SUPPLEMENTAL FIGURES

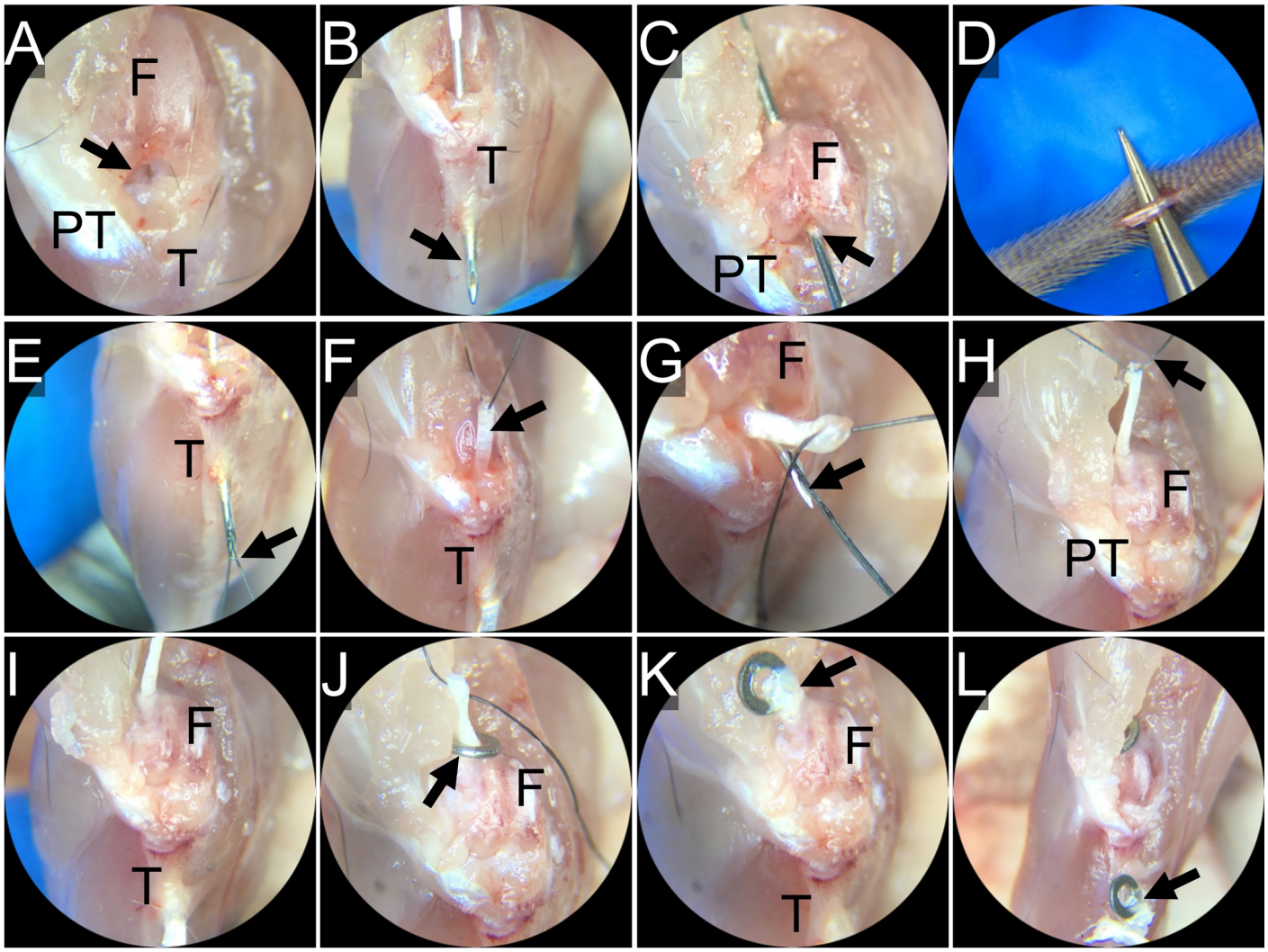

Fig. S1: Detailed surgical procedure.

