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# Differential requirement for *Hoxa9* in the development and differentiation of B, NK, and DC-lineage cells from *Flt3*<sup>+</sup> multipotential progenitors

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## Abstract

*Hoxa9* is a homeodomain transcription factor important for the generation of *Flt3*<sup>+</sup>*hi**IL-7R*<sup>-</sup> lymphoid biased-multipotential progenitors, *Flt3*<sup>+</sup>*IL-7R*<sup>+</sup> common lymphoid progenitors (CLPs), and B cell precursors (BCP) in bone marrow (BM). In addition to B-cell, *Flt3*<sup>+</sup>*IL-7R*<sup>+</sup> CLPs possess NK and DC developmental potentials, although DCs arise from *Flt3*<sup>+</sup>*IL-7R*<sup>-</sup> myeloid progenitors as well. In this study, we investigated the requirement for *Hoxa9*, from *Flt3*<sup>+</sup> or *Flt3*<sup>-</sup> progenitor subsets, in the development of NK and DC lineage cells in BM. *Flt3*<sup>+</sup>*IL-7R*<sup>+</sup>*Ly6D*<sup>-</sup> CLPs and their *Flt3*<sup>+</sup>*IL-7R*<sup>+</sup>*Ly6D*<sup>+</sup> B lineage-restricted progeny (BLP) were significantly reduced in *hoxa9*<sup>-/-</sup> mice. Interestingly, the reduction in *Flt3*<sup>+</sup>*IL-7R*<sup>+</sup> CLPs in *hoxa9*<sup>-/-</sup> mice had no impact on the generation of NK precursor (NKP) subsets, the differentiation of NKP into mature NK cells, or NK homeostasis. Similarly, percentages and numbers of common dendritic progenitors (CDP), as well as their plasmacytoid or conventional dendritic cell progeny in *hoxa9*<sup>-/-</sup> mice were comparable to wildtype. These findings reveal distinct requirements for *Hoxa9* or *Hoxa9*/*Flt3* molecular circuits in regulation of B versus NK and DC development in BM.

**Keywords:** *Hoxa9*, *Flt3*, *IL-7R*, B-cell, NK-cell, DC, Development

## Background

Cell fate decisions in the hematopoietic system are contingent upon the concerted activities of transcription factors that activate and/or repress lineage specific developmental programs and signaling molecules that regulate the temporal expression and activation status of lineage instructing regulatory proteins. *Hoxa9* is a homeobox transcription factor with key roles in embryogenesis, hematopoiesis, and leukemogenesis [1]. *Hoxa9* is highly expressed in primitive blood cells in mouse and man, and downregulated as multipotent hematopoietic progenitors undergo lineage restriction and commitment [2-4]. The nonredundant role of *Hoxa9* in hematopoietic progenitor biology has been determined through a variety of loss- and gain-of-function strategies. *Hoxa9*<sup>-/-</sup> mice are viable, but exhibit multiple hematopoietic defects including impaired recovery from hematopoietic stress, poor competitive reconstitution upon

transplantation, impaired responses to lineage determining cytokines, and significant reductions in select LSK + subsets, myeloid precursor subsets, common lymphoid progenitors (CLP), thymic progenitors, and B cell precursors (BCP) [5-7]. *Hoxa9* regulates the hematopoietic progenitor and BCP pool, in part, through transcriptional activation of the gene encoding *Flt3* [4]. The *Flt3* signaling pathway is critical for regulation of the hematopoietic progenitor pool and is essential at various stages for B, DC, and NK development and/or homeostasis [4,8,9].

Downregulation of *c-kit*, the receptor for the hematopoietic cytokine stem cell factor, accompanies lymphoid and DC lineage restriction from *Flt3*<sup>+</sup>*hi* LMPP [10-12]. *Lin*-*c-kit*<sup>+</sup>*Flt3*<sup>+</sup> *IL-7R*<sup>+</sup>CLPs are a heterogeneous population that exhibit B, NK, T, and DC lineage differentiation potentials. Gene-targeted deletion of *hoxa9* significantly reduces numbers of *Flt3*<sup>+</sup> *IL-7R*<sup>+</sup>CLPs and BCP in BM [8,13-15]. NKP are also the progeny of *Flt3*<sup>+</sup> *IL-7R*<sup>+</sup>CLPs. Importantly, two independent groups using different experimental approaches recently showed that

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hematopoietic progenitors enriched for NK potential were IL-7R+, but Flt3-. In both studies, downregulation of Flt3 and upregulation of CD122 (IL-15R $\alpha$ ) were sequential steps in NK commitment from Flt3+ IL-7R+CLPs [16,17]. CD122 is a critical component of the IL-15R complex and IL-15 signaling is essential for NK development [18]. Lin-CD122+ NKP expressed high levels of Id2, a transcription factor important for NK differentiation [16]. Flt3 signaling has been suggested to complement IL-15 in regulation of the BM NK cell pool as well as NK homeostasis in the spleen [19]. At present, there is no information regarding the consequence of Hoxa9-deficiency, through Flt3-dependent or Flt3-independent regulatory circuits, on NK commitment, differentiation, or homeostasis.

Dendritic cells (DC) are essential innate immune effector cells. DCs are generated in BM from both the myeloid and lymphoid progenitor pathways. Common dendritic progenitors (CDPs) originate from Flt3+ myeloid progenitors that express Flt3+ but lack expression of the IL-7R [12]. IL-7R+Flt3+ CLP primarily give rise to plasmacytoid DC [20]. Flt3 signaling is essential in regulation of DC development in BM as well as DC homeostasis in the peripheral lymphoid organs [9,12,21]. The Ets-family transcription factor PU.1 regulates Flt3 in DCs [22]. Interestingly, a recent study characterizing Hoxa9 target genes found many Hoxa9 binding sites were also bound by PU.1, notably *flt3* [23]. Therefore, a molecular contribution of Hoxa9 to the development and/or homeostasis of DC lineage cells, merits further investigation.

The goal of this study was to examine the role of Hoxa9 in regulation of NK and DC development and differentiation. We found that, in contrast to B cells, Hoxa9 is largely dispensable for the development or peripheral homeostasis of NK or DC lineage cells. These findings uncover a distinct requirement for Hoxa9 in regulation of B versus NK and DC development from Flt3+ MPPs.

## Methods

### Mice

C57Bl6 mice (8–16 weeks of age) were purchased from The Jackson Laboratory (Bar Harbor, ME). *Hoxa9*<sup>-/-</sup> and *flt3l*<sup>-/-</sup> mice have been described [4] and were bred and maintained at the Mayo Clinic animal facility and used between 8–16 weeks of age. All mice were aged matched in individual experiments and the experimental data did not vary as a function of age or sex. Bone marrow cells from individual male or female mice were collected from the four hind limb bones after carbon dioxide asphyxiation and single cell suspensions made in preparation for flow cytometric analysis. All experiments were carried out in accordance with Mayo Clinic Institutional Animal Care and Use Committee guidelines under Protocol A17509.

