

Figure S1. DOX caused cardiomyocytes ferroptosis and reduced the expression of CREG1

A. The effect of DOX (0, 2, 5 and 10 μ M) on the viability of HL-1 cardiomyocytes was examined using by CCK8 assay. **B.** The effect of different cell death inhibitor on the cell viability of HL-1 cells induced by DOX (5 μ M). **C.** The mRNA of *Creg1* and *Ptgs2* in the NCMCs with DOX treatment. **D-E.** Western blotting of CREG1 and ferroptosis-related proteins in the NCMCs with DOX treatment. n = 3 for each group. Z-VAD-FMK: apoptosis inhibitor, 40 μ M; CQ: chloroquine, autophagy inhibitor, 10 μ M; Fer-1: ferrostatin-1, ferroptosis inhibitor, 10 μ M; Nec-1: Necrostatin-1, necroptosis inhibitor, 30 μ M. DOX: doxorubicin, NCMCs: neonatal mouse cardiomyocytes. *p<0.05, **p<0.01 vs. control; ##p<0.01 vs. DOX group; &p < 0.01 vs. Fer-1+DOX group.

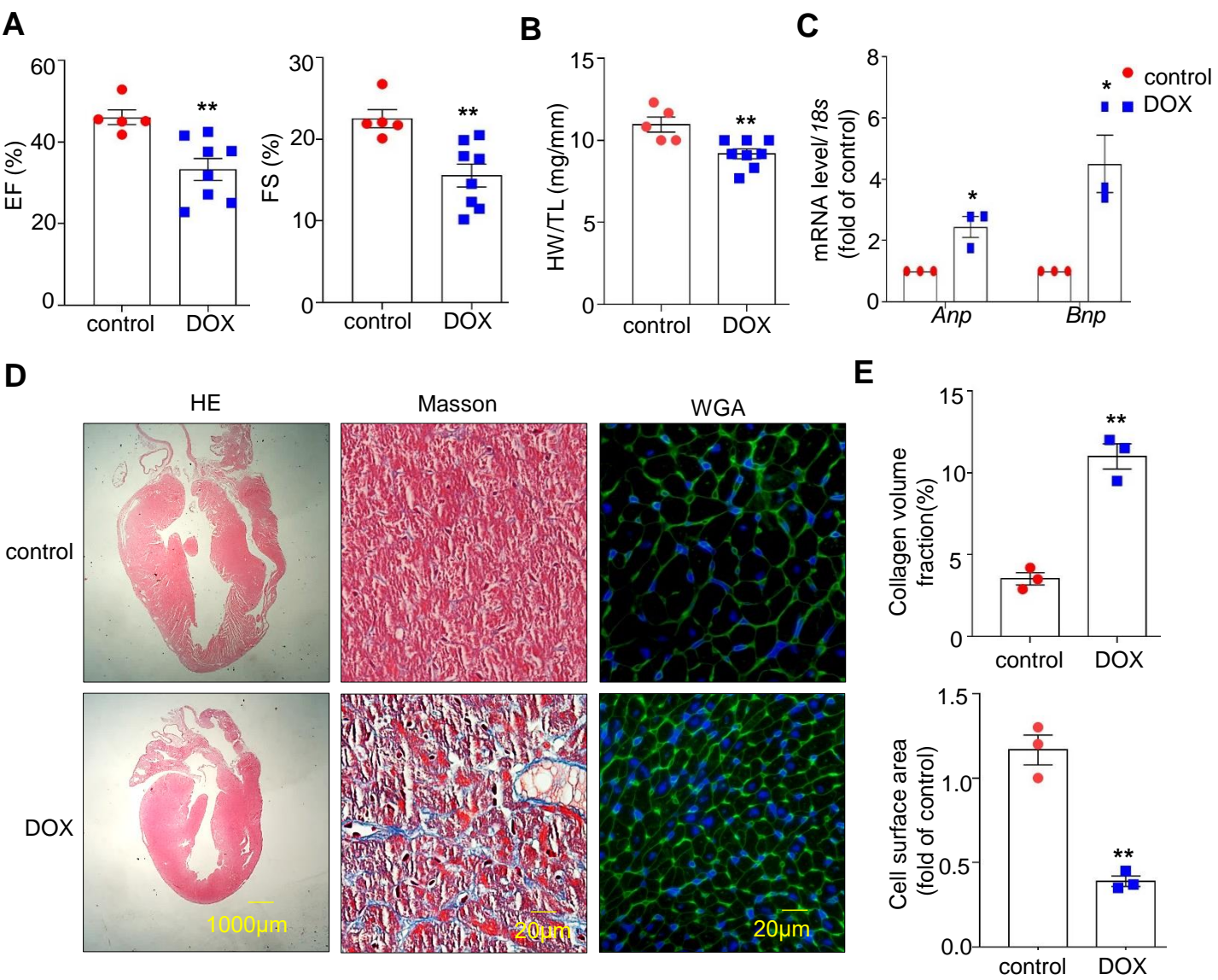


Figure S2. The establishment of DOX-induced cardiotoxicity

A. EF% and FS% in the control and DOX group of C57BL/6J mice (n = 5 for control group, n = 8 for DOX group). **B.** The ratio of heart weight (HW) to tibial length (TL) in the control and DOX group of C57BL/6J mice (n = 5 for control group, n = 8 for DOX group). **C.** The mRNA of *Anp* and *Bnp* in the myocardium after DOX treatment (n = 3). **D-E.** HE staining, Masson's trichrome staining, and WGA staining in the control and DOX group of C57BL/6J mice (n = 3). DOX: doxorubicin, *p<0.05, **p<0.01 vs. control.

