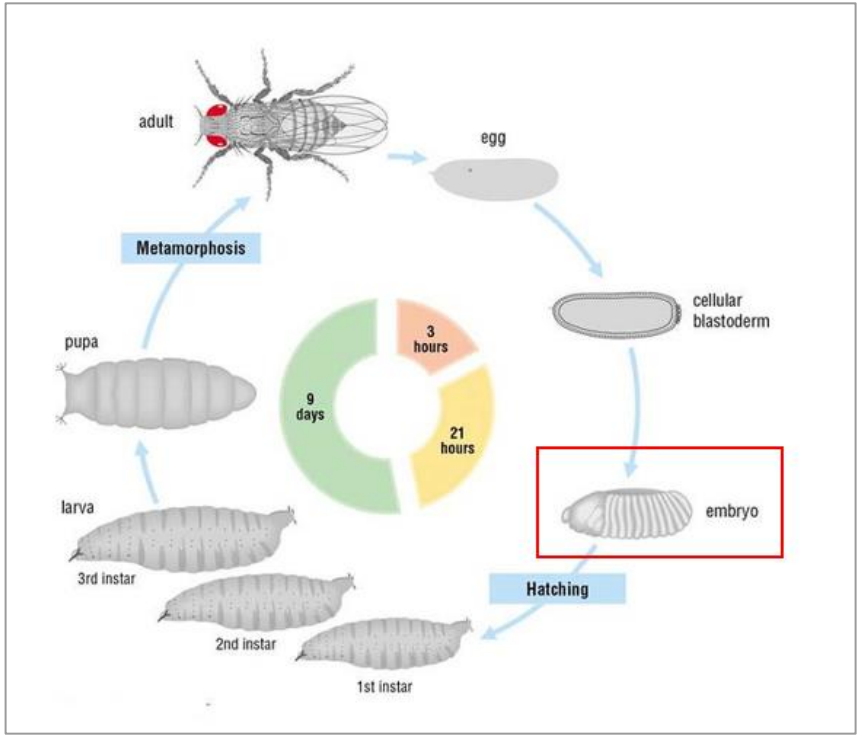
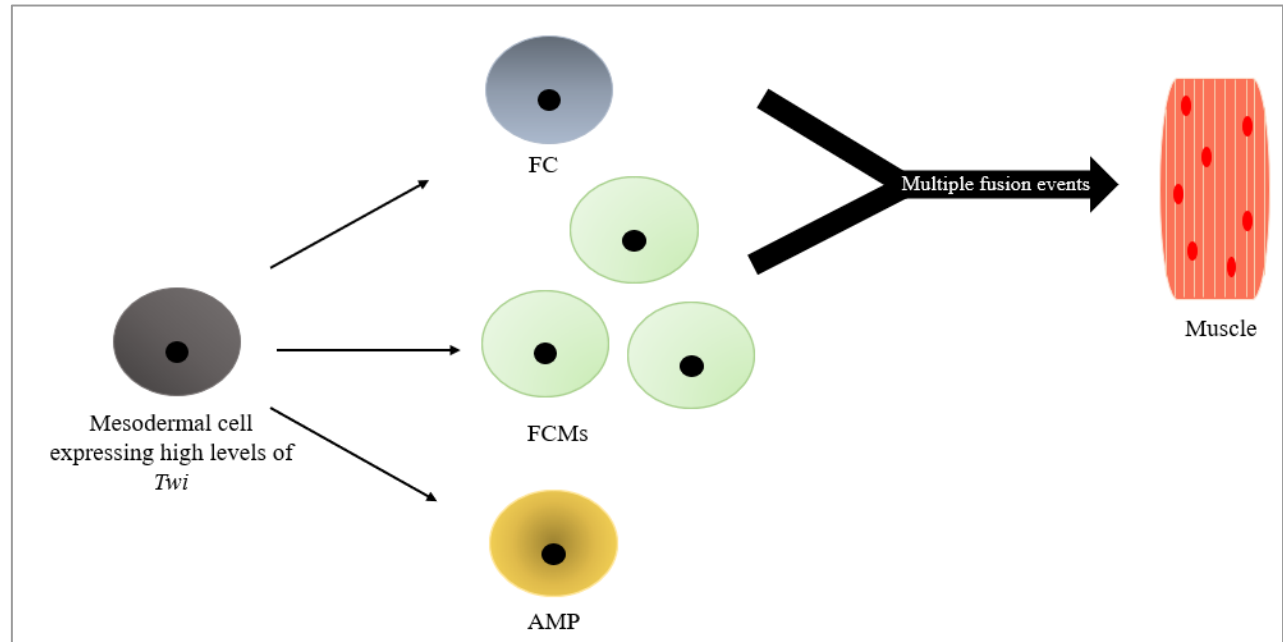


