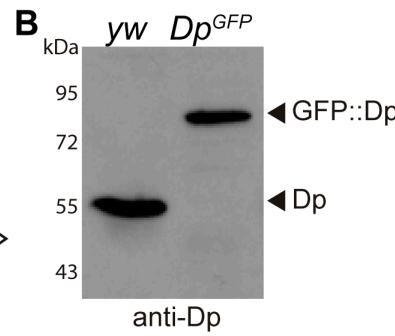
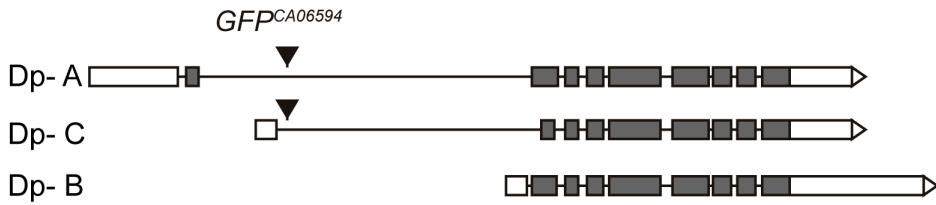
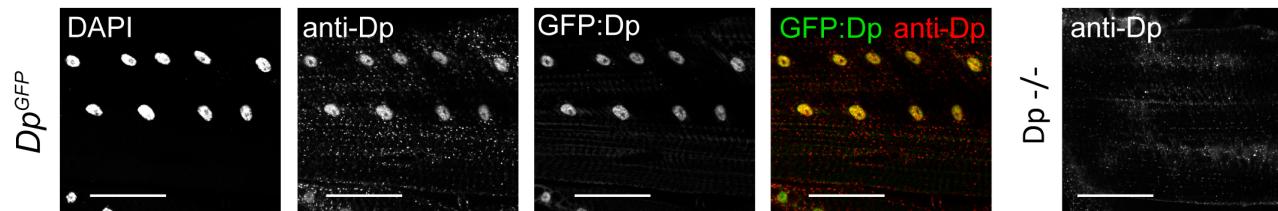
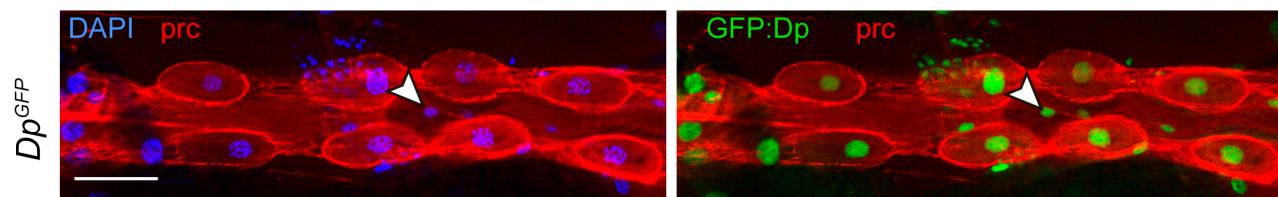
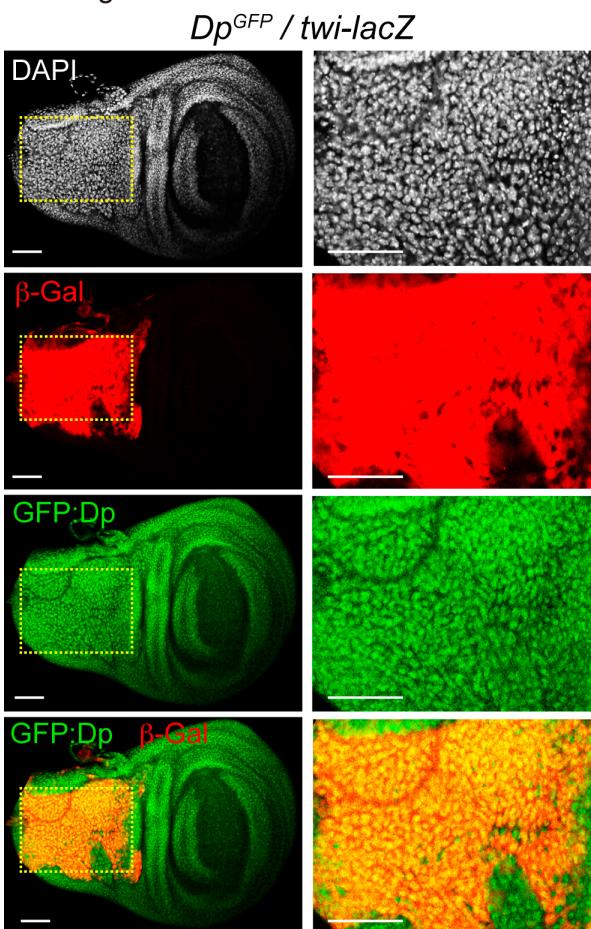
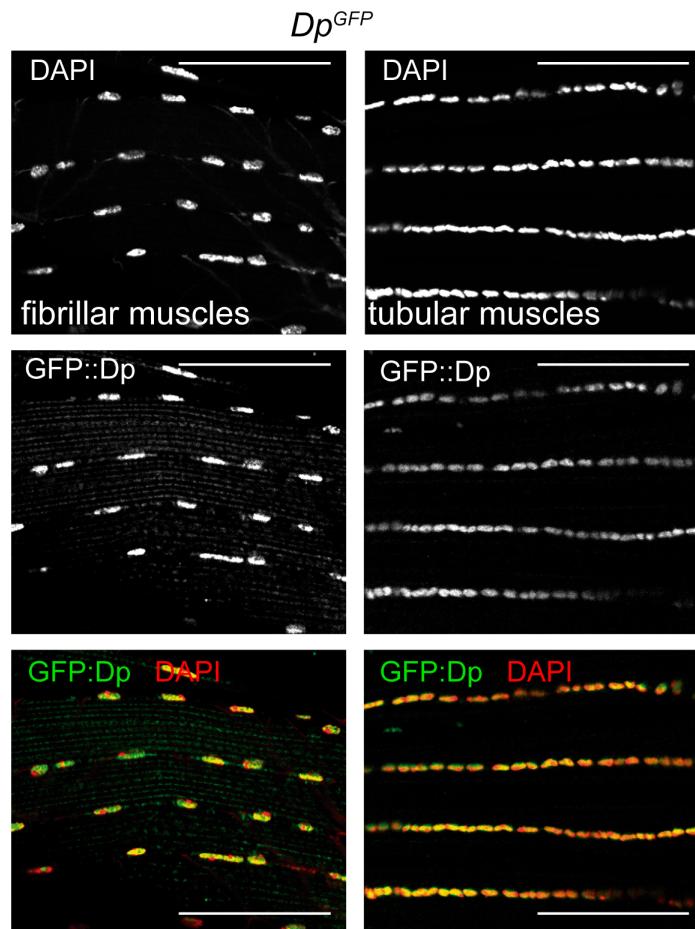


