Berliner DialyseSeminar

Darlegung potentieller Interessenskonflikte

Der Inhalt des folgenden Vortrages ist Ergebnis des Bemühens um größtmögliche Objektivität und Unabhängigkeit.

Als Referent versichere ich, dass in Bezug auf den Inhalt des folgenden Vortrags **keine Interessenskonflikte** bestehen, die sich aus einem Beschäftigungsverhältnis, einer Beratertätigkeit oder Zuwendungen für Forschungsvorhaben, Vorträge oder andere Tätigkeiten ergeben.

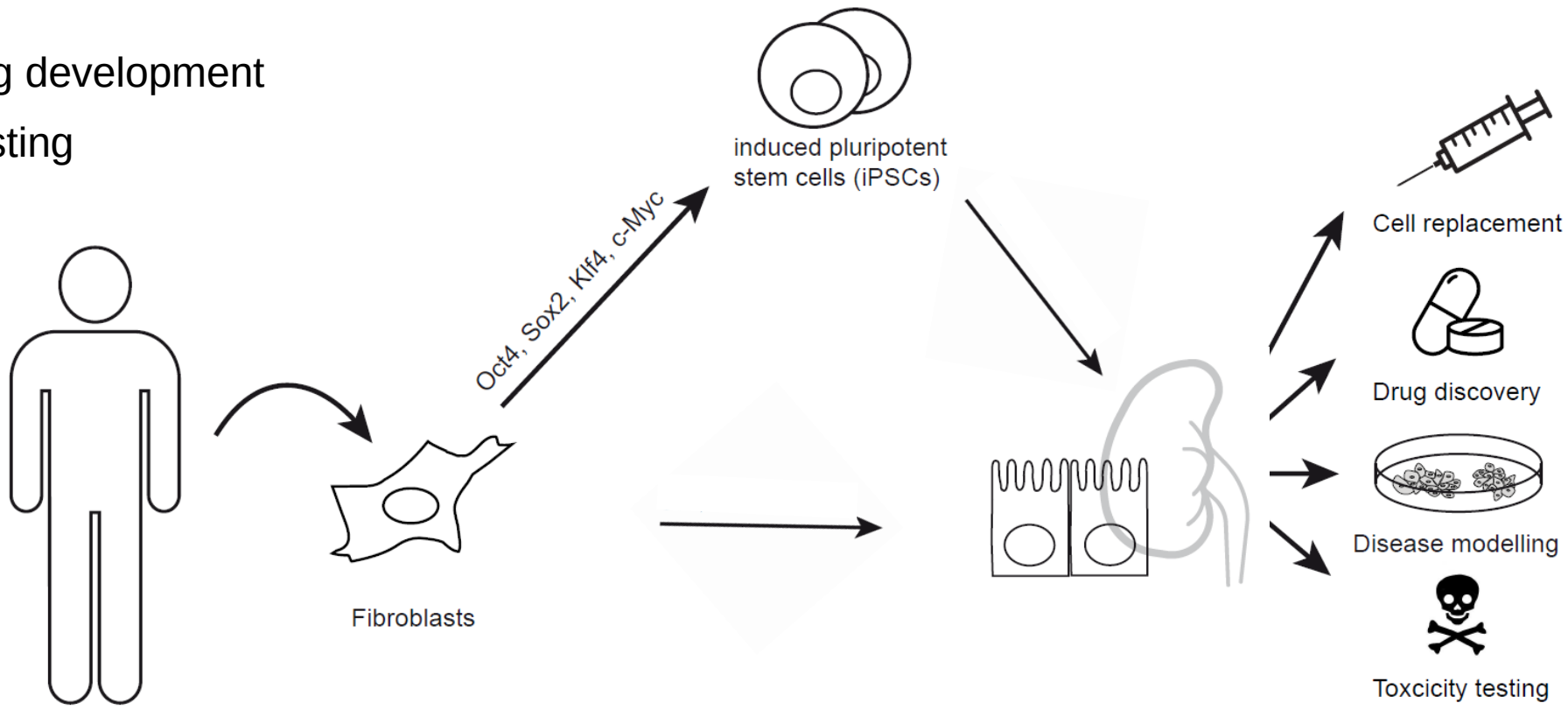
Grant Vision: Kidney Regeneration

→ organ replacement

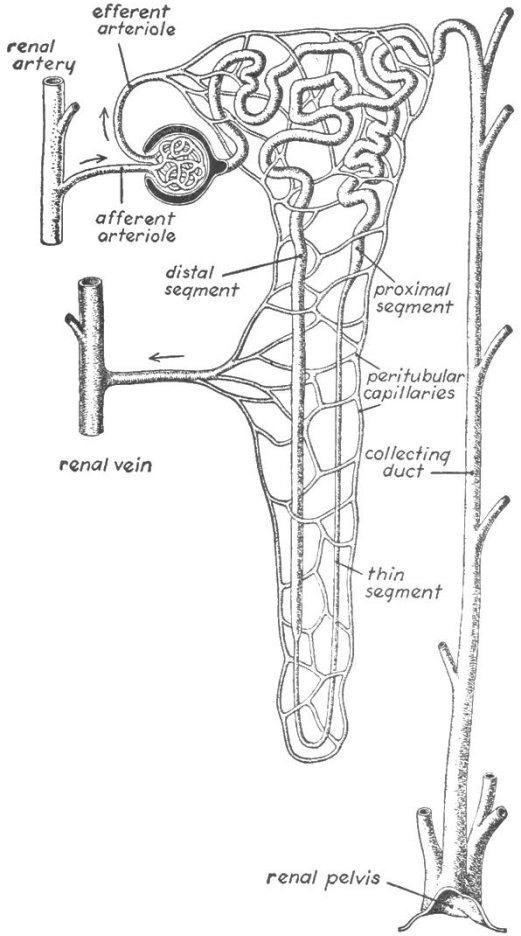
- dialysis can bridge the time to transplant
- high demand / low supply

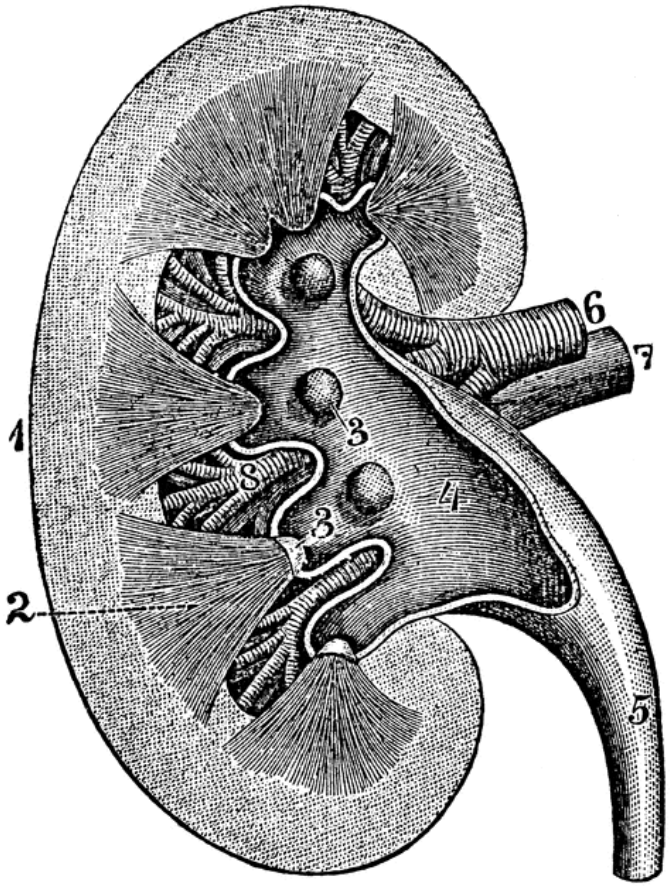
→ in vitro models

- personalized drug development
- nephrotoxicity testing



The kidney - as complex as a watch





I. C. S. Reference Library Arithmetic, Mensuaration, Principles of Mechanics

Homer W.Smith, From Fish to Philosopher, 1953

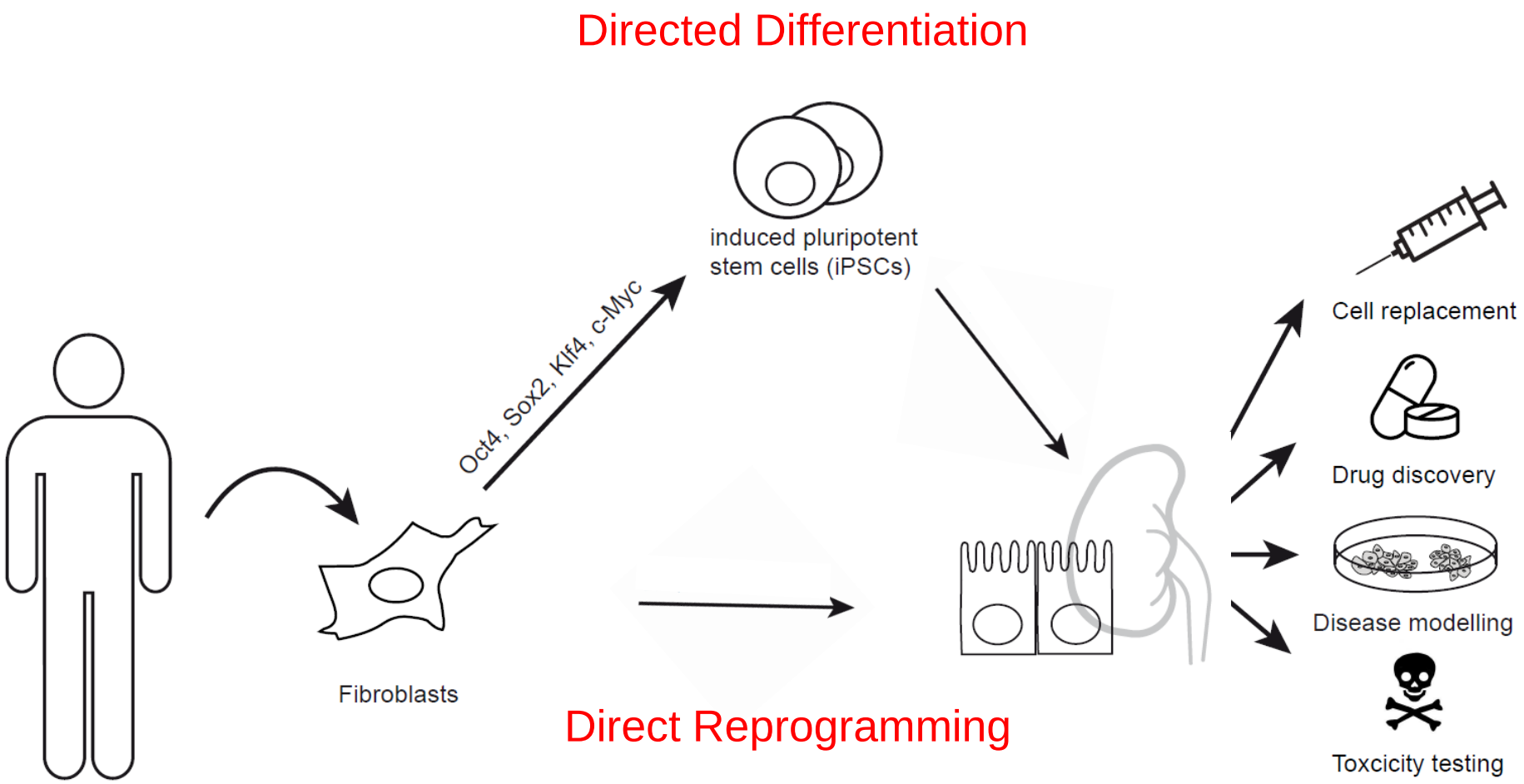
The kidney / watch – easy to destroy

The kidney / watch – easy to destroy, hard to put back together

Re-creating kidneys will help us understand them.

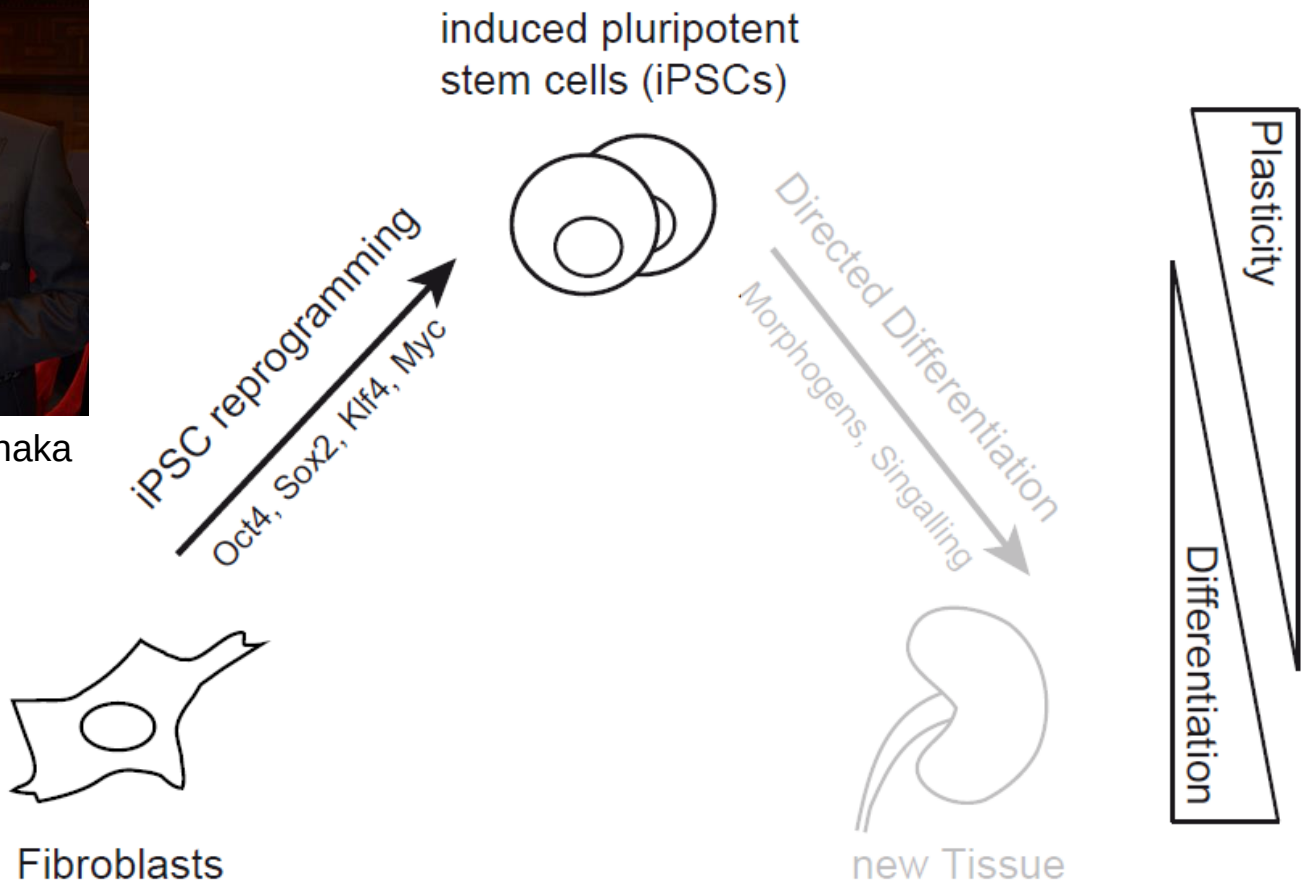
Richard Feynman

Two modes of creating kidney tissue in vitro:



