Supplemental Material.

Supplemental methods.

Generation of MAO-A^{neo} mice. MAO-A^{neo} mouse was generated by alteration of a 9.0kb Bam HI fragment of MAO-A gene containing exon 10 to exon 13 of mouse MAO-A gene. A 0.28kb SmaI-EcoRI loxP fragment was blunt and ligated into a unique SphI site in intron 11 of MAO-A and a 1.6kb EcoRI-BglII floxed neomycin cassette was directionally cloned into in the same sites within the intron 12 of MAO-A gene (Online Figure V). MAO-A^{neo} mice do not have MAO-A activity. The full MAO-A activity was restored by crossing MAO-A^{neo} mice with CMV-cre mice and selectively deleting the 1.6 kb floxed neomycin insert in intron 12.

Genotyping of MAO-A^{neo} mice. Primers flanking the loxP insert in intron 11 were designed for genotyping: forward primer (F): 5'-CCTCTCTTCCAAGTATTAGG-3'; reverse primer (R): 5'-GGAAAAGAGGGAGGAGTAAG-3'. Tail clipped genomic DNA was used as template, DNA fragment was amplified by a 4 min hot start at 94°C followed by 35 cycles of 30 sec at 94°C, 40 sec at the optimal annealing temperature (50°C), 45 sec at 72°C for elongation and a final 5 min extension at 72°C. The PCR product size is 300 bp and 500 bp for WT and MAO-A^{neo} mouse, respectively.

TAC procedure. TAC was performed following a previously reported protocol[1, 2]. After induction of anesthesia and intubation, mice were placed on a volume ventilator (120 breaths/min, 1.2 ml/g/min) and anesthesia was maintained with 5% isofluorane. The aortic arch was isolated and tied against a 27-gauge needle, resulting in a 65-70% constriction after the removal of the needle. The chest and skin were closed and animals extubated and allowed to fully recover. Sham-operated mice underwent the same operation except that after the aortic arch was isolated, there was no ligature placed.

Echocardiography. In vivo cardiac morphology and function were assessed by serial M-mode echocardiography (Acuson Sequioa C256, 13 MHz transducer, Siemens, PA) performed in conscious mice. LV end-systolic and end-diastolic dimensions were averaged from 3-5 beats. LV percent FS, EF and LV mass were calculated as described previously[3]. Thickness of posterior free wall and interventricular septum were averaged.

In Vivo Pressure-Volume Loop Studies. In vivo cardiac function was assessed by pressure-volume catheter in anesthetized mice employing a four-electrode pressure-volume catheter (model SPR-839, Millar Instruments, TX, USA) as previously described[2, 4, 5]. Anesthesia was maintained with i.p. etomidate (250 µg), urethane (30mg) and morphine (15 µg). Mouse was placed in supine position on a thermoregulated surgical table maintained at 37°C. Ventilation via endotracheal tube was maintained with 100% oxygen using a custom-designed constant flow ventilator delivering a tidal volume of 6.7 µl/g at 120 breaths/min. An internal jugular venous line was placed to provide a fluid and drug delivery port. The thorax was opened and a miniature PV catheter inserted into the left ventricle via the apex for continuous LV pressure-volume data. Calibration of the signal was performed using injection of hypertonic saline and direct measurement of cardiac output via an aortic flow probe (Transonic Instruments) placed around thoracic descending aorta or a pulse wave Doppler signal at the aortic outflow (Indus Instruments, Houston, TX). Pressure-volume loop analysis was made using a custom analysis program (WinPVAN 3.3).

Neonatal rat cardiomyocytes studies. Myocytes were isolated from 1-2 days old Sprague Dawley rats, as previously described[6]. After 2 days of culture, myocytes were pretreated with

