

# Nephrology and Transplantation Update Course



## Epigenetics and CKD

**Masaomi Nangaku**

Division of Nephrology and Endocrinology  
the University of Tokyo Graduate School of Medicine

# COI disclosure

*presenter: Masaomi Nangaku*

**I have the following relationships to disclose.**

**Potential Financial Conflicts of Interest**

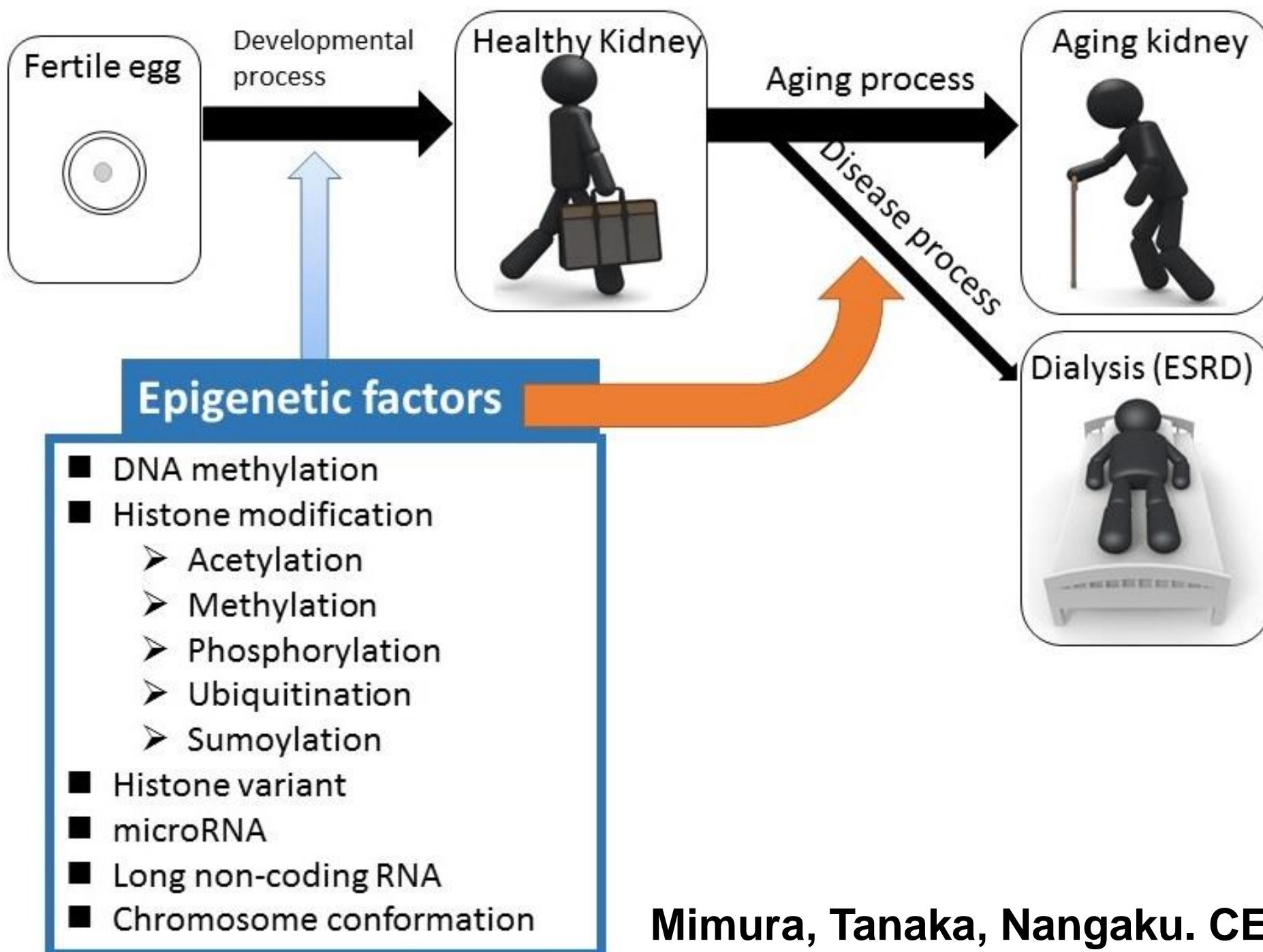
**(1)Employment:** No

**(2)Stock ownership or options:** No

**(3)Patent royalties/licensing fees:** No

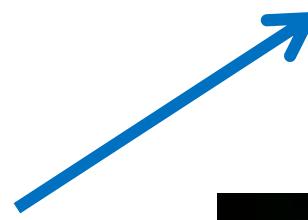
**(4)Honoraria and advisory fees:** Kyowa-Hakko-Kirin, Astellas, Chugai, GSK, JT, Tanabe-Mitsubishi

**(5)Research funding:** Kyowa-Hakko-Kirin, Daiichi-Sankyo, Alexion, Astellas, Takeda, JT



**Epigenetics: the study of persistent changes in gene expression that does not involve mutations of the underlying DNA**

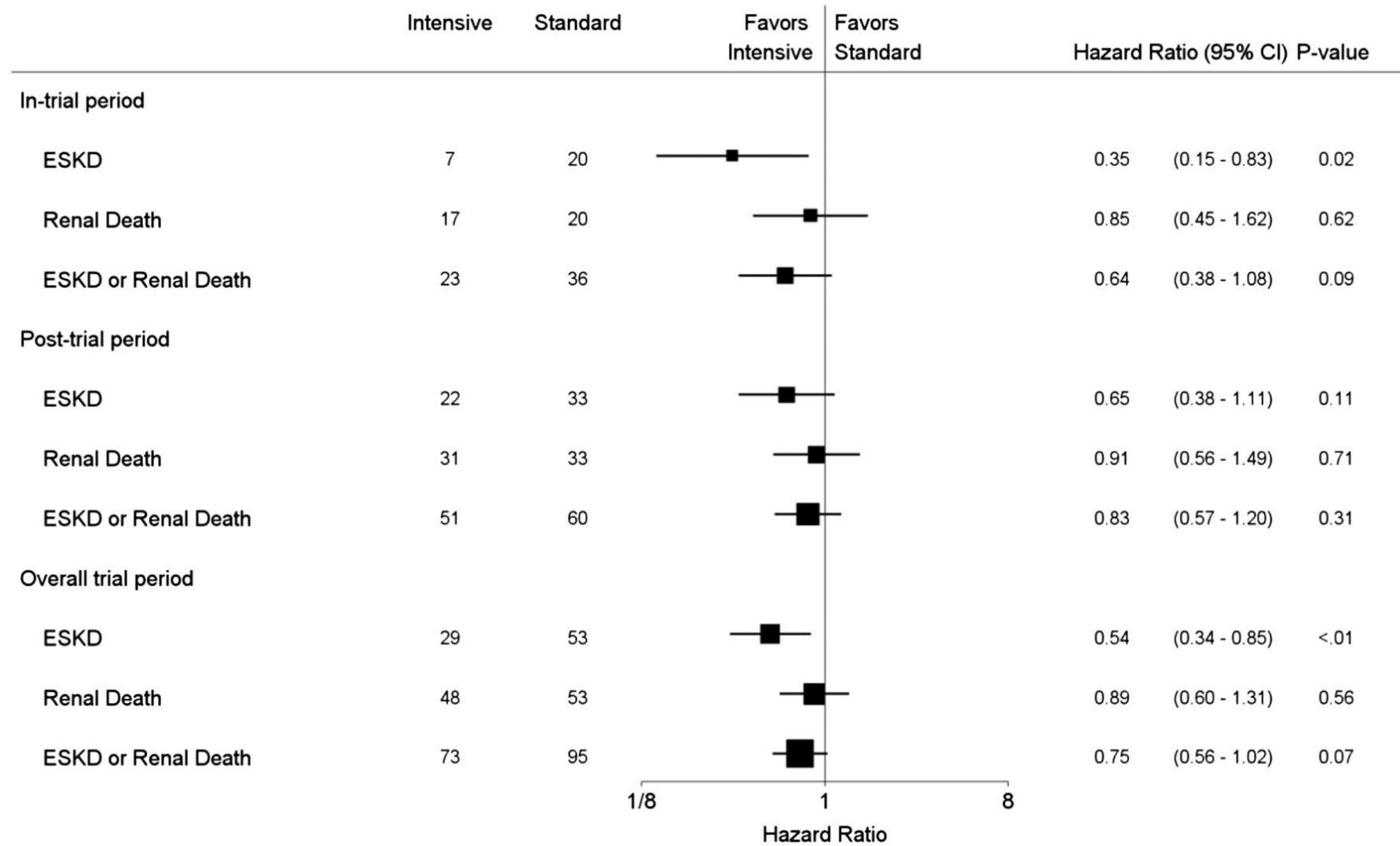




# **Metabolic memory**

# **Epigenetic changes**

# Long-term benefits of intensive glucose control for preventing ESKD: ADVANCE-ON



**5<sup>th</sup> September**

**Seminar 2: Basic Science; Diabetes**



**Chairs: Josephine Forbes & Melinda Coughlan**

**Karin Jandeleit-Dahm**

**Masaomi Nangaku**

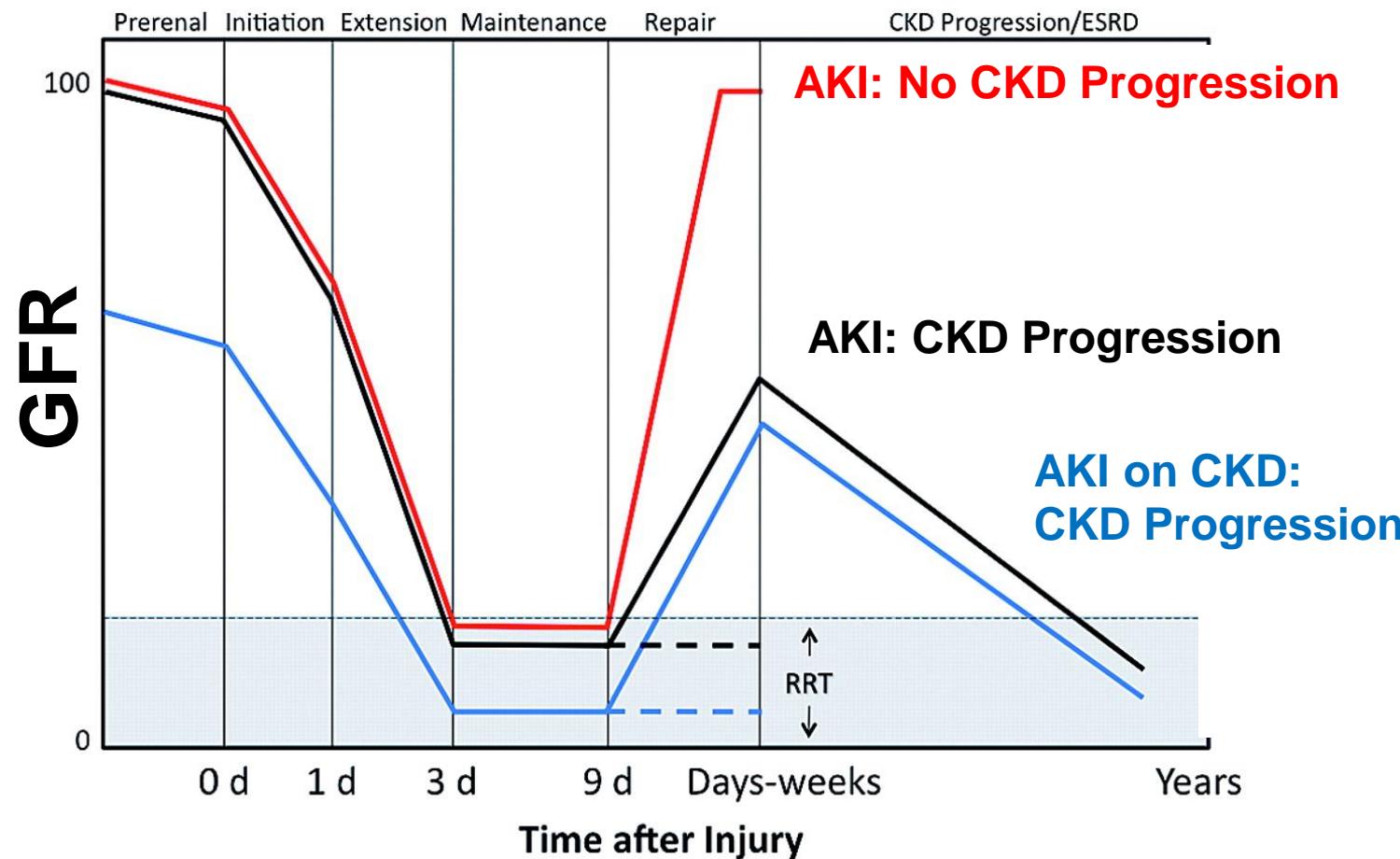
**Carol Pollock**

# Hypoxic memory

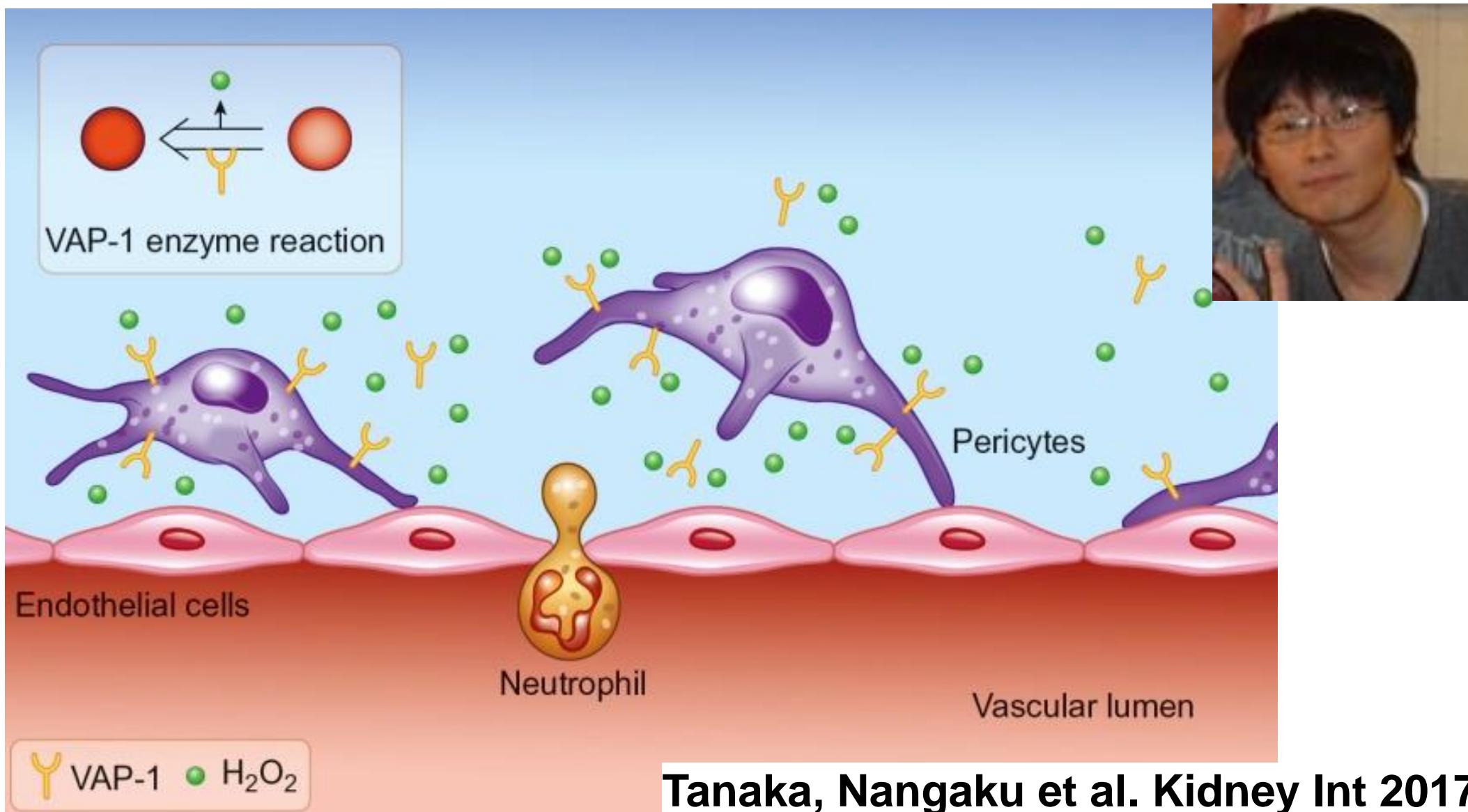
# Epigenetic changes

# The Nexus of Acute Kidney Injury, Chronic Kidney Disease, and World Kidney Day 2009

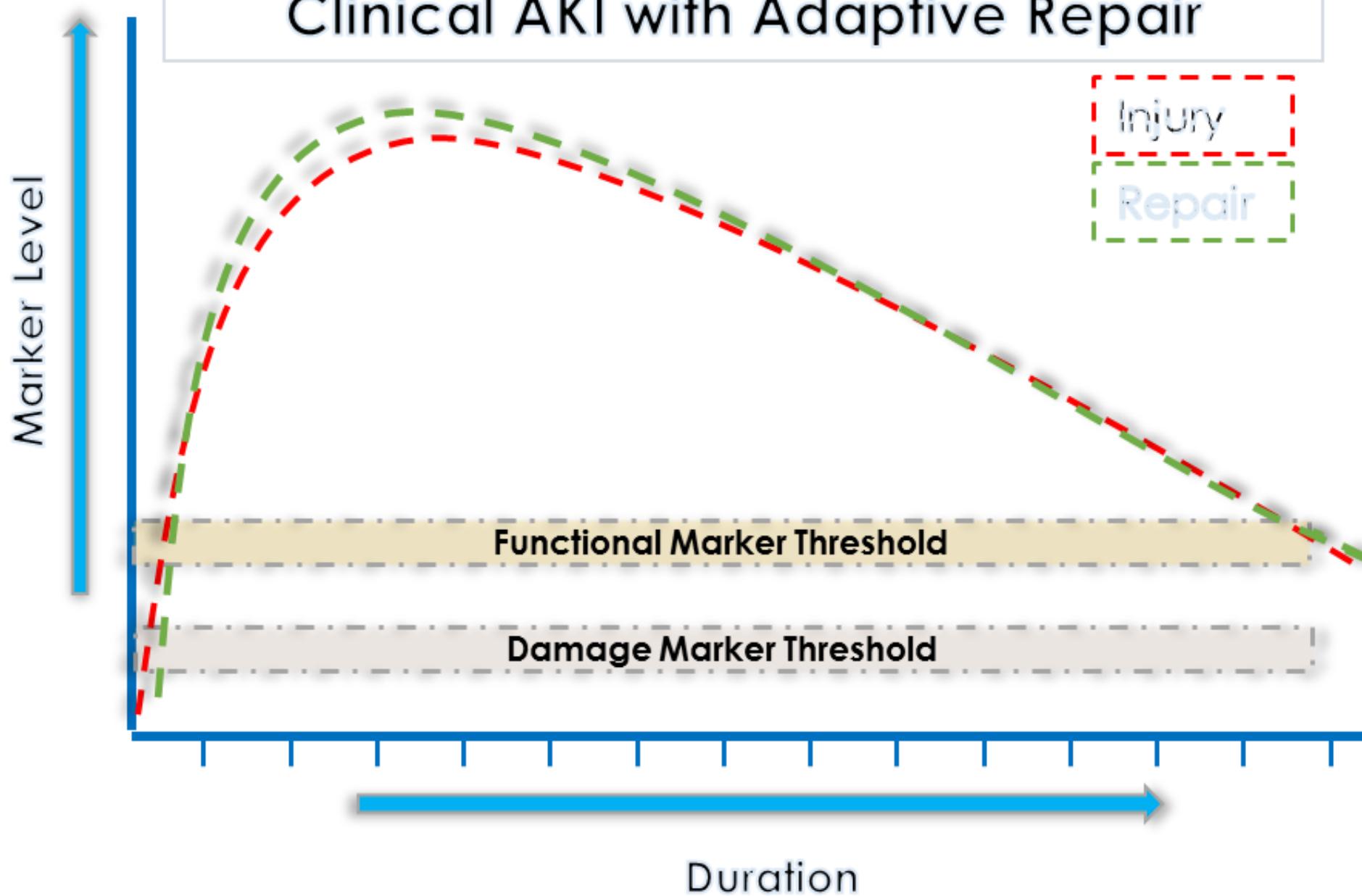
Mark D. Okusa,\* Glenn M. Chertow,<sup>†</sup> and Didier Portilla,<sup>‡</sup> for the Acute Kidney Injury Advisory Group of the American Society of Nephrology



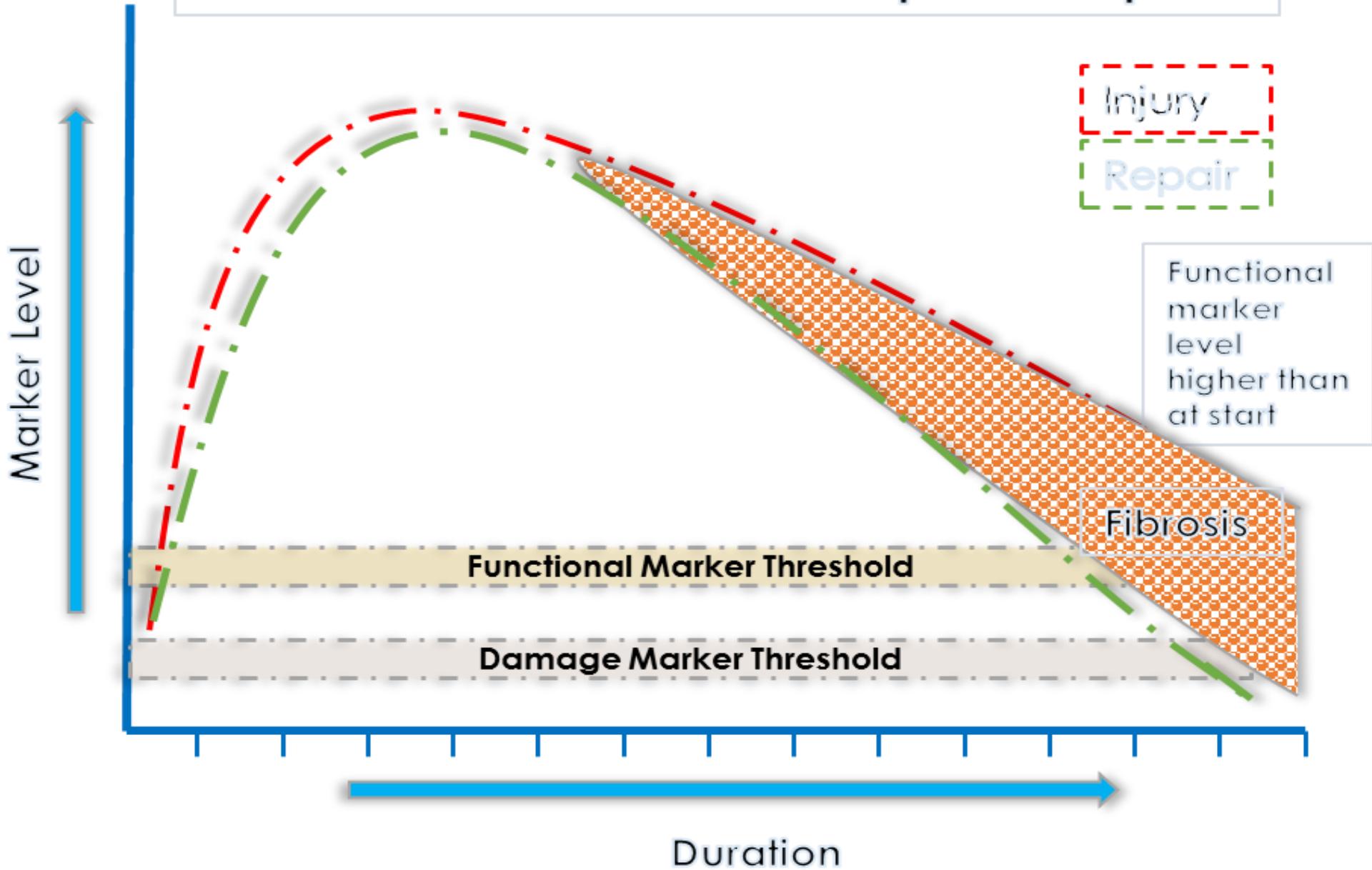
# VAP-1 in pericytes enhances neutrophil infiltration into the IR-injured kidney by generating H<sub>2</sub>O<sub>2</sub>



