Supplementary Data

Impaired NF-κB Signaling Underlies Cyclophilin D Mediated Mitochondrial Permeability Transition Pore Opening in Doxorubicin Cardiomyopathy.

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Supplementary figure legends

Supplementary figure 1. Doxorubicin Induces Mitochondrial Defects in Cardiac Myocytes.

Panel A: Histogram represents quantitative data for mitochondrial calcium by Rhod2- AM staining shown in Figure 1 panel C, data is normalized to control (CTRL). Fluorescence intensity was assessed using image j software and data are expressed as Mean ± SEM. Unpaired two tailed Students-t test was used to compare mean differences between groups.Statistically significant difference of Dox condition from CTRL, *p value< 0.0001. **Panel B:** Epifluorescence microscopy of cardiomyocytes assessed for Reactive oxygen species (ROS) by Dihydroethidium (Red) see methods for details,scale bar 40μm. **Panel C:** Mitochondrial membrane potential ($\Delta\Psi m$) by TMRM, see methods for details, scale bar 10μm. **Panel D:** Quantitative data for mPTP opening images shown in Figure 1 panel D. Statistically significant difference of Dox condition from CTRL, *p value< 0.0001 analyzed by unpaired two tailed Students-t test.

Supplementary figure 2. Impaired NF-κB Signaling Provokes mPTP Opening

Panel A: Real time qPCR analysis of NF-κB mRNA level in CTRL or Dox treated cardiomyocytes. Unpaired two tailed Students-t test was used to compare mean differences between groups. Statistically significant difference of Dox condition from CTRL, *p value< 0.002. **Panel B:** Western blot analysis of NF-κB protein in the hearts derived from saline or Dox treated mice. **Panel C:** Quantitative data for mPTP opening images shown in Figure 3 panel E. Data are expressed as Mean± SEM. Statistical significance between the groups analyzed by one way ANOVA and Bonferroni Post-Hoc test; $IKK\beta_{K\text{-}M}$ vs CTRL,*p value=0.0025; $IKK\beta_{K\text{-}M}$ vs $IKK\beta_{K\text{-}M}$ + CSA, *p value 0.0449; CTRL vs CSA (ns) p value > 0.999. **Panel D:** Quantitative data for mPTP opening images shown in Figure 5 panel A. Data are expressed as Mean± SEM.

Statistical significance between the groups analyzed by one way ANOVA and Bonferroni Post-Hoc test. CTRL vs Dox *p value=0.0192; Dox vs Dox +CSA*p value=0.0069; CTRL vs CSA (ns) p value> 0.999