A. An anteromedial incision was made along the patellar tendon and the patella was subluxed laterally to reveal the joint space and ACL (arrow).

B. A 27G needle (arrow) was used to hand drill the tibial tunnel retrograde originating as close to the native ACL footprint as possible.

C. A new 27G needle was used to hand drill the femoral tunnel originating at the native ACL footprint (arrow).

D. Incisions were made in the tail to acquire the tail tendon graft which consisted of 7-8 tail tendons in a bundle.

E. A suture was passed around the mid-portion of the tendon such that the graft was folded in half. The needle was re-inserted into the tibial tunnel and the suture was passed through the needle (arrow). The needle was then removed.

F. The suture was used to pull the graft (arrow) through the tibial tunnel.

G. The needle was inserted into the femoral tunnel and the suture was passed through the needle (arrow).

H. The suture (arrow) was used to pull the graft through the femoral tunnel.

I. Graft can be seen entering the tibial tunnel and exiting the femoral tunnel.

J. The graft was passed through the center of the stainless steel washer (arrow) at the femoral end.

K. The tendon was wrapped around the outside of the washer, resulting in a cow hitch knot (arrow).

L. The free ends of the tail tendon graft were then tied to another washer at the exit of the tibial tunnel on the outer cortex of the tibia (arrow).

Abbreviations: F – femur, T – tibia, and PT – patellar tendon.

