Evidence that hematopoietic stem cell function is preserved during aging in long-lived S6K1 mutant mice

Supplementary Material

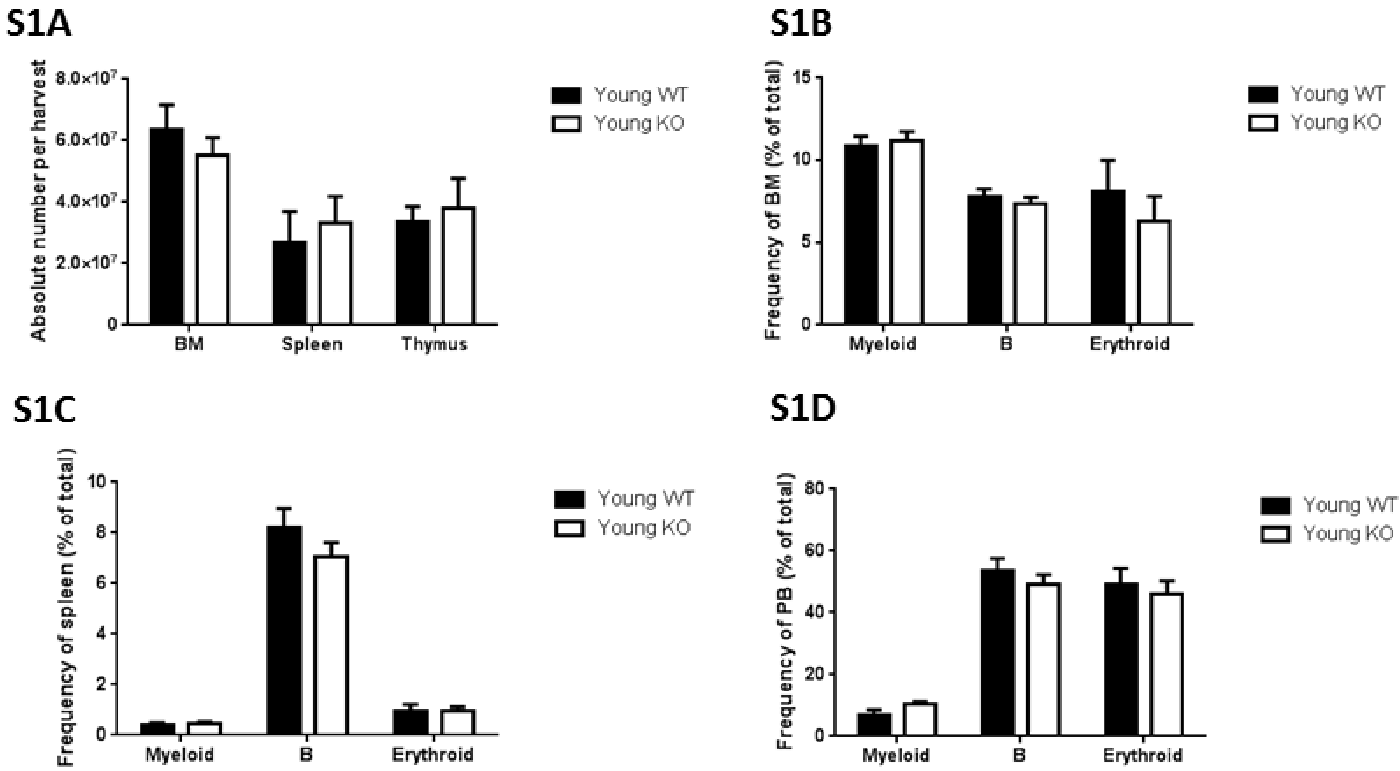

Figure S1A-D: Young mice. S1A. Cellularity per harvest in the bone marrow (BM), spleen and thymii of WT and *S6K1*$^{-/-}$ mice (n = 9-11, mixed gender). S1B. Frequency of myeloid (Gr-1+, CD11-b+), B (CD19+) and Erythroid (Ter-119+) cells in BM of (n = 9-11, mixed gender). S1C. Frequency of myeloid (Gr-1+, CD11-b+), B (CD19+) and Erythroid (Ter-119+) cells in spleen of (n = 9-11, mixed gender). S1D. Frequency of myeloid (Gr-1+, CD11-b+), B (CD19+) and Erythroid (Ter-119+) cells in PB of (n = 9-11, mixed gender).