### Antibodies and flow cytometry

Methods for flow cytometry and progenitor isolation have been extensively described [4,13]. Flow cytometric analysis was performed on the FACS-Canto or LSRII cytometers (BD Biosciences, San Jose, CA) and analyzed using FlowJo software (Tree Star, Ashland, OR). All antibodies used in this study were purchased from eBioscience, BioLegend, or Pharmingen. Antibody conjugations and combinations used to delineate progenitor subsets were the following: ALP/BLP stain Lin+ cocktail (FITC conjugated CD45R/B220, CD11b/Mac1, Ly6G/Gr1, TER119, CD3 $\epsilon$ , CD8 $\alpha$ , CD11c, NK1.1, Ly6C), c-kit APCefluor 780, Scal-PerCP Cy5.5, biotin IL-7R (visualized with streptavidin PeCy7), Flt3 PE, and Ly6D APC; NKP stain Lin+ cocktail (APC conjugated CD11b/Mac1, CD3 $\epsilon$ , NK1.1, CD19, Ly6D), biotin CD244 (visualized with streptavidin PeCy7), CD27 APCefluor 780, IL-7R PE, Flt3 PerCPefluor 710, and CD122 FITC; and CDP stain Lin+ cocktail (FITC conjugated CD45R/B220, CD11b/Mac1, Ly6G/Gr1, TER-119, CD3 $\epsilon$ , CD8 $\alpha$ , NK1.1), c-kit APCefluor 780, Flt3-PE, bio-IL-7R (visualized with streptavidin PeCy7), and CD115-APC; NK subsets, CD3 $\epsilon$  PeCy7, CD122 FITC, NK1.1 AF780, and Dx5 APC; DC subsets, B220 AF780, MHCII APC, and CD11c PerCPCy5.5. All flow cytometric analysis depicts progenitor subsets within the lymphocyte light scatter gate (LLS) of BM (Figure 1A).

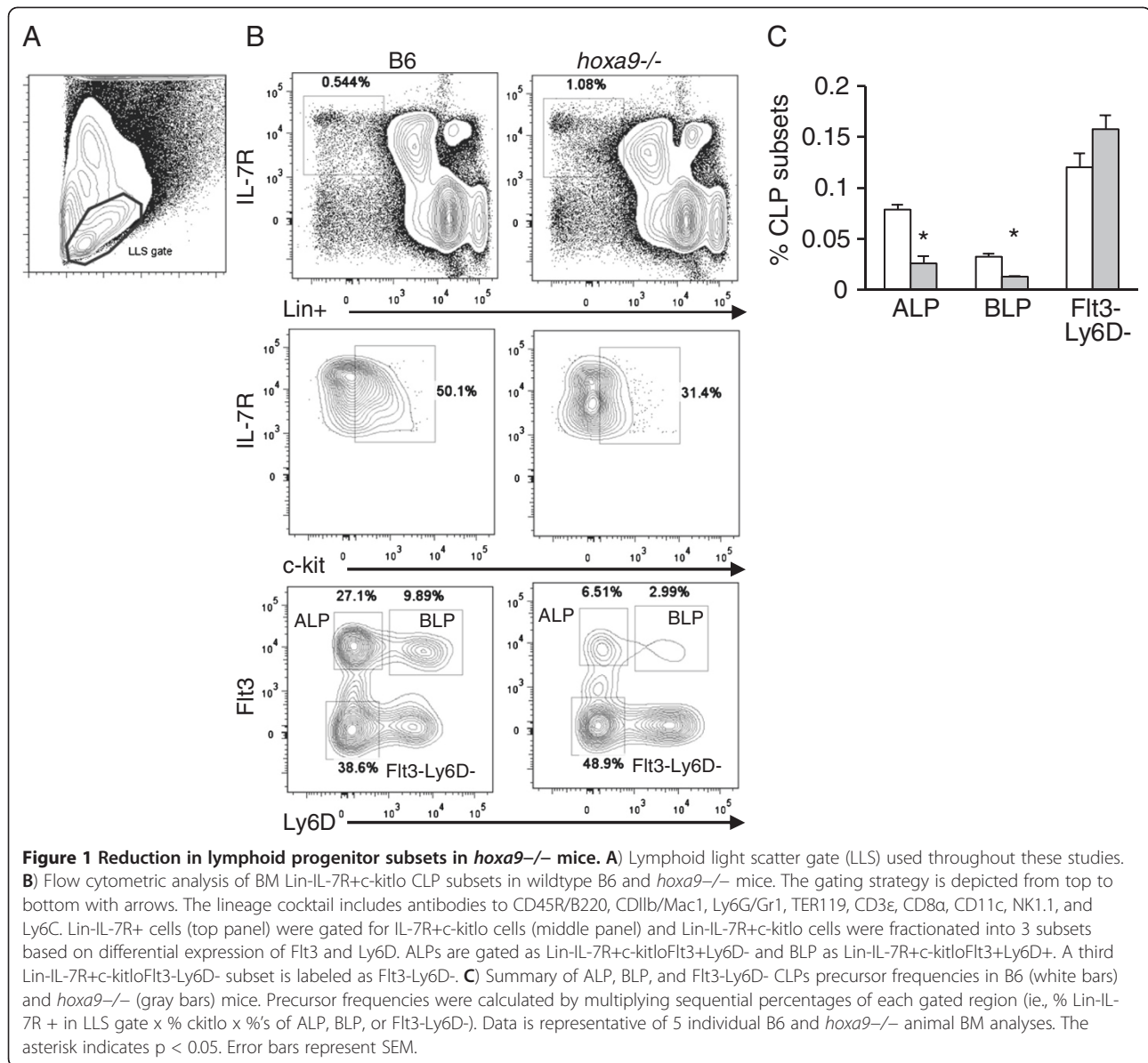
### Statistics

Statistical significance was determined using the Student-t test and p-values less than 0.05 were considered significant.

## Results

### Selective reduction in Flt3+IL-7R+, but not Flt3-IL-7R+CLPs in *hoxa9*<sup>-/-</sup> mice

We previously showed that Lin-c-kitloIL-7R+Flt3+ CLPs are significantly reduced in *hoxa9*<sup>-/-</sup> mice [4]. Lin-c-kitloFlt3+IL-7R+ cells can be fractionated into Flt3+IL-7R+Ly6D- all lymphoid progenitors (ALP) and Flt3+IL-7R+Ly6D+B lineage restricted progenitors (BLP) [24]. Therefore, we employed this flow cytometric analysis to further define the lymphoid/B lineage developmental block in *hoxa9*<sup>-/-</sup> mice [4,15]. The Lin+ cocktail used was extended to include antibodies to CD11c, NK1.1, and Ly6C to eliminate contamination of CLPs with mature NK1.1+ NK or CD11c/Ly6C + DCs. First, Lin-IL-7R+ cells within the lymphocyte light scatter gate (Figure 1A) were gated for low expression of c-kit (Figure 1B, gated region, middle panel). We note that using the extended Lin+ cocktail and gating on IL-7R+ cells, that overall percentages of Lin-IL-7R+ were not reduced in *hoxa9*<sup>-/-</sup> mice ( $0.71 \pm 0.20\%$  vs.  $0.83 \pm 0.29\%$  in *hoxa9*<sup>-/-</sup> vs. wildtype, respectively, n = 5 mice). The Lin-IL-7R+c-kitlo subset was then fractionated into ALP and BLP using differential expression of Flt3 and Ly6D (Figure 1B, bottom panels). This gating strategy revealed that ALP, as well as



