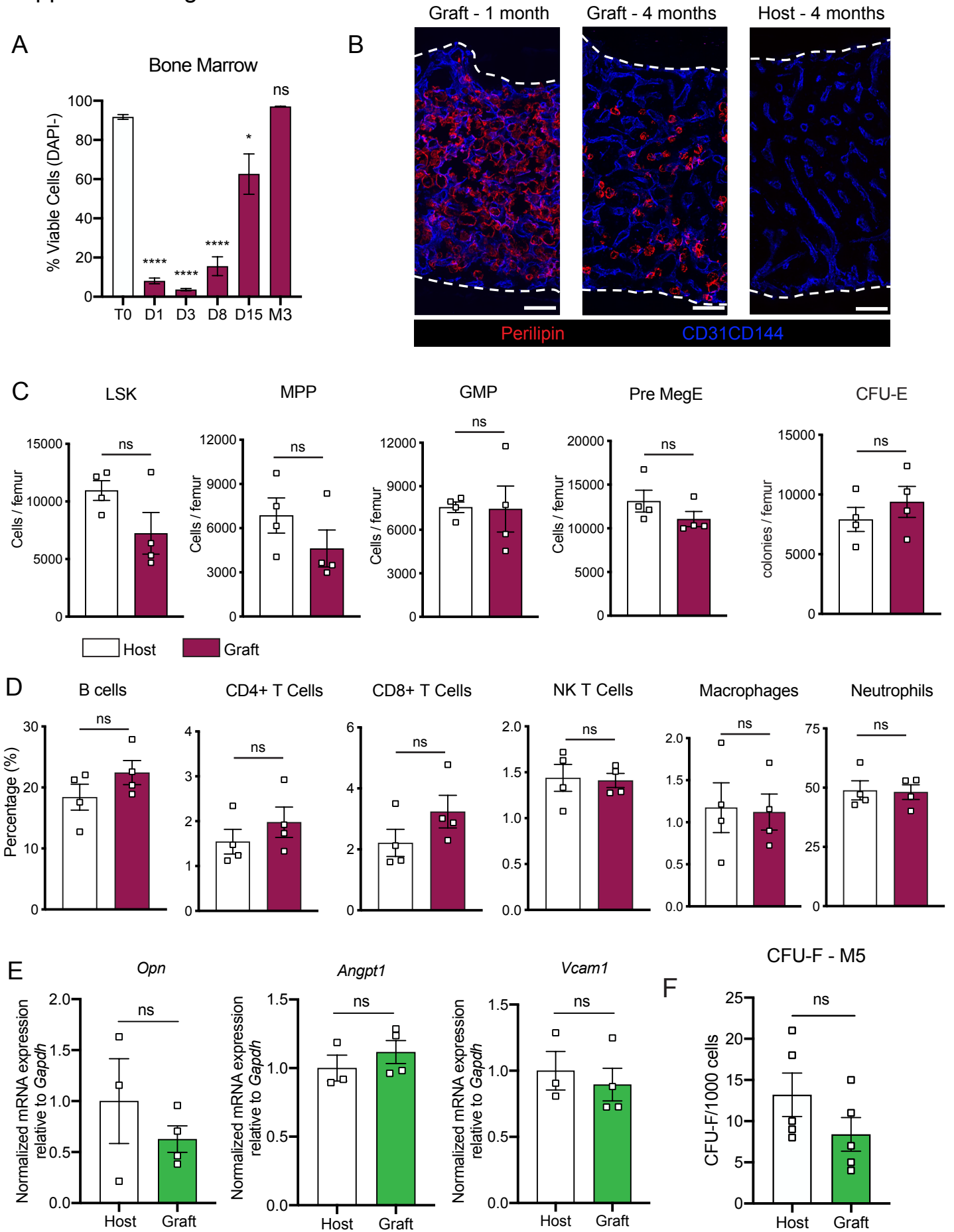