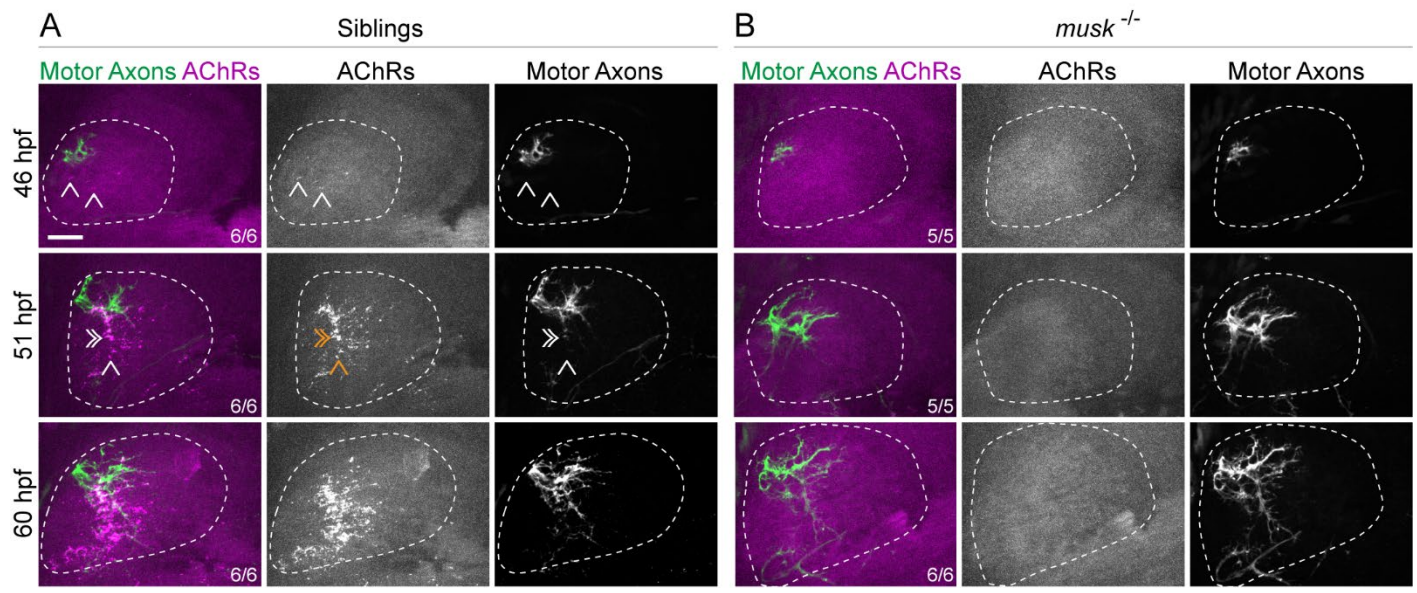


Fig. S1. Musk is required for pectoral fin prepatterning. Lateral view of maximum projections through pectoral fins of *Tg(mnx1:GFP)* larvae to label all motor neurons. Fins were stained with α-bungarotoxin to label acetylcholine receptors (AChRs). Dotted line outlines pectoral fin musculature. Single arrows point to aneural AChR clusters in siblings (A) that are absent in *musk* mutants (B). The double arrows at 51 hpf point to neural clusters. Brightness and contrast was individually adjusted across images so that background fluorescence was comparable but raw images were scored blinded. Scale bar is 25 microns.

