Identification of new therapy for kidney disease by systems approach

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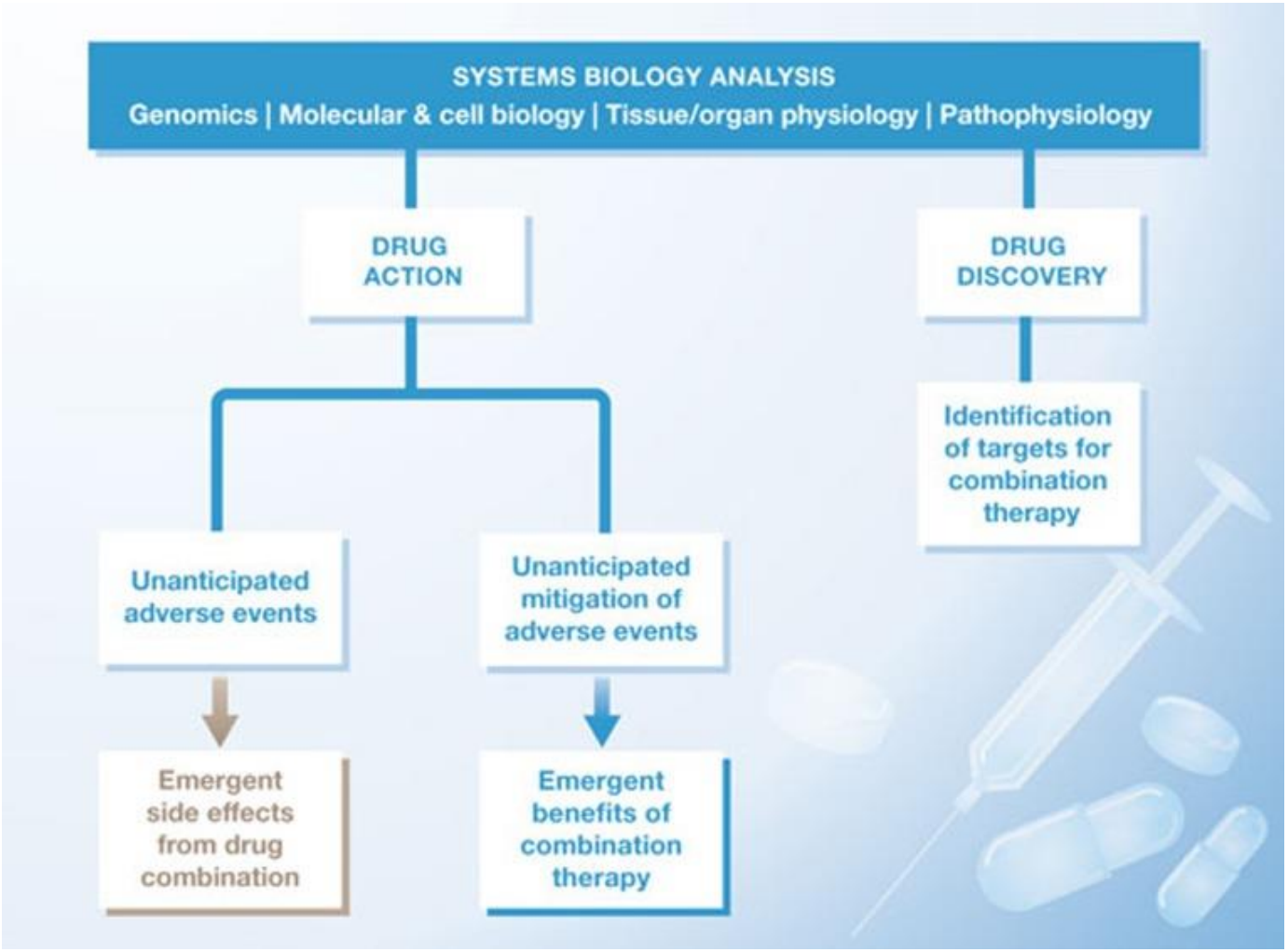
Treatment of Kidney Disease

- Steroids and Immunosuppressive therapies:
  - Significant side effects
  - Resistant and recurrent
- ACEI/ARBs:
  - Only classes of medications have renal protection.
  - Partial effect
  - Slow progression rate but not for the cure of disease
- New therapies:
  - Good data from Phase II clinical trials
  - Phase III clinical trials failed.

Complex diseases require complex therapies

Ravi Iyengar
Author Affiliations



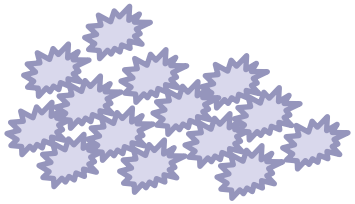
Systems Pharmacology

Systems approach to identify combinational drug therapy and drug repurposing for kidney disease

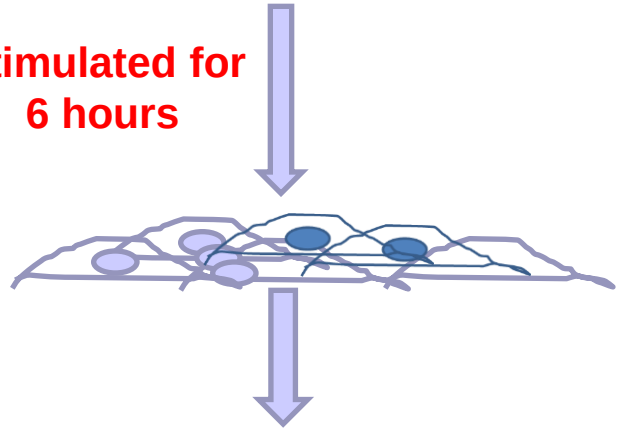
- Combination therapy is likely more effective than monotherapy for complex disease such as HTN and kidney disease.
- However, combination therapy targeting the renin-angiotensin system (RAS) fails to provide additional benefits.
- We developed here a new approach by applying CMAP to deduce the best drug combination therapy for kidney disease based on gene expression profile (Zhong et al JASN 2013).

Connectivity Map (CMAP)

1300 drugs

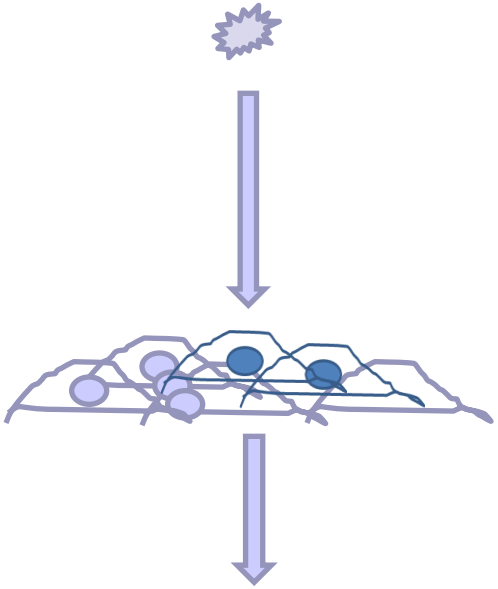


Stimulated for 6 hours



6000 Microarray studies

Each drug



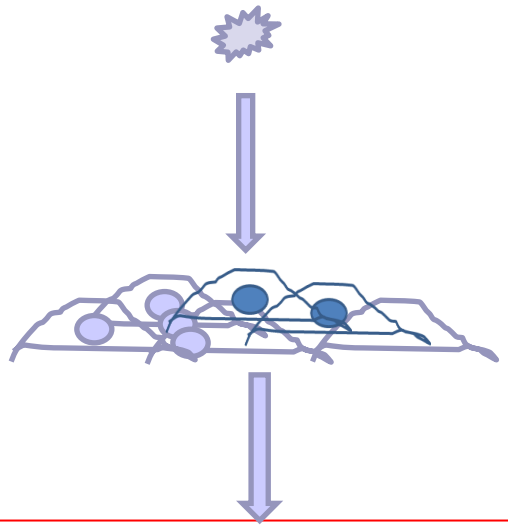
500 up-regulated genes and 500 down-regulated genes

The idea behind the CMAP study is to promote signature based drug profiling, instead of specific drug targets.

Broad Institute, Boston

Identification of pairs of drugs

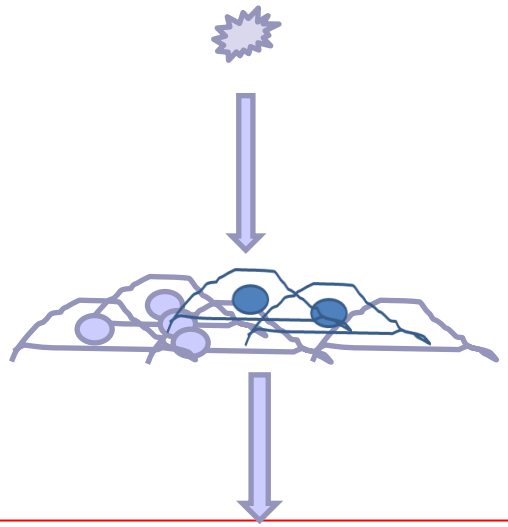
Drug A



500 up-regulated genes and 500 down-regulated genes by each drug

+

Drug B



500 up-regulated genes and 500 down-regulated genes by each drug

=

Compare with the genes altered in the disease kidney

A pair of drugs that reverse the maximal number of the genes that altered in the disease kidney while aggravating the minimal number of the genes.

The best combination is ACEI and HDACi

Total coverage Trichostatin A and Captopril	Coverage by Trichostatin A	Coverage by Captopril	Total Conflicts Trichostatin A and Captopril	Conflict by Trichostatin A	Conflict by Captopril
119 genes	70 genes	51 genes	51 genes	22 genes	31 genes

This is obtained by analyzing potential combination of any two drugs among 1200 FDA-approved drugs in a unbiased manner.

