Supplemental Data

Supplemental Figure 1. Expression of Gabbr1 and Gad1 in mouse hematopoietic populations. Related to Figure 1. (A) Published microarray analysis of pooled RNA shows higher relative expression of *Gabbr1* in HSCs compared to more differentiated hematopoietic populations[1,2]. *Gabbr1* is represented by one probeset on the Affymetrix Mouse Gene 1.0 ST Array Transcript Version microarray. The platform "Gene Expression Commons" compares sample data through meta-analysis to a common reference and generates dynamic range and threshold between low and high expression for each probeset. The highest relative expression level (+100% or "active") is purple, threshold (0%) is white, lowest (−100% or "inactive") in dark green. The log2 dynamic range for this probeset is 6.01 with the highest level in HSC (light green) and the lowest level in MEP and GMP (dark green). Details of bioinformatics analysis are published[2]. (B) Haemopedia RNA-seq database[3,4] search for mouse *Gad1* in hematopoietic populations shows there are only high levels in B cell lineages. (C) Representative intracellular flow cytometry data (left panel) for Gad1 expression in gated BM $CD93^{-}B220^{+}$ cells from WT and *Rag1*$^{-/-}$ mice. Bar graph in right panel represents n=4 WT versus n=4 *Rag1*$^{-/-}$; ***p<0.001 vs WT controls. (D) Immunofluorescent antibody staining of sternum BM showing Gad1 (green) is expressed in WT B220+ (red) B cells (arrowheads point to cells that express both markers), but not in *Rag1*$^{-/-}$ mice. WT images are representative of n=9 sternum regions from n=4 mice. *Rag1*$^{-/-}$ images are representative of n=5 sternum regions from n=2 mice). Scale bar=20μm.

Supplemental Figure 2. IMS data from independent samples of bone sections showing GABA and heme are both detectable by IMS in positive mode and have specialized localization. Related to Figure 2. (A-C) GABA is detected as two ions both in the commercial standard and in the endosteal regions of bone sections. Using the dried droplet technique on a MALDI-TOF mass spectrometer, both the protonated ($[M + H]^{+}$) molecule at *m/z* 104 and the

dehydrated ion ($[M - H_2O + H]^+$) at *m/z* 86 of the standard (10 mM) are detected with high intensity. (A) Averaged MS^1 spectrum from the IMS experiment of the GABA standard and bone sections. (B) Zoom of IMS spectra in (A) showing GABA ions at *m/z* 86 and *m/z* 104. (C) Dried droplet of matrix (yellow) compared to GABA standard (purple; 10 mM). (D-F) 3 regions of bone from n=2 mice to make one composite femur section. GABA is detected as both a protonated and dehydrated ion (GABA standards not shown). GABA localization to the endosteum replicates results from independent samples in Figure 1E-F. (E) Heme is detected in two forms: unbound (*m/z* 567) and iron-bound (*m/z* 616). The intensity of the iron-bound *m/z* 616 form is much higher and shows spatial specificity to the BM. Endogenous heme is present at high levels in the central sinus region of the marrow, where there is the largest volume of red blood cells. (F) Overlay of both GABA (green) and iron-bound heme (purple) signals indicate definitive separation in localization. (G-H) Comparison of GABA levels in femur sections between (G) WT and (H) *Rag1*$^{-/-}$ mice. These are representative images of IMS data quantified in Figure 2F. Shown are the bone sections (top), the dehydrated ion ($[M - H_2O + H]^+$) at *m/z* 86 (middle), and the protonated ($[M + H]^+$) molecule at *m/z* 104 (bottom).

Supplemental Figure 3. *Gabbr1* null mutant mouse model is generated on a C57/BL6 background by CRISPR/Cas9 genome editing. Related to Figure 3. (A) The *Gabbr1* locus shown on chromosome 17 (Mus musculus genome build mm10). Guide RNA was designed to target exon 5 because it is common to all isoforms (3' to 5' orientation is shown; cut site shown by ^^^^^; PAM sequence is bold). The single base pair insertion allele (*em59*; +1 base pair A), was selected to generate a predicted nonsense mutation. Next Generation Sequencing was used to confirm the frequency of mutant alleles, and that the mutant allele appeared at a predicted 50% Mendelian inheritance in heterozygotes. Color codes represent the percentage of the different alleles, so 50% WT and 50% mutant alleles in the heterozygote (orange), and 100% WT allele in the control (brown). (B) Appearance of postnatal day P15 *Gabbr1*$^{+/+}$ and

Gabbr1$^{-/-}$ mice. (C) Quantification of body weight data (n=5). (D) Brain tissues were collected from *Gabbr1*$^{+/+}$ and *Gabbr1*$^{-/-}$ mice. Protein was extracted using RIPA lysis buffer and separated with SDS-PAGE. Gabbr1 protein were detected by western blot. GAPDH was used as a loading control. (E) Numbers of red blood cells (RBCs), concentration of hemoglobin (Hb), and numbers of platelets (PLTs), and were determined in the PB from *Gabbr1*$^{+/+}$ and *Gabbr1*$^{-/-}$ mice at P15 (n=5). (F) Numbers of LSK progenitors and HSCs in *Gabbr1*$^{+/+}$ and *Gabbr1*$^{-/-}$ fetal livers were analyzed at embryonic day (E) 14.5 (n=4). Data are presented as mean ±SD (***p<0.001). (G) Presence of GABA ion is significantly higher in extracts of whole BM from femurs compared to extracts of fetal liver. Using liquid chromatography–mass spectrometry (LC-MS), GABA (m/z 104.0712) was detected in whole bone marrow extracts at a significantly higher abundance (**p<0.001 calculated by unpaired Welch's t-test) based on Extracted Ion Chromatogram (EIC) peaks compared to fetal liver extracts. Error bars indicate standard deviation of data sets based on n=4 measurements: 2 technical replicates each from 2 biological samples.

Supplemental Figure 4. There are less multipotent progenitors in *Gabbr1*$^{-/-}$ mutants than those in WT controls. Related to Figure 3. (A) Numbers of MPP1, MPP2 and MPP3/4 in BM from WT and *Gabbr1*$^{-/-}$ mice (n=5 WT versus n=5 mutants). (B) Numbers of GMP, MEP and MKP in BM from WT and *Gabbr1*$^{-/-}$ mice (n=5 WT versus n=5 mutants). (C) The gating strategy of Hardy B cell Fractions in BM from WT and *Gabbr1*$^{-/-}$ mice. *p<0.05 vs WT controls.

Supplemental Figure 5. Recipients of *Gabbr1*$^{-/-}$ mutant whole BM or sorted HSCs are delayed in their reconstitution of hematopoiesis, while *Gabbr1*$^{-/-}$ mutant HSCs in competitive transplants never fully restore hematopoiesis to WT levels. Related to Figure 4. (A) Whole BM transplantation. 1×10^6 BM cells (CD45.2) were isolated from *Gabbr1*$^{+/+}$ and *Gabbr1*$^{-/-}$ mice and transplanted into 9 Gy γ–ray irradiated CD45.1 recipient mice. Engraftment ability was analyzed at 1, 2, and 3 months after transplantation (n=5 recipients/group). (B) HSC

transplantation. 350 HSCs from *Gabbr1*$^{+/+}$ or *Gabbr1*$^{-/-}$ mice were sorted and transplanted into irradiated CD45.1 recipients. Mice were analyzed at 1, 2, and 3 months after transplantation (n=5 recipients/group). (C-D) Additional data from competitive BM transplantation (see Figure 3). 350 HSCs from *Gabbr1*$^{+/+}$ or *Gabbr1*$^{-/-}$ mice were sorted and transplanted into irradiated CD45.1 recipients with 350 HSCs from CD45.1/2 mice (n=5 recipients/group). (C) Percentages of indicated populations in PB were analyzed at 1, 2, and 3 months after transplantation and did not show significant differences. (D) 3 months after transplantation, absolute numbers of BM CD45.2^{+} CLP, CMP, GMP, and MEP were reduced. (E) Secondary transplantation. 1×10^{6} *Gabbr1*$^{+/+}$ and *Gabbr1*$^{-/-}$ BM cells from primary recipient mice were transplanted into irradiated CD45.1 recipients. Mice were analyzed at 2 and 4 months after secondary transplantation. (F) 4 months after transplantation, numbers of BM CD45.2^{+}, CD45.2^{+} LSK progenitors, and CD45.2^{+} HSCs were significantly reduced (n=5 recipients/group). Data are presented as mean ± SD (*p<0.05, **p<0.01, ***p<0.001).

Supplemental Figure 6. *Gabbr1*$^{-/-}$ mutant LSK progenitors have reduced proliferative capacity. Related to Figure 5. (A) Annexin V apoptosis assay. BM cells were harvested from P15 *Gabbr1*$^{+/+}$ and *Gabbr1*$^{-/-}$ mice and stained with Lin-PE, c-Kit-APC-Cy7, Sca1-Percp Cy5.5, CD150-PE-Cy7, CD48-Alex 700 and Annexin V-FITC antibodies. Percentages of Annexin V^{+} cells from LSK progenitors and HSCs were analyzed using Flowjo software (n=6). There are comparable percentages of apoptotic cells in LSK progenitors and HSCs from *Gabbr1*$^{+/+}$ and *Gabbr1*$^{-/-}$ mice. (B) Caspase 3 activity assay. Left panel, representative flow chart for Caspase 3 activity in LSK cells. Right panel, mean fluorescent intensity (MFI) of Caspase 3 activity in LSK cells and HSCs. (C) Apoptotic cell analysis in LSK progenitors at 48 hours after poly(I:C) treatment shows no significant differences. (D) Experimental design: 4,000 sorted LSK progenitors from *Gabbr1*$^{+/+}$ and *Gabbr1*$^{-/-}$ mice were seeded onto OP9 cells with medium containing IL-3, IL-6, IL-7 and Flt-3L. At day 14, all cells were collected, counted, and stained

with CD45, CD11b and Gr-1 antibodies. The absolute numbers were presented as mean ± SD and no significant differences were observed between *Gabbr1*$^{+/+}$ and *Gabbr1*$^{-/-}$ mice (data were from two mice in each genotype with 3 replicates).

