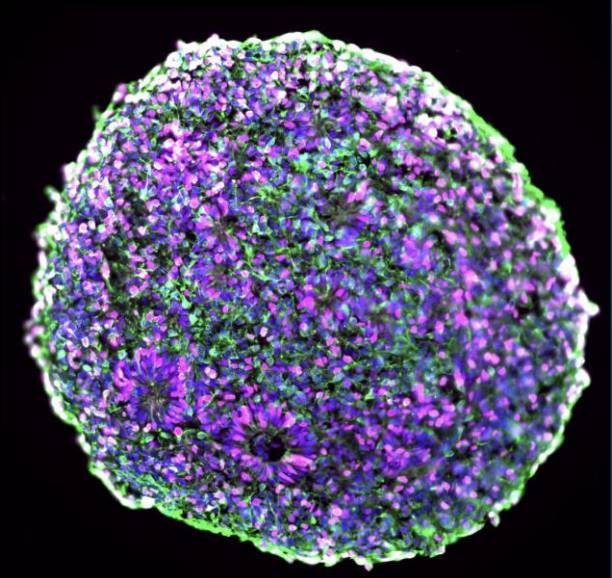


# Human Cellular Models and Their Application in RARE Drug Development

Angels Almenar-Queralt, Ph.D.
Project Scientist, UC San Diego
aalmenar@ucsd.edu

*Image credit: Surya Venogupal*

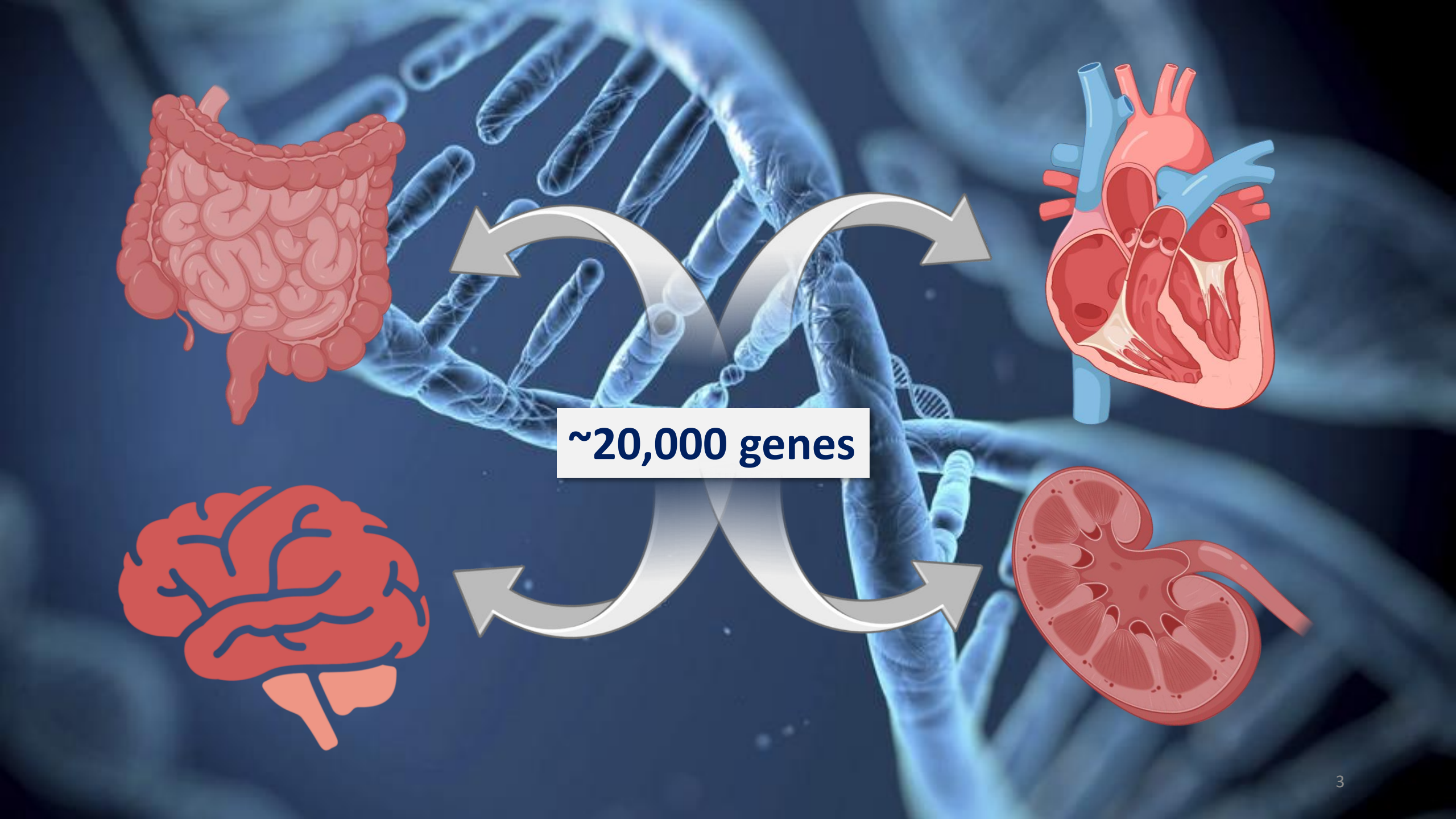
# Agenda

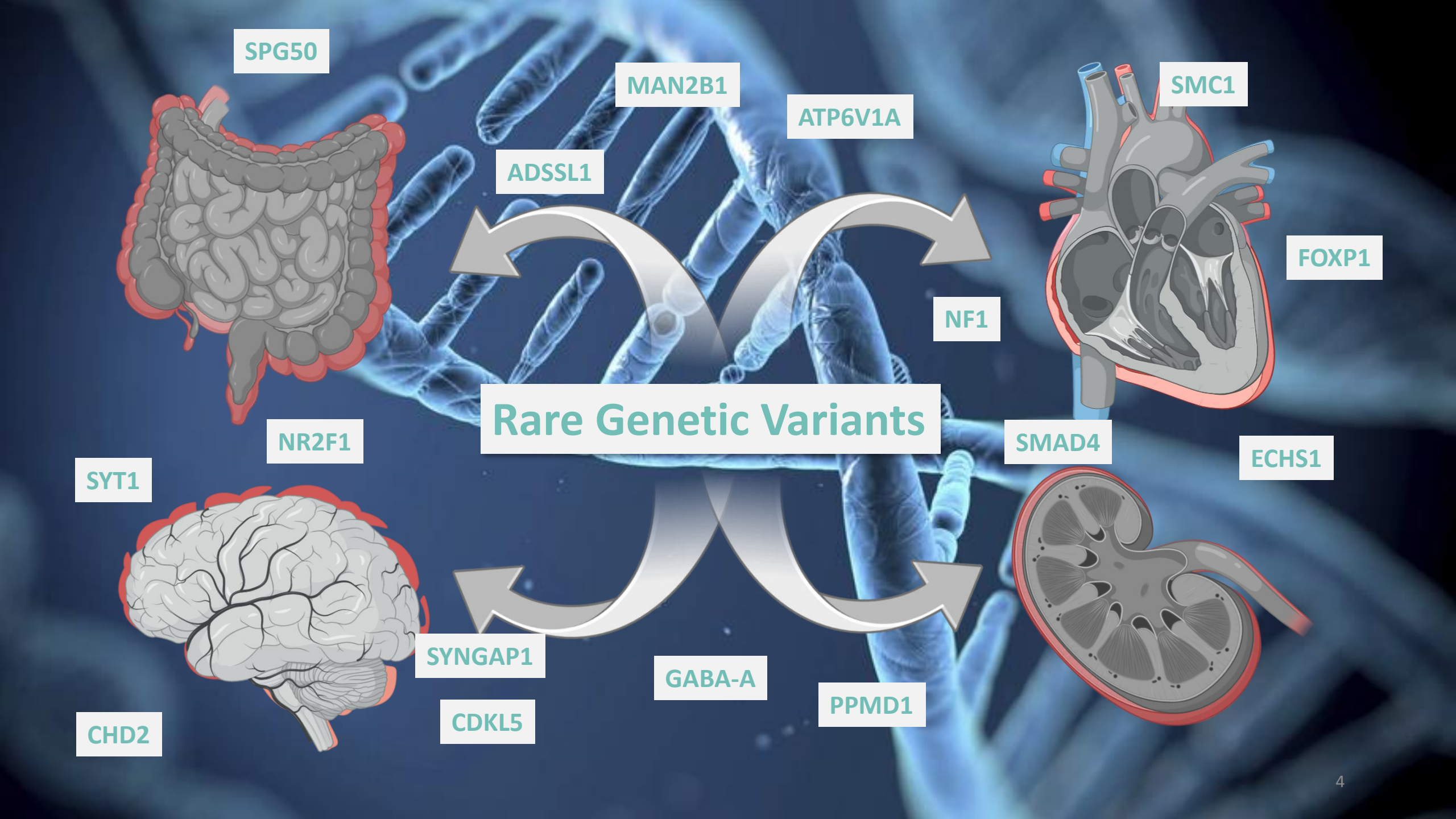
INTRODUCTION

OVERVIEW OF HUMAN CELL CULTURES

MODELING DISEASES IN BRAIN ORGANOIDS

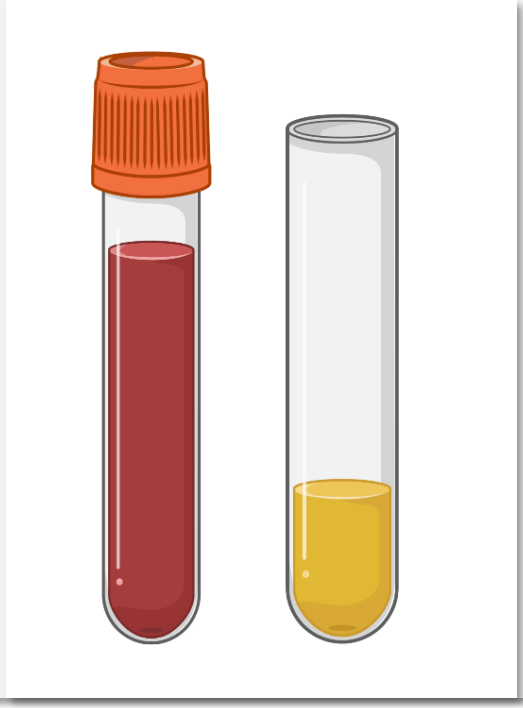
