Summary of Supplemental Figures and Tables

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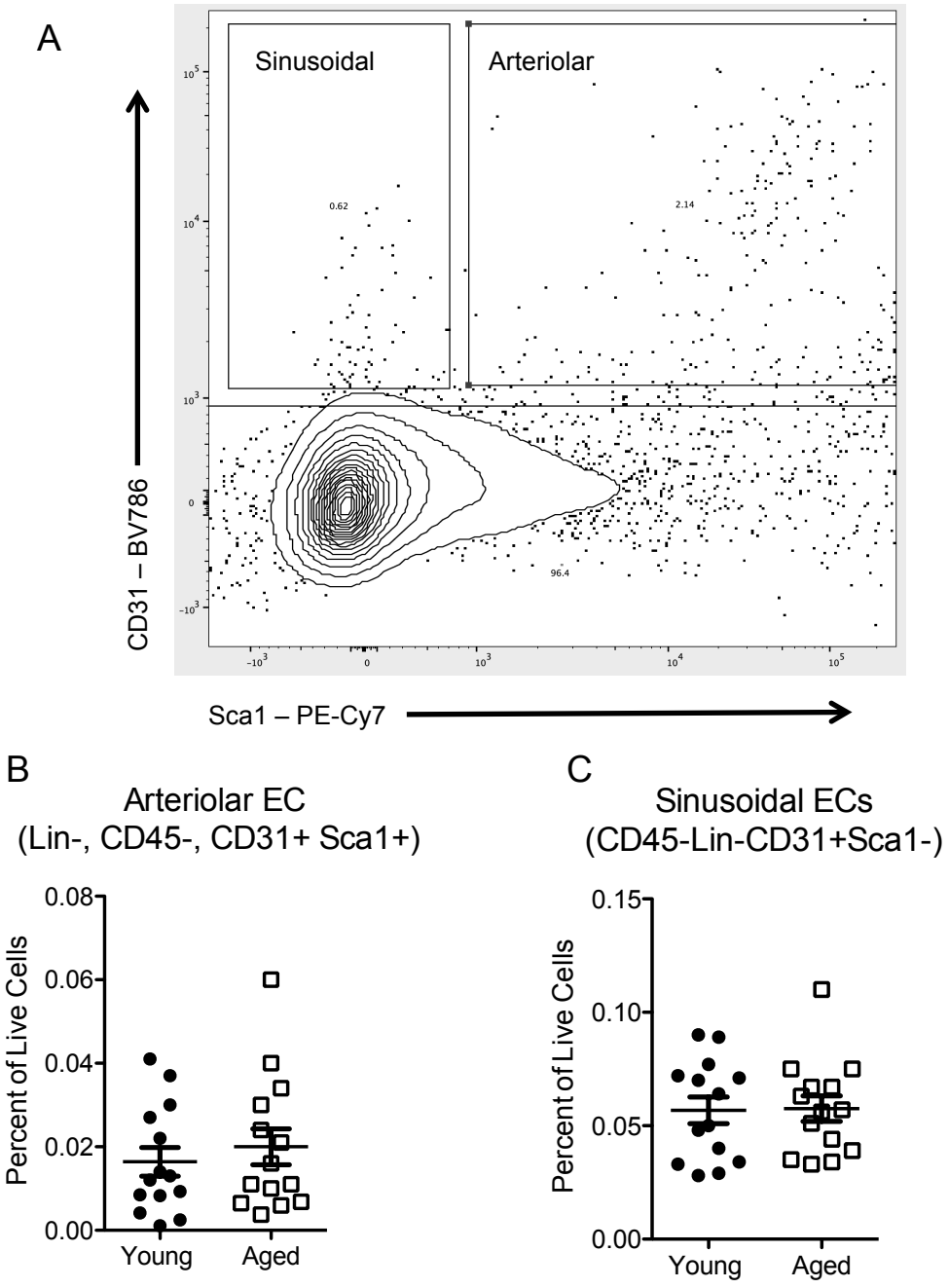
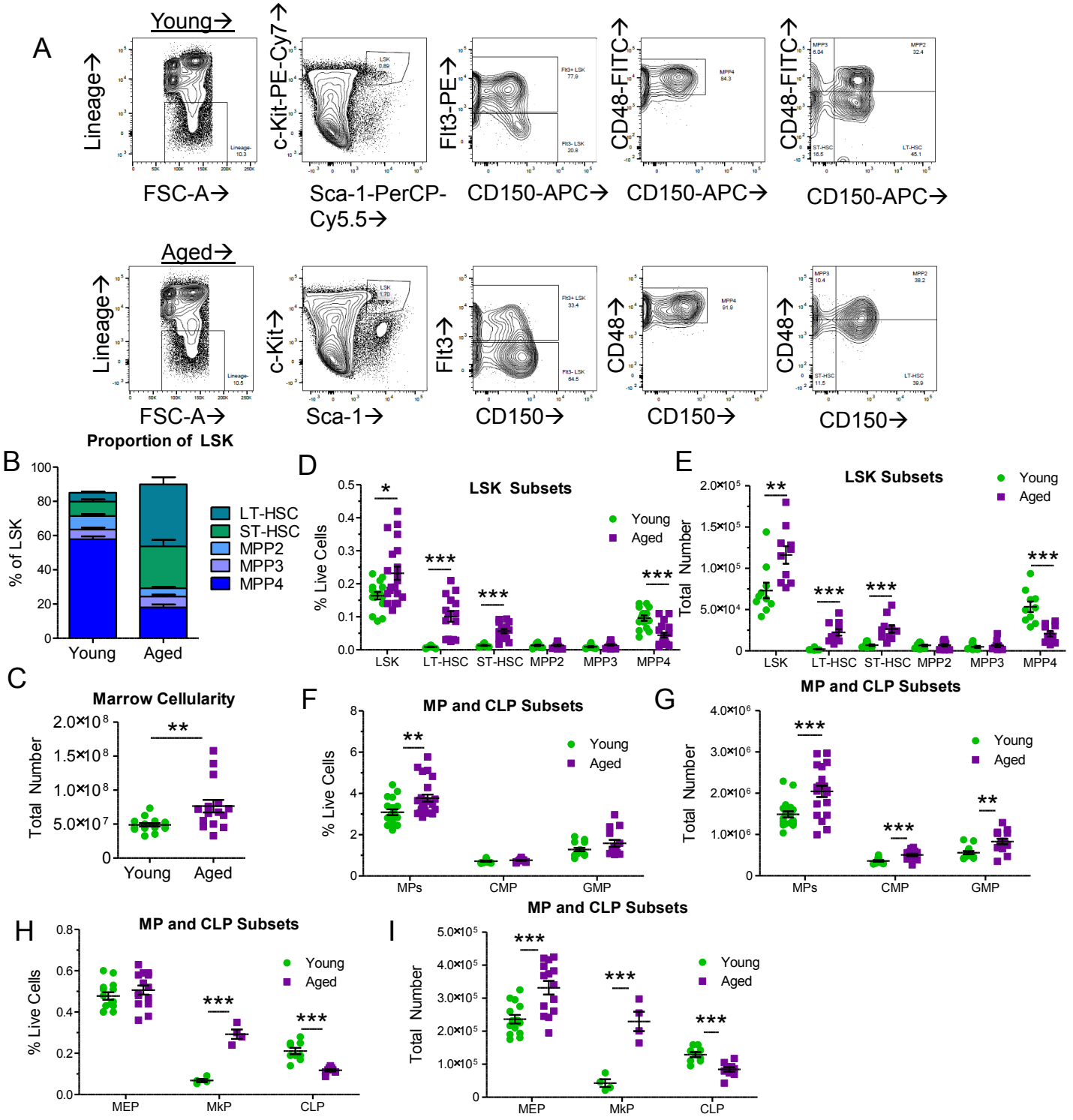
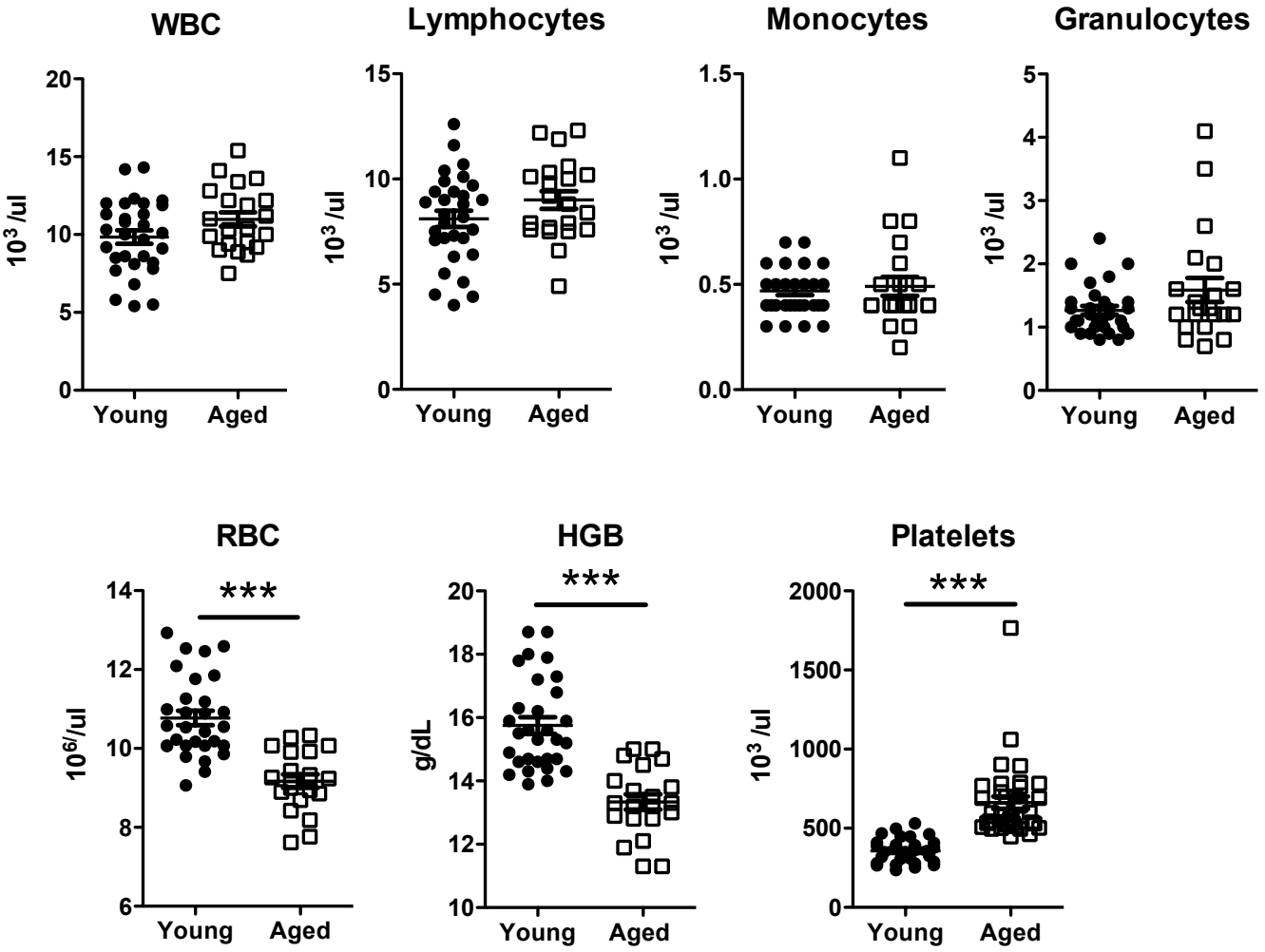