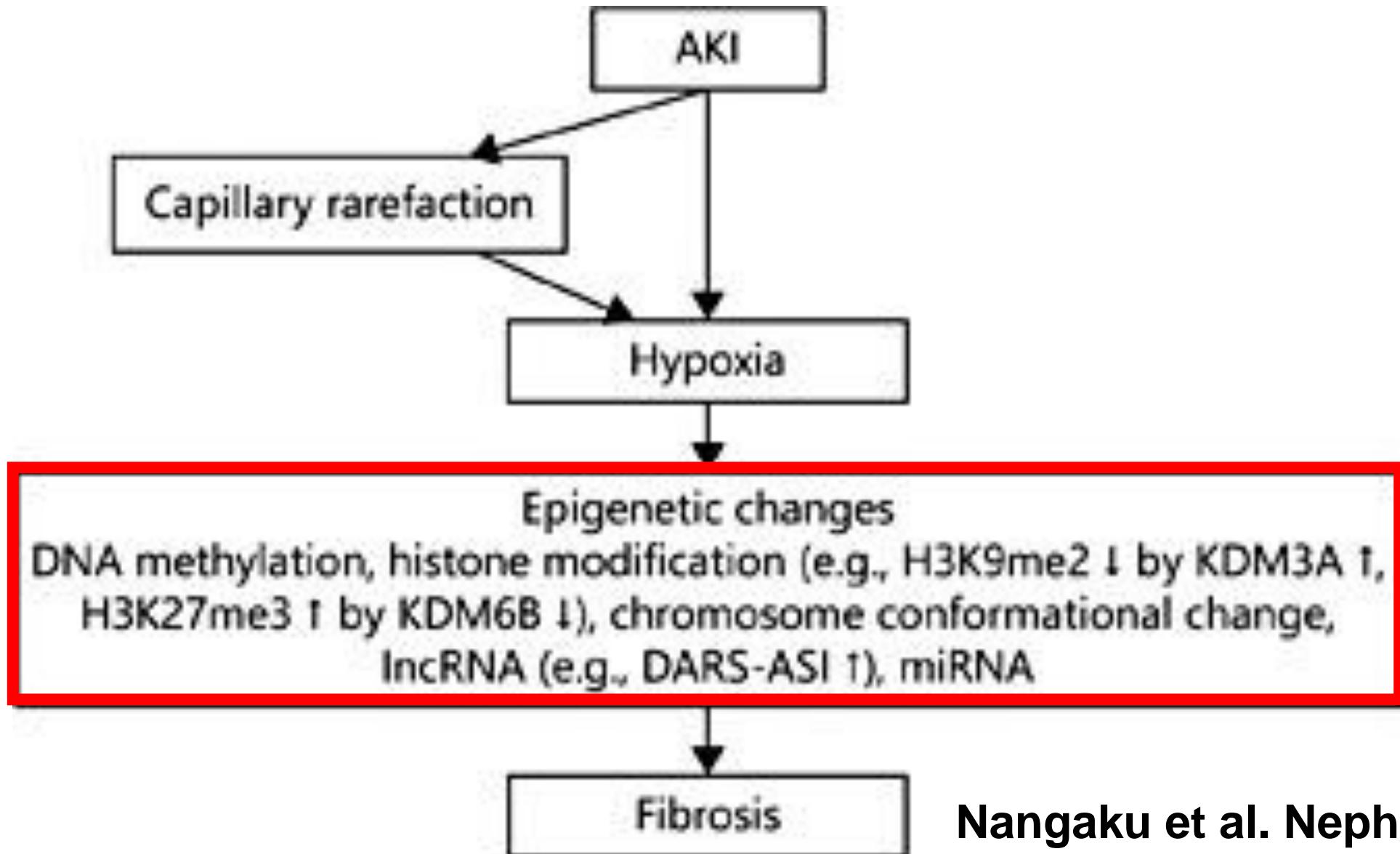
## Clinical AKI with Adaptive Repair



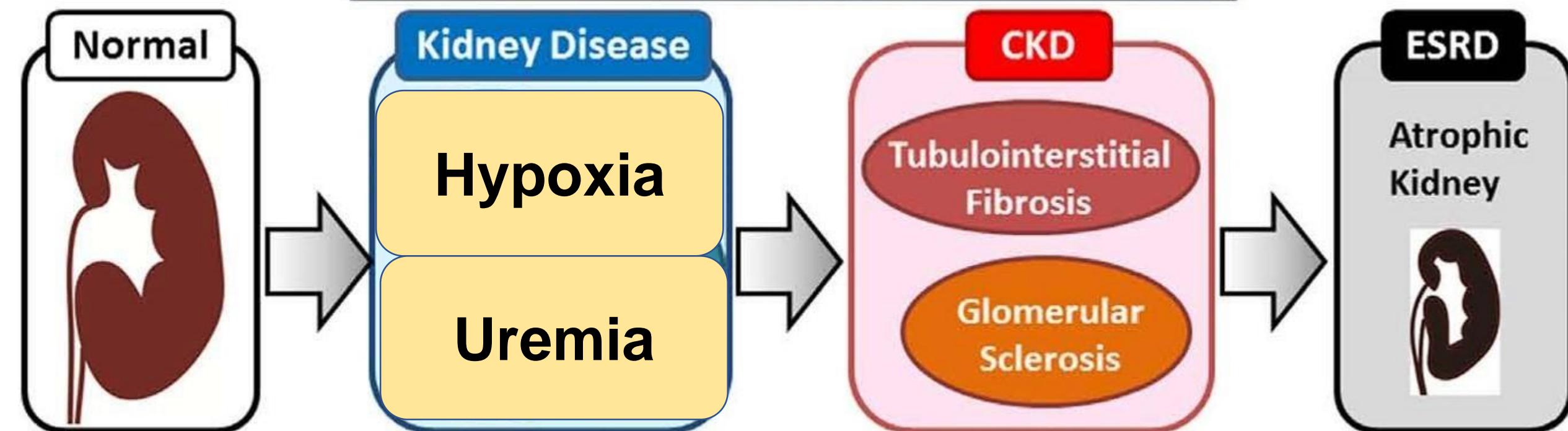
# Clinical AKI with Maladaptive Repair



# Renal hypoxia in the pathophysiology of the AKI-to-CKD transition

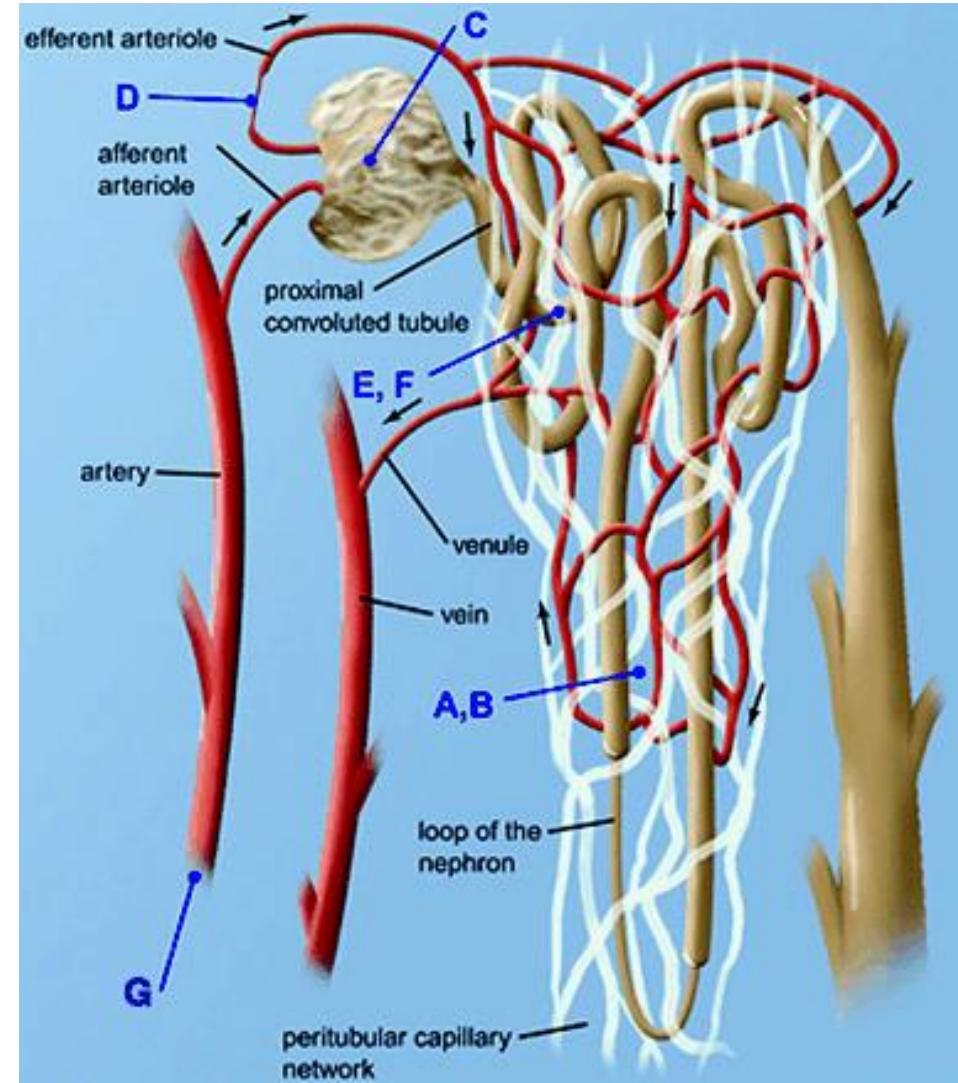
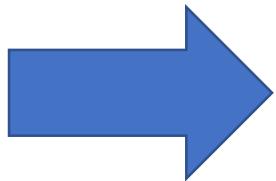
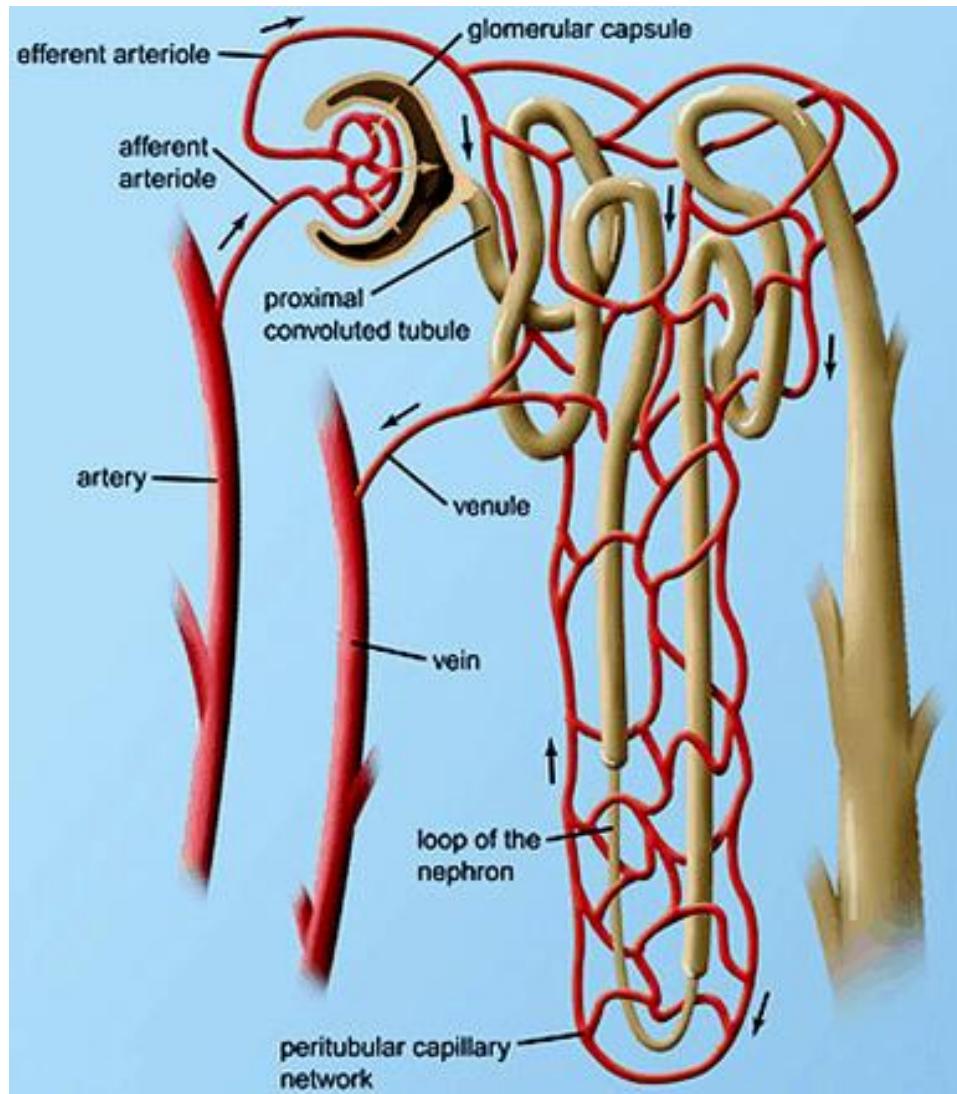


## Progression of kidney disease to ESRD.



Modified from Mimura, Tanaka, & Nangaku. Semin Nephrol 2013

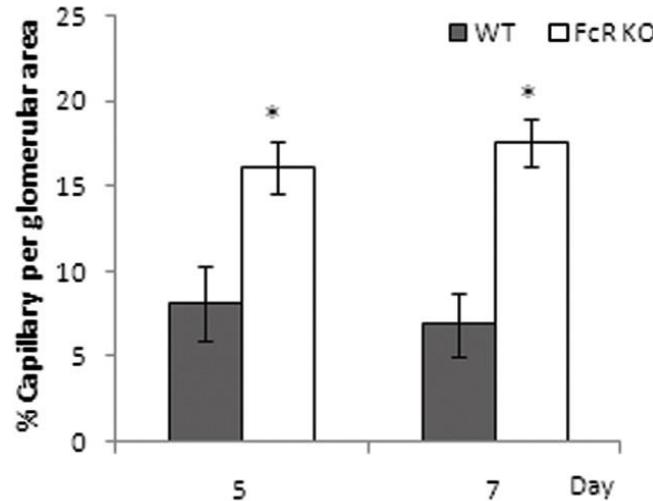
# Hypoxia as the final common pathway to ESKD



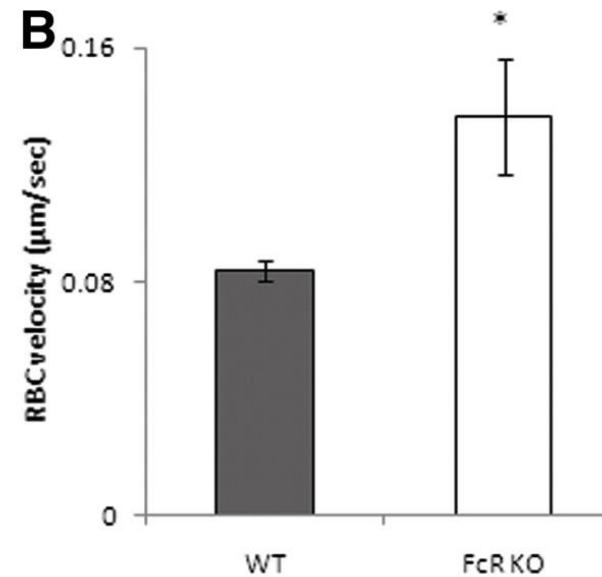
Nangaku. J Am Soc Nephrol 2006

# Peritubular ischemia contributes more to tubular damage than proteinuria in immune-mediated glomerulonephritis

A

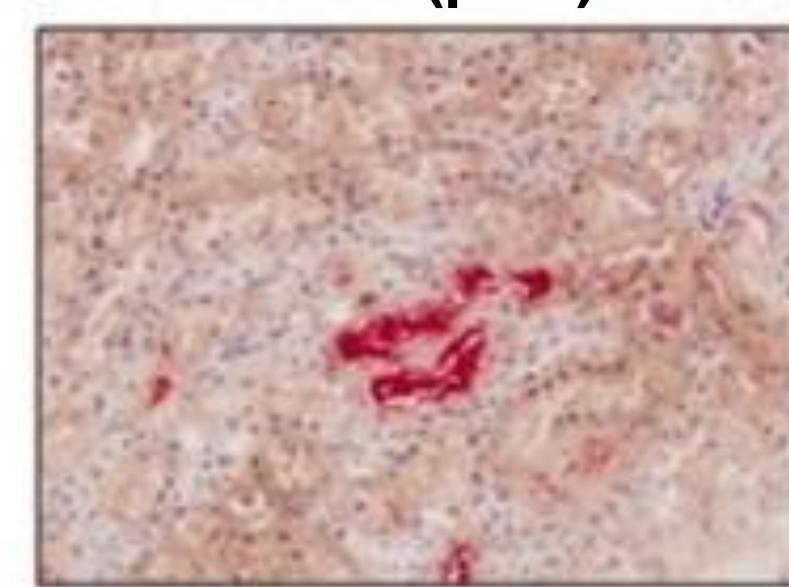
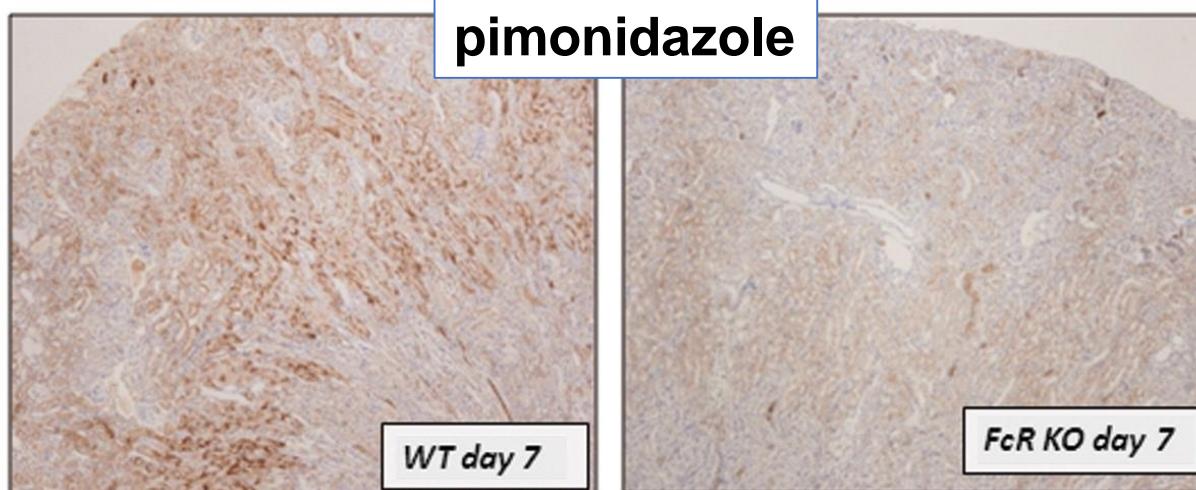


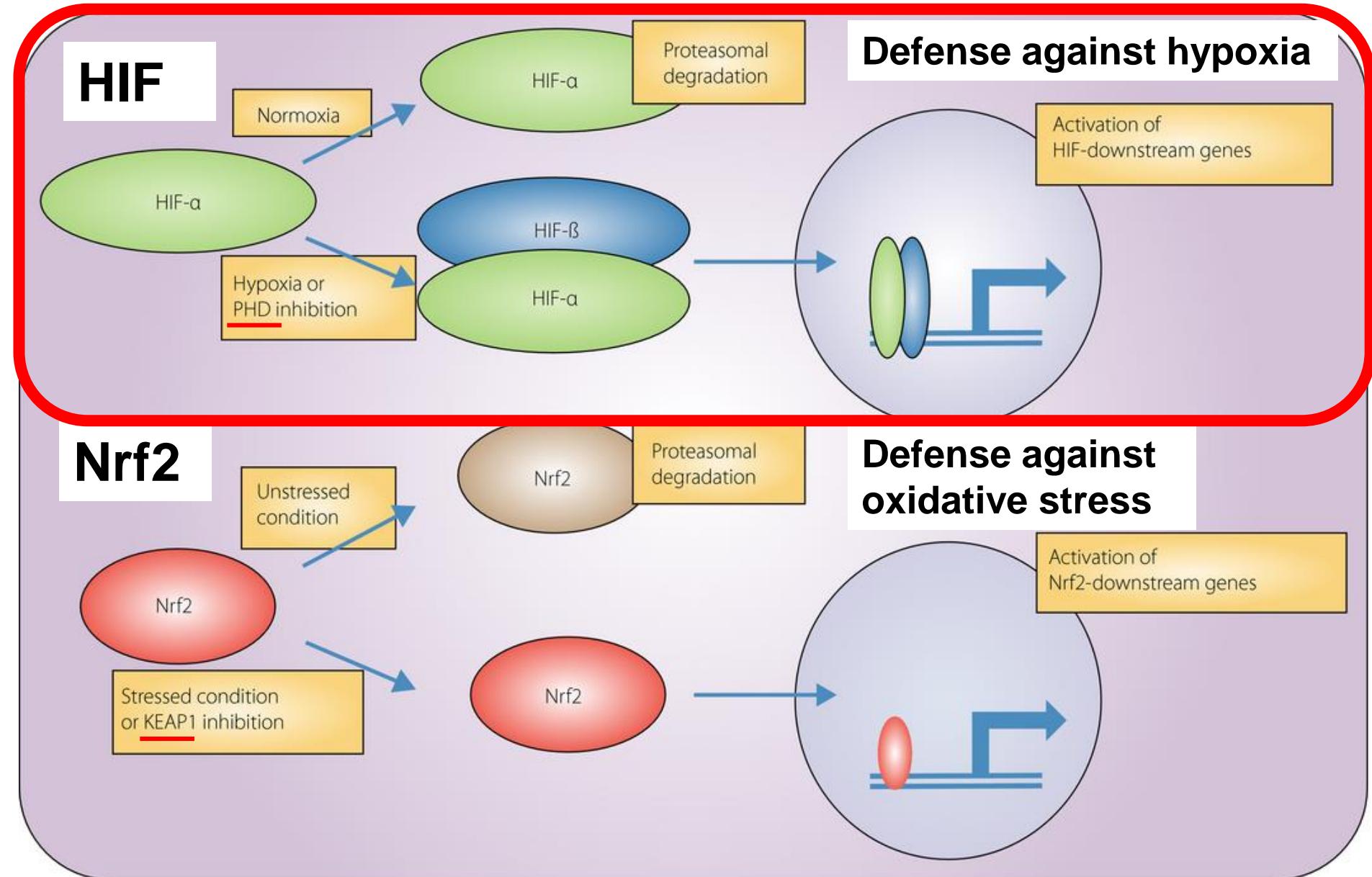
B



Double immunostaining showed pimonidazole-positive tubules (brown) located close to Kim-1-positive tubules (pink).

C





# **2016 Albert Lasker Basic Medical Research Award**

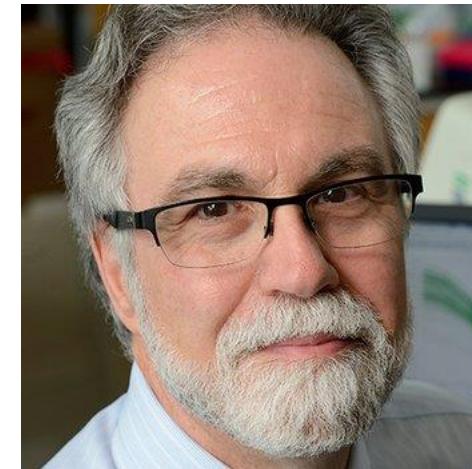
## **Oxygen sensing – an essential process for survival**



