Tet2 promotes pathogen infection–induced myelopoiesis through mRNA oxidation

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Varieties of RNA modification form the epitranscriptome for post-transcriptional regulation1. 5-Methylcytosine (5-mC) is a sparse RNA modification in messenger RNA (mRNA) under physiological conditions2. The function of RNA 5-hydroxymethylcytosine (5-hmC) oxidized by ten-eleven translocation (Tet) proteins in *Drosophila* has been revealed more recently3,4. However, the turnover and function of 5-mC in mammalian mRNA have been largely unknown. Tet2 suppresses myeloid malignancies mostly in an enzymatic activity-dependent manner5, and is important in resolving inflammatory response in an enzymatic activity-independent way6. Myelopoiesis is a common host immune response in acute and chronic infections; however, its epigenetic mechanism needs to be identified. Here we demonstrate that Tet2 promotes infection-induced myelopoiesis in an mRNA oxidation-dependent manner through Adar1-mediated repression of Socs3 expression at the post-transcriptional level. Tet2 promotes both abdominal sepsis–induced emergency myelopoiesis and parasite-induced mast cell expansion through decreasing mRNA levels of Socs3, a key negative regulator of the JAK–STAT pathway that is critical for cytokine–induced myelopoiesis. Tet2 represses Socs3 expression through Adar1, which binds and destabilizes Socs3 mRNA in a RNA editing-independent manner. For the underlying mechanism of Tet2 regulation at the mRNA level, Tet2 mediates oxidation of 5-mC in mRNA. Tet2 deficiency leads to the transcriptome-wide appearance of methylated cytosines, including ones in the 3′ untranslated region of Socs3, which influences double-stranded RNA formation for Adar1 binding, probably through cytosine methylation–specific readers, such as RNA helicases. Our study reveals a previously unknown regulatory role of Tet2 at the epitranscriptomic level, promoting myelopoiesis during infection in the mammalian system by decreasing 5-mCs in mRNAs. Moreover, the inhibitory function of cytosine methylation on double-stranded RNA formation and Adar1 binding in mRNA reveals its new physiological role in the mammalian system.

During infection, sensing pathogen and inflammatory cytokines skews haematoipoiesis towards myeloid development; however, the epigenetic mechanism for this pathogen infection–induced myelopoiesis is unclear7. We investigated the role of Tet2, a myeloid tumour suppressor, in pathogen infection–induced myelopoiesis. First, we subjected significant varied genes in RNA sequencing (RNA-seq) data (Supplementary Table 1) from Tet2-deficient bone-marrow–derived mast cells (BMMCs) and control cells to Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis; and the expression variation of the genes in JAK–STAT and PI3K–Akt pathways, which are critical for inflammatory cytokine–induced myelopoiesis8,9, were labelled near scatter plots (Fig. 2a). Among these genes, Socs3, a key suppressor of JAK–STAT signalling, was significantly increased in the Tet2-deficient group. Upregulation of Socs3 in both BMMCs and Lin+Kit+ haematopoietic stem and progenitor cells (HSPCs) stimulated by interleukin-3 (IL-3), a critical cytokine for both acute infection–induced myelopoiesis and parasite infection–induced mast cell expansion8, was observed at both mRNA and protein levels in Tet2-deficient groups (Fig. 2b–d; see Supplementary Fig. 1 for gel Source Data). We also found decreased expression of IL-3 signal–induced genes in Tet2-deficient BMMCs (Extended Data Fig. 2a–c) and the impaired phosphorylation of Akt and STAT5 in both IL-3–stimulated HSPCs and bone marrow cells from Tet2-deficient mice (Extended Data Fig. 2d, e), further validating the defective JAK–STAT signalling in Tet2-deficient myeloid cells. Silencing of Socs3 in Tet2-deficient BMMCs increased IL-3 signalling (Extended Data Fig. 2f), indicating that Tet2 promoted infection–induced myelopoiesis by repressing Socs3 expression for efficient cytokine signalling.

To reveal the molecular mechanism of Tet2–mediated Socs3 suppression, we first detected the DNA methylation levels of CpGs in two predicted CpG islands near the Socs3 promoter, and found that all of these CpGs were hypomethylated in both wild-type and Tet2-deficient BMMCs (Extended Data Fig. 3a, b); loss of Tet2 still increased the mRNA level of Socs3 when *de novo* transcription was inhibited (Extended Data Fig. 3c), indicating the potential role of Tet2 in regulating Socs3 at post-transcriptional level.
Tet2 was recently identified as an RNA-binding protein, implying its general role in post-transcriptional regulation. We performed three biological replicates of enhanced cell-linking and immunoprecipitation followed by high-throughput sequencing (eCLIP-seq) for obtaining potential Tet2-binding RNAs in BMMCs (Extended Data Fig. 3d, e), and biological replicates correlated well with each other (Extended Data Fig. 3f). Among thousands of peaks identified in the three replicates, more than 80% were located in genic regions (Fig. 2e), including Socs3 loci (Supplementary Table 2), and about 50–60% of CLIP peaks overlapped with each other in at least two replicates (Fig. 2f).

In RNA-seq data, we found more A-to-G mutations transcriptome-wide in the wild-type group than in the Tet2-deficient group, which contained an A-to-G mutation in the 3′ UTR of Socs3 (Supplementary Table 3). Most of the group-specific A-to-G mutants showed low mutation rates (Fig. 2g), and were more distributed in 3′ UTRs in mature mRNA elements (Fig. 2h), consistent with RNA editing preferences in mRNAs. Furthermore, top motifs across the mutation sites contained an A-to-G mutation in the 3′ UTR of Socs3 (Supplementary Table 2), and about 50–60% of CLIP peaks overlapped with each other in at least two replicates (Fig. 2f).

