HHS Public Access

Author manuscript

Exp Hematol. Author manuscript; available in PMC 2023 March 27.

Published in final edited form as:

Exp Hematol. 2021 February ; 94: 37–46. doi:10.1016/j.exphem.2020.12.004.

Arid2 regulates HSC differentiation in normal hematopoiesis

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Abstract

The Switch/Sugar Non-Fermenting (SWI/SNF) family of chromatin remodeling complexes have been implicated in normal hematopoiesis. The ARID2 protein is a component of the Polybromo-associated BAF (PBAF), one of the two main SWI/SNF complexes. In the current study, we used a conditional Arid2 knockout mouse model to understand its role in normal hematopoiesis. We showed that the loss of Arid2 has no discernable effects on steady state hematopoiesis, with the exception of a modest effect on erythropoiesis. Whereas, upon bone marrow transplantation, the loss of Arid2 affects HSC differentiation in a cell-autonomous manner, resulting in significant decreases in the ability to reconstitute the lymphoid lineage. Gene expression analysis of Arid2 knockout cells showed enrichment of myeloid-biased multipotent progenitor (MPP) cell signatures, while the lymphoid-biased MPP are enriched in the wild type, consistent with the observed phenotype. Moreover, Arid2 knockout cells showed enrichment of inflammatory pathways with up-regulation of TLR receptors, as well as, downstream signaling cascade genes. Furthermore, under lymphocyte biased growth conditions *in*-vitro, Arid2 null bone marrow cells have significantly impaired proliferation which decreased further upon LPS stimulation. Overall, these data suggest that the loss of Arid2 impairs HSC differentiation ability and this effect may be mediated through up regulation of inflammatory pathways.

Keywords

Chromatin remodeling; SWI/SNF; hematopoietic stem cell

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Author Contribution: T.B. and N.Z. designed the study and wrote the manuscript; T.B., N.Z., J.S., Y.Z., Y.Z., Y.Z., and J.D. performed research and analyzed data; R.B. and S.Z. analyzed RNA-seq transcriptome data; T.B., N.Z., D.W., J.I-C., and L.C. provided conceptual input and edited the manuscript.

Competing Interests Statement

The authors declare no competing interests.

INTRODUCTION

Adult hematopoiesis originates from the hematopoietic stem cells (HSCs) which reside in the bone marrow. HSCs are multipotent, adult stem cells with the hallmark ability to self-renew and differentiate into all blood lineages. Epigenetic regulators have been shown to play important roles in HSC function and their dysregulation leads to hematological malignancies [1]. These includes the Switch/Sugar Non-Fermenting (SWI/SNF) family of chromatin remodeling complexes [2-4].

In humans, the SWI/SNF family of chromatin remodelers consists of two main protein complexes, BRG1 associated factors (BAF) and Polybromo-associated BAF (PBAF). These protein complexes are evolutionary conserved from *S. cerevisiae* and *D. melanogaster* to humans [5,6]. The human orthologous complexes share a conserved core set of nine proteins, including the catalytic ATPases, Brahma-related gene 1 (BRG1) or Brahma (BRM) and are distinguished by unique AT-rich interactive domain-containing (ARID) proteins. ARID1A and ARID1B (BAF250A or B, respectively) are mutually exclusive to BAF, while ARID2 (BAF200) is unique to PBAF. Protein Polybromo 1 (PBRM1/BAF180) and bromodomain containing 7 (BRD7) additionally distinguish PBAF from BAF [7-13]. SWI/SNF complexes bind to nucleosomes and utilize ATP to disrupt nucleosome-DNA binding interactions. This disruption alters a nucleosome position by sliding or ejecting the nucleosome from chromatin, affecting biological processes using chromatin as template, including that of gene transcription [2,14,15].

SWI/SNF subunits have been shown to play important roles in normal hematopoiesis, including that of BAF53, BAF45A and BAF180 in HSC function [16-18], BRG1 in erythroid development and myeloid differentiation [19,20], SMARCD2 in granulopoiesis [21] and BRG1, BAF57 and BAF155 in lymphopoiesis [22]. In this study, we characterized the hematopoietic phenotype of the loss of Arid2, one of proteins that distinguishes the PBAF from the BAF complex. Whole-body homozygous loss of Arid2 is embryonic lethal at E12.5-E14.5 due to congenital heart defects [23]. Arid2 has been shown to have tissue-specific functions by modulating specific gene expression important in osteoblast differentiation [24]. Additionally, ARID2 alters cell cycle entry of hematoma cells. ARID2 knockdown in HepG2 cells leads to accelerated G1 to S transition [25]. Using a conditional Arid2 knockout mouse model, we showed that loss of Arid2 resulted in impairment of lymphoid lineage regeneration and correlated with transcriptional up-regulation of inflammatory pathways.

METHODS

A full description of mouse experiments, bone marrow transplantation, flow cytometry and sorting, ^{3}H-thymidine incorporation, LPS stimulation and immunoblotting, colony formation assay, RNA-sequencing, RT-qPCR, data analysis and statistical methods is included in the supplemental Methods.

