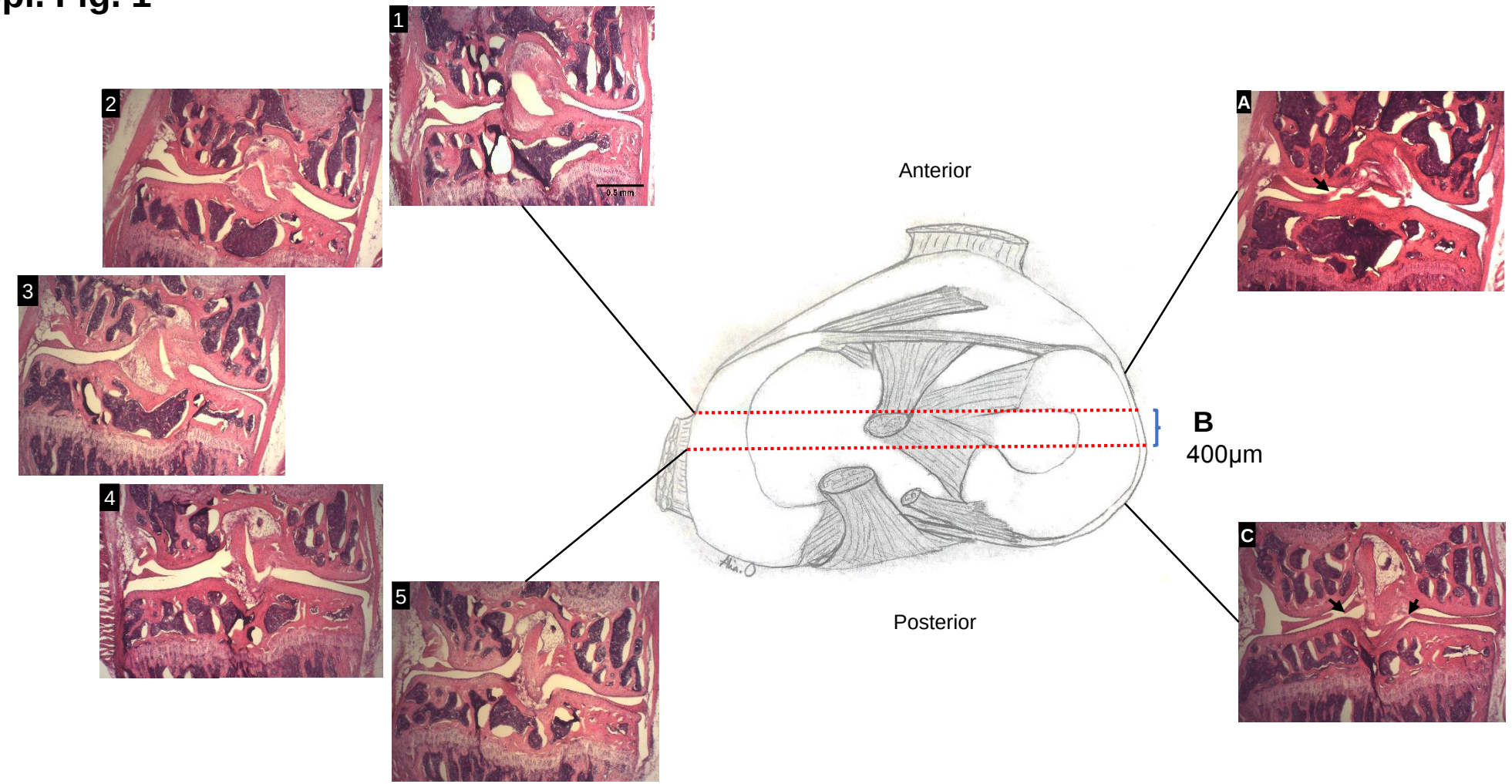
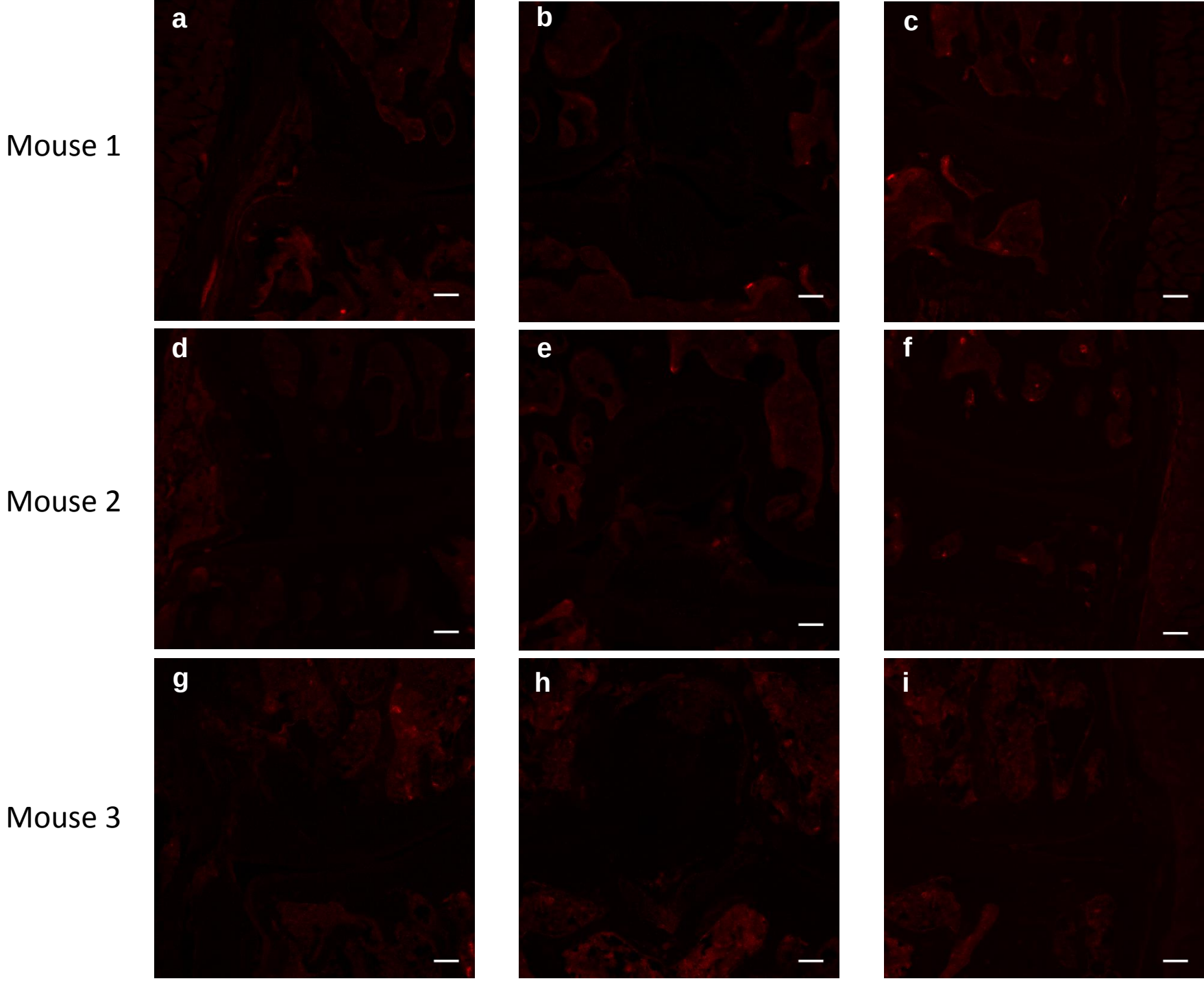
Suppl. Fig. 1



