

Figure S1

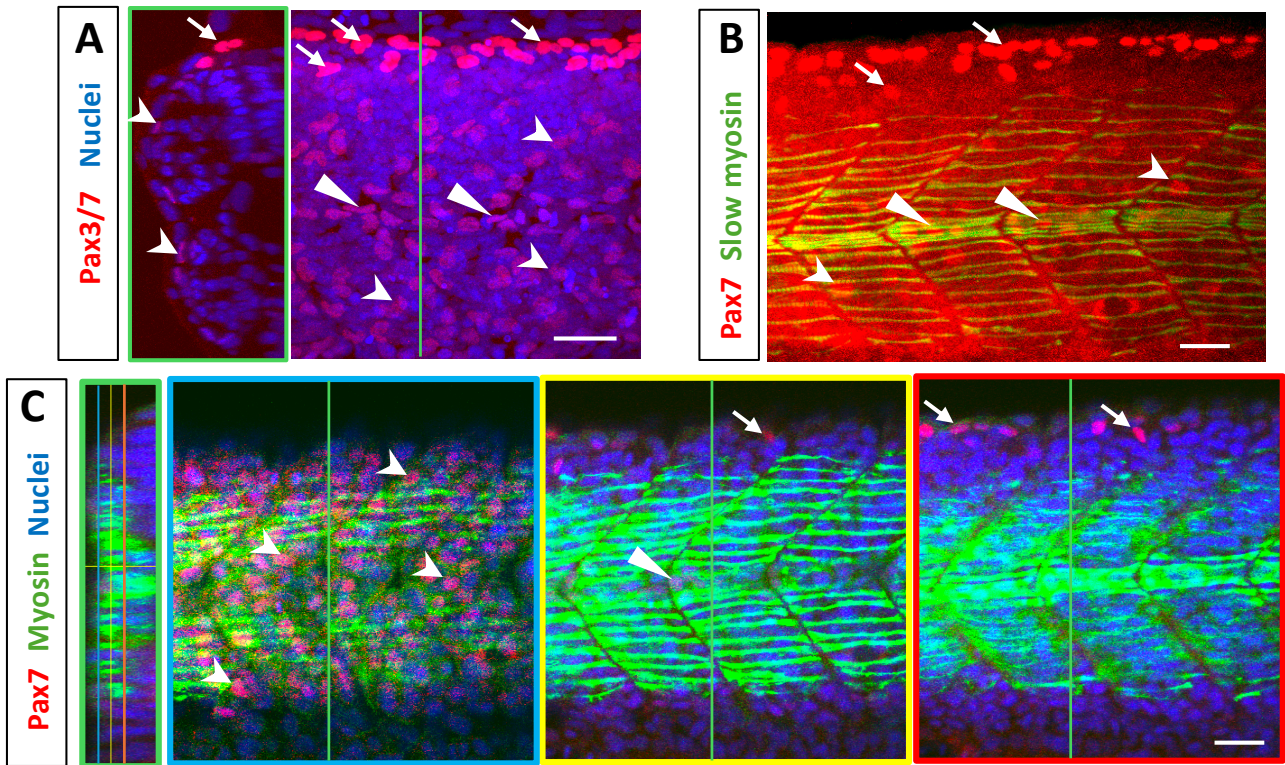


Figure S1. Pax7 immunoreactivity in the zebrafish trunk.

Immunodetection of the indicated proteins and nuclear counterstain in lateral YX views, dorsal to top, anterior to left, of 24 hpf zebrafish embryos. YZ transverse views are shown at left (A,C). XY maximal intensity projections (A,B) contrast with XY optical slices (C) at distinct mediolateral myotomal depths (coloured boxes/lines). Strong signal in neural crest-derived xanthophore precursors in dorsal medial regions (arrows), contrasts with weaker expression in superficial dermomyotome cells (chevron arrowheads) and contrasting morphology of some nuclei at the horizontal myoseptum (long arrowheads). Scale bars = 25 μ m.

