ACADEMY OF ATHENS
BIOMEDICAL RESEARCH FOUNDATION

ΕΛΛΗΝΙΚΗ ΝΕΦΡΟΛΟΓΙΚΗ ΕΤΑΙΡΕΙΑ 1970

ΜΕΙΩΜΕΝΗ ΕΚΦΡΑΣΗ ΤΟΥ ΜΑΚΡΟΥ ΜΗ ΚΩΔΙΚΟΠΟΙΟΥ RNA 01187 (LONG NON-CODING RNA 01187) ΓΟΝΙΔΙΟΥ ΣΧΕΤΙΖΕΤΑΙ ΜΕ ΝΕΦΡΟΠΑΘΕΙΕΣ ΣΤΟΝ ΑΝΘΡΩΠΟ

Δώρα Μανωλάκου, Μεταπτυχιακή Φοιτήτρια

Ερευνητικές Ομάδες Δρα Πολίτη και Δρα Χαρώνη

Ίδρυμα Ιατροβιολογικών Ερευνών Ακαδημίας Αθηνών

20ο Πανελλήνιο Συνέδριο Νεφρολογίας

3-6 Μαΐου 2018

The work to be presented is a collaborative effort

- **Biomedical Research Foundation of the Academy of Athens, Greece;** *Dora Manolakou, Valeria Kaltezioti, Elena Arvaniti, Panos Politis, Aris Charonis*
- **Department of Pathological Anatomy, University of Athens Medical School, Greece;** *Hara Gakiopoulou*
- **INSERM UMRS 1155, Tenon Hospital, Paris, France;** *Panagiotis Kavvadas, Christos Chatziantoniou*
- **Department of Nephrology, University Hospital of Regensburg, Germany;** *Simone Reichelt-Wurm, Miriam Banas*
- **Nephrology Center, Medical Clinic and Polyclinic IV, University of Munich, Germany;** *Maja Lindenmeyer, Clemens Cohen*
- **Institute of Pathology, RWTH, Aachen, Germany;** *Sonja Djudjaj, Peter Boor*

Non coding Genome: Junk DNA or critical regulator?

Long non-coding RNAs: why bother?

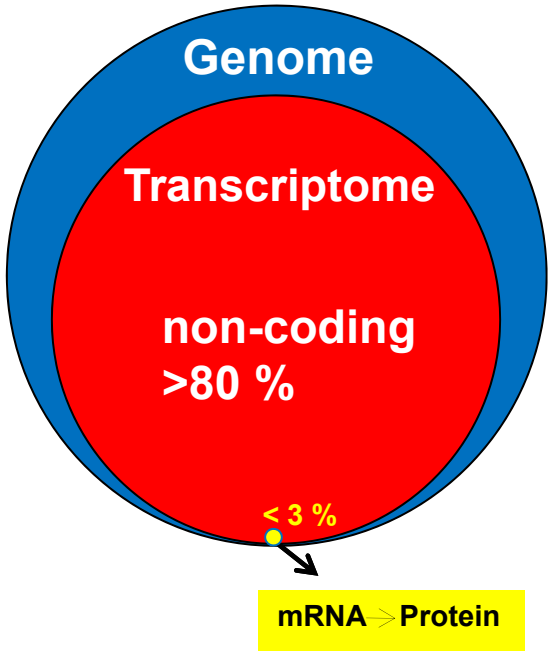
- **Non-protein coding**

(or lack > 100 amino acid open reading frame)

- **>200 nts**
- **Post-transcriptional processing**

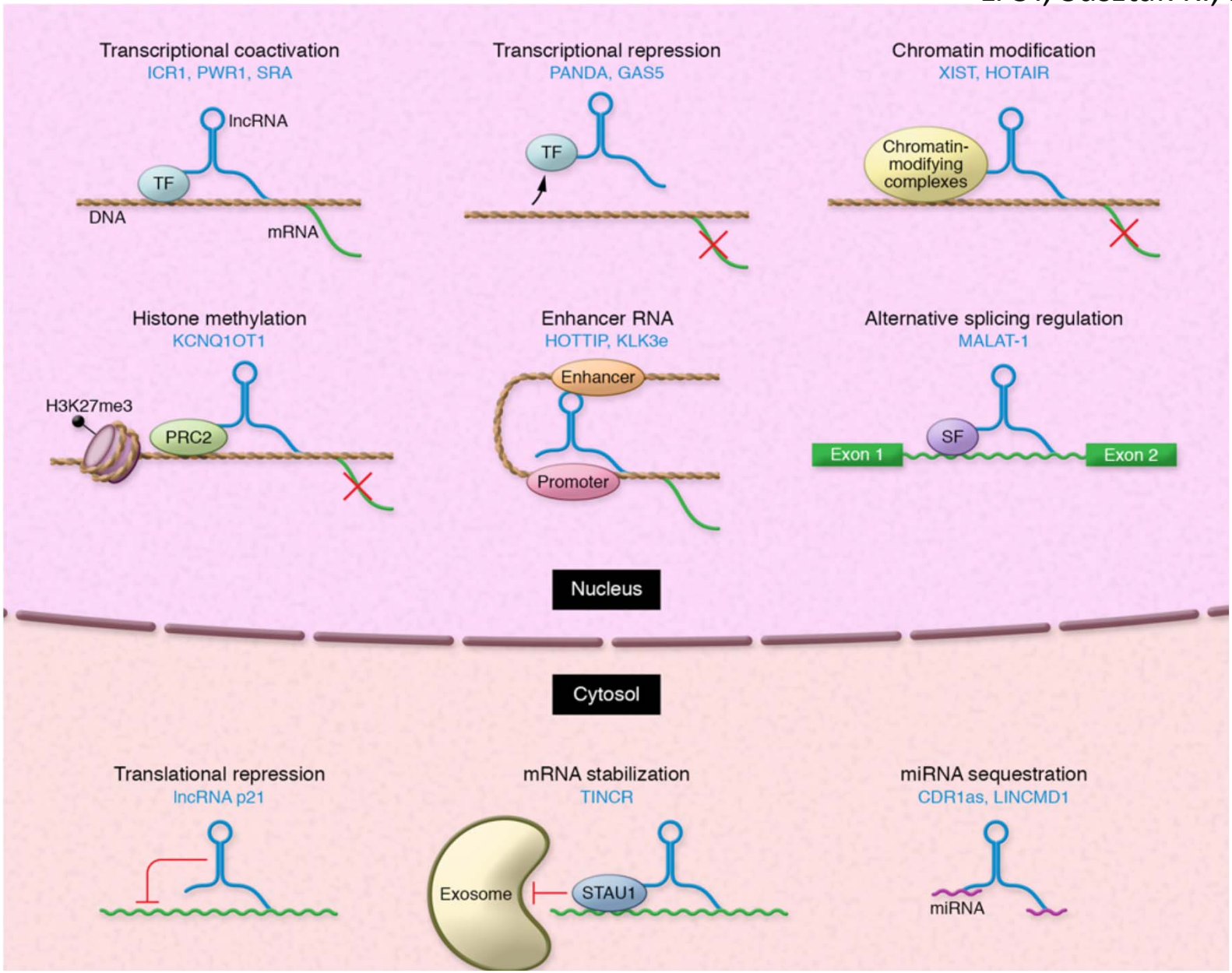
i.e. 5' cap, polyadenylation, splicing

- **Promoter conservation**
- **Tissue- / cell-type specific**
- **Nuclear and/or cytoplasmic functions**



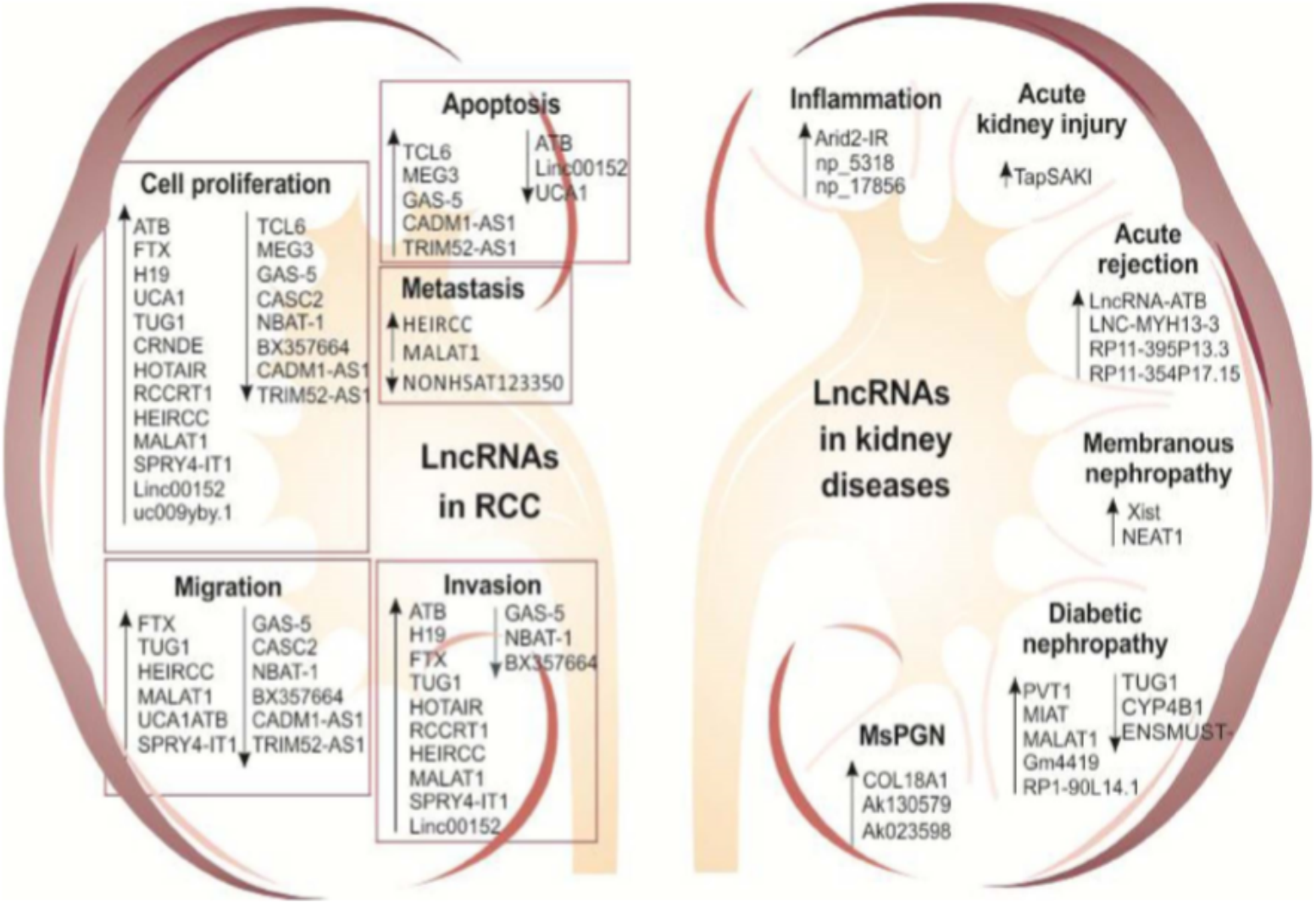
LncRNAs can interfere with gene function at multiple levels

