

# Activation of BK $\alpha$ channel Prevented TGF-induced Oxidative Stress in H9C2 Cardiomyocytes

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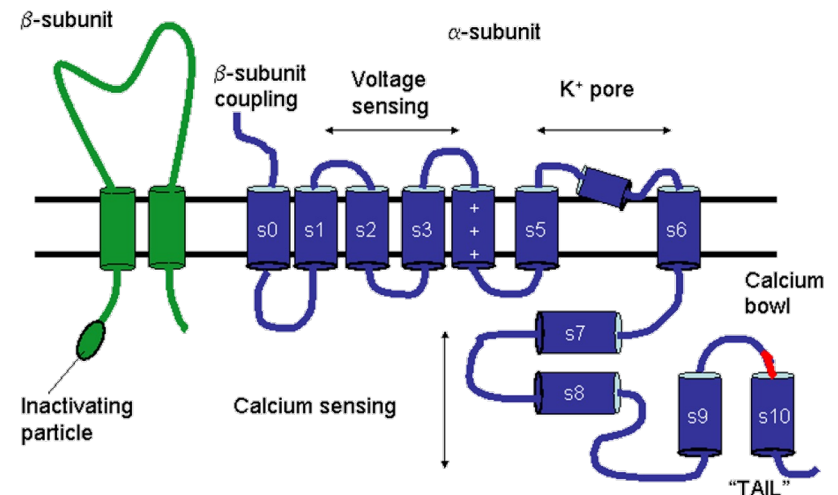
**Emory University 16<sup>th</sup> Annual Department of Medicine (DOM) Research Day**  
**Nov 2, 2023**

# Epidemiology

- Chronic kidney disease (CKD) affects 26–30 million U.S population.
- Prevalence of cardiovascular disease in CKD patients reaches 65% in patients 66 and older (Kuma et al. 2020).
- Uremic cardiomyopathy contributes significantly to CKD-induced morbidity and mortality. Most death occurred within 3 years from diagnosis of uremic cardiomyopathy (Trespalacios et al. 2003).

# Our target: BK Channel

- Large-conductance, calcium and voltage-activated (Maxi-K, or BK) potassium channels are widely distributed in the brain, smooth muscle, and apical membrane of renal epithelial cells.
- BK channels also function as calcium sensors and contribute to the control of cellular excitability and the regulation of neurotransmitter release.



# Prior Research

- Opening BK channels attenuated renal fibrosis in mice (Wang et al, Kidney Int, 2021).
- BK channel is a promising target for limiting acute cardiac damage and adverse long-term events that occur post myocardial infarction (Frankenreiter, Circulation, 2017).
- Activation of BK channels ameliorates liver fibrosis (Yang et al, Front. Pharmacol. 2020).

# Hypothesis

Activation of BK channel activity attenuates cardiac fibrosis in CKD by suppressing oxidative stress.

# Methodology

1. **In vivo** : 5/6 Nephrectomy mice physiology data
2. **In vitro** : Cardiac Myoblasts (H9C2) cultured in DMEM with 10% FBS and 1% P/S
  1. Tissue Level: Western Blots  
BK $\alpha$  channel activated with NS1916 (10-20  $\mu$ M).
  2. Organelle Level: Electrophysiology  
Single channel recordings
  3. Molecular Level: Peroxidase:
    - I. Hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) was tested by Amplex Red Hydrogen Peroxide Kit (Molecular Probes, A22188):
    - II. Superoxide (ROS) was detected by DHE (Dihydroethidium) Assay (Abcam, ab236206)
    - III. Superoxide Dismutase (SOD) was measured using colorimetric activity kit (Invitrogen, EIASODC).

*\* CKD mice induced by 5/6 nephrectomy, BMS-191011 (10 mg/kg BW) administered IP daily for 8 weeks.*

