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Supplemental Information

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SUPPLEMENTAL INFORMATION

Megakaryocyte Progenitor Cell Function is Enhanced Upon Aging Despite the Functional Decline of Aged Hematopoietic Stem Cells

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SUPPLEMENTAL EXPERIMENTAL PROCEDURES

Mouse lines

All animals were housed and bred in the AAALAC accredited vivarium at UC Santa Cruz and maintained under approved IACUC guidelines. The following mice were utilized for these experiments: C57Bl6 (JAX, cat# 664), aged C57Bl6 (NIH-ROS), BoyJ (JAX, cat# 2014), Ubc-GFP (JAX, cat# 4353).

Flow Cytometry

Bone marrow stem and progenitor cell populations and mature cell subsets were prepared and stained as previously described (Beaudin et al., 2014, 2016; Leung et al., 2019; Martin et al., 2021; Smith-Berdan et al., 2015, 2019; Ugarte et al., 2015). Briefly, the long bones (femur and tibia) from mice were isolated, crushed with a mortar and pestle, filtered through a 70μm nylon filter and pelleted by centrifugation to obtain a single cell suspension. APC-conjugated spherobeads (BD Bioscience) were added to the cell suspension prior to staining cells with fluorescently labeled antibodies to cell surface antigens. Cell labeling was performed on ice in 1X PBS with 5 mM EDTA and 2% serum. The stem and progenitor populations within the bone marrow were characterized as: HSC: $Lin^-/ckit^+/Sca1^+/Flk2^-/Slam^+$, MPP: $Lin^-/ckit^+/Sca1^+/Flk2^+/Slam^-$, MkP: $Lin^-/ckit^+/Sca1^-/CD41^+/Slam^+$, CMP: $Lin^-/ckit^+/Sca1^-/Fc\gamma R^{mid}/CD34^{mid}$, GMP: $Lin^-/ckit^+/Sca1^-/Fc\gamma R^{high}/CD34^{high}$, MEP: $Lin^-/ckit^+/Sca1^-/Fc\gamma R^-/CD34^-$, Pre-GM (Pronk): $Lin^-/cKit^+/Sca1^-/CD41^-/ Fc\gamma R^-/CD150^-/Endoglin^-$, Pre-Meg (Pronk): $Lin^-/cKit^+/Sca1^-/CD41^-/ Fc\gamma R^-/CD150^+/Endoglin^-$, Pre-CFU-E (Pronk): $Lin^-/cKit^+/Sca1^-/CD41^-/ Fc\gamma R^-/CD150^+/Endoglin^+$. Mature cells were characterized by: erythroid cells as circulating erythrocyte progenitors: (FSC^{lo-mid}/Ter-119^+/$CD71^+$/$Mac1^-$/$Gr1^-$/$B220^-$/CD3) or erythrocytes: (FSC^{lo-mid}/Ter-119^+/$CD61^-$/$Mac1^-$/$Gr1^-$/$B220^-$/$CD3^-$), platelets: (SSC^{lo}/ Ter-119^-/$CD61^+$/$Mac1^-$/$Gr1^-$/$B220^-$/$CD3^-$), GM (Ter-119^-/$CD61^-$/$Mac1^+$/$Gr1^+$/$B220^-$/$CD3^-$), B-cell (TER-119^-/$CD61^-$/$Mac1^-$/$Gr1^-$/$B220^+$/$CD3^-$), T-cell (Ter-119^-/$CD61^-$/$Mac1^-$/$Gr1^-$/$B220^-$/$CD3^+$). Cell suspensions were analyzed for specific cell populations using FACS Aria III (Becton Dickinson, San Jose, CA).

