Supplementary Information

Critical Role of *Lama4* for Hematopoiesis Regeneration and Acute Myeloid Leukemia progression

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Running title: *Lama4* in hematopoiesis and AML

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Supplementary Methods

Animal models

Wild type (*Lama4*$^{+/+}$) and *Lama4*$^{-/-}$ CD45.2 C57BL/6J mice aged 7-14 weeks were used for this study. For inducing myelosuppression by sublethal irradiation, the mice were irradiated (6-7Gy) using Gammacell® 40 Exactor (Best Theratronics, CA). Transplantation-induced MLL-AF9 AML mouse model was generated as described.[1] For therapeutic transplantation experiments, in *Lama4*$^{-/-}$ and *Lama4*$^{+/+}$ mice which had developed leukemia after injection of MLL-AF9 AML cells, were lethally irradiated (9.5-10Gy). 4-6 hours later normal BM mononuclear cells from C57BL/6-Tg (CAG-EGFP) mice (The Jackson Laboratory, USA) were transplanted via tail vein. For Ara-C treatment, the mice were first sublethally irradiated with 6Gy prior to the AML cell injection. The AML engraftment in PB was analyzed by FACS at 14 days after AML cell injection. The mice were then injected with normal saline (NS) or Ara-C (700mg/Kg body weight) for 5 days starting at day 15. The AML development was monitored by observing general health conditions such as movement, fur and breathing. The survival rate was counted based on the dates when mice were found dead or moribund mice were terminated due to ethical reasons. The time difference from the actual date of death could be 0.5-1 days in some of the mice according to previous studies. Blood cell counts were analysed by hematology analyzer Sysmex-XP300 and FACS.

Isolation of mononuclear cells from mouse BM

Mononuclear cell isolation was done as previously described.[1] Briefly, femurs, tibias and iliac crests were crushed in PBS+10% FBS. The marrow cells were collected. The cells adjacent to bone were obtained by subsequent treatment of bone fragments with 0.1% Collagenase II (CLS II Worthington Biochemical) for 30-45 min and with 0.05% trypsin-EDTA for 10 min at 37°C. Both cell fractions were pooled and spun down at 300g for 5-10 min and then resuspended in PBS+10% FBS for MSC isolation.

Multi-color fluorescence activated cell sorting (FACS) of MSCs and their progeny

Mouse BM MSCs were isolated as described.[1,2] The BM hematopoietic cells were first depleted by staining the cells with purified rat anti-mouse CD45 and hematopoietic lineage (LIN) markers TER119, CD19, CD3, GR1, NK1.1, CD11B and subsequently using sheep anti-rat Dynabeads (Invitrogen). The remaining hematopoietic cells were visualized by fluorescence-conjugated CD45 and TER119 for further removal of the residual hematopoietic cells.

Fluorochrome-conjugated antibodies CD31, SCA1, CD51 and CD44 were used to sub-fractionate the stromal cells into $CD45^-TER119^-$ $CD31^-CD44^-CD51^+SCA1^+$ MSCs, $CD45^-TER119^-CD31^-CD44^-CD51^+SCA1^-$ MPCs, $CD45^-TER119^-CD31^-CD44^-CD51^-SCA1^-$ mature stromal cells and $CD45^-TER119^-CD31^+$ endothelial cells. Dead cells were excluded by propidium iodide (PI) staining. The BM stromal cell subsets were sorted or analyzed on FACS Aria III (BD). The cells were gated according to fluorescence minus one (FMO). See Table S1 for detailed information about the antibodies used in the study.

Confocal imaging

Tibias and femurs were fixed with 4% paraformaldehyde overnight, then decalcified in 0.5 M EDTA, pH 7.2~3 in distilled water for 3-5 days at 4°C on a rotator. Subsequently, the samples were dehydrated with 30% sucrose in PBS at 4°C for overnight, then embedded in Tissue-Tek OCT Compound (4583, Sakura Finetek, Tokyo, Japan) and immediately frozen on dry ice and stored in -80°C freezer. The frozen blocks were thermally equilibrated in cryostat before sectioning. Bone tissues were sectioned at 50-70µm thick with cryostat (CM3050, Leica, Wetzlar, Germany), placed on positively charged slides (Superfrost Plus, VWR, Radnor, PA), air-dried at room temperature for >1hour. The sections were then permeabilized with 0.1% Triton-X with PBS for 10 min. For the staining of LAMA4, samples were warmed in antigen retrieval solution (S1699, Agilent Technologies, Santa Clara, CA) with microwave until they were hot but not boiling. After incubation with Background Eraser (BE965 H, Biocare Medical, Pacheco, CA*)*, primary antibodies were applied for overnight at 4°C. After washing with PBS, the sections were incubated with fluorescently labeled secondary antibodies (1:500) in 1% donkey serum in PBS for 60 min. Nuclei were visualized with 0.1µg/mL of 4',6-diamidino-2-phenylindole (DAPI). Subsequently, the sections were washed and sealed with aqueous mounting reagent (TA-030-FM, Thermo Fisher Scientific, MA). Images were taken with Eclipse Ti-E Inverted Point Scanning Confocal Microscope A1R Si (Nikon, Tokyo, Japan) in Nikon LCI facility at Karolinska Institute (https://ki.se/en/bionut/equipment-at-the-lci-facility) and maximum projection images were obtained. See supplementary table S2 for the antibodies used for confocal imaging.

Quantification of vessel-associated megakaryocytes (Mks) in mouse femur

CD41 (Mks), laminin-111 (all blood vessels), and SCA1 (arteries) in the BM were visualized according to confocal imaging protocol described above. Total- and vessel-associated

megakaryocytes were counted by co-localization of $CD41^+$ cells and vascular walls marked by laminin-111 staining using ImageJ, then the ratio of vessel-associated megakaryocytes was calculated. N = 4 for stressed state, two for steady state; average values from 2-5 images from 4 samples of each genotype were analyzed in two independent experiments.

Quantification of vessel density

The BM vessel density was assessed by quantifying the vascular fraction based on the area defined by fluorescence staining of laminin-111 using ImageJ v1.53c using 4 representative areas randomly selected from each femoral section. Briefly, wand tool ("Legacy mode" and "tolerance" at scale '4' was used to define the vascular area by laminin-111 staining signal (red). The vessel density was presented as percentage of vessel area in whole imaging area from each sample. Four samples in each genotype were analyzed in two independent experiments.

Transplantation-induced MLL-AF9 AML mouse model

The AML mouse model was generated by transplanting mouse BM KIT^+ CD45.1 expressing cells transduced with *MLL-AF9* retrovirus into non-irradiated immune competent C57BL/6 mice, as described.[1,3] The MLL-AF9-expressing cells sorted by FACS from the primary recipient mice that developed AML were expanded in culture in presence of IL3 (5-10ng/mL) in RPMI and 10% fetal bovine serum for the transplantation to induce AML. Hundred thousand to 1 million of the expanded MLL-AF9-expressing cells were intravenously transplanted into non-irradiated *Lama4*$^{+/+}$ and *Lama4*$^{-/-}$ mice. In some of the experiments, *MLL-AF9* AML cells expressing *DsRed* [4,5] were intravenously transplanted into sub-lethally irradiated (6Gy) recipient mice.

Analysis of blood, spleen and BM hematopoietic cells

Peripheral blood (PB) samples were collected from tail vein in EDTA-coated tubes (Sarstedt) from the mice at steady state or after irradiation or transplantation. PB was analyzed by an automated cell counter (Sysmex XP-300) for counting different blood components including red blood cells (RBC), white blood cells (WBC) and hemoglobin (HGB), platelets (PLT). The PB mononuclear cells (MNCs) were isolated using 2% Dextran T-500 (Pharmacia, Uppsala, Sweden) and 0.8% Ammonium Chloride (Stem Cell Technologies.). PB and BM myeloid lineage ($CD11B^+GR1^+$ granulocytes, $CD11B^+F4/80^+$ monocytes/macrophage and $CD11B^+GR1^-$ monocytes), B cells ($CD19^+$) and T cells ($CD4^+CD8^+$ or $CD3^+$) were analyzed within the CD45.1 or CD45.2 cells. BM cells from femurs, tibias and iliac crest, and spleens

were harvested for FACS and histological analysis. All samples were stained with propidium iodide (PI) to exclude dead cells prior to analysis on FACS ARIA III (BD). Data analysis was carried out with FlowJo software (TreeStar Inc.).

HSC and myeloid progenitors in recipient BM and spleen were analyzed as described.[6] Briefly, BM MNCs were stained with anti-mouse CD16/32-PECY7, CD105-PE, lineage (LIN) cocktail CD3/GR1/TER119/CD11B/NK1.1-PECY5, CD117-APCCY7, CD34-FITC, CD150-APC, SCA1-Alexa-700 and CD41-brilliant violet 421 antibodies. All samples were stained with PI prior to analysis on FACS Fortessa LSRII (BD) or FACS ARIA III (BD). The BM $LIN^-SCA1^+KIT^+$ (LSK), HSC ($LSKCD150^+$), megakaryocyte progenitors (MkP, $LIN^-SCA1^-KIT^+CD41^+$), granulocyte-macrophage progenitor (GMP, $LIN^-SCA1^-KIT^+CD41^-CD150^-FcγR^{high}$), Pre MK-erythrocyte progenitor (Pre-MegE, $LIN^-SCA1^-KIT^+CD41^-FcγR^-CD150^+CD105^-$), Pre-CFU-E, $LIN^-SCA1^-KIT^+CD41^-FcγR^-CD150^+CD105^+$ and Pre-GM ($LIN^-SCA1^-KIT^+CD41^-FcγR^-CD150^-CD105^-$) were defined as described.[7] The cells were gated according to fluorescence minus one (FMO). Data analysis was carried out with FlowJo software (TreeStar Inc.). See Table S1 for detailed information about the antibodies used in the study.

CFU-F assay was performed as described.[2,8] Briefly, 200,000 BM MNCs were plated in the complete MSC expansion media (CCM004, R&D System).

Histological analysis

Mouse bones (tibias or femurs) were fixed in 4% paraformaldehyde (PFA) and dehydrated in increasing ethanol concentrations, cleared in xylene and then embedded in paraffin. Tissue sections (4-µm thickness) were stained with hematoxylin and eosin (HE). HE images of tissue sections were scanned by using 3D Histech Slide Scanner using Pannoramic MIDI (3D HISTECH). Pannoramic Viewer (3D HISTECH) was used for taking images of bone sections.

