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p38α signaling programs dendritic cells to drive T_H17 cell differentiation and autoimmune inflammation

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Abstract

Dendritic cells (DCs) bridge innate and adaptive immunity, but how DC-derived signals regulate T cell lineage choices remains unclear. We report that p38α MAP kinase programs DCs to drive T_H17 differentiation. Deletion of p38α in DCs, but not macrophages or T cells, protects mice from T_H17-mediated autoimmune neuroinflammation. p38α orchestrates expression of cytokines and co-stimulatory molecules in DCs, and further imprints T cell IL-23R signaling to promote T_H17 differentiation. Moreover, p38α is required for tissue-infiltrating DCs to sustain T_H17 responses. This activity of p38α is conserved between mouse and human DCs, and is dynamically regulated by pattern recognition and fungal infection. Our results identify p38α as a central pathway to integrate instructive signals in DCs for T_H17 differentiation and inflammation.

Keywords

T_H17 cell; dendritic cell; MAPK; autoimmune disease; fungal infection

INTRODUCTION

Dysregulation of T cell responses is the cause of autoimmune disorders. T_H17 cells, a recently identified lineage of $CD4^+$ effector T cells, play a key role in the pathogenesis of many autoimmune conditions, including multiple sclerosis (MS), an inflammatory demyelinating disease of the central nervous system (CNS), and its murine model experimental autoimmune encephalomyelitis (EAE). While the involvement of T cell-

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AUTHOR CONTRIBUTIONS

G.H. designed and performed cellular and molecular experiments and *in vivo* models, and contributed to writing the manuscript; Y.W. designed and performed biochemical and gene expression analyses and contributed to cellular experiments; P.V. contributed to histopathology analysis; T.D.K. contributed to infectious models; K.O. contributed mouse models; H.C. designed experiments, wrote the manuscript, and provided overall direction.

intrinsic pathways in T_H17 differentiation has been described abundantly[1], how T_H17 development is triggered by extrinsic pathways and physiological and pathological stimuli remains poorly understood.

Dendritic cells (DCs) are the most important antigen-presenting cells (APCs) to bridge innate and adaptive immunity by triggering activation and differentiation of naïve T cells[2]. DCs express a repertoire of pattern recognition receptors (PRRs) that sense microbial pathogen products and endogenous ligands to initiate a signaling cascade culminating in the activation of DCs and induction of adaptive immunity. Toll-like receptors (TLRs) and downstream Myd88 signaling typically induce IFN-γ-producing T_H1 cells[2]. A distinct group of PRRs, the C-type lectin-like receptors (CLRs), are coupled to Syk-CARD9 signaling and preferentially polarize a T_H17 response[3, 4]. While CLR-mediated T_H17 responses are important for the protective anti-fungal immunity, little evidence exists for the involvement of CLR signaling in autoimmune diseases. Consequently, how the diverse innate immune signals are sensed and transduced to shape T_H17 responses and autoimmune pathogenesis remains to be established. Moreover, DCs are recruited into CNS in EAE to reactivate primed myelin-reactive T cells, thereby propagating local inflammation and disease development during EAE[5, 6]. The function mediated by CNS DCs is associated with their ability to mediate a T_H17-polarized response[7], but how this process is regulated is poorly defined.

Among the central pathways activated by innate signals are the MAP kinases (MAPKs). MAPKs, comprised of Erk, Jnk and p38, mediate an evolutionarily conserved mechanism for cellular responses to extracellular signals[8]. Excessive activation of MAPKs is associated with autoimmune diseases, and inhibitors of these pathways have been evaluated as new therapeutic strategies[9]. Despite the rapid and reversible actions, the nonspecific effects inherent in drug inhibitors make these approaches unlikely to provide definitive mechanistic insights[10]. Instead, genetic dissection has been highly instrumental in our understanding of the specific roles of MAPKs in the immune system. Recent work has identified functions of p38α, the most widely expressed isoform among p38 family members, in T cell intrinsic responses[11, 12], including an involvement in T_H17 differentiation[13–17]. Tissue-specific deletion in macrophages and epithelial cells also demonstrates that p38α regulates innate responses and acute inflammation[18, 19]. However, it remains unknown whether p38α regulates the crosstalk between innate and adaptive immunity, and whether this function is important for the pathogenesis of autoimmune disorders.

Here we report that p38α acts in DCs to drive T_H17 differentiation and inflammation. p38α in DCs mediates reciprocal regulation of interleukin 6 (IL-6) and IL-27, arguably the most potent positive and negative regulators of T_H17 polarization, respectively[1, 20, 21], and further imprints IL-23 receptor (IL-23R) signaling in responding T cells. Not only is p38α signaling in DCs essential for the induction of T_H17 differentiation, it is also required for CNS-infiltrating DCs to maintain the T_H17 population at sites of inflammation. Furthermore, in contrast to previous conclusions obtained from pharmacological and dominant negative approaches[13–17], we exclude a significant involvement of T cell-intrinsic p38 signaling in T_H17 differentiation. Altogether, our studies identify a key mechanism of DC-mediated programming of T_H17 differentiation.

RESULTS

Deletion of p38α in DCs protects mice from EAE

Although p38α is a major drug target for inflammatory disorders, the specific role for p38α in autoimmune diseases remains poorly understood. In mice that developed EAE following immunization with myelin oligodendrocyte glycoprotein peptide ($MOG_{35–55}$), activity of p38 was markedly elevated in myeloid cells infiltrating the spinal cord, as compared with CNS resident cells (Fig. 1a). To determine the role of p38 in EAE, we crossed mice bearing the floxed p38α allele with Rosa26-Cre-ER^{T2} mice (in which a Cre-ER fusion gene was recombined into the ubiquitously expressed Rosa26 locus) to generate $p38\alpha^{fl/fl}$Rosa26-Cre-ER^{T2} mice (called "$p38\alpha^{CreER}$ mice" thereafter). After tamoxifen-mediated deletion of the p38α gene, we immunized mice for the induction of EAE. As compared with wild-type controls (including Cre^+ mice) that developed a severe disease, $p38\alpha^{CreER}$ mice were completely resistant to EAE (Fig. 1b).

Development of EAE is dependent upon multiple components of the immune system. Aside from T cells, macrophages and DCs contribute to the pathogenesis by functioning as APCs as well as effector cells to cause CNS lesion. In these three cell types, p38α was expressed much higher than the other p38 isoforms (Supplementary Fig. 1a–c). To dissect the selective roles of p38α in the different immune compartments, we deleted p38α in T cells, macrophages and monocytes, and DCs by crossing $p38\alpha^{fl/fl}$ mice with CD4-Cre, LysM-Cre, and CD11c-Cre mice (called $p38\alpha^{\Delta T}$, $p38\alpha^{\Delta Mac}$ and $p38\alpha^{\Delta DC}$ mice, respectively). Efficient deletion of p38α was observed in these immune compartments (Supplementary Fig. 1d–g). In particular, deletion of p38α abrogated the expression and phosphorylation of p38 in DCs (Supplementary Fig. 1h), indicating that the total p38 activity in DCs is predominantly ascribed to p38α. No obvious defects in the development or homeostasis of the immune system were noticed in these mice (Supplementary Fig. 2 and data not shown). Following MOG immunization, $p38\alpha^{\Delta T}$ and $p38\alpha^{\Delta Mac}$ mice showed comparable disease development as wild-type mice (Fig. 1c,d). In contrast, $p38\alpha^{\Delta DC}$ mice exhibited marked reduction of disease progression (Fig. 1e). Histological analysis revealed significantly decreased inflammation and demyelination (as revealed by H&E and Luxol fast blue staining, respectively), associated with fewer numbers of $CD3^+$ T cells, $Iba1^+$ macrophages and microglia and $GFAP^+$ astrocytes in the CNS of $p38\alpha^{\Delta DC}$ mice (Fig. 1f,g). Flow cytometry analysis confirmed the reduced infiltration of $CD4^+$ T cells and $CD11b^+$ myeloid cells into the spinal cord of $p38\alpha^{\Delta DC}$ mice (Fig. 1h). Thus, p38α activity in DCs is required to precipitate the autoimmune CNS disease.

p38α is required in DCs for the generation of T_H17 cells *in vivo*

While EAE was classically considered as a T_H1-dependent disease, recent studies have identified a key role for T_H17 cells in disease development. CNS infiltrating T cells from $p38\alpha^{\Delta DC}$ mice had decreased *Il17a*, *Il17f* and *Il23r* mRNA expression, but normal levels of *Ifng*, *Foxp3* and *Il4*, at the peak of disease (Fig. 2a). Intracellular staining revealed lower frequency of IL-17^+ $CD4^+$ T cells in CNS from $p38\alpha^{\Delta DC}$ mice, whereas the percentages of IFN-γ^+ and $Foxp3^+$ $CD4^+$ T cells were comparable between the two groups of mice (Fig. 2b).

To test whether the diminished T_H17 generation was secondary to attenuated CNS inflammation, we analyzed T cell responses in the draining lymph nodes (LNs) at the preclinical stage of EAE (day 7 post-immunization). T cells from p38α$^{\Delta DC}$ mice expanded normally after *ex vivo* MOG stimulation (data not shown) but were deficient in IL-17 secretion (Fig. 2c). Also, there was a reduced frequency of IL-17$^+$ cells among the CD4$^+$ T cells that had proliferated in response to MOG stimulation (CFSE^{lo} cells) in immunized p38α$^{\Delta DC}$ mice in comparison to wild-type mice (Fig. 2d). These results identify a key role for p38α in DCs to promote MOG-induced T_H17 responses *in vivo*.

