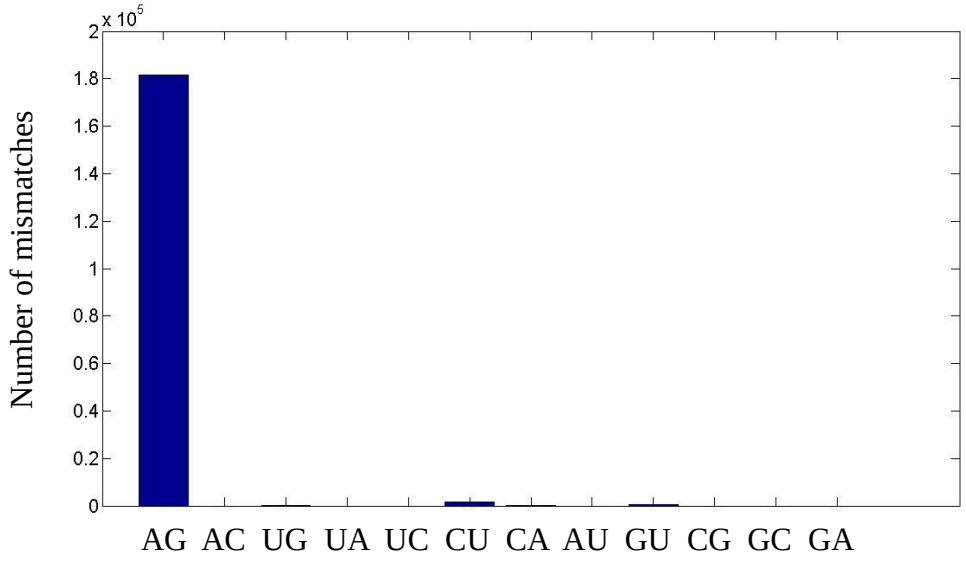
C)



Supplementary Figure 4: Western blot of the endogenous ADARB1 and EGFP-ADARB1 confirms the elevated levels of EGFP-ADARB1 in the *in-vitro* U118-ADARB1 cell-line experiments.



Supplementary Figure 5: A clear A-to-I editing signal in mature miRNAs from mouse brain tissues (cortex, cerebellum and hippocampus). The total number of mismatches of any type is given in absolute numbers.



Supplementary Tables

Supplementary Table 1: The sequence properties of the statistically significant A-to-G modifications sites detected in the human pooled brain sample and the frontal lobe samples.

miRNA	Location	Surrounding sequence	Opposing sequence
let-7d	star position 5	5' AUACG 3'	3' UACGU 5'
let-7e	star position 5[1]	5' AUACG 3'	3' UAUGU 5'
miR-27a	mature position 6	5' ACAGU 3'	3' GUUCG 5'
miR-27b	mature position 4	5' UCACA 3'	3' AGUGG 5'
miR-99a	mature position 1	5' UAAAC 3'	3' GUCUG 5'
miR-130a	mature position 2	5' GCAGU 3'	3' CAUCG 5'
miR-151	3p mature position 3[1]	5' CUAGA 3'	3' GAUCU 5'
miR-200b	mature position 5[1]	5' AUACU 3'	3' UACGA 5'
miR-340	star position 13	5' UUACU 3'	3' AC.GA 5'
miR-376b	mature position 6	5' AUAGA 3'	3' UAUCU 5'
miR-376b	mature position 13	5' AAAAU 3'	3' UUAUA 5'
miR-376c	mature position 6	5' AUAGA 3'	3' UAUCU 5'
miR-376a-1	star position 3	5' GUAGA 3'	3' CACCU 5'
miR-379	mature position 5	5' GUAGA 3'	3' CACCU 5'
miR-381	mature position 4	5' AUACA 3'	3' UAUGU 5'
miR-411	mature position 5	5' GUAGA 3'	3' CACCU 5'
miR-421	mature position 7[1]	5' ACAGA 3'	3' UGUUU 5'
miR-421	mature position 14	5' UUAAU 3'	3' AAAUU 5'
miR-455	5p mature position 17	5' CUACA 3'	3' GACGU 5'
miR-497	mature position 2	5' CCAGC 3'	3' GAUUG 5'
miR-539	mature position 10	5' UUAUC 3'	3' AACAG 5'
miR-589	star position 6	5' GAACA 3'	3' CUCGU 5'
miR-598	mature position 2	5' CUACG 3'	3' AGUGU 5'
miR-641	mature position 3[1]	5' AAAGA 3'	3' UUUCU 5'