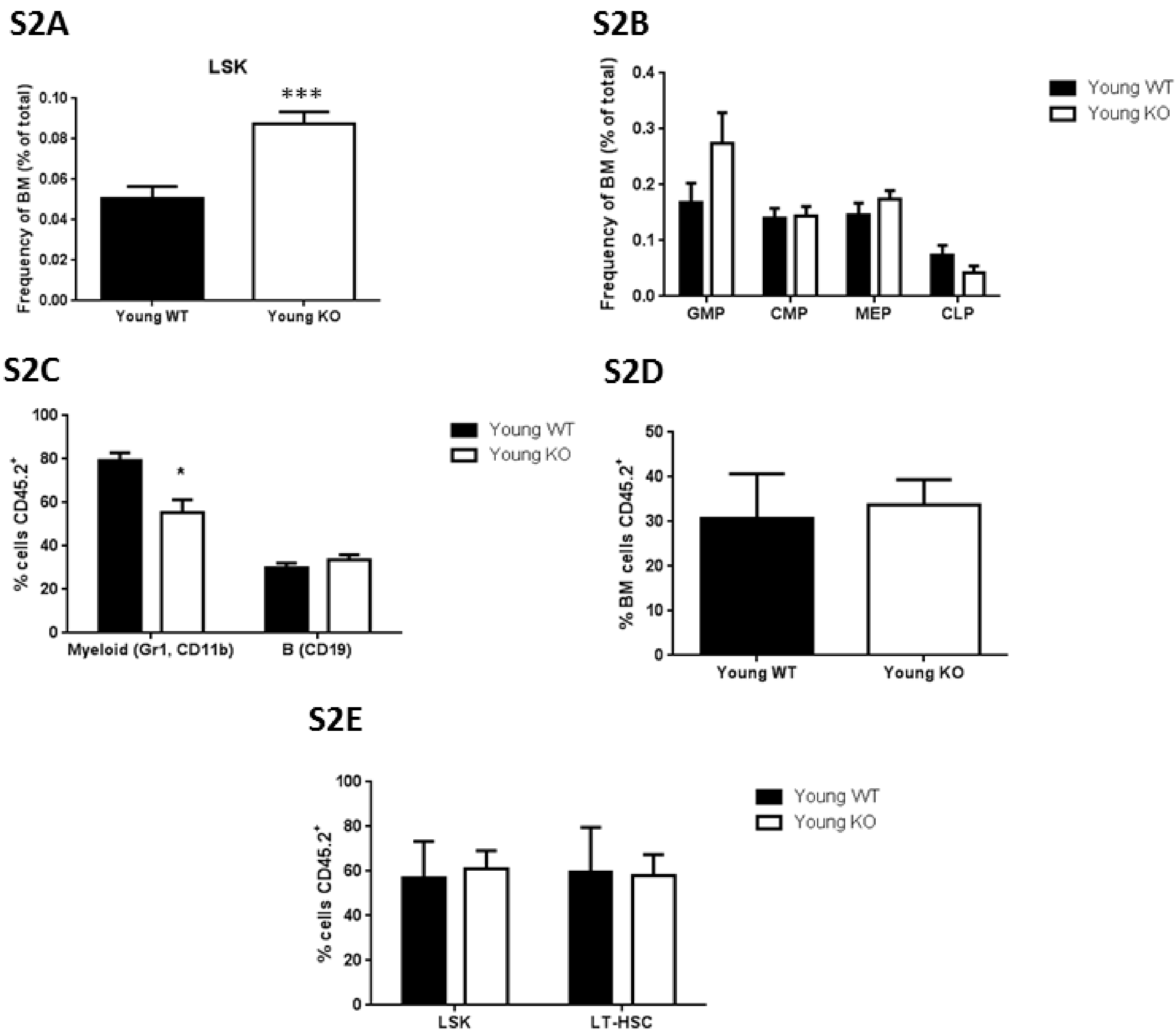

Figure S2A-E: Young mice. S2A. Frequency of cells positive for lineage-, c-Kit+, Sca-1+ cells (LSK) in the BM of WT and *S6K1*$^{-/-}$ mice (n = 9-11, mixed gender) *** *P*<0.001. S2B. Frequency of cells positive for progenitor cells (GMP (CD34^{+}, CD16/32^{+}), CMP (CD34^{+}, CD16/32^{-}), MEP (CD34^{-}, CD16/32^{-}) and CLP (CD 127^{+}) in the BM of WT and *S6K1*$^{-/-}$ mice (n = 9-11, mixed gender). S2C. Percentage of myeloid or B lymphoid cells donor derived (CD45.2^{+}) in the BM of transplanted mice (n=5-6 per group). S2D. Percentage of CD45.2^{+} in the BM of recipient mice transplanted with young WT or *S6K-1*$^{-/-}$ cells (donor cells female, recipients mixed gender, n=5-6 per group). S2E. Percentage of LSK and LT-HSC donor derived (CD45.2+) in BM of 16 week post-transplant recipient mice (n=5-6 per group).

