Hôpitaux Universitaires Paris Est
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LA SCIENCE A PARIS

Inserm
UMR_S1155

Acute Tubular Diseases: Genomic Imprinting

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Necker Seminars in Nephrology, April 27, 2015

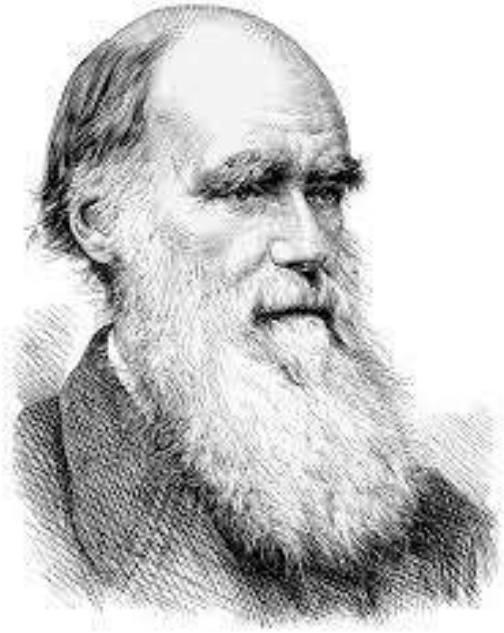
A short introduction to Epigenetics

Epigenetic impact of acute tubular injury

XIXth century. Fixism is dead. Evolution happens.

Charles Darwin

1809-1882



Jean-Baptiste de Lamarck

1744-1829

Organisms don’t adapt much

Natural Selection (random mutations)

Evolution is very slow

Organisms adapt to their environment

Inheritance of acquired characteristics

Evolution can be fast

Adrian Bird

Nature, 2007

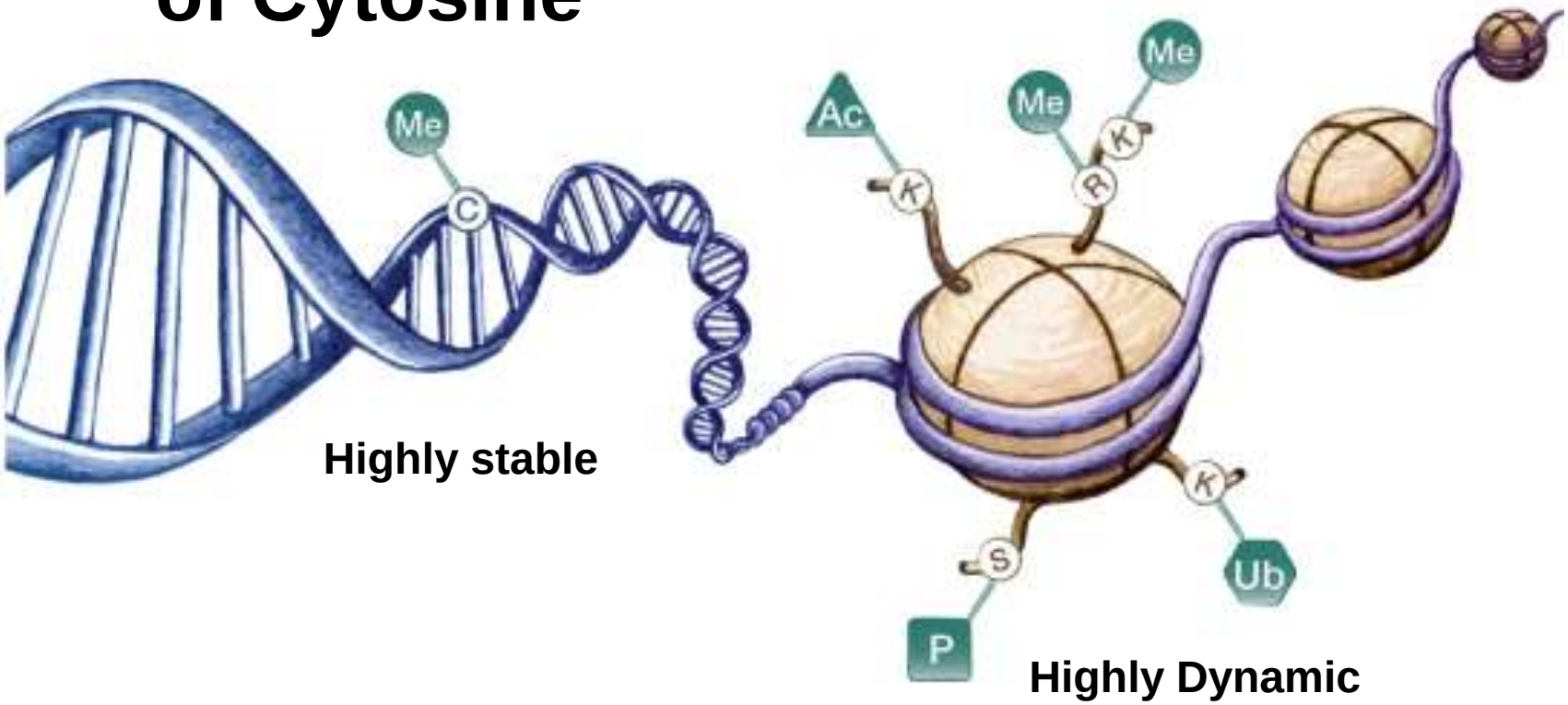
The latest definition of Epigenetics

The structural adaptation of chromosomal regions so as to register, signal, or perpetuate altered activity states

« The Triple Helix »

Methylation of Cytosine

Histone Modifications



Highly stable

Highly Dynamic

The Toadflax flower: one genome, two phenotypes

Lcyc silencing

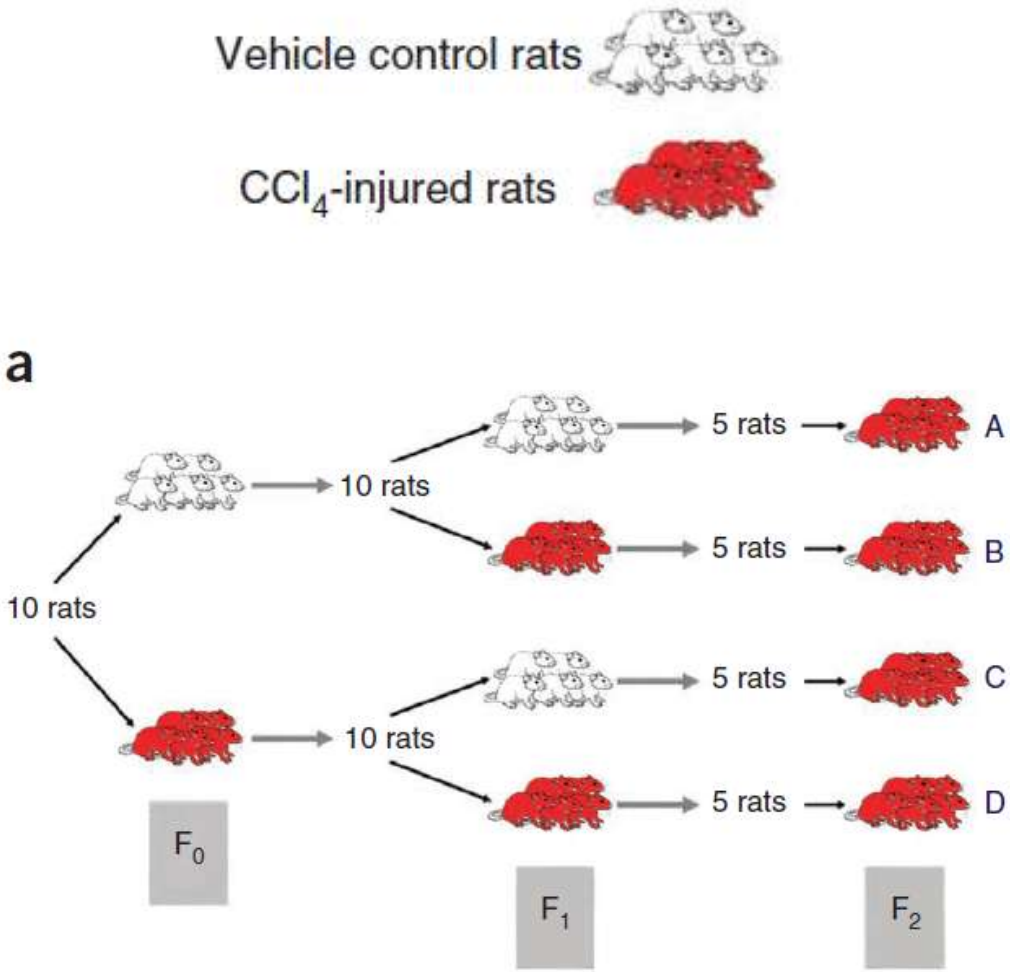
Wild-type

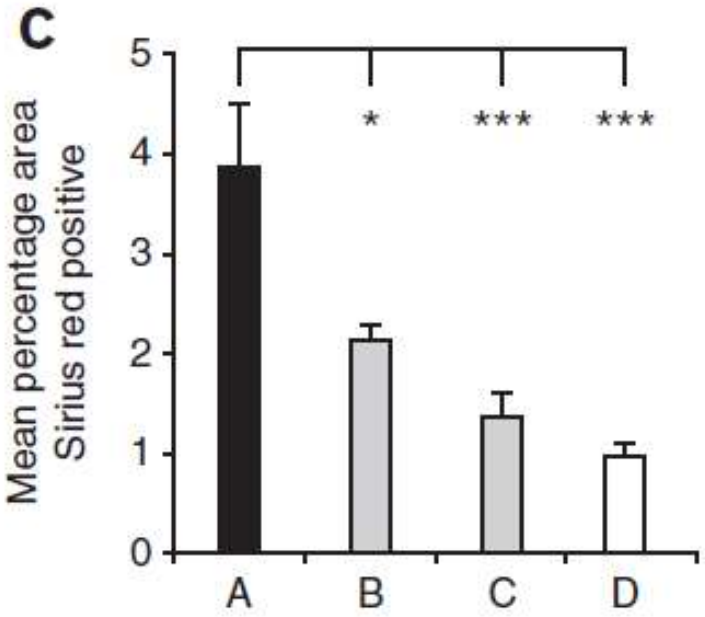
Peloric Epimutant

Peloria : *greek* for « Monster »

Linnaeus: "This is certainly no less remarkable than if a cow were to give birth to a calf with a wolf's head,"

Multigenerational Epigenetic Adaptation of the Hepatic Wound-Healing Response

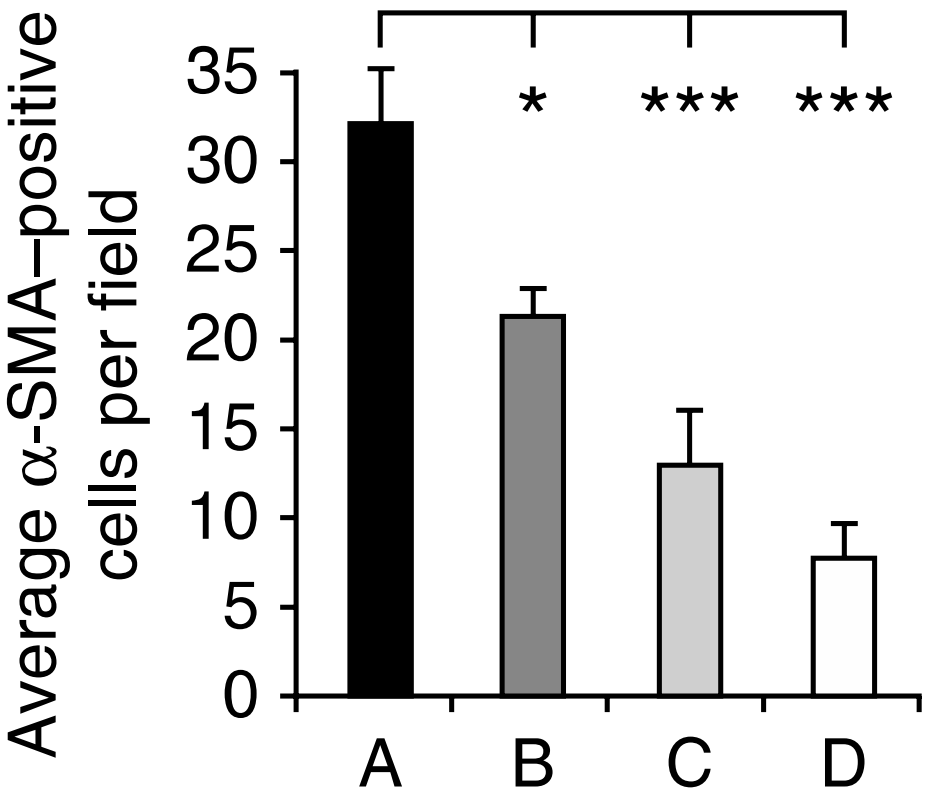




Zeybel M et al, Nat Med 2012

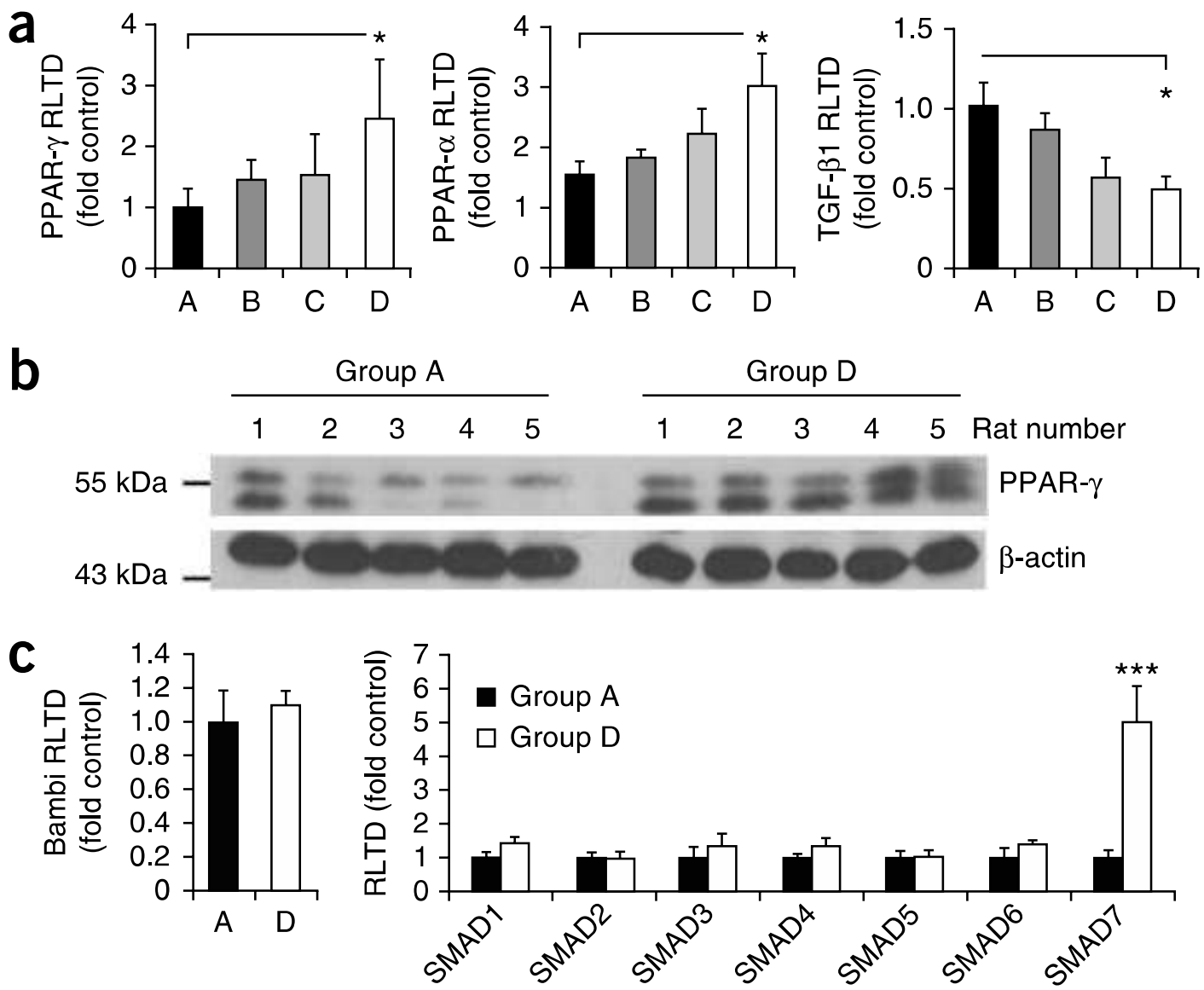
Same number of precursors of myofibroblasts before injury

