m6A mRNA methylation controls T cell homeostasis by targeting the IL-7/STAT5/SOCS pathways

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N6-methyladenosine (m6A) is the most common and abundant messenger RNA modification, modulated by ‘writers’, ‘erasers’ and ‘readers’ of this mark1,2. In vitro data have shown that m6A influences all fundamental aspects of mRNA metabolism, mainly mRNA stability, to determine stem cell fates3,4. However, its in vivo physiological function in mammals and adult mammalian cells is still unknown. Here we show that the deletion of m6A ‘writer’ protein METTL3 in mouse T cells disrupts T cell homeostasis and differentiation. In a lymphopaenic mouse adoptive transfer model, naive Mettl3-deficient T cells failed to undergo homeostatic expansion and remained in the naive state for up to 12 weeks, thereby preventing colitis. Consistent with these observations, the mRNAs of SOCS family genes encoding the STAT signalling inhibitory proteins SOCS1, SOCS3 and CIS3 were marked by m6A, exhibited slower mRNA decay and showed increased mRNAs and levels of protein expression in Mettl3-deficient naive T cells. This increased SOCS family activity consequently inhibited IL-7-mediated STAT5 activation and T cell homeostatic proliferation and differentiation. We also found that m6A has important roles for inducible degradation of Socs mRNAs in response to IL-7 signalling in order to reprogram naive T cells for proliferation and differentiation. Our study elucidates for the first time, to our knowledge, the in vivo biological role of m6A modification in T-cell-mediated pathogenesis and reveals a novel mechanism of T cell homeostasis and signal-dependent induction of mRNA degradation.

T cell differentiation and proliferation represent an exceptionally simple and tractable model system with which to understand the general principles of cellular specification and gene regulation. naïve T cells can differentiate and proliferate into distinct functional T helper effector subset cells in response to defined cytokines in vitro and different micro-environmental signals in vivo3,6. As m6A plays an essential role during the cell fate patterning of embryonic stem cells in vitro, we hypothesized that m6A might be an important regulator of T helper differentiation. To study the in vitro functions of m6A, we generated conditional knockout mice for the m6A writer protein Mettl3 (Extended Data Fig. 1a), as Mettl3 knockout (Mettl3-KO) mice are embryonic lethal3. CD4+ T cells from CD4-Cre conditional Mettl3lox/lox mice displayed absence of both Mettl3 and its associated Mettl14 proteins (Extended Data Fig. 1b). Concomitantly, the overall mRNA m6A methylation levels in knockout cells were decreased to roughly 28% of that in wild-type cells (Extended Data Fig. 1c). Characterization of mouse immune cell populations in the steady state revealed that T cell homeostasis was abnormal in spleen and lymph nodes, but not thymus, exhibited by the fact that naïve T cell numbers from lymph nodes were increased (Extended Data Fig. 1d–g).

To characterize the possible defects of the Mettl3-KO naive T cells, we used the defined in vitro TCR-dependent T cell differentiation system and found that Mettl3-deficient naive T cells exhibited reduction of Treg1 and Treg17 cells, an increase in Treg2 cells, and no changes in Tregc relative to wild-type naive T cells (Extended Data Fig. 2a, b). We also saw no significant differences in proliferation and apoptosis between the wild-type and knockout naive T cells in these cultures (Extended Data Fig. 2c, d). Together, these findings suggest that m6A modification has an important role during CD4+ T cell differentiation, but not in T cell apoptosis and TCR-mediated proliferation.

Upon adoptive transfer into lymphopaenic mice, naive T cells normally undergo homeostatic expansion in response to the elevated IL-7 levels in such mice and differentiate into effector T cells, causing colitis7. To study how Mettl3 regulates naive T cell homeostasis in vivo, we adoptively transferred CD4+CD25+CD45RBhi naive T cells into Rag2−/− mice, and found that mice receiving knockout naive T cells

Figure 1 | Mettl3-KO naive T cells do not promote disease in CD45RBhi adoptive transfer colitis mouse model. a. Body weight changes after naive T cell adoptive transfer into Rag2−/− host mice (n = 10), two-way ANOVA. b, c. Endoscopic colitis scores and representative pictures of haematoxylin and eosin staining of the colon from Rag2−/− receiving wild-type and knockout naive T cells 8 weeks after transfer (n = 10), unpaired t-test. d, FACS analysis of transferred T cells in colon tissues (n = 3), unpaired t-test. n = number of biological replicates. Repeated 3 times. ***P < 0.001, ****P < 0.0001.

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(Mettl3−/− recipients) showed no signs of disease for up to 12 weeks after transfer. Mettl3−/− recipients continued to gain weight throughout the experiment, whereas control mice that received wild-type naive T cells (Mettl3+/+ recipient) began losing weight in the fifth week after transfer (Fig. 1a). Mettl3−/− recipients exhibited no colitis upon endoscopy, displayed normal colon length, and were found to have reduced spleen and lymph node sizes compared to wild-type control mice at the eighth week after transfer (Fig. 1b, Extended Data Fig. 3a, b). When analysed by FACS, Mettl3−/− CD45.2 CD4+ donor T cells were nearly undetectable in recipient colons (Fig. 1d), as well as in the spleen, but instead remained in peripheral and mesenteric lymph nodes, whereas wild-type donor T cells were found in large numbers in all lymphoid organs of the recipients (Extended Data Fig. 3c, d). Haematoxylin and eosin staining and analysis of colons also confirmed that the Mettl3−/− KO T cells caused no T cell infiltration and inflammation, whereas wild-type T cells caused severe colonic inflammation and disrupted colonic structure (Fig. 1c). Remarkably, FACS analysis further revealed that the vast majority of transferred Mettl3−/− T cells recovered from the peripheral lymph nodes still displayed their original naive T cell marker CD45RBhi as long as 12 weeks after transfer into Rag2−/−/uni mice, whereas the transferred wild-type cells differentiated into the effector/memory T cells (CD45RBlo) that mediated the pathology in this model (Fig. 2a).