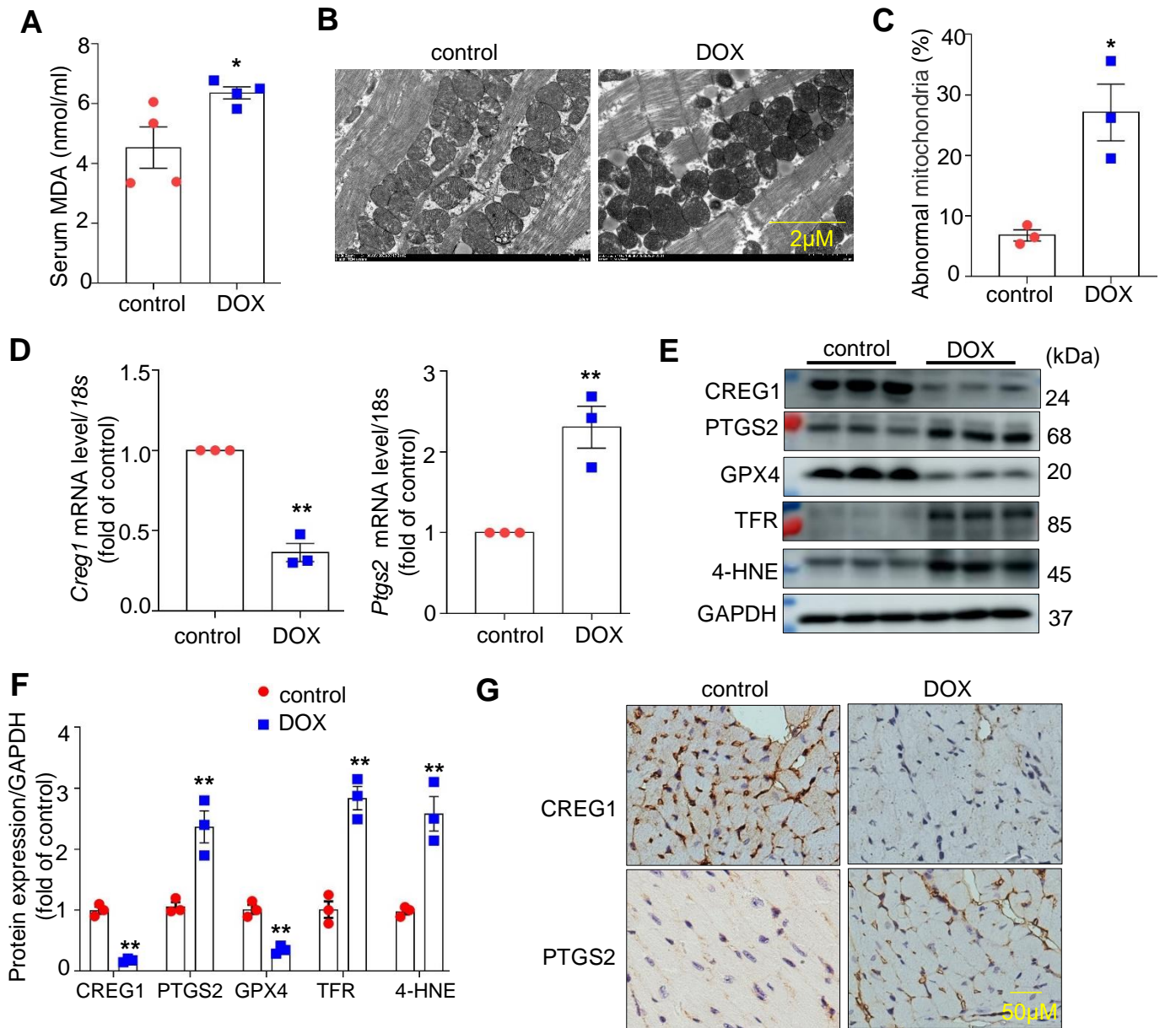


Figure S3. CREG1 expression was reduced in the myocardium after DOX treatment

A. Serum MDA content in DOX-treated C57BL/6J mice. **B-C.** Transmission electron microscope for mitochondria in the myocardium of DOX-treated C57BL/6J mice. **D.** The mRNA of *Creg1* and *Ptgs2* in the myocardium of DOX-treated C57BL/6J mice. **E-F.** Western blotting of CREG1 and ferroptosis-related proteins in the myocardium of DOX-treated C57BL/6J mice. **G.** Immunohistochemical staining of CREG1 and PTGS2 in the myocardium of DOX-treated C57BL/6J mice. n = 3 for each group. DOX: doxorubicin, MDA: Malondialdehyde. *p<0.05, **p<0.01 vs. control.

