Supplement

Additional Methods

1. Echocardiography

Echocardiography was performed according to the American Society of Echocardiography guidelines, using a Vevo 2100™ High-Resolution Imaging System (Visual Sonics, Toronto, Canada). Mice were exposed to isoflurane (1.5-2% in oxygen) inhalation in order to induce general anesthesia. A heated electrical platform was used to maintain body temperature at 37°C, which was monitored by rectal thermostat. Body temperature, heart rate, and respiratory rate were continuously monitored throughout the procedure. Each animal was maintained for 5 min under anesthesia and on the heated platform in a supine position until stabilization of vital signs. B-mode and M-mode echocardiography images were obtained in the parasternal long-axis view in a supine position while the transducer was placed on the left thorax and ultrasound beam was directed at the mid-papillary muscle level. All images were acquired with heart rate between 400 and 500 BPM. Ejection fraction (EF) was measured at baseline, at day one, and right before sacrifice in all experimental groups.

2. Enzyme-linked immunosorbent assays (ELISAs)

2.1. Assessment of plasma tumor necrosis factor-alpha (TNF-α) levels. Plasma TNF-α was detected using an ELISA STEMCELL Technologies kit (#02030), according to the manufacturer's instructions. Plasma samples 1:1 were added to ELISA diluent to reach a total minimum volume of 250 µL. One hundred µL of diluted sample was loaded in duplicate in the ELISA strip plate pre-coated with capture antibodies specific for the cytokine TNF-α and incubated 2h at room temperature. Five rinses with 300 µL washing buffer were performed,

biotinylated detection antibody was added, followed by incubation for 1 h. After 5 washes (300 µL each), 100 µL/well diluted streptavidin horseradish peroxidase (SA-HRP) was added, and the plate incubated for 1 h at room temperature. Wells were washed, and 100 µL of 3,3',5,5'-tetramethylbenzidine (TMB) substrate was added to each well and the plate incubated at room temperature for 15 min. Finally, 100 µL/well of stopping solution was added and the absorbance measured at 450 nm in a microplate reader.

2.2. Assessment of plasma 17β estradiol. Levels of plasma 17β estradiol were measured using the ELISA Abcam kit (#108667) in order to verify that female mice were in the premenopausal phase (22) and to confirm success of ovariectomy **(Suppl. Table 1).** Twenty-five µL of samples were loaded in duplicate. Afterward, 17β estradiol-HRP conjugated was added, and the plate incubated for 2 h at room temperature. After 3 washes with 100 µL washing buffer, 100 µL/well of TMB substrate was added to all wells and the plate incubated at room temperature for 30 min. Finally, 100 µL stopping solution was added per well and absorbance measured at 450 nm in a microplate reader.

Female group	Plasma 17β estradiol levels (Mean ± SEM) pg/mL	P value
Control	32.219 ± 2.489	0.5525
FMI	34.742 ± 1.336	
FMIOVX	Under detection limit <10	

Suppl. Table 1: Plasma estrogen levels in female mice

3. Immunofluorescence (IF)

IF was performed to detect accumulation of α-smooth muscle actin (α-SMA) in kidney tissues. Unstained kidney slides were placed in antigen retrieval buffer for fifteen min at 95°C. Slides were washed 2x with Tris-buffered saline (TBS) with 0.025% Triton (TBS-T 0.025%), 5 min

each and incubated with 10% normal goat serum (NGS) + 1% bovine serum albumin (BSA) in TBS at room temperature for 2 h to block non-specific binding of α-SMA antibody. Incubation with α-SMA antibody (1:200, Abcam, ab5694) in TBS was performed overnight at 4°C, followed by two 5 min washes with TBS-T 0.025%. Kidney slides were incubated with the fluorescein isothiocyanate (FITC) conjugated secondary antibody (Abcam, ab97050) in TBS (1:100) for 1 h and washed 2x 5 min with TBS. Finally, DAPI was added and slides were washed 2x with phosphate-buffered saline (PBS), 5 min each time, and observed with a Zeiss Axio under 20x magnification.

4. Twenty-four hour urine collection

Mice of both sexes were individually housed in clean dry metabolic cages 24 hours before sacrifice with free access to water replacement pouches, in order to collect 24 hour urine. Urine samples were used to measure urine output, proteinuria, and creatinine clearance.

5. Proteinuria measurement

Proteinuria was measured using Bradford reagent in a 96-well plate. Briefly, protein standards were prepared with BSA ranging from 0.1-1.4 mg/ml. Five µL of the urine samples were added to separate wells in the 96-well plate followed by 250 µL of Bradford reagent and the plate incubated at room temperature for 30 min. Absorbance was measured at 595 nm.