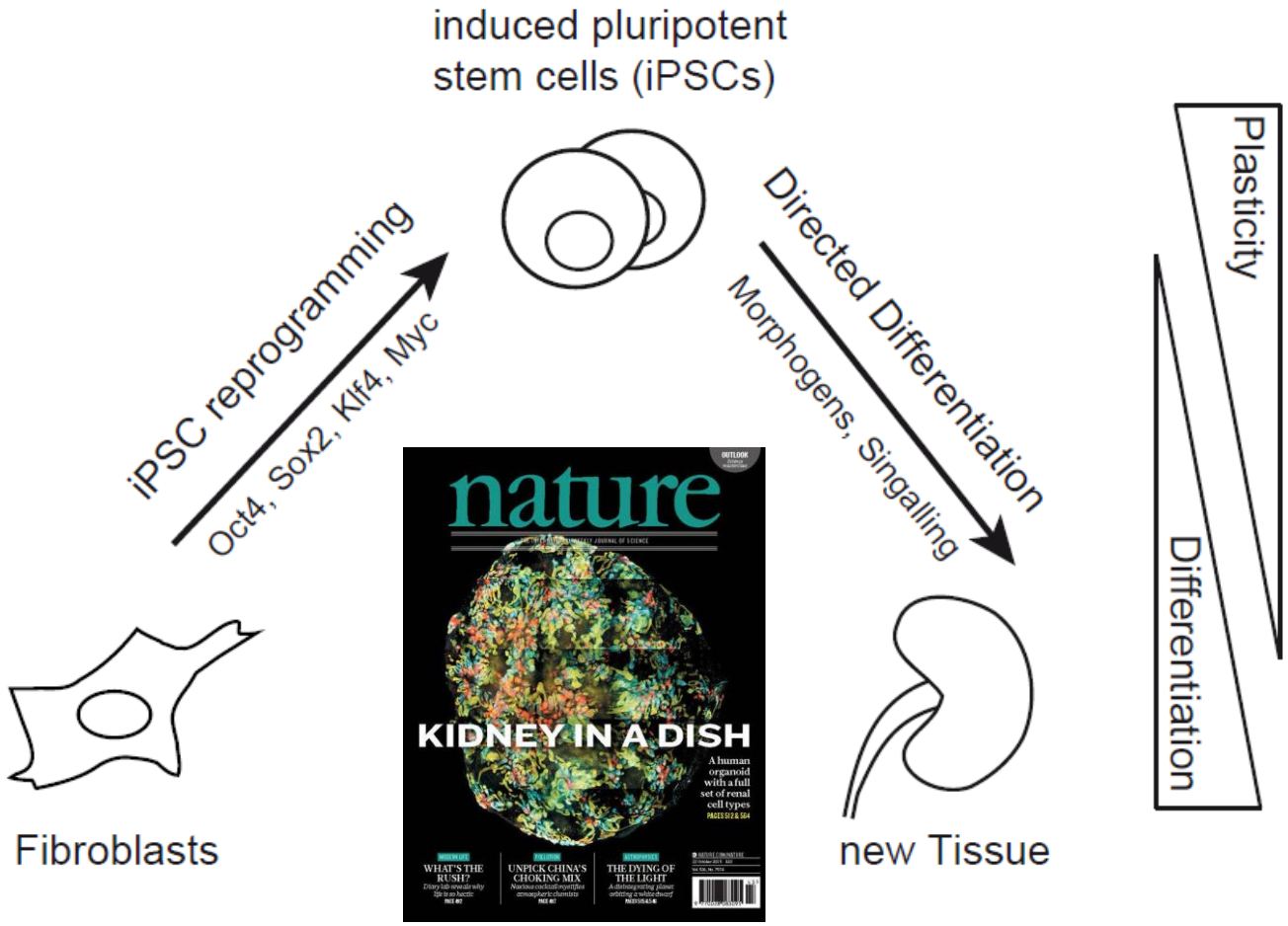
Induced pluripotent stem cells (iPS-cells)

Shinya Yamanaka



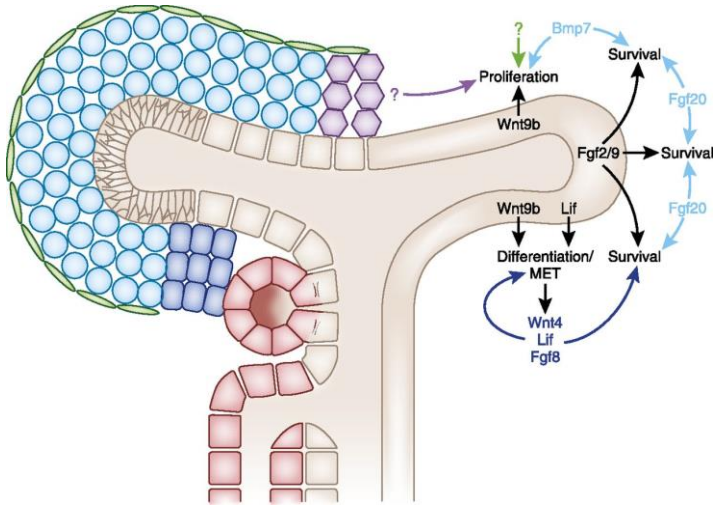
Cell. 2006 Aug 25;126(4):663-76.

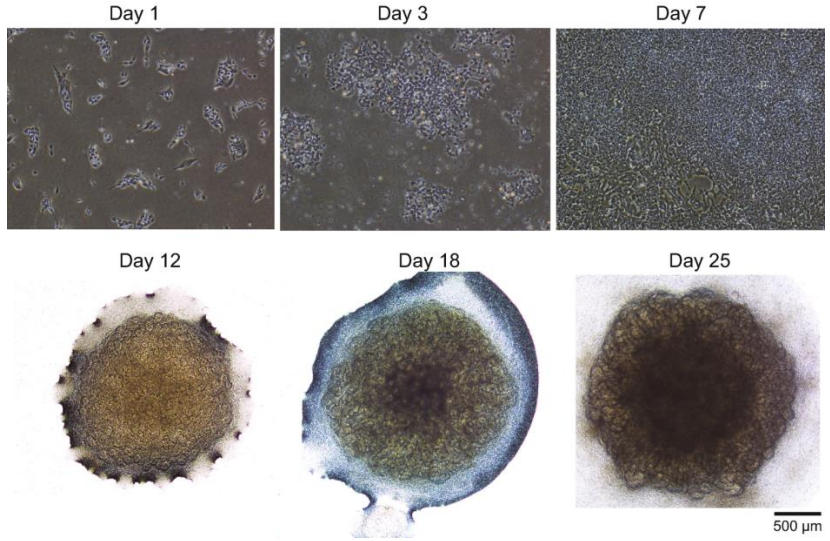
Directed differentiation – iPS-cells to kidney organoids

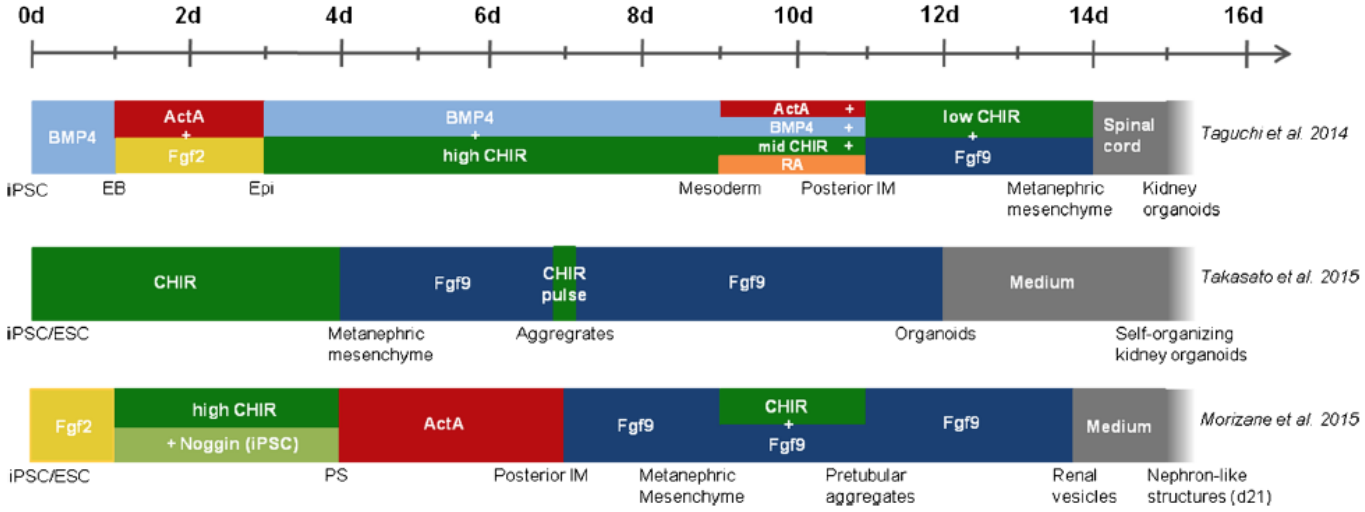


Takasato et al., Nature 2015
Morizane et al., Nat. Biotech 2015

Commonly used protocols for iPSC differentiation

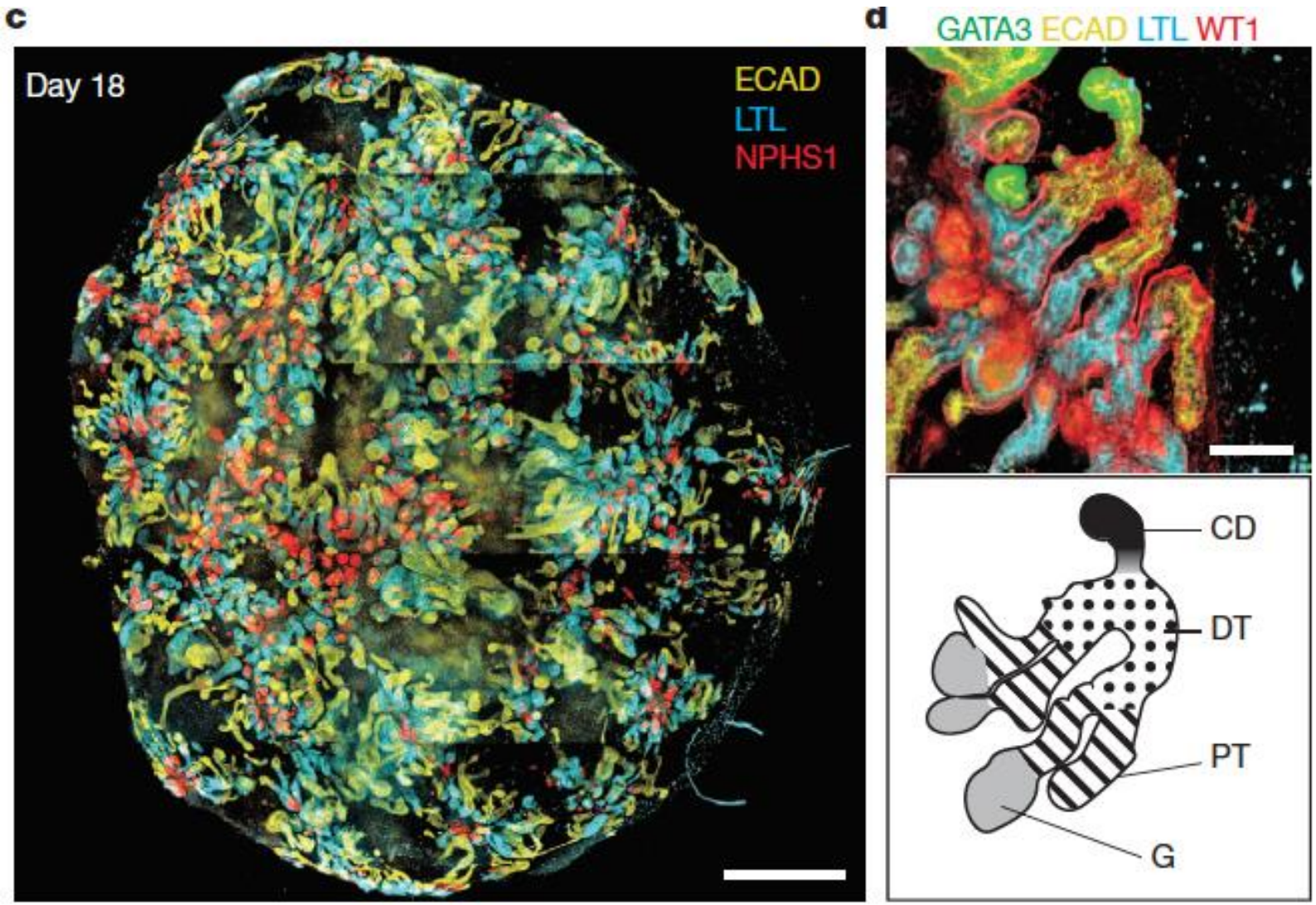






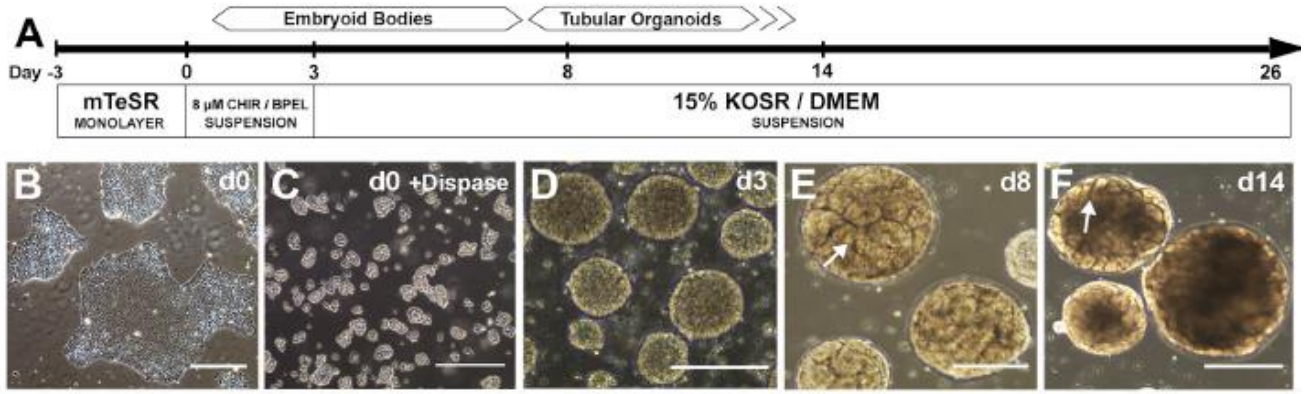
Kaminski et al., 2017, Cell Tiss Research

