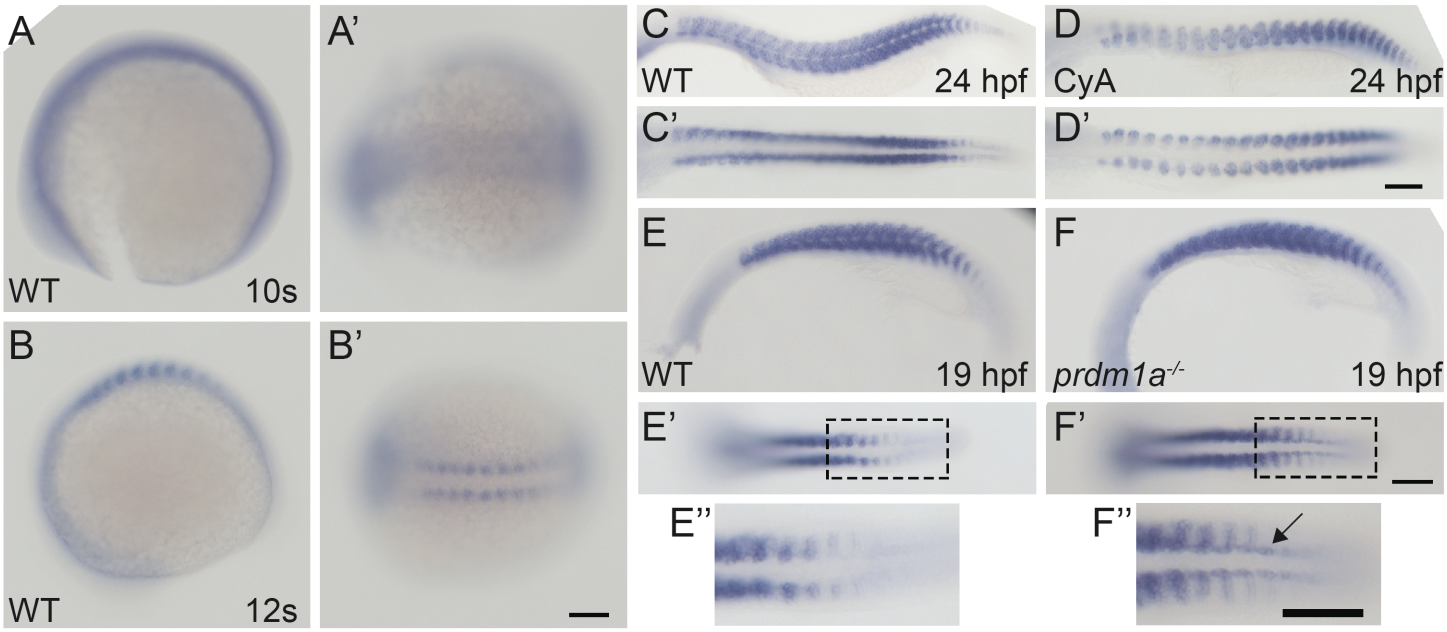
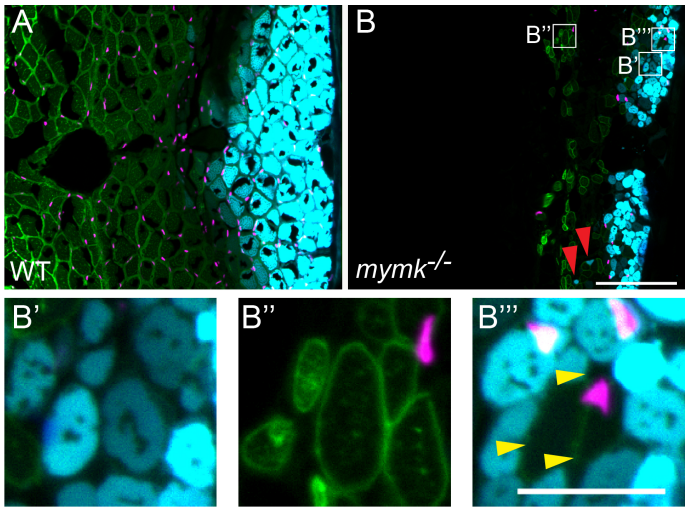
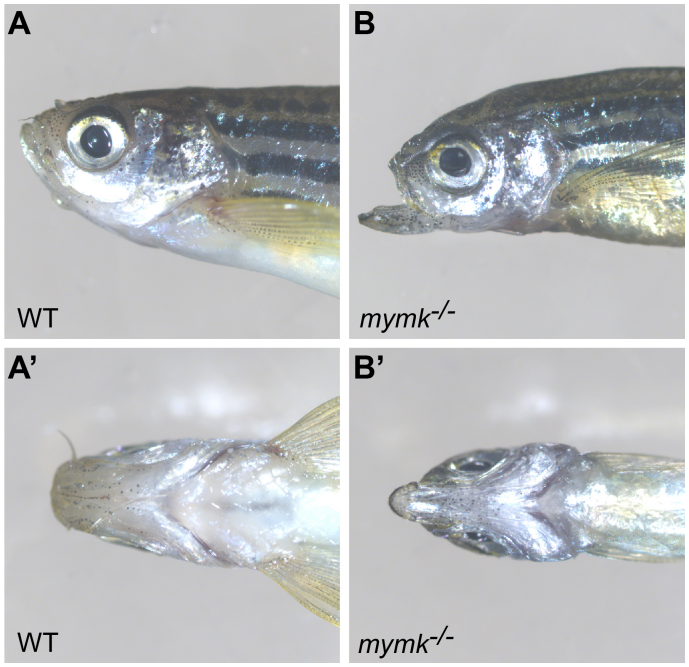
Supplemental Figures and Legends



