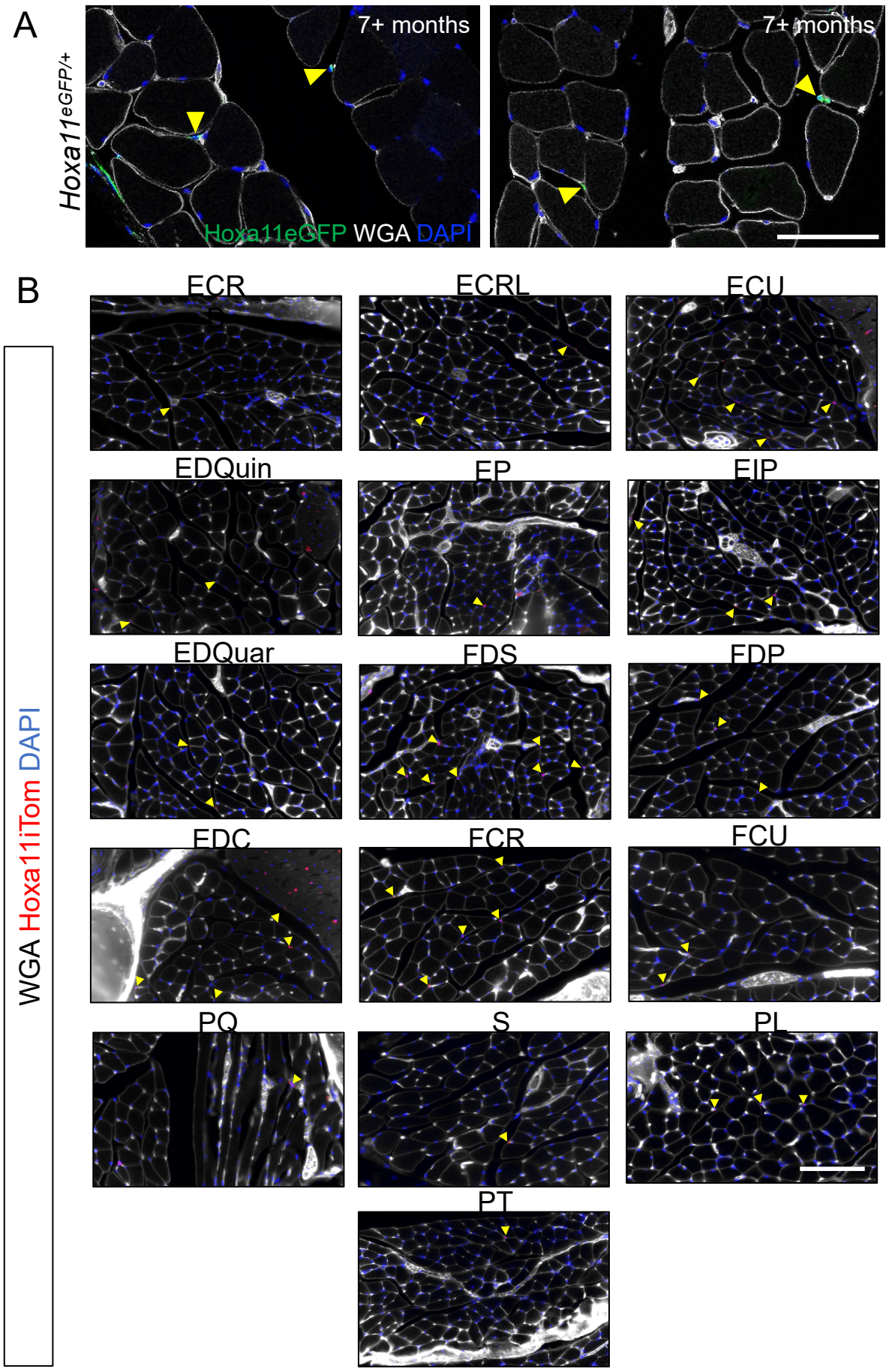

Figure S1. Hoxa11 expression in muscle interstitial cells is observed in aged mice and Hoxa11 lineage labeling of interstitial cells is observed in all forelimb muscles. (**A**) High magnification confocal microscopy shows Hoxa11eGFP-positive cells (green, yellow arrowheads) are observed in the muscle interstitium, defined by WGA, in animals 7 month of age - 1year old. Scale bar = 75 μm; N = 3 animals. (**B**) Expression of Hoxa11 in interstitial cells of individual forelimb muscles collected from *Hoxa11*$^{CreERT2/+}$ *; ROSA*$^{LSL\text{-}tdTomato/+}$ animals 3 days after tamoxifen treatment. High magnification images of Extensor Carpi Radialis Brevis (ECRB), Extensor Carpi Radialis Longus (ECRL), Extensor Carpi Ulnaris (ECU), Extensor Digiti Quinti (EDQuin), Extensor Pollicis (EP), Extensor Indicis Proprius (EIP), Extensor Digiti Quarti (EDQuar), Flexor Digitorum Sublimis (FDS), Flexor Digitorum Profundus (FDP), Extensor Digitorum Communis (EDC), Flexor Carpi Radialis (FCR), Flexor Carpi Ulnaris (FCU), Pronator Quadratus (PQ), Supinator (S), Palmaris Longus (PL), and Pronator Teres (PT). Scale bar = 100 μm; N = 4 animals.