To test if the requirement for DC-derived p38α signals is restricted to MOG-induced responses, we immunized wild-type and p38α$^{\Delta DC}$ mice with the protein antigen keyhole limpet hemocyanin (KLH). Draining LN cells from p38α$^{\Delta DC}$ mice had no defects in proliferation (data not shown) but showed decreased IL-17 expression in the recall response (Fig. 2e). We also examined the IL-17$^+$ CD4$^+$ T cells in several gut-associated lymphoid tissues (GALTs) that are known to contain a sizable population of T_H17 cells in response to commensal microbiota[22]. CD4$^+$ T cells from p38α$^{\Delta DC}$ mice contained a significantly decreased percentage of IL-17$^+$ cells but normal percentage of IFN-γ$^+$ cells (Fig. 2f). We conclude that p38α signaling in DCs is crucial to promote development of T_H17 responses *in vivo*.

p38α in DCs impacts IL-23R expression in responding T cells

These results prompted us to examine whether p38α mediates the DC–T cell crosstalk by driving lineage differentiation of antigen-specific naïve precursors. To test this, we transferred naïve T cells (CD62L^{hi}CD44^{lo}; Thy1.1$^+$) from 2D2 TCR-transgenic mice (specific for $MOG_{35–55}$)[23] into wild-type and p38α$^{\Delta DC}$ mice, followed by immunization with the cognate antigen emulsified in complete Freund's adjuvant (CFA). Donor T cells isolated from p38α$^{\Delta DC}$ hosts contained fewer IL-17$^+$ cells and expressed less *Il17a* mRNA in the recall response, whereas IFN-γ expression was unaltered (Fig. 3a,b). We further used T cells from a second TCR-transgenic model – the OT-II TCR-transgenic mice (specific for $OVA_{323–339}$). Donor T cells development into T_H17 cells was decreased in p38α$^{\Delta DC}$ hosts relative to wild-type hosts in response to OVA immunization in the presence of CFA (Fig. 3c) or incomplete Freund's adjuvant (IFA) (Supplementary Fig. 3). Therefore, deletion of p38α in DCs impairs differentiation of antigen-specific T_H17 cells *in vivo*.

We next co-cultured naïve OT-II T cells with splenic DCs in the presence of antigen and LPS, but without any exogenous cytokines, to model the physiological interaction between DCs and T cells. T cells stimulated with p38α$^{\Delta DC}$ DCs contained a significantly lower frequency of IL-17$^+$ cells (Fig. 3d). This was associated with reduced *Il17a* mRNA expression (Fig. 3e) and impaired upregulation of the chemokine receptor CCR6, a selective surface marker for T_H17 cells, but not of the T_H1-specific receptor CXCR3 (Supplementary Fig. 4a). Similar defect was observed when a synthetic ligand for TLR2 was used instead of LPS (Supplementary Fig. 4b,c). Importantly, diminished T_H17 differentiation was observed in different DC subsets lacking p38α, with the CD11b$^+$ DC subset exhibiting a greater defect (Fig. 3f). We noticed that IFN-γ expression in OVA-specific T cells was modestly upregulated by incubation with p38α$^{\Delta DC}$ DCs *in vitro* (Fig. 3d–f). To test whether defective

T_H17 differentiation was due to IFN-γ upregulation, we added a neutralizing anti-IFN-γ antibody. This resulted in an expected overall increase of IL-17 expression, but T cells stimulated with p38α$^{\Delta DC}$ DCs remained deficient in T_H17 differentiation (Fig. 3g). Moreover, lower IL-17 production was also observed in IFN-γ-deficient T cells activated with p38α$^{\Delta DC}$ DCs (Fig. 3h), thereby excluding a contribution from IFN-γ production to the p38α-dependent T_H17 differentiation. Altogether, p38α has a direct role in mediating DC–T cell crosstalk for T_H17 differentiation.

We next determined the mechanisms by which p38α induces T_H17 differentiation following DC-T cell interaction. T cell proliferation and IL-7Rα expression (which is important for T_H17 expansion[24]) were undisturbed when cultured with p38α$^{\Delta DC}$ DCs (data not shown). Expression of T_H17-associated factors was examined by real-time PCR analysis of DC-activated T cells. T cells cultured with p38α$^{\Delta DC}$ DCs showed substantial reduction of the T_H17 family cytokines including *Il17a*, *Il17f*, *Il21* and *Il22*, and the transcription factors *Rorc* (encoding RORγt) and *Rora* during the differentiation process (Fig. 3i). Development of T_H17 cells requires IL-23R, which is induced by T_H17-polarizing cytokines and confers enhanced responsiveness to IL-23 to facilitate T_H17 terminal differentiation and expansion[25–27]. T cells activated with p38α$^{\Delta DC}$ DCs were impaired to induce *Il23r* expression, whereas expression of other receptor chains including *Il12rb1*, *Il12rb2* and *Il6ra* was unaltered (Fig. 3j). To examine the role of IL-23R in mediating DC-dependent T_H17 response, we first used an IL-23R blocking antibody and found that it diminished IL-17 expression in a dose-dependent manner in T cells stimulated with wild-type DCs (Supplementary Fig. 5). To further determine whether decreased induction of IL-23R contributed to the T_H17 phenotype mediated by p38α$^{\Delta DC}$ DCs, we introduced IL-23R into T cells by retroviral transduction. Overexpression of IL-23R increased *Il17a* expression in T cells activated with both wild-type and p38α$^{\Delta DC}$ DCs when compared with control virus-transduced cells, however the difference between wild-type and p38α$^{\Delta DC}$ DCs was only partially rescued (Fig. 3k). These results indicate that induction of IL-23R expression in responding T cells may represent one mechanism by which p38α mediates the DC–T cell crosstalk, although additional mechanisms are likely to play a role.

p38α affects IL-6, IL-27 and CD86 expression in DCs

We explored the cellular mechanisms by which p38α acts in DCs to regulate T_H17 differentiation. To determine whether p38α regulates DC cytokine expression, we isolated DCs from mice at day 7 after immunization. Among the cytokines known to potentiate T_H17 differentiation, *Il6* was selectively decreased in splenic DCs from p38α$^{\Delta DC}$ mice after MOG immunization (Fig. 4a). This selective reduction of IL-6 was also observed in DCs after *in vivo* and *in vitro* stimulation with LPS (Fig. 4b,c). Accordingly, phosphorylation of STAT3 but not STAT4 was reduced in T cells activated with p38α$^{\Delta DC}$ DCs (Fig. 4d). Therefore, p38α activates the IL-6-STAT3 axis at the DC–T cell interface.

We tested the functional significance of p38α-dependent IL-6 expression. IL-6-deficient DCs, similar to p38α-deficient DCs, showed impaired ability to drive *Il17a* and *Il23r* expression in T cells (Supplementary Fig. 6a). Addition of IL-6 to p38α$^{\Delta DC}$ DCs nearly completely restored the defective *Il23r* expression in T cells (Fig. 4e), and increased the

frequency IL-17$^+$ cells in co-cultured T cells, although the rescue of IL-17 expression was not complete (Fig. 4f). When compared with IL-6-deficient DCs, DCs deficient in both IL-6 and p38α exhibited modestly reduced ability to induce *Il17a* expression (Supplementary Fig. 6b). Therefore, p38α-dependent IL-6 expression in DCs mediates T_H17 generation, although additional mechanisms seem to contribute as well.

T_H17 differentiation is shaped by both positive and negative polarizing cytokines. IL-27 is a potent cytokine to limit T_H17 differentiation[20, 21], whose expression can be repressed by osteopontin (encoded by *Spp1*) in DCs[28, 29]. Compared to wild-type cells, *Il27* expression was increased and *Spp1* was decreased in p38α-deficient DCs following MOG immunization (Fig. 4g). Although IL-27 can activate multiple STAT proteins, blocking IL-27 substantially restored the defective STAT3 activation in T cells induced by p38α$^{\Delta DC}$ DCs (Fig. 4h), consistent with the contrasting effects of IL-27 and IL-6 on T_H17 development[20, 21]. Further, whereas exogenous IL-6 and blockade of IL-27 each had a modest effect to promote *Il17a* expression mediated by p38α$^{\Delta DC}$ DCs, a combination of both considerably restored T_H17 differentiation (Fig. 4i). In addition to affecting cytokine production, p38α was required for LPS-induced expression of the DC co-stimulatory molecule CD86, but not CD80 (Fig. 4j). Blocking CD86 function in wild-type DCs diminished T_H17 differentiation but did not significantly affect IFN-γ production (Fig. 4k and Supplementary Fig. 7), which was in agreement with a role for CD86 in T_H17 responses[30, 31]. Collectively, these data illustrate that p38α exerts opposing effects on the positive and negative regulators of T_H17 generation and further shapes the strength of the co-stimulatory signals, thereby orchestrating a program for DC-dependent T_H17 differentiation.

DCs integrate T_H17-instructive signals through p38α

We next investigated the nature of the upstream signals that induced p38α activation in DCs to instruct T_H17 differentiation. Among PRRs, TLRs and CLRs mediate strong T_H1 and T_H17 responses, respectively[2, 3, 32]. Curdlan (an agonist for Dectin-1, a CLR that recognizes β-glucans) had a stronger *in vivo* adjuvant activity for the T_H17 response (Fig. 5a) and induced greater upregulation of p38 activity in DCs (Fig. 5b) when compared with LPS. Deficiency of p38α in DCs diminished T_H17 differentiation mediated by both LPS and curdlan, with a stronger impact on curdlan-induced differentiation (Fig. 5a). Following curdlan stimulation *in vivo*, antigen-specific donor T cells from p38α$^{\Delta DC}$ hosts showed reduced expression of *Il17a*, *Il17f*, *Il21*, *Il22*, *Rorc* and *Il23r* (Fig. 5c), indicating that p38α mediates CLR-induced T_H17 differentiation signals in DCs.

