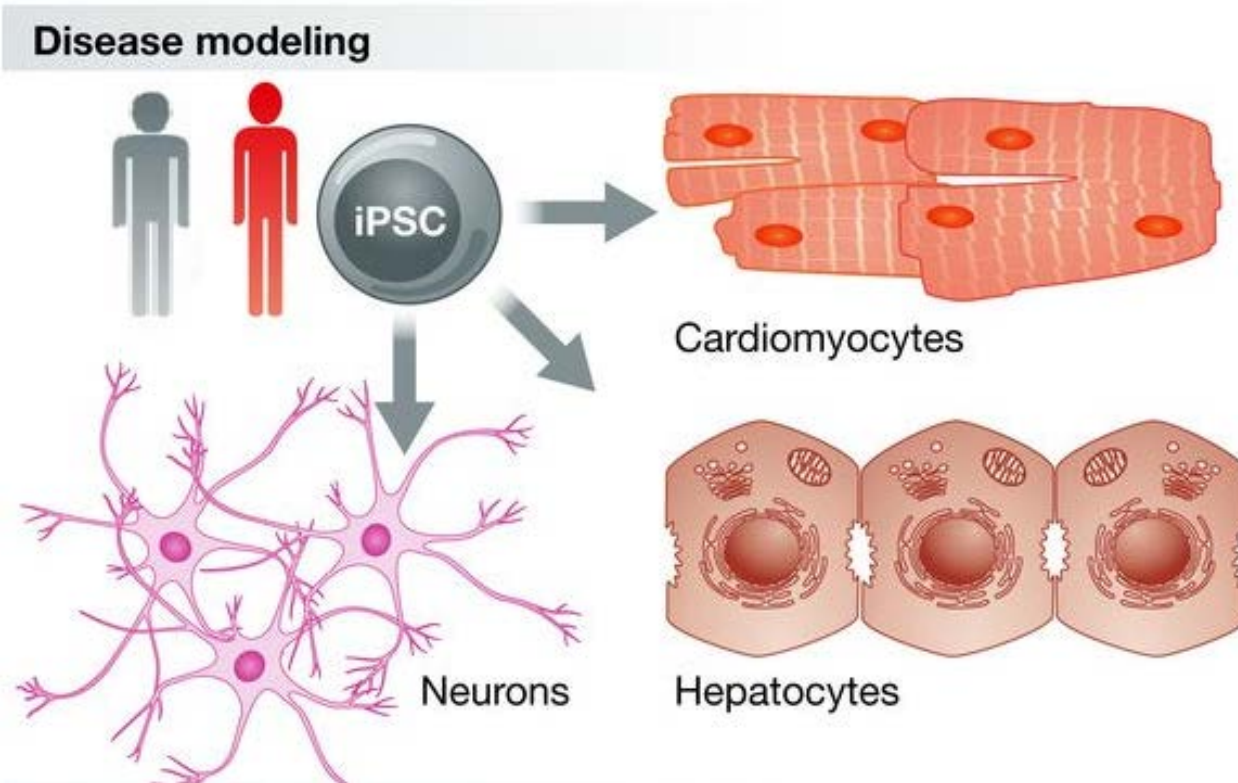


A 3D fluorescence micrograph of patient-derived mammary tissue. The tissue is stained with red and blue dyes, showing a complex, branching structure with numerous small, rounded nodules. The red staining highlights the cellular structure, while the blue staining likely represents nuclei. The overall appearance is that of a highly branched, tree-like structure with many small, spherical components.

3D Culture of Patient Derived Mammary Tissue: Implications for personalized medicine

Ethan Sokol
Gupta Lab
April 4, 2016

Patient Derived Cells can Inform our Understanding of Disease Biology



- Compare diseased and non-diseased patients
- Validate disease associated mutations
- Identify specific changes in the biology of disease cells

Long QT Syndrome (LQTS)



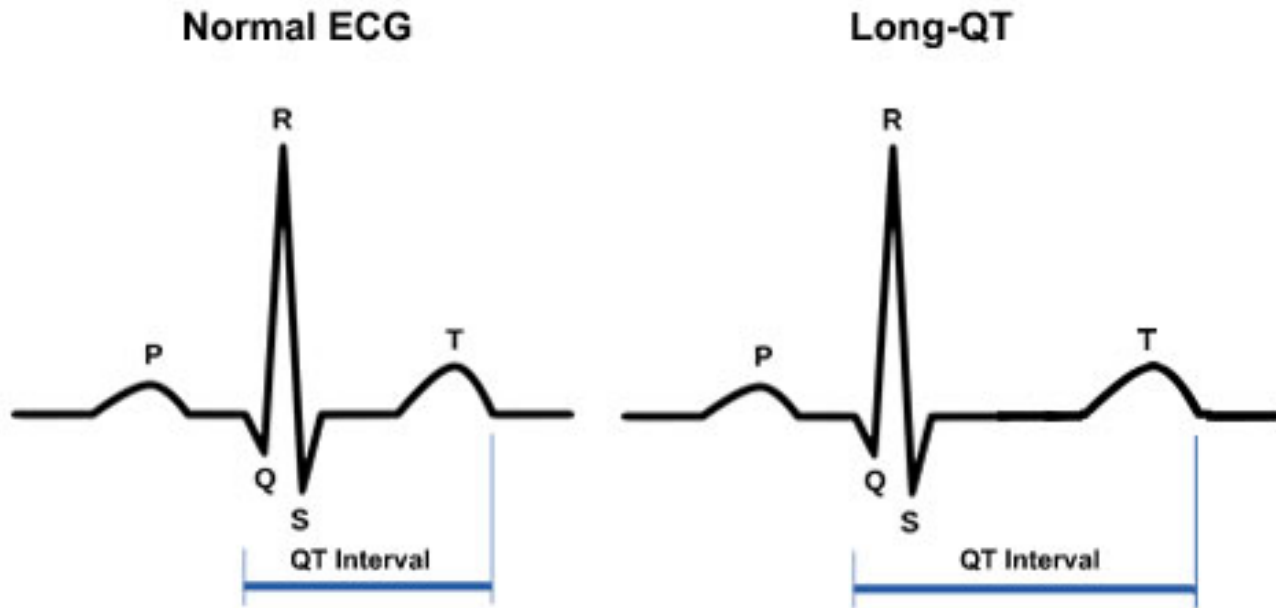
Long QT syndrome (LQTS)

Affects 1 in 7000 people

One of the leading causes of sudden cardiac arrest in adolescents

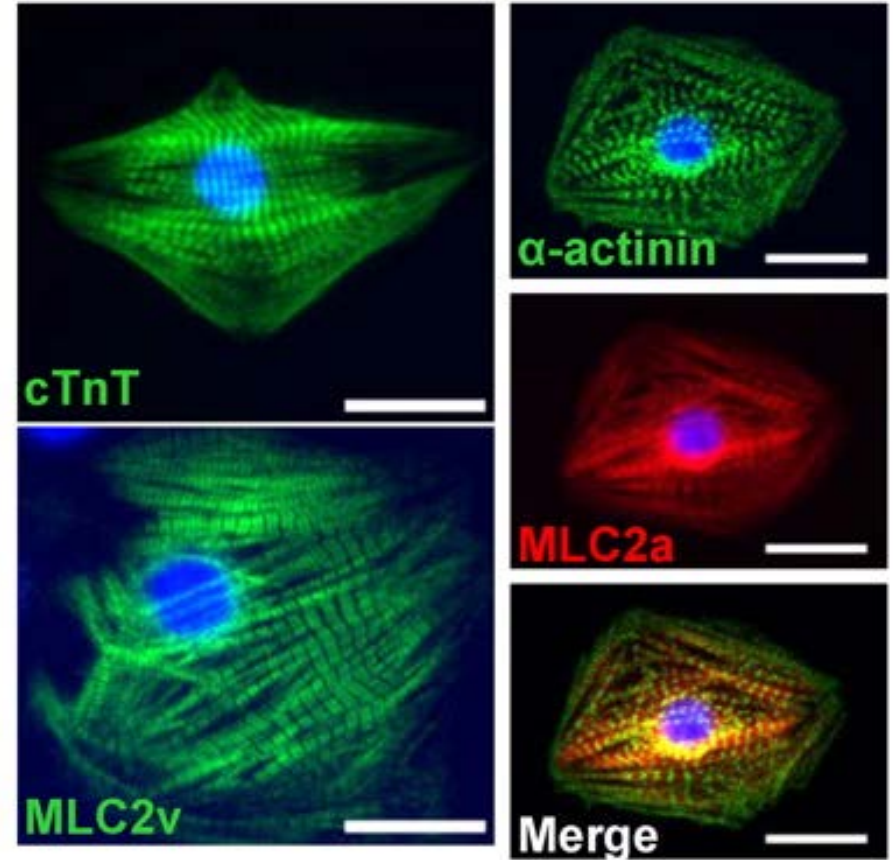
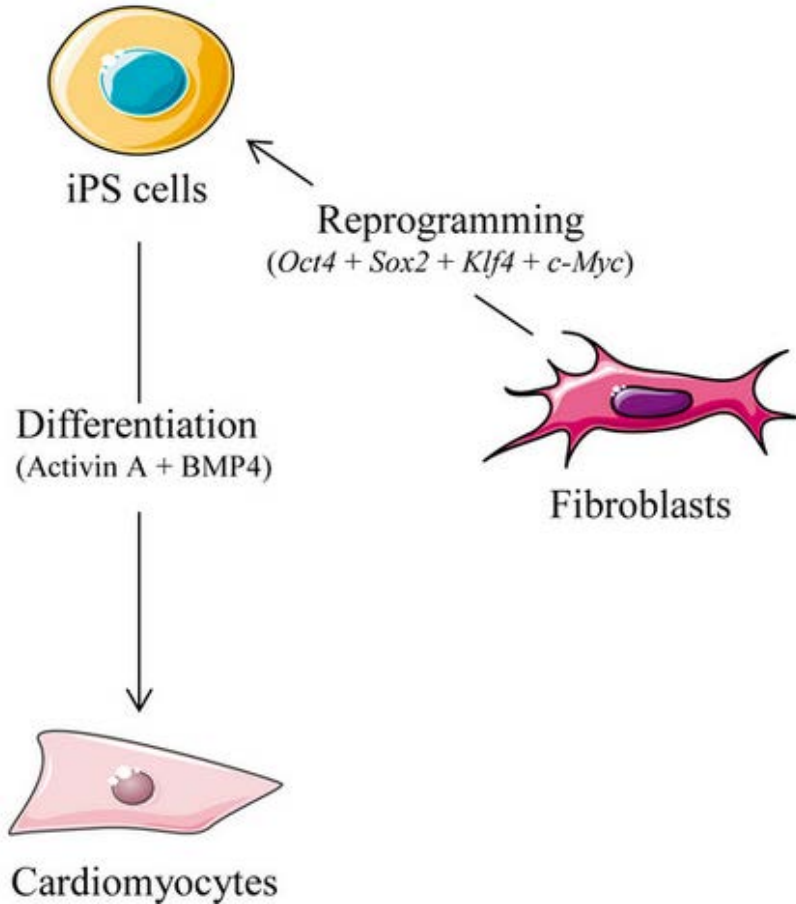
Caused by mutations in cardiac ion channels

Long QT Syndrome (LQTS)

