

Supplemental information for the manuscript:

Gelsolin* and *dCryAB* act downstream of muscle identity genes and contribute to preventing muscle splitting and branching in *Drosophila

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Supplementary Figure legends and Supplementary figures:

Figure S1. Sensitivity assessment of translational profiling by TRAP.

A-B. Volcano plots showing distribution of up- and down-regulated genes in Slou- and Lms-positive muscle subsets at the T3 time point. Note that genes known to be specifically expressed in the slou population (*slou*, *org-1*) and the lms population (*lms*) are consistently up-regulated. **C-D.** Distribution of Slou and Lms up-regulated and down-regulated genes from the T2 time window with respect to modEncode RNA expression level based on whole embryo RNA sequencing (stage 12–16).

Figure S2. *Gel* is expressed in LTs from stage 14 and is present in other mesodermal tissues. *Gel* expression in stage 13 (**A,A'**) and stage 14 (**B,B'**) embryos revealed by *in situ* hybridisation with the *Gel* RNA probe. Muscles are stained with anti-actin antibodies. *Gel* transcripts are first detected in LT muscles at stage 14 and are also found (**C,C'**) in visceral muscle (VM) and fat body (FB) (arrows).

Figure S3. Localisation of dCRyAB and *Gel* proteins in LT muscles. (A, A') In stage 15 embryos dCRyAB displays a dotted cytoplasmic pattern in LTs and is detected close to the myotube membrane (arrows) and at LTs extremities (arrowheads). (**A'**) As revealed by muscle-specific Mef>LifeAct-GFP dCRyAB partially co-localizes with Actin in LTs. (**B,B'**) In stage 15 embryos *Gel* is highly enriched at the extremities of LT myotubes (arrowheads) but discrete *Gel* spots are also detected at sub-membrane LT areas (arrows). (**B'**) Colocalization of *Gel* and F-Actin is seen at the LT myotube ends.

Figure S4. Branched and split phenotypes in LT iTF mutant embryos. (A-C) Lateral views of four hemisegments of stage 16 embryos stained with anti-Actin (A, B) and anti- β 3-Tubulin antibodies. (A) wild type muscle pattern. (B) An example of split (arrows) LT muscles in apterous *ap^{UGO35}* mutant embryos. Asterisks point to lacking LT muscles (C) An example of branched LT muscle phenotype (arrowheads) in *lms^{S95}* mutant embryo.

Figure S5. *In vivo* visualisation of LT splitting in *Gel* mutants.

Time lapse movie (3 h 30 min, with 3 min frame interval) from (A) control *lms>LAGFP* and (B) *Gel;lms>LAGFP* embryos showing one abdominal segment (A5) with developing LT muscles. The films begin at late stage 14 and end at late stage 16 and were generated using Imaris software version 9.2; URL: <https://imaris.oxinst.com> (B) Splitting of LT3 and LT4

muscles could be observed. It is initiated at stage 15 and fully apparent with split fibre morphology and duplicated ends at stage 16.

