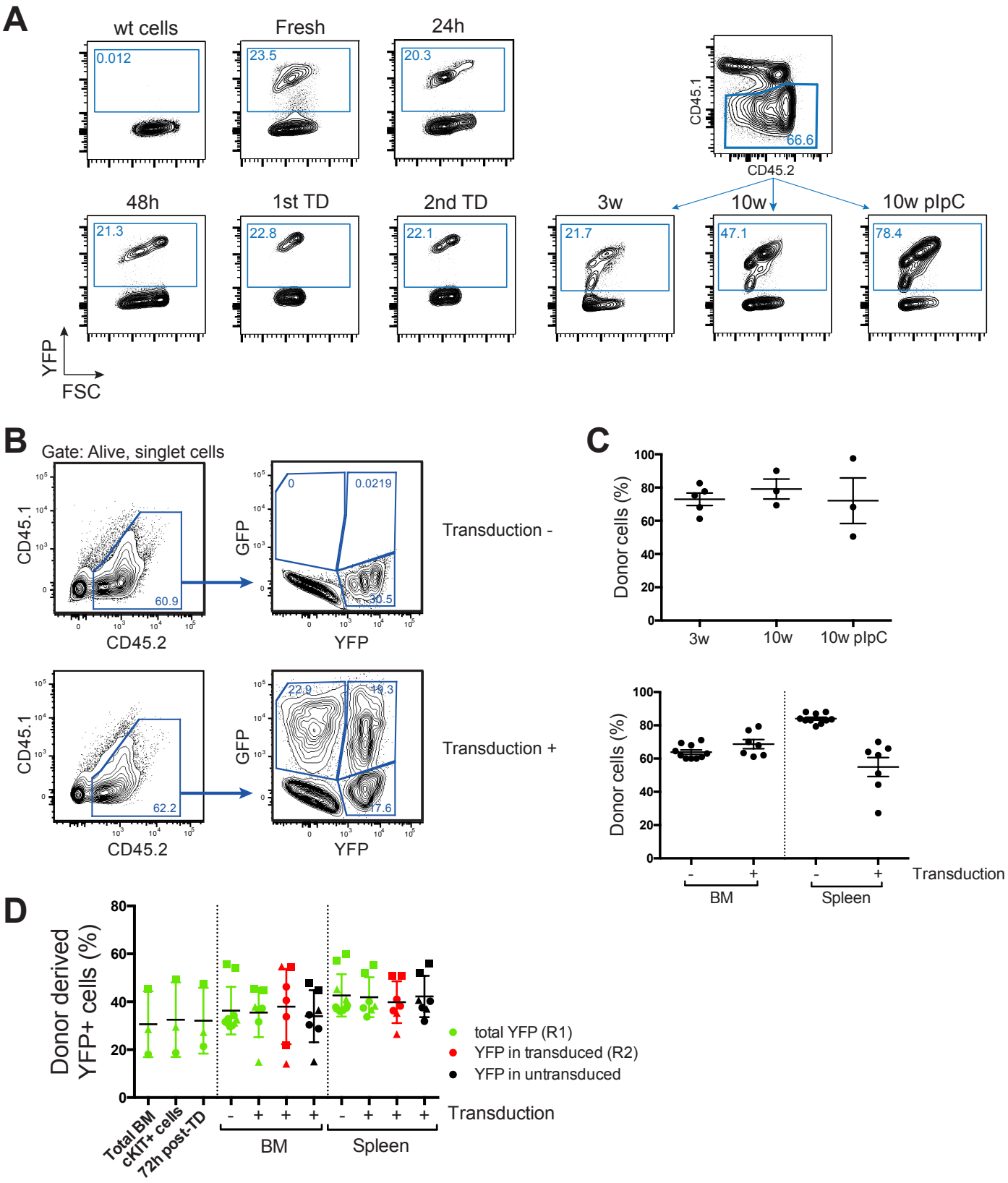
Stem Cell Reports, Volume *7*

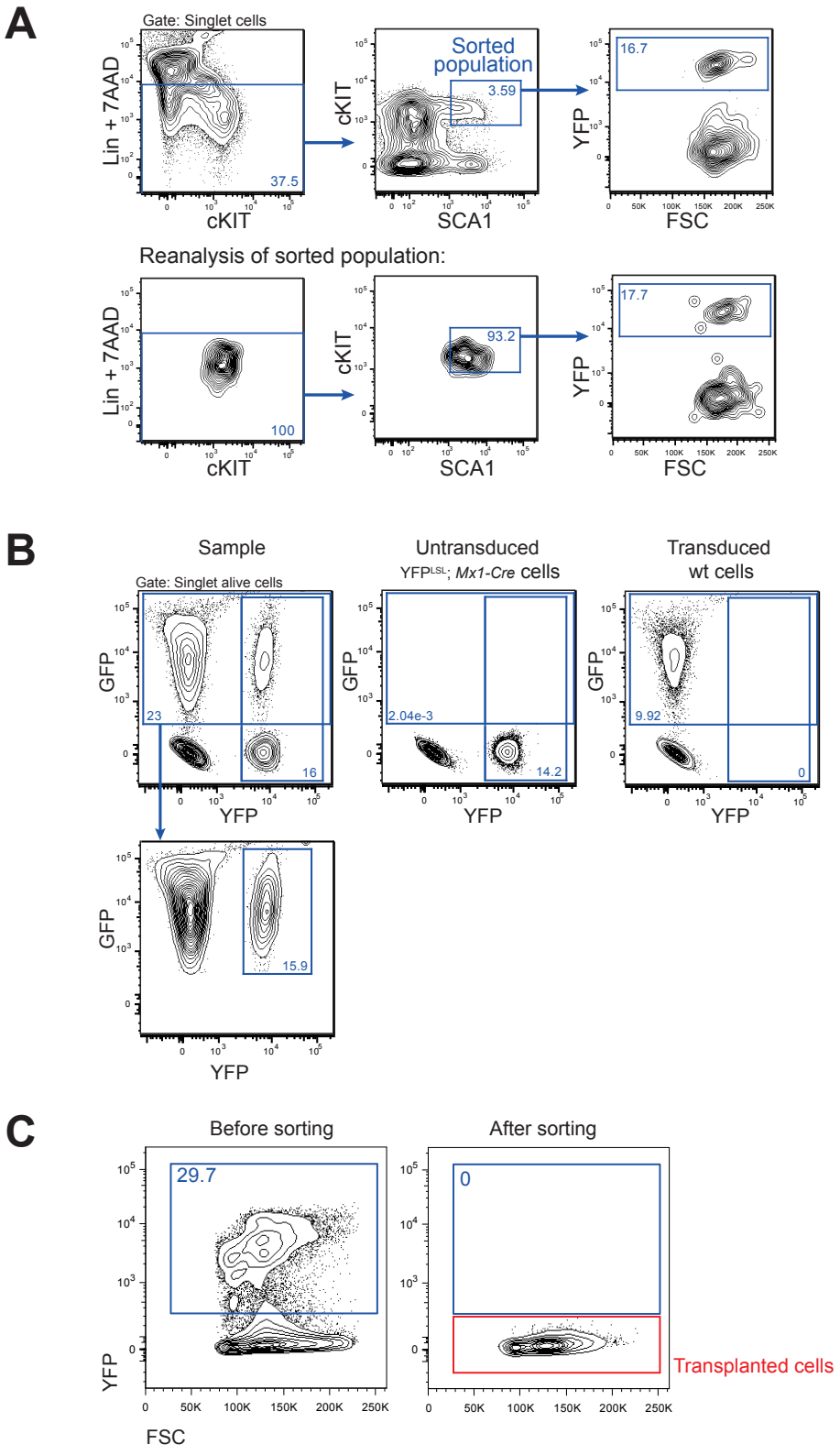
Supplemental Information

Potential Pitfalls of the *Mx1-Cre* System: Implications for Experimental Modeling of Normal and Malignant Hematopoiesis

