# Supporting Information

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### SI Materials and Methods

**Inhibitors and Agonists.** Reagents were purchased or synthesized in-house and used at the indicated final concentrations. Histone deacetylase (HDAC) inhibitors: trichostatin A (TSA) (Sigma; 100 nM), MGCD0103 (Selleck; 1 μM), diphenylacetohydroxamic acid (DPAH) [Sigma (D6071); 10 μM], tubastatin A (Selleck; 1μM), MS-275 (Selleck; 1μM). Mitogen-activated protein kinase (MAPK) inhibitors: U0126 (Calbiochem; 10 μM), PD98059 (Calbiochem; 10 μM), SP600125 (Calbiochem; 10 μM), SB203580 (Calbiochem; 10 μM). Stress stimuli: phenylephrine (PE) (Sigma; 10 μM), norepinephrine (NE) (Sigma; 10 mg/kg), sorbitol (Sigma; 100 mM), $H_2 0_2$ (Sigma; 1 mM), phorbol myristate acetate (PMA) (Sigma; 50 nM), leukemia inhibitory factor (LIF) (Millipore; 50 nM). BA-60 (100–1000 nM) and BRD3308 (1 μM) were synthesized in-house, and their purity was confirmed to be greater than 95%. Actinomycin D was purchased from Sigma and used at 1 μg/mL.

**Cell Isolation and Culture.** Neonatal rat ventricular myocytes (NRVMs) were prepared from hearts of 1- to 3-d-old Sprague–Dawley rats, as previously described (1). Cells were cultured overnight on 10-cm or 96-well plates coated with gelatin (0.2%; Sigma) in Dulbecco's Modified Eagle's Medium (DMEM) containing calf serum (10%), L-glutamine (2 mM), and penicillin–streptomycin. After overnight culture, cells were washed with serum-free medium and maintained in DMEM supplemented with L-glutamine, penicillin–streptomycin, and Neutridoma-SP (0.1%; Roche Applied Science), which contains albumin, insulin, transferrin, and other defined organic and inorganic compounds. Short interfering RNAs (siRNAs) for two independent rat HDAC3-specific sequences (Sigma) and a scrambled control (Sigma) were transfected into NRVMs using Lipofectamine Plus (Invitrogen), as previously described (2). Adult rat ventricular myocytes (ARVMs) were obtained from female Sprague–Dawley rats, as described previously (3). ARVMs were plated at a density of 100–150 cells/$mm^2$ on laminin-coated 60-mm plates and maintained in serum-free DMEM supplemented with albumin (2 mg/mL), 2,3-butanedione monoxime (1 mg/mL), L-carnitine (2 mM), creatine (5 mM), penicillin–streptomycin (100 μg/mL), triiodothyronine (1 pM), and taurine (5 mM).

**Operetta High Content Imaging.** Fixed and stained cells on 96-well, clear-bottomed plates (Greiner) were imaged on an automated fluorescence microscopy system (Operetta; Perkin-Elmer) using a 20× objective. Thirty fields were imaged in each well of the 96-well plates. Three channels were collected for each field, corresponding to fluorescein (α-actinin), Cy3 [atrial natriuretic factor (ANF)], and DAPI (nuclei). Images were analyzed using a custom algorithm in the Harmony high-content analysis software package (Perkin-Elmer). Briefly, objects were initially defined using the nuclear channel, then cytoplasm was segmented using the fluorescein channel. Mean fluorescein intensity was calculated for each cell. Cells were selected using threshold values for mean fluorescein fluorescence to filter out residual fibroblasts or outlying bright objects. Perinuclear masks were defined for each valid cell, and total Cy3 fluorescence was calculated for each mask. Finally, cell area was calculated for each valid cell based on the fluorescein channel.