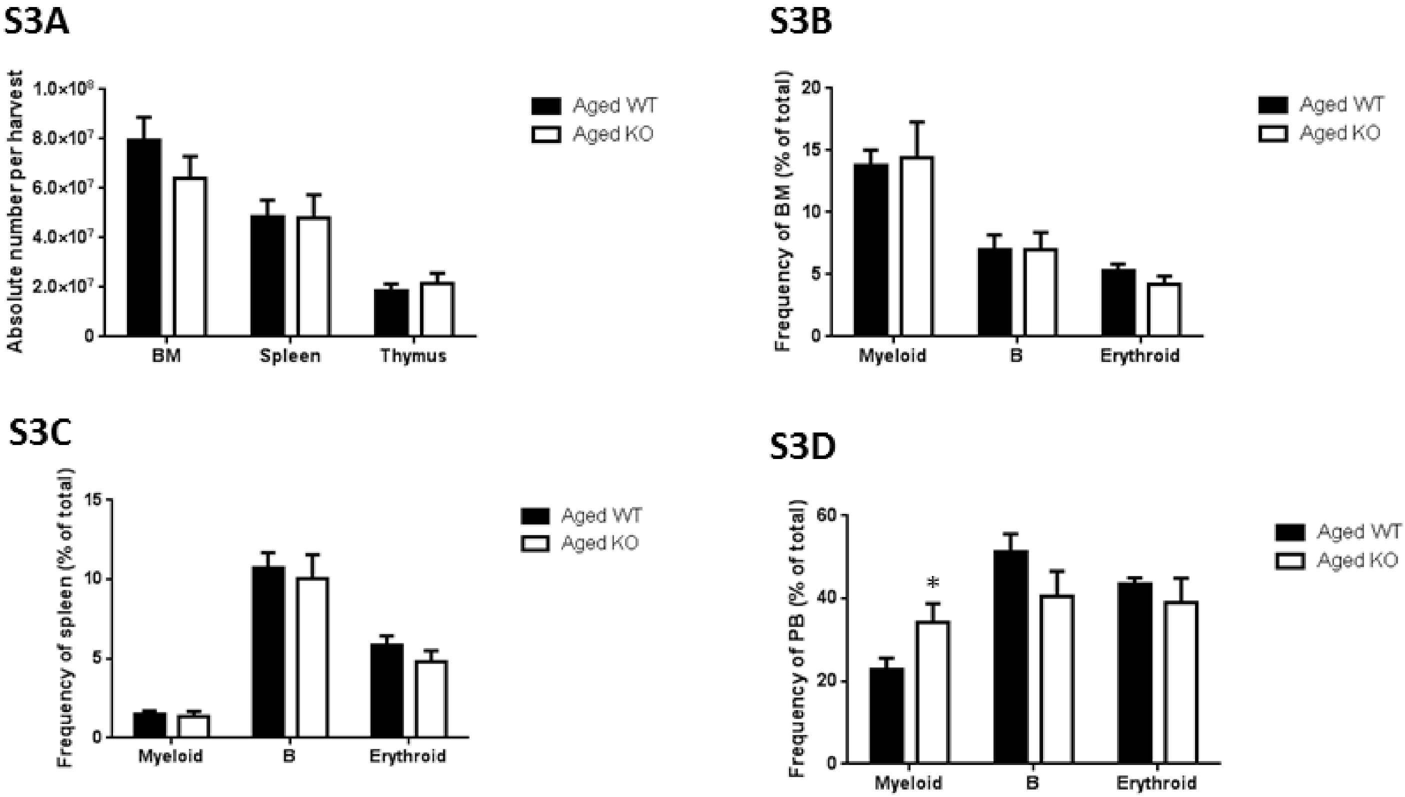

Figure S3A-D: Aged mice. S3A. Cellularity per harvest in the BM, spleen and thymii of aged WT and *S6K1*$^{-/-}$ mice (n = 11-18, mixed gender). S3B. Frequency of myeloid (Gr-1+, CD11-b+), B (CD19+) and Erythroid (Ter-119+) cells in BM of aged WT and *S6K-1*$^{-/-}$ mice (n = 11-15, mixed gender). S3C. Frequency of myeloid (Gr-1+, CD11-b+), B (CD19+) and Erythroid (Ter-119+) cells in spleen of aged WT and *S6K-1*$^{-/-}$ mice (n = 11-15, mixed gender). S3D. Frequency of myeloid (Gr-1+, CD11-b+), B (CD19+) and Erythroid (Ter-119+) cells in the PB of aged WT and *S6K-1*$^{-/-}$ mice (n = 5-11, mixed gender * P<0.05).

