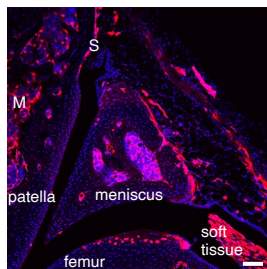


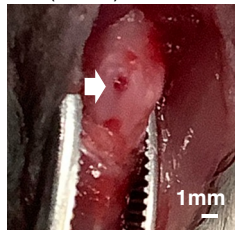
Leptin receptor Cre;tdTomato constitutive Cre euthanasia
 (no tamoxifen given) 2.5 month



A



B Injury using needle
 (time 0)



C Injury using biopsy
 punch (time 0)



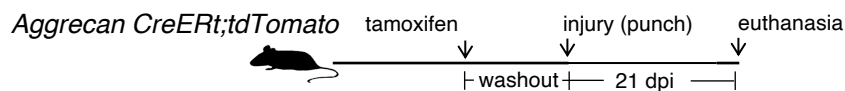
Supplemental figure 1 Diverse tissue labeling with constitutive (non-inducible) Leptin Receptor Cre, and injury models used in this study

A. homeostasis: fluorescent images of the knee of *Leptin Receptor*

Cre;rosatdTomato mice, 2.5 months of age. S, synovium; M, bone marrow. Red: tdTomato. Blue: DAPI nuclei stain. Scale Bar: 50 μm.

B-C. mouse knee surgical wounds at time 0; (B), using needle; (C), using core biopsy punch. Arrow points to the surgical wound. Scale Bar: 1 mm.

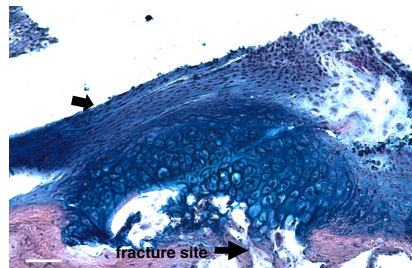
Individual panels might be adjusted for brightness and contrast and displayed using Fiji. The original images may be cropped to display the articular cartilage for adequate visualization.



A 21 dpi tam 8mg using punch



B



Supplemental figure 2 Wound architecture 21 dpi

A,B. Alcian Blue staining of a nearby section to that shown in figure 1G (given tamoxifen 8mg and using punch).

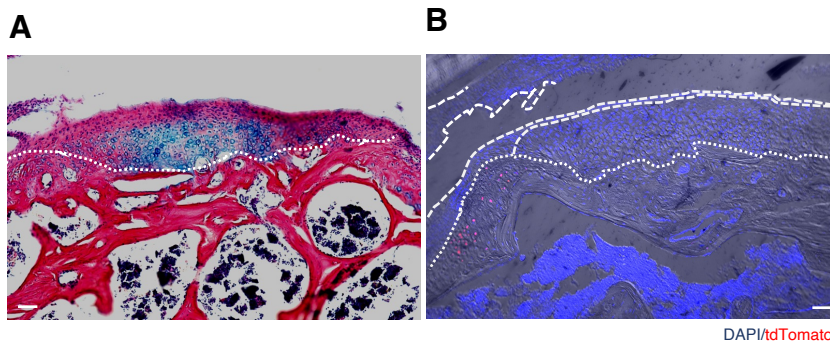
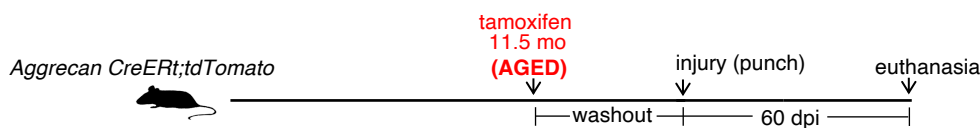
Arrow: superficial soft tissue surrounding the wound proper.

Long Arrows: site of subchondral bone fracture.

Patella tendon was cut during tissue processing to ensure penetration of tissue processing reagents.

