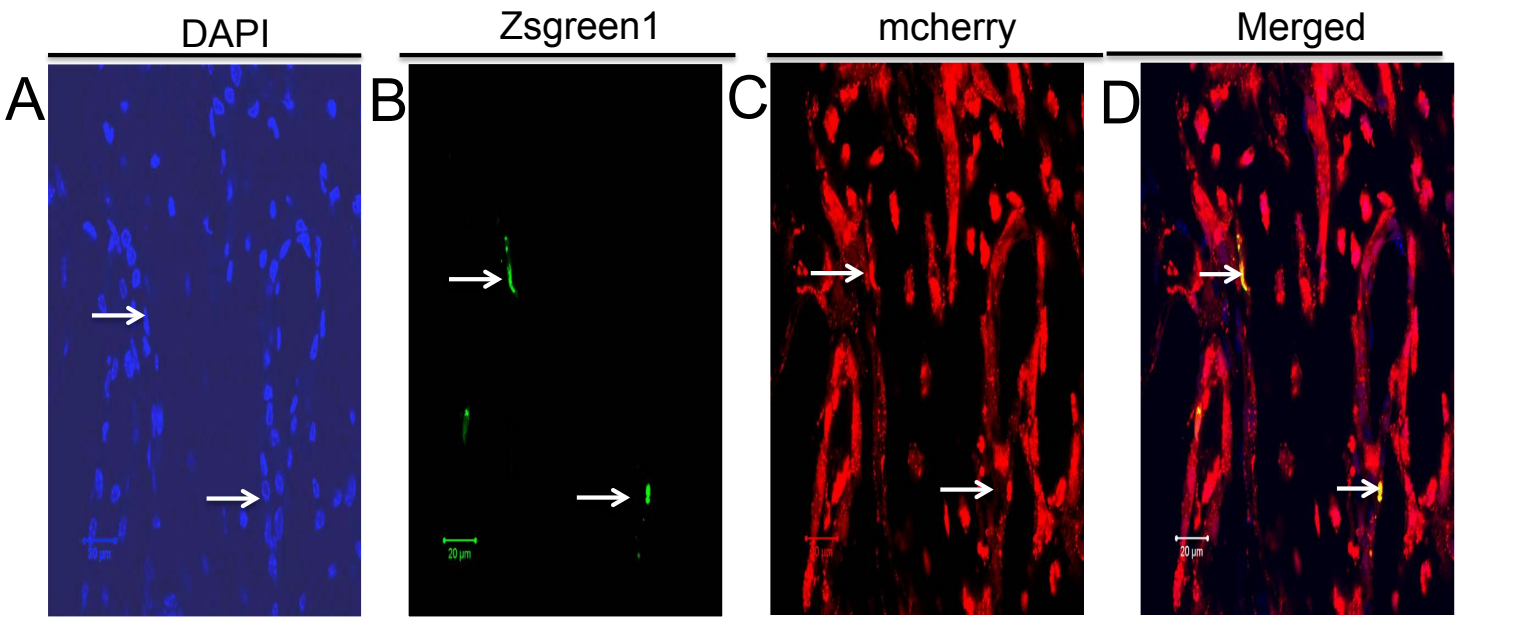
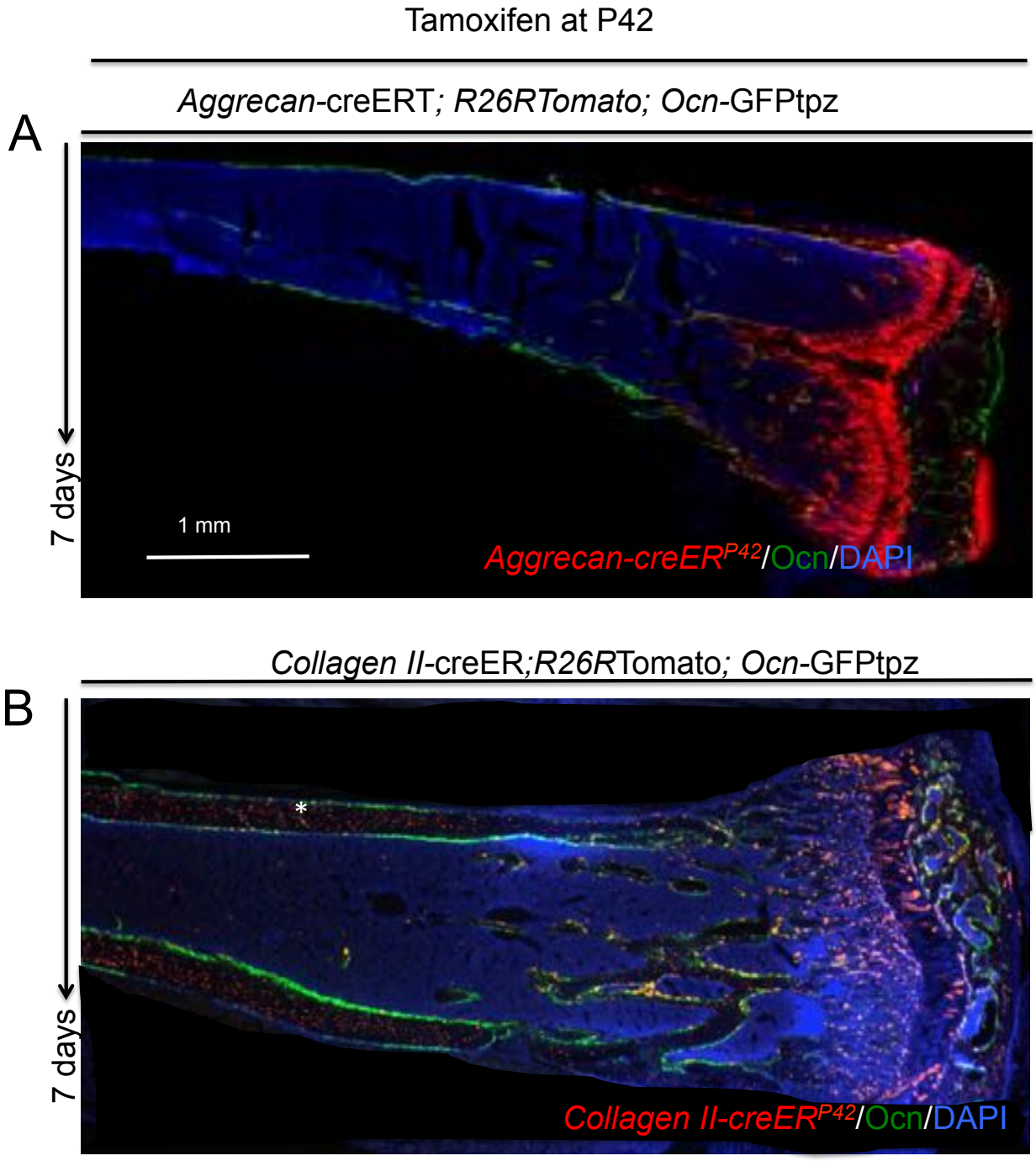
Supplementary figure: S1

