

# GATA2 deficiency elevates interferon regulatory factor-8 to subvert a progenitor cell differentiation program

Kirby D. Johnson, Alexandra A. Soukup, and Emery H. Bresnick

Wisconsin Blood Cancer Research Institute, Department of Cell and Regenerative Biology, Carbone Cancer Center, University of Wisconsin School of Medicine and Public Health, Madison, WI

## Key Points

- Elevated IRF8 in granulocyte-monocyte progenitors from *Gata2*  $-77$  enhancer mutant embryos cause macrophage-biased differentiation.
- Genetically ablating *Irf8* reverses fetal liver hematopoiesis defects of *Gata2* enhancer mutant embryos, thus establishing a GATA2-IRF8 axis.

Cell type-specific transcription factors control stem and progenitor cell transitions by establishing networks containing hundreds of genes and proteins. Network complexity renders it challenging to discover essential versus modulatory or redundant components. This scenario is exemplified by GATA2 regulation of hematopoiesis during embryogenesis. Loss of a far upstream *Gata2* enhancer ( $-77$ ) disrupts the GATA2-dependent transcriptome governing hematopoietic progenitor cell differentiation. The aberrant transcriptome includes the transcription factor interferon regulatory factor 8 (IRF8) and a host of innate immune regulators. Mutant progenitors lose the capacity to balance production of diverse hematopoietic progeny. To elucidate mechanisms, we asked if IRF8 is essential, contributory, or not required. Reducing *Irf8*, in the context of the  $-77$  mutant allele, reversed granulocytic deficiencies and the excessive accumulation of dendritic cell committed progenitors. Despite many dysregulated components that control vital transcriptional, signaling, and immune processes, the aberrant elevation of a single transcription factor deconstructed the differentiation program.

## Introduction

The cloning of sequence-specific DNA binding proteins unveiled master regulators including GATA1, which promotes erythrocyte, megakaryocyte, mast cell, and basophil development, and GATA2, which mediates hematopoietic stem and progenitor cell (HSPC) genesis/function.<sup>1-4</sup> During mouse embryogenesis, GATA2 ablation disrupts multilineage hematopoiesis, yielding lethality.<sup>3,5</sup> Human GATA2 heterozygous mutations cause GATA2 deficiency syndrome involving cytopenias, immunodeficiency, bone marrow failure, leukemia, and lymphedema.<sup>6-10</sup> These mutations alter the GATA2 coding region or “+9.5” intronic enhancer.<sup>11-13</sup> Another pathogenic enhancer resides upstream of GATA2 (77 kb in mice; 110 kb in humans).<sup>14,15</sup> A 3q21;q26 inversion relocates this enhancer from 1 allele to *MECOM1*, inducing *EVI1* expression and acute myeloid leukemia.<sup>16-19</sup> Homozygous deletion of the  $-77$  enhancer reduces *Gata2* messenger RNA (mRNA) levels by nearly fivefold in Lin<sup>-</sup>Sca1<sup>-</sup>Kit<sup>+</sup> progenitors, with reductions occurring in both common myeloid progenitors (CMPs) and granulocyte-monocyte progenitors (GMPs).<sup>14</sup> Though hematopoietic stem cell (HSC) production in the aorta-gonad mesonephros of  $-77^{-/-}$  embryos is largely unaffected, the mutation depletes megakaryocyte-erythroid progenitors (MEPs), yielding embryonic lethality after embryonic day 14.5 (E14.5).<sup>14,20</sup> In addition,  $-77^{-/-}$  myeloerythroid progenitors lose multilineage differentiation and generate excessive macrophages *ex vivo*.<sup>14</sup> Although monocytopenia is common in GATA2 deficiency syndrome that emerges in children or adults, bone marrow macrophages persist.<sup>21</sup>

Submitted 17 September 2021; accepted 17 December 2021; prepublished online on *Blood Advances* First Edition 10 January 2022; final version published online 2 March 2022. DOI 10.1182/bloodadvances.2021006182.

For original data, please contact the lead author at ehbresni@wisc.edu.

The full-text version of this article contains a data supplement.

© 2022 by The American Society of Hematology. Licensed under Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International (CC BY-NC-ND 4.0), permitting only noncommercial, nonderivative use with attribution. All other rights reserved.

Many GATA2-regulated genes have been described in diverse systems.<sup>1,20-24</sup> However, the proteins operating downstream of GATA2 that mediate vital GATA2 functions, epistatic relationships governing HSPC functions, and disease phenotypes are incompletely defined. Discovering functionally critical downstream factors and networks has been challenging because of context-dependent permutations of GATA2 mechanisms.<sup>1,12,13</sup> In addition, once GATA2-mutant HSCs populate the bone marrow, and bone marrow failure is triggered via ill-defined mechanisms, the pleiotropic and often vast changes complicate mechanistic analyses and interpretations. To circumvent these complexities, we have focused on elucidating hematopoietic mechanisms operational during embryonic development that are sensitive to alterations in GATA2 levels. Furthermore, in the context of bone marrow failure and leukemia predisposition syndromes, it is instructive to consider how variably penetrant genes that are pathogenic in children and adults dysregulate molecular/cellular processes during embryogenesis. Proteomics and single-cell transcriptomics with  $-77^{-/-}$  fetal liver progenitors revealed losses of proteins mediating erythroid, megakaryocyte, basophil and granulocyte differentiation, and elevated innate immune proteins, including interferon responsive factor 8 (IRF8).<sup>21</sup>

IRF8 interacts with other IRFs, PU.1, AP-1, and BatF3 at composite binding sites to control immune cell development, and IRF8 levels dictate target gene selection by regulating transcription factor complex composition.<sup>25</sup> Balanced production of monocytes and granulocytes is regulated by IRF8 in lineage-restricted progenitors. GMPs ( $\text{Lin}^{-}\text{Sca-1}^{-}\text{cKit}^{+}\text{CD34}^{+}\text{CD16/32}^{\text{high}}$ ) are a mix of multipotent and lineage-restricted myeloid progenitors that develop from CMPs ( $\text{Lin}^{-}\text{Sca-1}^{-}\text{cKit}^{+}\text{CD34}^{+}\text{CD16/32}^{\text{low}}$ ), which exhibit a broader lineage potential, including erythroid and dendritic cells (DCs). Differentiation of multipotent  $\text{Ly6C}^{-}$  GMPs into lineage-restricted  $\text{Ly6C}^{+}$  progenitors involves upregulation of macrophage colony-stimulating factor receptor (M-CSFR). M-CSFR expression is high in monocyte progenitors (MPs) and low in granulocyte progenitors (GPs).<sup>26</sup> GPs have been further characterized as CD81-expressing, committed neutrophil progenitors.<sup>27</sup> Bone marrow MPs and GPs are produced in the absence of IRF8, although high IRF8 levels in MPs are required for further monocytic differentiation.<sup>26</sup> IRF8 levels also direct differentiation of DC progenitors within the CMP population. In the absence of IRF8, monocyte-DC progenitors (MDPs) fail to differentiate into common DC progenitors (CDPs).<sup>28</sup> Production of dendritic cell subtypes from CDPs is also regulated by the level of IRF8. The generation of type 1 conventional DCs (cDC1) requires more IRF8 than cDC2.<sup>25,29</sup> IRF8 levels increase during the commitment of monocytic and DC progenitors, and GATA2 and IRF8 are inversely expressed at the single-cell level.<sup>21,30</sup>

Because IRF8 promotes monocyte development, and its loss favors neutrophil generation,<sup>28,31</sup> it is attractive to propose that IRF8 upregulation in  $-77^{-/-}$  progenitors unrestrains monocytic differentiation and GATA2 downregulation of IRF8 is critical for multilineage differentiation. Because expression correlations often do not reflect causation, and IRF8 function in GATA2 networks is not understood, IRF8 might be functionally nonredundant, redundant, or insignificant. We asked if *Irf8* loss, in the context of the  $-77^{-/-}$  allele, rescues the diverse differentiation potential of progenitors. We demonstrate that simultaneous ablation of the  $-77$  enhancer and *Irf8* reversed the loss of GPs and promoted granulocytic differentiation of  $\text{Ly6C}^{-}$  GMPs. *Irf8* ablation also reversed the elevated CDP production of  $-77$  mutants and blocked maturation of CMP-derived DCs.

Because ectopically high IRF8 resulting from GATA2 deficiency in embryonic progenitor cells disrupted hematopoiesis, this study illustrates how dysregulation of a single component among many dysregulated components of a transcriptome derails a multilineage differentiation process.

