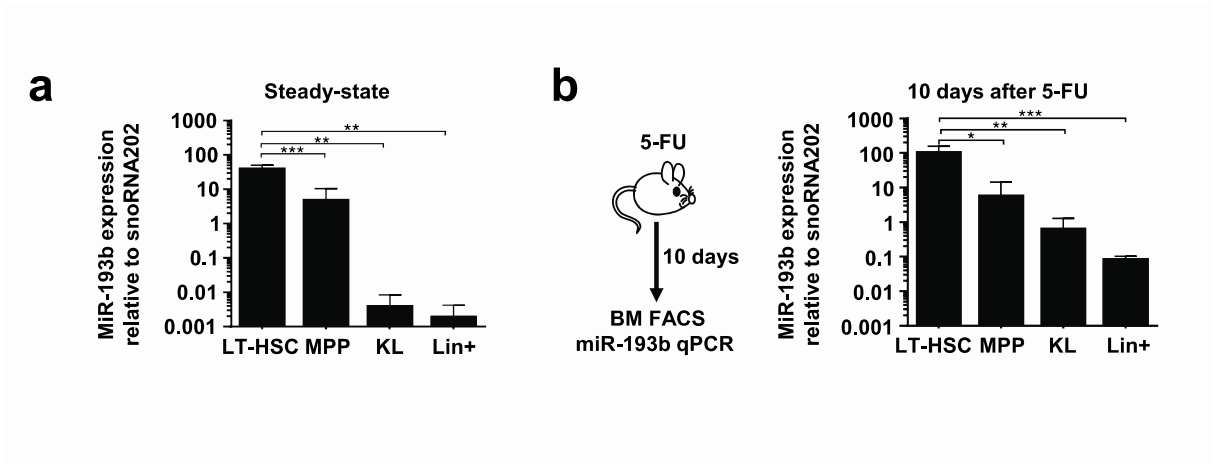
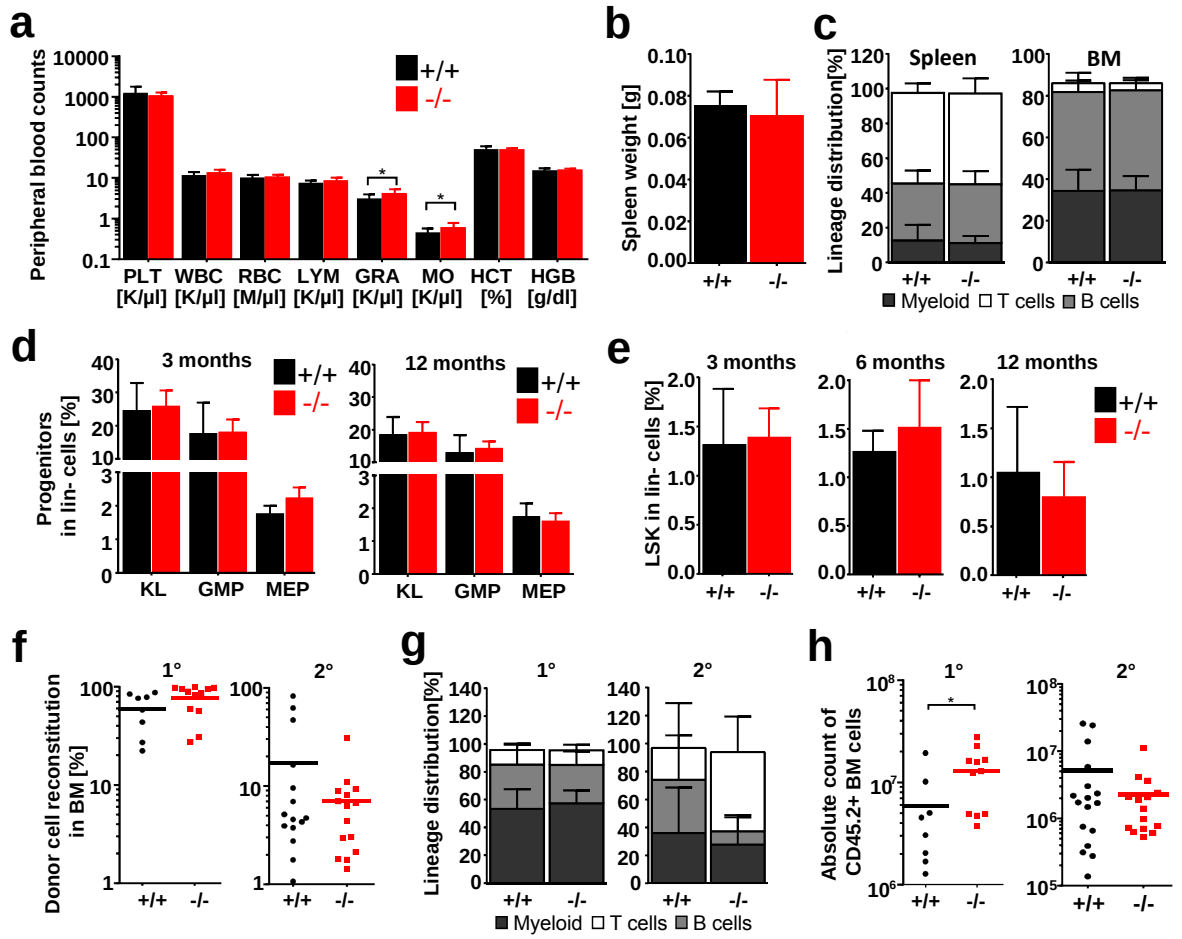
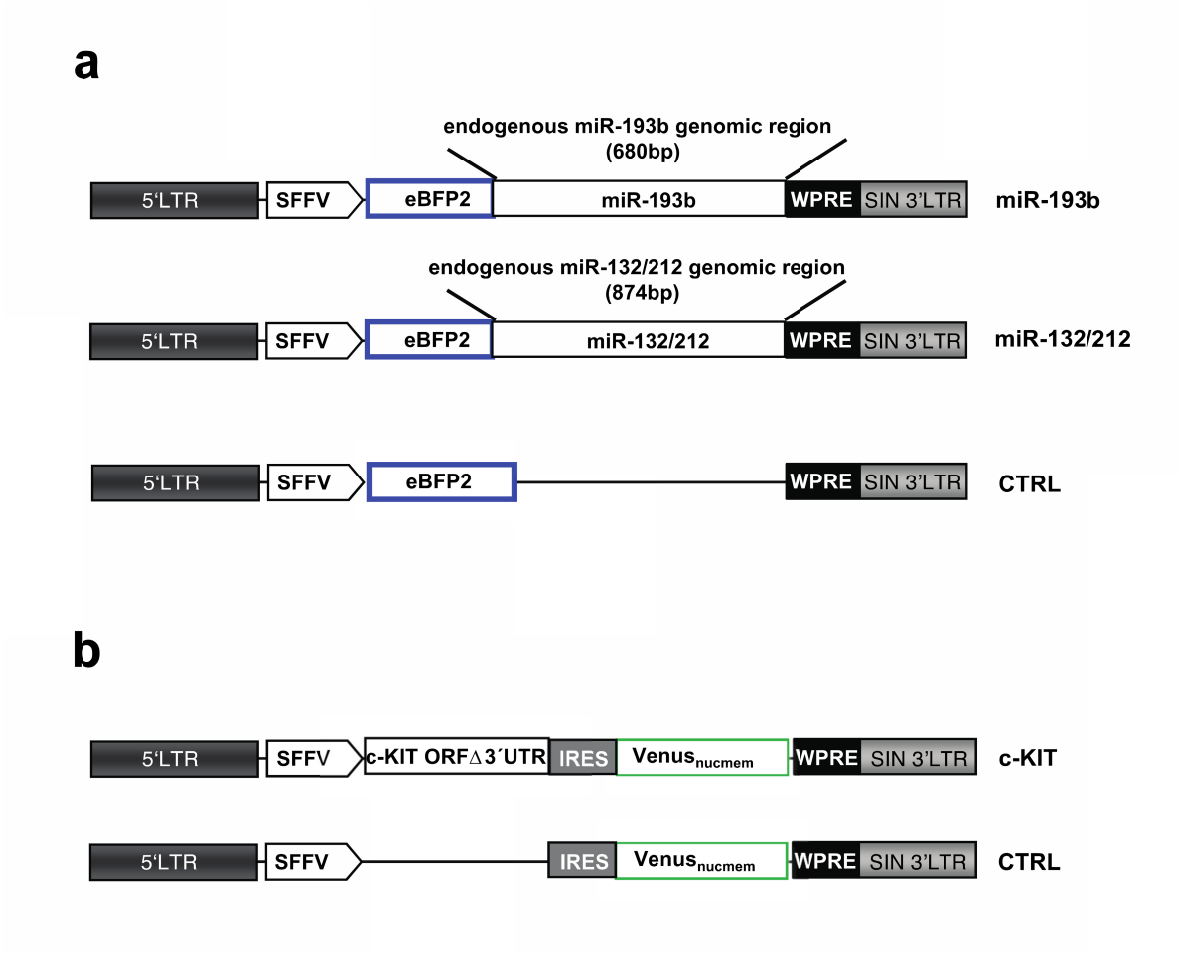


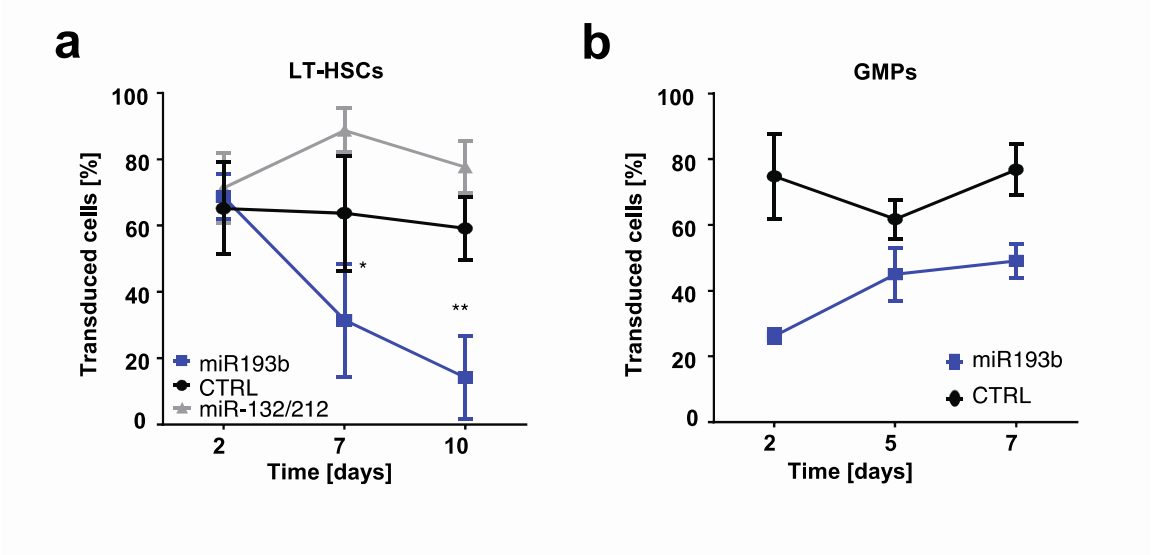
Supplementary Figure 1. MiR-193b expression in HSPC populations in steady-state and during haematopoietic stress. (a) Quantification of endogenous miR-193b in FACS-sorted LT-HSCs, MPPs, c-KIT$^+$ Sca1$^-$ Lin$^-$ (KL) cells and Lin$^+$ cells of adult wildtype mice in steady-state hematopoiesis, determined via miR-specific quantitative RT-PCR. MiR-193b levels were normalized to snoRNA-202 expression. (b) Quantification of endogenous miR-193b in FACS-sorted LT-HSCs, MPPs, c-KIT$^+$ Sca1$^-$ Lin- (KL) cells and Lin$^+$ cells of adult wildtype mice 10 days after a single 5-fluorouracil (5-FU) injection, determined via miR-specific quantitative RT-PCR. MiR-193b levels were normalized to snoRNA-202 expression. N=4 mice for MPPs, KL and Lin$^+$, N=3 mice for LT-HSCs. The bar diagrams represent the mean and SD. The significance was only determined between the LT-HSCs and the respective cell populations. *, P<0.05; **, P<0.01; and ***, P<0.001.



