Supplemental Figures

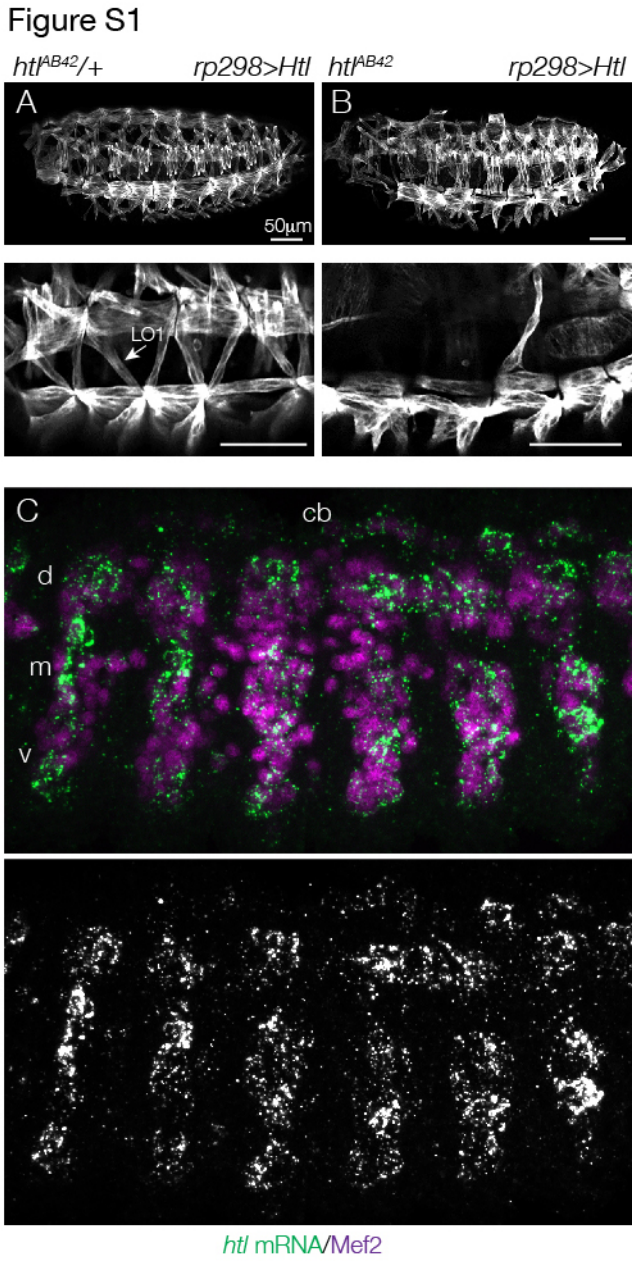

Figure S1. LO1 muscle morphogenesis is distinct from VL1 morphogenesis. (A,B) St16 *rp298>Htl* embryos labeled for Tropomyosin. Compared to controls (A), *htl*^{AB42} embryos that expressed Htl in all nascent myotubes showed LO1 muscle morphogenesis defects (B). A number of muscles in St16 *htl*^{AB42} *rp298>Htl* embryos were also absent. (C) St12 embryo labeled for *htl* mRNA (green) and Mef2 (violet). *htl* is strongly expressed in myoblasts, but there does not appear to be consistent variation in *htl* expression among the dorsal (d), medial (m), and ventral (v) myoblast populations.

