MicroRNA-127-3p controls murine hematopoietic stem cell maintenance by limiting differentiation

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Supplementary Methods

Mice

Animals used in this study, all fully backcrossed onto a C57BL/6 background, were housed and bred in the Stanford animal facility or in the specific-pathogen-free animal facility at Humanitas Clinical and Research Center. Wild type (wt) C57BL/6 Ly5.2 (CD45.2) and congenic Ly5.1 (CD45.1) mice were purchased from Charles Rivers Laboratories and used respectively as donors and recipients in transplantation experiments. All experimental procedures were performed with the approval of and in accordance with Stanford's and Humanitas Clinical and Research Center's Administrative Panel on Laboratory Animal Care, in compliance with Italian (4D.L. N.116, G.U., suppl. 40, 18-2-1992) and international (EEC Council Directive 2010/63/EU, OJ L 276/33, 22-09-2010; National Institutes of Health Guide for the Care and Use of Laboratory Animals, US National Research Council, 2011) law and policies. The study was approved by the Italian Ministry of Health (approval no. 122/2012-B). All efforts were made to minimize the number of animals used and their suffering.

Supplemental bioinformatic analysis

Unsupervised hierarchical clustering was performed using Pearson's correlation as distance measure and average linkage as agglomerative algorithm. For generation of DE miRNA lists, miRNAs have been renamed according to miRBase v18. For the endogenous normalization, the algorithms normFinder, gNorm, Delta CT and BestKeeper were applied for the selection of the best endogenous control. MammU6_average resulted the most stable control after comprehensive ranking of all four methods. Data have been deposited in NCBI's Gene Expression Omnibus (GEO)[1] and are accessible through GEO Series accession number GSE113062. To generate lists of DE miRNA predicted targets (PT)

several databases were used: DianaMicroT (loose and strict), miRanda-mirSVR (S_0 and S_C), miRecords, mirTarBase, MirTarget2, picTar (13sp and 7sp), PITA_miR18_mm9_flank_0.9_noGU, starBase and TargetScan_v6.0.

Hematopoietic stem and progenitor cells isolation

For isolation of murine HSC, MPP or Lineage-negative (Lin^-) cells, mice were euthanized by CO_2 inhalation, and BM was harvested by crushing of multiple bones (femurs, tibias, humeri, ulnae, sternum, hips and vertebral column). Hematopoietic progenitor cells were purified through lineage marker negative selection using the mouse Lineage Cell Depletion Kit (Miltenyi Biotec) followed by automated magnetic enrichment with AutoMACS Pro cell separator (Miltenyi Biotec) according to the manufacturer instructions. Lin^- cells where then subjected to lentiviral transduction (see below) or incubated with fluorescent-conjugated antibodies for analysis or sorting of HSC.

Flow Cytometry

BM cell suspension was obtained as described above or by flushing of femurs and tibiae in sterile FACS buffer. Splenocyte suspension was obtained by smashing freshly isolated spleen on a 40μm filter followed by red blood cell (RBC) lysis. Peripheral blood withdrawal was performed from tail vein and blood was collected in K_2EDTA spray coated microtainer (BD). Fresh whole blood was analyzed with Hemocytometer (Mythic18 VET, Orphée) for obtaining cell counts prior to RBC lysis and further processing for flow cytometry.
Dead cell discrimination was performed with LIVE/DEAD™ Fixable Dead Cell Stain Kit (Aqua or Yellow, ThermoFisher Scientific), following manufacturer instructions. Staining of cells for FACS analysis was performed in FACS buffer (PBS pH 7.2, 2% FBS, 1mM EDTA for analysis or PBS pH 7.2, 0,5% BSA, 2mM EDTA for sorting). For sorting of BM populations RBC lysis was always performed except for erythrocyte precursors. Data were

acquired using either a FACSCanto II (equipped with three lasers) or a LSR Fortessa (four lasers) or a FACS-Symphony (five lasers) (all instruments BD Bioscience), according to the complexity of multicolor staining. Cell sortings were performed using a FACSAria III (BD Biosciences). All the instruments were equipped with BD FACSDIVA™ software (BD), and data were analyzed using FlowJo (Tree Star).

All the analyzed and/or sorted subpopulations, with all fluorochrome-conjugated monoclonal antibodies used to identify them, are listed in Table 1 and in Supplemental Table 4, respectively.