Sox9-creER*; R26R*Zsgreen1*; Osx*-mCherry, Tamoxifen at P42, Chase 21 days



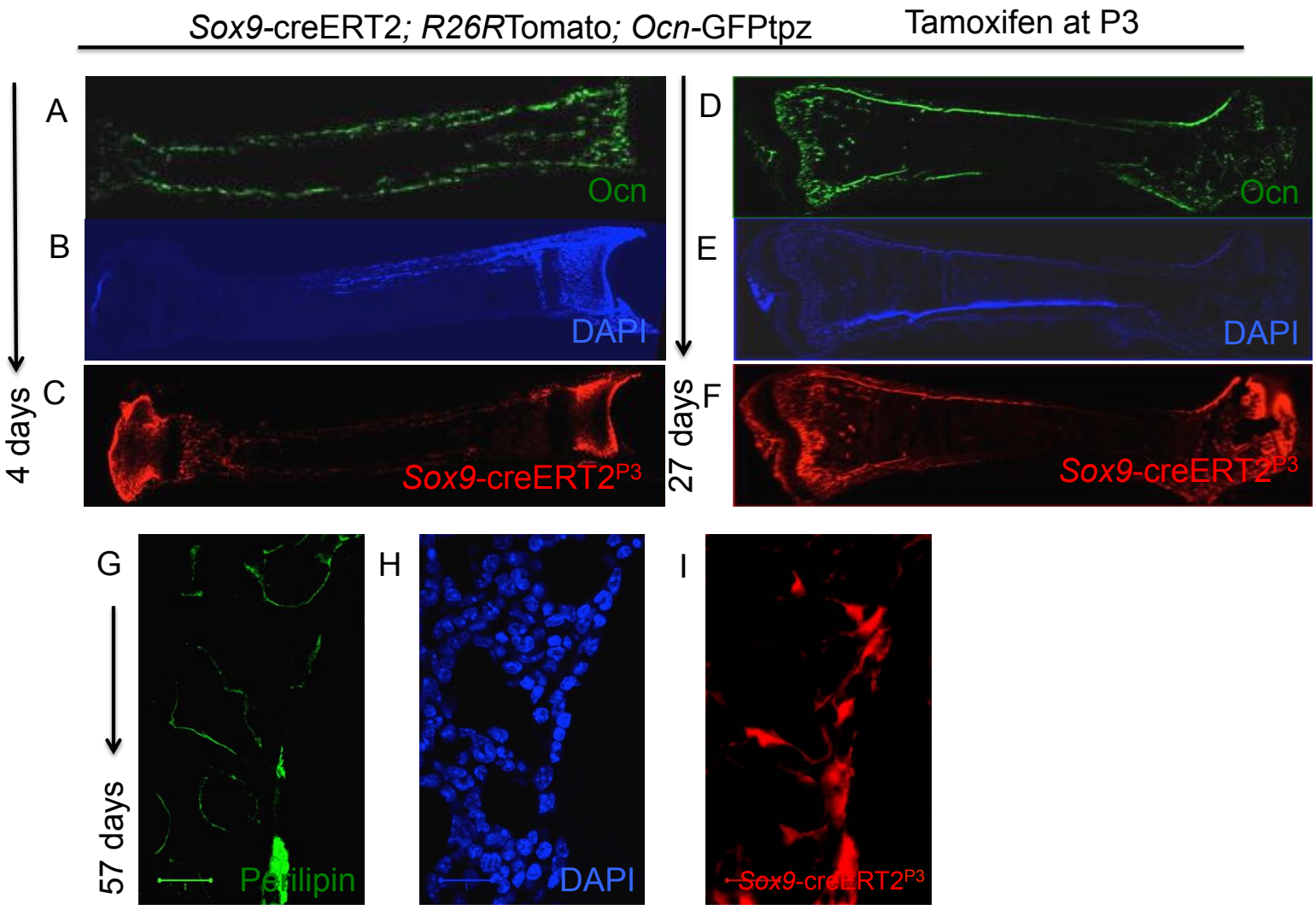
Supplementary Figure S1: *Sox9*-creERT2+ cells differentiate into *Osx*-mCherry+ cells in several weeks: Representative figures showing co-localization of Zsgreen1 protein and mcherry protein to the same cell (indicated by arrows) in the trabecular bone of tibia. Co-localization of two different proteins to the same cell indicate differentiation of *Sox9*-creERT2+ cells into Osterix+ cells. Scale bars 20μm. These data represent sections from 3 separate experiments.