Li SY, Susztak K., 2016



LncRNAs in renal diseases and cancer

Biomedicine & Pharmacotherapy 99 (2018) 755–765



Our aim

Based on:

www.nature.com/scientificreports

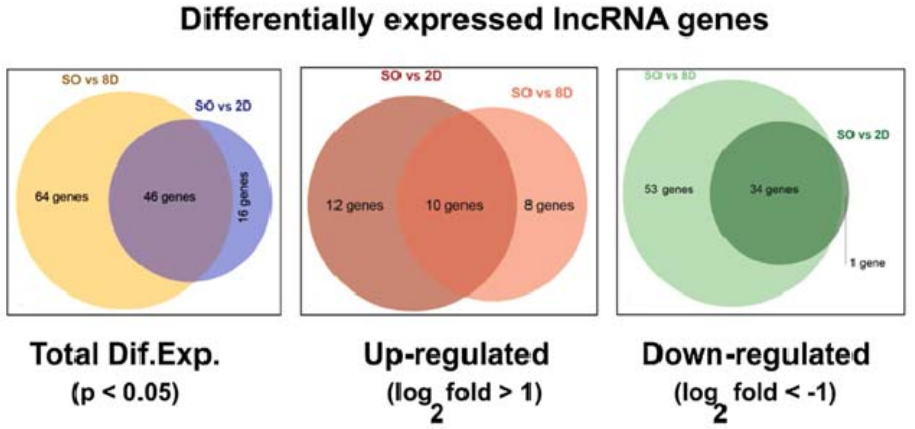
SCIENTIFIC REPORTS

OPEN

Whole-transcriptome analysis of UUO mouse model of renal fibrosis reveals new molecular players in kidney diseases

Received: 18 December 2015
Accepted: 28 April 2016
Published: 18 May 2016

Eleni Arvaniti[1], Panagiotis Moulos[2], Athina Vakrakou[1], Christos Chatziantoniou[3], Christos Chadjichristos[3], Panagiotis Kavvadas[3], Aristidis Charonis[1,*] & Panagiotis K. Politis[4,*]



Our goals:

- Unravel the role of lncRNAs in the pathophysiology of renal diseases
- Identify lncRNAs as potential prognostic and diagnostic biomarkers for renal diseases

Our strategy

from Mice to Men

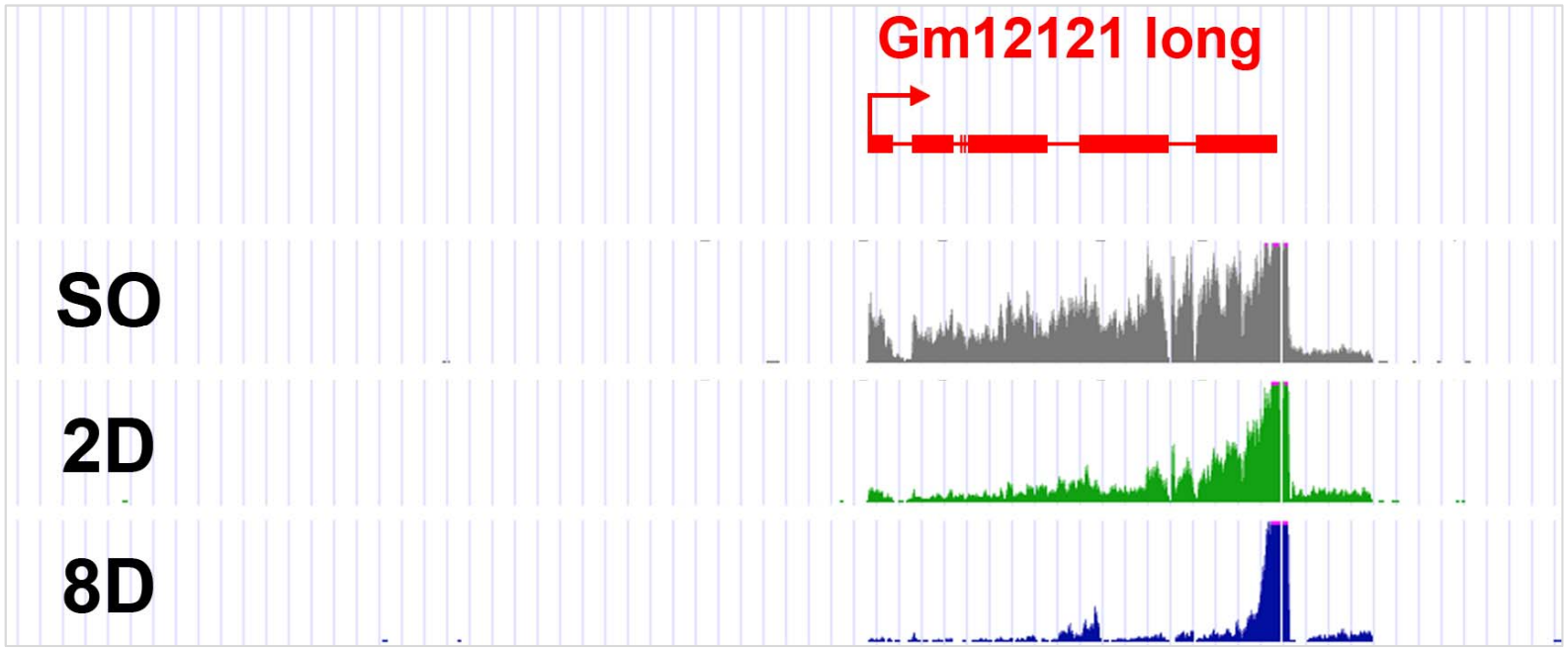
~80 mouse lncRNAs
with altered expression
in mouse animal models of renal diseases

18 human lncRNAs
- high expression in renal tissue
- high sequence conservation at the promoter region

Data on Gm12121long

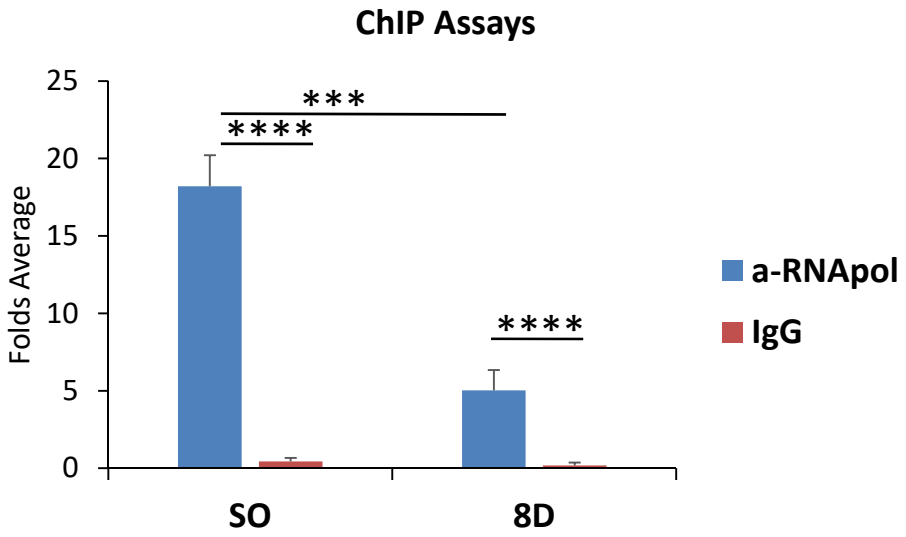
❑RNA-Seq Data from UUO mouse model

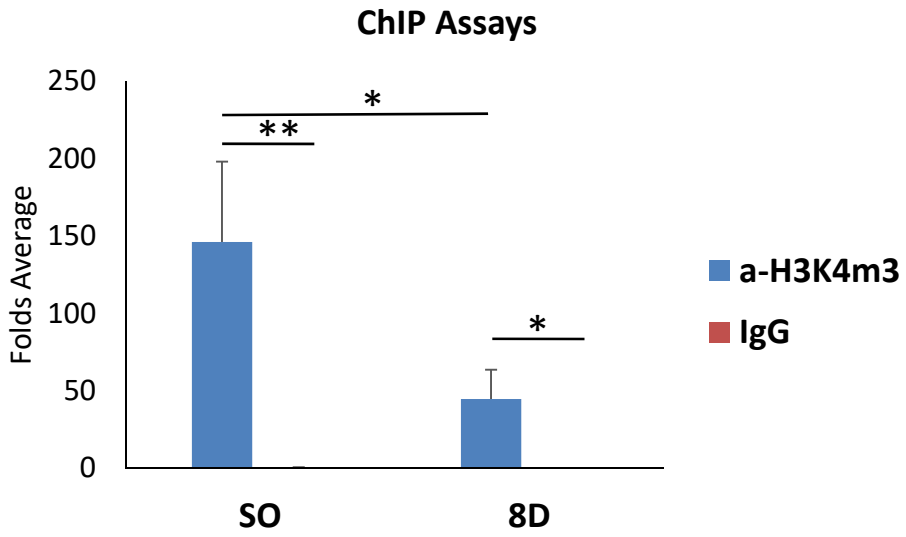
✓Gm12121long expression is reduced in diseased mice (2D and 8D) compared to the healthy ones (SO).



