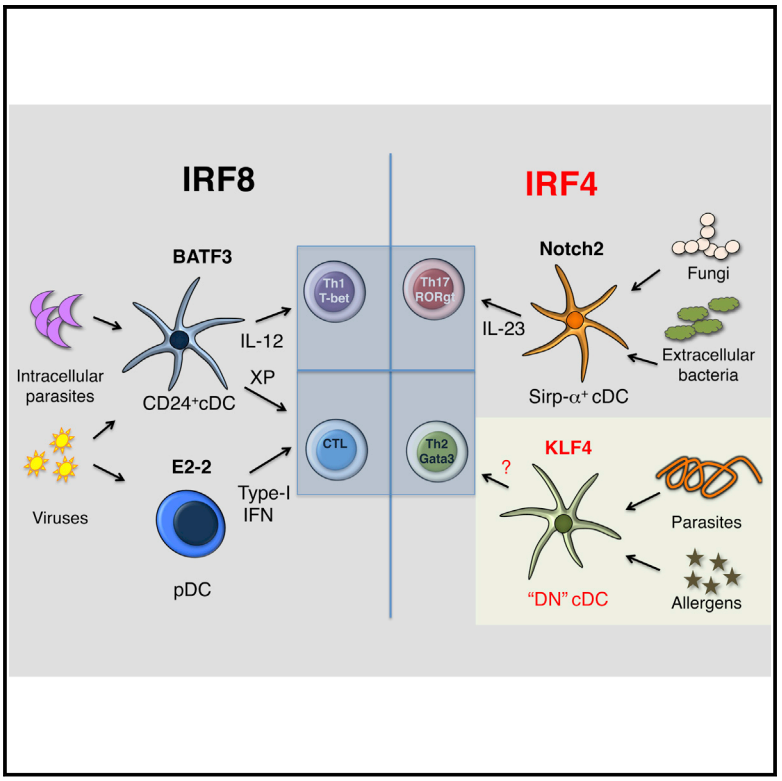
Article

Immunity

Klf4 Expression in Conventional Dendritic Cells Is Required for T Helper 2 Cell Responses

Graphical Abstract



Authors

Roxane Tussiwand, Bart Everts, ..., Edward J. Pearce, Kenneth M. Murphy

Correspondence

kmurphy@wustl.edu (K.M.M.), r.tussiwand@unibas.ch (R.T.)

In Brief

The IRF4-expressing cDCs subset is highly heterogeneous and has been implicated in priming Th17, as well as Th2 cell immunity. Murphy and colleagues dissect this heterogeneity showing a developmental requirement for Klf4 on specific subsets across several tissues. Moreover, Klf4 guides a transcriptional program necessary for Th2 cell immunity.

Highlights

- *Klf4* is required for the development of a subset of IRF4-expressing cDCs
- *Klf4* deletion results in reduced pre-cDCs
- A specific *Klf4*-dependent subset can be identified in several tissues
- *Klf4*$^{fl/fl}$ *Itgax*-cre mice have selectively impaired Th2 cell immunity

Accession Numbers

GSE68590

Tussiwand et al., 2015, Immunity *42*, 916–928
May 19, 2015 ©2015 Elsevier Inc.
http://dx.doi.org/10.1016/j.immuni.2015.04.017

Klf4 Expression in Conventional Dendritic Cells Is Required for T Helper 2 Cell Responses

Roxane Tussiwand,[1,3,*] Bart Everts,[1,4] Gary E. Grajales-Reyes,[1] Nicole M. Kretzer,[1] Arifumi Iwata,[1] Juhi Bagaitkar,[5] Xiaodi Wu,[1] Rachel Wong,[1] David A. Anderson,[1] Theresa L. Murphy,[1] Edward J. Pearce,[1] and Kenneth M. Murphy[1,2,*]

[1]Department of Pathology and Immunology, Washington University School of Medicine, 660 South Euclid Avenue, St. Louis, MO 63110, USA
[2]Howard Hughes Medical Institute, Washington University School of Medicine, St. Louis, MO 63110, USA
[3]Department of Biomedicine, University of Basel, Mattenstrasse 28, 4058, Basel, Switzerland
[4]Department of Parasitology, Leiden University Medical Center, Albinusdreef 2, 2333 Leiden, the Netherlands
[5]Department of Pediatrics, Washington University School of Medicine, 1 Children's Place, St. Louis, MO 63110, USA
*Correspondence: kmurphy@wustl.edu (K.M.M.), r.tussiwand@unibas.ch (R.T.)
http://dx.doi.org/10.1016/j.immuni.2015.04.017

SUMMARY

The two major lineages of classical dendritic cells (cDCs) express and require either IRF8 or IRF4 transcription factors for their development and function. IRF8-dependent cDCs promote anti-viral and T-helper 1 (Th1) cell responses, whereas IRF4-expressing cDCs have been implicated in controlling both Th2 and Th17 cell responses. Here, we have provided evidence that Kruppel-like factor 4 (*Klf4*) is required in IRF4-expressing cDCs to promote Th2, but not Th17, cell responses in vivo. Conditional *Klf4* deletion within cDCs impaired Th2 cell responses during *Schistosoma mansoni* infection, *Schistosoma* egg antigen (SEA) immunization, and house dust mite (HDM) challenge without affecting cytotoxic T lymphocyte (CTL), Th1 cell, or Th17 cell responses to *herpes simplex virus*, *Toxoplasma gondii*, and *Citrobacter rodentium* infections. Further, *Klf4* deletion reduced IRF4 expression in pre-cDCs and resulted in selective loss of IRF4-expressing cDCs subsets in several tissues. These results indicate that *Klf4* guides a transcriptional program promoting IRF4-expressing cDCs heterogeneity.

INTRODUCTION

Conventional dendritic cells (cDCs) are professional antigen-presenting cells that play a key role in shaping appropriate immune responses (Banchereau and Steinman, 1998; Merad et al., 2013; Satpathy et al., 2012b; Mildner and Jung, 2014). Several transcription factors have been implicated in cDCs development, but the basis for specification and commitment of cDC subsets is still incompletely understood (Belz and Nutt, 2012; Murphy, 2013). One major subset of cDCs identified by the expression of CD8α in spleen, and CD24 or CD103 in the periphery, requires the transcription factors IRF8 (Hambleton et al., 2011; Tailor et al., 2008), BATF3 (Edelson et al., 2010; Hildner et al., 2008; Ginhoux et al., 2009), NFIL3 (Kashiwada et al., 2011), and ID2 (Hacker et al., 2003; Spits et al., 2000). Selective loss of CD8α^+ and CD103$^+$ cDCs in *Batf3*$^{-/-}$ mice demonstrated the specialization of this cDC subset in promoting anti-viral immunity, tumor rejection, and protection against *Toxoplasma gondii* infection (Mashayekhi et al., 2011; Hildner et al., 2008; Tussiwand et al., 2012; Pinto et al., 2011; Torti et al., 2011). The second major branch of cDCs is characterized by the expression of IRF4 and CD11b and is developmentally impacted by the transcription factors *RelB*, *Traf6*, *Notch2*, *Irf2*, and *Irf4* (Mildner and Jung, 2014).

The function of CD11b$^+$ cDCs in controlling different classes of immune responses has been recently examined (Lewis et al., 2011; Satpathy et al., 2013; Persson et al., 2013; Schlitzer et al., 2013; Williams et al., 2013; Gao et al., 2013; Kumamoto et al., 2013; Zhou et al., 2014). Conditional *Notch2* deletion in cDCs impaired development of CD11b$^+$ cDCs expressing CD4 and the endothelial cell-selective adhesion molecule (ESAM) (Lewis et al., 2011; Satpathy et al., 2013). These mice are susceptible to infection with *Citrobacter rodentium*, resulting from a requirement for interleukin-23 (IL-23) production by *Notch2*-dependent cDCs early during infection (Satpathy et al., 2013). Similarly, conditional deletion of *Irf4* in cDCs causes a reduction in the numbers of CD11b$^+$ cDCs, and reduced IL-23 production leading to impaired Th17 cell development in both lung and intestine (Persson et al., 2013; Schlitzer et al., 2013). Consistently, mice lacking IRF4 expression in cDCs are therefore susceptible to pulmonary infection with *Aspergillus fumigatus* (Schlitzer et al., 2013). Subsequent studies showed that *Irf4*-dependent CD11b$^+$ cDCs are involved in Th2 cell responses in skin and lung (Gao et al., 2013; Kumamoto et al., 2013; Williams et al., 2013; Zhou et al., 2014). However, other reports have demonstrated that IRF4 acts in both migration of CD11b$^+$ cDCs (Bajaña et al., 2012) and expression of major histocompatibility complex (MHC)-II and co-stimulatory molecules in cDCs (Vander Lugt et al., 2014). As a result, it is unclear whether there is a specific cDC subset dedicated to Th2 cell priming that is selectively dependent on IRF4, or whether IRF4 acts in all CD11b$^+$ cDCs, affecting their migration and maturation in both Th17 and Th2 cell responses.

We identified Kruppel-like factor 4 (*Klf4*) as a potential candidate for regulating the development of the IRF4-expressing CD11b$^+$ cDCs. *Klf4* can act as a repressor or activator of transcription and regulates development in several epithelial tissues, including skin, lung, and intestine (Segre et al., 1999; Dang et al., 2000; Katz et al., 2002; Dang et al., 2000; Ghaleb et al., 2005; Feinberg et al., 2007; Alder et al., 2008; Zheng et al., 2009; McConnell and Yang, 2010; Yamanaka, 2008; Yoshida and

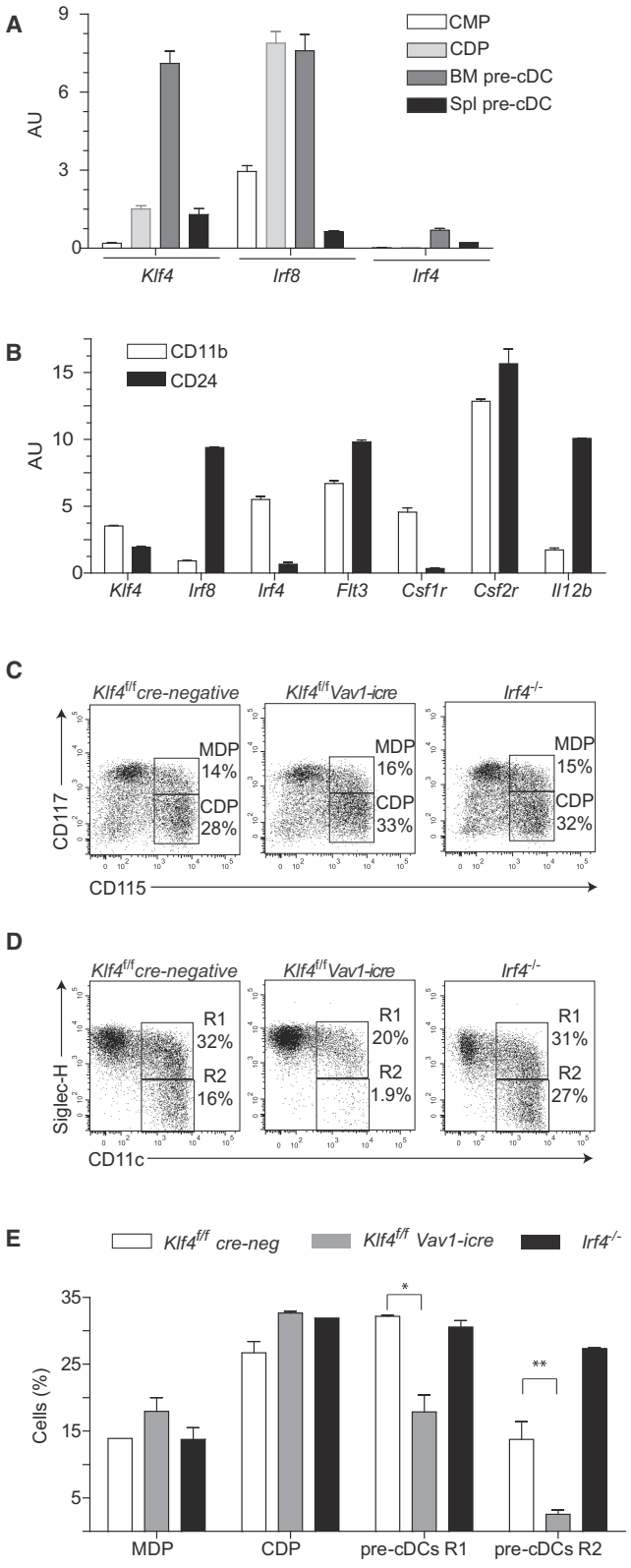


Figure 1. KLF-4 Deletion Impairs Development of Bone-Marrow pre-cDC Progenitors

(A) Relative expression of *Klf4*, *Irf8*, and *Irf4* determined by microarray analysis is shown for the indicated stages of DC progenitors.