[1] Detected in the frontal lobe samples

Supplementary Table 2: Summary of the sequence properties of the statistically significant A-to-G modifications sites detected.

Dataset	Number of editing sites	U in the nucleotide upstream the editing position	G in the nucleotide upstream the editing position	C or U in the nucleotide opposing the editing position	G in the nucleotide downstream the editing position
Human pooled brain sample[1]	19	12	0	17	8
Human frontal lobe samples[1]	5	3	0	5	3
Cell-line over-expression[2]	11	2	1	10	4
Mouse brain samples[3]	32	14	1	30	20

[1] Supplementary Table 1
[2] Supplementary Table 4
[3] Supplementary Table 9

Supplementary Table 3: Comparison between validated editing sites in mature human miRNAs as reported in the literature and the sites detected using the pooled brain sample and the frontal lobe samples in this work.

miRNA	Location	Reference	The pooled brain samples	The frontal lobe samples
let-7g	mature positions 7,10	[2]	Not detected	Not detected
miR-7-2	mature position 10	[2]	Not expressed	Not expressed
miR-27a	star positions 1,8	[2]	Not expressed	Not expressed
miR-33a	mature position 10	[2]	Not detected	Not detected
miR-99a	mature position 1	[1]	Detected	Detected
miR-99b	star position 3	[2]	Not detected	Not detected
miR-142	5p position 9	[2]	Not detected	Not detected
miR-151	5p position 19	[2]	Not expressed	Not expressed
miR-151	3p position 3	[2]	Not detected	Detected
miR-153-1	mature position 7	[2]	Not expressed	Not expressed
miR-153-2	mature position 7	[2]	Not expressed	Not expressed
miR-203	mature position 21	[2]	Not expressed[3]	Not detected
miR-376b	mature position 6	[2]	Detected	Not expressed
miR-376c	mature position 6	[2]	Detected	Not detected
miR-376a-1	star position 3	[2]	Detected	Not detected
miR-376a-1	mature position 6	[2]	Not expressed	Not expressed
miR-376a-2	mature position 6	[2]	Not expressed	Not expressed
miR-379	mature position 5	[1]	Detected	Detected
miR-411	mature position 5	[2]	Detected	Detected
miR-532	5p position 15	[2]	Not detected	Not detected
miR-607	mature positions 6, 17 and 20	[2]	Not expressed	Not expressed

[1] Blow et al. 2006
[2] Kawahara et al. 2008
[3] The expressed isomir in the pooled brain sample end at position 20 according to the miRBase definitions.

Supplementary Table 4: The sequence properties of the statistically significant A-to-G modifications sites detected in the *in-vitro* ADAR over-expression experiments but not in the human pooled brain sample or in the human frontal lobe.

miRNA	Location	Surrounding sequence	Opposing sequence
miR-22	mature position 15	5' GAAGA 3'	3' CUUCU 3'
miR-24-2	star position 6	5' CUACU 3'	3' CUUGA 5'
miR-27a	star position 1	5' GCAGG 3'	3' CGCCU 5'
miR-100	mature position 1	5' CAAAC 3'	3' GUAUG 5'
miR-106b	mature position 18	5' GCAGA 3'	3' CGCC. 5'
miR-130b	mature position 16	5' AAAGG 3'	3' UUUCU 5'
miR-138-1	star position 12	5' CAACA 3'	3' GUUGU 5'
miR-374b	mature position 11	5' CAACC 3'	3' GUUGG 5'
miR-374a	mature position 16	5' UGAUA 3'	3' ACUAU 5'
miR-641	mature position 2	5' GAAAG 3'	3' CUUUC 5'
miR-3176	mature position 15	5' CUACC 3'	3' GACGG 5'

