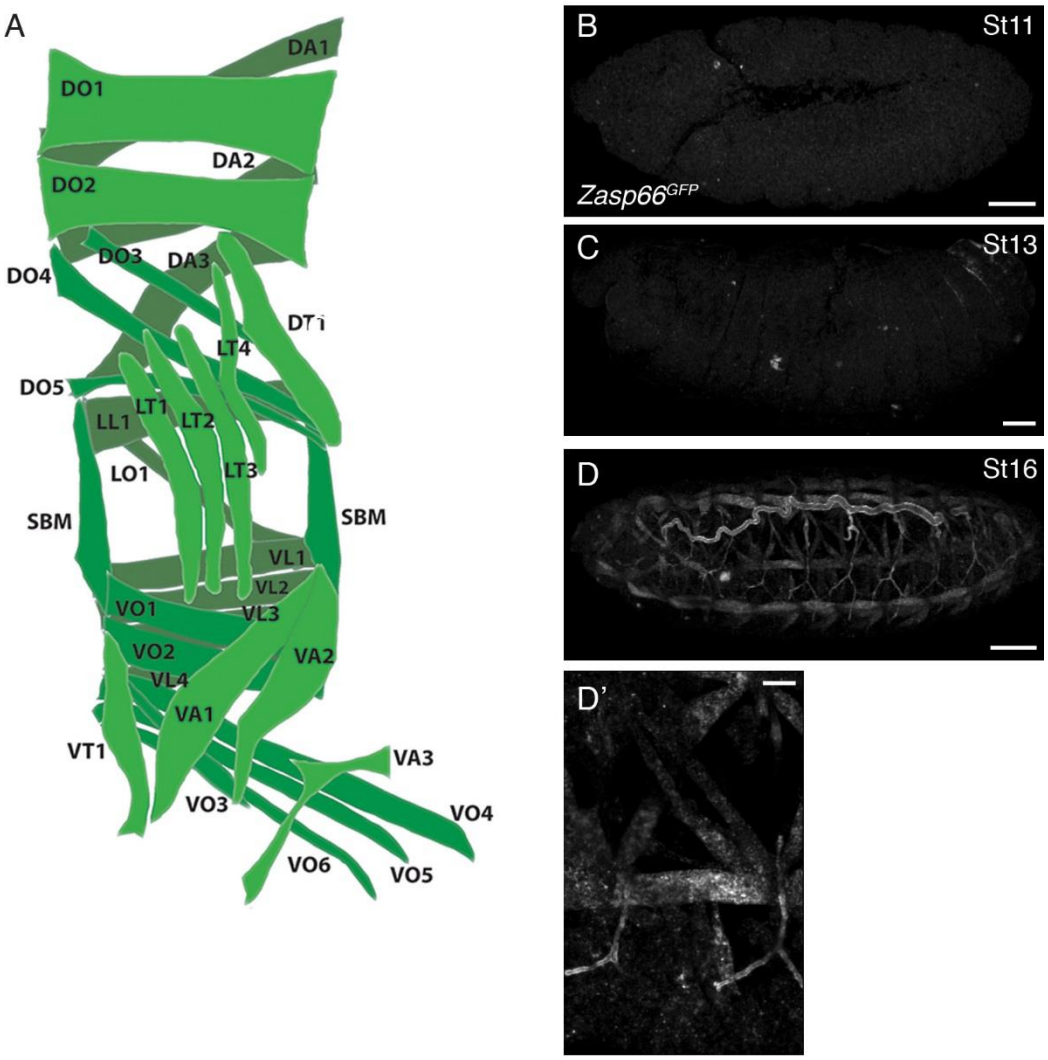

Figure S1. Zasp66 expression initiates late in myogenesis. (A) Somatic muscle diagram for one embryonic segment. Adapted from (Ruiz-Gomez et al., 1997). (B-D) *Zasp66*^{GFP} (protein trap) embryos labeled with α-GFP. Robust GFP expression is not detected until St16. Scale bars represent 50μm (B-D) and 10μm (D').

