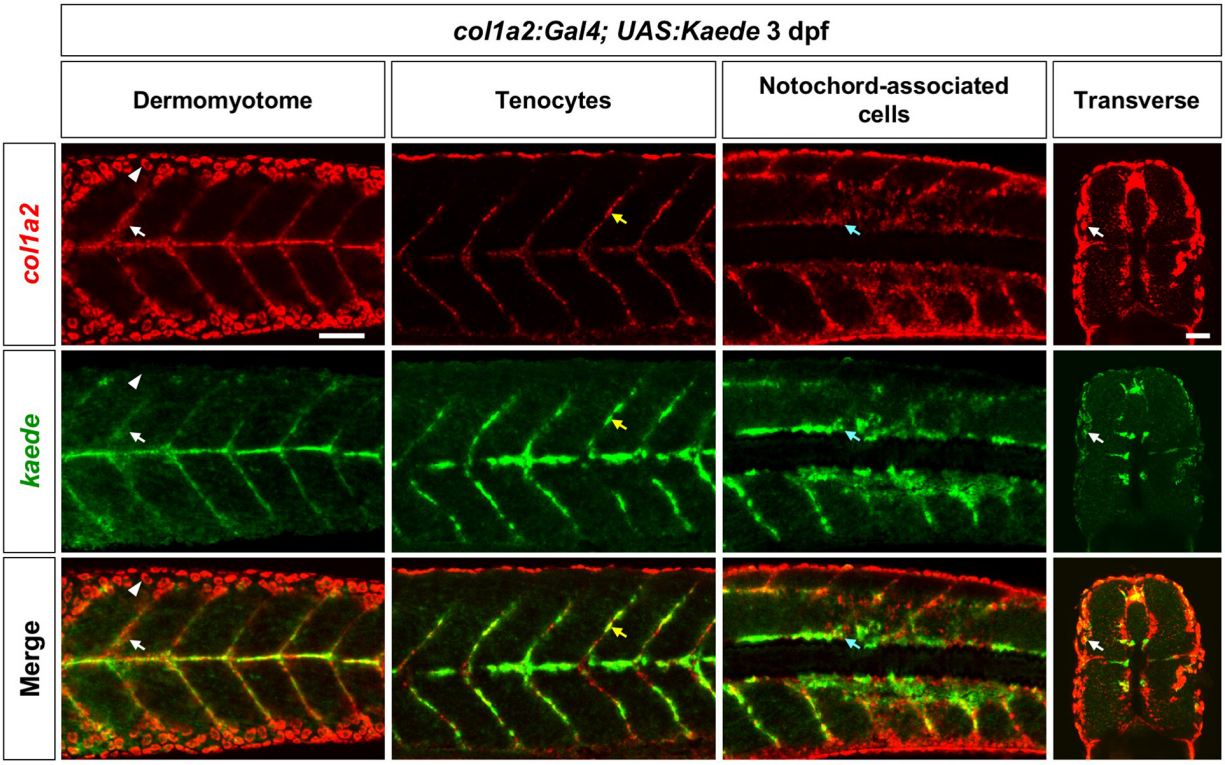


Figure S1. Validation of the *col1a2*^{Kaede} line. Double fluorescent in situ hybridization using *kaede* and *col1a2* probes were performed in *col1a2*^{Kaede} embryos at 3 dpf. Co-expression of *kaede* (green) and the endogenous *col1a2* (red) can be observed in dermomyotome cells (white arrows), tenocytes along the vertical myoseptum (yellow arrows), and deep interstitial cells around the notochord (cyan arrows). Note that *col1a2*^{Kaede} was not expressed in the epidermis (arrowheads) as *col1a2*. *n* = 19 embryos. Scale bar: 50 μm.