Figure S2

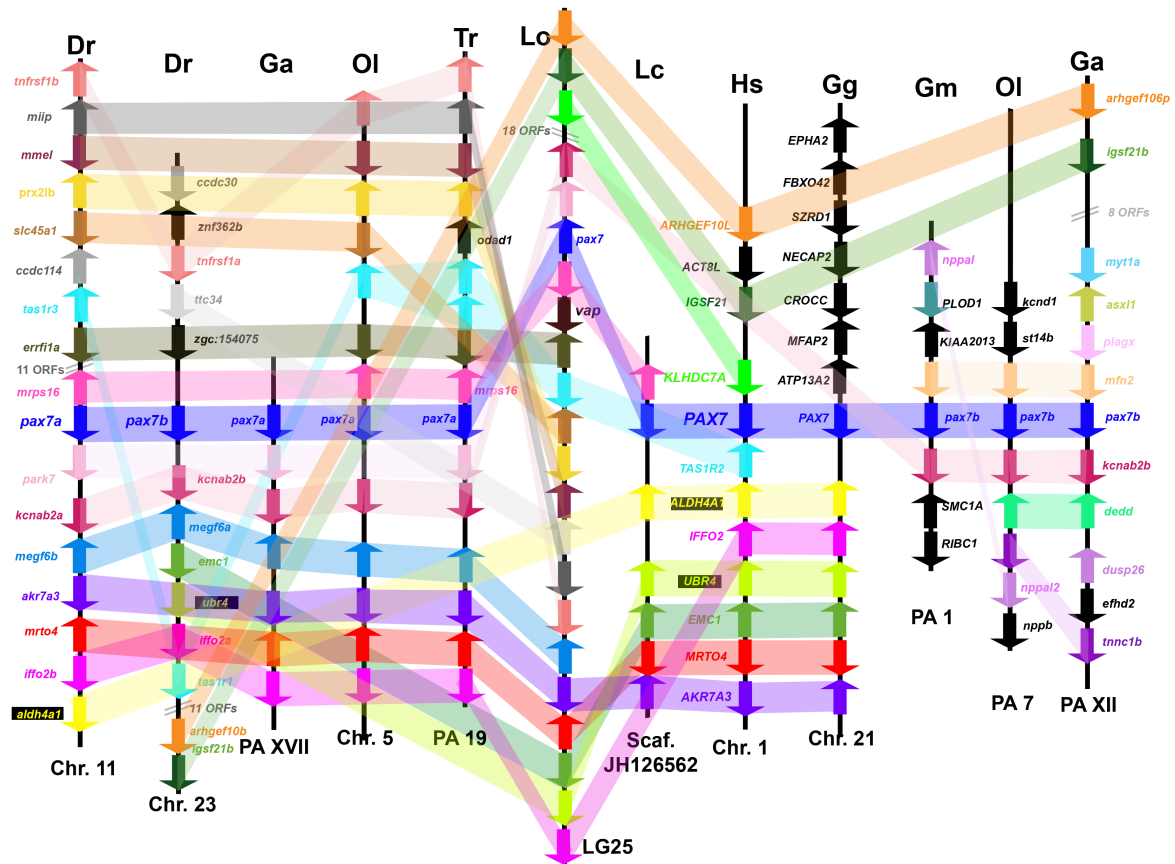


Figure S2. Synteny of PAX7 genes.

Synteny of genes adjacent to *PAX7* genes, taken from ENSEMBL 19 June 2026. Colour coding indicates orthologous genes, linked by pastel coloured lines to indicate synteny. Arrows shows read direction with respect to *pax7*. Black arrows represent genes lacking close synteny to *pax7* genes in other species analysed. Species are indicated above: Dr *Danio rerio*; Ga *Gasterosteus aculeatus* (Stickleback); Ol *Oryzias latipes* (Japanese Medaka); Tr *Takifugu rubripes* (Fugu); Lo *Lepisosteus oculatus* (Spotted Gar); Lc *Latimeria chalumnae* (Coelacanth); Hs *Homo sapiens*; Gg *Gallus gallus* (Chicken); Gm *Gadus morhua* (Cod). Depending on the state of assembly, Chromosome (Chr.), Linkage Group (LG), Primary Assembly (PA) or Scaffold (Scaf.) numbers are indicated below. ORFs show number of non-homologous open reading frames occupying locus.

