LETTER OPEN

Check for updates

ANIMAL MODELS

Il-1r1 drives leukemogenesis induced by *Tet2* loss

Sarah S. Burns[1,2,3], Ramesh Kumar[3,4], Santhosh Kumar Pasupuleti[3,4], Kaman So[5], Chi Zhang[2,6] and Reuben Kapur[2,3,4,7,8,9]✉

Leukemia (2022) 36:2531–2534; https://doi.org/10.1038/s41375-022-01665-3

Loss of the *ten-eleven translocation methylcytosine dioxygenase 2* (*Tet2*) gene, which is commonly mutated in hematological malignancies, dysregulates inflammatory pathways, including the interleukin-1 (IL-1) pathway [1–3]. Roles for IL-1 signaling have been reported in terminally differentiated hematopoietic cells and in non-cell autonomous contexts [3, 4]. However, our group demonstrated that inhibition of inflammatory pathways can suppress clonal hematopoiesis (CH), indicating potential direct roles for hematopoietic stem and progenitor cells (HSPCs) in inflammation [5]. As *TET2* mutations are often present in HSPCs and provide these cells with a competitive advantage, dysregulation of the IL-1 pathway in HSPCs may contribute to leukemogenesis and may catalyze the progression of preleukemic states to malignancy [6].

Mutations in the *TET2* gene are detected in a variety of myeloid malignancies, including acute myeloid leukemia (AML) [6]. Similarly, $Tet2^{-/-}$ transgenic mice and recipient mice transplanted with $Tet2^{-/-}$ bone marrow (BM) exhibit splenomegaly, monocytosis, extramedullary hematopoiesis, and expansion of the $Lin^{-}Sca1^{+}c\text{-}Kit^{+}$ (LSK) population [7]. Acute and chronic IL-1 exposure expands myeloid cells at the expense of lymphoid cells; however, chronic exposure ultimately depletes the ability of hematopoietic stem cells (HSCs) to self-renew [8]. While previous studies have investigated the exogenous effects of IL-1α and IL-1β on hematopoiesis and on mature hematopoietic cells, IL-1R1, the primary of ten IL-1 receptors, binds to multiple proteins, including IL-1α, IL-1β, IL-1 receptor antagonist, IL-38, and its co-receptor IL-1 receptor accessory protein, underscoring that the full spectrum of the consequences of IL-1R1-dependent signaling in HSPCs is not yet known [9].