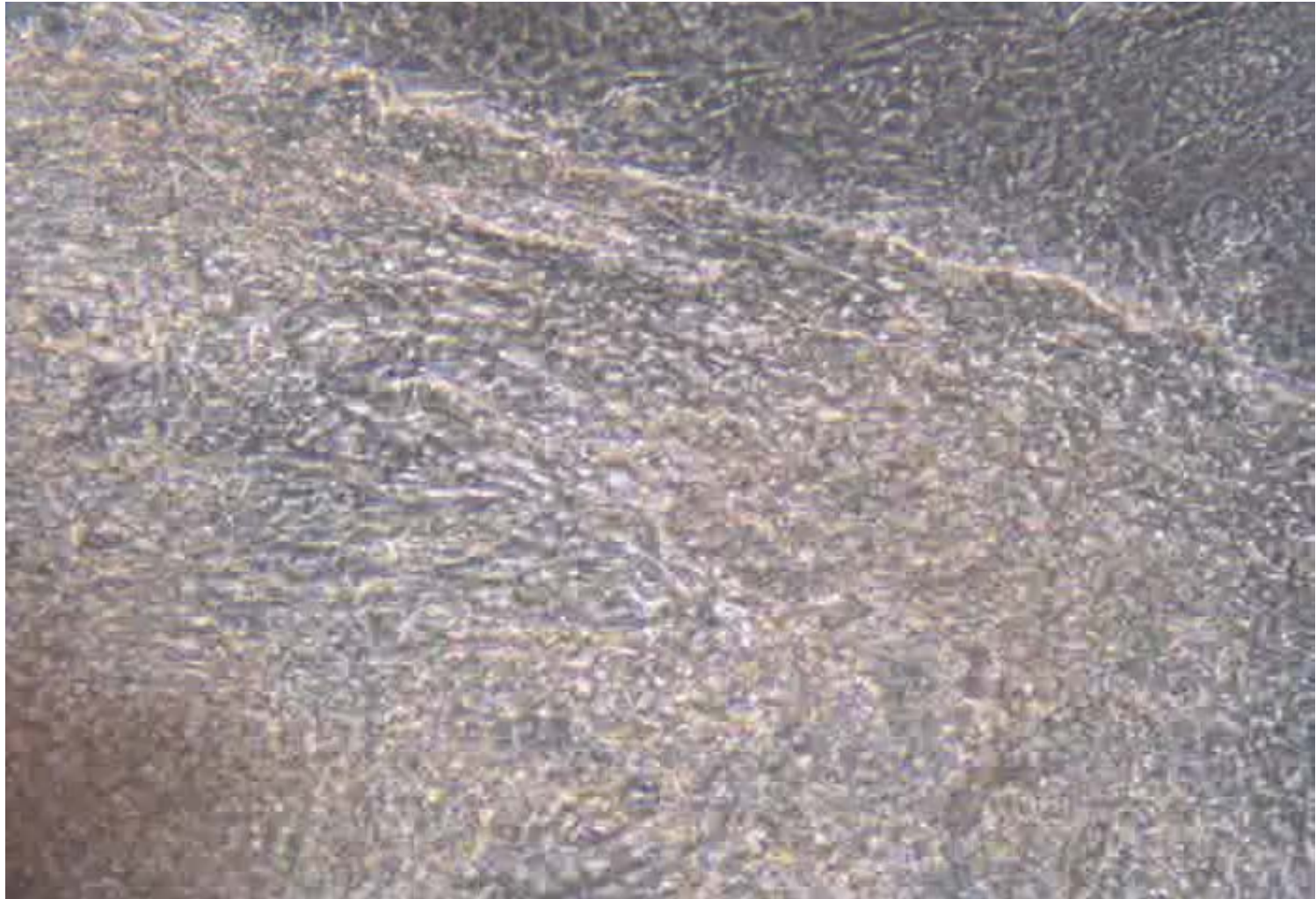


Patients are prone to ventricular fibrillation and cardiac arrest

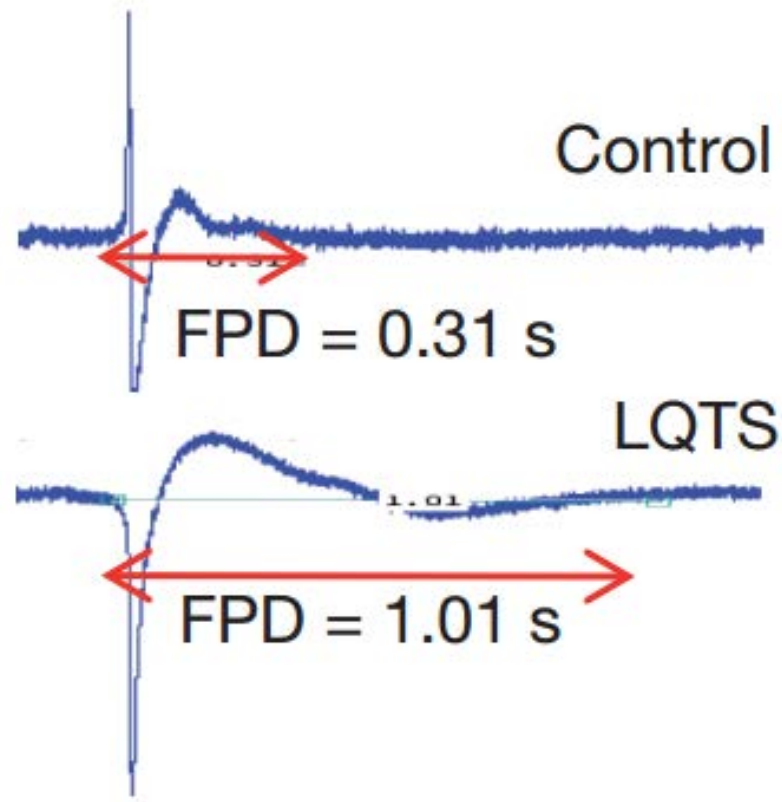
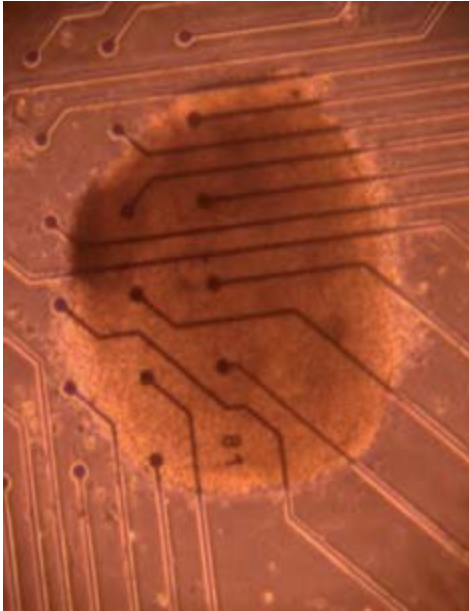
iPS Cells to Study LQTS



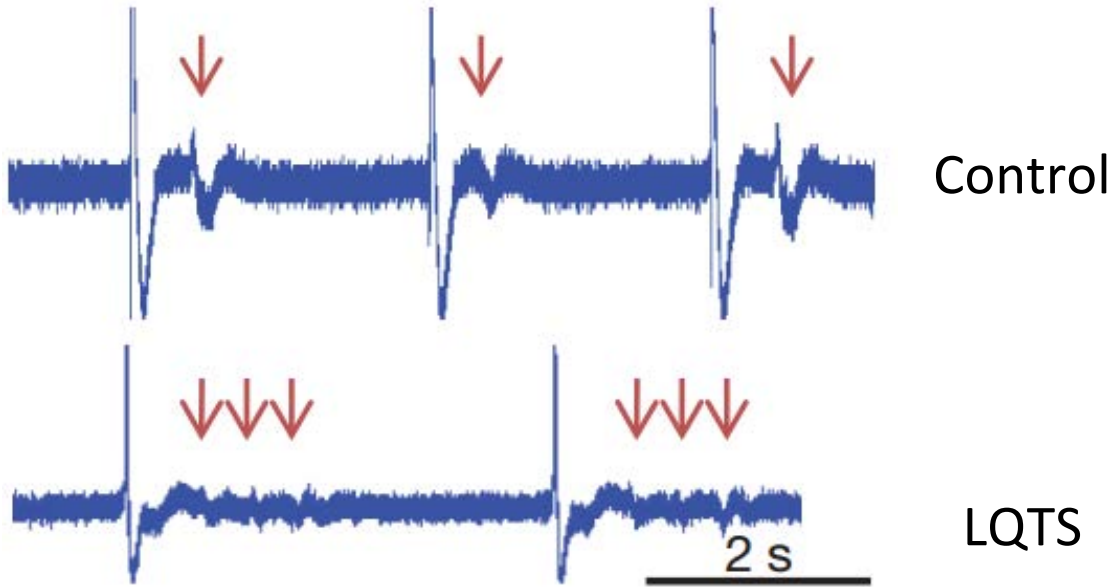
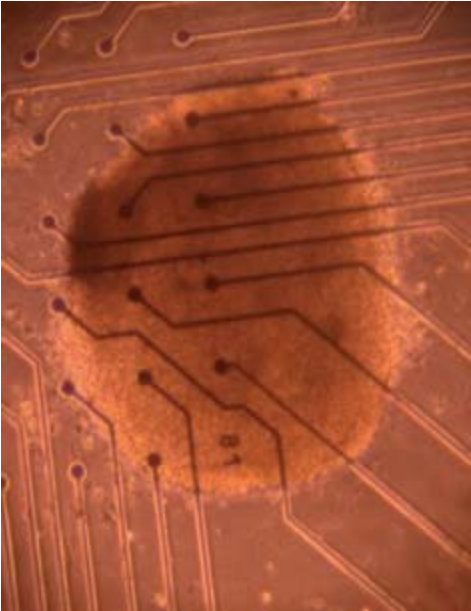
iPS Cells to Study LQTS



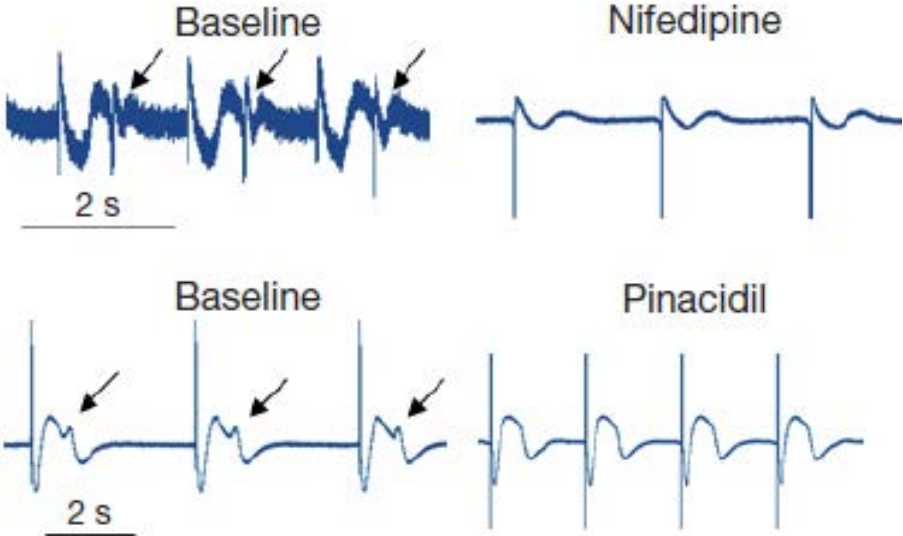
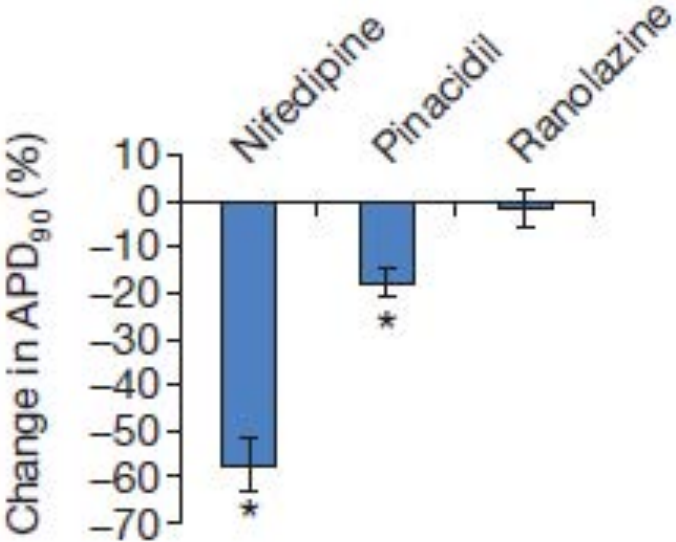
LQTS Patient Derived Cells Exhibit long QTs and Arrhythmias



LQTS Patient Derived Cells Exhibit long QTs and Arrhythmias

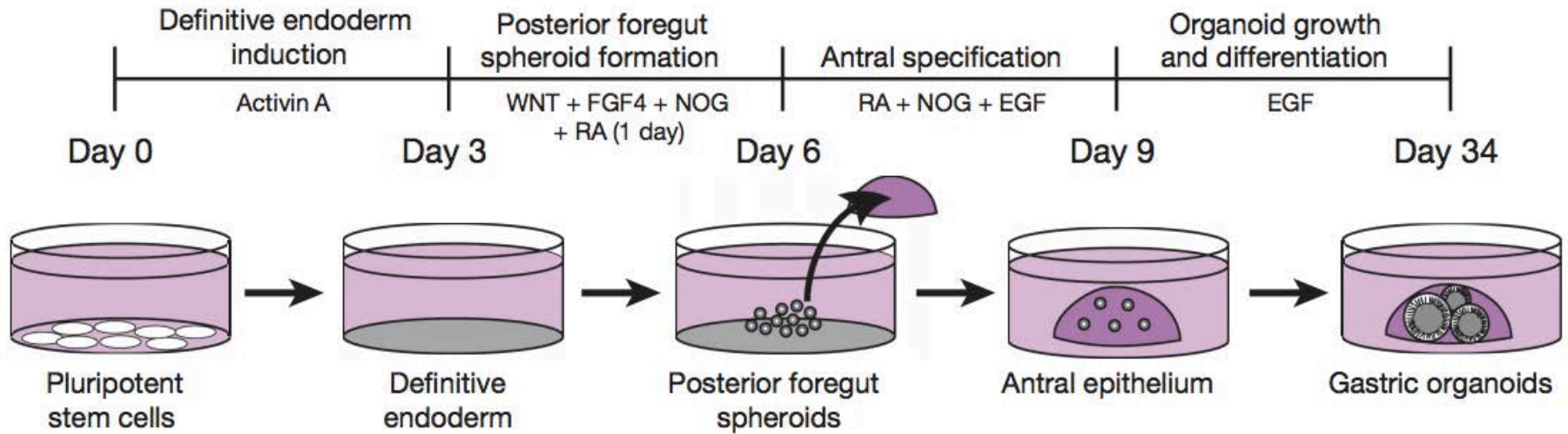


LQTS Derived Cells were used to screen for drugs that alleviate the syndrome

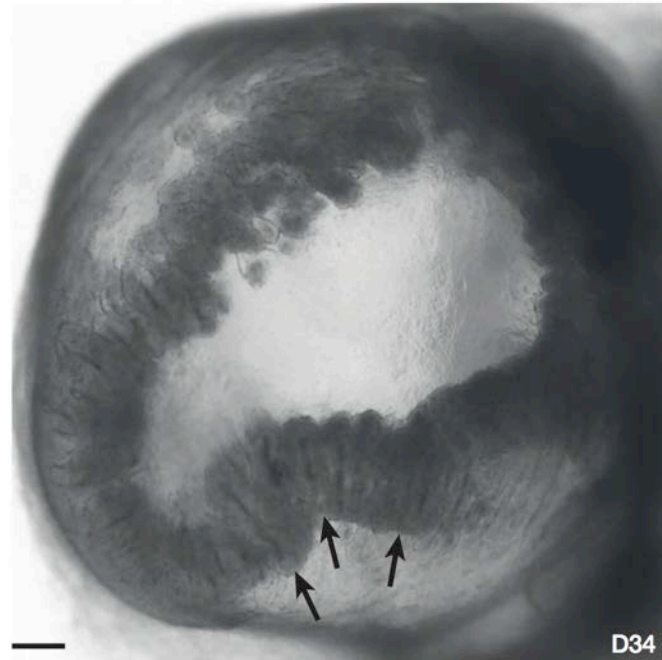
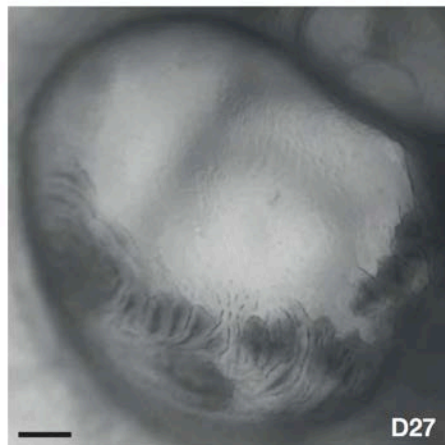
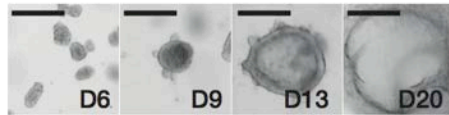


Gastric organoid production

a

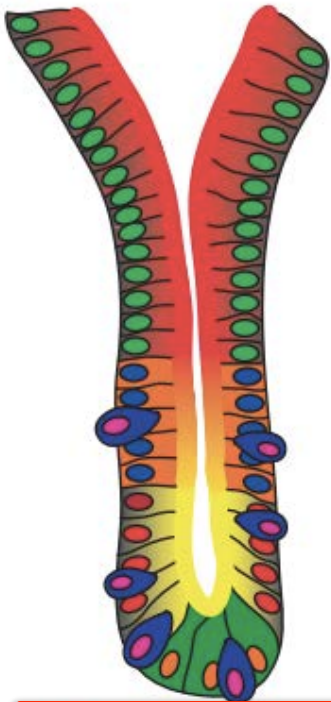


e



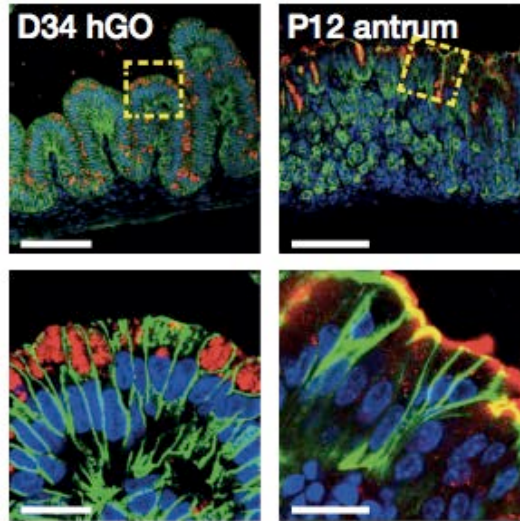
hGOs contain all stomach epithelial cell types