Supplementary Figure 1. *Mef2-GAL4* and *24B-GAL4* are specific drivers for somatic, cardiac and visceral muscles

Mef2-GAL4 and *24B-GAL4* are specific drivers for somatic, cardiac and visceral muscles, but leaked in salivary glands and some neurons of third instar larvae. GAL4 expression was reported using *UAS-GFP* (nuclear localization signal, bottom panel). Tissues were dissected and counterstained for DAPI (top panel). Confocal sections showed (A) body wall muscle, (B) gut at two different planes, muscle layer (left) and epithelial layer (right), (C) salivary gland, (D) eye disc, (E) fat body, (F) brain, (G) wing disc, and (H) cardiac tube of *Mef2-GAL4;UAS-GFP/* (left panel) and *24B-GAL4; UAS-GFP* (right panel). Scale bar is 50 μm . Images were taken and processed using the same parameters for comparative purpose.

A *Dp* transcripts (CG4654)**C** Body wall muscles**D** Aorta**E** Wing disc**F** Thoracic muscles

Supplementary Figure 2. Dp is localized in nuclei of larval and adult muscles

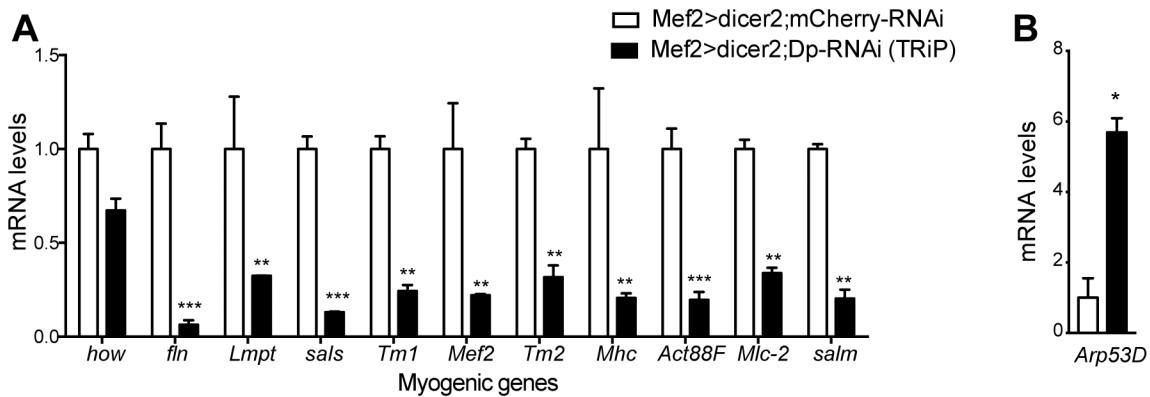
(A) Schematic diagram of the *Dp* transcripts (*Dp-A*, *Dp-B*, and *Dp-C*), which contains alternative transcription start sites. Exons and introns are indicated by boxes and lines, respectively. White boxes represent untranslated regions (UTR) and grey boxes highlight coding regions. The insertion location of the GFP-protein trap element (CA06594) is indicated by black arrowhead. Only transcripts *Dp-A* and *Dp-C* were predicted to contain GFP element and generate GFP-tagged proteins.

