

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 (<i>n</i>) 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 <i>Only common tests should be described solely by name; describe more complex techniques in the Methods section.</i>
☒	☐ 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. <i>F</i> , <i>t</i> , <i>r</i>) with confidence intervals, effect sizes, degrees of freedom and <i>P</i> value noted <i>Give P values as exact values whenever suitable.</i>
☒	☐ 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 <i>d</i> , Pearson's <i>r</i>), 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	Next-generation sequencing data were generated by Illumina Novaseq 6000 and Novaseq PE150.FASC data were generated by BD FACSAria II flow cytometry. RT-qPCR data were collected using LightCycler® 96 Instrument.
Data analysis	Deep-seq and RNA-seq data were analyzed with the online methods in this study.We use conda: 23.3.1; Bowtie2: 2.5.1; STAR: 2.7.9a; samtools: 1.17; Cutadapt: 1.15; TrimGalore: 0.6.7; FastQC: 0.11.9.We use the following Python packages with python 3.8.15:CRISPResso: 2.2.12; matplotlib: 3.7.3; scanpy 1.9.6; seaborn: 0.13.2We use the following R packages with R 4.1.2:DESeq2: 1.34.0; limma: 3.50.3; FactoMineR: 2.6; pheatmap: 1.0.12; stats: 4.1.2; clusterProfiler: 4.2.2

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 sgRNAs, linkers and primers sequences in this study are available in the Supplementary table. RNA-seq and Deep-seq data have been deposited on the National Center for Biotechnology Information database (accession nos. GSE261105 for RNA-seq; accession nos. GSE260967 for Deep -seq)

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	n/a
Reporting on race, ethnicity, or other socially relevant groupings	n/a
Population characteristics	n/a
Recruitment	n/a
Ethics oversight	n/a

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	Samples size are described in figure legends. Sample sizes were determined based on other researches that generate reproducible results in similar experimental contexts.
Data exclusions	No data were excluded.
Replication	Experiments in the article were reliably reproduced, replication were described in the figure legends.
Randomization	The samples were allocated randomly into experimental group in the in vivo experiments.
Blinding	Blinding was not performed for all experiments and data analyses, since no differential clinical intervention was performed or was being compared in 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

Methods

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

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) HEK293T cells (ATCC, CRL-3216 TM), MCF7 cells (ATCC, HTB-22), THP1 cells(ATCC, TIB-202), MV4-11(ATCC, CRL-9591)

Authentication Cell line identities were confirmed by STR profiling (ATCC).

Mycoplasma contamination Supernatant was analyzed for the presence of Mycoplasma and every test was negative.

Commonly misidentified lines (See [ICLAC](#) register) No commonly misidentified cell lines were used in this work.

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 C57BL/6 female mice, aged 6 weeks, were housed in ventilated cages in a temperature-controlled facility with a 12 hours (h) light/12 h dark cycle (lights on 6:00-18:00), room temperature of 25±2°C, ambient humidity range from 50 to 60%, and free access to food and water.

Wild animals We did not use any wild animals in this study.

Reporting on sex Only female animals were used in this study because sex of the animals is not considered relevant for the studies.

Field-collected samples We did not use any field-collected samples in this study.

Ethics oversight All animal studies were approved by the Scientific Investigation Board of Southern Medical University.

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

Plants

Seed stocks n/a

Novel plant genotypes n/a

Authentication n/a

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	Cell culture, transfection, harvest and resuspend cells in 500 µl FACS buffer, analysis
Instrument	BD LSRFortessa™ flow cytometer
Software	BD FACSDiva Software(BD Biosciences) and FlowJo software (TreeStar, USA).
Cell population abundance	Percentage of mNeonGreen positive was calculated as the proportion of mNeonGreen positive cells of total cells. Activation efficiency in BFP+ was calculated as the proportion of mNeonGreen positive cells within the transfected cells (BFP positive).
Gating strategy	Gates were performed by the untransfected control cells.

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