Adult haematopoietic stem cells lacking Hif-1α self-renew normally

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Running title: HSCs self-renew without Hif-1α

Key points:

- *Hif-1α* is dispensable for cell-autonomous HSC survival
- HSCs do not require intrinsic *Hif-1α* to respond to haematopoietic injury

Abstract

The haematopoietic stem cell (HSC) pool is maintained under hypoxic conditions within the bone marrow (BM) microenvironment. Cellular responses to hypoxia are largely mediated by hypoxia-inducible factors, Hif-1 and Hif-2. The oxygen-regulated alpha subunits of Hif-1 and Hif-2 (namely, Hif-1α and Hif-2α) form dimers with their stably expressed beta subunits, and control the transcription of downstream hypoxia-responsive genes to facilitate adaptation to low oxygen tension. An initial study concluded that *Hif-1α* is essential for HSC maintenance, whereby *Hif-1α*-deficient HSCs lost their ability to self-renew in serial transplantation assays. In another study, we demonstrated that *Hif-2α* is dispensable for cell-autonomous HSC maintenance, both under steady-state conditions and following transplantation. Given these unexpected findings, we set out to revisit the role of *Hif-1α* in cell-autonomous HSC functions. Here we demonstrate that inducible acute deletion of *Hif-1α* has no impact on HSC survival. Notably, unstressed HSCs lacking *Hif-1α* efficiently self-renew and sustain long-term multilineage haematopoiesis upon serial transplantation. Finally, *Hif-1α*-deficient HSCs recover normally after hematopoietic injury induced by serial administration of 5-fluorouracil. We therefore conclude that despite the hypoxic nature of the BM microenvironment, *Hif-1α* is dispensable for cell-autonomous HSC maintenance.

Introduction

Haematopoietic stem cells (HSCs) reside in hypoxic bone marrow (BM) niches where they self-renew and sustain life-long multilineage haematopoiesis[1-3]. Cellular responses to hypoxia are predominantly mediated by hypoxia-inducible factors, Hif-1 and Hif-2 that facilitate the transcription of hypoxia-responsive genes. Several studies employed a conditional gene knockout strategy to determine the role of *Hif-1α* and *Hif-2α* in HSC functions[4,5]. An initial study concluded that inducible *Hif-1α* deletion from the mouse haematopoietic system results in the progressive loss of HSCs upon serial transplantation[4], indicating that *Hif-1α* is required for HSC maintenance[4]. We demonstrated that constitutive or inducible haematopoiesis-specific *Hif-2α* deletion did not affect HSC maintenance[5]. Surprisingly, co-deletion of *Hif-1α* and *Hif-2α* had no impact on HSC numbers, steady-state haematopoiesis nor reconstitution upon transplantation[5]. These unexpected findings gave rise to the hypothesis that *Hif-1α* may not be as essential for HSC maintenance as previously suggested[4]. Here, using serial transplantation assays, we demonstrate that unstressed HSCs do not critically require *Hif-1α* to survive and self-renew.

Materials and Methods

Mice. *Hif-1α*$^{fl/fl}$, *Mx1-Cre* and *Vav-iCre* mice have been described previously[6-8] and were of C57BL/6 genetic background. Sex-matched 8 to 12 week old mice were used. Animal experiments were authorised by UK Home Office.

FACS analysis. Analysis and sorting was done using BD LSRFortessa™ cell analyzer and BD FACSAriaII cell sorter. Antibodies (Suppl. Table 1) were used as described previously[9-11]. Representative gating is shown in Suppl. Fig. 1.

Transplantation assays. 500,000 CD45.2$^+$ test donor unfractionated BM cells were injected intravenously into lethally irradiated (10 Gy) CD45.1$^+$/CD45.2$^+$ congenic recipients alongside 500,000 CD45.1$^+$ competitor unfractionated BM cells. Conditional gene deletion was achieved by administration of six intraperitoneal pIpC injections over the period of 10 days, every other day (GE Healthcare; 0.3 mg per dose). For LSK cell transplantation assays, 3,000 LSK cells sorted from donor BM were transplanted together with 200,000 CD45.1$^+$ unfractionated BM cells.

Statistical analysis. Statistical significance was determined using Mann-Whitney or Mantel-Cox tests.

Results and Discussion

We acutely deleted *Hif-1α* specifically within the haematopoietic system using *Mx1-Cre*[5,9] (Fig. 1A). Given that *Mx1-Cre* recombines within the haematopoietic system, BM microenvironment and extramedullary tissues[7,12], we first transplanted unfractionated BM from untreated CD45.2$^+$ *Hif-1α$^{fl/fl}$;Mx1-Cre* and control (*Hif-1α$^{+/+}$* without *Mx1-Cre* or *Hif-1α$^{fl/fl}$* without *Mx1-Cre*) mice into wild-type (WT) lethally irradiated syngeneic recipients (Fig. 1A). Following reconstitution (Fig. 1B), recipients received pIpC 8 weeks after transplantation, and were analysed 2 weeks later. Peripheral blood (PB) analyses of the pIpC-treated recipients revealed that deletion of *Hif-1α* (Fig. 1C) had no impact on CD45.2$^+$ donor-derived chimerism (Fig. 1D) and multilineage haematopoiesis in the BM (Fig. 1E), and spleen (Suppl. Fig. 2). The contribution of donor-derived cells to Lin$^-$Sca-1$^-$c-Kit$^+$ (LK), Lin$^-$Sca-1$^+$c-Kit$^+$ (LSK), LSKCD48$^-$CD150$^+$ HSC, LSKCD48$^-$CD150$^-$ multipotent progenitor (MPP), LSKCD48$^+$CD150$^-$ haematopoietic progenitor cell-1 (HPC-1) and LSKCD48$^+$CD150$^+$ HPC-2 compartments was also comparable (Fig. 1F-G). Therefore, acute deletion of *Hif-1α* has no immediate impact on HSC survival and multilineage haematopoiesis.

