

Supplementary Data

Supplementary Methods and Materials

Measurement of monoamine oxidase activity

Cardiac monoamine oxidase (MAO)-A and -B activity (expressed as nmol per 20 min per milligram of protein) was determined by use of ^{14}C labeled serotonin (5-HT) and ^{14}C labeled β -phenylethylamine as substrates, respectively. Assays were performed as previously described (1).

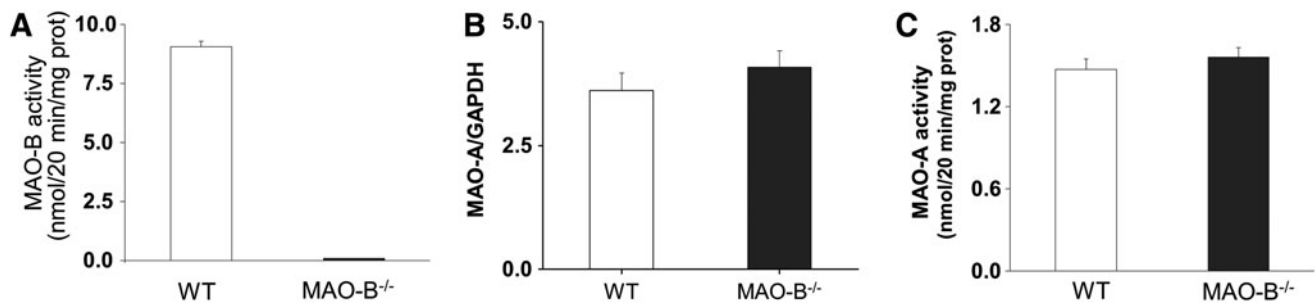
RT-PCR and real-time RT-PCR

RNA was isolated from left ventricle tissue and reversely transcribed using commercially available kits (Qiagen, In-

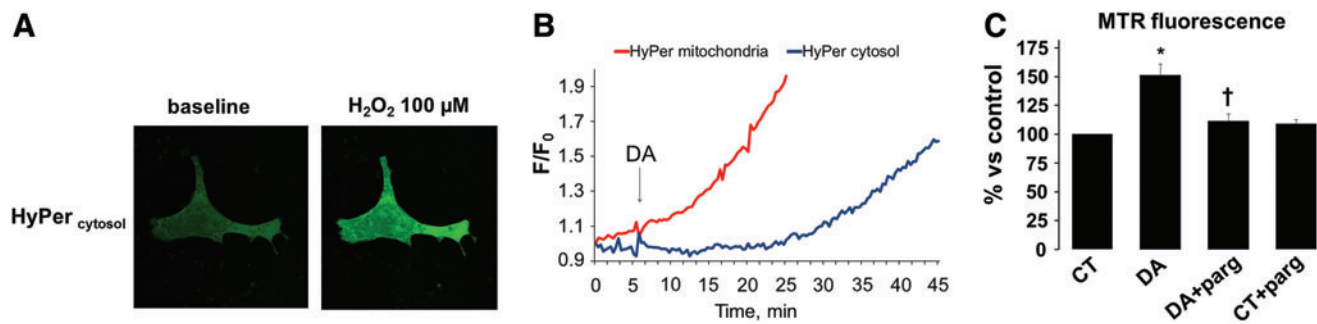
vitrogen). cDNA was subjected to real time PCR amplification (Abi Prism 7000 Detection System; Applied Biosystems) using SYBR Green dye (Applied Biosystems). Primer sets for the specific target genes were designed to span one or more introns (IDT Technologies).

Supplementary Reference

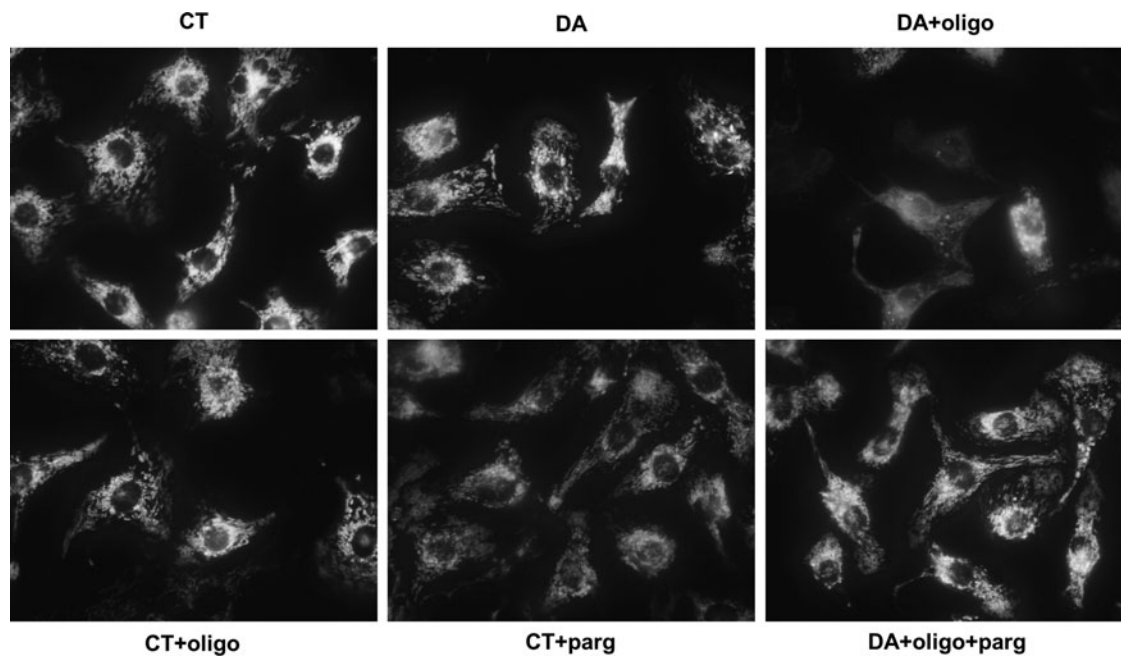
1. Chen K, Wu HF, and Shih JC. Influence of C terminus on monoamine oxidase A and B catalytic activity. *J Neurochem* 66: 797–803, 1996.



SUPPLEMENTARY FIG. S1. MAO-B and MAO-A activity in MAO-B^{-/-} mice. (A) *In vitro* activity of MAO-B in WT and MAO-B^{-/-} cardiac tissue. **(B)** MAO-A gene expression normalized to GAPDH and **(C)** MAO-A activity measured in hearts from WT and MAO-B^{-/-} mice. MAO, monoamine oxidase; WT, wild type.



SUPPLEMENTARY FIG. S2. H₂O₂ generation following MAO activation occurs early and predominantly in the mitochondria. (A) Distribution pattern and fluorescence intensity of cytosol-targeted HyPer in neonatal cardiomyocytes at baseline and after exogenous H₂O₂ addition. (B) Time course for H₂O₂ formation between mitochondria and cytosol upon MAO activity stimulation with DA. (C) Increase in mitochondrial reactive oxygen species generation upon MAO-B stimulation is also detectable with Mitotracker Red CM-H₂XRos. **p* < 0.005 versus CT, †*p* < 0.05 versus DA. DA, dopamine; F/F₀, fluorescence intensity relative to resting fluorescence. To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/ars



SUPPLEMENTARY FIG. S3. Representative images for TMRM staining in neonatal cardiomyocytes after 3 h of incubation with DA, oligomycin and without or with pargyline. CT, control; oligo, oligomycin; parg, pargyline; TMRM, tetramethylrhodamine methyl ester.