RESULTS

Steady state hematopoiesis is largely unaffected upon the loss of Arid2

We examined expression of Arid2 mRNA level using a published database and it shows that Arid2 is expressed relatively evenly across all hematopoietic lineages (Fig. S1) [26]. To understand the role of Arid2 during normal hematopoiesis, we obtained a transgenic Arid2 knockout-first allele mouse (derived on the C57/BL6 background) from The European Mouse Mutant Archive (EMMA) in which exon 4 of ARID2 ($Arid2^{tm1a/+}$) is flanked by LoxP sites [27,28]. These mice were bred with ACTB-FLPe mice, which express the Flp recombinase, to remove the gene trap cassette flanked by FRT sites. The resultant mice were then crossed to Cre-recombinase (Cre) expressing mice driven the Vav1 or the Mx1 promoter (Fig. 1A). Excision of Arid2, exon 4 results in a frame shift and introduction of an in-frame stop codon. The Vav1 promoter becomes constitutively active during embryonic development of hematopoietic and endothelial cells [29]. The $Arid2^{fl/fl}$ $VavCre^{+}$ mice are born with normal Mendelian ratios and we did not observe any overt phenotypes (data not shown). Mx1-driven Cre expression (MxCre) is interferon inducible in hematopoietic and bone marrow niche cells by injection of polyinosinic:polycytidylic acid (pIpC) [30]. Excision efficiency in the whole bone marrow is approximately 65-95% by VavCre, 80-99% excision by MxCre and in the LSK (LIN^{-} $cKit^{+}$ $Sca1^{+}$) population by MxCre is 75-99% (Fig. 1B). Total bone marrow (BM) cellularity is unchanged upon deletion of Arid2 (Fig. 1C).

To investigate the effect of loss of Arid2 on steady state hematopoiesis, we analyzed peripheral blood (PB), spleen (SPL) and BM from $Arid2^{fl/fl}$ $VavCre^{+}$ (experimental) and $VavCre^{+}$ (control) mice for mature blood cell counts and frequency. The BM of these mice was further assessed for changes in hematopoietic stem and progenitor cell (HSPC). Blood counts in Arid2 null mice revealed a modest, but significant, decrease in red blood cell number (10.3 to 9.6 $10^{6}/mm^{3}$), along with, decreased hemoglobin (15.4 to 14.1 g/dL) and hematocrit levels (49% to 46%), suggesting a mild anemia (Fig. 1D). B-cell ($B220^{+}$) frequency is significantly decreased (45% to 37%) in the PB, while there were no changes in mature cell lineages in the SPL and BM (Fig. 1D and Fig. S2). There were no significant differences in the frequency or cell count of HSPCs including LSK and all CD150 and CD48 LSK subsets ($LSKCD150^{+}CD48^{-}$, $LSKCD150^{+}CD48^{+}$, $LSKCD150^{-}CD48^{+}$, and $LSKCD150^{-}CD48^{-}$), and downstream progenitors, including that of, CMP (LIN^{-} $cKit^{+}$ $Sca1^{-}$ $CD34^{+}$ $FcγRII/III^{lo}$), GMP (LIN^{-} $cKit^{+}$ $Sca1^{-}$ $CD34^{+}$ $FcγRII/III^{+}$) MEP (LIN^{-} $cKit^{+}$ $Sca1^{-}$ $CD34^{-}$ $FcγRII/III^{-}$) and CLP (LIN^{-} $cKit^{+}$ $Sca1^{+}$ $IL7rα^{+}$ $Flt3^{+}$) in the BM between Arid2 knockouts and controls in the VavCre model (Fig. 1D). Inducible deletion of Arid2 in the MxCre model was consistent with the VavCre model, with the addition of a significant decrease in T cells ($CD3^{+}$) in the PB (36% to 24%) and SPL (30% to 19%) and a significant decrease in $LSKCD150^{+}CD48^{+}$ (0.016% to 0.007%) and $LSKCD150^{-}CD48^{+}$ (0.13% to 0.07%) cells in the BM (Fig. 1E). Overall, loss of Arid2 led to mild anemia, with decreases in mature lymphoid and progenitor populations during steady state hematopoiesis in adult mice.

Arid2 loss results in impairment of HSPC differentiation in hematopoietic regeneration

To examine the regenerative function of Arid2 null HSCs, we performed non-competitive and competitive transplantation experiments. Serial non-competitive transplantation, up to tertiary transplant, was performed by transplanting $Arid2^{fl/fl}$ $VavCre^{+}$ (Arid2 null, experimental) or $VavCre^{+}$ (control) whole BM into lethally irradiated C57/BL6 recipient mice. Experimental and control donor mice were $CD45.2^{+}$ and recipient mice were congenic, $CD45.1^{+}$ mice. Chimerism was assessed by flow cytometry analysis of $CD45.2^{+}$ versus $CD45.1^{+}$. In the primary transplant, overall reconstitution, as determined by $CD45.2^{+}$ percentage over the time course of 20 weeks, of Arid2 null primary recipients was approximately 80% in the PB and there is a very modest (90% to 80% at week 20) but significant difference in chimerism between Arid2 null and control recipients (Fig. 2A, Fig. S3A). There was no significant difference in the BM or SPL in total $CD45.2^{+}$, mature T and myeloid cell ($Mac1^{+}$ $Gr1^{+}$) chimerism, except a modest, but significant, decrease in B cell chimerism (72% to 65%) in the BM (Fig. 2A). Additionally, there were modest, but significant, decreases in $LSKCD150^{+}CD48^{+}$ (99% to 94%) and $LSKCD150^{-}CD48^{-}$ (96% to 87%) in the BM (Fig. 2A). Whole BM from primary recipients was serially transplanted into conditioned secondary and, iteratively, into tertiary recipients. In the secondary and tertiary transplants, overall PB chimerism was significantly decreased in Arid2 null recipients compare to controls and the differences further widened in the tertiary (74% to 56% at week 20) compare to the secondary transplants (95% to 86% at week 20) (Fig.2B, Fig. S3B and Fig. 2C, Fig. S3C). In the SPL, there was a significant decrease or a trend to decrease in overall chimerism in the secondary (90% to 82%) and tertiary (82% to 66%) transplants, respectively, and a consistent significant decrease in T cell chimerism (88% to 73% secondary, 68% to 43% tertiary) (Fig. 2B and Fig. 2C). We did not observe any significant changes in overall chimerism or mature cell lineages in the BM, in either, the secondary or tertiary transplant (Fig. 2B and Fig. 2C). In the tertiary transplant, there was a significant increase in mature myeloid chimerism (85% to 94%) in the SPL, as well as, a significant increase in CMP (86% to 91%) in the BM (Fig. 2C). Furthermore, there was a significant decrease in $LSKCD150^{-}CD48^{-}$ (73% to 51%) in the BM (Fig. 2C).

