Figure S1

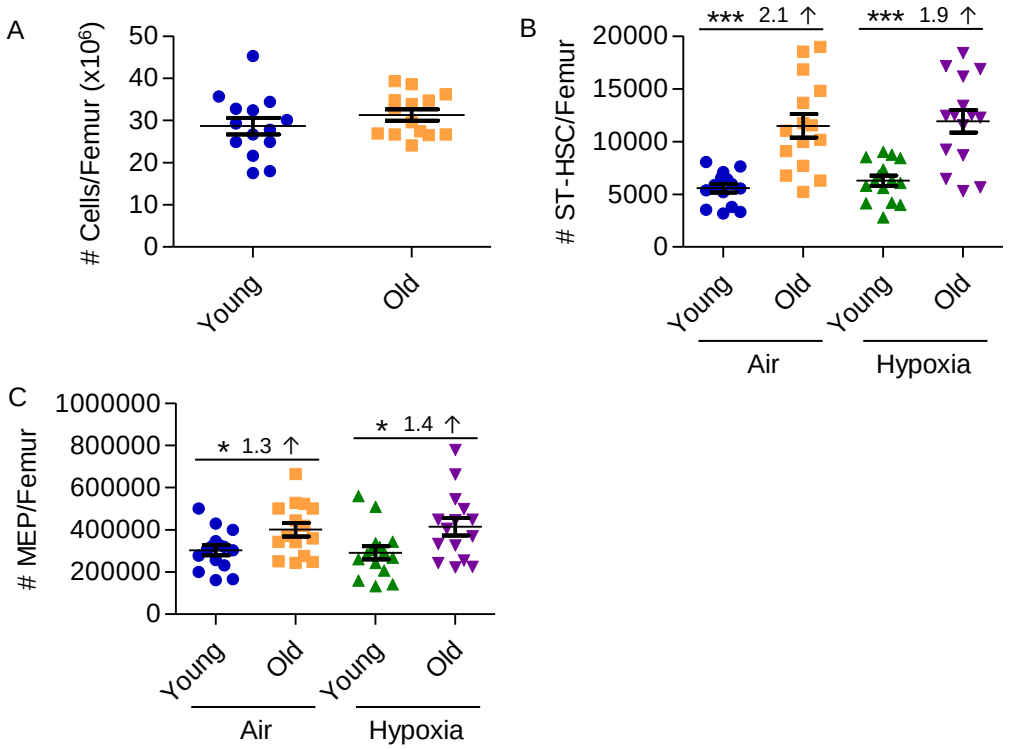

Figure S1. BM HSC and HPC phenotyping of young and old C57BL/6 mice collected in hypoxia (3% O_2) and processed under ambient air (21% O_2) versus hypoxia. In a hypoxic chamber (acclimated for 18 hours to 3% O_2), femurs of young (8-12 week old) and old (20-28 month old) male and female C57BL/6 mice were flushed in sterile PBS, counted, and then split in half. One half remained under hypoxic conditions (indicated as 'Hypoxia') and the other was removed from the hypoxic glove box and acclimated for 1 hour to ambient air conditions (indicated as 'Air'). Nucleated BM cells from each group were analyzed by flow cytometry (see Figure 1A for experimental setup). (A) Absolute nucleated cell counts of femurs isolated from young and old male and female C57BL/6 mice. (B) Short-term (ST)-HSC number per femur. (C) Megakaryocyte-erythrocyte progenitor (MEP) number per femur. (A-C) Data represents SEM of 11-15 C57BL/6 mice from 3-4 experiments. * $p<0.05$ and *** $p<0.001$ as determined by One-way ANOVA with post-hoc Tukey's Multiple Comparison Test.