Based on these findings, we hypothesized that loss of the *Il-1r1* gene would rescue the hematological abnormalities associated with *Tet2* deficiency at the HSPC level. Both *Il-1r1* and *Tet2* are expressed in multiple hematopoietic cell types, including high expression in HSPCs (Supplementary Fig. 3A, B) [10]. To determine whether loss of *Il-1r1* can ameliorate malignancy, we generated $Tet2^{-/-}$;$Il\text{-}1r1^{-/-}$ mice and analyzed peripheral blood (PB) counts in a large cohort. The frequencies of myeloid cells were elevated in $Tet2^{-/-}$ mice; however, these cell types were restored to wild-type (WT) levels in $Tet2^{-/-}$;$Il\text{-}1r1^{-/-}$ mice (Fig. 1A). In addition to an increase in myeloid cells, lymphocyte frequency was reduced in $Tet2^{-/-}$ mice, demonstrating a myeloid shift at the expense of lymphocytes (Fig. 1A). Higher red cell distribution width (RDW-CV) was recently reported as a measure of pro-inflammatory states and correlated with an increased risk of AML in humans [11]. Consistent with a pro-inflammatory state due to *Tet2* loss, RDW-CV was increased in $Tet2^{-/-}$ mice and was relieved by inactivation of *Il-1r1* (Fig. 1B). In representative mice from this larger cohort, $Tet2^{-/-}$ mice had larger spleen sizes and weights, which were corrected by loss of *Il-1r1* (Fig. 1C and Supplementary Fig. 1A). These mice showed similar alleviations of elevated myeloid frequencies and suppressed lymphocyte frequencies, supporting a role for *Il-1r1* at the stem-cell level (Supplementary Fig. 1B–D). To examine this possibility, the levels of HSPCs were measured by flow cytometry. Elevated levels of LSKs, long-term HSCs (LT-HSCs), short-term HSCs (ST-HSCs), multipotent progenitor (MPP) pools 2, 3/4, 3, and 4, and common myeloid progenitors (CMPs) were detected in $Tet2^{-/-}$ mice, and these increases were rescued in $Tet2^{-/-}$;$Il\text{-}1r1^{-/-}$ mice, suggesting that loss of *Il-1r1* rescues the expansion of HSPCs associated with *Tet2* deficiency (Fig. 1D; Supplementary Fig. 1E–K). Comparable to other progenitor populations, $Lin^{-}Sca1^{+}$ cells, which represent a subset of lymphoid progenitors that differ from common lymphoid progenitors (CLPs), were also increased in $Tet2^{-/-}$ mice (Fig. 1D) [12]. However, $Lin^{-}CD127^{+}$ progenitors within this $Lin^{-}Sca1^{+}$ population were suppressed, indicating the presence of a block in lymphopoiesis at this stage (Fig. 1D). The increases in LSKs, LT-HSCs, ST-HSCs, and MPPs may represent a compensatory response to this blockage. *Il-1r1* inactivation relieved inhibition of $Lin^{-}CD127^{+}$ cells and normalized the levels of mature lymphoid cell types (Fig. 1D). These findings show that *Il-1r1* loss can rescue *Tet2*-associated HSPC abnormalities. Together, they support roles for IL-1R-dependent signaling at the level of HSPCs, in the correction of myeloid disease, in the modulation of the pro-inflammatory state associated with *Tet2* deficiency, and in the balance of myeloid and lymphoid cell types.

To investigate whether *Il-1r1* deficiency rescues *Tet2*-associated hematological malignancies in a cell autonomous manner, we

[1]Medical Scientist Training Program, Indiana University-Purdue University, Indianapolis, USA. [2]Medical and Molecular Genetics Graduate Program, Indiana University-Purdue University, Indianapolis, USA. [3]The Herman B. Wells Center for Pediatric Research, Indiana University-Purdue University, Indianapolis, USA. [4]Department of Pediatrics, Indiana University-Purdue University, Indianapolis, USA. [5]Department of Biostatistics and Health Data Science, Indiana University-Purdue University, Indianapolis, USA. [6]Center for Computational Biology and Bioinformatics, Indiana University School of Medicine, Indiana, USA. [7]Molecular Biology and Biochemistry, Indiana University School of Medicine, Indiana, USA. [8]Medical and Molecular Genetics, Indiana University School of Medicine, Indiana, USA. [9]Microbiology and Immunology, Indiana University School of Medicine, Indiana, USA. ✉email: rkapur@iu.edu

Received: 14 March 2022 Revised: 15 July 2022 Accepted: 21 July 2022
Published online: 12 August 2022

