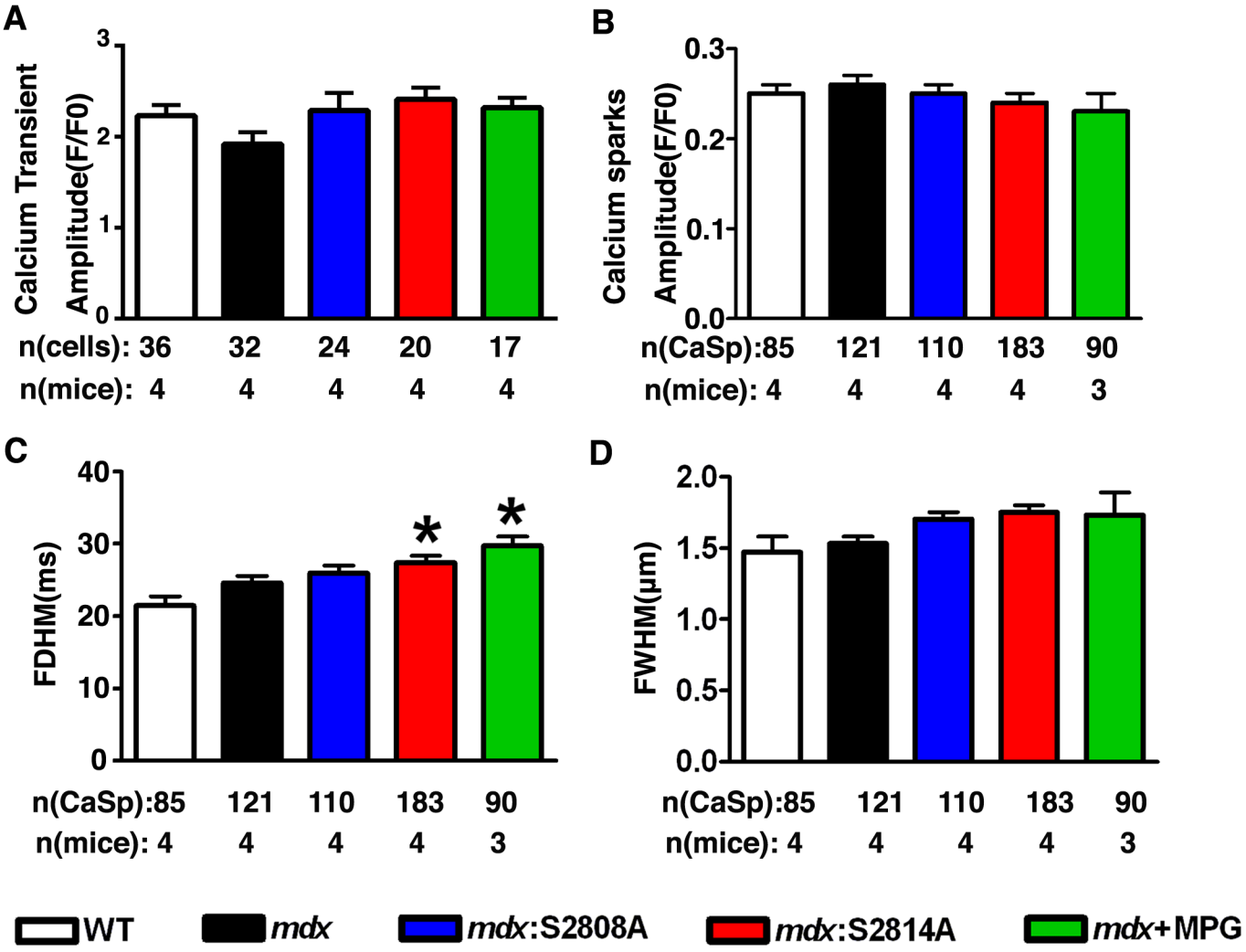
Crosstalk between RyR2 Oxidation and Phosphorylation Contributes to Cardiac dysfunction in Mice with Duchenne Muscular Dystrophy

Qiongling Wang PhD, Wei Wang PhD, Guoliang Wang PhD, George Rodney PhD, Xander H.T. Wehrens, MD PhD

