CYP2J2 expression in adult ventricular myocytes protects against ROS toxicity

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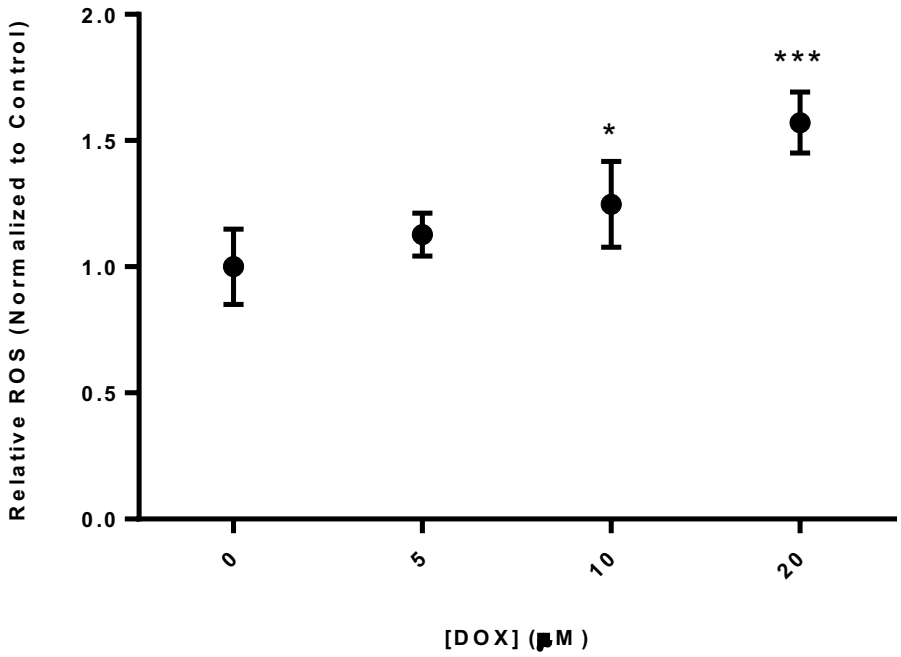
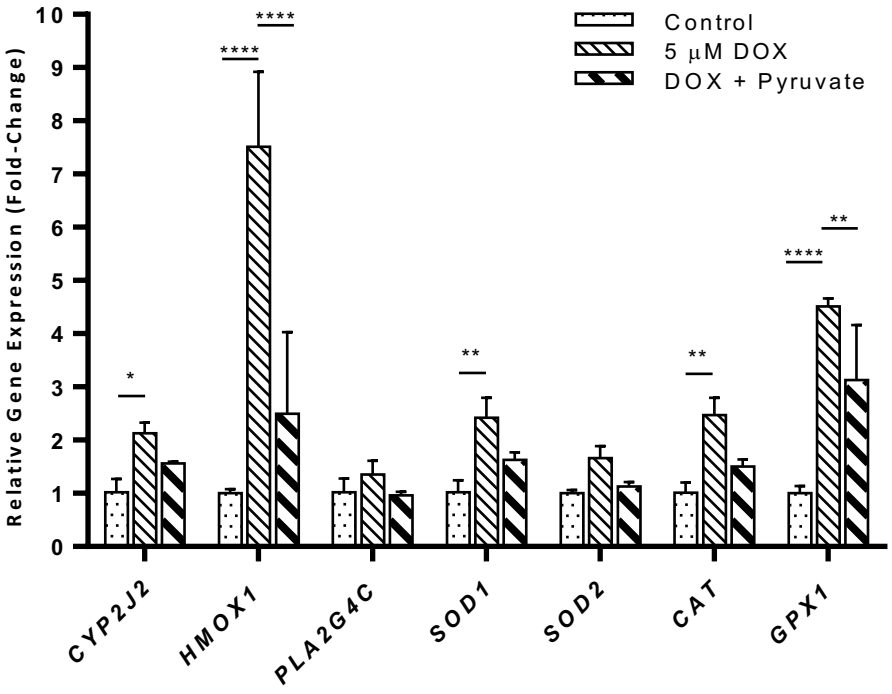
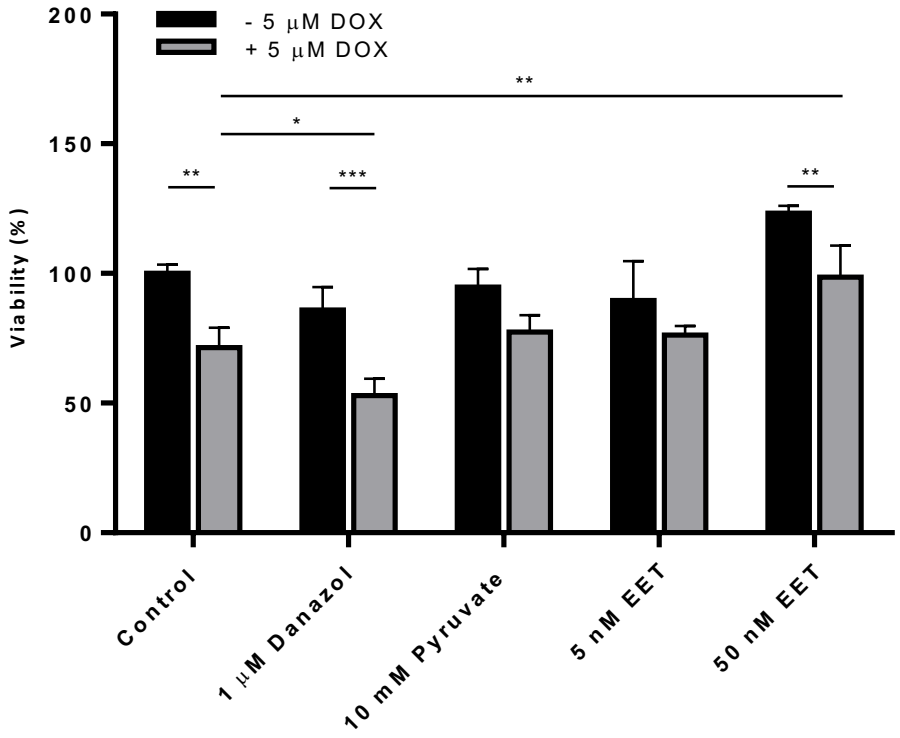