Supplementary Table 5: Comparison between the sequencing counts of the human pooled brain sample data, the frontal lobe data and the *in-vitro* ADAR over-expression data. Raw counts are presented in the format: 'Counts of mismatch' / 'Total number of counts'. The statistically significant modifications, as detected by our analysis, are marked with bold.

miRNA	Location	Pooled brain Sample	Human frontal lobe, sample A	Human frontal lobe, sample B	U87 control/ ADAR/ ADARB1	U118 control cell-line	U118 ADARB1 over-expression
let-7d	star position 5	**12/987**	**21/2879**	8/803	0/630 0/574 0/407	0/203	2/197
let-7e	star position 5	2/188	**6/355**	3/95	0/278 0/248 0/193	0/123	1/151
miR-27a	mature position 6	**15/1225**	**13/572**	4/286	10/ 18999 3/13919 1/7857	0/3114	17/7546
miR-27b	mature position 4	**39/9709**	138/ 193349	42/27325	9/52120 13/42923 3/28646	0/ 13859	3/ 30189
miR-99a	mature position 1	**9402/ 187273**	**197/ 17065**	**136/9451**	1/2495 0/1846 **7/896**	0/1948	**533/3077**
miR-130a	mature position 2	**20/2738**	8/1765	2/1177	0/3469 1/2884 0/2744	0/1811	**24/4435**
miR-151	3p mature position 3	1/111	**69/12469**	**25/1431**	7/8135 6/7138 11/5587	3/4611	**17/3652**
miR-200b	mature position 5	1/21	**5/118**	2/8	0/7 0/10 0/10	0/6	**4/55**
miR-340	star position 13	**9/482**	1/1321	0/606	0/3 0/1 0/5	0/42	0/21
miR-376b	mature position 6	**12/814**	0/9	0/10	0/0 0/0 0/0	0/0	0/0
miR-376b	mature position 13	**15/816**	0/9	0/10	0/0 0/0 0/0	0/0	0/0
miR-376c	mature position 6	**385/4553**	1/44	1/68	0/3 0/2 0/0	0/0	0/0
miR-376a-1	star position 3	**177/935**	1/184	0/37	0/0 0/0 0/1	0/0	0/0
miR-379	mature position 5	**108/1058**	**4/28**	4/140	0/2 0/4 0/0	0/0	0/0
miR-381	mature position 4	**357/5712**	**277/8324**	**195/ 11283**	1/151 **4/114** 0/92	0/2	0/2