**William G. Kaelin, Jr.**

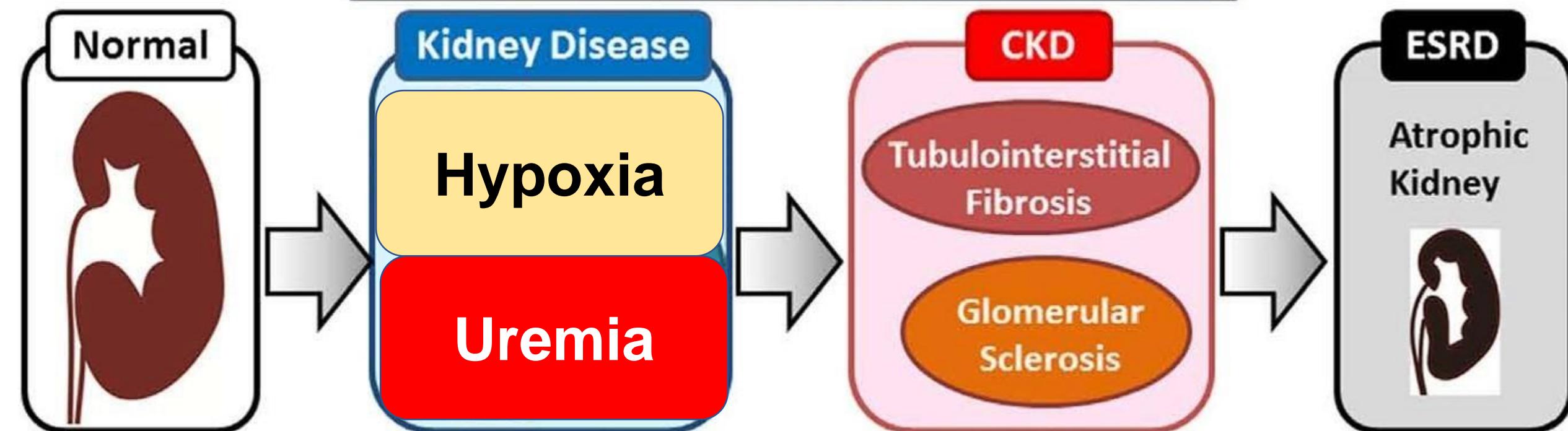


**Peter J. Ratcliffe**



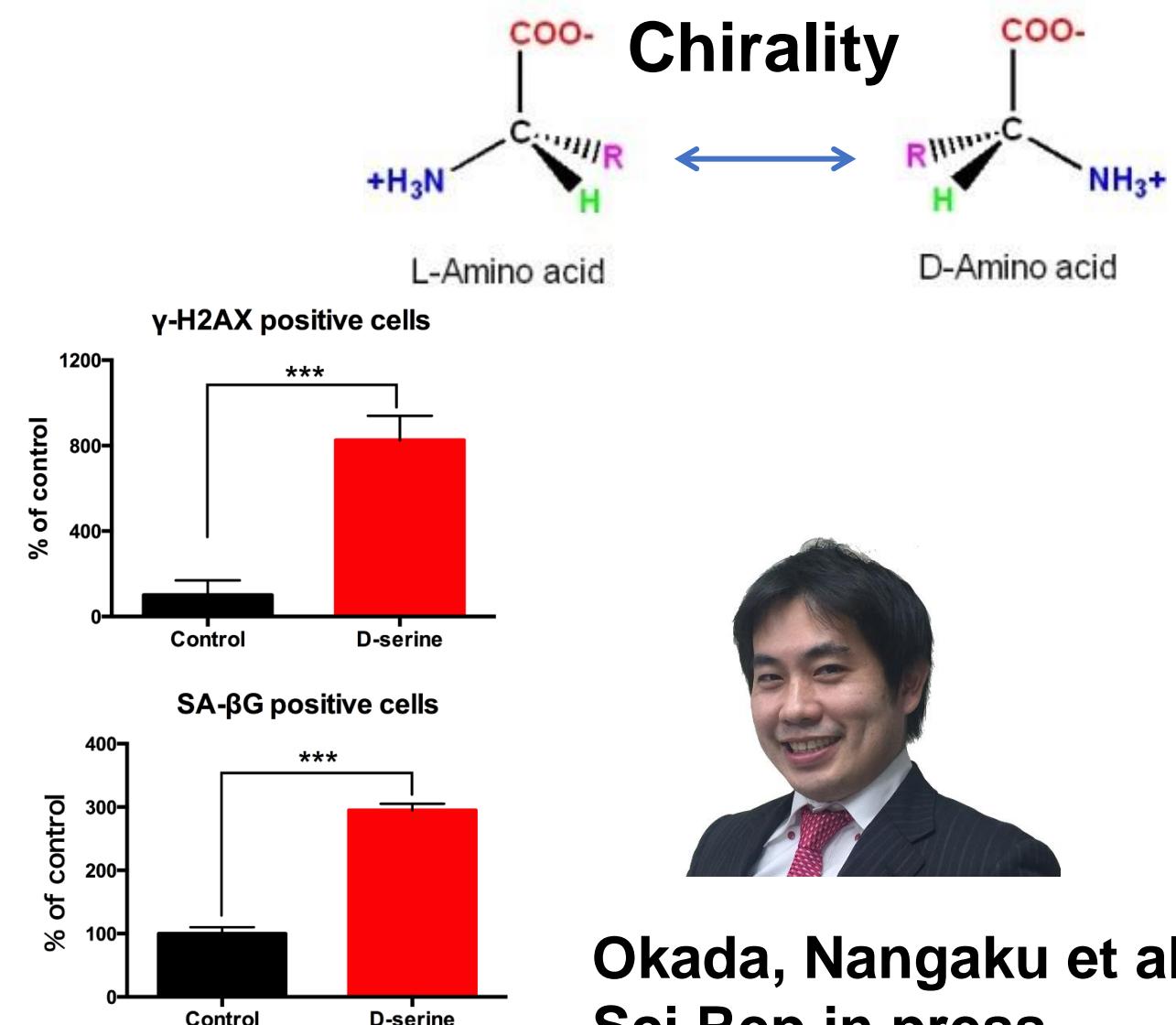
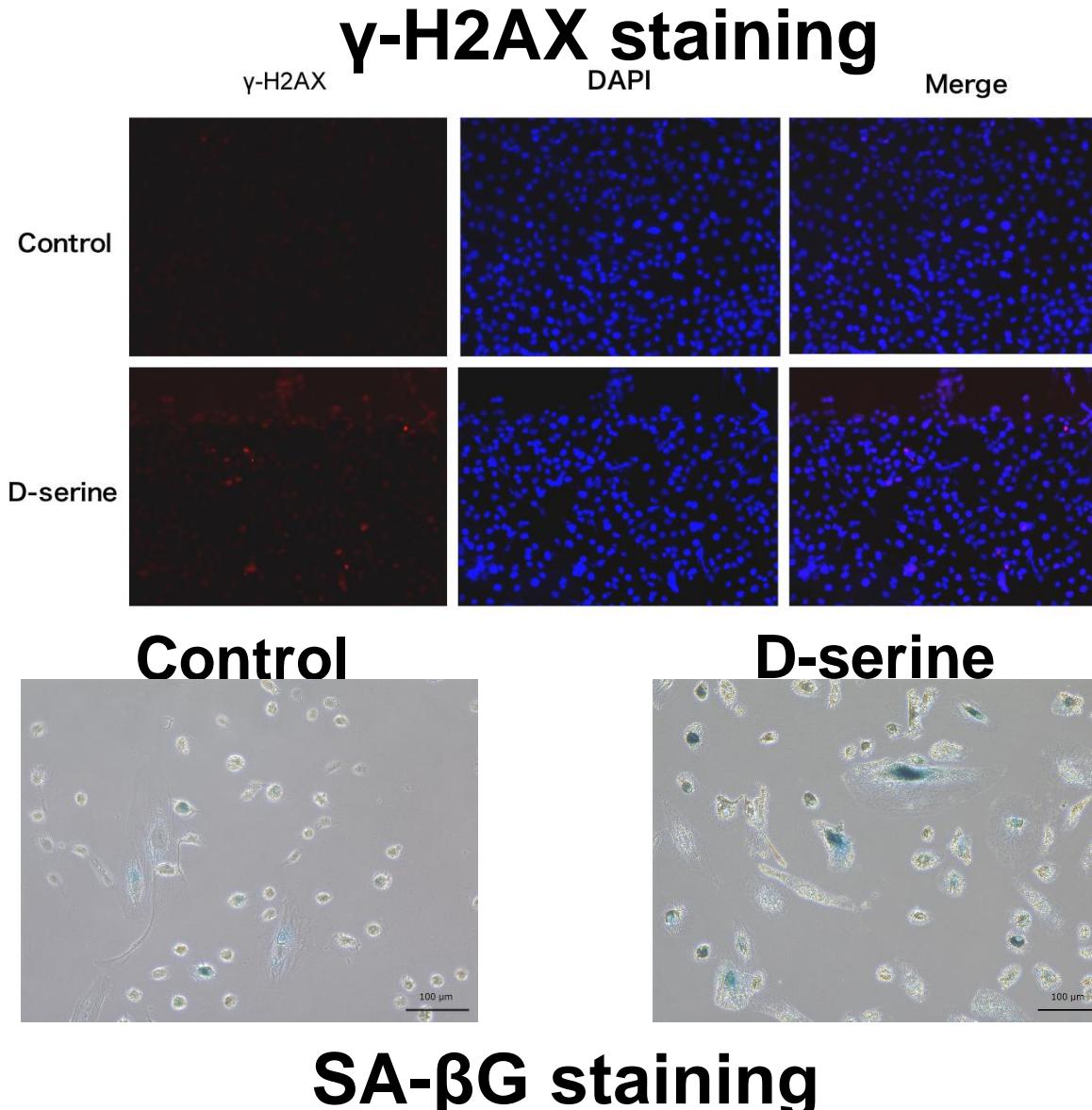
**Gregg L. Semenza**

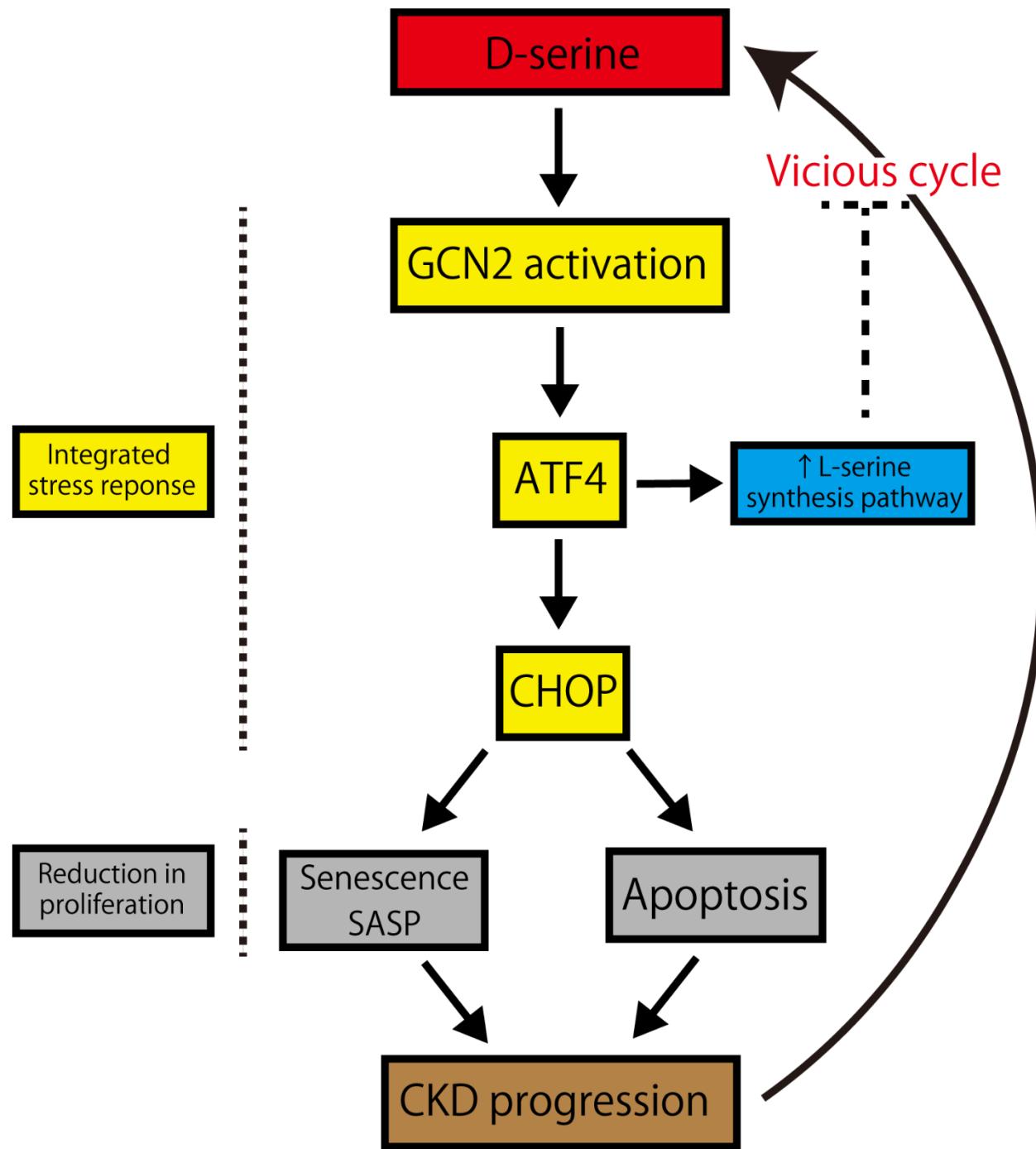
## Progression of kidney disease to ESRD.



Modified from Mimura, Tanaka, & Nangaku. Semin Nephrol 2013

# D-serine accelerates tubular senescence

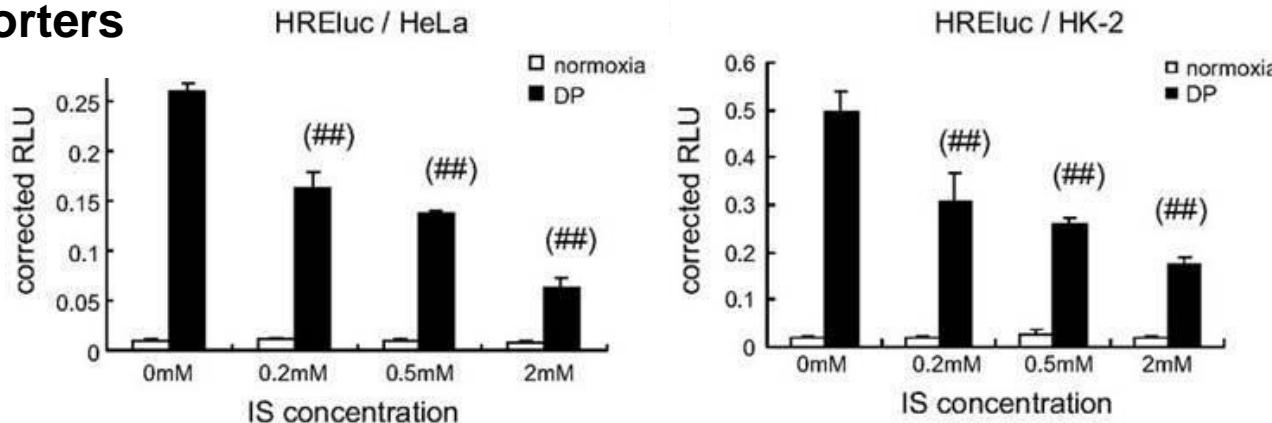




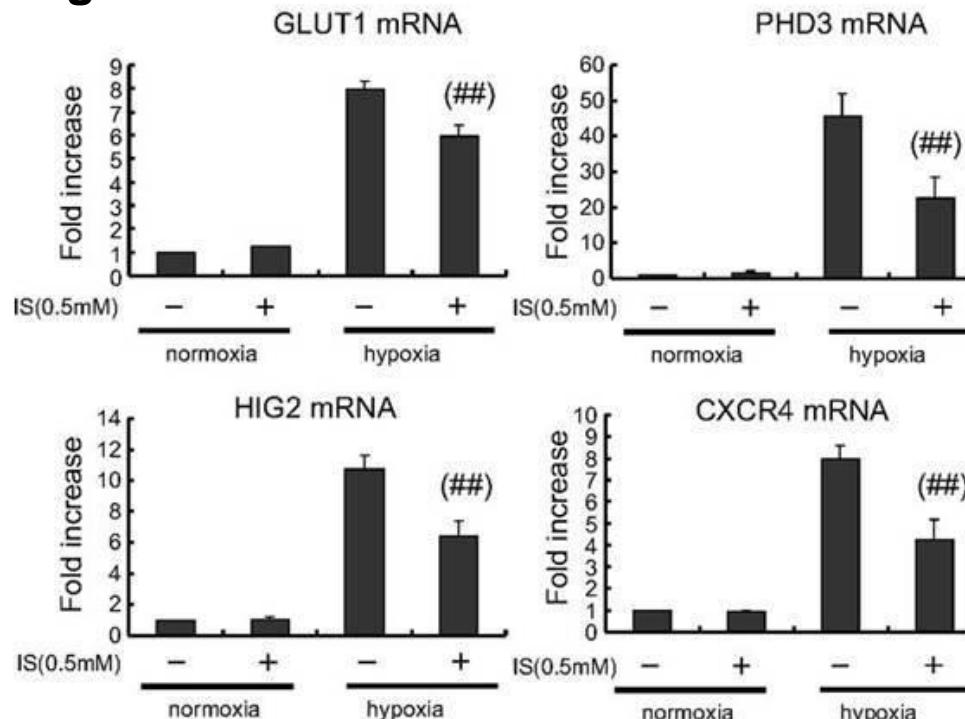
Okada, Nangaku et al.  
Sci Rep in press

# Indoxyl sulfate suppresses HIF activity

## reporters



## HIF targets

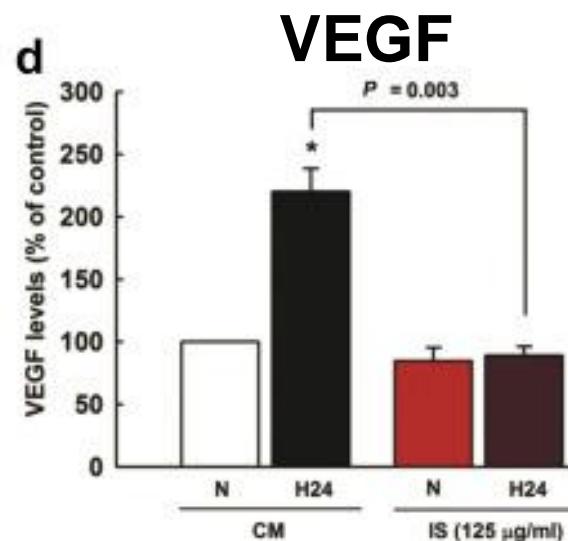
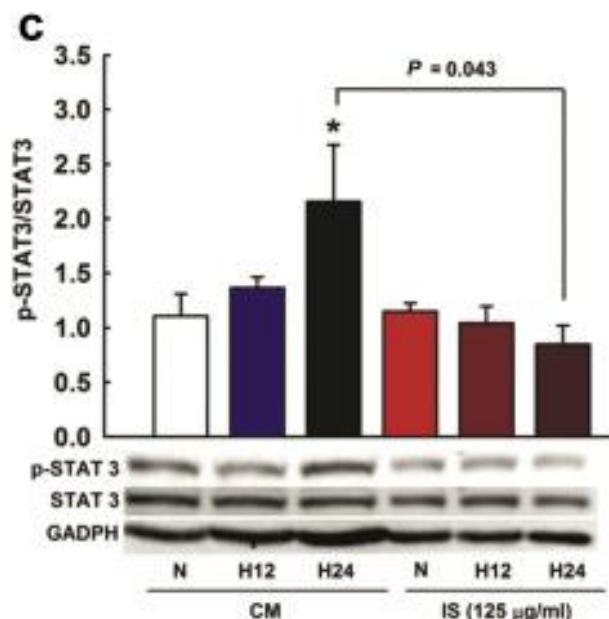
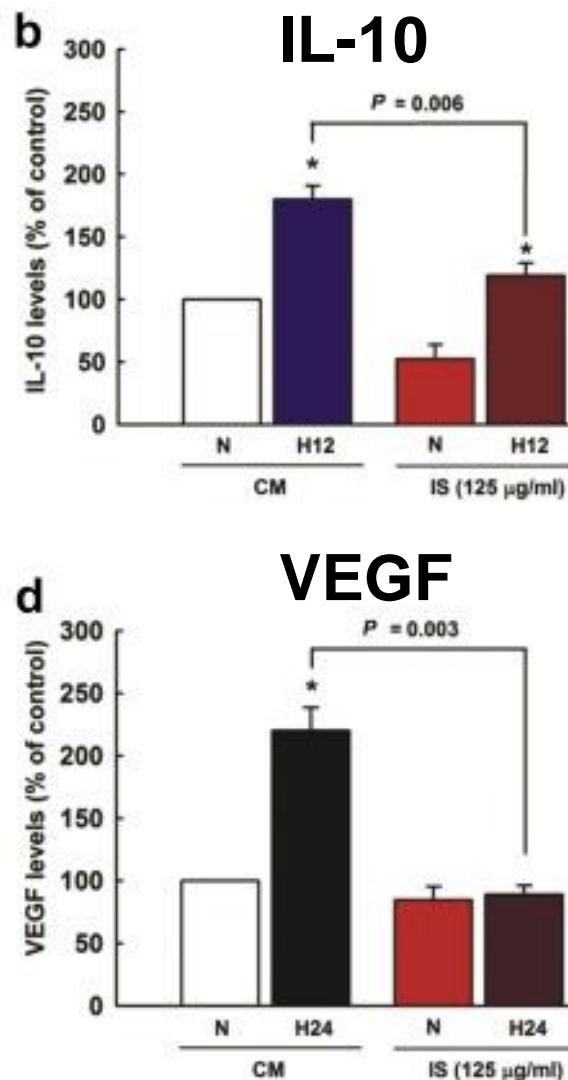
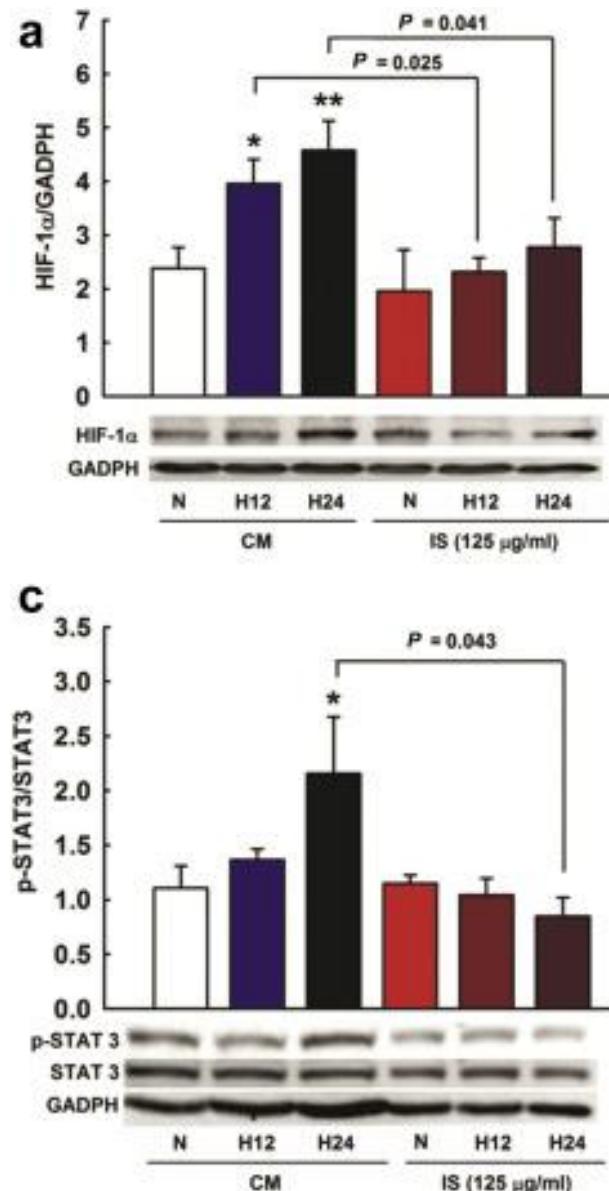