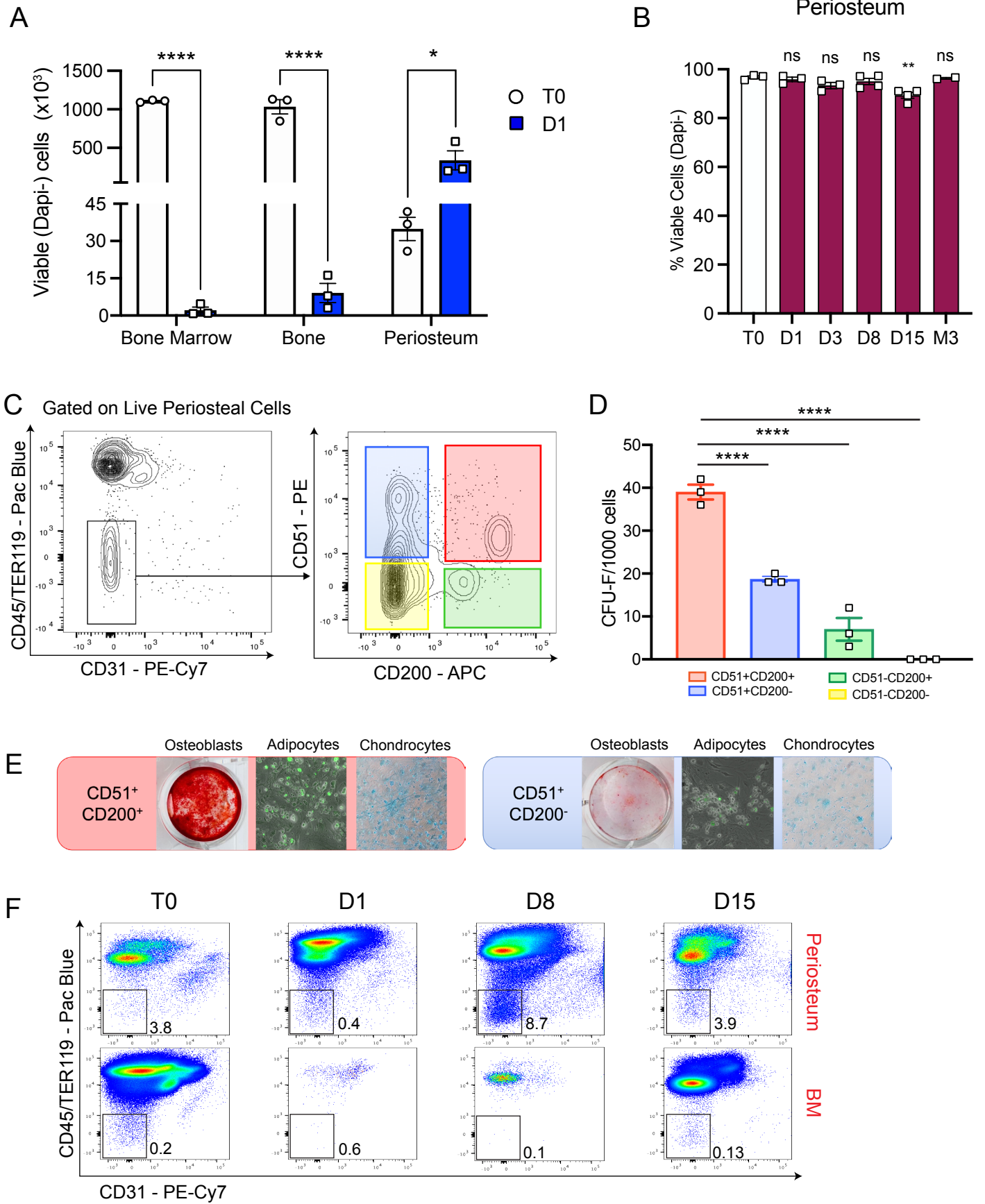# Supplemental Figure 1

