Utilisation des organoïdes rénaux en néphrologie

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Laboratoire des Maladies Rénales Héréditaires
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Université Paris Cité

Actualités Néphrologiques
Jean Hamburger– Hôpital Necker
IMAGINE 10 Mai 2022

Pourquoi et comment utiliser les organoïdes dans le cadre des maladies rénales héréditaires ?

- Développement d'organoïdes rénaux à partir de cellules hPSCs
  - Définition « actuelle » d'un organoïde: Structure 3D formée de types cellulaires spécifiques d'un organe et obtenue à partir de cellules souches qui s'auto-organisent en mimant les étapes du développement de l'organe
- Utilisation des organoïdes rénaux:
  - Modélisation des pathologies rénales (Impact des mutations dans le développement de la maladie)
  - Criblage de molécules (néphrotoxiques/thérapeutiques)
  - (Re)construire un rein

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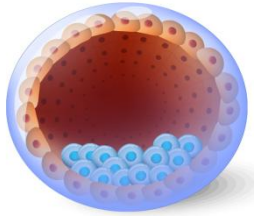
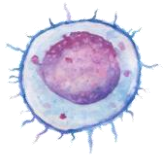
Cellules souches pluripotentes induites (iPSC)

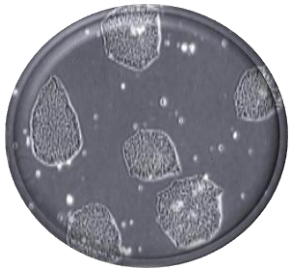
Blastocyste



Cellules souches embryonnaires humaines (hESC)

Cellule somatique:
- Peau (fibroblaste)
- Sang (lymphocytes)
- Urine





Facteurs de pluripotence

Reprogrammation génique

Cellules souches pluripotentes induites (iPSC)

Nobel Price **2012**
Shinya Yamanaka

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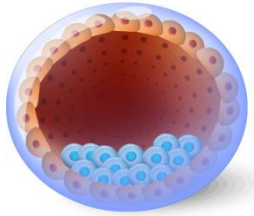

Cellules souches embryonnaires humaines (hESC)

- ✓ Indifférenciée
- ✓ Auto-renouvelement illimité
- ✓ Pluripotence

Cellule somatique:
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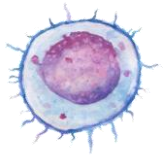

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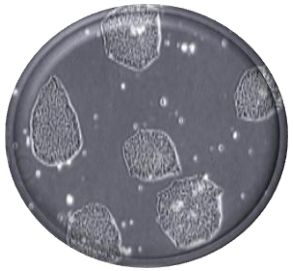
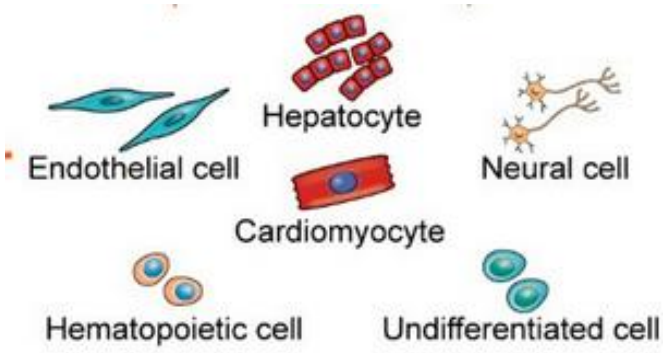
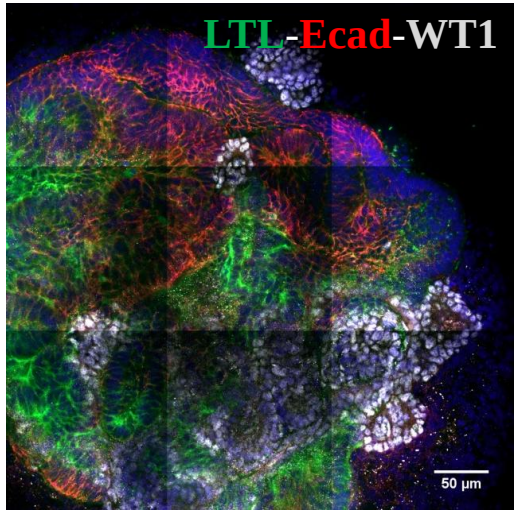


Différenciation

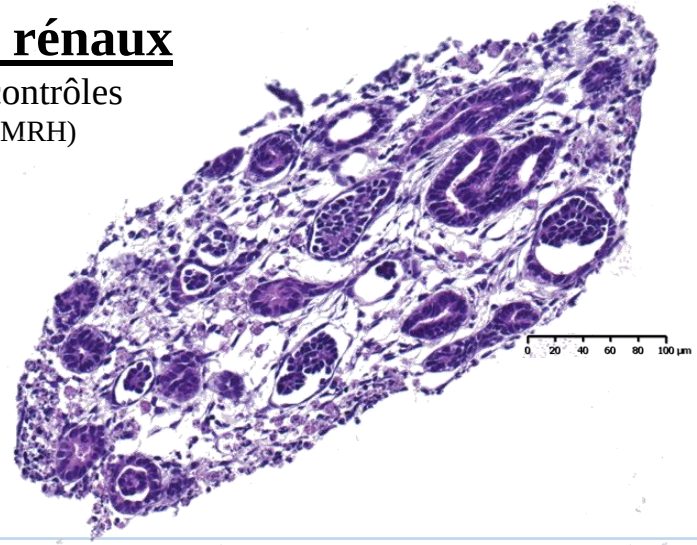
Différents types cellulaires



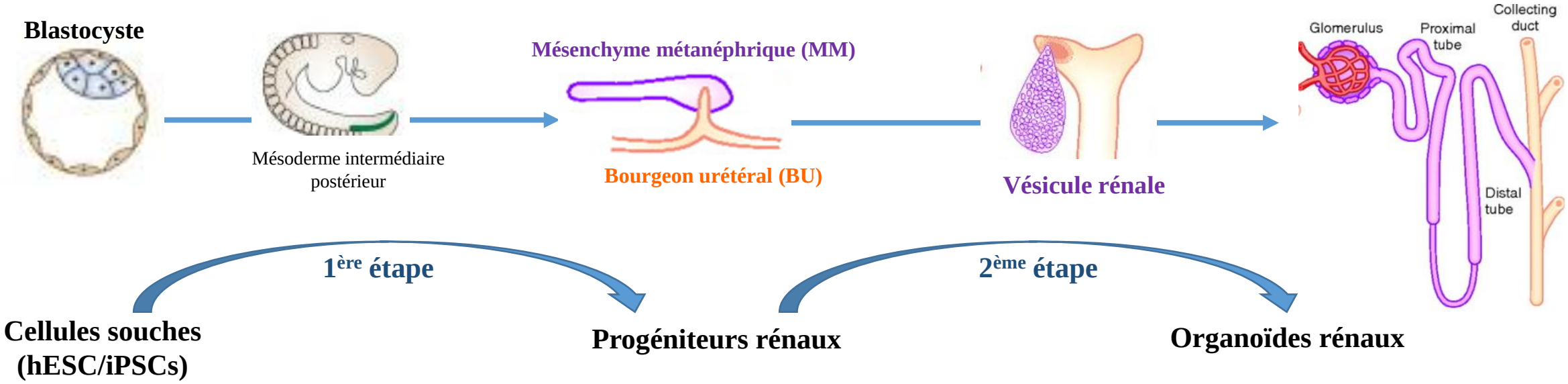


Organoïdes rénaux

issus d'iPSC contrôles
(G.Menara, LMRH)



Reproduire le développement rénal *in vitro*



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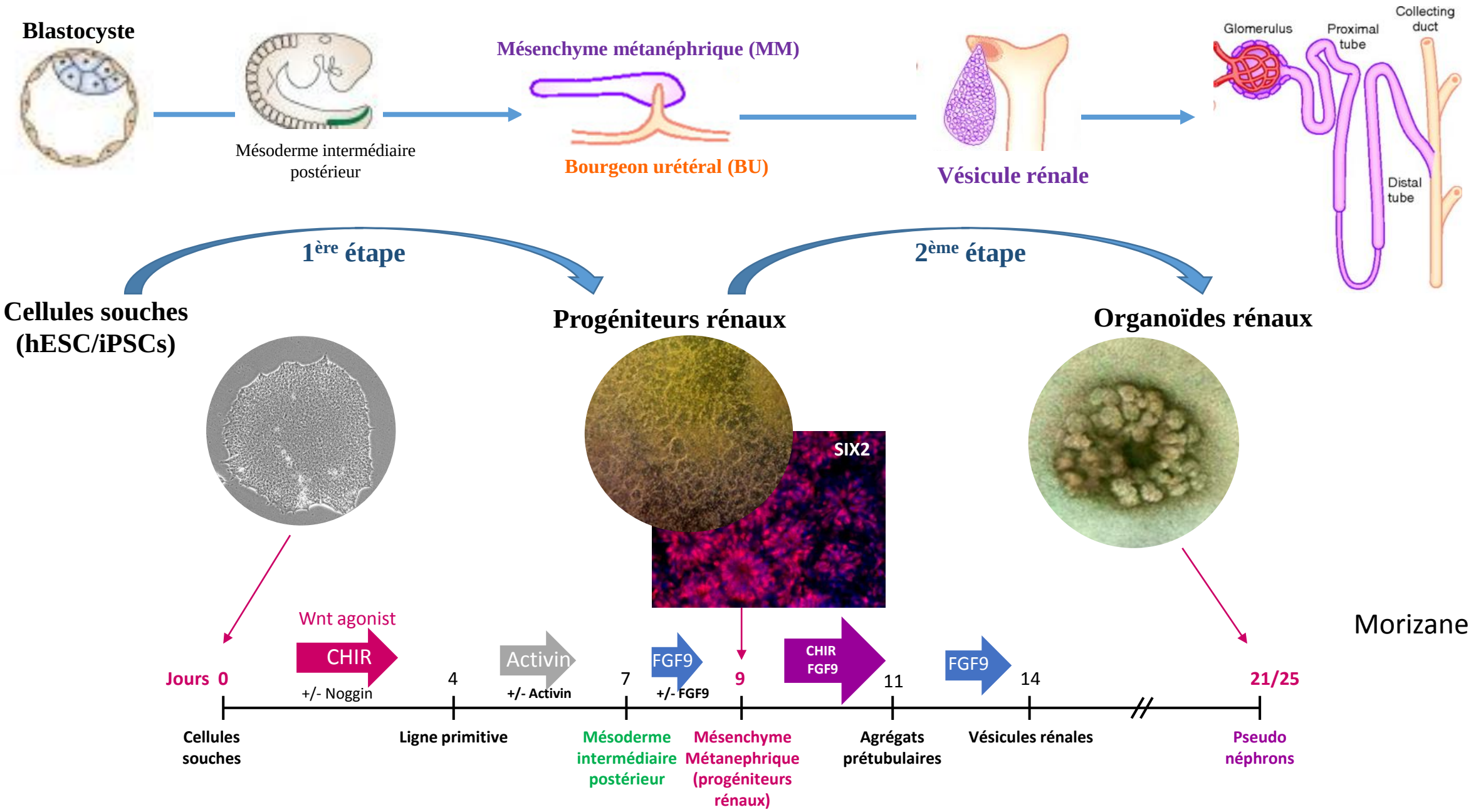

Morizane 2015

Différents protocoles de différenciation

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(Taguchi et al, 2014)

M.Little/M.Takasato
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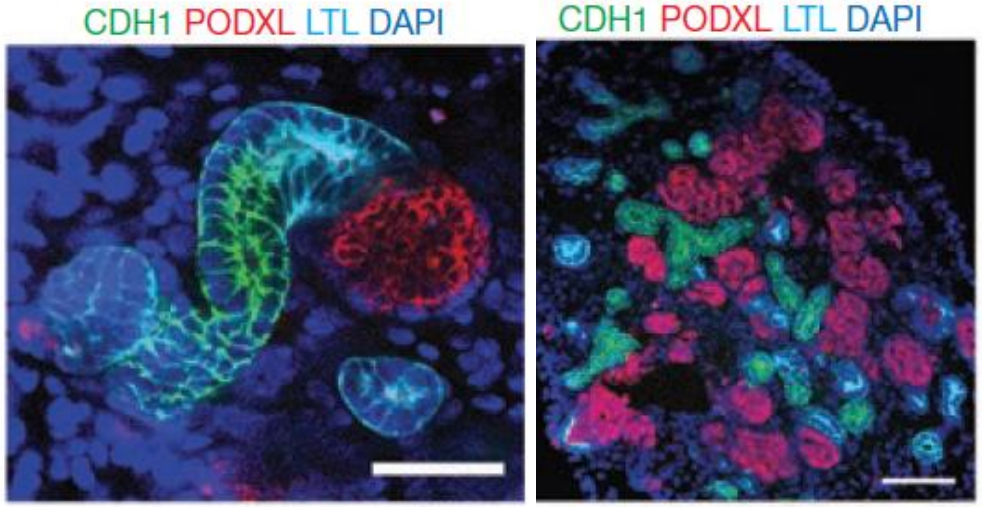
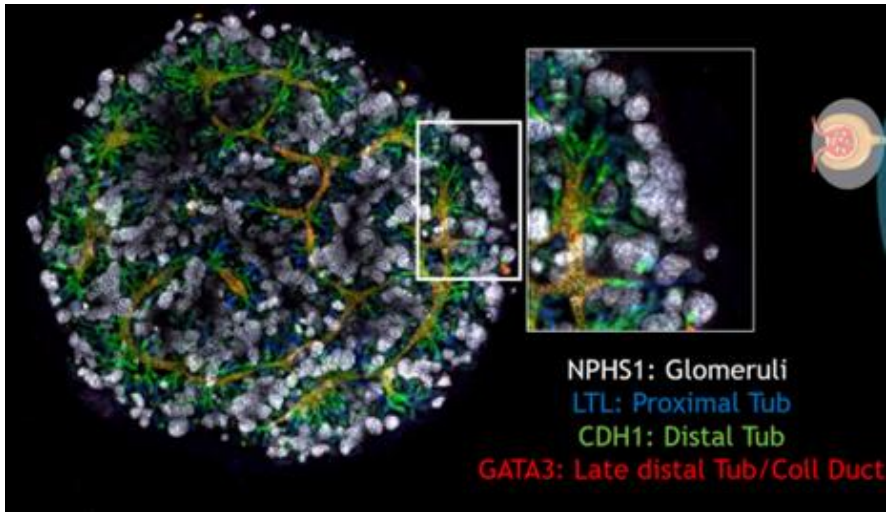
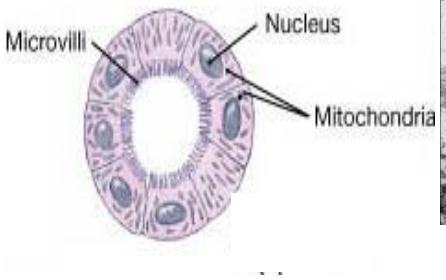
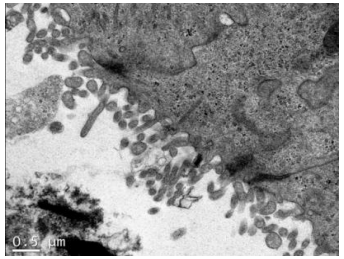
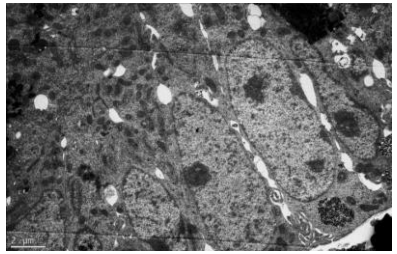
Tubes distaux/proximaux/ podocytes