clorgyline for 1 hour and then subjected to stimulation with norepinephrine or tyramine (10 and 20 μmol/L respectively). After 24 hours, cells were harvested for RNA isolation using Trizol (Invitrogen). For cell area measurements, cells were fixed, incubated with α-actinin primary antibody (1:500 dilution, Sigma) and fluorescent secondary antibody (Molecular probes) and visualized by confocal microscopy (Zeiss, LSM 510 Meta). Approximately 150-200 cells were analyzed per condition in each experiment. All the experiments were repeated at least four times. For mitochondrial ROS production measurements, the reduced form of Mitotracker Red (Mitotracker Red CM-H_2XRos, Molecular probes) was used. Cells were loaded with 100 nmol/L Mitotracker Red CM-H_2XRos for 30 minutes at 37˚C, washed and then incubated with NE or vehicle for 2 hours. After this incubation time, cells were analyzed and images acquired by confocal microscopy. Images were analyzed and fluorescence intensities determined using computer-assisted image analysis systems (ImageJ, NIH). For the mitochondrial membrane potential measurements, cells were loaded with 20 nmol/L tetramethylrodamine (TMRM) for 30 minutes and then treated with NE for 2 hours. Images were acquired and analyzed using computer-assisted image analysis systems (ImageJ, NIH).

Measurement of MAO-A activity. MAO-A activity in the heart (expressed as nmol per 20 min per milligram of protein) was determined by use of ^{14}C labeled serotonin (5-HT) as a substrate[7]. Alternatively, MAO-A activity was determined by Amplex Red assay (Invitrogen), measuring hydrogen peroxide formation.

Catecholamine measurement. In order to remove any contaminating blood from the LV specimens, after the excision the hearts were retrogradely perfused in a Langerdorff apparatus for 10 min using K-H solution. Then, LV specimens were dissected and homogenized in 0.4 mol/L perchloric acid containing 0.5 mmol/L EDTA, centrifuged at 3 000 rpm for 5 minutes and the supernatant stored for alumina extraction. CA and 5-HT were determined by HPLC as described previously[8]. CA/5-HT content is expressed as a function of protein content.

Histology. Hearts were fixed in 10% formalin overnight, embedded in paraffin, sectioned at 5 μm thickness and stained using H&E and Masson's trichrome[6]. Photomicrographs of the sections were evaluated for interstitial collagen fractions using computer-assisted image analysis systems (ImageJ, NIH).

Apoptosis detection by TUNEL staining. The assay was performed using a commercially available kit according to manufacturer's instructions (CardioTACs). TUNEL-positive nuclei were visualized using a light microscope equipped with a camera. The percentage of TUNEL-positive cardiomyocytes is expressed as a percentage of total nuclei.

Measurement of oxidative stress. Oxidative stress was determined by MDA formation measurement and DHE staining. MDA, an end product of lipid peroxidation, was determined spectrophotometrically by measurement of 2-thiobarbituric acid reactive substances (TBARS), as described previously[9]. For DHE staining, 10 μm thick cryosections were incubated with 5 μmol/L DHE (Sigma) for 30 minutes at 37˚C, washed twice with PBS, mounted and visualized using a confocal microscope (Zeiss, LSM 510 Meta).

MMP activity measurement. In vitro gelatin lysis by MMP-2 and MMP-9 was assessed by zymography as previously described[10]. Briefly, protein concentration was determined and equal amounts of lysed tissue samples were loaded on a 10% gelatin gels (Invitrogen Corp.). After electrophoresis, gels were washed twice with renaturing buffer at room temperature followed by developing buffer (Invitrogen Corp.) and then stained to visualize lytic bands (SimplyBlue;

Invitrogen Corp.). Lytic bands corresponding to active forms of MMP-2 and MMP-9 were quantified using the BioRad analysis software.

Western Blot and real time PCR. Western blot was performed as previously described[6]. RNA was isolated, purified and reverse transcribed using commercially available kits (Qiagen, Invitrogen). cDNA was subjected to PCR amplification (Abi Prism 7000 Detection System, Applied Biosystems) using SYBR Green dye (Applied Biosystems). Primer sets for the specific target genes were designed to span one or more introns (IDT Technologies).

Adult mouse ventricular myocytes (AMVM) isolation and culture. AMVMs were isolated from the hearts of adult (12 weeks) male MAO-A^{neo} mice and their WT littermates as described previously[11]. Cells were plated at a nonconfluent density of 25 000 rod shaped cells/ml on plastic culture dishes or glass coverslips precoated with laminin (20 µg/ml) and kept at 37°C in the culture medium (DMEM, Joklik modified MEM, $NaHCO_3$ 2 g/l, BSA 5 g/l, L-carnitine 1.5 mmol/L, creatine 5 mmol/L, taurine 7.5 mmol/L, ITS 1%, penicillin 100 IU/mL, streptomycin 10 µg/mL) for 1 hour before being used for the experiments. For mitochondrial ROS production measurements, cells were loaded with 100 nmol/L Mitotracker Red CM-H_2XRos for 30 minutes at 37˚C, washed and then incubated with NE (10 µmol/L) or vehicle for 2 hours. After this incubation time, cells were analyzed and images acquired by confocal microscopy. Images were analyzed and fluorescence intensities determined using computer-assisted image analysis systems (ImageJ, NIH). For cell area measurements, cells were incubated with NE (1 µmol/L) for 24 hours and visualized by confocal microscopy (Zeiss, LSM 510 Meta). Approximately 150-200 cells were analyzed per condition in each experiment and all the experiments were repeated at least three times.

