HHS Public Access

Author manuscript

Exp Hematol. Author manuscript; available in PMC 2023 February 06.

Published in final edited form as:

Exp Hematol. 2021 April ; 96: 35–43.e7. doi:10.1016/j.exphem.2021.02.001.

TLR7/8 Agonist Treatment Induces an Increase in Bone Marrow Resident Dendritic Cells and Hematopoietic Progenitor Expansion and Mobilization

Sidan Li[1,2], **Juo-Chin Yao**[1], **Justin T. Li**[1], **Amy P. Schmidt**[1], **Daniel C. Link**[1]

[1]Division of Oncology, Department of Medicine, Washington University School of Medicine, Saint Louis, MO, USA.

[2]Hematology Oncology Center, Beijing Children's Hospital, National Center for Children's Health, Capital Medial University, Beijing, China.

Toll-like receptors (TLRs) are a family of pattern recognition receptors (PRRs) which play a critical role in innate immunity [1]. There is accumulating evidence that TLRs also regulate hematopoiesis and contribute to the pathogenesis of certain hematopoietic malignancies [2, 3]. Chronic stimulation with TLR4 or TLR2 ligands results in an expansion of phenotypic HSCs with reduced self-renewal capacity and enhanced myeloid differentiation [4-6]. Of particular relevance to this study, Ignatz-Hoover et al. showed that stimulation of TLR8 signaling in AML cells induces their differentiation, suggesting a potential therapeutic benefit of TLR8 agonist therapy in AML [7]. TLR7 and TLR8 are endosomal TLRs that recognize single stranded ribonucleic acids, including viral RNAs and micro RNAs [3]. TLR7 and TLR8 are primarily expressed on myeloid cell populations, including monocytes, neutrophils, and dendritic cells (DCs), with undetectable expression on HSPCs [8]. TLR7/8 stimulation of human $CD34^+$ cells induces their differentiation into DCs in vitro [9]. However, the effect of TLR8 stimulation in vivo on normal hematopoiesis is largely unknown.

Methods

Mice

Zbtb46^{gfp} (129S-Zbtb46^{tm1Kmm}/J) and B6.Cg-*Zbtb46*$^{tm3.1(cre)Mnz}$/J mice were obtained from The Jackson Laboratory. B6.Cg-*Zbtb46*$^{tm3.1(cre)Mnz}$/J mice were bred with B6.129P2(SJL)-*Myd88*^{tm1Defr}/J mice to generate *Zbtb46-Cre, Myd88*$^{f/f}$ mice. *CX3CR1*$^{gfp/+}$ mice were kindly provided by Dr. David Littman (New York University School of

Corresponding Author: Daniel C. Link, M.D., Division of Oncology, Department of Medicine, 660 South Euclid Avenue, Campus Box 8007, Saint Louis, MO, 63110, Phone: (314) 362-8871, danielclink@wustl.edu.

Disclosures

The authors have no financial conflicts of interest.

Medicine). All animal handling and experimental procedures were approved by the Animal Studies Committee at Washington University.

R848 administration

R848 (tlrl-pms, Invivogen) was diluted in H_2O and administered intraperitoneally at a dose of 100μg per day every other day for 3 doses.

Bone marrow transplantation, quantitative RT-PCR, and RNA sequencing

Standard approaches were used and are outlined in detail in supplementary methods. The RNA sequencing data have been deposited at GEO (GSE163600).

Flow cytometry and sorting

Detailed methods for flow cytometry analysis are provided in supplementary methods. For DC sorting, bone marrow cells were harvested from *CX3CR1*$^{gfp/+}$ mice and stained with a biotin-conjugated lineage marker cocktail (Gr-1, B220, Ter119, and CD3e). Following lineage depletion using the Auto-MACS Pro Separator (Miltenyi Biotec) with anti-biotin microbeads (Miltenyi Biotec, 130-090-485), cells were stained with appropriate antibodies and sorted using a Sony SY3200 “Synergy” high-speed cell sorter (Sony).

Statistical analyses

The statistical analysis used for each experiment is provided in the figure legends. All data are presented as mean ± standard error of the mean (S.E.M.).

Results and Discussion

To assess the in vivo hematopoietic response to TLR8 stimulation, we treated C57Bl/6J mice with the TLR7/8 ligand R848 every other day for 3 doses. Prior studies showed that systemic treatment with TLR2 or TLR4 agonists results in the expansion of mature myeloid cells (neutrophils and monocytes) at the expense of B lineage cells in the bone marrow [10-12]. In contrast, R848 treatment had no significant effect on peripheral blood counts or the number of neutrophils or monocytes in the blood, bone marrow or spleen, except for an increase in splenic monocytes (Fig. 1A-D). Moreover, although B cells were reduced in the blood and bone marrow, they were increased in the spleen (Fig. 1B-D). As reported previously, DCs were identified as B220^{-} GR1^{-} MHCII^{hi} CD11c^{hi} cells (Fig.S1A) [13]. Interestingly, a significant (nearly 3-fold) increase in bone marrow resident DCs was observed after R848 treatment (Fig. 1C). To confirm this finding, we used *Zbtb46*^{GFP} mice, in which DCs and endothelial cells, but not monocytes or macrophages, express GFP [14]. By flow cytometry, we again observed a significant increase in B220^{-} GR1^{-} MHCII^{hi} CD11c^{hi} GFP^{+} DCs in the bone marrow (Fig. 1E-F). Of note, a modest increase in GFP^{+} DCs in the blood but not spleen also was observed (Fig. 1G-H). We next analyzed femur sections from *Zbtb46*^{GFP} mice. DCs were identified as GFP^{dim} perivascular cells that were located near GFP^{bright} VE-Cadherin^{+} endothelial cells (Fig. S1B). Consistent with the flow cytometry data, histomorphometry showed a significant increase in perivascular DCs (Fig. S1C). The impact of more prolonged R848 treatment on DC homeostasis remains an important open question.