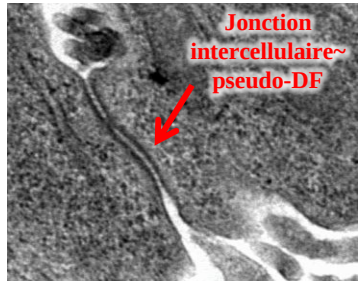


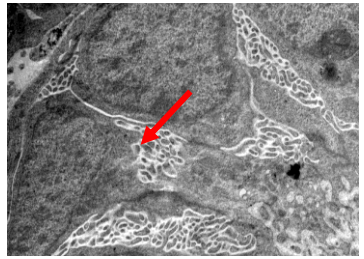




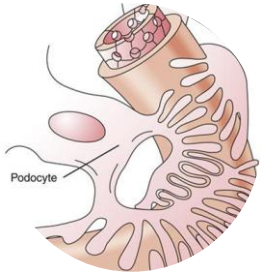


Cellules tubulaires (microvillis)





Podocytes (pieds I^{aires} /2^{aires})



Images de microscopie électronique obtenues au LMRH

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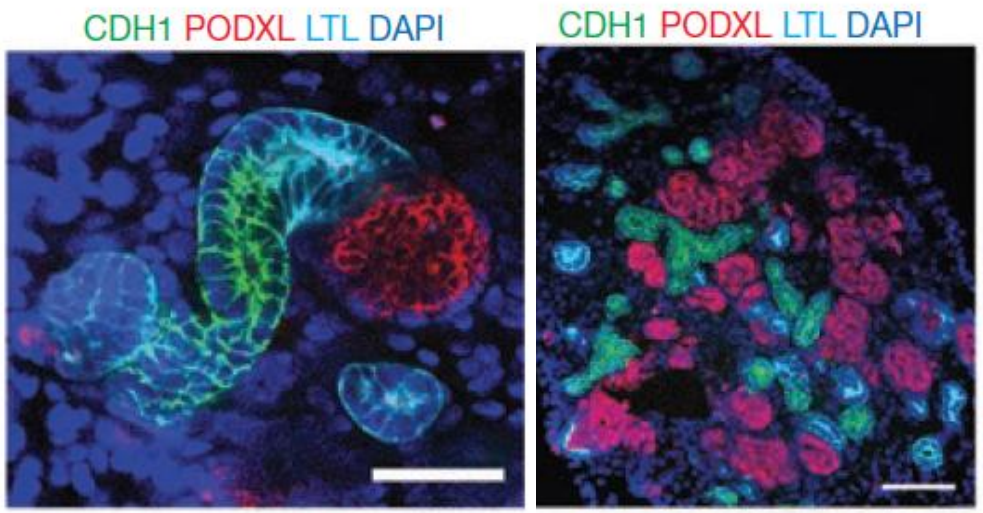
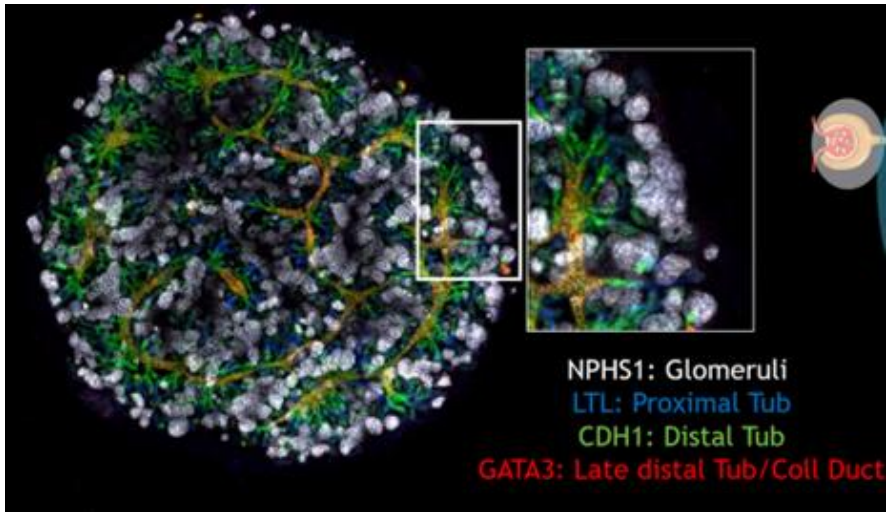
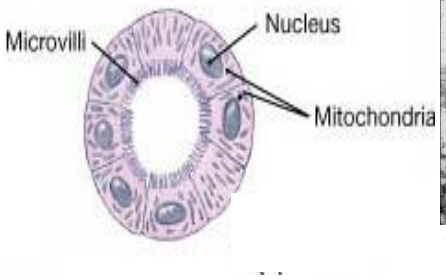
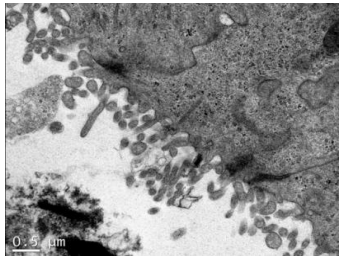
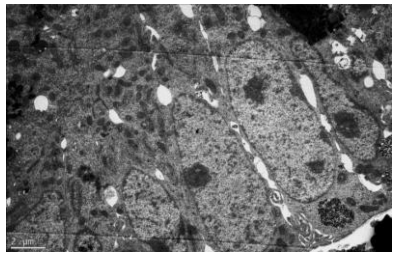




Cellules endothéliales +/-
Tubes collecteurs +/-
Diaphragme de fente absent
=
Organoïdes immatures







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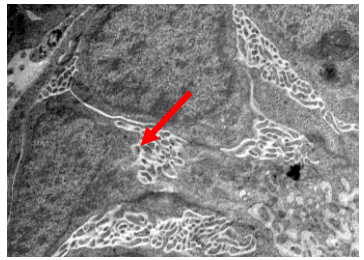

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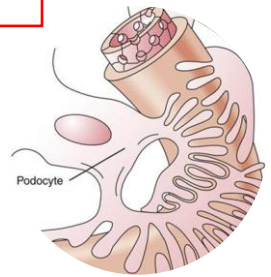

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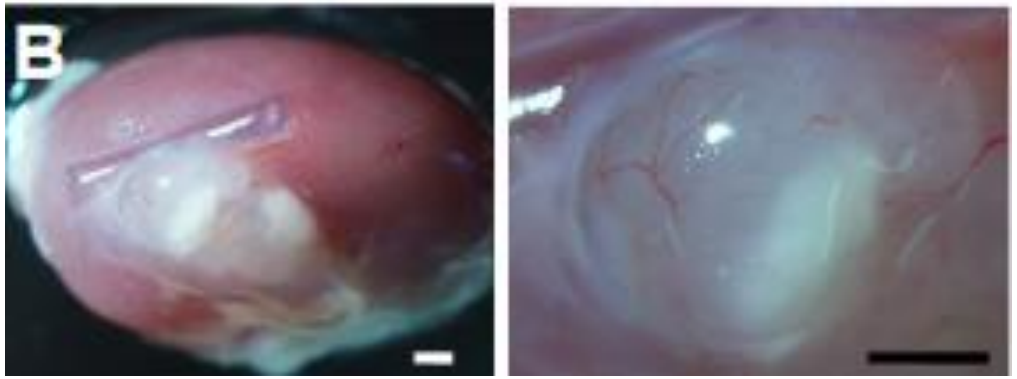
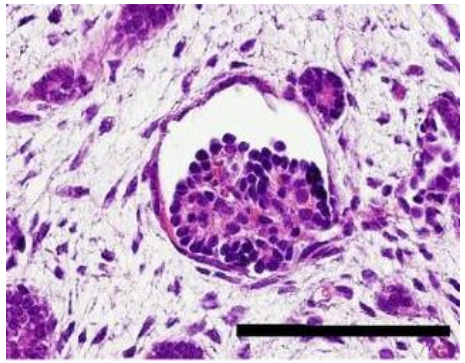
Vascularisation/maturation des organoïdes rénaux

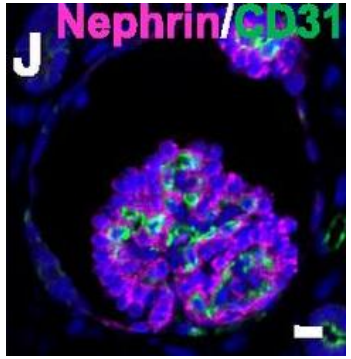
In vivo

- Transplantation dans l'espace sous-capsulaire du rein de souris (progéniteurs rénaux ou organoïdes)
  (Sharmin et al, 2016; van den Berg et al, 2018)
- Transplantation sous-cutanée (progéniteurs rénaux) (Bantounas et al, 2018)

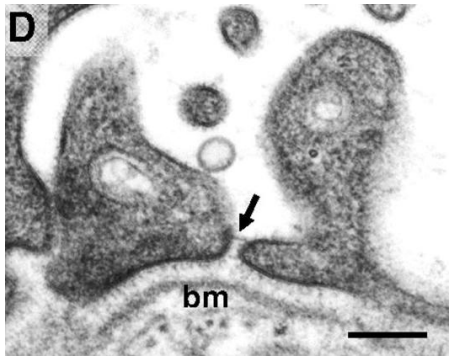
Transplantation sous-capsulaire



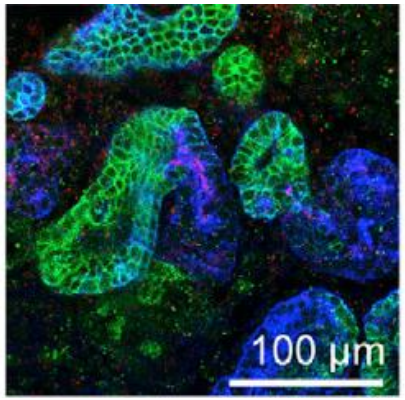




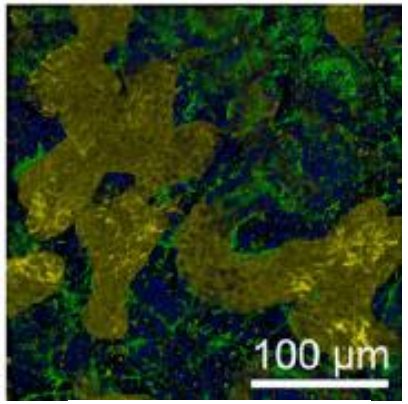
Cellules endothéliales murines dans le glomérule



diaphragme de fente



Tubes proximaux et distaux



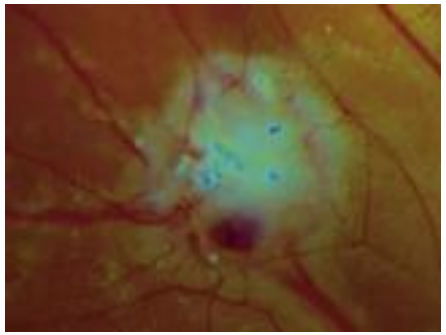
Tubes collecteurs

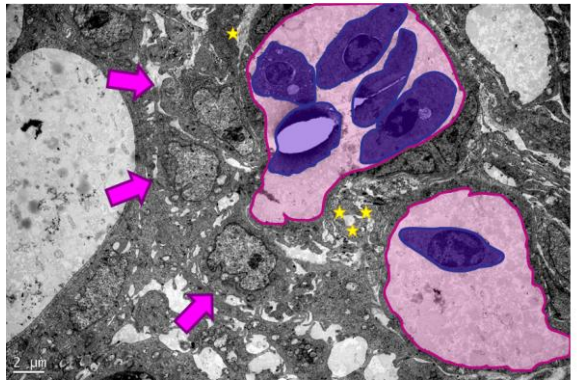
Vascularisation/maturation des organoïdes rénaux

In ovo

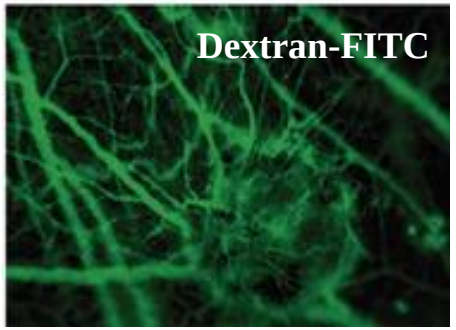
Transplantation d'organoïdes dans la membrane chorioallantoique de l'œuf de poule

(Garreta et al, 2019)



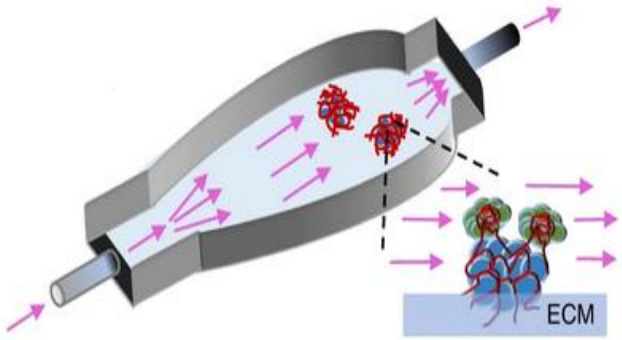


G. Menara, doctorante LMRH (2017-2018)

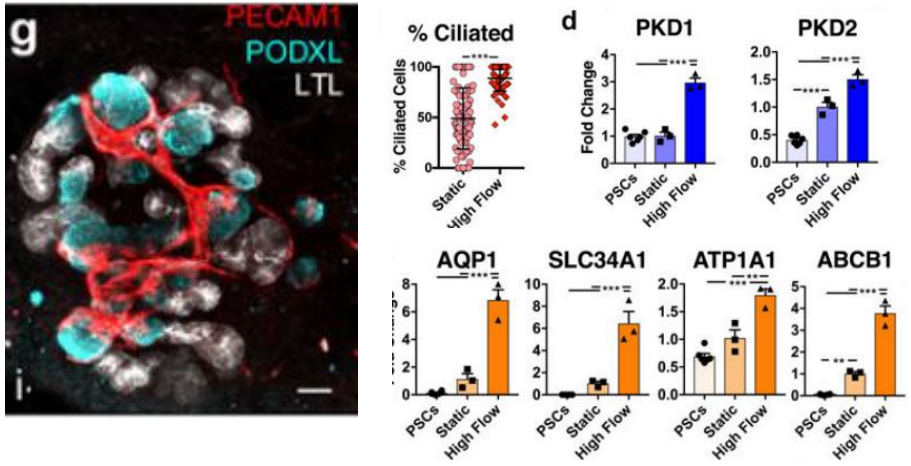


In vitro

- Puce millifluidique (organoïdes)
  (Homan et al, 2019; Bonventre and Morizane Lab)



