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## Introduction

Mutations in **Uromodulin** cause autosomal dominant tubulo-interstitial kidney disease (ADTKD-UMOD).

- Heterozygous mutations in **Uromodulin (UMOD)** cause autosomal dominant tubulo-interstitial kidney disease (ADTKD-UMOD) (Hart TC et al, J Med Genet 39:882, 2002).
- ADTKD is the second most common genetic cause for end-stage renal disease (ESRD) (Gast C et al, BMC Nephrol 19: 301,2018).
- ADTKD is characterized by ESRD at the age of 40-50 years, hyperuricemia, gout, urinary concentration defect, and salt wasting.

Fig. 1. In ADTKD-UMOD histology shows focal patchy tubular atrophy, interstitial fibrosis, and interstitial inflammation (Nasr et al, Kidney Int 73:971, 2008).

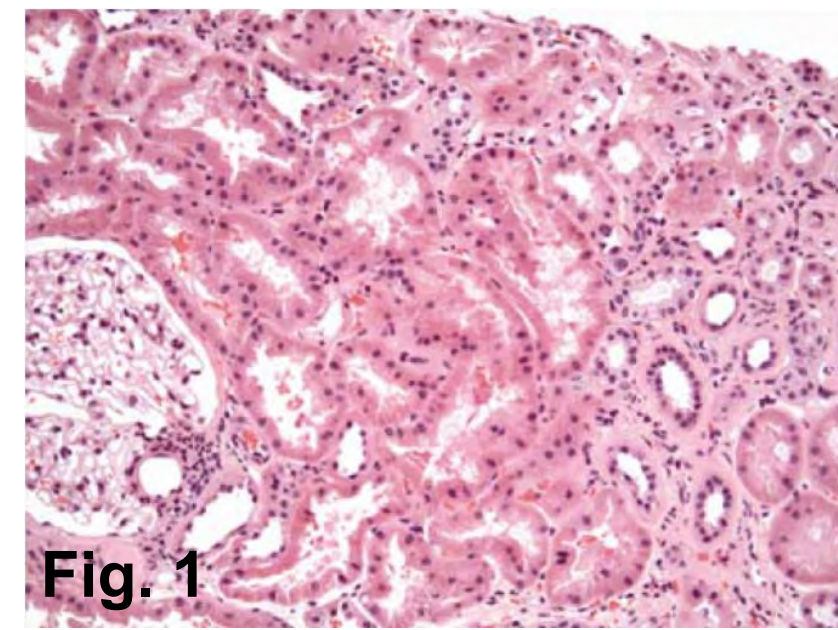


Fig. 1

- This condition was previously also known as medullary cystic kidney disease type 2, familial juvenile hyperuricemic nephropathy, or glomerulocystic kidney disease (Rampoldi L et al, Hum Mol Genet 12:3369, 2003).

Fig. 2. Cellular processing of UMOD. UMOD is a transmembrane protein which is cleaved from the apical membrane by the enzyme hepsin and forms polymers in urine.

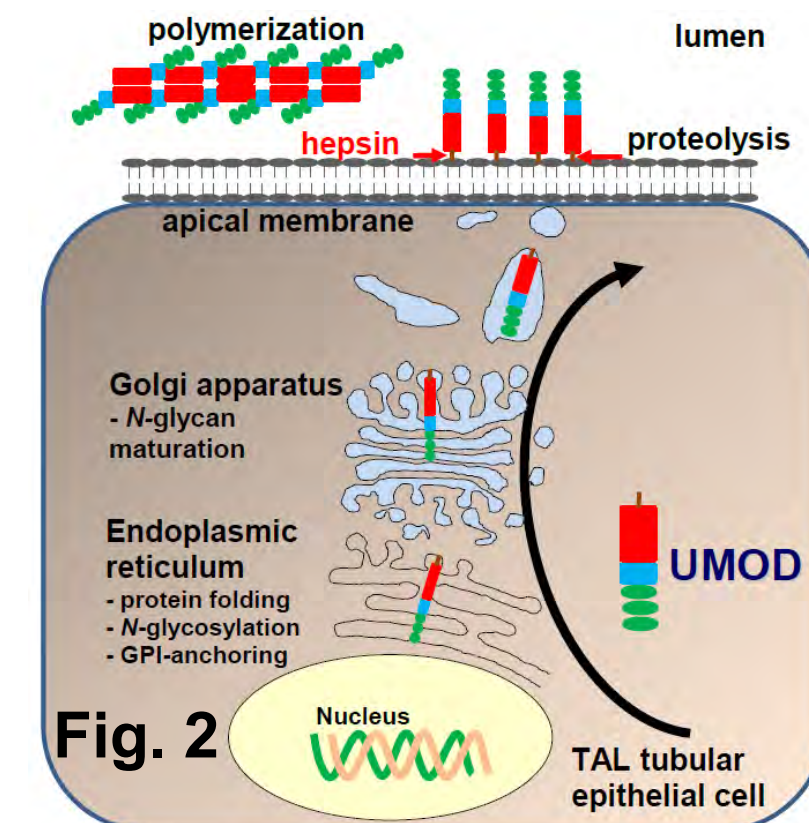


Fig. 2

- **UMOD** mutations result in protein misfolding. The abnormal protein accumulates in the endoplasmic reticulum and causes cellular apoptosis.
- So far no specific therapies are available for ADTKD-UMOD.

**Klotho is a transmembrane protein highly expressed in the DCT and alleviates different forms of nephropathies**

- Klotho is a transmembrane protein highly expressed in the distal convoluted tubule and functions as an anti-aging hormone.
- Klotho functions as a co-receptor with FGFR1c and reduces so tubular phosphate absorption.
- Klotho regulates different signaling pathways (e.g. insulin/IGF1, Wnt, TGFβ).
- Klothos interferes with apoptosis, fibrosis, senescence, and stimulates autophagy.
- Klotho is known to improve acute kidney injury, hypertension, chronic kidney disease, vascular calcification, proteinuria, glomerulopathy, obstructive uropathy, and renal fibrosis in mouse models.
- Very little information is known about the effect of Klotho on tubulopathies.

## Aims

1. Does overexpression of Klotho alleviate the progression of ADTKD-UMOD?
2. If so, what is the mechanism how Klotho may improve ADTKD-UMOD outcome?

## Methods

- We utilized the **Umod<sup>C93F</sup>** mutant mouse and used a homozygous model to produce a stronger phenotype (Kemter E et al, Hum Mol Genet 22:4148, 2013).
- We crossed the **Umod<sup>C93F/C93F</sup>** mouse on a transgenic Klotho mouse (TgKI).
- We studied wild-type (WT), TgKI, **Umod<sup>C93F/C93F</sup>**, and **Umod<sup>C93F/C93F</sup> xTgKI** at 6 and 13 months.