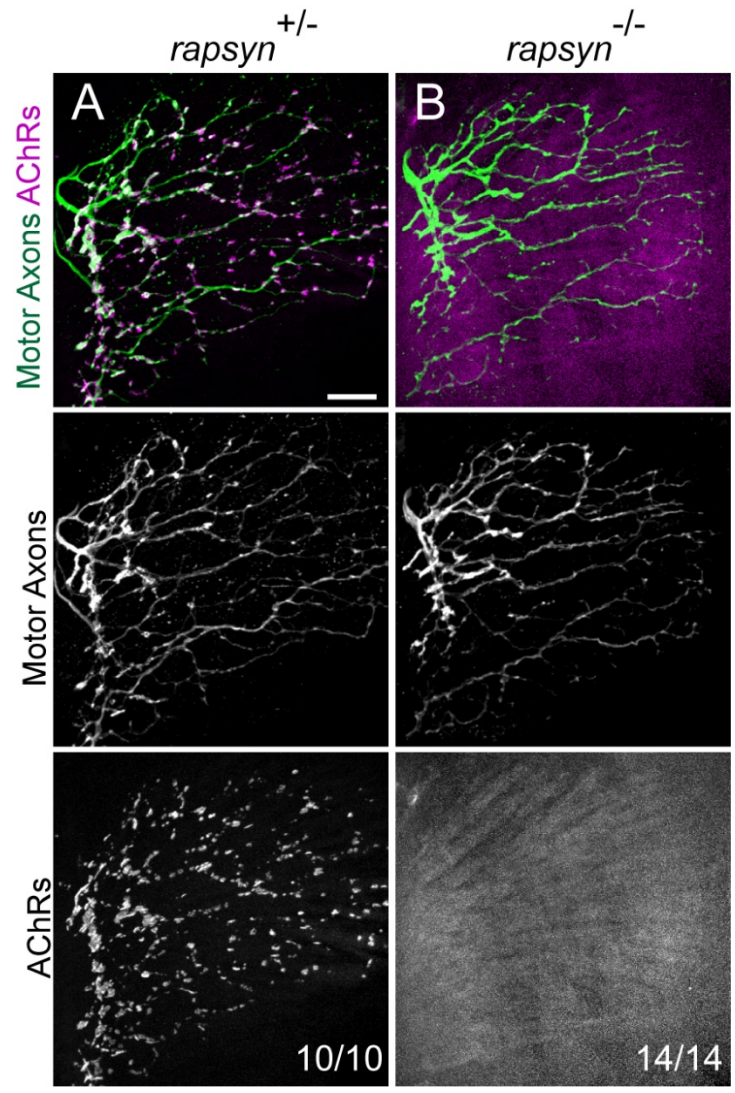


Fig. S2. Rapsyn is required for neuromuscular synapse formation. Abductor muscle innervation in the pectoral fin from 120 hours post fertilization *Tg*(*mnx1:GFP*) larvae expressing GFP to label motor neurons and stained with α-bungarotoxin to label acetylcholine receptors (AChRs). A) *rapsyn* sibling animals have numerous small AChR clusters while *rapsyn* mutants have diffuse AChR signal throughout muscle fibers in the fin. Images are maximum intensity projections. n = 10 (siblings), 14 (mutants). Scale bar is 25 microns.

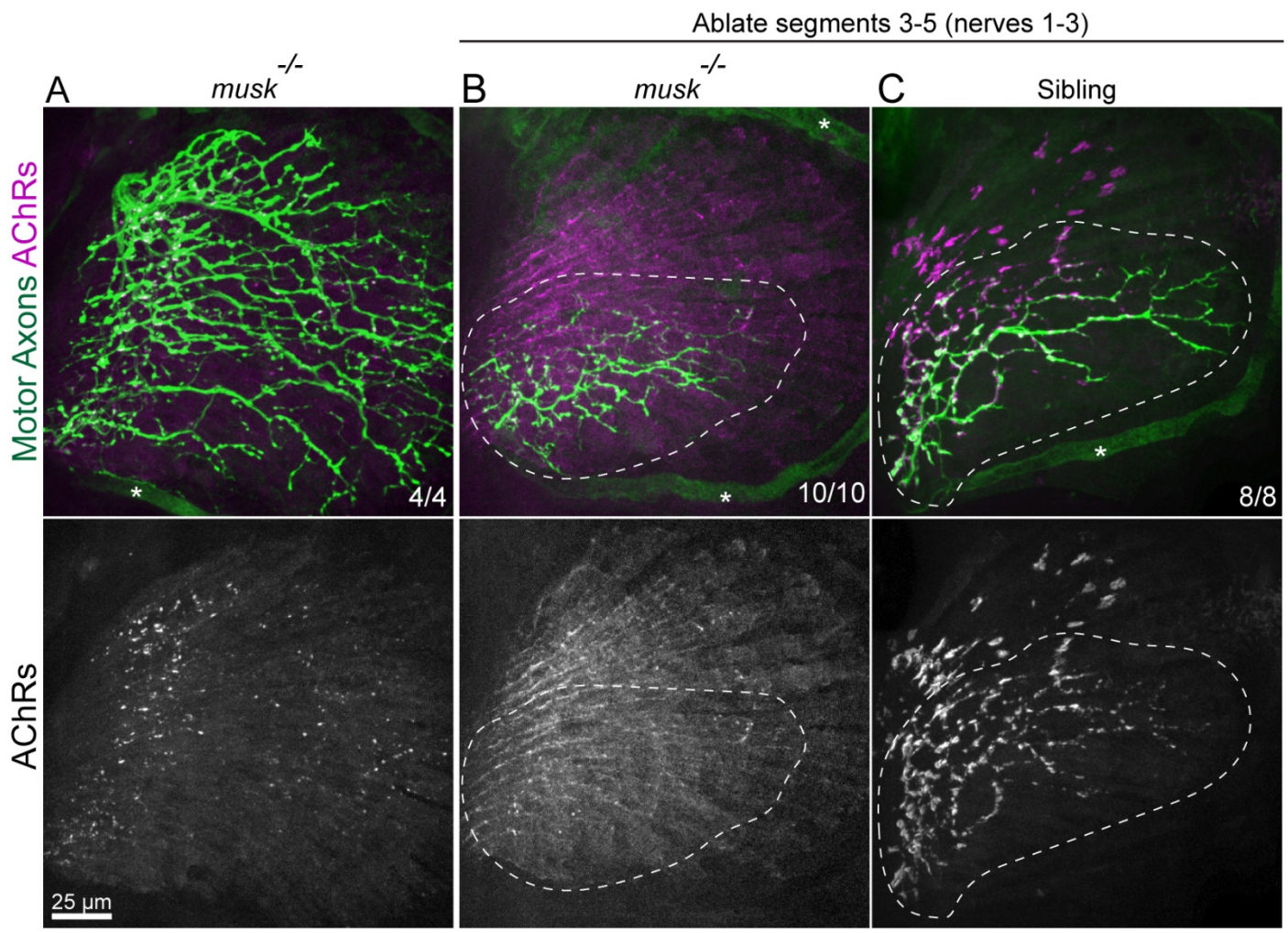


Fig. S3. MuSK is required to form large clusters in denervated pectoral fins. Spinal segments 3-5 were ablated in *Tg(mnx1:GFP)* embryos at 2, 3, and 4 dpf to prevent dorsal innervation of pectoral fins from nerves 1-3. At 5 dpf, both (A) unablated control *musk* mutant and (B) ablated *musk* mutant pectoral fins display diffuse AChR localization. C) In contrast, the non-innervated region of sibling pectoral fins displays abnormally large AChR clusters (see figure 4) while the innervated region exhibits small axon-induced AChR clusters. The innervated region in motoneuron-ablated fins is outlined in the white dotted line. The asterisks point to pectoral fin vasculature.

