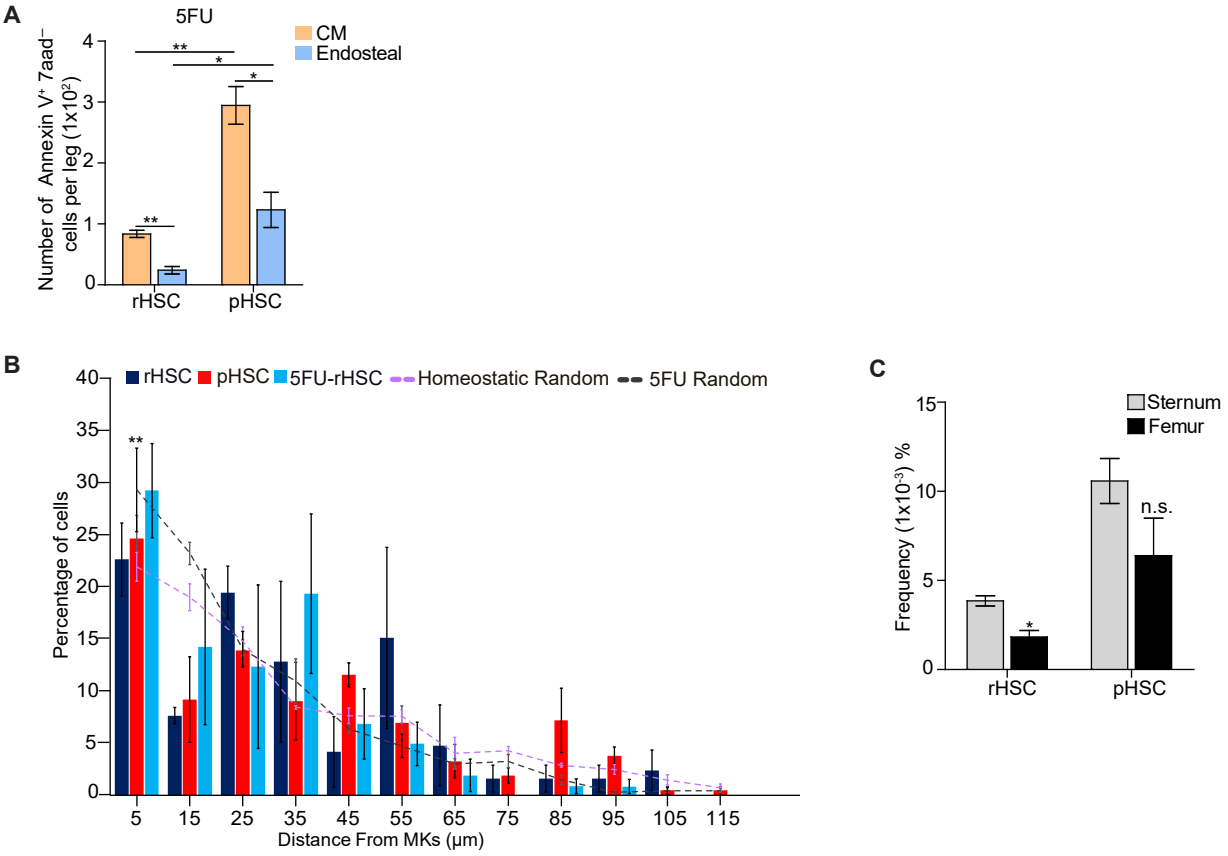


Figure S1. Apoptosis assay and distance quantification of rHSCs and pHSCs. Related to Figure 1.

(A) Frequency of Annexin V⁺7AAD⁻ rHSCs and pHSCs in the central marrow (CM) and endosteal region at day 1 after 5FU treatment (n=4).
(B) Relative distance between rHSCs, pHSCs and 5FU-rHSCs from MKs (n=51 rHSCs, n=144 pHSCs, n=102 5FU rHSCs).
(C) Flow cytometric analysis of frequency of rHSC and pHSC in sternum marrow and CM from femur. * P<0.05, ** P<0.01, ***P<0.001. Error bars, s.e.m.

