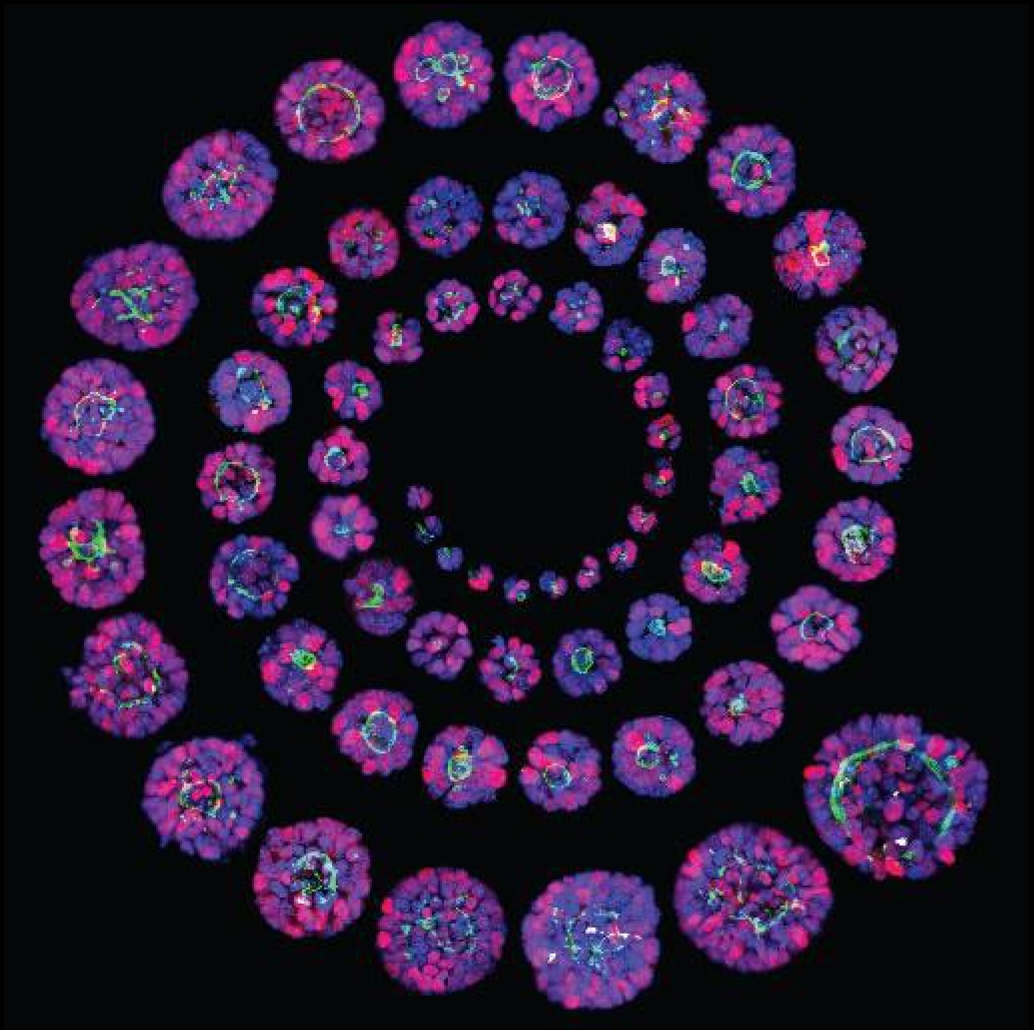
Organoides pancréatiques, modèles du développement humain normal et pathologique

Image credit
@Phil Seymour



MAX-PLANCK-GESELLSCHAFT

CBG
Max Planck Institute of Molecular Cell Biology and Genetics

Anne Grapin-Botton (MPI-CBG, Dresden)
GDR Organoides 2 decembre 2022

Organoids at MPI-CBG, Dresden

- Early stages of development
- From pluripotent stem cells


Jesse Veenvliet


Claudia Gerri

- Organogenesis
- From pluripotent stem cells
- From foetal cells


Anne Grapin-Botton


Wieland Huttner


Alf Honigmann

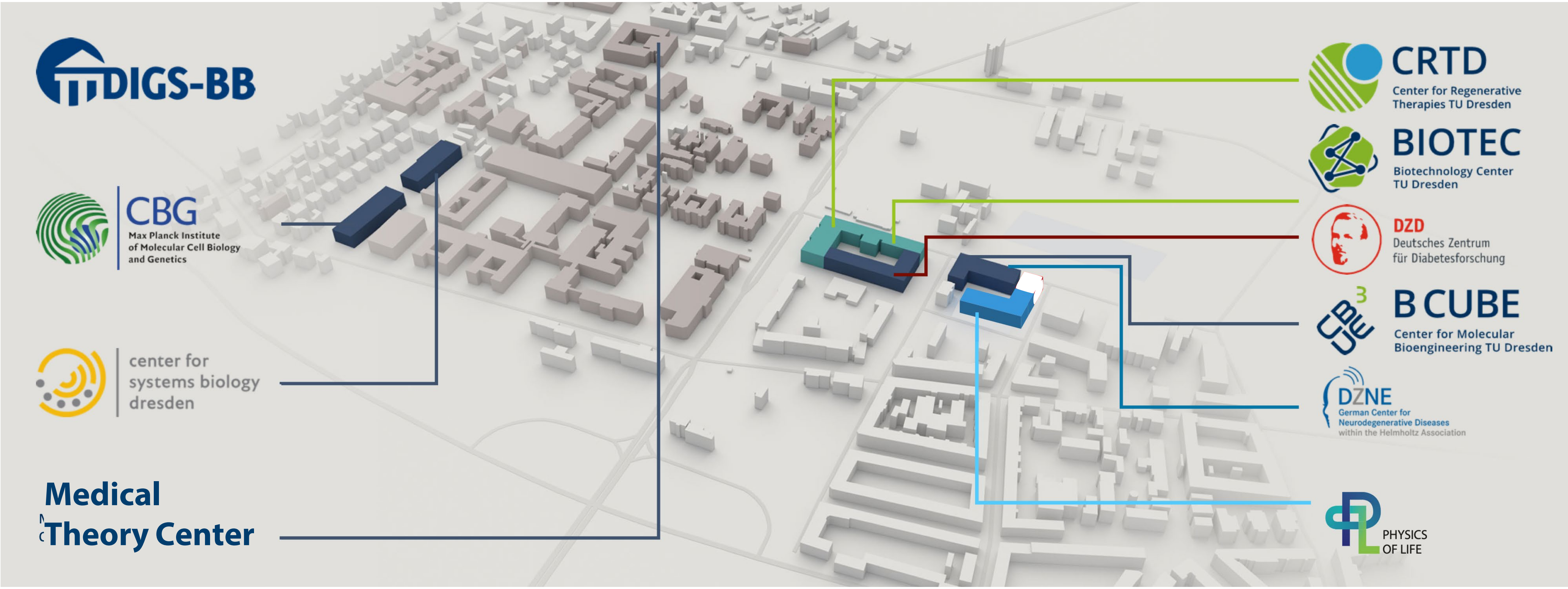
- Regeneration and disease
- From adult cells