We previously reported that bone marrow resident DCs display a type 2 classical DC (cDC2)-like immunophenotype, expressing CD11b but not XCR1 [13]. Of note, the expression pattern of certain inflammatory chemokines/cytokines and their receptors in bone marrow resident DCs is distinct from cDC2s in the spleen, suggesting that bone marrow DCs may represent a unique dendritic cell population with distinct functional properties [13]. To explore mechanisms for the increase in bone marrow DCs, we quantified common dendritic progenitors (CDPs) and pre-DCs in the bone marrow (Fig. 2A). A striking reduction in both cell populations was observed after R848 treatment (Fig. 2B). Of note, a prior study showed that TLR7/8 agonist treatment of human $CD34^+$ cells induced their differentiation into DCs [9]. Together, these observations suggest the possibility that TLR7/8 stimulation induces the differentiation of CDPs (and preDCs) into mature DCs in vivo. Alternatively, Schmid et al showed that treatment with agonists for TLR1/2, TLR4, or TLR9 results in modest mobilization of preDCs, suggesting that mobilization also may contribute to the decrease in CDPs and preDCs in the bone marrow [15].

To characterize the phenotype of the expanded pool of bone marrow resident DCs, we performed RNA sequencing on sorted DCs following R848 treatment. Principal component analysis segregated R848-treated DCs from control DCs (Fig 2C). A total of 192 differentially expressed genes were identified that met the following criteria: 1) a false discovery rate ≤ 1%; 2) a fold change of ≥ 4; and 3) minimum FPKM of ≥ 5 for the higher expressed cohort (Table S1). Significantly differentially expressed chemokines were CCL5, CCL9, and CXCL16 (Fig. 2D). In particular, CCL5 expression in bone marrow DCs increased 47-fold after R848 treatment. This is relevant, since CCL5 has been implicated in the expansion of myeloid-biased HSCs [16]. Significantly increased expression of CD83, a marker of DC activation, with a trend to increased CD40, CD80, or CD86 expression, also was observed (Fig. 2D) [17]. Of note, cell surface expression of MCHII was not changed after R848 treatment (Fig. 2E). To further assess DC phenotype, flow cytometry was performed to measure the cell surface expression of certain receptors involved in DC migration. Marked decreased expression of CCR2 and a significant, but modest, decrease in CCR7 were observed in R848 treated bone marrow DCs (Fig. 2E). Since CCR7 and CCR2 have been implicated in the tracking of DCs to draining lymph nodes, these data raise the possibility that TLR7/8 stimulation may reduce the migratory capacity of DCs [18-21]. On the other hand, a marked increase in CD62L (encoding L-selectin) was observed. Although its role in DC migration is unclear, there is strong evidence that CD62L is required for the efficient transendothelial migration of monocytes [22]. The impact of altered gene expression on DC subtype (e.g., cDC1 versus cDC2), activation, migratory capacity, and ability to activate T cells is unclear and will require further study.

Prior studies showed that treatment with TLR2 or TLR4 ligands results in an expansion of phenotypic HSPCs in the bone marrow and mobilization of HSPCs to blood and spleen [2, 11, 12]. Likewise, we observed that treatment with R848 induced a modest expansion of $lineage^-$ $Sca1^+$ Kit^+ (LSK) cells and phenotypic HSCs ($CD150^+$ $CD48^-$ LSK cells) in the bone marrow (Fig. 3A,C and Fig. S2A). Since Sca1 expression is upregulated in response to certain inflammatory stimuli [23], we also quantified phenotypic HSCs using EPCR instead of Sca1 (Fig. S2B). Again, a significant, increase in $CD150^+$ $CD48^-$ $Lineage^-$ $EPCR^+$ Kit^+ cells was observed in the bone marrow after R848 treatment (Fig. 3D).

Interestingly, a modest, but significant, decrease in common myeloid progenitors (CMP) and megakaryocyte-erythroid progenitors (CEP) was observed (Fig. 3E). Treatment with R848 also induced modest HSPC mobilization, with significant increases in phenotypic HSCs, LSK cells, and colony-forming cells (CFU-C) in the spleen and LSK cells in the blood (Fig. 3A-C). Of note, prior studies showed that HSPC mobilization induced by treatment with TLR4 or TLR2 ligands is mediated in part, by increasing G-CSF expression [4, 24]. G-CSF-induced HSPC mobilization is primarily mediated by decreasing CXCL12 and stem cell factor expression in the bone marrow [25]. However, no change in total bone marrow CXCL12 or stem cell factor mRNA was observed after R848 treatment (Fig. 3F). These data suggest that increased G-CSF signaling is not a major driver of HSPC mobilization in response to TLR7/8 stimulation.

TLR7/8 is expressed at low levels in murine and human HSPCs, raising the possibility of a non-cell autonomous mechanism of HSPC mobilization [26, 27]. Since bone marrow resident DCs express a relatively high level of TLR7 (FPKM ± SD: 9.4 ± 3.6) and TLR8 (21.9 ± 6.3), we next determined whether TLR7/8 signaling in DCs contributes to HSPC expansion or mobilization by generating *Zbtb46-Cre Myd88*$^{fl/fl}$ mice. Of note, *Zbtb46-Cre* selectively targets dendritic cells but not monocytes or macrophages [28]. Since, MYD88 is required for TLR7/8 signaling, dendritic cells in these mice should be unresponsive to R848 stimulation. Endothelial cells also express *Zbtb46* [14]; thus, we generated *Zbtb46-Cre Myd88*$^{f/f}$ or *Myd88*$^{f/f}$ (control) bone marrow chimeras to restrict the *Myd88* loss to hematopoietic cells (Fig. 4A). Surprisingly, the TLR7/8 agonist-induced increase in DCs and decrease in B cells was preserved in *Zbtb46-Cre Myd88*$^{f/f}$ mice (Fig. 4B-E). Likewise, the TLR7/8 agonist-induced increase in bone marrow HSPCs and HSPC mobilization were similar in control and *Zbtb46-Cre Myd88*$^{f/f}$ mice (Fig. 4G-J). These data show that TLR7/8 signaling in DCs is not required for the increase in DCs or HSPCs or HSPC mobilization. We previously showed that G-CSF induced HSPC mobilization is secondary to monocyte and/or macrophage activation [29]. Of note, both TLR7 and TLR8 are expressed on bone marrow resident monocytes (average FPKM ± SD: 12.8 ± 6.1 and 25.2 ± 7.1, respectively) and macrophages (11.5 ± 2.7 and 6.0 ± 4.2, respectively). These observations suggest the possibility that TLR7/8-induced monocyte or macrophage activation mediates the HSPC expansion and/or mobilization.