To further examine the effect of the loss of Arid2 on HSC function, we performed competitive transplantation of Arid2 null BM. Whole BM from $Arid2^{fl/fl}$ $VavCre^{+}$ (Arid2 null, experimental) or $VavCre^{+}$ (control) mice was mixed at a 1:1 ratio with whole BM from $CD45.1^{+}$ C57/BL6 and transplanted into congenic $CD45.1^{+}$ C57/BL6 mice. As shown in Fig. 2D, Arid2 null cells have decreased repopulation potential, as evident by a ~2-fold decrease in overall chimerism in the PB compare to controls over the time course of 20 weeks (Fig. 2D, Fig. S3D). There was a significant decrease in B and T (~2 fold) cell chimerism in the SPL and BM, along with a decrease in overall chimerism in the SPL (Fig. 2D). The overall chimerism in the BM, at the endpoint, showed a trend in decrease, but was not statistically significant, due to a large variation in the data. There is a decrease in LSK chimerism (68% to 50%) and a significant decrease in $LSKCD150^{-}CD48^{+}$ cells (64% to 42%), while the other HSPC and progenitor populations did not show significant changes (Fig. 2D). Together, these data suggest that the loss of Arid2 results in a decreased regenerative ability of the HSCs, compare to wild type cells, leading to a diminished ability to differentiate into lymphoid lineage.

To rule out that impaired HSC regenerative function in Arid2 null BM is due to an engraftment defect, we performed competitive and non-competitive transplantations using the inducible, hematopoietic specific, Mx1-driven Cre expression (MxCre) mouse model [30]. Arid2 was excised by pIpC administration 4 weeks post transplantation (week 0), allowing for stable engraftment prior to excision. We monitored PB chimerism over the course of 20 weeks post pIpC and analyzed chimerism in the SPL and BM at the endpoint. Defects in the impaired regenerative HSC function seen in the MxCre model were similar, yet exacerbated, to the VavCre model. Similar to the results from non-competitive serial transplantation in the VavCre model, we observed significant decreases in chimerism between Arid2 null and control recipients in the PB (93% to 81%) over the time course (Fig. 3A, Fig. S4A). Moreover, there were significant decreases in overall chimerism in the SPL (92% to 75%) and BM (84%-74%), as well as, in mature T and B cell chimerism in the SPL (93% to 83% and 98% to 95% respectively) and BM (85% to 68% and 78% to 60% respectively) at the end time point (Fig. 3A). In addition, the MxCre model showed HSPC populations: $LSKCD150^{-}CD48^{+}$ (98% to 96%), LSK (97% to 93%), CMP (96% to 88%) and MEP (55% to 23%) to be significantly reduced (Fig. 3A). In competitive transplantation, the results showed consistency with that observed in the VavCre model: a ~2 fold decrease in overall chimerism in PB compare to controls over the time course of 20 weeks, a significant decrease in overall $CD45.2^{+}$ chimerism (50% to 30%) and T (45% to 16%) cell chimerism in the SPL, significant decrease in T (43% to 24%) and B (47% to 27%) cell chimerism in the BM and significant decrease in $LSKCD150^{-}CD48^{+}$ (82% to 16%) in the BM (Fig. 3B, Fig. S4B). Unlike in the VavCre model, there was also, a significant decrease in overall $CD45.2^{+}$ chimerism in the BM (65% to 37%) and a significant decrease in $LSKCD150^{+}CD48^{+}$ (89% to 27%), $LSKCD150^{-}CD48^{-}$ (77% to 38%), and MEP (52% to 25%) chimerism in the BM (Fig. 3B).

To understand the cellular effects of the loss of Arid2, we performed cell cycle analysis, by Ki67 and DAPI, and apoptosis analysis, by Annexin V, in HSPC populations at steady state and at the end point of the competitive transplantation in the MxCre model. We did not observe any significant difference in Arid2 null versus control recipients during steady state or during the competitive transplantation (Fig. S5). This data suggests that the effect of the loss of Arid2 on the regenerative ability of HSPCs is not due to changes in apoptosis or cell cycle status.

To summarize, these data suggests that the loss of Arid2 impairs the regenerative function of HSCs. Arid2 null HSCs have decreased differentiation potential compare to that of the wild type while, HSC self-renewal is relatively unperturbed. This decreased differentiation potential is reflected in decreased progenitors with lymphoid potential and decreased ability to differentiate into mature lymphoid lineages. The effects on the regenerative ability of the HSPCs are not due to impairment in the engraftment potential of these cells, as evident by the comparable phenotypes observed in the VavCre and MxCre models.