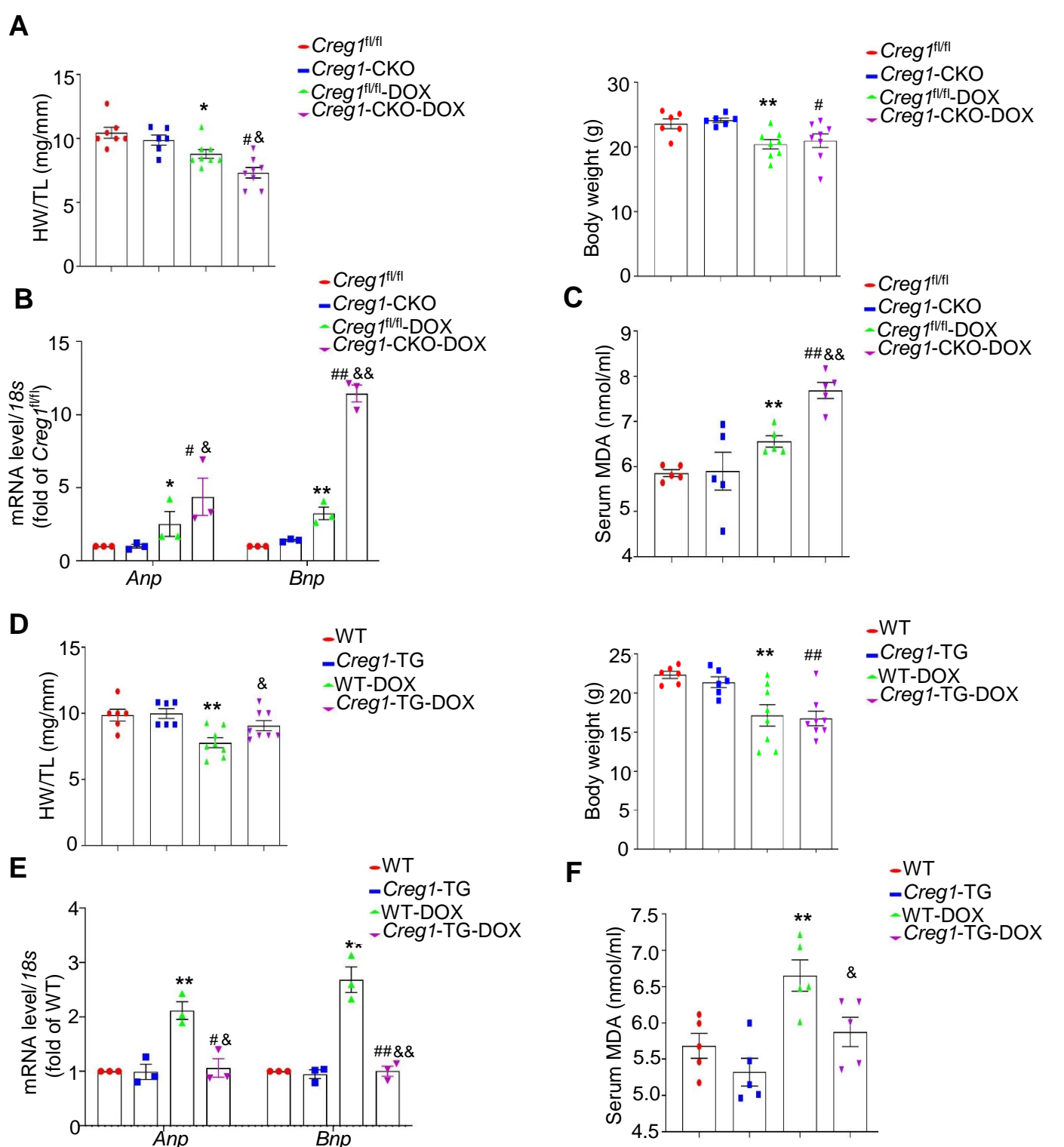


Figure S4. CREG1 deficiency aggravated DOX-induced cardiotoxicity and CREG1 overexpression alleviated DOX-induced cardiotoxicity

A. The ratio of heart weight (HW) to tibial length (TL) and body weight in the *Creg1*-CKO mice after DOX treatment (n = 6 for the control group, n = 8 for the DOX group). **B.** The mRNA of *Anp* and *Bnp* in the myocardium of *Creg1*-CKO mice after DOX treatment (n = 3). **C.** Serum MDA content in *Creg1*-CKO mice after DOX treatment (n = 5). **D.** The ratio of HW to TL and body weight in the *Creg1*-TG mice after DOX treatment (n = 6 for the control group, n = 8 for the DOX group). **E.** The mRNA of *Anp* and *Bnp* in the myocardium of *Creg1*-TG mice after DOX treatment (n = 3). **F.** Serum MDA content in *Creg1*-TG mice after DOX treatment (n = 5). DOX: doxorubicin; *Creg1*-CKO: *Creg1* cardiac-specific knockout mice; *Creg1^{fl/fl}* mice: littermate control mice. *Creg1*-TG: *Creg1* transgenic mice; WT mice: wild type mice. *p<0.05, **p <0.01 vs. *Creg1^{fl/fl}* mice or WT mice; #p<0.05, ##p<0.01 vs. *Creg1*-CKO or *Creg1*-TG; &p<0.01 vs. *Creg1^{fl/fl}*-DOX or WT-DOX.