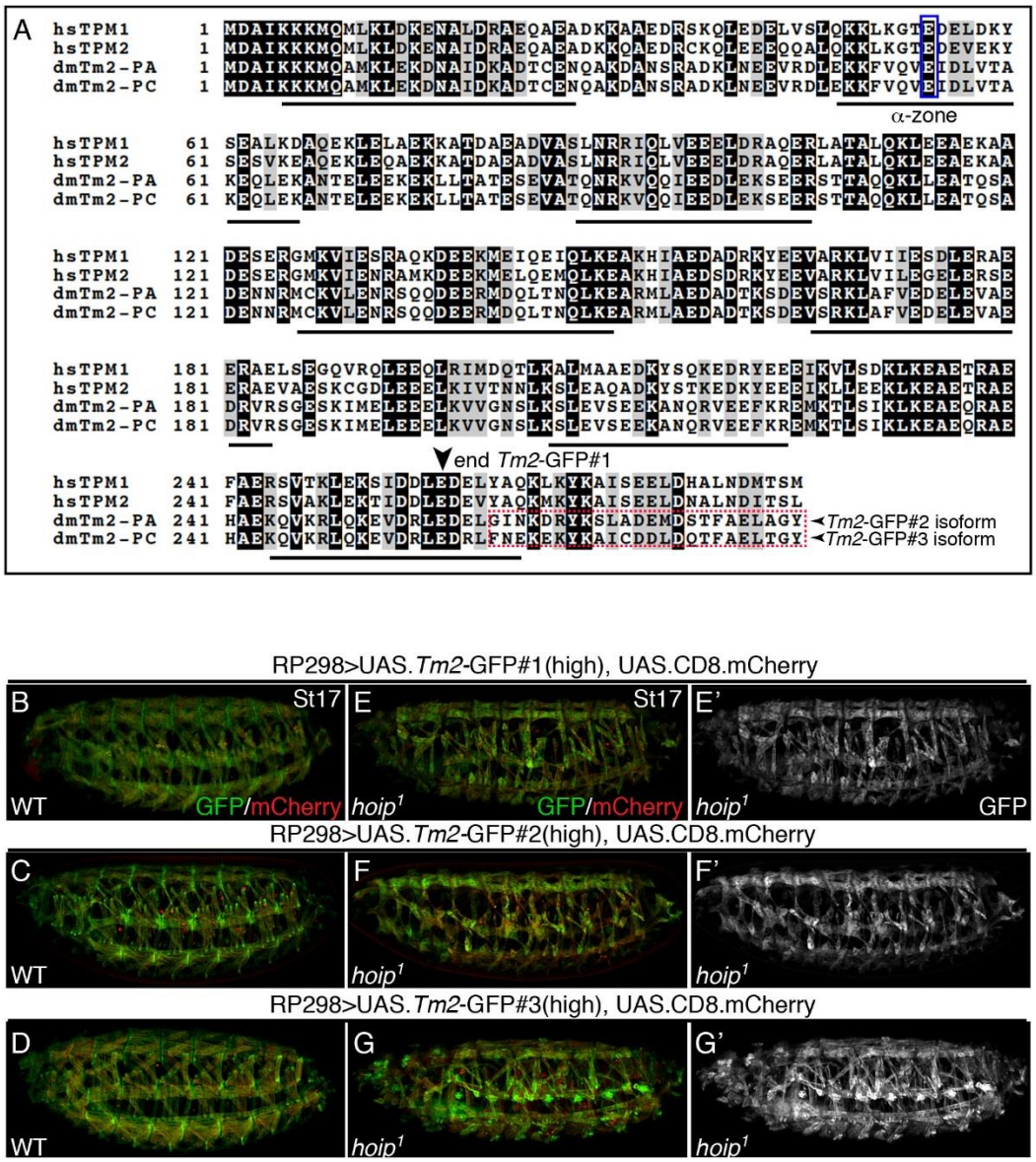

Figure S2. High-level *Tm2-GFPs* direct Tm2-GFP protein expression in *hoip*^{1} embryos. (A) Protein alignment of human TPM1 and TPM2 with two isoforms of *Drosophila* Tm2 (dmTm2). *Tm2* encodes two alternative final exons (5a/b). Isoform PC contains exon 5a; isoform PA contains exon 5b. The C-termini of the Tm2-GFPs are noted. Tropomyosin is a coiled coil protein comprised of 7 pseudo repeat domains, each of which contains an α-zone (underlined, adapted from Marttila et al., 2014). The E54K mutation is conserved and falls within the second α-zone. (B-G) Live St16 embryos. The