**IS: indoxyl sulfate**

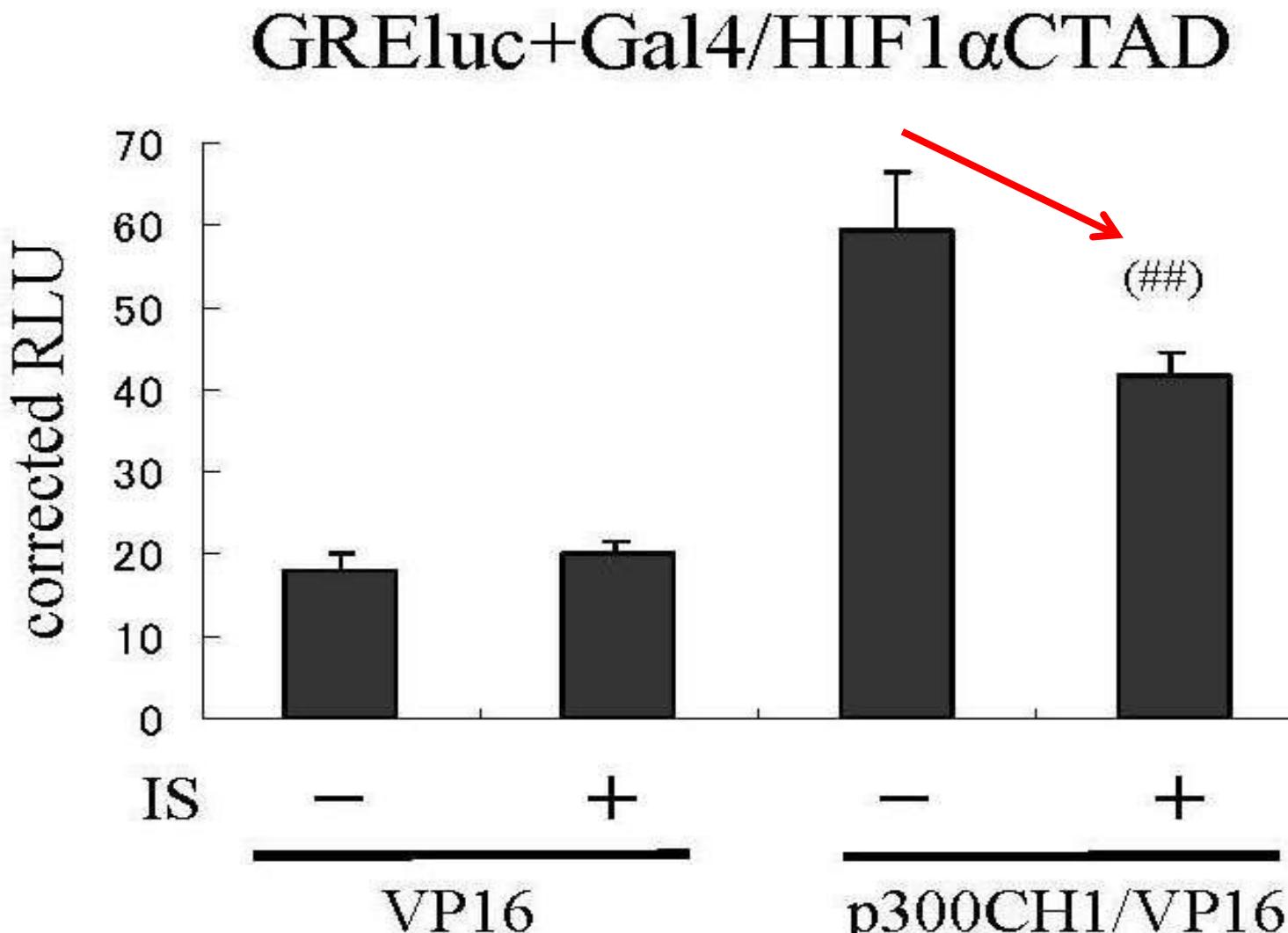


Tanaka, Nangaku et al.  
FASEB J 2013

# Suppression of HIF and its targets by indoxyl sulfate

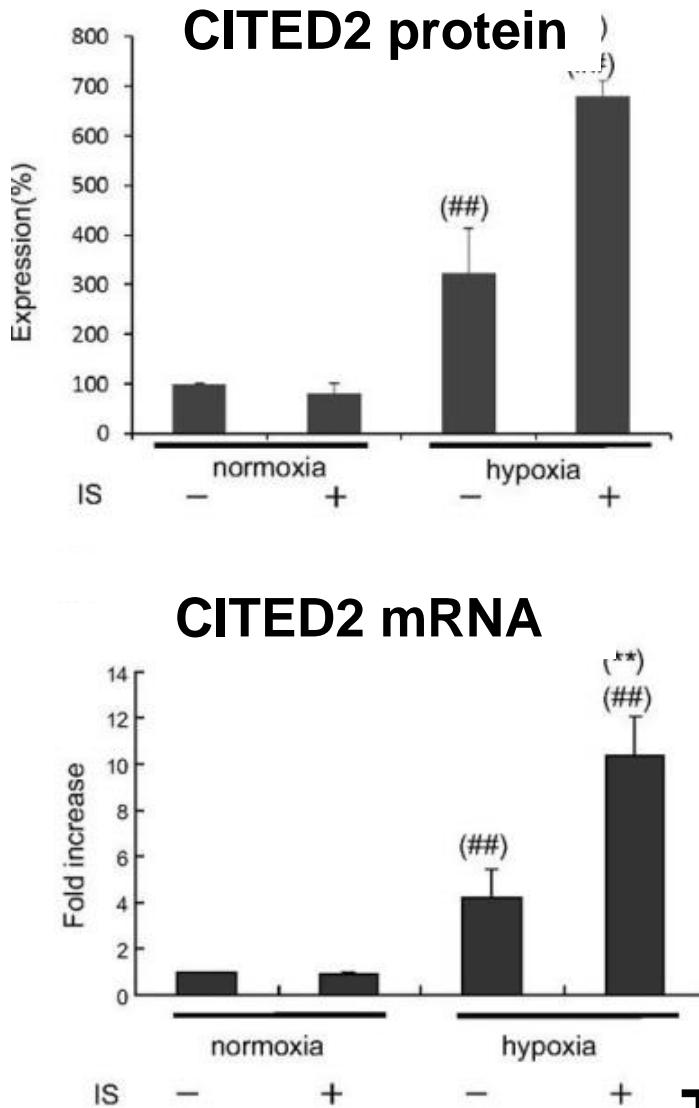


# Indoxyl sulfate inhibits binding of HIF to its co-factor, p300

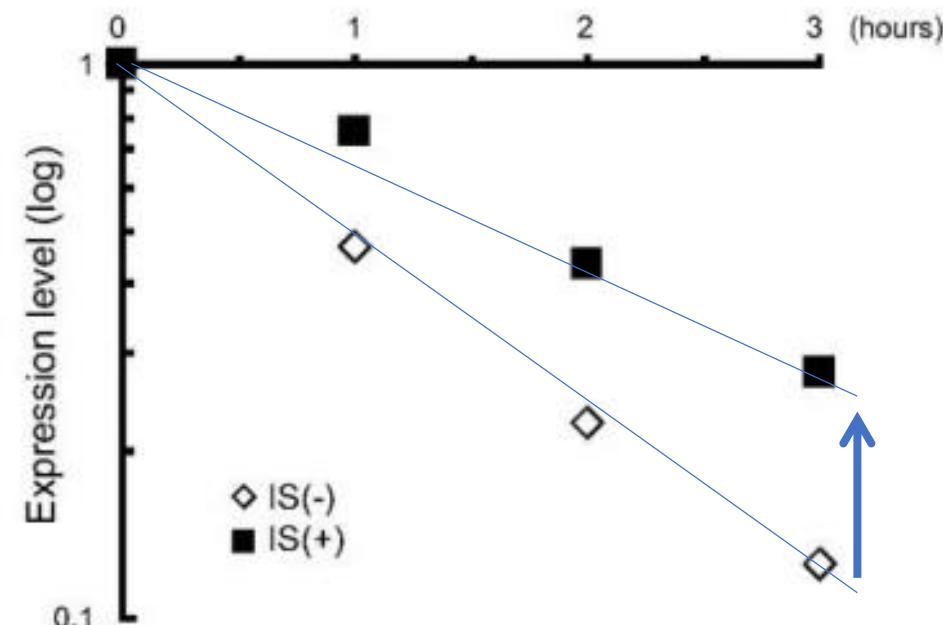


Tanaka, Nangaku et al.  
FASEB J 2013

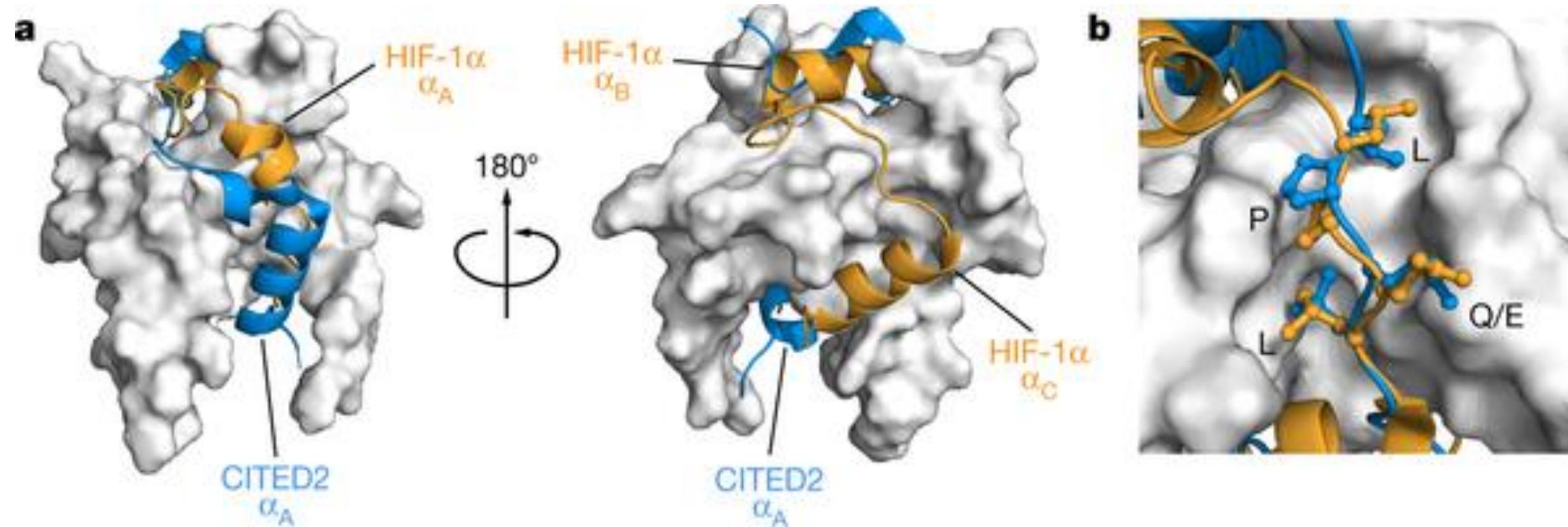
# Indoxyl sulfate induces HIF-inhibiting CITED2 protein



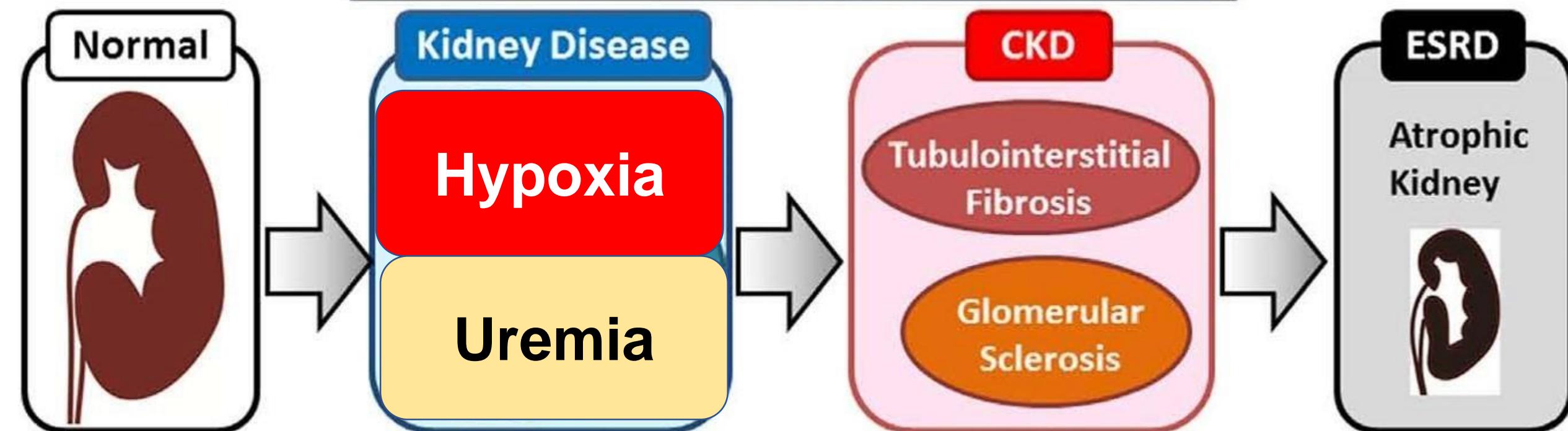
**Increased CITED2 mRNA stability by IS**



# The efficient displacement of HIF-1 $\alpha$ from its complex with CBP/p300 by CITED2 is kinetically driven and proceeds through a transient ternary intermediate

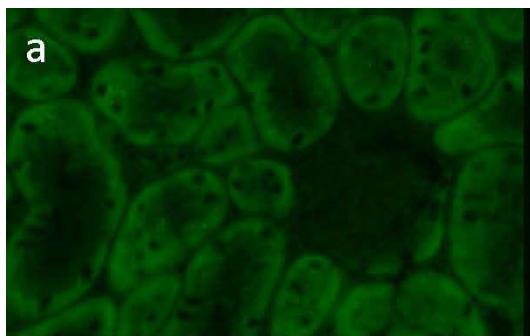


## Progression of kidney disease to ESRD.

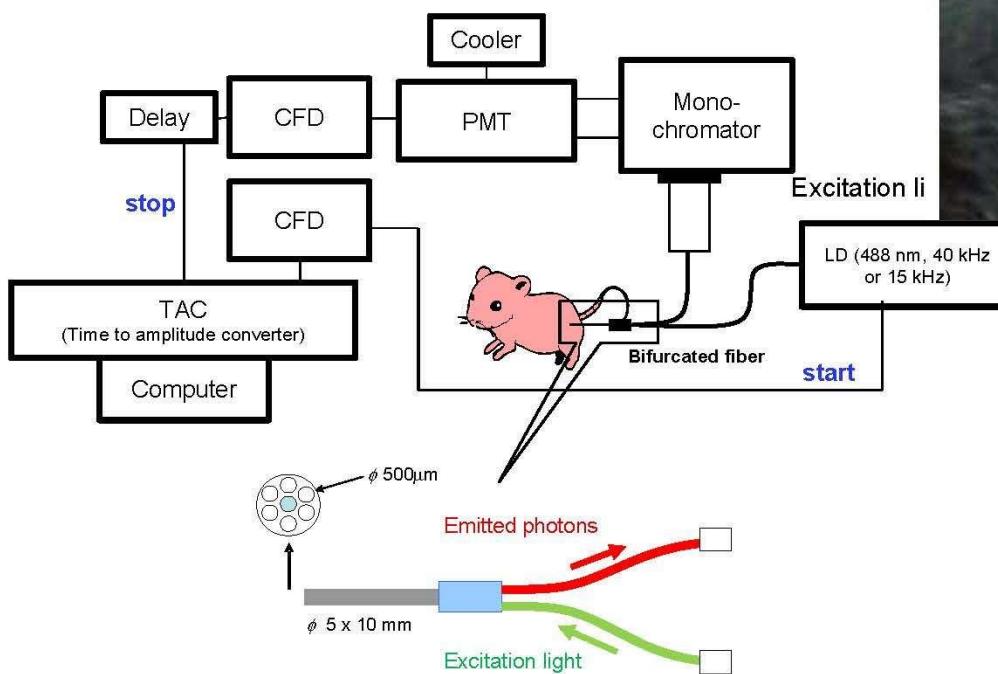


Modified from Mimura, Tanaka, & Nangaku. Semin Nephrol 2013

# Measurement of oxygen tension in the kidney utilizing a novel phosphorescence probe

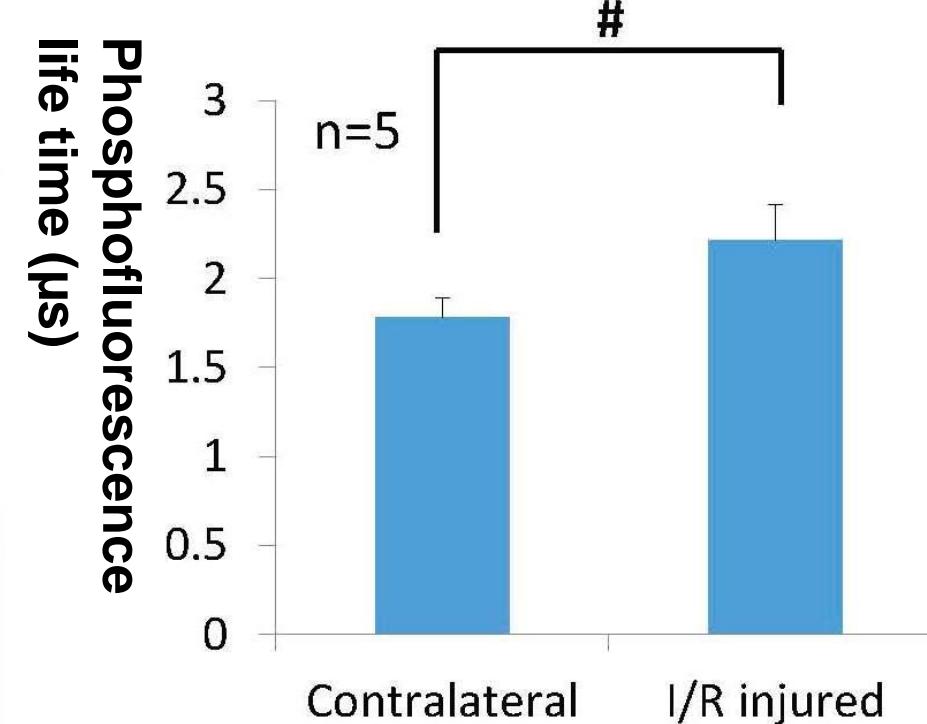
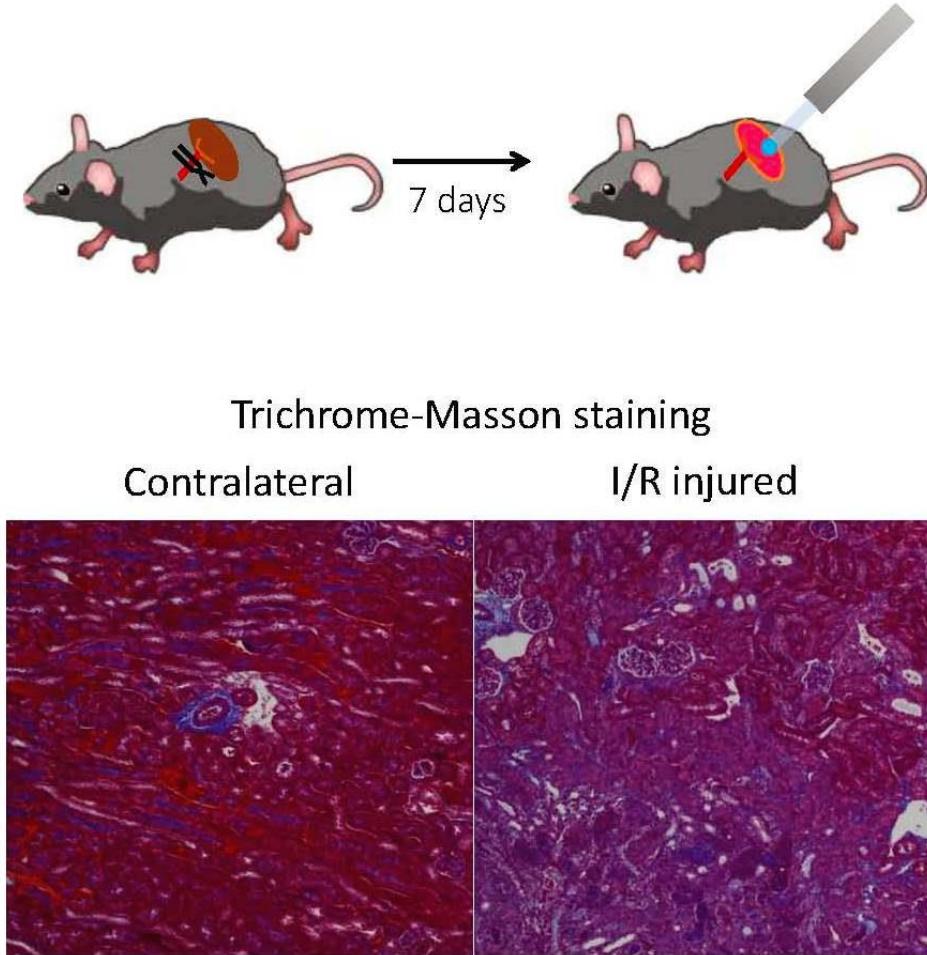


c  
(TCSPC system)



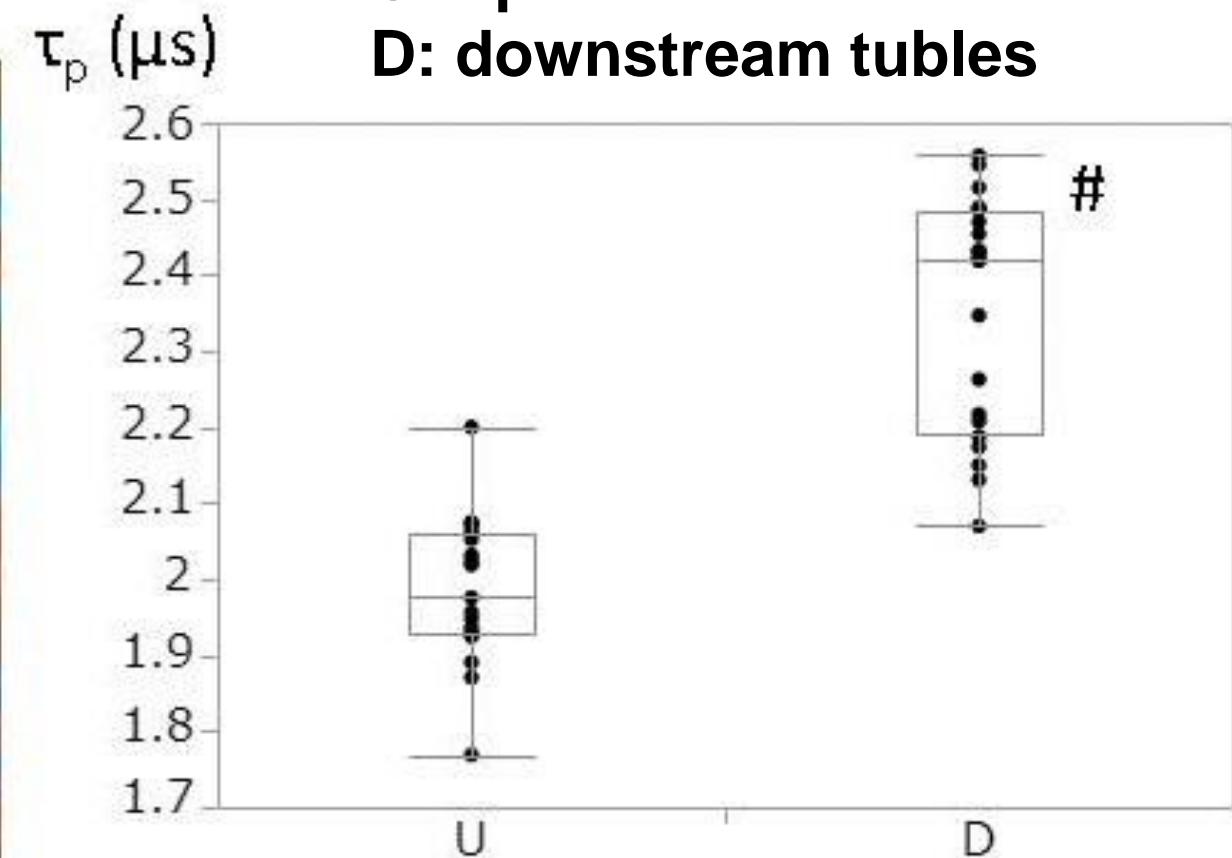
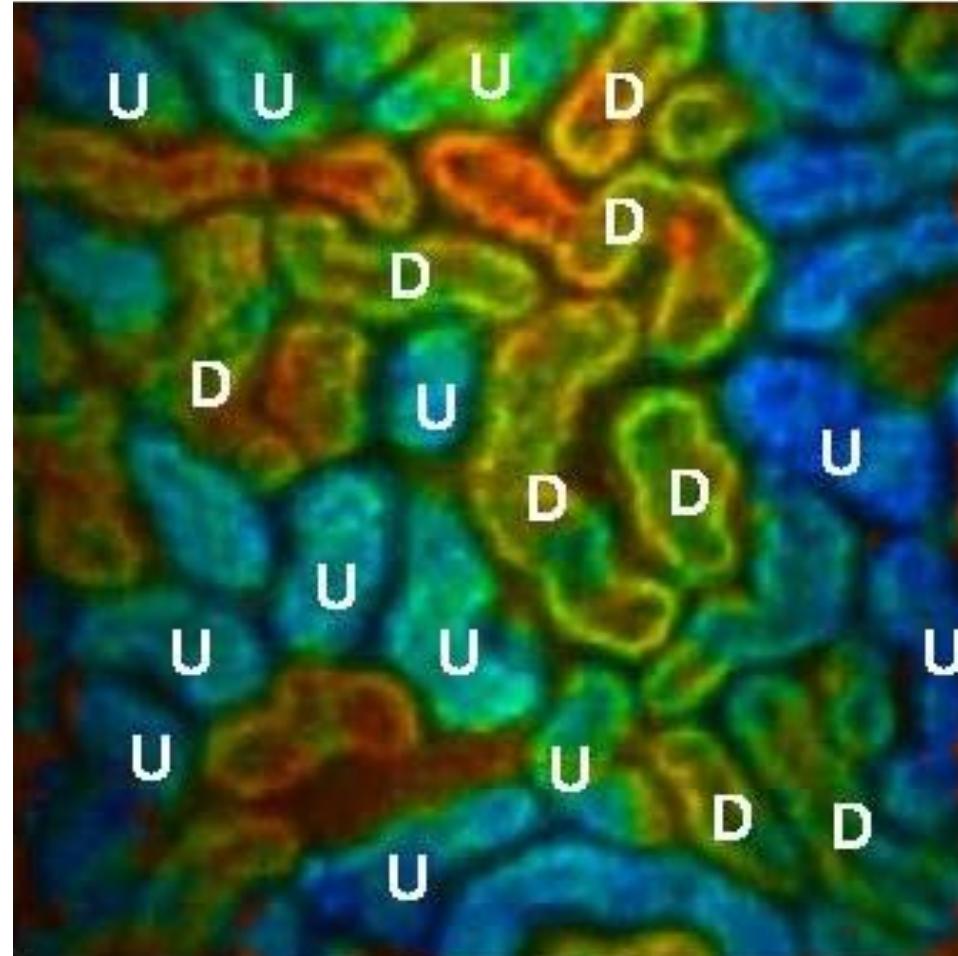
Hirakawa, Nangaku et al. Sci Rep 2015