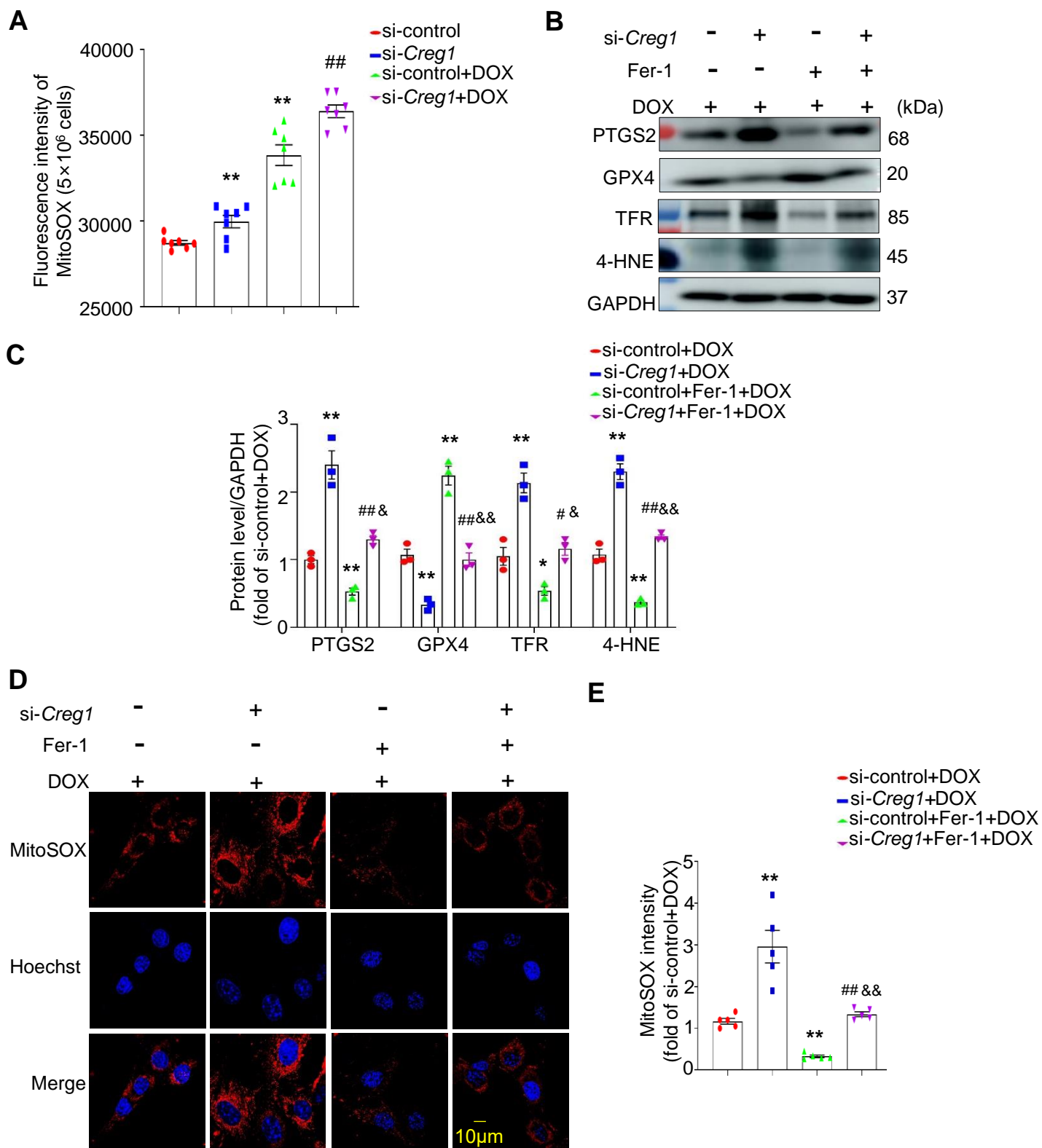


Figure S5. Ferroptosis inhibitor attenuated the effect of CREG1 knockdown on cardiomyocytes ferroptosis

A. The effect of CREG1 knockdown on the fluorescence intensity of MitoSOX-stained cells was examined using a full-wavelength enzyme-linked immunosorbent assay reader ($n = 7$). **B-C.** Western blotting of CREG1 and ferroptosis-related proteins in the CREG1-knockdown NMCMs after ferroptosis inhibitor Fer-1 treatment ($n = 3$). **D-E.** Effects of Fer-1 on mitochondrial oxidation in the CREG1-knockdown HL-1 cardiomyocytes using by MitoSOX staining ($n = 5$). DOX: doxorubicin, NMCMs: neonatal mouse cardiomyocytes. * $p < 0.05$, ** $p < 0.01$ vs. si-control group or si-control+DOX group; # $p < 0.05$, ## $p < 0.01$ vs. si-control+DOX group or si-Creg1+DOX group; & $p < 0.05$, && $p < 0.01$ vs. si-control+Fer-1+DOX group.

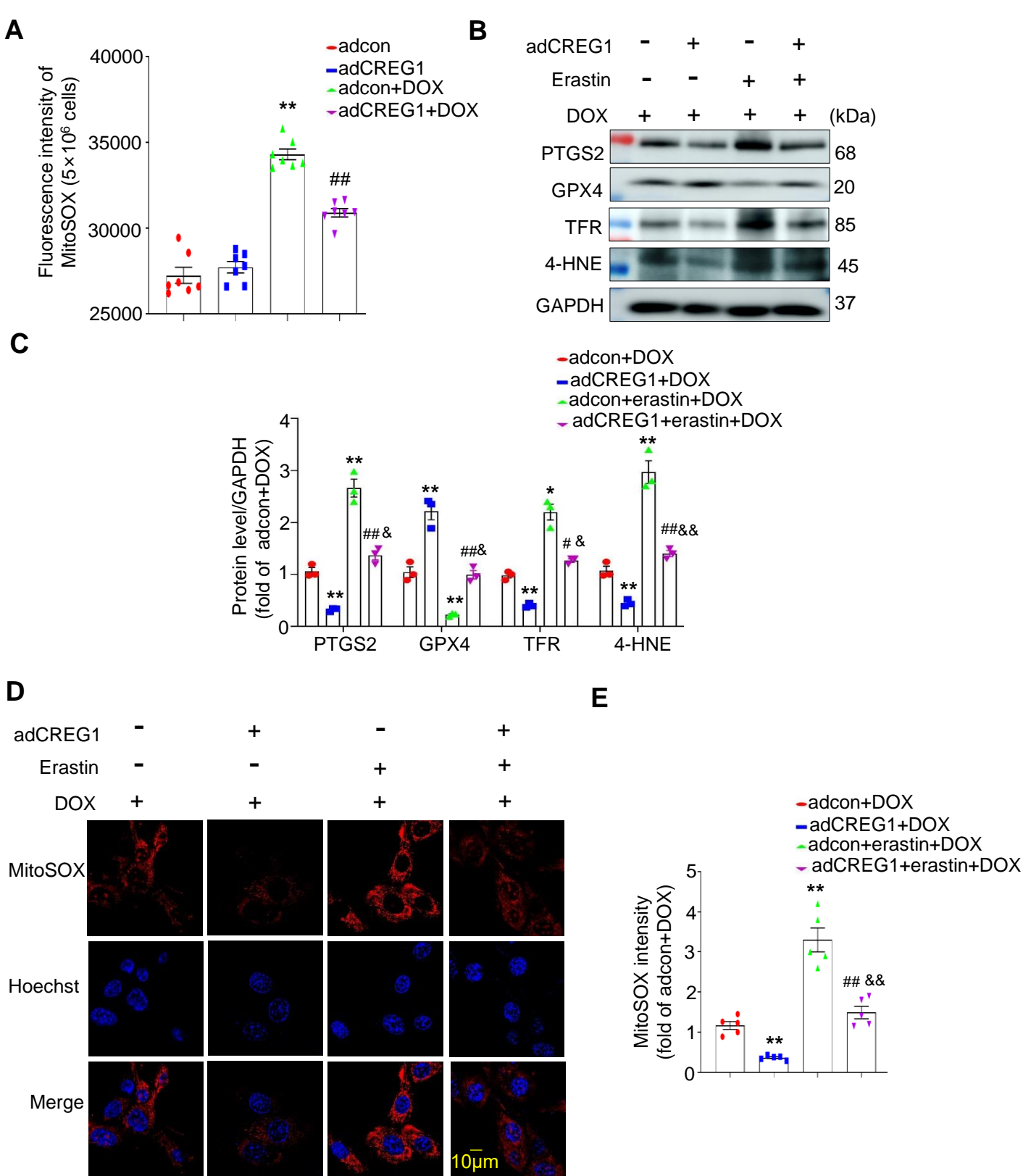


Figure S6. Ferroptosis inducer attenuated the effect of CREG1 overexpression on cardiomyocytes ferroptosis

A. The effect of CREG1 overexpression on the fluorescence intensity of MitoSOX-stained cells was examined using a full-wavelength enzyme-linked immunosorbent assay reader ($n = 7$). **B-C.** Western blotting of CREG1 and ferroptosis-related proteins in the CREG1-overexpressed NCMCs after ferroptosis inducer erastin treatment ($n = 3$). **D-E.** Effects of erastin on mitochondrial oxidation in the CREG1-overexpressed HL-1 cardiomyocytes using by MitoSOX staining ($n = 5$). DOX: doxorubicin, NCMCs: neonatal mouse cardiomyocytes. * $p < 0.05$, ** $p < 0.01$ vs. adcon group or adcon+DOX group; # $p < 0.05$, ## $p < 0.01$ vs. adcon+DOX group or adCREG1+DOX group; & $p < 0.05$, && $p < 0.01$ vs. adcon+erastin+DOX group.