Figure S3

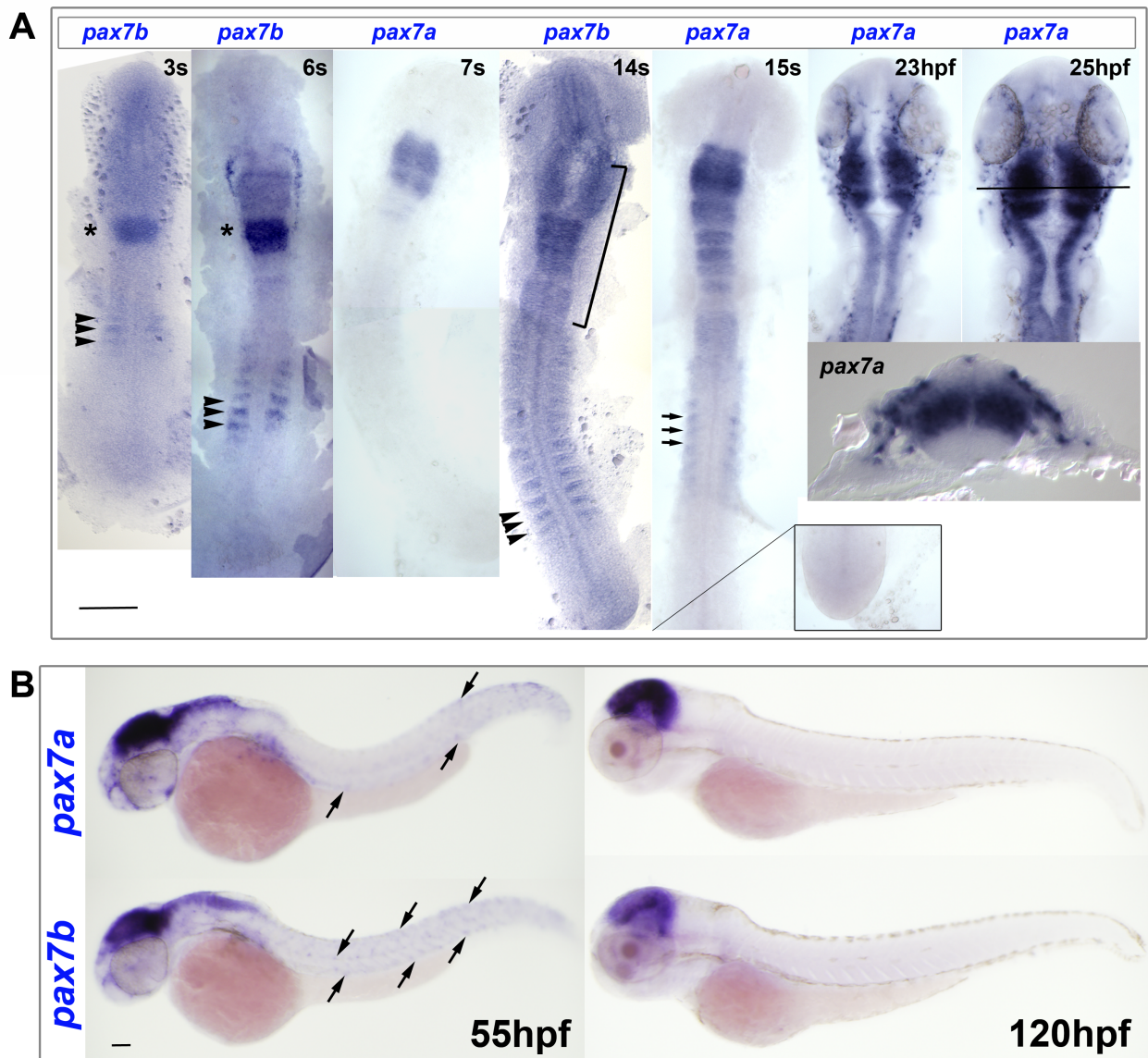


Figure S3. *Pax7b* mRNA is expressed before *pax7a* mRNA in central nervous system and somites. In situ mRNA hybridization for *pax7a* and *pax7b* are in flatmount dorsal views (A; anterior to top) and lateral wholemount (B; anterior to left, dorsal to top) in lower panels. **A.** *Pax7b* mRNA is detected in hindbrain stripes (asterisks) and anterior somite borders (arrowheads) as they form at the 3 somite stage (3s), before accumulating in brain regions (bracket) and spinal cord by 15s. In contrast, *pax7a* mRNA is first detected in more anterior brain regions and subsequently expresses in maturing somites (arrows) at 15s in similar cells to those expressing *pax7b* mRNA. These panels can be compared with others in the same experimental series in Figs 1A-D,R-E', 3Q,S, 4A-L, S1C-K of (Minchin and Hughes, 2008); from which two panels (*pax7b* 14s and *pax7a* 25hpf) are reproduced to aid comparison. **B.** At later stages (lower panels), expression of both genes persists in the brain but is detected at diminishing levels in somitic cells (long arrows) possibly due to their small size and flattened morphology. Scale bars = 100 mm.

Figure S4

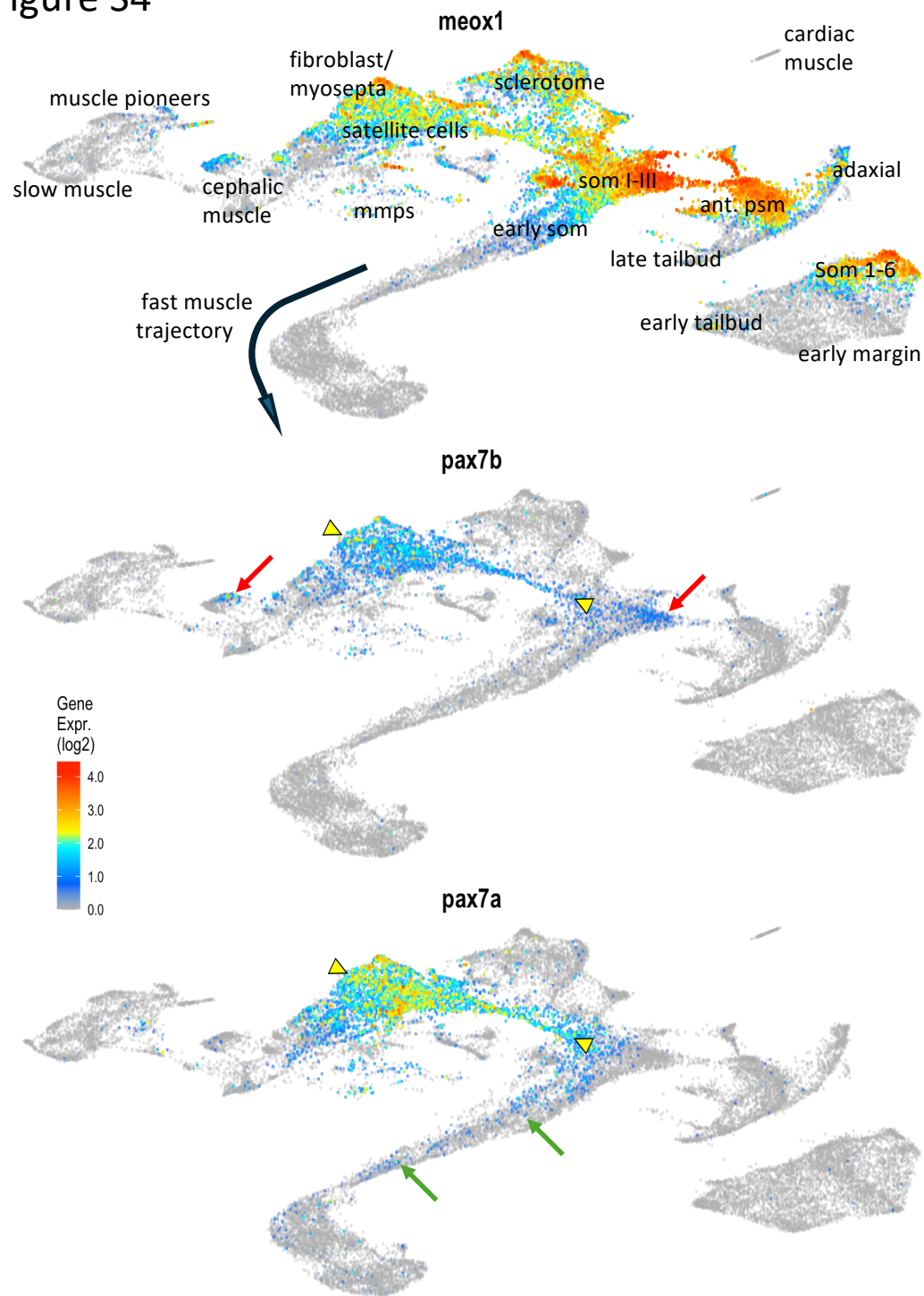


Fig. S4. Single cell RNA sequencing analysis confirms subset heterogeneity in *pax7a* and *pax7b* expression. UMAP comparison of scRNAseq data for *meox1*, *pax7b* and *pax7a* in the pre-defined ‘Muscle’ cell cohort. Defined Muscle cell subpopulations are labelled on the *meox1* plot. Cell groups expressing *pax7a*-only (green arrows), *pax7b*-only (red arrows), and both *pax7a* and *pax7b* (row between yellow arrowheads) are indicated. Mmps, migratory muscle precursors; ant. psm, anterior presomitic mesoderm; som I-III, the newest three somites during somitogenesis; Som 1-6, the anteriormost 6 somites. Plot created using DaniocellDesktop (Evans and Farrell, 2025).

