

## **SUPPLEMENTAL MATERIAL**

**Trehalose, a Natural Disaccharide, Reduces Cardiac Remodeling After Myocardial Infarction Through Autophagy Activation**

## **Supplemental Figure Legends**

### **Supplemental Figure 1**

In vivo study design.

### **Supplemental Figure 2**

**A-B.** Mice with and without LAD ligation received placebo, sucrose or trehalose for 4 weeks. Representative echocardiographic pictures (A) and PV loop diagrams (B) are shown.

### **Supplemental Figure 3**

Tg-GFP-LC3 mice were subjected to LAD ligation and received placebo (saline) or trehalose for 4 weeks. In some animals, chloroquine was also administered before sacrifice. Representative pictures of myocardial GFP-LC3 dots are shown (A), together with quantification of the number of dots/field (B). Data are expressed as fold vs. placebo without chloroquine (CT). Scale bar: 50  $\mu$ m. \*  $p < 0.05$ .

### **Supplemental Figure 4**

**A-B.** Mice with LAD ligation received placebo (CT) or trehalose (TRE). After 4 weeks, cardiac levels of cathepsin D and GAPDH were analysed by immunoblot. A representative immunoblot is shown (A), together with the densitometric analysis of normalized cathepsin D levels (B). Data are expressed as fold vs. CT. N=3. \* $p < 0.05$ .

### **Supplemental Figure 5**

**A-B.** Mice with LAD ligation received placebo (CT) or trehalose (TRE). After 4 weeks, cardiac levels of cleaved caspase 3, SERCA2, ubiquitin, tubulin and GAPDH were analysed by immunoblots. Representative immunoblots are shown (A-B).

### **Supplemental Figure 6**

**A-B.** Lysosomal degradation of mitochondria was examined in cardiomyocytes transduced with adenovirus expressing mito-Keima. Cardiomyocytes were incubated with or without trehalose (100 mM) for 24 hours and then subjected to 4 hours of glucose deprivation (GD). Representative images showing high ratio (560 nm/440 nm) dots representing mitophagy are shown. Enlarged images of the areas delineated by dashed rectangles are shown below (scale bar, 50  $\mu$ m). Quantitation of the area of high ratio dots per cell area (%), evaluated as a measure of mitophagy, is also shown. The cell numbers in each group were 35 (CT), 41 (TRE), 30 (CT/GD) and 41 (TRE/GD) in 3-4 independent experiments. Error bars represent SD. \* $p < 0.05$  vs. CT; # $p < 0.01$  vs. CT; † $p < 0.01$  vs. CT/GD.

### **Supplemental Figure 7**

**A.** Neonatal rat ventricular cardiomyocytes were incubated with or without trehalose (100

mM) for 24 hours. TFEB nuclear localization was then assessed. Scale bar: 100  $\mu$ m. **B.** C57BL/6J WT mice received placebo (saline) or trehalose (1 mg/g/day i.p.) for 48 hours. Hearts were then harvested and left ventricles were subjected to subcellular fractionation. Cytosolic and nuclear levels of TFEB, GAPDH and histone H3 (HH3) were assessed. **C-E.** Neonatal rat ventricular cardiomyocytes were transduced with an adenovirus expressing a short-hairpin sequence targeting TFEB or with a control adenovirus for 72 hours (C). Cells were then treated with and without trehalose for 24 hours and LC3 levels were assessed. A representative immunoblot is shown (D). After 72 hours of adenovirus transduction, cells were also treated or not treated with H<sub>2</sub>O<sub>2</sub> (150  $\mu$ M) with and without trehalose for 24 hours. Survival was assessed by MTT assay (E). Results are shown as a percentage of the relevant baseline control not treated with H<sub>2</sub>O<sub>2</sub> (CT). A representative baseline control bar set at 100% is shown. N=4. \*\*p<0.01 and \*p<0.05. **F.** Cardiomyocytes were exposed to H<sub>2</sub>O<sub>2</sub> with and without trehalose and/or bafilomycin (50 nM) treatment for 24 hours. Cleaved caspase 3 and GAPDH levels were then evaluated by immunoblot.

### **Supplemental Figure 8**

**A-B.** Mice with LAD ligation received placebo (CT) or trehalose (TRE). After 4 weeks, cardiac levels of P-ERK and ERK were analysed by immunoblot. Representative immunoblots are shown (A), together with the densitometric analysis of normalized P-ERK levels (B). Data are expressed as fold vs. CT. N=5.

