Supplementary Materials

Methods

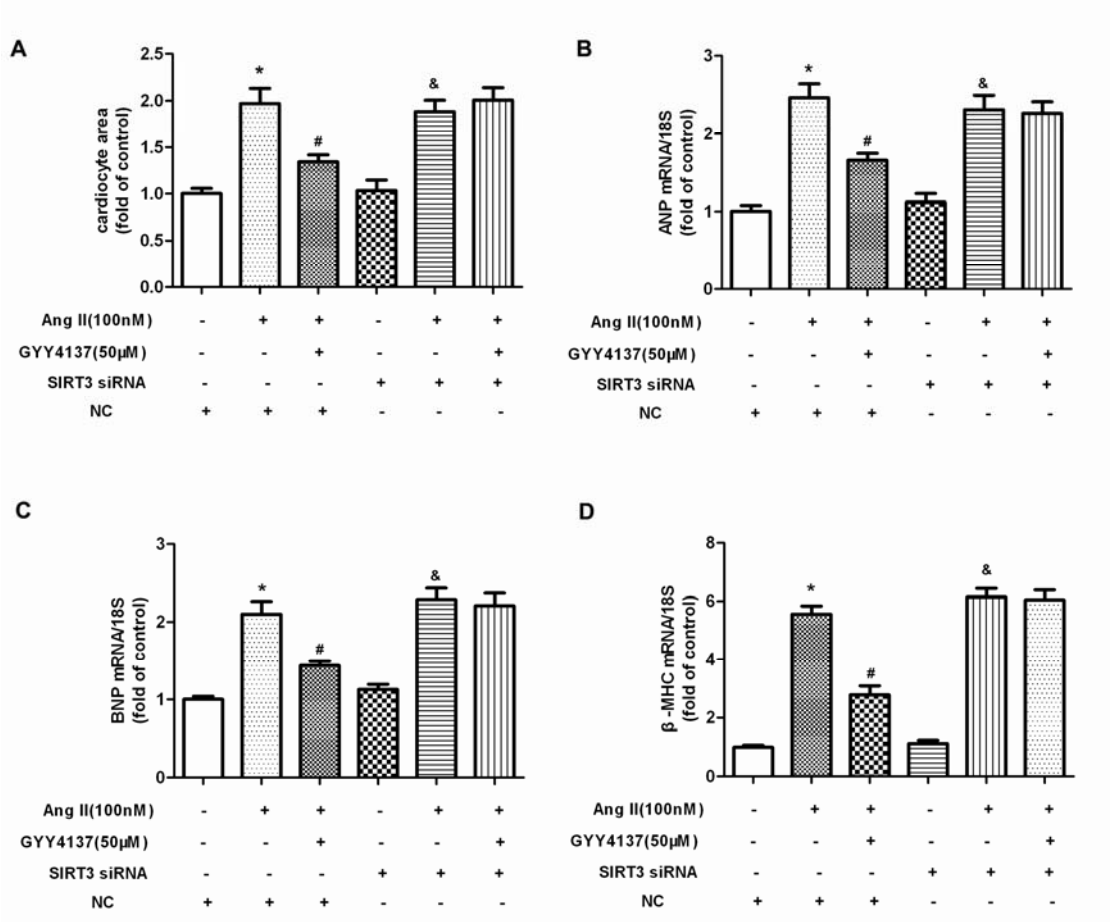
Adult mice cardiomyocyte isolation and culture

Mice after TAC with or without H_2S administration were used for adult cardiomyocytes isolation and culture as previously described (Zhou *et al.*, 2010). Mice were heparinized (5000 U/Kg) and anesthetized with a mixture of ketamine (100 mg/kg) and xylazine (5 mg/kg) by intraperitoneal injection. The heart was quickly removed from the chest and retrogradely aortic perfused as a constant pressure (100 cmH_2O) at 37 ^oC for 3 min with a Ca^{2+} free bicarbonate-based buffer (composition in mM: NaCl 120, KCl 5.4, $MgSO_4$ 1.2, NaH_2PO_4 5.6, $NaHCO_3$ 20, glucose 5.6, 2,3-butanedion monoxime 10, taurine 5), grassed with 95% O_2-5% CO_2. The enzymatic digestion was initiated by adding collagenase type II (0.5 mg/ml, Gibco) and protease type XIV (0.02mg/ml, Sigma) to the perfusing solution. When the heart became swollen and hard after about 3 min of digestion, 50 μM Ca^{2+} was added into the enzyme solution. About 7 min later, the left ventricle was quickly removed, cut into several chunks, and further digested in a shaker (60-70 rpm) for 10 min at 37 ^oC in the same enzyme followed by recovering calcium gradually. Total RNA were extracted from some of the cardiomyocytes and subjected to PCR analysis. Some other cardiomyocytes were seeded to the culture dishes (pre-coated with 10 μg/ml laminin) in Modiied Eagle's Medium, supplemented with 2 mM ATP, 2 mM glutamine, 10 % fetal bovine serum (FBS), 10 mM 2,3-butanedione monoxime (BDM), 100 U/ml penicillin and 100 μg/ml streptomycin for 1 h and photographed using an inverted microscope.