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Deficiency of Tet2 leads to increased transcripts and decreased A-to-G RNA editing for Socs3. a, Scatter plot of mRNA levels for a pair of Tet2-deficient (KO) and the control (WT) BMMCs. Upregulated (red) and downregulated (green) genes are coloured, varied genes in JAK–STAT and PI3K–AKT pathways are labelled near bigger plots. FRKM, fragments per kilobase of exon per million fragments mapped. b–d, qPCR and immunoblot assays of Socs3 in BMMCs (b, c) and IL-3–stimulated LinKit+ HSPCs for 8 h (c, d). e, f, Genomic distribution of CLIP peaks and their overlapping rates among the three biological replicates. g, h, Mutation levels and intragenic distribution of A-to-G mutants. CDS, coding sequence. i, Top motifs in 200 bp across A-to-G mutants. CDS, coding sequence. j, k, Unpaired two-sided Student’s t-test. Mean and s.e.m. of triplicate biological (b, d) replicates. Plots are representative of three independent experiments (c).

(Extended Data Fig. 6a, b). More methylcytosines were consistently identified, and with higher methylation levels in Tet2-deficient replicates than in controls (Fig. 4d and Extended Data Fig. 6c). There were many more group–specific methylcytosines in Tet2-deficient cells than in controls, more than half of which were located in the genic region, mostly in introns (Fig. 4e and Supplementary Table 4). As cell-specific alternative splicing leads to cell-specific intron retention, Tet2 may regulate 5-mC turnover in a cell-specific manner. Expression of genes bearing specific methylcytosines in the Tet2-deficient group or Tet2 CLIP peaks was more upregulated in Tet2-deficient cells (Extended Data Fig. 6d), implying an important role for Tet2-mediated mRNA oxidation in decreasing mRNA levels of genes such as Socs3. In mature mRNA-related elements, more methylcytosines located in the 3′ UTR of the Tet2-deficient group than in controls (Fig. 4f), further confirming the regulatory role of Tet2 in the 3′ UTR of mRNAs. When connecting the CLIP–seq data to specific methylcytosines in the Tet2-deficient group, we found that over 60% of genes or 3′ UTRs bearing these methylcytosines were associated with CLIP peaks (Fig. 4g). Methylocytosines and CLIP peaks in exons located close to each other in a mature mRNA view (Extended Data Fig. 6e). Among the 3′ UTRs bearing several methylcytosines, Socs3 was indeed in the list, and we validated that these methylcytosines appeared in Tet2-deficient cells (Extended Data Fig. 6f). We also found two other genes: Zfp65 with an A-to-G mutant near methylcytosines in the 3′ UTR where it was enriched in repeat elements; and Tmed10 with a predicted stable dsRNA structure in the 3′ UTR (Extended Data Fig. 6g–i). Tet2-deficient cells showed more Tmed10, partly depending on Tet2 (Fig. 4h). We further validated increased RNA methylation levels in 3′ UTRs of the three genes in Tet2-deficient BMMCs (Extended Data Fig. 6j).

We further investigated the underlying mechanism for the repressive role of 5-mC in Adar1 function, and found that overexpressed wild-type Tet2 and the Tet2ΔDNA indeed decreased 5-mC levels of overexpressed Socs3 mRNAs (Extended Data Fig. 7a). Oxidation forms of 5-mC in Socs3 3′ UTR were not detected in wild-type BMMCs (Extended Data Fig. 7b). Mutating the 5-mCs in the 3′ UTR of Socs3 mRNA decreased the mRNA levels of Socs3, and overexpression of Tet2 barely synergistically repressed the mRNA levels of mutated Socs3 with Adar1, compared with the wild-type Socs3 (Extended Data Fig. 7c).
According to our data, amounts of 5-hmC were much lower than 5-mC in mRNA from the tested mammalian cells. Thus, additional enzymatic steps by an unknown protein may convert 5-hmC back to cytosine in mRNA. Further study will be needed to reveal this unknown mechanism, probably involving the members of the ALKB family.

Moreover, our study implies that Tet2-mediated mRNA oxidation may be the critical step for RNA demethylation.

As a tumour suppressor gene, mutations of Tet2 were largely found in myeloid malignancies and some solid cancers. For haematopoiesis, loss of Tet2 leads to increased abnormal myeloid cells in ageing. And our study linked Tet2 to pathogen-infection-induced myelopoiesis in an immunological way, and found that Tet2 strengthens cytokine signalling in myeloid differentiation by suppressing the repressor. Furthermore, mutations with a deficiency in enzymatic activity and downregulation of Tet2 in types of disease can lead to dysregulation of 5-mC in mRNA, which may be critically involved in the pathogenesis of myeloid disorders, such as myeloid tumours. This needs further investigation.

Online Content Methods, along with any additional Extended Data display items and Source Data, are available in the online version of the paper; references unique to these sections appear only in the online paper.

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Author Contributions X.C. designed and supervised the study. X.C., Q.Z. and Q.She. analysed the data and wrote the manuscript. Q.She. established pathogen infection mouse models. Q.She. and Q.Z. confirmed and genotyped mice, performed RNA methylation- and RNA editing-related experiments and analysed all the data of this study. Q.She., and Q.Z. performed CLIP, bisulfite sequencing and analysed the sequencing data. Y.S. performed the dot plot assays. Q.She. and Q. Shi constructed plasmids with aid from Q.Z. Y.J. performed parasite infections of mice. Y.G. and Z.L. sorted and analysed immune cells. X.L., K.Z., C.W. and N.L. provided reagents and advice.