Quantitative real-time PCR (Q-PCR)

BM stromal cell subsets were sorted directly into RLT buffer (Qiagen) containing 2-Mercaptoethanol (143mM, Sigma) and kept at -80°C. RNA extraction and DNase treatment were performed with the RNeasy Micro Kit (Qiagen) according to the manufacturer's instructions. Eluted RNA samples were reverse transcribed using SuperScript® III Reverse Transcriptase (Invitrogen, catalogue number: 18080044), RNase OUT (ThermoFisher) and

random primers (Invitrogen) according to the protocol supplied by the manufacturer. Q-PCR reactions were performed by mixing 2 x TaqMan universal PCR master mix, 20 x TaqMan primer/probe mix, RNase-free H_2O and 2µl of cDNA for a final reaction volume of 8µl in 384-well plates in CFX384 Touch™ Real-Time PCR Detection System. See Table S3 for the information about Assays-on-Demand probes.

RNA sequencing and data analysis

About 7-20 million reads/sample were obtained. Sample quality was assessed using FastQC (v0.11.8) and MultiQC (v1.7). Reads were aligned to a reference built from Ensembl GRCm38 genome sequences using STAR (v2.6.1d).

The raw sequence counts in a FastQ format were mapped to mouse genome (GRCm38) by STAR, which were further calculated by FeatureCounts function from Subread package in R. Genes with Reads Per Kilobase of transcript per Million mapped reads (RPKM) values more than 0.1 were considered as being actively transcribed and proceeded to the analysis of Differential Gene Expression (DGE) between *Lama4*$^{-/-}$ and *Lama4*$^{+/+}$ phenotype.[9] The normalized read counts assigned to each sample were generated by Deseq2. The differentially expressed genes were identified by adjusted *P* value (*p*adj < 0.05) using Benjamini-Hochberg correction for multiple testing, together with thresholds at $\log_2$fold changes >1 (up-regulated) or <-1 (down-regulated). Gene sets enriched in either *Lama4*$^{-/-}$ or *Lama4*$^{+/+}$ phenotype were further analyzed via gene set enrichment analysis (GSEA) (v4.0.3) platform sourced from Broad Institute (http://www.broadinstitute.org/gsea/index.jsp). Three gene sets, including gene ontology (c5.all.v5.symbols.gmt), hallmark (h.all.v5.symbols.gmt) and KEGG (c2.kegg.v5.symbols.gmt), were utilized on normalized counts of each sequenced sample. Gene sets tested were considered to be statistically enriched in either *Lama4*$^{-/-}$ or *Lama4*$^{+/+}$ phenotype at nominal *P* value < .01 and FDR < .25. The leading-edge subsets contain genes occurring in the ranked gene list that contribute to the maximal GSEA enrichment score. The RNA sequencing data are available at GEO under accession number GSE167404 and GSE185846.

Cobblestone area forming cell (CAFC) assay for testing proliferation and drug response of AML cells in co-culture with MSCs

MLL-AF9 AML cells that were expanded in RPMI with IL3 (5-10 ng/mL) and 10% fetal bovine serum were used for the assay. BM MSCs ($CD45^-LIN^-CD31^-CD44^-CD51^+SCA1^+$) were sorted from *Lama4*$^{+/+}$ and *Lama4*$^{-/-}$ mice and expanded in StemXVivo® MSC Expansion Media (CCM004, R&D system) in hypoxic incubator (1% O_2). Twenty-four hours prior to co-culture,

30,000 MSCs were seeded in a 24-well plate in complete Dulbecco modified Eagle Medium (DMEM, GIBCO) containing 10% FBS, 10 mM HEPES, 100U of penicillin / streptomycin, and 10^{-4}M 2-Mercaptoethanol (Sigma) and maintained in a hypoxic incubator (1% O_2). The day after, the media was removed and 150-300 MLL-AF9 AML cells were added to each well in Myelocult medium (M5300, Stem Cell Technologies) with 10^{-6} M hydrocortisone (07904, Stem Cell Technologies), 1% penicillin streptomycin and 1ng/mL IL-3 (R&D) in a volume of 500µl/well. Co-cultures were maintained for 2 days to allow for spontaneous migration and adhesion before cytarabine (Ara-C) was added to the co-cultures at a concentration of 0.5 µM. The co-cultures were then incubated at 32-33°C, 5%CO_2 for additional 5-7 days. The CAFCs were counted under inverted microscope (CKX41, Olympus) and images were taken using *Infinity Analyze* 6.1.0. Lumenera, Otawa, Canada). A CAFC colony was defined as a cluster of more than 3 cells underneath the MSCs. For FACS analysis of AML stem cells (LSCs), the cells from each well were detached by 0.05% trypsin-EDTA (Gibco) and harvested for cell counting and staining with antibodies including CD36-APC, CD117-APC-CY7 and CD45.1-PE.

Lentiviral shRNA transduction

Lentivirus particles carrying Lama4 shRNA (Santa Cruz, sc-43147-V) or scrambled control shRNA (Santa Cruz, sc-108080) were produced by transfecting HEK293T cells with packaging plasmids psPAX2, the envelope vector (pCMV-VSVG), and the corresponding shRNA plasmids as described previously[10]. Culture-expanded healthy human BM MSCs in a 6-well plate (60,000 /well) in 2.5ml of complete DMEM +10%FBS w/o Pencillin/Streptomycin were transduced with 1 µL of lentivirus 100x concentrate (LentiX, PT4421-2) particles for 48h, followed by puromycin (1.5 µg/mL, Sigma) selection for 2-3 days to generate stable clones. The MSC clones were further expanded in completed DMEM+10%FBS and the expression of Lama4 in the MSCs was validated by Q-PCR.

LAMA4 Antibody inhibition of human BM MSCs in co-culture with human AML cells

Human BM MSCs from young (30–45-year-old) healthy volunteers were used for co-culture with patient-derived THP1 cell line. Human MSCs at passage 2-4 were first expanded from FACS-sorted CD45-LIN-CD31-CD44$^-$ cells[2] or culture-selected mesenchymal stromal cells. The THP1 cells were maintained in RPMI containing 10% FBS. Twenty-four hours prior to co-culture, 10,000 MSCs per well were seeded in a 96-well plate in complete Dulbecco modified

Eagle Medium (DMEM, GIBCO) containing 10% FBS, 10mM HEPES, 100U/mL of penicillin / streptomycin (HyClone), and 10^{-4}M 2-Mercaptoethanol (Sigma) and maintained in a hypoxic incubator (1% O_2). The day after, after the media was removed and 5-10 μg/ml mouse anti-human LAMA4 monoclonal antibody (R&D System, cat#MAB7340) or mouse IgG1κ control antibody (Clone # 11711 Cat#: MAB002) were added to the MSCs and incubated at 37°C for 1hour prior to adding THP1 cells (10,000-25,000/well). The media for antibody dilution and the co-culture was complete Myelocult (H5100, Stem Cell Technologies) with 10^{-6}M hydrocortisone (07904, Stem Cell Technologies), 100 U/mL penicillin streptomycin in a volume of 100 μl/well. One day later, the cells from each well were collected after dissociation with 0.05% Trypsin-EDTA. The total number of THP1 cells were counted with Trypan blue. Some of the wells were treated with cytarabine (Ara-C) at a concentration of 1.0 μM 4-6hours post seeding of THP1 cells.

Homing assay of AML cells

The homing of the AML cells was determined as previously described.[1] The $CD45.1^+$ MLL-AF9 AML cells were injected into the mice (10 million per mouse) via tail vein without prior irradiation. Peripheral blood, femurs and spleen of the recipients were harvested 3 hours after transplantation and the homing of $CD45.1^+$ AML cells were examined by FACS based on CD45.1 expression. A non-transplanted mouse was included as a negative control in the analysis.

Marrow culture and MkP isolation

Marrow pieces from *Lama4*$^{-/-}$ mouse femurs were flushed out by 2ml-Syringe and 23G needle in StemSpan media (Stem Cell Technologies. Cat#09650). Then the marrow was transferred to a U-bottom 96-well plate (non-cell culture treated) and 100μl/well of StemSpan media supplemented with 10ng/mL of recombinant human thrombopoietin (THPO), mouse stem cell factor (mSCF) and 10 μg/ml LAMA4 protein (3837-A4, R&D System) or PBS was added to the marrow. At day4, the marrow was washed with PBS in an Eppendorf tube and spun down at 300g 5 min, and thereafter treated with 100 μl 0.05% trypsin-EDTA for 5 min at 37°C to isolate single cells. The MNCs were filtered through 70 μm cell strainers and stained with PECY5-antibodies against blood lineages including CD19, GR1, CD3, TER119, and CD41-BV421, CD150-Alexa647, as mentioned above. Subsequently, $LIN^-SCA1^-KIT^+CD41^+CD150+/-$ cells were analyzed by FACS ARIA SORP (BD).

In some experiments, the $LIN^-SCA1^-KIT^+CD41^+$ cells were sorted for *in vitro* culture with recombinant LAMA4 or vehicle in the StemSpan media mentioned above for 4-5 days. The differentiated MKs were counted at day 3 and analyzed by FACS for $CD41^+CD150^+$ cells at day 4-5.

ELISA

EPO protein level in blood plasma was detected by mouse EPO Quantikine ELISA Kit (R&D system, cat#MEP00B) according to the manual from the company. The blood plasma was collected by using heparin as an anticoagulant and centrifuged for 20 min at 2000 x g within 30 minutes of collection. Serum and plasma samples were diluted by 2 times with Calibrator Diluent RD6Z prior to assay.

Transwell assay

MLL-AF9 cell culture with and without direct interactions with BM MSCs were performed in a 12-well plate containing Transwell inserts with a pore size of 0.45um. The day after plating BM MSCs in the bottom wells (30,000 /well), the MSCs were stained with MitoTracker™ Green FM (M7514, ThermoFisher) at 37°C for 60 minutes, according to the manufacturer's instructions. The cells were washed twice with PBS, then incubated for 3-4 hours to remove unbound probe before a final wash. Subsequently, 30,000 MLL-AF9 $CD45.1^+$ cells in Myelocult (M5300, StemCell Technologies) were plated in both transwell insert and lower chamber to coculture with MSCs for 24 hours. Mitochondrial transfer was quantified in $CD45.1^+$ AML cells by FACS.

