In Vivo Generation of Bone Marrow from Embryonic Stem Cells in Interspecies Chimeras

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ABSTRACT

Generation of bone marrow (BM) from embryonic stem cells (ESCs) promises to accelerate the
development of future cell therapies for life-threatening disorders. However, such approach is
limited by technical challenges to produce a mixture of functional BM progenitor cells able to
replace all hematopoietic cell lineages. Herein, we used blastocyst complementation to
simultaneously produce BM cell lineages from mouse ESCs in a rat. Based on FACS analysis
and single-cell RNA sequencing, mouse ESCs differentiated into multiple hematopoietic and
stromal cell types that were indistinguishable from normal mouse BM cells based on gene
expression signatures and cell surface markers. Receptor-ligand interactions identified Cxcl12-
Cxcr4, Lama2-Itga6, App-Itga6, Comp-Cd47, Col1a1-Cd44 and App-Il18rap as major signaling
pathways between hematopoietic progenitors and stromal cells. Multiple hematopoietic
progenitors, including hematopoietic stem cells (HSCs) in mouse-rat chimeras derived more
efficiently from mouse ESCs, whereas chondrocytes predominantly derived from rat cells. In the
dorsal aorta and fetal liver of mouse-rat chimeras, mouse HSCs emerged and expanded faster
compared to endogenous rat cells. Sequential BM transplantation of ESC-derived cells from
mouse-rat chimeras rescued lethally-irradiated syngeneic mice and demonstrated long-term
reconstitution potential of donor HSCs. Altogether, a fully functional bone marrow was
generated from mouse ESCs using rat embryos as “bioreactors”.

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INTRODUCTION

The bone marrow (BM) is a remarkably complex organ consisting of multiple mesenchymal, immune, endothelial, and neuronal cell types which together comprise a highly specialized microenvironment required to support life-long blood regeneration or hematopoiesis (Baccin et al., 2020; Baryawno et al., 2019; Rowe et al., 2016; Tikhonova et al., 2019; Vo and Daley, 2015). Hematopoiesis occurs in a stepwise manner and is initiated by a heterogeneous, multipotent, population of hematopoietic stem cells (HSCs), located at the apex of the hematopoietic differentiation tree. Long-term HSCs (LT-HSCs) remain quiescent to maintain their undifferentiated state within the bone marrow niche. When necessary, LT-HSCs can either undergo differentiation or self-renewal, to maintain the HSC pool. Conversely, short-term HSCs (ST-HSCs) are restricted in their self-renewal capacity and primed for differentiation into multipotent progenitors (MPPs), initiating the process of blood cell development. MPPs further differentiate into common myeloid progenitors (CMPs), lymphoid-primed multipotent progenitors (LMPPs) and common lymphoid progenitors (CLPs) that become increasingly lineage restricted with subsequent cell divisions, ultimately yielding all mature blood cell types (Haas et al., 2018).

The complexities of the hematopoietic system have been studied extensively in vitro, utilizing paired-daughter and colony forming unit (CFU) assays (Rowe et al., 2016; Vo and Daley, 2015). Fluorescence-activated cell sorting (FACS) has allowed for precise isolation and characterization of HSCs and progenitor populations based on cell surface markers. Classically, the most biologically relevant way to test HSC function remains to be through serial transplantation and hematopoietic reconstitution of irradiated recipient mice (Purton and Scadden, 2007; Rowe et al., 2016; Vo and Daley, 2015). Recent advances in single cell RNA sequencing (scRNAseq) have made it possible to further explore heterogeneity of the bone marrow niche (Baryawno et al., 2019; Tikhonova et al., 2019), and identify gene expression signatures of hematopoietic progenitor cells as they differentiate into mature blood cell types (Baccin et al., 2020; Nestorowa et al., 2016).
Generation of functional bone marrow from embryonic stem cells (ESCs) or induced pluripotent stem cells (iPSCs) promises to provide new therapeutic opportunities for hematologic and autoimmune disorders. However, this approach is limited by technical challenges to produce functional HSCs or the mixture of hematopoietic progenitors capable of replacing all mature blood cell types after cell transplantation. HSC-like cells have been generated from mouse and human ESCs and iPSCs using in vitro differentiation protocols (Amabile et al., 2013; Doulatov et al., 2013; Grigoriadis et al., 2010; Kitajima et al., 2011; Ledran et al., 2008; Sugimura et al., 2017; Vodyanik et al., 2006). Likewise, ESCs and iPSCs have been used to produce myeloid and lymphoid progenitor cells as well as differentiated hematopoietic cells, including neutrophils, monocytes, erythroid cells, T and B lymphocytes (Doulatov et al., 2013; Elcheva et al., 2014; Galic et al., 2006; Kennedy et al., 2012; Montel-Hagen et al., 2019; Nafria et al., 2020; Vodyanik et al., 2005). When transplanted into irradiated animals, ESC/iPSC-derived hematopoietic progenitor cells undergo differentiation and engraft into the bone marrow niche, providing an important source of renewal and regeneration for various blood cell lineages (Rowe et al., 2016; Sugimura et al., 2017; Vo and Daley, 2015). While ESC/iPSC-derived hematopoietic cells often express appropriate cell markers, gene expression and functional studies indicate significant differences between ESC/iPSC-derived cells and endogenous cells that have undergone normal morphogenesis in the bone marrow (Lin et al., 2019; Lu et al., 2016; Sugimura et al., 2017).

In vivo differentiation of ESCs into multiple cell lineages can be achieved using blastocyst complementation, in which donor ESCs are injected into blastocysts of recipient animals to create chimeras. Fluorescently labeled ESCs undergo differentiation in recipient embryos that serve as “biological reactors” by providing growth factors, hormones, and cellular niches to support ESC differentiation in the embryo. In mouse and rat apancreatic Pdx1-/- embryos, donor ESCs formed an entire pancreas in which both exocrine and endocrine cells were almost entirely derived from ESCs or iPSCs (Kobayashi et al., 2010; Yamaguchi et al.,
Mouse ESC/iPSC-derived β-cells from mouse-rat chimeras were fully differentiated and successfully rescued syngeneic diabetic mice (Yamaguchi et al., 2017). ESCs generated pancreatic cell lineages in apancreatic pigs (Matsunari et al., 2013), kidney in Sall1-deficient rats (Goto et al., 2019), endothelial cells in Flk1<sup>−/−</sup> mice (Hamanaka et al., 2018), lymphocytes in immunodeficient mice (Muthusamy et al., 2011) and neuronal progenitors in mice with forebrain-specific overexpression of diphtheria toxin (Chang et al., 2018). Recently, mouse ESCs were used to generate lung and thyroid tissues in embryos deficient for Fgf10, Nkx2-1, Fgfr2 or β-catenin (Kitahara et al., 2020; Mori et al., 2019; Wen et al., 2021). Using blastocyst complementation, mouse ESCs effectively produced hematopoietic cells in mice deficient for Kit or Flk1 (Hamanaka et al., 2018; Jansson and Larsson, 2010). ESC-derived endothelial progenitor cells from mouse-rat chimeras were indistinguishable from endogenous endothelial progenitor cells based on gene expression signatures and functional properties (Wang et al., 2021), indicating that ESC/iPSC-derived progenitors can be used for tissue regeneration (Bolte et al., 2020a; Bolte et al., 2018; Dharmadhikari et al., 2015; Kolesnichenko et al., 2021). While all these studies support the effectiveness of blastocyst complementation for differentiation of multiple cell types from ESCs/iPSCs <em>in vivo</em>, generation of functional bone marrow from ESCs in interspecies chimeras has not yet been achieved.

Herein, we used blastocyst complementation to produce mouse bone marrow in a rat. ESC-derived cells from multiple hematopoietic and stromal cell lineages were indistinguishable from normal mouse BM cells based on gene expression signatures and cell surface markers. Transplantation of ESC-derived BM cells into lethally irradiated syngeneic mice prevented mortality and resulted in a long-term contribution to BM and mature blood cell types. Our data demonstrate that interspecies chimeras can be used as “bioreactors” for <em>in vivo</em> differentiation and functional studies of ESC-derived BM hematopoietic and stromal cells.
RESULTS

Generation of bone marrow from pluripotent embryonic stem cells in interspecies mouse-rat chimeras. To determine whether mouse ESCs can differentiate into multiple hematopoietic cell lineages in the bone marrow of a rat, blastocyst complementation was performed by injecting GFP-labeled mouse C57BL/6 ESCs (ESC-GFP) into rat SD blastocysts to create interspecies mouse-rat chimeras. Chimeric embryos were transferred into surrogate female rats for subsequent development in utero (Figure 1A). While mouse-rat chimeras were viable, they were smaller than age-matched rats (Figure 1B). Consistent with the presence of mouse ESC-derived cells (black) in the skin tissue (Wang et al., 2021), mixed black and white pigmentation distinguished the mouse-rat chimeras from juvenile rats (Figure 1B). The average body weight of mouse-rat chimeras was smaller than rats, but larger than mice of similar age (Figure 1C). ESC-derived cells were abundant in femur and tibia bones of the chimeras as evidenced by GFP fluorescence (Figure 1D). FACS analysis of BM cells obtained from juvenile mouse-rat chimeras revealed that the percentage of ESC-derived cells was 15-50% (Figure 1E-F). Thus, ESCs contribute to the bone marrow of mouse-rat chimeras.