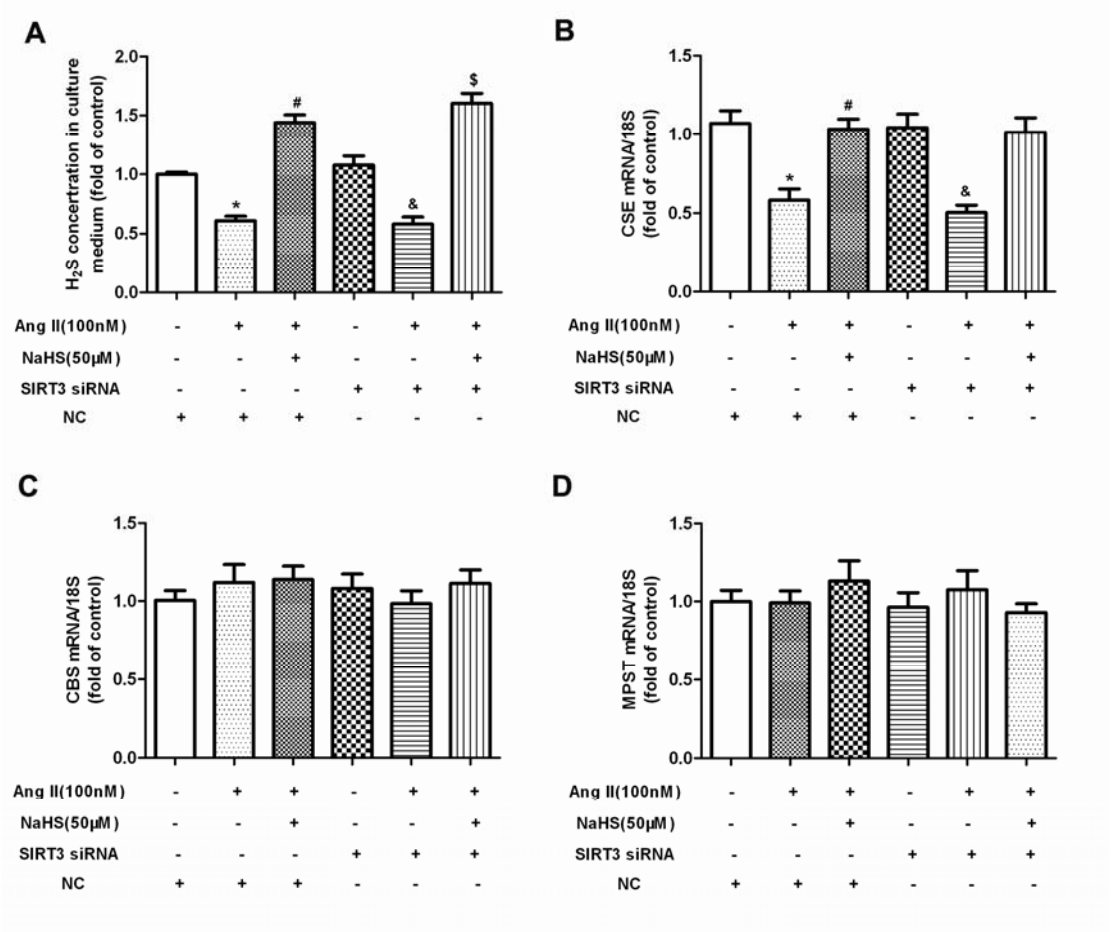
References

Zhou YY, Wang SQ, Zhu WZ, Chruscinski A, Kobilka BK, Ziman B *et al* (2000). Culture and adenoviral infection of adult mouse cardiac myocytes: methods for cellular genetic physiology. Am J Physiol Heart Circ Physiol 279: H429-H436.

