Online Data Supplement

Primary culture of neonatal rat ventricular myocytes

Primary cultures of ventricular cardiomyocytes were prepared from 1-day-old Crl:(WI) BR-Wistar rats (Harlan). A cardiomyocyte-rich fraction was obtained by centrifugation through a discontinuous Percoll gradient as described.[1]

Adenoviruses

Adenovirus vectors harboring HDAC4, (Ad-HDAC4), Nox4 (Ad-Nox4), shRNA-Nox4 (Ad-shNox4), shRNA-Nox2 (Ad-shNox2), Hyper-nuc (Ad-Hyper-nuc), Prx-3 (Ad-Prx-3), or Catalase-nuc (Ad-Catalase-nuc) were generated using the AdMax system (Clontech) as described.[2] In brief, the recombinant adenoviruses were generated in HEK293 cells by co-transfection with a cosmid (pBHGloxΔE1,3Cre) containing the adenovirus type 5 genome (devoid of E1 and E3) and pDC316, a shuttle vector, containing a gene of interest. Adenovirus vectors harboring LacZ (Ad-LacZ) and sh-Scramble (Ad-shScr) were used as controls. Ad-HDAC5 was purchased from ABM Inc.

Aortic banding

The methods used to impose pressure overload in mice have been described.[3] Mice were anesthetized with a mixture of ketamine (0.065 mg/g), xylazine (0.013 mg/g), and acepromazine (0.002 mg/g) and mechanically ventilated. The left side of the chest was opened at the second intercostal space. Aortic constriction was performed by ligation of the transverse thoracic aorta between the innominate artery and left common carotid artery with a 28-gauge needle using a 7-0 braided polyester suture. Sham operation was performed without constricting the aorta. To measure arterial pressure gradients, high-fidelity micromanometer catheters (1.4 French; Millar Instruments Inc., Houston, Texas, USA) were used.

Echocardiography

Mice were anesthetized using 12 μl/g BW of 2.5% avertin (Sigma-Aldrich), and echocardiography was performed using ultrasonography (Acuson Sequoia C256; Siemens Medical Solutions). A 13-MHz linear ultrasound transducer was used. M-mode measurements of left ventricular (LV) internal diameter were taken from more than three beats and averaged. LV end-diastolic diameter (LVEDD) was measured at the time of the apparent maximal LV diastolic dimension, while LV end-systolic diameter (LVESD) was measured at the time of the most anterior systolic excursion of the posterior wall. LV percent fractional shortening (%FS) was calculated as follows: %FS=(LVEDD-LVESD)/LVEDD x 100.[3]

Injection of phenyelphrine

The jugular vein was catheterized for drug delivery under anesthesia with a mixture of ketamine (0.065 mg/g), xylazine (0.013 mg/g), and acepromazine (0.002 mg/g). 10mg/kg of phenylephrine was injected intravenously.

Fractionation

To isolate crude nuclear fractions, we used procedures previously described.[4] Briefly, cultured neonatal rat myocytes were resuspended in hypotonic lysis buffer (10 mM K-HEPES (pH 7.9), 1.5 mM $MgCl_2$, 10 mM KCl, 1 mM DTT, 0.2 mM Na_3VO_4, 1% Protease Inhibitor Cocktail), incubated for 15 min on ice and homogenized. Whole-cell lysates were centrifuged at 60x*g* for 5 minutes to collect unbroken cells. The supernatant was collected, and the homogenization and centrifugation was repeated on the pellet. The total homogenate was

centrifuged at 1,200x*g* to separate crude nuclei and unbroken cells (pellet) from cell membrane and cytosolic proteins (supernatant). The supernatant of the total homogenate was centrifuged at 3,500x*g* for 20 min to separate mitochondrial fractions (pelleted in tube) from cytosolic and microsomal fractions (supernatant). The supernatant was further centrifuged at 100,000x*g* for 60 minutes to separate microsomal fractions (pelleted in tube) from cytosolic fractions (supernatant). Mitochondrial and microsomal fractions were resuspended in PBS containing protease inhibitors. The nuclei and cell pellet from the total homogenate were resuspended in 500 μl of hypotonic lysis buffer, combined with 5.5 ml of 2.4 M sucrose and layered on top of 6 ml of a 2.4 M sucrose cushion and centrifuged at 100,000x*g* for 90 min to purify nuclei. The pelleted nuclei were resuspended in storage buffer and protein content was determined for all fractions. A nuclear fraction from mouse hearts was prepared with NE-PER Extraction Reagent (Thermo Scientific).

Immunostaining

Neonatal rat cardiomyocytes grown on chamber slides (Lab-Tek) were washed three times with PBS. The cells were fixed with 4% paraformaldehyde and washed four times with PBS containing 0.1% Triton X-100. The cells were boiled for 10 min with a pressure-cooker to allow the antigen to be better exposed to the antibody. Then the cells were blocked with PBS containing 5% normal goat serum for 60 min and stained with antibodies as indicated. Images of HDAC4 staining with Troponin T and DAPI staining were obtained with regular microscopy. On the other hand, images of Nox4 staining with Troponin T and DAPI staining were obtained with confocal microscopy.

Immunoblot analyses

Heart homogenates and cardiomyocyte lysates were prepared in RIPA lysis buffer containing 50 mM Tris (pH7.5), 150 mM NaCl, 1% IGEPAL CA-630, 0.1% SDS, 0.5% deoxycholic acid, 10 mM $Na_4P_2O_7$, 5 mM EDTA, 0.1 mM Na_3VO_4, 1 mM NaF, 0.5 mM 4-(2-Aminoethyl) benzenesulfonyl fluoride hydrochloride (AEBSF), 0.5 μg/ml aprotinin, and 0.5 μg/ml leupeptin. Samples were subjected to SDS-PAGE. Proteins were transferred onto polyvinylidene fluoride microporous membranes (Bio Rad) and probed with primary antibodies. These included monoclonal antibodies raised against Nox4, GAPDH, actin, and COXIV (Sigma), and polyclonal antibodies raised against HDAC4, Histone H3 (Sigma), NF-κB, phosphorylated NF-κB, and IκB (Cell Signaling).

Histological analyses

The LV accompanied by the septum was cut into base, middle portion, and apex, fixed with 10% formalin, embedded in paraffin, and sectioned at 6 μm thickness. The sections were incubated in 3% H_2O_2 in PBS to prevent endogenous peroxidation and blocked with 5% BSA in PBS. Myocyte cross-sectional area was measured from images captured from sections stained with anti-wheat germ agglutinin (WGA) antibody as previously described.[3] The outlines of 200 myocytes were traced in each section. Interstitial fibrosis was evaluated by Masson Trichrome staining. Specimens were also stained with HDAC4 antibody using the N-Histofine DAB-3S kit (Nichirei Biosciences Inc).

Assays for apoptosis

TUNEL staining was conducted as described.[5, 6] Deparaffinized sections were incubated with proteinase K, and DNA fragments were labeled with fluorescein-conjugated dUTP using TdT (Roche Molecular Biochemicals). Nuclear density was determined by manual counting of DAPI-stained nuclei in six fields for each animal using the 40x objective.

TUNEL-positive nuclei in the entire section were identified and counted using the same power objective. Histone-associated DNA fragments were quantified by the Cell Death ELISA (Roche) according to the manufacturer's instructions.

Lucigenin assay

Nuclear fractions from hearts and myocytes were suspended in 200 μl of an assay buffer composed of 100 mM potassium phosphate (pH 7.0), 10 μM flavin adenine dinucleotide (FAD), 1 mM NaN_3, and 1 mM EGTA. After preincubation with 5 μM lucigenin, NADPH was added to a final concentration of 500 μM.[7] The chemiluminescence was continuously monitored using a luminometer. The reaction was terminated by the addition of superoxide dismutase (SOD) (100 μg/ml).

Quantitative real-time PCR reaction

Methods of quantitative RT-PCR have been described previously.[8] In brief, after preparing total RNA, first-strand cDNA was synthesized. Real-time PCR was then carried out on a DNA Engine Opticon 2 system (MJ Research Inc.) using the Maxima SYBR Green/ROX qPCR Master Mix (Fermentas). The specific oligonucleotide primers for GAPDH, ANF, and Nox4 were selected using Vector NTI (Invitrogen). The following oligonucleotide primers were used in this study: GAPDH, sense 5'-TGAACGGGAAGCTCACTGG-3' and antisense 5'-TCCACCACCCTGTTGCTGTA-3'; ANF, sense 5'-ATGGGCTCCTTCTCCATCAC-3' and antisense 5'-ATCTTCGGTACCGGAAGCTG-3'; Nox4, sense 5'-AGTCAAACAGATGGGATA-3' and antisense 5'-TGTCCCATATGAGTTGTT-3'.

Measurement of the myocyte cell surface area and total protein/DNA content

Cardiomyocyte size, total protein content and DNA content were determined as described previously.[9]

H_2O_2 measurement

Myocytes were transduced with Ad-Hyper-nuc and Ad-LacZ, Ad-Nox4, or Ad-shNox2 in the presence or absence of PE for 5 minutes. Imaging of Hyper-nuc was achieved by excitation at 488 nm and emission at 516 nm with confocal microscopy. The signal of Hyper-nuc (516 nm) indicates the level of H_2O_2 in the nucleus. Nuclei were counter-stained with DAPI.