We next sought to investigate whether Mettl3−/− naïve T cells were capable of proliferation and could demonstrate normal survival after transfer into Rag2−/−/uni mice. Using CellTrace labelling of the transferred naive T cells, proliferation of wild-type cells was observable beginning from week 2, whereas Mettl3−/− KO T cells retained a naive phenotype from week 2, whereas Mettl3−/− KO T cells remained in peripheral lymph nodes still displaying their original naive T cell marker CD45RBhi as long as 12 weeks after transfer into Rag2−/−/uni mice, whereas the transferred wild-type cells differentiated into the effector/memory T cells (CD45RBlo) that mediated the pathology in this model (Fig. 2a).

To establish that m6A directly controls T cell homeostatic expansion, we re-introduced the wild-type Mettl3 gene or m6A catalytic-mutant Mettl3 gene back into Mettl3−/− KO naive T cells, and showed that the wild-type Mettl3 gene, but not m6A catalytic-mutant Mettl3, could largely rescue the differentiation defects of Mettl3−/− KO naive T cells in vivo (Extended Data Fig. 3f, g). Furthermore, we also generated an additional CD4-Cre conditional mouse line for Mettl14, an essential m6A catalytic partner for Mettl3 in the same m6A ‘writer’ complex. Mettl14−/− KO mice showed an identical phenotype to Mettl3−/− KO mice. Specifically, 4 weeks after transfer into Rag2−/−/uni mice, Mettl14−/−/uni naive T cells remained in the naive state (Fig. 2e), the mice displayed smaller lymphoid organs, and the cells expanded less than transferred wild-type naive T cells (Extended Data Fig. 4a–d). Taken together, our data suggest that m6A RNA modification is required not only for T helper cell differentiation and proliferation in vivo, but also for T cells to properly exit the naive ‘progenitor’ state.

Peripheral T cell pools are maintained by complex mechanisms, and naïve T cell homeostasis and survival is mainly sustained by the IL-7/STAT5 and self-peptide–MHC/TCR signalling axes9. It is well established that the elevated levels of IL-7 in lymphoepithelial mice induce extensive homeostatic proliferation and differentiation of naïve T cells after adoptive transfer10. We hypothesized that the IL-7 receptor and its downstream molecular pathway were compromised in Mettl3−/− KO cells. Consistently, we observed markedly decreased JAK1 and STAT5 phosphorylation levels in knockout naïve T cells upon IL-7 stimulation (Fig. 3a). Moreover, basal ERK and AKT, but not NFκB, phosphorylation was found to be elevated in knockout naïve T cells, with TCR stimulation only minimally enhancing ERK and AKT signalling. These findings are consistent with the observation that Mettl3−/− naïve T cells proliferate normally ex vivo after TCR stimulation, indicating that the limited proliferation found after adoptive transfer to lymphoepithelial mice was driven by defects in STAT5 phosphorylation, whereas their long-term survival in these recipients was maintained by elevated basal ERK and AKT signalling. We conclude that m6A controls the balance of the two essential signalling pathways to control the T cell homeostasis, IL-7–mediated JAK–STAT signalling and TCR-mediated ERK/AKT signalling, thus uncoupling T cell proliferation from cell survival.

To explore further the molecular mechanism underlying the IL-7–signalling pathway defects, we performed RNA sequencing (RNA-seq) analysis on the naïve T cells isolated from Mettl3−/− KO mice and littermate control wild-type mice. Consistent with our biochemical observations, the JAK–STAT and TCR signalling pathways were among the top upregulated KEGG pathways (Extended Data Fig. 5a, b, Supplementary Table 3). Notably, three Suppressor of cytokine signalling (SOCS) family genes (Socs1, Socs3 and Cish) were among the most significantly upregulated genes. RNA-seq results were validated by qPCR and western blot, 

Figure 2 | Mettl3−/− naïve T cells are locked in the naive state and proliferate much more slowly than wild-type cells after transfer into Rag2−/−/uni mice. a. Most of the Mettl3−/− KO donor cells are retained in lymph nodes (LN) and are locked in naive states 12 weeks after transfer. b. The wild-type donor naive T cells start to differentiate from the second week after transfer (CD45RBlo), whereas the Mettl3−/− KO donor naive T cells always stay in naive states (CD45RBhi). c, d. The wild-type donor naive T cells are driven to proliferate rapidly from the second week, whereas the Mettl3−/− T cells proliferate slowly, with the total number of cells recovered from pLN shown in d. e. Mettl14−/− KO donor naive T cells recapitulate the phenotype of Mettl3−/− KO donor cells. At least six animals in each group were analysed, each experiment was repeated twice, and representative images are shown.
confirming that Socs1, Socs3 and Cish were overexpressed and Socs1 in particular showed even higher relative expression in Mettl3-KO naive T cells (Fig. 3c, d), whereas other important genes in IL-7 signalling pathways did not change (Extended Data Fig. 5c). Socs proteins are the key physiological inhibitors of Jak–Stat5 signalling pathways and have important roles in T cell proliferation and differentiation.\(^1\)\(^2\),\(^11\)\(^12\). In particular, Socs1 is a well-known negative regulator of IL-7 signalling, and Socs3 and Cish also inhibit Stat5 phosphorylation and T cell proliferation, while promoting ERK activity by binding to RasGap.\(^13\)\(^–\)\(^15\). Consistently, siRNA-mediated Socs1 knockdown partially rescued the in vivo differentiation defects of Mettl3-KO naive T cells 4 weeks after adoptive transfer into Rag2\(^{−/−}\) mice.\(^f\) m\(^6\)A peaks are enriched in the 3'UTRs of Socs1 and Socs3 mRNAs from m\(^6\)A RIP-seq data with wild-type CD4\(^+\) T cells,\(^g\) while siRNA knockdown in Socs1, Socs3 and Cish mRNA is overexpressed in Mettl3-KO versus wild-type naive T cells, unpaired t-test.\(^e\) Socs1 siRNA knockdown in Mettl3-KO naive T cells partially rescues the differentiation defects (see Methods and Extended Data Fig. 6a–d, Supplementary Table 4).

