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m^{6}A mRNA modification potentiates Th17 functions to inflame autoimmunity

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N^6-methyladenosine (m^{6}A), the most common and abundant epigenetic RNA modification, governs mRNA metabolism to determine cell differentiation, proliferation and response to stimulation. m^{6}A methyltransferase METTL3 has been reported to control T cell homeostasis and sustain the suppressive function of regulatory T cells (Tregs). However, the role of m^{6}A methyltransferase in other subtypes of T cells remains unknown. T helper cells 17 (Th17) play a pivotal role in host defense and autoimmunity. Here, we found that the loss of METTL3 in T cells caused serious defect of Th17 cell differentiation, and impeded the development of experimental autoimmune encephalomyelitis (EAE). We generated *Mettl3*$^{f/f}$*Il17a*^{Cre} mice and observed that METTL3 deficiency in Th17 cells significantly suppressed the development of EAE and displayed less Th17 cell infiltration into central nervous system (CNS). Importantly, we demonstrated that depletion of METTL3 attenuated IL-17A and CCR5 expression by facilitating SOCS3 mRNA stability in Th17 cells, leading to disrupted Th17 cell differentiation and infiltration, and eventually attenuating the process of EAE. Collectively, our results highlight that m^{6}A modification sustains Th17 cell function, which provides new insights into the regulatory network of Th17 cells, and also implies a potential therapeutic target for Th17 cell mediated autoimmune disease.

m^{6}A modification, Th17 cells, EAE, SOCS family

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INTRODUCTION

N^6-methyladenosine (m^{6}A) is a reversible chemical modification widely distributed on messenger RNA (mRNA) and non-coding RNA (ncRNA), and it has been reported to regulate multiple aspects of RNA metabolism, including RNA decay, splicing and translation (Boriack-Sjodin et al., 2018; Chen et al., 2022; Liu et al., 2019; Roundtree et al., 2017; Shi et al., 2019). m^{6}A modification is catalyzed by m^{6}A methyltransferase complex, mainly composed of the

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catalytic subunit methyltransferase-like protein 3 (METTL3), methyltransferase-like protein 14 (METTL14), and the adaptor proteins Wilms tumor I associated protein (WTAP). METTL3 and METTL14 constitute the heterodimeric catalytic core, in which the catalyzing enzyme METTL3 recognizes substrate RNAs with assistance from METTL14. WTAP and other adaptors recruit core enzymes and form the complex (Liu et al., 2019; Shi et al., 2019). There is overwhelming evidence showing that m^{6}A modification plays important roles in the immune system, and our previous studies also systematically clarified the regulatory role of m^{6}A RNA methyltransferase METTL3 in the homeostasis, differentiation and function of T cells. Previously, we constructed the conditional knockout mice to lineage-specifically deplete METTL3 in CD4$^+$ T cells (*Mettl3*$^{f/f}$*Cd4*^{Cre}), and discovered that METTL3 maintained naïve T cell homeostasis by targeting the interleukin 7 (IL-7)/signal transducer and activator of transcription 5 (STAT5)/suppressor of cytokine signaling (SOCS) pathway (Li et al., 2017). CD4$^+$ regulatory T cell (Treg) is a critical subset of effector T cells involved in inflammation suppression, and we also observed that depleting METTL3 in Tregs restrains their suppressive function by interfering with IL-2/STAT5 signaling pathway (Tong et al., 2018). However, the potential role of m^{6}A mRNA modification in other T cell subsets is still unclear.

Multiple sclerosis (MS) is an autoimmune inflammatory disease of the central nervous system (CNS) characterized by inflammatory lesions, demyelination and axonal damage, and experimental autoimmune encephalomyelitis (EAE) is a commonly used animal model for studying the pathogenesis and potential therapeutic agents of MS (Barclay and Shinohara, 2017; Glatigny and Bettelli, 2018; Kipp et al., 2017). T helper cells 17 (Th17), as one of the crucial autoreactive T cells, their activation and function strongly impact the occurrence and development of EAE, in which Th17 cells recruit other inflammatory cells into CNS lesions by producing multiple proinflammatory cytokines (e.g., IFN-γ, IL-1β, IL-2, GM-CSF and IL-17A) (Kaskow and Baecher-Allan, 2018; McGinley et al., 2018; Yasuda et al., 2019). These pro-inflammatory factors activate macrophages, B cells and other T cells, stimulate CNS inflammatory cascade, and further cause demyelination and axonal damage. Previous studies have shown that the differentiation and function of Th17 cells are under regulation of STAT3 signaling pathway, and abnormal phosphorylation of STAT3 in Th17 cells elicits the pathogenesis of EAE (Chen and Shannon, 2013; Cho et al., 2019; Harris et al., 2007; Yang et al., 2007), yet it is still unknown whether m^{6}A RNA modification plays a role in Th17 cells and their mediated inflammatory diseases.

In this study, we utilized both *Mettl3*$^{f/f}$*Cd4*^{Cre} and *Mettl3*$^{f/f}$*Il17a*^{Cre} mice that deleted METTL3 either in total CD4$^+$ T cells or in IL-17A$^+$ T cells, and demonstrated that METTL3 regulates Th17 cell differentiation and migration ability through IL-6/STAT3/SOCS3 signaling pathway, which then ameliorated the pathogenesis of EAE inflammatory response. Our findings uncovered the key function of METTL3 in Th17 cell subsets, and pointed to m^{6}A as a potential therapeutic target for immunotherapy in CNS autoimmune disease.