somatic muscle driver *RP298.gal4* was used to co-express *CD8.mCherry* and high-level *Tm2-GFPs*. GFP fluorescence is comparable between WT (B-D) and *hoip*^{1} embryos (E-G). (E'-G') GFP.

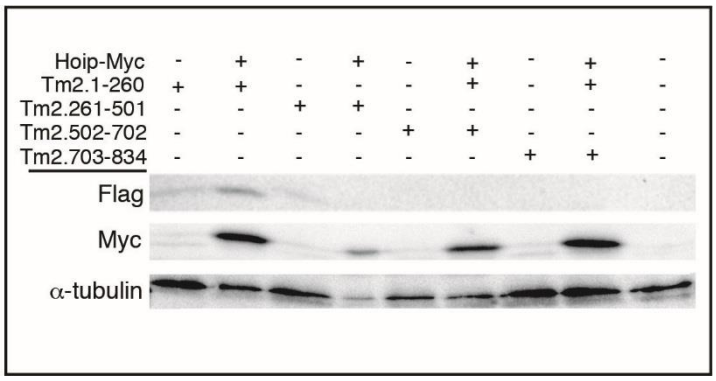

Figure S3. Mapping Hoip-responsive sequences in the *Tm2* coding region. Western blots from S2 cells transfected with Hoip.Myc and a series of *Tm2* coding region fragments. All *Tm2* constructs have a start codon in frame with a C-terminal Flag tag. The base pairs from the *Tm2* coding region used to generate each construct are given. Only the first 260bp of *Tm2* are Hoip responsive.

