JEM

SUPPLEMENTAL MATERIAL

Takizawa et al., http://www.jem.org/cgi/content/full/jem.20101643/DC1

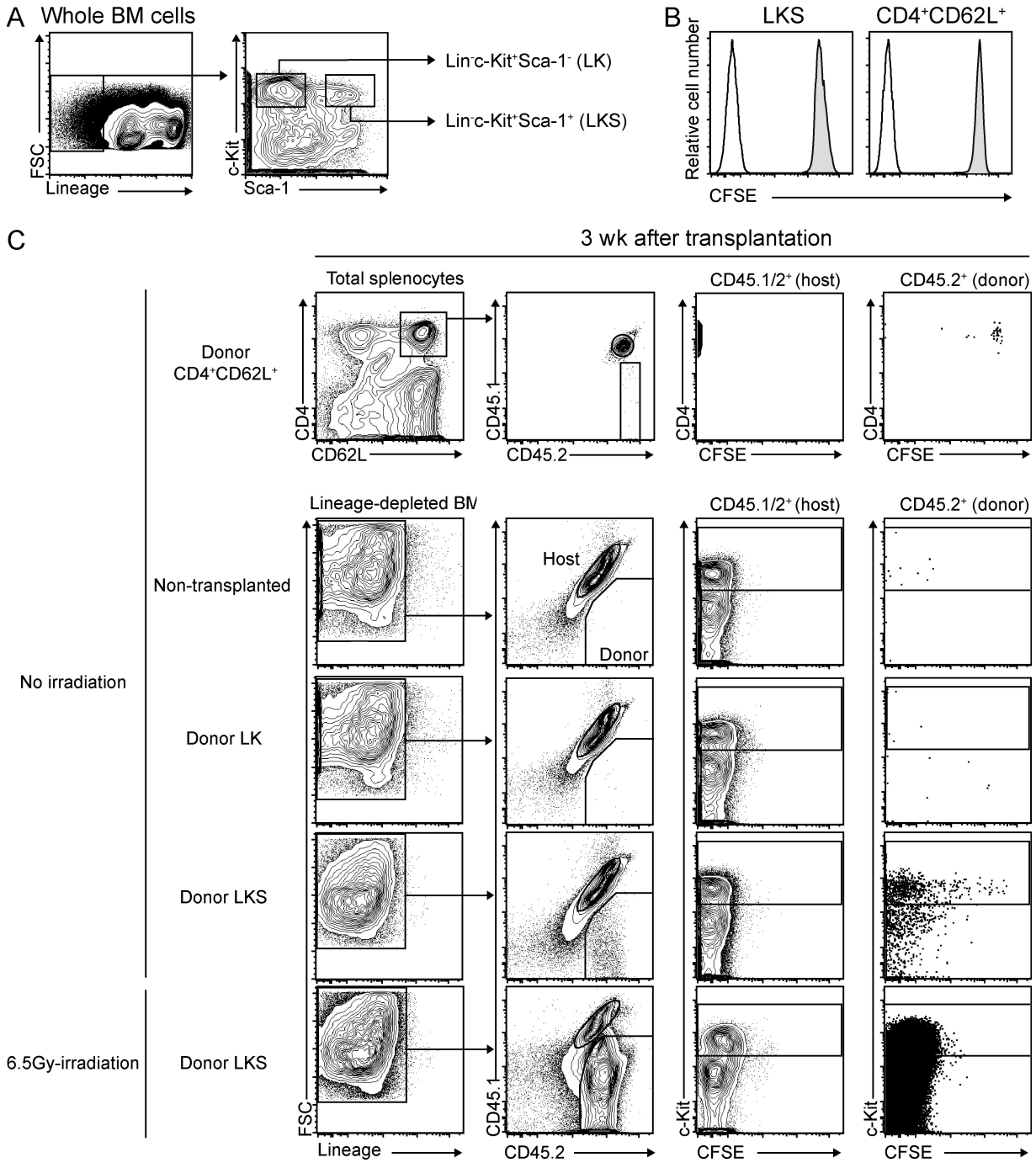

Figure S1. CFSE label retention in LKS cells but not LK cell populations upon transplantation into nonirradiated recipients. (A) Sorting gates for BM LKS and LK cells before transplantation. (B) Representative histogram of CFSE in LKS and $CD4^+CD62L^+$ cells with (closed) or without (open) ex vivo stain. (C) Representative BM dot plots 3 wk after cell transplantation in nonirradiated ($CD4^+CD62L^+$ cells, LKS cells, and LK cells) and 6.5 Gy sublethally irradiated (LKS cells) mice. 10^5 LKS, 10^6 LK, or 1–2 × 10^6 $CD4^+CD62L^+$ cells sorted from $CD45.2^+$ donor mice were labeled with CFSE and i.v. transplanted into $CD45.1/2^+$ hosts. At 3 wk after transplantation, BM or spleen cells were analyzed for division history analysis by FACS. Expression of CD4 or c-Kit against CFSE are shown gated on $CD45.1/2^+$ host or $CD45.2^+$ donor Lin^- cells. Four independent experiments were performed with one or two animals each.