To identify ESC-derived hematopoietic stem cells (HSCs), we used GFP fluorescence and mouse-specific antibodies recognizing multiple cell surface antigens (Figure 1E and Figure 1 – figure supplement 1A-B). First, ESC-derived GFP+ BM cells were subdivided into lineage-positive (Lin+) and lineage-negative (Lin-) subpopulations (Figure 1E and Figure 1 – figure supplement 1A-B). The percentage of ESC-derived Lin- cells in the bone marrow of mouse-rat chimeras was similar to the percentage of Lin- cells in the bone marrow of age-matched C57BL/6 mice (Figure 1E and 1G). Next, we used Sca1 and CD117 (c-KIT) antibodies to identify Lin-Sca1+c-KIT+ cells (LSKs) (Figure 1E). The percentage of LSKs was higher in the bone marrow of mouse-rat chimeras compared to the control (Figure 1G). Based on cell surface expression of CD150 and CD48, the percentage of LT-HSCs among LSKs were also higher in mouse-rat chimeras (Figure 1E and 1H). While changes in ST-HSCs were not significant
(Figure 1H), total numbers of HSCs (LT-HSCs + ST-HSCs) were higher in mouse-rat chimeras compared to mice of the same age (Figure 1I). Thus, mouse ESCs can differentiate into hematopoietic progenitor cells in the bone marrow of mouse-rat chimeras.

**Single cell RNA sequencing identifies multiple subpopulations of ESC-derived hematopoietic cells in the bone marrow of mouse-rat chimeras.** To identify ESC-derived cells in the bone marrow, single cell RNAseq (the 10X Chromium platform) of FACS-sorted GFP⁺ BM cells was performed. Mouse ESC-derived cells from P10 mouse-rat chimeras were compared to ESC-derived cells from P10 mouse-mouse (control) chimeras, the latter of which were produced by complementing mouse blastocysts with mouse ESCs from the same ESC-GFP cell line. Based on GFP fluorescence, contribution of ESCs to BM cells in both chimeras was similar (Figure 2 – figure supplement 1A-B). Since the numbers of HSCs and other hematopoietic progenitor cells in the bone marrow are low compared to numbers of differentiated hematopoietic cells, we enriched for BM progenitor cell populations prior to single cell RNA sequencing by combining 90% of FACS-sorted GFP⁺Lin⁻ cells and 10% of GFP⁺Lin⁺ cells in each experimental group. BM cells from 3 animals per group were combined prior to FACS sorting. Based on published gene expression signatures of mouse BM cells (Baccin et al., 2020), 11,326 cells from 14 major cell subtypes were identified: 5308 cells from control mouse-mouse chimeras and 6018 cells from mouse-rat chimeras. These include lymphoid, erythroid, myeloid and neutrophil progenitors, Pro-B, Pre-B, B and T lymphocytes, megakaryocytes, dendritic cells, neutrophils, basophils/eosinophils, monocytes and lymphoid-primed multipotent progenitor cells (LMPPs) (Figure 2A and Figure 2 – figure supplement 2A). Analysis of BM cells from mouse-rat and mouse-mouse chimeras demonstrated similar distributions of hematopoietic cell lineages derived from common myeloid progenitor (CMP) and common lymphoid progenitor (CLP) (Figure 2A), indicating identical cell types in mouse-rat and control chimeras. For selected genes, we used violin plots to confirm cell specificity and
expression levels of *Ptprc* (Cd45), *Pclaf*, *Vpreb1*, *Tmpo*, *Ebf1*, *Ms4a4b*, *Vamp5*, *Elof1*, *Elane*, *Ms4a2*, *Siglech*, *Ngp*, *Clec4d*, *Ctss* and *Ftl1-ps1* in the combined dataset (Figure 2 – figure supplement 3). Markers of endothelial cells, adipocytes, osteocytes and neuronal cells were undetectable in BM cell suspensions from both chimeras (Figure 2 – figure supplement 2B). Percentages CLP-derived lymphoid progenitors, Pro-B, Pre-B, and B cells were lower in mouse-rat chimeras compared to the control (Figure 2A-B). In contrast, percentages of CMP-derived erythroid, myeloid and neutrophil progenitors, dendritic cells, and basophils/eosinophils were higher (Figure 2B). Monocytes and neutrophils were similar, whereas megakaryocytes were decreased in the bone marrow of mouse-rat chimeras (Figure 2B). The percentage of lymphoid-primed multipotent progenitors (LMPPs) in mouse-rat chimeras was increased compared to the control (Figure 2A-B). Hematopoietic stem cells, identified by co-expression of *Kit*, *Ly6a(Sca1)* and *Flt3* mRNAs (Rowe et al., 2016; Vo and Daley, 2015), clustered together with myeloid and erythroid progenitors (Figure 2 – figure supplement 4A-B). The number of ESC-derived HSCs was higher in BM of mouse-rat chimeras compared to the control (Figure 2 – figure supplement 4C), findings consistent with FACS analysis (Figure 1H-I). Only 6 out of 6018 bone marrow cells (0.1%) in mouse-rat chimeras contained both mouse and rat mRNA transcripts (Supplementary files 1 and 2), indicating that the fusion of mouse and rat BM cells is rare. Thus, although the cellular composition of ESC-derived hematopoietic BM cells was similar in mouse-rat and mouse-mouse chimeras, mouse-rat bone marrow was enriched in HSCs, LMPPs and CMP-derived erythroid, myeloid and neutrophil progenitors.

**Single cell RNA sequencing identifies close similarities in gene expression signatures between ESC-derived hematopoietic cells in mouse-rat and mouse-mouse chimeras.** Comparison of gene expression signatures between mouse-rat and mouse-mouse chimeras revealed significant similarities among ESC-derived hematopoietic cell types. Lymphoid progenitors and pro-B cells isolated from mouse-rat and control chimeras expressed *Mif*, *Rcsd1*...
and Tspan13, whereas pre-B cells expressed Hmgb2 and Pgls (Figure 2 – figure supplement 5A). Cd79a and CD79b transcripts were detected in B cells of mouse-rat and control chimeras, whereas Cd3g and Lck were restricted to T cells (Figure 2 – figure supplement 5A). Based on the correlation analysis, gene expression profiles of all lymphoid cell types were similar between mouse-rat and control chimeras (Figure 2 – figure supplement 5B). Likewise, gene expression signatures of myeloid, erythroid and neutrophil progenitors and their derivatives in the bone marrow were similar in both experimental groups (Figure 2 – figure supplement 6A-B). Furthermore, single cell RNAseq identified close similarities in gene expression signatures of ESC-derived HSCs and LMPPs in both chimeras (Figure 2 – figure supplement 7A-B). Thus, gene expression signatures of ESC-derived hematopoietic cells were similar in mouse-rat and control mouse-mouse chimeras.

**Chimeric bone marrow is enriched in mouse hematopoietic progenitor cells and rat chondrocytes.** To examine the composition and origin of stromal cells in mouse-rat chimeras, we used an enzymatic digestion to obtain both hematopoietic and stromal cells from BM of P5 mouse-rat chimeras and compared them to BM cells of mice and rats of the same age. Flow sorting for GFP was performed to separate donor mouse cells (GFP+) and recipient rat cells (GFP−) in the chimeric BM. BM from control P5 mice and rats were also FACS-sorted for GFP− BM cells to ensure similar conditions of cell preparations prior to single cell RNAseq. Based on published gene expression signatures (Baccin et al., 2020), 6,375 mouse and 5,495 rat cells were identified in the chimeras, which were compared to 6,418 cells from control mice and 7,016 cells from control rats. Similar hematopoietic and stromal cell clusters were present in BM of mice, rats, and mouse-rat chimeras (Figure 3A-C). These included stromal cell clusters (endothelial cells, fibroblasts, myofibroblasts and chondrocytes) and hematopoietic cell clusters with various progenitor and differentiated hematopoietic cell types. Since we did not enrich BM cell populations for Lin− cells, some rare BM cell subsets, such as HSCs, LMPPs and dendritic
cells, were not detected as separate cell clusters. Compared to normal BM from P5 mice, chimeric BM was enriched in mouse ESC-derived hematopoietic progenitor cells, such as myeloid, granulocyte and erythroid progenitors, whereas mouse-derived B cell lineages were reduced (Figure 3A), findings consistent with single cell RNAseq comparison of P10 bone marrow from mouse-rat and mouse-mouse chimeras (Figure 2). The percentage of mouse endothelial cells was increased in mouse-rat BM, whereas the percentages of mouse chondrocytes and fibroblasts were reduced compared to mouse control (Figure 3A). In contrast, mouse-rat BM was enriched in rat-derived chondrocytes and fibroblasts, but the percentages of endothelial and most hematopoietic cells were reduced compared to age-matched rats (Figure 3B). Thus, mouse cells preferentially contributed to hematopoietic progenitors and endothelial cells, whereas rat cells contributed to the majority of chondrocytes and fibroblasts.

Direct comparison of mouse and rat cells within chimeric BM demonstrated significant similarities between gene expression signatures of hematopoietic and stromal cell lineages (Figure 3 – figure supplement 1A-D). To examine cell signaling between hematopoietic progenitors and stromal cells in BM of mouse-rat chimeras, we generated the map of potential ligand-receptor interactions using P5 single cell RNAseq datasets. There were remarkable similarities in major receptor-ligand interactions between stromal and erythro-myeloid progenitor cells (EMPs) (Figure 4). Regardless of mouse and rat origins of BM cells, endothelial cells interacted with EMPs through the Cxcl12-Cxcr4 receptor-ligand signaling pair. The main signaling circuit between fibroblasts and EMPs was Lama2-Itga6, whereas chondrocytes signaled to EMPs through App-Itga6 and Comp-Cd47 pathways (Figure 4). Major receptor-ligand interactions between granulocyte-monocyte progenitor cells (GMPs) and stromal cells were also similar in BM cells of mouse and rat origin (Figure 4 – figure supplement 1). These include Cxcl12-Cxcr4 signaling between endothelial cells and GMPs, Col1a1-Cd44 signaling between fibroblasts and GMPs, and App-Il18rap signaling between chondrocytes and GMPs (Figure 4 – figure supplement 1). Analysis of expression patterns for several ligands and their
receptors revealed no obvious differences between mouse and rat cells (Figure 4 – figure supplement 2). These results demonstrate that mouse and rat BM cells use similar signaling pathways between stromal and hematopoietic progenitor cells.