Flux: favorise le nombre de progéniteurs endothéliaux endogènes

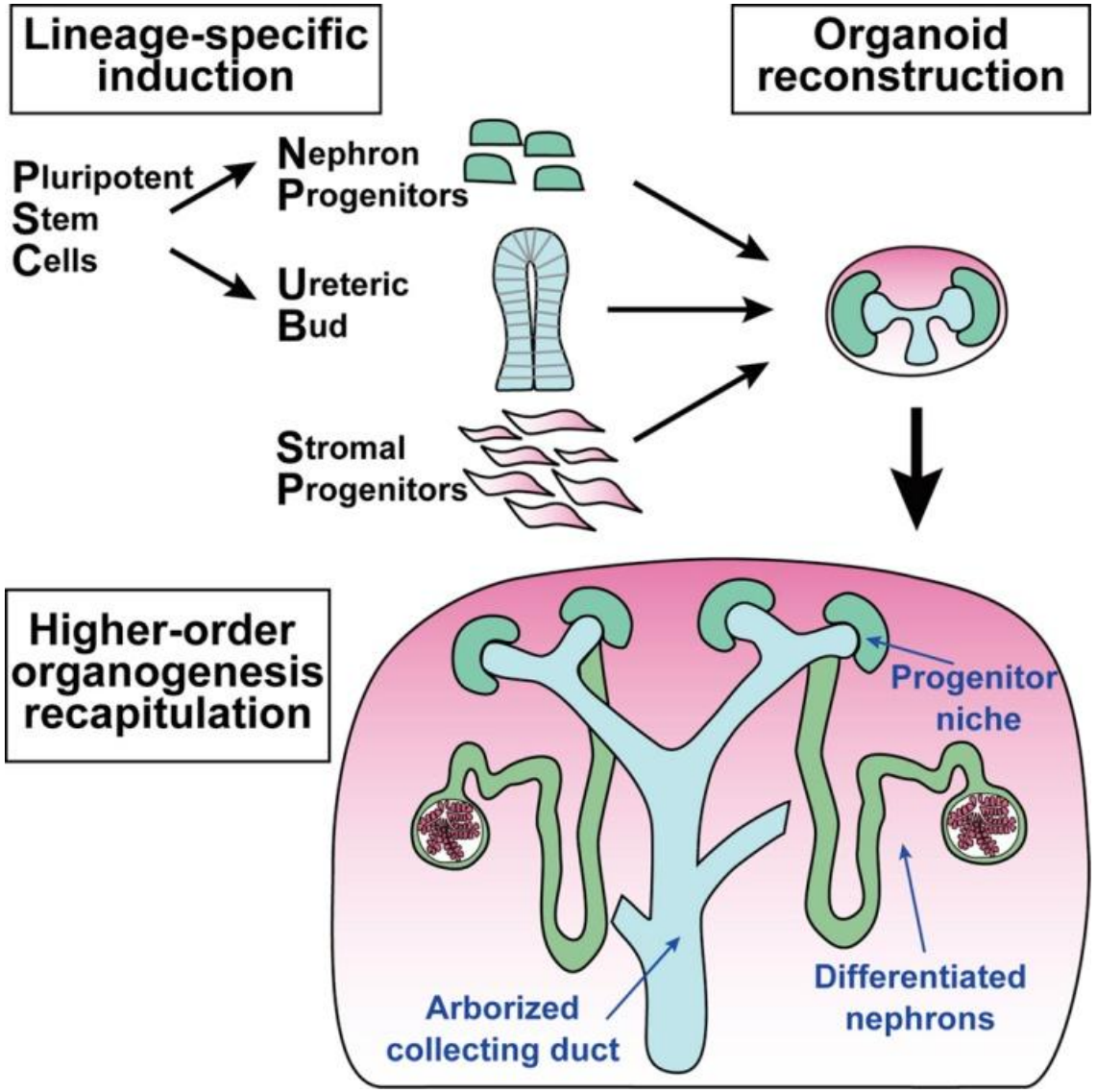


Homan K et al., Nat Method 2019

- Maturation de l'organoïde
- Apparition d'une « vascularisation »
- Meilleure différenciation des cellules tubulaires
- **Problème persistant: jonction avec le système excréteur**

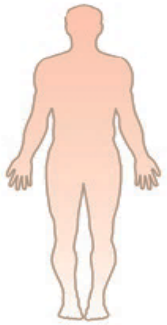
Comment se rapprocher des mécanismes de la néphrogenèse?

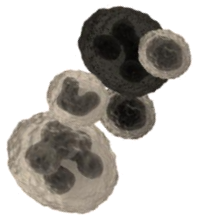
➢ Combinaison des protocoles de différenciation

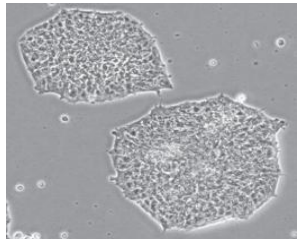


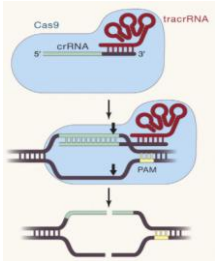
Taguchi & Nishinakamura, Cell Stem Cell 2017

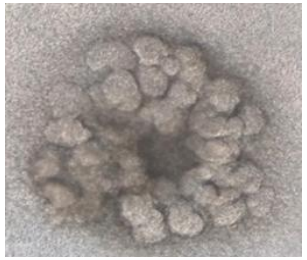
La modélisation des maladies rénales héréditaires

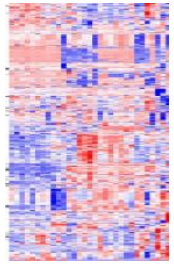








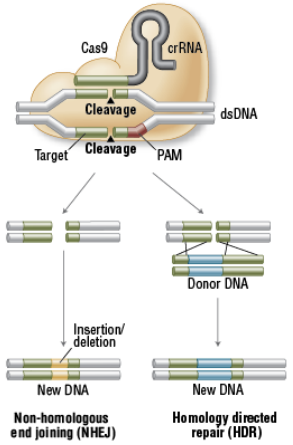




Patient → PBMC → hiPSC → KO/KI CRISPR/Cas9 → Kidney organoid → Phenotypic analysis

« insertion » de mutations ou «correction» des mutations

CRISPR/Cas9 genome editing: controles isogéniques & cellules IPS mutées



KO cell lines

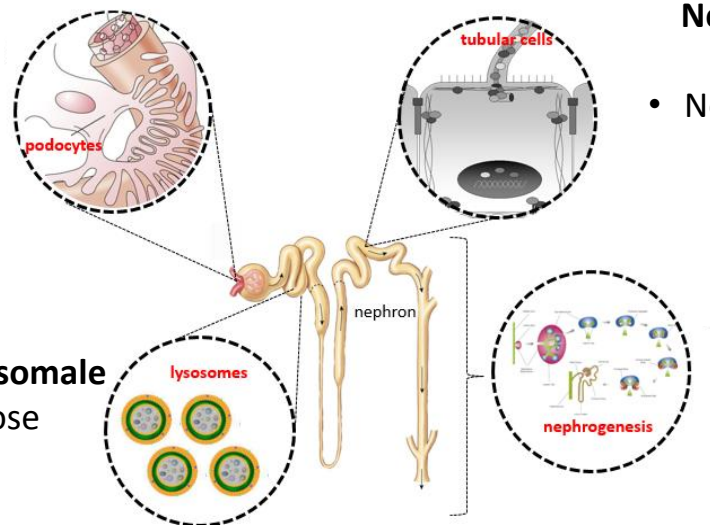
NPHS2
KIF14
NPHP1
NPHP4
CEP290
CTNS
…….

Néphropathies glomérulaires

- Syndrome néphrotique cortico-résistant
- Syndrome de Galloway-Mowat

Maladie Lysosomale

- Cystinose



Néphropathies tubulaires

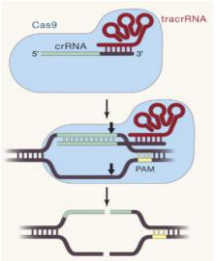
- Néphronophtise/ Ciliopathies

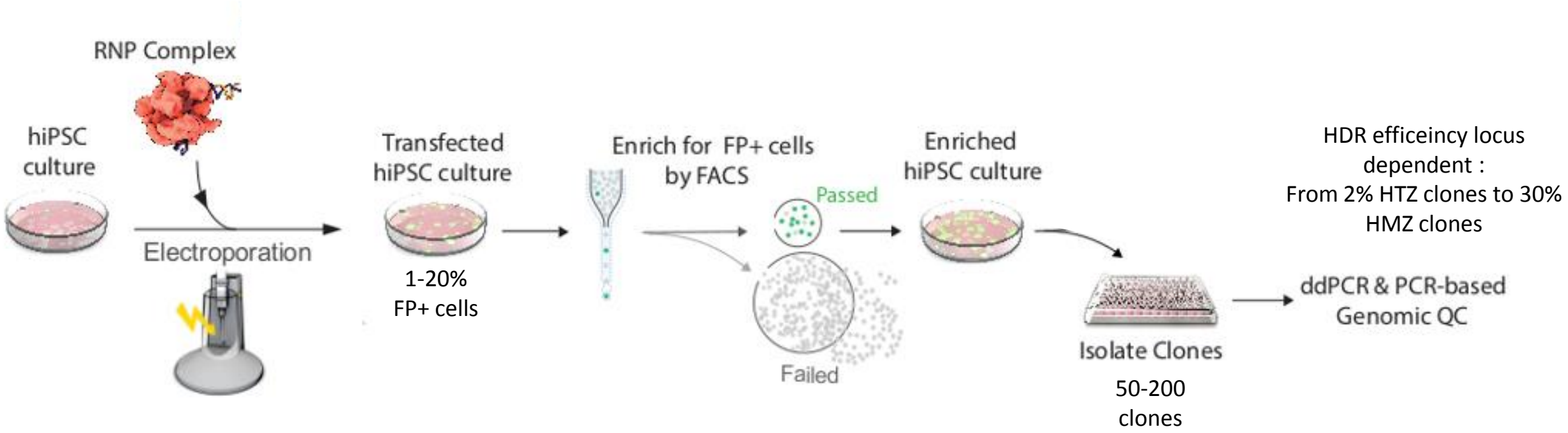
CAKUT

LMRH_IMAGINE

Bruno Estebe

La technique de CRISPR par nucleofection (KO et KI)





HDR efficeincy locus dependent :
From 2% HTZ clones to 30% HMZ clones

Reducing off target =

RiboNucleoProtein complex
(Cas9 protein + RNA cr and trac RNA)
+
High Fidelity Cas9 (R691A) or Nickase Cas9 (D10A)

On and off target screening :
PCR + sanger sequencing

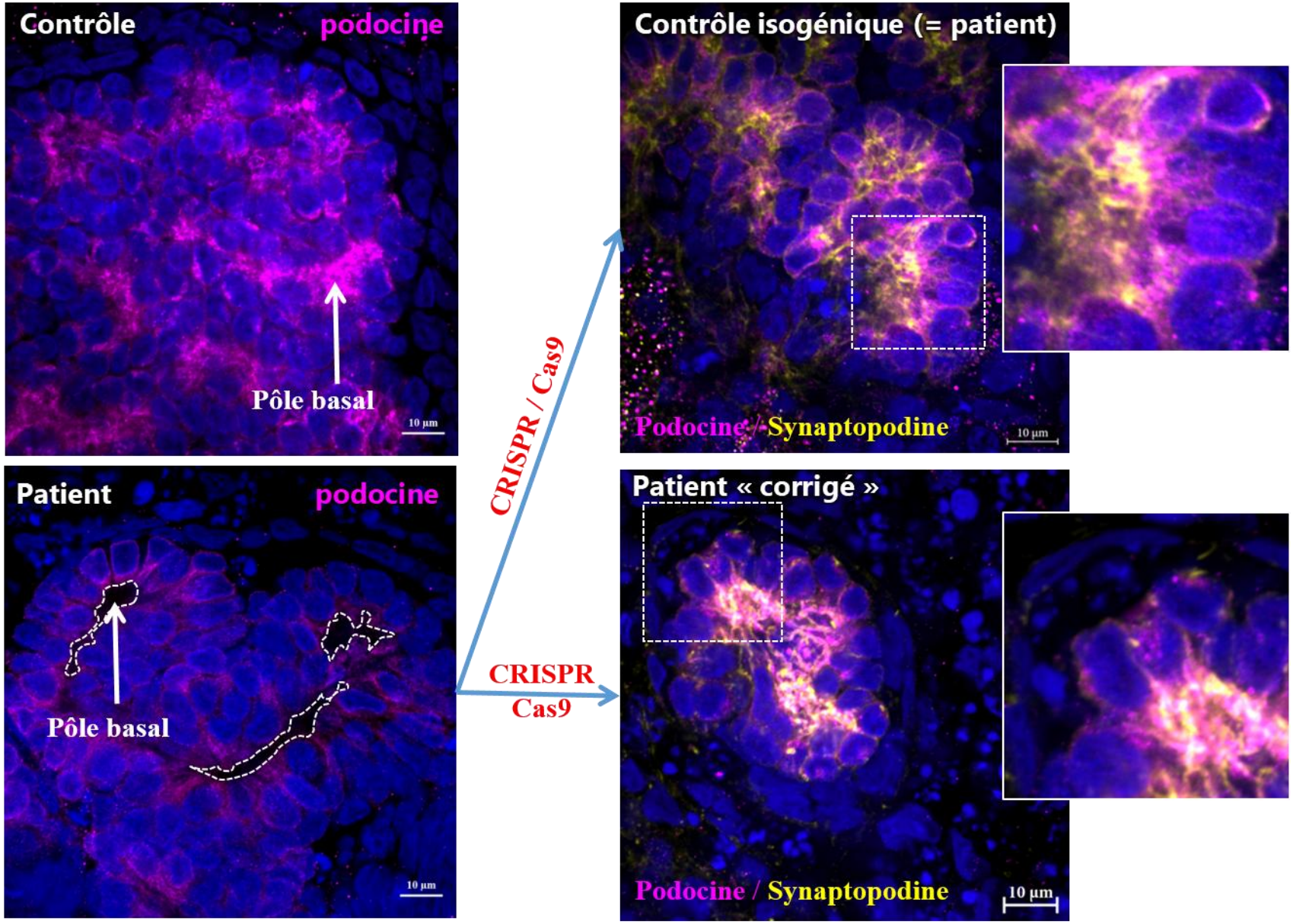
Modéliser les maladies rénales héréditaires