Meri Huch

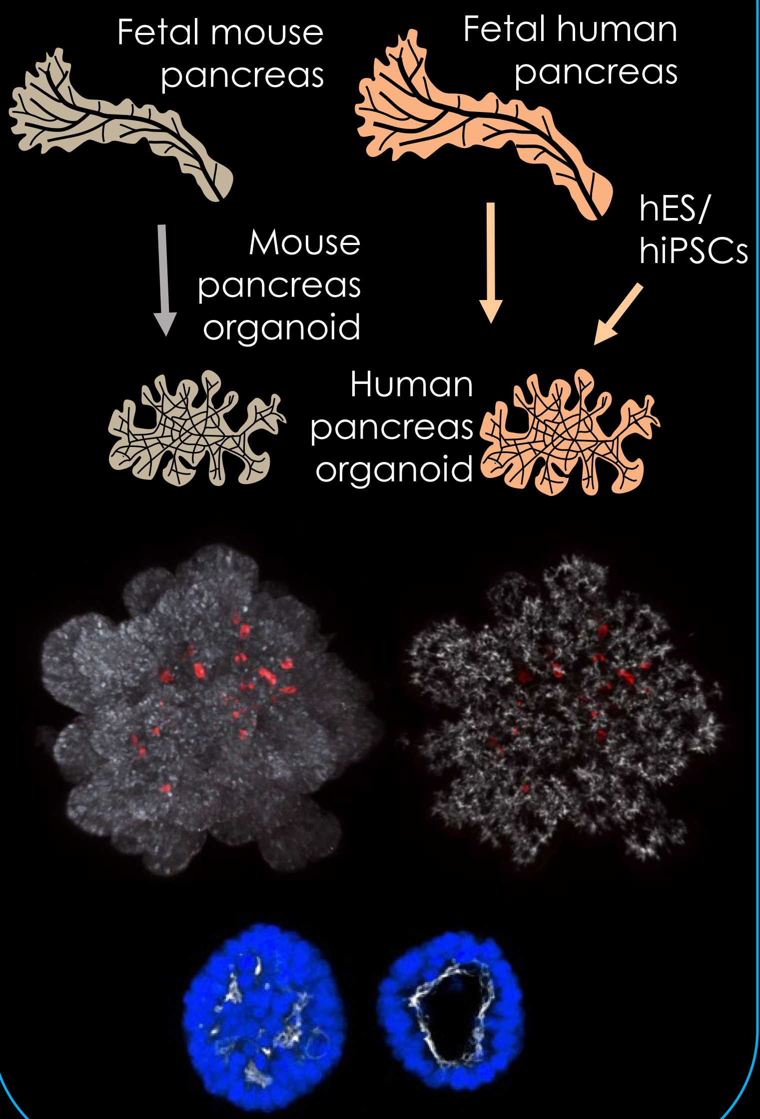
- Stem cell & organoid facility

Christina Eugster-Oegema
Organoid and Stem Cell Facility

Technische Universität Dresden

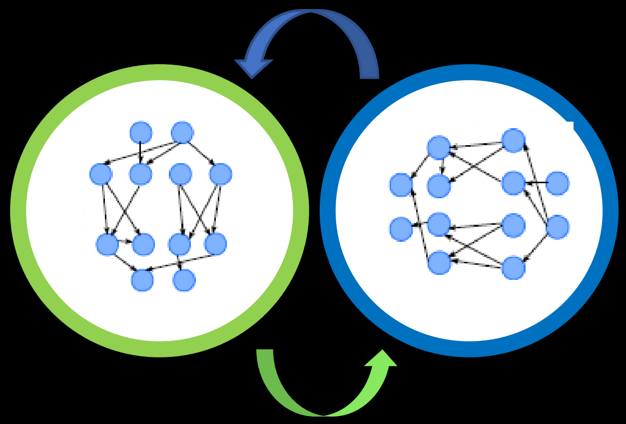


System development



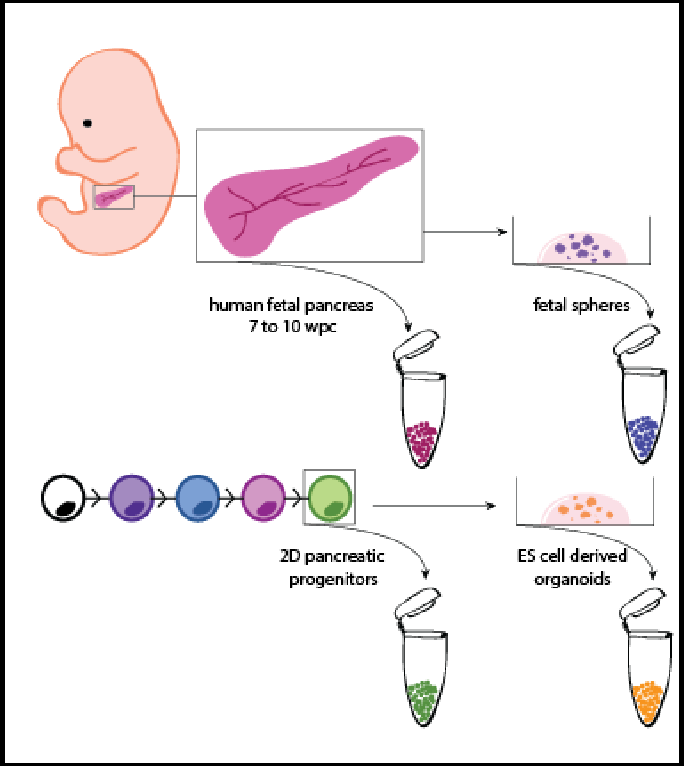
Self-organization

- Initial conditions
- Feedback loops
- External control



- Morphogenesis
- Tissue dynamics
- Tissue mechanics

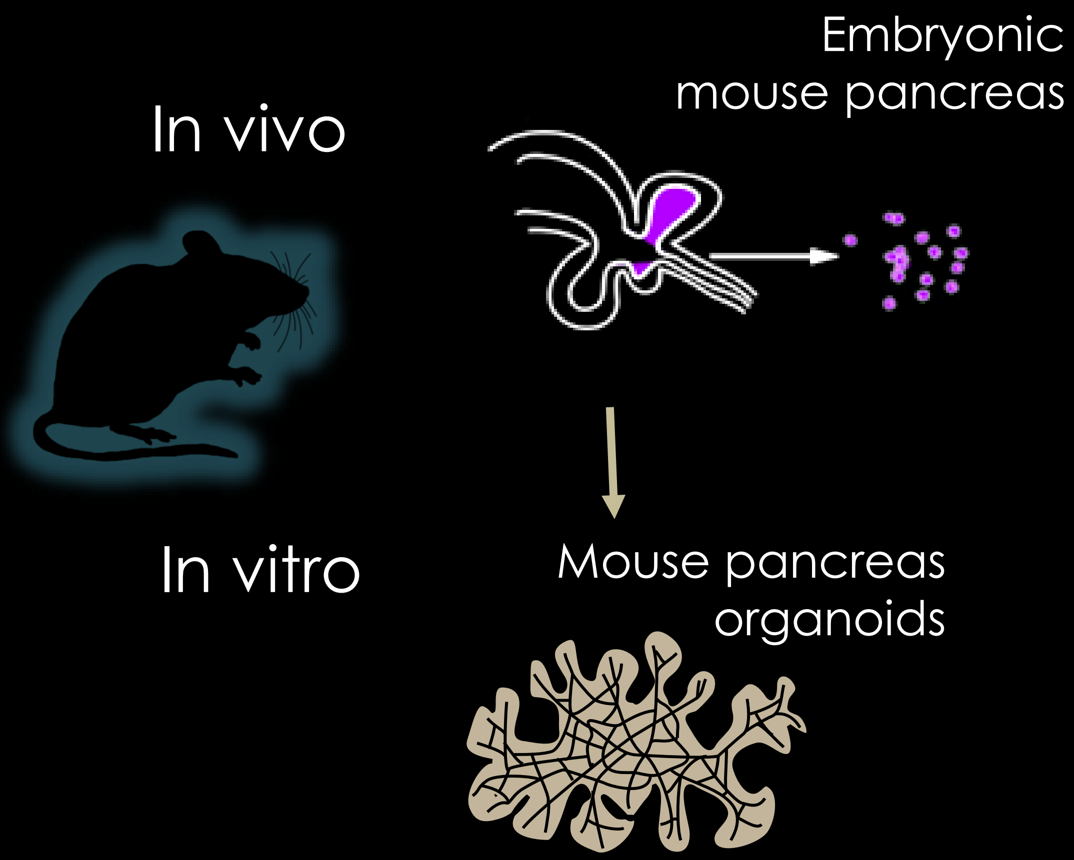
Human development and diabetes



- Human development
- GWAS functional studies

Pancreas development

Mouse pancreas organoids

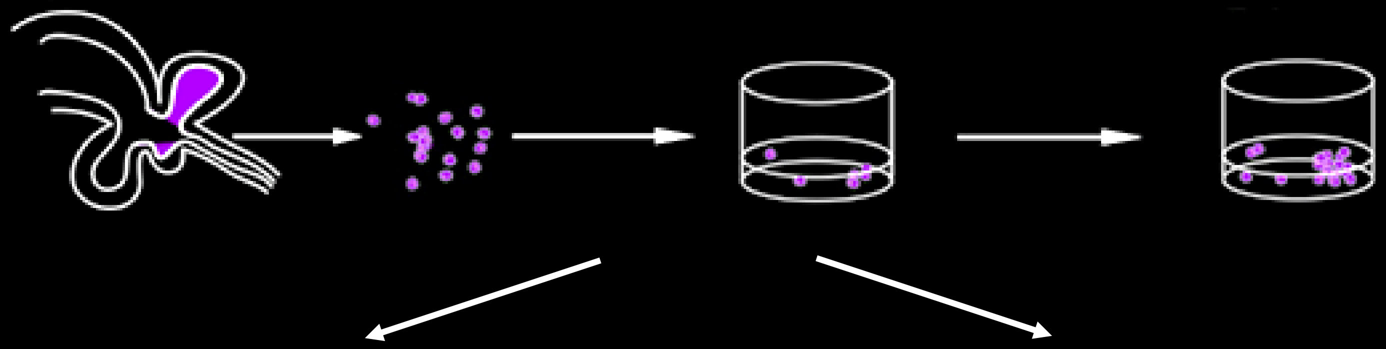


Greggio et al., Development, 2013
Dahl-Jensen et al., PLOS Biology 2018
Flasse et al., Cell Reports 2020

Why do we use organoids to study development?

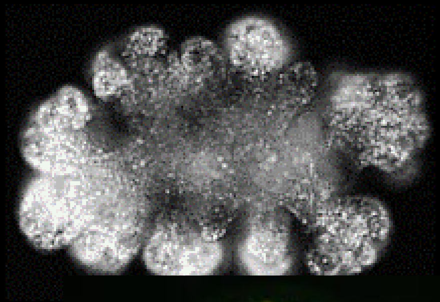
To control cell assemblies and external signals to understand emergent properties

From mouse pancreas progenitors to spheres and organoids: different media



"Organoid" medium

Pdx1-nGFP

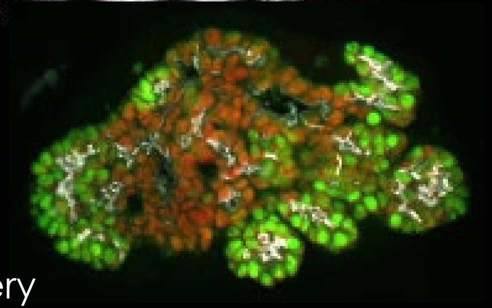


EGF
RSPO1
FGF1
FGF10
Y-27632
KOSR

Progenitors in the center

Network of ducts

Acinar cells at the periphery



Day 7

"Sphere" medium

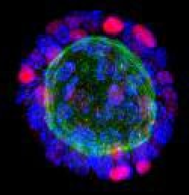
FGF2
Y-27632
B27

Phase

Pdx1-nGFP

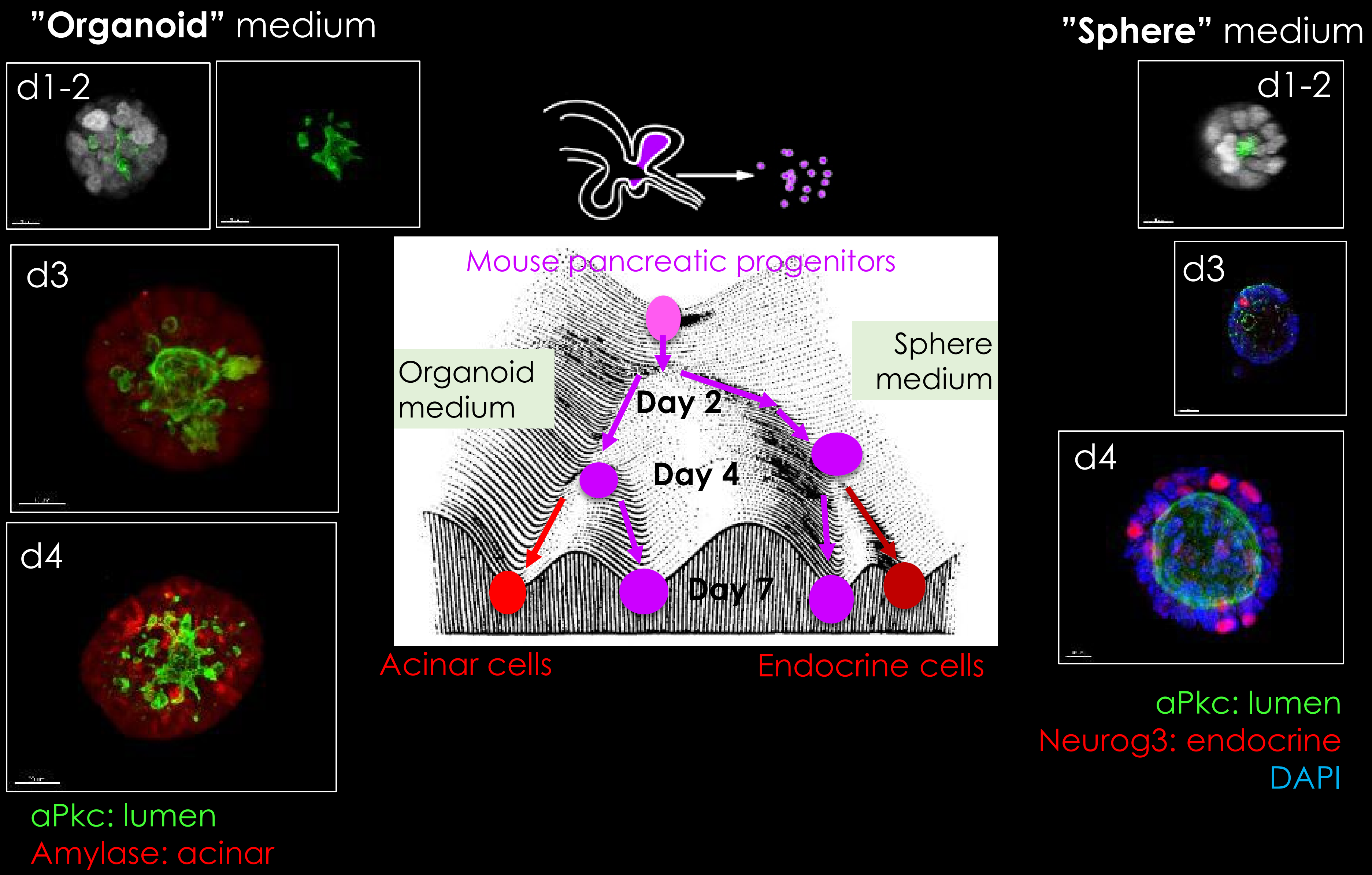
Day 7

aPKC (apical)
Neurog3 (endocrine)
DAPI (nuclei)

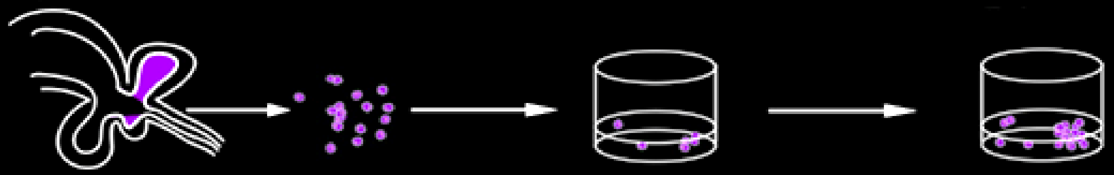


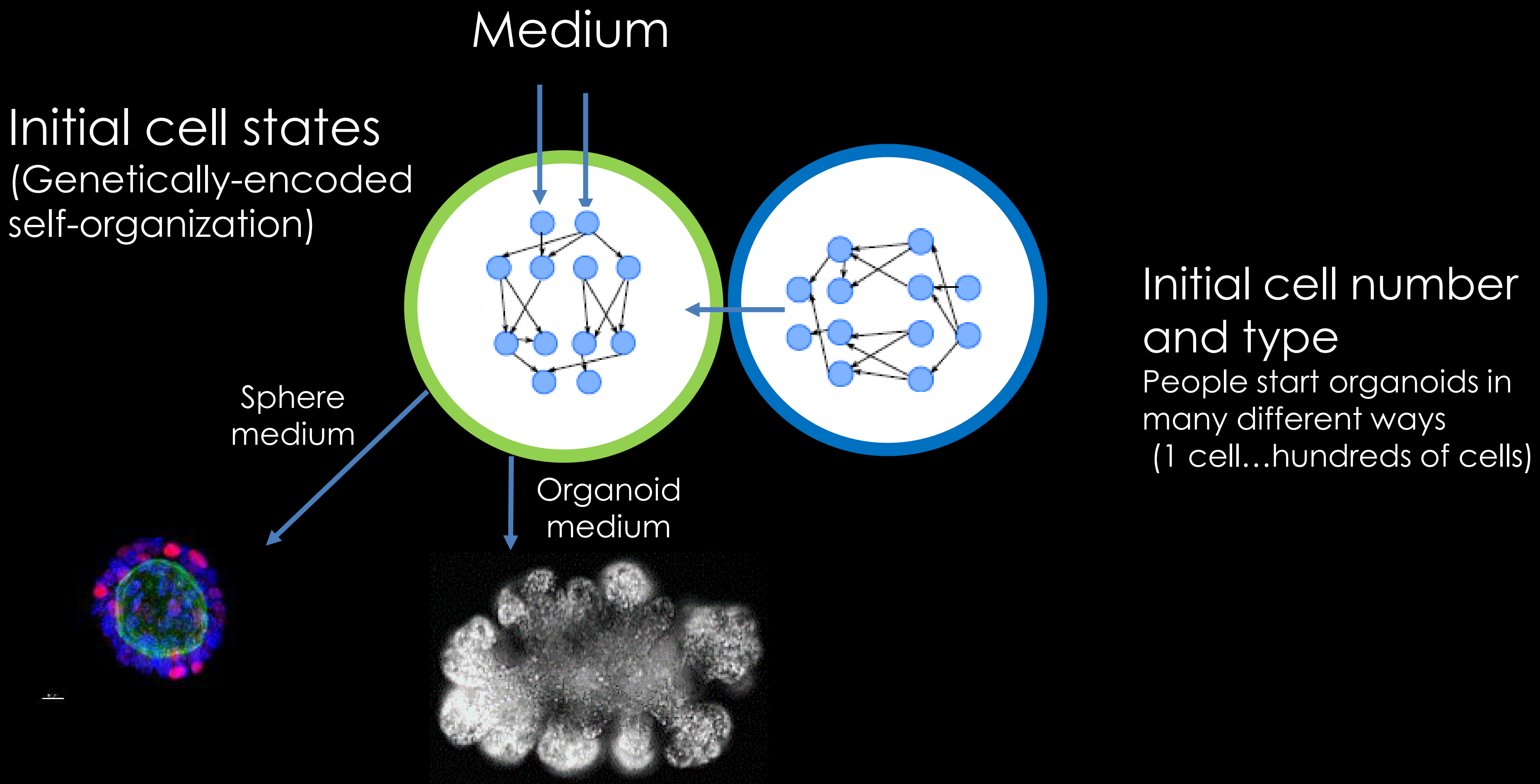
Greggio et al., Development, 2013

Same initial cells, different trajectories controled by the medium



Control over the initial cell assemblies to control self-organization



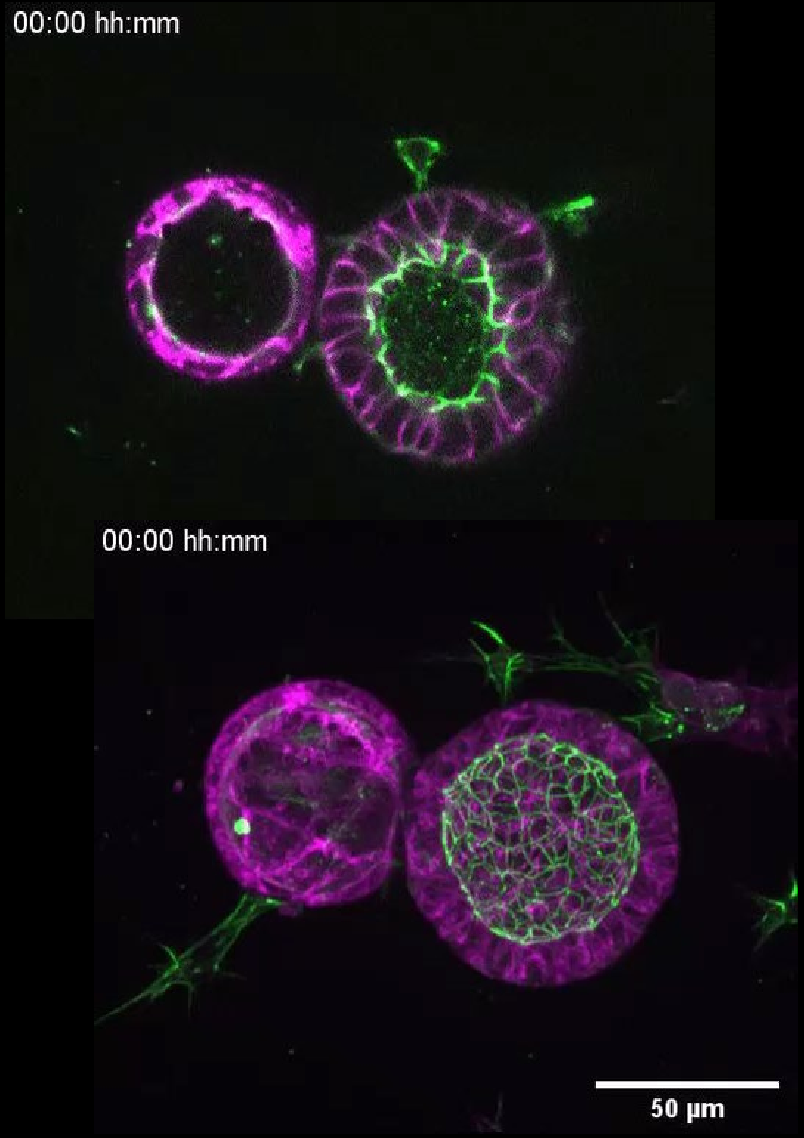


Why do we use organoids to study development?