Lentiviral constructs

For pre-mir-127 PCR amplification: mu127fwd primer, aactcgagTGCCTGGCTTTCTCTTGCAT; mu127rev primer, taacgcgtCTCCACCATGCATCGCACTA. miR-127 target sequence: 5' - AGCCAAGCTCAGGATCACGGATCCGA-3'. Control vectors for miR-127 OE and miR-127 DR contain the empty intron sequence linked to the corresponding fluorescence reporter (OFP and dGFP respectively).

miRNA expression analysis

For profile validation and individual miRNA analysis, RNA was extracted from sorted populations with RNeasy Micro Kit (Qiagen), according to the manufacturer's protocol for recovery long and short RNA molecules. RNA was then retrotranscribed using the same MegaPlex TaqMan® Assays system with PreAmplification step (Applied Biosystems, ThermoFisher Scientific) used for miRNA profiling generation. Individual Taqman® microRNA Assays (Applied Biosystems, ThermoFisher Scientific) were used to measure miRNA expression levels by real time PCR. Reactions were performed in duplicate using a

7900HT or Viia7 (both Applied Biosystems, Thermo Fisher). miRNA expression levels were normalized to mamm-U6.

Transduction of hematopoietic stem and progenitor cells and BM transplantation

Lin^- BM cells or HSC from 6-10 weeks old $CD45.2^+$ wt mice were transduced overnight with 1 x 10^8 transducing units/ml of lentiviral particles (multiplicity of infection = 100) in serum free StemSpan medium (StemCell Technologies) supplemented with 100 ng/ml murine stem cell factor (SCF), 50 ng/ml murine thrombopoietin (TPO), 20 ng/ml human Flt-3 Ligand and of murine interleukin 3 (IL-3) (all Peprotech). An aliquot of Lin^- infected cells was cultured for up to 14 days to assess stability of transduction efficacy through FACS analysis of reporter protein expression, and expression of miR-127 by qRT-PCR (for the OE studies). For *in vitro* experiments, HSC were kept in culture up to 9 days post infection under the same culture conditions used for transduction.

Eight to ten weeks old CD45.1 wt recipient mice were lethally irradiated (9 Gy) and transplanted with a total of 4-9x10^5 transduced Lin^- cells via retro-orbital vein injection. For competitive transplantations transduced Lin^- were mixed in a 1:1 or 9:1 ratio with not transduced cells immediately before transplantation. For secondary transplants, 3x10^6 BM cells freshly isolated from mice euthanized 20-24 weeks after primary transplant were injected in secondary lethally irradiated recipients. Two and four sets of independent noncompetitive and competitive transplantation experiments were performed, respectively, with three to nine recipient mice per group in each experiment.

Transduction of K562 cells and mRNA expression analysis

K562 cells were grown in DMEM in the presence of 10% FBS, glutamine and antibiotics. Cells were split the day before transduction, and 1x10^6 cells were transduced either with the sponge (miR127DR) or with empty (EV) lentiviral vector at the same multiplicity of

infection used for Lin^- cells. Stable transduction was assessed by FACS analysis several days after infection through the expression of the GFP reporter. RNA was purified 14 days post-infection using PureZOL RNA Isolation Reagent (BIO-RAD) followed by cDNA preparation with High Capacity cDNA Reverse Transcription Kit (Applied Biosystems, ThermoFisher Scientific). Real-time PCR was performed using Taqman probes (Applied Biosystems, ThermoFisher Scientific).

mRNA analysis on cultured HSC was performed after RNA purification with RNeasy Plus Micro Kit (Qiagen), cDNA preparation with High Capacity cDNA Reverse Transcription Kit, and pre-amplification with SSoAdvanced PreAmp Supermix (BIO-RAD). Real-time PCR was perfomed using Viia7 system (Thermo Fisher Scientific) with the SsoAdvanced Universal SYBR Green Supermix (BIO-RAD) using the following primers: Spi1 (AKA PU.1) F: 5'-CCTCAGTCACCAGGTTTCCTACA-3', R: 5'-CTCTCACCCTCCTCCTCATCTG-3'; , Lyz F: 5'-ATGGCAAACACAATGTCAAA-3', R: 5'-GCCCTGTTTCTGCTGAAGTC-3'; Cybb F: 5'-TGCCAACTTCCTCAGCTACA-3', R: 5'-GTGCACAGCAAAGTGATTGG-3; Actb F: 5'-CTAAGGCCAACCGTGAAAAG-3', R: 5'- ACCAGAGGCATACAGGGACA-3'.

Cell cycle analysis

The method for intracellular Ki67 and DNA content staining was modified from Flach J et al. [2]. Briefly, $1\text{-}2\times10^7$ unfractionated BM cells were first subjected to surface staining for HSC, then fixed in Cytofix/Cytoperm buffer (BD Biosciences) for 2 up to 18 h at 4°C, washed in PermWash (BD Biosciences), and stained with either FITC- or eFluor 450-conjugated anti-Ki67 (Clone SolA15, eBioscience) for 1 h at room temperature (RT), protected from light. Cells were washed in PermWash, resuspended in PBS and stained for DNA content just before analysis. For DNA content detection SYTOX® RED DNA dye (final concentration 250mM) plus RNase A (final concentration 100μg/ml) (Invitrogen,

ThermoFisher) were subsequently added to samples and incubated for 30 min RT protected from light. Samples were analyzed without washing.

