**Suliman *et al.* Data Supplement**

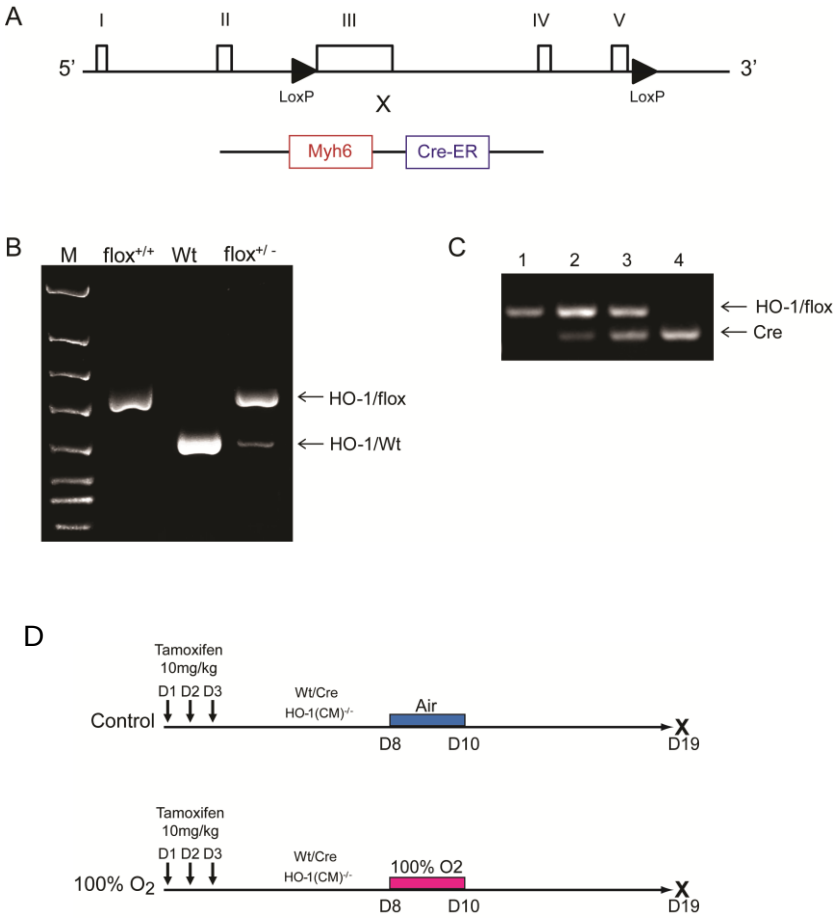


**Figure 1S**. **Generation of cardiac specific HO-1 knockout mice and experimental profile.** (**A**) The HO-1 locus and location of the loxp inserts crossed to schematic of the transgenic constructs in Myh6-Cre mice that were used to generate HO-1(CM)-/- mice (bottom).

(**B**) PCR of genomic DNA from floxed HO-1 and wild type HO-1.

(**C**) PCR from genomic DNA confirms disruption of the Hmox-1 gene showing lane 1, upper band of HO-1/f/f mice. Lane 2 and 3, shows upper band of HO-1/f/f and lower band of Cre+. Lane 4 shows the lower band only for Cre+ (n= 5 in each group).

(**D**) **Protocols.** To examine the effect of tamoxifen and Cre recombinase in Wt and HO-1 $(CM)^{-/-}$ mice, tamoxifen was injected for 3 consecutive days to induce Cre recombinase and result in deletion of *Hmox1* in HO-1$(CM)^{-/-}$ mice only. To examine the effect of hyperoxia in Wt/Cre and HO-1$(CM)^{-/-}$, 8 days following tamoxifen administration, the mice were exposed to 100%$O_2$ for 48 h, and then left to breathe ambient air for another 8 days. Hearts were harvested at the end of the 100% $O_2$ period (n=4 per group) and at day 19 (n=10 per group).

