Optineurin (OPTN) has important functions in diverse biological processes and diseases, but its effect on dendritic cell (DC) differentiation and functionality remains elusive. Here we show that OPTN is upregulated in human and mouse DC maturation, and that deletion of Optn in mice via CD11c-Cre attenuates DC maturation and impairs the priming of CD4\(^+\) T cells, thus ameliorating autoimmune symptoms such as experimental autoimmune encephalomyelitis (EAE). Mechanistically, OPTN binds to the JH1 domain of JAK2 and inhibits JAK2 dimerization and phosphorylation, thereby preventing JAK2-STAT3 interaction and inhibiting STAT3 phosphorylation to suppress downstream transcription of IL-10. Without such a negative regulation, Optn-deficient DCs eventually induce an IL-10/JAK2/STAT3/IL-10 positive feedback loop to suppress DC maturation. Finally, the natural product, Saikosaponin D, is identified as an OPTN inhibitor, effectively inhibiting the immune-stimulatory function of DCs and the disease progression of EAE in mice. Our findings thus highlight a pivotal function of OPTN for the regulation of DC functions and autoimmune disorders.
Dendritic cells (DCs) are the most potent antigen-presenting cells (APCs) in mammalian immune systems, playing a crucial role in the development and maintenance of immune responses and tolerance. In steady state, immature DCs (iDCs) undergo partial or homeostatic maturation, characterized by low surface expression of co-stimulatory molecules, such as CD80, CD86, and CD40, and increased anti-inflammatory cytokines secretion, which is important for maintaining peripheral immune tolerance by inducing T cells anergy and promoting regulatory T cells (Treg cells) production. During inflammation or exposure to danger signals (such as toll-like receptor signaling), DCs are stimulated for maturation to trigger T-cell immune activation. Mature DCs (mDCs) can migrate from peripheral tissue to draining lymph nodes (DLN), where they can present peptide of antigens by high-expressed major histocompatibility complex (MHC) molecules and co-stimulatory molecules for full T-cell activation. Besides, DCs can be generally divided into two major subsets, conventional DCs (cDCs) and plasmacytoid DCs (pDCs). cDCs are classical antigen-presenting cells, while pDCs are the main producers of type I IFNs further serving innate immunity. Therefore, many efforts have been made to exploit the potential of DC therapies, ranging from the suppression of autoimmunity, the establishment of transplant tolerance, to the induction of tumor immunity. The adoptive transfer of immature DCs (iDCs) emerged as a new strategy to restore immune tolerance, with a therapeutic implication in treating autoimmune diseases. The iDCs generated in vitro inhibited the experimental autoimmune encephalitis (EAE) in mice. However, successful pursuit of these applications requires fully understanding the factors regulating DC differentiation and function. Identifying the critical factors that control DC maturation is crucial for a better understanding of the immune response system and the discovery of novel clinical interventions for T-cell-mediated autoimmune diseases.

Optineurin (OPTN) is a multifunctional protein associated with various neuronal diseases. Recently, OPTN has attracted considerable attention for being identified differentially expressed in several immune disorders, including Crohn's disease (CD) and Paget's disease of bone. The well-characterized functions of OPTN are selective autophagy through its LC3 interaction region and ubiquitin binding that depends on its ubiquitin (Ub) binding domain (UBD). OPTN controls immunity mainly through nuclear factor-kappaB (NF-κB) and interferon (IFN) signaling pathways, which are also related to ubiquitination. It was shown to act as a negative regulator of tumor necrosis factor (TNF) induced NF-κB activation by competing with NF-κB essential modulator (NEMO) for ubiquitinated receptor-interacting protein (RIP). And the production of type I IFNs in the host defense against infections required the interaction between ubiquitin chains and OPTN. However, it remains elusive whether OPTN plays a specific role during DC maturation and consequently mediates the immune system.

Herein, we report that depletion of Optn in mice by CD11c-Cre promotes JAK2-STAT3 phosphorylation and the forming of IL-10/CD44+ T cells to prevent the development of autoimmunity in mice. Moreover, the natural product, Saikosaponin D (SSD), attenuates EAE symptom by inhibiting OPTN expression, suggesting that interfering OPTN could be beneficial for the therapeutic treatment of autoimmune diseases.

**Results**

**OPTN is required for DC maturation.** To explore the expression pattern of OPTN during DC maturation or activation, human monocyte-derived dendritic cells (MoDCs) were stimulated with bacterial lipopolysaccharide (LPS), a toll-like receptor 4 (TLR4) agonist, for maturation. Array analysis showed that Optn gene expression was significantly increased in mature MoDCs, similar to the dramatically upregulated DC maturation-related genes, such as CD40, TNF, IL-1β, CXCL9, and CXCL10 (Fig. 1a). Next, we cultured murine bone marrow-derived dendritic cells (BMDCs) by GM-CSF (50 ng/mL) and interleukin (IL)-4 (20 ng/mL), and then acquired mature BMDCs by LPS stimulation. Be different from the common GM-CSF (20 ng/mL) and IL-4 (5 ng/mL) system that comprised both of DCs and macrophages, our system acquired quite a low number of CD11c+ MHC-II+ CD11bhigh cells and almost no CD115+ macrophages (Supplementary Fig. 1), thus representing an effective BMDCs culture system in vitro. As expected, qRT-PCR verified the elevated expression of Optn in mature BMDCs (Fig. 1b). Immunostaining and western blotting analysis also indicated that OPTN was accumulated in CD11c+ human MoDCs and BMDCs after LPS or PolyI:C treatment (Fig. 1c), suggesting that OPTN may be involved in the regulation of DC maturation or activation.

To understand the biological role of OPTN in DC maturation, we generated CD11cΔ− cells conditional Optn knockout mice (OptnΔ−/−, CD11c-Cre mice, hereafter called OptnΔ−CD11c mice) and confirmed the total deletion of Optn in BMDCs by qRT-PCR and western blotting analysis (Fig. 1d). The number of CD11c+ DCs from bone marrow and spleen were comparable between OptnΔ−CD11c mice and control mice (Fig. 1e), and the expression levels of classic co-stimulatory molecules CD80 and CD86 were also changed faintly in Optn− deficient BMDCs (Fig. 1f), suggesting that OPTN was dispensable for the generation of DCs. Interestingly, the phosphorytic maturation of LPS challenged Optn− deficient BMDCs was inhibited obviously compared to that of controls (Fig. 1g). These data indicate that OPTN deficiency blocks DC maturation.