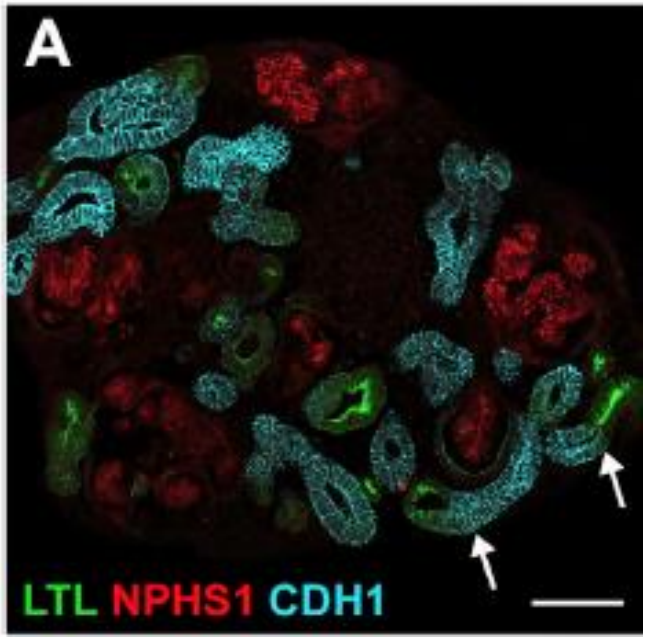
iPSC derived kidney organoids



Takasato et al., Nature 2015

Bioreactor based organoid culture



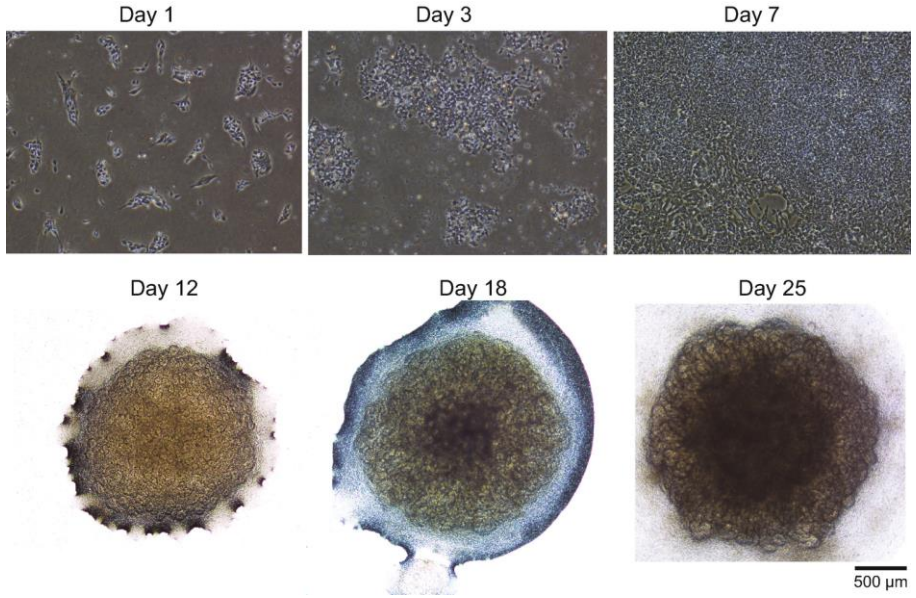


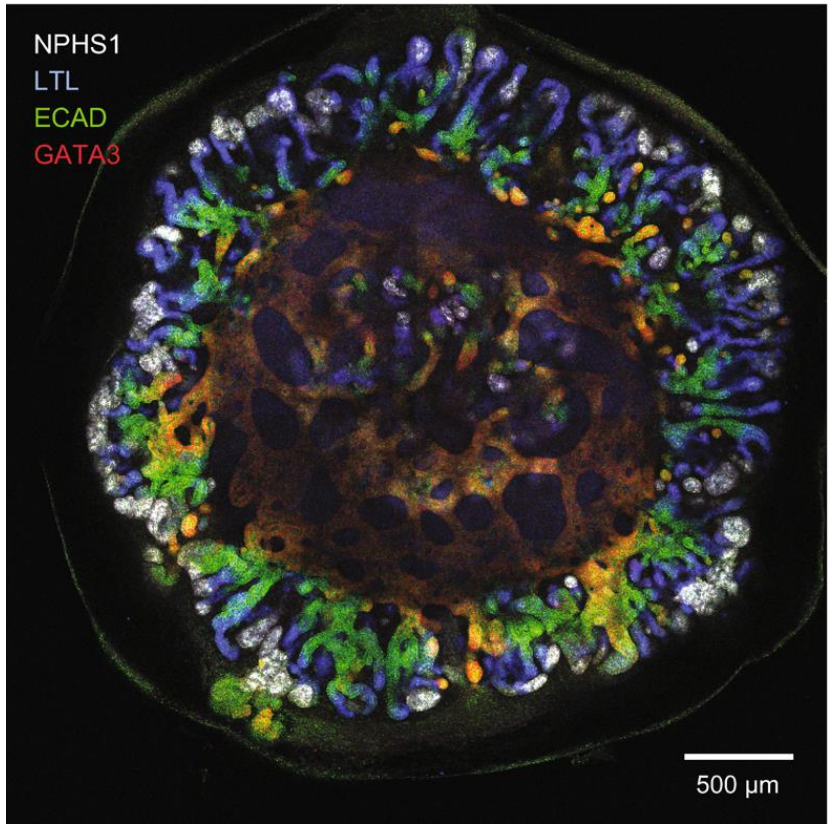
- Increased organoid production efficiency (1000 organoids in 125 ml)
- Low cost of reagents
- Higher robustness / less variability

Przepiorski A et al., Stem Cell Reports, 2018

Refined organoid induction protocols

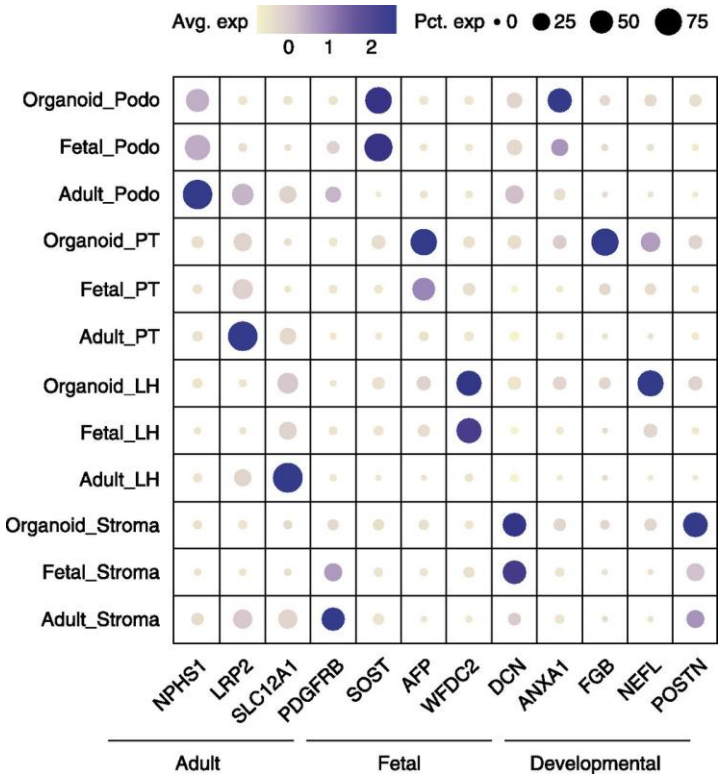
	Stage	Medium	Procedure	Method
day 0	Pluripotent	Essential 8	Seed cells on LN521	*Section 3.1* Steps 1-6
day 1-7	Primative streak	Essential 6 + CHIR99021 (4-8 µm)	Start differentiation	*Section 3.3* Steps 1-2
	Intermediate mesoderm	Essential 6 + FGF9 (200 ng/µl)	Swith to FGF9 between days 3-5. An ealier switch promotes more distal nephron patternig whereas a later switch promotes more proximal nephron	*Section 3.3* Steps 3-4
day 7-12	Nephrogenesis		On day 7 harvest and aggregate cells and transfer to transwell to promote 3D organisation and nephrogenesis. A short (1h) CHIR99021 pulse promotes nephron formation	*Section 3.4* Steps 1-13
day 13-25	Maturation	Essential 6	Remove FGF9 from medium on day 12 and culture organoids for additional ~2 weeks to promote further maturation. Additional factors may be also be added to effects nephron patterning/maturation. For example, retionic acid promotes podocyte formation	*Section 3.4* Step 14
	Endpoint		Harvest organoids for downstream applications/analyses	*Section 3.4* Step 15

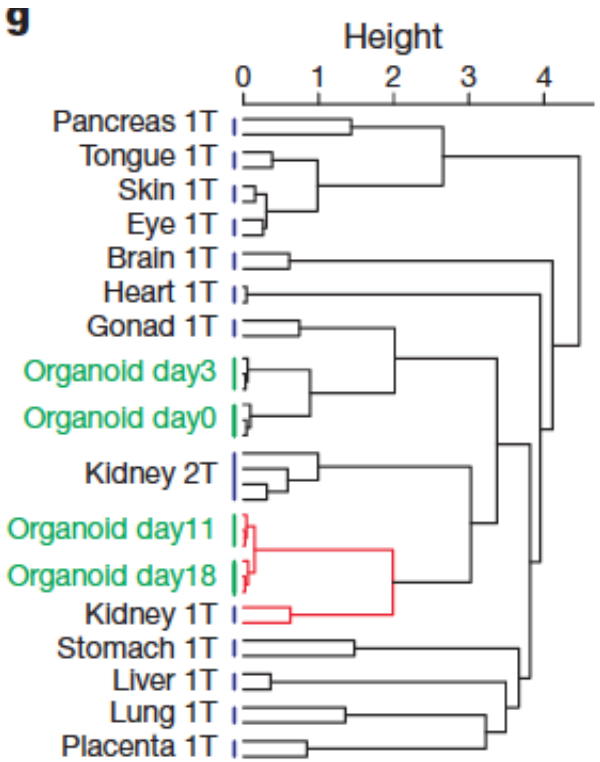




Howden A et al., Stem Cell and Tiss Repair, 2020

How similar are organoids to the real thing?

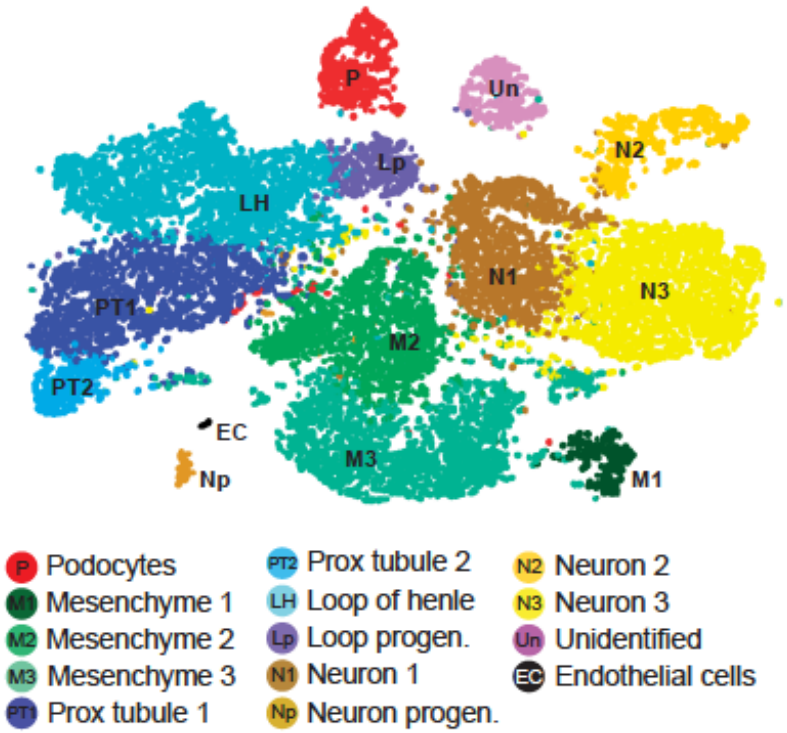


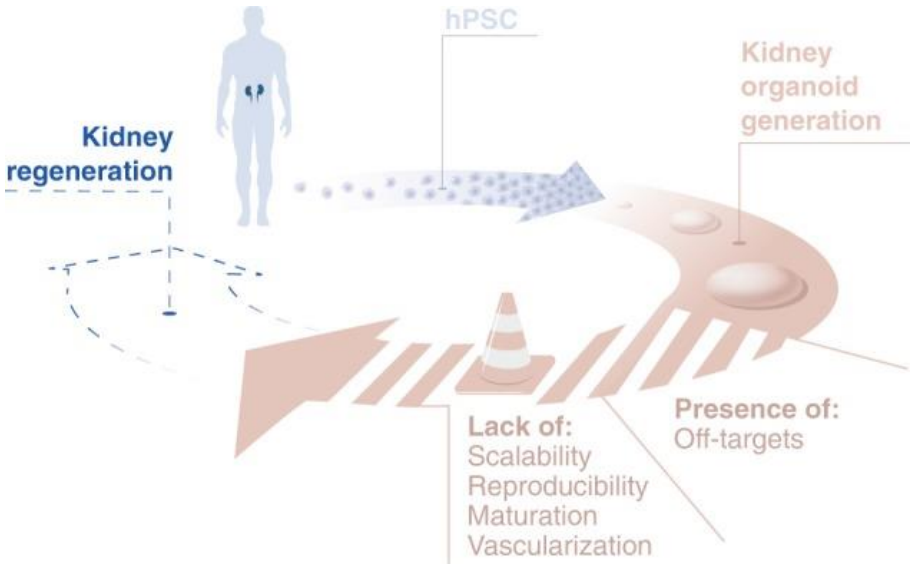


Wu et al., CJASN 2020

Takasato et al., Nature 2015

How similar are organoids to the real thing?

