Supplemental Information for: Identification of a Prg4-positive articular cartilage progenitor cell population

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Supplemental Materials and Methods

RNA-Seq

Mice carrying *Prg4*$^{GFPCreERt2/GFPCreERt2}$ (n=1) and *Prg4*$^{+/+}$ (n=1) alleles were sacrificed with CO_2 asphyxiation according to protocols approved by the Boston Children's Hospital Institutional Animal Care and Use Committee. Immediately after euthanasia, the right knee joints of mice were extracted with excisions at the tibial and femoral growth plates. Muscular tissue surrounding the joint capsule was quickly cleaned, and the knee joints were snap-frozen in liquid nitrogen. Total RNA was extracted from each specimen with phenol-chloroform separation, on-column purification and DNase treatment. Bar-coded cDNA libraries were generated using the TruSeq RNA Sample Preparation Kit (v2, Illumina, San Diego, CA). Libraries were multiplexed and sequenced on a single flowcell of an Illumina MiSeq desktop sequencer with 100 bp paired-end reads. 3.4 and 4.2 million read pairs were generated with the WT and GFPCreERt2 libraries, respectively. Reads were mapped to the mouse genome (mm9) and to the *GFPCreERt2* sequence (1) using Tophat2 (2). We estimated *GFPCreERt2* expression in the knee joint of the *Prg4*$^{GFPCreERt2/GFPCreERt2}$ mouse by counting the reads that mapped to the *GFPCreERt2* sequence and dividing this number by the sequence length. This yielded a measure of reads/bp. Similarly, we estimated the expression of endogenous *Prg4* mRNA in the *Prg4*$^{+/+}$ mouse knee by counting the total number of reads that mapped to exons 2 or 4

of *Prg4* and dividing this number by the length of each exon; we used these two individual exons rather than the entire *Prg4* transcript, because these 2 exons are in most *Prg4* isoforms and because exon 7 of *Prg4* encodes the highly repetitive mucin-like domain that is difficult to sequence and map with RNAseq. We both normalized the *GFPCreERt2* and *Prg4* ratios with other cartilage-specific transcripts (e.g., *Col2a1, Acan, Comp, Col9a2*), and also calculated the relative RPKM values for *GFPCreERt2* and *Prg4*, assuming that each knee joint library contained comparable relative abundances of cartilage and synovium.

Supplemental References

1. Mugford JW, Sipila P, McMahon JA, McMahon AP. Osr1 expression demarcates a multi-potent population of intermediate mesoderm that undergoes progressive restriction to an Osr1-dependent nephron progenitor compartment within the mammalian kidney. Dev Biol. 2008;324(1):88-98.
2. Kim D, Pertea G, Trapnell C, Pimentel H, Kelley R, Salzberg SL. TopHat2: accurate alignment of transcriptomes in the presence of insertions, deletions and gene fusions. Genome Biol. 2013;14(4):R36.

Supplemental Table 1.

	Normalized to *Prg4 and to ..*	*..Col2a1*	*..Acan*	*..Comp*	*..Col9a2*	RPKM
CRE/WT	**Exon 2**	7.1%	3.3%	4.9%	8.3%	4.3%
	Exon 4	3.4%	1.6%	2.4%	3.9%	2.1%

Supplemental Table 1: Relative expression level of the *Prg4*^{eGFPcreERT2} allele with respect to the *Prg4*$^{+}$ allele. Displayed are the relative ratios for *GFPCreERt2* and *Prg4* transcripts (CRE/WT for either exons 2 or 4 of WT *Prg4*), which have been normalized to levels of either *Col2a1, Acan, Comp, or Col9a2*. In addition we also display the relative Reads Per Kilobase of transcript per Million mapped reads (i.e., RPKM values) for *GFPCreERt2* and *Prg4*, normalized to the total number of reads for each library. Expression levels for the *GFPCreERt2* allele relative to the endogenous *Prg4* allele varied based on the normalization method. However, the data are consistent with *GFPCreERt2* expression being 12 to 50-fold lower than endogenous *Prg4* expression.