# 1. In-vivo Experiments

## A. Molecular

- i. Protein Expression Level
- ii. mRNA Expression Level

## B. Tissue

- i. Blood Pressure
- ii. Heart Size
- iii. Masson Staining for Fibrotic Markers

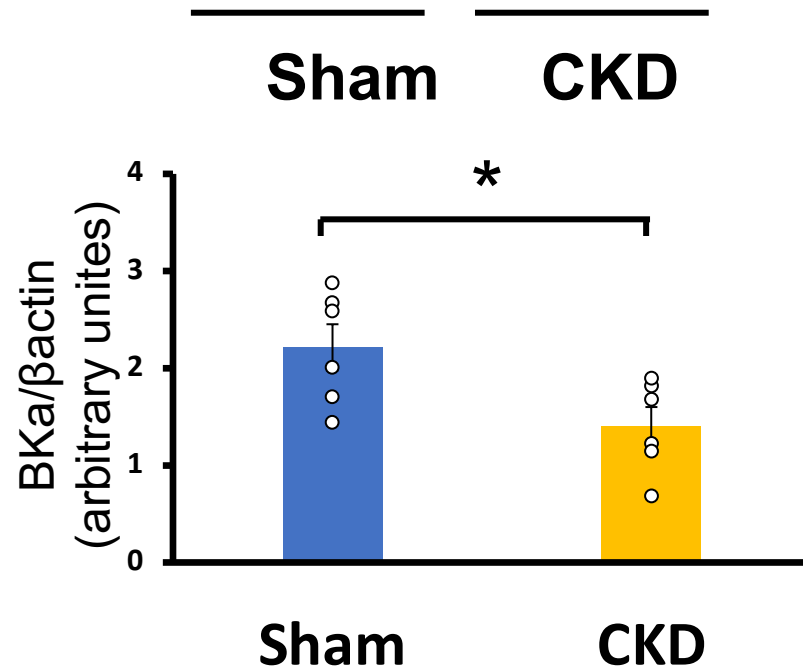
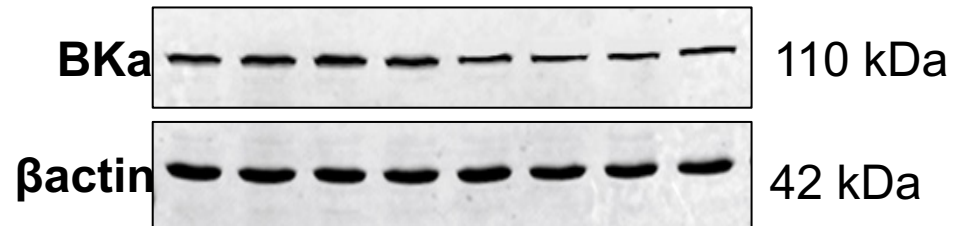
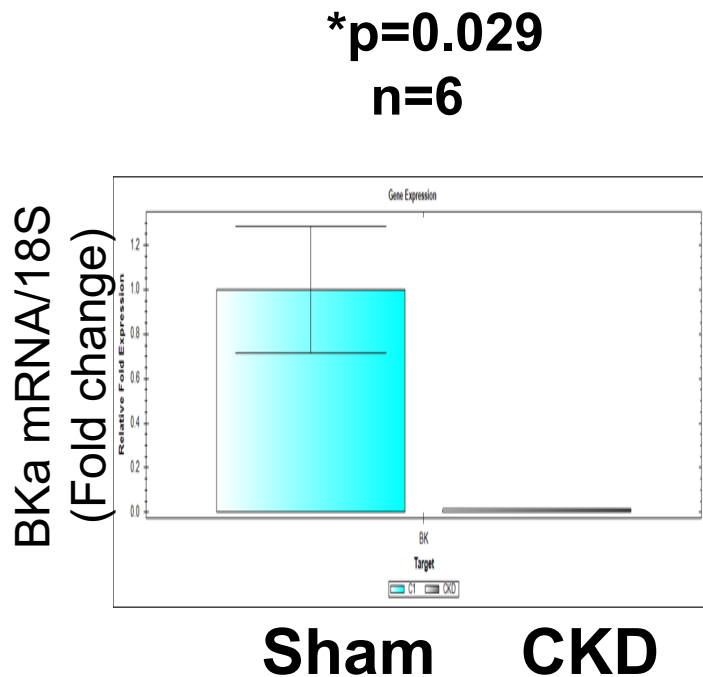
# BK $\alpha$ mRNA and protein were decreased in the heart of CKD mice

## A. Molecular

- ➔ i. Protein Expression Level
- ii. mRNA Expression Level

## B. Tissue

- i. Blood Pressure
- ii. Heart Size
- iii. Masson Staining for Fibrotic Markers





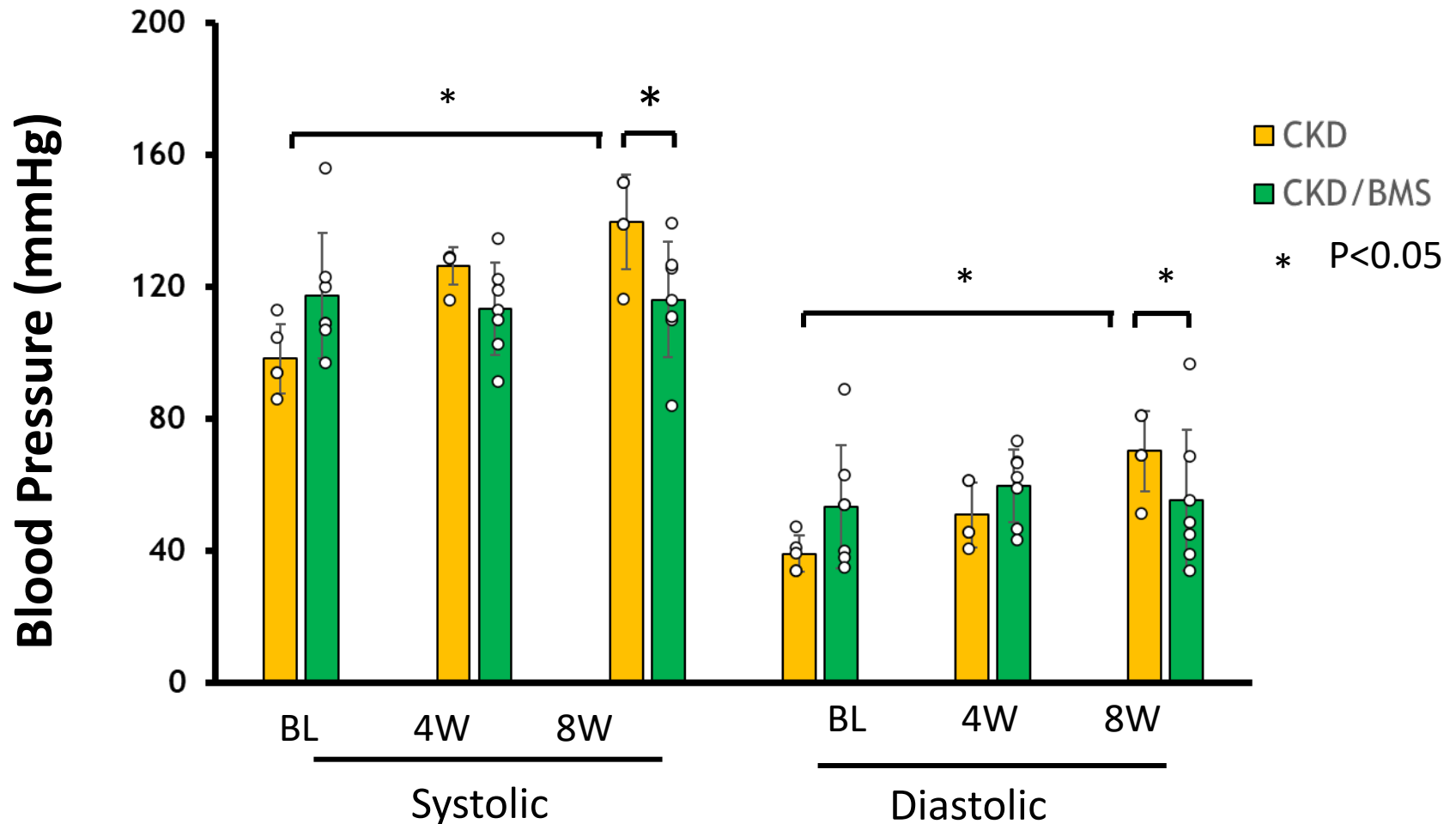
# Activation of BK $\alpha$ limits CKD-induced hypertension in 5/6 Nx mice