Figure S2

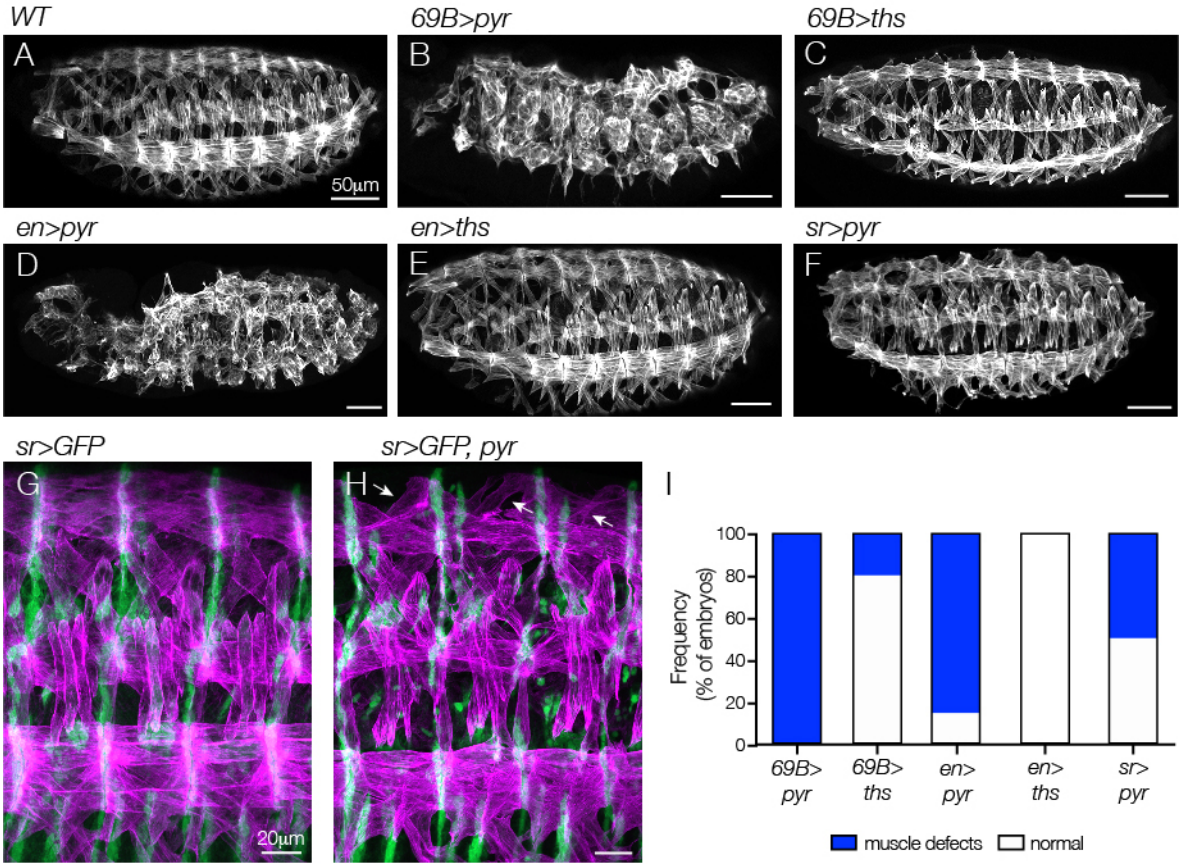

Figure S2. Pyr expression outside tendon cells disrupts muscle patterning. (A-F) St16 embryos labeled for Tropomyosin. Embryos that expressed Pyr broadly in the ectoderm (B,D) showed severe muscle phenotypes; embryos that expressed Ths showed largely normal muscle patterning (C,E). Embryos that expressed Pyr in tendon cells showed mild defects in muscle morphogenesis (F). (G,H) *sr>GFP* embryos labeled for GFP (green) and Tropomyosin (violet). Embryos that expressed Pyr in tendon cells showed largely normal muscle attachments, although DA1 and DO1 muscles often made incorrect muscle attachments (arrows). (I) Histogram of muscle morphology. Percent was calculated as the number of embryos showing any muscle phenotype divided by the total number of embryos imaged (n≥8 embryos per genotype).

