

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
<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. F , t , r) with confidence intervals, effect sizes, degrees of freedom and P 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 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

1. RNA/ tRNA sequencing data were sequenced by the Illumina HiSeq X10 platform.
2. RiboTag RNA sequencing data were sequenced using the Illumina Novaseq6000 platform.
2. Liquid chromatography-mass spectrometry (LC-MS) /MS data were collected with triple-quadrupole mass spectrometer (AB SCIEX QTRAP 5500).
3. RT-qPCR data were collected with Bio-Rad CFX Connect Real-Time System (Bio-Rad CFX Maestro 1.1).
4. Immunoblots were collected with Bio-Rad ChemiDoc™ Imaging System (12003153).
5. Flow cytometry data were collected with BD LSRFortessa X20.

Data analysis

1. For flow cytometry, FlowJo (vX.0.7) was used. Numerical data were exported to Excel (16.54) and were further analyzed by using GraphPad Prism (Version 6.0c).
2. For RNA-seq, Index of the reference genome was built using Hisat2 (v2.0.5) and paired-end clean reads were aligned to the mm10 genome using Hisat2 (v2.0.5), and then featureCounts (v1.5.0-p3) was used to count the reads numbers mapped to each gene. For Ribo-seq, the rRNA removed reads of each sample were mapped to the reference genome by Bowtie2 (v2.4.1) allowing no mismatches. For m1A-seq, clean reads were mapped to mm10 tRNA genome by bowtie2. For RiboTag-seq, the Index of the reference genome was built using Hisat2 (v2.2.1) and paired-end clean reads were aligned to the mm10 genome using Hisat2 (v2.2.1), and then Stringtie (v2.2.1) was used to count the reads numbers mapped to each gene.
3. For tRNA m1A-seq analysis. Raw reads adapter sequences were trimmed by trim galore (version 0.6.5), the minimum quality threshold was set to 20, and the minimum length required for reads after trimming was 30 nt. The remaining reads were further processed by removing the first 10 nt random barcode in the 5' end. Processed reads were mapped to mouse Transfer RNA (tRNA) reference (<http://gtrnadb.ucsc.edu/genomes/eukaryota/Mmus10/Mmus10-seq.html>) using BWA-MEM with default parameters.
4. Graphpad prism 6 (Version 6.0c) and Excel (16.54) was used to analyze the RT-qPCR data.
5. Sequencing data was analyzed using the Snappgene (4.1.6).

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 the high-throughput sequencing data generated for this study has been deposited in the NCBI Gene Expression Omnibus (GEO) (<https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE184909>).

The Chip-seq data are accessible from Gene Expression omnibus (GEO) with the following accession ID: GSE39756, GSE40918, GSE54191, GSE58075, GSE102317.

The mass spectrometry proteomics data are available via the PRIDE database (<http://www.proteomexchange.org>) under accession numbers PXD004367 and PXD005492.

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 statistical methods were used to pre-determine sample sizes but our sample sizes are similar to those reported in previous publications.
Data exclusions	No data were excluded from the analyses.
Replication	Replication are indicated in the figure legends. Independent experiment and biological replicate were used to ensure the reproducibility of results.
Randomization	Samples or mice were grouped according to treatment or genotypes, and thus not randomized.
Blinding	Data collection and analysis were not performed blind to the conditions of the experiments.

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
☒	☐ Human research participants
☒	☐ Clinical data
☒	☐ Dual use research of concern