Supplementary Table 1 Antibody used for FACS

Antibody	Conjugate	Clone	Source	Catalog No	Dilution
Anti-mouse TER-119	Purified	TER-119	Biolegend	116202	1/200
Anti-mouse CD45	Purified	30-F11	Biolegend	103102	1/200
Anti-mouse CD3	Purified	17A2	Biolegend	100202	1/200
Anti-mouse Ly-6G/Ly-6C (Gr-1)	Purified	RB6-8C5	Biolegend	108402	1/200
Anti-mouse B220	Purified	6D5	Biolegend	103202	1/200
Anti-mouse CD11B	Purified	M1/70	Biolegend	101202	1/400
Anti-mouse CD16/32	Purified	93	Biolegend	101302	1/50
Anti-mouse/Human CD44	APC/Cy7	IM7	Biolegend	103028	1/100
Anti-mouse CD31	PE/Cy7	390	Biolegend	102418	1/50
Anti-mouse CD45	PE/Cy5	30-F11	Biolegend	103110	1/200
Anti-mouse TER-119	PE/Cy5	TER-119	Biolegend	116210	1/200
Anti-mouse CD11b	PE/Cy5	M1/70	Biolegend	101210	1/800
Anti-mouse Ly-6A/E (Sca-1)	Pacific Blue	D7	Biolegend	108120	1/200
Anti-mouse Ly-6A/E (Sca-1)	Alexa Fluor 700	D7	eBioscience	e025615	1/100
Anti-mouse CD150	Alexa Fluor 647	TC1512F12.2	Biolegend	115918	1/200

Anti-mouse CD19	PE/Cy5	eBio1D3	eBioscience	12-480-180	1/200
Anti-mouse/Human CD11b	APC/Cy7	M1/70	Biolegend	101225	1/800
Anti-mouse Ly-6G/Ly-6C (Gr-1)	Brilliant Violet 421	RB6-8C5	Biolegend	108434	1/400
Anti-mouse CD45.1	PE	A20	Biolegend	553776	1/50
Anti-mouse CD45.1	BV510	A20	Biolegend	110741	1/50
Anti-mouse CD45.2	Alexa Fluor 700	104	Biolegend	109822	1/200
Anti-mouse CD3ε	PE/Cy5	145-2C11	Biolegend	100310	1/200
Anti-mouse Ly-6G/Ly-6C (Gr-1)	PE/Cy5	RB6-8C5	Biolegend	105409	1/200
Anti-mouse CD117	APC/Cy7	2B8	Biolegend	105825	1/200
Anti-mouse CD34	FITC	RAM34	eBioscience	11-0341-85	1/200
Anti-mouse CD135	PE	A2F10	Biolegend	135306	1/200
Anti-mouse CD140A	APC	APA5	eBioscience	17-1401-81	1/50

Supplementary Table 2 Antibody used for confocal imaging

A Primary antibodies:

Target	Host	Conjugate	Clone/ID	Dilution	Company/ producer	Cat #
Laminin-111	Rabbit	Purified	Polyclonal	1:50	Sigma	L9393
Laminin α4	Rabbit	Purified	1100+E	1:50	Dr. Takako Sasaki	-
Laminin α5	Rabbit	Purified	1113+E	1:400	Dr. Takako Sasaki	-
CD41	Rat	FITC	MWReg30	1:200	BD	553848
SCA1	Rat	FITC	E13 161-7	1:100	BioLegend	122505
SCA1	Rat	BV421	D7	1:100	BioLegend	108127
Endomucin	Rat	Alx647	V.7C7	1:50	Santa Cruz	sc-65495
CD45.1	Mouse	FITC	A20	1:50	BD	553775
Isotype	Rabbit	Purified	-	Same as the specific antibody	CST	3900S
Isotype	Rat	PB	-	Same as the specific antibody	BioLegend	RTK2758
Isotype	Mouse	FITC	-	Same as the specific antibody	BD	553929
Isotype	Rat	Alx647	-	Same as the specific antibody	Bio-Rad	MCA1212 A647

B. Secondary antibodies

Target	Host	Host	Conjugate	Dilution	Company	Cat #
Rabbit	Goat	Goat	Alx546	1:500	Invitrogen	A11035
Rabbit	Donkey	Donkey	Alx647	1:500	Invitrogen	A31573
Mouse	Goat	Goat	Alx488	1:500	Invitrogen	A11001

Supplemental Table 3 List of the probes used for Q-PCR.

Gene name	Assay ID	Full name
Cxcl12	Mm00445552_m1	C-X-C motif chemokine ligand 12
Kitl	Mm00442972_m1	Kit ligand
Angpt1	Mm00456503_m1	Angiopoietin-1
Il7	Mm00434291_m1	Interleukin 7
Runx2	Mm00501584_m1	Runt related transcription factor 2
Hprt	Mm01545399_m1	Hypoxanthine guanine phosphoribosyl transferase
Spp1	Mm00436767_m1	Secreted phosphoprotein 1
Il6	Mm00446190_m1	Interleukin 6
Lama4	Mm00496902_m1	Laminin subunit alpha-4
Cxcl14	Mm00444699_m1	Chemokine (C-X-C motif) ligand 14
Col1a1	Mm00801666_m1	Collagen, type I, alpha 1
Lama5	Mm01222029_m1	Laminin subunit alpha-5
Fabp4	Mm00445880_m1	Fatty acid binding protein 4
Thpo	Mm00437040_m1	Thrombopoietin
Igf1	Mm00439560_m1	Insulin-like growth factor 1 (IGF-1)
Jag1	Mm00496902_m1	Jagged 1
Tgfb1	Mm01178820_m1	Transforming growth factor beta 1
Epo	Mm01202755_ml	Erythropoietin
Cxcl10	Mm00445235_ml	C-X-C motif chemokine ligand 10

Supplementary Figure and Figure legends

Figure S1 Slow recovery of hematopoiesis in adult *Lama4*$^{-/-}$ mice following irradiation-induced myelosuppression.

(A) Experimental setup, *Lama4*$^{+/+}$ and *Lama4*$^{-/-}$ mice were sub-lethally irradiated (7Gy) and hematopoietic lineages in blood and BM were analyzed by FACS and CFU-C assay at steady state and at 2, 4, 6 weeks post irradiation (IR).

(B) White blood cells, red blood cells, platelets and hemoglobin (WBC, RBC, PLT and HGB and mean corpuscular volume (MCV) in PB of *Lama4*$^{+/+}$ and *Lama4*$^{-/-}$ mice.

(C) The numbers of hematopoietic lineages in PB of *Lama4*$^{+/+}$ and *Lama4*$^{-/-}$ mice.

(D) FACS analysis of phenotypically defined hematopoietic stem and progenitor cells (HSPC) in *Lama4*$^{+/+}$ and *Lama4*$^{-/-}$ mouse BM at 4 and 6 weeks (wk) after the irradiation. CFU-E, erythrocyte precursors, MEP, megakaryocyte-erythrocyte progenitors.

(E) Colony-forming units (CFU-C) in *Lama4*$^{+/+}$ and *Lama4*$^{-/-}$ mouse BM at 4 and 6 weeks after irradiation. Data are mean ± SD from 2-4 independent experiments. Each dot represents data from an individual mouse. The numbers in the panels are *p* values determined by Mann-Whitney test.