SN cortico-résistant lié à des mutations du gène *NPHS2*

Géraldine Mollet
Bruno Estebe

1 Patient avec SNCR

- Mutation *NPHS2* (podocine): **p.R138Q** (mutation la plus fréquente)
- Rétention dans le réticulum endoplasmique (+ dégradation protéasomale massive)
- Absence de localisation à la membrane plasmique (pôle basal)

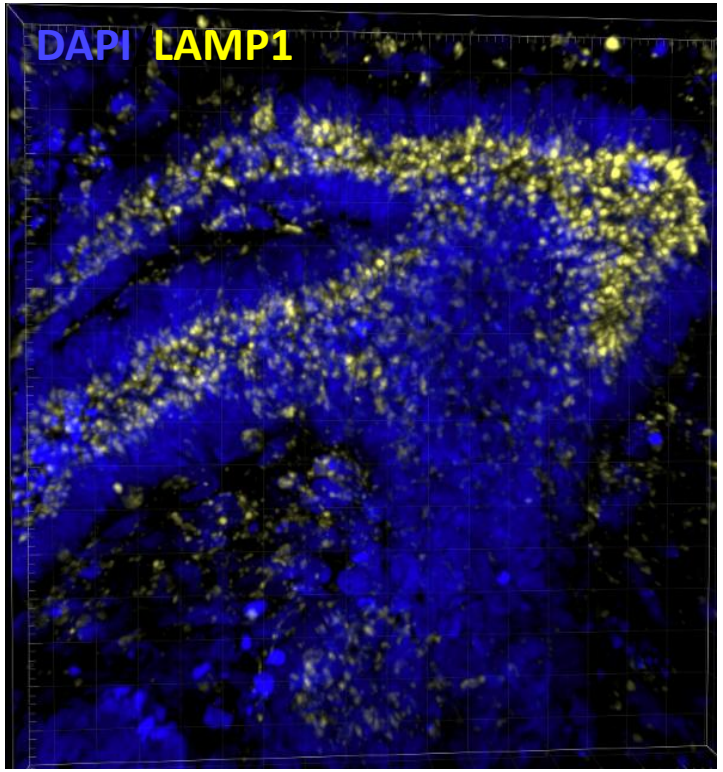


G. Menara, doctorante au laboraoire LMRH-2016-2020

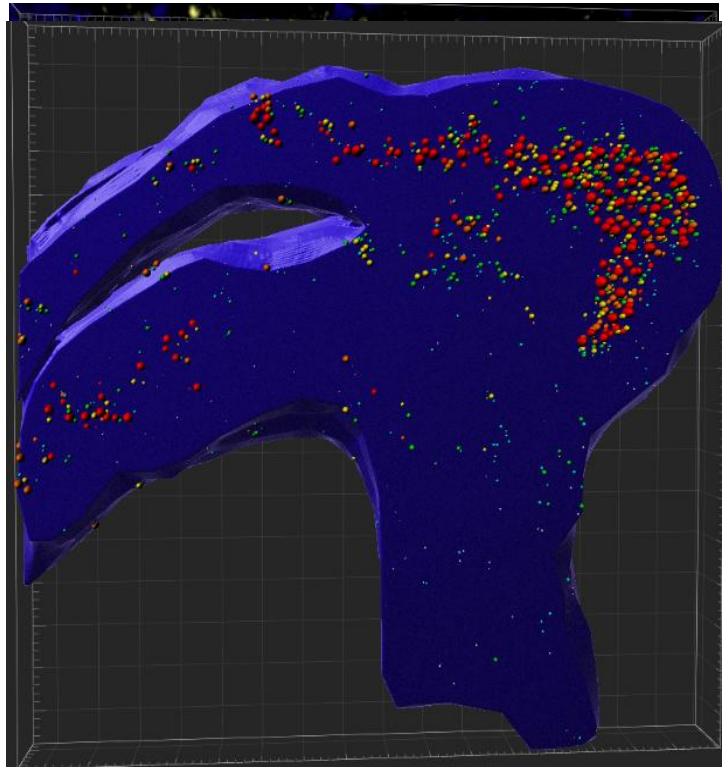
Bruno Estebe

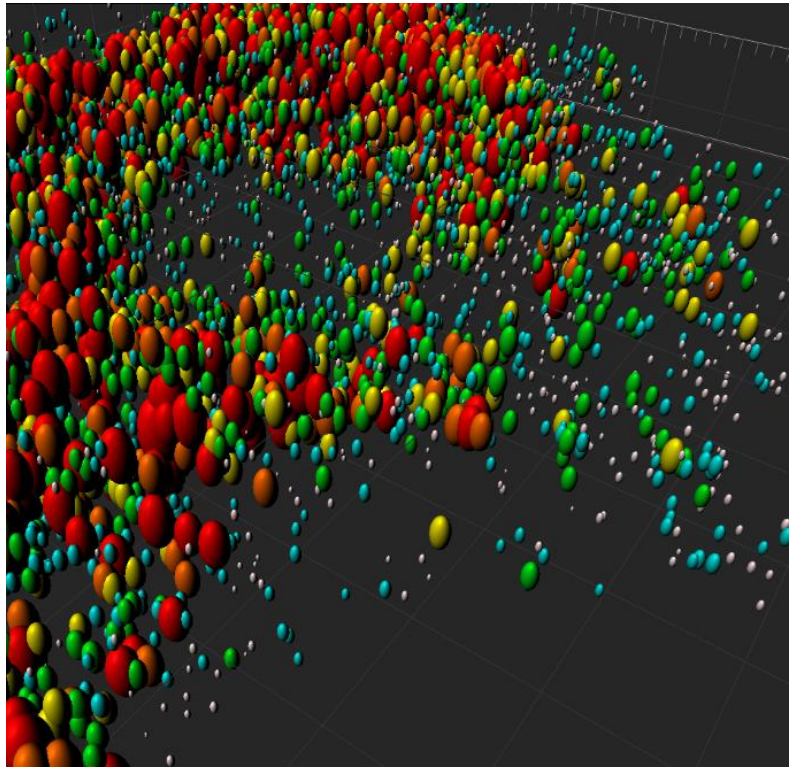
Cystinose: quantification de la taille des lysosomes par imagerie

Z stack 3D



Modélisation des lysosomes (LAMP1) dans les tubules proximaux (Imaris)





Classification Spot on area parameter:

Red = area>20 µm2

Orange = 20 µm2>area>15 µm2

Yellow = 15 µm2>area>10 µm2

Green = 10 µm2>area>5 µm2

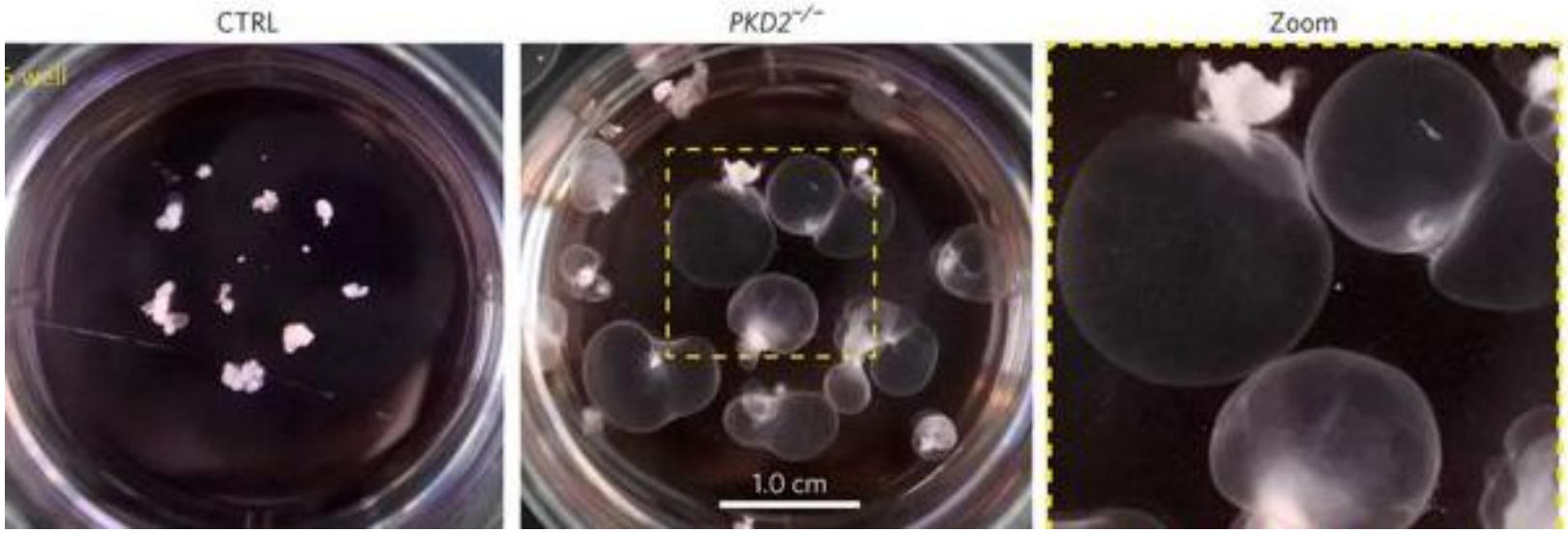
Bleu = 5 µm2>area>1,4 µm2

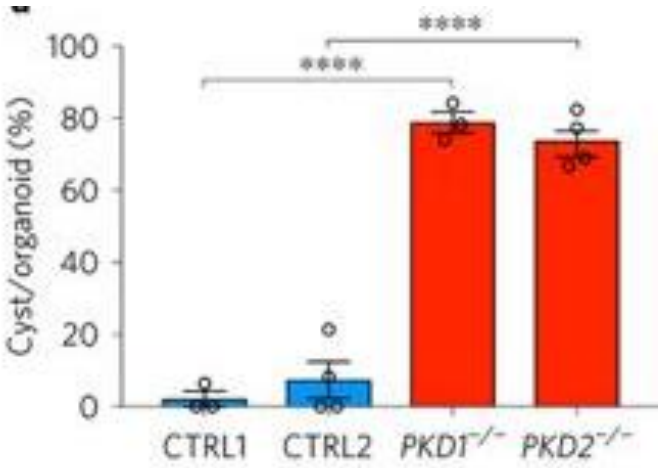
Grey = 1,4 µm2>area (=Background noise)

Modéliser les maladies rénales

Polykystose rénale autosomique dominante

- Obtention d'iPSC PKD1$^{-/-}$ et PDK2$^{-/-}$ par CRISPR/Cas9
- Formation de kystes quand les iPSCs PKD1$^{-/-}$ et PDK2$^{-/-}$ sont cultivées en suspension
- Caractéristiques retrouvées dans les kystes isolés de biopsies de patients avec ADPDK (marqueurs proximaux/distaux et prolifération)



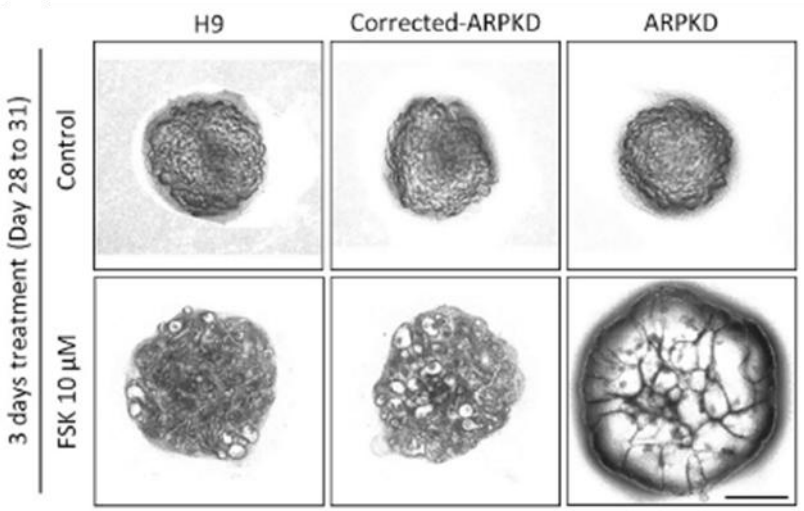


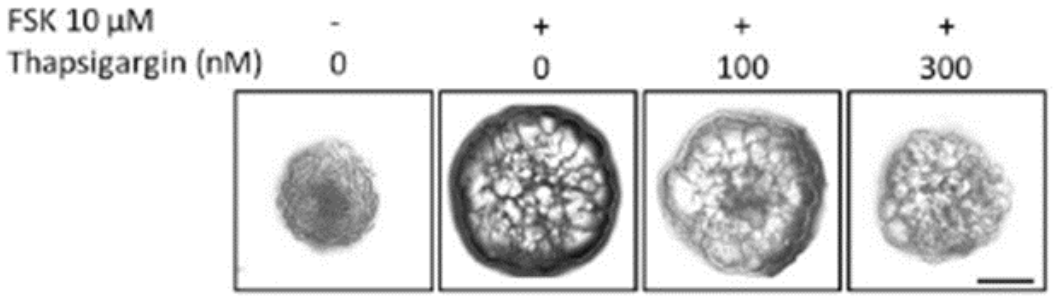
Cruz N. et al., Nature Materials, 2017 (Freedman's lab)

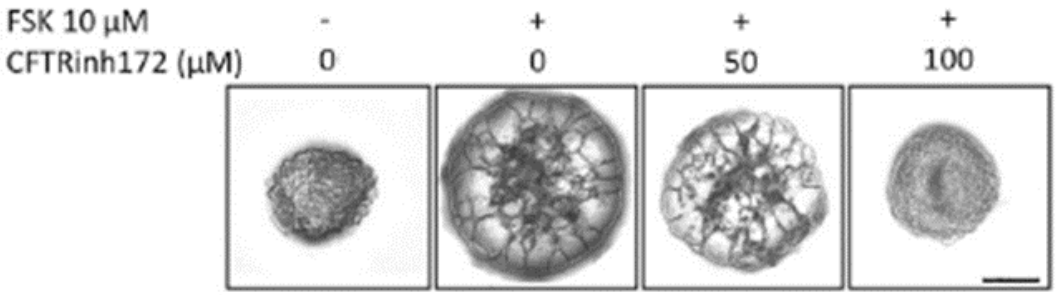
Modéliser les maladies rénales

Polykystose rénale autosomique récessive

- Obtention d'iPSC dérivé des fibroblastes d'un patient avec ARPKD (mutations hétérozygote composite dans le gène *PKHD1*)
- Correction d'une des mutations par CRISPR/Cas9
- Après traitement par la forskoline, les organoïdes ARPKD développent des kystes alors que les organoïdes « corrigés » n'en développent pas
- Tester des composés chimiques capables d'inhiber la kystogenèse dans les organoïdes ARPKD