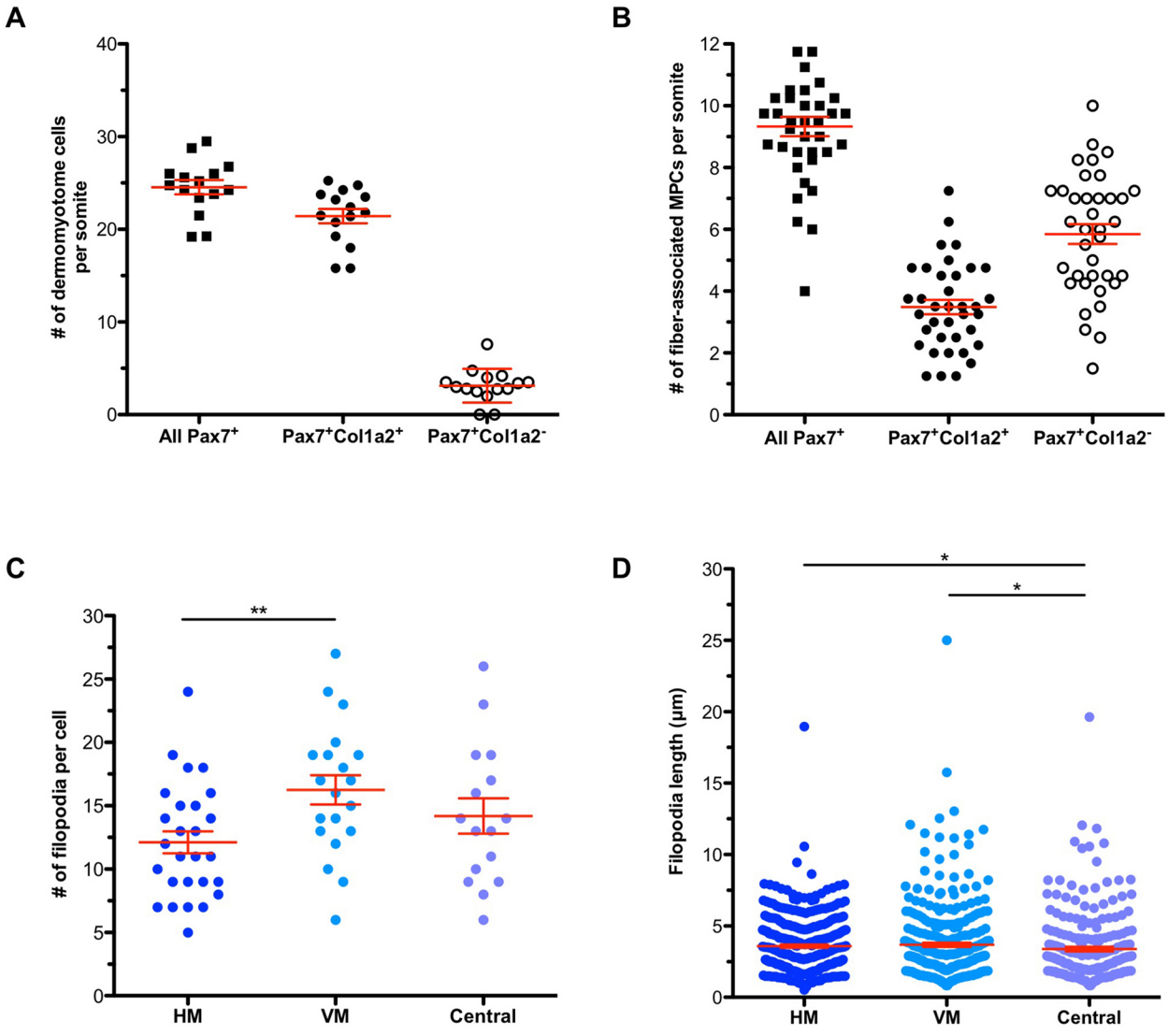


Figure S2. Characterization of *col1a2*$^{+}$ MPCs. (A) Quantification of dermomyotome cells per somite in *col1a2*$^{NTR\text{-}mCherry}$ fish stained with anti-Pax7 antibody at 2 dpf (n = 15 embryos). (B) Quantification of fiber-associated MPCs per somite in *col1a2*$^{NTR\text{-}mCherry}$ fish stained with anti-Pax7 antibody at 5 dpf (n = 37 embryos). (C-D) Single *col1a2*$^{+}$ MPCs in mosaic *col1a2*^{Kaede} fish were imaged to quantify the number (C) and length (D) of their filopodia (n = 36 embryos). HM: 27 cells; VM: 20 cells; Central: 16 cells. All data are plotted with mean ± SEM indicated. Statistics: Mann-Whitney U test. Asterisks representation: p-value < 0.05 (*) and p-value < 0.01 (**).