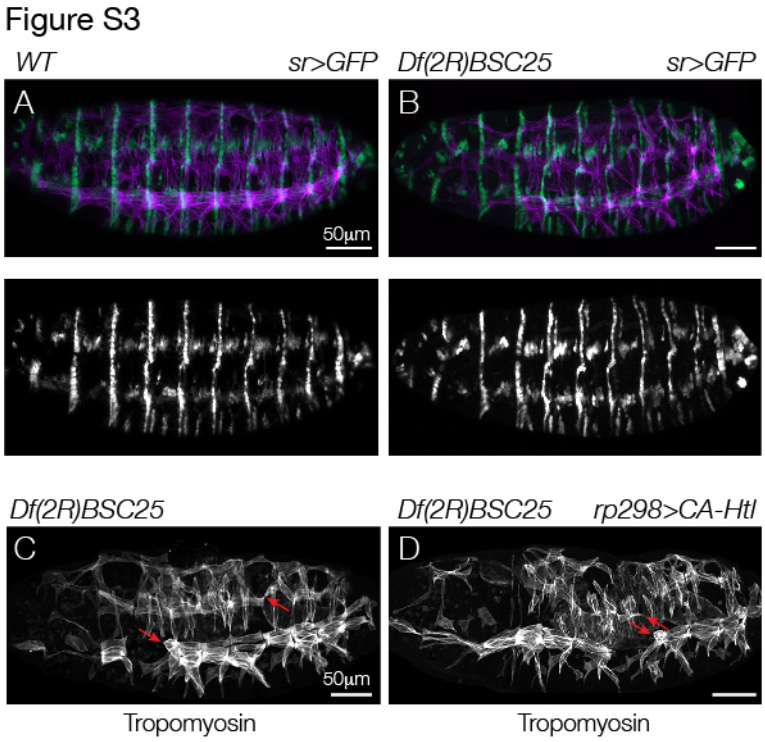

Figure S3. FGF signaling appears to be instructive. (A,B) St16 *sr>eGFP* embryos labeled for GFP (green) and Tropomyosin (violet). Tendon cells (marked by *sr>eGFP*) were correctly specified and maintained in *Df(2R)BSC25* embryos. (C,D) St16 embryos labeled for Tropomyosin. *Df(2R)BSC25* embryos that constitutively active Htl in nascent myotubes did not show an appreciable improvement in muscle morphology.