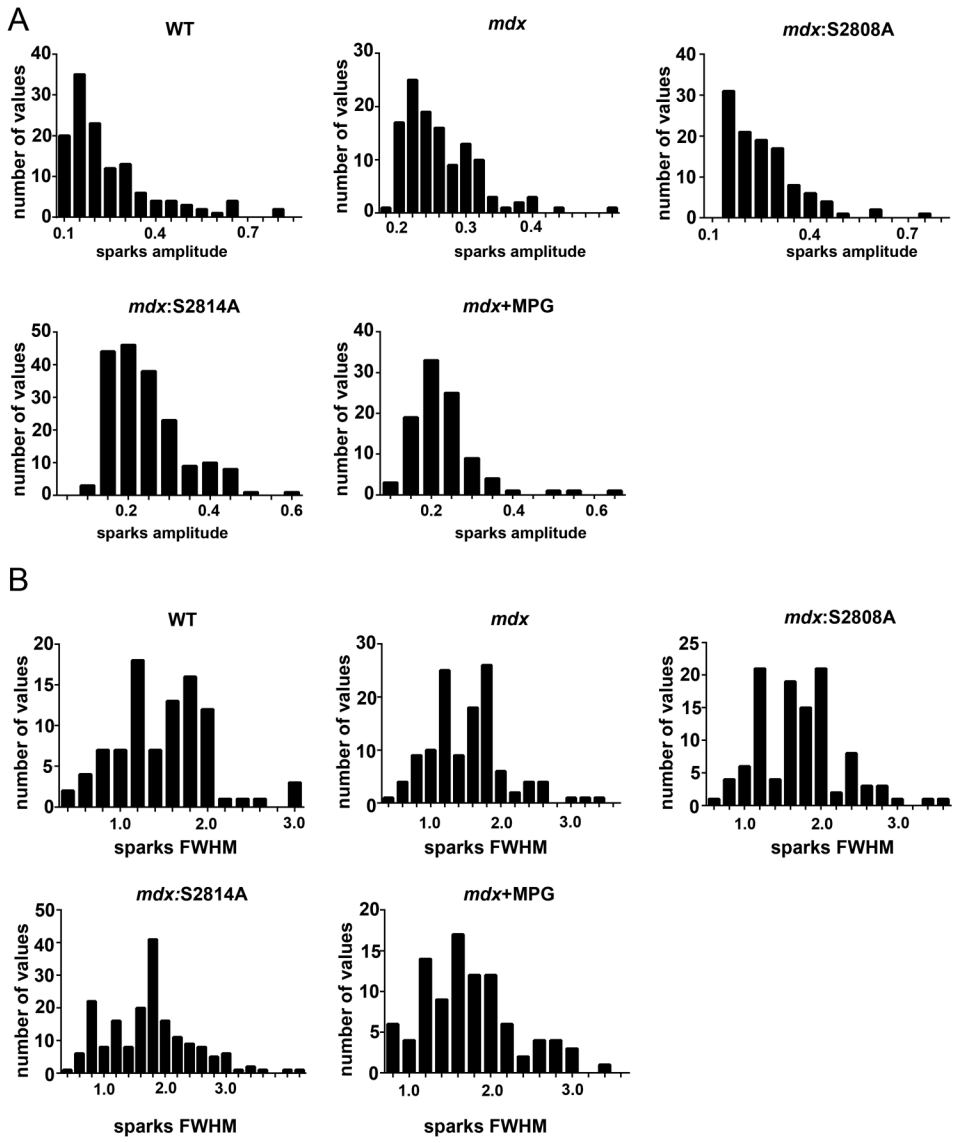
SUPPLEMENTAL METHODS

ROS measurement by DHE. Reactive oxygen species production was also determined using the fluorescent dye dihydroethidium (DHE). Cardiomyocytes were incubated with 5 μM DHE at for 30 min. Images were captured on a Zeiss LSM 510 microscope and pixel intensity of the cells was measured using ImageJ software.