a



b

MUC5AC CDH1 DAPI



Surface mucous (pit) cell
MUC5AC, TFF1, GKN1

Mucous gland (neck) cell
MUC6, TFF2

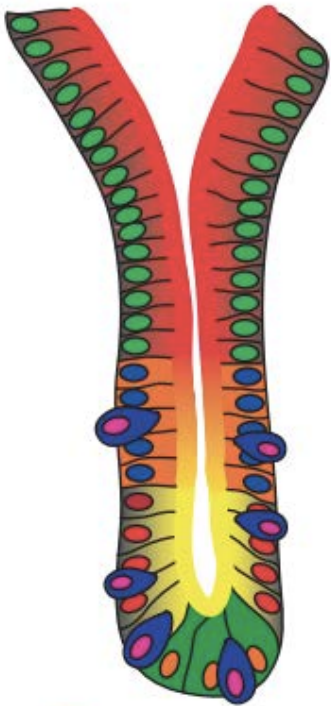
Endocrine cell
GAST, GHRL, SST, 5-HT

Progenitor cell
SOX9

Stem cell
LGR5

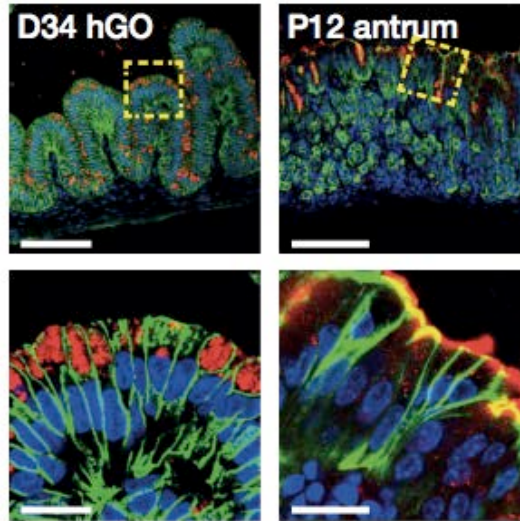
hGOs contain all stomach epithelial cell types

a



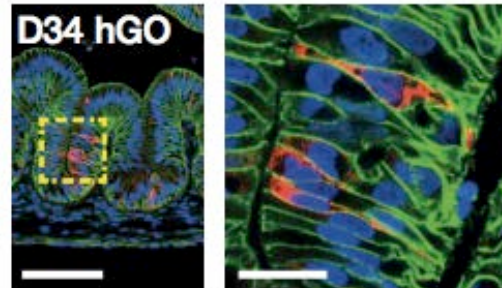
b

MUC5AC CDH1 DAPI



c

CTNNB1
MUC6 DAPI



Surface mucous (pit) cell
MUC5AC, TFF1, GKN1

Mucous gland (neck) cell
MUC6, TFF2

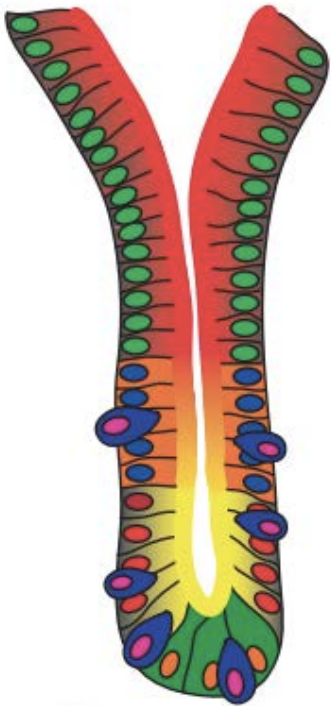
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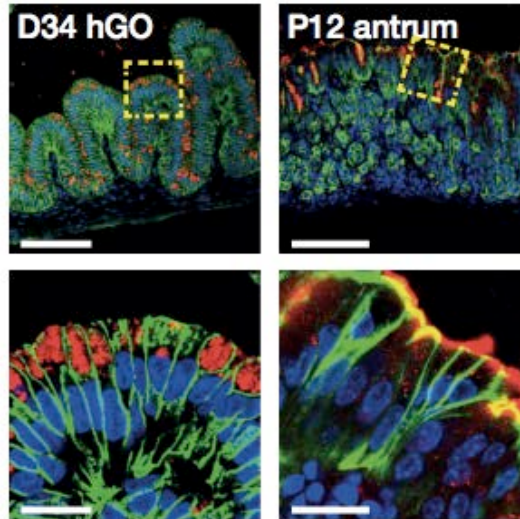
Endocrine cell
GAST, GHRL, SST, 5-HT