Figure S5

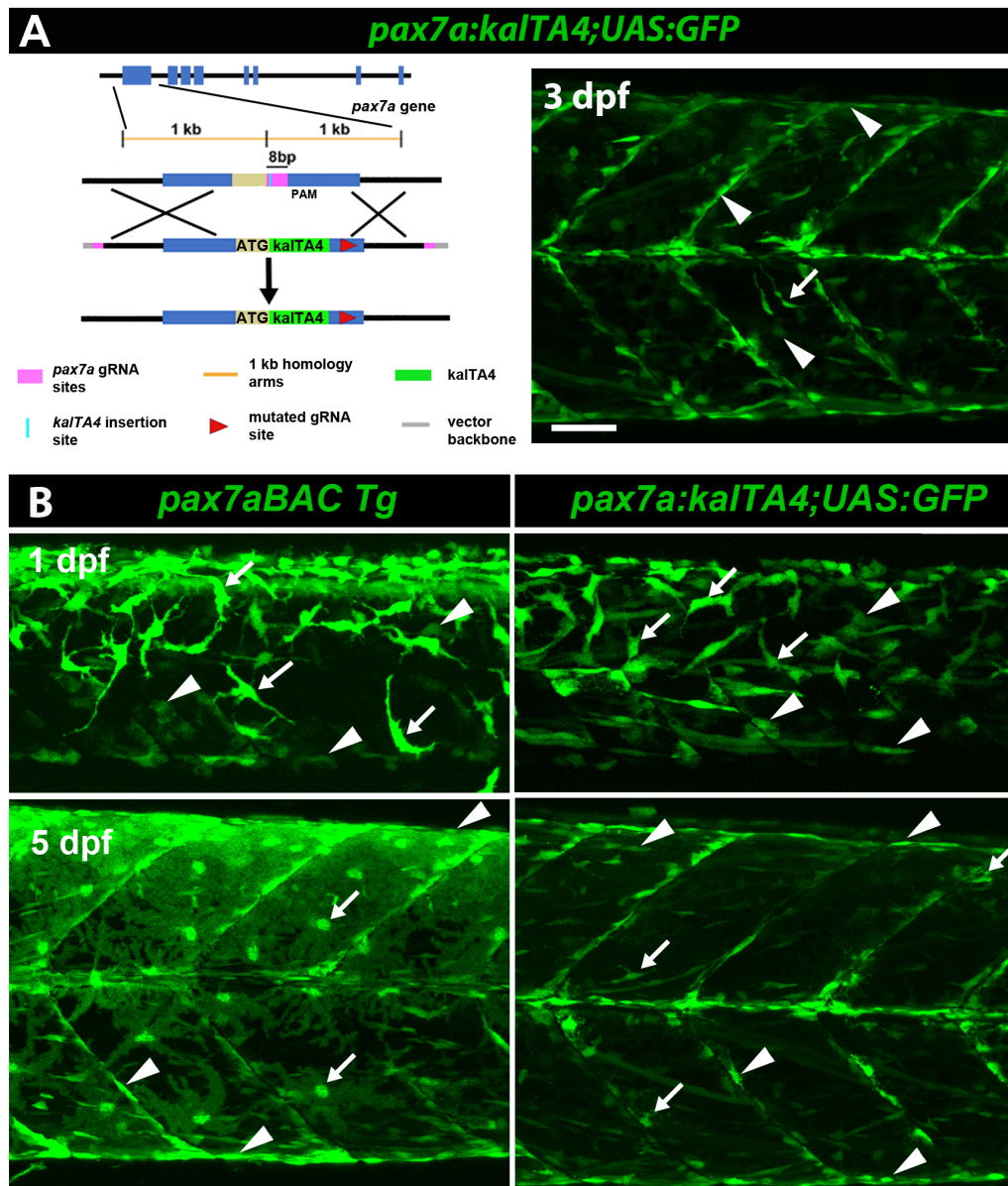


Figure S5. A *pax7a:kalTA4* knock-in allele largely recapitulates the *pax7a* BAC expression pattern. **A.** Diagram of the targeting of *pax7a* with *kalTA4* and expression of UAS:GFP in somites at 3 dpf, lateral view anterior to left dorsal up. **B.** Comparison of expression location and level of reporter GFP signal between *pax7a:GFP* BAC transgenic and *pax7a:kalTA4;UAS:GFP* knock-in fish, each showing the same individual at 1 and 5 dpf. Note the weaker xanthophore (arrows) and the mpc (arrowheads) expression in the knock-in, a difference consistent across six fish of each line. Scale bar = 50 μ m.