Systemic treatment with TLR4 or TLR2 ligands results in reduced HSC repopulating activity and enhanced myeloid differentiation of HSCs [2, 5, 10, 12]. To determine if TLR7/8 stimulation had a similar effect on HSCs, we performed a competitive repopulation assay using bone marrow from R848 or vehicle-alone treated mice. Multi-lineage engraftment similar to that observed with control cells was observed (Fig. 5A-B). Moreover, the contribution of donor cells to the LSK and LSK-SLAM populations was comparable to control cells (Fig. 5C). The similar engraftment of R848-treated bone marrow cells despite the increase in phenotypic HSCs (Figure 3C) raised the possibility that R848 treatment reduces the repopulating activity of individual R848-treated HSCs. Indeed, donor engraftment after transplantation of forty sorted LSK-SLAM cells was significantly reduced compared with control HSCs (Fig. 5D). Together, these data show that in vivo stimulation of TLR7/8 results in a modest expansion of phenotypic HSCs with reduced repopulating activity. Serial transplantations assays are needed to rigorously determine

whether R848 affects HSC self-renewal capacity. Of note, a prior study showed G-CSF selectively mobilizes dormant HSCs [30]. Thus, it is possible that TLR7/8 also selectively mobilizes HSCs with increased engraftment potential, contributing to the observed reduced repopulating activity of bone marrow resident HSCs.

To assess HSC lineage bias, the contribution of donor cells to myeloid and lymphoid lineages in the blood was assessed 16 weeks after transplantation of sorted HSCs (Fig. 5E). A similar percentage of myeloid (neutrophil or monocyte) and lymphoid (T-cell or B-cell) donor cells was observed in mice transplanted with control or R848-treated HSCs (Fig. 5E). Indeed, the mean percentage of donor cells that were myeloid was comparable, and the majority of mice showed a balanced HSC lineage output pattern (Fig. 5F-G). Thus, in contrast to a chronic (4-6 week) low-dose TLR4 stimulation, short-term (5 day) TLR7/8 stimulation does not alter the lineage bias of HSCs [5]. The impact of more prolonged treatment with a TLR7/8 agonist on HSC lineage output remains an open question.

Our data show that in vivo stimulation of TLR7/8 results in a significant expansion of perivascular DCs in the bone marrow. Of note, recent studies show that DCs are an important component of the perivascular niche in the bone marrow, providing signals that regulate HSPC function and mature B cell survival [31, 32]. The impact of increasing bone marrow DCs on the ability of the perivascular niche to support HSPCs and respond to inflammatory stimuli are open and important questions. Sioud et al. showed that DCs generated from human $CD34^+$ cells by TLR7/8 stimulation were able to induce potent alloreactive T-cell responses in vitro [9]. Thus, TLR7/8 agonist treatment, by expanding bone marrow DCs, may augment responses to certain immunotherapies, such as cancer vaccines or immune checkpoint blockade.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

This study was supported by the National Institute of Health, grant RO1HL131655 (DCL) and by the Taub Foundation Grants Program for MDS Research.

References

[1]. Fitzgerald KA, Kagan JC. Toll-like Receptors and the Control of Immunity. Cell. 2020;180:1044–1066. [PubMed: 32164908]

[2]. Monlish DA, Bhatt ST, Schuettpelz LG. The Role of Toll-Like Receptors in Hematopoietic Malignancies. Front Immunol. 2016;7:390. [PubMed: 27733853]

[3]. Cannova J, Breslin SJP, Zhang J. Toll-like receptor signaling in hematopoietic homeostasis and the pathogenesis of hematologic diseases. Front Med. 2015;9:288–303. [PubMed: 26297301]

[4]. Herman AC, Monlish DA, Romine MP, Bhatt ST, Zippel S, Schuettpelz LG. Systemic TLR2 agonist exposure regulates hematopoietic stem cells via cell-autonomous and cell-non-autonomous mechanisms. Blood Cancer J. 2016;6:e437. [PubMed: 27315114]

[5]. Esplin BL, Shimazu T, Welner RS, et al. Chronic exposure to a TLR ligand injures hematopoietic stem cells. J Immunol. 2011;186:5367–5375. [PubMed: 21441445]

[6]. Zhao Y, Ling F, Wang HC, Sun XH. Chronic TLR signaling impairs the long-term repopulating potential of hematopoietic stem cells of wild type but not Id1 deficient mice. PLoS One. 2013;8:e55552. [PubMed: 23383338]

[7]. Ignatz-Hoover JJ, Wang H, Moreton SA, et al. The role of TLR8 signaling in acute myeloid leukemia differentiation. Leukemia. 2015;29:918–926. [PubMed: 25283842]

[8]. Ohto U, Tanji H, Shimizu T. Structure and function of toll-like receptor 8. Microbes Infect. 2014;16:273–282. [PubMed: 24513445]

[9]. Sioud M, Floisand Y. TLR agonists induce the differentiation of human bone marrow CD34+ progenitors into CD11c+ CD80/86+ DC capable of inducing a Th1-type response. Eur J Immunol. 2007;37:2834–2846. [PubMed: 17853407]