Apoptosis and intracellular ROS detection

Lin$^-$ cells were isolated from the BM of primary transplanted mice. 2,5x10^4-2,5x10^5 cells were cultured for 24h in the same conditions used during transduction. Apoptosis and ROS production were evaluated before and after cytokine stimulation. To measure ROS levels, cells were incubated with 1μM CellROX® Deep Red Reagent (Invitrogen, ThermoFisher Scientific) for 30' at 37°C followed by surface staining for HSC markers and FACS analysis. The excitation/emission peak wavelength for the oxidized dye was 644 nm and 665 nm respectively. Apoptosis was measured after surface staining for HSC markers followed by incubation with PI/Annexin V (Biolegend) according to manufacturer instructions.

Colony forming Unit assay

1000 Lin$^-$ cells isolated from the BM of primary transplanted mice or 100 cells harvested from cultures initiated three days earlier from transduced sorted HSC were seeded into methylcellulose-based medium (MethoCult GF M3434; StemCell Technologies). CFU assay was performed in duplicate in 35mm dishes. Colonies were scored at day 9 before and after the 1-Step™ Ultra TMB-ELISA Substrate Solution (ThermoFisher Scientific) specific red-colony colorimetric assay [3].

Supplementary Table S1. DE miRNA in the physiological HSC-to-MPP transition.

For each miRNA FD obtained with quantile normalization (q-norm) and U6 normalization (U6 norm) is reported.

Down-regulated miRNA	FD *(q-norm)*	FD *(U6-norm)*		Up-regulated miRNA	FD *(q- norm)*	FD *(U6-norm)*
miR-127-3p	49.75	126.19		miR-221-3p	591.41	242,59
miR-184-3p	38.88	135.91		miR-107-3p	12.63	3.74
miR-411-5p	21.05	72.78		miR-467a-5p	10.88	6.41
miR-434-3p	10.66	23.88		rno-miR-207	10.12	5.92
miR-146a-5p	8.01	12.31		miR-23b-3p	9.70	5.07
miR-682	7.40	10.89		miR-532-5p	8.93	5.51
miR-1a-3p	7.14	9.80		miR-450a-5p	8.66	3.71
miR-197 (1)	7.12	12.36		miR-18a-5p	7.66	4.71
miR-451	6.55	12.28		miR-224-5p	7.52	3.97
miR-133a-3p	6.52	10.14		miR-671-3p	6.96	9.53
rno-miR-1	6.19	9.50		miR-15a-5p	6.89	4.38
miR-148a-3p	5.73	9.01		miR-324-3p	6.84	3.98
miR-7a-5p	5.60	9.31		miR-339-5p	5.86	ns
miR-34b-3p	5.51	10.35		miR-223-3p	5.48	3.24
miR-145-5p	5.21	9.20		miR-340-3p	5.10	3.27
miR-188-5p	4.73	7.33		rno-miR-351	5.09	3.25
miR-342-3p	3.73	7.03		miR-652-3p	5.06	4.58
miR-667-3p	3.45	6.00		miR-322-5p	4.21	2.84
miR-409-3p	3.21	5.43		miR-542-3p	3.48	ns
miR-152-3p	3.11	4,80		let-7i-5p	3.44	2.16
miR-203-3p	3.05	4.52		let-7b-5p	3.20	2.13
miR-31-5p	2.89	4.38		miR-100-5p	3.07	1.93
miR-128-3p	2.83	ns		let-7e-5p	3.05	2.23
miR-150-5p	2.76	4.20		miR-181c-5p	2.89	3.03
miR-365-3p	2.48	4.23		miR-103-3p	2.83	2.37
miR-99b-5p	2.19	3.11		miR-20b-5p	2.51	1.52
miR-193b-3p	1.91	3.14		miR-93-5p	2.27	1.40
miR-29a-3p	1.59	2.33		miR-744-5p	2.17	ns
miR-26b-5p	1.58	2.29		miR-196b-5p	2.02	ns
miR-101a-3p	1.51	2.29		let-7g-5p	1.96	ns
miR-191-5p	1.40	1.60		miR-19a-3p	1.94	ns
miR-140-5p	1.38	1.96		rno-miR-196c	1.77	1.30
miR-30e-5p	1.26	2.00		miR-222-3p	1.67	ns
miR-484	1.24	1.77		miR-130a-3p	1.53	ns
				miR-92a-3p	1.34	ns
				let-7d-5p	1.33	ns
				miR-331-3p	1.22	ns

(1) miR-197 was removed from miRBase after the generation of the profile. It was therefore not considered for subsequent analysis.