Supplemental Figure 7. Representative GSEA enrichment plots for 4 of the top 10 significant terms for genes that positively correlate with *Gabbr1* expression. Gene set enrichment analysis (GSEA) of published single cell RNA-seq data from HSPCs[5]. Enrichment plots correspond to GO terms in Supplemental Table 2. (A) GO_RESPONSE_TO_INTERFERON_GAMMA (Adjusted FWER *P* value=0). (B) GO_CELLULAR_RESPONSE_TO_INTERFERON_GAMMA (Adjusted FWER *P* value=0). (C) GO_INTERFERON_GAMMA_MEDIATED_SIGNALING_PATHWAY (Adjusted FWER *P* value=0.001). (D) GO_RESPONSE_TO_TYPE_I_INTERFERON (Adjusted FWER *P* value=0.001).

Supplemental Figure 8. Brief treatment of human UCB HSPCs with GABBR1 agonist Baclofen gives a sustained increase in progenitor numbers from bone marrow after long-term engraftment in NSG mice. Related to Figure 7. Colony-forming unit assays were used to assess progenitor input (A; 2 hour treatment of UCB prior to transplant), and progenitor output (B; 2 hour treatment of UCB followed by 16 week engraftment of xenotransplant BM). (A) There is no change in UCB progenitor numbers after a 2 hour treatment with GABBR1 agonist Baclofen (Bac). The GABBR1 antagonist Saclofen (Sac) produces a small but significant decrease in progenitor numbers at a 1 μM dose. (n=3 replicates per drug dose; unpaired *t* test of treatments versus control; **p*<0.03; ns=not significant; mean=S.E.M.). (B) All Bac doses produce higher colony numbers in the BM of xenotransplant mice engrafted for 16 weeks, with the 10 μM Bac dose being the most significant. Sac doses of 1 μM and 10 μM, but not 50 μM,

produce some increase in colony numbers after 16 weeks. Data points represent individual engrafted mice (n=3 per treatment group), shown as the total number of colonies from 1-3 dishes (unpaired *t* test of treatments versus control; *p<0.05, **p<0.01-0.001; ***p<0.0007; ****p<0.0001; mean=S.E.M.).

Supplemental Table 1. List of antibodies used

Markers	Clone	Antibody isotype	Conjugate
CD45R/B220[1]	RA3-6B2	IgG_{2a}	PE
CD45R/B220[1]	RA3-6B2	IgG_{2a}	Alexa Fluor® 700
CD3[1]	17A2	IgG_1	PE
CD11b[1]	M1/70	IgG_{2b}	PE
Gr-1[1]	RB6-8C5	IgG_{2b}	PE
Ter-119[1]	Ter-119	IgG_{2b}	PE
CD45R/B220[1]	RA3-6B2	IgG_{2a}	FITC
CD45R/B220[1]	RA3-6B2	IgG_{2a}	APC
CD3e[1]	17A2	IgG_1	FITC
CD11b[1]	M1/70	IgG_{2b}	FITC
Gr-1[1]	RB6-8C5	IgG_{2b}	FITC
Ter-119[1]	Ter-119	IgG_{2b}	FITC
CD16/CD32[1]	93	IgG_{2a}	purified
CD16/CD32[1]	93	IgG_{2a}	PE-CY7
CD34[1]	HM34	IgG	PE
CD45.2[1]	104	IgG_{2a}	Alexa Fluor 700
CD45.1[1]	A20	IgG_{2a}	PerCP-Cy5.5
CD43[1]	S11	IgG_{2b}	PerCP-Cy5.5
CD24[1]	M1/69	IgG_{2b}	APC/Fire™ 750
Bp-1[1]	6C3	IgG_{2a}	PE/Cyanine7

IgM[1]	RMM-1	IgG_{2a}	APC
IgD[1]	11-26c.2a	IgG_{2a}	PE
Sca-1[1]	D7	IgG_{2a}	PerCP-Cy5.5
c-kit[1]	2B8	IgG_{2b}	APC-CY7
CD150[1]	9D1	IgG_{2a}	PE-CY7
CD150[1]	TC15-12F12.2	IgG2a	Brilliant Violet 605™
CD48[1]	HM481	IgG_{2a}	APC
CD48[1]	HM481	IgG_{2a}	Alexa Fluor 700
CD127[1]	A7R34	IgG_{2a}	APC
CD135[1]	A2F10	IgG_{2a}	PE
CD4[1]	GK1.5	IgG_{2b}	PE-CY7
CD8[1]	53-6.7	IgG_{2a}	APC-CY7
Ki-67	16A8	IgG_{2a}	FITC
Annexin V[1]	-	-	FITC
GAD1/GAD-67[2]	F-6	IgG_3	none
$GABA_B$ R1[2]	D-2	IgG_1	none
Donkey anti-Mouse IgG Secondary Antibody, Alexa Fluor 488[3]			

Footnotes: [1] Biolegend, San Diego, CA; [2] Santa Cruz Biotechnology; [3] Invitrogen

Note: PerCP: peridinin-chlorophyll protein. APC: allophycocyanin. FITC: fluorescein isothiocyanate. PE: phycoerythrin.

Supplemental Table 2. Gene set enrichment analysis (GSEA) of published single cell RNA-seq data from HSPCs[5]. Top 10 significant terms for genes that positively correlate with *Gabbr1* expression.

GO TERM	**Adjusted FWER *P* value**
GO_IMMUNE_RESPONSE	0
GO_RESPONSE_TO_INTERFERON_GAMMA	0
GO_ADAPTIVE_IMMUNE_RESPONSE	0
GO_CELLULAR_RESPONSE_TO_INTERFERON_GAMMA	0
GO_REGULATION_OF_CYCLIC_NUCLEOTIDE_METABOLIC_PROCESS	0
GO_REGULATION_OF_RHO_PROTEIN_SIGNAL_TRANSDUCTION	0
GO_POSITIVE_REGULATION_OF_RESPONSE_TO_WOUNDING	0.001
GO_NEGATIVE_REGULATION_OF_LYMPHOCYTE_DIFFERENTIATION	0.001
GO_INTERFERON_GAMMA_MEDIATED_SIGNALING_PATHWAY	0.001
GO_RESPONSE_TO_TYPE_I_INTERFERON	0.001

Supplemental Table 3. Ranked correlation coefficients of the likelihood that a gene in single-cell RNA-seq data for HSPCs[5] is co-expressed with Gabbr1 (Attached).

Supplemental Table 4. Ingenuity Pathway Analysis (IPA) results of genes up or down regulated in *Gabbr1*$^{-/-}$ mutant LSKs (Attached).

Supplemental Methods

Imaging mass spectrometry (IMS)

Femurs were harvested from female 6–8 week old C57/BL6 mice, frozen fresh in 2% carboxymethylcellulose (217277, EMD Millipore) in pre-chilled hexane on dry ice, and stored at -80 °C. Bones were sectioned at 10 microns using MX35 blades (Thermo Scientific) on a Leica CM 1850 UV cryostat. We adhered the copper tape to the slides prior to collection and a roll plate was necessary to keep the samples from fracturing. After femurs were adhered to indium tin-oxide (ITO) coated glass sides (Bruker Daltonics) using double sided copper conductive tape (Electron Microscopy Sciences), the samples were prepared for matrix-assisted laser desorption/ionization (MALDI) time-of-flight (TOF) IMS. A 1:1 mixture of 2,5-dihydroxybenzoic acid (DHB (98%), Sigma) and α-cyano-4-hydroxycinnamic acid (CHCA (98%), Sigma) was applied via a HTX TM Sprayer. The solution for the sprayer was prepared as follows: 5 mg/mL of a 1:1 mixture of DHB and CHCA was dissolved in 90:10 ACN:H_2O + 0.1% TFA and sonicated to ensure solubility. The matrix mixture was recrystallized in house as previously reported.[6] The following instrument parameters were used to apply the matrix: Flow rate = 0.2 mL/min, Velocity = 1100 mm/min, Temperature: 30˚C, Track spacing = 3 mm, Passes = 8, Nitrogen Pressure = 10 psi, Spray Pattern = CC, Drying time = 0 sec, and Nozzle Height = 40 mm. Matrix was only applied to areas of the slide with bone sample.