**OPTN regulates the T cells priming function of DC.** It is well known that mature DCs can secrete anti-inflammatory cytokines to complete immunogenicity. Our results showed that Optn− deficient BMDCs secreted more anti-inflammatory cytokines like IL-10 and transforming growth factor (TGF)-β, although failed to inhibit the production of pro-inflammatory cytokines like IL-6 and IL-12 (Fig. 2a). Encouraged by these results, we further found that cDCs were decreased, while pDCs were increased in OptnΔ−CD11c mice (Fig. 2b). Meanwhile, the percentages of peripheral tissue-derived migratory DCs (M-DCs) but not resident DCs (R-DCs) were overtly reduced in the DLN of OptnΔ−CD11c mice (Fig. 2c). These data represent a potential role of OPTN in regulating the immunogenic phenotype of DCs, which may further control the T-cell activation.

Given that the function of DCs switches from capturing and processing antigens to predominantly presenting them to T cells after maturation, we next examined if Optn ablation in DCs affects the ability of T-cell priming in vitro. Flow cytometry results showed that Optn− deficient BMDCs had a much better ability than control cells to ingest more fluorescein isothiocyanate-dextran (FITC-dextran), suggesting that Optn− deficiency in DCs improved its ability of antigen uptake (Fig. 2d). Then, we applied an in vitro T-cell priming model which involved the activation of OT-II CD4+ T cells co-incubated with DCs pulsed with a specific peptide, chicken OVAa323–339. As expected, Optn− deficient DCs inhibited the proliferation of OT-II CD4+ T cells, as measured by CFSE staining (Fig. 2e). In addition, Optn− deficient DCs exhibited an impaired ability to initiate the production of autoimmune factors IFN-γ and IL-17 in OT-II CD4+ T cells (Fig. 2f). Thus, these results suggest that Optn deletion suppresses the T-cell priming function of DCs in vitro.
Mice lacking Optn in CD11c+ cells are resistant to autoimmunity. We next employed a classical central nervous system (CNS) autoimmunity animal model EAE to explore the role of OPTN in regulating inflammatory injuries in vivo. Western blotting results showed that BMDCs from EAE mice expressed a higher level of OPTN than those from control mice (Fig. 3a). OptnΔCD11c mice displayed substantially decreased sensitivity to EAE induction and milder symptom compared with control mice, as indicated by lower clinical scores (Fig. 3b). As expected, OptnΔCD11c mice exhibited a less demyelinated...
Fig. 2 OPTN regulates the T cells priming function of DC. a ELISA of cytokines in supernatants of LPS treated Ctrl and Optn null BMDCs. n = 3 independent experiments. P(IL-10) = 0.0029, P(IFN-γ) = 0.0239, P(IL-6) = 0.6114, P(IL-12) = 0.8559. b Flow cytometry analysis of the frequency of total DC (CD11c+), conventional DC (cDC, CD11cMHCII+), plasmacytoid DCs (pDC, CD11c+PDCA-1+) in the spleen of Ctrl and Optn<sup>−/−</sup> mice. n = 3 independent experiments, P(DC) = 0.4925, P(cDC) = 0.0271, P(pDC) = 0.0216. c Flow cytometry analysis of the frequency of total DC (CD11c+), migratory (M-DC, MHCI<sup>+</sup>CD11c<sup>+</sup>) and resident (R-DC, MHCI<sup>-</sup>CD11c<sup>+</sup>) in the draining lymph nodes of Ctrl and Optn<sup>−/−</sup> mice. n = 3 independent experiments, P(DC<sub>total</sub>) = 0.0295, P(DC<sub>M</sub>) = 0.2297, P(DC<sub>R</sub>) = 0.0394, P(MR-DC) = 0.0776. d Uptake of FITC-dextran was measured by flow cytometry in Ctrl and Optn null BMDCs. n = 3 independent experiments, P = 0.0007. e Proliferation rate of CFSE-labeled OT-II CD4<sup>+</sup> T cells incubated with Ctrl or Optn null BMDCs pulsed with OVA<sub>223-239</sub>, n = 3 independent experiments, P = 0.0055. f The percentages of differentiated Th1 and Th17 subsets of OT-II CD4<sup>+</sup> T cells incubated with Ctrl or Optn null BMDCs pulsed with OVA<sub>223-239</sub>, n = 3 independent experiments, P(Th1) = 8.6E-05, P(Th17) = 0.0028. Data are presented as means ± SD. *P < 0.05; **P < 0.01; ***P < 0.001; NS, not significant. Unpaired two-tailed Student’s t-test. Source data are provided in Source data file.

**Optn<sup>−/−</sup> mice may have normal functions of T cells and macrophages.** Although DNA recombination in CD11c-cre line is mainly present in Dcs, previous reports have shown that CD11c-Cre line has germline recombination and off-target problems, which may cause partial knockout of target genes in T cells and macrophages<sup>18,19</sup>. Consistently, we also found a weak deletion of OPTN in CD4<sup>+</sup> T cells but similar expression of OPTN in bone marrow-derived macrophages (BMDMs) from Optn<sup>−/−</sup> mice (Supplementary Fig. 2a). Considering the significant role of Dcs in T-cell activation, we then want to define the intrinsic impact of T cells in Optn<sup>−/−</sup> mice. Optn knockout by CD11c-Cre did not influence T cells in the spleen and DLN, as the

area and an increased number of CC1<sup>+</sup> mature oligodendrocytes in the white matter of spinal lesions at the peak of EAE (Fig. 3c, d), which could ultimately promote remyelination. In addition, Optn<sup>−/−</sup> mice displayed a reduced frequency of inflammatory Th1 (CD4<sup>+</sup>IFN-γ<sup>+</sup>) and Th17 (CD4<sup>+</sup>IL-17<sup>+</sup>) cells as well as an increased amount of Th2 (CD4<sup>+</sup>IL-4<sup>+</sup>) and Treg (CD4<sup>+</sup>Foxp3<sup>+</sup>) cells in both spleen and DLNs (Fig. 3e, f). Likewise, the absolute numbers of mononuclear cells (MNCs) infiltrating into the CNS were lower in Optn<sup>−/−</sup> mice than that in control mice (Fig. 3g). Taken together, these data indicate that depletion of Optn in CD11c<sup>+</sup> cells protects mice from CNS autoimmunity.
comparative numbers of CD4+ and CD8+ T cells in OptnΔCD11c and control mice (Supplementary Fig. 2b). In addition, no infiltration of lymphocytes in the liver, lung, and spleen from OptnΔCD11c mice was observed (Supplementary Fig. 2c), coupled with the similar frequency of activated (CD44hiCD62Llo) CD4+ and CD8+ T cells in the spleen (Supplementary Fig. 2d, e). These results demonstrate that Optn knockout by CD11c-Cre does not influence T cells homeostasis in the immune system.