Supplemental Figure Legends

Supplemental Figure 1. *Prg4*^{GFPCreERt2} allele generation and genotyping. (A) Agarose gel electrophoresis depicting PCR amplimers (42+37 and 39+57) and their digestion products (42+37/E and 39+57/S) from a correctly targeted ES cell clone. **(B)** Agarose gel electrophoresis depicting PCR amplimers from wild-type (WT), homozygous knock-in (Homo; *Prg4*$^{GFPCreERt2/GFPCreERt2}$), and heterozygous knock-in (Het; *Prg4*$^{+/GFPCreERt2}$) mice. Primer pair F1/R1 produces a 337 bp amplimer from the *Prg4*^{GFPCreERt2} allele and primer pair F1/R2 produces a 258 bp amplimer from the *Prg4*$^{+}$ allele (F1-TCAGGAATTCAAGCTGATTGC; R1-AACTTGTGGCCGTTTACGTC; R2-CCTTGAGATGAAACCTGTTGAATC). **(C)** Hematoxylin & eosin stained sections of knee articular cartilage from 9-month-old *Prg4*$^{+/+}$ and *Prg4*$^{GFPCreERt2/GFPCreERt2}$ mice. Note that the superficial-most chondrocyte layer, which is present in *Prg4*$^{+/+}$ cartilage (left arrow), is absent from the *Prg4*$^{GFPCreERt2/GFPCreERt2}$ cartilage (right arrow).

Supplemental Figure 2. *Prg4*^{GFPCreERt2} drives recombination and expression of mGFP from the *Rosa26*^{mTmG} allele. Either *Prg4*$^{+/GFPCreERt2}$ mice, *Rosa26*$^{+/mTmG}$ mice, or *Prg4*$^{+/GFPCreERt2}$;*Rosa26*$^{+/mTmG}$ mice were injected with tamoxifen at P21 for 10 days and harvested 3 days after the last injection. Sections of knee joints of these animals were assayed for either mGFP fluorescence **(A, B, C),** mTomato fluorescence **(D, E, F),** or DAPI staining **(G, H, I).** Note that *Prg4*$^{+/GFPCreERt2}$ mouse (which does not contain the *Rosa26*^{mTmG} allele) did not display detectable levels of GFP fluorescence.

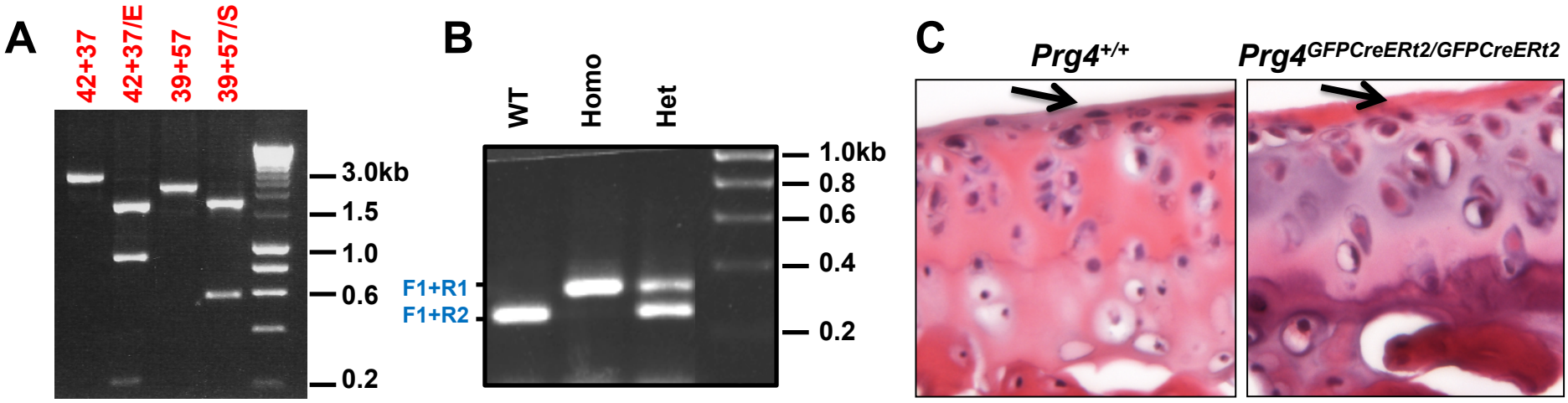
Supplemental Figure 3. β-galactosidase expression in a 15-month-old *Prg4* gene-trap mouse *(Prg4$^{+/lacZgenetrap}$)* is observed in all zones of the femoral head articular cartilage, extending nearly to the subchondral bone. Displayed is a section of the femoral head of a 15-month-old *Prg4* gene-trap mouse *(Prg4$^{+/lacZgenetrap}$)* that has been stained with X-Gal/fast red.