## A. Molecular

- i. Protein Expression Level
- ii. mRNA Expression Level

## B. Tissue

- i. Blood Pressure
- ii. Heart Size
- iii. Masson Staining for Fibrotic Markers



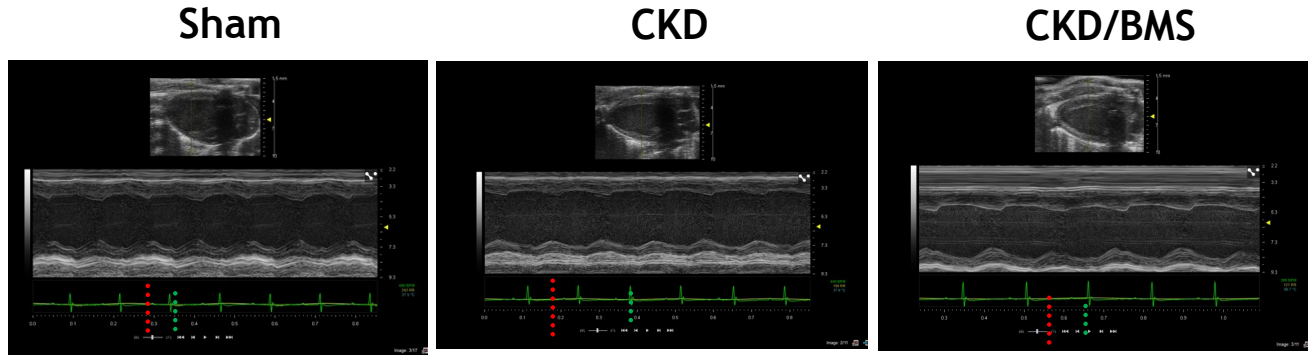
# Activation of BK $\alpha$ promotes heart function in CKD mice

## A. Molecular

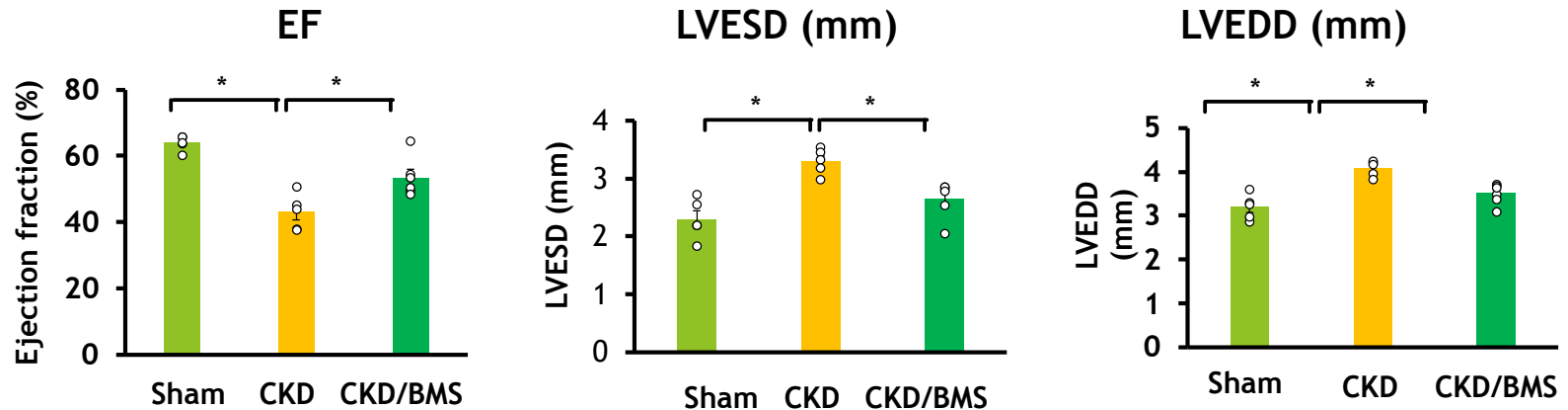
- i. Protein Expression Level
- ii. mRNA Expression Level

