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Supplemental Information

A Novel GATA2 Protein Reporter Mouse Reveals Hematopoietic Progenitor Cell Types

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Supplemental information:

Supplemental Figures:

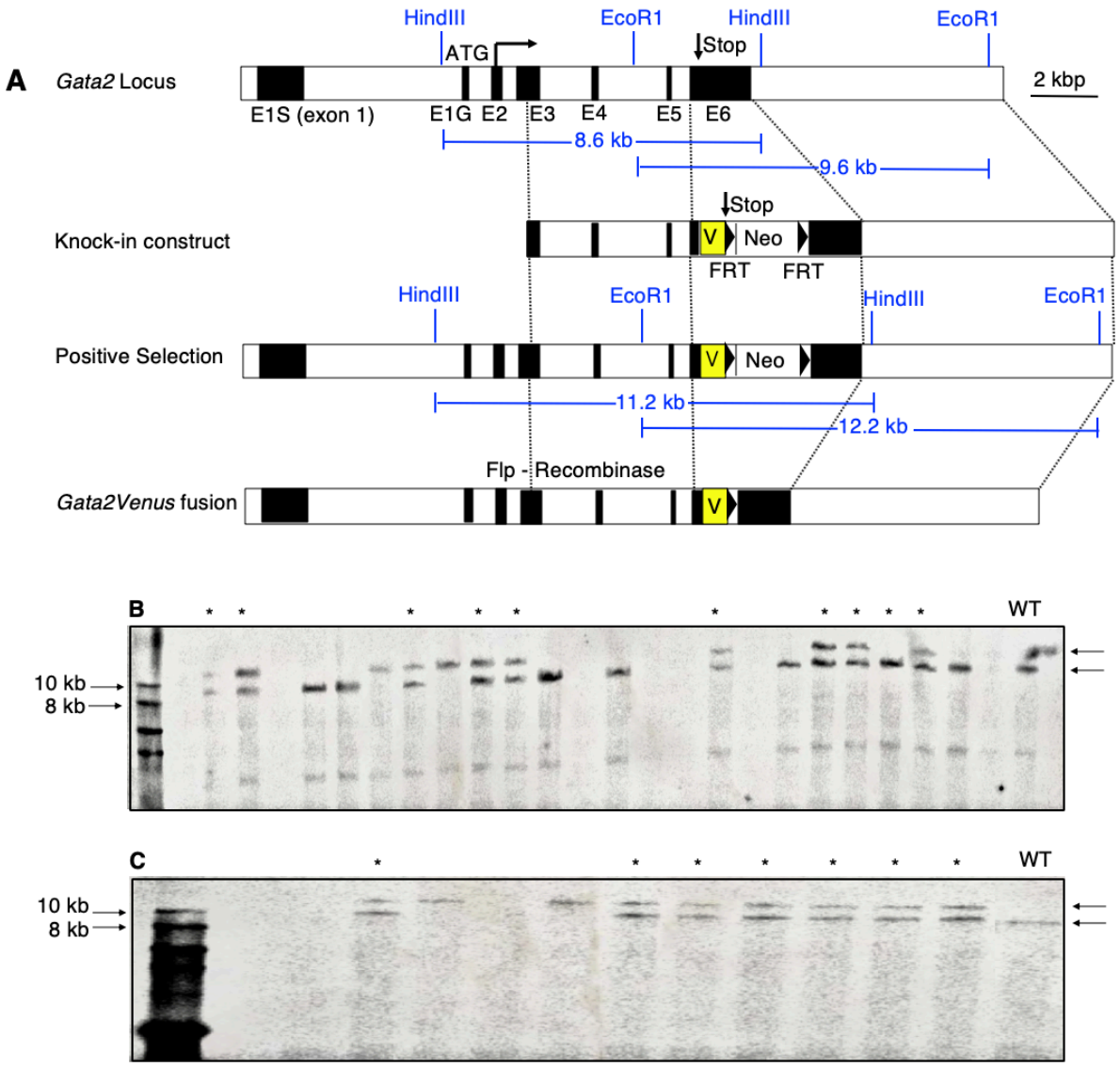


Figure S1. Correct integration of *Gata2Venus* cassette (see main figure 1). (A) Schematic illustration of generation of *Gata2* allele with *Venus* fusion before STOP codon in Exon VI. Restriction sites and expected band size for 5′ and 3′ southern blot probes are shown in blue. **(B)** 5′ end southern blot image to show correct integration of *Gata2Venus* cassette in mouse genome. The genomic DNA was digested with HindIII and hybridized with a 5′ probe. Correct 5′ end integration was confirmed in 10 ESC clones (indicated with stars). Top band (11261 bp) indicates knock-in allele and bottom band (8643 bp) indicates wild-type allele. **(C)** 3′ end southern blot image to highlight correct integration of *Gata2Venus* cassette in mouse genome. The genomic DNA was digested with EcoRI and hybridized with a 3′ probe (grey bar). 7 out of 10 clones exhibited correct 3′ integration (indicated with stars). Top band (12286 bp) indicates knock-in allele and bottom band (9671 bp) indicates wild-type allele. Arrows in **B** and **C** indicate position of DNA marker (left) and genomic DNA bands (right). (see main figure 1)