More myofibroblasts after liver injury



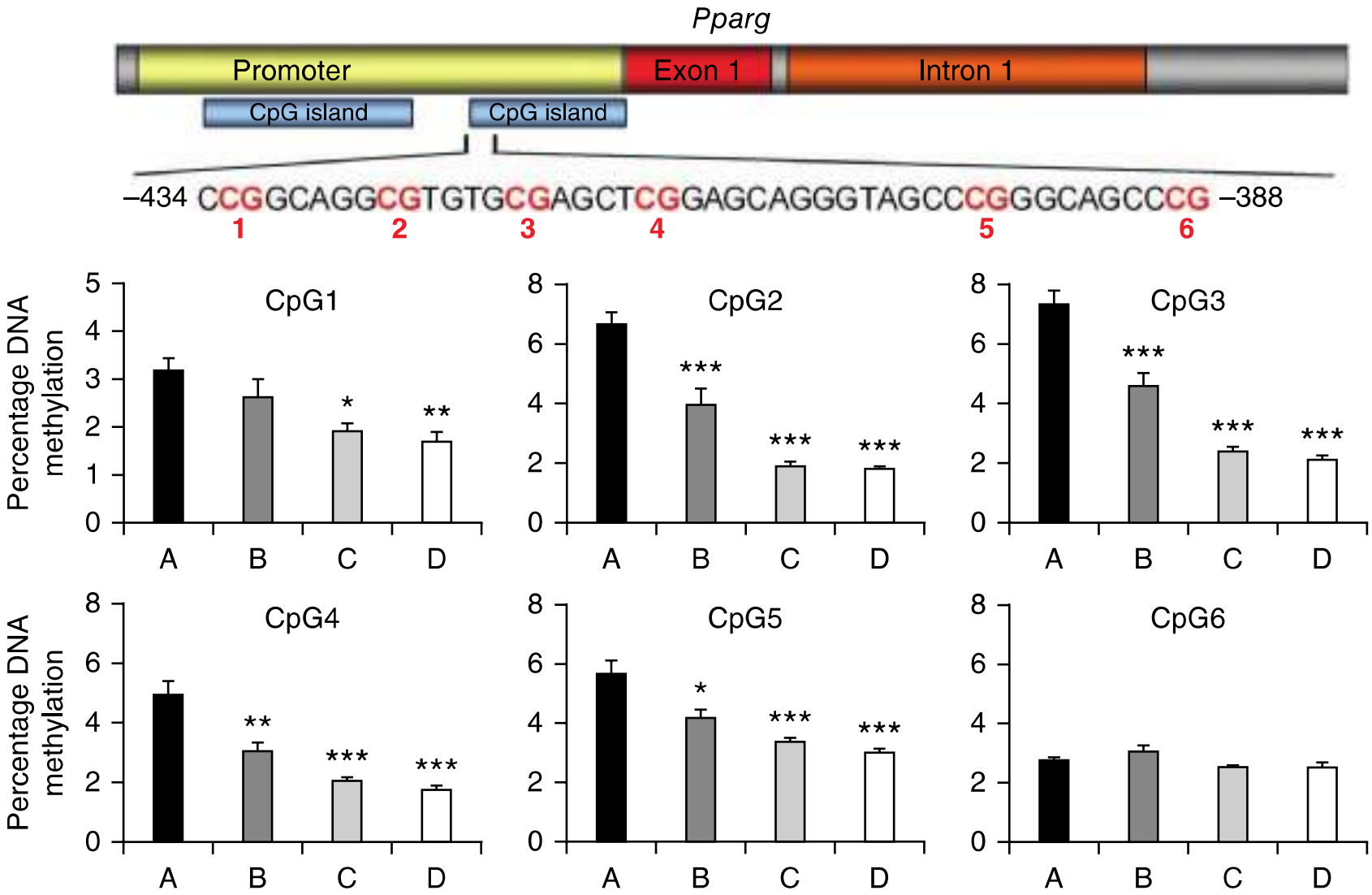
Zeybel M et al, Nat Med 2012

Rats born from exposed ancestors use PPAR-γ gene more easily



Zeybel M et al, Nat Med 2012

Hereditary transmission of hypomethylation of PPAR-γ promoter



Zeybel M et al, Nat Med 2012

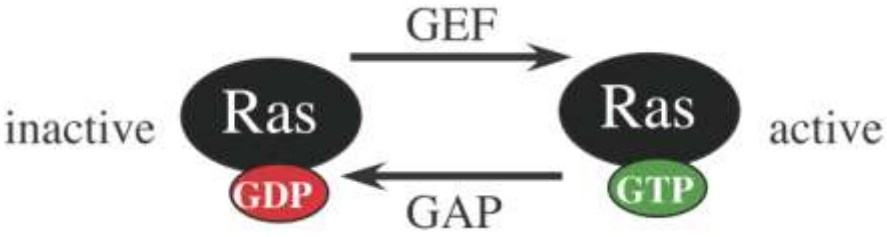
WELCOME TO MY WORLD

Genome wide analysis of methylation profile in fibroblasts isolated from normal vs fibrotic human kidneys

BGS

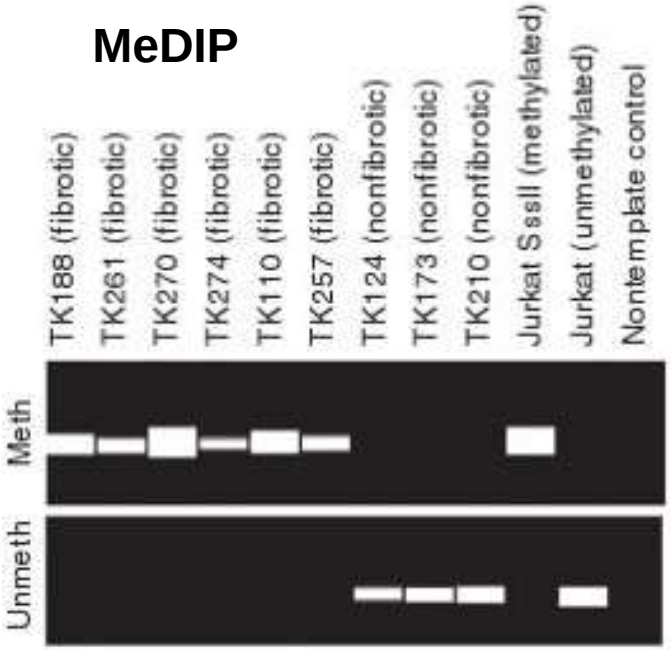
12 genes were found to be systematically methylated in fibrotic kidneys out of which 3 have orthologs in mice