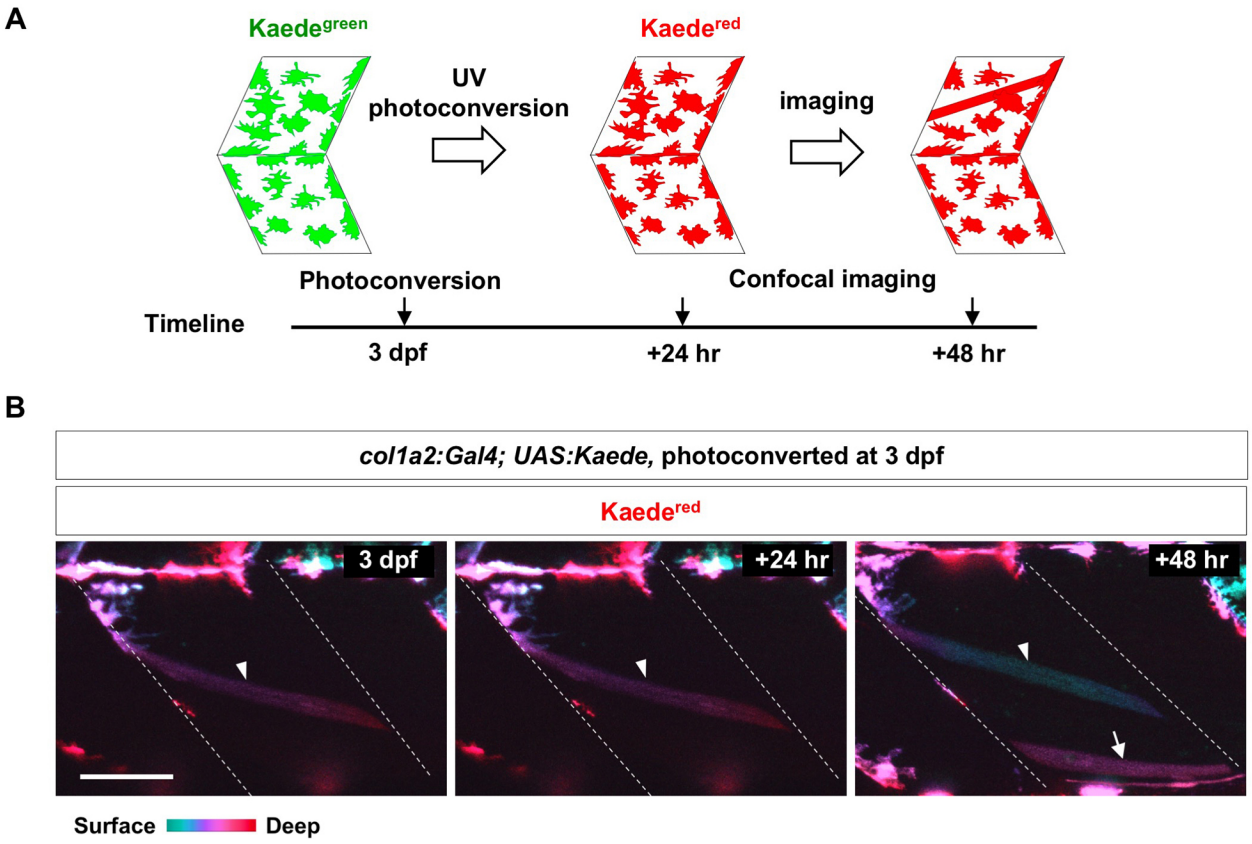


Figure S3. *col1a2*$^{+}$ cells contribute to muscle growth. (A) Schematics of photoconversion-based lineage tracing. (B) *col1a2*^{Kaede} embryos were photoconverted at 3 dpf, and imaged at indicated time points. Color coded depth projections (green corresponds to superficial slices, while red denotes deep slices) of converted *Kaede*^{red} showed that new *Kaede*^{red} muscle fibers (arrow) emerged at 48-hour post conversion. An existing muscle fiber present through all 3 time points is indicated by arrowheads. n = 15 embryos. Scale bar: 50 μm.

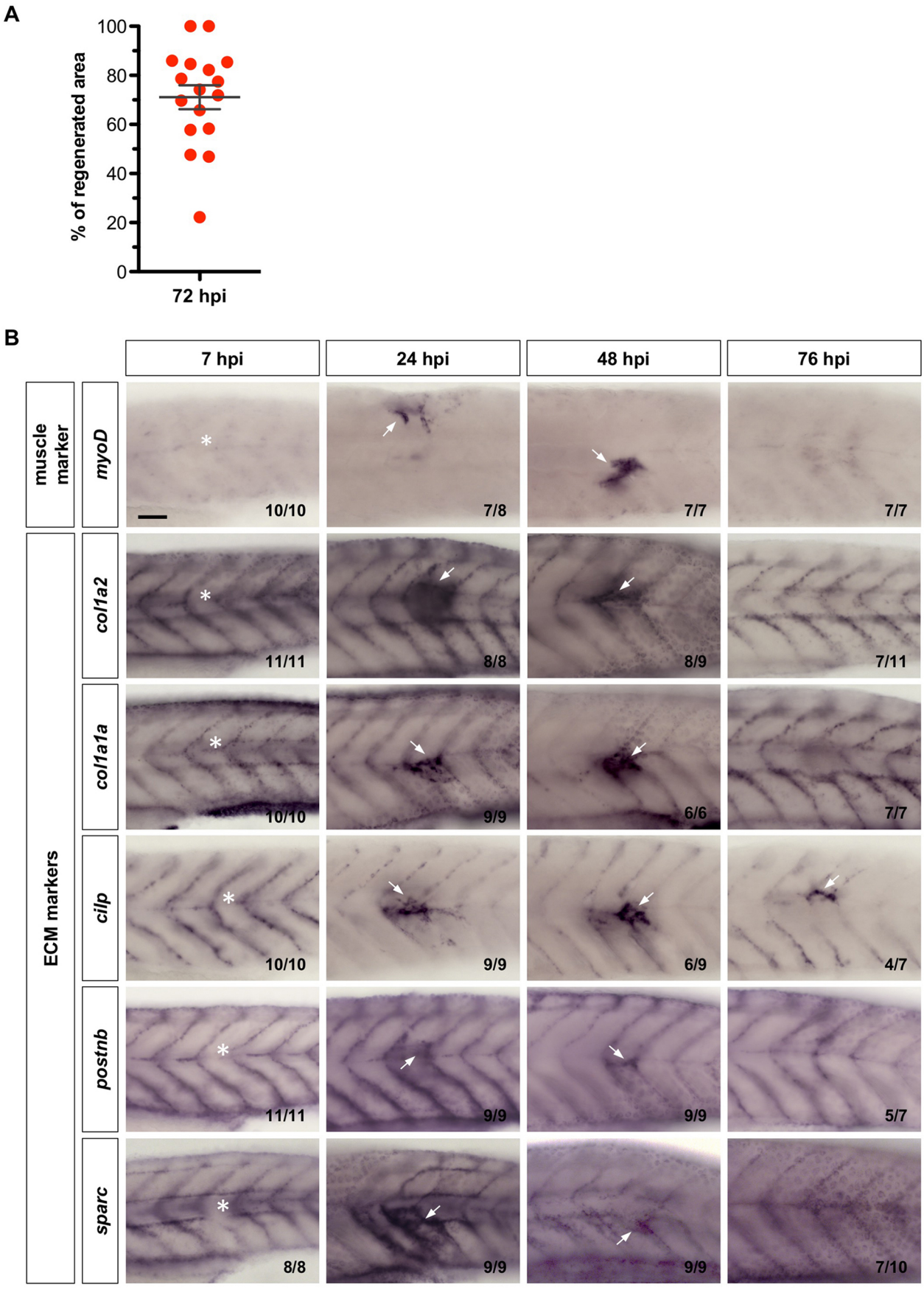


Figure S4. ECM molecules are upregulated during muscle regeneration. (A) Quantification of *col1a2*$^{+}$ MPC contribution to muscle regeneration in *col1a2*$^{NTR\text{-}mCherry}$*; α-actin:GFP* embryos at 72 hpi. The corresponding experiment is shown in Figure 4A. The percentage of contribution was calculated by dividing the area of *mCherry*$^{+}$ muscle fibers at the injury site by the total regenerated area marked by slightly elevated *α-actin:GFP* expression. Data are plotted with mean ± SEM indicated. *n* = 17 embryos. (B) Wild-type embryos were needle stabbed to injure a somite near the end of yolk extension (asterisks) at 3dpf, and fixed at 7, 24, 48, and 76 hpi. Embryos were then stained with the myogenic marker (*myoD*) and ECM markers (*col1a2*, *col1a1a*, *cilp*, *postnb*, and *sparc*). All markers showed upregulation at the site of injury starting from 24 hpi (arrows). Representative images are shown with the *n* number indicated for each staining. Scale bar: 50 *μ*m.