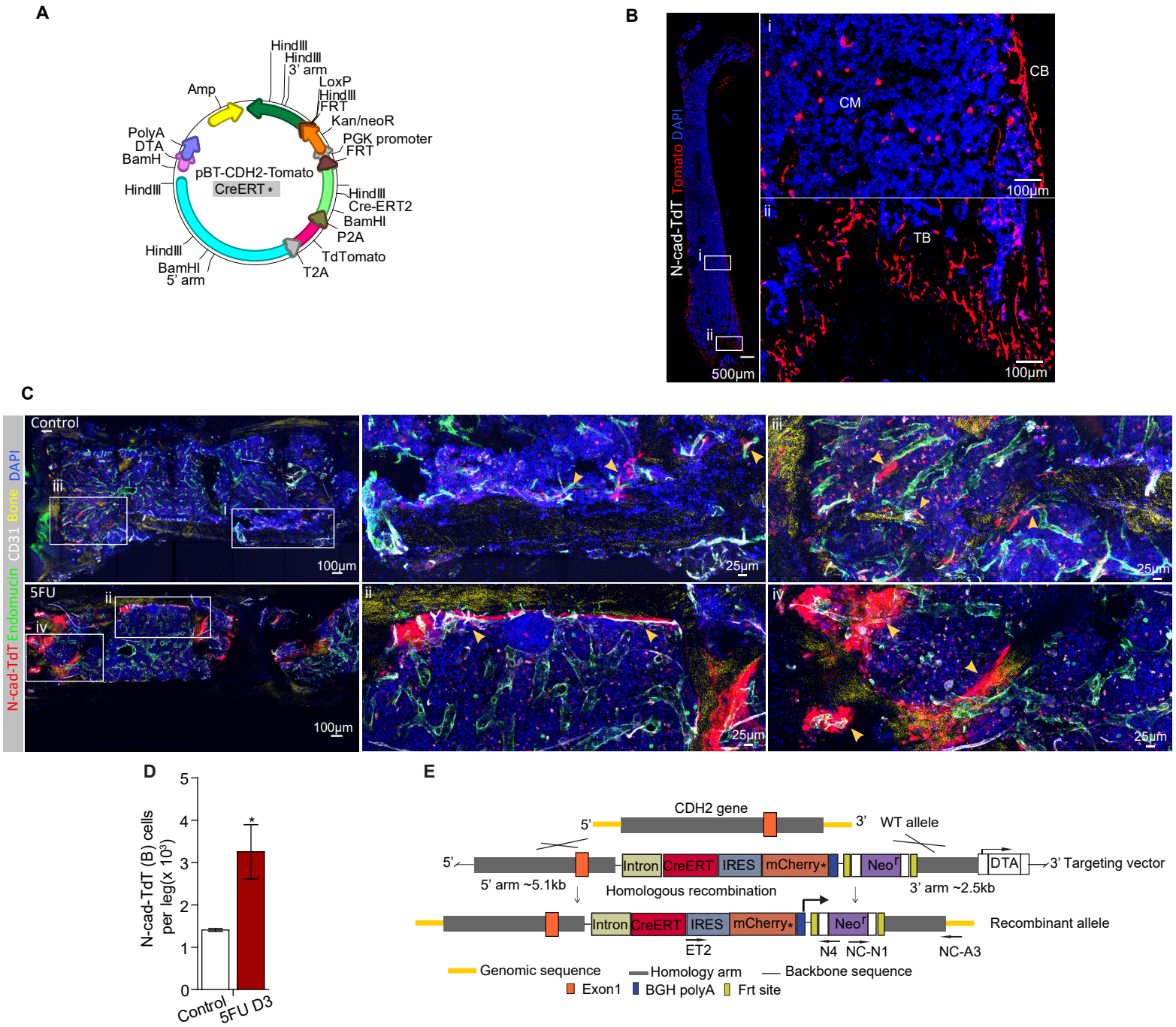


Figure S2. N-cad⁺ cells in the *N-cad-TdT* and *N-cad-CreER^T* mice strains. Related to Figure 2.

(A) CDH2-Tomato-CreER^T targeting Construct for generating *N-cad-TdT* mouse strain. The asterisk indicates inactive Cre recombinase post TMX induction. (B) Representative whole femur images from the *N-cad-TdT* mouse strain showing *N-cad* driven Tomato⁺ cells in CM, TB and CB. (C) Representative whole-mount images of sternum in *N-cad-TdT* mice showing Tomato⁺ cells with Endomucin⁺CD31⁺ vasculature and SHG signal (arrowhead) in homeostatsis and 3 days post 5FU. (D) Absolute number of Tomato⁺ cells in endosteal region of *N-cad-TdT* mice in homeostasis control and 3 days post 5FU (n=3). * P<0.05, ** P<0.01, ***P<0.001. Error bars, s.e.m. (E) Generation of N-cad-CreER^T mouse strain. The asterisk indicates no mCherry expression detected from *N-cad* promoter.