Supplementary Table S2. DE miRNA in Pbx1-deficient HSC *vs* control HSC.

In bold miRNA DE also in the normal HSC-to-MPP transition (see supplementary Table S1); in colored box the concordantly DE.

Down-regulated miRNA	FD *(q-norm)*	FD *(U6-norm)*		Up-regulated miRNA	FD *(q- norm)*	FD *(U6-norm)*
miR-127-3p	91.95	241.7		**miR-532-5p**	18.1	7.9
miR-411-5p	13.11	39.7		miR-182-5p	14.3	5.1
mmu-miR-1a-3p	12.95	30.6		miR-200c-3p	13.2	ns
rno-miR-1	11.80	27.5		**miR-324-3p**	9.0	4.8
miR-682 [(2)]	8.18	11.6		**miR-224-5p**	8.9	4.5
miR-872-5p	6.09	9.7		miR-680	7.2	6.2
miR-34b-3p	5.99	11.2		miR-34a-5p	6.7	ns
miR-434-3p	4.73	8.7		**miR-15a-5p**	6.4	ns
miR-203-3p	3.27	7.3		miR-124-3p	5.3	ns
miR-188-5p	2.65	4.7		**miR-146a-5p**	3.3	ns
miR-27a-3p	2.64	5.6		miR-139-5p	3.2	ns
miR-125b-5p	2.61	5.0		**miR-342-3p**	2.8	ns
miR-99b-5p	2.55	5.0		**let-7e-5p**	2.7	ns
miR-19b-3p	2.54	4.6		**let-7i-5p**	2.7	ns
miR-667-3p	2.40	4.3		**miR-148a-3p**	2.6	ns
miR-223-3p	2.32	4.6		miR-194-5p	2.6	ns
miR-25-3p	2.29	4.7		**miR-150-5p**	2.5	ns
rno-miR-381	2.08	4.1		**miR-671-3p**	2.4	ns
miR-197	1.94	3.7		miR-29c-3p	2.4	ns
miR-376b-3p	1.87	4.8		miR-126-5p	2.3	ns
miR-101a-3p	1.70	3.4		**let-7g-5p**	2.1	ns
miR-19a-3p	1.57	4.4		**miR-365-3p**	2.0	ns
miR-140-5p	1.50	3.1		**miR-331-3p**	1.7	ns
miR-93-5p	1.49	3.2				
miR-24-3p	1.42	2.8				

[(2)] miR-682 had Ct<35 only in one of the biological replicates (it was not filtered out in the analysis used for unsupervised hierarchical clustering shown in Figure 1B since it was expressed in Flk2$^-$MPP from Pbx1-cKO mice). It was therefore not considered among miRNAs fulfilling our criteria for selecting miRNA candidates potentially involved in HSC self-renewal.

Supplementary Table S3. Selected miRNA.

Selected DE miRNA (all down-regulated)			
miRNA	***FD (q-norm)***	***FD (U6-norm)***	***Predicted Targets among anticorrelated DE mRNA***
miR-127-3p	91.95	241.7	Gp1bb;Nek2
miR-411-5p	13.11	39.7	Cyb5r3;Alox5;Itgb1;P2rx7;Themis;Cep76;Prc1;Skp2;Fam188a
miR-34b-3p	5.99	11.2	Espl1;Ccnd2;Nek2;Themis;Prc1;Skp2;Mia3;Mad2l1;Lrr1