## Methods

### Experimental model

*Gata2*  $-77^{+/-}$  mice<sup>14</sup> and *Irf8*<sup>-/-</sup> strain B6(Cg)-*Irf8*<sup>tm1.2Hm/J</sup> (Jackson Labs, Bar Harbor, ME) were bred to generate  $-77^{+/-};Irf8^{+/-}$  males and females for timed matings. Animal protocols were approved by the University of Wisconsin–Madison Institutional Animal Care and Use Committee in accordance with the Association for Assessment and Accreditation of Laboratory Animal Care International regulations.

### Flow cytometry

E14.5 fetal liver hematopoietic progenitors were quantified using the LSR Fortessa (BD Biosciences) and Attune (Thermo Fisher Scientific) flow cytometers or sorted using a FACSAria (BD Biosciences). Antibodies were from BioLegend unless indicated. Fluorescein isothiocyanate-conjugated B220 (#103206), TER-119 (#116206), CD3 (#100306), CD5 (#11-0193-85; Thermo Fisher Scientific), CD11b (#101206), CD11c (#117306), CD49b (#108906), Ly6G (#127606), and Sca1 (#108106) antibodies were used for lineage exclusion. Other antibodies were Blue Violet (BV) 605-conjugated CD16/CD32 (#563006; BD Biosciences), BV711-conjugated Ly6C (#128017), phycoerythrin-conjugated CD115 (#135506), eFluor 660-conjugated CD34 (#50-0341; Thermo Fisher Scientific), peridinin chlorophyll-eFluor710-conjugated CD135 (#46-1351; Thermo Fisher Scientific), and phycoerythrin-Cy7-conjugated cKit (#105814). Markers for DC analysis were Super Bright 780-conjugated CD11c (#78-0114; Thermo Fisher Scientific) and BV605-conjugated CD86 (105037). Stained cells were washed with phosphate-buffered saline, 2% fetal bovine serum, 10 mM glucose, and 2.5 mM EDTA. Cells were resuspended in the same buffer containing 100 ng/mL 4',6-diamidino-2-phenylindole for live-dead discrimination.

### Quantitative real-time RT-PCR

Total RNA was purified with TRIzol (Invitrogen) and treated with DNase (Invitrogen). Complementary DNA was prepared with a mixture of random hexamer and oligo (dT) primers incubated with *m*-MLV Reverse Transcriptase (Invitrogen). Complementary DNA was analyzed by real-time reverse transcriptase polymerase chain reaction (RT-PCR) with a Vii7 real-time RT-PCR cyclor (Applied Biosystems). *Irf8* primers (GGCAAGCAGGATTACAATCAG and CCACACTCC ATCTCAGGAAC) detected exon loss in *Irf8*<sup>-/-</sup> embryos. *Gata2* primers (GCAGAGAAGCAAGGCTCGC and CAGTTGACACACT CCCGGC) were described previously.<sup>14</sup> Values were normalized to the standard curve and 18S control (CGCCGCTAGAGGTGAA ATTCT and CGAACCTCCGACTTTCGTTCT).

### Cell culture

All primary cell cultures were incubated at 37°C and 5% CO<sub>2</sub>. Colony assays were performed using M3434 methylcellulose media (StemCell Technologies). Sorted  $\text{Ly6C}^{-}$  GMPs were plated in duplicate at 1200 cells/plate and cultured for 7 days. Using a

blinded strategy, colonies were visually scored as having small, large, or mixed cell content; granulocyte colony-forming unit (CFU-G), macrophage CFU (CFU-M), and granulocyte and macrophage CFU (CFU-GM), respectively. For DC differentiation, sorted fetal liver CMPs (Lin<sup>-</sup>Sca-1<sup>-</sup>cKit<sup>+</sup>FcγR<sup>o</sup>CD34<sup>+</sup>) were cultured using the CellXVivo Mouse Dendritic Cell Differentiation Kit (#CDK008; R&D Systems). Cultures of 35 000 to 75 000 CMPs were initiated on the day of sorting in 1 mL of media containing interleukin-4 (IL-4) and granulocyte-macrophage colony stimulating factor (GM-CSF) according to the manufacturer's protocol. Fresh media was supplied on day 4. On day 6, the media was replaced with fresh media containing IL-4, GM-CSF, and tumor necrosis factor α (TNF-α). Following 24-hour culture in TNF-α-containing media, cells were recovered and analyzed by flow. DC differentiation was assessed using CD11c and CD86 antibodies.

## Quantification and statistical analysis

Statistical analyses were performed using GraphPad Prism, version 9.1. Multiple independent cohorts were used in each experiment. Data were evaluated for outliers before analysis with 2-sided Grubbs test. Statistical comparisons were performed using Brown-Forsythe and Welch analysis of variance tests with Welch's unequal variance *t* tests. A significance cutoff of *P* < .05 was used for all analyses. The results are presented as mean ± standard error of the mean.

## Results

### IRF8 mediates myeloid phenotypes in GATA2-deficient mouse embryos

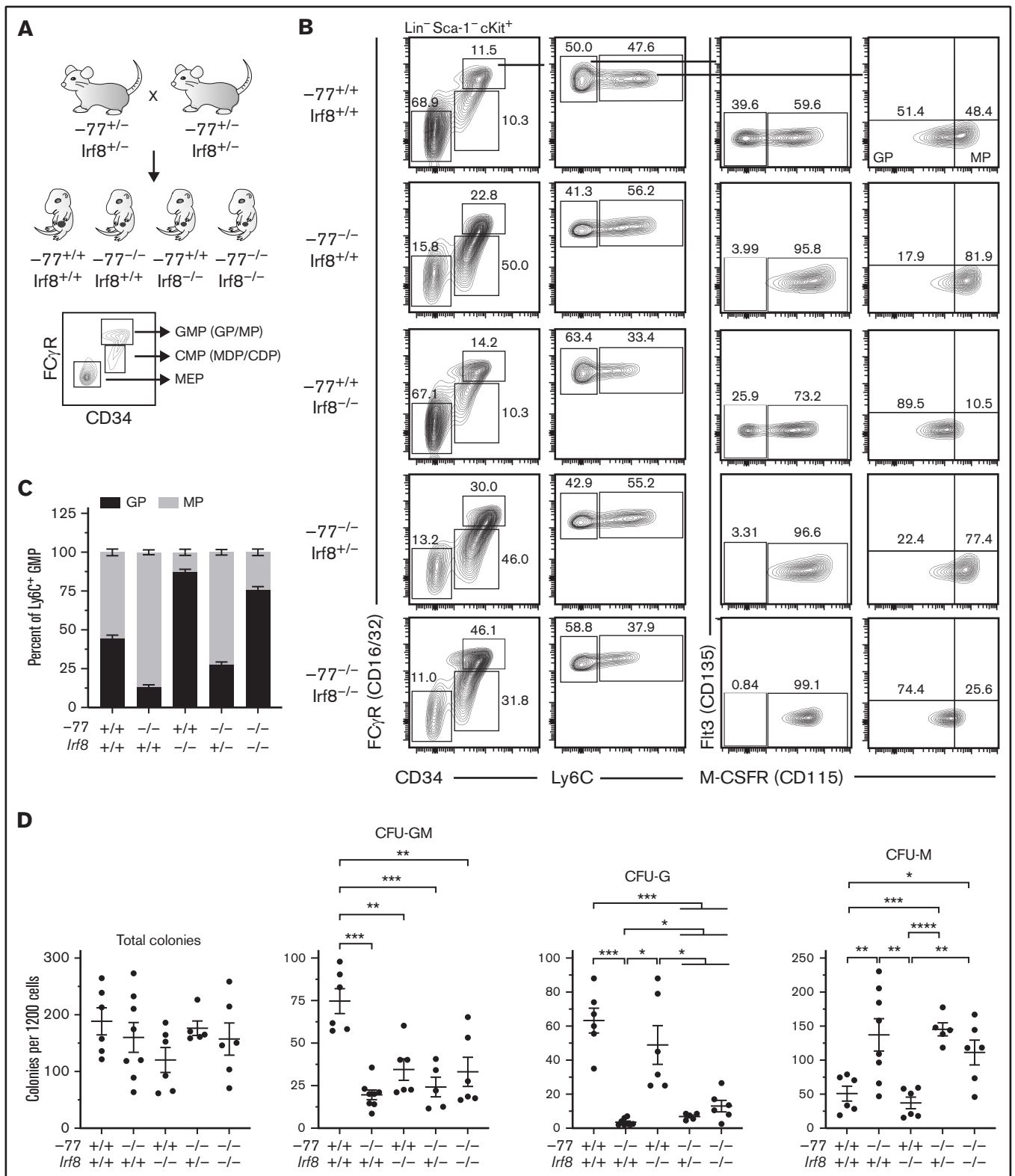
Previously, we demonstrated that the GP:MP ratio of -77<sup>-/-</sup> fetal liver is skewed toward MPs, and -77<sup>-/-</sup> progenitors generate excessive macrophages *ex vivo*.<sup>14,21</sup> IRF8, which is upregulated in GATA2-deficient -77<sup>-/-</sup> progenitors,<sup>21</sup> controls survival and differentiation, but not production, of these lineage-committed bone marrow progenitors.<sup>32</sup> To determine if elevated IRF8 resulting from -77 enhancer loss causes the GP:MP imbalance, GPs and MPs, and the populations from which they arise (supplemental Figure 1), were analyzed in -77 and *Irf8* double-mutant (-77<sup>-/-</sup>;*Irf8*<sup>-/-</sup>) embryos (Figure 1A; supplemental Figure 2A). Bone marrow GPs and MPs comprise the Ly6C<sup>+</sup> subset of GMPs and arise from multipotential (monocyte, neutrophil, eosinophil, basophil) Ly6C<sup>-</sup> GMPs that have little cell surface expression of M-CSFR (CD115) and produce predominantly CFU-GM colonies *in vitro*.<sup>26,27</sup> In wild-type fetal liver, only 43% of Ly6C<sup>-</sup> GMPs were M-CSFR<sup>-</sup> by flow cytometry; the remaining cells expressed low to moderate levels of M-CSFR (Figure 1B; supplemental Figure 2B). Colony assays performed with wild-type Ly6C<sup>-</sup> GMPs yielded 42% CFU-GM, comparable to the proportion of Ly6C<sup>-</sup> GMPs that are M-CSFR-deficient (supplemental Figure 2C, first stacked graph). The remaining colonies comprised solely granulocytes (38%) or macrophage (20%). The progression of GMPs from Ly6C<sup>-</sup> to Ly6C<sup>+</sup> is accompanied by increased surface expression of M-CSFR. M-CSFR<sup>-</sup> cells are absent, and there are similar proportions of low- and high-receptor-expressing GPs (44%) and MPs (56%), respectively (Figure 1B-C); comparable to what was reported in bone marrow.<sup>26</sup>