Introduction



Drosophila life cycle



Formation of muscle from fusion of FC and FCMs

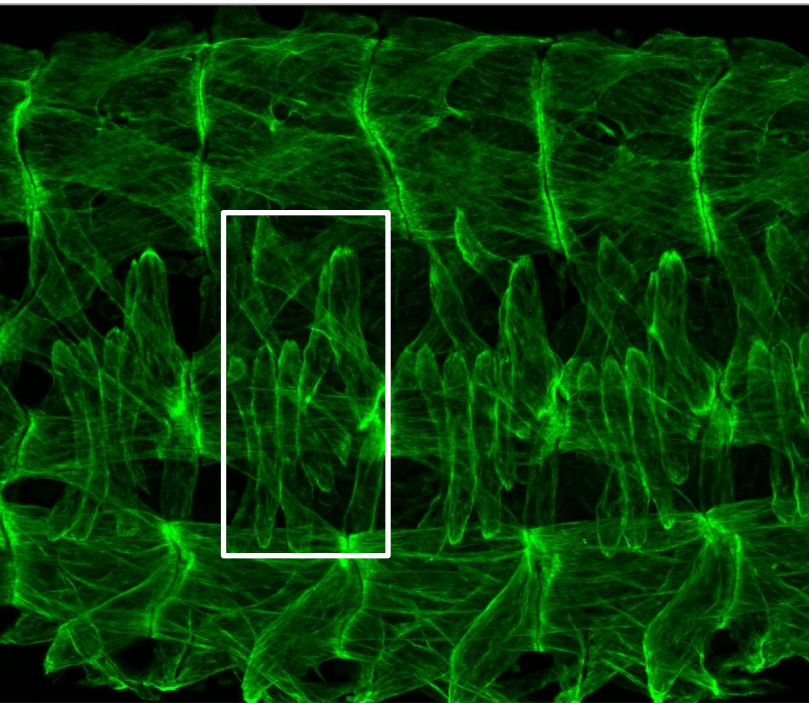
Larval Somatic Muscles

- Develop during embryogenesis¹
- Identity genes transcription factors specify muscle properties^{1,2}
- Unique characteristics such as size, shape, attachment sites, and innervation patterns by motor neurons¹