To control cell assemblies and external signals to understand emergent properties

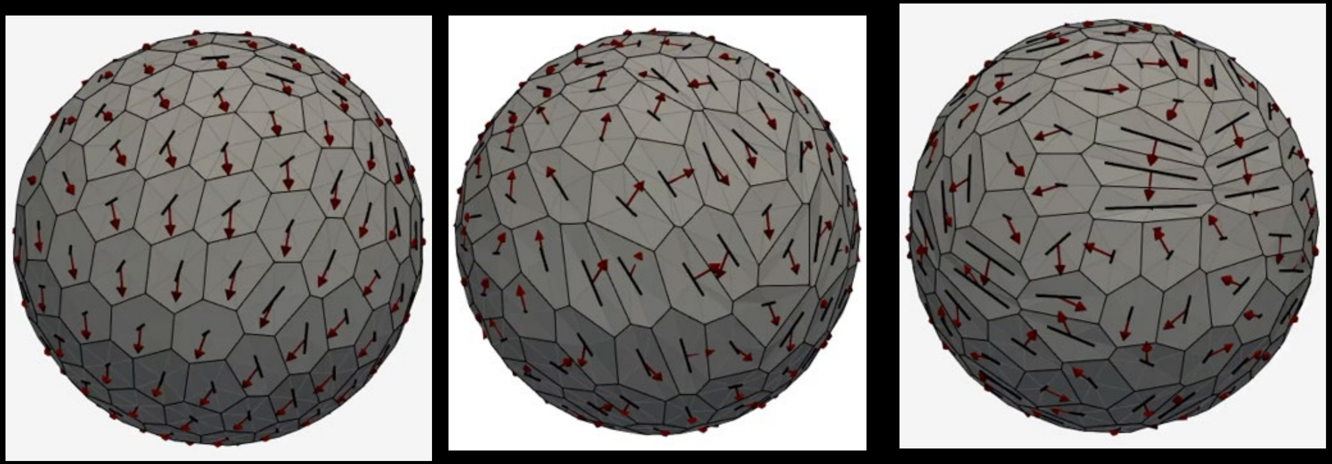
To study the mechanical control of development

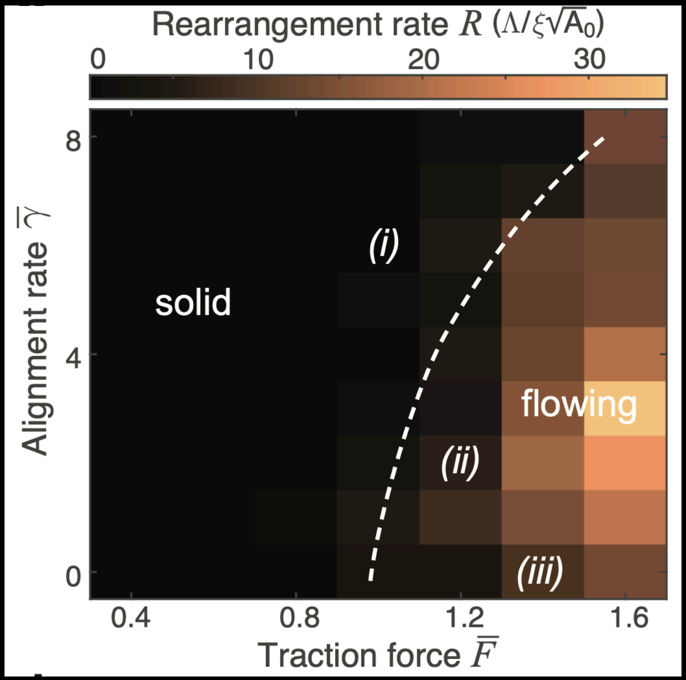
Live imaging reveals collective polarized movements in 3D spheres



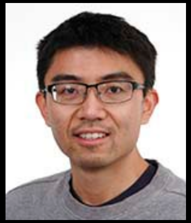
SiR-Actin; Membrane (mT/mG)

Tqn et al., BioRxiv 2022





Irene Seijo Barandiaran

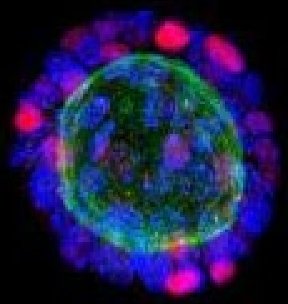


Tzer Han Tan

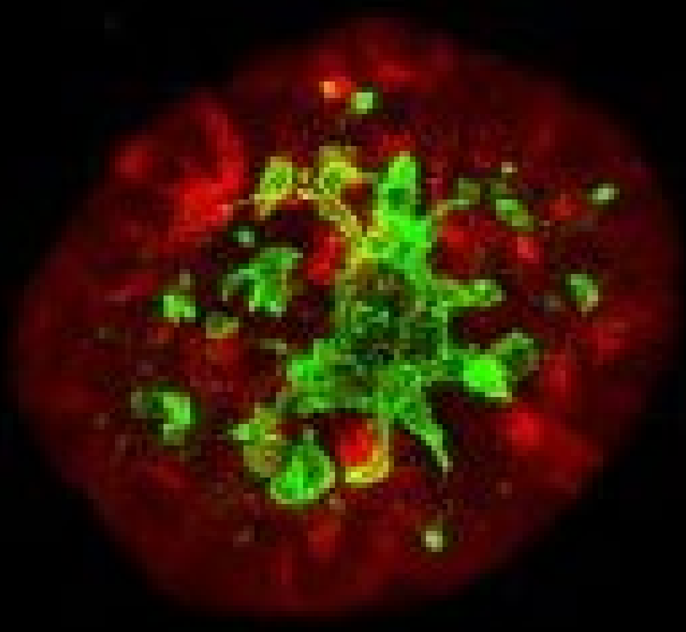
Aboutaleb Amiri
Frank Jülicher

Formation of the ductal network

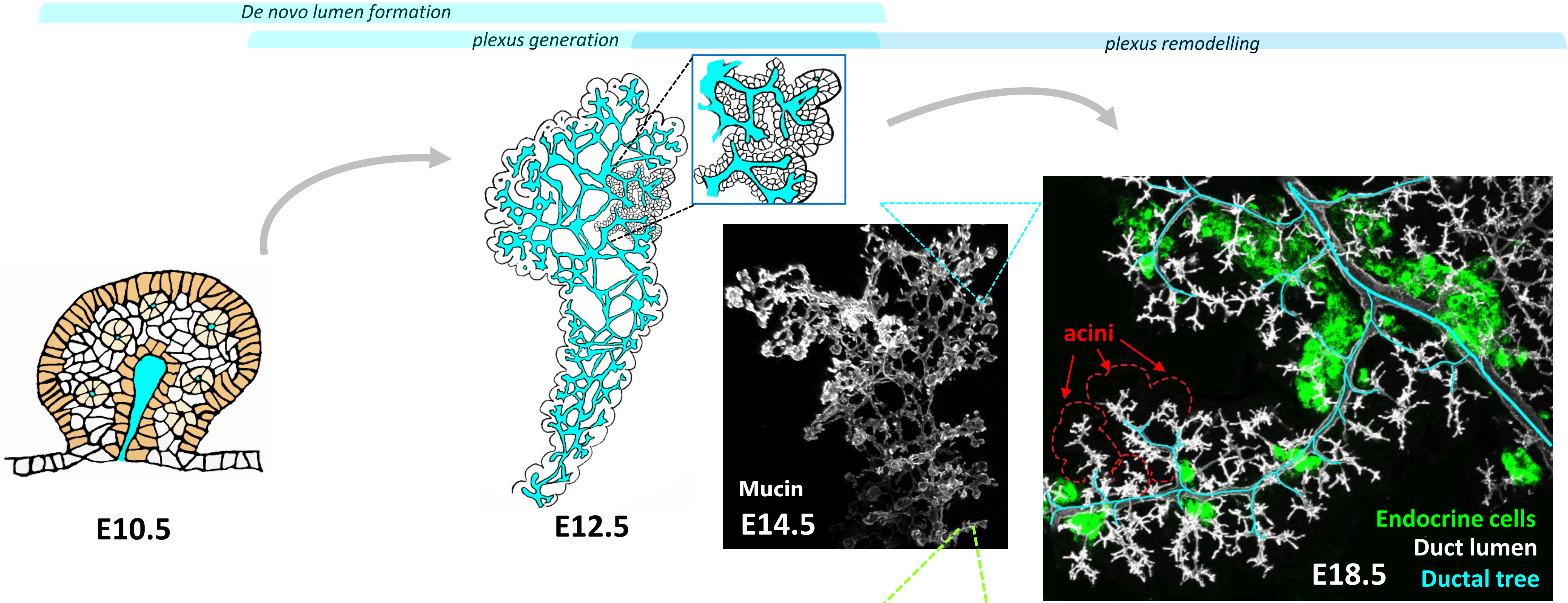
One lumen or many?
Spherical lumen or network?

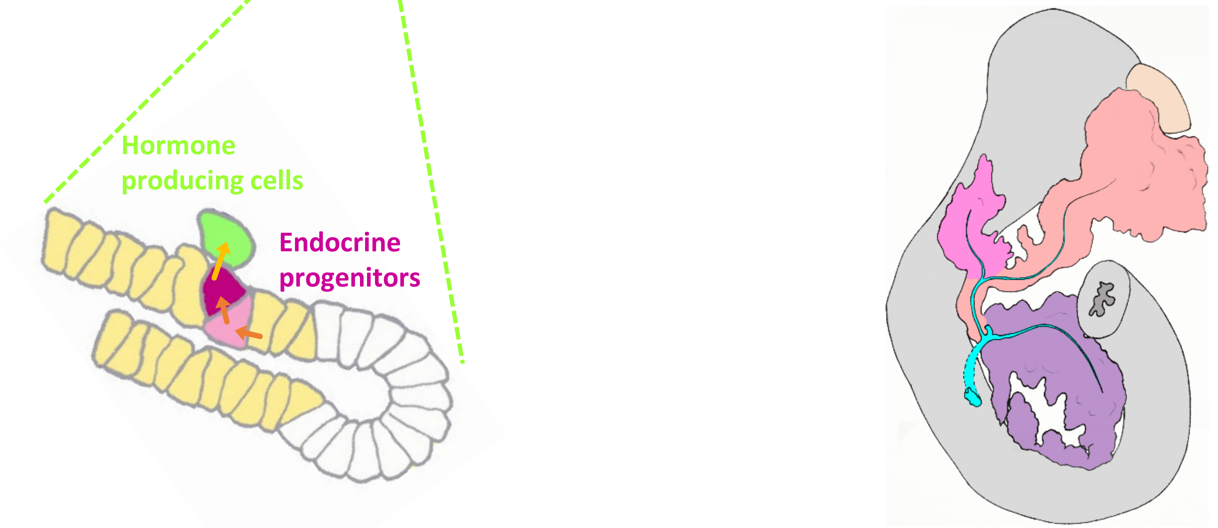


aPKC (apical)
Neurog3
(endocrine)
DAPI (nuclei)



aPKC (apical)
Amylase
(acinar)