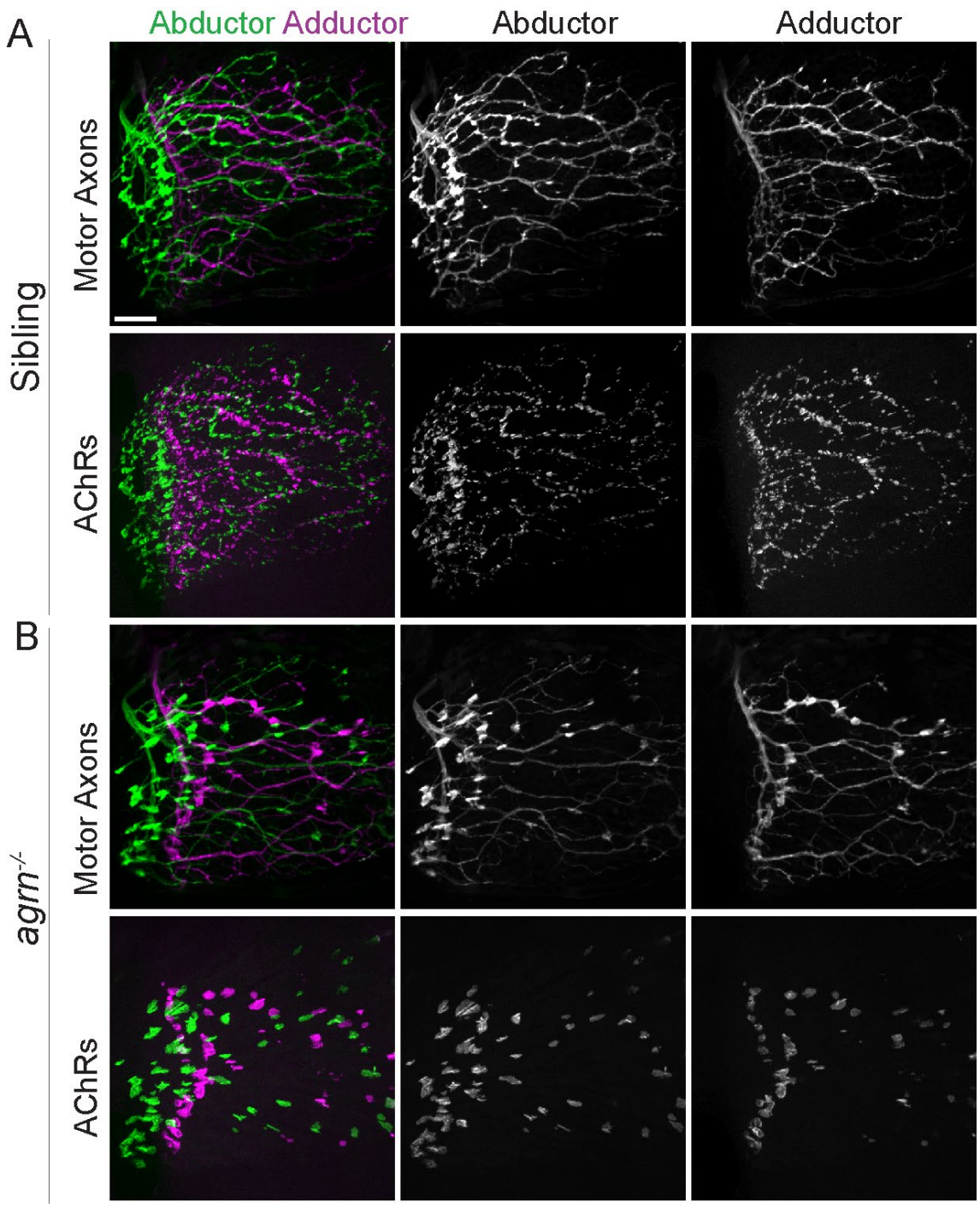
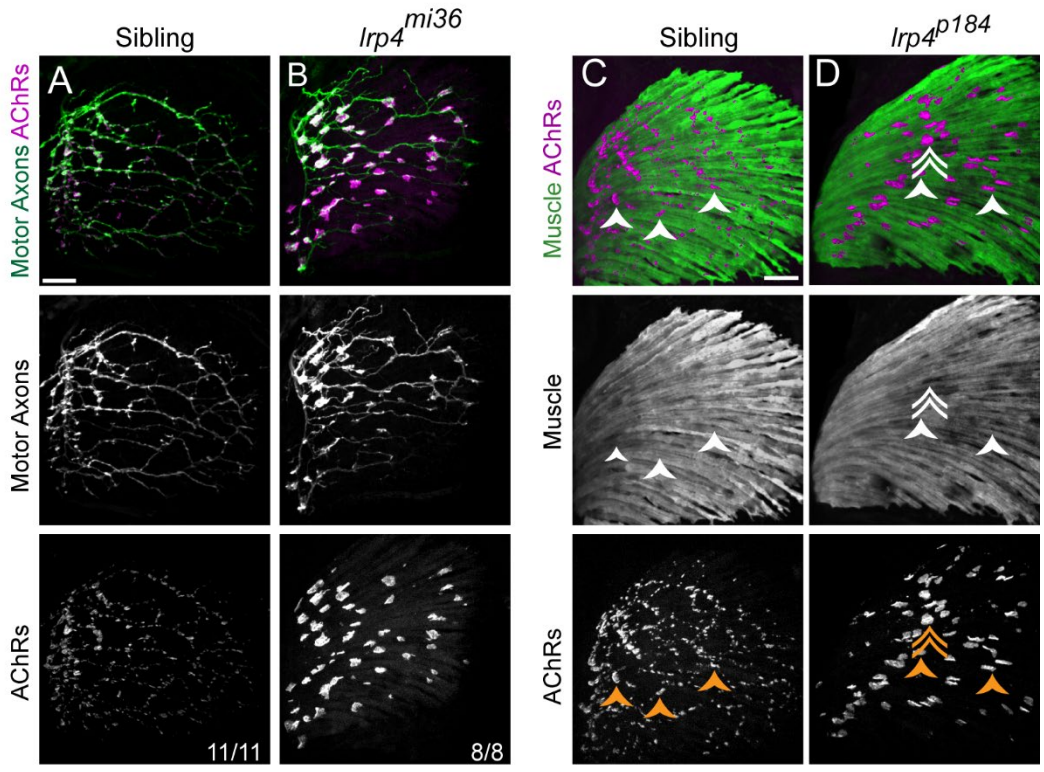


