NIK signaling axis regulates dendritic cell function in intestinal immunity and homeostasis

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Dendritic cells (DCs) play an integral role in regulating mucosal immunity and homeostasis, but the signaling network mediating this function of DCs is poorly defined. We identified the noncanonical NF-κB-inducing kinase (NIK) as a crucial mediator of mucosal DC function. DC-specific NIK deletion impaired intestinal immunoglobulin A (IgA) secretion and microbiota homeostasis, rendering mice sensitive to an intestinal pathogen, Citrobacter rodentium. DC-specific NIK was required for expression of the IgA transporter polymeric immunoglobulin receptor (pIgR) in intestinal epithelial cells, which in turn relied on the cytokine IL-17 produced by Th17 cells and innate lymphoid cells (ILCs). NIK-activated noncanonical NF-κB induced expression of IL-23 in DCs, contributing to the maintenance of Th17 cells and type 3 ILCs. Consistent with the dual functions of IL-23 and IL-17 in mucosal immunity and inflammation, NIK deficiency also ameliorated colitis induction. Thus, our data suggest a pivotal role for the NIK signaling axis in regulating DC functions in intestinal immunity and homeostasis.

Immuno globulin A (IgA) is a major immune component in the mucosa that plays an important role in mediating host defenses against pathogen infections, shaping the commensal communities, and maintaining intestinal homeostasis. After its production by B cells in gut-associated lymphoid tissues, IgA is secreted into the intestinal lumen via transcytosis that involves binding of dimeric IgA to polymeric immunoglobulin receptor (pIgR) on intestinal epithelial cells (IECs). Secreted IgA interacts with surface antigens of bacteria in the intestinal lumen, which serves as a mechanism to prevent invasion by pathogenic species and maintain a healthy composition of microbiota.

Being the major type of antigen-presenting cells, dendritic cells (DCs) play a crucial role in regulating mucosal immunity and homeostasis. DCs uptake and present gut microbial antigens to promote the differentiation and expansion of helper T cells, particularly the Th17 and Th1 subsets of helper T cells, as well as regulatory T (Treg) cells. Intestinal DCs also serve as an important regulator of type 3 innate lymphoid cells (ILCs). The DC-derived cytokine IL-23 promotes expansion and activation of Treg cells and ILC3s, which resemble Th17 cells in expressing the transcription factor RORγt and producing the pro-inflammatory cytokines IL-17A and IL-22. DCs sense bacterial components via Toll-like receptors (TLRs) and other pattern-recognition receptors and produce various immunoregulatory factors, such as transforming growth factor-β and the B cell survival factors B cell-activating factor (BAFF), a member of the tumor-necrosis factor (TNF) superfamily, and a proliferation-inducing ligand (APRIL), which are involved in induction of IgA class switching in B cells. Despite these important findings, the signaling network that mediates the functions of DCs in intestinal immunity is poorly defined.

The NF-κB family of transcription factors regulates diverse aspects of immune functions. Two different signaling pathways, the canonical and noncanonical pathways, mediate activation of distinct NF-κB members. The noncanonical NF-κB pathway is based on inducible processing of p100, a protein that serves as both the precursor of the NF-κB2 subunit p52 and an inhibitor of NF-κB (IκB)-like molecule that predominantly regulates the NF-κB member RelB. Proteasome-mediated p100 processing not only generates p52 but also disrupts the IκB-like function of p100, leading to nuclear translocation of the noncanonical NF-κB members p52 and RelB. A central signaling component of the noncanonical NF-κB pathway is NF-κB-inducing kinase (NIK), which responds to signals from specific immune receptors, predominantly members of the TNF receptor (TNFR) superfamily. After activation, NIK stimulates its downstream kinase IKKα, which in turn phosphorylates specific C-terminal serines of p100 to trigger its ubiquitin-dependent processing. The steady amount of NIK is extremely low due to its constant degradation mediated via ubiquitination by an E3 ubiquitin ligase complex composed of TNF receptor–associated factor 2 (TRAF2), TRAF3, and cellular inhibitor of apoptosis (cIAP), and noncanonical NF-κB activation involves TRAF3 degradation and NIK accumulation.

The noncanonical NF-κB pathway is known to regulate lymphoid organ development and B cell maturation. However, the role of this NF-κB pathway in the regulation of mucosal immunity and homeostasis is largely elusive. In this study, we demonstrated a
DC-intrinsic role for NIK in regulating IgA secretion and microbiota regulation.

Results
NIK is dispensable for DC development and homeostasis. To examine the role of NIK in DCs, we specifically deleted the NIK-encoding gene Map3k14 by crossing Map3k14-flox mice with Cd11c-Cre mice to generate Map3k14 DC-conditional knockout (Map3k14-cKO) and wild-type mice (Supplementary Fig. 1a). Real-time quantitative reverse-transcription PCR (qRT-PCR) detecting the deleted exon of Map3k14 confirmed the Map3k14 gene deletion in DCs, but not in T cells (Supplementary Fig. 1b). We further confirmed the NIK protein deficiency in Map3k14-cKO DCs by immunoblot using cells treated with a CD40 agonistic antibody together with the proteasome inhibitor MG132 known to inhibit NIK degradation (Supplementary Fig. 1c). The Map3k14-cKO and wild-type mice had similar frequencies of CD11c+ lymphoid tissues (Supplementary Fig. 1d). Consistent with a previous study, the NIK deficiency also did not influence the expression of DC maturation markers CD80 and CD86 (Supplementary Fig. 1e). The wild-type and Map3k14-cKO mice also did not display significant differences in LP DC subpopulation frequencies or surface marker expression (Supplementary Fig. 1f–h). These results suggest that NIK deletion in DCs had no obvious effect on the development or maturation of DCs.

NIK deletion in DCs alters intestinal microbiota. To examine the role of DC-specific NIK in regulating microbiota, we analyzed the profile of commensal bacteria in the Map3k14-cKO and wild-type mice housed under specific pathogen-free conditions. Selective-medium cultivation analyses revealed that most of the commensal species had similar abundance in the Map3k14-cKO and wild-type mice (Fig. 1a). However, the number of Enterococcus spp. was significantly higher in the Map3k14-cKO mice than in the control mice (Fig. 1a). Enterococci are low abundant gut commensals of humans and mice but can cause opportunistic infections, and increased Enterococcus infections have been associated with intestinal inflammation and other disorders. To further examine the role of DC-specific NIK in regulating microbiota, we performed 16S rRNA sequencing analyses. While the overall microbiota profile of the wild-type and Map3k14-cKO mice was similar, the Map3k14-cKO mice had significantly higher abundance of Candidatus Savagella (Fig. 1b), segmented filamentous bacteria (SFB) from the gut known to be involved in dynamic interaction between mucosal immunity and microbiota. Consistent with its low abundance, gut known to be involved in dynamic interaction between mucosal immunity and microbiota. As expected, the fecal IgA and IgG defects of Map3k14-cKO were not rescued by their co-housing with wild-type mice (Supplementary Fig. 4c,d), thus suggesting a role for NIK in regulating IgA secretion independently of microbiota.