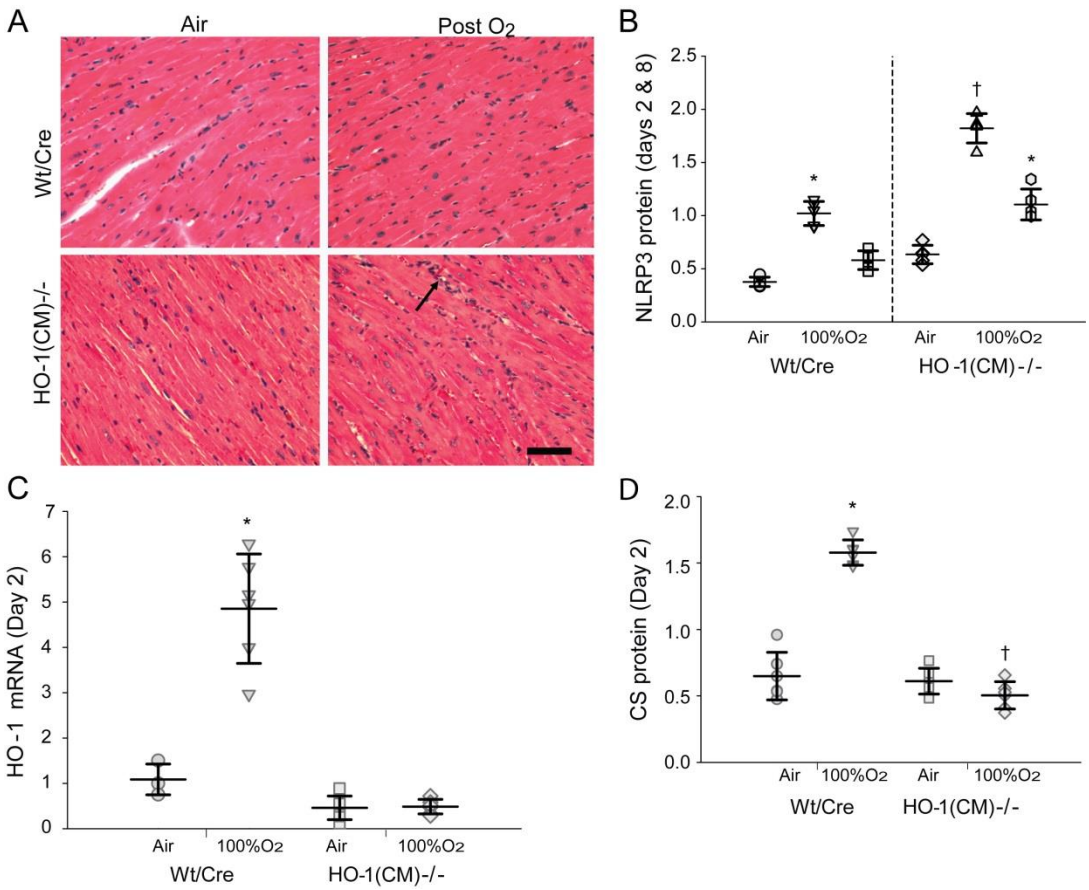


**Figure S2. Cardiomyocyte-specific ablation of HO-1 and immediate post-$O_2$ responses in adult mice.**

(**A**) Photomicrographs of LV sections stained with hematoxylin and eosin (H&E) of control Wt/Cre mice post-tamoxifen and pre 100% $O_2$; Wt/Cre mice immediately post 48h 100% $O_2$; control HO-1(CM)-/- mice pre-100% $O_2$, and immediately post 48h $O_2$. Hyperoxia after HO-1 ablation caused increased foci of inflammation (arrow). Original magnification ×250; scale bar = 10 μm.

(**B**) NLRP3 inflammasome protein expression in Wt/Cre and HO-1(CM)-/- mice immediately after 48h and 8days after 100% $O_2$. Results are expressed in arbitrary units as Mean ± SEM. Horizontal bars represent mean values. (* $P<0.05$ for pre- vs. post-100% $O_2$); †$P<0.05$ for Wt/Cre vs. HO-1(CM)-/-) (n= 6 per group).

(**C**) qRT-PCR analysis of cardiac HO-1 in Wt/Cre, and HO-1(CM)-/- mice immediately after 48h of 100% $O_2$. Results are expressed Mean ± SEM. Horizontal bars represent mean values. *$P<0.05$ for pre- vs. post-hyperoxia; †$P<0.05$ for Wt/Cre vs. HO-1(CM)-/-) (n= 6 per group).

(**D**) CS protein expression in Wt/Cre and HO-1(CM)-/- mice immediately after 48h 100% $O_2$. Results are expressed in arbitrary units as Mean ± SEM. Horizontal bars represent mean values. (* $P<0.05$ for pre- vs. post-100% $O_2$) (n=6 per group).