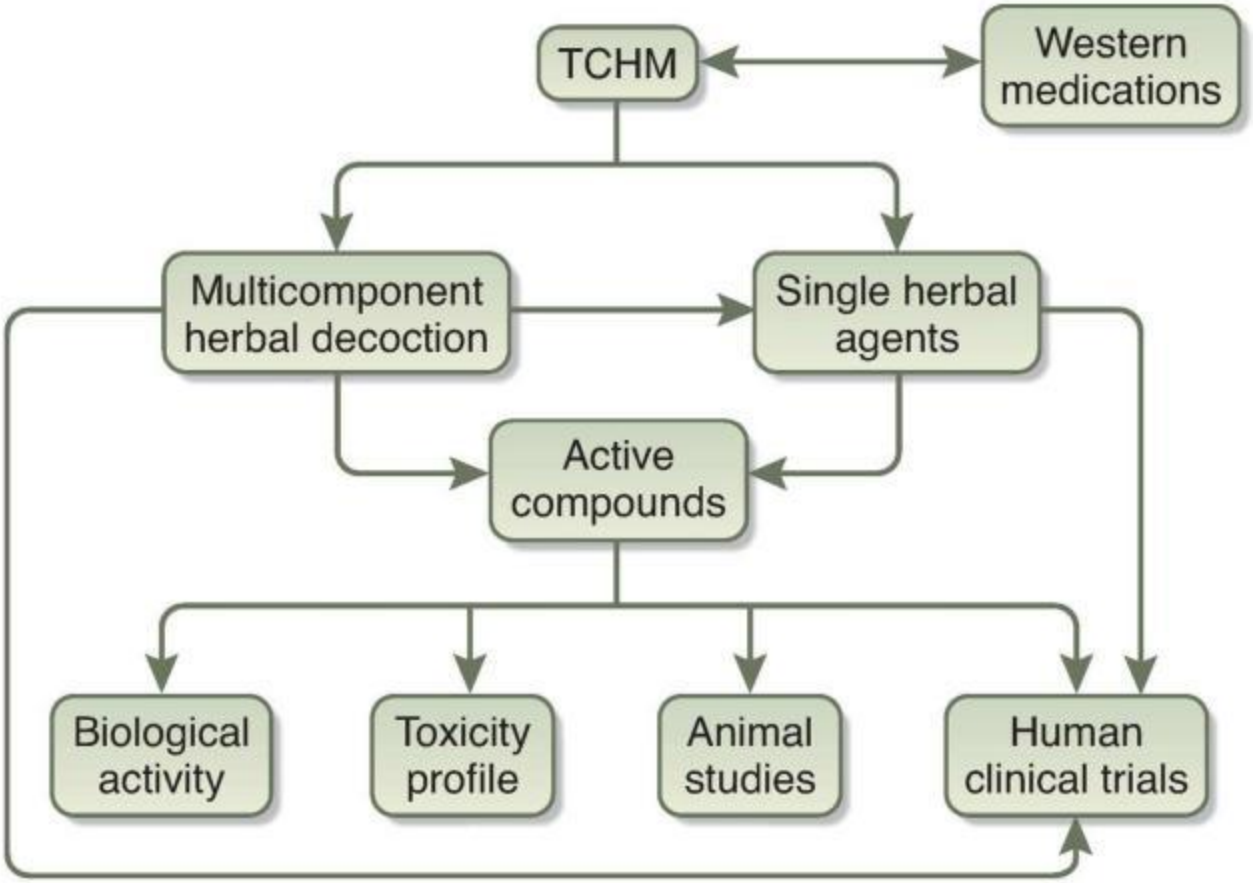
Zhong Y JASN 2013

Systems pharmacology analysis reveals that adverse event of one drug could be mitigated by 2nd drug, a new strategy for drug combination therapy.

- Rosiglitazone is associated with increased myocardial infarctions (MIs).
- Searching of a second drug ("drug B") in the FDA's Adverse Event Reporting System (FAERS) that could mitigate the risk of rosiglitazone ("drug A")-associated MI.
- Rosiglitazone usage is associated with increased occurrence of MI, but its combination with exenatide significantly reduces rosiglitazone-associated MI.
- Clinical data from the Mount Sinai Data Warehouse support the observations from FAERS.
- Analysis of cell biological networks predicted that the mitigating effect of exenatide on rosiglitazone-associated MI could occur through clotting regulation.
- Experimental data from the db/db mouse model validated the prediction.

Zhao S et al Science Translational Med 2014

Role of Chinese Medicine in the treatment of kidney disease



Zhong Y et al Kidney int. 2013

Systems Biology Approach to Identify New Drug Targets

Systems Biology Approach identifies JAK-STAT pathway in Diabetic Nephropathy

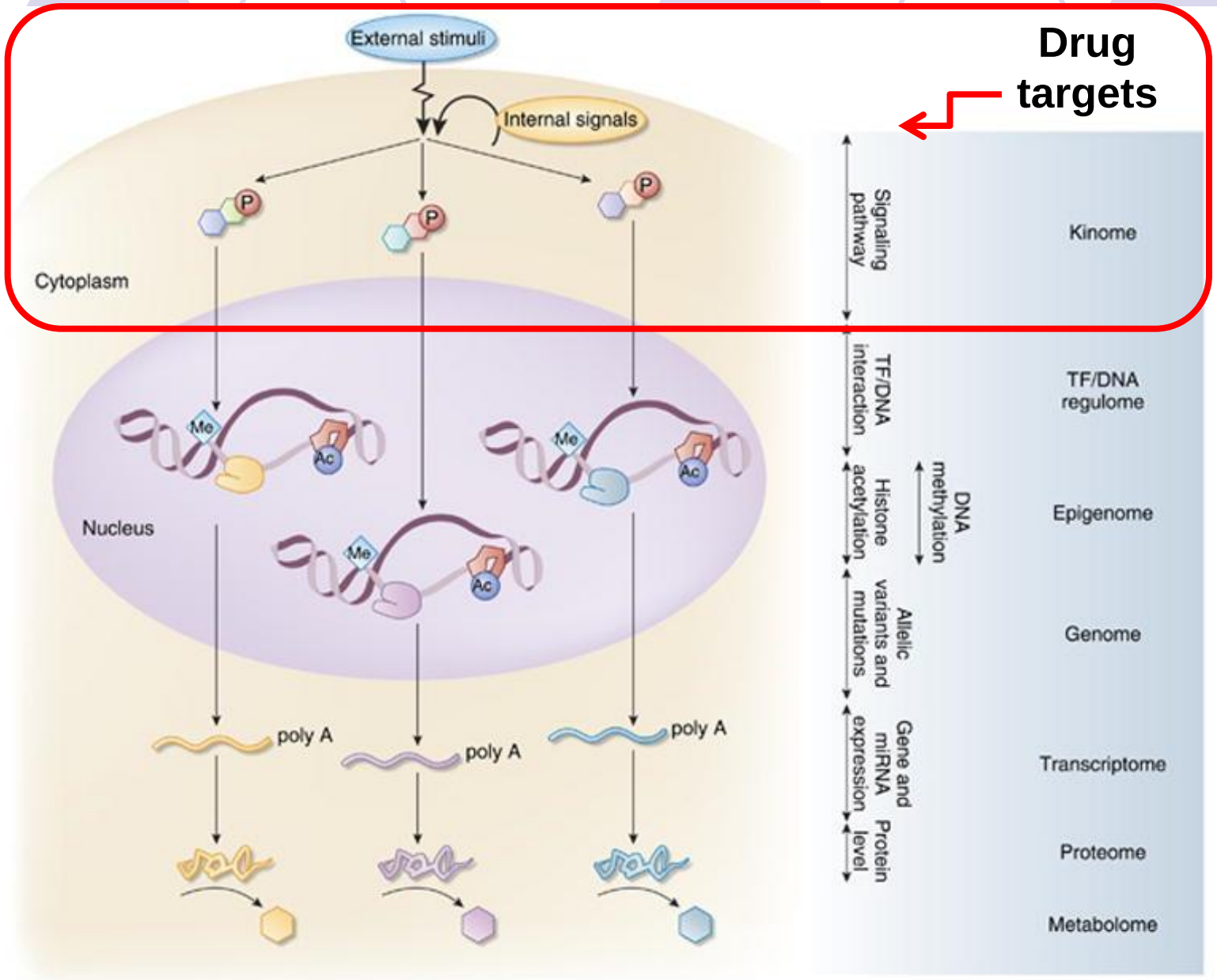
Diabetes. 2009 Feb;58(2):469-77. doi: 10.2337/db08-1328. Epub 2008 Nov 18.

Enhanced expression of Janus kinase-signal transducer and activator of transcription pathway members in human diabetic nephropathy.

Berthier CC[1], Zhang H, Schin M, Henger A, Nelson RG, Yee B, Boucherot A, Neusser MA, Cohen CD, Carter-Su C, Argetsinger LS, Rastaldi MP, BrosiusFC, Kretzler M.