Given that EAE is mainly driven by CD4+ T-cell activation, to rule out the role of Optn in CD4+ T cells from OptnΔCD11c mice, we isolated CD4+ T cells from WT and OptnΔCD11c mice, co-incubated with DCs from WT mice pulsed with MOG35-55 for 2 days. FACS analysis showed that the percentage of Th1, Th2, Th17, and Treg cells were comparable between WT and OptnΔCD11c mice-derived CD4+ T cells (Supplementary Fig. 2f), indicating that slight deletion of Optn had no effect on CD4+ T-cell activation. To further clarify the role of OptnΔCD11c mice-derived CD4+ T cells in the in vivo phenotype of EAE mice, we then adoptively transferred CD4+ T cells that were treated with WT DCs pulsed with MOG35-55 to induce EAE. As expected, results indicated that WT and OptnΔCD11c mice-derived CD4+ T cells had similar abilities to induce EAE pathogenesis (Supplementary Fig. 2g-i). Collectively, although we cannot completely rule out the role of OPTN in T cells, our results suggest that CD4+ T cells derived from OptnΔCD11c mice do not affect EAE progression.

On the other hand, BMDMs from WT and OptnΔCD11c mice had comparable capacities on M1/M2 polarization and pro/anti-inflammatory cytokine expression (Supplementary Fig. 3a-g). Inspired by the findings that BMDMs can also express CD11c21, our flow cytometry results showed that BMDMs from OptnΔCD11c mice had similar expression of CD11c to that of WT mice (Supplementary Fig. 3h). Besides, the polarization ability of F4/80+CD11c+ BMDMs from WT and OptnΔCD11c mice was also comparable (Supplementary Fig. 3i). Thus,
macrophages from OptnΔCD11c mice may have similar function to that from WT mice in vitro. Even so, there are still drawbacks that the potential function of OPTN in CD11c+ macrophages in vivo is still unknown and whether CD11c+ macrophages in OptnΔCD11c mice influence EAE pathology is also undefined. Nonetheless, our data at least clarify the function of OPTN in DCs and prove that Optn deficiency in CD11c+ cells mitigates EAE effectively.

STAT3 signaling pathway participates in Optn deficient DC maturation. STAT3, among all the members of the STAT family, has been well documented to promote the abnormal DC differentiation and function. Consistently, we also found that phosphorylated STAT3 (p-STAT3), a critical transcription factor for STAT3 transduction, was elevated after LPS treatment for 16 h (Fig. 4a). However, a subsequent western blotting analysis showed that Optn deficiency activated STAT3 transduction prominently after LPS treatment for just 8 h (Fig. 4b), which was further confirmed by the increased p-STAT3 level in nuclear subfractions of Optn null BMDCs (Fig. 4c). Although STAT3 in macrophages was found to limit the inflammatory function of EAE mice, our results showed that BMDMs from OptnΔCD11c mice had similar expression of (p-)STAT3 to WT mice, and the level of STAT3, among all the members of the STAT family, limits EAE. Adoptive transfer study showed that Stat3 deficient BMDCs aggravated MOG35,55 induced EAE progression when compared with control BMDCs (Supplementary Fig. 5). To further determine whether the immune tolerance of OptnΔCD11c mice is dependent on STAT3 signaling, we crossed the OptnΔCD11c mice with Stat3fl/fl mice (OptnΔCD11c; Stat3fl/fl; CD11c-Cre mice, hereafter called DKO mice) and verified the knockout efficiency by western blotting (Fig. 5a). Results showed that DKO mice displayed much more severe autoimmune symptoms than OptnΔCD11c mice, as evidenced by higher clinical score, more severe demyelination, and less CC1+ mature oligodendrocytes (Fig. 5b–d). In addition, the percentages of Th1 and Th17 cells were much higher in both the spleen and DLN of DKO mice during EAE than those of OptnΔCD11c mice (Fig. 5e, f). Conversely, the amounts of Th2 and Treg cells in DKO mice were significantly decreased when compared with OptnΔCD11c mice (Fig. 5e, f). Together, these results suggest that STAT3 in CD11c+ cells is a negative regulator of autoimmune response, and OPTN can manipulate STAT3 activity in CD11c+ cells to control autoimmune progress.

OPTN restrains the phosphorylated activation of JAK2 in DCs. Encouraged by the findings, we proceeded to study how OPTN regulates STAT3 signaling pathway. We found JAK2, the classical upstream signal of STAT3, was also activated observably after LPS treatment for only 8 h (Fig. 6a). To find out whether OPTN modulates JAK2 phosphorylated activation, we overexpressed OPTN in control and Optn deficient BMDCs. Results showed that OPTN abundance could inhibit the expression of p-JAK2 in both control and Optn knockout BMDCs stimulated with LPS or poly I:C (Fig. 6b, c). Considering that IL-6 and IL-10, classical stimulators of JAK2, are rapidly released in response to TLR4/NF-κB signals stimulated by LPS, we therefore suspected that IL-6/IL-10 may be involved in the regulation of JAK2-STAT3 in DCs. Despite the fact that both anti-IL6 and anti-IL10 eliminated LPS induced JAK2-STAT3 activation in Optn-deficient DCs (Fig. 6d), there was no direct interaction between TLR4 and JAK2 or STAT3 in DCs after LPS treatment and no marked difference in NF-κB signals between control and Optn-deficient DCs (Fig. 6e, f). In consequence, these data indicate that LPS activated JAK2/STAT3 is dependent on the IL-6/IL-10 but not the recruitment of JAK2 or STAT3 to TLR4, and OptN deletion cannot interfere the NF-κB signals which manipulate the initial transcription of IL-6/IL-10 in LPS treatment.