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

Antibodies

Antibodies used

Anti-mouse CD3 ϵ (145-2C11) FITC Biolegend Cat# 100306; Lot: B241616; RRID:AB_312671; 1:200
 Anti-mouse CD25 (3C7) PE Biolegend Cat# 101904; Lot: B238380; RRID:AB_312847; 1:200
 Anti-mouse CD62L (MEL-14) PE Biolegend Cat# 104408; Lot: B242686; RRID:AB_313095; 1:200
 Anti-mouse CD8 (53-6.7) PE Biolegend Cat# 100708; Lot: B243039; RRID:AB_312747; 1:200
 Anti-mouse CD8 (53-6.7) PE-Cy7 Biolegend Cat# 100722; Lot: B239088; RRID:AB_312761; 1:200
 Anti-mouse CD4 (GK1.5) PE-Cy7 Biolegend Cat# 100422; Lot: B224943; RRID:AB_312707; 1:200
 Anti-mouse CD3 (145-2C11) BV785 Biolegend Cat# 100355; Lot: B346637; RRID:AB_2565969; 1:200
 Anti-mouse TCR- β (H57-597) FITC Biolegend Cat# 109206; RRID:AB_313429; 1:200
 Anti-mouse TCR- β (H57-597) BV421 Biolegend Cat# 109234; Lot: B302703; RRID:AB_2562350; 1:200
 Anti-mouse CD4 (GK1.5) Alexa Fluor 700 Biolegend Cat# 100430; Lot: B331561; RRID:AB_493699; 1:200
 Anti-mouse CD4 (RM4-5) FITC Biolegend Cat# 100510; Lot: B324527; RRID:AB_312713; 1:200
 Anti-mouse CD4 (RM4-4) PE Biolegend Cat# 116006; Lot: B211830; RRID:AB_313691; 1:200
 Anti-mouse CD25 (3C7) FITC Biolegend Cat# 101908; RRID:AB_961212; 1:200
 Anti-mouse CD69 (H1.2F3) BV421 Biolegend Cat#104528; RRID:AB_2562328; 1:200
 Anti-mouse IFN- γ (XMG1.2) APC Biolegend Cat# 505810 ; Lot: B299238; RRID:AB_315404; 1:200
 Anti-mouse IL-17A (TC11-18H10.1) FITC Biolegend Cat# 506908 ; Lot: B259285; RRID:AB_536010; 1:200
 Anti-mouse CD44 (IM7) APC Biolegend Cat# 103012 ; Lot: B240355; RRID:AB_312963; 1:200
 Anti-mouse IL-2 (JES6-5H4) FITC Biolegend Cat# 503806 ; Lot: B272441; RRID:AB_315300; 1:200
 Anti-mouse CD127 (A7R34) BV421 Biolegend Cat# 135024 ; Lot: B277509; RRID:AB_11218800; 1:200
 Anti-mouse CD45RB (C363-16A) APC Biolegend Cat# 103320 ; Lot: B210345 RRID:AB_2565229; 1:200
 Anti-mouse CD45 (30-F11) APC-CY7 Biolegend Cat# 103116; RRID:AB_312981; 1:200
 Anti-mouse CD44 (IM7) PE-Cy7 Biolegend Cat# 103030; Lot: B220566; RRID:AB_830787; 1:200
 Anti-mouse CD62L (MEL-14) BV421 Biolegend Cat# 104436; RRID:AB_2562560; 1:200
 Anti-mouse CD45RB (C363-16A) PerCP-Cy5.5 Biolegend Cat# 103314; RRID:AB_2284707; 1:200
 Anti-mouse TCR- β (H57-597) PE-CY7 Biolegend Cat# 109222; Lot: B336462; RRID:AB_893625; 1:200
 Anti-mouse TCR- β (H57-597) APC-CY7 Biolegend Cat# 109220; RRID:AB_893624; 1:200
 Anti-mouse CD3 ϵ (145-2C11) PE-CY7 Biolegend Cat# 100320; Lot: B316240; RRID:AB_312685; 1:200
 Anti-mouse CD3 ϵ (145-2C11) APC-CY7 Biolegend Cat# 100330; RRID:AB_1877170;
 Anti-mouse CD4 (GK1.5) BV711 Biolegend Cat# 100447; RRID:AB_2564586; 1:200