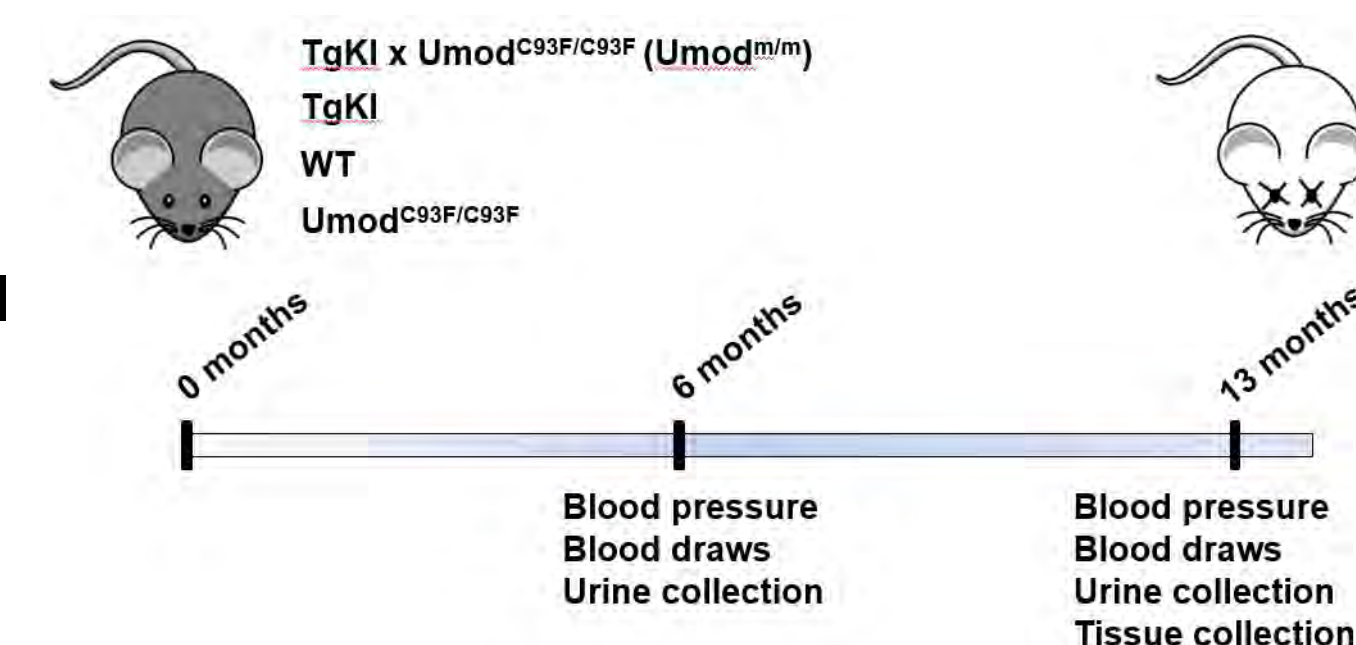


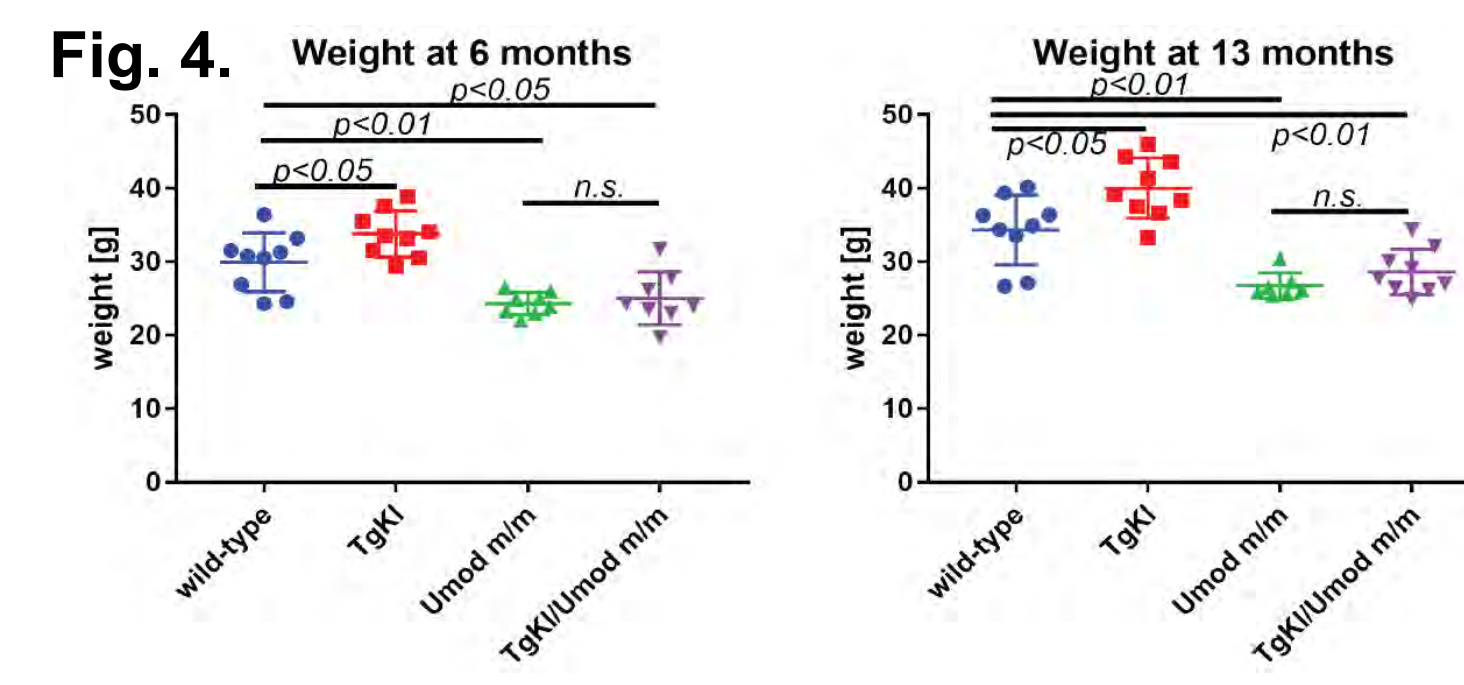
Fig. 3. Study design for the analysis of **Umod<sup>C93F/C93F</sup> xTgKI** mice.

- Animals were assessed for weight and blood pressures.
- Biomarkers of renal function and chronic kidney disease were analyzed.
- Urinary UMOD secretion was tested.
- Quantitative analysis of renal and cardiac mRNA expression was studied.
- To identify the mechanism of improved outcome in **Umod<sup>C93F/C93F</sup> xTgKI** mice an unbiased proteomics approach was performed.

## Results

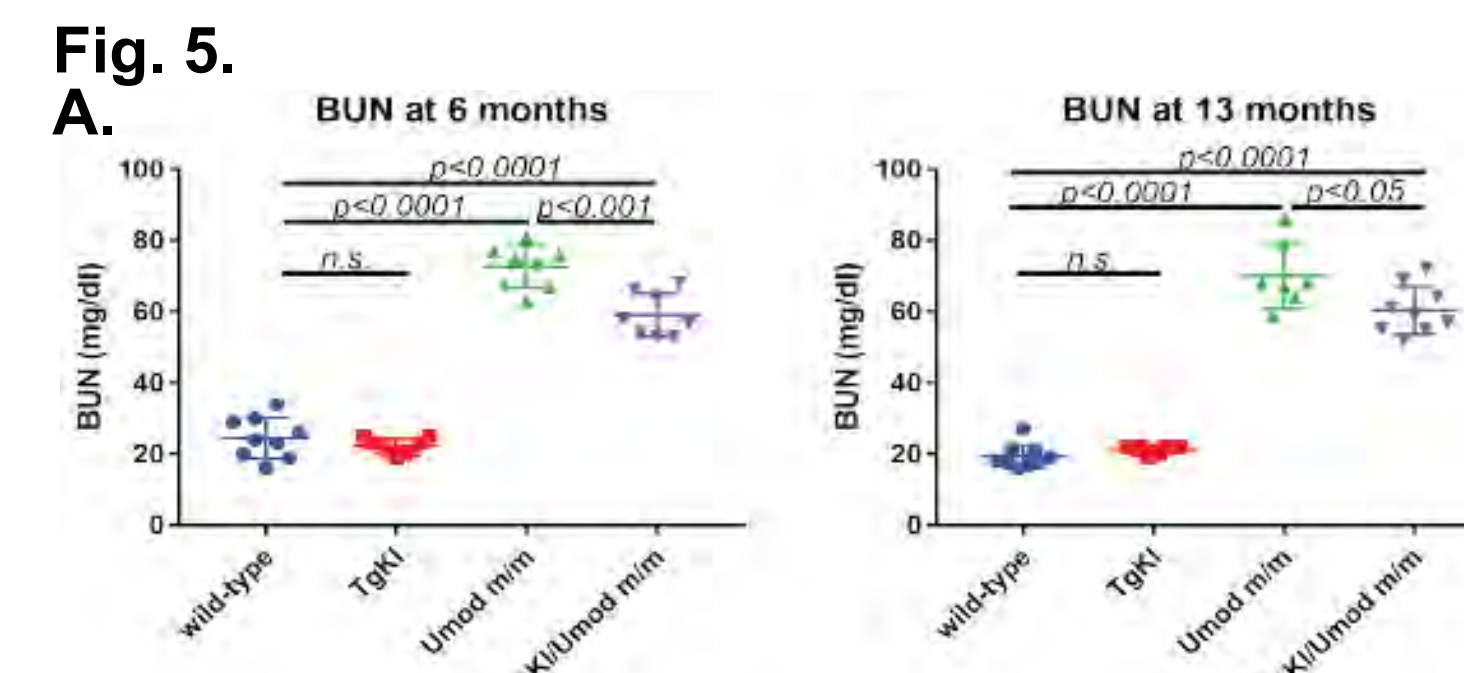
**Does Klotho overexpression increase body weight in Umod<sup>C93F/C93F</sup> mice?**

- 1) At 6 and 13 months TgKI mice had mildly higher body weight compared to WT mice. **Umod<sup>C93F/C93F</sup>** had lower weight than WT or TgKI mice. Klotho overexpression did not increase body weight of **Umod<sup>C93F/C93F</sup>** mice.