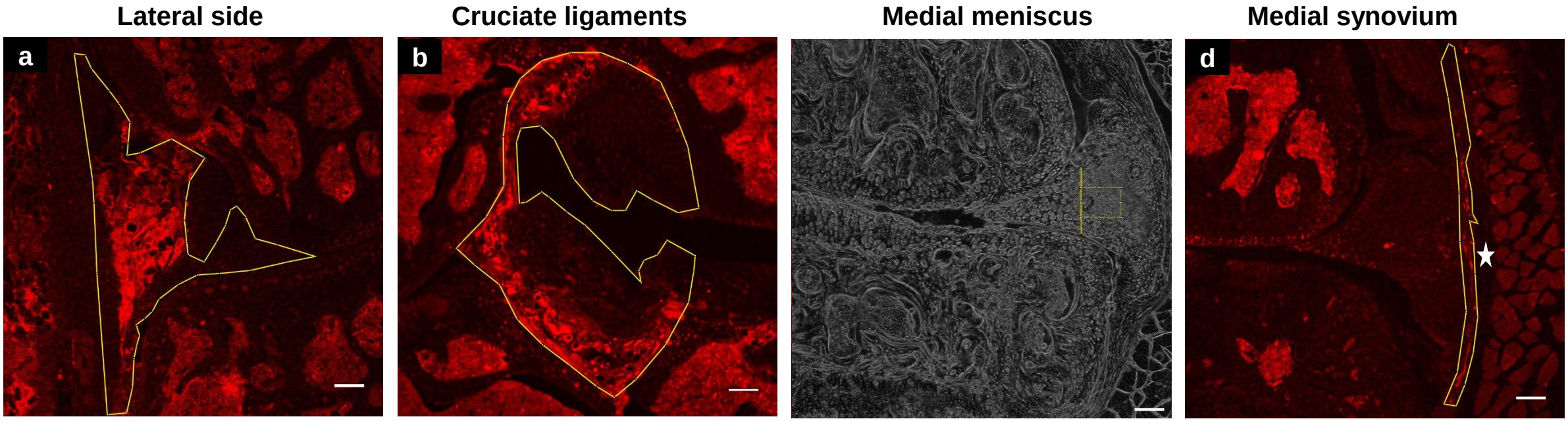
Supplemental Fig. 1 Twenty-µm thick coronal sections were cut throughout the whole joint as follows: the first 400-µm thick area comprises the anterior covering of the knee joint and includes the patella and attached patellar tendon. The next fifty sections (1000-µm thick area) comprises the anterior region of the articular cavity and is defined as the area where at least one of the meniscal horns (medial, lateral or both) have a ligament attached to it. (A) Shows a representative H&E stained coronal section depicting the end of the anterior region of the articular cavity where the lateral meniscal horn is still attached to a ligament (shown by arrow); (B, 1-5) The mid joint region comprises the next twenty sections from an approximately 400-µm thick region, and this region is the focus of this study. The mid-joint is the region where the meniscal horn is devoid of ligament attachments; (C) Shows the beginning of the posterior region of the articular cavity. Arrows in (C) indicate the start of the ligamentous attachments to the posterior horn of the medial meniscus