miR-411	mature position 5	**939/6141**	**878/6334**	**644/ 10239**	0/88 0/50 1/50	0/5	0/9
miR-421	mature position 7	1/274	24/7626	**24/3389**	1/657 0/781 0/566	0/542	2/1996
miR-421	mature position 14	**5/275**	**78/7635**	**31/3388**	0/658 3/781 **6/566**	3/541	**336/1987**
miR-455	5p mature position 17	**19/1569**	1/104	0/52	0/627 2/519 **14/435**	0/176	**54/281**
miR-497	mature position 2	**351/5691**	8/1413	**11/404**	0/285 0/265 2/190	0/53	**22/82**
miR-539	mature position 10	**5/75**	0/46	1/51	0/0 0/0 0/0	0/0	0/0
miR-589	star position 6	**7/10**	**18/27**	0/1	**6/310** 1/229 **4/285**	0/15	**15/158**
miR-598	mature position 2	**28/11249**	2/1443	1/1168	0/177 0/122 0/91	0/15	0/35
miR-641	mature position 3	0/27	**5/138**	**5/37**	0/277 0/233 0/173	0/80	2/142
miR-22	mature position 15	2/20560	25/ 283597	19/ 160831	44/625101 42/492269 184/358714	4/ 113303	**467/ 345496**
miR-24-2	star position 6	1/125	2/85	0/47	0/1055 0/922 **19/632**	0/584	**230/1630**
miR-27a	star position 1	0/0	0/84	0/19	0/1306 3/1113 **29/926**	2/890	**480/2346**
miR-100	mature position 1	52/82328	32/ 256298	4/30525	23/323711 25/315260 48/110446	19/ 524901	**1957/ 1030145**
miR-106b	mature position 18	0/1864	0/505	0/134	0/1788 0/1582 2/1111	0/501	**12/1065**
miR-130b	mature position 16	0/202	0/1437	3/386	0/2952 0/2648 0/1651	0/1583	**14/3702**
miR-138-1	star position 12	2/96	3/1350	1/922	1/744 0/656 1/795	0/2522	**40/4603**
miR-374b	mature position 11	4/1269	0/744	0/284	0/338 0/335 0/188	0/66	**5/254**
miR-374a	mature position 16	0/1070	0/307	0/95	0/321 0/315 0/110	0/55	**3/113**
miR-641	mature position 2	1/27	0/139	2/37	0/277 1/233 1/173	0/80	**22/143**

miR-3176	mature position 15	0/7	0/2	0/18	0/105 0/70 **3/53**	0/52	**30/127**

Supplementary Table 6: The predicted change in the mRNA targets of the human miRNAs as a result of the editing.

miRNA	Location	Number of mRNA targets before the editing	Number of mRNA targets after the editing	Number of overlapping targets
let-7d	star position 5	9	1	0
let-7e	star position 5	5	3	0
miR-27a	mature position 6	921	12	2
miR-27b	mature position 4	921	9	2
miR-130a	mature position 2	724	173	27
miR-151	3p mature position 3	76	77	3
miR-200b	mature position 5	796	321	66
miR-376b	mature position 6	143	162	6
miR-376c	mature position 6	157	193	11
miR-376a-1	star position 3	131	167	4
miR-379	mature position 5	63	81	4
miR-381	mature position 4	639	303	48
miR-411	mature position 5	65	59	0
miR-421	mature position 7	272	4	1
miR-497	mature position 2	969	486	89
miR-589	star position 6	334	275	20
miR-598	mature position 2	12	10	0
miR-641	mature position 3	355	129	11

Supplementary Table 7: The statistically significant A-to-G modifications sites detected in mature miRNAs from publicly available NGS of mouse brain tissues. The editing levels in the edited miRNAs previously detected in the mouse brain (Chiang et al. 2010) are also presented. Sites in which the modifications were not statistically significant are marked by N.S. Sites in miRNAs with low expression levels (≤10 reads) are marked with N.E. The editing levels are given in percentage.

miRNA	Location	Editing levels in mouse cerebellum [1]	Editing levels in mouse cortex [2]	Editing levels in mouse cortex [3]	Editing levels in mouse hippo-campus [4]	Editing levels in mouse hippo-campus [5]	Editing levels in mouse brain [6]	Editing levels in human brain [7]
let-7c-1	mature position 17	0.7	N.S	N.S	N.S	N.E	N.S	N.S
miR-24-2	star position 7	2.4	3.3	N.S	N.S	N.S	N.S	N.S
miR-27a	star position 1[12]	4.3	11.4	N.S	N.S	N.E	N.S	20.5[8], 3.1[9]
miR-27b	star position 1	N.S	1.1	N.S	N.S	N.E	N.S	N.S
miR-34b	5p mature	N.S	N.S	36.7	27.2	N.S	N.S	N.S