DC NIK promotes IgA secretion via Tfh17 cell induction. Our finding that DC-specific NIK regulates IgA secretion in IECs raised the question regarding the functional link between DCs and IECs. In this regard, DCs regulate mucosal T cell subsets, particularly the CD4+ Tfh1 and Tfh17 cells. Flow cytometric analysis revealed that the IL-17-producing Tfh17 cells were abundant in the LP, but scarce in the spleen and mesenteric lymph nodes (MLNs), whereas the interferon-γ (IFN-γ)-producing Tfh1 cells were readily detected in all three types of lymphoid tissues (Fig. 3a,b). Importantly, DC-specific NIK deficiency greatly reduced the frequency of Tfh17, but not Tfh1, cells in LP, and this phenotype was not seen in the spleen or MLNs (Fig. 3a,b). This finding provided important insight into the mechanism by which NIK regulates intestinal immunity and homeostasis. Specifically, the cytokine IL-17 has been shown to stimulate IECs for IgA production and IgA secretion.

To assess the functional connection between NIK-mediated Tfh17 cell regulation and IgA secretion, we injected the wild-type and Map3k14-cKO mice intraperitoneally with an IL-17-specific neutralizing antibody and analyzed IgA expression. Administration of anti-IL-17 efficiently inhibited the expression of IgA in IECs of wild-type mice to a level similar to that detected in the IECs of Map3k14-cKO mice (Fig. 3c). Consistent with fewer Tfh17 cells in the Map3k14-cKO LPs, injection of the anti-IL-17 to these mutant mice only moderately reduced IgA expression (Fig. 3c). Injection with anti-IL-17 also profoundly reduced the concentration of IgA and IgG in the feces of wild-type mice (Fig. 3d,e). Furthermore, on injection with anti-IL-17, the number of fecal enterococci in wild-type and Map3k14-cKO mice became comparable, as a result of substantial increase in the wild-type mice (Fig. 3f). In line with these findings, injection of Map3k14-cKO mice with a recombinant IL-17 rescued the abundance of fecal IgA and IgG (Fig. 3g,h). These results suggest that DC-specific NIK maintains the homeostasis of gut Tfh17 cells, which in turn promotes IgA secretion and IgA secretion via IL-17 secretion.

DC NIK regulates intestinal ILC3s. Like Tfh17 cells, ILC3s express the signature cytokines IL-17 and IL-22 and play a role in regulating intestinal immunity and homeostasis. ILC3-derived IL-22 has been shown to mediate mucosal immunity against intestinal pathogens, such as Citrobacter rodentium, as well as opportunistic pathogens.
from the microbiota, including enterococci\textsuperscript{26,27}. Compared with wild-type control mice, the Map3k14-cKO mice had a significantly reduced frequency of ILC3s in both the LP cells and intraepithelial lymphocytes (IELs) (Fig. 3i). Furthermore, the ILCs from Map3k14-cKO mice expressed less IL-22 and IL-17 (Fig. 3j,k). Thus, NIK is crucial for DC functions in regulating the homeostasis of both Th17 cells and ILC3s in the intestine.

The transcription factor retinoic acid receptor-related orphan nuclear receptor gamma t (ROR\textgamma t) is required for the generation of both Th17 cells and ILC3s as well as several other subsets of T cells\textsuperscript{28}. To further emphasize the role of IL-17-producing cells in IgA regulation, we examined IgA secretion in mice, the Rorc\texttextsuperscript{-cKO (Fig. 5a–c). Furthermore, like the SFB–/– mice had a drastically reduced fecal IgA and pIgR.

NIK mediates IL-23 induction by TLRs in DCs. To understand the molecular mechanism by which DC-specific NIK regulates the abundance of Th17 cells and ILC3s in the intestine, we analyzed the cytokine-encoding gene expression profile of LP DCs freshly isolated from wild-type or Map3k14-cKO mice. The expression of Il23a, encoding the IL-23p19 subunit, was drastically reduced in the NIK-deficient LP DCs (Fig. 4a). The Il12a gene, encoding the IL-12p35 subunit, was also downregulated, albeit less profoundly than the downregulation of Il23a (Fig. 4a). This phenotype was not detected in DCs derived from the MLNs (Fig. 4a). Since LP DCs are involved in dynamic interactions with commensal bacteria, this result suggested an important role for NIK in mediating induction of Il23a and Il12a genes by bacterial products.

In vitro studies revealed that TLR ligands stimulated the expression of several pro-inflammatory cytokine genes in bone marrow DCs (BMDCs) (Fig. 4b). In agreement with the results obtained from freshly isolated LP DCs, NIK deficiency attenuated TLR-mediated induction of Il23a and Il12a without significantly affecting the induction of the other cytokine genes analyzed (Fig. 4b). Parallel ELISA further confirmed the crucial role for NIK in mediating TLR-stimulated production of Il-23p19 (Fig. 4c). To examine TLR-stimulated cytokine gene expression in LP DCs, we resected the LP DCs to reduce their spontaneous cytokine gene expression and then stimulated them with TLR ligands. NIK deficiency also attenuated TLR-stimulated expression of Il23a and Il12a in the LP.
enterococci CFUs (Fig. 4f–h). Given the previous finding that IL-23, a pivotal function of NIK in regulating TLR-stimulated expression of NF-κB in mediating TLR signalalthough the role of noncanonical NF-κB pathways12,33,34. Consistently, the TLR-stimulated RelB nuclear expression was partially inhibited in the NIK-deficient DCs which typically responds to signals from the TNFR superfamily12, which is a central component in the noncanonical NF-κB signaling pathway, although the role of noncanonical NF-κB in mediating TLR signaling is poorly defined. We found that stimulation of BMDCs with several TLR ligands resulted in the induction of nuclear p52, which was largely, although not completely, dependent on NIK (Fig. 5a). The TLR ligands also induced the nuclear translocation of RelB, an NF-κB member activated by both the canonical and noncanonical NF-κB pathways12,33,34. Consistently, the TLR-stimulated RelB nuclear expression was partially inhibited in the NIK-deficient DCs, but not IL-12, is required for maintenance of intestinal T\(\text{T}_{17}\) cells29–32, these studies emphasize an important role for IL-23 in mediating the mucosal function of NIK.

Noncanonical NF-κB mediates IL-23 induction in DCs. NIK is a pivotal function of NIK in regulating TLR-stimulated expression of NF-κB member activated by both the canonical and noncanonical NF-κB pathways12,33,34. Consistently, the TLR-stimulated RelB nuclear expression was partially inhibited in the NIK-deficient DCs.

To examine the contribution of IL-23 and IL-12 to the impaired intestinal immune homeostasis of the Map3k14-cKO mice, we employed an antibody neutralization approach using the commercially available IL-12–IL-23 p40–neutralizing antibody. Injection of anti-p40, but not an isotype-matched control antibody, every other day for two weeks profoundly reduced the frequency of LP T\(\text{T}_{17}\) cells and ILC3s in the wild-type mice, making the frequencies of these immune cells comparable between the wild-type and Map3k14-cKO mice (Fig. 4d,e). The IL-12–IL-23 p40 neutralization also erased the differences between wild-type and Map3k14-cKO mice in the fecal IgA concentration, pIgR expression in IECs, and enterococci CFUs (Fig. 4f–h). Given the previous finding that IL-23, DCs (Supplementary Fig. 6). Collectively, these data demonstrated a pivotal function of NIK in regulating TLR-stimulated expression of IL-23 and, to a lesser extent, IL-12.

