Supplemental Material

Systematic Characterization of A-to-I RNA Editing Hotspots in MicroRNAs across Human Cancers

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Supplemental Fig S1. Overexpression of ADAR1 and ADAR2 in 3 cell lines. Western blot of ADAR enzymes overexpression in Hs578T, 786O and HeyA8 cell lines (NC: negative control, WT: wild-type, MUT: mutated).
Supplemental Fig S2. The editing levels of 15 validated A-to-I editing hotspots in 20 cancer types.
Each box plot represents the average editing level of a miRNA editing hotspot across the edited samples within a cancer type.
Supplemental Fig S3. The correlations of miRNA editing level with WT miRNA expression across different cancer types. Color depicts the correlation coefficient, and black box highlights correlations that are significant (FDR < 0.05).
Supplemental Fig S4. The correlations of miRNA editing levels with significantly mutated genes and copy number alterations in different cancer types

(A) miRNAs are depicted in orange, and mutated genes are in red or blue. Given a mutated gene, the edge color reflects the fold-change direction of the mean miRNA editing level in the mutated sample group relative to the wild-type sample group: red, increase; and blue, decrease. Color of each gene node reflects the fold change (log transformed). The specific cancer types showing the significant correlations are listed below gene names. (B) correlations of miRNA editing hotspots with frequent SCNAs. Red for positive correlations and blue for negative correlations.
Supplemental Fig S5

Supplemental Fig S5. Correlations of ADAR mRNA expression with patient survival times
Dot size depicts log-rank P value, and color represents the correlation direction (os = overall survival; pfs = progression free survival).
Supplemental Fig S6

(A) The over-expression amounts of miR-200b upon 24-hr transfection with 50nm wide-type miR-200b mimics in MCF10A, MDAMB-231 and SLR25 cell lines by qRT-PCR. The expression amounts transfected with negative controls were set as 1.

(B) The expression amount distribution of wide-type miR-200b (RPM) in all TCGA patient cancer samples. The expression amount of miR-200b in MDAMB-231 was calculated based on a miRNA sequencing dataset (NCBI SRA SRX004030) using the same analytic procedure. The arrow indicates the fold increase of miR-200b expression in MDAMB-231 relative to the highest expression level observed among patients.

Supplemental Fig S6. Quantitative assessment of transfected miR-200b expression amount in cell lines

(A) The over-expression amounts of miR-200b upon 24-hr transfection with 50nm wide-type miR-200b mimics in MCF10A, MDAMB-231 and SLR25 cell lines by qRT-PCR. The expression amounts transfected with negative controls were set as 1. (B) The expression amount distribution of wide-type miR-200b (RPM) in all TCGA patient cancer samples. The expression amount of miR-200b in MDAMB-231 was calculated based on a miRNA sequencing dataset (NCBI SRA SRX004030) using the same analytic procedure. The arrow indicates the fold increase of miR-200b expression in MDAMB-231 relative to the highest expression level observed among patients.
Supplemental Fig S7.

Effects of miR-200b mimics on cell viability in MCF10A, MDAMB-231 and SLR25 cells

Two-sided $t$-test was used to assess the difference. Error bars denote +/- SEM; *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$. 

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Supplemental Fig S7
Supplemental Fig S8

(A) **p < 0.001.

(B) Negative control

Supplemental Fig S8. Effect of wild-type miR-200b on target gene ZEB2
(A) 3' UTR representations of WT miR-200b target genes ZEB1; (B) qRT-PCR of ZEB2 upon 24-hr transfection with WT miR-200b mimics in MCF10A, MDAMB-231 and SLR25 cells. Two-sided t-test was used to assess the difference. Error bars denote +/- SEM; *p < 0.05; **p < 0.01; ***p < 0.001.
Supplemental Fig S9

(A) Summary of correlations between LIFR mRNA expression and patient survival times across cancer types. Circle size indicates statistical significance; color indicates correlation direction. In general, high expression of miR-200b is associated with better patient survival. (B) Kaplan-Meier plots of patients grouped by LIFR mRNA expression in individual cancer types.
Supplemental Fig S10. Correlation between edited miR-200b expression level and LIFR expression across cancer types