Supplemental Figure 1. Bone marrow regeneration after transplantation.
A. Cell viability determined by flow cytometry and expressed as percentage of total DAPI- alive cells in the BM graft at steady state, 1, 3, 8, 15 days and 3 months after bone transplantation (n=3-4). One-way ANOVA with Dunnett's multiple comparisons test used to determine statistical significance.
B. Representative whole-mount confocal z-stack projections of host and graft femurs at 1 and 4 months after transplantation. Adipocytes are stained using an anti-perilipin antibody. Scale bar = 50µm. Three independent experiments yielded similar results.
C. Comparison of different hematopoietic progenitors' populations between graft and host at 5 months after bone transplantation (n=4 per group).
D. Comparison of different hematopoietic populations between graft and host at 5 months after bone transplantation (n=4 per group).
E. Quantitative RT-PCR analysis of mRNA expression of *Opn*, *Angpt1*, and *Vcam1* relative to *Gapdh* in host and graft *Nes*-GFP$^+$ BM-MSCs at 5 months after transplantation (n=3-4 mice).
F. Number of CFU-F colonies formed by flushed CD45$^-$Ter119$^-$*Nes*-GFP$^+$ BM-MSCs sorted from host and graft femurs 5 months after transplantation and plated at equal number and clonal densities under CFU-F culture conditions (n=5 mice per group). Data represented as mean ± SEM. Unless otherwise noted, statistical significance was determined using unpaired two-tailed Student's t test. *p<0.05. ** p<0.01. *** p<0.001. ****p<0.0001.
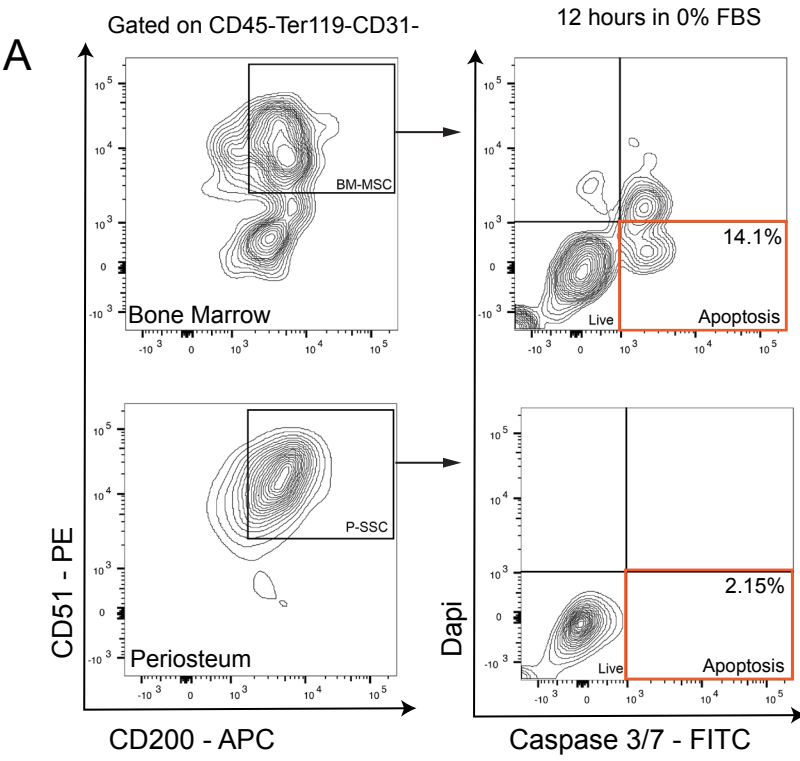
Supplemental Figure 2