Phosphorus red was used as a calibrant; 0.5 µL of a 1 mg/µL solution in MeOH was spotted onto the glass directly adjacent to the copper tape. Standards of γ-aminobutyric acid (GABA, Sigma) were spotted in the following concentrations directly onto areas of bone that were not selected for IMS analysis: 10 mM, 1 mM, 100 µM, 10 µM, and 1 µM. Each standard was dissolved in H_2O and mixed with matrix (1:1 CHCA:DHB in 70:30 ACN:H_2O + 0.1% TFA) in a 1:1 ratio and 1 µL of each was spotted. Once dry, the slide was scanned at 1200 dpi. Three sections across the four bone slices were imaged to represent one complete bone: ‘top,’

‘middle,’ and ‘bottom.’ The regions that were most intact (i.e. not fractured during sectioning) were chosen for IMS, and data was acquired using flexControl v 3.4 at 20 μm spatial resolution on an Autoflex Speed LRF MALDI-TOF mass spectrometer (Bruker Scientific, Billerica, MA) over the mass range 40−700 Da. In positive reflectron mode, laser power was set to 40%, laser width to 3 and reflector gain to 10×. For each raster point 500 laser shots at 2000 Hz were shot in a random walk method. Data was subsequently analyzed in flexImaging v 4.1 × 64 (Bruker Daltonics, Billerica, MA). All spectra were normalized to root mean square (RMS). SCiLS statistical analysis software (Bruker Daltonics) was used to validate GABA is in significantly higher abundance in the endosteal region than in other regions of the bone, and the colocalization algorithm was employed to detect *m/z*’s that were significantly correlated between the highest concentrations of GABA standard (10 mM and 1 mM, as the limit of detection sits just below this range), and the endosteum. For statistical significance the Pearson correlation coefficient was set to $p<0.05$, and no de-noising was performed.

Dried drop analysis of GABA standards (Figure 1B) was performed by mixing 1 μL of matrix (1:1 CHCA:DHB in 70:30 ACN:H_2O + 0.1% TFA) with 1 μL of 20 mM GABA in H_2O (to make a final concentration of 10 mM) and spotting on a steel MALDI plate. 2000 laser shots were summed to generate the spectrum in Figure 1E-F.

Detection of GABA in extractions of fetal liver and bone marrow samples by liquid chromatography–mass spectrometry (LC-MS)

Samples were received as homogenized tissue in 5 mL PBS. Suspensions were transferred to microcentrifuge tubes and spun at 10k rpm for 2 min to pellet tissue. PBS was removed from microcentrifuge tube and tissue was submerged in 1 mL DI H_2O, then sonicated for 60 min. Extract in DI H_2O was transferred to new centrifuge tube with 300 μL of $CHCl_3$ and the tube was inverted 5x to separate polar compounds from lipids. $CHCl_3$ was transferred to new tube, and process was repeated 2x more. DI H_2O was spun at 10k rpm for 2 min and solvent was

transferred to pre-weighed microcentrifuge tube. Extract was dried in vacuo at 30°C for 2 h. For Q-ToF analysis, samples were resuspended in DI H_2O to 10 mg/mL and 10 µL injected for analysis. LC parameters were 2-10% B (A: DI H_2O + 0.1% TFA, B: ACN + 0.1% TFA) over 5 min, 98% B for 2 min, and 2% B for 2 min to reequilibrate. Tuning Mix ES-TOF (Bruker) was used as an internal calibrant. MS parameters were to detect molecules 100-1000 Da in positive mode, and 9 precursors were automatically selected for fragmentation per scan. Intensity of GABA was evaluated by selecing an Extracted Ion Chromatogram of m/z 104.0712 (protonated GABA), based off of measured precursor ion from GABA standard (250 µM). Samples were run in duplicate.

Peripheral blood (PB) counts

Blood was obtained through retro-orbital bleeding and transferred to EDTA-coated tubes. PB cell numbers were determined using a Vet Abc Hematological analyzer (Scil Animal Care).

Analysis of different phenotypically-defined hematopoietic populations

For isolation of BM hematopoietic cells, both femora and tibiae were harvested from postnatal day 15 (P15) mice after they were euthanized with 3-5% isoflurane. BM cells were collected from bones into PBS containing 2% FBS. For isolation of fetal liver hematopoietic cells, fetal livers were dissected from embryonic day E14.5 embryos. Red blood cells were removed by ACK lysing buffer (Invitrogen) to have mononuclear cells for the following experiments.

Mononuclear cells from BM or fetal liver were incubated with anti-CD16/32 antibody (except CMP, GMP and MEP) to block the Fcγ receptors. The cells were then stained anti-CD3e, anti-CD45R/B220, anti-Gr-1, anti-CD11b, and anti-Ter-119 antibodies (CD11b antibody was excluded in fetal liver cells), and stained with various antibody combinations as shown in Supplemental Table 1 to analyze LSK progenitors ($Lin^-Sca1^+c\text{-}kit^+$ cells), HSCs ($CD150^+CD48^-$ LSK progenitors), MPP1 ($CD150^-CD48^-$LSK progenitors), MPP2 ($CD150^+CD48^+$LSK

progenitors), MPP3/4 ($CD150^-CD48^+$LSK progenitors), common lymphoid progenitors (CLP, $Lin^-Sca1^{low}c\text{-}kit^{low}CD135^+CD127^+$), common myeloid progenitors (CMPs, $CD16/32^-CD34^+Lin^-Sca1^-c\text{-}kit^+$ cells), granulocyte-macrophage progenitors (GMPs, $CD16/32^+CD34^+Lin^-Sca1^-c\text{-}kit^+$ cells), and megakaryocyte-erythrocyte progenitors (MEPs, $CD16/32^-CD34^-Lin^-Sca1^-c\text{-}kit^+$ cells). 4′,6-diamidino-2-phenylindole (DAPI, Sigma, St. Louis, MO) was used to exclude dead cells. For each mouse sample, approximately 1 x 10^6 cells were acquired, and the data were analyzed using BD FACSDiva 6.0 (BD Biosciences, San Jose, CA) and FlowJo (FlowJo, Ashland, OR) software.

The numbers of the different hematopoietic cell populations in each mouse were calculated by multiplying the total numbers of BM cells or fetal liver cells from each mouse or embryo with the frequencies of each population.

Hardy B cell fractions: Mononuclear cells from BM were incubated with anti-CD16/32 antibody to block the Fcγ receptors. The cells were then stained lineage antibodies anti-CD3e, anti-Gr-1, anti-CD11b, and anti-Ter-119, and stained with anti-B220, anti-CD43, anti-CD24, anti-BP-1, anti-IgM and anti-IgD as shown in Supplemental Table 1 to analyze different B cell fractions. DAPI was used to exclude dead cells. Fraction A: $Lin^-CD43^+B220^+CD24^-Bp\text{-}1^-$, Fraction B: $Lin^-CD43^+B220^+CD24^+Bp\text{-}1^-$, Fraction C: $Lin^-CD43^+B220^+CD24^-Bp\text{-}1^+$, Fraction D: $Lin^-CD43^-B220^+IgM^-IgD^-$, Fraction E: $Lin^-CD43^-B220^+IgM^+IgD^-$, Fraction F: $Lin^-CD43^-B220^+IgM^{+/-}IgD^+$. All antibody information used in the present study is provided in Supplemental Table 1.

Secondary HSC transplantation

Three months after transplantation, 1 × 10^6 BMCs from the recipient mice were used to analyze donor-derived LSK progenitors and HSCs using flow cytometer. 1 × 10^6 BMCs from the recipient mice were directly injected into lethally irradiated CD45.1 recipients to perform secondary transplantation. In addition, 350 WT and *Gabbr1*$^{-/-}$ HSCs from primary recipients were sorted and mixed with 350 competitive HSCs pooled from CD45.1/2 mice. Mixed cells

were then retro-orbitally transplanted into lethally irradiated CD45.1 recipients with 5×10^5 CD45.1 spleen cells. The donor cell engraftment ability was determined at 2 and 4 months after secondary transplantation using peripheral blood. 4 months after secondary transplantation, donor-derived LSK progenitors and HSCs in bone morrow were analyzed using flow cytometer. All antibody information used in the present study is provided in Supplemental Table 1.

Poly(I:C) treatment and BrdU incorporation assay for phenotypically-defined cells

To measure the responses of WT and *Gabbr1*$^{-/-}$ mice to interferon signaling, P15 mice were peritoneally injected with polyriboinosinic:polyribocytidylic acid (poly(I:C); InvivoGen, San Diego, CA). 48 hours later, mice were euthanized with 3-5% isoflurane and BM cells were utilized for cell cycle and apoptotic analysis in LSK progenitors and HSCs.

To assess proliferating status of hematopoietic cells from WT and *Gabbr1*$^{-/-}$ mice, BrdU (50 mg/kg, BD Biosciences, San Diego, CA) was IP injected into P15 animals. 48 hours later, BrdU incorporation was measured by flow cytometry using the APC BrdU Flow Kit from BD Biosciences (San Diego, CA), following the protocol from the manufacturer, after the cells were stained with antibodies against various cell surface markers. The percentages of $BrdU^+$ cells within the LSK and LSK $CD48^-$ populations were calculated.

Apoptosis assay for phenotypically-defined cells

Fc-γ receptors in BMCs were blocked with anti-CD16/32 antibody at 4°C for 15 minutes. Cells were then stained with various antibodies against cell surface markers. Annexin V staining was performed with a kit from BD Pharmingen (San Diego, CA) according to the manufacturer's protocol. The ratios of Annexin V positive cells were analyzed within different hematopoietic cell populations using a Fortessa flow cytometer.

Caspase 3 activity assay

Mononuclear cells from BM were incubated with anti-CD16/32 antibody to block the Fcγ receptors. The cells were then stained anti-CD3e, anti-CD45R/B220, anti-Gr-1, anti-CD11b, and anti-Ter-119 antibodies, and stained with c-Kit, Sca-1, CD150 and CD48 antibody combinations. Caspase 3 antibody was subsequently added to measure caspase 3 activity using CellEvent™ Caspase-3/7 Green Flow Cytometry Assay Kit (ThermoFisher Scientific Inc. USA). Caspase 3 activity was expressed as mean fluorescent intensity (MFI).