**Animal Studies.** Animal experiments were conducted in accordance with the National Institutes Health Guide for the Care and Use of Laboratory Animals, and were approved by the Institutional Animal Care and Use Committee at the University of Colorado Denver. For norepinephrine treatment, adult, male Sprague–Dawley rats (Charles River Labs) weighing ∼300 grams were pretreated with MGCD0103 (10 mg/kg) or vehicle control (50:50 DMSO:PEG-300) by daily i.p. injection for 3 d. Norepinephrine (NE; 10 mg/kg) was subsequently administered by i.p. injection and animals were killed 2 h post-NE treatment. For transverse aortic constriction (TAC) surgery, a left thoracotomy was performed through the fourth intercostal space and the aorta was isolated. A 4–0 silk suture was then placed around the descending aorta and tied against the appropriate gauge needle according to the rat's body weight to reach a fixed diameter.

**Adenovirus Production.** Recombinant adenoviruses encoding short hairpin (sh) RNAs to target rat dual-specificity phosphatase 5 (DUSP5) were prepared using the BLOCK-it Adenoviral RNAi Expression system (Invitrogen). Oligonucleotide targeting sequences are shown in Table S1. Top and bottom strands were annealed and ligated into the pENTR/U6 vector (Invitrogen). Positive subclones were recombined with the pAd/BLOCK-it–Dest vector (Invitrogen) and then transfected into 293A cells using Fugene 6 (Roche). For DUSP5 knockdown, NRVMs were simultaneously infected with adenoviruses encoding two distinct DUSP5 targeting sequences. Complementary DNA encoding human DUSP5 with an animo-terminal Myc tag was cloned into pENTR2B (Invitrogen) and was subsequently recombined into pAD/CMV/V5-DEST (Invitrogen). Viruses were amplified and recovered from 293A cells via multiple freeze/thaw cycles. Viral titers were determined using Adeno-XTM Rapid Titer kit (Clontech).

**Quantitative PCR.** Total RNA was harvested using TRI Reagent (Life Technologies) 48 h after treatment. All RNA samples were diluted to 100 ng/μL, and 5 μL (500 ng) of RNA was converted to cDNA using the Verso cDNA Synthesis kit (Thermo Scientific). Quantitative PCR (qPCR) was performed using Absolute QPCR SYBR Green ROX mix (Thermo Scientific) on a StepOne qPCR instrument (Applied Biosystems). PCR primers for DUSP5, brain natriuretic peptide (BNP), sarcoendoplasmic reticulum calcium ATPase (SERCA2a), α-skeletal actin, α- and β-myosin heavy chain (MyHC) and 18S as an endogenous control are shown in Table S2. Relative transcript levels were determined by measuring Ct values off of a standard curve made from serial dilutions of pooled cDNA. PCR array analysis was performed using the SABioscience RT2 Profiler Protein Phosphatase PCR array (Qiagen; PARN-045C-2).

**Immunoblotting.** Tissue extracts were prepared in PBS (pH 7.4) containing 0.5% Triton X-100, 300 mM NaCl and protease/phosphatase inhibitor mixture (Thermo Fisher) using a Bullet Blender homogenizer (Next Advance). Cultured cells were suspended in the same buffer and sonicated before clarification by centrifugation. Nuclear and cytoplasmic protein fractions were prepared using a NE-PER extraction kit (Pierce). Protein concentrations were determined using a BCA Protein Assay kit (Pierce). Proteins were resolved by SDS/PAGE, transferred to nitrocellulose membranes (BioRad) and probed with antibodies for phospho-ERK1/2 (Cell Signaling Technology; 4370), phospho-p38 (Cell Signaling Technology; 4631), phospho-JNK (Cell Signaling Technology; 4668), phospho-MEK1/2 (Cell Signaling Technology; 9121), total ERK (Santa Cruz Biotechnology; sc-154), total JNK (Santa Cruz Biotechnology; sc-7345), phospho-cytosolic phospholipase A2 (cPLA2) (Cell Signaling Technology; 2831), phospho-ETS domain-containing protein (ELK-1) (Cell Signaling Technology; 9181), calnexin (Santa Cruz Biotechnology; sc-11397), Sp1 (Santa Cruz Biotechnology; sc-59), c-Myc (Santa Cruz Biotech-

nology; sc-40), HDAC3 (Cell Signaling Technology; 3949), or glyceraldehyde phosphate dehydrogenase (GAPDH) GAPDH (Santa Cruz Biotechnology; sc-20358). Proteins were detected using a SuperSignal West Pico chemiluminescence system (Thermo Scientific) and a FluorChem HD2 imager (Alpha Innotech).