We then asked whether Optn knockdown could cause the constitutive activation of JAK2 and employed IL-6 to activate the IL-6 receptor/JAK2 signaling pathway. As a result, OPTN overexpression decreased the phosphorylation level of JAK2, while Optn depletion augmented the JAK2 activation (Fig. 6g). More importantly, this effect was strengthened after IL-6 treatment (Fig. 6g). Finally, the treatment of RAPA did not alter the level of p-JAK2 and p-STAT3 (Fig. 6h), proving that the activation of JAK2/STAT3 in Optn knockout BMDCs was independent of the OPTN-mediated autophagy deficiency.
**Fig. 4** STAT3 participates in OPTN-regulated DC maturation. a Expression of p-STAT3 in BMDCs stimulated with LPS for different time. n = 3 independent experiments. b Expression of (p)-STAT3 in Ctrl or Optn knockout BMDCs stimulated with LPS for 8 h. n = 3 independent experiments. c Cytoplasmic and nuclear proteins from Ctrl and Optn knockout BMDCs were analyzed by western blotting. The nuclear marker Lamin B and the cytoplasmic marker GAPDH were used to demonstrate the purity of the fractions. n = 3 independent experiments. d qRT-PCR analysis of STAT3 target genes in Ctrl and Optn knockout BMDCs. n = 3 independent experiments, \( P_{\text{Vega}} = 0.0238, P_{\text{Hilfa}} = 0.0023, P_{\text{Hilh}} = 1.8 E - 05, P_{\text{Tgfb2}} = 2.1 E - 06, P_{\text{Tgfb3}} = 0.0137, P_{\text{Tgfb3}} = 0.0076, P_{\text{Tgfb3}} = 8.9 E - 07, P_{\text{Tgfb3}} = 3.1 E - 05. e, f Ctrl BMDCs were transfected with STAT3-overexpression (OE) plasmid. mRNA level of CD80, CD86, H2-K1, and HI-dra were analyzed by qRT-PCR. \( n = 3 \) independent experiments. p-\(CD80\) = 0.0056, p-\(CD80\) = 0.0031, p-\(CD86\) = 6.4E - 05, p-\(HI-dra\) = 0.0034. g, j Ctrl and Optn knockout BMDCs were treated with static (10μM) before LPS stimulation. Expression of CD80, HI-dra (h) and CD86, MHC-II (i, j) were analyzed by qRT-PCR and flow cytometry, respectively. n = 3 independent experiments. \( P_{\text{Optn}} = 0.9612, P_{\text{Optn}} = 0.0025, P_{\text{Optn}} = 0.9157, P_{\text{Optn}} = 0.0030 \) of CD80 in (h); \( P_{\text{Optn}} = 0.9002, P_{\text{Optn}} = 0.0031, P_{\text{Optn}} = 0.3368, P_{\text{Optn}} = 0.0024 \) of CD86 in (j); \( P_{\text{Optn}} = 0.0005, P_{\text{Optn}} = 0.0015, P_{\text{Optn}} = 0.0002 \) of CD86 in (j); \( P_{\text{Optn}} = 2.2 E - 05, P_{\text{Optn}} = 4.7 E - 05 \) of MHC-II in (j). Data are presented as means ± SD. \( * P < 0.05; ** P < 0.01; *** P < 0.001; \) NS, not significant. Unpaired two-tailed Student’s t-test for (d, f); one-way ANOVA Tukey’s post hoc analysis for (h, j). Source data are provided in Source data file.

Overall, our results confirm that OPTN negatively modulates JAK2 phosphorylated activation in DCs.

**OPTN inhibits JAK2 dimerization and subsequent STAT3 activation.** Next, we wonder how OPTN regulates JAK2/STAT3 signaling. Immunoprecipitation analysis showed that ectopically expressed OPTN was reciprocally precipitated with JAK2, but not JAK1 or JAK3, from HEK293 cells overexpressing the proteins (Fig. 7a). A subsequent co-immunoprecipitation further confirmed the direct interaction between endogenous OPTN and JAK2 in mouse primary BMDCs (Fig. 7b). JAK2 kinase is composed of 7 JAK homology (JH) domains, termed JH1-7. The JH region functions as the kinase domain of JAK2, while JH2 can physically interact with JH1 and inhibit its kinase activity.
activity. And the JH3-7 region of JAK2 is essential for receptor interactions36. Accordingly, we generated a series of truncated JAK2 mutants to represent these domains. Analysis of the interaction between OPTN and the JAK2 mutants identified JH1, rather than JH2 or JH3–7, as the site for OPTN binding (Fig.7c). On the other hand, the interaction between OPTN and JH1 did not rely on its UBD, as OPTNΔUBD could also interact with JAK2 normally (Fig.7d). Therefore, these results suggest that OPTN may regulate JAK2 directly.

Previous studies suggested that JH1-JH1 trans-interaction mediates the dimerization and activation of JAK237, which prompted us to investigate whether OPTN inhibits JAK2 dimerization. The results showed that the ability of FLAG-tagged JAK2 to co-immunoprecipitate with HA-tagged JAK2 was markedly hampered by the overexpression of OPTN (Fig.7e). Moreover, the disuccinimidyl suberate-mediated cross-linking assays indicated that the dimer/monomer ratio of JAK2 was decreased in the presence of OPTN in both HEK293 and BMDCs (Fig.7f, g), further showing the inhibitory ability of OPTN to JAK2 dimerization. Given that once activated, JAK2 recruits and phosphorylates STAT3, we then wondered whether OPTN could affect JAK2 downstream activation. As expected, the JAK2-STAT3 interaction was abrogated by OPTN overexpression in both HEK293 and BMDCs (Fig.7f, i), implying that OPTN antagonized the binding of JAK2 with STAT3, further interfering STAT3 activation.

STAT3, a classical transcription factor, can transfer to the nucleus and bind to its target genes like Il-6 after phosphorylated activation38. To further explore the transcriptional regulatory role of STAT3, we analyzed the public STAT3 ChIP-seq data from GEO database38. Peak tracking results revealed that STAT3 had quite high bindings with Il-6 gene in DCs, while STAT3 showed weak bindings with genes like Il-6, Cd80, Cd86, Mhc-i (H2-K1), Mhc-ii (H2-Aa), Vegfa, Hif1a, Hgf, Ptg2, Il-13, Tgfβ2, or Tgfβ3 (Fig.7j and Supplementary Fig. 6), suggesting IL-10 might be the effective target gene of STAT3 in DCs. Consistent with the previous studies33,39, we found that the expression of Il-6 and Il-10 was increased and then went down after LPS treatment in control BMDCs (Fig. 7k). Intriguingly, knocking out of Optn in BMDCs facilitated and maintained the expression of IL-10 but not IL-6 (Figs. 2a and 7k), thus exaggerating the immunosuppressive function of IL-1031, and resulting in the evident activation of JAK2/STAT3 in Optn knockout BMDCs. Collectively, our data demonstrate that OPTN negatively regulates JAK2/STAT3 activation by interacting with JAK2 and inhibiting its dimerization, and Optn deficiency hinders DC maturation via activating IL-10/JAK2/STAT3/IL-10 positive feedback loop.

In addition to IL-10 target, we also found STAT3 could bind to its canonical target Socs3 gene in DCs by STAT3 ChIP-seq data peak tracking (Supplementary Fig. 6), and our qPCR results showed the gene expression of Socs3 was significantly upregulated in Optn deficient BMDCs (Fig. 4d). Given that Socs3 is found to inhibit DC maturation together with subsequent Th17 response38, these data indicate that Optn deficiency can inhibit DC maturation at least partially via activating STAT3 target Socs3.

SSD impairs DC function and alleviates EAE by reducing OPTN expression. The promising effect of Optn deficiency on DCs dictated immune activation shed creative lights on finding treatments for autoimmune diseases. We screened the natural product library for small molecule inhibitors of OPTN expression and found that Saikosaponin D (SSD), a triterpenoid saponin derived from Bupleurum falcatum L, significantly downregulated
OPTN in BMDCs after LPS stimulation (Fig. 8a). To investigate how SSD inhibits OPTN expression, we next analyzed the binding profiles of SSD and OPTN by using surface plasmon resonance (SPR) assay. Results revealed that SSD specifically binds to OPTN (KD = 5.115 x 10^{-5} M), with rapid on-rate and off-rate (Supplementary Fig. 7a, b), but not ubiquitin-like modifier activating enzyme 3 (UBA3) (Supplementary Fig. 7c, d). Then we performed a cycloheximide (CHX) chase assay in SSD-treated BMDCs to determine whether SSD affects the stability of OPTN protein. Western blotting results indicated that the stability of OPTN was decreased in the presence of SSD (Fig. 8b), suggesting that the interaction of SSD with OPTN promotes the degradation of OPTN, thus reducing OPTN expression.