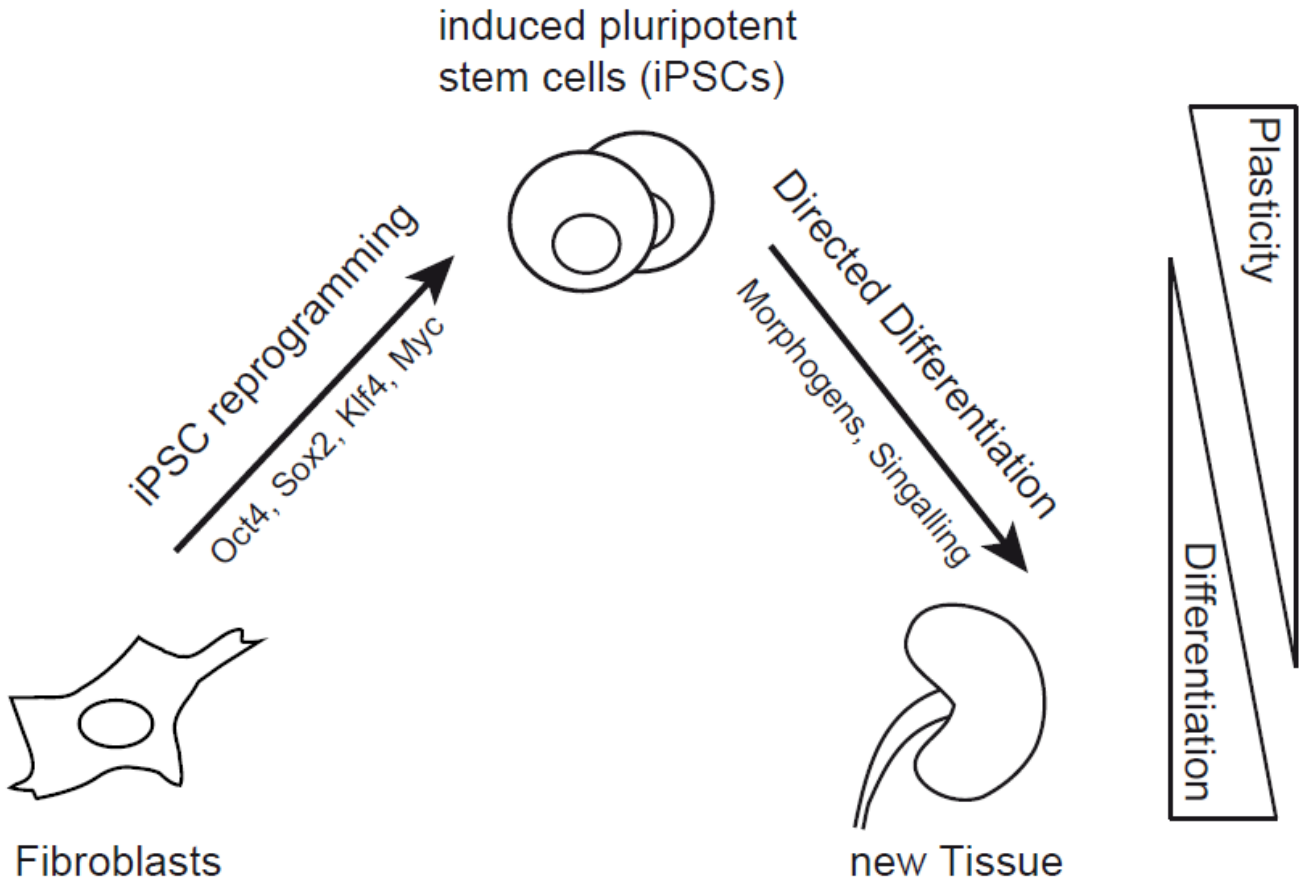




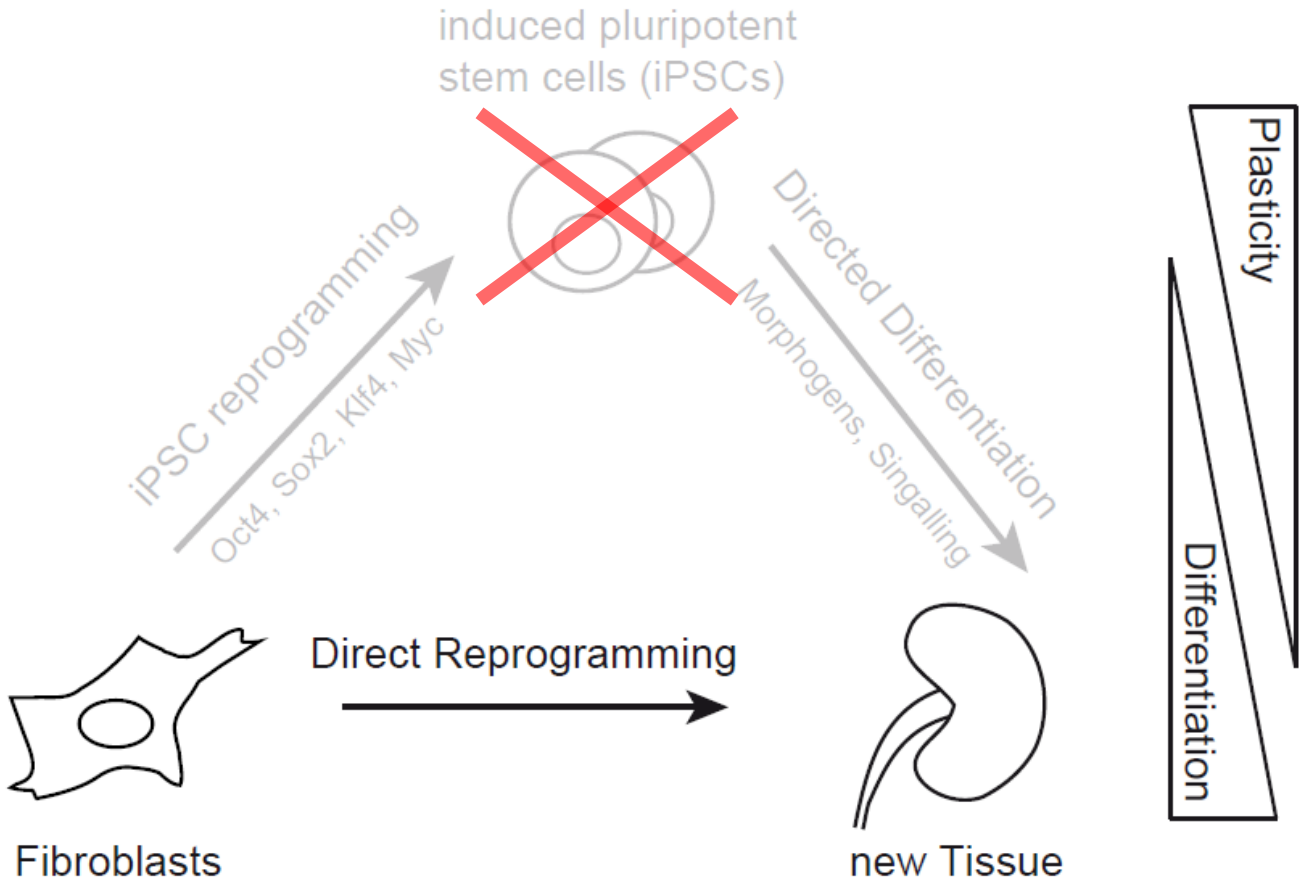
Wu et al., Cell Stem Cell 2018

Geuens T, NPJ Regen Med 2020

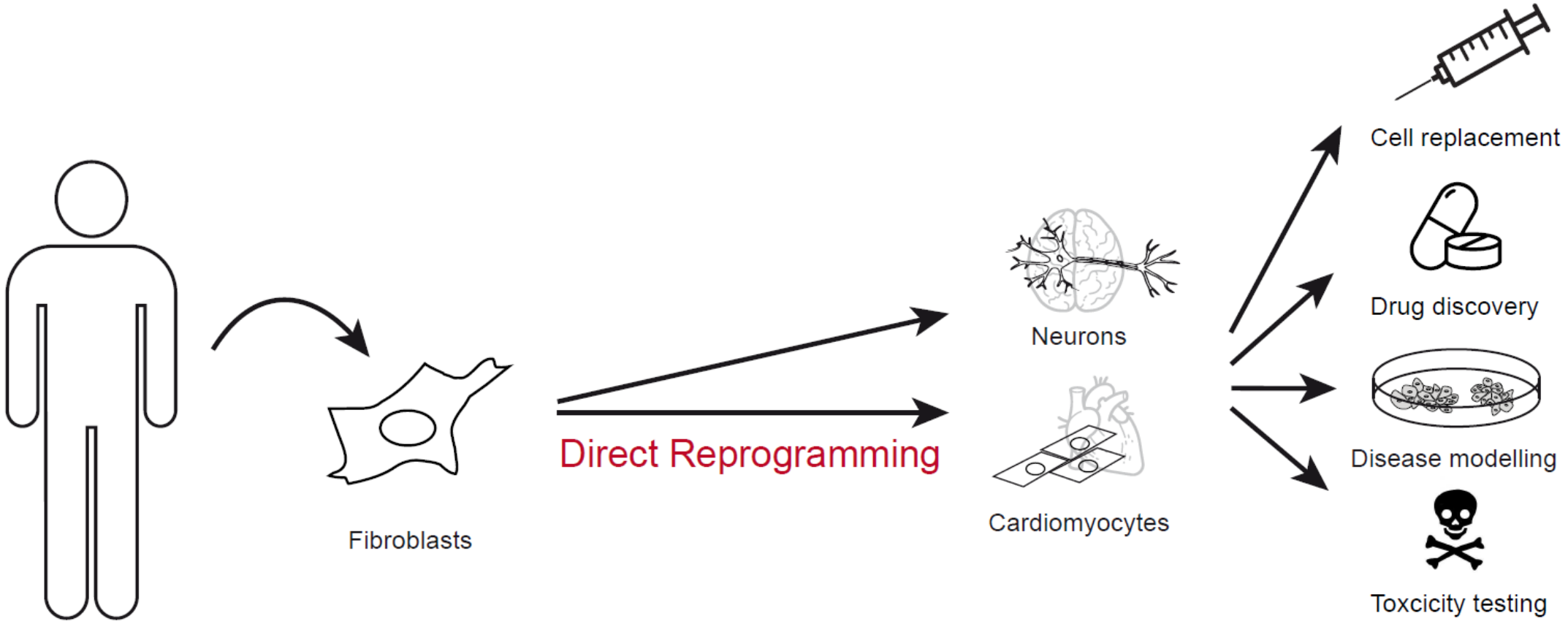
Directed differentiation - iPS-cells to kidney organoids



