Supplementary Data

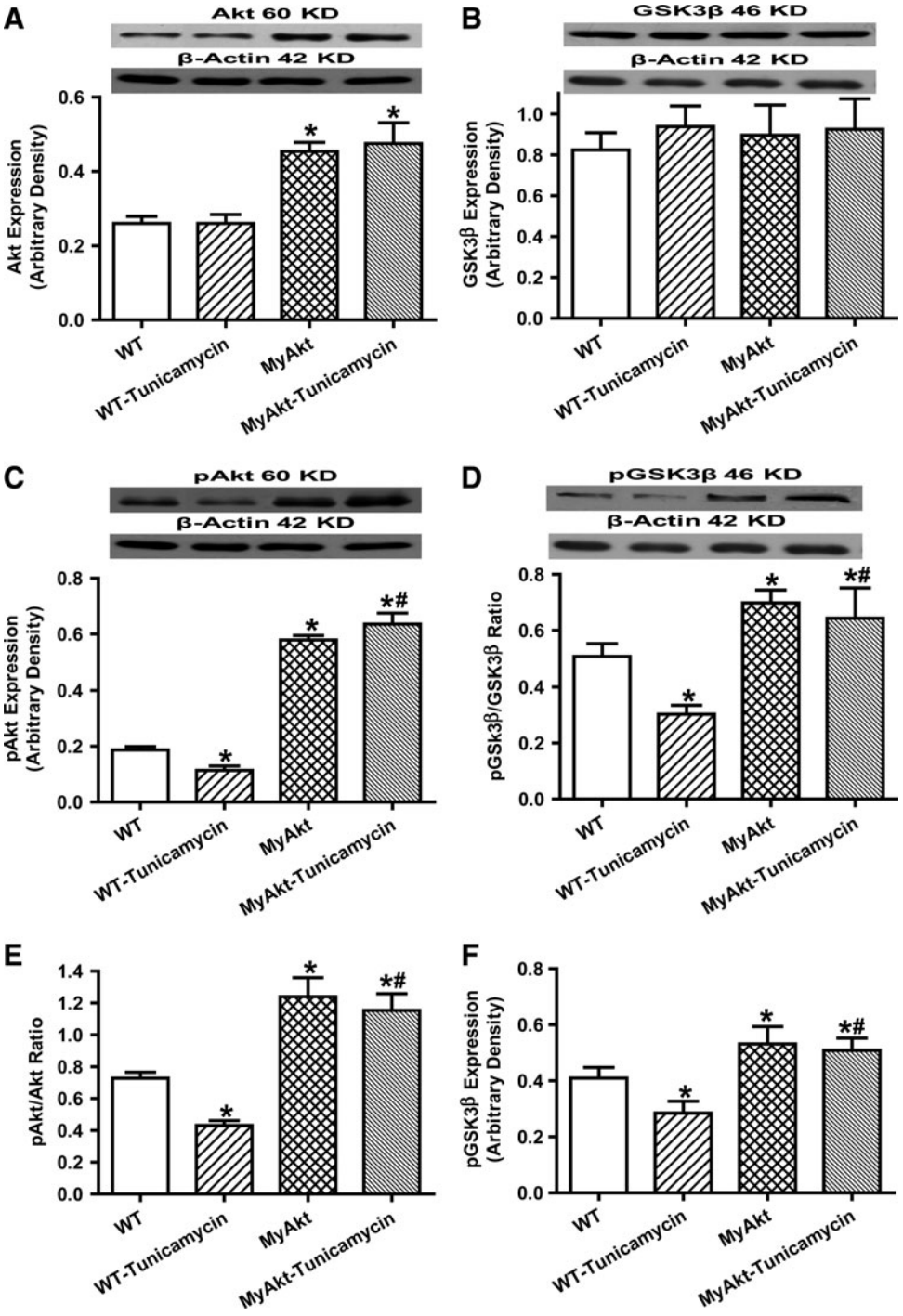
Materials and Methods

Genotyping of transgenic mice with cardiac-specific overexpression of Akt

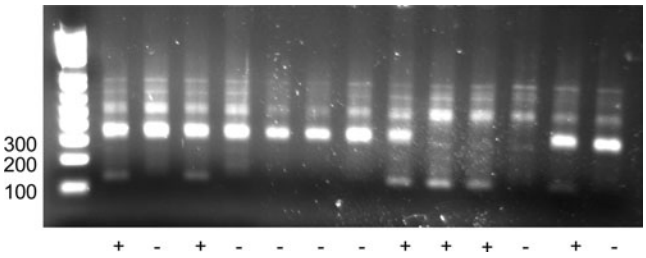
Production of MyAkt mice was described in detail previously (1). Cardiac-specific Akt transgenic mice (TG564 line) were genotyped by polymerase chain reaction using the following primer pair: forward; 5′-AGCCTAGCCCACACCAG AAA; reverse; 5′-GCCAGCCCTCCTTCACAAT (Supplementary Fig. S1). Transgenic positive mice and their transgenic negative littermates (wild-type) were used for experiments.

Reference

1. Matsui T, Li L, Wu JC, Cook SA, Nagoshi T, Picard MH, *et al.* Phenotypic spectrum caused by transgenic overexpression of activated Akt in the heart. *J Biol Chem* 277, 22896–22901, 2002.



SUPPLEMENTARY FIG. S1. Effect of tunicamycin on the expression of pan and phosphorylated Akt and glycogen synthase kinase 3β (GSK3β) in isolated cardiomyocytes from wild-type (WT) and MyAkt mice. Isolated murine cardiomyocytes were incubated with tunicamycin (3 μg/ml) for 5–6 h *in vitro* before assessment of protein abundance. **(A)** Pan Akt; **(B)** Pan GSK3β; **(C)** phosphorylated Akt (pAkt); **(D)** phosphorylated GSK3β; **(E)** pAkt-to-Akt ratio; and **(F)** pGSK3β-to-GSK3β ratio. *Insets*: Representative gel blots depicting expression of pan and phosphorylated Akt and GSK3β using specific antibodies. All protein expression was normalized to that of the loading control β-actin. Mean ± SEM, $n = 5$–7 isolations per group, $^{*}p < 0.05$ *versus* WT group; $^{\#}p < 0.05$ *versus* respective WT-tunicamycin group.



SUPPLEMENTARY FIG. S2. Polymerase chain reaction identification of MyAkt transgenic mice. Genomic DNA prepared from tails was employed as a template for polymerase chain reaction using primers to detect amplicon, respectively. The positive band is the one between 100 and 200 bp. The primers are forward, 5′-AGCCTAGCCCA CACCAGAAA; and reverse, 5′-GCCAGCCCTCCTTCA CAAT.