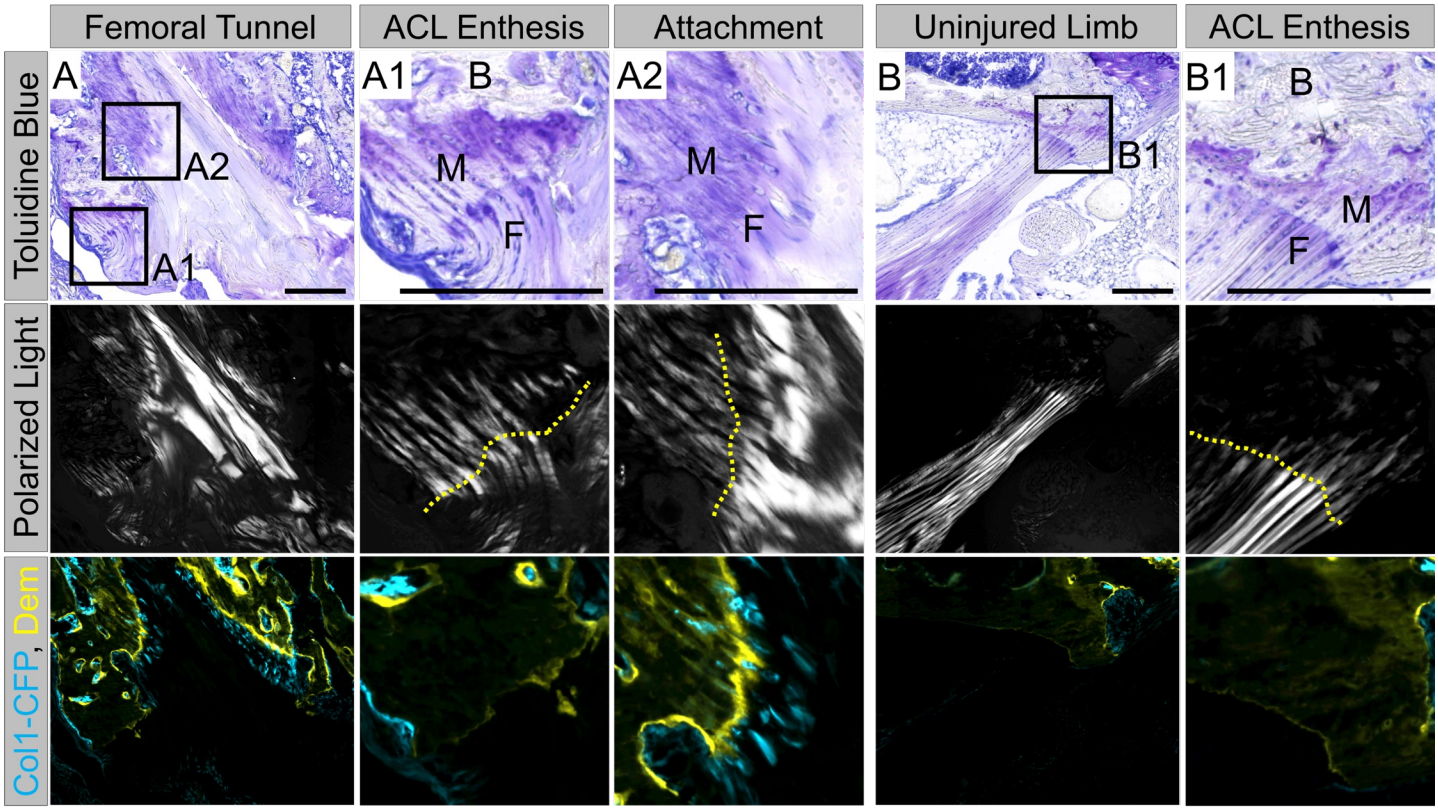

Fig. S2: Comparison of zonal tendon-to-bone attachments to uninjured ACL enthesis. The zonal attachment in figure 5 (panel A2 in this figure) was directly compared to the adjacent enthesis of the injured ACL (A1) because these structures were within the same section (A). However, the ACL was injured and therefore changes to the enthesis could occur. Therefore, panel B in the current figure includes the ACL enthesis from the uninjured limb. Similar to the enthesis from the injured ACL (A1), the uninjured ACL (B) includes proteoglycan staining within unmineralized and mineralized zones of fibrocartilage (first row), collagen fibers (second row) spanning across an inactive tidemark with minimal Col1-CFP expression (third row). As stated in figure 5, the zonal attachments share these features. Scale bars = 200μm.