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METHODS

Mice and reagents. C57BL/6 mice were obtained from Joint Ventures Sipper BK Experimental Animal Company (Shanghai). Tet2-deficient mice on a C57BL/6 × 129/SvEv background were provided by R. L. Levine, and backcrossed to the C57BL/6 background in our laboratory. Kitsilvl1/− or Kit−/− mice were obtained from The Jackson Laboratory. All animal experiments were undertaken in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals, with the approval of the Scientific Investigation Board of Second Military Medical University, Shanghai. Recombinant SCF and IL-3 were from PeproTech. Antibodies used were as follows: Anti–5-mC antibody (A3001, 10G4, lot: 2RC180672, Zymo Research), Anti–5-hmC antibody (Mab-31HMC, lot: 001, Diagenode), Anti–5-ICF antibody (61227, lot: 34711001, Active Motif), Anti–5-cAC antibody (61225, lot: 34711001, Active Motif), Anti–Adar1 antibody (A303-8844, Bethyl Labs), Anti–Sos3 (2923, lot: 2, Cell Signaling Technology), Anti–STAT3 (9363P, lot: 3, Cell Signaling Technology), Anti–STAT5 phospho-rylated (Tyr694) (9359P, C11CS, lot: 4, Cell Signaling Technology), Anti–Akt (pan) (4685, 11E7, lot: 3, Cell Signaling Technology), Anti–Akt phosphorylated (Ser473) (4069, D9YE, lot: 19, Cell Signaling Technology), Anti–ERK phospho-rylated (Thr202/Tyr204) (4370, D13 14E, lot: 6, Cell Signaling Technology), Anti–ERK (9102, lot: 23, Cell Signaling Technology), Anti–IKK phosphorylated (Thr183/Tyr185) (4668, 81E11, lot: 9, Cell Signaling Technology), Anti–IKNZ phosphorylated (92S958, 56GS, lot: 11, Cell Signaling Technology), Anti–Myo (2276, 9B11, lot: 24, Cell Signaling Technology), Anti–Flag (F1804, lot: SLB4607V, Sigma–Aldrich), Anti–HA (3724, lot: 3, Cell Signaling Technology), Anti–LaminA/C (4777, 4C11, lot: 9, Cell Signaling Technology), Anti–GAPDH (2118, 14C10, lot: 6, Cell Signaling Technology), Anti–Tez2 (MABE462, lot: Q1141878, Millipore), Anti–J2 monoclonal antibody (10010200, IgG2a, lot: No.121611 Scines), Anti–3-actin (3700, 8H1D01, lot: 13, Cell Signaling Technology), Anti–rabbit IgG–HRP (34660, lot: RR230194, Thermo Fisher Scientific), Anti–mouse IgG–HRP (34140, lot: RA230188, Thermo Fisher Scientific), Anti–human IgG (ab6643, lot: GR99377–4, Abcam), APC–anti–Kit (17-1171-81, 288, lot: E07202-1631, Bioscience), PerC pcyanine5.5–CD11b (45-0112-82, M1/70, lot: 4301974, eBioscience), PE cyanine7 anti–Fc R1 (25-2868-80, MAR-1, lot: E16926-105, Bioscience), FITC anti–Ly6G (127606, I-A8, lot: B164314, Biolegend), Alexa Fluor 700 anti–Ly6c (128024, HK.1.4, lot: B224165, Biolegend), BV42–anti–F4–H1 (120317, BM8, lot: B202010, Biolegend). All the antibodies are all commercially available and validated by the suppliers according to the validation statements on the manufacturers’ websites.

Cell purification and cultures. For BMMCs, mouse bone marrow cells were isolated from femurs and cultured in RPMI1640 medium plus 10% (v/v) FBS (Gibco) with 10 ng ml−1–actin or 1% input RNAs in each individual sample. The 2′−actin method was used to calculate relative expression changes. With the help of dissociation curves and a 3′UTR of Sos3 were obtained from in vitro transcription by using T7 RNA polymerase (Thermo Fisher Scientific). The reaction was incubated at 37 °C for 60 min in a thermocycler and the enzyme was removed immediately afterwards by a Nase MiniElute Cleanup Kit (Qiagen) according to the manufacturer’s instructions. DNase-treated and purified RNAs were used for subsequent analysis.

Quantification of 5-mC by LC–MS/MS. Quantification of 5-mC by LC–MS/MS was performed as previously reported10, with the following modifications: 0.5 U rSAP (NEB) was used instead of alkaline phosphatase (Sigma–Aldrich). The mass transitions of m/z 258.0–126.1 (5-mC), m/z 274.0–142.1 (5-mHC, m/c 271.2–140.1 (5-IC), m/z 286.1–156.1 (5-caC) and m/z 244.1–112.0 (cytosine), were monitored and recorded, a series of concentrations of pure authentic nucleoside standards (C, from 50 nM to 2.00 nM, Sigma–Aldrich; 5-mC, from 0.5 nM to 50 nM, Sigma–Aldrich; 5-hmC, Santa Cruz, 5-IC and 5-caC, Berry & Associates, from 0.1 nM to 50 nM) were run for every batch of experiments to obtain their corresponding standard curves. Concentrations of nucleosides in mRNA samples were deduced by fitting the signal intensities into the standard curves. The ratios of 5-mC or 5-mC/U were subsequently calculated. Relative oxidation amounts were compared with 5-mC/U%, 5mim as 100%.