### **Supplemental Figure 9**

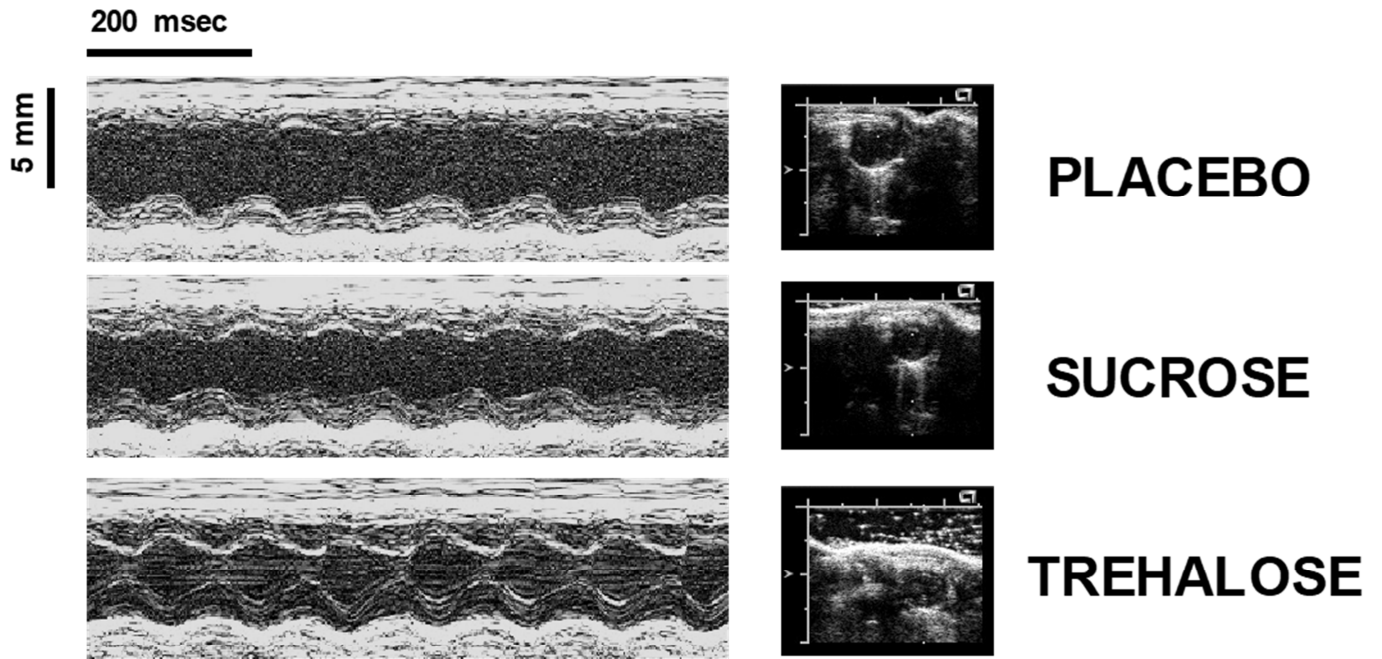
A scheme summarizing the results of the study. Trehalose reduces post-infarction cardiac remodeling and autophagy activation is involved in this beneficial effect.

