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Supplemental Information

Elevated Oxidative Stress Impairs Hematopoietic Progenitor Function in C57BL/6 Substrains

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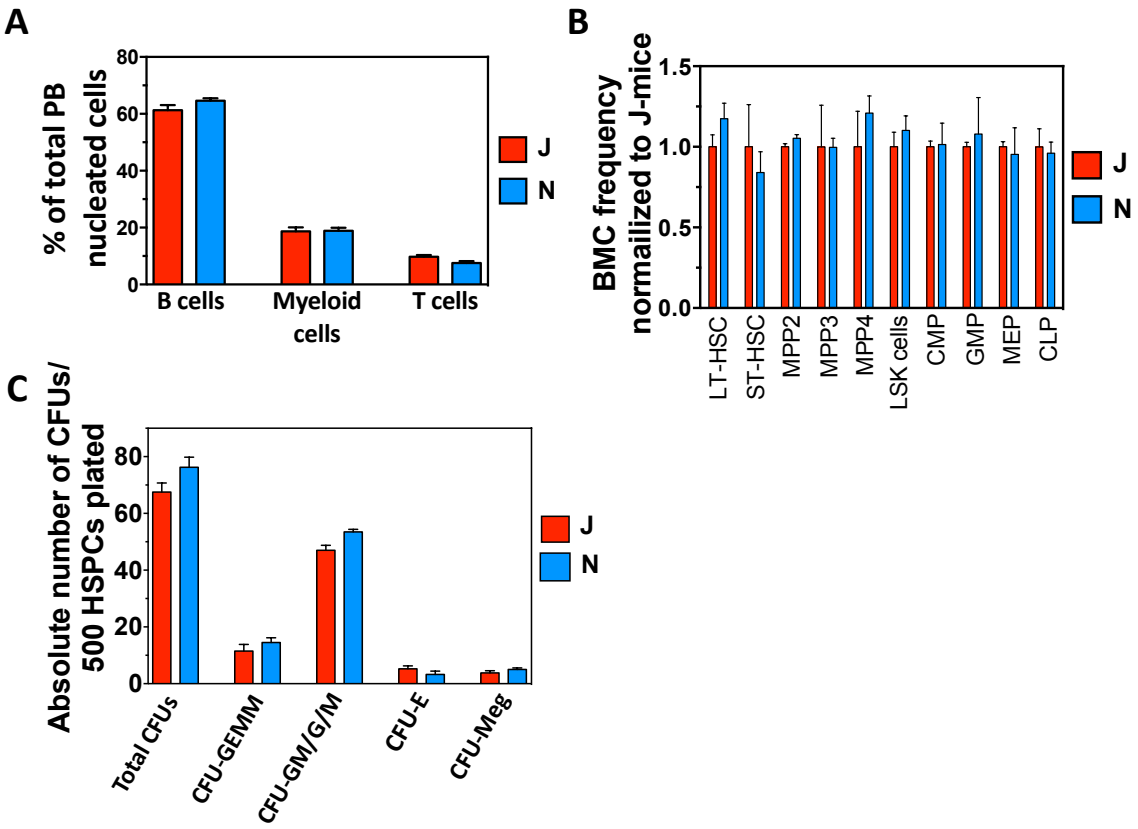

Figure S1. N and J-mice display a similar hematopoietic system under homeostatic conditions.
A. Percentage of myeloid (GR1+CD11B+) and lymphoid (B cells as B220+ and T cells as CD4+CD8+) lineage cells in the PB of N and J-mice. **B.** Absolute frequency of BMC (HSC-LT; HSC-ST; MPP; LSK cells; CMP; GMP; MEP; CLP) of N and J-mice. Data presented are normalized to the absolute levels in J-mice. **C.** LSK cells from N and J-mice were assayed for CFU potential. Data represented as mean ± SEM. ≥ 4 mice were examined in A-C.