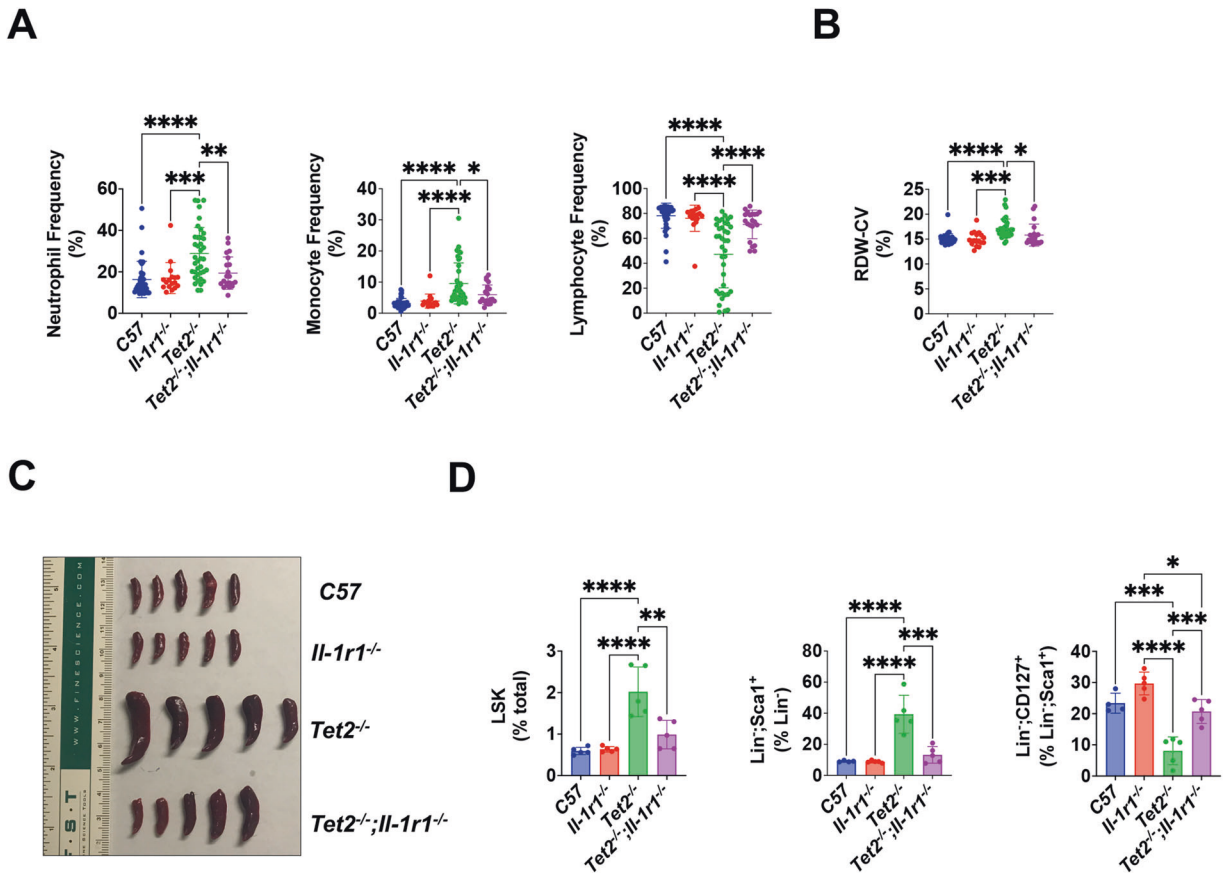

Fig. 1 ***Tet2*$^{-/-}$*;Il-1r1*$^{-/-}$ **mice demonstrated a correction of myeloid cell elevation, lymphocyte suppression, RDW-CV, spleen size, and HSPC levels. A**, **B** Means for neutrophil, monocyte, and lymphocyte frequencies and RDW-CV are displayed for a large cohort of mice over a range of ages. $n = 17–38$ per group. **C** Gross photographs of spleens for representative mice are presented. **D** Mean frequencies for LSK, Lin$^-$;Sca1$^+$, and Lin$^-$;CD127$^+$ cells are shown for representative mice. *$p \le 0.05$, **$p \le 0.01$, ***$p \le 0.001$, ****$p \le 0.0001$. Error bars represent standard deviation. $n = 4–5$ per group.