Next, the effect of SSD on DC activation was examined, and flow cytometry results showed that SSD markedly reduced the expression of CD80, CD86, and MHC-II in BMDCs (Fig. 8c, d). In addition, SSD-treated BMDCs exhibited an impaired ability to initiate the proliferation and differentiation of OT-II CD4+ T cells (Fig. 8e–g). These data indicate that SSD inhibits DC maturation and activation, which may be further linked to the potential therapeutic role of SSD on autoimmune disease. Our results showed that SSD treatment daily protected mice against the EAE challenge in a dose-dependent way (Fig. 8h). Luxol fast blue (LFB) and CC1 staining further confirmed that demyelination was markedly decreased and mature oligodendrocytes were dramatically increased after SSD treatment (Fig. 8i). Elsewhere, the amount of the inflammatory Th1 and Th17 cells in peripheral lymphoid organs of SSD-treated mice were reduced significantly during disease development (Fig. 8j, k). All together, these results suggest that SSD effectively inhibits not only the immunostimulatory function of DCs but also the disease progression of EAE.

We then asked whether SSD specifically depends on OPTN to regulate DC maturation and EAE progression. Western blotting results showed that SSD had no effect on TLR4 signaling, as shown by the equivalent expression of TLR4, MyD88, (p-)NF-κB, (p-)IRF3, and (p-)p38 MAPK upon SSD treatment (Supplementary Fig. 7e). Meanwhile, qRT-PCR analysis revealed the comparable mRNA levels of C180 and C186 in Optn knockout BMDCs with or without SSD incubation (Supplementary Fig. 7f). Finally, SSD administration exhibited a similar therapeutic effect as Optn deletion in effectively inhibiting the progression of EAE, and had no effect on Optn knockout mice undergoing EAE progress (Supplementary Fig. 7g). In aggregate, these results suggest that SSD ameliorates experimental autoimmunity through OPTN modulation.

**Discussion**

Immunostimulatory properties of DCs are closely associated with their maturation state. Tolerogenic immature DCs weaken T-cell functions and inhibit immune responses in an autoimmune disease, while mature DCs promote the immune activities in pathogenic conditions. However, the signaling network that mediates the maturation of DCs has not been fully understood. Meanwhile, emerging evidence highlight the pivotal role of OPTN in the immune system, but the mechanisms underlying the effects of OPTN on DC activation and immunogenicity...
HEK293 cells were transfected with FLAG-JAK2 and HA-OPTN or empty vector. Interaction between OPTN and JAKs was determined by immunoprecipitation (IP) with anti-HA antibody followed by immunoblotting with anti-Flag antibody. Interaction between endogenous OPTN and JAK2 in BMDCs was analyzed by IP. Schematic illustration of the truncated JAK2. Interactions between OPTN and truncation JAK2 in transiently transfected HEK293 cells were determined by IP. Interactions between HA-JAK2 and FLAG-JAK2 in transiently transfected HEK293 cells was determined by IP. f, g HEK293 cells were transfected with FLAG-JAK2 and HA-OPTN or empty vector. Whole-cell lysates were incubated with disuccinimidyl suberate (DSS). JAK2 antibody marked two bands: the upper band referring to the dimer and the lower band representing the monomer of JAK2.

Fig. 7 OPTN inhibits the dimerization of JAK2 and subsequent STAT3 activation. a HA-OPTN expression plasmid was transfected with Flag-JAK1, Flag-JAK2, or Flag-JAK3 expression plasmid into HEK293 cells. Expression of OPTN and JAKs was confirmed by immunoblotting. Interaction between OPTN and JAKs was determined by immunoprecipitation (IP) with anti-HA antibody followed by immunoblotting with anti-Flag antibody. b Interaction between endogenous OPTN and JAK2 in BMDCs was analyzed by IP. c Schematic illustration of the truncated JAK2. Interactions between OPTN and truncation JAK2 in transiently transfected HEK293 cells were determined by IP. d Interactions between JAK2 with OPTN or OPTNAUBD in transiently transfected HEK293 cells were determined by IP. e Interactions between HA-JAK2 and FLAG-JAK2 in transiently transfected HEK293 cells was determined by IP. f, g HEK293 cells were transfected with FLAG-JAK2 and HA-OPTN or empty vector (f). BMDCs were transfected with HA-OPTN or empty vector (g). Whole-cell lysates were incubated with disuccinimidyl suberate (DSS). JAK2 antibody marked two bands: the upper band referring to the dimer and the lower band representing the monomer of JAK2. h Interaction of FLAG-JAK2 and HA-STAT3 in transiently transfected HEK293 cells was determined by IP. i BMDCs were transfected with HA-OPTN or empty vector. Interaction between endogenous STAT3 and JAK2 in BMDCs was analyzed by IP. j ChIP-seq analysis of the binding between STAT3 and Il-10 or Il-6 from GSE27161. k Il-10 and Il-6 mRNA level in Ctrl and Optn deficient BMDCs stimulated with LPS for different times. n = 3 independent experiments, P_{(a)} = 3.2E-06, P_{(b)} = 0.0497, P_{(c)} = 1.2E-05, P_{(d)} = 3.9E-07, P_{(e)} = 0.0060 of Il-10. Data in (a-i) are representative of three independent experiments. Data are presented as means ± SD. *P < 0.05; **P < 0.01; ***P < 0.001. Unpaired two-tailed Student’s t-test. Source data are provided in Source data file.

Our present study demonstrated that Optm deficiency hampered the antigen presenting function of DCs and their ability to activate CD4+ T cells and differentiate into Th1 and Th17 subtypes, and Optn conditional knockout via CD11c-Cre in mice model could effectively disrupt the progression of EAE, a T cells dependent experimental autoimmune disease (Supplementary Fig. 8).

First of all, culture of DC precursors from bone marrow with GM-CSF or FLT3L are two common systems used in vitro. Of note, although both GM-CSF and FLT3L stimulated murine bone marrow cultures comprise a heterogeneous population of CD135+ DCs and CD115+ macrophages or monocyte progeny, our GM-CSF/IL-4 stimulated BMDC culture system contained almost no CD115+ macrophages (Supplementary Fig. 1), which may be relevant to the use of high concentration of IL-4 (20 ng/ml). Importantly, it has been also reported that GM-CSF/IL-4 derived DCs are the equivalents of in vivo DCs that emerge after inflammation, whereas FLT3L derived DCs better represent the steady-state resident DCs. Taken together, GM-CSF/IL-4 is supposed to be a better one in our study for DC culture experiments.