# Phosphofluorescent probe to detect hypoxia



# :  $P < 0.05$  by paired two-tailed t-test

# S1 segment has higher oxygen tension compared with S2 segment



Hirakawa & Nangaku.  
*manuscript in submission*

# **Epigenetics**

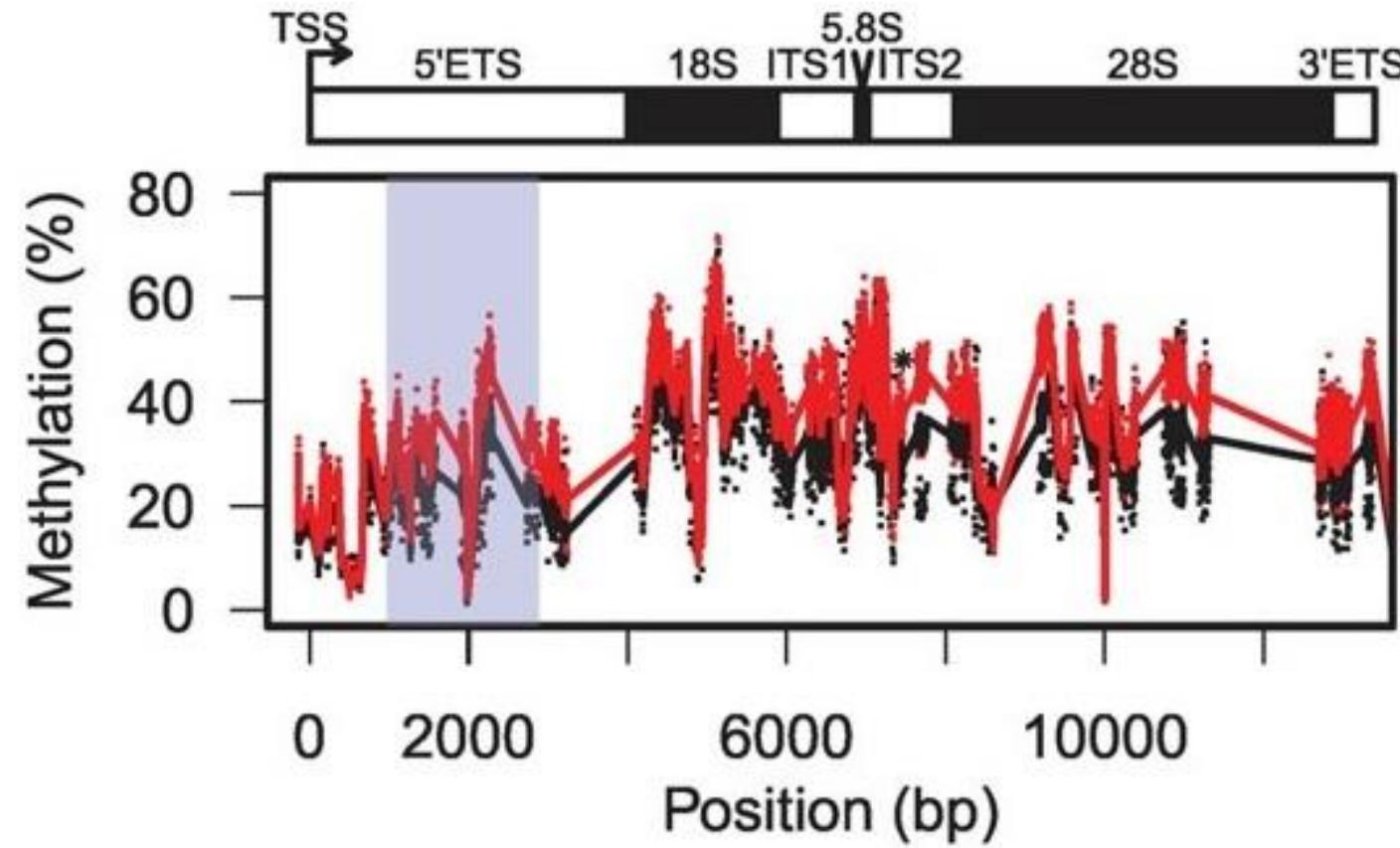
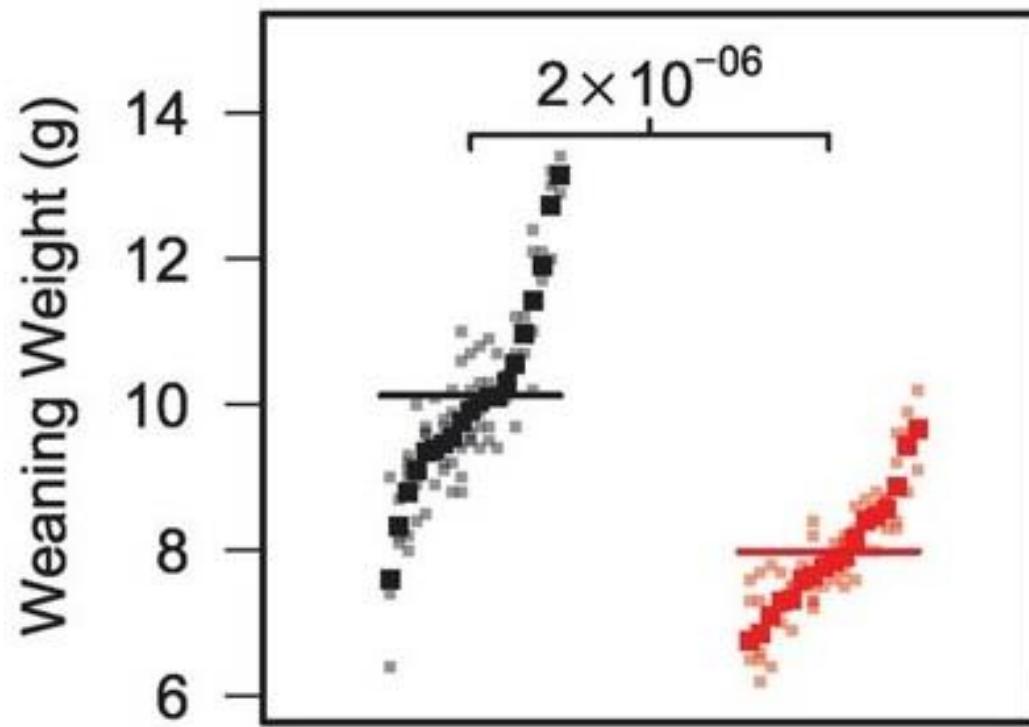
**DNA methylation**

**miRNA and lncRNA**

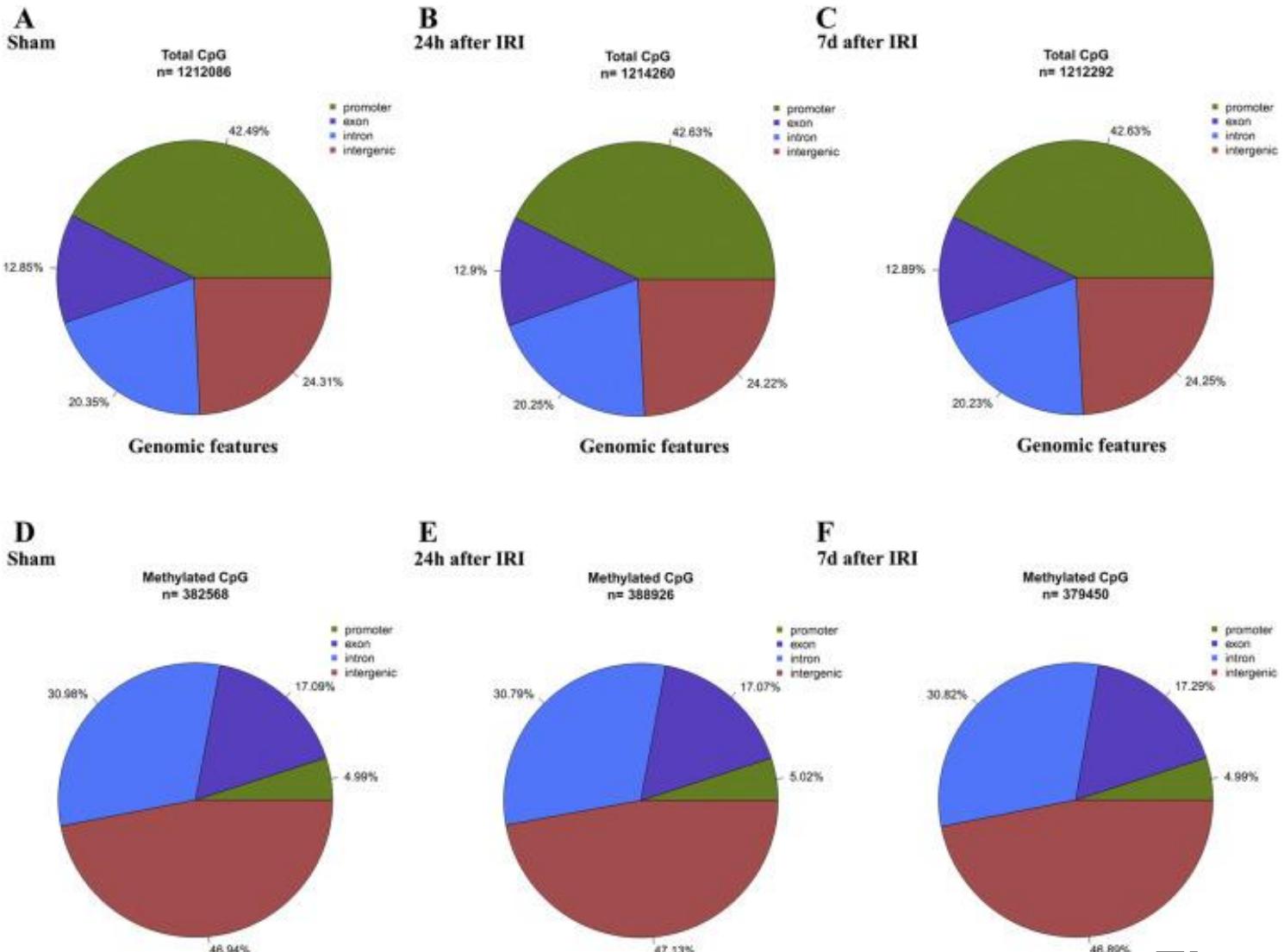
**Histone modification**

**Chromosomal conformational change**

# Maternal protein restriction diet induced less weaning weight and hypermethylation of ribosomal DNA in offspring

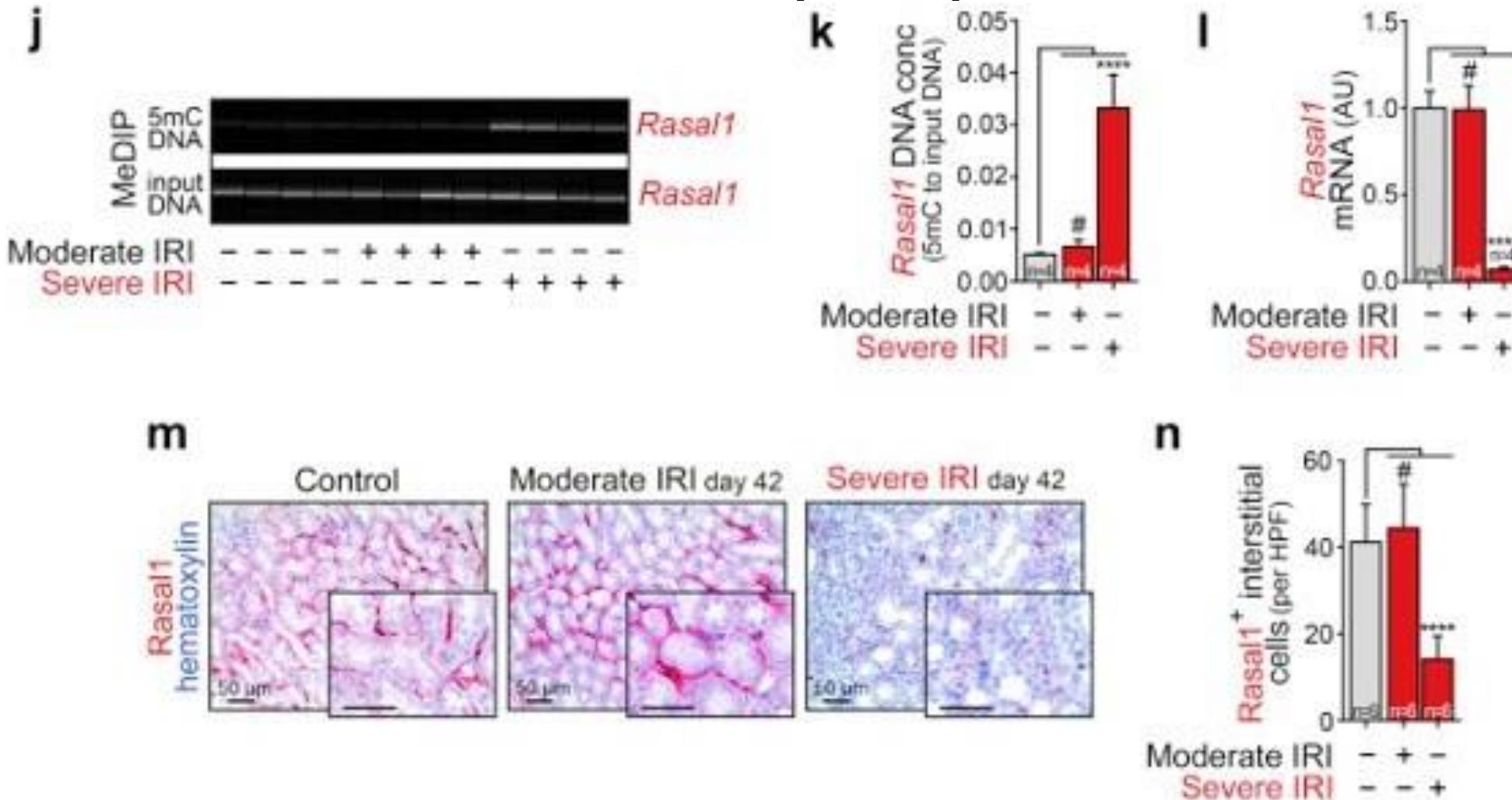


# Ischemia-reperfusion injury decreased the genome-wide methylation level and the CpG methylation level



# Aberrant *Rasal1* promoter methylation contributes to sustained fibroblast activation and AKI-to-CKD progression

## *Rasal1*: Ras-Gap-like protein-1



# **Epigenetics**

DNA methylation

**miRNA and lncRNA**

Histone modification

Chromosomal conformational change

# Long non-coding RNAs – towards precision medicine in diabetic kidney disease?

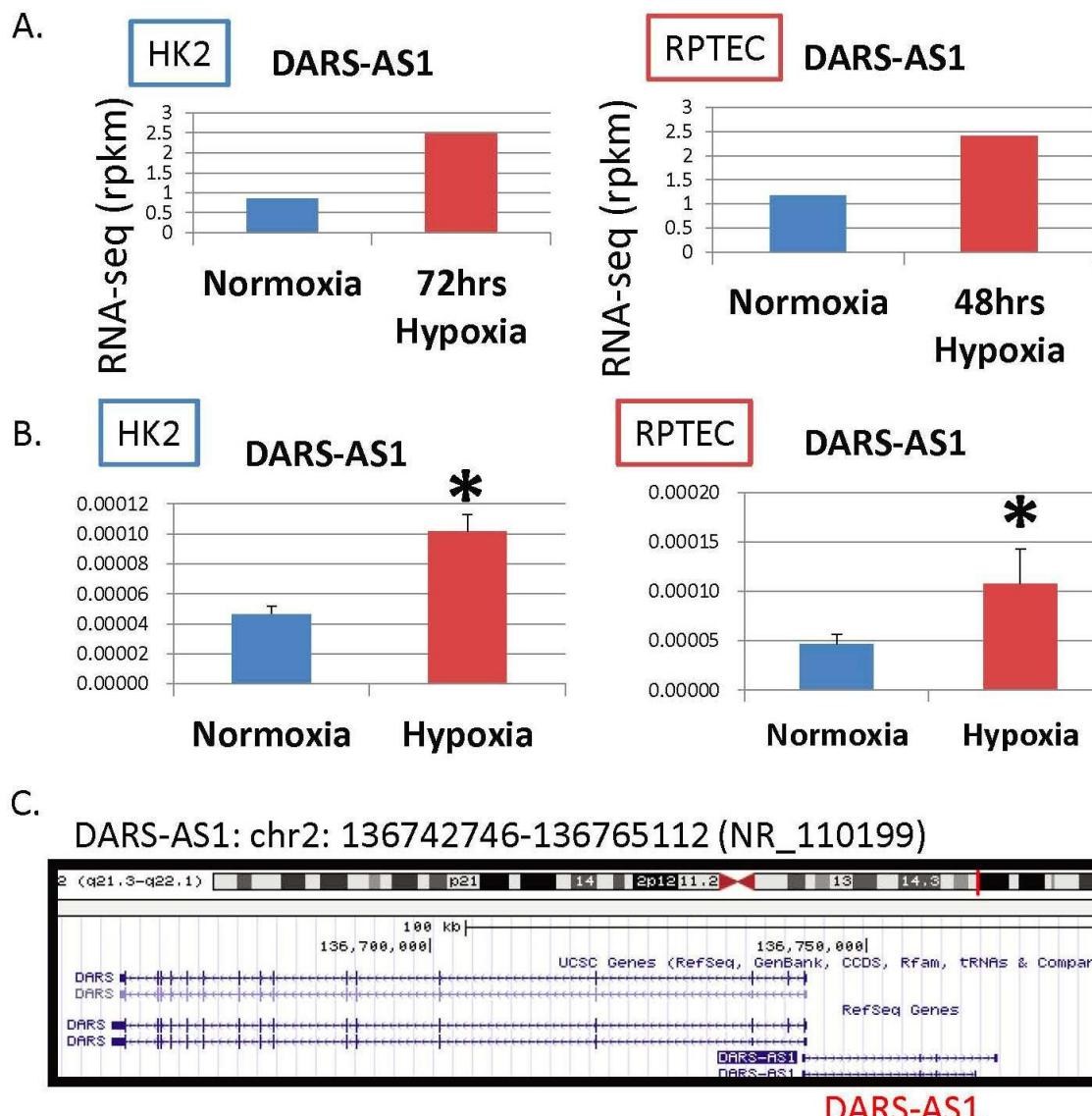
Usha Panchapakesan\* and Carol Pollock\*

\*Renal Research Group, Kolling Institute of Medical Research, Royal North Shore Hospital, University of Sydney, NSW 2065, Australia

**A clear advantage of targeting lncRNA rather than epigenetic-related enzymes or other non-coding RNA such as miRNA is the direct sequence specificity of the target site.**

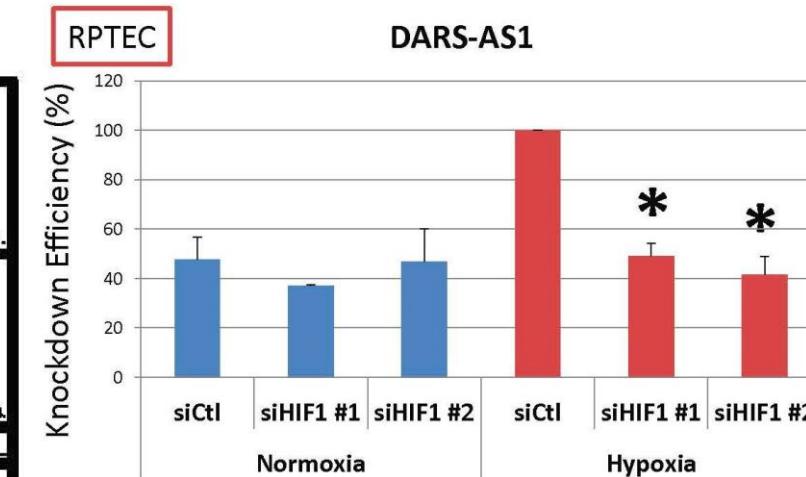
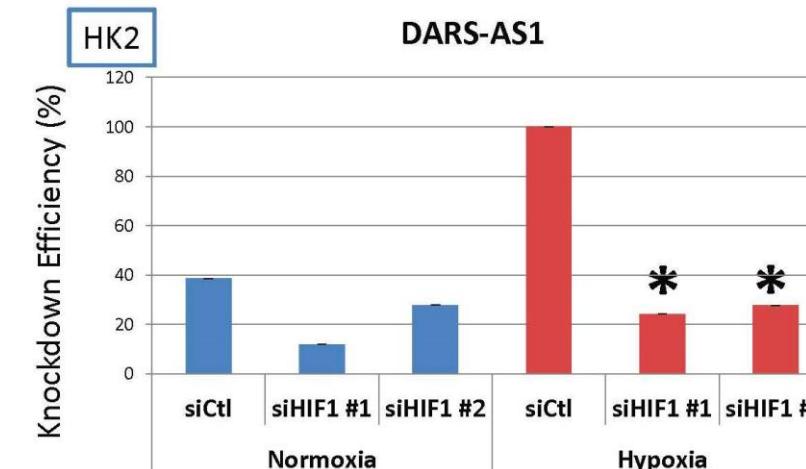
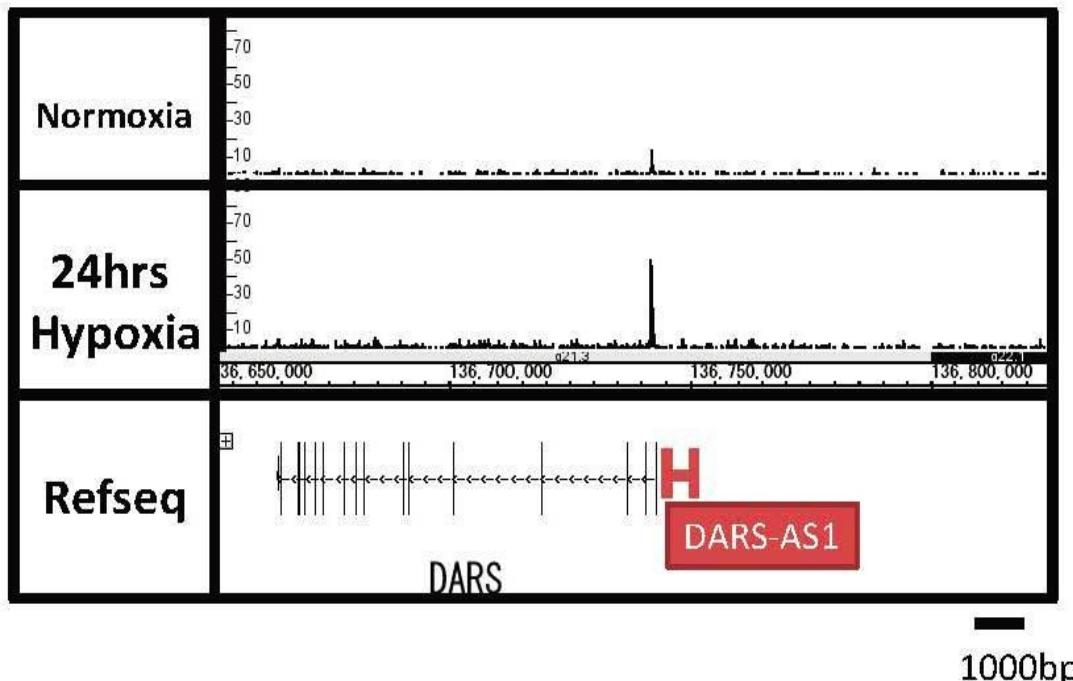
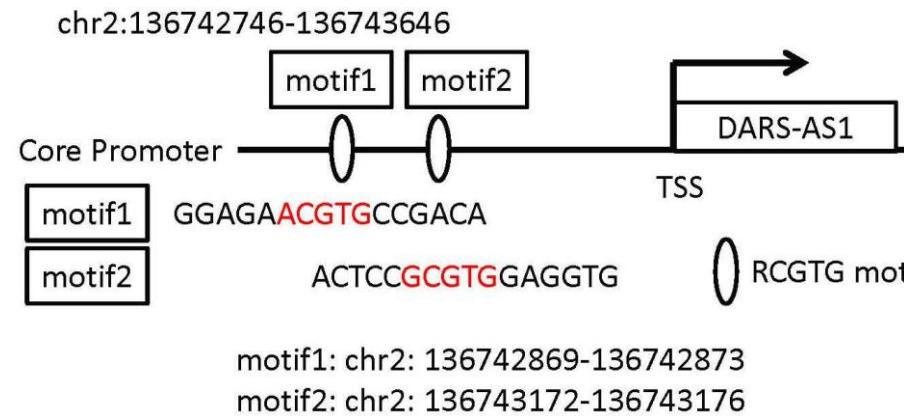
	<b>mRNA</b>	<b>Epigenetic enzymes</b>	<b>LncRNA</b>
Distribution	Ubiquitous	Ubiquitous	Cell/tissue specific
Expression	High	High	Low
Therapeutic targeting	Not specific to a target gene	Not specific to a target gene	Sequence specific

# RNA-seq revealed *DARS-AS1* as a hypoxia-induced lncRNA



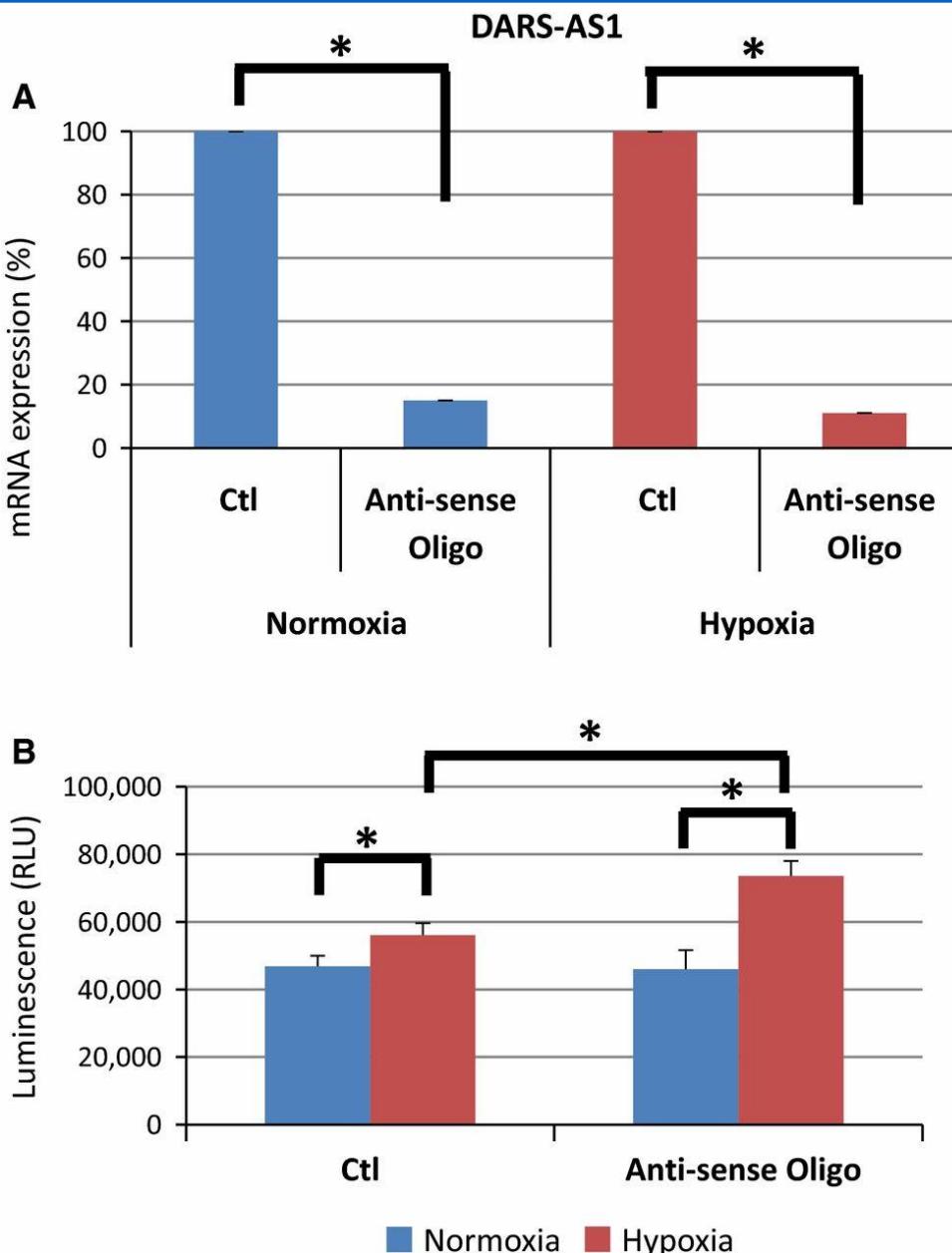
Mimura, Nangaku et al.  
Physiol Rep 2017