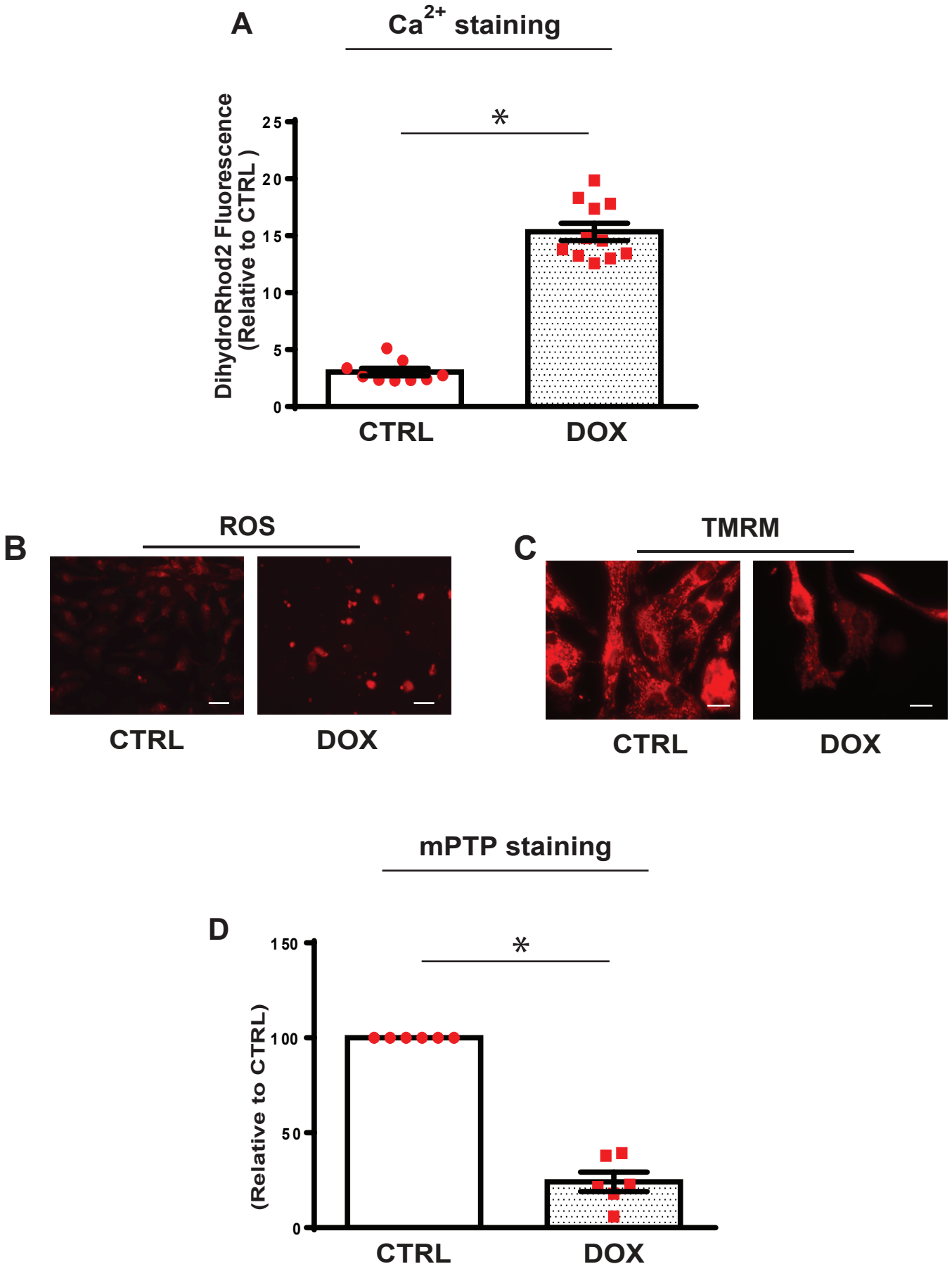
Supplementary figure 3. Cyclophilin D is Involved in Bnip3 Mediated Reactive Oxygen Species (ROS) Production in Cardiac Myocytes.

Panel A: Representative fluorescent images for ROS production. Cardiomyocytes expressing Bnip3 in the presence of siRNA directed against CypD (CypD siRNA 40 nM) or scrambled siRNA were assessed for ROS production, scale bar 40μm. **Panel B:** Quantitative data for mPTP opening images shown in Figure 7 panel A. Data are expressed as Mean± SEM. Statistical significance between the groups analyzed by one way ANOVA and Bonferroni Post-Hoc test; CTRL vs Bnip3,*p value=0.0022; Bnip3 vs Bnip3 + Cypd siRNA, *p value 0.017. **Panel C:** Quantitative analysis of Bnip3 protein showed in Figure 7 panel C (top panel). Statistical significance determined using one way ANOVA and Bonferroni Post-Hoc test. CTRL vs Dox *p value= 0.001; Dox vs Dox +IKKβ *p value=0.0053; CTRL vs Dox + IKKβ (ns) p value= 0.465