To examine the contribution of IL-23 and IL-12 to the impaired intestinal immune homeostasis of the Map3k14-cKO mice, we employed an antibody neutralization approach using the commercially available IL-12–IL-23 p40–neutralizing antibody. Injection of anti-p40, but not an isotype-matched control antibody, every other day for two weeks profoundly reduced the frequency of LP T\(\text{T}_{17}\) cells and ILC3s in the wild-type mice, making the frequencies of these immune cells comparable between the wild-type and Map3k14-cKO mice (Fig. 4d,e). The IL-12–IL-23 p40 neutralization also erased the differences between wild-type and Map3k14-cKO mice in the fecal IgA concentration, pIgR expression in IECs, and enterococci CFUs (Fig. 4f–h). Given the previous finding that IL-23, but not IL-12, is required for maintenance of intestinal T\(\text{T}_{17}\) cells29–32, these studies emphasize an important role for IL-23 in mediating the mucosal function of NIK.

Noncanonical NF-κB mediates IL-23 induction in DCs. NIK is a central component in the noncanonical NF-κB signaling pathway, which typically responds to signals from the TNFR superfamily12, although the role of noncanonical NF-κB in mediating TLR signaling is poorly defined. We found that stimulation of BMDCs with several TLR ligands resulted in the induction of nuclear p52, which was largely, although not completely, dependent on NIK (Fig. 5a). The TLR ligands also induced the nuclear translocation of RelB, an NF-κB member activated by both the canonical and noncanonical NF-κB pathways12,33,34. Consistently, the TLR-stimulated RelB nuclear expression was partially inhibited in the NIK-deficient DCs.
NIK deficiency did not affect the activation of canonical NF-κB members, RelA, c-Rel, and p50 (Fig. 5a). TLR-stimulated early phase activation of canonical NF-κB signaling events, including phosphorylation of IκBα and RelA, was also not affected by the NIK deficiency (Fig. 5b).

NIK-mediated activation of noncanonical NF-κB members is based on the induction of p100 processing41. TLR ligands induced p100 processing in wild-type DCs, as revealed by enhanced generation of p52 (Fig. 5c). Although NIK deficiency did not block the basal p52 expression, TLR-induced production of p52 was inhibited in the NIK-deficient DCs (Fig. 5c). Furthermore, the induction of p100 processing by TLR ligands was coupled with robust accumulation of NIK in wild-type DCs (Fig. 5d). These results suggest that TLR signals activate NIK, thereby inducing noncanonical NF-κB activation in DCs.

The induction of p100 processing requires phosphorylation of two specific serine residues (S866 and S870)36,35. A mutant mouse, Nfkβ2<sup>−/−</sup>, carries a point mutation in the Nfkb2 gene that lym1, carries a point mutation in the Nfkb2 gene that

Fig. 3 | DC-specific deletion of NIK reduces the frequency of intestinal Th17 cells and ILC3s. a, b, Flow cytometric and ICS analysis of IFNγ-producing T<sub>H</sub>1 cells and IL-17-producing T<sub>H</sub>17 cells in the CD3<sup>+</sup>CD4<sup>+</sup> lymphocyte population of LP, spleen (Spl), and MLNs of wild-type or Map3k14-cKO mice. Data are presented as a representative plot (a) and summary graph (b) based on multiple mice (T<sub>H</sub>17: LP, n = 8 mice per group; spleen, n = 10 mice per group; MLN, wild-type, n = 5; cKO, n = 4; T<sub>H</sub>1: LP, wild-type, n = 7; cKO, n = 6; spleen and MLN, n = 6 mice per group). c–f, qRT-PCR analysis of Pigr mRNA in IECs (c, n = 5 mice per group), ELISA of fecal pIgR (d, n = 5 mice per group), and IgA concentration (e, n = 5 mice per group), and selective agar analysis of fecal Enterococcus spp. CFUs (f, n = 5–6 mice per group) in wild-type and Map3k14-cKO mice injected intraperitoneally with an IL-17-neutralization antibody (10 μg/kg bodyweight) or an IgG isotype-matched control antibody every other day for two weeks. g–i, ELISA analysis of IgA (g) and pIgR (h) concentrations in fecal extracts of age-matched wild-type (n = 5) and Map3k14-cKO (n = 5) mice that had been injected intraperitoneally with recombinant IL-17 (+; 2 μg in 100 μl of PBS) or PBS control (−) every other day for two weeks (n = 5 mice per group). j, Flow cytometric analysis of RORγ<sup>+</sup>-CD127<sup>−</sup> ILC3s in LP lymphocytes (LPL) and intraepithelial lymphocytes (IEL) of wild-type (n = 4) and Map3k14-cKO (n = 4) mice, gated on CD90<sup>+</sup>- and lineage-marker-negative (Lin<sup>−</sup>) cells. k, Flow cytometry and ICS analysis of IL-22 and IL-17-expressing ILCs (gated on CD90<sup>+</sup>-Lin<sup>−</sup> cells) in LP lymphocytes of wild-type and Map3k14-cKO mice (n = 3 mice per group). Each symbol represents an individual mouse, and small horizontal lines indicate the mean ± s.e.m. (b–k). Data are analyzed by one-way ANOVA followed by Tukey’s multiple-comparisons test (c–e) or unpaired two-tailed Student’s t-test (b, f, k), and P values are shown above plots. Data are representative of three independent experiments. NS, not significant.
previously in B cells\(^4\), the BMDCs derived from \(NfkB_{2\text{ym}1/2}\) heterozygous mice predominantly expressed the mutated p100, which had smaller molecular size than wild-type p100 due to the loss of its C-terminal region (Fig. 5e). Therefore, for the convenience of breeding, we employed the \(NfkB_{2\text{ym}1/2}\) heterozygous mice. TLR ligands stimulated p100 processing to generate p52 in wild-type BMDCs but not in the \(NfkB_{2\text{ym}1/2}\) DCs (Fig. 5e). Furthermore, the TLR-stimulated nuclear translocation of p52 and RelB was also diminished in the \(NfkB_{2\text{ym}1/2}\) DCs (Fig. 5f).

Consistent with the results obtained with NIK-deficient DCs, \(I23a\) gene expression was significantly attenuated in TLR-stimulated \(NfkB_{2\text{ym}1/2}\) BMDCs as well as freshly isolated \(NfkB_{2\text{ym}1/2}\) LP DCs (Fig. 5g and Supplementary Fig. 7a). Furthermore, as seen with the \(Map3k14\) cKO mice, the \(NfkB_{2\text{ym}1/2}\) mice had significantly reduced concentration of fecal IgA and plgR compared with the wild-type control mice (Supplementary Fig. 7b,c). Similarly, the \(NfkB_{2\text{ym}1/2}\) mice also had reduced frequencies of LP T\(_h\)17 cells and ILCs (Supplementary Fig. 7d,e), associated with significantly increased Enterococcus spp. and C. Savagella (Supplementary Fig. 7f,g).

To examine whether \(I23a\) was a direct target gene of noncanonical NF-kB, we performed chromatin immunoprecipitation (ChIP) assays. The TLR ligands CpG and Pam3CSK4 stimulated the association of several NF-kB members to the \(I23a\) promoter in wild-type DCs (Fig. 5h). Consistent with its NIK-independent activation, RelA bound to the \(I23a\) promoter to a similar level in wild-type and NIK-deficient DCs in response to TLR stimulation (Fig. 5h). In contrast, the association of p52 and RelB with \(I23a\) promoter was significantly reduced in NIK-deficient DCs (Fig. 5h), which was in line with the defect of these cells in TLR-stimulated p52/RelB

![Image](60x364 to 138x442)
nuclear translocation (Fig. 5a). Together, these results suggest that TLR stimulates NIK-dependent activation of noncanonical NF-κB, which in turn is required for the induction of Il23a gene expression and mucosal immune homeostasis.