their B lineage restricted progeny BLP, are significantly reduced in *hoxa9*<sup>-/-</sup> mice, consistent with our previous findings [4]. Precursor frequency analysis revealed that ALP represent  $0.061 \pm 0.013\%$  vs.  $0.024 \pm 0.01\%$  of BM cells within the lymphoid light scatter gate in B6 vs. *hoxa9*<sup>-/-</sup> mice, respectively ( $p = 0.0009$ ). Similarly, BLP represent  $0.026 \pm 0.002\%$  vs.  $0.01 \pm 0.002\%$  of BM cells within the lymphoid light scatter gate in B6 vs. *hoxa9*<sup>-/-</sup> mice, respectively ( $p = 0.0005$ ). Less BLP resulted in reductions in CD19+IgM- B cell precursors ( $11.5 \pm 2\%$  vs.  $20.03 \pm 2.0\%$  of BM cells within the lymphoid light scatter gate,  $p = 0.0009$ , in *hoxa9*<sup>-/-</sup> vs. B6, respectively), consistent with our previous report [4,15]. In contrast, a conspicuous population of IL-7R+FIt3-Ly6D- CLPs was not reduced ( $0.087 \pm 0.22\%$  vs.

$0.11 \pm 0.06\%$  of BM cells within the lymphoid light scatter gate in B6 vs. *hoxa9*<sup>-/-</sup> mice, respectively,  $p = 0.11$ ). Percentages of ALP, BLP, and IL-7R+FIt3-Ly6D- CLPs in B6 control and *hoxa9*<sup>-/-</sup> mice are summarized in Figure 1C (data represents mean and SEM). We conclude from these flow cytometry analyses that Hoxa9-deficiency preferentially reduces FIt3+IL-7R+, but not FIt3-IL-7R+ CLP subsets.

#### NK development and differentiation in *hoxa9*<sup>-/-</sup> mice

CD27+ and CD244+ are cell surface markers expressed on IL-7R-FIt3+ multipotential progenitors (MPP) and CLPs, and maintained on mature NK cells [25,26]. A small subset of CD27+CD244+IL-7R+ progenitors were identified in BM that lacked expression of surface

markers displayed by lineage committed precursor subsets, including CD19, Mac1, Ly6D, NK1.1, and CD3. CD122 (IL-2R $\beta$ ) is a critical component of the IL-15R complex (comprised of IL-15R $\alpha$ , IL-2R $\gamma$  and IL-2R $\beta$ ) [27]. Expression of the IL-15R is acquired as CLP differentiate into NK cells and is associated with loss of B, T, and DC lineage differentiation potentials. Fathman, et al., evaluated NK precursor potential in Lin- (CD19-Mac1- Ly6D- NK1.1- CD3-) CD27+CD244+IL-7R+ cells [17]. Within the Lin-CD27+CD244+IL-7R+ subset, differential expression of Flt3 and CD122 distinguished three progenitor subsets, Flt3+CD122-, Flt3-CD122-, and Flt3-CD122+. Transplantation studies revealed that the Lin- CD27+CD244+ IL-7R+Flt3+ subset were found to functionally largely overlap CLPs, while the Lin-CD27+ and Lin-CD244+ subsets gave rise to NK cells. *In vitro* differentiation studies confirmed that the Lin-CD27+CD244+IL-7R+Flt3-CD122- and CD122+ subsets generated only NK cells. Based on differential expression of CD122 and the NK lineage bias exhibited by these cells in functional assays, the Lin-CD27+CD244+IL-7R+Flt3-CD122- subset was designated PreNKP and the Lin-CD27+CD244+IL-7R+Flt3-CD122+ subset rNKP [17]. Previous studies showed that loss of Flt3-L significantly reduced the mature BM NK pool [9,19]. However, a role for Hoxa9, through Flt3 dependent or independent mechanisms, in regulation of NK commitment and differentiation from the CLP stage has not been determined.

We showed in Figure 1B-C that a subset of IL-7R+ CLPs that lack Flt3- and Ly6D- are spared by Hoxa9-deficiency. Since IL-7R+Flt3-Ly6D- CLPs are enriched for NK precursors we speculated that Hoxa9 might be dispensable for NK commitment and differentiation from CLPs. To make this determination, we employed the flow cytometric analysis described above to examine the NK precursor compartment in *hoxa9*<sup>-/-</sup> mice. Consistent with our findings in Figure 1B, percentages and absolute numbers of Lin- CD27+CD244+IL-7R+Flt3+ CLPs were significantly reduced in *hoxa9*<sup>-/-</sup> mice (Figure 2A-C). Importantly, shown in Figure 2A and summarized in Figures 2B-C, neither frequencies nor numbers of PreNKP or rNKP within the lymphoid light scatter gate in BM were diminished by Hoxa9-deficiency. The reduction in CLPs reinforces the requirement for Hoxa9 in regulation of the IL-7R+Flt3+ CLP subset. Surprisingly, the reduction in IL-7R+Flt3+ CLPs did not impact the generation or maintenance of Pre-NKP or rNKP. These data suggest that commitment to the NK fate is not dependent on Hoxa9 function within CLPs.

Flt3 signaling has been implicated in regulation of the mature BM NK compartment. Specifically, it was shown that mature Lin-CD122+NK1.1+Dx5+ NK cells are reduced in *flt3l*<sup>-/-</sup> mice and our results concur with those findings (Figure 2D-E) [19]. Until the identification