Figure S1

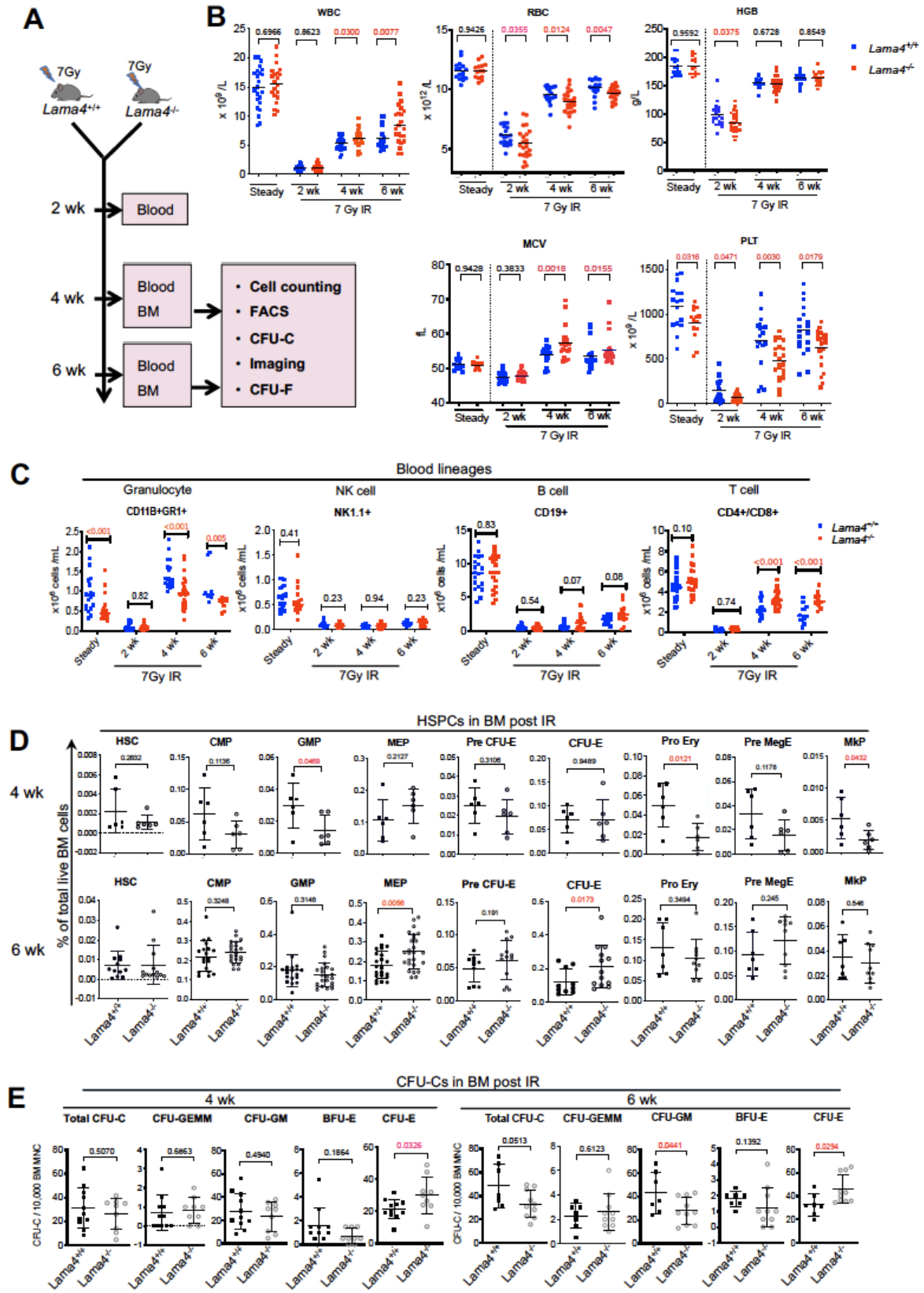

A
7Gy
7Gy
Lama4+/+
Lama4-/-
2 wk
Blood
4 wk
Blood
BM
6 wk
Blood
BM
• Cell counting
• FACS
• CFU-C
• Imaging
• CFU-F
B
WBC
RBC
HGB
MCV
PLT
Steady
2 wk
4 wk
6 wk
7 Gy IR
Lama4+/+
Lama4-/-
C
Blood lineages
Granulocyte
CD11B+GR1+
NK cell
NK1.1+
B cell
CD19+
T cell
CD4+/CD8+
7Gy IR
D
HSPCs in BM post IR
4 wk
6 wk
% of total live BM cells
HSC
CMP
GMP
MEP
Pre CFU-E
CFU-E
Pro Ery
Pre MegE
MkP
E
CFU-Cs in BM post IR
4 wk
6 wk
Total CFU-C
CFU-GEMM
CFU-GM
BFU-E
CFU-E
CFU-C / 10,000 BM MNC

Figure S2 Hematopoietic stem and progenitor cells (HSPCs) in adult *Lama4*$^{-/-}$ mice under steady state and 6 weeks post irradiation. Hematopoiesis in 3-4 months old *Lama4*$^{+/+}$ and *Lama4*$^{-/-}$ mice was analyzed by Sysmex, FACS and CFU-C assay. Each dot represents data from an individual mouse. Horizontal bars are mean ± SD.

(A) Total BM cellularity in the *Lama4*$^{+/+}$ and *Lama4*$^{-/-}$ mice during steady state and after irradiation.

(B) Representative FACS profiles showing the gating strategy for analysis of the HSPCs.

(C) The HSPC frequencies in *Lama4*$^{+/+}$ and *Lama4*$^{-/-}$ mouse BM at steady state.

(D) CFU-C frequencies in *Lama4*$^{+/+}$ and *Lama4*$^{-/-}$ mouse BM at steady state.

Data are mean ± SD from 2 independent experiments. Each dot represents an individual mouse. The numbers in the panels are *p* values determined by unpaired nonparametric *t* test.

Figure S2

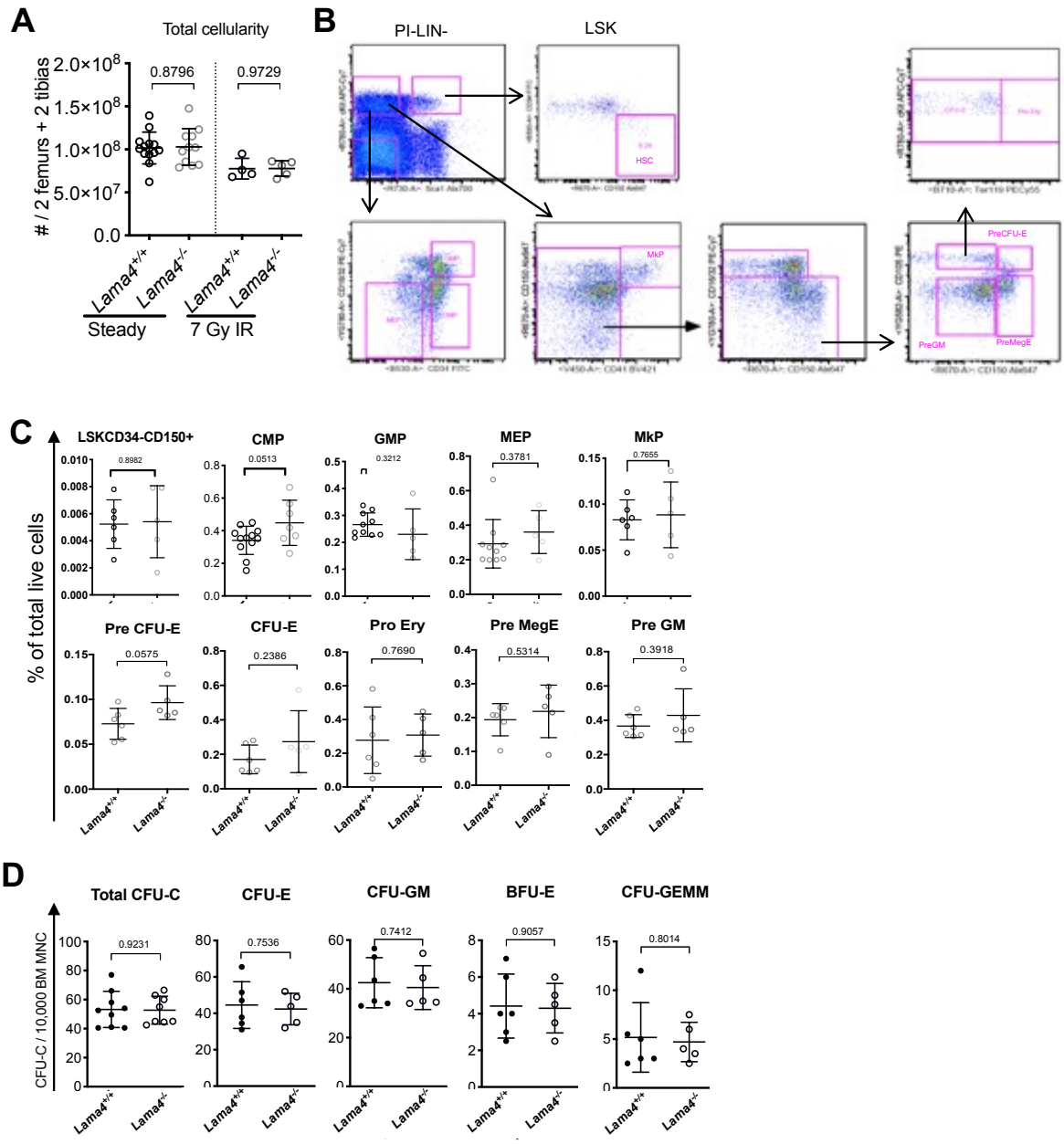

A
Total cellularity
/ 2 femurs + 2 tibias
2.0×10^8
1.5×10^8
1.0×10^8
5.0×10^7
0.0
0.8796
0.9729
Lama4+/+
Lama4-/-
Lama4+/+
Lama4-/-
Steady
7 Gy IR
B
PI-LIN-
LSK
HSC
MkP
PreCFU-E
PreGM
PreMegE
C
% of total live cells
LSKCD34-CD150+
0.8982
CMP
0.0513
GMP
0.3212
MEP
0.3781
MkP
0.7655
Pre CFU-E
0.0575
CFU-E
0.2386
Pro Ery
0.7690
Pre MegE
0.5314
Pre GM
0.3918
Lama4+/+
Lama4-/-
D
CFU-C / 10,000 BM MNC
Total CFU-C
0.9231
CFU-E
0.7536
CFU-GM
0.7412
BFU-E
0.9057
CFU-GEMM
0.8014
Lama4+/+
Lama4-/-

Figure S3 CFU-Cs in *Lama4*$^{+/+}$ and *Lama4*$^{-/-}$ mouse spleen at 4 weeks after sublethal irradiation (IR). The hematopoietic progenitors in the spleens were estimated by the frequencies of CFU-GM, CFU-GEMM, BFU-E and CFU-E in spleen MNCs.

(A) Illustration of normal erythrocyte and megakaryocyte developmental pathway from HSCs to enucleated erythrocytes.

(B) Total cellularity in the spleens of *Lama4*$^{+/+}$ and *Lama4*$^{-/-}$ mice 4 weeks after IR.

(C) CFU-C frequencies in *Lama4*$^{+/+}$ and *Lama4*$^{-/-}$ mouse spleens after IR.

Data are mean ± SD. Each dot represents an individual mouse. The numbers in the panels are *p* values determined by unpaired Mann-Whitney test. Data were from 2 independent experiments.