Progenitor cell
SOX9

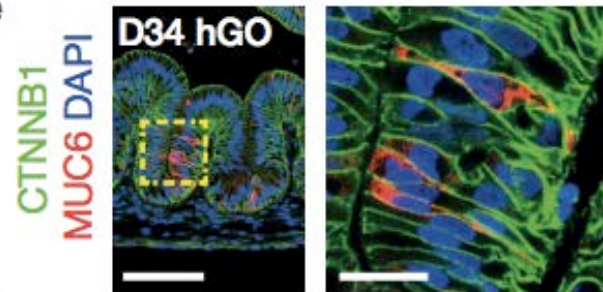
Stem cell
LGR5

b

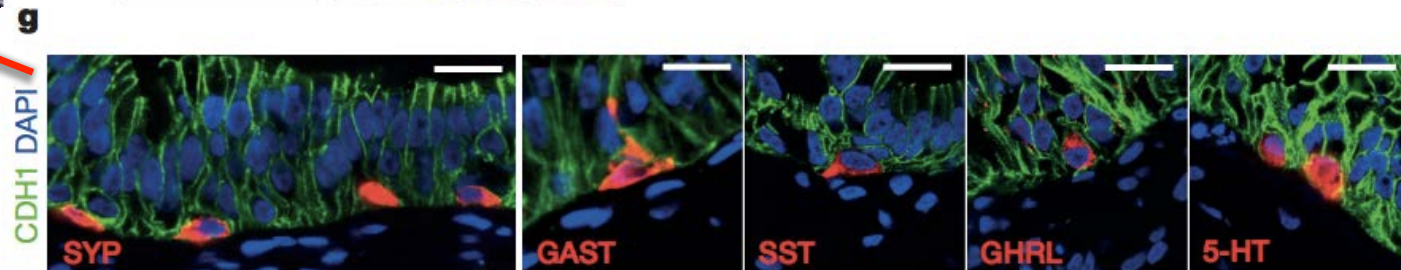
MUC5AC CDH1 DAPI



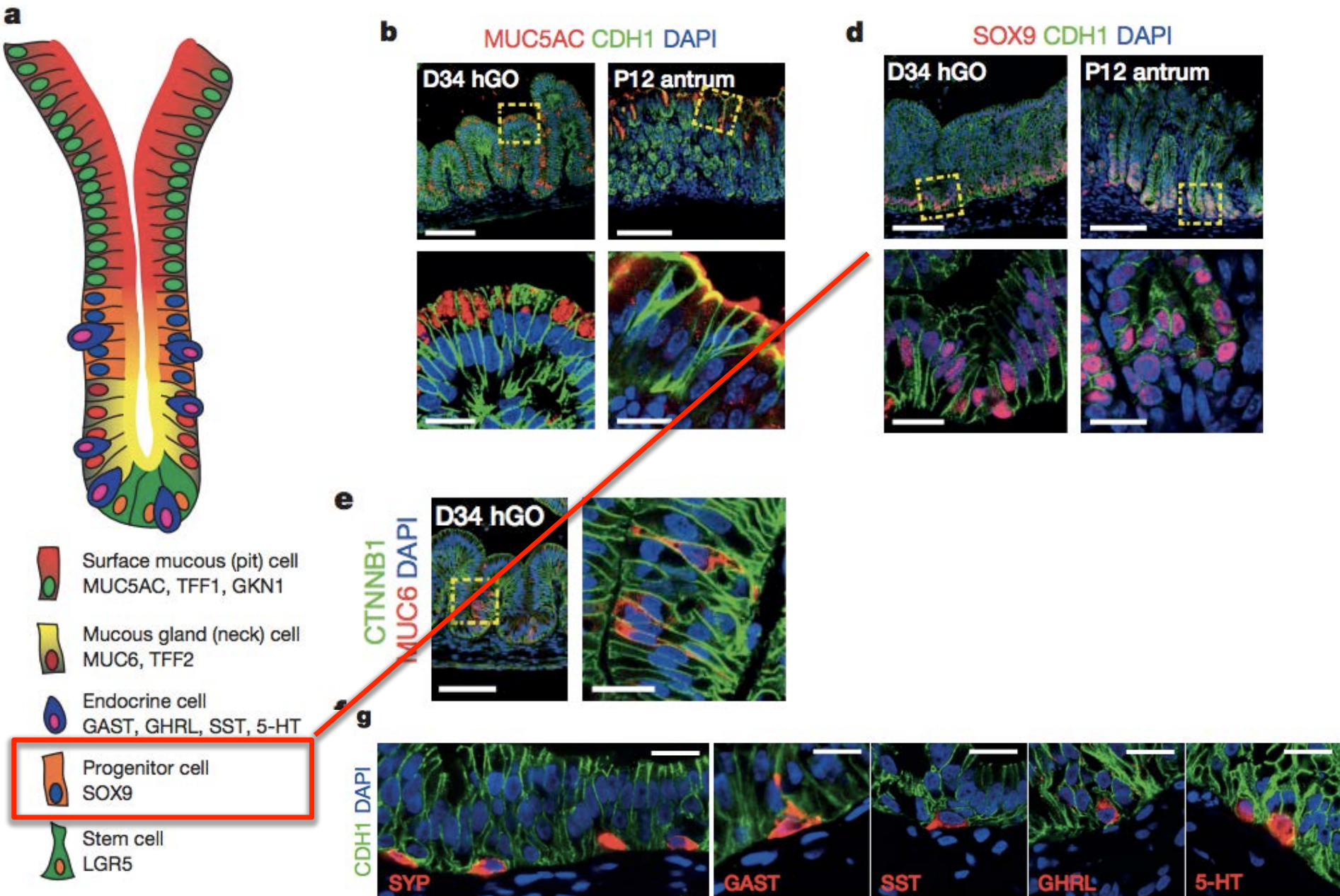
c



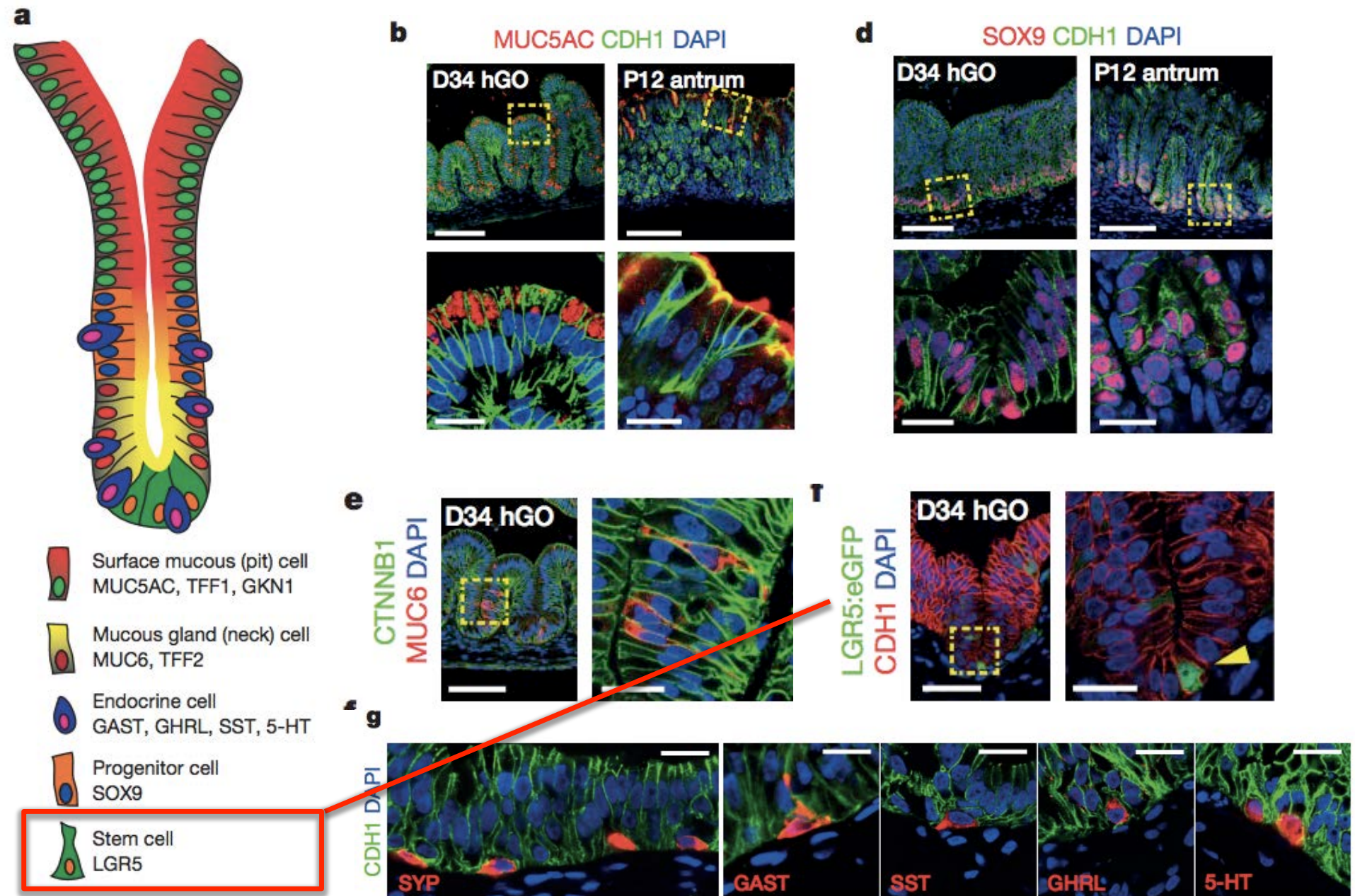
d



hGOs contain all stomach epithelial cell types

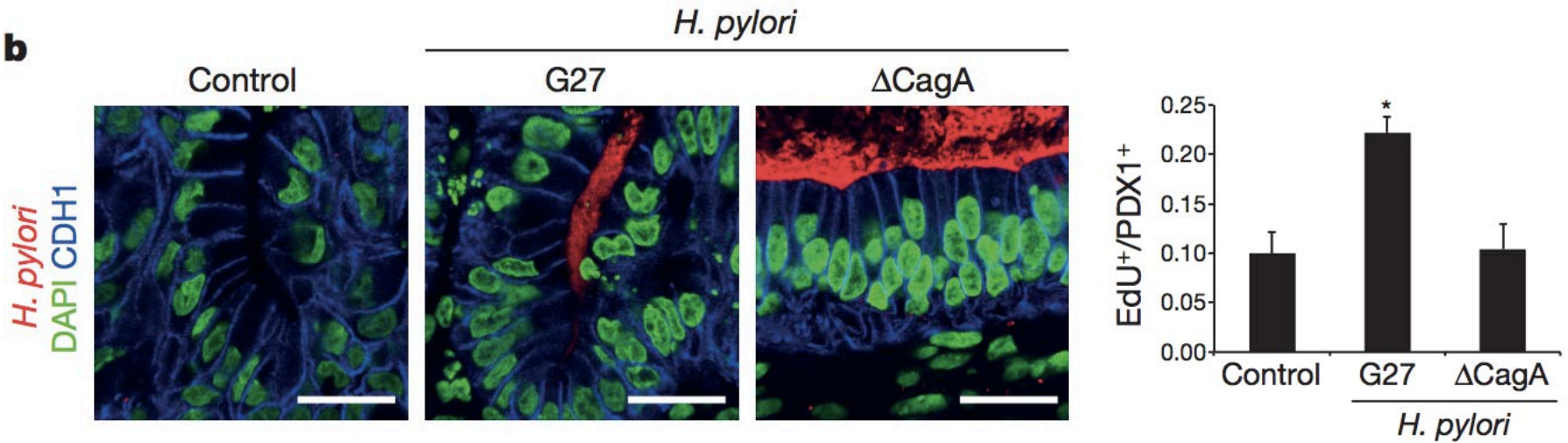


hGOs contain all stomach epithelial cell types



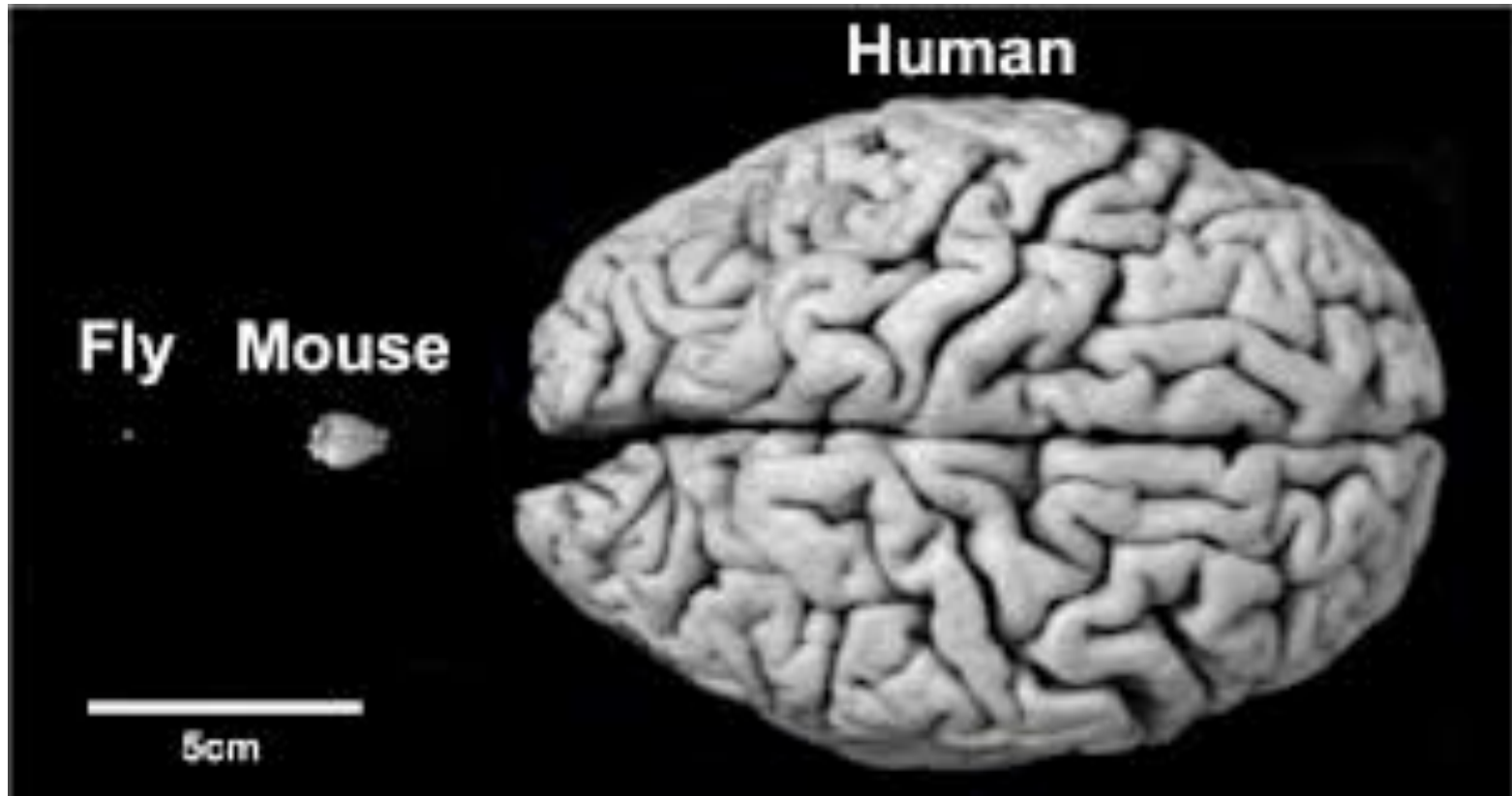
Disease Application (*H. pylori*)

b



Activates proliferation of the gastric cells through the activation of MET signaling

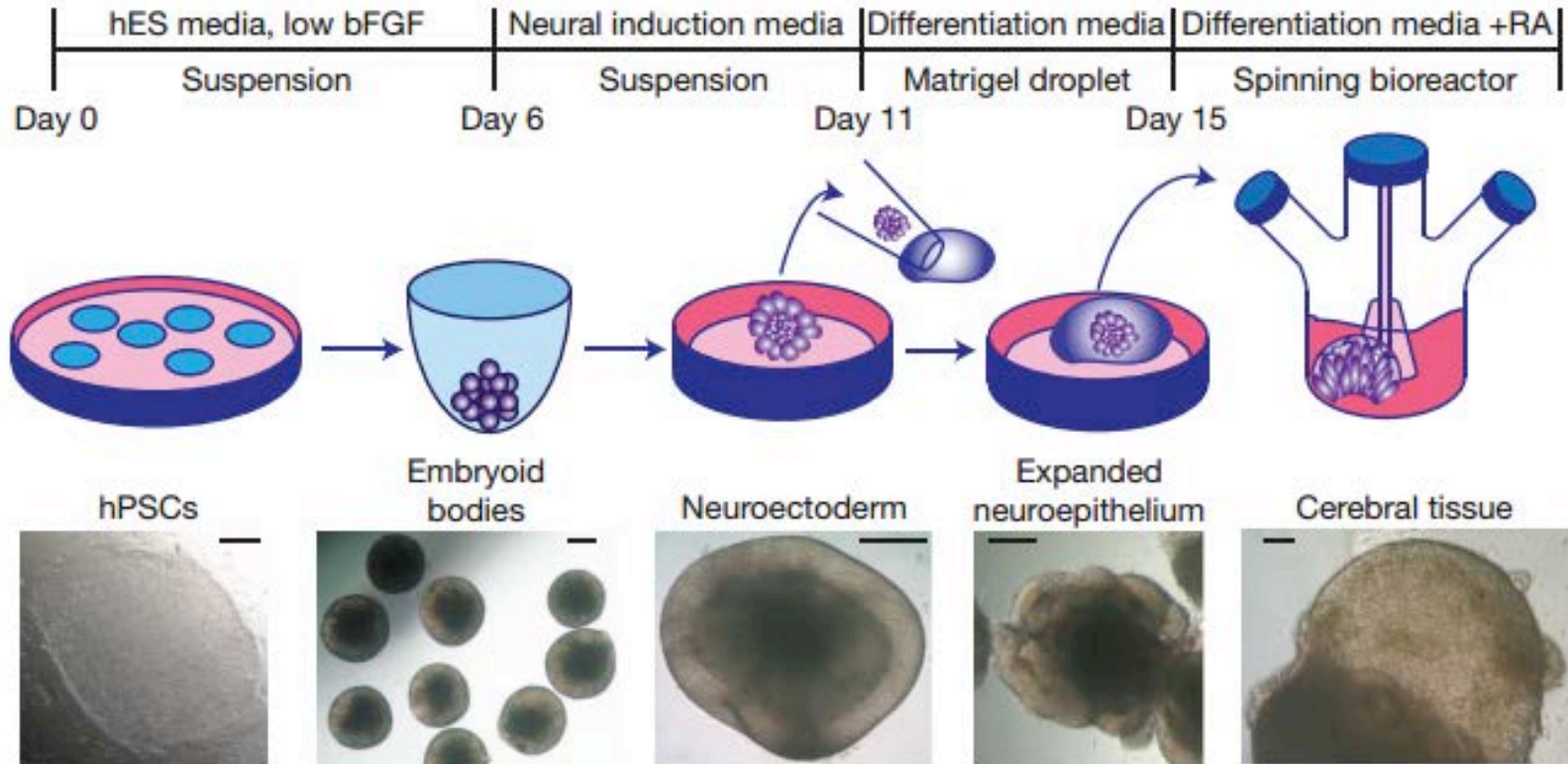
Human brain diseases are difficult to study in mice



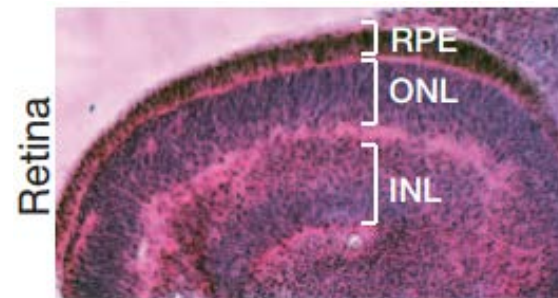
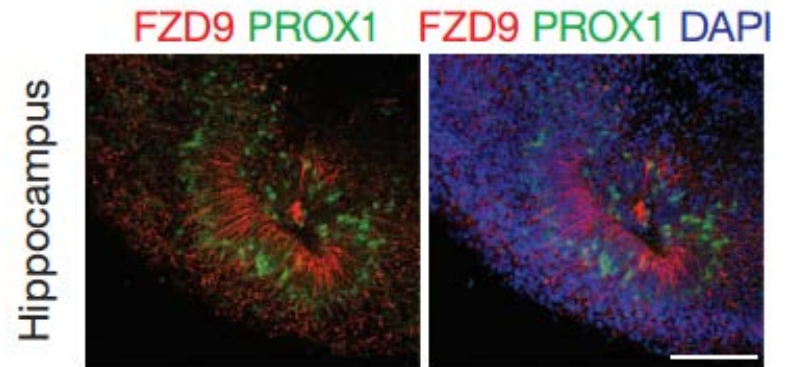
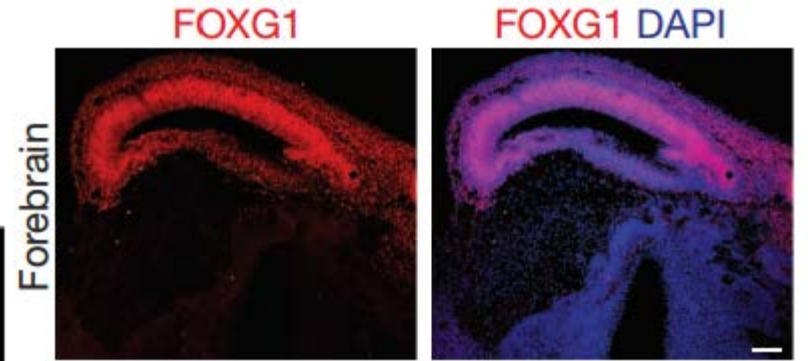
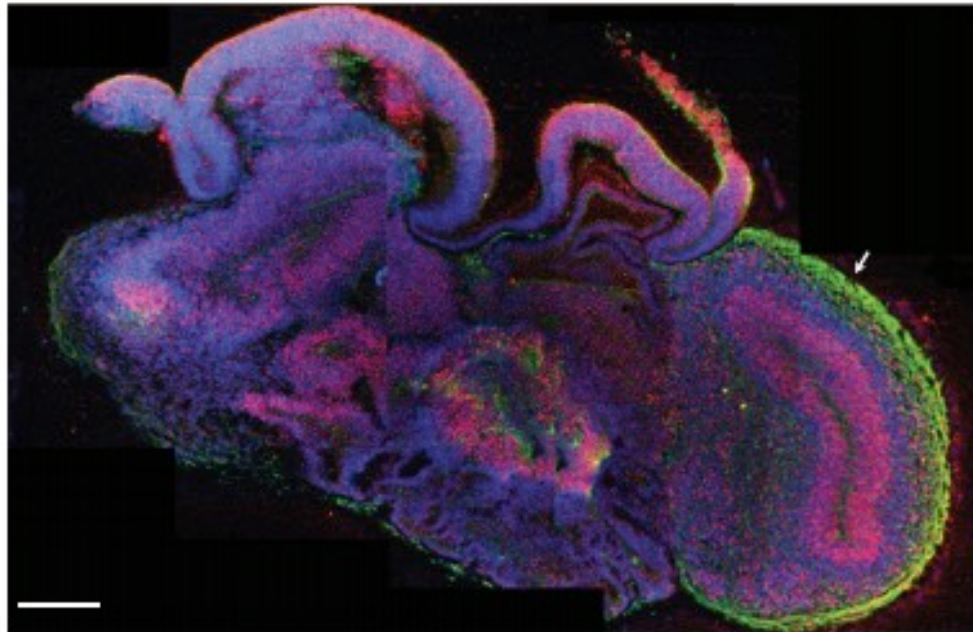
Brain Disorders are Prevalent but Difficult to Study

- Nearly half of all people will be diagnosed with a mental disorder in their lifetime (ADHD, mental retardation, Alzheimer's, major depression, Parkinson's)
- Most disorders do not have a good model