[10]. De Luca K, Frances-Duvert V, Asensio MJ, et al. The TLR1/2 agonist PAM(3)CSK(4) instructs commitment of human hematopoietic stem cells to a myeloid cell fate. Leukemia. 2009;23:2063–2074. [PubMed: 19641520]

[11]. Burberry A, Zeng MY, Ding L, et al. Infection mobilizes hematopoietic stem cells through cooperative NOD-like receptor and Toll-like receptor signaling. Cell Host Microbe. 2014;15:779–791. [PubMed: 24882704]

[12]. Megias J, Yanez A, Moriano S, O'Connor JE, Gozalbo D, Gil ML. Direct Toll-like receptor-mediated stimulation of hematopoietic stem and progenitor cells occurs in vivo and promotes differentiation toward macrophages. Stem Cells. 2012;30:1486–1495. [PubMed: 22511319]

[13]. Zhang J, Supakorndej T, Krambs JR, et al. Bone marrow dendritic cells regulate hematopoietic stem/progenitor cell trafficking. J Clin Invest. 2019;129:2920–2931. [PubMed: 31039135]

[14]. Satpathy AT, Kc W, Albring JC, et al. Zbtb46 expression distinguishes classical dendritic cells and their committed progenitors from other immune lineages. J Exp Med. 2012;209:1135–1152. [PubMed: 22615127]

[15]. Schmid MA, Takizawa H, Baumjohann DR, Saito Y, Manz MG. Bone marrow dendritic cell progenitors sense pathogens via Toll-like receptors and subsequently migrate to inflamed lymph nodes. Blood. 2011;118:4829–4840. [PubMed: 21908421]

[16]. Ergen AV, Boles NC, Goodell MA. Rantes/Ccl5 influences hematopoietic stem cell subtypes and causes myeloid skewing. Blood. 2012;119:2500–2509. [PubMed: 22289892]

[17]. Li Z, Ju X, Silveira PA, et al. CD83: Activation Marker for Antigen Presenting Cells and Its Therapeutic Potential. Front Immunol. 2019;10:1312. [PubMed: 31231400]

[18]. Forster R, Schubel A, Breitfeld D, et al. CCR7 coordinates the primary immune response by establishing functional microenvironments in secondary lymphoid organs. Cell. 1999;99:23–33. [PubMed: 10520991]

[19]. Jimenez F, Quinones MP, Martinez HG, et al. CCR2 plays a critical role in dendritic cell maturation: possible role of CCL2 and NF-kappa B. J Immunol. 2010;184:5571–5581. [PubMed: 20404272]

[20]. Nakano H, Lyons-Cohen MR, Whitehead GS, Nakano K, Cook DN. Distinct functions of CXCR4, CCR2, and CX3CR1 direct dendritic cell precursors from the bone marrow to the lung. J Leukoc Biol. 2017;101:1143–1153. [PubMed: 28148720]

[21]. Ohl L, Mohaupt M, Czeloth N, et al. CCR7 governs skin dendritic cell migration under inflammatory and steady-state conditions. Immunity. 2004;21:279–288. [PubMed: 15308107]

[22]. Ivetic A, Hoskins Green HL, Hart SJ. L-selectin: A Major Regulator of Leukocyte Adhesion, Migration and Signaling. Front Immunol. 2019;10:1068. [PubMed: 31139190]

[23]. Baldridge MT, King KY, Boles NC, Weksberg DC, Goodell MA. Quiescent haematopoietic stem cells are activated by IFN-gamma in response to chronic infection. Nature. 2010;465:793–797. [PubMed: 20535209]

[24]. Boettcher S, Gerosa RC, Radpour R, et al. Endothelial cells translate pathogen signals into G-CSF-driven emergency granulopoiesis. Blood. 2014;124:1393–1403. [PubMed: 24990886]

[25]. Greenbaum AM, Link DC. Mechanisms of G-CSF-mediated hematopoietic stem and progenitor mobilization. Leukemia. 2011;25:211–217. [PubMed: 21079612]

[26]. Rapin N, Bagger FO, Jendholm J, et al. Comparing cancer vs normal gene expression profiles identifies new disease entities and common transcriptional programs in AML patients. Blood. 2014;123:894–904. [PubMed: 24363398]

[27]. Chambers SM, Boles NC, Lin KY, et al. Hematopoietic fingerprints: an expression database of stem cells and their progeny. Cell Stem Cell. 2007;1:578–591. [PubMed: 18371395]

[28]. Loschko J, Rieke GJ, Schreiber HA, et al. Inducible targeting of cDCs and their subsets in vivo. J Immunol Methods. 2016;434:32–38. [PubMed: 27073171]

[29]. Christopher MJ, Petti AA, Rettig MP, et al. Immune Escape of Relapsed AML Cells after Allogeneic Transplantation. N Engl J Med. 2018;379:2330–2341. [PubMed: 30380364]

[30]. Bernitz JM, Daniel MG, Fstkchyan YS, Moore K. Granulocyte colony-stimulating factor mobilizes dormant hematopoietic stem cells without proliferation in mice. Blood. 2017;129:1901–1912. [PubMed: 28179275]

[31]. Zhang J, Supakorndej T, Krambs JR, et al. Bone marrow dendritic cells regulate hematopoietic stem/progenitor cell trafficking. J Clin Invest. 2019;130.

