SUPPLEMENTARY MATERIAL

Multimerization of the GATA4 transcription factor regulates transcriptional activity and cardiomyocyte hypertrophic response

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Supplementary figure and figure legend

figure S1. Both GATA4 C-terminal zinc finger domain and GATA4 multimerization region were required for DNA binding of GATA4

A biotin-labeled ET-1 probe, which include GATA4 binding sequence, was mixed with GST alone or GST-GATA4 mutants, and then bound to streptavidin beads. The DNA-bound GST fusion GATA4 mutants were detected by Western blotting using anti-GST antibody. The arrow indicates GST alone or GST-GATA4 mutants.

Supplementary method

DNA binding assay

The GST fusion proteins were expressed in *E. coli* BL21 DE3 and mixed with a biotin-labelled ET-1 probe (sense, 5'-BioCCTCTAGAGCCGGGTCTTATCTCCGGCTGCACGTTGC-3'; anti-sense, 5'-GCAACGTGCAGCCGGAGATAAGACCCGGCTCTAGAGG-3') in DNA binding buffer (0.3 mg/ml bovine serum albumin (BSA), 0.1 mg/ml salmon sperm, 20 mM Hepes pH 7.9, 1.5 mM MgCl₂, 400 mM NaCl, 0.2 mM EDTA, 25% glycerol, 0.02% Tween20)
and incubated for 2 h at 4 °C. Streptavidin Sepharose High Performance Beads were 
added to the protein-DNA mixtures and incubated for 2 h at 4°C. The beads were then 
washed four times with wash buffer (20 mM Tris pH 8.0, 2.5 mM MgCl₂, 100 mM KCl, 
5% glycerol, and 0.1% Tween20), resuspended in 0.1 M glycine (pH 2.5 at 4 °C for 5 
min), and subsequently analyzed by Western blotting using anti-GST antibody (Medical 
& Biological Laboratories, Tokyo, Japan).