Supplementary Table S4. Antibody List

	CLONE	FLUOROCHROME	SUPPLIER
CD3	17A2	FITC; PE; APC; PerCP-Cy5.5	eBioscience
CD4	RM4-5	FITC; PE-Cy7; eFluor 450	eBioscience
CD8	53-6.7	APC	eBioscience
CD11b	M1/70	APC; PerCP-Cy5.5; eFluor 450	eBioscience
CD16/32	93	PerCP-Cy5.5	eBioscience
CD19	1D3	PerCP-Cy5.5; PE-Cy7; eFluor 450	eBioscience
CD31	390	PE	eBioscience
CD34	RAM34	FITC	eBioscience
CD41	MWReg30	FITC eFluor 450	BD Pharmingen eBioscience
CD43	eBioR2/60	FITC	eBioscience
CD44	IM7	FITC	BD Pharmingen eBioscience
CD45.1	A20	APC-eFluor 780	eBioscience
CD45.2	104	eFluor 450; PerCP-Cy5.5 BV510	eBioscience BioLegend
CD45R (B220)	RA3-6B2	APC-eFluor 780; PerCP-Cy5.5	eBioscience
CD48	HM48.1	PE-Cy7 BV510	eBioscience BD Pharmingen
CD105	MJ7/18	PE	eBioscience
CD117 (cKit)	2B8	PE; APC-eFluor 780 BV786	eBioscience BD Pharmingen
CD127	A7R34	APC	eBioscience
CD135	A2F10	PE	eBioscience
CD150	TC15-12F12.2	APC; BV605	BioLegend
CD201	eBio1560	PE	eBioscience
IgM	II/41	APC	eBioscience
Ly-6A/E (Sca-1)	D7	PE-Cy7; APC BV711; BV421; PE	eBioscience BD Pharmingen
Ly6C	AL-21	FITC	BD Pharmingen
Ly6G	1A8	PE-Cy7; BV421	BD Pharmingen
Ly-6G (Gr-1)	RB6-8C5	PerCP-Cy5.5; eFluor 450	eBioscience
NK1.1	PK136	PE-Cy7	eBioscience
Ter119	TER-119	Pe-Cy7; PerCP-Cy5.5	eBioscience
Lineage cocktail: a mixture of the above mentioned antibodies against CD3, B220, CD19 TER-119, CD11b and Gr-1 was used for lineage marker exclusion	17A2; RA3-6B2; 1D3; TER-119; M1/70; RB6-8C5	eFluor 450; PerCP-Cy5.5	eBioscience

Legends to Supplementary Figures

Supplementary Figure S1. miR-221 is the most up-regulated miRNA in the normal HSC-to-MPP transition. qRT-PCR analysis of miR-221 expression in sorted HSC and MPP from 12 weeks old wt mice. miRNA expression level is shown as FD relative to HSC (n=3 pools of a total of 8 mice; 2-3 mice/pool).

Supplementary Figure S2. Analysis of 127DR mice. (A) Effect of miR-127 lentiviral sponge vector transduction on *BLIMP-1* and *XBP-1* expression. K562 cells were infected overnight with either miR-127-3p sponge vector (127DR) or empty vector (EV). dGFP (left panel) and target expression (right) were measured >14 days after transduction. Histogram on the right shows fold difference of *BLIMP-1* and *XBP-1* expression relative to untransduced cells. (B) Survival curves of 127DR- and EV-transduced primary recipients, and of mice transplanted with not infected Lin^- cells, cultured overnight in the same conditions used for transduced cells. (C) Analysis of vector integration in *ex-vivo* isolated B- and T-lymphocytes with different brightness in the green channel. dGFP negative, low and positive fractions of donor $CD19^+$ B and $CD3^+$ T lymphocytes were FACS-sorted from the spleen of individual 127DR- and EV-transduced recipient mice. The sorting strategy is shown with representative FACS plots (top panels). A mouse transplanted with not infected cells was used as negative control for dGFP expression level in each population. The presence of integrated vector was evaluated by PCR analysis of GFP sequence on genomic DNA (50 and 10ng) extracted from sorted cells (bottom).

Supplementary Figure S3. Analysis of miR127DR secondary recipients. (A) Analysis of chimerism in the PB over time of the two 127DR secondary recipients that remained alive (red: mouse #1.2; black: mouse #2.5). Vertical axis shows the percentage of total chimerism (donor $CD45.2^+$ cells within total $CD45^+$ cells; dotted lines) and of myeloid chimerism (donor $CD45.2^+$ cells within $CD11b^+$ cells; plain lines). (B) FACS analysis of 127DR stem and progenitor cells in mouse #1.2. Dot plots refer to the LKS population; a representative sample is showed for the EV group. Untransplanted CD45.1 recipient is shown as control. Numbers represent the percentage of the indicated HSC gate. Histogram overlay shows CD45.2 expression within the HSC gate.

Supplementary Figure S4. Immunophenotypic analysis of in vitro cultured transduced HSCs. (A) FACS analysis (representative of 4 individual mice) performed at days 3 and 6 of culture of transduced HSC, gating on the GFP^+cKit^+ population, showing that gradual acquisition of CD48 and loss of CD150 occur to a similar extent in the 127DR and EV experimental groups. (B) Representative FACS analisys at day 6 after gating on $GFP^+cKit^+CD48^-CD150^+$ cells.

Supplemental Figure 5. Stable over expression of miR-127 *in vitro* and *in vivo*. (A) Experimental workflow of the non-competitive BM transplantation experiments. (B) qRT-PCR analysis of miR-127 (n=2) and miR-16 (n=1) expression in Lin^- cells immediately after *ex-vivo* isolation and after five days of culture in infected and not-infected cells. Bars indicate the range. (C) qRT-PCR analysis of miR-127 and miR-16 expression in splenocytes of 127OE and EV transduced mice (n=3-4) harvested 22 weeks after

transplantation. (D) Experimental workflow of the competitive BM transplantation experiments.