(B) Relative expression determined by microarray analysis of the indicated genes is shown for splenic CD24$^+$ Sirp-α^- CD11b$^-$ (CD24) and CD24$^-$ Sirp-α^+ CD11b$^+$ (CD11b) DCs.

(C and D) DC progenitors in BM were analyzed from wild-type mice (WT), *Klf4*$^{fl/fl}$ (*cre*-neg) or *Klf4*$^{fl/fl}$ mice crossed onto *Vav1-icre* (Harker et al., 2002), or *Irf4*$^{fl/fl}$ mice crossed into B6.C-Tg(CMV-cre)1Cgn/J (The Jackson Laboratory) backgrounds. CDP, MDP, and pre-cDC were gated as described (Liu et al., 2009) in the Experimental Procedures and analyzed for the indicated markers.

(E) The percent of the progenitors from the indicated genotype analyzed in (C and D) are shown. The experiment was repeated four times, and two mice per genotype were analyzed. Error bars ± SD, n = 5, Student's t test. *p < 0.05; **p < 0.001.

See also Figure S1.

Hayashi, 2014). In hematopoietic cells, *Klf4* is expressed on myeloid cells, is required for monocyte development (Feinberg et al., 2007; Alder et al., 2008; Kurotaki et al., 2013), as well as for in vitro M2 macrophage polarization (Feinberg et al., 2007; Kurotaki et al., 2013; Terry and Miller, 2014). *Klf4* conditional deficient mice have reduced CD11b$^+$ cDCs in spleen; however, the nature of the defect was not further analyzed with respect to cDC subsets or function (Park et al., 2012). Here we showed that *Klf4* is required within IRF4-expressing cDC subsets for normal priming of Th2 cell responses. Our results indicated that the IRF4-expressing cDC lineage is functionally heterogeneous, with *Klf4* promoting a DC transcriptional program controlling Th2 cell responses.

RESULTS

Conditional Deletion of *Klf4* Alters Development of IRF4-Expressing pre-cDCs

Klf4 expression was transiently upregulated at the bone marrow (BM) pre-cDC stage, whereas *Irf8* was induced in common DC progenitors (CDPs) (Liu et al., 2009; Akashi et al., 2000 ; Onai et al., 2007) (Figure 1A). *Klf4* expression within mature splenic cDC subsets was reduced compared to *Irf8* and *Irf4* (Figure 1B). We crossed the *Klf4*$^{fl/fl}$ allele (Katz et al., 2002) onto *Vav1-icre*, *Itgax-cre*, and *Lyz2-cre* deleter strains (Caton et al., 2007; de Boer et al., 2003; Clausen et al., 1999). *Vav1-icre* induced general hematopoietic *Klf4* deletion as expected, whereas *Itgax-cre* deleted *Klf4* only within cDCs (Figure S1A). Deletion of *Klf4* by *Vav1-icre* resulted in loss of Ly6C^{hi} monocyte development (Figures S1B and S1C), as previously reported (Feinberg et al., 2007). Neither *Lyz2-cre*- nor *Itgax*-cre-mediated deletion of *Klf4* impaired Ly6C^{hi} monocyte development, confirming an early developmental requirement for *Klf4* in monocyte differentiation and validating the use of *Itgax-cre* mice for a cDC restricted deletion of *Klf4* (Figures S1B and S1C). *Klf4* deletion by *Vav1-icre* reduced the expression of IRF4 on pre-cDC (Figure S1E) and impaired development of SiglecH$^-$ pre-cDCs (Figures 1C–1E), which also had reduced IRF4 expression (Figure S1E). Macrophage and DC precursors (MDPs) and CDPs were unaltered in *Klf4*$^{fl/fl}$ *Vav1-icre* mice (Figures 1C–1E, S1I). CD11c is induced at the pre-cDC stage (Naik, 2010; Liu et al., 2007) and comparison of *Klf4*$^{fl/fl}$ *Itgax-cre* and *cre*-negative progenitors showed few changes in gene expression in CDPs, but increased differences in pre-cDCs (Figures S1F–S1I). Deletion of *Klf4* by *Vav1-icre* or *Itgax-cre* reduced IRF4 expression in progenitors but still

allowed the divergence of DC progenitors into two major subsets of IRF8$^+$ and IRF4$^+$ cDCs in BM cultures (Figure S1D).

Klf4 Regulates Development of Specific Subsets of IRF4-Expressing cDCs in Peripheral Tissues

We next examined various lymphoid and peripheral tissue-resident DC subsets under conditional deletion of *Klf4* (Figures 2 and S2). Consistent with a previous report (Park et al., 2012), there was approximately 50% reduction in total CD11c$^+$ MHC-II$^+$ cells in spleen (Figures 2D and S2G), mostly compromising Sirp-α^+ CD24$^-$ cDCs (Figures 2D and S2G). Approximately a 50% reduction of CD11b$^+$ cDCs occurred across all lymphoid and peripheral tissues examined (Figures 2 and S2). In skin draining lymph nodes (sLN), one subset within the migratory cDCs was completely absent (Figures 2A, 2B, and S2B). In skin, Langerhans cells (LCs) and at least four other cDCs subsets can be distinguished on the basis of CD207, CD103, and CD11b (Henri et al., 2010). The CD103$^+$CD207$^+$ cDC subset represents the *Batf3*-depedendent cDC lineage, but three other subsets (CD103$^-$CD207$^+$, CD207$^-$CD11b$^+$, and CD103$^-$CD11b$^-$CD207$^-$ cDCs) are independent of *Batf3* (Edelson et al., 2010; Ginhoux et al., 2009) and have distinct transcriptional identities (Miller et al., 2012; Robbins et al., 2008; Bar-On and Jung, 2010). Among these migratory cDC subsets, deletion of *Klf4* completely impaired development of CD11b$^-$ CD24$^-$ cDCs, previously called "double negative" (DN) cDCs (Malissen et al., 2014; Henri et al., 2010) and characterized as CD207$^-$ CD11b$^-$ Sirp-α^+ CX3CR1$^+$ (Figures 2B, S2A, and S2B and data not shown). In the dermis, deletion of *Klf4* reduced CD11b$^+$ cDCs and increased CD24$^+$ and CD103$^+$ cDCs (Figure S2D).

In the lung, *Klf4*-deficiency selectively eliminated a Sirp-α^+ CD24$^+$ subset (Figures 2E and S2J and S2K), which comprised 20% of cDCs (Figures S2J and S2K), and reduced Sirp-α^+ CD24$^-$ cDCs. Further, Mgl-2 expression identified the *Klf4*-dependent Sirp-α^+ CD24$^+$ cDCs in lung (Figure 2E), but not in sLN (Figure S2C). In mediastinal lymph nodes, *Klf4* deficiency severely reduced Sirp-α^+ cDCs, which expressed PD-L2, but not Mgl2 (Figures S2L and S2M and data not shown). In the liver, *Klf4* deficiency reduced the frequency of Sirp-α^+ CD24$^-$ cDCs, which also expressed Mgl-2 (Figure S2H). In mesenteric lymph nodes, lamina propria, and thymus, Sirp-α^+ cDCs were reduced and CD24$^+$ CD103$^+$ respectively increased in the absence of *Klf4* (Figures S2E and S2F; Figure 2F and S2I). These results highlight the requirement for *Klf4* on the IRF4-expressing, but not IRF8-dependent cDCs, and suggest heterogeneous expression of PD-L2 and Mgl2 across the different peripheral tissues (Figures 2E and S2 and data not shown). Expression amounts of IRF4 and IRF8 were analyzed on *Klf4*$^{fl/fl}$ cre-negative mice (Figure 2C and data not shown). Among the different IRF4 expressing cDCs, the *Klf4*-dependent DN subset showed the highest expression level for IRF4 (Figure 2C).

A Cell-Intrinsic Requirement for *Klf4* in Development of CD11b$^-$ cDCs in sLNs

Previous studies concluded that dermal CD11b$^-$MHC-II^{hi} cells were conventional DCs based on hematopoietic origin and dependence on *Flt3L* (Henri et al., 2010; Mollah et al., 2014), although these properties also identify progenitors of other hematopoietic lineages (McKenna et al., 2000; Mackarehtschian et al., 1995). Thus, we independently assessed the identity of the sLN *Klf4*-dependent cells (Figure 3). *Klf4*-dependent CD11b$^-$ cells had morphology similar to other migratory and resident cDC subsets (Figures 3A and S3A), were not affected by *Lyz2-cre*-mediated *Klf4* deletion, and were present in *Csf2rb*$^{-/-}$ mice (Figures 3B and 3C). In contrast, IRF8-dependent CD103$^+$ cDCs develop in *Csf2rb*$^{-/-}$ mice but have reduced surface CD103 expression (Figure 3C) (Edelson et al., 2011). Moreover, the *Klf4*-dependent CD11b$^-$ cells in sLNs were absent in *Flt3l*$^{-/-}$ and *Irf4*$^{-/-}$ mice (Figures 3B and 3C), confirming their identity as IRF4-dependent cDCs. Among migratory cDCs, IRF4 expression was highest on the *Klf4*-dependent DCs (Figure S1W). *Klf4* deficiency did not impair the development of CD24$^+$ cDCs, which were absent in *Flt3l*$^{-/-}$ mice (Figures 3B and 3C), nor the development of LCs, which were severely reduced in *Il34*$^{-/-}$ mice (Wang et al., 2012; Greter et al., 2012) (Figure S3F). *Klf4*-dependent CD11b$^-$ cells were most similar in gene expression to migratory CD11b$^+$ cDCs and more distantly related to resident CD11b$^+$ cDCs and LCs (Figures 3D and S3G). In *Zbtb46*^{gfp} mice, the *Klf4*-dependent CD11b$^-$ cells expressed GFP at amounts similar to migratory CD11b$^+$ cDCs and resident cDCs in sLN (Figures 3E and S3H–S3J). These results suggest that *Klf4*-dependent CD11b$^-$ cells are bona fide dendritic cells.

To test for a non-cell intrinsic action of Klf4, we analyzed single- and mixed-BM chimeras reconstituted with *Klf4*$^{fl/fl}$*Itgax-cre* and *Zbtb46*^{gfp} BM (Figures 3F and S3B–S3D). A hematopoietic requirement of *Klf4* was confirmed by the selective loss of migratory CD11b$^-$ cDCs in sLN (Figure 3F) and the reduction of splenic CD11b$^+$ cDCs (Figure S3B) in chimeras reconstituted with *Itgax-cre Klf4*$^{fl/fl}$ BM. We also excluded an indirect action of *Klf4* deficiency on other hematopoietic lineages in regulating development of migratory CD11b$^-$ cDCs, because this subset was positive for GFP expression in chimeras reconstituted with a 10:1 mixture of *Itgax-cre Klf4*$^{fl/fl}$ BM and *Zbtb46*^{gfp} BM (Satpathy et al., 2012a) (Figures S3C and S3D). As a control, we observed that similar ratios of GFP-positive and -negative cells developed within the CD24$^+$ and CD11b$^+$ migratory DC subsets (Figure S3D). Collectively, these results argue for a cell-intrinsic requirement for *Klf4* acting on migratory CD11b$^-$ cDCs.