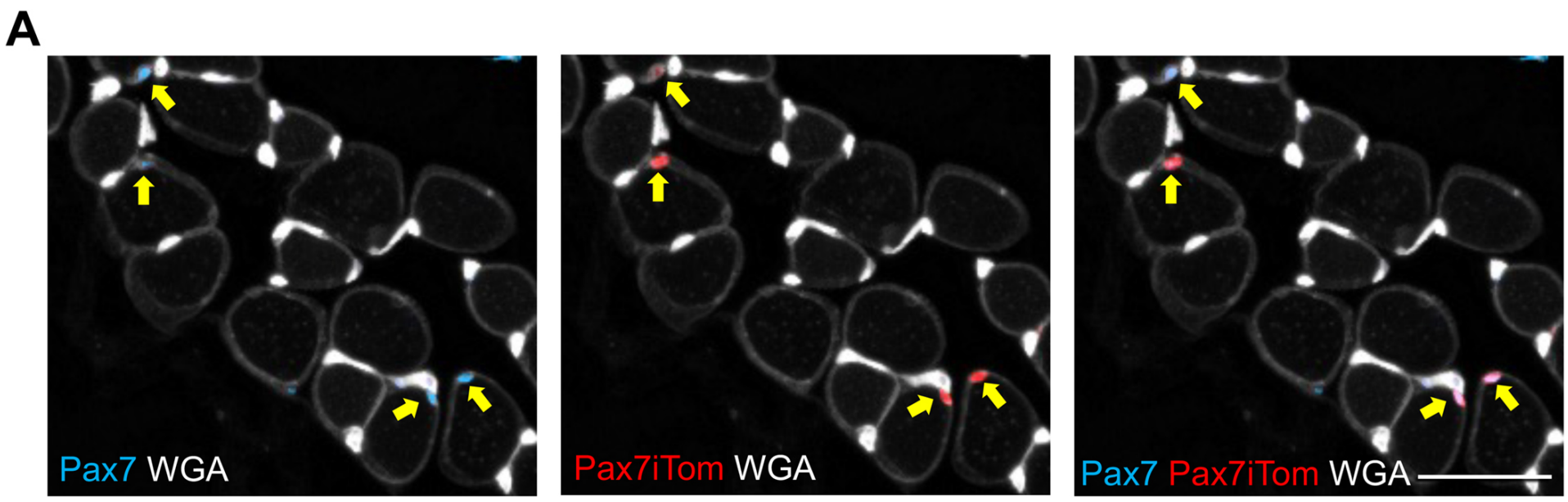

Fig. S2. Both *Hoxa11-CreERT2* and *Pax7-CreERT2* show approximately 90% efficiency with lineage reporter ROSA-LSL-tdTomato. (A) Images show Pax7 IF staining (blue) overlaps with tdTomato (red) in Pax7 lineage reporter animals (Pax7iTom) 4 days after tamoxifen treatment. Scale Bar = 50 μm. **(B)** Quantification of Pax7-CreERT2 efficiency was assessed by counting the number of Pax7 antibody-stained cells and tdTomato labeled cells at 48hrs (n= 2 animals) and 4 days (n= 2 animals) after Tamoxifen treatment. **(C)** Quantification of Hoxa11-CreERT2-induced recombination of ROSA-LSL-tdTomato with a single 5mg bolus of tamoxifen was assessed by counting the number of Hoxa11eGFP and tdTomato labeled cells at 48hrs (n=2 animals) and 7 days (n=2 animals) after tamoxifen administration. Both Cres resulted in approximately 90% efficiency.

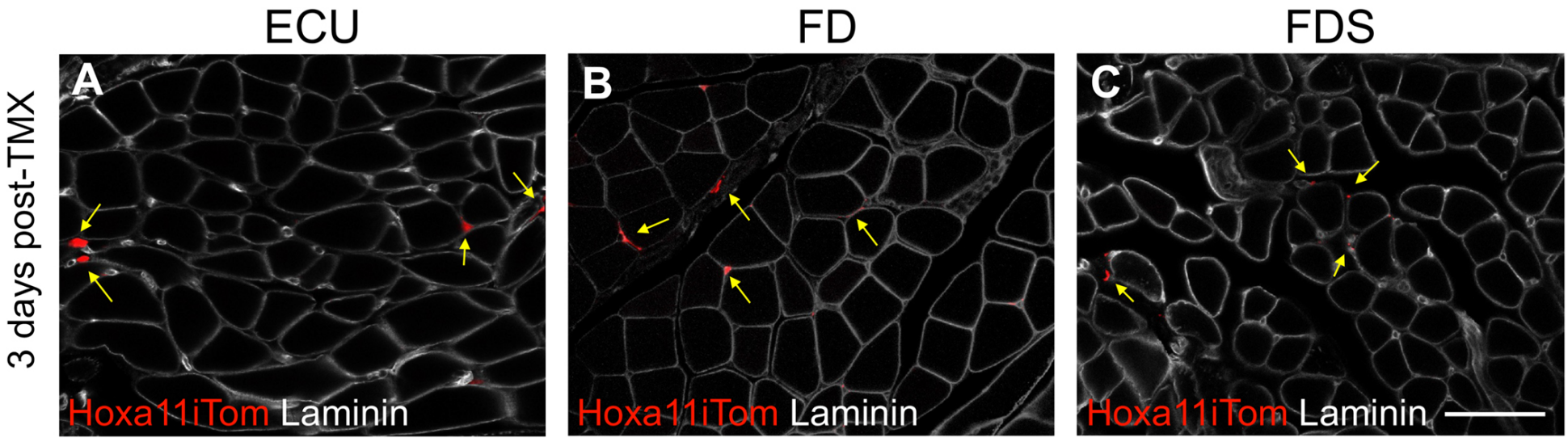

Fig. S3. Hoxa11 expressing cells are only in the interstitium of zeugopod-attached muscles after initial recombination. Hoxa11 lineage labeling (Hoxa11iTom, red) is observed 3 days following tamoxifen administration. (**A-C**) Hoxa11 lineage-labeled cells are only seen in the interstitium (yellow arrows) of the Extensor Carpi Ulnaris (ECU), Flexor Digitorum Profundus (FDP) and Flexor Digitorum Sublimis (FDS) as shown by laminin (white). Scale bar = 100 μm. N = 4 animals.

