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**Supplemental Information**

**C-KIT Expression Distinguishes Fetal from Postnatal Skeletal Progenitors**

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1 **Supplementary Figure Legends**

2 **Figure S1. Analyses of *Kit*<sup>MerCreMer</sup>; *R26*<sup>tdTomato</sup> mice.**

3 (A) Representative confocal images of femur sections from *Kit*<sup>MerCreMer</sup>; *R26*<sup>tdTomato</sup>;  
4 *Col2.3-GFP* mice showed no Cre activity without tamoxifen treatment. (n=3 mice from  
5 3 independent experiments)

6 (B) Representative confocal images of femur sections from *Kit*<sup>MerCreMer</sup>; *R26*<sup>tdTomato</sup>;  
7 *Col2.3-GFP* mice showed co-staining of endosteal Tomato<sup>+</sup> stromal cells by anti-TRAP  
8 antibody. (n=3 mice from 3 independent experiments)

9

10 **Figure S2. In vitro differentiation of c-kit<sup>+</sup> cell-derived CFU-Fs.**

11 (A) Representative confocal images of femur sections from *Kit*<sup>MerCreMer</sup>; *R26*<sup>tdTomato</sup> mice  
12 at E13.5. Mice were treated with tamoxifen at E12.5. Arrows indicated Tomato<sup>+</sup> cells at  
13 the growth cartilage. (n=3 mice from 3 independent experiments)

14 (B) Representative bright field image of colonies derived from Tomato<sup>+</sup> stromal cells  
15 of *Kit*<sup>MerCreMer</sup>; *R26*<sup>tdTomato</sup>; *Col2.3-GFP* mice. (n=3 mice from 3 independent experiments)

16 (C and D) *Kit*<sup>MerCreMer</sup>; *R26*<sup>tdTomato</sup>; *Col2.3-GFP* were tamoxifen-treated at E12.5/14.5  
17 and euthanized at 2 months of age. Tomato<sup>+</sup> stromal cells were sorted into culture for  
18 10 days before in vitro differentiation. Colonies were cultured in osteogenic medium  
19 for 7 days and then *Col2.3-GFP* expression was detected by fluorescent microscopy

20 (C). Colonies were cultured in adipogenic medium for 7 days, fixed by PFA and then  
21 stained by anti-perilipin antibody (D). (n=3 mice from 3 independent experiments)

22 (E) A schematic diagram showing the procedure of the ossicle formation assay.

1

2 **Figure S3. Conditional deletion of *Pparg* from fetal c-kit<sup>+</sup> cells reduced bone**  
3 **marrow adiposity.**

4 (A and B) Representative confocal images of femur sections from *Pparg*<sup>fl/fl</sup> and  
5 *Kit*<sup>MerCreMer</sup>; *Pparg*<sup>fl/fl</sup> mice that were stained by anti-perilipin antibody. (n=3 mice per  
6 genotype from 3 independent experiments)

7 (C) Quantification of perilipin<sup>+</sup> adipocyte numbers on femur sections from *Pparg*<sup>fl/fl</sup> and  
8 *Kit*<sup>MerCreMer</sup>; *Pparg*<sup>fl/fl</sup> mice. (n=3 mice per genotype from 3 independent experiments)

9 (A and B) Representative confocal images of femur sections from *Pparg*<sup>fl/fl</sup> and *Prx1-*  
10 *cre*; *Pparg*<sup>fl/fl</sup> mice that were stained by anti-perilipin antibody. (n=3 mice per genotype  
11 from 3 independent experiments)

12 (C) Quantification of perilipin<sup>+</sup> adipocyte numbers on femur sections from *Pparg*<sup>fl/fl</sup> and  
13 *Prx1-cre*; *Pparg*<sup>fl/fl</sup> mice. (n=3 mice per genotype from 3 independent experiments)

14

15 **Figure S4. *Lepr*-Cre efficiently targeted *Kitl*-expressing cells in young adult bone**  
16 **marrow stroma**