Next, we analysed our deep RNA-seq data, and did not find any splicing differences between Mettl3-KO and wild-type cells, suggesting that the naive T cell homeostasis defect is mainly due to m\(^6\)A-mediated degradation, rather than splicing or translation. Taken together, these data show that loss of m\(^6\)A modification in Mettl3-KO naive T cells leads to increased Socs1, Socs3 and Cish mRNA half-life and protein levels, thus suppressing the IL-7/Stat5 signalling pathway.

mRNA levels are tightly regulated by both transcription and degradation.\(^20\) While the abundance of most transcripts is mainly controlled by transcription rate, it has been shown that the mRNA levels of a minority of genes (~17%) are significantly regulated by mRNA degradation rates, notably immediate-early inducible genes.\(^21\)\(^22\). Socs genes are well-known immediate-early genes induced upon IL-7 stimulation,\(^11\)\(^12\), thus we hypothesized that m\(^6\)A specifically targets ‘signal-dependent immediate-early genes’ for degradation. Interestingly, we found that upregulated genes (including Socs1 and Socs3) in Mettl3-KO naive T cells were significantly enriched in the degradation-controlled group of genes from LPS-stimulated dendritic cells\(^20\)\(^,\)\(^21\) (chi square test, \(P < 0.0001\), Extended Data Fig. 7a). In addition, using the RNA decay assay we found that the mRNAs of all three Socs genes were degraded faster upon IL-7 stimulation, as early as 10 min after IL-7 stimulation, compared to control treatment in wild-type cells, whereas the accelerated mRNA degradation upon IL-7 stimulation was abrogated in Mettl3-KO naive T cells (Extended Data Fig. 7b). To extend our observation genome-wide and estimate the rates of synthesis and degradation, we conducted a time-course s\(^4\)U-seq with IL-7 induction and found a cluster of 34 transcripts including Cish, Socs1 and Socs3 that were increased in Mettl3-KO relative to wild-type cells and show similar kinetics of induction\(^23\) (Fig. 4a, b; see also Methods, Extended Data Fig. 8a–c, Supplementary Table 5). This analysis also confirmed that the estimated degradation rates were lower in Mettl3-KO cells for Socs transcripts after IL-7 induction (Fig. 4c, d). We conclude that m\(^6\)A targets a group of immediate-early inducible genes including Socs1, Socs3 and Cish for rapid mRNA degradation upon IL-7 stimulation, allowing IL-7/JAKs signalling to activate the
m6A specifically targets a group of immediate-early genes for degradation upon IL-7 stimulation. a, s4U-seq experiment overview. b, Heatmap showing the results of clustering that normalizes transcript expression levels with significant changes after IL-7 induction and differences between wild-type and Mettl3−/−. Cluster 3 contains 34 transcripts with similar expression profiles including Cish, Socs3 and Socs1. c, Computed RNA degradation rates from s4U-seq data. d, Read density for total RNA and s4U-enriched RNA at the indicated genes for wild-type and Mettl3−/− samples after IL-7 stimulation.

downstream target STAT5, to initiate the re-programming of naive T cells for differentiation and proliferation.

T cell homeostasis is essential for maintaining the T cell pool size and forms the basis for adaptive immunity. Rather than the findings of m6A functions in ES cells, here we have revealed a different strategy, whereby m6A targets mRNAs encoding signalling proteins that control the ‘gatekeeper’ IL-7 signal in naive T cells, the progenitors of the adaptive immune system. Thus these targets expand the scope of m6A biology and enable RNA modification to affect critical dynamic signalling systems and responses to external stimuli that maintain homeostasis. Specifically, using Mettl3 and Mettl14 conditional knockout mice, our current study demonstrates a novel mechanism whereby m6A functions in control T cell homeostasis by inducible degradation of Socs gene family mRNA, and consequently relieves the block on IL-7 signalling and T cell proliferation (Extended Data Fig. 9). Our study implies that m6A represents an evolutionarily conserved mechanism to specifically control the degradation rates of a group of immediate-early response genes in response to various environmental stimuli. Our study illustrates not only how this important epitranscriptomic marker has an essential role in development, but also that m6A modifications act as a critical regulator of immune cell homeostasis and function, opening new avenues of investigation into the function of m6A in human health and disease. As T cells regulate the entire adaptive immune response, this has broad implications. These findings further suggest that T cell-specific delivery of m6A-modifying agents might be an effective treatment to alleviate various autoimmune diseases.

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.

Received 3 October 2016; accepted 30 June 2017.

Published online 9 August 2017.

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Supplementary Information is available in the online version of the paper.