# DARS-AS1 is induced by HIF-1



Mimura, Nangaku et al.  
Physiol Rep 2017

# Knockdown of *DARS-AS1* aggravates apoptotic cell death



Mimura, Nangaku et al.  
Physiol Rep 2017

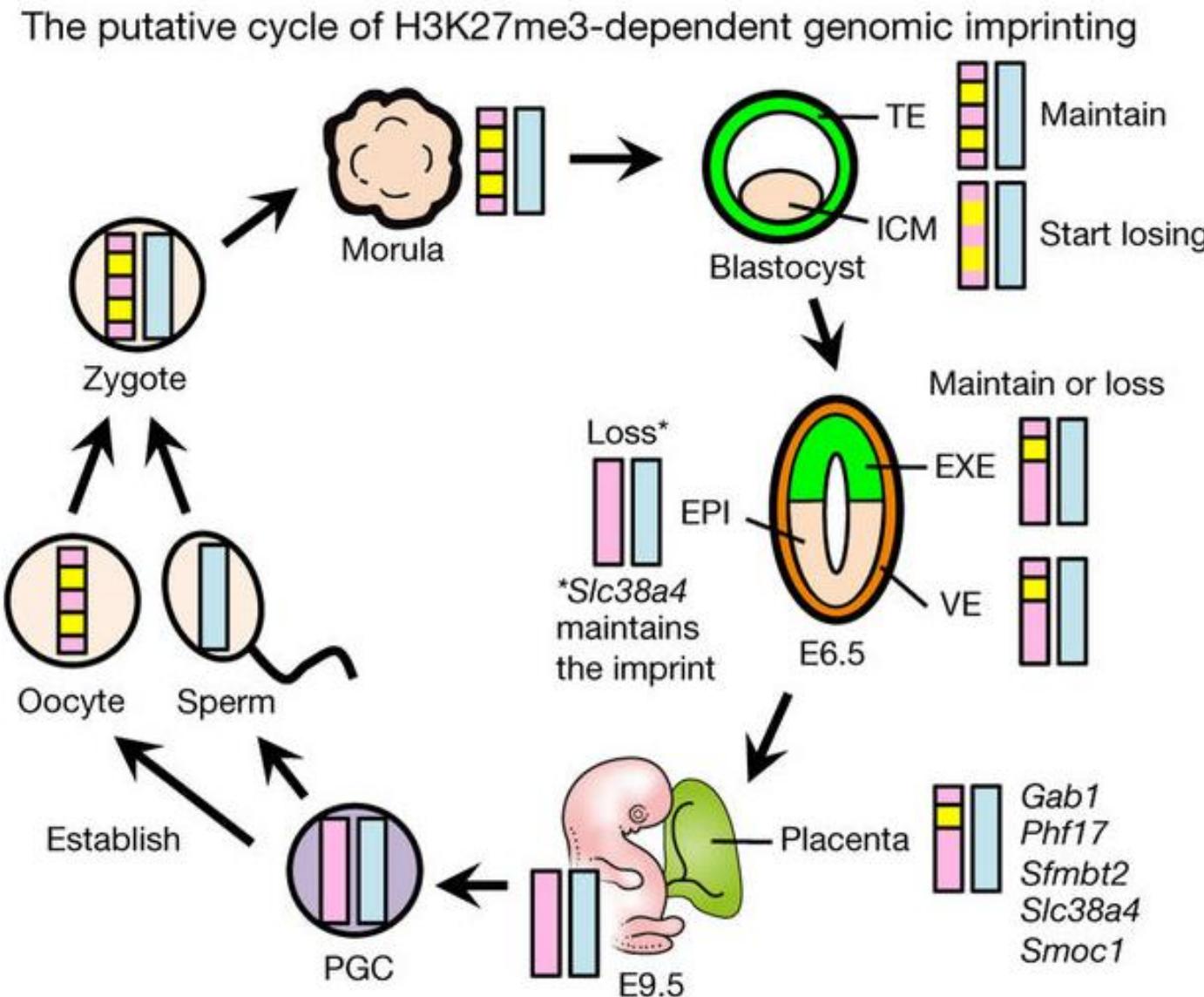
# **Epigenetics**

**DNA methylation  
miRNA and lncRNA**

**Histone modification**

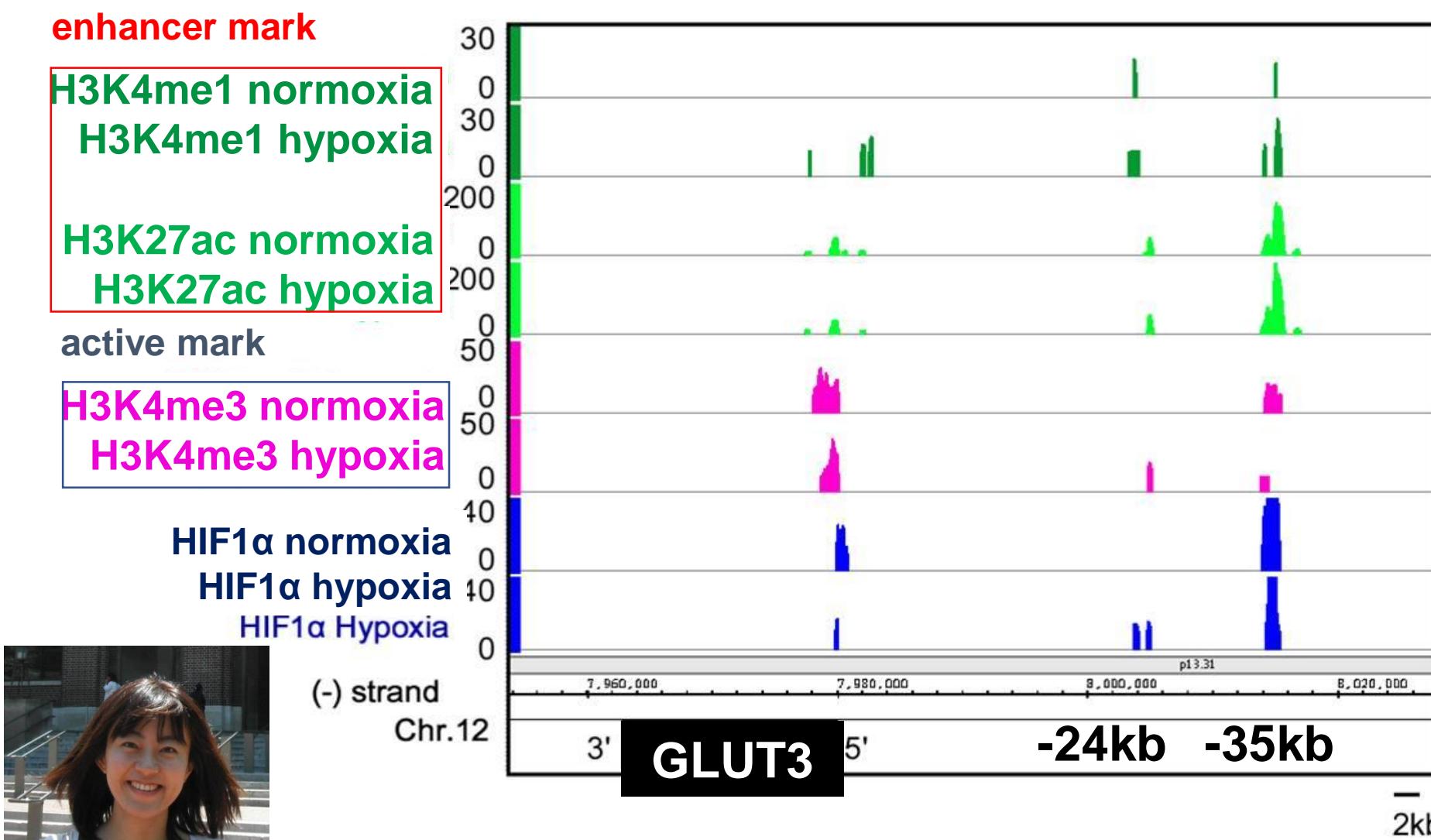
**Chromosomal conformational change**

# Maternal H3K27me3 controls DNA methylation-independent imprinting

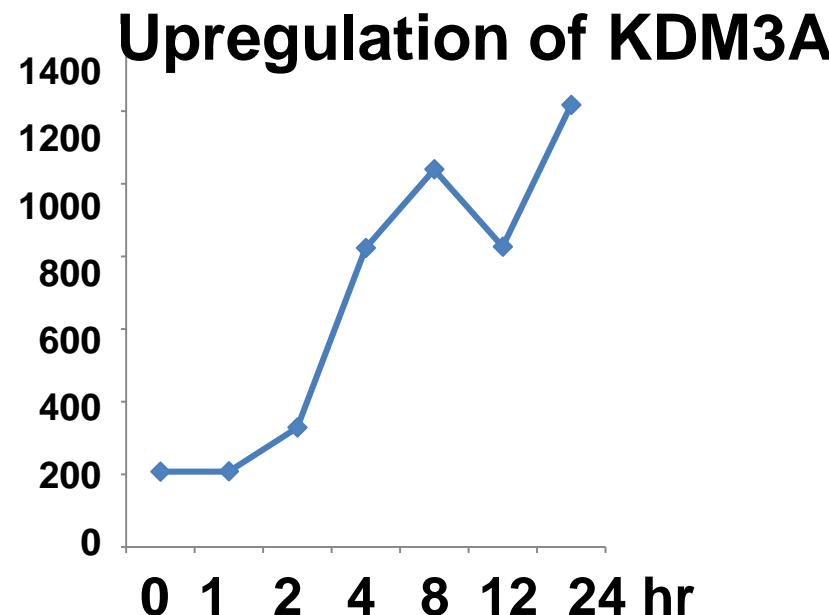


Inoue et al. Nature 2017

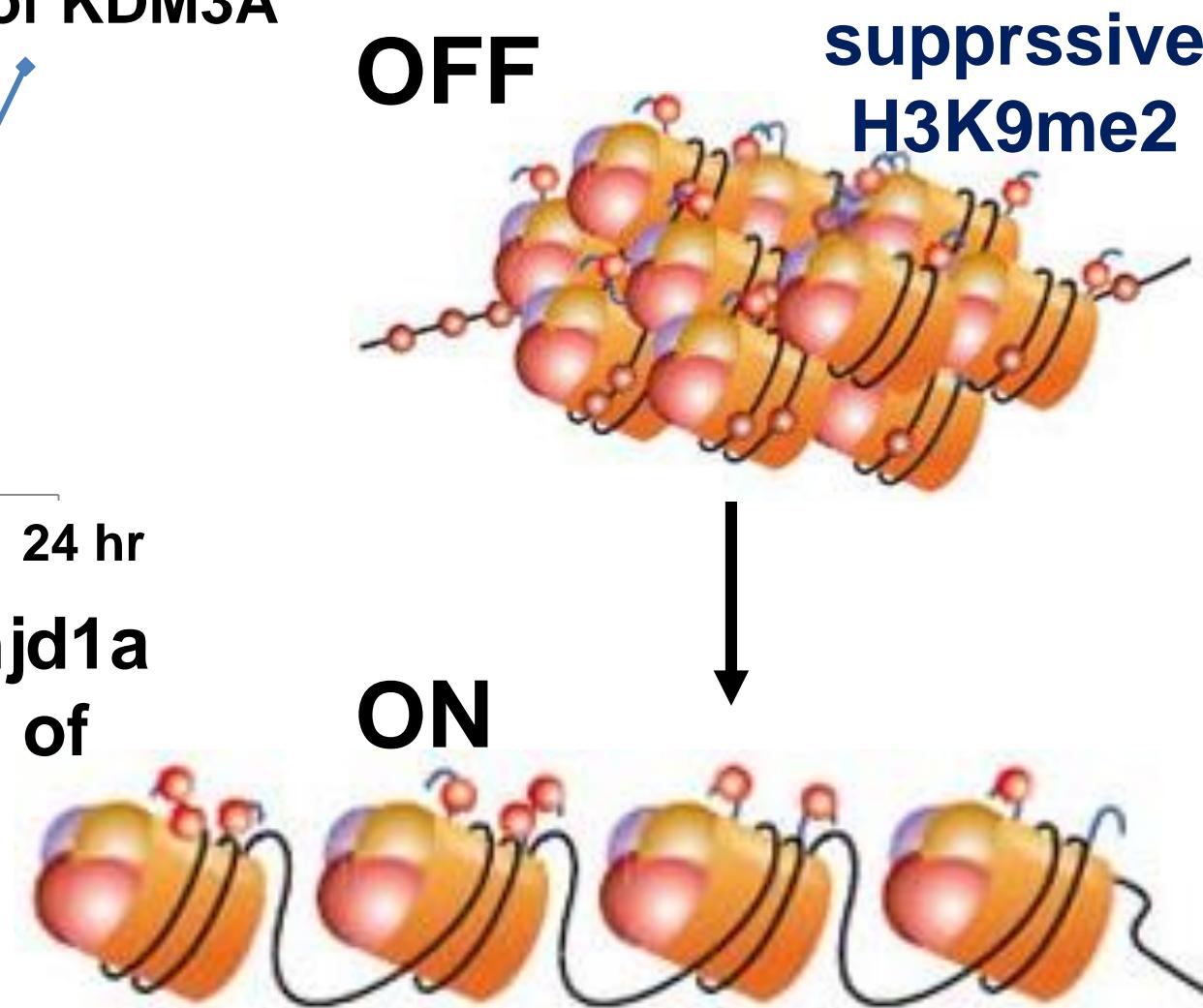
# Epigenetic modification of expression of glucose transporter 3 (GLUT3) by hypoxia



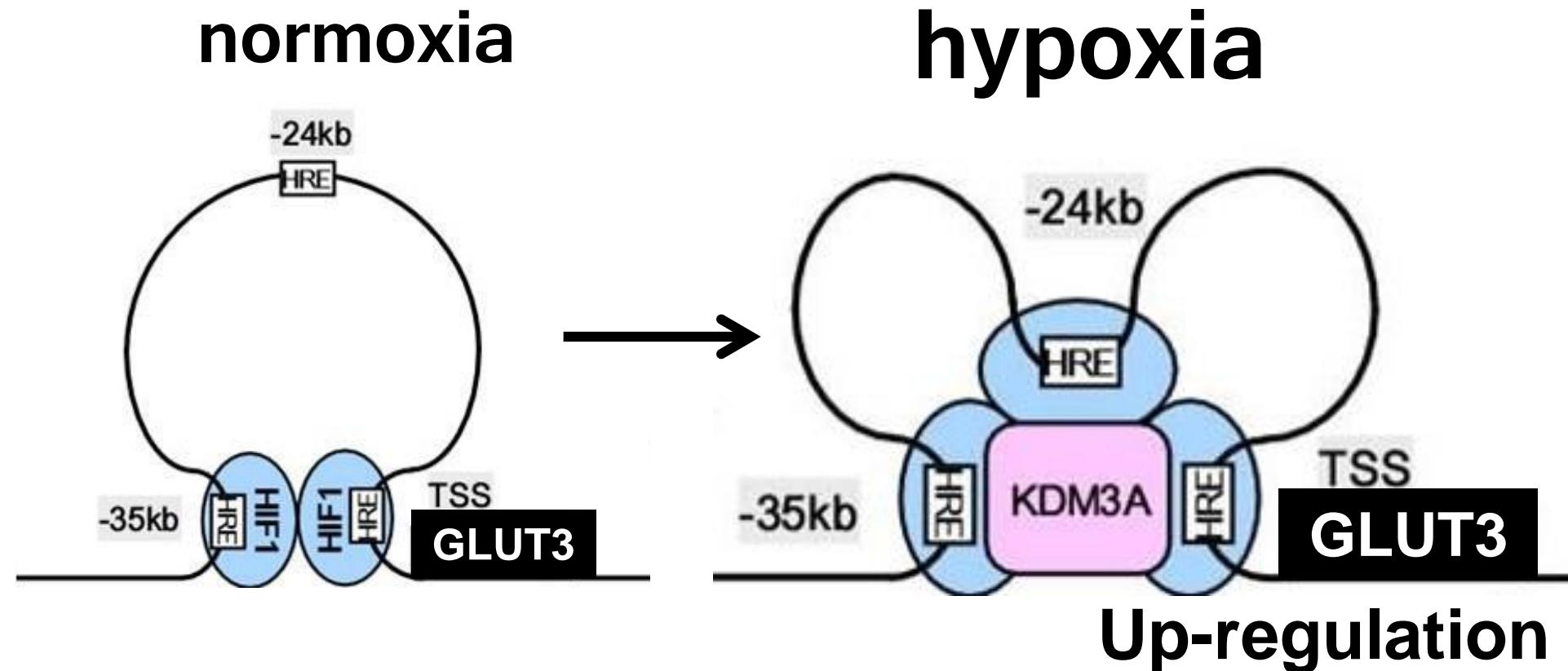
# Histone demethylation by hypoxia



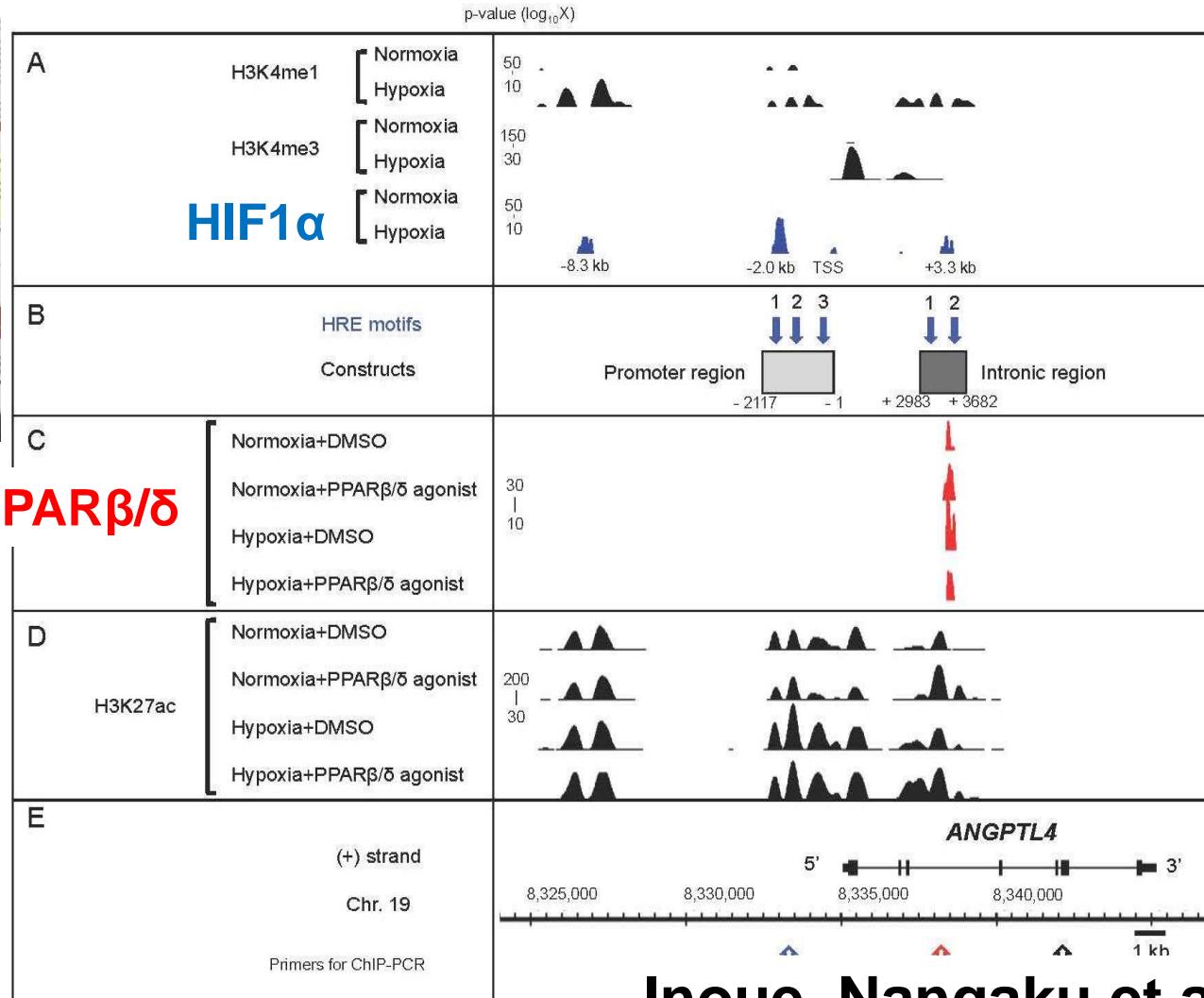
KDM3A = Jmjd1a  
demethylase of  
H3K9me2

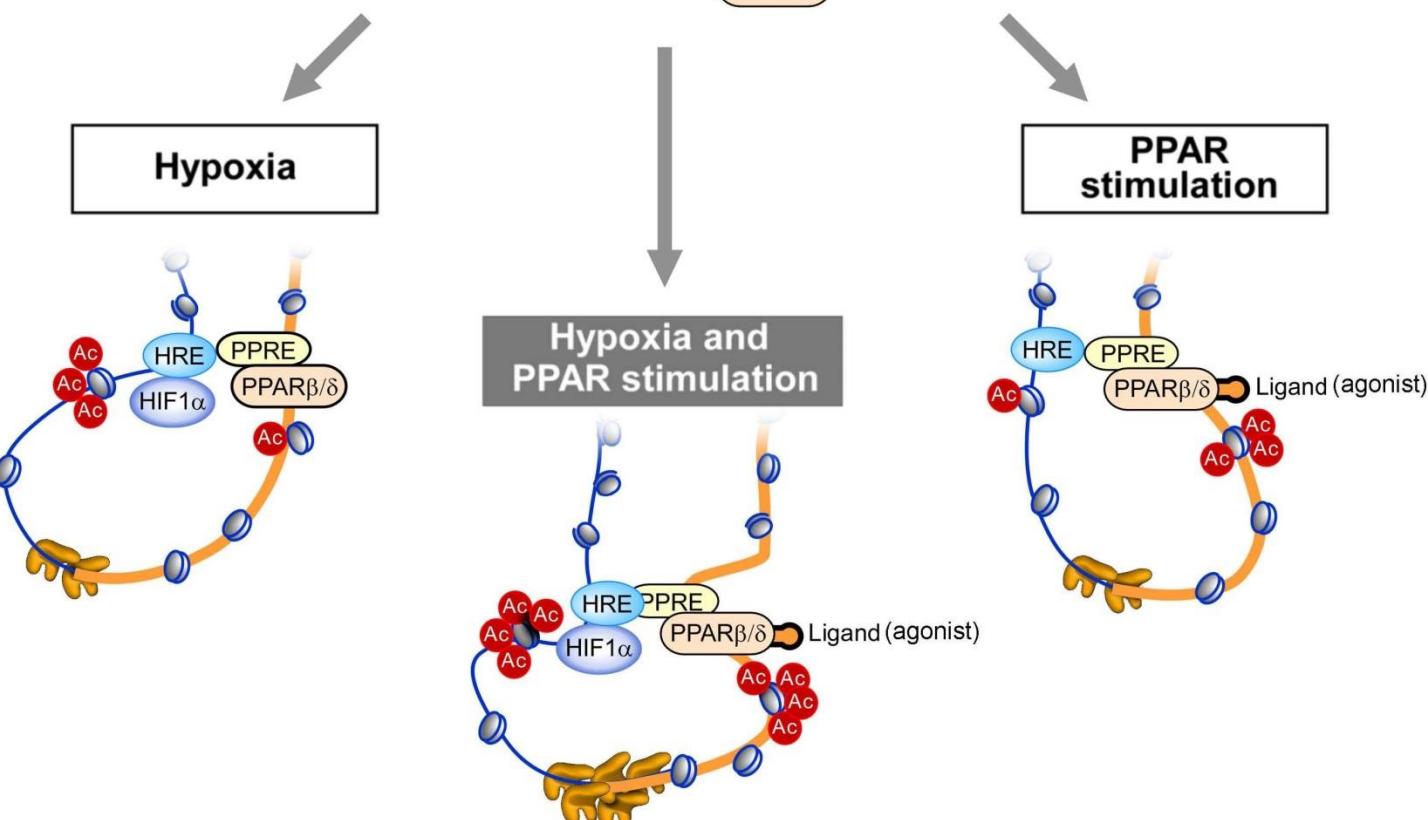
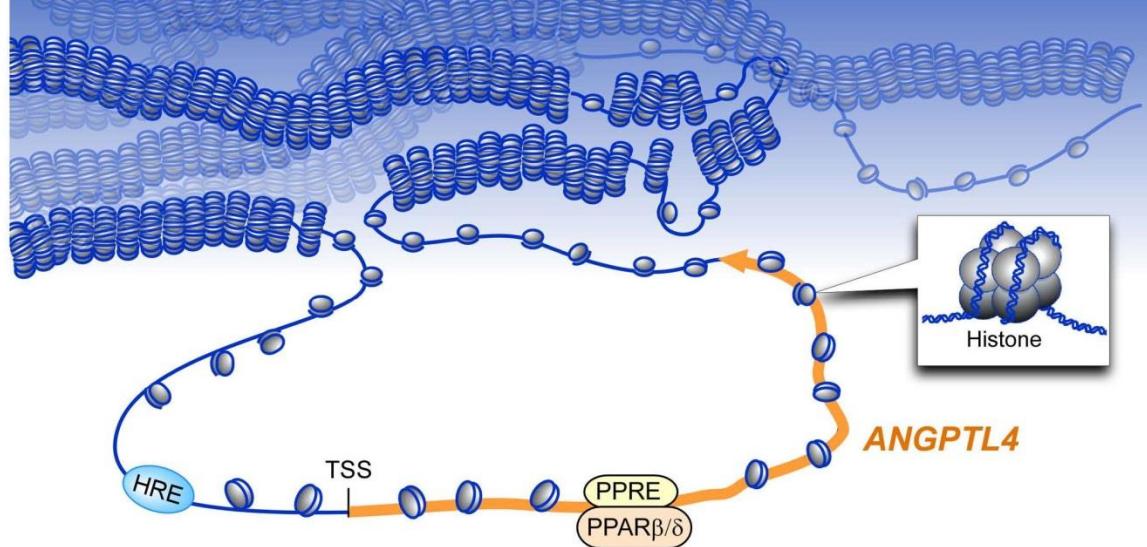