Cerebral Organoids Recapitulate Brain Differentiation

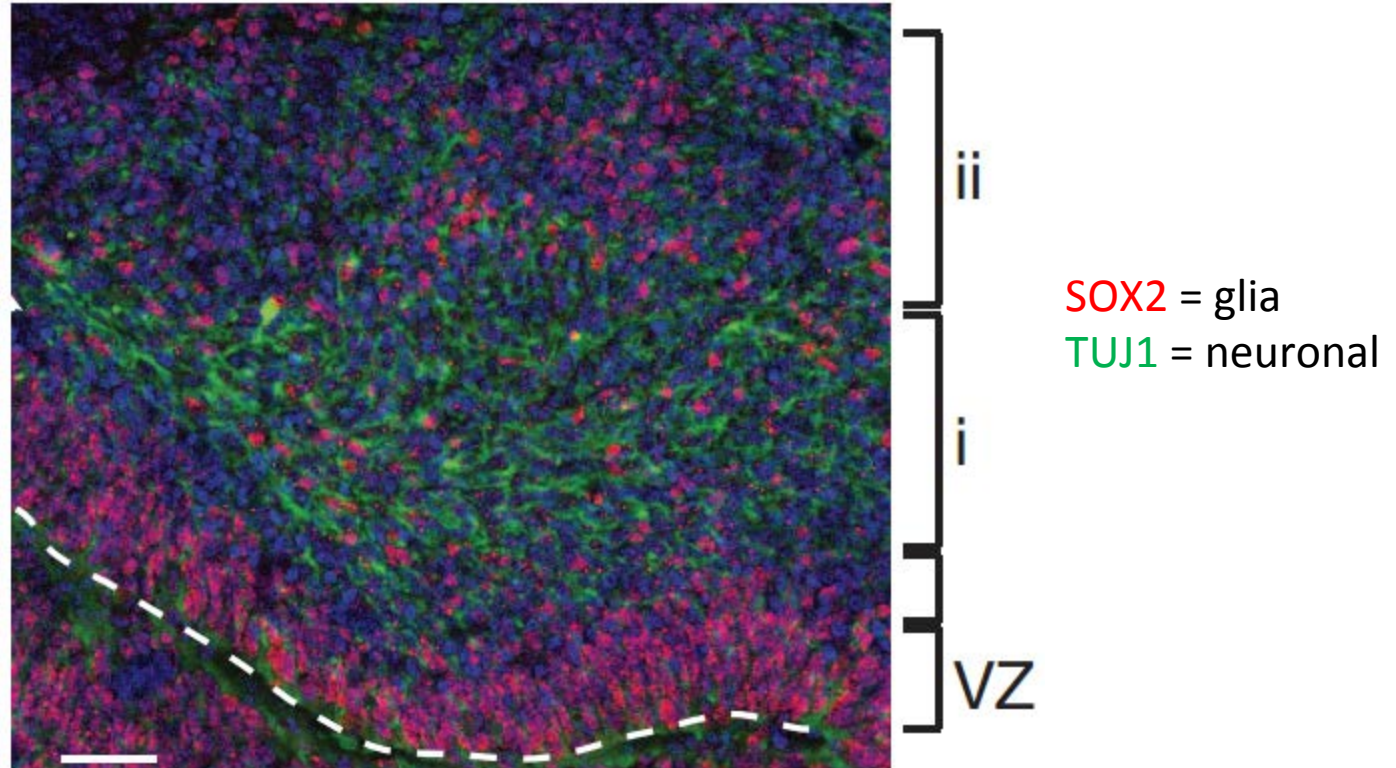


Cerebral Organoids Recapitulate Brain Differentiation



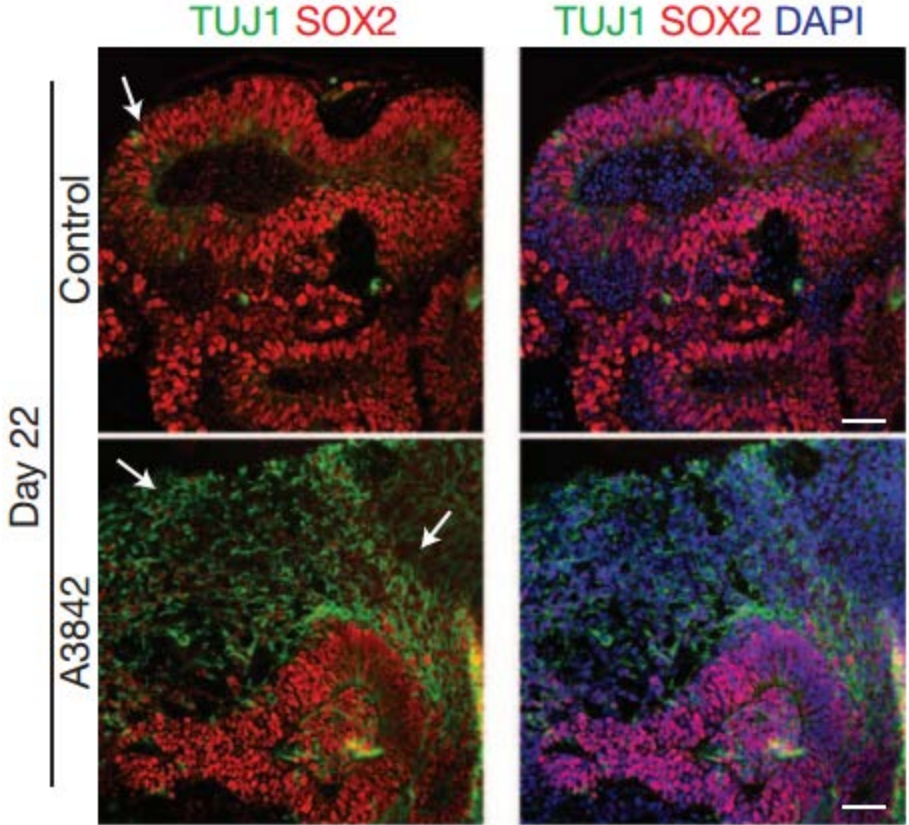
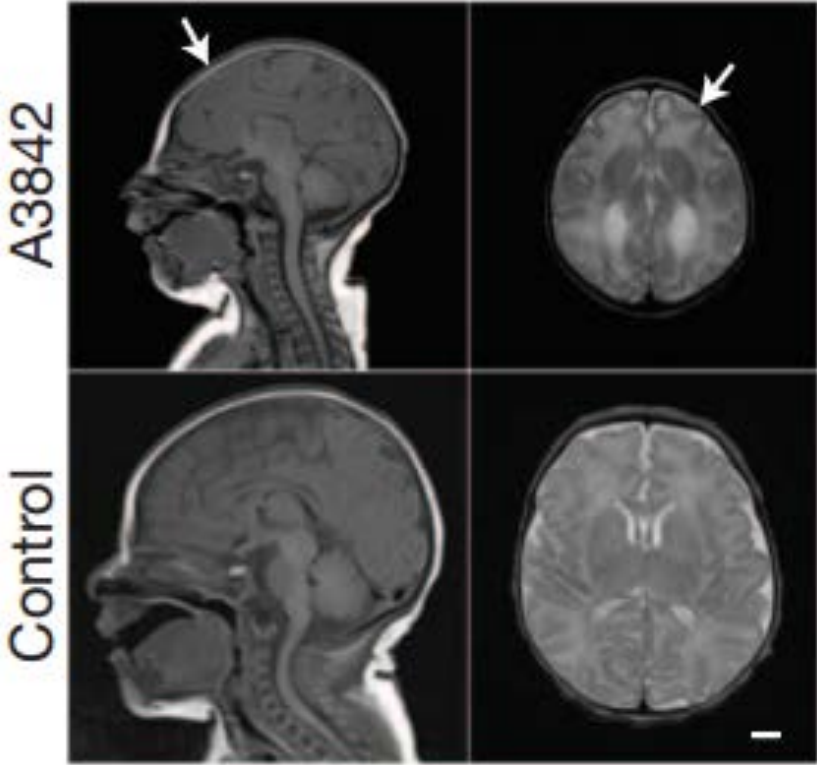
Cerebral Organoids Recapitulate Human Brain Architecture

TUJ1 SOX2 Hoechst

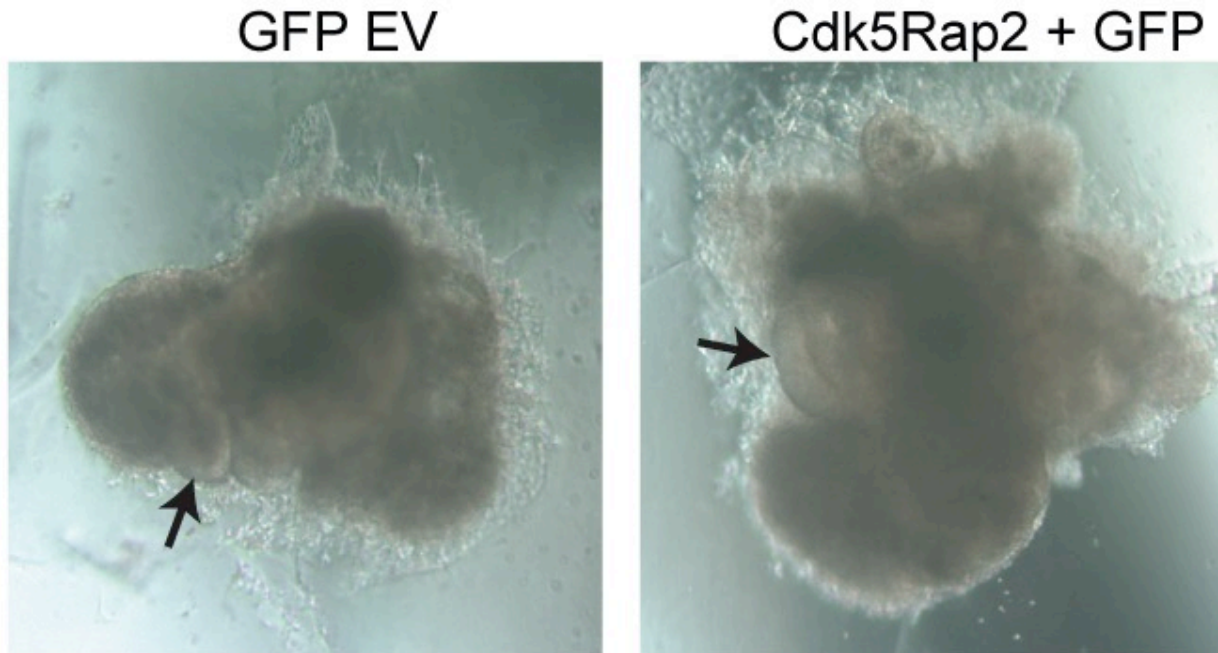


OSVZ region in human cerebral organoids

Cerebral Organoids Model Microcephaly

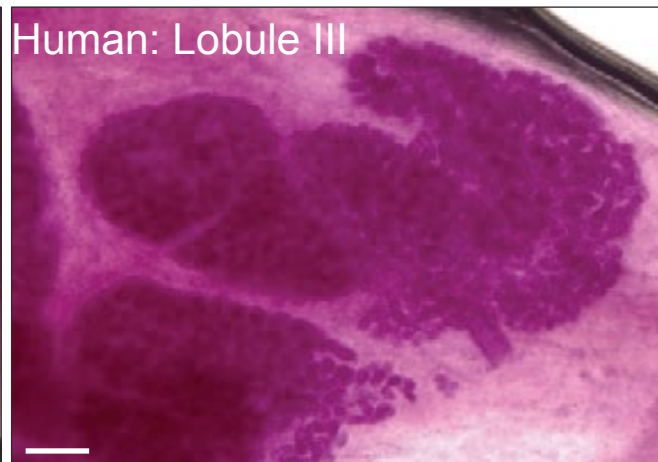
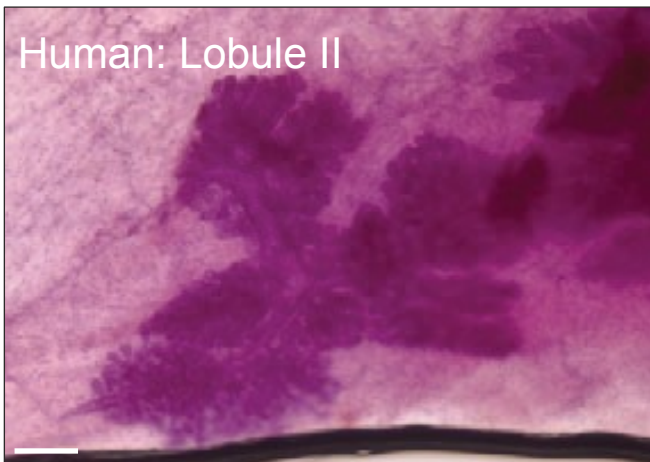
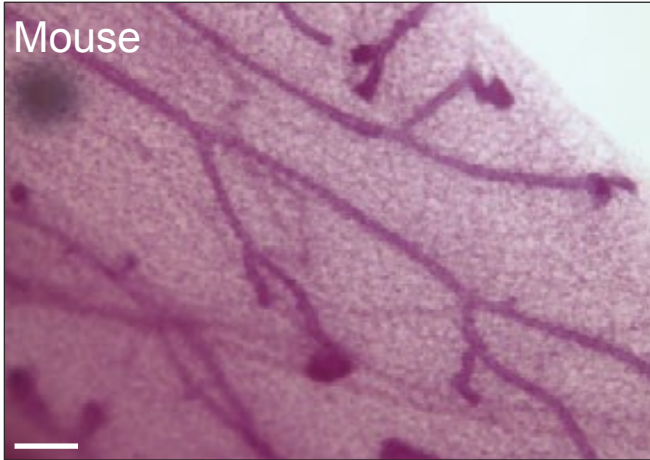


Developmental Phenotypes Rescued with CDK5RAP2

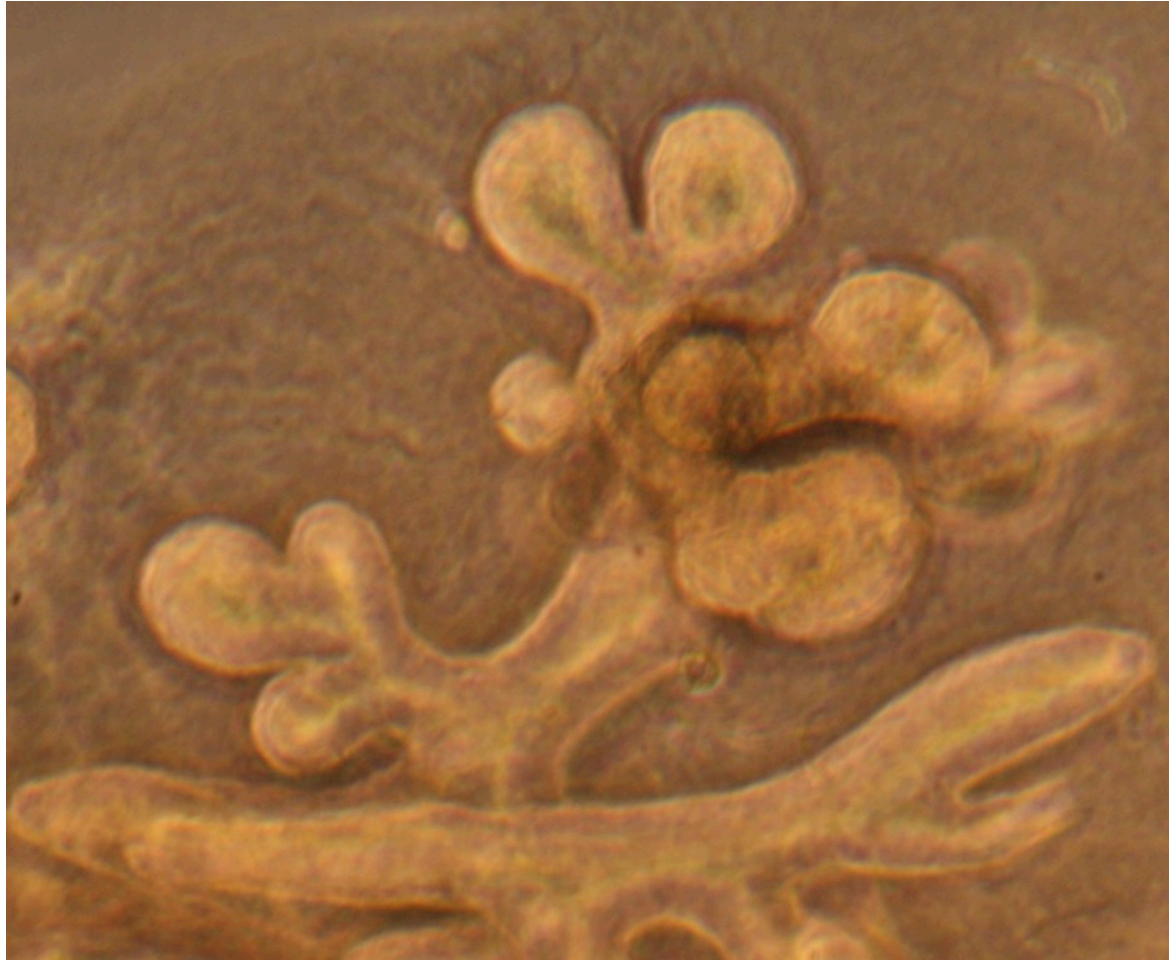


Larger organoids, more stem and progenitor regions

Mouse v Human Mammary Gland



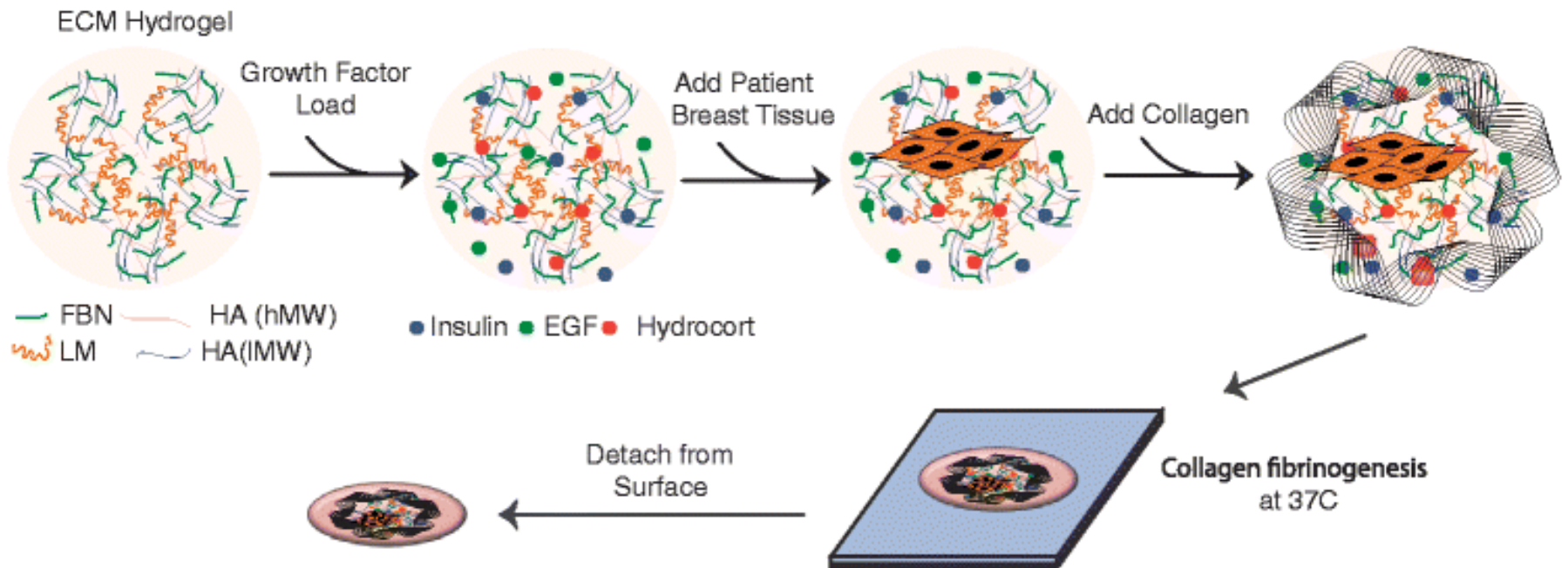
Immortalized Mammary Cell Lines can Grow into Ductal Structures in Collagen Matrices