Suppl. Fig. 2



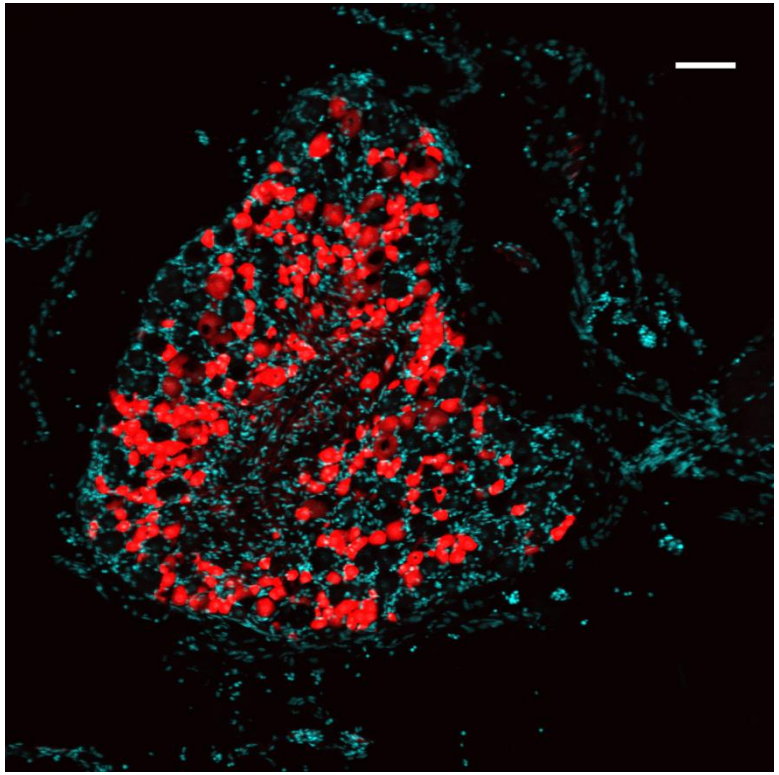
Supplemental Fig. 2. Confocal images of 3 right knees from 3 wild type male mice (10 weeks old) showing the lateral (a,d,g), middle (b,e,h) and medial (c,f,i) compartments of each knee. Sections were imaged and processed applying the same setting used in $Na_V1.8$-tdTomato knees. Scale bar = 100 μm.

Suppl. Fig. 3



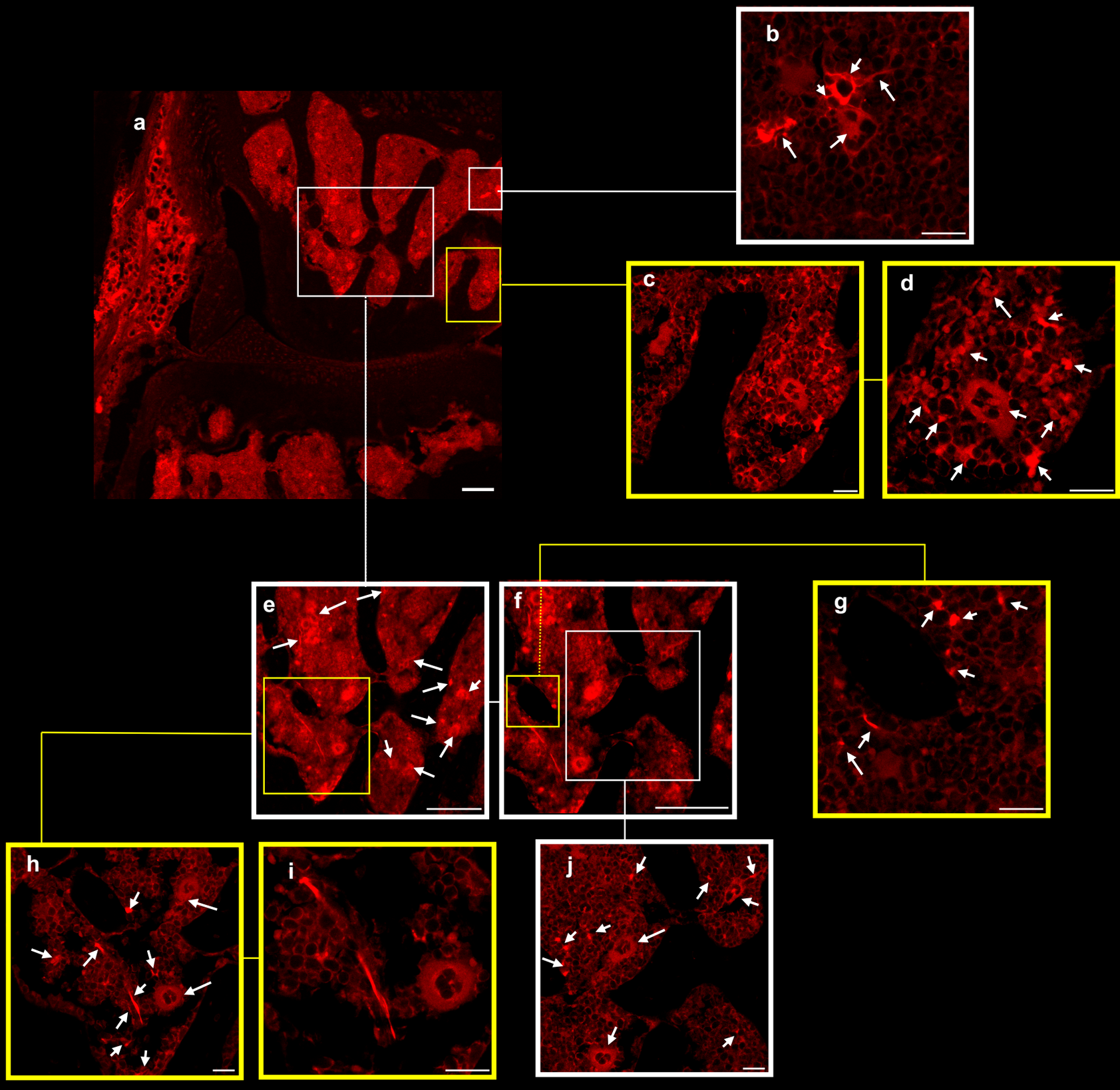
Supplemental Fig. 3 Outline of regions of interest for quantification of signal in mid-joint coronal sections. (a) The lateral meniscus and synovium outlined in a 10-week old knee; (b) The cruciate ligaments outlined in a 10-week old knee; (c) The outer region of the medial meniscus close to the junction of inner and outer region is outlined in the medial meniscus of knee 16 weeks after DMM. The junction between outer and inner region of the meniscus is indicated by the yellow line; (d) The medial synovium adjacent to the capsule outlined in a knee, 16 weeks after DMM. The star points to the capsule. Scale bar = 100 μm