# Regulation of GLUT3 expression by hypoxia



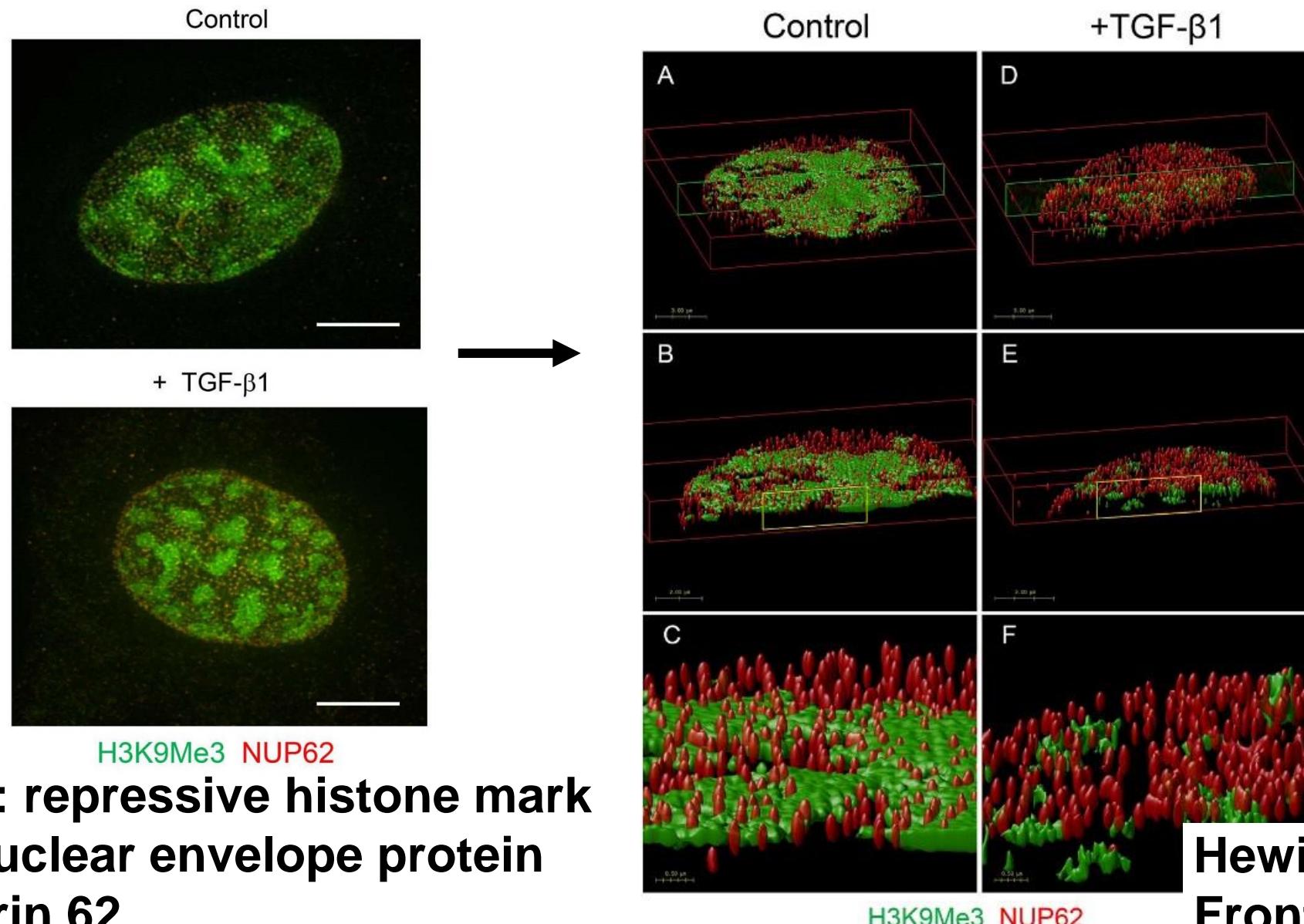
# Cross-enhancement of ANGPTL4 transcription by HIF1 and PPAR $\beta/\delta$





Inoue, Nangaku et al. Genome Biol 2014

# OMX super-resolution microscopy showed changes of the distribution of H3K9Me3 marks in NRK-52e cells by TGF- $\beta$ 1

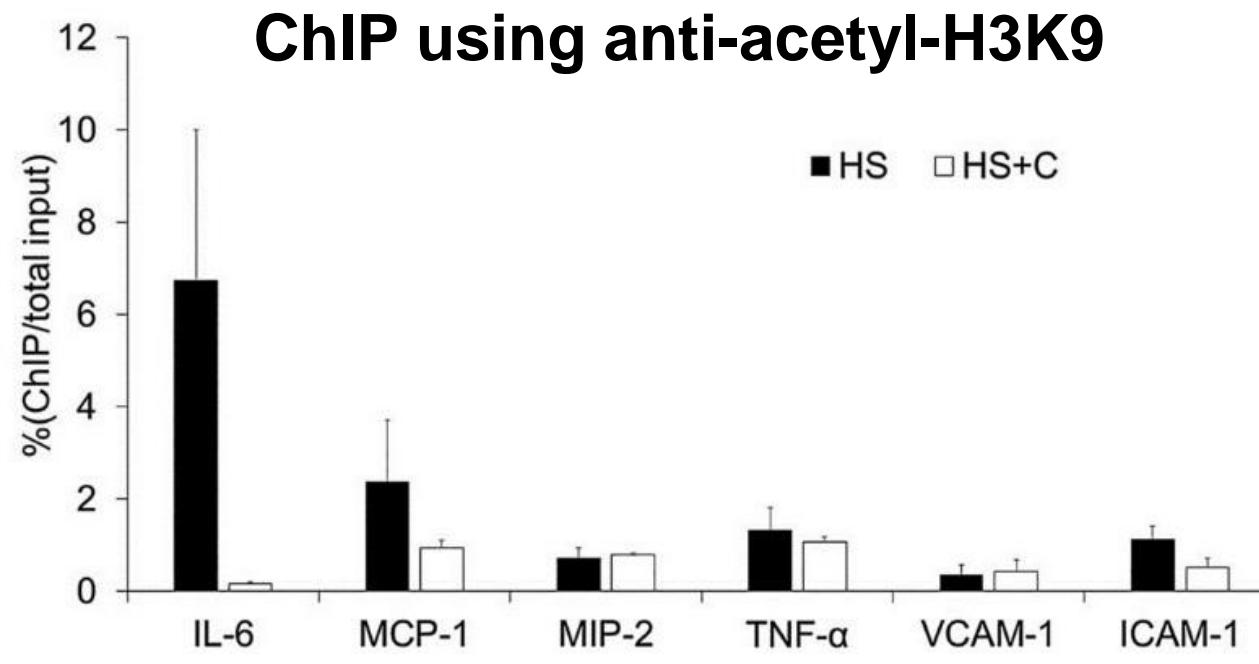
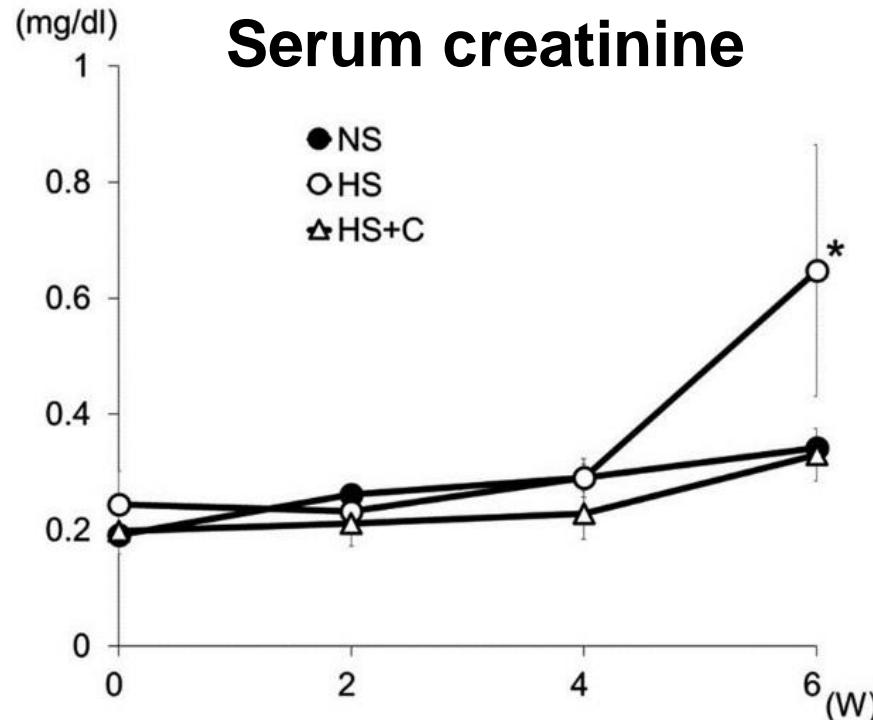
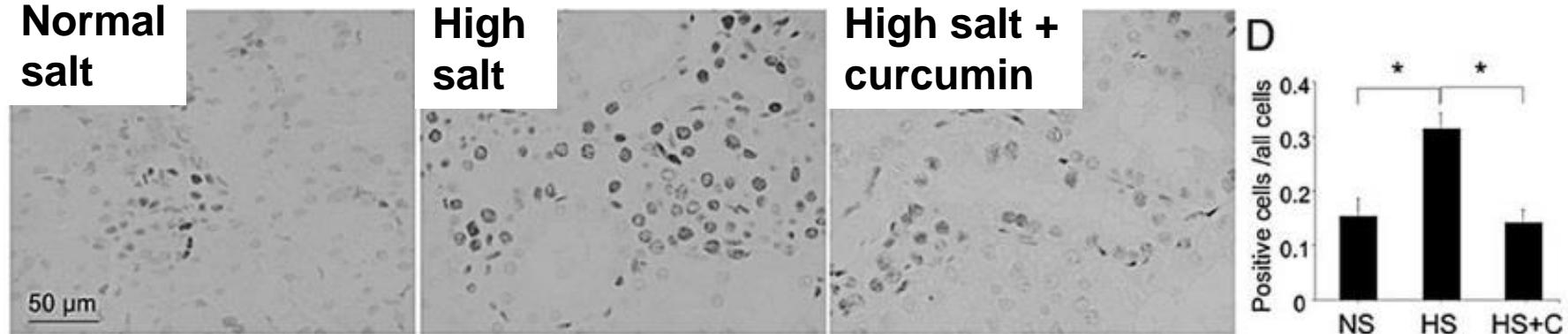


H3K9Me3: repressive histone mark  
NUP62: nuclear envelope protein  
nucleoporin 62

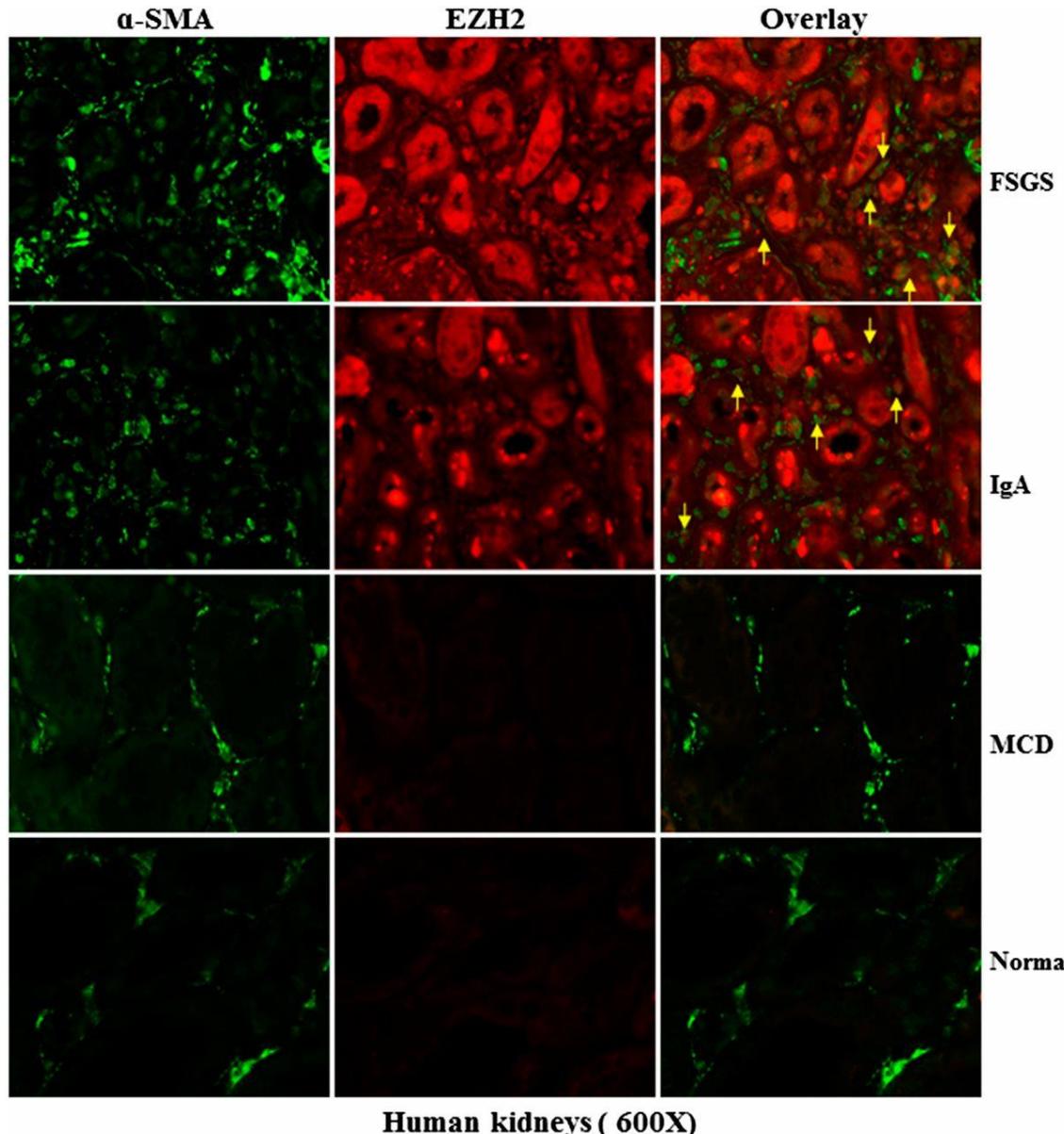
Hewitson et al.  
Front Pharmacol 2017

# curcumin improves nephrosclerosis via suppression of histone acetylation

acetylated H3K9

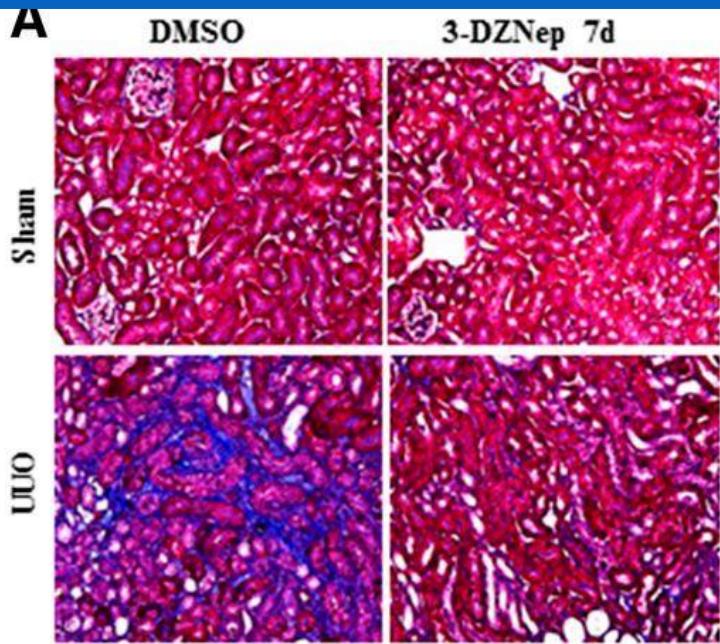


# increased EZH2 in the human fibrotic kidney

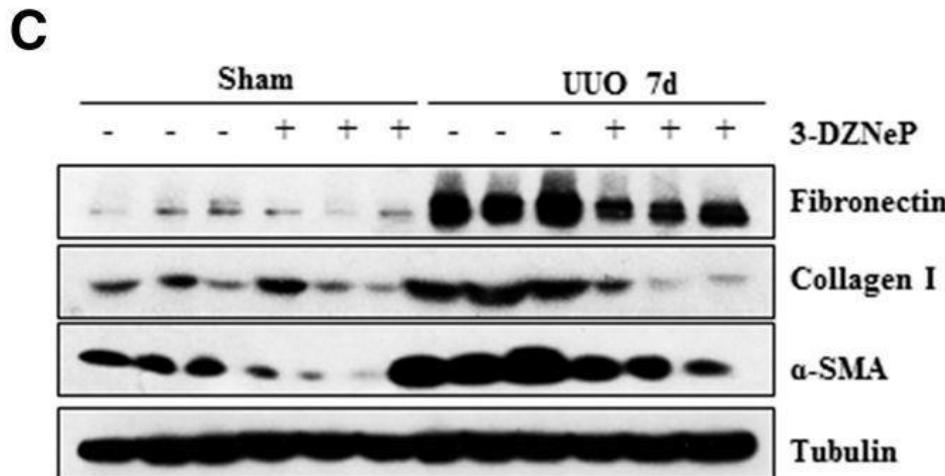


**EZH2 (Enhancer of zeste homolog 2): histone methyltransferase of H3K27me**  
**EZH2 mediates kidney fibrosis by downregulating expression of Smad7 and PTEN**

# Dz nep attenuates renal fibrosis in obstructed kidneys



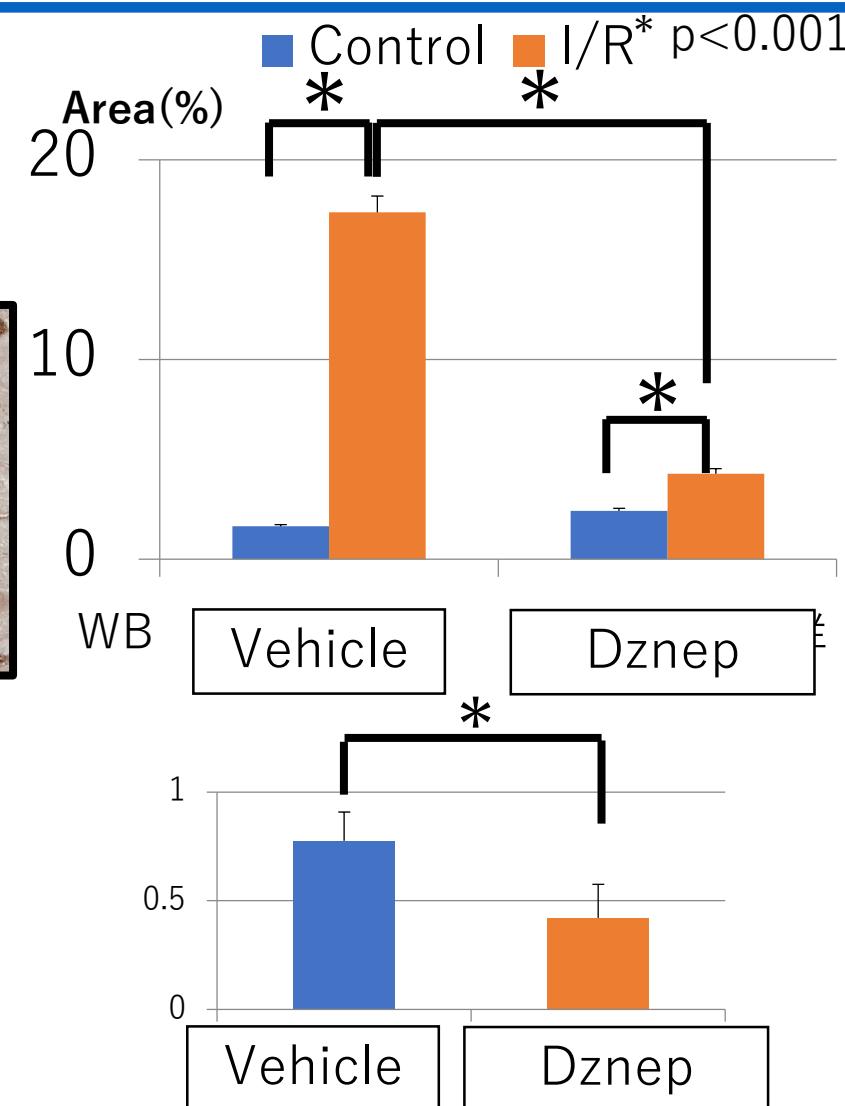
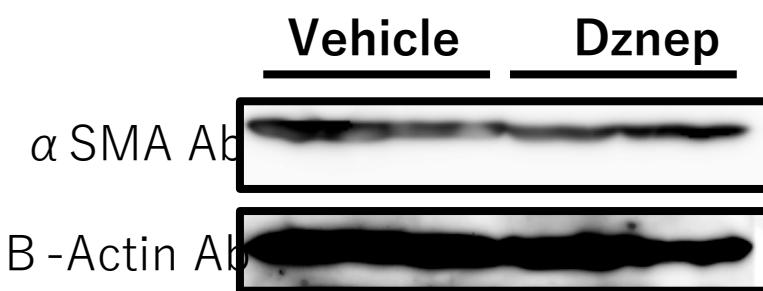
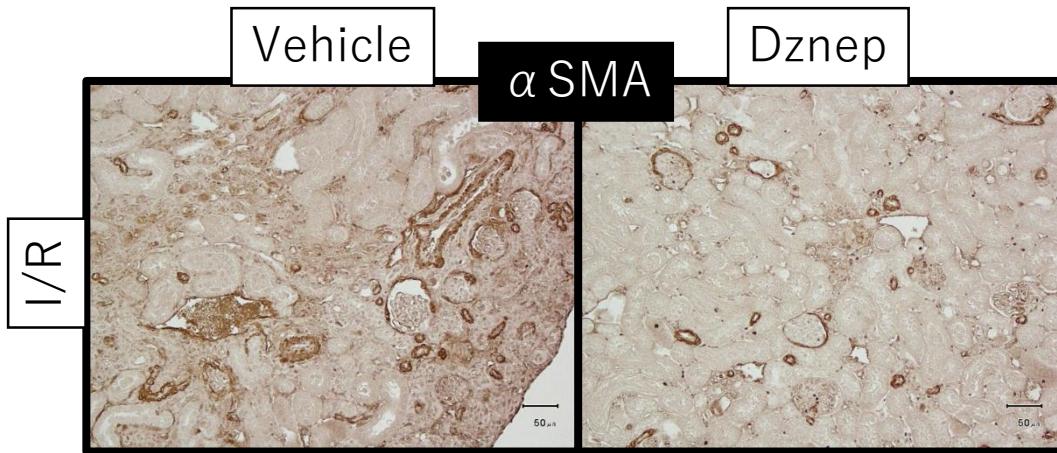
Dz nep: inhibitor of EZH2