Unlike wild-type MPs, -77 enhancer deletion mutant (-77<sup>-/-</sup>;*Irf8*<sup>+/+</sup>) MPs were proportionally more abundant than GPs comprising 87% (*P* < .0001) of the Ly6C<sup>+</sup> population (Figure 1B-C), corroborating our

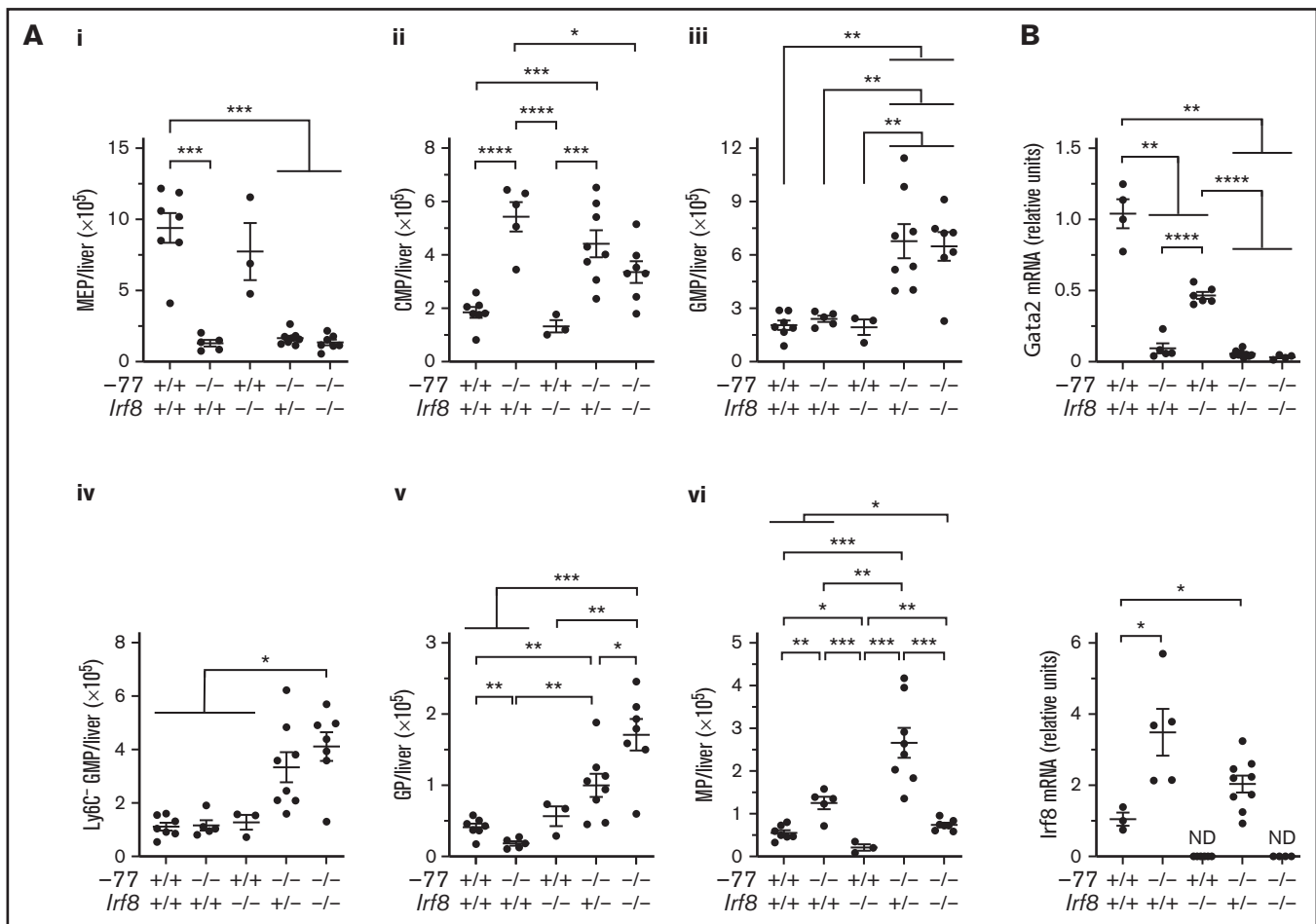
prior findings.<sup>21</sup> In addition, M-CSFR-deficient Ly6C<sup>-</sup> GMPs were nearly absent from -77<sup>-/-</sup>;*Irf8*<sup>+/+</sup> fetal liver (7.1-fold lower than wild-type; *P* < .0001) (Figure 1B; supplemental Figure 2B), and these cells exhibited reduced CFU-GM and CFU-G colony formation relative to wild-type (Figure 1D; supplemental Figure 2C). Unlike -77<sup>-/-</sup>;*Irf8*<sup>+/+</sup> Ly6C<sup>-</sup> GMPs, 77<sup>+/+</sup>;*Irf8*<sup>-/-</sup> Ly6C<sup>-</sup> GMPs retain M-CSFR<sup>-</sup> and M-CSFR<sup>+</sup> populations (Figure 1B). However, M-CSFR levels were reduced in -77<sup>+/+</sup>;*Irf8*<sup>-/-</sup> Ly6C<sup>+</sup> GMPs, thereby increasing the proportion of immunophenotypic GPs to 87% (*P* < .0001) relative to wild-type (Figure 1B-C). Because -77 and *Irf8* mutations had opposing effects on the GP:MP ratio, and -77<sup>-/-</sup> progenitors exhibited elevated levels of *Irf8*, we tested whether reducing *Irf8* expression rescues the -77 mutation-induced defects in GMP populations. Like the -77<sup>-/-</sup>;*Irf8*<sup>+/+</sup> cells, Ly6C<sup>-</sup> GMPs from -77<sup>-/-</sup>;*Irf8*<sup>+/-</sup> or -77<sup>-/-</sup>;*Irf8*<sup>-/-</sup> fetal livers predominantly expressed M-CSFR and, in colony assays, CFU-GMs were not rescued (Figure 1B,D; supplemental Figure 2C). However, reducing *Irf8* (-77<sup>-/-</sup>;*Irf8*<sup>+/-</sup> or -77<sup>-/-</sup>;*Irf8*<sup>-/-</sup>) increased the proportion of immunophenotypic GPs to levels intermediate between either single mutant (Figure 1B-C).