Supplemental Figure 1. Frequency of phenotypically defined endothelial cells is unchanged in aged mice. (A) Representative flow cytometry gating for Arteriolar (Lin-, CD45-, CD31+, Sca1+) and Sinusoidal (Lin-, CD45-, CD31+, Sca1-) Endothelial cells from the bone marrow of young and aged mice. (B, C) Quantification of Arteriolar (B) and Sinusoidal (C) endothelial cell populations.

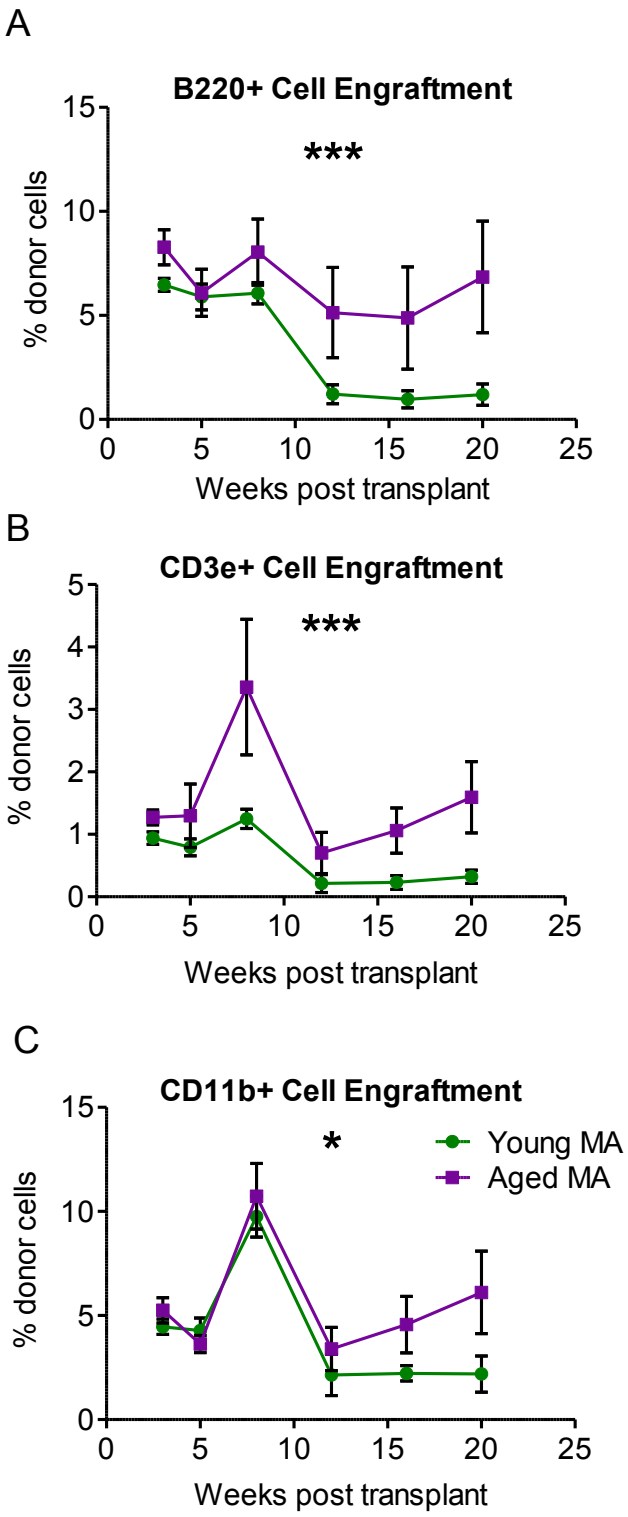


Supplemental Figure 2. Aged LSKs have increased myeloid/megakaryocytic bias. (a,b) Flow cytometry strategy **(a-**representative plots from young and aged mouse**)** and percent contribution **(b)** of lineage$^-$Sca1$^+$ c-Kit$^+$ (LSK) subsets to HSPC pool of young and aged mice (N=10-15). **(c)** Marrow cellularity of young and aged mice (N=15). **(d-i)** Flow cytometry of percent live **(d,f,h)** and total number **(e,g,i)** of LSKs and subsets **(d,e)** and of myeloid (MP, CMP, GMP, MEP), lymphoid (CLP) and megakaryocytic (MkP) progenitors **(f-i)** in young and aged mice (N=10-15). Data represent mean±s.e.m. (*p*-values: two-tailed Student's t-test; *p<0.05; **p<0.01; ***p<0.001).



Supplemental Figure 3. RBCs are decreased and Platelets are increased in peripheral blood of aged mice. CBC analysis of peripheral blood demonstrates no change in the numbers of (A) WBC, (B) Lymphocytes, (C) Monocytes, (D) Granulocytes, a decrease in the number of (E) RBCs, and (F) HGB, and an increase in the number of (G) Platelets. N=25-30 mice/group. ***P<0.001.