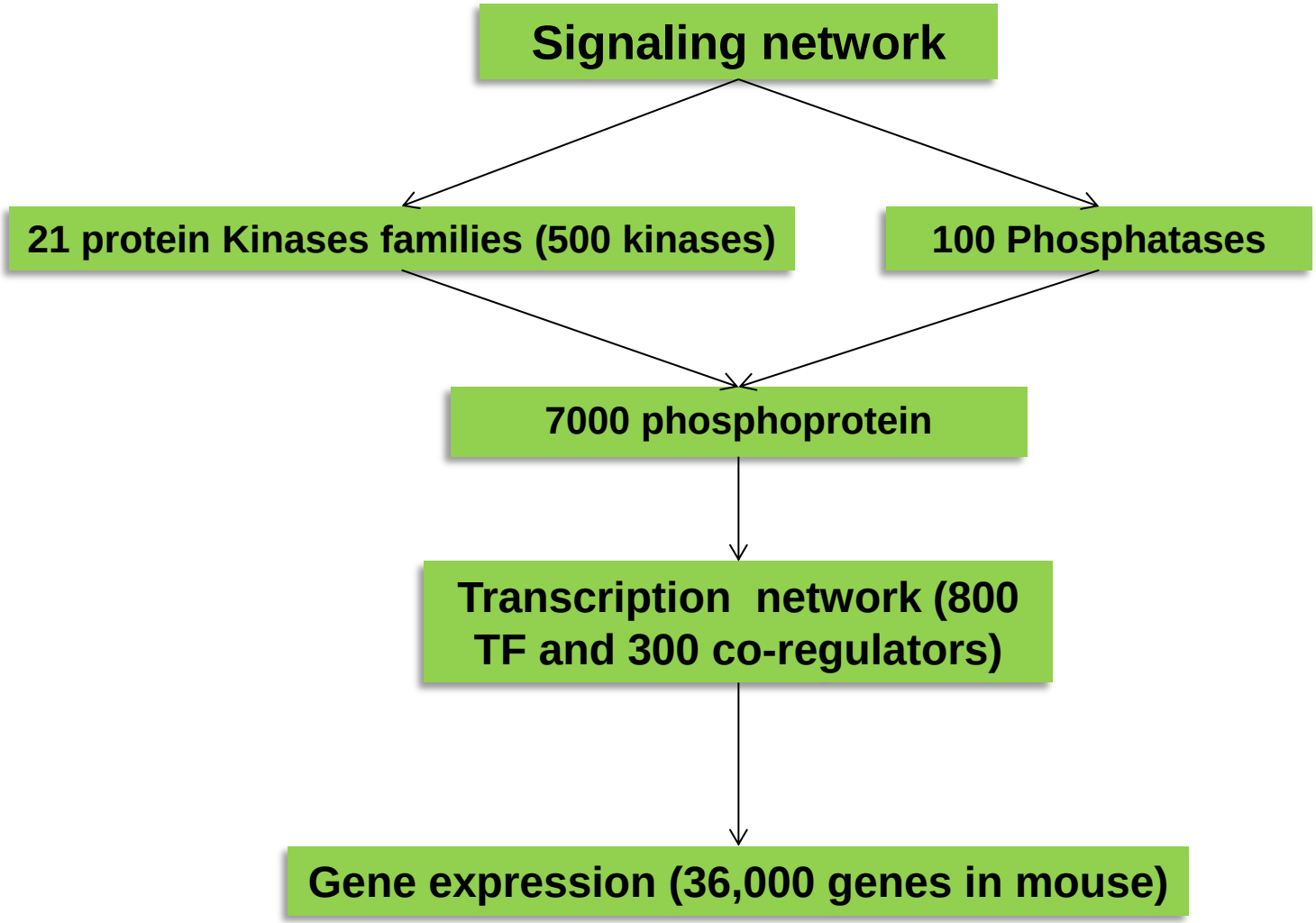
A phase 2 clinical trial will determine whether a JAK1/JAK2 inhibitor (Baricitinib from Eli Lilly) will be effective in patients with progressive diabetic nephropathy (ClinicalTrials.gov identifier: NCT01683409).

Systems Biology Analysis of Drug Targets



He et al Kidney Int. 2012

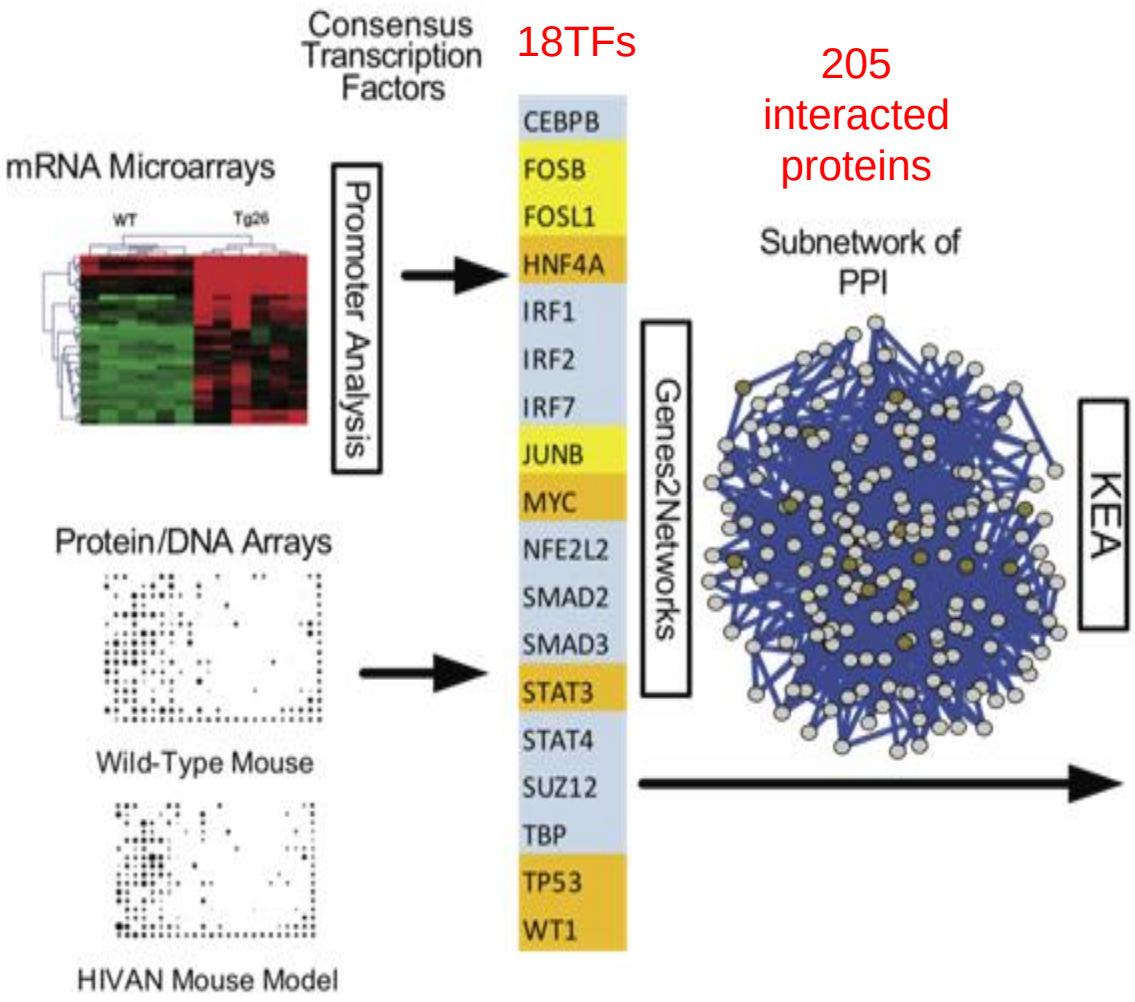
Analysis of cellular network



Study of Signaling Network

- Signaling pathway activation usually occurs at the post-translational level such as protein phosphorylation. Therefore, it is difficult to deduce simply from gene expression datasets.
- It is technically challenged to screen protein kinase activity and protein phosphorylation profile using proteomic approach.
- We developed a combined computational and experimental approach to deduce upstream signaling pathways from gene expression datasets.

Identification of HIPK2 as a key regulator of gene network in kidney disease



Kinases Most Specific for the Subnetwork

Kinase	Total Substrates	Subnetwork Substrates	p-value
MAPK1	229	38	1.11E-12
MAPK3	170	30	1.06E-10
HIPK2	34	14	2.18E-09
TAF1	42	15	2.77E-09
CHEK1	22	11	2.61E-08
CSNK2A1	194	27	9.79E-08
MAPK14	377	39	1.64E-07
TGFBR1	164	24	2.40E-07
MAPK8	234	29	2.99E-07
PAK1	72	15	1.16E-06
PRKDC	200	25	1.91E-06
AKT1	176	23	2.62E-06
TRRAP	12	7	4.72E-06

Jin et al Nature Medicine 2012

HIPK2

A nuclear serine-threonine protein kinase acting as a transcription regulatory factor.

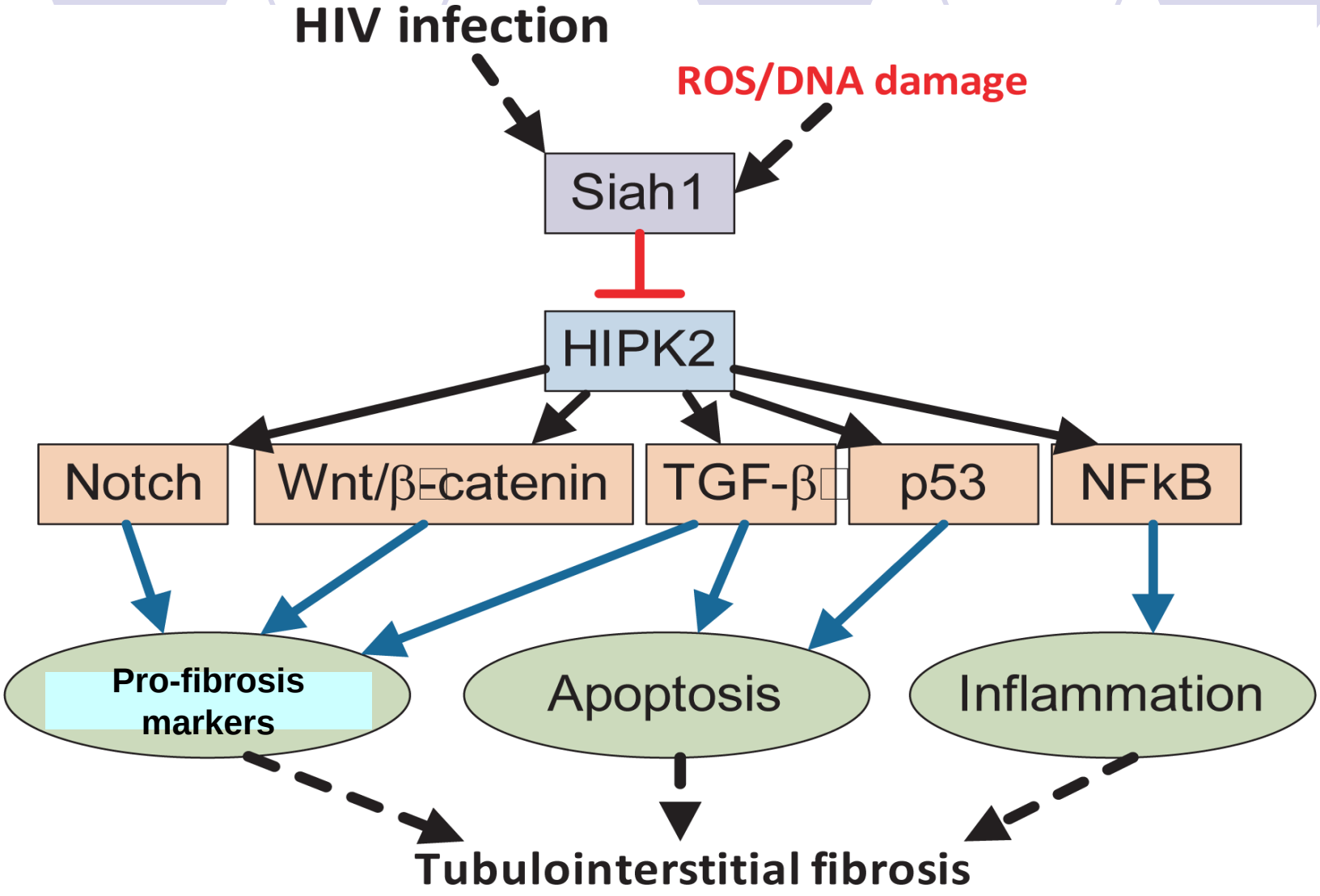
It mediates apoptosis by interacting with p53 pathway.