We further compared the effects of different pathogens on p38 activation in DCs and T_H17 differentiation. Prototypic bacteria such as Gram-positive *Listeria monocytogenes* and Gram-negative *Legionella pneumophila* are known to induce strong T_H1-polarized responses, whereas fungi (including *Candida albicans* and *Saccharomyces cerevisiae*) appear to be the most potent pathogens characterized to elicit T_H17 responses[33]. When the heat-killed bacteria and fungi (to avoid the variance due to differential pathogen clearance) were used as adjuvants to stimulate antigen-specific T cells *in vivo*, *C. albicans* and *S. cerevisiae* generated a much greater T_H17 response than *L. monocytogenes* and *L.*

pneumophila (Fig. 5d). This was associated with the ability of *C. albicans* and *S. cerevisiae* to strongly activate p38 in DCs (Fig. 5e). Notably, splenocytes from p38α$^{\Delta DC}$ mice produced lower amounts of IL-17 after infection with *C. albicans*, while IFN-γ expression was normal (Fig. 5f). Thus, fungal stimulation represents a robust activator of p38 in DCs for the induction of T_H17 responses.

In agreement with reports suggesting that activation of CD40 by T cell-derived CD40L signals can promote T_H17 differentiation[34, 35], we detected defective T_H17 differentiation induced by DCs deficient in CD40 (Supplementary Fig. 8a). Importantly, anti-CD40 strongly upregulated p38 activity in DCs (Supplementary Fig. 8b), and p38α deficiency in DCs diminished T_H17 differentiation following anti-CD40 stimulation (Supplementary Fig. 8c). We conclude that p38α is a convergent point in DCs to integrate infectious and inflammatory signals to instruct T_H17 differentiation.

p38α-dependent effector pathways in DC signal integration

We investigated p38α-dependent effector pathways that could mediate these diverse upstream inputs. p38α deficiency led to decreased IL-6 production from DCs stimulated with curdlan and anti-CD40 *in vivo* (Fig. 6a,b) and *in vitro* (Fig. 6c), while IL-1β and TGF-β1 levels were not altered. DC expression of *Il27* and *Spp1* was also affected by p38α deficiency after stimulation with LPS and curdlan, while their expression was independent of p38α in response to anti-CD40 (Supplementary Fig. 9). These results indicate that IL-6 is a shared target of p38α in DCs activated with different stimuli.

To examine the signaling and transcriptional pathways involved in IL-6 expression, we assessed activation of two known p38 targets, MAPKAP kinase 2 (MK2) and MSK1[8], and noted that it was diminished in LPS-stimulated p38α$^{\Delta DC}$ DCs as compared with wild-type cells (Fig. 6d). Additionally, activation of two transcription factors important for IL-6 production, CREB and c-Fos[36], was decreased, whereas phosphorylation of CCAAT/enhancer binding protein β (C/EBPβ) and IκBα (indicative of NF-κB activity) was not affected (Fig. 6d and Supplementary Fig. 10). MK2, MSK1, CREB and c-Fos activation was similarly diminished in p38α-deficient DCs after curdlan and anti-CD40 stimulation (Fig. 6e), indicating that a core set of molecular targets is activated by p38α in response to diverse upstream inputs. Further, treatment of wild-type DCs with an MK2 inhibitor reduced IL-6 production after stimulation with LPS, curdlan and anti-CD40 (Fig. 6f), and diminished their capacity to drive T_H17 generation after stimulation with LPS (Fig. 6g,h), curdlan or anti-CD40 (data not shown). Finally, p38α and MK2 were also required for IL-6 production in DCs following stimulation with heat-killed *C. albicans* (Supplementary Fig. 11). These results collectively establish a p38α-MK2 signaling axis to integrate upstream signals in DCs for T_H17 differentiation.

Effects of p38α in CNS DCs and therapeutic targeting

During EAE, DCs are recruited into the CNS where they present antigens to myelin-specific T cells and polarize the local T_H17 response[5, 7]. To test if CNS DCs require p38α to actively maintain the proinflammatory T_H17 response, we immunized p38α^{CreER} mice with MOG, and started tamoxifen treatment at day 7 to bypass the requirement of p38α in T cell priming

in the periphery. Such treatment markedly ameliorated disease severity (Fig. 7a) and inflammation and demyelination of CNS (Fig. 7b). DCs isolated from CNS following acute deletion of p38α were impaired to induce *Il17a* and *Il23r* expression from co-cultured MOG TCR-transgenic T cells *in vitro*, indicating a key function for p38α in CNS DCs to mediate T_H17 responses (Fig. 7c).

Using a T_H17-polarized transfer model of EAE[37], we next investigated the contribution of p38α in DCs to the effector phase of CNS inflammation. Upon adoptive transfer of T_H17 effector cells into recipient mice, wild-type recipients rapidly developed EAE, whereas $p38\alpha^{\Delta DC}$ mice showed delayed onset and reduced severity (Fig. 7d). Expression of *Il17a* and *Il23r* mRNA was lower in CNS-infiltrating cells from $p38\alpha^{\Delta DC}$ mice than wild-type mice (Fig. 7e). These results suggest a continuous requirement for p38α activity in DCs to sustain IL-23R expression and T_H17 responses at sites of inflammation.

To further evaluate the role of p38α in DCs as a therapeutic target for T_H17-mediated diseases, we investigated if these regulatory pathways apply to human cells as well. DCs derived from human peripheral blood monocytes were treated with a p38 inhibitor and then cultured with naïve T cells. Similar to the mouse DCs that were used as control (Fig. 7f), blocking p38 in human DCs diminished IL-17 expression from T cells (Fig. 7g,h), as well as IL-6 production from DCs (Fig. 7i). Thus, p38 activity represents an evolutionarily conserved pathway to shape DC-dependent T_H17 differentiation.

p38 in T cells is dispensable for T_H17 differentiation

Several recent studies suggest an important role of p38 in T cells during T_H17 generation[13–17]. Although deletion of p38α in T cells did not protect mice from EAE (Fig. 1c), we further tested whether p38 regulates T_H17 differentiation in a T cell-intrinsic manner. Naïve T cells from $p38\alpha^{\Delta T}$ mice differentiated normally into IL-17^+ cells under T_H17-polarizing conditions *in vitro* (Fig. 8a,b), despite the loss of p38 expression and phosphorylation in these cells (Supplementary Fig. 12a). To exclude compensation from other p38 family members, we generated mice lacking both p38α and p38β isoforms ($p38\alpha^{\Delta T}p38\beta^{KO}$), because these two p38 isoforms are detectable in T cells (Supplementary Fig. 1a). T_H17 differentiation *in vitro* was normal in T cells from $p38\alpha^{\Delta T}p38\beta^{KO}$ mice (Fig. 8c,d). In addition, $MOG_{35–55}$ peptide immunization of wild-type, $p38\alpha^{\Delta T}$, $p38\beta^{KO}$ and $p38\alpha^{\Delta T}p38\beta^{KO}$ mice followed by *ex vivo* recall response to antigen or polyclonal stimulation showed comparable levels of IL-17 expression in T cells (Fig. 8e and Supplementary Fig. 12b). In addition, transfer of naïve T cells from $p38\alpha^{\Delta T}p38\beta^{KO}$ OT-II TCR-transgenic mice into wild-type mice followed by OVA immunization showed that T cells lacking p38α and p38β were as efficient as wild-type cells in mounting IL-17 expression (Fig. 8f). Altogether, our results exclude a T cell-intrinsic function of p38 signaling in T_H17 differentiation.

DISCUSSION

How instructive signals derived from the innate immune system trigger T_H17 responses and inflammation, in contrast to T cell-intrinsic transcriptional mechanisms[1], remains poorly understood. Here we describe that p38α integrates a diverse array of immunostimulatory

signals in DCs to direct T_H17 generation under autoimmune, inflammatory and infectious conditions, thereby establishing a previously unappreciated pathway of DC-dependent programming of T_H17 differentiation. We show that p38α directs IL-6 and IL-27 and further shapes CD86 expression in DCs and imprints STAT3 signaling and IL-23R expression in responding T cells. Although p38α deficiency in DCs does not completely abrogate DC-dependent IL-17 production from T cells, the pathway has a critical role in disease pathogenesis and may represent an attractive therapeutic target, as both the induction and effector phases of autoimmune inflammation are highly dependent upon p38α activity in DCs.

Adaptive immunity is controlled by DC-derived signals at multiple checkpoints that dictate the activation and differentiation of T cell-mediated immune responses[2]. The few identified innate immune pathways that potentiate the T_H17 response, such as Dectin-Syk-CARD signaling[3, 4], transduce a specific type of pathogen-derived signals. In addition, their involvements in autoimmune diseases are unclear. Additional DCs signaling pathways, such as MKP-1 and Wnt-β-catenin signaling, have been shown to downregulate T_H17 differentiation, although they also influence multiple other T cell lineages[32, 38]. Here we show that p38α is potently activated in DCs by T_H17-instructive innate signals via engagement of PRRs, as well as T cell-dependent CD40 signals, to induce IL-6 production. Moreover, p38α regulates IL-27 downstream of innate (TLR and CLR) but not adaptive (CD40) signals, and further shapes co-stimulatory signals. Therefore, p38α orchestrates a program for DC-dependent T_H17 differentiation under inflammatory conditions, although its role under steady state requires additional testing.