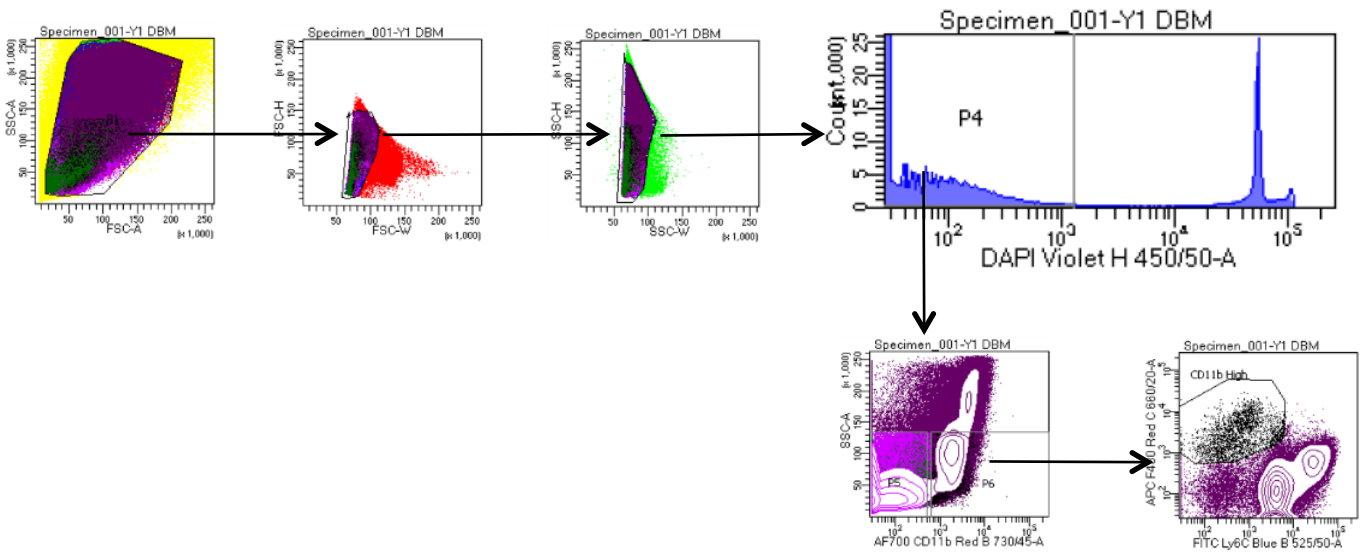
Supplemental Figure 4. Increased MSC populations, and decreased osteoblastic cells in aged BMME tissue cultures. Aged BMME cultures are enriched for MSC populations recapitulating what is seen in vivo in aged mice. Arteriolar and Sinusoidal endothelial cells make up less than 1% of total cells in BMME cultures and are not changed in aging. Data represent mean±s.e.m. (*p*-values: two-tailed Student's t-test; **p*<0.05; ****p*<0.001).



Supplemental Figure 5. Aged BMME cells increase cell engraftment following competitive transplantation. (a-c) Aged BMME cells demonstrate increase engraftment of B220+ **(a)**, CD3e+ **(b)**, and CD11b+ **(c)** cells compared to young BMME cells. (N= 5 Recipients/group). Data represent mean±s.e.m. (*p*-values: two-tailed Student's t-test; *$p<0.05$; **$p<0.01$; ***$p<0.001$).