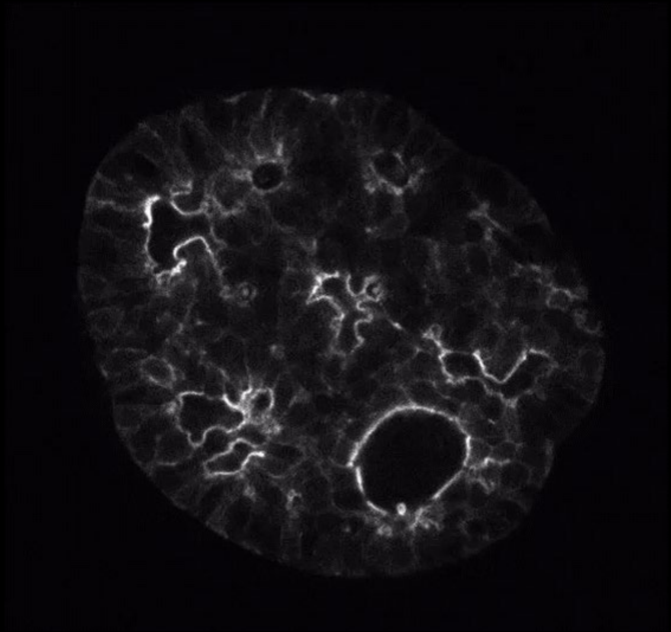
Formation of the ductal network

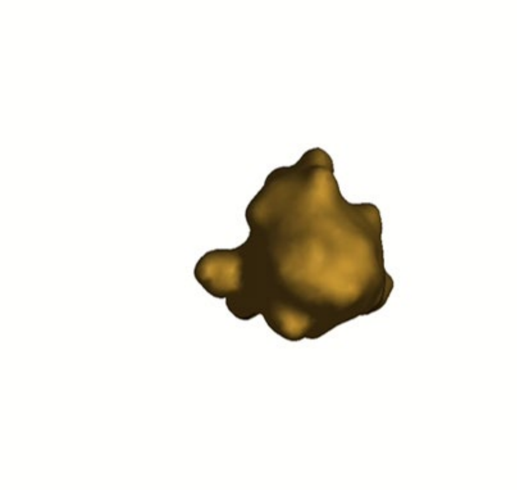
Formation of the ductal network

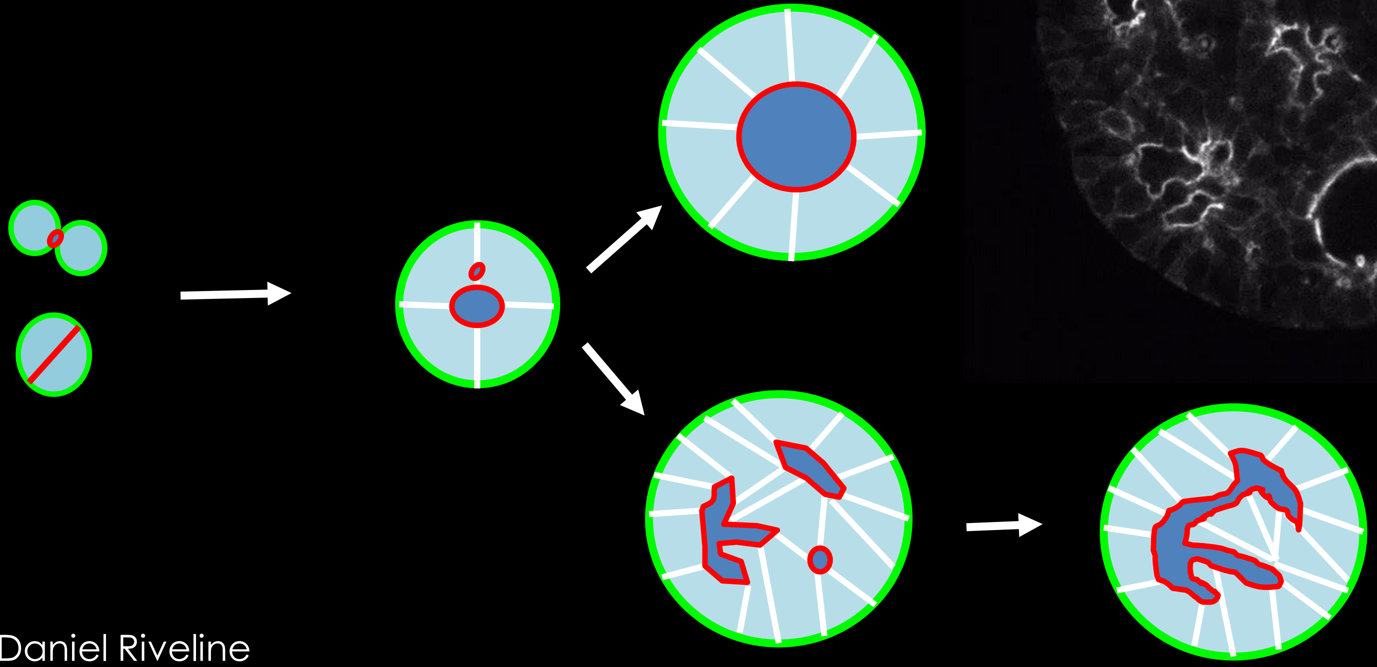
3 phases:
- Microlumen formation (cell polarization)
- Connection into a network
- Optimization of network into tree

Timelapse

GFP-LifeAct







Daniel Riveline
Alf Honigmann
Masaki Sano

Byung Ho Lee
Heike Petzold
Phil Seymour

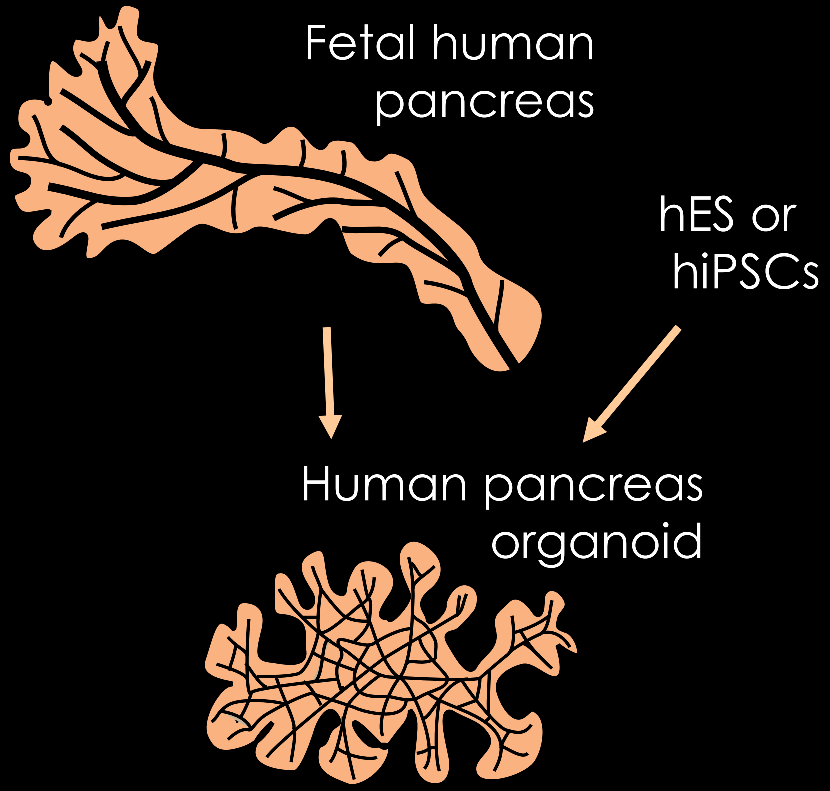
Why do we use organoids to study development?

To control cell assemblies and external signals to understand emergent properties

To study the mechanical control of development

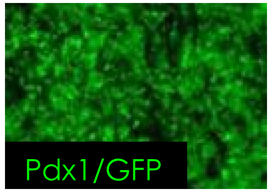
To study human development

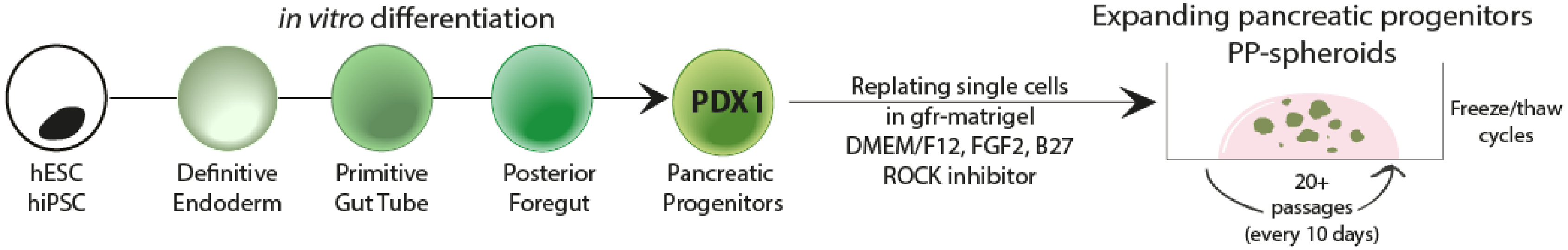
Human pancreas organoids

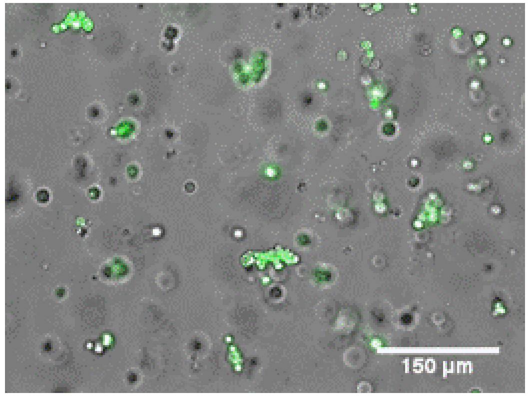


Goncalves et al., Nat. Comm., 2021

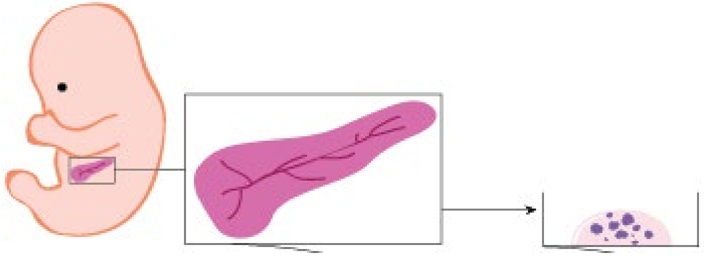
Development of pancreas spheroids from hESCs and iPSCs





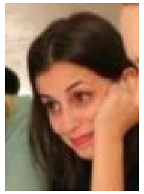


Rezzania et al. 2014 protocol or Semb/Ameri;
Pdx-GFP line from H. Semb- works with a variety of lines.



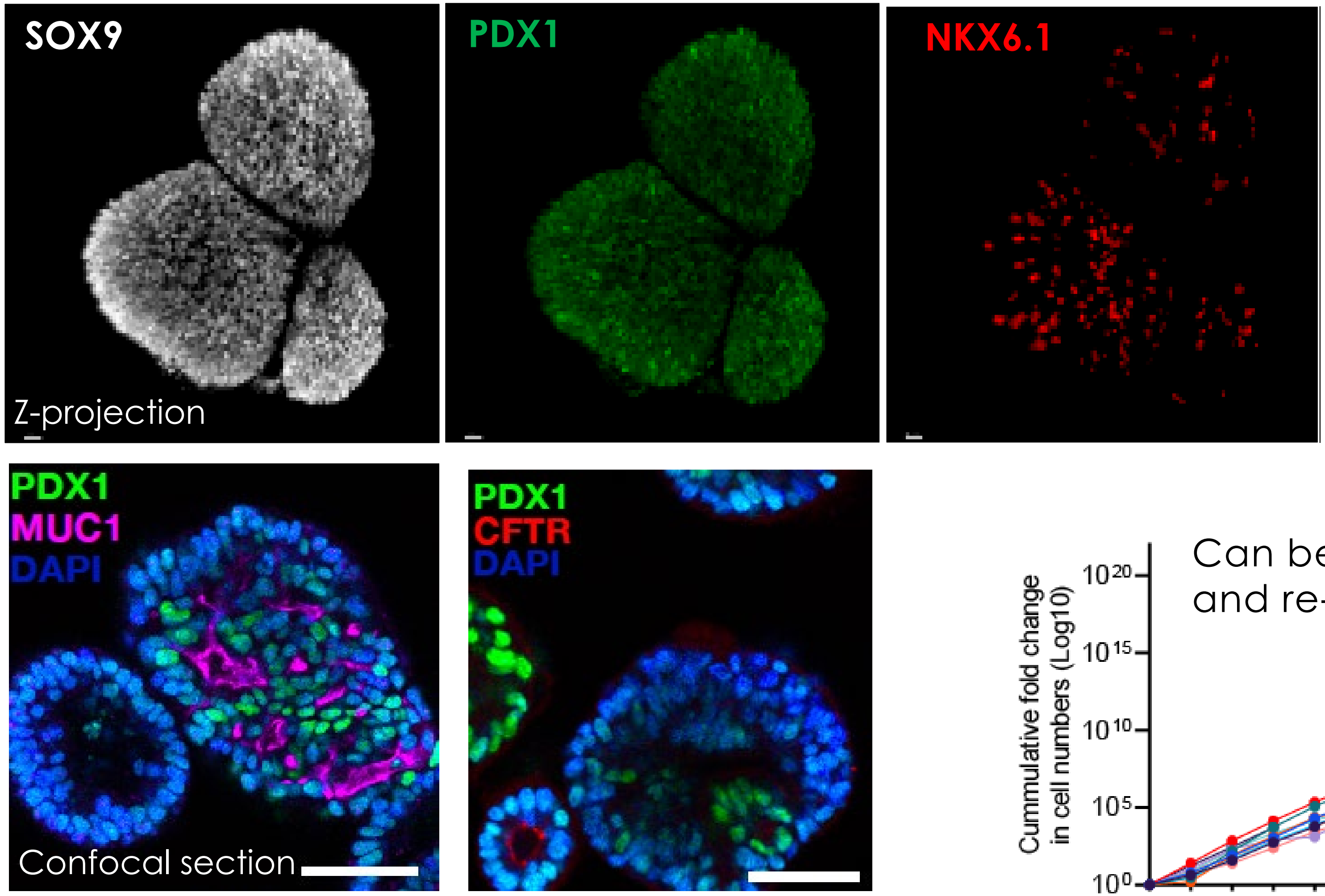
Same medium

Akiko Nakamura Carla Gonçalves



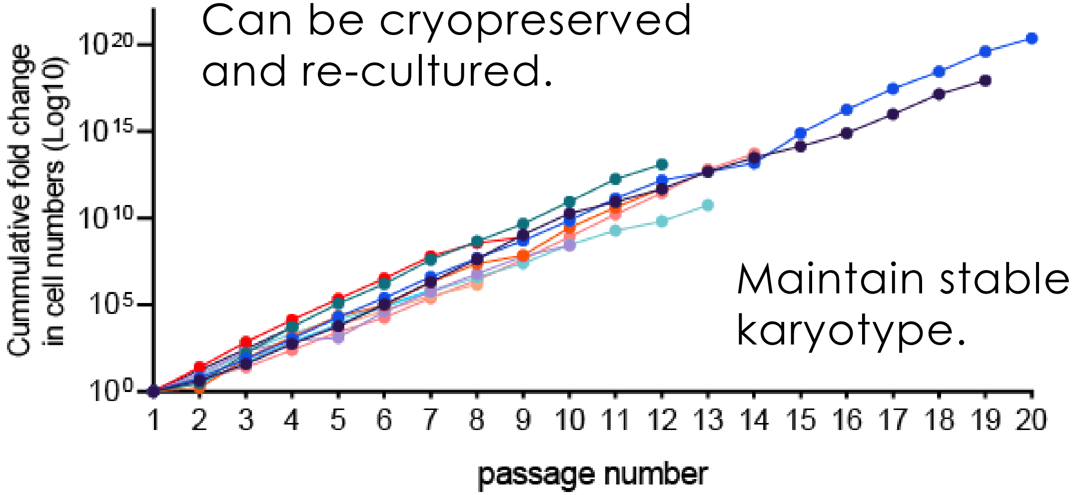
Goncalves et al., Nat. Comm., 2021

3D expansion of pancreas progenitors



A majority of pancreas progenitors.

Heterogeneity in shape and expression.

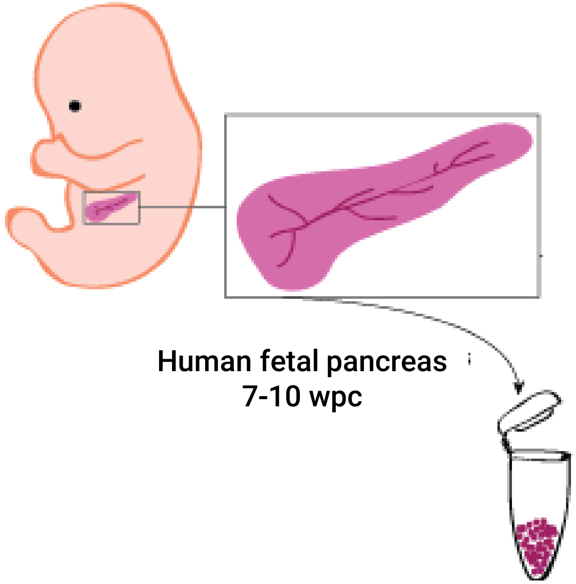


Can be cryopreserved and re-cultured.