Supplementary figure: S2



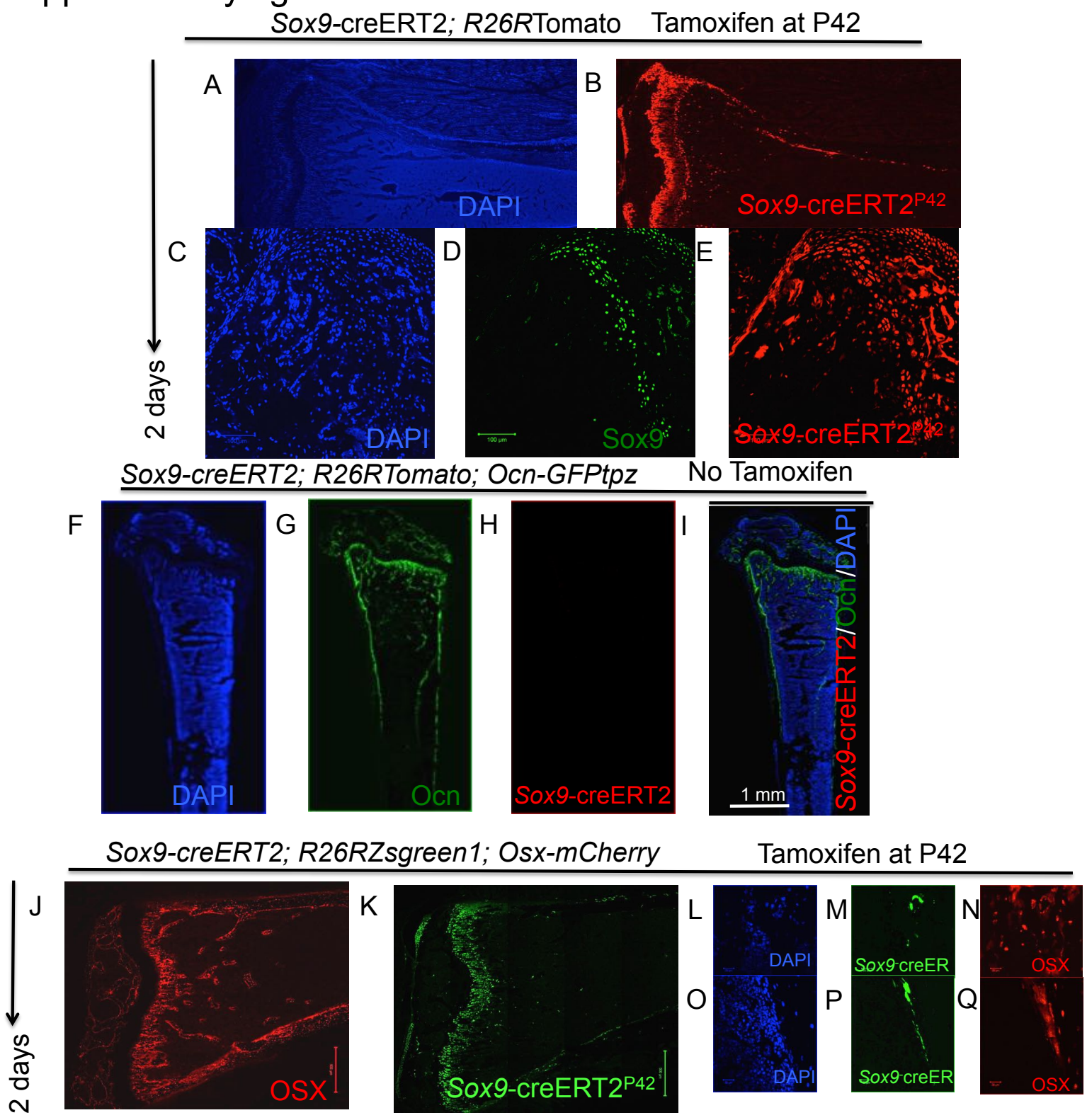
Supplementary Figure S2: Representative figures from Aggrecan-creER; *R26R*Tomato and *Collagen II*-creER; *R26R*TdTomato mice showing *Aggrecan*-creER+ and Collagen II-creER+ cells also label very early cells of the osteoblast lineage in adult mice in vivo. (A, B) Representative sections following lineage tracing of Aggrecan-creER+ and Collagen II-creER+ cells after injecting 2 mg tamoxifen into P42 mice. Aggrecan-creER; *R26R*Tomato; *Ocn*-GFPtpz and *Collagen II*-creER; *R26R*Tomato; *Ocn*-GFPtpz triple transgenic mice 7 days post-tamoxifen. Both: *Aggrecan*-creERT+ and *Collagen II*-creERT exhibit similar patterns of distribution as compared to *Sox9*-creERT2 mice. However, Collagen II-creERT; *R26R*Tomato show leakiness of cre, which is evident by presence of numerous TdTomato+ osteocytes at day 7 (asterisk), that were not present in the *Sox9*-creERT2 model and Aggrecan-creERT2; *R26R*Tomato mice. These data represent sections from 3 separate experiments.

Supplementary figure: S3



Supplementary Figure S3: Single color images of Figure 1. Panels A, D, G represents the FITC channel. Panel B, E, H represents the DAPI channel. Panels C, F and I represents the rhodamine channel.

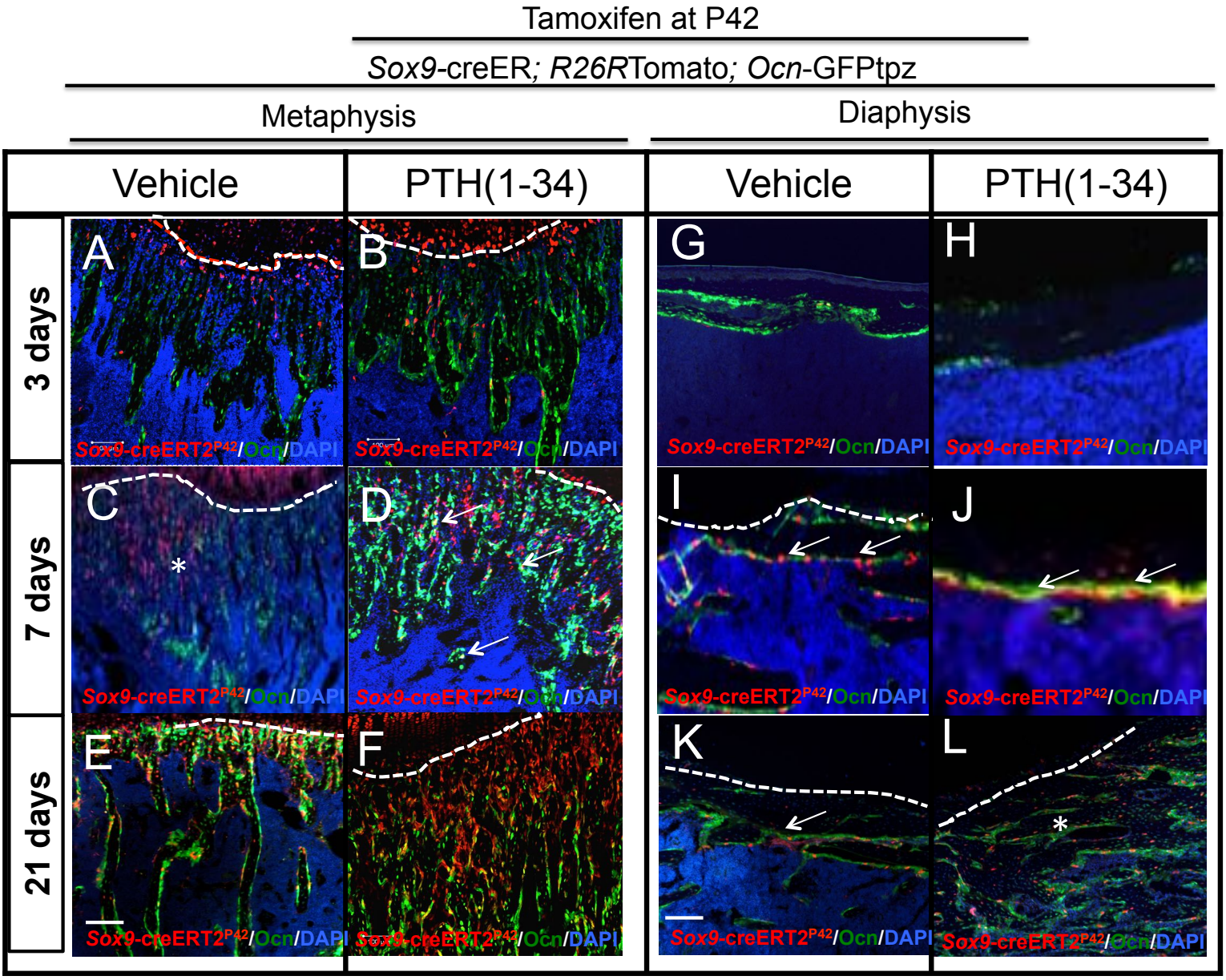
Supplementary figure: S4



Supplementary Figure S4: Single color images from figure 2. A, C, L, O panels represent the DAPI channel. Panels B, E, J represents the rhodamine channel and panels D, K, M, P represents the FITC channel.