Cell cycle assay for phenotypically-defined cells

1×10^6 BMCs were stained with various antibodies against cell-surface markers and fixed and permeabilized using the Fixation/Permeabilization Solution from BD Biosciences (San Diego, CA). The cells were then stained with anti-Ki67-FITC antibody and 7-AAD (Sigma, St. Louis, MO) for 30 min on ice. Cell cycle was analyzed by flow cytometry and Flowjo software.

GAD1 immunostaining of sternum BM

Whole mount sternum imaging was performed as previously described[7]. Mouse sternums were dissected immediately after euthanasia and connective tissue was removed. Sternum segments were sectioned along the frontal plane and each half was transferred to individual wells in a 96-well plate and incubated with PE-anti mouse B220 (1:200, BioLegend) in 100μl of ice-cold PBS + 2% FBS for 30 minutes. Following staining, specimens were fixed in 4% PFA for 30 minutes, blocked and permeabilized for 45 minutes in PBS containing 0.25% Triton X-100 and 5% donkey serum before incubating with anti-mouse GAD1/GAD-67 (dilution 1:50, Clone F-9, Santa Cruz Biotechnology, Catalog# sc-28376) overnight at 4C. After washing, sternum slices were then incubated with donkey anti-Mouse IgG Secondary Antibody, Alexa Fluor 488 (1:2000, Invitrogen) for 1 hour at room temperature followed by DAPI nuclei staining. Confocal images

were obtained on a Zeiss LSM710 confocal microscope. Images were processed using ImageJ. All antibody information used in the present study is provided in Supplemental Table 1.

GAD1 intracellular flow cytometry

1×10^6 BMCs were blocked with CD16/32 antibody and stained with anti-CD93, anti-B220 and anti-CD11b antibodies. Cells were fixed and permeabilized using the Fixation/Permeabilization Solution from BD Biosciences (San Diego, CA). The cells were then stained with anti-GAD1 antibody (Clone F-9, Santa Cruz Biotechnology, Catalog# sc-28376) for 30 min on ice. GAD1 expression was analyzed by flow cytometry and Flowjo software. All antibody information used in the present study is provided in Supplemental Table 1.

Quantitative RT-PCR

Various populations were directly sorted into RLT Plus buffer. RNA was extracted using the Qiagen RNeasy minikit or microkit (Qiagen, USA). cDNA was synthesized from RNA with the Super Script III kit (ThermoFisher Scientific Inc. USA). Transcripts were amplified with SYBR Green PCR master mix (Applied Biosystems, USA), and qPCR for Gabbr1 and GAD1 genes was performed on the ABI Prism 7900HT system (Applied Biosystems, USA). GAPDH was used as a housekeeping gene. *Gabbr1* primers: F, 5'-CAAAACAGACAAGTGGATCGGAGG-3'; R, 5'-CTGGGAGTTCTGGATATAACGAAC-3'. *Gad1* primers: F, 5'-TCGATTTTTCAACCAGCTCTCTACT-3'; R, 5'-GTGCAATTTCATATGTGAACATATT-3'.

Ionizing irradiation

CD45.1 C57BL/6 Female were used for recipients in BM transplantation assay. Six to eight-week-old females were loaded on a rotating platform and exposed to 9.0 Gy of ^{137}Cesium γ-ray irradiation in an irradiator (J. L. Shepherd, Glendale, CA) at a dose rate of 6.38Gy/min.

Western blot

Brain tissues from WT and *Gabbr1*$^{-/-}$ mice were lysed in lysis buffer (20 mM Tris–HCl, pH 7.4, 150 mM NaCl, 1 mM EDTA, 1 mM EGTA, 10% glycerol, 1.0% NP-40, 0.1 M NaF, 1 mM DTT, 1 mM PMSF, 1 mM NaVO4, 2 μg/ml leupeptin and aprotinin) for 30 minutes on ice. Protein concentration was determined with the Bio-Rad protein assay dye reagent (Bio-Rad). Proteins were separated using SDS-PAGE and transferred to PVDF membranes. Antibodies used for Western blot: GABBR1 (mouse monoclonal antibody specific for an epitope mapping between amino acids 929-958 at the C-terminus of $GABA_B$ R1 of rat origin; clone D-2; #sc-166408 Santa Cruz Biotechnology, Santa Cruz, CA); GAPDH (Sigma, St. Louis, MO); secondary anti-rabbit-HRP or anti-goat-HRP or anti-mouse-HRP (Pierce). Blots were visualized with SuperSignal west femto chemilumenscence substrate (Thermo Fisher Scientific. Waltham, MA).

In vitro B cell differentiation assay

4,000 LSK progenitors from WT and *Gabbr1*$^{-/-}$ mice were directly sorted onto pre-prepared OP9 stromal cells with 1 ml complete DMEM medium containing 10% FBS, 1 ng/ml IL-7, and 5 ng/ml Flt-3L. 3 and 6 days later, 1 ml complete DMEM medium was added, respectively. One week after initial culture, all cells including OP9 cells were mechanically detached and filtered with a 70 μm cell strainer. Cells were passaged onto fresh OP9 stromal cells with complete medium. Medium was changed every 3 days. At 14 days after initial culture, all cells were detached and stained with CD45.2, CD19, B220 and CD11b antibodies. The numbers of the different hematopoietic cell populations in each well were calculated by multiplying the total cell numbers from each well with the frequencies of each population. To assess the effects of Gabbr1 on myeloid cell differentiation from LSK cells, the DMEM medium containing 10% FBS, 1 ng/ml IL-7, 5 ng/ml Flt-3L, 5 ng/ml IL-6, 6 ng/ml IL-3 was used. 14 days after sorted LSK cells were cultured on OP9 cells, numbers of $CD11b^+$ and $Gr\text{-}1^+$ cells were measured via flow cytometry. All antibody information used in the present study is provided in Supplemental Table 1.

Mouse Hematopoietic Progenitor Cell Colony Assays

Assays were performed as previously described[8-11]. Bone marrow cells were harvested from 2 tibias by flushing with DPBS (Hyclone). Splenocytes were isolated from spleens by homogenizing individual spleens through a 70uM mesh (Fisher) using DPBS. Colony forming units (CFU)-granulocyte macrophage, burst forming units (BFU)-erythroid (E), granulocyte, erythrocyte, macrophage, megakaryocyte (GEMM) colonies were scored after 7 days in culture by plating whole bone marrow at $5x10^4$ cells/ml and splenocytes at $2x10^5$ cells/ml in 1% methylcellulose/Iscoves Modified Dulbeccos Medium (IMDM) with 30% FBS (Corning), 1U/ml erythropoietin (Amgen), 5%(v/v) pokeweed mitogen spleen cell conditioned medium (PWMSCM), 50ng/ml recombinant mouse stem cell factor (rmSCF, R&D Systems), and 0.1mM Hemin (Sigma). Cultures were incubated at 37^0C with 5% CO2/O2. Absolute numbers of progenitors were calculated based on nucleated cell counts. Colonies were identified by the type of cells they contained: BFU-E contained only erythroid cells, as defined by their characteristic red color, with hemin enhancing this color; CFU-GEMM contained GM cells, erythroid cells, and usually have megakaryocytes. The tritiated thymidine cell kill procedure was performed as previously described[8-12]. Briefly, to calculate percent of progenitors in S-Phase of the cell cycle (an estimate of cell cycling) cells were pulsed with or without high specific activity tritiated thymidine (1mCi/ml, Perkin Elmer) for 30 minutes at 37^0C. Cells were then washed and plated, and the differences in number of each type of colony between cells pulsed with control media and high specific activity tritiated thymidine was used to calculate the percent of each type of colony in S-phase of the cell cycle.

Human UCB colony forming assays

Colony forming assays were set up with either CD34+ UCB progenitors treated as above prior to xenotransplant (i.e. input cells), or from xenotransplant recipient BM after 16 weeks (i.e. output cells). Input cells were plated at a density of 1 x 10^3 cells/dish. Output cells were plated at a density of 2 x 10^5 cells/dish. Cells were mixed with MethoCult M3434 (STEMCELL Technologies), and plated into each of three 35 mm gridded dishes. Dishes were incubated at 37°C for 12 days, and colonies enumerated under a bright light microscope. A clonogenic colony contained at least 50 cells or greater.

CD34+ UCB cell xenotransplants into NSG mice

All NSG studies were carried out in accordance with, and approval of the Institutional Animal Care and Use Committee of Indiana University School of Medicine and the Guide for the Care and Use of Laboratory animals. Female NOD.Cg-PrkdcscidIl2rgtm1Wjl/SzJ (NSG) mice were obtained from the In Vivo Therapeutics Core of the Indiana University Simon Cancer Center. Animals were maintained under pathogen-free conditions and maintained on Irradiated Global 2018 (Uniprim 4,100 ppm; TD.06596, Harlan Laboratories USA) food pellets with ad libitum access to autoclaved, acidified tap water under a 12-hour light–dark cycle at 22°C to 24°C. Mice were irradiated 4 hours prior to transplant (3.0 Gy of 137Cesium γ-ray irradiation in an irradiator (J. L. Shepherd, Glendale, CA) at a dose rate of 88 cGy/min). CD34+ UCB cells were thawed in 1% human serum albumin in StemSpan media (STEMCELL Technologies) with human SCF 100 ng/ml and allowed to sit for 2 hours. Cells were then counted and plated at 0.5 x 10^6 cells/ml in StemSpan media with 1% HSA. Cells were plated and treated with the following for 2 hours at 37°C: 1, 10, or 50 μM (RS)-Baclofen (Tocris Cat. No. 0417/1G); 1, 10, or 50 μM 2-hydroxy-saclofen (Tocris Cat. No. 0245/10); vehicle control (DMSO). Cells were harvested

and immediately was with PBS by centrifugation at 300xg for 10 minutes. Mice were transplanted with 20,000 cells each in 200 μl PBS (n=7 mice per group). PB and BM were harvested at 16 weeks post-transplant.