To test the self-renewal capacity of *Hif-1α*-deficient (*Hif-1α$^{\Delta/\Delta}$*) HSCs we performed competitive serial transplantation assays with sorted CD45.2$^+$LSK cells from primary recipients 2 weeks after pIpC treatment (Fig. 1A). LSK cells from primary recipients efficiently reconstituted short-term and long-term haematopoiesis in secondary recipients (Fig. 1H). 16 weeks after transplantation, sustained *Hif-1α* deletion was confirmed (Fig. 1I), and *Hif-1α$^{\Delta/\Delta}$* LSK cells efficiently contributed to all differentiated lineages in the BM and spleen (Fig. 1J and Suppl. Fig. 3), and LK, LSK, HSC, MPP, and HPC BM compartments of the secondary recipient mice (Fig. 1K-L). Finally, sorted *Hif-1α$^{\Delta/\Delta}$* LSK cells from secondary recipients (16 weeks after secondary transplantation) successfully reconstituted long-term multilineage haematopoiesis in tertiary recipients (Fig. 1M-N). Complete *Hif-1α* deletion was maintained for the duration of these experiments (Fig. 1O). Therefore, self-

renewing long-term HSCs do not critically require *Hif-1α* to maintain their pool upon the stress of serial transplantation.

To test the cell-autonomous role of *Hif-1α* in HSC stress responses, we employed *Hif-1α*$^{fl/fl}$*;Vav-iCre* mice in which *Hif-1α* is conditionally deleted specifically from haematopoietic cells using a codon-improved Cre (iCre)[5,8]. *Hif-1α*$^{fl/fl}$*;Vav-iCre* and control mice received 3 doses of 5-fluorouracil (5-FU) and were analysed 10 days after the last 5-FU administration (Fig. 1P). We also analysed untreated *Hif-1α*$^{fl/fl}$*;Vav-iCre* and control mice that did not receive 5-FU. While 5-FU-treated mice had decreased survival compared to untreated mice, we observed no differences in survival between 5-FU-treated *Hif-1α*$^{fl/fl}$*;Vav-iCre* and control mice (Fig. 1Q). 5-FU-treated *Hif-1α*$^{fl/fl}$*;Vav-iCre* and control mice had comparable total BM cellularity, and BM LSK, HSC and LK cell numbers (Fig. 1R). Thus *Hif-1α* is not essential for cell-autonomous HSC maintenance following serial 5-FU administration.

We next determined the long-term consequences of *Hif-1α* deletion from the haematopoietic system. We transplanted unfractionated BM cells from untreated *Hif-1α*$^{fl/fl}$*;Mx1-Cre* or control mice, administered pIpC and analysed the primary recipients 32 weeks after pIpC treatment (Fig. 2A). PB and BM analyses indicated that loss of *Hif-1α* did not affect long-term multilineage haematopoiesis (Fig. 2B-C), or donor-derived chimerism in the stem and progenitor cell compartments of the BM (Fig. 2D-E) and spleens (data not shown) of the recipient mice 32 weeks after pIpC treatment. Moreover, LSK cells of both genotypes equally reconstituted long-term multilineage haematopoiesis in secondary recipients (Fig. 2F-G) and evenly contributed to the stem and progenitor cell compartments of the recipients (Fig. 2H). Efficient *Hif-1α* deletion was confirmed by PCR on genomic DNA from donor-derived cells (Fig. 2I). Given that donor-derived HSCs in this experiment have undergone long-term stress and are very rare in secondary recipients, to perform tertiary transplantation assays, instead of re-transplanting purified LSK cells we transplanted unfractionated BM cells harvested from secondary recipients 16 weeks post transplantation. BM cells of both genotypes equally generated multilineage haematopoiesis in tertiary recipients (Fig. 2J-K). Efficient *Hif-1α* gene deletion was maintained for the duration of the serial transplantation experiment (Fig. 2L). Thus, HSCs with chronic *Hif-1α* deficiency sustain

normal steady-state multilineage haematopoiesis and display normal regenerative capacity upon serial transplantation.

Finally, we set out to delete *Hif-1α* more broadly from the BM using *Mx1-Cre*, which in addition to haematopoietic cells, also recombines in the BM microenvironment[13]. *Hif-1α$^{fl/fl}$;Mx1-Cre* and control mice received 6 doses of pIpC and were analysed 30 days after the last injection (Suppl. Fig. 4A). pIpC-treated *Hif-1α$^{fl/fl}$;Mx1-Cre* and control mice had comparable numbers of nucleated BM cells, LSK cells, and HSC, MPP, HPC-1 and HPC-2 populations (Suppl. Fig. 4B). We next treated *Hif-1α$^{fl/fl}$;Mx1-Cre* and control mice with 6 doses of pIpC followed by 2 doses of 5-FU (Suppl. Fig. 4C). We found that a combined stress of pIpC treatment and subsequent 5-FU administration resulted in substantially decreased survival of these mice (Suppl. Fig. 4D). Importantly however, there was no difference in survival of pIpC- and 5-FU-treated *Hif-1α$^{fl/fl}$;Mx1-Cre* and control mice. Therefore, *Mx1-Cre*-mediated deletion of *Hif-1α* had no impact on HSC numbers or their ability to respond to 5-FU.

HSCs are constantly exposed to local hypoxia within the BM[14,15] and the low oxygen tension has been proposed to protect HSCs[16]. Takubo et al.[4] found a defect in HSC self-renewal upon *Hif-1α* deletion and suggested that *Hif-1α* is an important mediator of HSC functions. Here we set out to acutely induce deletion of *Hif-1α* in the haematopoietic system and determine its short-term and long-term impact on HSC maintenance. We found that inactivation of *Hif-1α* did not compromise survival of HSCs 2 weeks after gene ablation and unstressed *Hif-1α$^{\Delta/\Delta}$* HSCs self-renewed equally efficiently compared to control counterparts upon serial transplantation. Furthermore, deletion of *Hif-1α* had no long-term consequences for maintenance of unstressed HSCs within 32 weeks after pIpC administration and did not compromise their ability to repopulate recipient mice upon serial transplantation. Different conclusions from our study and that of Takubo et al. may result from discrepancies in experimental designs. While they serially transplanted LSK cells from pIpC-treated *Hif-1α$^{fl/fl}$;Mx1-Cre* mice, we conducted our serial transplantation assays using *Hif-1α$^{\Delta/\Delta}$* LSK cells sorted from pIpC-treated WT chimeric recipient mice harbouring *Hif-1α$^{fl/fl}$;Mx1-Cre* BM cells. Given that *Mx1-Cre* recombines also within the BM microenvironment[12] and that *Hif-1α* is required for BM mesenchymal progenitor cell functions[17], *Hif-1α* deletion from the BM microenvironment