(F-I) Representative figures of no tamoxifen control of *Sox9*-creERT2; *R26R*Tomato; *Ocn*-GFPtpz mice at 6 weeks of age. Panel H clearly shows that the reporter expression is dependent on tamoxifen at 6 weeks of age.

Supplementary figure: S5

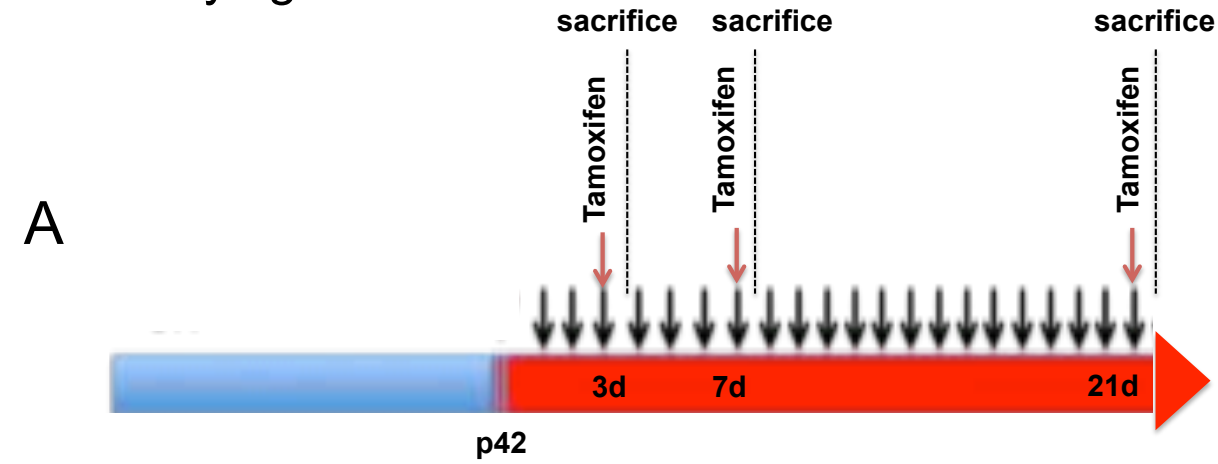


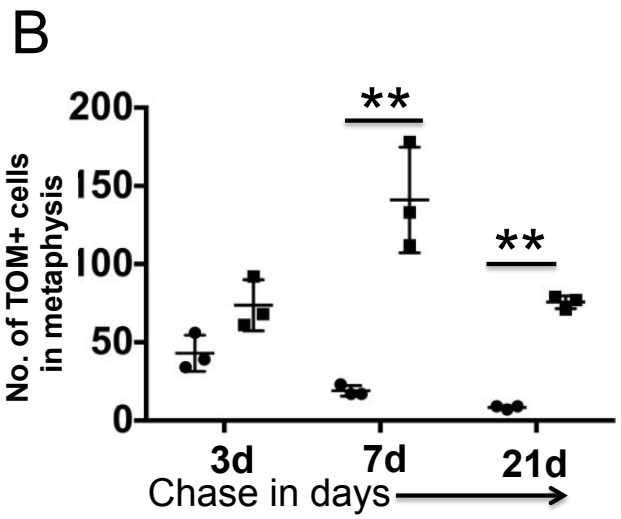
Supplementary Figure S5:

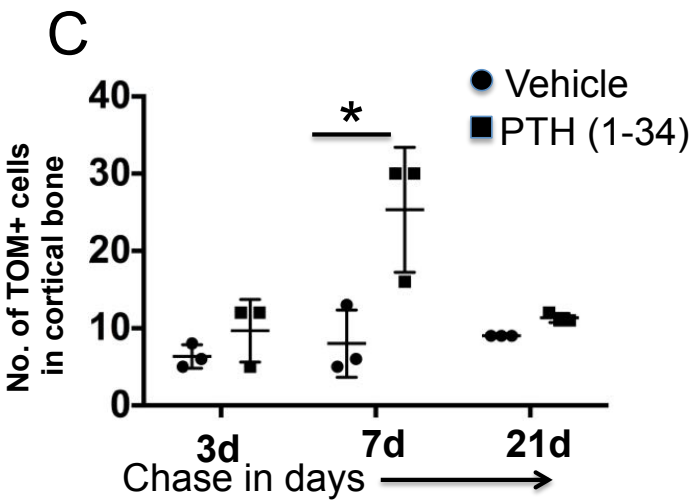
(A-F) Representative confocal images of metaphyseal region from sections of vehicle and teriparatide-treated mice. Note that metaphysis shows TdTomato+ cells on day 7 (asterisk), but PTH (1-34) shows several double-positive cells (arrows). Each panel reflects data from three mice/genotype from three independent experiments.

(G-L): Representative confocal images from sections of vehicle and teriparatide treated mice in the cortical region. Each panel reflects data from three mice/genotype from three independent experiments. Arrows point to TdTomato+ cells on the endocortical surfaces of long bones. Note, endocortical bone shows TdTomato+ cells on day 7, but PTH (1-34) show TdTomato and *Ocn*-GFPtpz cells (arrows) appearing as bright yellow. Notice the merging of trabecular bone with the cortical bone on day 21 (asterisk) in teriparatide-treated mice.

Supplementary figure: S6







Supplementary Figure S6:

(A) The protocol used in the experiment to study if PTH (1-34) affects very early *Sox9*-creERT2-expressing cells. In this experiment mice were subjected to either vehicle or PTH (1-34) once daily for 3, 7 and 21 days and received a single tamoxifen injection at p45, p49 and p63 and 24 hours later, mice were euthanized.