The lineage cocktail was comprised of CD3 (Biolegend cat #100306), CD4 (Biolegend cat #100423), CD5 (Biolegend cat #100612), CD8 (Biolegend cat #100723), Ter-119 (Biolegend cat #116215), Mac1 (Biolegend cat #101217), Gr1 (Biolegend cat #108417), and B220 (Biolegend cat #103225). Antibodies used in sorting were:

cKit(Biolegend cat #105826), Sca1 (Biolegend cat #122520), CD150 (Biolegend cat #115914), FLK2 (ebiosciences cat #12-1351- 83), CD34 (ebiosciences cat #13-0341-85), CD41 (Biolegend cat #133914), CD105 (Biolegend cat #120402). Antibodies used in peripheral blood were: CD71 (Biolegend cat # 113803), CD61 (Biolegend cat # 104314), CD3 (Biolegend cat #100306), TER-119 (Biolegend cat #116215), Mac1 (Biolegend cat #101217), Gr1 (Biolegend cat #108417), and B220 (Biolegend cat #103225).

HSC and MkP sorts by Flow Cytometry

HSC ($Lin^-/cKit^+/Sca1^+/Flk2^-/Slam^+$) or MkP ($Lin^-/cKit^+/Sca1^-/CD41^+/Slam^+$) from young or old mice were prospectively isolated using a FACS ARIA III (Becton Dickinson, San Jose, CA) as previously described (Boyer et al., 2019; Leung et al., 2019; Martin et al., 2021). Cells were harvested from long bones as mentioned above and stained with CD117-microbeads (Miltenyi), then passed over a magnetic column to enrich for $CD117^+$ stem and progenitor cells. HSC and MkP cell populations were double-sorted on low pressure with a 100μm nozzle into PBS, 2% serum.

