nature portfolio

Corresponding author(s): Haifeng Wang

Last updated by author(s): May 19, 2026

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	For the flow cytometry analysis, the data were collected by BD LSRFortessa SORP. RT-qPCR data was collected by ABI-QuantStudio 1 Real-Time PCR system. Confocal images were acquired with Andor Dragonfly 200 High-Speed Platform, using an Andor Sona-2BV11 camera mounted on a Nikon TiE inverted microscope. Cytotoxicity was calculated based on luminescence signals (counts per second), which were measured using a Spark™ multimode microplate reader (Tecan). Incucyte® SX5 Live-Cell Analysis System (Sartorius) was used for in vitro Incucyte cell killing assay. The IVIS Spectrum Imaging System (PerkinElmer) was used for bioluminescence imaging with Living Image software (v4.8.0).
Data analysis	FlowJo v10.8.1 was used for all FACS data analysis. Imaris v10.0.1 was used for confocal image analysis. GraphPad Prism v10.1.1 was used for statistical analysis and graphing. The on-target indel rates were analyzed using CRISPResso2 v2.2.14 (http://crispresso.pinellolab.org/submission). On-target editing outcomes and genome-wide off-target insertion sequencing were analyzed using PEM-Q (https://github.com/liumz93/PEM-Q).

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

All relevant data supporting the findings of this study are available in the manuscript and supplementary information files of this article. HTS data are available at https://doi.org/10.6084/m9.figshare.32358084. Additional datasets are available at https://www.wanglab-thu.org.cn/knit.

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	No human participants involved.
Reporting on race, ethnicity, or other socially relevant groupings	No human participants involved.
Population characteristics	No human participants involved.
Recruitment	No human participants involved.
Ethics oversight	No human participants involved.

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

Life sciences study design

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

Sample size	For each experimental condition included in each dataset, the final results were generated from at least two or three independent biological replicates. Sample sizes were determined according to established norms and prior studies within the relevant discipline, and are generally considered adequate for obtaining a reliable estimate of the mean value for each measured parameter.
Data exclusions	No data were excluded for analysis.
Replication	The biological replicates were stated in the figure legends or method section.
Randomization	Mice were randomly assigned to groups based on baseline tumor radiance, with comparable body weight and age across groups. Eukaryotic cell cultures were maintained under identical experimental conditions.
Blinding	Blinding was not performed, as it was not relevant given that baseline/reference levels were established using appropriate control conditions.

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

Antibodies

Antibodies used

Antibody information used in the study.
LIPA monoclonal antibody, clone 9G7F12 (Abnova, Cat #MAB5527, 1:500)
GAPDH antibody (Proteintech, Cat #10494-1-AP, 1:5000)
Horseradish peroxidase (HRP) labeled Goat anti-Mouse IgG (H+L) (Beyotime, A0216, 1:1000)
Horseradish peroxidase (HRP) labeled Goat anti-Rabbit IgG (H+L) (Beyotime, A0208, 1:1000)
Myc-Tag (9B11) Mouse mAb (Pacific Blue™ Conjugate) (Cell Signaling Technology, Cat #64584, 1:50)
Myc-Tag (9B11) Mouse mAb (Alexa Fluor® 488 Conjugate) (Cell Signaling Technology, Cat #2279, 1:50)
Myc-Tag (9B11) Mouse mAb (Alexa Fluor® 647 Conjugate) (Cell Signaling Technology, Cat #2233, 1:50)