Dot blot. Dot blot analysis was performed for 5-mC, 5-hmC and 5-caC quantification in 90 °C heat treated RNAs or DNAs were conducted as previously reported11. In brief, RNAs or DNAs were spotted onto a nylon membrane (GE Healthcare). The membrane was dried and crosslinked twice with 20000 cGy–1 ultraviolet light. The membrane was blocked in 5% BSA in PBS + 0.1% Tween-20 for 1 h before transfer into blocking solution supplemented with 5-mC or other modification antibody and incubated overnight at 4 °C. After secondary antibody incubation and wash, dot blots were visualized using SuperSignal West Femto Chemiluminescent Substrate (Thermo Fisher Scientific) by a chemiluminescent imaging system. The same amounts of denatured RNAs were degradation by TURBO DNase (Thermo Fisher Scientific) negative control. To remove possible contaminating genomic DNAs, all RNA samples were treated by the DNase.

Plasmid constructs. Full-length mouse Sos3 or Adar1 was PCR amplified using reverse transcribed RNAs from BMCCs. Tet2 eukaryotic expression vector was obtained as previously described9. Mouse Tet2 mutant forms were as follows: Tet2Δm1, HS(H/Y)R(D/A)IQ; Tet2Δm2–m3, TR(M/J)(S/F)LVLYRH, CT/R(G) RCSQ; and Tet2ΔcaN, (W/R)SMYNYNGK(E/F)ESARN(S/N). They were generated by PCR-based amplification of the construct coding the wild–type protein and subcloned into the pcCMV-Myc–N (Clontech). Wild–type Adar1, Adar1ΔsDNA and Adar1ΔdsDNA11 (delete N456–G473) ampiclons were subcloned into the pcDNA3.1–Flag–C (Invitrogen) vector. The Sos3 and Sos3ΔcaN (chromosome 11: 117967529, 522, 518, 507, 485) full length were constructed into pcDNA3.1–HA–N (Thermo Fisher Scientific) vector. The 3′ UTR of Sos3 and the editing mutants Sos3ΔcaN (chromosome 11: 117967036) was subcloned into pMIR–REPORT Luciferase (Thermo Fisher Scientific) vector. All constructs were confirmed by DNA sequencing.

eCLIP–seq. Biological replicates of BMCCs that had different culture start dates were crosslinked end dates were collected. eCLIP was conducted as previously reported12, with the following modifications. Ultraviolet–crosslinked (first 400 mJ cm−2 ultraviolet light. Beads were washed three times using lysis buffer and incubated with proteins lysates for 4 h at 4 °C. A 3′ RNA adapter was ligated onto the RNA with T4 RNA ligase (NEB). Protein–RNA complexes were run on a 4–12% gradient Bis-Tris Gels (Invitrogen), transferred to PVDF membranes, and RNA was isolated off the membrane by using eCLIP. A fraction of sample was used for western blot of CLP, followed by secondary antibody incubation and washed, western blots were visualized using Unichem Luminescent Substrate (Thermo Fisher Scientific) by a chemiluminescent imaging system. The same amounts of denatured RNAs were degradation by TURBO DNase (Thermo Fisher Scientific) negative control. To remove possible contaminating genomic DNAs, all RNA samples were treated by the DNase.

Biochemical assay of Tet2-mediated oxidation of 5-mC in RNA. The Tet2-meditated RNA oxidation assay was conducted with the experimental workflows as previously reported24, with the presence of RNAin Plus RNase Inhibitor (Promega). A reaction mixture contained 250 ng of 5-mC-bearing 3′ UTR of Sos3, the catalytic domain of recombinant human Tet2 (active motif) or immunoprecipitated Tet2 mutants overexpressed in HEK293T cells, oxidation reagent (1.5 mM Fe(NH4)2(SO4)2–6H2O), with (α–K–Ig– group) or without (α–K– group) oxidation reagent (333 mM NaCl, 167 mM HEPEs (pH 8.0), 4 mM ATP, 8.3 mM buffer (50 mM Tris–HCl, 1 M NaCl)); the 3′ UTRS of Sos3 were obtained from in vitro transcription by using T7 RNA polymerase (Thermo Fisher Scientific). The reaction was incubated at 37 °C for 60 min in a thermocycler and the enzyme was removed immediately afterwards by a Nase MiniElute Cleanup Kit (Qiagen) according to the manufacturer’s instructions. DNase-treated and purified RNAs were used for subsequent analysis.
primers. Sequencing reads were processed and mapped according to cECLIP procedure. Peaks were identified using a ‘valley seeking’ algorithm, in which a peak is called if the valley of certain depth are found on both sides39. Peaks were filtered with peak height above 5 as cut off. Enriched motifs in CLIP peaks were analysed by homer software using strand-specific sequences from peak regions as inputs.

**RIP assay.** RIP was conducted as previously reported40. For endogenous RIP, cell lysates were made from wild-type or Tet2 knockout BMMCs in polysome lysis buffer (100 mM KCl, 5 mM MgCl₂, 10 mM HEPES pH 7.0, 0.5% NP40) supplemented with DTT, the immunoprecipitation cocktails and 1% Triton X100 (Promega) on ice. Lysates were sonicated and stored at 80 °C. The Protein G magnetic beads were pre-blocked for 1 h with rotation in NT2 buffer supplemented with 5% BSA (50 mM Tris-HCl pH 7.4, 150 mM NaCl, 1 mM MgCl₂, 0.05% NP40). The antibody was then added for 4 h at 4 °C with rotation, followed by wash anti-body-coated beads with 1 ml of ice-cold NT2 buffer four or five times. The cell lysate was thawed on ice and the insolubles were removed by centrifugation at 4 °C; 1% of total lysate was saved for input. Incubated cleared lysate and antibody were mixed for 2 h at 4 °C with rotation. Beads were washed four times with NT2 buffer and the RNA released by proteinase K for 30 min at 55 °C. Then RNA was isolated by adding TRizol to the beads and glycogen (Thermo Fisher Scientific) added as a carrier to aid the precipitation reaction. For overexpression RIP experiments, the Myc-tagged Tet2 and its mutants, or haemagglutinin-tagged Socs3 or the mutant and Flag-tagged Adar1 were transfected into HEK293T cells or Tet2 knockout BMMCs for 36–48 h. Cells were collected in ice-cold polysome lysis buffer supplemented with protease inhibitor cocktail and RNase Inhibitor. For quantification in the RIP assay, cDNA amplicons signals were normalized by those from the reserved 1% input and used for comparison between experimental samples. When antibody against target was initially used in one type of cell, normalized signals in IgG groups were used for comparison with experimental samples. RNAs from both the reserved 1% input and the immunoprecipitated samples were treated with TURBO DNase, purified by TRIzol LS, and then reverse transcribed using random hexamers and Protocol II Reverse Transcriptase (NEB). PCR was used to amplify the target regions. The qPCR primers for the assay of the association of Adar1 with Socs3 3′ UTR are in Extended Data Table 1.