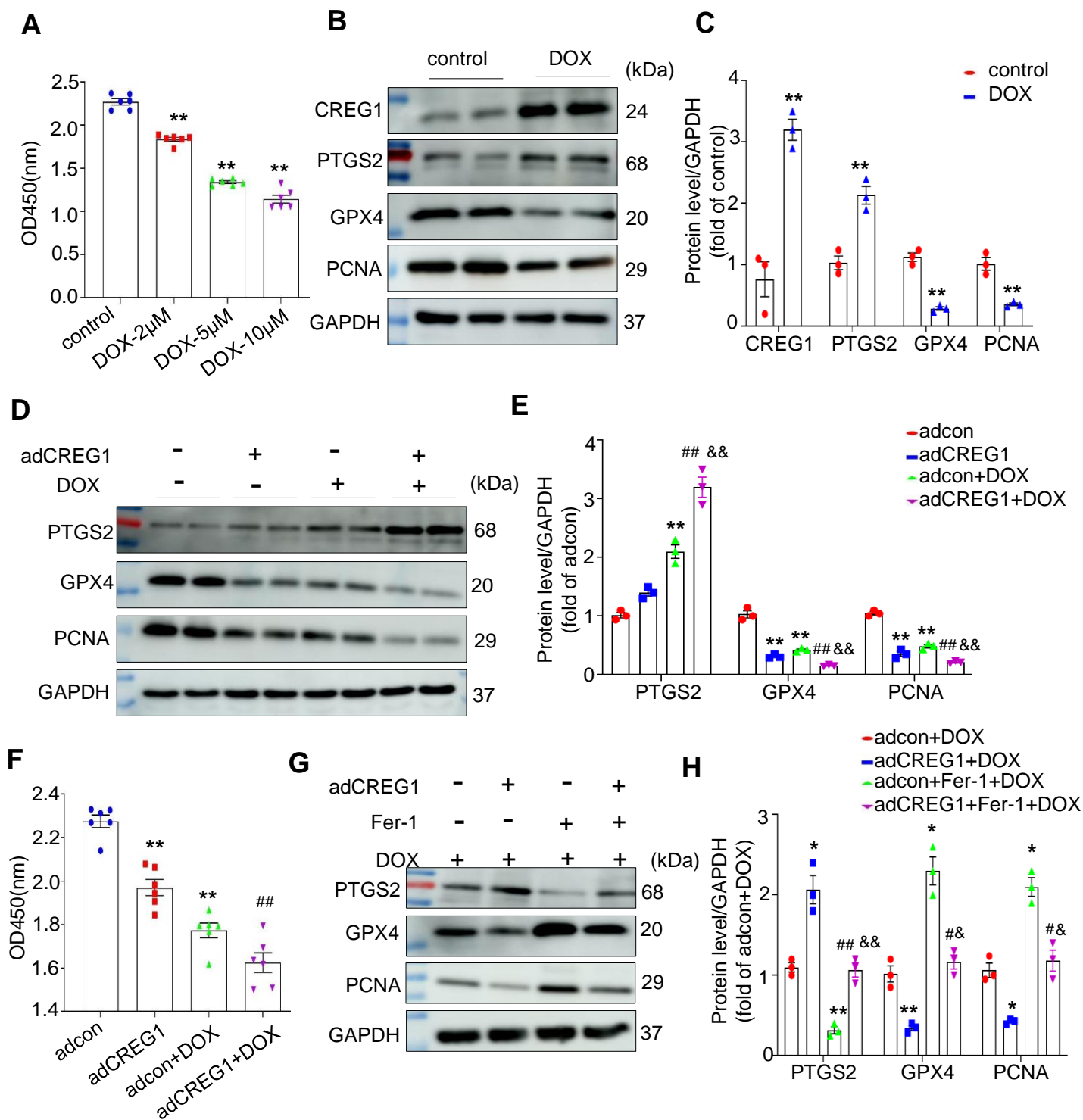


Figure S7. CREG1 inhibited the proliferation of breast cancer cell by regulating the ferroptosis

A. The effect of DOX (0, 2, 5 and 10 μ M) on the proliferation of MDA-MB-231 cells was examined using by CCK8 assay. **B-C.** The effect of DOX (5 μ M) on the expressions of CREG1, ferroptosis-related protein and PCNA in MDA-MB231 cells were examined by western blotting. **D-E.** Effects of CREG1 overexpression on ferroptosis and PCNA in MDA-MB-231 cells were examined by western blotting. **F.** The effect of CREG1 overexpression on the proliferation of MDA-MB-231 cells was examined using by CCK8 assay. **G-H.** Effects of Fer-1 on the ferroptosis and PCNA in MDA-MB-231 cells were examined by western blotting. $n = 3$. MDA-MB-231: breast cancer cell, DOX: doxorubicin. Fer-1: ferroptosis inhibitor. ** $p < 0.01$ vs. control or adcon group or adcon+DOX group; # $p < 0.05$, ## $p < 0.01$ vs. adCREG1 group or adCREG1+DOX group; & $p < 0.05$, && $p < 0.01$ vs. adcon+DOX group or adcon+Fer-1+DOX group.