Figure S4

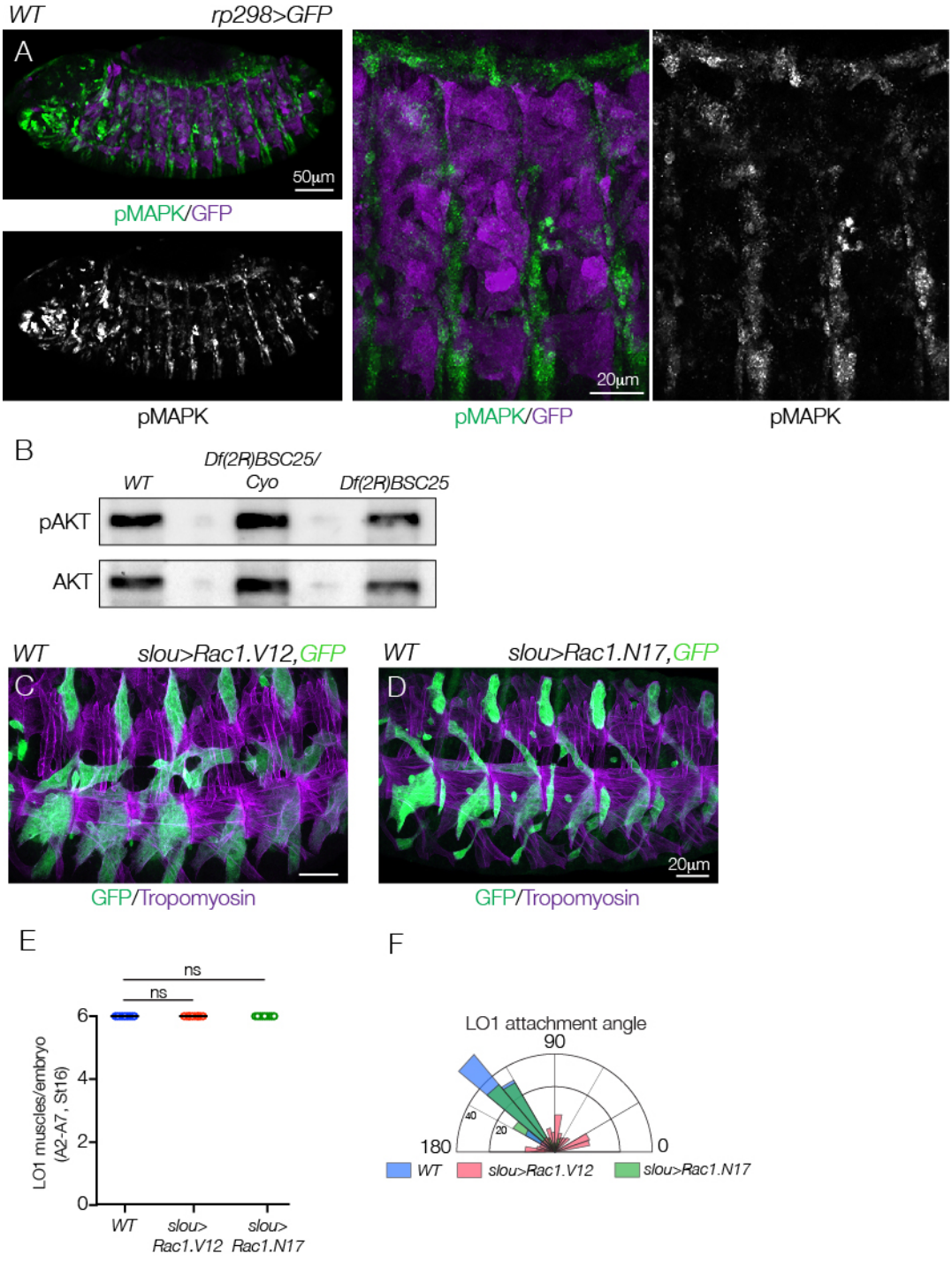

Figure S4. Constitutively active Rac1 induces myotube guidance defects. (A) St12 *rp298>eGFP* embryo labeled for pMAPK (green) and GFP (violet). Nascent myotubes did not show significant accumulation of pMAPK. (B) pAKT Western blot of St12 embryo lysates. pAKT levels were unchanged in *Df(2R)BSC25* embryo lysates compared to controls. (C,D) St16 *slou>eGFP* embryos labeled for GFP (green) and Tropomyosin (violet). LO1 myotubes that expressed constitutively active Rac1 (Rac1.V12, C) showed severe guidance defects. LO1 myotubes that expressed dominant-negative Rac1 (Rac1.N17, D) showed normal morphology. (E) Number of LO1 muscles in St16 embryos [(ns) not significant; error bars represent SEM]. (F) Radial density plot of LO1 muscle attachment angles in St16 embryos ($n \geq 34$ muscles per genotype). See Fig. 3 legend for details.

Figure S5

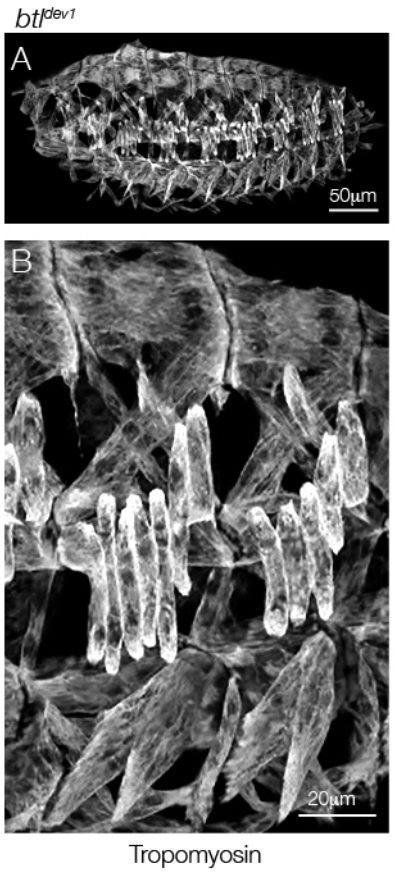

Figure S5. *btl* embryos show largely normal muscle patterning. St16 *btl*^{dev1} embryo labeled for Tropomyosin shown at low (A) and high (B) magnification.