# Study Design

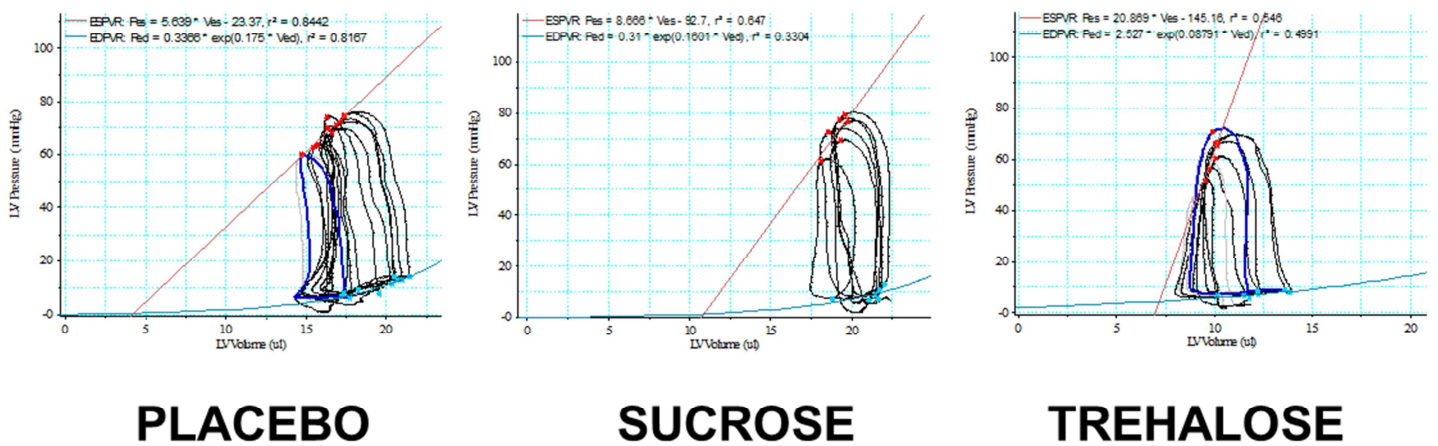


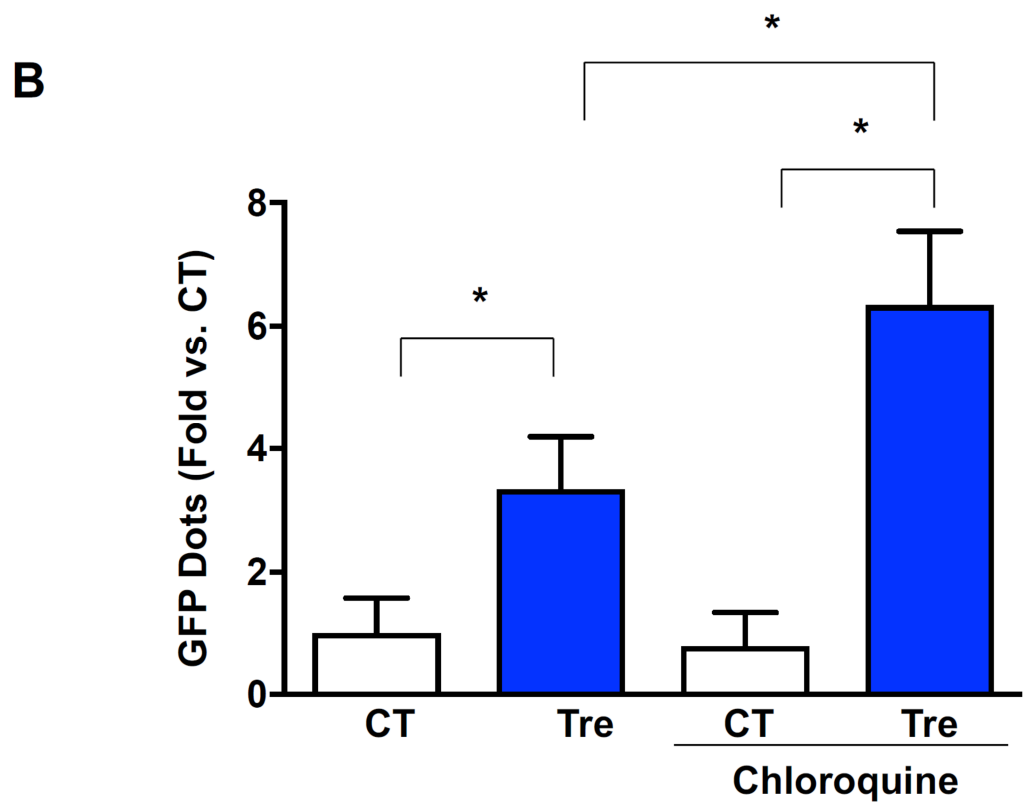
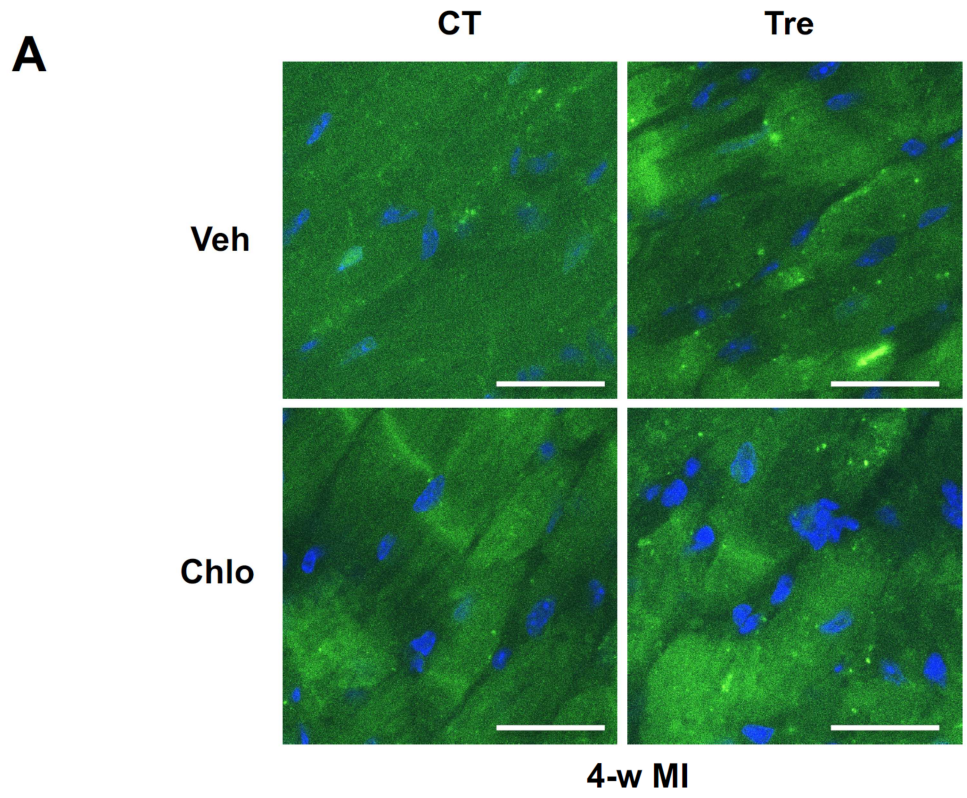