What’s more, although CD11c-Cre line is associated with germeline recombination or off-target problem in T cells as the presence of CD11c on the minor subpopulation of them, our validation nonetheless showed that Optm deletion via CD11c-Cre did not affect the function of T cells. Besides, more and more studies have found that CD11c is also expressed in a portion of macrophages in many tissues like liver, adipose tissue, intestinal, and alveolar macrophages, the ratio of which will be significantly elevated in inflammatory conditions. Thus, CD11c+ macrophages are claimed as activated, pro-inflammatory or M1-like monocytes derived macrophages. Consistent with previous findings, we found that bone marrow monocytes derived BMDCs also contain CD11c+ macrophages, the percentage and function of which was similar in BMDCs from Ctrl and OptmACD11c mice.
Fig. 8 OPTN inhibitor SSD impairs DC function and attenuates EAE. a Western blotting analysis of BMDCs that treated with SSD of different concentrations for 24 h before LPS stimulation. n = 3 independent experiments. b Western blotting analysis of 293T cells that treated with cycloheximide (CHX) for different time after SSD (1 μM) treatment. n = 3 independent experiments. c, d BMDCs were cultured with SSD (1 μM) for 24 h before LPS stimulation. Flow cytometry analysis for the expression of CD80, CD86, and MHC-II. Data are presented as means ± SD. *P < 0.05; **P < 0.01; ***P < 0.001. e, f Proliferation of CFSE-labeled OT-II CD4+ T cells that incubated with BMDCs treated with or without SSD and then pulsed with OVA323–339. n = 3 independent experiments, P = 0.0009. g, h Differentiation of OT-II CD4+ T cells that incubated with BMDCs treated with or without SSD and then pulsed with OVA323–339. n = 3 independent experiments, P = 0.0028. i C57BL/6 mice (8-week-old, female) were immunized with MOG35–55 and administered daily with SSD or placebo solution intragastrically from the day of immunization. Mean clinical scores are shown. n = 8 independent animals, P = 0.0047. j, k Flow cytometry analysis of Th1 and Th17 cells in spleen (SP) (j) and draining lymph nodes (DLN) (k) from vehicle and SSD treated EAE mice at day 20. n = 3 independent experiments, P = 0.0148, P = 0.0004 in (j); P = 0.0095, P = 0.0004 in (k). Data are presented as means ± SD. *P < 0.05; **P < 0.01; ***P < 0.001. Unpaired two-tailed Student’s t-test. Source data are provided in Source data file.
Then, our data showed that the expression of OPTN was augmented in both LPS stimulated MoDCs and BMDCs. The Optn−/− deficient DCs had lower levels of CD80, CD86, MHC-I, and MHC-II, along with impaired ability to promote CD4+ T-cell proliferation. In the light that the activated DCs, by TLR4 agonist LPS, could be used as a credible model system to dissect mechanisms in the TLR4 associated EAE85,35,36, we employed EAE to further explore the biological role of DCs specific OPTN in regulating immune responses in vivo. As a result, we found that CD11c-Cre mediated deletion of Optn in CD11c+ cells protected mice from EAE by reducing the amount of proinflammatory Th1 and Th17 cells in the peripheral lymph system. Mechanistically, we found that OPTN did not interfere TLR4 or NF-κB pathway but negatively regulated the JAK2-STAT3 signaling pathway through direct interaction with the JH1 domain of JAK2 and subsequently II-10 transcription signals. Literature has reported that OPTN plays a critical role in selective autophagy and ubiquitination through its LC3 interaction region and Ub-binding domain11. However, our study showed that Optn ablation did not reverse the maturation of autophagy activated BMDCs, and OPTN without Ub-binding domain could promote DC maturation like OPTN but still retained the ability to bind to JAK2, indicating that the ubiquitination or autophagy function of OPTN does not participate in the regulation of JAK2-STAT3 signaling pathway in LPS stimulated BMDCs.

The process of DC maturation is facilitated by multiple signaling transductions, such as JAK-STAT3, NF-κB, MAPK, and Hippo-Mst pathways47–49. Although OPTN was reported to suppress TNF-mediated NF-κB activation12, our data, however, demonstrated that Optn deficiency exerted no obvious effect on the NF-κB signaling in DCs, which was consistent with the previous report that Optn−/− did not affect NF-κB activation in BMDCs50. Our results revealed that JAK2-STAT3 signaling pathways were strongly changed in LPS stimulated Optn deficient BMDCs. In parallel, it has been reported that cyclic helix B peptide inhibited TLR activation induced DC maturation by upregulating the suppressor of cytokine-signaling-1 (SOCS1) through JAK2-STAT3 signaling51. SOCS1 is the most potent JAK2 inhibitor through binding to the tyrosine residue (Y1007) in the activation loop of JH1 and recruiting the E3 ubiquitin ligase scaffold (Cullin5) to catalyze the ubiquitination/degradation of activated JAK252–54. Here, we reported that OPTN also acted as a negative feedback inhibitor of JAK2 by directly binding to its JH1 domain. Moreover, such binding enabled OPTN to inhibit the interaction of STAT3 with JAK2. This finding accords with previous reports that TBK1, which could bind to and phosphorylated OPTN55, directly phosphorylated STAT3 at serine 754 in the transactivation domain upon cytosolic DNA stimulation56. However, whether TBK1, OPTN, and JAK2 could form a complex is still unknown. Furthermore, consistent with the previous study showing that STAT3 deletion in DCs breaks immune tolerance and causes T-cell activation in mice, STAT3 inhibitor static rescued the expression of CD80 and CD86 in Optn deficient BMDCs19,33,37.

Aberrant JAK2 activation induces abnormal haematopoiesis58, which can nonetheless be utilized to ameliorate inflammatory diseases. For instance, JAK2-STAT3 activation is linked to improved cardiac remodeling and reduced myocardial apoptosis in the metformin-treated type 2 diabetic model59. The phosphorylation of JAK2-STAT3 could also inhibit inflammation in non-alcoholic steatohepatitis60. Our study found that JAK2 activation, mediated by OPTN deprivation, effectively promoted the function of JAK2 in the immune system while suppressing DC activity.

It is worth noting that the relationship between TLRs and JAK2-STAT3 signaling pathway is complicated. On one hand, TLR regulates JAK/STAT pathway, as it has been reported that LPS treatment can activate JAK2 and STAT352. On the other hand, STAT3 could downregulate TLR ligand-mediated gene expression, despite unclear mechanisms62. In the present study, we found that the levels of p-JAK2 and p-STAT3 were elevated 16 h after LPS stimulation (50 ng/mL), which may be related to the release of IL-6 and IL-10 by LPS-TLR4 pathway. In union, we conjectured that OPTN-regulated JAK2/STAT3 activation and DC maturation through the direct interaction with JAK2 instead of TLR4 signals.