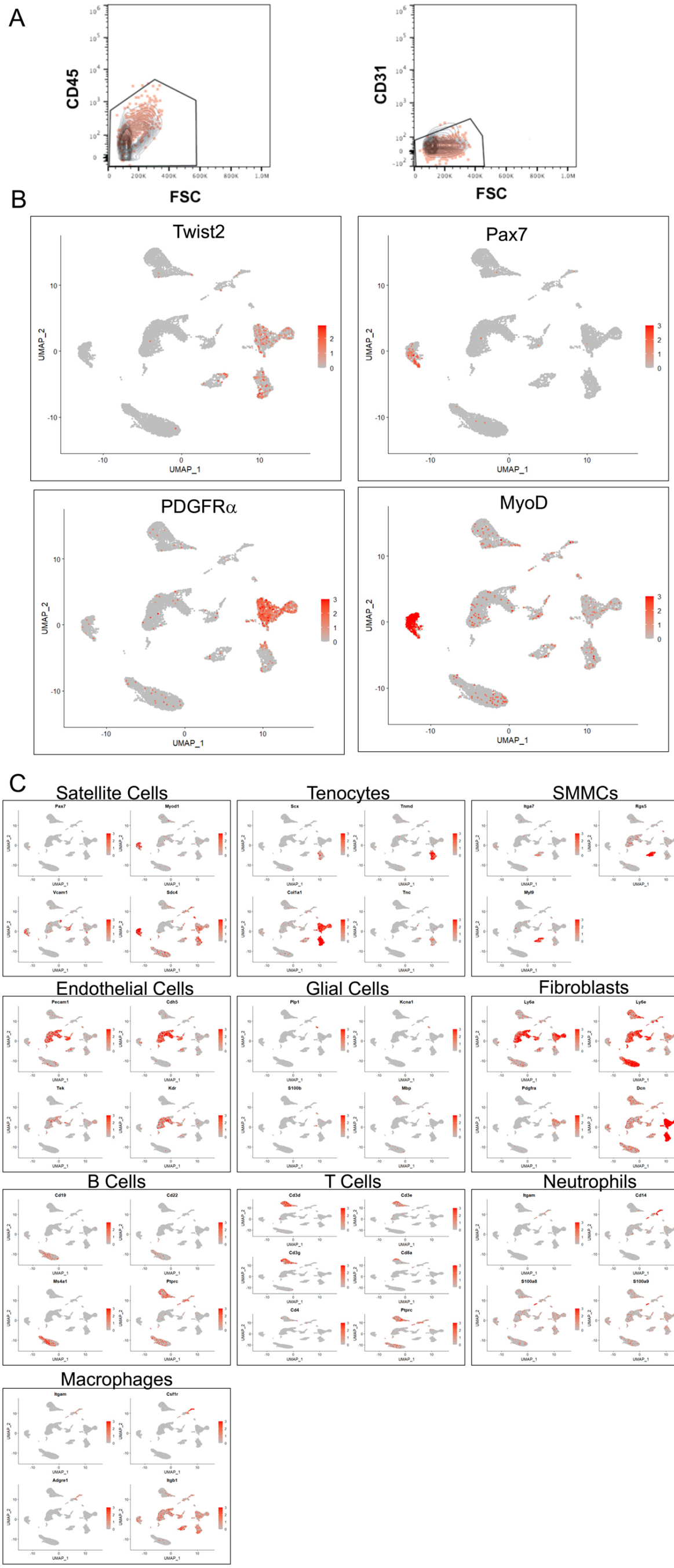

Fig. S4. Further flow cytometry and Sing-cell RNA-sequencing analyses. (A) Hoxa11iTom+ cells dotted (red) over contour plot of non-tdTom cells. The mononuclear subsets analyzed by flow cytometry were first gated to exclude hematopoietic and endothelial cells; Hoxa11iTom+ cells are non-hematopoietic and no-endothelial. **(B)** Plotting single gene expressions onto a UMAP projection show Twist2 and PDGFRα predominantly in fibroblasts, and Pax7 and MyoD predominantly found in satellite cells though low representations of each of these four genes are found in other clusters, highlighting the imprecision of statistical clustering with limited representation of expressed gene sets that is characteristic of single cell sequencing. **(C)** Additional feature plots for additional markers used to define subsets.