performed a competitive transplantation of BM containing HSPCs from C57 (CD45.2), Boy/J (CD45.1), *Tet2*$^{-/-}$ (CD45.2), *Il-1r1*$^{-/-}$ (CD45.2), and *Tet2*$^{-/-}$*;Il-1r1*$^{-/-}$ (CD45.2) donor mice into CD45.1- and CD45.2-expressing F1 recipient mice and evaluated the effects of *Il-1r1* loss on engraftment and on *Tet2*$^{-/-}$ HSPCs and mature hematopoietic cell types (Supplementary Fig. 2A). Inactivation of *Il-1r1* reduced the increased engraftment of CD45.2-expressing cells and the high numbers and frequencies of white blood cells (WBC) and myeloid cells detected in mice transplanted with *Tet2*$^{-/-}$ BM (Fig. 2A; Supplementary Figs. 2B–G and 4A, B). Similar corrections of myeloid cells were observed in PB smears and Gr-1$^+$ myeloid cells (Fig. 2A, B). As in the transgenic mice, loss of *Il-1r1* corrected spleen sizes and weights (Fig. 2C; Supplementary Fig. 2H). Consistent with relief of lymphocyte suppression, an increased lymphocyte frequency and elevated percentages of CD4$^+$ T cells, CD8$^+$ T cells, natural killer (NK) cells, and plasmacytoid dendritic cells (pDCs), all cells of lymphoid origin, were detected in mice transplanted with *Tet2*$^{-/-}$*;Il-1r1*$^{-/-}$ BM, demonstrating that loss of *Il-1r1* at the HSPC level can restore the levels of multiple lymphoid cell types (Supplementary Fig. 4C–N). Similar to the transgenic mice, increased levels of LSK, MPP2, and MPP3/4 cells associated with *Tet2* deficiency were rescued in mice transplanted with *Tet2*$^{-/-}$*;Il-1r1*$^{-/-}$ BM, corroborating a role for *Il-1r1* in the regulation of cell populations that contain leukemia-initiating cells (Supplementary Fig. 3C–E). In addition, in mice transplanted with *Tet2*$^{-/-}$ BM, Lin$^-$;c-Kit$^+$ cells were reduced, while Lin$^-$;Sca1$^+$ cells were significantly elevated (Supplementary Fig. 3F, G). These changes were reversed in mice transplanted with *Tet2*$^{-/-}$*;Il-1r1*$^{-/-}$ BM, further supporting profound shifts in myeloid and lymphoid populations (Supplementary Fig. 3F, G). Collectively, these findings suggest that *Il-1r1* loss abrogates hematological malignancy and corrects disruption of the myeloid-lymphoid balance via cell autonomous mechanisms in HSPCs.

To investigate whether inactivation of IL-1 signaling in BM cells alleviates systemic inflammation associated with *Tet2* deficiency, serum cytokine levels were measured. Consistent with previous studies, loss of *Tet2* led to increases in multiple cytokines and chemokines, including tumor necrosis factor α (TNFα) and the interferon-γ (IFN-γ)-inducible genes IFN-γ-induced protein 10 (IP-10/CXCL10) and monokine induced by IFN-γ (MIG/CXCL9) (Supplementary Fig. 5A–C) [1, 2]. These cytokines and chemokines were restored to WT levels in mice transplanted with *Tet2*$^{-/-}$*;Il-1r1*$^{-/-}$ BM (Supplementary Fig. 5A–C). TNFα promotes the expansion of *Tet2*$^{-/-}$ cells in vitro, indicating a non-cell autonomous role [2]. However, we demonstrated that TNFα levels were also elevated in mice transplanted with *Tet2*$^{-/-}$ BM and that this increase was rescued by *Il-1r1* loss in a cell autonomous manner. TNFα and IFNγ can control the levels of Lin$^-$;Sca1$^+$ cells and Sca1 expression and can promote myeloid expansion and regeneration, providing opportunities for antagonistic regulation of lymphoid and myeloid populations [13–15]. Together, these results support roles for IL-1 signaling in HSPCs in modulating the myeloid-lymphoid balance and in determining the pro-inflammatory status of *Tet2*$^{-/-}$ mature hematopoietic cells. Based