References to supplementary information

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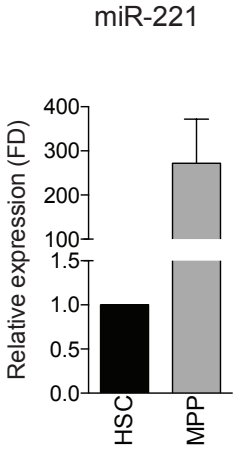
Figure S1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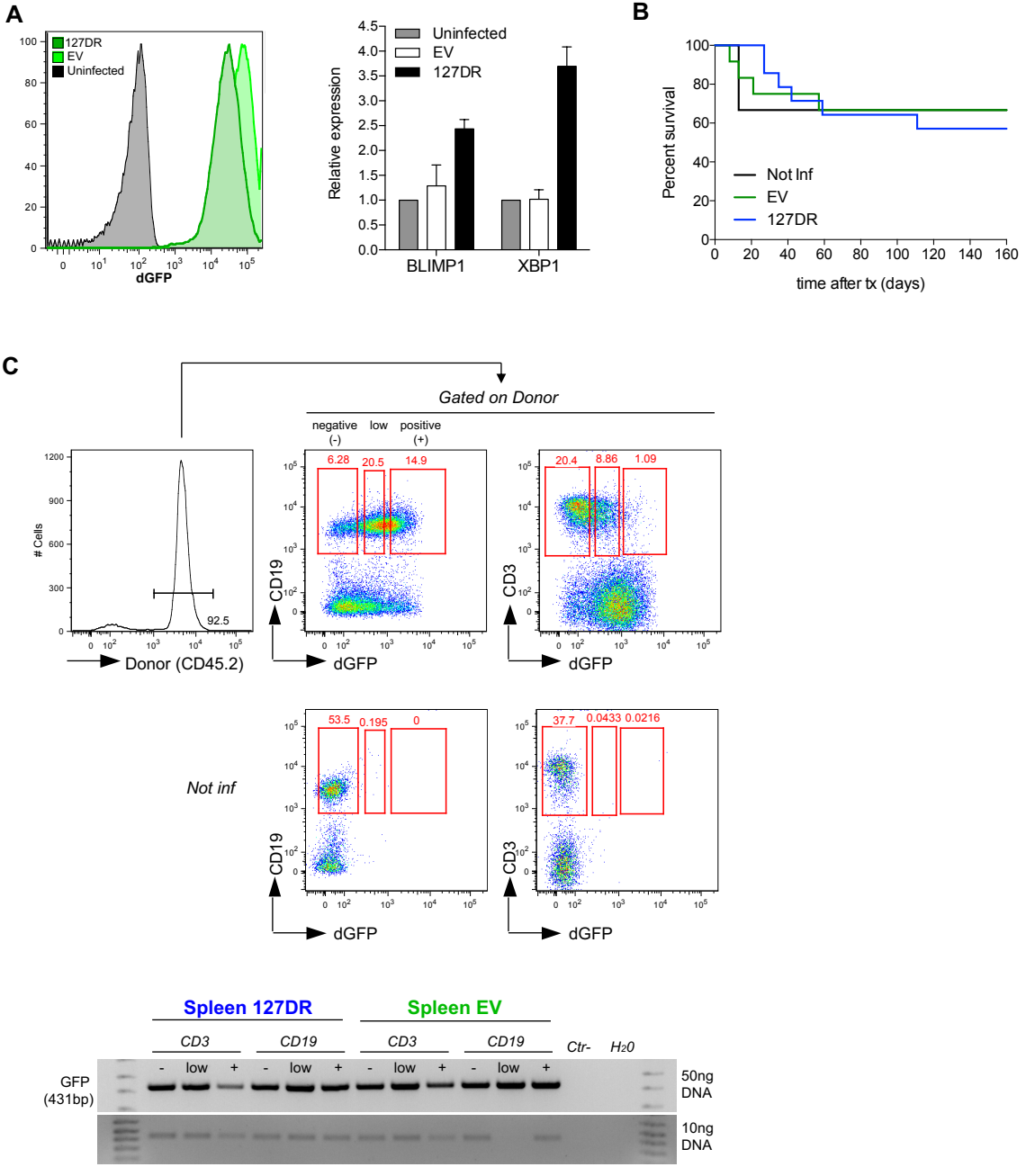
1.0
0.5
0.0
HSC
MPP