Anti-mouse CD69 (H1.2F3) APC Biolegend Cat# 104514; Lot: B244020;
 RRID:AB_492843; 1:200
 Anti-mouse Foxp3 (FJK-16s) APC Thermo Fisher Scientific Cat# 17-5773-80; Lot: 2152040;
 RRID:AB_469456; 1:200
 Anti-mouse IFN- γ (XMG1.2) Alexa Fluor 700 Biolegend Cat# 505824;
 RRID:AB_2561300; 1:200
 Anti-mouse IL-17A (TC11-18H10.1) Alexa Fluor 647 Biolegend Cat# 506912;
 RRID:AB_536014; 1:200
 Anti-mouse Ki-67 (16A8) BV421 Biolegend Cat# 652411;
 RRID:AB_2562663; 1:200
 Anti-mouse CD8a (53-6.7) BV605 Biolegend Cat# 100744;
 RRID:AB_2562609; 1:200
 Anti-mouse CD44 (IM7) PE Biolegend Cat# 103008; Lot: B334370;
 RRID:AB_312959; 1:200
 Anti-mouse CD8a (53-6.7) PerCP-Cy5.5 Biolegend Cat# 100734; Lot: B313041;
 RRID:AB_2075238; 1:200
 Anti-mouse IFN- γ (XMG1.2) PE Biolegend Cat# 505808; Lot: B295804;
 RRID:AB_315402; 1:200
 Anti-mouse TRMT61A antibody Thermo Fisher Scientific Cat# PA5-76553; Lot:31134A07;
 RRID:AB_2720280; 1:2000
 Anti-mouse TRMT6 antibody Proteintech Cat# 16727-1-AP;
 RRID:AB_2878306; Lot: 00008101; 1:2000
 Anti-mouse MYC antibody Proteintech Cat# 10828-1-AP;
 RRID:AB_2148585; 1:2000
 Anti-mouse c-JUN antibody Cell Signaling Technology Cat# 9165; Lot: 13;
 RRID:AB_2130165; 1:2000
 Anti-mouse FOSL2 antibody abcam Cat# ab216838; 1:2000
 Anti-mouse RHOA antibody Cell Signaling Technology Cat# 2117;
 RRID:AB_10693922; Lot: 5; 1:2000
 Anti-mouse Cyclin-E1 antibody Proteintech Cat# 11554-1-AP; Lot: 00047453;
 RRID:AB_2071066; 1:2000
 Anti-mouse Cdk2 antibody abcam Cat# ab32147;
 RRID:AB_726775; 1:2000
 Anti-mouse P27 antibody Proteintech Cat# 25614-1-AP;
 RRID:AB_2880161; 1:2000
 Anti-mouse p-STAT5 antibody Cell Signaling Technology Cat# 4322;
 RRID:AB_10544692; 1:2000
 Anti-mouse STAT5 antibody Cell Signaling Technology Cat# 94205;
 RRID:AB_2737403; 1:2000
 Anti-mouse p-Erk antibody Cell Signaling Technology Cat# 4370;
 RRID:AB_2315112; 1:2000
 Anti-mouse Erk antibody Cell Signaling Technology Cat# 4695;
 RRID:AB_390779; 1:2000
 Anti-mouse Zap70 antibody Cell Signaling Technology Cat# 3165;
 RRID:AB_2218656; 1:2000
 Anti-mouse p-Zap70 antibody Cell Signaling Technology Cat# 2717;
 RRID:AB_2218658; 1:2000
 Anti-mouse p-4EBP1 antibody Cell Signaling Technology Cat# 2855;
 RRID:AB_560835; 1:2000
 Anti-mouse p-S6 antibody Cell Signaling Technology Cat# 4858;
 RRID:AB_916156; 1:2000
 Anti-mouse Actin antibody Cell Signaling Technology Cat# 3700; Lot: 18;
 RRID:AB_2242334; 1:2000
 Anti-rabbit IgG, HRP-linked Antibody Cell Signaling Technology Cat# 7074;
 RRID:AB_2099233; 1:10000
 Anti-mouse IgG, HRP-linked Antibody Biodragon Cat# BF03001; Lot: KIA9033285; 1:10000

Validation

The validation of all of the antibodies depends on product datasheet and published literature.

Eukaryotic cell lines

Policy information about [cell lines](#)

Cell line source(s)

HEK293T cells were purchased from ATCC (ATCC CRL-3216).

Authentication

No method of cell line authentication was used.

Mycoplasma contamination

All cell lines are tested negative for mycoplasma contamination.

Commonly misidentified lines
(See [ICLAC](#) register)

No such cell lines were used in this study.

Animals and other organisms

Policy information about [studies involving animals](#); [ARRIVE guidelines](#) recommended for reporting animal research

Laboratory animals

Trmt61a-floxed mice were generated at the Shanghai Biomodel Organism Science & Technology Development Co., Ltd using the CRISPR–Cas9-based genome-editing system. The guide RNA (gRNA) and donor oligonucleotides used were listed as follows. For Trmt61a left side loxP: 5'-TGCATGAACCATGTTGTCGG-3', and 5'-AGCACACCTTTAAGCACAGTATTTGTTAGGCAGAGCAGGCAGATCTCTGGGCTGAGACCAGCCTGATCTACATAGTGAGTTCCAGGCAGCCAAGGCTATATAGGGAGGCTGTCTGAAAGACAAAATATAGCCCTGCATGAACCATGTTGTCGGTATGATGTTACACAAATTAGCTTTCTGTTCCCATCTGTAAAGTGGTCATAAAATTGTAAGGAAGAGGATTCAATGACATGAGAG-3'; for Trmt61a right side loxP: 5'-GTGCCCTATGAGGTCGGAGC-3', and 5'-TGGGAGCTAACCTGGGTTGGAGAAGAAAGAGGAGGAGGTGCCCTATGAGGTCGGAGCTGGGATTGCTTGATGCTGGCCAAGGTGCTGACTGCTTTTCTAGGCCAGCTGCCCTTCTGGAGGGAGGCTGGATACCTCAGAGCTCATTGCAGAGAGAAGTCTGTCCACACTTACAGCCAGGCCCTTCCCTAGTTCATCGAAGATTAGCACTGTCACTGATACGAGGCCAGGTGGTA-3'.