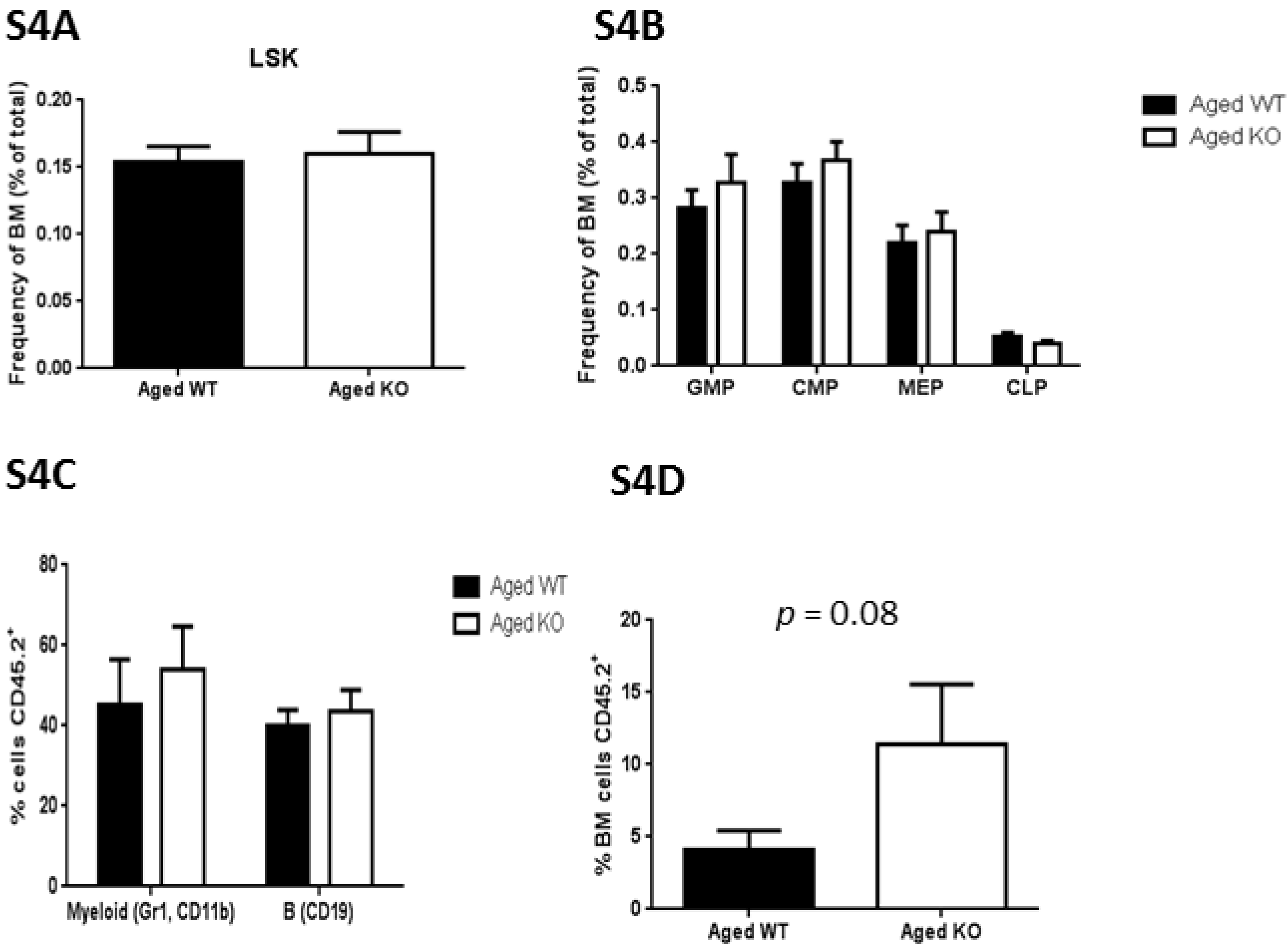

Figure S4A-D: Aged mice. S4A. Frequency of cells positive for lineage-, c-Kit+, Sca-1+ cells (LSK) in the BM of aged WT and *S6K1*$^{-/-}$ mice (n = 7-15, mixed gender). S4B. Frequency of cells positive for progenitor cells (GMP, CMP, MEP and CLP) in the BM of WT and *S6K1*$^{-/-}$ aged mice (n =7-14, mixed gender). S4C. Percentage of myeloid or B lymphoid cells donor derived (CD45.2^{+}) in the BM of transplanted mice (n=6 per group).S4D. Percentage of CD45.2^{+} in the BM of recipient mice transplanted with aged WT or *S6K-1*$^{-/-}$ cells (donor cells female, recipients mixed gender, n=6 per group).

