The interplay of mitochondrial oxidative stress and endoplasmic reticulum stress in cardiovascular fibrosis in obese rats

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6 supplemental figures

SUPPLEMENTAL TABLES

Table S1. Primers used in real time PCR analysis

Gene	Primer	Sequence (5´to 3´)
Col 1a1	Forward	GCCTCCCAGAACATCACCTA
	Reverse	ATGTCTGTCTTGCCCCAAGT
Tgf-β	Forward	CAGAAGTTGGCATGGTAGCC
	Reverse	TGCTTCAGCTCCACAGAGAA
Hprt	Forward	AGGACCTCTCGAAGTGT
	Reverse	ATTCAAATCCCTGAAGTACTCAT

Col1a1: Collagen type I; *Tgf-β*: Transforming growth factor-beta and *Hprt*: hypoxanthine-guanine phosphoribosyltransferase.

SUPPLEMENTAL FIGURES

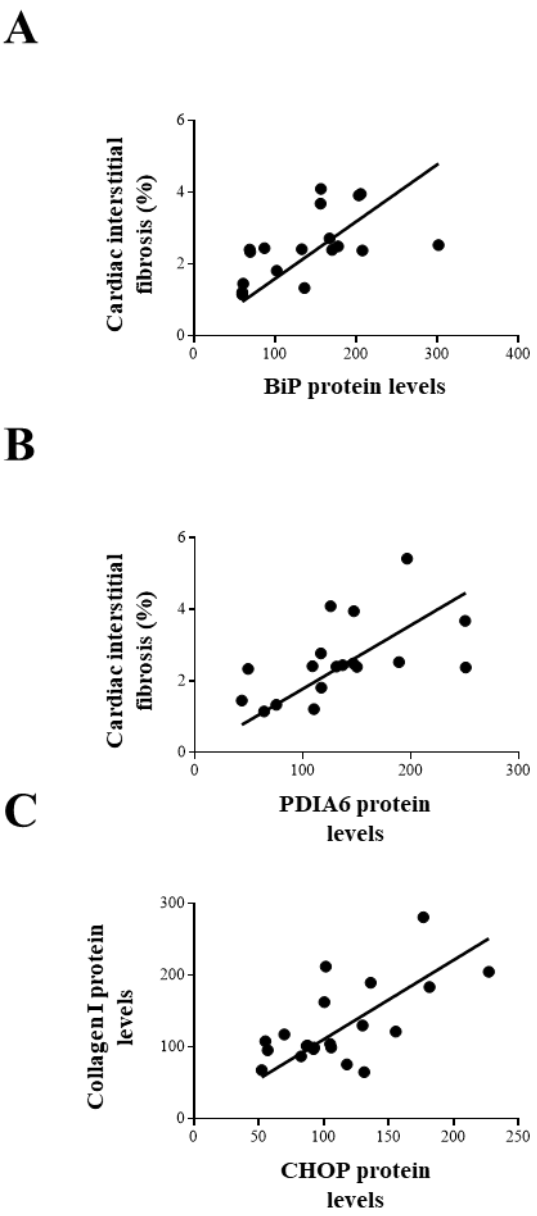

Figure S1. Correlations observed between cardiac fibrosis and endoplasmic reticulum stress. Direct correlation between cardiac interstitial fibrosis and **(A)** immunoglobulin binding protein (BiP; r=0.5475; p=0.0187); **(B)** protein disulfide isomerase family A member 6 (PDIA6; r=0.5534; p=0.0172) protein expression in all animals. **(C)** Direct correlation between cardiac CCAAT-enhancer-binding protein homologous protein (CHOP) and collagen type I protein expression in all animals (r=0.6385; p=0.0018).