**Mouse HSCs in mouse-rat chimeras develop earlier than rat HSCs.** Fetal HSCs emerge from hemogenic endothelium in the aorta-gonad-mesonephros region (AGM) and later undergo expansion in the embryonic liver (Gao et al., 2018; Weijts et al., 2021). To examine the development of HSCs in mouse-rat chimeras, mouse-derived (GFP⁺) and rat-derived (GFP⁻) hemogenic endothelial cells were visualized in the dorsal aorta by colocalization of FLK1 with RUNX1 transcription factor (Figure 5A-B). At E11, mouse embryos were significantly larger than rat and mouse-rat chimeric embryos (Figure 5 – figure supplement 1), consistent with previous studies demonstrating that the main stages of mouse embryonic development occur approximately 1.5 days faster compared to embryonic development in the rat (Farrington-Rock et al., 2008; Marcela et al., 2012; Takahashi and Osumi, 2005; Torres et al., 2008). Therefore, we compared E11 mouse embryos with E12.5 rat and chimeric embryos which were in similar developmental stages. In the dorsal aorta of mouse-rat chimeras, the majority of FLK1⁺RUNX1⁺ cells expressed GFP, indicating the mouse origin of these cells (Figure 5B). Later in development, percentages of mouse Lin⁻ cells, LSKs and ST-HSCs were higher in fetal livers of mouse-rat chimeras as demonstrated by FACS analysis for Lin, CD117, Sca1, CD48 and CD150 (Figure 5C and Figure 5 – figure supplement 2). The percentage of LT-HSC in fetal livers was unchanged (Figure 5C). Thus, ESC complementation causes the earlier development of donor HSCs in the dorsal aorta and increases percentages of donor-derived Lin⁻ cells, LSKs and ST-HSCs in the fetal liver.

**Transplantation of ESC-derived bone marrow cells from interspecies mouse-rat chimeras rescues lethally irradiated syngeneic mice.** To test functional properties of mouse BM
hematopoietic progenitor cells derived through a rat, cells were FACS-sorted for GFP from the bone marrow of juvenile mouse-rat chimeras and transferred into the tail vein of syngeneic C57BL/6 adult mice that received the lethal dose of whole-body gamma-irradiation three hours prior to the bone marrow transplant (Figure 6A). Consistent with published studies (Rowe et al., 2016; Sugimura et al., 2017; Vo and Daley, 2015), all mice without bone marrow transplant died between 9 and 12 days after irradiation (Figure 6B). In contrast, all 20 mice transplanted with GFP+ BM cells from mouse-rat chimeras survived after lethal irradiation (Figure 6B-C). Histological assessment of femur bones confirmed the presence of GFP+ donor cells in the BM compartment of transplanted mice (Figure 6D). Blood analysis of mice harvested 8 days after irradiation showed significant decreases in white blood cells (WBC), red blood cells (RBC), platelets (PLT), hemoglobin (Hb) as well as numbers of granulocytes, monocytes and lymphocytes (Figure 7A and Figure 7 – figure supplements 1 and 2). Transplantation of ESC-derived BM cells from mouse-rat chimeras increased WBC and the numbers of granulocytes, monocytes and lymphocytes in the peripheral blood at day 8 (Figure 7A and Figure 7 – figure supplements 1 and 2). Contribution of ESC-derived BM cells to granulocytes, monocytes and B cells was higher compared to erythroid and T cells (Figure 7B and Figure 7 – figure supplement 3). At 5 months after BM transplantation, ESC-derived cells completely restored blood cell numbers, PLT and Hb in lethally irradiated mice (Figure 7C and Figure 7 – figure supplements 1 and 2). Long-term contributions of ESC-derived BM cells to all hematopoietic cell lineages in the peripheral blood were between 49% and 96% (Figure 7C and Figure 7 – figure supplement 3). Thus, transplantation of ESC-derived bone marrow cells from mouse-rat chimeras prevented mortality and restored hematopoietic blood lineages in lethally irradiated syngeneic mice.

Transplantation of ESC-derived bone marrow cells from interspecies mouse-rat chimeras resulted in the long-term contribution of donor cells to hematopoietic progenitor cells. Based on FACS analysis of irradiated mice at day 8, whole-body irradiation decreased the
number of hematopoietic progenitor cells in the bone marrow, including LSKs, ST-HSCs and LT-HSCs (Figure 7D and Figure 7 – figure supplement 4A-B). Transplantation of ESC-derived BM cells significantly increased LSKs but did not affect the numbers of ST-HSCs and LT-HSCs in irradiated mice (Figure 7D). Contribution of ESC-derived BM cells to Lin⁻ and LSK cell subsets was high, whereas ESC contribution to ST-HSCs and LT-HSCs at day 8 was low (Figure 7E and Figure 7 – figure supplement 5). At 5 months after BM transplantation, percentages of LSKs, ST-HSCs and LT-HSCs in the bone marrow were increased (Figure 7D and Figure 7 – figure supplement 4B). Long-term contribution of ESC-derived BM cells to LSKs, ST-HSCs and LT-HSCs was between 92% and 95% (Figure 7F and Figure 7 – figure supplement 5). Finally, we performed BM transplantation again in secondary recipients to establish the functional potential and self-renewal capacity of the chimeric HSCs (Figure 7 – figure supplement 6A). The secondary BM transplantation rescued lethally irradiated mice and resulted in long-term engraftment of ESC-derived HSCs into hematopoietic cell lineages in the bone marrow and peripheral blood (Figure 7 – figure supplement 6B-E). Altogether, transplantation of ESC-derived bone marrow cells from mouse-rat chimeras resulted in efficient, long-term contribution of donor cells to the bone marrow and blood of lethally irradiated mice.
DISCUSSION

Recent single cell RNA sequencing studies identified remarkable diversity of hematopoietic cell types in the bone marrow (Baccin et al., 2020). Generation of functional bone marrow cells from pluripotent ESCs or iPSCs in a dish or in organoids represents a formidable challenge (Rowe et al., 2016; Vo and Daley, 2015). In the present study, we used blastocyst complementation to generate a diversity of hematopoietic cell types from mouse ESCs in rat embryos. Interspecies mouse-rat chimeras were viable and contained approximately 25% of ESC-derived mouse cells in the bone marrow. It is possible that inactivation of genes critical for hematopoiesis in rat embryos prior to blastocyst complementation can improve the integration of mouse ESCs into the bone marrow of mouse-rat chimeras. This approach was supported by recent studies with mouse-mouse chimeras, in which ESCs contributed to more than 90% of hematopoietic cells in mice deficient for either Kit or Flk1 (Hamanaka et al., 2018; Jansson and Larsson, 2010). While ESCs contributed to all hematopoietic cell lineages in interspecies bone marrow, the percentage of lymphoid progenitors was lower, whereas the percentages of myeloid progenitor cells and HSCs were higher in mouse-rat chimeras compared to control mouse-mouse chimeras. Since both chimeras were produced by complementing blastocysts with mouse ESCs from the same ESC-GFP cell line, it is unlikely that these changes are dependent on donor ESCs. It is possible that the observed differences in BM cellular composition between mouse-rat and mouse-mouse chimeras are due to interactions of donor ESCs with the host embryo. Structural and functional differences between hormones, growth factors and their receptors in rats and mice can contribute to the efficiency or timing of differentiation of mouse ESCs into hematopoietic cell lineages in BM of chimeras.

Our data demonstrate that chimeric HSCs develop more efficiently from donor mouse cells in the dorsal aorta, fetal liver, and bone marrow, whereas rat cells are less efficient to differentiate into HSCs. Since we observed high numbers of mouse hemogenic endothelial cells in the chimeric dorsal aorta, it is likely that donor hemogenic endothelium undergoes direct
transition to functional HSCs in the fetal liver, whereas endogenous (non-GFP+) hemogenic
endothelium can be a source of rat HSCs. Since mouse embryos develop faster compared to
rat embryos by approximately 1.5 days (Farrington-Rock et al., 2008; Marcela et al., 2012;
Takahashi and Osumi, 2005; Torres et al., 2008), it is possible that mouse ESC-derived
progenitor cells migrate faster into developing hematopoietic niches in the mouse-rat chimeras,
leading to preferential development of HSCs from cells of mouse origin and contributing to
increased numbers of mouse-derived hematopoietic progenitors in the bone marrow of mouse-
rat chimeras. These data suggest that using donor ESCs from species with less gestational time
in interspecies “bioreactors” can lead to larger quantities of ESC-derived hematopoietic
progenitors in the chimeric bone marrow. Our single cell RNAseq analysis enabled us to identify
potential signaling pathways and receptor-ligand interactions between hematopoietic
progenitors and stromal cells in the bone marrow. These pathways include \textit{Cxcl12-Cxcr4}
signaling between hematopoietic progenitors and endothelial cells, which plays a critical role in
maintenance of HSCs during BM homeostasis and promotes niche regeneration and
hematopoietic reconstitution after BM transplantation (Baccin et al., 2020; Singh et al., 2020;
Sugiyama et al., 2006). Other pathways identified in our studies, including \textit{Lama2-Iltga6}, \textit{App-
Iltga6}, \textit{Comp-Cd47}, \textit{Col1a1-Cd44} and \textit{App-Ill18rap}, have not been extensively studied in the BM
microenvironment but are implicated in regulation of cell adhesion, migration, oncogenesis,
fibrosis, and inflammatory responses (Kiratipaiboon et al., 2020; Mk et al., 2019; Rock et al.,
2010; Strelnikov et al., 2021; Yang et al., 2017). Notably, our data suggest that some of these
signaling pathways can be targeted to modulate the development and expansion of donor ESC-
derived hematopoietic progenitor cells in the bone marrow of interspecies chimeras.