Figure S1

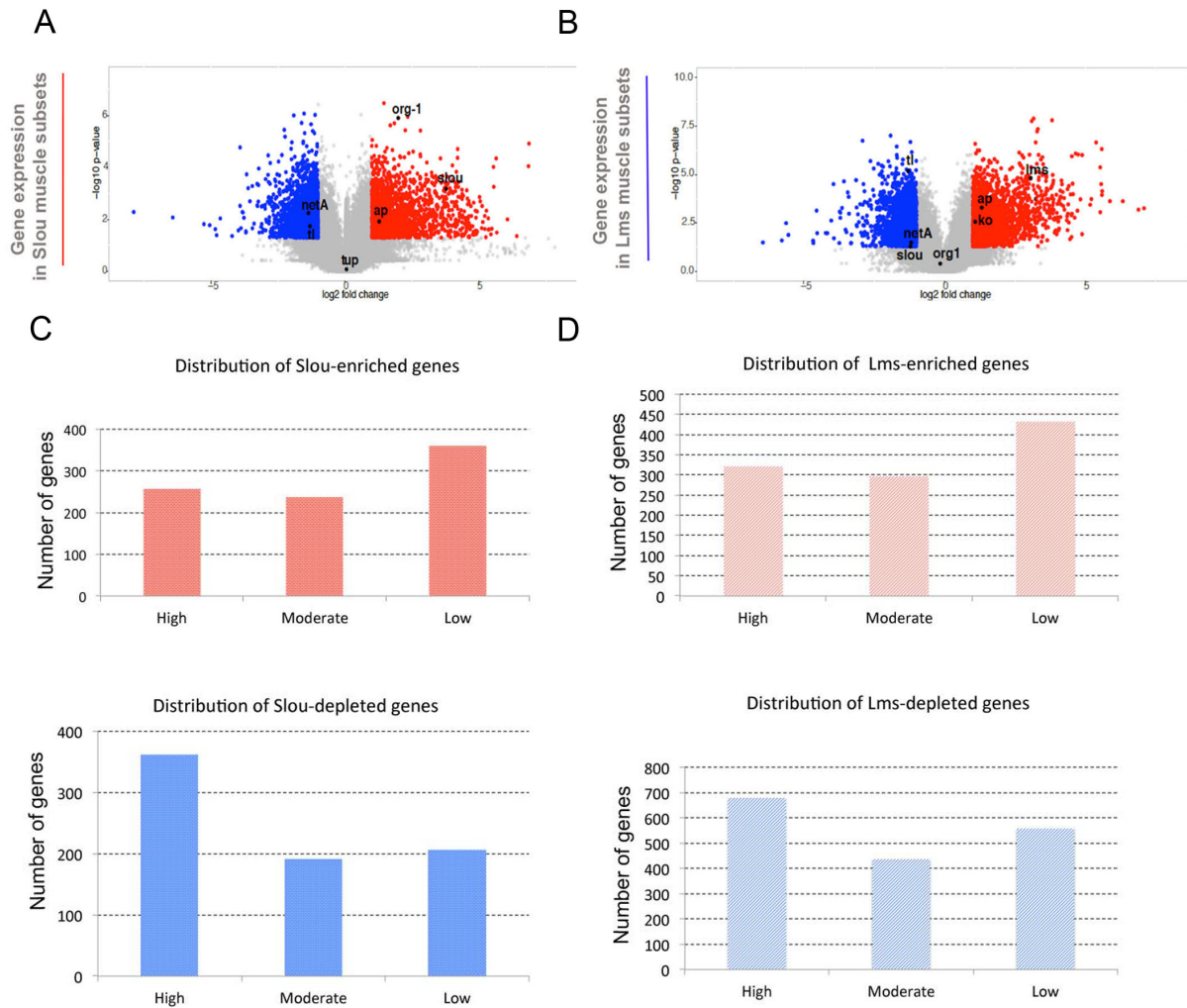


Figure S2.