Table S1. FACS-seq results.

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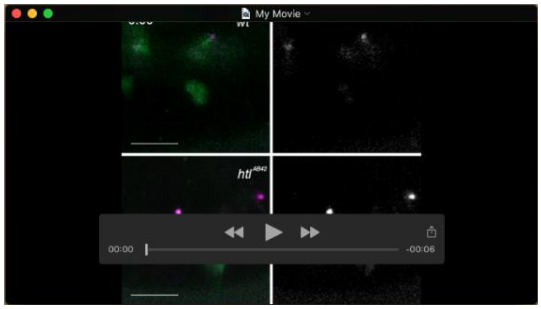
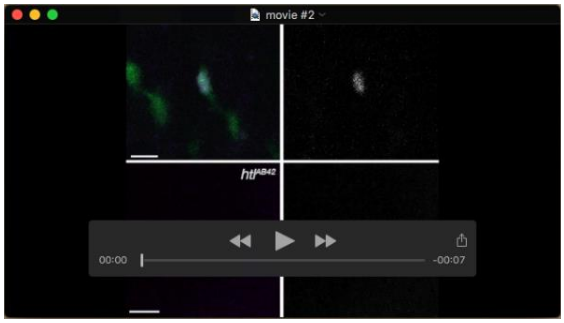
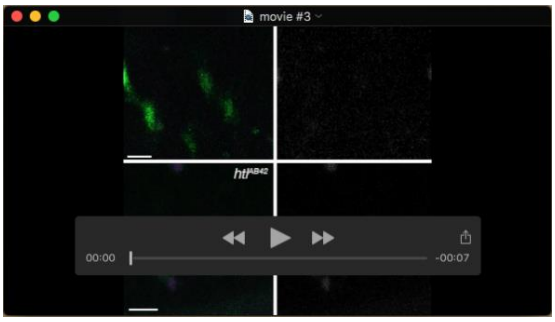


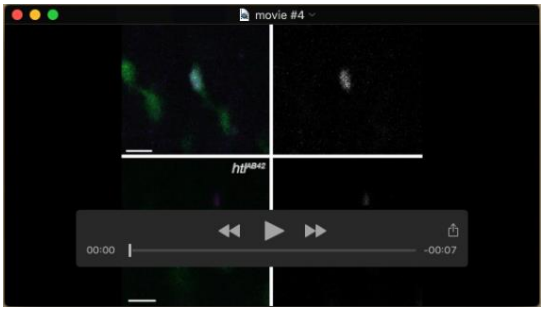
Movie 1. Htl regulates VL1 myotube guidance. Stage 12 *5053>eGFP,nRFP* embryos were live imaged at 5 min intervals to visualize VL1 muscle development. Rounded VL1 muscles in *htl* embryos often failed to elongate across the segment.



Movie 2. Htl regulates LO1 myotube guidance. Stage 12 *slou>eGFP,nRFP* embryos were live imaged at 5 min intervals to visualize LO1 muscle development. LO1 muscles in *htl* embryos occasionally fail to turn toward the anterior.



Movie 3. *Htl* myotubes that reach an incorrect attachment site migrate towards alternative attachment sites. Stage 12 *slou>eGFP,nRFP* embryos were live imaged at 5 min intervals to visualize LO1 muscle development. This *htl* LO1 muscle elongated to the posterior segment border, and then migrated dorsally.



Movie 4. A second example of *htl* myotubes migrating towards alternative attachment sites. Stage 12 *slou>eGFP,nRFP* embryos were live imaged at 5 min intervals to visualize LO1 muscle development. This *htl* LO1 muscle elongated to the anterior segment border, and then migrated dorsally.