Klf4-Dependent Migratory cDCs Transport Antigen from Skin to sLNs

cDCs residing in the migratory gate transport the majority of fluorescein isothiocyanate (FITC) from skin to sLNs and the *Klf4*-dependent CD11b$^-$ migratory cDC subset accounted for a substantial fraction of total antigen transport (Figures 4A–4C). *Itgax-cre Klf4*$^{fl/fl}$ and *Vav1-icre Klf4*$^{fl/fl}$ mice showed reduced percentage and absolute numbers of FITC$^+$ cells present in sLNs after FITC painting (Figures 3A, 3B, and S4A). In control mice, direct examination of FITC$^+$ cells showed roughly similar contributions to antigen transport by CD11b$^+$ and *Klf4*-dependent CD11b$^-$ cDCs, which together accounted for about 90% of FITC$^+$ cells (Figures 4B, 4C, and S4B). In the absence of migratory CD11b$^-$ cDCs, the relative contribution to antigen transport by CD24$^+$ cDCs was increased from 10% to roughly 40% (Figures 4B, 4C, and S4B). In contrast to *Irf4*$^{-/-}$ mice (Bajaña et al., 2012; Gao et al., 2013), deletion of *Klf4* did not affect antigen transport, as *Klf4*-independent migratory CD11b$^+$ cDCs

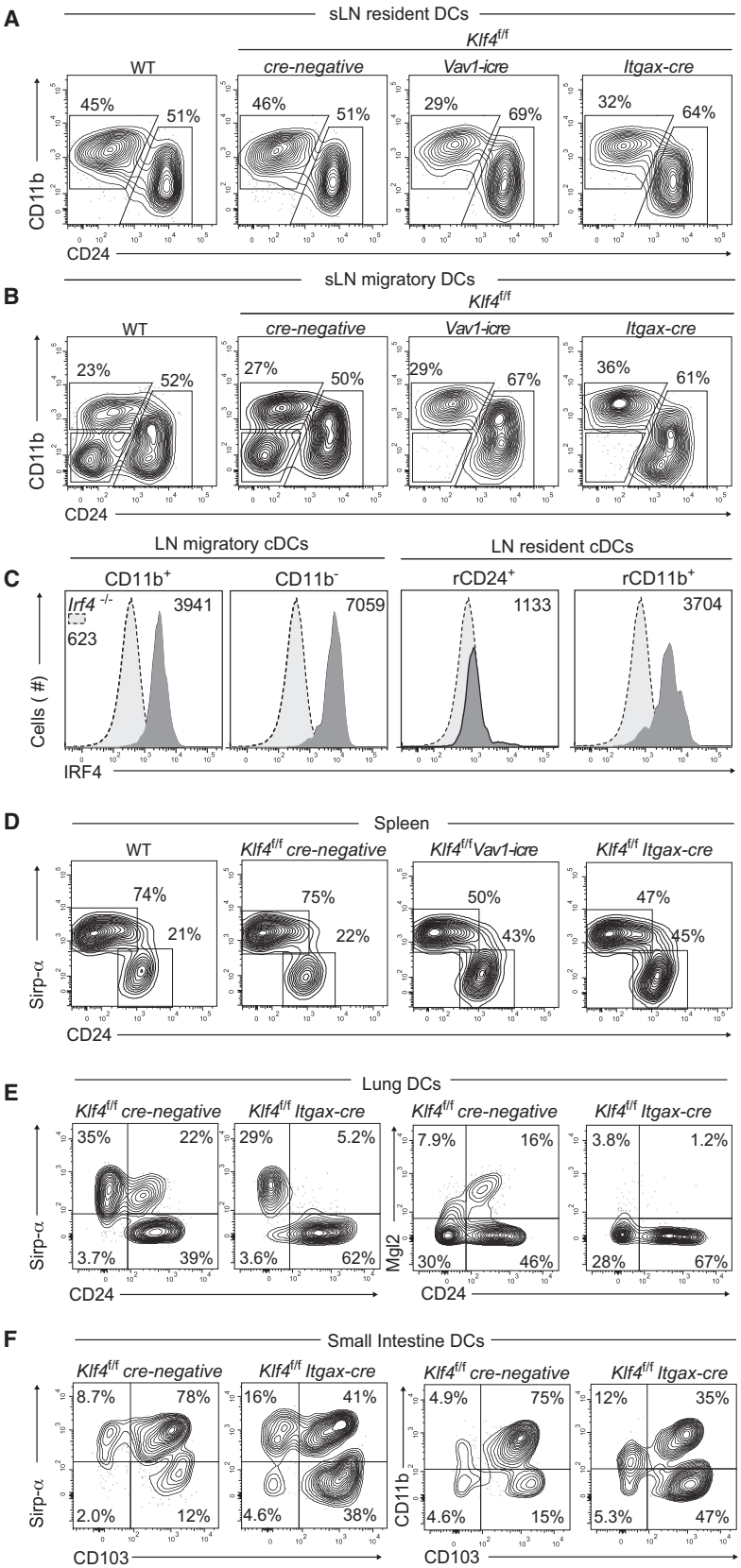


Figure 2. A Discrete Subset of Migratory cDCs in Skin-Draining Lymph Nodes Requires *Klf4*

(A and B) Two-color histograms for CD11b and CD24 expression are gated on live cells for the resident (A) and migratory (B) gates shown in (S2A).

(C) Single color histograms for intracellular IRF4 expression of the indicated subsets gated as in Figures 2A and 2B for resident and migratory DCs isolated from $Klf4^{fl/fl}$ cre-negative mice. Dotted line shows $Irf4^{-/-}$ control cDCs. Numbers represent geometrical mean values of IRF4 expression on the gated subsets.

(D) Splenocytes from mice of the indicated genotypes were pre-gated as cDCs (MHC-II^{hi} $CD11c^{hi}$) and analyzed for CD24 and Sirp-α expression. Numbers indicate the percent of cells in the gate.

(E and F) Two-color histograms for Sirp-α, CD24, CD11b, CD103, and Mgl2 as indicated in the plots, are shown for live cells from lung (E) and liver (F) of mice of the indicated genotype. Cells were pre-gated as $CD45^+$ MHC-II^{hi} $CD11c^+$ $CD64^-$. Numbers indicate percent cells in the gates. All stainings were repeated in at least three independent experiments using at least two mice per genotype.

See also Figure S2.

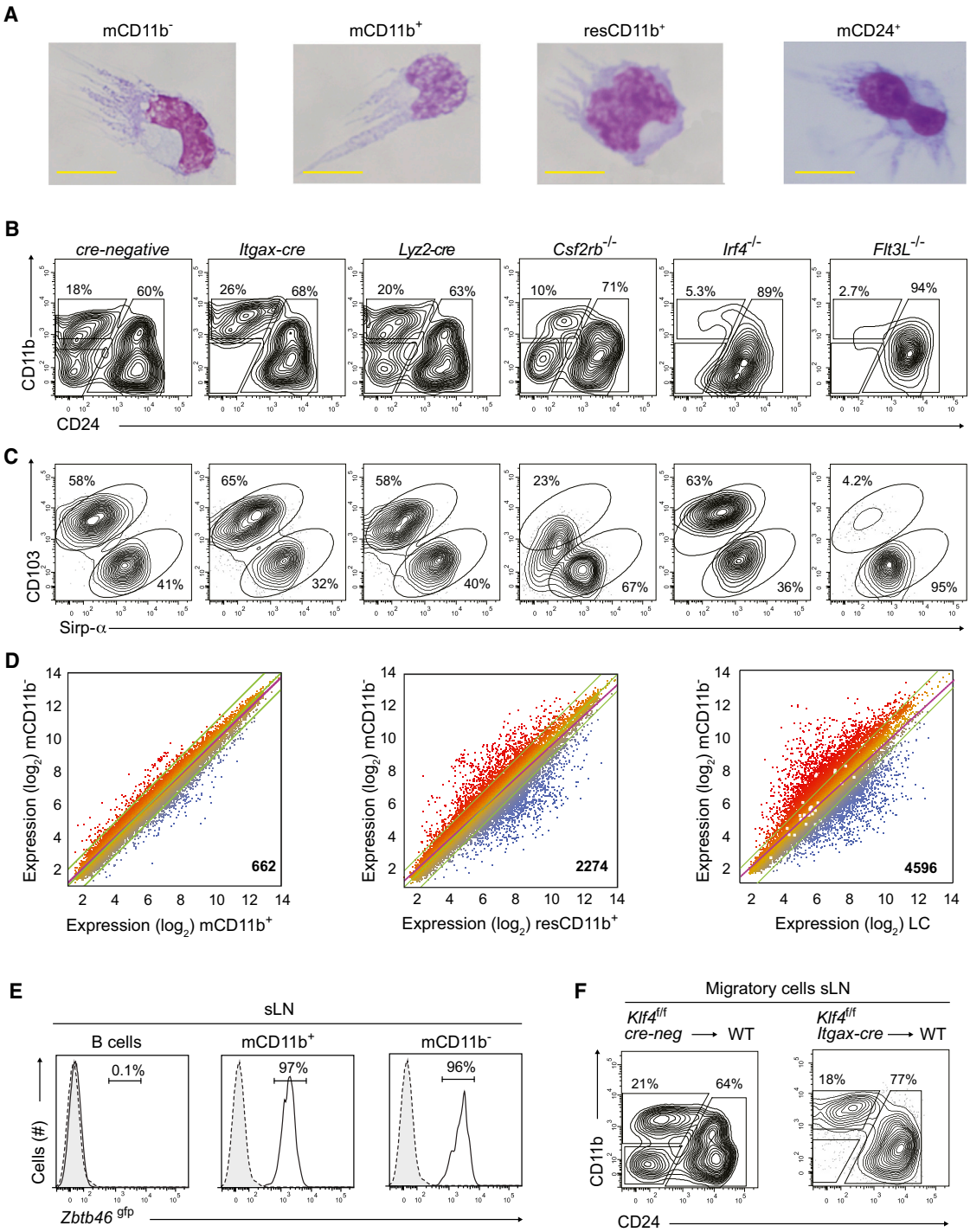


Figure 3. *Klf4*-Dependent CD11b⁻ cDCs Require IRF4 and FLT3L, Express *Zbtb46*^{gfp}, and Most Closely Resemble CD11b⁺ cDCs

(A) Wright's stain of sort-purified migratory CD11b⁻ cDCs (mCD11b⁻), CD11b⁺ (mCD11b⁺), or CD24⁺ (mCD24⁺) and resident CD11b⁺(resCD11b⁺) cDCs. Images were collected from two independent experiments, cells from two mice were pooled and at least ten cells were evaluated per subset.

(B and C) Representative two-color histograms for CD11b, CD24 (B), and CD103, Sirp-α (C) for cells pre-gated as migratory CD24⁺ cDCs as in Figure 2B are shown for sLN cells harvested from mice of the indicated genotypes. Analysis was performed in at least two independent experiments and at least three mice per genotype were analyzed.

(D) Microarray analysis of gene expression presented as M-plots for sort-purified mCD11b⁺ cDCs, mCD11b⁻ cDCs, CD24⁺ migratory cDCs, and resident CD11b⁺ cDCs harvested from sLN. Colors indicate higher (red) or lower (blue) expression. Shown at the bottom right is the number of genes differing between paired samples by more than 2-fold.