Acknowledgements We thank R. Flynn, R. Jackson, Y. Yang, M. Vesely, R. Paiva, N. Palm and all the other members of the Flavell laboratory for discussions and comments. We thank J. Alderman, C. Lieber, C. Hughes and J. Stein for technical support. H.-B.L. was supported by NIH T32 TR320K007356. S.Z was supported by a fellowship from Helen Hay Whitney Foundation-Howard Hughes Medical Institute. This work was supported by the Howard Hughes Medical Institute (R.A.F.), NSF Major International Joint Research Program of China - 31420103901 (Z.Y. and R.A.F.) and ‘111’ project (Z.Y.), R01-HG004361 (H.Y.C.), NIH New Innovator Award DP2 HD083992-01 (M.D.S.), and a Searle scholarship (M.D.S.).

Author Contributions H-B. L. conceived the project. H.-B. L., J. T., S. Z., P.B., E.E.D., W.B., G.C., Y. C., G.W., J.P.B. and Y.G.C. performed the experimental work. J.Z., L.K., M.D.S. and P.B. analysed the RNA-seq, ribo-profiling, s^4U-seq and m^6A-seq data and performed the statistical analysis. H.Y.C., M.D.S., Y.K., and Z.Y. provided key suggestions. H.-B. L. and R.A.F designed the study, analysed the data and wrote the manuscript. R.A.F. supervised the study.

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Reviewer Information Nature thanks F. Fuks, J. H. Hanna and the other anonymous reviewer(s) for their contribution to the peer review of this work.
METHODS
Mice. Metll3 and Metll14 conditional knockout mice were both generated by inserting two lox sites into the first and the last introns using the CRISPR–Cas9-based genome-editing system as previously described24 (Extended Data Fig. 1a). The gRNA and donor oligonucleotides used were as follows. For Metll3 left side: 5′-AGTCGTCCATGTGATGAAAGG-3′, and 5′-AGGTAAT*GTTCTCTGGAAATCCCAATAGTATTTGGGAAACAAGGCTGCCTGAATGATCAGATGACCTTATGCTGTCGGCTCAGC*GAA*TGG-G-3′; for Metll3 right side: 5′-ATACATTCGTGTTACGGTACAGTATGTCGCTGCTGCAGC*GAA*TGG-G-3′; for Metll4 left side: 5′-CAATATCAGATGATGAAAACAAGGCTGCCTGAATGATCAGATGACCTTATGCTGTCGGCTCAGC*GAA*TGG-G-3′; and 5′-AAATG*TGGAAATCCCAATAGTATTTGGGAAACAAGGCTGCCTGAATGATCAGATGACCTTATGCTGTCGGCTCAGC*GAA*TGG-G-3′; for Metll4 right side: 5′-TACATATCAGATGATGAAAACAAGGCTGCCTGAATGATCAGATGACCTTATGCTGTCGGCTCAGC*GAA*TGG-G-3′, and 5′-ATT*TGGAAATCCCAATAGTATTTGGGAAACAAGGCTGCCTGAATGATCAGATGACCTTATGCTGTCGGCTCAGC*GAA*TGG-G-3′. The donor oligonucleotides were synthesized by IDT with 5′- and 3′-ends modified by phosphorothioate bonds (denoted *) to increase the oligonucleotide stability after delivered into mouse embryos. The pups born were genotyped and the PCR products were sequenced to validate intact integration of the lox sequences into the right genome loci.

We crossed floxed Metll3 or Metll4 mice with CD4-Cre mice to obtain conditional knockout mice. The CD4-Cre mice were purchased from the Jackson Laboratory and have been fully backcrossed to C57BL/6 mice from the Charles River laboratories (over than 10 generations).

Metll3−/− or CD4-Cre, or only CD4-Cre mice had both been used as wild-type controls for Metll3−/−, CD4-Cre mice, and we found there were no signs of differences using either one regarding our observed phenotypes. Thereafter, we only used Metll3−/− as wild-type controls for Metll14−/−, CD4-Cre knockout mice for most of the experiments reported here. All of the knockout and wild-type mice were littermates and co-housed for any experiments described. All the mice were bred and maintained under specific-pathogen-free conditions at the animal facility of Yale University School of Medicine. Animal procedures were approved by the Institutional Animal Care and Use Committee of Yale University. Both female and male mice were used in experiments. Wherever possible, preliminary experiments were performed to determine requirements for sample size, taking into account resources available and ethical, reductionist animal use. Exclusion criteria such as inadequate staining or low cell yield due to technical problems were pre-determined. Animals were assigned randomly to experimental groups. Each cage contained animals of all the different experimental groups.

Reagents and antibodies. The detailed information on all reagents and antibodies used in this study are listed in Supplementary Table 1.

CD45RBb+ adoptive transfer colitis, endoscopic and histologic analysis. We performed the experiment as described25. Briefly, pure CD4+ CD25− CD45RBb naïve T cells were sorted from wild-type and Metll3−/− or Metll14−/− KO mice by FACS, washed twice with PBS, counted, and intravenously injected 0.5 million cells into each Rag2−/− recipient mice. The recipient mice were monitored and weighed each week.

At week 7, colon colitis was visualized using Colonview system (Karl Storz). Briefly, colitis score was evaluated considering the consistence of stools, granularity of the mucosal surface, transluency of the colon, fibrin deposit and vascularization of the mucosa (0–3 points for each parameter). Haematoxin and eosin staining was performed on paraffin sections of colon previously fixed in Bouin’s fixative solution.