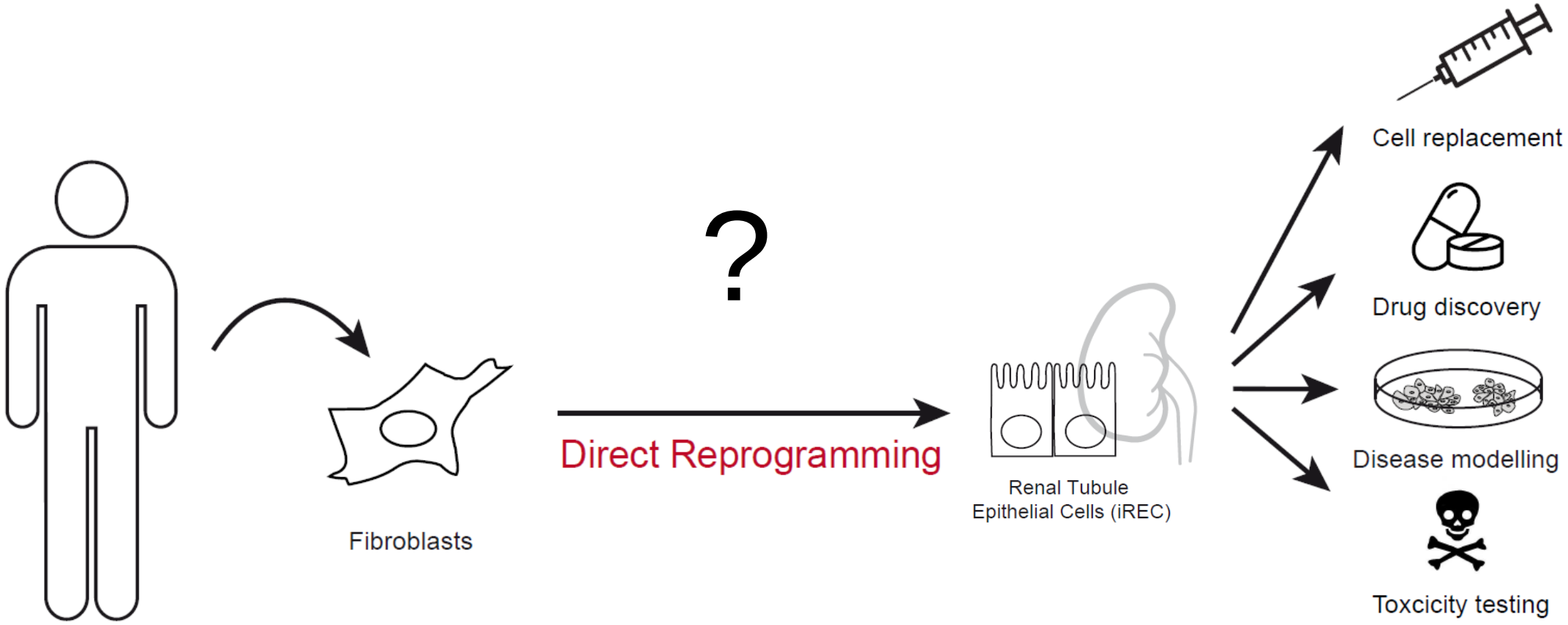
Direct reprogramming



Direct reprogramming - Vision

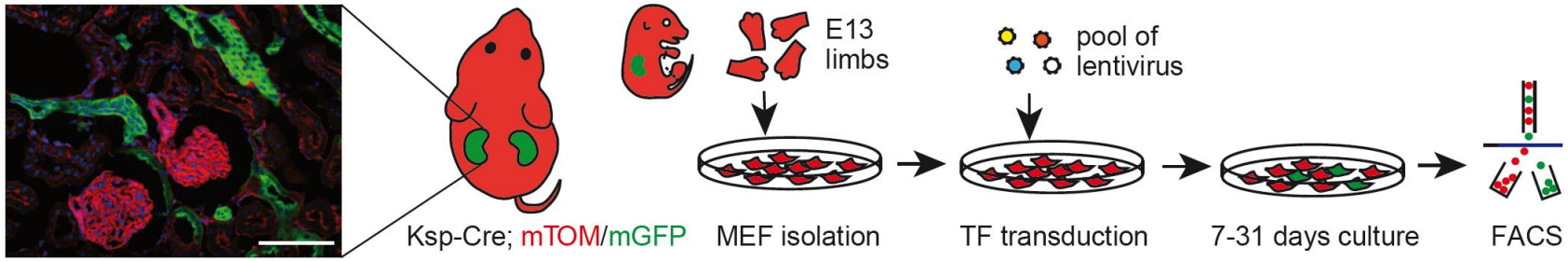


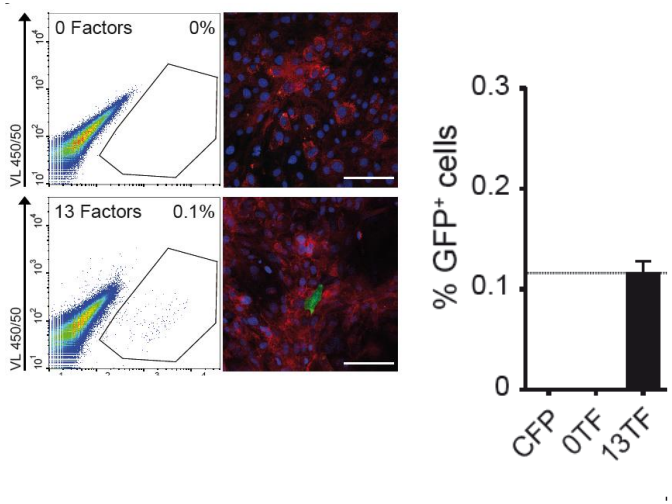
Direct reprogramming to kidney cells



Kaminski et al., Nat Cell Bio, 2016

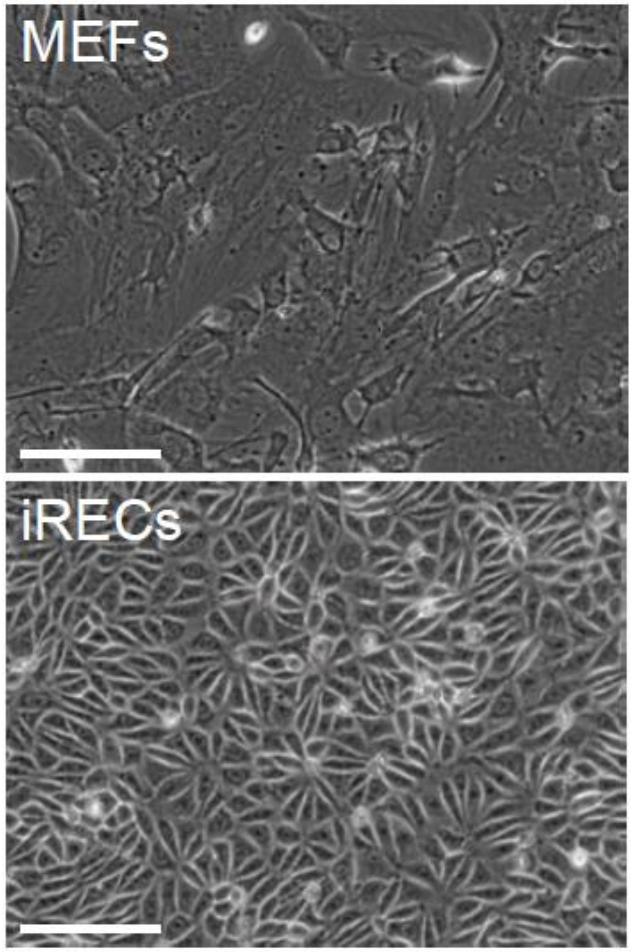
Mouse model / screen for reprogramming factors

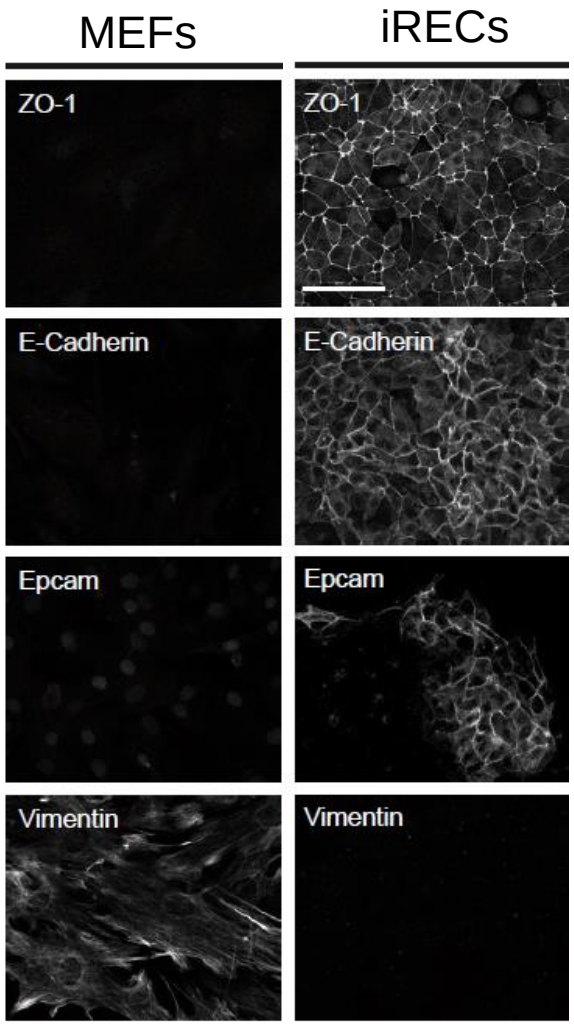




Kaminski et al., Nat Cell Bio, 2016

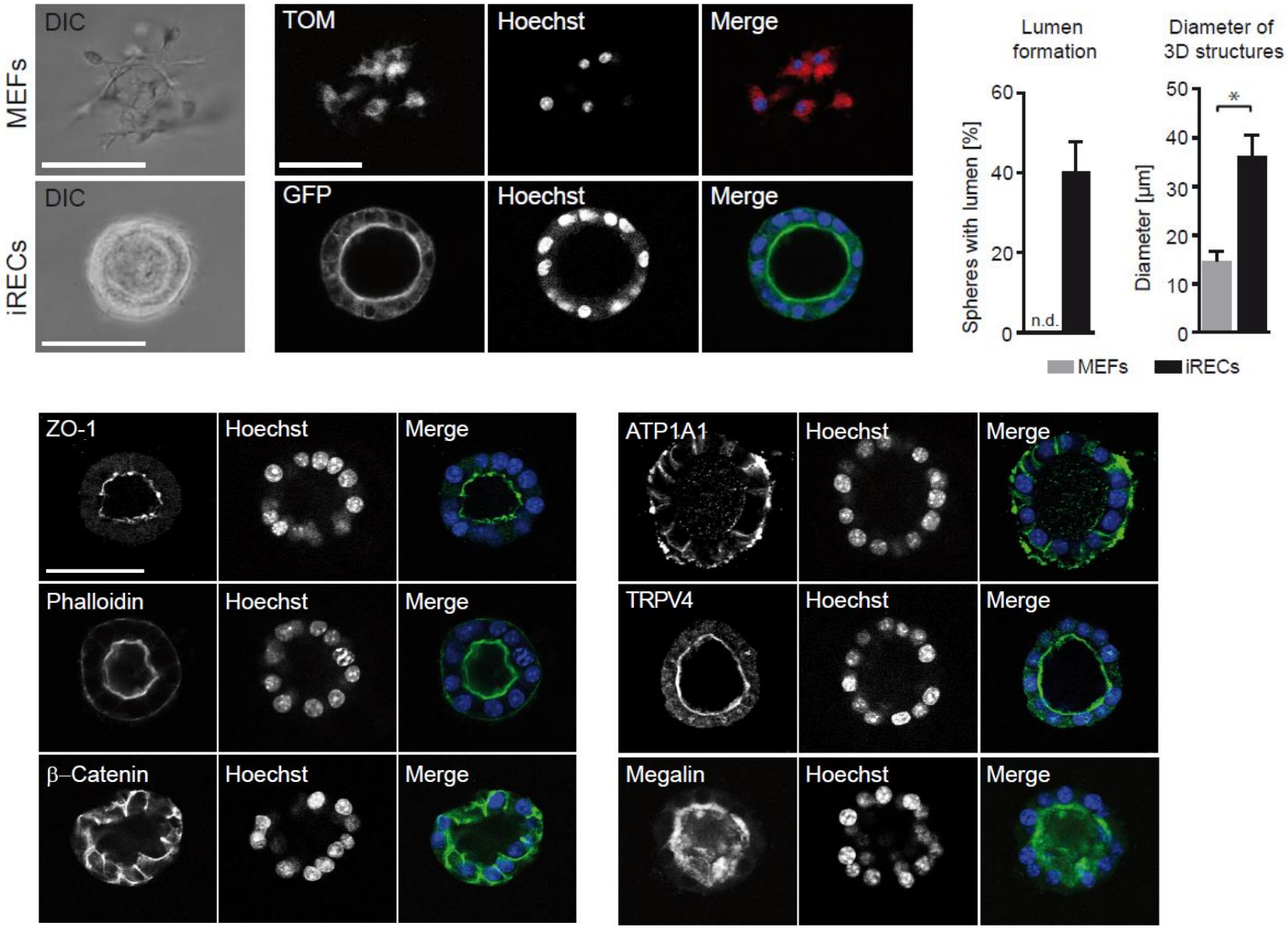
induced renal tubular epithelial cells (iRECs) have epithelial characteristics





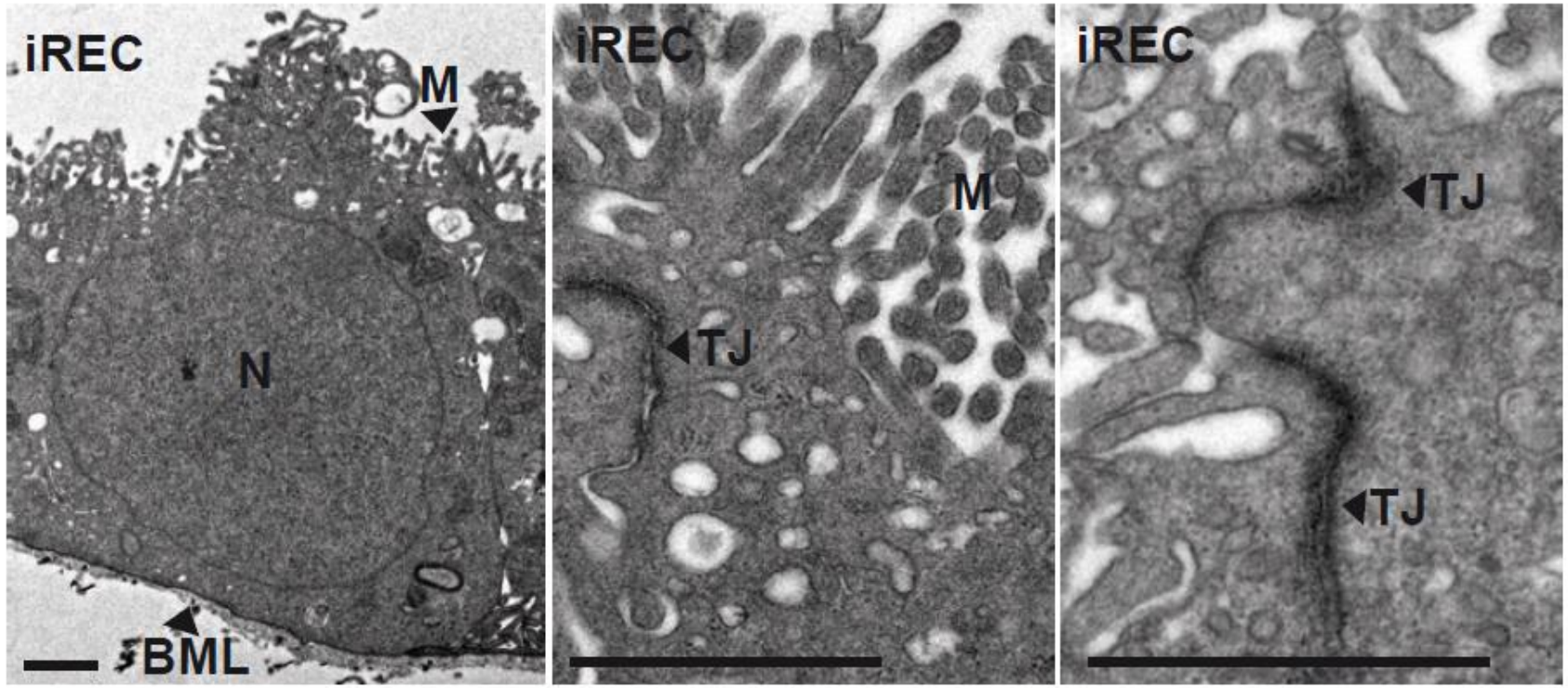
Kaminski et al., Nat Cell Bio, 2016

iRECs form polarized spheres in 3D-culture



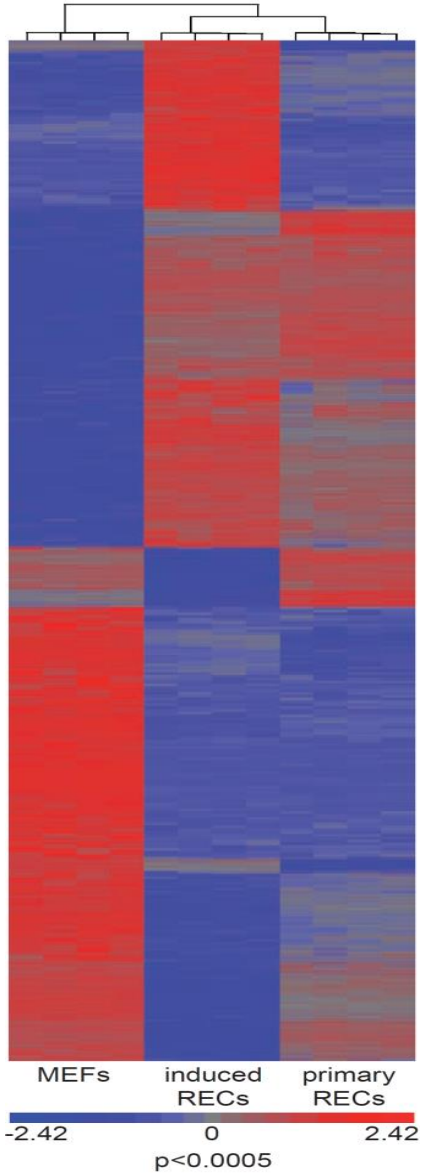
Kaminski et al., Nat Cell Bio, 2016

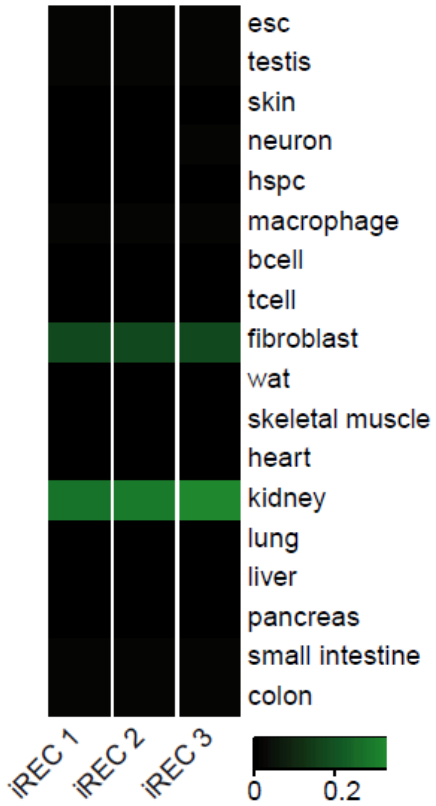
iRECs have ultrastructural features of tubule cells