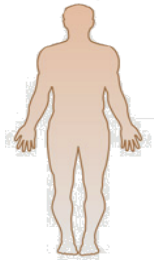


> Possibilité de criblages à haut débit de chimiothèques

Low J et al., Cell Stem Cell 2019

Modéliser les maladies rénales héréditaires

Ciliopathie due à des mutations du gène *IFT140*

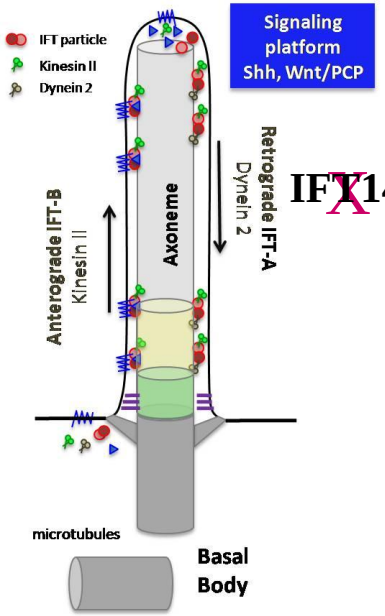


Patient

iPSC « muté »

CRISPR Cas9

iPSC « corrigé »

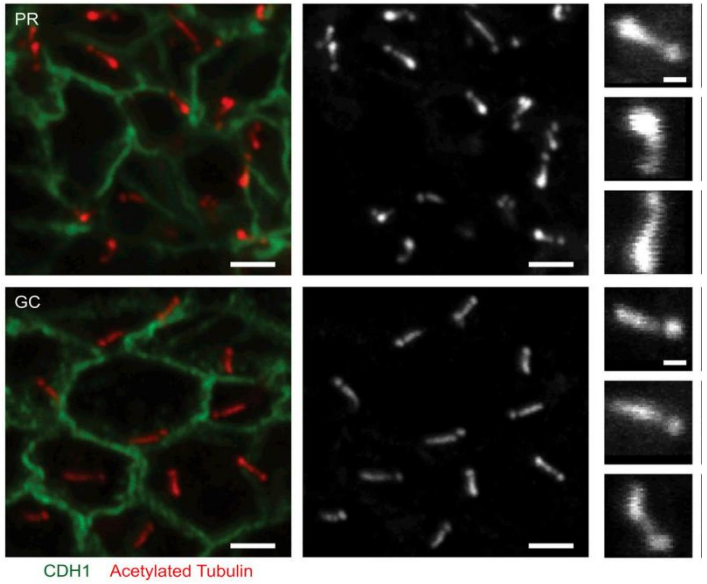


IFT140

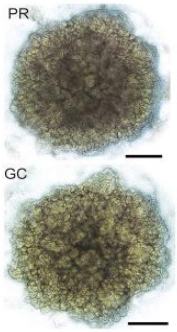
- ✓ Néphronophtise
- ✓ Epiphyses en cônes
- ✓ Rétinite pigmentaire

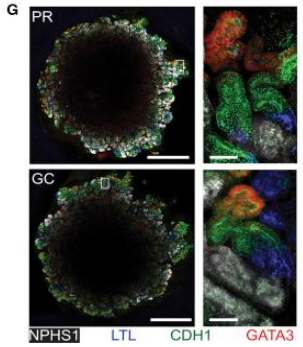
✓ <u>Défauts de ciliogenèse</u>

(cils primaires raccourcis et en forme de club

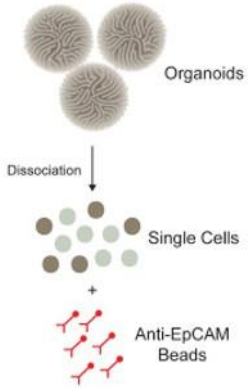


✓ Différentiation en organoïdes rénaux





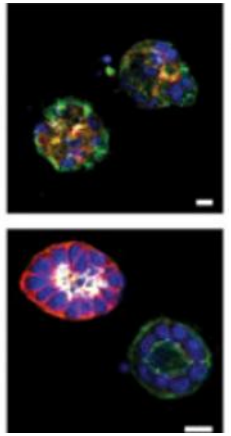
✓ <u>Défauts de polarité apico-basale & jonctions cellulaires</u>



Analyse RNA-seq

muté

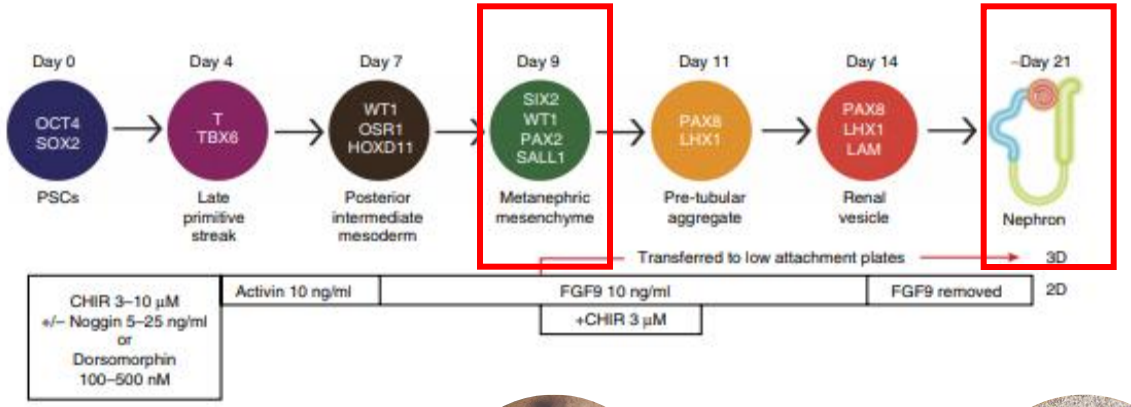
corrigé

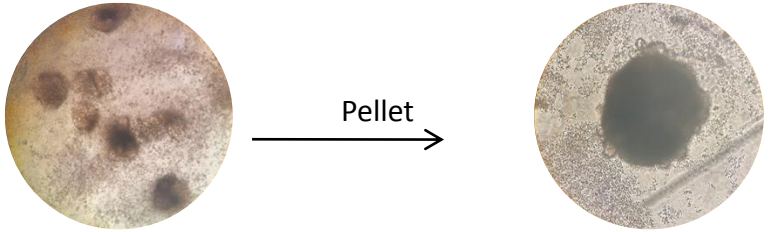


Forbes T et al., Am J Hum Genet, 2018 (M.Little's lab)

Nephronopthise: mutant iPSC-derived kidney organoids

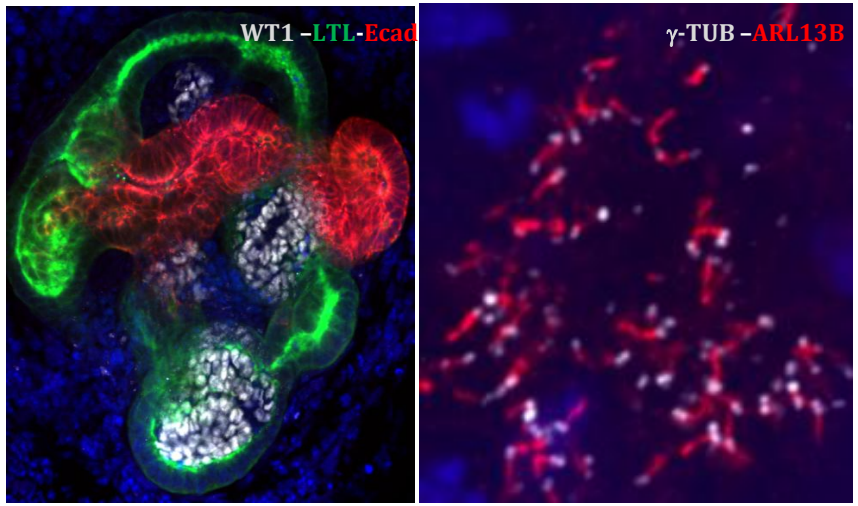
Damelys Calderon
Julie Pernel





Pre-tubular aggregate

> Renal organoids

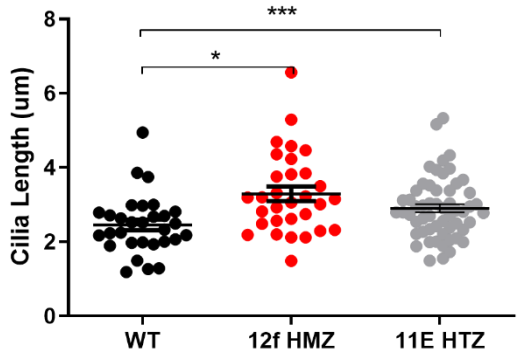


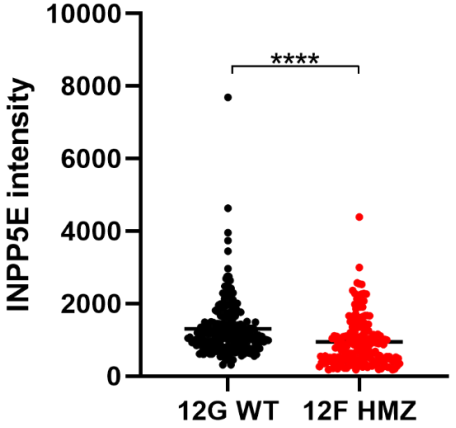
PBMC from patients for reprogramming iPSc
NPHP1/NPHP4/CEP290/candidat genes…

Gene invalidation by CRISPR-Cas in iPCS
➢ NPHP1

- Ciliogenesis and ciliary composition

ARL13b – yTub staining





12G= 213
12F= 184

Nephronopthise: mutant iPSC-derived kidney organoids

imagine
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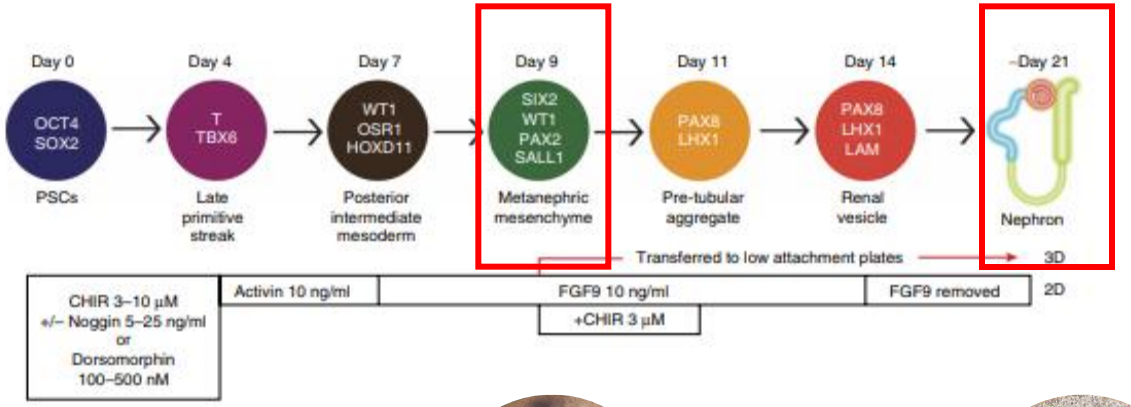
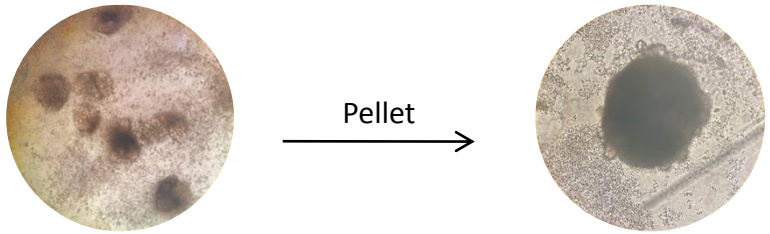
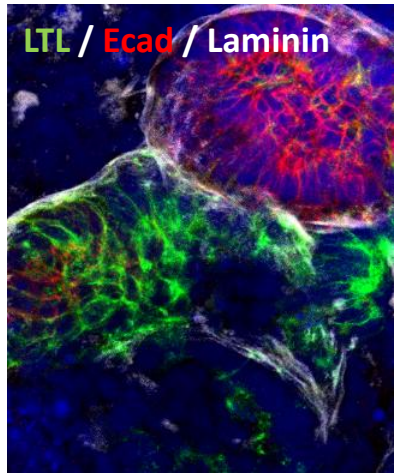
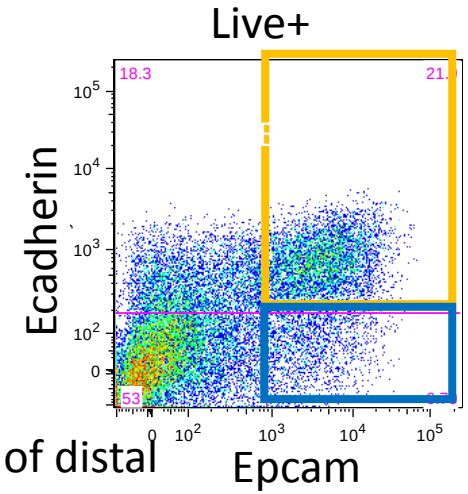