Trmt6-floxed mice were generated at the Cyagen Biosciences Inc. using the CRISPR–Cas9-based genome-editing system. The guide RNA (gRNA) and donor oligonucleotides used were listed as follows. For Trmt6 left side loxP: 5'-AAGAGACTGAGATCTCCGATAGG-3', and 5'-GCTTGCTTTGAAGTTGCTCTAAGAGACTGAGATCTCCGATAGGAAGGCTAATGCCTGACCCTTGGCAGTACTTCATTAGTTCTACATCCATTTCCAA TGTGTAGATTGCCAATAATGTTTATTCTGACACAGGCTTTTGGAAATTTGCTTTTCTAATAGAGTAGCCAATTAGACAGA-3'; for Trmt6 right side loxP: 5'-ATGCTAGAGAATTAGCCCAACGG-3', and 5'-CTTATCTCAAAAAGGATCTTTCAGGATGCTAGAGAATTAGCCCAACGGTAAAGTACTTGTTGCTCTGAGAAGATCCAGGTTTCAGTTCTGAACAC CCACATGGTGGCTACAACCATCTGTTACTTCAGTTCAGGGATCTGATGCCCTTCTGAGCTCAGGCACAGGCATGCATCTGGTACTCATACATG CACACAGGCAAAACACTCAA-3'.

Trmt6/61aflox/flox mice were crossed with Cd4Cre mice to obtain conditional knockout mice. Cd4Cre mice were purchased from the Jackson laboratory and have been fully backcrossed to C57BL/6 mice (over more than 10 generations).

RiboTag (B6N.129 strain) mice are viable and fertile, with a targeted mutation of the ribosomal protein L22 (Rpl22) locus harboring a loxP-flanked wildtype C-terminal exon 4 followed by an identical C-terminal exon 4 that is tagged with three copies of the hemagglutinin (HA) epitope before the stop codon. Prior to exposure to Cre recombinase, RiboTag mice express the wildtype RPL22 protein (15 kDa). When the RiboTag mice are bred to cre-expressing mice, offspring will have the floxed wildtype exon 4 deleted in the cre-expressing tissue and subsequent use of the downstream HA epitope-tagged exon 4 as the terminal exon. RiboTag mice were purchased from the Jackson laboratory (Strain #:011029).

Mice were housed in specific pathogen-free facility under 12h light/dark cycle at 25°C and analyzed between six to twelve-weeks of age unless described otherwise. Female and male mice were used.

Wild animals

No wild animals were used in the study.

Field-collected samples

No field-collected samples were used in this study.

Ethics oversight

Animal procedures were approved by the Institutional Animal care and Use Committee (IACUC) of Shanghai Jiao Tong University School of Medicine.

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

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

Thymus, spleen, peripheral and mesenteric lymph nodes were collected and pressed through a 200- gauge mesh. Spleen cells were prepared by lysing the erythrocytes with red blood cell lysis buffer (Thermo Fisher Scientific). Primary T cells were isolated from the spleen and lymph nodes of age-matched WT and conditional knockout mice (6-12 weeks old). Naive CD4+ or total CD4+ T cells were purified by using EasySep Mouse Naive CD4+ T or CD4+ T Cell Isolation Kit (STEMCELL Technologies) according to the instructions, respectively.

Instrument

Flow cytometry data were collected with a BD Fortessa X20 (BD FACSDiva Software v8.0.1). Flow sorting was performed using an Arial III (BD Biosciences)

Software

Flow cytometry data were analyzed using the FlowJo (vX.0.7).

Cell population abundance

20,000-100,000 cells were acquired and analyzed for each independent experiment.

Gating strategy

Cells were first gated on CD45+ population, and then gated on FSC/SSC, doublets excluded by

Gating strategy

T cells were identified by gating CD3+TCRb+ cells. CD4 and CD8 T cells were identified by gating CD4+ and CD8+ events within the CD3+TCRb+ gate.

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