Suppl. Fig. 4



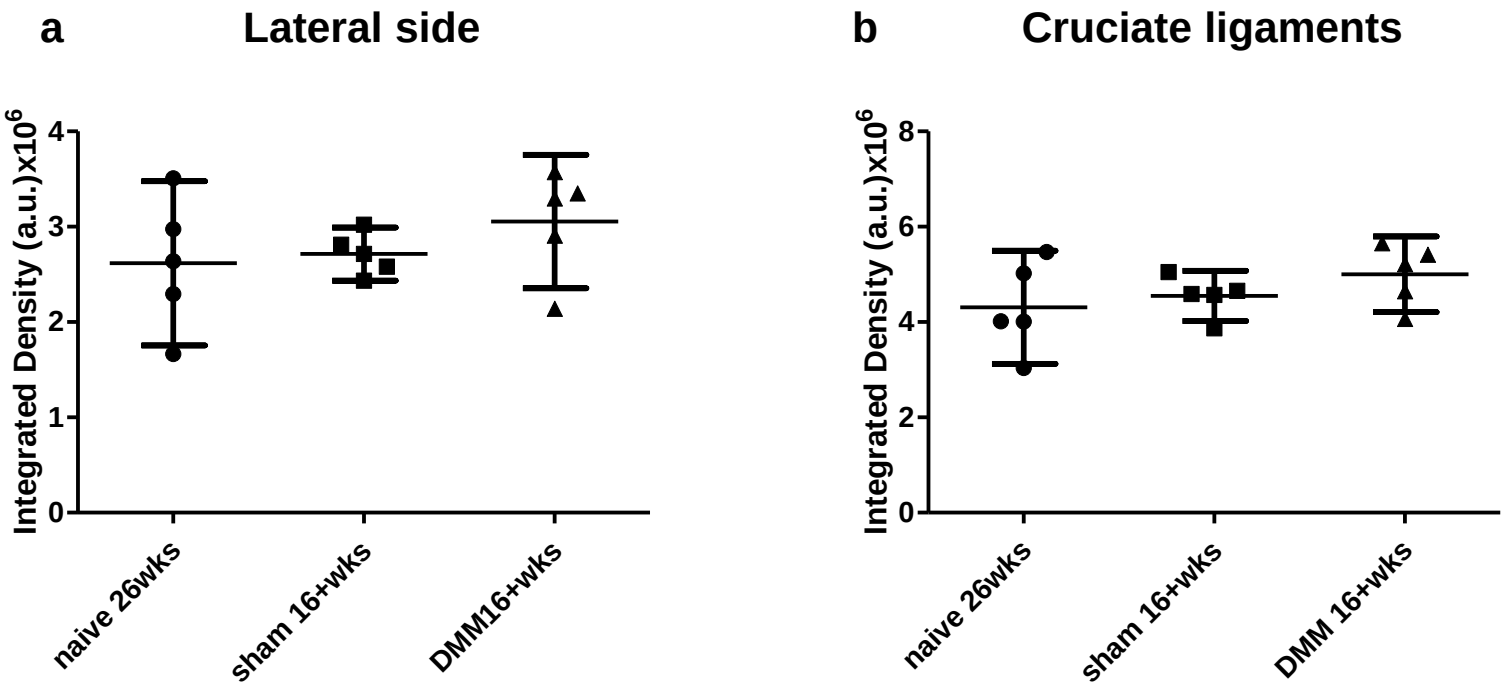
$Na_V1.8$-tdTomato L4 DRG

Supplemental Fig. 4 Confocal image of an L4 DRG harvested from a $Na_V1.8$-tdTomato mouse, showing $Na_V1.8$-positive cell bodies, which are all small-to-medium sized, consistent with nociceptors. Scale bar = 100μm.