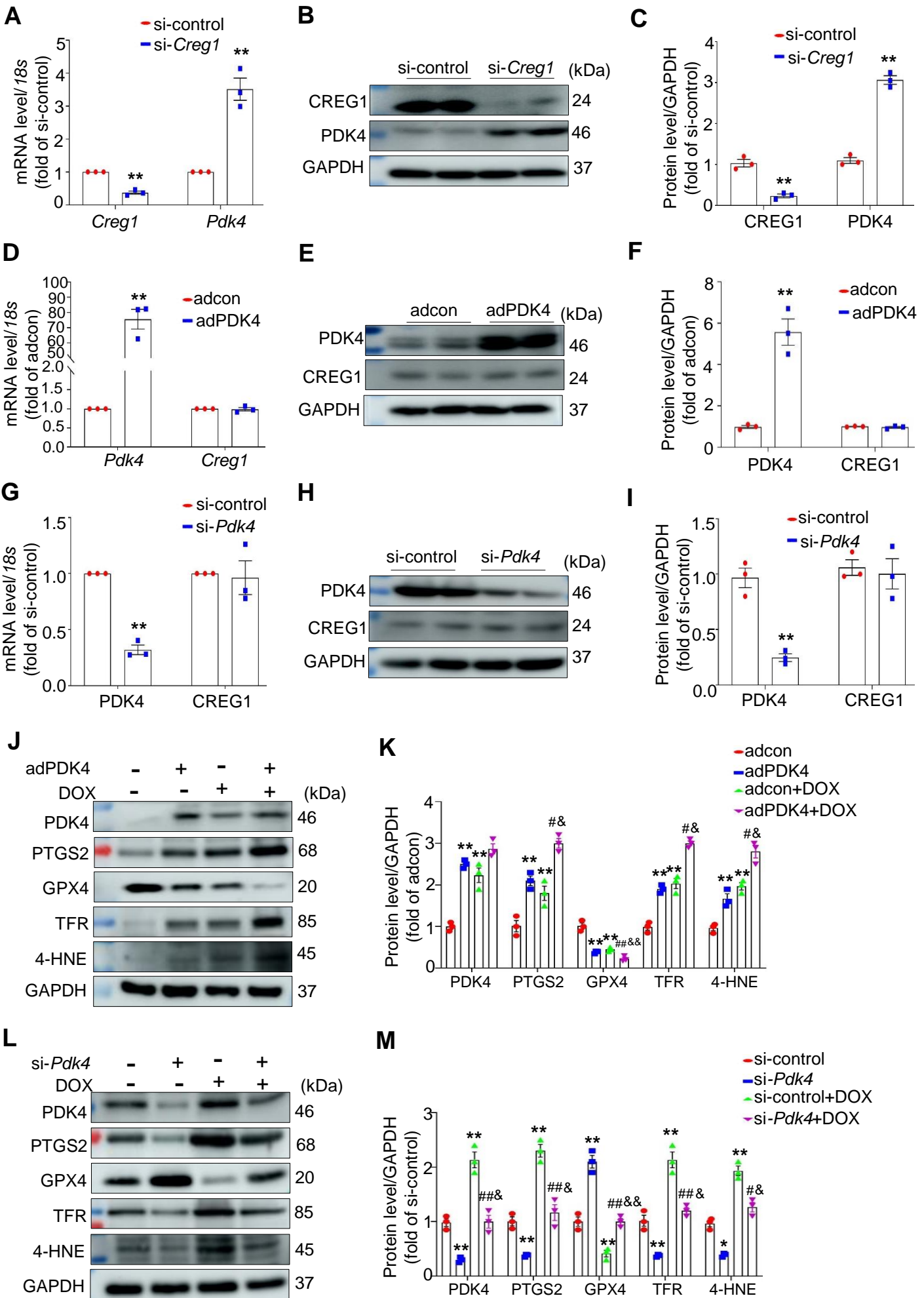


Figure S8. CREG1 knockdown increased the PDK4 expression in cardiomyocytes

A-C. Real-time PCR and western blotting analysis of PDK4 expression in CREG1-knockdown NMCMs. **D-F.** Real-time PCR and western blotting analysis of CREG1 expression in PDK4-overexpressed NMCMs. **G-I.** Real-time PCR analysis of CREG1 expression in PDK4-knockdown NMCMs. **J-K.** Effects of PDK4 overexpression on the expression of ferroptosis-related proteins in NMCMs, as determined by western blotting. **L-M.** Effects of PDK4 knockdown on the expression of ferroptosis-related proteins in NMCMs, as determined by western blotting. n = 3 for each group. DOX: doxorubicin, NMCMs: neonatal mouse cardiomyocytes. *p<0.05, **p<0.01 vs. adcon or si-control group; #p<0.05, ##p<0.01 vs. adPDK4 or si-*Pdk4* group; &p<0.05, &&p<0.01 vs. adcon+DOX or si-control+DOX group.