Arid2 loss leads to transcriptional up-regulation of inflammatory pathways

To understand the molecular mechanism of Arid2 function in HSPC biology, we performed transcriptome analysis by RNA-seq in LSK cells isolated from $Arid2^{fl/fl}$ $MxCre^{+}$ (KO) or

MxCre^{+} (control, Ctrl) mice six weeks after pIpC administration. Gene expression analysis showed 2,021 of genes are differentially expressed between KO LSKs versus control using an absolute $\log_2$ fold-change threshold of >1.5 and adjusted p value of < 0.05. Amongst the differentially expressed genes, 1,104 are up-regulated and 917 are down-regulated (Fig. 4A, 4B, and Table S4). Excision of Arid2 in the mRNA was confirmed for exon 4 via RNA-seq (Fig. 4C, Fig S6A). The overall normalized expression of Arid2 was unchanged (adj. p-value 0.97) between the control and the conditional knockout; it is likely that non-sense mediated mRNA decay did not take place (Fig. S6A,B). We performed GSEA of the expression data using previously described HSPC gene signatures [31] and found enrichment of the myeloid-biased MPP signature (MPP3) in KO cells, while the lymphoid-biased MPP (MPP4) signatures are enriched in the control cells (Fig. 4D). Moreover, GSEA using the REACTOME database showed enrichment of, predominately, inflammatory pathways in KO cells including, IL-1 and TLR signaling pathways and the signaling cascade mediated by MYD88 and TRIF, which lead to NFκB activation (Fig. 5A). Indeed, most of the genes within those pathways, including multiple TLRs, are up-regulated in KO cells, while the IL-1 receptor is downregulated (Fig. S6D,E, Fig S7). Additionally, we used i-Cis Target to identify overrepresented transcription factor motifs in the significant differentially expressed genes and found enrichment of NFκB, Rela, and Rel (Fig. 5B,C) amongst the top enriched motifs [32,33]. Furthermore, there is an enrichment of NFκB target genes and significant upregulation of those genes in the KO cells (Fig. 5D,E) [34]. Transcription factor motifs for Spib, Spi1, and Spic, as well as, an enrichment and upregulation of positively regulated PU.1 genes were identified in the KO cells (Fig. 5B,C, Fig. S6F,G) [35]. This is consistent with the observed erythroid and lymphoid lineage impairment we observed, since previous studies have shown that continuous high PU.1 expression level impairs erythroid differentiation [36], as well as, B and T cell development [37,38]. Finally, we investigated whether dysregulation of inflammatory signaling underlies the mechanism of Arid2 null cell impairment. To this end, we compared NFκB and MAPK signaling transduction, as well as, cell proliferation in the presence of IL-7 and LPS (a TLR4 agonist) stimulation between control and Arid2 null cells. We examined the level of IKKβ and p38 phosphorylation following LPS stimulation in Lin-c-Kit^{+} cells. We did not find any significant changes in IKKβ (Fig. S8A,B) and the result for p38 was inconclusive due to high background level of p38 at zero time point in the absence of LPS. Nonetheless, our ^{3}H-thymidine incorporation assay showed that Arid2 null cells have significantly decreased proliferation compared to wildtype counterparts in the presence of IL-7 alone. There was further decrease in cell proliferation of Arid2 null cells with the addition of LPS, whereas, introduction of LPS has no effect on the wildtype cells (Fig 5F). Moreover, this effect of LPS is lymphoid specific, since we did not observe any difference in myeloid colony formation between Arid2 null and control cells (Fig S8B). Given that these inflammatory pathways, in general, impair HSC self-renewal [39-43] and promote myeloid differentiation, at the expense of lymphocyte development [44], these findings are consistent with our observed phenotype of impaired lymphoid repopulation ability in the transplantation experiments.

DISCUSSION

SWI/SNF components have widespread roles in hematopoiesis and function as tumor suppressors in various malignancies [2-4]. Here we used hematopoietic specific mouse models to understand the role of Arid2, a unique subunit of PBAF, in normal hematopoiesis and HSPC function. Our study demonstrated that the loss of Arid2 results in mild anemia and a decrease in mature B and T cells in adult mice (Fig. 1D and Fig. 1E). Upon hematopoietic stress, examined by transplantation, Arid2 null BM exhibit impaired, cell-autonomous regenerative potential, as evident by decreased PB chimerism in serial non-competitive transplantations (Fig. 2, Fig. S3), as well as, a two-fold reduction in chimerism in competitive reconstitution (Fig. 3, Fig. S4). Moreover, we did observe decreases in various HPC chimerisms in both non-competitive (Fig. 2) and competitive (Fig. 3) transplantations. By the tertiary non-competitive transplantation, there was evidence of myeloid-biased hematopoiesis: increased Mac1/Gr1 chimerism in the SPL and increased chimerism in CMP in the BM (Fig. 2C). Taken together, these data suggest HSC differentiation is impaired upon the loss of Arid2. The differentiation impairment is largely attributed to a decrease in LSK CD48+, lymphoid restricted, progenitor populations. Consistent with this, changes in chimerism of mature cells, observed in transplantation experiments, are largely within the B and T cell compartment (Fig. 2, Fig. S3 and Fig. 3, Fig. S4). Similar observations between the VavCre and MxCre models suggest that the observed differences between Arid2 null and controls are not due to an engraftment defect of Arid2 null cells (Fig. 2D and Fig. 3B). While this study was undergoing, Liu et.al. published their characterization of the hematopoietic phenotype using Arid2 knockout mouse models [45]. Our data agree with their finding in that, both groups observed anemia in steady state hematopoiesis and showed decreased differentiation ability of Arid2 null HSPCs in BM transplantations during competitive transplantation. However, in contrast with their data, we did not observe reduced HSC frequency in the VavCre model at steady state. Additionally, our study assessed HSC self-renewal by serial non-competitive transplantation and showed that, at least out to the tertiary transplant, self-renewal remains relatively intact, as evident by similar chimerism in LSK $CD48^{-}$ $CD150^{+}$ between ARID2 null and controls cells. Liu et.al. did not address HSC self-renewal in their published study.