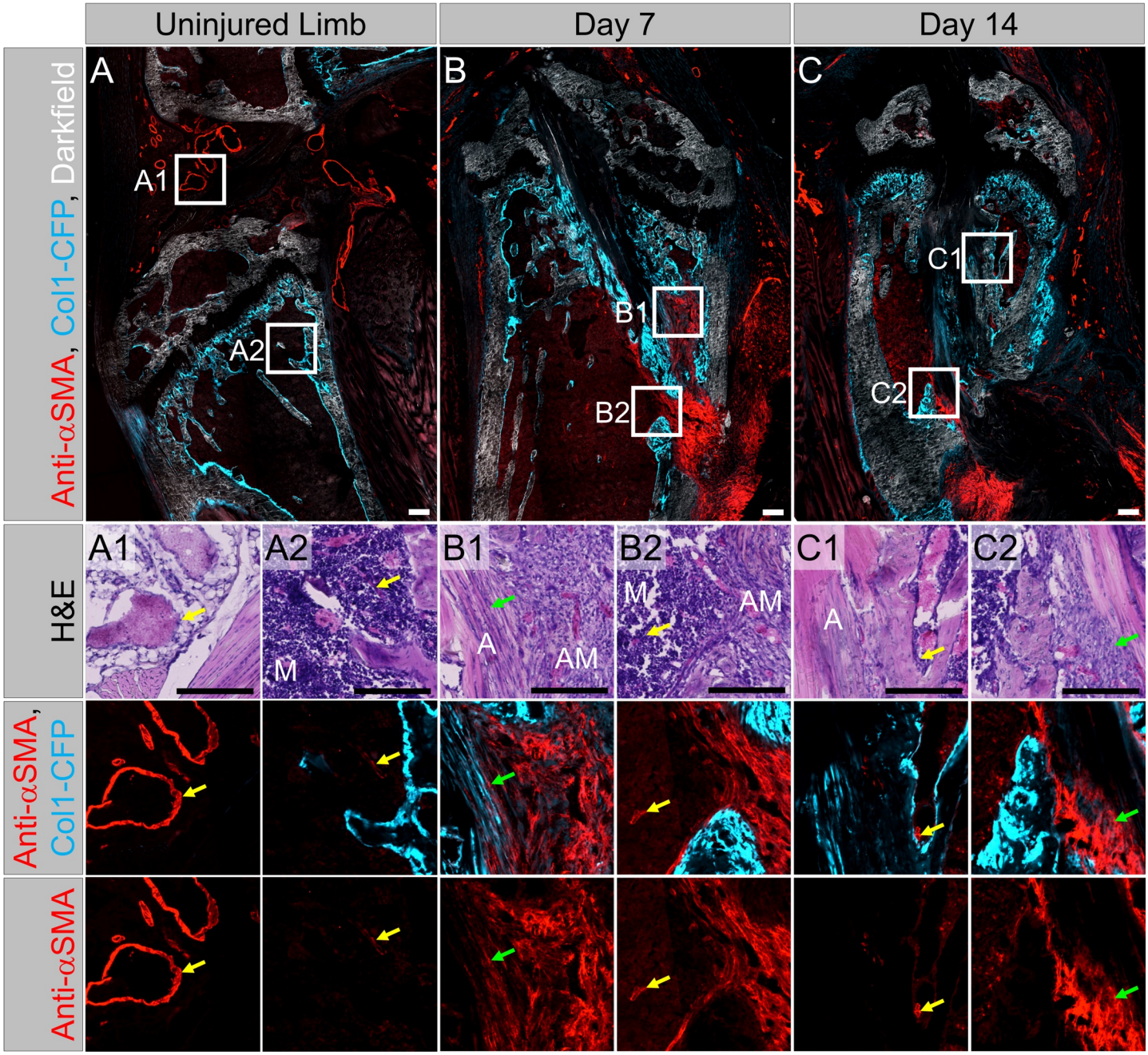

Fig. S3: αSMA is transiently expressed in the activated bone marrow and early tendon-to-bone attachments. Cre-negative double transgenic mice were stained with an antibody against αSMA as well as hematoxylin and eosin and imaged alongside the Col1-CFP reporter in uninjured left limbs and reconstructed right limbs at 7 and 14 days post-surgery. αSMA staining was concentrated within vascular and perivascular regions of the uninjured limbs (A1-A2), as well as in the bone marrow adjacent to the tunnels in the repaired tissue (AM in B1-B2). Cells infiltrating the graft expressed αSMA at day 7 but staining was reduced in mineralized attachments at day 14 (B1 vs. C1). M represents uninjured marrow, green arrows are αSMA-Col1-CFP co-expressing cells, and yellow arrows are vascular and perivascular cells. Scale bars = 200μm.