To assess the differentiation potential of -77 and *Irf8* single- and double-mutant progenitors and determine if lowering IRF8 rescues the granulocyte deficiency of -77 mutants, Ly6C<sup>-</sup> GMPs were scored for colony formation. Though similar numbers of colonies were formed regardless of genotype (Figure 1D), fewer mixed lineage CFU-GMs were produced by -77 (3.8-fold) and *Irf8* (2.2-fold) single mutants and CFU-GM were not rescued in -77<sup>-/-</sup>;*Irf8*<sup>-/-</sup> cultures. Consistent with our prior results,<sup>14</sup> fewer CFU-G and more CFU-M were produced by -77<sup>-/-</sup>;*Irf8*<sup>+/+</sup> compared with wild-type. CFU-G and CFU-M from -77<sup>+/+</sup>;*Irf8*<sup>-/-</sup> Ly6C<sup>-</sup> GMPs were not significantly different than wild-type. Lowering IRF8 levels in -77<sup>-/-</sup> progenitors increased CFU-G colonies by 2.0-fold (*P* = .011) and 3.7-fold (*P* = .036) in cultures of -77<sup>-/-</sup>;*Irf8*<sup>+/-</sup> and -77<sup>-/-</sup>;*Irf8*<sup>-/-</sup> Ly6C<sup>-</sup> GMPs, respectively, compared with -77<sup>-/-</sup>;*Irf8*<sup>+/+</sup>. This demonstrates that elevated IRF8 levels in -77<sup>-/-</sup> progenitors causes the loss of granulocytic potential of GMPs, and reducing IRF8 can partially restore granulocytic differentiation of -77<sup>-/-</sup> GMPs.

The -77<sup>-/-</sup> embryos have smaller livers because of loss of Ter119<sup>+</sup> erythroid cells and erythroid progenitors, resulting in proportionally higher numbers of CMPs and GMPs.<sup>14</sup> To determine if the -77 mutation, with or without loss of IRF8, perturbs absolute cell numbers of these progenitor populations, progenitors were quantified in E14.5 livers. -77<sup>-/-</sup>;*Irf8*<sup>+/+</sup> embryos exhibited a 7.2-fold (*P* = .0002) reduction in MEPs, and *Irf8* loss did not rescue MEPs in -77<sup>-/-</sup>;*Irf8*<sup>+/-</sup> or -77<sup>-/-</sup>;*Irf8*<sup>-/-</sup> fetal livers (Figure 2Ai). Relative to wild-type, -77<sup>-/-</sup>;*Irf8*<sup>+/+</sup> CMPs increased by 2.9-fold, whereas -77<sup>+/+</sup>;*Irf8*<sup>-/-</sup> CMP levels were unaffected (Figure 2Aii). Elevated CMP levels were retained by -77<sup>-/-</sup>;*Irf8*<sup>+/-</sup> embryos, whereas -77<sup>-/-</sup>;*Irf8*<sup>-/-</sup> CMPs decreased by 38% relative to the -77 single mutant. GMP levels in -77 and *Irf8* single-mutant embryos were unchanged relative to wild-type (Figure 2Aiii). However, loss of 1 or both alleles of wild-type *Irf8* increased GMPs in -77 mutants by 2.8-fold and 2.7-fold, respectively. Within the GMP compartment, -77<sup>-/-</sup>;*Irf8*<sup>+/+</sup> Ly6C<sup>-</sup> GMPs, were unchanged (Figure 2Aiv), despite the loss of M-CSFR<sup>-</sup> cells (Figure 1B). Of the -77<sup>-/-</sup>;*Irf8*<sup>+/+</sup> Ly6C<sup>+</sup> GMPs, a 2.2-fold (*P* = .003) decrease in GPs was balanced by a 2.3-fold increase (*P* = .006) in MPs, thereby retaining normal numbers of total GMPs in -77 mutants. Compared with -77 single-mutant GMPs, -77<sup>-/-</sup>;*Irf8*<sup>-/-</sup> GMPs



**Figure 1. Elevated IRF8 causes aberrant myeloid differentiation potential of GATA2-deficient progenitor cells.** (A) Experimental strategy for double mutant in vivo rescue system. Mice heterozygous for  $-77$  enhancer deletion or  $Irf8^{tm1.2^{tm}}$  were bred to generate E14.5 embryos. Fetal liver progenitor populations with select genotypes were analyzed by flow cytometry. (B) Representative flow cytometry analysis of progenitor populations from GMPs ( $Lin^{-} Sca-1^{-} cKit^{+} CD34^{+} CD16/32^{high}$ ). GPs and MPs are distinguished from multipotential GMPs by expression of Ly6C and from each other by M-CSFR (CD115). (C) Quantitation of the frequency of MPs and GPs within the Ly6C<sup>+</sup> GMP populations from 7 litters ( $-77^{+/+} Irf8^{+/+}$ ,  $n = 7$ ;  $-77^{-/-} Irf8^{+/+}$ ,  $n = 5$ ;  $-77^{+/-} Irf8^{+/+}$ ,  $n = 3$ ;  $-77^{-/-} Irf8^{+/-}$ ,  $n = 8$ ;  $-77^{+/-} Irf8^{-/-}$ ,  $n = 7$ ). Relevant statistical comparisons for panel C are described in "Results." (D) Quantitation of colonies from sorted Ly6C<sup>+</sup> GMPs plated in M3434 methylcellulose media (STEMCELL Technologies) at 1200 cells/plate. Colonies were scored as mixed-lineage CFU-GM, or single-lineage granulocyte CFU-G, or CFU-M colonies. Ly6C<sup>+</sup> GMPs were obtained from 4 litters ( $-77^{+/+} Irf8^{+/+}$ ,  $n = 6$ ;  $-77^{-/-} Irf8^{+/+}$ ,  $n = 8$ ;  $-77^{+/-} Irf8^{+/+}$ ,  $n = 6$ ;  $-77^{-/-} Irf8^{+/-}$ ,  $n = 5$ ;  $-77^{+/-} Irf8^{-/-}$ ,  $n = 6$ ). Error bars for all plots represent mean  $\pm$  standard error of the mean (SEM). \* $P < .05$ , \*\* $P < .01$ , \*\*\* $P < .001$ , \*\*\*\* $P < .0001$ ; Welch's unequal variance  $t$  tests.



**Figure 2. GATA2-IRF8 axis differentially regulates fetal liver progenitor populations.** (A) Quantitation of progenitor populations in E14.5 fetal livers obtained from 7 litters ( $-77^{+/+}; Irf8^{+/+}$ ,  $n = 7$ ;  $-77^{-/-}; Irf8^{+/+}$ ,  $n = 5$ ;  $-77^{+/+}; Irf8^{-/-}$ ,  $n = 3$ ;  $-77^{-/-}; Irf8^{+/+}$ ,  $n = 8$ ;  $-77^{-/-}; Irf8^{-/-}$ ,  $n = 7$ ). (B) mRNA quantitation from sorted Ly6C<sup>-</sup> GMPs from 4 to 9 embryos obtained from 10 litters. Real-time PCR primers for quantitation of *Irf8* transcripts selectively amplified the wild-type, but not mutant, allele. ND, not detected. Error bars for all plots represent mean  $\pm$  SEM. \* $P < .05$ , \*\* $P < .01$ , \*\*\* $P < .001$ , \*\*\*\* $P < .0001$ ; Welch's unequal variance *t* tests.

were increased for Ly6C<sup>-</sup> cells (3.5-fold) (Figure 2Aiv) and GPs (9.3-fold) (Figure 2Av) and reduced in MPs (59%) (Figure 2Avi). By contrast,  $-77^{-/-}; Irf8^{+/+}$  GMPs exhibited elevated GPs (5.4-fold) and MPs (2.1-fold). These results demonstrate that high levels of IRF8 dictate the fate of fetal liver progenitors in  $-77$  mutants.