Gm12121long is reduced in kidney diseases

Promoter activity is reduced in **UUO** mice (8D) compared to the healthy ones (SO)

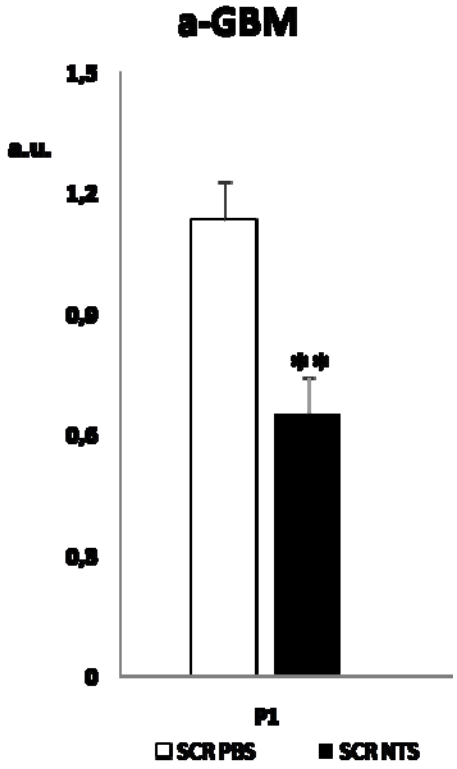




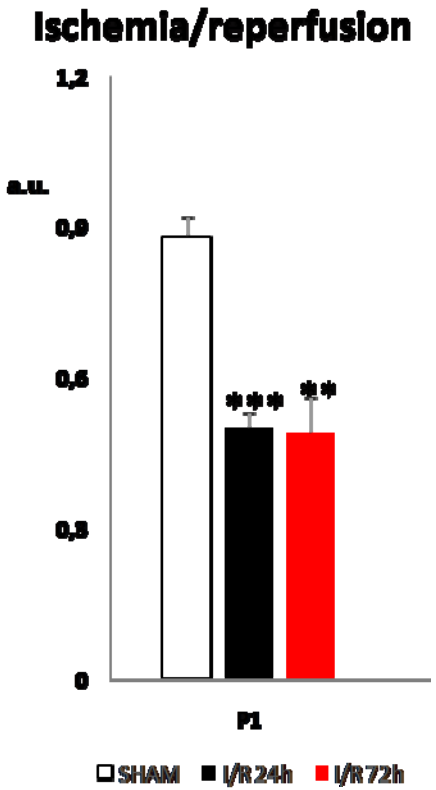
*p<0.05, **p<0.01, ***p<0.001, ****p<0.0001

Gm12121long is reduced in kidney diseases

Gm12121long expression is reduced in **anti-GBM** and **ischemia/reperfusion** models mice compared to the healthy ones



** P < 0.01 vs scr pbs

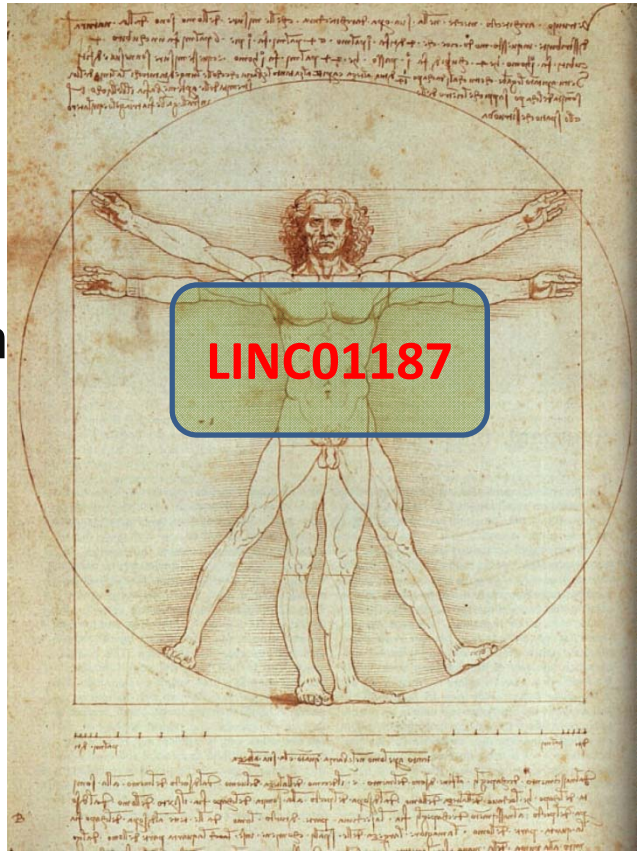


** P < 0.01, *** P < 0.001 vs sham

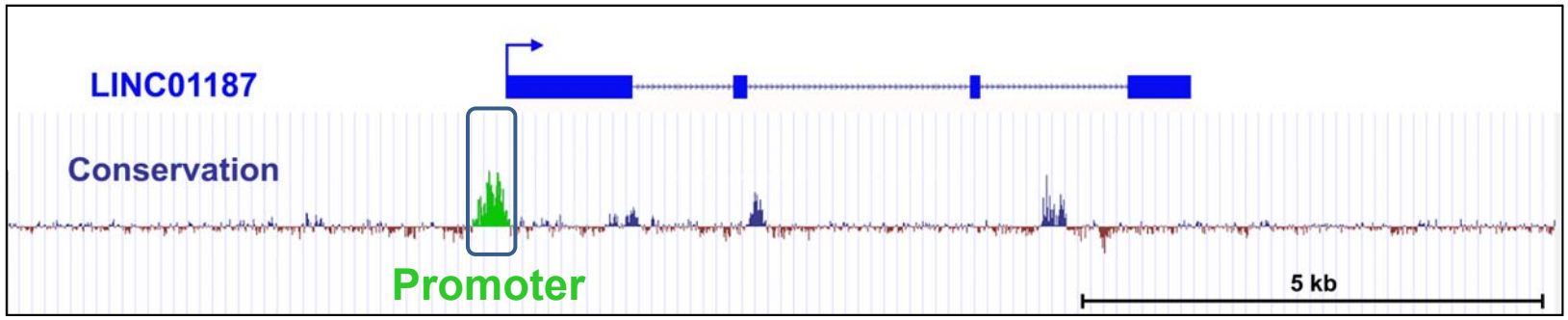
I/R 24 hours: peak of damage
I/R 72 hours: repair