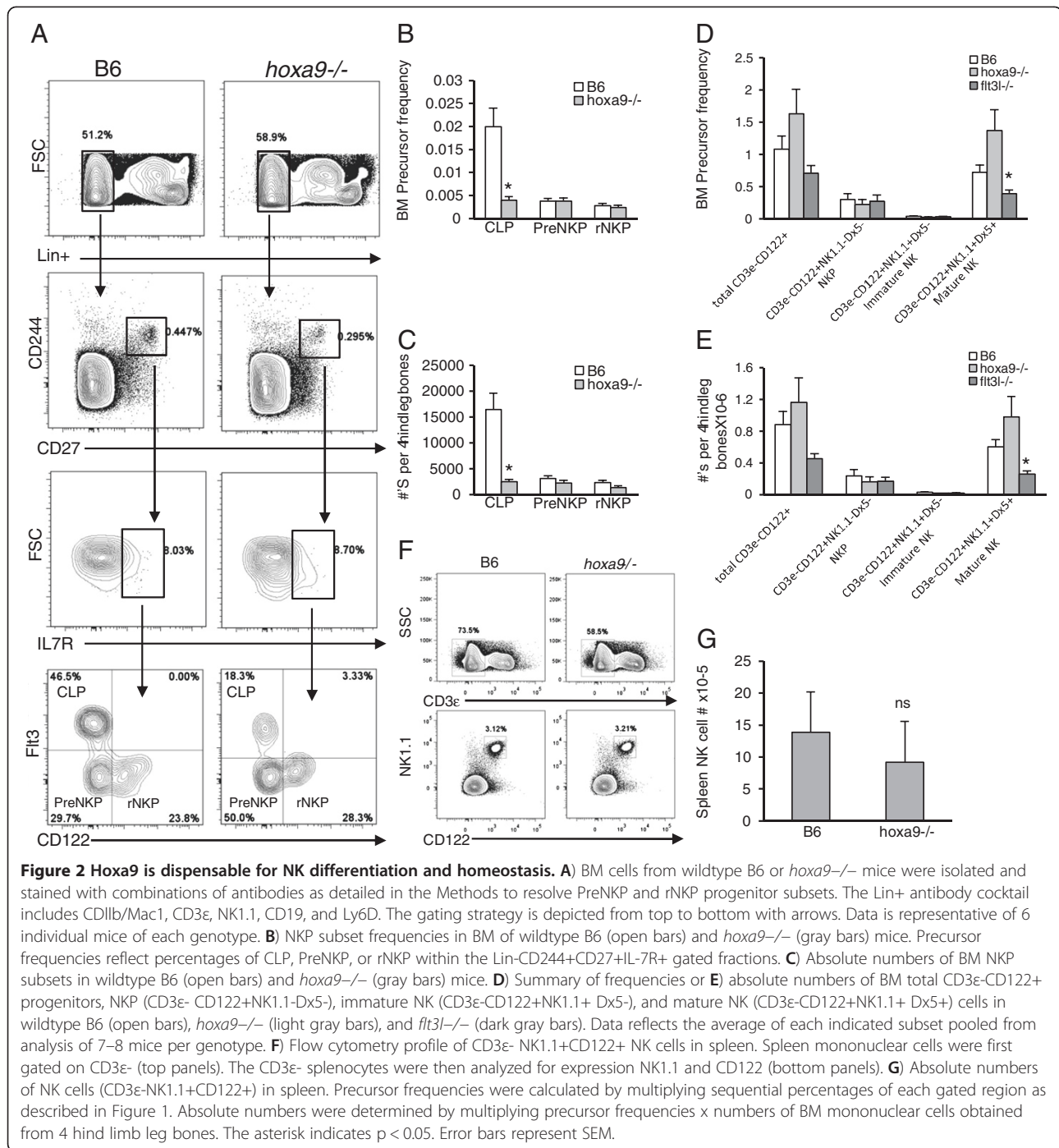
of NKP, BM NK transitional subsets were distinguished by differential expression of CD3 $\epsilon$ , CD122, NK1.1, and CD49b/Dx5 [28]. To determine whether Hoxa9 regulates NK differentiation from NKP, BM cells from B6 wildtype, *hoxa9*<sup>-/-</sup>, and *flt3*<sup>-/-</sup> mice were stained with antibodies to CD3 $\epsilon$ , CD122, NK1.1, and CD49b (Dx5) [28]. As shown in Figure 2D-E, percentages and numbers of CD3 $\epsilon$ -CD122+NK1.1-Dx5- NKP and CD3 $\epsilon$ -CD122+NK1.1+Dx5- immature NK cells were similar between the 3 genotypes. These results suggest that Hoxa9 is dispensable for NK differentiation from rNKP. However, in contrast to Flt3 signaling, Hoxa9 is also dispensable for NK homeostasis in BM and spleen as neither mature NK cell frequencies nor numbers are reduced in BM or spleen in *hoxa9*<sup>-/-</sup> mice (Figure 2D-G).

#### DC differentiation in *hoxa9*<sup>-/-</sup> mice

Lineage-restricted precursors of DCs are enriched within Lin-ckit+loFlt3+ MPPs [12]. Flt3 signaling is essential for DC differentiation and homeostasis [21,29]. Lineage-restricted common dendritic progenitors CDP express the CD115/M-CSFR, but lack expression of the lymphoid-lineage-associated cytokine receptor IL-7R [12]. Lin-ckit+loFlt3+ progenitors are reduced in *hoxa9*<sup>-/-</sup> mice (Figure 3A, middle panel). However, percentages of Lin-c-kit+lo Flt3+ cells expressing the CD115/M-CSFR were comparable between wildtype and *hoxa9*<sup>-/-</sup> mice (Figure 3A and summarized in Figure 3B). Next we compared percentages and absolute numbers of DC-lineage progeny. CD11c+MHCII+B220- conventional DC (cDC) and CD11c+MHCII+B220+ plasmacytoid DC (pDC) are the progeny of CDP, although there is experimental evidence that DCs develop from CLP [20,29-31]. As shown in Figure 4A-C, frequencies and absolute numbers of cDC (gray bars) and pDC (white bars) in *hoxa9*<sup>-/-</sup> mice were largely comparable to WT. Furthermore, percentages and absolute numbers of cDC and pDC were also found at comparable frequencies to B6 wildtype mice in the spleen (data not shown). Consistent with previous findings, cDC and pDC were reduced in BM and spleen of *flt3l*<sup>-/-</sup> animals (Figure 4B-C and data not shown) [9]. These data suggest that Hoxa9 is not a critical component of the regulatory circuitry that orchestrates DC commitment and/or differentiation from Flt3+ MPPs.