We performed bulk RNA-seq to understand the mechanism underlying the impairment of Arid2 null HSPCs. Our RNA-seq analysis identified some, but not all, of the differentially expressed genes between Arid2 null HSPC and controls that were highlighted in Liu et al.'s study by RT-qPCR. We were not able to compare, at a global level, the extent of the differences between our transcriptome analysis and theirs, because Liu et al. did not use replication as is required for a statistical analysis of differential gene expression. Our RNA-seq data, additionally, identified enrichment of the myeloid-biased MPP signature (MPP3) in Arid2 knockout cells, but not those of lymphoid-biased MPP (MPP4) signatures. This result is consistent with the observed decrease in the progenitors with lymphoid potential and the mature lymphoid cells in the reconstitution transplantations. Likewise, we identified inflammatory pathways, including multiple TLR pathways, as the most affected in Arid2 knockout cells. Furthermore, we showed that stimulation by LPS, a TLR4 agonist, functionally impairs Arid2 null cell proliferation under lymphocyte promoting conditions.

Up-regulation of inflammatory pathways provides a possible explanation for our observed lymphoid phenotype, as activation of TLR pathways pathway have been shown to lead to a similar phenotype [39-44] as we observed in our Arid2 knockout model, namely the impaired regeneration ability of HSPCs especially towards the lymphoid lineage. In addition, up-regulation of PU.1 may also contribute to the phenotype we observed. Further studies are required to validate the functional importance of the inflammatory pathways and elevated PU.1 expression in mediating the Arid2 knockout phenotype, as well as, whether Arid2 directly regulates those pathways through transcriptional regulation.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgement

The authors thank Benedetta Bonacci for assistance with cell sorting.

This work was supported by the Versiti Blood Research Institute Foundation and in part by Institutional Research Grant IRG #16-183-31 from the American Cancer Society and the Medical College of Wisconsin Cancer Center.

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HIGHLIGHTS

- Loss of Arid2 has largely no effect on steady state hematopoiesis
- Arid2 loss leads to impairment in lymphoid lineage regeneration
- Arid2 null HSPCs show enrichment of myeloid-biased MPP signature
- Loss of Arid2 leads to up-regulation of inflammatory pathways
- Arid2 null cells have proliferation defects under lymphoid condition with LPS

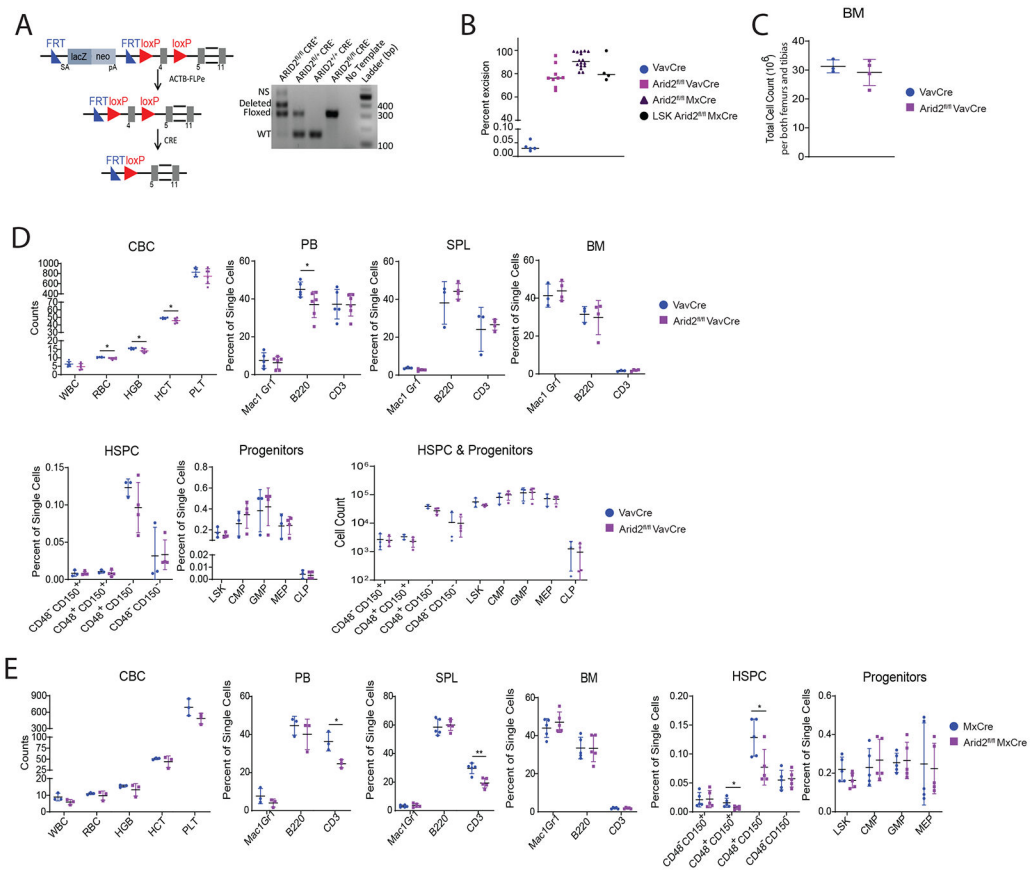

Figure 1. Effect of loss of Arid2 on steady state hematopoiesis.