Cell proliferation and apoptosis assay. To trace T cell homeostatic proliferation in vivo, we labelled the FACS-purified CD4+ CD25− CD45RBb naïve cells with CellTrace (ThermoFisher Scientific, C34557) before intravenous injections2. We then intravenously injected 0.5 million cells into each Rag2−/− recipient mouse. We analysed the mice at 1 week, 2 weeks, 4 weeks and 10 weeks after injection by FACS using the naive T cell marker CD45RB for differentiation, and CellTrace violet retaining ratio for proliferation.

For apoptosis assay, we stained the in vitro cultured cells or cells from mice with annexin V and 7-AAD and analysed by FACS, in which double-negative cells are viable cells, whereas annexin V+ or double-positive cells are apoptotic cells.

T cells ex vivo differentiation. We FACS sorted CD4+ CD62L− CD44hi cells with FACSaria II Cell Sorter (BD Biosciences) and activated them with plate-bound monomeric antibodies to CD3 (10 μg ml−1, 145-2C11) and CD28 (1–2 μg ml−1, PV-1) in the presence of mouse recombinant cytokines and blocking antibodies. Specifically, TGF-β1 direction with IL-2 (10 ng ml−1) and antibody to IL-4 (11B11, 10 μg ml−1); TGF-β2 direction with IL-4 (10 ng ml−1) and antibody to IFN-γ (XMG1.2, 10 μg ml−1); TGF-β17 with IL-6 (20 ng ml−1), IL-23 (20 ng ml−1), and antibodies to IFN-γ (XMG1.2, 10 μg ml−1) and IL-4 (11B11, 10 μg ml−1); TGF-β1 with TGF-β (2 ng ml−1). IL-23 (20 ng ml−1) and IL-4 (11B11, 10 μg ml−1). All cytokines were purchased from R&D. Click’s (Irvine Scientific) or RPMI (SIGMA-ALDRICH) (when indicated) media were supplemented with 10% FBS, 1-glutamine (2 mM), penicillin (100 U ml−1) and β-mercaptoethanol (40 μM). After 4 days of culture, the cells were analysed by FACS.

Signalling assay. Naïve T cells were isolated by FACS, counted, and 1 million cells in cell culture media were plated on each well of 48-well plate. 10 μg of IL-7 or IL-2, or 0.2 μl of CD3/CD28 beads (ThermoFisher Scientific, 11452D) were added into the media, mixed and incubated at 37°C. Cells were collected before adding cytokines or antibody beads (t=0), or 30 min (t=30) or 60 min (t=60) after adding the cytokines or antibody beads. The cells were lysed on ice for 30 min in RIPA buffer (ThermoFisher Scientific, 89901) with protease inhibitor cocktails (ThermoFisher Scientific, 78437) and phosphatase inhibitor cocktails (ThermoFisher Scientific, 78428). The supernatants were then used for western blot.

RNA-seq. We isolated CD4+ cells using StemCell mouse CD4+ Kit (StemCell Technologies, catalogue 19852) from spleen and lymph nodes. Then pure CD4+ CD25− CD45RBb naïve T cells were isolated by FACS sorting. We used two pairs of wild-type and knockout mice for the experiment. Total RNAs were isolated with Direct-zol RNA MicroPrep kits (Zymo Research, R2062). Yale Center for Genome Analysis (YCGA) processed the total RNA by Ribo-Zero rRNA removal kit, constructed the libraries, and subject them to standard illumine HiSeq2000 sequencing, and obtained >40 million reads for each sample.

Raw RNA-sequencing reads were aligned to the mouse genome (mm10, GRCm38) with TopHat26. Gene expression levels were measured by Cufflinks and differential analysis was performed with Cuffdiff27. Genes were considered significantly differentially expressed if showing ≥1.5 fold change and <0.01 P value. Gene set analysis was performed and enriched KEGG pathways were obtained through online bioinformatics tools28. Volcano plot and pathway plot were generated with R package ggplot229. We used CuffDiff and rMATS to analyse possible splicing difference events, and did not find any significant difference between Metll3-KO and wild-type samples20.

RT-qPCR. Total RNA was isolated from naïve T cells as described in the ‘RNA-seq’ section, then reverse transcribed using High-Capacity cDNA Reverse Transcription Kit (ThermoFisher Scientific, 4368814). Primers used for qPCR are included in Supplementary Table 2. All qPCRs were run on Bio-Rad CFX96 real-time system using iTaq Universal SYBR Green Supermix (Bio-Rad, #1725124). Actin-β was used as internal control to normalize the data across different samples.

RNA degradation assay. Naïve T cells purified by FACS sorting were plated on 96-well plates with 0.5-million cells per well. Actinomycin-D (Sigma-Aldrich, A1410) was added to a final concentration of 5 μg ml−1, and cells were collected before or 2 h after adding actinomycin-D. Then the cells were processed as described in RT-qPCR, except that the data were normalized to the t=0 time point.

For signalling-dependent degradation assay, we first treated the naïve T cells with actinomycin-D for 1 h to fully inhibit transcription, then added IL-7 (10 μg ml−1) or PBS as control into each well. Cells were lysed with Trizol LS at 0, 10 min, 20 min, 30 min, or 45 min after IL-7 addition.