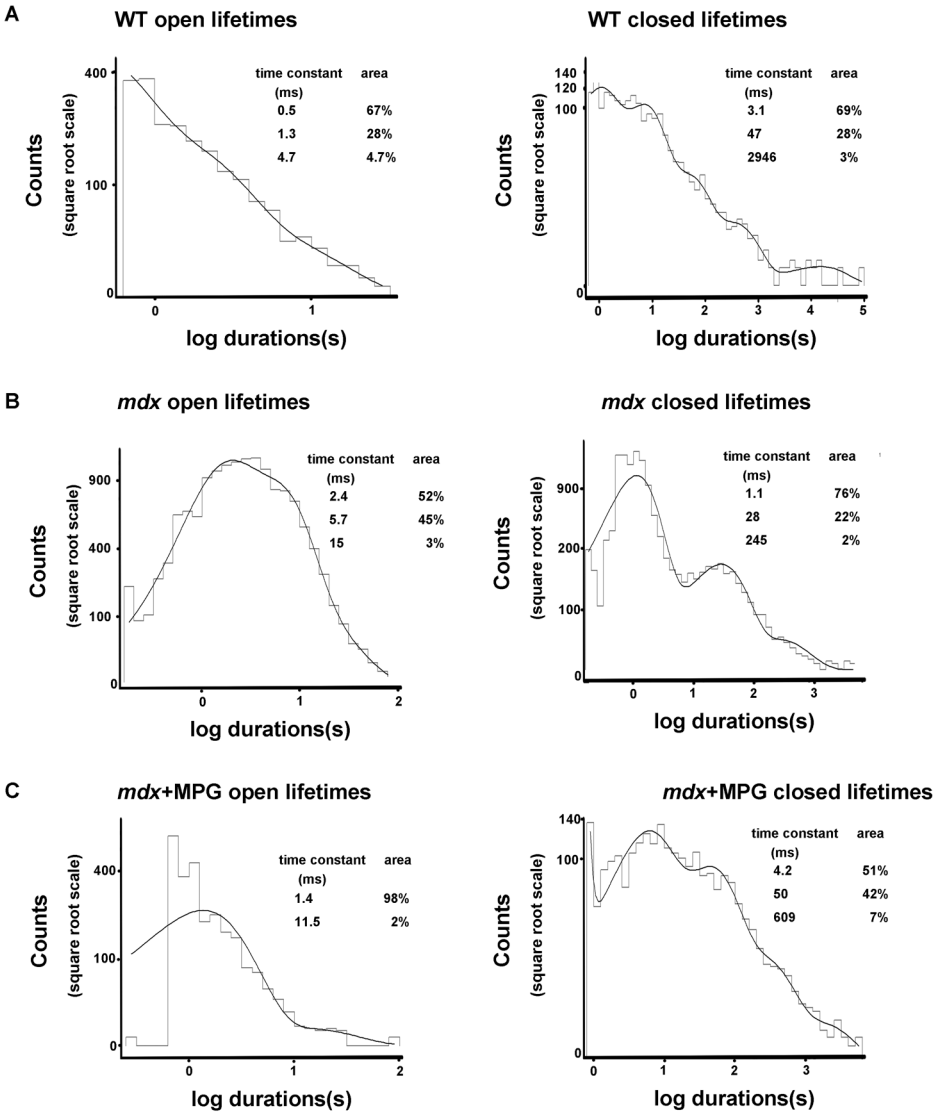
SUPPLEMENTAL FIGURES



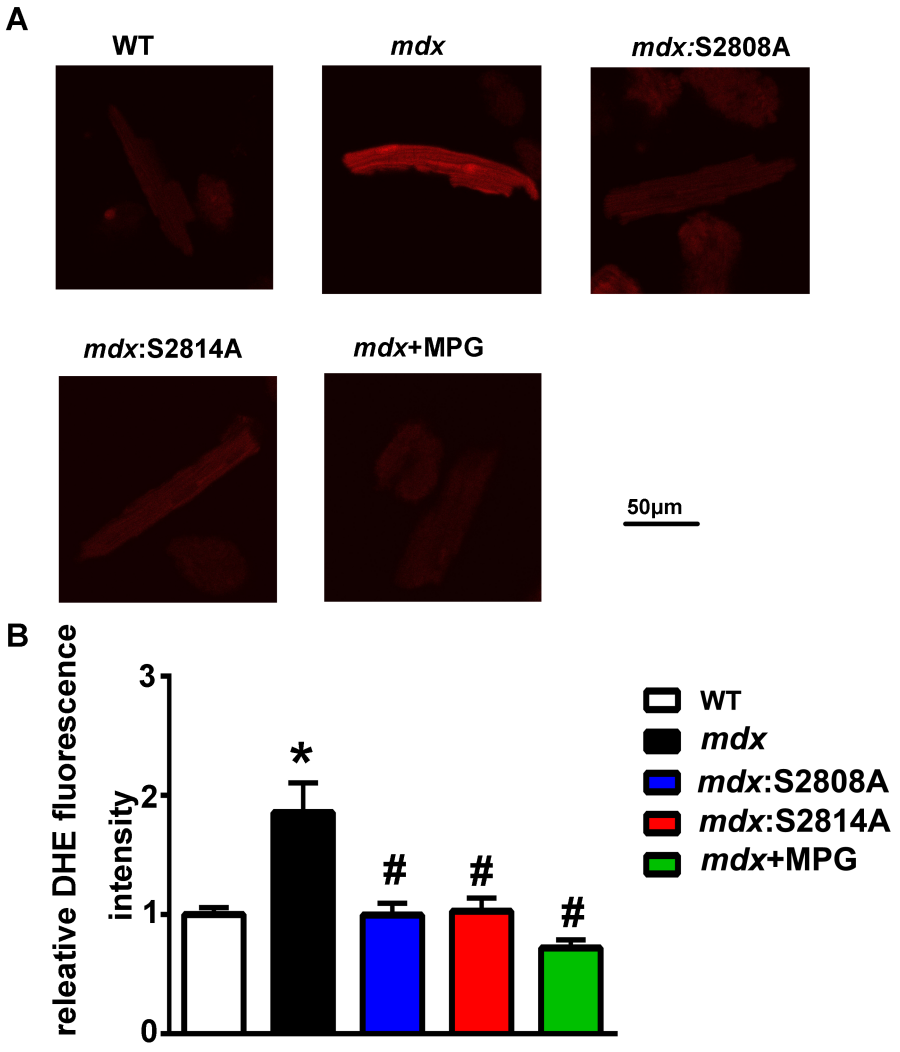
Supplemental Fig 1. ROS scavenger (MPG) as well as inhibition of RyR2 phosphorylation both inhibit Ca sparks in *mdx* mice. Bar graph showing **(A)** Calcium transients amplitude, **(B)** Spark amplitude, **(C)** Full diameter half maximum (FDHM), and **(D)** Full width half maximum (FWHM) following 1 Hz pacing. *p<0.05 vs. WT.