(A) Schematic of Arid2 conditional knockout mouse and representative PCR genotyping. NS- non-specific, WT- wildtype (B) Deletion efficiency in Vav and Mx model in whole BM and LSK cells by qPCR. (C) Total BM cellularity of both tibias and femurs per mouse. (D-E) CBC counts in PB, mature blood cell frequency in PB, SPL, and BM and HSPC cell frequency and cell count in BM in VavCre (D) and MxCre models (E). Analysis were performed on mice 8 weeks old for VavCre model. For MxCre model, mice were pIpCed at 8 weeks of age and were analyzed 6 weeks later. n=3-5 mice per genotype p-value *< 0.05 parametric, two tailed t-test.

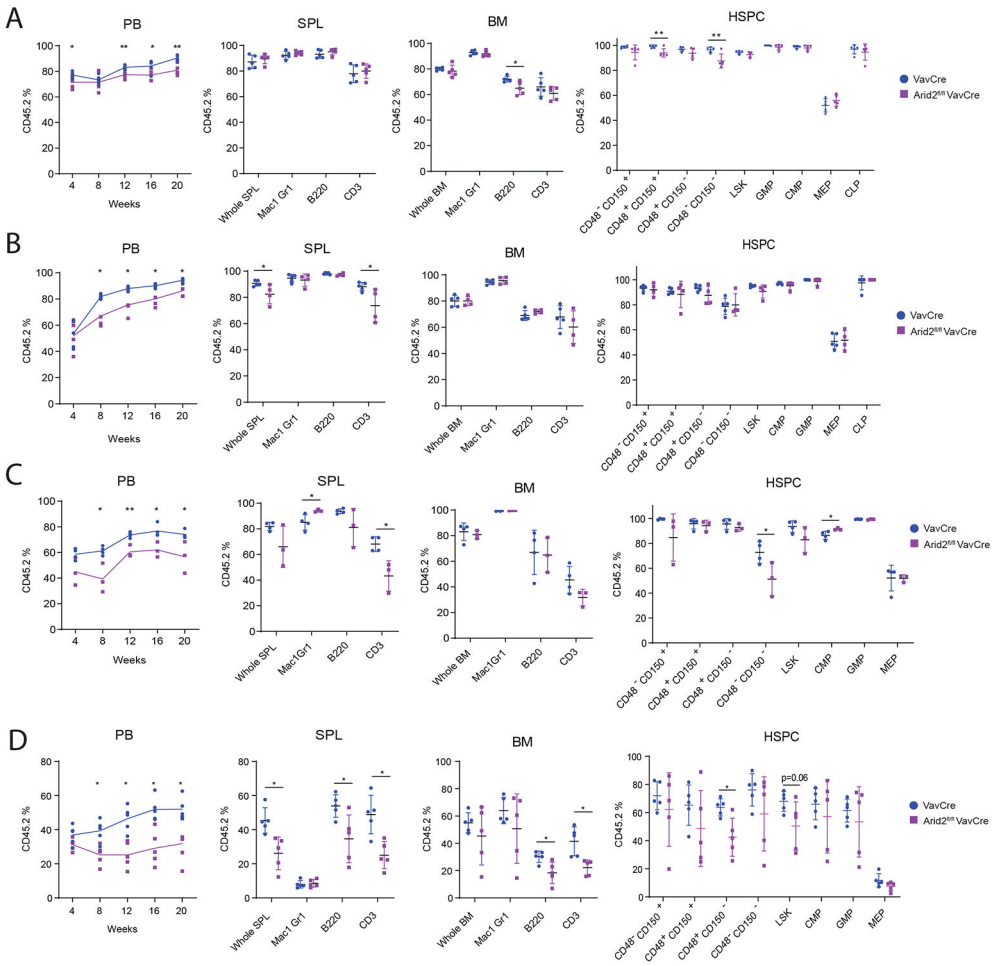

Figure 2. Assessment of the regenerative potential of Arid2 null HSPCs in the VavCre model.

(A-C) VavCre serial non-competitive transplantation. 2 million whole BM cells from CD45.2^{+} VavCre^{+} or Arid2$^{fl/fl}$ VavCre^{+} donor mice were serially transplanted into lethally irradiated CD45.1^{+} recipients. Frequency of CD45.2^{+} cells in PB 4-20 weeks post-transplant, mature cell compartments in SPL and BM and HSPC cell compartments in BM at endpoint (20 weeks) in primary (A), secondary (B) and tertiary (C) recipients. (D) VavCre competitive transplantation. 1 million whole BM cells from CD45.2^{+} VavCre^{+} or Arid2$^{fl/fl}$ VavCre^{+} donor mice were mixed 1:1 with CD45.1^{+} competitor cells and transplanted into lethally irradiated CD45.1^{+} recipients. Frequency of CD45.2^{+} cells in PB 4-20 weeks post-transplant, mature cell compartments in SPL and BM and HSPC cell compartments in BM at endpoint (20 weeks). n=3-5 mice per genotype. p-value *< 0.05, **< 0.01, ***< 0.001 parametric, two tailed t-test