Supplemental Figure 1: *mymk* is expressed in embryonic zebrafish fast muscle cells. In situ hybridization using an antisense *mymk* (*tmem8c)* probe at 10-somite **(A, A')** and 12-somite **(B, B')** stages reveals that *mymk* expression in fast muscle myoblasts begins sometime between these two stages (around 14.5-15 hpf). **(C-D')** Fast muscle-specific *mymk* expression appears the same in DMSO-treated wild-type (WT) control embryos (C, C') and embryos treated with the Smoothened inhibitor cyclopamine between 5.5-19 hpf to prevent slow muscle formation (Barresi *et al.* 2001, Peterson and Henry 2009) (D, D') (lateral views in C, D; dorsal views in C' and D'). **(E-F'')** In 19 hpf WT embryos, *mymk* expression is restricted to fast muscle cells (E-E''), but in *prdm1a*^{nrd} mutant embryos (F-F"), *mymk* expression is also detected in cells located adjacent to the midline (black arrow). These adaxial cells are normally fated to become slow muscle (Devoto *et al.* 1996), but become fast muscle instead in *prdm1a*^{nrd} mutant embryos (von Hofsten et al., 2008) (lateral views in E and F; dorsal views in E'-F''). The boxed region in E' and F' is magnified in E'' and F'', respectively. Scale bars in B' (for A-B'), D' (for C-D'), F' (for E-F'), and F'' (for E'' and F'') are 100 μm.



Supplemental Figure 2: Myofiber size is variable and myofiber types are intermingled in adult *mymk* mutants. (A-B) Transverse sections of wild-type (WT) (A) and *mymk* mutant (B) 3MuscleGlow transgenic adults. *myog:H2B-mRFP* (magenta) labels myonuclei, *mylfpa:lyn-cyan* (green) marks fast myofiber membranes, and *smyhc1:EGFP* (aqua) marks slow myofibers. Red arrowheads indicate slow myofibers located within the fast muscle domain. **(B'-B''')** Magnified views of the boxed regions in B reveal variable myofiber size in *mymk* mutant slow (B') and fast (B'') myofibers, as well as fast myofibers located within the slow muscle domain (yellow arrowheads, B'''). Scale bar in B (for A, B) is 100 µm and in B''' (for B'-B''') is 20 µm.



Supplemental Figure 3: Adult *mymk* mutants have severe jaw malformations. Compared to adult wild-type (WT) siblings **(A, A')**, *mymk*^{oz17} mutants **(B, B')** have malformed jaws. About 20% of the time, the lower jaw in *mymk* mutants remains open and immobile throughout adulthood. Fish shown are 3 months old.

Supplemental Movie Legends

Movie 1: Fast muscle fibers shift anteriorly before fusing. Time-lapse movie of a *six1b:lyn-GFP* (fast muscle cells; green); *smyhc1:lyn-tdTomato* (slow muscle cells; blue) double transgenic embryo injected with mRNA encoding H2B-CFP (magenta; nuclei), beginning at 19 hpf. Cell tracking in a single z-plane reveals that some fast myoblasts migrate and/or elongate anteriorly within the somite before fusing to more posterior fast myoblasts. White dots indicate tracked nuclei. Images are taken every 5 minutes. Channels from this time-lapse were used for Figure 1 A-F'. Scale bar is 50 μm.

Movie 2: The plasma membrane breaks down and disperses during cell-cell fusion. Time-lapse movie of a *six1b:lyn-GFP* (fast muscle cells; green) and *smyhc1:lyn-tdTomato* (slow muscle cells; blue) double transgenic embryo injected with mRNA encoding H2B-CFP (magenta; nuclei), beginning at 20 hpf**.** Nuclei of fusing cells are tracked with white dots. At the fusion interface (arrowhead), the plasma membrane breaks down and disperses into the cytoplasm. Images are taken every 5 minutes. Stills from this time-lapse are shown in Figure 1 G-N. Scale bar is 25 μm.

Movie 3: Slow muscle migration coordinates fast muscle and anterior border cell morphogenesis. Time-lapse movie showing a single confocal section of a *six1b:lyn-GFP* (fast muscle cells; green); *smyhc1:lyn-tdTomato* (slow muscle cells; magenta) double transgenic embryo, beginning at 18 hpf. Embryo is oriented with anterior to left, lateral up. As slow muscle cells pass fast myoblasts, the fast myoblasts elongate and fuse together. Slow muscle cell migration also corresponds with lateral displacement of an anterior border cell (white dot), leading to its movement into the external cell layer. Images are taken every 3 minutes. Scale bar is 50 μm.