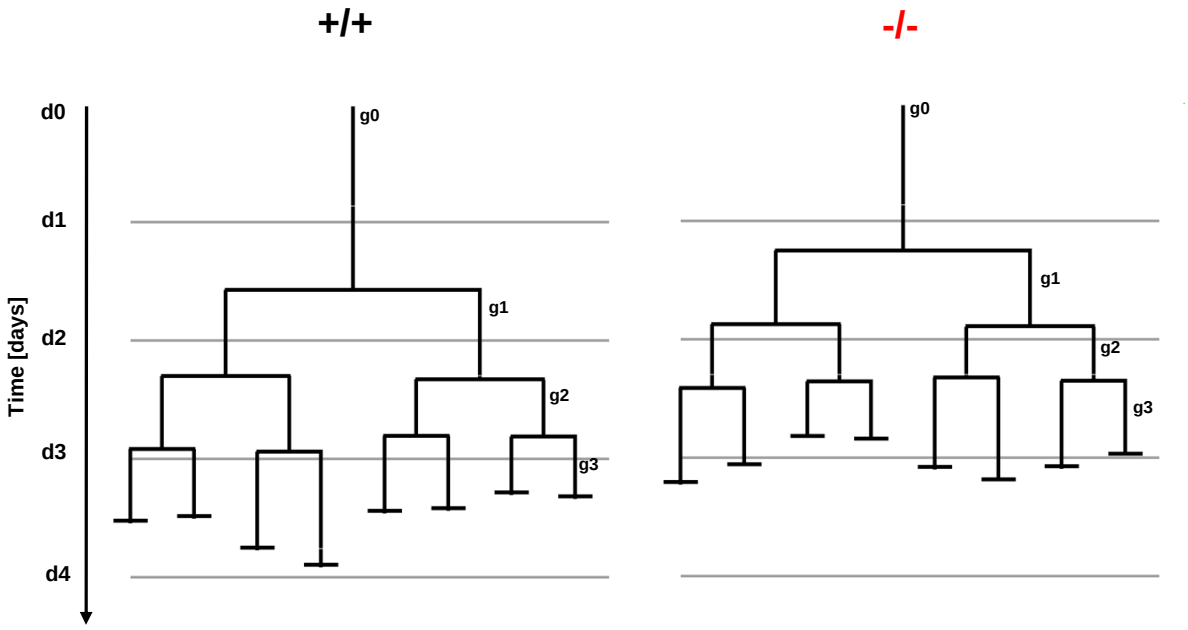
Supplementary Figure 2. Haematopoietic cell distribution in miR-193b-deficient mice and in recipient mice transplanted with *miR-193b*$^{-/-}$ LT-HSCs. (a) Peripheral blood counts in 3-4-month-old mice. N=11 *miR-193b*$^{+/+}$ mice, N=17 *miR-193b*$^{-/-}$ mice. (b) Spleen weights of 3-month-old mice, N=6 *miR-193b*$^{+/+}$ mice, N=14 *miR-193b*$^{-/-}$ mice. (c) Distribution of mature cell lineages in the spleen and bone marrow of 3-month-old mice, N=6 *miR-193b*$^{+/+}$ mice, N=7 *miR-193b*$^{-/-}$ mice. (d) Progenitor cell distribution in lineage enriched bone marrow in 3 months old mice, N=4 *miR-193b*$^{+/+}$ mice, N=6 *miR-193b*$^{-/-}$ mice. (e) Percentage of LSK cells in in 3-month-old mice, N=4 *miR-193b*$^{+/+}$ mice, N=6 *miR-193b*$^{-/-}$ mice. (f) Donor cell reconstitution in the bone marrow of primary and secondary transplanted recipients g) Multilineage engraftment in the bone marrow of primary and secondary transplanted recipients. (h) Absolute number of donor cells in in the bone marrow of primary and secondary transplanted recipients. Mann-Whitney test. The bar diagrams represent the mean and SD. *, P<0.05; **, P<0.01; and ***, P<0.001.