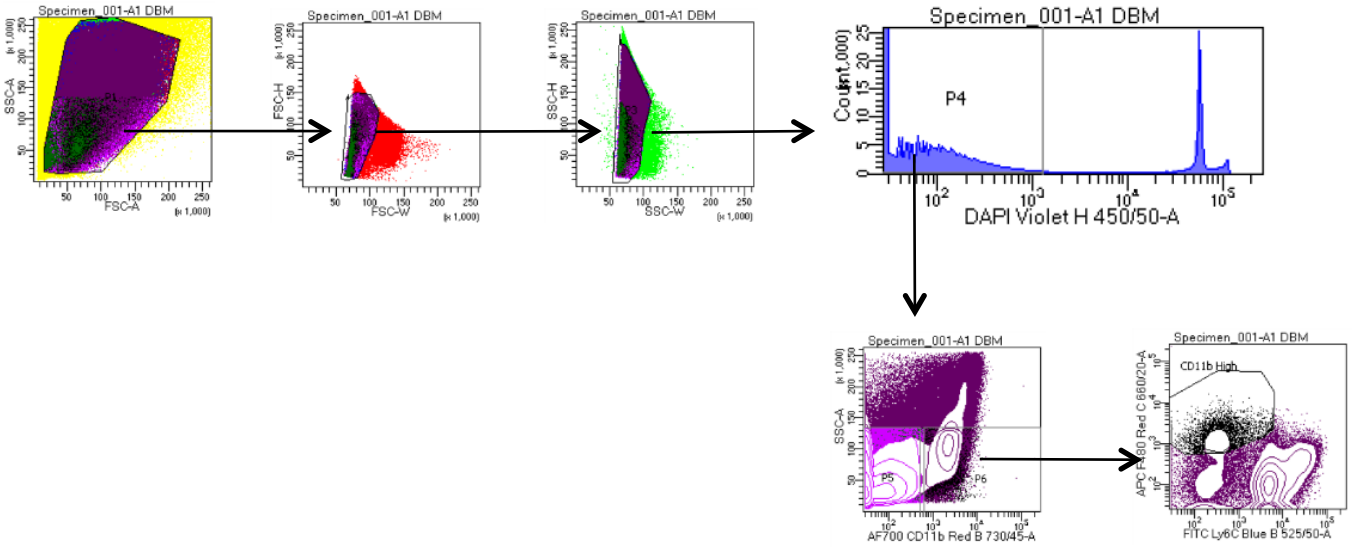
A

Young murine bone marrow



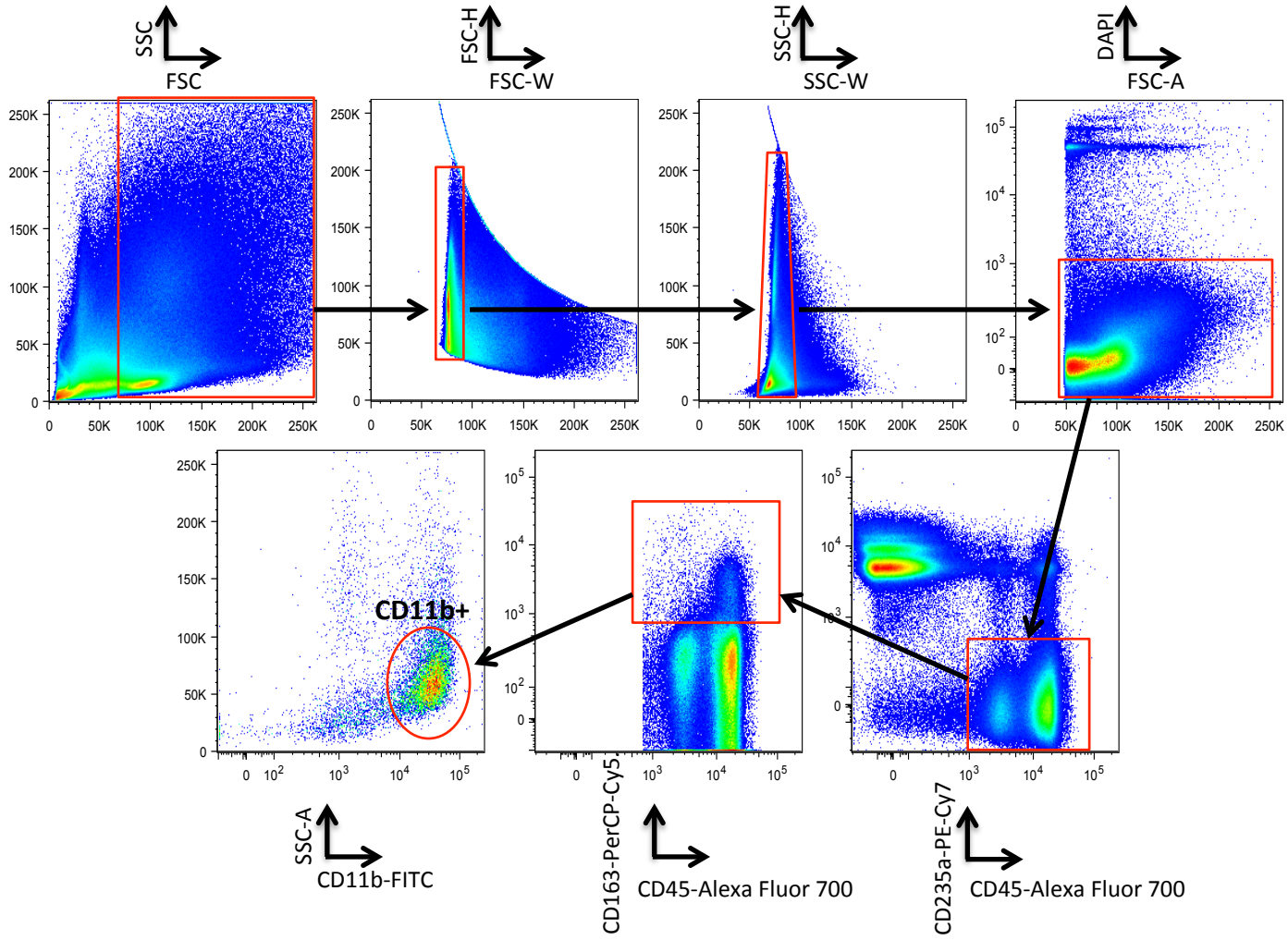
B

Aged murine bone marrow

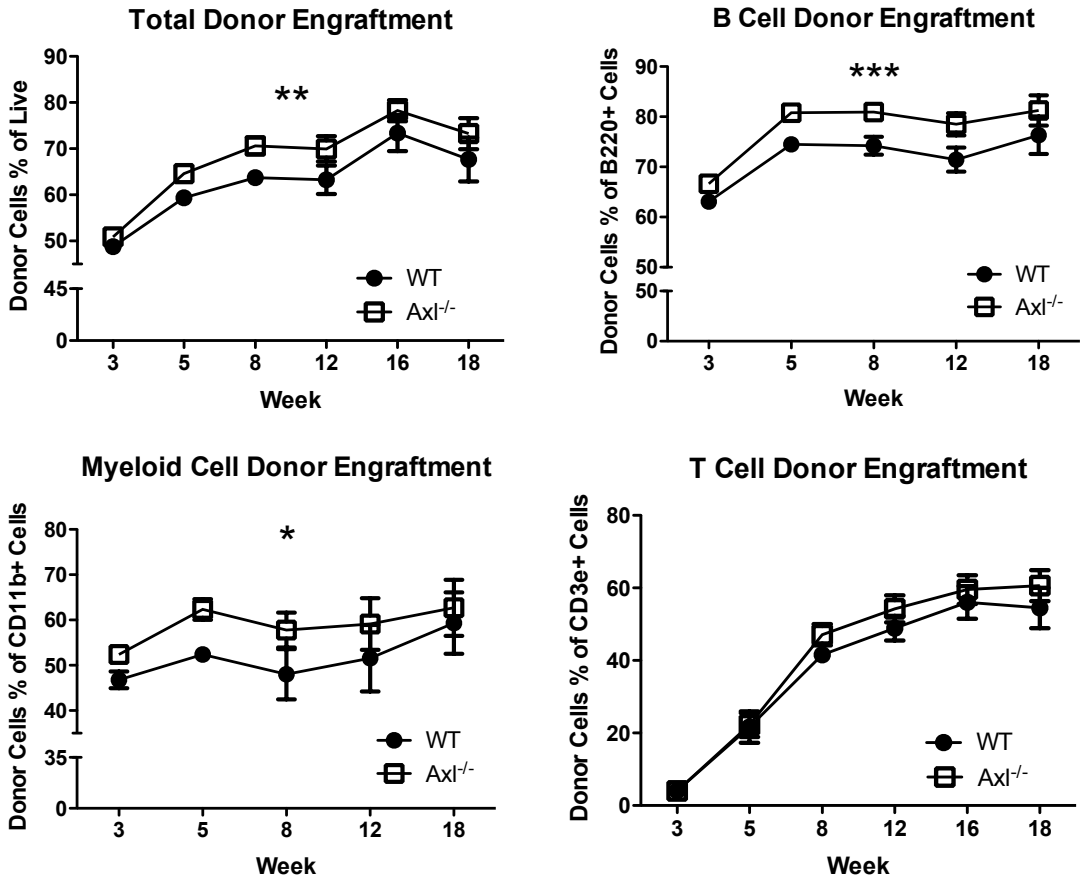


Supplemental Figure 6. Flow cytometry gating strategy for young and aged murine Mφs. Representative gating strategy used to analyze murine Mφs. F4/80$^+$, Ly6C$^-$, CD11b$^+$ Mφs were selected for analysis.

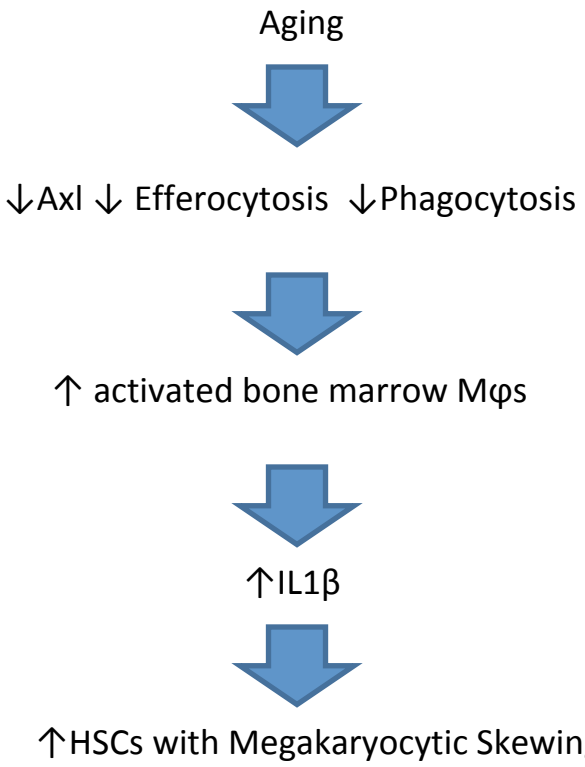
A



Supplemental Figure 7. Flow cytometry gating strategy for human Mφs. Representative gating strategy used to analyze Mφs in samples from human volunteers. $CD45^+$, $CD163^+$ and $CD45^+$, $CD11b^+$ Mφs were selected for analysis.



Supplemental Figure 8. Axl-/- LT-HSCs have increased cell engraftment following competitive transplantation. (a-c) Axl-/- LT-HSCs demonstrate increase engraftment of B220+ **(a)**, CD3e+ **(b)**, and CD11b+ **(c)** cells compared to wildtype littermates. (N= 5 Recipients/group). Data represent mean±s.e.m. (*p*-values 2-way ANOVA; **p*<0.05; ***p*<0.01; ****p*<0.001).



