Table S2 Compilation of screening data for Tribolium orthologs of Drosophila genes known to regulate various aspects of muscle development with no annotated muscle phenotypes, uninterpretable muscle phenotypes, or no injected dsRNAs.

No muscle phenotypes with pupal injections and larval injections (if performed) were annotated for iB dsRNA injections for the following muscle development-related genes (Drosophila gene name (alphabetical)/Tribolium gene name/iB dsRNA fragment tested):

```
aret/TC012080/iB 05164 (not done with larval injection)
CLIP190/TC031243/iB 01909
H15 */TC013513/iB_05442
Hand */TC004726/iB_03827
how #/TC000827/iB_03083 (all animals died after pupal injection) (not done with larval injection)
IIk #/TC002476/ iB_00385 (but wing blisters & possible muscle phenotype w. larval injections) (lethal after pupal injection)
kette # (hem)/TC001541/iB 00258 (animals sterile after pupal injection)
KIc/TC007992/iB 09878 (animals sterile after pupal injection)
Ibe */TC011748/iB_05096
mid */TC014296/iB_05605 (not done with larval injection)
mp20/TC007135/iB_01129
msh * * (Dr)/TC011744/05095
msh * * (Dr)/TC012748/iB_05308 (embryonic muscle defects potentially obscured by early defects)
Msp300 #/TC033655/iB 04879
Msp300 #/TC034351/iB_04893 (not done with larval injection)
parvin #/TC000078/iB_00014 (but wing blisters w. larval injections; animals sterile after pupal injection)
paxillin/TC010609/iB 01717
poxm * #/TC008838/iB 04548
Ptx1 */TC032174/iB 07879 (not done with larval injection)
rhea #/TC033529/iB_01537 (for pupal injections; no adults obtained with larval injections)
rols #/TC002665/iB_07027 (not done with larval injection)
Scar #/TC006152/iB 01003 (strongly reduced egg lays after pupal injection, no embryonic cuticle)
sd/ TC032219/09689 (disrupted embryo morphology, > 80% inside-out)
sing #/TC012091/iB 05167
six4 * #/TC003852/iB 03634
slou */TC012332/iB 05216
slow/TC005670/iB_07509 (not done with larval injection)
sns *, hbs/ TC032336/iB 03544
Tsp #/TC033883/iB 01372 (> 80% not fertilized/not developed and no cuticle)
```

Not injected (---), or not screenable for muscle phenotypes due to lethality prior to muscle development (pupal, adult, or early embryonic) upon larval and pupal injections, were dsRNAs for the following muscle development-related genes:

```
abba/TC031245/iB_01986 (not screenable with pupal injections; --- with larval injections)
ara */caup */TC031040/iB 03594 (not screenable)
Arp2/TC000144/iB_00026 (not screenable)
Arp3/TC002963/iB_00483 (not screenable)
blow/TC014242/---
col * (kn)/TC001270/---
dia/TC006029/iB_00980 (no embryonic cuticle)
Dhc64C/TC008801/---
DIc90F/TC010513/---
DIc90F/TC009745/---
ens/TC010873/---
Fit1/Fit2/TC010123/iB_01652 (all animals died in both screens after injection)
Grip/TC034397/---
hoip/TC002918/---
Ims */TC033532/---
mbc/TC012454/iB_10628 (early embryonic lethality + no cuticle)
Mef2/TC010850/iB_04920 (not screenable)
mew/TC034177/---
mys/TC011707/---
mys/TC013706/---
stck/TC011050/---
Wasp/TC013303/---
```

- muscle identity genes
- genes used for estimating the recovery rate of the screen for identifying Tribolium muscle regulatory genes. The marked genes have been reported and judged to give sufficiently conspicuous embryonic muscle phenotypes in Drosophila zygotic or germline clone mutants so that they should be detectable under conditions similar to those used in our pupal RNAi injection screen. Among muscle founder genes only those are included that affect more than ~15% of the muscles in each segment.