Luciferase assay

Transfection of plasmids into myocytes was performed with Fugene 6 (Roche). Luciferase activity was measured with a luciferase assay system (Promega). The method used for *in vivo* reporter gene assays has been described.[9]

Chromatin immunoprecipitation (ChIP) assay

ChIP assays were performed using the SimpleChIP™ Enzymatic Chromatin IP Kit (Cell Signaling Technology) according to the manufacturer's instructions with modifications. Briefly, cultured cardiac myocytes treated with or without PE were fixed using a 1% concentration of formaldehyde for 10 minutes. Then, cross-linking was stopped by adding glycine to a final concentration of 125 mM. Anti-NF-κB-p65 (Abcam) or rabbit IgG (Cell Signaling Technology) antibody was used to immunoprecipitate chromatin-protein complexes, which were subsequently isolated with protein G agarose beads. These ChIP samples were analyzed by PCR. Primers were designed to amplify the fragment from -1755 to -1562 of the mouse Nox4 gene-promoter region containing the NF-κB-binding motif (Online Figure VII).

The forward primer sequence was 5'- TGGAGGAGCAAACCTCTCAG-3' and the reverse primer sequence was 5'- CTGCCGCACACATTTCTTTC-3'. A pair of primers designed to amplify the fragment of the mouse Nox4 gene-promoter region from -1955 to -1655 that does not contain the NF-κB-binding motif was used in the ChIP assay as a negative control; the forward primer sequence was 5'- TGGGGTCCTGGGAGGCTC-3' and the reverse primer sequence was 5'- CCCTTAATCTTCCTTTCCAT-3'.

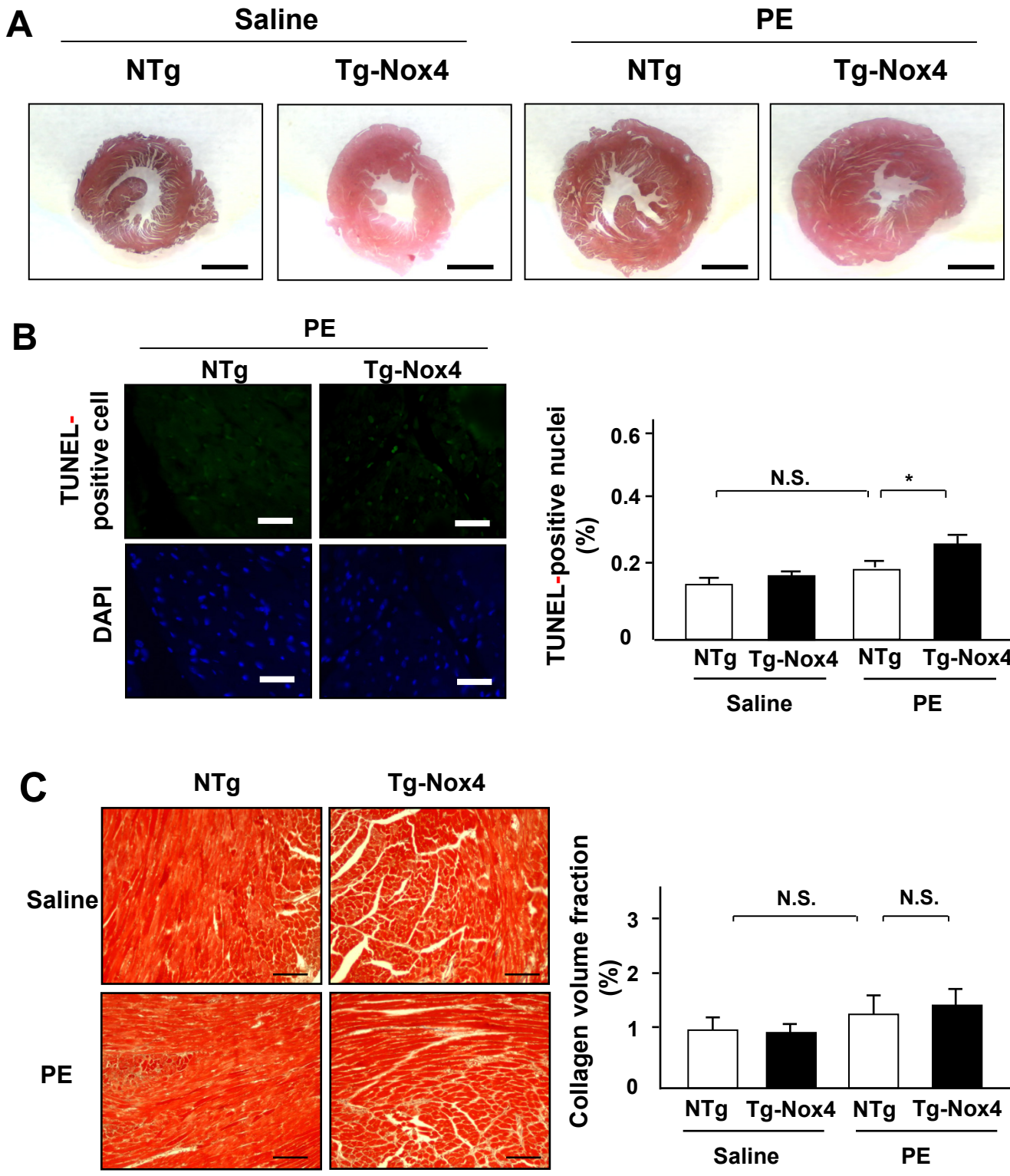
References

1. Morisco C, Zebrowski D, Condorelli G, Tsichlis P, Vatner SF, Sadoshima J. The akt-glycogen synthase kinase 3beta pathway regulates transcription of atrial natriuretic factor induced by beta-adrenergic receptor stimulation in cardiac myocytes. *J Biol Chem*. 2000;275:14466-14475
2. Ago T, Kuroda J, Pain J, Fu C, Li H, Sadoshima J. Upregulation of nox4 by hypertrophic stimuli promotes apoptosis and mitochondrial dysfunction in cardiac myocytes. *Circ Res*. 2010;106:1253-1264
3. Matsuda T, Zhai P, Maejima Y, Hong C, Gao S, Tian B, Goto K, Takagi H, Tamamori-Adachi M, Kitajima S, Sadoshima J. Distinct roles of gsk-3alpha and gsk-3beta phosphorylation in the heart under pressure overload. *Proc Natl Acad Sci U S A*. 2008;105:20900-20905
4. Wright CD, Chen Q, Baye NL, Huang Y, Healy CL, Kasinathan S, O'Connell TD. Nuclear alpha1-adrenergic receptors signal activated erk localization to caveolae in adult cardiac myocytes. *Circ Res*. 2008;103:992-1000
5. Yamamoto S, Seta K, Morisco C, Vatner SF, Sadoshima J. Chelerythrine rapidly induces apoptosis through generation of reactive oxygen species in cardiac myocytes. *J Mol Cell Cardiol*. 2001;33:1829-1848
6. Yamamoto S, Yang G, Zablocki D, Liu J, Hong C, Kim SJ, Soler S, Odashima M, Thaisz J, Yehia G, Molina CA, Yatani A, Vatner DE, Vatner SF, Sadoshima J. Activation of mst1 causes dilated cardiomyopathy by stimulating apoptosis without compensatory ventricular myocyte hypertrophy. *J Clin Invest*. 2003;111:1463-1474
7. Ago T, Kitazono T, Ooboshi H, Iyama T, Han YH, Takada J, Wakisaka M, Ibayashi S, Utsumi H, Iida M. Nox4 as the major catalytic component of an endothelial nad(p)h oxidase. *Circulation*. 2004;109:227-233
8. Zhai P, Yamamoto M, Galeotti J, Liu J, Masurekar M, Thaisz J, Irie K, Holle E, Yu X, Kupershmidt S, Roden DM, Wagner T, Yatani A, Vatner DE, Vatner SF, Sadoshima J. Cardiac-specific overexpression of at1 receptor mutant lacking g alpha q/g alpha i coupling causes hypertrophy and bradycardia in transgenic mice. *J Clin Invest*.