## B. Tissue

- i. Blood Pressure
- ii. Heart Size
- iii. Masson Staining for Fibrotic Markers



----- LVEDD (mm): left ventricular end-diastolic dimension  
----- LVESD (mm): left ventricular end-systolic dimension



LVEDD (mm): left ventricular end-diastolic dimension

LVESD (mm): left ventricular end-systolic dimension

EF: ejection fraction, refers ventricle pumps blood with each heartbeat

(Results obtained at: 8 wk)

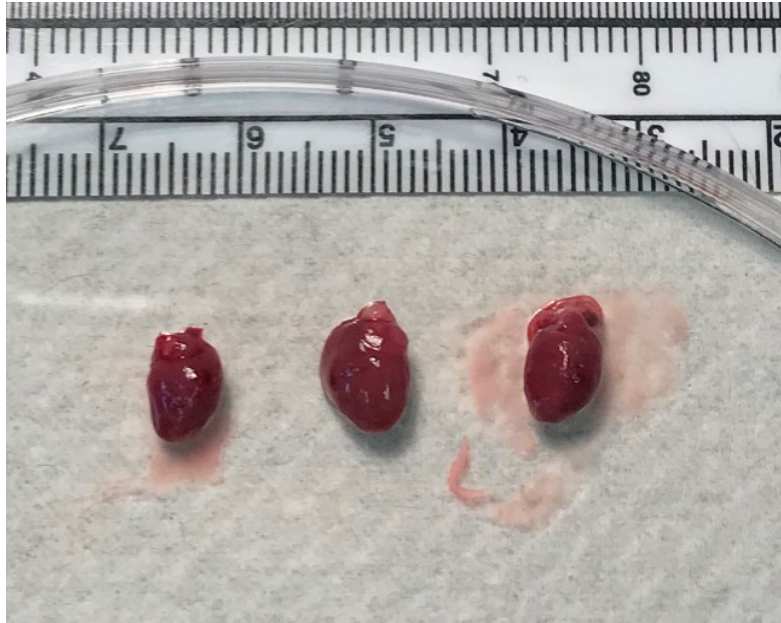
# Activation of BK $\alpha$ limits CKD-induced cardiac hypertrophy

## A. Molecular

- i. Protein Expression Level
- ii. mRNA Expression Level

## B. Tissue

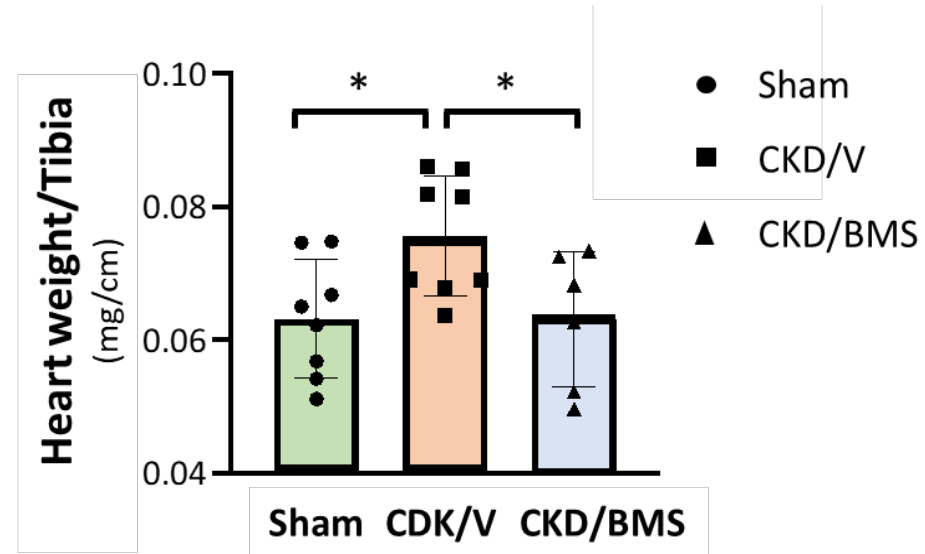
- i. Blood Pressure
- ii. Heart Size
- iii. Masson Staining for Fibrotic Markers