#### Discussion

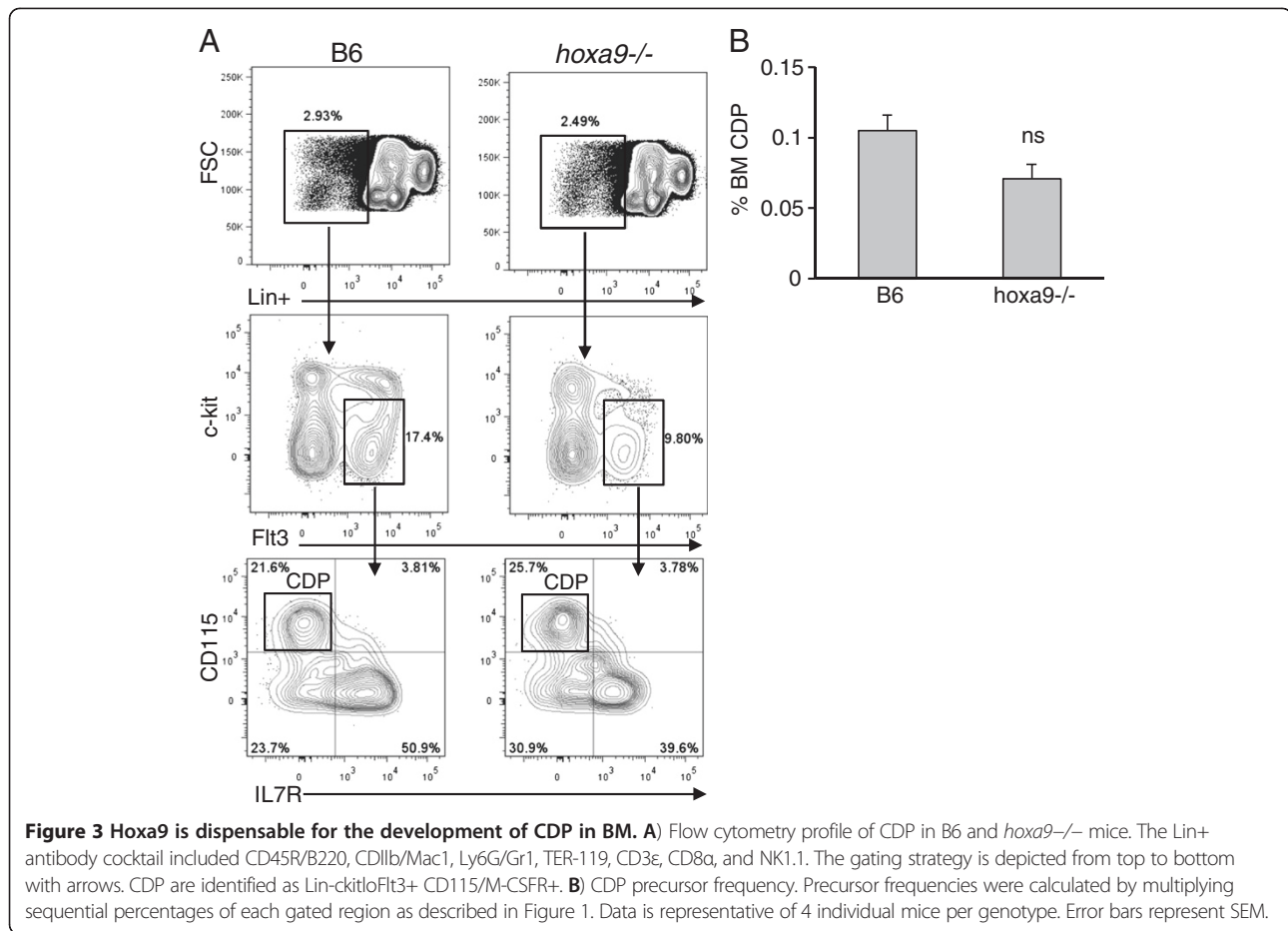
In this study we investigated the role of Hoxa9 in regulation of NK- and DC-lineage commitment and differentiation in BM. Hoxa9-deficient mice exhibit reductions in select hematopoietic progenitor subsets, including Flt3+ MPPs, Flt3+IL-7R+ CLPs, myeloid progenitor subsets, and BCPs, in addition to Pro-T subsets in the thymus [4,15]. Hoxa9 regulates these hematopoietic progenitor subsets, at least in part, through transcriptional regulation



of Flt3 [4]. Flt3 signaling is important for the development and/or maintenance of Flt3+ MPPs, CLPs, B, T, NK, and DC lineage cells [9,19]. Previously, a role for Hoxa9, through Flt3-dependent or independent regulatory circuits, in regulation of NK- or DC-lineage commitment and differentiation in BM had not been established. Here we show that Hoxa9 is dispensable for the generation and differentiation of NK and DC lineage cells in BM. In contrast, Hoxa9 plays a non-redundant role in regulation of

Flt3+IL-7R+ CLPs, as well as their Flt3+IL-7R+Ly6D+ B-lineage restricted progeny. Importantly, the reduction in Flt3+IL-7R+ CLPs in *hoxa9*<sup>-/-</sup> mice does not compromise the generation of NKP. These findings distinguish a critical function for Hoxa9 in Flt3+IL-7R+ CLPs in regulation of B, but not NK lineage commitment.

The differentiation of DC lineage cells from multipotent hematopoietic progenitors is driven by the combinatorial activities of cytokines, notably Flt3L, M-CSF,