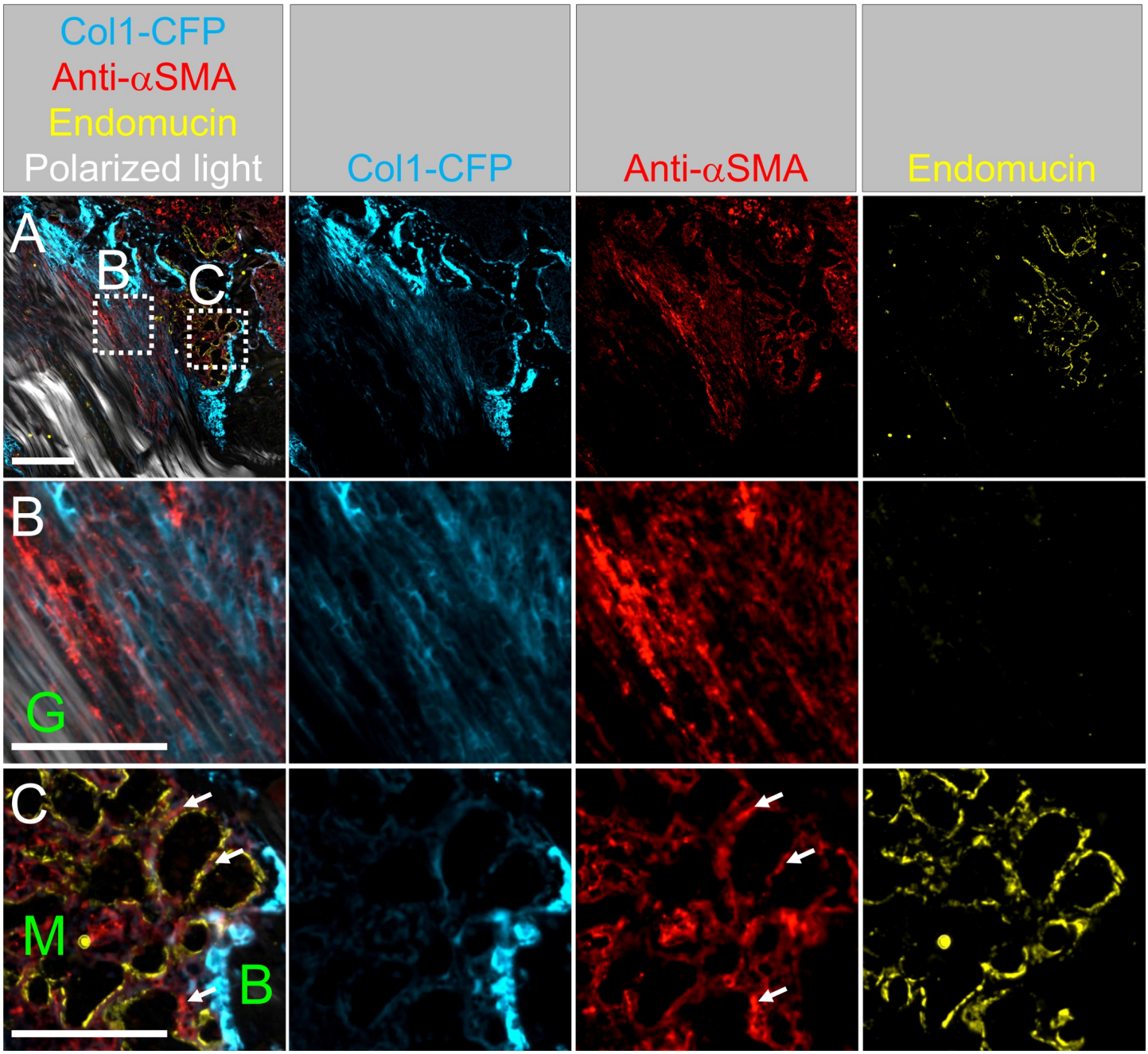

Fig. S4: A subset of αSMA-expressing cells are in perivascular regions. A subset of sections were stained for antibodies against αSMA and endomucin and imaged alongside the Col1-CFP reporter (A). Cells infiltrating the graft (G) expressed αSMA but did not express Endomucin (B). However, some cells (arrows in C) in the perivascular regions of the adjacent marrow (M) expressed αSMA. B denotes bone. Scale bars = 200μm (A) and 100μm (B and C).