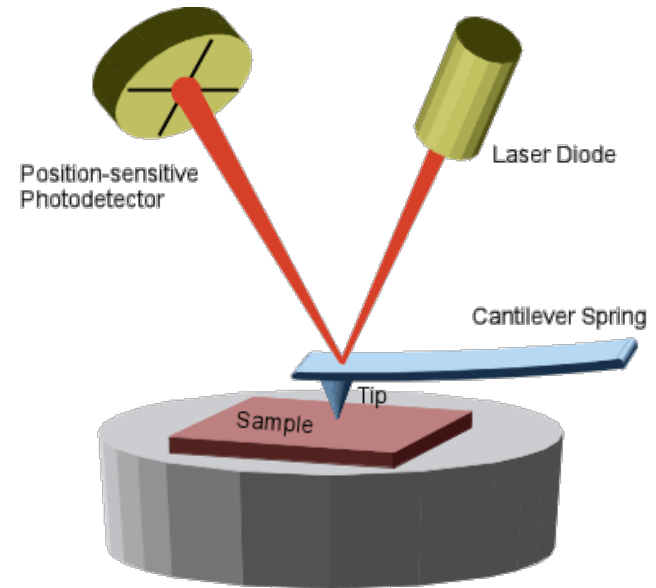
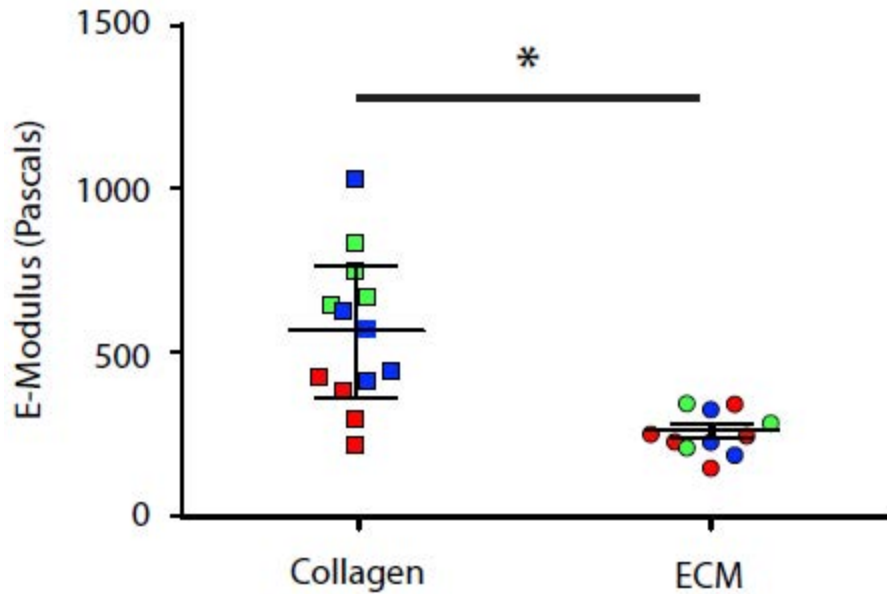
Design of 3D Culture System

- Extracellular proteins and carbohydrates in the mammary gland
- Growth factors for mammary development
- Physical elasticity similar to the mammary gland

Design of 3D Culture System: ECM Hydrogels



Engineered ECM Hydrogels Exhibit Similar Elasticities to Human Mammary Tissue



Day 4 tissues



Day 5 tissues



Day 6 tissues



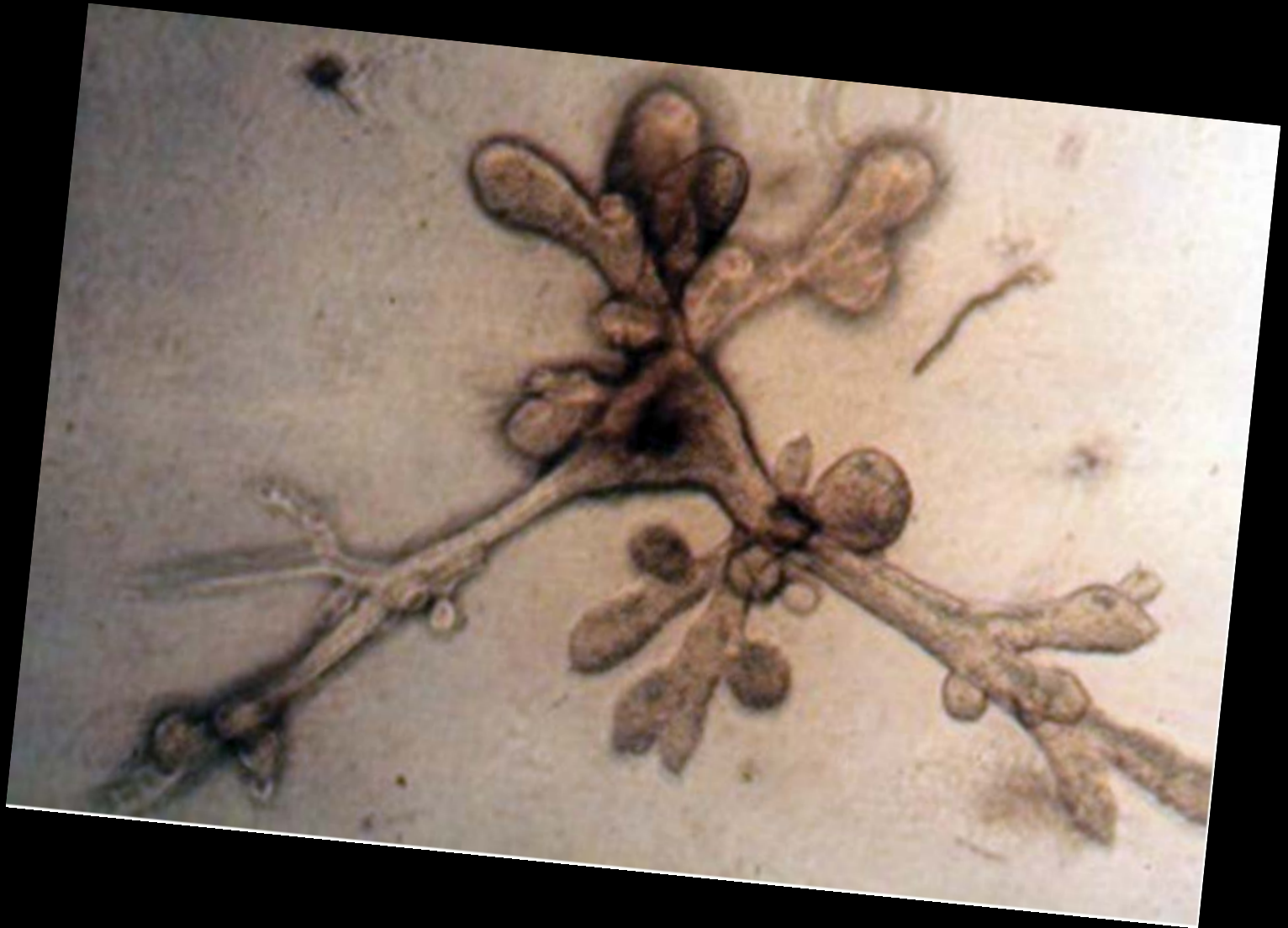
Day 7 tissues



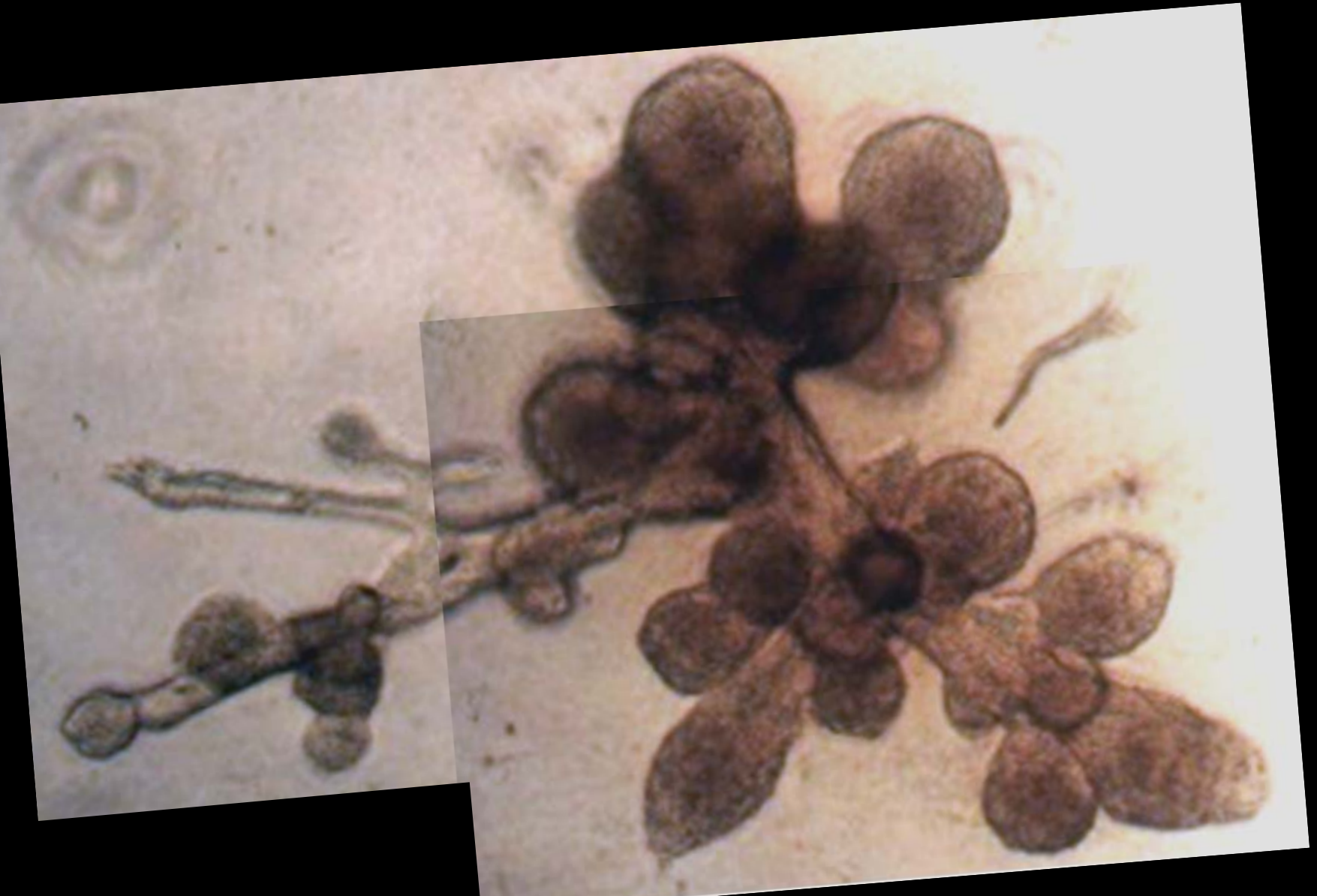
Day 8 tissues



Day 10 tissues

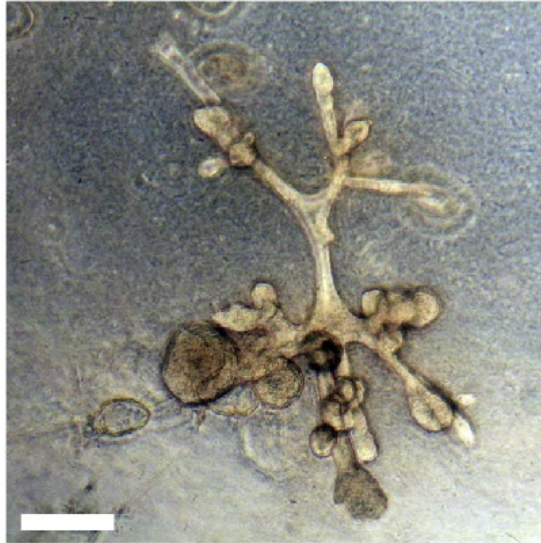


Day 12 tissues

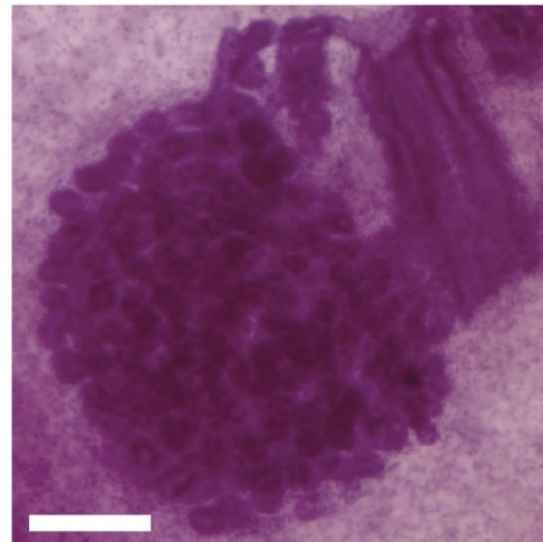
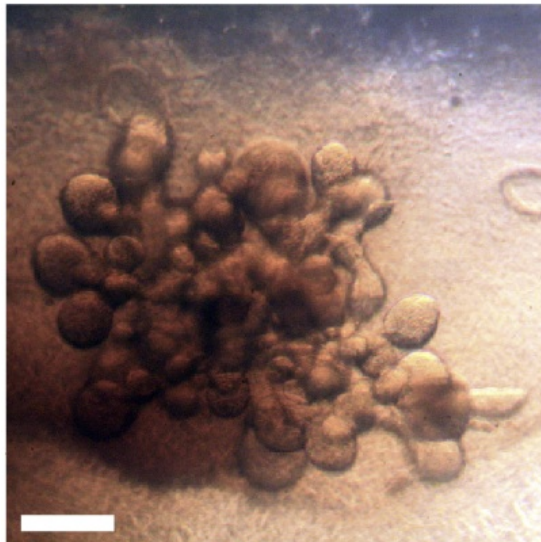
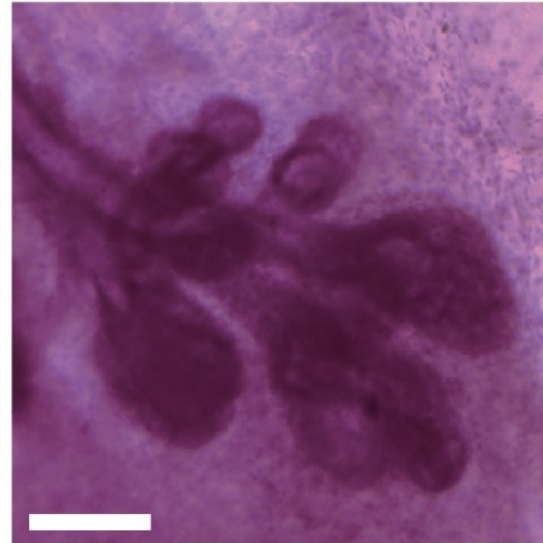