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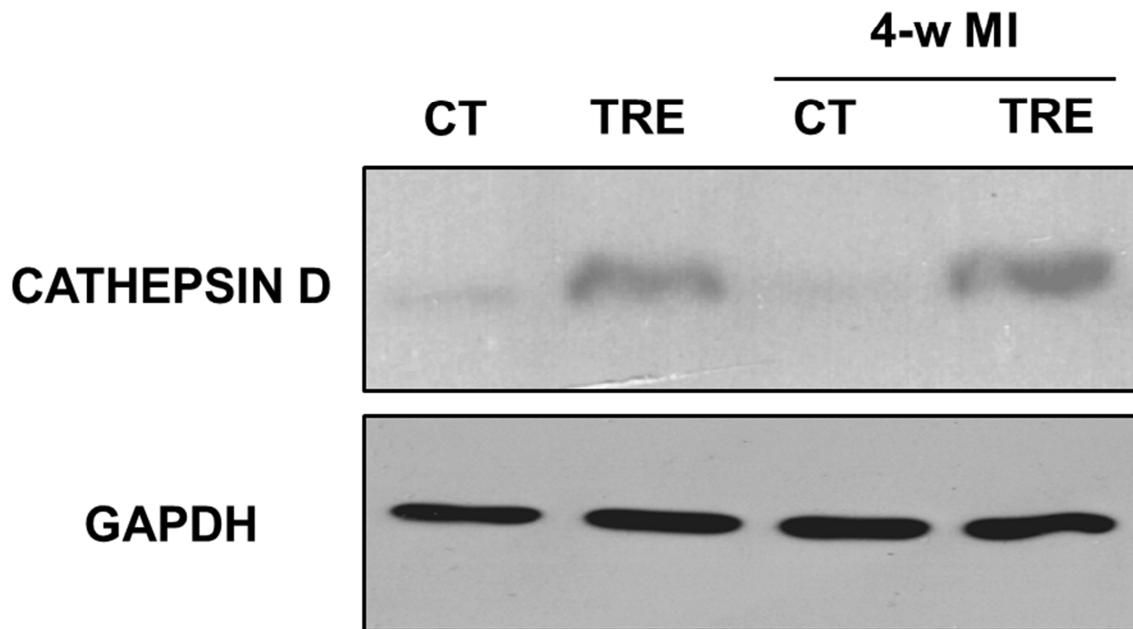