References

(1) Takimoto E, Yao A, Toko H, Takano H, Shimoyama M, Sonoda M, Wakimoto K, Takahashi T, Akazawa H, Mizukami M, Nagai T, Nagai R, Komuro I. Sodium calcium exchanger plays a key role in alteration of cardiac function in response to pressure overload. *FASEB J* 2002;16:373-8.

(2) Isoda T, Paolocci N, Haghighi K, Wang C, Wang Y, Georgakopoulos D, Servillo G, Della Fazia MA, Kranias EG, Depaoli-Roach AA, Sassone-Corsi P, Kass DA. Novel regulation of cardiac force-frequency relation by CREM (cAMP response element modulator). *FASEB J* 2003;17:144-51.

(3) Takimoto E, Champion HC, Li M, Ren S, Rodriguez ER, Tavazzi B, Lazzarino G, Paolocci N, Gabrielson KL, Wang Y, Kass DA. Oxidant stress from nitric oxide synthase-3 uncoupling stimulates cardiac pathologic remodeling from chronic pressure load. *J Clin Invest* 2005;115:1221-31.

(4) Georgakopoulos D, Kass DA. Protocols for hemodynamic assessment of transgenic mice in vivo. *Methods Mol Biol* 2003;219:233-43.

(5) Murphy AM, Kogler H, Georgakopoulos D, McDonough JL, Kass DA, Van Eyk JE, Marban E. Transgenic mouse model of stunned myocardium. *Science* 2000;287:488-91.

(6) Takimoto E, Champion HC, Li M, Belardi D, Ren S, Rodriguez ER, Bedja D, Gabrielson KL, Wang Y, Kass DA. Chronic inhibition of cyclic GMP phosphodiesterase 5A prevents and reverses cardiac hypertrophy. *Nat Med* 2005;11:214-22.

(7) Wu HF, Chen K, Shih JC. Site-directed mutagenesis of monoamine oxidase A and B: role of cysteines. *Mol Pharmacol* 1993;43:888-93.

(8) Eisenhofer G, Goldstein DS, Stull R, Keiser HR, Sunderland T, Murphy DL, Kopin IJ. Simultaneous liquid-chromatographic determination of 3,4-dihydroxyphenylglycol, catecholamines, and 3,4-dihydroxyphenylalanine in plasma, and their responses to inhibition of monoamine oxidase. *Clin Chem* 1986;32:2030-3.

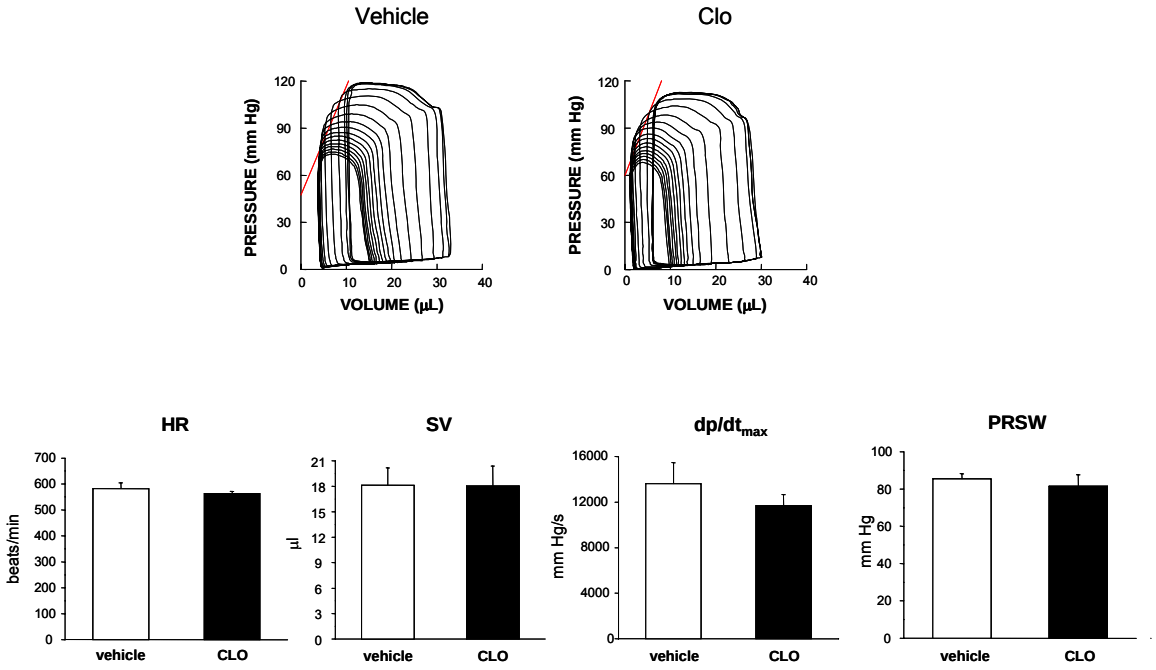
(9) Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Anal Biochem* 1979;95:351-8.