Scale Bar: 500 μ m in the uppermost panel (a), and 50 μ m elsewhere.

Individual panels might be adjusted for brightness and contrast and displayed using Fiji. The original images may be cropped to display the articular cartilage for adequate visualization.



Supplemental figure 3 Wound architecture (aged mice), 60 dpi

A,B. Aged (11.5 months) AggreCanCreERT2;rosatdTomato, 60 dpi wound (punch); tamoxifen 4mg, 13 weeks washout to allow observation of any proliferation of aggrecan-expressing marrow stromal cells, and microfracture using 0.3mm biopsy punch (n=2).

(A): Alcian Blue.

(B): fluorescent confocal image of an adjacent section.

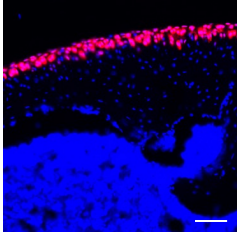
Dashed contours outline the superficial soft tissue surrounding the wound.

Dotted contours: the wound border.

Red: tdTomato. Blue: DAPI nuclei stain.

Scale Bar: 50 μ m.

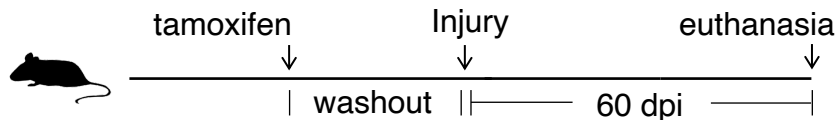
Individual panels might be adjusted for brightness and contrast and displayed using Fiji. The original images may be cropped to display the articular cartilage for adequate visualization.

A

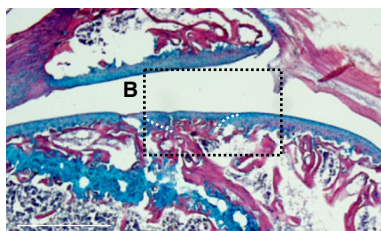
1 Supplemental figure 4

- 2 **A.** fluorescent images of *Prg4 creERT;rosa tdTomato* mouse knee following similar
3 protocol as in figure 2A (homeostasis), given higher tamoxifen at 12mg.