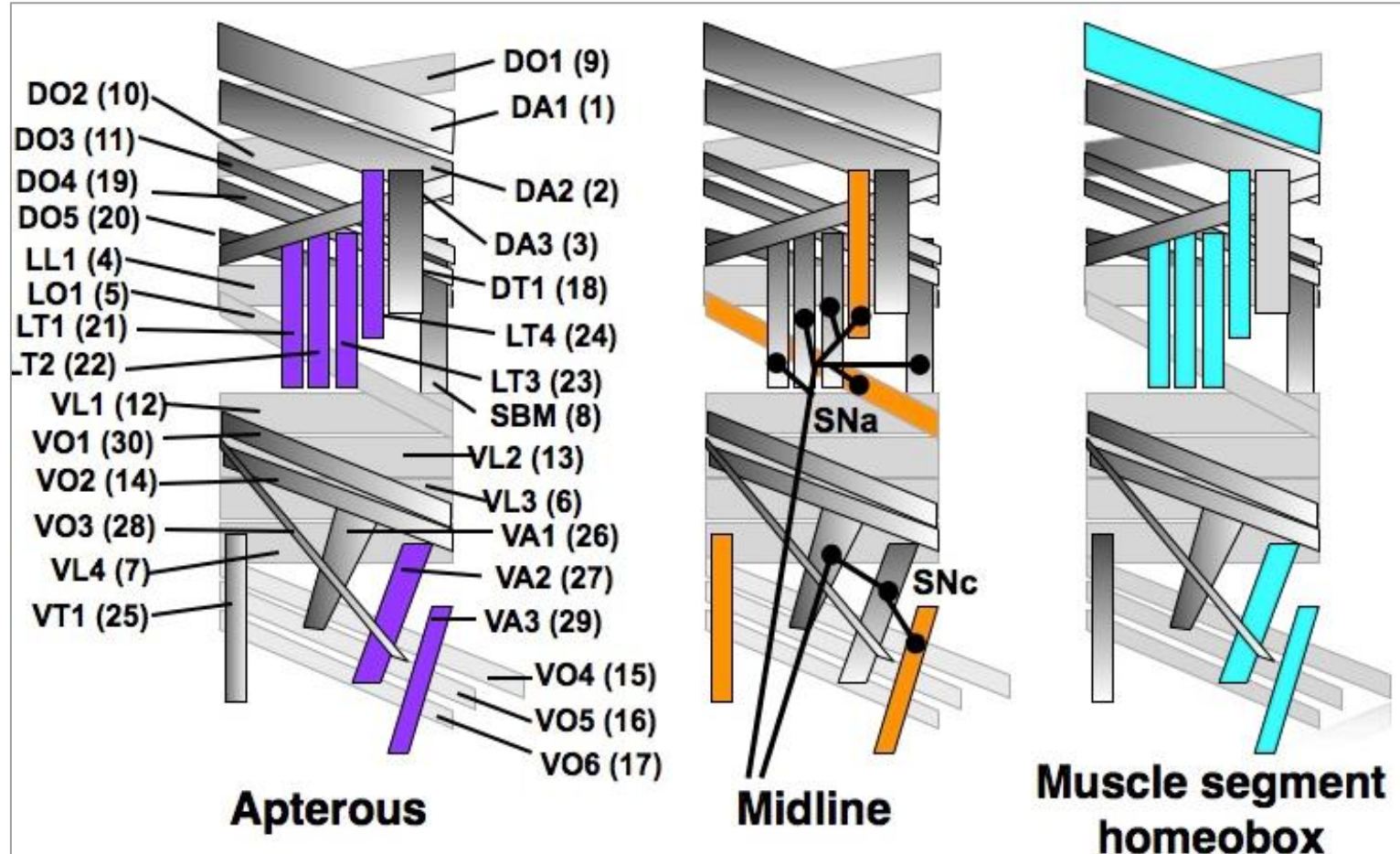
Motor Neuron Innervation

- Three types of myoblasts³
 - Founder cells (FCs) regulate muscle formation AND defasciculation of motor axons⁴
 - Fusion-competent myoblasts (FCMs)
 - Adult muscle precursors (AMPs)
- Motor axons exit central nervous system via Segmental nerve (SN), Intersegmental nerve (ISN), and Transverse nerve (TN)⁵

Focus: Motor neuron innervation by SNa in abdominal muscles specifically lateral transverse (LTs) muscles (shown in white box)



Abdominal muscles in *wild-type* embryo with each hemisegment made up of four LTs



Expression of muscle identity transcription factors

Methodology

Genetics

- Well-characterized loss-of-function mutants; heterozygotes excluded using marked balancer chromosomes

Embryo Collection and Fixing

- Embryos collected on apple juice agar plates
- Dechorionated in bleach
- Fixed in 4% paraformaldehyde and heptane
- Devitellinized using heptane and methanol

Staining

- Antibodies
 - Muscle: myosin heavy chain and tropomyosin
 - Motor neurons: FITC-HRP and Fas-II

Imaging

- Zeiss LSM880 Confocal Plan Apochromat 40X/1.2 NA
- Images processed Fiji/Image J