Figure S3

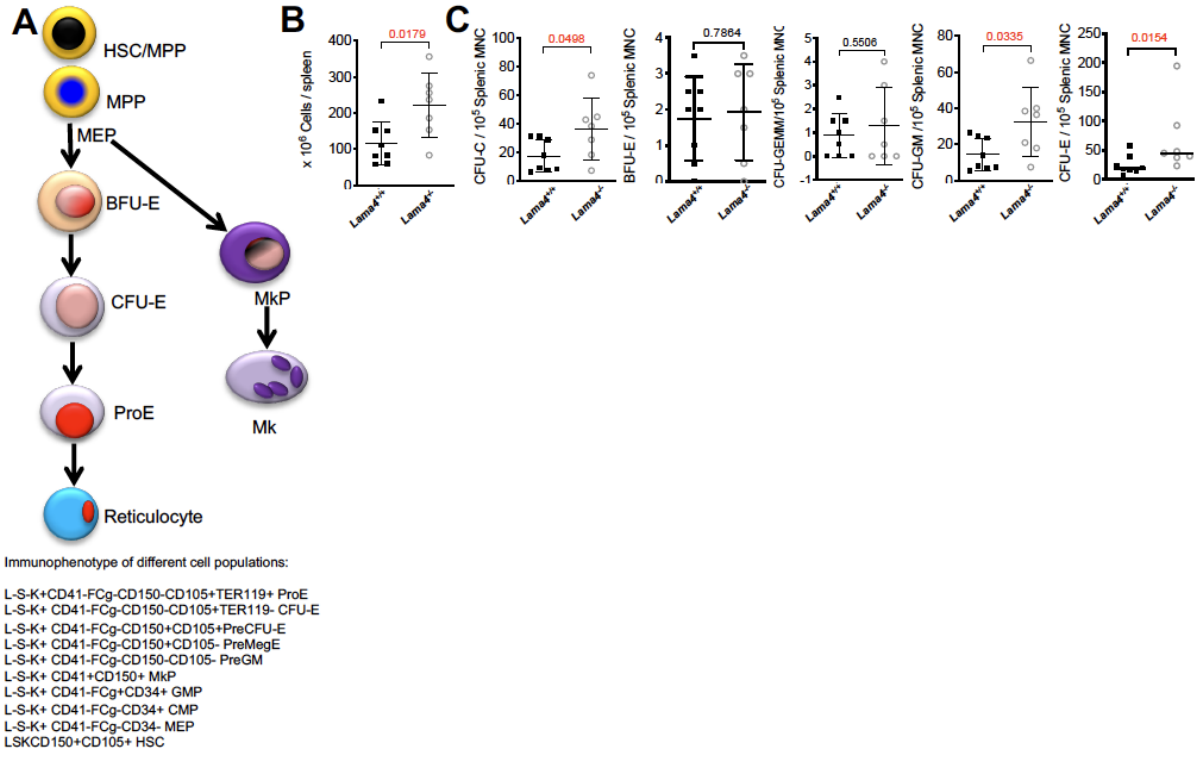


Figure S4 Lower expression of LAMA4 in BM MNCs is associated with poor prognosis in patients with AML. The overall survival of AML patients with high or low expression levels of *LAMA4* (GSE5122,[11] high $n = 46$, low $n = 4$) in patient BM MNCs. *P* value by log-rank (Mantel–Cox) test

Related to Figure 2.

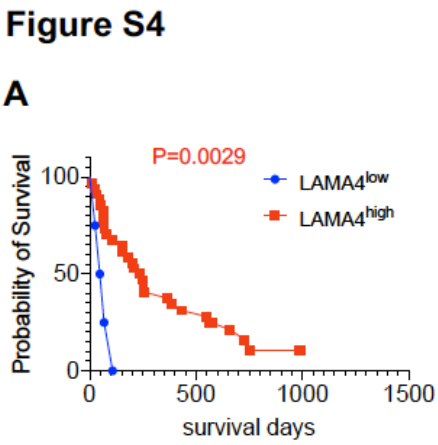


Figure S5 Loss of *Lama4* accelerates AML progression. The number of AML or host cells were calculated based on the frequency of each subset detected by FACS.

(A) BM cellularity in *Lama4*$^{+/+}$ and *Lama4*$^{-/-}$ recipient mice after AML cell injection. The mice were terminated at day 17 or 24 post AML injection.

(B) The absolute numbers of AML cells (CD45.1^{+}) in *Lama4*$^{+/+}$ and *Lama4*$^{-/-}$ recipient mouse BM post AML cell injection.

(C) The absolute numbers of AML LSCs (CD45.1^{+}KIT^{+}) in *Lama4*$^{+/+}$ and *Lama4*$^{-/-}$ recipient mouse BM post AML cell injection.

(D) The absolute numbers of host cells (CD45.2^{+}) in *Lama4*$^{+/+}$ and *Lama4*$^{-/-}$ recipient mouse BM post AML cell injection.

(**E-F**) The numbers of host-derived stem and progenitor cells (CD45.2^{+}GR1^{-}KIT^{+} and GR1^{-}SCA1^{+}KIT^{+} (LSK) in *Lama4*$^{+/+}$ and *Lama4*$^{-/-}$ mouse PB (**E**) and BM (**F**) at day 24 post AML injection.

(**G**) The numbers CD45.1^{+} AML cells in *Lama4*$^{+/+}$ and *Lama4*$^{-/-}$ mouse PB and BM after AML relapse following therapeutic BM transplantation.

(**H**) Representative FACS profiles showing the AML cells in PB, BM, spleen 3 hours after transplantation. The numbers in the panels are frequencies of the AML cells of total live cells in each organ.

The statistical differences were determined by one-tailed or two tailed Mann-Whitney or Welch's test depending on the data distribution.

Related to Figure 2.

Figure S5

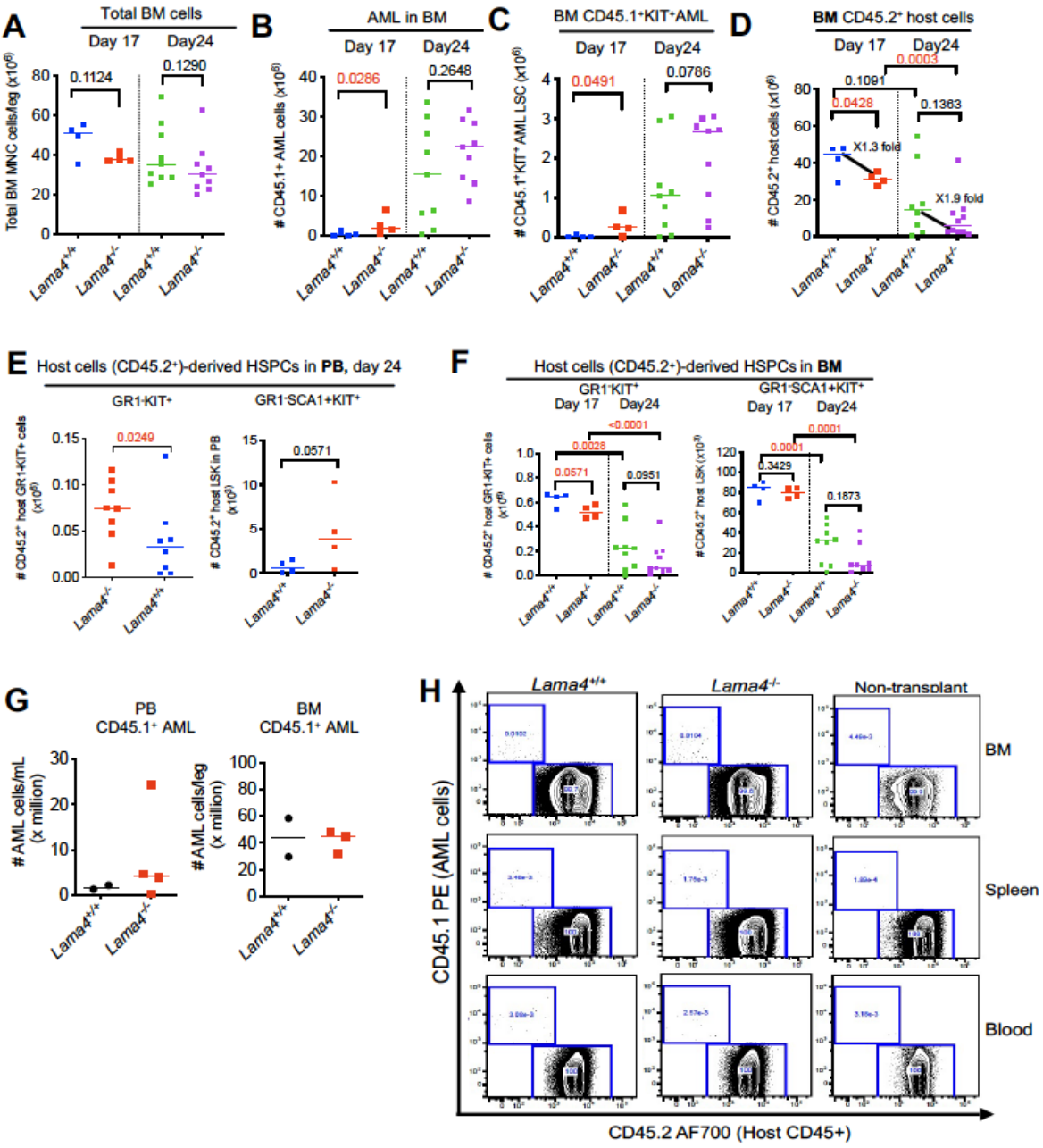

A
Total BM cells
Day 17
Day24
Total BM MNC cells/leg (x10^6)
0.1124
0.1290
B
AML in BM
Day 17
Day24
CD45.1+ AML cells (x10^6)
0.0286
0.2648
C
BM CD45.1+KIT+AML
Day 17
Day24
CD45.1+KIT+ AML LSC (x10^6)
0.0491
0.0786
D
BM CD45.2+ host cells
Day 17
Day24
CD45.2+ host cells (x10^6)
0.0003
0.1091
0.0428
0.1363
X1.3 fold
X1.9 fold
Lama4+/+
Lama4-/-
E
Host cells (CD45.2+)-derived HSPCs in PB, day 24
GR1-KIT+
GR1-SCA1+KIT+
CD45.2+ host GR1+KIT+ cells (x10^6)
0.0249
CD45.2+ host LSK in PB (x10^3)
0.0571
F
Host cells (CD45.2+)-derived HSPCs in BM
GR1-KIT+
Day 17
Day24
GR1-SCA1+KIT+
Day 17
Day24
CD45.2+ host GR1+KIT+ cells (x10^6)
<0.0001
0.0028
0.0571
0.0951
CD45.2+ host LSK (x10^3)
0.0001
0.0001
0.3429
0.1873
G
PB
CD45.1+ AML
BM
CD45.1+ AML
#AML cells/mL (x million)
#AML cells/leg (x million)
H
Lama4+/+
Lama4-/-
Non-transplant
BM
Spleen
Blood
CD45.1 PE (AML cells)
CD45.2 AF700 (Host CD45+)

Figure S6 Hematopoiesis reconstitution at 18 weeks after transplantation of WT cells into *Lama4*$^{+/+}$ and *Lama4*$^{-/-}$ recipient mice. 0.5-1.0 million BM cells from CD45.2^{+} actin-EGFP reporter mice were transplanted into lethally irradiated *Lama4*$^{+/+}$ and *Lama4*$^{-/-}$ mice. Hematopoiesis reconstitution in blood and BM was determined by Sysmex and FACS.

(A) Total donor and donor-derived myeloid cell reconstitution in blood from *Lama4*$^{+/+}$ and *Lama4*$^{-/-}$ mice 3 weeks after transplantation of 0.5 or 1.0 million cells.