A

Viable (Dapi-) cells (x10³)

○ T0
■ D1

**** **** *

Bone Marrow   Bone   Periosteum

B Periosteum

% Viable Cells (Dapi-)

ns ns ns ** ns

T0 D1 D3 D8 D15 M3

C Gated on Live Periosteal Cells

CD45/TER119 - Pac Blue
CD31 - PE-Cy7

CD51 - PE
CD200 - APC

D

CFU-F/1000 cells

**** **** ****

CD51+CD200+   CD51-CD200+
CD51+CD200-   CD51-CD200-

E

CD51⁺ CD200⁺   Osteoblasts   Adipocytes   Chondrocytes

CD51⁺ CD200⁻   Osteoblasts   Adipocytes   Chondrocytes

F

T0   D1   D8   D15

CD45/TER119 - Pac Blue

Periosteum
3.8   0.4   8.7   3.9

BM
0.2   0.6   0.1   0.13

CD31 - PE-Cy7

Supplemental Figure 2. Periosteal stem cells expansion after transplantation.
A. Number of viable cells within bone marrow, bone and periosteum at steady state and 24 hours post transplantation (n=3 per group).
B. Cell viability determined by flow cytometry and expressed as percentage of total DAPI- alive cells in the periosteum graft at steady state and at 1, 3, 8, 15 days and at 3 months after bone transplantation (n=2-4 per group). One-way ANOVA with Dunnett's multiple comparisons test was used to determine statistical significance.
C. Representative FACS plot showing gating of $CD51^+CD200^+$ P-SSCs after gating on stromal ($CD45^-Ter119^-CD31^-$) fraction.
D. Absolute CFU-F number from 1000 sorted cells within various cell populations of the periosteum. One-way ANOVA with Dunnett's multiple comparisons test was used to determine statistical significance.
E. Representative trilineage differentiation pictures of $CD51^+CD200^+$ compared to the group with the next highest CFU-F activity ($CD51^+CD200^-$ cells). Differentiation into osteoblasts, adipocytes and chondrocytes observed using Alizarin Red, Bodipy, and Alican Blue staining, respectively.
F. Representative FACS plots of graft periosteum and bone marrow analysis gated on CD45-Ter119-CD31- fraction at steady state and at one-, eight- and fifteen-days post transplantation. Data represented as mean ± SEM. Unless otherwise noted, statistical significance was determined using unpaired two-tailed Student's t test. *p<0.05. ** p<0.01. *** p<0.001. ****p<0.0001.