Type of cross	Pups born	Expected genotype frequency			Observed genotype frequency		
		homo	het	wt	homo	het	wt
homo X wt	24	0	1	0	0	1	0
het X wt	29	0	0.5	0.5	0	0.45	0.55
het X het	22	0.25	0.5	0.25	0.27	0.5	0.23
homo X het	26	0.5	0.5	0	0.5	0.5	0
homo X homo	41	1	0	0	1	0	0

Figure S2. Mendelian frequency is not altered in GATA2VENUS mouse line (see main figure 1). Mice with different genotype backgrounds of GATA2VENUS allele were crossed and resulting frequency of offspring genotypes was quantified and compared to expected frequency for > 6 litters born. Different types of crosses include (1) GATA2VENUS homozygous (homo) crossed with wild-type (wt), (2) GATA2VENUS heterozygous (het) crossed with wild-type (wt), (3) GATA2VENUS heterozygous (het) crossed with GATA2VENUS heterozygous (het), (4) GATA2VENUS homozygous (homo) crossed with GATA2VENUS heterozygous (het) and (5) GATA2VENUS homozygous (homo) crossed with GATA2VENUS homozygous (homo) mice. (see main figure 1)

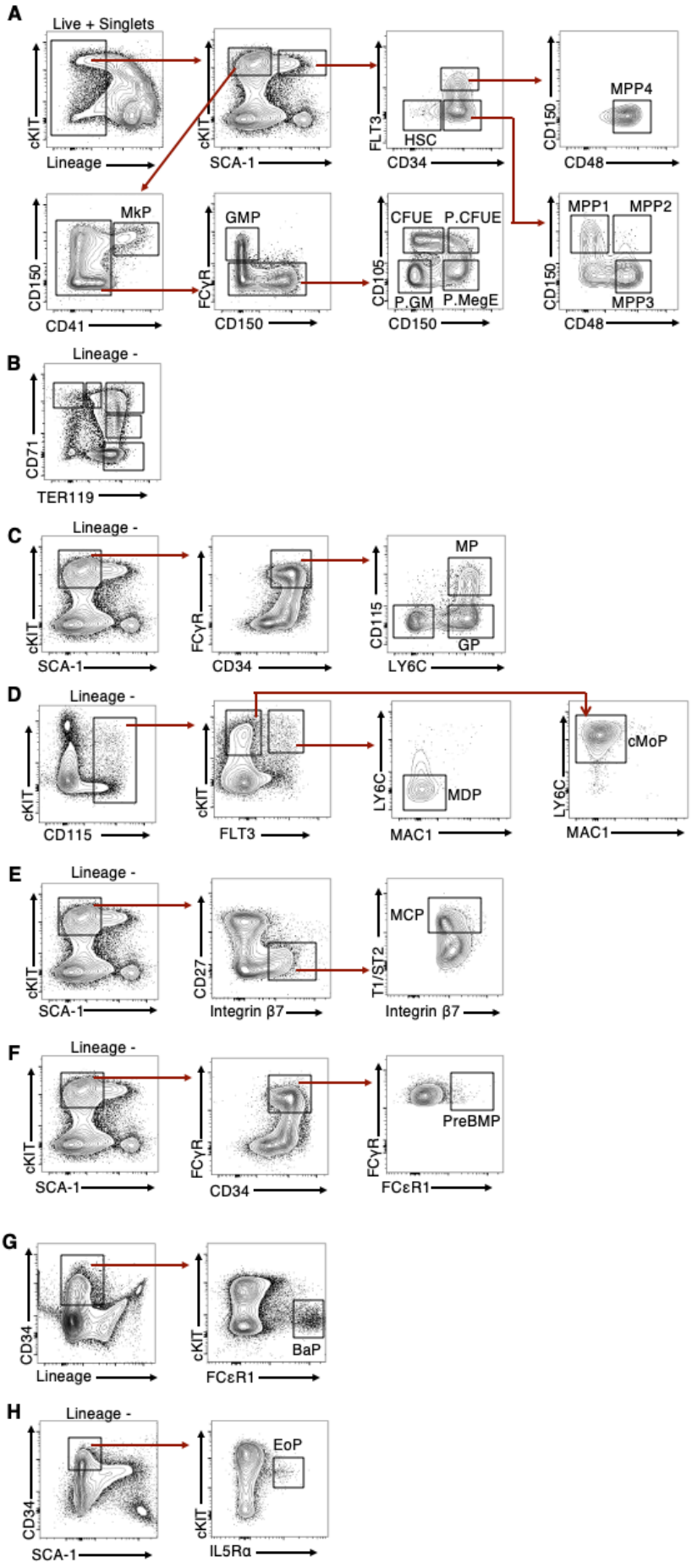
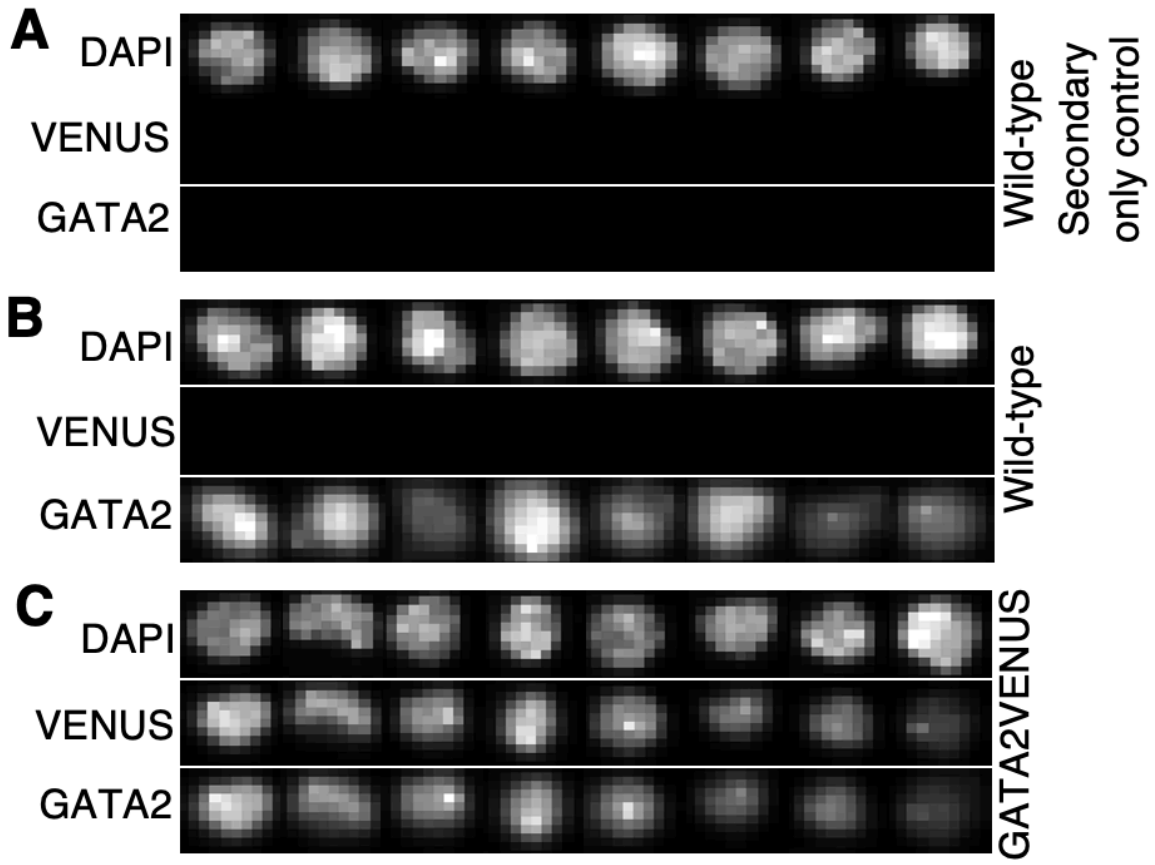