**TLR activation of noncanonical NF-κB requires TNFRII.** NIK is constantly degraded through its ubiquitination by an E3 ubiquitin ligase complex composed of cIAP (cIAP1 or cIAP2), TRAF2, and TRAF3. Induction of noncanonical NF-κB signaling by TNFR superfamily members involves disruption of the cIAP–TRAF2–TRAF3 ubiquitin ligase complex, typically mediated through degradation of TRAF3\(^{12}\). Stimulation of DCs with TLR ligands did not induce appreciable loss of TRAF3, cIAP1, or cIAP2, but triggered a dramatic loss of TRAF2 (Fig. 6a,b). This result was unexpected, since TRAF2 typically functions in the TNFR signaling pathways, instead of the TLR pathways. We noticed that the TLR-stimulated TRAF2 degradation involved delayed kinetics (Fig. 6c). Thus, we surmised that TLR-stimulated TRAF2 degradation and noncanonical NF-κB activation might be mediated by an indirect mechanism, likely involving the autocrine action of a TNFR superfamily member. One TNFR family member known to stimulate TRAF2 degradation is TNFRII (also called CD120b)\(^ {12}\). We found that TNFRII was abundantly expressed on DCs and further induced by TLR ligands (Fig. 6d). Because TLR ligands are also strong inducers of TRAF3\(^{12}\), we examined the role of TNFRII in DCs. We first determined the role of TNFRII in TLR-stimulated noncanonical NF-κB signaling and Il23a gene expression. A TNFRII neutralizing antibody profoundly inhibited CpG-stimulated TRAF2 degradation and p52 nuclear translocation as well as induction of IL-23 p19 by CpG and Pam3CSK4 (Fig. 6e,f). Parallel studies using BMDCs prepared from mice deficient in the TNFRII encoding gene Tnfsf11b (Tnfsf11b\(^{-/-}\)) revealed a complete blockade of TLR-stimulated TRAF2 degradation, associated with impaired p52 nuclear expression and Il23a gene induction (Fig. 6g,h). Consistently, Tnfsf11b\(^{-/-}\) mice had a significant reduction in the concentration of fecal IgA and plgR, plgR expression in IECs, and the frequency of T\(_{17}\) cells and ILCs in LP compared with...
TNFRII, highlighting a dynamic mechanism triggering noncanonical IL-6 expression. We infected the wild-type and Tnfrsf1b−/− mice with C. rodentium, particularly the mouse intestinal bacterial pathogen C. rodentium, to investigate its role in the late phase induction of IL-22-producing T H22 and TH17 subtypes. C. rodentium infection and higher colon shortening characteristics of colon inflammation (Fig. 7b), indicating a severe bodyweight loss (Fig. 7a), significant colon shortening characteristics of colon inflammation (Fig. 7b), and higher C. rodentium titer in the spleen, liver, and feces (Fig. 7c). Consistently, histological analysis revealed much more severe damage in the colon tissue of the Map3k14-cKO mice compared with the wild-type mice. Each symbol represents an individual mouse, and small horizontal lines indicate the mean (± s.e.m.) (f,h,i). Data are analyzed by unpaired Student’s t-test with P values shown above the plots (f,h,i). Data were representative of two independent experiments (h,i) or three independent experiments (a-g).

Wild-type controls (Fig. 6i-1). These data suggest that induction of noncanonical NF-κB activation and Il23a gene expression involves TNFRII, highlighting a dynamic mechanism triggering noncanonical NF-κB signaling in DCs.

Nik regulates host defense against pathogen infections. In addition to regulating intestinal homeostasis, IL-23 and IL-22 play an important role in promoting mucosal immunity against pathogens, particularly the mouse intestinal bacterial pathogen C. rodentium. Early phase of host defense against C. rodentium infection requires IL-23-dependent induction of IL-22 in ILC3s, although the late phase induction of IL-22-producing T H22 and T H17 subsets of CD4+ T cells is largely independent of IL-23 and dependent on IL-6. We infected the wild-type and Map3k14-cKO mice with C. rodentium and monitored inflammation caused during the early phase of infection. With the dose used in our experiment, the wild-type mice were largely resistant to C. rodentium infection and did not display substantial bodyweight loss (Fig. 7a). In contrast, the Map3k14-cKO mice were much more sensitive to C. rodentium infection, showing a severe bodyweight loss (Fig. 7a), significant colon shortening characteristics of colon inflammation (Fig. 7b), and higher C. rodentium titer in the spleen, liver, and feces (Fig. 7c). Consistently, histological analysis revealed much more severe damage in the colon tissue of the Map3k14-cKO mice compared with the wild-type mice (Fig. 7d). Studies using the Nkbp1−/− mice revealed similar results (Supplementary Fig. 8), emphasizing the function of NIK-dependent noncanonical NF-κB pathway in the regulation of mucosal immunity against C. rodentium infection.

In response to C. rodentium challenge, the Map3k14-cKO mice had a significantly lower level of IL-22-producing ILCs than wild-type controls (Fig. 6i-1). These data suggest that induction of noncanonical NF-κB activation and Il23a gene expression involves TNFRII, highlighting a dynamic mechanism triggering noncanonical NF-κB signaling in DCs.
wild-type control mice (Fig. 7c), which was in line with the critical role of ILC-derived IL-22 in mediating the early phase of host defense against C. rodentium infections.\(^{26,27}\) However, the Map3k14-cKO and wild-type mice had comparable frequencies of CD4^+ T cells expressing IL-22 (T_H17 cells), IL-17, or both IL-17 and IL-22 (T_H17 cells) on C. rodentium infection (Fig. 7f). Parallel qRT-PCR analysis revealed reduced expression of Il23a and Il22 in the colonic tissue of Map3k14-cKO mice (Fig. 7g). The phenotype of Il23a expression became more striking when we used purified LP DCs (Fig. 7h). DC-specific NIK deficiency also reduced the expression of bacterial genes, RegIIIb and RegIIIg, known to be induced by IL-22 (Fig. 7i). These results demonstrate an important role for the DC-specific NIK in regulating mucosal immunity against C. rodentium infection and further emphasized the role of NIK in mediating IL-23 induction in DCs and generation of IL-22-producing ILCs.

DC-specific NIK deletion ameliorates colonic inflammation. While IL-23 and IL-17 are important for host defense against infections, these cytokines also contribute to colon inflammation under conditions that disrupt intestinal homeostasis and have been associated with human inflammatory bowel disease (IBD).\(^{30,31,40,41}\) A well-defined animal model of chronic intestinal inflammation is the IL10^−/− mouse, which spontaneously develops colitis associated with bodyweight loss, rectal prolapse, and increased mortality.\(^{32,43}\) The chronic colon inflammation of IL10^−/− mice is dependent on IL-23, which appears to act through promoting T_H17 cell differentiation and expansion.\(^{31}\) As expected, the IL10^−/− mice spontaneously developed signs of colitis by three months of age, including rectal prolapse, body weight loss, and histological features of inflammation characterized by the thickening of mucosa (Fig. 8a–c). Remarkably, DC-specific deletion of NIK in the IL10^−/− mice largely prevented the development of these symptoms of inflammation.
inflammation (Fig. 8a–c). Consistently, DC-specific deletion of NIK in Il10–/– mice inhibited the expression of IL-23 in LP DCs (Fig. 8d), coupled with reduced frequencies of IL-17-producing T cells (Fig. 8e). Interestingly, however, the NIK deficiency did not significantly affect the frequency of ILC3s (Fig. 8f). The spontaneous colitis of Il10–/– mice is also associated with an increase in the level of secreted IgA. We found that DC-specific NIK deletion drastically reduced the level of fecal IgA in Il10–/– mice (Fig. 8g). Thus, NIK has a crucial role in the regulation of colonic inflammation in the Il10–/– mouse model.