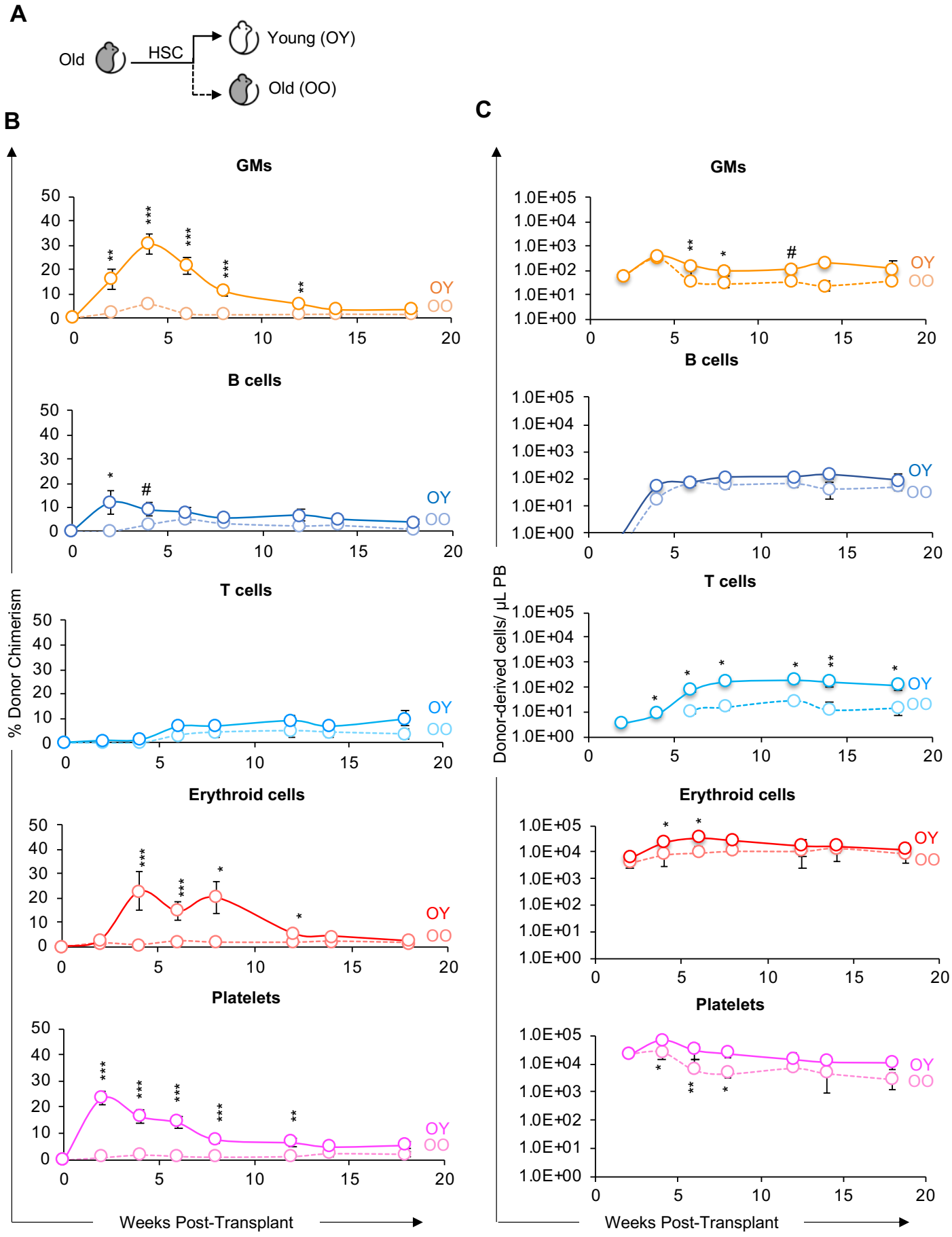
Transplantation Reconstitution assays

Reconstitution assays were performed by transplanting double-sorted HSCs (200 per recipient) from young or old Ubc-GFP^+ whole bone marrow and transplanting into congenic C57Bl6 mice via retro-orbital intravenous transplant. We also transplanted double-sorted MkPs (22,000 per recipient) from C57Bl6 into Ubc-GFP^+ hosts. Hosts were preconditioned with sub-lethal radiation (~750 rads) using a Faxitron CP160 X-ray instrument (Precision Instruments). Recipient mice were bled via the tail vein at the indicated intervals post-transplantation for analysis of peripheral blood donor chimerism. APC-labeled spherobeads were added to whole peripheral blood prior to staining with B220-APCy7, CD3-A700, Mac1-PECy7, Ter119-PECy5, Gr1-PB, CD71-Biotin/STA-BV605, and CD61-Alexa-647 (Biolegend) to detect mature lineage subsets for both host and donor mice. Cell suspensions were analyzed by FACS Aria III or LSR II (Becton Dickinson, San Jose, CA) for whole blood phenotypes and again post red blood cell lysis with a hypotonic alkaline lysis solution (ACK). Cells counts were calculated based on the number of cells analyzed and known number spherobeads added per sample.

In vitro Proliferation

Megakaryocyte progenitors were prospectively isolated using CD117-Microbead enrichment (Miltenyi) and sorted by fluorescence-activated cell sorting (FACS) from young (6-12 weeks old) and old WT (20-24 months old) mice as previously described (Smith-Berdan et al., 2015). 2000 cells were plated per well into a 96-well U-bottom tissue culture plate. Sorted cells were cultured for 3 days (MkP) in 200μl/well containing IMDM medium (Fisher) supplemented with 10% FBS, 50ng/ml rmTPO, 20ng/ml rmIL-6, 20ng/ml of rmIL-11, 50ng/ml of rmSCF, and 10ng/ml rmIL-3 (Cytokines from Peprotech) and Primocin (Invivogen). On days 3 APC-labeled spherobeads (BD Bioscience) were added to appropriate wells with cells and triplicate samples were analyzed using a FACSAria (Becton Dickinson, San Jose, CA). Cell expansion rates were calculated based on the number of beads recovered per beads added per well.

SUPPLEMENTAL FIGURE 1



Supplemental Figure 1: Old HSCs exhibited lower reconstitution potential in old compared to young recipients

(A) Schematic of transplantation of HSCs from old mice into separate cohorts of young or old mice.

(B) Old HSCs displayed lower donor-host-chimerism in the old recipients compared to in young recipients. Analysis of donor-derived mature cells (GMs, B cells, T cells, erythroid cells, and platelets) in peripheral blood of recipients presented as percent donor chimerism.

(C) Old HSCs produced fewer total mature cells in old recipients compared to in young recipients. Reconstitution data from (B) replotted as absolute number of donor-derived GMs, B cells, T cells, erythroid cells, and platelets per microliter of peripheral blood.