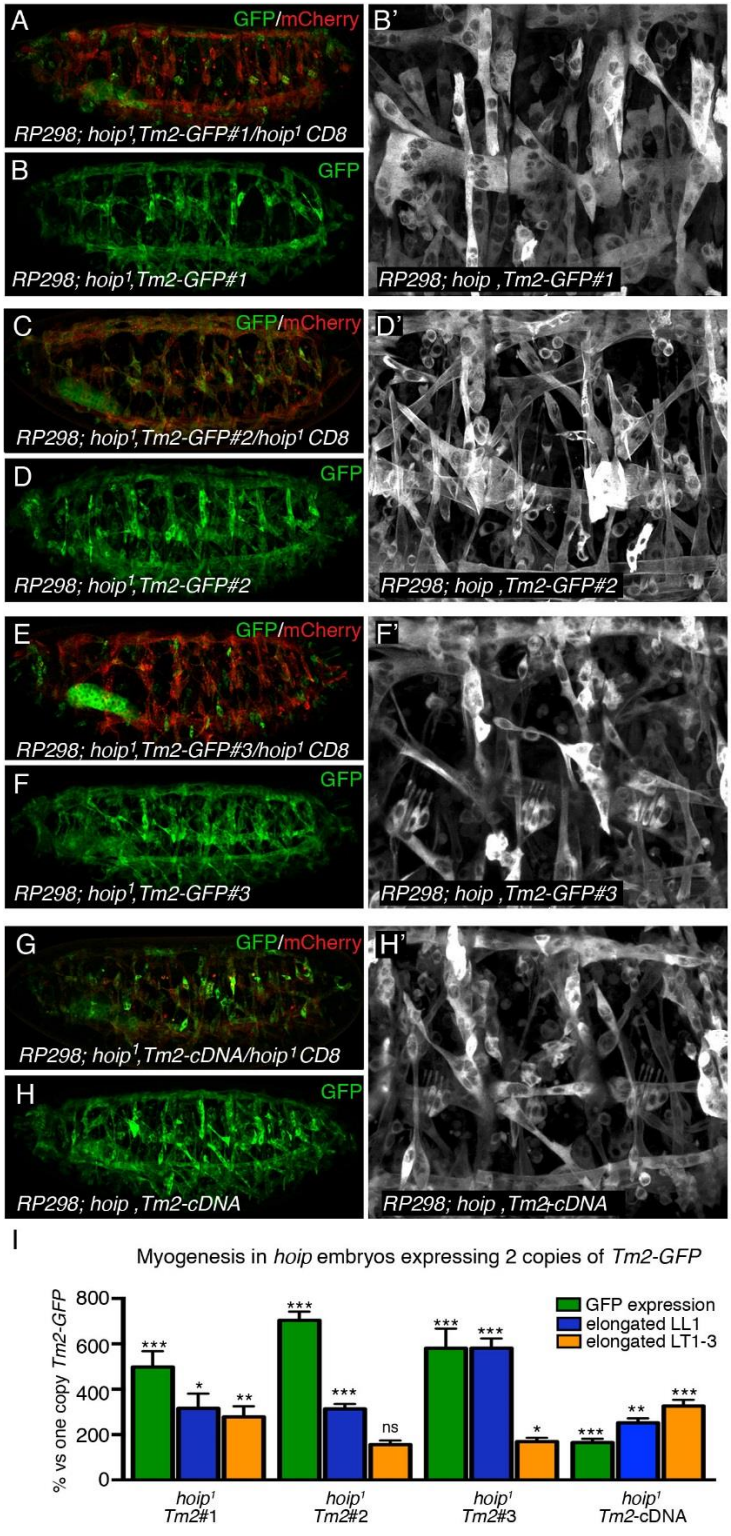

Figure S4. Rescue of the *hoip*^{1} myotube elongation phenotype by Tm2. (A-H) Live St16 embryos. *RP298.gal4* was used to express low-level *Tm2-GFPs* from a single copy of each transgene (A,C,E,G) or from two copies of each transgene (B,D,F,H) in *hoip*^{1} embryos. Single

copy embryos co-expressed CD8.mCherry to normalize Gal4 availability. *hoip*^{1} embryos that expressed *Tm2-GFPs* from a single copy showed reduced GFP expression compared to *hoip*^{1} embryos that expressed *Tm2-GFPs* from two copies. In addition, two copy embryos showed improved myotube elongation. (B',D',F',H') High magnification views of the embryos shown in (B,D,F,H). (I) Quantification of GFP fluorescence (DO2) and muscle morphology in *hoip*^{1} embryos that expressed *Tm2-GFP* transgenes. Significance was determined between *hoip*^{1} embryos that expressed one copy of the low-level *Tm2-GFPs* versus *hoip*^{1} embryos that expressed two copies. (*) $p<0.05$, (**) $p<0.01$, (***) $p<0.001$. Error bars represent SEM.