The tissues mimic the organization of the human gland

ECM hydrogel



Human tissue



The tissues differentiate into **luminal** and **basal** lineages



Luminal cells (CK8/18)
are cells that
produce milk and line
the ducts

Basal cells (CK14)
contract and push
milk toward the nipple

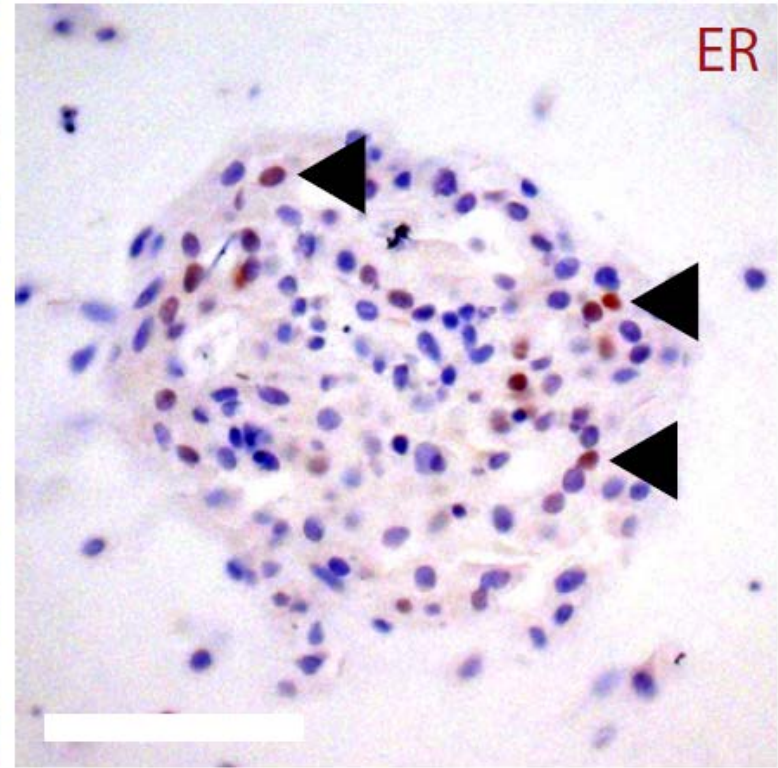
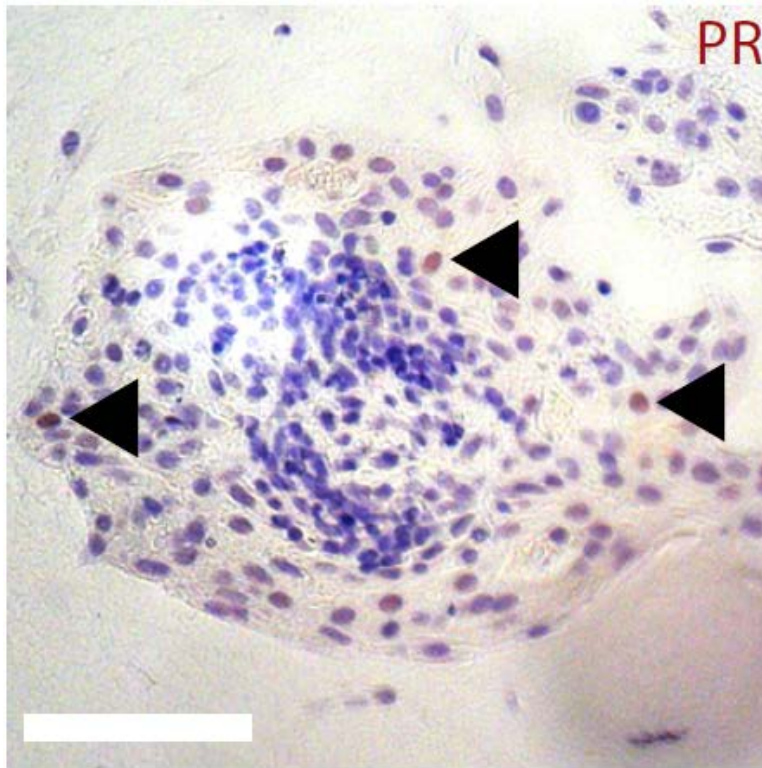
The tissues differentiate into **luminal** and **basal** lineages



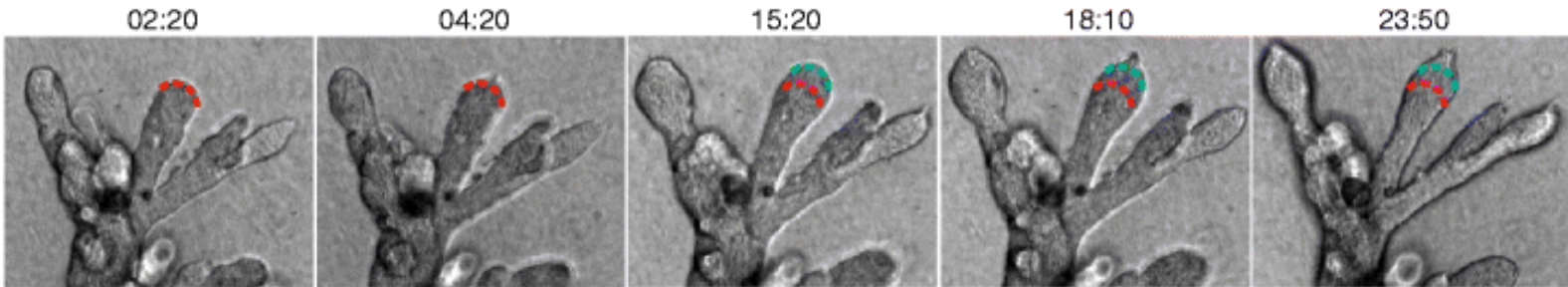
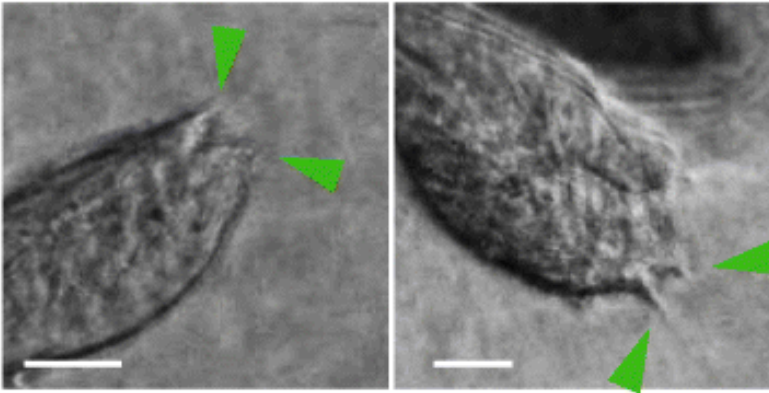
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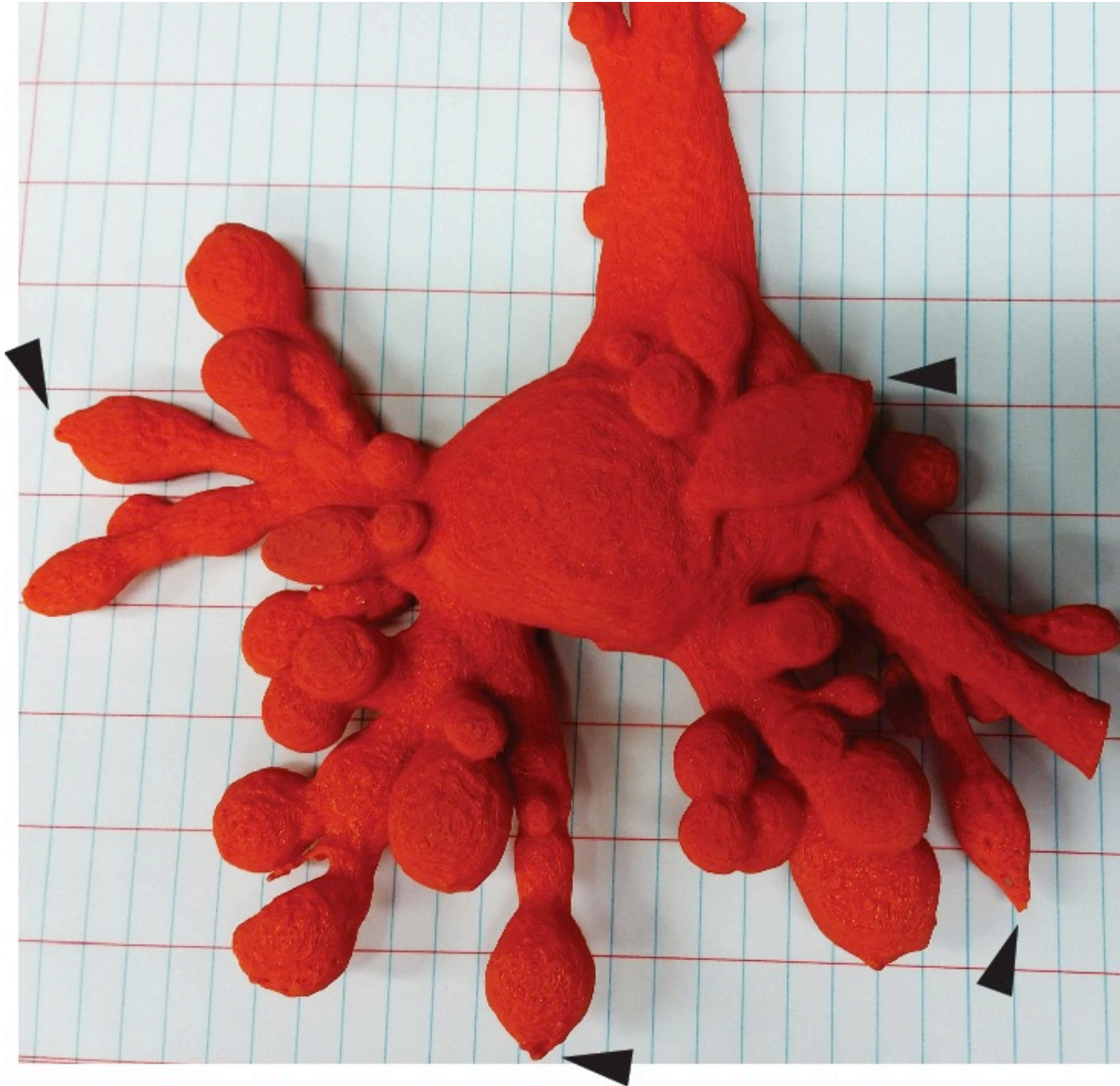
The tissues maintain hormone receptor (ER, PR) expression