RESULTS

METTL3 controls the EAE development and T cell differentiation

Our previous results demonstrated that the loss of m^{6}A methyltransferase METTL3 in CD4$^+$ T cells leads to the disruption of their homeostatic proliferation and differentiation into effector cells, indicating that METTL3 is essential to trigger T cell derived autoimmune disease. In order to identify the effect of m^{6}A methyltransferase METTL3 on the regulation of autoimmune disease *in vivo*, we used the *Mettl3*$^{f/f}$*Cd4*^{Cre} mouse line and generated the EAE model, the most commonly used experimental model of human inflammatory demyelinating disease multiple sclerosis. During the induction of EAE, myelin specific CD4$^+$ T cells are activated in the periphery, cross blood brain barrier and migrate into CNS where CD4$^+$ T cells can secrete proinflammatory cytokines and chemokines to attract more pathogenic cells, thereby starting an inflammatory cascade (Barclay and Shinohara, 2017; McGinley et al., 2018).

Here, we found that *Mettl3*$^{f/f}$*Cd4*^{Cre} mice were resistant to EAE induction compared with their wild-type (WT) littermates (Figure 1A). There was decreased infiltration of total CD4$^+$ T cells in spinal cord, lymphoid node and spleen of *Mettl3*$^{f/f}$*Cd4*^{Cre} mice, whereas infiltrated activated T cells (CD4$^+$CD44$^+$) were significantly decreased in the spinal cord but increased in the spleen than WT littermates (Figure 1B and C). Considering the significance of pathogenic CD4$^+$ T cells in triggering inflammatory cascades, we measured the expression of critical proinflammatory cytokines derived from CD4$^+$ T cells, such as IFN-γ and IL-17A in EAE model. We observed that METTL3 deficient CD4$^+$ T cells displayed robust reduce of IFN-γ and IL-17A secretion in the spinal cord than CD4$^+$ T cells from WT littermates (Figure 1D–F). These results demonstrate that the deletion of METTL3 in T cells mediates protection against experimental neuroinflammation. Since autoreactive Th17 cells, the main source of IL-17A, are implicated in mediating inflammation in CNS and other tissues, and GO enrichment analysis also showed that METTL3 deficiency impeded CD4$^+$ T cell differentiation including Th17 subset differentiation (Figure 1G; Figure S1 in Supporting Information), consistent to our previous observation (Li et al., 2017). Therefore, we speculated that