(legend continued on next page)

subset transported substantial antigen from skin to sLNs, in both *Itgax-cre Klf4*$^{fl/fl}$ and *Vav1-icre Klf4*$^{fl/fl}$ mice (Figures 4B, 4C, and S4B).

Deletion of *Klf4* in cDCs Selectively Impairs Th2 Cell Responses to Pathogens

IRF4-expressing DCs produce IL-23 that drives ILC3 and Th17 cell activation and protect against *Citrobacter rodentium* and *Aspergillus fumigatus* infection (Schlitzer et al., 2013; Satpathy et al., 2013; Persson et al., 2013). We tested the role of *Klf4*-dependent cDCs in IL-23 production and Th17 cell response by infecting *Itgax*-cre *Klf4*$^{fl/fl}$ mice with *C. rodentium.* We confirmed that susceptible to *C. rodentium* infection was increased in mice where *Notch2* was deleted by *Itgax-cre* (c*Notch2*$^{-/-}$). These mice lost 10%–15% of their body weight and succumbed to infection around day 12 (Figures 5A and 5B). In contrast, *Itgax*-cre *Klf4*$^{fl/fl}$ mice did not lose weight and survived infection similar to wild-type *Klf4*$^{fl/fl}$ *cre*-negative control mice, suggesting normal in vivo activation of ILC3 and Th17 cell responses. While c*Notch2*$^{-/-}$ mice have severely reduced splenic ESAM^{+} cDCs, *Klf4* deficiency did not compromise development of this subset (Figures S5A–S5C).

Protection against the helminth pathogen *Schistosoma mansoni* requires a Th2 cell response (Brunet et al., 1997; Fallon et al., 2000; Herbert et al., 2004). We infected *Klf4*$^{fl/fl}$ *Itgax*-cre and *Il4*$^{-/-}$ mice with *S. mansoni* (Figure 5C). *Il4*$^{-/-}$ mice succumbed to infection after 50 days with an overall mortality of approximately 75% at 60 days after infection (Figure 5C), as expected (Brunet et al., 1997; Fallon et al., 2000; Pearce et al., 1996). *Klf4*$^{fl/fl}$ *Itgax*-cre mice succumbed to infection with a similar rate and kinetic as *Il4*$^{-/-}$ mice, while wild-type control mice survive beyond 100 days after challenge (Figure 5C).

Because *Itgax*-cre can induce deletion in cells other than DCs, including T cells (Figure S1A) (Abram et al., 2014), we tested Th2 and Th17 cell polarization after *Klf4* deletion. Deletion of *Klf4* by either *Vav1-icre* or *Itgax*-cre did not decrease IL-17, IL-4, and IL-10 production in Th17 or Th2 cells compared to cre-negative controls (Figures 5D and 5E), but *Batf*$^{-/-}$*Batf3*$^{-/-}$ T cells were strongly impaired in all of these cytokines, as expected (Tussiwand et al., 2012). Further, deletion of *Klf4* by either *Vav1-icre* or *Itgax*-cre also did not reduce intracellular expression of GATA3 (Figures 5D and 5E). Thus, deletion of *Klf4* in T cells by *Vav-icre* leaves Th2 cell polarization intact, suggesting that susceptibility to *S. mansoni* infection occurred through a mechanism other than a T cell intrinsic action.

We also immunized mice with *S. mansoni* egg antigen (SEA) (Sabin et al., 1996; Everts et al., 2012; Kane et al., 2008; Pearce et al., 1991). IL-4 and IL-5 were both produced by T cells harvested from sLNs 7 days after SEA immunization of wild-type *cre*-negative control mice, but this was substantially and selectively reduced in *Klf4*$^{fl/fl}$ *Itgax*-cre mice (Figures S5D–S5G). Moreover, switching of germinal center B cells from surface immunoglobulin M (IgM) to IgG1 after SEA-immunization was reduced in *Klf4*$^{fl/fl}$ *Itgax*-cre mice relative to *cre*-negative control mice (Figures S7H and S7I).

To test whether *Klf4* deletion within cDCs impaired the function of the IRF8- and *Batf3*-dependent cDCs, we examined infections by *T. gondii* and *herpes simplex virus* (HSV) (Mashayekhi et al., 2011; Tussiwand et al., 2012). Control mice *cre*-negative *Klf4*$^{fl/fl}$ and *Itgax*-cre *Klf4*$^{fl/fl}$ mice were similarly resistant to *T. gondii* infection, while *Batf3*$^{-/-}$ mice were as expected highly susceptible (Figures S5J and S5K). Second, *Itgax*-cre *Klf4*$^{fl/fl}$ mice, but not *Batf3*$^{-/-}$ mice, generated a robust Th1 cell response specific for the Gb2 peptide of *herpes simplex virus* (HSV) 7 days after infection (Figures S5L and S5M). Also, cross-presentation of cell-associated antigens was preserved in *Itgax*-cre *Klf4*$^{fl/fl}$ mice (Figure S5N) suggesting that IRF8-expressing DC were phenotypically and functionally fully preserved in *Itgax*-cre *Klf4*$^{fl/fl}$ mice.

We also excluded that *klf4* deletion resulted in a general priming defect in the remaining IRF4-dependent cDCs by performing several DC-T cell cultures, where we did not observe any difference using either BM derived or isolated DCs from control mice *cre*-negative, *Itgax*-cre or *Vav1-icre Klf4*$^{fl/fl}$ mice (Figure S5O). Further, *Klf4*-dependent DCs did not show superior in vitro priming ability, but only increased expression of the co-stimulatory molecules CD80 and CD86 after SEA (data not shown).

Collectively, because deletion of *Klf4* in *Itgax*-cre *Klf4*$^{fl/fl}$ mice did not compromise the development of other myeloid subsets other than cDCs and these mice had normal Th17, Th1, and CTL cell response to several infections and antigens, *Klf4* appears to be selectively required on DCs for inducing Th2 immunity.

Deletion of *Klf4* in cDCs Prevents House Dust Mite-Induced Allergic Inflammation

Development of asthma is associated with a Th2 cell type of immunity characterized by the accumulation of eosinophils (Robinson, 2000; Robinson et al., 1992). In mice, intra-nasal challenge with extracts of house dust mite (HDM) causes acute allergic peribronchial Th2 cell inflammation mediated by CD11b^{+} cDCS (Hammad et al., 2009; Plantinga et al., 2013; Williams et al., 2013). Because pulmonary macrophages express CD11c, we used *Klf4*$^{fl/fl}$ *Lyz2-cre* mice as a control for potential requirements for Klf4 in pulmonary macrophages. *Klf4*$^{fl/fl}$ *Itgax*-cre mice showed a dramatic reduction in pulmonary accumulation of eosinophils compared to both *cre*-negative *Klf4*$^{fl/fl}$ control mice and *Klf4*$^{fl/fl}$ *Lyz2-cre* mice, which had robust eosinophilia (Figures 6A and 6B). Control cre-negative and *Klf4*$^{fl/fl}$ *Lyz2-cre* mice, but not *Klf4*$^{fl/fl}$ *Itgax*-cre mice, had typical asthmatic features with peribronchial inflammation with substantial leukocyte infiltrations (Figure 6C).

DISCUSSION

Klf4 regulates DCs development beginning in the pre-cDC, where it impacts the IRF4-expressing branch of cDCs. Deletion

(E) Shown are single-color histograms for *Zbtb46*^{gfp} expression for B cells, mCD11b^{+}, or mCD11b^{-} cDCs from sLN of *Zbtb46*$^{+/gfp}$ mice (Satpathy et al., 2012a). Numbers are the percent of live cells within the indicated gate.

(F) Chimeras were generated by reconstitution of lethally irradiated C57BL/6 mice (WT) using BM from *Klf4*$^{fl/fl}$ *cre*-negative or *Klf4*$^{fl/fl}$ *Itgax*-cre mice as indicated. Shown are two-color histograms as in (B) for cells pre-gated as migratory cDCs as in Figure S2A. Experiment was performed twice and at least four mice per group were analyzed. See also Figure S3.

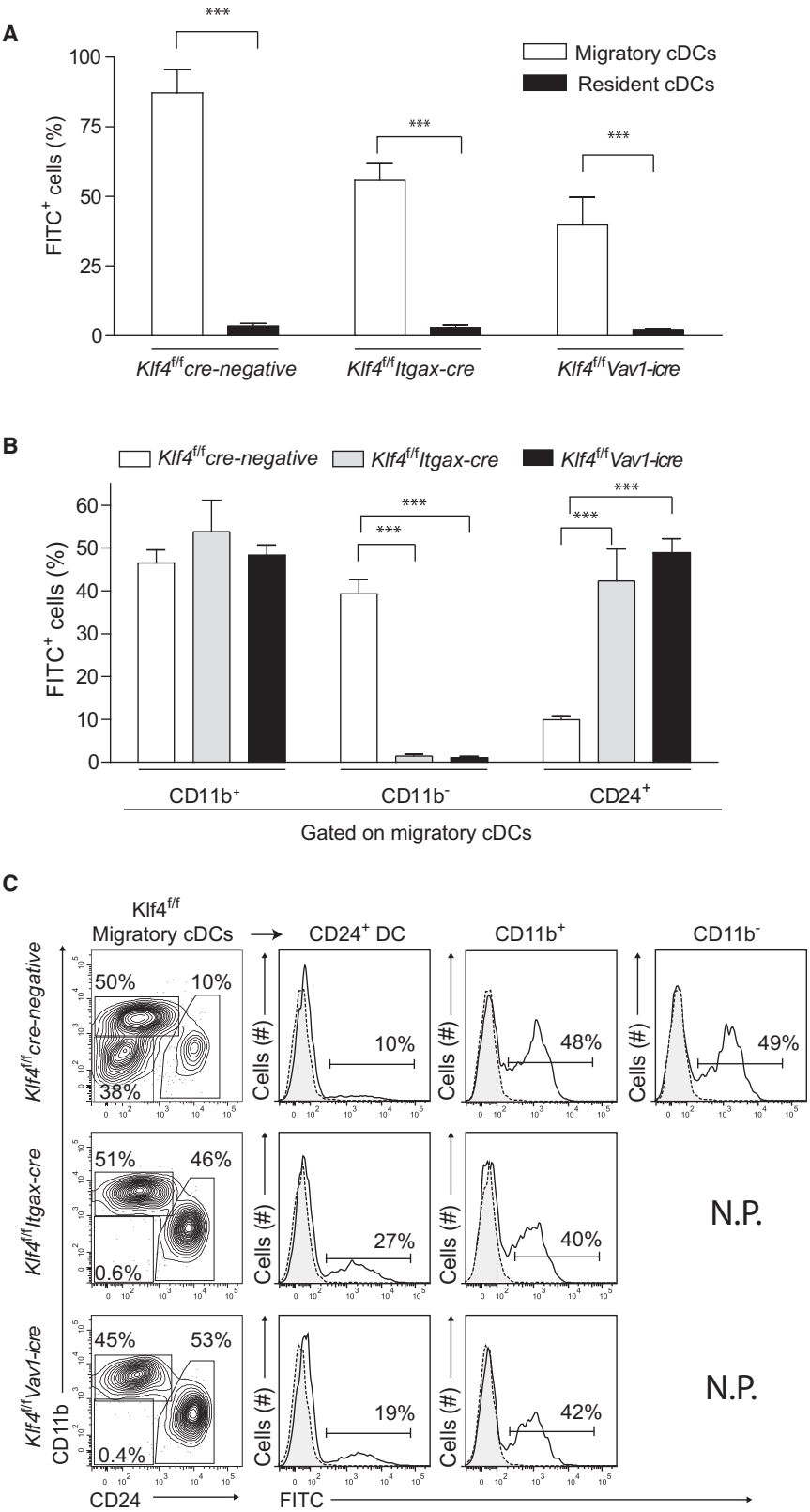


Figure 4. *Klf4*-Dependent CD11b⁻ cDCs Account for Substantial Antigen Transport from Skin to Lymph Nodes

(A) Shown is the contribution to total FITC$^+$ cells by migratory (open bars) and resident (closed bars) cDCs in skin draining lymph nodes (sLN) 16 hr after FITC painting, as a percentage of total FITC$^+$ sLN cells. Error bars ± SD, n = 6, Student's t test. ***p < 0.001.