Figure S3. FACS gating scheme used for analysis and isolation of hematopoietic stem and progenitor cells (see main figure 3).
(A) HSC – Hematopoietic Stem Cell, MPP 1-4 – Multipotent Progenitor 1-4, P.MegE – pre Megakaryocyte Erythrocyte, MkP – Megakaryocyte progenitor, P.CFUE – pre colony forming unit Erythrocyte, CFUE – colony forming unit Erythrocyte, preGM – pre Granulocyte Monocyte, GMP – Granulocyte Monocyte Progenitor. **(B)** Erythrocyte differentiation stages based on CD71 and TER119 expression. **(C)** MP – Monocyte progenitor, GP – Granulocyte progenitor. **(D)** MDP – Monocyte Dendritic cell progenitor, cMoP – common Monocyte progenitor. **(E)** MCP – Mast cell progenitor. **(F)** preBMP – pre Basophil Mast cell progenitor. **(G)** BaP – Basophil progenitor. **(H)** EoP – Eosinophil progenitor. (see main figure 3)



Supplementary Figure S4. Colocalization of GATA2 and VENUS signal inside GATA2VENUS nuclei (see main figure 1). Localization of GATA2 and VENUS in P.MegE from wild-type mouse (VENUS negative control) without **(A)** and with **(B)** primary anti-GATA2 antibody. **(C)** With primary anti-GATA2 antibody in GATA2VENUS P.MegE. See Figure 1K. Image tile: 10 µm.