Analysis and multi-parametric flow cytometry immunostaining of xenotransplant mice

For peripheral blood counts, blood was obtained through retro-orbital bleeding and transferred to EDTA-coated tubes. Peripheral blood cell numbers were determined using a Vet Abc Hematological analyzer (Scil Animal Care). For PB, BM, and spleen analysis of the target populations in xenotransplant mice, the cells were collected using a red blood cell lysis (eBioscience). The following primary conjugated monoclonal antibodies were used: anti-human CD34 PE (BD Pharmingen), anti-human AC133 APC (Miltenyi Biotec), anti-human CD19 PECy7 (BD Pharmingen), anti-human CD33 PerCP-Cy5.5 (BD Pharmingen), anti-human CD38 FITC (BD Pharmingen), anti-human CD45 APC-AlexaFluor (AF) 750 (Invitrogen), and the fixable amine reactive viability dye, LiveDead (Violet, Invitrogen).

The lysed PB, BM, and spleen cells were incubated with both human and murine Fc blocking reagent (Miltenyi Biotec) for 10 minutes on ice. Briefly, cells were incubated with the titred antibodies listed above for 30 minutes at 4˚C, washed twice in PBS with 2% fetal bovine serum, fixed in 1% paraformaldehyde (Tousimis), and run within 24 hours on a BD Fortessa flow cytometer (BD, Franklin Lakes, NJ, USA) equipped with a 405nm violet laser, 488nm blue laser, 640nm red laser, and 561nm yellow-green laser. Approximately 100,000 events per sample were acquired uncompensated and exported as FCS 3.1 files, and analyzed utilizing FlowJo software, version 9.9.6 (Tree Star, Inc). “Fluorescent minus one” (FMO) gating controls were also used to ensure proper gating of positive events[13,14].

Statistical analysis

The data were presented as mean ± SD. Analysis of variance (ANOVA) was used for data analysis. For experiments, single experimental and control groups were used, the differences were analyzed by an unpaired Student's t test. Differences among the groups were analyzed by Student-Newman-Keuls multiple comparisons test after two-way ANOVA. $p<0.05$ was considered a significant difference. GraphPad Prism from GraphPad Software were used for all analyses.

Analysis of published single cell RNA-seq gene expression profiles of HSPCs

The count data for the previously published single cell gene expression data set [5] was downloaded directly from http://blood.stemcells.cam.ac.uk/data/normalisedCountsVariableGenes.txt.gz. Data was processed by the Scatter library from the R Bioconductor resource. *Gabbr1* expression was assessed for contribution to different clusters. To identify genes with expression correlated to *Gabbr1*, we used a linear correlation and ranked the correlated genes by their correlation co-efficient. Next, we applied gene set enrichment analysis (GSEA) software using the *biological process* sets from the Gene Ontology[15] (http://www.broad.mit.edu/gsea/) on a ranked list of genes using the correlation scores. In this way, genes with high positive correlation were co-expressed and co-regulated with *Gabbr1*, while negatively correlated genes were not expressed and showed opposite expression regulation (repressed when *Gabbr1* is expressed). Enriched gene sets were assessed by the FEWER adjusted p-value for significance and those with a value of <0.05 were accepted as significant.

Bulk RNA-seq sample preparation

LSK cells (~12,000) from P15 wild-type (n=4) or *Gabbr1* null (n=4) BM were sorted (MoFlo Astrios) and collected in Trizol LS (Invitrogen). Volumes were adjusted to a final ratio of 3:1 for Trizol LS and nuclease-free water. Samples were stored at -80°C until total RNA was isolated. Then samples were thawed, equilibrated to room temperature, and divided into 800 µl aliquots. 5 µg of Sigma "GenElute" linear polyacrylamide (LPA; Cat. No. 56575-1ML), diluted in nuclease-free water, was added to each aliquot and vortexed for 30 seconds. Chloroform extraction and 3 ethanol washes were performed as per the Trizol product insert (Invitrogen protocol Cat. No. 10296-010). The pellet was resuspended in 6 µl RNAse-free water.

Bulk RNA-seq library preparation and sequencing

RNA samples were quantified and purity and integrity were checked using NanoDrop™ One Spectrophotometer (Thermo Scientific) and Agilent 4200 TapeStation. NGS library preparation was completed using NuGen's Ovation® SoLo RNA-Seq Systems, per their User Guide M01406 v4, including a targeted depletion with SoLo AnyDeplete Mouse Probe Mix. RNA input into library preparation was between 3.6 - 5 ng. The number of cycles for the first library PCR amplification were optimized using qPCR, as outlined in the SoLo User Guide, with the Prime Pro 48 Real-Time PCR system (Techne).

Completed libraries were quantified using Qubit fluorometer (Invitrogen) and size was determined using TapeStation. Libraries were pooled based on Qubit dsDNA concentration and run on MiSeq for index balancing. The second pool, with corrected library inputs based on the %Reads Identified (PF) results from the MiSeq run, was purified with 1:1 Agencourt AMPure XP beads. The final, purified pool was quantified using Qubit and pool size was determined using TapeStation. Sequencing was run on a NovaSeq6000 SP flow cell, 2x50 bp, two lanes, at the

University of Illinois Roy J. Carver Biotechnology Center High-Throughput Sequencing and Genotyping Unit.

Bulk RNA-seq data analysis

After library preparation and sequencing, raw reads were aligned to reference genome mm10 using STAR[16]. ENSEMBL genes were quantified using FeatureCounts[17]. Differential expression statistics were computed using edgeR[18,19] on raw expression counts with the exactTest function. Principal component analysis (PCA) identified outlier samples that were removed from further analysis. The final analysis included n=2 wild-type samples and and n=3 *Gabbr1* null samples. P-values were adjusted for multiple testing using the false discovery rate (FDR) correction of Benjamini and Hochberg[20]. Data were analyzed through the use of IPA to find “Ingenuity Canonical Pathways” that were associated with genes expressed at higher or lower levels in *Gabbr1* null relative to wild-type (QIAGEN). For pathway enrichments, up and down-regulated genes were determined based on a FDR cutoff of 0.1 and compared to single-cell RNA-seq gene lists from Miyai et al.[21] using Fisher’s Exact Test. RNA-seq data will be deposited in the NCBI GEO database.