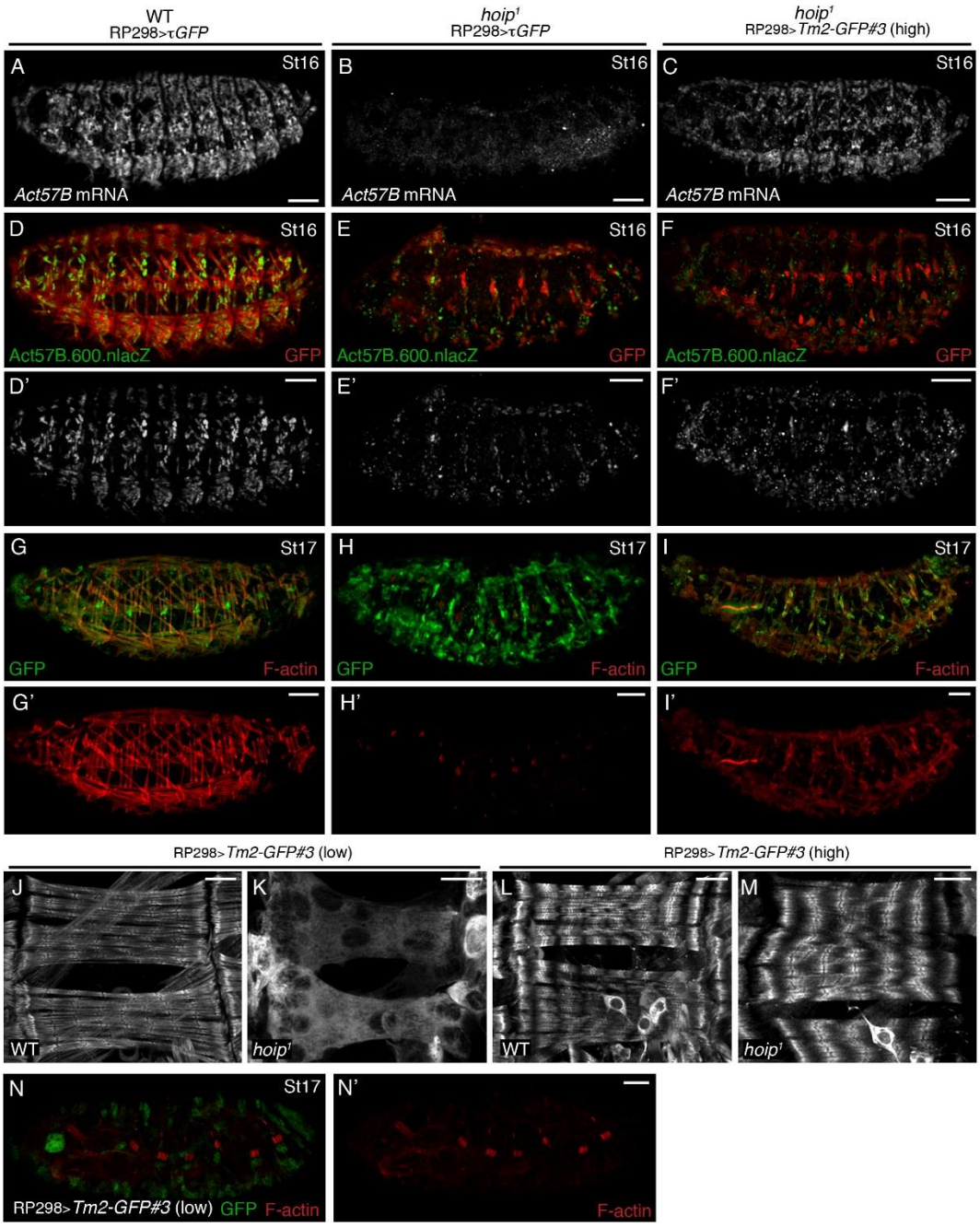

Figure S5. *Tm2* restores *Act57B* expression, F-actin assembly, and sarcomerogenesis in *hoip*^{1} embryos. (A-C) St16 embryos that expressed *τ.GFP* (A,B) or one copy of *Tm2-GFP#3* (C) labeled for *Act57B* mRNA. *hoip*^{1} embryos showed reduced SM *Act57B. hoip*^{1} embryos that expressed *Tm2-GFP#3* showed improved *Act57B* expression. (D-F) St16 *Act57B.600.nlacZ* embryos that expressed *τ.GFP* (D,E) or one copy of *Tm2-GFP#3* (F). *hoip*^{1} embryos showed