Figure S6

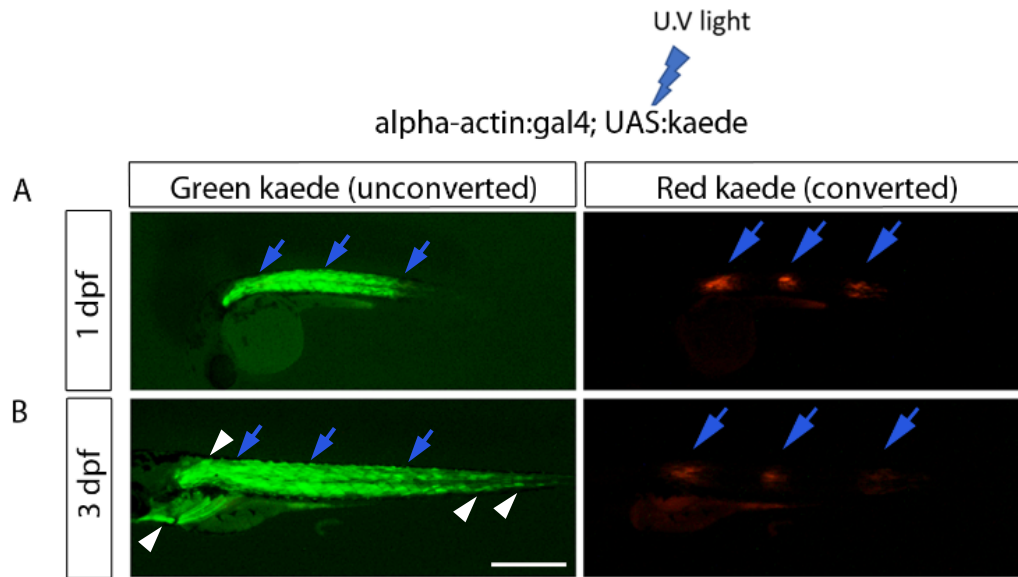


Figure S6. Expression and perdurance of photoconverted Kaede in myotomal muscle. *Tg(α -actin:GAL4);Tg(UAS:Kaede)* embryos were generated by crossing parents carrying each transgene and expressed unconverted green Kaede in terminally differentiated muscle fibres. **A.** Photoconversion with UV light was performed at 1 dpf at three separated locations (blue arrows). Photoconversion was non-saturating, as shown by persistence of green unconverted Kaede in the red regions at 1 dpf. **B.** Red converted Kaede perdured at 3 dpf (blue arrows) as newly-expressed green Kaede continued to accumulate (white arrowheads). Scale bar = 500 μ m .

Figure S7

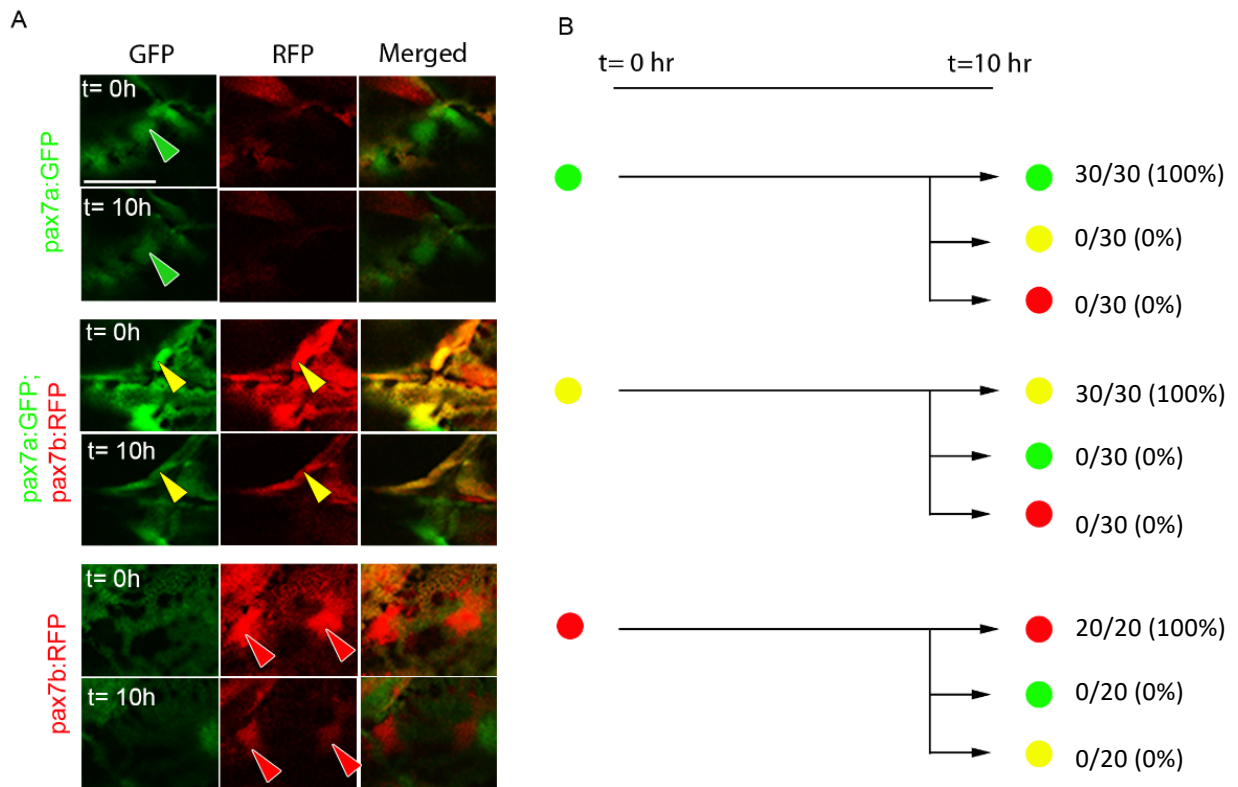


Figure S7. Little interconversion between mpc populations. LMA embedded lightly-anaesthetised *pax7a:GFP;pax7b:RFP* larvae at 3 dpf were subjected to timelapse confocal analysis by repeated capture of stacks of somites 15-17. **A.** Single confocal slices of identified mpcs marked by *pax7a:GFP*-only, *pax7b:RFP*-only, or both at t=0 and t=10 hr. Coloured arrowheads indicate mpcs of each type. **B.** Schematic summary of fate of 30 *pax7a:GFP* (top), 30 *pax7a:GFP;pax7b:RFP* (middle) and 20 *pax7b:RFP* (bottom) mpcs. Scale bar = 20 μ m.