[32]. Sapoznikov A, Pewzner-Jung Y, Kalchenko V, Krauthgamer R, Shachar I, Jung S. Perivascular clusters of dendritic cells provide critical survival signals to B cells in bone marrow niches. Nat Immunol. 2008;9:388–395. [PubMed: 18311142]

HIGHLIGHTS

1. Systemic TLR7/8 agonist treatment induces an increase in phenotypic hematopoietic stem cells with reduced repopulating activity.
2. Systemic TLR7/8 agonist treatment induces hematopoietic stem/progenitor cell mobilization.
3. Systemic TLR7/8 agonist treatment is associated with a marked increase in mature classical dendritic cells and decrease in dendritic cell progenitors/precursors

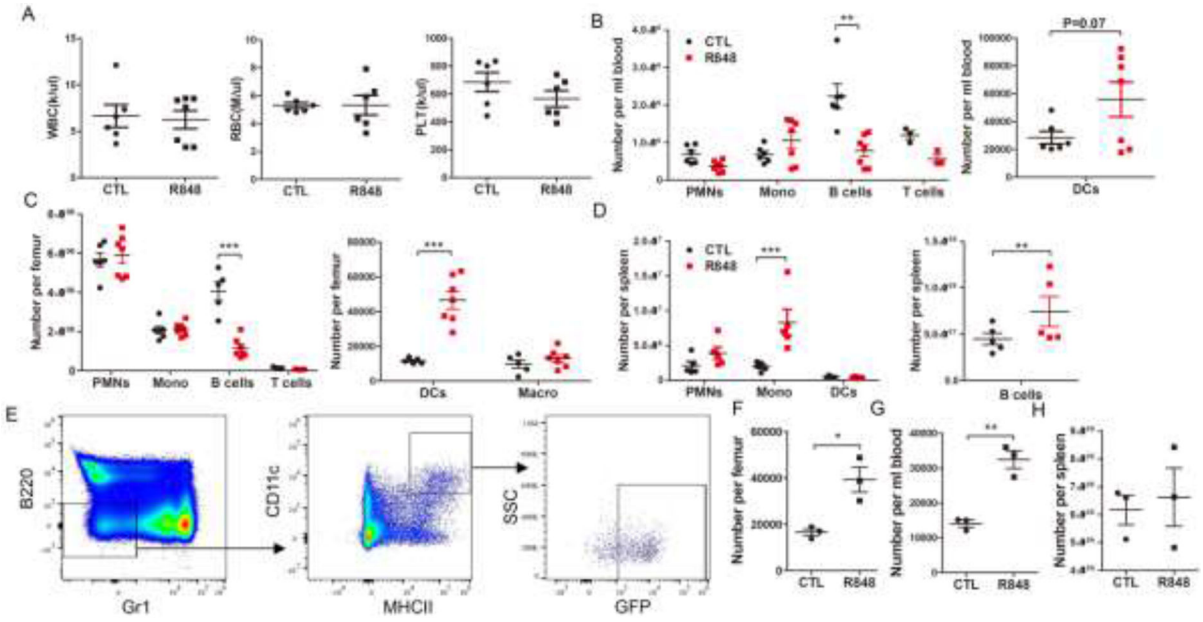

Figure 1. TLR7/8 agonist treatment is associated with an increase in bone marrow dendritic cells.

Wild-type or *Zbtb46*^{gfp} (129S-Zbtb46^{tm1Kmm}/J) mice were treated with R848 (100μg, every other day x 3 doses) and analyzed 24 hours after the final dose. **A**. White blood cell (WBC), red blood cell (RBC), and platelet counts (PLT) are shown. **B-D**. Number of the indicated cell type in blood **(B)**, bone marrow **(C)**, or spleen **(D). E.** Representative flow plots showing the gating strategy using *Zbtb46*^{gfp} mice to identify DCs (MHCII^{high} CD11c^{high} GFP^{high} Gr1^{-} B220^{-}) in the bone marrow. **F-H**. Number of GFP^{+} DCs in bone marrow **(F)**, blood **(G)**, or spleen **(H)** of *Zbtb46*^{gfp} mice. Data represent the mean ± SEM. Statistical significance determined using an unpaired t-test. n = 3-7, from two independent experiments.*P<0.05, **P < 0.01, ***P < 0.001.