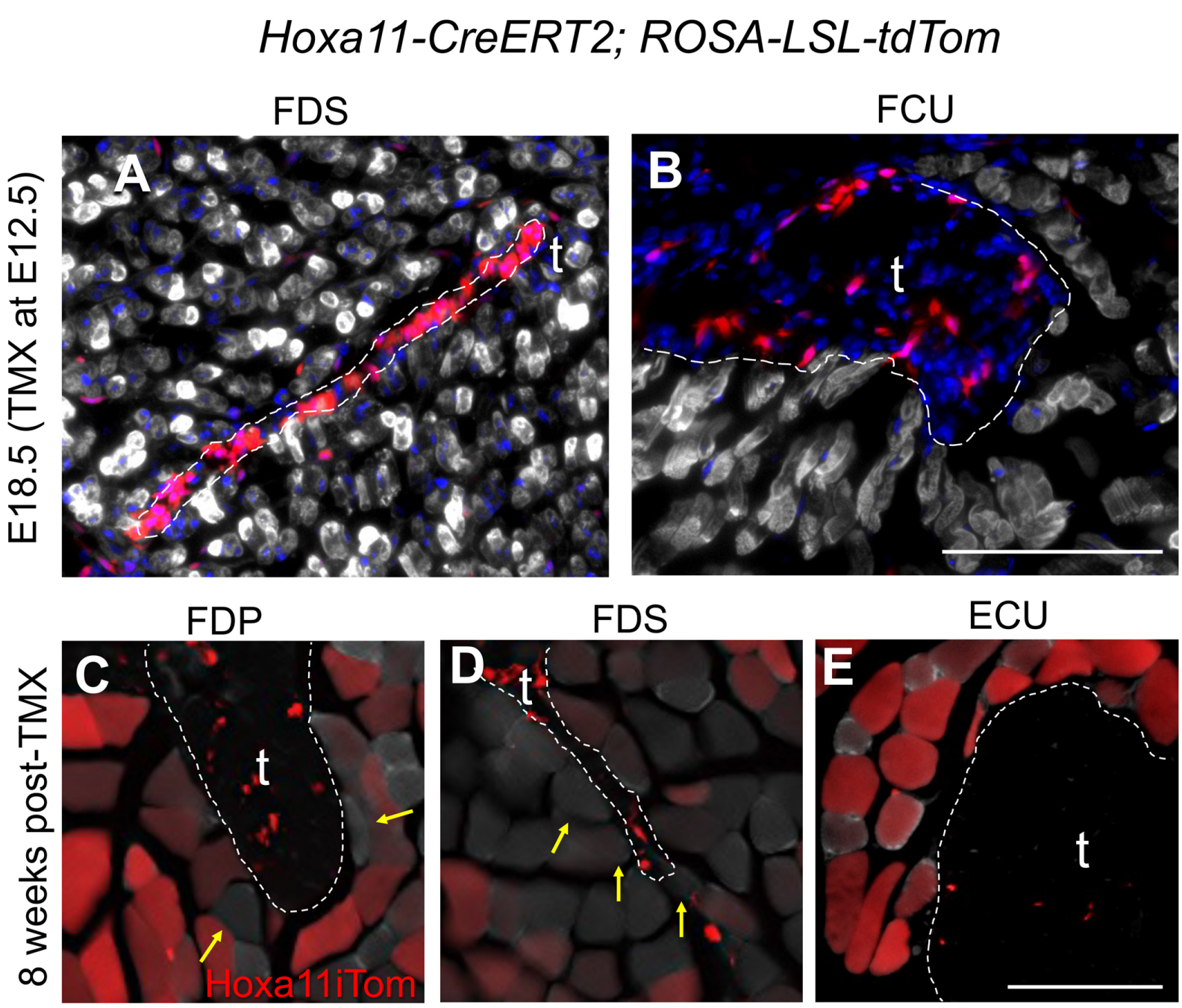

Fig. S5. Hoxa11 lineage shows no preferential contribution to myofibers near the myotendinous junctions during embryogenesis or homeostasis. (A,B) Sections of embryonic forelimb muscles were analyzed for myofiber lineage contribution at E18.5. N = 3 animals. **(C-E)** Images of the Flexor Digitorum Sublimis (FDS) and Flexor Carpi Ulnaris (FCU) muscles at the myotendinous junction show no Hoxa11iTom (red) overlap with My32 (white), consistent with lineage labeling throughout the rest of the muscle body. Sections from adult Hoxa11iTom muscle of the Flexor Digitorum Profundus (FDP), Flexor Digitorum Sublimis (FDS), and Extensor Carpi Ulnaris were analyzed for Hox11 lineage contribution at the myotendinous junction 8 weeks after the induction of lineage reporting. High magnification images show similar lineage labeling near the myotendinous junction of the FDP **(C)**, FDS **(D)**, or ECU **(E)** as in the mid-body of the muscle (Figure 4). N = 5 animals. Scale bars = 100 μm. Tendons are outlined with dashed line and marked by t.