Fig. S4. Abductor and adductor muscles exhibit identical innervation phenotypes.
Tg(mnx1:GFP) pectoral fins stained with α-bungarotoxin to label acetylcholine receptors (AChRs) in siblings (A) or *agrn* mutants (B). Innervation pattern or α-bungarotoxin stain from abductor (green) or adductor (magenta) muscle pseudo-colored in merged maximum projection. Signal from each muscle is also shown individually. As the phenotype is similar between the abductor and adductor muscles, for most figures we have only shown the abductor innervation. N = 25 (siblings), 22 (mutants). Scale bar is 25 microns.



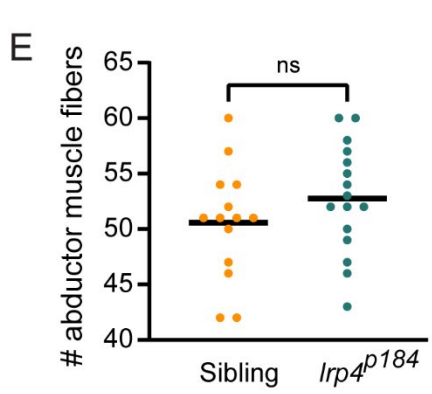


Fig. S5. Lrp4 acts through a ligand-dependent mechanism and muscles develop normally. Maximum projection of abductor innervation of 120 hours post fertilization *Tg(mnx1:GFP)* pectoral fins expressing GFP in all motor neurons and stained with α-bungarotoxin to label acetylcholine receptors (AChR). Compared with siblings (A), *lrp4*^{mi36} (ennui) mutants lacking the intracellular domain (B) have swellings in the motor neuron innervation pattern and enlarged AChR clusters, identical to presumptive null *lrp4*^{p184} mutants. Pectoral fin muscles labeled by *α-actin:GFP* are indistinguishable between siblings and *lrp4*^{p184} mutants. (C) AChR clusters labeled with α-bungarotoxin reveal hundreds of small clusters (arrowheads) distributed across muscle fibers in siblings. (D) In contrast, giant AChR clusters in *lrp4* mutants nestle between adjacent muscle fibers (arrowheads) or can span across multiple muscle fibers (double arrowhead). A minimum of 10 samples were screened per condition. Scale bars are 25 microns. (E) There is no difference in the number of abductor muscle fibers in *lrp4* mutants compared to sibling controls.