	position 11							
miR-99b	star position 3[12]	4.5	3	N.S	N.S	N.S	N.S	N.S
miR-99a	mature position 1[12]	8.1	6.7	N.S	N.S	2.8	N.S	5
miR-100	mature position 1	0.3	1.4	N.S	N.S	N.S	N.S	0.2[8]
miR-132	star position 2	1.2	N.S	N.S	N.S	N.S	N.S	N.S
miR-137	mature position 11	0.7	0.4	N.S	N.S	N.S	N.S	N.S
miR-137	mature position 16	2.8	1.3	N.S	N.S	N.S	N.S	N.S
miR-151	3p mature position 3[12]	0.6	2.5	1.7	0.6	N.S	N.S	0.6[15], 1.7[16]
miR-154	mature position 2	2.2	1.2	N.S	N.S	N.S	N.S	N.S
miR-195	mature position 5	0.7	N.S	N.S	N.S	N.E	N.S	N.S
miR-212	3p mature position 3	1.1	0.9	N.S	N.S	N.S	N.S	N.S
miR-300	mature position 7	0.8	0.6	N.S	N.S	N.S	N.S	N.E
miR-376a	star position 4	7.2	4.8	15.1	9.8	N.E	29.7	18.9[10]
miR-376b	star position 7[13]	2.3	0.9	7.3	7	N.E	N.S	N.E
miR-376c	mature position 6	2.6	4	1.4	N.S	N.S	31.1	8.5
miR-377	mature position 10[14]	5.2	4.1	N.S	N.S	N.E	N.S	N.S
miR-378	mature position 12	1.6	0.5	N.S	N.S	N.S	N.S	N.E
miR-378	mature position 16	18.9	4.7	N.S	8.7	6.6	8.7	N.E
miR-378	mature position 1	0.2	0.8	N.S	N.S	9.7	N.S	N.E
miR-379	mature position	17.2	9.6	N.S	N.S	3.5	9.5[11]	10.2

miR-381	5 mature position 7	N.S	0.4	N.S	N.S	N.S	N.S	N.S
miR-384	5p mature position 4	0.9	1.1	N.S	N.S	N.S	N.S	N.E
miR-411	mature position 5	88	73.4	1.1	0.9	12.4	23.9	15.3
miR-421	mature position 14	11	N.S	N.S	N.S	N.E	5.4	1.8
miR-467d	mature position 3	10.6	15.9	N.S	N.S	N.S	9.4	Not defined
miR-497	mature position 2	0.8	N.S	4.6	1.5	N.S	10.4	6.2
miR-1251	mature position 6	42.7	21.2	N.S	N.S	14.8	43.1	N.E
miR-3099	mature position 7	3.4	2.3	N.S	N.S	N.S	20.9	Not defined

[1] SRA dataset SRR346417; [2] SRA dataset SRR346423; [3] SRA dataset SRR038744
[4] SRA dataset SRR038741; [5] SRA dataset SRR345196; [6] Chiang et al. 2010
[7] The pooled brain sample, from Table 1
[8] Detected in the U118 ADARB1 over-expression experiment (Supplementary Table 5)
[9] Detected in the U87 ADARB1 over-expression experiment (Supplementary Table 5)
[10] The equivalent of mouse miR-376a in star position 4 is human miR-376a-1 in star position 3
[11] Chiang et al. (2010) indicate editing in the star of miR-379, but they probably mean the mature as the star do not have A in position 5.
[12] Known editing site (Kawahara et al. 2008)
[13] Known editing site (Kawahara et al. 2007)
[14] Known editing site in Rat (Linsen et al. 2010)
[15] Detected in the human frontal lobe, sample A (Table 1)
[16] Detected in the human frontal lobe, sample B (Table 1)