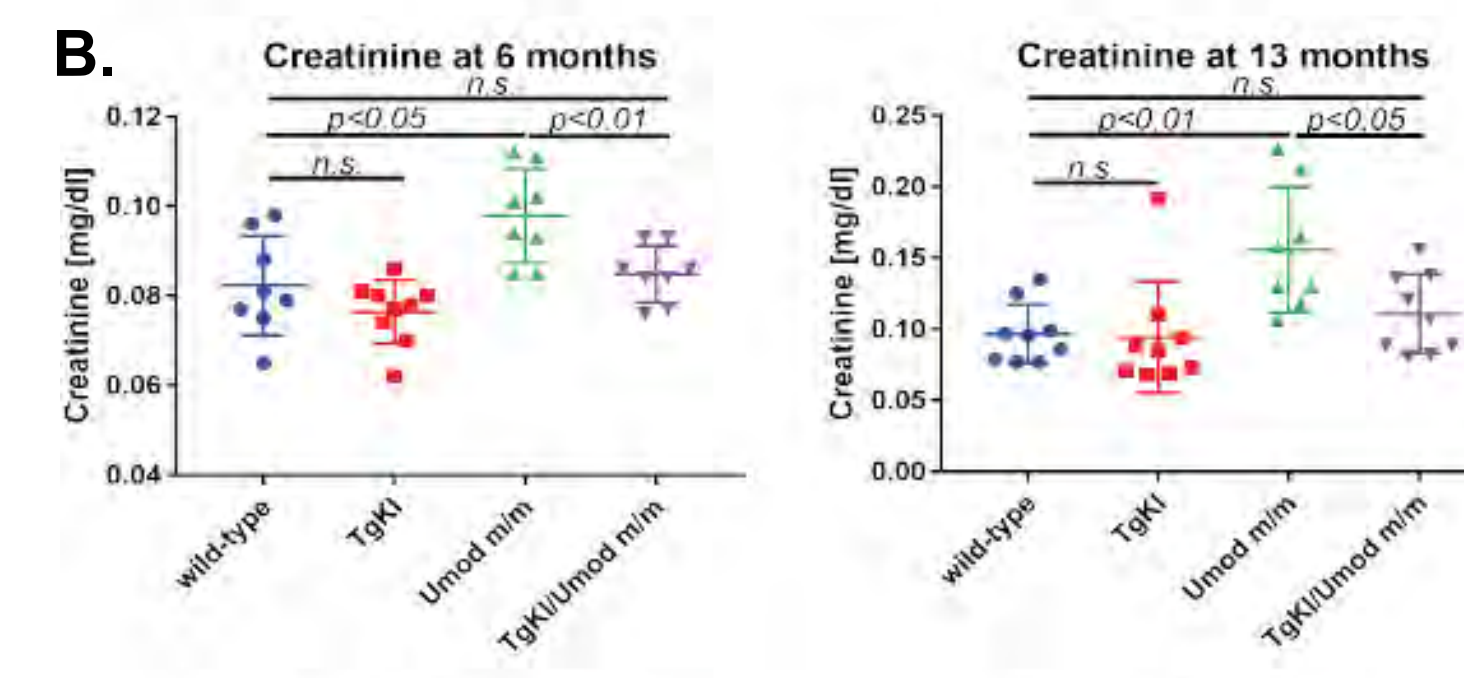


**Does Klotho overexpression affect renal function and progression of kidney disease in Umod<sup>C93F/C93F</sup> mice?**

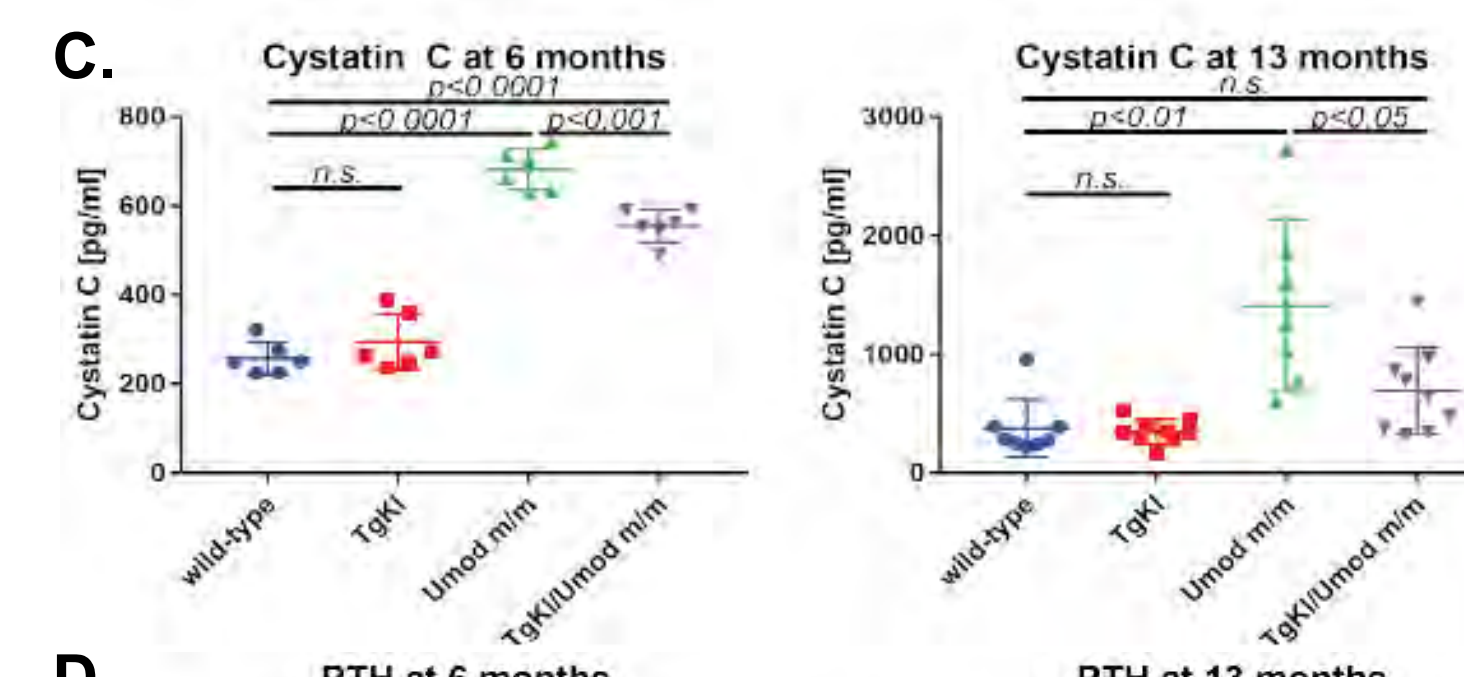
- 1) At 6 and 13 months TgKI/**Umod<sup>C93F/C93F</sup>** mice had significantly lower BUN, creatinine, and cystatin C values compared to **Umod<sup>C93F/C93F</sup>** mice.



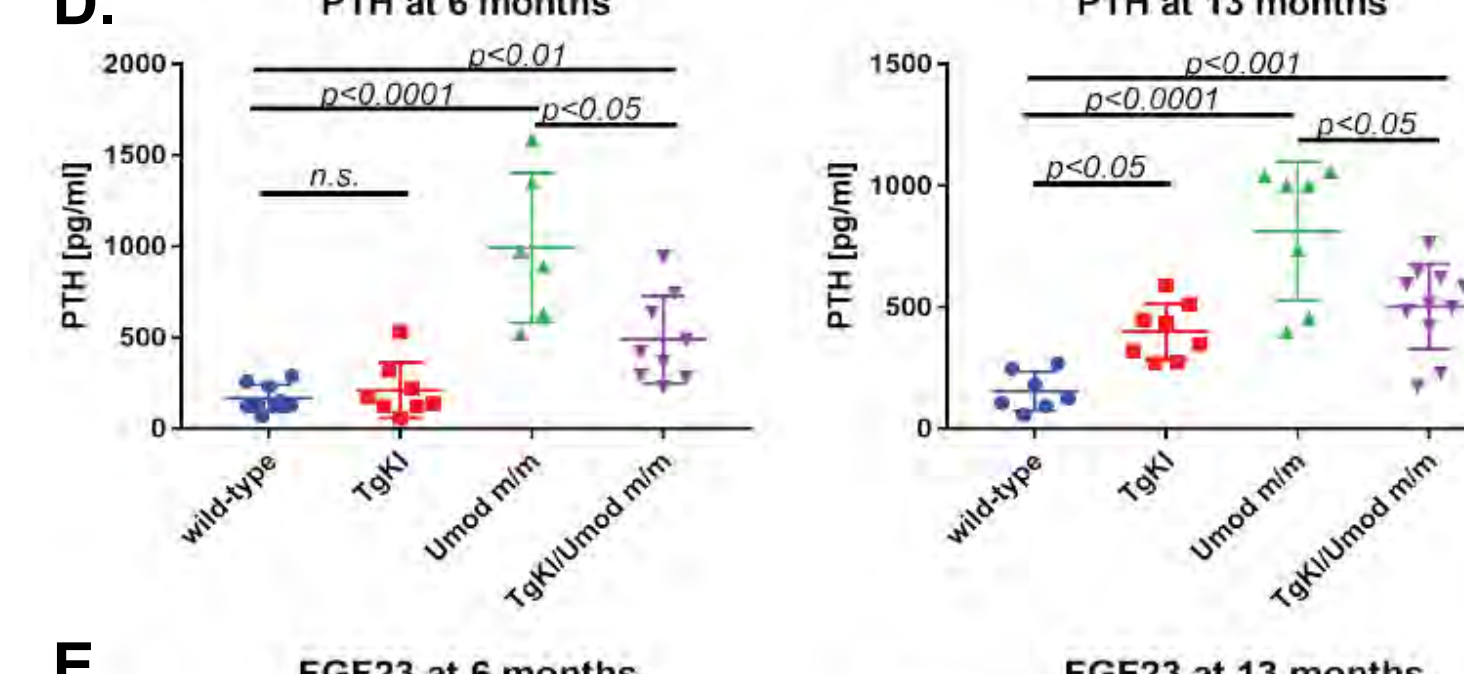
- 2) BUN, creatinine, and cystatin C were between 15-30% lower in the TgKI/**Umod<sup>C93F/C93F</sup>** group.