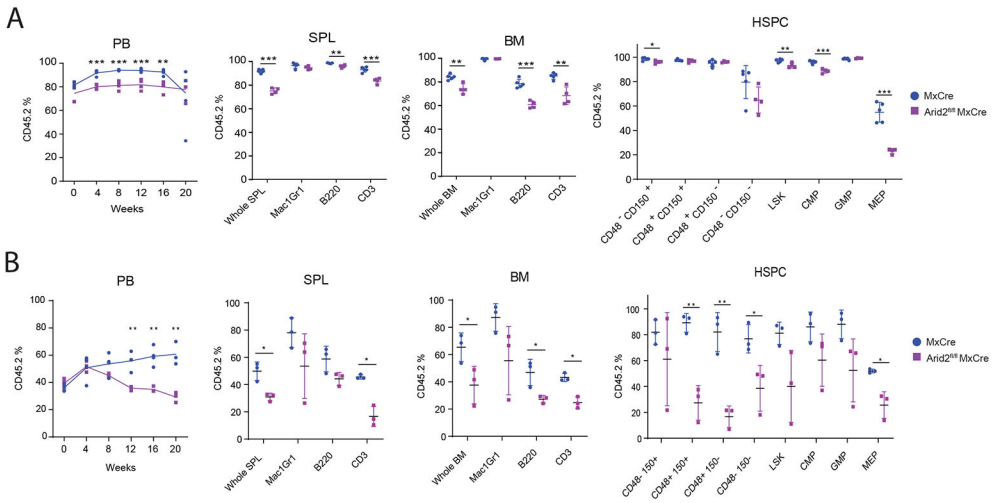

Figure 3. Bone marrow transplantation of Arid2 null HSPCs using the MxCre model.

(A) MxCre primary non-competitive transplantation. 4 weeks post transplantation (week 0) recipient mice received pIpC injection. Frequency of CD45.2$^+$ cells in PB 0-20 weeks post-transplant, mature cell compartments in SPL and BM and HSPC cell compartments in BM at endpoint (20 weeks) (B) MxCre competitive transplantation. 4 weeks post transplantation (week 0) recipient mice received pIpC injection. Frequency of CD45.2$^+$ cells in PB 0-20 weeks post-transplant, mature cell compartments in SPL and BM and HSPC cell compartments in BM at endpoint (20 weeks). n=3-5 mice per genotype. p-value *< 0.05, **< 0.01, ***< 0.001 parametric, two tailed t-test

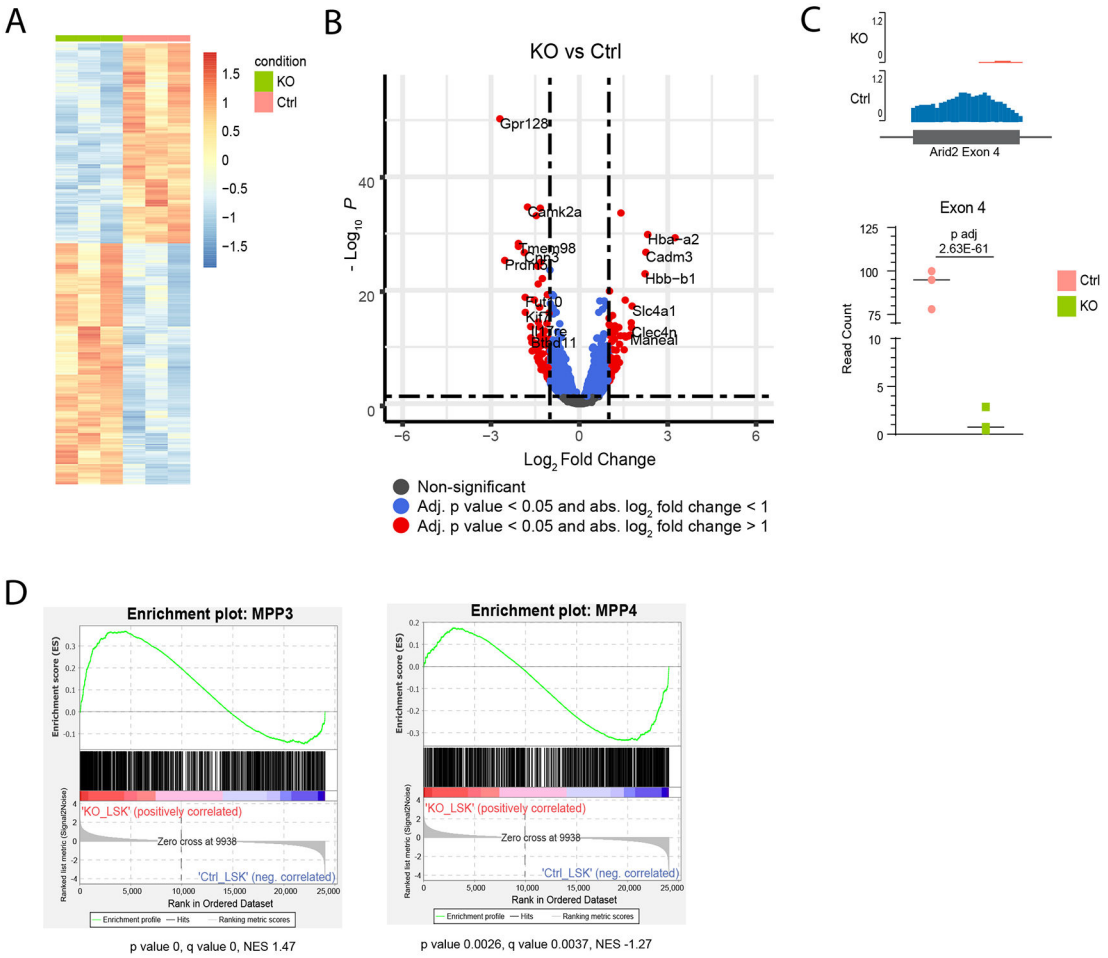

Figure 4. Transcriptome analysis of Arid2 null HSPCs.