We used edited miR-200b expression (log₂RPM) and the corresponding LIFR mRNA expression level (log₂RSEM) to evaluate their correlation. Each dot represents a sample; different colors depict different cancer types. A significant negative correlation was detected by both Spearman rank correlation ($Rs = -0.189, p = 0$); and two-factor ANOVA test, with the cancer type being the fixed effect and miR-200b being an independent variable ($p = 4.3\times10^{-4}$).
Supplemental Table S1. Overview of TCGA miRNA sequencing data.

| TCGA code | Cancer type                | # Sample | Tumor sample | Normal sample | Average tumor mappable reads (millions) | Average normal mappable reads (millions) | # Confident A-to-I editing events |
|-----------|----------------------------|----------|--------------|---------------|----------------------------------------|------------------------------------------|----------------------------------|
| BLCA      | Bladder                    | 414      | 395          | 19            | 5.96 ± 3.85                            | 15.39 ± 9.99                           | 929                              |
| BRCA      | Breast                     | 890      | 801          | 89            | 3.76 ± 2.74                            | 3.80 ± 2.49                            | 2021                             |
| CESC      | Cervical                   | 301      | 298          | 3             | 5.33 ± 2.46                            | 15.3 ± 1.74                            | 682                              |
| COAD      | Colon                      | 399      | 391          | 8             | 4.48 ± 3.82                            | 1.22 ± 0.36                            | 883                              |
| HNSC      | Head and neck              | 562      | 518          | 44            | 5.05 ± 2.34                            | 6.34 ± 2.13                            | 1189                             |
| KICH      | Kidney (chromophobe)       | 90       | 65           | 25            | 6.35 ± 1.60                            | 8.08 ± 2.25                            | 103                              |
| KIRC      | Kidney (clear)             | 587      | 516          | 71            | 3.57 ± 2.41                            | 3.73 ± 1.39                            | 1032                             |
| KIRP      | Kidney (papillary)         | 325      | 291          | 34            | 6.74 ± 2.94                            | 9.00 ± 2.64                            | 551                              |
| LAML      | Leukemia                   | 188      | 188          | 0             | 0.85 ± 0.31                            | NA                                      | 311                              |
| LGG       | Low-grade glioma           | 511      | 511          | 0             | 2.41 ± 1.08                            | NA                                      | 2620                             |
| LIHC      | Liver                      | 421      | 371          | 50            | 5.12 ± 2.27                            | 5.53 ± 1.61                            | 935                              |
| LUAD      | Lung (adeno)               | 529      | 483          | 46            | 5.47 ± 2.73                            | 5.99 ± 2.75                            | 1250                             |
| LUSC      | Lung (squamus)             | 519      | 474          | 45            | 3.83 ± 2.10                            | 8.22 ± 2.88                            | 1137                             |
| OV        | Ovarian                    | 489      | 489          | 0             | 4.01 ± 1.99                            | 8.34 ± 4.70                            | 976                              |
| PRAD      | Prostate                   | 545      | 493          | 52            | 6.71 ± 3.56                            | NA                                      | 1233                             |
| READ      | Rectum                     | 160      | 157          | 3             | 5.32 ± 4.17                            | 1.10 ± 0.34                            | 347                              |
| SKCM      | Melanoma                   | 100      | 98           | 2             | 4.20 ± 2.24                            | 1.94 ± 0.11                            | 219                              |
| STAD      | Stomach                    | 430      | 389          | 41            | 5.31 ± 4.16                            | 9.70 ± 6.57                            | 879                              |
| THCA      | Thyroid                    | 587      | 518          | 69            | 5.65 ± 2.09                            | 7.16 ± 2.12                            | 1057                             |
| UCEC      | Uterus                     | 548      | 515          | 33            | 5.12 ± 3.74                            | 16.69 ± 7.57                           | 1092                             |
| Total     |                           | 8595     | 7961         | 634           | 4.76 ± 7.50                            | 7.50 ± 19446                           | 19446                            |
### Supplemental Table S2. Summary of high-confidence RNA editing events and RNA editing hotspots