Supplemental Figure 3

A

Gated on CD45-Ter119-CD31-

12 hours in 0% FBS

CD51 - PE

CD200 - APC

Dapi

Caspase 3/7 - FITC

BM-MSC

Bone Marrow

P-SSC

Periosteum
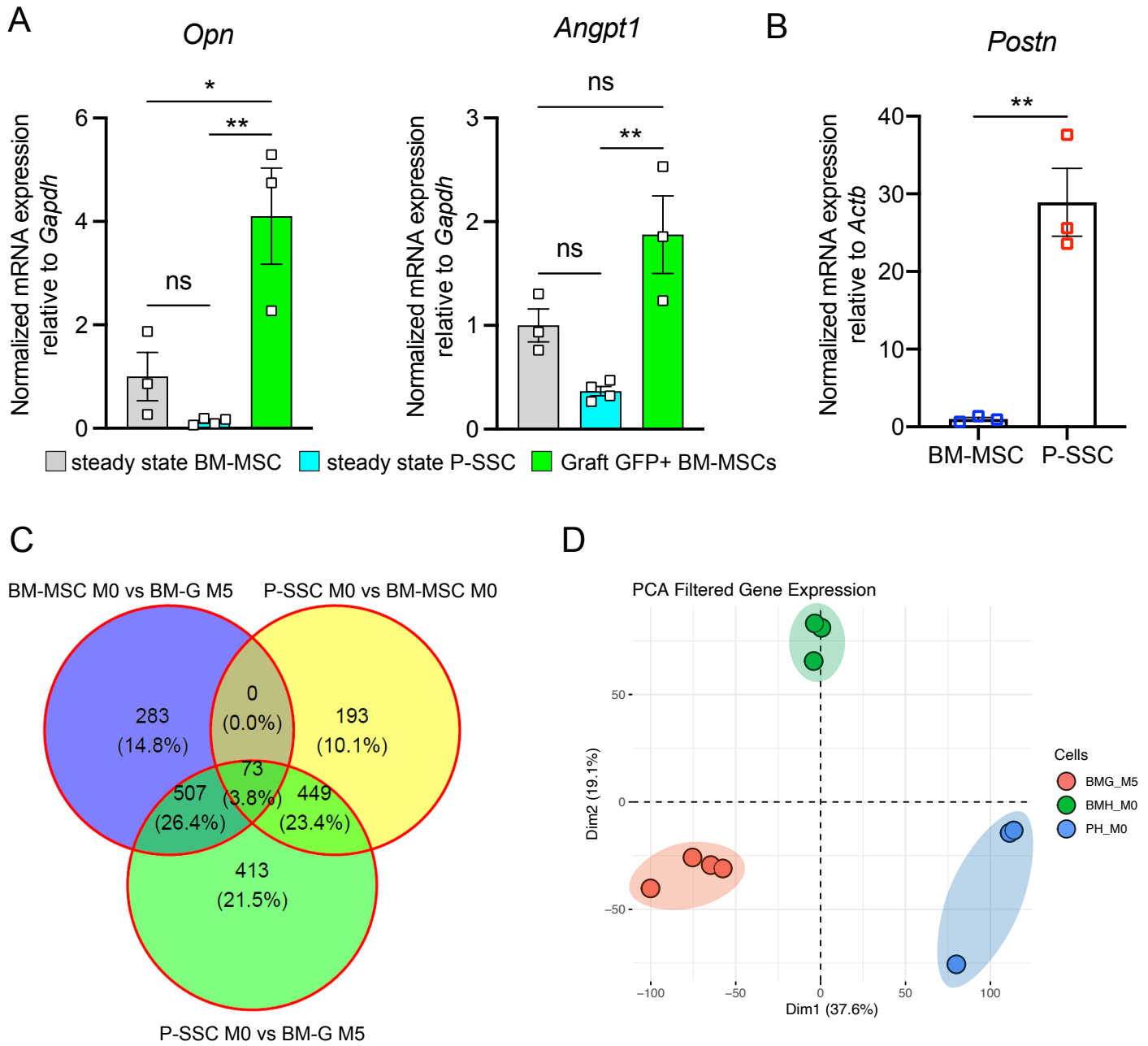
Live

Apoptosis

14.1%

Live

Apoptosis

2.15%

Supplemental Figure 3. Comparison of periosteal SSCs and bone marrow MSCs.
A. Representative FACS plots showing gating strategy for analyzing apoptosis at12 hours after switching from 20% to 0% FBS media.

Data represented as mean ± SEM. Unless otherwise noted, statistical significance was determined using unpaired two-tailed Student's t test. *p<0.05. ** p<0.01. *** p<0.001. ****p<0.0001.

Supplemental Figure 4

A

Opn

Angpt1

☐ steady state BM-MSC ☐ steady state P-SSC ☐ Graft GFP+ BM-MSCs

B

Postn

C

BM-MSC M0 vs BM-G M5   P-SSC M0 vs BM-MSC M0

P-SSC M0 vs BM-G M5

D

PCA Filtered Gene Expression

Supplemental Figure 4. Periosteal SSCs migrate into the bone marrow and upregulate HSC-niche factors.

A. Quantification of *Opn* and *Angpt1* mRNA level relative to *Gapdh* in sorted control bone marrow CD45⁻Ter119⁻CD31⁻Nestin-GFP⁺ BM-MSCs, CD45⁻Ter119⁻CD31⁻CD51⁺CD200⁺ steady-state P-SSCs, and CD45⁻Ter119⁻CD31⁻CD51⁺CD200⁺GFP⁺ graft BM-MSCs (n=3-4 per group). One-way ANOVA was used to determine statistical significance.

B. *Postn* expression in sorted, steady-state CD51⁺CD200⁺ BM-MSCs and P-SSCs from CD45.2 WT mice (n=3).

C. Venn diagram of RNA sequencing analysis comparing the numbers of differentially expressed genes between the groups

D. 2D principal component analysis (PCA) plot showing the variance between graft BM-MSCs at five months post transplantation, and BM-MSCs and P-SSCs at steady state (n=3-4).

Data represented as mean ± SEM. Unless otherwise noted, statistical significance was determined using unpaired two-tailed Student's t test. *p<0.05. ** p<0.01. *** p<0.001. ****p<0.0001.