Despite mosaicism in interspecies bone marrow, mouse ESC-derived cells from multiple
hematopoietic cell lineages were highly differentiated and indistinguishable from the normal
mouse bone marrow cells based on gene expression signatures and cell surface proteins.
Consistent with functional competency of ESC-derived bone marrow, transplantation of BM cells
into lethally irradiated syngeneic mice prevented mortality and resulted in long-term contribution of ESC-derived cells to all hematopoietic cell lineages in the bone marrow and peripheral blood. One of the limitations of our studies is that the functional potential of chimeric HSCs was established from whole BM transplants and not from transplantation of purified HSCs. While these experiments are technically challenging, transplantation of FACS-sorted donor HSCs into lethally irradiated mice will be needed in our future studies to investigate whether chimeric HSCs are fully functional to restore all hematopoietic cell lineages after irradiation. Our results are consistent with recent studies demonstrating the ability of mouse ESCs to generate functional pancreatic, endothelial and kidney cells in interspecies mouse-rat chimeras (Goto et al., 2019; Wang et al., 2021; Yamaguchi et al., 2017). Interestingly, long-term contribution of donor BM cells to ST-HSCs and LT-HSCs of irradiated mice was high, supporting the ability of donor HSCs to self-renew. In contrast, the short-term contribution of donor BM cells to ST-HSCs and LT-HSCs of irradiated mice was low. Low contribution of donor BM to HSCs at day 8 is not surprising considering an acute hematopoietic deficiency in lethally irradiated mice. It is possible that most donor-derived HSCs undergo rapid differentiation into other hematopoietic cell types to compensate for the loss of injured hematopoietic cells after irradiation.

Generation of intraspecies chimeras through blastocyst complementation creates an interesting opportunity to use patient-derived iPSCs to produce tissues or even organs in large animals, for example, pigs or sheep, which can serve as “biological reactors”. However, at this stage of technological advances it is impossible to restrict the integration of ESC/iPSC-derived cells into selected organs or cell types. Off-target integration of ESCs and iPSCs into the brain, testes and sensory organs raises important ethical concerns for the use of human-animal chimeras in regenerative medicine (Masaki and Nakauchi, 2017; Wu et al., 2016). To improve the selectivity of ESC/iPSC integration into chimeric tissues, various genetic modifications can be introduced into the host embryos to advance the technology. Harvest of tissues from chimeric embryos instead of adult chimeras can alleviate some of the ethical concerns,
suggesting a possibility of using chimeric embryos as a potential source of patient-specific hematopoietic progenitor cells.

In summary, blastocyst complementation of rat embryos with mouse ESCs was used to simultaneously generate multiple hematopoietic and stromal cell lineages in the bone marrow. ESC-derived cells in mouse-rat chimeras were indistinguishable from normal mouse BM cells based on gene expression signatures and cell surface markers. Transplantation of ESC-derived BM cells rescued lethally irradiated syngeneic mice and resulted in long-term contribution of donor cells to hematopoietic cell lineages. Thus, the interspecies chimeras could be considered for in vivo differentiation of patient-derived iPSCs into hematopoietic cell lineages for future cell therapies.
METHODS

Mice, rats and generation of mouse-rat and mouse-mouse chimeras through blastocyst complementation. C57BL/6 mice were purchased from Jackson Lab. Interspecies mouse-rat chimeras were generated using blastocyst complementation as described (Li et al., 2021; Wang et al., 2021). Briefly, blastocysts from SD rats were obtained at embryonic day 4.5 (E4.5), injected with fifteen GFP-labeled mouse ESC cells (ESC-GFP, C57BL/6 background) (Sun et al., 2021; Wen et al., 2021) and transferred into pseudo pregnant SD rat females. Mouse-mouse chimeras were generated by complementing CD1 blastocysts with fifteen mouse ESC-GFP cells. For FACS analysis and bone marrow transplantation, BM cells were collected from chimeric pups that were harvested between postnatal day 4 (P4) and P10. For single cell RNA sequencing, BM cells were prepared from P10 and P5 mice, rats and chimeras. To perform BM transplantation, BM cells from 2 tibias and 2 fibulas of mouse-rat chimeras were collected and FACS-sorted for ESC-derived (GFP+) cells. 500,000 FACS-sorted GFP+ BM cells were intravenously (i.v.) injected into lethally irradiated C57BL/6 male mice (6-8 weeks of age) via the tail vein. Three hours before BM transplantation, whole-body irradiation was performed using 11.75 Gy. Mice were harvested after 8 days or 5 months after BM transplantation. For the second BM transplantation, GFP+ BM cells were FACS-sorted from irradiated mice 5 months after the first BM transplantation and then i.v. injected into new irradiated recipients. Tissue dissection, processing and preparation of single cell suspensions were carried out as described (Bolte et al., 2011; Kalin et al., 2008; Kalinichenko et al., 2003; Kim et al., 2005; Wang et al., 2003). Blood analysis was performed in animal facility of Cincinnati Children’s Hospital Research Foundation.

Single Cell RNAseq analysis of ESC-derived bone marrow cells. Prior to scRNAseq (10X Chromium platform), BM cells were pooled from three P10 mouse-rat chimeras and three P10 mouse-mouse (control) chimeras and then FACS-sorted for GFP and the lineage (Lin) marker.
Since the numbers of HSCs and other hematopoietic progenitors in BM are significantly low compared to numbers of differentiated hematopoietic cells, the cell mixtures were enriched for BM progenitor cell populations by combining 90% of FACS-sorted GFP\(^{+}\)Lin\(^{-}\) cells and 10% of GFP\(^{+}\)Lin\(^{+}\) cells in each experimental group. This enrichment enabled us to obtain enough progenitor cells for UMAP clustering analysis. In separate scRNAseq experiments, all BM cells (including hematopoietic, vascular and stromal cells) were prepared from P5 mice, rats and mouse-rat chimeras using enzymatic digestion and cell purification as described (Baccin et al., 2020). BM cells from five animals were pooled together prior to single cell RNAseq. All raw data and the processed count matrix of BM datasets were uploaded to the GEO database (accession number GSE184940). Read alignments, quality controls and false discovery rates were described previously (Guo et al., 2019; Ren et al., 2019; Wang et al., 2022). Identification of cell clusters and quantification of cluster-specific gene expression in BM scRNAseq datasets was performed as described (Baccin et al., 2020; Wang et al., 2021; Wen et al., 2021). To assess the transcriptomic similarity of ESC-derived and endogenous BM cells, the scRNAseq datasets were normalized with SCTransform and then integrated utilizing the canonical correlation analysis (CCA). In the integrated scRNAseq datasets, the SelectIntegrationFeatures in Seurat package (version 4.0.0 in R 4.0 statistical environment) was used to identify anchors for integration. The RunPCA function was used for Principal component analysis (PCA) of scRNAseq datasets, and the PCElbowPlot function was used to calculate the standard deviations of the principal components (PCs). PCs with standard deviation > 3.5 were chosen as input parameters for non-linear UMAP clustering analysis. Next, the FindNeighbors function was used to compute the k.param nearest neighbors, and BM cell clusters were identified by a shared nearest neighbor (SNN) modularity optimization clustering algorithm implemented in the FindClusters function with resolution set at 0.4 (Guo et al., 2019; Wang et al., 2021; Wen et al., 2021).
Analysis of potential receptor-ligand interactions using single cell RNAseq data sets. The R package *NicheNet* was used to analyze the information about expression of cognate ligands and receptors to identify intercellular communication patterns between hematopoietic progenitors and stromal cells as described (Browaeys et al., 2020). Erythro-myeloid (EMP) and granulocyte-monocyte progenitor cells (GMP) were chosen as potential sources of receptors, whereas BM stromal cell types were chosen as potential sources of ligands. The background expression of genes was specified with default approach used in the *NicheNet* pipeline, and expressed genes were identified based on >10% detection in specific clusters. To identify ligand-receptor interactions between EMPs/ GMPs and stromal cells, we selected the top 20 ligands predicted to drive hematopoietic cell differentiation based on the Pearson correlation coefficient between the ligand-receptor regulatory potential score of each ligand and the target indicator vector. Using the *NicheNet* pipeline, the Circos plots were generated to show common ligand-receptor interactions between EMPs/ GMPs and stromal cells in the bone marrow.

