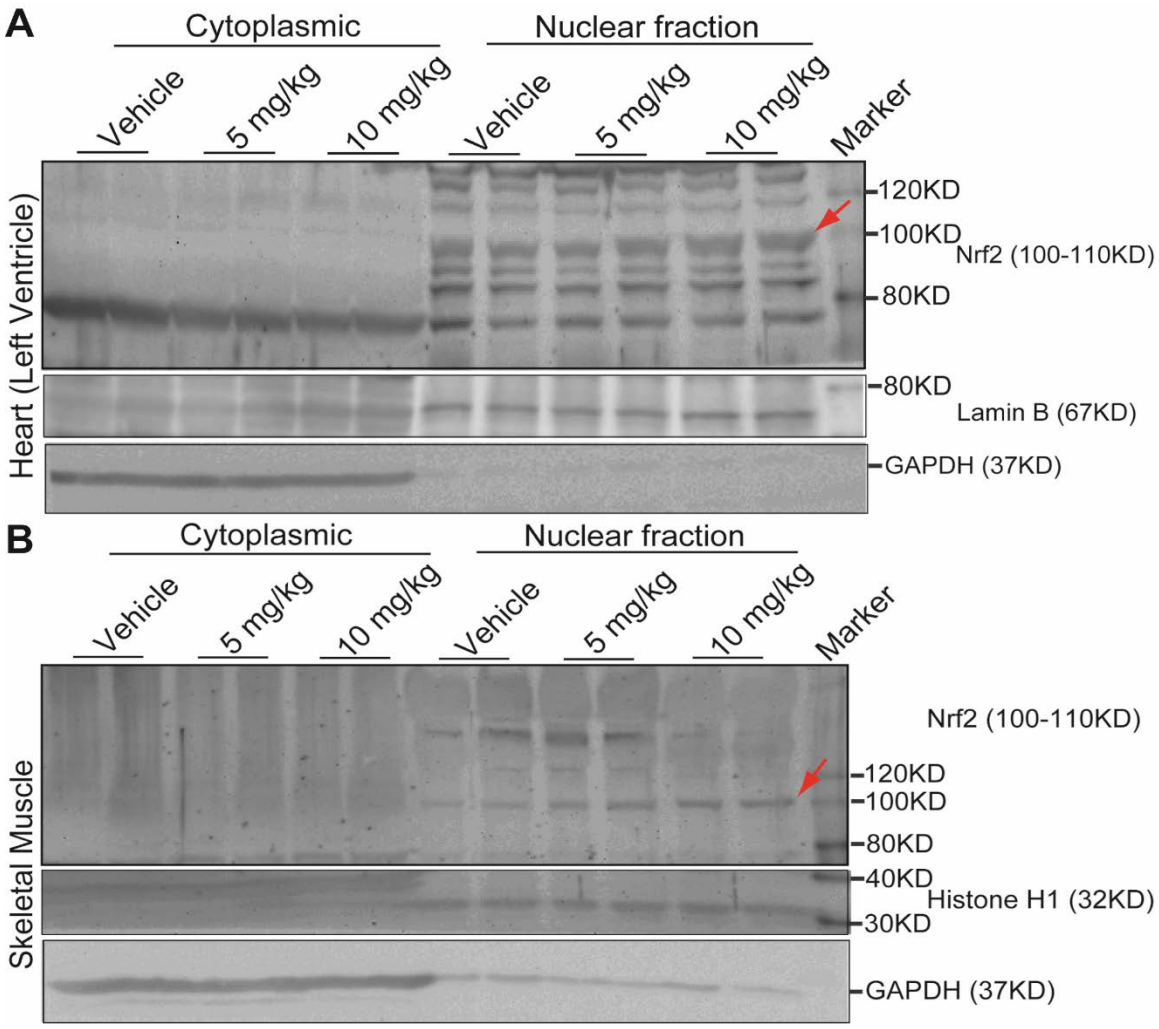
Supplemental Data

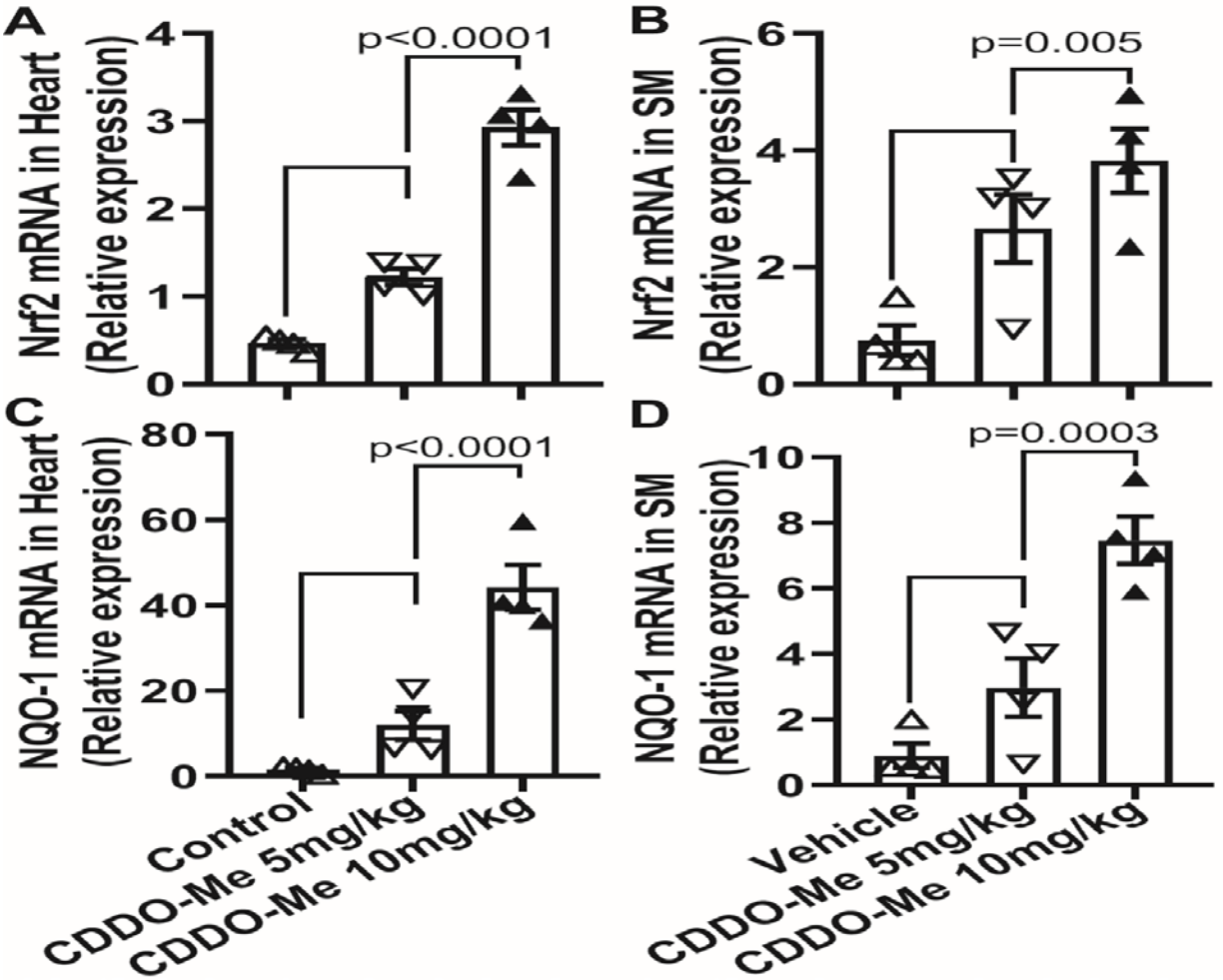
Therapeutic effects of Nrf2 activation by Bardoxolone methyl in Chronic Heart Failure