Supplementary Table 1: Flow Cytometry Antibodies

Antibody	Clone	Fluorochrome (Manufacturer)
anti-CD45	30-F11	APC-Cy7 (eBioscience), Pacific Blue (Biolegend)
anti-Ter119	Ter-119	APC-Cy7 (eBioscience), Pacific Blue (Biolegend), AF700 (Biolegend), biotin (BD Biosciences)
anti-CD31	390	PE-Cy7 (Biolegend; eBioscience), AF647 (Biolegend)
anti-CD51	RMV-7	biotin (Biolegend), PE (eBioscience)
anti-CD200	OX-90	APC (Biolegend)
anti-CD140α	APA5	PE-Cy7 (eBioscience), APC (Biolegend)
anti-strepavidin	(n/a)	FITC (eBioscience), APC-Cy7 (eBioscience), BV570 (Biolegend)
anti-CD11b	M1/70	AF647 (Biolegend), biotin (BD Biosciences)
anti-CD4	GK1.5	PE-Cy7 (eBioscience)
anti-CD8a	53-6.7	PE-Cy7 (eBioscience)
anti-B220	RA3-6B2	PerCP-Cy5.5 (eBioscience), biotin (BD Biosciences)
anti-CD45.1	A20	PE (Biolegend)
anti-CD45.2	104	FITC (Biolegend)
anti-Ki67	SolA15	FITC (eBioscience)
anti-CD144	BV13	AF647 (Biolegend)
anti-Gr-1	RB6-8C5	APC-Cy7 (eBioscience), FITC, biotin (BD Biosciences)
anti-CD150	TC15-12F12.2	PE (Biolegend)
anti-CD117	2B8	BV421 (Biolegend)
anti-Sca-1	D7	FITC (eBioscience)
anti-CD105	MJ7/18	PE-Cy7 (Biolegend)
anti-CD41	MWReg30	PerCP-e710 (eBioscience)
anti-CD16/32	93	APC-Cy7(Biolegend)
anti-CD48	HM48-1	AF647 (Biolegend)
anti-CD3e	145-2C11	biotin (BD Biosciences)
anti-F4/80	BM8	AF647 (Biolegend)
anti-NK1.1	PK136	BV421 (Biolegend)
anti-CD115	AFS98	PE (eBioscience)

Supplementary Table 2: RT-PCR primer sequences

Primer	Forward Sequence (5' → 3')	Reverse Sequence (5' → 3')
Cxcl12	5'-CGCCAAGGTCGTCGCCG-3'	5'-TTGGCTCTGGCGATGTGGC-3'
Kitl	5'-CCCTGAAGACTCGGGCCTA-3'	5'-CAATTACAAGCGAAATGAGAGCC-3'
Opn	5'-AGCAAGAAACTCTTCCAAGCAA-3'	5'-GTGAGATTCGTCAGATTCATCCG-3'
Angpt1	5'-CTCGTCAGACATTCATCATCCAG-3'	5'-CACCTTCTTTAGTGCAAAGGCT-3'
Vcam1	5'-GACCTGTTCCAGCGAGGGTCTA-3'	5'-CTTCCATCCTCATAGCAATTAAGGTG-3'
Cdkn1a	5' CCTGGTGATGTCCGACCTG-3'	5' CCATGAGCGCATCGCAATC-3'
Cdkn1c	5-CGAGGAGCAGGACGAGAATC-3'	5' GAAGAAGTCGTTCGCATTGGC-3'
Cdk4	5'-ATGGCTGCCACTCGATATGAA 3'	5' TCCTCCATTAGGAACTCTCACAC-3'
Sod1	5'-TGGTGGTCCATGAGAAACAA-3'	5'-GTTTACTGCGCAATCCCAAT-3'
Gls	5' TTCGCCCTCGGAGATCCTAC-3'	5' CCAAGCTAGGTAACAGACCCT-3'
Gpx1	5'-AGTCCACCGTGTATGCCTTCT-3'	5'- GAGACGCGACATTCTCAATGA-3'-3'
Postn	5'-CCTGCCCTTATATGCTCTGCT-3'	5'-AAACATGGTCAATAGGCATCACT-3'
Gapdh	5'-TGTGTCCGTCGTGGATCTGA-3'	5'-CCTGCTTCACCACCTTCTTGA-3'
Actb	5' GCTTCTTTGCAGCTCCTTCGT-3'	5' ATCGTCATCCATGCCGAACT-3'