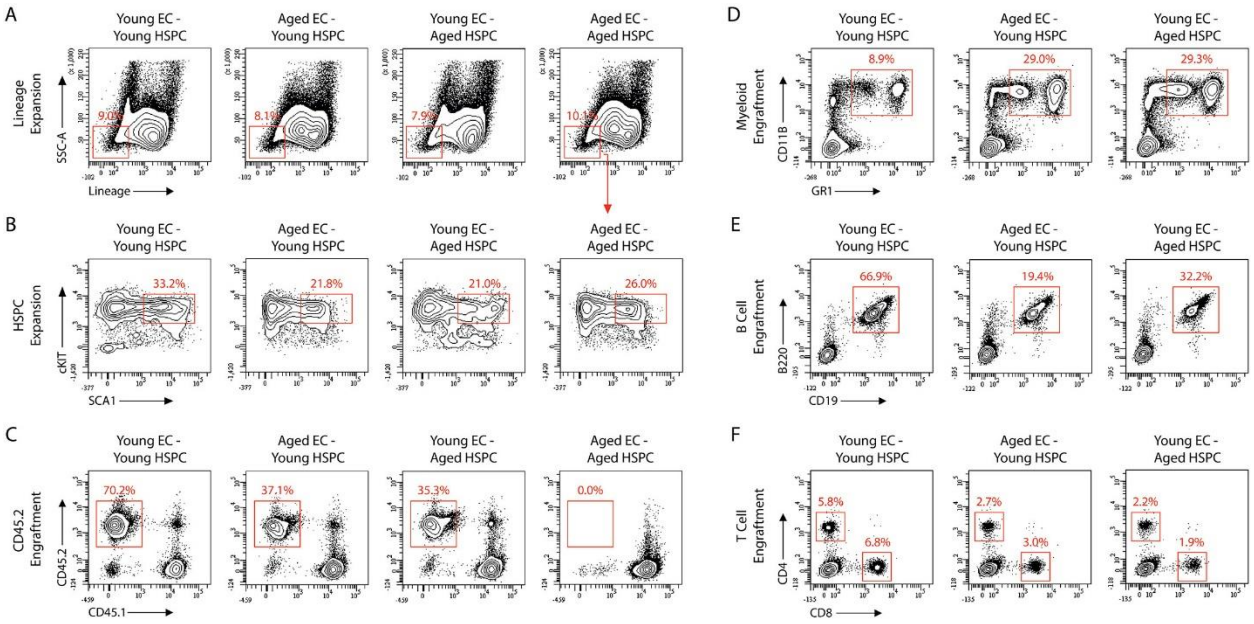
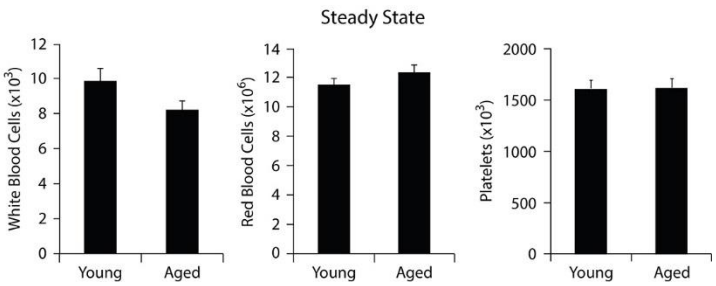
Supplemental Material

Poulos et. al.
Endothelial transplantation rejuvenates aged hematopoietic stem cell function