Data are representative means ± SEM from 12 young recipient mice (7 independent experiments) and 8 old recipient mice (3 independent experiments). P values were determined using unpaired two-tailed t-test. $*p < 0.05$, $**p < 0.005$, $***p < 0.0005$.

SUPPLEMENTAL TABLE 1

Table 1: Top 50 differentially expressed genes between young and old MkPs				
Gene symbol	Log2 Fold Change	P-value	Basemean Young	Basemean Old
Tubb6	-1.5234473	8.40E-08	3.90E+02	1.35E+02
AI506816	-1.4245791	8.12E-04	7.89E+03	2.94E+03
Tmem97	-1.3199598	7.64E-04	2.94E+03	1.18E+03
Atp5k	-1.2412058	1.39E-03	1.23E+03	5.21E+02
Emid1	-1.2234213	5.17E-03	1.74E+03	7.44E+02
Dnajc15	-1.1961287	5.12E-03	2.48E+03	1.08E+03
Bckdhb	-1.1225232	1.72E-03	4.86E+02	2.24E+02
Phgdh	-1.094522	5.00E-03	9.78E+03	4.58E+03
Nup37	-1.0609982	6.96E-04	8.68E+02	4.17E+02
Tbrg4	-1.0608148	6.07E-04	9.99E+02	4.79E+02
Uqcc2	-1.0583517	8.25E-04	1.77E+03	8.51E+02
Hspe1	-1.0582077	2.34E-03	4.33E+03	2.08E+03
Timm8a1	-1.0572596	3.59E-03	2.14E+03	1.03E+03
Tmem258	-1.0394034	2.15E-03	1.29E+03	6.30E+02
Tysnd1	-1.0359832	4.98E-04	2.15E+03	1.05E+03
Rpf2	-1.0327924	1.70E-03	9.38E+02	4.59E+02
Mecr	-1.0214637	2.34E-07	6.37E+02	3.15E+02
Yars	-1.0198991	3.54E-03	1.97E+03	9.71E+02
F10	-1.0169751	1.05E-04	1.13E+03	5.60E+02
Gchfr	-1.0166502	6.24E-03	3.92E+02	1.94E+02
Asns	-1.0166478	3.58E-03	3.50E+03	1.73E+03
Wdr18	-1.0000778	7.12E-04	5.85E+03	2.93E+03
Mrps18a	-0.9960708	2.40E-04	3.19E+03	1.60E+03
Coq7	-0.9954788	2.70E-03	1.26E+03	6.32E+02
Atp5e	-0.9929881	2.03E-03	2.24E+03	1.13E+03
Pik3ip1	2.1552087	1.66E-03	2.42E+02	1.08E+03
AK165889	2.1835855	1.01E-03	1.50E+02	6.81E+02
Selp	2.2508835	8.79E-04	4.96E+02	2.36E+03
Krt18	2.2564015	3.52E-04	2.25E+02	1.07E+03
Nfkbia	2.3271007	1.32E-06	4.50E+02	2.26E+03
Selm	2.4368411	1.28E-03	1.22E+02	6.59E+02
Selm	2.4466799	1.21E-03	1.19E+02	6.48E+02
Hbb-bt	2.4514855	1.05E-03	2.92E+02	1.60E+03
Gstm2	2.4781945	2.66E-03	1.74E+02	9.67E+02
Clu	2.6034299	6.48E-03	7.73E+02	4.70E+03
Hbb-bs	2.8180971	5.94E-04	6.86E+02	4.83E+03
Lcp1	2.8224804	1.14E-06	1.75E+02	1.24E+03
Tgm2	2.8253432	2.47E-04	1.10E+02	7.81E+02
Nupr1	2.8873895	6.77E-05	1.92E+02	1.42E+03
H2-Ab1	2.9240602	3.72E-10	2.63E+02	1.99E+03
Galnt6	2.9439361	2.55E-05	1.02E+02	7.87E+02
C1qb	3.0211859	4.90E-03	3.14E+02	2.55E+03
C1qc	3.1118748	4.54E-03	1.30E+02	1.12E+03
Aldh1a1	3.8871329	2.06E-15	1.29E+02	1.91E+03
S100a8	4.7393422	2.40E-04	2.05E+02	5.47E+03
Ngp	5.4444377	7.87E-05	8.30E+02	3.61E+04
Cd74	5.5091778	3.86E-25	1.61E+02	7.35E+03
Camp	5.9138395	2.85E-05	1.02E+02	6.13E+03
Chil3	6.0448889	5.37E-05	1.02E+02	6.77E+03
Ltf	6.1725772	2.75E-05	2.76E+02	1.99E+04