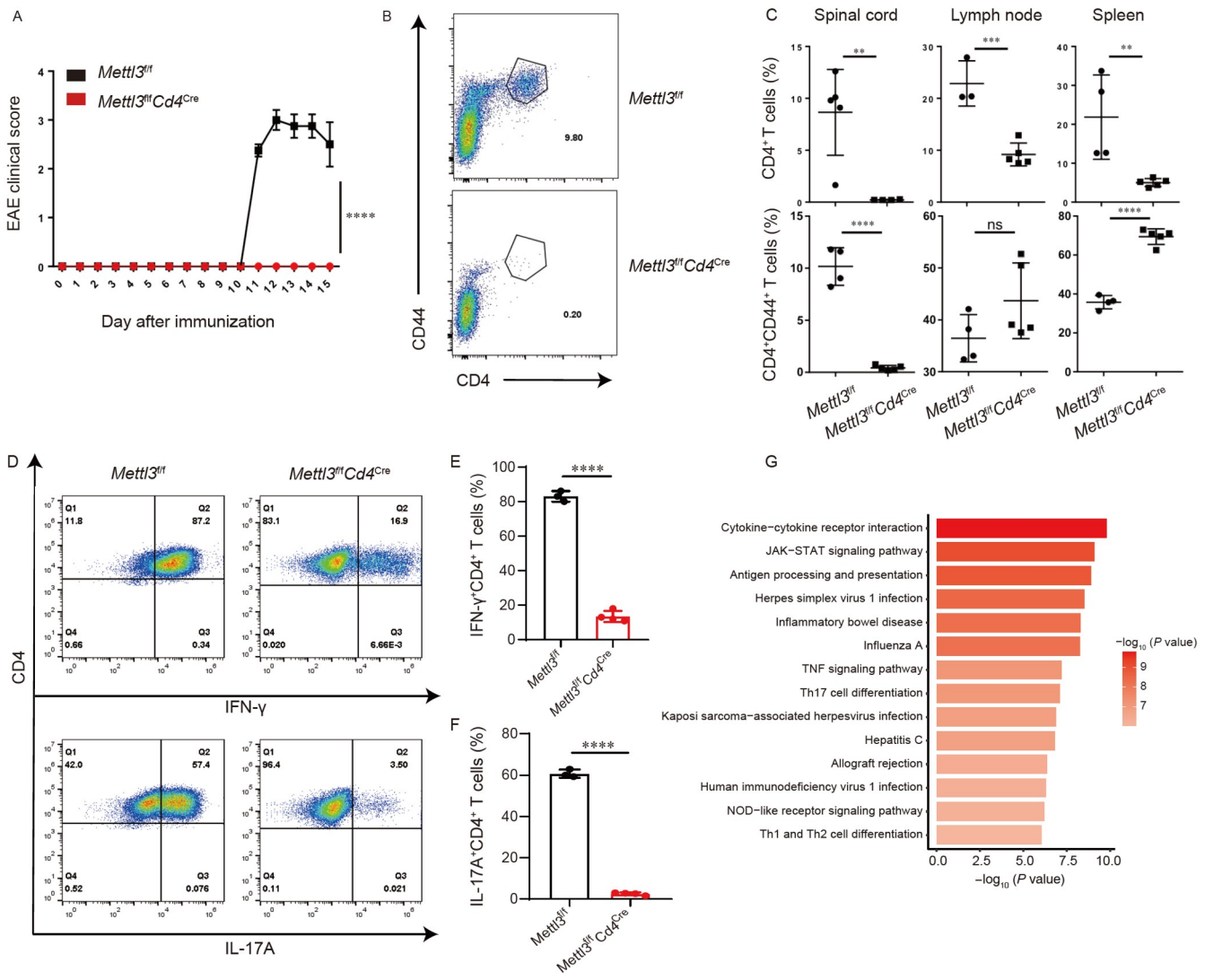

Figure 1 METTL3 deficiency in T cells suppresses the EAE development and T cell differentiation. A, *Mettl3*$^{f/f}$*Cd4*^{Cre} mice and WT littermates were immunized with the $MOG_{35–55}$ peptide emulsified in a complete Freund's adjuvant, and clinical EAE scores obtained daily were shown (n=4). B, Representative dot plots showed the infiltrated activated T cells ($CD4^+CD44^+$) in the spinal cord of *Mettl3*$^{f/f}$*Cd4*^{Cre} mice and WT littermates after EAE induction. C, The percentage of total $CD4^+$ T cells and activated T cells ($CD4^+CD44^+$) in spinal cord, lymph node and spleen of *Mettl3*$^{f/f}$*Cd4*^{Cre} mice and WT littermates after induced EAE were analyzed. D, Representative dot plots showed the expression of IFN-γ and IL-17A in $CD4^+$ T cells from spinal cord of *Mettl3*$^{f/f}$*Cd4*^{Cre} mice and WT littermates after EAE induction. E and F, The percentage of IFN-γ^+ (E) and IL-$17A^+$ (F) $CD4^+$ T cells described under (D) were calculated. G, GO enrichment analysis of down-regulated transcripts in naïve $CD4^+$ T cells isolated from *Mettl3*$^{f/f}$*Cd4*^{Cre} mice compared with WT control cells (GSE100048). Data are shown as representative results of three independent experiments and are shown as the means±SEM. **, P<0.01; ***, P<0.001; ****, P<0.0001; ns, not significant.

METTL3 plays critical regulatory roles for Th17 cell function.

METTL3 deficiency impedes Th17 cell differentiation

To clearly assess the regulatory effect of METTL3 on Th17 cells, we additionally constructed a conditional knockout mouse line by crossing *Mettl3*$^{f/f}$ mice with *Il17a*^{Cre} deleter tool mouse line, in which the METTL3 expression is specifically deleted in IL-$17A^+$ T cells (*Mettl3*$^{f/f}$*Il17a*^{Cre}). Under the homeostatic condition, the absolute number and proportion of $CD4^+$, $CD8^+$, double positive and double negative T cells in thymus and spleen of *Mettl3*$^{f/f}$*Il17a*^{Cre} mice were similar to that in WT littermates (Figure 2A–C). These results indicate that depletion of METTL3 in Th17 cells did not affect T cell development at a steady state. Then, we isolated naïve $CD4^+$ T cells from *Mettl3*$^{f/f}$*Il17a*^{Cre} mice and WT littermates, and examined the T cell differentiation ability *in vitro*. The expression of IFN-γ, IL-17A and FOXP3 was detected as the effector marker of Th1, Th17 and Treg cells respectively. We observed that naïve $CD4^+$ T cells from *Mettl3*$^{f/f}$*Il17a*^{Cre} mice exhibited limited potential for Th17 cell differentiation relative to the naïve $CD4^+$ T cells from WT mice, whereas their differentiation ability to become

