# Supplement Material

## 1 Supplementary Methods

### 1.1 Rat *in vivo* Myocardial Ischemia/Reperfusion (IR) Model

Male Sprague-Dawley rats (with body weight of 200-250g) were maintained and housed in the Center for Experimental Animals (an AAALAC accredited experimental animal facility) at Peking University, Beijing, China.

Rats were anesthetized with pentobarbital (40 mg/kg, i.p.) and ventilated *via* a tracheostomy on a Harvard rodent respirator. A midline sternotomy was performed, and a reversible coronary artery snare occluder was placed around the left anterior descending coronary artery. Myocardial IR was performed by tightening the snare for 45 min and then loosening it for different period of time. For mRNA or protein collection, at the indicated time point, rats were euthanatized with over-dosage pentobarbital. Hearts were excised and put into ice cold saline. Ischemic areas of the hearts were dissected and put into liquid nitrogen for following analysis. Myocardial infarction was assessed at 24 h after reperfusion. Blood samples for lactate dehydrogenase (LDH) measurement were collected 4 h after the reperfusion.

### 1.2 Measurement of Myocardial Infarct Size

To measure the infarct size of rat hearts in vivo, at the end-point, the animals were anesthetized with sodium pentobarbital (50 to 100 mg/kg i.p. to effect) and heparinized (400 USP U/kg, i.p.). The heart was excised and the ascending aorta was cannulated (distal to the sinus of Valsalva), then perfused retrogradely with saline to remove blood. The coronary artery was re-occluded at the site of occlusion before perfusion with Alcian blue solution (0.05%) to visualize the area at risk. Hearts were frozen at -80°C for 10 min and cut into slices (5-6 slices/heart), which were then incubated in a sodium phosphate buffer containing 1 % 2,3,5-triphenyl-tetrazolium chloride for 15 min to visualize the unstained infarcted region.

### 1.3 LDH Release

Blood samples were collected 4 h after reperfusion from rats subjected to IR, and centrifuged for 10 min at 3000 rpm for serum. LDH was spectrophotometrically assayed using a kit from Sigma Chemical Co. LDH activity was expressed as units per liter.

### 1.4 Primer Sequences

The following primer pairs were used: 18S RNA, 5'-GGA AGG GCA CCA CCA GGA GT-3' (forward) and 5'-TGC AGC CCC GGA CAT CTA AG-3' (reverse). The primers for CaMKII-δB and CaMKII-δC were used as reported previously [1]. The primers for iHSP70 (or HSP72) and Hst70 were used, as described previously [2, 3]. Amplification was performed as follows: 94°C for 30 s and 30 cycles at 94°C for 30 s, 55°C for 30 s, and 72°C for 30 s. The cycle number at which the emission intensity of the sample rises above the baseline was referred to as Ct (threshold cycle) and was

proportional to target concentration.

### 1.5 cDNA Microarray Analysis

Total RNA (2 μg) was reverse-transcribed to synthesize double stranded cDNA with T7-oligo dT primer. All the reverse transcription products were linearly amplified to produce biotin-labeled cRNA using the MessageAmpTM II aRNA Amplification Kit. The cRNA targets were fragmented to strands of 35-200 bp in length, then hybridized to Affymetrix GeneChip rat genome array which contains 30,000 transcripts. Hybridization was performed at 45$^{o}$C for 16 h. We washed and stained the arrays on Affymetrix Fluidics Station 450, followed by scanning on GeneChip Scanner 3000.

The hybridization data were analyzed employing GeneChip Operation software (GCOS 1.4) and normalized using dChip software for the different arrays. In a comparison analysis, we applied a two-class unpaired method in the Significant Analysis of Microarray software (SAM) to identify significantly and differentially expressed genes. Differentially expressed genes were identified with a selection threshold of fold change > 1.5 or < 0.5 and a false discovery rate of 5%.

### 1.6 Small Interfering RNA (siRNA) and Sequences of siRNA

For gene silencing assay, siRNAs comprising 19 nucleotides and a dTdT overhang at each 3-terminus were designed using Invitrogen's website (with Gene ID # 294254 for Hspa1b, heat shock 70 protein 1B, encoding iHSP70, Rattus norvegicus; and Gene ID # 60460 for Hspa2, heat shock protein 2, encoding Hst70, Rattus norvegicus). Cardiomyocytes were transfected with siRNA by Lipofectamine™ RNAiMAX (Invitrogen) following the manufacturer's instructions. The efficiency of gene knockdown was detected by real-time PCR and Western blot at 60 h and 72 h after siRNA transfection, respectively. For DNA laddering experiments, siRNA was delivered 36 h before adenoviral infection.