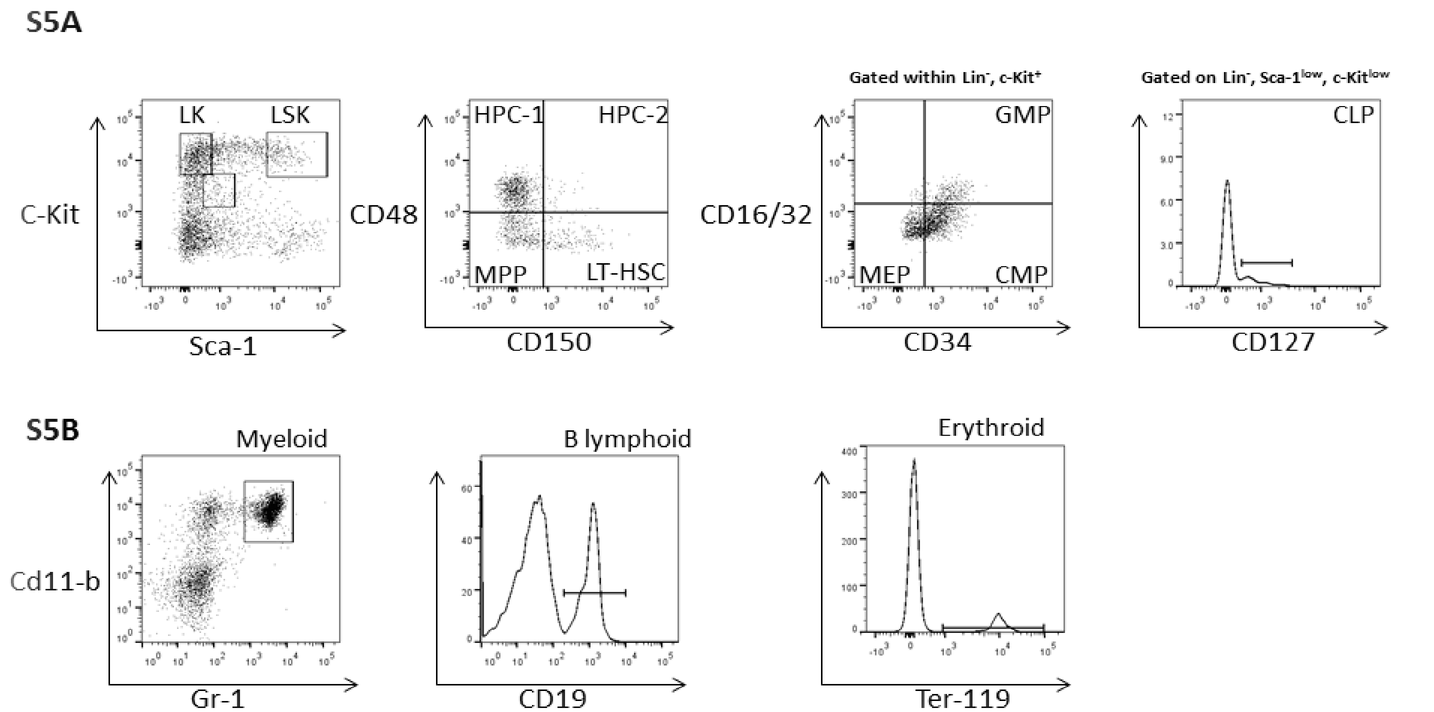

Figure S5A and B. S5A shows a representative BM plot displaying cell distribution for Sca-1 and c-Kit within lineage negative cells. Within the LSK population the middle dot plot shows representative staining for CD150 and CD48 to identify HSC sub populations. The right dot plot displays CD34 and CD16/32 staining within the LK population to identify GMP, MEP and CMP populations. Within lineage negative, c-Kit^{low} and Sca-1^{low} cells, cells were identified as CLP based on CD127^{+} expression. S5B shows a representative BM plot displaying cell distribution for Gr-1 and Cd11-b to identify myeloid cells. The middle and right histograms respectively display representative plots displaying CD19^{+} cells and Ter-119^{+} cells displaying gating strategy for B lymphoid and erythroid cells respectively.