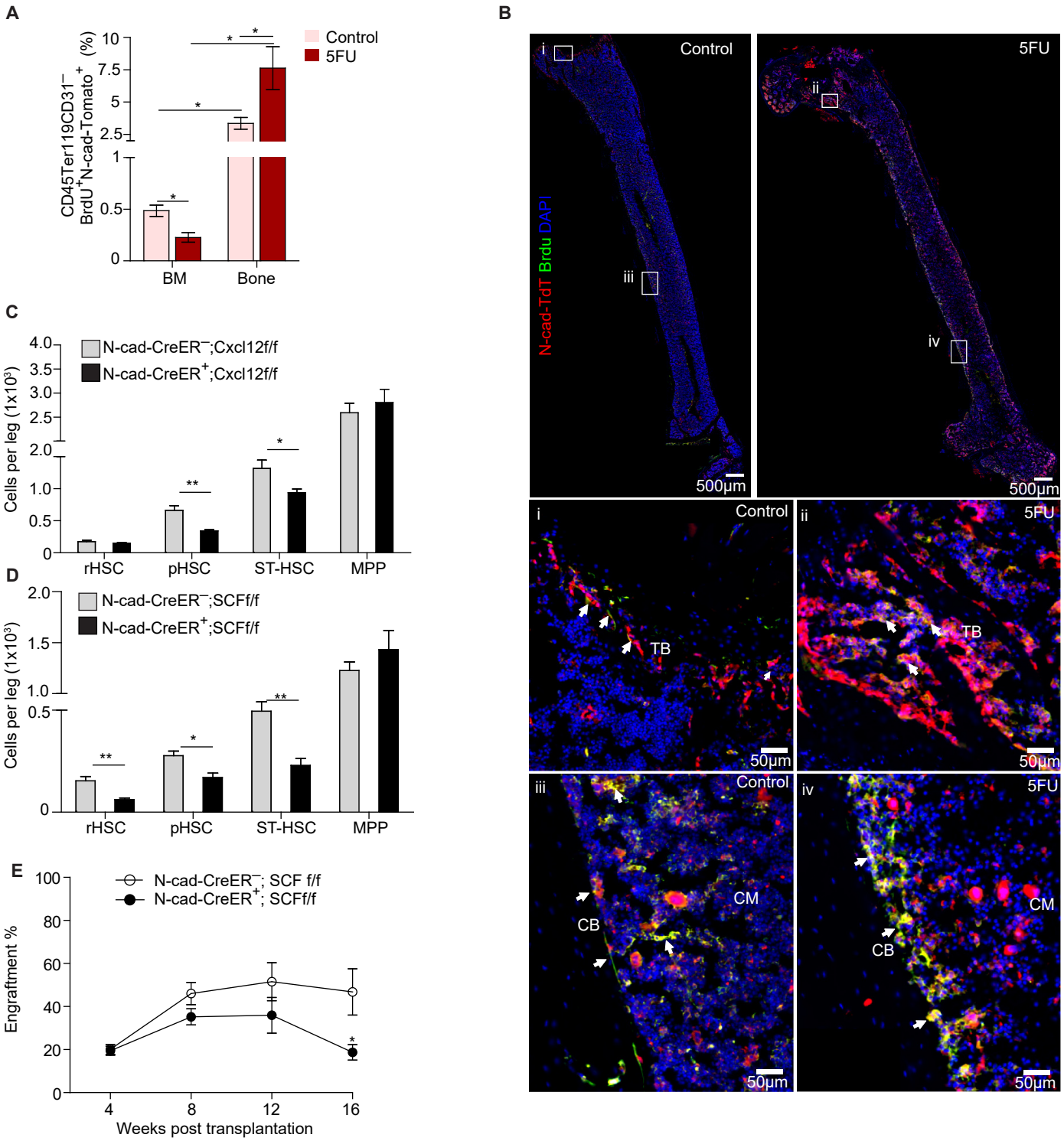


Figure S3. BrdU assay in N-cad-tomato⁺ cells and HSPCs analysis in N-cad-CreER^T mediated *Cxcl12* or *SCF* knockout mice. Related to Figure 2 and 3.