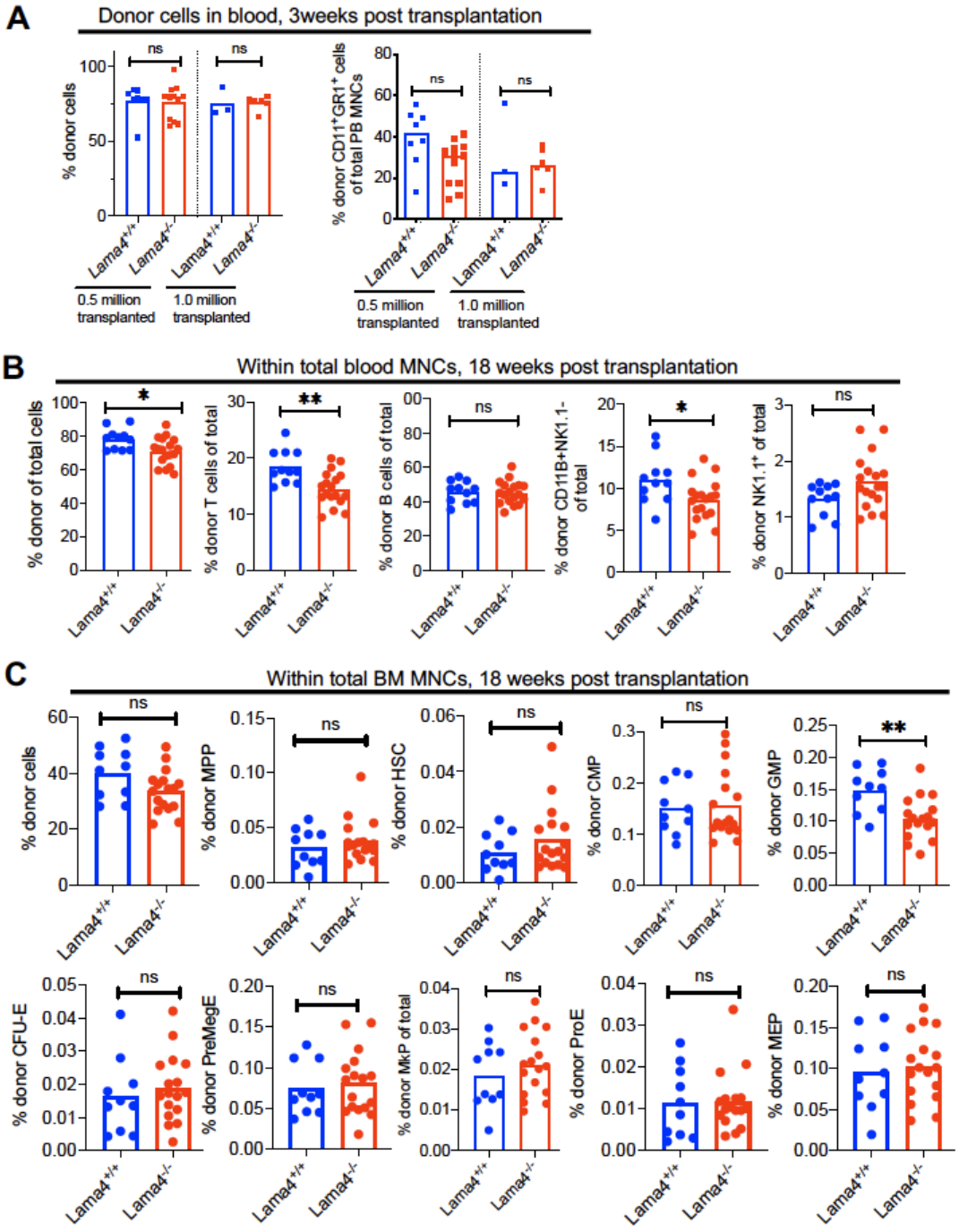
(B) Donor-derived lineage reconstitution in blood of *Lama4*$^{+/+}$ and *Lama4*$^{-/-}$ mice at 18 weeks post transplantation.

(C) Donor-derived total BM MNCs and HSPC reconstitution in *Lama4*$^{+/+}$ and *Lama4*$^{-/-}$ mice at 18weeks post transplantation. The donor (EGFP^{+}) derived BM HSPC subsets in the BM were analyzed by FACS based on their immunophenotype. The surface antigen expression profiles of each HSPC subsets are defined as listed.

Each dot represents data from a single recipient mouse. *p<.05, **p<.01, analyzed by unpaired Welch's t test. Horizontal bars indicate mean values**.** Data were from 2 independent experiments.

Related to Figure 2.

Figure S6



Immunophenotype of HSPCs:

LIN-KIT+SCA1-CD41-FCg-CD150-CD105+TER119+ ProE
LIN-KIT+SCA1-CD41-FCg-CD150-CD105+TER119- CFU-E
LIN-KIT+SCA1-CD41-FCg-CD150+CD105+PreCFU-E
LIN-KIT+SCA1-CD41-FCg-CD150+CD105- PreMegE
LIN-KIT+SCA1-CD41-FCg-CD150-CD105- PreGM
LIN-KIT+SCA1-CD41+CD150+ MkP
LIN-KIT+SCA1-CD41-FCg+CD34+ GMP
LIN-KIT+SCA1-CD41-FCg-CD34+ CMP
LIN-KIT+SCA1-CD41-FCg-CD34- MEP
LIN-KIT+SCA1+CD150+CD105+ HSC
LIN-KIT+SCA1+CD150-CD105+ MPP

Figure S7 BM niche alteration in *Lama4*$^{-/-}$ mice at steady state and after sublethal irradiation

(A) Representative Hematoxylin-Eosin staining of diaphysis of *Lama4*$^{+/+}$ and *Lama4*$^{-/-}$ femur before and 6 weeks after irradiation. Data were from n = 3-5 mice, 2 independent experiments.

(B) Vessel fraction in the *Lama4*$^{+/+}$ and *Lama4*$^{-/-}$ mouse femur before (steady state) and after sublethal irradiation (IR). Each dot represents a mean value of two measurements in different areas of the stained bone sections. The data were from 2-3 mice of each genotype.

(C) FACS analysis showing reduced mean fluorescence intensity (MFI) of CD31 staining in *Lama4*$^{-/-}$ BM at steady state and following the irradiation. Data shown are mean ± SD. Each dot represents data from an individual mouse.

(D) Histograms showing CD31 MFI in *Lama4*$^{+/+}$ and *Lama4*$^{-/-}$ BM.

(E) FACS analysis of BM stromal cells in *Lama4*$^{+/+}$ and *Lama4*$^{-/-}$ mice at steady state. Representative FACS profile shows analysis of the CD44^{+} and CD44^{-}CD51^{-}SCA1^{-} mature stromal cells and the right panels show % of the CD44^{+} and CD44^{-}CD51^{-}SCA1^{-} stromal cells within total live BM MNCs. Data were from 2 independent experiments. Each dot represents data from an individual mouse. The *p* values were determined by unpaired Mann-Whitney test.

(F) FACS analysis showing % of BM MSCs (CD45^{-}TER119^{-}CD31^{-}CD44^{-}CD51^{+}SCA1^{+}), MPCs (CD45^{-}TER119^{-}CD31^{-}CD44^{-}CD51^{+}SCA1^{-}) and endothelial cells CD45^{-}TER119^{-}CD31^{+}) in *Lama4*$^{+/+}$ and *Lama4*$^{-/-}$ mice 6 weeks after the irradiation. Data are mean. Each dot represents data from an individual mouse. The numbers in the panels are *p* values determined by unpaired Mann-Whitney test.

(G) EPO protein level detected by ELISA in blood plasma of *Lama4*$^{+/+}$ and *Lama4*$^{-/-}$ mice 6 weeks after the irradiation. Each dot represents data from an individual mouse. The numbers in the panels are *p* values determined by unpaired Mann-Whitney test.

Related to Figure 3 and Figure 4.