Results

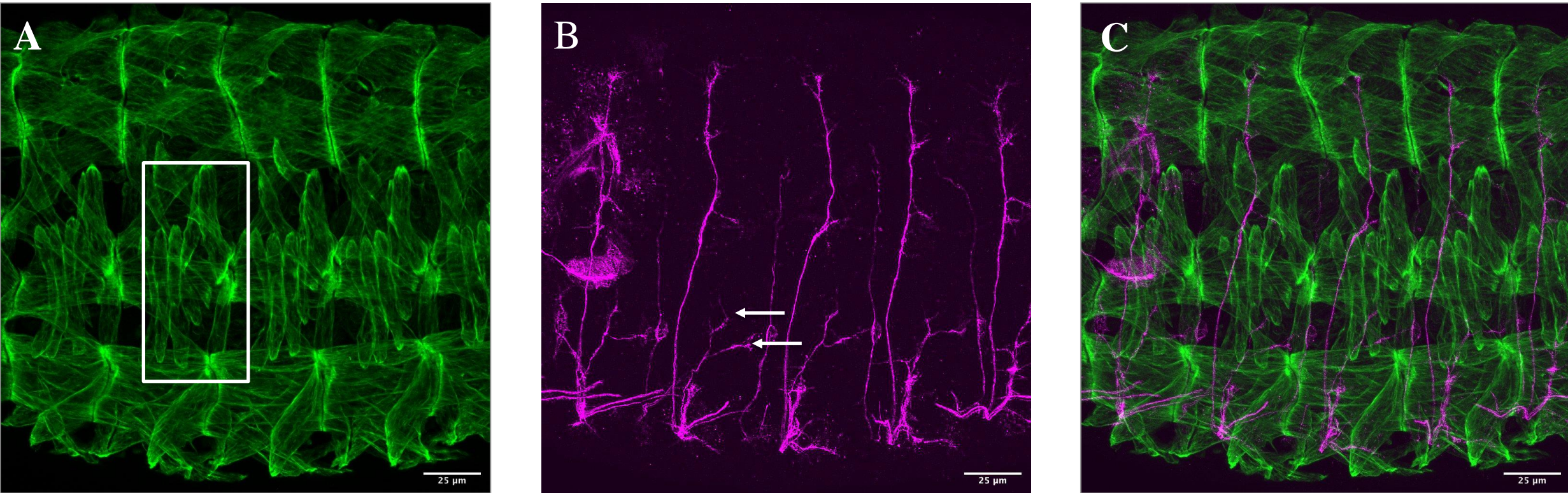
OreR and loss-of-function mutants stained with tropomyosin and Fas-II