Natural product SSD, with its anti-inflammatory and immune-modulatory properties, is one of the main bioactive components in traditional Chinese medicine, Bupleurum falcatum L63. Our data showed that SSD could effectively block OPTN expression through interfering with its stability, consistent with previous reports that certain compounds bound to a protein to induce its misfolding or degradation64. Furthermore, we found that SSD specifically depends on OPTN to modulate DC maturation and EAE progression, potentially representing an OPTN targeting drug for autoimmune disorders.

In conclusion, we demonstrated a critical role of OPTN in regulating DC maturation and hence autoimmune responses (Supplementary Fig. 8). Inhibition of OPTN via genetic down-regulation in CD11c+ cells can activate the JAK2-STAT3 signaling by extricating JAK2 and facilitating its dimerization, thus motivating the transcription function of STAT3, expediting the expression of II-10, and finally impeding DC maturation and EAE injury. Together with the fact that pharmacological inhibition of OPTN by SSD can also prevent the progression of the experimental autoimmune disease, we highlight potentially promising DCs based immunotherapy for the treatment of autoimmune disease.

**Methods**

**Mice.** C57Bl/6 mice were obtained from Beijing Vital River Laboratory Animal Technology Co., Ltd (stock: 219). CD11c-Cre mice were acquired from the Jackson Laboratory (stock: 008088). OT-II mice were gifted by Prof. Lie Wang65. Optn+/+ mice were gifted by Prof. Ronggui Hu66. The DCs specific Optn-null mice were generated by breeding Optn−/− mice with CD11c-cre transgenic mice (Optn+/−; CD11c-Cre, Optn−/−). Stat3−/− mice were obtained from the Shanghai Model Organisms Center, Inc (stock: NM-CKO-200050), and intercrossed with OptnCreCD11c mice to generate Optn+/+, Stat3−/−, CD11c-cre (DKO) mice. All mice were housed in specific pathogen-free environment at 21 ± 1 °C and 60 ± 5% humidity, with a 12-h light/dark cycle. Experimental and control animals were bred separately. All mice were used at 6–8 weeks old, and euthanized with carbon dioxide. All animal use and studies were performed in compliance with all relevant ethical regulations, and were approved by the Institutional Animal Care and Use Committee (IACUC) at Zhejiang University.

**Human materials and MoDCs culture.** Blank human peripheral blood was collected from the Second Affiliated Hospital of School of Medicine, Zhejiang University. Samples from healthy people who showed no history of major diseases and had normal biochemical indicators and blood routine after physical examination were involved in this cohort. Volunteers provided informed consent for this study. All experiments were approved by the Human Subject Research Ethics Committee of the Second Affiliated Hospital of School of Medicine, Zhejiang University (No. Yan2016-003).

For human monocyte-derived-dendritic cells (MoDCs) culture, peripheral blood mononuclear cells (PBMCs) were isolated from blood through Ficoll-Hypaque (Mediatech Cellgro) density gradient centrifugation. Monocytes were purified from PBMCs using anti-CD14 microbeads (Miltenyi Biotech) and introduced into MoDCs in the presence of hGM-CSF (30 ng/mL; Peprotech) and hIL-4 (10 ng/mL; Peprotech). Half medium was replaced by fresh medium with GM-CSF and IL-4 at day 3 and day 5, and matured by LPS (300 ng/mL; Sigma-Aldrich) for 8 h at day 7.

**Cell isolation and culture.** For bone marrow-derived dendritic cells (BMDCs) preparation, bone marrow from 6 to 8-week-old C57Bl/6 mice was cultured in RPMI-1640 medium (Gibco) supplemented with 10% fetal bovine serum (FBS)
(Gibco), recombinant mouse GM-CSF (50 ng/mL; Peprotech), and IL-4 (20 ng/mL; Peprotech). Half medium was replaced by fresh medium with GM-CSF at day 3. BMDMs were then stimulated with LPS (50 ng/mL; Sigma-Aldrich) for M1 polarization, or IL-4 (20 ng/mL; Peprotech) for M2 polarization for 24 h at day 6. CD11c+ DCs were isolated from the spleen using CD11c positive selection kit (STEMCELL Technologies), and CD4+ T cells were isolated from the lymph nodes using CD4 positive selection kit (STEMCELL Technologies).

**Cell transfection.** Transient transfection was performed using Lipofectamine 2000 (Invitrogen), according to the manufacturer’s instructions, in 60-mm dishes with 2–3 μg of total DNA per transfection. HA-tagged OPTN and OPTN plasmids were transfected by Pro. Ronggui Hu. FLAG-tagged JAK1, FLAG-tagged JAK2, and FLAG-tagged JAK3 plasmids were obtained from OriGene. The truncation mutations of JAK2 were generated by PCR and subcloned into pCMV-Flag (Sigma-Aldrich). OPTN[ΔUBD] plasmid was constructed by GenScript. The siRNAs of mouse OPTN (S-5′-CUG UGU UUG AAG AUA CATT-3′) were synthesized by GenePharma. pLenti-GFP-mCherry-LC3B plasmid was obtained from Applied Biological Materials Inc.

**Drug screening.** HEK293 were cultured in DMEM medium (Gibco) supplemented with 10% fetal bovine serum (FBS) (Gibco), recombinant mouse M-CSF (50 ng/mL; Peprotech). All supernate was replaced by fresh medium with M-CSF at day 3. BMDMs were then stimulated with LPS (50 ng/mL; Sigma-Aldrich) for M1 polarization, or IL-4 (20 ng/mL; Peprotech) for M2 polarization for 24 h at day 6.

**Flow cytometry.** The phenotypes of DCs and BMDMs were determined by flow cytometry. For cell surface staining, single-cell suspensions were incubated with PE-cy7-anti-CD11c (117308, 0.2 μg/mL), FITC-anti-CD11b (104076, 0.2 μg/mL), PE/Cy7-anti-CD68 (105014, 0.2 μg/mL), FITC-anti-MHC-I (114606, 0.2 μg/mL), PerCP/Cy5.5-anti-MHC-II (107626, 0.2 μg/mL), FITC-anti-F4/80 (123108, 0.2 μg/mL), PerCP/Cy5.5-anti-CD11b (101228, 0.3 μg/mL), PE-anti-CD40 (141706, 0.2 μg/mL), APC-anti-CD115 (135509, 0.4 μg/mL), FITC-anti-CD11c (107709, 0.3 μg/mL) (eBioscience), anti-CD44 (100019, 0.05 μg/mL), PE-anti-CD26L (104047, 0.2 μg/mL) (all purchased from BioLegend). Samples were washed and then analyzed by FACS versus flow cytometry (BD Biosciences).