(10) Paolocci N, Tavazzi B, Biondi R, Gluzband YA, Amorini AM, Tocchetti CG, Hejazi M, Caturegli PM, Kajstura J, Lazzarino G, Kass DA. Metalloproteinase inhibitor counters high-energy phosphate depletion and AMP deaminase activity enhancing ventricular diastolic compliance in subacute heart failure. *J Pharmacol Exp Ther* 2006;317:506-13.

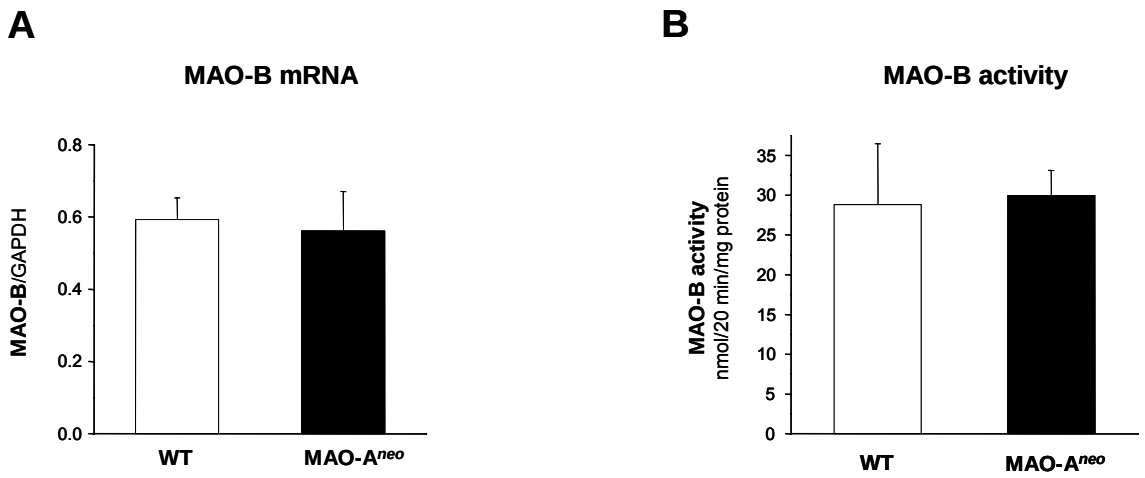
(11) Tocchetti CG, Wang W, Froehlich JP, Huke S, Aon MA, Wilson GM, Di Benedetto G, O'Rourke B, Gao WD, Wink DA, Toscano JP, Zaccolo M, Bers DM, Valdivia HH, Cheng H, Kass DA, Paolocci N. Nitroxyl improves cellular heart function by directly enhancing cardiac sarcoplasmic reticulum Ca2+ cycling. *Circ Res* 2007;100:96-104.