(B-C) Dp fusion to GFP was confirmed by western blot **(B)** and immunofluorescence **(C)**. **(B)** A shift of approx 27 kDa was observed in *Dp[GFP]* compared to wild type (*yw*) larval homogenates using α -DP antibody. **(C)** Dp was localized in nuclei of body wall muscles at third instar larva. Dp::GFP colocalized with α -DP antibody and DAPI. The specificity of α -Dp antibody was analyzed by staining *Dp* null mutant (*Dp*^{a3}/*Df(2R)Exel7124*), which is lethal at pupal stage. Scale bar is 50 μ m.

(D) Dp::GFP was detected in nuclei of cardiac cells (white arrowhead) and in pericardial cells, labeled with α -pericardin, in third instar larva cardiac tube. Scale bar is 50 μ m.

(E) Confocal images of third instar larva wing disc showed Dp::GFP localized in nuclei of adult myoblasts labeled with *twi-lacZ* using β -Gal antibody (red). Magnifications of yellow dashed boxes are shown on the right panels. Scale bar is 50 μ m.

(F) Dp::GFP was localized in nuclei of indirect flight muscles of adult flies. Fibrillar and tubular muscles are shown on the left and right panels, respectively. Scale bar is 50 μ m.



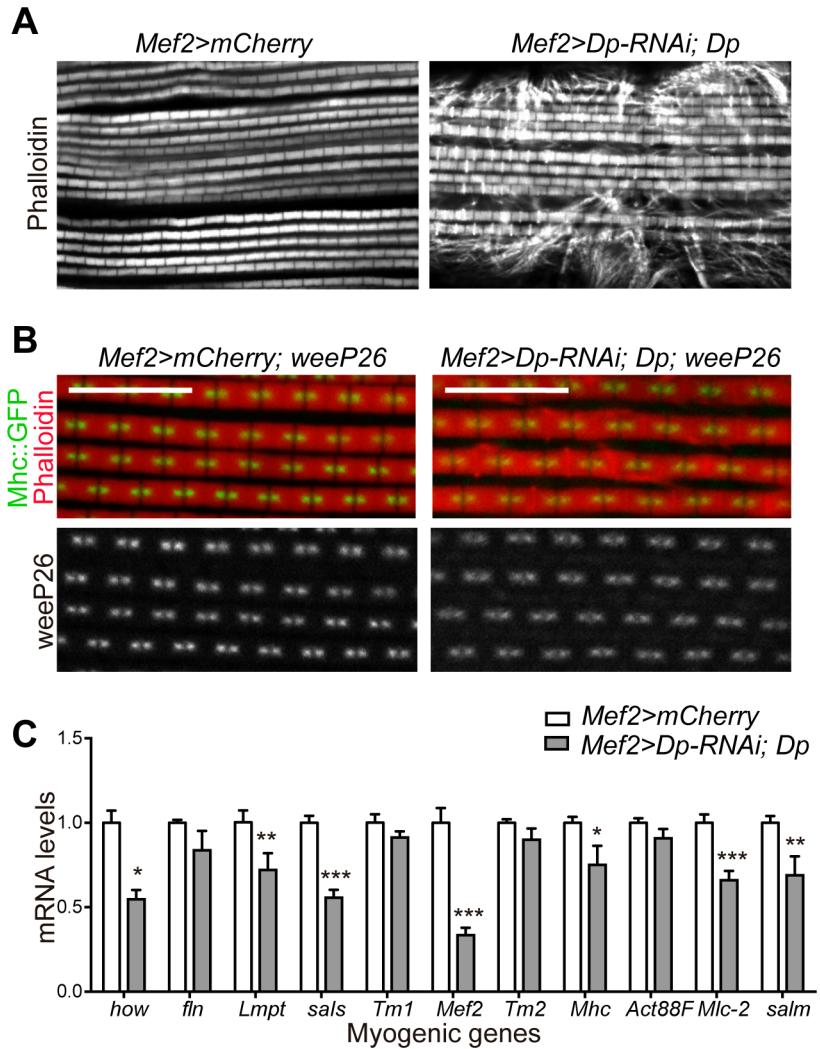
Supplementary Figure 3. Dp-depleted flight muscles displayed reduced expression of myogenic genes in pharates