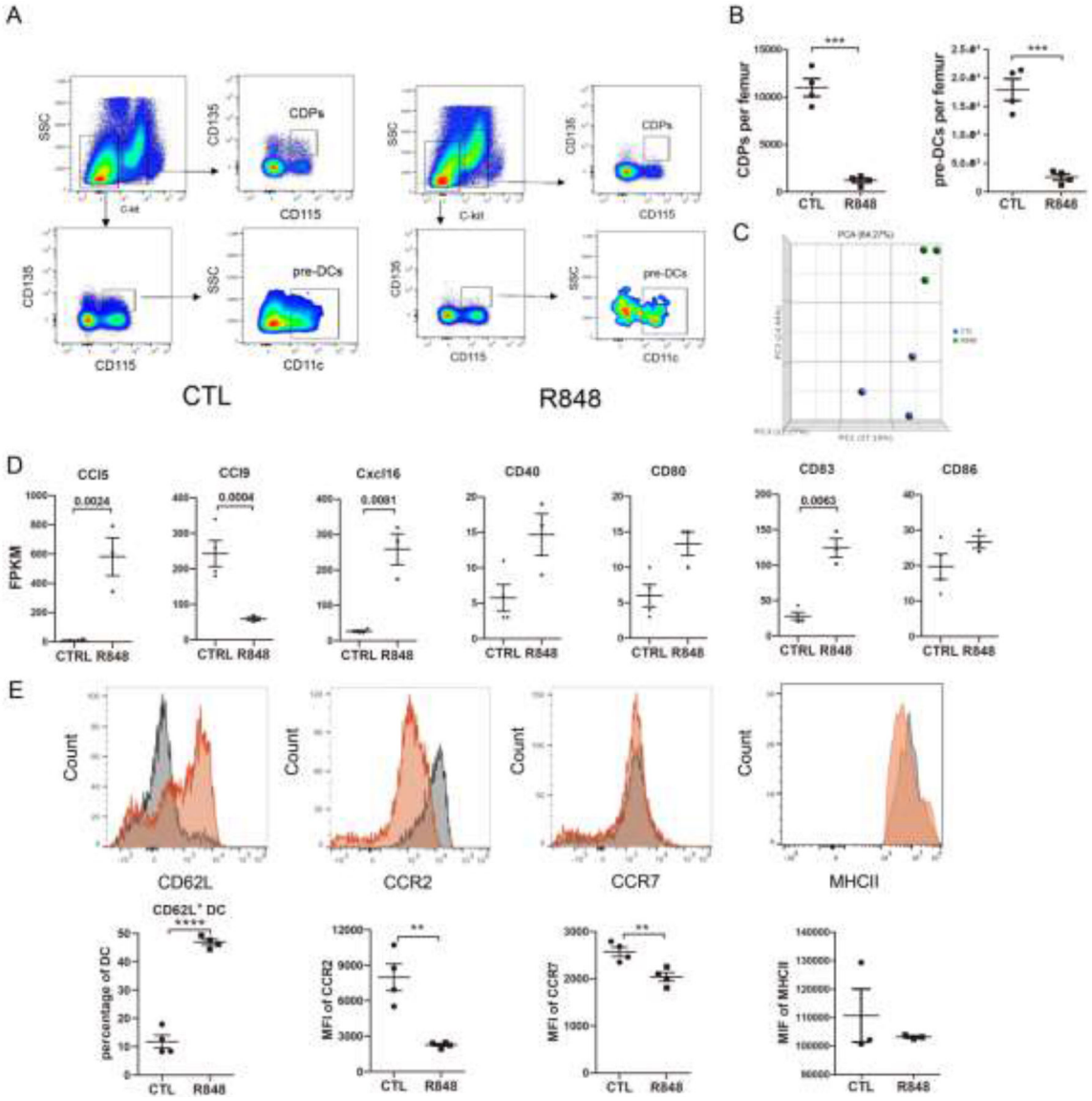

Figure 2. DC progenitor/precursors in the bone marrow are reduced following TLR7/8 agonist treatment.

Wild-type mice were treated with R848 (100μg, every other day x 3 doses) and analyzed 24 hours after the final dose. **A**. Representative flow plots showing gating strategy to identify common dendritic progenitors (CDPs) and pre-DCs. **B**. Number of CDPs and pre-DCs in the bone marrow. **C-D**. *Cx3cr1*$^{gfp/+}$ mice were treated with a single dose of R848 (100 μg), and RNA sequencing was performed on sorted bone marrow DCs (MHCII^{high} CD11c^{high} CX3CR1-GFP^{high} Gr-1^{-} B220^{-}). **C**. Principal component analysis **D**. Expression of the indicated gene (FPKM, fragments per kilobase) with false discovery rates shown. **E**. Representative histograms showing cell surface expression of the indicated protein (upper panel), with quantification of the data provided in the lower panel. The gray and orange histograms represent control and R848-treated DCs, respectively. Statistical significance determined using an unpaired t-test (B, E) or false discovery rate (D). **P < 0.01, ***P < 0.001.