Dr Chatziantoniou group, Paris

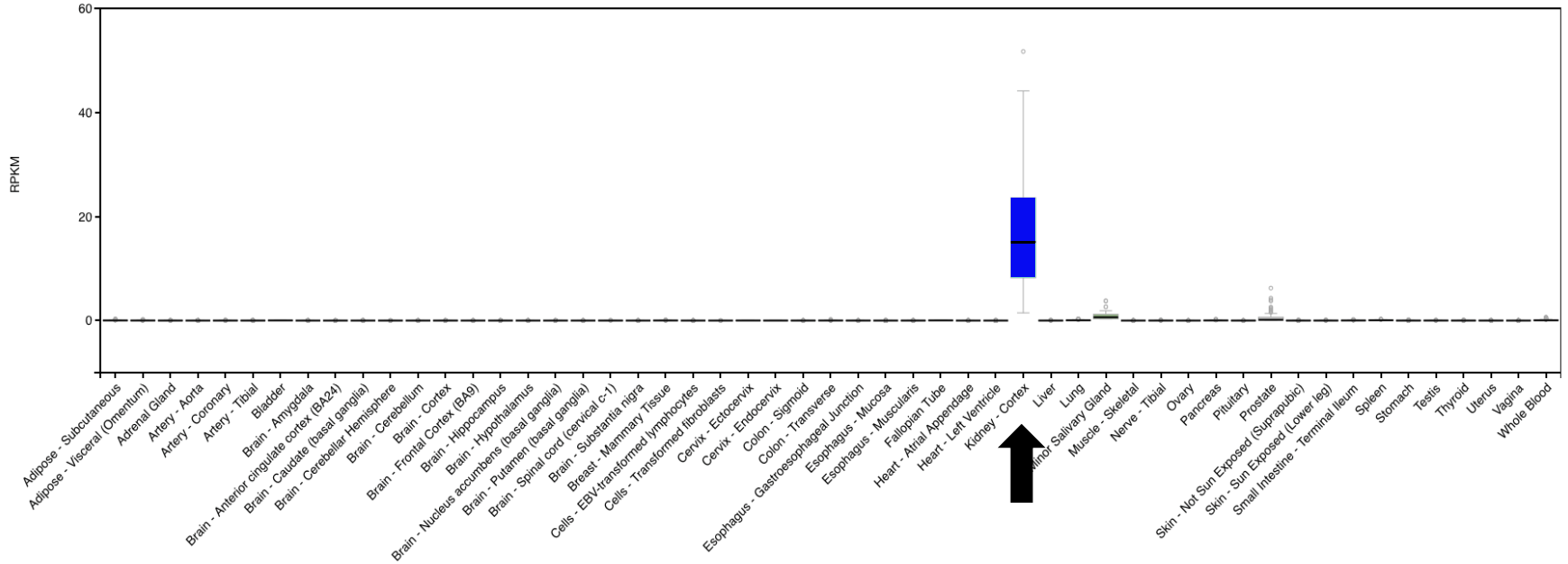
from Mice to Men



Gene architecture of long non coding RNA LINC01187

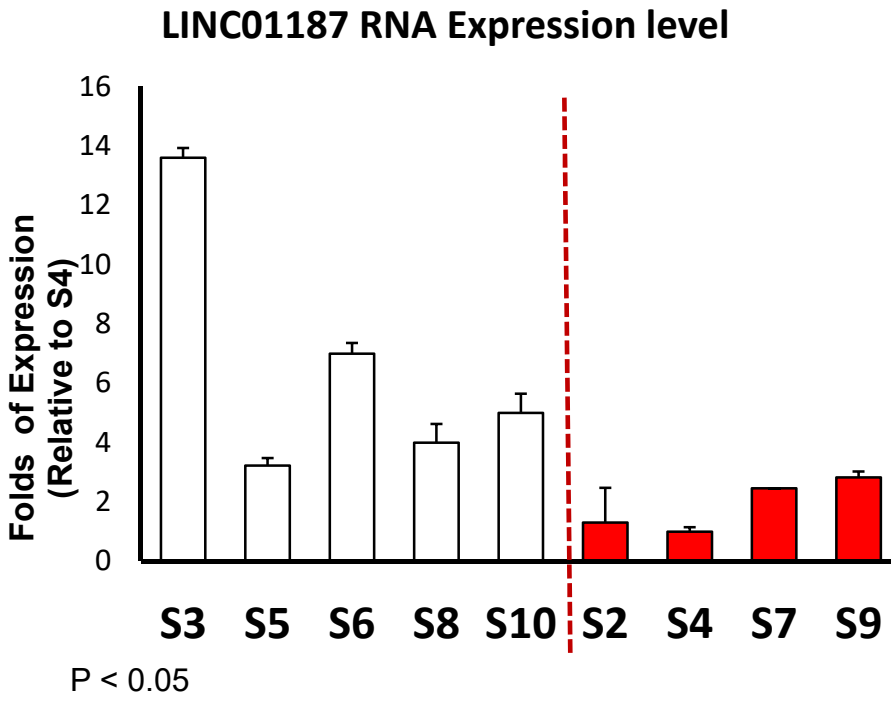


Expression of LINC01187 in human tissues (GTEX analysis)



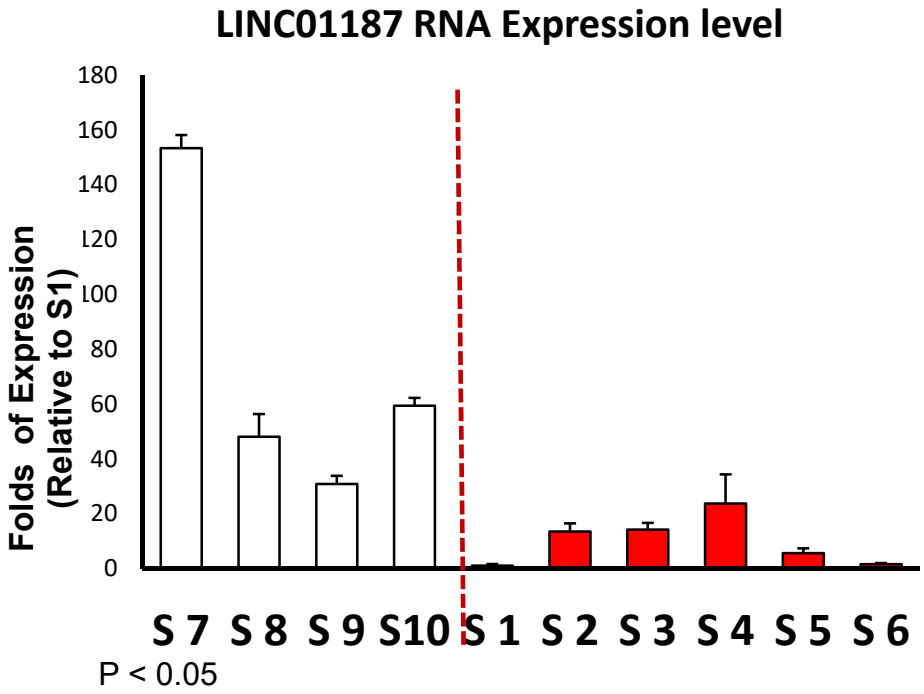
LINC01187 expression is reduced in renal pathological samples (A)

Renal cDNA from Aachen



S3-S5-S8-S10: Control samples
S2-S4-S7-S9 : Patient samples

Renal cDNA from Regensburg



S7-S10 : Control samples
S1-S6: Patient samples

LINC01187 expression is reduced in pathological samples (B)

Microdissected renal biopsies of the
European Renal cDNA Bank (ERCB)

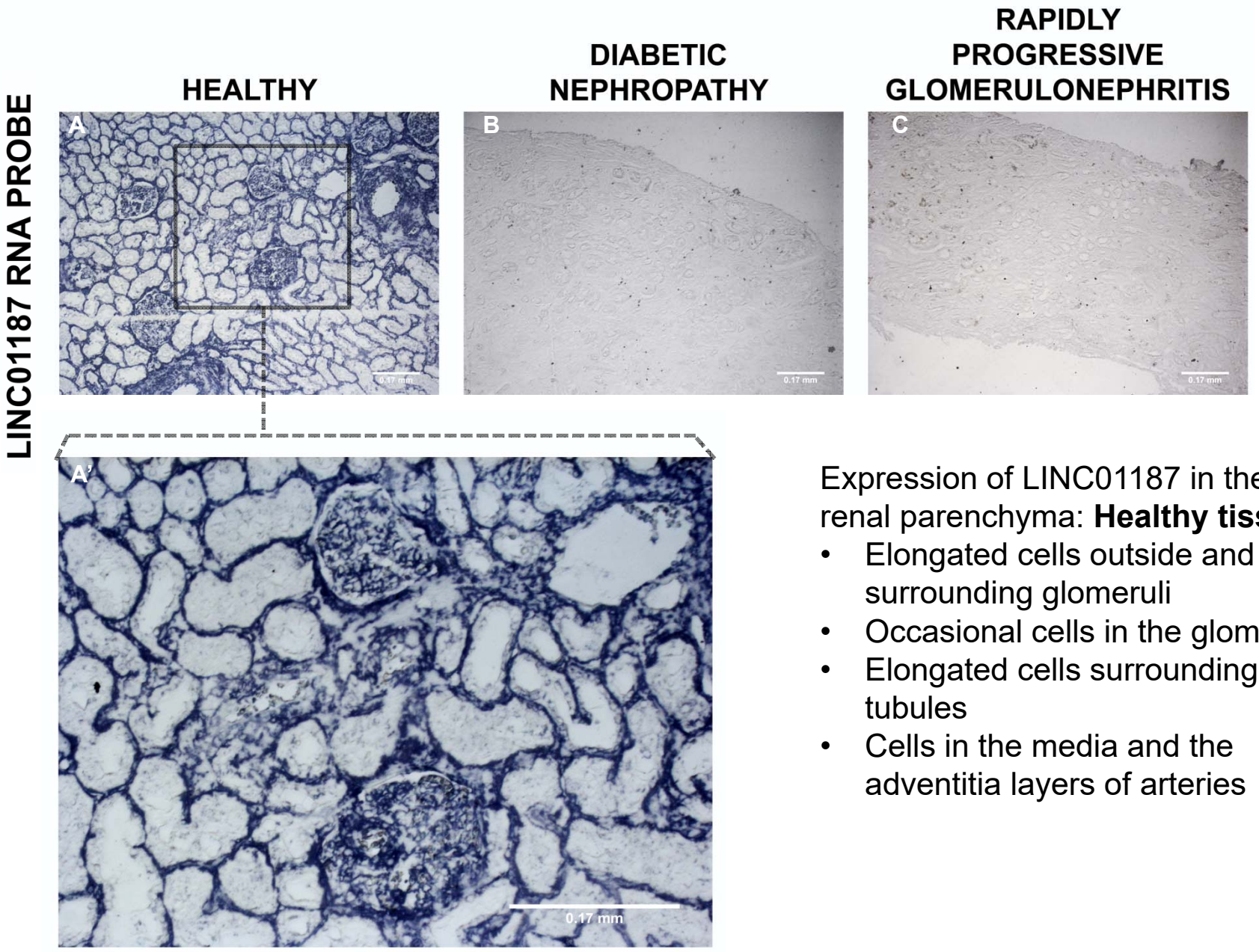
LINC01187 expression **REDUCTION**

- Diabetic Nephropathy
- Rapidly Progressive Glomerulonephritis

both in the glomerular and the tubular compartment

Maja Lindenmeyer and Clemens Cohen, Munich

LINC01187 expression is reduced in pathological samples (C)



Expression of LINC01187 in the renal parenchyma: **Healthy tissue**

- Elongated cells outside and surrounding glomeruli
- Occasional cells in the glomeruli
- Elongated cells surrounding tubules
- Cells in the media and the adventitia layers of arteries

What do these cells have in common?