An alternative *UAS-Dp-RNAi* from TRiP collection was used to knockdown Dp expression.

(A) Gene expression was quantified by RT-qPCR in indirect flight muscles of pharate pupa. Muscle genes are *held out wings* (*how*), *flightin* (*fln*), *Limpet* (*Lmpt*), *sarcomere length short* (*sals*), *Tropomyosin 1* (*Tm1*), *Myocyte enhancer factor 2* (*Mef2*), *Tropomyosin 2* (*Tm2*), *Myosin heavy chain* (*Mhc*), *Actin 88F* (*Act88F*), *Myosin light chain 2* (*Mlc2*), and *spalt major* (*salm*). Mean \pm SEM, n=2 independent samples, two-way ANOVA, ** p < 0.01, *** p < 0.001.

(B) *Arp53D* gene expression. Mean \pm SEM, n=2 independent samples, t-test with Welch's correction, * p < 0.05.

Genotypes are *Mef2-GAL4/UAS-mCherry-RNAi* (white bar) and *UAS-Dp-RNAi TRiP;Mef2-GAL4* (black bar).



Supplementary Figure 4. Myofibril assembly is partially rescued in the few adults *Mef2>Dp-RNAi; UAS-Dp*

(A) Myofibrils at the edge of the Dorsal Longitudinal muscles are slightly defrayed in the partially rescued adult flies, thus indicating a mild yet persistent defect in myofibril morphology. Confocal images of DLMs stained with Phalloidin (red) in sagittal view.

(B) Both the reduced myofibril width and the defect in labeling of sarcomeric marker were rescued in partially rescued adult flies. Confocal images of DLMs in a sagittal view stained with Phalloidin (red) in a GFP-tagged splice variant Mhc-IFM19 background (*weeP26*), to mark A-band structure. Scale is 5 μ m.

(C) The expression of whole set of myogenic genes was not fully restored in the partially rescued adult flies. Muscle genes are *held out wings* (*how*), *flightin* (*fln*), *Limpet* (*Lmpt*), *sarcomere length short* (*sals*), *Tropomyosin 1* (*Tm1*), *Myocyte enhancer factor 2* (*Mef2*), *Tropomyosin 2* (*Tm2*), *Myosin heavy chain* (*Mhc*), *Actin 88F* (*Act88F*), *Myosin light chain 2* (*Mlc2*), and *spalt major* (*salm*). Mean \pm SEM, N = 3 independent samples, two-way ANOVA, * p < 0.05, ** p < 0.01, *** p < 0.001.

Genotypes are *Mef2-Gal4/UAS-mCherry* (left panel, white bar), and *UAS-Dp-RNAi; Mef2-GAL4/UAS-Dp* (right panel, grey bar).