DCs are sparse in the healthy CNS, but are markedly increased in number during CNS inflammation. Whereas CNS DCs have been implicated in driving T_H17 responses and precipitating inflammation[5–7], opposing evidence also exists[39, 40]. Among distinct subsets of DCs, the $CD11b^+$ myeloid DCs are most efficient at driving T_H17 differentiation in the CNS[7]. Consistent with this observation, we found that $CD11b^+$ DCs are strongly dependent on p38α activity to direct T_H17 differentiation as compared with $CD8^+$ DCs. By identifying a molecular pathway in CNS DCs to drive T_H17 responses and disease pathogenesis, our findings provide critical genetic evidence supporting the key proinflammatory function of CNS DCs *in vivo*.

p38 has been shown to act in a T cell-intrinsic manner for T_H17 development, as IL-17 expression was diminished after pharmacological inhibition of p38 or expression of a dominant negative p38 transgene[13–17]. However, by using T cell-specific deletion of p38α in multiple *in vitro* and *in vivo* systems, and after excluding genetic redundancy among the two p38 isoforms expressed by T cells (p38α and p38β), we found no evidence for a T cell-intrinsic function of p38 in T_H17 responses. The function of p38 described in T cells previously could result from nonspecific actions of the experimental approaches, or the inability to distinguish the effects in T cells from those in APCs. Our results support a selective role of p38α in DC-mediated, but not T cell-intrinsic, T_H17 differentiation, although our results do not exclude the function of p38 in T cells under other conditions[11, 12]. Moreover, p38α activity in other cell types also contributes to EAE, as

systemic ablation but not DC-specific deletion of p38α results in complete protection from EAE.

p38α is by far the most extensively investigated protein kinase target for the development of anti-inflammatory drugs in the pharmaceutical industry, but severe side effects have prevented clinical advancement of p38α inhibitors[9]. Our findings have potential implications for the use of the vast number of p38α inhibitors that are already available. First, our results indicate that p38α inhibitors would be effective for T_H17-mediated diseases. Heterogeneity in T cell effector responses is a common feature of autoimmune diseases, and both T_H17 and T_H1 responses can mediate MS and EAE. Notably, IFN-β, the most frequently prescribed drug for relapsing-remitting MS, is ineffective in 30–50% patients with a prevalent T_H17 response[41]. Second, our results suggest that selectively targeting DCs is sufficient for therapeutic efficacy, and the use of novel drug-delivery vehicles to target p38α inhibitors to specific tissues or cell types is a promising strategy to avoid undesired side effects[42]. Therefore, p38α-dependent regulation of DC functions and T_H17 responses can be further explored for innovative autoimmune therapies.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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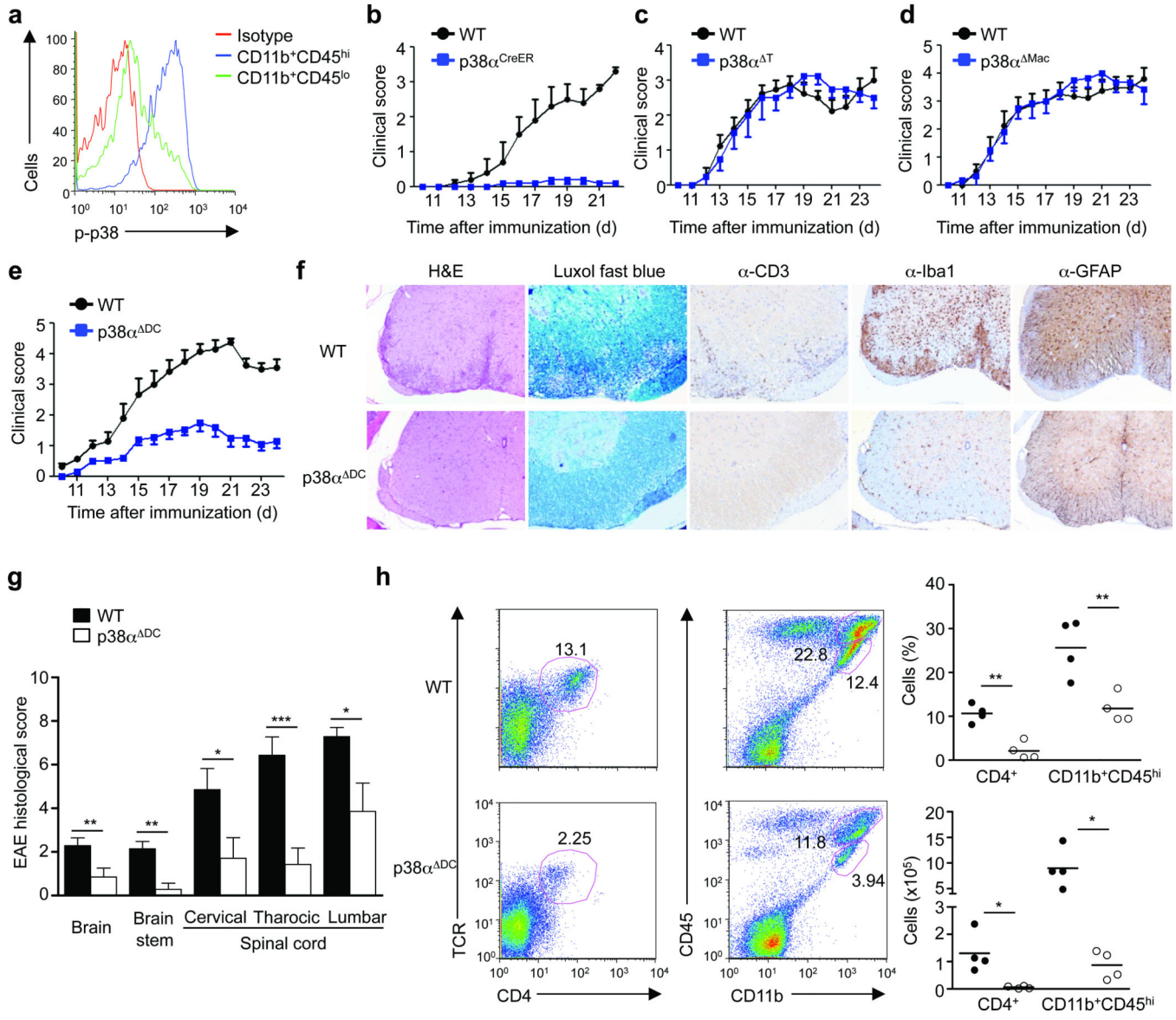

Figure 1. Deletion of p38α in DCs protects mice from EAE

(a) p38 activity in infiltrating myeloid ($CD11b^{+}CD45^{hi}$) and CNS resident cells ($CD11b^{+}CD45^{lo}$) in the spinal cord of EAE mice (day 16). **(b–e)** EAE disease course in tamoxifen-treated wild-type (WT) and $p38\alpha^{CreER}$ mice (**b**), wild-type and $p38\alpha^{\Delta T}$ mice (**c**), wild-type and $p38\alpha^{\Delta Mac}$ mice (**d**), and wild-type and $p38\alpha^{\Delta DC}$ mice (**e**). **(f–h)** Histopathology (**f**), histological scores (**g**) and flow cytometry (**h**) of spinal cord of wild-type or $p38\alpha^{\Delta DC}$ EAE mice (day 16). Images are 10× (H&E, α-Iba1 and α-GFAP) and 20× (Luxol fast blue and α-CD3) original magnification (**f**). Each symbol represents an individual mouse and small horizontal lines indicate the mean (**h**). $*P < 0.05$; $**P < 0.01$; $***P < 0.001$ (Student's *t*-test). Data are representative of 2 (**a,h**, n=4 mice per group; **d,f,g**, n≥5 mice per group), 3 (**b,c**, n≥5 mice per group) and 4 (**e**, n≥5 mice per group) independent experiments. Error bars indicate SEM.