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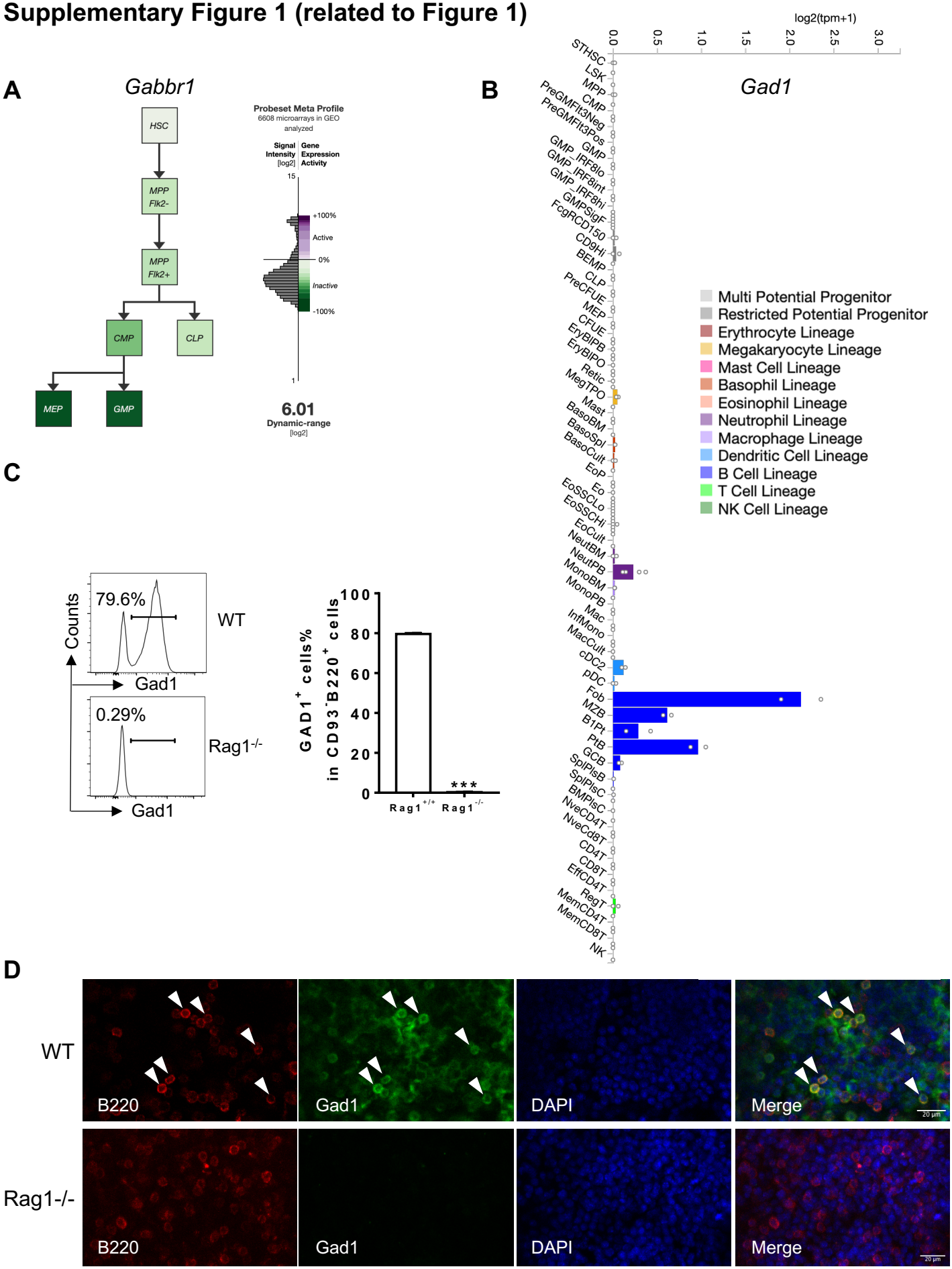
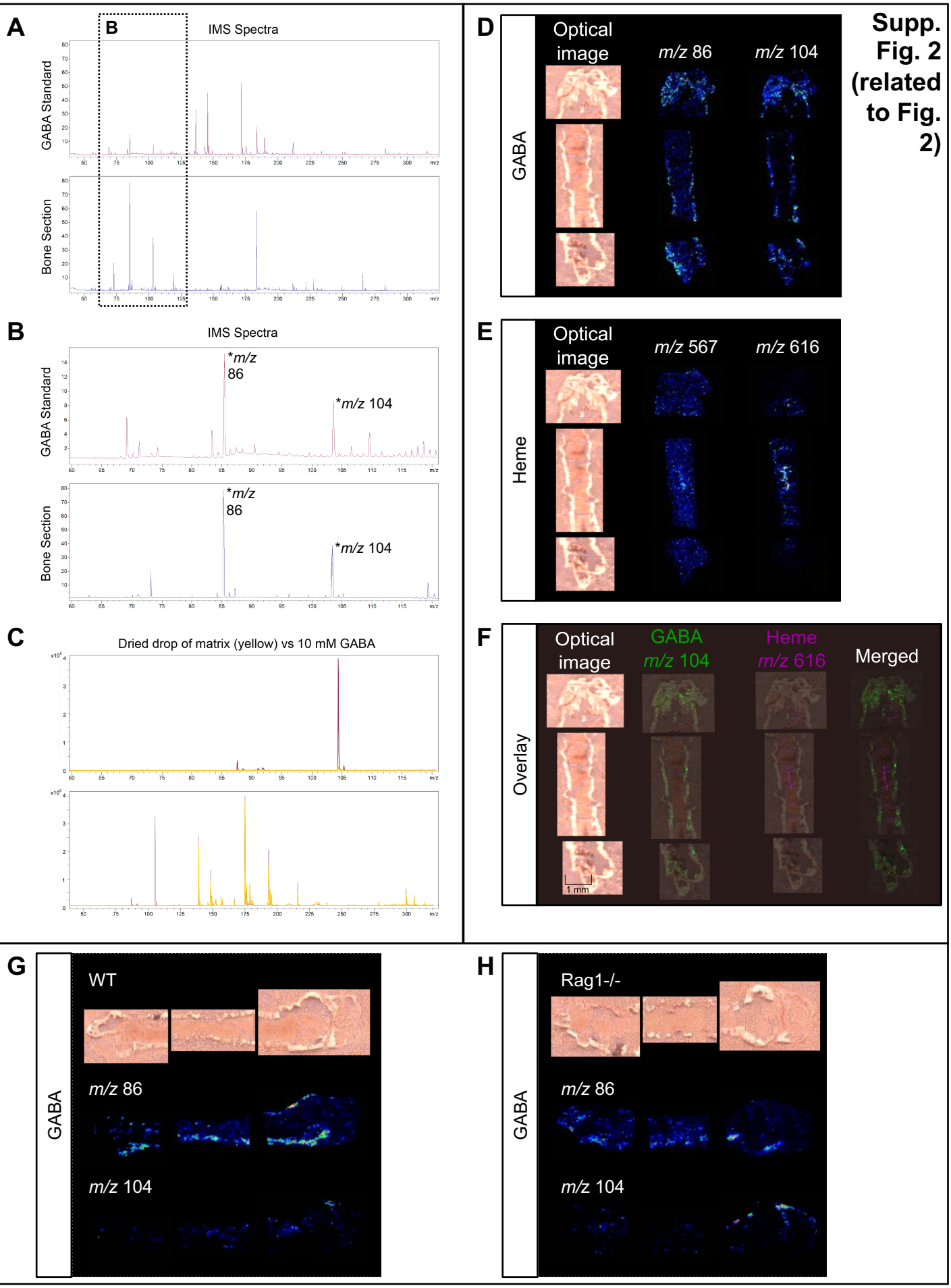
Supplementary Figure 1 (related to Figure 1)




A
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Dried drop of matrix (yellow) vs 10 mM GABA
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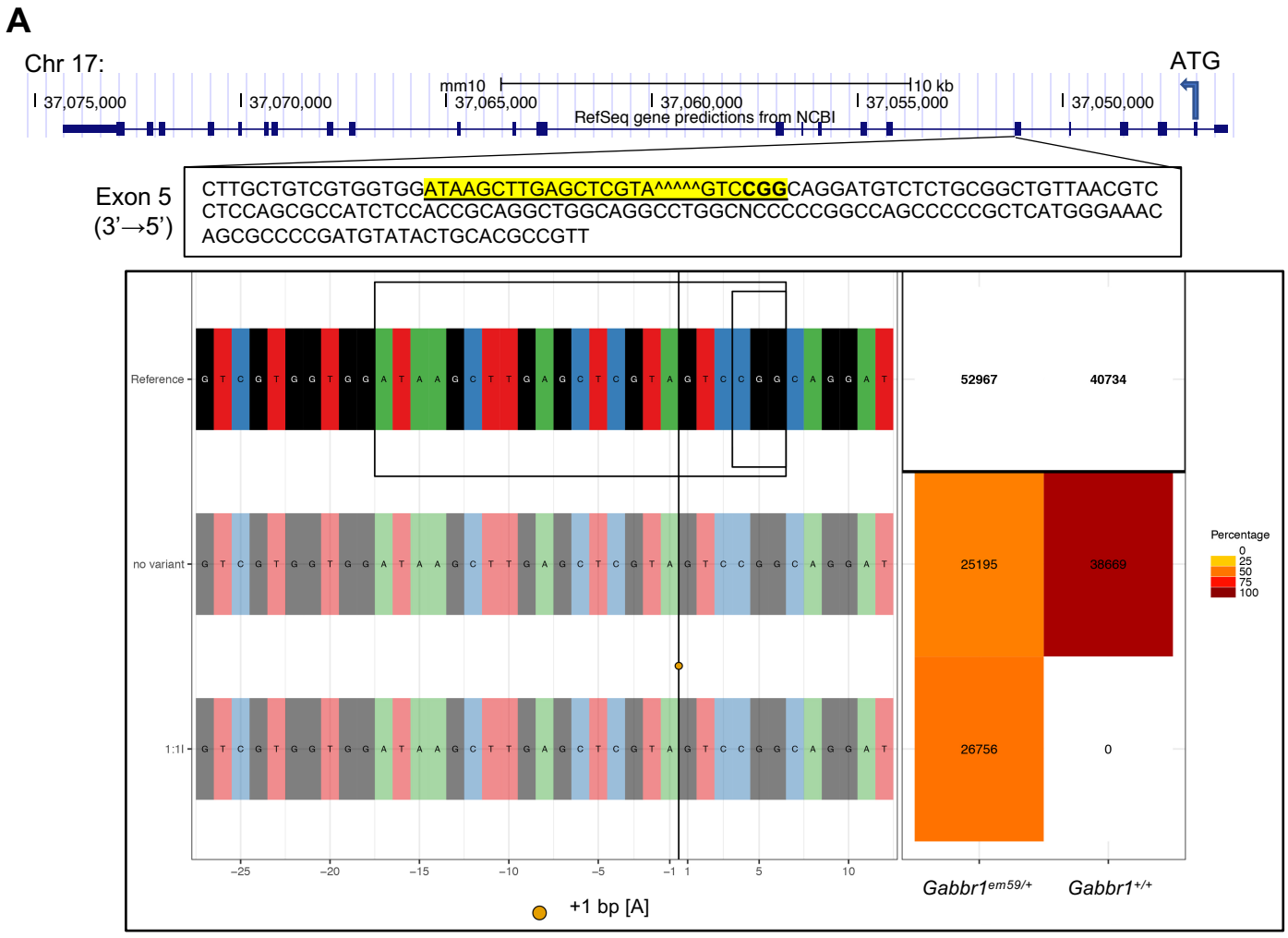
Supp. Fig. 2 (related to Fig. 2)

Supplementary Figure 3 (related to Figure 3)