Do they have a common origin?

Is LINC01187 acting in cis-?

- Genes close to LINC01187 in human chromosome 5?
  - ➢ Short list for protein coding genes

- **Forkhead Box I1 (FOXI1)** is expressed almost exclusively in renal tissue

- Transcriptional activator required for the development of normal hearing, sense of balance and kidney function

Conclusions

- Gm12121long is reduced in mouse models for kidney diseases
- LINC01187 is potentially involved in the pathogenesis of human kidney diseases
  - ✓ Specific spatial expression pattern around/ inside glomeruli, around tubules, arteries of healthy kidney tissue
  - ✓ Reduced expression is correlated to cases of kidney pathogenesis (DN, RPGN)
  - ✓ It could potentially act in cis with FOXI1 transcription factor

Thank you!

❖ **Dr Charonis lab, BRFAA**

Valeria Kaltezioti, MSc, lab technician

Myrto Rizou, PhD candidate

George Barkas, PhD candidate

Elena Arvaniti, PhD

❖ **Dr Politis lab, BRFAA**

Valeria Kaltezioti, MSc, lab technician

Nikos Malissovas, PhD

Daphne Antoniou, PhD candidate

Elpinickie Ninou, PhD candidate

Dimitris Gkikas, PhD candidate

Tina Tsampoula, PhD candidate

Artemis Michail, PhD candidate

Efstathia Tetringa, MSc Student

❖ **Dr Hara Gakiopoulou, Med School**

❖ **Dr Periklis Makrythanasis, BRFAA**

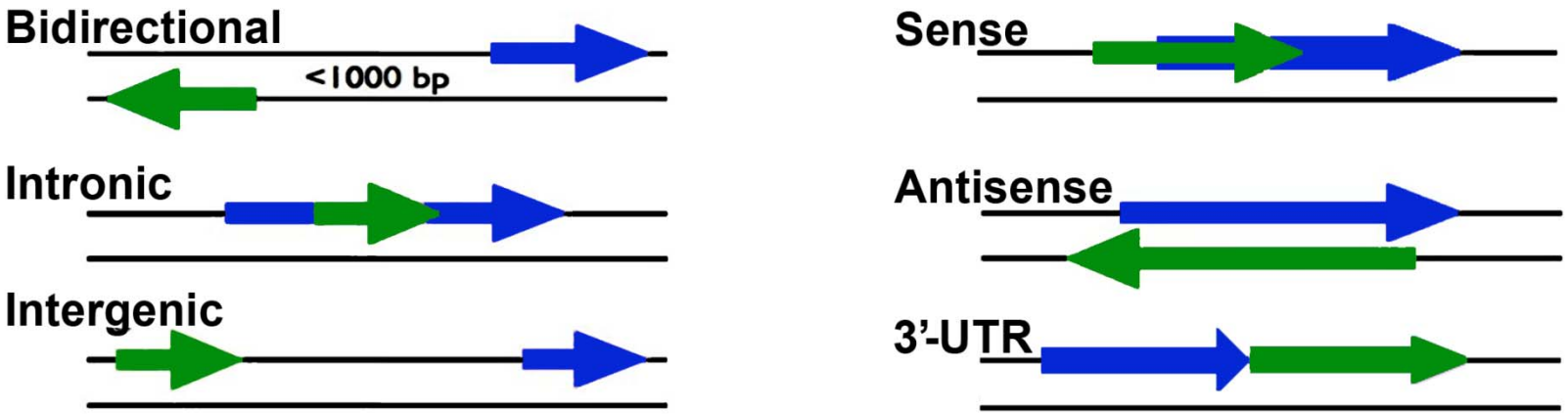
❖ **All the collaborators from abroad**

Supplementary

Non coding Genome: Junk DNA or critical regulator?

: genes encoding for lncRNAs

: genes encoding for Proteins



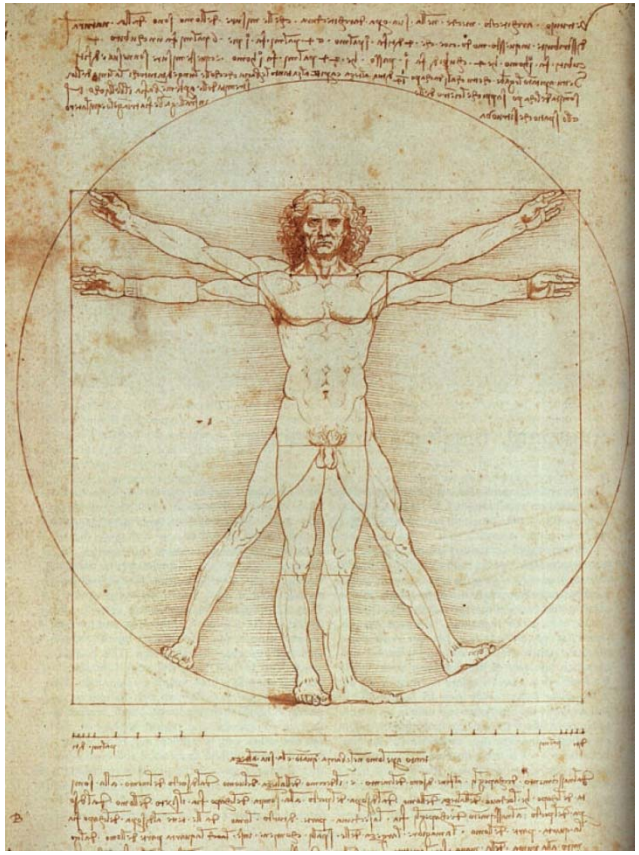
Long non coding RNAs in renal diseases

UP-REGULATED lncRNAs in UUO

-AI504432
-Gm13889
-A430104N18Rik
-Gm20645
-Snhg5
-Snhg1
-Snhg6
-Neat1
-Mir17hg
-Malat1
-Snhg7

DOWN-REGULATED lncRNAs

-1500016L03Rik (LHX1os)
-Gm17750
-1700022N22Rik
-Fam120aos
-9130409J20Rik
-2500002B13Rik
-Gm12121

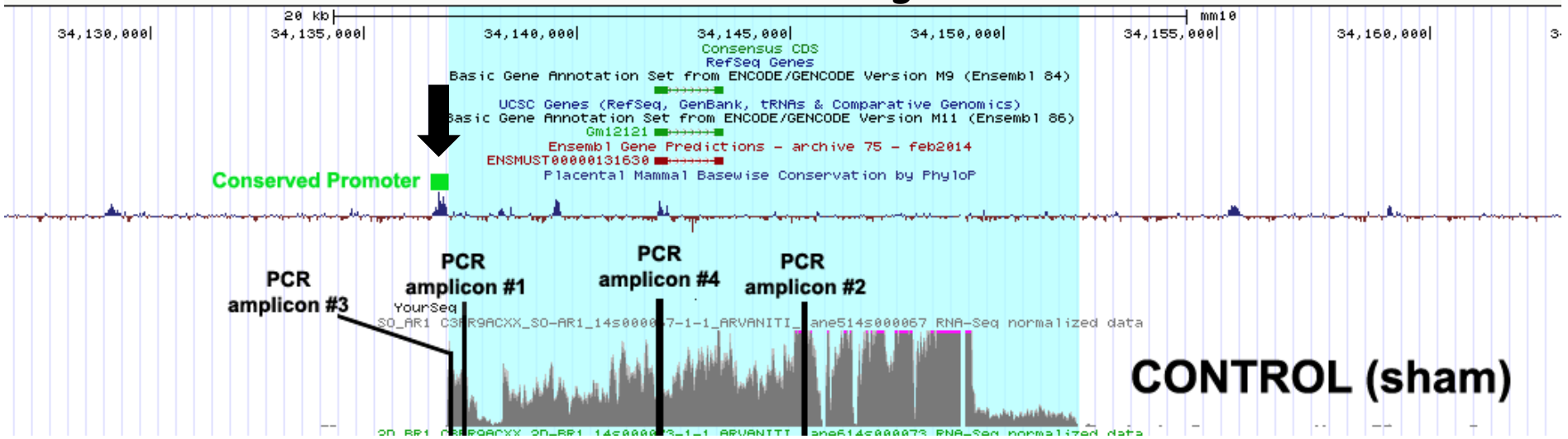


18 human lncRNAs
(based on conservation)

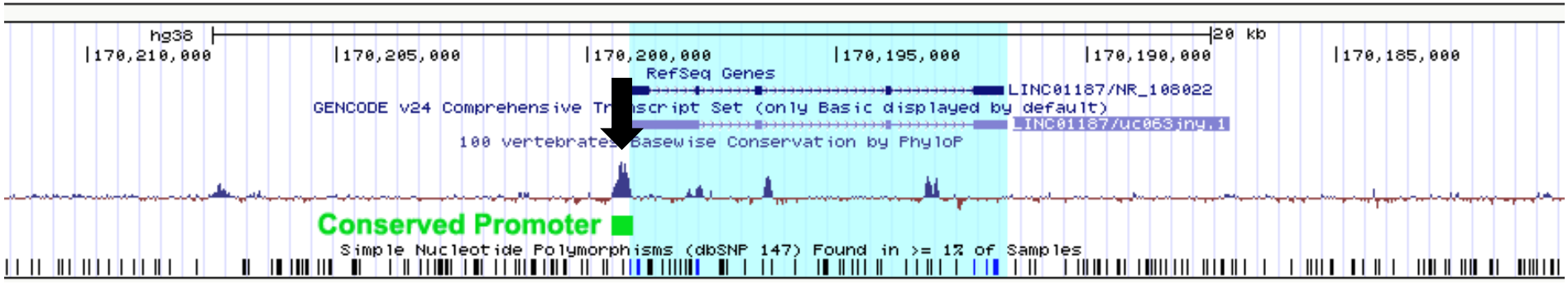
Expression of Gm12121-long in mouse tissues (ENCODE DATA)