Maintain stable karyotype.

How similar are pancreas organoids to the human fetal pancreas?

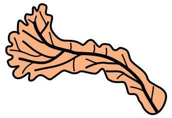
Cells expanding in 3D are more similar to *in vivo* than 2D



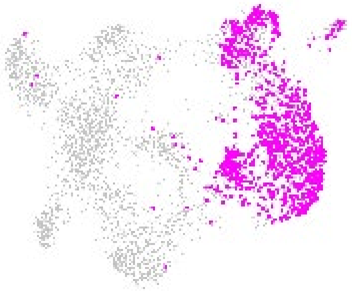
Fetal spheres

**Pancreatic progenitors
in 2D**

Human fetal
organoid
s



Fetal human
pancreas

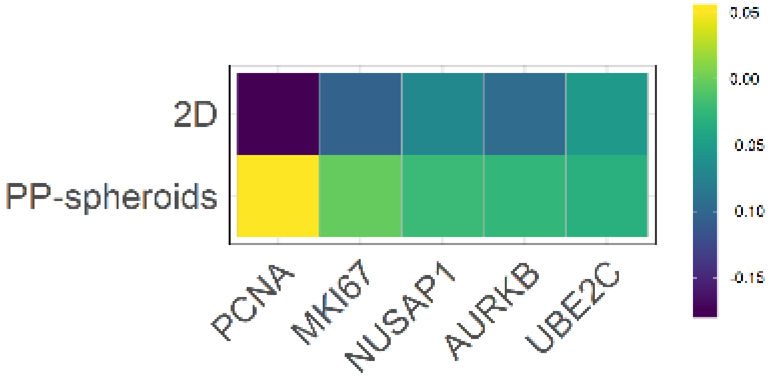


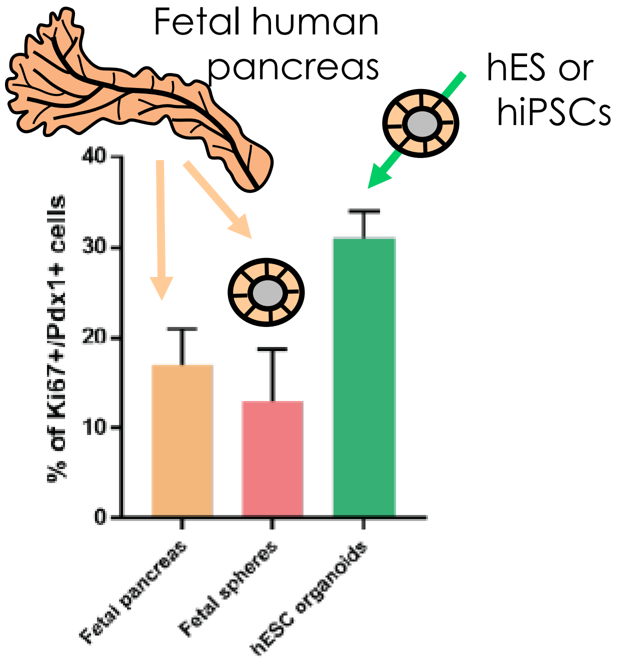
Data available in UCSC Cell browser.
Raw data available at EGA

Goncalves et al., Nat. Comm., 2021

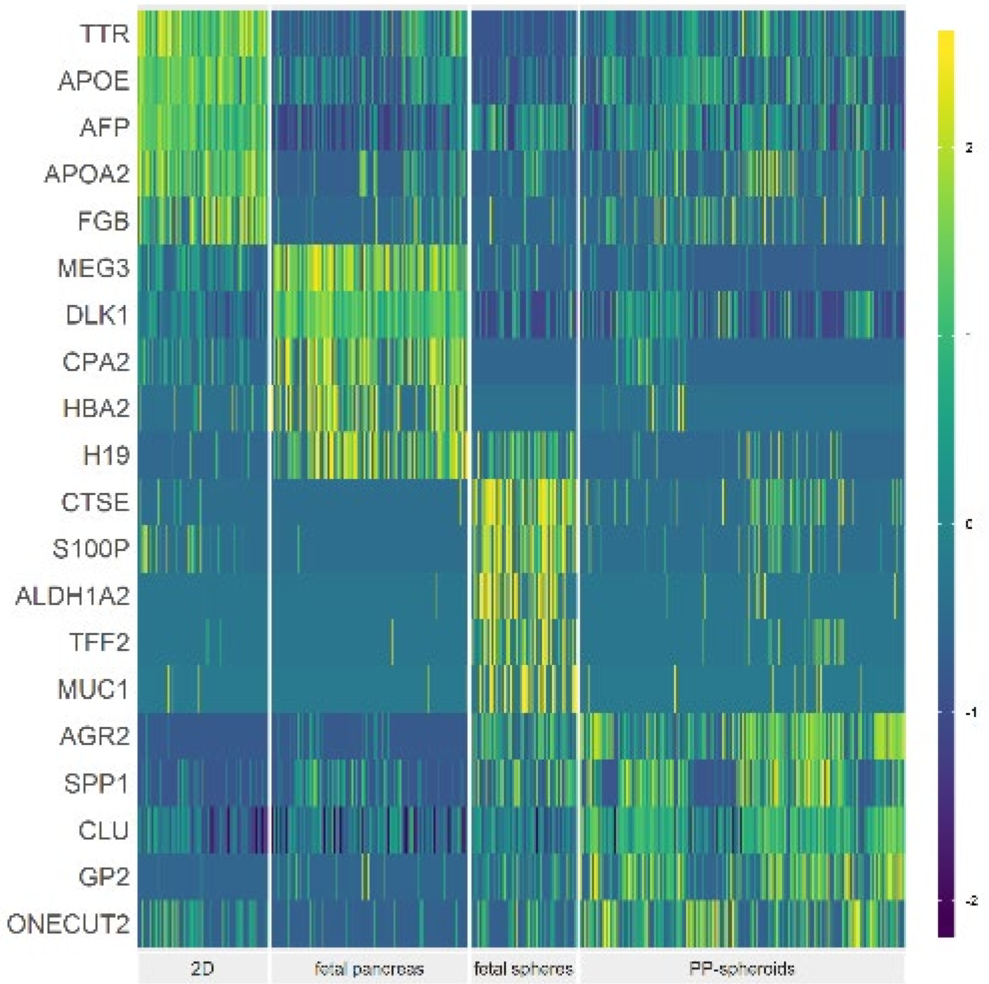
Major differences between 2D and 3D

More proliferation in 3D

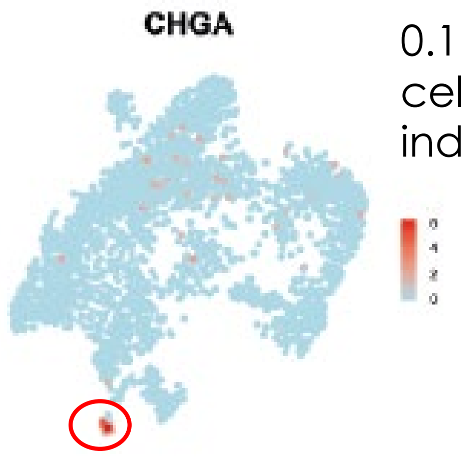




Fate more mixed pancreas/liver in 2D

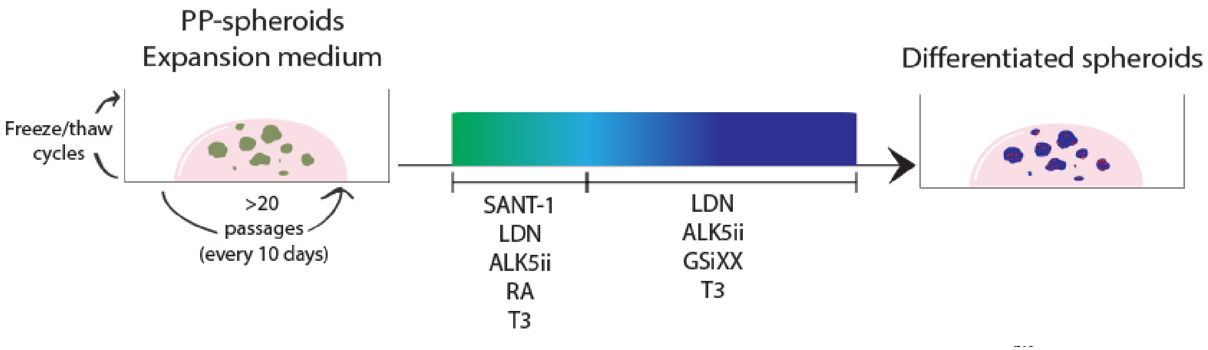


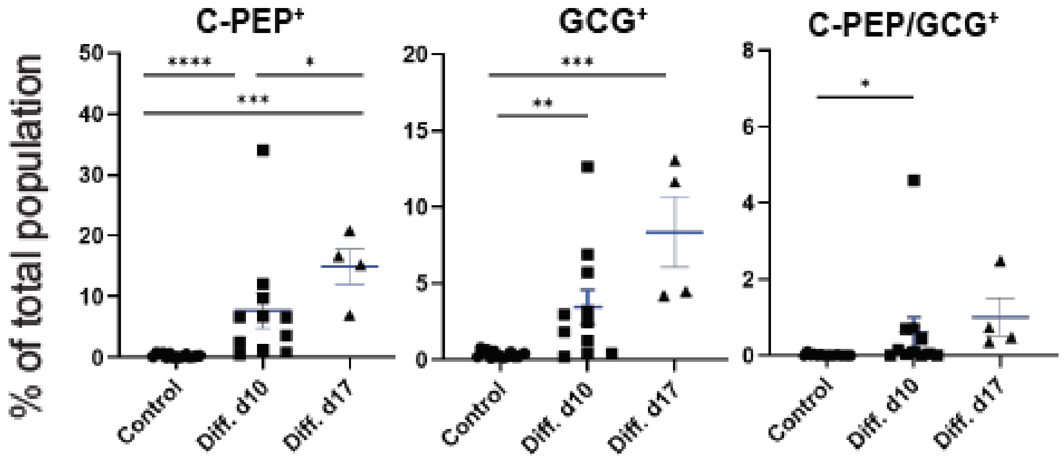
Pancreas progenitors in vitro keep functional features of the cells in vivo
Endocrine differentiation

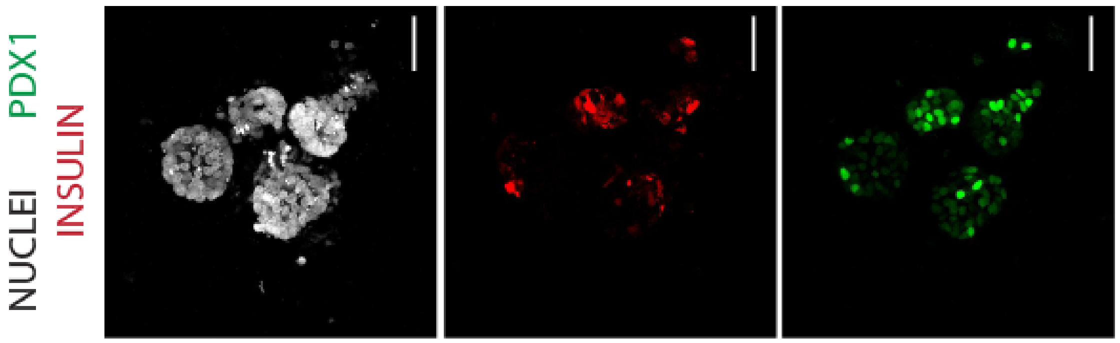


0.1 % Endocrine cells without induction

Induction of differentiation (Rezania st5/6)







Retain endocrine differentiation capacity at all passages. Any ability to differentiate into acinar cells?

Why do we use organoids to study development?

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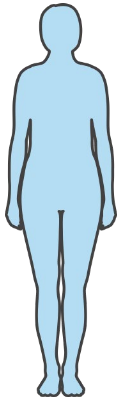
Reporters- comparing species

Using organoids to study genes impairing human development: Neurogenin 3 is critical for pancreatic endocrine commitment

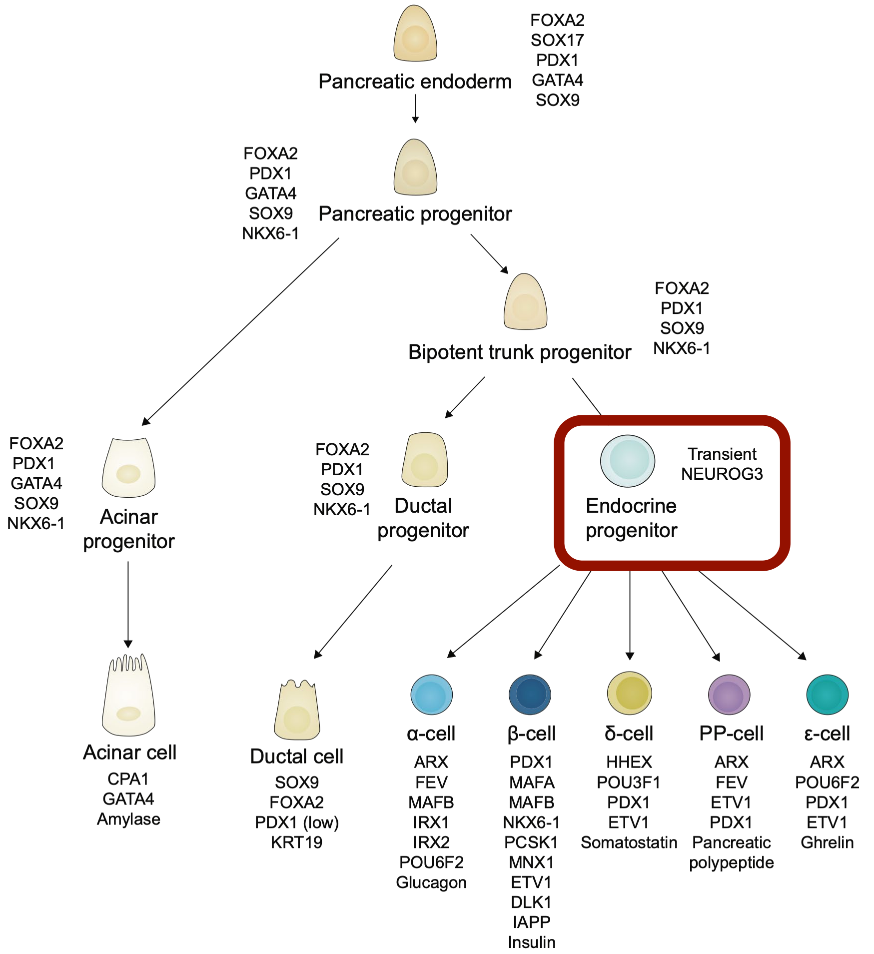
Neurog3 KO

No pancreatic endocrine cells
Mice die postnatally from diabetes mellitus



severe *NEUROG3* mutations

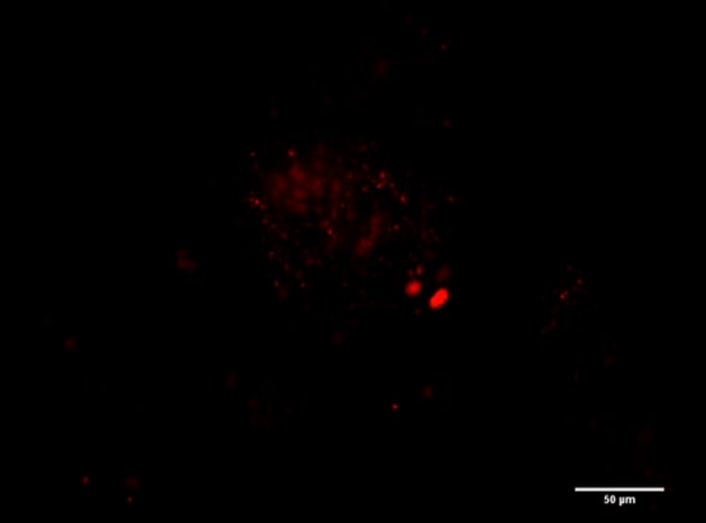
Most patients develop diabetes mellitus early in life