may have affected HSC functions, thus contributing to the phenotypes observed by Takubo et al. Finally, to test the cell-autonomous role of *Hif-1α* in ageing, Takubo et al. transplanted *Hif-1α*$^{fl/fl}$*;Mx1-Cre* BM cells into recipient mice and administered pIpC 4 months later. While they did not observe any differences 4 months after pIpC treatment, 11 months after pIpC treatment they found that the level of repopulation by *Hif-1α*-deficient BM cells was decreased. This raises a possibility that the cell-autonomous *Hif-1α* deficiency causes HSC defects only when exposed to an ageing BM microenvironment.

In conclusion, our data presented here, taken together with our previous findings that *Hif-2α* is dispensable for HSC maintenance[5], suggest that hypoxia impacts on HSC functions in a Hif-1- and Hif-2-independent manner. We conclude that despite the hypoxic nature of the BM microenvironment, self-renewing HSCs do not critically require intrinsic *Hif-1α* to maintain their pool and sustain long-term multilineage haematopoiesis.

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Author Contribution

K.R.K. designed experiments and wrote the manuscript. P.J.R. and T.L.H. helped with experimental designs. M.V., C.Sepulveda and C.Subramani performed all experiments. A.V.G., J.M., L.A., T.I.P., J.P., H.L., A.V., A.D.D., and D.G. helped with experiments.

Conflict of interest

The authors declare no conflict of interest.

Figure Legends

Figure 1. Haematopoiesis-specific deletion of *Hif-1α* does not affect HSC survival and maintenance, and their ability to respond to 5-FU-induced stress. (**A**) Experimental design. 500,000 donor-derived (CD45.2$^+$) unfractionated BM cells from untreated *Hif-1α$^{fl/fl}$;Mx1-Cre* and control (without *Mx1-Cre*) mice were transplanted into lethally irradiated syngeneic CD45.1$^+$/CD45.2$^+$ recipient mice (together with 500,000 CD45.1$^+$ competitor BM cells). Two independent donors were used per genotype. 8 weeks post-transplantation, the mice received 6 sequential doses of pIpC over a period of 10 days (every alternate day) and were analysed 2 weeks after last dose of pIpC. CD45.2$^+$ LSK cells from the primary recipients were serially transplanted into secondary and tertiary recipients. (**B**) Percentage of CD45.2$^+$ donor-derived cells 8 weeks post-transplantation in the peripheral blood (PB) of primary recipient mice prior to pIpC treatment (n=5-6 per group). (**C**) Representative gel showing PCR amplification of genomic DNA from donor-derived CD45.2$^+$ fraction of the PB of pIpC-treated recipient mice 2 weeks after last pIpC injection. WT – wild-type allele, Δ – excised allele, fl – undeleted conditional allele. For PCR controls we used genomic DNA from c-Kit$^+$ cells from BM of *Hif-1α$^{+/+}$*, *Hif-1α$^{fl/fl}$* and *Hif-1α$^{fl/fl}$;Vav-iCre* mice. (**D**) Percentage of CD45.2$^+$ donor-derived cells in PB 2 weeks after the last pIpC injection (n=5-6 per group). (**E**) Percentage of CD45.2$^+$ cells in the unfractionated BM (total BM cells), and myeloid (CD11b$^+$Gr1$^+$), B lymphoid (CD19$^+$B220$^+$) and erythroid (Ter119$^+$) cell compartments of the primary recipient mice. Data are mean ± SEM (n=5-6 per group). (**F**) Percentage of CD45.2$^+$ donor-derived cells measured in BM Lin$^-$Sca-1$^-$c-Kit$^+$ (LK) and Lin$^-$c-Kit$^+$Sca-1$^+$ (LSK) cell compartments of the primary recipients. Data are mean ± SEM (n=5-6 per group). (**G**) Percentage of CD45.2$^+$ donor-derived cells in LSK CD48$^-$CD150$^+$ HSC, LSK CD48$^-$CD150$^-$ MPP, and primitive progenitor (LSK CD48$^+$CD150$^-$ HPC-1 and LSK CD48$^+$CD150$^+$ HPC-2) cell compartments of primary recipients. Data are mean ± SEM (n=5-6 per group). (**H**) PB chimerism in secondary recipients of control and *Hif-1α$^{Δ/Δ}$* LSK cells sorted from BM of the primary recipients. Data are mean ± SEM (n=6 per group). (**I**) Representative gel showing efficient deletion of the conditional alleles of *Hif-1α* in the CD45.2$^+$ BM cells of secondary recipients 16 weeks after transplantation. For PCR controls we used genomic DNA from c-Kit$^+$ cells from BM of *Hif-1α$^{+/+}$*, *Hif-1α$^{fl/fl}$* and *Hif-1α$^{fl/fl}$;Vav-iCre* mice. (**J**) Percentage of CD45.2$^+$ cells in total BM, myeloid, B lymphoid,