Supplementary Table 8: The raw counts of the statistically significant A-to-G modifications sites detected in mature miRNAs from publicly available NGS of mouse brain tissues. Raw counts are presented in the format: 'Counts of mismatch' / 'Total number of counts'. The statistically significant modifications, as detected by our analysis, are marked with bold.

miRNA	**Location**	**Editing levels in mouse cerebellum[1]**	**Editing levels in mouse cortex [2]**	**Editing levels in mouse cortex [3]**	**Editing levels in mouse hippo-campus [4]**	**Editing levels in mouse hippo-campus [5]**
let-7c-1	mature position 17	**14/1894**	7/2348	5/21328	12/33844	0/0
miR-24-2	star position 7	**30/1245**	**74/2265**	1/991	0/619	0/49
miR-27a	star position	**4/93**	**20/175**	3/76	1/41	0/1

miR-27b	1 star position 1	5/767	**8/711**	2/94	0/97	0/0
miR-34b	5p mature position 11	0/601	0/84	**58/158**	**212/779**	0/14
miR-99b	star position 3	**63/1385**	**37/1216**	0/99	1/95	0/39
miR-99a	mature position 1	**4366/53647**	**3337/49522**	6/13727	6/18256	**88/3175**
miR-100	mature position 1	**492/177723**	**1919/134097**	0/7907	0/7047	1/495
miR-132	star position 2	**10/856**	11/4120	3/2778	0/876	0/454
miR-137	mature position 11	**52/7805**	**321/71670**	10/58267	9/44222	0/242
miR-137	mature position 16	**216/7802**	**937/71646**	25/58269	14/44219	0/242
miR-151	3p mature position 3	**113/20183**	**533/21336**	**36/2100**	**11/1903**	0/135
miR-154	mature position 2	**24/1075**	**14/1185**	0/2664	0/1297	0/24
miR-195	mature position 5	**75/10063**	9/1969	2/889	1/540	0/9
miR-212	3p mature position 3	**11/1012**	**48/5223**	1/2259	1/647	0/20
miR-300	mature position 7	**264/33147**	**372/57511**	2/15040	1/8055	0/1096
miR-376a	star position 4	**265/3678**	**265/5525**	**541/3586**	**186/1905**	0/7
miR-376b	star position 7	**99/4286**	**51/5716**	**373/5136**	**180/2574**	0/0
miR-376c	mature position 6	**6/230**	**12/301**	**12/852**	4/452	0/17
miR-377	mature position 10	**40/773**	**51/1254**	3/1293	1/677	0/0
miR-378	mature position 12	**1191/76438**	**140/30125**	0/74	0/48	0/122
miR-378	mature position	**14341/75697**	**1427/30087**	3/73	**4/46**	**8/121**

miR-378	16 mature position 1	**115/67100**	**197/24630**	0/69	0/45	**9/93**
miR-379	mature position 5	**25672/149116**	**34782/362543**	43/27643	23/15555	**32/922**
miR-381	mature position 7	92/40619	**166/38384**	1/39049	0/24647	1/67
miR-384	5p mature position 4	**52/5986**	**30/2654**	1/2817	0/1741	0/27
miR-411	mature position 5	**45261/51456**	**40540/55269**	**96/8838**	**50/5256**	**1249/10048**
miR-421	mature position 14	**14/127**	3/128	0/46	0/32	0/1
miR-467d	mature position 3	**17/161**	**10/63**	1/58	0/47	0/34
miR-497	mature position 2	**40/4869**	0/1243	**27/582**	**8/548**	0/31
miR-1251	mature position 6	**96/225**	**14/66**	0/121	0/47	**4/27**
miR-3099	mature position 7	**15/442**	**10/431**	1/198	0/125	0/22

[1] SRA dataset SRR346417; [2] SRA dataset SRR346423; [3] SRA dataset SRR038744
[4] SRA dataset SRR038741; [5] SRA dataset SRR345196;

Supplementary Table 9: The sequence properties of the statistically significant A-to-G modifications sites detected in mature miRNAs from publicly available NGS of mouse brain tissues.