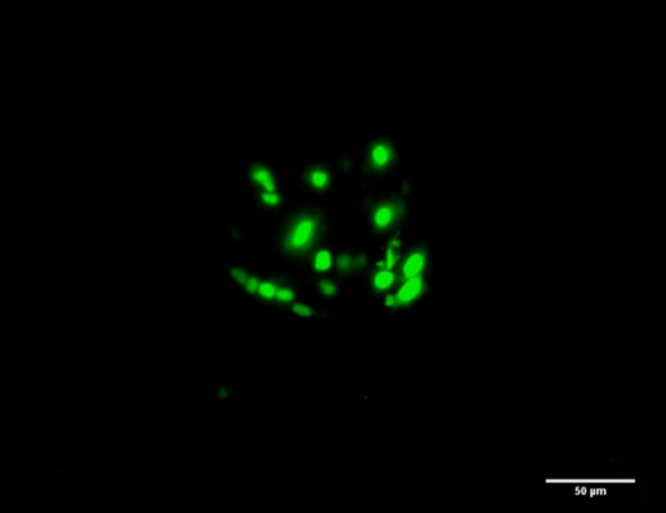
Gradwohl *et al*. 2000
Pinney *et al*. 2011
Rubio-Cabezas *et al*. 2011
Solorzano-Vargas *et al*. 2020

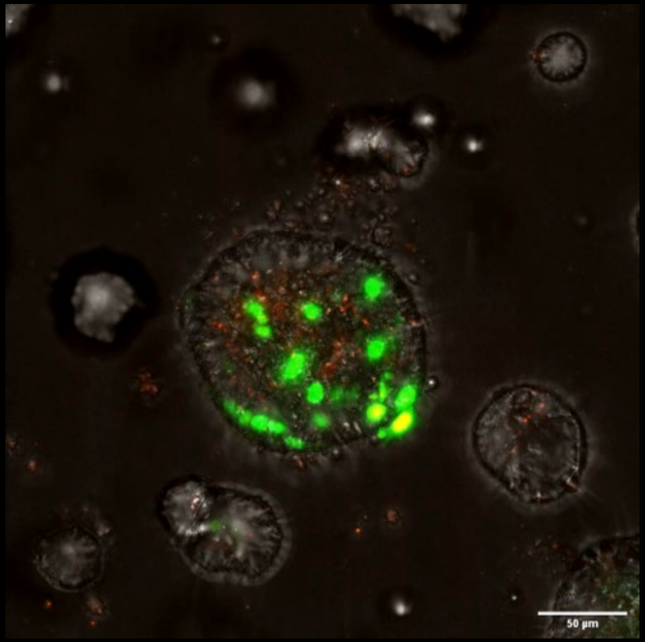
Following endocrine progenitor cell birth live

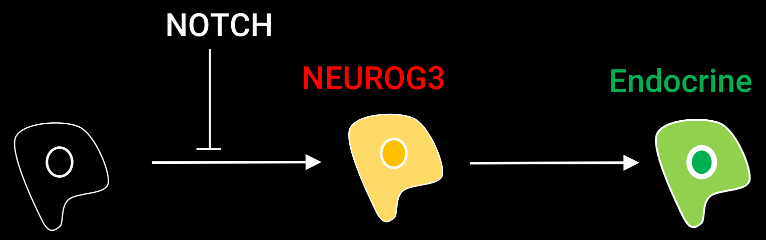
NEUROG3 protein fusion



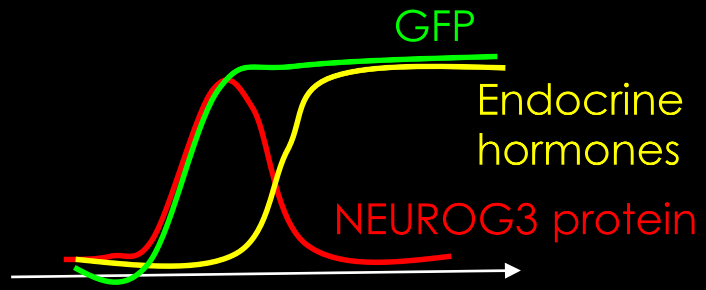
GFP from NEUROG3 promoter- long half-life- follow endocrine cells







Beydag-Tasöz *et al*. 2021, bioRxiv

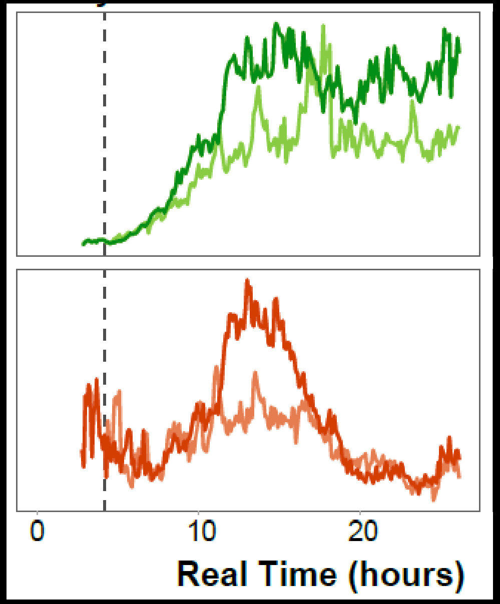


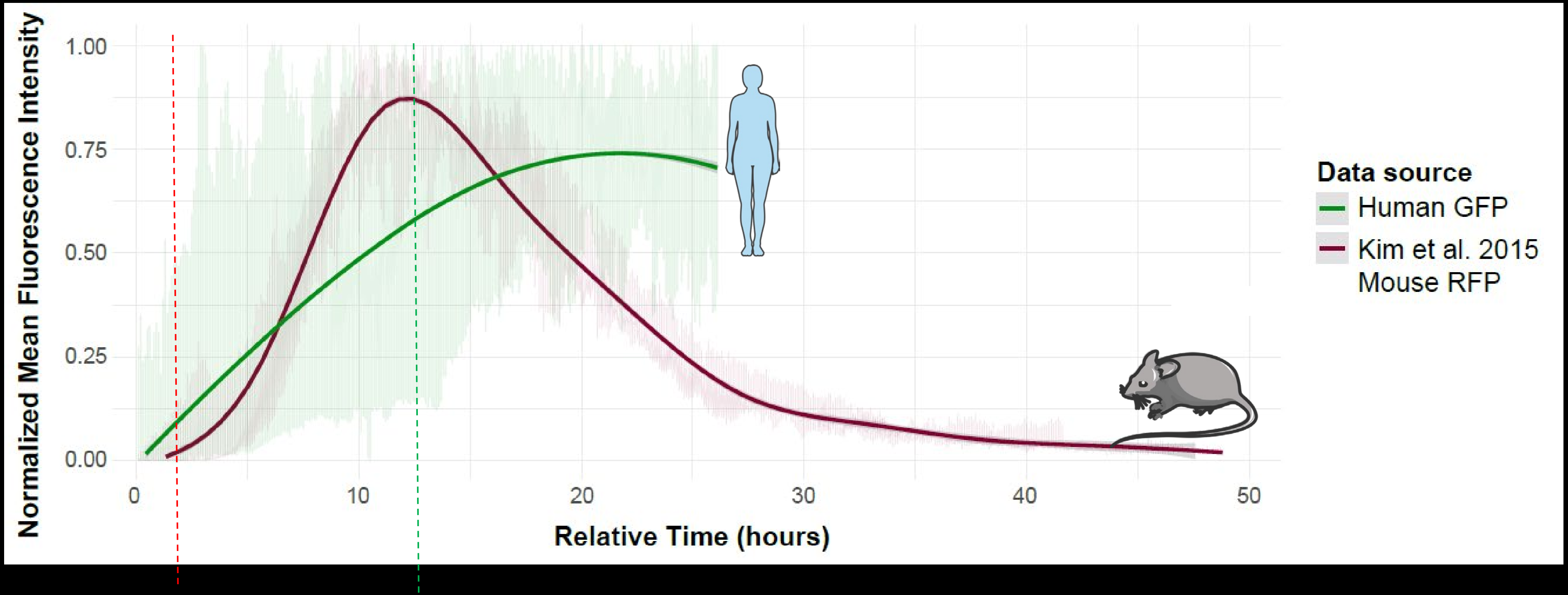
Selcen Beydag-Tasöz

Joyson DCosta

Yung Hae Kim

Slower process of differentiation than in mice

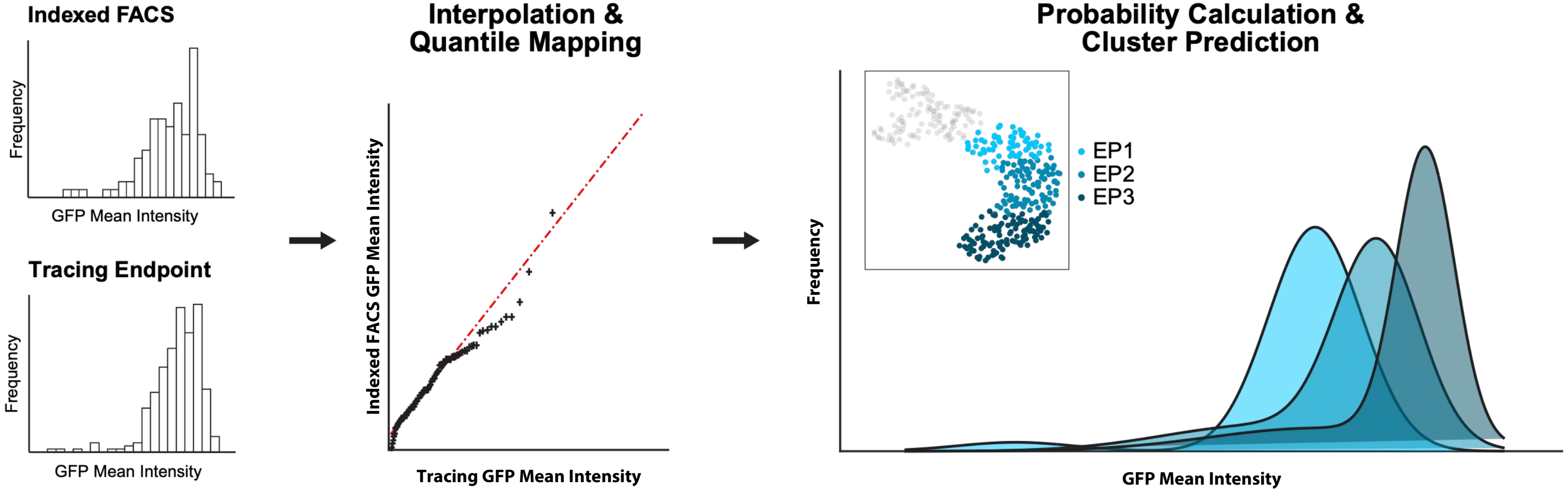




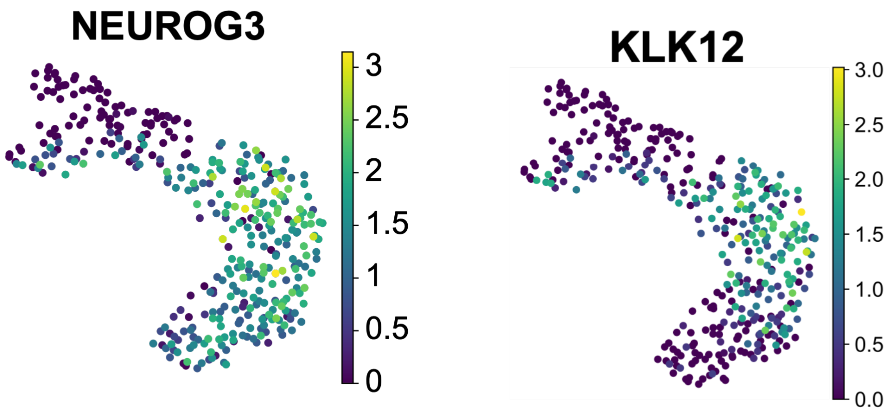
- Heterogeneity of peak levels of NEUROG3 (Stochasticity in Notch ligand inputs?)
- Transcription 2x slower in mouse than in human (also cell cycle length of progenitors)

Our mouse data in Kim et al., PLoS Biol. 2015 (live) and Larsen et al., Nat. Comm. 2017 (in vivo clonal analysis)