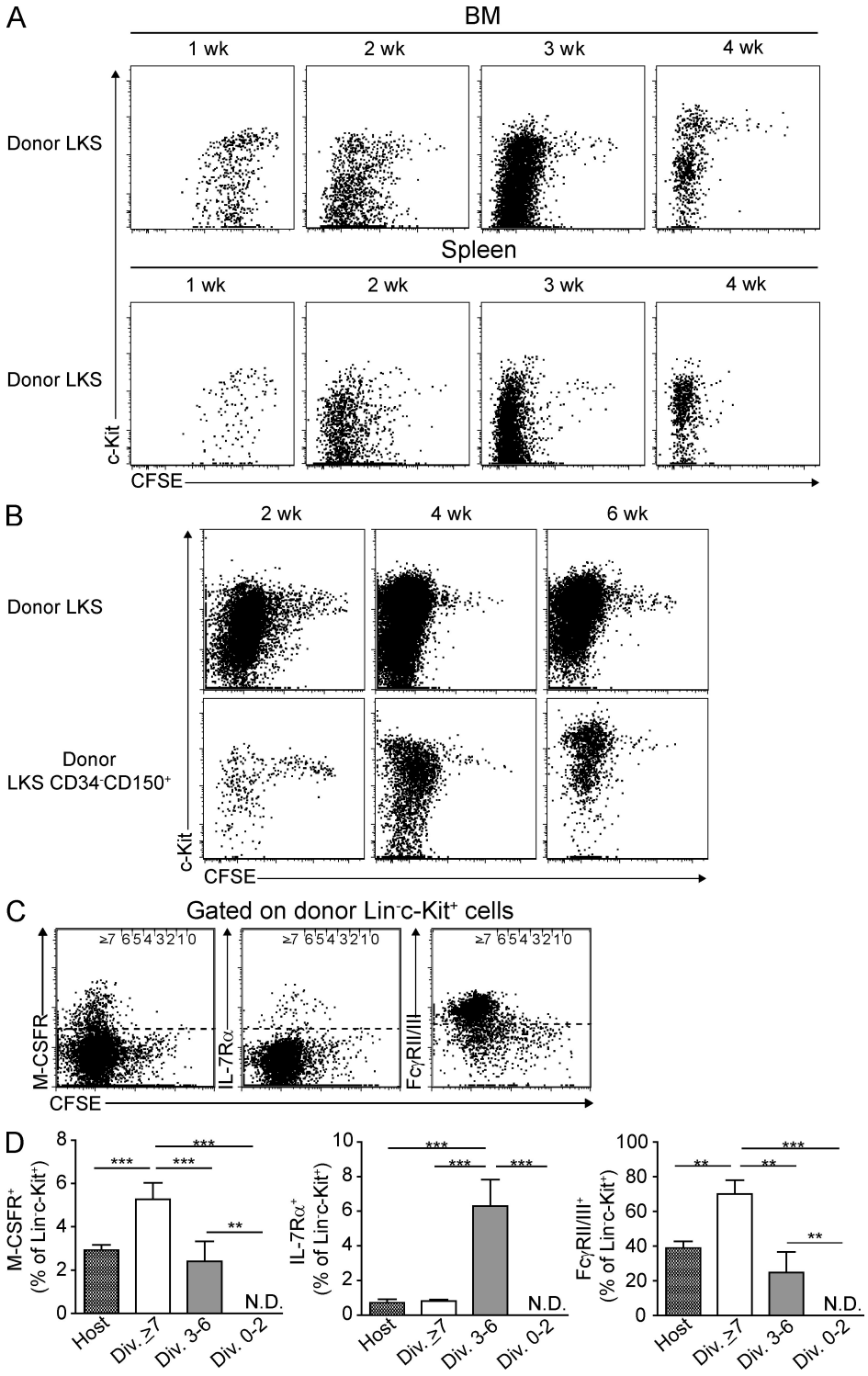

Figure S2. 0×–divided cells are maintained in BM, but not spleen, are contained in HSC-enriched LKS CD34$^-$CD150$^+$ cells, and are negative for early lineage differentiation markers. (A) Representative dot plots of BM and spleen from the same mice engrafted with 10^5 LKS cells at weeks 1–4 after transplantation. Two independent experiments were performed (n = 2 mice per each experiment). (B) Representative dot plots of BM from nonirradiated mice transplanted with CFSE-labeled 10^5 LKS or 6×10^3 LKS CD34$^-$CD150$^+$ cells at weeks 2, 4, and 6 after transplantation. Seven independent experiments were performed (n = 5 mice per each time point). Expression of c-Kit against CFSE is shown gated on donor Lin$^-$ cells in BM and spleen. (C) Representative dot plots gated on BM donor Lin$^-$c-Kit$^+$ cells depicting CFSE label versus M-CSFR, IL-7Rα, and FcγRII/III 2 wk after transplantation. Dashed lines represent the cutoff regarded as positive expression. (D) Percentage of Lin$^-$c-Kit$^+$ cells positive for indicated antigens within total host cells or 0–2×-, 3–6×-, and ≥7×-divided donor cells (shown in C). Mean ± SD is shown (n = 4–6 from three to six independent experiments for each stain). **, $P < 0.01$; ***, $P < 0.001$. ND, not detected.