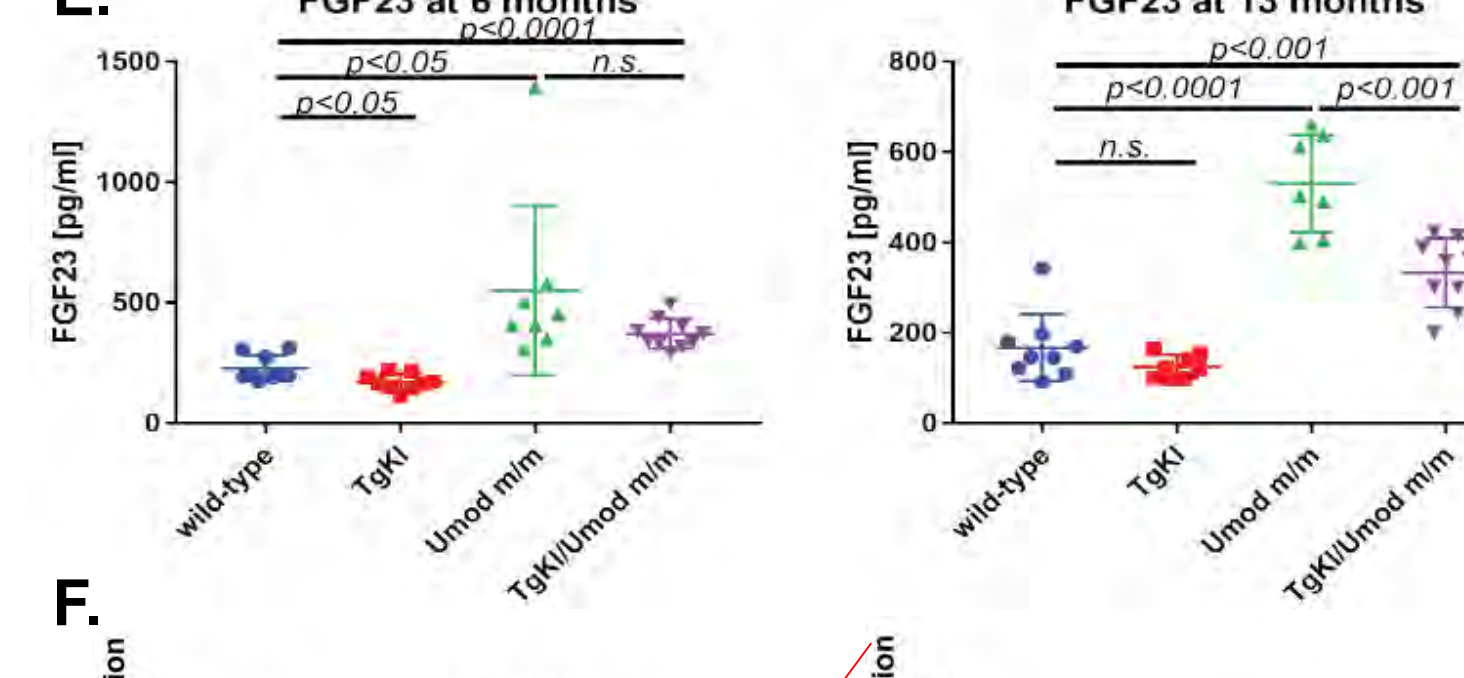
- 3) At 6 and 13 months TgKI/**Umod<sup>C93F/C93F</sup>** mice had significantly lower PTH values compared to **Umod<sup>C93F/C93F</sup>** mice.



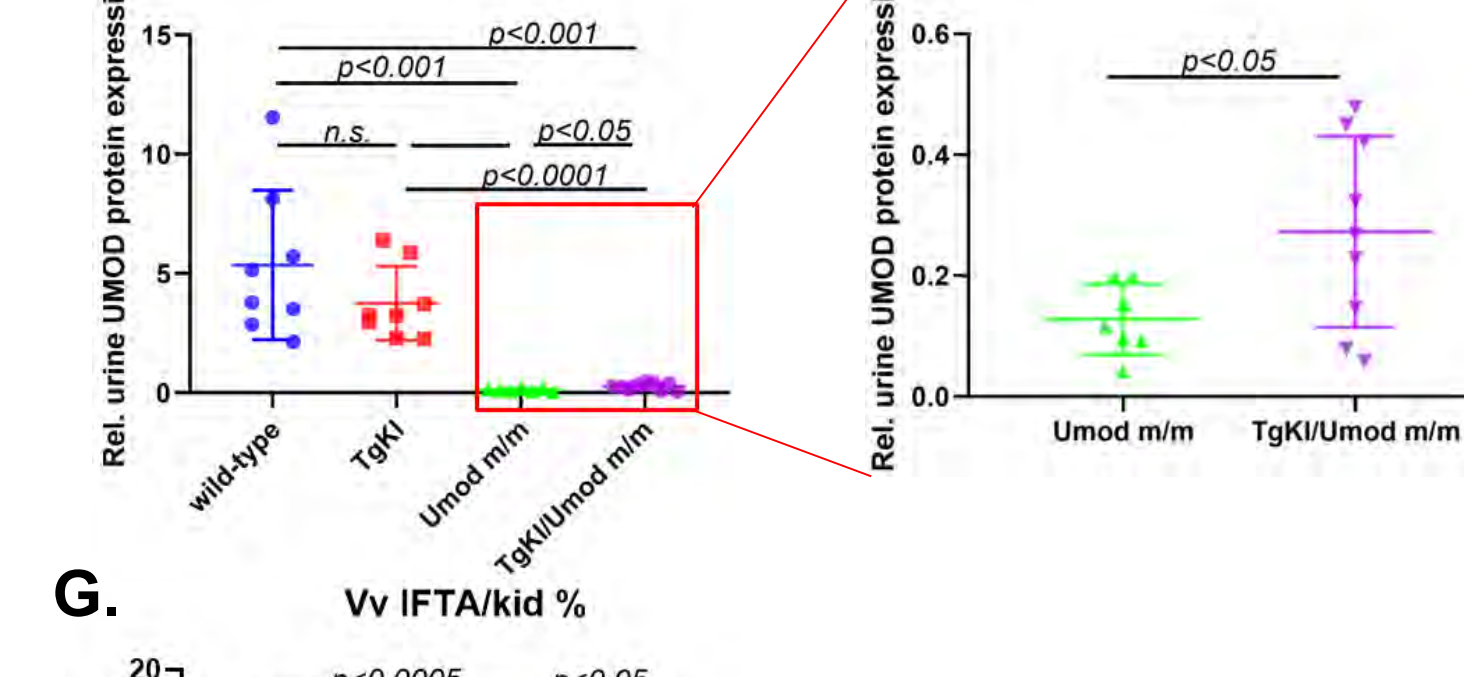
- 4) At 13 months TgKI/**Umod<sup>C93F/C93F</sup>** mice had significantly lower FGF23 values compared to **Umod<sup>C93F/C93F</sup>** mice.