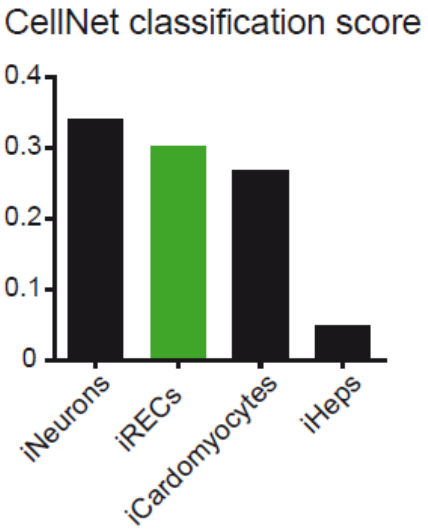


Kaminski et al., Nat Cell Bio, 2016

RNA profiling of iRECs – similar to primary tubules



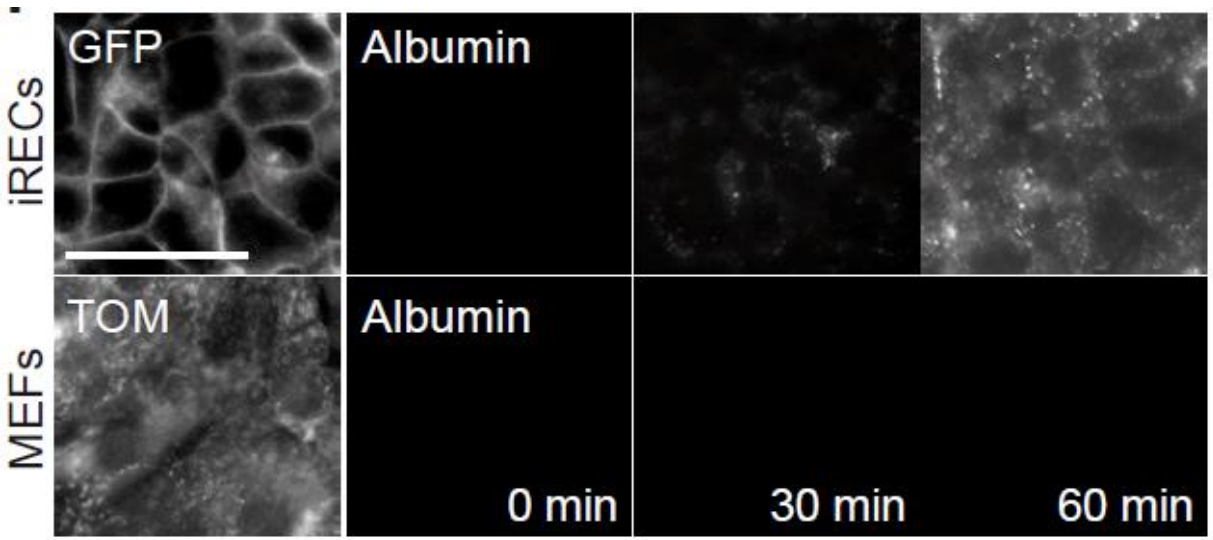


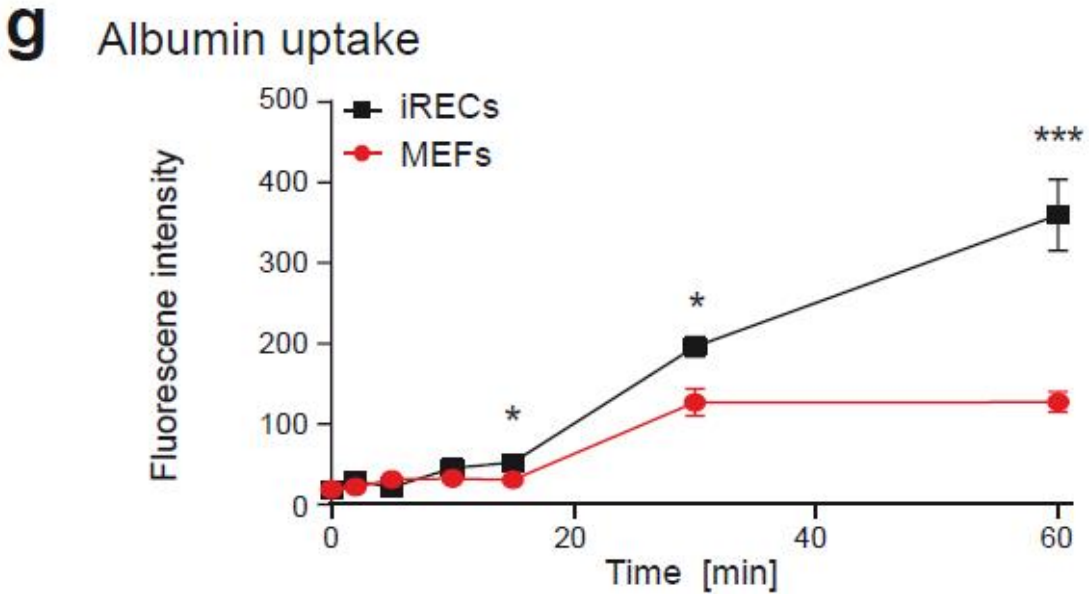


CellNet based analysis of conversion efficiency

Kaminski et al., Nat Cell Bio, 2016

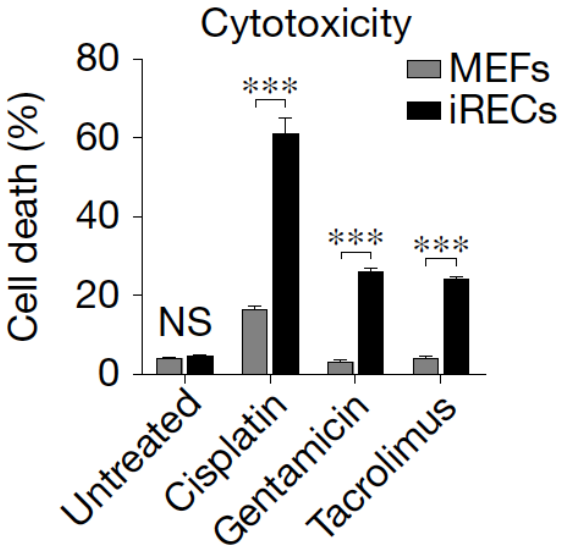
iRECs have endocytotic activity

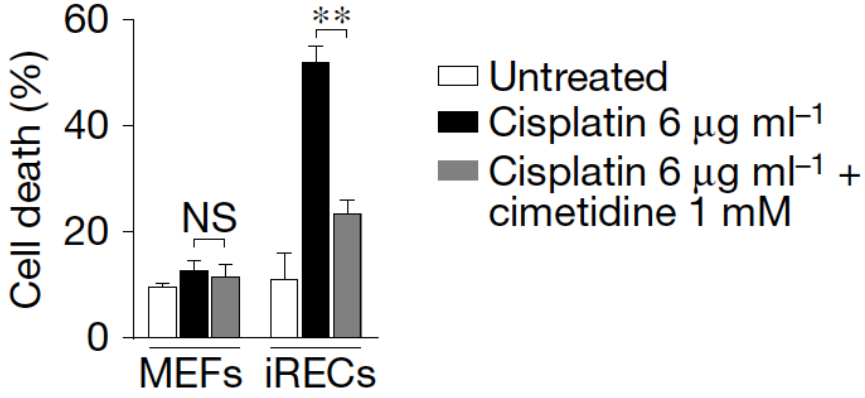


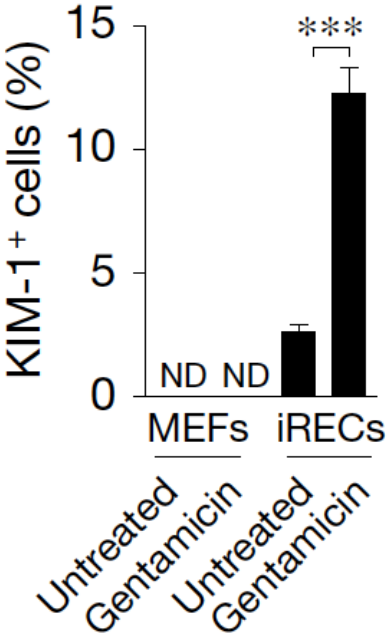


Kaminski et al., Nat Cell Bio, 2016

iRECs respond to nephrotoxic challenge

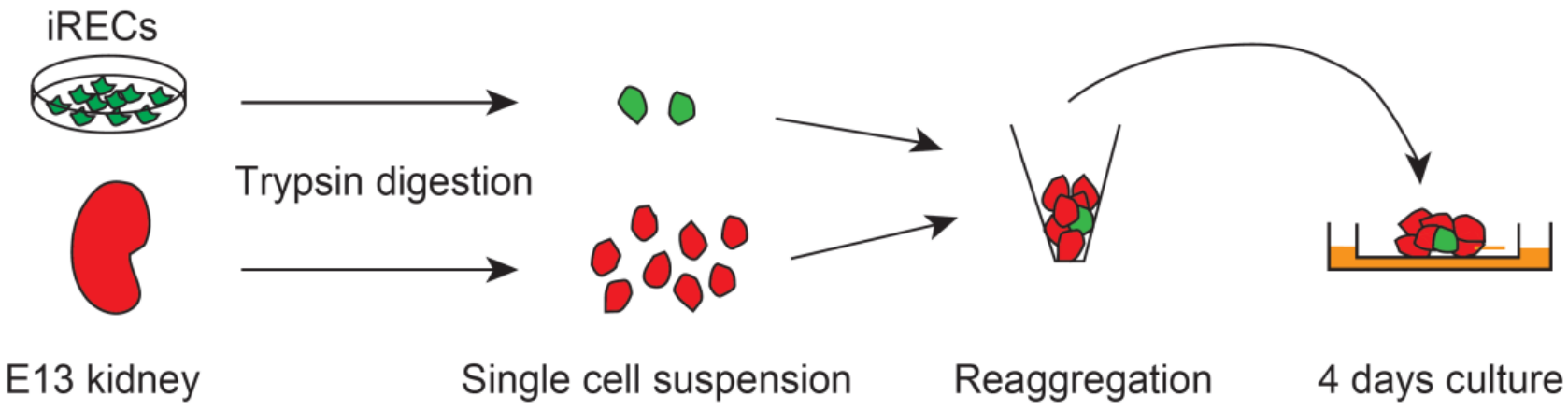


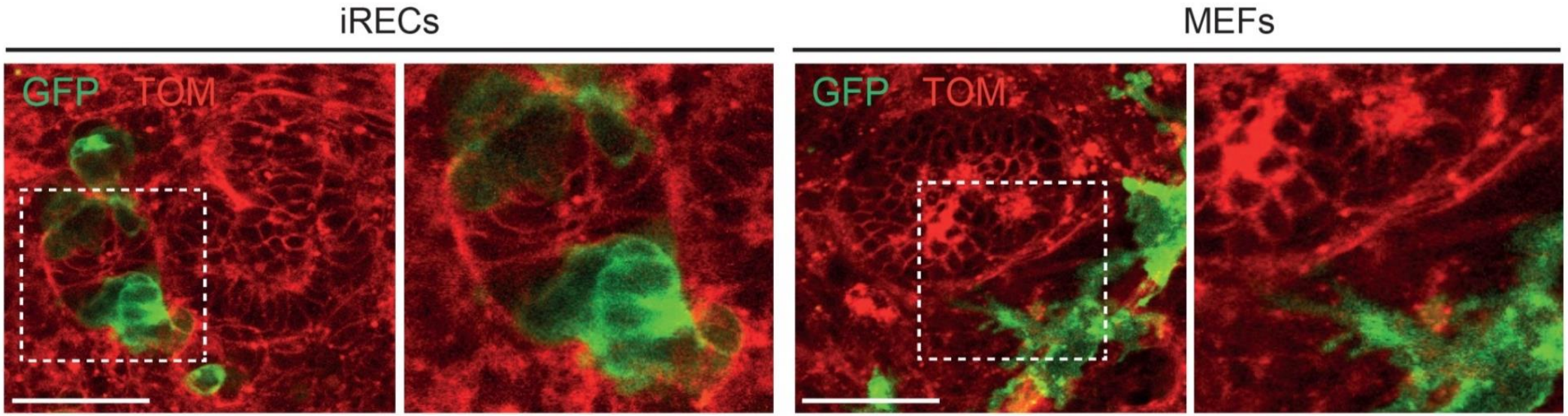




Kaminski et al., Nat Cell Bio, 2016

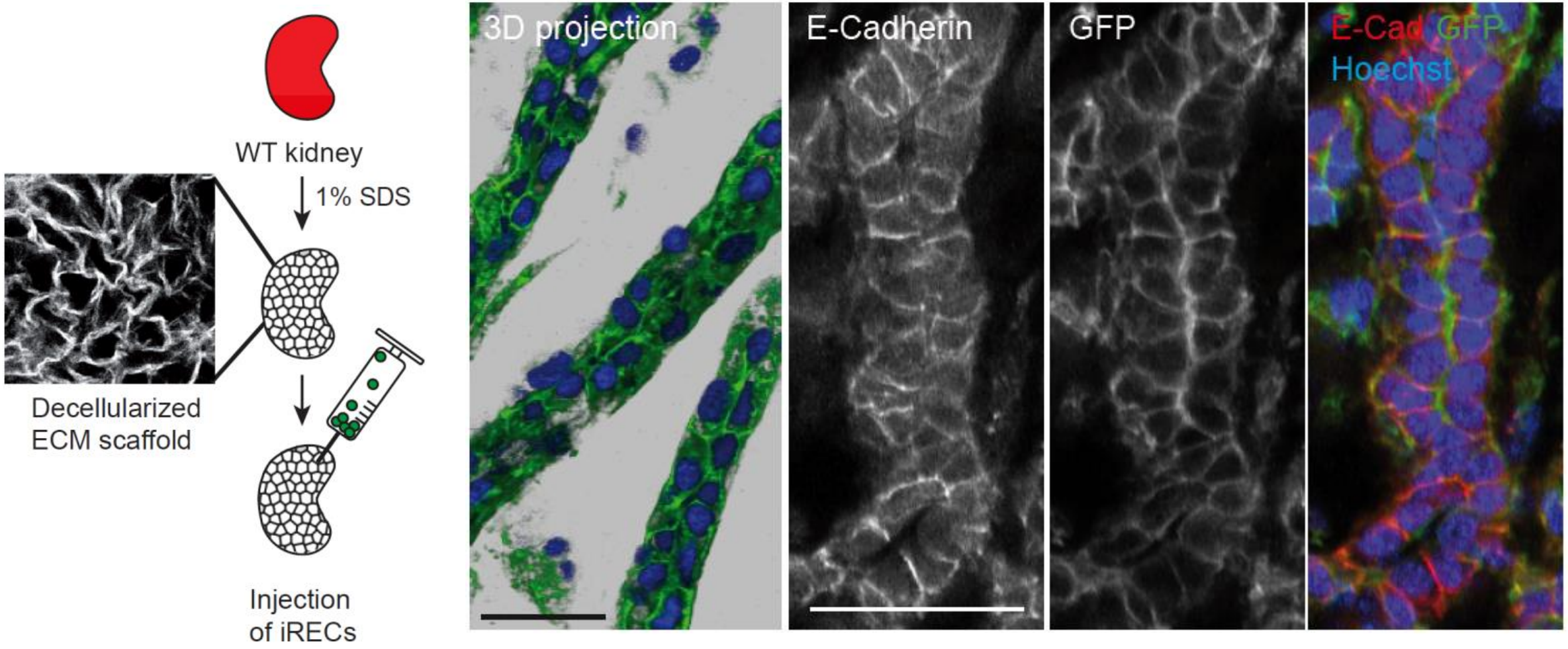
iRECs integrate into reaggregated kidneys