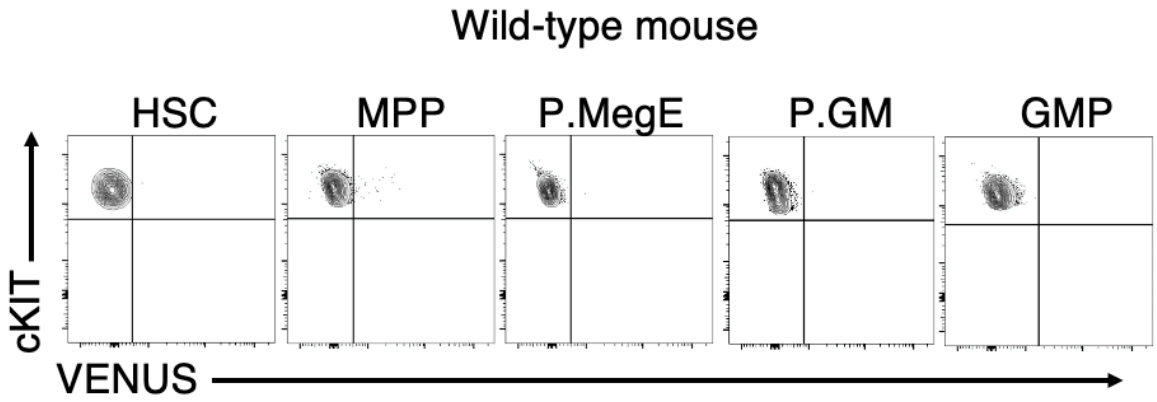


Figure S5. VENUS fluorescence channel background in wild-type HSPCs (see main figure 3). FACS of VENUS channel in HSPCs from a 12-week old wild-type mouse. Used to set background levels of GATA2VENUS expression in cells from GATA2VENUS mouse line in Figure 3.
Abbreviations: HSC – Hematopoietic Stem Cell, MPP – Multipotent Progenitor, P.MegE – pre Megakaryocyte Erythrocyte, preGM – pre Granulocyte Monocyte, GMP – Granulocyte Monocyte Progenitor. (see main figure 3)

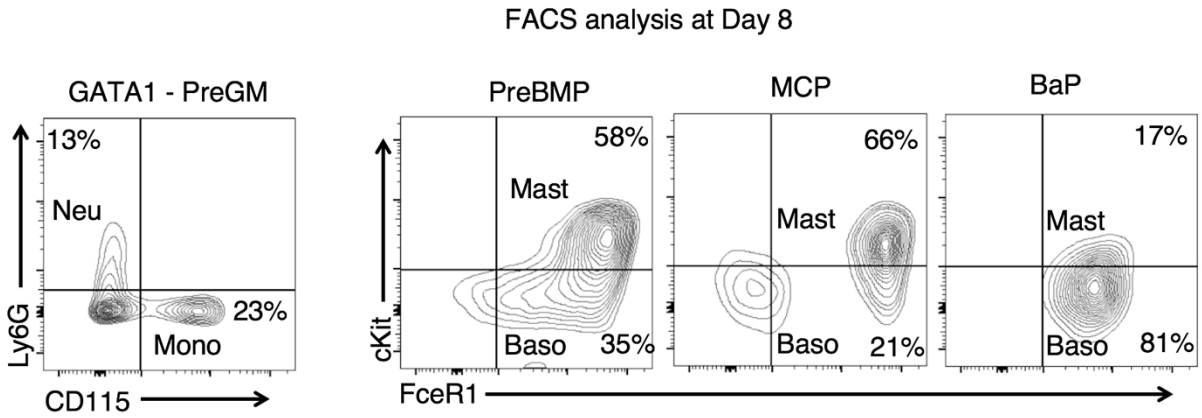


Figure S6. Pan myeloid media conditions support survival, proliferation and differentiation of different granulocyte and monocyte lineages (see main figure 5 & 6). GATA1-low preGMs, preBMPs, MCPs and BaPs from PU.1eYFP/GATA1mCHERRY mice(Hoppe et al., 2016) were sorted and cultured for 8 days in pan myeloid media supporting survival, proliferation and differentiation of monocytes, neutrophils, basophils and mast cells. Mature cell types were detected at day 8 by flow cytometry analysis of lineage specific markers including $cKIT^{+}$ $FC\varepsilon R1^{+}$ cells (mast cells), $cKIT^{-}$ $FC\varepsilon R1^{+}$ cells (basophils), $LY6G^{+}$ cells (neutrophils) and $CD115^{+}$ cells (monocytes). Positive gates were set using unstained control cells.
Abbreviations: preGM – pre Granulocyte Monocyte, preBMP – pre Basophil Mast cell progenitor, MCP – Mast cell progenitor and BaP – Basophil progenitor. (see main figure 5 & 6)

Table S1: FACS gating scheme used for analysis and isolation of hematopoietic stem and progenitor cells. (Related to main figure 3)