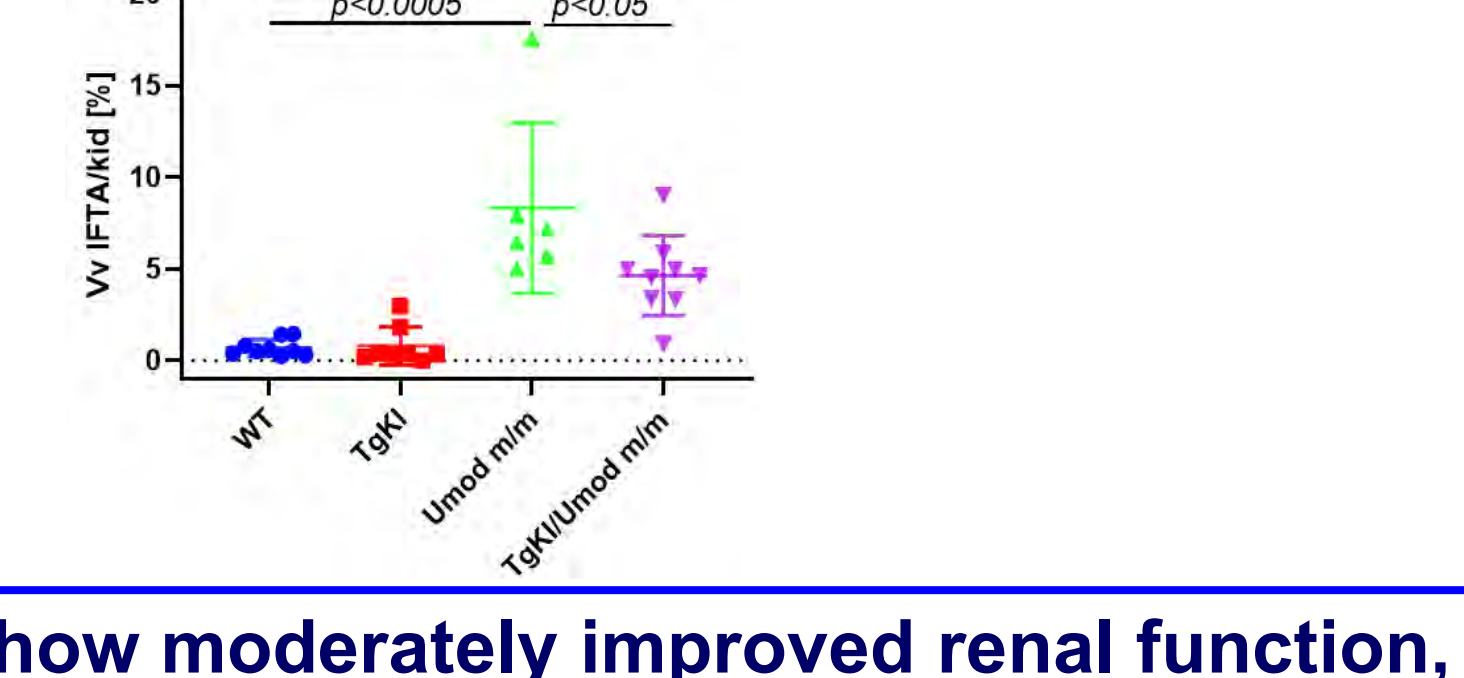
- 5) PTH and FGF23 were approximately 40% lower in the TgKI/**Umod<sup>C93F/C93F</sup>** group.



- 6) Consistent with cellular retention of mutant UMOD in **Umod<sup>C93F/C93F</sup>** mice, these animals secret significantly less urinary UMOD compared to WT and TgKI mice (left). TgKI/**Umod<sup>C93F/C93F</sup>** mice secret more urinary UMOD than **Umod<sup>C93F/C93F</sup>** mice (inlet, right). However, compared to WT and TgKI, TgKI/**Umod<sup>C93F/C93F</sup>** mice secreted relatively low urinary UMOD levels.



- 7) TgKI/**Umod<sup>C93F/C93F</sup>** mice have less interstitial fibrosis and tubular atrophy (Vv IFTA/kid %) than **Umod<sup>C93F/C93F</sup>** mice.

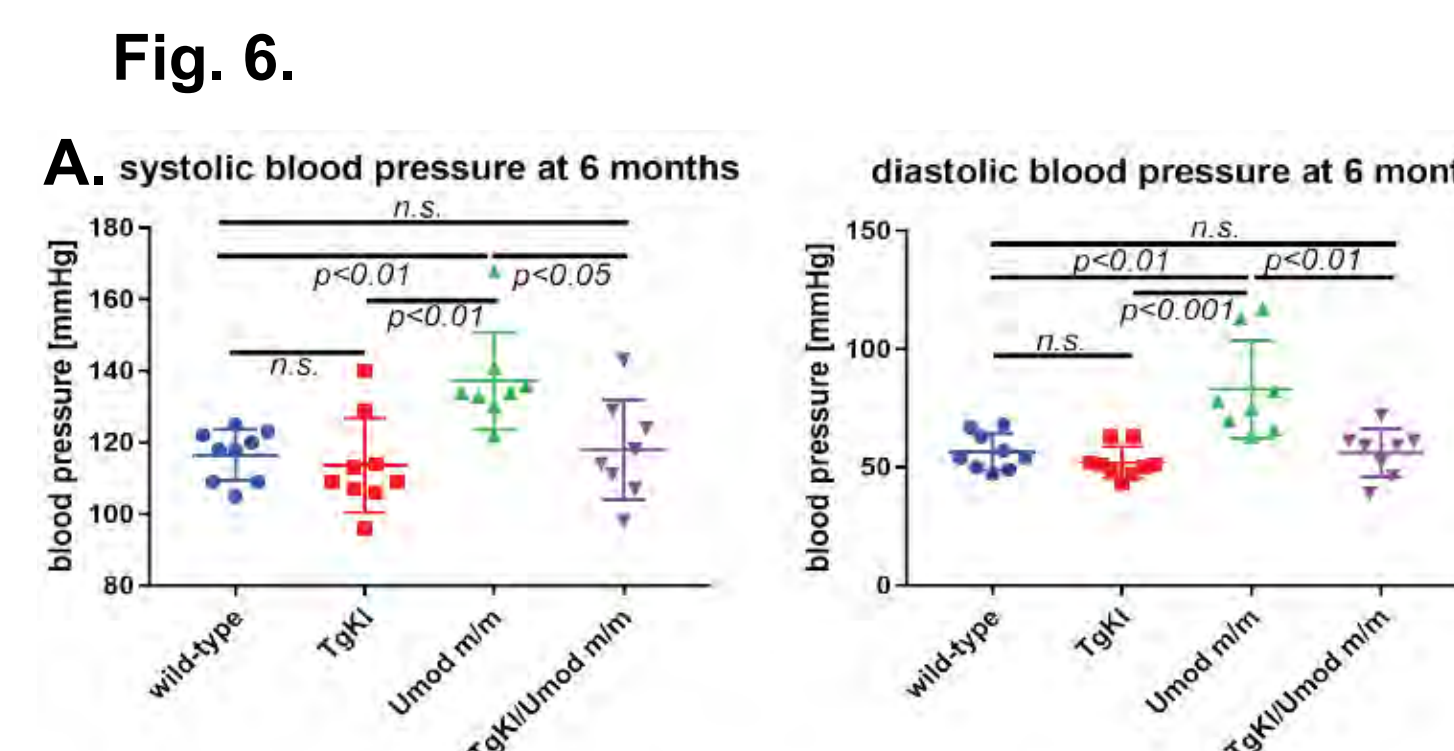


**TgKI/**Umod<sup>C93F/C93F</sup>** mice show moderately improved renal function, renal fibrosis, urinary UMOD secretion, and CKD progression.**

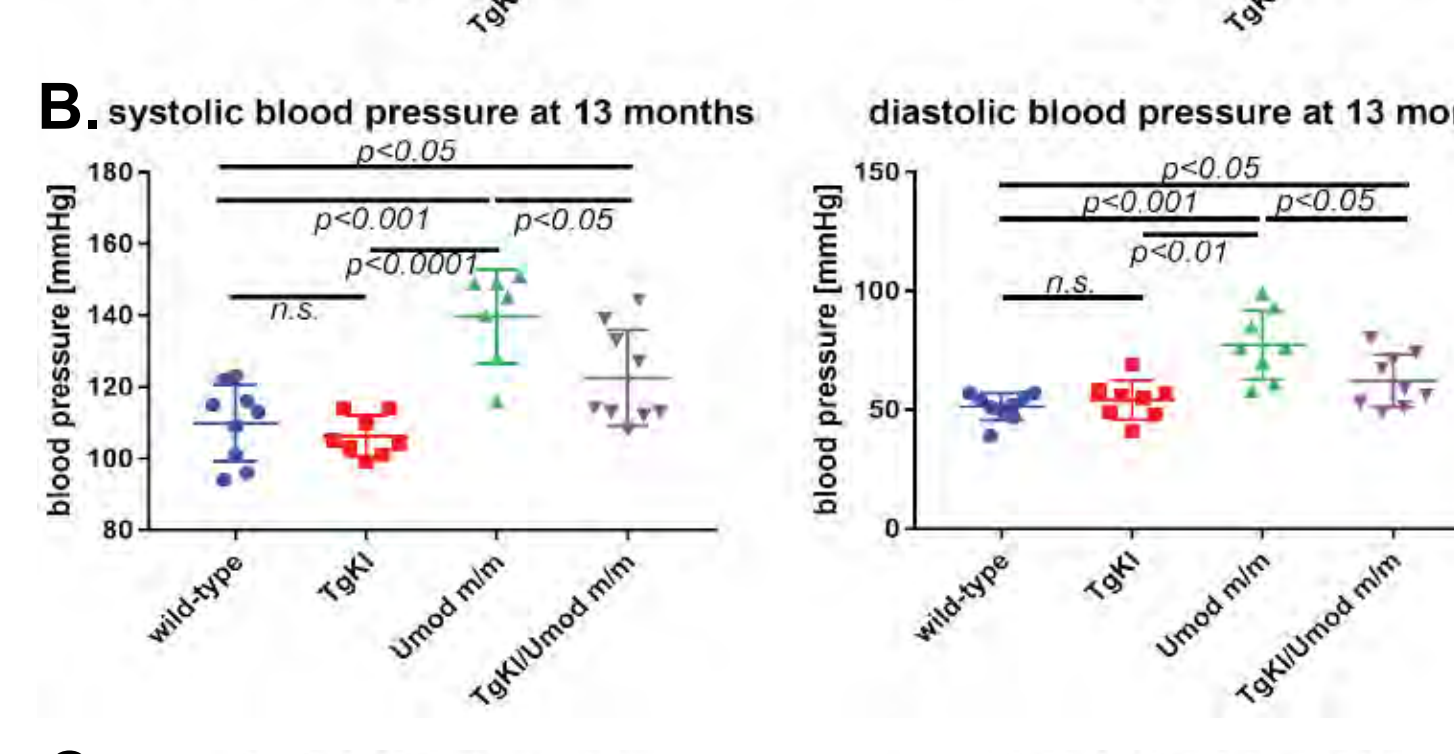
**Does Klotho overexpression improve blood pressures and CKD-related heart disease in Umod<sup>C93F/C93F</sup> mice?**

- Klotho is known to provide cardioprotection in animal models of chronic kidney disease (Xie J et al, Nat Commun 3:1238, 2012). Therefore, we asked if Klotho overexpression would result in lower blood pressures in TgKI/**Umod<sup>C93F/C93F</sup>** mice.