| Tumor Type | #RNA editing events | #RNA editing hotspots |
|------------|---------------------|-----------------------|
| **BRCA**   |                     |                       |
| AG         | 929                 | 5                     |
| AC         | 355                 | 16                    |
| UA         | 153                 | 1                     |
| GU         | 28                  | 2                     |
| CU         | 25                  | 1                     |
| UG         | 16                  | 1                     |
| **CEC**    |                     |                       |
| AG         | 2021                | 7                     |
| UA         | 81                  | 1                     |
| AC         | 58                  | 3                     |
| CU         | 23                  | 1                     |
| GU         | 11                  | 1                     |
| **COAD**   |                     |                       |
| AG         | 682                 | 3                     |
| UA         | 107                 | 1                     |
| AC         | 27                  | 1                     |
| **HNSC**   |                     |                       |
| AG         | 1189                | 3                     |
| AC         | 801                 | 20                    |
| GU         | 499                 | 15                    |
| UA         | 66                  | 1                     |
| **KICH**   |                     |                       |
| AG         | 103                 | 2                     |
| GU         | 13                  | 1                     |
| **KIRC**   |                     |                       |
| AG         | 1032                | 5                     |
| UA         | 24                  | 1                     |
| CU         | 11                  | 1                     |
| UG         | 301                 | 1                     |
| **LAML**   |                     |                       |
| AG         | 311                 | 3                     |
| **LGG**    |                     |                       |
| AG         | 2620                | 14                    |
| AC         | 760                 | 44                    |
| UA         | 171                 | 1                     |
| GU         | 32                  | 1                     |
| UG         | 21                  | 1                     |
| CU         | 18                  | 1                     |
| UC         | 15                  | 1                     |
| **LIHC**   |                     |                       |
| AG         | 935                 | 7                     |
| GU         | 236                 | 18                    |
| AC         | 199                 | 11                    |
| UA         | 105                 | 1                     |
| **LUAD**   |                     |                       |
| AG         | 1250                | 7                     |
| AC         | 1141                | 22                    |
| GU         | 591                 | 15                    |
| UA         | 43                  | 1                     |
| **LUSC**   |                     |                       |
| AG         | 1137                | 6                     |
| AC         | 535                 | 15                    |
| GU         | 259                 | 11                    |
| UA         | 49                  | 1                     |
| **OV**     |                     |                       |
| AG         | 976                 | 5                     |
| GC         | 282                 | 1                     |
| CU         | 59                  | 2                     |
| UG         | 30                  | 1                     |
| UA         | 11                  | 1                     |

| Tumor Type | #RNA editing events | #RNA editing hotspots |
|------------|---------------------|-----------------------|
| **KIRP**   |                     |                       |
| AG         | 551                 | 4                     |
| AC         | 239                 | 12                    |
| GU         | 214                 | 11                    |
| UA         | 96                  | 1                     |
| **LAML**   |                     |                       |
| AG         | 311                 | 3                     |
| **LGG**    |                     |                       |
| AG         | 2620                | 14                    |
| AC         | 760                 | 44                    |
| UA         | 171                 | 1                     |
| GU         | 32                  | 1                     |
| UG         | 21                  | 1                     |
| CU         | 18                  | 1                     |
| UC         | 15                  | 1                     |
| **LIHC**   |                     |                       |
| AG         | 935                 | 7                     |
| GU         | 236                 | 18                    |
| AC         | 199                 | 11                    |
| UA         | 105                 | 1                     |
| **LUAD**   |                     |                       |
| AG         | 1250                | 7                     |
| AC         | 1141                | 22                    |
| GU         | 591                 | 15                    |
| UA         | 43                  | 1                     |
| **LUSC**   |                     |                       |
| AG         | 1137                | 6                     |
| AC         | 535                 | 15                    |
| GU         | 259                 | 11                    |
| UA         | 49                  | 1                     |
| **OV**     |                     |                       |
| AG         | 976                 | 5                     |
| GC         | 282                 | 1                     |
| CU         | 59                  | 2                     |
| UG         | 30                  | 1                     |
| UA         | 11                  | 1                     |
| TCGA  | #RNA editing events | #RNA editing hotspots |
|-------|---------------------|-----------------------|
| PRAD  |                     |                       |
| AG    | 1233                | 4                     |
| UA    | 301                 | 1                     |
| AC    | 177                 | 10                    |
| CU    | 139                 | 2                     |
| READ  |                     |                       |
| AG    | 347                 | 3                     |
| AC    | 28                  | 1                     |
| SKCM  |                     |                       |
| AG    | 219                 | 4                     |
| UA    | 33                  | 1                     |
| UC    | 28                  | 1                     |
| GC    | 17                  | 1                     |
| GU    | 10                  | 1                     |
| STAD  |                     |                       |
| AG    | 879                 | 3                     |
| UA    | 152                 | 1                     |
| AC    | 126                 | 5                     |
| CA    | 54                  | 3                     |
| UC    | 13                  | 1                     |
| THCA  |                     |                       |
| AG    | 1057                | 6                     |
| AC    | 537                 | 23                    |
| UA    | 71                  | 1                     |
| GU    | 36                  | 2                     |
| UC    | 35                  | 1                     |
| UCEC  |                     |                       |
| AG    | 1092                | 4                     |
| AC    | 106                 | 7                     |
| UA    | 44                  | 1                     |
| GU    | 20                  | 2                     |
Supplemental Table S3. Information about 19 A-to-I RNA editing hotspots identified in miRNAs