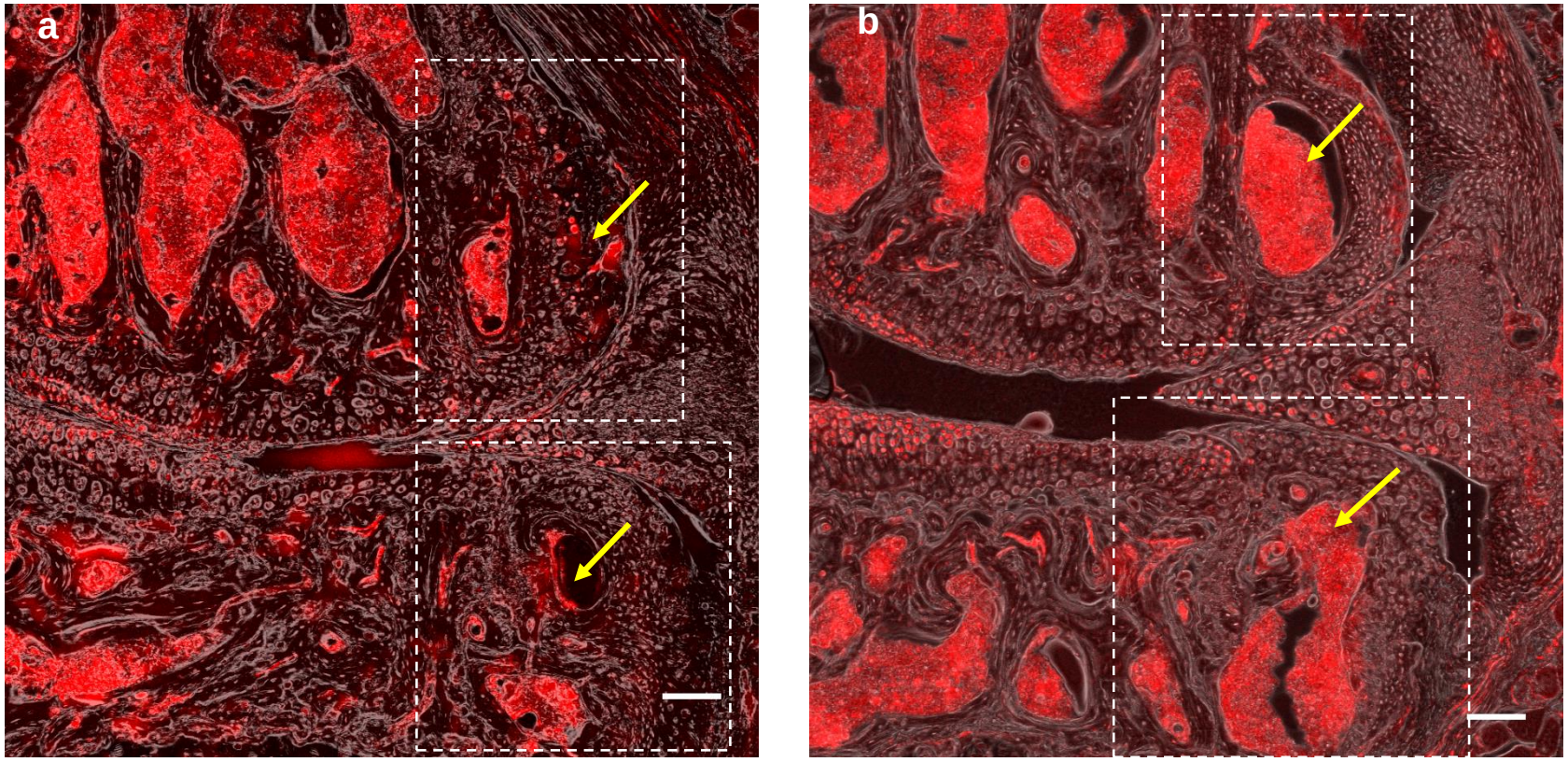
Supplemental Fig. 5 Confocal images of the right knee of a 10-week old male naïve $Na_V1.8$-tdTomato mouse. (a) overview of $Na_V1.8$-tdTomato signal in the lateral compartment of the knee; magnified area of top white inset in (b) showing an area of bone marrow with $Na_V1.8$ signal; magnified areas of yellow inset in (c,d); magnified area of white inset in (e,f); (g) yellow inset of (f) magnified; (h,i) magnified area of the yellow inset of (e); (j) magnified area of the white inset of (f); the white arrows show examples of different $Na_V1.8$+ nerve structures inside the bone marrow. Scale bar=100μm for (a,e,f). scale bar =20μm for (b-d) and (g-j).

Suppl. Fig. 6



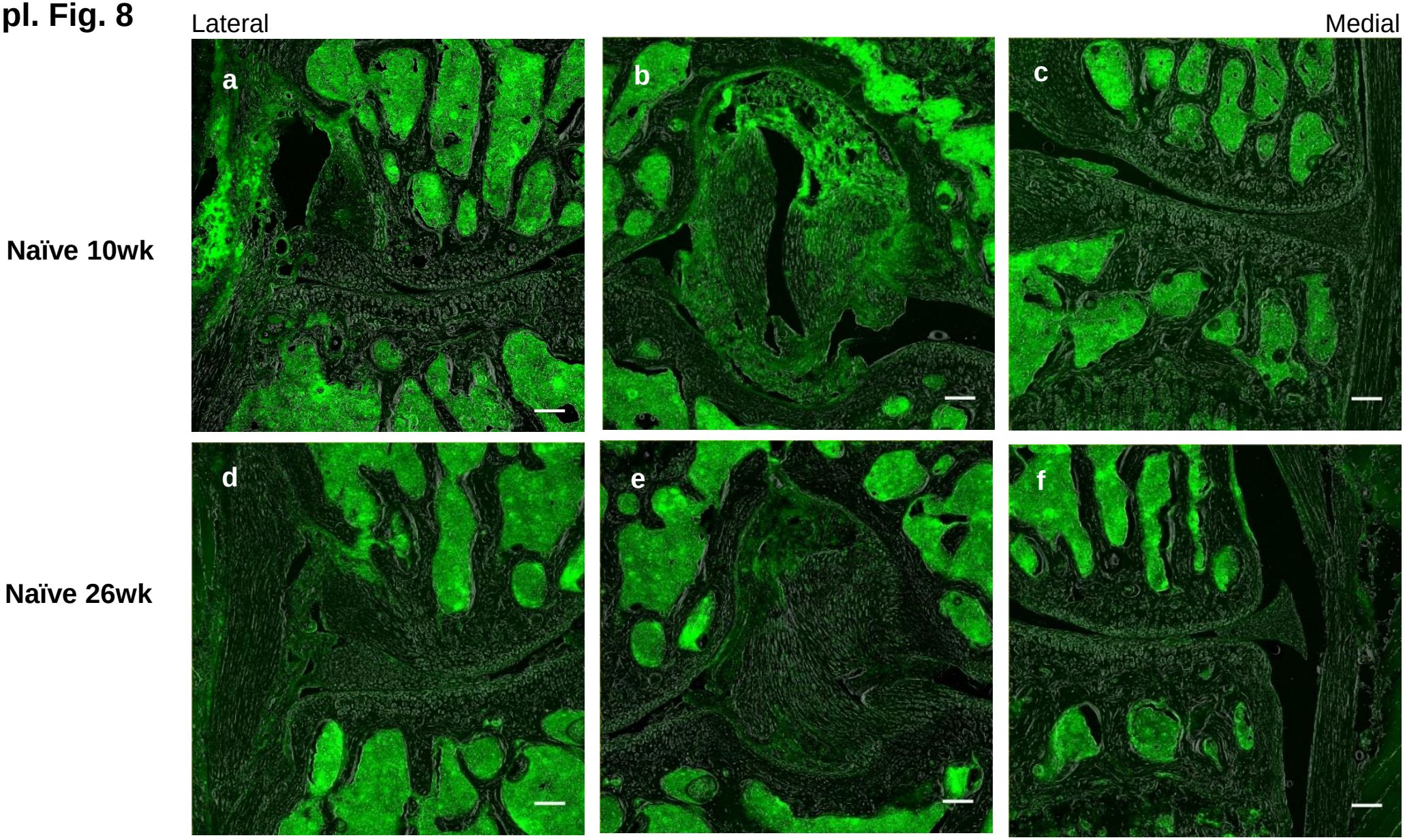
Supplemental Fig. 6 Quantification of $Na_V1.8$-tdTomato signal in knees from n=5 mice for each different group, 26-week old naïve mice and 16-week after sham or DMM surgery; in (a) the lateral synovium and meniscus; (b) the cruciate ligaments. No statistical significance. mean ± 95% CI.

