

Reporting Summary

Nature Portfolio 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 Portfolio 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.

n/a Confirmed

- ☐ ☒ 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 suitable.
- ☒ ☐ 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 computer code](#)

Data collection

Quantification data for gel image collected using Image Lab v6.0.1. Human miRNAs sequences were collected from miRbase v22. Protein structures were obtained from Protein Data Bank. Cryo-EM data was collected at Biological Cryo-EM Center in The Hong Kong University of Science and Technology

Data analysis

Cutadapt v1.15, fastq-join v1.3.1, FASTX-Toolkit v0.0.13, BWA v 0.7.17, RNAfold from ViennaRNA Package v2.4.9, Python 3.7.1, , Pymol-2.5.4, Image Lab v6.0.1, Chimera 1.17.3, Coot, Phenix, Spyder.

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 Portfolio [guidelines for 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 description of any restrictions on data availability
- For clinical datasets or third party data, please ensure that the statement adheres to our [policy](#)

The raw sequencing data and processed data for massively parallel dicing assay are deposited at Gene Expression Omnibus (GEO), accession number: GSE296721,

with accession token for reviewers of spcdgkycjhdzgf.

Structural models of the DICER/26S-GU, DICER/26S-UG, DICER-D991G-H992G/26S-GU, DICER/pre-mir-517a-GU in pre-dicing state and in dicing-state complexes have been deposited in the Protein Data Bank (PDB) under the accession codes 9V42, 9V43, 21CQ, 21CB, 21CN, respectively. The density maps for the above models have been deposited in the Electron Microscopy Data Bank (EMDB) under the following accession codes: EMD-64764 (DICER/26S-GU), EMD-64765 (DICER/26S-UG), EMD-67575 (DICER-D991G-H992G/26S-GU), EMD-67570 (DICER/pre-mir-517a_GU in the pre-dicing state), and EMD-67573 (DICER/pre-mir-517a_GU in the dicing state).

Research involving human participants, their data, or biological material

Policy information about studies with [human participants or human data](#). See also policy information about [sex, gender \(identity/presentation\), and sexual orientation](#) and [race, ethnicity and racism](#).

Reporting on sex and gender	Not applicable to this study
Reporting on race, ethnicity, or other socially relevant groupings	Not applicable to this study
Population characteristics	Not applicable to this study
Recruitment	Not applicable to this study
Ethics oversight	Not applicable to this study

Note that full information on the approval of the study protocol must also be provided in the manuscript.

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](https://www.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	No sample size calculation was performed.
Data exclusions	No data was excluded.
Replication	03 replicate for RNA sequencing of high-throughput pre-miRNA processing library and small RNA sequencing library, at least 03 replicate for other quantitative experiments. See method section and figure legends.
Randomization	Not applicable to this study
Blinding	Not applicable 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 item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

n/a	Involved in the study
☒	☐ Antibodies
☐	☒ Eukaryotic cell lines
☒	☐ Palaeontology and archaeology
☒	☐ Animals and other organisms
☒	☐ Clinical data
☒	☐ Dual use research of concern
☒	☐ Plants

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☒	☐ Flow cytometry
☒	☐ MRI-based neuroimaging

Eukaryotic cell lines

Policy information about [cell lines and Sex and Gender in Research](#)

Cell line source(s)	The Sf9 cell lines was the gifts from Dr. Shangyu DANG's lab (The Hong Kong University of Science and Technology). HCT116 DICER-KO cells, HEK293E cell lines was 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

Plants

Seed stocks	Not applicable to this study
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Novel plant genotypes	Not applicable to this study
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Authentication	Not applicable to this study
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