17 (A) Representative flow cytometry plots of enzymatically dissociated bone marrow  
18 cells from 2-month-old *Lepr*<sup>cre</sup>; *R26*<sup>tdTomato</sup>; *Kitl*<sup>GFP</sup> mice showed that most GFP<sup>+</sup> cells  
19 were Tomato<sup>+</sup> and vice versa. (n=3 mice from 3 independent experiments)

20 (B) Real-time PCR analyses of *Kitl* mRNA level (normalized to  $\beta$ -actin) of CD45<sup>+</sup>Ter119<sup>-</sup>  
21 CD31<sup>-</sup>PDGFR $\alpha$ <sup>+</sup> bone marrow stromal cells from 2-month-old *Kitl*<sup>fl/fl</sup> and *Lepr*<sup>cre</sup>; *Kitl*<sup>fl/fl</sup>  
22 mice. *Kitl* mRNA level in *Kitl*<sup>fl/fl</sup> mice was set as 1. (n=4 mice per genotype from 3

1 independent experiments)

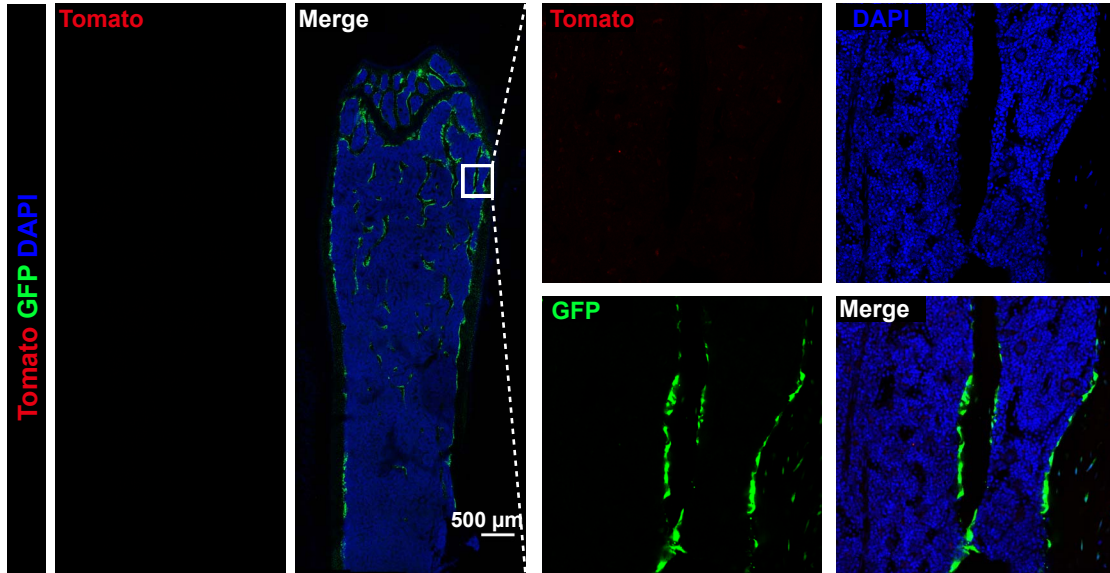
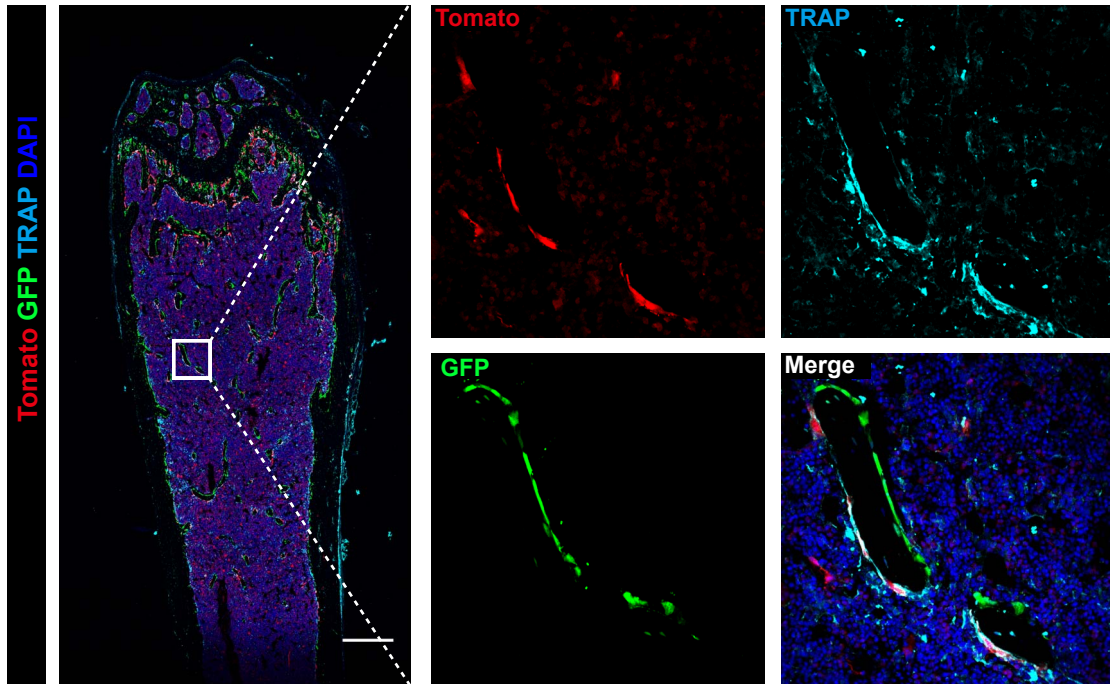
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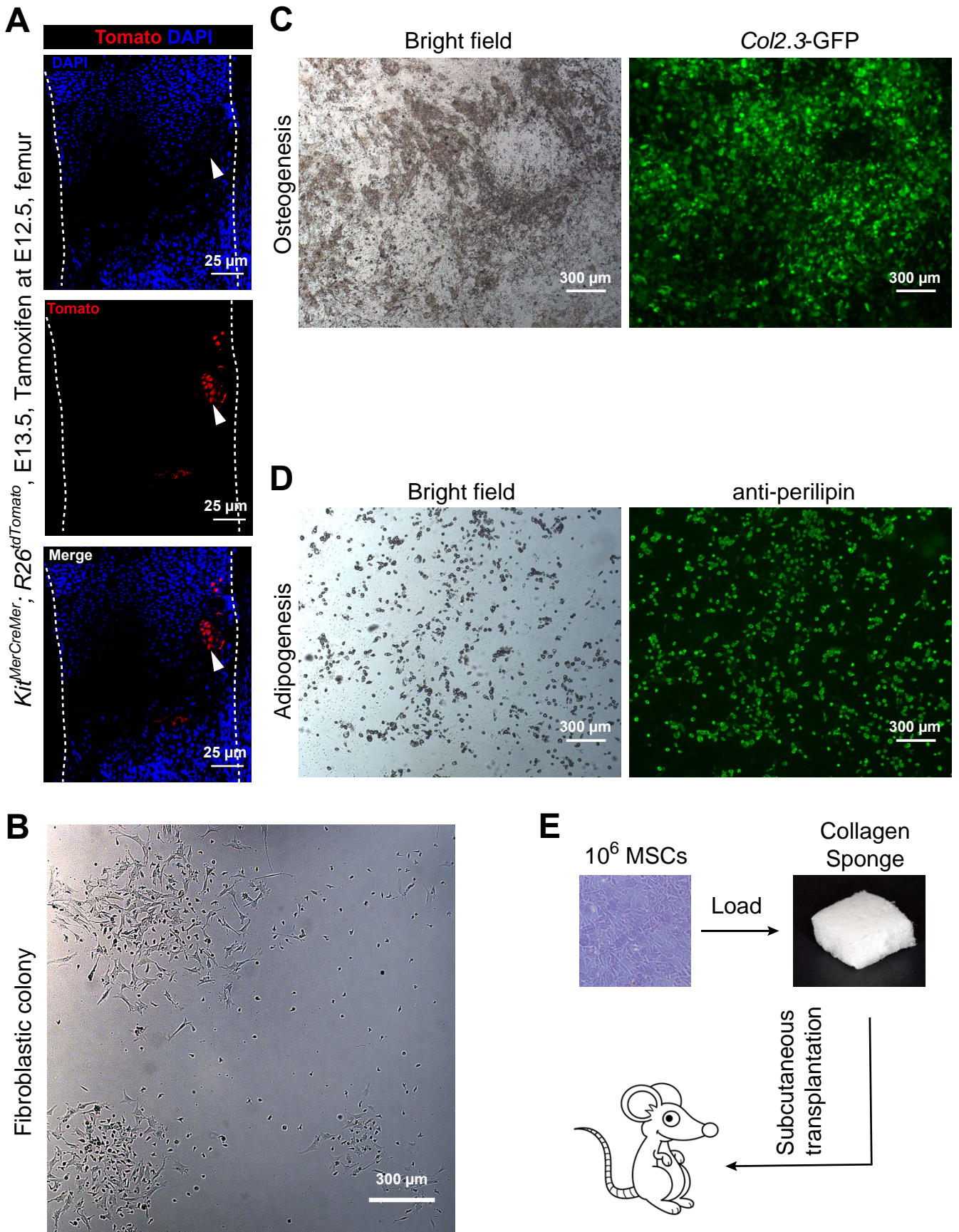
3 **Figure S5. *Prx1*-Cre efficiently targeted *Kitl*-expressing cells in young adult bone**  
4 **marrow stroma**