It mediates TGF-beta signaling pathway.

It interacts with Notch and Wnt/β-catenin signaling pathway.

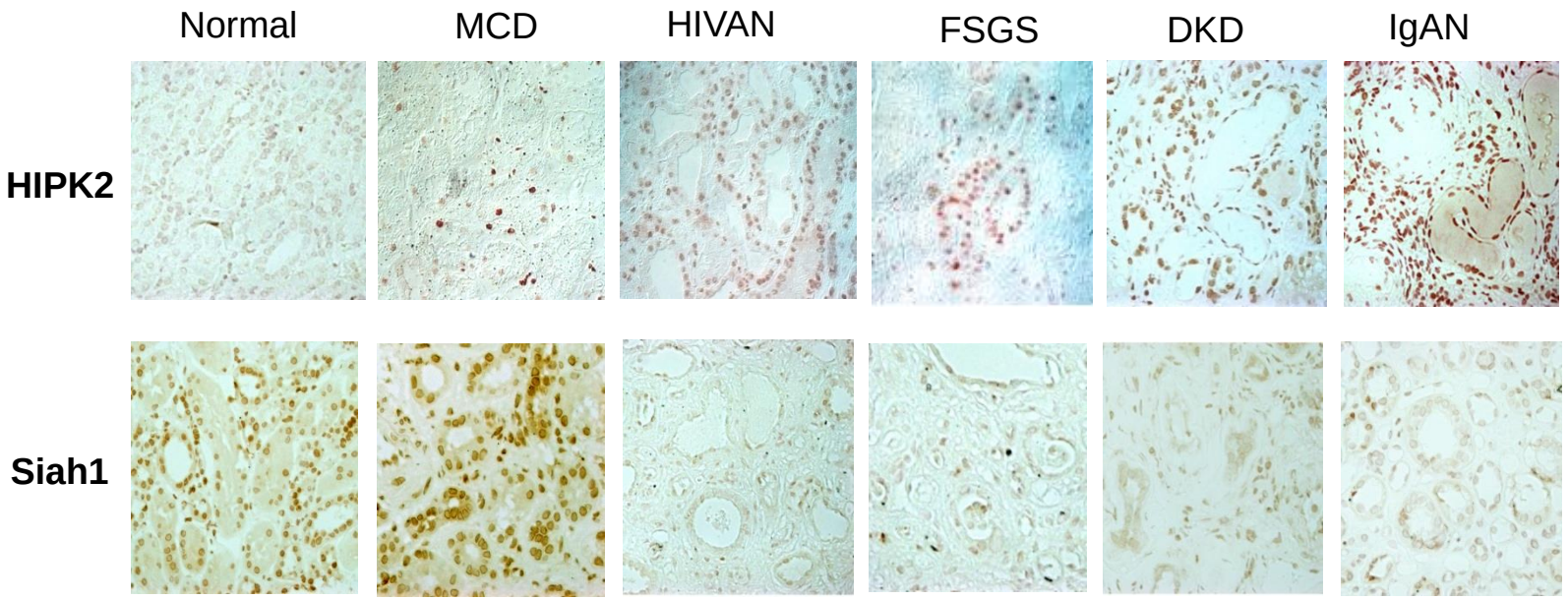
http://www.genecards.org/cgi-bin/carddisp.pl?gene=HIPK2

Summary of HIPK2 pathway in kidney cells



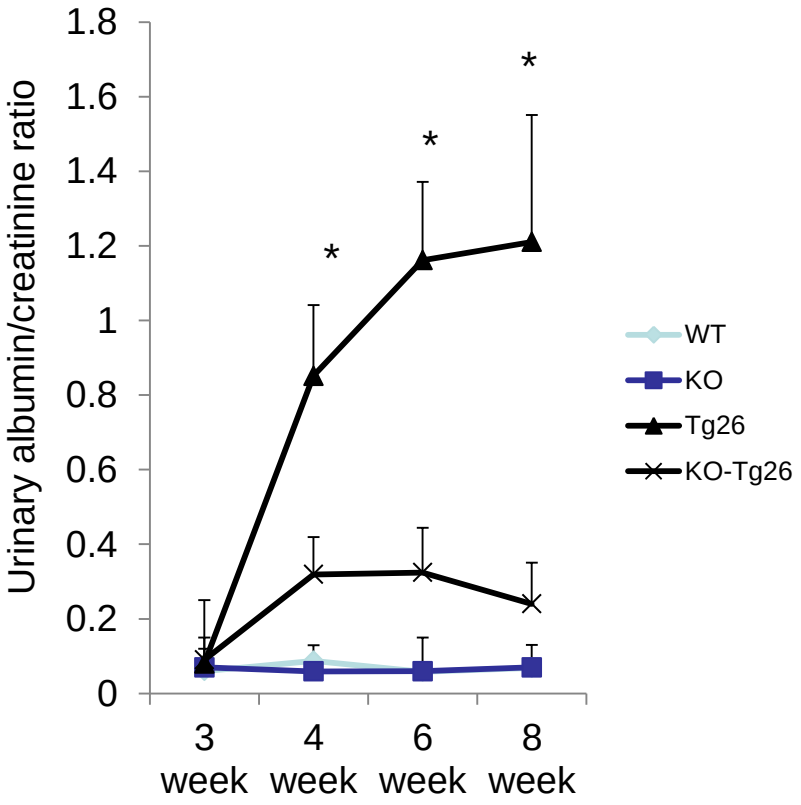
Jin et al Nature Medicine 2012

There is an inverse relationship between HIPK2 and Siah1 expression in disease kidneys

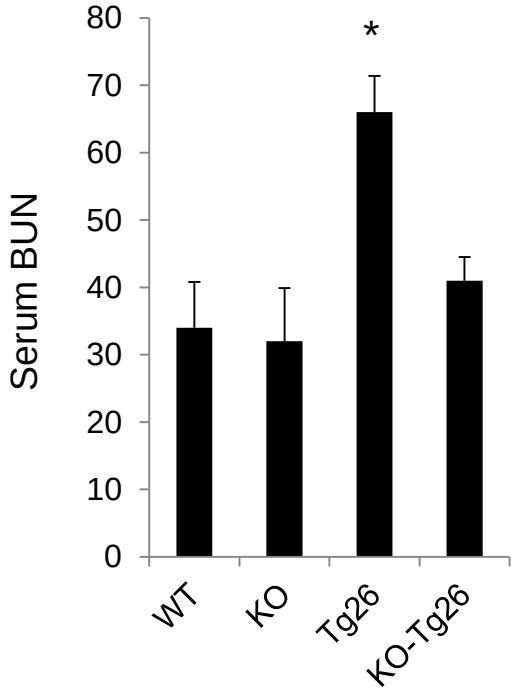


Knockout of HIPK2 improves proteinuria and renal function in Tg26 mice

A.