**Statistical Analysis.** Statistical analyses were conducted using GraphPad Prism software. Knockdown data were analyzed using ANOVA with Tukey's post hoc analysis, used when the $P$ value for the respective parameter was statistically significant ($P < 0.05$). Inhibitor data were analyzed using analysis of variance, with Tukey's post hoc analysis conducted when the $P$ value for the respective parameter was statistically significant ($P < 0.05$). Animal studies were analyzed using ANOVA with Tukey's post hoc analysis used when the $P$ value for the respective parameter was statistically significant ($P < 0.05$).

1. Palmer JN, Hartogensis WE, Patten M, Fortuin FD, Long CS (1995) Interleukin-1 beta induces cardiac myocyte growth but inhibits cardiac fibroblast proliferation in culture. *J Clin Invest* 95(6):2555–2564.
2. Harrison BC, et al. (2006) Regulation of cardiac stress signaling by protein kinase d1. *Mol Cell Biol* 26(10):3875–3888.
3. Satoh N, Suter TM, Liao R, Colucci WS (2000) Chronic alpha-adrenergic receptor stimulation modulates the contractile phenotype of cardiac myocytes in vitro. *Circulation* 102(18):2249–2254.

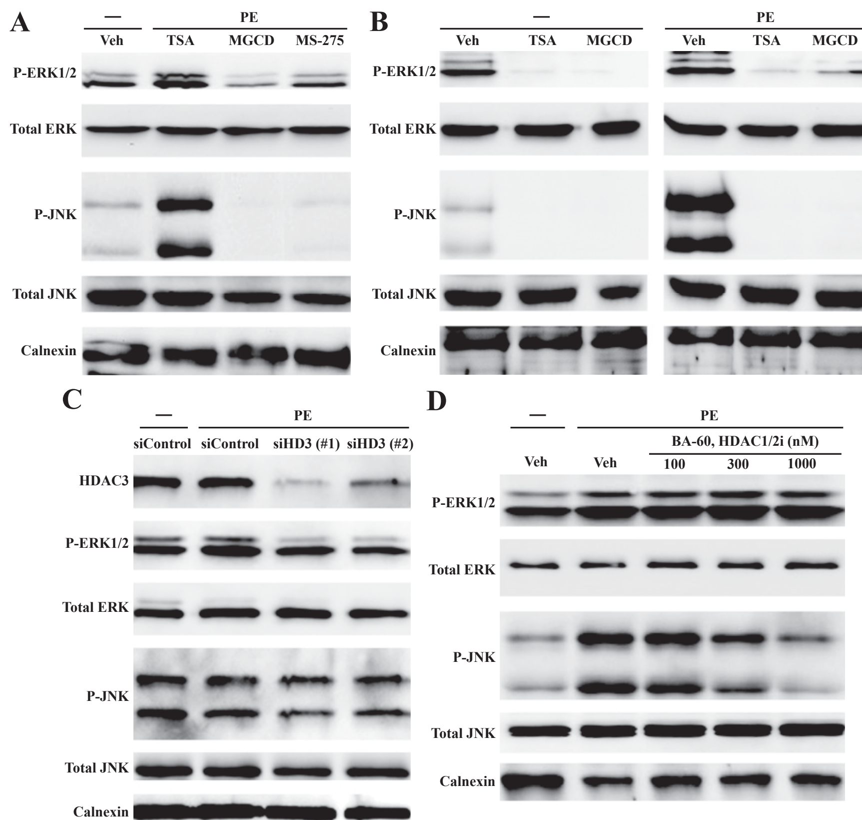


**Fig. S1.** Cardiac ERK1/2 and JNK are regulated by HDAC3 and HDAC1/2, respectively. (*A*) NRVMs were treated with PE for 48 h in the absence or presence of the class I HDAC inhibitors MGCD0103 or MS-275 (1 μM) before immunoblotting. (*B*) NRVMs were left untreated or stimulated with