Discussion

In this study, we identified the noncanonical NF-κB kinase NIK as a pivotal mediator of DC functions in the regulation of IgA secretion and microbiota homeostasis. Our data suggest that DCs control plgR expression and IgA secretion through a signaling mechanism that requires NIK and its downstream noncanonical NF-κB pathway. The NIK signaling axis facilitates TLR-stimulated IL-23 production and, thereby, maintains the abundance of Th17 cells and ILC3s, major sources of IL-17. IL-17 in turn induces the expression of plgR in IECs to support IgA secretion.

Consistent with its involvement in mucosal immunity, the NIK pathway played a role in regulating the homeostasis of intestinal microbiota. DC-specific NIK deletion or Nfkβ2α–/– mutation increased the abundance of Enterococcus spp. and the SFB C. Savagella. SFB induce mucosal immunity, including IgA production, whereas the secretory IgA is required for controlling SFB expansion in the intestine23,24. Enterococci are known as low abundance gut commensals that are induced to cause opportunistic infections under pathogenic conditions and are associated with intestinal inflammation18–20. A recent report suggests that ILC-derived IL-22 is crucial for controlling the expansion and invasion by enterococci25. Interestingly, we found that the Enterococcus expansion in Map3k14-cKO mice was associated with a severe reduction in IL-22-producing ILCs, supporting the involvement of IL-22 in Enterococcus spp. control. However, we believe that the defective IgA secretion may also contribute to the microbiota deregulation in Map3k14-cKO mice. We found that Enterococcus spp., like C. Savagella, are IgA-coated bacteria. Interference of IgA secretion by antibody-mediated IL-17 neutralization causes an increase in Enterococcus numbers. Although the relative contribution of IgA and IL-22 to the regulation of enterococci is unclear,
it is apparent that IL-23 plays an important role in controlling these enteric bacteria, since antibody-mediated IL-23 neutralization also causes Enterococcus upregulation.

Noncanonical NF-κB activation is typically mediated by signals from the TNFR superfamily members14. Our current study suggests that the noncanonical NF-κB pathway may be stimulated by TLR ligands in intestinal DCs. However, it appeared that this novel function of TLRs is coupled with specific members of the TNFR superfamily, particularly TNFRII. TNFRII was highly expressed on DCs and further upregulated on TLR stimulation. We obtained strong evidence that TNFRII is involved in TLR-stimulated TRAF2 degradation and noncanonical NF-κB activation. Consistently, the TNfsf11b<sup>−/−</sup> mice had reduced frequency of T<sub>H</sub>17 cells and ILCs and impaired IgA secretion. It is possible that TLRs stimulate TNFRII-mediated noncanonical NF-κB activation through upregulation of TNFRII and induction of its ligand, TNF. However, our data do not exclude the involvement of additional TNFR members. In fact, another TNFR member, CD40, was also strongly induced in DCs by lipopolysaccharide (LPS), although not by another TLR ligand, Pam3CSK4. Unlike the ligand of TNFRII, which is expressed in DCs, the CD40 ligand is expressed on T cells and is involved in T cell-dependent CD40 stimulation. Another TNFR member that may be involved in noncanonical NF-κB activation in DCs is lymphotoxin beta receptor (LTβR), since it has been implicated in the regulation of intestinal DCs<sup>15</sup>.

The function of noncanonical NF-κB in DCs has been poorly studied, since the DC-conditioned NIK knockout mice were not available until recently16. Our finding that DC-specific NIK is crucial for intestinal homeostasis is novel and unexpected. We identified Il23a as a major target gene of the noncanonical NF-κB pathway in DCs stimulated with different TLR ligands. The requirement of noncanonical NF-κB for IL-23 expression was also demonstrated in freshly isolated LP DCs, thus emphasizing the physiological relevance of this finding. IL-23 and its downstream cytokine IL-17 have paradoxical roles in the regulation of intestinal homeostasis. While these cytokines promote host defenses against infections and shape the microbiota, they are also important players in colon inflammation and have been associated with human IBD<sup>13,21,40</sup>. In agreement with its role in mediating Il23a gene induction, NIK plays a crucial role in the Il10<sup>−/−</sup> animal model of IBD.

In summary, our data provide genetic evidence that the NIK signaling axis is crucial for the functions of DCs in regulating intestinal immunity and homeostasis. The DC-specific NIK is required for maintaining IgP expression in IECs and, thus, IgA secretion, as well as involved in modulation of microbiota composition. Our work identifies Il23a as a novel target gene of the NIK-regulated noncanonical NF-κB pathway in DCs. Given the crucial role of IL-23 in regulating mucosal immune homeostasis and inflammation, this finding provides important insight into the molecular mechanism that mediates the mucosal function of DCs. Our work also has important implications for preventive and therapeutic approaches for colon inflammation.

**Online content**

Any methods, additional references, Nature Research reporting summaries, source data, statements of data availability and associated accession codes are available at [https://doi.org/10.1038/s41590-018-0206-z](https://doi.org/10.1038/s41590-018-0206-z).

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**Author contributions**

Z.J. and J.-Y. Y. designed and performed the research, prepared the experiments, and wrote part of the manuscript; M.G., H.W., X.L., Y.L., T.L., L.Zhu, J.S., L.Zhang, X.Z., D.J., D.L., and X.C. contributed experiments; H.D.B. contributed critical reagents; Y.C. made supervising contributions to intestinal immune cells analyses; and S.-C.S. supervised the work and wrote the manuscript.

**Competing interests**

H.D.B. is an employee of Genentech, Inc. The other authors declare no competing interests. Genentech, Inc. provided the Map3k14-flox mice.

**Additional information**

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Methods

Mice. Map3k14-Flox mice (on C57BL/6 background), provided by Genentech, were generated using loxP system targeting exon 2 of the Map3k14 gene. The Map3k14-Flox mice were crossed with Cdl1-Cre transgenic mice (B6 genetic background, Jackson Laboratories) to create Map3k14Δ/Δ conditional KO or Map3k14Δ-CKO (Map3k14Δ/Δ+Cdl1-Cre) and wild-type (Map3k14Δ/Δ+Cdl1-Cre) mice. B10Δ mice were purchased from Jackson Lab and crossed with Map3k14Δ-CKO mice to produce l10Δ-Map3k14-ko and control l10Δ mice. Rorcα and Tnfrsf1bΔ mice were purchased from Jackson Lab. Nfkb2Δ mice were provided by R. Starr (Walter and Eliza Hall Institute of Medical Research) and Eliza Hall Institute of Medical Research)36. Nfkb2Δ heterozygous mice were used in experiments since they display strong phenotype in impaired noncanonical NF-kB activation and function37. All KO and conditional KO mice were crossed using heterozygous breeders to generate littermate KO and wild-type control mice for experiments. Mice were maintained in a specific pathogen-free facility, and all animal experiments were in accordance with protocols approved by the Institutional Animal Care and Use Committee of the University of Texas MD Anderson Cancer Center.