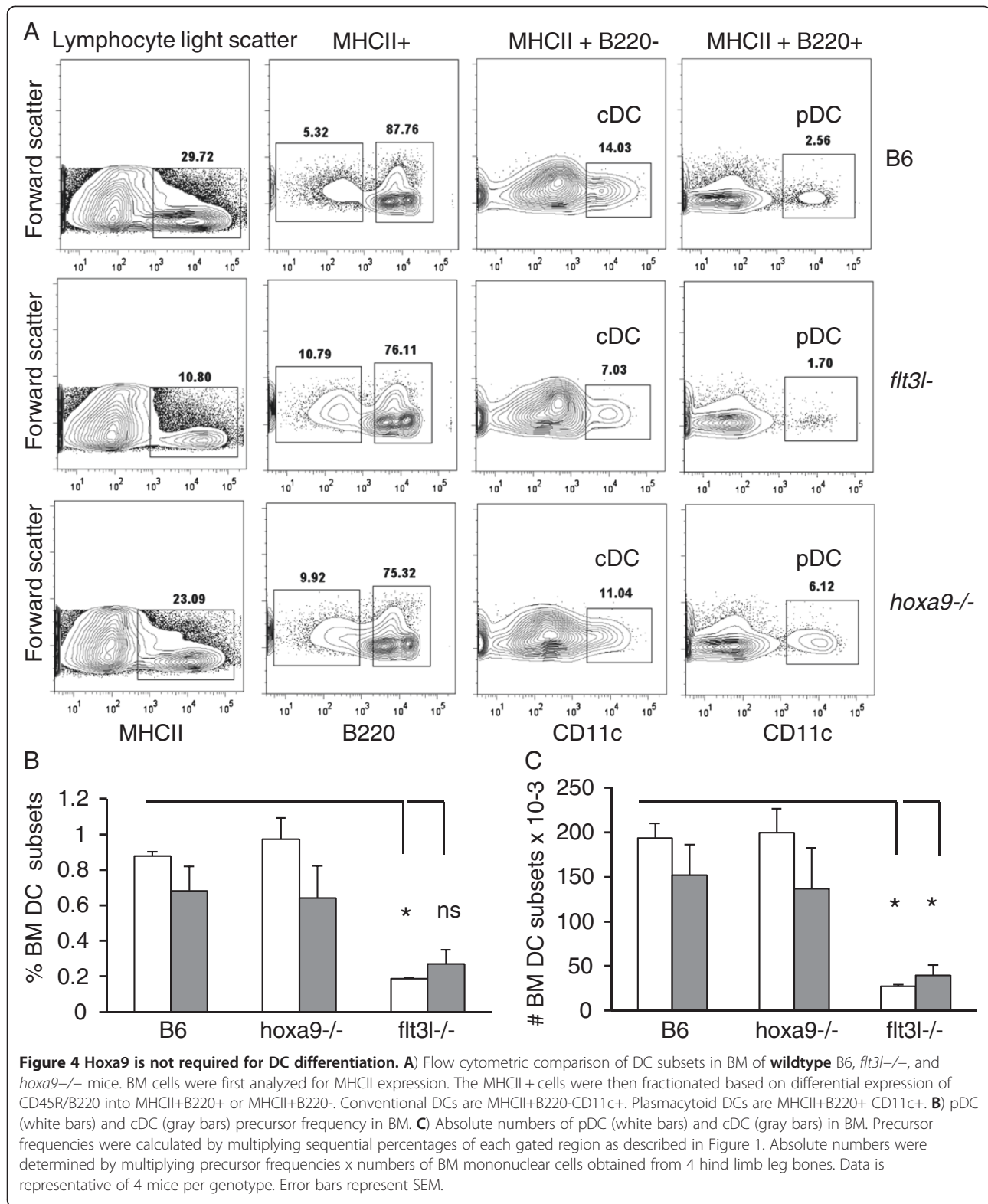


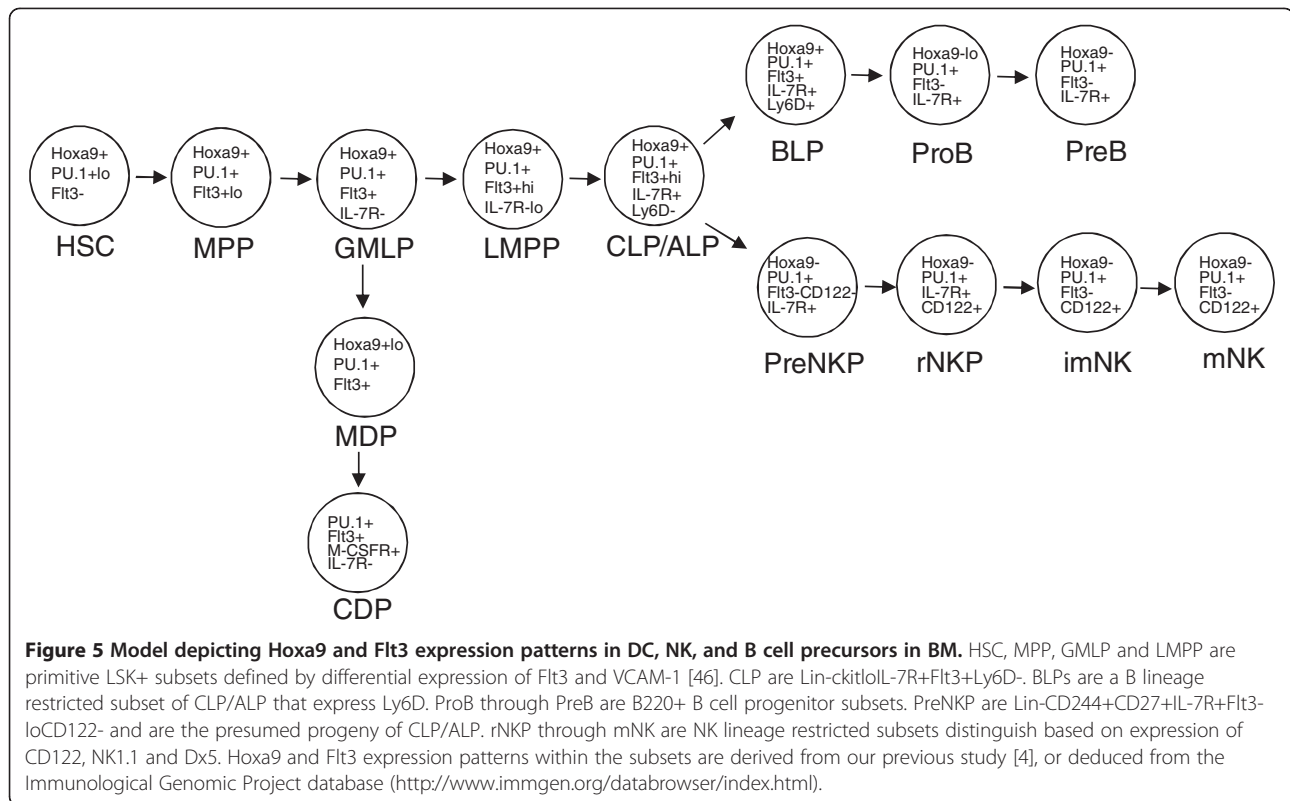
and GM-CSF [32]. Importantly, DCs develop from both myeloid and lymphoid precursors. Myeloid progenitors are minimally affected by *Hoxa9*-deficiency, thus providing an alternate route for DC production [6]. Although *Hoxa9* is a critical regulator of *flt3*, *Flt3* is expressed, albeit at lower levels, in *Hoxa9*-deficient hematopoietic progenitors [4]. In addition to *Hoxa9*, the Ets-family transcription factor PU.1 is a key regulator of *flt3*. It was previously established that PU.1 regulation of *Flt3* is essential for DC development [22]. *Hoxa9* transcripts are low/negative in CDP and Macrophage Dendritic Progenitors (MDP), compared to *Flt3*<sup>+</sup> MPP or CLPs. In contrast, PU.1 transcripts are elevated in CDP and MDP (Figure 5) [33]. Hierarchically, MDP give rise to CDP, and CDP are the lineage restricted precursors of conventional and plasmacytoid dendritic cells [34]. Interestingly, MDP numbers are not reduced in *flt3*<sup>-/-</sup> mice, suggesting that *Flt3* expression is not required for commitment to the DC fate [21]. Our data extend these results and establish that *Hoxa9* function is dispensable for commitment and differentiation in the DC lineage.

A recent fate mapping study of an IL-7R reporter mouse suggested that all NK-lineage cells progress through an IL-7R<sup>+</sup> stage [35]. IL-7R<sup>+</sup> hematopoietic

progenitors are generated in *flt3*<sup>-/-</sup> and *flt3l*<sup>-/-</sup> mice, albeit at reduced frequencies [8,13]. Thus, *Flt3* signaling is not essential for induction of IL-7R expression, consistent with our current findings. CD122 is upregulated in *Flt3*<sup>-</sup> CLPs uncoupling *Flt3* signaling from induction of CD122 expression. PreNKP have been proposed to be developmentally positioned downstream of *Flt3*<sup>+</sup> IL-7R<sup>+</sup> CLPs (Figure 5). We note that the few B220<sup>+</sup> cells identified in *FL*<sup>-/-</sup> *x* *IL-7Ra*<sup>-/-</sup> mice are NK1.1<sup>+</sup> [36]. Furthermore, *il7*<sup>-/-</sup> mice exhibit no deficiencies in NK cells in BM [19,37]. These experimental results together with our findings herein suggest that commitment to the NK fate is not dependent on the combinatorial activities of *Flt3* and/or IL-7R signaling or *Hoxa9* function.

We show significantly reduced percentages of *Flt3*+IL-7R<sup>+</sup> CLP/ALPs and *Flt3*+IL-7R<sup>+</sup> BLPs in *hoxa9*<sup>-/-</sup> mice. The combinatorial activities of *Flt3* and IL-7R signaling are indispensable for B cell genesis [36]. IL-7R signaling in CLPs promotes the generation of BLP and their subsequent differentiation into BCP through Stat5-mediated induction of the B-cell fate determinant EBF [38,39]. EBF in turn suppresses *Id2* transcription [40,41]. *Id2* is critical for NK differentiation and transcript levels are increased in IL-7R<sup>+</sup>*Flt3*<sup>-</sup> rNKP [16,42-44]. The observation that





frequencies and numbers of PreNKP and rNKP are essentially unchanged while BLP are reduced suggest that Hoxa9, alone or in combination with Flt3 signaling, regulates a critical molecular circuitry that dictates the B vs. NK fate decision in Flt3+IL-7R+ CLP/ALPs.