(A) Frequency of CD45Ter119CD31⁻BrdU⁺Tomato⁺ cells by flow cytometric analysis. Both groups (*N-cad-CreER^T;R26-tdT* mice) were induced with 3 TMX injections followed by 5FU and pulse-chased with BrdU for 3 days (n=3 per group). (B) Representative images of BrdU⁺ N-cad-Tomato⁺ cells in *N-cad-TdT* mice in homeostasis control at day 3 after 5FU. Arrow indicates BrdU⁺N-cad-Tomato⁺ cells in TB, CB and CM. (C-D) Flow cytometric analysis showing the absolute number of HSPCs in the BM from *N-cad-CreER^{T}Cxcl12 f/f* (*N-cad-CreER⁻;Cxcl12 f/f*, n=3, *N-cad-CreER⁺;Cxcl12 f/f*,n=6) and *N-cad-CreER^T;SCF f/f* (*N-cad-CreER⁻;SCF f/f*, n=4, *N-cad-CreER⁺; SCF f/f*, n=6) post TMX. (E) Percentage of engraftment in transplantation assay. 50K total BM cells from *N-cad-CreER⁻;SCF f/f* and *N-cad-CreER⁺;SCF f/f mice* were transplanted. Total engrafted donor cells measured at the indicated number of weeks post transplantation by PB analysis. * P<0.05, ** P<0.01, ***P<0.001. Error bars, s.e.m.

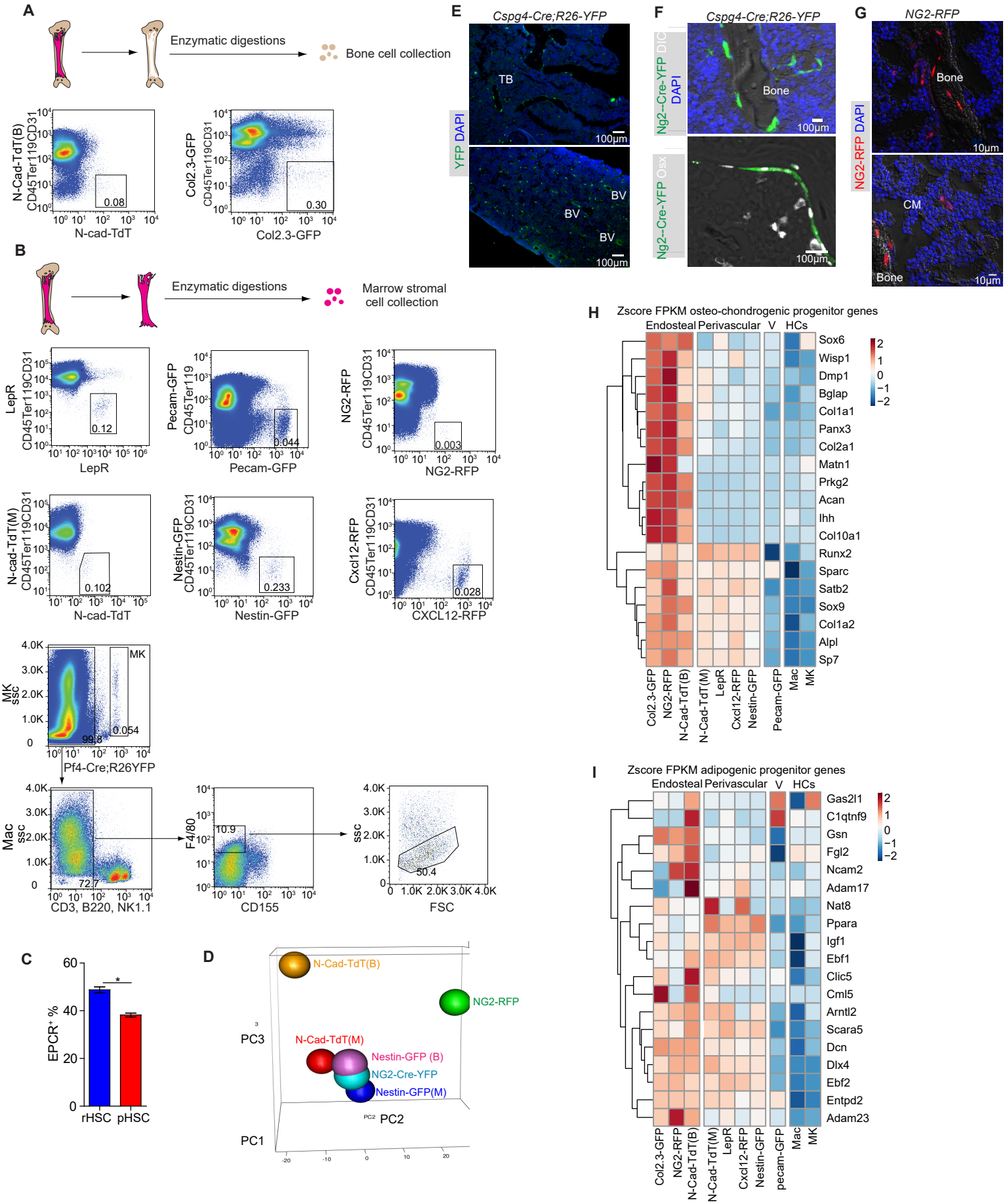
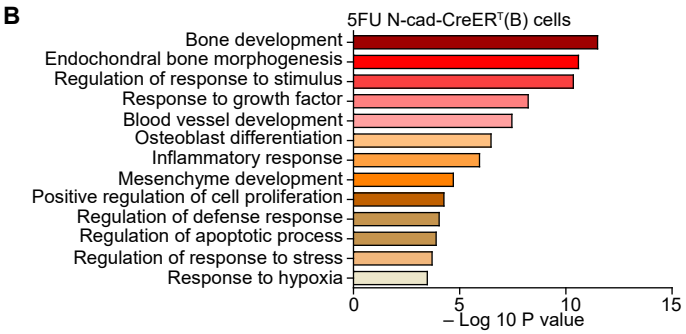