Time lapse movies to discover how these tissues grow

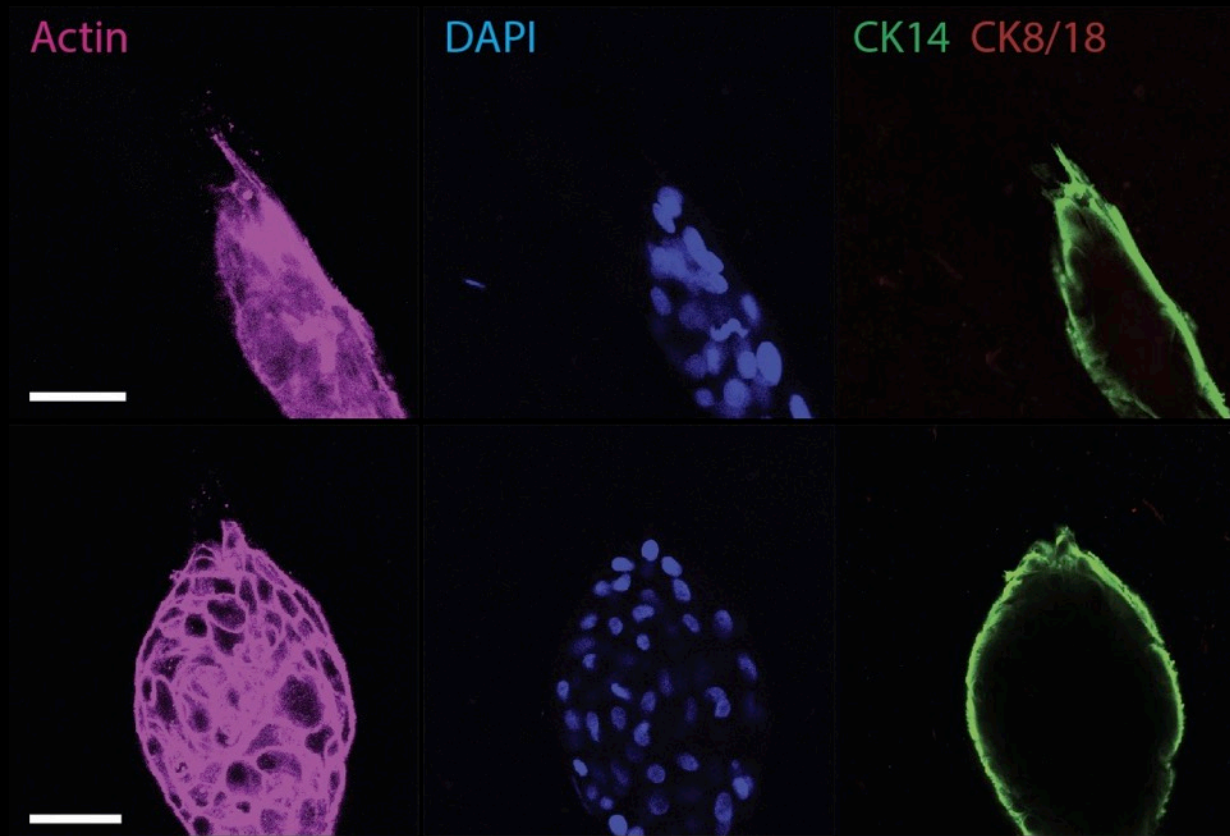


Leader cells drive ductal elongation

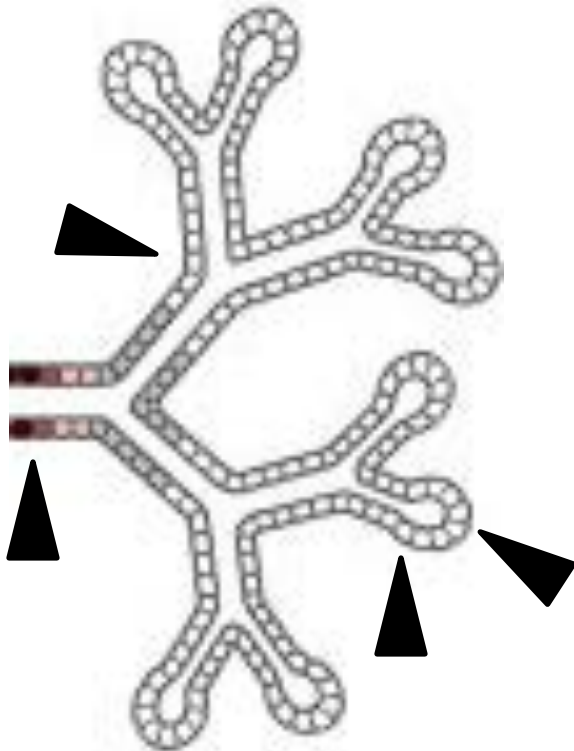


Evidence of leader cells
in 3D printed model

These leader cells are **actin**-rich **basal** cells



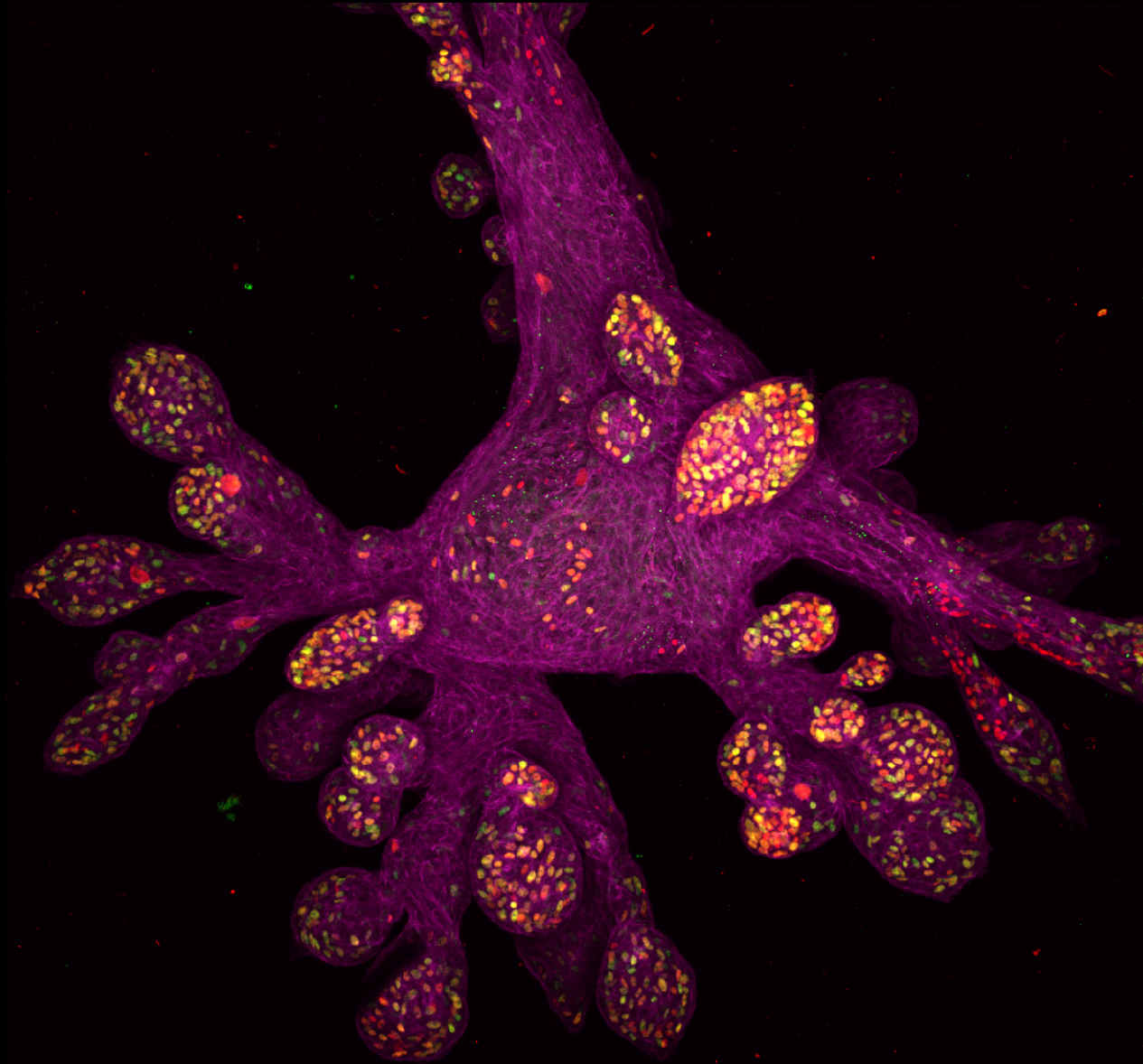
Where are the stem cells localized?



Stem cells may feed the pool of leader cells at the growing tip

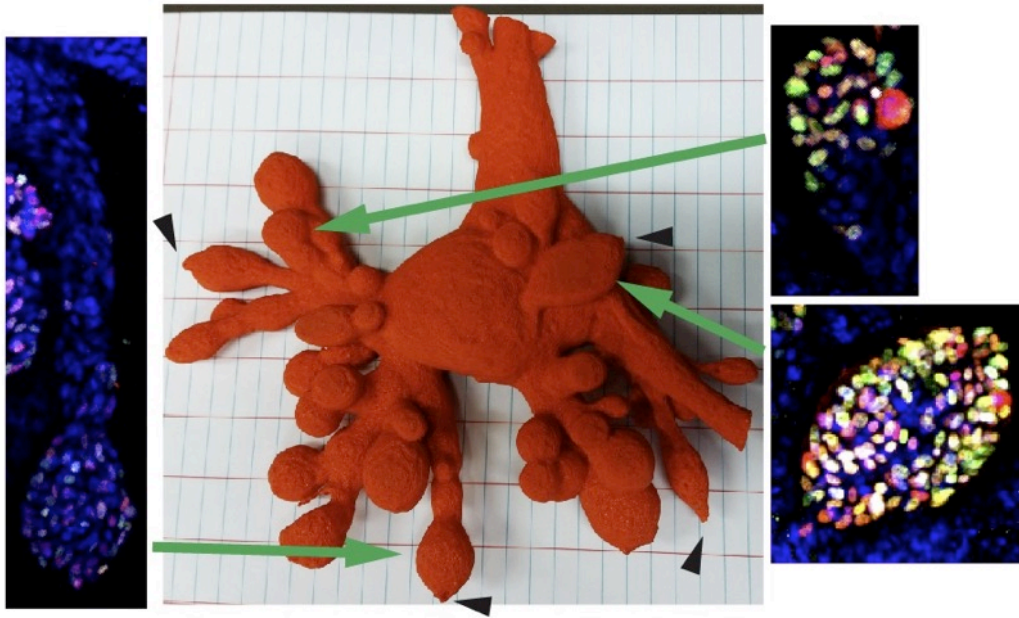
Could be localized behind the growing bud, at branch points, or at the base of the structures

Stem cells are localized to the growing tips



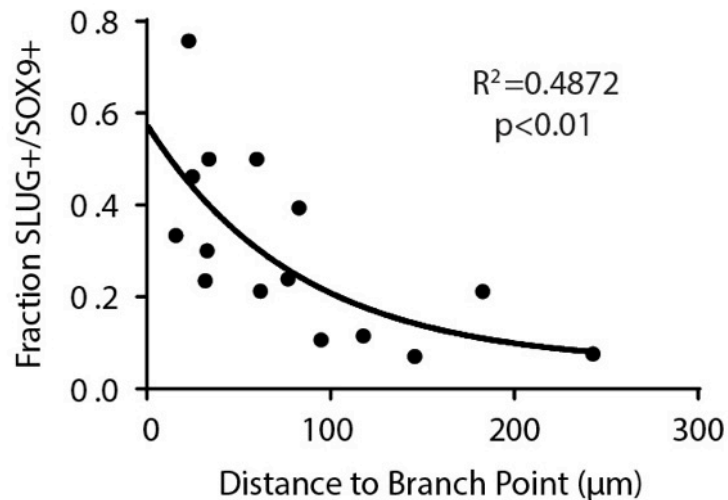
SLUG and **SOX9** – stem cell markers in the mouse mammary gland

Stem cells are localized to the growing tips



New outgrowths are strongly enriched for stem cells

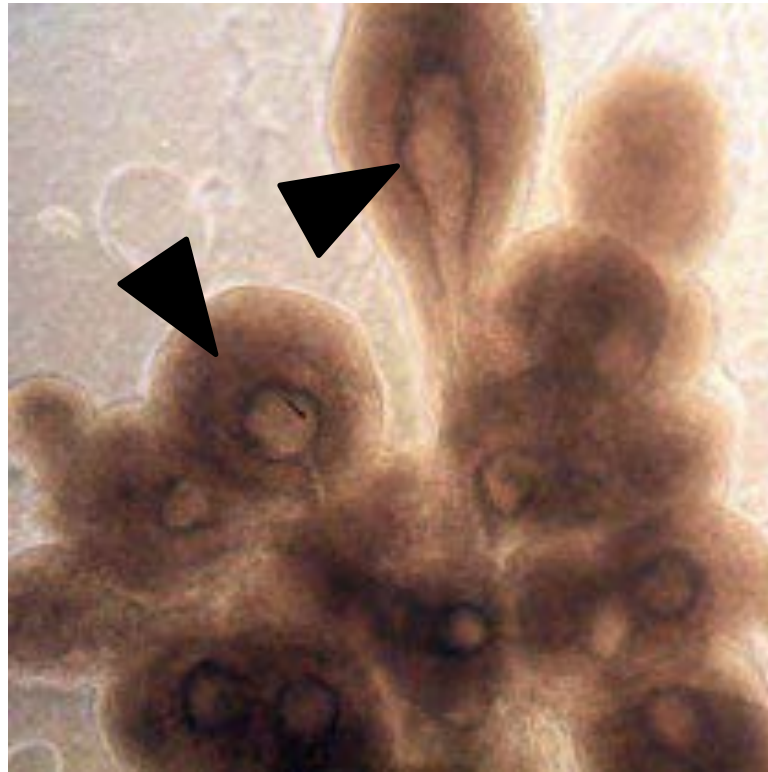
Longer, more differentiated ducts have fewer stem cells



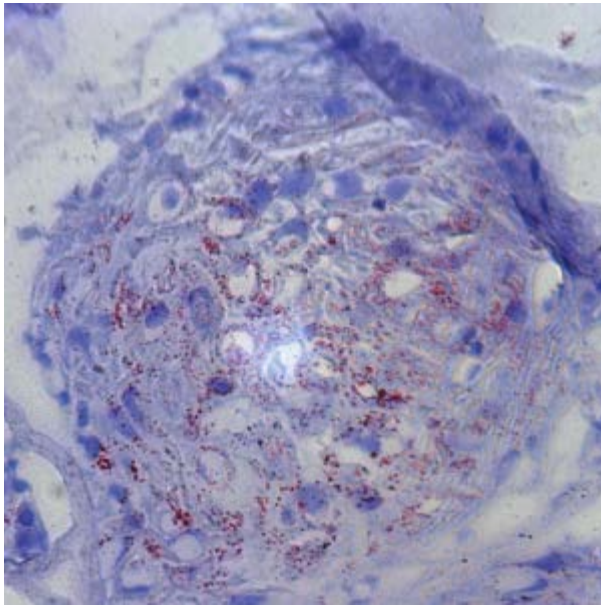
Next Steps

- Explore the hormone responsiveness of the tissues to understand on an epithelial-intrinsic basis how hormones are functioning

Estrogen and Progesterone Lead to Differentiation and Hollowing of the Tissues



Prolactin addition promotes human milk production

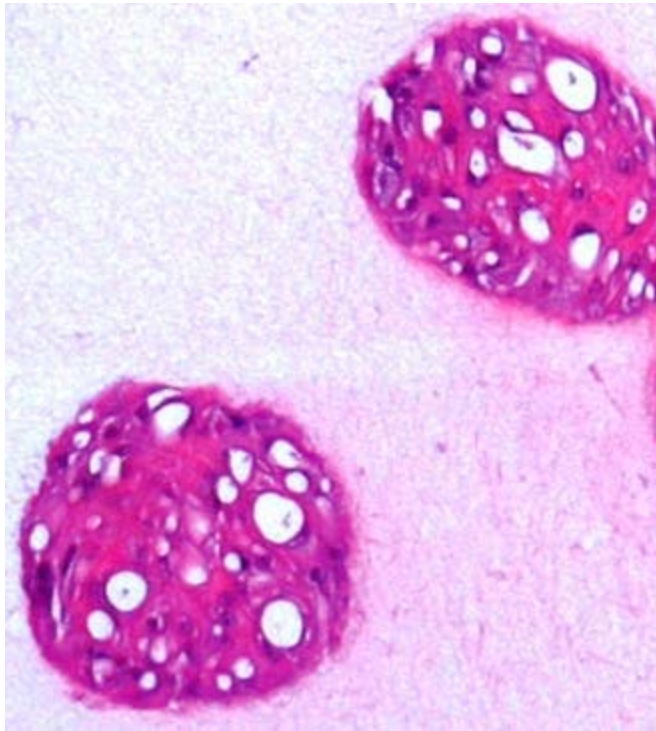


Oil Red O

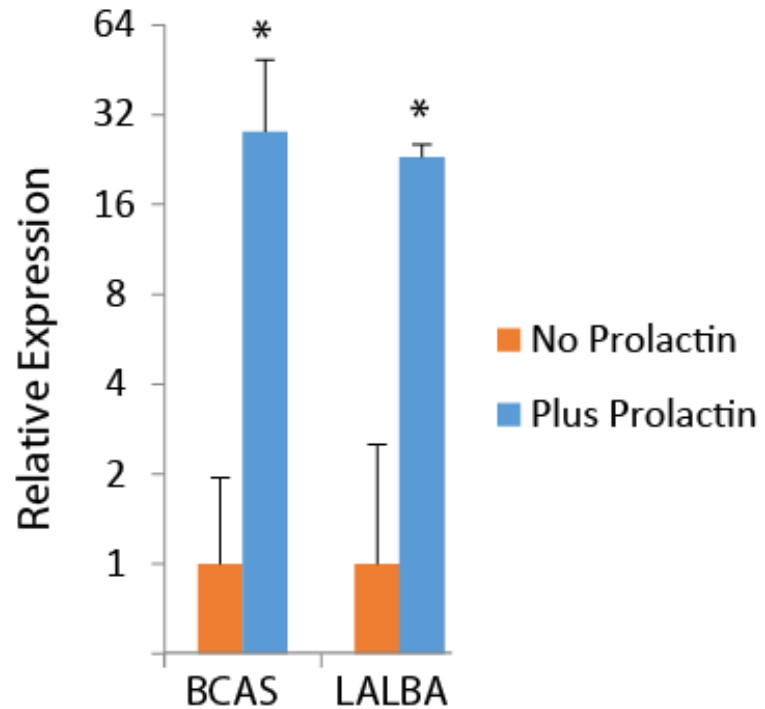


Brightfield (Milky substance in lobules)

Prolactin addition promotes human milk production



H&E: Lipid Droplets



qPCR: Beta Casein and Lactalbumin

Acknowledgements

Gupta Lab

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Cima Lab

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Tom DiCesare

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