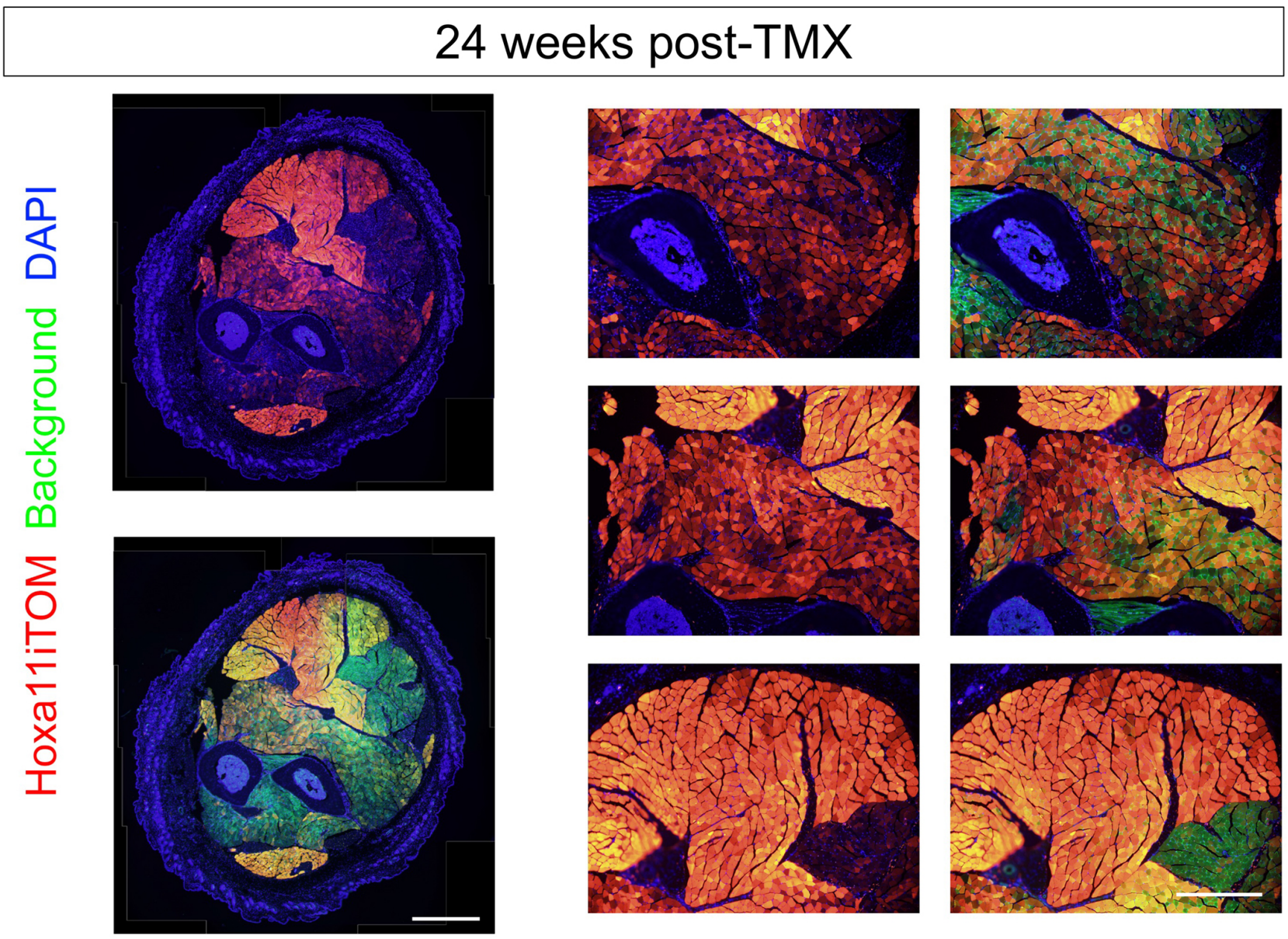

Fig. S6. Hoxa11 lineage 24 weeks after reporter induction. Hoxa11$^{CreERT2/+}$; ROSA$^{LSL\text{-}tdTomato/+}$ mice were given 5mg tamoxifen at 8 weeks of age and collected 24 weeks (6 months) later. A full cross-section with and without background (green) show many Hoxa11iTom^{+} (red) muscle fibers. Close-ups with and without background depict high levels of tdTomato within muscle fibers. Scale bars = 1000 μm and 500 μm, respectively. N = 2 animals.