2005;115:3045-3056

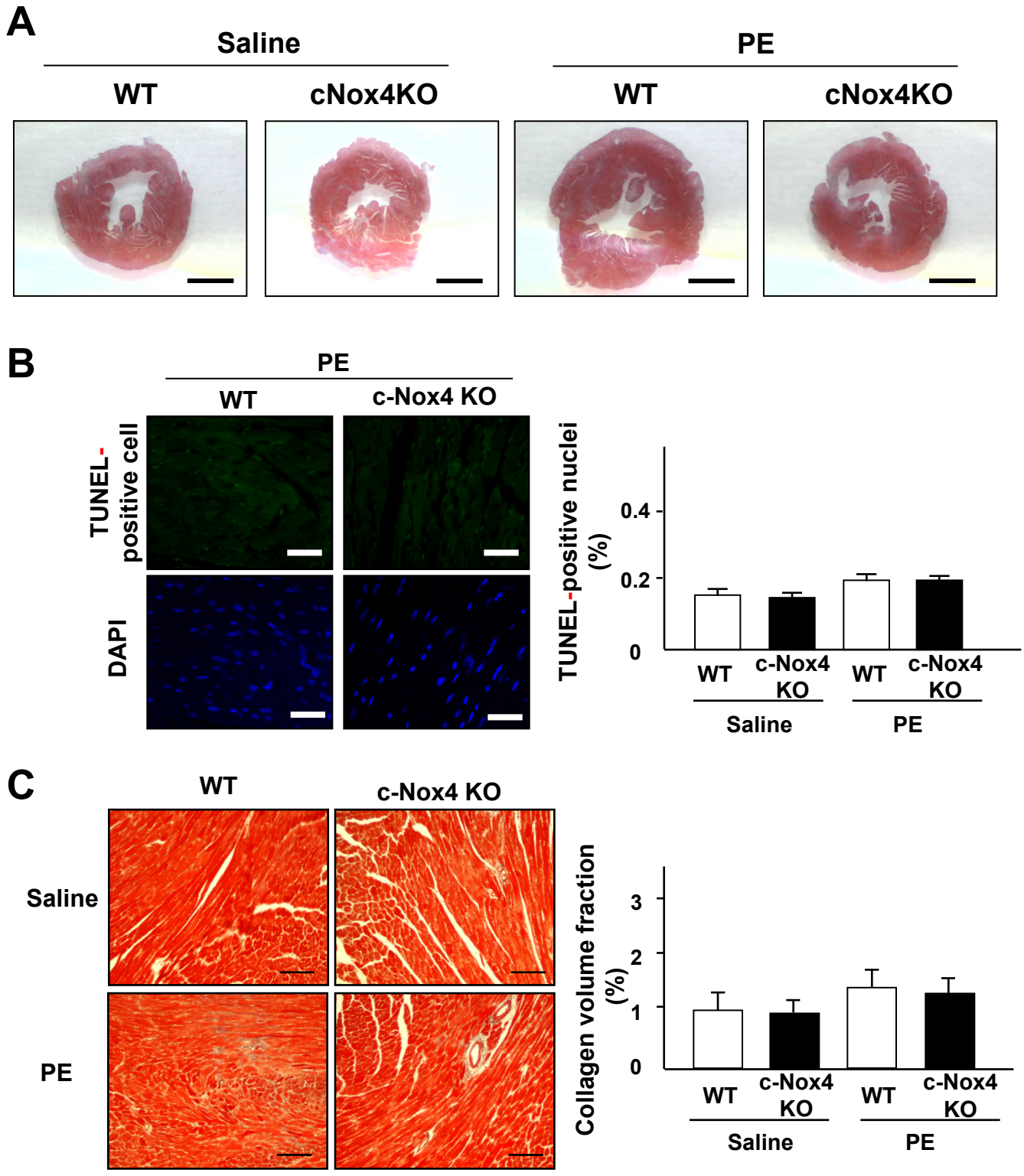
9. Morisco C, Zebrowski DC, Vatner DE, Vatner SF, Sadoshima J. Beta-adrenergic cardiac hypertrophy is mediated primarily by the beta(1)-subtype in the rat heart. *J Mol Cell Cardiol*. 2001;33:561-573

Online Figure I



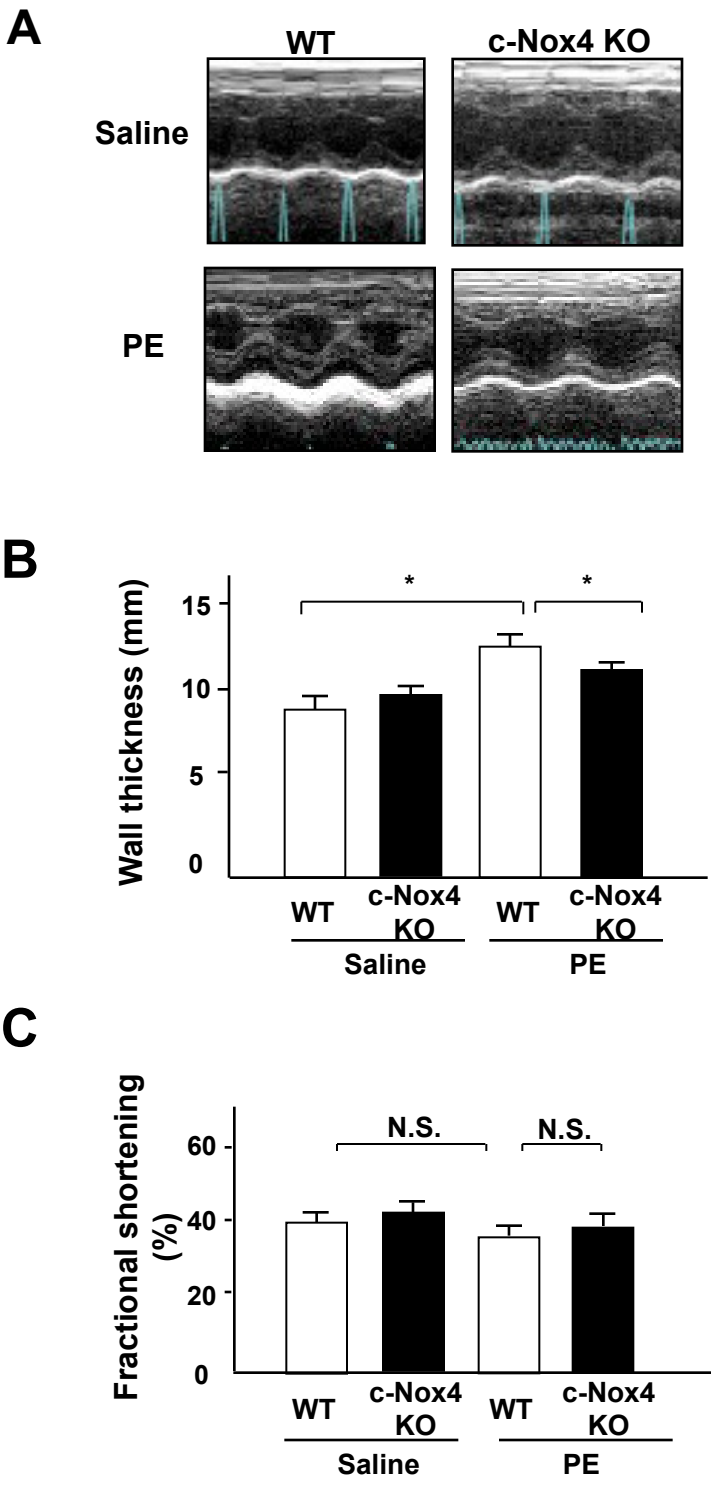
Online Figure I The effect of overexpression of Nox4 on apoptosis and fibrosis in the heart in response to PE. NTg and Tg-Nox4 mice were subjected to either saline or PE infusion for 2 weeks. A. LV morphology was evaluated with Masson-Trichrome staining. Bar=1mm. **B.** Apoptosis in LV sections was evaluated with TUNEL staining. The number of TUNEL-positive cells was increased in Tg-Nox4 mice after PE treatment (n=4). Bar=20 μm. *P<0.05. N.S.: not significant. **C.** Collagen volume fraction, evaluated using Masson-Trichrome staining, was similar among all groups (n=4). Bar=50 μm.

Online Figure II



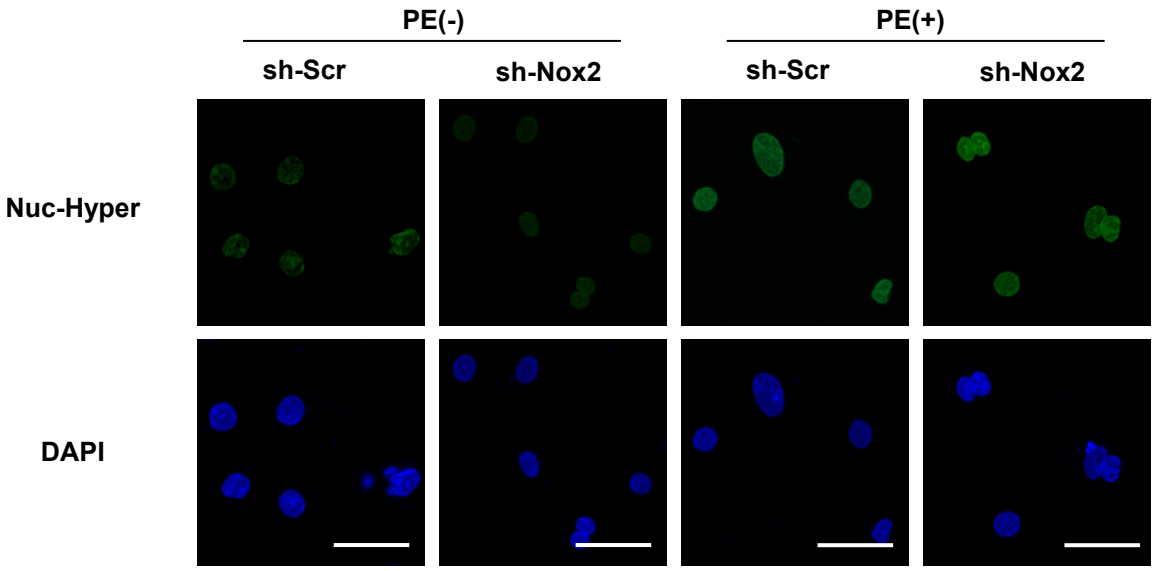
Online Figure II The effect of downregulation of Nox4 on apoptosis and fibrosis in the heart in response to PE. WT and c-Nox4 KO mice were subjected to either saline or PE infusion for 2 weeks. **A.** LV morphology was evaluated with Masson-Trichrome staining. Bar=1mm. **B.** Apoptosis in LV sections was evaluated with TUNEL staining. The number of TUNEL-positive cells was similar among all groups (n=4). Bar=20 μm. **C.** Collagen volume fraction, evaluated using Masson-Trichrome staining, was similar among all groups (n=4). Bar=50 μm.