and erythroid cell compartments of the secondary recipient mice 16 weeks post-transplantation. Data are mean ± SEM (n=6 per group). (**K**-**L**) Percentage of CD45.2^{+} cells in BM stem and progenitor cell compartments of the secondary recipient mice 16 weeks post-transplantation. Data are mean ± SEM (n=6 per group). (**M**) Results of the tertiary transplantation assay. The graph shows the percentage of tertiary recipients with long-term multilineage reconstitution (>0.5% of donor-derived myeloid and lymphoid cells in BM) 16 weeks after tertiary transplantation of *Hif-1α*$^{\Delta/\Delta}$ and control LSK cells sorted from BM of the secondary recipients. Data are mean ± SEM (n=12-19 per group) (**N**) Percentage of CD45.2^{+} cells in the BM haematopoietic compartments of tertiary recipient mice 16 weeks after transplantation (n=12-19 per group). (**O**) Representative gel showing efficient deletion of the conditional alleles of *Hif-1α* in the CD45.2^{+} BM cells of tertiary recipients 16 weeks after transplantation. For PCR controls we used genomic DNA from c-Kit^{+} cells from BM of *Hif-1α*$^{+/+}$, *Hif-1α*$^{fl/fl}$ and *Hif-1α*$^{fl/fl}$*;Vav-iCre* mice. (**P**) Experimental design. *Hif-1α*$^{fl/fl}$*;Vav-iCre* and control mice received 3 sequential doses of 5-FU (150 mg/kg; 10 days apart) and were analysed 10 days after the last 5-FU administration. In parallel, untreated *Hif-1α*$^{fl/fl}$*;Vav-iCre* and control mice that did not receive 5-FU were also analysed. (**Q**) Kaplan-Meier survival curve of *Hif-1α*$^{fl/fl}$*;Vav-iCre* and control mice treated with 5-FU (i.e. +5-FU) (n=7-8 per group) or those that were not treated with 5-FU (i.e. -5-FU) (n=4-6 per group). Arrows in the graph indicate 5-FU administration. (**R**) Total numbers (per 2 femurs and 2 tibias) of BM white blood cells, LSK cells, HSCs and LK cells from the mice described in Fig. 1P-Q. Data are mean ± SEM (n=4-6 per group). At least two independent experiments were performed for all analyses.

Figure 2. Prolonged *Hif-1α* deficiency does not affect the steady-state maintenance of HSCs and their regenerative capacity.

(**A**) Experimental design. Primary recipient mice were transplanted with 500,000 donor-derived unfractionated BM cells from untreated *Hif-1α$^{fl/fl}$;Mx1-Cre* and control mice (together with 500,000 CD45.1^{+} competitor BM cells). 8 weeks post-transplantation, the mice received 6 doses of pIpC. The mice were analysed 32 weeks after the last dose of pIpC. CD45.2^{+} LSK cells from the primary recipients were transplanted into secondary recipients. 2x10^{6} total BM cells from secondary recipients were transplanted into tertiary recipients together with 200,000 CD45.1^{+} unfractionated BM cells. (**B**) CD45.2^{+} donor-derived PB chimerism 20, 24, 28 and 32 weeks after the last dose of pIpC. Mean ± SEM (n=5-7 per group). (**C**) Percentage of CD45.2^{+} cells in total BM, myeloid, B lymphoid, and erythroid cell compartments of primary recipients 32 weeks after pIpC treatment. Data are mean ± SEM (n=5-7 per group). (**D-E**) Percentage of CD45.2^{+} cells in the indicated haematopoietic compartments of the primary recipients. Data are mean ± SEM (n=5-7 per group). (**F**) LSK cells of indicated genotypes were pooled from primary recipients 32 weeks after pIpC treatment and transplanted into secondary recipients. The graph shows CD45.2^{+} donor-derived PB chimerism in secondary recipient mice 4, 12 and 16 weeks after transplantation. Data are mean ± SEM (n=4-7 per group). (**G**) CD45.2^{+} donor-derived chimerism within the total BM cell fraction, and myeloid, B lymphoid and erythroid cell compartments of secondary recipient mice 16 weeks after transplantation. Data are mean ± SEM (n=4-7 per group). (**H**) Percentage of CD45.2^{+} cells in the indicated haematopoietic stem and progenitor cell compartments of the secondary recipients. Mean ± SEM (n=4-7 per group). (**I**) PCR amplification of genomic DNA from donor-derived CD45.2^{+} cell fraction of the PB of secondary recipient mice 12 weeks post-transplantation. For PCR controls we used genomic DNA from c-Kit^{+} cells from BM of *Hif-1α$^{+/+}$*, *Hif-1α$^{fl/fl}$* and *Hif-1α$^{fl/fl}$;Vav-iCre* mice. (**J**) Results of the tertiary transplantation assays. The graph depicts the percentage of tertiary recipients with long-term multilineage reconstitution (>0.5% of donor-derived myeloid and lymphoid cells in PB) 16 weeks after tertiary transplantation of 2x10^{6} total BM cells obtained from the secondary recipients (n=5-10 per genotype). (**K**) Percentage of CD45.2^{+} cells in white blood cell (total) compartment, and myeloid, B lymphoid, and T lymphoid cell compartments of PB in tertiary recipients 16 weeks after transplantation. Data are mean ± SEM (n=5-10 per

group). (**L**) *Hif-1α* gene deletion was confirmed by PCR on genomic DNA from donor-derived CD45.2^{+} cell fraction of the BM of tertiary recipients 16 weeks post-transplantation. For PCR controls we used genomic DNA from c-Kit^{+} cells from BM of *Hif-1α*$^{+/+}$, *Hif-1α*$^{fl/fl}$ and *Hif-1α*$^{fl/fl}$*;Vav-iCre* mice. At least two independent experiments were performed for all analyses.