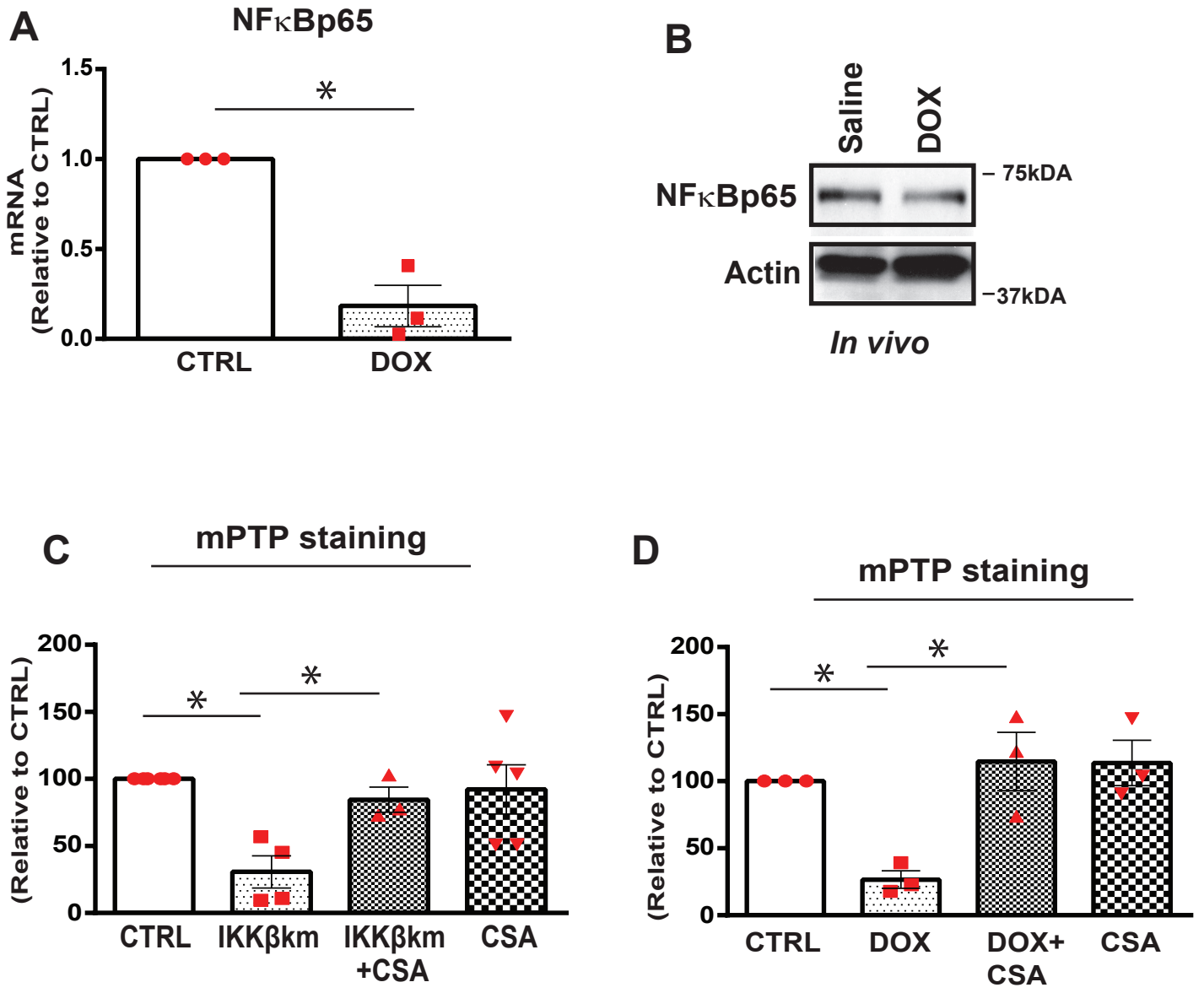
Supplementary figure 4. Restoration of NF-κ B Signaling by IKKβwt Suppress Dox Induced mPTP Opening and ROS Production via Inhibition of Bnip3 in Ventricular Myocytes.