(B) Shown is contribution to total FITC$^+$ cells by each migratory DC subset as a percentage of total FITC$^+$ sLN cells in mice of the indicated genotypes 16 hr after FITC painting. Error bars ± SD, n = 6, Student's t test. ***p < 0.001.

(C) FITC transport by individual migratory cDC subsets is show as single color histogram from mice of the indicated genotypes and gates (left panels). N.P., not present. Numbers indicate the percent of cells in the indicated gates. The experiment was performed three times, and two mice per group were used.

See also Figure S4.

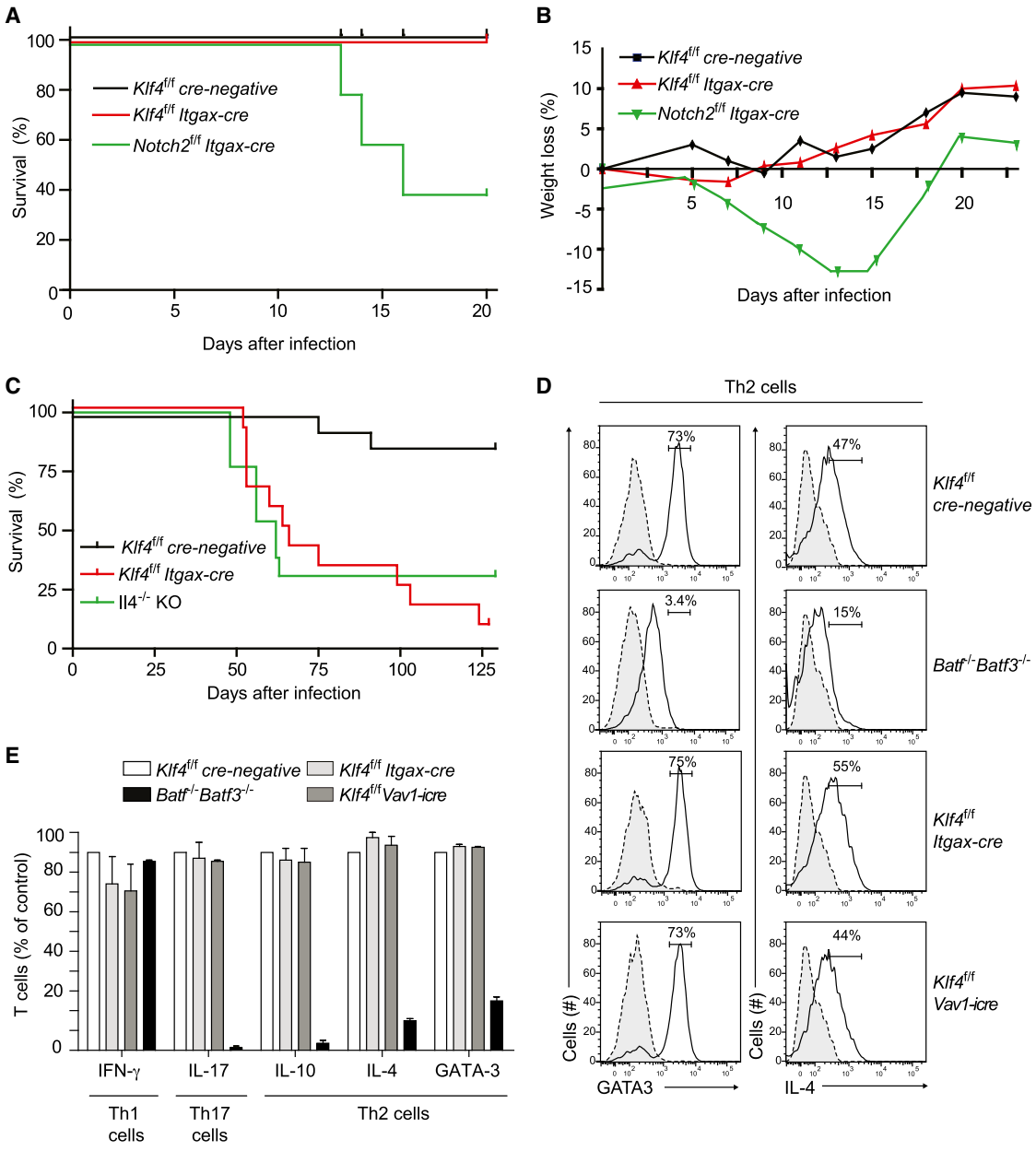


Figure 5. Conditional Deletion of *Klf4* in DCs Selectively Impairs Th2 Cell Responses to *S. mansoni*

(A and B) Survival (A) and weight loss (B) of mice after oral inoculation of *C. rodentium* (2×10^9 colony-forming units). Mice used were $Klf4^{fl/fl}$ (n = 20) or $Notch2^{fl/fl}$ (n = 5) (Satpathy et al., 2013) crossed to *Itgax*-cre (n = 10) (Caton et al., 2007) ($Notch2^{c-/-}$) or $Klf4^{fl/fl}$ *cre*-negative (*cre*-neg) (n = 10) as indicated. Experiment was repeated two times.

(C) Survival of mice after skin inoculation with 100 *Schistosoma mansoni* cercariae. Mice used were $Klf4^{fl/fl}$ crossed to *Itgax*-cre (n = 7) or a *cre*-negative background, (n = 11), or were $Il4^{-/-}$ (Kopf et al., 1993) (n = 7) experiment was repeated three times.

(D) Shown are histograms for intracellular GATA3 and IL-4 staining for $CD4^+$ T cells isolated from mice of the indicated genotypes purified by MACS beads and passed twice in vitro with anti-CD3 and anti-CD28 under Th2-inducing (solid lines) or Th1-inducing conditions (dotted and shaded) as described (Schraml et al., 2009). Mice used were $Klf4^{fl/fl}$ crossed to *Itgax*-cre, *Vav1-icre*, *cre*-negative, or $Batf^{-/-}Batf3^{-/-}$ double-deficient mice (Tussiwand et al., 2012). Numbers are the percent of cells in the indicated gates.

(E) MACs purified T cells from mice of the indicated genotypes as in (D) were polarized under Th1-, Th17-, or Th2-inducing conditions as described (Schraml et al., 2009). Shown are the percent of T cells positive for intracellular expression of the indicated cytokine or factor for each genotype as a percent of the maximum expression obtained for *cre*-negative control T cell samples. Two mice per genotype were used per experiment and the experiment was repeated four times. Error bars ± SD, n = 8, Student's t test.

See also Figure S5.

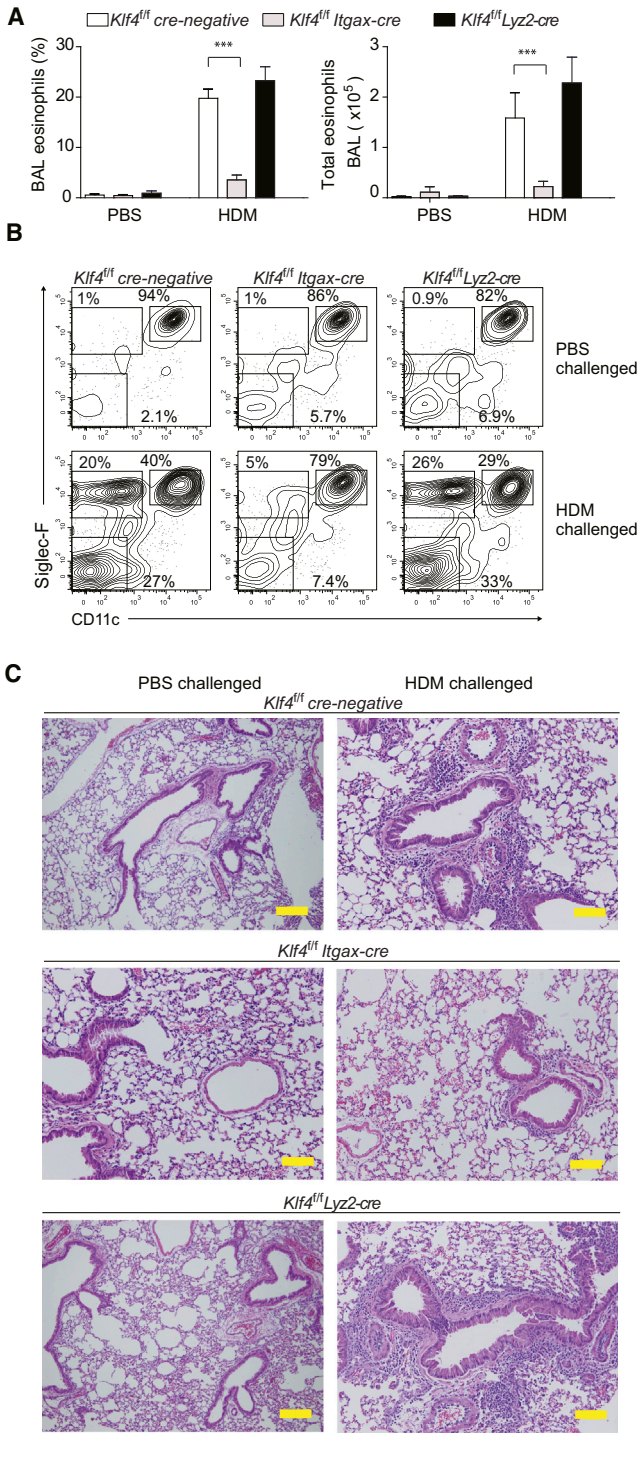


Figure 6. Conditional Deletion of *Klf4* in DCs Prevents Development of HDM-Induced Allergic Inflammation

(A) HDM sensitized mice of the indicated genotypes were challenged intranasally with PBS or HDM extract. After 3 days, bronchial alveolar lavage (BAL) was analyzed for eosinophils by FACS. Shown are eosinophils as a percentage (left panel) or total numbers recruited into BAL (right panel) and pre-gated as $CD45^+$ live cells. Mice used were $Klf4^{fl/fl}$ crossed to *Itgax*-cre (n = 8) or *Lyz2-cre* (n = 8) or a *cre*-negative background (n = 8) 3 mice per genotype used per experiment. Experiment was repeated three times. Error bars, ± SD, n = 8, Student's t test. ***p < 0.001.

(B) Mice of the indicated genotypes were challenged as in (A) and BAL analyzed by flow cytometry after 3 days. Shown are two-color histograms pre-gated on $CD45^+$ BAL cells. Numbers indicate percentage of cells in the indicate gates for eosinophils ($SiglecF^+$ $CD11c^-$) or pulmonary macrophages ($SiglecF^+$ $CD11c^+$), respectively.

(C) Shown are hematoxylin and eosin stained sections of lungs from mice described in (A) as indicated. Scale bars represent 200 μm.

of *Klf4* by *Vav1-icre* altered the expression of relatively few genes, but among these, IRF4 was severely downregulated as measured by microarray expression and intracellular staining. Reduction of IRF8 and induction of IRF4 occurs at the $SiglecH^-$ pre-cDCs stage, a stage that was severely reduced in *Vav1-icre* $Klf4^{fl/fl}$ mice. However, IRF4 expression was not altered in mature cDCs or in *Flt3l* generated BM-derived cDCs implying a developmental rather than an absolute requirement for *Klf4* in the induction of IRF4 expression.