Suppl. Fig. 7



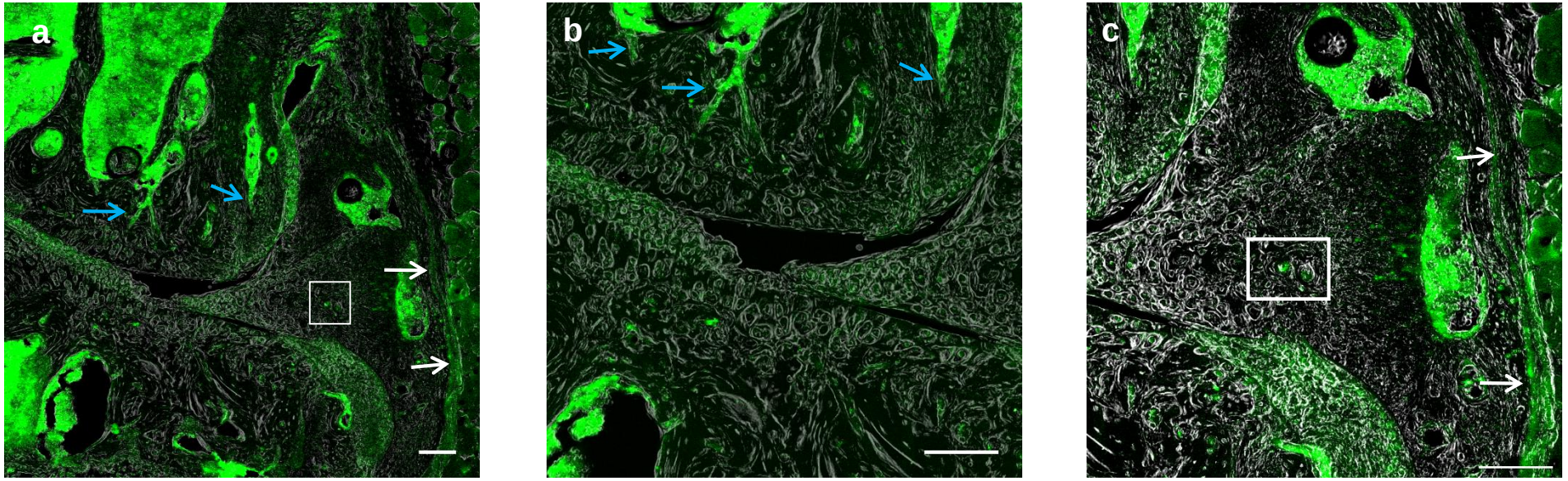
Supplemental Fig. 7 Confocal images showing osteophytes in the medial compartment, 16 weeks after DMM; (a, dashed box) shows an example of less mature and (b, dashed box) an example of more mature osteophytes. Yellow arrows indicate the $Na_V1.8$ fluorescent signal within the osteophytes. Scale bar = 100 μm.