**FACS Analysis.** FACS analysis was performed using cells obtained from the bone marrow and blood. Antibodies for FACS analysis are listed in Supplementary file 3. Immunostaining of cell suspensions were performed as described (Bolte et al., 2017; Xia et al., 2015). Identification of hematopoietic cell types based on multiple cell surface markers is described in (Bolte et al., 2020b; Pradhan et al., 2019; Ren et al., 2013; Ren et al., 2010; Sun et al., 2017). To identify ESC-derived HSCs, we used GFP fluorescence and mouse-specific antibodies recognizing multiple cell surface antigens. First, ESC-derived GFP* BM cells were subdivided into Lin* and Lin− cell subsets. Second, we used Sca1 and CD117 (c-KIT) antibodies to identify Lin−Sca1*c-KIT* cells (LSKs). Third, CD150 and CD48 antibodies were used to identify ST-HSCs and LT-HSCs among LSKs. Stained cells were analyzed using a five-laser FACSArria II (BD Biosciences) (Cai et al., 2016; Sun et al., 2021).
Histology and immunostaining. Frozen or paraffin-embedded sections of tissue samples were stained with hematoxylin and eosin (H&E) for histological evaluation (Kalinichenko et al., 2002) or to visualize GFP (Ustiyan et al., 2018; Ustiyan et al., 2016). Frozen sections from embryos were used for immunofluorescent staining as described (Black et al., 2018; Ustiyan et al., 2012; Wang et al., 2010). Primary antibodies for immunostaining are listed in Supplementary file 3. Secondary antibodies were conjugated with Alexa Fluor 488, Alexa Fluor 594 or Alexa Fluor 647 (Invitrogen and Jackson ImmunoResearch Laboratory) to visualize specific staining as described (Bolte et al., 2012; Hoggatt et al., 2013; Milewski et al., 2017a). DAPI (Vector Laboratory) was used to counterstain cell nuclei (Milewski et al., 2017b). Histological and immunofluorescent images were obtained using a Zeiss Axioplan2 microscope (Carl Zeiss Microimaging) as described (Bolte et al., 2015; Kalin et al., 2008; Pradhan et al., 2016).

Statistical Analysis. Statistical significance was determined using non-parametric Mann-Whitney U test, one-way ANOVA, and Student's t-test. Multiple means were compared using one-way analysis of variance with the post-hoc Tukey test. \( p \leq 0.05 \) was considered statistically significant. Data were presented as mean ± standard error of mean (SEM).
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ADDITIONAL INFORMATION

Competing interests

Authors of this manuscript have no conflicts of interest.

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Ethics and data sharing statement

Bone marrow single cell RNA-seq datasets were uploaded to the Gene Expression Omnibus (GEO) database (accession number GSE184940) and made available to other investigators for purposes of replicating the procedures or reproducing the results. All animal studies were reviewed and approved by the Institutional Animal Care and Use Committee of the Cincinnati Children’s Research Foundation.
ADDITIONAL FILES

Supplementary files

**Supplementary file 1.** The number and percentage of hematopoietic BM cells containing both mouse and rat mRNAs (hybrid cells).

**Supplementary file 2.** The number of counts and features (genes) in 6 hybrid cells identified in mouse-rat chimera.

**Supplementary file 3.** Antibodies used for Flow Cytometry (FC) and Immunofluorescence staining (IF).

**Transparent reporting form**

**Data availability**

Raw data and the processed count matrix of single cell RNAseq datasets have been deposited in GEO database under accession code GSE184940.
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FIGURE LEGENDS

Figure 1. Mouse ESCs contribute to hematopoietic stem cells in the bone marrow of mouse-rat chimeras. A, Schematic shows blastocyst complementation of rat embryos with mouse ESCs to generate interspecies mouse-rat chimeras. GFP-labeled mouse ESCs (mESCs) were injected into rat blastocysts, which were implanted into surrogate rat females to undergo embryonic development in utero. Femur and tibia bones of the chimeras were used to obtain bone marrow (BM) cells. B, Photographs of mouse-rat chimeras are taken at postnatal (P) days P3, P8, P13 and P28. Mixed black and white pigmentation distinguishes the mouse-rat chimeras from juvenile rats and mice. C, Weights of mouse-rat chimeras are shown at different time points and compared to rats and mice of similar ages. Chimeras are significantly smaller than rats, but larger than mice (n=7-18 in each group), p<0.01 is **, see also Figure 1 – source data 1. D, Fluorescence microscopy shows GFP and bright field images of femur and tibia bones from P4 rat, mouse and mouse-rat chimera. E, FACS analysis of mouse ESC-derived (GFP-positive) cells in the bone marrow of P10 mouse-rat chimeras. Lineage-negative (Lin⁻), LSK, ST-HSC and LT-HSC cell subsets were identified in the bone marrow of mouse-rat chimeras (n=10) and control mice (n=8), see also Figure 1 – figure supplement 1A-B. F, Histograms show GFP fluorescence of BM cells from chimeras and control mice. G-H, FACS analysis shows increased percentages of mouse LSKs and LT-HSCs in BM of mouse-rat chimeras (n=10) compared to control mice (n=8), p<0.01 is **, N.S. indicates no significance. I, FACS analysis shows increased numbers of HSCs (ST-HSCs + LT-HSCs) in BM of mouse rat chimeras (n=10) compared to control mice (n=8), p<0.01 is **.

The online version of this article includes the following figure supplement(s) for figure 1:

Source data 1. Excel spreadsheet containing quantitative data for Figure 1.

Figure supplement 1. Identification of lineage⁻ cells, LSKs, ST-HSCs and LT-HSCs in the bone marrow.
Figure 2. Single cell RNAseq analysis identifies ESC-derived hematopoietic cell lineages in the bone marrow of mouse-rat chimeras. A, Parallel dimension UMAP plots show identical hematopoietic cell clusters in the bone marrow of mouse-mouse chimera (5308 cells) and mouse-rat chimera (6018 cells). ESC-derived BM cells were obtained from the bone marrow of P10 chimeras using FACS sorting for GFP+ cells, see Figure 2 – figure supplement 1A-B. Cells from n=3 animals per group were pooled together prior to FACS sorting. Cell clusters were identified from single cell RNAseq datasets using Uniform Manifold Approximation and Projection (UMAP) method, see also Figure 2 – figure supplements 2A-B and 3. HSCs were identified by co-expression of Kit, Ly6a (Sca1) and Flt3 (Flk2), see Figure 2 – figure supplement 4. Heatmaps and linear regression analysis identified significant similarities in gene expression signatures of lymphoid and myeloid progenitor cells obtained from mouse-rat (R) and mouse-mouse chimeras (M), see Figure 2 – figure supplements 5A-B and 6A-B. Gene expression profiles of ESC-derived hematopoietic stem cells and lymphoid-primed multipotent progenitor cells is shown in Figure 2 – figure supplements 7A-B. B, Table shows percentages of cells in individual clusters in mouse-mouse and mouse-rat chimeras. Blue color indicates decreased percentages of cells in mouse-rat chimeras compared to mouse-mouse chimeras. Red color indicates increased percentages of cells in mouse-rat chimeras.

The online version of this article includes the following figure supplement(s) for figure 2:

Figure supplement 1. Purification of mouse ESC-derived cells from bone marrow of mouse-rat and mouse-mouse chimeras before scRNAseq.

Figure supplement 2. Single cell RNAseq analysis identifies hematopoietic cell sub-sets in the bone marrow of mouse-rat chimeras.
**Figure supplement 3.** Violin plots confirm expression of hematopoietic marker genes in BM cell clusters.

**Figure supplement 4.** Single cell RNAseq analysis identifies genes expressed in hematopoietic stem cells in chimeric bone marrow.

**Figure supplement 5.** ESC-derived lymphoid cell types in mouse-rat and mouse-mouse chimeras exhibit identical gene expression profiles.

**Figure supplement 6.** ESC-derived myeloid cell types in mouse-rat and mouse-mouse chimeras exhibit similar gene expression profiles.

**Figure supplement 7.** Heatmaps identify gene expression profile of ESC-derived hematopoietic stem cells and lymphoid-primed multipotent progenitor cells from mouse-rat and mouse-mouse chimeras.

**Figure 3.** Single cell RNAseq analysis shows increased percentages of ESC-derived hematopoietic progenitors and endothelial cells but decreased percentages of ESC-derived chondrocytes in the bone marrow of mouse-rat chimeras. **A-B,** Parallel dimension UMAP plots show identical hematopoietic and stromal cell clusters in the bone marrow of P5 mice, rats, and mouse-rat chimeras. BM cells were obtained from P5 animals using an enzymatic digestion (n=5 animals per group) and pooled prior to single cell RNAseq. Cell clusters were identified from single cell RNAseq datasets using Uniform Manifold Approximation and Projection (UMAP) method. Red color in the tables indicates increased percentages of cells in mouse-rat chimeras compared to either mice or rats of the same age. Blue color indicates decreased percentages of cells in mouse-rat chimeras. Gene expression signatures of mouse and rat hematopoietic and stromal cells are shown in **Figure 3 – figure supplement 1A-D.**
bar graph shows relative percentages of ESC-derived mouse cells (green) and endogenous rat cells (blue) in the bone marrow of P5 mouse-rat chimeras.

The online version of this article includes the following figure supplement(s) for figure 3:

**Figure supplement 1.** Heatmaps compare gene expression profile in mouse and rat hematopoietic and stromal cells that form bone marrow in mouse-rat chimeras.

**Figure 4.** Single cell RNAseq analysis shows remarkable similarities in major receptor-ligand interactions between erythro-myeloid progenitors and stromal cells of mouse and rat origins. BM cells were obtained from P5 animals using an enzymatic digestion (n=5 animals per group). Single cell RNAseq was performed to identify BM stromal and erythro-myeloid progenitor cells (EMPs) based on gene expression signatures. The R package *NicheNet* was used to analyze the expression of ligands and receptors to identify intercellular communication patterns between EMPs and BM stromal cells. Receptor-ligand interactions between stromal and granulocyte-monocyte progenitor cells (GMPs) are shown in **Figure 4 – figure supplement 1**. Violin plots were used to identify expression of ligands and their receptors in hematopoietic and stromal BM cells, see **Figure 4 – figure supplement 2**.