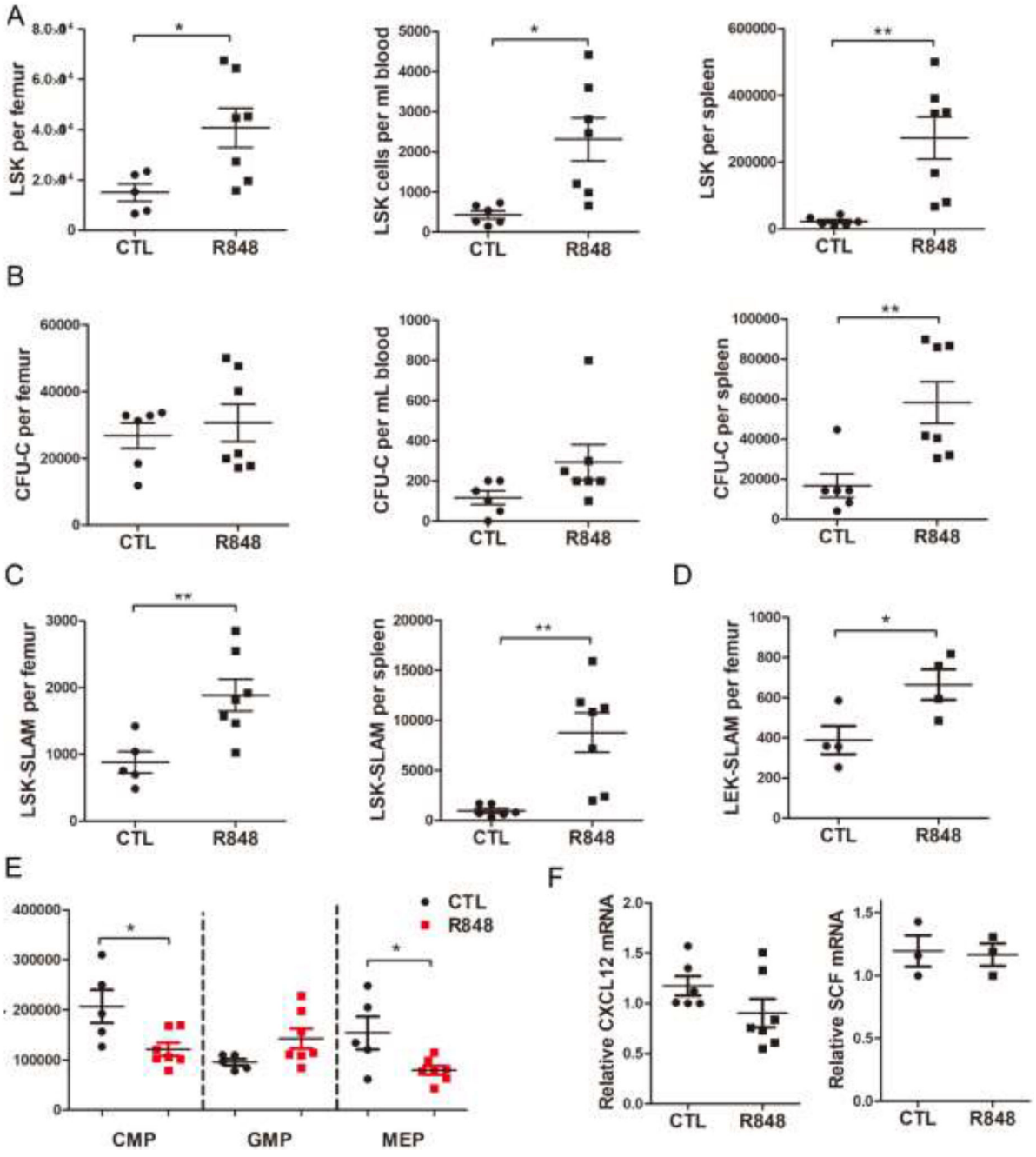

Figure 3. TLR7/8 agonist treatment induces HSPC expansion and mobilization.

Wild-type mice were treated with R848 (100μg, every other day x 3 doses) and analyzed 24 hours after the final dose. **A**. Number of lineage$^-$ sca1$^+$ c-kit$^+$ (LSK) in the bone marrow, blood and spleen. **B**. Number of colony-forming cells (CFU-C) in bone marrow, blood and spleen. **C**. Number of CD150$^+$ CD48$^-$ LSK (LSK-SLAM) in the bone marrow and spleen. **D**. Number of lineage$^-$ EPCR$^+$ c-kit$^+$ CD150$^+$ CD48$^-$ (LEK-SLAM) cells per femur. **E**. Number of common myeloid progenitors (CMPs), granulocyte-macrophage progenitors (GMPs), and megakaryocyte-erythroid progenitors (MEPs) in bone marrow. **F**. RNA expression of the indicated gene relative to β-actin from total bone marrow RNA.

Data represent the mean ± SEM. Statistical significance determined using an unpaired t-test. *P<0.05, **P < 0.01.

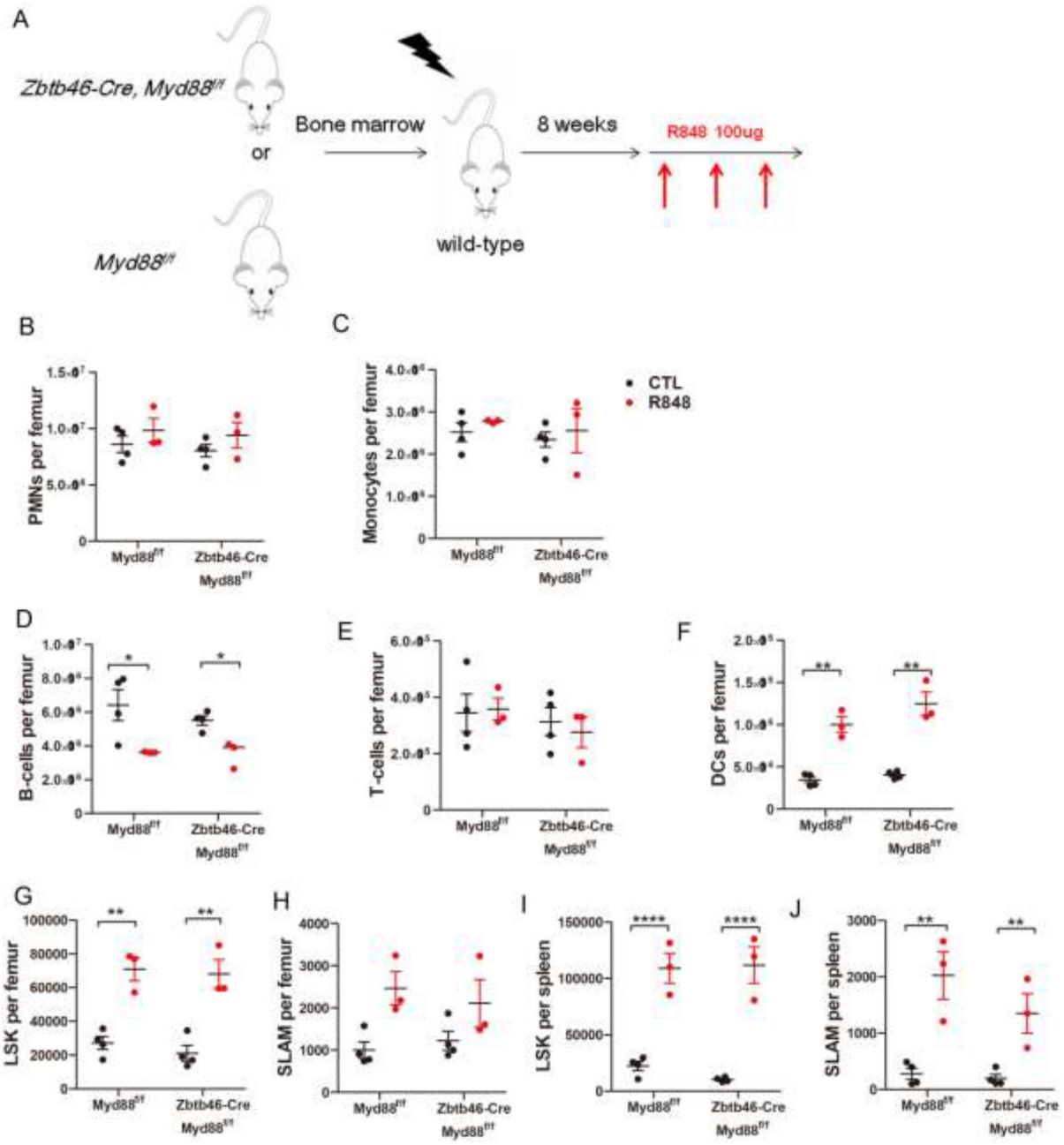

Figure 4. TLR7/8 signaling in DCs is dispensable for TLR7/8 agonist-induced HSPC expansion and mobilization.