Changhai Tian, Lie Gao, Andi Zhang, Bryan T. Hackfort, Irving H. Zucker

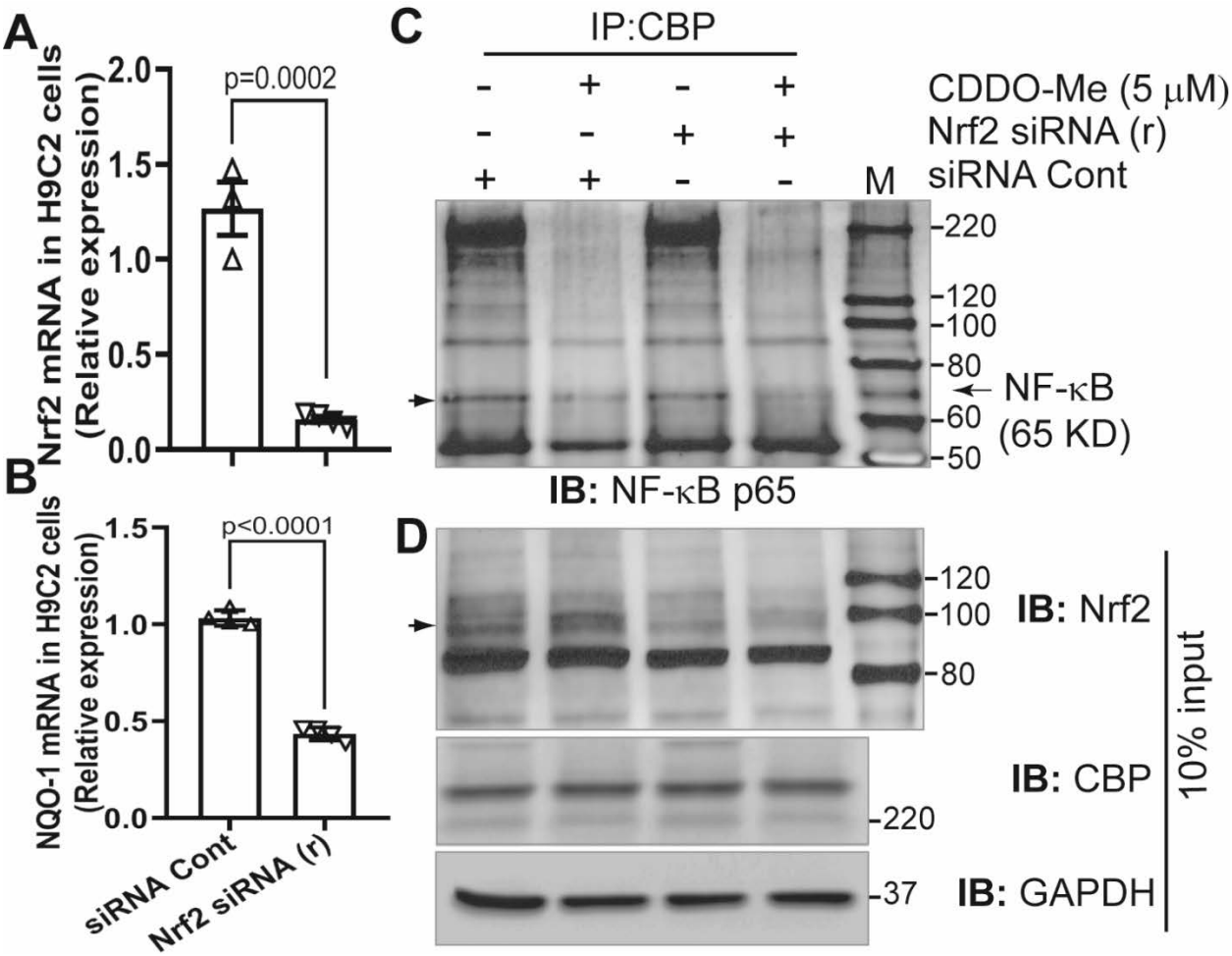
Journal of Pharmacology and Experimental Therapeutics



Supplemental figure 1 Sprague-Dawley rats were treated with two different doses of CDDO-Me as indicated for five days. The left ventricle of hearts (A) and gastrocnemius of legs (B) were freshly collected, and then subjected to subcellular fractionation using NE-PER™ Nuclear and Cytoplasmic Extraction Reagents supplemented with Halt™ Protease Inhibitor Cocktail (100X) (ThermoFisher Scientific, Rockford, lL) following the manufacturer's instructions, and western blotting analysis with a primary Nrf2 antibody. Lamin B (C-20) (1:1,000, sc-6216) and Histone H1 (1:1,000, ab181977) were used as loading controls for the cardiac tissue and skeletal muscle nuclear fraction, respectively. Histone H1 was used for skeletal muscle nuclear fraction, and GAPDH was used for whole tissue lysates.



Supplemental figure 2. Sprague-Dawley rats were treated with two different doses of CDDO-Me as indicated for five days. The left ventricle of hearts and gastrocnemius were freshly collected, and then subjected to RNA extraction using RNeasy Mini Kit (QIAGEN Inc., Valencia, CA) per the manufacturer's recommendations, and cDNA synthesis using reverse transcription MasterMix (Applied Biological Materials, Richmond, Canada). Nrf2 and NQO-1 mRNA level were determined by qRT-PCR using SYBR® Select Master Mix (Life Technologies, Los Angeles, CA) and the specified primer pairs (Integrated DNA Technologies, USA). GAPDH was used as an internal control (n=4, ±SEM). The quantification of mRNA expression was performed with the $2^{(-\Delta\Delta Ct)}$ method.



Supplemental figure 3, H9C2 rat cardiomyocytes were transfected with Control siRNA-A (sc-37007) and Nrf2 siRNA (r) (sc-156128), respectively, using Lipofectamine™ RNAiMAX Transfection Reagent per the manufacturer's recommendations (ThermoFisher Scientific, Cat. 13778030). Cells were subjected to RNA extraction using RNeasy Mini Kit (QIAGEN Inc., Valencia, CA) and then cDNA synthesis using reverse transcription MasterMix (Applied Biological Materials, Richmond, Canada). qRT-PCR was carried out using SYBR® Select Master Mix (Life Technologies, Los Angeles, CA) and the specified primer pairs, including Nrf2 (A), NQO-1 (B) and GAPDH as an internal control; H9C2 cells cultured on 100 mm dish were transfected with Control siRNA and Nrf2 siRNA, respectively. After 24 hours, transfected cells were treated with vehicle

(DMSO) and 5 μM CDDO-Me, respectively, for another 24 hours. Cell lysates were subjected to Co-Immunoprecipitation with CBP antibody (sc-7300), and subsequent western blotting analysis with NF-kB (p65) antibody (ab16502) (C). Nrf2 and CBP levels in 10% input were determined by Nrf2 (ab31163) and CBP antibodies (D), and GAPDH (MA5-15738) was used as a loading control.