The sequences of siRNA for iHSP70 are as follows: siRNA-1: 5'-C C A A G G U G C A G G U G A A C U A dTdT-3'; siRNA-2: 5'-C C U G A A C A A G A G C A U C A A U dTdT-3' and scramble siRNA: 5'-C U A C A A G A A C G C U G A A A U dTdT-3'. The sequences of siRNA of Hst70 are as follows: siRNA-1: 5'-C C A U C G A G G A U G G C A U C U U dTdT-3'; siRNA-2: 5'-G C U C A A C G C C G A U C U C U U U dTdT-3'; siRNA-3: 5'-G G A G G U G A U C A A C U G G C U U dTdT-3'; and the scramble siRNA is the same as described above.

### 1.7 Real-time PCR

Quantitative real-time PCR was performed on a Bio-Rad iQ5 Multicolor Real-time PCR Detection System in combination with SYBR Green (Roche Applied Science, Mannheim, Germany), as described previously.[4].

### 1.8 Western Blot Analysis and Confocal Immunocytochemical Imaging of Hemagglutinin (HA)-tagged CaMKII-δB or CaMKII-δC

Western blot to assay the abundance of CaMKII-δ in cytosolic and nuclear extracts was performed with an anti-CaMKII-δ antibody (Santa Cruz Biotechnology, Inc., sc-5392), as previously described [5]. The antibodies reacting with total and phosphorylated CaMKII were purchased from Cell Signaling Technology (#3362 and #3361, respectively), whereas the antibody reacting with iHSP70 was purchased from Santa Cruz Biotechnology, Inc. (sc-66048). Antibody for HSF1 or phosphorylated HSF1 was from Santa Cruz Biotechnology, Inc. Confocal immunocytochemical imaging was performed as previously described [6].

### 1.9 Isolation, Culture and Adenoviral Infection of Rat Ventricular Myocytes

All procedures involving experimental animals were performed in accordance with protocols approved by the Committee for Animal Research of Peking University, China, and conformed to the Guide for the Care and Use of Laboratory Animals (NIH publication No. 86-23, revised 1985).

Neonatal ventricular myocytes were isolated from 1-day-old Sprague-Dawley rats and cultured by methods described previously [4]. Adult ventricular myocytes were isolated and infected with adenovirus as described previously [6]. Adenovirus mediated gene transfer was implemented after 24 h quiescence in serum free Dulbecco's modified Eagle medium (DMEM) following 48 h culture in DMEM containing 10% FBS. Adenoviral vector expresses CaMKII-δB or CaMKII-δC or β-gal were described previously [6].

### 1.10 Cell Viability

DNA fragmentation was visualized by DNA laddering assay, as previously described [4]. Cell apoptotic nuclear morphological feature was detected by Hoechst staining by incubation of fixed cells (70% alcohol and 30% acetone) in 10 mM Hoechst 33342. The percentage of Hoechst staining-positive cells was determined by randomly counting 500-800 cardiac myocytes over 20 randomly chosen fields in each culture dish. 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay was used as another cell viability index, which was performed as described previously [4]..

## Supplementary References


**1.** Xu X, Yang D, Ding JH, Wang W, Chu PH, Dalton ND, Wang HY, Bermingham JR, Jr., Ye Z, Liu F, Rosenfeld MG, Manley JL, Ross J, Jr., Chen J, Xiao RP, Cheng H, Fu XD. ASF/SF2-regulated CaMKIIdelta alternative splicing temporally reprograms excitation-contraction coupling in cardiac muscle. *Cell.* 2005;120:59-72.

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**3.** Scieglinska D, Widlak W, Rusin M, Markkula M, Krawczyk Z. Expression of the testis-specific HSP70-related gene (hst70 gene) in somatic non-testicular rat tissues revealed by RT-PCR and transgenic mice analysis. *Cell Biol Int.* 1997;21:813-821.