Figure S2

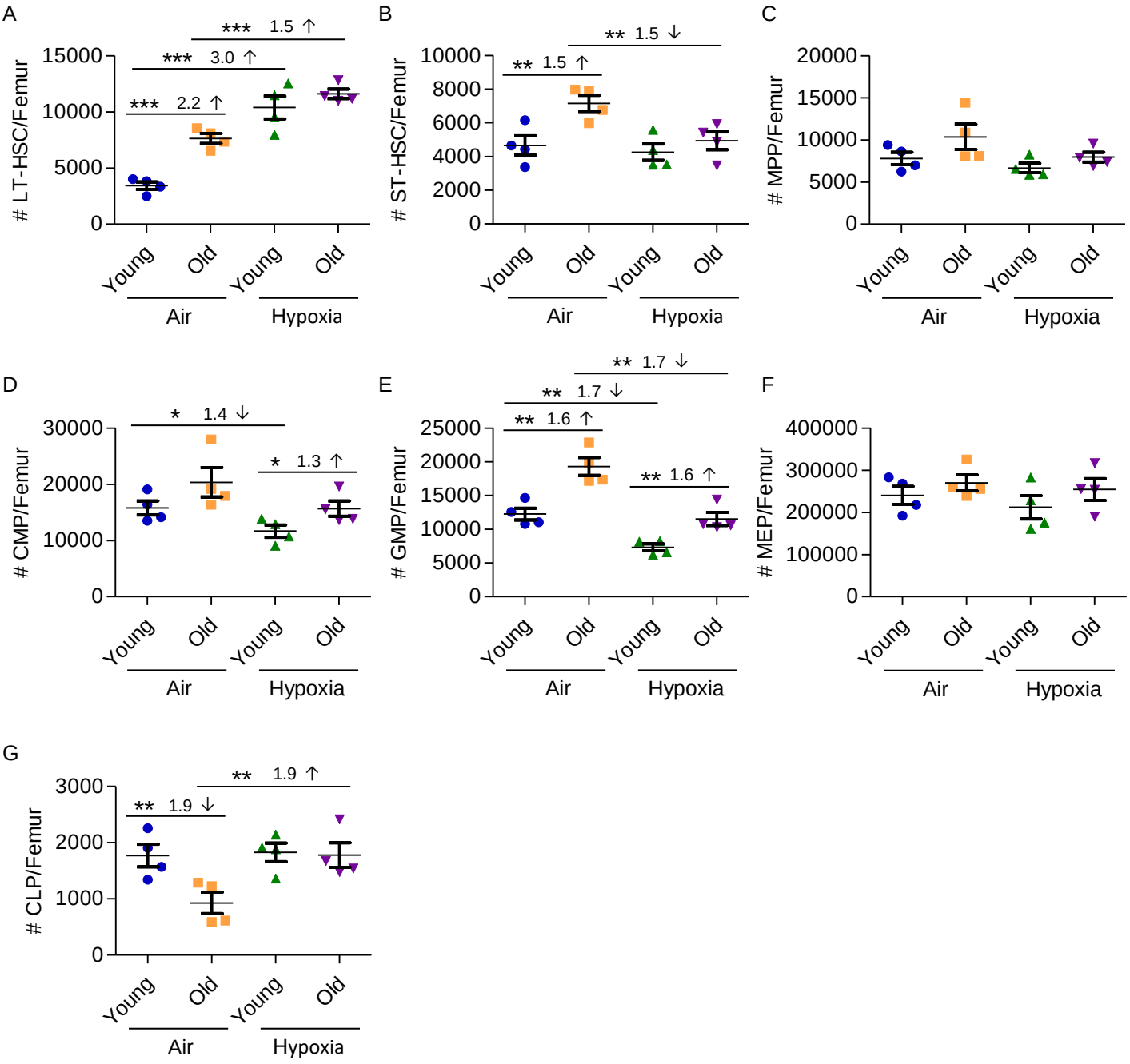
A
LT-HSC/Femur
15000
10000
5000
0
*** 1.5 ↑
*** 3.0 ↑
*** 2.2 ↑
Young
Old
Young
Old
Air
Hypoxia
B
ST-HSC/Femur
10000
8000
6000
4000
2000
0
** 1.5 ↓
** 1.5 ↑
Young
Old
Young
Old
Air
Hypoxia
C
MPP/Femur
20000
15000
10000
5000
0
Young
Old
Young
Old
Air
Hypoxia
D
CMP/Femur
30000
20000
10000
0
* 1.4 ↓
* 1.3 ↑
Young
Old
Young
Old
Air
Hypoxia
E
GMP/Femur
25000
20000
15000
10000
5000
0
** 1.7 ↓
** 1.7 ↓
** 1.6 ↑
** 1.6 ↑
Young
Old
Young
Old
Air
Hypoxia
F
MEP/Femur
400000
300000
200000
100000
0
Young
Old
Young
Old
Air
Hypoxia
G
CLP/Femur
3000
2000
1000
0
** 1.9 ↑
** 1.9 ↓
Young
Old
Young
Old
Air
Hypoxia

Figure S2. BM HSC and HPC phenotyping of young and old CB6 mice collected in hypoxia and processed under ambient air versus hypoxia. BM cells from young and old CB6 donor mice were collected/processed as noted in Figure 1A then flow cytometry was performed. (A) Long-term (LT)-HSC numbers per femur. (B) ST-HSC numbers per femur. (D) multipotent progenitor (MPP) numbers per femur. (E) Common myeloid progenitor (CMP) numbers per femur. (F) Granulocyte macrophage progenitor (GMP) numbers per femur. (G) MEP numbers per femur. (H) Common lymphoid progenitor (CLP) numbers per femur. Data represents SEM of 4 CB6 mice. * $p<0.05$, ** $p<0.01$, and *** $p<0.001$ as determined by One-way ANOVA with post-hoc Tukey's Multiple Comparison Test.