The online version of this article includes the following figure supplement(s) for figure 4:

**Figure supplement 1.** Single cell RNAseq analysis shows similar receptor-ligand interactions between stromal and granulocyte-monocyte progenitor cells.

**Figure supplement 2.** Violin plots show expression of ligands and their receptors in hematopoietic and stromal BM cells.
Figure 5. Mouse HSCs in mouse-rat chimeras develop earlier than rat HSCs. A-B, Immunostaining for RUNX1 (white) and FLK1 (red) shows that hemogenic endothelium in the dorsal aorta (DA) of mouse-rat chimeras develops mostly from ESC-derived mouse cells. GFP (green) was used to identify ESC-derived cells, whereas DAPI (blue) was used to stain cell nuclei. Frozen sections were obtained from E11 mouse embryos and E12.5 embryos from rats and mouse-rat chimeras since these embryos are in similar developmental stages, see also Figure 5 – figure supplement 1A-C. DA indicates the lumen of dorsal aorta. Yellow dashed line indicates the luminal surface of DA wall. Inserts show high magnification of hemogenic endothelial cells expressing both RUNX1 and FLK1. Scale bars are: A, 200μm; B, 20μm; inserts in B, 5μm. Abbreviations: DA, dorsal aorta; Li, liver. C, FACS analysis shows increased percentages of mouse ESC-derived Lin− cells, LSKs and ST-HSCs in fetal livers of mouse-rat chimeras (n=6) compared to control mouse embryos (n=4), see also Figure 5 – figure supplement 2. Fetal livers were obtained from E15.5 mouse-rat chimeras and E14 mouse embryos since these embryos are in similar developmental stages. p<0.05 is *, p<0.01 is **, N.S. indicates no significance, see also Figure 5 – source data 1.

The online version of this article includes the following figure supplement(s) for figure 5:

Source data 1. Excel spreadsheet containing quantitative data for Figure 5.

Figure supplement 1. Mouse embryonic development occurs faster than embryonic development in the rat and mouse-rat chimera.

Figure supplement 2. FACS analysis identifies mouse hematopoietic progenitor cells in fetal livers of mouse-rat chimeras.

Figure 6. Transplantation of mouse ESC-derived BM cells from interspecies mouse-rat chimeras rescues lethally irradiated syngeneic mice. A, Schematic diagram shows
transplantation of ESC-derived bone marrow cells (BMC) into lethally irradiated (IR) mice. ESC-derived cells were obtained from the bone marrow of juvenile mouse-rat chimeras using FACS-sorting for GFP+ cells. Bone marrow and peripheral blood were harvested 8 days and 5 months after BM transplantation. **B,** Kaplan-Meier survival analysis shows a 100% mortality in irradiated mice. Survival is dramatically improved after transplantation of irradiated mice with ESC-derived BM cells obtained from mouse-rat chimeras (IR + BMC). Survival in untreated wild type (wt) mice is shown as a control (n=12-20 mice in each group). **C,** Photograph shows irradiated C57BL/6 mice 5 months after successful bone marrow transplantation. Untreated C57BL/6 mouse is shown as a control. Grey color of irradiated mice (arrows) is consistent with large doses of whole-body radiation treatment. **D,** H&E staining shows increased amounts of hematopoietic cells in femur bones after BM transplantation into irradiated mice (top panels). GFP+ donor cells (green) are abundant in the BM compartment of transplanted mice (bottom panels). DAPI (blue) was used for counterstaining. Scale bars are: D, 200μm; inserts in D, 5μm.

**Figure 7.** Transplantation of mouse ESC-derived BM cells from interspecies mouse-rat chimeras restores hematopoietic cell lineages in the blood and bone marrow of lethally irradiated syngeneic mice. **A,** Blood analysis shows that transplantation with ESC-derived BM cells from mouse-rat chimeras increases white blood cell (WBC) counts and red blood cell (RBC) counts in the peripheral blood of irradiated recipients. Blood samples were obtained from untreated mice (no IR), lethally irradiated mice without bone marrow transplant (IR), and lethally irradiated mice with bone marrow transplant (IR+BMC). BM transplantation was performed using ESC-derived BM cells obtained from juvenile mouse-rat chimeras. FACS analysis of the peripheral blood to identify granulocytes, B cells, monocytes, T cells and erythroid cells is shown in **Figure 7 – figure supplement 1.** Concentrations of lymphocytes, monocytes and neutrophil in the blood were increased after BM transplantation (n=9-15 mice in each group),
p<0.01 is **, N.S. indicates no significance, see also *Figure 7 – source data 1*. BM transplantation also increased concentrations of platelets, hemoglobin, basophils and eosinophils in the peripheral blood, see *Figure 7 – figure supplement 2*. B-C, FACS analysis for GFP* cells in each cell subset shows that ESC-derived BM cells from mouse-rat chimeras contribute to multiple hematopoietic cell lineages in the peripheral blood of lethally irradiated mice (n=9-16 mice in each group), see also *Figure 7 – figure supplement 3*. D, FACS analysis shows that transplantation with ESC-derived BM cells from mouse-rat chimeras increases percentages of LSKs, ST-HSCs and LT-HSCs in the bone marrow of irradiated mice 5 months after BM transplantation (n=9-16 mice in each group), see also *Figure 7 – figure supplement 4A-B*. p<0.01 is **, N.S. indicates no significance, see also *Figure 7 – source data 1*. E-F, FACS analysis for GFP* shows that ESC-derived BM cells from mouse-rat chimeras contribute to multiple hematopoietic progenitor cells in the bone marrow of irradiated mice (n=9-16 mice in each group), see also *Figure 7 – figure supplement 5*. For secondary transplantation of mouse ESC-derived BM cells into lethally irradiated syngeneic mice, see *Figure 7 – figure supplement 6A-E*.

The online version of this article includes the following figure supplement(s) for figure 7:

*Source data 1*. Excel spreadsheet containing quantitative data for *Figure 7*.

*Figure supplement 1*. FACS analysis identifies granulocytes, B cells, monocytes, T cells and erythroid cells in the peripheral blood after BM transplantation.

*Figure supplement 2*. Transplantation of irradiated mice with ESC-derived BM cells from mouse-rat chimeras increases concentrations of platelets, hemoglobin, basophils and eosinophils in the peripheral blood.

*Figure supplement 3*. Identification of ESC-derived cells in the peripheral blood of irradiated mice after BM transplantation.
Figure supplement 4. Transplantation of mouse ESC-derived BM cells from interspecies mouse-rat chimeras results in reconstitution of BM hematopoietic and progenitor cells after irradiation.

Figure supplement 5. Identification of ESC-derived hematopoietic cells in the bone marrow of irradiated mice after BM transplantation.

Figure supplement 6. Secondary transplantation of mouse ESC-derived BM cells from interspecies mouse-rat chimeras rescues lethally irradiated syngeneic mice.
SUPPLEMENTAL FIGURE LEGENDS

Figure 1 – figure supplement 1. Identification of lineage\(^-\) cells, LSKs, ST-HSCs and LT-HSCs in the bone marrow. 

A, FACS gating strategy shows the identification of lineage\(^-\) (Lin\(^-\)) cells, LSKs, ST-HSCs and LT-HSCs in the bone marrow of wild type (\(wt\)) mice. 

B, Histograms show specificity of antibodies against Lineage antigens, CD117 (c-KIT), Sca1, CD150 and CD48. To identify mouse Lin\(^-\) cells, LSKs, ST-HSCs and LT-HSCs, cell suspensions from BM of \(wt\) mouse, mouse-rat chimera and \(wt\) rat were compared to determine the gating.

Figure 2 – figure supplement 1. Purification of mouse ESC-derived cells from bone marrow of mouse-rat and mouse-mouse chimeras before scRNaseq. FACS gating strategy shows identification of ESC-derived Lin\(^-\) and Lin\(^+\) cell subsets in the bone marrow of mouse-rat (A) and mouse-mouse chimeras (B). Chimeric BM cells were harvested at P10.

Figure 2 – figure supplement 2. Single cell RNAseq analysis identifies hematopoietic cell sub-sets in the bone marrow of mouse-rat chimeras. 

A, The integrated projection of ESC-derived BM hematopoietic cells from mouse-rat and mouse-mouse (control) chimeras. Cells were obtained from the bone marrow of P10 chimeras. Cell clusters were identified using Uniform Manifold Approximation and Projection (UMAP) method. Expression of marker genes shows different hematopoietic cell clusters in the bone marrow. 

B, Genes enriched in neurons, endothelial cells, adipocytes and osteocytes are not detected in cell clusters from chimeric bone marrow.
Figure 2 – figure supplement 3. Violin plots confirm expression of hematopoietic genes in BM cell clusters. Single cell RNAseq was performed using ESC-derived BM hematopoietic cells from mouse-rat and mouse-mouse P10 chimeras. Cell clusters were identified using UMAP. Violin plots show expression of selected hematopoietic genes in BM cell clusters. *Ptprc (Cd45)* mRNA is expressed in all hematopoietic cell types.