5 (A) Representative flow cytometry plots of enzymatically dissociated bone marrow  
6 cells from 2-month-old *Prx1-cre; R26<sup>GtdTomato</sup>; Kitl<sup>GFP</sup>* mice showed that most GFP<sup>+</sup> cells  
7 were Tomato<sup>+</sup> and vice versa. (n=3 mice from 3 independent experiments)

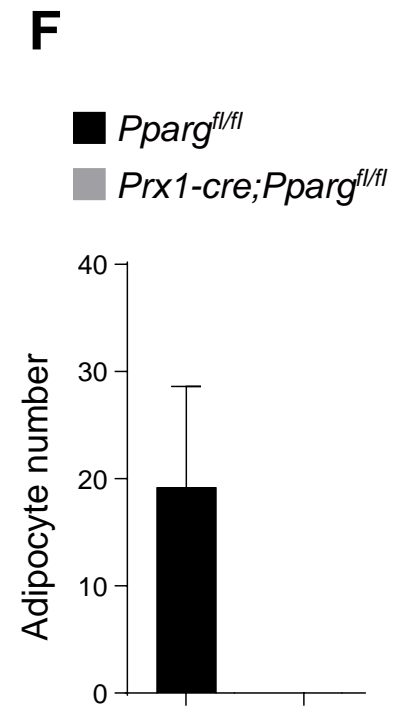
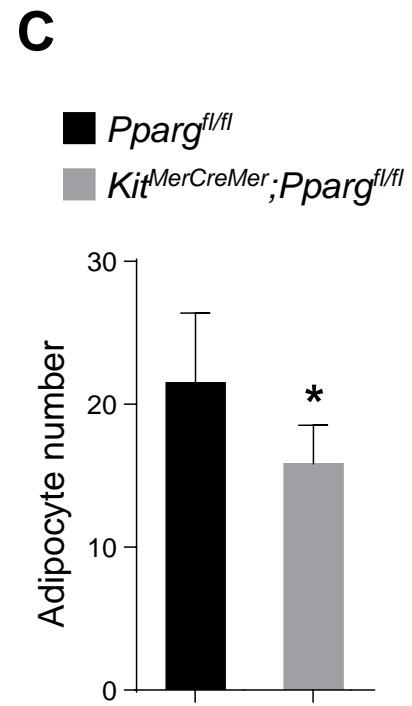
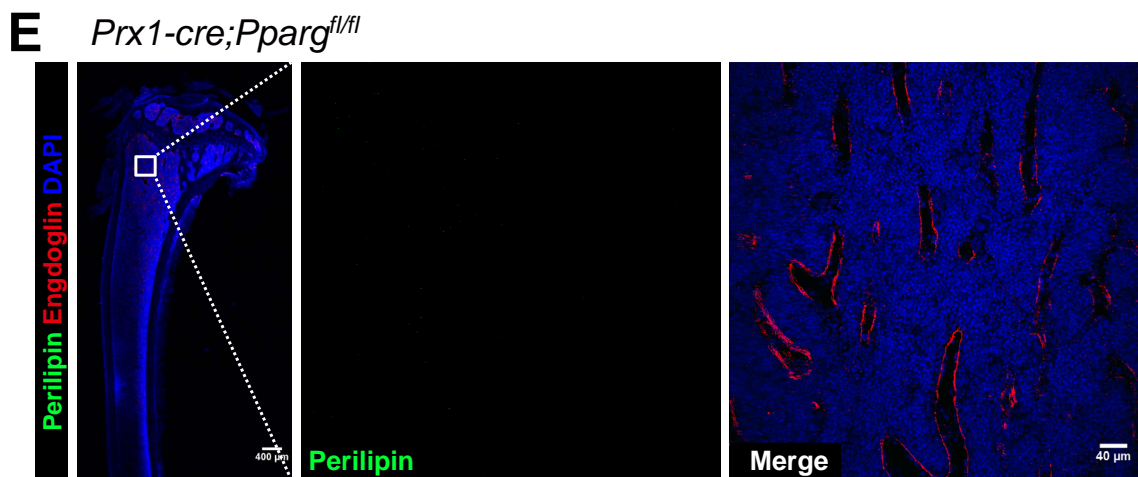
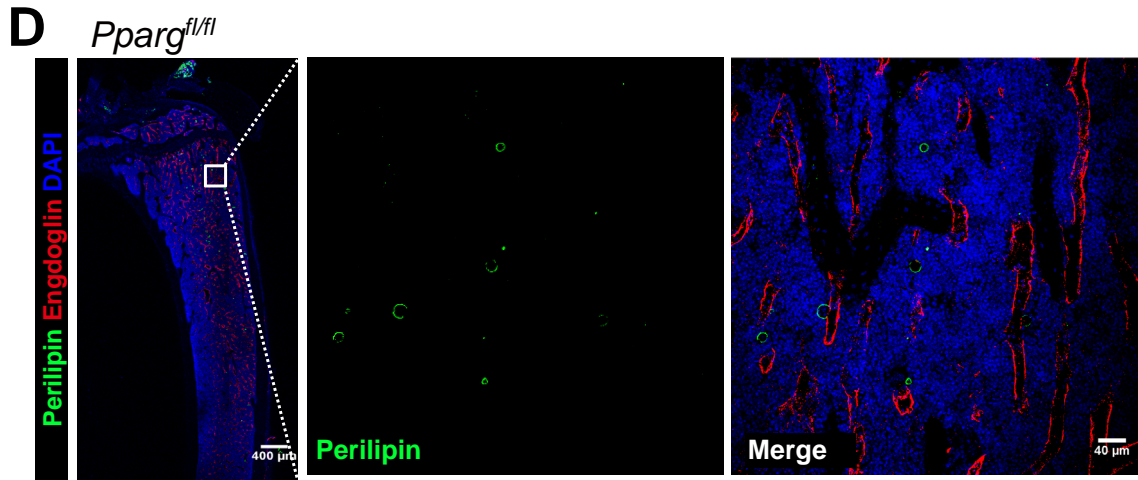
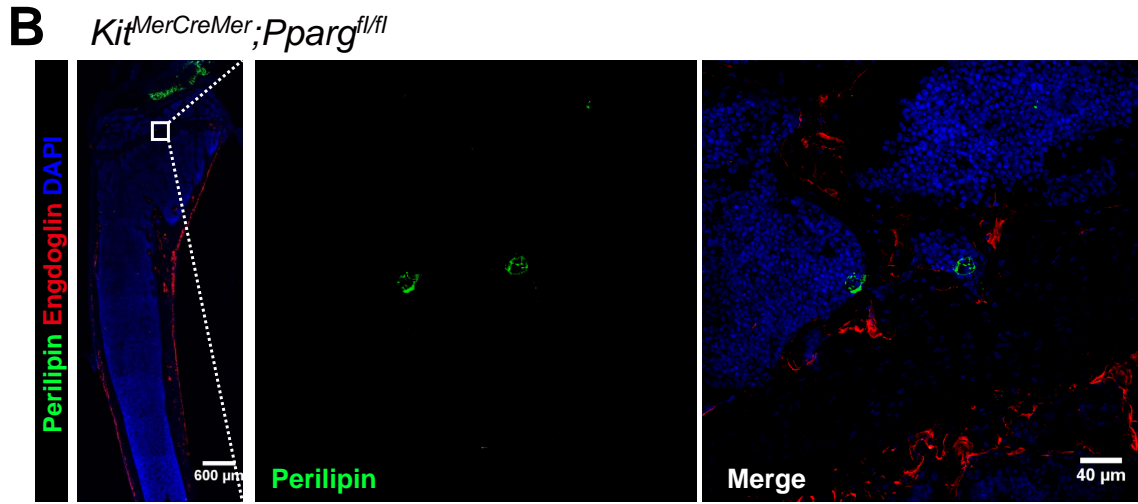
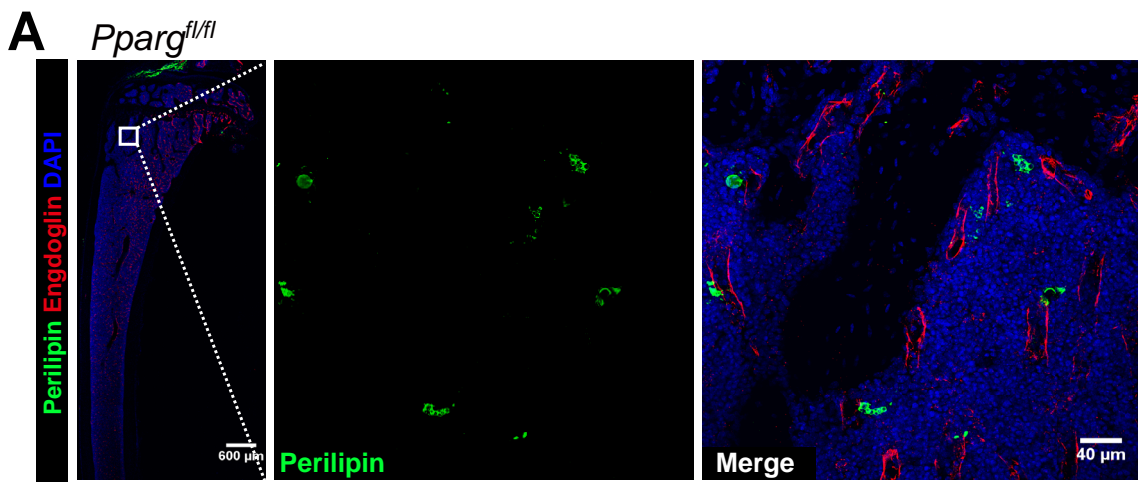
8 (B) Real-time PCR analyses of *Kitl* mRNA level (normalized to  $\beta$ -actin) of CD45<sup>-</sup>Ter119<sup>-</sup>  
9 CD31<sup>-</sup>PDGFR $\alpha$ <sup>+</sup> bone marrow stromal cells from 2-month-old *Kitl<sup>fl/fl</sup>* and *Prx1-cre; Kitl<sup>fl/fl</sup>*  
10 mice. *Kitl* mRNA level in *Kitl<sup>fl/fl</sup>* mice was set as 1. (n=4 mice per genotype from 3  
11 independent experiments)