Identification of LINC01187 as human homolog of Gm12121-long

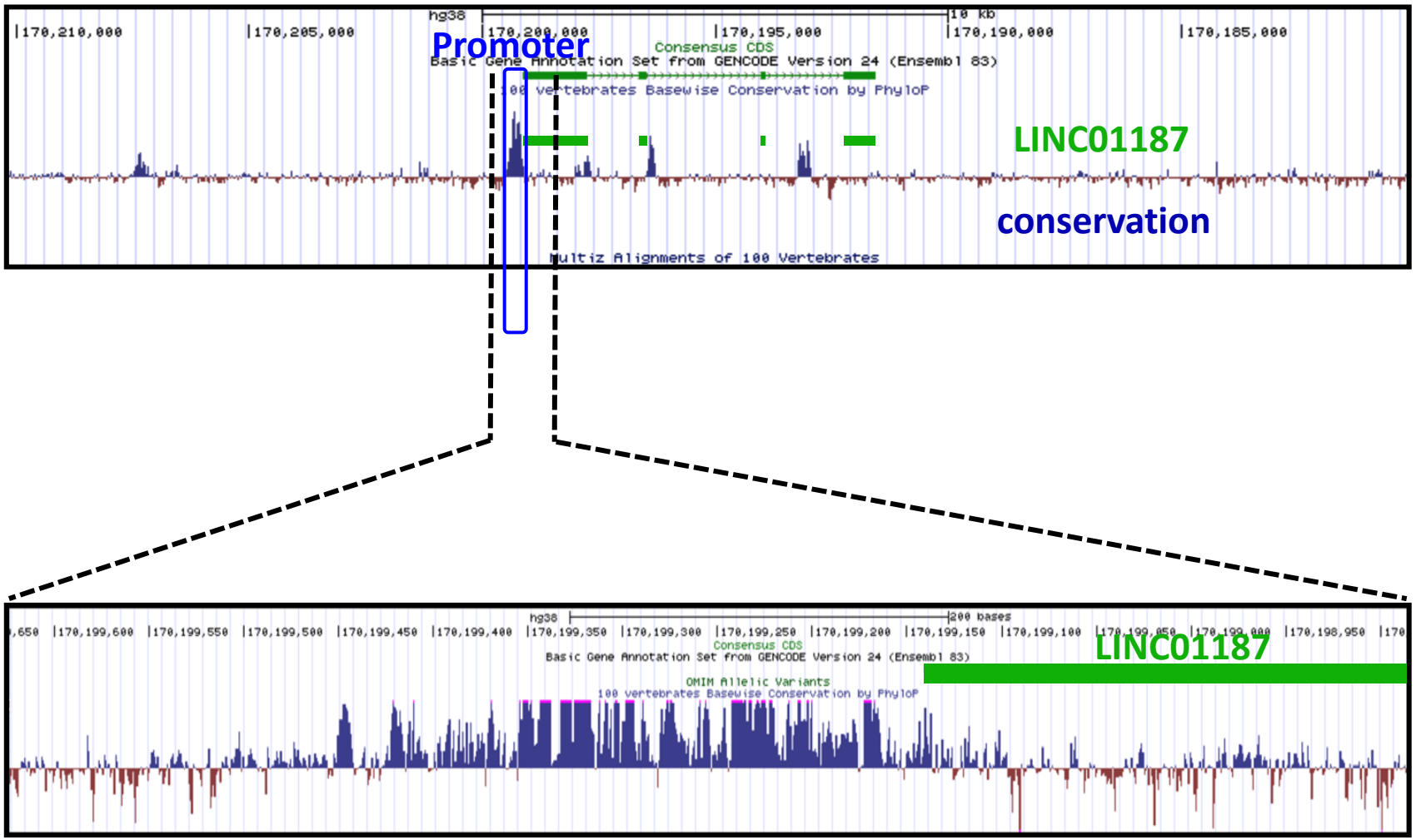
Gm12121-long



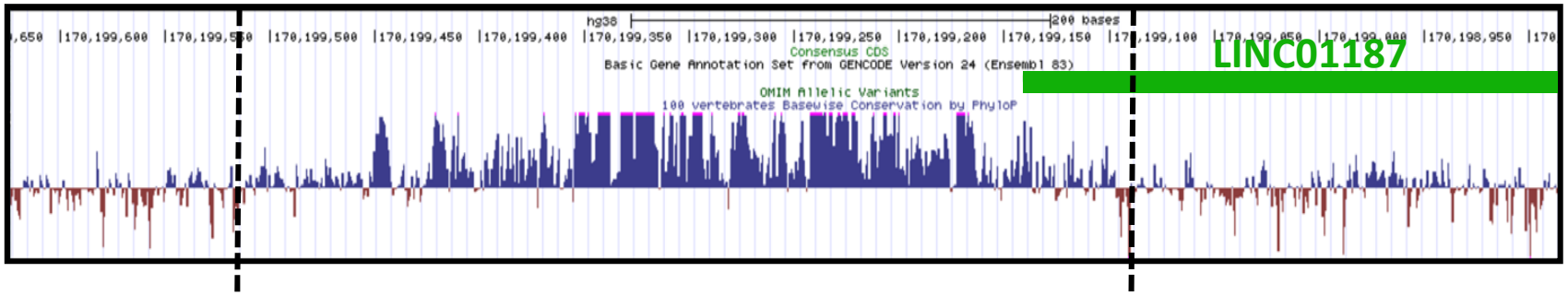
human LINC01187



Gene architecture of long non coding RNA LINC01187



Alignment of human vs mouse LINC01187 promoters




```
hLINC01187          GTCCGGCCCTATGATAAAATGCATTGTTTCTCTCTGAGTGAAGCTTAATGCACCTCAGCC
mGM12121-long       -----CAGAGAGG--------------AAGGCTGGCAGTGACCTTCTGTGACTCTGGGTC
                             * *                 **   *****   *   **   **  * *

hLINC01187          TGGGGATTTATACTAAGCAATAAAAT-------------------------CCACAGGA
mGM12121-long       ATGGGTTTGTTTGCAAGAACACAGACACACAAGTGGCCACTGACAATCTGCGCTCCAACA
                      *** **  *   *** *   * *                            *  **  *

hLINC01187          TAGAGAAATGCAAATAGATTGTAGAATATAATATTATCATTGTCAACATAAAAGACTTTT
mGM12121-long       TCACATATGGGGCATATTTTGCTCAGAAAC---ATGCCATGTTAGTTGTTGAAACCTT-T
                    *     *  *   ***  ***   *  *      *  ***  *     *  **  *** *

hLINC01187          ATTTACTGCCCCCCAAAAGAAAATACACCATAGTGTCGTTTGTTTCAGGGAATGCAAAAA
mGM12121-long       TTT--GCATTCCCTGAAACAAATGGCACCATGGGGTATTTTCTTTTAAGGAGCAGTAAAT
                     **       ***  *** ***   ****** * **  *** *** * ***     ***

hLINC01187          AG--GTTTCAACAACTAACAGGGCATGTTTCTGAGCAAAATATACCCCGTATATAATGTT
mGM12121-long       AAAAGTCTTTTATGTTGACA----------ATGATAATATTATATTCCACAATT------
                    *   ** *       * ***           ***  * * ****  **  *  *

hLINC01187          GGAACACATATTGTCAGTGGCCACTC--GTGTGTCTGTGTTCTTGCAAACAAACCTGTGG
mGM12121-long       --------GATTTGCATTTTCCTATTCTGTGGATCTTTCTGCTTGGTATAAATCCTCAGG
                            ***  ** *  **  *   ***  *** * * ****  *  ** ***  **

hLINC01187          AAT-------------TGAGTCATGGAAGGTCACTT-----------
mGM12121-long       TTGAAATGCACTGGGCCTCACTCAGGGATCAGCAATGGCTTTTGTCTCT
                               * * *** ***    ** *