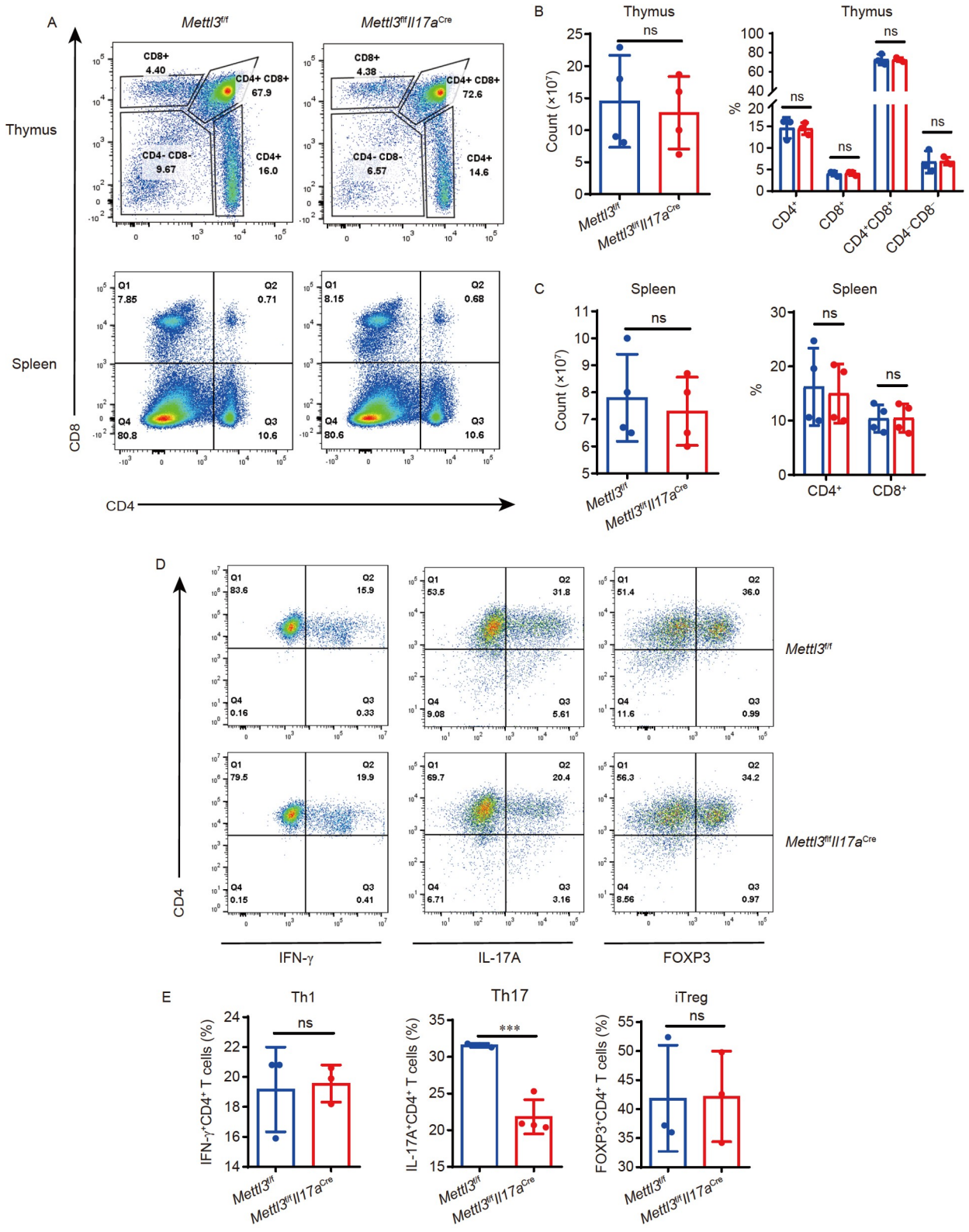

Figure 2 Depletion of METTL3 in Th17 cells has no effect on T cell development at steady state but impedes Th17 cell differentiation *in vitro*. A, Representative dot plots showed the composition of $CD4^+$, $CD8^+$, double positive and double negative T cells in thymus and spleen from *Mettl3*$^{f/f}$*Il17a*^{Cre} mice and WT littermates at steady state. B and C, The number of total T cells and proportion of $CD4^+$, $CD8^+$ and double positive cell in total T cells from thymus (B) and spleen (C) were analyzed. D, Naïve $CD4^+$ T cells isolated from *Mettl3*$^{f/f}$*Il17a*^{Cre} mice and WT littermates were differentiated into Th1, Th17 and Treg subsets, and representative dot plots showed the expression of IFN-γ, IL-17A and FOXP3 in the indicated cells. E, Percentage of IFN-γ$^+$, IL-17A$^+$ and FOXP3$^+$ $CD4^+$ T cells described under (D) were calculated. Data are shown as representative results of three independent experiments and are shown as the means±SEM. ***, P<0.001; ns, not significant.

Th1 and Treg subsets was not affected (Figure 2D and E). These results suggested that METTL3 is required for Th17 cell differentiation, and METTL3 deficiency could elicit an indispensable impact on Th17 cell derived autoimmune disease. In addition, we observed that there was only a slight increase of *Mettl3* upon anti-CD3/28 antibodies stimulation, indicating less effect of METTL3 on early activation of T cells (Figure S2 in Supporting Information).

Loss of METTL3 in Th17 cells suppresses the development of EAE

According to our results above, we further generated the EAE model in *Mettl3*$^{f/f}$*Il17a*^{Cre} mice to investigate the effect of METTL3 deficient Th17 cells in autoimmune disease *in vivo*. Similar to *Mettl3*$^{f/f}$*Cd4*^{Cre} mice, *Mettl3*$^{f/f}$*Il17a*^{Cre} mice showed significant resistance to the induction of EAE (Figure 3A). Histological staining revealed reduced lymphocyte infiltration and demyelination in the spinal cord of *Mettl3*$^{f/f}$*Il17a*^{Cre} mice compared with WT littermates (Figure 3B). The reduction of CD4$^+$ T cells infiltration in the spinal cord of *Mettl3*$^{f/f}$*Il17a*^{Cre} mice was also exhibited by flow cytometry (Figure 3C). The number and proportion of total CD4$^+$ T cells in the spinal cord of *Mettl3*$^{f/f}$*Il17a*^{Cre} mice and WT littermates during the development of EAE were analyzed. Results showed that the total number of CD4$^+$ T cells in the spinal cord was comparable between *Mettl3*$^{f/f}$*Il17a*^{Cre} mice and WT littermates, whereas its proportion was reduced in *Mettl3*$^{f/f}$*Il17a*^{Cre} mice (Figure 3D). There was also a significant reduction of IL-17A$^+$ and IFN-γ^+ CD4$^+$ T cells in the spinal cord of *Mettl3*$^{f/f}$*Il17a*^{Cre} mice compared with WT littermates, whereas the IFN-γ^+IL-17A$^+$ T cells remained unchanged (Figure 3E and F). Together, these results indicated that the resistance to EAE in *Mettl3*$^{f/f}$*Il17a*^{Cre} mice is due to a failure of encephalitogenic Th17 cell differentiation and infiltration into CNS.