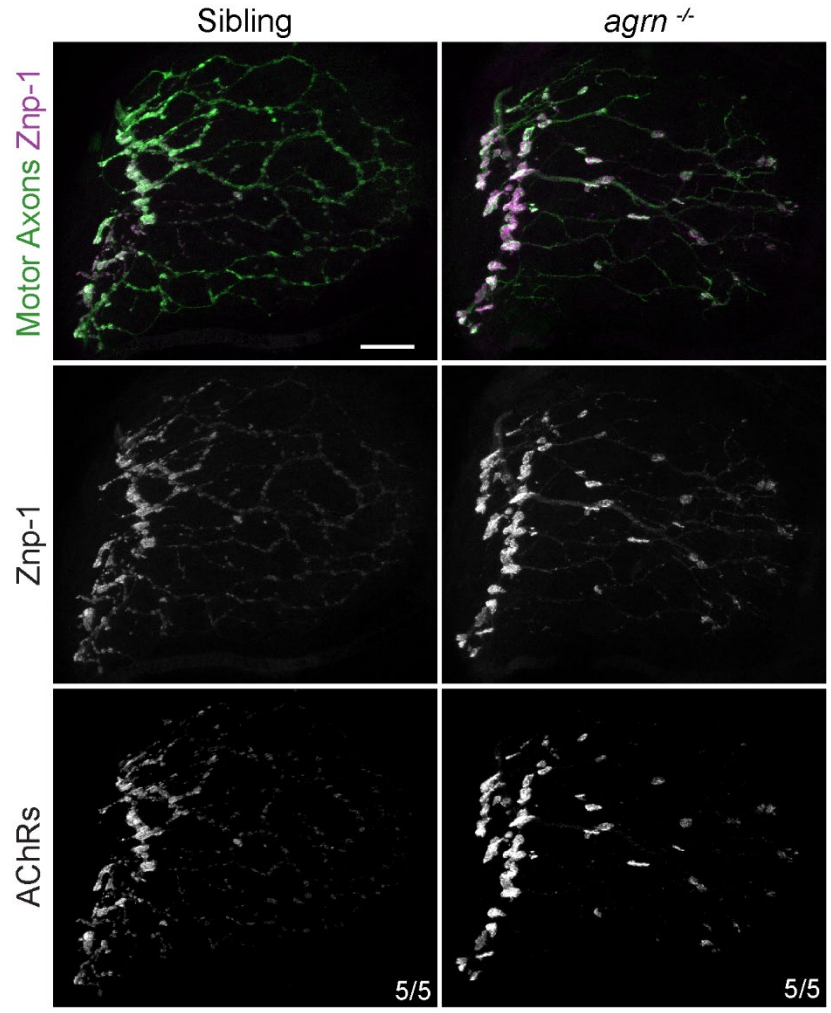


Fig. S6. Presynaptic marker Znp-1 colocalizes with AChR clusters in the pectoral fin. At 120 hours post fertilization, sibling control and *agrn* mutant pectoral fins exhibit postsynaptic AChR clusters labeled with α-bungarotoxin that colocalize with the presynaptic marker Znp-1, which labels Synaptotagmin 2. Motor axons were labeled with *mnx1:GFP*. Scale bar is 25 microns.

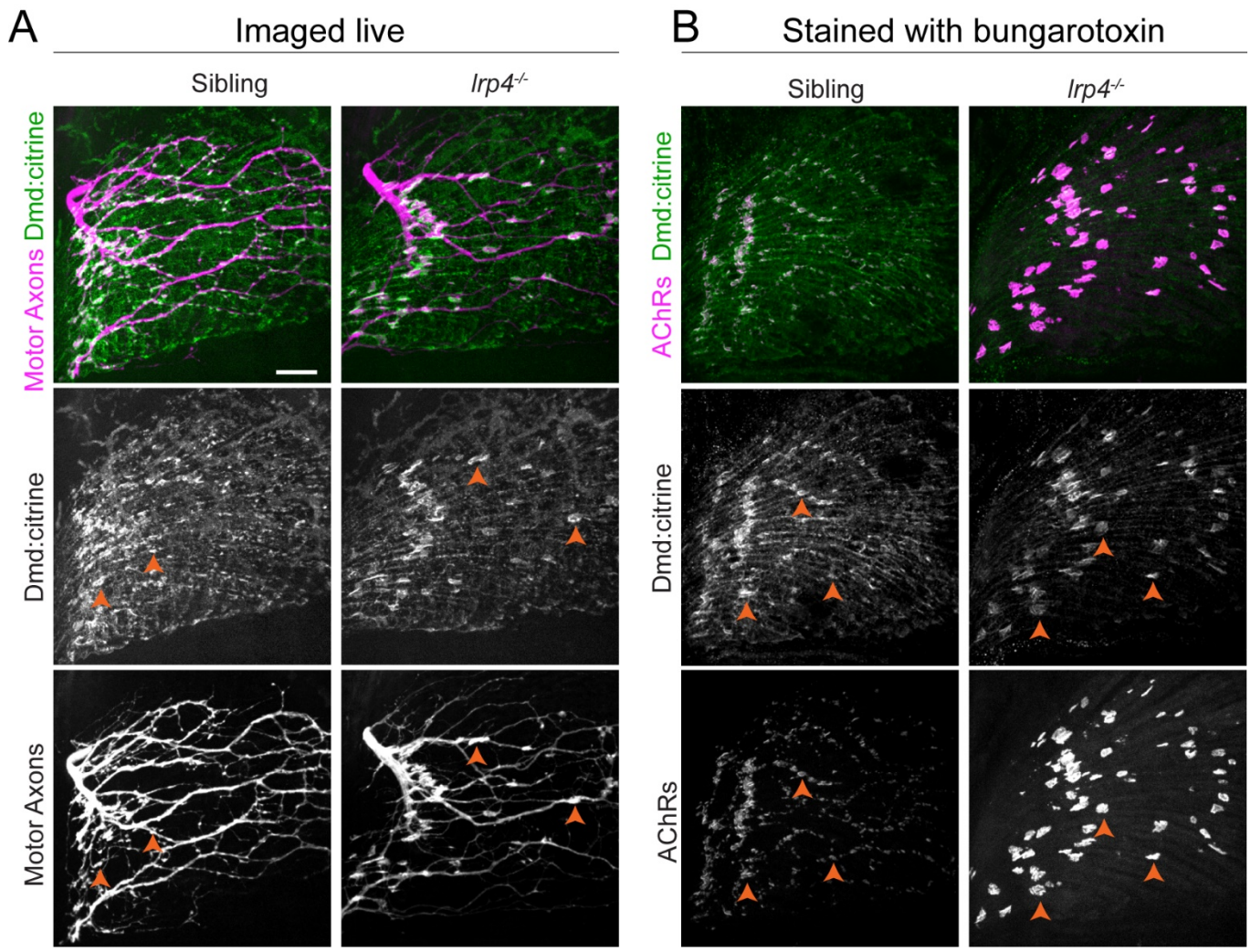


Fig. S7. Giant clusters co-localize with postsynaptic marker Dystrophin. Endogenous Dystrophin, labeled with *Gt(dmd-citrine)*, is expressed diffusely in the pectoral fin but concentrates between muscle fibers and at synaptic regions. A) Co-labeling with motor axons (labeled with *Tg(Xla.tubb:dsRed)* shows that concentrated Dmd-citrine co-localizes with axons in sibling controls and with presynaptic swellings in *lrp4* mutants. B) Dmd-citrine signal concentrates in regions marked with α-bungarotoxin to label acetylcholine receptors (AChRs) in both sibling controls and *lrp4* mutants. A minimum of 10 samples were screened per condition. Scale bar is 25 microns.