Antibodies and reagents. The following fluorochrome-labeled antibodies specific to mouse (m) proteins and their corresponding isotype controls were purchased from ebioscience: Allophycocyanin (APC) - or phycocerythrin (PE) - conjugated anti-mIL-17A (ebio778), fluorescein isothiocyanate (FITC) - or APC-conjugated anti-mIFN-γ (XMGI1.2), PE-conjugated anti-mIL-22 (H18PSWR), PE-Cy7-conjugated anti-mCD3 (17A4), Pacific blue-conjugated anti-mCD4 (GK1.5), PerCP-Cy5.5-conjugated anti-mCD8 (53-6.7), PE-Cy7-conjugated anti-mNK1.1 (PK1.3), PE- or PerCP-Cy5.5-conjugated anti-mR202 (RA3-β2), PE-Cy7-conjugated anti-mCD11b (M1/70), PE-Cy7-conjugated anti-mCD11c (N418), PE-Cy7-conjugated anti-mGr1 (RB6-8C5), PE-Cy7-conjugated anti-mIer (TER-119), FITC-conjugated anti-mCD45 (30-F11), APC-conjugated anti-mRORγt (B2D), APC- or PE-Cy7-conjugated anti-mMHCII (M5/114.15.2), PE- or FITC-conjugated anti-mCD80 (16–10A1), PE- or FITC-conjugated anti-mCD86 (GL1), PE- or Pacific blue-conjugated anti-mCD11c (A20), PE-Cy7-conjugated anti-mCD103 (2E7), APC-conjugated anti-mCD62L (MEL-14), PE-conjugated anti-mCD44 (IM7), APC-conjugated anti-mEpCAM (G8.8), APC-conjugated anti-mCD317 (B72T), PerCP-Cy5.5-conjugated anti-mCCR7 (4B12), and eFluor 506 conjugated fixable viability dye. Purified anti-mCD16/32 (C3/130.13) conjugated anti-mlgA (C10-3), FITC-conjugated anti-mCD90.2 (30–H12), PE-Cy7-conjugated anti-mCD8CRX (G8), and APC-conjugated anti-mPd1–I43 were purchased from BD Pharmingen. PE-conjugated anti-mAPRIL (A3D8) and Alexa Fluor647-conjugated anti-mTLR5 (ACT5) were purchased from BioLegend. PE-conjugated anti-mTLR9 (5G11b) was purchased from Bio-Rad. PE-conjugated anti-mBAFF (121808) was purchased from R&D Systems. Anti-Actin (C-4, 1:10,000) was purchased from Sigma, and anti-p100/p65 (E24, 1:8,000) was purchased from National Cancer Institute Preclinical Repository. Antibodies for RbEl (C-19, 1:1,000), Lamin B (C-20, 1:1,000), NIK (H24, 1:1,000), TRAF2 (C-20, 1:1,000), TRAF3 (H-122, 1:1,000), C/EBP (H85, 1:1,000), phospho-p53 (Ser25, 1:1,000), and c-Rel (sc71, 1:3,000) as well as a control rabbit IgG (sc-2027, 1:1,000) were purchased from Santa Cruz Biotechnology. Antibodies for phospho-h-cRel (Ser32, 1:1,000), and eFluor 506 conjugated fixable viability dye were purchased from Thermo Fisher Scientific. The entire colon was removed, opened longitudinally, rinsed to clean internal contents, and rolled over the full length to obtain a ‘Swiss roll’, which was then fixed in 4% formaldehyde in PBS for 1 h at 25°C. The samples were dehydrated by gradually soaking in alcohol and xylene and then embedded in paraffin. The paraffin-embedded specimens were cut into 5 μm sections, stained with hematoxylin–eosin (H&E), and viewed with a digital inverted light microscope (EVOS, Thermo Fisher Scientific).

Confocal analysis. For pglr staining, small intestinal tissue was cut longitudinally and rolled over a ‘Swiss roll’, which was then fixed in 4% formaldehyde in PBS for 1 h at 25°C. Fixed tissues were dehydrated by gradually soaking in sucrose (10–30%) and then embedded in cryomatrix (Thermo Scientific). The frozen sections were sequentially reacted with goat anti-pglr IgG Antibody (R&D Systems) at 4°C overnight, and then incubated with FITC-conjugated donkey anti-goat antibody (Jackson ImmunoResearch) at room temperature for 2 h and viewed under a confocal scanning laser microscope (Zeiss). The pglr* dots were counted on the intestinal villi that were randomly selected in five regions per slide. Data shown are mean values ± s.e.m. of individual mice from two independent experiments with three mice per experiment.

Histology analysis. The entire colon was removed, opened longitudinally, rinsed to clean internal contents, and rolled over the full length to obtain a ‘Swiss roll’, which was then fixed in 4% formaldehyde in PBS for 1 h at 25°C. Fixed tissues were dehydrated by gradually soaking in alcohol and xylene and then embedded in paraffin. The paraffin-embedded specimens were cut into 5 μm sections, stained with hematoxylin–eosin (H&E), and viewed with a digital inverted light microscope (EVOS, Thermo Fisher Scientific).

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C. rodentium infection. C. rodentium preparation and infection as were previously described. Briefly, C. rodentium strain DSS100 (ATCC 31459) was grown in LB broth at 37°C overnight with shaking. The number of bacteria was measured by checking optical density (OD) at 600 nm and further confirmed as CFUs by spreading 10 μl agar plates at 37°C with shaking for 18 h. In the previous washing step was centrifuged (10,000 rpm, 10 min, 5°C), and then sorted by MACS. After MACS separation, each 50 μl of the positive and negative fraction was collected for clarification of cell separation and for cell counting. Each sample was diluted and smeared on several selective agar media to detect IgA-positive commensal bacteria. The sorted IgA-positive and negative fractions were further analyzed by 16S RNA gene sequencing.

Histology analysis. The entire colon was removed, opened longitudinally, rinsed to clean internal contents, and rolled over the full length to obtain a ‘Swiss roll’, which was then fixed in 4% formaldehyde in PBS for 1 h at 25°C. Fixed tissues were dehydrated by gradually soaking in alcohol and xylene and then embedded in paraffin. The paraffin-embedded specimens were cut into 5 μm sections, stained with hematoxylin–eosin (H&E), and viewed with a digital inverted light microscope (EVOS, Thermo Fisher Scientific).

Confocal analysis. For pglr staining, small intestinal tissue was cut longitudinally and rolled over a ‘Swiss roll’, which was then fixed in 4% formaldehyde in PBS for 1 h at 25°C. Fixed tissues were dehydrated by gradually soaking in sucrose (10–30%) and then embedded in cryomatrix (Thermo Scientific). The frozen sections were sequentially reacted with goat anti-pglr IgG Antibody (R&D Systems) at 4°C overnight, and then incubated with FITC-conjugated donkey anti-goat antibody (Jackson ImmunoResearch) at room temperature for 2 h and viewed under a confocal scanning laser microscope (Zeiss). The pglr* dots were counted on the intestinal villi that were randomly selected in five regions per slide. Data shown are mean values ± s.e.m. of individual mice from two independent experiments with three mice per experiment.