(B, C) The number of *Sox9*-creERT2; *R26R*Tomato+ cells counted blindly in the metaphysis and cortical bone in diaphysis in a standard region described in supplementary methods 1 on day 4, 8 and day 22 after either vehicle or PTH (1-34)-administration with 2 mg tamoxifen injected intraperitonealy on day 3, 7 and 21 respectively. Data represent mean ± SD from 3 independent experiments with at least three-mice/ experiment). *p<0.01, **p<0.001. Statistical evaluation was done by non-parametric 2-tailed student t-tests

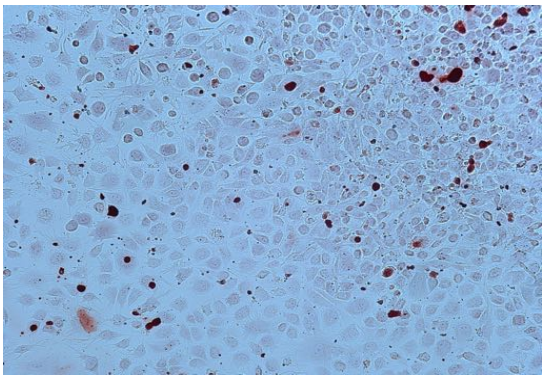
Supplementary figure: S7

Control

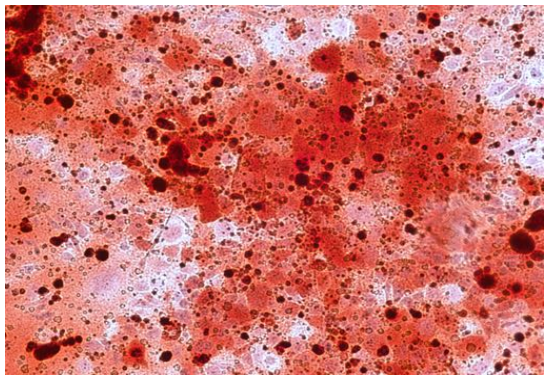
Differentiation media

Alizarin red

A

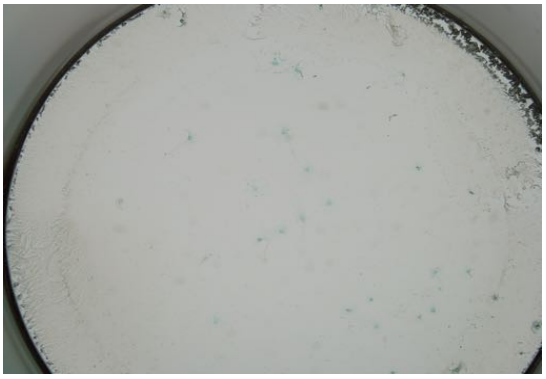


B

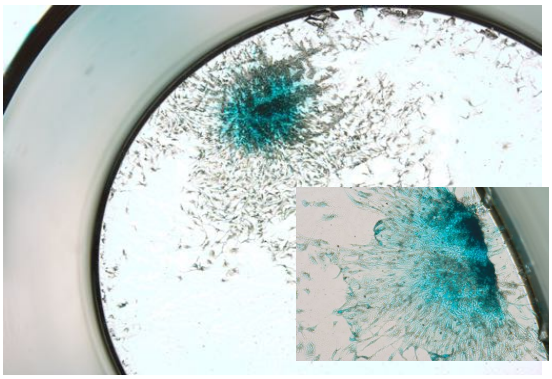


Alcian Blue

C