Online Figure III



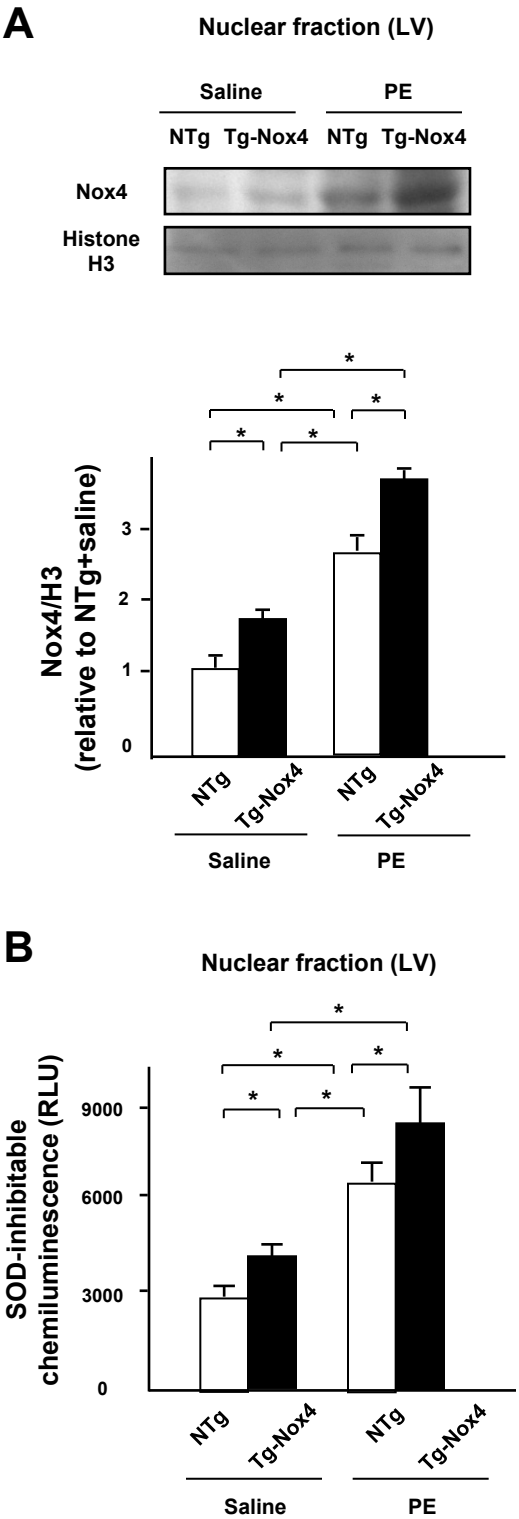
Online Figure III Echocardiographic data obtained from WT and c-Nox4 KO mice subjected to saline or PE infusion. A. Representative pictures of M-mode echocardiography. **B and C.** LV wall thickness and fractional shortening were evaluated with echocardiographic measurements (n=6). *P<0.05. N.S.: not significant.

Online Figure IV



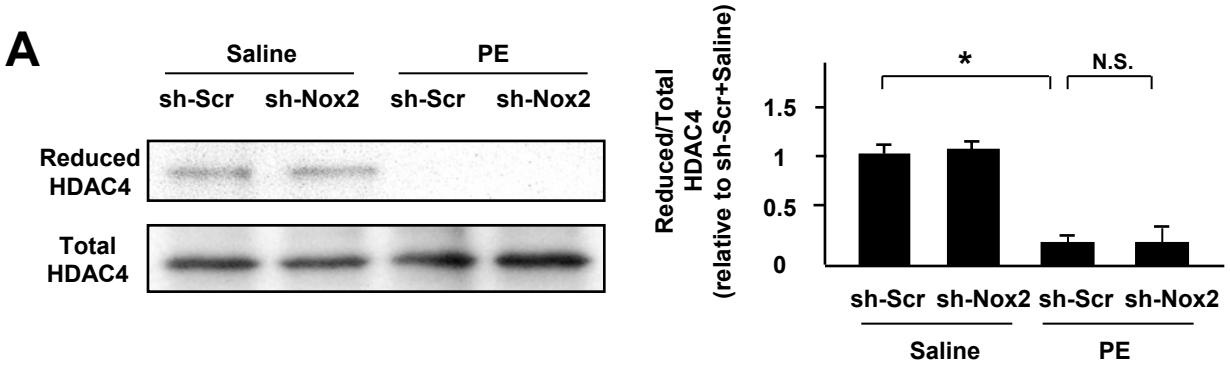
Online Figure IV Nox4 plays an important role in mediating PE-induced increases in oxidative stress in the nucleus. Myocytes were transduced with Ad-Hyper-nuc and Ad-LacZ or Ad-shNox2 in the presence or absence of PE for 5 minutes. Hyper-nuc is an indicator of hydrogen peroxide localized in the nucleus. **Green staining** indicates H_2O_2 production (n=8). Nuclei were stained with DAPI. Bar=20 μm.

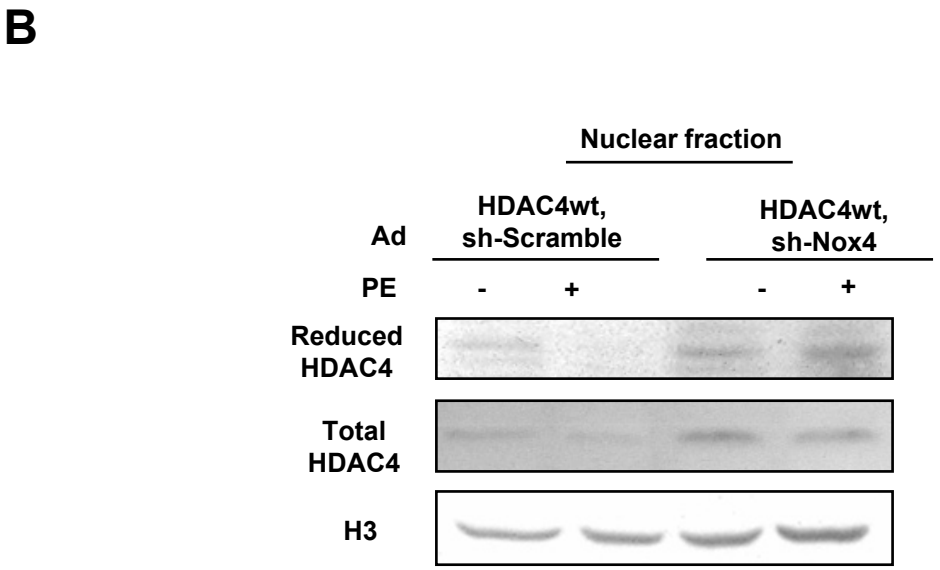
Online Figure V



Online Figure V Upregulation of Nox4 enhances PE-induced increases in oxidative stress in the nucleus. Nuclear fractions were prepared from NTg and Tg-Nox4 mouse hearts. **A.** Expression levels of Nox4 and histone H3 in the nuclear fraction from NTg and Tg-Nox4 mouse hearts (n=5). **B.** NADPH-dependent and SOD-inhibitable O_2^- release was measured using the lucigenin method (n=5). *P<0.05.

