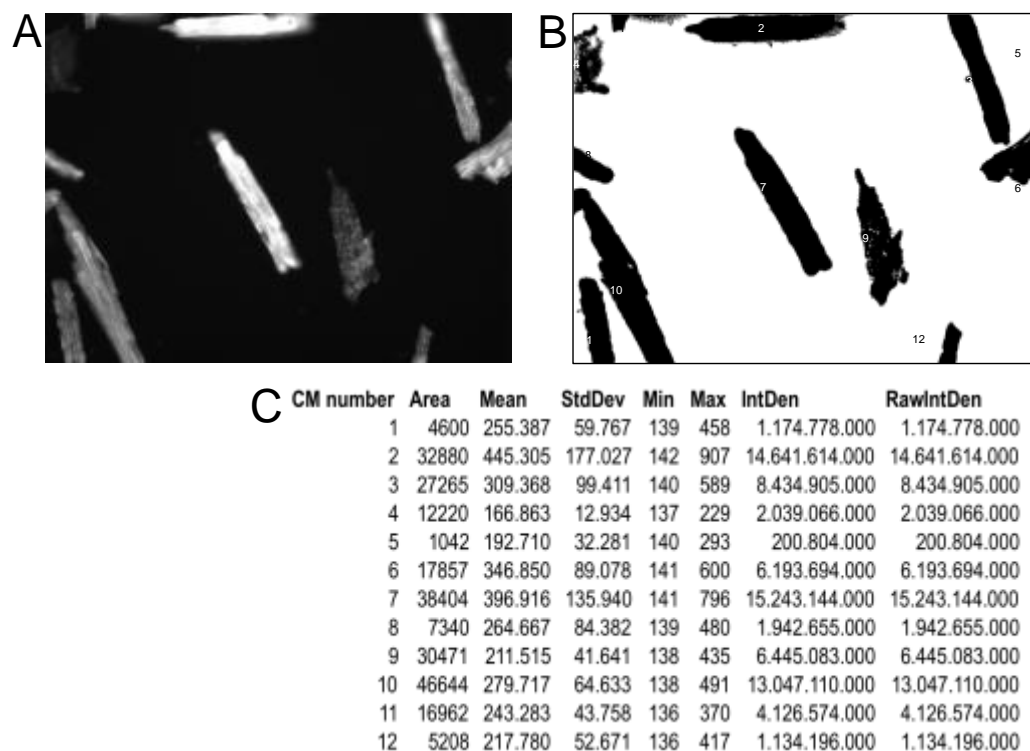


## Supplementary data

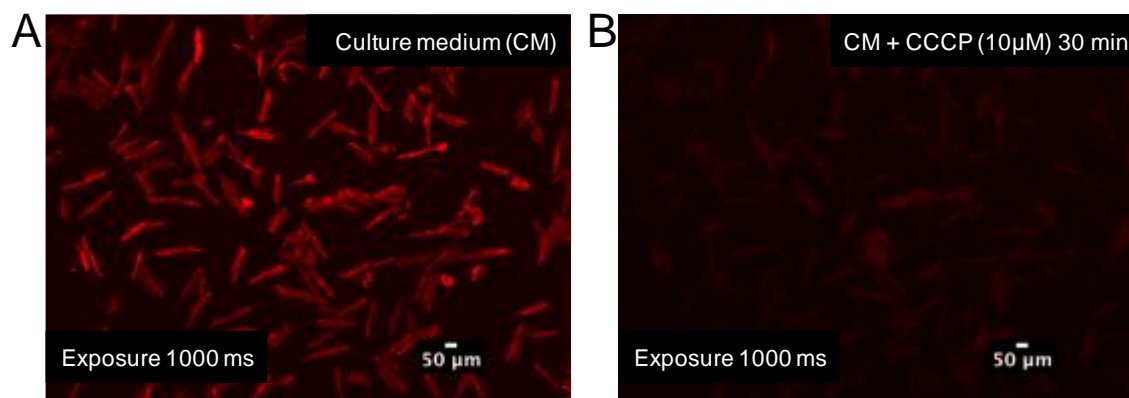
### **NOX2 amplifies acetaldehyde-mediated cardiomyocyte mitochondrial dysfunction in alcoholic cardiomyopathy**

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**Figure S1: Image quantification to measure MitoSOX- or TMRM<sup>+</sup>-enhanced fluorescence per cardiomyocyte.** After background subtraction to account for uneven illumination, raw images (A) were subjected to particle analysis algorithms (B) yielding multiple parameters per CM (C).