Figure S3

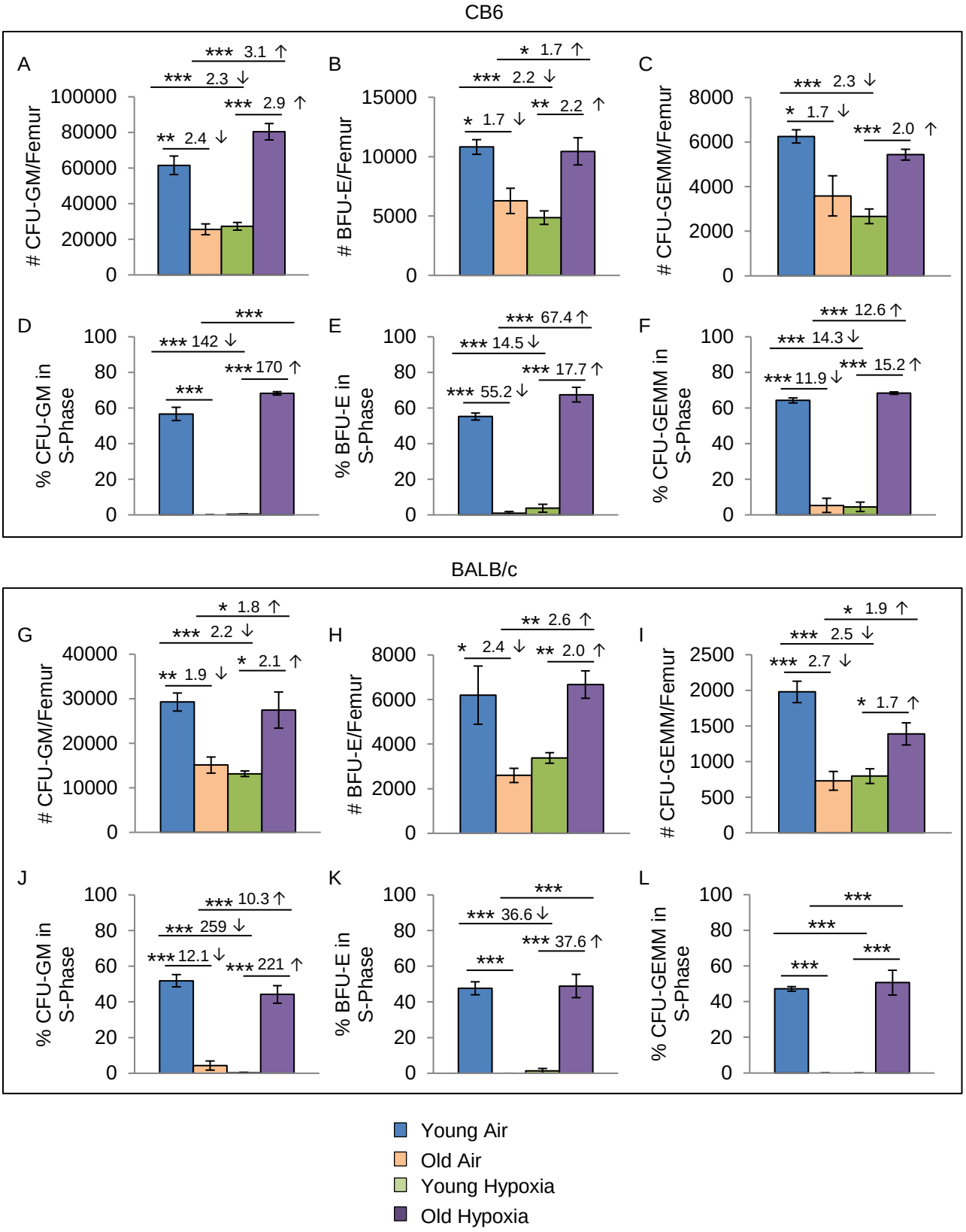
CB6
A
CFU-GM/Femur
100000
80000
60000
40000
20000
0
** 2.4 ↓
*** 2.3 ↓
*** 3.1 ↑
*** 2.9 ↑
B
BFU-E/Femur
15000
10000
5000
0
* 1.7 ↓
*** 2.2 ↓
* 1.7 ↑
** 2.2 ↑
C
CFU-GEMM/Femur
8000
6000
4000
2000
0
* 1.7 ↓
*** 2.3 ↓
*** 2.0 ↑
D
% CFU-GM in S-Phase
100
80
60
40
20
0
*** 142 ↓
***
***
*** 170 ↑
E
% BFU-E in S-Phase
*** 14.5 ↓
*** 55.2 ↓
*** 67.4 ↑
*** 17.7 ↑
F
% CFU-GEMM in S-Phase
*** 14.3 ↓
*** 11.9 ↓
*** 12.6 ↑
*** 15.2 ↑
BALB/c
G
CFU-GM/Femur
40000
30000
20000
10000
0
** 1.9 ↓
*** 2.2 ↓
* 1.8 ↑
* 2.1 ↑
H
BFU-E/Femur
8000
6000
4000
2000
0
* 2.4 ↓
** 2.6 ↑
** 2.0 ↑
I
CFU-GEMM/Femur
2500
2000
1500
1000
500
0
*** 2.7 ↓
*** 2.5 ↓
* 1.9 ↑
* 1.7 ↑
J
% CFU-GM in S-Phase
*** 12.1 ↓
*** 259 ↓
*** 10.3 ↑
*** 221 ↑
K
% BFU-E in S-Phase
*** 36.6 ↓
***
***
*** 37.6 ↑
L
% CFU-GEMM in S-Phase
***
***
***
***
Young Air
Old Air
Young Hypoxia
Old Hypoxia