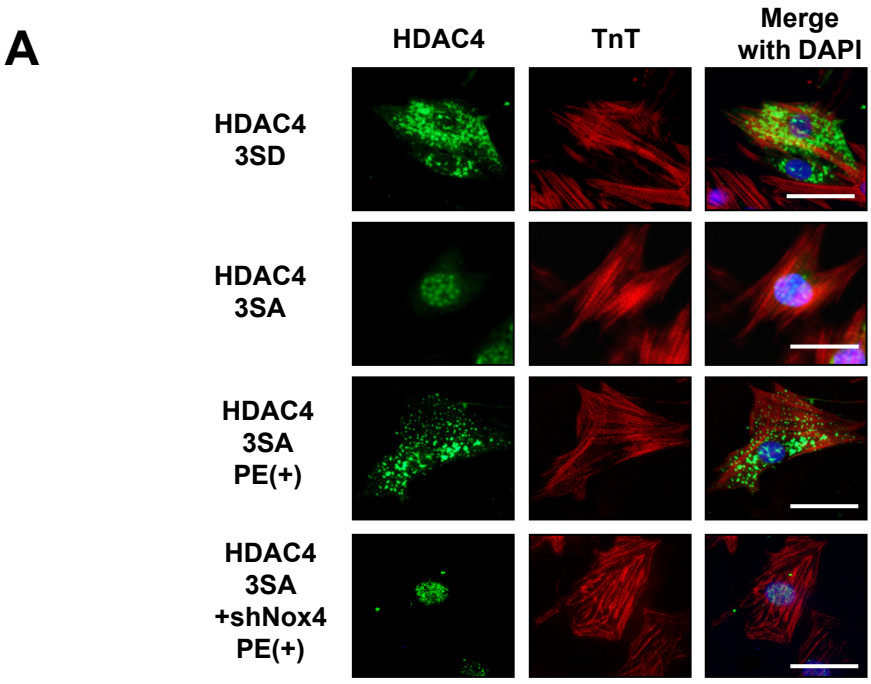
Online Figure VI

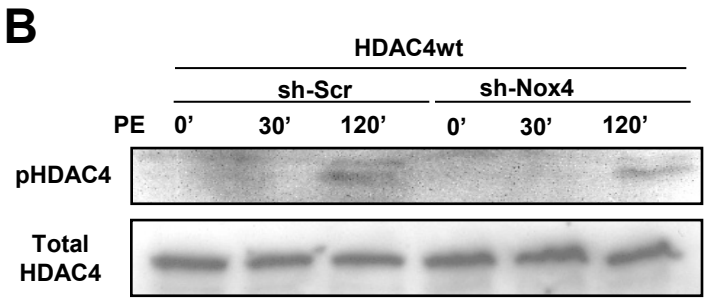


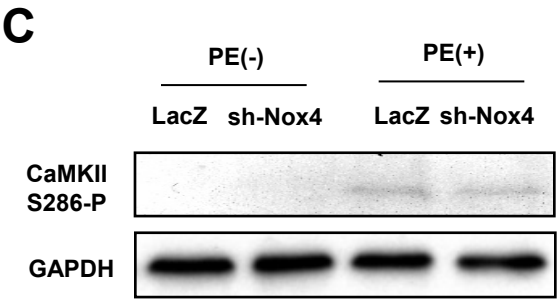


Online Figure VI A. The extent of cysteine reduction in HDAC4 in myocytes transduced with the indicated adenovirus was detected by biotinylated IAM pulldown assay.(n=3)**. B.** Myocytes transduced with the indicated adenoviruses were treated with or without PE. The extent of cysteine reduction in HDAC4 in the nuclear fraction was evaluated with biotinylated-iodoacetamide. The level of Histone H3 indicates equal loading of the samples.

Online Figure VII

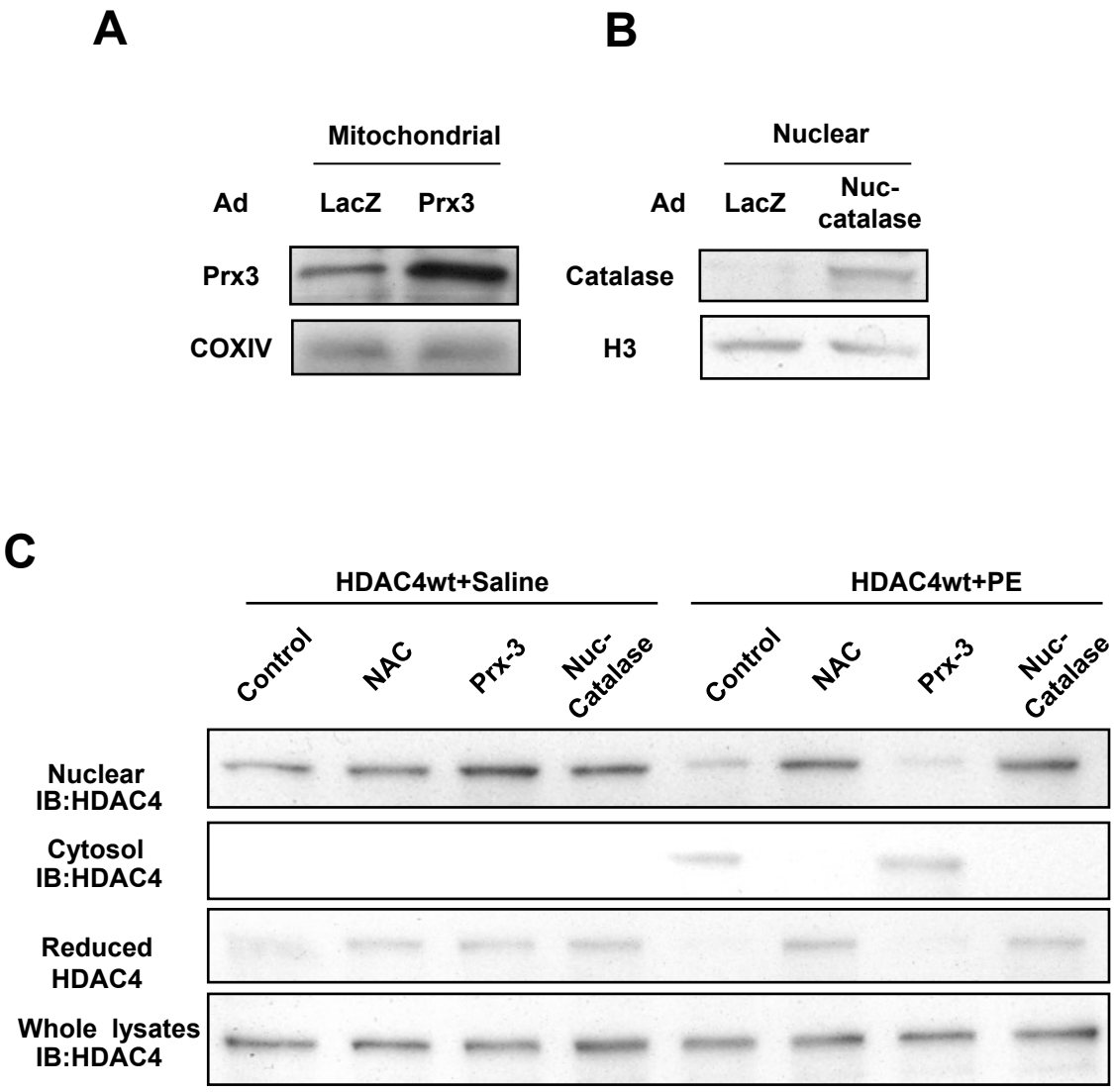






Online Figure VII The roles of Nox2 and Nox4 in mediating PE-induced oxidation and nuclear exit of HDAC4. A. Myocytes transduced with the indicated adenoviruses were stained with anti-HDAC4 antibody (green), anti-Troponin T antibody (red), and DAPI (Blue). Bar=10 μm. The results shown are representative of 8 experiments. **B**. Myocytes transduced with the indicated adenovirus were treated with PE for the indicated time periods. The expression level of HDAC4 and phosphorylation of HDAC4 were examined by immunoblot. **C.** Myocytes transduced with the indicated adenovirus were treated with PE for 2 hours. The degree of phosphorylation of CaMKII and GAPDH expression were examined by immunoblot. In **C** and **D**, the results shown are each representative of 3 experiments.

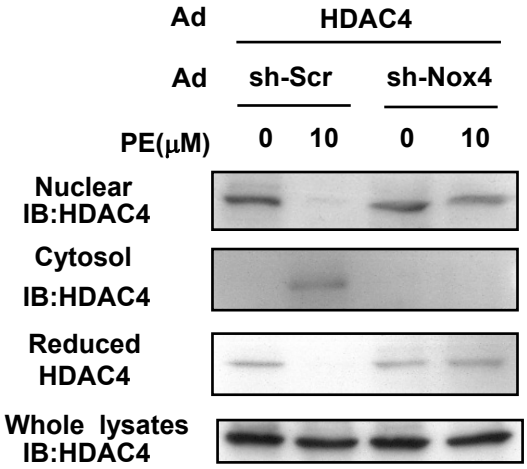
Online Figure VIII



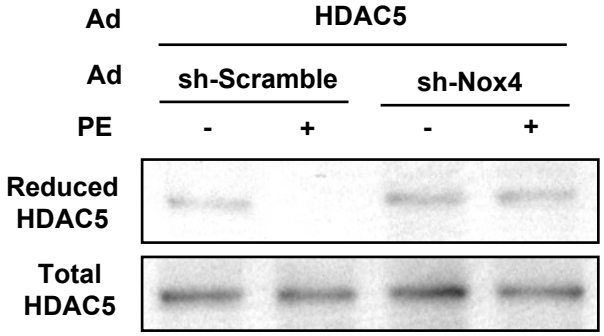
Online Figure VIII Nuclear oxidative stress mediates PE-induced oxidation and nuclear exit of HDAC4. A. Myocytes were transduced with the indicated adenoviruses. The expression level of Prx3 and COXIV in the mitochondrial fraction of myocytes was examined by immunoblots. **B.** Myocytes were transduced with the indicated adenovirus. The expression level of catalase and histone H3 in the nuclear fraction of myocytes was examined by immunoblots. **C**. Myocytes treated with NAC (100μM) or transduced with the indicated adenoviruses were treated with saline alone or PE (100μM). The expression levels of nuclear, cytosol, and total HDAC4 were examined by immunoblot. The extent of cysteine reduction in HDAC4 was evaluated with biotinylated IAM pulldown assays.