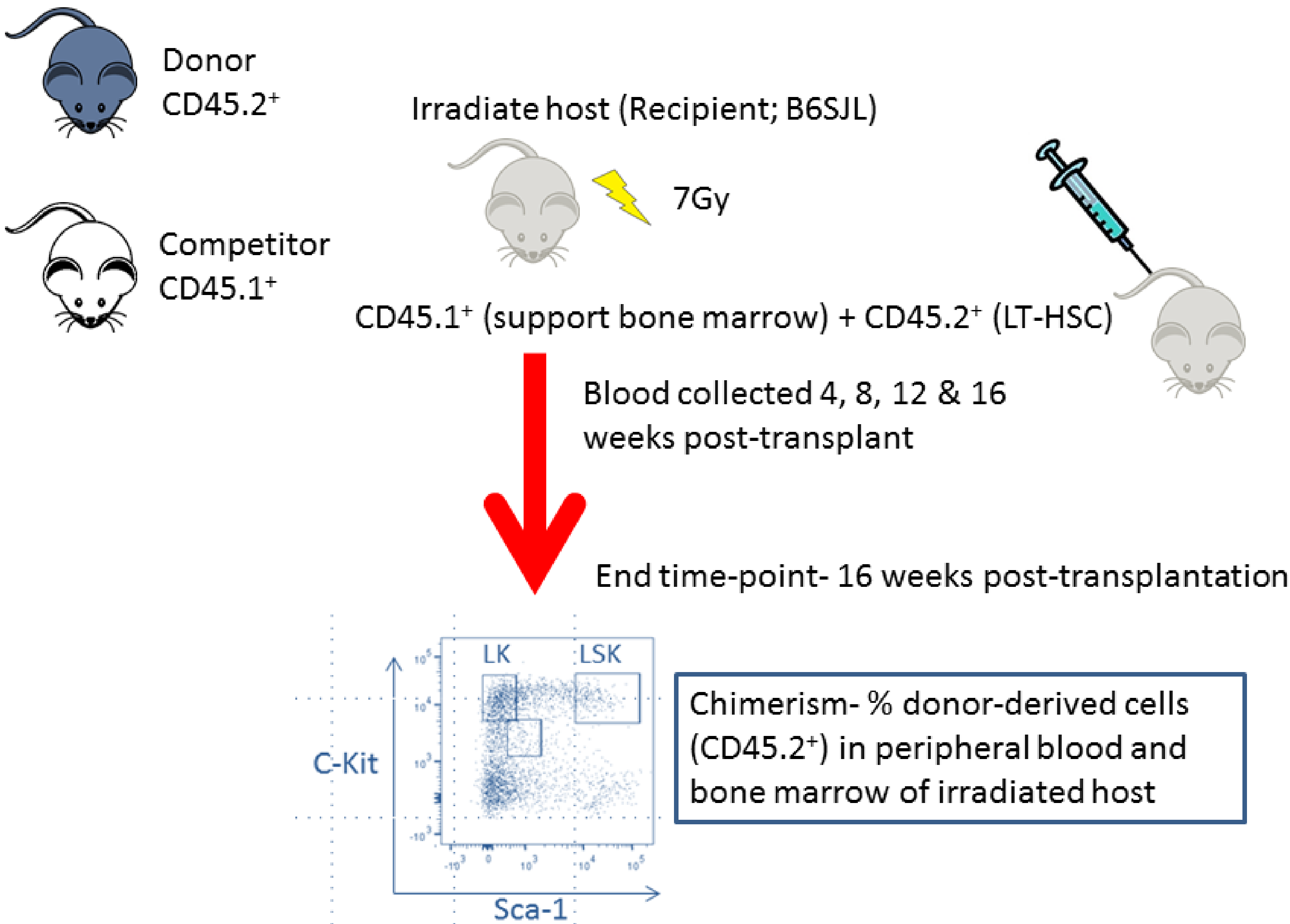

Figure S6. Schematic representation of the transplantation experimental protocol employed for both young and aged mice.

Table S1. mRNA levels of candidate genes linked to HSC function in c-Kit^{+} cells. All data analysed using a general linear model, where all non-significant (NS) interaction effects (p>0.05) were subsequently removed from each analysis in order to obtain the best-fitted model in each case. Genotype effects (WT vs *S6K1*$^{-/-}$) and age effects (young 12 wk vs. old 80 weeks). All female mice, where n=3-5.

Gene symbol	**Genotype effect**	**Age effect**	**Interaction effect**
Cdkn2a	0.502	**0.046**	NS
Bmi1	0.192	0.335	NS
Cdkn1a	0.673	0.091	NS
Ccdnd1	**0.011**	0.216	NS
Ccnd2	**0.033**	0.050	NS
Cdkn1b	0.355	**0.009**	NS
Cknnb1	0.761	0.153	NS
Rb1	**0.045**	0.698	NS
Eprs1	0.272	0.405	NS
Dnajb9	0.610	0.303	NS