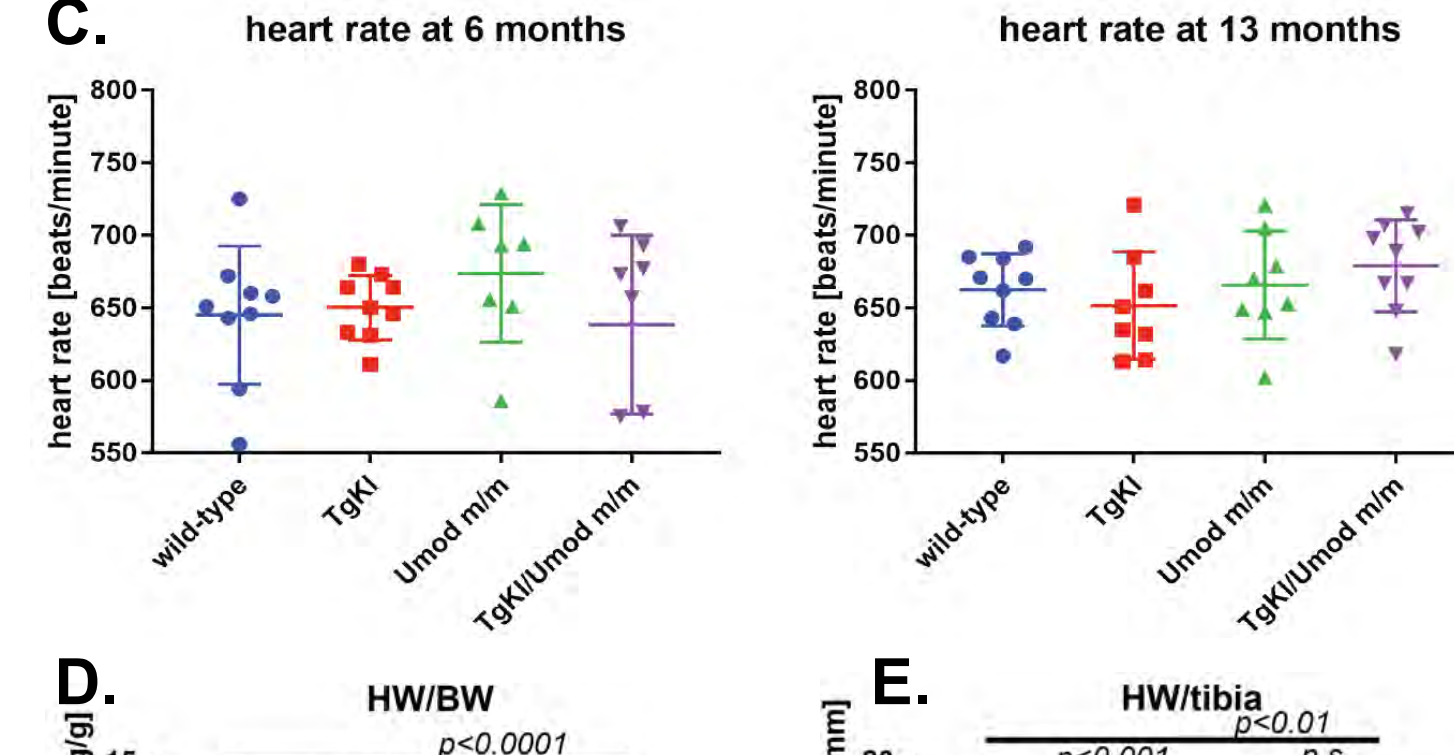
- 1) At 6 and 13 months TgKI/**Umod<sup>C93F/C93F</sup>** mice had significantly lower systolic and diastolic blood pressures compared to **Umod<sup>C93F/C93F</sup>** mice.



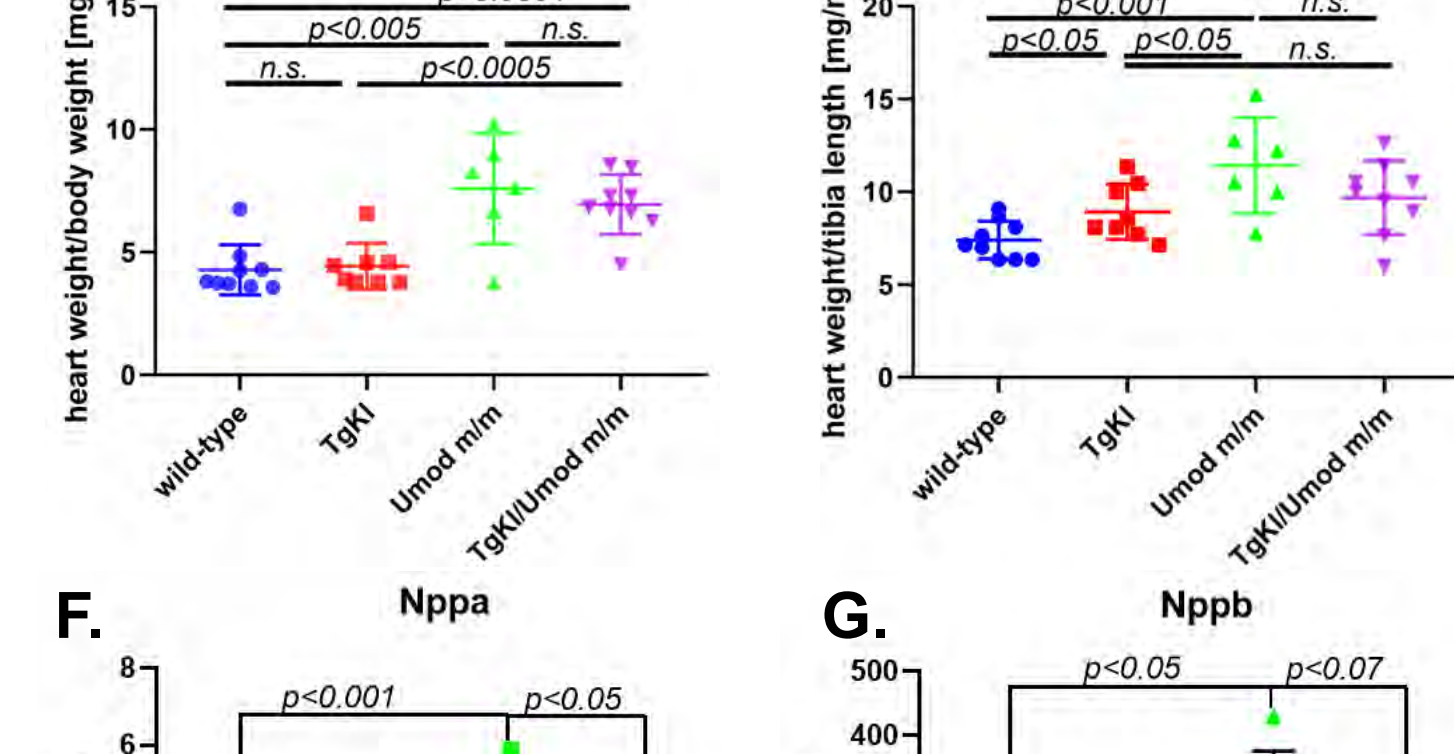
- 2) No effect of Klotho overexpression was seen on heart rate (Fig. 5C).