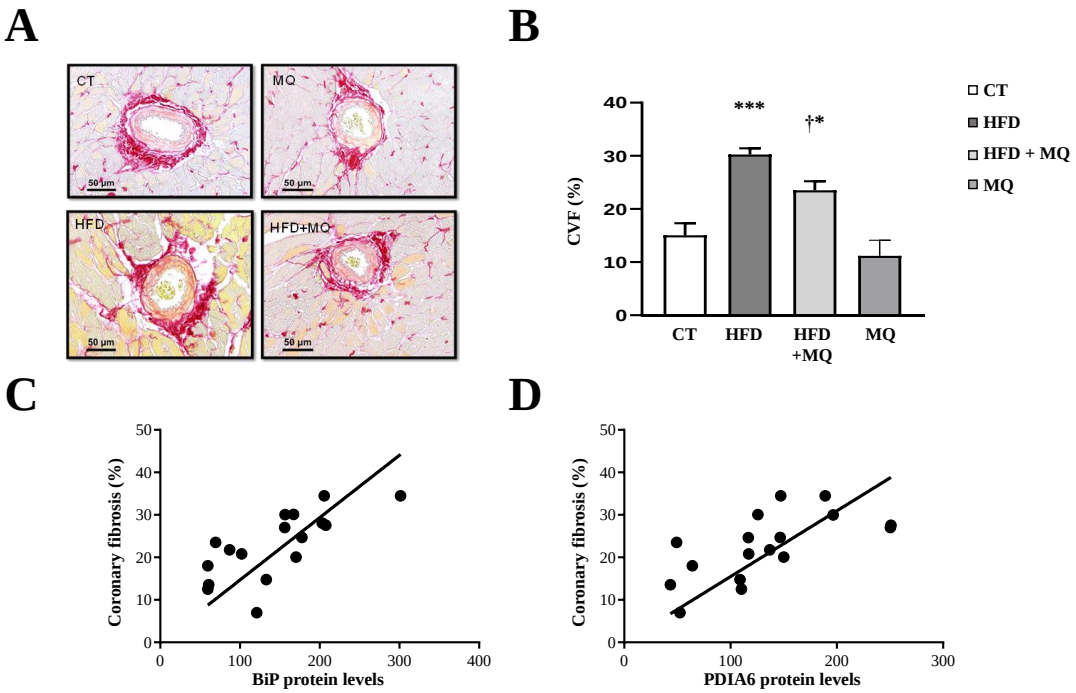

Figure S2. Mitochondrial oxidative stress mediates the fibrosis of the media of the descending coronary artery. (A) Representative microphotographs of cardiac sections staining with picrosirius red and **(B)** quantification of collagen volume fraction in the media of the coronary artery from control rats fed a normal chow (CT) and rats fed a high fat diet (HFD) treated with vehicle or with the mitochondrial antioxidant MitoQ (MQ; 200 µM). Scale bar: 50 µm. Bars graphs represent the mean ± SEM of 6-8 animals. **p<0.01; ***p<0.001 vs. control group. †p<0.05 vs. HFD group. Direct correlation between coronary media fibrosis and **(C)** immunoglobin binding protein (BiP; r=0.7207; p=0.0011); **(D)** protein disulfide isomerase family A member 6 (PDIA6; r=0.6460; p=0.0051) protein expression in all animals.