Panel A: Quantitative data for mPTP opening images shown in Figure 8 panel A. Data are expressed as Mean± SEM. Statistical significance between the groups analyzed by one way ANOVA and Bonferroni Post-Hoc test; CTRL vs Dox *p value= 0.0004; Dox vs Dox +IKKβ *p value=0.0006; CTRL vs Dox + IKKβ (ns) p value> 0.999. **Panel B:** Quantitative data for reactive oxygen species (ROS) production for the images shown in Figure 8 panel B. Data are expressed as Mean± SEM. Statistical significance between the groups analyzed by one way ANOVA and Bonferroni Post-Hoc test; CTRL vs Dox *p value= 0.0384; Dox vs Dox +IKKβ *p value=0.0362; CTRL vs Dox + IKKβ p value> 0.999. **Panel C:** Cardiomyocytes overexpressing IKKβwt only or IKKβwt in combination with Bnip3 expression adenovirus were treated with Dox and assessed for viability, scale bar- 40μm**. Panel D:** Histogram presents quantitative data for panel C. Statistical significance between the groups marked in the histogram, analyzed by one way ANOVA and Bonferroni Post-Hoc test, CTRL vs Dox *p value= 0.0048; Dox vs Dox +IKKβ *p value=0.013; Dox vs Dox + IKKβ+Bnip3 (ns) p value =0.265