miRNA	Location	Surrounding sequence	Opposing sequence
let-7c-1	mature position 17	5' GUAUG 3'	3' CAUGU 5'
miR-24-2	star position 7	5' CUACU 3'	3' GACGA 5'
miR-27a	star position 1	5' GCAGG 3'	3' CGCCU 5'
miR-27b	star position 1	5' GCAGA 3'	3' CGUCU 5'
miR-34b	5p mature position 11	5' UAAUU 3'	3' CUCAA 5'
miR-99b	star position 3	5' CAAGC 3'	3' GUUCC 5'
miR-99a	mature position 1	5' UAAAC 3'	3' GUCUG 5'
miR-100	mature position 1	5' CAAAC 3'	3' GUAUG 5'
miR-132	star position 2	5' CAACC 3'	3' GCUGG 5'
miR-137	mature position 11	5' UAAGA 3'	3' GUUCU 5'
miR-137	mature position 16	5' AUACG 3'	3' UAUGG 5'
miR-151	3p mature position 3	5' CUAGA 3'	3' GAUCU 5'
miR-154	mature position 2	5' AUAGG 3'	3' UAUCC 5'
miR-195	mature position 5	5' GCAGC 3'	3' CGUCG 5'
miR-212	3p mature position 3	5' UAA.C 3'	3' AUUCG 5'
miR-300	mature position 7	5' CAAGG 3'	3' GUUUC 5'

miR-376a	star position 4	5' GUAGA 3'	3' CACCU 5'
miR-376b	star position 7	5' AUAUU 3'	3' UACAA 5'
miR-376c	mature position 6	5' AUAG. 3'	3' UAUCU 5'
miR-377	mature position 10	5' AAAGG 3'	3' UUCCC 5'
miR-378	mature position 12	5' GGAGU 3'	3' CCUCA 5'
miR-378	mature position 16	5' UCAGA 3'	3' AGUCC 5'
miR-378	mature position 1	5' GCACU 3'	3' UGUGU 5'
miR-379	mature position 5	5' GUAGA 3'	3' CACCU 5'
miR-381	mature position 7	5' .AAGG 3'	3' UUUCC 5'
miR-384	5p mature position 4	5' GUAAA 3'	3' CACUU 5'
miR-411	mature position 5	5' GUAGA 3'	3' CACCU 5'
miR-421	mature position 14	5' UUAAU 3'	3' AAAUU 5'
miR-467d	mature position 3	5' UAAGU 3'	3' .UCCA 5'
miR-497	mature position 2	5' CCAGC 3'	3' GAUUG 5'
miR-1251	mature position 6	5' CUAGC 3'	3' GACCG 5'
miR-3099	mature position 7	5' CUAGA 3'	3' GACCU 5'

Supplementary Table 10: The predicted change in the mRNA targets of the mouse miRNAs as a result of the editing.

miRNA	**Location**	**Number of mRNA targets before the editing**	**Number of mRNA targets after the editing**	**Number of overlapping targets**
miR-24-2	star position 7	38	78	0
miR-99b	star position 3	13	7	0
miR-132	star position 2	10	7	0
miR-151	3p mature position 3	52	54	2
miR-154	mature position 2	48	38	1
miR-195	mature position 5	691	12	1
miR-212	3p mature position 3	200	97	9
miR-300	mature position 7	180	115	12
miR-376a	star position 4	54	48	2
miR-376b	star position 7	70	47	3
miR-376c	mature position 6	127	109	10
miR-379	mature position 5	44	26	0
miR-381	mature position 7	462	226	32
miR-384	5p mature position 4	868	94	20
miR-411	mature position 5	36	32	0
miR-467d	mature position 3	102	11	1
miR-497	mature position 2	691	261	36
miR-1251	mature position 6	46	146	3
miR-3099	mature position 7	37	121	1

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