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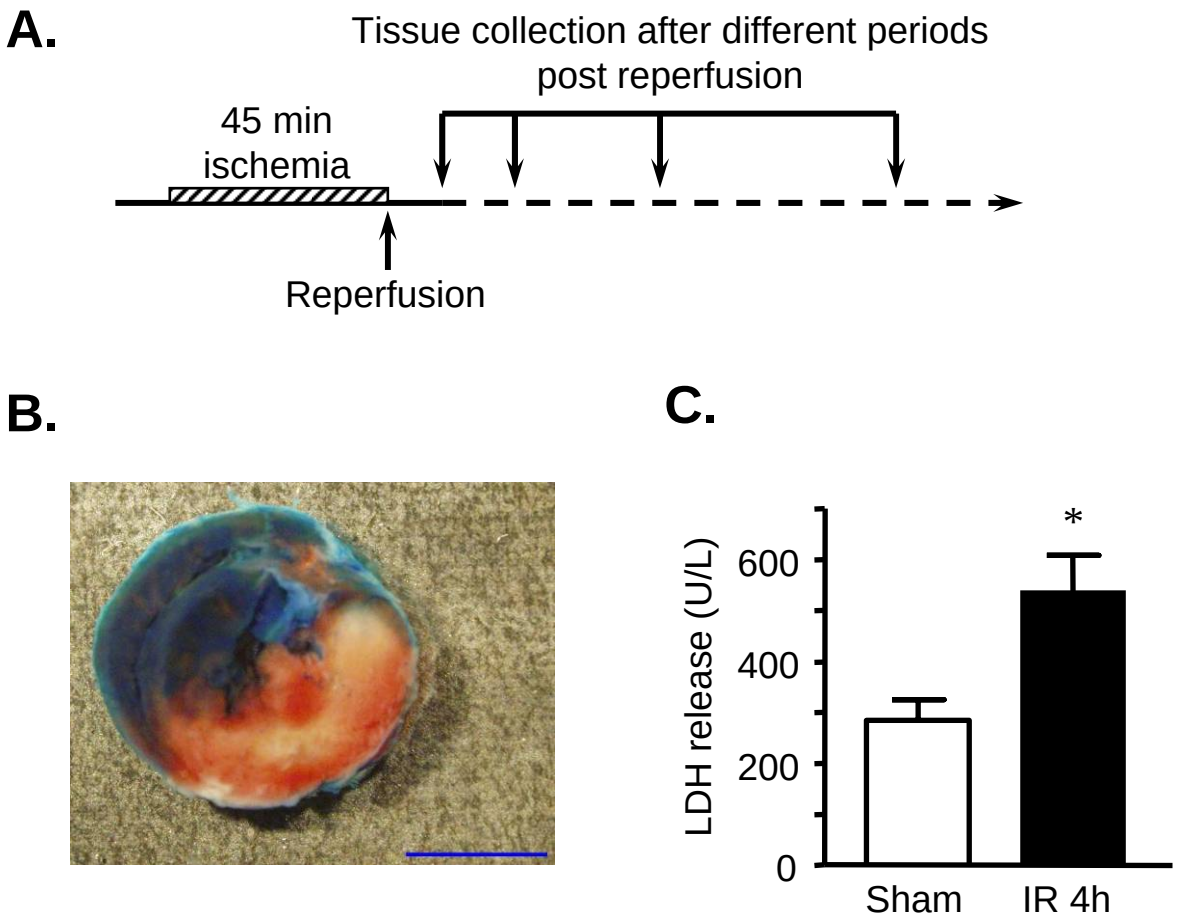
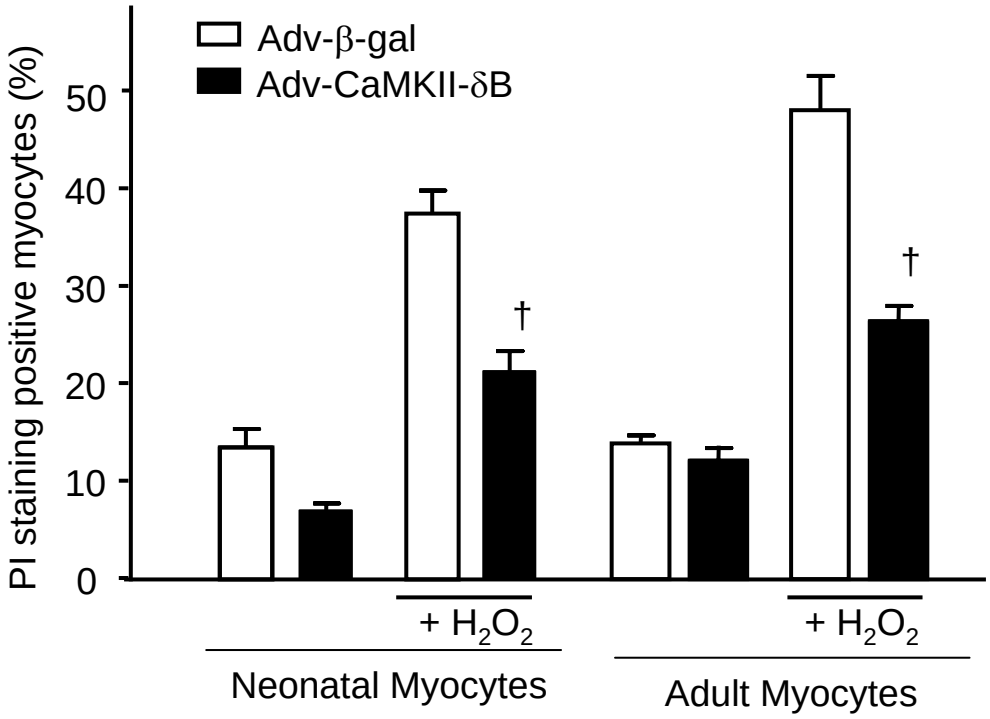
**5.** Zhu W, Woo AY, Yang D, Cheng H, Crow MT, Xiao RP. Activation of CaMKIIdeltaC is a common intermediate of diverse death stimuli-induced heart muscle cell apoptosis. *J Biol Chem.* 2007;282:10833-10839.