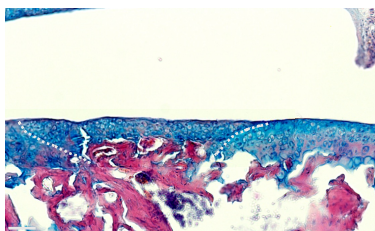
Prg4 CreERt; tomato



A 60 dpi, using needle



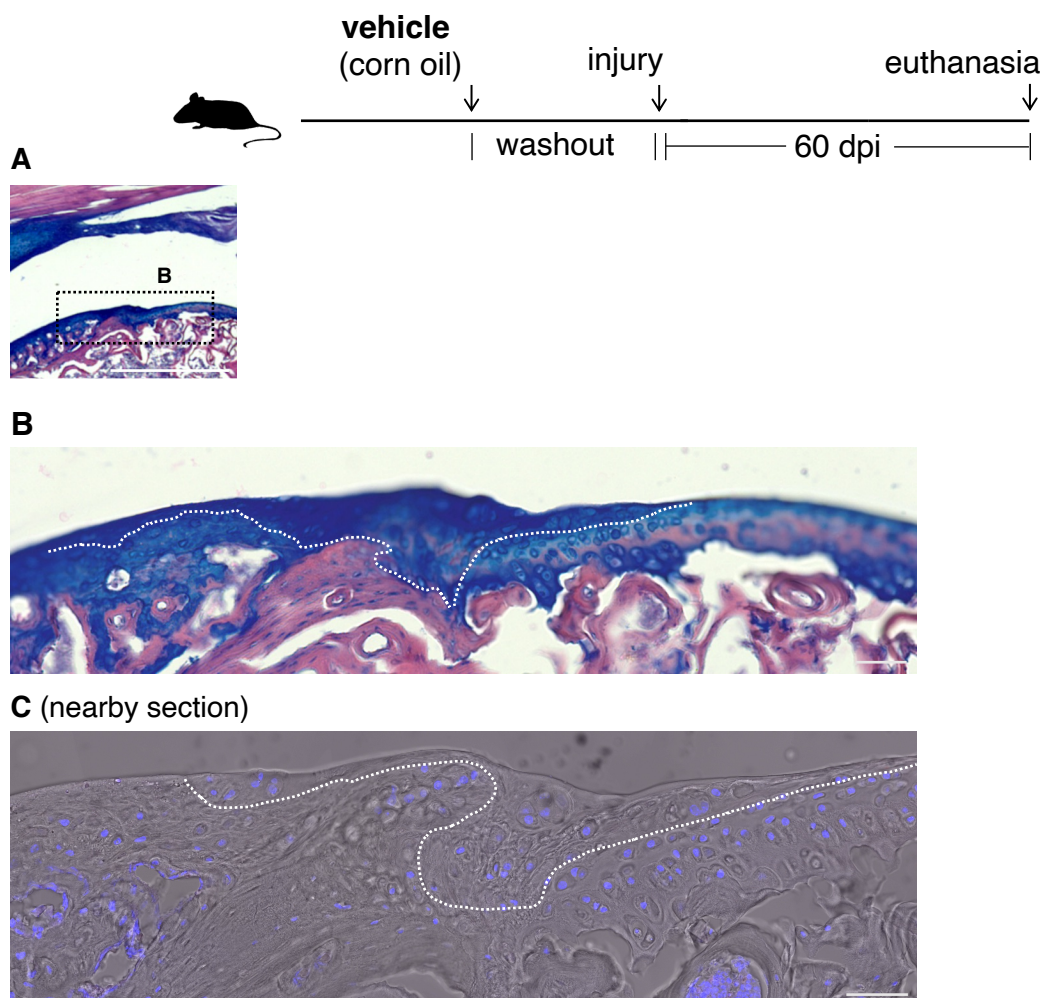
B



Supplemental figure 5 Wound architecture, 60 dpi

A,B. Alcian Blue stained nearby section to figure 2I, with mice injured using needle and euthanized at 60 dpi. Dotted contours: the wound border.