6. Creatinine clearance determination

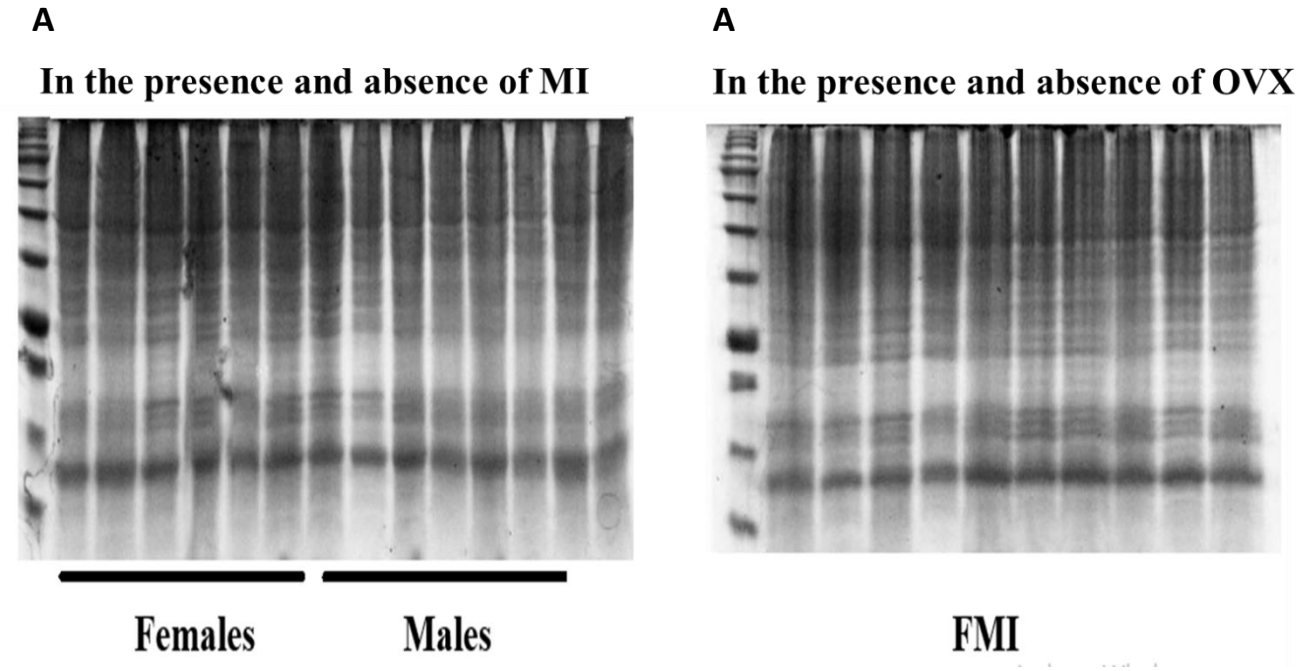
Creatinine concentration in plasma and urine samples was measured by the Jaffé-method using a Fluitest® CREA kit (Analyticon) according to the manufacturer's protocol. Creatinine clearance

(Crcl) was expressed as ml/min and obtained from the following equation: (concentration of urine creatinine (mg/mL) / concentration of plasma creatinine (mg/mL)) x urine volume (mL) in 24 hour.

Supplementary Figures

Total protein quantification

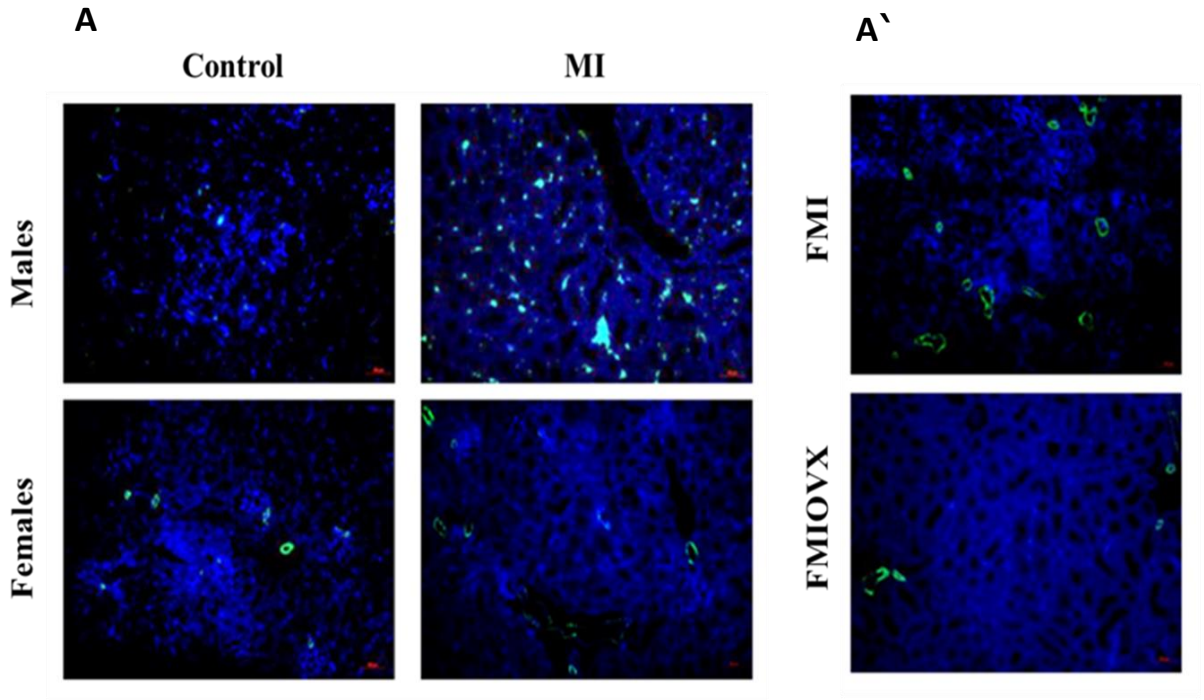
IL-1β and uncleaved caspase-3 protein levels were normalized to total protein (**Supplemental Figure 1A and 1A`**)