Zhou et al. JASN 2016



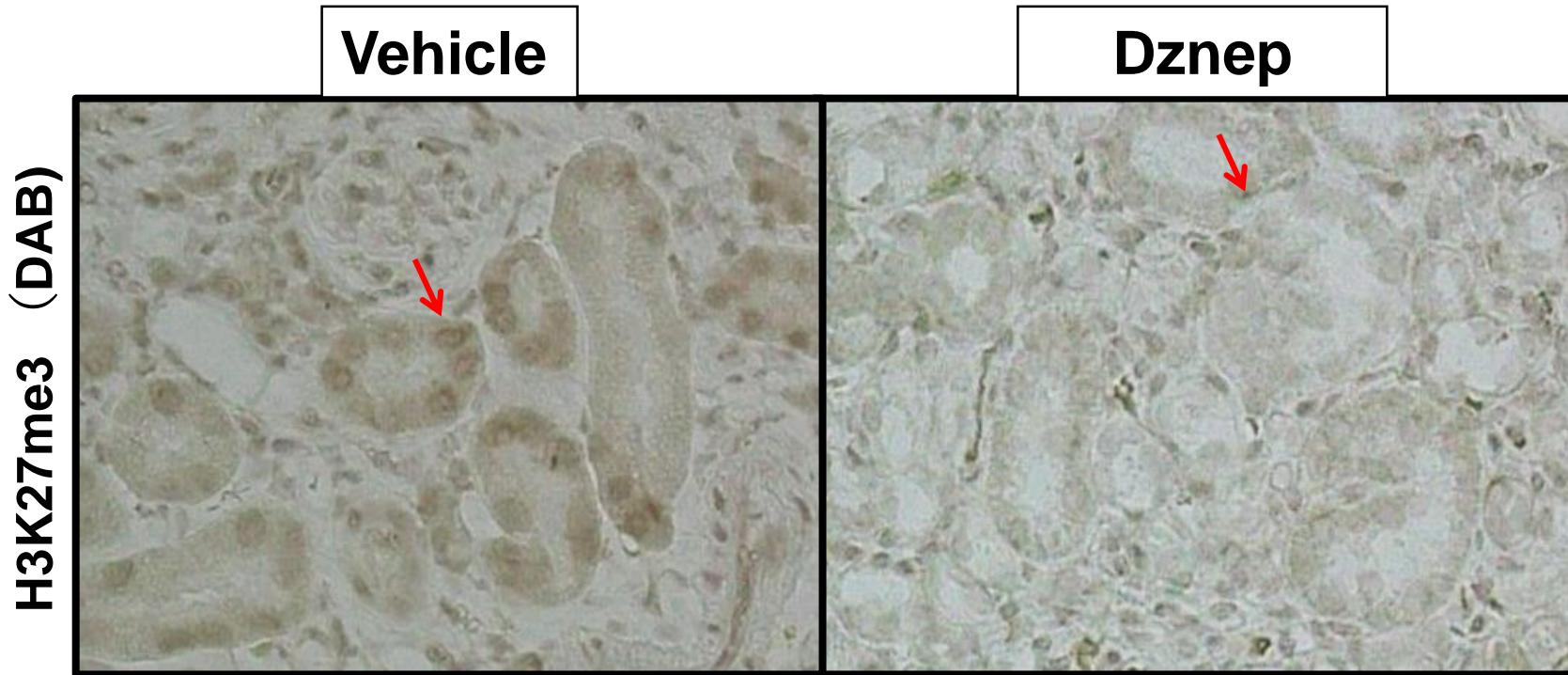
# Amelioration of AKI-to-CKD transition by Dznep



Mimura, Hirakawa, Nangaku. *manuscript in submission*

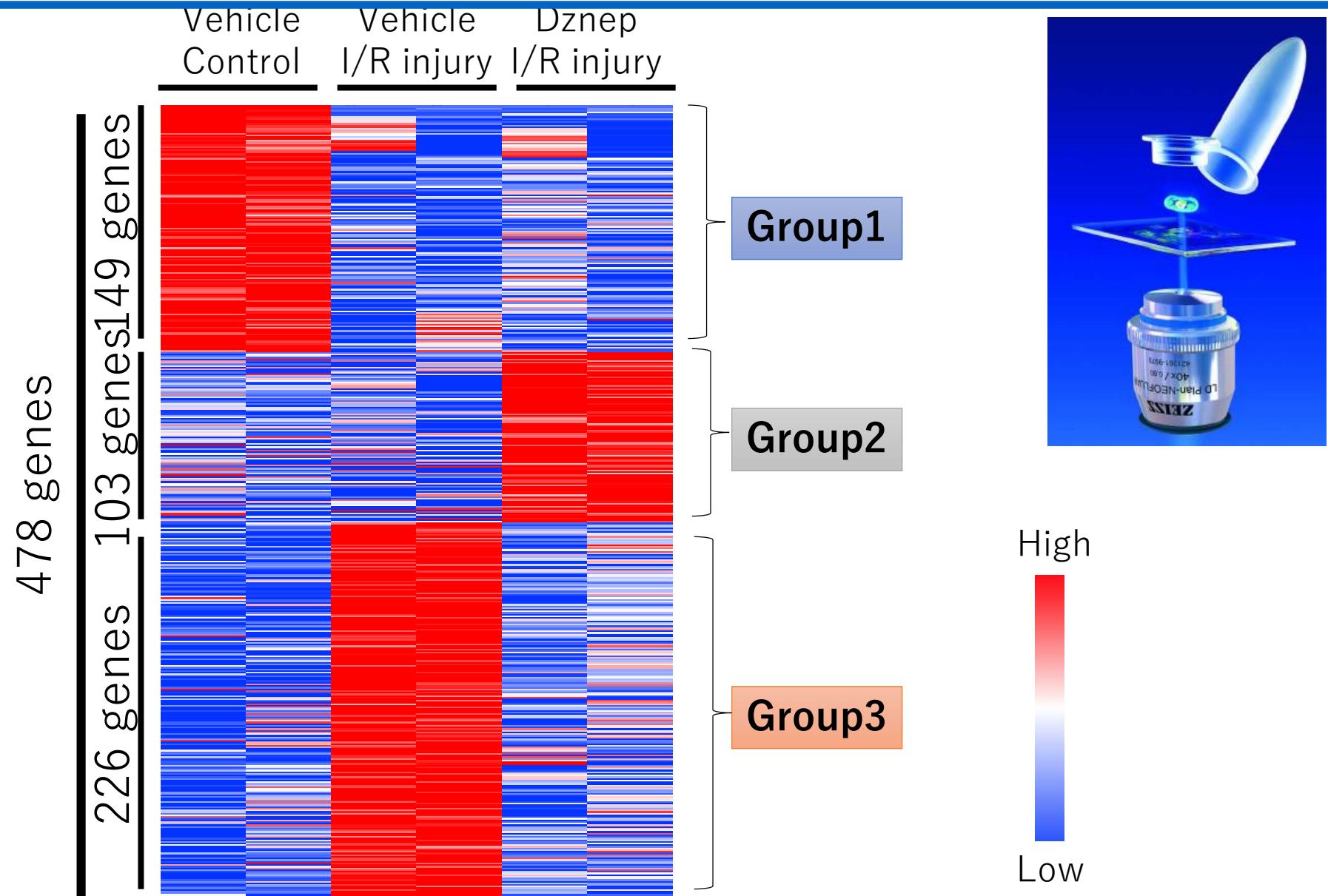
# Suppression of H3K27me3 by Dznep

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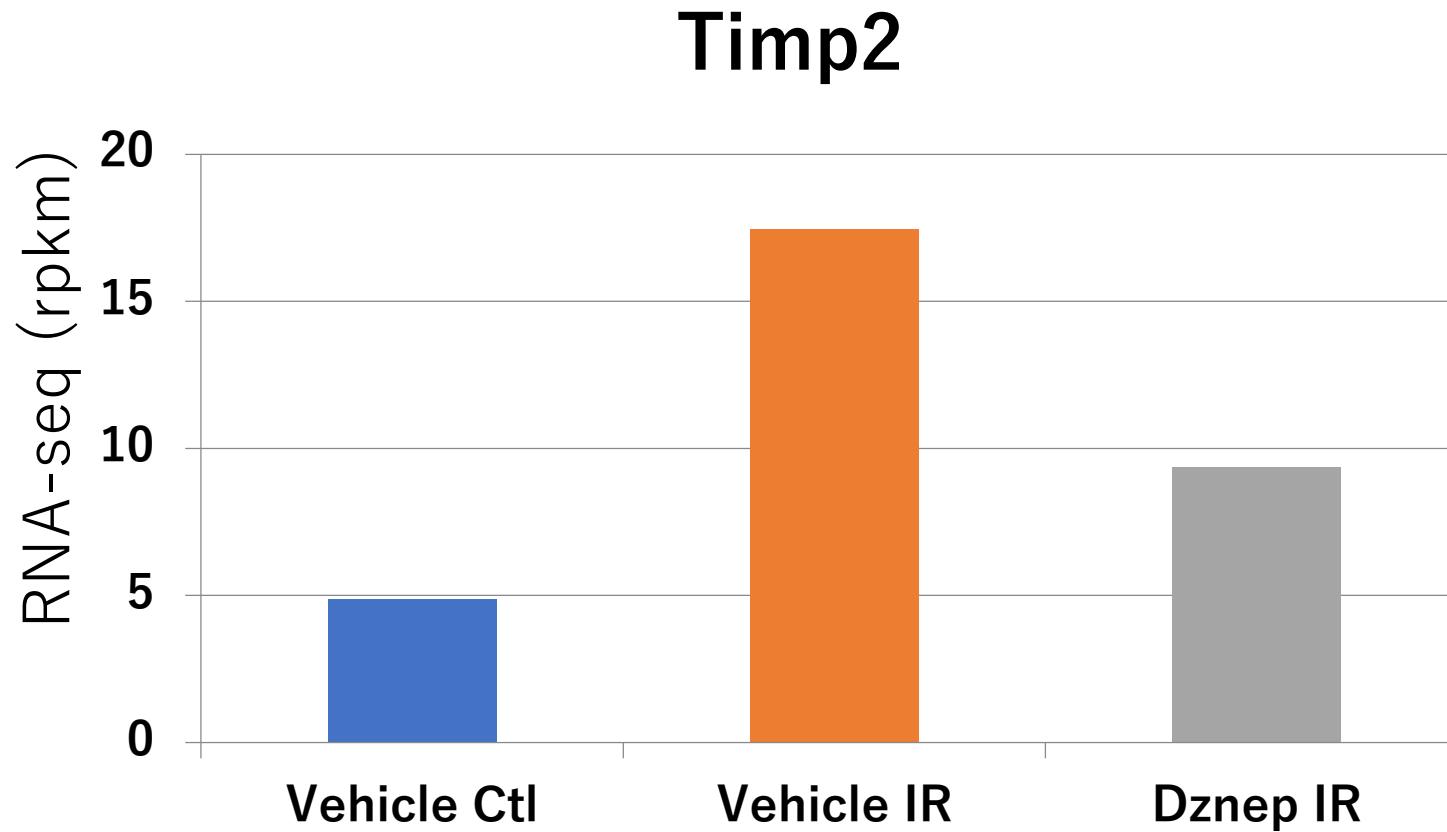
Nuclear staining of tubular cells showed decreased staining of H3K27me3.

# RNA-seq of tubules isolated by laser capture microdissection

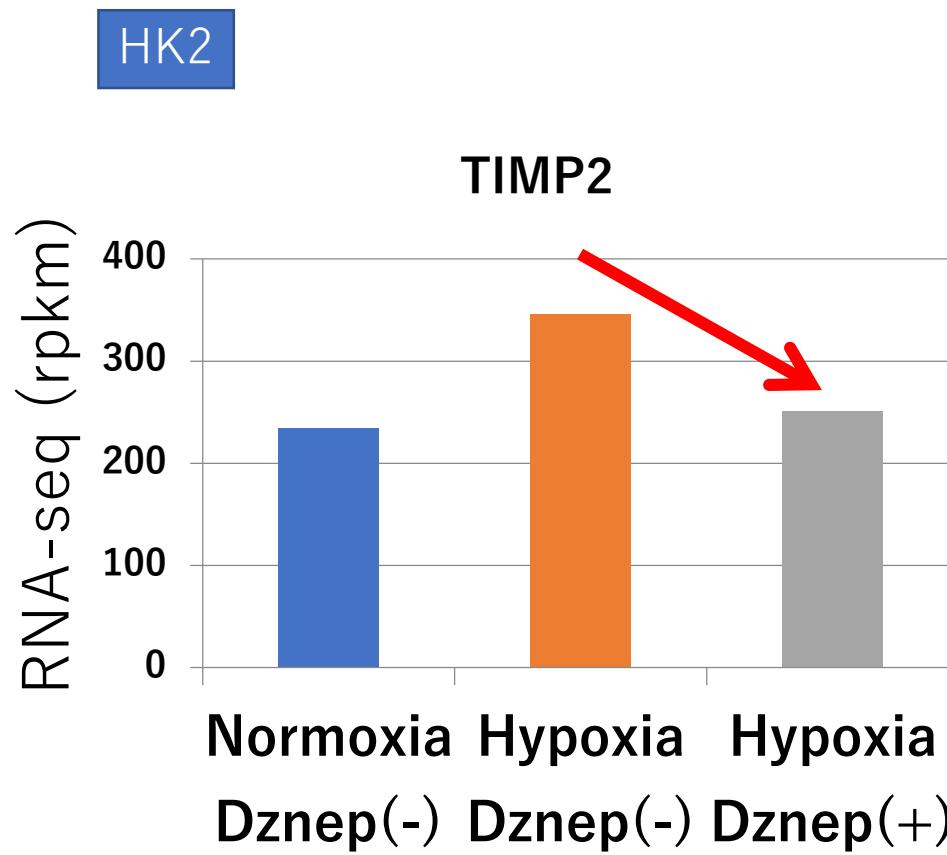


# RNA-seq of the kidney

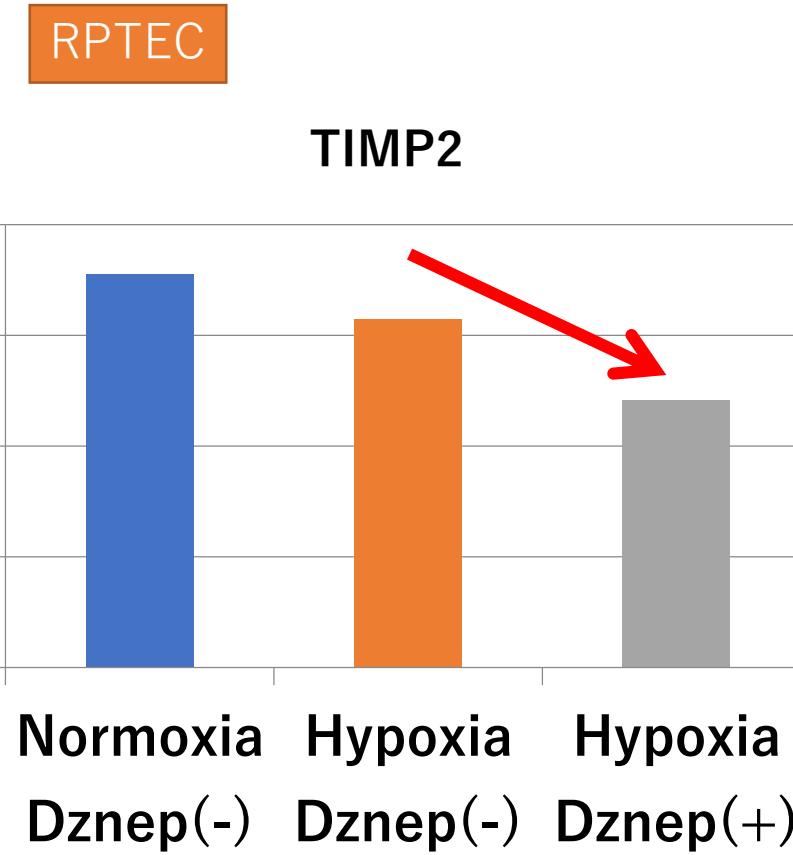
Dznep suppressed TIMP2 expression in the kidney



# RNA-seq: in vitro

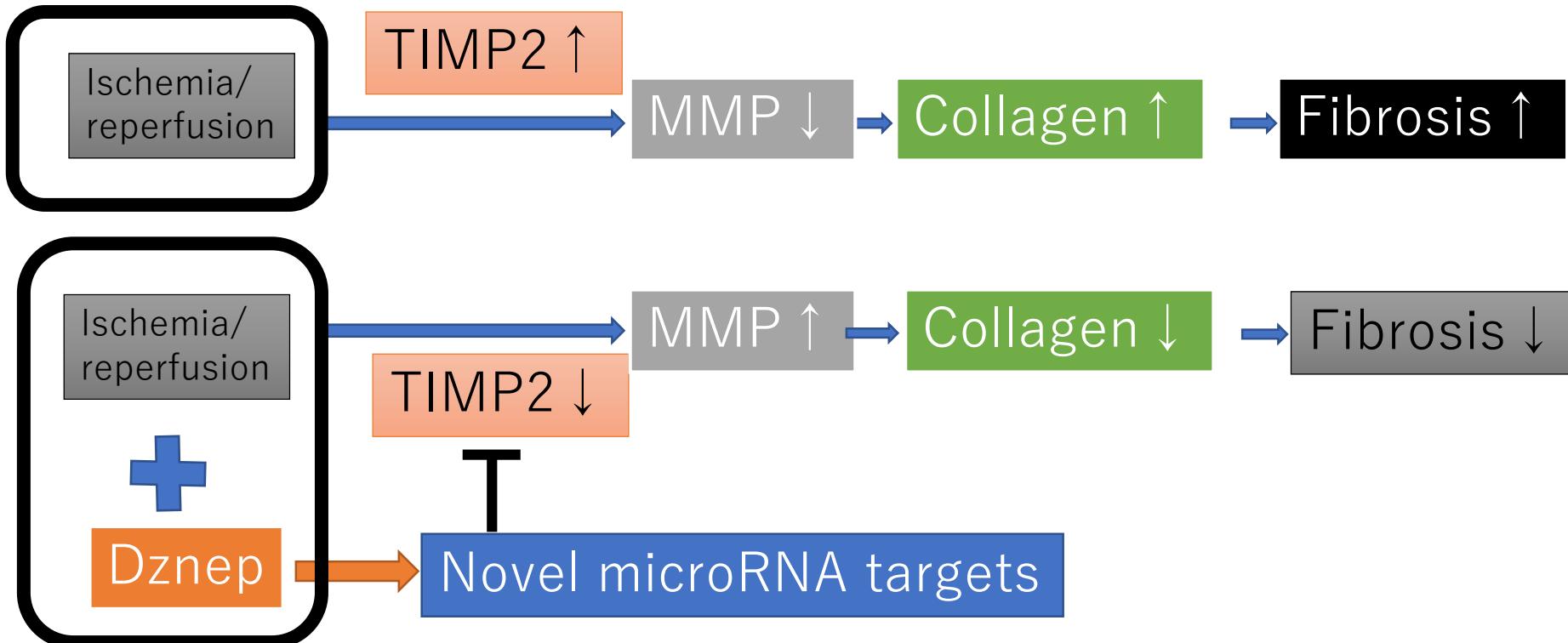


HK2: human kidney-2



RPTEC: renal proximal tubular epithelial cell  
(human primary culture cells)

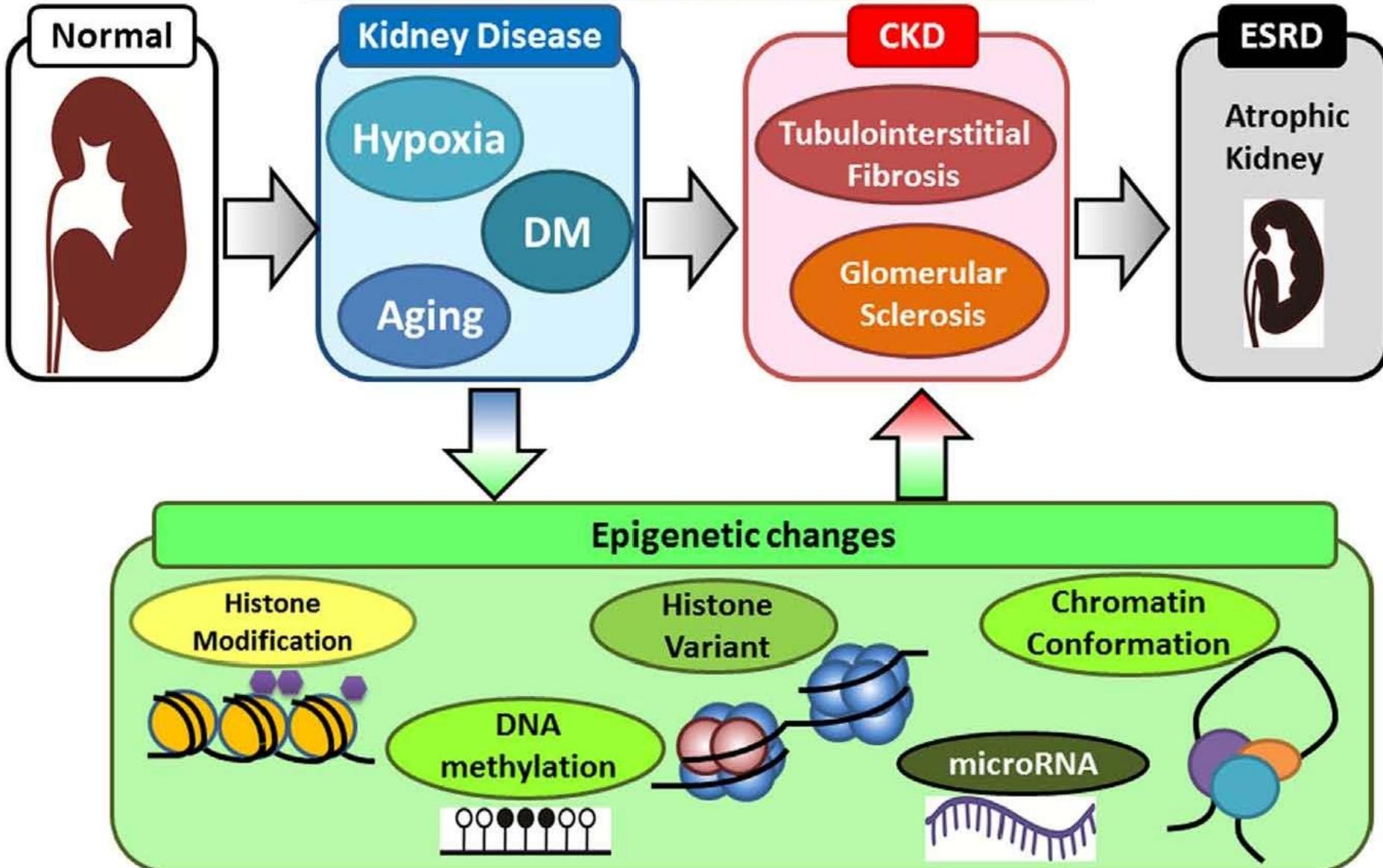
# Dz nep suppresses expression of TIMP2 via microRNA



Identified by small RNA-seq

Mimura, Hirakawa, Nangaku. *manuscript in submission*

## Progression of kidney disease to ESRD.



Mimura, Tanaka, & Nangaku. Semin Nephrol 2013



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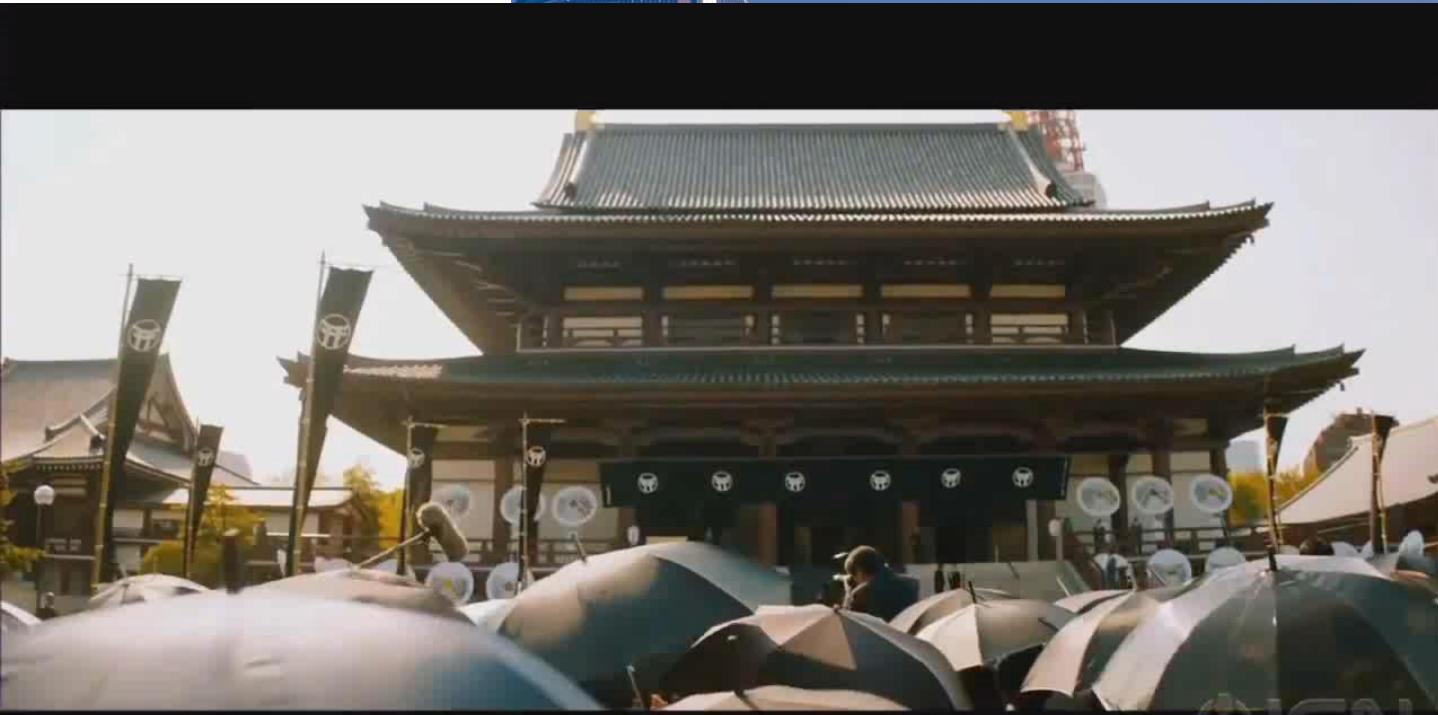
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# ISN FRONTIERS MEETINGS



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**KIDNEY DISEASE &  
CARDIOVASCULAR DISEASE**  
**FEBRUARY 22-25, 2018**  
**TOKYO, JAPAN**  
**VENUE: KEIO PLAZA HOTEL**  
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