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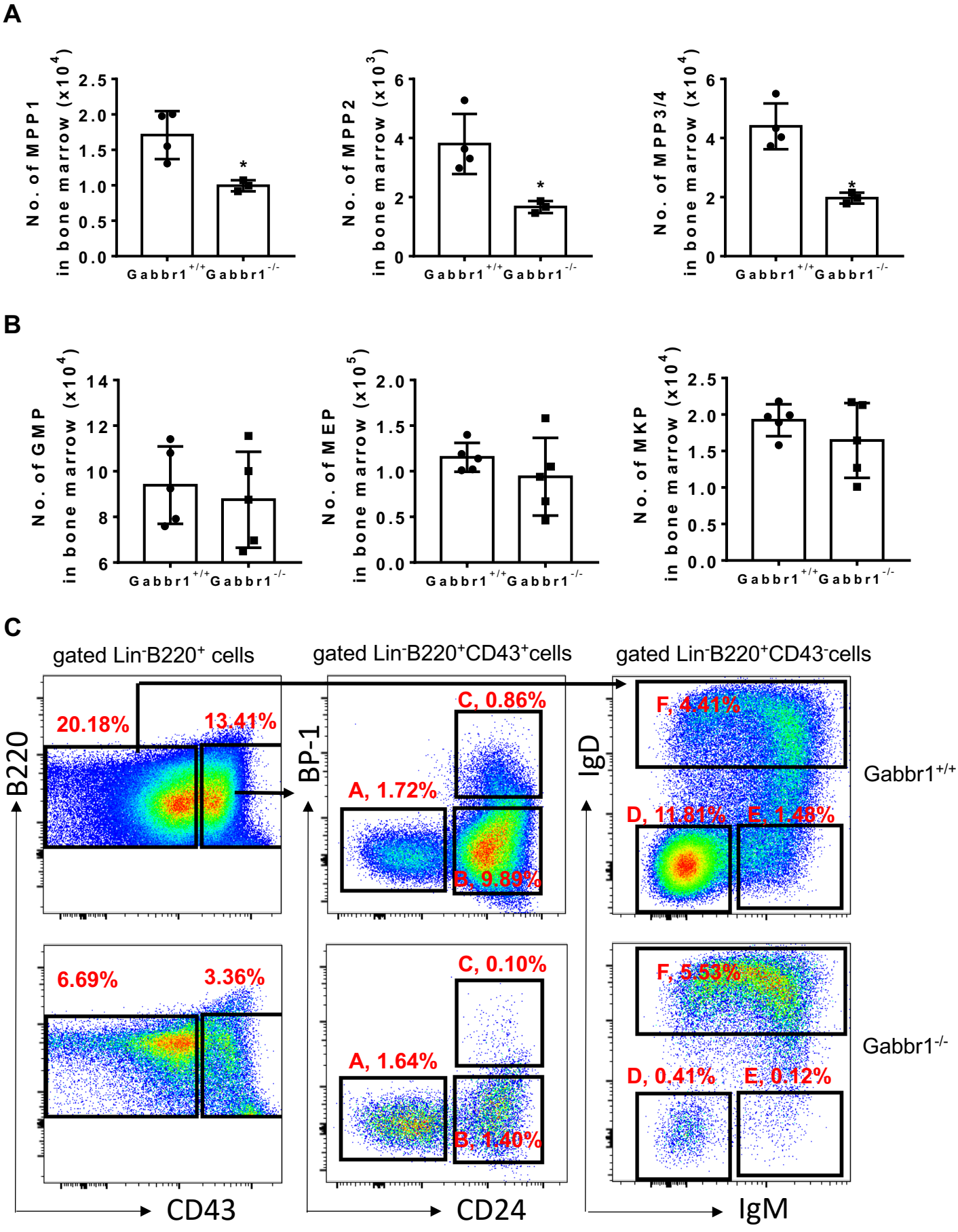
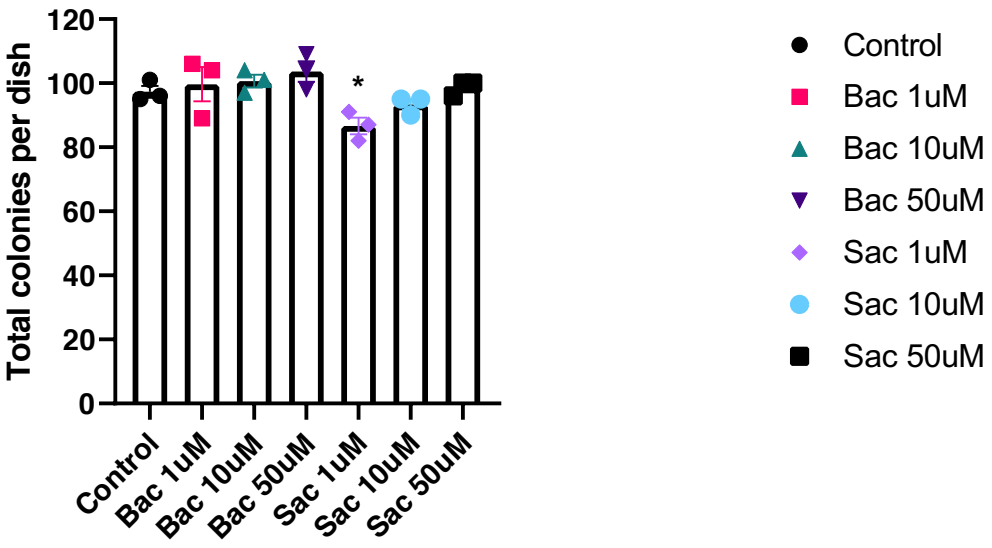
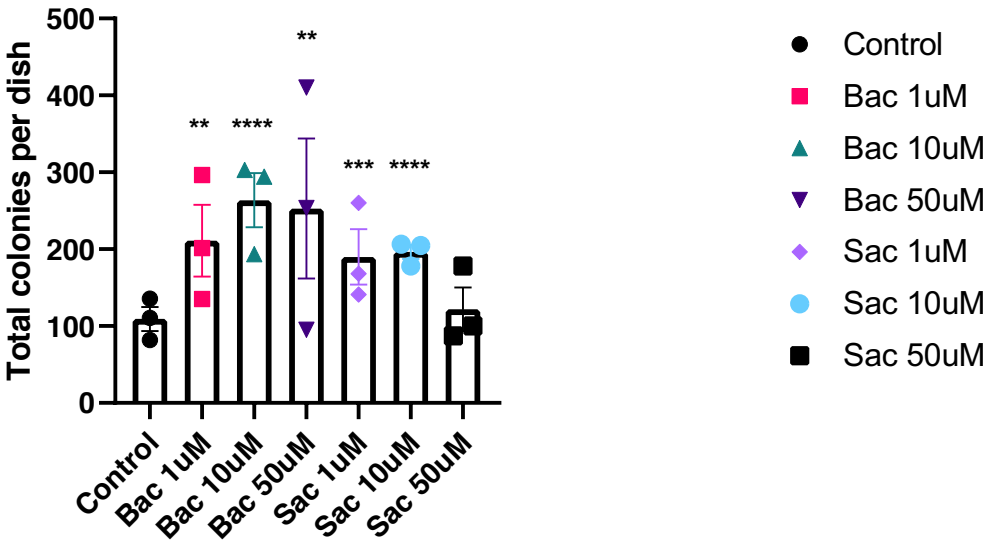


Supplementary Figure 5 (related to Figure 6)

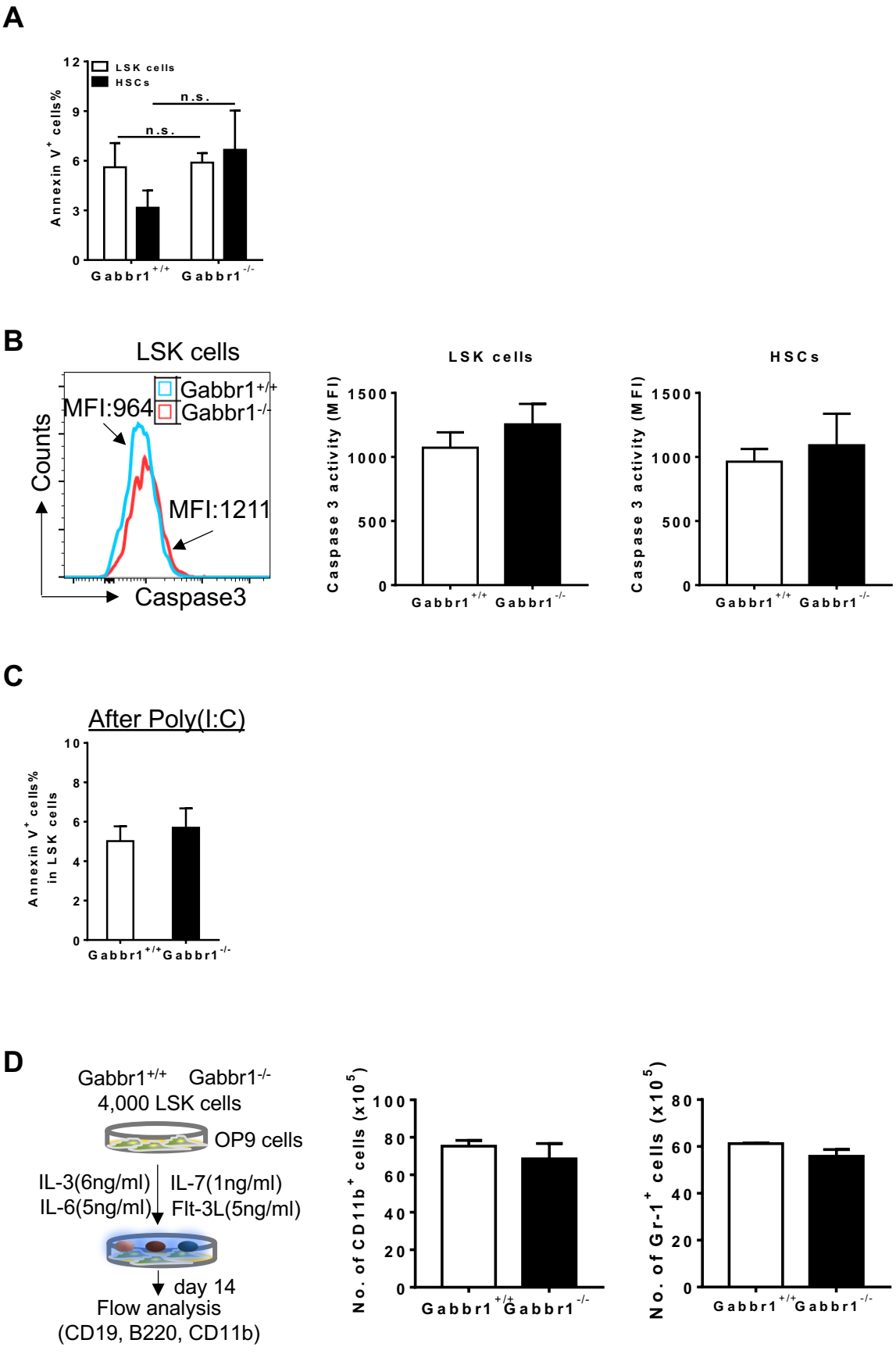
A **Progenitor input: UCB HSPCs after 2 hour treatment**