Ddit4	0.725	0.725	NS
Ezh2	0.981	**0.003**	NS
Ddit3	0.605	0.137	NS
Cxcl1	0.244	0.098	NS
Fox01	0.203	0.660	NS
Atf4	**0.045**	0.572	NS
Atg7	0.298	**0.004**	NS
Bcl2	0.930	**0.004**	NS
Ccdn3	0.070	0.206	NS
Cxcl5	0.159	0.471	NS
Dppa5a	0.248	0.943	NS
Fox03a	0.625	0.447	NS
Gata2	0.174	0.547	NS
Gfi1	0.653	0.408	NS
Gsk3b	0.183	0.433	NS
Irs1	**0.020**	**0.005**	**0.040**
Irs2	0.254	0.464	NS
Mc11	0.750	0.328	NS
Mi67	0.718	0.691	NS
Nfe212	0.234	0.915	NS
Nrf1	0.367	0.873	NS
Pck3r1	0.238	0.880	NS
Pik3ca	0.407	0.094	NS
Psmd11	0.420	0.110	NS
Pten	0.157	0.403	NS
Rps6kb2	0.187	**0.013**	**0.010**
Sod1	0.754	0.750	NS
Sod2	0.576	0.922	NS

Tgfb1	0.224	0.300	NS
Trp53	0.184	0.276	NS
Ulk1	0.586	0.118	NS
Xbp1	0.278	**0.001**	NS
Sk2b3	0.655	0.360	NS
18S (control)	0.462	0.133	NS

Table S2. List of probes used for qPCR analysis. Table displays gene name and assay ID of each Taqman™ probe used.