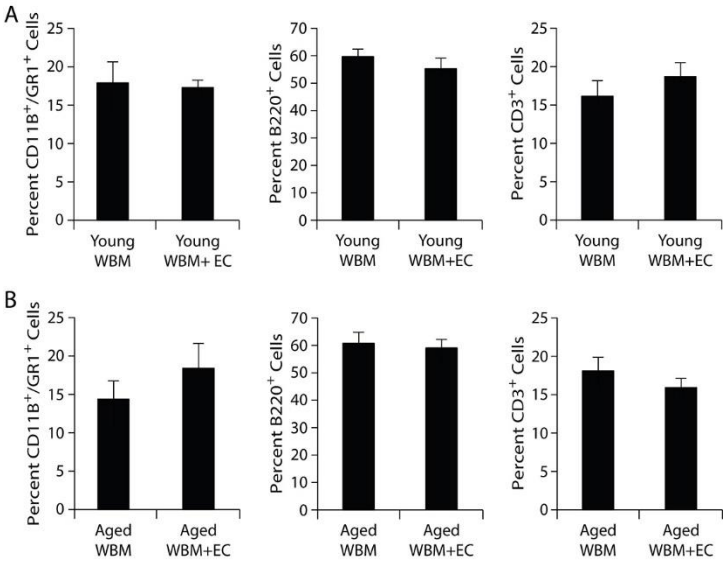
Supplemental Figures and Legends



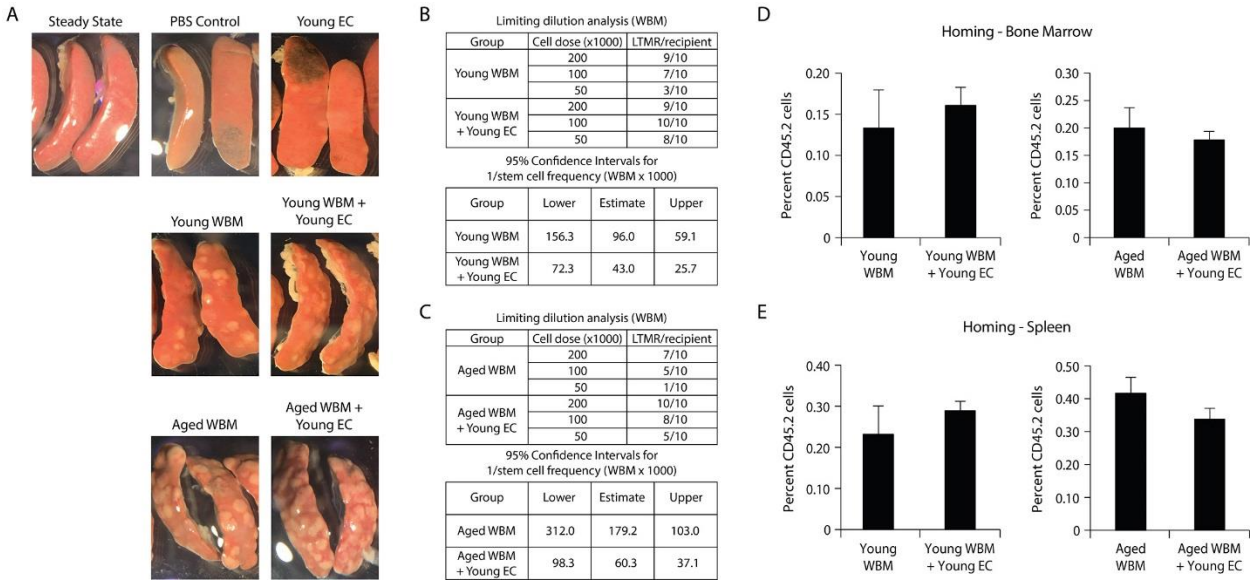
Supplemental Figure 1. Representative contour plots of hematopoietic expansion and multi-lineage engraftment. **(A-B)** Hematopoietic populations following 14 days of expansion on endothelial cells (ECs). Hematopoietic populations were enriched using CD45 microbeads following expansion. **(A)** Lineage$^-$ (GR1$^-$CD11B$^-$B220$^-$CD3$^-$CD41$^-$TER119$^-$) cell populations. Red box/arrow indicates gating strategy for phenotypic hematopoietic stem and progenitor cells (HSPCs). **(B)** Phenotypic lineage$^-$cKIT$^+$SCA1$^+$ (LKS) HSPCs (red box). Lineage$^-$cKIT$^+$SCA1^{High} populations are indicated (dashed blue box). **(C-F)** Long-term multi-lineage reconstitution of HSPC/EC *ex vivo* expansions four months post-transplantation. **(C)** Representative plots of CD45.2$^+$CD45.1$^-$ peripheral blood hematopoietic engraftment. All engraftment plots were gated on TER119$^-$ cell populations. **(D)** Representative plots of GR1$^+$CD11B$^+$ myeloid cell, **(E)** B220$^+$CD19$^+$ B Cell, and **(F)** CD8$^+$/CD4$^+$ T Cell reconstitution. All multi-lineage plots were gated on total CD45.2$^+$ cell populations.



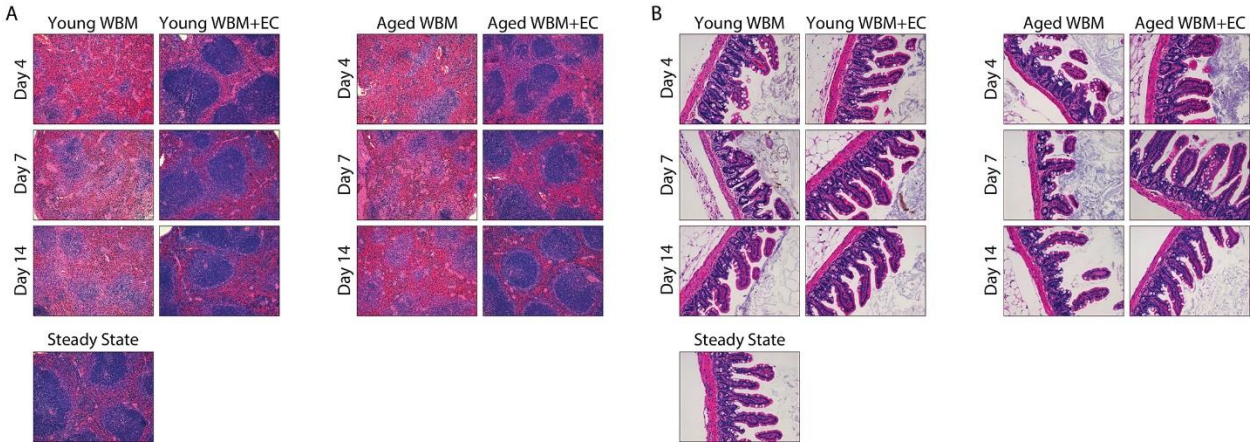
Supplemental Figure 2. Complete blood counts of young and aged control mice. Steady state (pre-irradiation) young (3 month) and aged (24 month) C57BL/6 mice were bled and analyzed for peripheral blood counts (n = 5). Results demonstrate no observable differences between pre-irradiation young or aged animals. Unpaired two-tailed Student's t-tests were performed to determine significance (* $P<0.05$; ** $P<0.01$; *** $P<0.001$).



