Reporting Summary

Nature Research wishes to improve the reproducibility of the work that we publish. This form provides structure for consistency and transparency in reporting. For further information on Nature Research policies, see our Editorial Policies and the Editorial Policy Checklist.

Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

- The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement
- A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
- The statistical test(s) used and whether they are one- or two-sided
  - Only common tests should be described solely by name; describe more complex techniques in the Methods section.
- A description of all covariates tested
- A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
- A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) and variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
- For null hypothesis testing, the test statistic (e.g. F, t, r) with confidence intervals, effect sizes, degrees of freedom and P value noted
  - Give P values as exact values whenever possible.
- For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
- For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
- Estimates of effect sizes (e.g. Cohen’s d, Pearson’s r), indicating how they were calculated

Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of code

- Data collection: Quantification data for gel image collected using Image Lab v6.0, miRNA annotation collected from mirBase.org v22, DROSHA-dependent pri-miRNAs collected from MiRgeneDB v2.0, RNA sequencing data collected using Illumina CASAVA

- Data analysis: Cutadapt v1.15, fastq-join v1.3.1, FASTX-Toolkit v0.0.13, Bowtie2 v2.2.9, Python v3.7.1

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors and reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research guidelines on submitting code & software for further information.

Data

Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A list of figures that have associated raw data
- A description of any restrictions on data availability

The raw data of small RNA sequencing were deposited to the Gene Expression Omnibus (GEO: GSE140209). The source data underlying Figs. 1d-e, 2b-c, e-f, 3c-d, 4b-c, d-f, 5a-g, 6a-e are provided as a Source Data file.
Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

- [ ] Life sciences
- [ ] Behavioural & social sciences
- [ ] Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see nature.com/documents/nr-reporting-summary-flat.pdf

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

- **Sample size**: 3 replicates for EMSA assay, 3 replicates for each cleavage assay. See method section and figure legends.
- **Data exclusions**: No data was excluded.
- **Replication**: 3 replicates for EMSA assay, 3 replicates for each cleavage assay. See method section and figure legends.
- **Randomization**: Not applicable, sample randomization is not relevant to this study.
- **Blinding**: Not applicable, blinding is also not relevant to this study.

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list applies to your research, read the appropriate section before selecting a response.

| Materials & experimental systems | Methods |
|----------------------------------|---------|
| n/a                              | n/a     |
| □ Antibodies                     | □ ChiP-seq |
| □ Eukaryotic cell lines          | □ Flow cytometry |
| □ Palaeontology and archaeology  | □ MRI-based neuroimaging |
| □ Animals and other organisms    |         |
| □ Human research participants    |         |
| □ Clinical data                  |         |
| □ Dual use research of concern   |         |

Antibodies

- **Antibodies used**: α-DGCR8 antibody (4D4) for DGCR8, and the α-DROSHA antibody (21D3) for DROSHA. Both α-DROSHA and α-DGCR8 were gifts from Dr. Narry KIM’s lab, Seoul National University
- **Validation**: The α-DROSHA antibody (21D3) previously used in Kwon, S., Baek, S., Choi, Y., Yang, J., Lee, Y., Woo, I., & Kim, V. (2019). Molecular Basis for the Single-Nucleotide Precision of Primary microRNA Processing. Molecular Cell, 73(3), 505-518.e5. doi:10.1016/j.molcel.2018.11.005

Eukaryotic cell lines

- **Policy information about celllines**
  - **Cell line source(s)**: The cell lines (HEK293E, HCT116, DGCR8&TT KO cells) were the gifts from Dr. Narry KIM’s lab, Seoul National University.
  - **Authentication**: None of the cell lines were authenticated by our facility.
  - **Mycoplasma contamination**: All the cell lines were tested negative for mycoplasma contamination.
  - **Commonly misidentified lines** (See ICLAC register): none used