**RNA-mediated interference.** BMMCs and HEK293T cells were transfected with siRNA (20 nM) through use of INTERFERin reagent (Polyplus). The mouse-specific siRNAs targeting Socs3 and Adar1 were designed and synthesized by GenePharma (Shanghai). Sequences of siRNAs were as follows: Socs3 siRNA 5′-GCCUCAAUACUCAAAGA-3′; Adar1 siRNA 5′-GCGCUAGCGAUAUUA8-3′; Tet2 siRNA 5′-CAGUGCAAAUUUUCUU-3′. J2 dsRNA pull-down. J2 antibody (1:50) was incubated with total nuclear extracts for 2 h at 4 °C (Lysis buffer: 50 mM HEPES pH 7.5, 50 mM KCl, 5 mM MgCl₂, 1 mM DTT, 1 × complete protease inhibitors and RNase Inhibitor). Protein G-magnetic beads (Thermo Fisher Scientific) pre-blocked for 1 h with 1% BSA were added and incubated on a wheel for an additional 1.5 h at 4 °C. dsRNA–antibody complexes were eluted and dsRNAs were extracted using TRizol LS Reagent (Invitrogen). RNA treatment and qPCR analysis were performed as in the RIP assay. The qPCR primers for validation of the Socs3 mRNA are in Extended Data Table 1. The methylated control RNA and modified control RNA were obtained from in vitro transcription by using T7 RNA polymerase (Thermo Fisher Scientific). Adar1–Flag-overexpressed HEK293T whole-cell lysates (1 mg) were incubated for 2 h at 4 °C with anti-Flag beads (Sigma–Aldrich). Adar1-containing immunocomplexes were washed twice with cell lysis buffer and incubated with 500 ng RNA substrate and 300 ng total extracts from BMMCs in 22 mM Tris–HCl (pH 7.5), 40 mM KCl, 10 mM NaCl, 6.5% glycerol, 0.5 mM DTT, 0.1 mM 2-mercaptoethanol, 0.05% NP-40 and RNase Inhibitor for 4 h at 30 °C. The RNA was recovered by TRizol extraction and ethanol precipitation. For the Adar1 binding assay, the beads from incubation with RNA substrate and nuclear extracts of BMMCs were washed three times with ice-cold NT2 buffer (50 mM Tris–HCl pH 7.4, 150 mM NaCl, 1 mM MgCl₂, 0.05% NP40). The beads were resuspended by TRizol reagent. RNA was precipitated followed by reverse transcription and qPCR analysis. 5′-mC affinity pull-down coupled with LC–MS/MS analysis. BMMCs (1 × 10⁶) were swelled onto 3D collagen in 50ml RSB buffer (10 mM Tris–HCl pH 8.0, 10 mM NaCl, 0.5% NP-40, 3 mM MgCl₂) and centrifuged at 2000g for 5 min at 4 °C. The pellets containing of nuclei were lysed by 90 min incubation in two volumes of nuclear lysis buffer (420 mM NaCl, 20 mM HEPES pH 7.9, 20% v/v glycerol, 2 mM MgCl₂, 0.2 mM EDTA, 0.1% NP40, protease inhibitor and 0.5 mM DTT). After centri-fugation, protein concentrations in the extractions were measured by BCA assay and stored at −80 °C. Ten micrograms of 5′-biotinylated cytosine or 5′-mC RNAs were immobilized on 75 μl of Dynabeads MyOne C1 (Invitrogen) by incubating for 1 h at room temperature in a total volume of 350 μl of binding buffer (1 μM NaCl, 10 mM Tris–HCl pH 8.1, 1 mM EDTA pH 8, and 0.05% NP-40 and RNase Inhibitor). Beads were then incubated with 1 μg of nuclear extracts of BMMCs in a total volume of 600 μl of protein binding buffer (50 mM Tris–HCl pH 8.5, 150 mM NaCl, 1 mM DTT, 0.25% NP-40 and complete protease inhibitors in the presence of RNase Inhibitor) for 2 h at 4 °C. Protein-complex-containing beads were washed extensively and eluted. LC–MS/MS analysis was conducted with the experimental workflows as previously reported41.

**Assay of luciferase reporter gene expression.** HEK293T cells were transfected with a mixture of the appropriate luciferase reporter plasmid, pRL-TK-renilla luciferase plasmid and the appropriate additional constructs using jetPEI (Polypus). The total amount of plasmid DNA was equalized by empty control plasmids measured with a Dual-Luciferase Reporter Assay System according to the manufacturer's (Promega) protocols after 24 h. Data were normalized for transfection efficiency by the division of firefly luciferase activity with that of renilla luciferase.