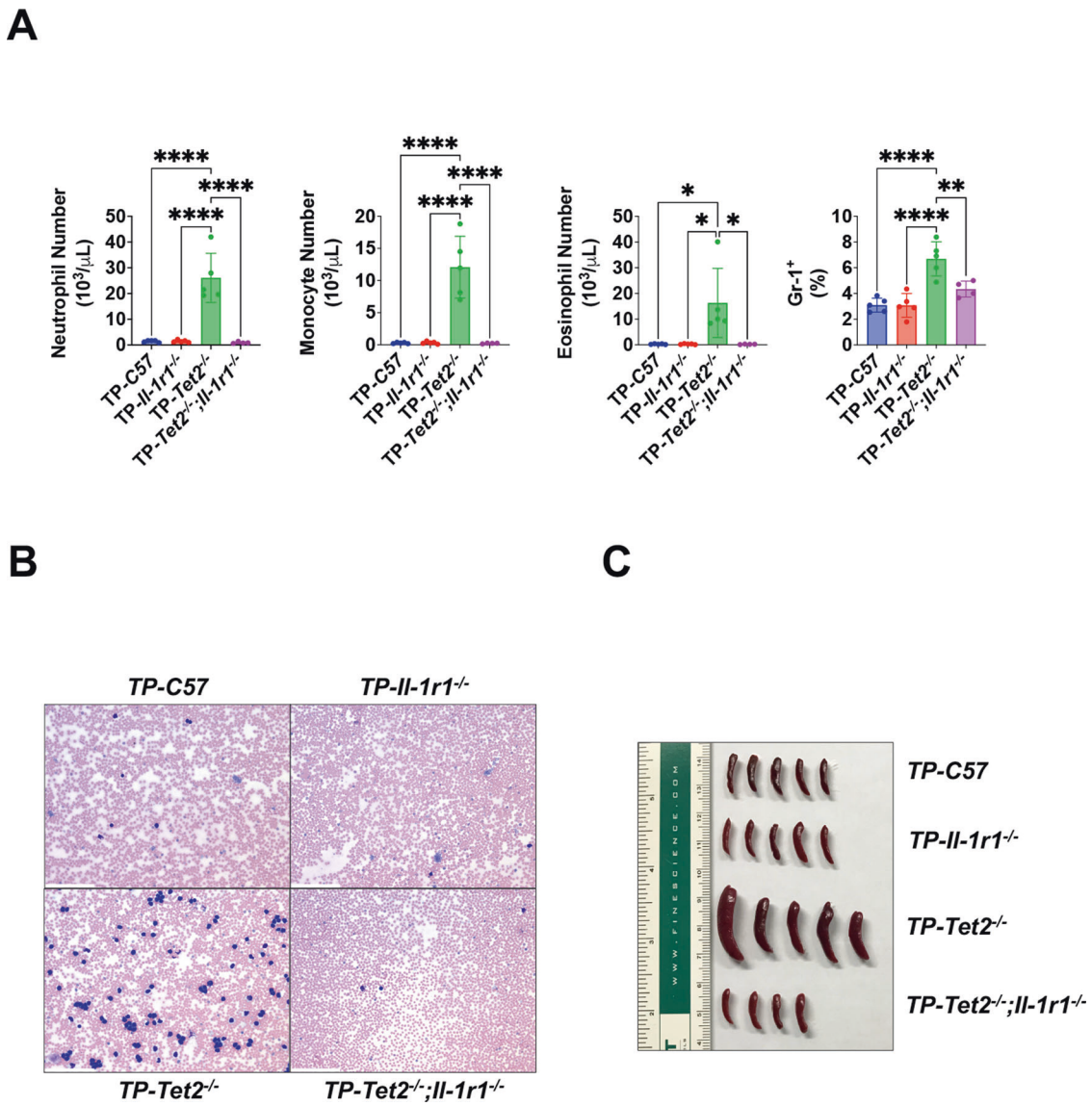

Fig. 2 Recipients of *Tet2*$^{-/-}$;*Il-1r1*$^{-/-}$ BM exhibited rescue of increased levels of myeloid cells and spleen size. A Mean absolute counts of neutrophils, monocytes, and eosinophils and the frequency of Gr-1^{+} myeloid cells at six-months post-transplant. **B** Photographs of representative blood smears are shown and were acquired at 20X magnification. The scale bar denotes 20 μm. **C** Gross photographs of spleens are presented. *$p \leq 0.05$, **$p \leq 0.01$, ***$p \leq 0.001$, ****$p \leq 0.0001$. Error bars represent standard deviation. $n = 4$–5 per group.