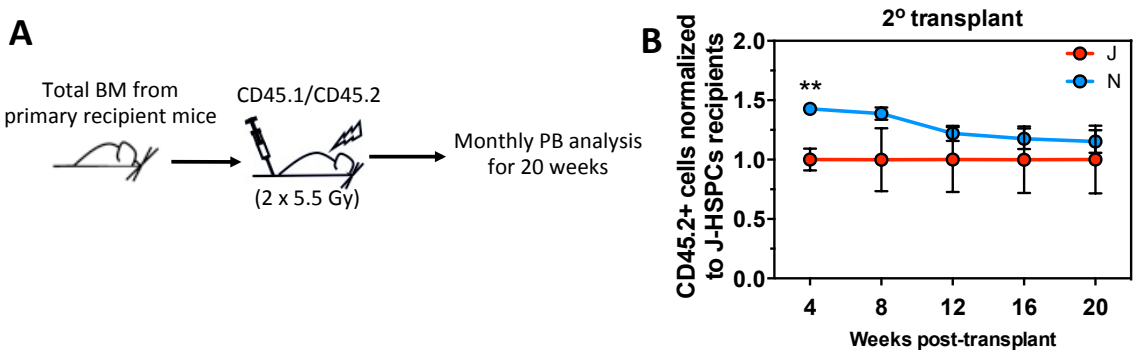

Figure S2. J-BM cells display short-term repopulating defect in secondary transplants. Related to Figure 1. A. 10^6 BM cells from primary transplant recipients were injected into lethally irradiated recipient mice (CD45.1/CD45.2). The PB of secondary recipients was examined every four weeks for 20 weeks post-transplant for CD45.2+ cells. **B.** Normalized CD45.2+ cells percentage in recipient mice PB after secondary transplant. Recipient mice CD45.2+ cells percentage was corrected to the original injected CD45.2+ cells percentage. Data presented are normalized to the CD45.2+ cells percentage observed in recipients of J-LSK cells. Data represented as mean ± SEM. Donor mice $N \geq 5$ per transplant. Recipient mice $N \geq 5$ per transplant. ** $p < 0.005$ significantly different to recipients of J-BM cells.

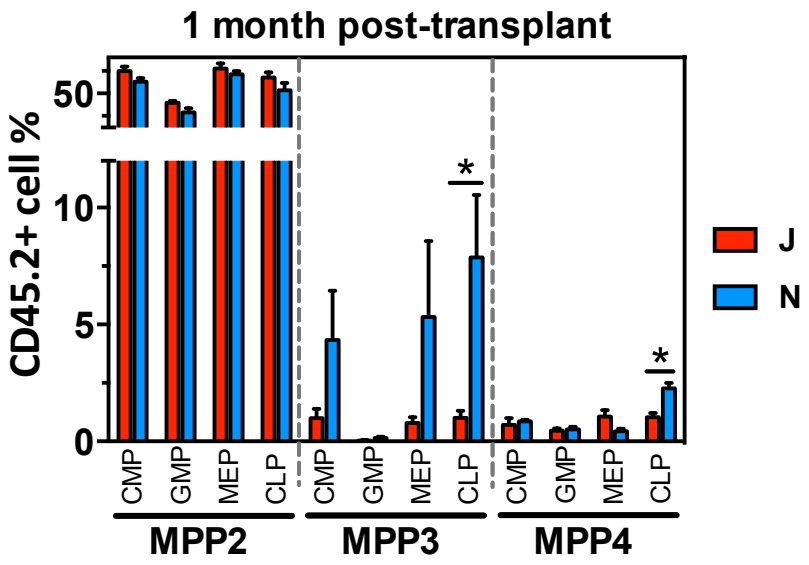

Figure S3. J-MMP3 and MPP4 cells display a CLP repopulating defect compared with N-MPP3 and MPP4 cells. Related to Figure 3. CD45.2+ MPP2, MPP3 or MPP4 cells from J or N mice were transplanted into sub-lethally irradiated recipients (CD45.1/CD45.2). Normalized CD45.2+ cells percentage of each BMC one month post-transplant. Data presented are normalized to the CD45.2+ cells percentage in J-mice. Data from two independent transplants represented as mean ± SEM. Donor mice $N \geq 3$ pooled per transplant. Recipient mice $N \geq 5$ per transplant. * $p < 0.05$ significantly different to recipients of J-HSPCs.

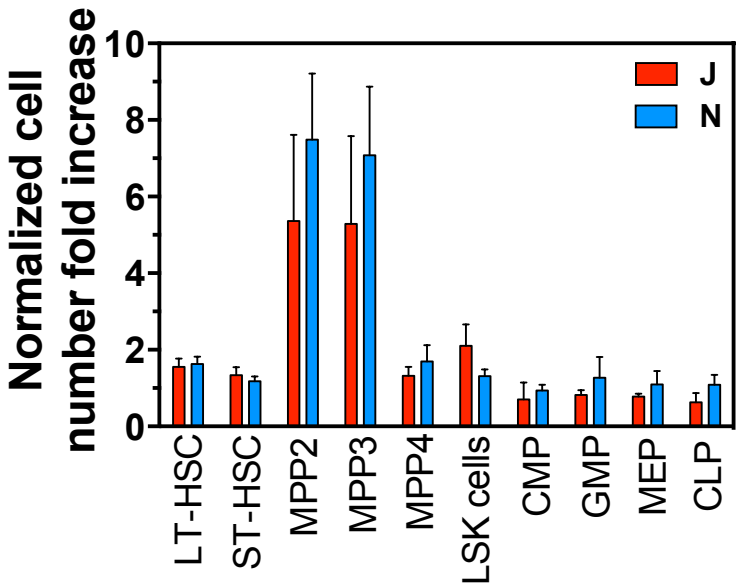

Figure S4. Similarly elevated BMC numbers in pI:pC treated J and N-mice. Related to Figure 6. 24 hours post-pI:pC injection, the absolute frequency of BMC (LT-HSC; ST-HSC; MPP; LSK cells; CMP; GMP; MEP; CLP) of N and J-mice was analyzed. The increase between control and pI:pC injected animals is shown. Data are presented as mean ±SEM. N = 3.