References

1. Ding L, Morrison SJ. Haematopoietic stem cells and early lymphoid progenitors occupy distinct bone marrow niches. *Nature*. 2013;495(7440):231-235.
2. Ding L, Saunders TL, Enikolopov G, Morrison SJ. Endothelial and perivascular cells maintain haematopoietic stem cells. *Nature*. 2012;481(7382):457-462.
3. Morrison SJ, Scadden DT. The bone marrow niche for haematopoietic stem cells. *Nature*. 2014;505(7483):327-334.
4. Takubo K, Goda N, Yamada W, et al. Regulation of the HIF-1alpha level is essential for hematopoietic stem cells. *Cell Stem Cell*. 2010;7(3):391-402.
5. Guitart AV, Subramani C, Armesilla-Diaz A, et al. Hif-2alpha is not essential for cell-autonomous hematopoietic stem cell maintenance. *Blood*. 2013;122(10):1741-1745.
6. Ryan HE, Poloni M, McNulty W, et al. Hypoxia-inducible factor-1alpha is a positive factor in solid tumor growth. *Cancer Res*. 2000;60(15):4010-4015.
7. Kuhn R, Schwenk F, Aguet M, Rajewsky K. Inducible gene targeting in mice. *Science*. 1995;269(5229):1427-1429.
8. de Boer J, Williams A, Skavdis G, et al. Transgenic mice with hematopoietic and lymphoid specific expression of Cre. *Eur J Immunol*. 2003;33(2):314-325.
9. Kranc KR, Schepers H, Rodrigues NP, et al. Cited2 is an essential regulator of adult hematopoietic stem cells. *Cell Stem Cell*. 2009;5(6):659-665.
10. Mortensen M, Soilleux EJ, Djordjevic G, et al. The autophagy protein Atg7 is essential for hematopoietic stem cell maintenance. *The Journal of Experimental Medicine*. 2011;208(3):455-467.
11. Calaminus SD, Guitart AV, Sinclair A, et al. Lineage tracing of Pf4-Cre marks hematopoietic stem cells and their progeny. *PLoS One*. 2012;7(12):e51361.
12. Walkley CR, Shea JM, Sims NA, Purton LE, Orkin SH. Rb regulates interactions between hematopoietic stem cells and their bone marrow microenvironment. *Cell*. 2007;129(6):1081-1095.
13. Joseph C, Quach JM, Walkley CR, Lane SW, Lo Celso C, Purton LE. Deciphering hematopoietic stem cells in their niches: a critical appraisal of genetic models, lineage tracing, and imaging strategies. *Cell Stem Cell*. 2013;13(5):520-533.
14. Spencer JA, Ferraro F, Roussakis E, et al. Direct measurement of local oxygen concentration in the bone marrow of live animals. *Nature*. 2014;508(7495):269-273.
15. Parmar K, Mauch P, Vergilio JA, Sackstein R, Down JD. Distribution of hematopoietic stem cells in the bone marrow according to regional hypoxia. *Proc Natl Acad Sci U S A*. 2007;104(13):5431-5436.
16. Mantel CR, O'Leary HA, Chitteti BR, et al. Enhancing Hematopoietic Stem Cell Transplantation Efficacy by Mitigating Oxygen Shock. *Cell*. 2015;161(7):1553-1565.
17. Guarnerio J, Coltella N, Ala U, Tonon G, Pandolfi PP, Bernardi R. Bone Marrow Endosteal Mesenchymal Progenitors Depend on HIF Factors for Maintenance and Regulation of Hematopoiesis. *Stem Cell Reports*. 2014;2(6):794-809.

