SUPPLEMENTARY MATERIAL

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

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Keywords: GATA4, Multimerization, Acetylation, Cardiomyocyte, Hypertrophy

Supplementary figure and figure legend

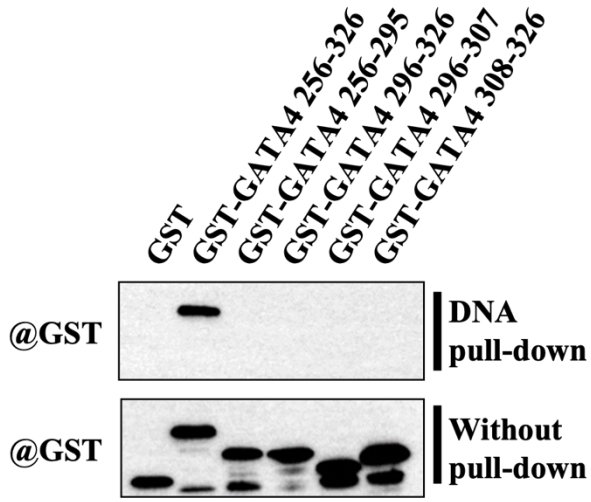

figure S1. Both GATA4 C-terminal zinc finger domain and GATA4 multimerization region were required for DNA binding of GATA4

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 $MgCl_2$, 400 mM NaCl, 0.2 mM EDTA, 25% glycerol, 0.02% Tween20)

and incubated for 2 h at 4 ℃. Streptavidin Sepharose High Performance Beads were added to the protein-DNA mixtures and incubated for 2 h at 4℃ . The beads were then washed four times with wash buffer (20 mM Tris pH 8.0, 2.5 mM $MgCl_2$, 100 mM KCl, 5% glycerol, and 0.1% Tween20), resuspended in 0.1 M glycine (pH 2.5 at 4 ℃ for 5 min), and subsequently analyzed by Western blotting using anti-GST antibody (Medical & Biological Laboratories, Tokyo, Japan).