Online Figure IX

A



B



Online Figure IX Effect of phenylephrine on HDAC isoforms. A. Myocytes transduced with the indicated adenoviruses were treated with or without 10μM of PE for 5min. The expression level of nuclear, cytosol, and total HDAC4 was examined by immunoblots. The extent of cysteine reduction in HDAC4 was evaluated with biotinylated-IAM pulldown assays. **B.** Myocytes transduced with the indicated adenoviruses were treated with or without10μM of PE for 5min. The extent of cysteine reduction in HDAC5 in whole-cell lysates was evaluated with biotinylated-IAM pulldown assays.

Online Figure X

A

-1829

TCTCTCTCTGTTTTCCCAGGGGATACTTTCTCTCTTTTCTTTGCAGGGGTCTTTGCAGGTT

sense primer

CAGGCTCTAGGTTTGGAGGAGCAAACCTCTCAGAGGCAAATGTCCTGCCCAACTTTCAC

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AAGGAAGATTAAGGGTTCAGTTTGAGGATGCAGTGATCTCTGGCTATGGATATTCAGTGT

TGACACTCCTGGGGGTTTCCATGTATGACTTTCCTGGTTTACACATAACAAGTATGGTTAG

NF-κB binding motif

GTATAAATAAGGTCTATTAACTGGCAATAAAATCTTAGTATTACTTACACATAATATGTAGAC

TATA box

AGCTATACTTTGTGAAGTGTTCTTGATTCACTCATGCTTGAGAAAGATAACACATTGAAAT

antisense primer

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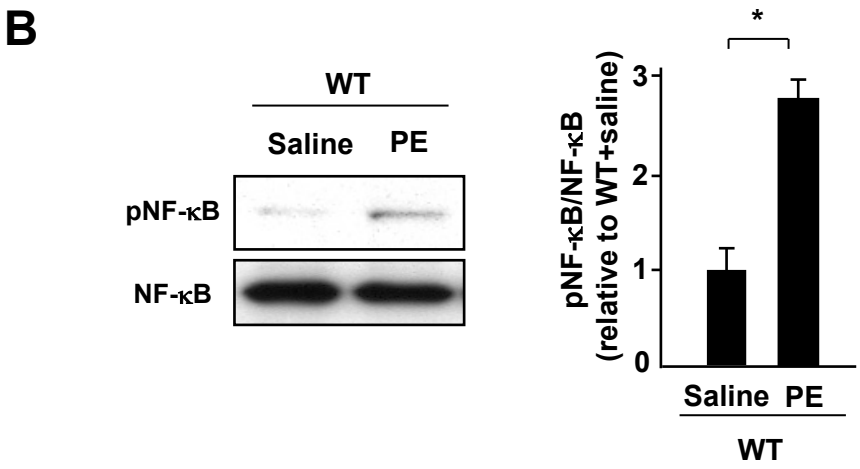
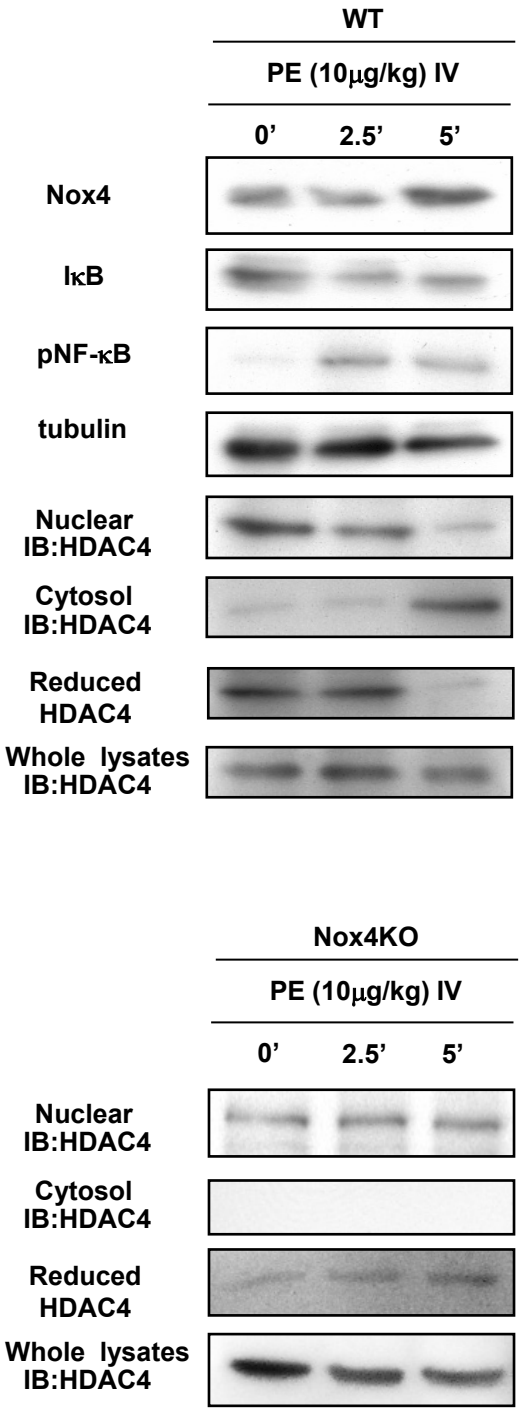
-1547

B



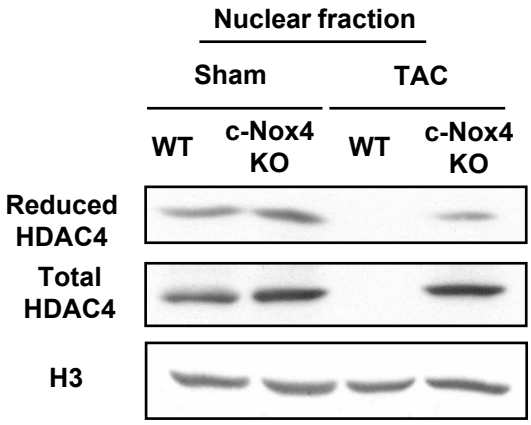
Online Figure X PE stimulates NF-κB in cardiomyocytes. A. The sequence of the rat Nox4 gene promoter containing the NF-κB-binding site and the primers used for the ChIP assay. **B.** Myocytes were treated with PE for 48 hours. The phosphorylation of NF-κB was examined by immunoblot (n=3).