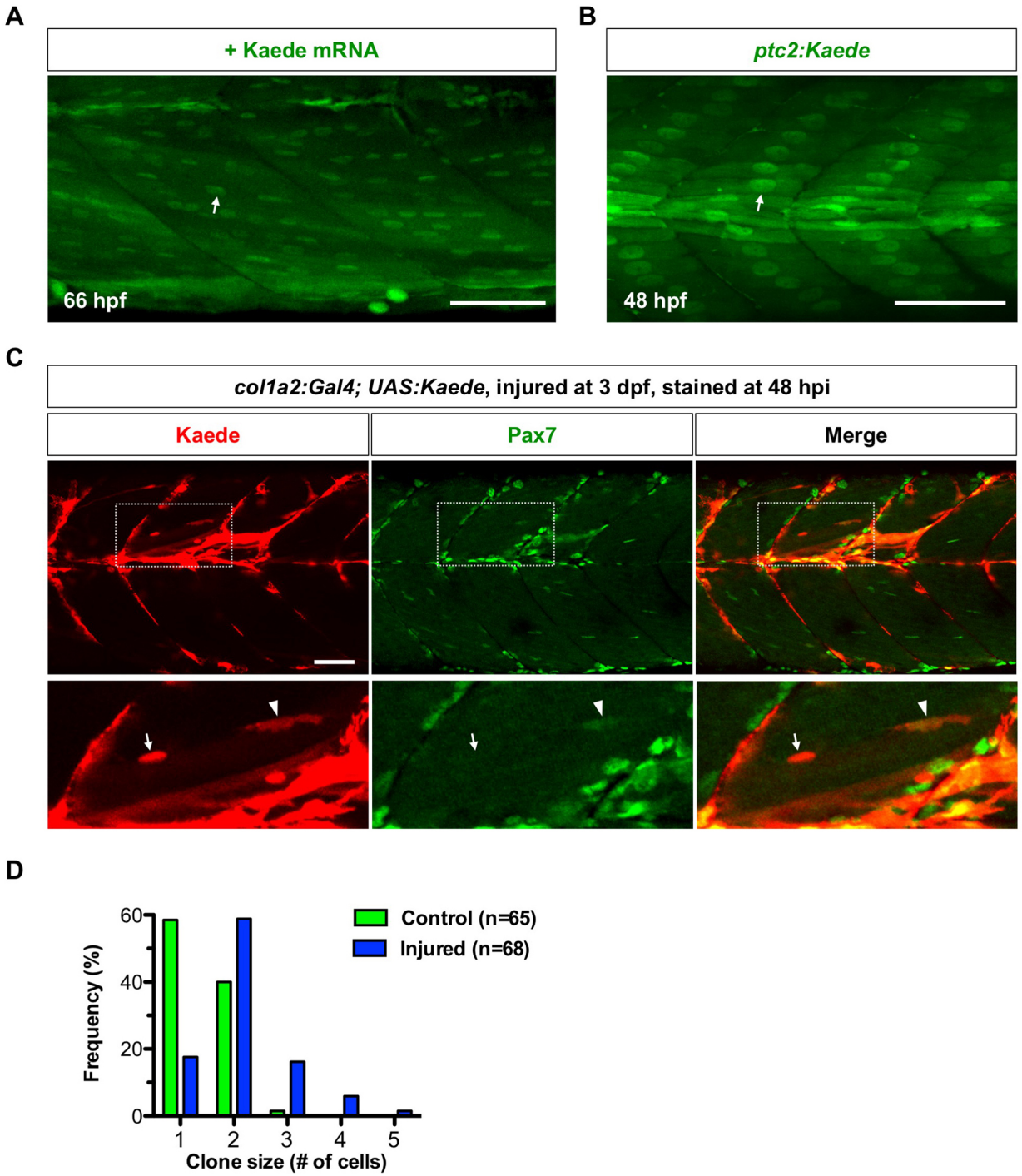


Figure S5. Kaede protein is preferentially localized in nuclei of muscle fibers. (A) Wild-type embryos were injected with Kaede mRNA, and imaged at 66 hpf. Kaede protein (green) is preferentially localized in the nuclei of muscle fibers (arrow). *n* = 40 embryos. (B) Kaede protein (green) is concentrated in the nuclei of slow myofibers (arrow) in *ptc2:Kaede* embryos at 48 hpf. *n* = 40 embryos. (C) *col1a2*^{Kaede} embryos were injured at 3 dpf, and stained at 48 hpi

with the anti-Pax7 antibody (green). *Kaede*$^{+}$ cells (red) contributed to muscle regeneration (boxed regions). The expanded views show that newly formed muscle fiber displayed strong Kaede expression in the nucleus (arrows), which was not labelled by Pax7. By contrast, a small elongated *Kaede*$^{+}$ cell between muscle fibers was Pax7 positive (arrowheads). *n* = 11 embryos. (D) Quantification of clone size in single cell clonal analysis described in Fig 5. *col1a2*$^{+}$ MPCs under the injury condition (blue, n=68) tend to generate larger clones compared to MPCs in the wild-type condition (green, n=65). Scale bars: 50 μm.