PE in the absence or presence of TSA (100 nM) or MGCD0103 (1 μM) for 48 h. Cells were harvested and protein lysates immunoblotted with the indicated antibodies. (*C*) NRVMs were transfected with short interfering RNA targeting two independent HDAC3 sequences (siHD3 1 and 2) or a scrambled control (siControl). Twenty-four hours posttransfection, cells were stimulated with PE for 48 h and lysates immunoblotted with the indicated antibodies. High basal phosphorylation of ERK1/2 and JNK was due to the transfection procedure. (*D*) NRVMs were stimulated with PE for 48 h in the absence or presence of increasing concentrations of BA-60, which is a highly selective inhibitor of HDAC1 and HDAC2. Cells were lysed and immunoblotting was performed with the indicated antibodies.

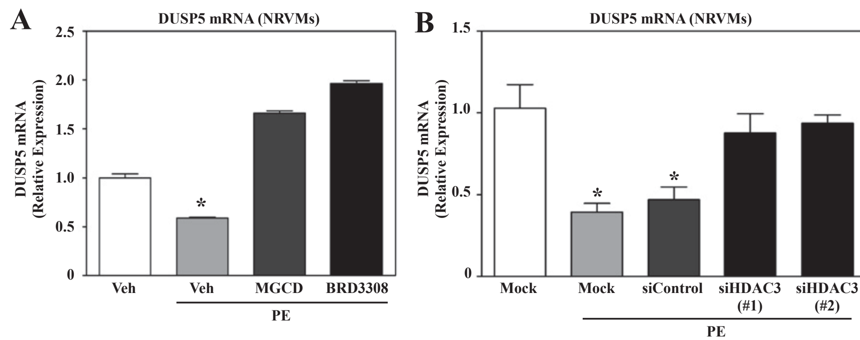


**Fig. S2.** HDAC3 regulates DUSP5 mRNA expression in cardiac myocytes. (*A*) NRVMs were left untreated or stimulated with PE for 48 h in the absence or presence of MGCD0103 or BRD3308 (1 μM), which is a selective HDAC3 inhibitor. DUSP5 mRNA expression was analyzed by qPCR. (*B*) NRVMs were mock transfected or transfected with short interfering RNA against two independent HDAC3 sequences (siHD3 1 and 2) or a scrambled control (siControl). Twenty-four hours posttransfection, cells were stimulated with PE for 48 h and DUSP5 mRNA expression analyzed by qPCR. $n = 3$ plates of NRVMs per condition; $*P < 0.05$ vs. unstimulated, vehicle-treated cells.