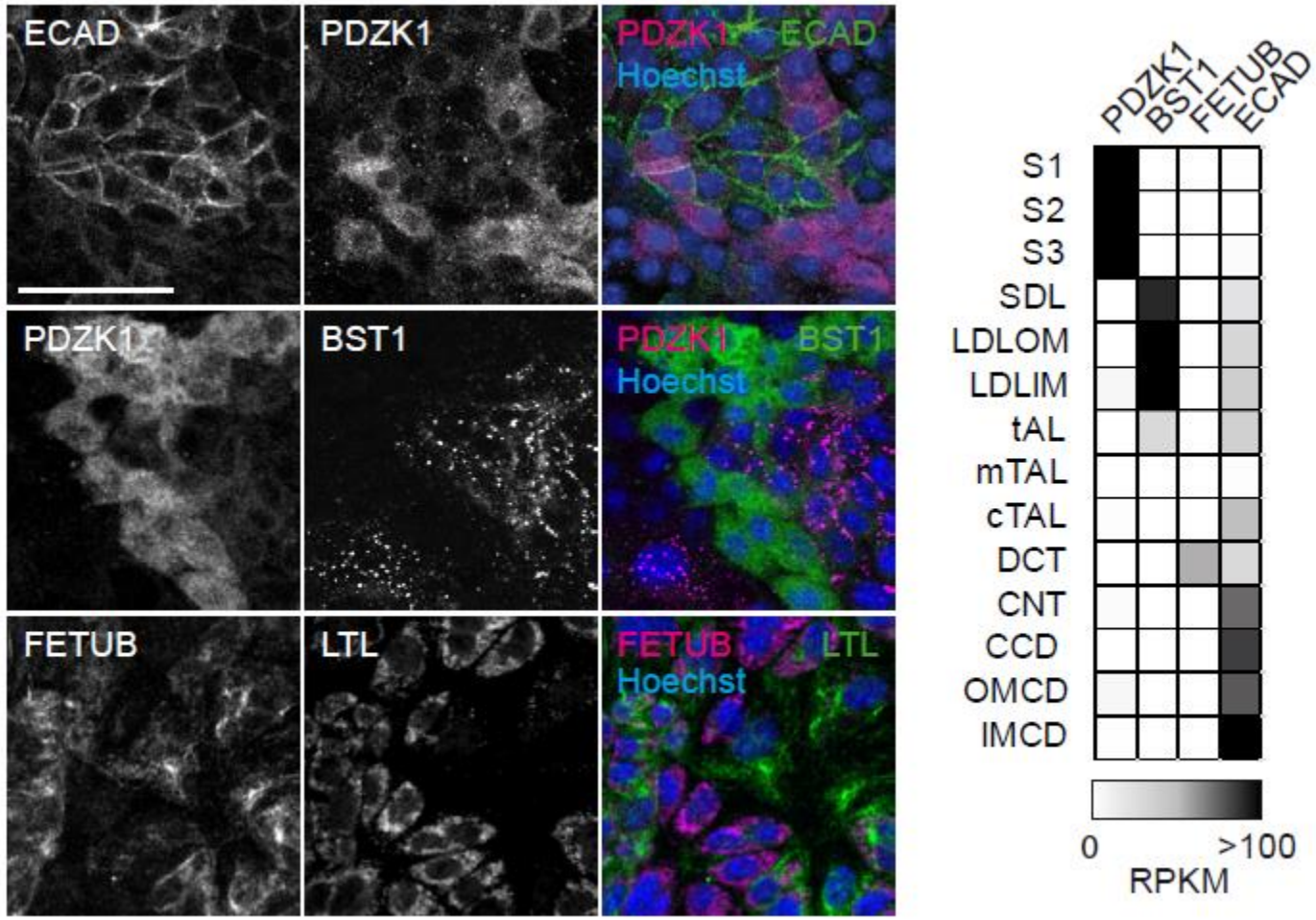
Kaminski et al., Nat Cell Bio, 2016

iRECs repopulate decellularized kidneys



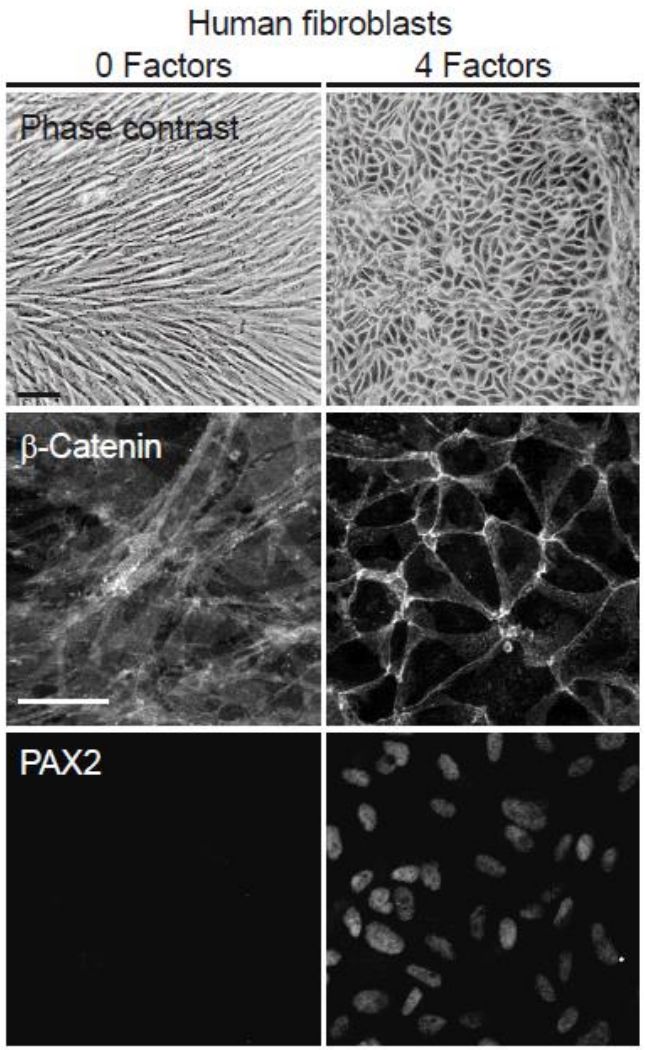
Kaminski et al., Nat Cell Bio, 2016

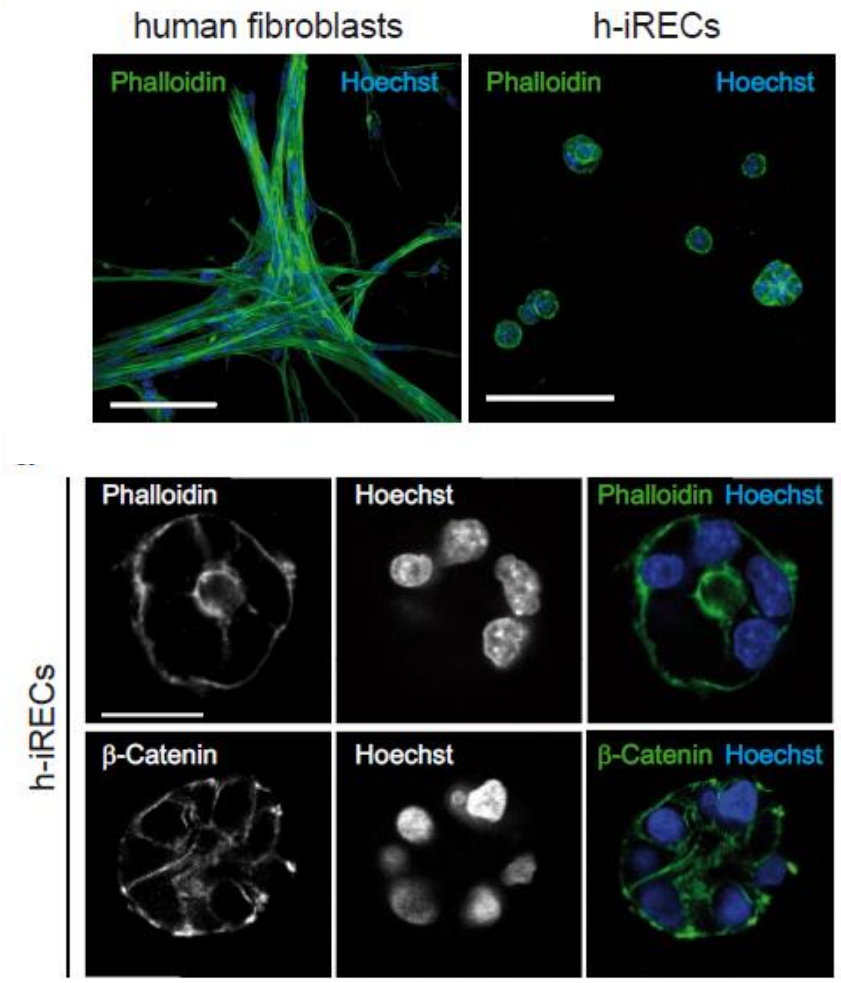
iRECs represent multiple tubule segments



Kaminski et al., Nat Cell Bio, 2016

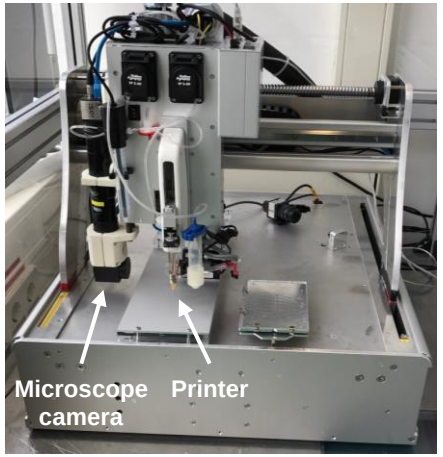
Reprogramming of human fibroblasts (h-iRECs)

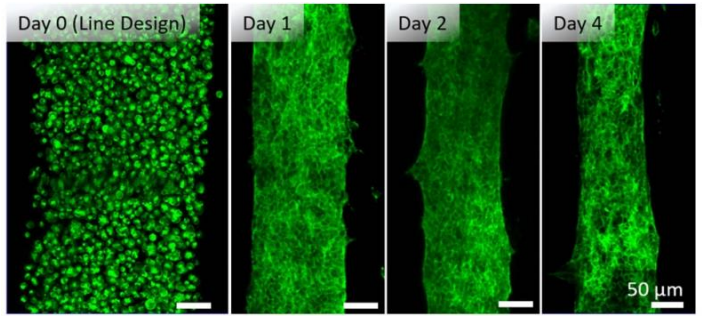


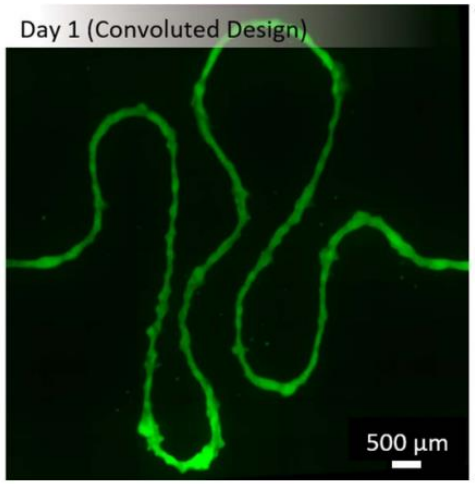


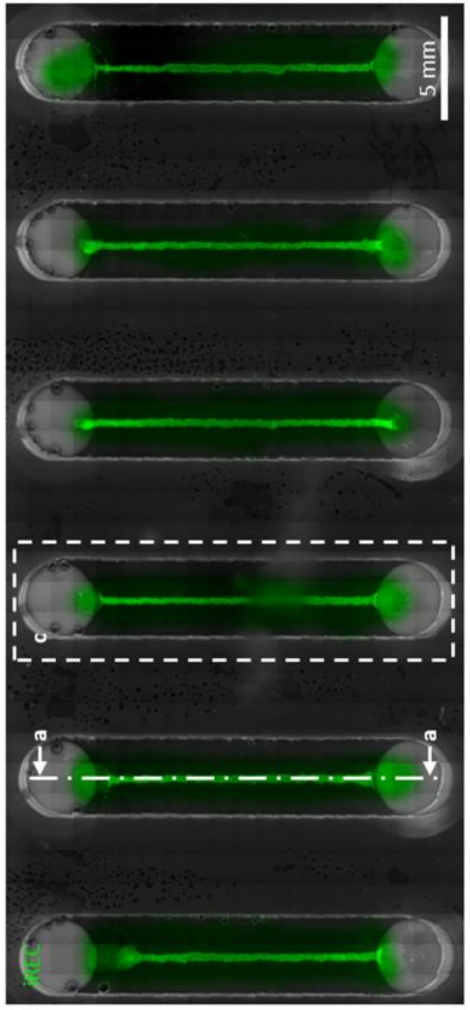
Kaminski et al., Nat Cell Bio, 2016

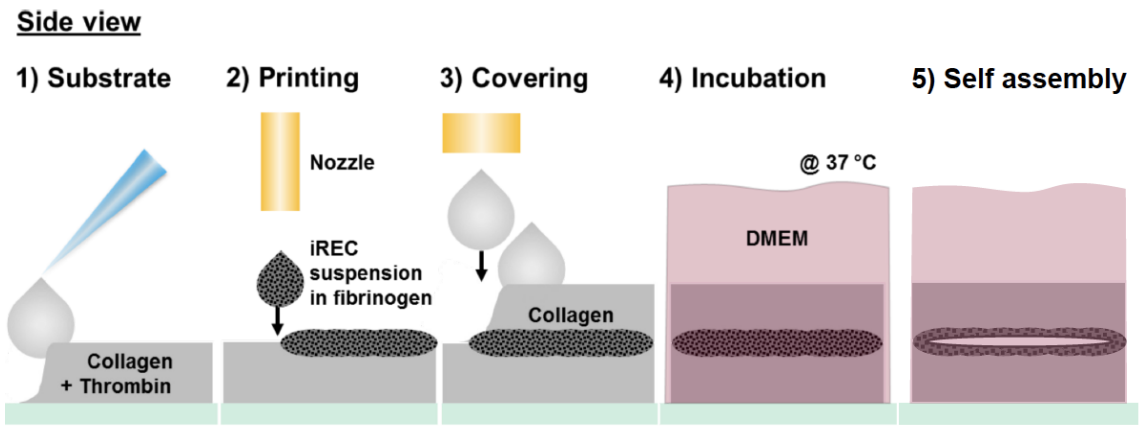
Bioprinting renal tubules

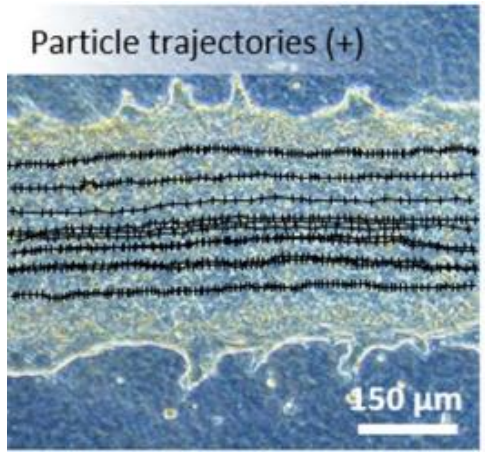








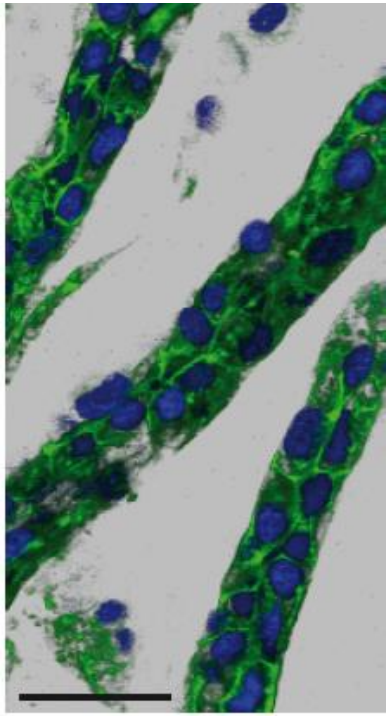




Kollaboration IMTEK, Uni Freiburg,
Peter Koltay, Stefan Zimmermann, Kevin Tröndle