**Figure S2: Addition of the ionophore/mitochondrial uncoupler carbonyl cyanide *m*-chlorophenyl hydrazone (CCCP) to CM loaded with TMRM<sup>+</sup> (A) resulted in an almost complete loss of TMRM<sup>+</sup>-enhanced fluorescence indicating a complete mitochondrial depolarization (B).**

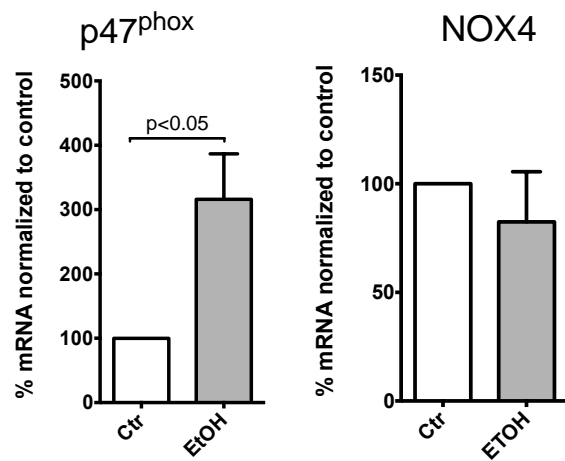


Figure S3: Effect of ethanol exposure of isolated cardiomyocytes on the expression of the NADPH subunit p47<sup>phox</sup> and NADPH oxidase 4 (NOX4). Data presented as the mean $\pm$ SEM.

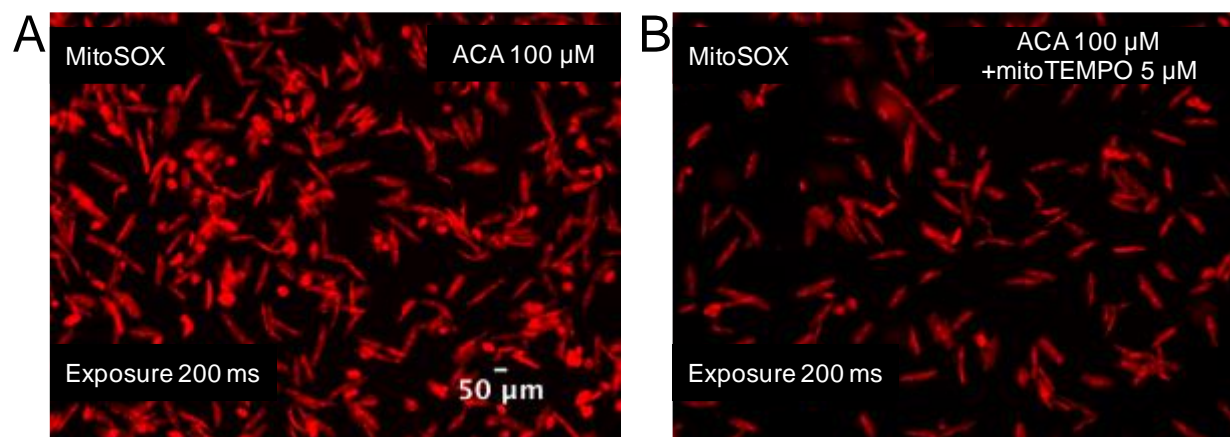


Figure S4: Effect of the mitochondrial specific antioxidant mitoTEMPO on CM ROS after exposure in culture media containing ACA 100  $\mu$ M.