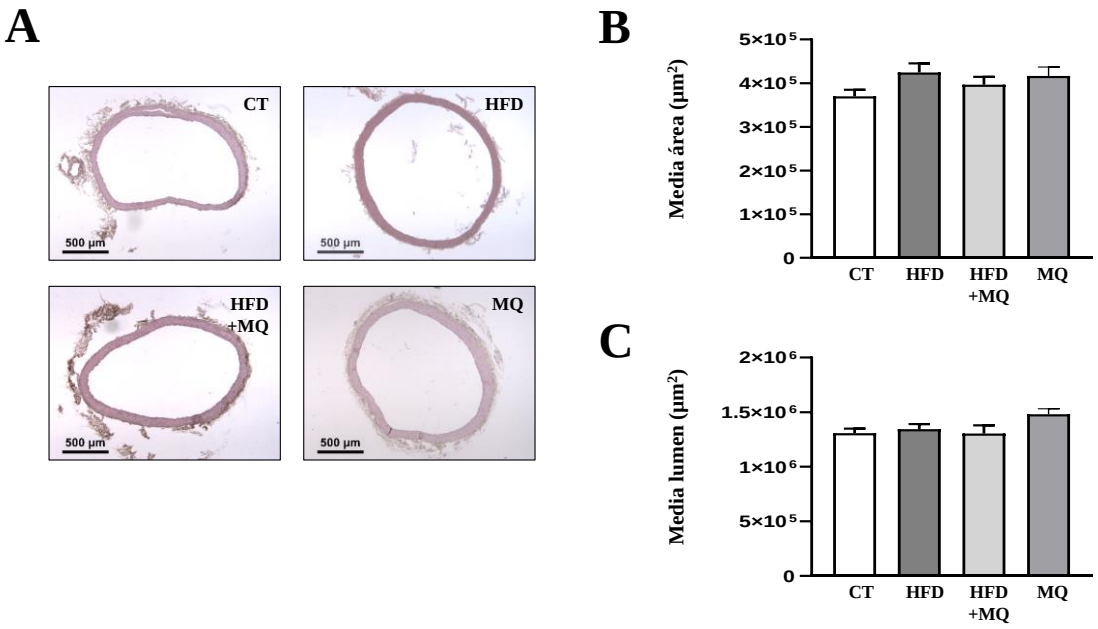

Figure S3. Vascular morphology of aorta in the animals. (A) Representative microphotographs of aortas stained with hematoxylin/eosin. **(B)** Media and **(C)** lumen area from control rats fed a normal chow (CT) and rats fed a high fat diet (HFD) treated with vehicle or with the mitochondrial antioxidant MitoQ (MQ; 200 µM). Scale bar: 500 µm. Bars graphs represent the mean ± SEM of 6-8 animals.

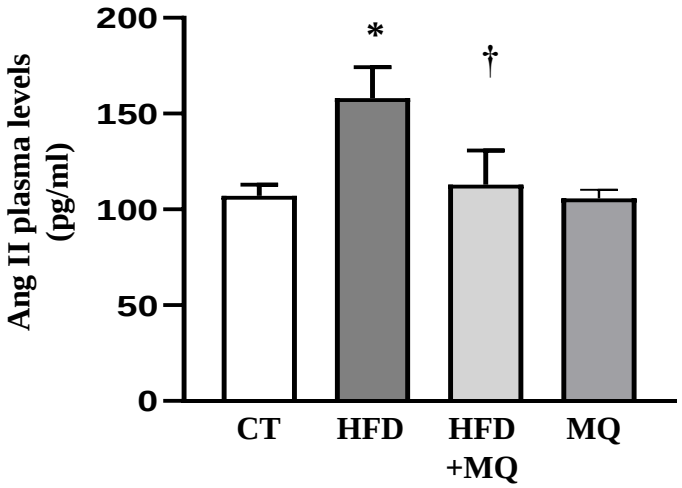

Figure S4. Angiotensin (Ang II) plasma levels from control rats fed a normal chow (CT) and rats fed a high fat diet (HFD) treated with vehicle or with the mitochondrial antioxidant MitoQ (MQ; 200 μM). Bars graphs represent the mean ± SEM of 5-8 animals. *p<0.05 vs. control group. †p<0.05 vs. HFD group.

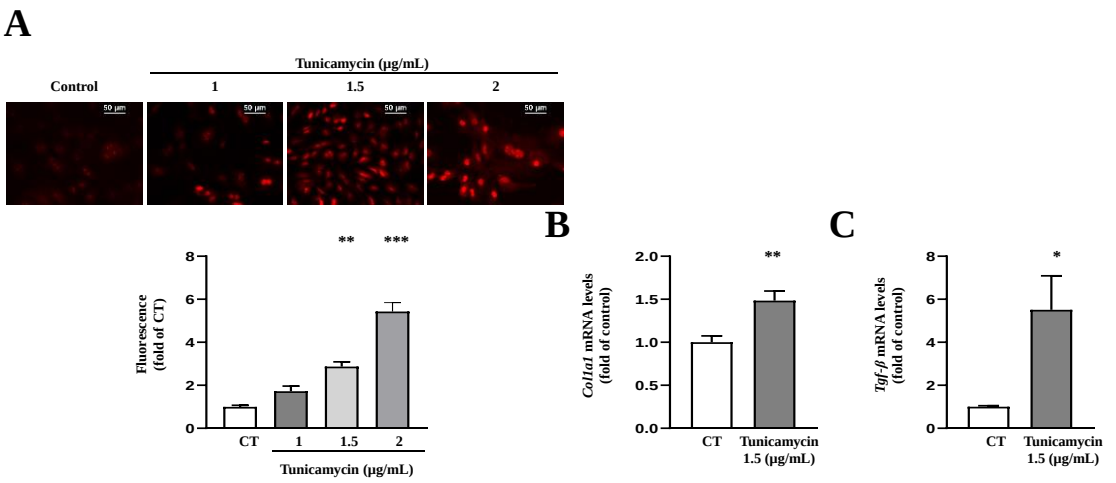

Figure S5. Endoplasmic reticulum stress increases oxidative stress and extracellular matrix markers in vascular smooth muscle cells. Effects of the endoplasmic reticulum stress inductor, tunicamycin (1-2 µg/mL) on superoxide anion production. **(A)** Representative microphotographs and quantification of cells labelled with the oxidative dye dihydroethidium (magnification 40X). mRNA levels of **(B)** collagen type I (*Col 1a1*), and **(C)** transforming growth factor-beta (*Tgf-β*) in vascular smooth muscle cells treated with tunicamycin (1.5 µg/mL)) for 24 hours. Bars graphs represent the mean ± SEM of four to six assays normalized for hypoxanthine phosphoribosyltransferase (HPRT). *p<0.05; **p<0.01, ***p<0.001 vs. control cells.