e.g. **RASAL1**



RASAL1 is a GAP protein

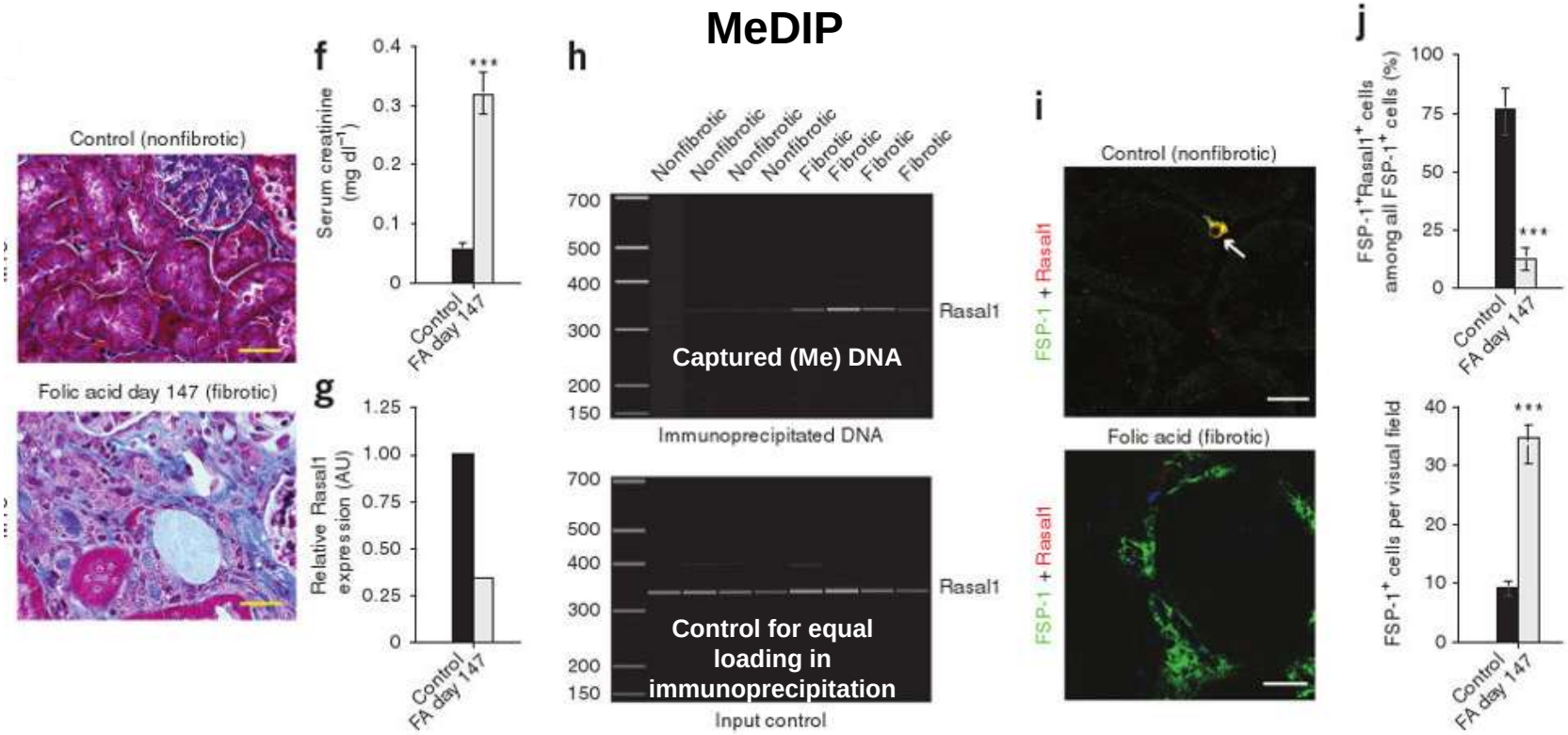
MeDIP



Méthylation of RASAL1 In fibroblasts extracted from fibrotic kidneys

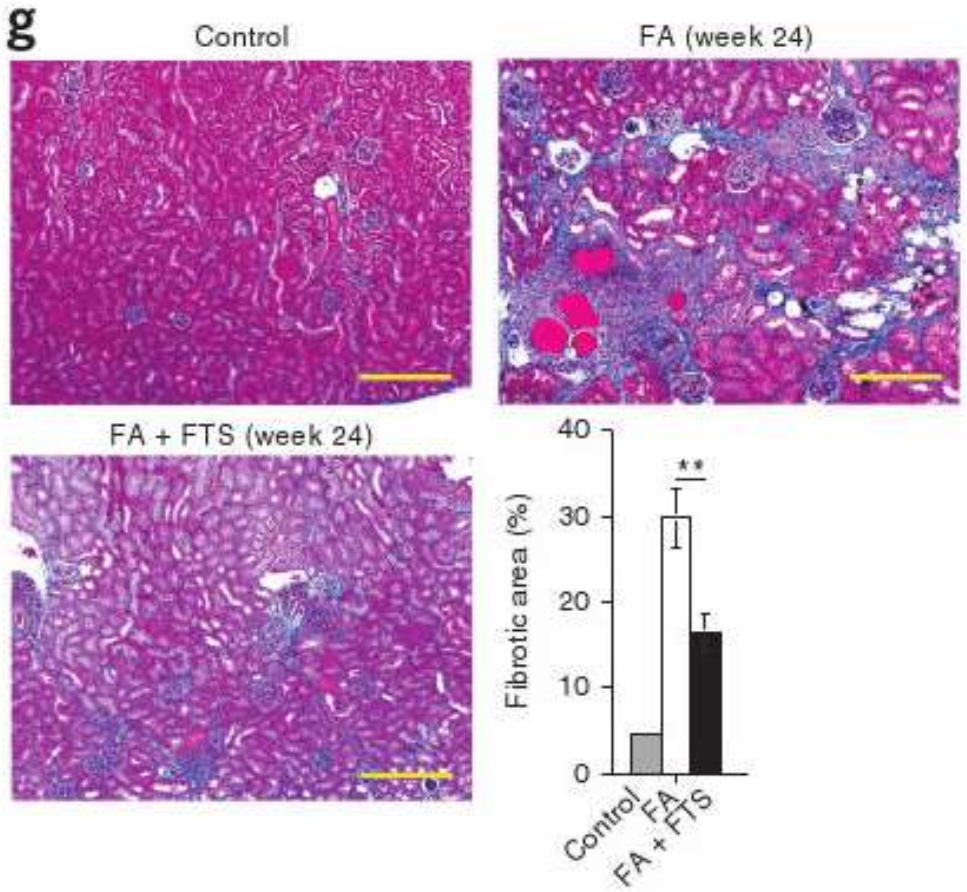
Bechtel W et al, Nat Med 2010

RASAL1 promoter is methylated in myofibroblasts from injured kidneys



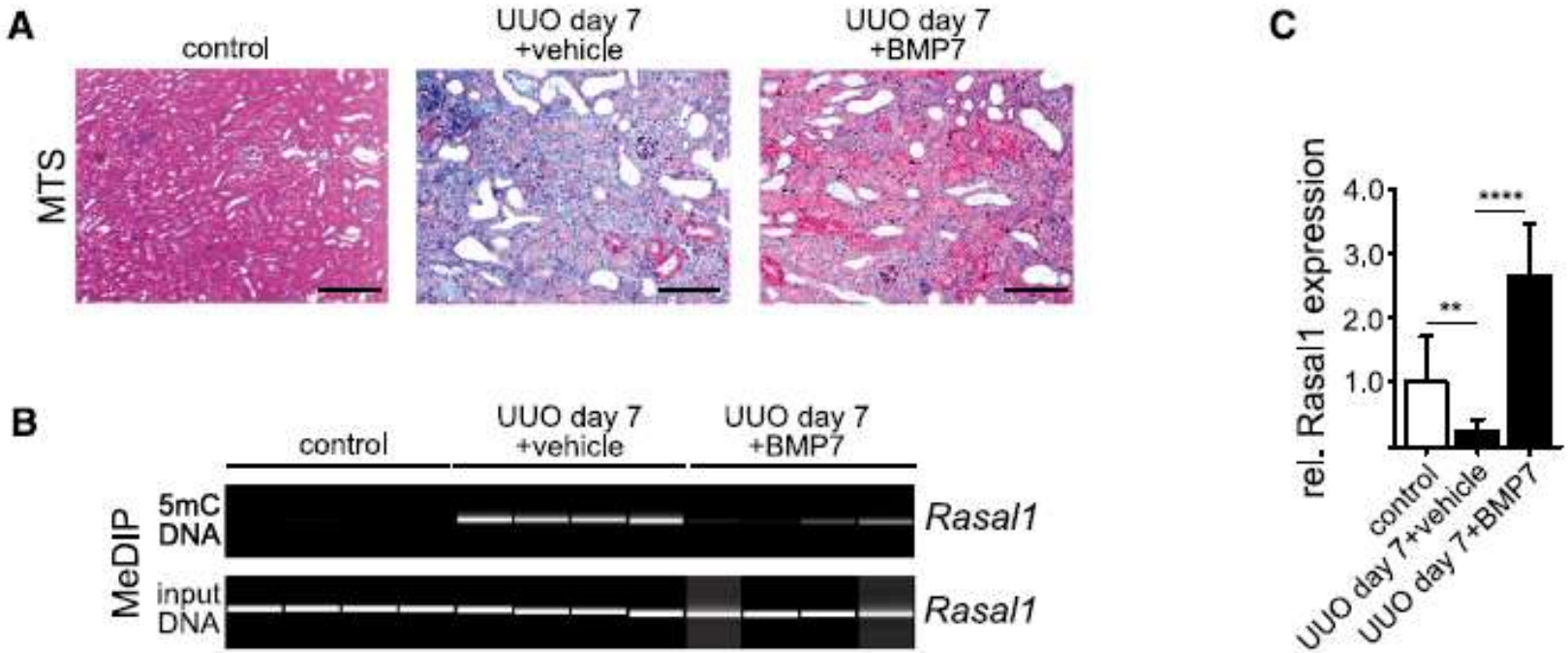
Bechtel W et al, Nat Med 2010

RAS inhibitors are potentially anti-fibrotic drugs



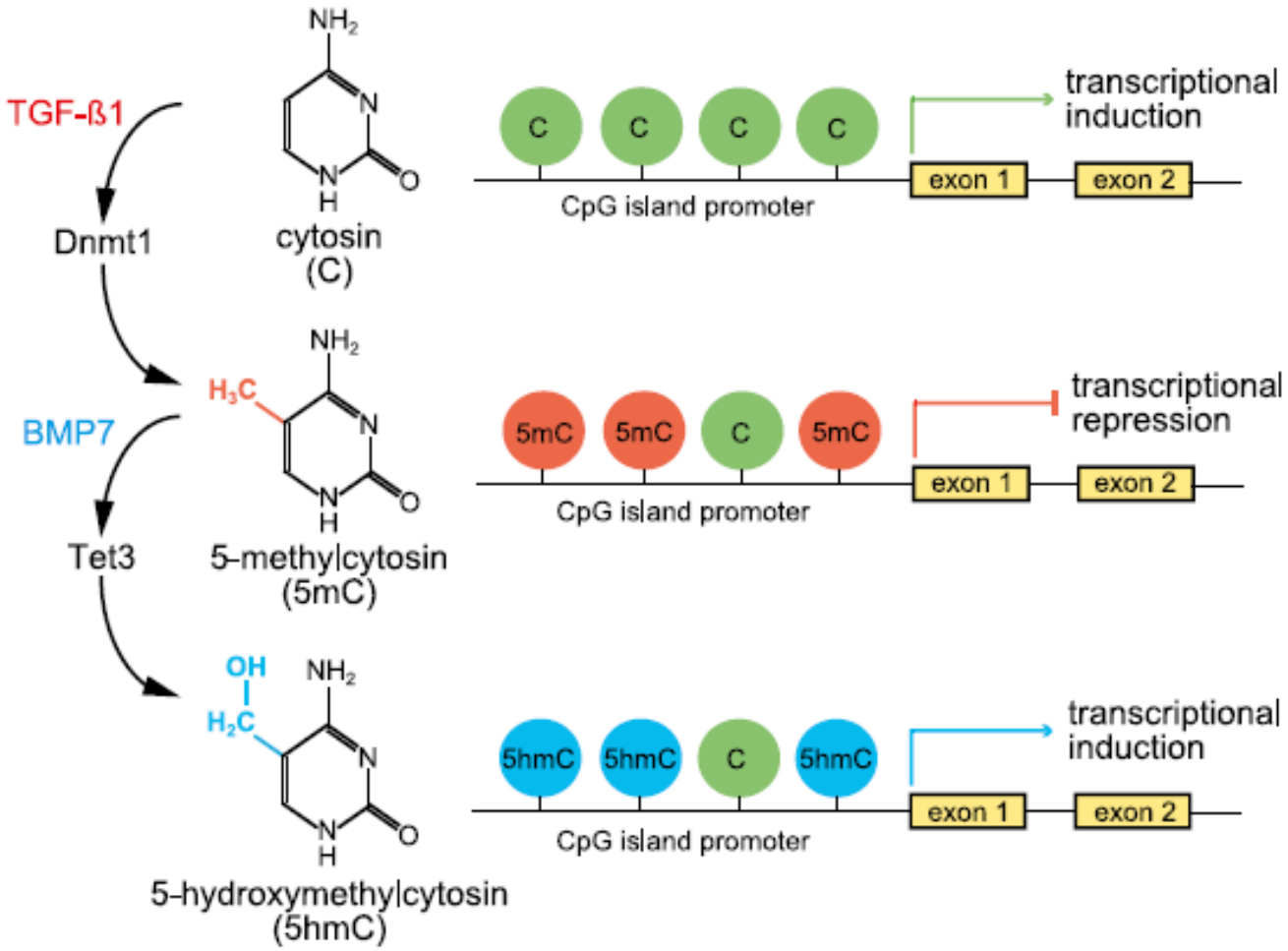
Bechtel W et al, Nat Med 2010

BMP7 decreases RASAL1 methylation in the UUO model



Tampe B et al, J Am Soc Nephrol 2014

BMP7 induces TET3

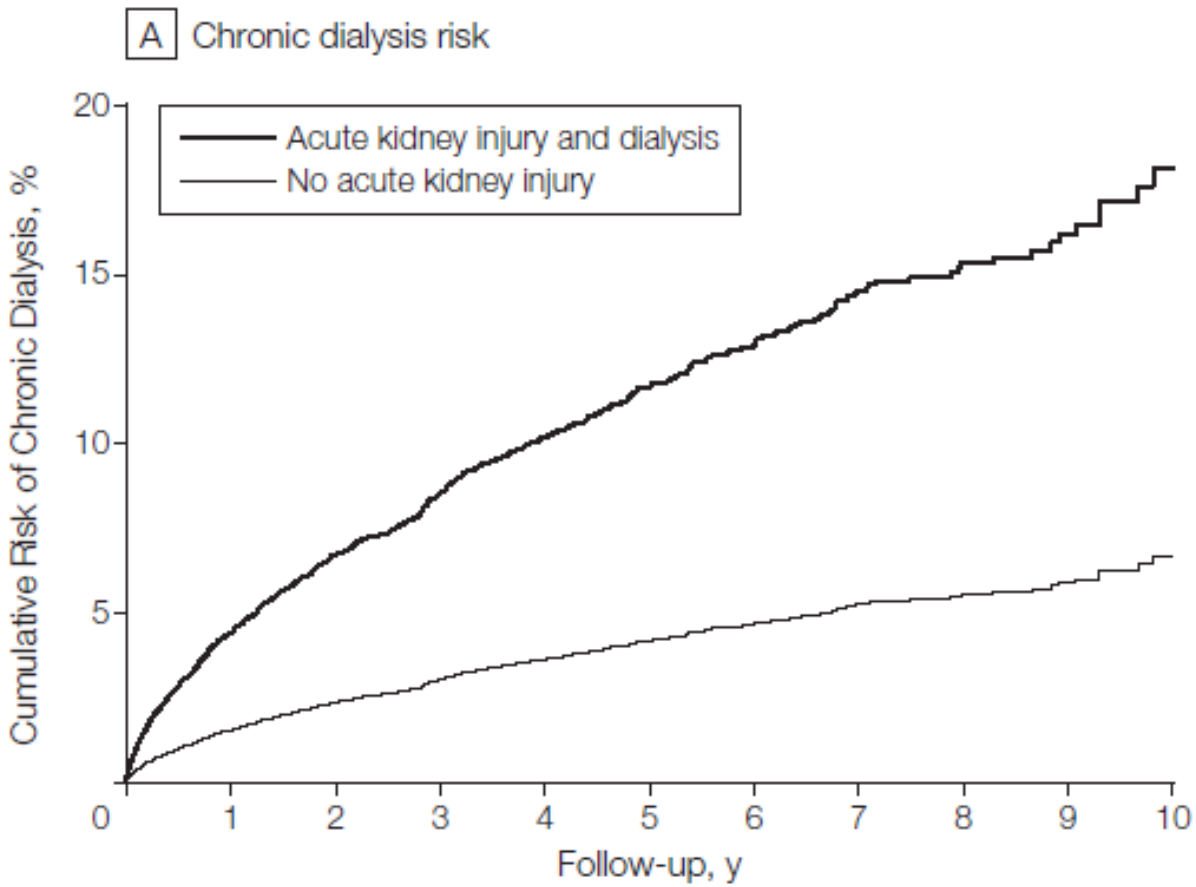


Tampe B et al, J Am Soc Nephrol 2014

Ischemic Acute Kidney Injury

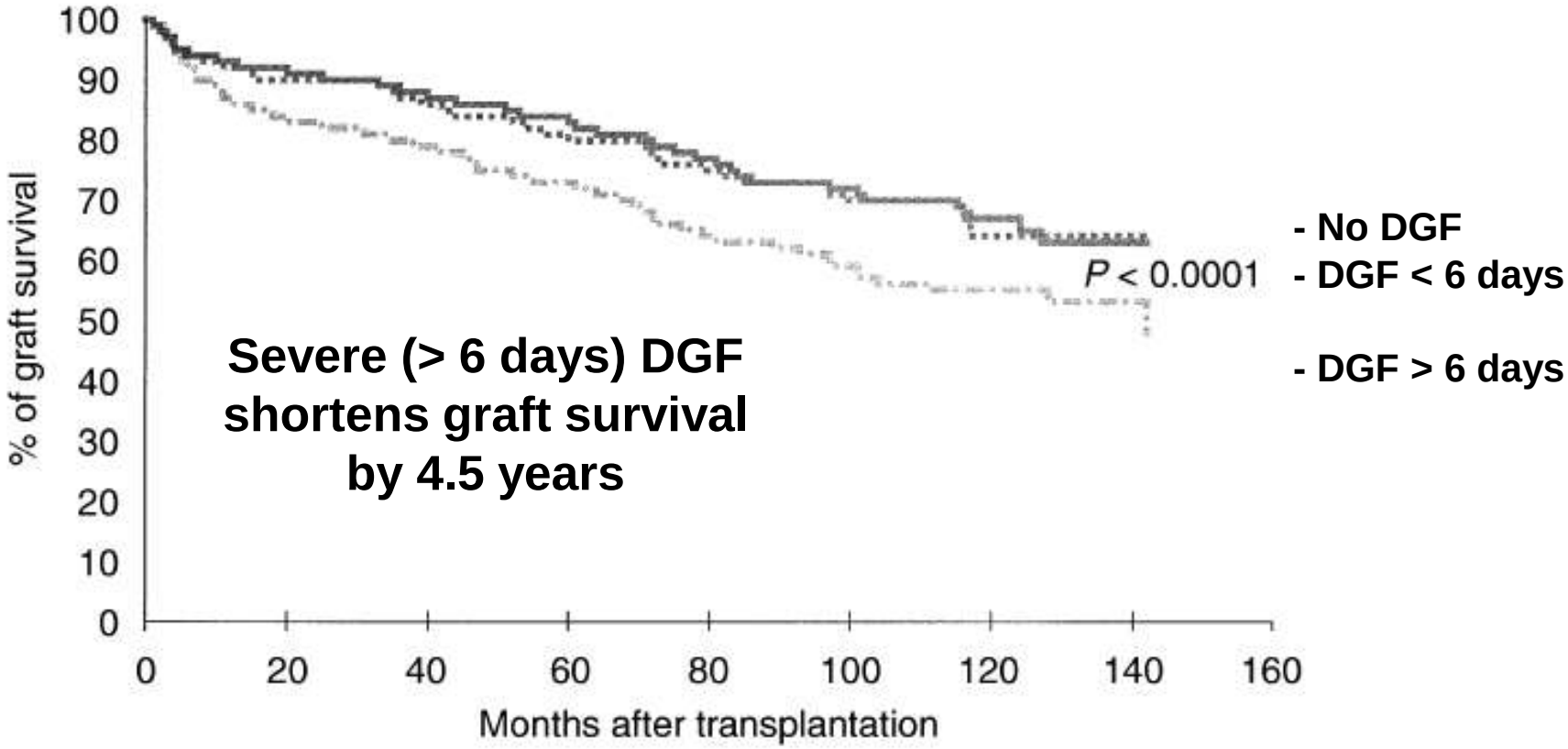
(no modification of RASAL1 promoter methylation status)