Sham

CKD

CKD/BMS



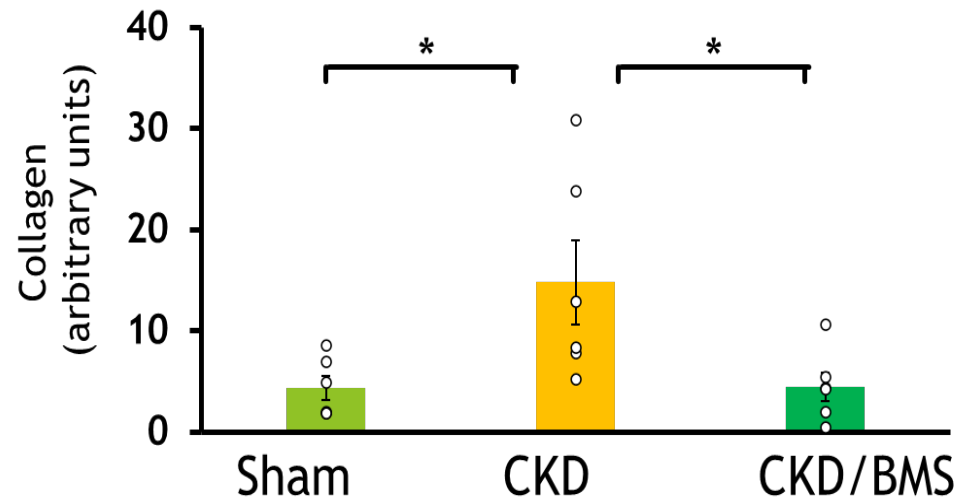
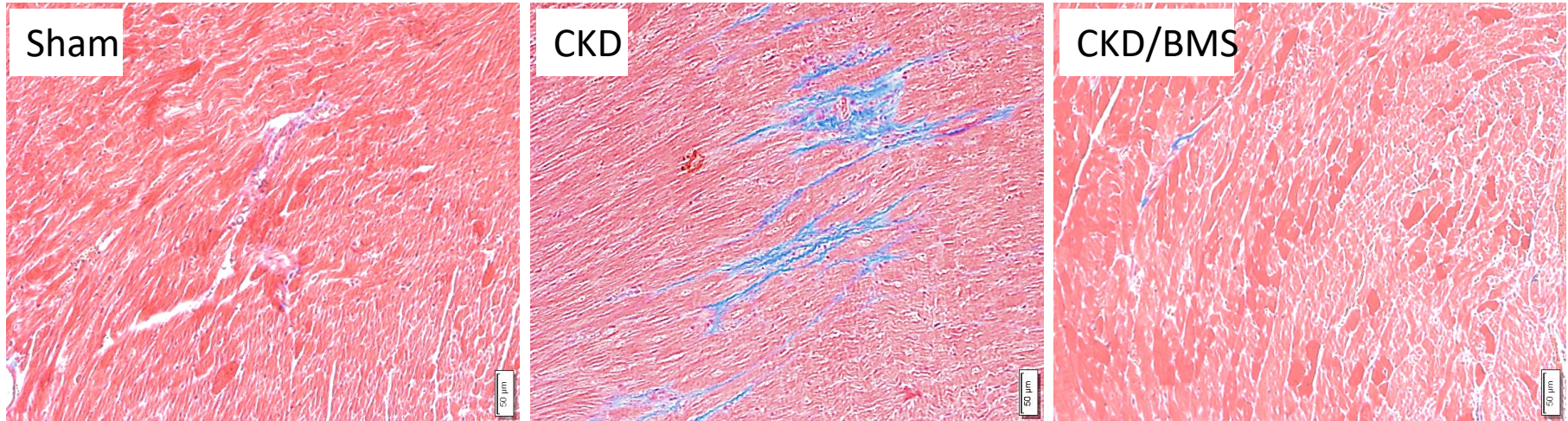
# Activation of BK $\alpha$ attenuated CKD-induced cardiac fibrosis