**Statistical analysis.** Error bars displayed throughout the paper represent s.e.m. or s.d. and were calculated from triplicate technical or triplicate biological
replicates described in figure legends. Sample sizes were chosen by standard methods to ensure adequate power, and no randomization of weight and sex or blinding was used for animal studies. Data shown are representative of three independent experiments, including histological images, blots and gels. No statistical method was used to predetermine sample size. Statistical significance was determined using unpaired two-sided Student’s t-tests; *P < 0.05; **P < 0.01.

**Data availability.** The data that support the findings of this study are available from the corresponding author upon reasonable request. The RNA sequencing data have been deposited in NCBI Sequence Read Archive under accession numbers GSE100559, GSE100560 and GSE100719.

Extended Data Figure 1 | Tet2 promotes mast cell expansion during parasite infection. a, In vivo experimental design of transplantation and infection studies with bone marrow cells from Tet2-deficient (knockout) and littermate control (wild-type) mice. b, Quantitative assessment of toluidine blue-positive mast cells in the intestinal tissues (n = 6 biologically independent mice). c, Representative photomicrographs of toluidine blue-stained tissue sections derived from Kit<sup>W-sh/W-sh</sup> mice transplanted with bone marrow cells from the indicated genotypes. Arrows indicate mast cells. Scale bars, 50 μm. *P < 0.05, **P < 0.01, unpaired two-sided Student’s t-test. Mean and s.d. of n samples (b). Data are representative of three independent experiments with similar results (c).
Extended Data Figure 2 | Impaired IL-3 signalling pathway in Tet2-deficient myeloid cells. a–c, qPCR analysis of mRNA levels of indicated genes in wild-type (WT) and Tet2-deficient (KO) BMMCs treated with IL-3 (10 ng ml⁻¹). d, e, Immunoblot assays of the phosphorylated (p-) or total proteins in lysates of wild-type and knockout BMMCs (d) and bone marrow cells (e) stimulated with IL-3 for the indicated time. Bone marrow cells were collected from Tet2-deficient (knockout) and littermate control (wild-type) mice and pre-stimulated with IL-3 for 12 h.
f, Immunoblot assays of the phosphorylated (p-) or total proteins in lysates of knockout BMMCs treated with non-targeting control siRNA (siCtrl) or Socs3-specific siRNA (siSocs3). Before being re-stimulated for the indicated times for subsequent analysis, BMMCs and bone marrow cells were starved for 12 h in the absence of cytokines. *P < 0.05, **P < 0.01, unpaired two-sided Student’s t-test. Mean and s.e.m. of triplicate biological replicates (a–c). Blots are representative of three independent experiments (d–f).
Extended Data Figure 3 | Tet2 binds and represses Socs3 mRNA.  

**a, b,** Bisulfite-PCR assay of methylation states of CG dinucleotides in DNA regions of chromosome 11: 117969004–258 (a) or chromosome 11: 117969363–777 (b) in Tet2-deficient BMMCs and the control cells.  

**c,** Wild-type and knockout BMMCs were starved for 12 h in the absence of cytokines, and then treated with 5 mg ml$^{-1}$ actinomycin D (actD) for 0, 15, 30, 60 min. Socs3 mRNA decay was quantified by qPCR and normalization by β-actin.  

**d,** Immunoblot of Tet2 immunoprecipitation during CLIP. Black line indicates region excised for CLIP library preparation.  

**e,** PCR amplification products from CLIP experiments before indexing. Red box indicates gel region where DNA products were extracted for further indexing and high-throughput sequencing. Biorep 1, 2 and 3 are biological replicates from three BMMC samples which have different culture start dates and crosslinked end dates.  

**f,** Pairwise correlation analysis between biological replicates with normalized tag numbers in common peaks (from left to right: biorep 1 versus biorep 2, biorep 1 versus biorep 3, biorep 2 versus biorep 3). *P < 0.05, **P < 0.01, unpaired two-sided Student’s t-test. Mean and s.d. of triplicate biological replicates (c). Blots are representative of three independent experiments (d).
Extended Data Figure 4 | Adar1 binds and represses Socs3 mRNA. 

a, b, ViennaRNA prediction of secondary structure of sequences near editing sites in Socs3 and Lrrc47 3′ UTR. Arrows highlight edited adenosines. 

c, Experimental validation of the A-to-G mutation (0) and the nearby adenosines (−2, −1, 1, 2) in control BMMCs. 

Extended Data Figure 4 | Adar1 binds and represses Socs3 mRNA. 

d, RIP-qPCR analysis of Socs3 or Lrrc47 transcripts in RNAs from anti-Adar1 immunoprecipitated wild-type and knockout BMMCs lysates. 

e, Immunoblot of ADAR1 immunoprecipitation during CLIP. Black line indicates region excised for CLIP RNA preparation. 

Extended Data Figure 4 | Adar1 binds and represses Socs3 mRNA. 

f, RT–PCR sequencing assay of A-to-G mutation frequencies in Socs3 mRNA from wild-type BMMCs at the indicated culture stages. 

Extended Data Figure 4 | Adar1 binds and represses Socs3 mRNA. 

g, Immunoblot of Adar1 protein expression in BMMCs from wild-type and Tet2-deficient (knockout) mice. 

Extended Data Figure 4 | Adar1 binds and represses Socs3 mRNA. 

h, Immunoblot of Adar1 among cytoplasm and nuclear proteins of BMMCs. 

Extended Data Figure 4 | Adar1 binds and represses Socs3 mRNA. 

i, Immunoblot of Adar1 protein expression in BMMCs treated with non-targeting control siRNA (siCtrl) or Adar1-specific siRNA (siAdar1). 