Chronic Dialysis and Death Among Survivors of Acute Kidney Injury Requiring Dialysis



Wald R et al, JAMA 2009

IMPACT OF DELAYED GRAFT FUNCTION

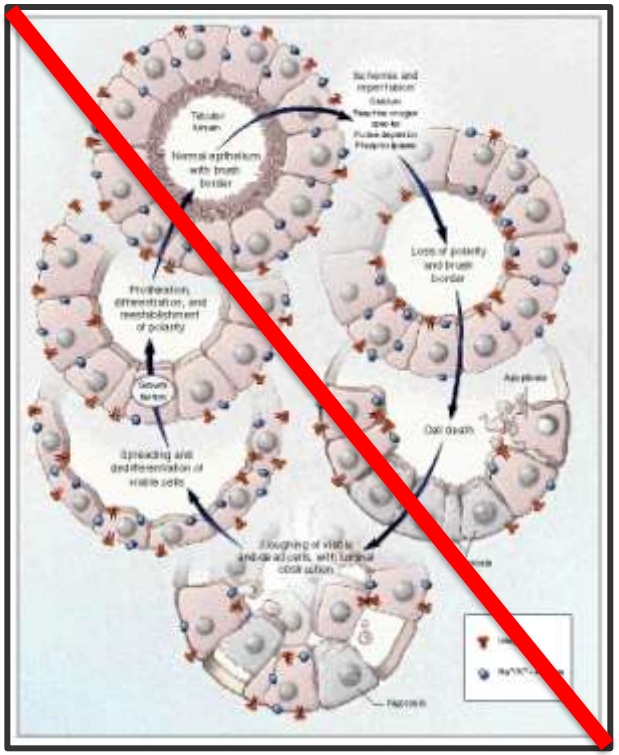


Giral M et al, Kidney Int 1998

USRDS DATA (2013): Odds Ratio for graft loss at 5 years: 1.7 (after exclusion of ECD)

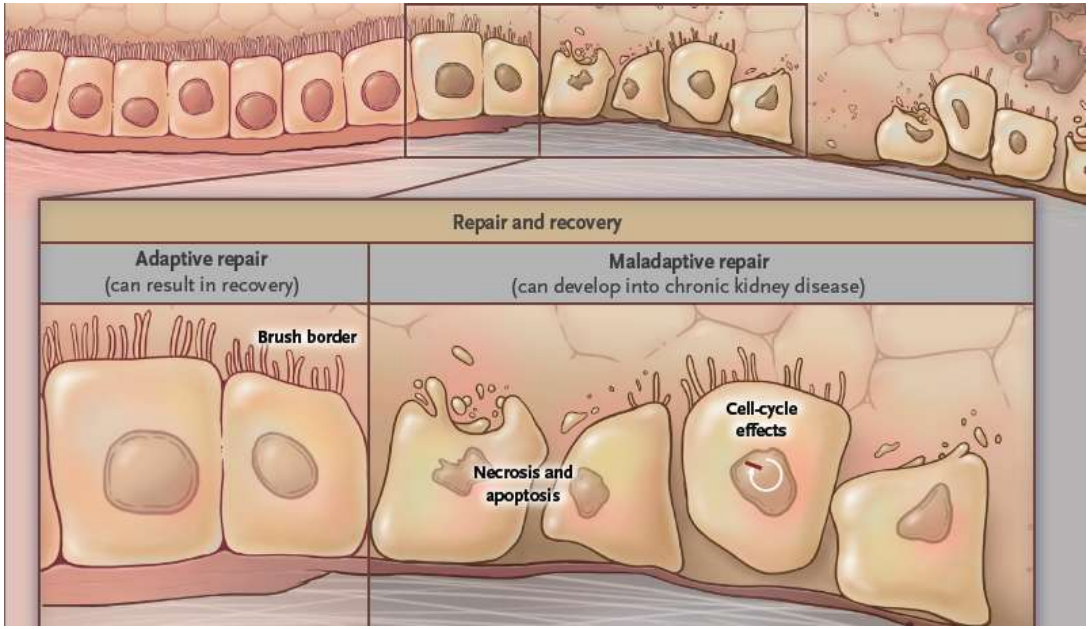
Butala et al, Transplantation 2013

Does Acute Tubular Necrosis really resolve *ad integrum* ?

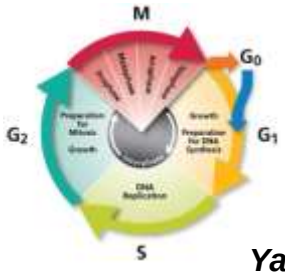


Thadhani R et al. N Engl J Med 1996

« Maladaptative repair »

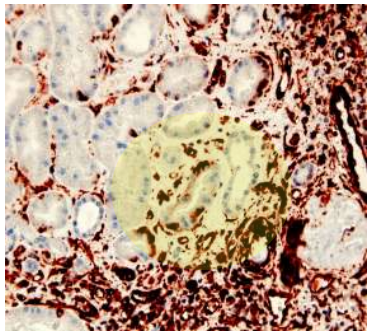


Chawla LS et al. N Engl J Med 2014



Yang L, Nat Med 2010

Certitem trial



Multicenter Randomized Trial in France

194 patients included

Therapeutic intervention to prevent fibrogenesis in EMT+ grafts

A history of delayed graft function is the stronger independent risk factor for the « mesenchymal transition » of tubular epithelial cells

OR 4.7, 95%IC [2.02-10.94], p<0.0003

Rostaing L et al, Am J Transplant 2015

HYPOTHESIS

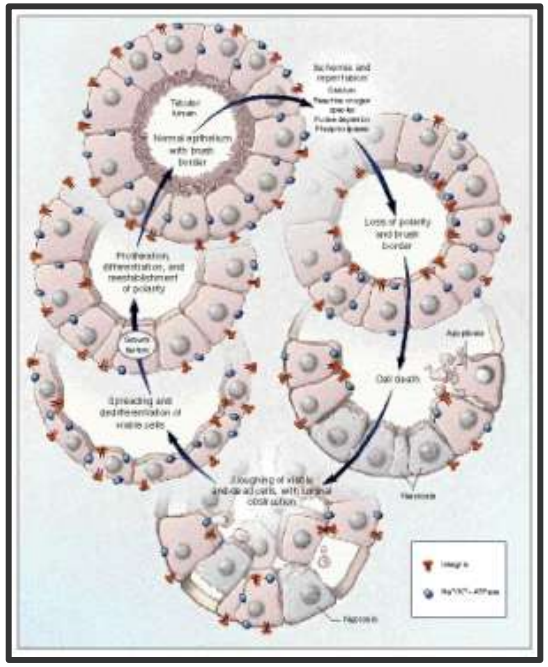
Renal fibrogenesis is influenced by previous injuries

and not only by current ones (hypertension, diabetes, etc)

Surviving epithelial cells

repare the tubule

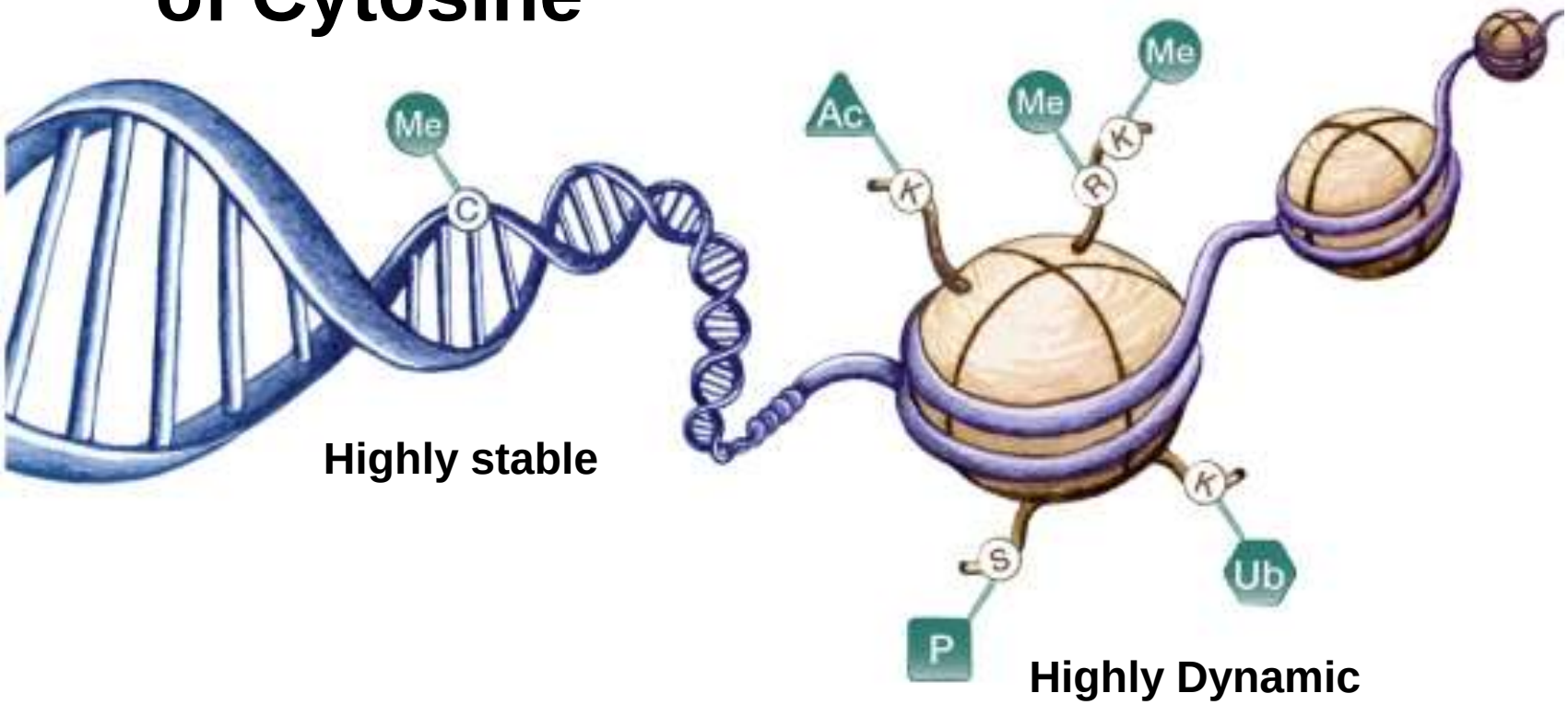
but have a « memory »



« The Triple Helix »

Methylation of Cytosine

Histone Modifications

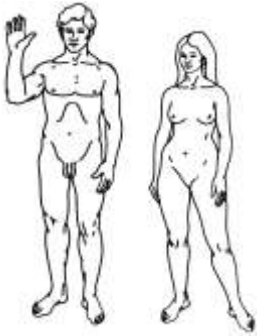


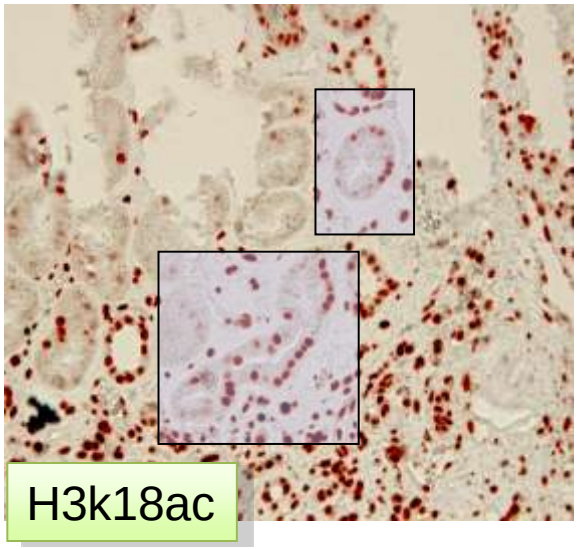
Highly stable

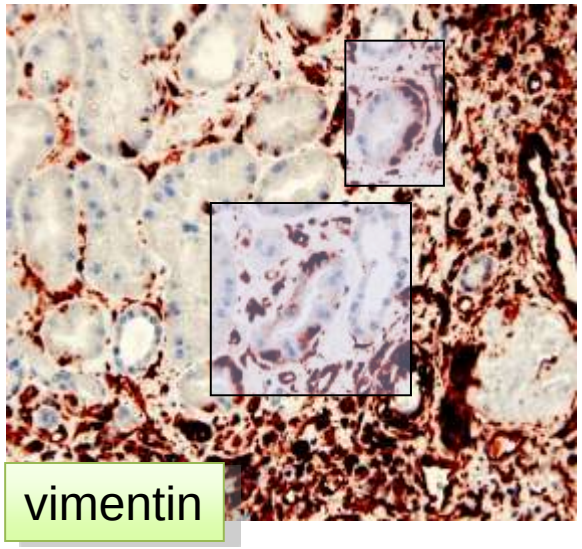
Highly Dynamic

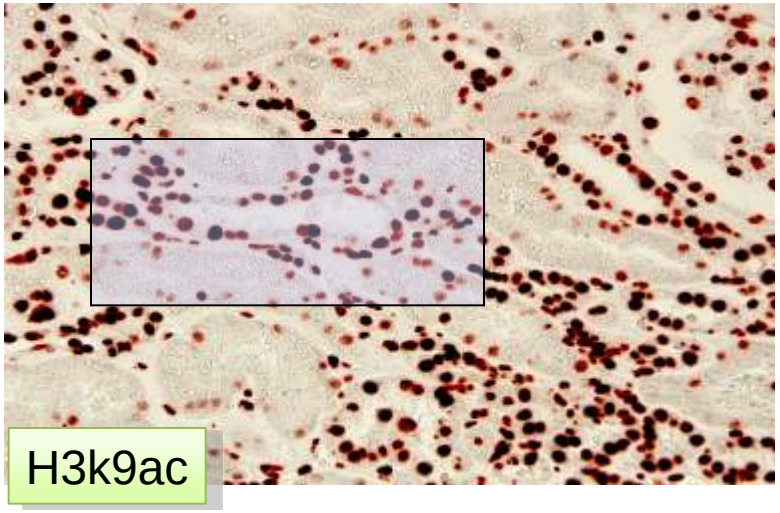
Maren Burbach

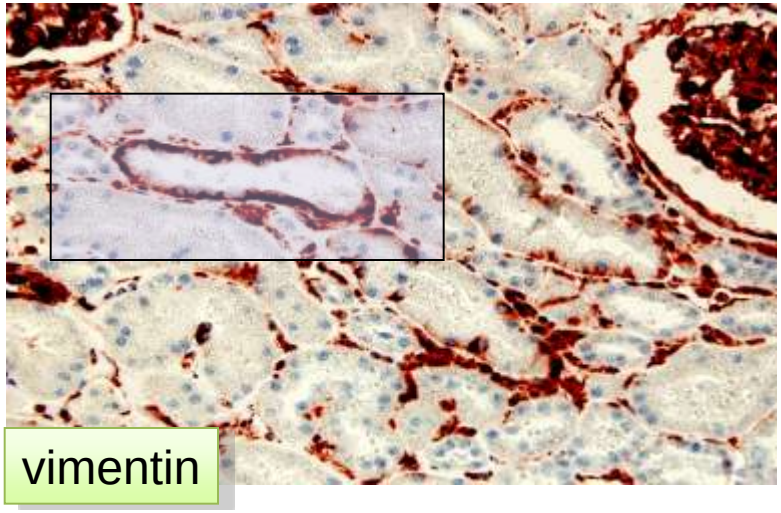
Histone modifications and epithelial reprogramming