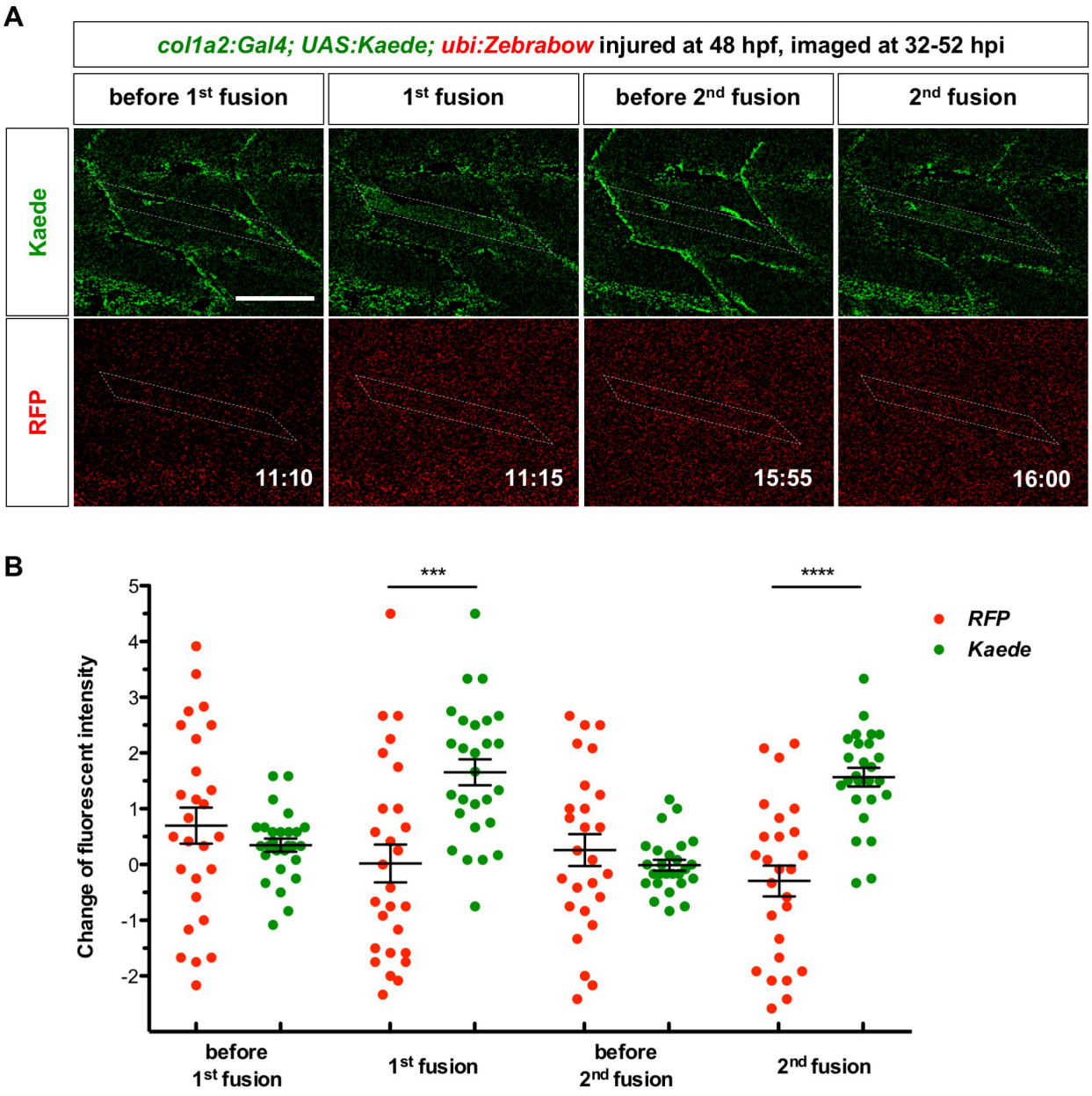
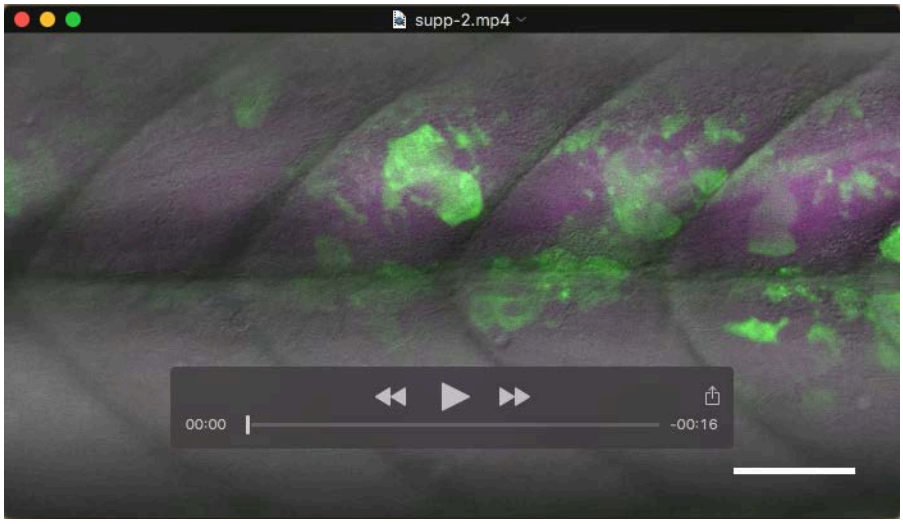
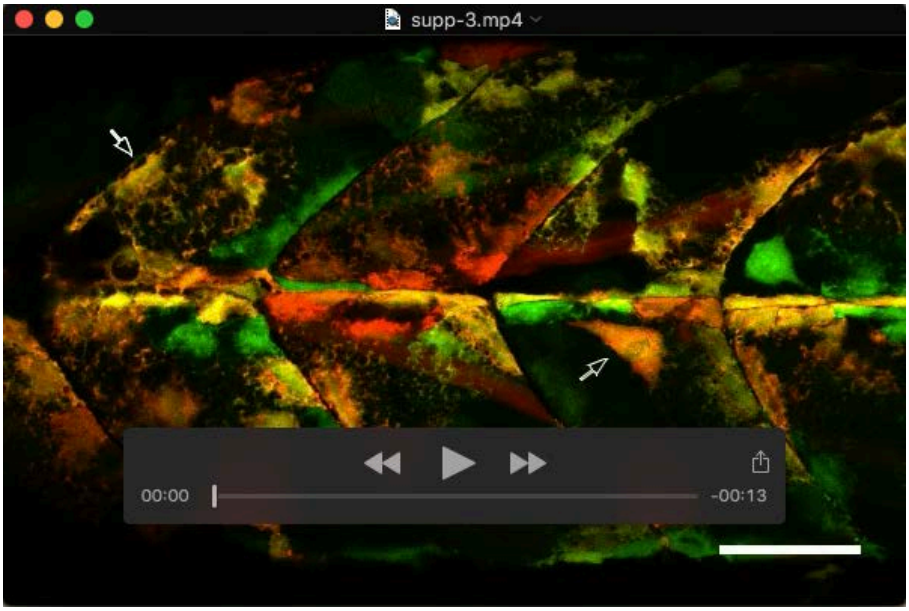