The  $-77$  enhancer deletion reduces *Gata2* expression in CMPs and GMPs and elevates *Irf8* levels in a CMP/GMP pool.<sup>14,21</sup> We analyzed the relationship between *Gata2* and *Irf8* expression in Ly6C<sup>-</sup> GMPs. *Gata2* levels were lower in  $-77^{-/-}$  single (11-fold) and  $-77^{-/-}; Irf8^{-/-}$  double (32-fold) mutant Ly6C<sup>-</sup> GMPs relative to wild-type (Figure 2B). *Gata2* levels were also decreased in *Irf8*<sup>-/-</sup> Ly6C<sup>-</sup> GMPs (2.2-fold). Regardless of genotype, *Gata2* expression was reduced in GPs and MPs relative to Ly6C<sup>-</sup> GMPs; with 33-fold and 46-fold lower expression in wild-type cells, respectively (supplemental Figure 3). As described previously,<sup>26,33</sup> *Irf8* expression was very low in wild-type Ly6C<sup>-</sup> GMPs and higher in MPs than GPs (Figure 2B; supplemental Figure 3). However,  $-77$  deletion increased *Irf8* expression in Ly6C<sup>-</sup> GMPs (3.3-fold) and GPs (2.7-fold) but not MPs (Figure 2B; supplemental Figure 3). Because the primers used to measure *Irf8* mRNA

annealed within the region deleted in the B6(Cg)-*Irf8*<sup>tm1.2Hm/J</sup> strain used in our analysis, no *Irf8* expression was detected in *Irf8*<sup>-/-</sup> embryos. Quantitation of progenitor populations revealed that the proportions of MPs and GPs remain responsive to IRF8 in  $-77$  mutant embryos, and the high-level *Irf8* expression, in both multipotential and lineage-restricted  $-77^{-/-}$  progenitors, suppresses the expansion of both populations.

### IRF8 elevation in GATA2-deficient embryos disrupts mechanisms that restrict production and differentiation of dendritic progenitor cells

In GATA2 deficiency syndrome, DCs are commonly depleted from bone marrow and peripheral blood of children and adults.<sup>6,34</sup> As with monocytes, IRF8 promotes DC generation.<sup>32,35</sup> IRF8 levels within CDPs determine the DC subtype produced; high and low IRF8 promote cDC1 and cDC2 production, respectively.<sup>25</sup> *Irf8*<sup>-/-</sup> mice exhibit increased bone marrow MDPs and reduced CDPs.<sup>32</sup>

Our prior transcriptomic analyses that identified *Irf8* upregulation in  $-77^{-/-}$  myeloid progenitors<sup>21</sup> also revealed upregulation of

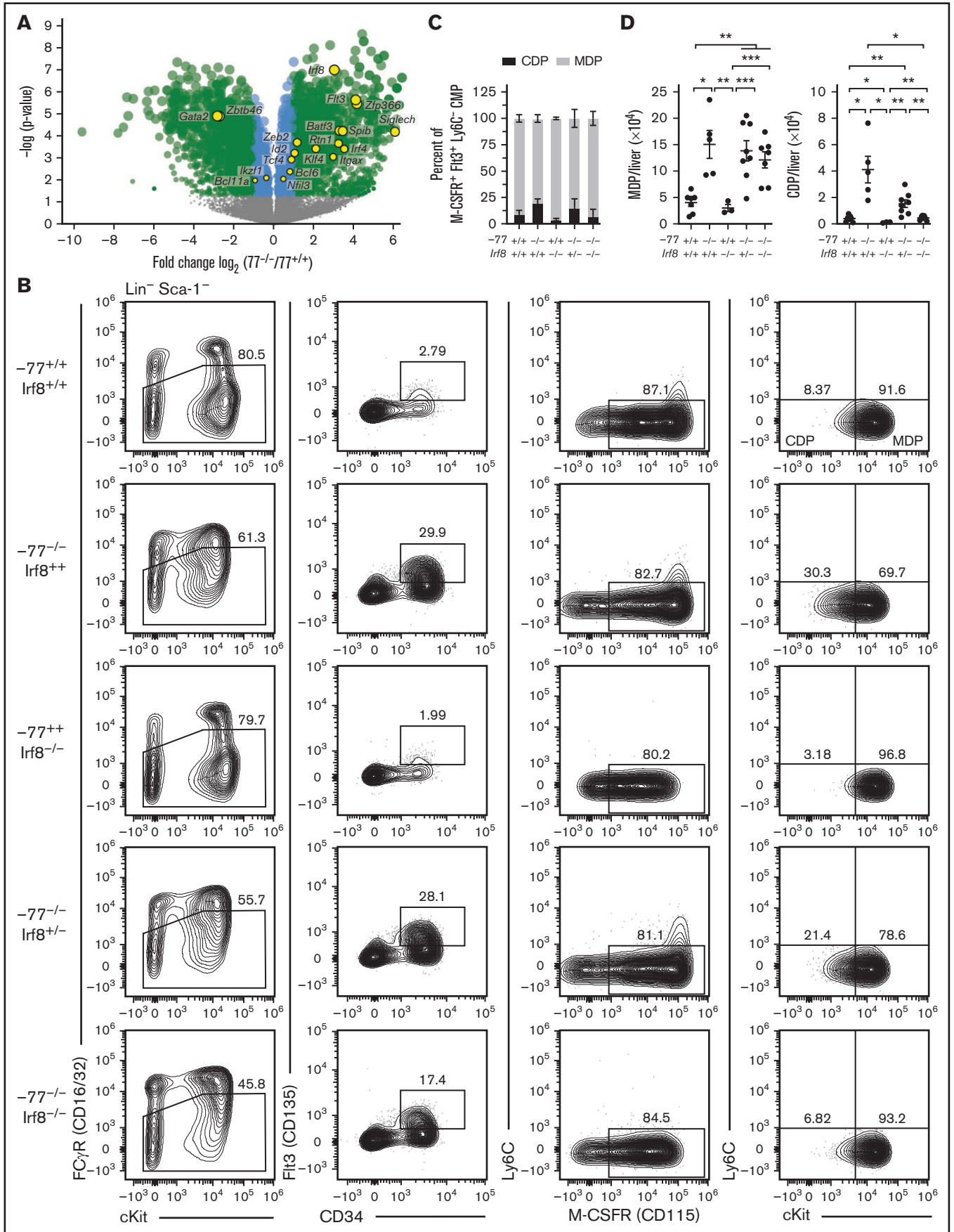
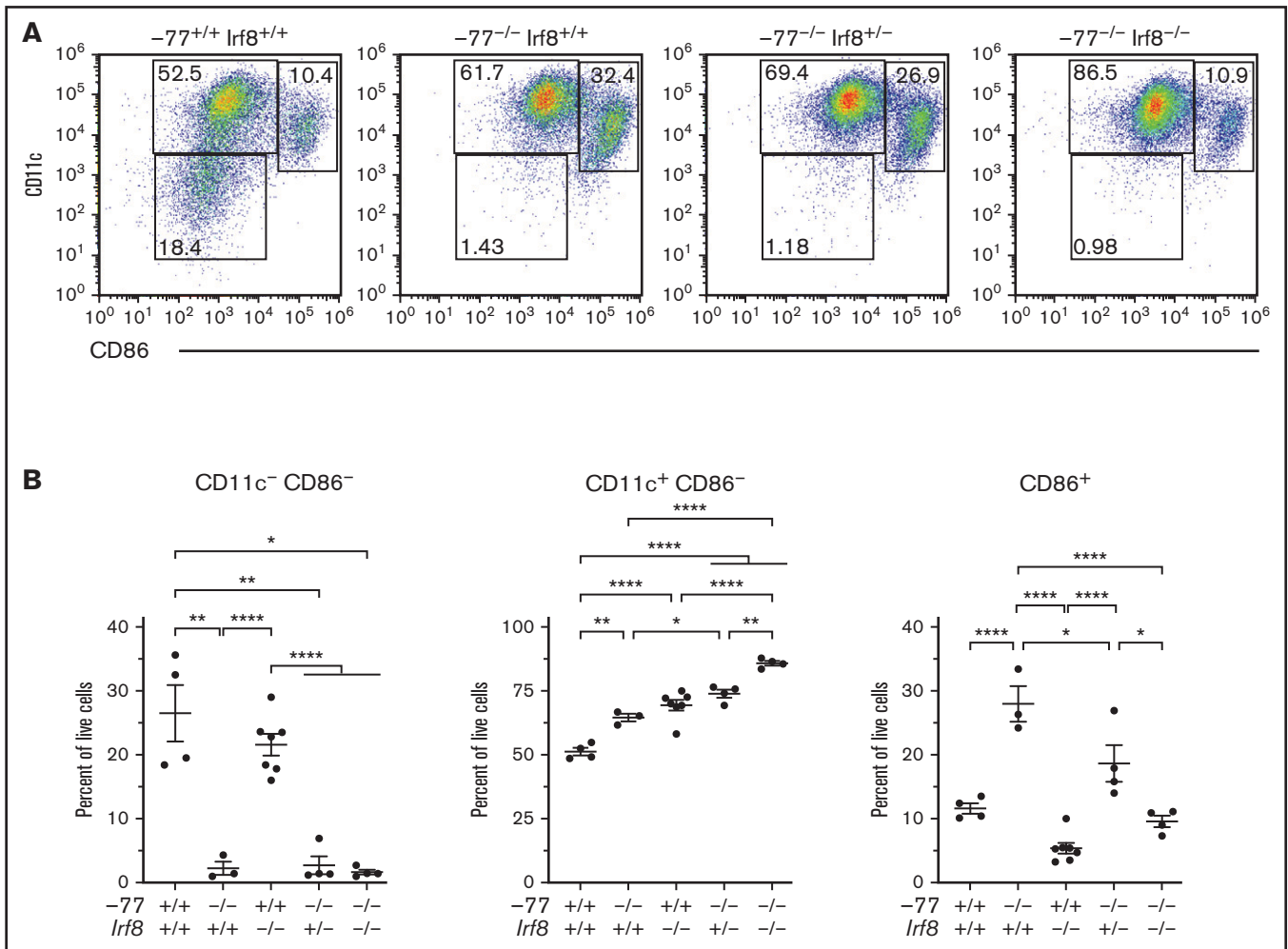


Figure 3.