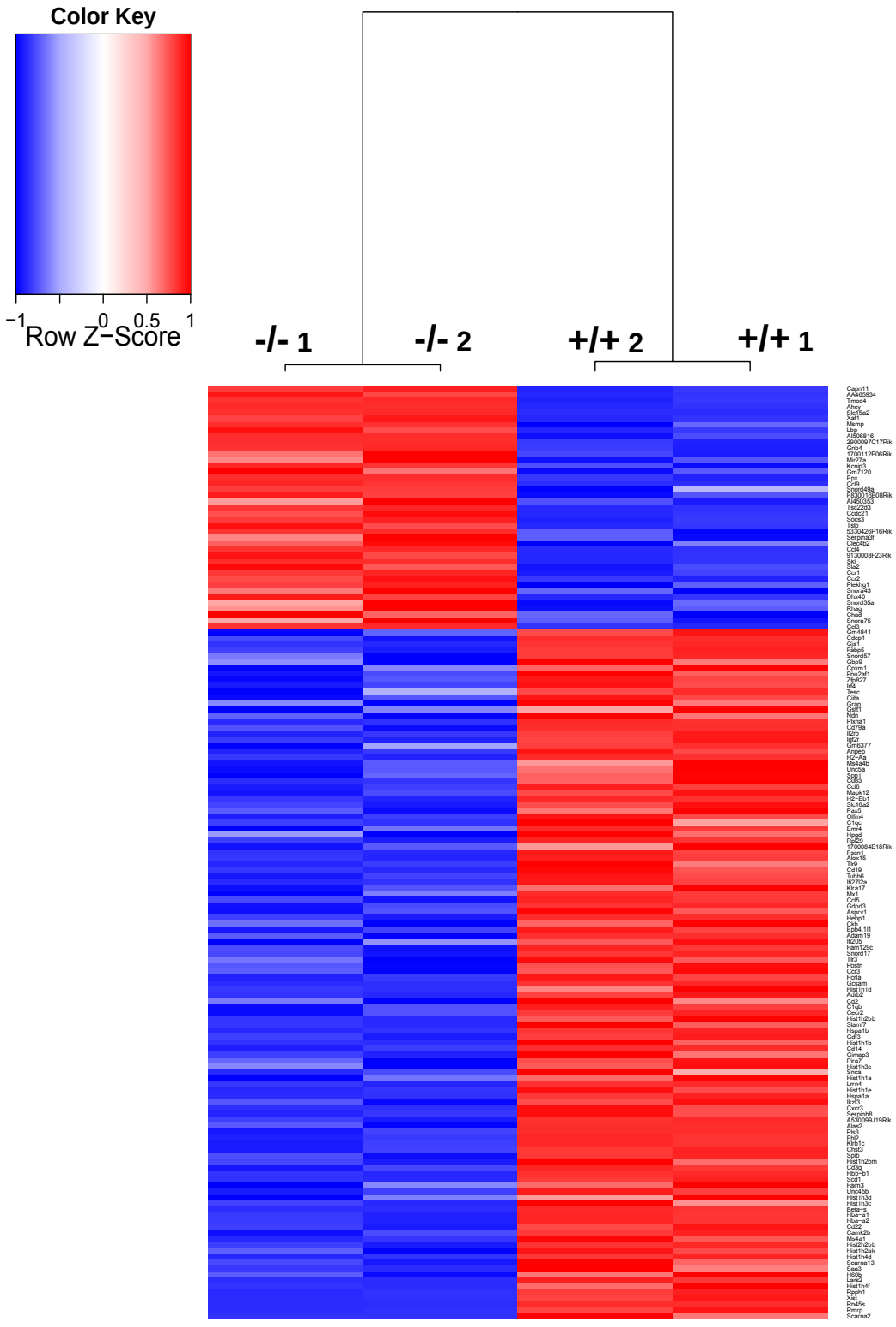
Supplementary Figure 3. Lentiviral expression vectors. (a) Schematic lentiviral vector maps used for ectopic miR-193b and miR-132/212 expression. The natural genomic loci encompassing the miR-193b sequence (680bp) and the miR-132/212 sequence (874bp) were cloned into the vector 3´of the open reading frame of eBFP2. (b) Schematic lentiviral vector maps used for ectopic c-KIT expression. The expression vector contains the open reading frame coding for c-KIT but not the 3´UTR containing the miR-193b targeting sequence.