Supplemental 4. *Prg4^{GFPCreERt2}* is expressed in the knee joint, ankle and tail tendons, heart and liver. P21 *Prg4$^{+/GFPCreERt2}$;Rosa26$^{+/floxlacZ}$* mice were injected with tamoxifen (Tam) or corn oil as indicated for 10 consecutive days and harvested 3 days after the last injection. Tissues and organs were harvested and X-Gal staining was performed on the knee joint **(A)**, tail tendons **(B)**, the heart **(C,D)** and the liver **(E, F)**. All X-Gal stained sections were counterstained with fast red.

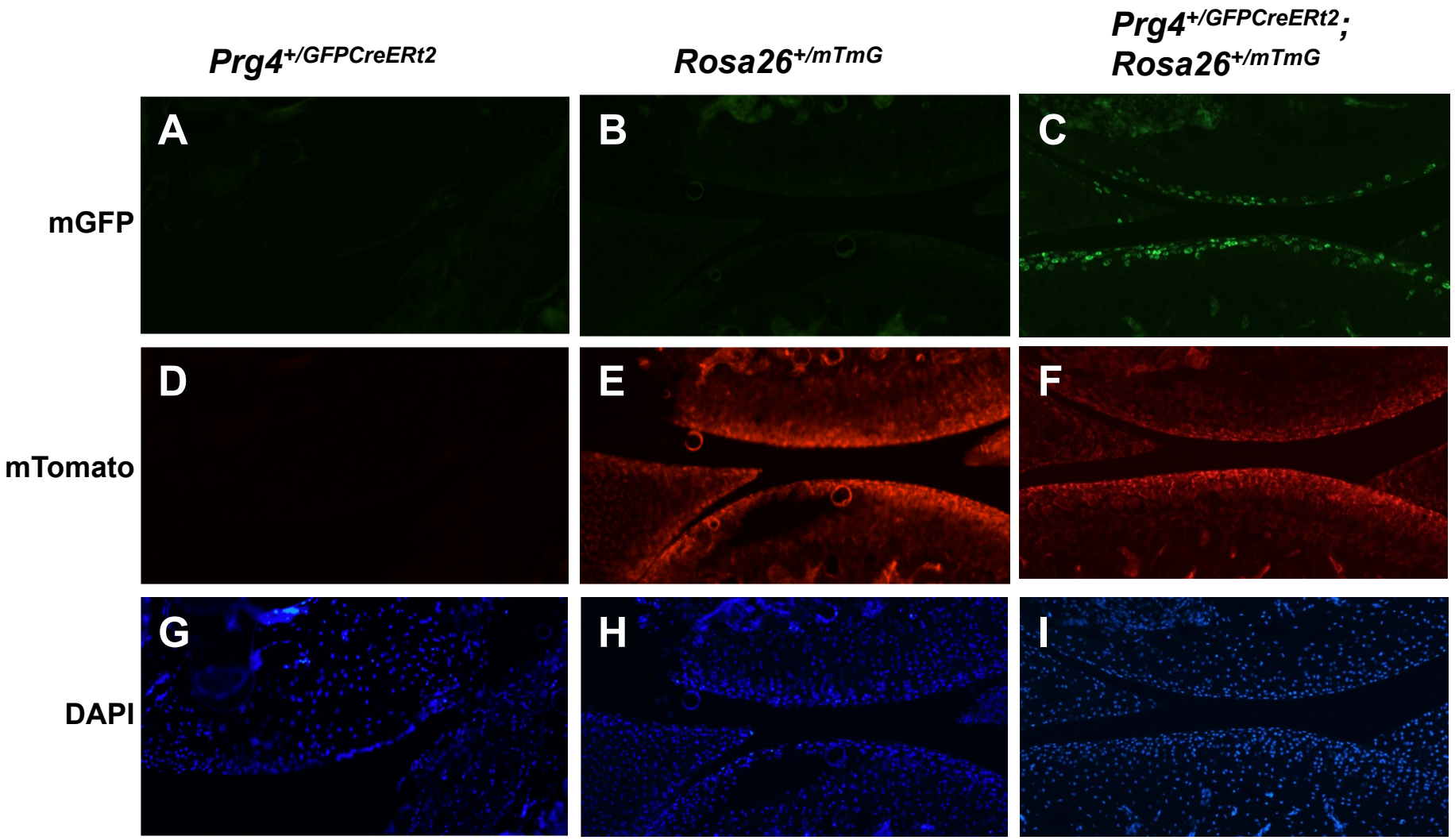
Supplemental Figure 5. *Prg4^{GFPCreERt2}* is expressed in hepatocytes in the liver. (A-C) Sections of liver from 1-month-old *Prg4$^{+/+}$* mice were immunostained for expression of liver cell-type specific proteins: ASMA (Ito cells), CD31 (endothelial cells), and albumin (hepatocytes)**. (D**) Sections of the liver from 1-month-old *Prg4$^{+/GFPCreERt2}$; Rosa26$^{+/mTmG}$* mice that had been injected with tamoxifen for 10 consecutive days and analyzed for mGFP and mTomato expression by fluorescence microscopy. GFP expressing cells in *Prg4$^{+/GFPCreERt2}$; Rosa26$^{+/mTmG}$* mouse liver have a morphology and location consistent with being hepatocytes.