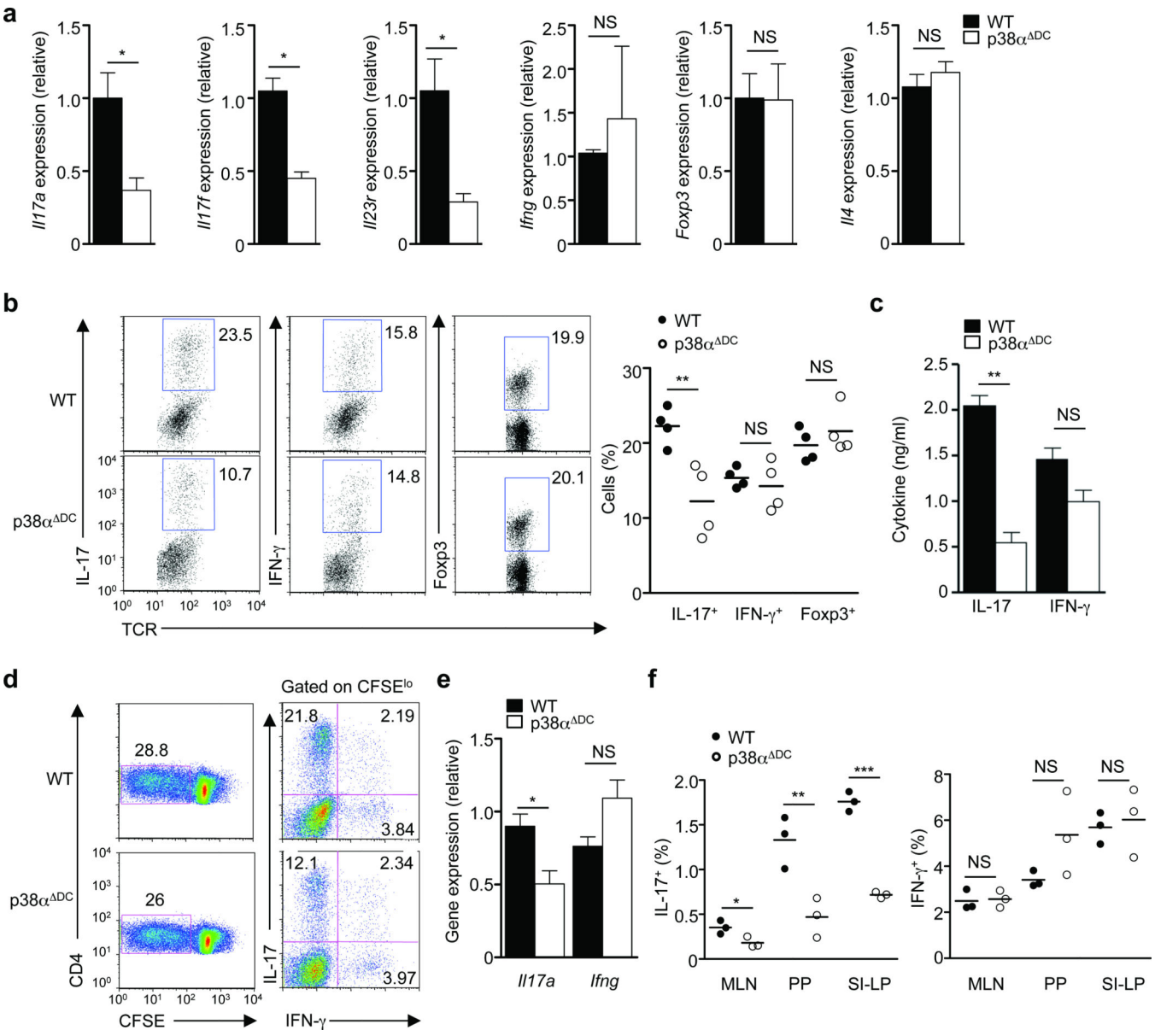

Figure 2. p38α deficiency in DCs impairs the generation of T_H17 cells *in vivo*

(a) RNA analysis of spinal cord cells of wild-type (WT) or p38α$^{\Delta DC}$ EAE mice (day 16). **(b)** Flow cytometry (left) and proportions (right) of IL-17$^+$, IFN-γ$^+$ (after PMA and ionomycin stimulation) and Foxp3$^+$ cells in CD4$^+$ T cells from spinal cord of wild-type or p38α$^{\Delta DC}$ EAE mice (day 16). **(c)** Cytokine secretion by draining LN cells from MOG-immunized wild-type or p38α$^{\Delta DC}$ mice (day 7) after *ex vivo* antigen stimulation for 3 days. **(d)** Expression of IFN-γ and IL-17 in CFSE^{lo} populations of draining LN CD4$^+$ T cells isolated from (**c**), followed by CFSE labeling and then MOG stimulation for 4 days. **(e)** Analysis of *Il17a* and *Ifng* mRNA in draining LN cells from KLH-immunized mice after *ex vivo* stimulation with KLH for 48 h. **(f)** Proportions of IL-17$^+$ and IFN-γ$^+$ cells among CD4$^+$ T cells from wild-type or p38α$^{\Delta DC}$ mouse GALTs. MLN, mesenteric lymph nodes; PP, Peyer's patches; SI-LP, small intestine lamina propia. Each symbol represents an individual

mouse and small horizontal lines indicate the mean (**b,f**). NS, not significant; **P* < 0.05; ***P* < 0.01; ****P* < 0.001 (Student's *t*-test). Data are representative of 3 independent experiments (**a–e**, n≥4 mice per group; **f**, n=3 mice per group). Error bars indicate SEM.

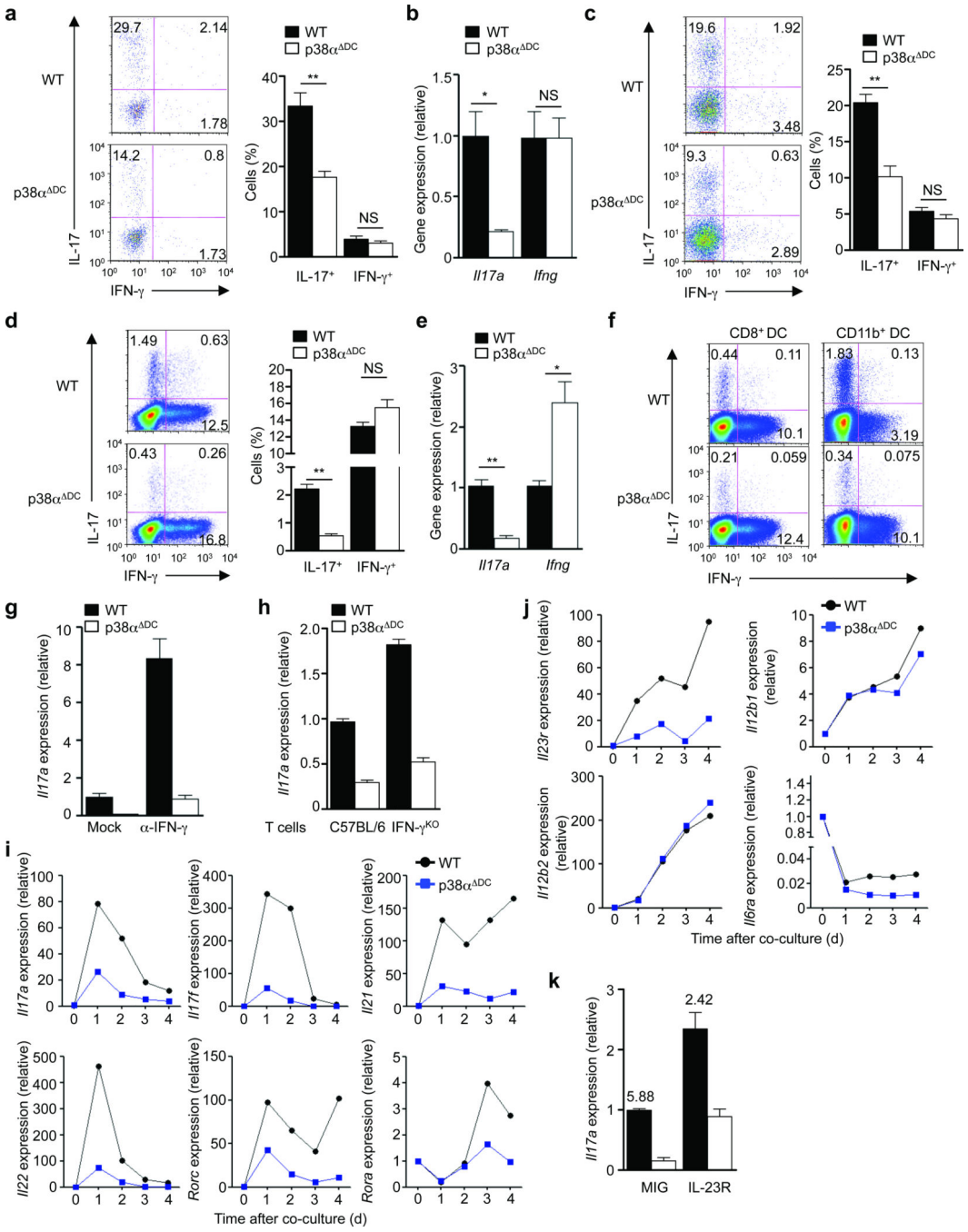

Figure 3. p38α signaling in DCs drives T_H17 differentiation *in vivo* and *in vitro* and imprints IL-23R expression in responding T cells

(a) Expression of IL-17 and IFN-γ in the MOG TCR-transgenic donor population (Thy1.1$^+$) of draining LN cells from wild-type (WT) or p38α$^{\Delta DC}$ mice immunized with MOG + CFA. Right, the proportions of IL-17$^+$ and IFN-γ$^+$ populations among donor CD4$^+$ T cells. **(b)** Analysis of *Il17a* and *Ifng* mRNA in draining LN cells isolated from (**a**) after stimulation with MOG for 48 h. **(c)** Expression of IL-17 and IFN-γ in the OT-II donor population (Thy1.1$^+$) of draining LN cells from mice immunized with OVA + CFA. Right, the

proportions of IL-17$^+$ and IFN-γ$^+$ populations among donor CD4$^+$ T cells. **(d)** Expression of IL-17 and IFN-γ in OT-II T cells activated with LPS-pulsed wild-type or p38α$^{\Delta DC}$ DCs for 5 days, followed by PMA and ionomycin stimulation. Right, the proportions of IL-17$^+$ and IFN-γ$^+$ populations. **(e)** Analysis of *Il17a* and *Ifng* mRNA in T cells from (**d**) after brief α-CD3 stimulation. **(f)** Expression of IL-17 and IFN-γ in OT-II T cells activated with antigen and LPS-pulsed CD8$^+$ DCs or CD11b$^+$ DCs for 5 days. **(g)** Analysis of *Il17a* mRNA in T cells activated with LPS-pulsed wild-type or p38α$^{\Delta DC}$ DCs in the presence or absence of α-IFN-γ for 5 days. **(h)** Analysis of *Il17a* mRNA in T cells from C57BL/6 or IFN-α^{KO} mice stimulated with α-CD3 and LPS-pulsed wild-type or p38α$^{\Delta DC}$ DCs for 5 days. **(i,j)** RNA analysis of T cells activated with wild-type or p38α$^{\Delta DC}$ DCs for various times for expression of cytokines, transcription factors (**i**) and cytokine receptors (**j**). **(k)** Analysis of *Il17a* mRNA in T cells activated with wild-type or p38α$^{\Delta DC}$ DCs and transduced with control (MIG) or IL-23R expressing retrovirus. The numbers above the bars indicate the ratios of *Il17a* mRNA expression between wild-type and p38α$^{\Delta DC}$ DC-stimulated T cells. NS, not significant; **P* < 0.05; ***P* < 0.01 (Student's *t*-test). Data are representative of 2 (**a–c**, n≥5 mice per group; **f–j**, n=4 mice per group), 3 (**k**, n=4 mice per group) and 4 (**d–e**, n=4 mice per group) independent experiments. Error bars indicate SEM.