Tropomyosin muscle pattern

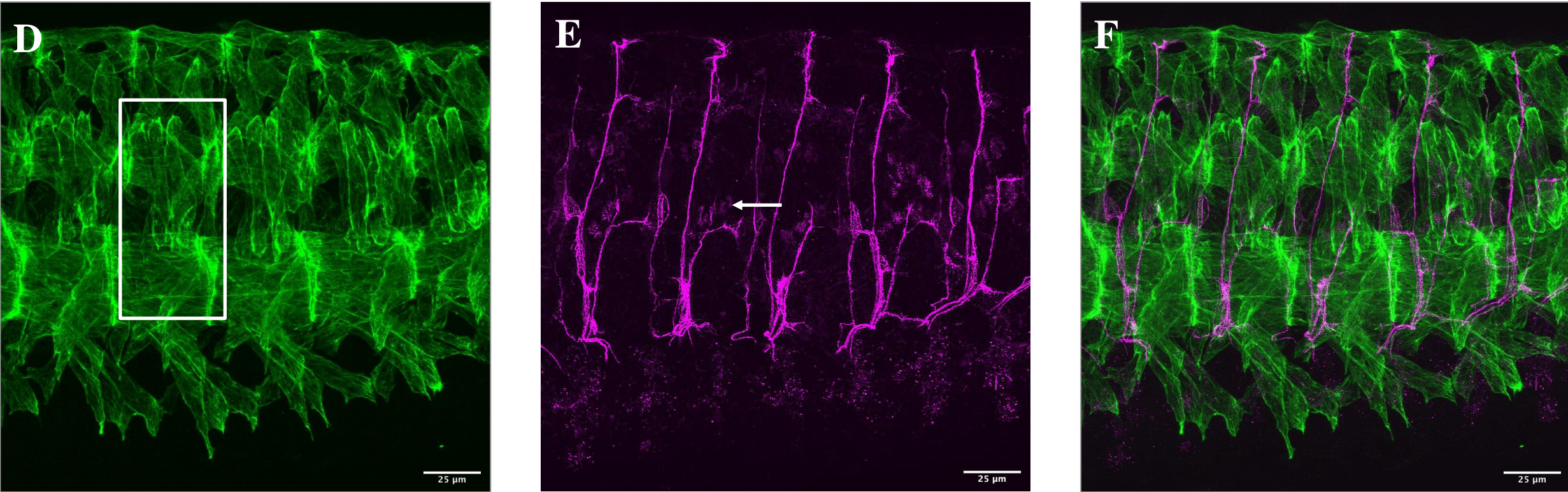
Fas-II motor neurons

Merged

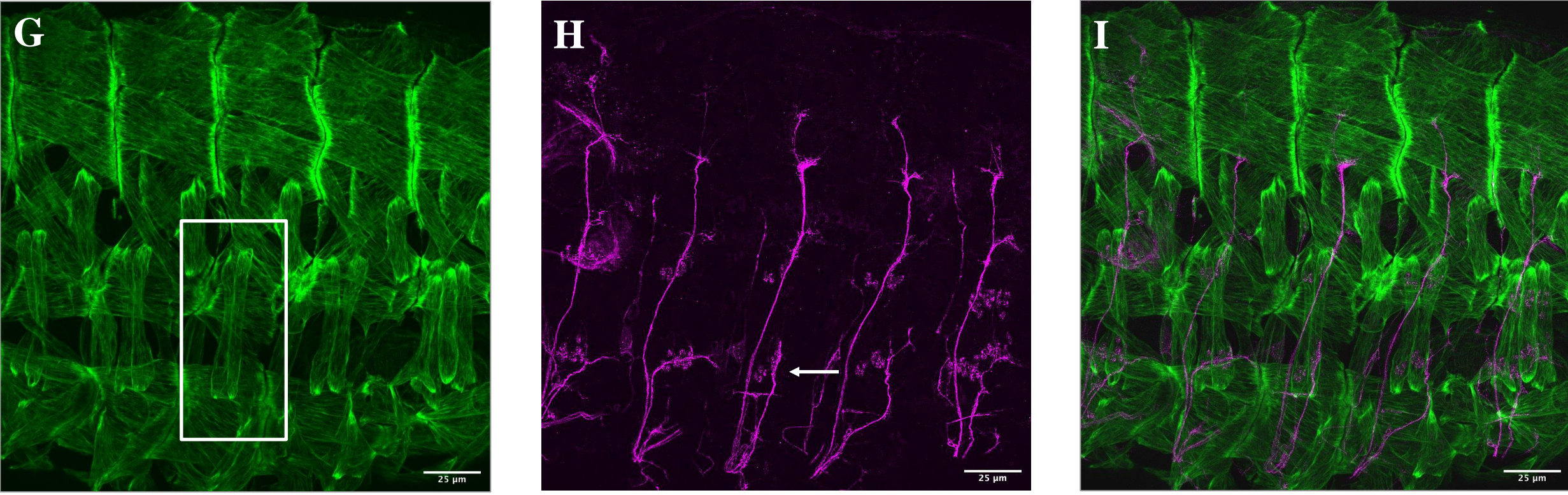
OreR