Figure S3. Numbers of functional HPC, assessed by colony assay, of young and old CB6 and BALB/c mice collected in hypoxia and processed under ambient air versus hypoxia. In a hypoxic glove box (3% O_2), femurs of young (8-12 weeks old) and old (24-25 months old) CB6 (A-F) and BALB/c (G-L) were processed as in Figure 1A. After acclimation time, nucleated BM cells from each group were utilized in a HPC colony forming cell assay stimulated *in vitro* with EPO, SCF, PWMSCM, and hemin (A-C and G-I) with the percent of HPC in S-phase defined by high specific activity tritiated thymidine kill assay (D-F and J-L). Numbers of colony-forming units granulocyte-macrophage (CFU-GM; A, D, G, and J), burst-forming unit erythroid (BFU-E; B, E, H, and K) and CFU granulocyte-erythrocyte-macrophage-megakaryocyte (CFU-GEMM; C, F, I, and L) were calculated per femur. Data represents SEM of 4 CB6 and BALB/c mice. * $p<0.05$, ** $p<0.01$, and *** $p<0.001$ as determined by One-way ANOVA with post-hoc Tukey's Multiple Comparison Test.

Figure S4

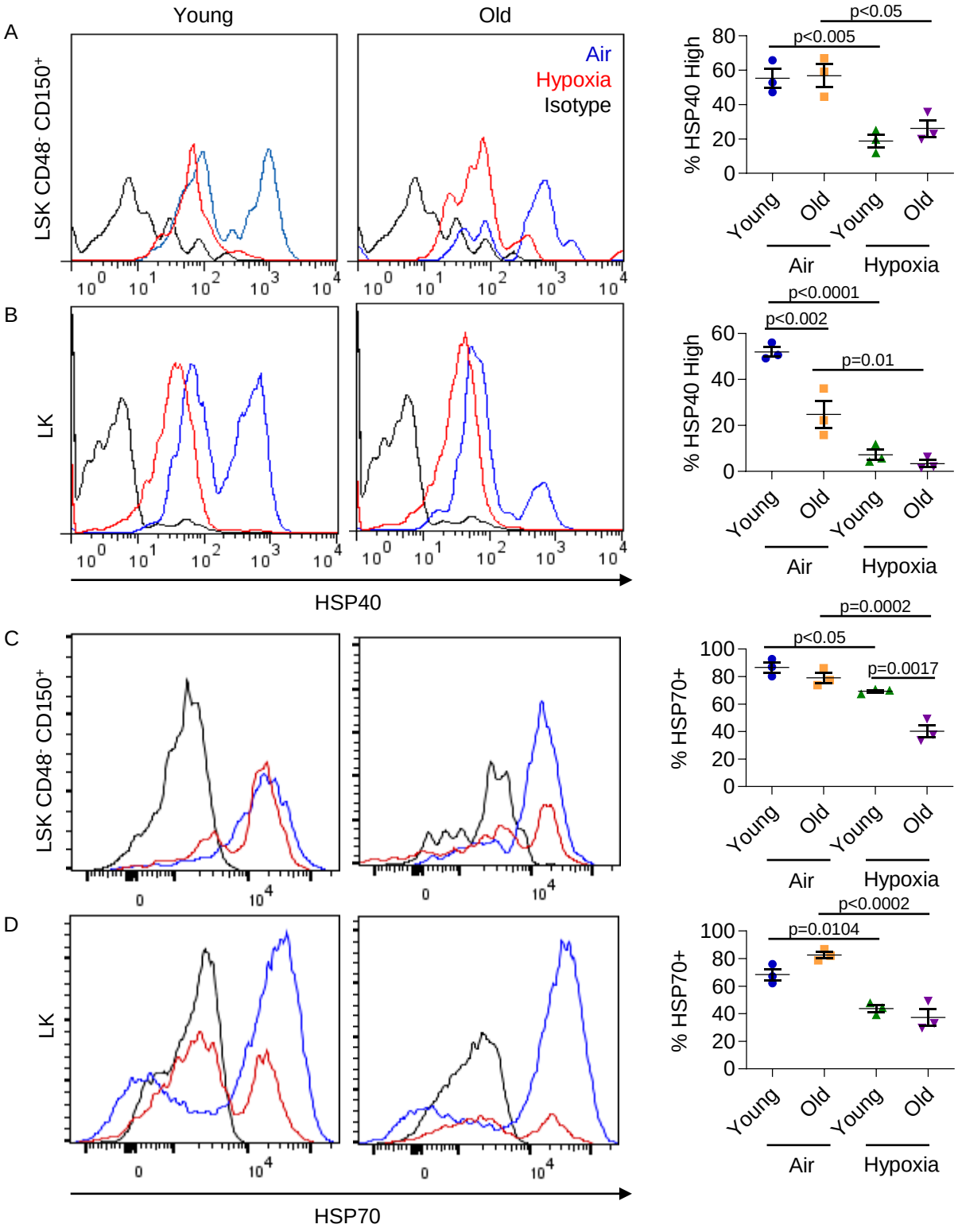
A
Young
Old
Air
Hypoxia
Isotype
LSK CD48- CD150+
% HSP40 High
p<0.05
p<0.005
Young
Old
Air
Hypoxia
B
LK
HSP40
p<0.0001
p<0.002
p=0.01
C
LSK CD48- CD150+
% HSP70+
p=0.0002
p<0.05
p=0.0017
D
LK
HSP70
p<0.0002
p=0.0104