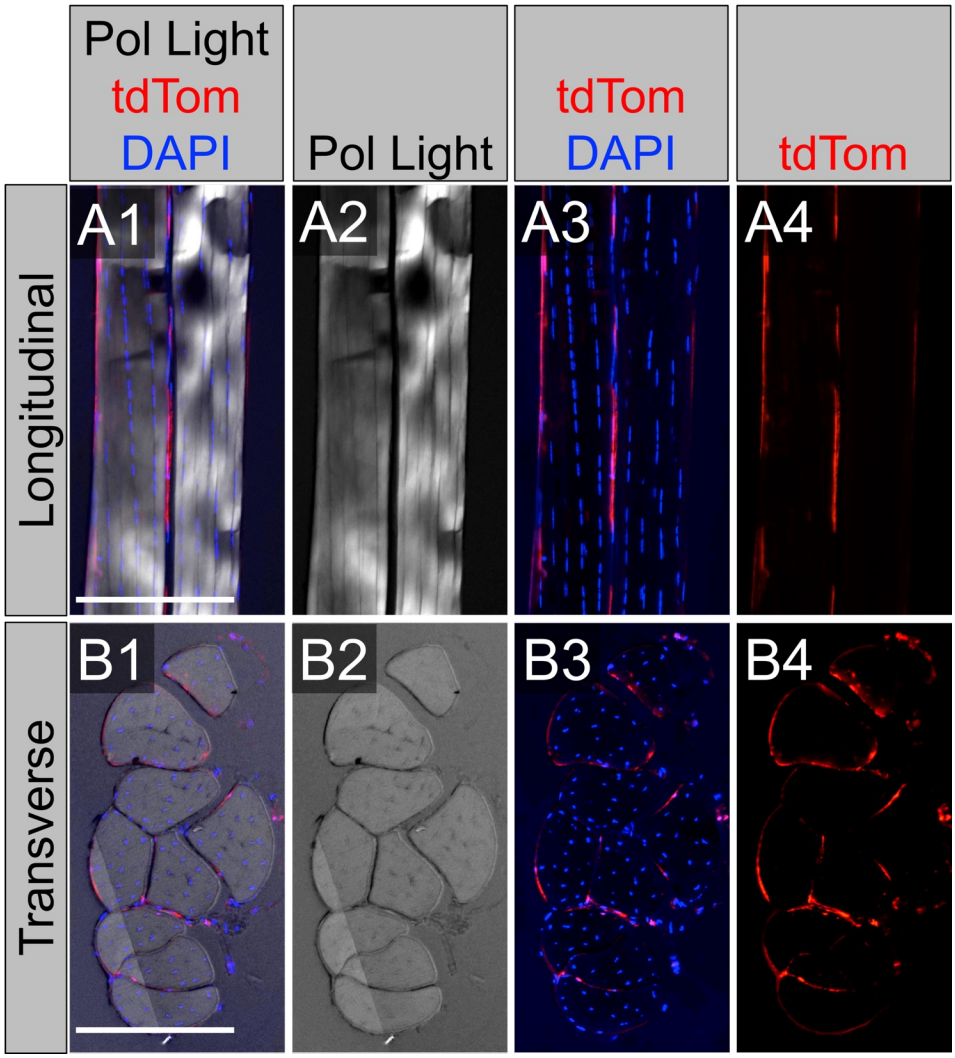

Fig. S5: SMA-labeling in tail tendons prior to reconstruction. Tail tendons were removed from the tail from the T_{-14} group on the day after the last injection. The tendons were fixed and then sectioned in longitudinal (A) and transverse (B) planes. While there was intense labeling on the epitenon, only 0.74±0.68% of cells within the tail tendon midsubstance were tdTomato+ (n = 4). Scale bars = 200μm.