**6.** Zhu WZ, Wang SQ, Chakir K, Yang D, Zhang T, Brown JH, Devic E, Kobilka BK, Cheng H, Xiao RP. Linkage of beta1-adrenergic stimulation to apoptotic heart cell death through protein kinase A-independent activation of Ca2+/calmodulin kinase II. *J Clin Invest.* 2003;111:617-625.



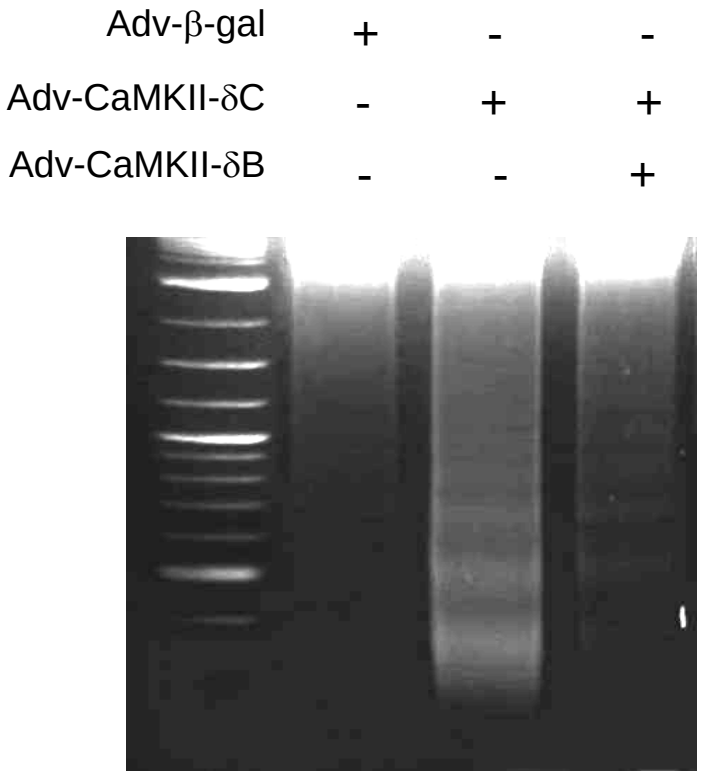

**Online Figure I: CaMKII-δB and CaMKII-δC expression in rat heart subjected to IR injury.** ***A.*** shows schematic figure of rat *in vivo* IR protocol. ***B.*** Rat heart slice after alcian blue perfusion and TTC staining 24 h after IR injury to show myocardial infarction, scale bar is 5 mm. ***C.*** shows serum LDH concentration in sham and IR rats. * P<0.01 (n = 5).