Extended Data Figure 4 | Adar1 binds and represses Socs3 mRNA. 

j, qPCR analysis of HEK293T cells transiently transfected with vectors coding haemagglutinin-tagged Socs3, Flag-tagged Adar1 and indicated Myc-tagged Tet2 mutants. 

Extended Data Figure 4 | Adar1 binds and represses Socs3 mRNA. 

k, Dot blot assays of 5-hmC levels in 10 ng DNA from Tet2- and Tet2 mutant-overexpressed HEK293T cells. Error bars, s.d. of triplicate technical replicates. 

Extended Data Figure 4 | Adar1 binds and represses Socs3 mRNA. 

Expression in BMMCs from wild-type and Tet2-deficient (knockout) mice. 

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Extended Data Figure 5 | Tet2 promotes cytosine demethylation of mRNA. **a,** One microgram of in vitro transcribed RNAs containing 1% 5-mC, or 3% mixture of 5-hmC, 5-IC and 5-caC was analysed by dot blots using 5-mC antibody. **b, c,** The 5-mC levels in mRNAs (b) and 5-hmC and 5-caC levels of DNAs (c) from in vitro Tet2 oxidation assay with or without α-KG were analysed by dot blots. Twofold gradient dilutions of 20 ng synthetic Socs3 mRNAs (b) and 10 ng DNAs (c) after oxidation were used for quantification. **d,** LC–MS for quantifying 5-mC levels of mRNAs from HEK293T cells overexpressing the indicated mutant forms of Tet2. **e, g,** Dot blot assays of 5-mC levels in 800 ng mRNAs (e) and 1 μg total RNA (g) from Tet2- and Tet2∆DNA mutant-overexpressed HEK293T cells. Twofold gradient dilutions of 800 ng in vitro transcribed Socs3 mRNAs containing 0.4% 5-mCs were used for the dilution curve of grey value-based quantification. **f,** In vitro RNA 5-mC oxidation assay of Tet2 mutants. The overexpressed Myc-tagged Tet2 mutants immunoprecipitated from HEK293T cells were subjected to in vitro oxidation. Oxidized RNAs pretreated with DNase were used for dot blot analysis of 5-hmC levels. **h,** Bisulfite-PCR assay of the 4th to 14th cytosines in tRNA Asp(GUC) in Tet2-overexpressed HEK293T cells or Tet2-deficient BMMCs and the control cells. **i, j,** Immunoblot of Tet2 protein expression and LC–MS for quantifying 5-mC levels of mRNAs in HEK293T cells treated with non-targeting control siRNA (siCtrl) or Tet2-specific siRNA (siTet2). Mean and s.d of triplicate technical replicates (b, d, e, g, j). Blots are representative of three independent experiments (a–c, e–g, i).
Extended Data Figure 6 | See next page for caption.
Extended Data Figure 6 | Specific profiles of mRNA 5-mCs in Tet2-deficient BMMCs. a, Overlap rates of methylcytosines with methylation levels above the indicated values in bisulfite sequencing assay between indicated technical replicates for Tet2-deficient (knockout, K1/2) and control (wild-type, W1/2) groups. b, Overlap rates of methylcytosines between the two biological replicates from common cytosines with read coverage above four. c, Methylcytosines in the knockout group were chosen, and mean methylation rates of these methylcytosine sites in both the wild-type and knockout groups were categorized with indicated variation folds and are presented in the scatter plot. Different colours indicate the variation of mean methylation levels of each of the methylcytosines in the knockout group compared with those in the wild-type group. d, Fraction of genes associated with knockout group-specific methylcytosines (mCgene) or CLIP peaks (CLIPgene) with variations of mRNA levels (>1.3-fold, up; <0.77-fold, down; P < 0.05) in the knockout group, compared with the control group. e, Exon-located CLIP peak and methylcytosine in the same gene were chosen, and the distance in mature mRNA between the CLIP peak boundary and the methylcytosine clusters with the shortest gap was calculated. These distances for each of the genes are presented in the box plot (centre, median; box boundaries, 25% and 75% percentiles; whiskers, 1.5-fold interquartile range; diamond, outlier; n = 11 distance values). f, Bisulfite-PCR sequencing assay of cytosine sites with methylation-supported reads in the 3′ UTR of Socs3. g, Genome browser views of gene loci containing 5-mCs in the Tet2-deficient group. Black signals indicate mean mC-supporting read numbers of all the replicates in the Tet2-deficient group. h, Genome browser view of the indicated region with RepeatMasker Viz containing the editing site in the 3′ UTR of Zfp65. i, ViennaRNA prediction of secondary structure of sequences containing the methylation sites in the 3′ UTR of Tmed10. j, qPCR analysis of gene transcripts from anti-5-mC immuno-selected RNAs from total RNAs of wild-type and knockout BMMCs. Unmethylated and methylated spike-ins as the negative and positive controls. Cytosine with coverage above 4, at least two reads supporting methylation and methylation level equal or above 0.1 was chosen as methylcytosine, considering both bioinformatic and biological significance. Mean and s.d of triplicate technical replicates (j).
Extended Data Figure 7 | Cytosine methylation in the 3′ UTR of Socs3 inhibits dsRNA structure. a, qPCR analysis of overexpressed Socs3 transcripts from anti-5-mC immuno-selected RNAs from total RNAs of HEK293T cells transfected with Tet2 or Tet2 mutants. b, qPCR analysis of Socs3 transcripts from specific-modification antibodies immuno-selected RNAs from total RNAs of wild-type BMMCs. Unmodified and modified spike-ins as the negative and positive controls. c, qPCR analysis of HEK293T cells transiently transfected for 24 h with vectors coding haemagglutinin-tagged wild-type Socs3 or C-to-G mutant Socs3 (Socs3C-to-G), with or without Flag-tagged Adar1 and Myc-tagged Tet2. d, RIP–qPCR analysis of Socs3 3′ UTR levels in RNAs from Flag-tagged Adar1-immunoprecipitated HEK293T cell lysates overexpressed with Socs3 or Socs3C-to-G together with Adar1. Lysates (1%) were used for normalization as input. e, A-to-I editing rates in Socs3 3′ UTR with cytosine or 5-mC after Adar1 editing in vitro. f, Socs3 transcript levels determined by RT-qPCR from J2 immuno-selected dsRNA; p1, primer 1; p2, primer 2. Mean and s.d. of triplicate technical replicates (a–d, f). Data are representative of three independent experiments (e).
Extended Data Figure 8 | Schematic illustration of Tet2-mediated repression of Socs3 via Adar1. a, Genome browser view of sequencing data on Socs3 locus. Blue, A-to-G mutant reads in wild-type BMMCs; red, mean mC-supporting read numbers in knockout BMMCs; black, CLIP tag coverage. b, Tet2 promotes mRNA cytosine demethylation for effective formation of dsRNA which is bound by Adar1, leading to the suppression of Socs3 expression at the post-transcriptional level.
Extended Data Table 1 | Sequences of PCR primers used in this study