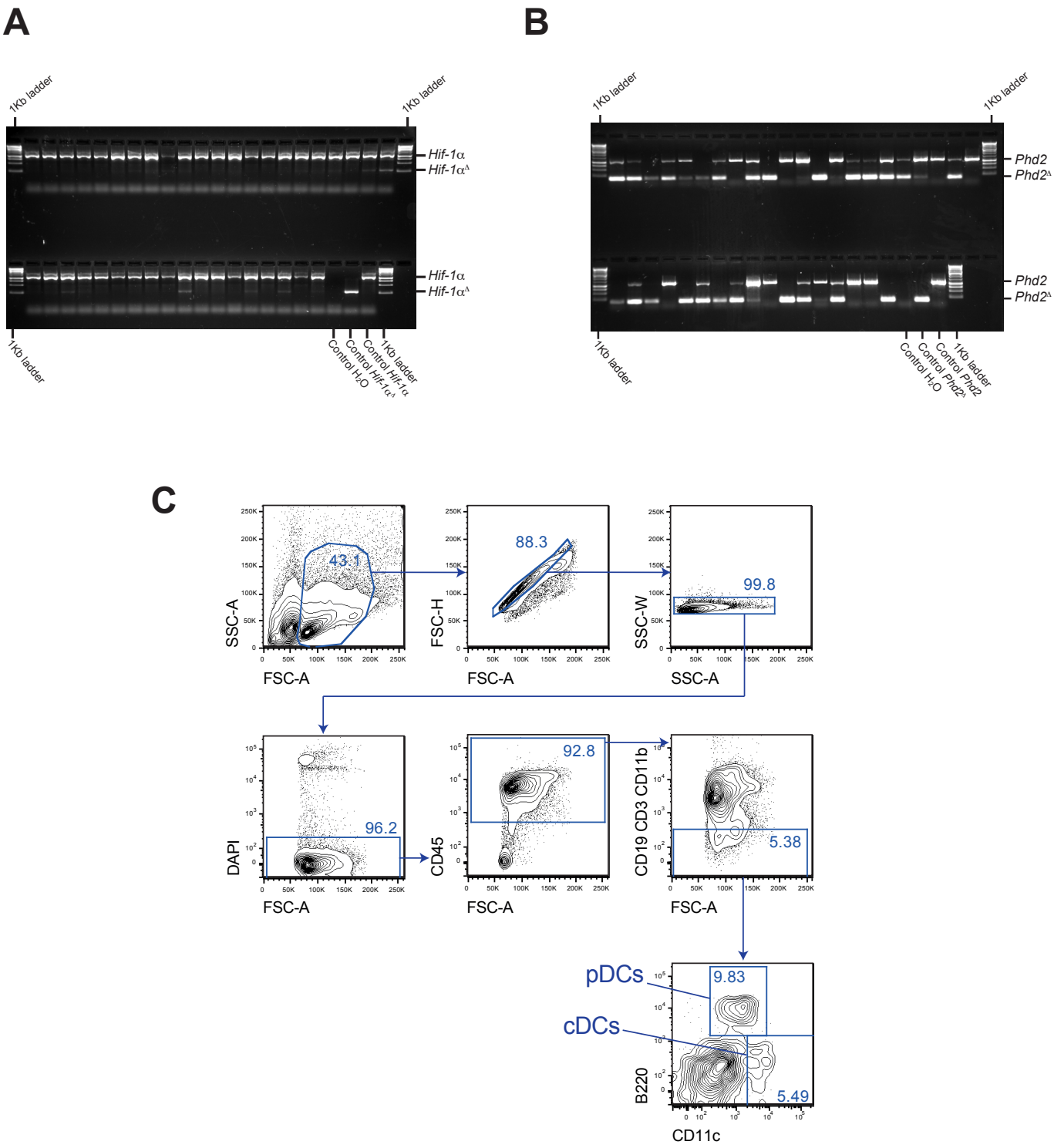
Talia Velasco-Hernandez, Petter Säwén, David Bryder, and Jörg Cammenga