**Online Figure I**



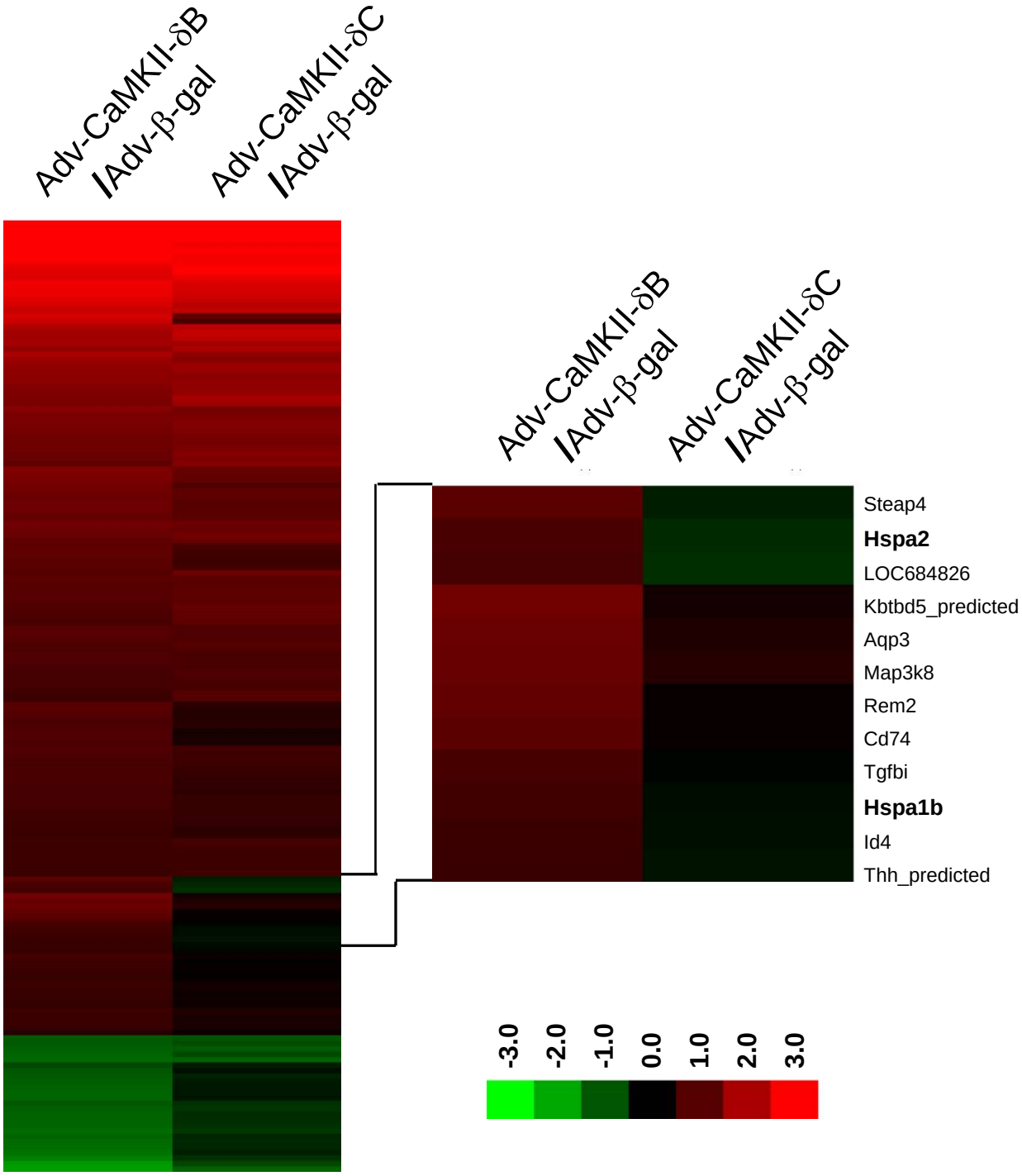

**Online Figure II: Overexpression of CaMKII-δB protects both neonatal and adult rat cardiomyocytes against $H_2O_2$ (200 μM for neonatal and 10 μM for adult cardiomyocytes)- induced cell death.** Average data of PI staining positive cell († P<0.01, *v.s.* untreated and β-gal $H_2O_2$; n = 3-4. Data are shown by mean ± SE).