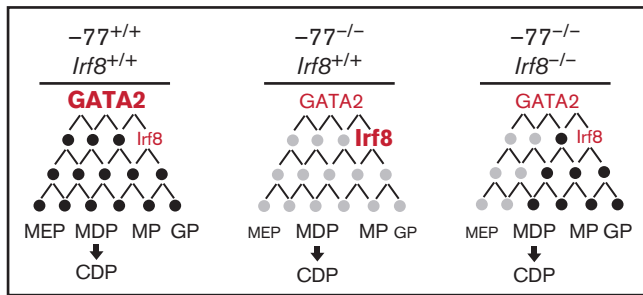


**Figure 4. Enhanced dendritic cell differentiation of  $-77^{-/-}$  CMPs requires IRF8.** (A) Representative flow plots of dendritic cells differentiated from flow-sorted CMPs cultured with GM-CSF, IL-4, and TNF $\alpha$ . (B) Quantitation of dendritic cell populations using early (CD11c) and late (CD86) DC maturation markers. Data were obtained from 3 to 8 embryos (7 litters). The populations were pregated to exclude cell doublets and dead (4',6-diamidino-2-phenylindole<sup>+</sup>) cells. Error bars represent mean  $\pm$  SEM. \* $P < .05$ , \*\* $P < .01$ , \*\*\*\* $P < .0001$ ; Welch's unequal variance  $t$  tests.

additional regulators of DC differentiation (eg, *Flt3*, *Irf4*, *Batf3*, *Id2*, *Zeb2*, *Zfp366* [*DC-Script*], and *Spib*) and/or transcripts enriched in DC progenitor or precursor cells (eg, *Siglech*, *Itgax* [CD11c], and *Rtn1*) (Figure 3A).<sup>36-39</sup> Transcripts for other DC-regulatory proteins were either unchanged (eg, *Bcl11a*, *Ikzf1*, *Nfil3*, *Bcl6*, and *Tcf4* [E2-2]) or decreased (*Zbtb46*), possibly because of broader expression in cell types lost in  $-77$  mutants, such as MEPs. Because IRF8 promotes DC development in adult mice and humans,<sup>32,35</sup> GATA2 suppresses IRF8 expression in mouse embryos at the single-cell level<sup>21</sup> and high IRF8 corrupts GATA2-deficient

( $-77^{-/-}$ ) progenitor differentiation (Figure 1D), we asked if the GATA2-IRF8 axis affects DC progenitors. We analyzed DC progenitors using established markers that segregate  $Flt3^{+}$  M-CSFR<sup>+</sup> CMPs into MDPs ( $Ly6C^{-}$  cKit<sup>high</sup>) and CDPs ( $Ly6C^{-}$  cKit<sup>low</sup>).<sup>40</sup> In wild-type E14.5 fetal livers, CDPs were 9.2% of  $CD16/32^{low}$ - $CD34^{+}$   $Flt3^{+}$  M-CSFR<sup>+</sup>  $Ly6C^{-}$  progenitors (Figure 3B-C), whereas the proportion of CDPs in *Irf8*<sup>-/-</sup> fetal liver was 3.9%. The  $-77^{-/-}$  CDPs increased to 20.2%, a 2.2-fold increase over wild-type ( $P = .007$ ). Biallelic *Irf8* and  $-77$  loss restored the proportion of CDPs to resemble wild-type embryos (7.2%,  $P = .979$ ). In addition

**Figure 3. GATA2 suppression of IRF8 levels restricts common dendritic cell progenitor production.** (A) Volcano plot of differentially expressed genes mined from transcriptome analysis of lineage-depleted wild-type and  $-77^{-/-}$  fetal livers cultured for 3 days as previously described.<sup>21</sup> GEO accession: GSE133606. DC genes included factors involved in DC differentiation and/or differentially expressed in DC progenitor populations.<sup>38</sup> *Gata2* downregulation, resulting from the  $-77$  enhancer deletion, is also depicted. (B) Representative flow cytometry analysis of monocyte-dendritic cell progenitor (MDP) and common dendritic cell progenitor (CDP) populations. Quantitation of the frequency of CDPs and MDPs within the  $Flt3^{+}$  M-CSFR<sup>+</sup>  $Ly6C^{-}$  CMP pools (C) and of total cells per liver (D) from 7 litters ( $-77^{+/+}$  *Irf8*<sup>+/+</sup>,  $n = 7$ ;  $-77^{-/-}$  *Irf8*<sup>+/+</sup>,  $n = 5$ ;  $-77^{-/-}$  *Irf8*<sup>+/-</sup>,  $n = 8$ ;  $-77^{-/-}$  *Irf8*<sup>-/-</sup>,  $n = 7$ ;  $-77^{+/+}$  *Irf8*<sup>-/-</sup>,  $n = 3$ ). Error bars represent mean  $\pm$  SEM. \* $P < .05$ , \*\* $P < .01$ , \*\*\* $P < .001$ ; Welch's unequal variance  $t$  tests. Relevant statistical comparisons for panel C are described in "Results."



**Figure 5. Model illustrating IRF8 function as an essential mediator within GATA2 genetic networks.** Physiological levels of GATA2 establish a transcriptome (genes, black circles) with low *Irf8* expression, which maintains a balance in myeloid and dendritic cell progenitors. Loss of the  $-77$  enhancer and consequent reduction in GATA2 disrupts the network (gray circles), upregulating *Irf8* and increasing proportions of MP, MDP, and CDP populations. Reducing *Irf8*, in the context of the  $-77$  mutant allele, reversed granulocytic deficiencies and the excessive accumulation of dendritic cell progenitors.

to the increased proportion of CDPs to MDPs in  $-77^{-/-}$  livers relative to wild-type, the absolute number of both MDPs and CDPs increased in  $-77$  single mutants; 3.7-fold ( $P = .012$ ) and 10.2-fold ( $P = .021$ ), respectively (Figure 3D). In  $-77$ ; *Irf8* double-mutants, MDP levels were unchanged relative to  $-77$  single mutants, whereas CDP levels decreased to wild-type levels, consistent with IRF8 promoting CDP production.<sup>28</sup>

To determine if mutant progenitors are competent to produce DCs, we analyzed DC differentiation using sorted fetal liver CMPs cultured for 7 days in media containing GM-CSF and IL-4 with TNF- $\alpha$  added for the final day of culture (Figure 4A). CD11c, an early DC marker, and CD86, a marker expressed on mature/activated DCs, segregate populations in  $-77^{+/+}$ ; *Irf8*<sup>+/+</sup> CMP cultures that define progressive DC maturation (Figure 4A-B).<sup>41</sup> In  $-77^{-/-}$ ; *Irf8*<sup>+/+</sup> CMP cultures, CD11c<sup>-</sup>; CD86<sup>-</sup> cells are 11.8-fold lower than in wild-type cultures, whereas the more differentiated population of CD86<sup>+</sup> cells comprised 28% of the total, a 2.4-fold increase relative to wild-type. Although *Irf8* loss in the  $-77$  mutants ( $-77^{-/-}$ ; *Irf8*<sup>+/+</sup> and  $-77^{-/-}$ ; *Irf8*<sup>-/-</sup>) did not rescue the double-negative population, the expanded CD86<sup>+</sup> compartment in  $-77$  mutants required *Irf8* as the proportion of CD86<sup>+</sup> cells in  $-77^{-/-}$ ; *Irf8*<sup>-/-</sup> CMP cultures was decreased to wild-type levels. Consequently, the majority of cells were CD11c<sup>+</sup>; CD86<sup>-</sup> (Figure 4A-B). This analysis defines a GATA2-regulated DC population within the CMP pool, and the double-mutant rescue system revealed a GATA2-IRF8 genetic interaction that controls the levels of these cells. GATA2 suppressed MDP levels independent of IRF8, and the elevated IRF8 level of  $-77$  mutants promoted differentiation into CDPs. In addition, progenitors retained the capacity for DC differentiation. Thus, the GATA2-IRF8 axis function extends beyond that of establishing a balance between MPs and GPs within the GMP population.