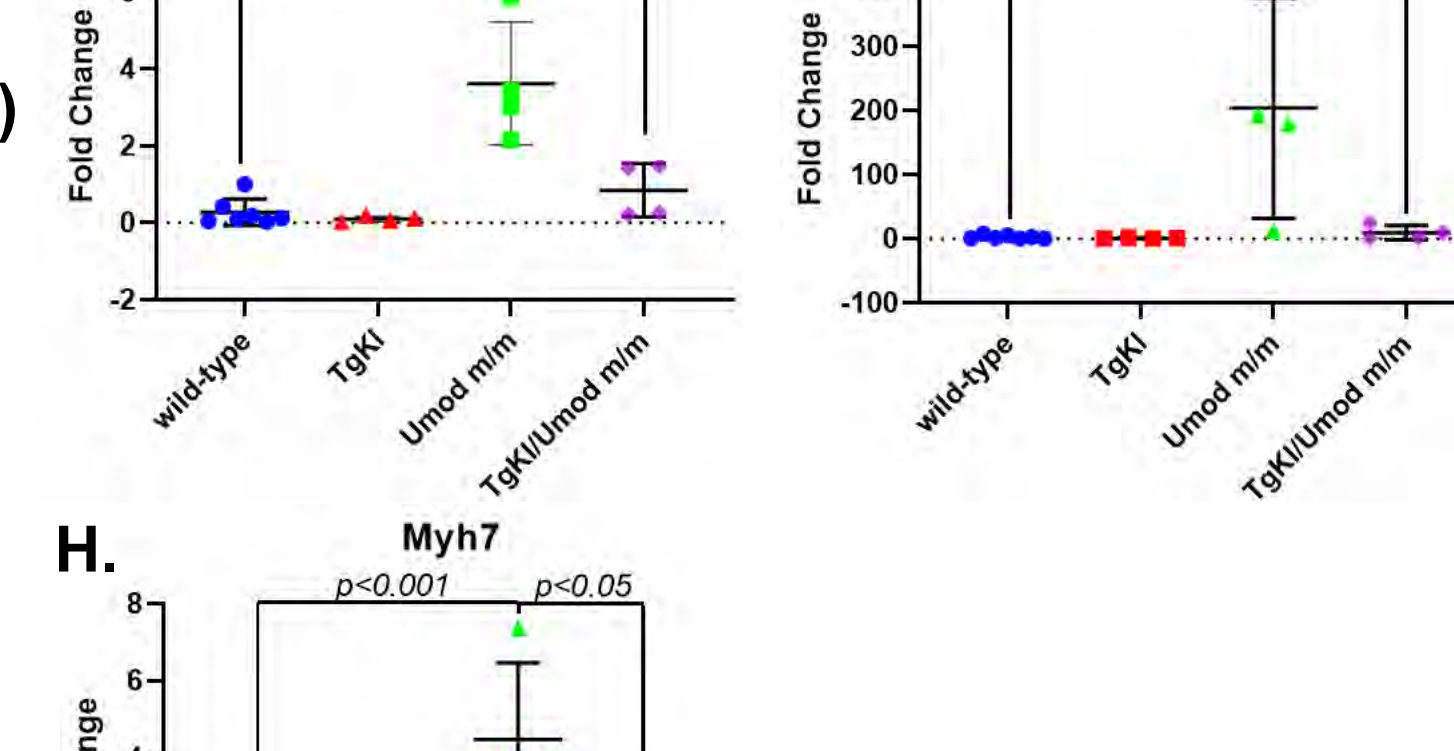
- 3) **Umod<sup>C93F/C93F</sup>** mice had significantly higher heart weight/body weight (HW/BW) ratio (D) and HW/tibia ratio (E) but no effect was seen with Klotho overexpression.



- 4) Expression of mRNA biomarkers for cardiac hypertrophy (Nppa encodes ANP) (F), (Nppb encodes BNP) (G), and (Myh7 encodes Myosin heavy chain 7) (H) were significantly lower in TgKI/**Umod<sup>C93F/C93F</sup>** compared to **Umod<sup>C93F/C93F</sup>** mice.



- 5) Myh7 mRNA expression was significantly lower in TgKI/**Umod<sup>C93F/C93F</sup>** compared to **Umod<sup>C93F/C93F</sup>** mice.



**Klotho overexpression improves systolic and diastolic blood pressures and mRNA expression of genes involved in cardiac hypertrophy but not heart/body weight or heart weight/tibia ratio.**

**What is the mechanism that Klotho overexpression improves ADTKD-UMOD?**

To identify the mechanism for better renal and cardiovascular outcome in TgKI/**Umod<sup>C93F/C93F</sup>** mice, we performed an unbiased proteomics approach applying LC-MS/MS using renal medulla from kidneys from 3 months old mice.

- 1) Analyzing protein expression we observed three distinct protein clusters with 1. WT and TgKI mice (shown in purple and blue as WT and KI), 2. for **Umod<sup>C93F/C93F</sup>** mice (shown in red as HST001), and 3. for TgKI/**Umod<sup>C93F/C93F</sup>** mice (green as HST001\_KI).

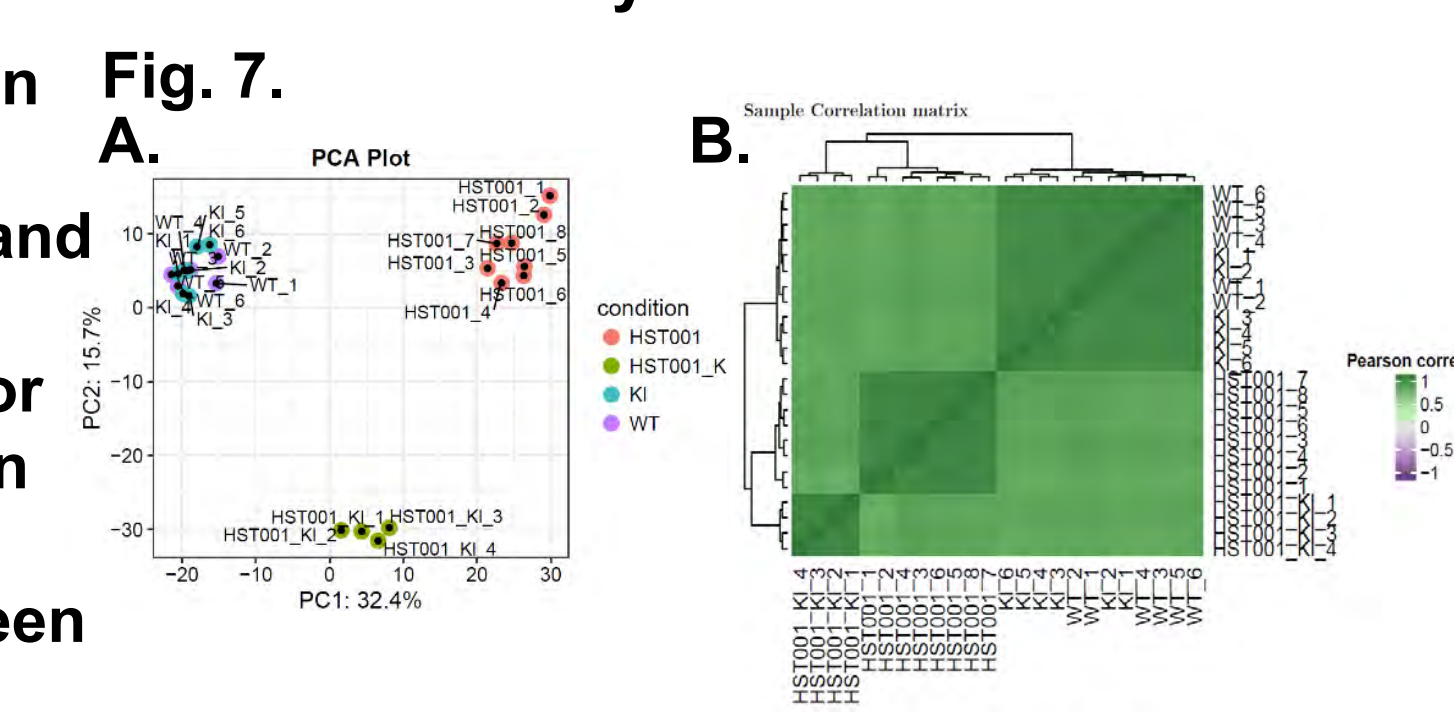


Fig. 8.