Figure S2


A
127DR
EV
Uninfected
dGFP
Relative expression
BLIMP1
XBP1
B
Percent survival
Not Inf
EV
127DR
time after tx (days)
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Gated on Donor
negative (-)
low
positive (+)
Cells
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Donor (CD45.2)
CD19
dGFP
CD3
6.28
20.5
14.9
20.4
8.86
1.09
Not inf
53.5
0.195
0
37.7
0.0433
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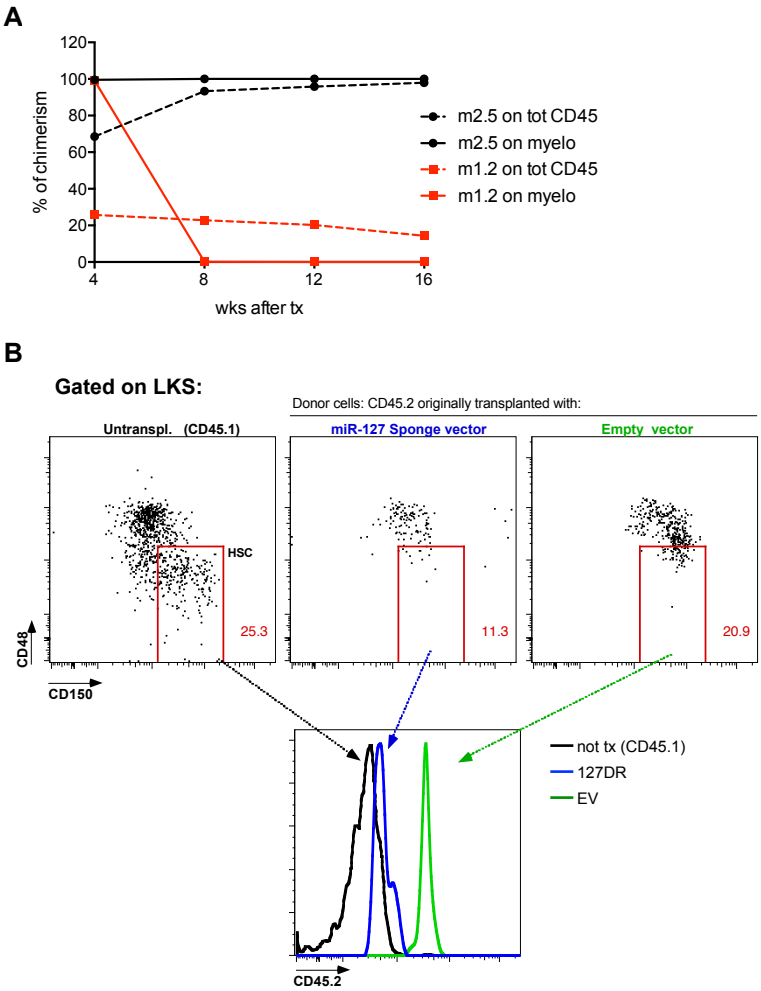
CD19
Ctr-
H2O
GFP (431bp)
50ng DNA
10ng DNA

Figure S3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120
100
80
60
40
20
0
% of chimerism
4
8
12
16
wks after tx
m2.5 on tot CD45
m2.5 on myelo
m1.2 on tot CD45
m1.2 on myelo
B
Gated on LKS:
Donor cells: CD45.2 originally transplanted with:
Untranspl. (CD45.1)
miR-127 Sponge vector
Empty vector
HSC
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11.3
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CD48
CD150
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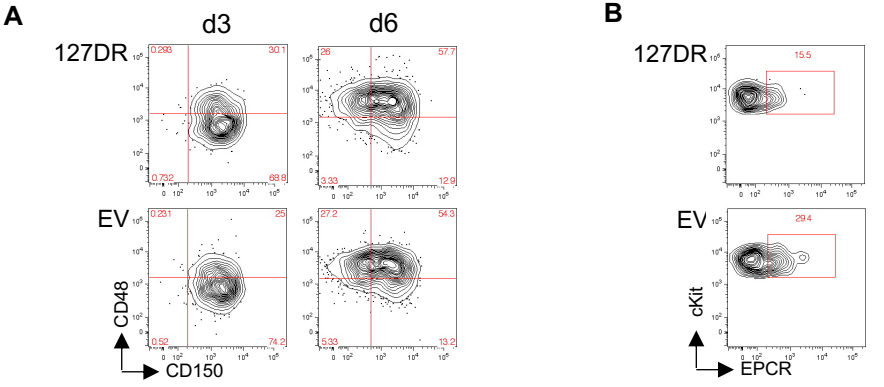
not tx (CD45.1)
127DR
EV