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<th>Name</th>
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<td>5' - CTTAACAACCTACCAAAAAAACC - 3'</td>
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Life Sciences Reporting Summary

Nature Research wishes to improve the reproducibility of the work that we publish. This form is intended for publication with all accepted life science papers and provides structure for consistency and transparency in reporting. Every life science submission will use this form; some list items might not apply to an individual manuscript, but all fields must be completed for clarity.

For further information on the points included in this form, see Reporting Life Sciences Research. For further information on Nature Research policies, including our data availability policy, see Authors & Referees and the Editorial Policy Checklist.

1. Experimental design

   1. Sample size
      Describe how sample size was determined.
      
      The sample size chosen for our animal experiments in this study was estimated based on our prior experience of performing similar sets of experiments.

   2. Data exclusions
      Describe any data exclusions.
      
      No data were excluded.

   3. Replication
      Describe whether the experimental findings were reliably reproduced.
      
      We at least independently repeated all the data once. All attempt to reproduce the results were successful.

   4. Randomization
      Describe how samples/organisms/participants were allocated into experimental groups.
      
      All animal- and cell-based samples in each of the group were included and no method of randomization was applied.

   5. Blinding
      Describe whether the investigators were blinded to group allocation during data collection and/or analysis.
      
      Blinding is not relevant to our study, as we need to know the genotypes of the mouse strains.

   Note: all studies involving animals and/or human research participants must disclose whether blinding and randomization were used.

2. Statistical parameters

   For all figures and tables that use statistical methods, confirm that the following items are present in relevant figure legends (or in the Methods section if additional space is needed).

   n/a | Confirmed
   --- | ---
   - [x] The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement (animals, litters, cultures, etc.)
   - [x] A description of how samples were collected, noting whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
   - [x] A statement indicating how many times each experiment was replicated
   - The statistical test(s) used and whether they are one- or two-sided (note: only common tests should be described solely by name; more complex techniques should be described in the Methods section)
   - [x] A description of any assumptions or corrections, such as an adjustment for multiple comparisons
   - [x] The test results (e.g. P values) given as exact values whenever possible and with confidence intervals noted
   - [x] A clear description of statistics including central tendency (e.g. median, mean) and variation (e.g. standard deviation, interquartile range)
   - [x] Clearly defined error bars

   See the web collection on statistics for biologists for further resources and guidance.
software

7. Software

Describe the software used to analyze the data in this study.

RNA-seq: Tophat (2.1.0) & Cufflinks (2.2.1) & HTseq (0.6.1) & DEseq (1.20.0);
Bisulfite-seq: BS-RNA (1.0); CLIP:gscripts (1.1) & STAR (2.4.0) & Homer (4.8)

For manuscripts utilizing custom algorithms or software that are central to the paper but not yet described in the published literature, software must be made available to editors and reviewers upon request. We strongly encourage code deposition in a community repository (e.g. GitHub). Nature Methods guidance for providing algorithms and software for publication provides further information on this topic.

Materials and reagents

8. Materials availability

Indicate whether there are restrictions on availability of unique materials or if these materials are only available for distribution by a for-profit company.

No restriction on availability of materials

9. Antibodies

Describe the antibodies used and how they were validated for use in the system under study (i.e. assay and species).

The detailed information on all antibodies were provided in the method section: Mice and reagents. Those antibodies are all commercially available, and have validation notes in the supplier’s websites.

10. Eukaryotic cell lines

a. State the source of each eukaryotic cell line used.

HEK293T cells were obtained from ATCC.

b. Describe the method of cell line authentication used.

None of the cell lines have been authenticated.

c. Report whether the cell lines were tested for mycoplasma contamination.

The cell lines were not tested for mycoplasma contamination.

d. If any of the cell lines used are listed in the database of commonly misidentified cell lines maintained by ICLAC, provide a scientific rationale for their use.

No commonly misidentified cell lines were used.

Animals and human research participants

11. Description of research animals

Provide details on animals and/or animal-derived materials used in the study.

Both male and female C57BL/6 mice of 6-8 weeks. The transgenic mouse lines: Tet2-/-(C57BL/6), Kitw-sh/w-sh(C57BL/6).

12. Description of human research participants

Describe the covariate-relevant population characteristics of the human research participants.

The research did not involve human research participants.