Cell type	Surface marker combination	Ref
Lineage*	CD3e B220 CD19 CD11b Gr-1 TER119	(Cabezas-Wallscheid et al., 2014; Kiel et al., 2005; Wilson et al., 2008)
HSC	Lin$^-$ SCA-1$^+$ cKIT$^+$ CD135$^-$ CD34$^-$ CD48$^-$ CD150$^+$	(Cabezas-Wallscheid et al., 2014; Kiel et al., 2005; Wilson et al., 2008)
MPP1	Lin$^-$ SCA-1$^+$ cKIT$^+$ CD135$^-$ CD34$^+$ CD48$^-$ CD150$^+$	(Cabezas-Wallscheid et al., 2014; Kiel et al., 2005; Wilson et al., 2008)
MPP2	Lin$^-$ SCA-1$^+$ cKIT$^+$ CD135$^-$ CD34$^+$ CD48$^+$ CD150$^+$	(Cabezas-Wallscheid et al., 2014; Kiel et al., 2005; Wilson et al., 2008)
MPP3	Lin$^-$ SCA-1$^+$ cKIT$^+$ CD135$^-$ CD34$^+$ CD48$^+$ CD150$^-$	(Cabezas-Wallscheid et al., 2014; Kiel et al., 2005; Wilson et al., 2008)
MPP4	Lin$^-$ SCA-1$^+$ cKIT$^+$ CD135$^+$ CD34$^+$ CD48$^+$ CD150$^-$	(Cabezas-Wallscheid et al., 2014; Kiel et al., 2005; Wilson et al., 2008)
P. MegE	Lin$^-$ SCA-1$^-$ cKIT$^+$ CD16/32$^-$ CD41$^-$ CD105$^-$ CD150$^+$	(Pronk et al., 2007)
MkP	Lin$^-$ SCA1-$^-$ cKIT$^+$ CD16/32$^-$ CD41$^+$ CD105$^-$ CD150$^+$	(Pronk et al., 2007)
P. CFUE	Lin$^-$ SCA-1$^-$ cKIT$^+$ CD16/32$^-$ CD41$^-$ CD105$^+$ CD150$^+$	(Pronk et al., 2007)
CFUE	Lin$^-$ SCA-1$^-$ cKIT$^+$ CD16/32$^-$ CD41$^-$ CD105$^+$ CD150-	(Pronk et al., 2007)
Ery-S1	Lin- CD71^{high} TER119^{low}	(Pop et al., 2010)
Ery-S2	Lin- CD71^{high} TER119^{mid}	(Pop et al., 2010)
Ery-S3	Lin- CD71^{high} TER119^{high}	(Pop et al., 2010)
Ery-S4	Lin- CD71^{mid} TER119^{high}	(Pop et al., 2010)
Ery-S5	Lin- CD71^{low} TER119^{high}	(Pop et al., 2010)
preGM	Lin$^-$ SCA-1$^-$ cKIT$^+$ CD16/32$^-$ CD41$^-$ CD105$^-$ CD150$^-$	(Pronk et al., 2007)
GMP	Lin$^-$ SCA-1$^-$ cKIT$^+$ CD16/32$^+$ CD41$^-$ CD105$^-$ CD150$^-$	(Pronk et al., 2007)
MP	Lin$^-$ SCA-1$^-$ cKIT$^+$ CD16/32$^+$ CD34$^+$ Ly6C$^+$ CD115$^+$	(Yanez et al., 2017)
GP	Lin$^-$ SCA-1$^-$ cKIT$^+$ CD16/32$^+$ CD34$^+$ Ly6C$^+$ CD115$^-$	(Yanez et al., 2017)
cMoP	Lin$^-$ cKIT$^+$ CD115$^+$ CD135$^-$ LY6C$^+$ CD11b$^-$	(Hettinger et al., 2013)
MDP	Lin$^-$ cKIT$^+$ CD115$^+$ CD135$^+$ LY6C$^-$ CD11b$^-$	(Hettinger et al., 2013)
EoP	Lin$^-$ SCA-1$^-$ cKIT^{mid} CD34$^+$ IL5Rα$^+$	(Iwasaki et al., 2005)
preBMP	Lin$^-$ SCA-1$^-$ cKIT$^+$ CD16/32$^+$ CD34$^+$ FCεR1$^+$	(Qi et al., 2013)
BaP	Lin$^-$ cKIT$^-$ CD34$^+$ FCεR1$^+$	(Arinobu et al., 2005)
MCP	Lin$^-$ SCA-1$^-$ cKIT$^+$ CD27$^-$ Integrin β7$^+$ T1/ST2$^+$	(Chen et al., 2005)

*TER119 lineage antibody was omitted during analysis of Erythroid stages S1 – S5.
*CD11b lineage antibody was omitted during analysis of Eosinophil progenitors (EoPs) and Basophil progenitors (BaPs).

Table S2: List of antibodies for FACS analysis and isolation of HSPCs. (Related to experimental procedures)

Antigen	**Conjugation**	**Clone**	**Company**	**Catalog no**
Strepavidin (Lineage)	BV711	N/A	BD Biosciences	563262
SCA-1	Pacific blue PerCP-CY5.5	D7 D7	Biolegend eBiosciences	108120 45-5981-82
cKIT	PE-CY7 BV510	2B8 ACK2	eBiosciences Biolegend	25-1171-82 135119
CD135	PerCPeFL710 PECF594	A2F10 A2F10.1	eBioscience BD Bioscience	46-1351-82 562537
CD34	eFL660 eFL450	RAM34 RAM34	eBioscience eBioscience	50-0341-82 48-0341-82
CD48	APCeFL780	HM48-1	eBioscience	47-0481-82
CD150	PE	TC15-12F12.2	Biolegend	115904
CD16/32	PE-CY7 APC-CY7	93 93	Biolegend Biolegend	101317 101328
CD41	BV605	MWReg30	BD Biosciences	563317
CD105	APC	MJ7/18	Biolegend	120414
CD71	APC	R17.217.1.4	eBioscience	17-0711-82
TER119	APCeFL780	Ter-119	eBioscience	47-5921-82
T1/ST2	PE	DIH9	Biolegend	145304
IL5Ra	PE	DIH37	Biolegend	153404
CD115	PE	AFS98	eBioscience	12-1152-82
Integrin B7	PE-CY7	FIB504	eBioscience	25-5867-41
FCeR1	APC	MAR-1	eBioscience	17-5898-82
CD11b	APC	M1/70	eBioscience	17-0112-81
CD27	APC-CY7	LG.3A10	Biolegend	124226
LY6C	APC-Fire 750	HK1.4	Biolegend	128026