12 (D and E) Anti-TRAP staining of femur sections from *Kitl<sup>fl/fl</sup>* and *Prx1-cre; Kitl<sup>fl/fl</sup>* mice.  
13 (n=3 mice per genotype from 3 independent experiments)

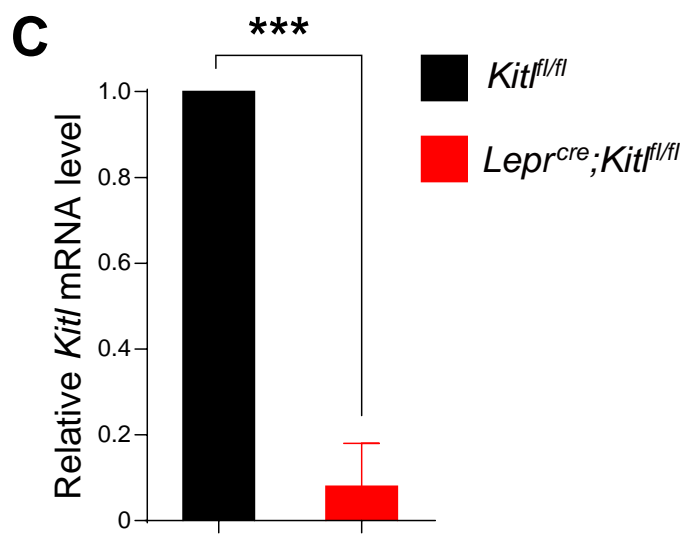
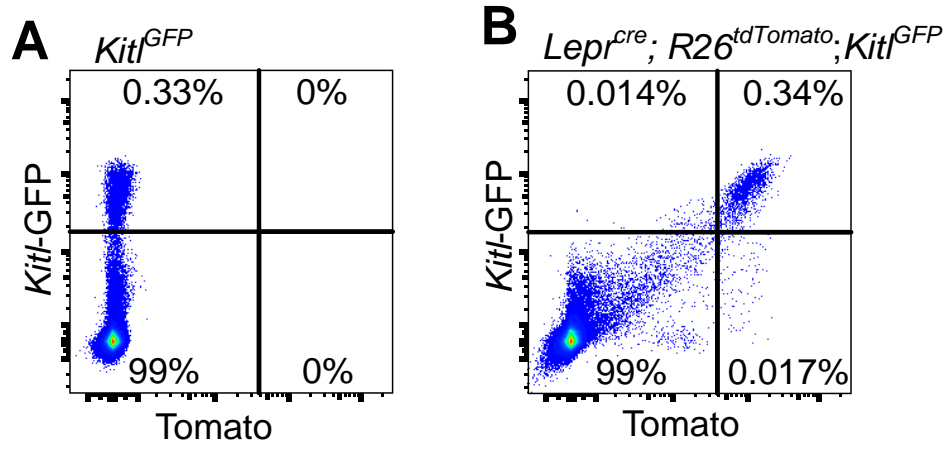
**A***Kit<sup>MerCreMer</sup>; R26<sup>tdTomato</sup>; Col2.3-GFP*, 2-month-old, no tamoxifen, femur**B***Kit<sup>MerCreMer</sup>; R26<sup>tdTomato</sup>; Col2.3-GFP*, 2-month-old, tamoxifen at P1-3, femur**Figure S1**