Gene name	Assay ID
18S	Mm03928990_g1
Ccnd1	Mm00432359_m1
Ccnd2	Mm00438070_m1
Ccnd3	Mm01612362_m1
Cdkn2a	Mm00494449_m1
Cdkn1a	Mm00432448_m1
Cdkn1b	Mm00438168_m1
Mki67	Mm01278617_m1
Rb1	Mm00485586_m1
Ezh2	Mm00468464_m1
Bmi1	Mm03053308_g1
Gata-2	Mm00492301_m1
Gfi1	Mm00515853_m1
Fox01	Mm00490671_m1
Fox03	Mm01185722_m1
Atf4	Mm00515324_m1
Xbp1	Mm00457357_m1
Ddit3	Mm00492097_m1
Bcl2	Mm00477631_m1
Mcl1	Mm01257351_g1
Irs1	Mm01278327_m1
Irs2	Mm03038438_m1
Pik3ca	Mm00435673_m1
Pik3r1	Mm01282781_m1
Sod1	Mm01344233_g1
Sod2	Mm01313000_m1
Tgfb	Mm01178820_m1
Pten	Mm00477208_m1

S6K1	Mm01310033_m1
S6K2	Mm00445440_m1
Eprs	Mm01315474_m1
Trp53	Mm01731290_g1
Sh2b3	Mm00833471_m1
Cxcl1	Mm04207460_m1
Cxcl5	Mm00436451_g1
Ulk1	Mm00437238_m1
Nrf1	Mm01135606_m1
Nfe2l2	Mm00477784_m1
Ddit4	Mm00512504_g1
Dnajb9	Mm01622956_s1
Dppa5	Mm01171664_g1
Psmd11	Mm00780758_sH
Ctnnb1	Mm00483039_m1
Gsk3b	Mm00444911_m1
Atg7	Mm00512209_m1
Gene name	Assay ID
18S	Mm03928990_g1
Ccnd1	Mm00432359_m1
Ccnd2	Mm00438070_m1
Ccnd3	Mm01612362_m1
Cdkn2a	Mm00494449_m1
Cdkn1a	Mm00432448_m1
Cdkn1b	Mm00438168_m1
Mki67	Mm01278617_m1
Rb1	Mm00485586_m1
Ezh2	Mm00468464_m1
Bmi1	Mm03053308_g1
Gata-2	Mm00492301_m1
Gfi1	Mm00515853_m1
Fox01	Mm00490671_m1
Fox03	Mm01185722_m1
Atf4	Mm00515324_m1
Xbp1	Mm00457357_m1
Ddit3	Mm00492097_m1
Bcl2	Mm00477631_m1
Mcl1	Mm01257351_g1
Irs1	Mm01278327_m1
Irs2	Mm03038438_m1
Pik3ca	Mm00435673_m1
Pik3r1	Mm01282781_m1
Sod1	Mm01344233_g1
Sod2	Mm01313000_m1

Tgfb	Mm01178820_m1
Pten	Mm00477208_m1
S6K1	Mm01310033_m1
S6K2	Mm00445440_m1
Eprs	Mm01315474_m1
Trp53	Mm01731290_g1
Sh2b3	Mm00833471_m1
Cxcl1	Mm04207460_m1
Cxcl5	Mm00436451_g1
Ulk1	Mm00437238_m1
Nrf1	Mm01135606_m1
Nfe2l2	Mm00477784_m1
Ddit4	Mm00512504_g1
Dnajb9	Mm01622956_s1
Dppa5	Mm01171664_g1
Psmd11	Mm00780758_sH
Ctnnb1	Mm00483039_m1
Gsk3b	Mm00444911_m1
Atg7	Mm00512209_m1