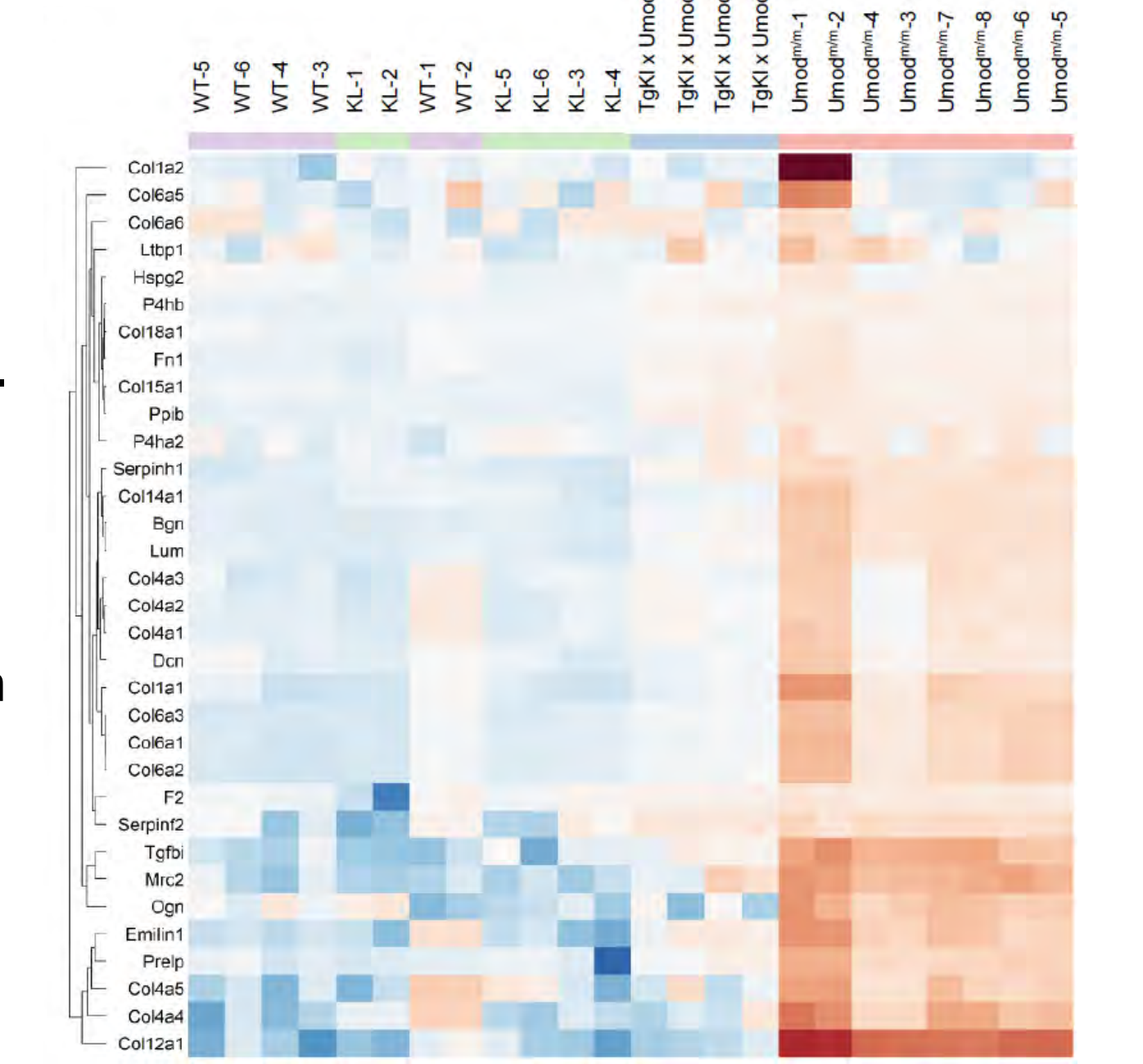
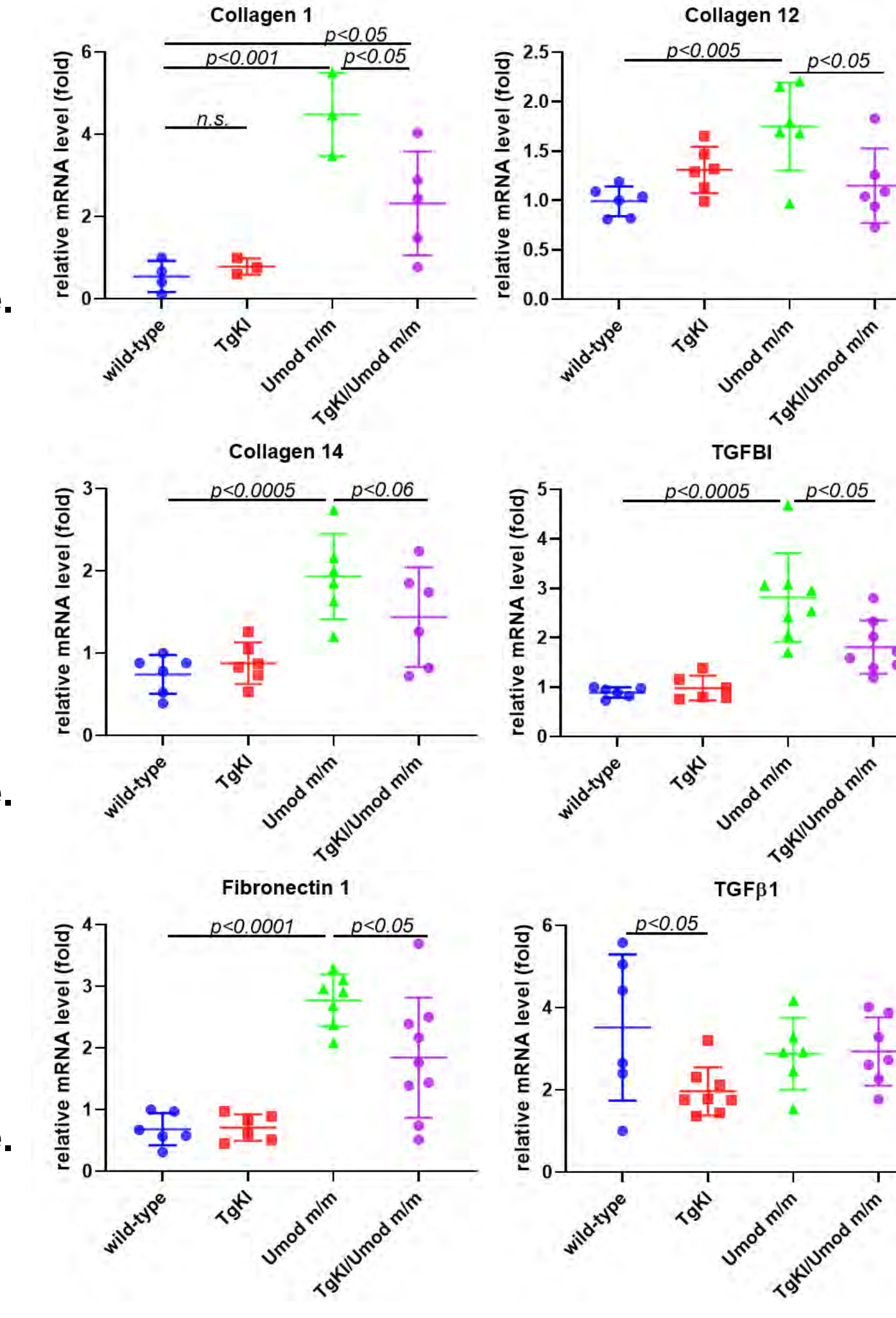


Fig. 9.



- 1) Applying an unbiased proteomics approach we identified downregulation of multiple collagens in TgKI/**Umod<sup>C93F/C93F</sup>** mice compared to **Umod<sup>C93F/C93F</sup>** mice.

- 2) Other downregulated proteins in TgKI/**Umod<sup>C93F/C93F</sup>** mice included interaction partners and modifiers of collagens such as Transforming growth factor-beta-induced protein (TGFBI), prolargin, biglycan, and osteoglycin/mimecan.

- Applying qPCR we confirmed downregulation of Collagen 1, Collagen 12, and Collagen 14 mRNA expression in TgKI/**Umod<sup>C93F/C93F</sup>** mice compared to **Umod<sup>C93F/C93F</sup>** mice.

- We also confirmed downregulation of Transforming growth factor-beta-induced protein (TGFBI) mRNA expression in TgKI/**Umod<sup>C93F/C93F</sup>** mice compared to **Umod<sup>C93F/C93F</sup>** mice.

- Fibronectin-1 is considered an interaction partner of TGFBI and is also downregulated in TgKI/**Umod<sup>C93F/C93F</sup>** mice compared to **Umod<sup>C93F/C93F</sup>** mice.
- In contrast to TGFBI, TGFβ1 mRNA expression was not significantly downregulated in TgKI/**Umod<sup>C93F/C93F</sup>** mice.

**Applying qPCR we confirmed downregulation of multiple collagens, TGFBI and Fibronectin 1 but not TGFβ1 in TgKI/**Umod<sup>C93F/C93F</sup>** mice.**

## Summary

1. Klotho overexpression improves progression of chronic kidney disease in TgKI/**Umod<sup>C93F/C93F</sup>** mice with improved creatinine, BUN, cystatin C, PTH, and FGF23 values.
2. Klotho overexpression reduces interstitial fibrosis and tubular atrophy in TgKI/**Umod<sup>C93F/C93F</sup>** mice.
3. Klotho overexpression increases urinary UMOD secretion.
4. Systolic and diastolic blood pressures are improved in TgKI/**Umod<sup>C93F/C93F</sup>** mice at 6 and 13 months.
5. Expression of genes involved in cardiac hypertrophy such as ANP, BNP, and MYH7 is lower in TgKI/**Umod<sup>C93F/C93F</sup>** mice compared to **Umod<sup>C93F/C93F</sup>** mice.
6. An unbiased proteomics approach shows that TgKI/**Umod<sup>C93F/C93F</sup>** mice have a lower protein expression of multiple collagens and TGFBI but not TGFβ1.
7. The lower TGFBI and collagen expression may explain the lower degree of renal fibrosis and better renal outcome in TgKI/**Umod<sup>C93F/C93F</sup>** mice.

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