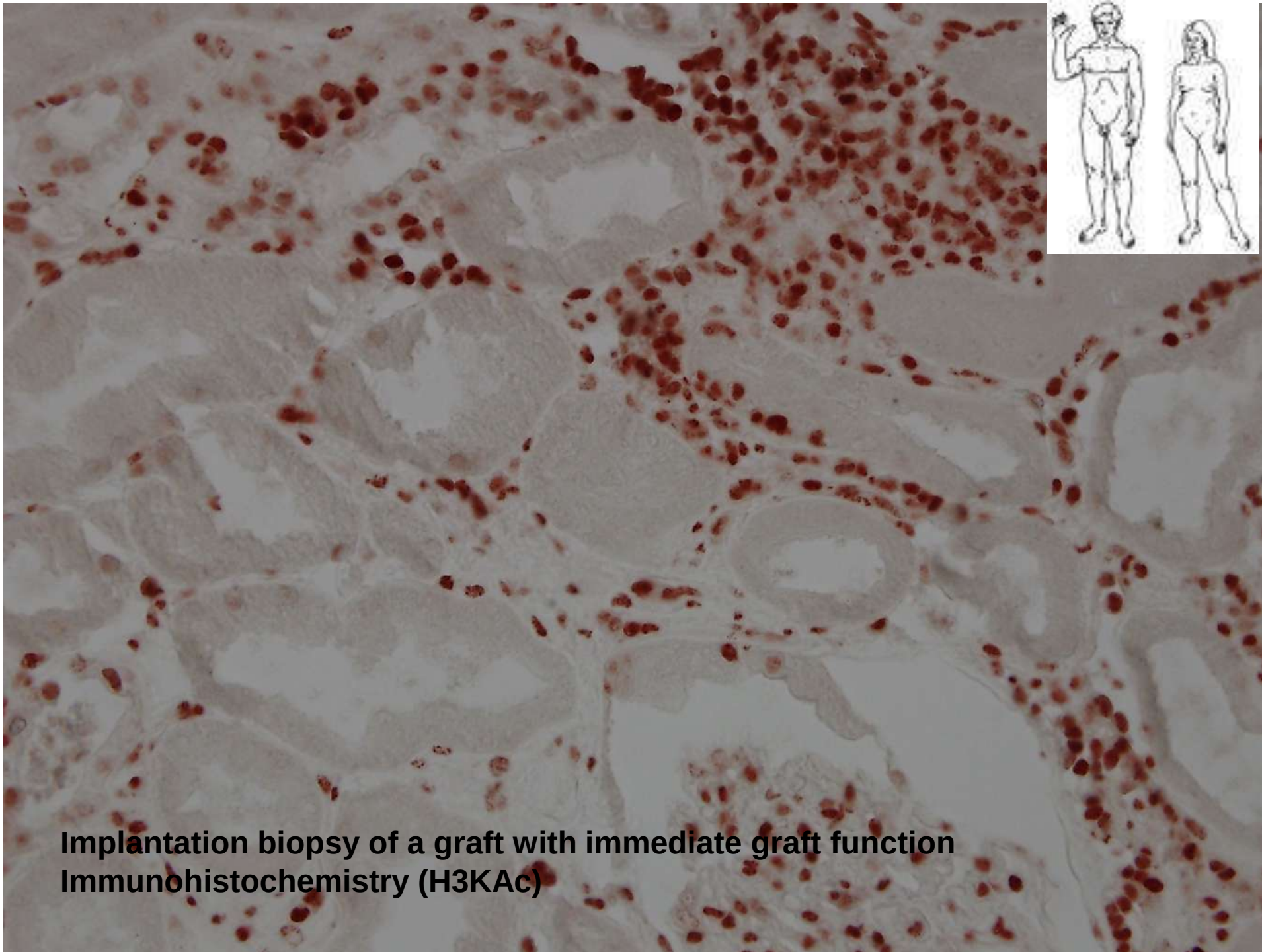




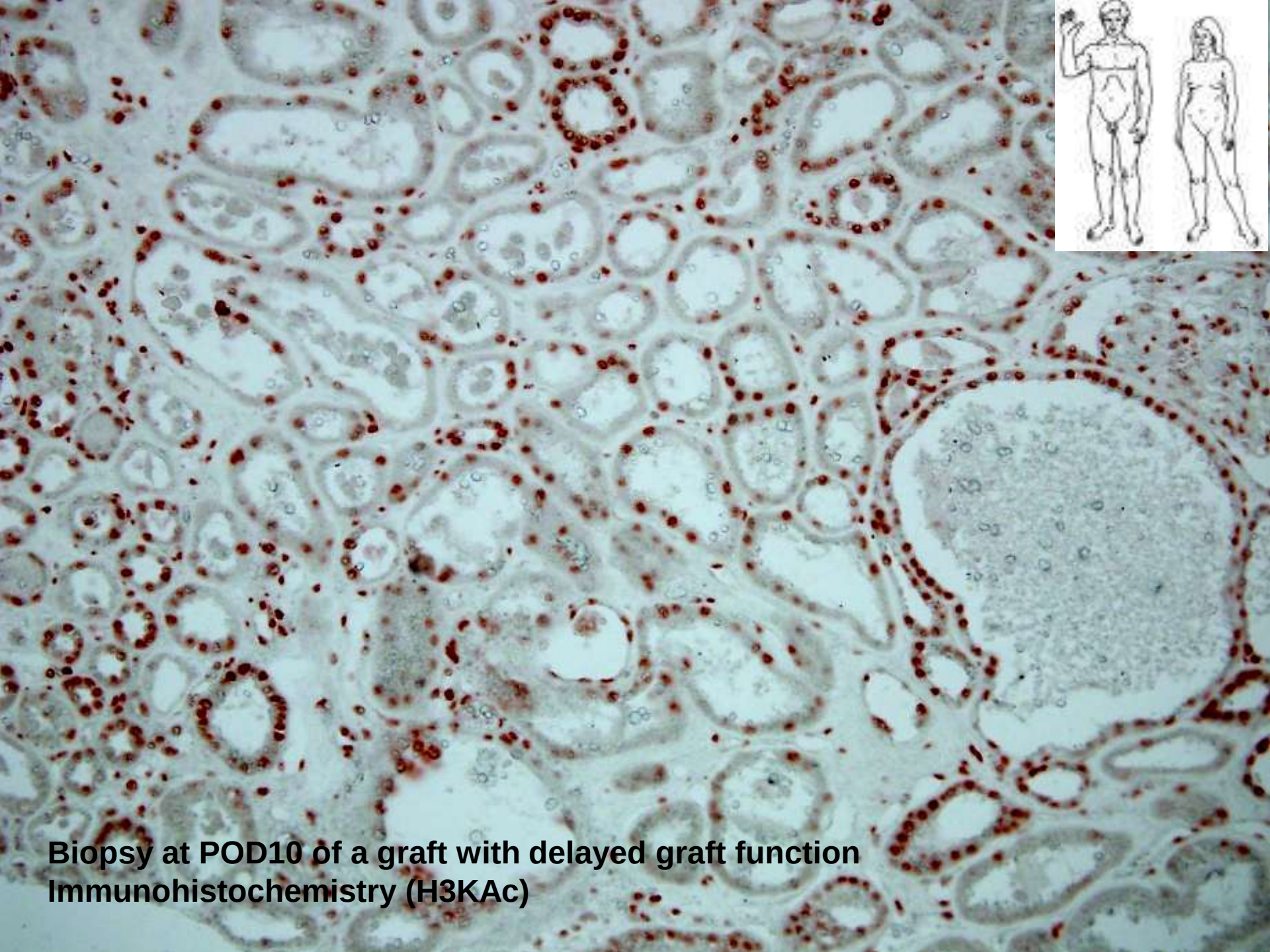






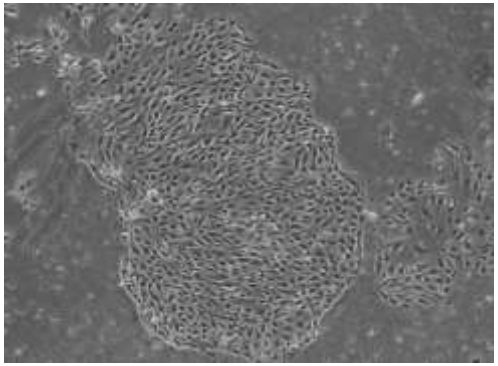


Implantation biopsy of a graft with immediate graft function
Immunohistochemistry (H3KAc)

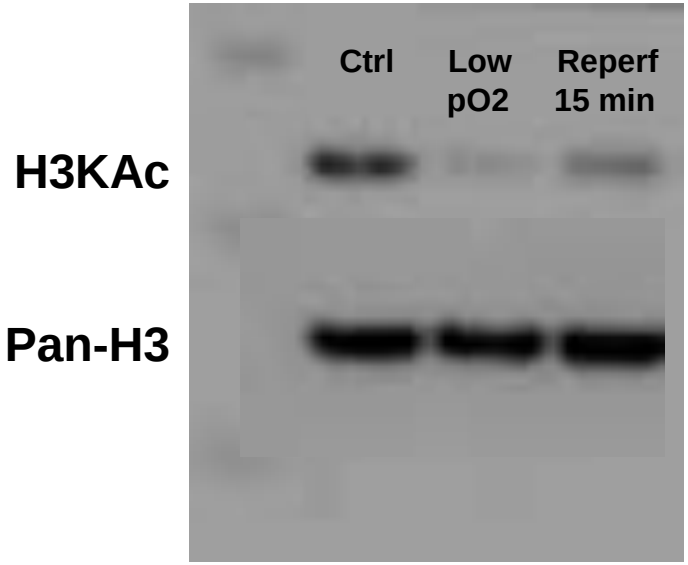


Biopsy at POD10 of a graft with delayed graft function
Immunohistochemistry (H3KAc)