Pre-tubular aggregate

- RNAseq analysis on EPCAM+ sorted cells

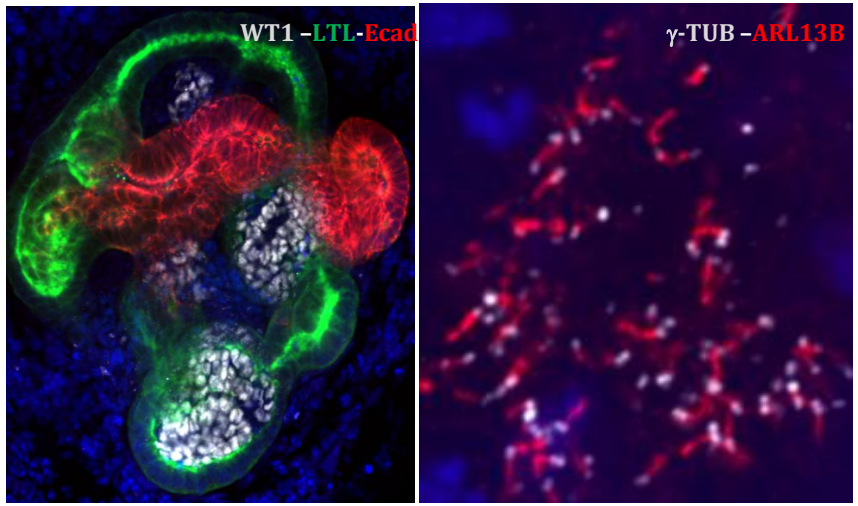




Cell sorting of distal tubular cells

Bulk RNAseq analysis
Single cell RNAseq

> Renal organoids



PBMC from patients for reprogramming iPSc
NPHP1/NPHP4/CEP290/candidat genes…

Gene invalidation by CRISPR-Cas in iPCS
➢ NPHP1

Damelys Calderon

Kidney organoids: 3D protocol to study fibrosis

imagine
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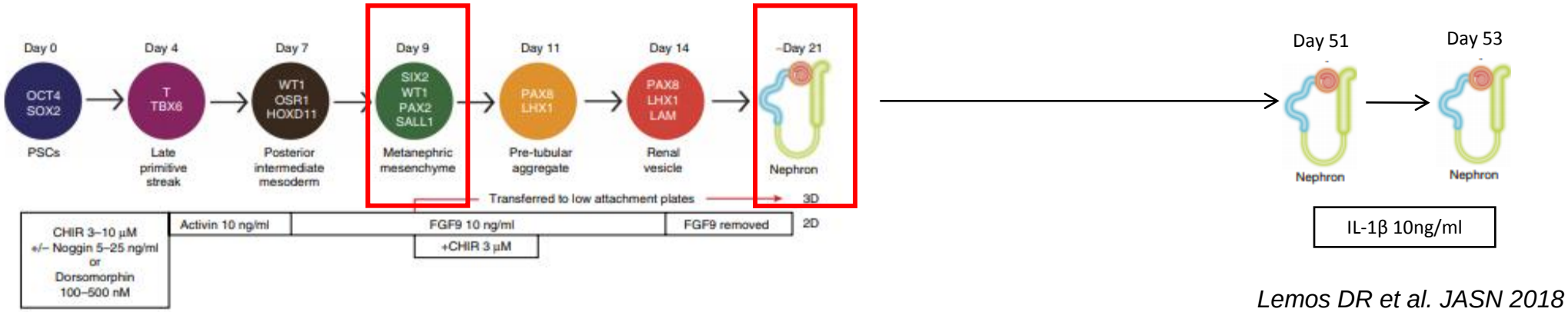
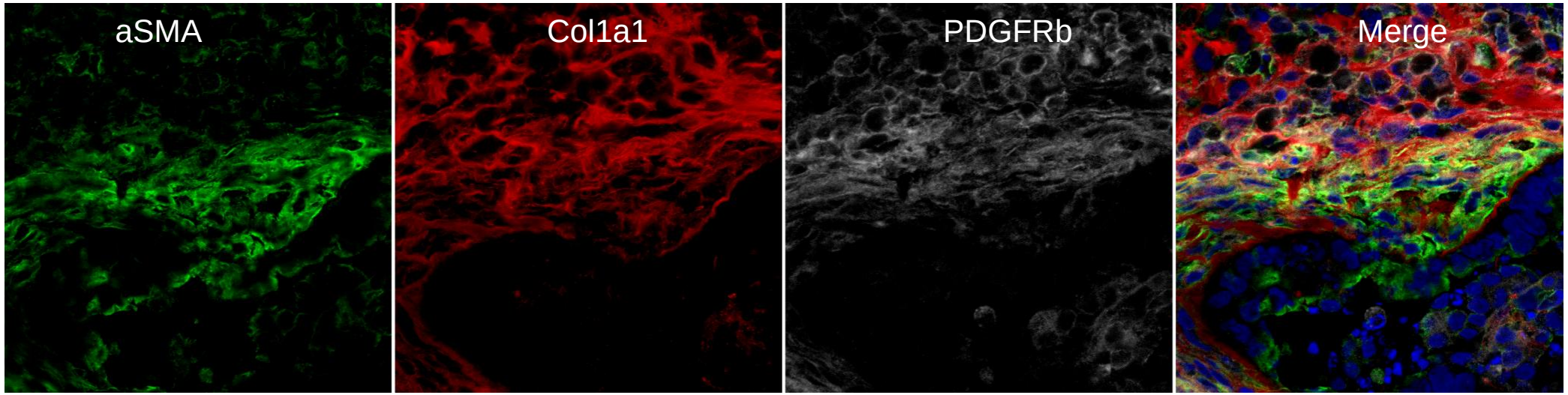


Lemos DR et al. JASN 2018

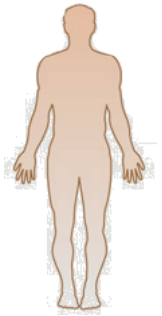


Readouts
- Fibrosis
- myofibroblast transition
- senescence,

Damelys Calderon, Giulia Ferri

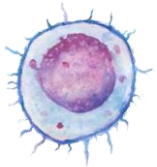
Modéliser les maladies rénales héréditaires

Formes syndromiques

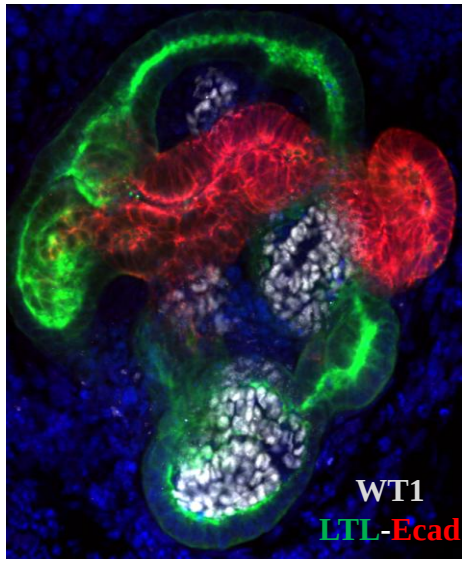


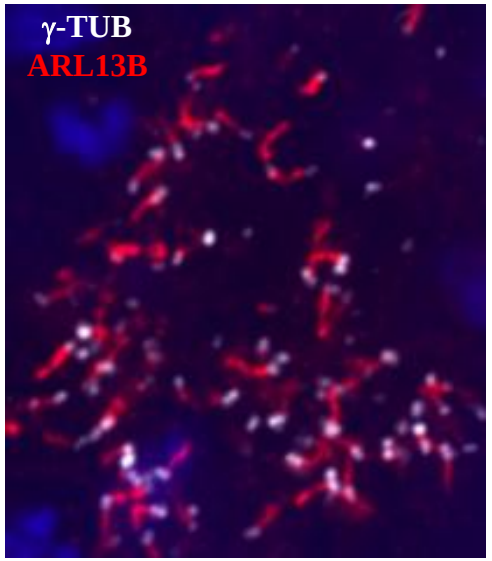
Patients ave c néphropathie +/- atteinte neurologique:

- 2 patients *NPHP1* / 1 patient *NPHP4* / 1 patient *CEP290*
- 3 patients avec syndrome de Galloway-Mowat (KEOPS complex)

→ **PBMC (ou urines)**  → **Cellules iPS**

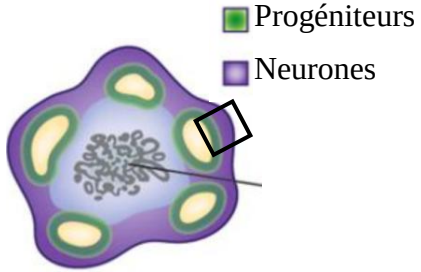
> Organoïdes rénaux

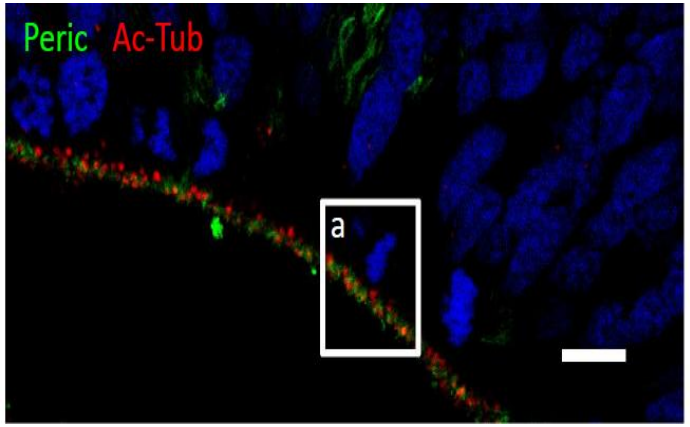


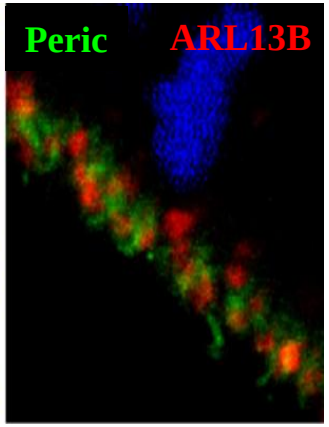


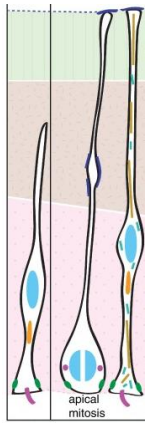
LMRH

> Organoïdes cérébraux









S. Thomas, UMR1163

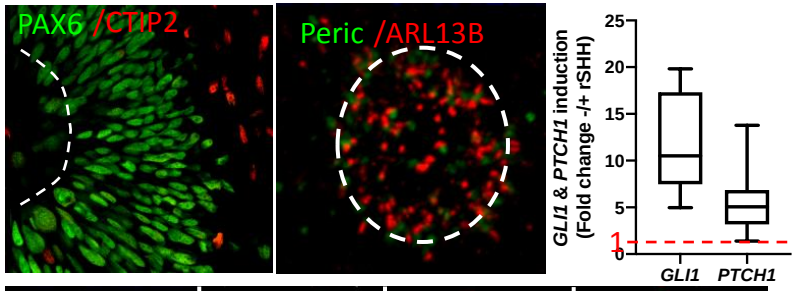
Caractérisation de mécanismes biologiques et moléculaires communs entre le rein et le cerveau

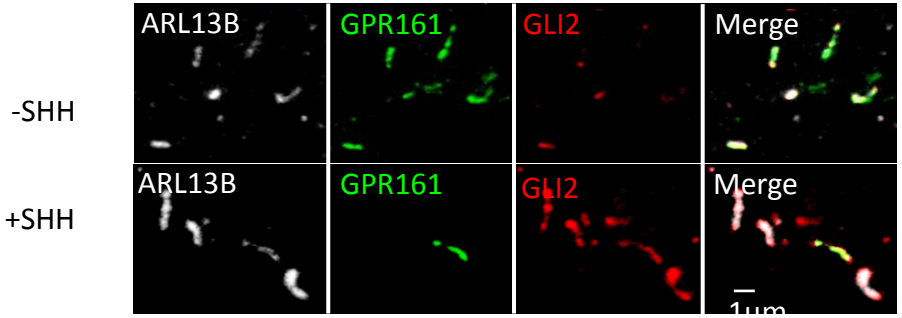
Modéliser les maladies rénales héréditaires

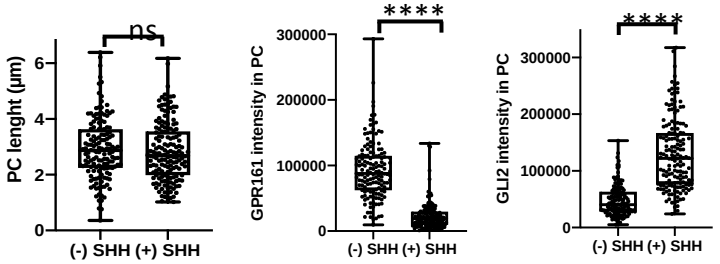
Formes syndromiques

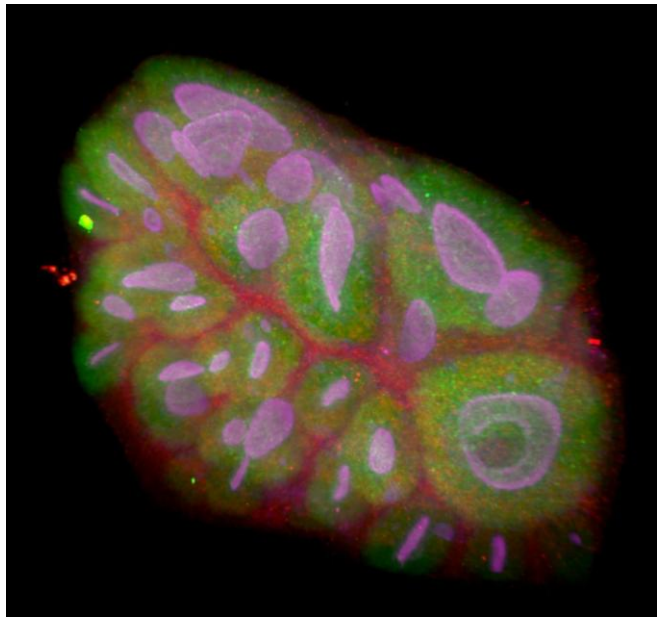
imagine
INSTITUT DES MALADIES GÉNÉTIQUES

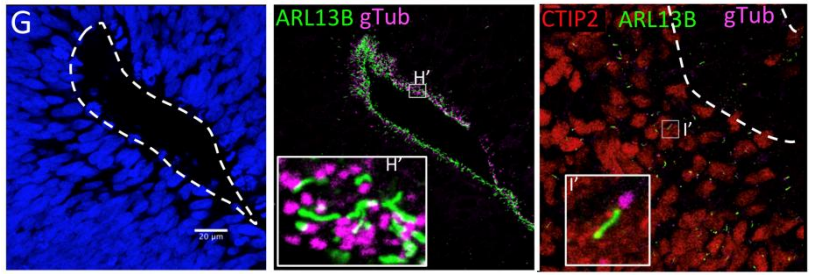
Sophie Thomas
Damelys Calderon











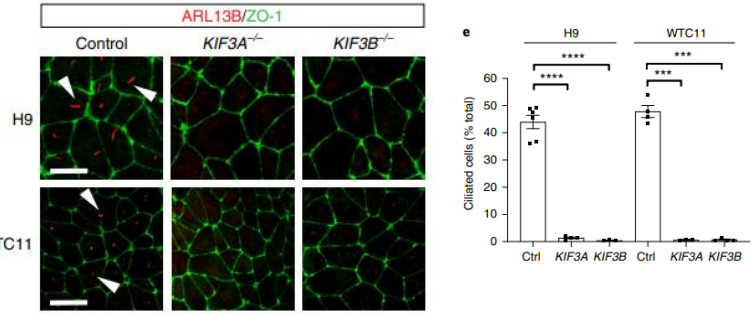
Boutaud et al. 2021

Modèles 2D et 3D pour disséquer l'implication du cilium primaire dans la pathophysiologie des anomalies du développement du cortex cérébral.