reduced lacZ expression. *hoip*^{1} embryos that expressed *Tm2-GFP#3* showed improved lacZ expression. (G-I, N) St17 embryos that expressed *τ.GFP* (G,H) or one copy of *Tm2-GFP#3* (I) under the control of *RP298.gal4* co-labeled for F-actin (phalloidin, red). (G) WT embryos showed robust F-actin in myofibrils. (H) F-actin was absent from the SM of *hoip*^{1} embryos. F-actin is detectable in the VM and unknown structures in the ectoderm. (I) *hoip*^{1} embryos that expressed high-level *Tm2-GFP#3* showed improved F-actin accumulation in the SM compared to *hoip*^{1} embryos. (J,K) DO1 and DO2 muscles from St17 embryos that expressed low-level *Tm2-GFP#3*. Low-level Tm2-GFP#3 incorporated into sarcomeres in WT but not *hoip*^{1} embryos. Confocal laser levels were increased between (J) and (K) to detect Tm2-GFP#3. (L,M) DO1 and DO2 muscles from St17 embryos that expressed high-level *Tm2-GFP#3*. High-level Tm2-GFP#3 incorporated into sarcomeres in both WT and *hoip*^{1} embryos. (N) *hoip*^{1} embryos that expressed low-level *Tm2-GFP#3* showed SM F-actin levels that were comparable to *hoip*^{1} embryos. (SM) somatic muscle, (VM) visceral muscle