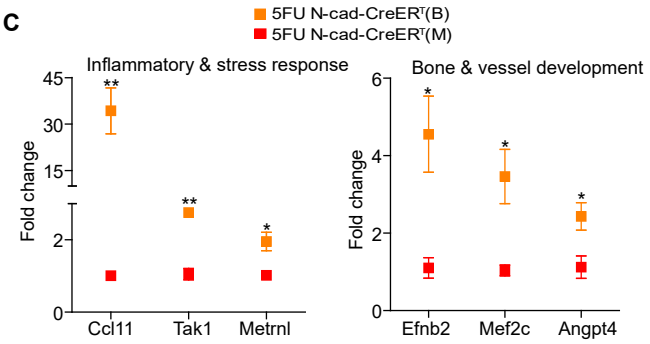
Figure S4. Sorting strategy and signature gene expression of niche cells. Related to Figure 4.

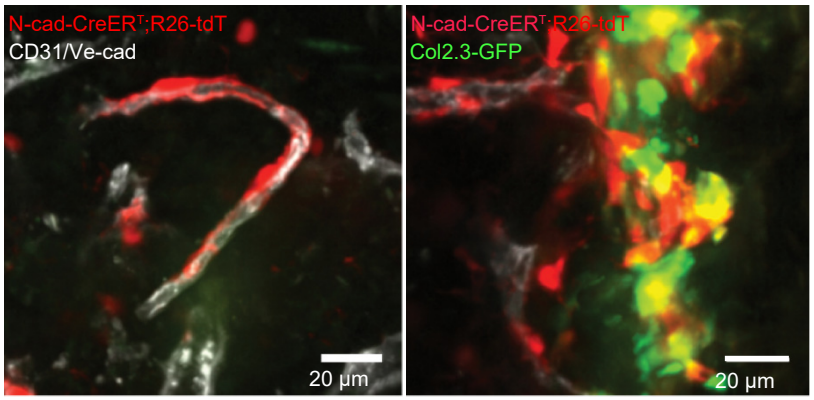
(A) Niche cells in endosteal zone harvested from digested bone cells. (B) Niche cells from perivascular and sinusoid zones harvested from digested BM cells.(C) Flow cytometric analysis of frequency of EPCR$^+$ cells in rHSCs and pHSCs (n=2).(D) PCA analysis of niche cells from BM and bone compartment. (E-F) YFP$^+$ cells in both endosteal (TB) and peri-arterial regions (BV, blood vessel) in *Cspg4-Cre;R26-YFP* mouse. Localization of YFP$^+$ cells in bone surface shown with differential interference contrast (DIC) or stained by Osterix (Osx)(F). (G) Localization of NG2-RFP$^+$ cells primarily in endosteal area in *NG2-RFP* mouse line. (H-I) Heatmap of osteo-chondrogenic progenitor gene and adipogenic progenitor gene expression in niche cells. * P<0.05, ** P<0.01, *** P<0.001. Error bars, s.e.m.