Validation

LIPA monoclonal antibody, clone 9G7F12, used for WB. Species Reactivity: Human. This antibody was validated by the company.
GAPDH antibody, used for WB. Species Reactivity: Human, Mouse, Rat, Porcine. This antibody was validated by the company.
Horseradish peroxidase (HRP) labeled Goat anti-Mouse IgG (H+L), used for WB. Species Reactivity: Mouse. This antibody was validated by the company.
Horseradish peroxidase (HRP) labeled Goat anti-Rabbit IgG (H+L), used for WB. Species Reactivity: Rabbit. This antibody was validated by the company.
Myc-Tag (9B11) Mouse mAb (Pacific Blue™ Conjugate), used for primary T cells surface immunostaining. Species Reactivity: All Species Expected. This antibody was validated by the company.
Myc-Tag (9B11) Mouse mAb (Alexa Fluor® 488 Conjugate), used for primary T cells surface immunostaining. Species Reactivity: All Species Expected. This antibody was validated by the company.
Myc-Tag (9B11) Mouse mAb (Alexa Fluor® 647 Conjugate), used for primary T cells surface immunostaining. Species Reactivity: All Species Expected. This antibody was validated by the company.

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s)

HEK293T cells (ATCC, CRL-3216)
NIH 3T3 cells (ATCC, CRL-1658)
Jurkat, Clone E6-1 cells (ATCC, TIB-152)
K562 cells (ATCC, CCL-243)
Nalm-6-luciferase-GFP cells (VITALSTAR)

Authentication

All cells were authenticated by the vendor via STR profiling.

Mycoplasma contamination

All cells were tested negative for mycoplasma contamination.

Commonly misidentified lines
(See ICLAC register)

No commonly misidentified cell lines are employed in this study.

Animals and other research organisms

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research, and Sex and Gender in Research

Laboratory animals

6–8-week-old NOD/SCID/IL-2Rγ-null NPG female mice (VITALSTAR) were used in this study.
All mice were maintained in a Specific Pathogen Free (SPF) facility equipped with individually ventilated cages under strictly controlled environmental conditions: temperature 23 ± 2°C, relative humidity 40%-70%, and a 12-hour light/dark cycle.

Wild animals

This study did not involve wild animals.

Reporting on sex

Female mice were used in this study.

Field-collected samples

This study did not involve samples collected from the field.

Ethics oversight	All animal care and experimental procedures were reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) of Tsinghua University, Beijing, China.

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

Plants

Seed stocks	Not used.
Novel plant genotypes	Not used.
Authentication	Not used.

Flow Cytometry

Plots

Confirm that:

☒ The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).

☒ The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a 'group' is an analysis of identical markers).

☒ All plots are contour plots with outliers or pseudocolor plots.

☒ A numerical value for number of cells or percentage (with statistics) is provided.

Methodology

Sample preparation	Cells were washed with phosphate-buffered saline (PBS) and digested with 0.25% Trypsin-EDTA (Gibco, Cat #25200072) at room temperature. The digestion was terminated using DMEM supplemented with 10% FBS. Cells were pelleted and resuspended with 200 µl of DMEM supplemented with 3% FBS. CAR-T cells were stained with Myc-Tag (9B11) Mouse mAb (Pacific Blue™ Conjugate), Myc-Tag (9B11) Mouse mAb (Alexa Fluor® 488 Conjugate) or Myc-Tag (9B11) Mouse mAb (Alexa Fluor® 647 Conjugate) (Cell Signaling Technology, Cat #2233). T cells were resuspended in 100µL of antibody diluted 1:50 and incubated for 1 hour at room temperature, protected from light. The cells were then washed with PBS and analyzed by flow cytometry.
Instrument	For analysis, BD LSRFortessa SORP flow cytometer (BD Biosciences) For cell sorting, BD FACSAria flow cytometer (BD Biosciences)
Software	FlowJo v10.8.1 for data analysis.
Cell population abundance	At least 10000 cells were collected for analysis, except for Extended Data Fig. 10g. For Extended Data Fig. 10g, equal proportions of Cas9- and KE-treated samples were analyzed.
Gating strategy	A figure exemplifying the gating strategy is shown in Supplementary Fig. 1. After data collection, FCS files were imported into FlowJo software for gating and analysis. The flow cytometry and FACS analyses in this study were performed using the indicated gating/sorting strategy. Positive cell populations were subsequently determined according to the corresponding negative control groups. This strategy was applied to all flow cytometry and FACS analyses in this study.

☒ Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information.