Supplemental Figure 3. Multi-lineage reconstitution of WBM-EC co-infused animals. (A-B) Whole bone marrow (10^5 cells) from **(A)** young (3 month) or **(B)** aged (24 month) C57BL/6 mice were co-infused with or without $5x10^5$ young endothelial cells (ECs) in a non-competitive setting following lethal total body irradiation (950 Rads). Surviving mice were assessed for multi-lineage reconstitution four months post-TBI (n = 10 mice/cohort). Unpaired two-tailed Student's t-tests were performed to determine significance (* $P<0.05$; ** $P<0.01$; *** $P<0.001$).



Supplemental Figure 4. Co-infusion of young endothelium supports HSPC activity. Whole bone marrow (WBM) from young (3 month) and aged (24 month) C57BL/6 mice were co-infused with or without $5x10^5$ young endothelial cells (ECs) into lethally-irradiated recipients (950 Rads) and assessed for hematopoietic stem and progenitor activity. **(A)** Representative images of colony-forming units-spleen (CFU-S) in mice eight days following transplantation with 10^5 WBM with or without young ECs. **(B-C)** Limiting dilution data displaying the frequency of long-term multi-lineage reconstitution (LTMR) of non-competitive WBM transplantations from **(B)** young and **(C)** aged mice (n = 10 mice/cohort). Stem cell frequency and 95% confidence intervals were determined using Extreme Limiting Dilution Analysis (ELDA). **(D-E)** Hematopoietic homing analysis of 10^6 young and aged CD45.2$^+$ WBM with and without $5x10^5$ young ECs infused/co-infused in lethally-irradiated (950 Rads) recipients. Data demonstrates no observable differences between hematopoietic homing in the **(D)** bone marrow or **(E)** spleen 16 hours post-infusion. Unpaired two-tailed Student's t-tests were performed to determine significance (* $P<0.05$; ** $P<0.01$; *** $P<0.001$).



Supplemental Figure 5. Endothelial cell co-infusion safeguards radiosensitive tissues. Following a lethal dose of total body irradiation (950 Rads), C57BL/6 mice infused with a single dose of young (3 month) or aged (24 month) whole bone marrow with or without $5x10^5$ young endothelial cells (ECs) and assessed for tissue damage. Representative Hematoxylin and Eosin (H&E) stained sections demonstrate distinct radioprotection of both **(A)** spleen and **(B)** intestine tissue morphology in EC co-infused groups (n = 10 mice/cohort; 100X and 200X magnification, respectively).

Gene	Primer Bank ID	Fwd Primer (5'-3')	Rev Primer (5'-3')
Vcam1	26326181a1	gttccagcgagggtctacc	aactcttggcaaacattaggtgt
Sele	6755452a1	atgcctcgcgctttctctc	gtagtcccgctgacagtatgc
Kitl	198596a1	gaatctccgaagaggccagaa	gctgcaacaggggtaacat
Cxcl12	7305465a1	tgcatcagtgacggtaaacca	ttcttcagccgtgcaacaatc
Jag1	7305197a1	cctcgggtcagtttgagctg	ccttgaggcacactttgaagta
Jag2	21553297a1	caatgacaccactccagatgag	ggccaaagacgtcgttgcg
Dll1	164565442c2	cccatccgattccccttcg	ggttttctgttgcgaggtcatc
Dll4	9506547a1	ttccaggcaaccttctccga	actgccgctattcttgtccc
Igfbp3	6680385a1	ccaggaaacatcagtgagtcc	ggatggaacttggaatcggtca
Csf1	166064045c1	gtgtcagaacactgtagccac	tcaaaggcaatctggcatgaag
Il6	13624311a1	tagtccttcctaccccaatttcc	ttggtccttagccactccttc
Actb	In-house	cgtgcgtgacatcaaagagaa	ggccatctcctgctcgaa

Supplemental Table 1. Primer sets used for young and aged endothelial gene expression analysis.