Figure S6. Quantification of sequential muscle fusion of *col1a2*$^{+}$ sibling cells. *col1a2*^{Kaede}*; ubi:Zebrabow* embryos were injured at 48 hpf, and imaged from 32 to 52 hpi. The same experiment is also shown in Figure 6E and Movie 6. (A) Image quantification. Change in fluorescent intensity at each time point was generated by subtracting the fluorescent intensity of corresponding channels at the previous time point (5 minutes earlier). Note that negative pixel values after image processing were set to zero. The muscle fiber that *col1a2*$^{+}$ sibling cells fused to is indicated by dotted lines. The increase of Kaede intensity but not the RFP signal in the muscle fiber can be observed at 11:15 (1st fusion event) and 16:00 (2nd fusion event). (B)

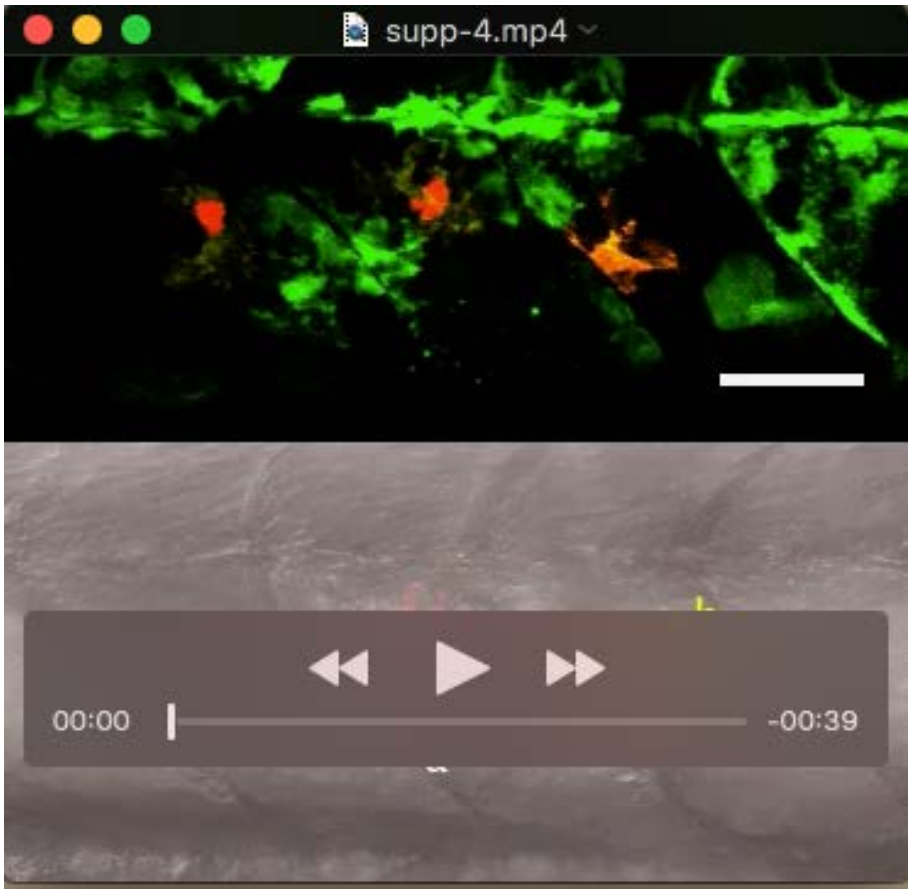
Quantification of fluorescence change before and after cell fusions. Small ROIs within the outlined muscle fiber were measured for fluorescent intensity at different time points ($n = 27$ for the 1st fusion event and $n = 26$ for the 2nd fusion event). Change in fluorescent intensity for each ROI in each channel was calculated and plotted for each time point. For example, change in fluorescent intensity for a given ROI at the 1st fusion (11:15) was generated by subtracting the fluorescent intensity at 11:10 from the intensity at 11:15. Significant increase in Kaede intensity but not the RFP signal in the muscle fiber can be observed at 11:15 (1st fusion event) and 16:00 (2nd fusion event), suggesting fusion events between a *Kaede*$^{+}$ MPC and a muscle fiber. All data are plotted with mean ± SEM indicated. Statistics: Mann-Whitney *U* test. Asterisks representation: p-value < 0.001 (***) and p-value < 0.0001 (****). Scale bar: 50 μm.