Mapping live imaging/cell behaviour to single-cell RNA sequencing

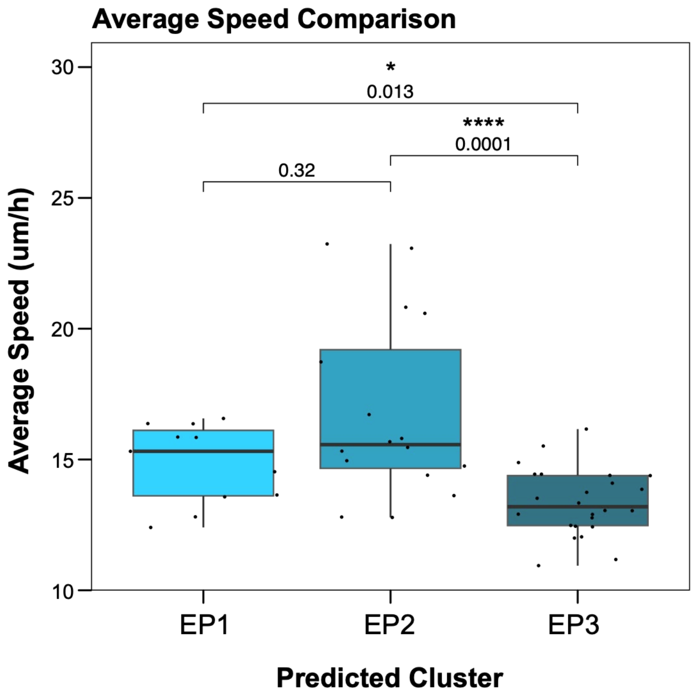


Federica Luppino Christoph Zechner



Kallikrein Related Peptidase 12

Serine protease involved in sprouting & migration of endothelial cells through ECM remodeling. Can efficiently cleave human fibronectin and tenascin. (Kryza *et al.* 2018)



Beydag-Tasöz *et al.* 2021, bioRxiv

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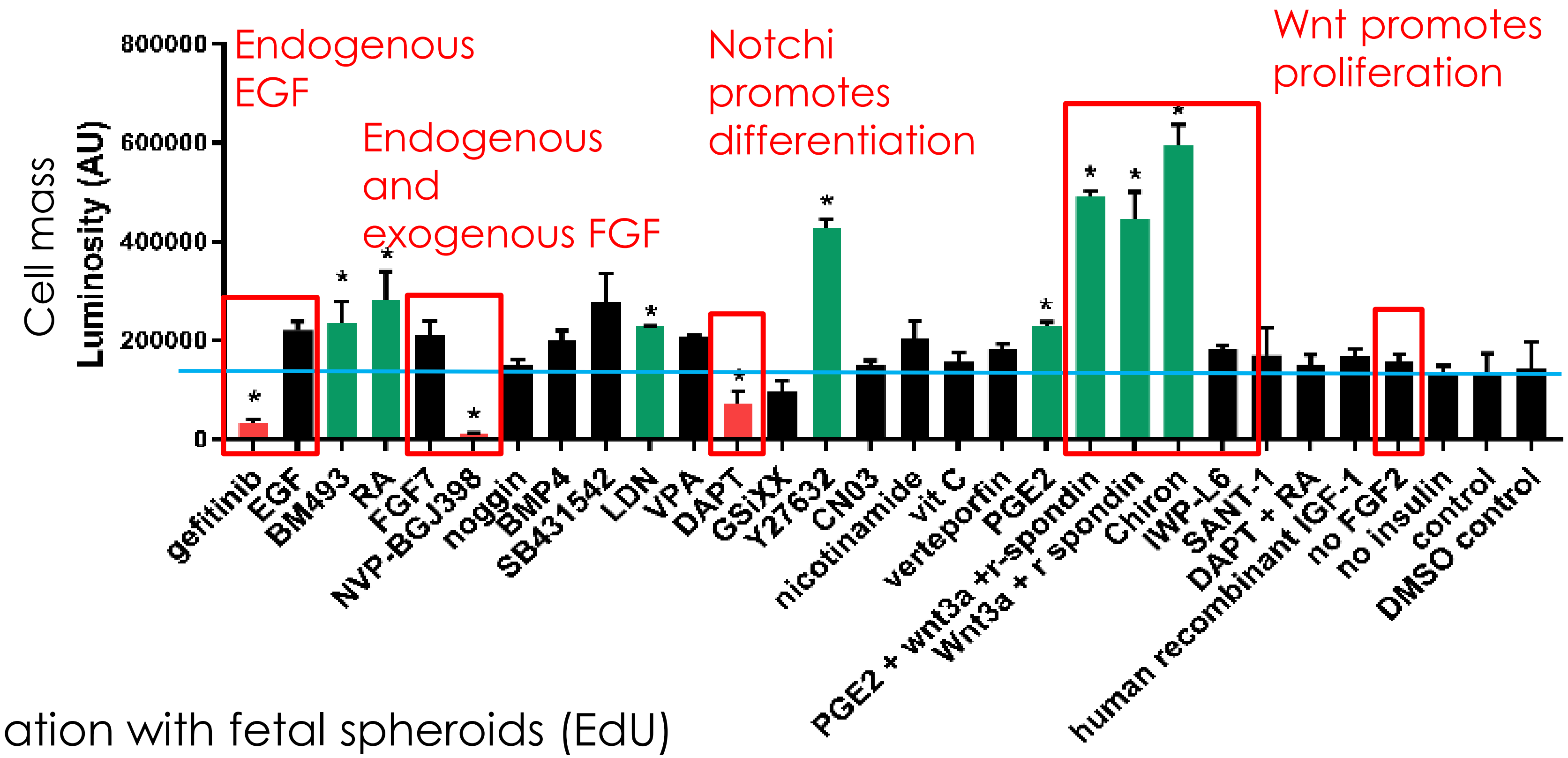
Reporters- comparing species

Interferences in screening formats

Screening for pathways controlling expansion and differentiation reveals different ligand-receptor pairing in human and mouse

hESC-derived PP in 96-well plates. Screening 30 compounds → Proliferation and differentiation



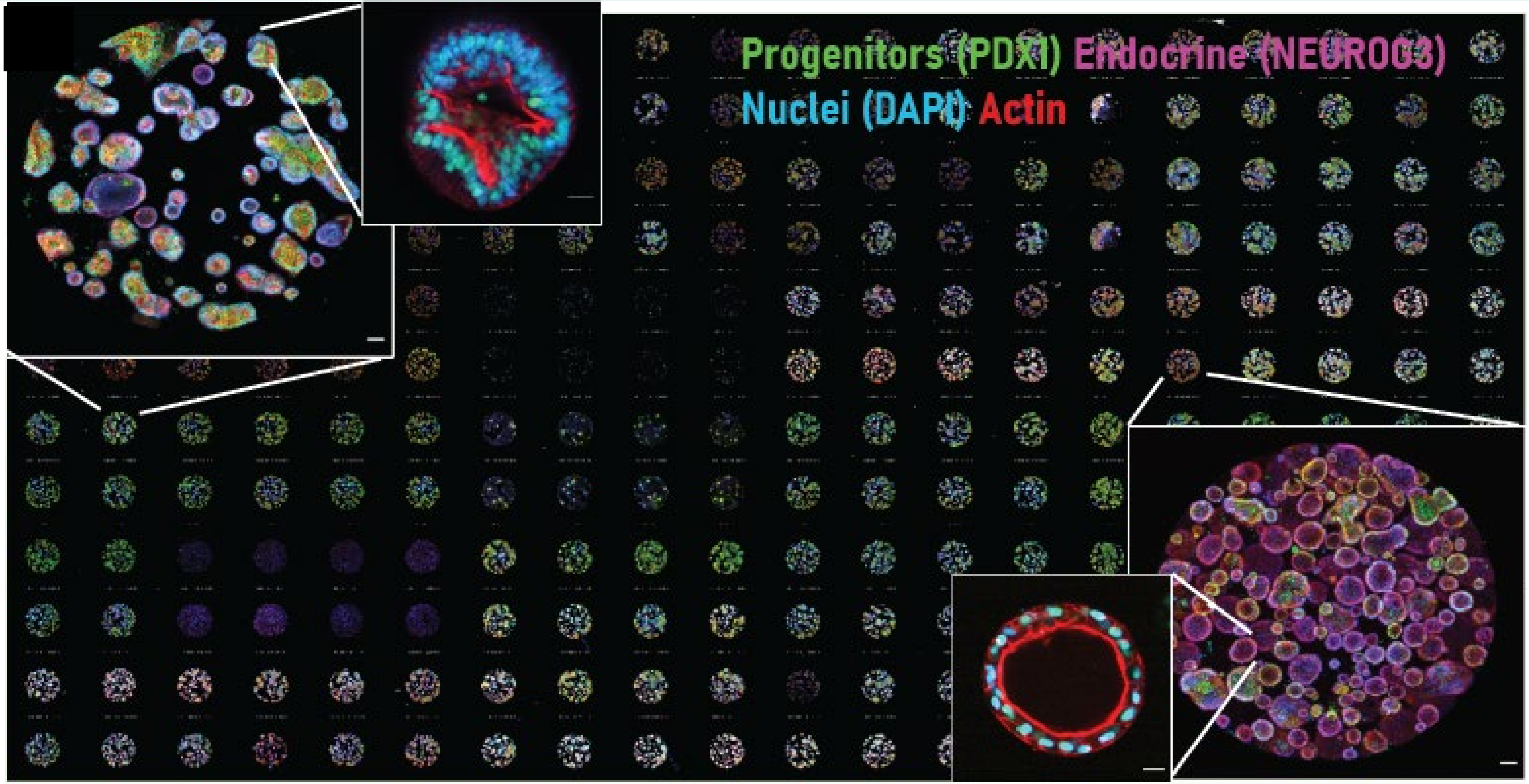
InterComm:
FGF2, 9 & 13 natural ligands in vivo and in organoids (Fgf10 in mouse)

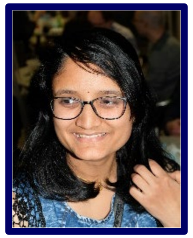
AREG & TGFa natural ligands in vivo and in organoids (BTC & EGF in mouse)

Validation with fetal spheroids (EdU)
Ligand-receptor pairing in vitro and in vivo suggests different ligands in human and mouse.

Goncalves et al., Nat. Comm., 2021

Drug screening to identify regulators of human pancreas development



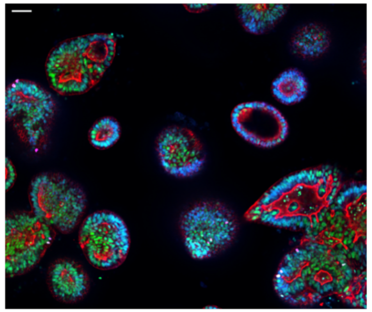


Rashmi Keshara

Image Analysis

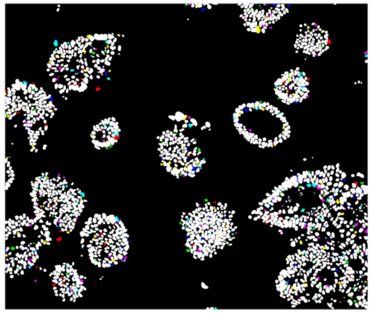

Marc Bickle

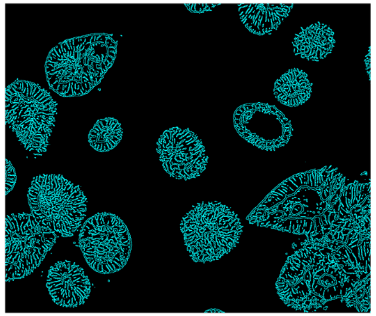

Antje Janosch

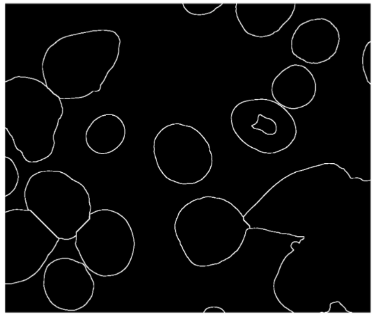

Raw image (20x)

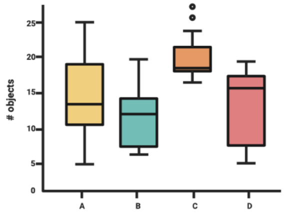

Rico Barsacchi

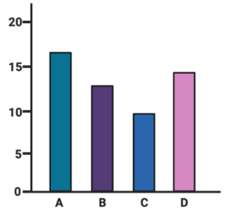

Martin Stöter

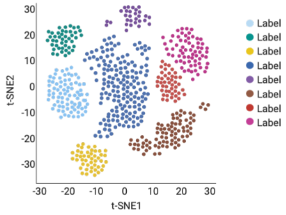

Nuclear Segmentation


Actin Segmentation


Organoid Segmentation







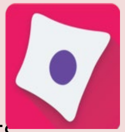
Open for Innovation KNIME

Created with Biorender.com

Hit Selection

Nuclear features

Total: 290



Intensity
Size&Shape
Number of objects
…

Organoid features

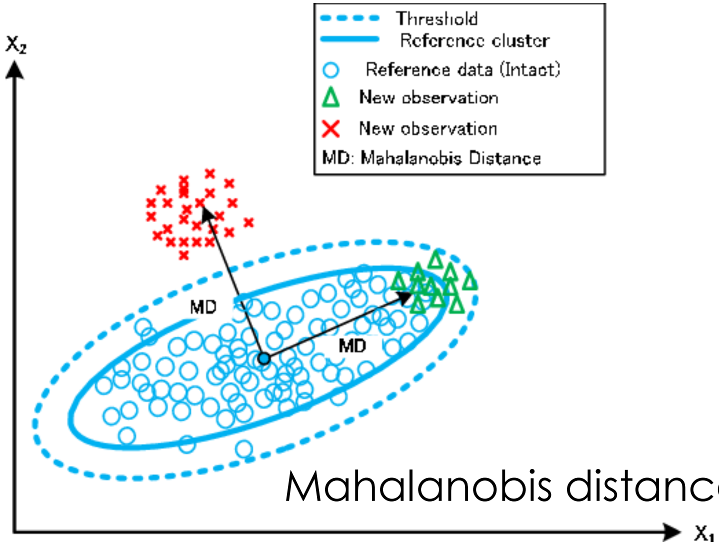
Total: about 300

Actin intensity
…

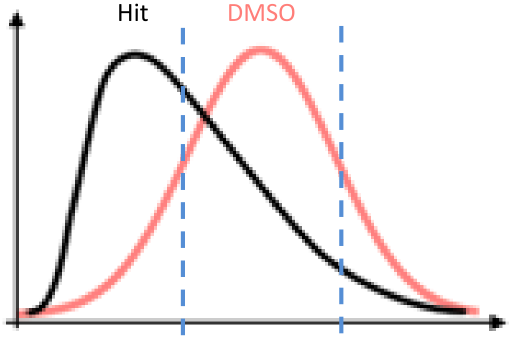
Parameter Grouping

Hierarchical clustering using Pearson Correlation Coefficient (PCC>0.5)
91 groups

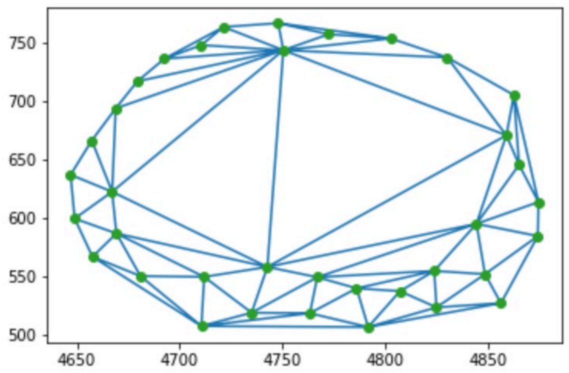
Org_Area
Org_EquivalentDiameter
Org_MajorAxisLength
Org_Perimeter



Development of morphometric indicators



Binning Analysis for shape and size parameters



Network Analysis based on Delaunay Triangulation

Types of hits:

- Less nuclei
- Decrease or increase of PDX1 intensity
- Decrease or increase of PDX1
- Less NEUROG3 nuclei
- More spherical
- Polarity inversion
- Reduced lumen

Antje Janosch

Rico Barsacchi

Kim CW, et al. 2013

Why do we use organoids to study development?