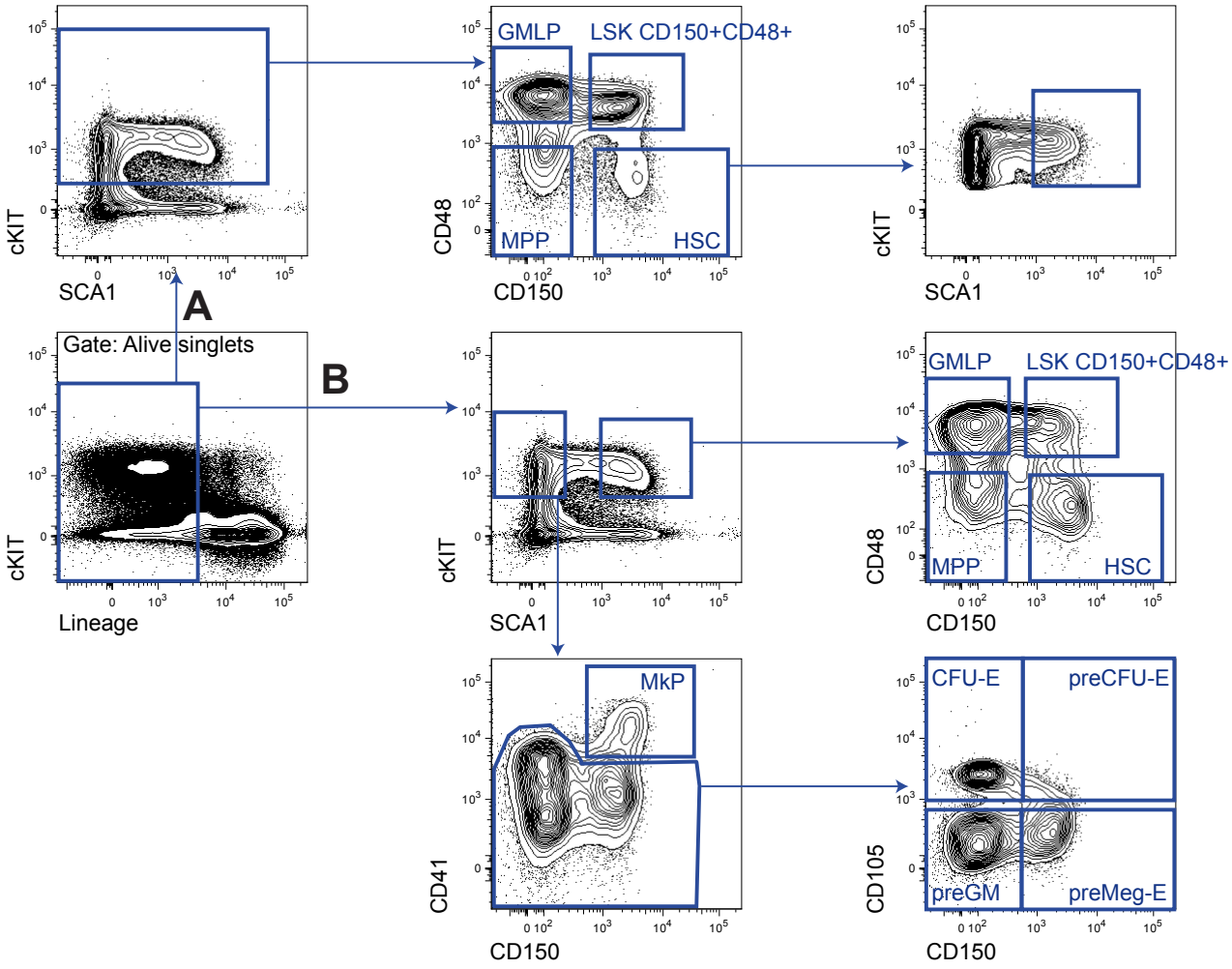
Supplemental Figure S1, related to Figure 1. (A) Flow cytometry gating used to identify YFP+ cells in Figure 1C. (B) Flow cytometry gating used to identify the different populations in Figure 1G. (C) Donor contribution in the analyzed samples in Figures 1C and 1G, respectively. (D) Recombined (YFP+) percentage of cells corresponding to normalized data of Figure 1H. Different shapes (squares, circles and triangles) indicate each used donor and the recipient mice transplanted with it. Plots show mean ± SEM. TD: transduction.