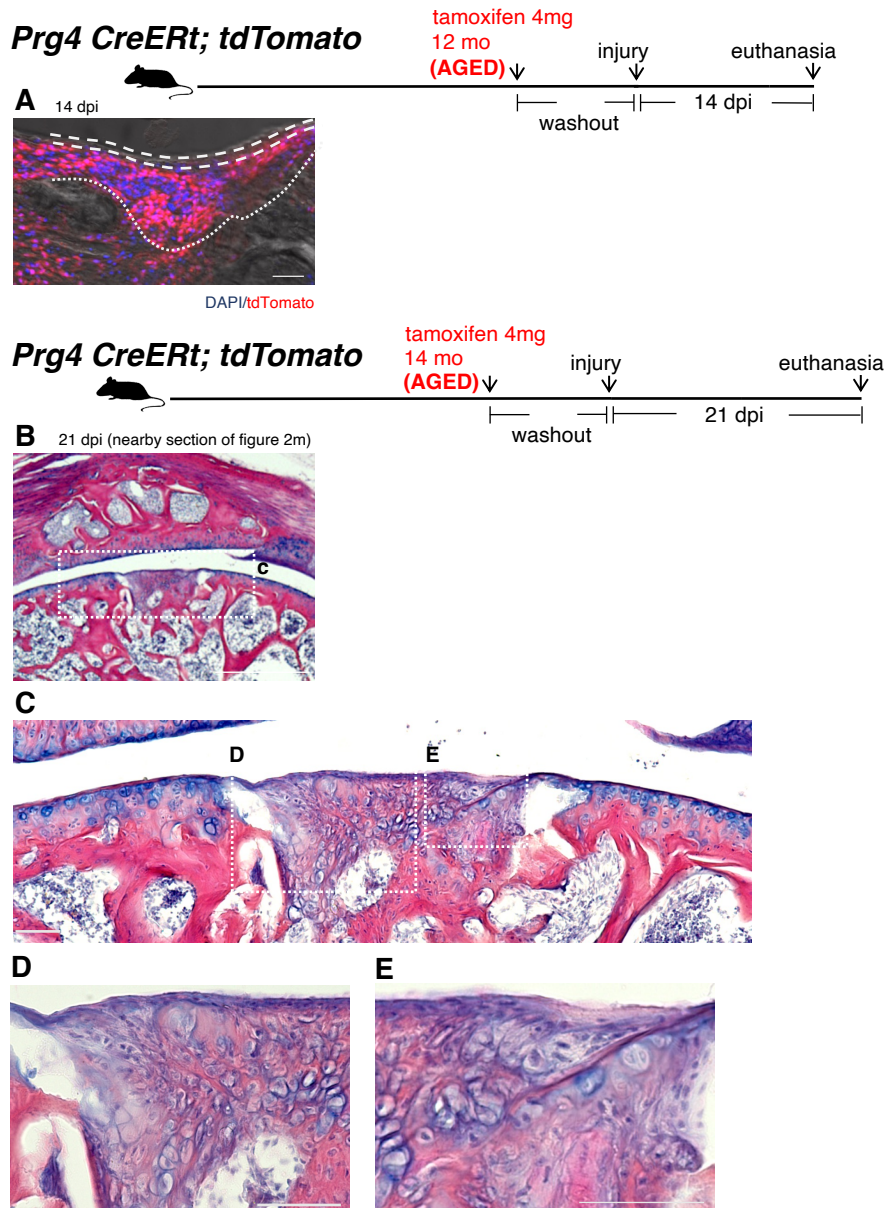
Scale Bar: 500 μ m (A), 50 μ m (B). Individual panels might be adjusted for brightness and contrast and displayed using Fiji. The original images may be cropped to display the articular cartilage for adequate visualization.

Prg4* CreERt; *tdTomato**Supplemental figure 6 Vehicle control**

A-C. Alcian blue (A,B) and fluorescent confocal (C) images of *Prg4* creERt; *rosa* *tdTomato* knee wound (using punch), receiving only corn oil and euthanized at 60 dpi (n=2).

Dotted contours: the wound border. Scale Bar: 500 μ m (A), 50 μ m (B).

Individual panels might be adjusted for brightness and contrast and displayed using Fiji. The original images may be cropped to display the articular cartilage for adequate visualization.



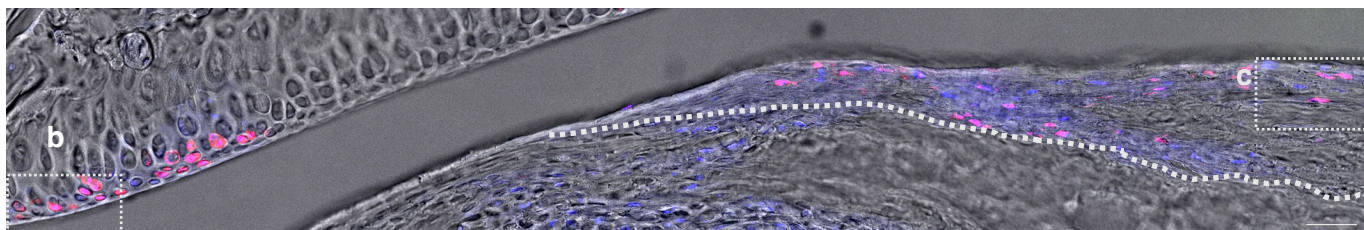
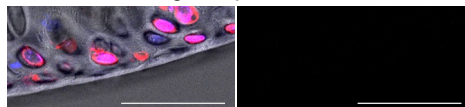
Supplemental figure 7: Wound architecture (aged mice)

A. 14 dpi: fluorescent image of *Prg4 creERT;tdTom* knee wound (needle), given 4mg tamoxifen, followed by washout, injury and euthanasia at 14 dpi .