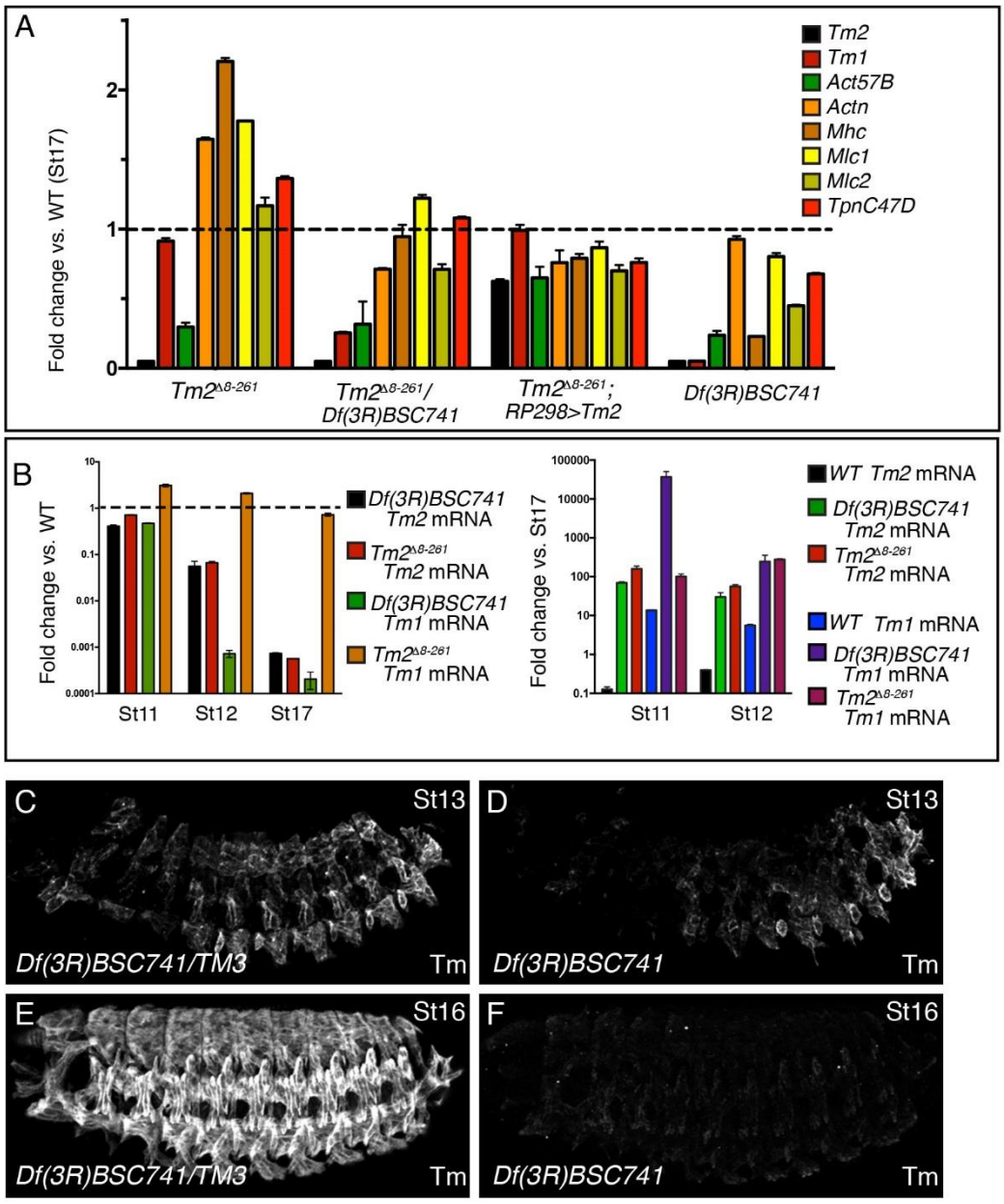

Figure S6. Maternally contributed *Tm1* and *Tm2* mRNAs promote Tropomyosin expression through St16. (A) qPCR of mRNA isolated from St17 embryos. *Tm2*$^{\Delta 8\text{-}261}$ is a genomic deletion that begins in the first coding exon and extends past the final coding exon. *Df(3R)BSC741* deletes *Tm1*, *Tm2,* and nineteen additional genes. *Tm2*$^{\Delta 8\text{-}261}$*/Df(3R)BSC741* transheterozygous embryos showed reduced *Act57B* abundance, which was partially rescued in mutant embryos that expressed *Tm2.GFP* under the control of *RP298.gal4.* (B) *Tm2* mRNA is detectable in *Tm2*$^{\Delta 8\text{-}261}$ and *Df(3R)BSC741* homozygous embryos at St11 and St12, but not St17. *Tm1* mRNA is enriched in St11-12 *Tm2*$^{\Delta 8\text{-}261}$ embryos suggesting *Tm1* can compensate for *Tm2*. (C-F) Embryos labeled with α-Tropomyosin (Tm). *Df(3R)BSC741* embryos expressed significant Tm protein at St13 (C,D), and Tm could still be detected in somatic muscle at St16.