| miRNA          | Edited sequence          | Cross mapping | Edited seed region | Shared seed region with miRNA |
|----------------|--------------------------|---------------|--------------------|-------------------------------|
| hsa-miR-376a-5p | guggauucuccuucuaugagua  | No            | uggauuc            | No                            |
| hsa-miR-381-3p  | uaugcaagggcaagcucucugu  | No            | augcaag            | No                            |
| hsa-miR-411-5p  | uaguggacguauagcguacg    | No            | aguggac            | No                            |
| hsa-miR-99a-5p  | gacccguagauccgaucuugug | No            | accgua             | not in seed region            |
| hsa-miR-379-5p  | ugguggacuaugacguagg     | No            | gguggac            | hsa-miR-8071                  |
| hsa-miR-376c-3p | aacauggagaaauuccacgu    | No            | acaugga            | hsa-miR-4802-3p               |
| hsa-miR-589-3p  | ucagagcaaagccguucccaga  | No            | cagacga            | hsa-miR-6501-3p               |
| hsa-miR-664a-5p | acuggcugggaaaauguuggau  | No            | cuggcug            | hsa-miR-3064-5p               |
| hsa-miR-497-5p  | cggcagcCACACACUGGUUUGU  | No            | gccagca            | No                            |
| hsa-miR-151a-3p | cuggacugaagcuccuuggagg | No            | uggacug            | hsa-miR-1269a                 |
| hsa-miR-200b-3p | uaaugcugcccucuuaugaga   | No            | aaugcug            | No                            |
| hsa-miR-3144-3p | auguaccuuucguucuuaua    | No            | uguaccu            | No                            |
| hsa-miR-1301-3p | uugcgccuggccuggagucuuc  | No            | ugcggcu            | No                            |
| hsa-miR-1251-5p | acucugccugccaaagcgcgu   | No            | cucuggc            | No                            |
| hsa-miR-6503-3p | gggacugggaugcagaccucc   | No            | ggacugg            | hsa-miR-4515                  |
| hsa-miR-1295b-3p| aauaggccgcaugcagggcaaa  | No            | auaggcc            | not in seed region            |
| hsa-miR-337-3p  | cucucuguauagccuucuuc     | No            | uccugua            | No                            |
| hsa-miR-1304-3p | ucucgcuguauccccuagccccc  | No            | cucgcug            | No                            |
| hsa-miR-3622a-3p| ugcaccugccuaccuaccuacc  | No            | gcaccuaga          | hsa-miR-6078                  |
Supplemental Table S4. Validation of the correlations of miRNA editing level with ADAR2 enzymes using a miRNA-seq dataset of ADAR2-perturbed cell lines

| miRNA | Editing site | TCGA LGG | U87 cell line | U118 cell line |
|-------|--------------|----------|---------------|----------------|
|       |              | Correlation Coefficient | FDR | control | ADAR2 over - expression | control | ADAR2 over - expression | ADAR2 E/A | siADAR2 |
| miR-99a | 1 | 0.64 | 0 | 0 | 0.786 | 0 | 13.815 | 0 | 6.913 |
| miR-379 | 5 | 0.51 | 0 | NE | NE | NE | NE | NE | NE |
| miR-497 | 2 | 0.42 | 8.36E-22 | 0 | 1.058 | 0 | 25.862 | 0 | 16.245 |
| miR-411 | 5 | 0.37 | 2.53E-16 | 0 | 2 | NE | 13.26 | NE | 5.882 |
| miR-1301 | 5 | 0.33 | 9.58E-14 | 0 | NE | 0 | 9.524 | 0 | 0 |
| miR-1251 | 6 | 0.31 | 5.79E-11 | NE | NE | NE | NE | NE | NE |