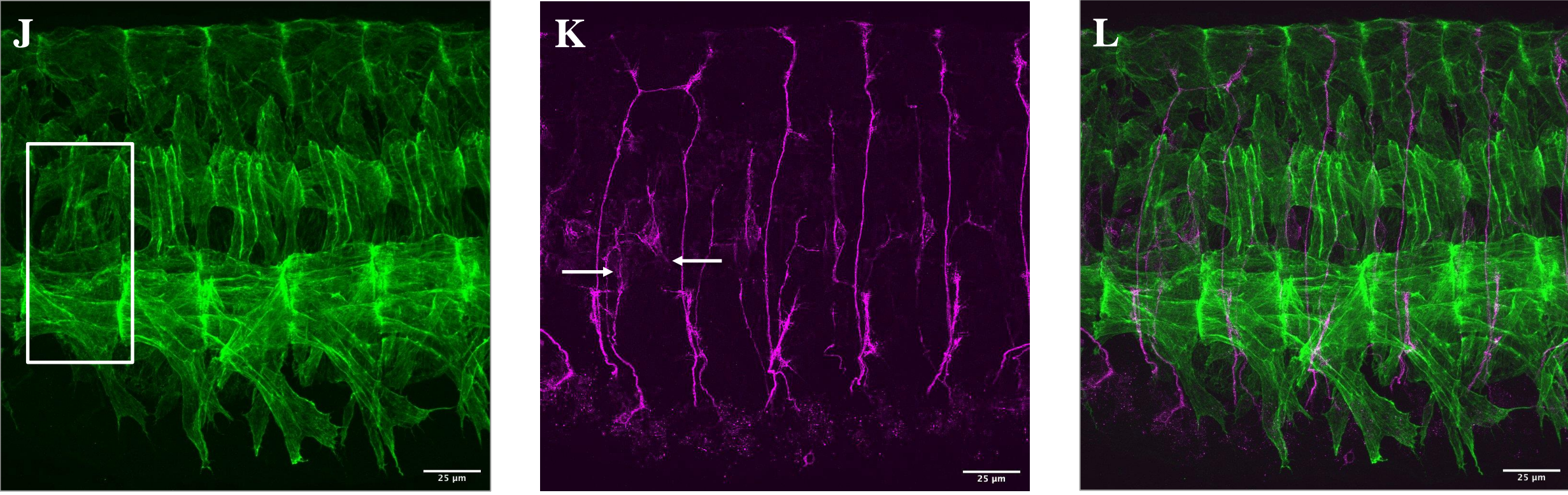
ap^{UGO35}



mid^l



mshΔ68-lacZ



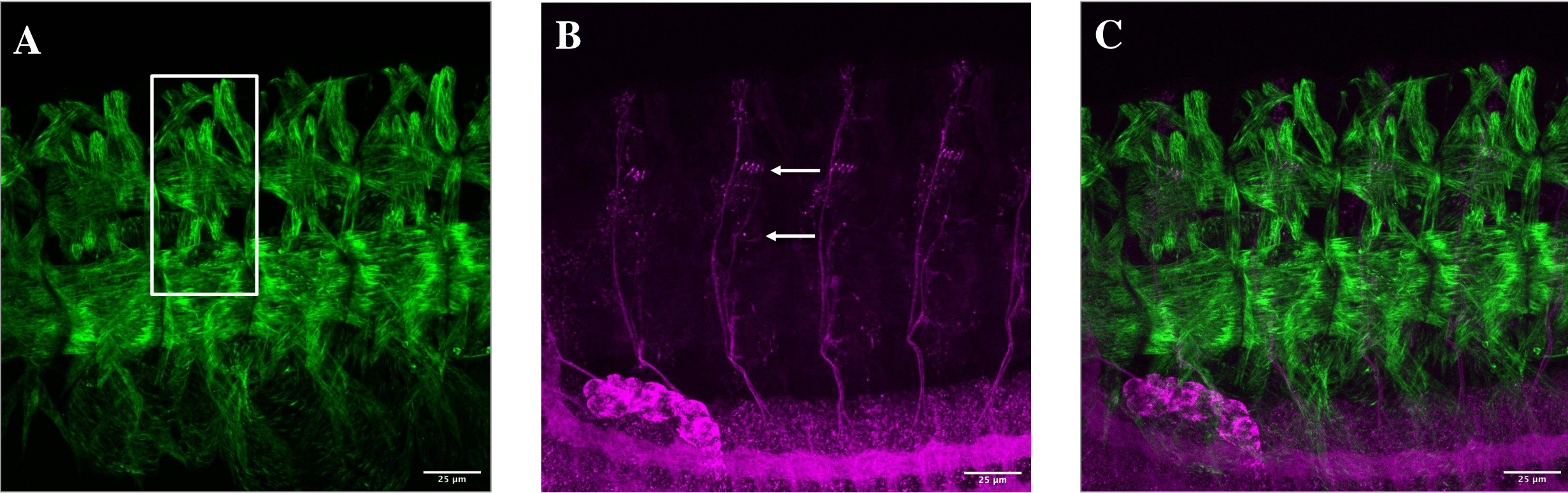
OreR and *ap^{UGO35}* stained with myosin heavy chain and FITC-HRP