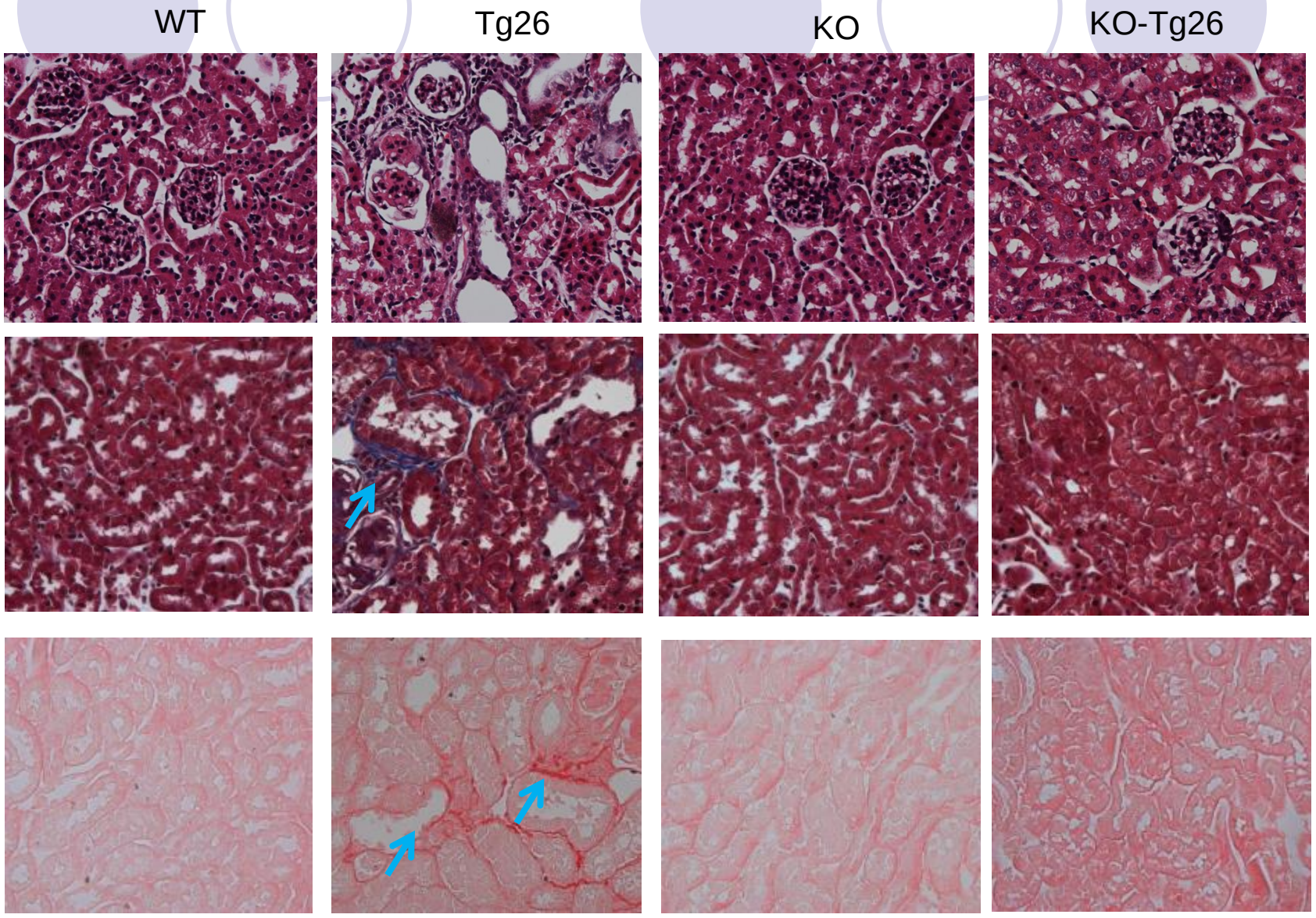


B.

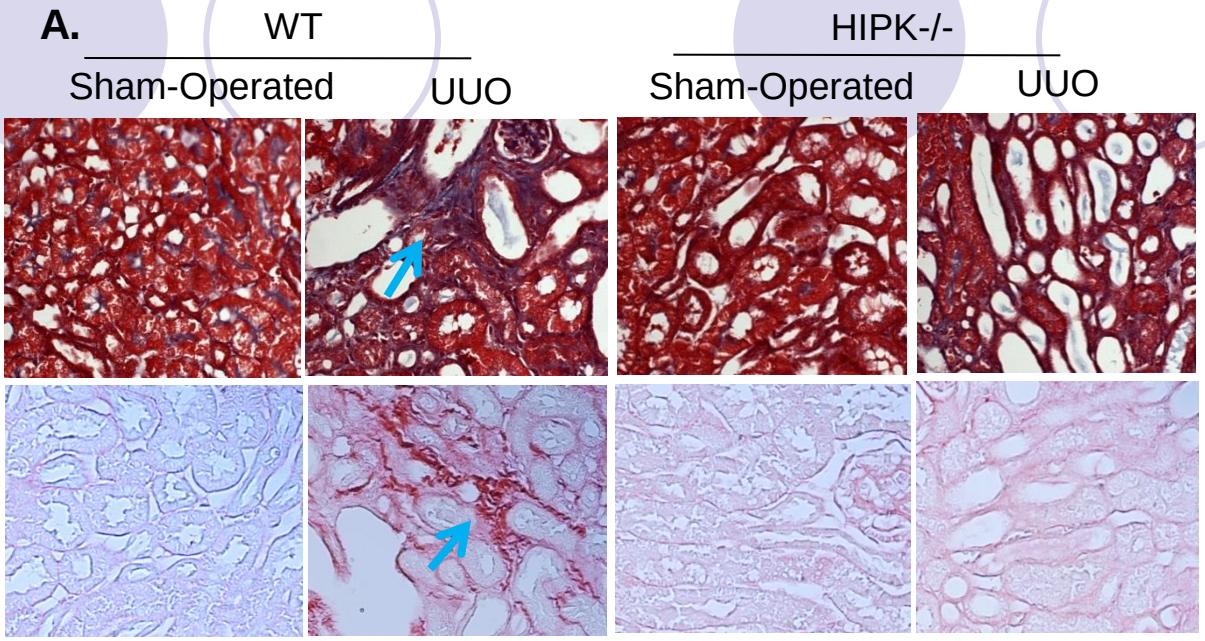


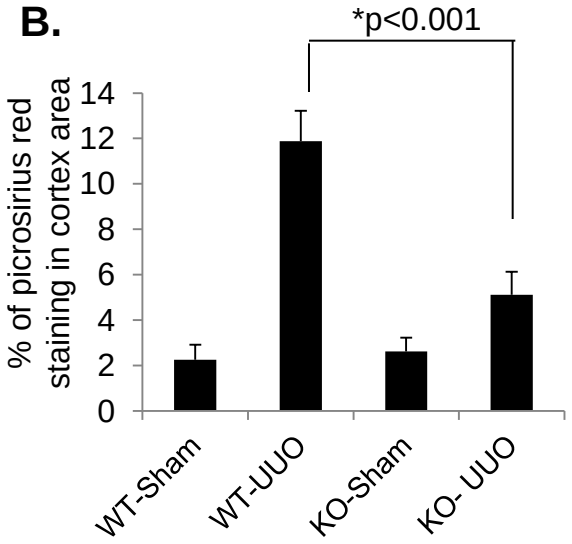
Jin et al Nature Medicine 2012

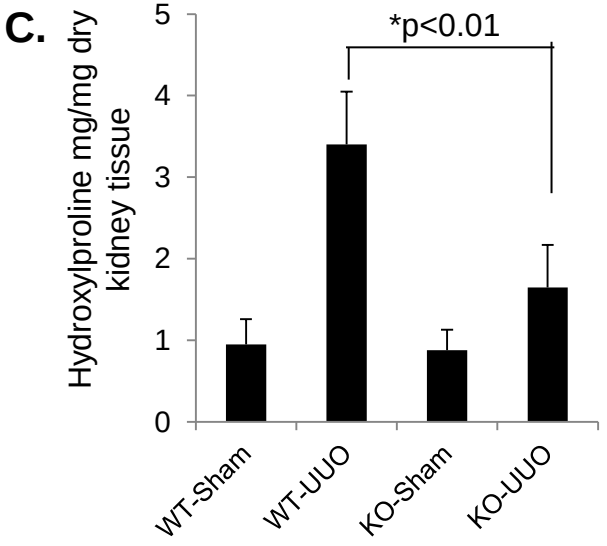
Knockout of HIPK2 attenuates renal fibrosis in Tg26



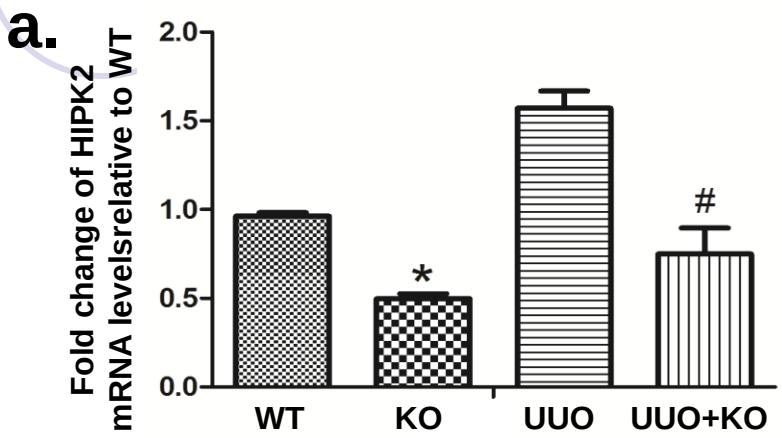
Knockout of HIPK2 attenuates renal fibrosis in UUO mice