Figure S7

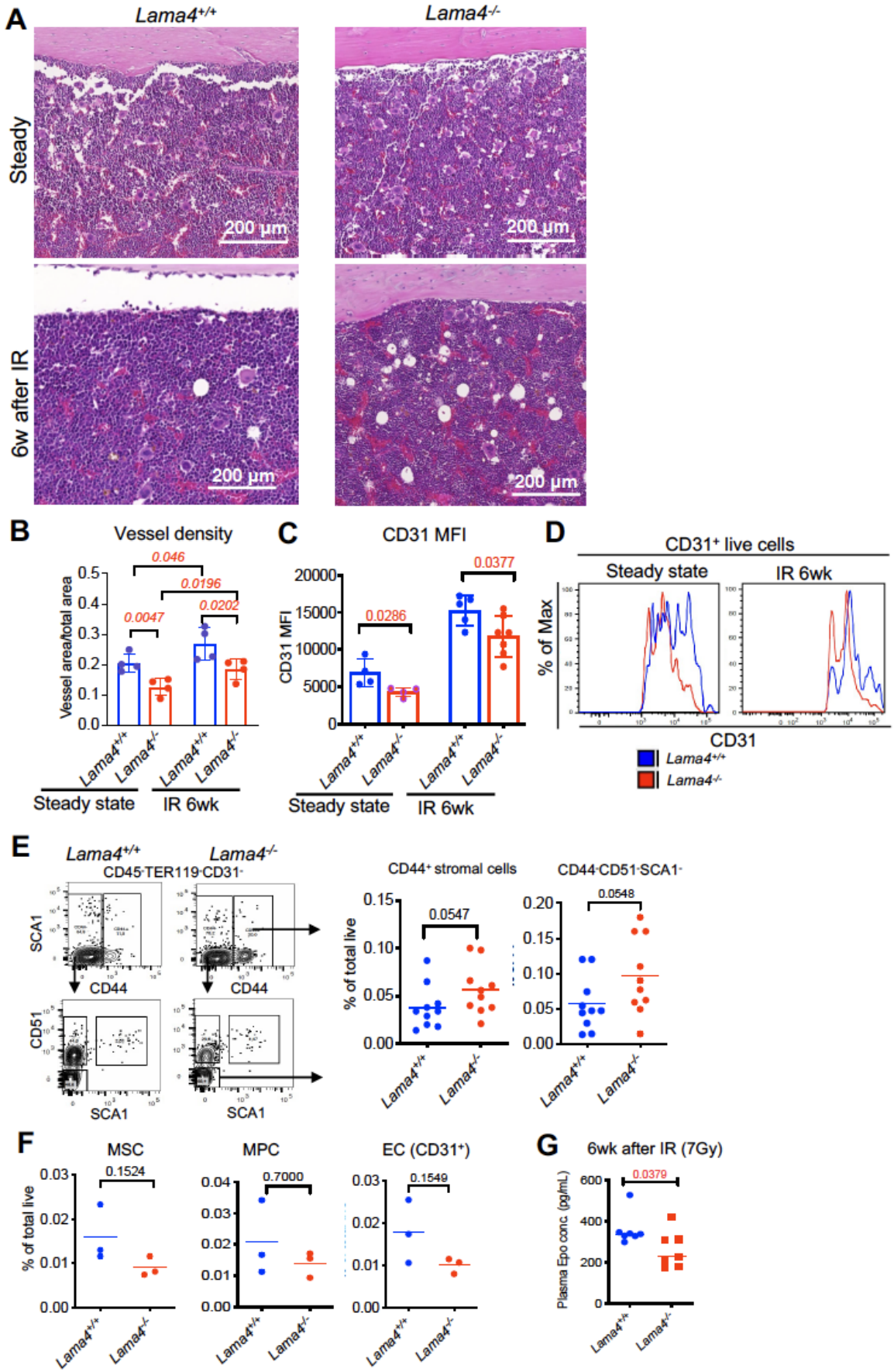

A
Lama4+/+
Lama4-/-
Steady
6w after IR
200 μm
B
Vessel density
Vessel area/total area
0.046
0.0196
0.0202
0.0047
Steady state
IR 6wk
C
CD31 MFI
CD31 MFI
0.0286
0.0377
D
CD31+ live cells
Steady state
IR 6wk
% of Max
CD31
E
CD45-TER119-CD31-
SCA1
CD44
CD51
CD44+ stromal cells
CD44-CD51-SCA1-
% of total live
0.0547
0.0548
F
MSC
MPC
EC (CD31+)
0.1524
0.7000
0.1549
G
6wk after IR (7Gy)
Plasma Epo conc (pg/mL)
0.0379

Figure S8 RNA-sequencing show altered molecular profiles of *Lama4*$^{-/-}$ BM MPCs. RNA sequencing was performed on sorted MPCs from *Lama4*$^{+/+}$ and *Lama4*$^{-/-}$ mice at 6 weeks post the irradiation.

(A) Unsupervised clustering of the *Lama4*$^{-/-}$ and *Lama4*$^{+/+}$ MPCs based on top 400 differentially expressed genes between the two types of samples, showing distinct transcriptional profiles of *Lama4*$^{-/-}$ MPCs.

(B) Gene set enrichment analysis (GSEA) shows up- and down-regulated gene sets related to various biological processes and functional pathways in the *Lama4*$^{+/+}$ and *Lama4*$^{-/-}$ MPCs.

(C) GSEA plot showing downregulation of the genes related ECM-receptor interactions in the *Lama4*$^{-/-}$ MPCs.

(D) GSEA plot showing upregulation of the genes related interferon alpha response in the *Lama4*$^{-/-}$ MPCs.

(E) Fold changes in expression of the genes involved in ROS scavenging in *Lama4*$^{-/-}$ MPCs compared with *Lama4*$^{+/+}$ MPCs.

(F) Normalized enrichment scores of gene sets associated with ROS generation (oxidative phosphorylation, lipid oxidation) and ROS-scavenging in *Lama4*$^{-/-}$ MPCs.

(G) Expression of pro-apoptotic genes in the *Lama4*$^{+/+}$ and *Lama4*$^{-/-}$ MPCs.

Each dot represents data from an independent cell sorting from 1-2 mice. The *p* values were determined by unpaired two-tailed or one-tailed *t* test. Data were from 2 sequencing experiments. * $P<0.05$, ** $P<0.01$.

Related to Figure 4.

Figure S8

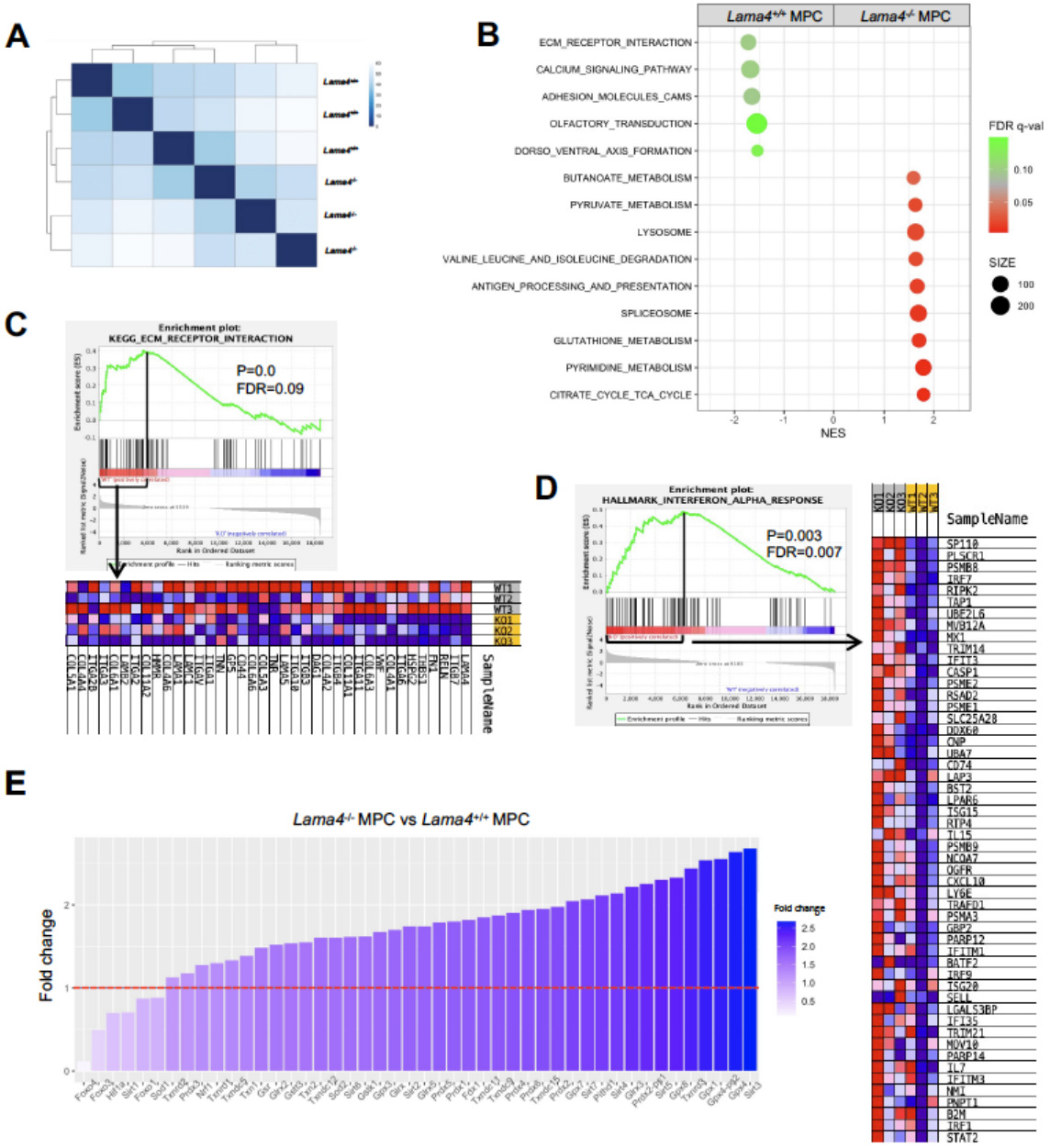

A
B
Lama4+/+ MPC
Lama4-/- MPC
ECM_RECEPTOR_INTERACTION
CALCIUM_SIGNALING_PATHWAY
ADHESION_MOLECULES_CAMS
OLFACTORY_TRANSDUCTION
DORSO_VENTRAL_AXIS_FORMATION
BUTANOATE_METABOLISM
PYRUVATE_METABOLISM
LYSOSOME
VALINE_LEUCINE_AND_ISOLEUCINE_DEGRADATION
ANTIGEN_PROCESSING_AND_PRESENTATION
SPLICEOSOME
GLUTATHIONE_METABOLISM
PYRIMIDINE_METABOLISM
CITRATE_CYCLE_TCA_CYCLE
NES
FDR q-val
SIZE
C
Enrichment plot:
KEGG_ECM_RECEPTOR_INTERACTION
P=0.0
FDR=0.09
D
Enrichment plot:
HALLMARK_INTERFERON_ALPHA_RESPONSE
P=0.003
FDR=0.007
SampleName
E
Lama4-/- MPC vs Lama4+/+ MPC
Fold change
F
ROS generation:
Oxidative phosphoprylation
Fatty acid oxidation
ROS scavenging:
Peroxidase activity
Antioxidant activity
Hydrogen peroxide catabolic process
Thioredoxin proxidase activity
Glutathione metabolic process
FDR
NES
G
Pro-apoptotic genes
(normalized counts)
Bak
Bax
Bid
Bim
Noxa
Puma
Bad
Bmf
ns

Figure S9 Laminin receptor expression in BM mesenchymal cells and endothelial cells in *Lama4*$^{-/-}$ mice under steady state and after sublethal irradiation.

(**A-B**) FACS analysis of CD29/integrin β1, CD49F/Integrin α6 and CD146 expressions in BM MSCs, MPCs, ECs from young adult *Lama4*$^{+/+}$ and *Lama4*$^{-/-}$ mice under steady state (**A**) and 6 weeks after sublethal irradiation (**B**). Each dot represents data from a single mouse. The *p* values shown in the panels are analyzed by unpaired student *t* test. Horizontal bars indicate mean values.

Related to Figure 4.