MHC muscle pattern

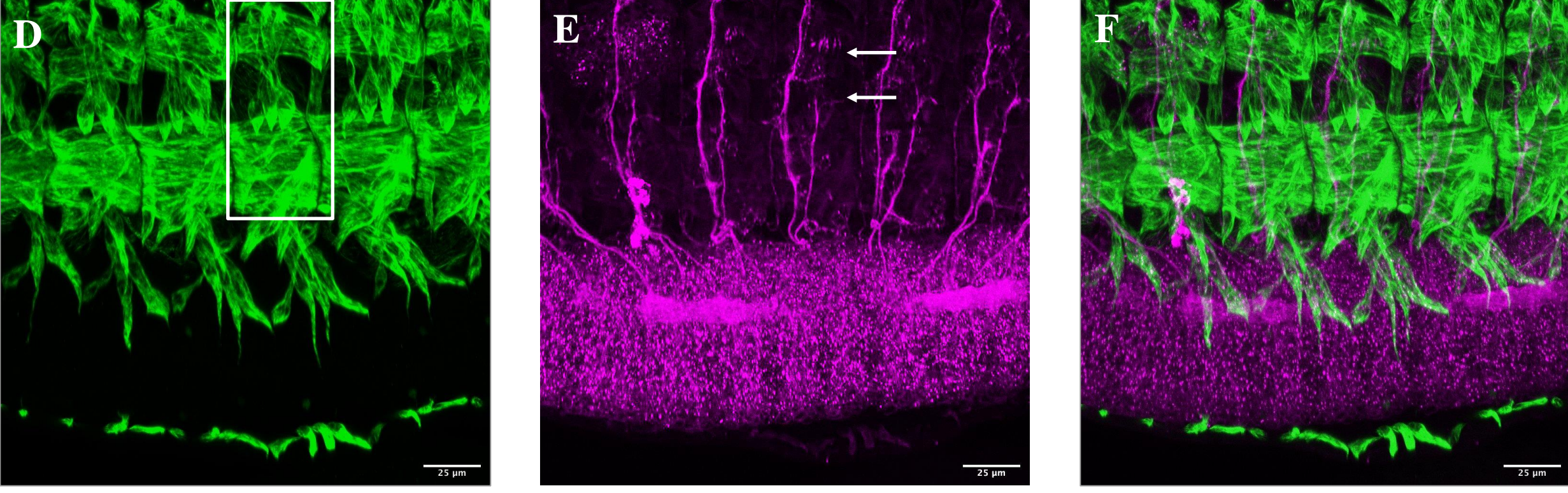
FITC-HRP motor neurons

Merged

OreR



ap^{UGO35}



Discussion

Visualization with Fas-II

- *OreR* shows four LTs, corresponding with proper defasciculation of SNa axons
- All mutants lack one or more of the LTs or develop abnormally shaped LTs
- Defects in LTs correspond to disruption in proper neuron innervation

Visualization with FITC-HRP

- Four distinct LTs in *OreR*, with proper nerve branching
- Three LTs or LTs incorrectly attached together at center in some hemisegments of *ap^{UGO35}*

Summary: Mutant genotypes with implications in muscle patterning also show disruption in neuron innervation

Future Directions

Genotypes

- Collect and observe more genotypes
- Gain-of-function mutants (Gal4/UAS system)

Antibodies

- Use different antibodies: Horse-radish peroxidase (HRP), Fasciclin III, Islet-1 and connectin

Staining

- Live imaging

References

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2. Schulman VK, Dobi KC, Baylies MK. Morphogenesis of the somatic musculature in *Drosophila melanogaster*. *WIREs Dev Biol* 2015. doi: 10.1002/wdev.180
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4. Landgraf M, Baylies M, Bate M. Muscle founder cells regulate defasciculation and targeting of motor axons in the *Drosophila* embryo. *Current Biology* 1999; 9:589-592
5. Landgraf M, Bossing T, Technau GM, Bate M. The origin, location, and projections of the embryonic abdominal motoneurons of *Drosophila*. *J Neurosci*. 1997;17(24):9642-9655. doi:10.1523/JNEUROSCI.17-24-09642.1997

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