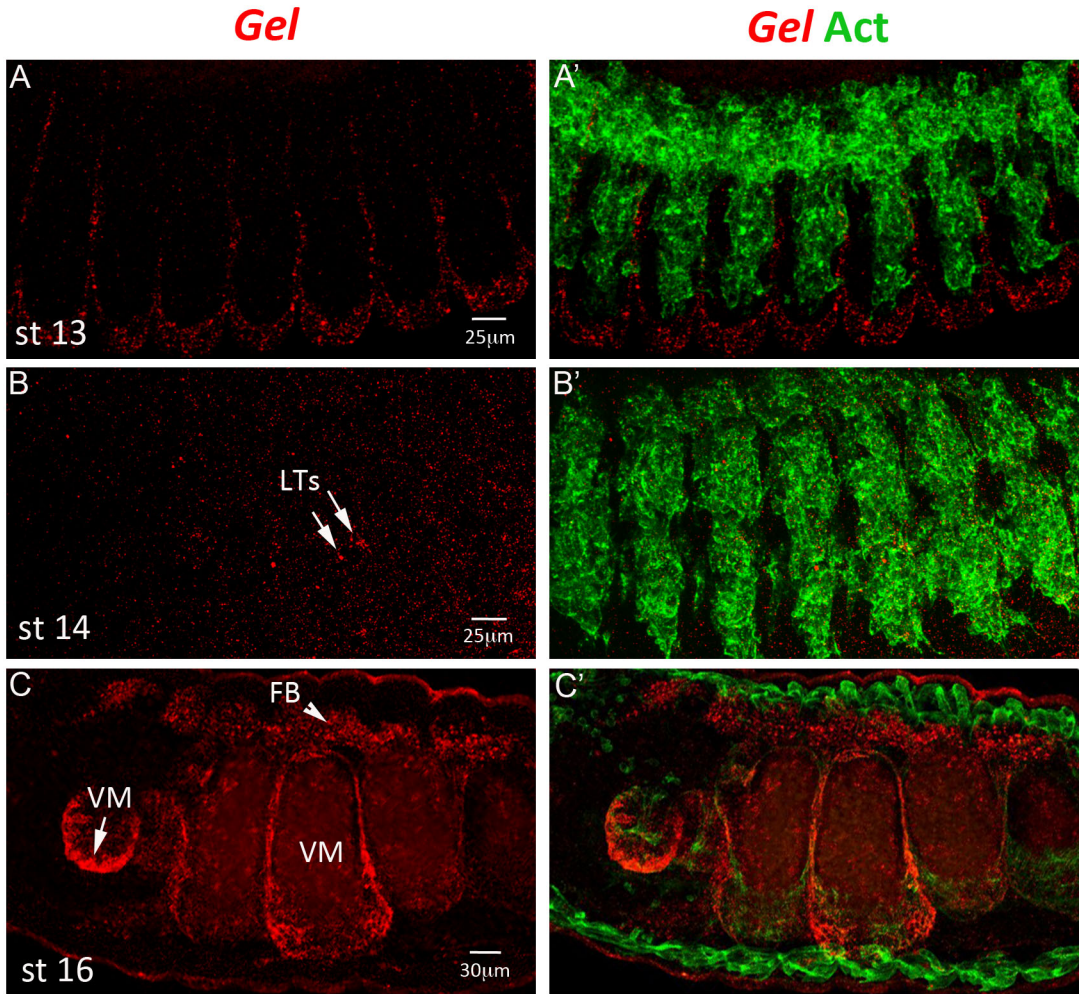


Figure S3

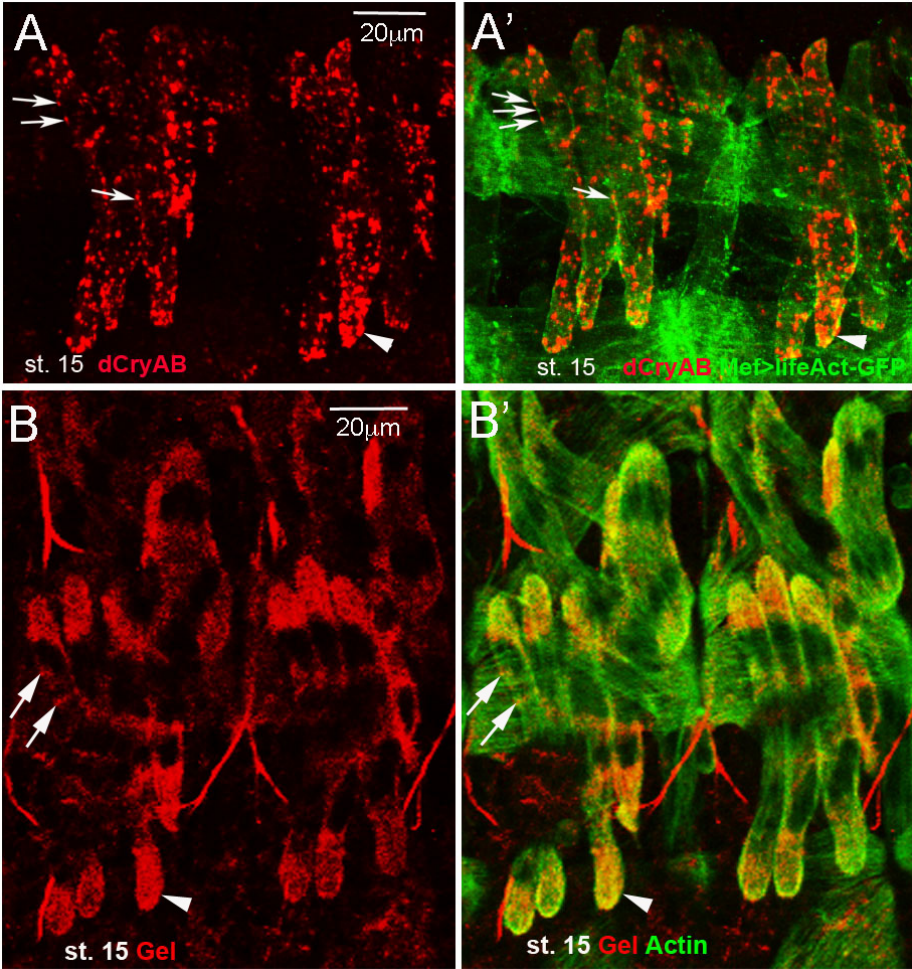


Figure S4