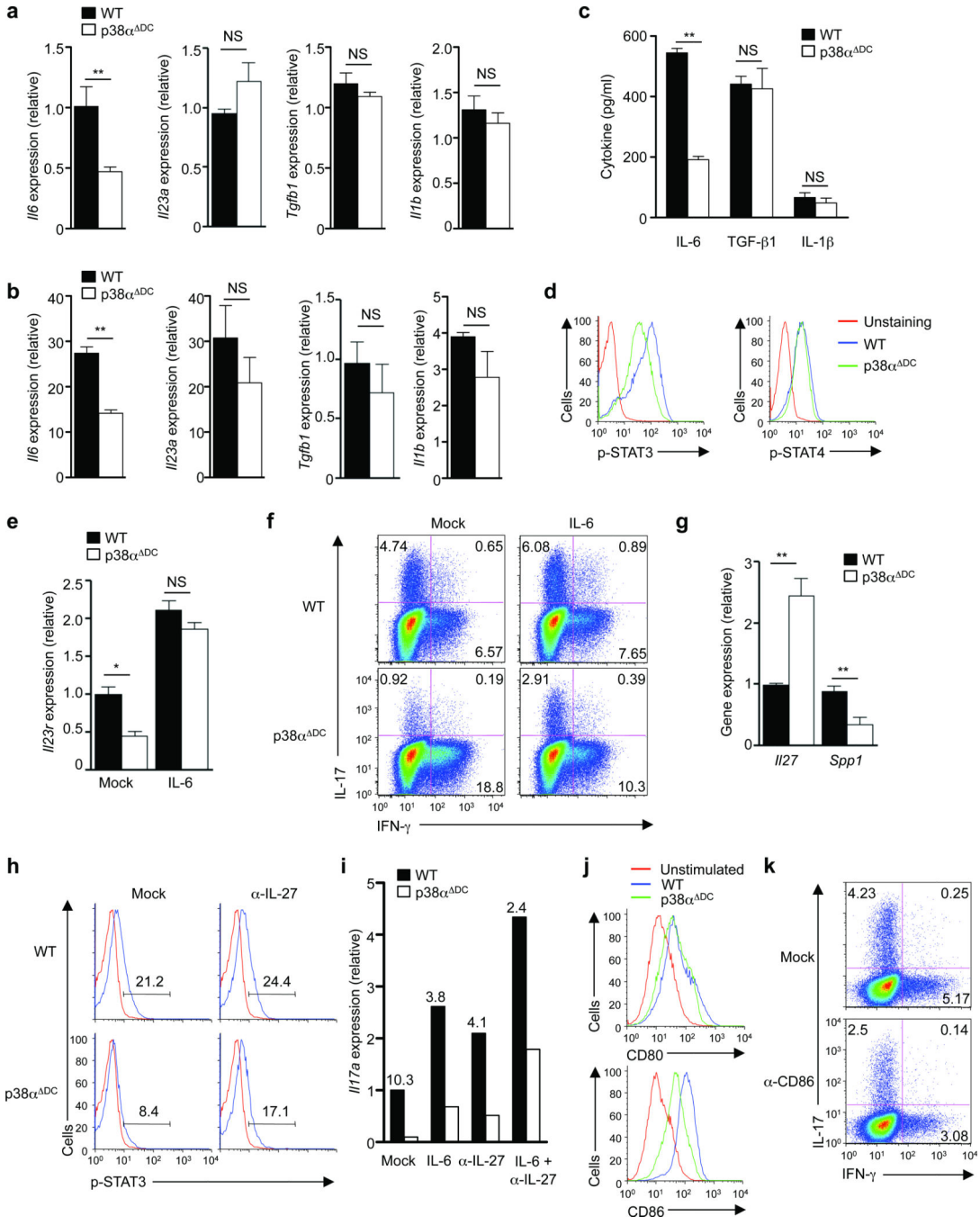

Figure 4. p38α signaling in DCs directs T_H17 differentiation by regulating expression of IL-6, IL-27 and CD86

(a) RNA analysis of wild-type (WT) or p38α$^{\Delta DC}$ splenic DCs isolated from MOG-immunized mice (day 7). **(b)** RNA analysis of wild-type or p38α$^{\Delta DC}$ DCs after *in vivo* stimulation with LPS for 5 h. The baseline level in unstimulated wild-type DCs was set as 1. **(c)** Cytokine production from wild-type or p38α$^{\Delta DC}$ DCs after *in vitro* stimulation with LPS for 24 h. **(d)** Levels of p-STAT3 and p-STAT4 in T cells activated with antigen and wild-type or p38α$^{\Delta DC}$ DCs for 2 days *in vitro*. **(e)** Analysis of *Il23r* mRNA in T cells activated

with wild-type or p38α$^{\Delta DC}$ DCs in the presence or absence of IL-6. **(f)** Expression of IL-17 and IFN-γ in T cells stimulated with wild-type or p38α$^{\Delta DC}$ DCs in the presence or absence of IL-6 for 5 days. **(g)** Analysis of *Il27* and *Spp1* mRNA in wild-type or p38α$^{\Delta DC}$ splenic DCs from MOG-immunized mice (day 7). **(h)** Levels of p-STAT3 in T cells activated with wild-type or p38α$^{\Delta DC}$ DCs in the presence or absence of α-IL-27 antibodies for 2 days. Red line, isotype control. **(i)** Analysis of *Il17a* mRNA in T cells stimulated with wild-type or p38α$^{\Delta DC}$ DCs in the presence or absence of IL-6, α-IL-27 or IL-6 + α-IL-27 for 5 days. The numbers above the bars indicate the ratios of *Il17a* mRNA expression between wild-type and p38α$^{\Delta DC}$ DC-stimulated T cells. **(j)** Expression of CD80 and CD86 on wild-type or p38α$^{\Delta DC}$ splenic DCs after *in vivo* LPS stimulation for 5 h. **(k)** Expression of IL-17 and IFN-γ in T cells activated with LPS-pulsed wild-type DCs in the presence or absence of α-CD86 for 5 days. NS, not significant; **P* < 0.05; ***P* < 0.01 (Student's *t*-test). Data are representative of 2 (**a,g**, n≥5 mice per group; **j,** n=4 mice per group), 3 (**f,h,i,k,** n=4 mice per group) and 4 (**b**,**c,d,e,** n=4 mice per group) independent experiments. Error bars indicate SEM.