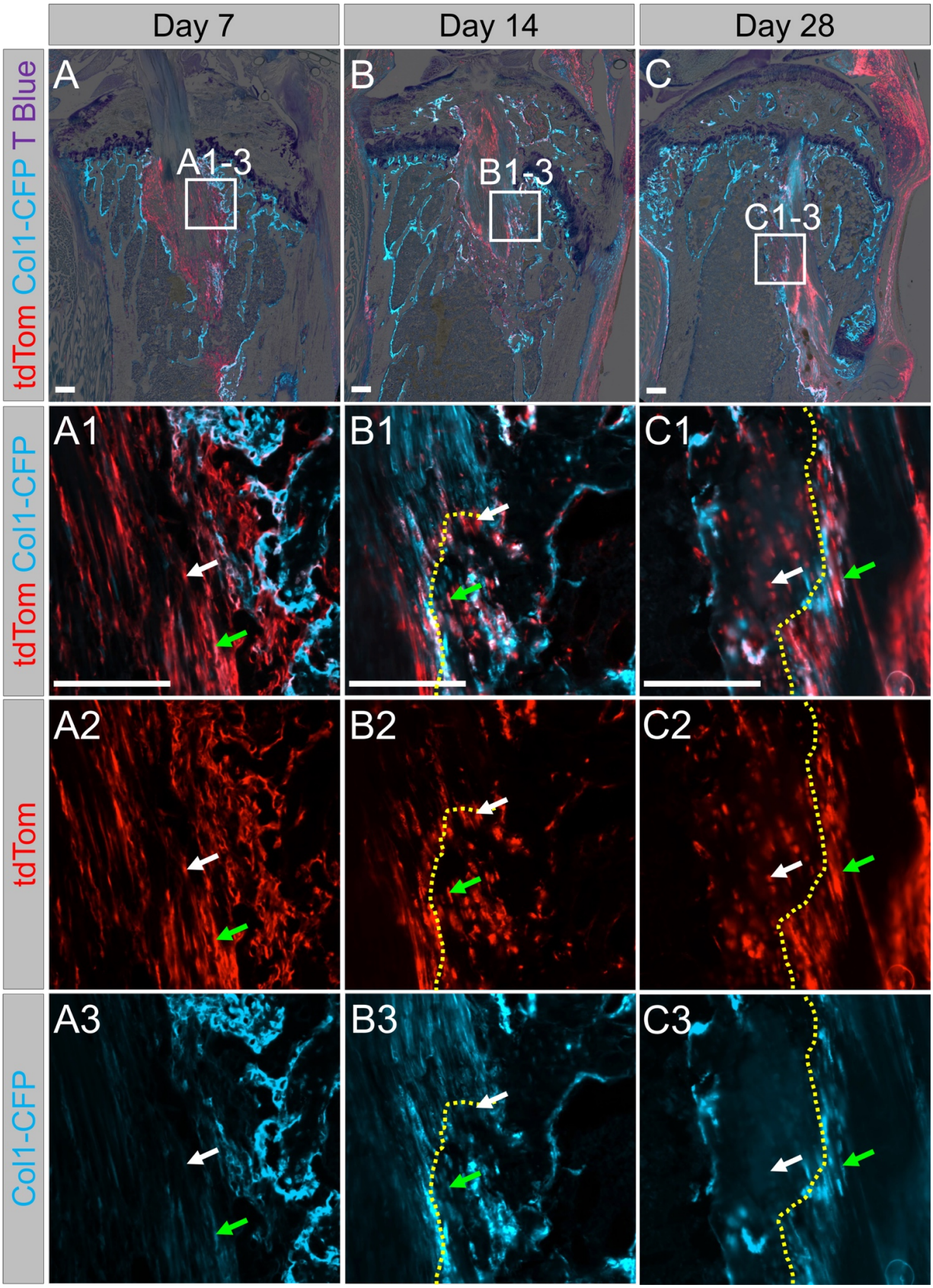

Fig. S6: A subset of SMA-labeled cells co-express Col1-CFP in the zonal attachments. Samples that were independently presented for Col1-CFP (Fig. 4A-C) or tdTomato (Fig. 6B-C and 7B) are presented in this figure as composite images with both Col1-CFP and tdTomato. SMA-labeled cells co-expressed Col1-CFP (green arrows) at day 7 (A), 14 (B), and 28 (C) days during the formation of attachments. The co-expressing cells were predominantly in unmineralized regions of the attachments. White arrows denote tdTomato+ that were negative for Col1-CFP. Dotted yellow lines indicate tidemarks. Scale bars = 200μm.