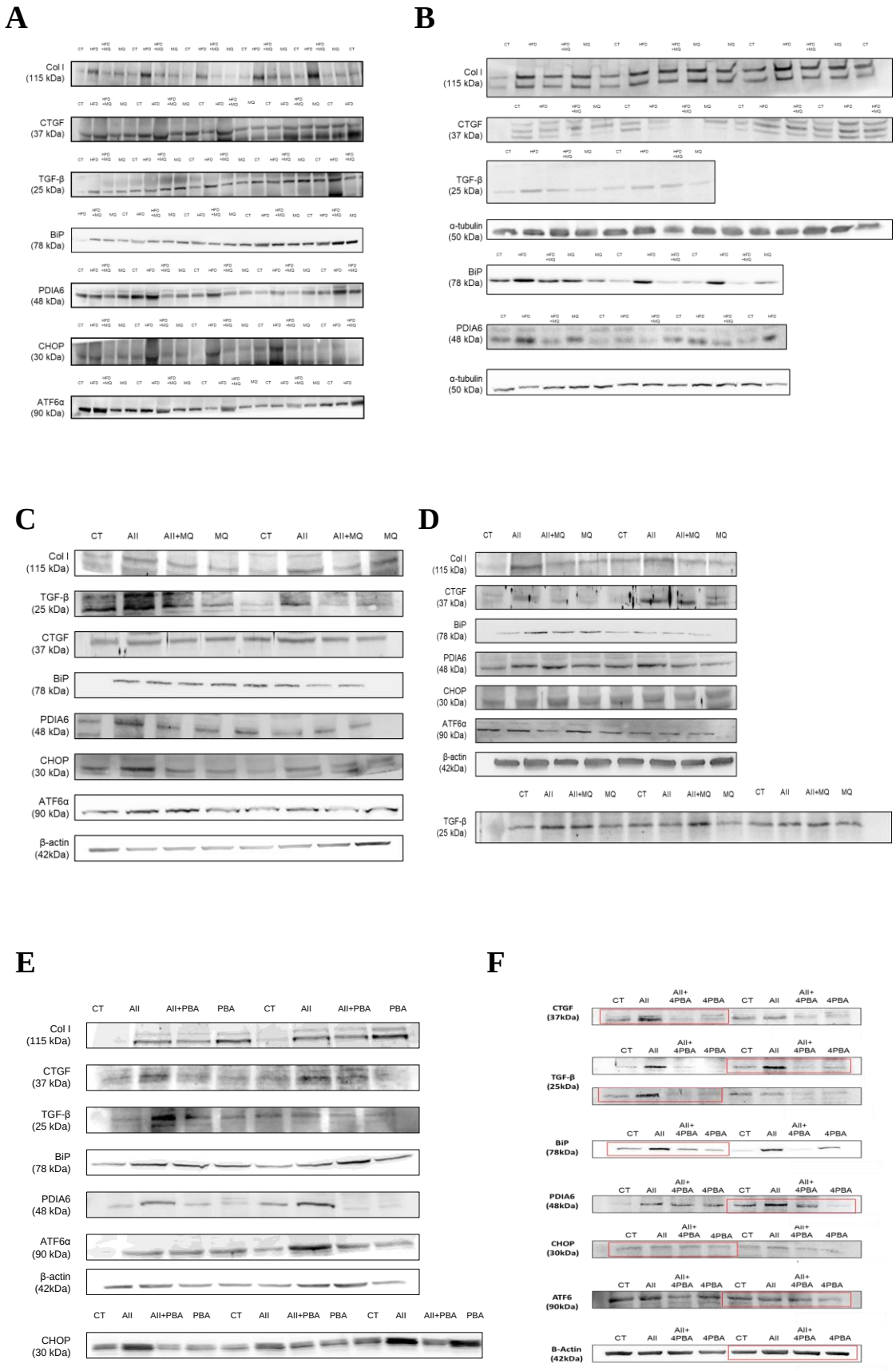

Figure S6. Original blots corresponding to (A) Figure 1; **(B)** Figure 2; **(C)** Figure 3; **(D)** Figure 4; **(E)** Figure 5 and **(F)** Figure 6.