In mature cDCs, *Klf4* deletion selectively compromised the development of particular subsets of IRF4-expressing cDCs. The subset most clearly dependent on *Klf4* was the previously described migratory subset of $CD11b^-CD24^-$ (DN) cDCs in sLN. This subset had the highest expression of IRF4 among migratory cDCs. However, it is still unclear whether impaired *Klf4*-dependent cDCs development is caused by reduced IRF4-expression at a progenitor stage or due to some other action of *Klf4*.

The DN migratory cDCs identified here as being *Klf4* dependent have been described recently to acquire fluorescently labeled antigen from non-viable *Nippostrongylus* larvae and suggested to be involved in Th2 cell responses based on in vitro analysis (Ochiai et al., 2014; Connor et al., 2014). Our results would support those conclusions by drawing a correlation between the loss of the DN cDC subset and reduced in vivo Th2 cell responses in *Klf4*-deficient mice. We also observed a higher co-stimulatory potential of the *Klf4*-dependent DN migratory cDCs relative to other cDC subsets after SEA stimulation. Nonetheless, despite the genetic requirement for *Klf4* for DN cDC development, it is still possible that *Klf4* controls Th2 responses by actions in other cDC subsets that are not seemingly affected by the deletion. Excluding such possibilities awaits the development of deleter strains with higher DC-subset specificity.

IRF4-expressing cDCs were recently shown to control Th2 cell responses induced in skin and lungs (Schlitzer et al., 2013; Gao et al., 2013; Kumamoto et al., 2013; Zhou et al., 2014; Williams et al., 2013), although no mechanism for this action was determined. $Irf4^{-/-}$ cDCs fail to migrate to sLN (Bajaña et al., 2012) and show reduced expression of co-stimulatory molecules (Vander Lugt et al., 2014), which could explain reduced T cell priming, at least in skin. *Klf4* deletion selectively impaired Th2 cell responses without preventing migration of the remaining cDCs.

IRF4-expressing cDCs also control Th17 cell responses in lungs and intestine (Schlitzer et al., 2013; Lewis et al., 2011; Satpathy et al., 2013). We find that in the lung, *Klf4* deletion selectively eliminated an IRF4-dependent cDC subset characterized by the expression of Sirp-α^+ $CD24^+$ Mgl-2^+. Conceivably, *Klf4*-dependent Sirp-α^+ $CD24^+$ lung cDCs might mediate Th2 cell responses to HDM, while *Klf4*-independent Sirp-α^+ $CD24^-$ cDCs mediate Th17 cell immunity to fungal infections.

Alternately, *Klf4* might act on both IRF4-expressing cDC subsets but selectively influence Th2, but not Th17, cell priming. Similarly, in the small intestine, Th17 cell immunity against *C. rodentium* infection has been attributed to the action of a *Notch2*-dependent subset of IRF4-expressing cDCs (Lewis et al., 2011; Satpathy et al., 2013). *Klf4* deletion did not increase susceptibility to *C. rodentium* infection and did not impair development of the splenic *Notch2*-dependent ESAM-expressing cDC subset. Thus, in the intestine as well, distinct transcriptional programs regulated by either *Notch2* or *Klf4* appear to control different types of immunity mediated by the IRF4-expressing cDCs.

Our results show a genetic requirement for *Klf4* in cDCs for Th2 cell immunity; however, they do not reveal the cellular mechanism mediating Th2 cell responses. The in vivo trigger for Th2 cell development has been elusive, with many mechanisms proposed over time. Conceivably, a particular cDC subset could directly induce Th2 development, for example by production of a cytokine or expressing a specific surface receptor. Alternately, the cDC subset could recruit an accessory cell that favors and creates the necessary microenvironment for Th2 cell priming. Indeed, recent evidence suggests the involvement of innate lymphoid cells type 2 (ILC2s) in the lung (Halim et al., 2014; Licona-Limón et al., 2013; Halim et al., 2012) and of eosinophils in the intestine (Chu et al., 2014). It will be important to determine whether *Klf4*-dependent cDCs utilize these cells, because such a mechanism would not be re-capitulated by in vitro studies using isolated co-cultures of cDCs and T cells.

EXPERIMENTAL PROCEDURES

Mice

All animals were bred and maintained in a specific pathogen-free animal facility according to institutional guidelines and with protocols approved by the Animal Studies Committee at Washington University in St. Louis. Mice of the following genotypes were purchased from Jackson Laboratories: wild-type mice C57BL6 (C57BL/6J), *Itgax*-cre (B6.Cg-Tg$^{(Itgax\text{-}cre)1\text{-}1Reiz/J}$) (Caton et al., 2007), *Vav1-icre* (B6.Cg-Tg$^{(Vav1\text{-}cre)A2Kio/J}$) (Harker et al., 2002), *Lyz2-cre* (B6.129P2-Lyz2$^{tm1(cre)Ifo/J}$) (Clausen et al., 1999), and *Cx3cr1*^{gfp} (B6.129P-Cx3cr1tm1Litt/J) (Jung et al., 2000). *Irf4*$^{-/-}$ were generated in our facility by backcrossing *Irf4*$^{fl/fl}$ mice (B6.129S1-Irf4tm1Rdf/J) to the cytomegalovirus (CMV) promoter expressing CMV-cre mice (B6.C-Tg(CMV-cre)1Cgn/J), both purchased from Jackson Laboratories. *Csf2rb*$^{-/-}$ (B6.129S1-Csf2rb$^{tm1Cgb/J}$) and *Flt3l*$^{-/-}$ (C57BL/6-*flt3L*^{tm1Imx}) were obtained from Taconic. *Irf8*$^{-/-}$ mice were obtained from the European Mutant Mouse Archive and maintained on the C57BL/6 background. *Notch2*$^{fl/fl}$*Itgax*-cre were generated and maintained as described (Satpathy et al., 2013). *Klf4*$^{fl/fl}$ mice were obtained from MMRC (MMRRC line 29877) and backcrossed to *Itgax*-cre, *Vav1-icre*, *Lyz2-cre*. *Zbtb46*^{gfp} were generated as previously described (Satpathy et al., 2012a). *Batf3*$^{-/-}$ were generated as previously described (Hildner et al., 2008). *Il4*$^{-/-}$(B6.129P2-*Il4*$^{tm1Cgn/J}$) (Kopf et al., 1993) mice were obtained from EJ Pearce (Pearce et al., 1996). For BM chimera experiments, the CD45.1+ B6.SJL (B6.SJL-PtprcaPepcb/BoyJ) mice were purchased from Jackson Laboratories. Unless otherwise indicated, experiments used sex- and age-matched littermates between 6 and 12 weeks of age.

Infection Models

All animal model studies described were done in accordance with institutional guidelines and with protocols approved by the Animal Studies Committee at Washington University in St. Louis.

For *Citrobacter rodentium* infection Mice were orally inoculated with 2 × 10^9 colony-forming units of *C. rodentium*, strain DBS100 (American Type Culture Collection) as described (Lee et al., 2012). Survival and weight loss were monitored over 30 days. Mice used for *C. rodentium* experiments weighed less than 22 g.

For *Toxoplasma gondii* infection, the type II Prugniaud strain of *T. gondii* expressing a firefly luciferase and GFP transgene (PRU-FLuc-GFP) (provided by J. Boothroyd, Stanford University) was used in all tachyzoite experiments. The parasites were grown in human foreskin fibroblasts cultures as described (Mashayekhi et al., 2011). For infection, freshly egressed parasites were filtered, counted, and 100 tachyzoites per mouse were injected intraperitoneally. Survival of mice was monitored over 30 days; parasite burden was measured every 2 days as described below.

Snails infected with *S. mansoni* (strain NMRI, NR-21962) were provided by the Schistosome Research Reagent Resource Center for distribution by BEI Resources, NIAID NIH. Mice were each infected by percutaneous exposure to 100 cercariae. *S. mansoni* Egg Antigen (SEA) was made from parasite eggs isolated from the livers of infected mice, as previously described (MacDonald et al., 2001). Mice were each immunized with 50 μg of SEA subcutaneously (s.c.) into a rear footpad. For Herpes simplex virus 1 (HSV-1) infection mice were infected with the KOS strain s.c. with 1.5 × 10^5 plaque-forming units (pfu) per mouse in the footpad.

In Vitro T Cell Re-stimulation after SEA and HSV Infection

One week after HSV infection, spleens and popliteal lymph nodes (LN) were collected and re-stimulated with the HSV peptide HSV-gB2 (498-505) (Anaspec). Briefly, 2 × 10^6 splenocytes or LN cells were re-stimulated for 5ch in the presence of brefeldin A at 1 μgcml−1 and analyzed by FACS for intracellular IFN-γ and TNF-α production as described later.

Similarly, 1 week after SEA injection, 3 × 10^5 cells harvested from popliteal lymph nodes were collected and restimulated under different conditions: SEA (20 μg/ml), plate-bound anti-CD3, PMA and Ionomycin (50ng/ml and 1 μg/ml), or media alone. For detection of IL-4, 2.5 μg /ml of anti-IL-4R antibody (M1) was added to the culture. Supernatants were collected after 72 hr and analyzed with the BD CBA mouse Th1/Th2 Kit (BD Biosciences), and data were analyzed with FCAP Array software (Soft Flow).

HDM-Induced Asthma

HDM (Dermatophagoidespteronyssinus extracts, Greer Laboratories) was dissolved in PBS. To induce allergic airway inflammation, we sensitized mice i.n. 10 μg HDM subsequently challenged with 50 μg HDM i.n. on days 7 and collected lungs, BAL, and mediastinal LNs 3 days after challenge.

Statistical Analysis

Differences between groups in survival were analyzed by the log rank test. Analysis of all other data was done with an unpaired, two-tailed Student's t test with a 95% confidence interval (Prism; GraphPad Software). p values less than 0.05 were considered significant. *0.01 < p < 0.05; **0.001 < p < 0.01; ***p < 0.001; ****p < 0.0001.

ACCESSION NUMBERS

The GEO accession number for the microarray data reported in this paper is GSE68590.

SUPPLEMENTAL INFORMATION

Supplemental Information includes five figures and Supplemental Experimental Procedures and can be found with this article online at http://dx.doi.org/10.1016/j.immuni.2015.04.017.

ACKNOWLEDGMENTS

This work was supported by the Howard Hughes Medical Institute (K.M.M.). NIH grant AI32573 to E.J.P. B.E. was supported by a VENI grant from the Netherlands Organization for Scientific Research. We thank Susan Gilfillan and Marco Colonna for *Il34*$^{-/-}$ mice (Wang et al., 2012). We thank Victor Cortez for help in processing LP tissues.

Received: September 9, 2014
Revised: February 15, 2015
Accepted: April 29, 2015
Published: May 19, 2015

REFERENCES

Abram, C.L., Roberge, G.L., Hu, Y., and Lowell, C.A. (2014). Comparative analysis of the efficiency and specificity of myeloid-Cre deleting strains using ROSA-EYFP reporter mice. J. Immunol. Methods *408*, 89–100.

Akashi, K., Traver, D., Miyamoto, T., and Weissman, I.L. (2000). A clonogenic common myeloid progenitor that gives rise to all myeloid lineages. Nature *404*, 193–197.

Alder, J.K., Georgantas, R.W., 3rd, Hildreth, R.L., Kaplan, I.M., Morisot, S., Yu, X., McDevitt, M., and Civin, C.I. (2008). Kruppel-like factor 4 is essential for inflammatory monocyte differentiation in vivo. J. Immunol. *180*, 5645–5652.

Bajaña, S., Roach, K., Turner, S., Paul, J., and Kovats, S. (2012). IRF4 promotes cutaneous dendritic cell migration to lymph nodes during homeostasis and inflammation. J. Immunol. *189*, 3368–3377.

Banchereau, J., and Steinman, R.M. (1998). Dendritic cells and the control of immunity. Nature *392*, 245–252.