SUPPLEMENTAL TABLE 2

Table 2: Lists of genes under GO terms from Figure 7C.

regulation of hemopoiesis	inflammatory response	cellular response to cytokine stimulus	mitochondrial translation	regulation of tumor necrosis factor production	regulation of innate immune response	regulation of reactive oxygen species metabolic process	regulation of leukocyte cell-cell adhesion	apoptotic signaling pathway	positive regulation of cell population proliferation
Tyrobp	Ctss	Snx10	Ptcd1	Tyrobp	Sec14l1	Tyrobp	Cd177	Eya2	Tyrobp
Rhoh	Lpl	Csf3r	Mrps11	Ifih1	Ifih1	Cd177	F11r	Mmp9	Nudt16
Tcf3	Tnfrsf1a	Ccl6	Mrps15	Slamf1	Cd177	Parl	S100a8	Ctsh	Slamf1
Csf3r	Alox5ap	Igtp	Tufm	Bcl3	Slamf1	Hspd1	Selplg	Parl	Bex1
Gfi1b	Nupr1	Hyal1	Mtif3	Ltf	Ccl6	Hbp1	S100a9	Plscr1	Atxn1
Evi2b	Nfkbia	Csf2ra	Mrps18b	Lpl	Camp	H2-M3	Selp	Mtch2	Mmp9
Trf	Ctsc	Plscr1	Mrps18a	Tnfrsf1a	Ltf	App	Icam1	Nupr1	Camp
Tesc	Hspd1	Trf	Gars	Zbtb20	H2-Eb1	Ogt		Pea15a	Lrg1
Jun	Gpsm3	Tnfrsf1a		Hspd1	Gbp2	Clu		Ctsc	Tacstd2
Nfkbia	S100a8	Ifi203		App	Ifitm6	Hp		Vdac2	Hyal1
Pglyrp2	App	Gbp2		Clu	C1rl	Icam1		Lgals3	S100a11
Prmt1	S100a9	Nfkbia		Axl	Irf1	Mmp8		S100a8	Ctsh
Irf1	Tgm2	Ifitm6		Mmp8	H2-Aa	Coq7		Erp29	Slfn2
H2-Aa	Il16	Hspd1			Gata3	Ier3		Clu	Trf
Spi1	Grn	Rps6ka4			Capg			S100a9	Ltf
Hax1	Mmp8	Irf1			Irgm2			Cd74	Jun
Gata3		Spi1			Phb2			Maged1	Nupr1
Hspa9		Hax1			Lgals3			Acsl5	Nfkbia
Mmp14		Rnf125			S100a8			Trap1	Ndn
H2-M3		Gata3			Unc93b1			Icam1	Irf1
Pglyrp1		Gpd1			Cfp			Grina	H2-Aa
Anxa1		Capg			Pglyrp1			Ier3	Gata3
Cd74		Irgm2			Usp14				Ecm1
Loxl3		Darc			App				Lgals3
Lag3		Cd74			Pld3				Sat1
Stat1		Lsp1			S100a9				Lgmn
Cd1d1		Krt18			Pstpip1				H2-M3
Axl		Il16			Anxa1				App
C1qc		Mrpl15			Polr3h				Clu
		Selp			C1qb				Tgm2
		Pirb			Trim28				Ccpg1
		Stat1			Stat1				Anxa1
		Icam1			Cd1d1				Cd74
		Stxbp4			Stxbp4				Prnp
		Axl			Axl				Maged1
		H2-Ab1			Padi4				Txnip
					Atad3a				Pim2
					Lcn2				Grn
					H2-Ab1				Col18a1
					C1qc				Ndrg2
					Fcnb				Stat1
									Cd1d1
									Stxbp4
									H2-Ab1
									Ctsl