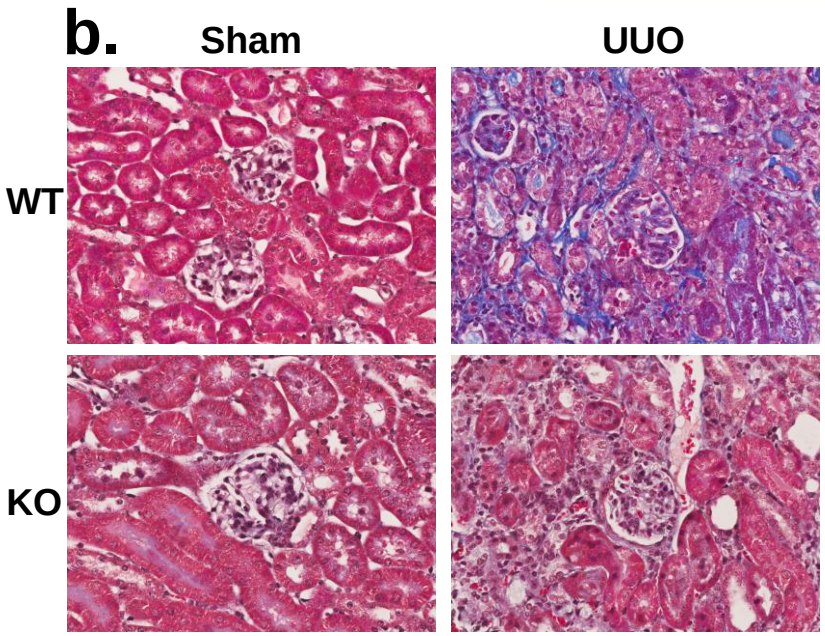


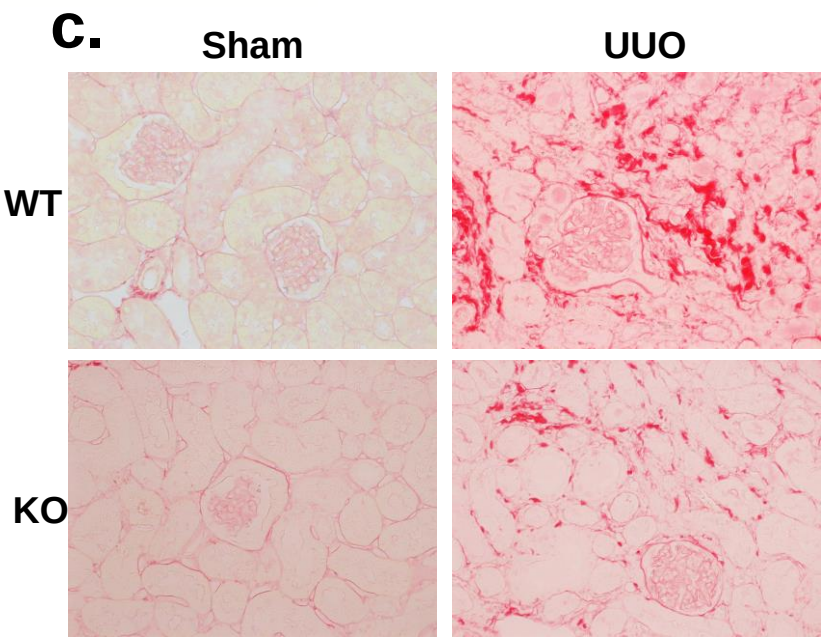




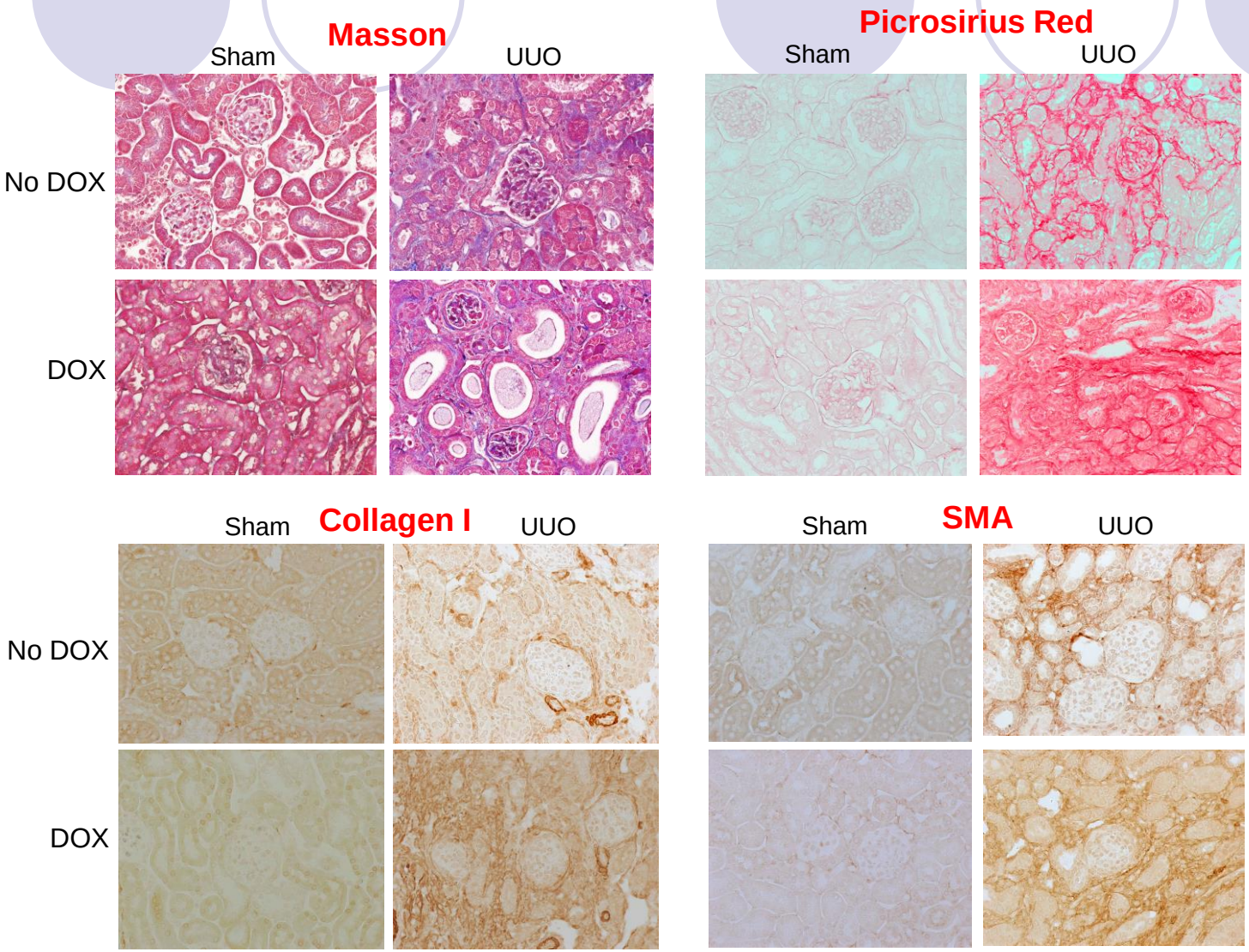
Knockout of Hipk2 expression in proximal tubular cells attenuates renal fibrosis in the UUO model







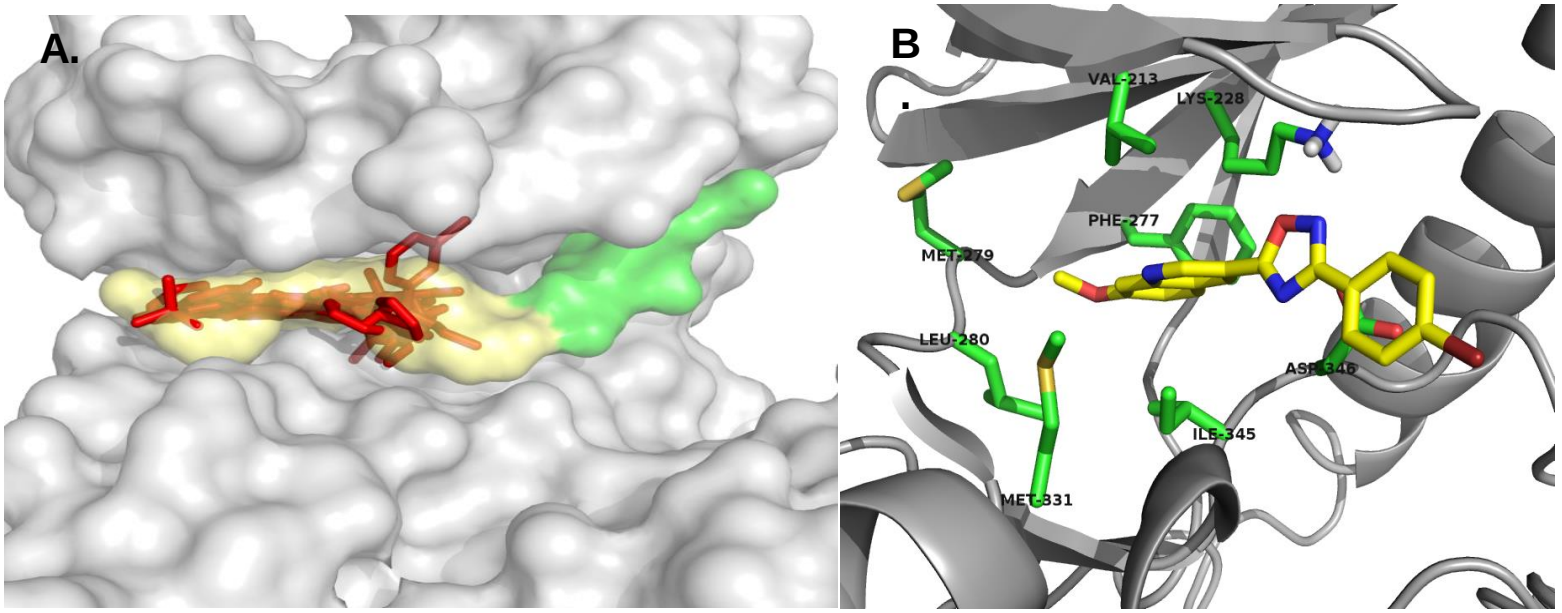
Induction of HIPK2 expression in renal tubular epithelial cells aggravates renal fibrosis in the UUO mice