Genome instability induced by ischemia

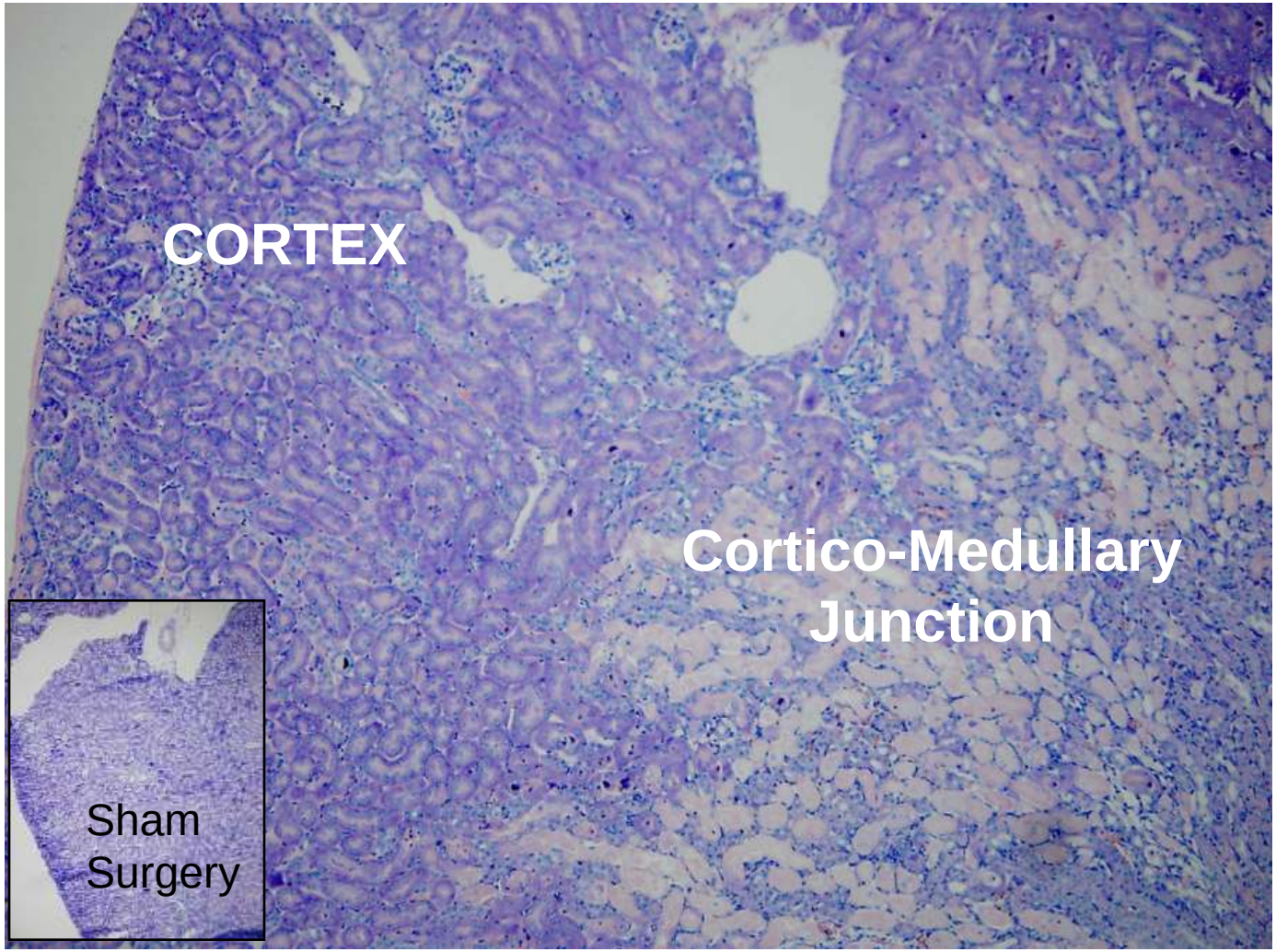


mouse PTEC
under low PO2



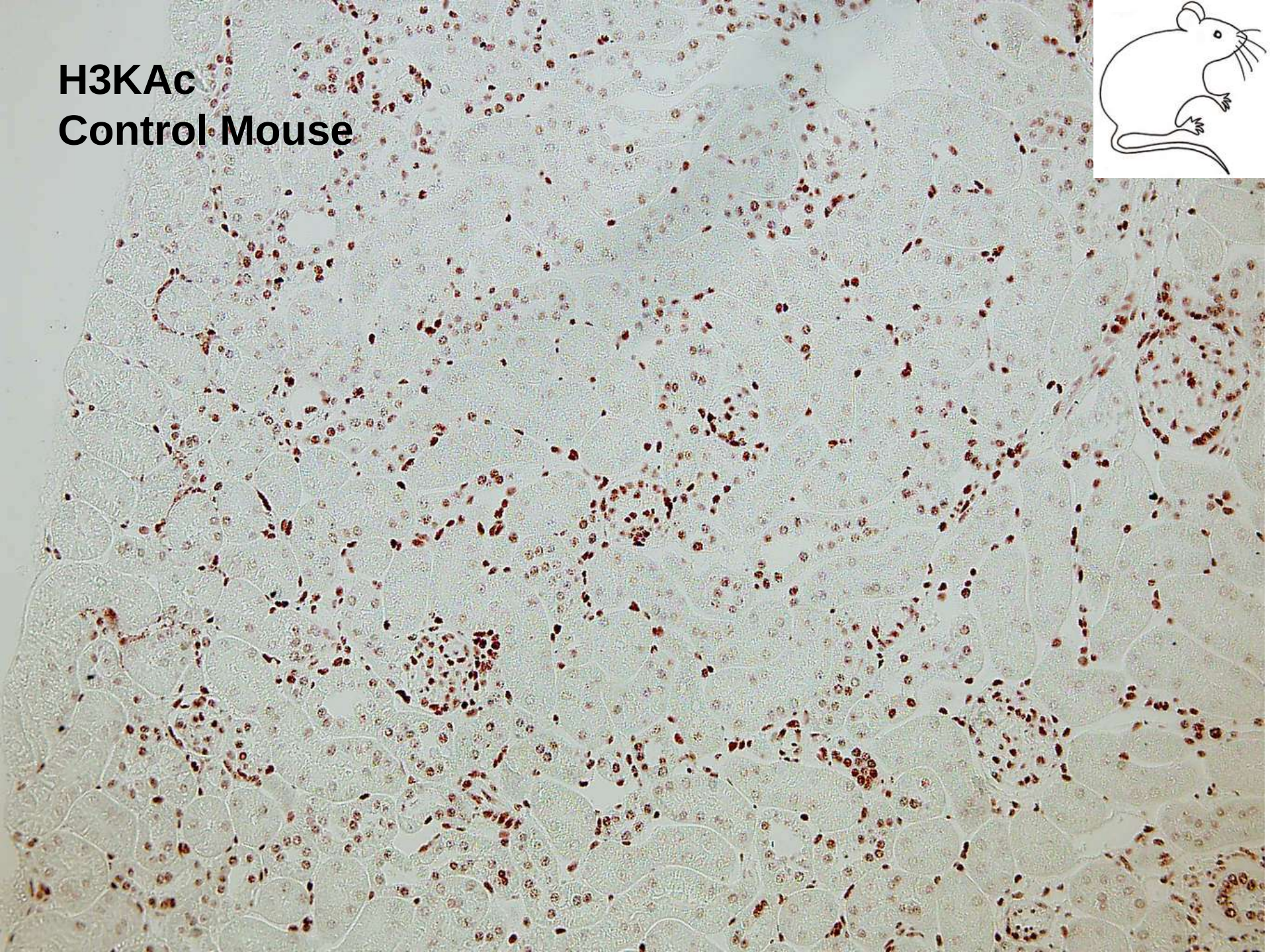
Histone deacetylation and reacetylation

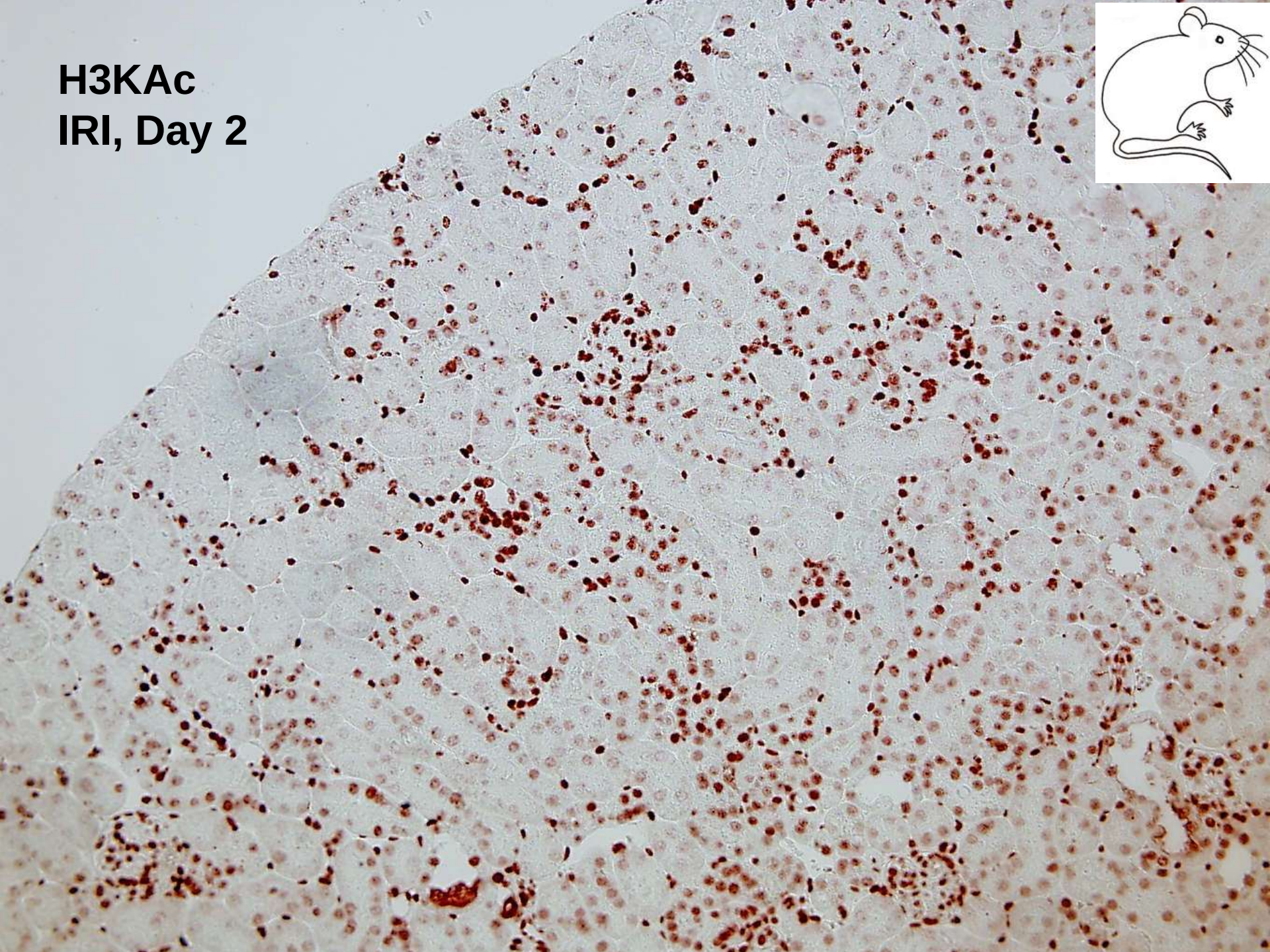
Acute tubular necrosis induced by ischemia reperfusion injury

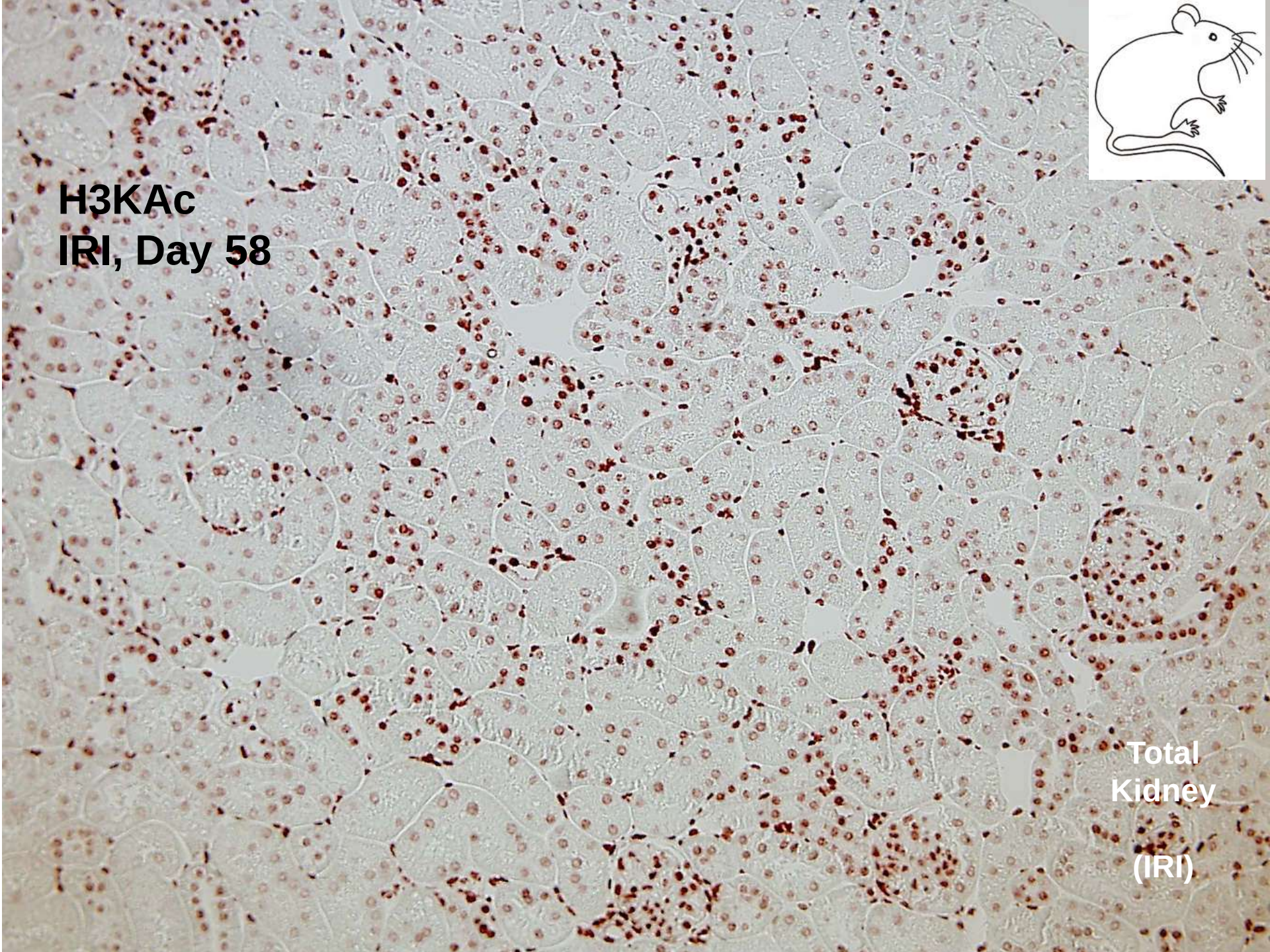


Giemsa

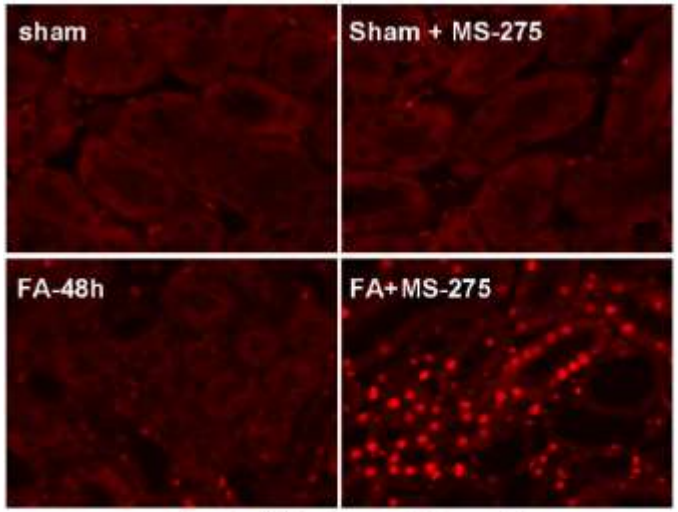
H3KAc
Control Mouse



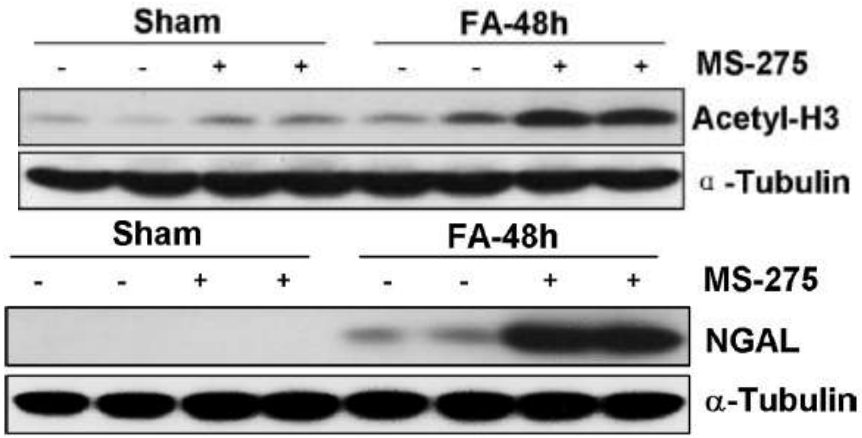

H3KAc
IRI, Day 2

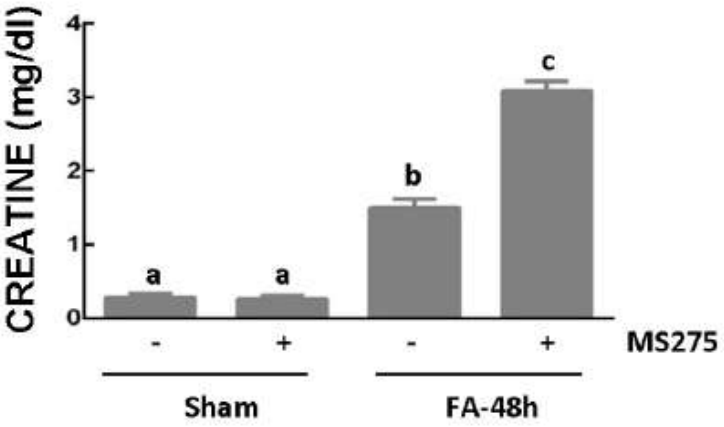

H3KAc
IRI, Day 58
Total
Kidney
(IRI)