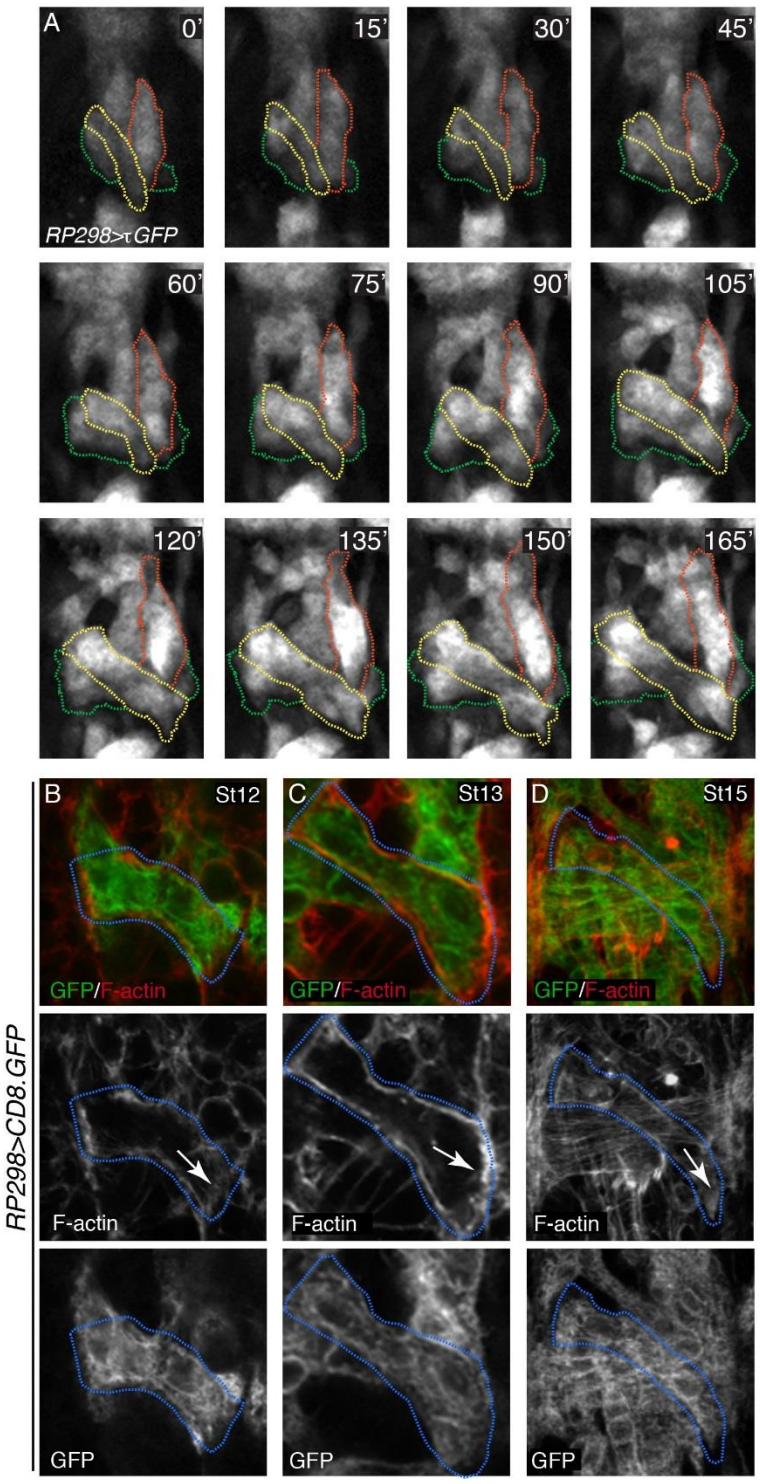

Figure S7. Time lapse microscopy. (A) Live myotube elongation in a single embryonic segment. *RP298.gal4* was used to express *τGFP*. Muscles shown include LL1 (green), DO5 (yellow), and DT1 (orange). (B-D) St12-15 embryos that expressed *CD8.GFP* under the control of *RP298.gal4* double labeled for GFP (green) and phalloidin (red). The DO5 muscle is outlined. Leading edge F-actin (arrows) corresponds with the boundary of CD8/membrane expression.

Table S1. Primers for qPCR

Primer Name	Sequence
TM2 F	5'-CAG CTG ACC AAC CAG TTG AA-3'
TM2 R	5'-GAC ACC TCC AGG GAC TTC AG-3'
TM1 F	5'-CAA GCG ATG AAA GTC GAC AA-3'
TM1 R	5'-CGG TCT GGA TCT TCT TCT GC-3'
Act57B F	5'-GCC TAG CAC CAA CAC TAG CA-3'
Act57B R	5'-CGC GAG CGA TTA ACA AGT GG-3'
Actn F	5'-CAA CGA GCT GAA GGC CCT AA-3'
Actn R	5'-CTT CTC CAG GAT TCG CTC GG-3'
Mhc F	5'-GTG CCG GAA AGA CTG AGA AC-3'
Mhc R	5'-GCA CCA GCC AGT TTA CCA GT- 3'
Mlc1 F	5'-CAA CTT CAC GCT TTG GAA CA-3'
Mlc1 R	5'-AGC TCA TCC GCG ATA CAG TT-3'
Mlc2 F	5'-ACC ACC CTC TTC CTT GGT CT-3'
Mlc2 F	5'-CAG CCA GAG ATT CAG TGT GC-3'
TpnC47D F	5'-TGG ACG AAC TCC TCG AAC TC-3'
TpnC47D R	5'-GGT GAT CGA TCT GGT GAA CA-3'