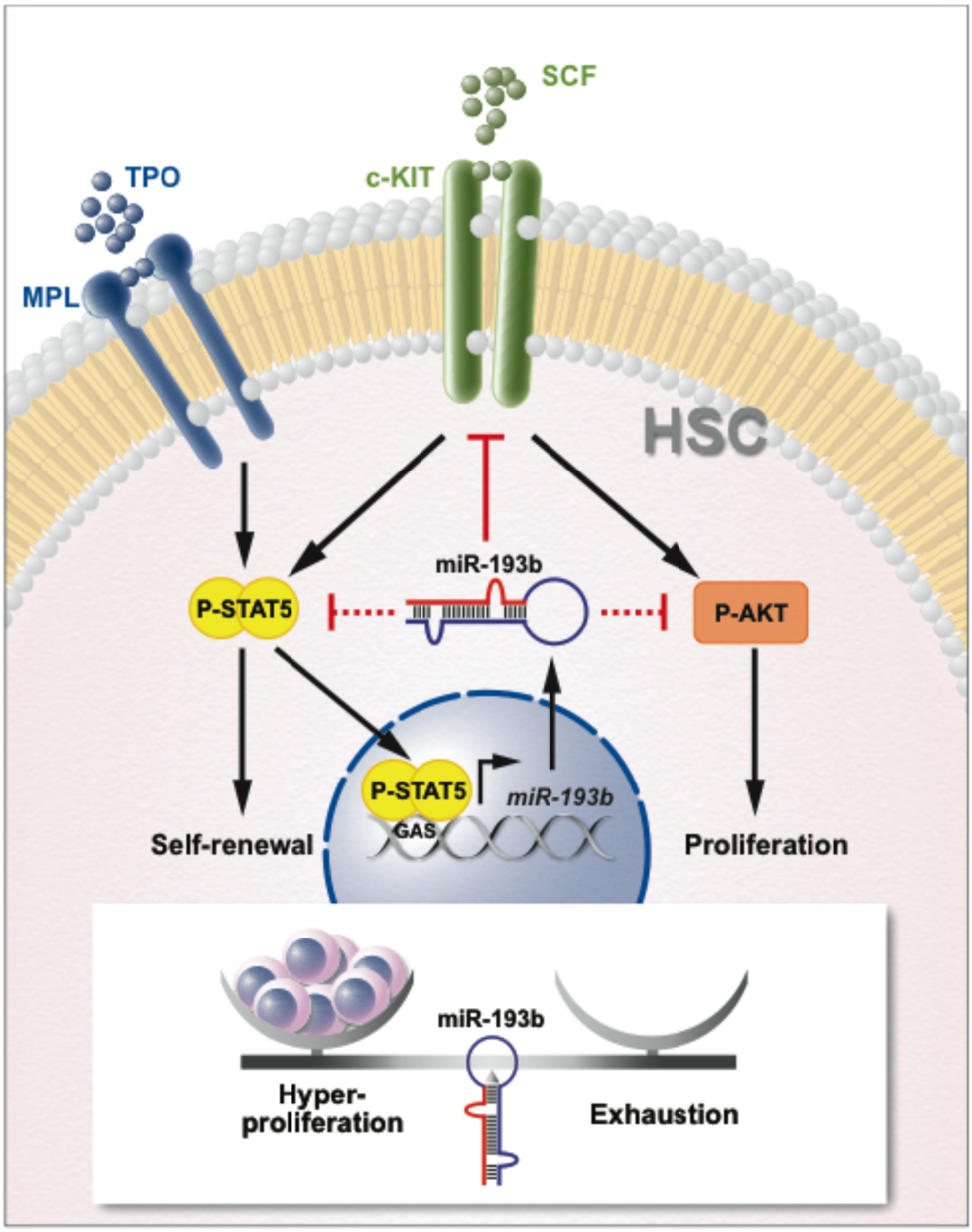
Supplementary Figure 4. The ectopic expression of an unrelated miR-132/212 did not lead to diminished expansion of LT-HSCs. (a) Lentiviral transduction of LT-HSCs with miR-193b, miR-132/212 or CTRL vectors and cell culture in SCF, TPO for 10 days. The percentage of transduced cells was determined via FACS at indicated time points. N=3 independent experiments. (b) Lentiviral transduction of granulocyte-macrophage progenitors (GMPs) with miR-193b or CTRL vectors and cell culture in SCF, IL3, IL6 for 7 days. The percentage of transduced cells was determined via FACS at indicated time points. N=3 independent experiments. The data is represented as mean and SD. *, $P<0.05$; **, $P<0.01$; and ***, $P<0.001$.