on these findings, we propose a mechanism by which loss of *Tet2* leads to a pro-inflammatory state that is characterized by high levels of TNFα and IFN-γ and that causes a myeloid bias at the expense of lymphoid cells (Supplementary Fig. 2I). This shift was evidenced by the elevation of CMPs and the suppression of Lin^{-};CD127^{+} lymphoid progenitors in *Tet2*-deficient contexts. The loss of IL-1R1-dependent signaling rescued these disruptions in normal hematopoiesis, abrogating myeloid disease and bolstering its potential as a therapeutic target (Supplementary Fig. 2J).

To determine the clinical relevance of *IL-1R1* expression in patients with myeloid malignancies, we examined two publically-available datasets for *IL-1R1* expression levels and correlated these levels with survival. Both pediatric and adult AML patients with higher levels of *IL-1R1* expression exhibited decreased survival, suggesting a role for *IL-1R1* in AML pathogenesis (Supplementary Fig. 6A, B). To evaluate whether the effects of *IL-1R1* on survival are specific to distinct AML subtypes, survival was analyzed in the context of high and low *IL-1R1* expression in ten adult AML subtypes. High expression of *IL-1R1* conferred reduced survival in subtypes containing mutations in the *CBFB-MYH11*, *NPM1*, or *p53C* genes (Supplementary Fig. 7A–K). IL-1 signaling has been implicated in the expansion of CD34^{+} human AML cells, further supporting its clinical relevance [4]. These results underscore the potential therapeutic implications of IL-1R-dependent signaling in myeloid malignancies and suggest that patient stratification may be needed.

In summary, we have shown that loss of *Il-1r1* in *Tet2*$^{-/-}$ HSPCs rescued several abnormalities associated with *Tet2* deficiency, including the elevation of LSK cells, the pro-inflammatory state, and the myeloid-lymphoid imbalance. Furthermore, high expression of *IL-1R1* had a clinically significant impact on AML survival. Collectively, these findings underscore a potential therapeutic role for IL-1 signaling in the myeloid aspects of hematological malignancies and preleukemic conditions at the stem-cell level.

DATA AVAILABILITY

The *IL-1R1* expression data are publically available. Other data generated in this study are available from the corresponding author on reasonable request.