## A. Molecular

- i. Protein Expression Level
- ii. mRNA Expression Level

## B. Tissue

- i. Blood Pressure
- ii. Heart Size
- iii. Masson Staining for Fibrotic Markers



## 2. In-vitro Experiments (H9C2)

A. Western Blots

B. Peroxidase

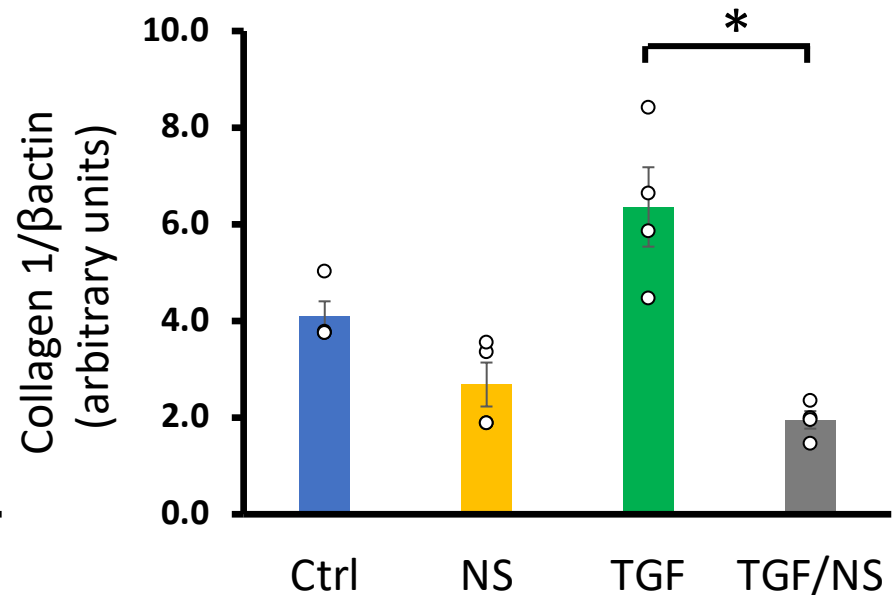
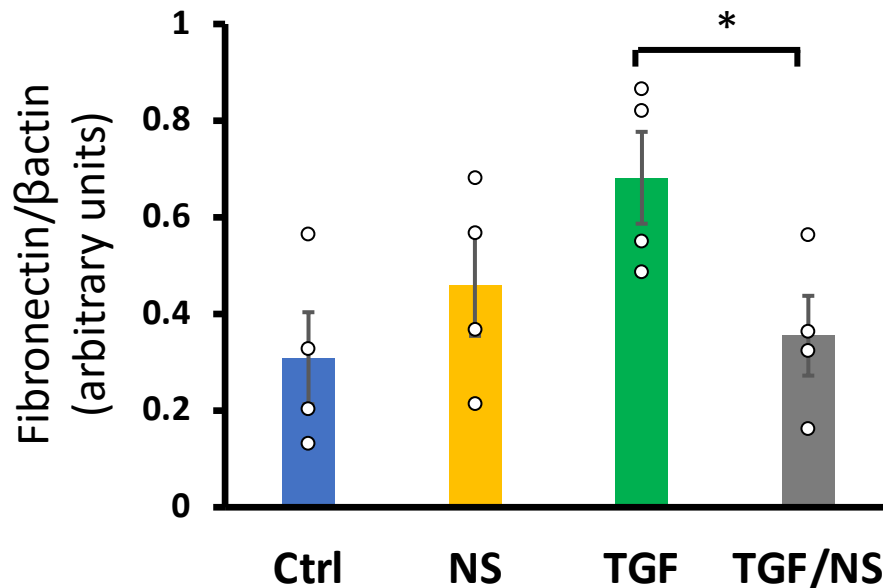
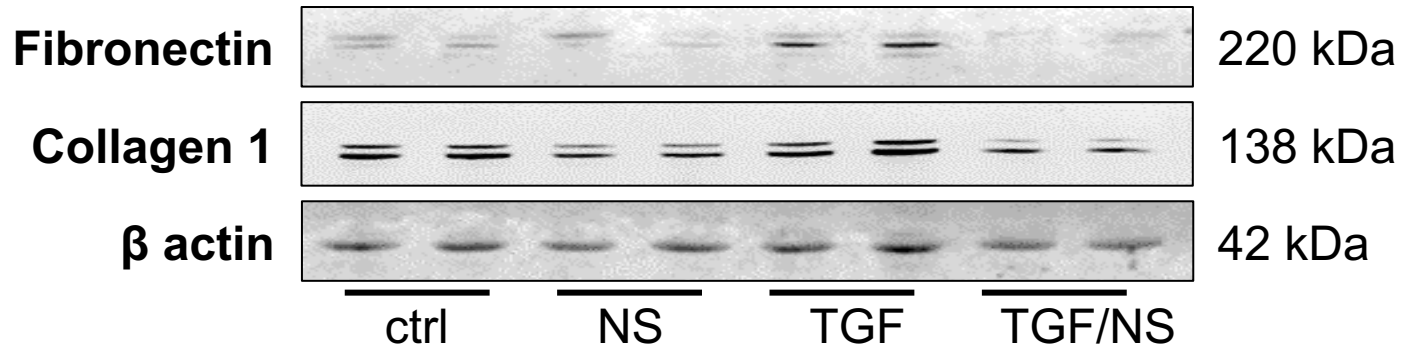
A.  $\text{H}_2\text{O}_2$