Figure S4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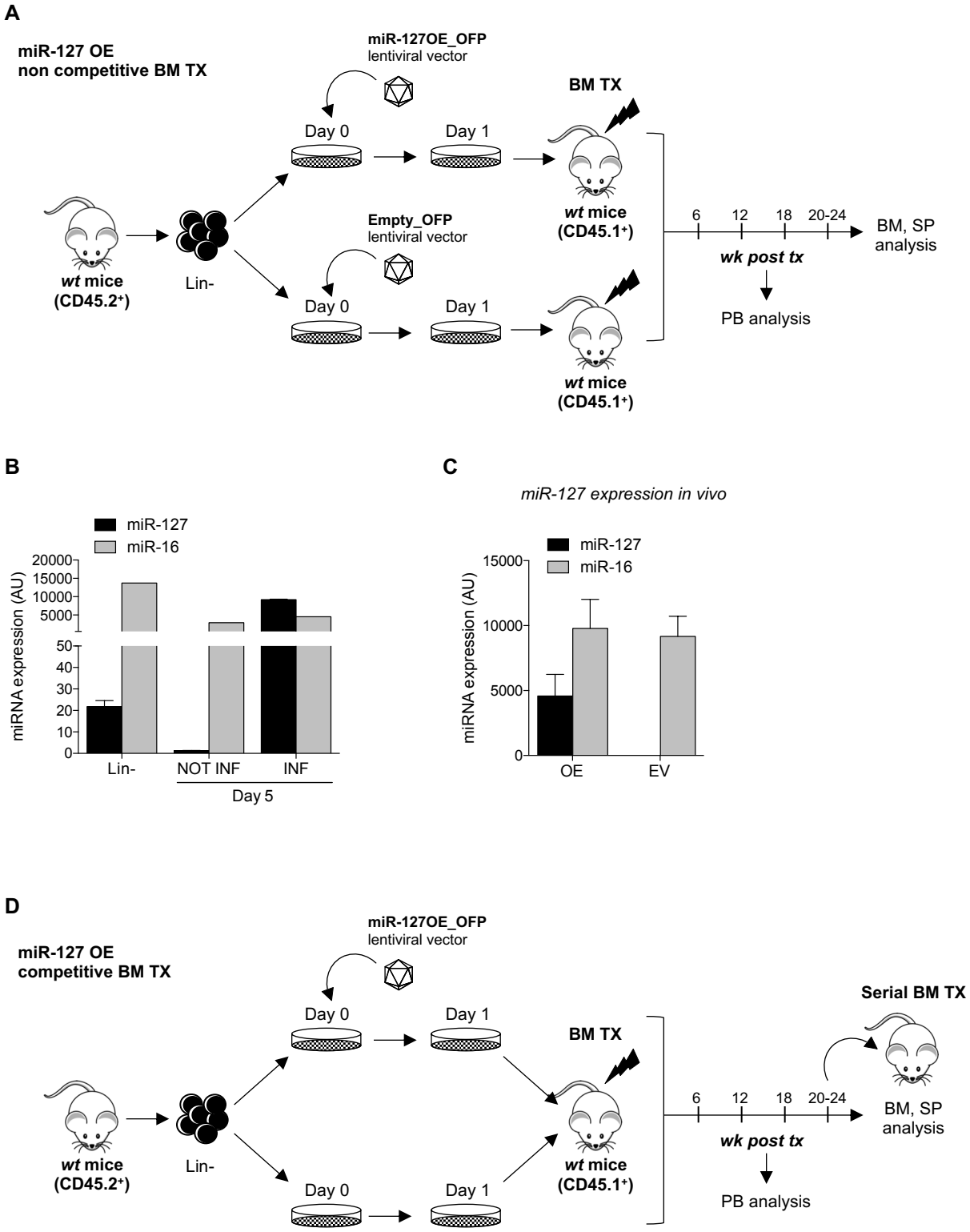
127DR
EV
0.293
30.1
0.732
68.8
26
57.7
3.33
12.9
0.231
25
0.52
74.2
27.2
54.3
5.33
13.2
CD48
CD150
B
127DR
EV
15.5
29.4
cKit
EPCR

Figure S5


A
miR-127 OE
non competitive BM TX
miR-127OE_OFP
lentiviral vector
Empty_OFP
lentiviral vector
Day 0
Day 1
BM TX
wt mice
(CD45.2+)
Lin-
wt mice
(CD45.1+)
6
12
18
20-24
wk post tx
BM, SP
analysis
PB analysis
B
miR-127
miR-16
miRNA expression (AU)
20000
15000
10000
5000
50
40
30
20
10
0
Lin-
NOT INF
INF
Day 5
C
miR-127 expression in vivo
miR-127
miR-16
miRNA expression (AU)
15000
10000
5000
0
OE
EV
D
miR-127 OE
competitive BM TX
miR-127OE_OFP
lentiviral vector
Day 0
Day 1
BM TX
wt mice
(CD45.2+)
Lin-
wt mice
(CD45.1+)
Serial BM TX
6
12
18
20-24
wk post tx
BM, SP
analysis
PB analysis