Figure S8

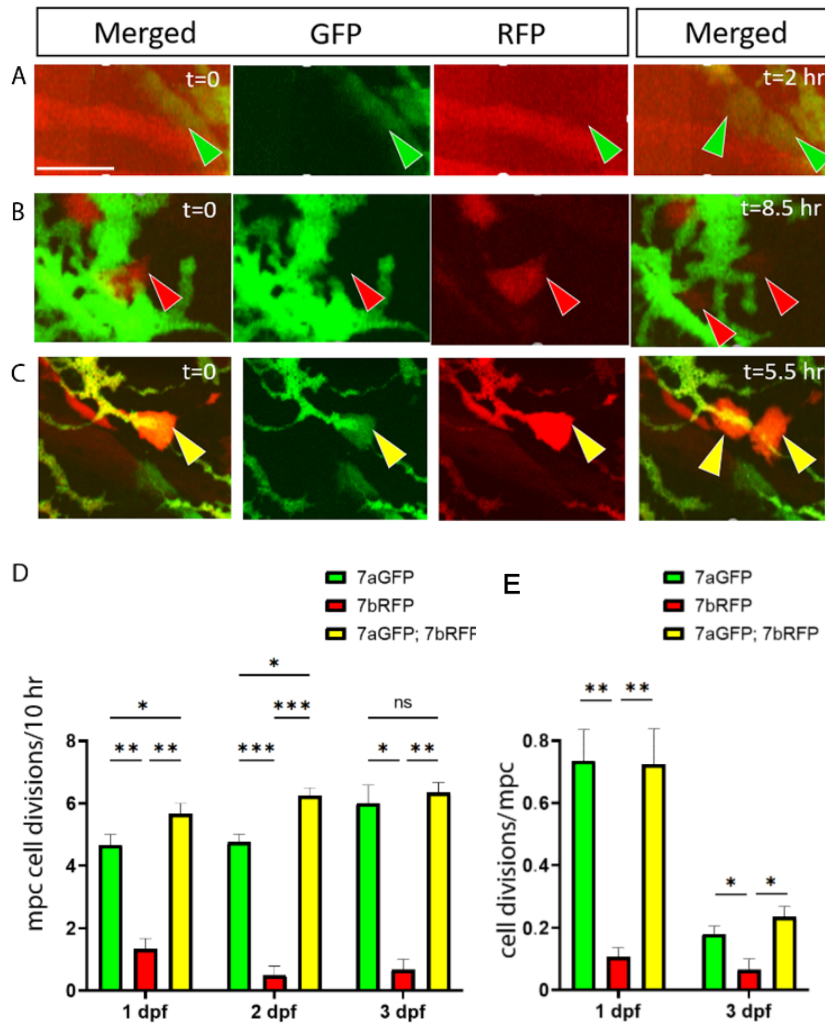


Figure S8. Each mpc population undergoes division. Time lapse images of *pax7:GFP;pax7b:RFP* fish at 3-3.5 dpf. **A-C.** Stills showing cell division of *pax7a:GFP*-only (A), *pax7b:RFP*-only (B) and *pax7a:GFP; pax7b:RFP* (C) mpcs and their paired daughters on somite 16 annotated with green, red and yellow arrowheads, respectively. Left and righthand panels show pre-division and post-division timepoints. **D.** Numbers of mpc mitotic divisions observed from each mpc population/10 hr from somite 15-16 (n=3 embryos). **E.** Mpc cell division rate in 10 hr calculated as ratio of data in Fig. S8D to that in Fig. 5A. Scale bar = 20 μ m.