Red: tdTomato. Blue: DAPI nuclei stain. Scale Bar: 50 μ m.

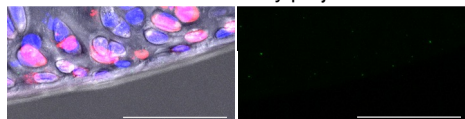
B-E. 21 dpi: Alcian blue staining of *Prg4 creERT;tdTom* knee wound of a nearby section to that shown in figure 3A, given 4mg tamoxifen, followed by washout, injury and euthanasia at 21 dpi. Scale Bar: 500 μ m (B), 50 μ m elsewhere.

Individual panels might be adjusted for brightness and contrast and displayed using Fiji. The original images may be cropped to display the articular cartilage for adequate visualization.

A DAPI/tdTomato/ negative control mRNA**B** single z optic section

DAPI/tdTomato/
negative control mRNA

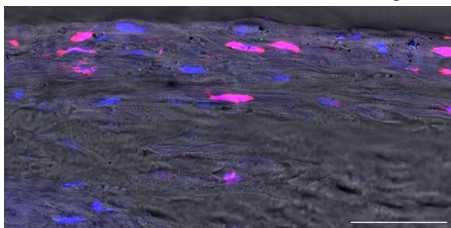
maximum intensity projection



DAPI/tdTomato/
negative control mRNA

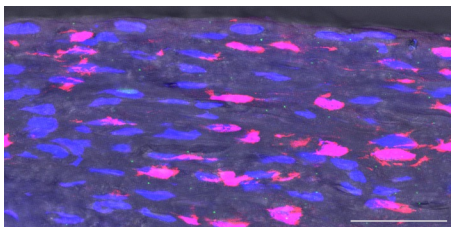
C

single z optic section

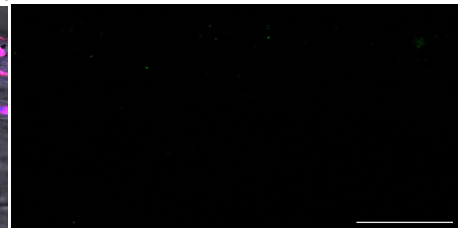


DAPI/tdTomato/negative control mRNA

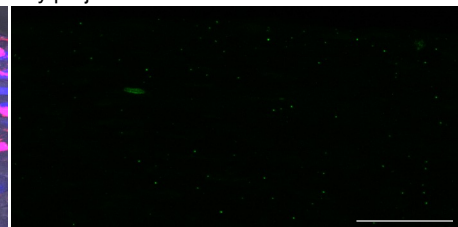
maximum intensity projection



DAPI/tdTomato/negative control mRNA



negative control mRNA



negative control mRNA

Supplemental figure 8 Aggrecan and Col2 mRNA in situ experimental controls related to figure 4

A-C: Aggrecan mRNA in situ hybridization negative controls using negative control mRNA probes and anti-RFP antibody.

(A): overview.

(B,C): high magnification insets.

Dotted contours: the wound border.