## Discussion

Our results unveiled a GATA2-IRF8 axis that orchestrates hematopoietic progenitor generation and function during mouse embryogenesis. Given the conservation of GATA2 and IRF8 mechanisms from mouse to man, and human GATA2- and IRF8-linked

hematologic pathologies, these findings inform human disease mechanisms. Because *Gata2* and *Irf8* mRNAs co-reside in single fetal liver progenitors at reciprocal levels, and reintroducing GATA2 into  $-77^{-/-}$  progenitors decreases IRF8,<sup>21</sup> GATA2 and IRF8 levels in a single cell are key determinants of this developmental mechanism. GATA2 and IRF8 also control basophil development because GATA2 expression in *Irf8*<sup>-/-</sup> bone marrow cells rescues basophil differentiation.<sup>42</sup> In this context, however, it is unclear if they function in the same or distinct cells to control differentiation.

Patients with GATA2 mutations can be asymptomatic, and with variable penetrance, the predisposition state can be triggered to progress into bone marrow failure and leukemia.<sup>6,34,43</sup> Epigenetic silencing of the wild-type GATA2 allele has been implicated in triggering,<sup>44</sup> and additional mutations are likely relevant.<sup>45-47</sup> Because clinical hallmarks of GATA2 deficiency syndrome in children and adults include monocyte, lymphocyte, and DC depletion,<sup>6,34,48</sup> it is instructive to consider whether GATA2 deficiency in the developing embryo recapitulates these cellular deficits. In a mouse GATA2 deficiency model involving a single-nucleotide variant in an Ets motif of the conserved +9.5 intronic enhancer,<sup>49</sup> mutant HSPCs exhibit a nearly normal transcriptome and activity until they are forced to regenerate, which reveals gross transcriptomic and HSC defects.<sup>50</sup> Despite the stress-instigated pathogenic activity of this variant, it has little to no impact on hematopoiesis during embryogenesis. Patients with GATA2 deficiency have monocytopenia, yet retain macrophages in bone marrow, and mouse  $-77^{-/-}$  GATA2-deficient embryonic progenitor cells generate excessive macrophages.<sup>21</sup> DC progenitors accumulate in  $-77^{-/-}$  GATA2-deficient fetal liver progenitors, and these cells can generate cells ex vivo with the immunophenotype expected for DCs (Figures 3 and 4). In patients with GATA2 deficiency, DCs are depleted,<sup>7,48,51</sup> although depletion has not been described in asymptomatic children or adults. The differential impact of GATA2 mutations in embryos and adults, with or without stressors, has important implications for pathogenesis, analogous to the resistance of embryonic mouse progenitors to the common adult leukemogenic gene *Ft3*-ITD.<sup>52</sup> One could envision GATA2 mutations inducing an indelible epigenomic alteration that serves as a pathogenic predisposition many years thereafter. Consistent with this model, allele-specific chromatin accessibility analysis revealed that a heterozygous +9.5 enhancer deletion in GATA2 deficiency syndrome<sup>11</sup> reverted accessible to inaccessible chromatin at the mutant *Gata2*, but not the wild-type, allele.<sup>53</sup>

The reduced GATA2 level of  $-77^{-/-}$  myelo-erythroid progenitors, which impedes erythroid, megakaryocyte, granulocyte, and basophil developmental trajectories, while maintaining monocyte progenitors, is associated with collapse of the GATA2 network (3161 differentially expressed genes and 434 differentially expressed proteins in  $-77^{-/-}$  vs  $-77^{+/+}$  progenitors).<sup>21</sup> Because the aberrant network includes many regulatory factors, one would assume that a complex multicomponent mechanism skews differentiation. Intrinsic to this type of mechanism, phenotypic normalization would likely require restoration of physiological expression patterns and protein levels of multiple components. Strikingly, our results with the double-knockout rescue system demonstrated that ablating *Irf8* partially normalized the skewed hematopoietic progenitor levels and granulocyte colony activity of  $-77^{-/-}$  progenitors (Figure 5), thus establishing a mechanism in which GATA2 suppresses IRF8 levels to ensure normal levels of specific types of hematopoietic progenitors. IRF8 elevation in GATA2-deficient embryos corrupts this mechanism.



Applying the strategy innovated to other network components will discriminate vital stem and progenitor cell regulators from those exhibiting intriguing expression patterns that represent inconsequential correlations.

## Acknowledgments

The authors thank Mabel M. Jung for graphic design.

This work was funded by NIH grants R01DK68634 and University of Wisconsin Carbone Cancer Center Support Grant P30CA014520), ASH Scholar Award (A.A.S.), Leukemia and Lymphoma Society Career Development Program (A.A.S.), and Edward Evans Foundation.

## References

1. Katsumura KR, Bresnick EH, GATA Factor Mechanisms Group. The GATA factor revolution in hematology. *Blood*. 2017;129(15):2092-2102.
2. Tsai SF, Martin DI, Zon LI, D'Andrea AD, Wong GG, Orkin SH. Cloning of cDNA for the major DNA-binding protein of the erythroid lineage through expression in mammalian cells. *Nature*. 1989;339(6224):446-451.
3. Tsai FY, Keller G, Kuo FC, et al. An early haematopoietic defect in mice lacking the transcription factor GATA-2. *Nature*. 1994;371(6494):221-226.
4. Evans T, Felsenfeld G. The erythroid-specific transcription factor Eryf1: a new finger protein. *Cell*. 1989;58(5):877-885.
5. Tsai F-Y, Orkin SH. Transcription factor GATA-2 is required for proliferation/survival of early hematopoietic cells and mast cell formation, but not for erythroid and myeloid terminal differentiation. *Blood*. 1997;89(10):3636-3643.
6. Spinner MA, Sanchez LA, Hsu AP, et al. GATA2 deficiency: a protean disorder of hematopoiesis, lymphatics, and immunity. *Blood*. 2014;123(6):809-821.
7. Dickinson RE, Griffin H, Bigley V, et al. Exome sequencing identifies GATA-2 mutation as the cause of dendritic cell, monocyte, B and NK lymphoid deficiency. *Blood*. 2011;118(10):2656-2658.
8. Hahn CN, Chong CE, Carmichael CL, et al. Heritable GATA2 mutations associated with familial myelodysplastic syndrome and acute myeloid leukemia. *Nat Genet*. 2011;43(10):1012-1017.
9. Hsu AP, Sampaio EP, Khan J, et al. Mutations in GATA2 are associated with the autosomal dominant and sporadic monocytopenia and mycobacterial infection (MonoMAC) syndrome. *Blood*. 2011;118(10):2653-2655.
10. Ostergaard P, Simpson MA, Connell FC, et al. Mutations in GATA2 cause primary lymphedema associated with a predisposition to acute myeloid leukemia (Emberger syndrome). *Nat Genet*. 2011;43(10):929-931.
11. Johnson KD, Hsu AP, Ryu MJ, et al. Cis-element mutated in GATA2-dependent immunodeficiency governs hematopoiesis and vascular integrity. *J Clin Invest*. 2012;122(10):3692-3704.
12. Soukup AA, Bresnick EH. GATA2 +9.5 enhancer: from principles of hematopoiesis to genetic diagnosis in precision medicine. *Curr Opin Hematol*. 2020;27(3):163-171.
13. Bresnick EH, Jung MM, Katsumura KR. Human GATA2 mutations and hematologic disease: how many paths to pathogenesis? *Blood Adv*. 2020;4(18):4584-4592.
14. Johnson KD, Kong G, Gao X, et al. Cis-regulatory mechanisms governing stem and progenitor cell transitions. *Sci Adv*. 2015;1(8):e1500503.
15. Grass JA, Jing H, Kim S-I, et al. Distinct functions of dispersed GATA factor complexes at an endogenous gene locus. *Mol Cell Biol*. 2006;26(19):7056-7067.
16. Yamazaki H, Suzuki M, Otsuki A, et al. A remote GATA2 hematopoietic enhancer drives leukemogenesis in inv(3)(q21;q26) by activating EVI1 expression. *Cancer Cell*. 2014;25(4):415-427.
17. Gröschel S, Sanders MA, Hoogenboezem R, et al. A single oncogenic enhancer rearrangement causes concomitant EVI1 and GATA2 deregulation in leukemia. *Cell*. 2014;157(2):369-381.
18. Smeenk L, Ottema S, Mulet-Lazaro R, et al. Selective requirement of MYB for oncogenic hyperactivation of a translocated enhancer in leukemia. *Cancer Discov*. 2021;11(11):2868-2883.
19. Kiehlmeier S, Rafiee MR, Bakr A, et al. Identification of therapeutic targets of the hijacked super-enhancer complex in EVI1-rearranged leukemia. *Leukemia*. 2021;35(11):3127-3138.
20. Mehta C, Johnson KD, Gao X, et al. Integrating enhancer mechanisms to establish a hierarchical blood development program. *Cell Rep*. 2017;20(12):2966-2979.
21. Johnson KD, Conn DJ, Shishkova E, et al. Constructing and deconstructing GATA2-regulated cell fate programs to establish developmental trajectories. *J Exp Med*. 2020;217(11):e20191526.
22. Linnemann AK, O'Geen H, Keles S, Farnham PJ, Bresnick EH. Genetic framework for GATA factor function in vascular biology. *Proc Natl Acad Sci USA*. 2011;108(33):13641-13646.