Supplementary Table 1: Phenotypes associated with knockdown of Dp by RNAi with Gal4 drivers

| GAL4 driver | GAL4 tissue expression | GAL4 expression developmental stage | Phenotype crossed to UAS-Dp-RNAi |
|-----------------------------|---|---|--|
| <i>Act5C</i> | Ubiquitous | Throughout dev. | Lethal |
| <i>elavC155</i> | Pan-neuronal | Throughout dev. | Viable, mild rough eye |
| <i>nSyb</i> | Pan-neuronal | Throughout dev. | Viable |
| <i>Mz1407</i> | Pan-neuronal | Throughout dev. | Semi-viable adults (30 % pharate) |
| <i>D42</i> | Motor neurons | Throughout dev. | Viable |
| <i>arm.S</i> | Epithelia | Throughout dev. | Viable |
| <i>AB1 c729 34B</i> | Salivary glands | Embryonic and larval dev. | Viable |
| <i>r4</i> | Fat body | Larval dev. | Viable |
| <i>twi.G twi.2xPE</i> | Mesoderm | Embryonic dev. | Viable |
| <i>24B</i> | Mesoderm | Embryonic dev. | Lethal (pupa) |
| | - Precursors of somatic muscles, visceral muscles and cardiac cells - All muscles | Embryonic and larval dev. | |
| | Adult muscle precursor cells | Adult myogenesis | |
| <i>Mef2.R</i> | Somatic, visceral and cardiac muscles | Embryonic and adult myogenesis | Lethal (pharate) |
| <i>rP298</i> | Progenitors and founders of somatic and visceral muscles | Embryonic dev. | Semi-lethal adults (54% pupa) |
| | - Thoracic and abdominal adult muscle founder cells - Developing adult muscles | Adult myogenesis | |
| <i>1151</i> | - Adult muscle precursors - Developing adult muscles - Leg tendon cells and precursors | Adult myogenesis | Semi-lethal adults (74% pharate) Flight/jump defect |
| <i>Mhc.F3-580</i> | Indirect Flight muscles at the onset of myofibrillogenesis (40 h APF at 22 °C) throughout adult | Late adult myogenesis | Flight/ jump defect |
| <i>Act88F</i> | Post-fusion Indirect Flight muscles throughout adult | Late adult myogenesis | Flight/ jump defect |
| <i>bap3</i> | Trunk visceral mesoderm | Embryonic dev. | Viable |
| <i>5053A</i> | Longitudinal musculature of the midgut | From end of embryogenesis throughout dev. | Viable |
| <i>Hand4.2</i> | Myocardium, epicardium, and visceral mesoderm | Embryonic dev. | Viable |
| <i>GMH5</i> | Myocardial cells | Larva and adult heart | Viable |
| <i>tinC.44</i> | Cardiomyoblasts | Embryonic dev. | Viable |