Figure S4. HSC and HPC HSP40 and HSP70 expression in the BM of young and old C57BL/6 mice collected in hypoxia and processed under ambient air versus hypoxia. BM cells from young and old C57BL/6 donor mice were collected/processed as noted in Figure 1A. HSC (LSK $CD48^-$ $CD150^+$; A&C), and LK (B&D) cells were analyzed by flow cytometry for HSP40 (A-B) and HSP70 (C-D) expression. Data are representative of 2 separate experiments. Data represents SEM of 3 C57BL/6 mice per group. Statistics were determined by One-way ANOVA with post-hoc Tukey's Multiple Comparison Test.

Figure S5

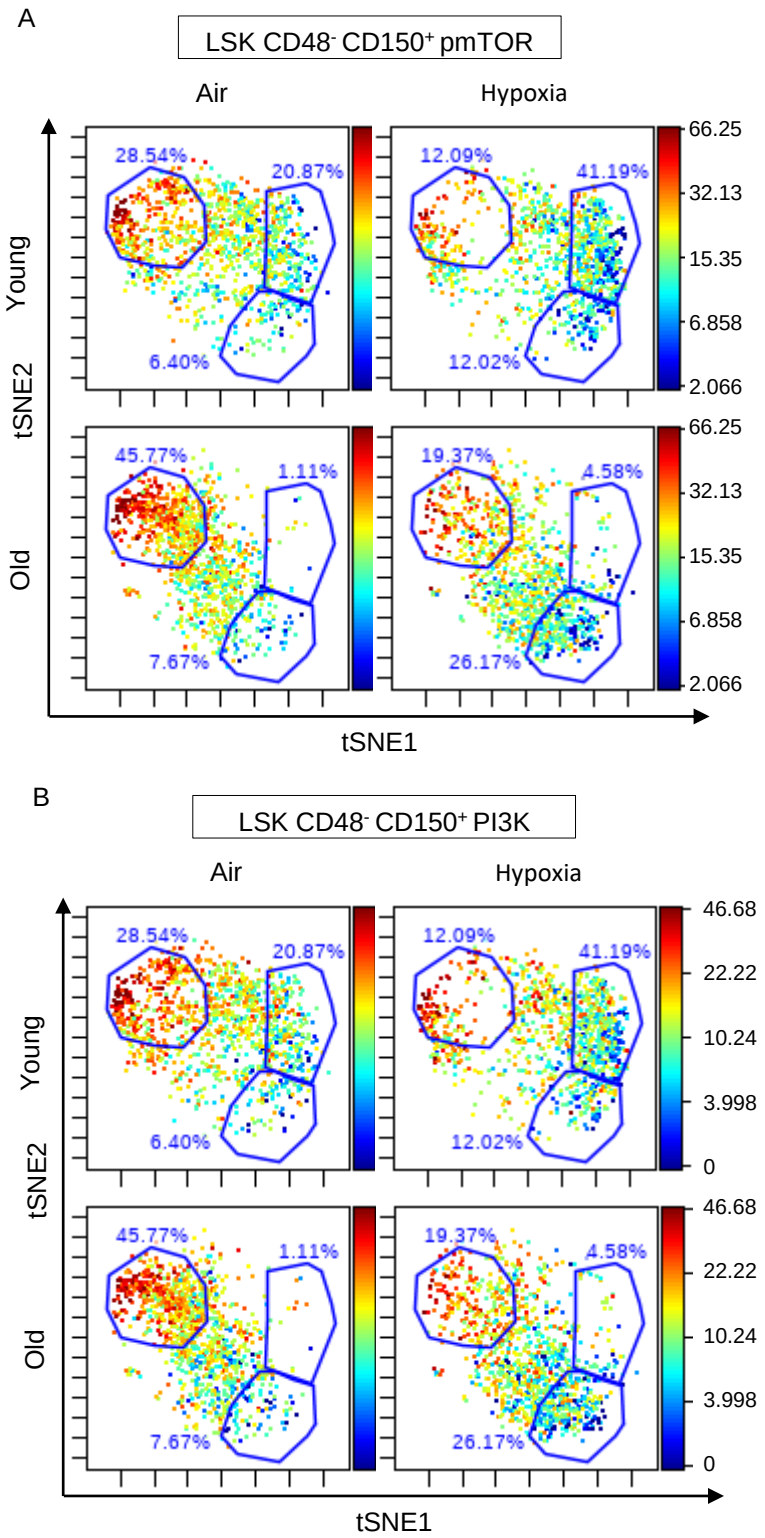
A
LSK CD48- CD150+ pmTOR
Air
Hypoxia
Young
Old
tSNE2
tSNE1
28.54%
20.87%
6.40%
12.09%
41.19%
12.02%
45.77%
1.11%
7.67%
19.37%
4.58%
26.17%
66.25
32.13
15.35
6.858
2.066
B
LSK CD48- CD150+ PI3K
Air
Hypoxia
Young
Old
tSNE2
tSNE1
28.54%
20.87%
6.40%
12.09%
41.19%
12.02%
45.77%
1.11%
7.67%
19.37%
4.58%
26.17%
46.68
22.22
10.24
3.998
0