Bar-On, L., and Jung, S. (2010). Defining in vivo dendritic cell functions using CD11c-DTR transgenic mice. Methods Mol. Biol. *595*, 429–442.

Belz, G.T., and Nutt, S.L. (2012). Transcriptional programming of the dendritic cell network. Nat. Rev. Immunol. *12*, 101–113.

Brunet, L.R., Finkelman, F.D., Cheever, A.W., Kopf, M.A., and Pearce, E.J. (1997). IL-4 protects against TNF-alpha-mediated cachexia and death during acute schistosomiasis. J. Immunol. *159*, 777–785.

Caton, M.L., Smith-Raska, M.R., and Reizis, B. (2007). Notch-RBP-J signaling controls the homeostasis of CD8- dendritic cells in the spleen. J. Exp. Med. *204*, 1653–1664.

Chu, D.K., Jimenez-Saiz, R., Verschoor, C.P., Walker, T.D., Goncharova, S., Llop-Guevara, A., Shen, P., Gordon, M.E., Barra, N.G., Bassett, J.D., et al. (2014). Indigenous enteric eosinophils control DCs to initiate a primary Th2 immune response in vivo. J. Exp. Med. *211*, 1657–1672.

Clausen, B.E., Burkhardt, C., Reith, W., Renkawitz, R., and Förster, I. (1999). Conditional gene targeting in macrophages and granulocytes using LysMcre mice. Transgenic Res. *8*, 265–277.

Connor, L.M., Tang, S.C., Camberis, M., Le Gros, G., and Ronchese, F. (2014). Helminth-Conditioned Dendritic Cells Prime CD4+ T Cells to IL-4 Production In Vivo. J. Immunol. *193*, 2709–2717.

Dang, D.T., Pevsner, J., and Yang, V.W. (2000). The biology of the mammalian Krüppel-like family of transcription factors. Int. J. Biochem. Cell Biol. *32*, 1103–1121.

de Boer, J., Williams, A., Skavdis, G., Harker, N., Coles, M., Tolaini, M., Norton, T., Williams, K., Roderick, K., Potocnik, A.J., and Kioussis, D. (2003). Transgenic mice with hematopoietic and lymphoid specific expression of Cre. Eur. J. Immunol. *33*, 314–325.

Edelson, B.T., Kc, W., Juang, R., Kohyama, M., Benoit, L.A., Klekotka, P.A., Moon, C., Albring, J.C., Ise, W., Michael, D.G., et al. (2010). Peripheral CD103+ dendritic cells form a unified subset developmentally related to CD8alpha+ conventional dendritic cells. J. Exp. Med. *207*, 823–836.

Edelson, B.T., Bradstreet, T.R., Kc, W., Hildner, K., Herzog, J.W., Sim, J., Russell, J.H., Murphy, T.L., Unanue, E.R., and Murphy, K.M. (2011). Batf3-dependent CD11b(low/-) peripheral dendritic cells are GM-CSF-independent and are not required for Th cell priming after subcutaneous immunization. PLoS ONE *6*, e25660.

Everts, B., Hussaarts, L., Driessen, N.N., Meevissen, M.H., Schramm, G., van der Ham, A.J., van der, H.B., Scholzen, T., Burgdorf, S., Mohrs, M., Pearce, E.J., Hokke, C.H., Haas, H., Smits, H.H., and Yazdanbakhsh, M. (2012). Schistosome-derived omega-1 drives Th2 polarization by suppressing protein synthesis following internalization by the mannose receptor. J. Exp. Med. *209*, 1753–1767, S1.

Fallon, P.G., Richardson, E.J., McKenzie, G.J., and McKenzie, A.N. (2000). Schistosome infection of transgenic mice defines distinct and contrasting pathogenic roles for IL-4 and IL-13: IL-13 is a profibrotic agent. J. Immunol. *164*, 2585–2591.

Feinberg, M.W., Wara, A.K., Cao, Z., Lebedeva, M.A., Rosenbauer, F., Iwasaki, H., Hirai, H., Katz, J.P., Haspel, R.L., Gray, S., et al. (2007). The Kruppel-like factor KLF4 is a critical regulator of monocyte differentiation. EMBO J. *26*, 4138–4148.

Gao, Y., Nish, S.A., Jiang, R., Hou, L., Licona-Limón, P., Weinstein, J.S., Zhao, H., and Medzhitov, R. (2013). Control of T helper 2 responses by transcription factor IRF4-dependent dendritic cells. Immunity *39*, 722–732.

Ghaleb, A.M., Nandan, M.O., Chanchevalap, S., Dalton, W.B., Hisamuddin, I.M., and Yang, V.W. (2005). Krüppel-like factors 4 and 5: the yin and yang regulators of cellular proliferation. Cell Res. *15*, 92–96.

Ginhoux, F., Liu, K., Helft, J., Bogunovic, M., Greter, M., Hashimoto, D., Price, J., Yin, N., Bromberg, J., Lira, S.A., et al. (2009). The origin and development of nonlymphoid tissue CD103+ DCs. J. Exp. Med. *206*, 3115–3130.

Greter, M., Lelios, I., Pelczar, P., Hoeffel, G., Price, J., Leboeuf, M., Kündig, T.M., Frei, K., Ginhoux, F., Merad, M., and Becher, B. (2012). Stroma-derived interleukin-34 controls the development and maintenance of langerhans cells and the maintenance of microglia. Immunity *37*, 1050–1060.

Hacker, C., Kirsch, R.D., Ju, X.S., Hieronymus, T., Gust, T.C., Kuhl, C., Jorgas, T., Kurz, S.M., Rose-John, S., Yokota, Y., and Zenke, M. (2003). Transcriptional profiling identifies Id2 function in dendritic cell development. Nat. Immunol. *4*, 380–386.

Halim, T.Y., MacLaren, A., Romanish, M.T., Gold, M.J., McNagny, K.M., and Takei, F. (2012). Retinoic-acid-receptor-related orphan nuclear receptor alpha is required for natural helper cell development and allergic inflammation. Immunity *37*, 463–474.

Halim, T.Y., Steer, C.A., Mathä, L., Gold, M.J., Martinez-Gonzalez, I., McNagny, K.M., McKenzie, A.N., and Takei, F. (2014). Group 2 innate lymphoid cells are critical for the initiation of adaptive T helper 2 cell-mediated allergic lung inflammation. Immunity *40*, 425–435.

Hambleton, S., Salem, S., Bustamante, J., Bigley, V., Boisson-Dupuis, S., Azevedo, J., Fortin, A., Haniffa, M., Ceron-Gutierrez, L., Bacon, C.M., et al. (2011). IRF8 mutations and human dendritic-cell immunodeficiency. N. Engl. J. Med. *365*, 127–138.

Hammad, H., Chieppa, M., Perros, F., Willart, M.A., Germain, R.N., and Lambrecht, B.N. (2009). House dust mite allergen induces asthma via Toll-like receptor 4 triggering of airway structural cells. Nat. Med. *15*, 410–416.

Harker, N., Naito, T., Cortes, M., Hostert, A., Hirschberg, S., Tolaini, M., Roderick, K., Georgopoulos, K., and Kioussis, D. (2002). The CD8alpha gene locus is regulated by the Ikaros family of proteins. Mol. Cell *10*, 1403–1415.

Henri, S., Poulin, L.F., Tamoutounour, S., Ardouin, L., Guilliams, M., de Bovis, B., Devilard, E., Viret, C., Azukizawa, H., Kissenpfennig, A., and Malissen, B. (2010). CD207+ CD103+ dermal dendritic cells cross-present keratinocyte-derived antigens irrespective of the presence of Langerhans cells. J. Exp. Med. *207*, 189–206.

Herbert, D.R., Hölscher, C., Mohrs, M., Arendse, B., Schwegmann, A., Radwanska, M., Leeto, M., Kirsch, R., Hall, P., Mossmann, H., et al. (2004). Alternative macrophage activation is essential for survival during schistosomiasis and downmodulates T helper 1 responses and immunopathology. Immunity *20*, 623–635.

Hildner, K., Edelson, B.T., Purtha, W.E., Diamond, M., Matsushita, H., Kohyama, M., Calderon, B., Schraml, B.U., Unanue, E.R., Diamond, M.S., et al. (2008). Batf3 deficiency reveals a critical role for CD8alpha+ dendritic cells in cytotoxic T cell immunity. Science *322*, 1097–1100.

Jung, S., Aliberti, J., Graemmel, P., Sunshine, M.J., Kreutzberg, G.W., Sher, A., and Littman, D.R. (2000). Analysis of fractalkine receptor CX(3)CR1 function by targeted deletion and green fluorescent protein reporter gene insertion. Mol. Cell. Biol. *20*, 4106–4114.

Kane, C.M., Jung, E., and Pearce, E.J. (2008). Schistosoma mansoni egg antigen-mediated modulation of Toll-like receptor (TLR)-induced activation occurs independently of TLR2, TLR4, and MyD88. Infect. Immun. *76*, 5754–5759.

Kashiwada, M., Pham, N.L., Pewe, L.L., Harty, J.T., and Rothman, P.B. (2011). NFIL3/E4BP4 is a key transcription factor for CD8α$^+$ dendritic cell development. Blood *117*, 6193–6197.

Katz, J.P., Perreault, N., Goldstein, B.G., Lee, C.S., Labosky, P.A., Yang, V.W., and Kaestner, K.H. (2002). The zinc-finger transcription factor Klf4 is required for terminal differentiation of goblet cells in the colon. Development *129*, 2619–2628.

Kopf, M., Le Gros, G., Bachmann, M., Lamers, M.C., Bluethmann, H., and Köhler, G. (1993). Disruption of the murine IL-4 gene blocks Th2 cytokine responses. Nature *362*, 245–248.

Kumamoto, Y., Linehan, M., Weinstein, J.S., Laidlaw, B.J., Craft, J.E., and Iwasaki, A. (2013). CD301b$^+$ dermal dendritic cells drive T helper 2 cell-mediated immunity. Immunity *39*, 733–743.

Kurotaki, D., Osato, N., Nishiyama, A., Yamamoto, M., Ban, T., Sato, H., Nakabayashi, J., Umehara, M., Miyake, N., Matsumoto, N., et al. (2013). Essential role of the IRF8-KLF4 transcription factor cascade in murine monocyte differentiation. Blood *121*, 1839–1849.

Lee, J.S., Cella, M., McDonald, K.G., Garlanda, C., Kennedy, G.D., Nukaya, M., Mantovani, A., Kopan, R., Bradfield, C.A., Newberry, R.D., and Colonna, M. (2012). AHR drives the development of gut ILC22 cells and postnatal lymphoid tissues via pathways dependent on and independent of Notch. Nat. Immunol. *13*, 144–151.

Lewis, K.L., Caton, M.L., Bogunovic, M., Greter, M., Grajkowska, L.T., Ng, D., Klinakis, A., Charo, I.F., Jung, S., Gommerman, J.L., et al. (2011). Notch2 receptor signaling controls functional differentiation of dendritic cells in the spleen and intestine. Immunity *35*, 780–791.

Licona-Limón, P., Kim, L.K., Palm, N.W., and Flavell, R.A. (2013). TH2, allergy and group 2 innate lymphoid cells. Nat. Immunol. *14*, 536–542.

Liu, K., Waskow, C., Liu, X., Yao, K., Hoh, J., and Nussenzweig, M. (2007). Origin of dendritic cells in peripheral lymphoid organs of mice. Nat. Immunol. *8*, 578–583.

Liu, K., Victora, G.D., Schwickert, T.A., Guermonprez, P., Meredith, M.M., Yao, K., Chu, F.F., Randolph, G.J., Rudensky, A.Y., and Nussenzweig, M. (2009). In vivo analysis of dendritic cell development and homeostasis. Science *324*, 392–397.