B. ROS

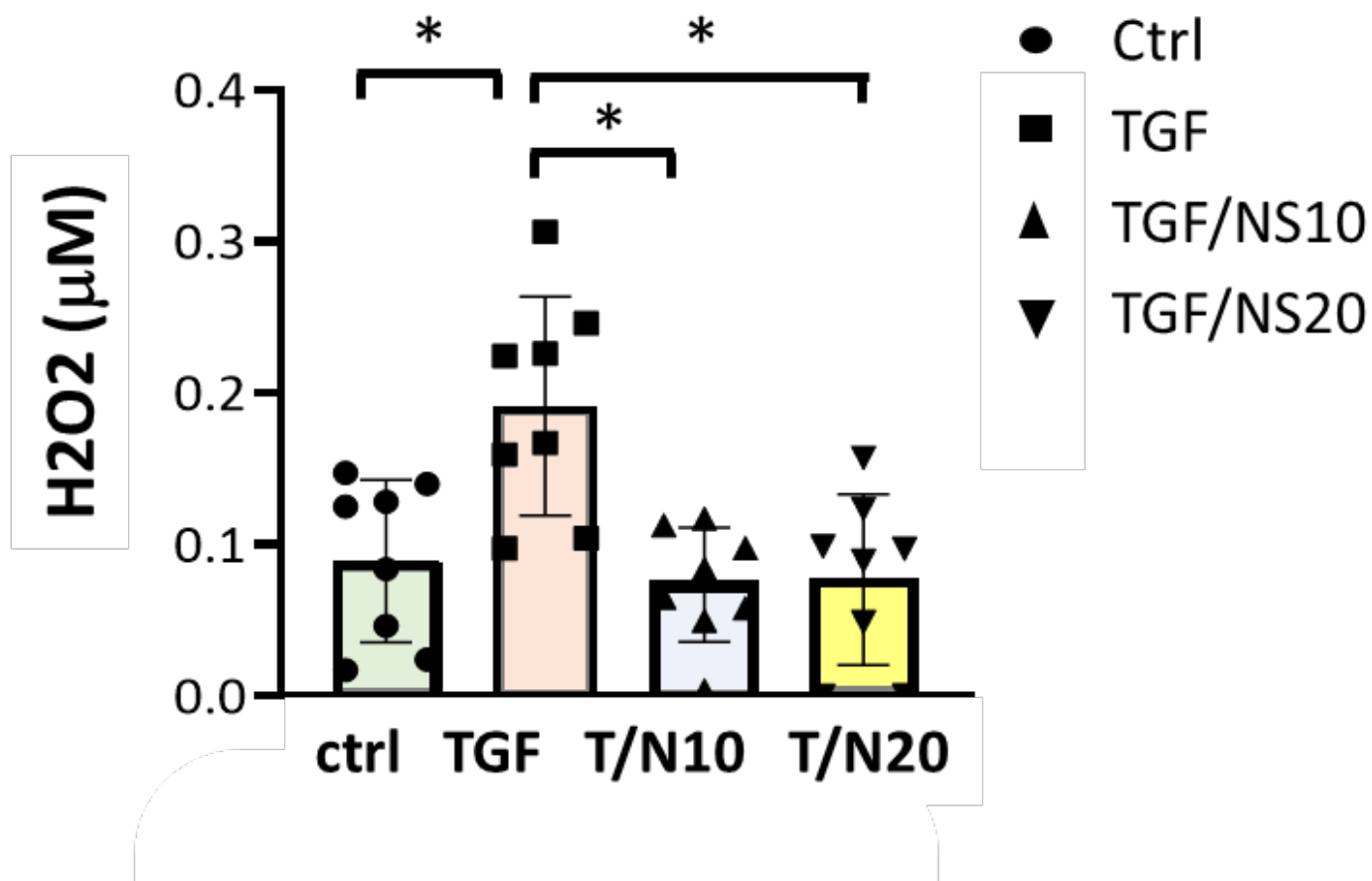
C. SOD

# BK $\alpha$ openers attenuated TGF-induced upregulation of fibrotic proteins in cardiac myoblasts

➔ A. Western Blots  
B. Peroxidase  
A. H<sub>2</sub>O<sub>2</sub>  
B. ROS  
C. SOD