Figure 1

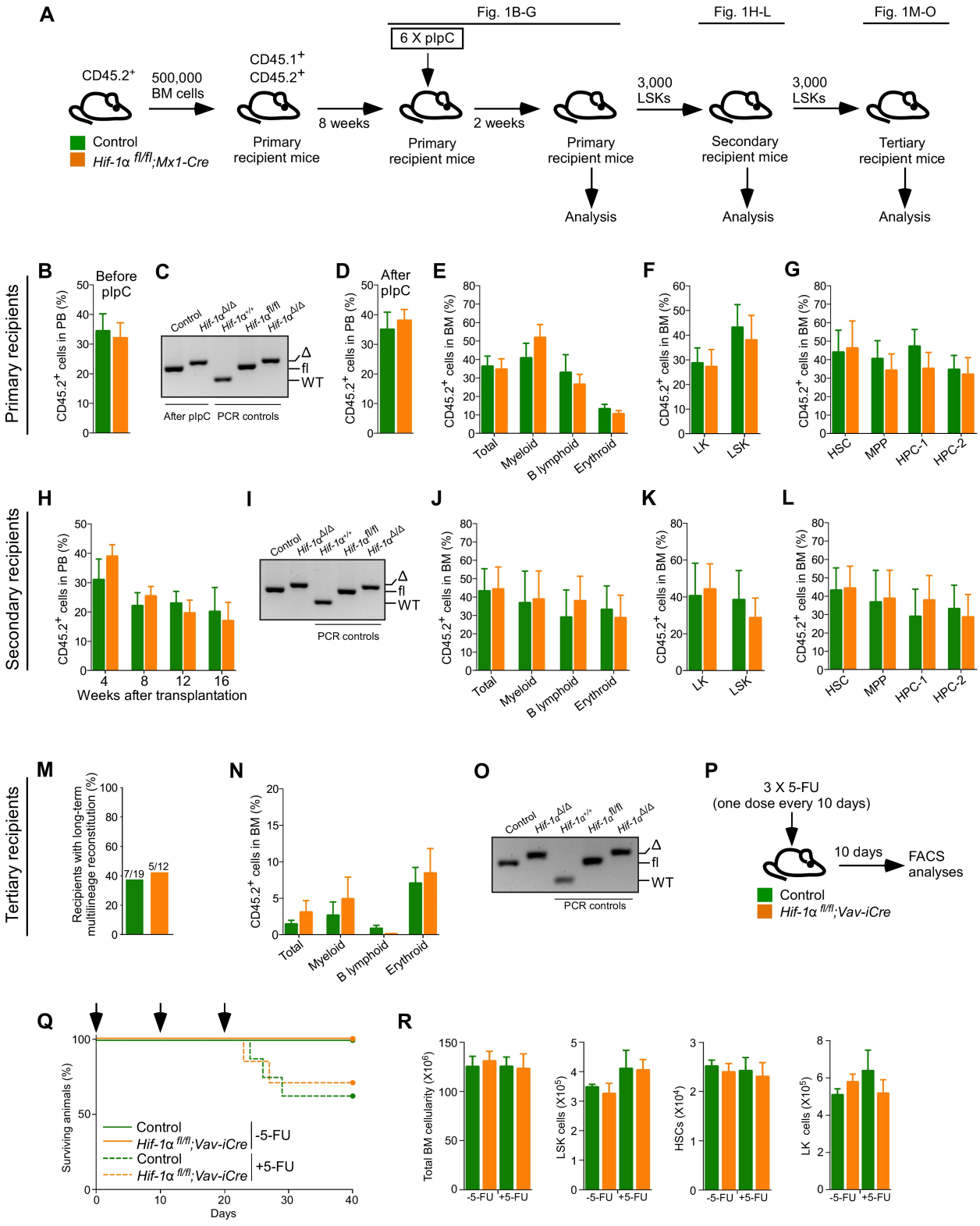
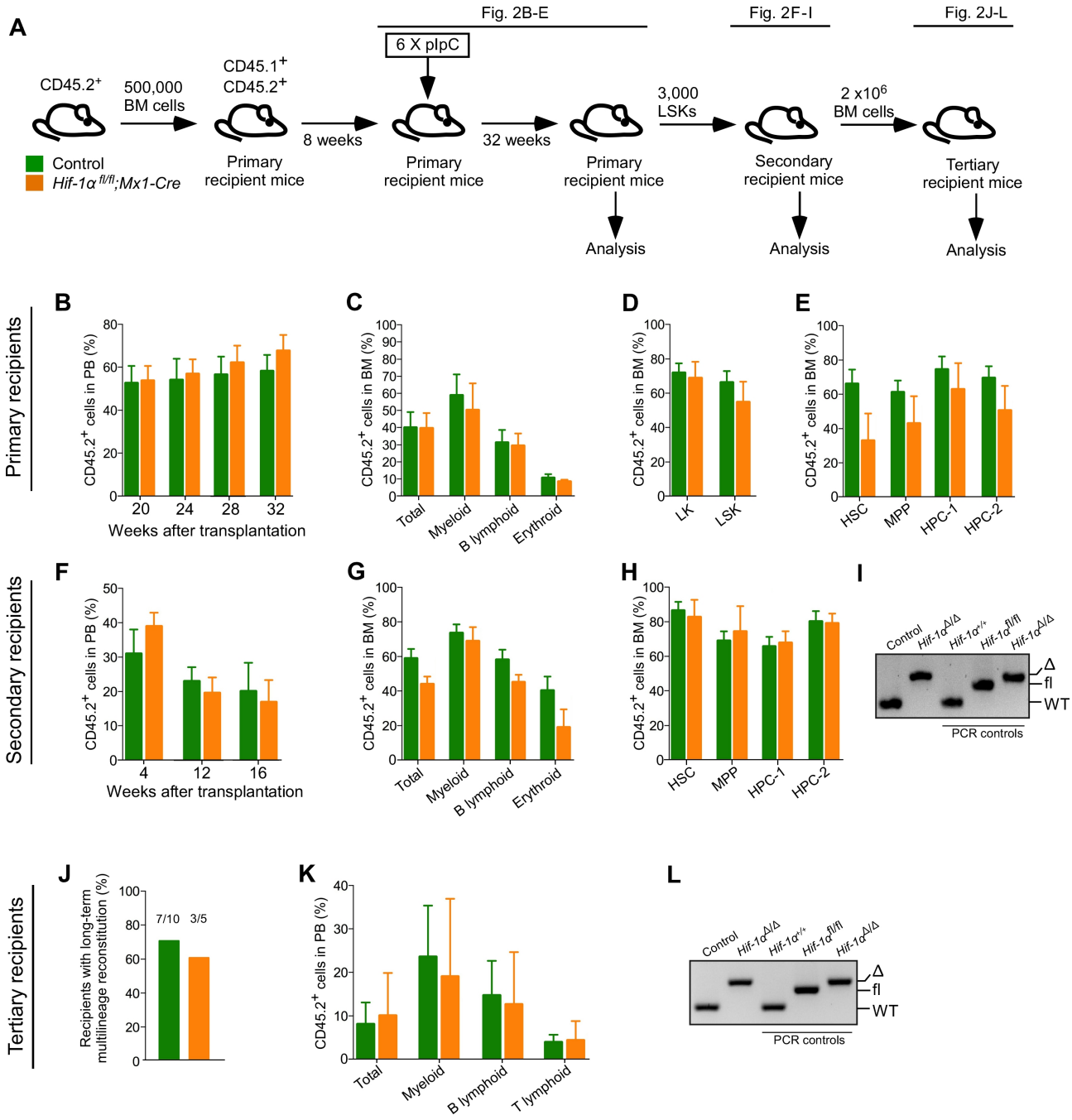
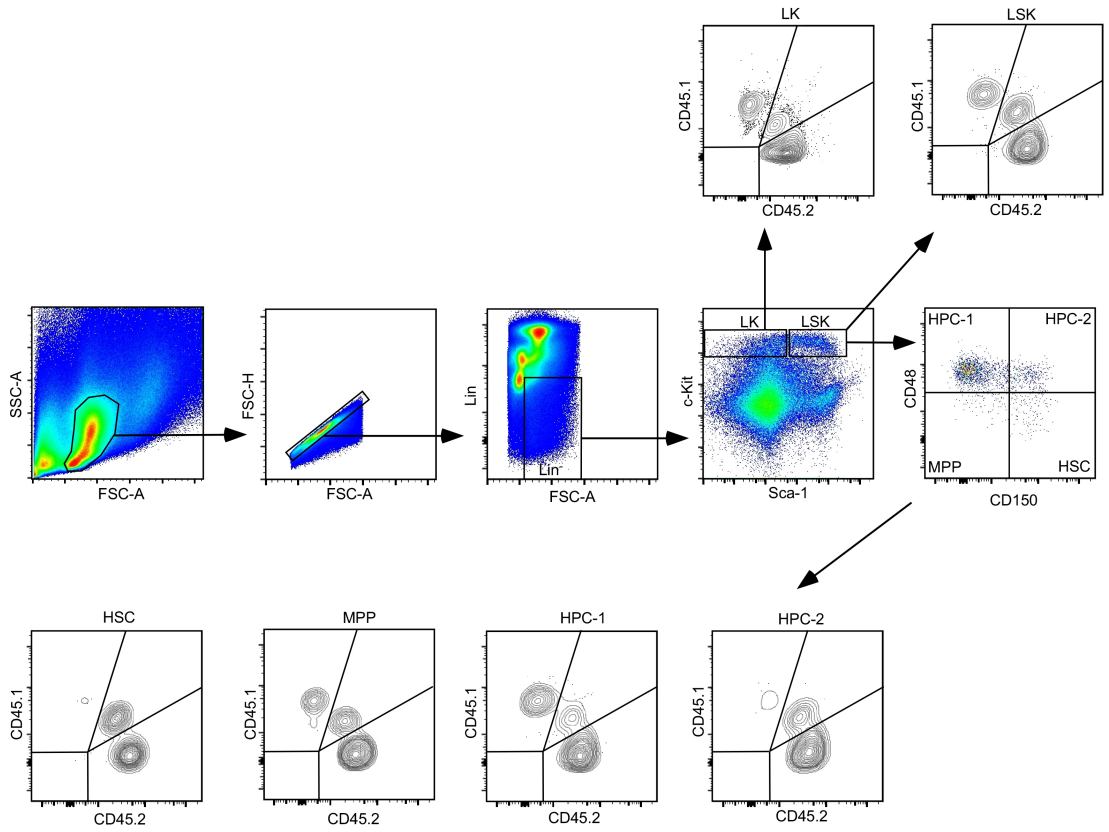