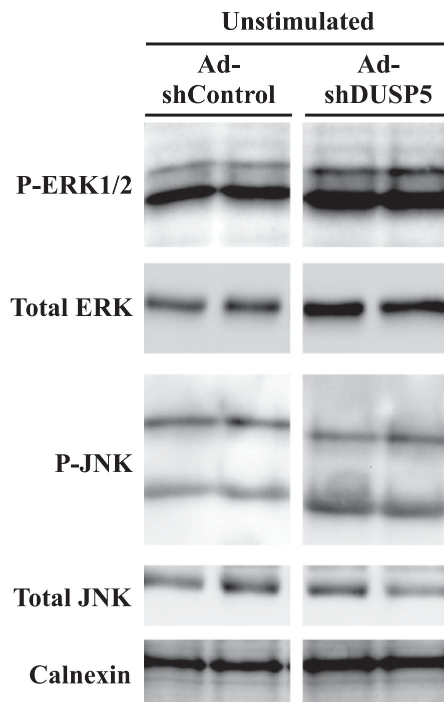


**Fig. S3.** DUSP5 knockdown increases basal ERK1/2 phosphorylation in NRVMs. NRVMs were infected with adenovirus encoding shRNA to knockdown expression of endogenous DUSP5 (Ad-shDUSP5) or a scrambled negative control (Ad-shControl); multiplicity of infection (MOI) = 50 for each virus. Cells were harvested 72 h postinfection and immunoblotting was performed with the indicated antibodies.

**Fig. S4.** DUSP5 regulates cardiomyocyte hypertrophy. (*A*) NRVMs were infected with adenovirus encoding β-galactosidase control (Ad-β-Gal) or Myc-tagged DUSP5 (Ad-Myc-DUSP5), at the indicated MOIs, and stimulated with PE for 48 h. Immunoblotting was performed with the indicated antibodies. NRVMs were left uninfected or infected with Ad-β-Gal or Ad-Myc-DUSP5, each at an MOI of 1.0, and stimulated with PE for 48 h. Quantitative PCR was performed to assess expression of BNP (*B*), α-skeletal actin (*C*), SERCA2a (*D*), β-MyHC (*E*), and α-MyHC (*F*) mRNA transcripts. (*G*) NRVMs cultured on 96-well plates were left uninfected or infected with Ad-shDUSP5 or Ad-shControl; MOI = 50 for each virus. After 24 h of infection, cells were treated with PE in the absence or presence of TSA (100 nM) for an additional 48 h. Cells were fixed and cell area was quantified by high content imaging. $n$ = 16 wells and >500 cells per condition; *$P < 0.05$ vs. unstimulated.

**Table S1. Oligonucleotide sequences for construction of two distinct shDUSP5 adenoviruses**

| Target | Top strand shRNA | Bottom strand shRNA |
|---|---|---|
| Rat DUSP5 1 | 5′-CACCGCGGGTCTACTTCCTTAAAGGCGAACCTTTAAGGAAGTAGACCCGC-3′ | 5′-AAAAGCGGGTCTACTTCCTTAAAGGTTCGCCTTTAAGGAAGTAGACCCGC-3′ |
| Rat DUSP5 2 | 5′-CACCGCACAACCCACCTACACTACACGAATGTAGTGTAGGTGGGTTGTGC-3′ | 5′-AAAAGCACAACCCACCTACACTACATTCGTGTAGTGTAGGTGGGTTGTGC-3′ |

**Table S2. Primer sequences for quantitative PCR**

| Target | Forward qPCR primer | Reverse qPCR primer |
|---|---|---|
| DUSP5 | 5′-GGCAAGGTCCTGGTTCACTGT-3′ | 5′-GTTGGGAGAGACCACGCTCCT-3′ |
| BNP | 5′-GGTGCTGCCCCAGATGATT-3′ | 5′-CTGGAGACTGGCTAGGACTTC-3′ |
| SERCA2a | 5′-GGCCAGATCGCGCTACA-3′ | 5′-GGGCCAATTAGAGAGCAGGTTT-3′ |
| α-Skeletal actin | 5′-CCACCTACAACAGCATCATGAAGT-3′ | 5′-GACATGACGTTGTTGGCGTACA-3′ |
| β-MyHC | 5′-CGCTCAGTCATGGCG GAT-3′ | 5′-GCCCCAAATGCAGCCAT-3′ |
| α-MyHC | 5′-CCTGTCCAGCAGAAAGAG-3′ | 5′-CAGGCAAAGTCAAGCATTCATATTTAT TGTG-3′ |
| 18S | 5′-GCCGCTAGAGGTGAAATTCTTG-3′ | 5′-CTTTCGCTCTGGTCCGTCTT-3′ |