B **Progenitor output: BM 16 weeks after transplant**

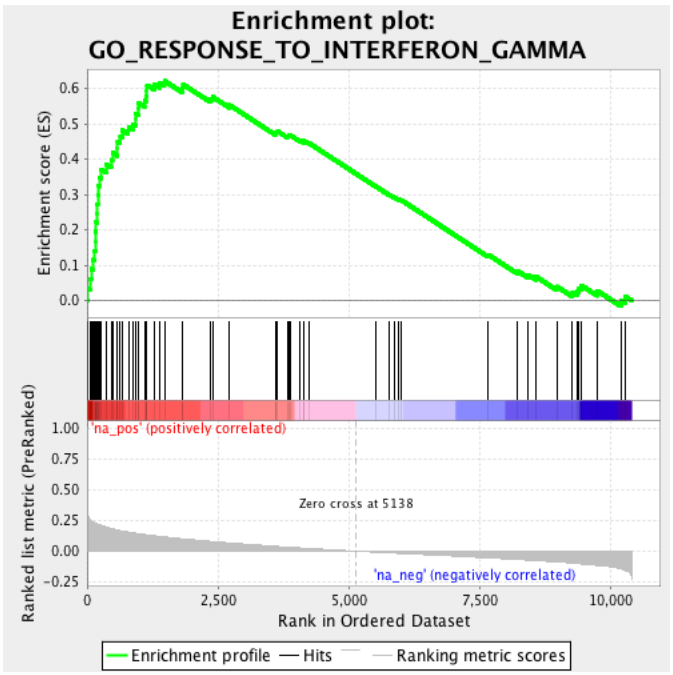


Supplementary Figure 6 (related to Figure 5)



Supplementary Figure 7 (related to Figure 6)

A



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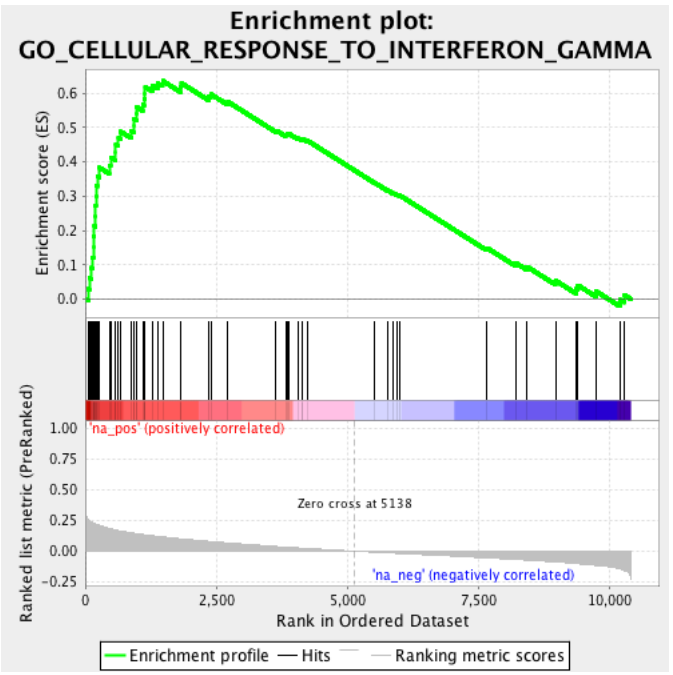

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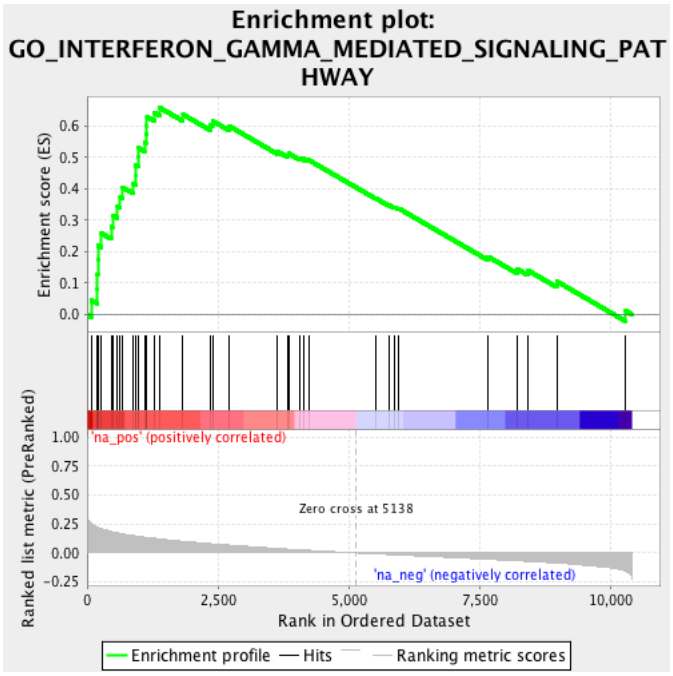

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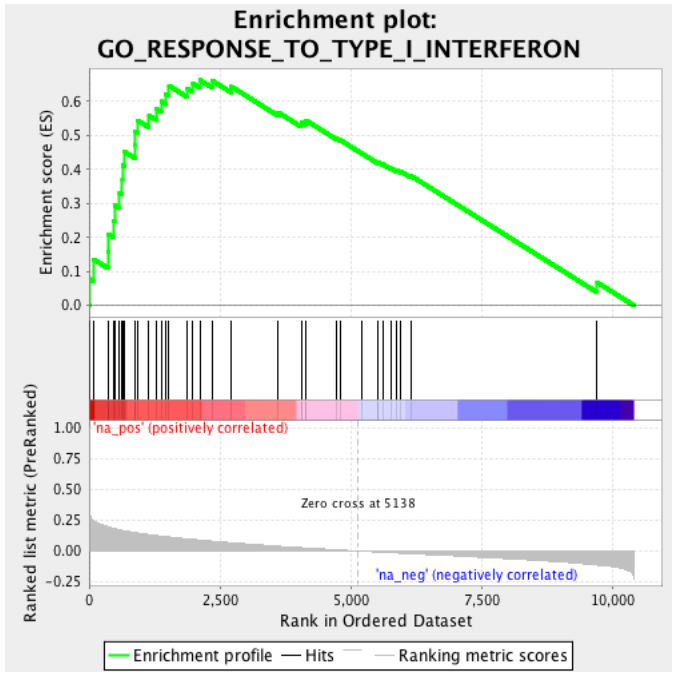
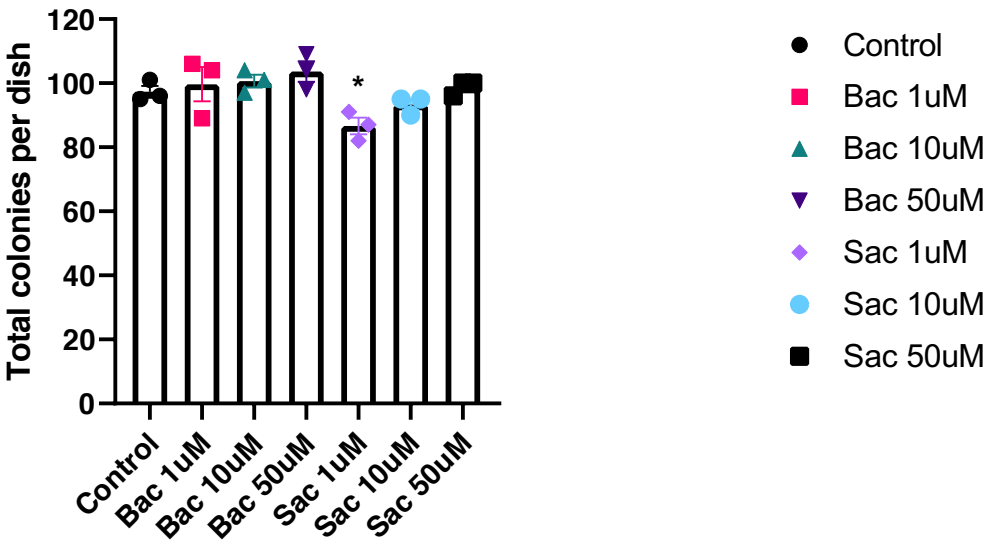


Supplementary Figure 8 (related to Figure 7)

A **Progenitor input: UCB HSPCs after 2 hour treatment**



B **Progenitor output: BM 16 weeks after transplant**