## Online Figure II



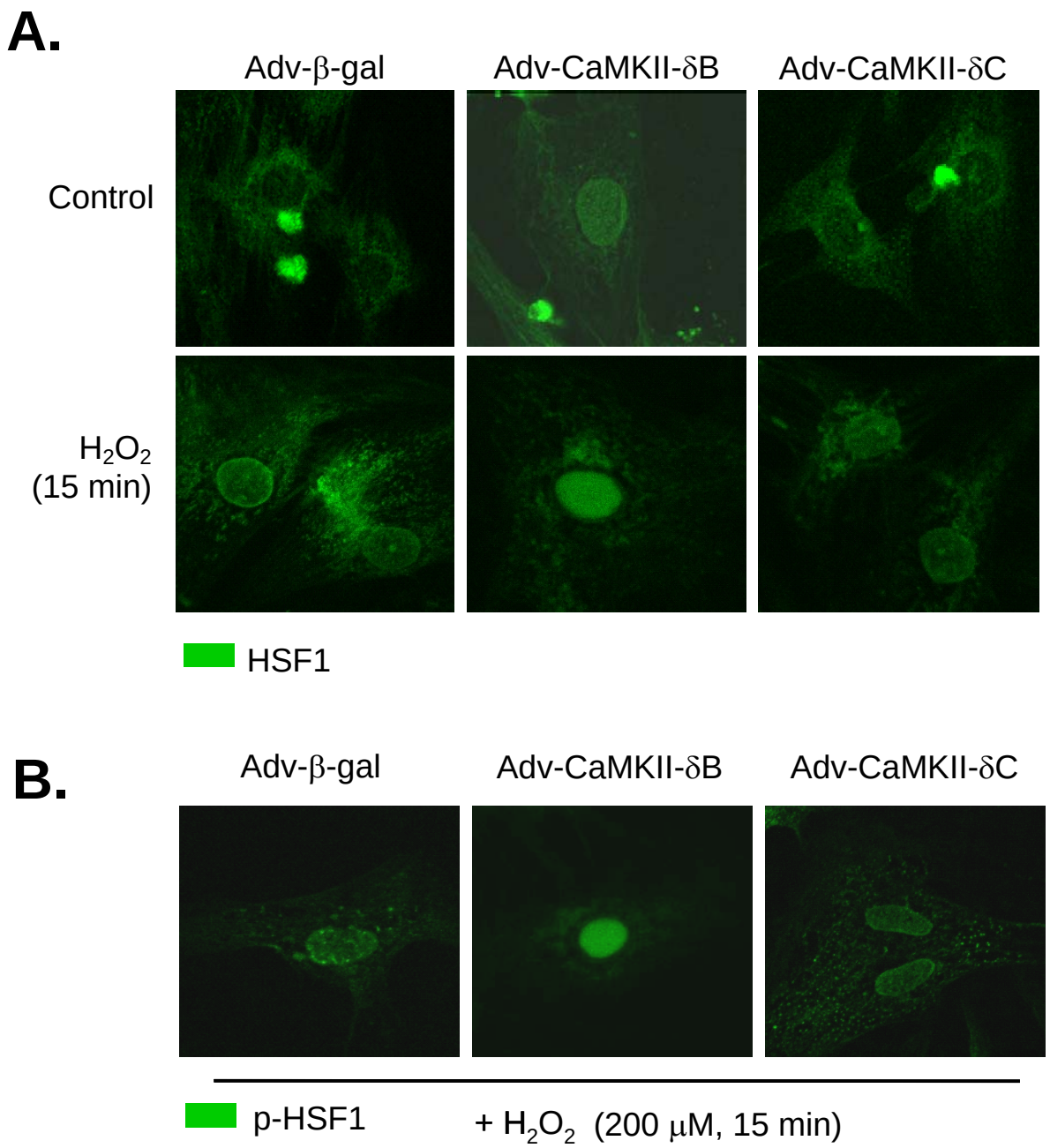

**Online Figure III: CaMKII-δB overexpression blocks CaMKII-δC-induced myocyte apoptosis.** Cardiomyocyte apoptosis assayed by DNA laddering in neonatal cardiomyocytes infected with Adv-β-gal or Adv-CaMKII-δC in the presence or absence of Adv-CaMKII-δB (all at 20 m.o.i. for 48 h). Similar results were obtained in three independent experiments.