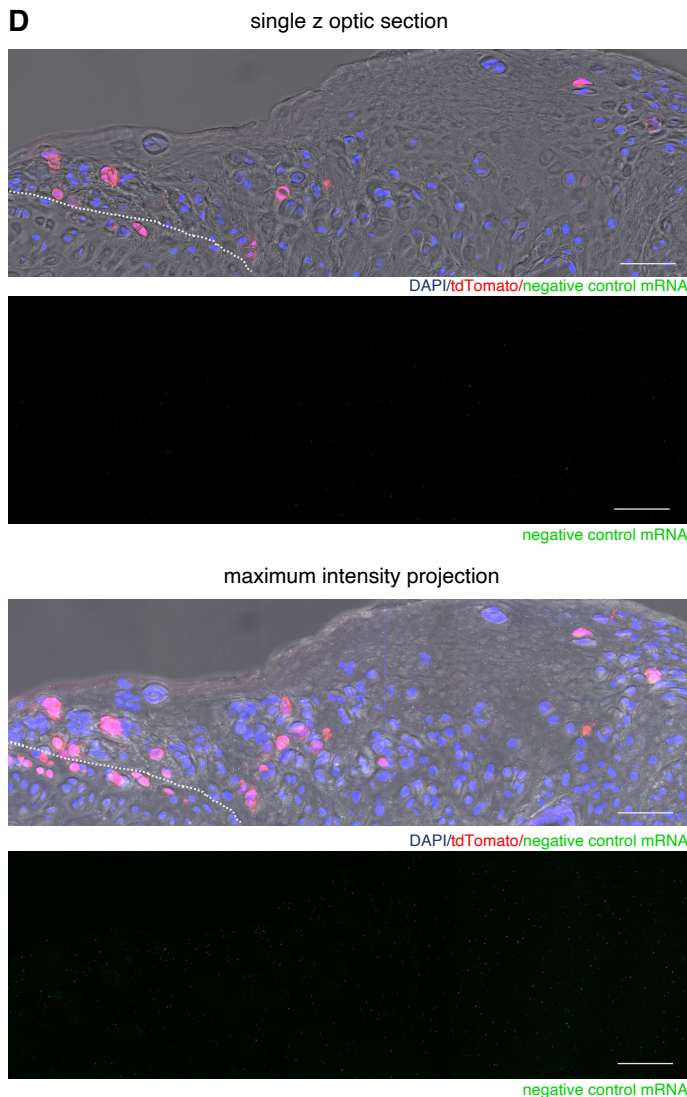
Blue: DAPI. Red: tdTomato. Red was pseudo-colored based on the staining

signals of anti-tdTomato secondary antibody. Green: ACD negative control

mRNA probe. Green was pseudo-colored based on the staining results of

fluorescent mRNA in situ hybridization using far-red fluorophores to eliminate

background associated with cartilage matrix auto-fluorescence.



Supplemental figure 8 (continued,)

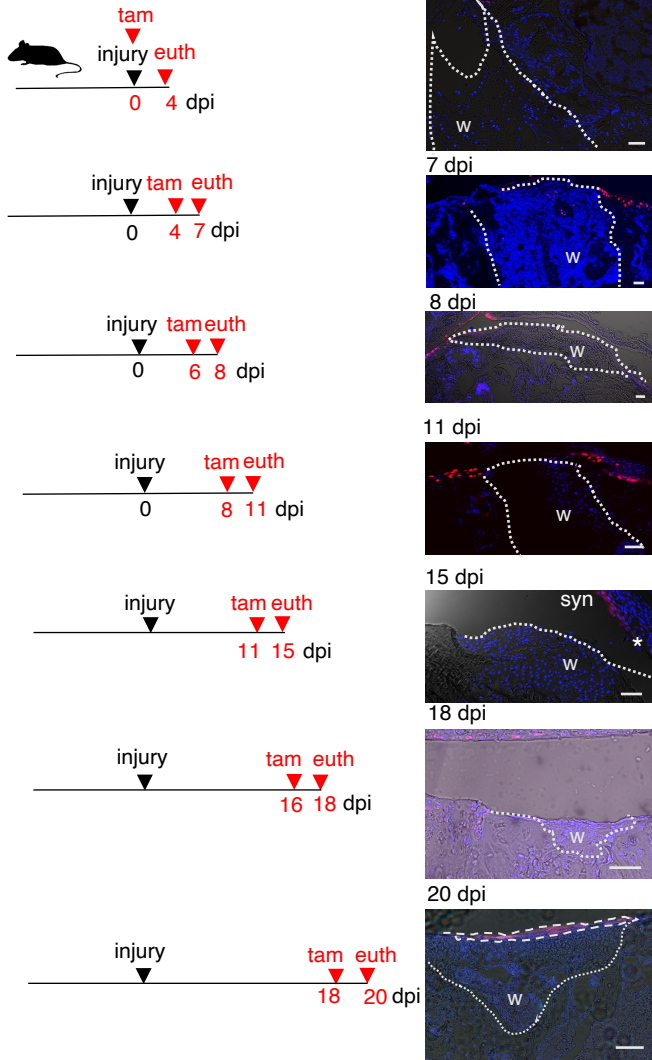
D: Col2 mRNA in situ hybridization negative controls, as in A-C.