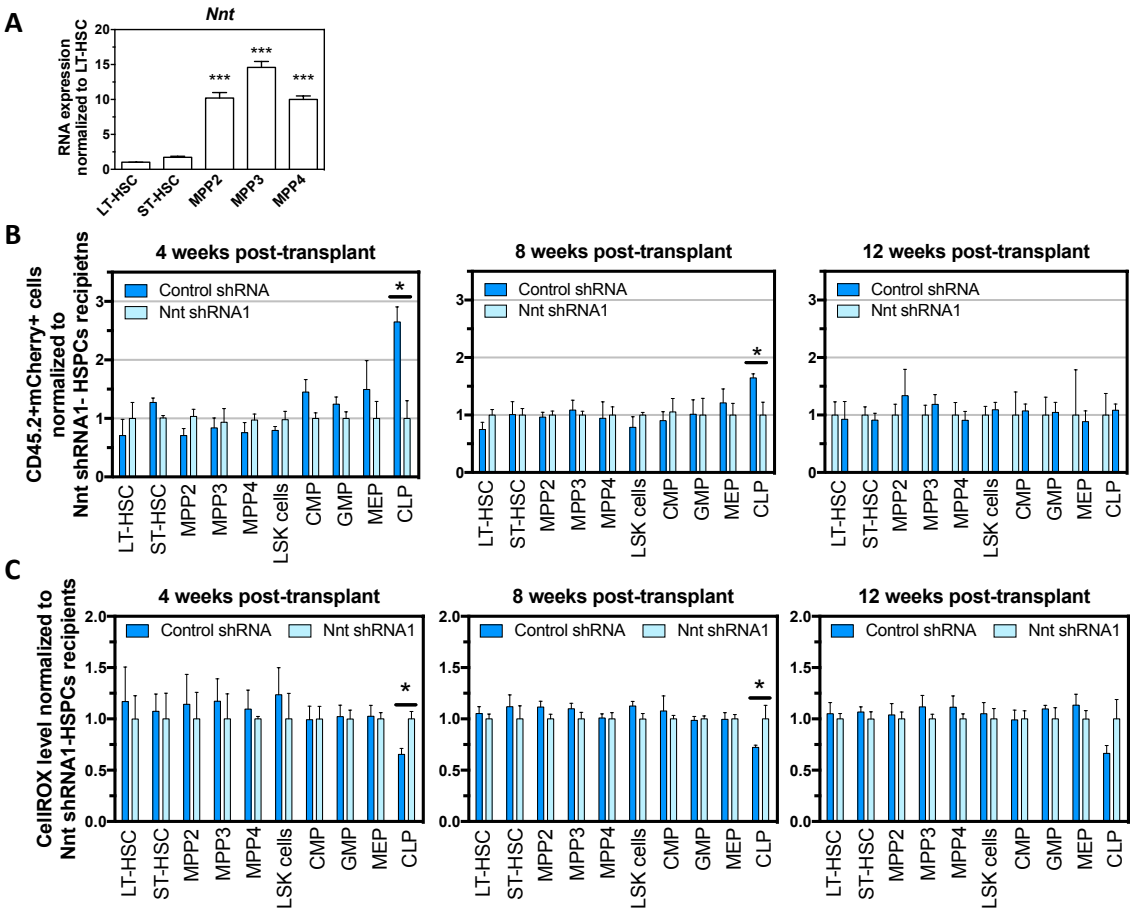

Figure S5. N-HSPCs express *Nnt* at different levels and *Nnt* knockdown in N-HSPCs recapitulates J-HSPC short-term repopulating defect. Related to Figure 7. A. LT-HSC, ST-HSC, MPP2, MPP3 and MPP4 were isolated from N-mice and *Nnt* expression was quantified by RT-qPCR. Data are presented as mean ±SEM. N = 2. *** $p < 0.001$ significantly different to LT-HSC. LSK cells isolated from N or J-mice (CD45.2+) were transduced with lentivirus containing control or *Nnt*-shRNAs. Transduced cells (i.e. mCherry+) were then isolated and transplanted with an equal number of competitor LSK cells (CD45.1+) into lethally irradiated recipients (CD45.2+/CD45.1+). The frequency (**B**) and ROS levels (**C**) of CD45.2+mCherry+ cells in the bone marrow of recipients was then analyzed by flow cytometry every four weeks for 12 weeks post-transplant. Data represented as mean ±SEM. Donor mice N = 3 per transplant. Recipient mice N = 5 per transplant. * $p < 0.05$ significantly different to recipients of N-LSK cells transduced with *Nnt*-shRNA.