Supplemental Figure 9. Schematic representation of mechanisms by which age-dependent defects in marrow Mφs induce megakaryocytic bias in HSCs

Supplemental Table 1. Frisch, Hoffman *et al*.

Murine Hematopoietic Stem and Progenitor Cell Panel			
Antibody	**Clone**	**Fluorophore**	**Supplier and Cat #**
Lineage			
α-Ter119	TER-119	Biotin (primary) PE-CF594-Streptavidin (secondary)	BD Biosciences; 560512
α-Gr1	RB6-8C5		BD Biosciences; 561103
α-B220	RA3-6B2		BD Biosciences; 561101
α-CD3e	145-2C11		BD Biosciences; 551163
α-cKit	2B8	PE-Cy5	eBioscience; 15-1171
α-Sca1	D7	PerCP-Cy5.5	BD Biosciences; 558162
α-Flt3	A2F10	PE	eBioscience; 12-1351
α-CD48	HM48-1	FITC	eBioscience; 11-0481
α-CD150	9D1	APC	eBioscience; 17-1501
α-CD34	RAM34	Alexa Fluor 700	BD Biosciences; 560518
α-CD41	MWReg30	BUV-395	BD Biosciences; 565980
α-FcγR	2.4G2	APC-Cy7	BD Biosciences; 560541
α-CD127	A7R34	PE-Cy7	Biolegend; 135013