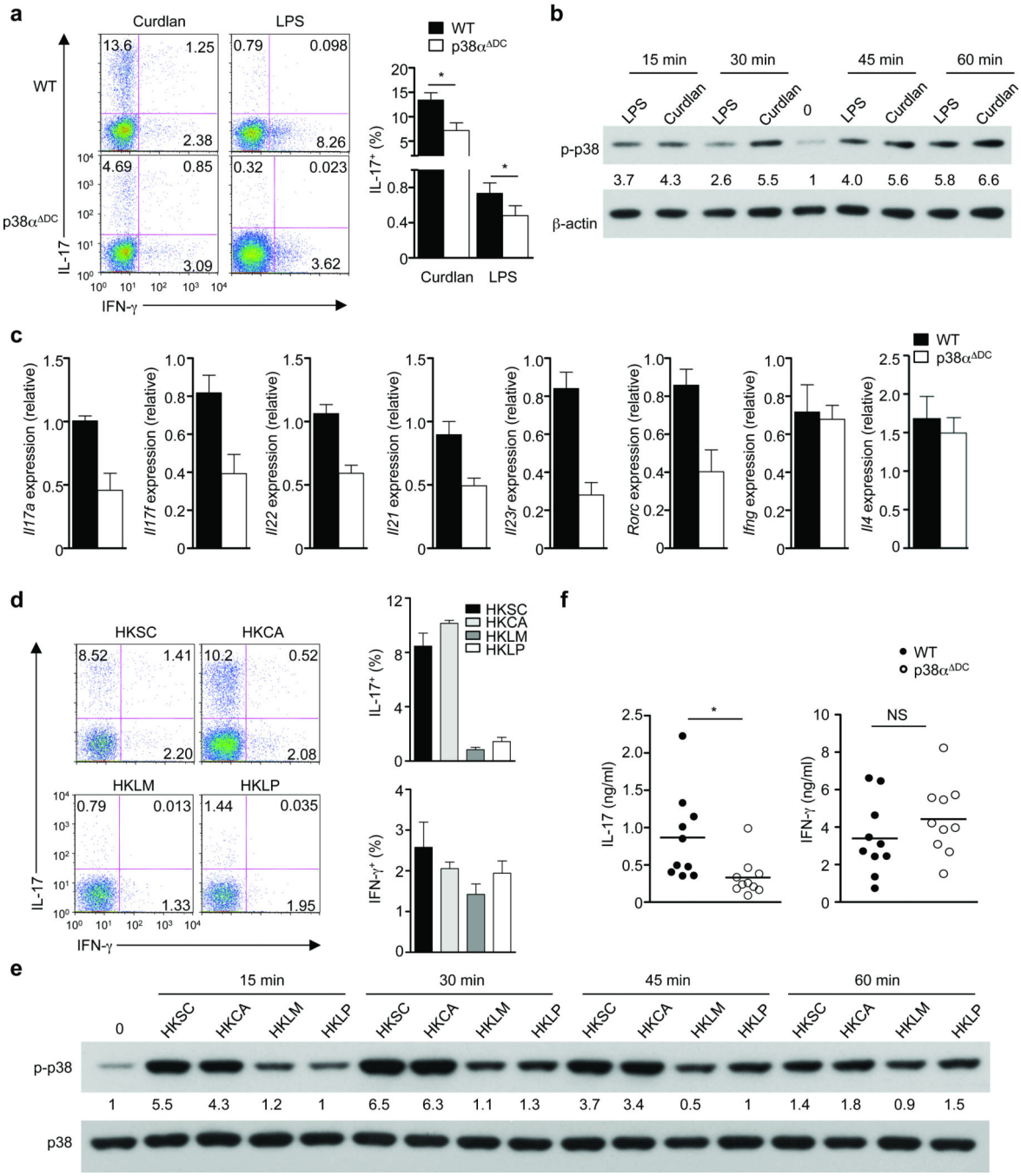

Figure 5. p38α signaling in DCs integrates T_H17-instructive signals derived from PRRs and infectious agents

(a) Expression of IL-17 and IFN-γ in the OT-II donor population (Thy1.1$^+$) of draining LN cells from wild-type (WT) or p38α$^{\Delta DC}$ mice immunized with OVA + LPS or curdlan. Right, the proportions of IL-17$^+$ populations among donor CD4$^+$ T cells. **(b)** Activation of p38 in splenic DCs stimulated with LPS or curdlan. Numbers below p-p38 lanes indicate band intensity relative to that of β-actin (loading control). **(c)** mRNA analysis of donor T cells isolated from draining LN cells of OVA + curdlan immunized mice as in (**a**). **(d)** Expression

of IL-17 and IFN-γ in the donor population (Thy1.1$^+$) of draining LN cells from mice immunized with antigen plus 2 × 10^7/mouse heat-killed *S. cerevisiae* (HKSC), *C. albicans* (HKCA), *L. monocytogenes* (HKLM) or *L. pneumophila* (HKLP). Right, the proportions of IL-17$^+$ and IFN-γ$^+$ populations among donor CD4$^+$ T cells. **(e)** Activation of p38 in splenic DCs stimulated with 10^7/ml HKSC, HKCA, HKLM or HKLP. Numbers below p-p38 lanes indicate band intensity relative to that of p38. **(f)** Cytokine secretion from splenocytes isolated from live *C. albicans*-infected mice, followed by stimulation with HKCA for 48 h. Each symbol represents an individual mouse and small horizontal lines indicate the mean. NS, not significant; **P* < 0.05 (Student's *t*-test). Data are representative of 2 (**a,c,d,f**, n≥5 mice per group) and 3 (**b,e**, n=5 mice per group) independent experiments. Error bars indicate SEM.

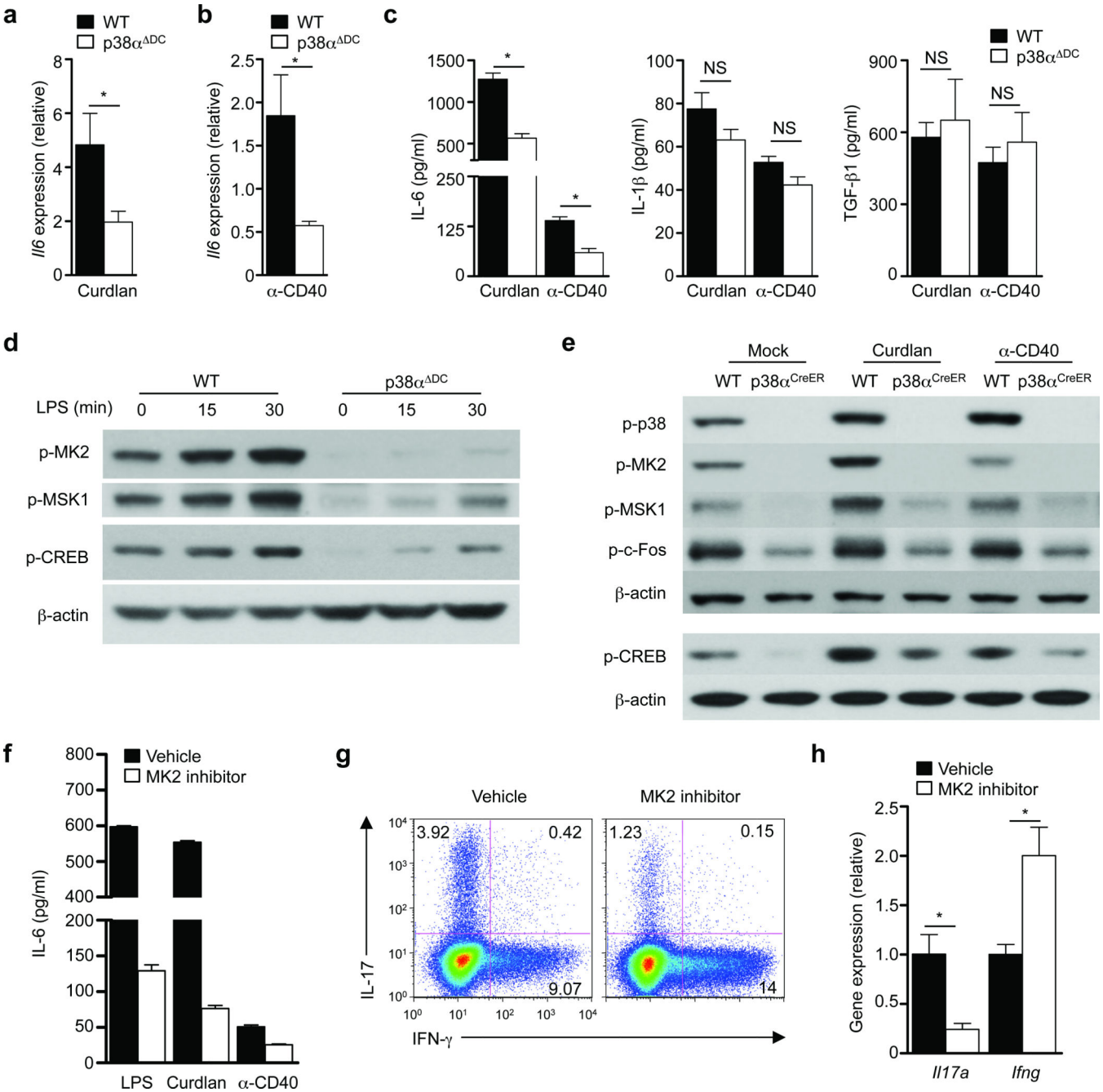

Figure 6. p38α regulates a core set of downstream effector pathways in DCs to mediate diverse upstream signals

(a,b) Analysis of *Il6* mRNA in wild-type (WT) or p38α$^{\Delta DC}$ DCs after *in vivo* stimulation with curdlan (**a**) or α-CD40 (**b**) for 5 h. **(c)** Cytokine production from wild-type or p38α$^{\Delta DC}$ DCs after *in vitro* stimulation with curdlan or α-CD40 for 24 h. **(d)** Immunoblot analysis of p38α downstream targets in LPS-stimulated wild-type or p38α$^{\Delta DC}$ DCs. p-, phosphorylated. β-actin, the loading control. **(e)** Immunoblot analysis of p38α and downstream targets in curdlan and α-CD40-stimulated DCs from wild-type or p38α^{CreER} mice after treatment with tamoxifen. **(f)** IL-6 production from DCs stimulated with LPS, curdlan or α-CD40 in the

presence of the MK2 inhibitor or vehicle for 24 h. **(g,h)** Intracellular staining (**g**) and RNA analysis (**h**) of IL-17 and IFN-γ expression from T cells incubated for 5 days with DCs previously pulsed with LPS in the presence of the MK2 inhibitor or vehicle. NS, not significant; **P* < 0.05; ***P* < 0.01 (Student's *t*-test). Data are representative of 2 (**a,b,** n≥5 mice per group), 3 (**c–e**, n≥4 mice per group) and 4 (**f–h**, n≥4 mice per group) independent experiments. Error bars indicate SEM.