Online Figures

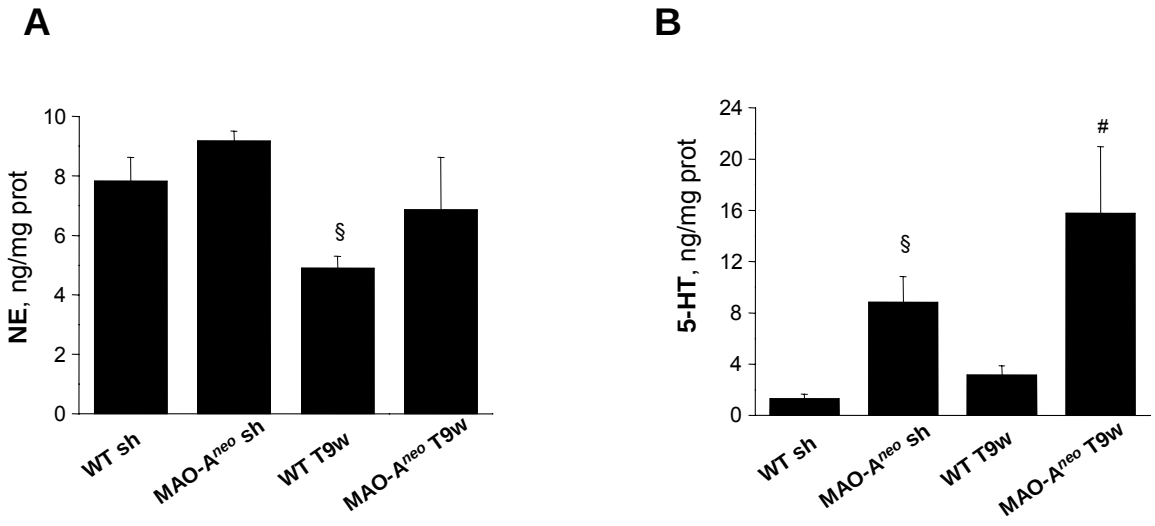
Online Figure I. Hemodynamic assessment of control (vehicle) and clorgyline- treated mice. A: Representative PV loops at baseline and after IVC occlusion from 6 weeks vehicle and clorgyline-treated mice. B: Heart rate (HR), stroke volume (SV), dp/dt_{max} and preload recruitable stroke work (PRSW) in vehicle and clorgyline treated mice. (n=5 each)



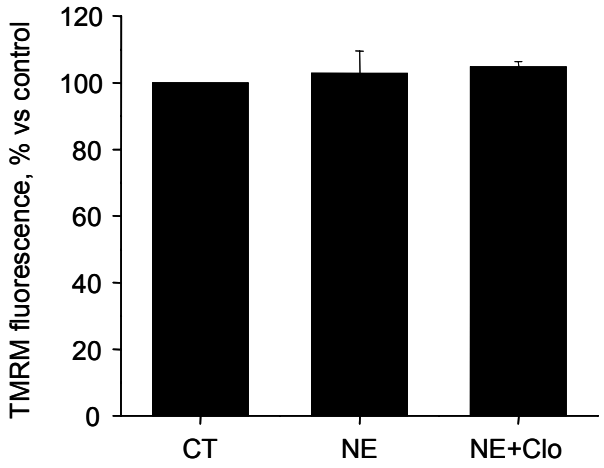
Online Figure II. MAO-B expression and activity in MAO-A^{neo} mice. A: MAO-B mRNA levels in hearts from WT and MAO-A^{neo} mice. B: MAO-B activity in hearts from WT and MAO-A^{neo} mice. (n=5 each)



Online Figure III. NE (panel A) and serotonin (panel B) levels in hearts from WT and MAO-A^{neo} mice at baseline an after 9 weeks of TAC. $^{\S}p<0.05$ *vs WT sham,* $^{\#}p<0.05$ *vs WT T9w.* (n=5 each)



Online Figure IV. Mitochondrial membrane potential determined by TMRM after 2 hours of incubation with 10 μmol/L NE in the absence or in the presence of clorgyline (Clo).



Online Figure V. Schematic representation of MAO-A^{neo} mice generation. The insertion of 0.28kb loxP and 1.6 kb loxP-pgk1-Neo-loxP cassette into intron 11 and intron 12 of MAO A gene. A loxP (0.28kb SmaI-EcoRI) fragment was blunt end ligated into a unique Sp site in intron 11. Another 1.6kb E-Bg floxed neo cassette was directionally cloned into the E-Bg site in intron 12 of MAO-A gene. (Sp: SphI; E; EcoRI; Bg: Bgl II restriction enzyme).