Suppl. Fig. 8



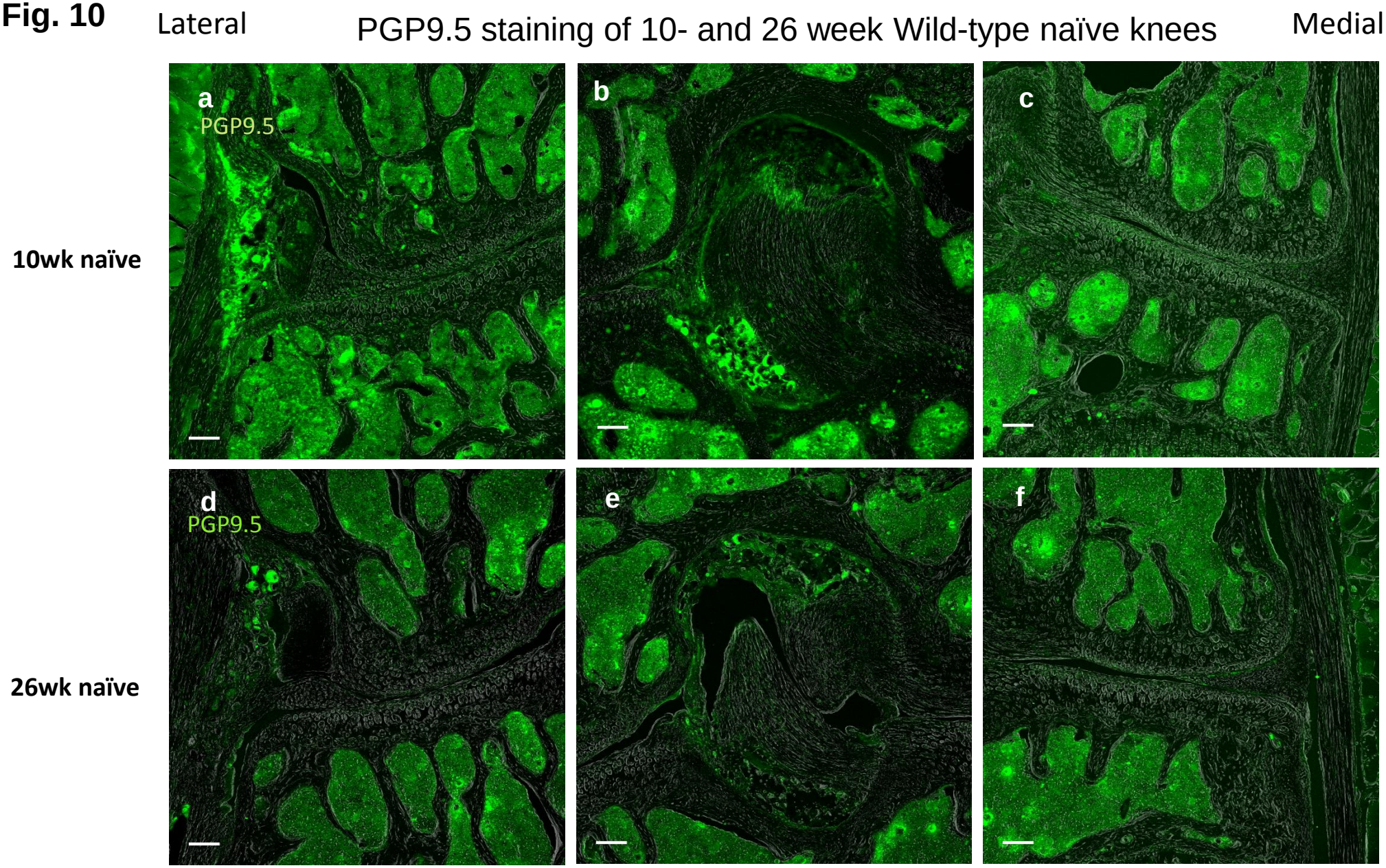
Supplemental Fig. 8 Representative confocal images with phase contrast overlays of the right knee joint of Pirt-GCaMP3 mice. (a-c) 10-week old naïve knee; (d-f) 26-week old naïve knee. Scale bar = 100 μm.

Suppl. Fig. 9



Supplemental Fig. 9 Representative confocal images with phase contrast overlays of the right knee joint of Pirt-GCaMP3 mice 16wks after DMM surgery. (a) the medial side showing Pirt-GCaMP3 positive nerve fibers in the medial synovium (white arrows) , medial meniscus (white box), and within femoral subchondral bone channels (blue arrows). (b) magnified image of some Pirt-GCaMP3 positive nerve fibers within femoral channels (shown in blue arrows). (c) magnified image of the medial meniscus showing the Pirt-GCaMP3 positive signal within the medial synovium (white arrows), and in the medial meniscus (inside the white box). Scale bar = 100 μm.