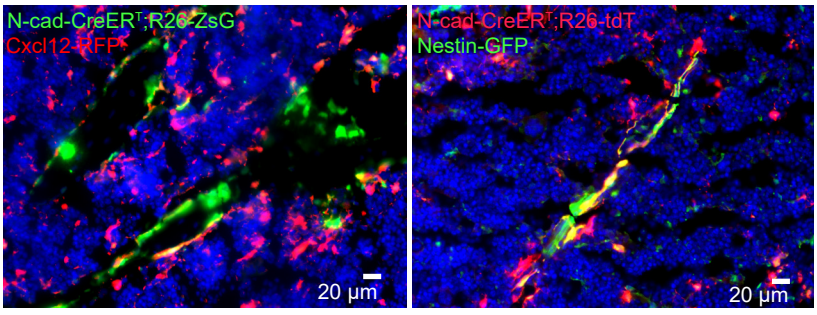
A

Stromal cell population	Cell dose	CFU-F colony per well	CFU-F frequency
N-cad-TdT(B)	1	4/32	1 in 8.79
	5	12/32	
	10	12/16	
LepR	1	1/8	1 in 9.7
	5	17/40	
	10	25/40	
NG2-RFP	1	1/2	1 in 6.72
	5	4/5	
	10	4/7	
Nestin-GFP	1	0/8	1 in 17.6
	5	8/40	
	10	19/40	
N-cad-CreER^T(B)	1	2/32	1 in 10.7
	5	12/32	
	10	20/32	
N-cad-CreER^T(M)	1	1/8	1 in 7.37
	5	18/40	
	10	31/40	









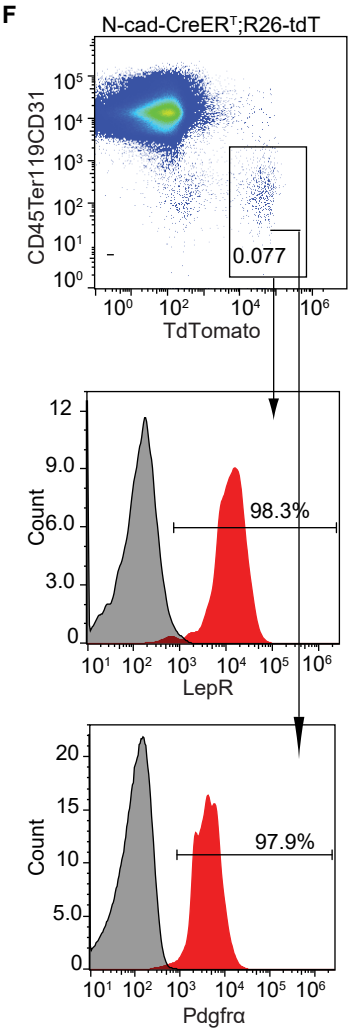


Figure S5. Overlap of N-cad$^+$ cells with other niche cells with MSC potential. Related to Figure 4 and 5.

(A) CFU-F activity in niche cells from the endosteal and perivascular zones. (B-C) GO term analysis of upregulated genes (B) and examples of fold change in stress and inflammatory genes, as well as bone and vessel development genes (C) in endosteal N-cad$^+$ cells compared to marrow N-cad$^+$ cells at day 3 after 5FU, from *N-cad-CreER^T; R26-tdT* mice post 3 TMX injections. * P<0.05, ** P<0.01, ***P<0.001. Error bars, s.e.m. (D-E) Lineage tracing of *N-cad-CreER^T;R26-tdT; Col2.3-GFP*, *N-cad-CreER^T; R26-ZsG; Cxcl12-DsR* and *N-cad-CreER^T; R26-ZsG; Nestin-GFP* mice after 3 TMX injections; blood vessels stained by CD31 and Ve-cadherin antibodies. (F) Enzymatically digested BM cells from *N-cad-CreER^T; R26-tdT* mice post 3 TMX injections. LepR and Pdfgrα stained by antibodies shown as red peaks. Isotype control shown as gray peaks.