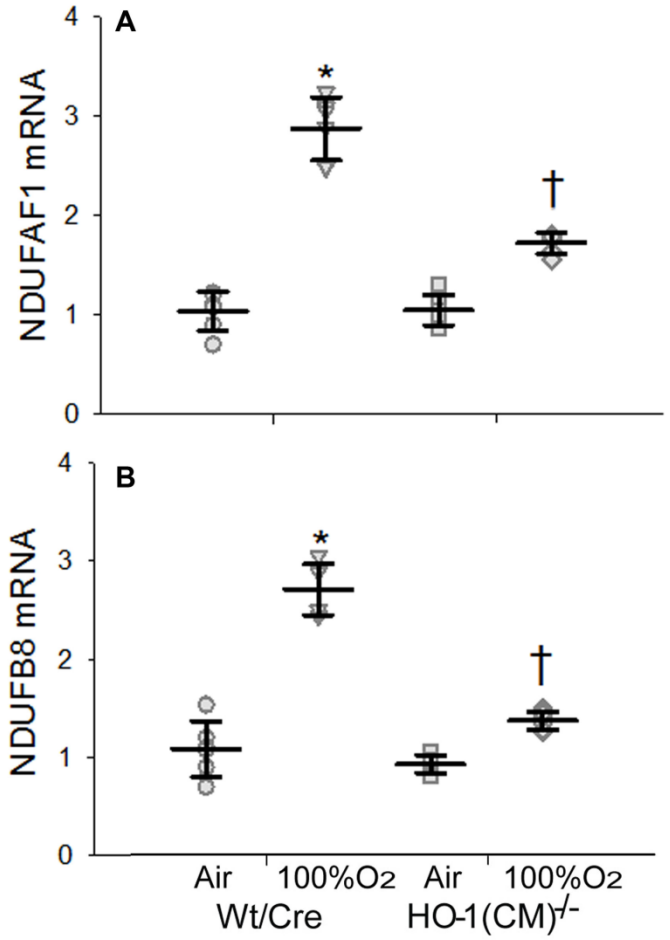


**Figure S3. Cardiomyocyte-specific ablation of HO-1 attenuate NDUFAF1 and NDUFB8 mRNA expression compared to Wt post-O2 responses in adult mice.**

(A) qRT-PCR analysis of cardiac NDUFAF1 in Wt/Cre, and HO-1(CM)-/- mice after 48h of 100% O2. Results are expressed as Mean ± SEM. Horizontal bars represent mean values. *P<0.05 for pre- vs. post-hyperoxia; †P<0.05 for Wt/Cre vs. HO-1(CM)-/-) (n= 6 per group).

(B) qRT-PCR analysis of cardiac NDUFB8 in Wt/Cre, and HO-1(CM)-/- mice after 48h of 100% O2. Results are expressed as Mean ± SEM. Horizontal bars represent mean values. *P<0.05 for pre- vs. post-hyperoxia; †P<0.05 for Wt/Cre vs. HO-1(CM)-/-) (n= 6 per group).

