Sh3bgr overexpressing embryos (**C**). Enah protein was labeled in green and MHC was labeled in red. The Z-line and M-band are indicated within a sarcomere. Both knockdown and overexpression of Sh3bgr delocalize Enah from the Z-lines. **D-E**. Enah staining in control (**D**) and Sh3bgr overexpressing (**E**) animal cap cells. In Sh3bgr overexpressing animal cap cells, the Enah protein is highly enriched in tri-junctional foci (asterisk). **F-G**. The western blot analysis showed that the endogenous Enah protein level was sharply increased in whole embryos (**F**) or animal cap tissues (**G**) upon overexpression of Sh3bgr.

Scale bar (A-C) = 2μm Scale bar (D, E) = 10μm

Figure S1. The expression pattern of *sh3bgr* and *sh3bgrl*

At stage 11, *sh3bgr* is broadly expressed in most of the dorsal mesoderm (**A**) and at stage 26, *sh3bgr* expression is observed in the somites and heart tissues (**C, C'**). In contrast, *sh3bgrl* expression is restricted to the posterior region of the dorsal mesoderm which develops into the presomitic tissues (**B**). **D**. *sh3bgr* is expressed as multiple splicing variants. The longer variant is marked by a red arrow and the blue arrow indicates the shorter variant. The sequence alignment for two major splicing variants are shown in (**E**).

Figure S2. The knockdown phenotypes of *sh3bgr*.

A. The myogenic regulatory factors are well expressed in the *sh3bgr* morphants. **B**. Microinjection of sh3bgr antisense morpholino (Sh3bgr-MO) effectively inhibited the mature mRNA formation of the gene. Lane1: control, Lane 2: Sh3bgr-MO 40ng, Lane 3: Sh3bgr-MO 50ng, Lane 4: Sh3bgr-MO 65ng. **C**. Knockdown of Sh3bgr caused a hypomorphic heart and somites. **D**. The Sh3bgr-flag protein localizes to the muscle fibers with regular intervals.

Figure S3. Knockdown of *sh3bgr* disrupted sarcomere formation.

A-B. Overexpression of sh3bgr altered the thick filament morphology (**B**) compared to control (**A**). **A'-B'**. The intensity plots show the intensity of yellow lines indicated in panels (A, B). **C**. The average length of thick filament in each samples is shown in the bar-graph. **D**. The western blot analysis revealed that knockdown or overexpression of Sh3bgr did not affect the expression level of MHC, myomesin, or α-actinin significantly. **E-G**. The Enah protein localized to the Z-lines in control (**E**) and either knockdown (**F**) or overexpression (**G**) of Sh3bgr disrupted the localization of Enah. Also, overexpression of Sh3bgr resulted in abnormally discontinuous thick filaments compared to control (**G'**). **H**. Overexpression of sh3bgr in the animal cap tissues increased the intensity of Enah protein signals. The Enah signals were normalized to the DAPI channel and shown in the graph. Scale bar = 5μm