For intracellular staining of cytokines, single-cell suspensions from DLN and spleen of the indicated EAE mice were stained with FITC-anti-CD4 (553047, BD Biosciences, 0.2 μg/mL) first, followed by staining with PE-anti-IFN-γ (554412, BD Biosciences, 0.3 μg/mL), PE-anti-IL17A (506904, BioLegend, 0.3 μg/mL) and PE-anti-IL4 (72-7041-82, eBioScience, 0.4 μg/mL) using Cytofix/Cytoperm kit (BD Biosciences) according to the manufacturer’s protocol. Intracellular staining with PE-anti-Foxp3 (126404, BioLegend, 0.4 μg/mL) was performed using a Fixation/Permeabilization kit (eBioScience) according to the manufacturer’s protocol.

**Western blotting.** Protein extracts were subjected to SDS-PAGE (8–12% gels) and blotted onto PVDF membranes. After blocking with 5% fat-free milk, the membranes were incubated with the following antibodies at 4°C overnight: anti-OPTN (sc-166576, 1:1000), anti-IL-12Rb1 (sc-293072, 1:1000), and anti-Lamin B (sc-6216, 1:1000) were purchased from Santa Cruz; anti-JAK2 (92320, 1:1000), anti-p-JAK2 (Tyr1007/1008) (3771, 1:1000), anti-STAT3 (91439, 1:1000), anti-p-STAT3(Y705) (9145, 1:1000), anti-MyD88 (84283, 1:1000), anti-p-STAT3 (9193, 1:1000), anti-ΔCD11c mice-derived CD4+ T cells were incubated with PE-anti-IFN-γ (554412, BD Biosciences, 0.3 μg/mL), PE-anti-IL17A (506904, BioLegend, 0.3 μg/mL) and PE-anti-IL4 (12-7041-82, eBioScience, 0.4 μg/mL) using Cytofix/Cytoperm kit (BD Biosciences) according to the manufacturer’s protocol. The bound antibodies were detected using horseradish peroxidase (HRP)-conjugated IgG (MULTI Sciences) and visualized with enhanced chemiluminescence (ECL, PerkinElmer) detection reagents (Thermo Scientific, USA). GAPDH or Lamin B was used as a loading control. Images were taken by GE AL600.

**Co-immunoprecipitation.** After transfection, HEK293 cells were lysed in lysis buffer (25 mM Tris (pH 8.0), 150 mM NaCl, 1% NP40) supplemented with protease inhibitor cocktail. Whole-cell lysates were incubated with the indicated tag-beads for 2 h. The beads were washed using wash buffer (25 mM Tris (pH 8.0), 150 mM NaCl, 0.2% NP40) for at least five times, and then boiled in SDS loading buffer before immunoprecipitation protein complex complexes. The protein complexes were analyzed by western blotting. Anti-HA magnetic beads were bought from Bimake. Anti-HA and Anti-Flag antibodies were bought from Santa Cruz. For co-immunoprecipitation of endogenous interaction between OPTN and JAK2, whole-cell lysates were incubated with OPTN (Santa Cruz) and control IgG (Santa Cruz), followed by immunoprecipitation using Protein A/G beads (Santa Cruz) and detection of JAK2 using JAK2-specific antibodies.

**RNA extraction and quantitative real-time PCR.** Total RNA was isolated from cells with Trizol reagent (Invitrogen), cDNA was transcribed using Transcript script kit (TransGen Biotech). qRT-PCR analysis was performed using the SYBR Green (Bio-Rad) method on the ABI Fast 7500 real-time PCR instrument (Perkin-Elmer Applied Biosystems). The gene expression was normalized to the expression of the internal reference gene using the 2−ΔΔCT method.

**Histological staining.** Spinal cords collected from PBS perfused EAE mice were fixed in 4% paraformaldehyde (PFA) overnight. For luxol fast blue (LFB) staining, tissues were embedded in OCT (SAKURA Finetek) and cut into 12-μm sections. Slides were stained with LFB solution (Sigma-Aldrich) overnight, rinsed with distilled water, and differentiated with 0.05% lithium carbonate solution followed by 70% ethanol. Differentiation was stopped by rinsing in distilled water. Liver, lung, and spleen from control and OPTN[ΔUBD] mice were fixed in 4% PFA overnight, then embedded in paraffin and cut into 8-μm thick. Slides were deparaffinized and stained with hematoxylin and eosin (HE) for evaluation of inflammation.

**Immunofluorescence staining.** Spinal cord sections were permeabilized in PBS containing 0.5% Triton X-100 for 10 min at room temperature. Then primary antibodies of CC1 (Oncogene Research) were incubated at 4°C overnight. Subsequently, slides were incubated with Alexa Fluor 488-coupled secondary antibodies (A-21202, Life Technologies, 1:1000). The nuclei were counterstained with DAPI. Images were taken with Leica TCS SP5 microscope.

**Fluo cytometry.** After 4 h incubation, cells were incubated with Alexa Fluor 488-coupled secondary antibodies (A-21103, Life Technologies, 1:1000) or 688-coupled secondary antibodies (A-21909, Life Technologies, 1:1000). The nuclei were counterstained with DAPI. MoDCs and pLenti-GFP-mCherry-LC3B transfected BMDMs were imaged by Leica SP8 microscope.
Statistical comparisons were performed using GraphPad Prism software (version 8.0.1) and Image J (version 1.8.0). Unpaired two-tailed Student’s t-test or one-way ANOVA Tukey’s post hoc analysis was performed to determine statistical significance between two samples or for multiple comparisons, respectively. All data are from at least three independent experiments and presented as means ± SD. The significance was considered to be when p values were <0.05 (represented as *p < 0.05, **p < 0.01, ***p < 0.001, or not significant (NS)).

**Reporting summary.** Further information on research design is available in the Nature Research Reporting Summary linked to this article.

**Data availability.** The LPS stimulated human MoDCs array analysis data downloaded and used in Fig. 1a are available in the NCBI Gene Expression Omnibus (GEO) database under accession code GSE2706. The STAT3 genome binding/occupancy profiling of splenic DCs data downloaded and used in Fig. 7 and Supplementary Fig. 6 are available in the NCBI GEO database accession code GSE27161. The reporting summary for this Article is available in the Supplementary Information file. All the other data supporting this study are available within this Article, Supplementary Information, Source data, or from the corresponding authors upon reasonable requests. Source data are provided with this paper.

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Author contributions
Q.J.W. and J.J.W. conceived and designed the study. J.J.W., J.Y.W., W.X.H., L.L.Z., L.Q.S., C.Y.F., and Y.P.Q. performed the experiments. J.J.W., J.Y.W., W.X.H., Q.S., Y.F.S., L.I.Y., Z.X.Y., and J.C.W. performed the data analysis. J.J.W., J.Y.W., W.X.H., Q.J.W., B.Y., and Q.I.H. contributed to writing the manuscript. G.F.H. and J.C. gave some critical advices. Q.I.W., B.Y., Q.I.H., Y.F.S., and G.F.H. contributed to the materials. All the authors read and approved the final version of the manuscript.

Competing interests
The authors declare no competing interests.

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