HPLC quantification of m6A levels. RNA was collected with RLT buffer (Qiagen) according to the manufacturer’s instructions. Two rounds of poly(A) selection were performed using PolyA purist Mag kit (Ambion), 200 ng of poly(A)-selected RNA was digested with 1 unit of nuclease P1 in 50 μM NH40Ac at 37°C for 1 h, and sample was cleared on a 0.22-μm filter. HPLC was performed on an Agilent 1290 Infinity UPLC system coupled to the Agilent 6490 Triple Quad mass spectrometer with the iFunnel. For the LC, the A solvent was water with 0.1% formic acid and the B solvent was acetonitrile with 0.1% formic acid. The MS was in positive mode, scanning for the AMP and m6A product ion of 136 and 150.1, respectively. Samples were run in duplicate, and m6A/A ratios calculated.

m6A RNA-IP-qPCR and m6A RNA-IP-seq. Total RNA was isolated with TRIzol, according to the manufacturer’s instructions, and subjected to RNA depletion with RiboMinus kit (Ambion). RNA was fragmented to ~100 nucleotide fragments with Ambion fragmentation reagent (40 s incubation at 94°C). 200 ng of RNA was denatured and incubated with 20 μl of protein A beads, previously bound to 1 μg of anti-m6A polyclonal antibody (Synaptic Systems) or rabbit IgG in 1× IPP.
buffer (150 mM NaCl, 10 mM TRIS-HCL and 0.1% NP-40). RNA was incubated with the antibody for 3 h at 4°C in 1× IFF buffer. Beads were washed twice with 1× IFF buffer, twice with low salt buffer (50 mM NaCl, 10 mM TRIS-HCL and 0.1% NP-40), twice with high salt buffer (500 mM NaCl, 10 mM TRIS-HCL and 0.1% NP-40) and once with 1× IFF buffer. RNA was eluted from the beads with 50 μl of RLT buffer, and purified with Qiagen RNeasy columns. RNA was eluted in 100 μl of RNase free water.

m2A enrichment was analysed on a LightCycler 480 by RT-qPCR with One-Step RT-PCR kit (E-043120-00-0005), and the experiments were carried out according to the manufacturer’s instructions. Input and enriched samples were multiplexed with Illumina bar codes and sequenced using paired-end 2 × 75 nt cycles on an Illumina HiSeq 2500 instrument. To process m2A-seq data, reads were aligned using TopHat2. Macs was then used for peak calling following the protocol in ref. 32. Samples were normalized to get pileup per million reads in each sample, using the number of reads which were left after filtering redundant tags from MACS33. Peaks were then visualized using IGV34.

siRNA knockdown. Socs1 siRNA and non-targeting control siRNA were purchased from GE-Dharmacon (E-043120-00-0005), and the experiments were carried out according to the manufacturer’s instructions. The naive T cells with siRNA transfection were incubated at 37°C for 3 h, then were transferred into Rag2−/− recipient mice by intravenous injection (1 million cells per mouse). Four weeks after transfer, the cells from spleen, peripheral lymph nodes, and mesenteric lymph nodes were analysed by FACS. Representative FACS images show that the naive marker CD45RB peaks were negative controls for m6A RIP–qPCR. Two tailed t-test for unequal, unpaired data sets with heteroscedastic variation was used to compare outcomes. We independently repeated the data at least once and all attempts to reproduce the results were successful. For all the bar graphs, data are expressed as mean ± s.e.m. Statistical analyses were performed using GraphPad Prism 6. Differences were analysed by Student’s t-test or two-way ANOVA test using GraphPad Prism 6. P values ≤ 0.05 were considered significant (∗ P < 0.05; ∗∗ P < 0.01; ∗∗∗ P < 0.001). P values > 0.05 were not considered significant (NS). FlowJo (TreeStar) was used to analyse all the flow cytometry data. The sample sizes (biological replicates), specific statistical tests used, and the main effects of our statistical analyses for each experiment were detailed in each figure legend.

Data availability. The data that support the findings of this study are available from the corresponding author upon reasonable request. RNA-seq, ribosome profiling, m2A-seq, and mIA RIP-seq data sets have been deposited in Gene Expression Omnibus under the accession number GSE100048.
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Extended Data Figure 1 | Abnormal T cell homeostasis in generated Mettl3 CD4-Cre conditional knockout mice. 

**a**. The two lox sites were inserted into the first and last introns by CRISPR technology. 

**b**. Protein levels of METTL3 and its associated METTL14 were analysed by western blot in the naive T cells and in in vitro differentiated T\(_{H1}\), T\(_{H2}\) and T\(_{H17}\) cells from Mettl3-KO and wild-type mice.

**c**. Overall levels of RNA m6A methylation in naive T cells from Mettl3-KO and wild-type mice (\(n = 3\), \(P = 0.0032\)).

**d**. Naive T cells increased in all lymphoid organs from Mettl3-KO mice compared to littermate control wild-type mice. Cells from spleen (SPL), mesenteric lymph node (mLN), and peripheral lymph node (pLN) were analysed by FACS by staining with CD4/CD44/CD62L.

**e**, **f**. The percentage of CD4\(^+\)CD44\(^{lo}\)CD62\(^{lo}\) naive T cells increased in all three lymphoid organs in Mettl3-KO mice (e) and the total number of naive T cells in mLN and pLN also increased in knockout mice (f; \(n = 3\)).

**g**. The cell population in the thymus did not change in Mettl3-KO (\(n = 3\)). The number of biological replicates. Repeated three times and one set of data is shown. **P** \(< 0.01\), ***P** \(< 0.001\).
Extended Data Figure 2 | Naive T cells from Mettl3-KO mice differentiated into fewer T_{H}1 and T_{H}17 cells and more T_{H}2 cells ex vivo compared to wild-type naive T cells. 

**a.** Naive T cells isolated from Mettl3 wild-type and knockout mice were differentiated into effector subsets under defined optimal conditions. 