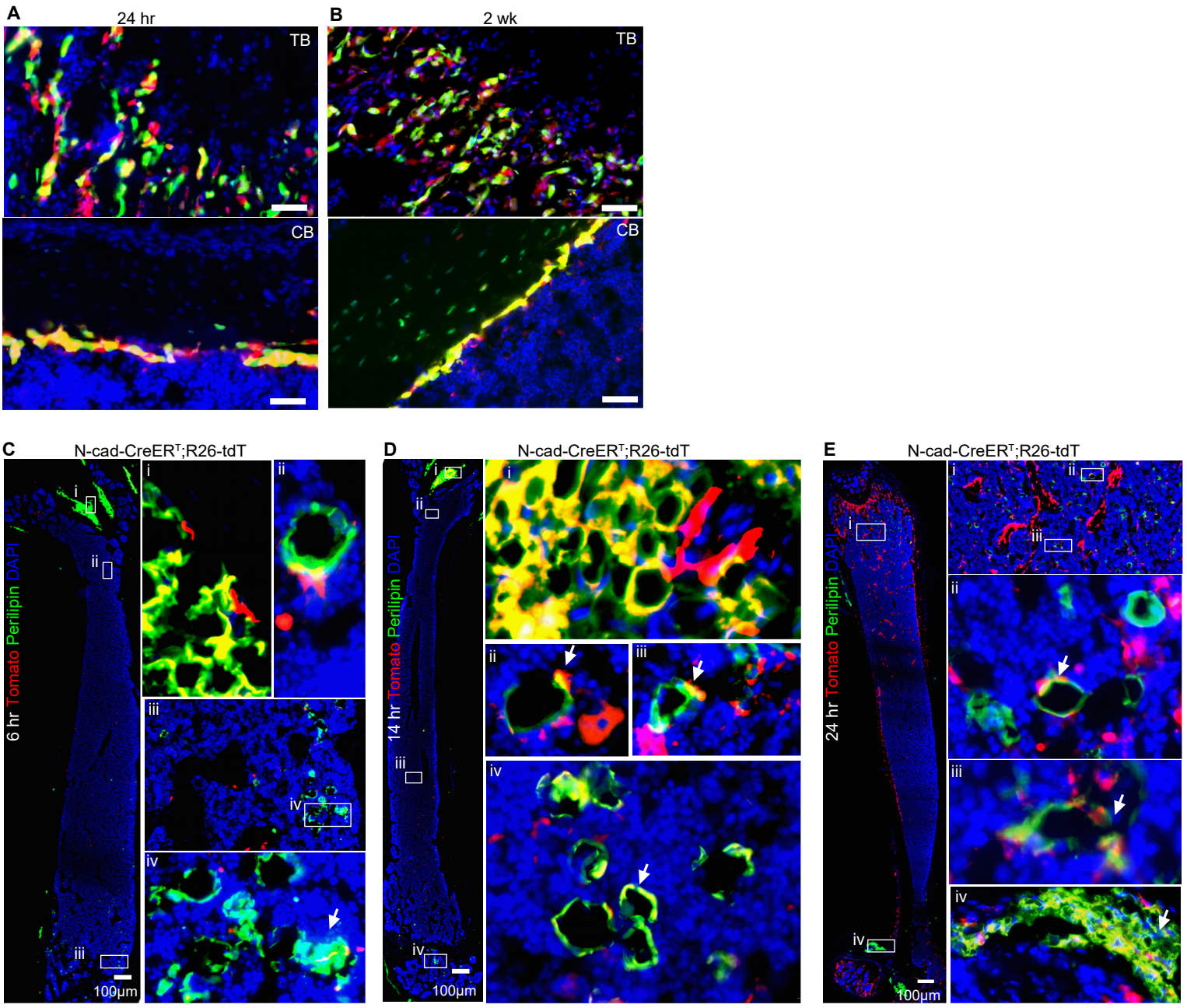


Figure S6. Lineage tracing in *N-cad-CreER^T;R26-tdT* mice at early time points post TMX induction. Related to Figure 6.

(A-B) Representative images of the TB and CB in *N-cad-CreER^T; R26-tdT;Col2.3-GFP* mice at 24 hours (A) and 2 weeks (B) post TMX induction. Scale bar, 20μm. (C-D) Representative whole femur section with high magnification (40x) images of *N-cad-CreER^T; R26-tdT* mice at 6,14 and 24 hours post one TMX induction. Adipocytes stained with perilipin antibody. Arrow indicates N-cad$^+$ cells derived perilipin$^+$ adipocytes.