The editing levels (%) in two glioblastoma cell lines U87 and U118 were presented for 6 miRNA editing hotspots that show significantly strong correlation (FDR < 0.05, spearman correlation Rs ≥ 0.3) with ADAR2 enzyme in TCGA LGG patient samples. ADAR2 E/A is the inactive form of ADAR2. “NE” depicts sites with not enough coverage (< 10) to quantify the editing level.
Supplemental Table S5. List of potential new targets of edited miR-200b

| Gene    | Transcript   | # Match to wt miR-200b | # Match to edited miR-200b | FDR       | Log2 fold change |
|---------|--------------|------------------------|---------------------------|-----------|-----------------|
| LIFR    | NM_002310    | 0                      | 2                         | 2.05E-12  | 0.940541692     |
| RAB5C   | NM_004583    | 0                      | 2                         | 7.05E-12  | 1.045945875     |
| MFAP3   | NM_005927    | 0                      | 2                         | 1.34E-11  | 1.178306516     |
| LIN9    | NM_173083    | 0                      | 2                         | 1.28E-10  | 1.418951397     |
| MARCH6  | NM_005885    | 0                      | 4                         | 2.66E-10  | 0.966610739     |
| GINS1   | NM_021067    | 0                      | 2                         | 2.66E-10  | 0.823375314     |
| SLC12A6 | NM_005135    | 0                      | 2                         | 1.93E-09  | 0.976012095     |
| BTBD3   | NM_014962    | 0                      | 2                         | 2.02E-09  | 1.224580566     |
| CSRP2   | NM_001321    | 0                      | 2                         | 1.65E-08  | 1.173966876     |
| C15orf41| NM_032499    | 0                      | 3                         | 1.02E-07  | 0.923243231     |
| SUN1    | NM_025154    | 0                      | 2                         | 1.49E-07  | 0.76629024      |
| PDS5A   | NM_001100399 | 0                      | 3                         | 1.97E-07  | 1.066848588     |
| ARL5B   | NM_178815    | 0                      | 2                         | 2.48E-07  | 0.880867339     |
| SNX13   | NM_015132    | 0                      | 2                         | 1.23E-06  | 0.753840947     |
| IL13RA1 | NM_001560    | 0                      | 2                         | 1.47E-06  | 0.691503253     |
| ZSCAN31 | NM_030899    | 0                      | 2                         | 3.54E-06  | 0.814574932     |
| TRPC1   | NM_003304    | 0                      | 2                         | 5.13E-06  | 1.08040322      |
| FAR1    | NM_032228    | 0                      | 2                         | 5.38E-06  | 0.960681537     |
| RUNDC1  | NM_173079    | 0                      | 2                         | 1.01E-05  | 0.697887552     |
Supplemental Table S6. List of 3’UTR binding sites of wild-type and edited miR-200b to their target genes ZEB1, ZEB2, and LIFR.

| Target Gene | 3’UTR binding sites |
|-------------|---------------------|
| **ZEB1**    |                     |
|             | 357…GTTTTATCTTATCGTATTA…376 |
|             | 451…CTAAATCCGCTTCAGTATTT…470 |
|             | 880…AGTGCCATTTCCTCGATTT…899 |
|             | 1231…ATTTTTACCTATCGTATTA…1250 |
|             | 1301…CTTCAAAACCTGGCAGTATTA…1320 |
|             | 1952…TTTCATCATTACGATTT…1971 |
| **ZEB2**    |                     |
|             | 380…ACTACCATACTACGATTT…399 |
|             | 443…ACTACAAATGCATCAGTATTA…462 |
|             | 801…AACGCCCATGTGAGTATTTG…820 |
|             | 886…CATTAATTTCACCAGTATTA…905 |
|             | 1017…TACTGTAGTGTACGATTT…1036 |
| **LIFR**    |                     |
|             | 500…CTCCTCTATCCACACGATTC…519 |
|             | 3255…CATTTTCCAAAAACCGATTT…3274 |