**b.** The percentages of each T cell subtype over total CD4^{+} T cells were analysed by FACS (n = 3). 

**c.** No apoptosis defects were found in ex vivo cultured cells from wild-type and Mettl3-KO naive T cells by FACS staining of annexin V and 7AAD. Double-negative stained cells are live cells, and the remaining are apoptotic cells. The percentage is listed in the right graph (n = 2). 

**d.** No proliferation differences were found in ex vivo cultured cells from wild-type and Mettl3-KO naive T cells. Naive T cells labelled with CellTrace were cultured ex vivo under different concentrations of anti-CD3/CD28 beads for 4 days. The percentages of proliferating cells are listed in the right graph (n = 2). n = number of biological replicates. Repeated three times and one set of data is shown. ***P < 0.001.
Extended Data Figure 3 | m^6_A methylation function of Mettl3 controls naive T cell homeostatic expansion. a, Mettl3^{-/-} recipients had normal colon length, and Mettl3^{+/+} recipients had shorter colon length. b, Mettl3^{+/+} recipients had enlarged spleens indicative of normal homeostatic expansion, while Mettl3^{-/-} recipients had very small spleens. c, All lymph organs had many fewer transferred knockout cells compared to wild-type cells analysed by FACS. d, The percentage of transferred Mettl3-KO and wild-type cells in Rag2^{+/+} host mice (n = 3). e, No apoptosis defects were found in in vivo cells recovered from peripheral lymph nodes of Mettl3 wild-type and knockout recipient mice by FACS staining of annexin V and 7AAD. Double-negative stained cells are live cells, and the remainder are apoptotic cells. The percentage is listed in the right graph (n = 3). f–g, Wild-type Mettl3 constructs, but not m^6_A catalytic dead Mettl3 constructs, could rescue Mettl3-KO phenotypes in vivo. Empty construct (N103), catalytic dead Mettl3 construct (N103-CD), and wild-type Mettl3 construct (N103-M3) were electroporated into Mettl3-KO naive T cells, and then transferred into Rag2^{-/-} mice. Four weeks after transfer, the cell number (proliferation) and CD45RB marker (differentiation) were analysed by FACS. Representative images are shown in f, and the numbers of cells are shown in g (n = 3). n = number of biological replicates. Repeated twice and one set of data is shown. **P < 0.01, ***P < 0.001.
Extended Data Figure 4 | Mettl14-KO naive T cell adoptive transfer pheno-copies Mettl3-KO cells. a, Mettl14-KO recipient mice have smaller lymphoid organs, including spleen, peripheral lymph nodes, and mesenteric lymph nodes. b, c, The percentage and the number of transferred Mettl14-KO cells in Rag2−/− recipient mice were much lower than those of wild-type cells 4 weeks after transfer in all lymphoid organs (n=3). d, The MFI (median fluorescence intensity) of naive cell marker CD45RB is much higher in knockout than wild type, suggesting Mettl14 naive T cells were locked in naive state, whereas wild-type naive T cells differentiated after 4 weeks in Rag2−/− mice (n=3). n = number of biological replicates. Repeated twice and one set of data is shown. ***P < 0.001.
Extended Data Figure 5 | Socs genes are the m^6A targets that contribute to the observed phenotypes. a, Upregulated KEGG pathways in Mettl3-KO cells over wild-type cells based on RNA-seq data.

b, Downregulated pathways in Mettl3-KO cells over wild-type cells.

c, RT–qPCR validated the RNA-seq data, showing that the mRNA expression levels of other genes and regulators in IL-7 pathways did not change in Mettl3-KO naive T cells compared to wild-type cells (n = 6).

d, Socs1 siRNAs knock down Socs1 gene expression by half in vitro (n = 3).

Naïve T cells were incubated with Socs1 or control siRNA in vitro for 3 days, and RT–qPCR was used to measure the mRNA levels of Socs1. e, Socs1, Socs3 and Cish mRNA 3′ UTRs are enriched with m^6A peaks from published ES cell and dendritic cell m^6A-RIP genome mapping. Red denotes the IP RNA counts, and grey denotes input. n = number of biological replicates. Repeated three times and one set of data is shown. **P < 0.01.
Extended Data Figure 6 | Ribosome profiling does not reveal any ribosome occupancy differences in IL-7 and TCR signalling related genes. a, Overall statistical analysis for all genes. Socs genes and other IL-7 pathway genes are highlighted. The y-axis is the log2 fold change of Mettl3-KO over wild type, and the x-axis plots the P value of the fold change value. b, Calculated translation efficiency for all genes, and the IL-7 and TCR pathway genes, do not show differences in translation efficiency between Mettl3-KO and wild-type naive T cells. c, Overall levels of RNA m6A methylation in naive T cells from Mettl3-KO and wild-type mice. c, d, Example ribosome profiles of Socs1 and Socs3 mRNAs, which do not show any significant differences between wild-type (right panel) and Mettl3-KO (left panel) samples. The RNA-seq for the inputs are shown below the ribosome profiles, which also demonstrate enhanced mRNA expression for Socs genes.
Extended Data Figure 7 | Socs genes are signal-inducible degradation-controlled genes. a, Upregulated genes in Mettl3-KO naive T cells are significantly enriched in the degradation-controlled group of genes from LPS-stimulated dendritic cells. We compared the genes that were differentially regulated by m^6^A in naive T cells to degradation-controlled genes in dendritic cells. We can assign cluster information to 5,784 genes in our sequencing data set. We looked at the clusters where fast degradation played a key role (clusters 2, 4 and 6), and tested whether the number of genes upregulated was significant with a chi square test. The P value was < 0.0001. 'Fast deg,' genes in clusters 2, 5, 6; 'not fast deg,' all other clusters; 'up,' genes upregulated (marked as significant and positive fold change); 'not up,' genes that did not change or were downregulated.