HIPK2 as a potential drug target for anti-fibrosis therapy: Pros and Cons

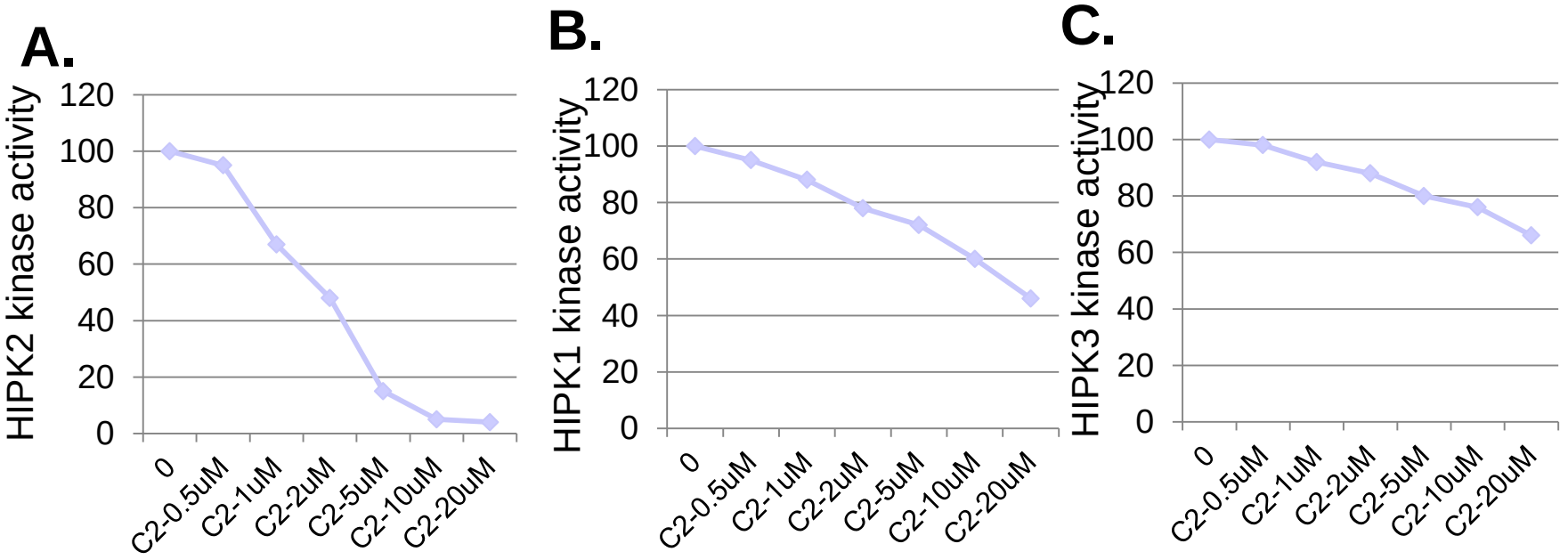
- Pros:
  1. Protein kinase is considered as good drug targets.
  2. HIPK2 knockout mice do not have obvious phenotype, suggesting that HIPK2 inhibitors should have low toxicity profile.
  3. HIPK2 affects multiple pro-fibrosis pathways and may have potent anti-fibrosis effects as compared to the drug targeting the individual pathway.
- Cons:
  - Most protein kinase inhibitors have off target effects.
  - HIPK2 and HIPK1 double knockout mice embryonic lethal.
  - Some previous studies suggest that inhibition of HIPK2 may have oncogenic effect through inhibition of p53.

Modeling of HIPK2

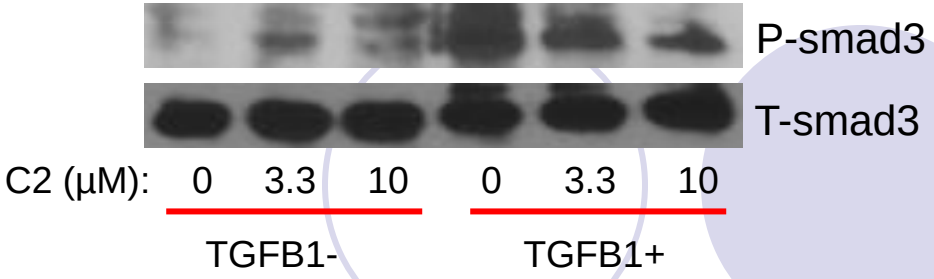


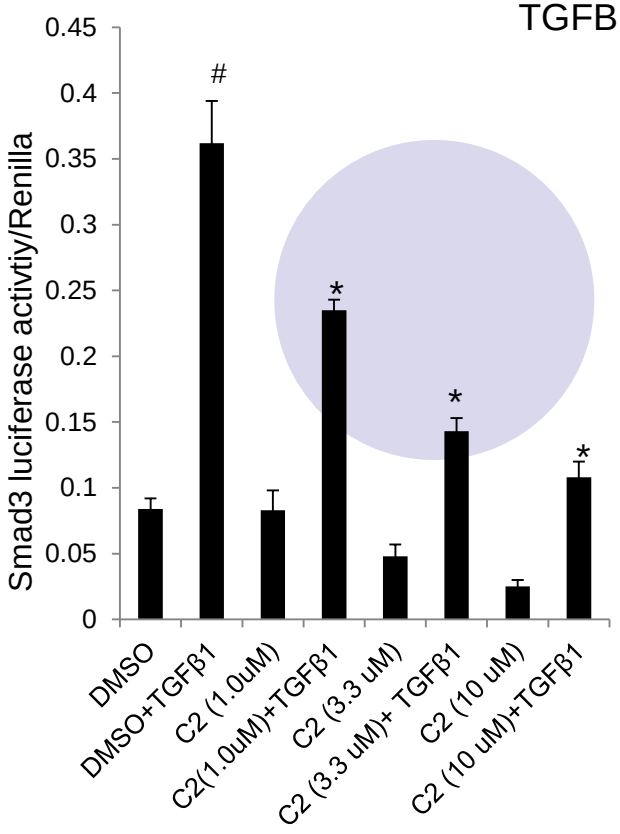
Modelling of HIPK2. A. Comparative model of HIPK2 (gray surface) is shown with the ATP-binding site highlighted in yellow and the structures of inhibitors of homologous kinases (DYRK1A, DYRK2, CLK1, CLK2, CLK3) shown in red. A region that can be exploited for enhancing the selectivity of HIPK2 inhibitors is highlighted in green. B. Stick model of HIPK2

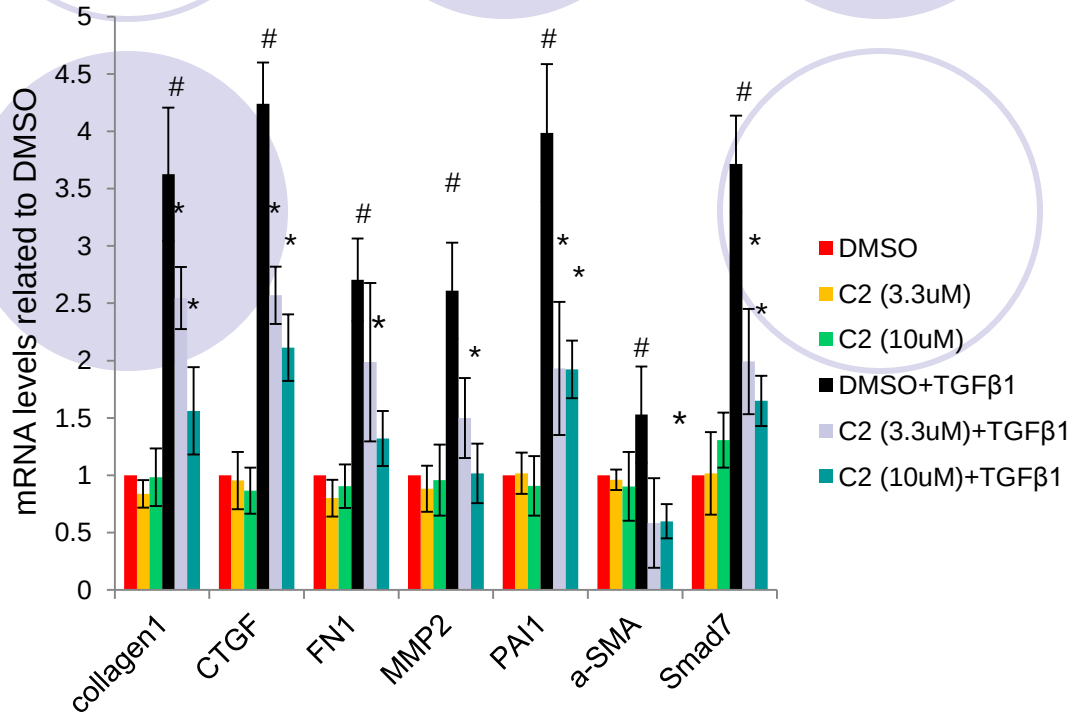
C2 has selective inhibition on HIPK2 kinase activity



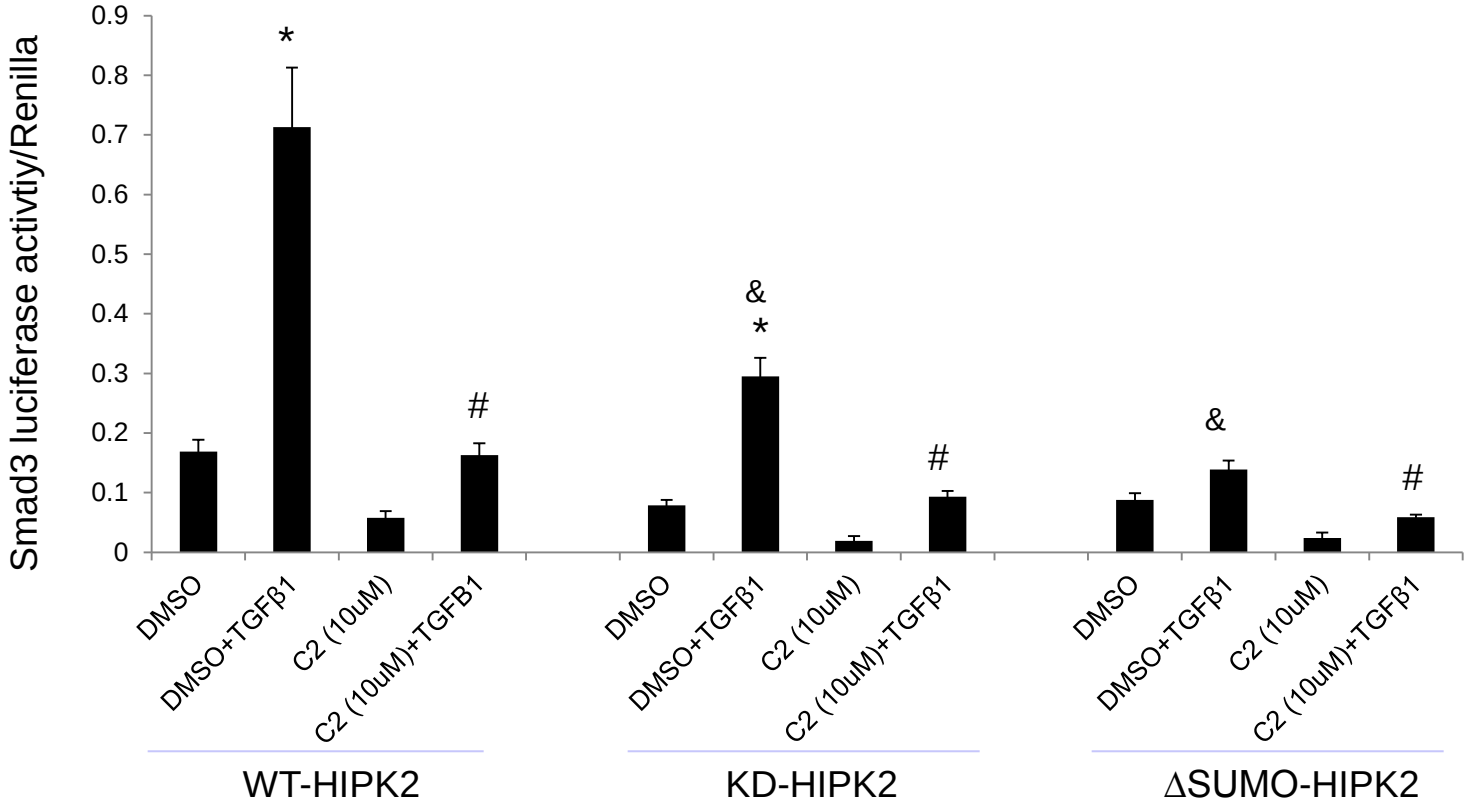
Inhibitory effects of compound #2 on TGF-β-induced Smad3 activation