METTL3 regulates IL-17A and CCR5 expression in Th17 cells through governing *Socs3* mRNA stability

To reveal the molecular mechanism by which METTL3 regulates Th17 cell function, we analyzed RNA sequencing (RNA-seq) data of METTL3 deficient and WT Th17 cells differentiated *in vitro*. RNA-seq analysis showed that the downregulated genes were mainly involved in the T cell differentiation and focal adhesion (Figure 4A and B). We found that the expression of *Il17a* and *Ccr5* were intensely disrupted in METTL3 deficient Th17 cells relative to WT Th17 cells (Figure 4A), and these results were further confirmed by quantitative real-time PCR (Figure 4C). IL-17A is a Th17 cell specifical cytokine which contributes to demyelination and neuronal loss during EAE pathogenesis, and CCR5 deficiency has also been reported to confer resistance to EAE (Gu et al., 2016). In particular, both IL-17A and CCR5 expression are under regulation of IL-6/STAT3 signaling pathway in Th17 cells (Harris et al., 2007; McLoughlin et al., 2005; Radojcic et al., 2010; Yang et al., 2007). We then carried out Western blotting experiment and verified that phosphorylation of STAT3 was indeed significantly decreased in METTL3 deficient Th17 cells (Figure 4D). These data indicated that METTL3 controls IL-17A and CCR5 expression in Th17 cells through the IL-6/STAT3 signaling pathway.

SOCS3 is a well-known negative regulator of STAT3 signaling (Carow and Rottenberg, 2014; Palmer and Restifo, 2009; Yoshimura et al., 2007). In our previous study, we performed m^{6}A-RIP-seq and demonstrated that the mRNAs of SOCS family genes, including *Socs1*, *Socs3* and *Cish*, were marked by m^{6}A (Li et al., 2017). These SOCS family genes exhibited slower mRNA decay and increased expression in METTL3 deficient naïve T cells, consequently inhibiting the IL-7/STAT5 pathway. Consistently, we performed RNA decay assay and found that the mRNA level of *Socs3* was increased in METTL3 deficient Th17 cells in comparison to WT Th17 cells after actinomycin-D treatment (Figure 4E). What's more, we also observed that *Socs1* but not *Cish* mRNA level was increased in Th17 cells from *Mettl3*$^{f/f}$*Il17a*^{Cre} mice compared with WT control cells (Figure S3 in Supporting Information). In summary, our results revealed that loss of METTL3 facilitated SOCS family RNA stability, and inhibited IL-6/STAT3 mediated IL-17A and CCR5 expression, which in turn impeded Th17 cells differentiation and infiltration, eventually attenuating the process of EAE (Figure 4F).

DISCUSSION

m^{6}A is the most extensively studied RNA modification across various species, and the important effect of m^{6}A modification in the immune system has been revealed in distinct contexts (Shulman and Stern-Ginossar, 2020; Wang et al., 2021). Despite our previous studies demonstrating that m^{6}A controls various aspects of T cell development and activity, the role of m^{6}A in T cell subsets such as T helper cell remains indistinct. In this study, we found that ablation of METTL3 caused serious disruption of Th17 cell differentiation. Lineage specific deletion of METTL3 in Th17 cells gave rise to less Th17 cell infiltration into CNS, and significantly suppressed the development of neuroinflammation. These findings uncovered the indispensable role of METTL3 mediated m^{6}A modification in the Th17 cell subset and its involvement in autoimmune disease.

m^{6}A modification has been reported to participate in RNA metabolism, predominantly affecting RNA stability. In our previous study, we documented that SOCS gene mRNAs are