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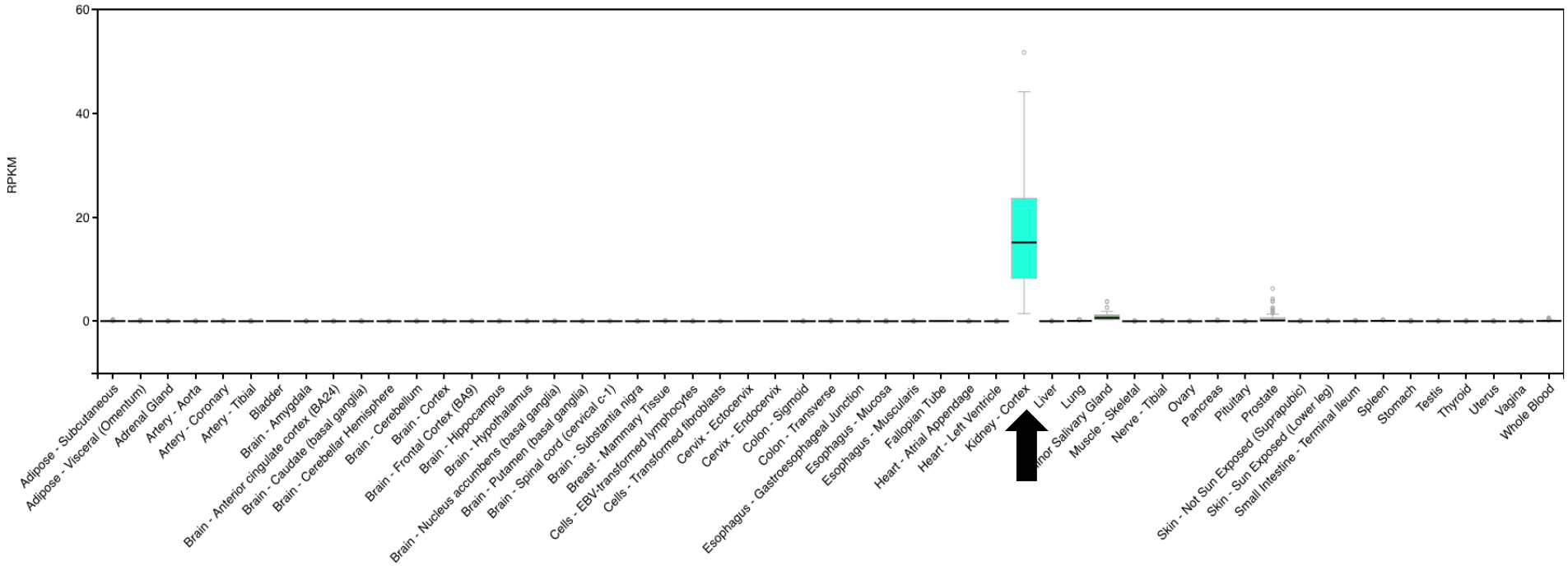
Expression of LINC01187 in human tissues (GTEX analysis)



Acidosis and Deafness in Patients with Recessive Mutations in FOXI1

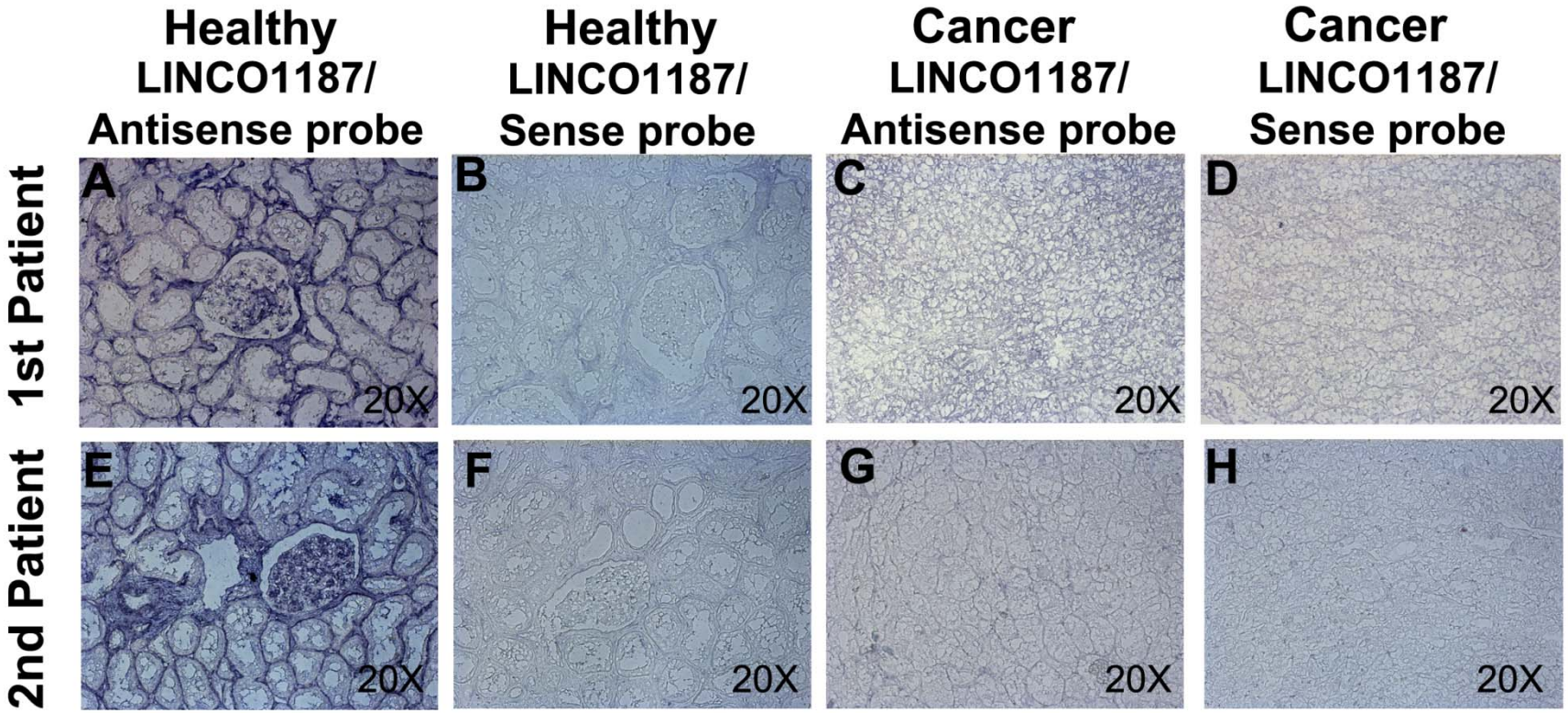
Sven Enerbäck,[1] Daniel Nilsson,[1] Noel Edwards,[2] Mikael Heglind,[1] Sumaya Alkanderi,[2] Emma Ashton,[3] Asma Deeb,[4] Feras E.B. Kokash,[5] Abdulrahim R.A. Bakhsh,[5] William van't Hoff,[6] Stephen B. Walsh,[7] Felice D'Arco,[5] Arezoo Daryadel,[8,9] Soline Bourgeois,[8,9] Carsten A. Wagner,[8,9] Robert Kleta,[6,7] Detlef Bockenhauer,[6,7] and John A. Sayer[2]

[1]Department of Medical Biochemistry and Cell Biology, Institute of Biomedicine, University of Gothenburg, Gothenburg, Sweden; [2]Institute of Genetic Medicine, Newcastle University, Newcastle Upon Tyne, United Kingdom; [3]North East Thames Regional Genetic Service Laboratories, London, United Kingdom; [4]Pediatric Services, Mafraq Hospital, Abu Dhabi, United Arab Emirates; [5]Department of Medicine, Medical School, Gulf University, Ajman, United Arab Emirates; [6]Great Ormond Street Hospital for Children, National Health Service Foundation Trust, London, United Kingdom; [7]University College London Centre for Nephrology, London, United Kingdom; [8]Institute of Physiology, University of Zürich, Zurich, Switzerland; and [9]National Center for Competence in Research, National Center in Competence in Research Kidney.CH, Zurich, Switzerland

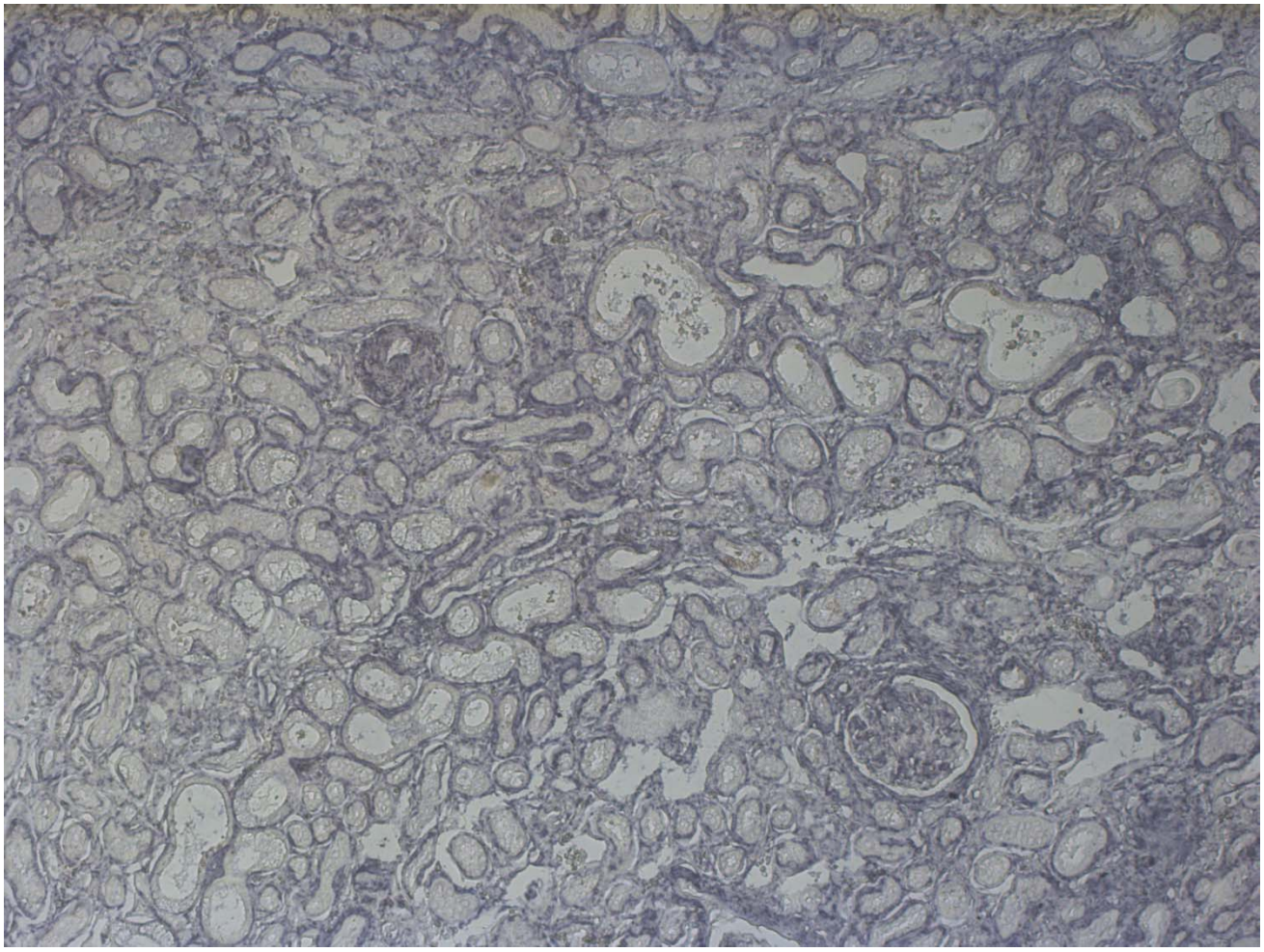
ABSTRACT

Maintenance of the composition of inner ear fluid and regulation of electrolytes and acid-base homeostasis in the collecting duct system of the kidney require an overlapping set of membrane transport proteins regulated by the forkhead transcription factor FOXI1. In two unrelated consanguineous families, we identified three patients with novel homozygous missense mutations in *FOXI1* (p.L146F and p.R213P) predicted to