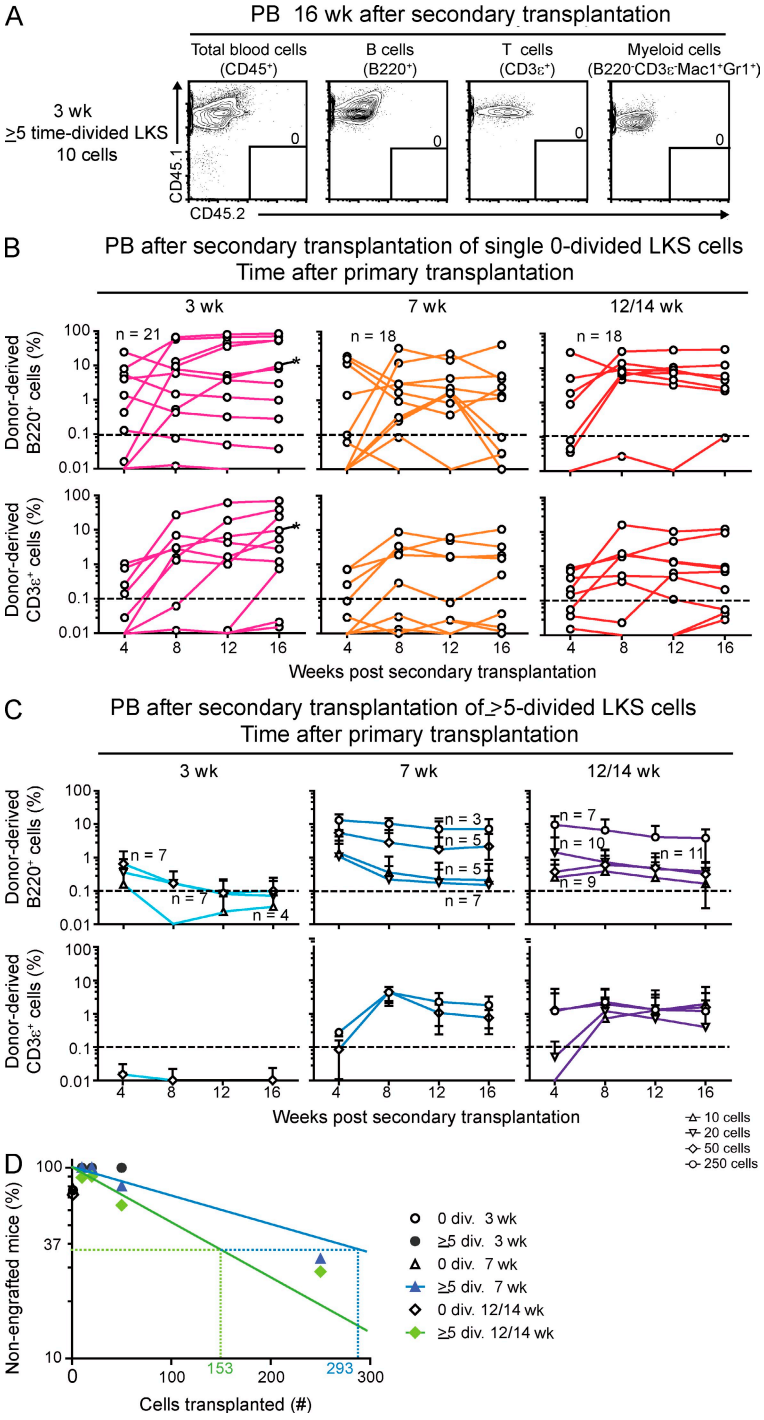

Figure S3. Time course PB analysis for donor contribution to lymphoid and myeloid lineages and HSC frequency based on limiting dilution transplantation. (A) Representative PB FACS dot plots 16 wk after secondary transplantation with 10 ≥5×-divided LKS cells sorted from primary recipients at 3 wk after LKS cell transfer. (B and C) Donor engraftment levels within B (B220$^+$) and T cells (CD3ε^+) was tested at 4, 8, 12, and 16 wk after secondary transplantation of single 0×-divided LKS cells (B) or 10–250 ≥5×-divided (open symbols and light blue, dark blue, and purple lines) LKS cells (C). (B) Each line represents data from an individual animal (n = 18–21 mice as indicated from four to five independent experiments). Asterisk indicates engraftment data of the animal shown in Fig. 3 C. (C) Each line represents data pooled from four independent experiments with error bars representing SD (numbers of mice are indicated at each line). The dashed line at 0.1% marks the cutoff determined for nonengraftment. (D) HSC frequency in 0×- and ≥5×-divided donor LKS cells determined by engraftment after limiting dilution secondary transplantation. Percentage of nonengrafted mice at 4 mo is plotted against number of transplanted LKS cells. Blue and green lines indicate the ≥5 division class at 7 or 12/14 wk after primary transplantation, respectively. Data were pooled from four to five independent experiments (n = 3–21 mice per each data point).