REFERENCES

1. Cull AH, Snetsinger B, Buckstein R, Wells RA, Rauh MJ. Tet2 restrains inflammatory gene expression in macrophages. Exp Hematol. 2017;55:56–70.
2. Abegunde SO, Buckstein R, Wells RA, Rauh MJ. An inflammatory environment containing TNFalpha favors Tet2-mutant clonal hematopoiesis. Exp Hematol. 2018;59:60–65.
3. Fuster JJ, MacLauchlan S, Zuriaga MA, Polackal MN, Ostriker AC, Chakraborty R, et al. Clonal hematopoiesis associated with TET2 deficiency accelerates atherosclerosis development in mice. Science 2017;355:842–7.
4. Carey A, Edwards DK, Eide CA, Newell L, Traer E, Medeiros BC, et al. Identification of Interleukin-1 by functional screening as a key mediator of cellular expansion and disease progression in acute myeloid leukemia. Cell Rep. 2017;18:3204–18.
5. Cai Z, Kotzin JJ, Ramdas B, Chen S, Nelanuthala S, Palam LR, et al. Inhibition of inflammatory signaling in Tet2 mutant preleukemic cells mitigates stress-induced abnormalities and clonal hematopoiesis. Cell Stem Cell. 2018;23:833–49.
6. Kaner J, Desai P, Menchia-Trinchant N, Guzman ML, Roboz GJ, Hassane DC. Clonal hematopoiesis and premalignant diseases. Cold Spring Harb Perspect Med. 2020;10:a035675.
7. Li Z, Cai X, Cai L-C, Wang J, Zhang W, Petersen BE, et al. Deletion of Tet2 in mice leads to dysregulated hematopoietic stem cells and subsequent development of myeloid malignancies. Blood 2011;118:4509–18.
8. Pietras EM, Mirantes-Barbeito C, Fong S, Loeffler D, Kovtonyuk L, Zhang S, et al. Chronic interleukin-1 exposure drives haematopoietic stem cells towards precocious myeloid differentiation at the expense of self-renewal. Nat Cell Biol. 2016;18:607–18.
9. Fields JK, Günther S, Sundberg EJ. Structural basis of IL-1 family cytokine signaling. Front Immunol. 2019;10:1412.
10. Choi J, Baldwin TM, Wong M, Bolden JE, Fairfax KA, Lucas EC, et al. Haemopedia RNA-seq: A database of gene expression during haematopoiesis in mice and humans. Nucleic Acids Res. 2019;47:D780–5.
11. Vucinic V, Ruhnke L, Sockel K, Röhnert MA, Backhaus D, Brauer D, et al. The diagnostic red blood cell distribution width as a prognostic factor in acute myeloid leukemia. Blood Adv. 2021;5:5584–7.
12. Kumar R, Fossati V, Israel M, Snoeck H-W. Lin-Sca1+Kit- bone marrow cells contain early lymphoid-committed precursors that are distinct from common lymphoid progenitors. J Immunol. 2008;181:7507–13.
13. Jacobsen FW, Veiby OP, Stokke T, Jacobsen SE. TNF-alpha bidirectionally modulates the viability of primitive murine hematopoietic progenitor cells in vitro. J Immunol. 1996;157:1193–9.
14. Khan KD, Lindwall G, Maher SE, Bothwell AL. Characterization of promoter elements of an interferon-inducible Ly-6E/A differentiation antigen, which is expressed on activated T cells and hematopoietic stem cells. Mol Cell Biol. 1990;10:5150–9.
15. Yamashita Y, Passegue E. TNF-α coordinates hematopoietic stem cell survival and myeloid regeneration. Cell Stem Cell. 2019;25:357–72.

ACKNOWLEDGEMENTS

The authors would like to thank the members of the Indiana University Melvin and Bren Simon Cancer Center Flow Cytometry Resource Facility for their outstanding technical support. The Indiana University Melvin and Bren Simon Comprehensive Cancer Center Flow Cytometry Resource Facility (FCRF) is funded, in part, by NCI grant P30 CA082709, NIDDK grant U54 DK106846, and by NIH instrumentation grant 1S10D012270. The authors would also like to acknowledge the In Vivo Therapeutics Core for support in irradiation of mice and Dr. Mingjiang Xu for supplying $Tet2^{-/-}$ mice. We would also like to thank Ms. Tracy Winkle for administrative support.

AUTHOR CONTRIBUTIONS

RKapur supervised all aspects of the project. SB generated the $Tet2^{-/-}$;$Il\text{-}1r1^{-/-}$ mice, performed all experiments, and wrote the manuscript. RKumar and SP assisted in the experiments. CZ and KS performed the gene expression stratification and survival analysis. All authors have read and approved the manuscript.

FUNDING

Grant Support: This work was supported by NIH grants R01CA173852, R01CA134777, R01HL146137, and R01HL140961, and Riley Children's Foundation (to RK). SB was supported by a T32 DK007519 "Regulation of Hematopoietic Cell Production" to Dr. Hal Broxmeyer, a Cancer Biology Training Program Fellowship from the Melvin and Bren Simon Cancer Center, a Ruth L. Kirschstein National Research Service Individual Fellowship (F30), and a Walter A. and Laura W. Deutsch Research Endowment from the Herman B. Wells Center for Pediatric Research.

COMPETING INTERESTS

The authors declare no competing interests.

ADDITIONAL INFORMATION

Supplementary information The online version contains supplementary material available at https://doi.org/10.1038/s41375-022-01665-3.

Correspondence and requests for materials should be addressed to Reuben Kapur.

Reprints and permission information is available at http://www.nature.com/reprints

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.