Cell preparation from intestine. For isolation of lymphocytes from small intestinal LP (SI-LP), tissue pieces were treated with RPMI 1640 containing 0.5 mM EDTA for 20 min at 37°C to remove epithelial cells. This washing step was repeated twice. Tissues were minced and then treated with 500 μl 1% collagenase D (Sigma-Aldrich) and 100 μg/ml DNase I (Roche Applied Science) in RPMI 1640 containing 10% FBS and digested for 30 min at 37°C with shaking. This enzyme reaction was also repeated twice. The lymphocytes of LP were enriched by using 40% and 75% Percoll gradients (Amersham Biosciences). For isolation of IECs and IELs from SI-LP, the filtered liquid in the previous washing step was centrifuged (700g, 20 min, 25°C) in 3 different Percoll gradients (25%/40%/75%) with no brake. IECs and IELs were enriched in the boundary between 25% and 40% and between 40% and 75% Percoll, respectively. For sorting LP-DCs and IECs, LP cells were isolated and stained with FITC-conjugated anti-mCD11c and APC-conjugated anti-mHICHI. LP-DCs were stained with CD11c+MHCII+ cells using FACS Aria. IECs were isolated as described previously and were further sorted as CD45 EpCAM+ population using FACS Aria.
Flow cytometry and intracellular cytokine staining. Single-cell suspensions of spleen, mesenteric lymph nodes, Peyer’s patches, LP, bone marrow, and thymus were subjected to flow cytometry using an LSRFortessa (Becton Dickinson). The cells were incubated with anti-CD16/CD32 (eBioscience) to block nonspecific antibody binding via Fc receptors and then stained with fluorochrome-labeled antibodies, and subsequently processed on an LSRFortessa. The data were analyzed using FlowJo software (Tree Star). Gating strategies are summarized in Supplementary Fig. 9. For intracellular cytokine staining (ICS) detection of IFN-γ, IL-17, and IL-22, cells were stimulated for 4–5 h with PMA (50 ng ml$^{-1}$), ionomycin (750 ng ml$^{-1}$), and brefeldin A (5 μg ml$^{-1}$), and fixed with 4% paraformaldehyde fixed, permeabilized, and stained for intracellular cytokines by using a fixation/permeabilization kit (BD Biosciences). Samples were processed on an LSRFortessa (Becton Dickinson) and analyzed by using FlowJo software (TreeStar).

**IL-17 and IL-12/IL-23 p40 neutralization.** For IL-17 neutralization, mice were intraperitoneally injected with anti-mouse IL-17 antibody (Clone: 17F3) or isotype control IgG1 (Clone: MOPC-21) (10 μg g$^{-1}$ mouse weight) every other day for 2 weeks. For IL-12/IL-23 p40 neutralization, mice were intraperitoneally injected with anti-mouse IL-12/IL-23 p40 antibody (Clone: C17.8) or isotype control IgG2a (Clone: 2A3) (100 μg per mice) every other day for two weeks. Anti-mouse IL-17 (Clone: 17F3), anti-mouse IL-12/IL-23 p40 (Clone: C17.8), mouse IgG1 isotype control (Clone: MOPC-21), and mouse IgG2a isotype control (Clone: 2A3) were bought from BioXcell company.

**Real-time qRT-PCR.** Total RNA was isolated from DC cells or colon tissue using TRI reagent (Invitrogen) and subjected to cDNA synthesis using MMLV reverse transcriptase (Invitrogen) and oligo (dT) primers. qRT-PCR was performed using iCycler Sequence Detection System (Bio-Rad) and iQ SYBR Green Supermix (Bio-Rad). The expression of individual genes was calculated by a standard curve method and was normalized to the expression of Actb. The primers used in qRT-PCR assays are shown in Supplementary Table 1.

**Immunoblot assays.** Whole-cell and subcellular extracts were prepared and subjected to IB essentially as described$^{49}$. In brief, protein samples were separated by SDS-PAGE, transferred to PVDF membranes (0.45 μm, Millipore), and blocked for 1 h with 5% BSA, 0.1% Tween-20 in TBS. The membranes were then washed and incubated with primary antibodies overnight. The goat anti-rabbit or goat anti-mouse IgG secondary antibodies (1:5,000 dilution) were used to incubate for 1 h. After washing, immunoreactive bands were visualized by chemiluminescent detection (ECL, Roche Diagnostics) and exposure to X-ray film (Thermo Fisher Scientific).

**ChIP assays.** BMDCs were stimulated for 12 h with CpG, Pam3CSK4, and LPS and then fixed with 1% formaldehyde and sonicated as previously described$^{54}$. Lysates (2× 10$^6$ cells in 3 ml) were subjected to IP with the indicated antibodies, and the precipitated DNA was then purified by Qiaquick columns (Qiagen) and quantified by qPCR using a pair of primers that amplify the Il23a promoter (Supplementary Table 1). The y axis is the percentage (%) of target transcript factor-binding DNA normalization for total input DNA.

**ELISA.** For IgA and pIgR ELISA analysis, fresh feces were collected from individual mice, homogenized in sterile PBS, and centrifuged to remove bacteria and insoluble debris. Fecal samples were diluted in sterile PBS as a twofold serial dilution and added to 96-well plates pre-coated overnight (at 4 °C) with 1 μg ml$^{-1}$ anti-mouse IgA (Southern Biotechnology) or 0.5 μg ml$^{-1}$ anti-mouse pIgR (R&D Systems). Samples were incubated at room temperature for 2 h. For IgA analysis, HRP conjugated anti-IgA (Southern Biotechnology) was added for 1 h. For pIgR ELISA analysis, biotinylated mouse pIgR antibody was added for 1 h, followed by incubation with avidin-HRP for 30 min. Plates were developed using dTMB substrate according to the manufacturer’s instructions (Thermo Fisher Scientific) and analyzed at 450 nm using a microplate reader.

**For ELISA analysis of cytokines (IL-23p19, IL-1β, and IL-6), equal numbers of wild-type and Mapt$^{−/−}$ BMDCs were stimulated with CpG or Pam3CSK4 for 48 h, and supernatants were collected for ELISA analysis using an ELISA kits (Thermo Fisher Scientific) according to the manufacturer’s instructions. Detection limits were 16 pg ml$^{-1}$ for IL-23p19, 1.2 pg ml$^{-1}$ for IL-1β, and 4 pg ml$^{-1}$ for IL-6.**

**Statistical analysis.** Statistical analysis was performed using GraphPad Prism software (GraphPad). Significant differences between two groups were analyzed with two-tailed unpaired t-test. Multiple groups were analyzed by one-way analysis of variance (ANOVA), where applicable, was performed to determine whether an overall statistically significant change existed before the multiple Student’s t-test to analyze the difference between any two groups. The body weight loss was analyzed by multiple t-tests, and in turn statistical significance was determined by using the Holm–Sidak method. The prolapse-free rate was compared using the Kaplan–Meier method, with differences determined by log-rank test.

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

**Data availability**

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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☐ The statistical test(s) used AND whether they are one- or two-sided

☐ Only common tests should be described solely by name; describe more complex techniques in the Methods section.

☐ A description of all covariates tested

☐ A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons

☐ A full description of the statistics including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)

☐ For null hypothesis testing, the test statistic (e.g. F, t, r) with confidence intervals, effect sizes, degrees of freedom and P value noted

☐ Give P values as exact values whenever suitable.

☐ For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings

☐ For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes

☐ Estimates of effect sizes (e.g. Cohen's d, Pearson's r), indicating how they were calculated

☐ Clearly defined error bars

☐ State explicitly what error bars represent (e.g. SD, SE, CI)

Our web collection on statistics for biologists may be useful.

Software and code

Policy information about availability of computer code

Data collection  No software were used for data collection in this study.