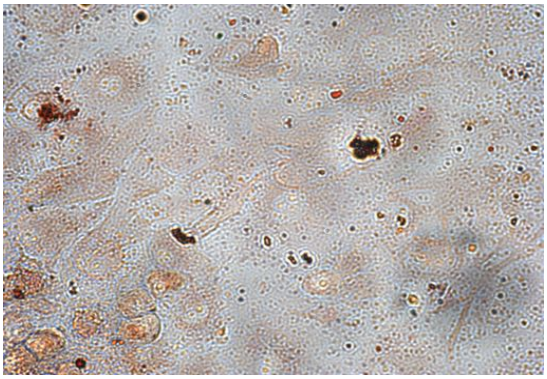


D

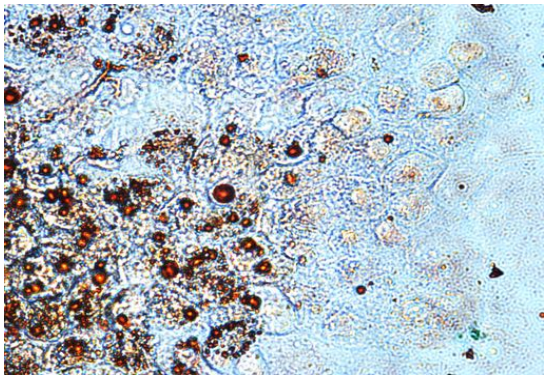


Oil red O

E

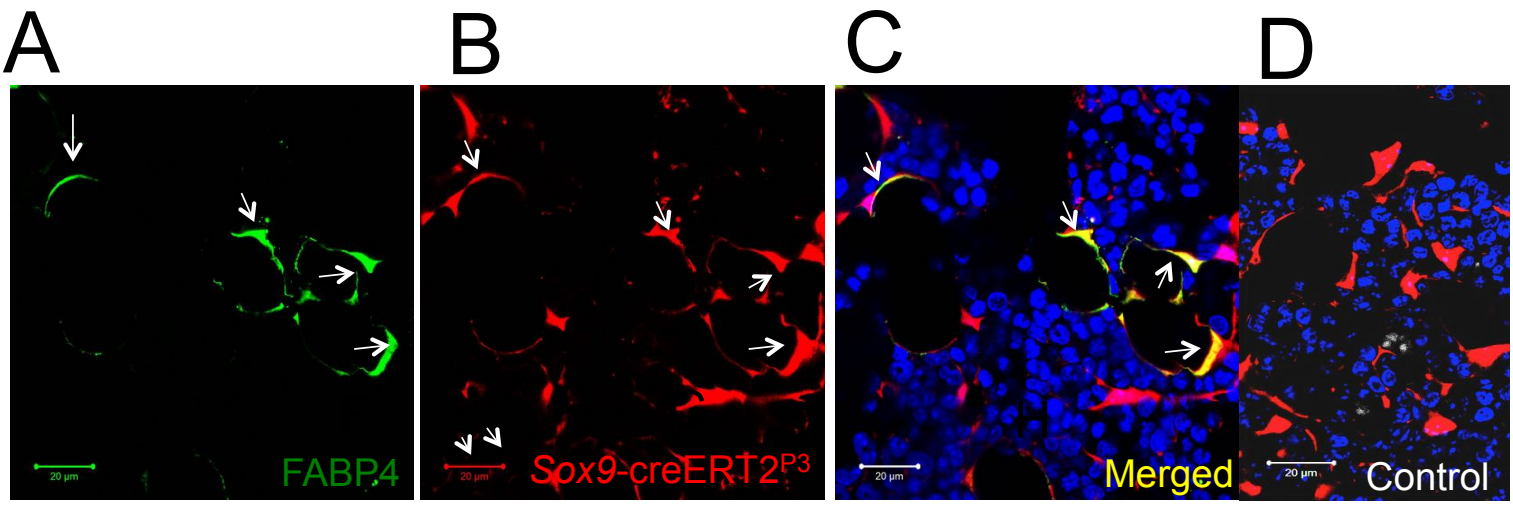


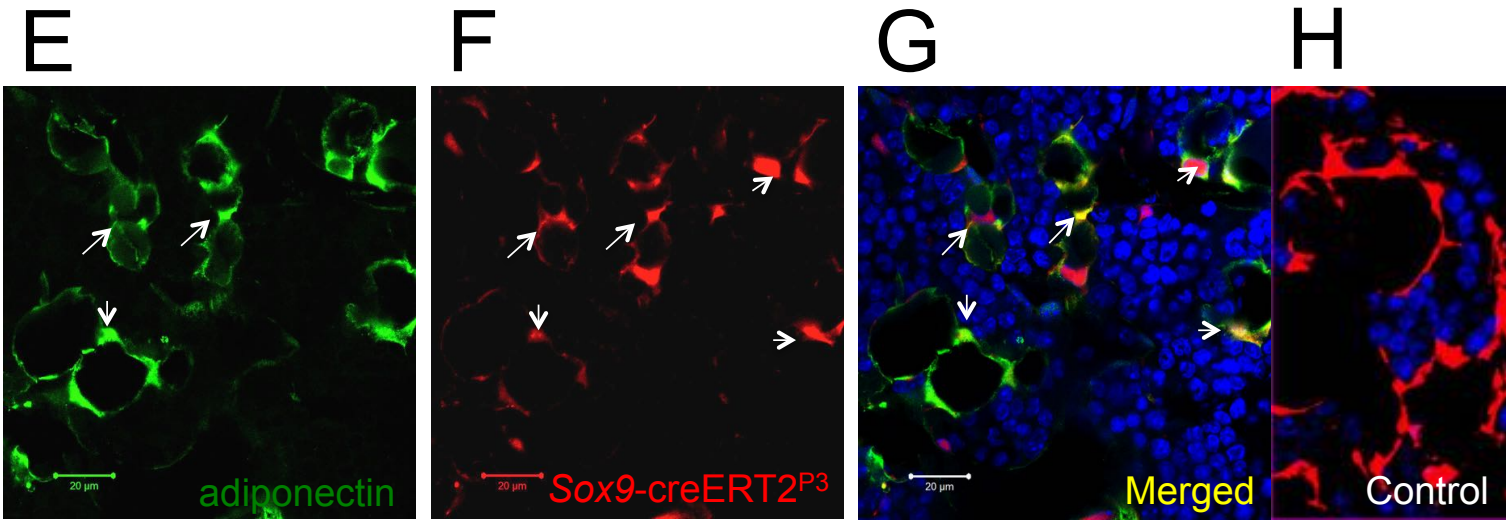
F

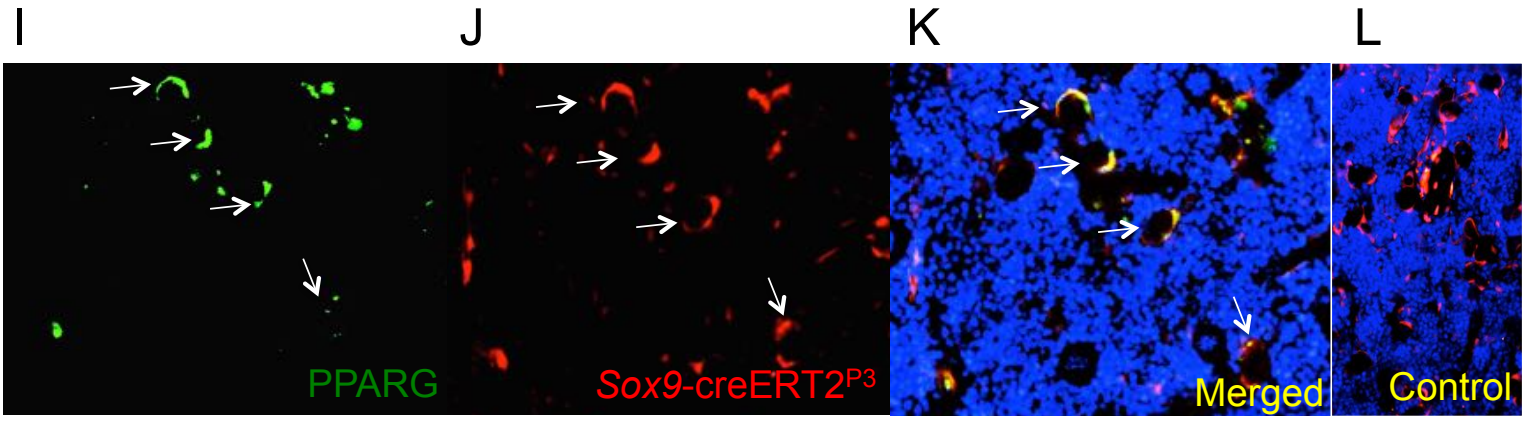


Supplementary Figure: S7: In vitro differentiation assay of *Sox9*-creERT2; *R26R*Tomato+ cells: 300 *Sox9*-creERT2; *R26R*Tomato+ cells were isolated by FACS and plated on a 96 well plate. Cells were grown for 7 days and subsequently changed into differentiation medium for osteoblasts, chondrocytes and adipocytes. 2 weeks later, the cells were fixed with 4% PFA and stained with alizarin red to detect osteoblasts (A, B), alcian blue to detect chondrocytes (C, D) and Oil red O to detect adipocytes (E and F). These data represent results from 3 separate experiments.