Figure S9

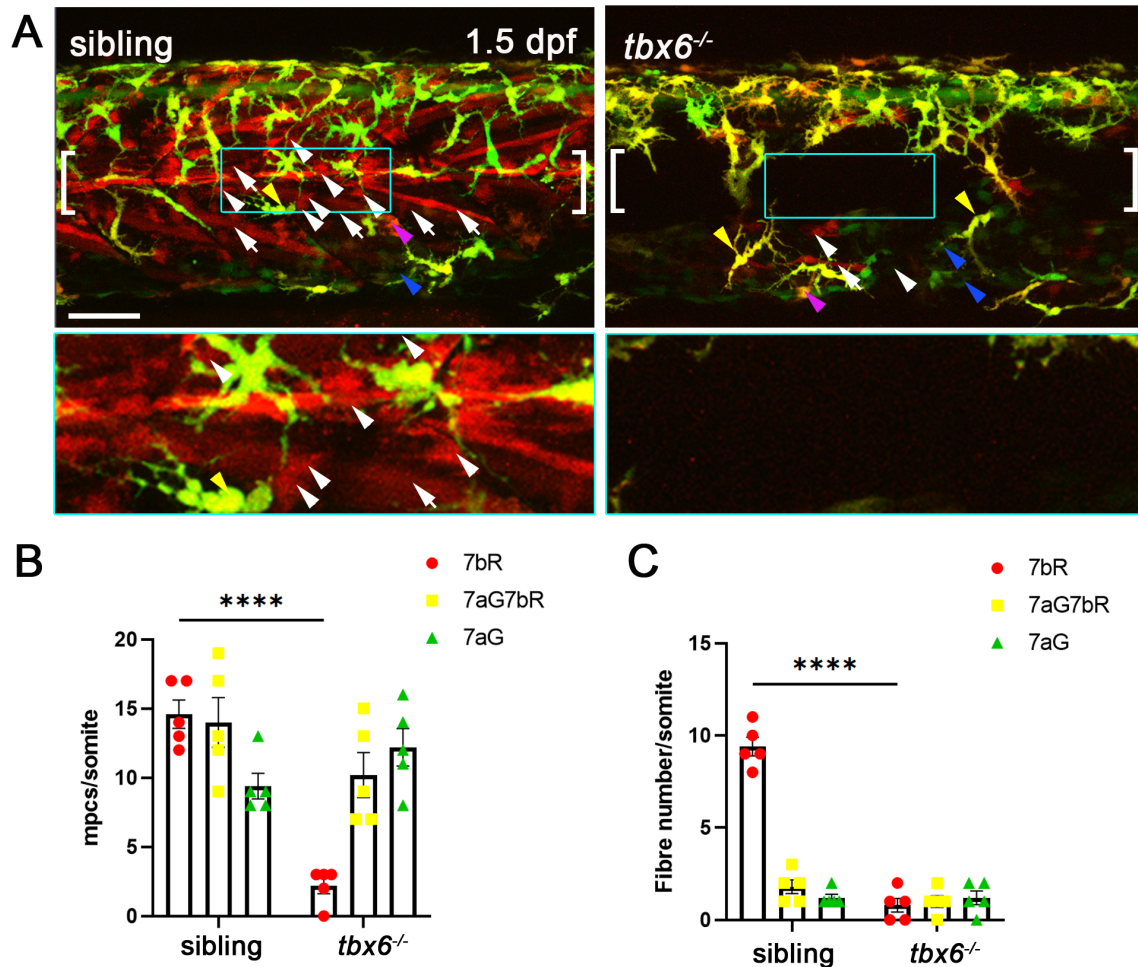


Figure S9. *Tbx6* mutants show selective reduction in *pax7b:RFP*-expressing mpcs and fibres within central region during early myotome development. *Tbx6* mutants and siblings were generated by crossing a *tbx6*^{+/-} female with a *tbx6*^{+/-};*pax7a:GFP;pax7b:gal4;UAS:RFP* male and analysed at 1.5 dpf. **A.** Lateral maximum intensity projection views (anterior to left, dorsal up) of confocal stacks of live *tbx6* sibling (left) and mutant (right). *Pax7b:RFP*-marked mpcs (white arrowheads), *pax7a:GFP;pax7b:RFP* mpcs (magenta arrowheads) and *pax7a:GFP* mpcs (blue arrowheads) present in both genotypes. *Pax7b:RFP*-only mpcs and fibres (white arrows) were more abundant in the central dermomyotomal region (brackets) in siblings than in mutants, whereas *pax7a:GFP*-only mpcs remained in the dorsal and ventral regions. Xanthophores (yellow arrowheads) persist in mutants. Cyan boxes are magnified beneath showing somite 16 central myotome region. **B,C.** Quantitative analysis of number of mpcs (B) and fibres (C) in entire somite 16 marked by each sub-population. Mean $\pm$ s.e.m, each symbol represents an individual fish (n=5). Statistics are two-way ANOVA with factors genotype and cell sub-population showing significant genotype post hoc tests. Scale bar = 50 μ m.