A
Fig. 1B-G
Fig. 1H-L
Fig. 1M-O
6 X pIpC
CD45.2+
500,000 BM cells
CD45.1+ CD45.2+
8 weeks
2 weeks
3,000 LSKs
3,000 LSKs
Control
Hif-1α fl/fl;Mx1-Cre
Primary recipient mice
Primary recipient mice
Primary recipient mice
Secondary recipient mice
Tertiary recipient mice
Analysis
Analysis
Analysis
Primary recipients
B
Before pIpC
CD45.2+ cells in PB (%)
C
Control
Hif-1α Δ/Δ
Hif-1α +/+
Hif-1α fl/fl
Hif-1α Δ/Δ
Δ
fl
WT
After pIpC
PCR controls
D
After pIpC
CD45.2+ cells in PB (%)
E
CD45.2+ cells in BM (%)
Total
Myeloid
B lymphoid
Erythroid
F
CD45.2+ cells in BM (%)
LK
LSK
G
CD45.2+ cells in BM (%)
HSC
MPP
HPC-1
HPC-2
Secondary recipients
H
CD45.2+ cells in PB (%)
4
8
12
16
Weeks after transplantation
I
Control
Hif-1α Δ/Δ
Hif-1α +/+
Hif-1α fl/fl
Hif-1α Δ/Δ
Δ
fl
WT
PCR controls
J
CD45.2+ cells in BM (%)
Total
Myeloid
B lymphoid
Erythroid
K
CD45.2+ cells in BM (%)
LK
LSK
L
CD45.2+ cells in BM (%)
HSC
MPP
HPC-1
HPC-2
Tertiary recipients
M
Recipients with long-term multilineage reconstitution (%)
7/19
5/12
N
CD45.2+ cells in BM (%)
Total
Myeloid
B lymphoid
Erythroid
O
Control
Hif-1α Δ/Δ
Hif-1α +/+
Hif-1α fl/fl
Hif-1α Δ/Δ
Δ
fl
WT
PCR controls
P
3 X 5-FU
(one dose every 10 days)
10 days
FACS analyses
Control
Hif-1α fl/fl;Vav-iCre
Q
Surviving animals (%)
Control
Hif-1α fl/fl;Vav-iCre
-5-FU
Control
Hif-1α fl/fl;Vav-iCre
+5-FU
Days
R
Total BM cellularity (X10^6)
LSK cells (X10^5)
HSCs (X10^4)
LK cells (X10^5)
-5-FU
+5-FU

Figure 2


A
Fig. 2B-E
Fig. 2F-I
Fig. 2J-L
6 X pIpC
CD45.2+
500,000 BM cells
CD45.1+ CD45.2+
8 weeks
32 weeks
3,000 LSKs
2 x10^6 BM cells
Control
Hif-1α fl/fl;Mx1-Cre
Primary recipient mice
Secondary recipient mice
Tertiary recipient mice
Analysis
Primary recipients
Secondary recipients
Tertiary recipients
B
C
D
E
F
G
H
I
J
K
L
CD45.2+ cells in PB (%)
CD45.2+ cells in BM (%)
Weeks after transplantation
Total
Myeloid
B lymphoid
Erythroid
LK
LSK
HSC
MPP
HPC-1
HPC-2
Recipients with long-term multilineage reconstitution (%)
7/10
3/5
T lymphoid
Control
Hif-1α Δ/Δ
Hif-1α +/+
Hif-1α fl/fl
Δ
fl
WT
PCR controls

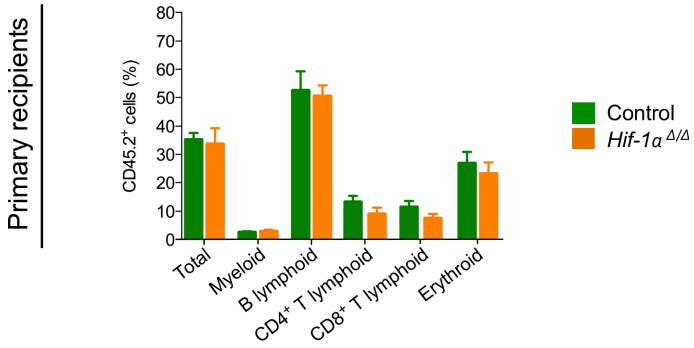
Antibody		Conjugate	Catalog #	Clone	Manufacturer
Lineage Cocktail	CD4	biotin	553649	H129.19	BD Biosciences
	CD5	biotin	553019	53-7.3	BD Biosciences
	CD8a	biotin	553029	53-6.7	BD Biosciences
	CD11b	biotin	553309	M1/70	BD Biosciences
	CD45R/B220	biotin	553086	RA3-6B2	BD Biosciences
	Ter119	biotin	553672	TER-119	BD Biosciences
	Gr-1/Ly-6G/C	biotin	553125	RB6-8C5	BD Biosciences
	CD45.1	FITC	110706	A20	Biolegend
	Ter119	FITC	116206	TER-119	Biolegend
	CD4	PE	130310	H129.19	Biolegend
	CD45R / B220	PE	103208	RA3-6B2	Biolegend
	CD48	PE	103406	HM48-1	Biolegend
	CD150	PE-Cy7	115914	TC15-12F12.2	Biolegend
	Gr-1/Ly-6G/C	PE-Cy7	108416	RB6-8C5	Biolegend
	CD8a	APC	100712	53-6.7	Biolegend
	CD11b	APC	101212	M1/70	Biolegend
	CD117/c-Kit	APC	105812	2B8	Biolegend
	Sca-1/Ly-6A/E	APC-Cy7	122513	E13-161.7	Biolegend
	CD19	APC-Cy7	115530	6D5	Biolegend
	CD45.2	Pacific Blue	109820	104	Biolegend
	CD16/CD32 (FC Block™)	None	553142	2.4G2	BD Pharmingen

Supplemental Table 1. List of antibodies used for flow cytometry.