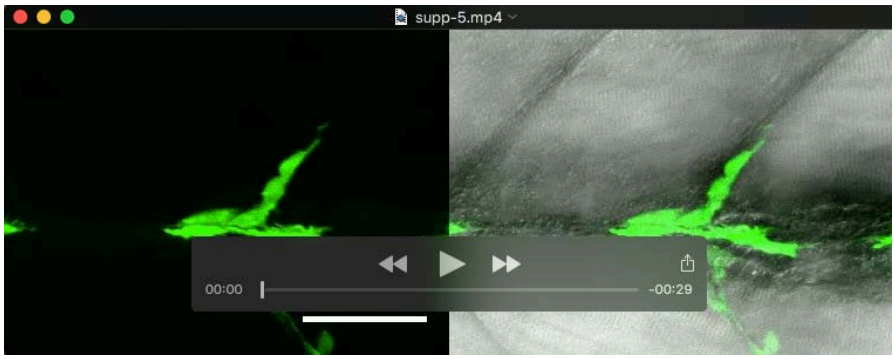
Movie 1. Expression pattern of the *col1a2*$^{NTR\text{-}mCherry}$ line. A confocal z-stack of *col1a2:Gal4; UAS:NTR-mCherry; α-actin:GFP* embryos at 3 dpf shows mCherry expression (green) in dermomyotome cells, some muscle fibers, tenocytes and notochord-associated cells (arrows). Muscle fibers are labeled with *α-actin:GFP* (magenta). Scale bar: 50 *μ*m.



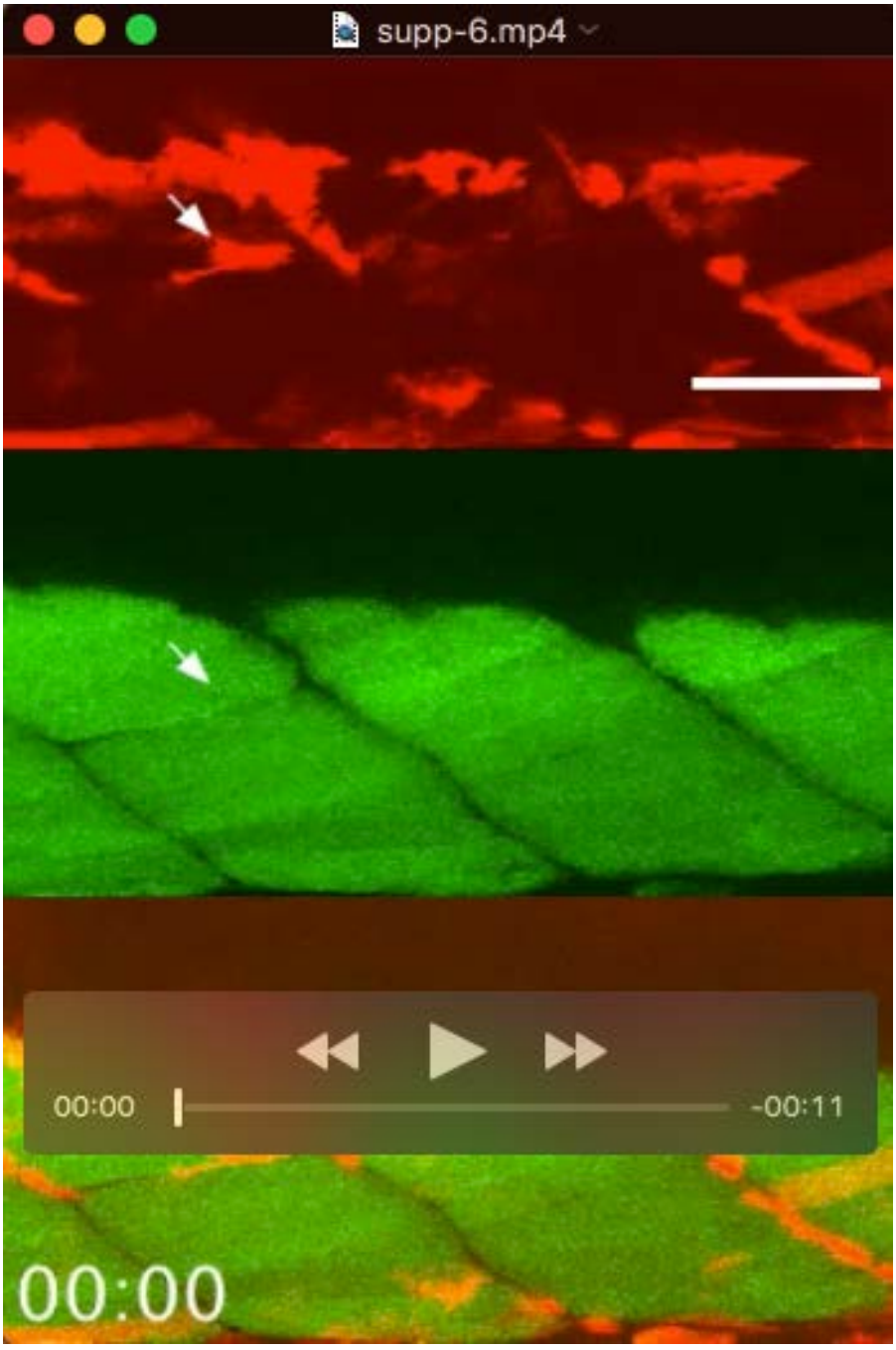
Movie 2. Dynamics *col1a2*$^{+}$ MPCs in quiescent state. *col1a2:Gal4; UAS:NTR-mCherry; UAS:Kaede* embryos were imaged at 2 dpf for 7.9 hours. *col1a2*$^{+}$ MPCs cover the surface of the somite. When a *col1a2*$^{+}$ MPC divides, daughter cells reclaim the same surface area soon after the division. Five different cell divisions are indicated by arrows. Representative snapshots are shown in Figure 2D. Scale bars: 50 μm.