MacDonald, A.S., Straw, A.D., Bauman, B., and Pearce, E.J. (2001). CD8- dendritic cell activation status plays an integral role in influencing Th2 response development. J. Immunol. *167*, 1982–1988.

Mackarehtschian, K., Hardin, J.D., Moore, K.A., Boast, S., Goff, S.P., and Lemischka, I.R. (1995). Targeted disruption of the flk2/flt3 gene leads to deficiencies in primitive hematopoietic progenitors. Immunity *3*, 147–161.

Malissen, B., Tamoutounour, S., and Henri, S. (2014). The origins and functions of dendritic cells and macrophages in the skin. Nat. Rev. Immunol. *14*, 417–428.

Mashayekhi, M., Sandau, M.M., Dunay, I.R., Frickel, E.M., Khan, A., Goldszmid, R.S., Sher, A., Ploegh, H.L., Murphy, T.L., Sibley, L.D., and Murphy, K.M. (2011). CD8α(+) dendritic cells are the critical source of interleukin-12 that controls acute infection by Toxoplasma gondii tachyzoites. Immunity *35*, 249–259.

McConnell, B.B., and Yang, V.W. (2010). Mammalian Krüppel-like factors in health and diseases. Physiol. Rev. *90*, 1337–1381.

McKenna, H.J., Stocking, K.L., Miller, R.E., Brasel, K., De Smedt, T., Maraskovsky, E., Maliszewski, C.R., Lynch, D.H., Smith, J., Pulendran, B., et al. (2000). Mice lacking flt3 ligand have deficient hematopoiesis affecting hematopoietic progenitor cells, dendritic cells, and natural killer cells. Blood *95*, 3489–3497.

Merad, M., Sathe, P., Helft, J., Miller, J., and Mortha, A. (2013). The dendritic cell lineage: ontogeny and function of dendritic cells and their subsets in the steady state and the inflamed setting. Annu. Rev. Immunol. *31*, 563–604.

Mildner, A., and Jung, S. (2014). Development and function of dendritic cell subsets. Immunity *40*, 642–656.

Miller, J.C., Brown, B.D., Shay, T., Gautier, E.L., Jojic, V., Cohain, A., Pandey, G., Leboeuf, M., Elpek, K.G., Helft, J., et al.; Immunological Genome Consortium (2012). Deciphering the transcriptional network of the dendritic cell lineage. Nat. Immunol. *13*, 888–899.

Mollah, S.A., Dobrin, J.S., Feder, R.E., Tse, S.W., Matos, I.G., Cheong, C., Steinman, R.M., and Anandasabapathy, N. (2014). Flt3L dependence helps define an uncharacterized subset of murine cutaneous dendritic cells. J. Invest. Dermatol. *134*, 1265–1275.

Murphy, K.M. (2013). Transcriptional control of dendritic cell development. Adv. Immunol. *120*, 239–267.

Naik, S.H. (2010). Generation of large numbers of pro-DCs and pre-DCs in vitro. Methods Mol. Biol. *595*, 177–186.

Ochiai, S., Roediger, B., Abtin, A., Shklovskaya, E., Fazekas de St Groth, B., Yamane, H., Weninger, W., Le Gros, G., and Ronchese, F. (2014). CD326(lo) CD103(lo)CD11b(lo) dermal dendritic cells are activated by thymic stromal lymphopoietin during contact sensitization in mice. J. Immunol. *193*, 2504–2511.

Onai, N., Obata-Onai, A., Schmid, M.A., Ohteki, T., Jarrossay, D., and Manz, M.G. (2007). Identification of clonogenic common Flt3+M-CSFR+ plasmacytoid and conventional dendritic cell progenitors in mouse bone marrow. Nat. Immunol. *8*, 1207–1216.

Park, C.S., Lee, P.H., Yamada, T., Burns, A., Shen, Y., Puppi, M., and Lacorazza, H.D. (2012). Kruppel-like factor 4 (KLF4) promotes the survival of natural killer cells and maintains the number of conventional dendritic cells in the spleen. J. Leukoc. Biol. *91*, 739–750.

Pearce, E.J., Caspar, P., Grzych, J.M., Lewis, F.A., and Sher, A. (1991). Downregulation of Th1 cytokine production accompanies induction of Th2 responses by a parasitic helminth, Schistosoma mansoni. J. Exp. Med. *173*, 159–166.

Pearce, E.J., Cheever, A., Leonard, S., Covalesky, M., Fernandez-Botran, R., Kohler, G., and Kopf, M. (1996). Schistosoma mansoni in IL-4-deficient mice. Int. Immunol. *8*, 435–444.

Persson, E.K., Uronen-Hansson, H., Semmrich, M., Rivollier, A., Hägerbrand, K., Marsal, J., Gudjonsson, S., Håkansson, U., Reizis, B., Kotarsky, K., and Agace, W.W. (2013). IRF4 transcription-factor-dependent CD103(+) CD11b(+) dendritic cells drive mucosal T helper 17 cell differentiation. Immunity *38*, 958–969.

Pinto, A.K., Daffis, S., Brien, J.D., Gainey, M.D., Yokoyama, W.M., Sheehan, K.C., Murphy, K.M., Schreiber, R.D., and Diamond, M.S. (2011). A temporal role of type I interferon signaling in CD8+ T cell maturation during acute West Nile virus infection. PLoS Pathog. *7*, e1002407.

Plantinga, M., Guilliams, M., Vanheerswynghels, M., Deswarte, K., Branco-Madeira, F., Toussaint, W., Vanhoutte, L., Neyt, K., Killeen, N., Malissen, B., Hammad, H., and Lambrecht, B.N. (2013). Conventional and Monocyte-Derived CD11b(+) Dendritic Cells Initiate and Maintain T Helper 2 Cell-Mediated Immunity to House Dust Mite Allergen. Immunity *38*, 322–335.

Robbins, S.H., Walzer, T., Dembélé, D., Thibault, C., Defays, A., Bessou, G., Xu, H., Vivier, E., Sellars, M., Pierre, P., et al. (2008). Novel insights into the relationships between dendritic cell subsets in human and mouse revealed by genome-wide expression profiling. Genome Biol. *9*, R17.

Robinson, D.S. (2000). The Th1 and Th2 concept in atopic allergic disease. Chem. Immunol. *78*, 50–61.

Robinson, D.S., Hamid, Q., Ying, S., Tsicopoulos, A., Barkans, J., Bentley, A.M., Corrigan, C., Durham, S.R., and Kay, A.B. (1992). Predominant TH2-like bronchoalveolar T-lymphocyte population in atopic asthma. N. Engl. J. Med. *326*, 298–304.

Sabin, E.A., Kopf, M.A., and Pearce, E.J. (1996). Schistosoma mansoni egg-induced early IL-4 production is dependent upon IL-5 and eosinophils. J. Exp. Med. *184*, 1871–1878.

Satpathy, A.T., Kc, W., Albring, J.C., Edelson, B.T., Kretzer, N.M., Bhattacharya, D., Murphy, T.L., and Murphy, K.M. (2012a). Zbtb46 expression distinguishes classical dendritic cells and their committed progenitors from other immune lineages. J. Exp. Med. *209*, 1135–1152.

Satpathy, A.T., Wu, X., Albring, J.C., and Murphy, K.M. (2012b). Re(de)fining the dendritic cell lineage. Nat. Immunol. *13*, 1145–1154.

Satpathy, A.T., Briseño, C.G., Lee, J.S., Ng, D., Manieri, N.A., Kc, W., Wu, X., Thomas, S.R., Lee, W.L., Turkoz, M., et al. (2013). Notch2-dependent classical dendritic cells orchestrate intestinal immunity to attaching-and-effacing bacterial pathogens. Nat. Immunol. *14*, 937–948.

Schlitzer, A., McGovern, N., Teo, P., Zelante, T., Atarashi, K., Low, D., Ho, A.W., See, P., Shin, A., Wasan, P.S., et al. (2013). IRF4 transcription factor-dependent CD11b+ dendritic cells in human and mouse control mucosal IL-17 cytokine responses. Immunity *38*, 970–983.

Schraml, B.U., Hildner, K., Ise, W., Lee, W.L., Smith, W.A., Solomon, B., Sahota, G., Sim, J., Mukasa, R., Cemerski, S., et al. (2009). The AP-1 transcription factor Batf controls T(H)17 differentiation. Nature *460*, 405–409.

Segre, J.A., Bauer, C., and Fuchs, E. (1999). Klf4 is a transcription factor required for establishing the barrier function of the skin. Nat. Genet. *22*, 356–360.

Spits, H., Couwenberg, F., Bakker, A.Q., Weijer, K., and Uittenbogaart, C.H. (2000). Id2 and Id3 inhibit development of CD34(+) stem cells into predendritic cell (pre-DC)2 but not into pre-DC1. Evidence for a lymphoid origin of pre-DC2. J. Exp. Med. *192*, 1775–1784.

Tailor, P., Tamura, T., Morse, H.C., 3rd, and Ozato, K. (2008). The BXH2 mutation in IRF8 differentially impairs dendritic cell subset development in the mouse. Blood *111*, 1942–1945.

Terry, R.L., and Miller, S.D. (2014). Molecular control of monocyte development. Cell. Immunol. *291*, 16–21.

Torti, N., Walton, S.M., Murphy, K.M., and Oxenius, A. (2011). Batf3 transcription factor-dependent DC subsets in murine CMV infection: differential impact on T-cell priming and memory inflation. Eur. J. Immunol. *41*, 2612–2618.

Tussiwand, R., Lee, W.L., Murphy, T.L., Mashayekhi, M., Kc, W., Albring, J.C., Satpathy, A.T., Rotondo, J.A., Edelson, B.T., Kretzer, N.M., et al. (2012). Compensatory dendritic cell development mediated by BATF-IRF interactions. Nature *490*, 502–507.

Vander Lugt, B., Khan, A.A., Hackney, J.A., Agrawal, S., Lesch, J., Zhou, M., Lee, W.P., Park, S., Xu, M., DeVoss, J., et al. (2014). Transcriptional programming of dendritic cells for enhanced MHC class II antigen presentation. Nat. Immunol. *15*, 161–167.

Wang, Y., Szretter, K.J., Vermi, W., Gilfillan, S., Rossini, C., Cella, M., Barrow, A.D., Diamond, M.S., and Colonna, M. (2012). IL-34 is a tissue-restricted ligand of CSF1R required for the development of Langerhans cells and microglia. Nat. Immunol. *13*, 753–760.

Williams, J.W., Tjota, M.Y., Clay, B.S., Vander Lugt, B., Bandukwala, H.S., Hrusch, C.L., Decker, D.C., Blaine, K.M., Fixsen, B.R., Singh, H., et al. (2013). Transcription factor IRF4 drives dendritic cells to promote Th2 differentiation. Nat. Commun. *4*, 2990.

Yamanaka, S. (2008). Induction of pluripotent stem cells from mouse fibroblasts by four transcription factors. Cell Prolif. *41* (1), 51–56.

Yoshida, T., and Hayashi, M. (2014). Role of Krüppel-like factor 4 and its binding proteins in vascular disease. J. Atheroscler. Thromb. *21*, 402–413.

Zheng, H., Pritchard, D.M., Yang, X., Bennett, E., Liu, G., Liu, C., and Ai, W. (2009). KLF4 gene expression is inhibited by the notch signaling pathway that controls goblet cell differentiation in mouse gastrointestinal tract. Am. J. Physiol. Gastrointest. Liver Physiol. *296*, G490–G498.

Zhou, Q., Ho, A.W., Schlitzer, A., Tang, Y., Wong, K.H., Wong, F.H., Chua, Y.L., Angeli, V., Mortellaro, A., Ginhoux, F., and Kemeny, D.M. (2014). GM-CSF-licensed CD11b+ lung dendritic cells orchestrate Th2 immunity to Blomia tropicalis. J. Immunol. *193*, 496–509.