## Authorship

Contribution: K.D.J. and E.H.B. conceived the project, designed the experiments, and wrote the paper; K.D.J. and A.A.S. performed the experiments.

Conflict-of-interest disclosure: The authors declare no competing financial interests.

ORCID profiles: K.D.J., 0000-0003-0236-7482; A.A.S., 0000-0002-4149-2393; E.H.B., 0000-0002-1151-5654.

Correspondence: Emery H. Bresnick, 4009 WIMR, University of Wisconsin-Madison, Madison, WI 53705; e-mail: ehbresni@wisc.edu.

23. Fujiwara T, O'Geen H, Keles S, et al. Discovering hematopoietic mechanisms through genome-wide analysis of GATA factor chromatin occupancy. *Mol Cell*. 2009;36(4):667-681.
24. Zwifelhofer NM, Cai X, Liao R, et al. GATA factor-regulated solute carrier ensemble reveals a nucleoside transporter-dependent differentiation mechanism. *PLoS Genet*. 2020;16(12):e1009286.
25. Kim S, Bagadia P, Anderson DA III, et al. High amount of transcription factor IRF8 engages AP1-IRF composite elements in enhancers to direct type 1 conventional dendritic cell identity [published correction appears in *Immunity*. 2021;54(7):1622]. *Immunity*. 2020;53(4):759-774.e9.
26. Yáñez A, Ng MY, Hassanzadeh-Kiabi N, Goodridge HS. IRF8 acts in lineage-committed rather than oligopotent progenitors to control neutrophil vs monocyte production. *Blood*. 2015;125(9):1452-1459.
27. Kwok I, Becht E, Xia Y, et al. Combinatorial single-cell analyses of granulocyte-monocyte progenitor heterogeneity reveals an early uni-potent neutrophil progenitor. *Immunity*. 2020;53(2):303-318.e5.
28. Becker AM, Michael DG, Satpathy AT, Sciammas R, Singh H, Bhattacharya D. IRF-8 extinguishes neutrophil production and promotes dendritic cell lineage commitment in both myeloid and lymphoid mouse progenitors. *Blood*. 2012;119(9):2003-2012.
29. Sichier D, Scott CL, Martens L, et al. IRF8 transcription factor controls survival and function of terminally differentiated conventional and plasmacytoid dendritic cells, respectively. *Immunity*. 2016;45(3):626-640.
30. Ahmed N, Kunz L, Hoppe PS, et al. A Novel GATA2 protein reporter mouse reveals hematopoietic progenitor cell types. *Stem Cell Reports*. 2020;15(2):326-339.
31. Li L, Jin H, Xu J, Shi Y, Wen Z. Irf8 regulates macrophage versus neutrophil fate during zebrafish primitive myelopoiesis. *Blood*. 2011;117(4):1359-1369.
32. Yáñez A, Goodridge HS. Interferon regulatory factor 8 and the regulation of neutrophil, monocyte, and dendritic cell production. *Curr Opin Hematol*. 2016;23(1):11-17.
33. Yáñez A, Coetzee SG, Olsson A, et al. Granulocyte-monocyte progenitors and monocyte-dendritic cell progenitors independently produce functionally distinct monocytes. *Immunity*. 2017;47(5):890-902.e4.
34. Dickinson RE, Milne P, Jardine L, et al. The evolution of cellular deficiency in GATA2 mutation. *Blood*. 2014;123(6):863-874.
35. Cytlak U, Resteu A, Pagan S, et al. Differential IRF8 transcription factor requirement defines two pathways of dendritic cell development in humans. *Immunity*. 2020;53(2):353-370.e8.
36. Schlitzer A, Sivakamasundari V, Chen J, et al. Identification of cDC1- and cDC2-committed DC progenitors reveals early lineage priming at the common DC progenitor stage in the bone marrow. *Nat Immunol*. 2015;16(7):718-728.
37. Murphy TL, Grajales-Reyes GE, Wu X, et al. Transcriptional control of dendritic cell development. *Annu Rev Immunol*. 2016;34(1):93-119.
38. Amon L, Lehmann CHK, Baranska A, Schoen J, Heger L, Dudziak D. Transcriptional control of dendritic cell development and functions. *Int Rev Cell Mol Biol*. 2019;349:55-151.
39. Cichoń MA, Klas K, Buchberger M, et al. Distinct distribution of RTN1A in immune cells in mouse skin and lymphoid organs. *Front Cell Dev Biol*. 2021;8:608876.
40. Liu Z, Gu Y, Chakarov S, et al. Fate mapping via Ms4a3-expression history traces monocyte-derived cells. *Cell*. 2019;178(6):1509-1525.
41. Cabeza-Cabrerizo M, Cardoso A, Minutti CM, Pereira da Costa M, Reis E Sousa C. Dendritic cells revisited. *Annu Rev Immunol*. 2021;39(1):131-166.
42. Sasaki H, Kurotaki D, Osato N, et al. Transcription factor IRF8 plays a critical role in the development of murine basophils and mast cells. *Blood*. 2015;125(2):358-369.
43. Churpek JE, Bresnick EH. Transcription factor mutations as a cause of familial myeloid neoplasms. *J Clin Invest*. 2019;129(2):476-488.
44. Al Seraihi AF, Rio-Machin A, Tawana K, et al. GATA2 monoallelic expression underlies reduced penetrance in inherited GATA2-mutated MDS/AML. *Leukemia*. 2018;32(11):2502-2507.
45. McReynolds LJ, Yang Y, Yuen Wong H, et al. MDS-associated mutations in germline GATA2 mutated patients with hematologic manifestations. *Leuk Res*. 2019;76:70-75.
46. Fisher KE, Hsu AP, Williams CL, et al. Somatic mutations in children with GATA2-associated myelodysplastic syndrome who lack other features of GATA2 deficiency. *Blood Adv*. 2017;1(7):443-448.
47. Churpek JE, Pyrtel K, Kanchi KL, et al. Genomic analysis of germ line and somatic variants in familial myelodysplasia/acute myeloid leukemia. *Blood*. 2015;126(22):2484-2490.
48. Bigley V, Cytlak U, Collin M. Human dendritic cell immunodeficiencies. *Semin Cell Dev Biol*. 2019;86:50-61.
49. Hsu AP, Johnson KD, Falcone EL, et al. GATA2 haploinsufficiency caused by mutations in a conserved intronic element leads to MonoMAC syndrome. *Blood*. 2013;121(19):3830-3837.
50. Soukup AA, Zheng Y, Mehta C, et al. Single-nucleotide human disease mutation inactivates a blood-regenerative GATA2 enhancer. *J Clin Invest*. 2019;129(3):1180-1192.
51. Bigley V, Haniffa M, Doulatov S, et al. The human syndrome of dendritic cell, monocyte, B and NK lymphoid deficiency. *J Exp Med*. 2011;208(2):227-234.
52. Porter SN, Cluster AS, Yang W, et al. Fetal and neonatal hematopoietic progenitors are functionally and transcriptionally resistant to *Ft3*-ITD mutations. *eLife*. 2016;5:5.
53. Sanalkumar R, Johnson KD, Gao X, et al. Mechanism governing a stem cell-generating cis-regulatory element. *Proc Natl Acad Sci USA*. 2014;111(12):E1091-E1100.