Murine Stromal Cell Panel			
Antibody	**Clone**	**Fluorophore**	**Supplier and Cat #**
Lineage			
α-Ter119	TER-119	PerCP-Cy5.5	BD Biosciences; 560512
α-Gr1	RB6-8C5		BD Biosciences; 561103
α-B220	RA3-6B2		BD Biosciences; 561101
α-CD3e	145-2C11		BD Biosciences; 551163
α-Sca1	D7	PE-Cy7	BD Biosciences; 558162
α-CD105	MJ7/18	PE-CF594	BD Biosciences; 562762
α-CD51	RMV-7	PE	BD Biosciences; 551187
α-CD31	390	FITC	BD Biosciences; 558738
α-CD45	30-F11	APC-Cy7	BD Biosciences; 557659
α-CD11b	M1/70	Alexa Fluor 700	BD Biosciences; 557960
α-PDGFRα	APA5	APC	BD Biosciences; 562777
α-LepR		Biotin (primary) BUV395-Streptavidin (secondary)	R&D Systems; BAF497/ BD Biosciences; 564176

Murine Macrophage Cell Panel (*in vitro*)			
Antibody	**Clone**	**Fluorophore**	**Supplier and Cat #**
α-CD86	GL-1	PE-Cy7	BD Biosciences; 560582
α-MHCII	M5/114.15.2	PerCP-Cy5.5	BD Biosciences; 562363
α-F4/80	BM8	FITC	eBioscience; 11-481
α-CD45	30-F11	APC-Cy7	BD Biosciences; 557659
α-CD11b	M1/70	Alexa Fluor 700	BD Biosciences; 557960

Supplemental Table 1 (continued). Frisch, Hoffman *et al*.

Murine Macrophage Cell Sorting			
Antibody	**Clone**	**Fluorophore**	**Supplier and Cat #**
α-CD45	30-F11	APC-Cy7	BD Biosciences; 557659
α-F4/80	BM8	FITC	eBioscience; 11-481
Murine Macrophage Cell Panel (*in vivo*)			
Antibody	**Clone**	**Fluorophore**	**Supplier and Cat #**
α-Ly6C	HK1.4	Pacific Blue	BioLegend; 128014
α-F4/80	CI:A3-1	APC-Cy7	BioLegend; 122614
α-CD11b	M1/70	PE	BioLegend; 101216
α-MHC-II	AF6-120.1	PE-Cy7	BioLegend; 116419
Murine Senescent Neutrophil Panel			
Antibody	**Clone**	**Fluorophore**	**Supplier and Cat #**
α-Ly6G	IA8	BUV-395	BD Biosciences; 563978
α-CXCR4	2B11	PE-CF594	BD Biosciences; 565019
α-CD62L	MEL-14	APC	BD Biosciences; 561919
Human Macrophage Cell Panel			
Antibody	**Clone**	**Fluorophore**	**Supplier and Cat #**
α-CD163	GHI/61	PerCP-Cy5.5	Biolegend; 333607
α-CD11b	ICRF44	FITC	BD Biosciences; 562793
α-CD45	HI30	Alexa Fluor 700	BD Biosciences; 560566
α-CD235	HIR2	PE-Cy7	BD Biosciences; 563666
Murine Transplants Panel			
Antibody	**Clone**	**Fluorophore**	**Supplier and Cat #**
α-CD45.1	A20	PE	BD; 561872
α-CD45.2	104	FITC	BD; 561874
α-CD3e	145-2C11	PerCP-Cy5.5	BD; 561108
α-CD11b	M1/70	APC-Cy7	BD; 561039
α-B220	RA3-6B2	APC	BD; 561880

Supplemental Table 1 (continued). Frisch, Hoffman *et al*.

Co-Culture Phenotyping Panel			
Antibody	**Clone**	**Fluorophore**	**Supplier and Cat #**
Lineage			
α-Ter119	TER-119	PerCP-Cy5.5	BD Biosciences; 560512
α-Gr1	RB6-8C5		BD Biosciences; 561103
α-B220	RA3-6B2		BD Biosciences; 561101
α-CD3e	145-2C11		BD Biosciences; 551163
α-Sca1	D7	PE-Cy7	BD Biosciences; 558162
α-c-Kit	2B8	PE-Cy5	eBioscience; 15-1171
α-CD41	MwReg30 2C9.G2	BUV-395	BD Biosciences; 565980
α-CD61		AF647	BD Biosciences; 563523

Table S1. List of antibodies used in the flow cytometric and cell sorting analyses described. These include panels for murine hematopoietic stem and progenitor cells, murine stromal cells, murine and human macrophages and the murine competitive transplant experiment.

Supplemental Table 2. Frisch, Hoffman *et al*.