Tröndle, et al. Biofabrication, 2021

Conclusions



- Regenerative Nephrology is a field of active research

- iPSC based kidney organoids
  - Replicate embryonic renal development
  - Contain all major cell types of the kidney
  - Issues of maturity and vascularization remain to be solved

- Direct reprogramming to renal tubular epithelial-like cells (iRECs)
  - Omits prior pluripotency
  - Generates primarily tubular cells that replicate tubule formation *in vitro*
  - Issues of segment specificity and optimal culture environments remain to be solved

- Dialysis / Transplants are unlikely to be replaced anytime soon

Acknowledgements

initial iREC project:

Michael Kaminski

Sebastian Arnold (Pharmakology, Freiburg)

Jelena Tosic (Pharmakology, Freiburg)

Gerd Walz

Team Zürich:
Maike Getwan
Thomas Naert
Ruth Röck
Julia Traversari
Kelli Grand
Ludovica Rizzo
Paulina Ogar
Daniel Invernot-Perez
Melanie Horn
Daniel Prata

Team Freiburg:

Roman Pichler

Martine Stolz

Alena Sammarco

Amos Spielmann

Angelina Bogus

Collaborators:
Anna Köttgen, Anselm Hoppmann, Pascall Schlosser (Genetische Epidemiolgie, Freiburg)
Ekkehart Lausch (Uni-Kinderklinik Freiburg)
Friedhelm Hildebrandt, Konstatin Deutsch (Boston Childrens's Hospital, Harvard Medical School, MA)
Thomas Brox, Ösgün Cicek (Dept. Computer Sciences, Freiburg)
Fritjof Helmchen, Fabian Voigt (Brain Research Center, UZH)
Johannes Loffing, Max Bürgi (Institute of Anatomy, UZH)

Emmy Noether-Programm
Deutsche Forschungsgemeinschaft DFG

KIDGEM
SFB 1140

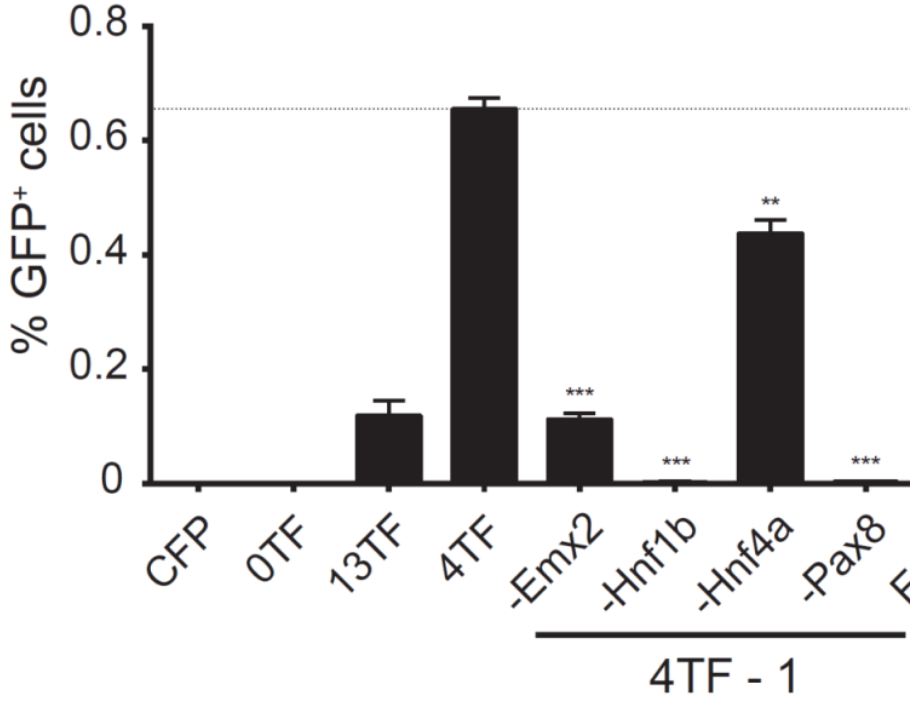
KIDNEY CONTROL OF HOMEOSTASIS
SWISS NATIONAL CENTRE OF COMPETENCE IN RESEARCH

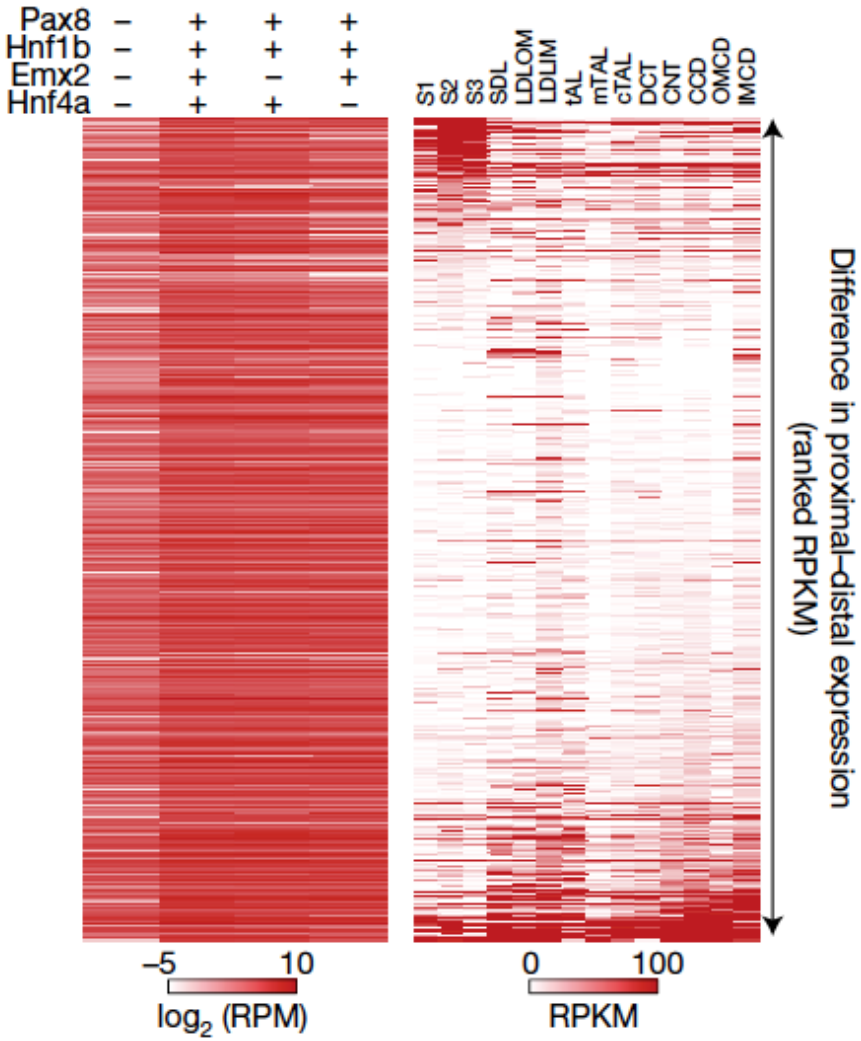
erc

European Commission

FNSNF
FONDS NATIONAL SUISSE
SCHWEIZERISCHER NATIONALFONDS
FONDO NAZIONALE SVIZZERO
SWISS NATIONAL SCIENCE FOUNDATION

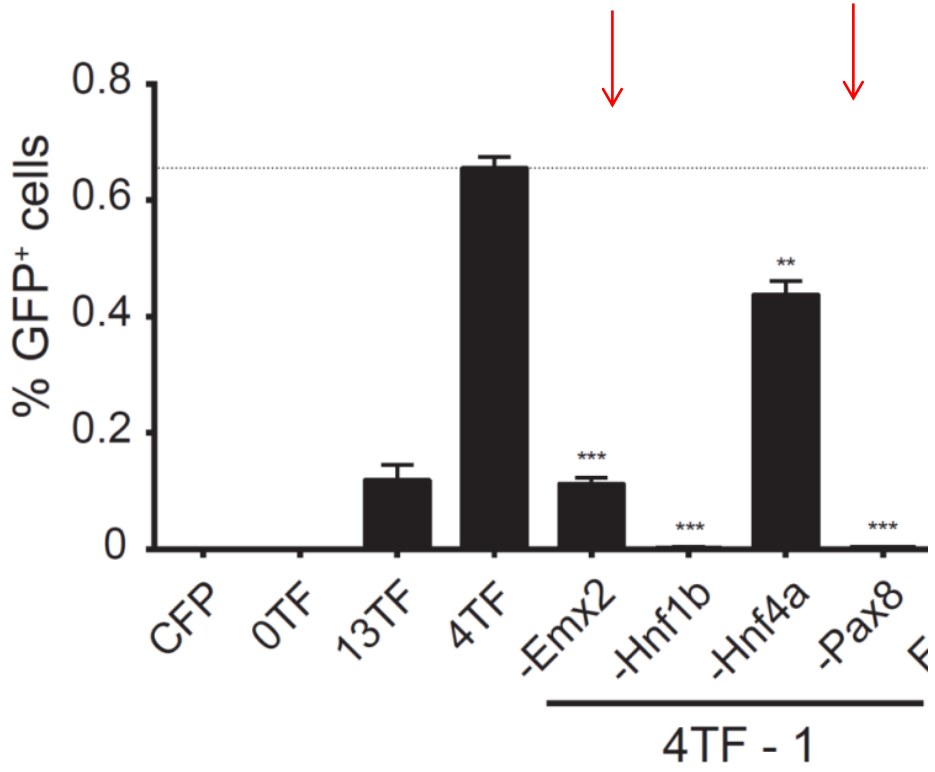
HNF4A confers proximal segment identity

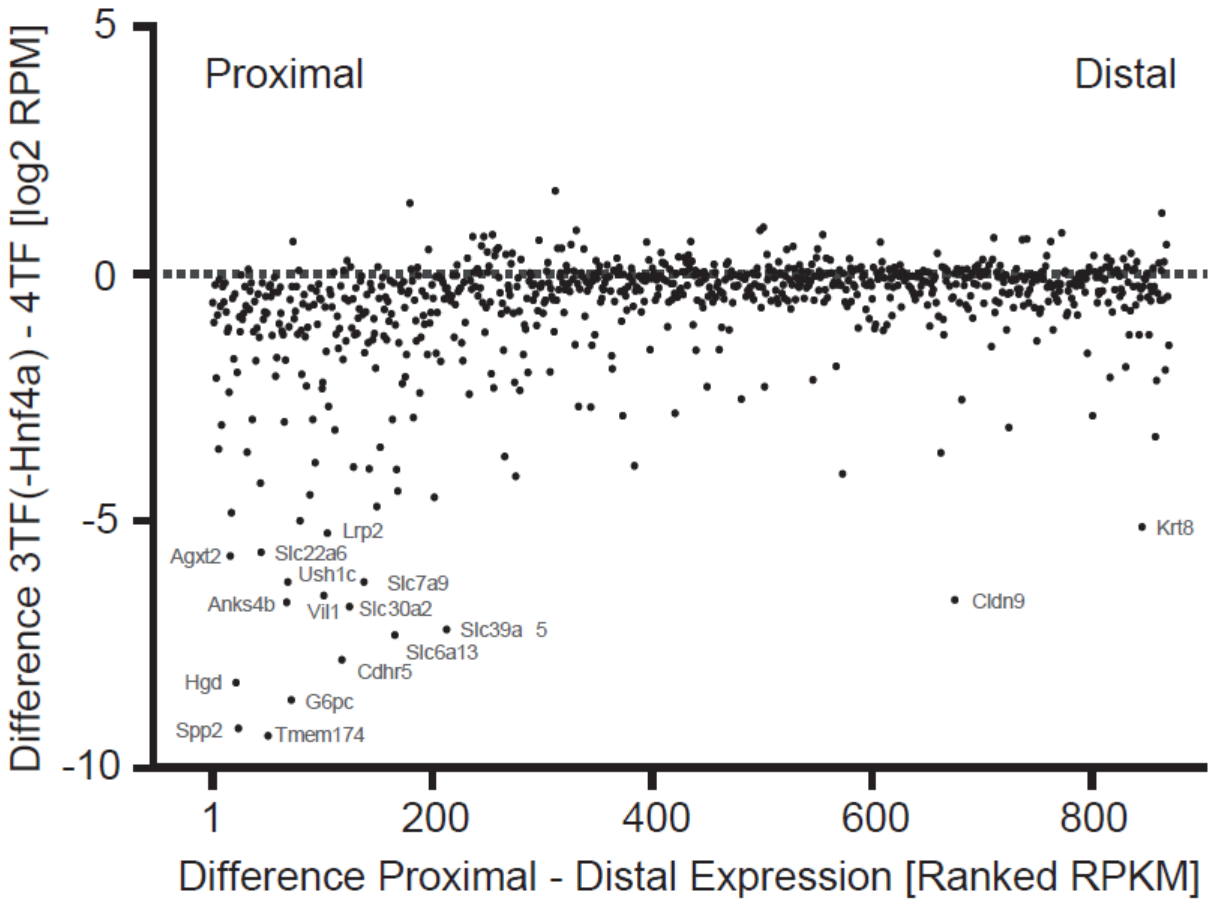




Kaminski et al., Nat Cell Bio, 2016

HNF4A confers proximal segment identity





Kaminski et al., Nat Cell Bio, 2016