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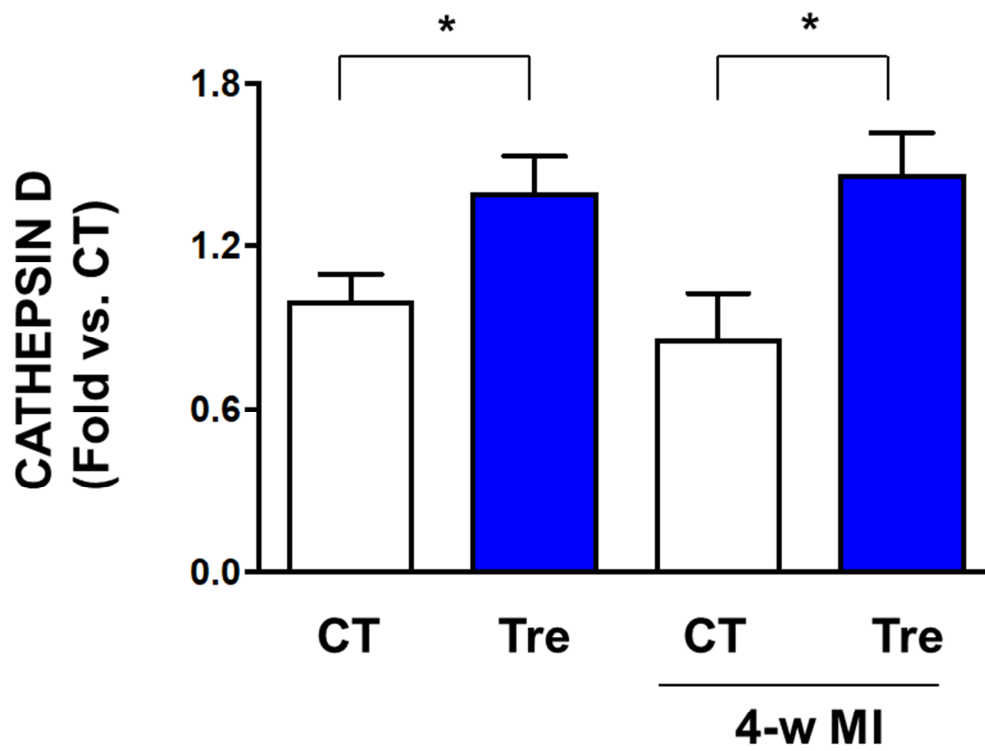


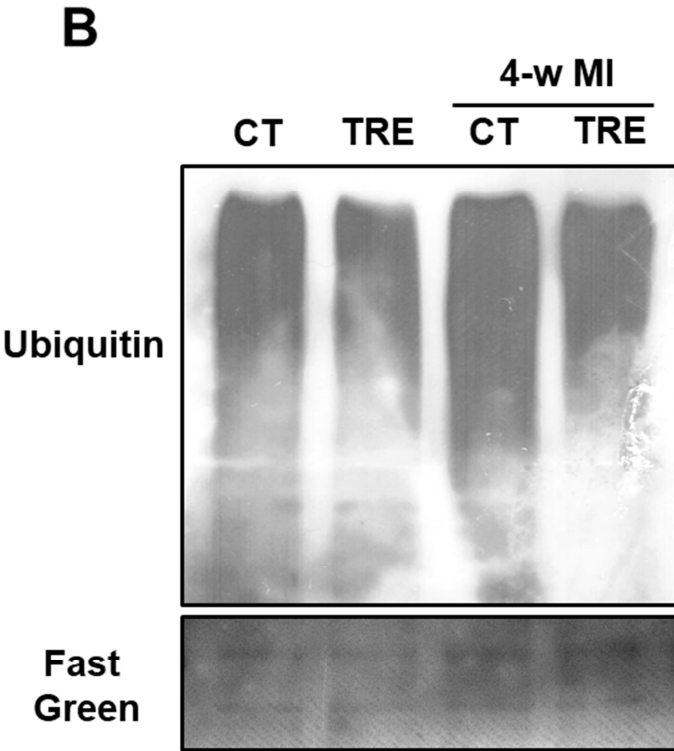
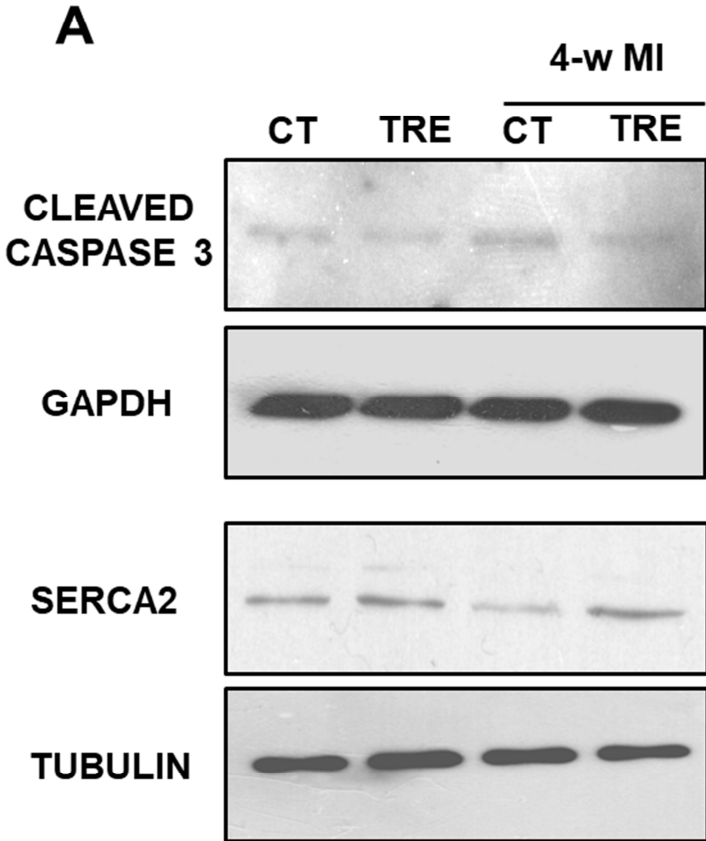