Supplemental Figure S2, related to Figure 1. (A) Flow cytometry gatings used to identify the LSK population during the sorting and reanalysis of the sorted cells in Figure 1D. (B) Flow cytometry gatings used to identify the recombined cells (YFP+) and transduced cells (GFP+) after the transduction and culture of the LSK cells. (C) YFP negative cells sorted and used for transplantation in Figure 1H. Cells derived from one donor mouse are shown.



Supplemental Figure S3, related to Figure 2. (A) Colony analysis derived from cKO-*Hif-1α* fetal liver cells. Fetal liver cells (E14.5) from *Hif-1α*$^{flox/flox}$; *Mx1-Cre*; *Flt-3*^{ITD} embryos were plated in methylcellulose medium. After 7 days, colonies were picked and genomic DNA from individual colonies analyzed by PCR for *Hif-1α* deletion (n=40). PCR results from colonies derived from one of the analyzed embryos are shown. (B) Colony analysis derived from cKO-*Phd2* BM cells. BM cells from 12-week-old *Phd2*$^{flox/flox}$; *Mx1-Cre*; *Flt-3*^{ITD} mice were plated in methylcellulose medium. After 7 days, colonies were picked and genomic DNA from individual colonies analyzed by PCR for *Phd2* deletion (n=39-40). PCR results from colonies derived from one of the analyzed mice are shown. (C) Gating strategy used for the identification of dendritic cells. For the identification of plasmocytoid dendritic cells (pDCs) and classical dendritic cells (cDCs) we used the above gating strategy: cell-size events, singlets (FSC-A vs FSC-H), singlets (SSC-A vs SSC-W), alive cells (DAPI^{-}), CD45^{+} cells, lineage negative cells (CD19^{-}, CD3^{-} and CD11b^{-}) and CD11c and B220 expression.



Supplemental Figure S4, related to Figure 3. Gating strategy used for the identification of the different subsets of HSPCs. FACS plots derived form a control mouse (day 1, no pIpC treatment) are shown. The strategy A was used in Figure 3D for the analysis of SCA1 expression in HSCs and GMLPs. The strategy B was used in Figure 3A-C for the identification of the different subsets of HSPCs. HSC, hematopoietic stem cell; GMLP, granulocyte/macrophage lymphoid progenitors; preGM, pre-granulocyte/macrophage progenitors; MkP, megakaryocyte progenitor; preMegE, pre-megakaryocyte–erythrocyte progenitors; preCFU-E, pre-colony forming units-erythrocytes; CFU-E, colony forming units-erythrocytes; MPP, multipotent progenitor; LSK, Lin-SCA1+cKIT+.

Supplemental Experimental Procedures

Cell culture

Colony assay and clonal analysis: Peripheral blood (PB), BM or fetal liver samples were harvested at indicated time points, erythrocytes lysed with ammonium chloride (Stem Cell Technologies) (PB samples), plated into methylcellulose (Methocult GM3434, Stem Cell Technologies) and incubated for 7 days at 37°C. Single colonies were picked and genomic DNA extracted. PCR was performed to identify the deletion of *Hif-1α* or *Phd2*.