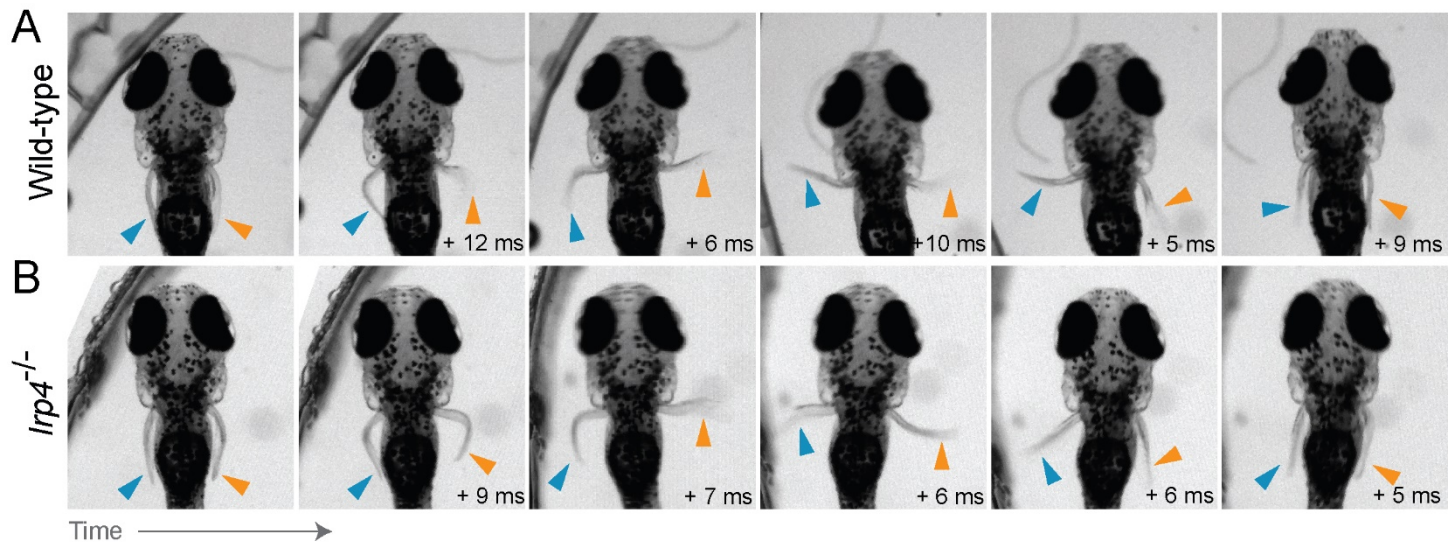


Fig. S8. Pectoral fin movements are grossly normal in *lrp4* mutants. High-speed imaging of pectoral fin movements in (A) wild-type (n=6) and (B) *lrp4* mutant (n=7) larvae at ~120 hpf. Despite their abnormal neuromuscular synapses, *lrp4* mutant pectoral fin movements are grossly normal compared to wild-type siblings. Movies were captured at 1000 frames per second. Time elapsed from the previous frame is reported in milliseconds (ms) in the bottom right of each still image. Blue arrows point to the left fin and orange arrows point to the right fin.