(A) Heat map of differentially expressed genes (adjusted $p < 0.05$ and fold change +/− 1.5) between MxCre^{+} (Ctrl) or Arid2$^{fl/fl}$ MxCre^{+} (KO) LSKs 6 weeks post pIpC injection. (B) Volcano plot of differentially expressed genes between KO and Ctrl. (C) Representative genomic tracks from normalized RNA-seq data for Arid2 exon 4 (top panel) and total normalized read counts of Arid2 exon 4 of Ctrl and KO (bottom panel). (D) GSEA enrichment plots of MPP gene signatures [31] between KO and Ctrl. n=3 litter matched mice per genotype.

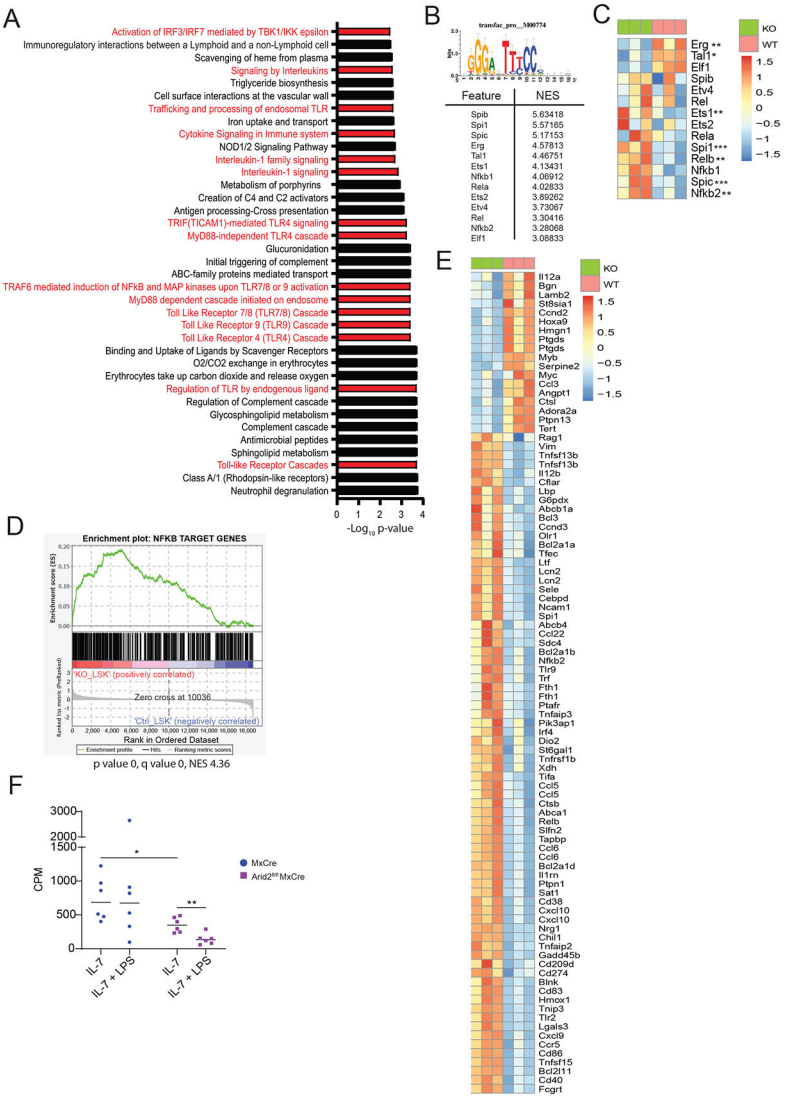

Figure 5. Pathway analysis of transcriptomics data in Arid2 null HSPCs.

(A) Significantly upregulated pathways from the REACTOME pathway database in MxCre^{+} (Ctrl) or Arid2$^{fl/fl}$ MxCre^{+} (KO) LSKs 6 weeks post pIpC injection. Inflammatory pathways are highlighted in red. (B) Enriched NFκB transcription factor motif (top), top significantly enriched transcription factor motif features with normalized enrichment score (NES) identified by i-CisTarget within the significant, differentially expressed genes in KO vs Ctrl LSK cells (bottom) (32,33). (C) Heat map of transcription factor genes corresponding to motifs identified by i-Cis Target in KO and Ctrl LSK cells. (D) GSEA enrichment plot of NFκB target genes between KO and Ctrl (34). (E) Heat map of significant, differentially expressed NFκB target genes in Ctrl or KO LSKs. n=3 litter matched mice per genotype. (F) ^{3}H-thymidine incorporation. 7.5x10^{4} whole BM cells from MxCre^{+} and Arid2$^{fl/fl}$ MxCre^{+} non-competitive primary transplanted recipient mice were cultured in IL-7 (10ng/ml) alone or IL-7 (10ng/ml) + LPS (100ng/ml) for 6 days and then pulsed with ^{3}H-thymidine for 18 hours. Non-competitive primary transplantation recipient mice received pIpC injection 4

weeks post transplantation. n=6 mice per genotype. n=6 mice per genotype p-value *< 0.05, **< 0.01 parametric, two tailed t-test