A. Bone marrow from *Zbtb46-Cre, Myd88*$^{f/f}$ or *Myd88*$^{f/f}$ mice were transplanted into irradiated wild-type recipient mice, and 8 weeks later, mice were treated with R848 (100μg, every other day x 3 doses) and analyzed 24 hours after the final dose. **B-E**. Number of the indicated in the cell type in bone marrow. **F**. Number of DCs (MHCII^{high} CD11c^{high} Gr-1^{-} B220^{-}) in the bone marrow. **G-H**. Number of lineage^{-} sca1^{+} c-kit^{+} (LSK) in the bone marrow. **I-J**. Number of CD150^{+} CD48^{-} LSK (LSK-SLAM) in the spleen. Data represent the mean ± SEM. Statistical significance determined using a one-way ANOVA with Newman-Keuls multiple comparison testing. *P<0.05, **P < 0.01, ***P < 0.001.

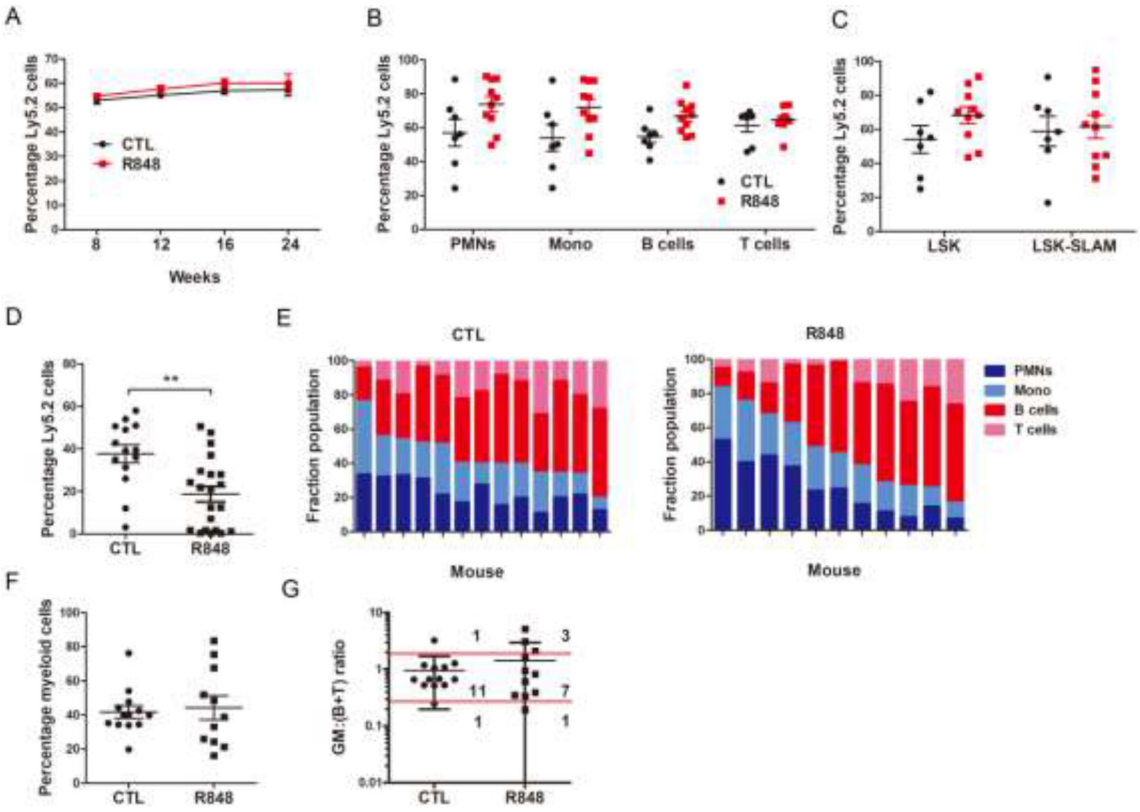

Figure 5. TLR7/8 agonist treatment reduces the repopulating activity of individual HSCs without affecting lineage output.

Bone marrow from wildtype (Ly5.2) mice treated with R848 or vehicle alone (CTL) were transplanted along with an equal number of competitor (Ly5.1/Ly5.2) bone marrow cells into irradiated Ly5.1 recipients (n=7-10 per cohort, from two independent experiments). **A**. Percentage of Ly5.2 peripheral blood leukocytes over time. **B-C**. Percentage of Ly5.2 cells in the indicated cell population 16 or 24 weeks after transplantation. PMNs: neutrophils; Mono: monocytes; LSK: lineage$^-$ Sca1$^+$ Kit$^+$ cells; LSK-SLAM: LSK-CD150$^+$ CD48$^-$ cells. **D-G**. Forty sorted LSK-SLAM cells were transplanted into recipient and peripheral blood chimerism assessed 16 weeks later (n = 11-13, from two independent experiments). **D**. Percentage of Ly5.2 peripheral blood leukocytes. **E**. Contribution of the indicated lineage to the total pool of Ly5.2$^+$ cells. Each column represents a different mouse. **F**. Percentage of cells in **E** that were myeloid (Gr1$^+$ or CD115$^+$). **G**. Quotient of the percentage donor contribution to the overall myeloid lineage (GM; Gr1$^+$ or CD115$^+$) over the sum of the percentage donor contributions to the overall B and T cell lineages (B+T) for individual mice. Myeloid-biased mice had a ratio >2, lineage balanced mice between 0.25 and 2, and lymphoid-biased mice <0.25. Statistical significance determined using a 2-way ANOVA (A) or an unpaired t-test (B-G). *P<0.05, **P < 0.01.