In vitro culture conditions: cKIT+ or LSK cells were stimulated for transduction in StemSpan SFEM medium (StemCell Technologies) supplemented with penicillin/streptomycin (Invitrogen), mIL3 (20 ng/mL), hIL6 (50 ng/mL), hTPO (50 ng/mL) and mSCF (50 ng/mL).

Tat-Cre recombination: For *in vitro* Cre recombination, cells were washed and incubated for 4 hours at 37°C in 900 ml StemSpan (StemCell Technologies) supplemented with mSCF (100 ng/ml) and hTPO (20 ng/ml) (Peprotech), 50 μM β-mercaptoethanol and penicillin/streptomycin (Invitrogen) with 50 μg/ml recombinant Tat-Cre (Excellgen). In the case of *Col1a1*-tetO-MLL/ENL x $KRAS^{LSL-G12D}$ cells, 1 mg/ml doxycycline (Sigma) was also added. In the case of $TdTomato^{LSL}$ cells, TdTomato expression was evaluated after 3 days in culture.

HSC transplantation

AML1-ETO9a and MLL-AF9: Transduction and transplantation of these models were performed as previously described (Velasco-Hernandez et al., 2014).

YFP^{LSL} cells: For analysis of spontaneous recombination after transplantation, BM c-kit+ cells were isolated using a magnetic separation system (MACS) (Miltenyi Biotech). Transduction of cells was performed as described previously (Velasco-Hernandez et al, 2014) with RFP viruses (generated from a producer cell line, 293GPG-pCAG-RFP-WPRE) or GFP viruses (MIGR1 empty retroviral vector). After transduction, $1x10^6$ cells were transplanted together with $1x10^5$ support cells (no supporting cells in figure 1G) by tail-vein injection into lethally irradiated wt mice (850-900 cGy). For analysis of Tat-Cre recombination, LSK cells were isolated by sorting and transplanted (10,000 LSKs/recipient) into CD45.1 irradiated recipients (850 cGy) without supporting cells.

MLL-ENL x $KRAS^{LSL-G12D}$ cells: sorted HSCs (lineage-negative, $SCA1^+$, $cKIT^+$, $CD150^+$ and $CD48^-$) from *Col1a1*-tetO-MLL/ENL x $KRAS^{LSL-G12D}$ mice (CD45.1-CD45.2) were transplanted (450 cells/recipient) together with $3x10^5$ unfractionated BM support/competitor cells (CD45.1) into lethally irradiated (950 cGy) C57BL/6 mice. Recipient mice were administered doxycycline containing food pellets 5 days prior to transplantation (2 g/kg, Ssniff Spezialdiäten GmbH) and throughout the experiment.

Flow cytometry

For YFP^{LSL} cell analysis, transduction rate and Cre-mediated recombination (YFP^+ cells) were monitored by flow cytometry of BM cells. For dendritic cell analysis the following antibodies were used: CD45.2-FITC (104), CD3e-PE (142-2C11), Gr-1-PE (RB6-8C5), CD11b-PE (M1/70), B220-APC (RA3-6B2) (BioLegend) and CD19-PE (1D3), CD11c-PECy7 (HL3) (BD Biosciences). DAPI (Sigma) was used to exclude dead cells. For HSPCs phenotype analysis and sorting, HSPCs were isolated as described (Ugale et al., 2014). Shortly, the following antibodies were used: CD4 (GK1.5), CD8a (53-6.7), Gr1 (RB6-8C5), CD11b (M1/70), B220 (RA3-6B2), Ter119 (Ter-119), cKIT (2B8), SCA1 (D7), CD150 (TC15-12F12.2), CD48 (HM 48-1), CD41 (MWReg30), CD105 (MJ7/18), CD45.1 (A20) (BioLegend), and CD45.2 (104) (eBioscience). Samples were analyzed using a FACSCantoII, a LSRII, a LSRFortessa (BD Biosciences) or a FACS Aria III cell sorter (BD Biosciences) and analyzed with FlowJo software (TreeStar).