Finally, the dramatic downregulation of Flt3 in PreNKP suggests that commitment to the NK fate might initiate a series of events that silences *flt3* expression. Induction of the B lineage commitment factor Pax5 silences Flt3 transcription in committed BCP [45]. Interestingly, failure to downregulate Flt3 impairs B cell differentiation. Ectopic expression studies examining the consequences of sustained expression of Flt3 or Hoxa9 on NK differentiation will be informative with regard to the requirement for regulated expression of Flt3 or Hoxa9 in NK development.

## Conclusion

In summary, Hoxa9 is a critical regulator of early hematopoietic differentiation, in part through regulation of Flt3. We uncovered differential requirements for a Hoxa9 in the development/maintenance of IL-7R+Flt3+ CLPs and BLP. Although NKP and CDP share a common Lin-c-kitloFlt3+ progenitor with BLP, Hoxa9 is dispensable for their development and differentiation. These findings reinforce the stringent requirement for Hoxa9 in regulation of B lymphopoiesis and support future studies aimed at delineating Hoxa9-dependent genetic circuits that dictate the B cell fate decision.

## Abbreviations

BM: Bone marrow; MPP: Multipotential progenitor; LSK: Lineage negative Sca-1+ c-kit + hi; GMLP: Granulocyte-macrophage-lymphoid progenitor; LMPP: Lymphoid biased multipotential progenitor; CLP: Common lymphoid progenitor; ALP: All lymphoid progenitor; BCP: B cell precursor; NK: Natural killer cell progenitor; rNKP: Restricted natural killer cell progenitor; CDP: Common dendritic progenitor; MDP: Macrophage dendritic progenitor; Flt3L: Flt3 ligand.

## Competing interests

The authors declare that they have no competing financial interests.

## Authors' contributions

KLM conceived and designed the experiments, performed data analysis, made figures and wrote the manuscript. KG, JJD, MBS designed and performed experiments and analyzed data. All authors read and approved the final manuscript.

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## References

1. Abramovich C, Humphries RK: **Hox regulation of normal and leukemic hematopoietic stem cells.** *Curr Opin Hematol* 2005, **12**(3):210-216.
2. Sauvageau G, Lansdorp PM, Eaves CJ, Hogge DE, Dragowska WH, Reid DS, Largman C, Lawrence HJ, Humphries RK: **Differential expression of homeobox genes in functionally distinct CD34+ subpopulations of human bone marrow cells.** *Proc Natl Acad Sci USA* 1994, **91**(25):12223-12227.
3. So CW, Karsunky H, Wong P, Weissman IL, Cleary ML: **Leukemic transformation of hematopoietic progenitors by MLL-GAS7 in the absence of Hoxa7 or Hoxa9.** *Blood* 2004, **103**(8):3192-3199.