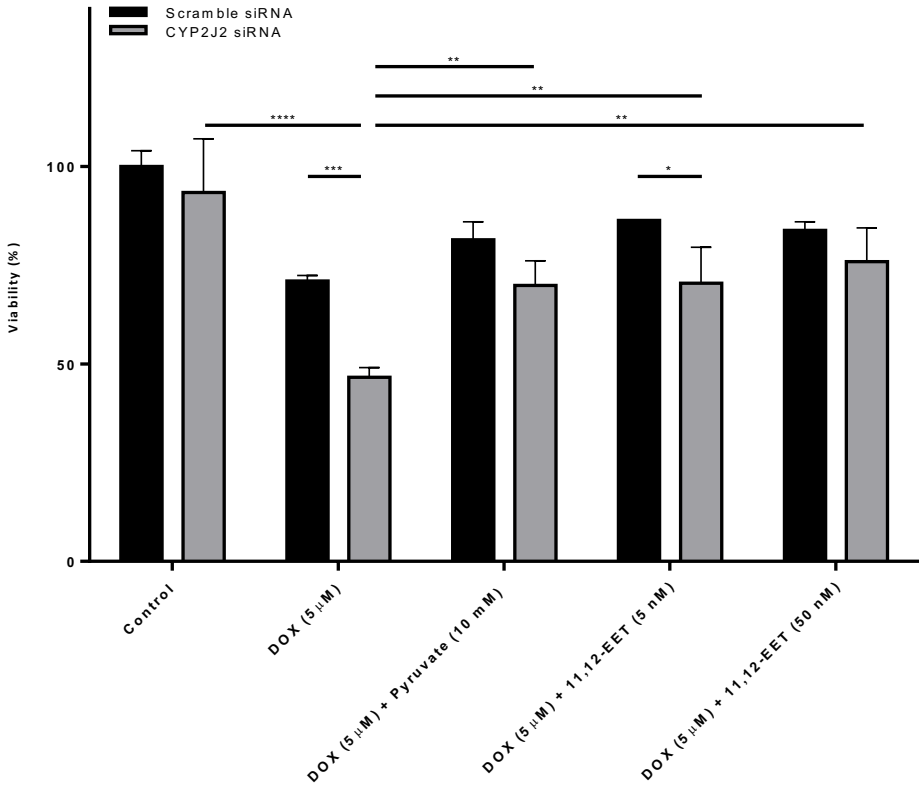
Supplemental Figure 1. Relative ROS levels in adult human ventricular myocytes after 24 hours of exposure to varying doxorubicin concentrations. Fluorescence values were normalized to vehicle control (0 μM DOX, 0.1% DMSO) to determine the relative intracellular ROS levels in cells with each concentration of DOX. The data demonstrate a dose-dependent increase in intracellular ROS levels, compared to the untreated control, with the maximum dose tested (20 μM) resulting in approximately 50% higher ROS levels than the vehicle control. Values reported are the mean ± SD of triplicates. * $p < 0.05$, *** $p < 0.001$.



Supplemental Figure 2. Fold changes in gene expression of a panel of genes following exposure of adult human ventricular myocytes to 5 μM doxorubicin, in the presence and absence of 10 mM pyruvate. Various genes, many of which are ROS sensitive and encode antioxidant enzymes, are upregulated in response to doxorubicin exposure. Additionally, this upregulation is mitigated when cells are exposed to doxorubicin (5 μM) and pyruvate (10 mM) simultaneously. Data shown are the mean ± SD of triplicates. * $p < 0.05$, ** $p < 0.01$, **** $p < 0.0001$.



Supplemental Figure 3. Cell viability of adult human ventricular myocytes treated with or without 5 μM DOX. Additional conditions (x-axis) were also tested. Data demonstrate that in the absence of additional stress, cell viability decreases 25% on average when exposed to doxorubicin (5 μM). When the CYP2J2 inhibitor danazol is added to this exposure, cell death is exacerbated. Finally, this decrease in cell viability is attenuated in the presence of either the antioxidant pyruvate or 11,12-EET (5 nM or 50 nM). The data presented on this figure are representative of the mean ± SD of triplicates. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.



Supplemental Figure 4. Cell viability of adult ventricular myocytes when *CYP2J2* is silenced prior to doxorubicin (5 μM) exposure. Gene silencing by itself does not affect cell viability, however exposure to doxorubicin results in increased cell death with greater cell death occurring among cells with *CYP2J2* expression silenced. Additionally, this decrease in cell viability can be mitigated by the addition of the antioxidant pyruvate (10 mM) or 11,12-EET (5 nM or 50 nM). The data presented are the mean ± SD of triplicates in a single experiment. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$.