Scale bars: 50 μ m. Individual panels might be adjusted for brightness and contrast and displayed using Fiji. The original images may be cropped for adequate visualization.

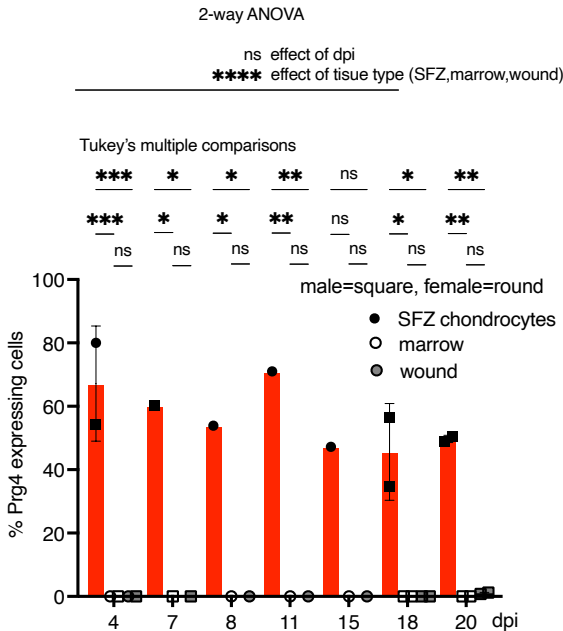
Dotted contours: the wound border.

Blue: DAPI. Red: tdTomato. Red was pseudo-colored based on the staining signals of anti-tdTomato secondary antibody. Green: ACD negative control mRNA probe. Green was pseudo-colored based on the staining results of fluorescent mRNA in situ hybridization using far-red fluorophores to eliminate background associated with cartilage matrix auto-fluorescence.

A *Prg4* CreERT;tdTomato



B *Prg4* expressing cells in post-injury marrow & wounds



Supplemental figure 9 % *Prg4* expressing cells in post-injury marrow and wounds

A. fluorescent images of *Prg4* CreERT; rosa tdTomato with injury first then tamoxifen (reversing the sequence of tamoxifen and injury used in figure 2), to detect *Prg4* expressing cells in post-injury marrow and wounds, showing post-injury marrow, wounds (“w”), articular chondrocytes, and synovium (“syn”). Tamoxifen 4 mg was given for all time points, except 6mg for 4,6,8,11 dpi with euthanasia within 2-4 days. Dashed contours outline the superficial soft tissue surrounding the wound. Dotted contours: the wound border. At 20 dpi, soft tissue surrounding the wound contained some tdTomato+ cells but these were not included in wound cell counts due to their locations outside the wound proper. Red: tdTomato. Blue: DAPI nuclei stain. Scale bars: 50 μ m. Individual panels might be adjusted for brightness and contrast and displayed using Fiji. The original images may be cropped to display the articular cartilage for adequate visualization.