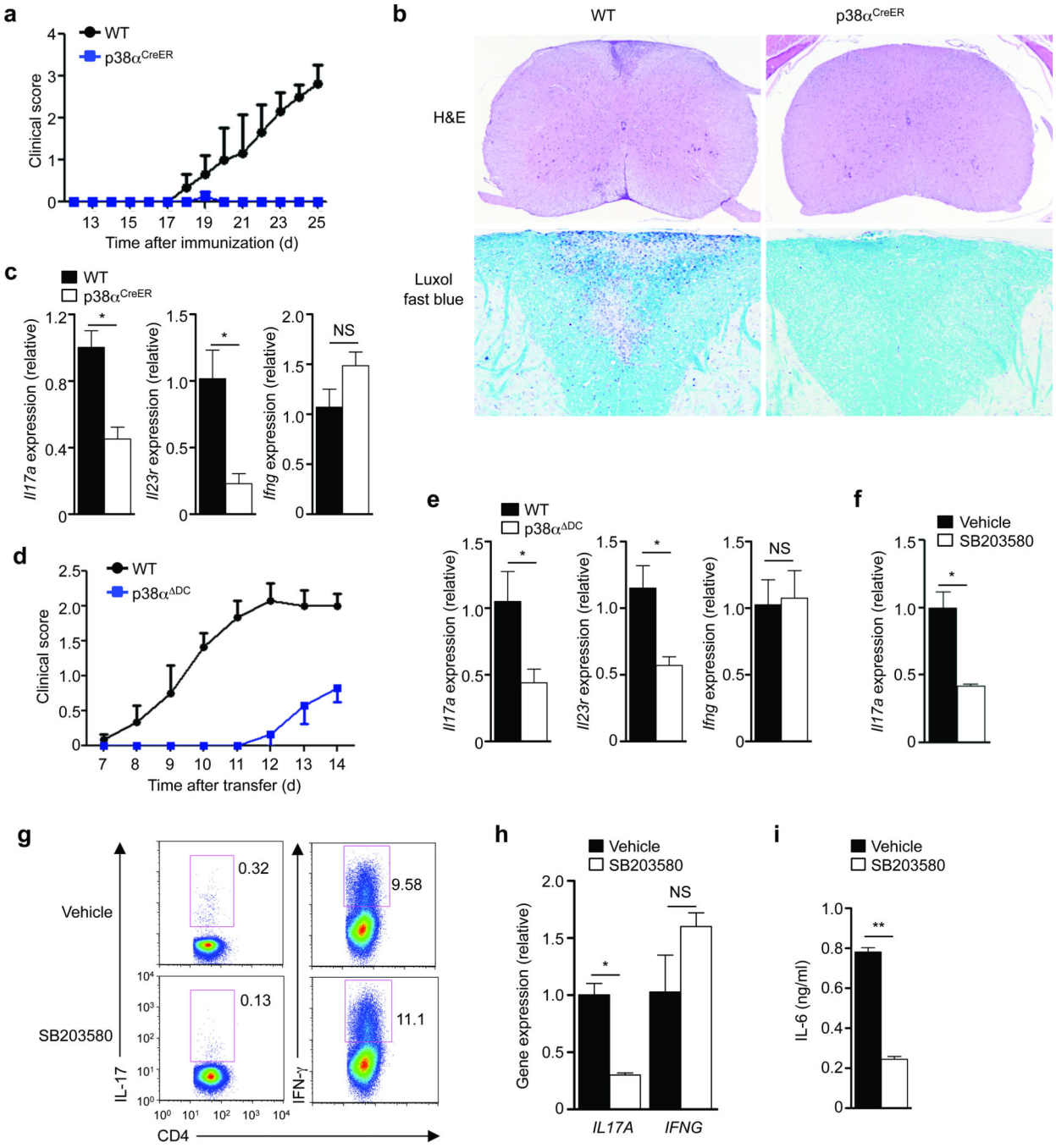

Figure 7. p38α activity is required for CNS-infiltrating DCs to maintain the T_H17 response and is evolutionarily conserved between mouse and human DCs

(a,b) EAE disease course (**a**) and histology (**b**) of wild-type (WT) or p38α^{CreER} mice treated with tamoxifen at day 7 after immunization. Images are 4× (H&E) and 20× (Luxol fast blue) original magnification (**b**). **(c)** RNA analysis of MOG TCR-transgenic T cells stimulated with antigen and DCs isolated at the peak of disease from the spinal cord of wild-type or p38α^{CreER} mice (treated with tamoxifen at day 13) for 5 days. **(d)** EAE disease course in wild-type or p38α$^{\Delta DC}$ mice transferred with *in vitro* derived T_H17 cells. **(e)** RNA analysis of

spinal cord cells from mice in (**d**). **(f)** Analysis of *Il17a* mRNA in T cells incubated for 5 days with mouse DCs previously pulsed with LPS in the presence of the p38 inhibitor SB203580 or vehicle. **(g,h)** Intracellular staining (**g**) and RNA analysis (**h**) of IL-17 and IFN-γ of human cord blood T cells incubated for 7 days with human DCs previously pulsed with LPS in the presence of SB203580 or vehicle. **(i)** IL-6 production from human DC stimulated with LPS in the presence of SB203580 or vehicle for 24 h. NS, not significant; **P* < 0.05; ***P* < 0.01 (Student's *t*-test). Data are representative of 2 (**b–f**: n≥ 4 mice per group) and 3 (**a**, n=5 mice per group; **g–i**) independent experiments. Error bars indicate SEM.

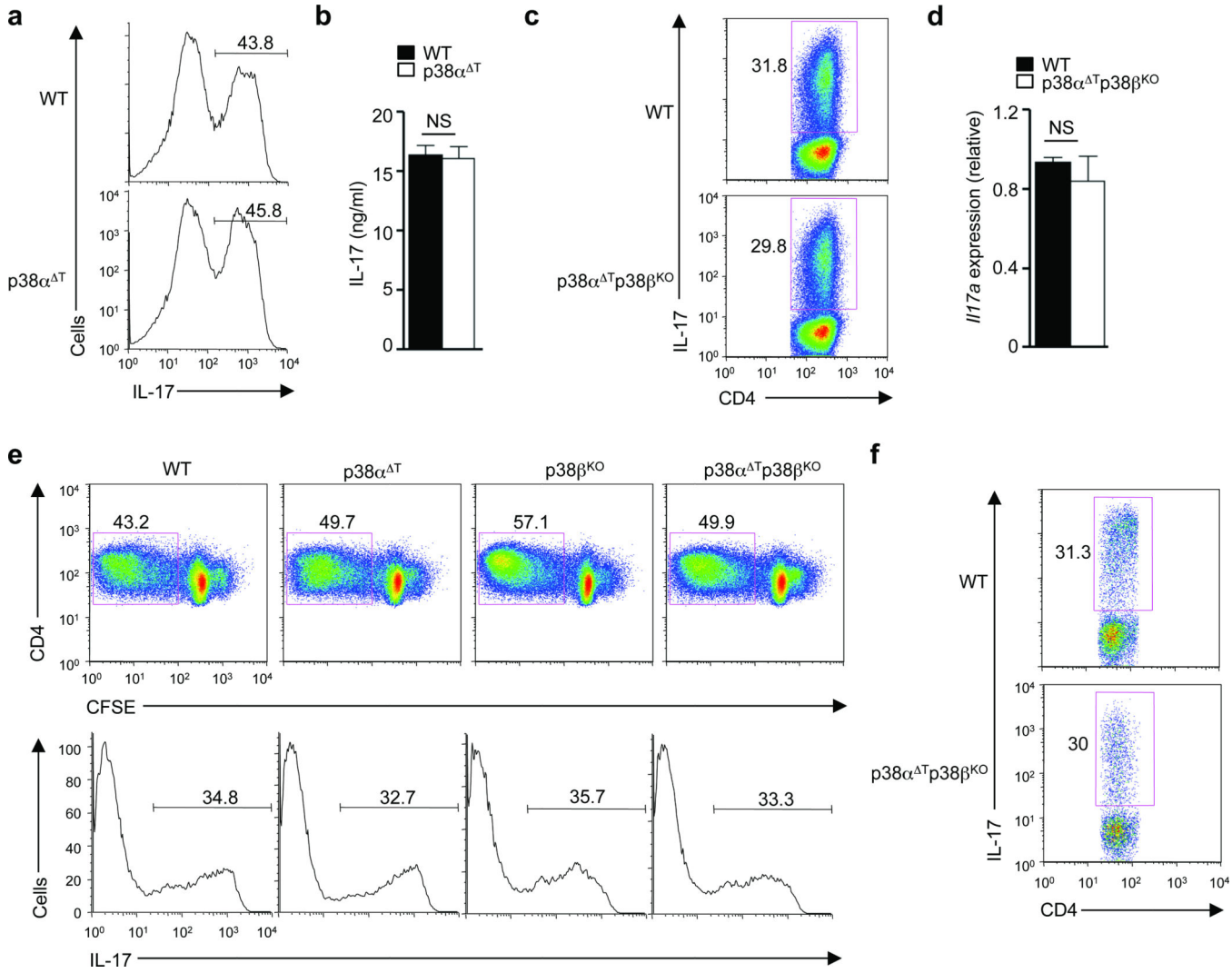

Figure 8. p38α and p38β are not required for regulating T cell-intrinsic T_H17 differentiation

(a) Expression of IL-17 in wild-type (WT) or p38α$^{\Delta T}$ naïve T cells differentiated under T_H17 conditions for 5 days, followed by PMA and ionomycin stimulation. **(b)** IL-17 secretion by T cells from (**a**) after α-CD3 stimulation for 24 h. **(c)** Expression of IL-17 in naïve T cells from wild-type or p38α$^{\Delta T}$p38β^{KO} mice simulated with LPS-pulsed DCs in the presence of IL-6 and TGF-β1 for 5 days, followed by PMA and ionomycin stimulation. **(d)** Analysis of *Il17a* mRNA in T cells from (**c**) after brief α-CD3 stimulation. **(e)** IL-17 expression in the CFSE^{lo} population of draining LN cells isolated from mice immunized with MOG + CFA, followed by CFSE labeling and then stimulation with MOG for 4 days. **(f)** Expression of IL-17 in the OT-II donor population of draining LN cells from recipients (CD45.1^{+}) immunized with OVA + CFA. NS, not significant (Student's *t*-test). Data are representative of 2–3 independent experiments (n≥3 mice per group). Error bars indicate SEM.