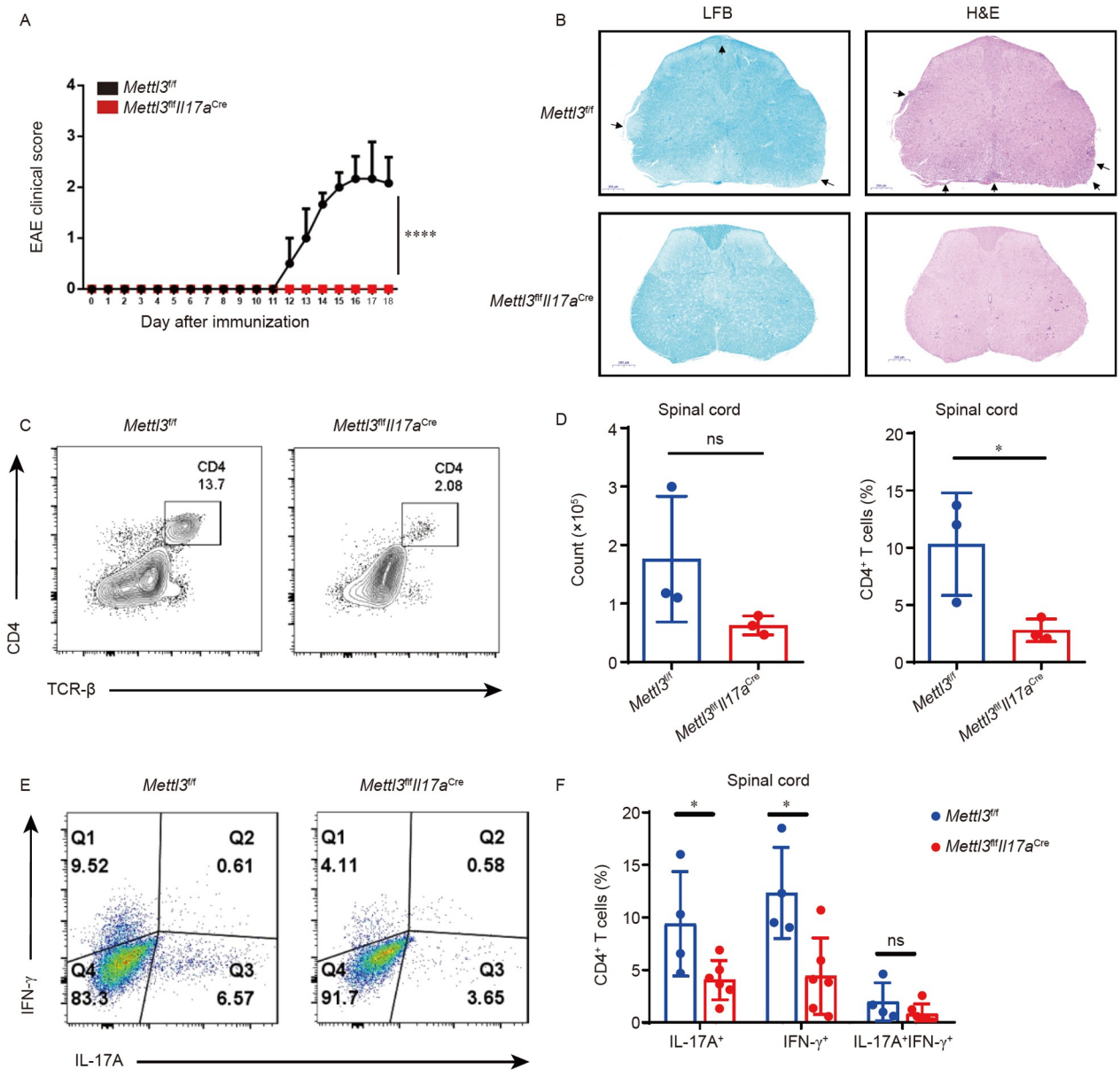

Figure 3 Loss of METTL3 in Th17 cells suppresses the development of EAE. A, *Mettl3*$^{f/f}$*Il17a*^{Cre} mice and WT littermates were immunized with the $MOG_{35–55}$ peptide emulsified in a complete Freund's adjuvant, clinical EAE scores obtained daily were shown (n=3). B, Representative images showed the luxol fast blue (LFB) staining (left) and hematoxylin and eosin (H&E) staining (right) of spinal cords of mice described under (A). Demyelination in LFB-stained sections and lymphocyte infiltration in H&E-stained sections were indicated with arrows. C, Representative dot plots showed the CD4$^+$TCRβ$^+$ T cells in the spinal cord of mice described under (A). D, The number and proportion of total CD4$^+$ T cells in the spinal cord of indicated mice were analyzed. E, Representative dot plots showed the expression of IFN-γ and IL-17A in the indicated cells. F, The percentage of IFN-γ$^+$, IL-17A$^+$ and IFN-γ$^+$IL-17A$^+$ CD4$^+$ T cells described under (E) were calculated. Data are shown as representative results of three independent experiments and are shown as the means±SEM. *, P<0.05; ns, not significant.

m^{6}A targets in CD4$^+$ T cells, and deletion of METTL3 led to attenuation of SOCS mRNA decay (Li et al., 2017). Here, we also verified that *Socs1* and *Socs3* mRNA stability are under control of METTL3 in Th17 cells, which further strengthens the mechanism of METTL3 mediated m^{6}A modification in regulating RNA stability in T cells.

In the dynamic balance of m^{6}A modification, m^{6}A demethylase the alkylated DNA repair protein AlkB homolog 5 (ALKBH5) and the fat mass and obesity-associated protein (FTO) are responsible for m^{6}A deletion. We have found that ALKBH5 decreases m^{6}A modification on *Cxcl2* and *Ifng* mRNA in CD4$^+$ T cells, increases their transcript stability and protein expression, and thus causes enhanced pathogenicity of CD4$^+$ T cells during neuroinflammation (Zhou et al., 2021). Additionally, we have observed that depletion of ALKBH5 in lymphocytes specifically induces an expansion of γδ T cells, and enhances protection against gastrointestinal *Salmonella typhimurium* infection (Ding et al., 2022). In