To control cell assemblies and external signals to understand emergent properties

To study the mechanical control of development

To study human development

Reporters- comparing species

Interferences in screening formats

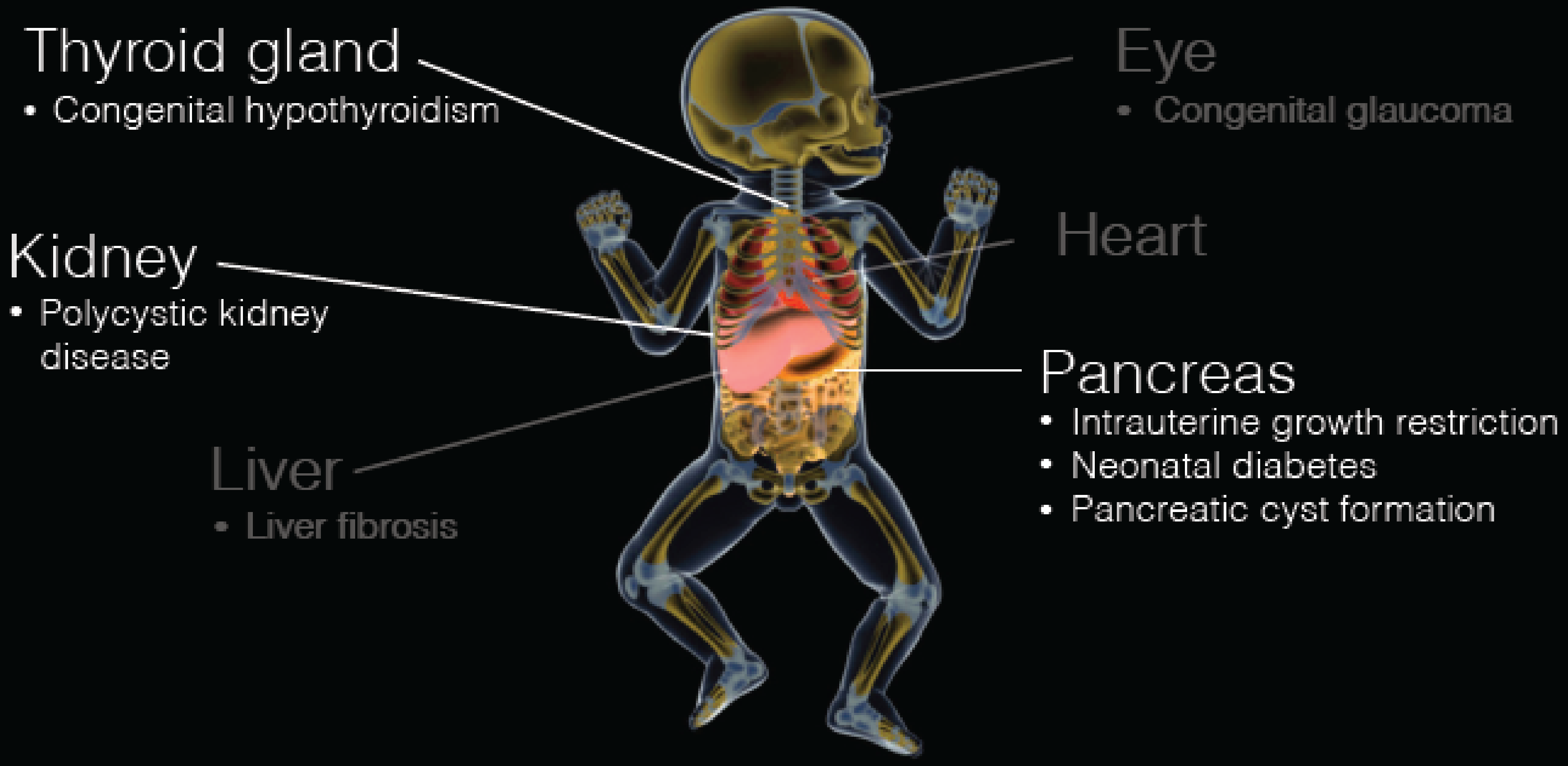
Interferences inspired from human genetics

Are human organoids useful to understand disease mechanisms?

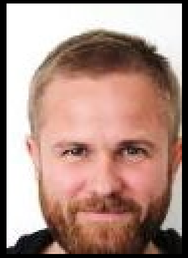
GLIS3 mutations cause neonatal diabetes and hypothyroidism syndrome

GLIS3

Heterozygous variants predispose to T1D &T2D



Taha *et al.*
American journal of medical genetics, 2003

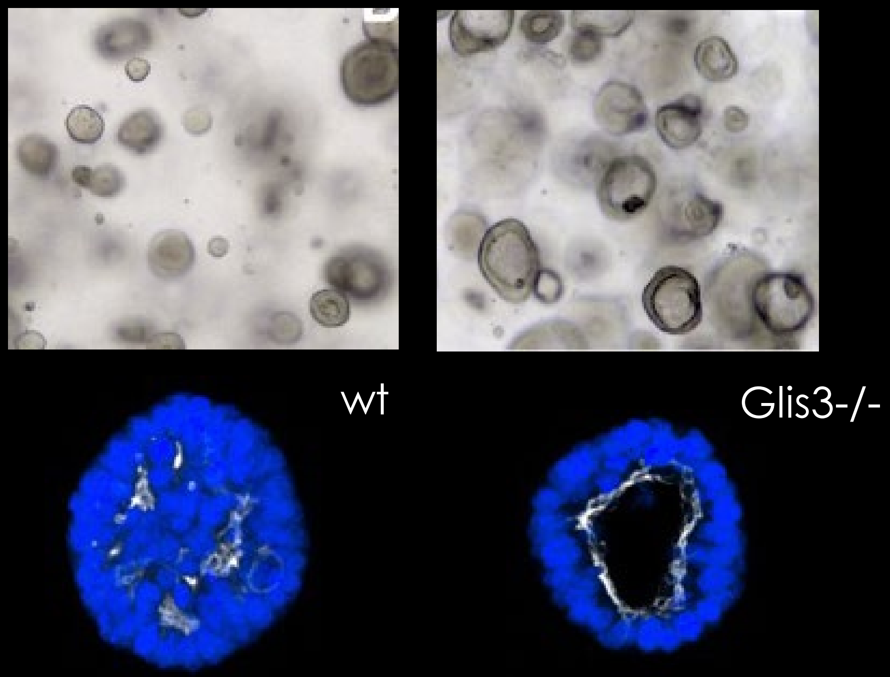


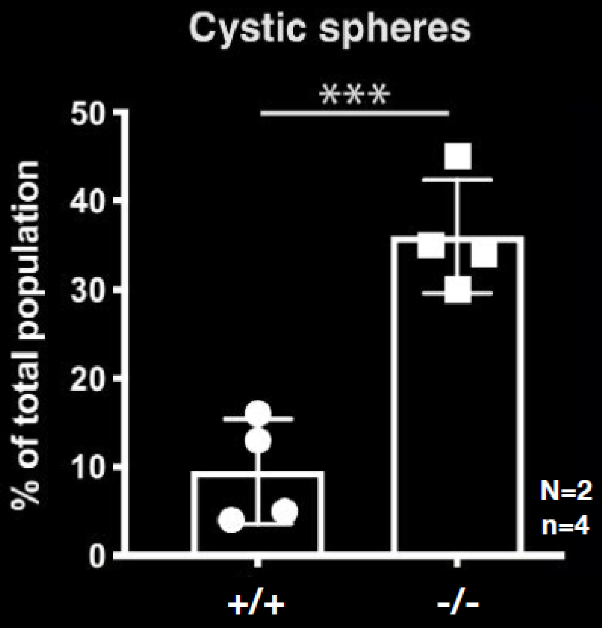
Michael Larsen

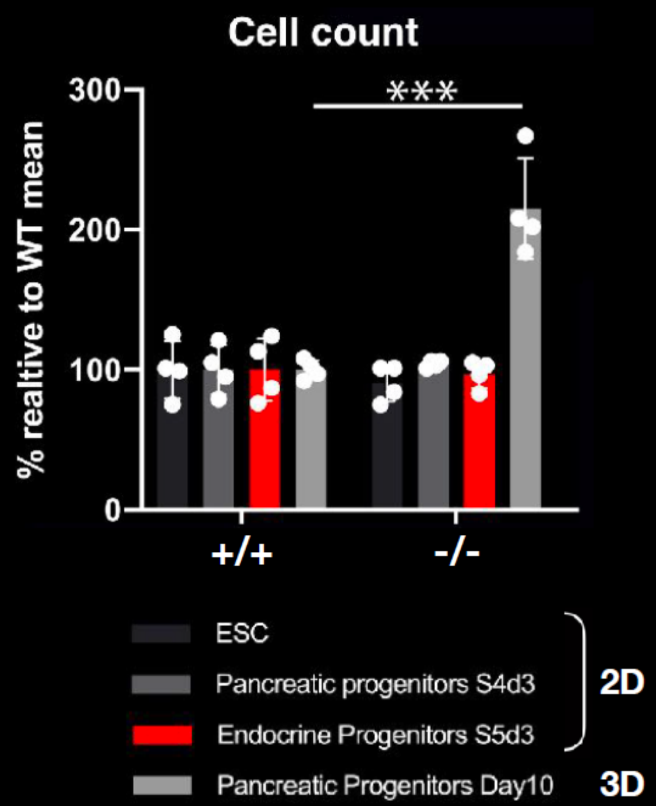
Glis3 causes a cystic phenotype in human spheroids

Developmental phenotype of mouse mutant: Cysts and 90% reduction of beta cell formation

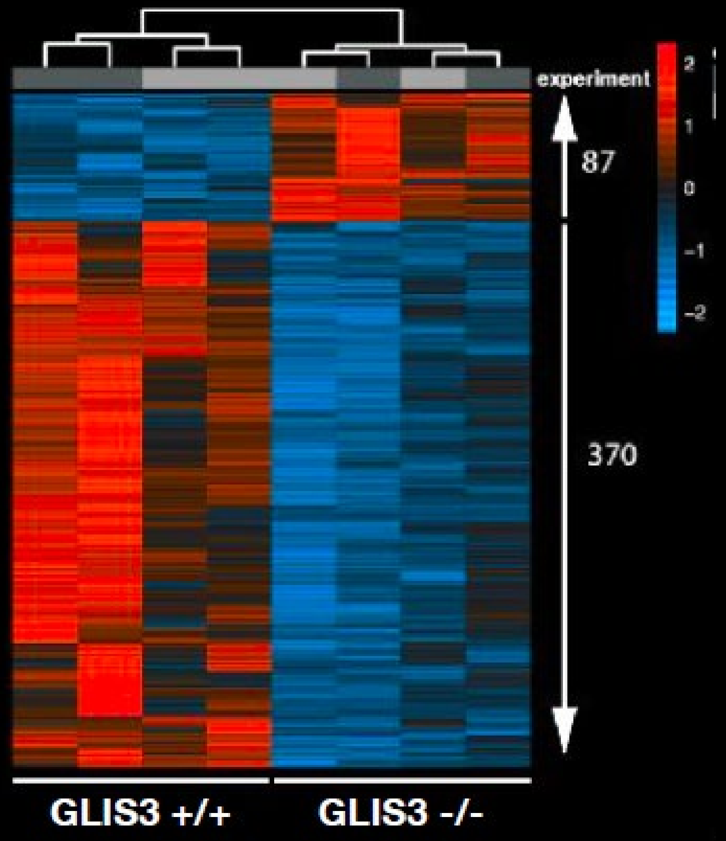
Crispr Inactivation of Glis3 in hES cell-derived pancreas spheres results in cystic spheres







Mechanisms of cyst formation



Bulk transcriptome day 5
Prior to cyst detection

Increase in proliferation and cell cycle terms

Decrease in ductal cell markers, extracellular matrix and cilia- terms

Effect on endocrine differentiation under evaluation.

Challenges

Benchmarking: there are always some molecular differences with cells in vivo. Do they matter?

Relevance of in vitro observations to the in vivo setting? (rotation, endocrine cell wandering)

Medium masking some effects when testing gene function

Heterogeneity of objects (screening)

Thanks to our collaborators on these projects

Anne Jørgensen (Rigshospitalet, Copenhagen)- Human embryos
Sascha Yung, Antonio del Sol (Univ. Luxemburg)- InterComm
Ido Amit (Weizmann institute)- Set up MARS-Seq
Federica Luppino & Christoph Zechner (MPI-CBG)- Quantile mapping
Helfpful, competent and creative MPI-CBG facility staff

DanStem

Former team members

Akiko Nakamura
Carla Gonçalves
Joyson DCosta

Team MPI-CBG Dresden

Irene Seijo Barandiaran
Byung Ho Lee
Heike Petzold
Keiichi Katsumoto
Selcen Beydag
Coline Schewin
Rashmiparvathi Keshara
Allison Lewis
Tzer Han Tan
Marit Leuschner
Yung Hae Kim
Jifeng Liu
Katharina Hilbig
Louis Hewitt

MAX-PLANCK-GESELLSCHAFT

CBG Max Planck Institute of Molecular Cell Biology and Genetics

DET FRIE FORSKNINGSRÅD

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Danmarks Grundforskningsfond Danish National Research Foundation

novo nordisk fonden

Post-doc positions available