**A**



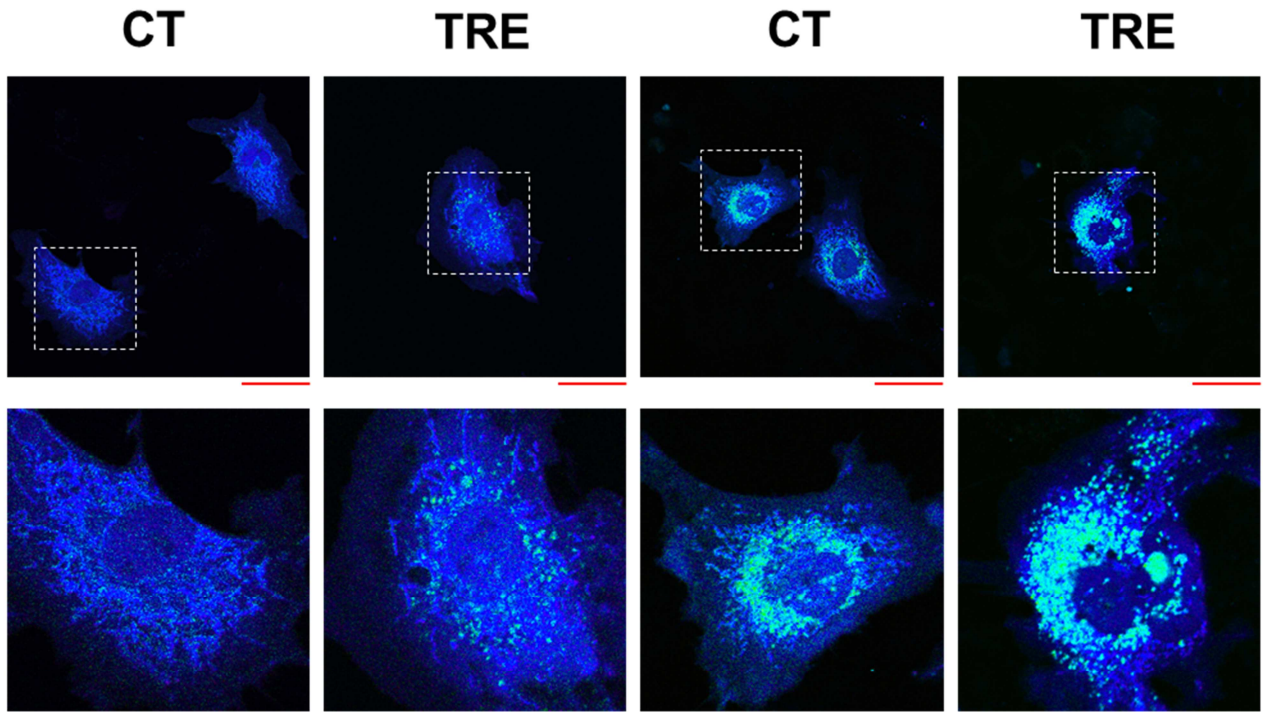
**B**



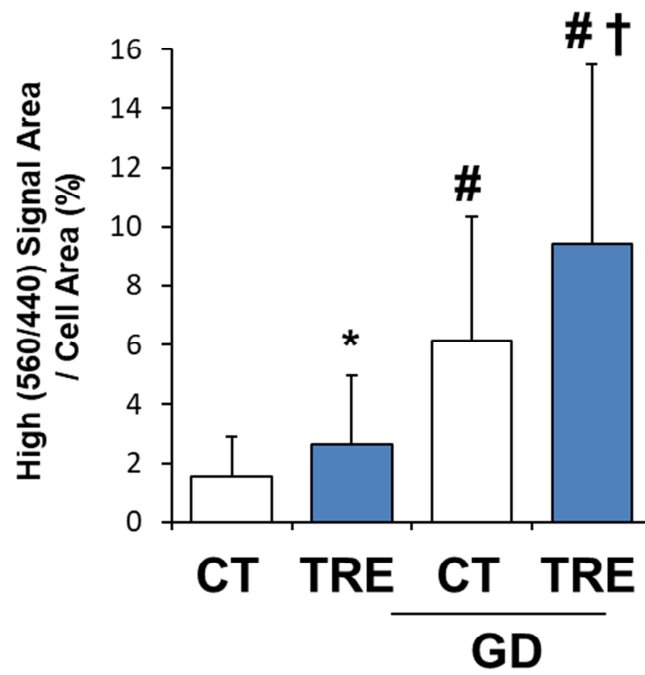


GD 4 h

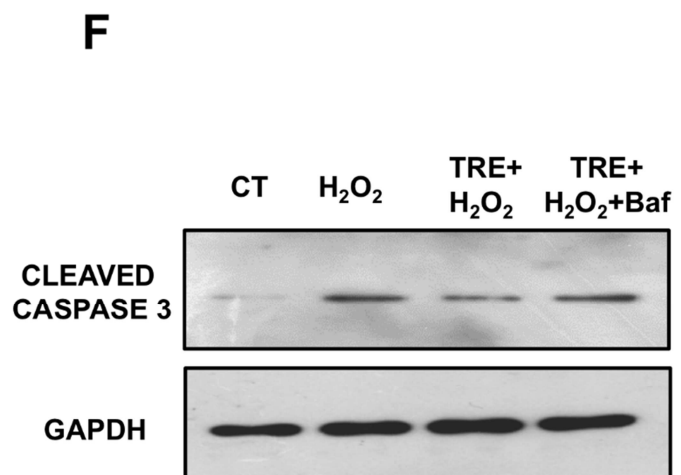
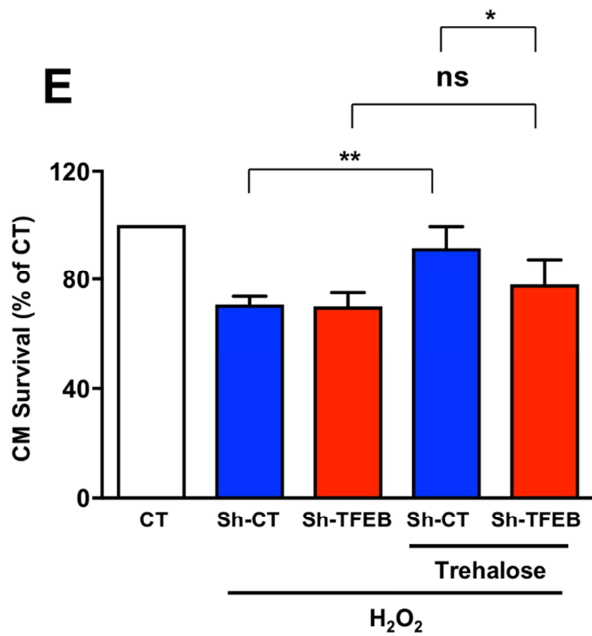