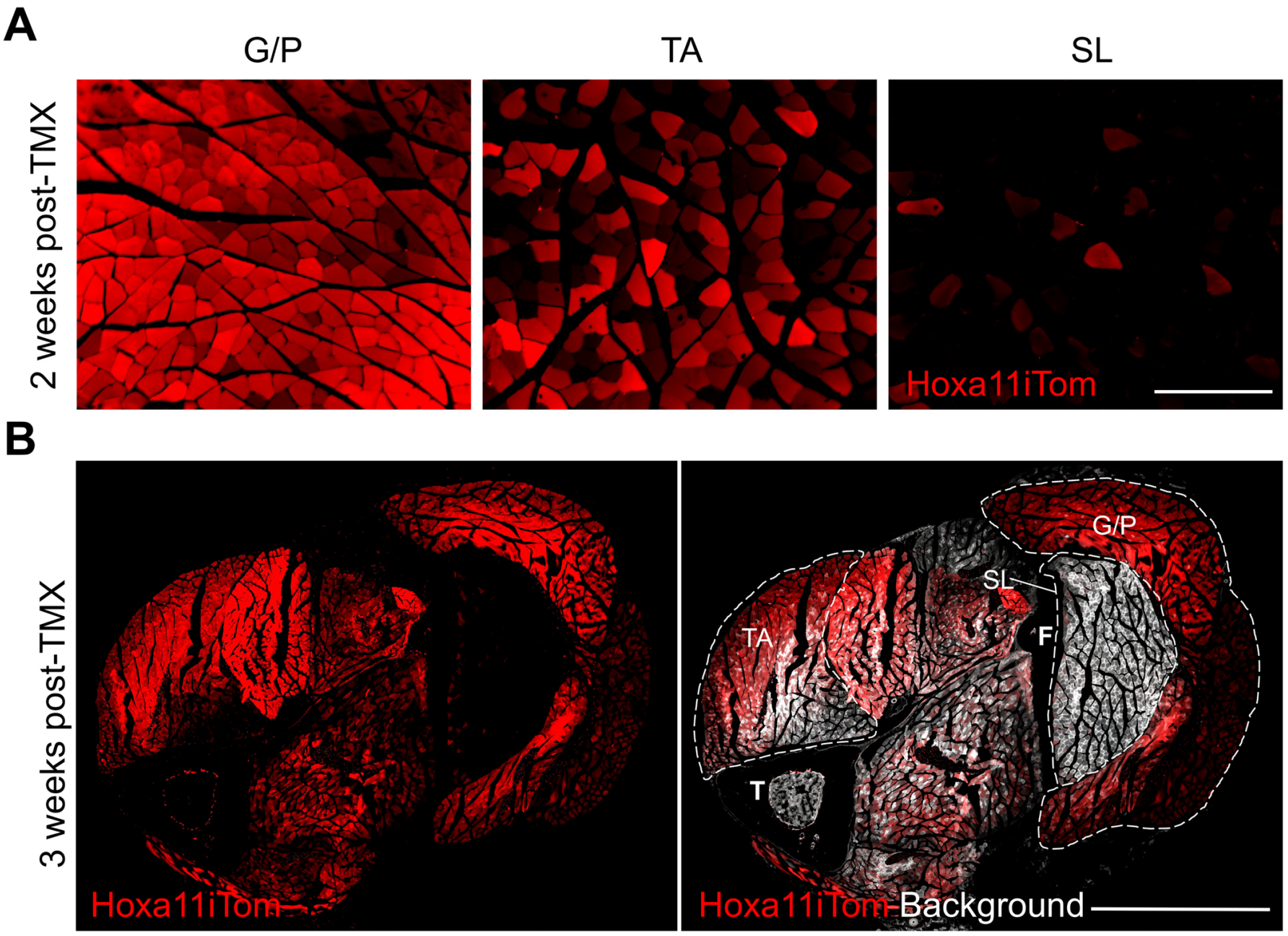

Fig. S7. Hoxa11 lineage differentially contributes to hindlimb muscles. (A) Hindlimb muscles taken from Hoxa11iTom animals 2 weeks after tamoxifen treatment show different levels of lineage contribution in the Gastrocnemius/Plantaris (G/P), Tibialis Anterior (TA), and Soleus (SL). Scale bar = 200 μm. N = 4 animals. **(B)** A whole hindlimb cross-section from an animal 3 weeks after the start of lineage labeling shows variable tdTom expression in muscles of the hindlimb. T and F mark the tibia and fibula, respectively. Scale bar = 1000 μm. N = 1 animal. Muscles shown in **A** are outlined and labeled in **B**.