Supplementary Table 2. Primer sequence

| Gene | Forward primer sequence | Reverse primer sequence | Application |
|----------------------|-------------------------------|-------------------------|-------------|
| <i>Arp53D</i> | TATAACAAATAAACGGGCACAGA AC | GCTCATGTTCCAGGAGAGG | RT-qPCR |
| <i>Him</i> | AACCAGGTCCAGAACATCACATCG | TGGTACTGCAGCTGAAACG | RT-qPCR |
| <i>twi</i> | AAGCTCAGCAAGATCCAGACC | GAGCTGCCGATCCATACG | RT-qPCR |
| <i>Mef2</i> | CGGATATCATGAGCCTAACAC | CGTGAACCATTGCTATTCTGC | RT-qPCR |
| <i>blow</i> | CGACTGTGTCGATAGCAAGG | TCGTAGAGCTGCTGATGTGG | RT-qPCR |
| <i>sing</i> | TACCATACGCCGATTCCATT | ACTGCCGATGTAGCCATACC | RT-qPCR |
| <i>mbc</i> | AAGACGCCAGATCCTCATGT | CTGAAGTGGAATCGGTGGTT | RT-qPCR |
| <i>lmd</i> | TAAGGCATTCTCGCGTTGG | GATCCGTAGCGCTTGGT | RT-qPCR |
| <i>ewg</i> | TTATCACACTGCCGGATGGTAC | ACATCAGTGAGACTCTAACCGG | RT-qPCR |
| <i>vg</i> | ACCAAAGAAGAGGACCTCATCG | GGATGGCTGTGCGAGTGG | RT-qPCR |
| <i>htl</i> | AACGCATCGAAACCGTTCAC | TGGTGCCTGTTCTGTATC | RT-qPCR |
| <i>stumps</i> | AACAAGGTGGTTGCTCTGCT | ACTGCAGGGTAGGGATTG | RT-qPCR |
| <i>Act88F</i> | ATGGTGGGTATGGTCAGAA | CTTCTCCATGTCGTCCCAGT | RT-qPCR |
| <i>Mhc</i> | AAGAACGACCTCGAGAACAG | TCGGCCTCTTCTGCTG | RT-qPCR |
| <i>fln</i> | GGCAAAGAGGGACAAACAAAC | ACTACGGAGTGCTCATCCGTT | RT-qPCR |
| <i>TnT</i> | CTTCACCATTGCCAAGAAGG | TTTGACAATGAGCTCCCACA | RT-qPCR |
| <i>Actn</i> | AGTACGGAGATGGCTACATGG | TGCGCAAATGGCTGTTACAC | RT-qPCR |
| <i>Cf2</i> | CACCACTCACCGTCAAGCTA | TGGCATAGACAGGCACAGAG | RT-qPCR |
| <i>b-tubulin</i> | ACATCCGCCCGTGGTC | AGAAAGCCTTGCCTAACATAG | RT-qPCR |
| <i>RpL32</i> | TACAGGCCAAGATCGTGAAG | GACGCACTCTGTTGTCGATACC | RT-qPCR |
| <i>RpL30</i> | GCAAATACTGCCTGGGCTAC | ACTTCAGTCTGGCCAGCAT | RT-qPCR |
| <i>COX5A</i> | AAGGCCACCCCTCATCCCTA | CATCGTACACGGACTTCAGG | RT-qPCR |
| <i>ND-B17.2</i> | GTAGTGCATCCAGCCGTACC | CTACTTCTACGGCCGCAATC | RT-qPCR |
| <i>Idh</i> | GAGTAGGAGGCACCAGCAAC | TTTCCAATCCAAACCAGTTC | RT-qPCR |
| <i>how</i> | ATCTGTCGATGACCTGCATG | TTAGCTCATCTTCGCCCTTCGG | RT-qPCR |
| <i>Lmpt-K</i> | AGTGGCTGCCCTAAAACAAC | ATTGCTCCTCTGCTGCGATAG | RT-qPCR |
| <i>sals</i> | GCAAGCCATGAAGAAGAACAG | TCGTCTCGTCTAGCTTCATGG | RT-qPCR |
| <i>Tm1</i> | TGCAAGCGATGAAAGTCGAC | TCTGGATCTTCTCTGCAAGCTG | RT-qPCR |
| <i>Tm2</i> | TCCAAGATCATGGAGCTGGAG | TTCATCTCGCGCTGAACTC | RT-qPCR |
| <i>Mlc-2</i> | TTCTCTGTGTTCTCCAGAACG | TTGTCGGCATCCATGAGTTG | RT-qPCR |
| <i>salm</i> | ACTACAGGAGGCCACACCAAAG | ATGTGTTGCTTCAGGTTGCC | RT-qPCR |
| <i>E2f1</i> | ACCTGCTCAACGTGGATCTC | AATCTCGCGCGCAATTTCG | RT-qPCR |
| <i>How-650</i> | GCGAAACAAAATGCCGCTTG | ATTAAGACACTGCGCTGTGG | ChIP-qPCR |
| <i>Lmpt+100</i> | CCAAAGCTGAAGCCAAAAGC | CACACACACAAAGACAGTCACTC | ChIP-qPCR |
| <i>Sals-500</i> | TTTGCCTTGTCAGCATCAGC | TAGCAATGGCCGTTAACG | ChIP-qPCR |
| <i>Mef2-220</i> | TTCAGAGCGTCGAACCTCTC | TGGAGTTCAAATGCCCTTC | ChIP-qPCR |
| <i>Tm2-130</i> | ACGAGAACATGCAGCCAAAAG | TATGTGGAATGGCGCTTCG | ChIP-qPCR |
| <i>Tm1-IC-280</i> | AGCGTTACCGTTAACAGCAAGG | TTTGCTGCGGCTGCTTATC | ChIP-qPCR |
| <i>Fln -610bp</i> | AACGGCGTGAAAACAATCGC | TACGAGCGCCTATCGATGTG | ChIP-qPCR |
| <i>Arp53D-108</i> | TAGCTGCTTACGTATCGACTGC | TGTTGTGCTGTGTTCCAG | ChIP-qPCR |
| <i>Negative site</i> | TGTGTATGCCTTGCTGCAC | TCTATGCACACGCTACTGAG | ChIP-qPCR |