Supplementary figure: S8

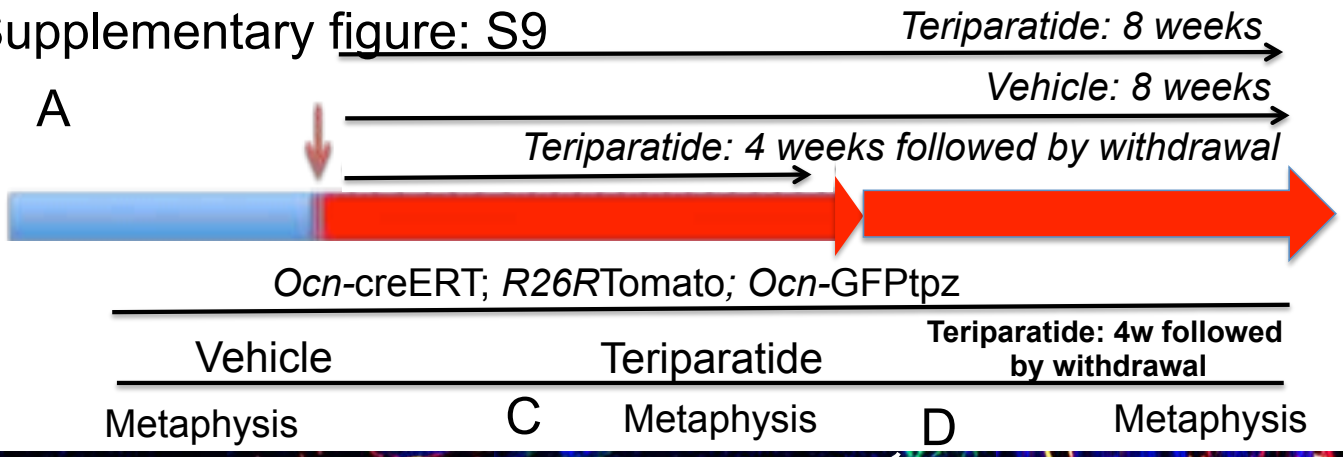


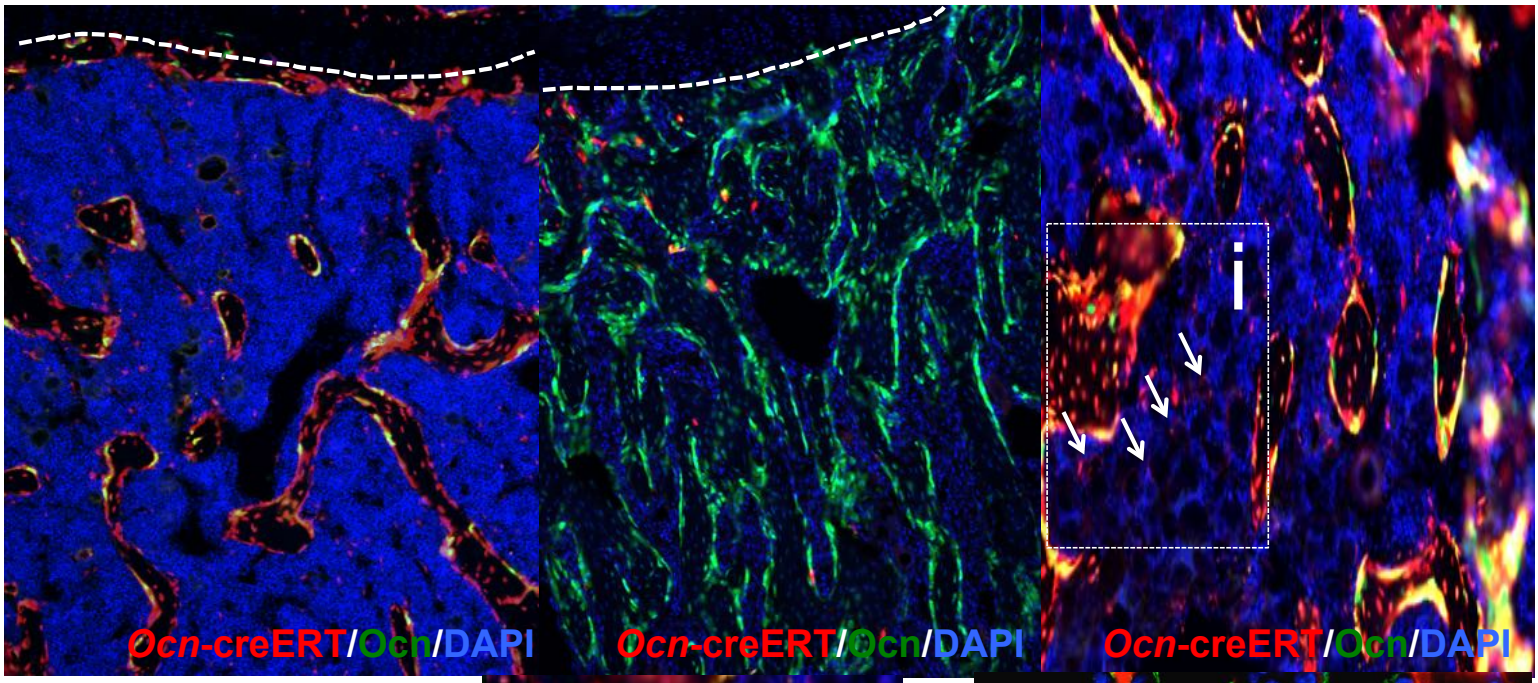


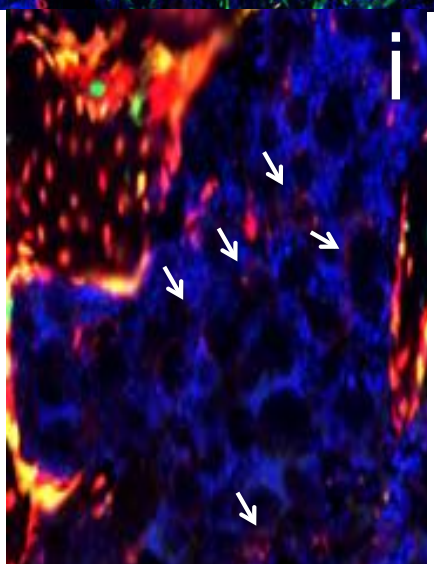

I
J
K
L
PPARG
Sox9-creERT2P3
Merged
Control

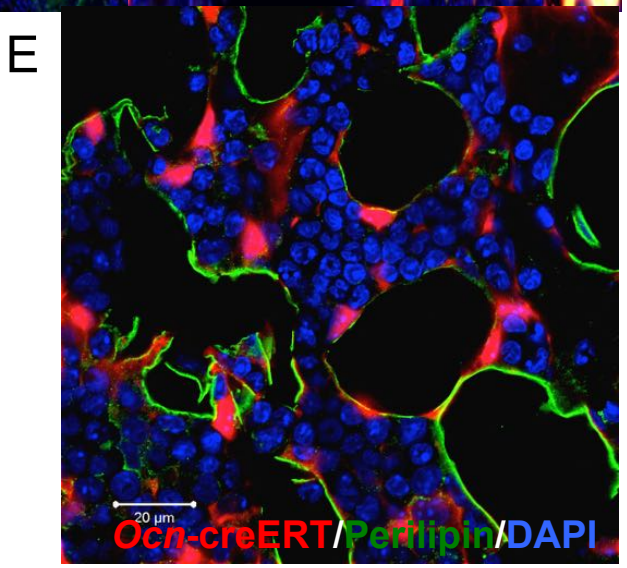
Supplementary figure S8: In order to ascertain if *Sox9*-creERT2; *R26R*Tomato+ adipocytes co-express classical markers of adipocytes besides Perilipin, sections were stained to assess for the co-expression of tomato with FABP4 (A-D), adiponectin (E-H) and PPARG (I-L). These data represent sections from 3 separate experiments.