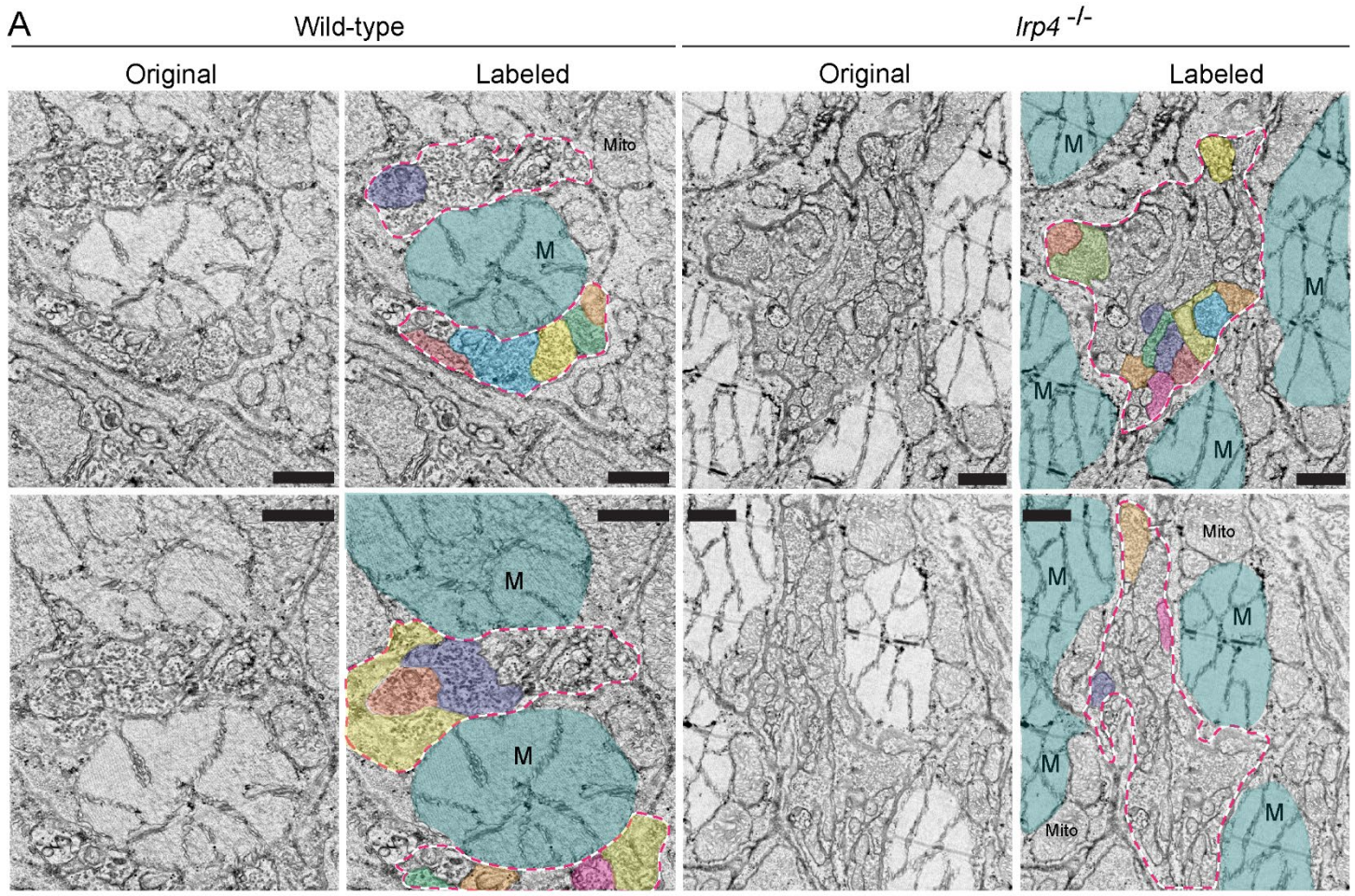


Fig. S9. Electron microscopy of pectoral fins.
A) Electron micrographs in wild-type and *lrp4* mutant pectoral fins at 5 dpf. Images are cross-sections across myofibrils. Original micrographs are shown to the left with the same micrograph pseudo-colored and labeled to the right. Some myofibrils (M) are pseudo-colored in teal and some mitochondria (Mito) are labeled. Regions with vesicle-positive axonal processes, as defined by structures with synaptic vesicles, are outlined in the pink dotted line and quantified in B. Individual vesicle-positive axonal processes are pseudo-colored in different colors when their borders could be defined. B) Quantification of the total area of each region with vesicle-positive axonal processes (pink outlined areas). The three *lrp4* mutant datapoints in magenta were cut off at the edge of the micrograph. Scale bars are 1 micron. N = 10 (wild-type) and 11 (*lrp4* mutant) regions with vesicle-positive axonal processes..