Figure S5. PI3K and pmTOR expression is decreased in hypoxia collected and processed LT-HSC in BM of young and old mice. pmTOR (A) and PI3K (B) levels within gated LSK $CD48^{-}$ $CD150^{+}$ plots using viSNE analysis. Scales are the mean marker intensity of ArcSinh transformed values. (A&B) Populations changed by ambient air exposure are labeled as gate I, II and III. Percent of cells within the gate are indicated. Data is a representative of three separate experiments.

Figure S6

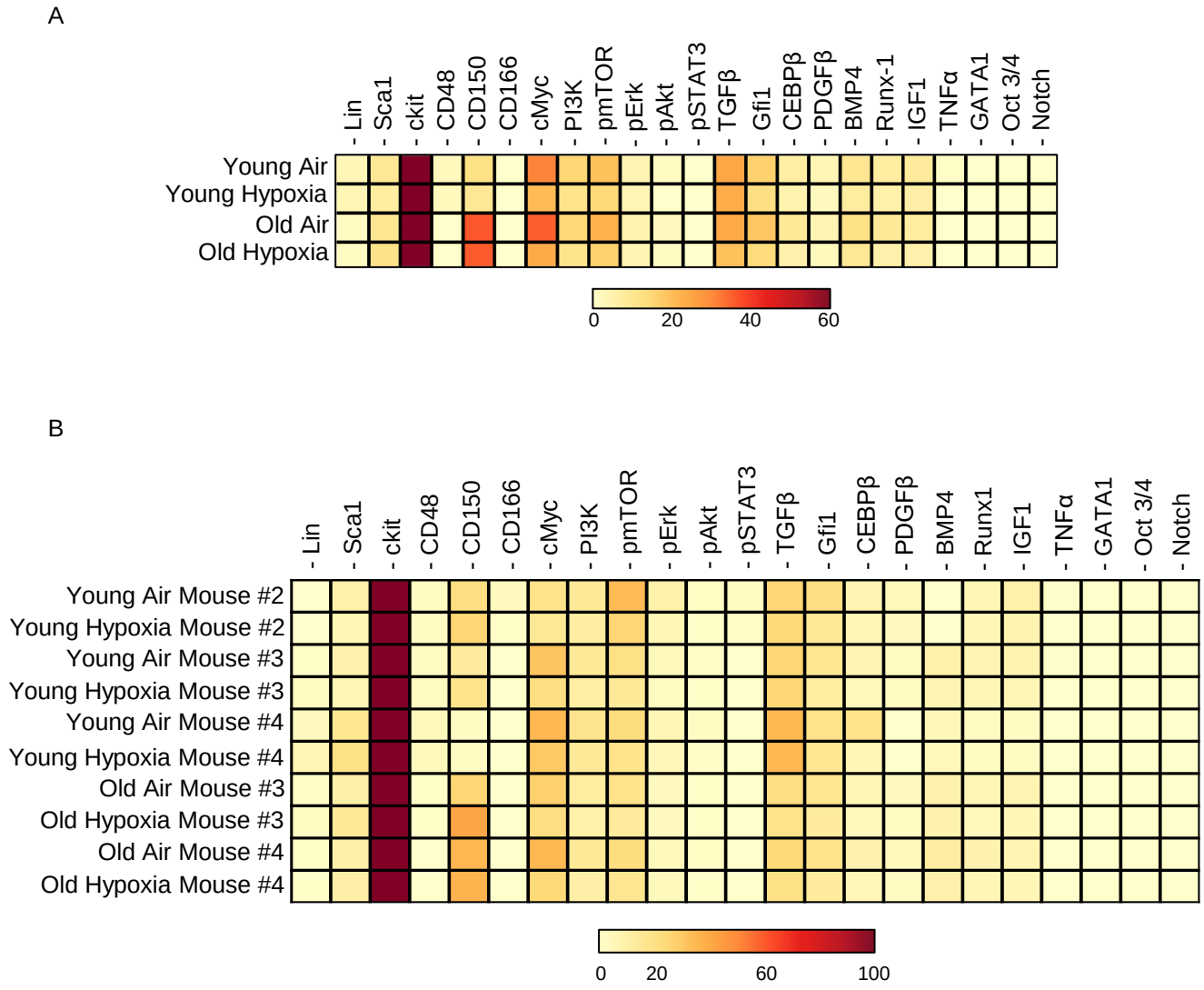

Figure S6. CyTOF analysis of gated LT-HSCs among ambient air-acclimated and hypoxia-collected and processed lineage negative BM cells. BM from young and old C57BL/6 donor mice were collected/processed as in Figure 1A. Samples were stained with indicated antibodies conjugated to metal (see Table S3), then analyzed by CyTOF 2 mass cytometer and Cytbank software. (A) Heatmap of total LSK CD48- CD150+ LT-HSC population protein concentrations shown in Figure 6 and Figure S5 of indicated mice determined through analysis of normalized CyTOF events. (B) Heatmap of replicate experiments shown in (A).

Table S1: Determination of CRU frequency by limiting dilution assay.

Group	Number of cells transplanted	Number of mice with >15% donor chimerism/total number of mice	CRU frequency	95% confidence interval	Number of CFU in $1x10^6$ BM cells
Young Air	25,000	0/5	1/51,471	1/106,096 to 1/24,970	19.4
	50,000	3/4			
	100,000	5/5			
Old Air	25,000	0/5	1/221,246	1/670,272 to 1/73,030	4.52[a]
	50,000	0/4			
	100,000	3/5			
Young Hypoxia	25,000	2/4	1/20,866	1/46,064 to 1/9,452	47.9[b]
	50,000	5/5			
	100,000	4/4			
Old Hypoxia	25,000	0/6	1/74,772	1/156,384 to 1/35,751	13.4[a,b]
	50,000	3/6			