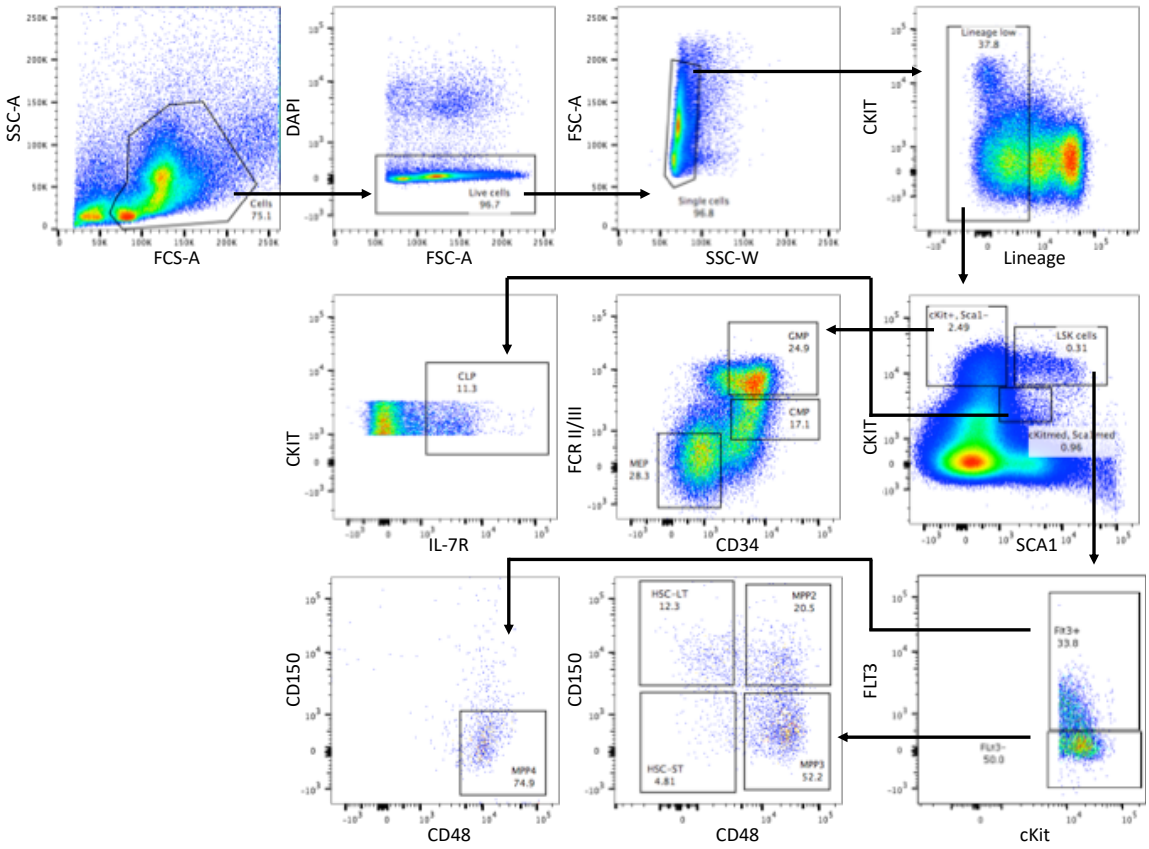

Figure S6. BMC gating strategy. Related to Figure 1-7, S1, S3, S4 and S5. Identification of each BMC according to their specific surface markers expression. LT-HSC (Lin$^-$SCA1$^+$CKIT$^+$FLT3$^-$CD150$^+$CD48$^-$); ST-HSC (Lin$^-$SCA1$^+$CKIT$^+$FLT3$^-$CD150$^-$CD48$^-$); MPP2 (Lin$^-$,Sca-1$^+$c-Kit$^+$Flt3$^-$CD150$^+$CD48$^+$); MPP3 (Lin$^-$SCA1$^+$CKIT $^+$FLT3$^-$CD150$^-$CD48$^+$); MPP4 (Lin$^-$SCA1$^+$CKIT $^+$FLT3$^-$CD150$^-$CD48$^+$); CMP (Lin$^-$SCA1$^-$ CKIT $^+$CD34$^+$FCR II/III^{med}); GMP (Lin$^-$SCA1$^-$CKIT$^+$CD34$^+$FCR II/III^{high}); MEP (Lin$^-$SCA1$^-$CKIT $^+$CD34$^-$FCR II/III$^-$); CLP (Lin$^-$SCA1^{med}CKIT^{med} IL-7R$^+$).