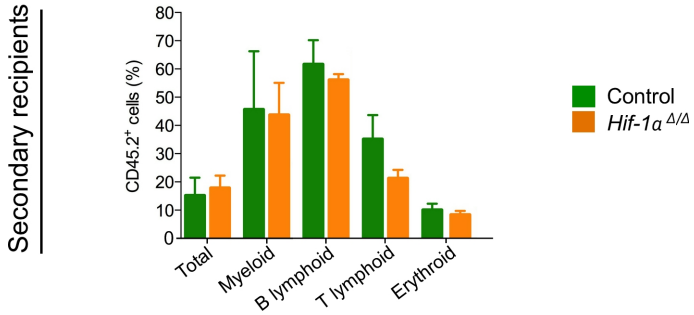
Supplemental Figure 1. Representative gating strategy for FACS analyses of recipient mice transplanted with BM or LSK cells.

Total BM cells were first live and size gated using forward scatter area (FSC-A) and side scatter area (SSC-A). Doublets were excluded using FSC-A versus FSC height (FSC-H). Lineage positive cells were excluded by gating on the lineage negative (Lin^-) population which contains the LK and LSK cells. LSK cells were further sub-gated on HSC, MPP, HPC-1, and HPC-2 fractions. Within each population, $CD45.2^+$ cells were gated by excluding the bone marrow support ($CD45.1^+$) and recipient-derived haematopoietic cells remaining after irradiation ($CD45.1^+/CD45.2^+$).



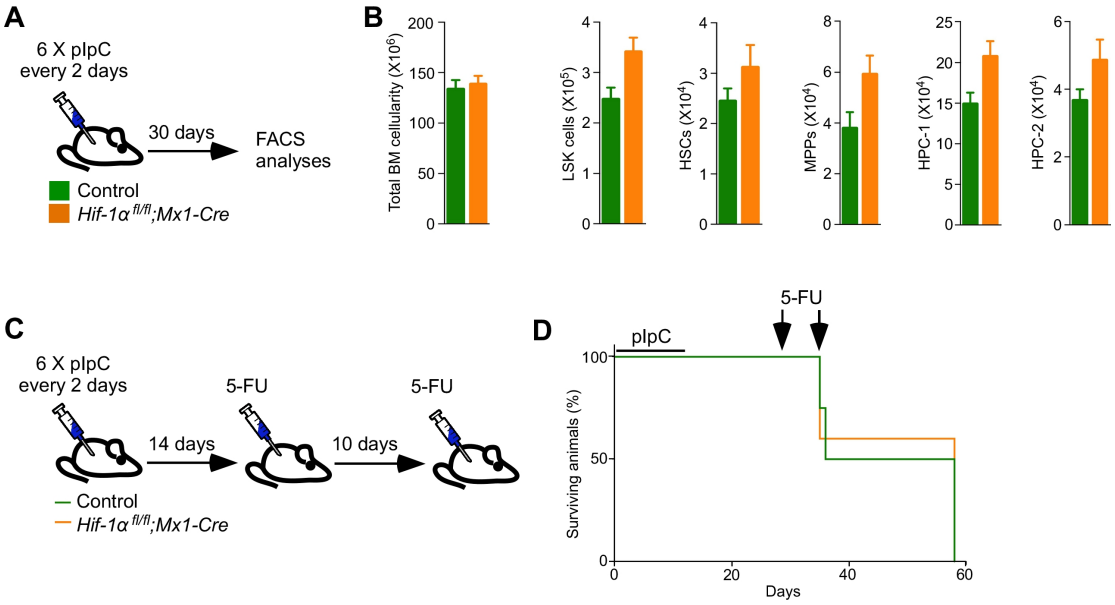
Supplemental Figure 2. Primary transplantation assay.

Percentage of CD45.2$^+$ cells in the unfractionated splenocytes (total), and myeloid (CD11b$^+$Gr1$^+$), B lymphoid (CD19$^+$B220$^+$), T lymphoid (CD4$^+$ and CD8$^+$) and erythroid (Ter119$^+$) cell compartments in spleens of the primary recipient mice. Data are mean ± SEM (n=5-6 per group).



Supplemental Figure 3. Secondary transplantation assay.

CD45.2$^+$ cell chimerism in the indicated spleen cell compartments of the secondary recipient mice 16 weeks post-transplantation. Data are mean ± SEM (n=6 per group).



Supplemental Figure 4. Deletion of *Hif-1α* in *Hif-1α$^{fl/fl}$;Mx1-Cre* mice.

(A) Experimental design. *Hif-1α$^{fl/fl}$;Mx1-Cre* and control mice received 6 doses of pIpC over a period of 10 days (every alternate day). 30 days after the last pIpC treatment mice were analysed. (B) BM analysis of pIpC-treated *Hif-1α$^{fl/fl}$;Mx1-Cre* and control mice showing total numbers of BM white blood cells, LSK cells, HSCs, MPPs, and HPC-1 and HPC-2 populations. Data are mean ± SEM (n=4-5 per group). (C) Experimental design. *Hif-1α$^{fl/fl}$;Mx1-Cre* and control mice received 6 doses of pIpC every alternate day. 14 days after the last pIpC treatment mice were injected with two doses of 5-FU, 10 days apart. (D) Kaplan-Meier survival curve of *Hif-1α$^{fl/fl}$;Mx1-Cre* and control mice treated with both pIpC and 5-FU (n=7-8 per group).