Figure 2 – figure supplement 4. Single cell RNAseq analysis identifies genes expressed in hematopoietic stem cells in chimeric bone marrow. **A**, UMAP analysis shows expression of genes enriched in hematopoietic stem cells (HSCs), including *Kit*, *Ly6a (Sca-1)* and *Flt3 (Flk2)*, using the integrated projection of ESC-derived BM hematopoietic cells from mouse-rat and mouse-mouse (control) chimeras. Cells were obtained from the bone marrow of P10 chimeras. **B**, *Kit*^+*Ly6a*^+*Flt3*^+^ triple positive cells are identified in myeloid and erythroid progenitor cell clusters in the combined scRNAseq dataset. **C**, Separate views of triple positive cells in individual scRNAseq datasets show increased number of HSCs in mouse-rat chimera compared to mouse-mouse chimera.

Figure 2 – figure supplement 5. ESC-derived lymphoid cell types in mouse-rat and mouse-mouse chimeras exhibit identical gene expression profiles. **A**, Heatmap shows significant similarities in gene expression signatures of lymphoid progenitor cells obtained from mouse-rat (R) and mouse-mouse chimeras (M). Single cell RNAseq was performed using BM cell suspensions that were FACS-sorted for GFP to identify ESC-derived cells. **B**, Linear regression analysis shows the correlation index (R) between gene expression profiles in individual lymphoid cell clusters from mouse-rat and mouse-mouse chimeras.
**Figure 2 – figure supplement 6.** ESC-derived myeloid cell types in mouse-rat and mouse-mouse chimeras exhibit similar gene expression profiles. **A,** Heatmap shows significant similarities in gene expression signatures of myeloid progenitor cells, megakaryocytes, erythroid progenitor cells, basophils, eosinophils, neutrophils, dendritic cells, monocytes and neutrophil progenitor cells obtained from mouse-rat (R) and mouse-mouse chimeras (M). Single cell RNAseq was performed using BM cell suspensions that were FACS-sorted for GFP to identify ESC-derived cells. **B,** Linear regression analysis shows the correlation index (R) between gene expression profiles in individual myeloid cell clusters from mouse-rat and mouse-mouse chimeras.

**Figure 2 – figure supplement 7.** Heatmaps identify gene expression profile of ESC-derived hematopoietic stem cells and lymphoid-primed multipotent progenitor cells from mouse-rat and mouse-mouse chimeras. Combined analysis of ESC-derived BM hematopoietic cells from mouse-rat and mouse-mouse chimeras compares gene expression signature of hematopoietic stem cells (HSCs) (**A**) and lymphoid-primed multipotent progenitor cells (LMPPs) (**B**) with gene expression signatures of other myeloid and lymphoid BM cells. Single cell RNAseq was performed using BM cell suspensions from P10 chimeras. ESC-derived cells were purified using FACS.

**Figure 3 – figure supplement 1.** Heatmaps compare gene expression profile in mouse and rat hematopoietic and stromal cells that form bone marrow in mouse-rat chimeras. **A-B,** Heatmaps show gene expression signatures of ESC-derived (mouse) and endogenous (rat) hematopoietic cells that were obtained from bone marrow of mouse-rat chimeras. Single cell RNAseq was performed using BM cells from P5 chimeras. GFP+ and GFP- cells were purified.
using FACS. **C-D**, Heatmaps show gene expression signatures of mouse and rat stromal BM cells that were obtained from P5 mouse-rat chimeras.

**Figure 4 – figure supplement 1.** Single cell RNAseq analysis shows similar receptor-ligand interactions between stromal and granulocyte-monocyte progenitor cells. Single cell RNAseq was performed using BM cells from P5 mice, rats, and mouse-rat chimeras. Stromal and granulocyte-monocyte progenitor cells (GMPs) were identified using gene expression signatures. The R package *NicheNet* was used to analyze the expression of ligands and receptors to identify intercellular communication patterns between GMPs and BM stromal cells.

**Figure 4 – figure supplement 2.** Violin plots show expression of ligands and their receptors in hematopoietic and stromal BM cells. Single cell RNAseq was performed using BM cells from P5 mice, rats, and mouse-rat chimeras. GFP\(^+\) and GFP\(^-\) cells were purified using FACS. Cell clusters were identified using UMAP. Violin plots show BM expression patterns of mouse and rat genes that were identified by the ligand-receptor interaction analysis.

**Figure 5 – figure supplement 1.** Mouse embryonic development occurs faster than embryonic development in the rat and mouse-rat chimera. **A**, Comparison of mouse and rat embryos at E11, E12.5, E14 and E15.5. Images show that rat embryos develop slower compared to mouse embryos of the same gestational time. **B**, Images show similar sizes of rat and mouse-rat chimeric embryos at E12.5. GFP fluorescence indicates the contribution of mouse ESCs to many tissues in mouse-rat chimeras. **C**, H&E staining of sagittal sections shows similar sizes of E11 mouse embryos and E12.5 rat and chimeric embryos. Scale bar is 200μm.
**Figure 5 – figure supplement 2.** FACS analysis identifies mouse hematopoietic progenitor cells in fetal livers of mouse-rat chimeras. Dot plots show the gating strategy to identify ESC-derived (GFP⁺) lineage-negative (Lin⁻), LSK, ST-HSC and LT-HSC cell subsets in fetal livers of E15.5 mouse-rat chimeras. Livers from E14 mouse embryos were used as controls for GFP.

**Figure 7 – figure supplement 1.** FACS analysis identifies granulocytes, B cells, monocytes, T cells and erythroid cells in the peripheral blood after BM transplantation. Blood samples were obtained from untreated mice (no IR), lethally irradiated mice without bone marrow transplant (IR), and lethally irradiated mice with bone marrow transplant (IR+BMC). BM transplantation was performed using ESC-derived BM cells obtained from juvenile mouse-rat chimeras. FACS analysis was performed 8 days and 5 months after BM transplantation.

**Figure 7 – figure supplement 2.** Transplantation of irradiated mice with ESC-derived BM cells from mouse-rat chimeras increases hemoglobin concentration and numbers of platelets, basophils and eosinophils in the peripheral blood. Blood samples were obtained from untreated mice (no IR), lethally irradiated mice without bone marrow transplant (IR), and lethally irradiated mice with bone marrow transplant (IR+BMC). BM transplantation was performed using ESC-derived BM cells obtained from juvenile mouse-rat chimeras. Mice were harvested 8 days or 5 months after BM transplantation. Concentrations of basophils and eosinophils in the peripheral blood were significantly increased 8 days after BM transplantation. Concentrations of platelets (PLT), hemoglobin (Hb), basophils and eosinophils were fully restored 5 months after BM transplantation (n=9-15 mice in each group), p<0.05 is *, p<0.01 is **, N.S. indicates no significance.
**Figure 7 – figure supplement 3. Identification of ESC-derived cells in the peripheral blood of irradiated mice after BM transplantation.** Histograms show the presence of ESC-derived (GFP-positive) granulocytes, B cells, T cells, monocytes and erythroid cells in the peripheral blood of irradiated mice after BM transplantation (green line). Blood of mice without BM transplantation is used to identify autofluorescence in GFP channel (blue line). Blood samples were harvested 8 days or 5 months after BM transplantation and used for FACS analysis.

**Figure 7 – figure supplement 4. Transplantation of mouse ESC-derived BM cells from interspecies mouse-rat chimeras results in reconstitution of BM hematopoietic and progenitor cells after irradiation.** A, Photographs show cell suspensions obtained from the bone marrow of untreated mice (no IR), lethally irradiated mice without bone marrow transplant (IR), and lethally irradiated mice with bone marrow transplant (IR+BMC). Mice were harvested 8 days (left image) or 5 months after BM transplantation (right image). ESC-derived BM cells from juvenile mouse-rat chimeras were used for BM transplantation. B, FACS analysis shows identification of Lineage− (Lin−) cells, LSKs, ST-HSCs and LT-HSCs in the bone marrow of irradiated mice 8 days and 5 months after BM transplantation.

**Figure 7 – figure supplement 5. Identification of ESC-derived hematopoietic cells in the bone marrow of irradiated mice after BM transplantation.** Histograms show the presence of ESC-derived (GFP-positive) Lin− cells, LSKs, ST-HSCs and LT-HSCs in the bone marrow of irradiated mice after BM transplantation (green line). For each cell subset, bone marrow of mice without BM transplantation is used to identify autofluorescence in GFP channel (blue line). Bone
marrow was obtained from one tibia and one fibula bones 8 days or 5 months after BM transplantation.

**Figure 7 – figure supplement 6. Secondary transplantation of mouse ESC-derived BM cells from interspecies mouse-rat chimeras rescues lethally irradiated syngeneic mice.**

*Figure 7 – figure supplement 6. Secondary transplantation of mouse ESC-derived BM cells from interspecies mouse-rat chimeras rescues lethally irradiated syngeneic mice. A,* Schematic diagram shows two sequential transplantations of ESC-derived bone marrow cells (GFP BMC) into lethally irradiated (IR) mice. ESC-derived cells were obtained from the bone marrow of juvenile mouse-rat chimeras using FACS-sorting of BM cells for GFP. Bone marrow and peripheral blood were analyzed by FACS five months after the second BM transplantation. *B,* ESC-derived BM cells increase survival of irradiated mice after two sequential BM transplantations (IR + BMC) compared to irradiated mice without BM transplantation (IR). Survival in untreated wild type (wt) mice is shown as a control. *C,* Photograph shows irradiated C57BL/6 mice 5 months after the second BM transplantation. Untreated C57BL/6 mouse is shown as a control. Grey color of irradiated C57BL/6 mice (arrows) is consistent with large doses of whole-body radiation treatment. *D-E,* FACS analysis for GFP in each cell subset shows that ESC-derived BM cells from mouse-rat chimeras contribute to multiple hematopoietic cell lineages in the peripheral blood and bone marrow of lethally irradiated mice 5 months after the second BM transplantation (n=3 mice).
Figure 1

A. GFP mESC

Rat blastocysts → Pseudopregant rat → Pup → Femur/Tibia

B. Rat Chimera  Rat Chimera  Rat Chimera  Rat Chimera (Mouse)

P3  P8  P13  P28

C. Weight/g

C. Rat Chimera Mouse

D. Bright GFP

E. Mouse SSC-W

F. GFP+ BM cells

G. % of Lin- (among BM cells)

H. % of ST-HSC (among LSK)

I. Number of HSCs

GFP+ % of LSK (among Lin- cells)

% of cells

Mouse  Chimera

% of cells

Mouse  Chimera

% of cells

Mouse  Chimera

Cell number in 500,000 cells

Mouse  Chimera
Figure 2

A.