Cell Population	Immunophenotype
LSK	Lin-/Sca1+/cKit+
LT-HSC	Lin-/Sca1+/cKit+/Flt3-/CD48-/CD150+
ST-HSC	Lin-/Sca1+/cKit+/Flt3-/CD48-/CD150-
MPP2	Lin-/Sca1+/cKit+/Flt3-/CD48+/CD150+
MPP3	Lin-/Sca1+/cKit+/Flt3-/CD48+/CD150-
MPP4	Lin-/Sca1+/cKit+/FLt3-/CD48+
MP	Lin-/Sca1-/cKit+
CMP	Lin-/Sca1-/cKit+/CD34+/FcγR-
GMP	Lin-/Sca1-/cKit+/CD34+/FcγR+
MEP	Lin-/Sca1-/cKit+/CD34-/FcγR-
MkP	Lin-/Sca1-/cKit+/CD41+/CD150+
CLP	Lin-/CD127+/Flt3+
MSCs	Lin-/CD45-/CD31-/Sca1+/CD51+
OBCs	Lin-/CD45-/CD31-/Sca1-/CD51+
PαS+	Lin-/CD45-/CD31-/Sca1+/PDGFRα+
PααV	Lin-/CD45-/CD31-/CD51+/PDGFRα+
LepR	Lin-/CD45-/CD31-/LepR+
Macrophage	CD11b+/F4/80+/Ly6C-Lo
Neutrophil	CD11b+/F4/80-/Ly6C-Int
Senescent Neutrophil	Ly6G+/CXCR4-Hi/CD62L-Lo

Table S2. List of cell populations analyzed and their respective immunophenotypes.

GO Term	Genes	Fold Enrichment	P-Value	FDR
Inflammatory response	PRKCZ, CCL3, TLR1, PPARG, CXCL2, TNFRSF8, NLRP1B, FPR2, CCL4, TLR8, IL10, TLR9, S1PR3, SLC11A1, CCL22, MYD88, SMPDL3B, CXCR6, TICAM1, PYCARD, IL1B, LTA, NFKBIZ, LYN, C4B, HCK, IL27, ACKR2, TNFRSF14, SMAD1, CD180, CHST1, CCR5, TRP73, THEMIS2, CD14	4.009115	6.09E-12	1.07E-08
Immune system process	CD244, LST1, CADM1, FGR, TLR1, PTPN22, TNFSF13, NLRP1B, GBP2B, TLR8, LGR4, TLR9, MYD88, SMPDL3B, SH2D1B1, TICAM1, OASL1, MAP3K8, PYCARD, CD8B1, LYN, CD3E, HCK, IL27, CD300E, H2-Q7, CD180, PRKCB, IFIT3, C1QB, IFIT1, LAT2, CD300LF, THEMIS2, CD14	3.500852	4.95E-10	8.69E-07
Innate immune response	CD244, FGR, TLR1, IGHG2C, JCHAIN, NLRP1B, IGHG2B, TRIM10, IGHM, TLR8, LGR4, TLR9, MYD88, SMPDL3B, SH2D1B1, PTK6, TICAM1, OASL1, PYCARD, FCER1G, IGKC, LYN, C4B, HCK, IL27, CD180, IFIT3, C1QB, IFIT1, IGLC2, SRMS, CD14	3.064746	6.97E-08	1.22E-04
Immune response	CD244, CCL3, LST1, TLR1, CXCL2, TNFRSF8, TNFSF13, CCL4, FTH1, IL10, TLR9, CCL22, MYD88, OASL1, IGKV4-55, IL1B, LTA, FYB, GZMC, CTSS, TNFSF9, CCR8, CBLB, CCR6, CXCL14, CCR5, CD274, TGFBR3, BMPR1A, LCP2	4.225293	1.36E-10	2.39E-07
Positive regulation of tumor necrosis factor production	ZBTB20, CCL3, MYD88, CCR5, TICAM1, CD2, PYCARD, FCER1G, IL12B, CCL4, CD14, TLR9	7.791727	3.02E-07	5.30E-04
B cell receptor signaling pathway	LAT2, LYN, BCL2, IGHG2C, IGHG2B, RFTN1, IGKC, IGLC2, IGHM, PRKCB	6.965331	1.12E-05	0.019694
Positive regulation of interferon-gamma production	SLC11A1, IL27RA, CD3E, PYCARD, BCL3, IL1B, IL12B, TNFSF9, LTA, CD14	7.367177	6.96E-06	0.012212
Release of cytochrome c from mitochondria	ATP7A, BCL2A1D, BCL2A1C, BCL2A1B, BCL2A1A, BCL2, PMAIP1, TRP73	11.78748	3.33E-06	0.005849
Immunoglobulin mediated immune response	MYD88, C4B, IGHG2C, FCER1G, TNFSF13, IGHG2B, IGHA, IGHM	13.93066	9.45E-07	0.00166

Table S3. List of top GO-Biological Process categories enriched for significantly upregulated genes from murine aged vs young marrow macrophages.

KEGG ID	Genes	Fold Enrichment	P-Value	FDR
Cytokine-cytokine receptor interaction	CCL3, IL9R, IL21R, TNFRSF8, TNFSF13, TNFRSF14, TNFSF9, CCL4, IL10, KDR, CCR8, CCR6, CXCL14, CCR5, CLCF1, IL10RA, CXCR6, IL1B, IL12B, CSF2RA, LTA, IFNLR1, BMPR1A	3.206791	2.35E-06	1.07E-08
Toll-like receptor signaling pathway	CCL3, TLR1, CCL4, TLR8, TLR9, IKBKE, MYD88, TICAM1, MAP3K8, IL1B, PIK3R5, IL12B, CD14	4.396741	3.38E-05	8.69E-07
NF-kappa B signaling pathway	TRAF1, LYN, CCL4, CARD11, BCL2A1D, MYD88, BCL2A1C, BCL2A1B, BCL2A1A, BCL2, TICAM1, GM11787, IL1B, CD14, LTA	5.282365	6.97E-07	1.22E-04

Table S4. List of significant KEGG Pathways enriched for significantly upregulated genes from murine aged vs young marrow macrophages.