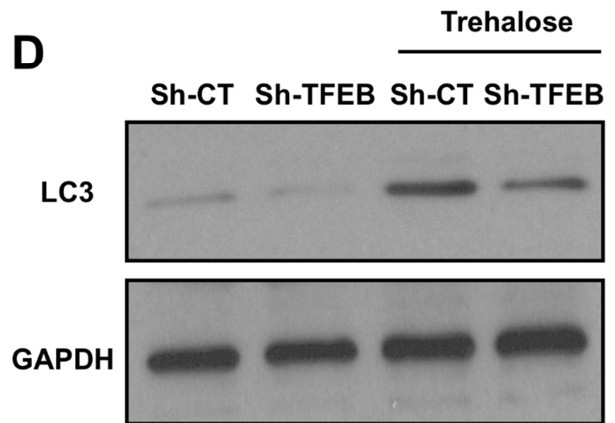
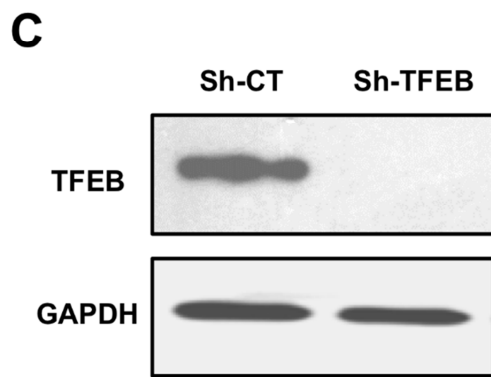
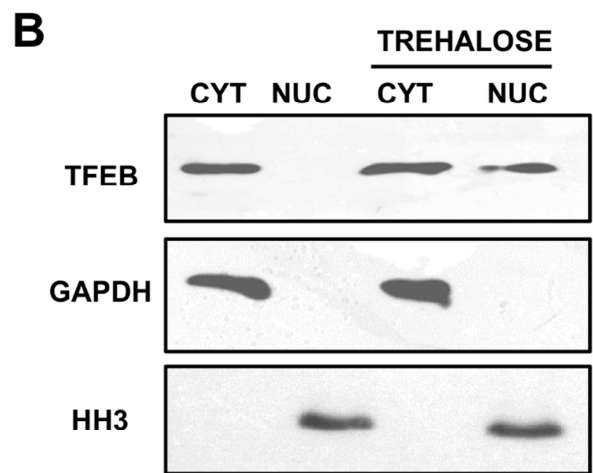
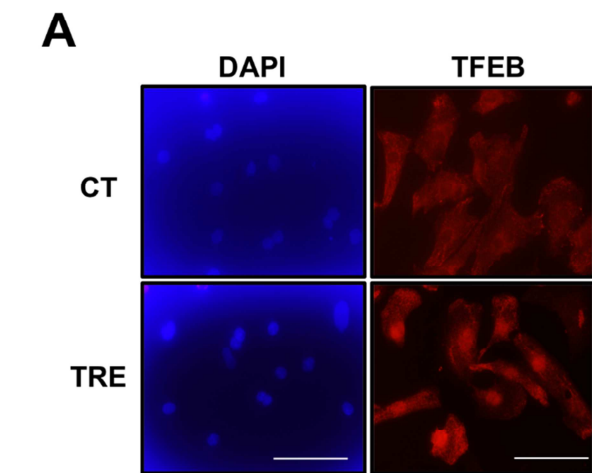
**A**