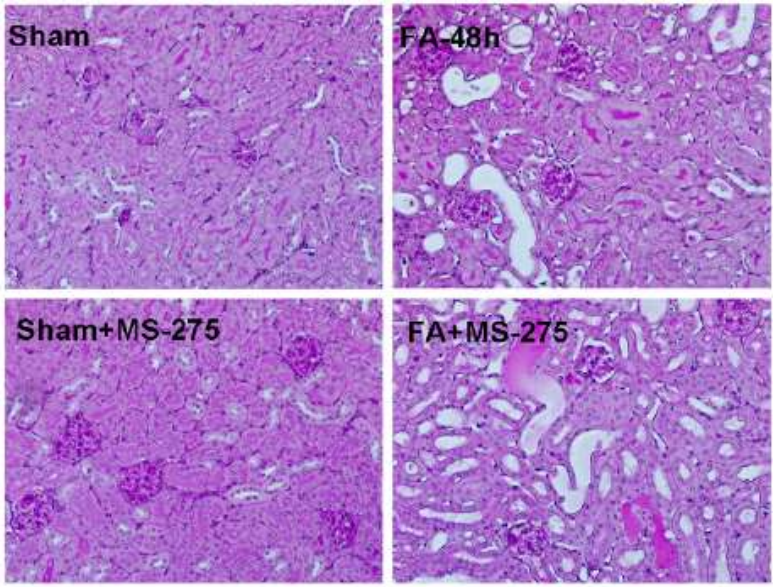
Class I HDAC inhibition potentiates renal damage in folic acid induced AKI



Acetyl-histone 3 (Lys 9)



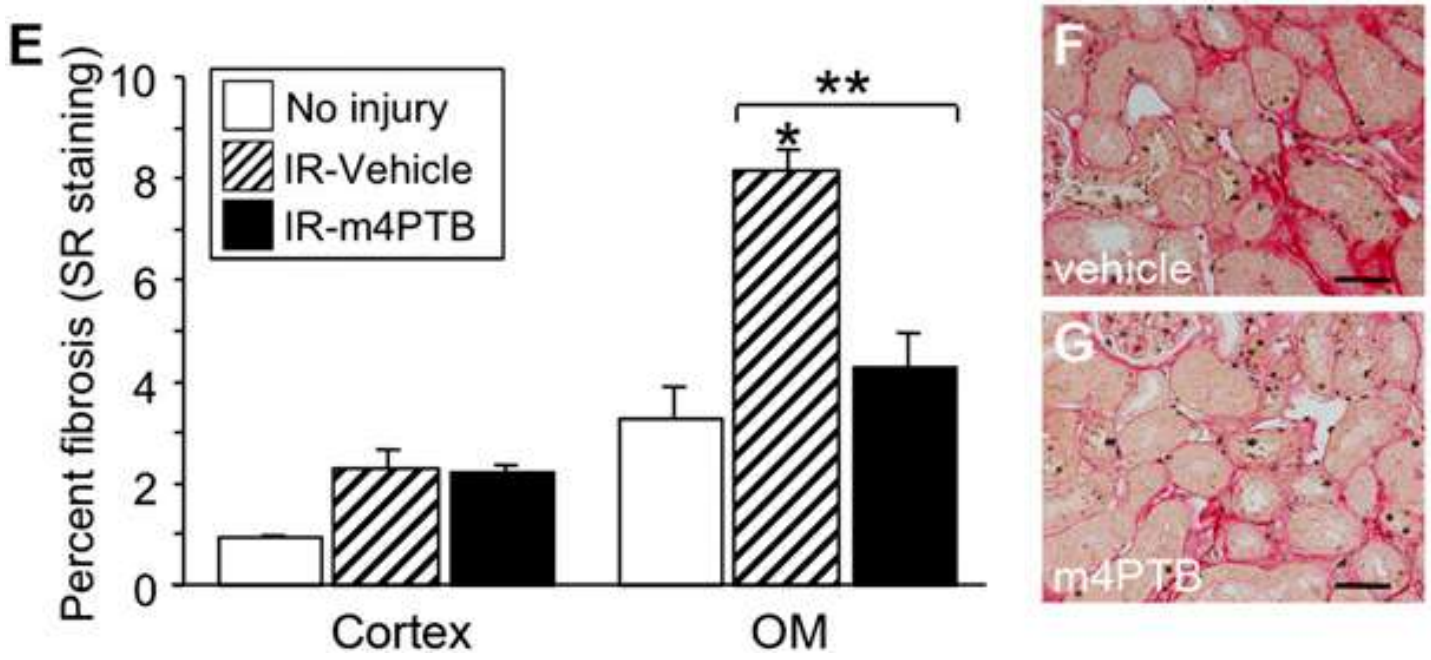




Tang J et al, Am J Physiol Renal Physiol 2014

Therapeutic effect of m4PTB, a Histone Deacetylase Inhibitor

Day O: Left IRI; Day 1 to 7 m4PTB, day 8 right Nx, Day 28: sacrifice



Cosentino CC et al, J Am Soc Nephrol 2013

Which genes are epigenetically imprinted

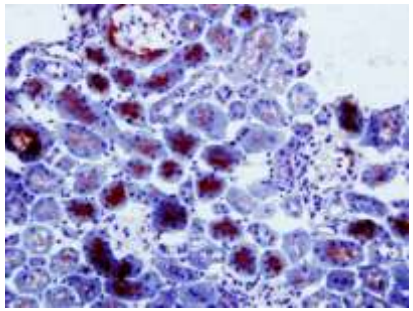
by H3KAc in tubular epithelial cells ?

David Legouis

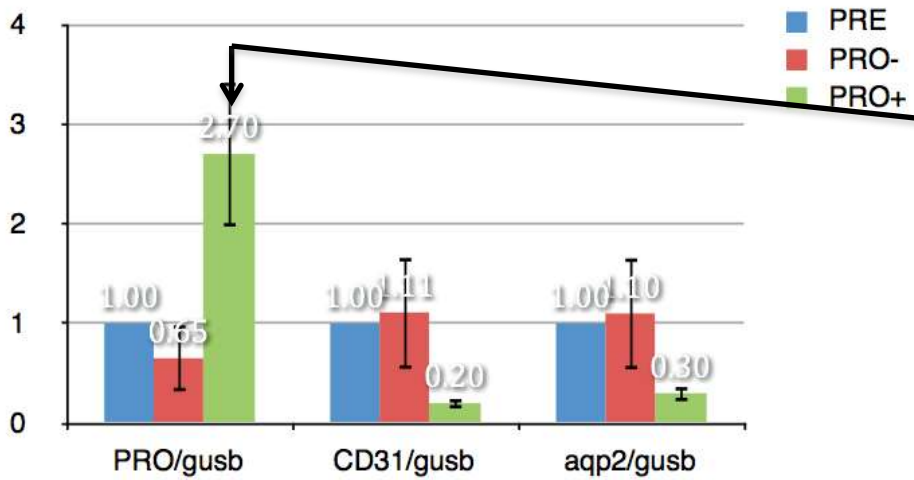
Renal dissociation

Incubation of cell suspension with magnetic beads coated with an antibody targeting PROMININ-1

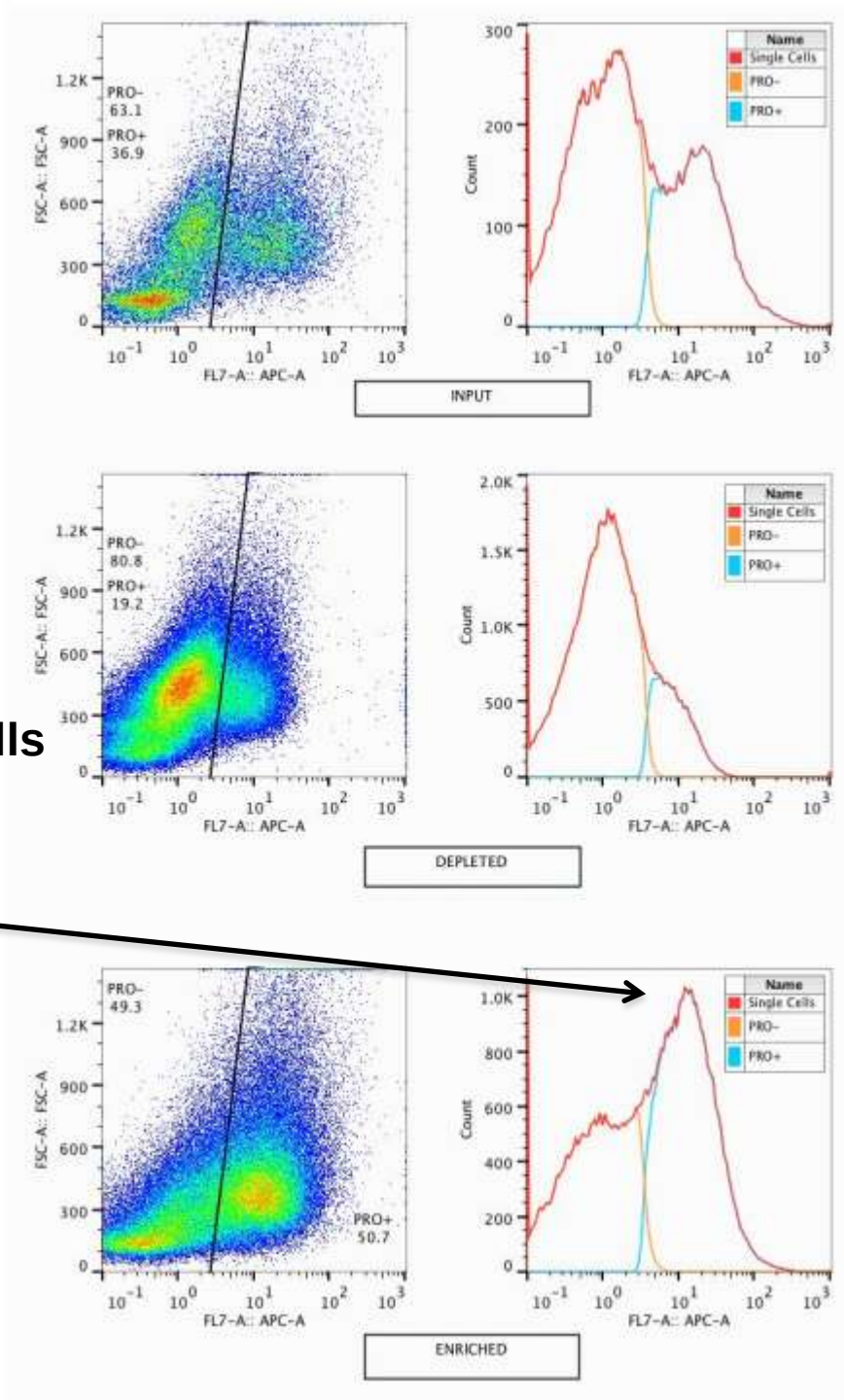
Prominin1 is expressed in the brush border (PTEC)



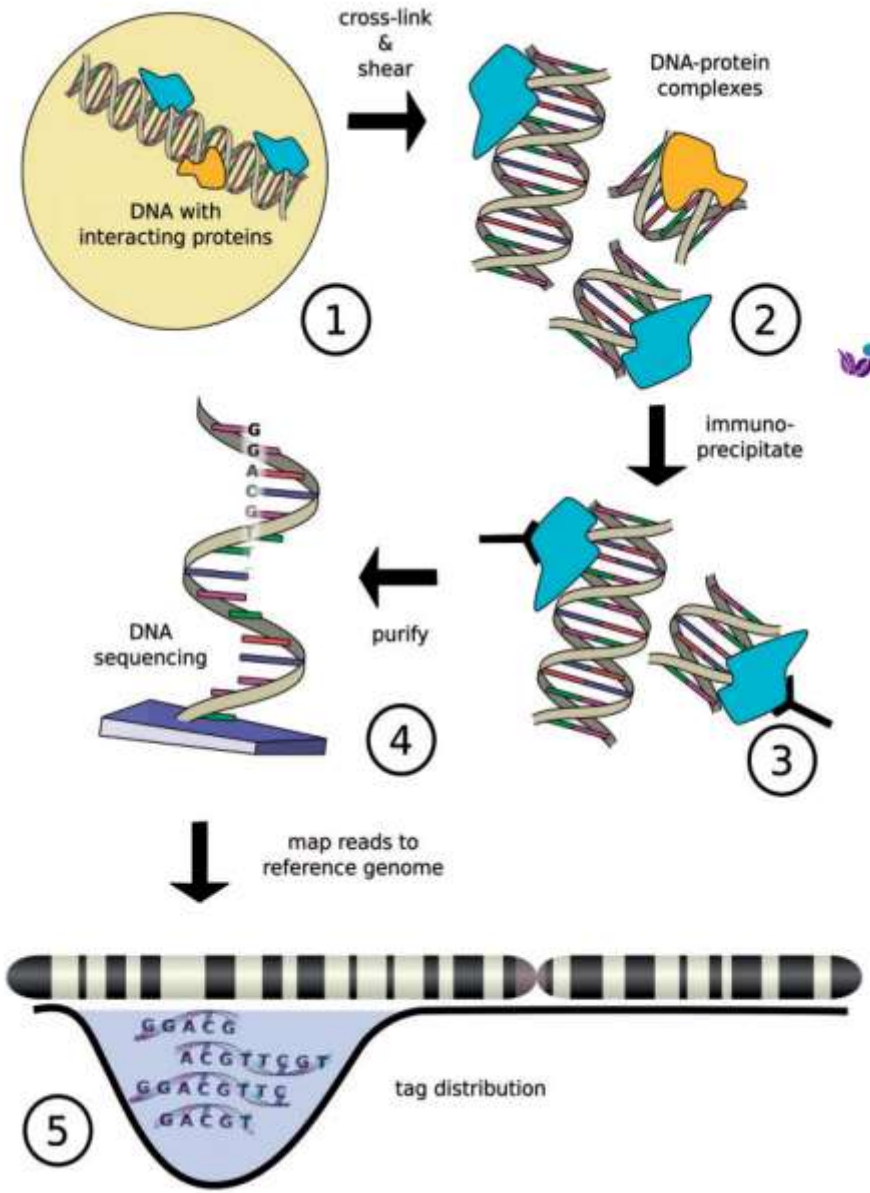
Enrichment in Proximal tubular epithelial cells