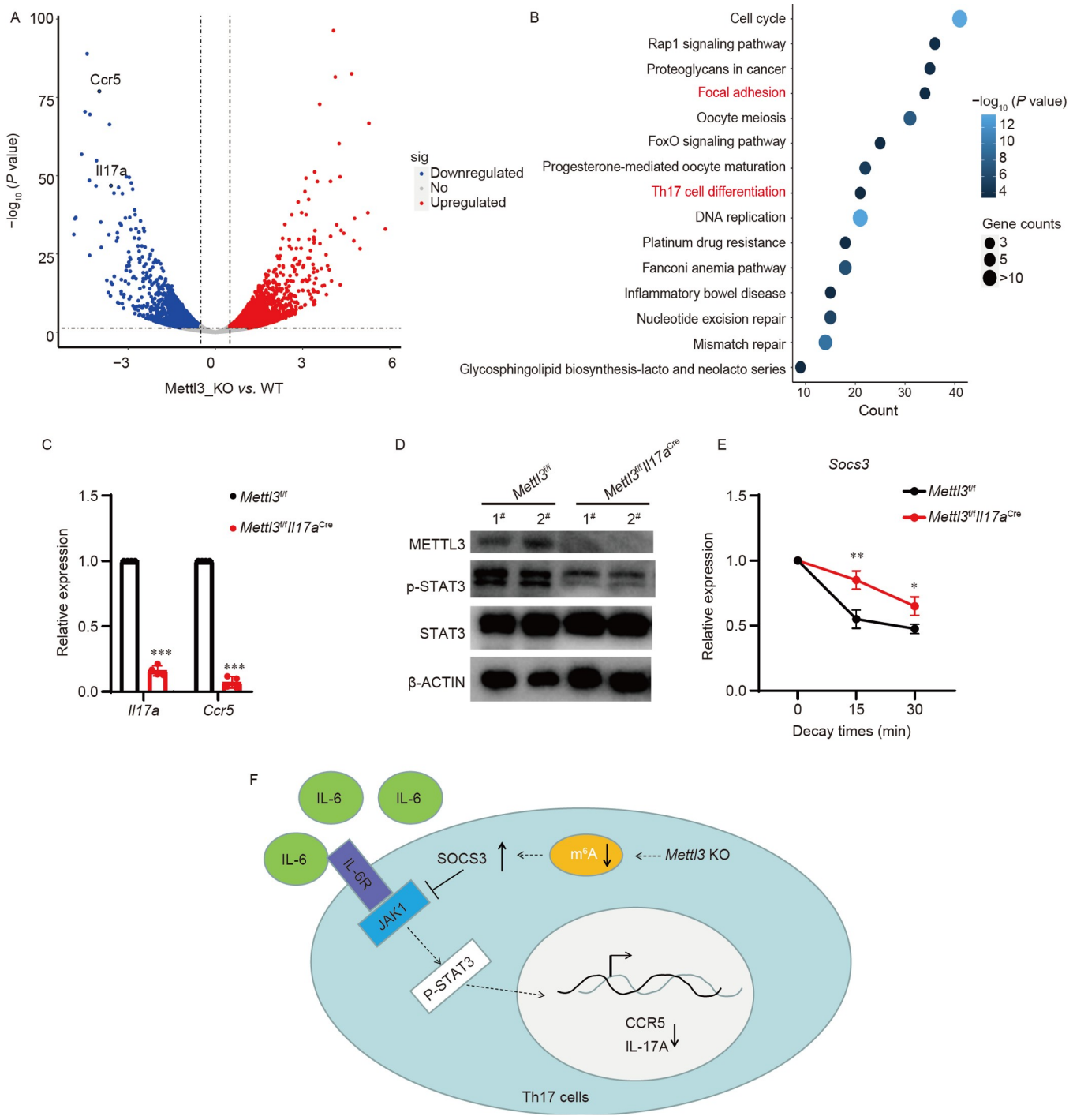

Figure 4 Downregulation of IL-17A and CCR5 is due to the facilitation of *Socs3* mRNA stability in METTL3 deficient Th17 cells. A, Volcano plot exhibited the differentially expressed genes (|fold change|>2; P<0.05; up-regulated genes, red; down-regulated genes, blue) in METTL3 deficient Th17 cells compared with WT Th17 cells. *Il17a* and *Ccr5* are highlighted. B, GO enrichment analysis showed down-regulated transcripts in METTL3 deficient Th17 cells compared with WT Th17 cells. C, The mRNA expression of *Il17a* and *Ccr5* was validated by real-time qPCR in METTL3 deficient and WT Th17 cells. D, Phosphorylation of STAT3 was detected in METTL3 deficient and WT Th17 cells after 5 days' differentiation *in vitro*. The 1# and 2# represented two reduplicative data from different *Mettl3*$^{f/f}$*Il17a*^{Cre} and *Mettl3*$^{f/f}$ mice. E, METTL3 deficient and WT Th17 cells were treated with the transcription inhibitor actinomycin-D, and the level of *Socs3* transcripts was measured over time. F, The diagram summarized the regulatory mechanism of METTL3 derived m^{6}A RNA modification in Th17 cell pathogenicity and autoimmunity during EAE. Data represent one of three independent experiments and are shown as the means±SEM. *, P<0.05; **, P<0.01; ***, P<0.001.

terms of the regulatory effect of ALKBH5 in Th17 cells, it still needs to be further explored.

Overall, our study reveals an important role of METTL3 in modulating Th17 cell function during EAE. Specifically, METTL3 catalyzes m^{6}A modification on *Socs3* mRNA for rapid degradation, allowing IL-6/STAT3 signaling to activate

the downstream IL-17A, CCR5 and other differentiation-related gene expression, and initiate Th17 cell differentiation and response to EAE. These findings suggest that blocking METTL3 expression is an effective way to resist the autoreactive Th17 cells and may become a promising immunotherapeutic strategy in autoimmune disease.

MATERIALS AND METHODS

Animals

Mettl3$^{f/f}$ mice were generated as previously described and crossed with *Cd4*^{Cre} and *Il17a*^{Cre} mice (the Jackson Laboratory, USA) to obtain conditional knockout mice. The animals were maintained under specific pathogen-free conditions and used according to protocols approved by Animal Care and Use Committees of the Shanghai Jiao Tong University School of Medicine.

Induction and assessment of EAE

EAE model was induced by subcutaneous injection with 200 μg of the mouse $MOG_{35–55}$ peptide emulsified with complete Freund's adjuvant (CFA) containing heat-killed *Mycobacterium tuberculosis* H37RA on day 0. Intravenous injection of Pertussis toxin (200 ng/mouse) was administered on the day of immunization and 2 days post immunization. Mice were evaluated by two independent, blinded examiners every day using the following clinical score assessment system as described previously: Score 0, no clinical signs; Score 1, limp tail; Score 2, paraparesis (weakness, incomplete paralysis of one or two hind limbs); Score 3, paraplegia (complete paralysis of two hind limbs); Score 4, paraplegia with forelimb weakness or paralysis; and Score 5, mori bund or death.