**Figure S2**

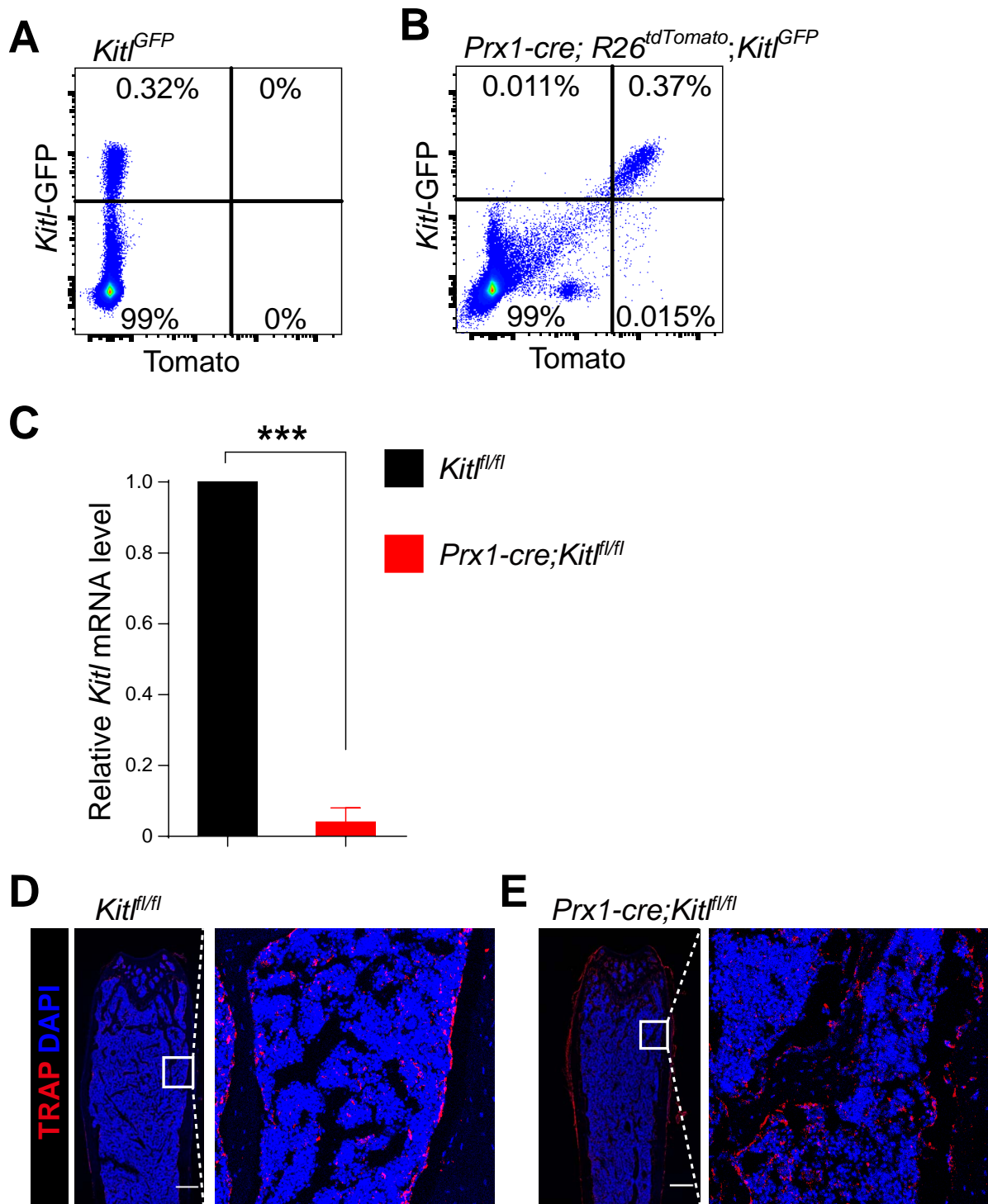


**Figure S3**



**Figure S4**





**Figure S5**