Data analysis  We used GraphPad Prism 7 for unpaired two-tail Student t-test, one/two-way ANOVA followed by multiple-comparisons test and Kaplan-Meier method. FlowJo 10.0 FACS software were used to analyze data obtained from Flow Cytometer.

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors/reviewers upon request. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research guidelines for submitting code & software for further information.

Data

Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A list of figures that have associated raw data
- A description of any restrictions on data availability

The datasets generated during the current study are available from the corresponding author on reasonable request.
Field-specific reporting

Please select the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☑ Life sciences ☐ Behavioural & social sciences ☐ Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see nature.com/authors/policies/ReportingSummary-flat.pdf

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size

For in vitro and mouse in vivo experiments, sample sizes were chosen according to the basis of previous publications in the immunology field, without prior power analysis. We usually used more than 4 mice per group to ensure the statistically significant difference could be obtained from unpaired two-tailed Student’s t-test or ANOVA analysis followed by multiple-comparisons test. On the other hand, we also tried to minimize the animal number to follow the guidelines of the animal experiments in our animal protocol. We described the exact numbers of animals/samples for each experiment in the figure legends.

Data exclusions

There were no excluded data or samples from the analysis in this study.

Replication

Experimental findings were reliably reproduced.

Randomization

During the study, we randomly chose the mice from the same littersmates for each experiment group. We also randomly chose the age- and sex- matched littersmates for control groups.

Blinding

As the gene-deficient mice (Map3k14-cKO, Tnfrsf1b−/−, Rorc−/−, Nfkb2lym1/+ ) were preliminary analyzed in other studies, and to definitely identify/distinguish the differences of immune functions among these genes, it was not necessary for us to be blinded to group allocation because this method is usually appropriate for the first investigation of a function-unknown gene.

Reporting for specific materials, systems and methods

Materials & experimental systems

n/a Involved in the study

☑ ☐ Unique biological materials
☑ ☐ Antibodies
☑ ☐ Eukaryotic cell lines
☐ ☐ Palaeontology
☐ ☐ Animals and other organisms
☐ ☐ Human research participants

Methods

n/a Involved in the study

☐ ☑ ChIP-seq
☐ ☑ Flow cytometry
☐ ☑ MRI-based neuroimaging

Antibodies

The following fluorochrome-labeled antibodies specific to mouse (m) proteins and their corresponding isotype controls were purchased from ebioscience: APC- or PE-conjugated anti-mIL-17A (eBio17B7), FITC- or APC-conjugated anti-mIFN-γ (XMG1.2), PE-conjugated anti-mIL-22 (1H8PWSR ), PE-Cy7-conjugated anti-mCD3 (17A2), Pacific blue-conjugated anti-mCD4 (GK1.5), PerCP-Cy5.5-conjugated anti-mCD8 (53-6.7), PE-Cy7-conjugated anti-mNK1.1 (PK136), PE-Cy7-conjugated anti-mB220 (RA3-6B2), PE-Cy7-conjugated anti-mCD11b (M1/70), PE-Cy7-conjugated anti-mCD11c (N418), PE-Cy7-conjugated anti-mGr-1 (RB6-8C5), PE-Cy7-conjugated anti-mTer-119 (TER-119), FITC-conjugated anti-mCD45 (30-F11), APC-conjugated anti-mRORyt (B2D), APC- or PE-Cy7-conjugated anti-mMHCII (M5/114.15.2), PE- or FITC-conjugated anti-mCD80 (16-10A1), PE- or FITC-conjugated anti-mCD86 (GL1), PE- or Pacific blue-conjugated anti-mCD127 (A7R34), PE-conjugated anti-mCD103 (2E7), APC-conjugated anti-mCD62L (MEL-14), PE-conjugated anti-mCD44 (IM7), APC-conjugated anti-mEpCAM (G8.8), APC-conjugated anti-mCD317 (BST2), PerCP-Cy5.5-conjugated anti-mCcr7 (4B12) and eFluor 506 conjugated fixable viability dye. Purified anti-mCD16/32 (2.4G2), PE conjugated anti-mCD120b (TR75-89), FITC-conjugated anti-mIgA (c10-3), FITC-conjugated anti-mCD90.2 (30-H12), PE-Cy7 conjugated anti-mILC2 (ASG8), and APC-conjugated anti-mPO-1 (J43) were purchased from BD Pharmingen. PE-conjugated anti-mAPRIL (A3D8), and Alexa Fluor647-conjugated anti-mTLR5 (ACT5) were purchased from BioLegend. PE-conjugated anti-mBAFF (121808) was purchased from R&D Systems. For in vivo blocking study, anti-mouse IL-17A (17F3), anti-mouse IgG1 (10 F8), and their isotype controls (mouse IgG1, rat IgG2a) were purchased from BioXcell. Anti-mouse pigR was purchased from R&D.

Anti-Actin (C-4:1:10,000) was from Sigma, and anti-p100/p52 (TB4, 1:8,000) was from National Cancer Institute Preclinical
Validation

All the antibodies used for flow cytometry, IB and CHIP assay were validated according to the manufacturers instructions and largely described in the literature.

Animals and other organisms

Policy information about studies involving animals: ARRIVE guidelines recommended for reporting animal research

Laboratory animals

Map3k14-flox mice (on C57BL/6 background), provided by Genentech, were generated using loxP system targeting exon 2 of the Map3k14 gene. The Map3k14-flox mice were crossed with Cd11c-Cre transgenic mice (B6 genetic background, Jackson Laboratories) to create Map3k14 DC-conditional KO or Map3k14-cKO (Map3k14 fl/flCd11c-Cre) and wild-type (Map3k14+/+) mice. Il10–/– mice were purchased from Jackson Lab and crossed with Map3k14-cKO mice to produce Il10–/–Map3k14-cKO and control Il10–/– mice. Rorc–/– and Tnfrsf1b–/– mice were purchased from Jackson Lab. Nfkb2lym1 mice were provided by R. Starr (Walter and Eliza Hall Institute of Medical Research). All KO and conditional KO mice were crossed using heterozygous breeders to generate littermate KO and wild-type control mice for experiments. Mice were maintained in a specific pathogen-free facility, and all animal experiments were in accordance with protocols approved by the Institutional Animal Care and Use Committee of the University of Texas MD Anderson Cancer Center. We usually used sex-matched mice with 8-10 weeks old for this study.

Wild animals

This study did not involve the use of wild animals.

Field-collected samples

This study did not involve the use of field-collected samples.

Flow Cytometry

Plots

- The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).
- The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a 'group' is an analysis of identical markers).
- All plots are contour plots with outliers or pseudocolor plots.
- A numerical value for number of cells or percentage (with statistics) is provided.

Methodology

Sample preparation

Cells were blocked with Fc blocker (CD16/32), and stained for specific surface markers. For intracellular staining, cells were fixed and permeabilized and stained for intracellular cytokines by fixation/permeabilization kit (BD bioscience).

Instrument

Flow cytometry data were collected by LSRII and FACSFortessa.

Software

Flow cytometry data were analyzed by FlowJo software (TreeStar, Ashland, OR).

Cell population abundance

For IEC sorting, we recovered 1-2 x 10^6 cells post sorting.
For intestine DC sorting, we recovered 0.5-1 x 10^6 cells post sorting.

Gating strategy

For immune cells, first we gated the lymphocytes based on the FSC-A and SSC-A. After that, we gated on the single cells, and then gated on CD45+ cells. The specific cell population was gated on the indicated surface markers as described in the manuscript. We added the gating strategy in Supplementary figure 10.

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