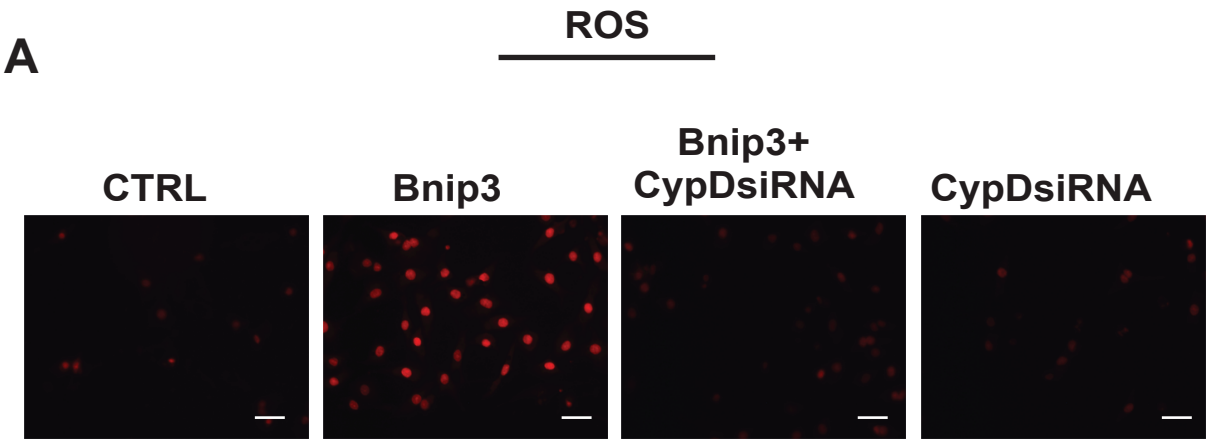
Supplementary figure 1

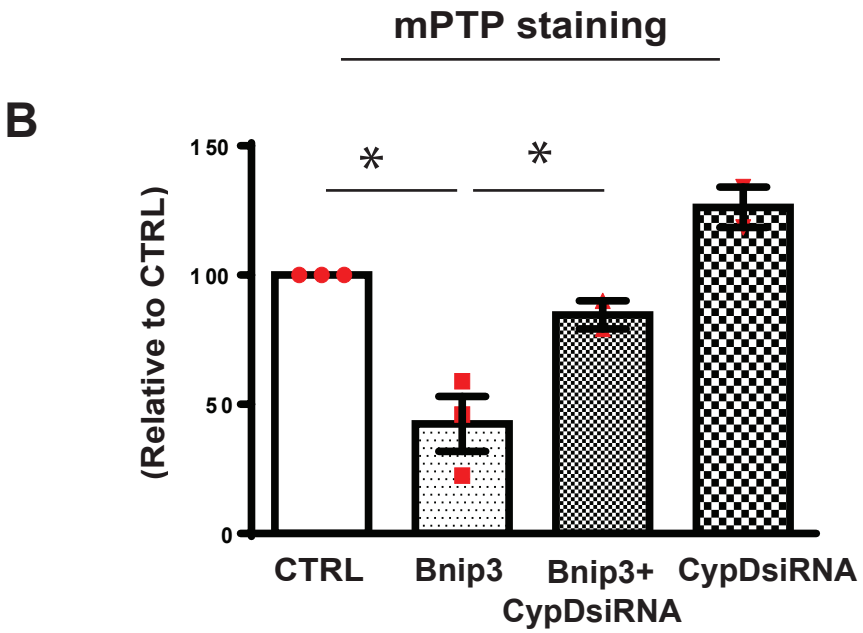


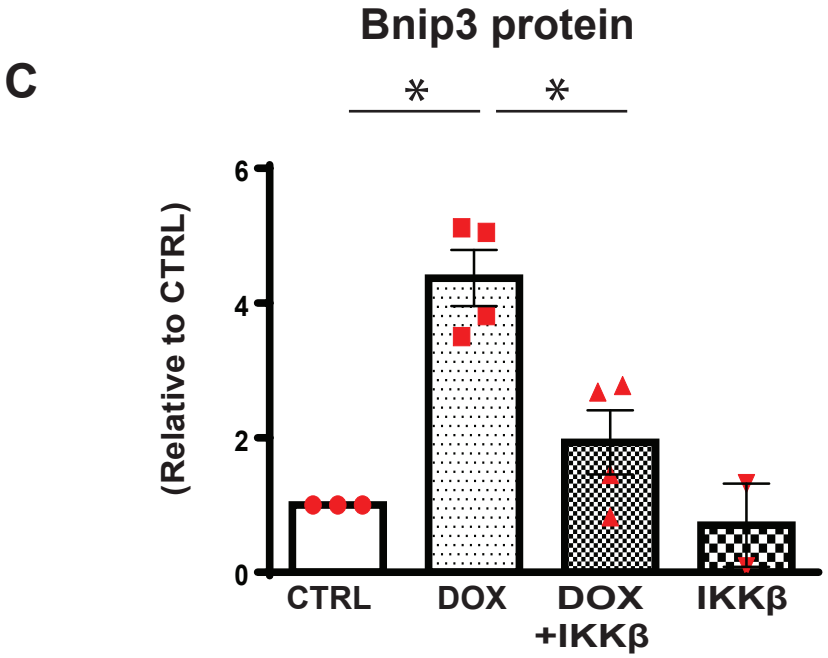
Supplementary figure 2



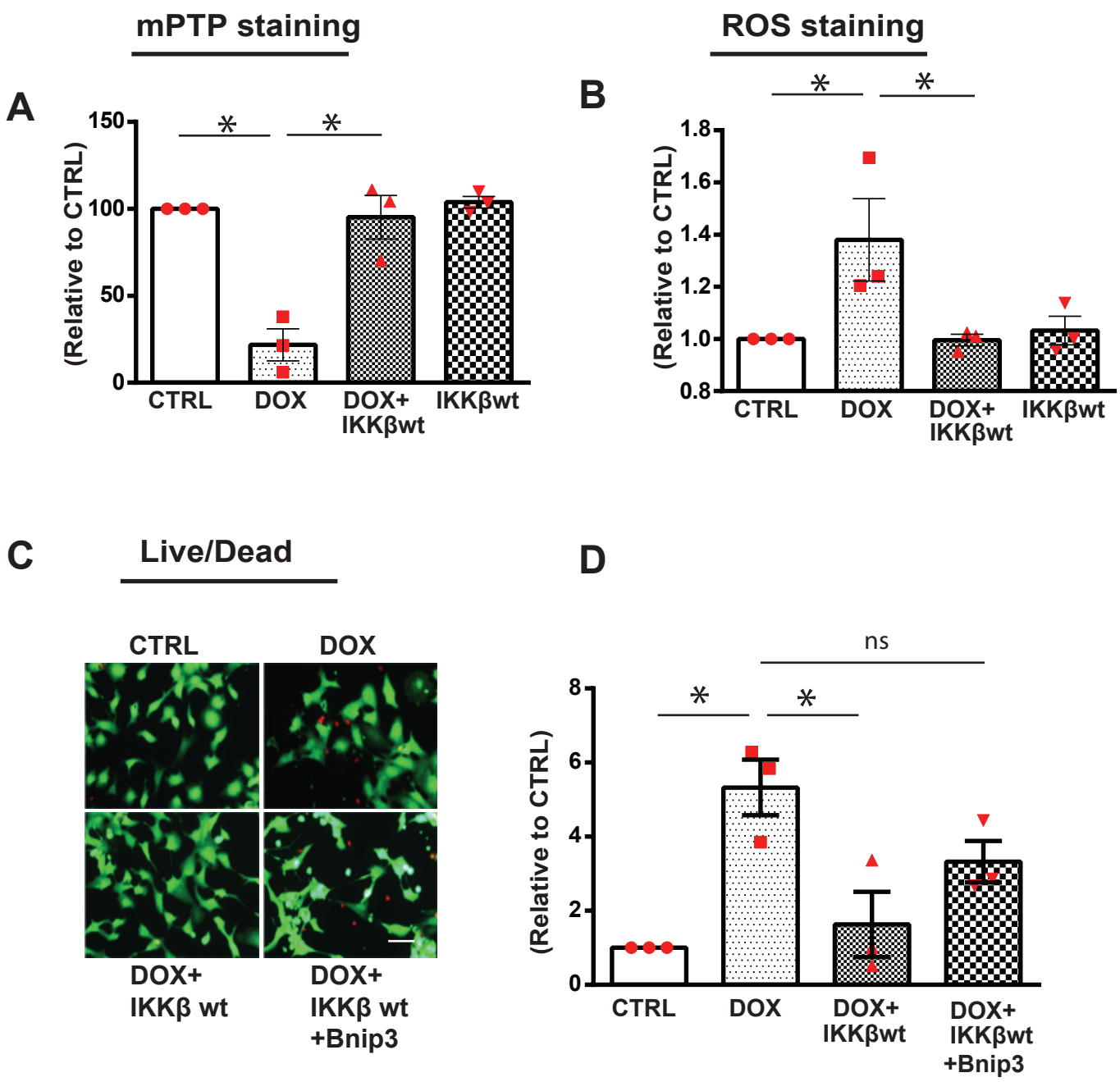
Supplementary figure 3







Supplementary figure 4



Supplementary Tables

siRNA sequence

The small interfering RNA (siRNA) against Cyclophilin D (*ppif)* and NF-κBp65 was custom designed and synthesized from Invitrogen.

Supplementary Table 1: Sequence for siRNA Cyclophilin D (*ppif)*

Cyclophilin D siRNA sequence	
Sense	GACAUCUAAGAAGAUUGUCAUCACA
Anti-sense	UGUGAUGACAAUCUUCUUAGAUGUC

Supplementary Table 2: Sequence for scrambled siRNA used as control for Cyclophilin D (*ppif)* si RNA

Scrambled Control siRNA sequence	
Sense	GACAAUCGAAGGUUAUACUCUAACA
Anti-sense	UGUUAGAGUAUAACCUUCGAUUGUC

Supplementary Table 3: Sequence for siRNA NF-κBp65

NF-κBp65 siRNA sequence	
Sense	GGACCAGGAACAGUUCGAATT
Anti-sense	UUCGAACUGUUCCUGGUCCTT

Supplementary Table 4: scrambled siRNA used as control for siRNA NF-κBp65

Scrambled Control siRNA sequence	
Sense	GGAAGGAGACACUUCCGAATT
Anti-sense	UUCGGAAGUGUCUCCUUCCTT