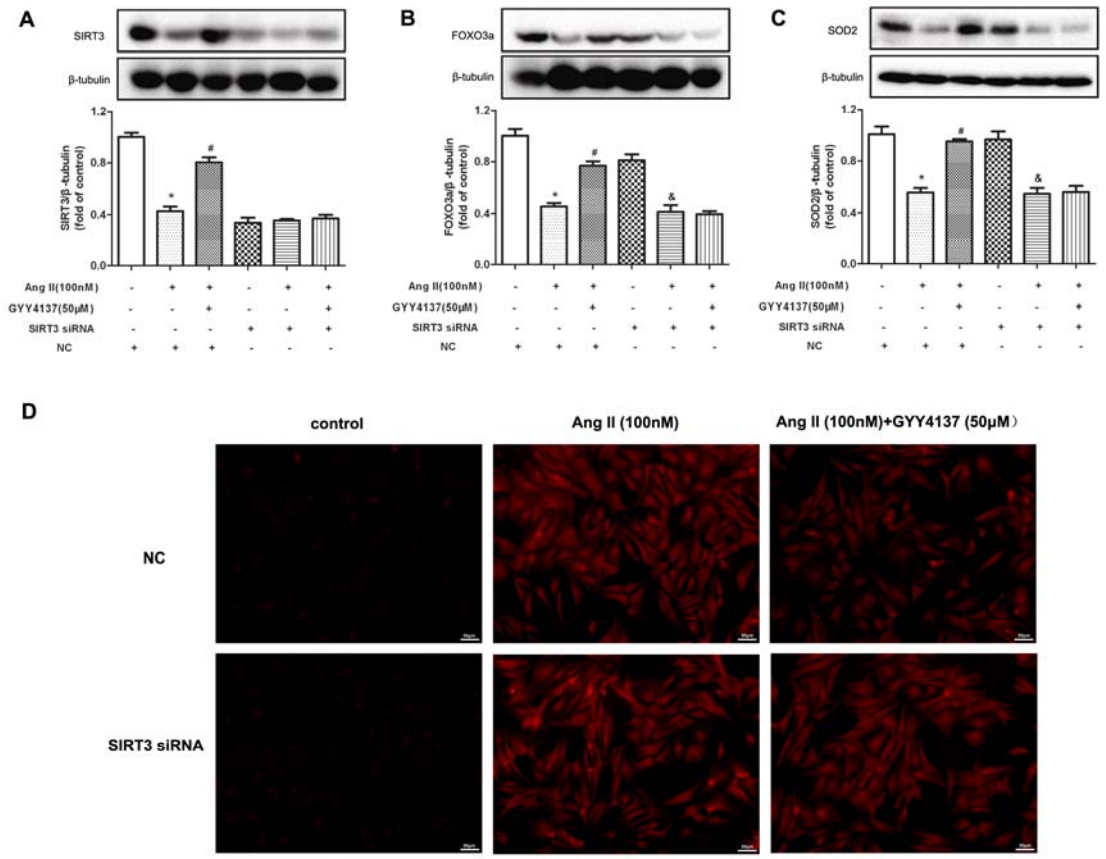
Supplementary Figures and legends



SFigure 1 Effect of GYY4137 on Ang II induced cardiomyocyte hypertrophy. After SIRT3 siRNA or NC siRNA was transfected into neonatal rat cardiomyocytes for 24 h, cells were pre-treated with GYY4137 (50 μM) for 4 h followed by Ang II (100 nM) for 24 h. (A) Cells were digested with 0.1% trypsin and photographed using an inverted microscope. Cell surface area of cardiomyocytes was calculated using Imagepro-Plus system and normalized to cells not undergoing any drug treatment (with NC siRNA transfection). (B-D) Quantification of atrial natriuretic peptide (ANP), brain natriuretic peptide (BNP) and β-myosin heavy chain (β-MHC) mRNA expression in cardiomyocytes was carried out by real-time PCR. $^{*}P<0.05$ versus cells not undergoing any drug treatment (with NC siRNA transfection); $^{\#}P<0.05$ verse cells treated with Ang II alone (with NC siRNA transfection); $^{\&}P<0.05$ versus cells not undergoing any drug treatment (with SIRT3 siRNA transfection). n= 6.