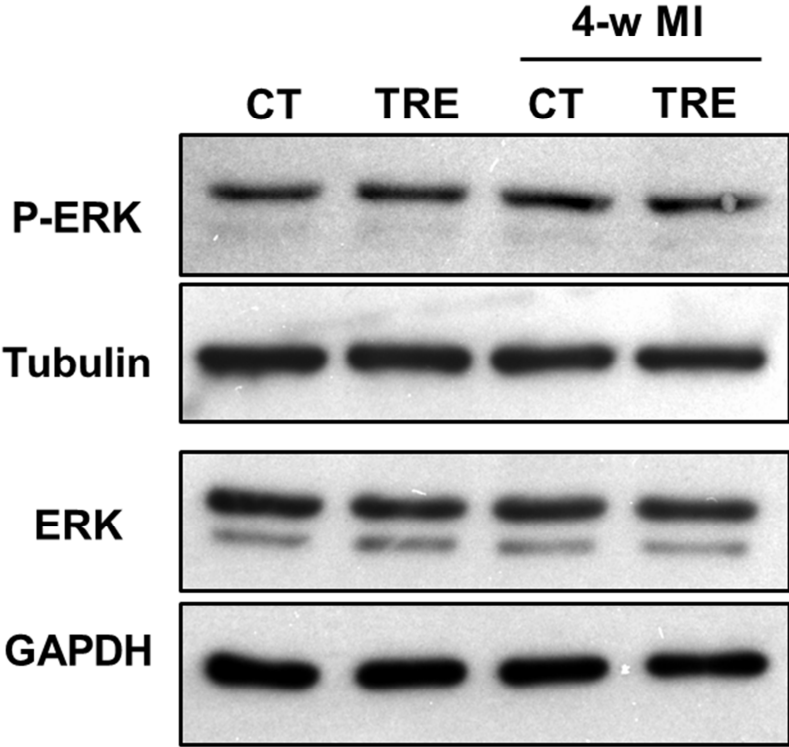
**B**







**A**



**B**