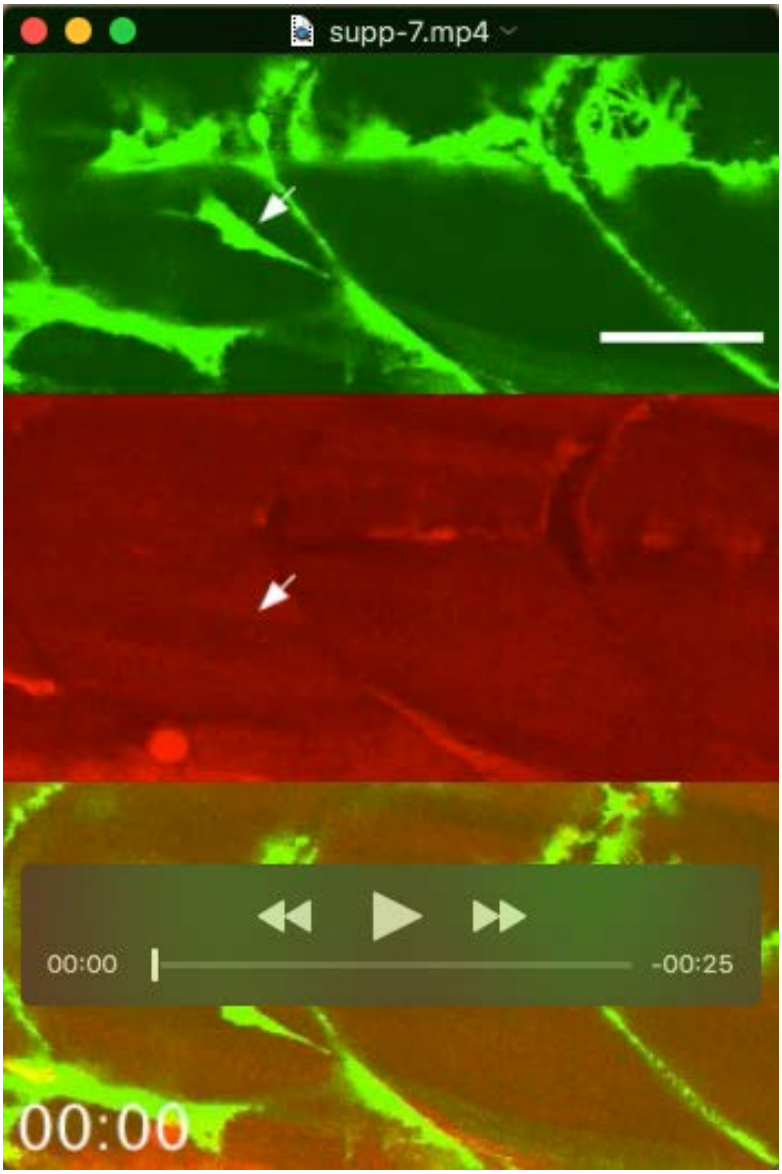
Movie 3. Dynamics *col1a2*$^{+}$ MPCs in injured condition. *col1a2*^{Kaede} embryos were injured, photoconverted at 59 hpf, and then imaged over 23 hours (0-23 hpi). Cells “a” and “b” were within the injured area while the cell “c” was in the uninjured area. Cell “a” (white arrows) maintained the ramified morphology, and divided once at 7 hpi generating two daughter cells with similar morphologies. By contrast, cell “b” (yellow arrows) extended to form an elongated morphology (arrowheads), and divided once at 16 hpi generating two polarized daughter cells. Cell “c” (cyan arrows) did not divide and remained its ramified morphology. Representative snapshots of the injured area (cell a and b) are shown in Figure 6B. Scale bar: 50 μm.



Movie 4. Generation of new muscle fibers by cell fusion. *col1a2*^{Kaede} fish was injured at 3 dpf and imaged from 29 hpi onwards. A *Kaede*$^{+}$ MPC (arrows) near the injury site elongated at 34 hpi (white arrowheads), formed protrusions at 39 hpi, and fused with a neighboring muscle fiber at 40 hpi. The newly formed muscle fiber can be identified by the weak Kaede expression throughout the muscle fiber and the strong Kaede expression in the nucleus (yellow arrowheads). Representative snapshots are shown in Figure 6D. Scale bar: 50 μm.



Movie 5. Generation of new muscle fibers by cell fusion. *col1a2*$^{NTR\text{-}mCherry}$*; α-actin:GFP* embryos were injured at 79 hpf and imaged from 24 to 36 hpi. The red channel (*col1a2*$^{NTR\text{-}mCherry}$), the green channel (*α-actin:GFP*), and the merged images are shown with time stamps indicated in the hh:mm format. A *mCherry*$^{+}$ MPC (arrows) near the injury site elongated and fused with a neighboring muscle fiber between 07:07 and 07:14. The fusion event was evident by the spreading of the mCherry signal in the muscle fiber and the mCherry signal in the nucleus (arrowheads) remained visible until the end of the movie at 12:15. A total of 23 fusion events were observed in movies from 12 embryos. Scale bar: 50 μm.



Movie 6. Sequential fusions of sibling cells derived from *col1a2*$^{+}$ MPCs. *col1a2*^{Kaede}*; ubi:Zebrabow* embryos were injured at 48 hpf, and imaged from 32 to 52 hpi. Representative snapshots and image quantification are shown in Figures 6E and S6, respectively. The green channel (*col1a2*^{Kaede}), the red channel (*ubi:Zebrabow*) and the merged images are shown with time stamps indicated in the hh:mm format. Note that *Kaede*$^{+}$ MPCs showed almost no RFP expression compared to muscle cells. A *Kaede*$^{+}$ cell (white arrows) divided between 00:55 and 01:00. The anterior daughter cell (cyan arrows) fused with a muscle fiber between 11:10 and 11:15, while the posterior daughter cell (yellow arrows) fused with the same muscle fiber between 15:55 and 16:00. Concentrated Kaede expression in the nucleus after cell fusion was clearly visible (arrowheads of corresponding colors). A total of 31 fusion events were observed in movies of 12 embryos. Scale bar: 50 μm.