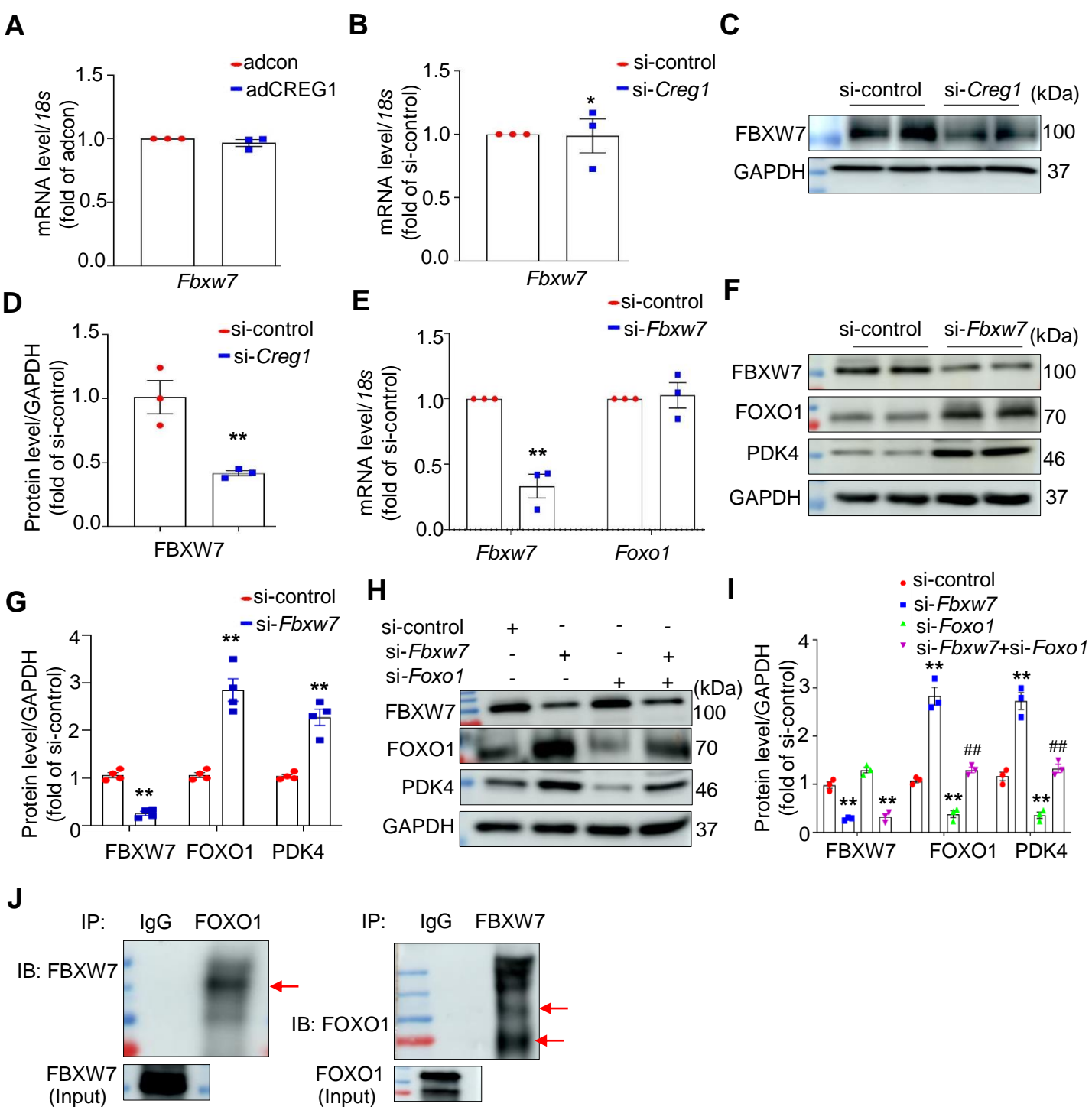


Figure S9. FBXW7 knockdown increased the protein expression of FOXO1 in cardiomyocytes

A. Real-time PCR analysis of *Fbxw7* mRNA expression in CREG1-overexpressed NMCMs (n = 3). **B-D.** Real-time PCR and western blotting analysis of FBXW7 expression in CREG1-knockdown NMCMs (n = 3). **E.** Real-time PCR of FOXO1 expression in FBXW7-knockdown NMCMs (n = 3). **F-G.** Western blotting of FOXO1 and PDK4 expression in FBXW7-knockdown NMCMs (n = 4). **H-I.** Western blotting of FOXO1 and PDK4 expression in FBXW7-knockdown together with FOXO1 knockdown NMCMs (n = 3). **J.** IP assays of the binding regions of FBXW7 and FOXO1 in HEK293T cells. NMCMs: neonatal mouse cardiomyocytes. *p<0.05, **p<0.01 vs. si-control group; ##p<0.01 vs. si-Fbxw7.