nature biomedical engineering
ARTICLES
https://doi.org/10.1038/s41551-022-00880-8
Check for updates

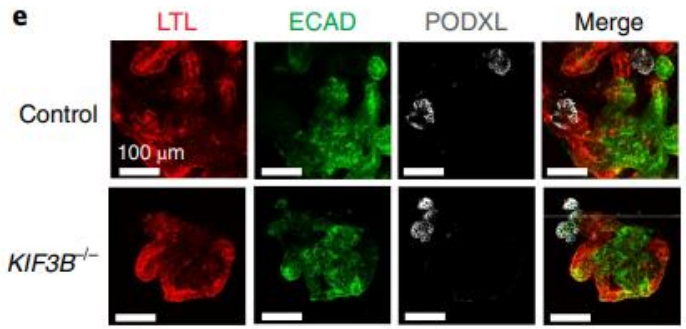
Modelling ciliopathy phenotypes in human tissues derived from pluripotent stem cells with genetically ablated cilia

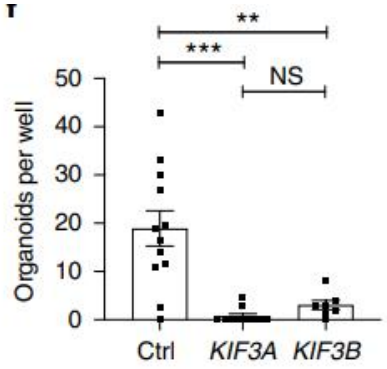
Nelly M. Cruz[1,2,3,4,9], Raghava Reddy[1,2,3,4,9], José L. McFaline-Figueroa[5], Christine Tran[1,2,3,4], Hongxia Fu[3,4,6,7] and Benjamin S. Freedman[1,2,3,4,7,8]



KIF3A$^{-/-}$ and KIF3B$^{-/-}$ hPSCs lacked cilia

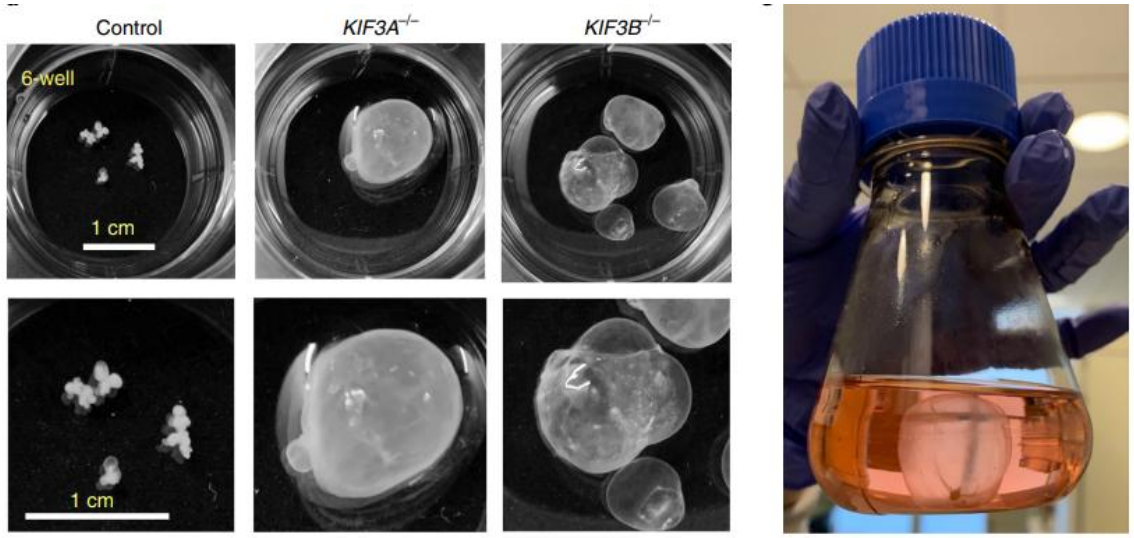
> cilia are dispensable in undifferentiated hPSCs.



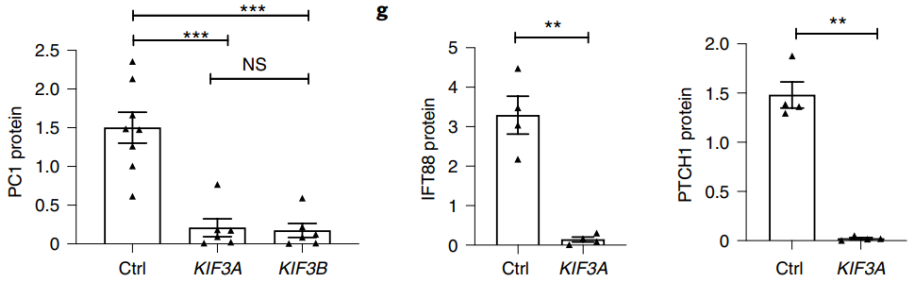


- a critical role for cilia in the induction of nephron cells
- **Increased hedgehog pathway activation**

~50% of kinesin-2 knockout organoids formed large translucent cysts after 2 weeks >1 cm



Kinesin-2 knockout hPSCs and organoids reveal EV secretion defects in disease-relevant signalling pathways.

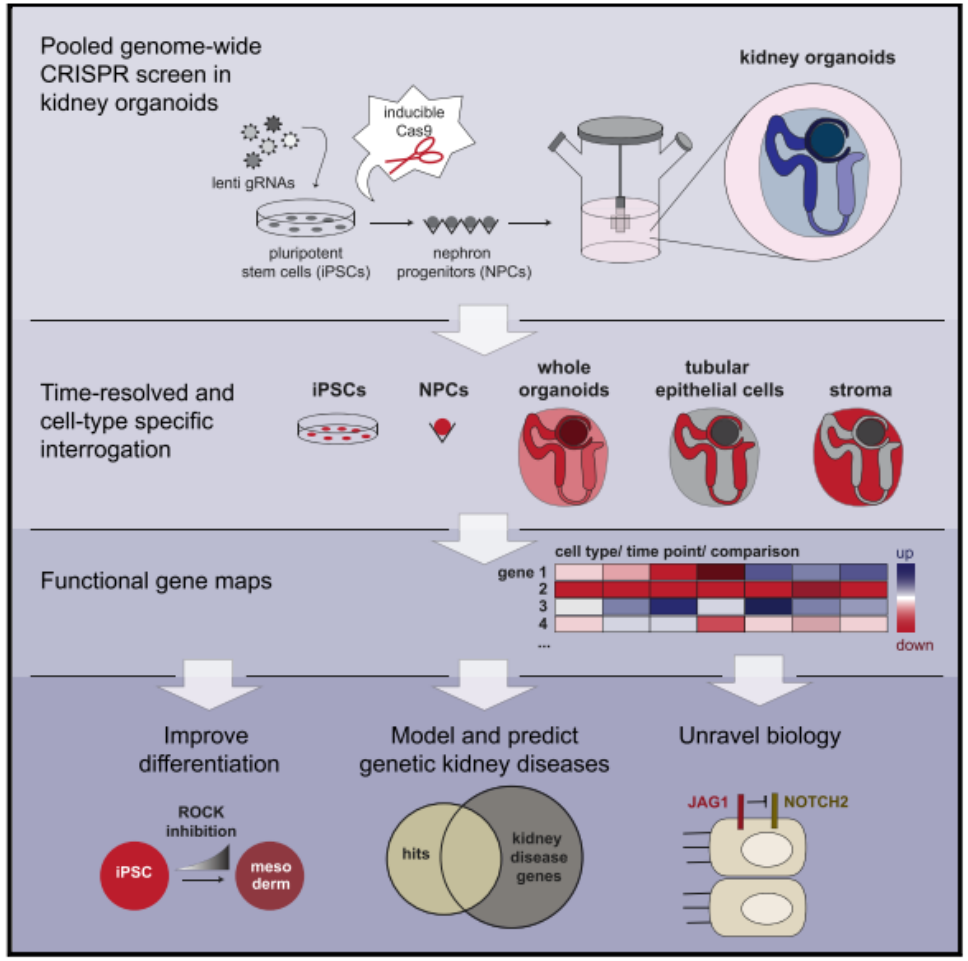


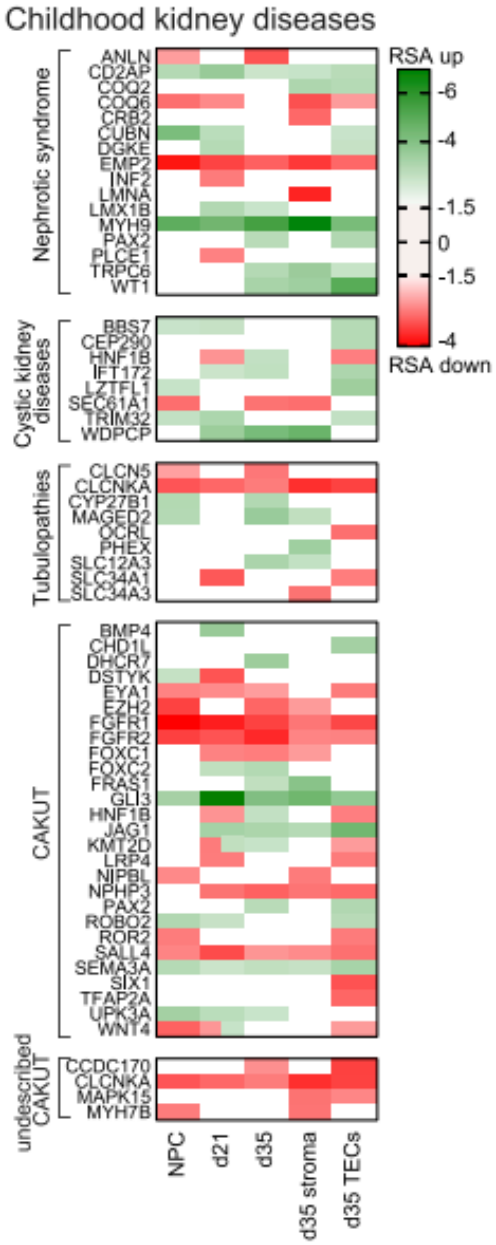
Cell Stem Cell

Resource

Genome-wide screening in human kidney organoids identifies developmental and disease-related aspects of nephrogenesis

Rosemarie Ungricht, Laure Guibbal, Marie-Christine Lasbennes, ..., Jan S. Tchorz, Dominic Hoepfner, Philipp S. Hoppe

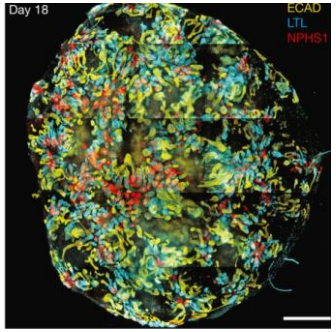




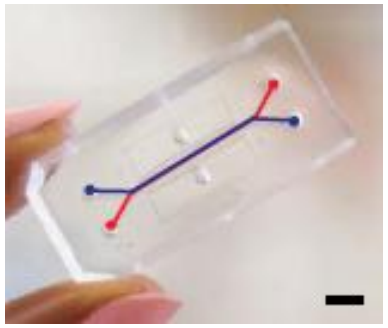
> Identifier des genes relevants pour les maladies rénales chroniques pédiatriques et les ciliopathies

Autres utilisations possibles des iPSC

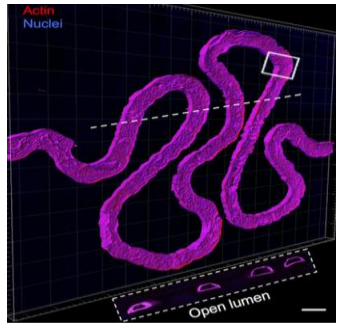
Cellules iPS



Organoïdes



Organ-on-chip



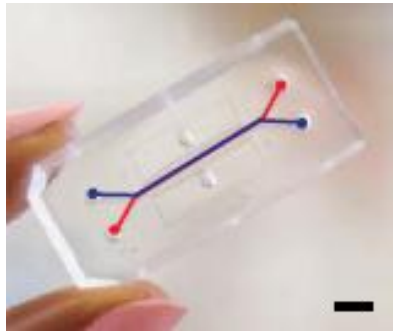
Impression 3D

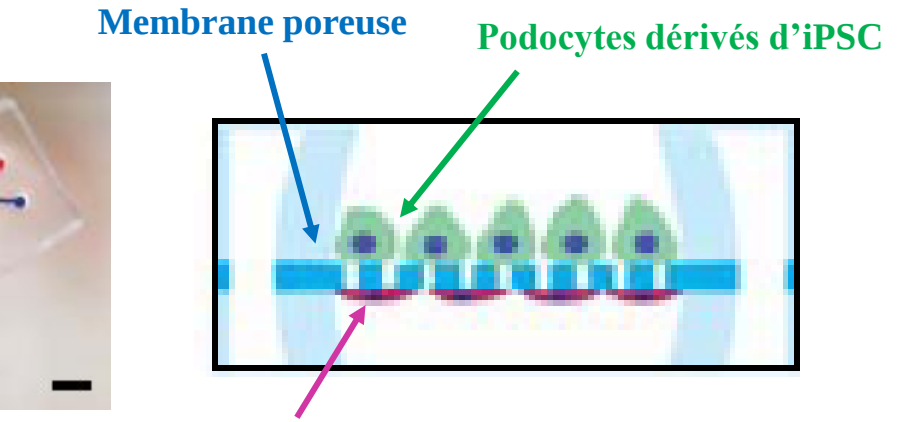
Reins décellularisés

Construction/reproduction de segments du néphron

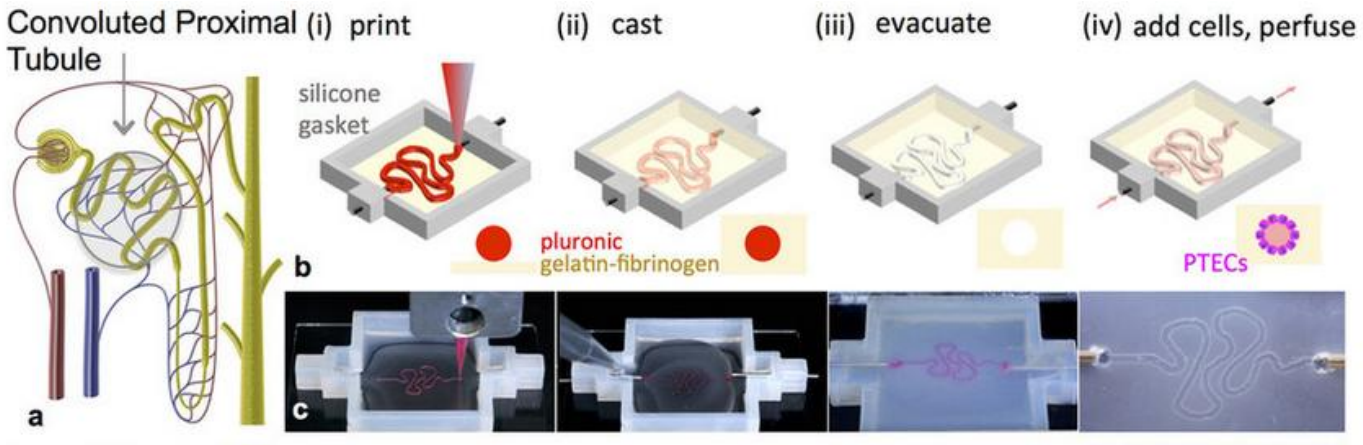
- Système microfluidique: « Glomerulus Chip » reproduisant la **barrière de filtration glomérulaire**