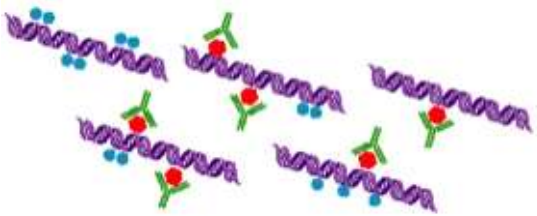


Legouis D et al, BMC Cell Biology 2015



Chromatin Immunoprecipitation



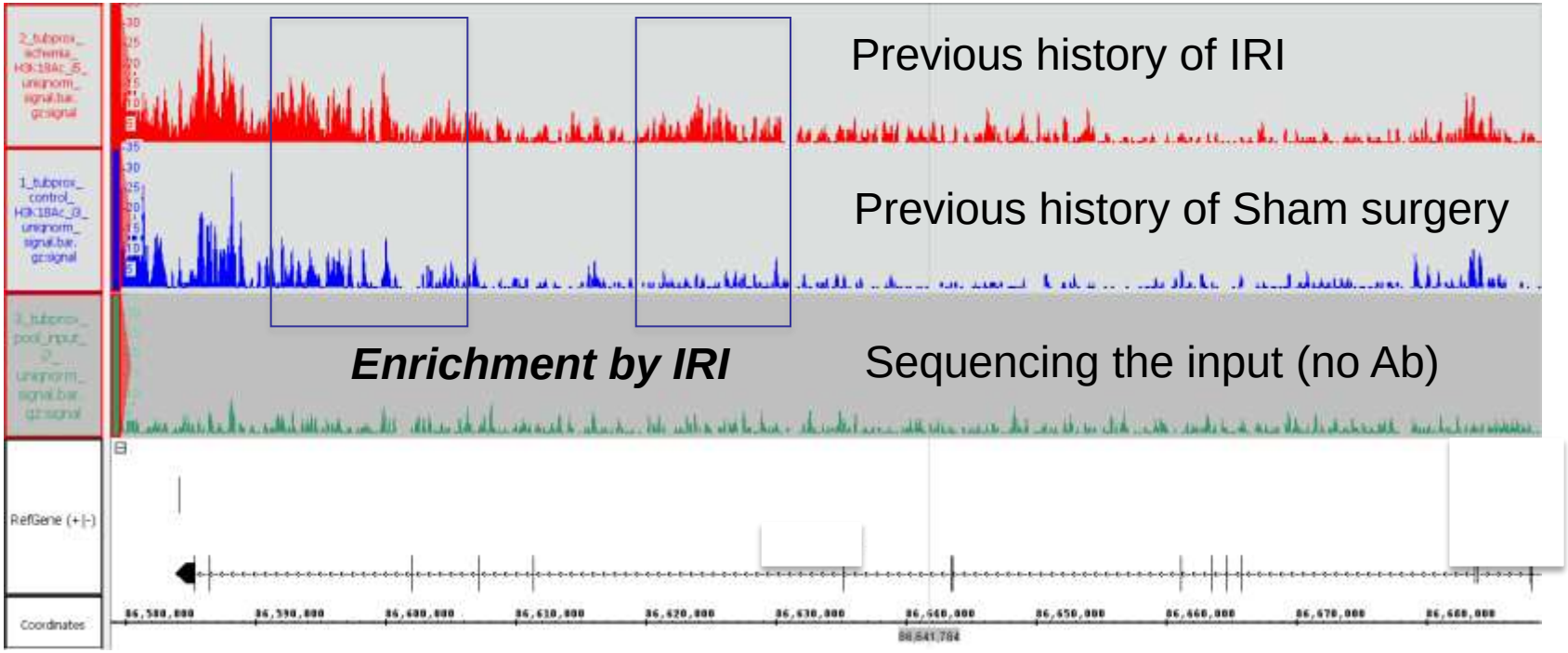


Ex: Anti-H3KAc AB

Sequencing

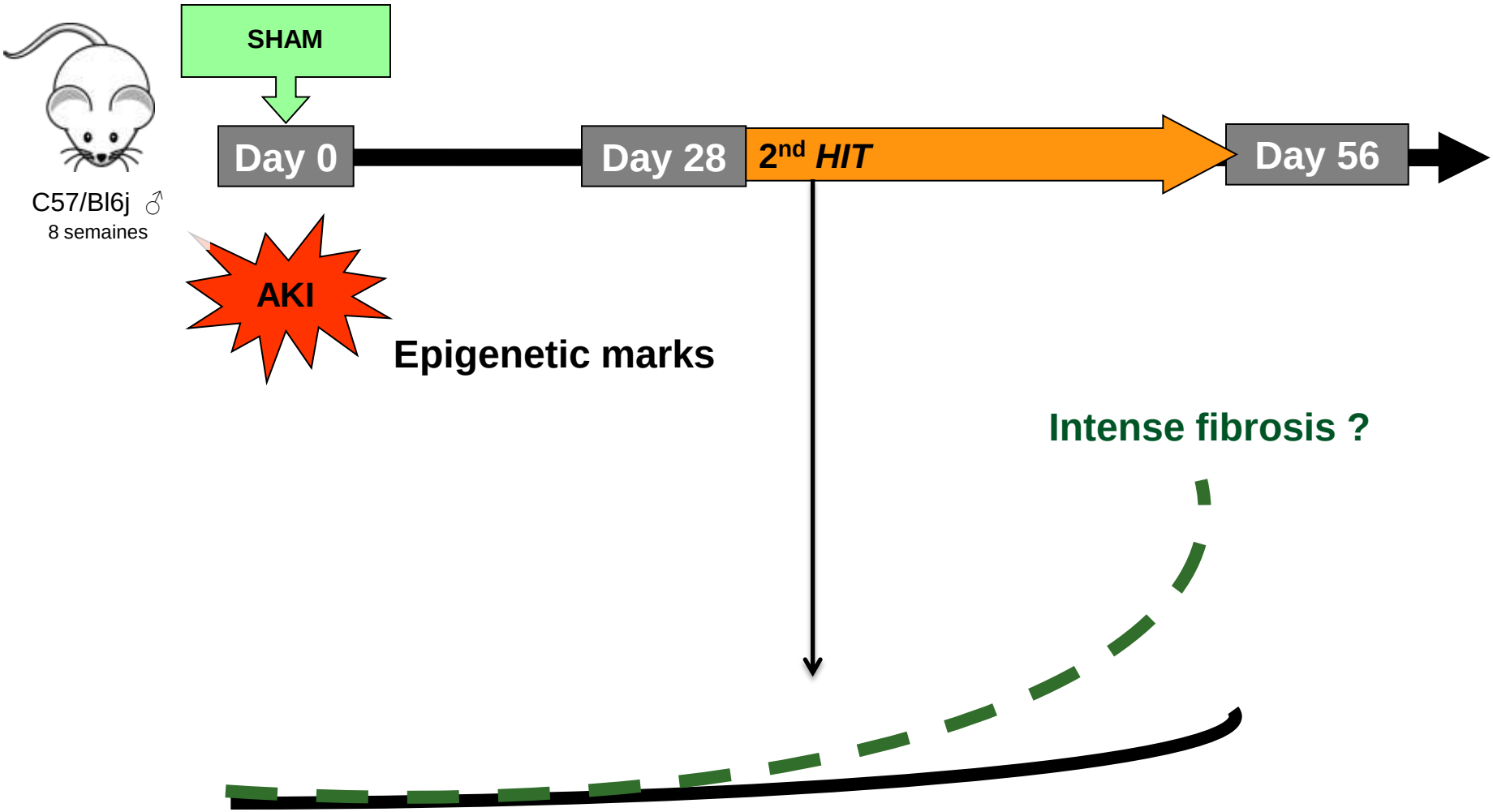
Intensity of the histone mark in the genome: differential analysis

Ischemia vs Sham surgery



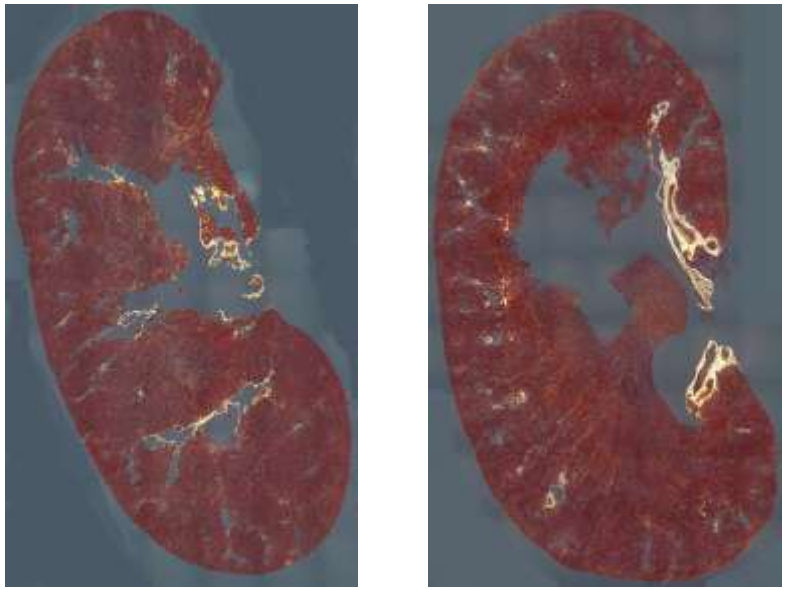
CHiP Seq H3KAc / Isolated Tubular Epithelium / IRI vs Sham

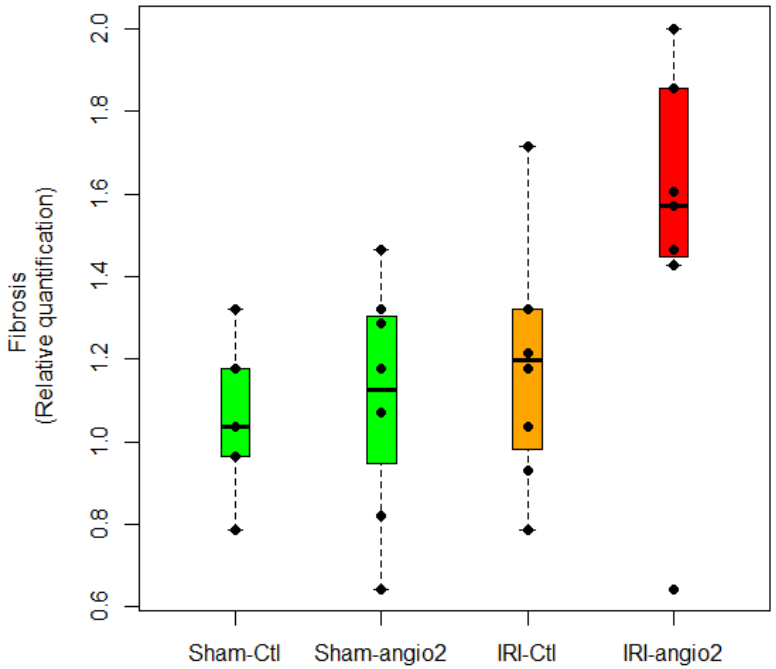
Which impact on renal fibrogenesis ?



Impact of a resolved AKI on fibrogenesis in the presence of a 2nd hit ?

One hit **Two hits**





Genome-wide analysis of the memory effect of AKI ongoing (RNA seq):

- **Before the second hit (are genes permanently activated ?)**
- **After the second hit (are genes « poised for activation »)**

P3S

CONCLUSIONS

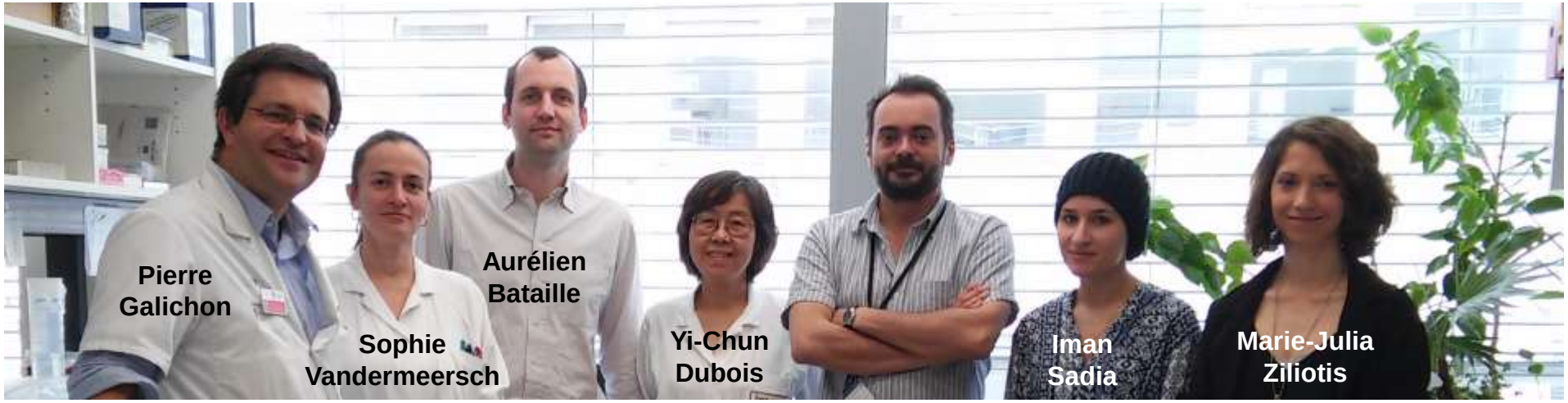
Acute and Chronic Kidney Diseases are interconnected

Auto-perpetuation of fibrosis after AKI is epigenetically driven:

- **Methylation status of PPARγ, TGFβ, RASAL1 in myofibroblasts**
- **Histone modifications in tubular epithelial cells**

New drugs on the way

The « Epigenetic Task Force » of INSERM UMRS_1155, Tenon Hospital



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astellas

SFAR Société Française d'Anesthésie et de Réanimation

« The only lasting truth is change »

Héraclite, *Fragments*

1901-1966

“I think of a child's mind as a blank book.

During the first years of his life, much will be written on the pages.

The quality of that writing will affect his life profoundly.”

Walt Disney