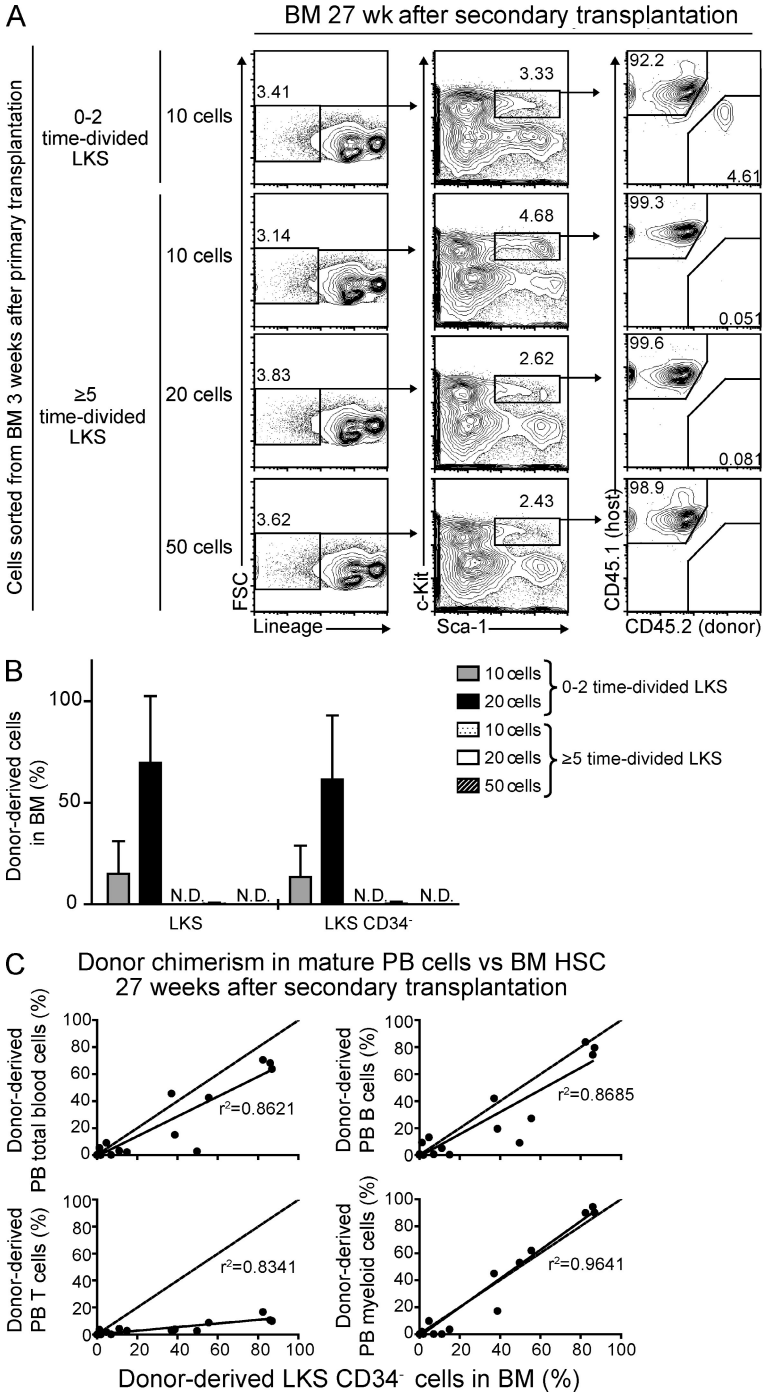

Figure S4. Linear correlation of donor-derived BM LKS CD34$^-$ cell engraftment with donor PB engraftment in secondary recipients. (A) Representative donor LKS cell chimerism analysis in BM of lethally irradiated animals transplanted with 2×10^5 autologous BM cells and indicated numbers of 0–2×- and ≥5×-divided LKS cells derived from primary recipients 3 wk after primary transfer (see scheme in Fig. 3 A). Percentage of donor chimerism (CD45.2$^+$) in LKS cells 27 wk after secondary transplantation is shown. (B) Percentage of donor-derived LKS or LKS CD34$^-$ cells in BM 27 wk after secondary transplantation is summarized from transplants with the indicated number of 0–2×- or ≥5×-divided cells at 3 wk after primary transfer (n = 4–6 mice per group from three independent experiments). Error bars show SD. ND, not detected. (C) Linear correlation between donor chimerism in BM LKS CD34$^-$ cells and in PB mature cells. Percentage of donor-derived total cells, B cells (B220$^+$), T cells (CD3ε^+), and myeloid cells (B220$^-$CD3ε^-CD11b$^+$Gr-1$^+$) in PB are plotted against that of donor-derived LKS CD34$^-$ cells in BM from the same experiments as shown in A and B. Solid line shows linear regression with confidence interval. The dashed line represents theoretical value if donor chimerism in PB corresponds to donor HSC chimerism in BM.