Supplemental Figure 1: Representative figure of total protein quantification using Coomassie blue in male and female MI mice in the presence and absence of MI (**1A)** and OVX (**1A`**); MI: Myocardial Infarction; OVX: Ovariectomy

The impact of MI and OVX on the accumulation of α-SMA in the kidneys

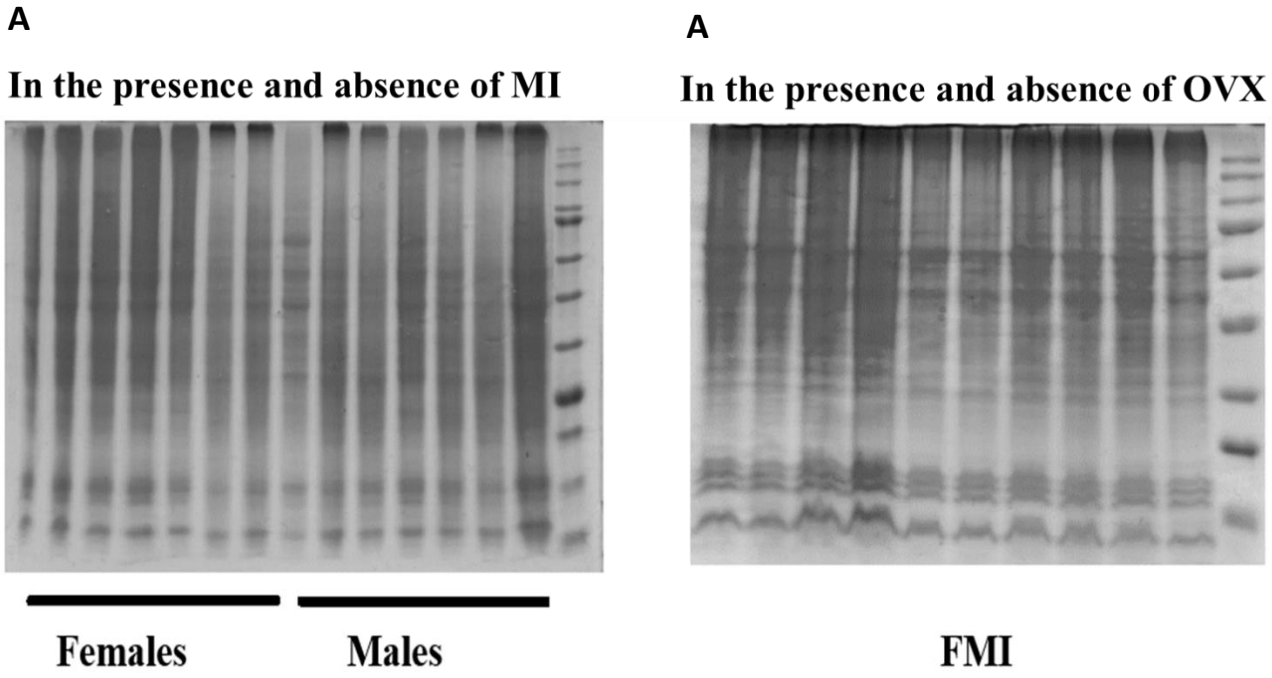
Supplemental Figure 2A shows an accumulation of α-SMA protein levels in the peritubular space in males post-MI, whereas no accumulation of α-SMA in the peritubular space in female mice following MI and OVX was observed **(2A`)**



Supplemental Figure 2: Representative figure of IF shows an accumulation of α-SMA in the peritubular space of the kidneys in MI male mice, only. MI: Myocardial Infarction; OVX: Ovariectomy

Total protein quantification

IL-4 and α-SMA protein levels were normalized to total protein (**Supplemental Figures 3A and 3A`**)



Supplemental Figure 3: Representative figure of total protein quantification using Coomassie blue in male and female MI mice in the presence and absence of MI (**3A**); and OVX (**3A`**); MI: Myocardial Infarction; OVX: Ovariectomy