Table S3: List of antibodies used in lineage depletion cocktail. (Related to experimental procedures)

Antigen	**Conjugation**	**Clone**	**Company**	**Catalog no**
B220	Biotin	RA3-6B2	eBioscience	13-0452-86
CD19	Biotin	MB19-1	eBioscience	13-0191-86
CD3e	Biotin	145-2C11	eBiosciences	13-0031-85
CD11b	Biotin	M1/70	eBioscience	13-0112-85
Gr-1	Biotin	RB6-8C5	eBioscience	13-5931-85
TER119	Biotin	Ter-119	eBioscience	13-5921-85

Table S4: List of antibodies used for live imaging of myeloid colonies. (Related to main figure 6)

Surface marker	**Conjuagte**	**Clone**	**Company**	**Catalog no**
CD115	PE	AFS98	eBioscience	12-1152-82
LY6G	BV480	1A8	BD Biosciences	746448
FCεR1	APC	MAR-1	eBioscience	17-5898-82

Table S5: List of antibodies for quantitative immunostaining of transcription factors. (Related to main figure 4)

Antigen	**Antibody**	**Dilution**	**Clone**	**Company**	**Catalog no**
GATA2	Rabbit anti-GATA2	1:50	H-116	SCBT	sc-9008
GATA1	Rat anti-GATA1	1:25	N6	SCBT	sc-265
FOG-1	Goat anti-FOG1	1:50	M-20	SCBT	sc-9361
PU.1	Rabbit anti-PU.1	1:50	T-21	SCBT	sc-352
CEBPα	Rabbit anti-CEBPα	1:50	14AA	SCBT	sc-61
IRF8	Goat anti-IRF8	1:50	C-19	SCBT	sc-6058
VENUS	chicken anti-GFP	1:1000	Polyclonal	Aves	GFP-1020
GATA2 (tissues)	rabbit anti-GATA2	1:200	Polyclonal	Novus	NBP1-82581

Secondary Antibody	**Conjugation**	**Dilution**	**Company**	**Catalog no**
Donkey anti-rabbit	Alexa 488	1:200	Invitrogen	A21206
Donkey anti-rat	Alexa 594	1:200	Invitrogen	A21209
Donkey anti-goat	Alexa 594	1:200	Invitrogen	A11058

Table S6: List of cytokines for culture of HSPCs. (Related to main figure 5 & 6)

Cytokine	**Concentration**	**Company**	**Catalog no**
SCF	100 ng/ml	Peprotech	250-03
EPO	2 units/ml	Peprotech	100-64
TPO	100 ng/ml	Peprotech	315-14
IL3	20 ng/ml	Peprotech	213-13
IL6	10 ng/ml	Peprotech	216-16
IL9	50 ng/ml	Peprotech	219-19
GM-CSF	20 ng/ml	Peprotech	AF-315-03

Table S7: List of filters used for imaging and quantification of transcription factor immunostainings. (Related to main figure 4)

Fluorophore	**Filter**	**Ex.spec**	**Em.spec**	**Beamsplitter**	**Company**	**Catalog no**
Alexa 488	EGFP ET	470/40	525/50	495 LP	AHF	F46-002
Alexa 594	mCherry	550/32	605/15	BS 585	AHF	F37-550 F38-585 F37-605
DAPI	DAPI	405/10	460/50	405 LPX	AHF	F39-404 F47-460 F48-404

Table S8: List of filters used for imaging of liquid culture colonies. (Related to main figure 6)

Fluorophore	**Filter**	**Ex.spec**	**Em.spec**	**Beamsplitter**	**Company**	**Catalog no**
GATA2VENUS	YFP ET	500/20	535/30	515 LP	AHF	F46-003
CD115-PE	mCherry	550/32	605/15	BS 585	AHF	F37-550 F38-585 F37-605
FCεR1-APC	Cy5	620/60	700/75	LPXR 660	AHF	F49-620 F48-660 F47-700
LY6G-BV480	CFP ET	436/20	480/40	455 LP	AHF	F46-001

Experimental procedures:

Generation of GATA2VENUS knock-in reporter mouse line:
The GATA2Venus knock-in construct consists of 5.0 *kbp* 5′-end homology arm lasting until the last codon of *Gata2* (skipping the endogenous stop-codon) followed by a 24 *bp*-short linker sequence (AGAGCATCAGGTACCAGTGGAGCT) encoding 8-amino acid peptide (Arg-Ala-Ser-Gly-Thr-Ser-Gly-Ala), the coding sequence of *Venus*, FRT (Flp recognition target)-flanked neomycin resistance gene cassette and 5 *kbp* 3′-end homology arm. Downstream of the 3′- homology arm there is a negative selection cassette consisting of herpes simplex virus thymidine kinase (HSV-TK) driven by the MC1 promoter. JM8.A3.N1 ES cells (C57BL/6J background) were electroporated (250 V, 500 µF; Gene Pulser Xcell, BioRad) with linear (*Gata2Venus*) construct followed by selection with 0.2 mg/ml G418 and 2 µM ganciclovir. 24 clones were picked, expanded and prepared for screening according to described protocols(Anastassiadis et al., 2013). Correct integration was confirmed in 9 clones by 5′- and 3′- end southern blot assay. In brief, genomic DNA was digested with HindIII (for 5′ end) or EcoRI (for 3′ end), separated on 0.8% agarose gels, blotted on nylon membranes (PALL) and hybridized with radioactively labeled (^{32}P-dCTP) by random priming (High Prime, Roche diagnostics) 5′- and 3′-probe respectively. Germline chimeras were generated from correct ES cell clone by ES-cell aggregation. Founders were identified by *Gata2Venus* PCR and FRT-flanked neo-selection cassette was excised *in vivo* using recombinase-mediated excision by crossing with Flpe deleter strain(Dymecki, 1996). Resulting GATA2VENUS offspring were backcrossed for >5 generations with C57BL/6J animals. Identification of homozygous *Gata2Venus* and wild-type animals was done by *Gata2Venus* specific genotyping protocol using the following set of primers: (a) *Gata2* forward primer: 5′- GAAGTCACCGCCCTTCAGTG -3′, (b) *Gata2* reverse primer: 5′- CTGCCAAACCACCCTTGATG -3′ and (c) *Venus* reverse primer: 5′- CGGACACGCTGAACTTGTGG -3′. *Gata2Venus* alleles is identified by a 290 bp band while wild-type *Gata2* allele by a 222 bp band.

Isolation of primary HSPCs:
Male mice for FACS analysis and HSPC isolation were euthanized at age of 12 – 16 weeks. Isolation of primary HSPCs was performed according to protocols described[27–37]. Briefly, femurs, tibiae, humeri and vertebrae of adult mice were isolated, crushed in FACS buffer (2% FCS (PAA) + 1mM EDTA (Invitrogen) in PBS (Sigma Aldrich)), subjected to ACK (Lonza) lysis buffer (for 2 min), lineage depletion using biotinylated antibodies (incubation for 15 min) followed by Streptavidin-conjugated beads (Roche) (incubation for 7 min) and immune-magnetic (Stem Cell Technologies) depletion (incubation for 7 min). Lineage depleted cells were finally stained with color-conjugated primary antibodies for 90 minutes on ice. FACS analysis and sorting was performed on FACS ARIA III (BD Biosciences).

Immunostaining of transcription factors in primary HSPCs:
Immunostaining of TFs in primary HSPCs was performed according to protocols as described(Etzrodt et al., 2018; Hoppe et al., 2016). Briefly, HSPCs were isolated, directly seeded on Poly-L Lysine (Sigma Aldrich) coated, plastic-bottom 384 well plates (Greiner Bio-one), stored for 30 min at 4°C, fixed with 4% paraformaldehyde (Sigma Aldrich) for 10 min at RT, washed thrice with PBS, permeabilized with PBS-T (0.2 % triton X (AppliChem) in PBS) for 5 min, washed twice with TBS-T (0.1 % Tween (Sigma Aldrich) in TBS buffer), incubated with blocking buffer (10 % donkey serum (Jackson Immuno research) in TBS-T) for 1h at RT, incubated with primary antibodies (1:50 dilution in blocking buffer) overnight at 4º C, washed thrice with blocking buffer, stained with secondary antibodies (1:200 dilution in blocking buffer) for 1h, incubated with DAPI (1:10000 in blocking buffer), washed thrice with blocking buffer, incubated with PBS and imaged. Images were acquired on a Nikon Eclipse Ti-E microscope using Lumencore light source with 0.7x camera adapter and 10X objective with 0.45 NA (Plan Apo) and analyzed using bioimaging pipeline described below.

Immunostaining of embryonic tissues:
The day of the vaginal plug was considered embryonic day (E) 0. Fresh isolated E14 embryos were immediately frozen, subsequently embedded on Optimal Cutting Temperature (OCT) (PolyFreeze, Sigma) and stored at -80°C until further processing. Full embryo cryosectioning (20 µm thick) was performed with a cryostat (CryoStar™ NX50 Cryostat, ThermoFisher) on superfrost glass slides, immediately fixed with 4% PFA for 20 min at room temperature and then stored in 1xPBS at 4°C. Tissue sections were immunostained with primary antibodies diluted in blocking solution (0.5% Triton-X in PBS with 10% NDS) overnight at 4°C. Sections were washed twice in PBS and fluorescent secondary antibodies were applied in blocking solution for 90 min at room temperature. DAPI was used to visualize nuclei. Sections were again washed two times and mounted in ProLong Diamond Antifade Mountant (ThermoFisher). Confocal images were taken on a Leica TCS SP5 equipped with three photomultiplier tubes, two HyD detectors, five lasers (405 nm, Argon Laser (458, 476, 488, 496 and 514 nm), 561, 594 and 633 nm) using 10X and 20x objective lens. All scans were acquired at 20–25 °C, 100 Hz, in the

bidirectional mode, with z-spacing of 0.3 - 1 μm at 1024x1024 pixel resolution. Images were acquired in 8-bit format.