Supplemental Figure 6. *Prg4*-expressing cells that were lineage-labeled in 1-month-old *Prg4*$^{+/GFPCreERt2}$;*Rosa26*$^{+/floxlacZ}$ mice gave rise to β-galactosidase expressing cells in the meniscus, synovium, and patella. Representative X-Gal stained knee joint from 18-months-old *Prg4*$^{+/GFPCreERt2}$;*Rosa26*$^{+/floxlacZ}$ mouse that had been given daily IP injections of tamoxifen from P21 to P31 (A). Higher magnification (20X) images showing meniscus (B), synovium (C) and patella groove (D) are displayed. Note that most β-galactosidase expressing superficial chondrocytes had been lost in these older animals.

Supplemental Figure 1



Supplemental Figure 2



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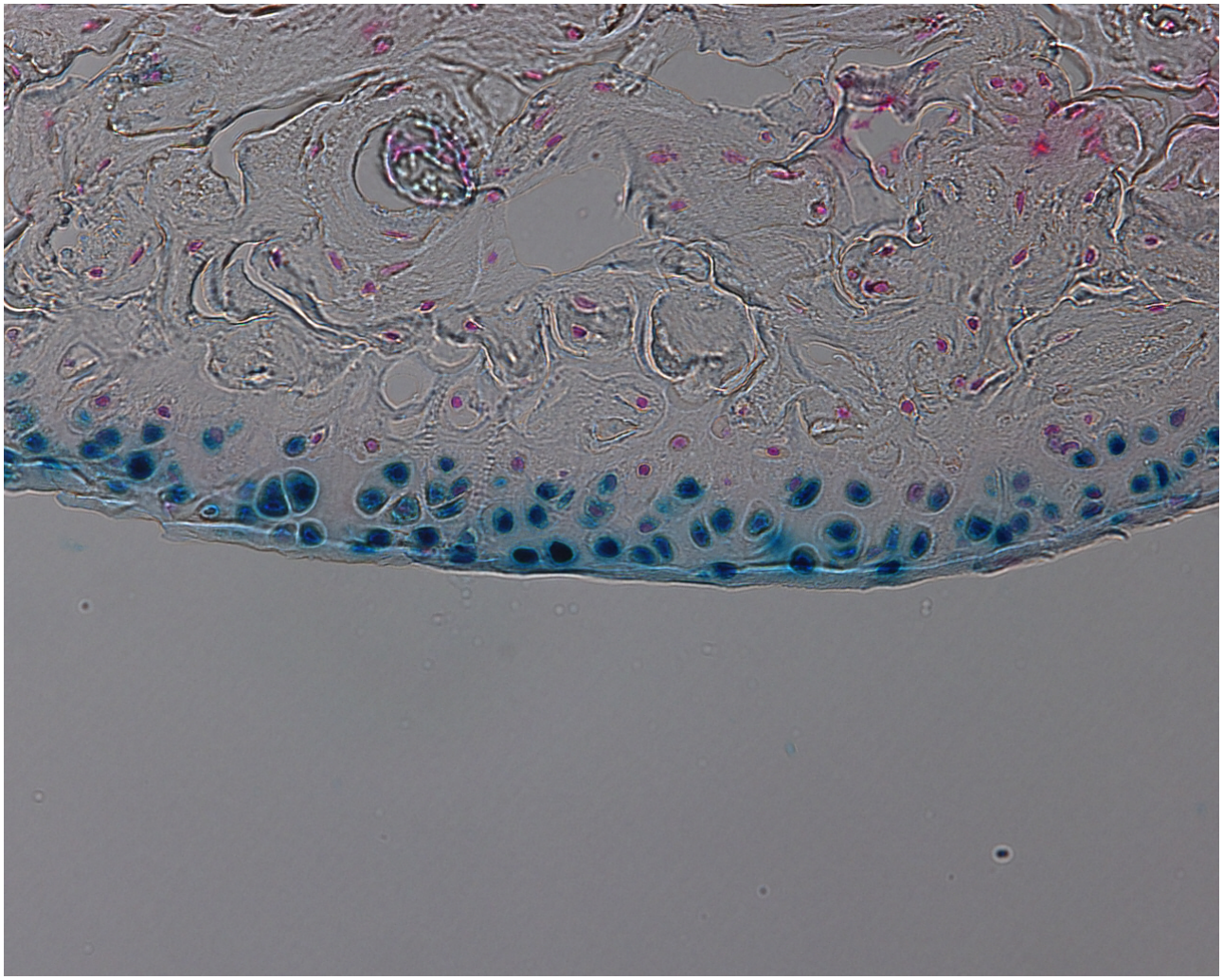
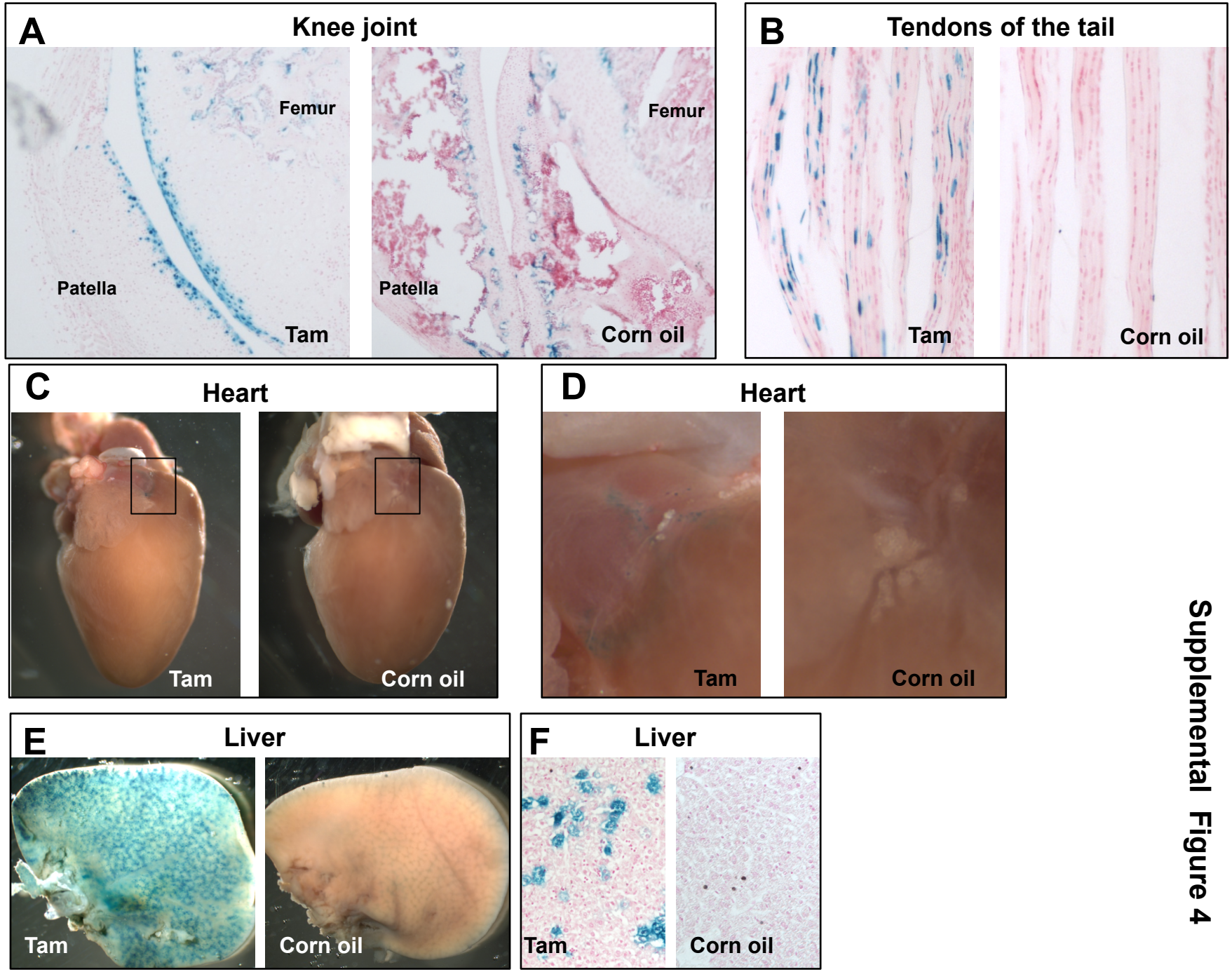
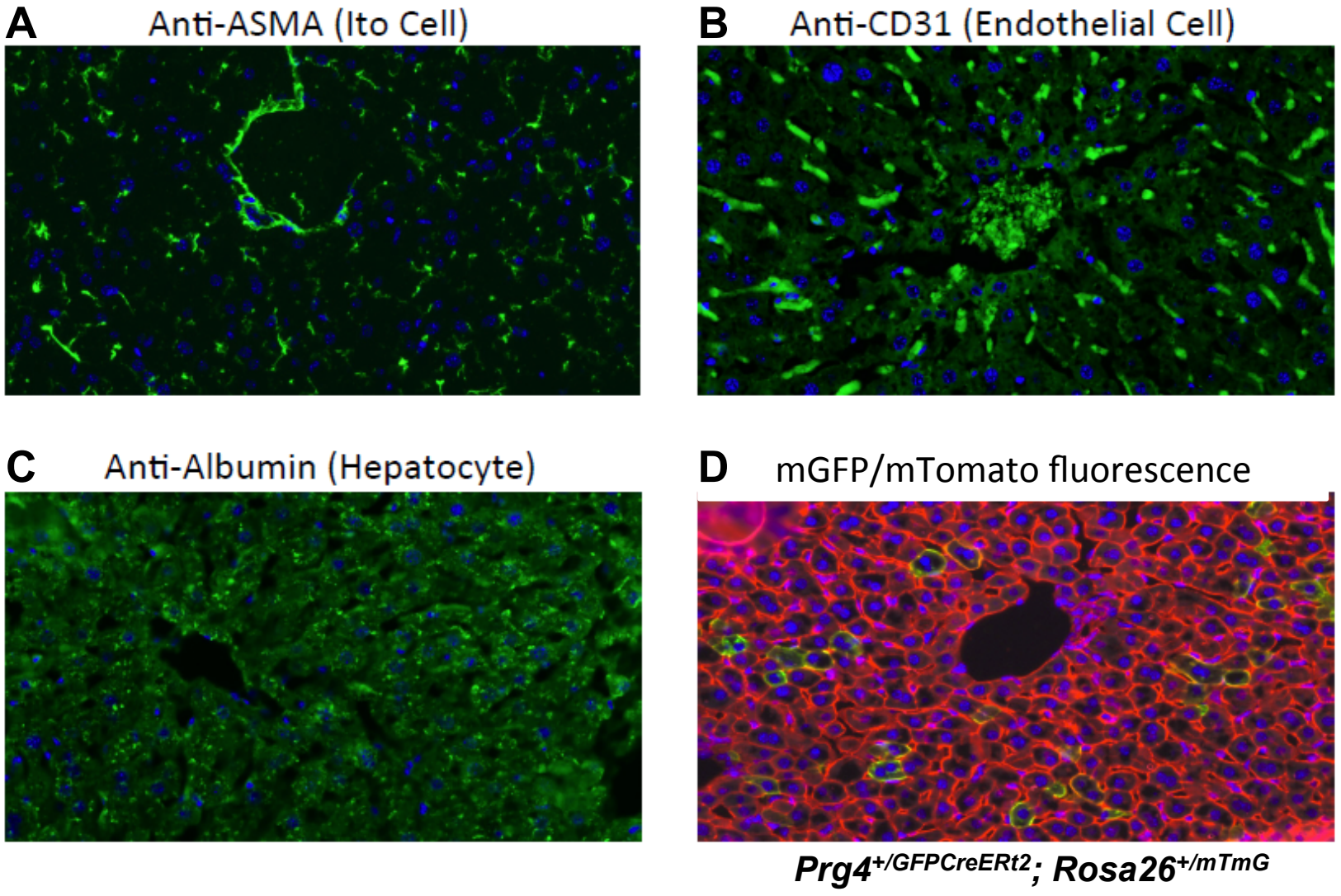




Supplemental Figure 4

Supplemental Figure 5



***Prg4*$^{+/GFPCreERt2}$*; Rosa26*$^{+/mTmG}$

Supplemental Figure 6