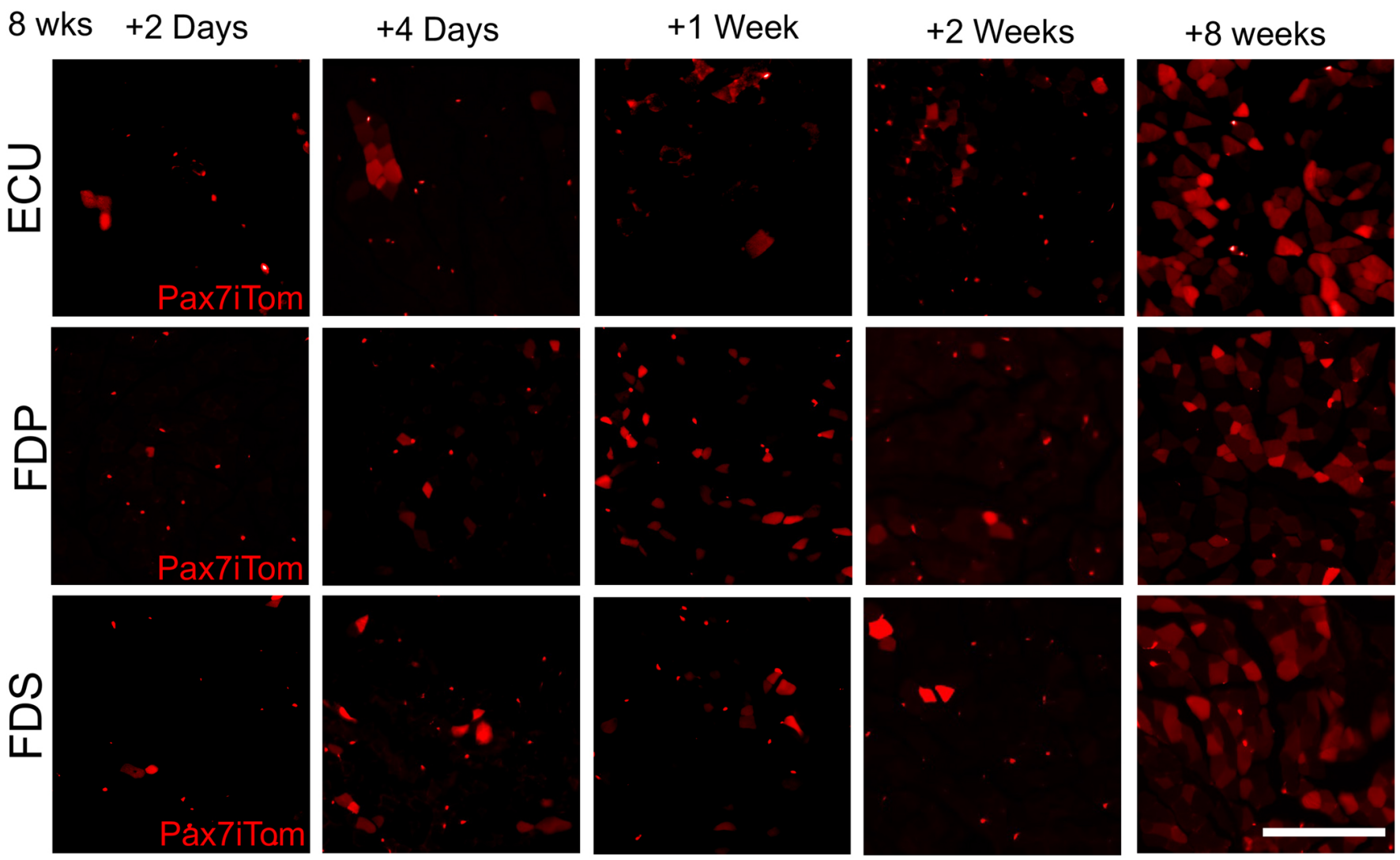

Fig. S8. Pax7iTom lineage in the forelimb muscles. *Pax7CreERT2; ROSALSL-TdTomato* (Pax7iTom) mice were given a single intraperitoneal injection of 5 mg tamoxifen at 8 weeks of age and collected at 2 days, 4 days, 1 week, 2 weeks, and 8 weeks after tamoxifen induction. Images of the Extensor Carpi Ulnaris (ECU), Flexor Digitorum Profundus (FDP) and Flexor Digitorum Sublimis (FDS) muscles show tdtom+ myofibers as well as tdTom+ satellite cells. N = 3 (2d, 4d, 7d, 2wk) and 4 (8wk) animals per timepoint. Scale bar = 200 μm.

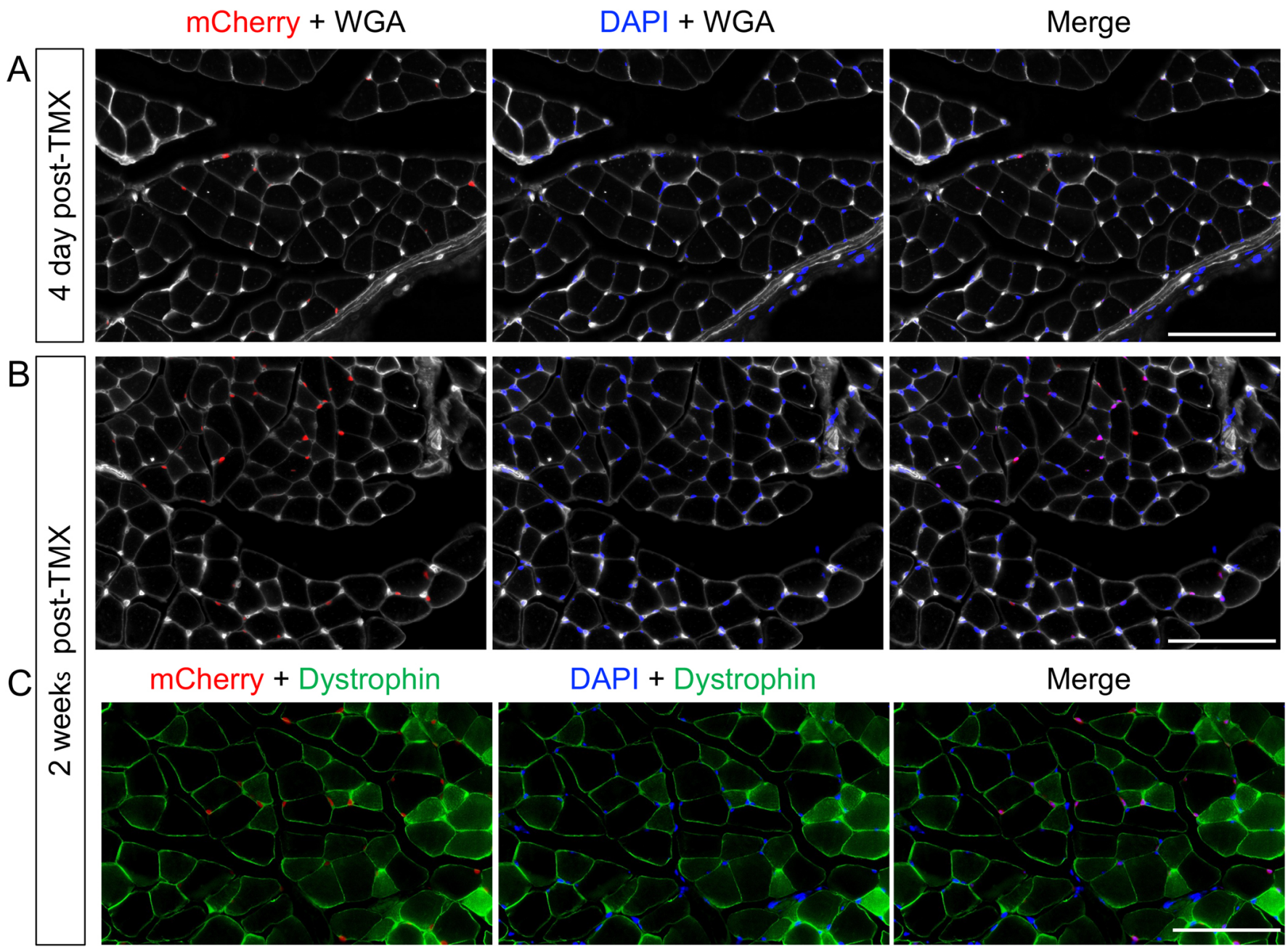

Fig. S9. Hoxa11iH2BmCherry-labeled nuclei overlap with nuclear stain. H2BmCherry expression in nuclei shown in Fig. 5 was confirmed with DAPI nuclear stain in images 4 days (**A**), 2 weeks (**B**) post tamoxifen, and dystrophin stain (**C**). Muscle fiber outer membranes were stained with WGA (**A,B**). Scale bars = 100 μm. N = 3 animals (4 day) and 2 animals (2 weeks).