Figure S10

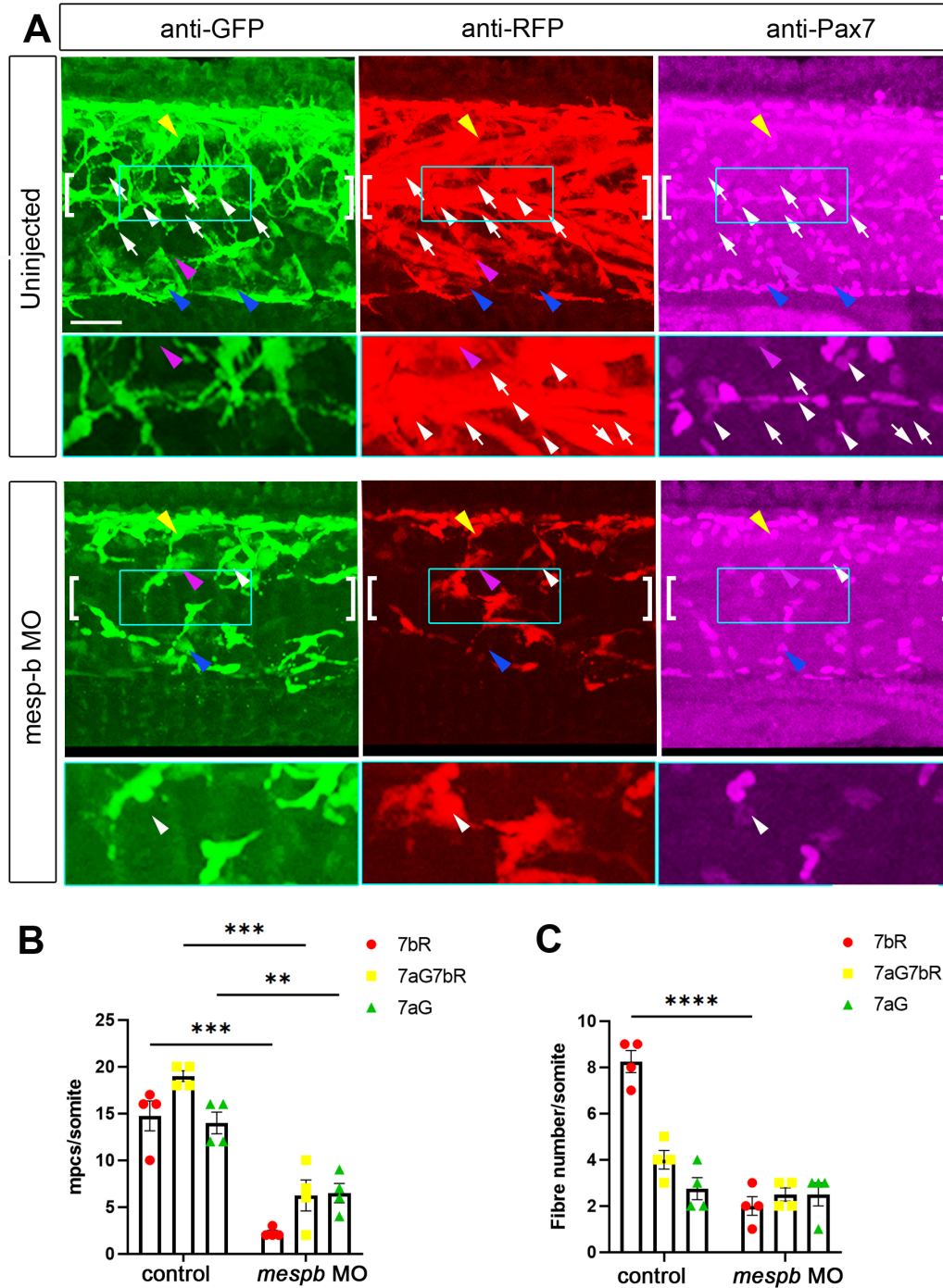


Figure S10. Mesp-bs promote formation of *pax7b:RFP*-expressing mpcs and fibres within central region during early myotome development. Some *pax7a:GFP;pax7b:gal4;UAS:RFP* embryos were injected with *mesp-b* MO and analysed at 1.5 dpf in comparison to control un-injected siblings by immunostaining for each marker and Pax7 protein. **A.** Lateral maximum intensity projection views (anterior to left, dorsal up) of confocal stacks of control (top) and *mesp-b* MO-injected (bottom) embryos. *Pax7b:RFP*-marked mpcs (white arrowheads), *pax7a:GFP;pax7b:RFP* mpcs (magenta arrowheads) and *pax7a:GFP* mpcs (blue arrowheads) present in both conditions. *Pax7b:RFP*-only mpcs and fibres (white arrows) were more abundant in the central dermomyotomal region (brackets) in siblings than in morphants, whereas *pax7a:GFP*-only mpcs remained in the dorsal and ventral regions. Xanthophores (yellow arrowhead) have stronger Pax7 and persist in mutants. Cyan boxes are magnified beneath showing somite 16 central myotome region. **B,C.** Quantitative analysis of number of mpcs (B) and fibres (C) labelled by *pax7b:RFP*, *pax7a:GFP;pax7b:RFP* and *pax7a:GFP* immunodetection in entire somite 16. Mean $\pm$ s.e.m, each symbol represents an individual fish ($n=4$). Statistics are two-way ANOVA with factors treatment and cell sub-population showing significant treatment post hoc tests. Scale bar = 50 μ m.

Figure S11

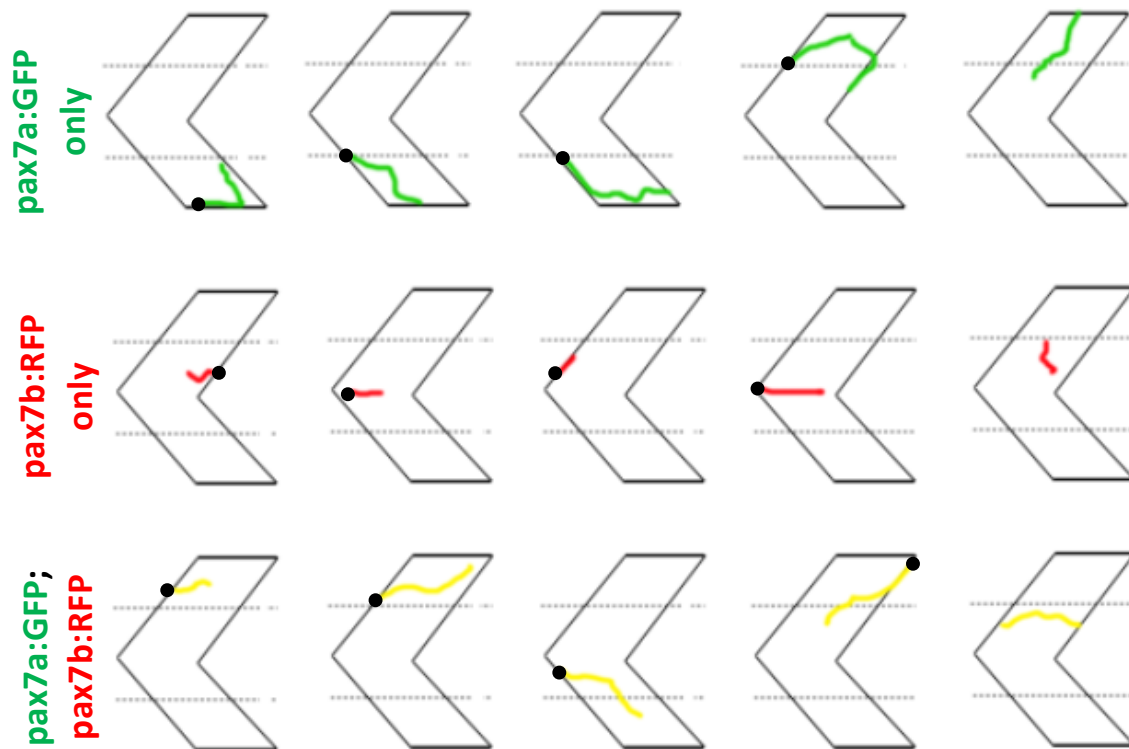


Figure S11. Mpc populations show difference in migration in the developing myotome. Confocal time lapse 3D stack images were captured in lateral view of somite 17 of *pax7a:GFP*; *pax7b:RFP* larvae between 3 and 4 dpf. Most mpcs did not migrate. For each mpc population, the trajectory of the centre of a selected migratory mpc was manually tracked in the 4D stack from its origin at the vertical myoseptum (black dot) for 10 h. Migration tracks of five mpcs from each population are shown projected onto the XY plane. Note the differing track lengths, even within migratory mpcs.