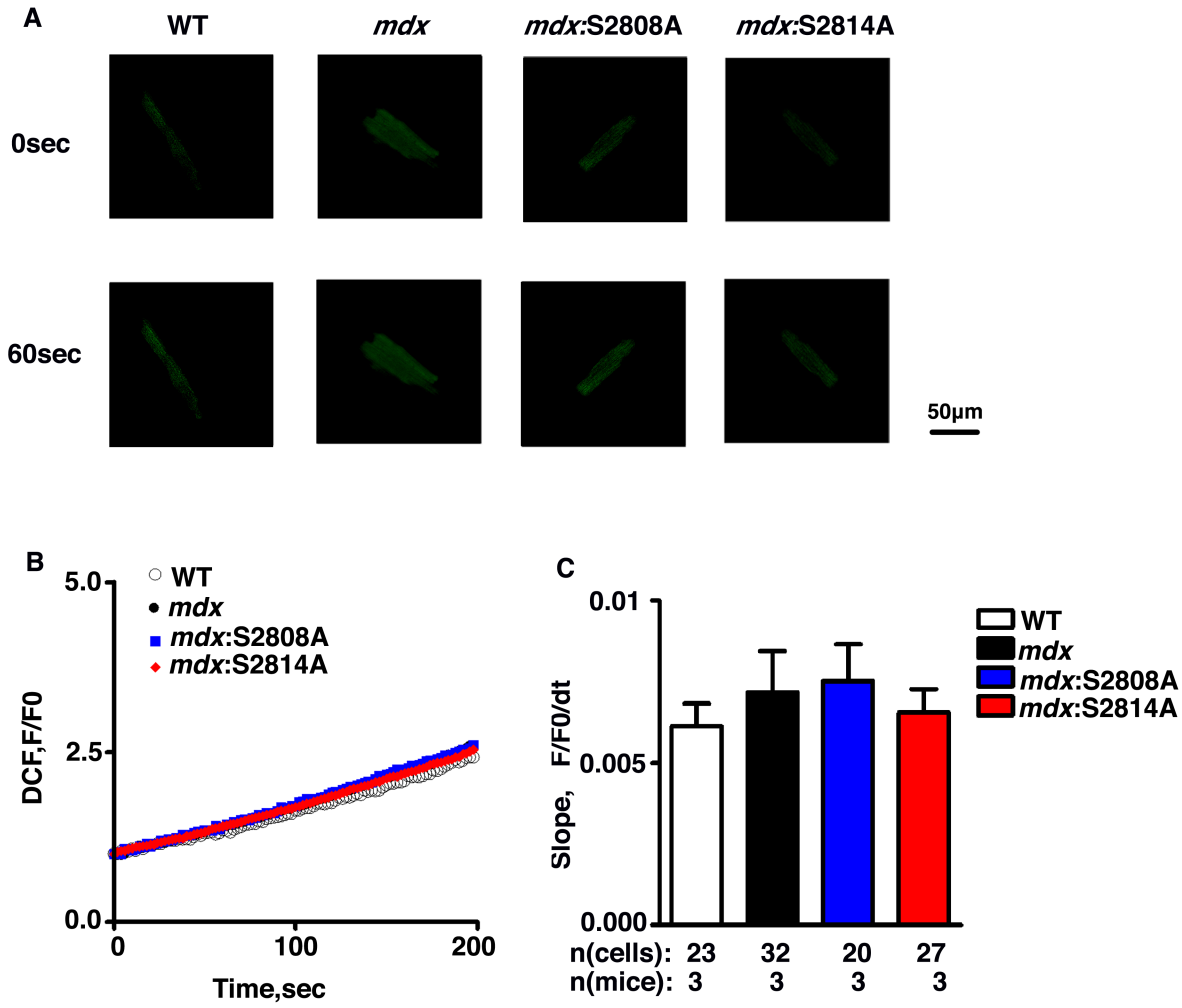
Supplemental Fig 2. Unaltered Calcium sparks amplitude and FWHM histogram. (A) Histogram of calcium spark amplitude in WT, *mdx*, *mdx*:S2808A, *mdx*:S2814A and *mdx*+MPG, **(B)** Histogram of calcium sparks FWHM in WT, *mdx*, *mdx*:S2808A, *mdx*:S2814A and *mdx*+MPG.



Supplemental Fig 3. Inhibition of RyR2 oxidation inhibits RyR2 channel lifetimes of open state and increases lifetimes of closed state in *mdx* mice. (A) Open and closed lifetime distributions and probability density functions (pdf) for a typical single RyR2 channel from WT, **(B)** Open and closed lifetime distributions and pdfs for a typical single RyR2 channel from *mdx*, **(C)** Open and closed lifetime distributions and pdfs for a typical single RyR2 channel from *mdx+MPG*.



Supplemental Fig 4. Inhibition of RyR2 phosphorylation reduces ROS production in *mdx* mice. (A) Representative DHE imaging of cardiomyocytes from WT, *mdx*, *mdx*:S2808A, *mdx*:S2814A mice, and MPG treated *mdx* mice. **(B)** Quantification of normalized DHE fluorescence intensity in cardiomyocytes from WT, *mdx*, *mdx*:S2808A, *mdx*:S2814A mice, and MPG treated *mdx* mice.



Supplemental Fig 5. Unaltered ROS production in *mdx* mice at 1 month of age. (A) Representative DCF imaging of cardiomyocytes from WT, *mdx*, *mdx*:S2808A, and *mdx*:S2814A mice. **(B)** Representative DCF fluorescence intensity tracing and **(C)** Quantification of normalized DCF slope in cardiomyocytes from WT, *mdx*, *mdx*:S2808A, and *mdx*:S2814A mice.