T cell *ex vivo* differentiation

Naïve $CD4^+$ T cells were sorted and purified from spleens by EasySep Mouse Naïve $CD4^+$ T Cell Isolation Kit (STEMCELL Technologies, USA), and then cultured with plate bound anti-CD3 mAb (10 μg mL^{-1}, 145-2C11) and anti-CD28 mAb (2 μg mL^{-1}, PV-1) in the presence of mouse recombinant cytokines and blocking antibodies. Specifically, Th1 was induced with IL-12 (10 ng mL^{-1}) and anti-IL-4 mAb (10 μg mL^{-1}, 11B11); Th17 was induced with IL-6 (20 ng mL^{-1}), IL-23 (20 ng mL^{-1}), anti-IFN-γ mAb (10 μg mL^{-1}, XMG1.2) and anti-IL-4 mAb (10 μg mL^{-1}, 11B11); iTreg was induced with TGF-β (2 ng mL^{-1}), IL-2 (50 U mL^{-1}), IL-23 (20 ng mL^{-1}), anti-IFN-γ mAb (10 μg mL^{-1}, XMG1.2) and anti-IL-4 mAb (10 μg mL^{-1}, 11B11). All cytokines were purchased from R&D Systems. RPMI (Sigma-Aldrich, USA) media was supplemented with 10% FBS, l-glutamine (2 mmol L^{-1}), penicillin (100 U mL^{-1}) and β-mercaptoethanol (40 nmol L^{-1}). After 4 days of culture, the cells were analyzed by FACS.

Flow cytometry

Monoclonal antibodies against CD3ε (145-2C11), CD4 (RM4-5), CD8 (53-6.7), TCR-β (GL3), CD44 (IM7), IFN-γ (XMG1.2), IL-17A (17B7) and Foxp3 (MF-14) were purchased from BioLegend (USA). Single-cell suspensions were prepared from CNS, lymphoid node and spleen, and performed incubation with mAbs cocktails against surface molecules for 30 min at 4°C. For intracellular cytokine staining, cells were stimulated with PMA (10 ng mL^{-1}, Sigma-Aldrich), Ionomycin (1 mg mL^{-1} Sigma-Aldrich) and Brefeldin A (5 mg mL^{-1}, Sigma-Aldrich) for 4 h, then fixed and permeabilized with BD Fixation/Permeabilization buffer (BD Biosciences, USA) for 30 min at 4°C, and subsequently stained with mAbs against intracellular molecules for 30 min at 4°C. For intracellular and intranuclear staining, cells were fixed and permeabilized using the Foxp3/Transcription Factor Staining Buffer Kit (eBioscience, USA) according to the manufacturer's protocol. All antibodies were used at a 1 in 200 dilution. Data was recorded on BD LSRFortessa X-20 and analyzed with FlowJo software (FlowJo 10.4).

RNA sequencing of Th17 cells

$CD4^+$ naïve T cells from spleen of *Mettl3*$^{f/f}$*Il17a*^{Cre} mice and WT littermates were differentiated into Th17 cells *in vitro* as above described, and total RNA was extracted using TRIzol reagent (Invitrogen, USA) based on the manufacturer's protocol. The RNA purity and quantification were evaluated by the NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, USA), and RNA integrity was assessed by the Agilent 2100 Bioanalyzer (Agilent Technologies, USA). RNA reverse transcription and library construction were performed using NEBNext® Ultra™ II Directional RNA Library Prep Kit for Illumina (NEB, USA) based on the manufacturer's instructions. Illumina HiSeq platform (150 bp paired-end reads) was adopted by OE Biotech Co. Ltd. (Shanghai, China) to sequence the libraries. Three independent biological replicates were performed for RNA sequencing.

Quantitative real-time PCR

Total RNA was extracted from Th17 cells after 4 days' differentiation *in vitro* with TRIzol (Invitrogen), then reverse transcribed using TransScript All-in-One First-Strand cDNA Synthesis SuperMix (TransGen Biotech, Beijing, China). iTaq Universal SYBR Green Supermix (Bio-Rad, USA) was used for real-time PCR.

RNA degradation assay

Th17 cells differentiated 4 days *in vitro* were seeded on 96-well plates with 0.5 million cells per well. Actinomycin-D (Sigma-Aldrich) was added to a final concentration of 5 μmol L^{-1}. Cells were collected after adding actinomycin-D at indicated time points. Then, total RNA was extracted for real-time qPCR. Data were normalized to the t=0 time point.

Histology analysis

Mice were sacrificed at 30 days after the EAE model, and their spinal cords were fixed in 10% neutral buffered formalin and embedded in paraffin. Serial paraffin sections (4 μm) were cut and stained with hematoxylin and eosin (H&E) staining and luxol fast blue (LFB).

Western blotting

Total proteins from indicated Th17 cells were extracted with radioimmunoprecipitation (RIPA) assay buffer (Beyotime, Beijing, China) supplemented with protease and phosphatase inhibitor cocktail (Thermo Fisher Scientific), loaded on SDS-PAGE gels, electrophoresed and transferred onto PVDF membranes (Millipore, USA). The membranes were incubated with TBS containing 5% skimmed milk and 0.1% Tween-20 for 1 h at room temperature. Antibodies against METTL3 (Abcam, UK), STAT3 (Cell Signaling Technology, USA), Phospho-STAT3 (Tyr705) (Cell Signaling Technology) and β-ACTIN (Cell Signaling Technology) were diluted in 5% no-fat milk buffer with the concentration of 1:1,000 and incubated at 4°C overnight. After washing three times with phosphate buffered saline (PBS) containing 0.1% Tween-20 buffer, horseradish peroxidase (HRP)-conjugated secondary antibody was added to the membranes and incubated at room temperature for 1 h. The blotting signal was detected by enhanced chemiluminescence (ECL) with pico ECL using ChemiDoc MP (Bio-Rad).

Statistics

Unpaired Student's t-test and two-way ANOVA were performed on datasets. All data were analyzed using GraphPad Prism 8. Error bars represent standard error of the mean (SEM). Values of P<0.05 were considered statistically significant. Details are provided in figure legends.

Compliance and ethics *The author(s) declare that they have no conflict of interest.*

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