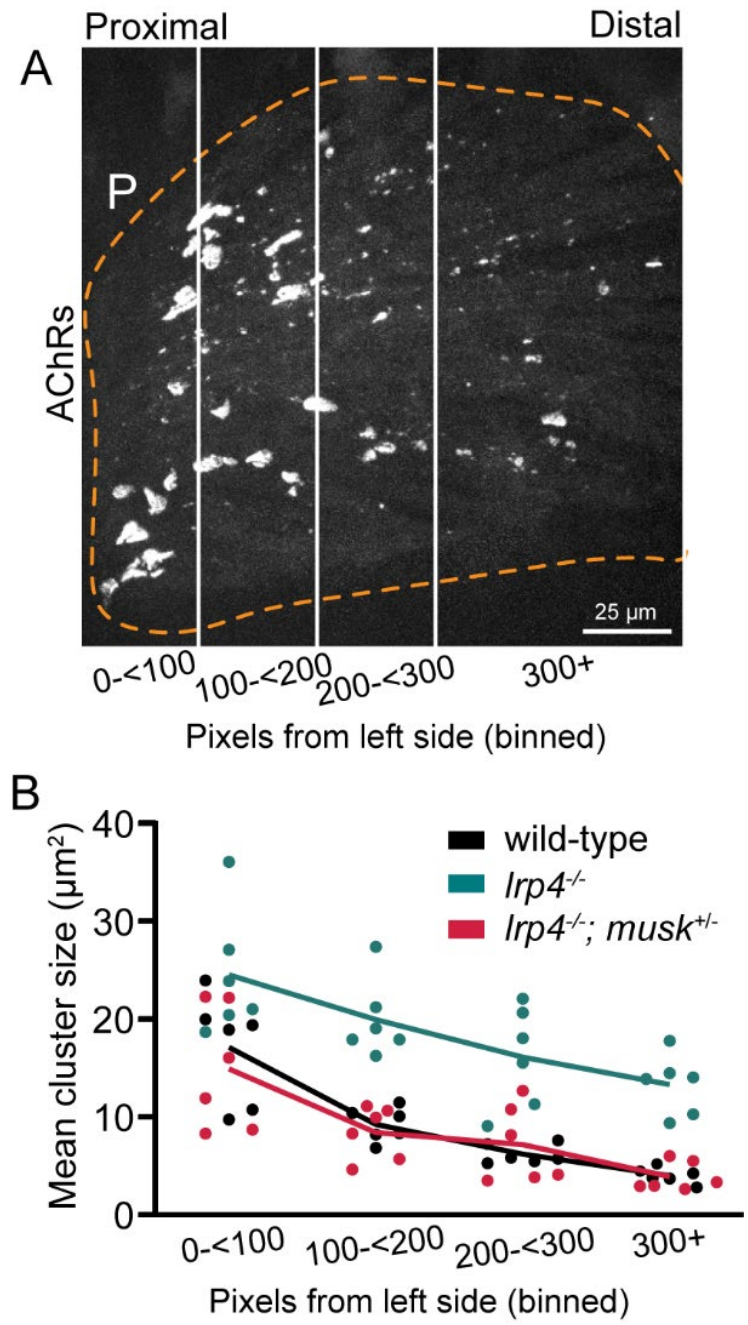
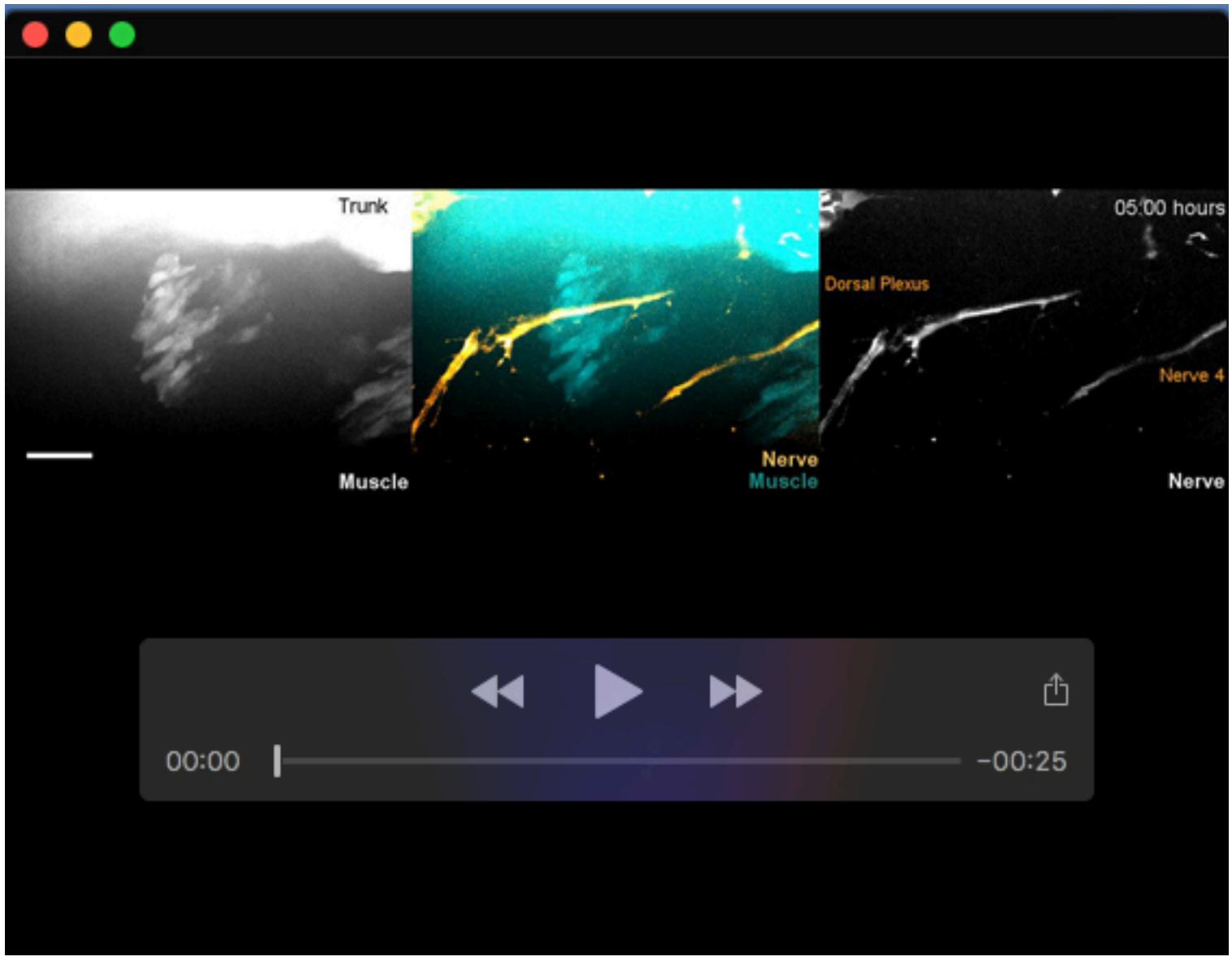


Fig. S10. Smaller AChR clusters localize to the distal tip of pectoral fins. A) Pectoral fin AChR clusters from Figure 7 were binned according to their position relative to the left side of images as shown in the example fin (from Figure 7D). The location of the dorsal plexus is marked with P. B) The mean area of individual clusters within each bin were plotted relative to their position. Each point per bin represents a different fin. Like wild-type pectoral fins, the largest AChR clusters in *lrp4* mutants that are heterozygous for *musk* (*lrp4*$^{-/-}$;*musk*$^{+/-}$) are found in the proximal fin base while smaller clusters localize to the distal fin.



Movie 1. Timelapse of pectoral fin development. Live imaging of *Tg(α-actin:GFP); Tg(Xla.Tubb:DsRed)* larvae to label muscles and axons, respectively. By approximately prim-25 (36 hpf; start of movie), motor axons from nerves 1-3 coalesce at what will form the dorsal plexus and nascent muscle fibers in the pectoral fin bud, located laterally to the axons, have just started expressing *α-actin*:*GFP.* Muscle fibers continue to divide and reorganize through the long-pec stage as the fin moves further medial, closer to the plane of the dorsal plexus, and motor axons begin to grow into the abductor and then adductor muscles beginning around the long-pec stage (approximately 13 hours in movie). Concurrently, axons in nerve 4 make a sharp turn dorsally to innervate the fin via the ventral plexus. Thick axon bundles first grow perpendicular to muscle fibers near the proximal fin base, but subsequently axons turn posteriorly to grow mostly parallel to muscle fibers and towards the fin tip. As muscle fibers elongate, branching motor axons follow close behind to form a diffuse innervation network. At the end of the movie (after approximately 37 hours), a simplified innervation pattern is established. Frames are maximum projections through the developing pectoral fin at 30 minute increments. Stills from this timelapse were used in figure 2B. The movie plays at 4 frames per second. Scale bar is 25 microns.