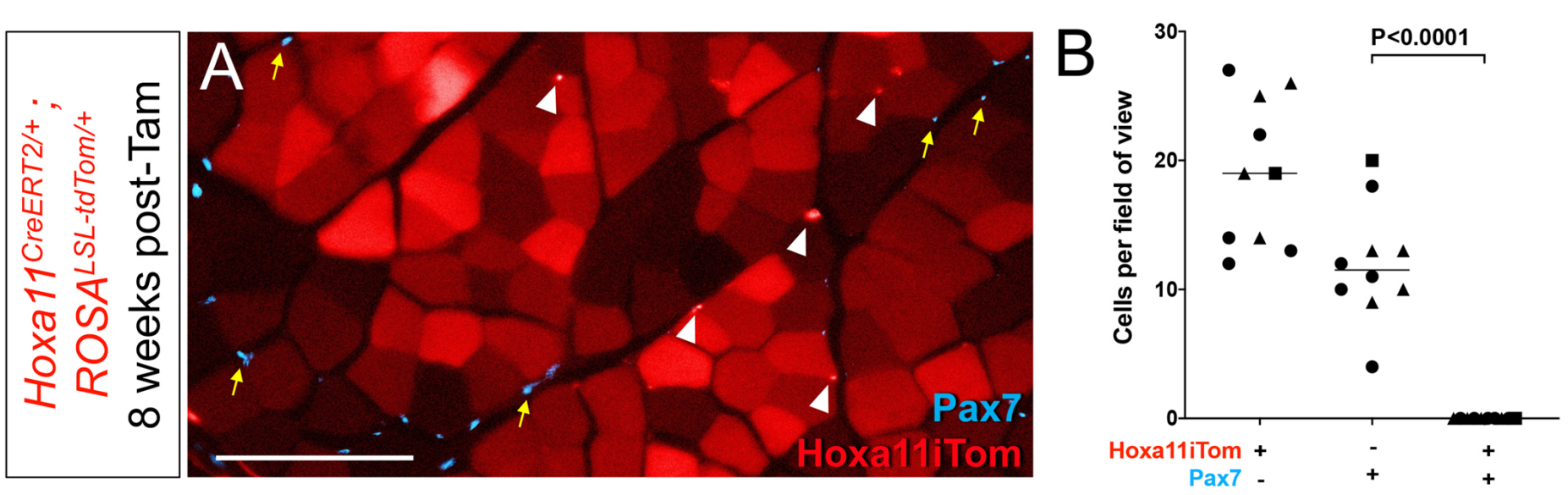

Fig. S10. Hoxa11 lineage does not label satellite cells 8 weeks post-induction of lineage reporter. (**A**) Forelimb muscle sections from animals 8 weeks (dosing at 8 weeks of age, collection at 16 weeks of age) after the start of lineage labeling were stained for Pax7 (cyan, yellow arrows) and analyzed for overlap of Hoxa11itom (red, arrowheads) and Pax7. (**B**) Quantification shows no Hoxa11iTom+/ Pax7+ cells were observed through forelimb muscles. Scale bar = 100 μm. N = 3 animals.

Table S1. Antibodies

Name	Source	Catalog Number	Dilution	Antigen retrival
anti-GFP	Aves Labs	GFP-1010	1:200	No
anti-Twist2	Abcam	ab66031	1:200	No
anti-PDGFRa	Abcam	ab61219	1:50	No
anti-Tcf4	Cell Signaling	2569S	1:100	No
WGA-647	Invitrogen	W32466	1:250	No
WGA-488	Invitrogen	W11261	1:250	No
anti-Pax7	DSHB	Pax7-S	1:10	Yes
anti-Laminin	Sigma	L9393	1:500	No
anti-My32	Sigma	M4276	1:400	Yes
anti-PECAM1	DSHB	2H8-S	1:10	No
anti-BF-F3	DSHB	BF-F3	1:10	Yes
anti-SC-71	DSHB	SC-71	1:10	Yes
anti-Myosin (Skeletal, Slow)	Sigma	M8421	1:250	Yes
anti-ScaI(Ly6A/E)-BV711	Biolegend	108131	1:100	No
anti-CD34-PE-Cy7	Biolegend	119325	1:100	No
anti-CD31-PE-CF594	BD-Horizon	563616	1:100	No
anti-ITGA7-AF647	AbLab	67-0010-05	1:100	No
anti-CD45-FITC	Biolegend	304005	1:100	No
anti-CD106(VCAM1)-PE/Cy7	Biolegend	105719	1:40	No
Alexa-488 Donkey anti-Chicken	Jackson ImmunoResearch Labs	016-580-084	1:500	No
Alexa-555 Donkey anti-Rabbit	Invitrogen	A31572	1:500	No
Streptavidin-AF647	Invitrogen	S21374	1:500	No
Anti-Dystrophin	Thermofisher	PA1-21011	1:100	Yes