## Online Figure III




**Online Figure IV**

**Online Figure IV: Gene expression profiling of cardiomyocytes infected with Adv-CaMKII-δB, Adv-CaMKII-δC, or Adv-β-gal.** The left panel shows a cluster analysis of 174 genes that are differentially and significantly (>1.5-fold or < 0.5-fold, n = 3) regulated by infection of cells with Adv-CaMKII-δB relative to those in cells infected with Adv-β-gal (all at 20 m.o.i. for 36 h). Green represents downregulation, while red is for upregulation relative to that in cells infected with Adv-β-gal. The right panel displays the cluster analysis and gene symbols of 12 differentially regulated genes, including Hspa2 and Hspa1b encoding Hst70 and iHSP70, respectively.




**Online Figure V: $H_2O_2$-induced nuclear translocation and transient increase in phosphorylation of HSF1 at Ser230. *A.-B.*** Confocal immunofluorescent imaging to visualize the localization of total HSF (**A**) or phosphorylated HSF1 (***B***) using site-specific antibodies in neonatal cardiomyocytes in the presence or absence of $H_2O_2$ (200 μM) treatment for 15 min. Similar results were obtained in other three independent experiments for both panels A and B.

**Online Figure V**

# Online Table I: Gene Expression Profiling of Neonatal Cardiomyocytes Infected with Adv-CaMKII-δB, Adv-CaMKII-δC or Adv-β-gal

| | Gene Title | Gene Symbol | Fold of Change (CaMKII-δB/β-gal) | Fold of Change (CaMKII-δC/β-gal) | Function |
|---|---|---|---|---|---|
| **1** | Kelch repeat and BTB (POZ) domain containing 5 (predicted) | Kbtbd5_ predicted | 2.505 | 1.164 | Involved in the adaption to nutritional deprivation |
| **2** | Aquaporin 3 | Aqp3 | 2.361 | 1.290 | Positive regulation of immune system process, Response to retinoic acid, Water transport |
| **3** | Rad and gem related GTP binding protein 2 | Rem2 | 2.235 | 1.070 | Regulation of transcription， small GTPase mediated signal transduction |
| **4** | Heat shock protein 2 | Hspa2 | 1.809 | 0.714 | Cell differentiation, Multicellular organismal development, Response to stress, Spermatogenesis |
| **5** | Transforming growth factor, beta induced | Tgfbi | 1.769 | 0.966 | Response to stimulus, Response to stress,Visual perception, Cell adhesion |
| **6** | Similar to Basic helix-loop-helix transcription factor scleraxis | LOC684826 | 1.743 | 0.685 | Regulation of transcription, Multicellular organismal development |
| **7** | Heat shock 70kD protein 1B | Hspa1b | 1.706 | 0.905 | DNA repair, Response to heat, Response to unfolded protein, Telomere maintenance |
| **8** | Aldehyde dehydrogenase 3 family, member B1 | Aldh3b1 | 1.667 | 1.048 | Metabolic process of lipid,alcohol and aldehyde |
| **9** | CDW92 antigen | Cdw92 | 1.654 | 1.309 | Choline transport |
| **10** | Inhibitor of DNA binding 4 | Id4 | 1.604 | 0.891 | Brain development， Positive regulation of cell proliferation, Regulation of transcription, et. al. |
| **11** | Nuclear receptor subfamily 4, group A, member 1 | Nr4a1 | 1.594 | 1.033 | Negative regulation of caspase activity, Regulation of transcription, Induction of apoptosis |
| **12** | Leucine zipper-EF-hand containing transmembrane protein 2 | Letm2 | 1.569 | 0.920 | A mitochondrial protein |
| **13** | Trichohyalin (predicted) | Thh_ predicted | 1.574 | 0.864 | Intermediate filament organization |
| **14** | Diacylglycerol kinase, alpha | Dgka | 1.554 | 0.932 | Activation of protein kinase C activity, Intracellular signaling cascade |
| **15** | Coiled-coil domain containing 109B | Ccdc109b | 1.543 | 1.027 | Involved in acute lymphoblastic leukemia |
| **16** | Procollagen, type IV, alpha 4 | Col4a4 | 0.385 | 0.780 | Phosphate transport |
| **17** | Actin-binding Rho activating protein | Abra | 0.282 | 0.566 | Positive regulation of transcription, Positive regulation of Rho protein signal transduction, Protein transport |