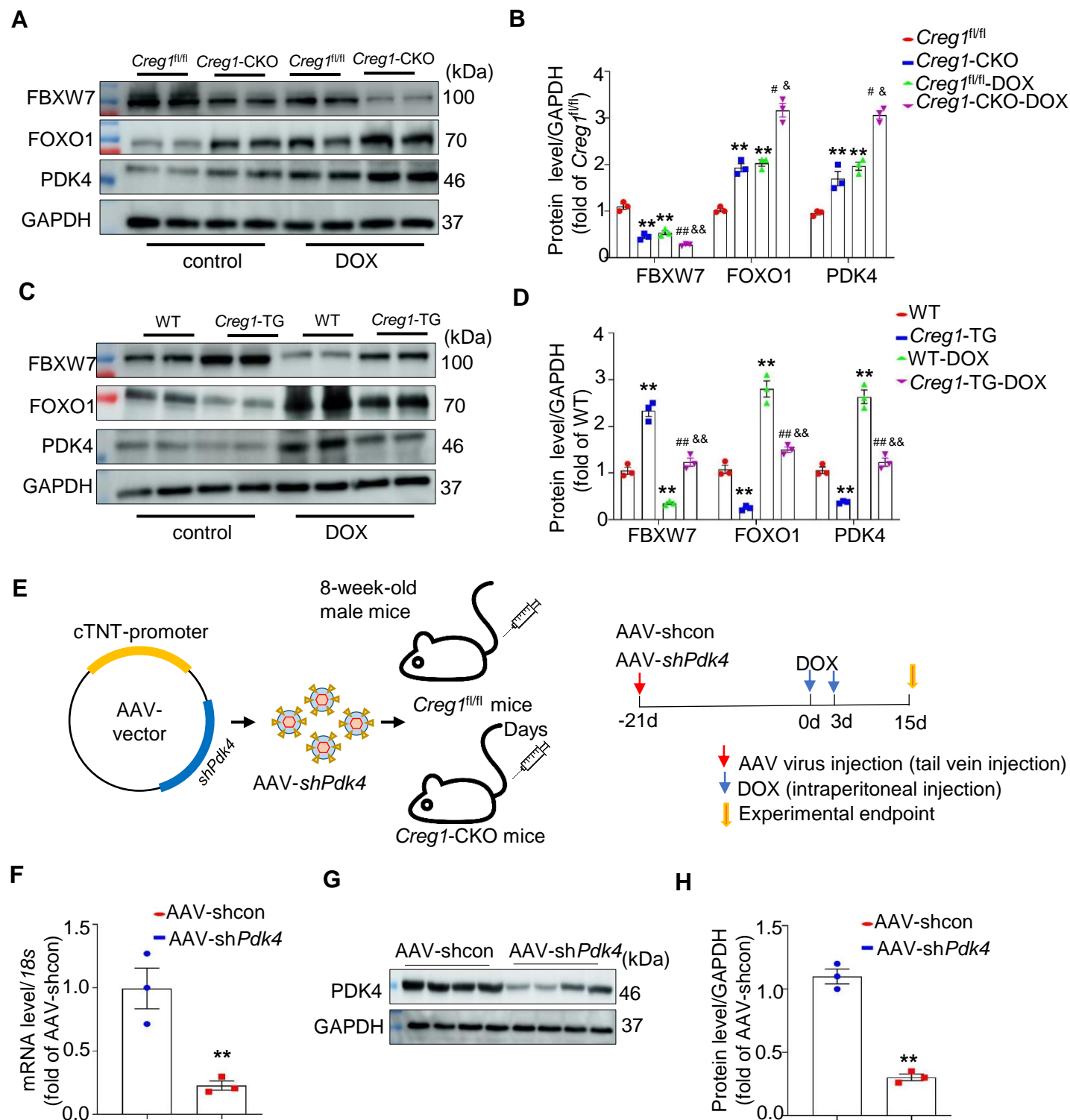


Figure S10. CREG1 overexpression inhibited PDK4 protein expression by regulating FBXW7-FOXO1 pathway

A-B. Western blotting of FBXW7, FOXO1 and PDK4 in the *Creg1-CKO* mice after DOX treatment (n = 3). **C-D.** Western blotting of FBXW7, FOXO1 and PDK4 in the *Creg1-TG* mice after DOX treatment (n = 3). **E.** Schematic diagram depicting the experimental strategy of PDK4 knockdown in *Creg1^{fl/fl}* mice and *Creg1-CKO* mice. **F.** Real-time PCR analysis of *Pdk4* mRNA expression in the myocardium of *Creg1^{fl/fl}* mice after 21days of AAV-sh*Pdk4* virus injection (n = 3). **G-H.** Western blotting of PDK4 protein in the myocardium of *Creg1^{fl/fl}* mice after 21days of AAV-sh*Pdk4* virus injection (n = 3). DOX: doxorubicin, *Creg1-CKO*: *Creg1* cardiac-specific knockout mice; *Creg1^{fl/fl}* mice: littermate control mice; *Creg1-TG*: *Creg1* transgenic mice; WT mice: wild type mice. **p<0.01 vs. *Creg1^{fl/fl}* mice or WT mice or AAV-shcon; #p<0.05, ##p<0.01 vs. *Creg1-CKO* or *Creg1-TG*; &p<0.05, &&p<0.01 vs. WT-DOX or *Creg1^{fl/fl}-DOX*.

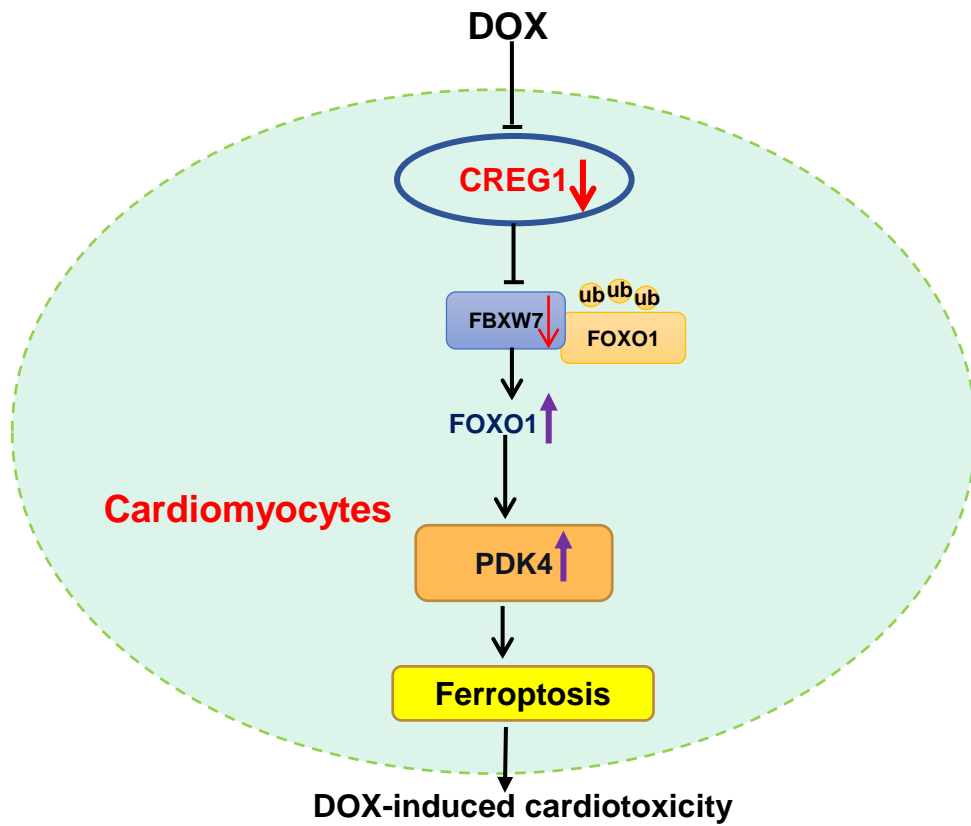


Figure S11. Mechanism diagram of CREG1 in DOX-induced cardiotoxicity

Table S1. Primers for real-time PCR

Primer name	Forward Primer (5'-3')	Reverse Primer (5'-3')
<i>Creg1</i>	CTTCGCGGACATCATCTCAAT	GTCAGCGTAGCCTCTGGATTT
<i>Ptgs2</i>	CTGCGCCTTTTCAAGGATGG	GGGGATACACCTCTCCACCA
<i>Pdk4</i>	CCGCTTAGTGAACACTCCTTC	TGACCAGCGTGTCTACAAACT
<i>Foxo1</i>	GGGTCCCACAGCAACGATG	CACCAGGGAATGCACGTCC
<i>Fbxw7</i>	GTTCCGCTGCCTAATCTTCCT	CCCTTCAGGGATTCTGTGCC
<i>Anp</i>	ACCTGCTAGACCACCTGGAG	CCTTGGCTGTTATCTTCGGTACCGG
<i>Bnp</i>	GAGGTCACCTCCTATCCTCTGG	GCCATTTCTCCGACTTTTCTC
<i>18s</i>	TTGACGGAAGGGCACCACCAG	GCACCACCACCCACGGAATCG