Immunostaining of adult kidney:
Tissue preparation: Tissue sections were prepared as described before(Coutu et al., 2018). Bones were fixed for 24 hours in 4% PFA at 4º C. Kidneys were fixed for 1 hour in 4% PFA at room temperature. The tissues were embedded in 4% low-gelling temperature agarose and subsequently sectioned (100 μm thick) using a vibratome (Leica VT1200 S).
Immunostaining: All steps were performed at room temperature with gentle rocking in double side adhesive silicon chambers (Grace Biolabs) glued on glass slides. Sections were blocked and permeabilized with TBS (final concentration 0.1M Tris, 0.15 M NaCl, pH 7.5) containing 0.05% Tween-20, 20% DMSO, 1% Triton X 100 and 10% donkey serum (Jackson Immuno Research) for a minimum of 2 hours. This buffer was also used to dilute all primary and secondary antibodies. Primary antibodies were applied overnight. Secondary antibodies were applied for 2 hours. After extensive washing (with TBS-T), sections were mounted in homemade mounting medium (80% Glycerol in TBS containing 0.2 M N-propyl gallate, pH 8.5) using size 1.5 coverslips.
Confocal microscopy: Confocal microscopy was performed on a Leica TCS SP8 equipped with three photomultiplier tubes, two HyD detectors, five lasers (405 nm, Argon Laser (458, 476, 488, 496 and 514 nm), 561, 594 and 633 nm) using Leica type G immersion liquid and a 63x glycerol immersion lens (NA 1.3, FWD 0.28 mm). All scans were acquired at 20–25 °C, 400 Hz, in the bidirectional mode, with z-spacing of 1 μm (the optical slice thickness of the optics was 0.99 μm) at 1024x1024 pixel resolution. Images were acquired in 8-bit format. For signal acquisition only HyD detectors were used.

Protein stability assay of transcription factors:
HSPCs were sorted (described above) and cultured in plastic-bottom 384 well plates in multi-lineage supporting media (IMDM (Gibco) + 5 % BIT + P/S (Gibco) + SCF + EPO + TPO + IL3 + IL6). Cells were treated with 50 μM cyclohexamide (Sigma Aldrich) for indicated time duration, fixed immediately with 4% paraformaldehyde (Sigma Aldrich) and proceeded with standard immunostaining protocol (described above)(Hoppe et al., 2016).

Single-cell liquid culture colony assay of HSPCs:
HSPCs were single-cell sorted in plastic-bottom 384 well plates (Greiner Bio-one) using FACS ARIA III with pan myeloid culture media as described (IMDM (Gibco) + 5 % BIT (Stem Cell Technologies) + 10% FCS (PAA) + P/S (Gibco) + SCF + GM-CSF + IL3 + IL9)(Drissen et al., 2016). Plates were incubated at 37° C and 5% CO2. At day 8, color-conjugated antibodies against lineage markers were added (1:5000) to wells, incubated for 3 hours at 37°C and 5% CO2 and imaged. Images were acquired on a Nikon Eclipse Ti-E microscope using Lumencore light source with 0.7x camera adapter and 10X objective with 0.45 NA (Plan Apo) and analyzed as described below.

FACS analysis of bulk-liquid culture colonies:
HSPCs were sorted using FACS ARIA III and 100 – 200 cells were seeded in each well of 24 well plates (Thermo scientific) with pan myeloid media (described above). Plates were incubated at 37° C and 5% CO2. At day 8, cells were taken, washed with FACS buffer (2% FCS in PBS), stained with color-conjugated antibodies against lineage markers, incubated for 90 min at 4°C and washed with FACS buffer. Finally, FACS analysis of cell types was performed using FACS ARIA III.

May-Grünwald and Giemsa staining of mature colonies:
May-Grünwald and Giemsa staining was performed according to protocol as described (Hettich). Briefly, cytospin hardware was arranged and 200 μl of cells were taken directly from day 8 culture of HSPCs and added on cytospin columns. Cells were centrifuged at 270g for 3 minutes. Supernatant was removed carefully followed by second round of centrifugation at 270g for 1 minute. Air dried the slides and 1 ml of May-Grünwald (Roth) solution was added for 4 minutes. Slides were washed twice with dist. H2O and 1 ml of 5% Giemsa solution (Sigma Aldrich) was added for 16 minutes. Slides were washed twice with dist. H2O. Air-dried the slides followed by imaging. All steps were performed at 4º C. Images were acquired using Nikon Eclipse Ti-E microscope.

Bioimaging pipeline for image acquisition, detection and quantification:
Fluorescence images (immunostaining of transcription factors and single-cell liquid colony assays) were acquired on Nikon Eclipse Ti-E microscope in an automated manner using custom written software. Single-cell colonies were analyzed based on fluorescence signal of surface markers and scored manually. To count the number of cells per colony, cells were segmented in brightfield based on morphology and quantified using FastER segmentation tool(Hilsenbeck et al., 2017). To quantify the signal of transcription factors in single cells, background signal was normalized by using BaSiC tool as previously described(Peng et al., 2017) and segmentation of cells was

performed on DAPI signal. Finally, quantification of transcription factor signal was performed using fastER segmentation tool as described(Hilsenbeck et al., 2017). Data was analyzed using custom written R scripts.

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