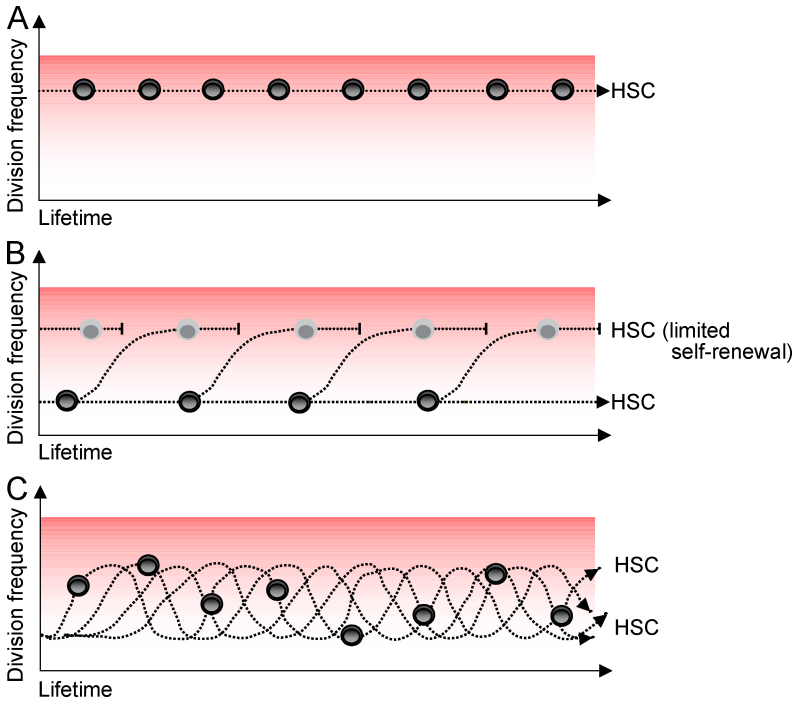

Figure S5. Hypothetical models for steady-state hematopoiesis. (A) Clonal maintenance model: all HSCs continuously divide and equally contribute to hematopoiesis. (B) Clonal succession model: quiescent HSCs start to divide and give rise to mature blood cells until they die or differentiate and, subsequently, other HSCs follow the same fate. (C) Dynamic repetition model: some HSCs or progeny that divide more frequently, dominate blood formation for a certain period of time, subsequently enter a resting slow-dividing phase in which other fractions take over, and get reactivated again and contribute to blood formation in repetitive cycles. Red indicates contribution to blood formation.

Table S1. Estimates of the parameters of the three-subpopulation model

Population and parameter	Estimate (95% CI per week)
First subpopulation	
f_1	0.70 (0.57−0.88)
λ_1	4.8e-9 (3.4×10^{-11} − 0.00023)
d_1	1.8 (0.66 − 6.3)
Second subpopulation	
f_2	0.22 (0.081 − 0.34)
λ_2	0.57 (0.48 − 0.72)
d_2	0.13 (0.036 − 0.19)
Third subpopulation	
$f_3 = 1 - f_1 - f_2$	0.077 (0.038 − 0.13)
λ_3	0.072 (0.059 − 0.10)
d_3	0.040 (1.3×10^{-9} − 0.086)

Table S2. Estimate of biologically functional HSC obtained by combining the LKS cell data with the repopulation assay data

Weeks	0×-divided HSC	≥5×-divided HSC
3	247.33	0.00
3	69.76	0.00
3	139.52	0.00
3	69.76	0.00
3	171.23	0.00
7	7.50	34.48
7	29.99	15.17
7	44.98	42.87
7	29.99	44.94
12	28.97	ND
12	19.31	153.11
12	77.26	450.21
14	32.62	64.74
14	18.64	103.40
14	9.32	23.58
14	37.28	98.53