affect the highly conserved DNA binding domain. Patients presented with early-onset sensorineural deafness and distal renal tubular acidosis. In cultured cells, the mutations reduced the DNA binding affinity of FOXI1, which hence, failed to adequately activate genes crucial for normal inner ear function and acid-base regulation in the kidney. A substantial proportion of patients with a clinical diagnosis of inherited distal renal tubular acidosis has no identified causative mutations in currently known disease genes. Our data suggest that recessive mutations in FOXI1 can explain the disease in a subset of these patients.

J Am Soc Nephrol 29: ●●●–●●●, 2018. doi: https://doi.org/10.1681/ASN.2017080840


Healthy
LINCO1187/
Antisense probe
Healthy
LINCO1187/
Sense probe
Cancer
LINCO1187/
Antisense probe
Cancer
LINCO1187/
Sense probe
1st Patient
2nd Patient
A
B
C
D
E
F
G
H
20X
20X
20X
20X
20X
20X
20X
20X

2nd-15360



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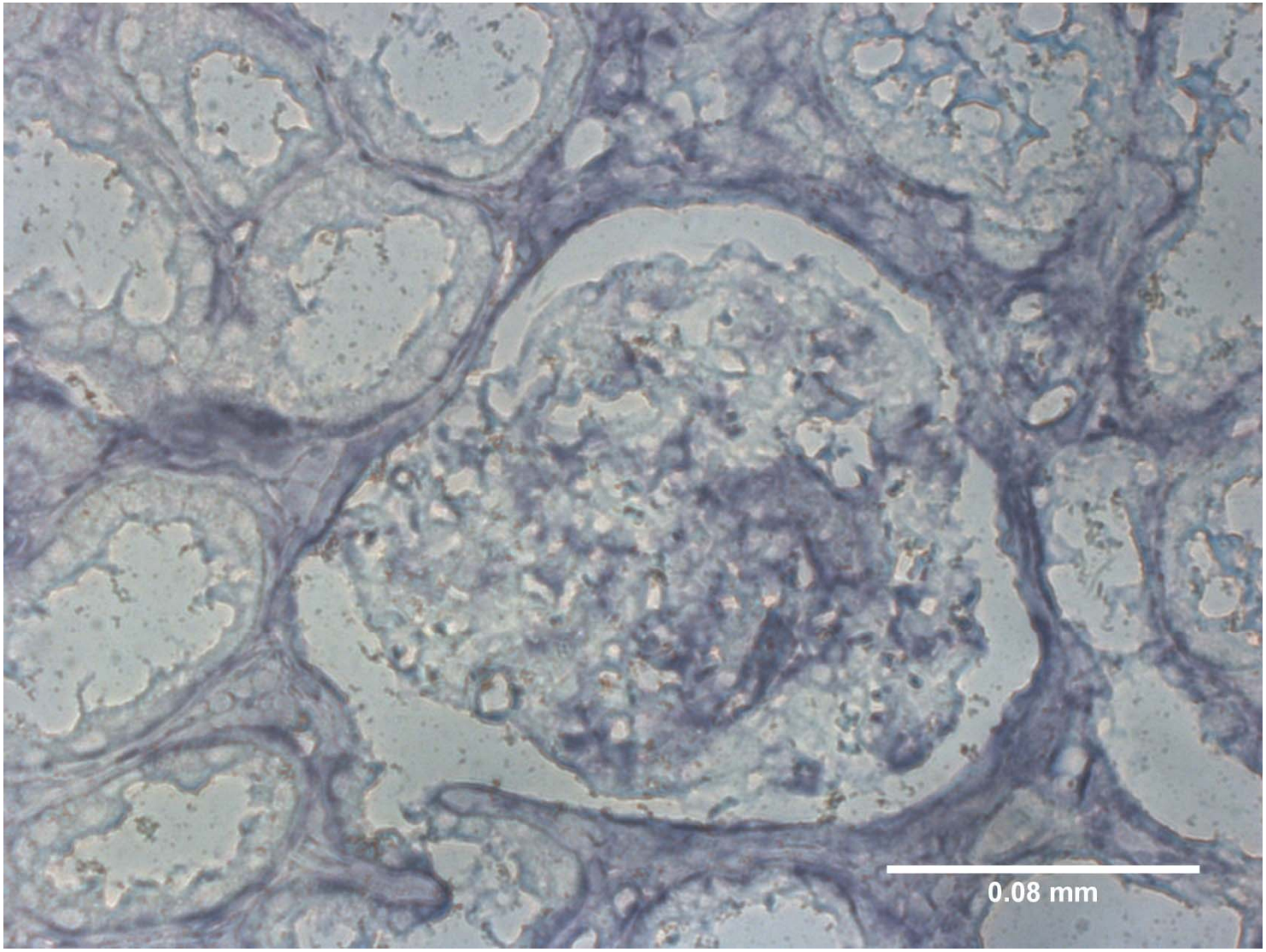

The cell biology of renal filtration

Rizaldy P. Scott and Susan E. Quaggin
Feinberg School of Medicine, Northwestern University, Chicago, IL 60611

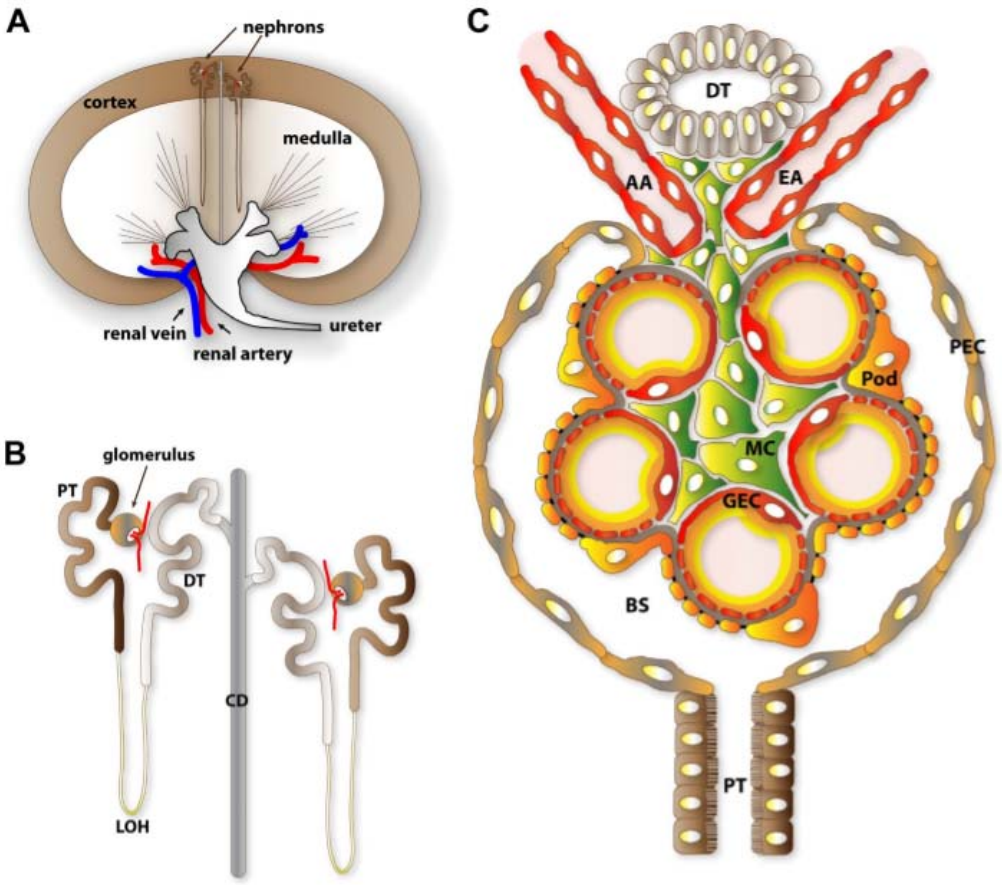

Figure 1. **Anatomical overview of renal filtration.** (A) Diagrammatic representation of nephron distribution in the kidney. Glomeruli, the filtration compartments of nephrons, are found within the kidney cortex. (B) Segmental structure of nephrons. The vascularized glomerulus is found at the proximal end and is connected through a series of renal tubules where urinary filtrate composition is refined through resorption and secretion. (C) Cellular organization of the glomeruli. GEC, glomerular endothelial cell; AA, afferent arteriole; EA, efferent arteriole; Pod, podocyte; MC, mesangial cell; PEC, parietal epithelial cell; PT, proximal tubule; DT, distal tubule; LOH, loop of Henle; CD, collecting duct; BS, Bowman's space.