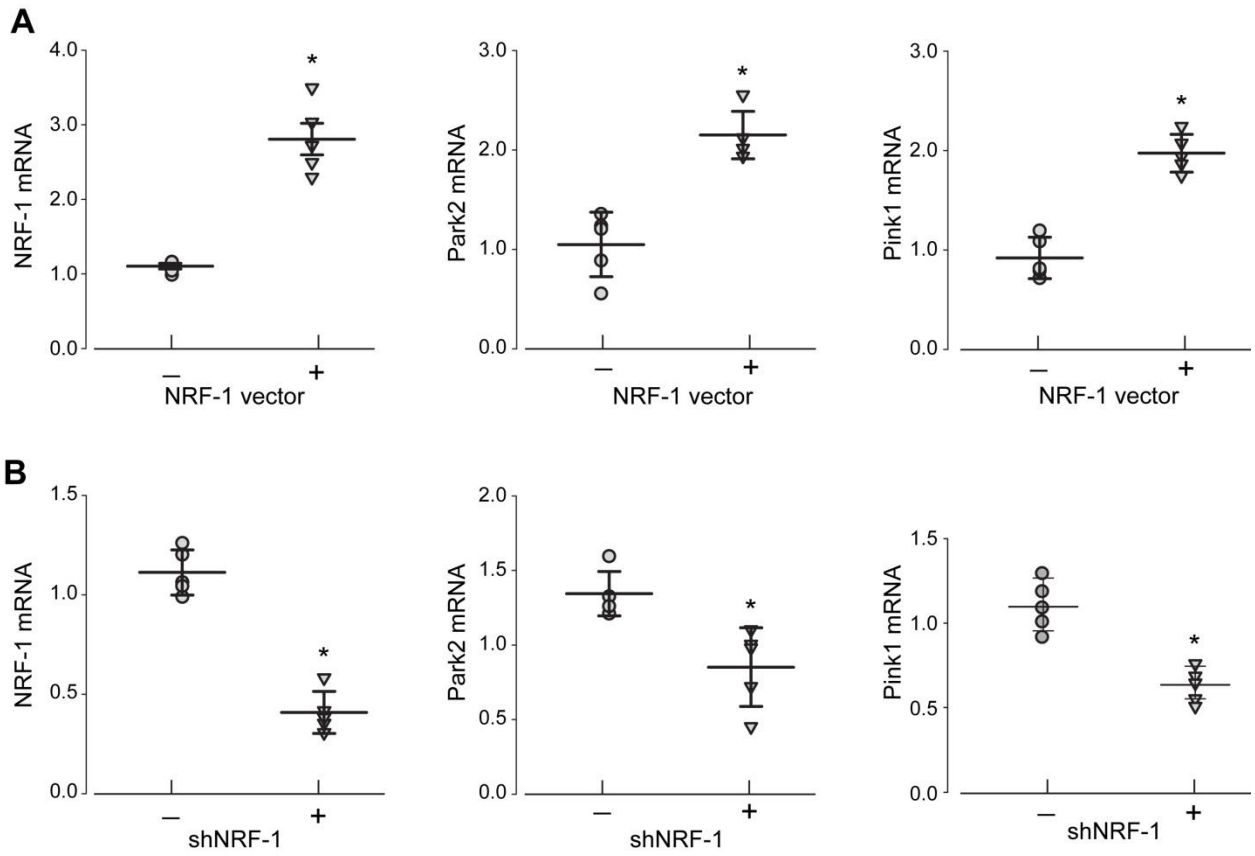


**Figure S4: NRF-1 regulate mitophagy genes in HL-1 cells.**

(**A**) Cardiomyocyte over-expression of NRF-1 and changes in Park2 and Pink1 mRNA levels in HL-1 beating cardiomyocytes. See methods for cell preparation. Horizontal bars represent mean values. Asterisks indicate P< 0.05 relative to control (-) values.

(**B**) Ablation of NRF-1 in HL-1 beating cardiomyocytes and changes in Park2 and Pink1 mRNA levels. Loss of NRF-1 expression results in lower levels of Park2 and Pink1 mRNA at 72 h. Horizontal bars represent mean values. Asterisks indicate P< 0.05 relative to control (-) values.

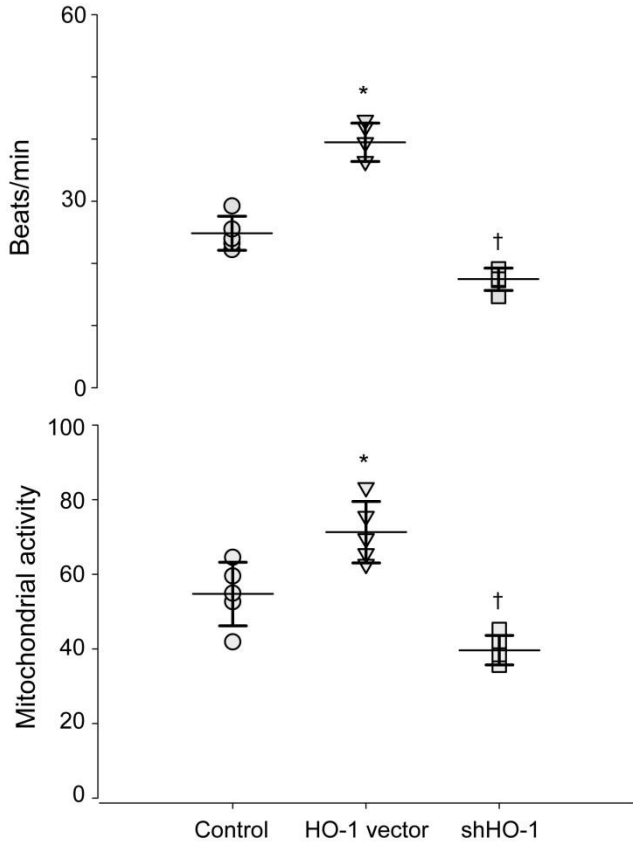


**Figure S5: HO-1 regulate mitochondrial activity in HL-1 cardiomyocytes.**

Top panel compares beating frequency of HL-1 cardiomyocytes after overexpression or silencing of HO-1 (See Methods). Beat rate was estimated by counting the number of beats per min in five different cell clusters in four blinded experiments. Bottom panel shows MTT assay to estimate total cellular metabolic activity based on the reduction of MTT compound to the formazan by mitochondrial dehydrogenases. Horizontal bars represent mean values. Asterisks and dagger symbols indicate $P< 0.05$ relative to control (-) values.