b, Socs1, Socs3 and Cish, but not Socs2, degraded faster upon IL-7 treatment in wild-type cells, and the faster degradation with IL-7 stimulation was abrogated in Mettl3-KO naive T cells. The naive T cells isolated from both wild-type and Mettl3-KO mice were pre-treated with actinomycin-D for 1 h to fully stop transcription before IL-7 stimulation, and the residual mRNAs at different time points were normalized back to t = 0 (100%).
Extended Data Figure 8 | Summary of s^4U-seq data. 

a, Analysis of reads mapping to introns demonstrates high intronic read density in s^4U-enriched samples. The ratio of reads mapping to introns is expressed as a ratio to the total number of reads that map to each transcript in each sample. 
b, Plot illustrating the Spearman correlations of the transcript-level read frequencies in total and s^4U-enriched samples for wild-type and Mettl3-KO cells at various times after IL-7 stimulation. 
c, Changes in transcript frequencies after IL-7 stimulation for wild-type or Mettl3-KO cells with and without s^4U enrichment on the basis of s^4U-seq data. Expression levels are presented relative to the transcript levels of wild-type cells before IL-7 stimulation. Shown are cluster-3 Socs genes and a control gene Xist.
Extended Data Figure 9 | Working model for m^6^A-controlled naive T cell homeostasis. a, Mettl3-KO naive T cell molecular mechanism: loss of m^6^A leads to slower Socs mRNA degradation and increased SOCS protein levels, which blocks the IL7 pathway. b, Revised T cell differentiation model: m^6^A targets Socs1, Socs3 and Cish for inducible and rapid mRNA degradation upon IL-7 stimulation, allowing IL-7-JAKs signalling to activate the downstream target STAT5, to initiate the re-programming of the naive T cells for differentiation and proliferation.
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Nature Research wishes to improve the reproducibility of the work we publish. This form is published with all life science papers and is intended to promote consistency and transparency in reporting. All life sciences submissions use this form; while some list items might not apply to an individual manuscript, all fields must be completed for clarity.

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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 from the analysis.

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

4. Randomization
   - Describe how samples/organisms/participants were allocated into experimental groups.
   - All animal results 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.
   - Binding 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.

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

   | n/a | Confirmed |
   |-----|-----------|
   | ☑ | ☑ | The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement (animals, litters, cultures, etc.) |
   | ☑ | ☑ | A description of how samples were collected, noting whether measurements were taken from distinct samples or whether the same sample was measured repeatedly. |
   | ☑ | ☑ | 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) |
   | ☑ | ☑ | A description of any assumptions or corrections, such as an adjustment for multiple comparisons |
   | ☑ | ☑ | The test results (e.g. p values) given as exact values whenever possible and with confidence intervals noted |
   | ☑ | ☑ | A summary of the descriptive statistics, including central tendency (e.g. median, mean) and variation (e.g. standard deviation, interquartile range) |
   | ☑ | ☑ | Clearly defined error bars |

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

Policy information about availability of computer code

7. Software

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

- RNA-seq was analyzed by Tophat & Cuffdiff & R package `ggplot2`
- Ribosome Profiling by tuxedo suit & R package
- s4U Seq by STAR & HTSeq-count & R package
- INSPEcT/corrplot/maSigPro/pheatmap. All those information has been detailed in the method part.

For all studies, we encourage code deposition in a community repository (e.g. GitHub). Authors must make computer code available to editors and reviewers upon request. The *Nature Methods* guidance for providing algorithms and software for publication may be useful for any submission.

### Materials and reagents

Policy information about availability of materials

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.

- All mouse lines generated in the paper are freely available upon reasonable request. No other unique materials used.

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 and extended data table 1. Those antibodies are all commercially available, and have been used routinely by other labs.

10. Eukaryotic cell lines

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

- No eukaryotic cell lines were used

b. Describe the method of cell line authentication used.

- No eukaryotic cell lines were used

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

- No eukaryotic cell lines were used

d. If any of the cell lines used in the paper 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

Policy information about studies involving animals; when reporting animal research, follow the ARRIVE guidelines

11. Description of research animals

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

- We used both male and female C57BL/6 mice of all ages, we isolate T cells from mouse spleen and lymph nodes. The transgenic mouse lines we used include: Mettl3-f/f, Mettl14-f/f, CD4-Cre, RAG2/-.

Policy information about studies involving human research participants

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.
Flow Cytometry Reporting Summary

Data presentation

For all flow cytometry data, confirm that:

1. The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).
2. 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).
3. All plots are contour plots with outliers or pseudocolor plots.
4. A numerical value for number of cells or percentage (with statistics) is provided.

Methodological details

5. Describe the sample preparation. Cells from thymus, peripheral lymph nodes, mesenteric lymph nodes and spleen were isolated and used for FACS analysis. For sample preparation from spleen, erythrocytes were lysed and removed before FACS analysis.
6. Identify the instrument used for data collection. The LSR II Flow Cytometer from BD Bioscience were used for FACS data collection.
7. Describe the software used to collect and analyze the flow cytometry data. The FlowJo Software (Version 7.6.1) was used for FACS data analysis.
8. Describe the abundance of the relevant cell populations within post-sort fractions. The purity of sorted cells was detected via flow cytometry immediately after sorting and samples with purity higher than 95% were used.
9. Describe the gating strategy used. A FSC-H/FSC-A gate was used to determine single-cell populations. The boundaries between "positive" and "negative" were determined by the clear cell subpopulations and unstained negative controls.

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