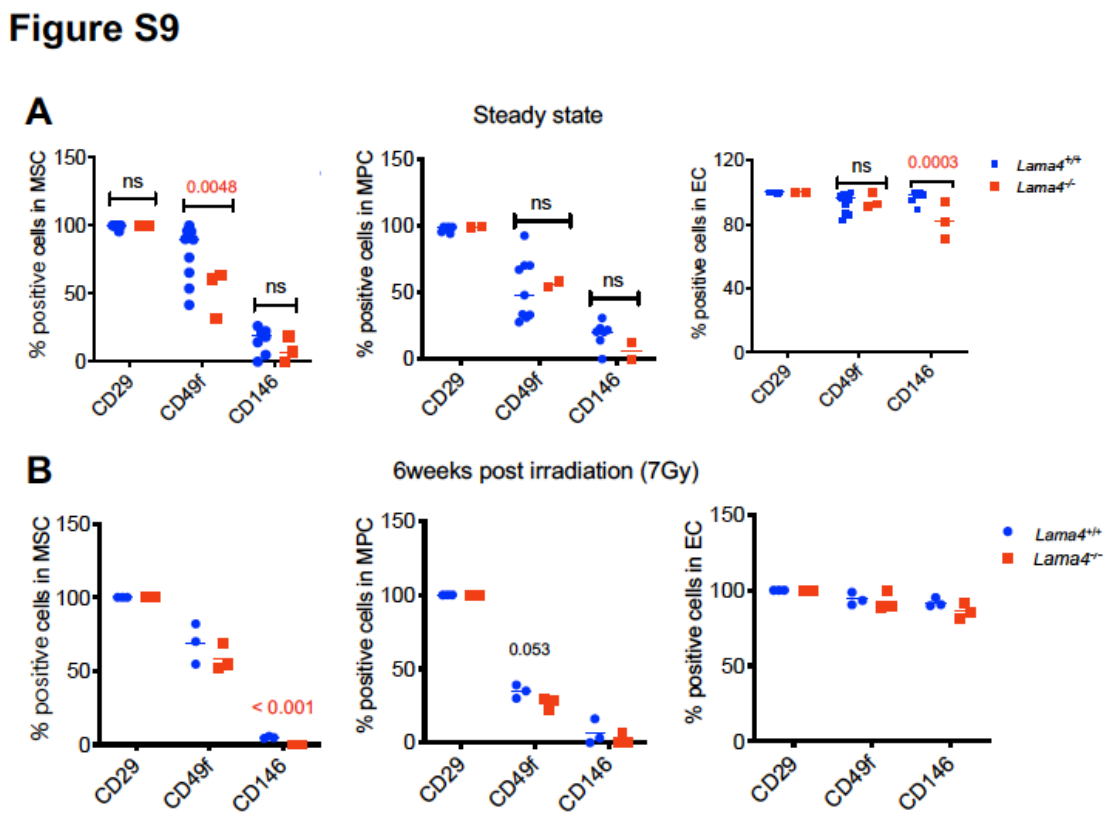


Figure S10 AML cell engraftment in *Lama4*$^{-/-}$ mice before and post Ara-C treatment.

(A) The AML cells (DsRed^{+}) in blood of *Lama4*$^{+/+}$ and *Lama4*$^{-/-}$ mice at 14 days post-AML transplantation. The data shown were from 2 experiments and the horizontal bars are median values.

(B) WBCs in PB at 1-3 days post-Ara-C or normal saline (NS) treatment. Data were from 2 experiments.

(C) Total BM cellularity in *Lama4*$^{+/+}$ and *Lama4*$^{-/-}$ mice at 1day post-Ara-C treatment. The cells were from one femur and one tibia.

(D) Representative FACS dot plots showing AML and host cells in BM and blood at 1-day after the last injection of NS or Ara-C. The AML cells were identified by expression of CD45 and DsRed. WT *Lama4*$^{+/+}$ mice; KO *Lama4*$^{-/-}$ mice

Related to Figure 7.

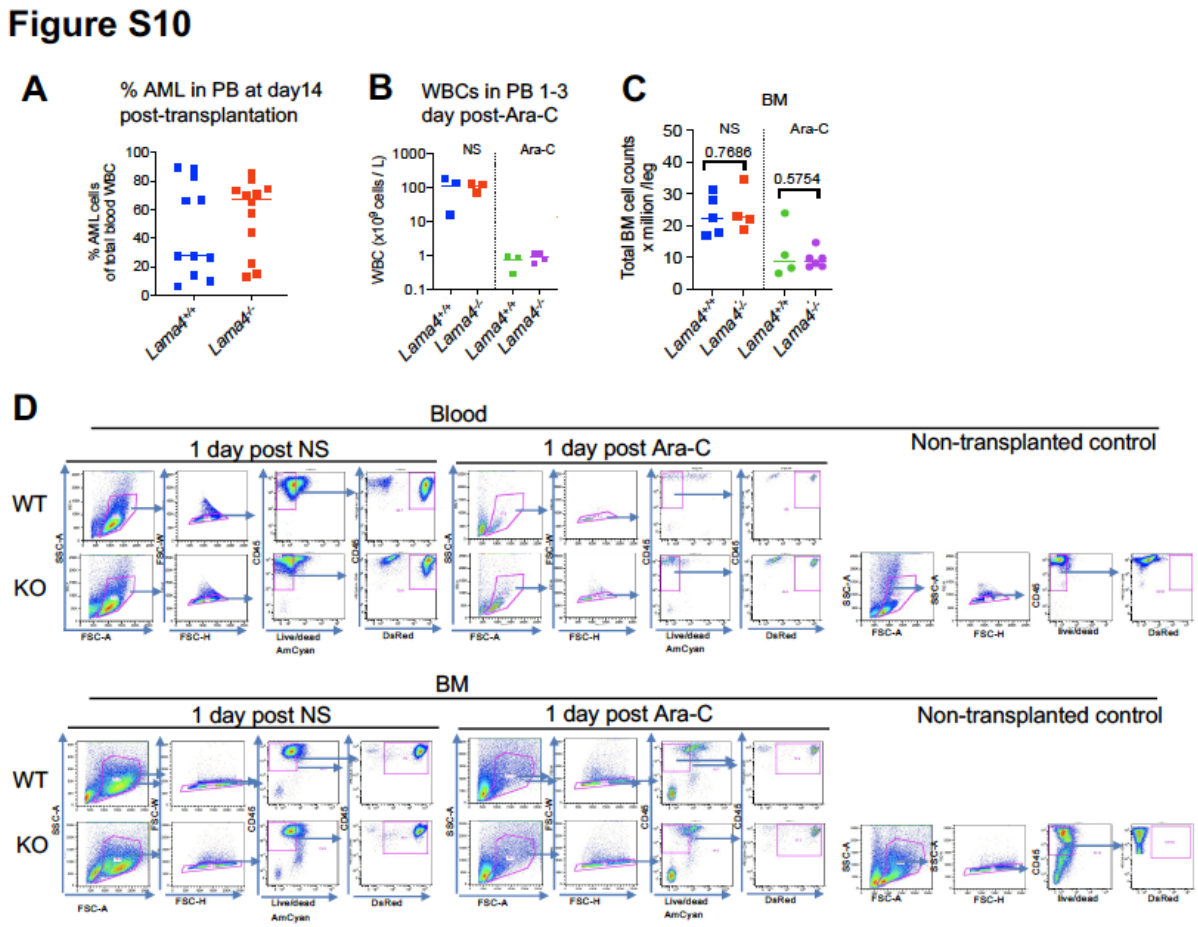


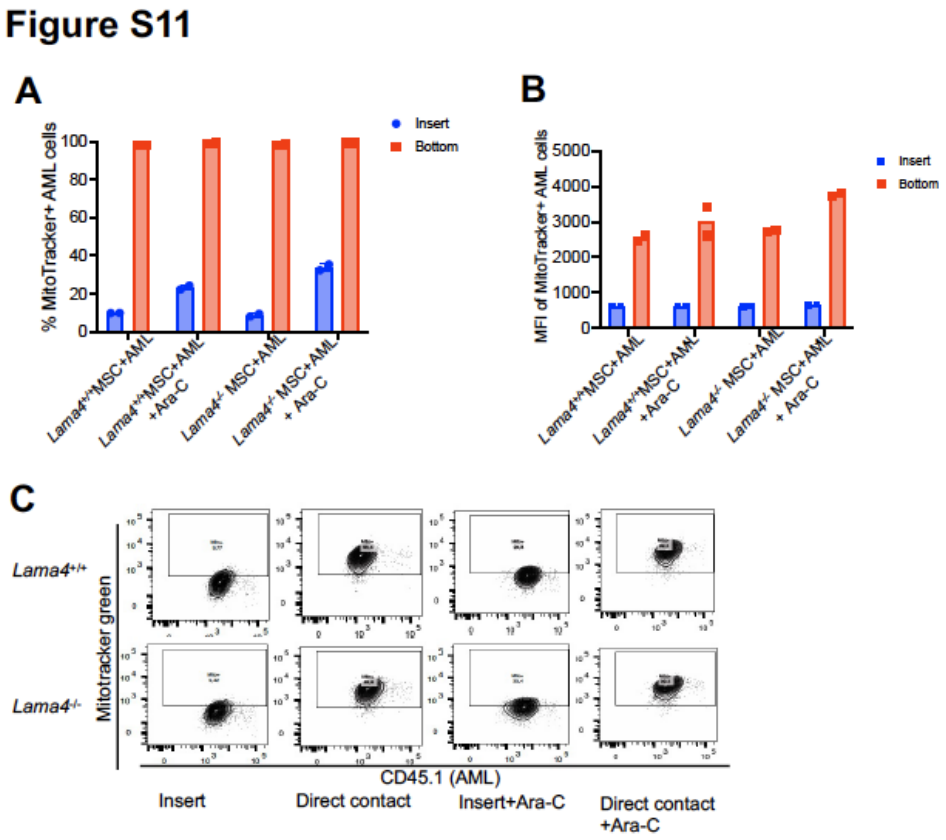
Figure S11 Mitochondrial transfer requires direct AML cell interactions with MSCs.

(A) The % of MitoTracker^{+} AML cells in transwell inserts and in bottom wells co-cultured with BM MSCs. Data shown are replicate values.

(B) MitoTracker MFI in the MitoTracker+ AML cells in the transwell inserts and bottom wells co-cultured with *Lama4*$^{+/+}$ or *Lama4*$^{-/-}$ MSCs.

(C) Representative FACS profiles showing mitoTracker^{+} AML cells 24h post culture in transwell inserts and bottom wells co-cultured with *Lama4*$^{+/+}$ and *Lama4*$^{-/-}$ MSCs.

Related to Figure 7.



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