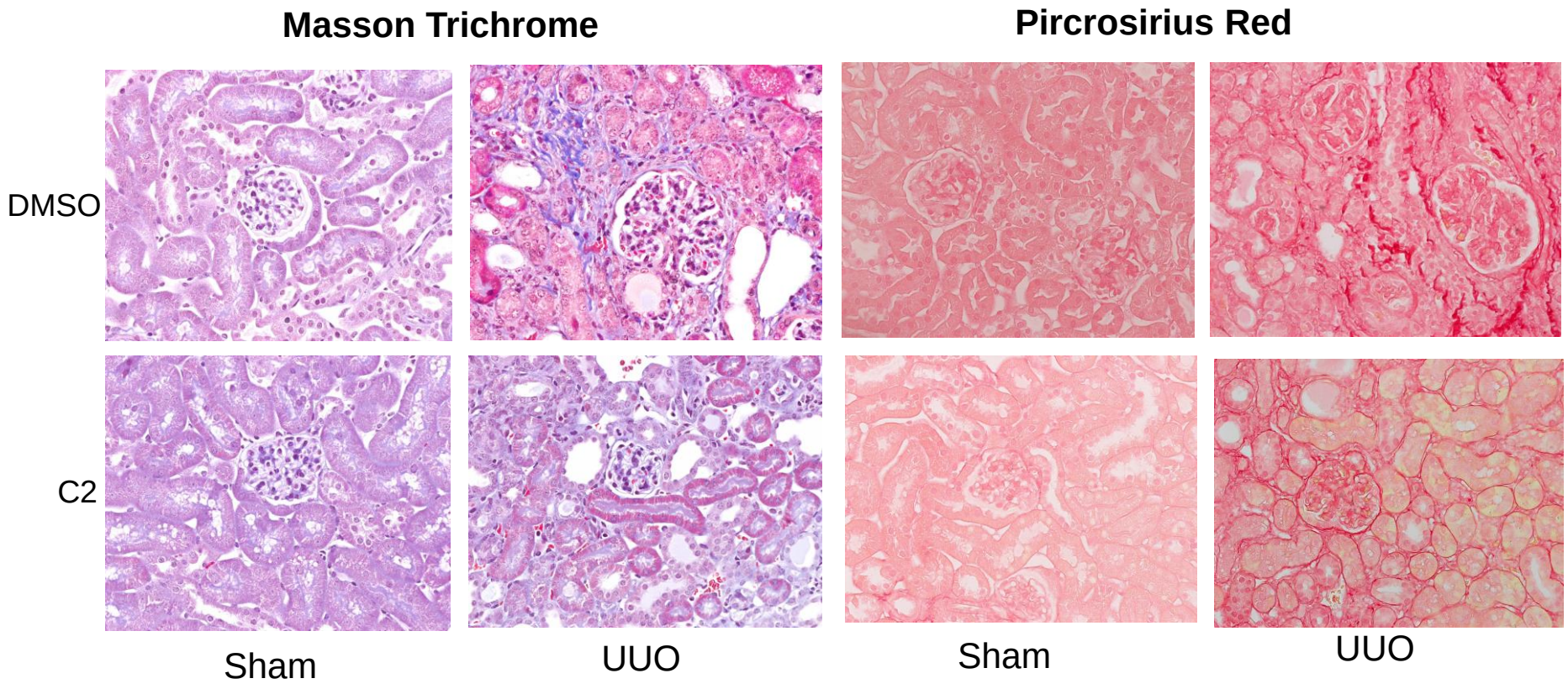




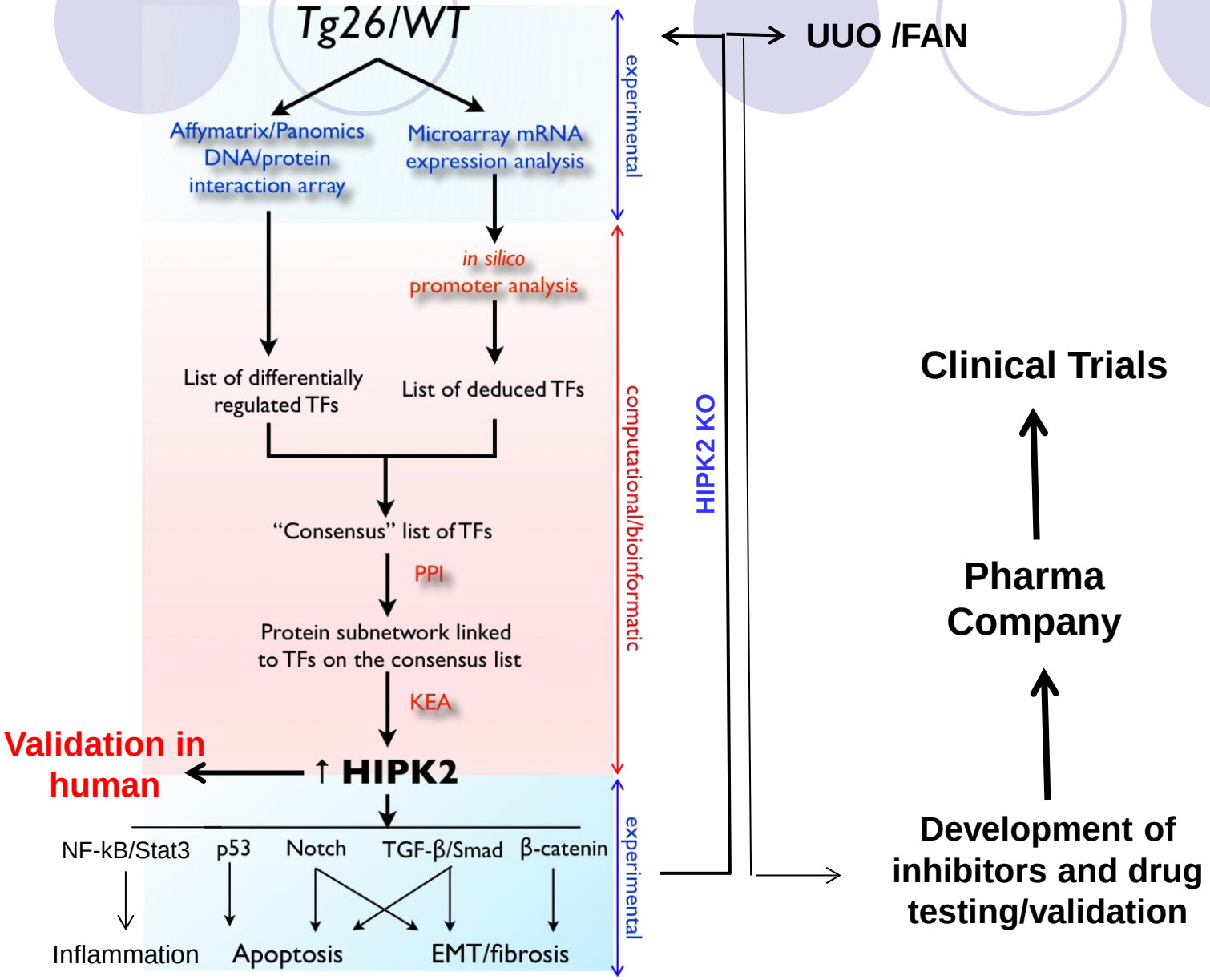
HIPK2 inhibitor (C2) suppressed TGF-β-induced Smad3 activation.



Treatment with HIPK2 inhibitor (C2) reduces renal fibrosis in the UUO mice.



Summary


Tg26/WT
Affymatrix/Panomics DNA/protein interaction array
Microarray mRNA expression analysis
experimental
in silico promoter analysis
List of differentially regulated TFs
List of deduced TFs
"Consensus" list of TFs
PPI
Protein subnetwork linked to TFs on the consensus list
KEA
computational/bioinformatic
Validation in human
↑ HIPK2
NF-kB/Stat3
p53
Notch
TGF-β/Smad
β-catenin
Inflammation
Apoptosis
EMT/fibrosis
experimental
UUO /FAN
HIPK2 KO
Clinical Trials
Pharma Company
Development of inhibitors and drug testing/validation

Acknowledgements

Members in the lab

Yumang Jin, MD/PhD

Ying Fan, MD

Wenzhen Xiao, MD

Ruijie Liu, PhD

Jia Fu, MD

Fang Zhong, MD

Peter Chaung, MD

Madhav Menon, MD

Zhengzhe Li, MD

Sandeep Mallipattu, MD

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Chengguo Wei, PhD

Ravi Iyenagr, PhD

Paul Klotman, MD

Funding: NIDDK/NIH/VA

1R01DK078897-01

1R01DK088541

P01-DK-56492

VA merit award