# Activation of $\text{BK}\alpha$ prevented TGF-induced upregulation of $\text{H}_2\text{O}_2$





A. Western Blots

B. Peroxidase

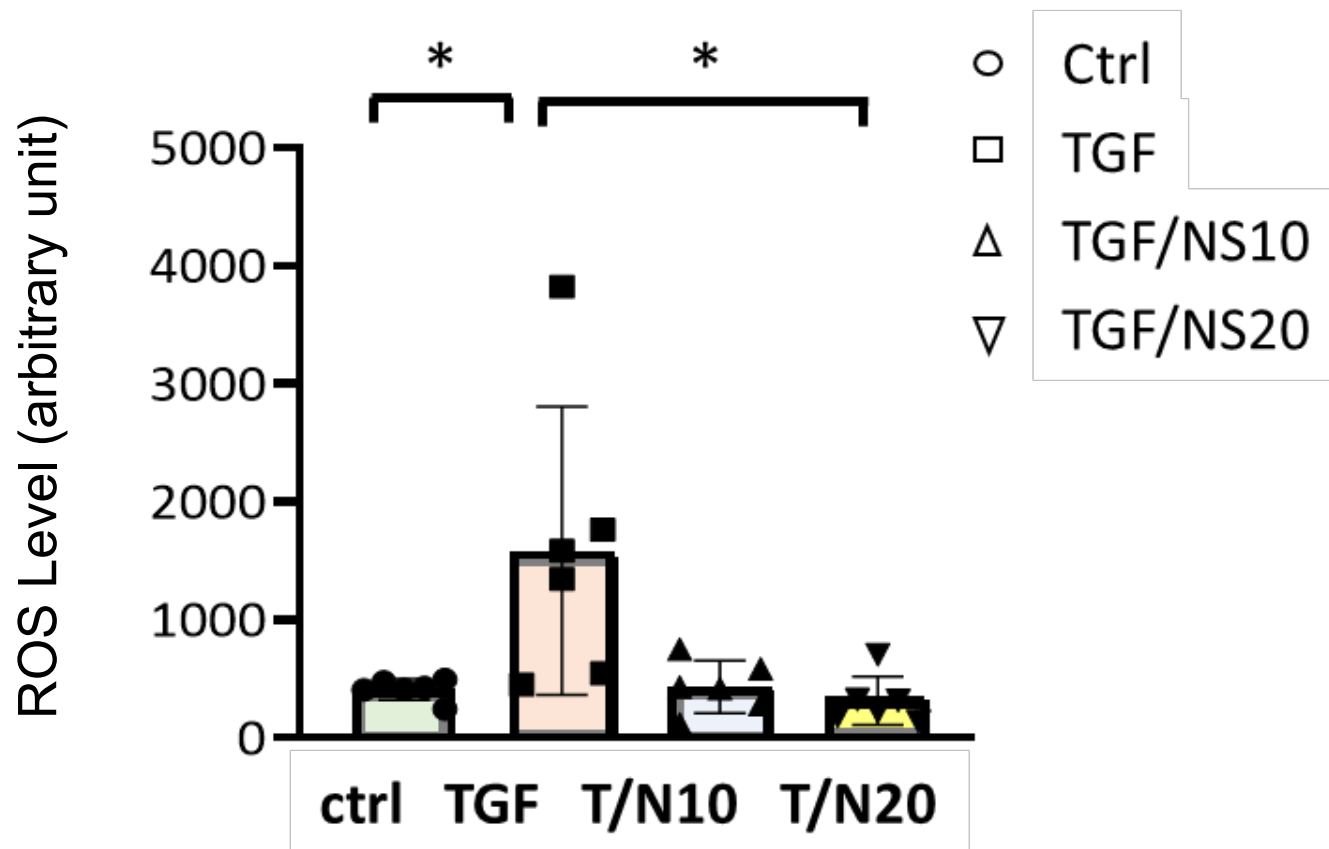
A.  $H_2O_2$

B. ROS

C. SOD



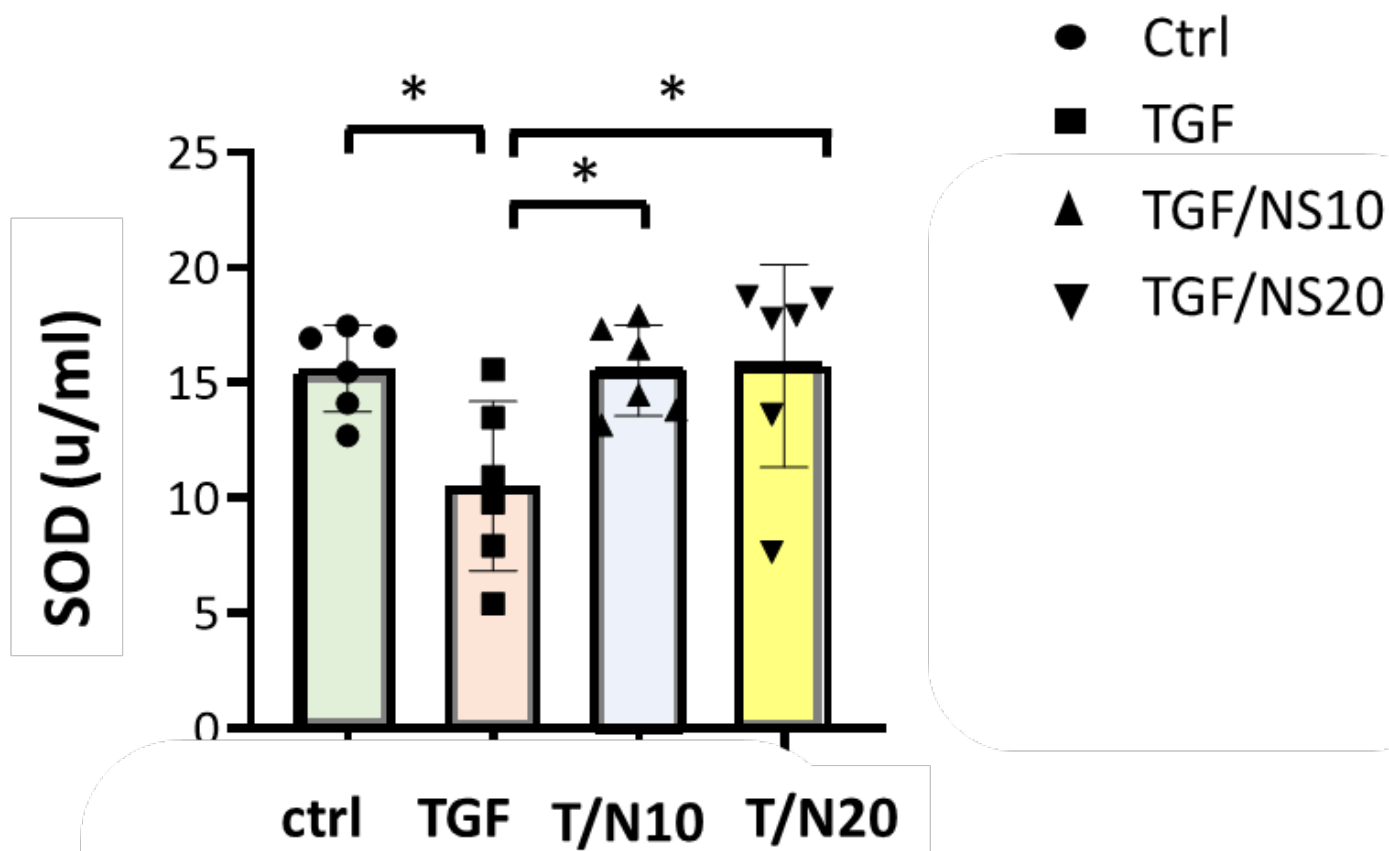
# Activation of $BK\alpha$ suppresses TGF-induced upregulation of reactive oxygen species (ROS)







# Activation of $\text{BK}\alpha$ restored TGF-induced down-regulation of Superoxide Dismutase (SOD)



# Conclusion

- BK $\alpha$  was down-regulated in hearts of CKD mice.
- Human uremic serum inhibited BK $\alpha$  activity.
- Activation of BK $\alpha$  channel attenuated TGF $\beta$ -induced upregulation of H<sub>2</sub>O<sub>2</sub> and ROS production in vitro.
- BK $\alpha$  activation restored the TGF  $\beta$ -induced down-regulation of Superoxide Dismutase (SOD).
- Our results provided a novel evidence on the protected effects of BK $\alpha$  activation on uremic cardiomyopathy by suppressing oxidative stress.

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