	100,000	4/4			

[a], $p<0.05$ when comparing results from transplants receiving young versus old BM donor cells isolated under the same O_2 condition.
[b], $p<0.05$ when comparing results from transplants receiving young versus old BM donor cells isolated under different O_2 conditions.

Table S2: Subpopulation definitions for Figure 6B.

Color	Subpopulation #	Phenotype
	1	$CD150^{+}$ $cMyc^{+}$ $IGF1^{+}$ $Runx1^{+}$ $PDGF\beta^{+}$ $Gfi1^{+}$ $pErk1/2^{+}$ $TGF\beta^{+}$ $PI3K^{+}$ $pmTOR^{+}$
	2	$CD150^{h}$ $cMyc^{h}$ $IGF1^{+}$ $Runx1^{+}$ $PDGF\beta^{h}$ $Gfi1^{h}$ $pErk1/2^{+}$ $TGF\beta^{h}$ $TNF\alpha^{+}$ $BMP4^{h}$ $pAkt^{+}$ $PI3K^{h}$ $pmTOR^{h}$ $CEBP\beta^{h}$
	3	$CD150^{+}$ $cMyc^{+}$ $IGF1^{low}$ $Runx1^{+}$ $Gfi1^{+}$ $TGF\beta^{low}$ $BMP4^{+}$ $PI3K^{+}$ $pmTOR^{+}$
	4	$CD150^{+}$ $cMyc^{+}$ $IGF1^{low}$ $Gfi1^{+}$ $TGF\beta^{low}$ $BMP4^{+}$ $PI3K^{+}$ $pmTOR^{+}$
	5	$CD150^{+}$ $cMyc^{+}$ $IGF1^{+}$ $Runx1^{+}$ $Gfi1^{h}$ $TGF\beta^{h}$ $BMP4^{+}$ $PI3K^{+}$ $pmTOR^{+}$
	6	$CD150^{+}$ $cMyc^{+}$ $IGF1^{+}$ $Runx1^{+}$ $Gfi1^{h}$ $TGF\beta^{+}$ $BMP4^{+}$ $pAkt^{+}$ $PI3K^{+}$ $pmTOR^{+}$ $CEBP\beta^{+}$
	7	$CD150^{h}$ $IGF1^{+}$ $Runx1^{+}$ $Gfi1^{h}$ $pErk^{+}$ $TGF\beta^{+}$ $BMP4^{+}$ $pAkt^{+}$ $PI3K^{+}$ $pmTOR^{+}$
	8	$CD150^{h}$ $cMyc^{+}$ $IGF1^{+}$ $Gfi1^{h}$ $pErk^{+}$ $TGF\beta^{+}$ $BMP4^{+}$ $PI3K^{+}$ $pmTOR^{+}$ $CEBP\beta^{+}$
	9	$CD150^{+}$ $cMyc^{h}$ $IGF1^{+}$ $Runx1^{+}$ $PDGF\beta^{+}$ $Gfi1^{h}$ $TGF\beta^{h}$ $BMP4^{low}$ $pAkt^{+}$ $PI3K^{h}$ $pmTOR^{h}$ $CEBP\beta^{h}$
	10	$CD150^{h}$ $cMyc^{h}$ $IGF1^{+}$ $Runx1^{+}$ $PDGF\beta^{+}$ $Gfi1^{h}$ $pErk^{+}$ $TGF\beta^{+}$ $BMP4^{+}$ $pAkt^{+}$ $PI3K^{h}$ $pmTOR^{h}$ $CEBP\beta^{h}$