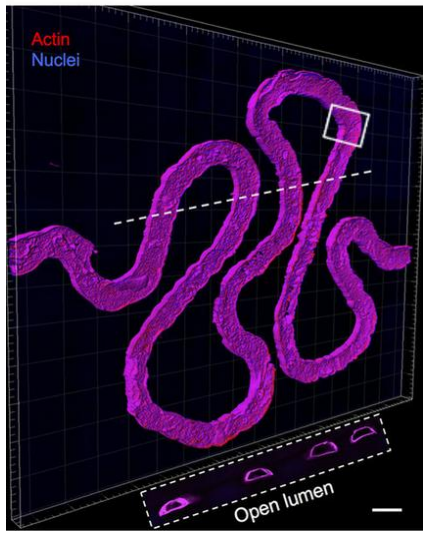
Musha S et al., Nature Protocols, 2018

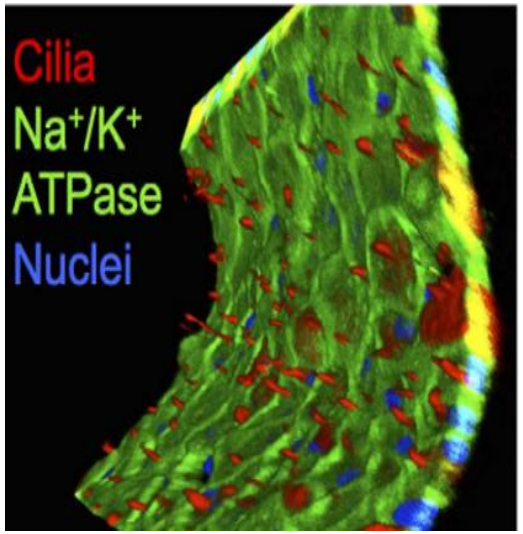




- « Bioprinting » de **tubules proximaux** en 3D sur des puces perfusables

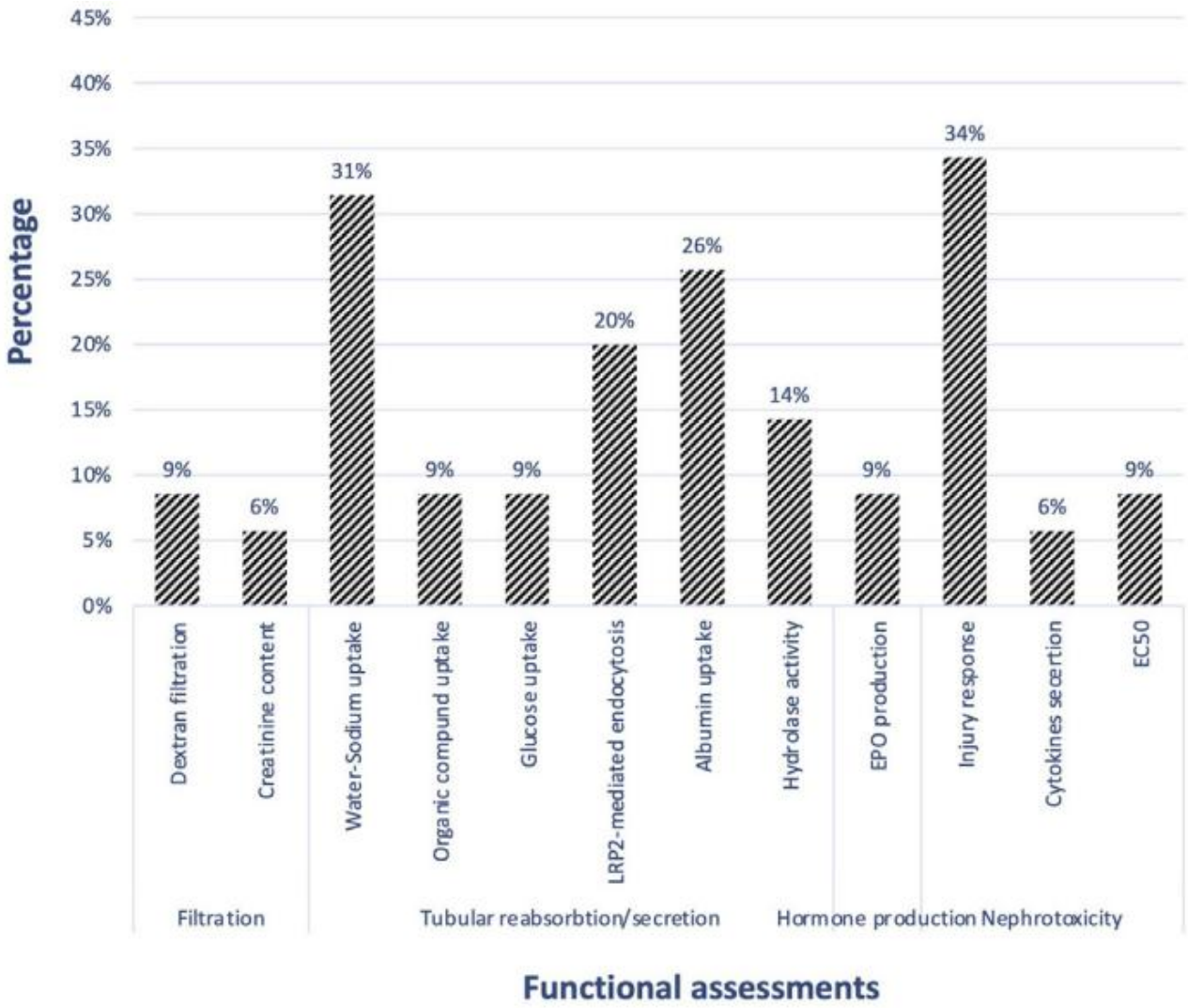




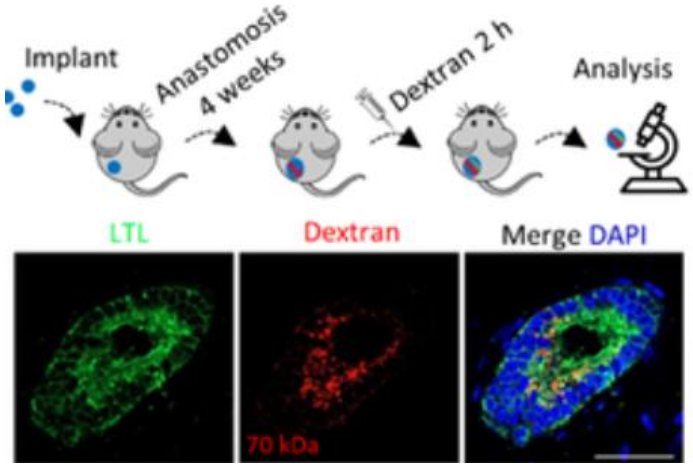


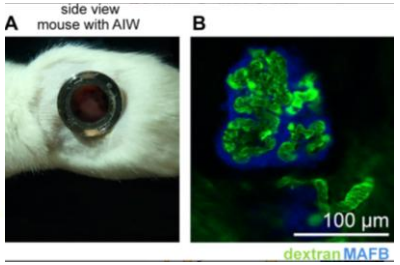
Homan K et al., Scientific Rep 2016

Essais fonctionnels à partir des organoïdes rénaux

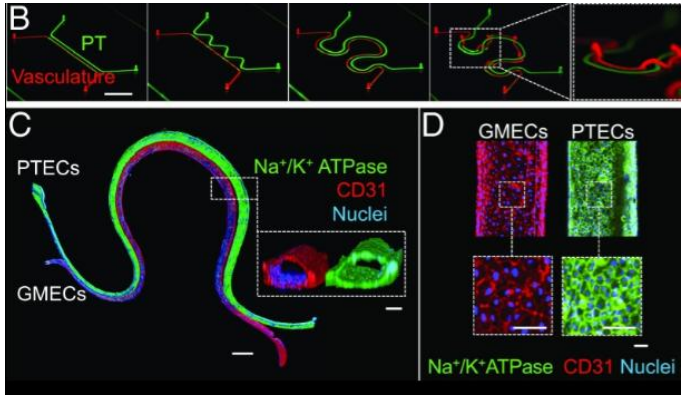


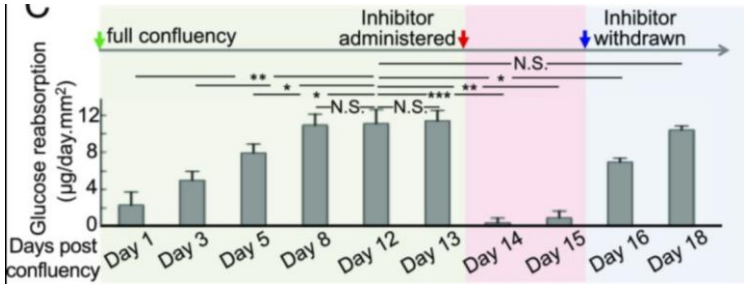
Rizki-Safitri et al. Function (Oxf), review 2021





Low J et al., Cell Stem Cell 2019





Neil Y C Lin et al. PNAS 2019

Essais fonctionnels à partir des organoïdes rénaux

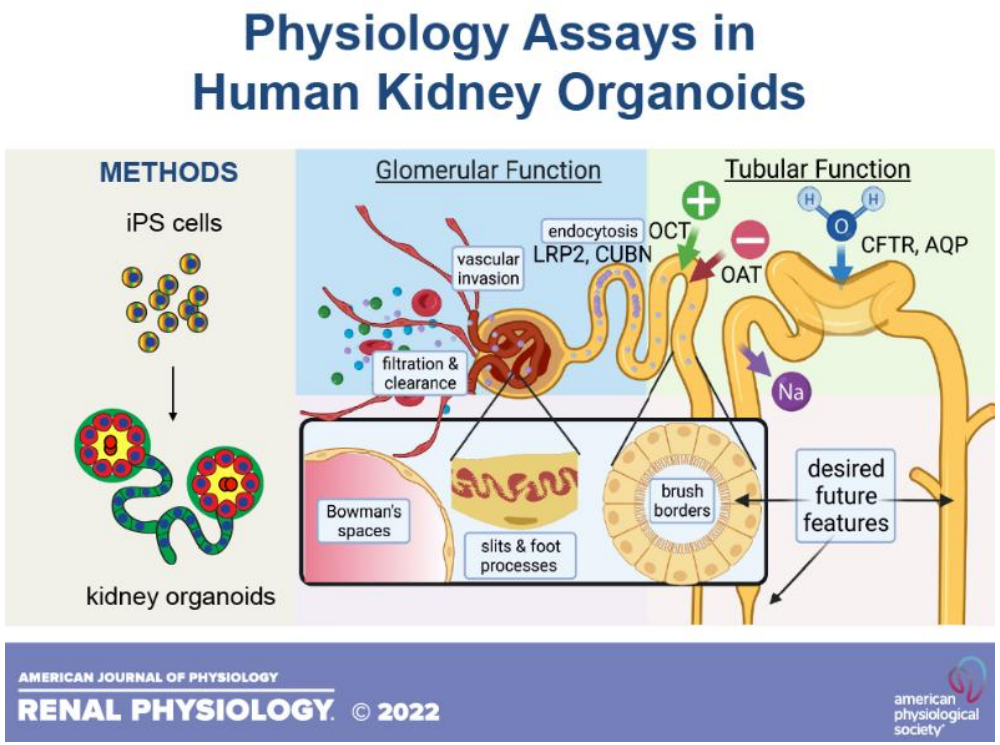


Table 2. *Physiology assays in human kidney organoids*

Function	Molecular Agent	Nephron Segment(s)	References
Accumulation	Dextran	Tubules	Freedman et al. (2); Takasato et al. (4); Low et al. (27)
	Fluorescein Methotrexate	Tubules	Freedman et al. (2)
	Fluorescein	Tubules	Shankar et al. (28)
	6-Carboxyfuorescein	Tubules	Przepiorski et al. (29)
Swelling	Forskolin	Tubules	Cruz et al. (6); Low et al. (27); Shimizu et al. (30); Kuraoka et al. (31)
	8-Bromo-cAMP	Tubules	Cruz et al. (6); Low et al. (27)
Filtration	Blood	Endothelium	Sharmin et al. (32); van den Berg et al. (33); Nam et al. (34); Kumar Gupta et al. (35)
	Dextran	Endothelium, podocytes	van den Berg et al. (36); Kumar Gupta et al. (35)
Endocrine	Renin	Stroma	Shankar et al. (28)
	Vitamin D	Undetermined	Shankar et al. (37)
Injury	Cisplatin	Tubules, stroma	Freedman et al. (2); Morizane et al. (3); Takasato et al. (4); Czerniecki et al. (7); Digby et al. (38); Gupta et al. (39)
	Gentamicin	Tubules	Freedman et al. (2); Morizane et al. (3); Lawrence et al. (40)
	Hemin	Tubules	Przepiorski et al. (29)
	SARS-CoV-2	Tubules	Helms et al. (41); Jansen et al. (42)

SARS-CoV-2, severe acute respiratory syndrome-coronavirus-2.

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Freedman, Renal Physiology, review 2022

Conclusion

- Avancées majeures dans le domaine de la génération de tissus à partir des cellules souches
- Application possible des nouvelles technologies: ‘genome editing ’, impression 3D et génération de chimères
- Utilisation possible des iPSC pour :
  - La caractérisation des mutations et de leur rôle dans le développement d’une maladie génétique
  - La découverte de médicaments
  - A plus long terme recréer un rein (ou des portions de celui-ci)

- A améliorer: systèmes immatures, vascularisation et système excréteur

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