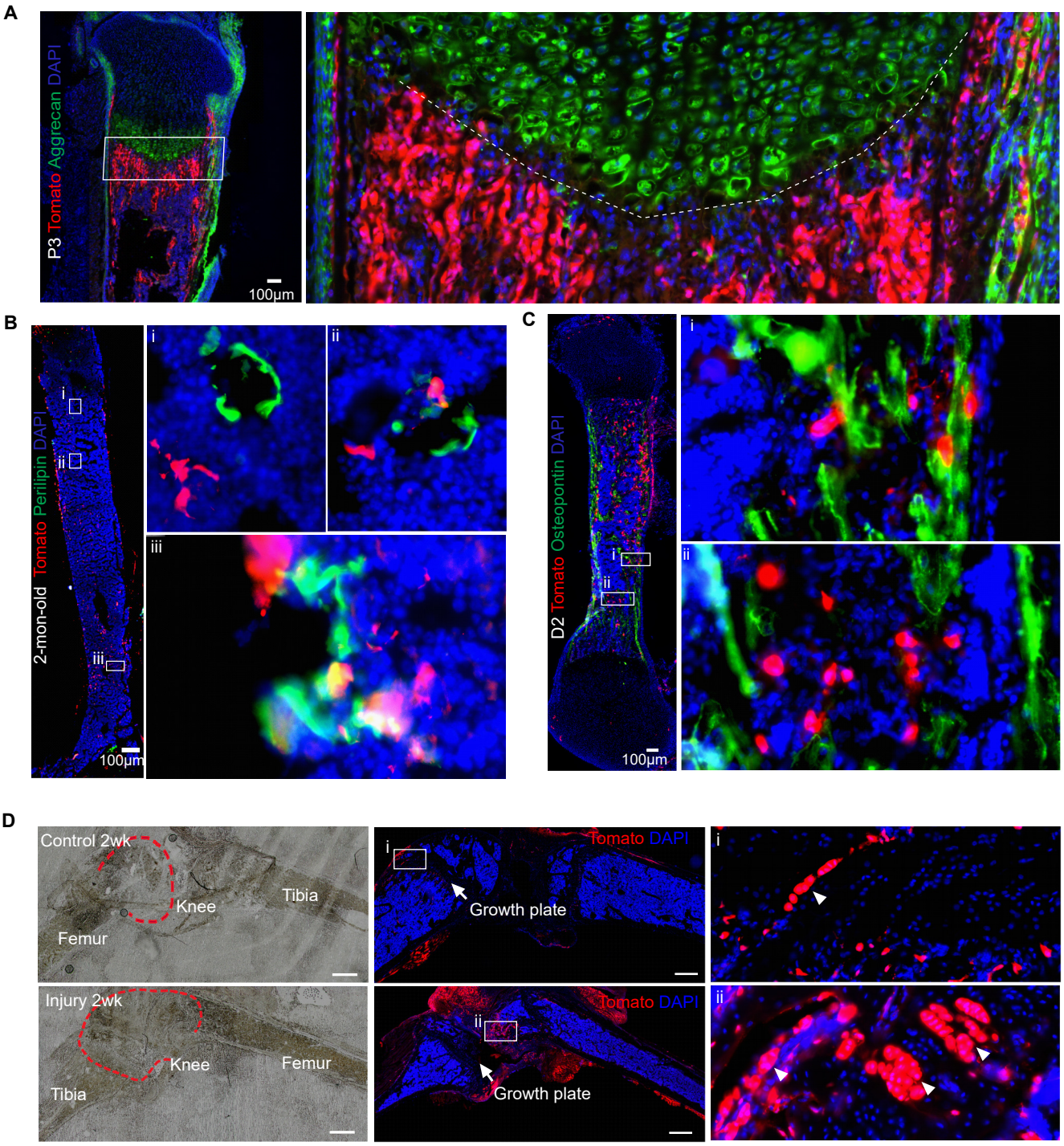


Figure S7. Characterization of N-cad⁺BMSPCs from early development and in injury repair. Related to Figure 7.

(A) Representative femur section showing N-cad⁺BMSPCs with Aggrecan staining in *N-cad-CreER^T; R26-tdT* at 24 hours post 1 TMX injection at post-natal D2. Black background area is outside the microscope tiling regions in stitched composite images. (B-C) Representative femur section from 2-month-old (B) and 2-day-old (C) *N-cad-CreER^T; R26-tdT* mice with 1 TMX injection at embryonic day 12.5 (E12.5). Adipocytes shown with Perilipin antibody staining. Developing bone cells shown with Osteopontin antibody staining. (D) Representative sagittal knee sections of *N-cad-CreER^T; R26-tdT* mice with 1 TMX injection at E12.5 showing left leg (control) and right leg (femoral groove injury at ~ 2-month-old). Images collected at 2 weeks after injury. Red dotted line indicates injured region in the patella groove of the lower femur. Arrow indicates the growth plate. Arrowhead indicates chondrocytes derived from N-cad⁺ cells. Scale bar, 1mm.