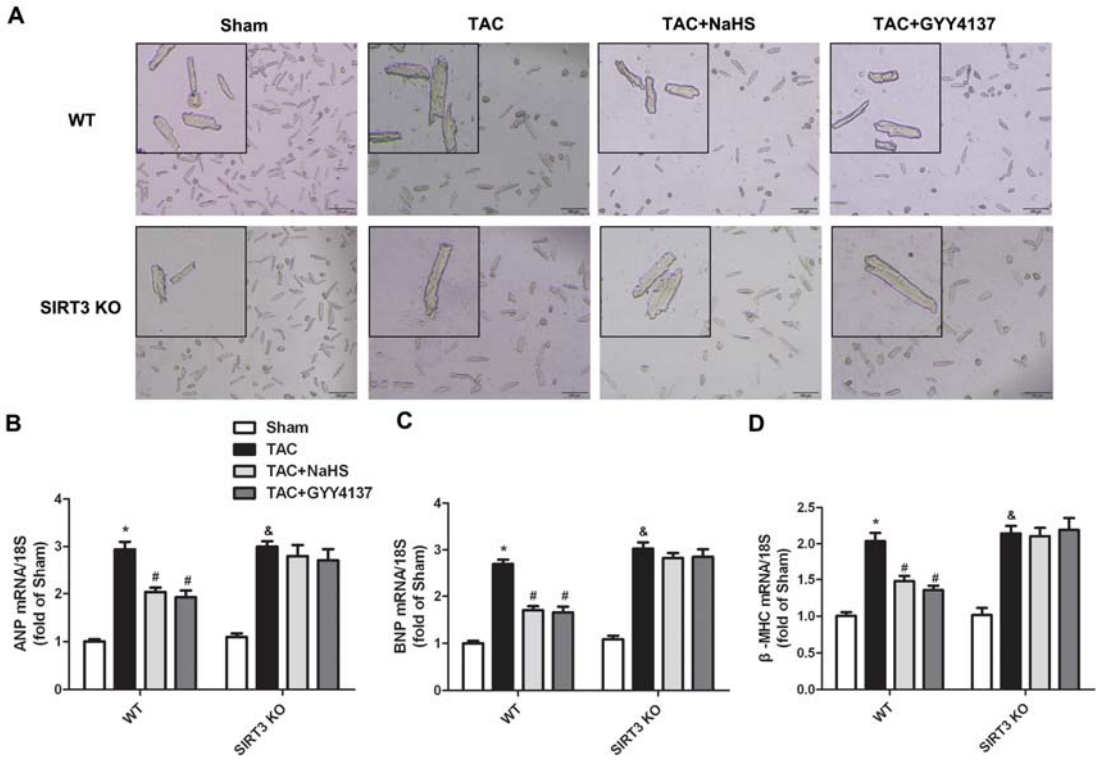


SFigure 2 Effect of NaHS on H_2S level in Ang II stimulated cardiomyocytes after SIRT3 was knocked down. After SIRT3 siRNA or NC siRNA was transfected into neonatal rat cardiomyocytes for 24 h, cells were pre-treated with NaHS (50 μM) for 4 h followed by Ang II (100 nM) for 24 h. (A) H_2S concentration in the culture medium was measured with a H_2S-specific microelectrode. (B-D) Quantification of cystathionine γ-lyase (CSE), cystathionine β-synthase (CBS) and 3-mercaptypyruvate sulfertransferase (MPST) mRNA expression in cardiomyocytes was carried out by real-time PCR. $^{*}P<0.05$ versus cells not undergoing any drug treatment (with NC siRNA transfection); $^{\#}P<0.05$ verse cells treated with Ang II alone (with NC siRNA transfection); $^{\&}P<0.05$ versus cells not undergoing any drug treatment (with SIRT3 siRNA transfection); $^{\$}P<0.05$ versus cells treated with Ang II alone (with SIRT3 siRNA transfection). n= 6.



SFigure 3

Effect of GYY4137 on SIRT3, FOXO3a, SOD2 expression and oxidative stress in Ang II stimulated cardiomyocytes. (A-C) After SIRT3 siRNA or NC siRNA was transfected into neonatal rat cardiomyocytes for 24 h, cells were pre-treated with GYY4137 (50 μM) for 4 h followed by Ang II (100 nM) for 24 h. SIRT3, FOXO3a and SOD2 protein expression was measured with western blot. $^{*}P<0.05$ versus cells not undergoing any drug treatment (with NC siRNA transfection); $^{\#}P<0.05$ verse cells treated with Ang II alone (with NC siRNA transfection); $^{\&}P<0.05$ versus cells not undergoing any drug treatment (with SIRT3 siRNA transfection). n= 5. (D) Superoxide production in cardiomyocytes was detected under a fluorescence microscope with dihydroethidium (DHE) fluorescent probe. Bar=50 μm.



SFigure 4 Effect of NaHS or GYY4137 on primary adult cardiomyocytes isolated from WT and SIRT3 KO mice after TAC. Male 129S1/SvImJ (WT) and SIRT3 KO mice were intraperitoneally injected with NaHS (50 μmol/kg/d) or GYY4137 (133 μmol/kg/d) or saline for 2 weeks. Then, mice were subjected with transverse aortic constriction (TAC) surgery. NaHS (50 μmol/kg/d) or GYY4137 (133 μmol/kg/d) or saline was administrated for another 2 weeks. (A) Primary adult cardiomyocytes were isolated from mice and photographed. Bar=200 μm. (B-D) Quantification of ANP, BNP and β-MHC mRNA expression in primary adult cardiomyocytes was carried out by real-time PCR. $^{*}P<0.05$ versus WT+SHAM; $^{\#}P<0.05$ versus WT+TAC; $^{\&}P<0.05$ versus SIRT3 KO+SHAM. n= 6.