Online Figure XI



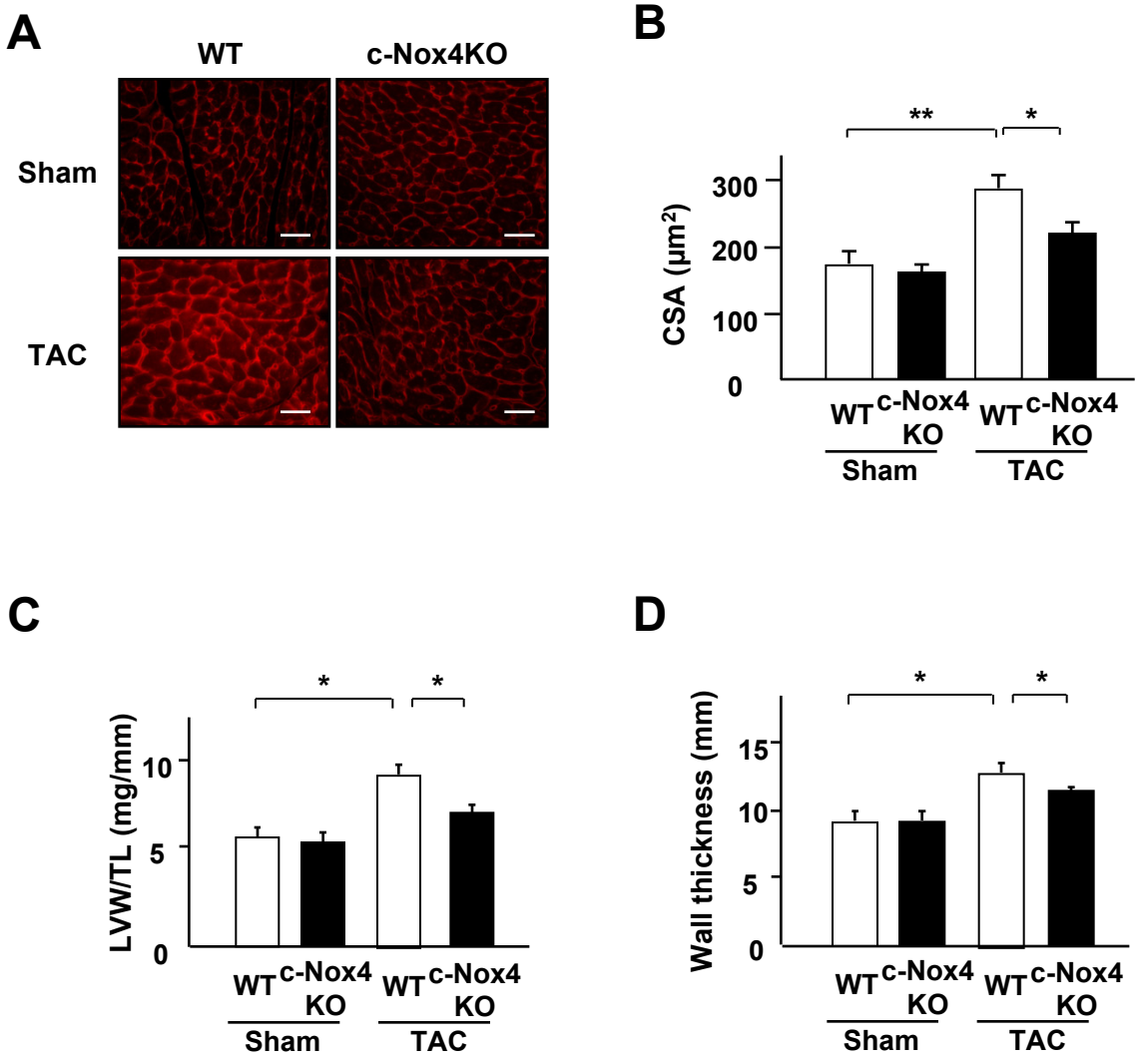
Online Figure XI Acute injection of phenylephrine induces nuclear exit of HDAC4 in the heart. WT and c-Nox4KO mice were injected with PE (10μg/kg) intravenously. The expression level of Nox4, IκB, phosphorylation of NF-κB, tubulin, and nuclear, cytosol, and total HDAC4 in mouse hearts at indicated time points as evaluated with immunoblot analyses. The extent of cysteine reduction in HDAC4 was evaluated with biotinylated IAM pulldown assays.

Online Figure XII



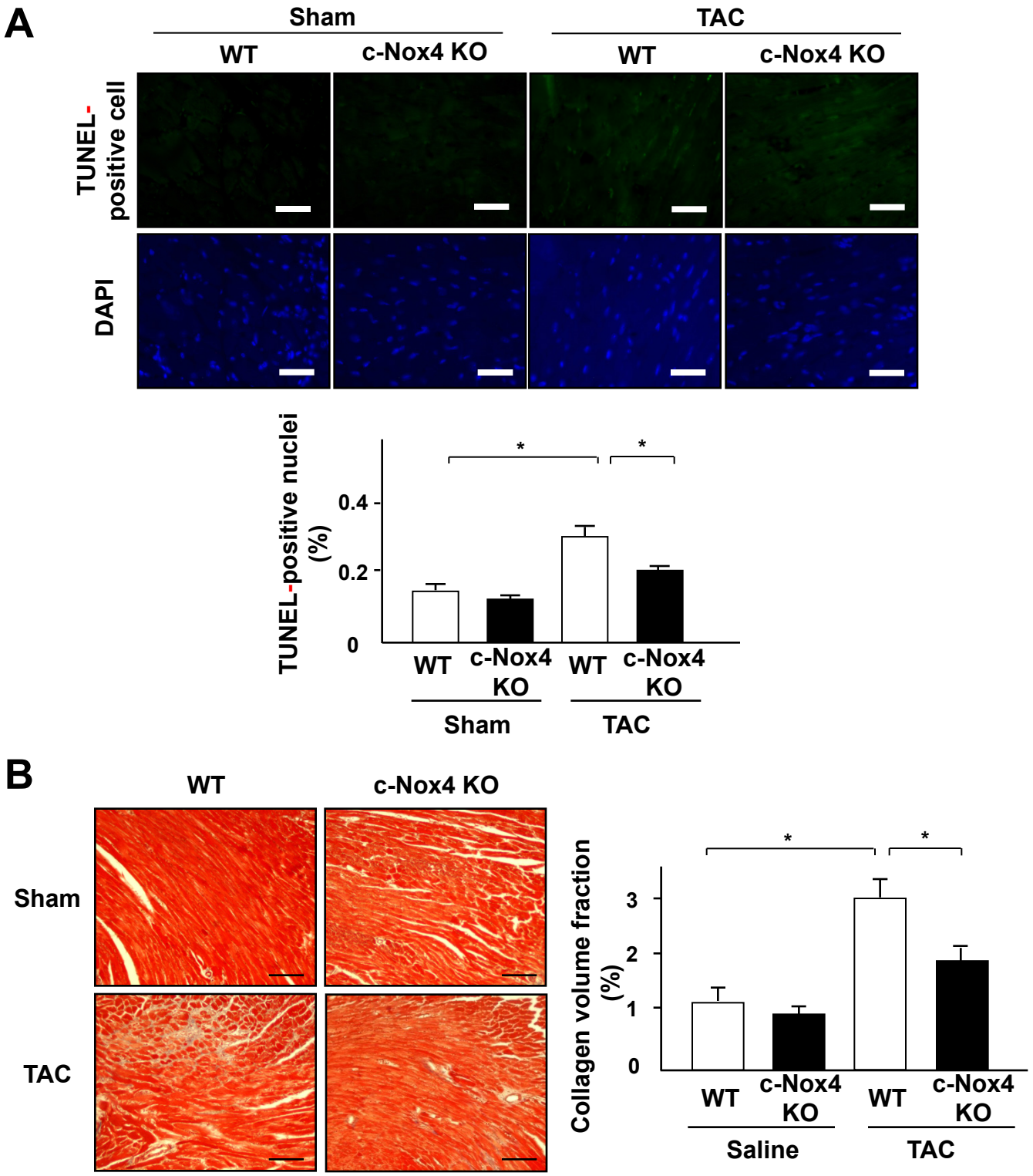
Online Figure XIV WT and c-Nox4KO mice were subjected to either sham or TAC operation. The expression level of HDAC4 and the extent of cysteine reduction in HDAC4 in the indicated mouse were examined by immunoblots and biotinylated IAM pulldown assays. The level of histone H3 indicates equal loading of the samples.

Online Figure XIII



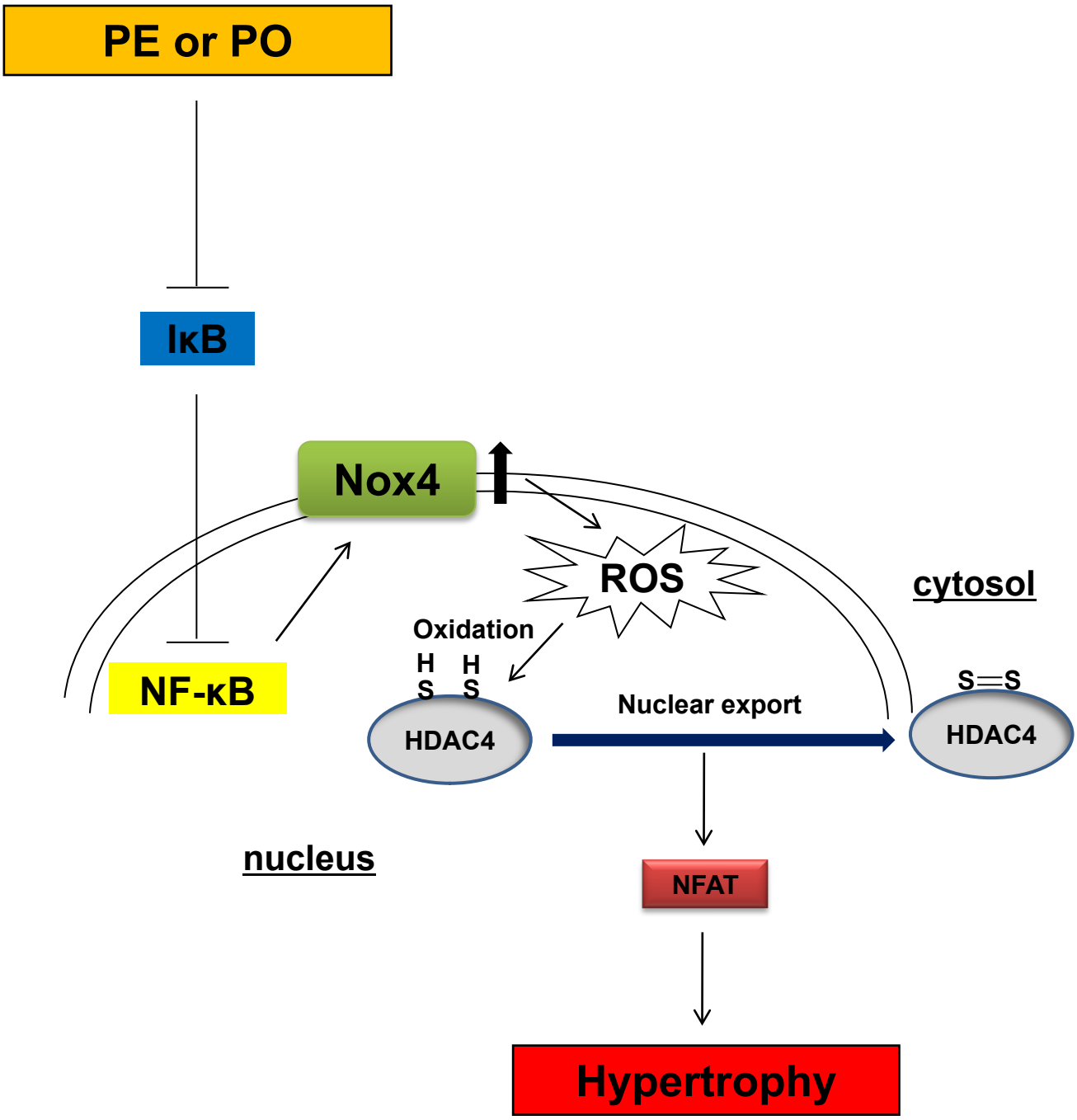
Online Figure XII The effect of cardiomyocyte-specific knockout of Nox4 on cardiac hypertrophy 2 weeks after TAC. A and B. LV myocyte cross-sectional area, as evaluated using WGA staining (n=4). Bar=20 μm. **C.** LVW/TL was determined 2 weeks after TAC operation (n=4-6). **D.** LV wall thickness was determined 2 weeks after TAC operation by echocardiography (n=4-6). *P<0.05, **P<0.01.