Supplementary Figure 5. Behaviour of HSCs and their progeny determined via cell tracking. Example pedigrees of individual HSCs and their progeny derived from *miR-193b*$^{+/+}$ and *miR-193b*$^{-/-}$ mice as assessed using video-microscopy-based cell tracking. The HSCs are represented at the apex of the pedigree; each cell division is indicated with a horizontal bar. All cells emerging from the mother HSC (g0) were tracked until generation 3 (g3).



Supplementary Figure 6. Heat map of up-regulated and down-regulated genes in *miR-193b*$^{-/-}$ LSKs. Gene expression in LSKs from two sets of *miR-193b*$^{-/-}$ and *miR-193b*$^{+/+}$ mice was quantified using RNA sequencing. Differentially expressed genes (1.5 fold up-regulated or down-regulated, $p<0.05$) were clustered and displayed as a heat map.



Supplementary Figure 7. Graphical abstract of the study. Here we show a regulatory circuit that prevents excessive HSC self-renewal by up-regulation of miR-193b driven by self-renewal promoting Thrombopoietin (TPO)–Mpl-STAT5 signalling. In turn, miR-193b restricts cytokine signalling by targeting the tyrosine kinase receptor c-KIT. The dashed lines indicate potential additional direct inhibition.

Supplementary Table 1 Antibodies used for flow cytometry

Antigen	Clone	Dosage	Conjugate	Company
CD3	145-2C11	1μl/1x10^7cells 1μl/test	Biotin, PE-Cy7	eBioscience
CD11b	M1/70	0.5μl/1x10^7cells	Biotin	eBioscience
CD11b	M1/70	1μl/test	PE, AF670	Biolegend
CD16/32	2.4G2	3μl/test	FITC, V450	BD
CD19	1D3	0.5μl/1x10^7cells	Biotin	eBioscience
CD34	RAM34	25μl/test	eFluor660	eBioscience
CD41	MWReg30	0.5μl/1x10^7cells	Biotin	eBioscience
CD48	HM48.1	2μl/test	PE, FITC	Biolegend
CD45.1	A20	1μl/test	eFluor450, FITC	eBioscience
CD45.2	104	1μl/test	PerCP-Cy5.5	eBioscience
CD45R (B220)	RA3-6B2	0.5μl/1x10^7cells 1μl/test	Biotin, PE	eBioscience
CD117 (c-KIT)	2B8	3μl/test	PE-Cy7, APC, BV421	eBioscience
CD150	TC15-12F12.2	3μl/test	PE	Biolegend
Sca-1	D7	2μl/test	PerCP-Cy5.5, BV510	eBioscience
Gr-1 Ly6G	RB6-8C5 1A8	0.5μl/1x10^7cells 1μl/test	Biotin, PE, AF647	eBioscience Biolegend
Ter119	TER-119	2μl/1x10^7cells 1μl/test	Biotin APC-eFlour780	eBioscience
Streptavidin		2μl/1x10^7cells	APC-eFluor780	eBioscience
P- STAT5	47	20μl/test	PE	BD
P-STAT3	4	20μl/test	AF647	BD
P-AKT	M89-61	5μl/test	BV421	BD
P-ERK1/2	20A	20μl/test	Alexa Fluor488	BD
Ki67	SolA15	1 μl/test	FITC	eBioscience
7-AAD		20μl/test		BD
BrdU	BU20A	1μl/test	PE	BD