Mouse-Mouse
5308 cells

Mouse-Rat
6018 cells

B.

<table>
<thead>
<tr>
<th>Cluster</th>
<th>Mouse-Mouse</th>
<th>Mouse-Rat</th>
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</thead>
<tbody>
<tr>
<td>Lymphoid progenitor</td>
<td>4.5%</td>
<td>3.31%</td>
</tr>
<tr>
<td>Pro.B cell</td>
<td>14.09%</td>
<td>2.59%</td>
</tr>
<tr>
<td>Pre.B cell</td>
<td>17.29%</td>
<td>8.99%</td>
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<tr>
<td>B cell</td>
<td>37.09%</td>
<td>13.43%</td>
</tr>
<tr>
<td>T cell</td>
<td>1.75%</td>
<td>4.04%</td>
</tr>
<tr>
<td>Erythroid progenitor</td>
<td>3.84%</td>
<td>18.64%</td>
</tr>
<tr>
<td>Myeloid progenitor</td>
<td>1.34%</td>
<td>7.73%</td>
</tr>
<tr>
<td>Neutrophil progenitor</td>
<td>2.51%</td>
<td>5.95%</td>
</tr>
<tr>
<td>Basophil-Eosinophil</td>
<td>1.02%</td>
<td>9.02%</td>
</tr>
<tr>
<td>Dendritic</td>
<td>1.83%</td>
<td>6.96%</td>
</tr>
<tr>
<td>Monocyte</td>
<td>2.52%</td>
<td>2.74%</td>
</tr>
<tr>
<td>Neutrophil</td>
<td>4.54%</td>
<td>4.99%</td>
</tr>
<tr>
<td>Megakaryocyte</td>
<td>7.2%</td>
<td>1.68%</td>
</tr>
<tr>
<td>LMPPPs</td>
<td>0.047%</td>
<td>0.994%</td>
</tr>
</tbody>
</table>
Figure 3

A. Mouse cells

Mouse

Mouse-Rat Chimera

Cluster | Mouse | Mouse-Rat
-------|-------|-----------
1       | 2.06% | 5.66%     
2       | 3.43% | 6.96%     
3       | 0.51% | 6.07%     
4       | 4.49% | 7.51%     
5       | 10.60%| 18.24%    
6       | 3.85% | 8.14%     
7       | 0.97% | 6.65%     
8       | 3.32% | 4.60%     
9       | 18.45%| 9.29%     
10      | 0.05% | 2.68%     
11      | 0.09% | 4.44%     
12      | 29.93%| 0.89%     
13      | 19.80%| 15.15%    
14      | 2.46% | 3.70%     

B. Rat cells

Rat

Mouse-Rat Chimera

Cluster | Rat | Mouse-Rat
-------|-----|-----------
1       | 8.14%| 1.80%     
2       | 6.07%| 6.39%     
3       | 5.30%| 2.42%     
4       | 5.19%| 0.58%     
5       | 9.26%| 29.97%    
6       | 2.12%| 11.34%    
7       | 14.17%| 8.48%    
8       | 3.15%| 12.99%    
9       | 21.42%| 0.42%    
10      | 7.41%| 0.05%     
11      | 1.33%| 0.80%     
12      | 2.67%| 9.79%     
13      | 6.26%| 9.68%     
14      | 7.51%| 5.28%     

C. Mouse-Rat Chimera

Endothelial
EMP
GMP
Monocyte
Neutrophil
Neutrophil progenitor
Erythroid progenitor
Erythroblast
B cell
T cell
Megakaryocyte
Chondrocyte
Fibroblast
Myofibroblast

Percentage of cells

Mouse cell | Rat cell
Figure 5

A. RUNX1  GFP  DAPI/Merge

Mouse
 DA Li
 DA Li
 DA Li

Rat
 DA Li
 DA Li
 DA Li

Chimera
 DA Li
 DA Li
 DA Li

B. Dorsal aorta

FLK1  RUNX1  GFP  DAPI/Merge

Mouse
 DA DA DA DA

Rat
 DA DA DA DA

Chimera
 DA DA DA DA

C. Fetal liver

GFP+ Liver cells

% of Lin- (among Liver cells)

% of LSK (among Lin- cells)

% of ST-HSC (among LSK)

% of LT-HSC (among LSK)

*Mouse  Chimera
Figure 6

A. Sorting GFP BMC

IR mice

Transplantation of GFP BMC

Harvest

8 days BMC, blood

5 months BMC, blood

B. Graph showing survival versus days after irradiation:

Harvest (20/20) (15/15)

(6/12)

(3/12)

(0/12)

Days after irradiation

C.

D. H&E

DAPI+GFP
Figure 2-figure supplement 1

A. Mouse-rat chimera

B. Mouse-mouse chimera
A. Cell lineage derived from common lymphoid progenitor (CLP)

M: mouse-mouse  R: mouse-rat

- Pclaf
- Mif
- Tsc22d1
- Vpreb1
- Rcsd1
- Tspan13
- Hmgb2
- Tmpo
- Pglis
- Ebf1
- Cd79b
- Cd79a
- Ms4a4b
- Cd3g
- Lck

Mouse-Mouse Scale

Mouse-Rat Scale

B.

- Lymphoid progenitor
- Pro.B cell
- Pre.B cell
- B cell
- T cell

Mouse-Rat

Mouse - Mouse

R = 0.94
R = 0.95
R = 0.95
R = 0.91
R = 0.93
Figure 2-figure supplement 6

A

Cell lineage derived from common myeloid progenitor (CMP)

M: mouse-mouse  R: mouse-rat

<table>
<thead>
<tr>
<th>Erythroid progenitor</th>
<th>Megakaryocyte</th>
<th>Myeloid progenitor</th>
<th>Basophil-Eosinophil</th>
<th>Dendritic</th>
<th>Monocyte</th>
<th>Neutrophil</th>
<th>Neutrophil progenitor</th>
</tr>
</thead>
</table>

Mouse-Mouse Scale

Mouse-Rat Scale

B

<table>
<thead>
<tr>
<th>Erythroid progenitor</th>
<th>Megakaryocyte</th>
<th>Myeloid progenitor</th>
<th>Basophil-Eosinophil</th>
</tr>
</thead>
</table>

Mouse-Rat

R = 0.89  R = 0.91  R = 0.93  R = 0.92

Mouse - Mouse

R = 0.94  R = 0.94  R = 0.85  R = 0.81
Figure 3-figure supplement 1

A. Mouse hematopoietic cells from Mouse-Rat Chimera

B. Rat hematopoietic cells from Mouse-Rat Chimera

C. Mouse Stromal cells from Mouse-Rat Chimera

D. Rat Stromal cells from Mouse-Rat Chimera

Cluster:
1. Endothelial
2. EMP
3. GMP
4. Monocyte
5. Neutrophil
6. Neutrophil progenitor
7. Erythroid progenitor
8. Erythroblast
9. B/Pro.B/Pre.B cell
10. T cell
11. Megakaryocyte
12. Chondrocyte
13. Fibroblast
14. Myofibroblast
Figure 5-figure supplement 2

Mouse

- Lin^+ Lin^+ 6.7% 93.3%
- Lineage
- CD117 LSK 1.66%
- Sca1
- CD48 ST-HSC 3.7% LT-HSC 36.9%
- CD150

Chimera

- Lin^- Lin^+ 0% 22.2%
- Lineage
- CD117 LSK 5.54%
- Sca1
- CD48 ST-HSC 30.9% LT-HSC 8.25%
- CD150
Figure 7-figure supplement 1

8 days

- no IR
  - Granulocytes 49.3%
  - B cells 25.7%
- IR
  - Granulocytes 6.47%
  - B cells 4.54%
- IR + BMC
  - Granulocytes 67.9%
  - B cells 3.18%

5 months

- no IR
  - Granulocytes 27%
  - B cells 40.7%
- IR + BMC
  - Granulocytes 22.7%
  - B cells 38%

- CD45R
- CD45R
- CD45R
- CD45R

- CD3e
- CD3e
- CD3e
- CD3e

- CD11b
- CD11b
- CD11b
- CD11b

- Ter119
- Ter119
- Ter119
- Ter119

- Erythroid cells
- Erythroid cells
- Erythroid cells
- Erythroid cells

- 45.5%
- 70.8%
- 73.5%
- 56.6%

- 49.1%
Figure 7-figure supplement 2

- PLT (10^3/μl)
- Hb (g/dl)
- Basophil (10^3/μl)
- Eosinophil (10^3/μl)

Legend:
- Red circles: no IR 8 days
- Orange squares: IR 8 days
- Green triangles: IR + BMC 8 days
- Blue triangles: no IR 5 months
- Purple circles: IR + BMC 5 months

Significance levels:
- N.S.: Not significant
- *: p < 0.05
- **: p < 0.01