Online Figure XIV



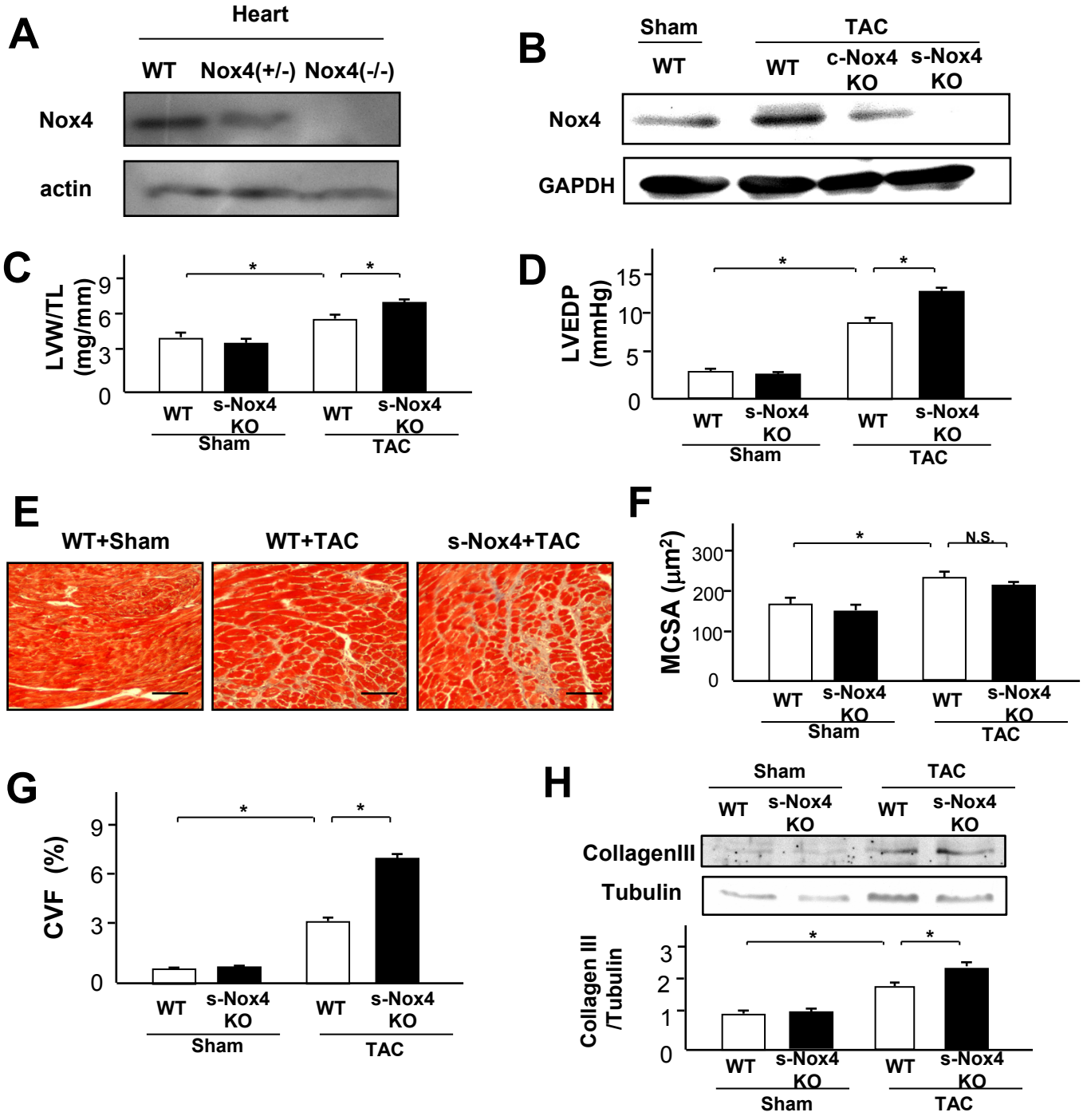
Online Figure XIII The effect of cardiomyocyte-specific knockout of Nox4 on apoptosis and fibrosis in the heart 2 weeks after TAC. A. Apoptosis in LV sections was evaluated with TUNEL staining. PE-induced increases in the number of TUNEL-positive cells were decreased in c-Nox4 KO mice (n=4). Bar=20 μm. **B.** PE-induced increases in collagen volume fraction, as evaluated with Masson-Trichrome staining, were decreased in c-Nox4 KO mice (n=4). Bar=50 μm.

Online Figure XV



Online Figure XV A hypothetical model of the role of Nox4 in mediating cardiac hypertrophy in the heart. PE upregulates Nox4 through activation of NF-κB, and then HDAC4 is oxidized by upregulated Nox4. Oxidized HDAC4 is exported from the nucleus to the cytosol and the hypertrophic signal is activated.

Online Figure XVI



Online Figure XVI Systemic knockout of Nox4 in mice does not attenuate cardiac hypertrophy after TAC. A. Expression levels of Nox4 and actin in WT, Nox4+/-, and systemic Nox4-/- mouse hearts. **B.** Expression levels of Nox4 and GAPDH in WT, c-Nox4 KO and systemic Nox4-/- (s-Nox4 KO) mouse hearts subjected to either sham or TAC operation for 4 weeks. The results shown are representative of 3 experiments. **C.** LVW/TL was determined 4 weeks after TAC operation (n=5). **D.** LVEDP was determined 4 weeks after TAC operation by hemodynamic measurement (n=3-5). **E, F, and G.** LV myocyte cross-sectional area and collagen volume fraction (CVF) were evaluated by Masson-Trichrome staining (n=4). Bar=50 μm.. **H.** Expression levels of collagen III and Tubulin in WT, c-Nox4 KO and s-Nox4 KO mouse hearts subjected to either sham or TAC operation for 4 weeks. The results shown are representative of 3 experiments. * $p<0.05$.

Online Table I

Hemodynamic analyses of c-Nox4 KO after PE treatment

	WT+Saline n=6	c-Nox4KO+Saline n=6	WT+PE n=6	c-Nox4KO+PE n=6
HR	480+53	520+35	530+21	530+15
sBP	108+4	111+7	104+10	111+10
dBP	80+2	84+4	78+6	79+5
mBP	89+3	92+5	89+7	87+7
sLVP	108+7	104+16	105+7	93+13
EDP	5.7+4.2	1.5+0.5	10.7+2.7	3.7+1.5*[1]
+dP/dt	7200+2318	9400+1000	7067+1537	7800+1101
-dP/dt	7933+2040	8400+400	4667+742	7867+933*[2]

Data are mean ± SEM, *[1] P=0.085 vs. WT+PE. *[2] P=0.055 vs. WT+PE.

Online Table II

Hemodynamic analyses of c-Nox4 KO in response to pressure overload

	WT+Sham n=4	c-Nox4KO+sham n=4	WT+TAC n=6	c-Nox4KO+TAC n=6
HR	501+26	491+41	453+70	509+12
sBP	110+5	113+7	138+22	148+12
dBP	79+3	83+3	71+4	73+6
mBP	88+2	91+4	93+7	98+6
sLVP	109+9	108+9	116+7	132+23
EDP	5.7+4.2	1.5+0.5	13.3+1.3*[1]	7.3+1.6*[2]
+dP/dt	7200+2318	7400+1000	5566+1411	5867+1162
-dP/dt	7933+1540	8400+400	4267+561	6566+411
PG	-	-	62+14	68+15

Data are mean ± SEM, *[1] P<0.05 vs. WT+Sham. *[2] P<0.05 vs. WT+TAC.