Table S3: Antibodies used for CyTOF.

Antibody Targets	Company	Clone(s)	Metal Conjugate
Lineage Cocktail (Lin; includes markers CD3/GR-1 CD11b/B220/Ter-119)[a]	BioLegend	Cat. #133301	160Gd
Stem Cell Antigen 1 (Sca1; a.k.a. Ly6a)[b]	Invitrogen	D7	145Nd
cKit (a.k.a. CD117 and Stem Cell Factor Receptor)	Fluidigm	2B8	166Er
CD48	Fluidigm	HM48.1	156Gd
CD150 (a.k.a. Signaling Lymphocytic-Activation Molecule or SLAM)[c]	Invitrogen	9D1	176Yb
CD166 (a.k.a. Activated Leukocyte Cell Adhesion Molecule or ALCAM)	eBioscience	eBioALC48	151Eu
Octamer-Binding Transcription Factor 3/4 (Oct3/4; a.k.a. POU5f1)	DVS Sciences	40/Oct-3	165Ho
Tumour Necrosis Factor α (TNFα)	Fluidigm	MP6-XT22	165Dy
Platelet-Derived Growth Factor β (PDGFβ)	Fluidigm	Polyclonal	169Tm
Insulin-Like Growth Factor 1 (IGF1)	Fluidigm	Polyclonal	174Yb
Transforming Growth Factor β (TGFβ)	Fluidigm	TW7-16B4	164Dy
CCAAT/Enhancer-Binding Protein β (CEBPβ)[d]	BioLegend	1H7	141Pr
Bone Morphogenetic Protein 4 (BMP4)[d]	Invitrogen	Polyclonal	159Tb
Runt-Related Transcription Factor 1 (Runx1)[d]	Invitrogen	3H2L6	170Er
GATA Binding Protein 1 (GATA1)[d]	R&D Systems	234737	149Sm
cMyc[d]	Invitrogen	9E10	175Lu
Notch1[d]	BioLegend	HMN1-12	171Yb
Growth Factor Independent 1 Transcriptional Repressor (Gfi1)[d]	Invitrogen	Polyclonal	168Er
Phosphoinositide 3-kinase p85α (PI3K)[d]	NOVUS	6G10	150Nd
Akt [pS473] (a.k.a. pAKT or Protein Kinase B)	Fluidigm	D9E	152Sm
Extracellular Signal-Regulated Kinase 1/2 [202/204] (a.k.a. pERK1/2)	Fluidigm	D13.14.4E	167Er
Signal Transducer and Activator of Transcription 3 [Y705] (a.k.a. pSTAT3)	Fluidigm	4/P-STAT3	158Gd
Mammalian Target of Rapamycin [Ser2448] (a.k.a. pmTOR)[d]	BioLegend	A17024A	143Nd

[a], Lineage cocktail antibodies were conjugated to FITC. A secondary anti-FITC antibody was used (Fluidigm; clone FIT-22).
[b], Anti-Sca1 antibody was conjugated to PE. A secondary anti-PE antibody was used (DVS Sciences; clone PE001).
[c], Anti-CD150 antibody was conjugated to APC. A secondary anti-APC antibody was used (Fluidigm; clone APC003).
[d], Antibodies were conjugated to metals in house using the Maxpar Antibody Labeling kit (Fluidigm).