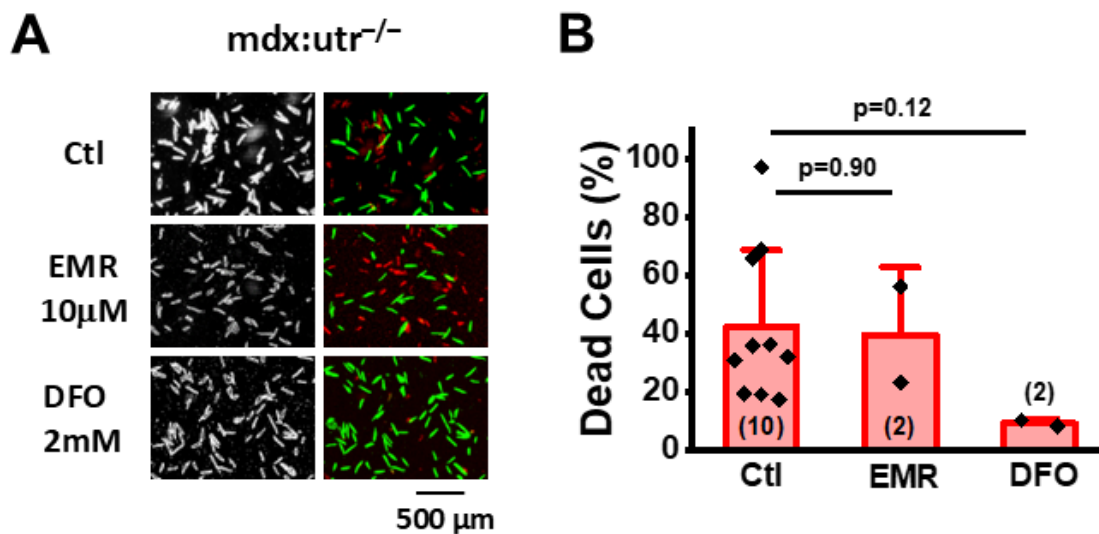


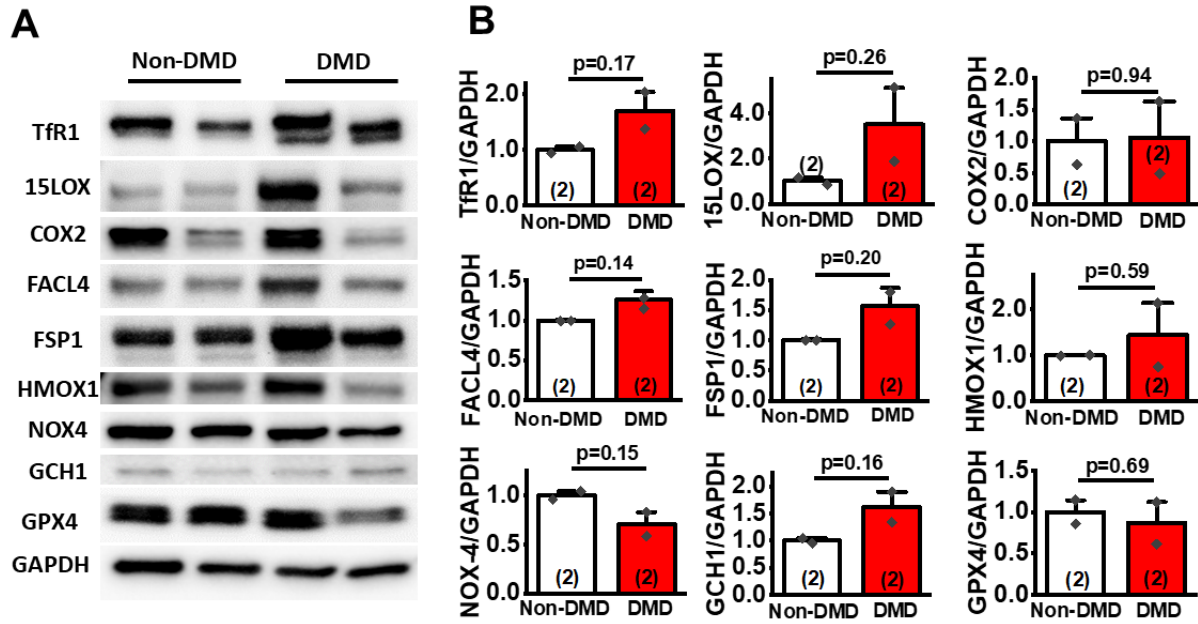
Potential Involvement of Ferroptosis in Duchenne Muscular Dystrophy-Associated Cardiomyopathy

Nadezhda Fefelova, Sri Harika Pamarthi, Satvik Mareedu, Andreas Ivessa, Diego Fraidenaich, Gopal J. Babu, Judith K. Gwathmey and Lai-Hua Xie *

Supplementary Data



Supplement Figure S1. Incidence of ferroptosis in *mdx:utr^{-/-}* cardiomyocytes in the absence (Ctl) and presence of the apoptosis blocker emricasan (EMR) or the iron chelator deferoxamine (DFO). **A:** Representative images of *mdx:utr^{-/-}* cardiomyocytes cultured for ~18 hours under control conditions (Ctl) or treated with 10 µM EMR or 2mM DFO, respectively. Brightfield (left) and Live/Dead (right) images are shown for each condition. Live cells were stained with calcein AM (green), and nuclei of dead cells were stained with ethidium homodimer-1 (red). **B:** Quantitation of cell death rates, which were calculated as dead cells/total cells. Note the Ctl data is the same as in Fig. 2B *mdx:utr^{-/-}* for comparison. P-Values are shown as determined by two-way ANOVA followed by Tukey's post hoc test. Cell death in *mdx:utr^{-/-}* cardiomyocytes showed a strong tendency to be prevented by DFO, but not by EMR, consistent with an iron dependency and ferroptotic nature.



Supplement Figure S2. Assessment of ferroptosis-related molecules in heart tissues from DMD patients compared to those of non-DMD controls. **A:** Western blot images of various ferroptosis-related molecules in the human ventricular biopsies of DMD patients compared to those of non-DMD controls. **B:** Quantification of ferroptosis-related molecule expression normalized to GAPDH loading control. Data were normalized to non-DCM control on the same gel (n=2 in each group).