Suppl. Fig. 10

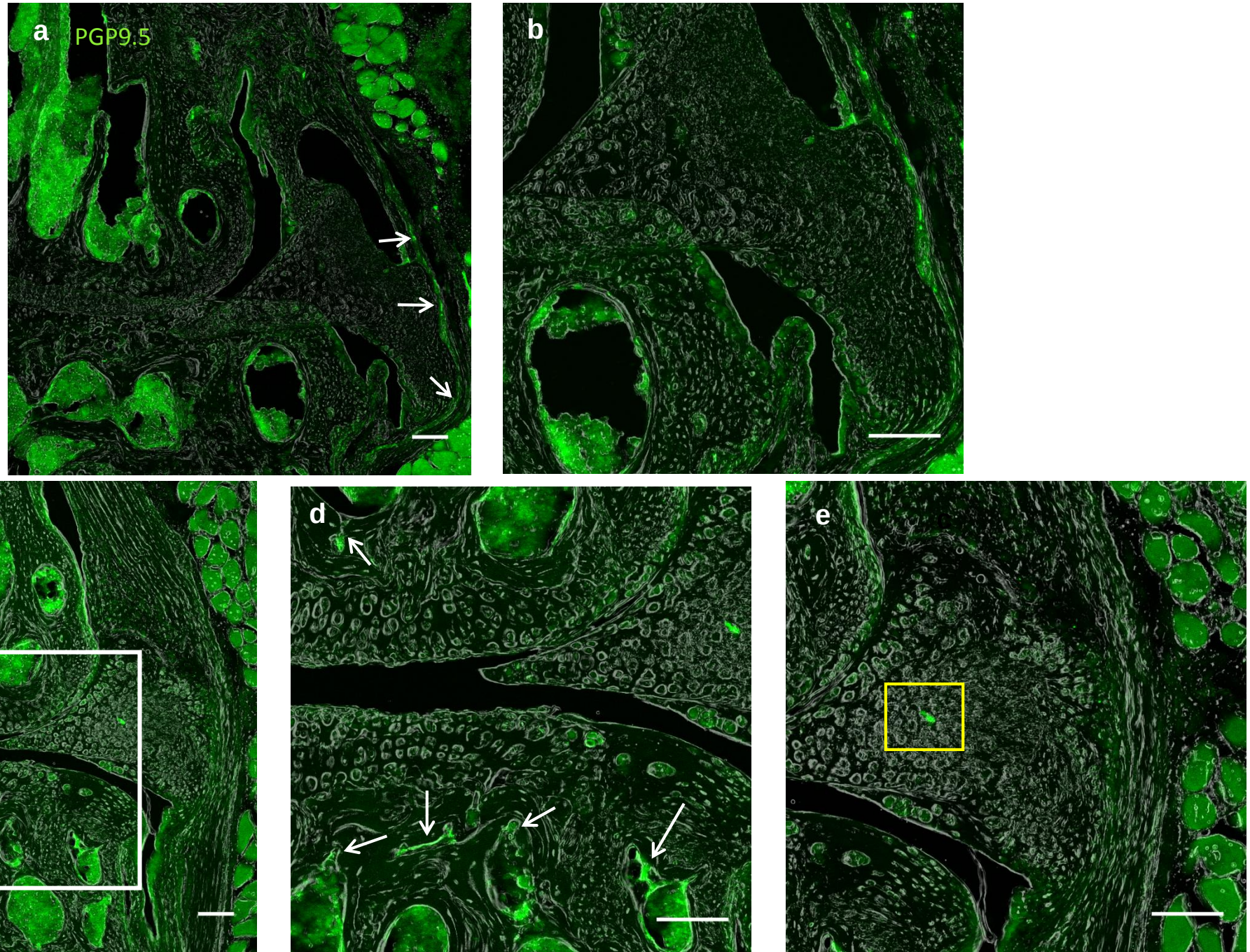


Supplemental Fig. 10 Representative confocal images with phase contrast overlays of PGP9.5 stained wild-type knees. (a-c) 10-week old naïve knee; (d-f) 26-week old naïve knee. Scale bar = 100 μm.

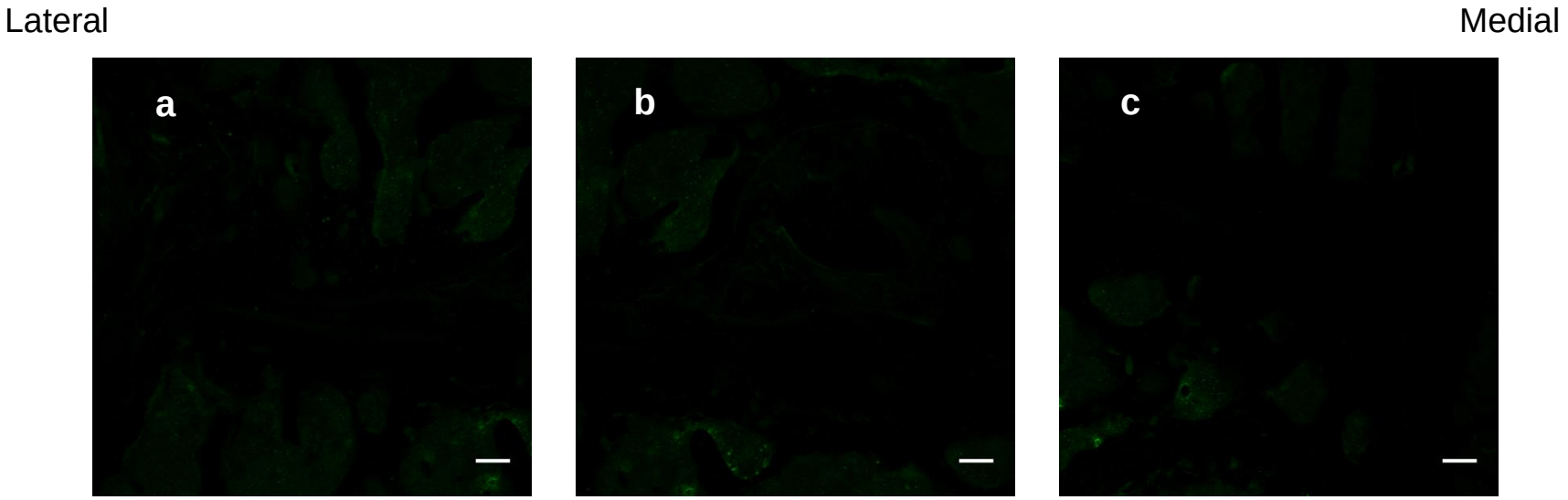
Suppl. Fig. 11

PGP9.5 staining of Wild-type knees16 weeks after DMM surgery

Supplemental Fig. 11 Representative confocal images with phase contrast overlays of PGP9.5 stained wild-type knees 16 weeks after DMM surgery. (a) Shows the nerve fibers in the medial synovium (shown in white arrows). (b) Magnified image of these fibers. (c) An overview of the medial side. (d) Magnified image of subchondral channels (shown in white arrows). (e) Shows the signal within the medial meniscus. Scale bar = 100 µm.



Suppl. Fig. 12



Supplemental Fig 12. Confocal images of the no primary antibody control for PGP9.5 immunostaining of a wild-type knee joint. Scale bar = 100 μm.