4. Gwin K, Frank E, Bossou A, Medina KL: **Hoxa9 regulates Flt3 in lymphohematopoietic progenitors.** *J Immunol* 2010, **185**(11):6572–6583.
5. Lawrence HJ, Christensen J, Fong S, Hu YL, Weissman I, Sauvageau G, Humphries RK, Largman C: **Loss of expression of the Hoxa-9 homeobox gene impairs the proliferation and repopulating ability of hematopoietic stem cells.** *Blood* 2005, **106**(12):3988–3994.
6. Lawrence HJ, Helgason CD, Sauvageau G, Fong S, Izon DJ, Humphries RK, Largman C: **Mice bearing a targeted interruption of the homeobox gene HOXA9 have defects in myeloid, erythroid, and lymphoid hematopoiesis.** *Blood* 1997, **89**(6):1922–1930.
7. Thorsteinsdottir U, Mamo A, Kroon E, Jerome L, Bijl J, Lawrence HJ, Humphries K, Sauvageau G: **Overexpression of the myeloid leukemia-associated Hoxa9 gene in bone marrow cells induces stem cell expansion.** *Blood* 2002, **99**(1):121–129.
8. Mackaretschian K, Hardin JD, Moore KA, Boast S, Goff SP, Lemischka IR: **Targeted disruption of the flk2/flt3 gene leads to deficiencies in primitive hematopoietic progenitors.** *Immunity* 1995, **3**(1):147–161.
9. McKenna HJ, Stocking KL, Miller RE, Brasel K, De Smedt T, Maraskovsky E, Maliszewski CR, Lynch DH, Smith J, Pulendran B, et al: **Mice lacking flt3 ligand have deficient hematopoiesis affecting hematopoietic progenitor cells, dendritic cells, and natural killer cells.** *Blood* 2000, **95**(11):3489–3497.
10. Kondo M, Weissman IL, Akashi K: **Identification of clonogenic common lymphoid progenitors in mouse bone marrow.** *Cell* 1997, **91**(5):661–672.
11. Payne KJ, Medina KL, Kincade PW: **Loss of c-kit accompanies B-lineage commitment and acquisition of CD45R by most murine B-lymphocyte precursors.** *Blood* 1999, **94**(2):713–723.
12. Onai N, Obata-Onai A, Schmid MA, Ohteki T, Jarrossay D, Manz MG: **Identification of clonogenic common Flt3 + M-CSFR + plasmacytoid and conventional dendritic cell progenitors in mouse bone marrow.** *Nat Immunol* 2007, **8**(11):1207–1216.
13. Dolence JJ, Gwin K, Frank E, Medina KL: **Threshold levels of Flt3-ligand are required for the generation and survival of lymphoid progenitors and B-cell precursors.** *Eur J Immunol* 2011, **41**(2):324–334.
14. Sitnicka E, Bryder D, Theilgaard-Monch K, Buza-Vidas N, Adolfsson J, Jacobsen SE: **Key role of flt3 ligand in regulation of the common lymphoid progenitor but not in maintenance of the hematopoietic stem cell pool.** *Immunity* 2002, **17**(4):463–472.
15. So CW, Karsunky H, Passegue E, Cozzio A, Weissman IL, Cleary ML: **MLL-GAS7 transforms multipotent hematopoietic progenitors and induces mixed lineage leukemias in mice.** *Cancer Cell* 2003, **3**(2):161–171.
16. Carotta S, Pang SH, Nutt SL, Belz GT: **Identification of the earliest NK-cell precursor in the mouse BM.** *Blood* 2011, **117**(20):5449–5452.
17. Fathman JW, Bhattacharya D, Inlay MA, Seita J, Karsunky H, Weissman IL: **Identification of the earliest natural killer cell-committed progenitor in murine bone marrow.** *Blood* 2011, **118**(20):5439–5447.
18. Puzanov IJ, Bennett M, Kumar V: **IL-15 can substitute for the marrow microenvironment in the differentiation of natural killer cells.** *J Immunol* 1996, **157**(10):4282–4285.
19. Cheng M, Charoudeh HN, Brodin P, Tang Y, Lakshminanth T, Hoglund P, Jacobsen SE, Sitnicka E: **Distinct and overlapping patterns of cytokine regulation of thymic and bone marrow-derived NK cell development.** *J Immunol* 2009, **182**(3):1460–1468.
20. Sathe P, Vremec D, Wu L, Corcoran L, Shortman K: **Convergent differentiation: myeloid and lymphoid pathways to murine plasmacytoid dendritic cells.** *Blood* 2013, **121**(1):11–19.
21. Waskow C, Liu K, Darrasse-Jeze G, Guermonprez P, Ginhoux F, Merad M, Shengelia T, Yao K, Nussenzweig M: **The receptor tyrosine kinase Flt3 is required for dendritic cell development in peripheral lymphoid tissues.** *Nat Immunol* 2008, **9**(6):676–683.
22. Carotta S, Dakic A, D'Amico A, Pang SH, Greig KT, Nutt SL, Wu L: **The transcription factor PU.1 controls dendritic cell development and Flt3 cytokine receptor expression in a dose-dependent manner.** *Immunity* 2010, **32**(5):628–641.
23. Huang Y, Sitwala K, Bronstein J, Sanders D, Dandekar M, Collins C, Robertson G, MacDonald J, Cezard T, Bilenky M, et al: **Identification and characterization of Hoxa9 binding sites in hematopoietic cells.** *Blood* 2012, **119**(2):388–398.
24. Inlay MA, Bhattacharya D, Sahoo D, Serwold T, Seita J, Karsunky H, Plevritis SK, Dill DL, Weissman IL: **Ly6d marks the earliest stage of B-cell specification and identifies the branchpoint between B-cell and T-cell development.** *Genes Dev* 2009, **23**(20):2376–2381.
25. Wiesmann A, Phillips RL, Mojica M, Pierce LJ, Searles AE, Spangrude GJ, Lemischka I: **Expression of CD27 on murine hematopoietic stem and progenitor cells.** *Immunity* 2000, **12**(2):193–199.
26. Kim S, Iizuka K, Kang HS, Dokun A, French AR, Greco S, Yokoyama WM: **In vivo developmental stages in murine natural killer cell maturation.** *Nat Immunol* 2002, **3**(6):523–528.
27. Budagian V, Bulanova E, Paus R, Bulfone-Paus S: **IL-15/IL-15 receptor biology: a guided tour through an expanding universe.** *Cytokine Growth Factor Rev* 2006, **17**(4):259–280.
28. Rosmaraki EE, Douagi I, Roth C, Colucci F, Cumano A, Di Santo JP: **Identification of committed NK cell progenitors in adult murine bone marrow.** *Eur J Immunol* 2001, **31**(6):1900–1909.
29. Karsunky H, Merad M, Cozzio A, Weissman IL, Manz MG: **Flt3 ligand regulates dendritic cell development from Flt3+ lymphoid and myeloid-committed progenitors to Flt3+ dendritic cells in vivo.** *J Exp Med* 2003, **198**(2):305–313.
30. Traver D, Akashi K, Manz M, Merad M, Miyamoto T, Engleman EG, Weissman IL: **Development of CD8alpha-positive dendritic cells from a common myeloid progenitor.** *Science (New York, NY)* 2000, **290**(5499):2152–2154.
31. Manz MG, Traver D, Miyamoto T, Weissman IL, Akashi K: **Dendritic cell potentials of early lymphoid and myeloid progenitors.** *Blood* 2001, **97**(11):3333–3341.
32. Belz GT, Nutt SL: **Transcriptional programming of the dendritic cell network.** *Nat Rev Immunol* 2012, **12**(2):101–113.
33. Miller JC, Brown BD, Shay T, Gautier EL, Jojic V, Cohain A, Pandey G, Leboeuf M, Elpek KG, Helft J, et al: **Deciphering the transcriptional network of the dendritic cell lineage.** *Nat Immunol* 2012, **13**(9):888–899.
34. Liu K, Vitorica GD, Schwickert TA, Guermonprez P, Meredith MM, Yao K, Chu FF, Randolph GJ, Rudensky AY, Nussenzweig M: **In vivo analysis of dendritic cell development and homeostasis.** *Science (New York, NY)* 2009, **324**(5925):392–397.
35. Schlenner SM, Madan V, Busch K, Tietz A, Laufle C, Costa C, Blum C, Fehling HJ, Rodewald HR: **Fate mapping reveals separate origins of T cells and myeloid lineages in the thymus.** *Immunity* 2010, **32**(3):426–436.
36. Sitnicka E, Brakebusch C, Martensson IL, Svensson M, Agace WW, Sigvardsson M, Buza-Vidas N, Bryder D, Cilio CM, Ahlenius H, et al: **Complementary signaling through flt3 and interleukin-7 receptor alpha is indispensable for fetal and adult B cell genesis.** *J Exp Med* 2003, **198**(10):1495–1506.
37. Vosshenrich CA, Ranson T, Samson SJ, Corcuff E, Colucci F, Rosmaraki EE, Di Santo JP: **Roles for common cytokine receptor gamma-chain-dependent cytokines in the generation, differentiation, and maturation of NK cell precursors and peripheral NK cells in vivo.** *J Immunol* 2005, **174**(3):1213–1221.
38. Kikuchi K, Kasai H, Watanabe A, Lai AY, Kondo M: **IL-7 specifies B cell fate at the common lymphoid progenitor to pre-proB transition stage by maintaining early B cell factor expression.** *J Immunol* 2008, **181**(1):383–392.
39. Dias S, Silva H Jr, Cumano A, Vieira P: **Interleukin-7 is necessary to maintain the B cell potential in common lymphoid progenitors.** *J Exp Med* 2005, **201**(6):971–979.
40. Pongubala JM, Northrup DL, Lancki DW, Medina KL, Treiber T, Bertolino E, Thomas M, Grosschedl R, Allman D, Singh H: **Transcription factor EBF restricts alternative lineage options and promotes B cell fate commitment independently of Pax5.** *Nat Immunol* 2008, **9**(2):203–215.
41. Thal MA, Carvalho TL, He T, Kim HG, Gao H, Hagman J, Klug CA: **Ebf1-mediated down-regulation of Id2 and Id3 is essential for specification of the B cell lineage.** *Proc Natl Acad Sci USA* 2009, **106**(2):552–557.
42. Boos MD, Yokota Y, Eberl G, Kee BL: **Mature natural killer cell and lymphoid tissue-inducing cell development requires Id2-mediated suppression of E protein activity.** *J Exp Med* 2007, **204**(5):1119–1130.
43. Ikawa T, Fujimoto S, Kawamoto H, Katsura Y, Yokota Y: **Commitment to natural killer cells requires the helix-loop-helix inhibitor Id2.** *Proc Natl Acad Sci USA* 2001, **98**(9):5164–5169.
44. Yokota Y, Mansouri A, Mori S, Sugawara S, Adachi S, Nishikawa S, Gruss P: **Development of peripheral lymphoid organs and natural killer cells depends on the helix-loop-helix inhibitor Id2.** *Nature* 1999, **397**(6721):702–706.
45. Holmes ML, Carotta S, Corcoran LM, Nutt SL: **Repression of Flt3 by Pax5 is crucial for B-cell lineage commitment.** *Genes Dev* 2006, **20**(8):933–938.
46. Lai AY, Lin SM, Kondo M: **Heterogeneity of Flt3-expressing multipotent progenitors in mouse bone marrow.** *J Immunol* 2005, **175**(8):5016–5023.

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