B. Quantitative summary: % *Prg4* expressing (tdTomato) cells in tissues post-injury. (n=2 for groups given tamoxifen at 0, 16 or 18 dpi, and n=1 for the other groups). Two-way ANOVA: interaction, F=0.7812 (df between groups=12 and within groups=9), p=0.6622; time effect (wound dpi), F=0.7492 (df between groups=6 and within groups=9), p=0.6255; tissue effect (SFZ chondrocytes, marrow, and wound), F=150.2 (df between groups=2 and within groups=9), p<0.0001; Tukey’s multiple comparisons shown. All data were presented as mean $\pm$ s.d.



Supplemental figure 10 Adult-marrow-dwelling stromal cells (peri-natal Sp7-lineage) are not major contributors to cartilage wound repair

A,B: Peri-natal tamoxifen labeling only.

(A). homeostasis: fluorescent images of knees of Sp7 (Osterix) creERT; rosa tdTomato mice showing tdTomato cells in adult bone marrow after receiving tamoxifen 0.1 mg/g during peri-natal time points (e18.5, n=1; e18.5+post-natal-day5, n=2) and euthanasia at 2.2m. High magnification views in insets (1-3). C: articular cartilage; M: marrow; S: synovium; L: lining; SL: synovium sublining.

(B). injury: mice received tamoxifen at peri-natal time points, followed by injury at 2m and euthanasia at 14 dpi (n=2). High magnification view of synovium in inset.

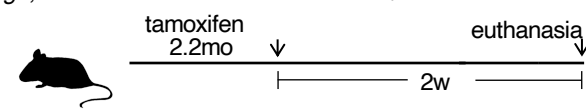
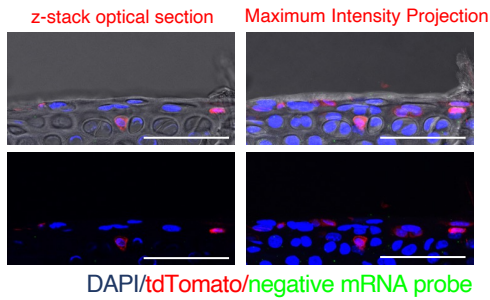
C,D: Peri- and post-natal tamoxifen labeling.

(C). homeostasis: fluorescent images of knee after receiving tamoxifen 0.1 mg/g at e18.5+post-natal-day5, 1mg at 3 weeks, 2mg at 6 weeks, and 2mg at 7 weeks of age (n=2), with euthanasia at 2.2m. High magnification views in insets (1-3). C: articular cartilage; M: marrow; S: synovium; L: lining; SL: synovium sublining.

(D). injury: mice followed the same tamoxifen protocol as in homeostasis (C) at e18.5, followed by 2 weeks washout after the last tamoxifen injection, injury at 2.4m, and euthanasia at 14 dpi (n=2) or 21 dpi (n=4). Dotted line outlines the cartilage wound.

Red: tdTomato. Blue: DAPI. Scale Bar: 50 μ m unless otherwise indicated.

E. Quantitative summary: the abundance of tdTomato cells in adult marrow in homeostasis (left bars) and wounds (right bars). Sample size: as described above. For marrow homeostasis control, unpaired two-tailed student's t-test: $t=1.489$, $df=3$, $p=0.2331$; for wounds, one-way ANOVA: $F = 4.974$ (df between groups=2 and within groups=5 , $p=0.0647$; Tukey's multiple comparisons shown. All data were presented as mean $\pm$ s.d.

**A** Negative control (negative mRNA probe)**Supplemental figure 11**

A: negative control in Aggrecan mRNA in situ hybridization assay: single z optic section vs. maximum intensity projection. Scale bars: 50 μ m. Blue: DAPI. Red: tdTomato. Red was pseudo-colored based on the staining signals of anti-tdTomato secondary antibody. Green: ACD negative control mRNA probe. Green was pseudo-colored based on the staining results of fluorescent mRNA in situ hybridization using far-red fluorophores to eliminate background associated with cartilage matrix auto-fluorescence.