Supplementary figure: S9







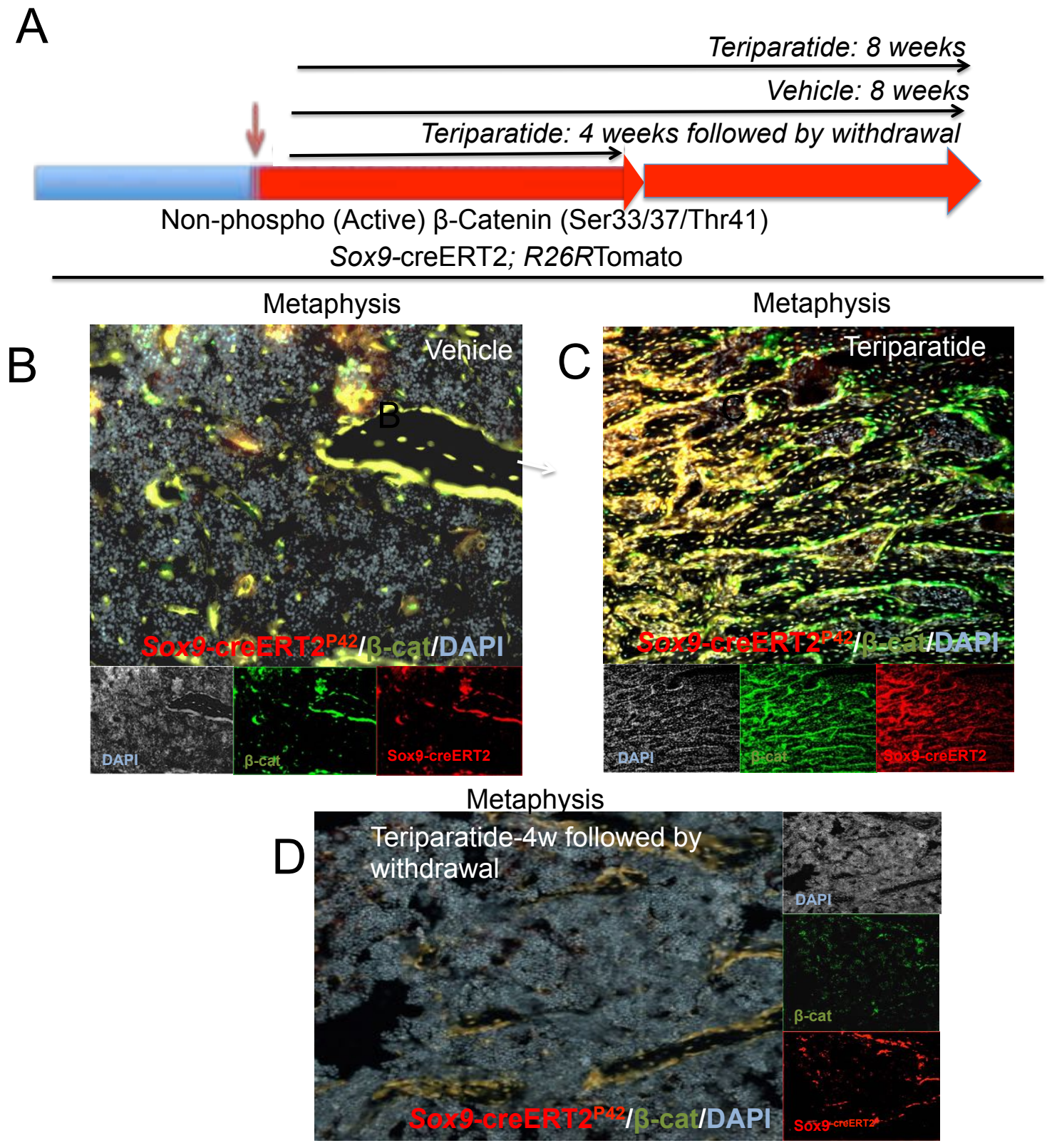


Supplementary figure. S9:

(A) The protocol used in the experiment to study the fate of *Ocn*-creER+ cells after teriparatide withdrawal. Mice received a single tamoxifen injection at P42. 24 hours later, mice were subjected to either vehicle or PTH (1-34) once daily for 30 days. Mice were sacrificed and long bones were harvested for evaluation by confocal microscopy. Each panel reflects data from three mice/genotype from three independent experiments.

(B-D) Confocal images from Vehicle (B), Teriparatide (C) and Teriparatide withdrawal mice (D), i) Magnified view of the box drawn in D and Perilipin co-expression visualized under confocal microscope (E). These data represent sections from 3 separate experiments.

Supplementary figure: S10

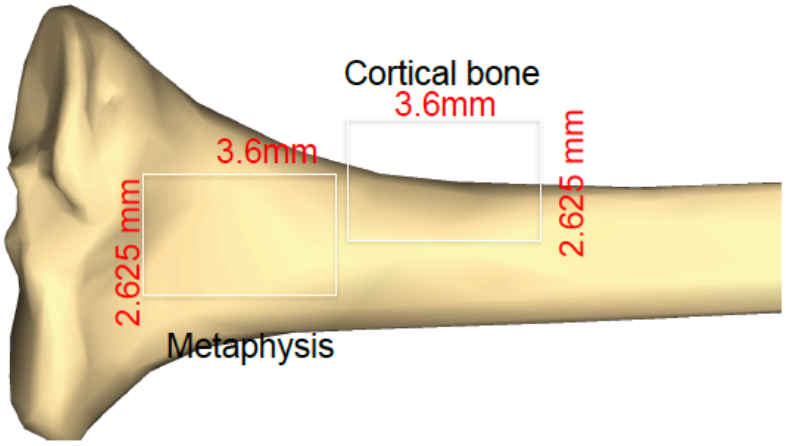


Supplementary figure S10:

(A) The protocol used in the experiment to study the fate of *Sox9*-creERT2+ cells after teriparatide withdrawal. Mice received a single tamoxifen injection at P42. 24 hours later, mice were subjected to either vehicle or PTH (1-34) once daily for 30 days. Mice were sacrificed and long bones were harvested for evaluation by confocal microscopy. Each panel reflects data from three mice/genotype from three independent experiments.

(B-D) To assess the expression of activated β-Catenin, sections were stained and the co-expression of TdTomato+ and β-Catenin was studied under epifluorescence microscope. Arrows point to activated β-Catenin in *Sox9*-creERT2+ cells.

Supplementary Figure 11



Supplementary Figure 11: Orientation of grid during blinded counting of the number of TdTomato+ cells with the help of a microscopic grid. The grid was kept consistent throughout our experiments. The grid on the microscope was aligned as showed. The dimensions were kept constant in all experiments. Length-3.6 mm and breath 2.626 mm.

Supplementary methods: In vitro differentiation of *Sox9*-creERT2; *R26R*Tomato+ cells: Cells were isolated by FACS as described in the section flow cytometry. 300 cells/ well were incubated in 96 well plate in a humidified chamber with 5% CO_2 in α-MEM and 10% FCS along with 1% Penicillin/ streptomycin for 7 days. Medium was changed every 2 days. After 7 days, cells were differentiated into osteoblasts using medium containing 10% FBS, β-glycerophosphate (10 mM), and ascorbic acid (50 µg/ml) and adipocytes using 10% FBS, 3-isobutyl-1-methylxanthine (IBMX, 0.5 µM), dexamethasone (1 µM) and insulin (10 ng/ml) and chondrocytes using a commercially available chondrocytes differentiation kit (Hemogenix, M-CDLS-100).