CONCLUSIONS


~20,000 genes


SPG50
MAN2B1
ATP6V1A
SMC1
ADSSL1
FOXP1
NF1
Rare Genetic Variants
NR2F1
SMAD4
ECHS1
SYT1
SYNGAP1
GABA-A
PPMD1
CDKL5
CHD2

# Inaccessibility to Living Human Tissue

| Biomarkers | Imaging | Postmortem Tissue |
| --- | --- | --- |
| Indirect Measurements | Low Resolution | Snapshots |



# Limited Predictivity of Animal Models

- **Species specific genetics**
- **Do not fully recapitulate disease complexity**
- **Shorter lifespans**
- **Size differences**
- **Controlled and limited experimental conditions**
- **Ethical concerns**

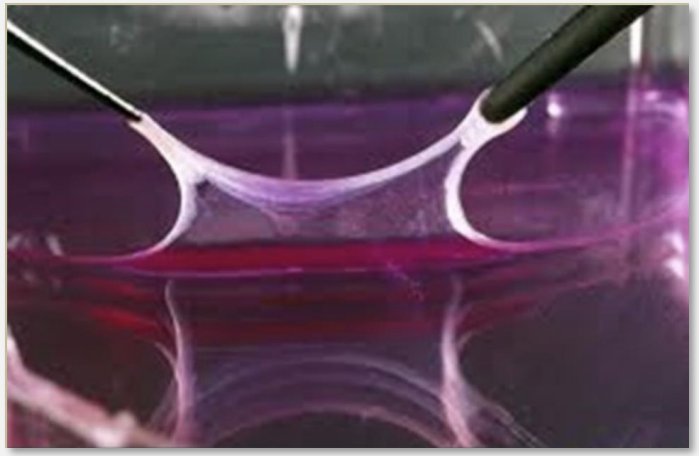
Low Predictivity:

Thalidomide
Alzheimer's vaccine
Traumatic brain injury
Aspirin

# Ex vivo Cultured Cells

## A century ago

Roux: Neural plate of chicken embryos for a few days

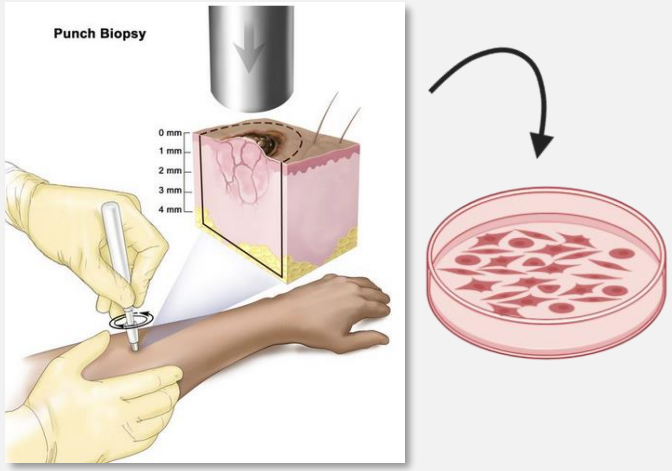


## Immortalized Cell Lines

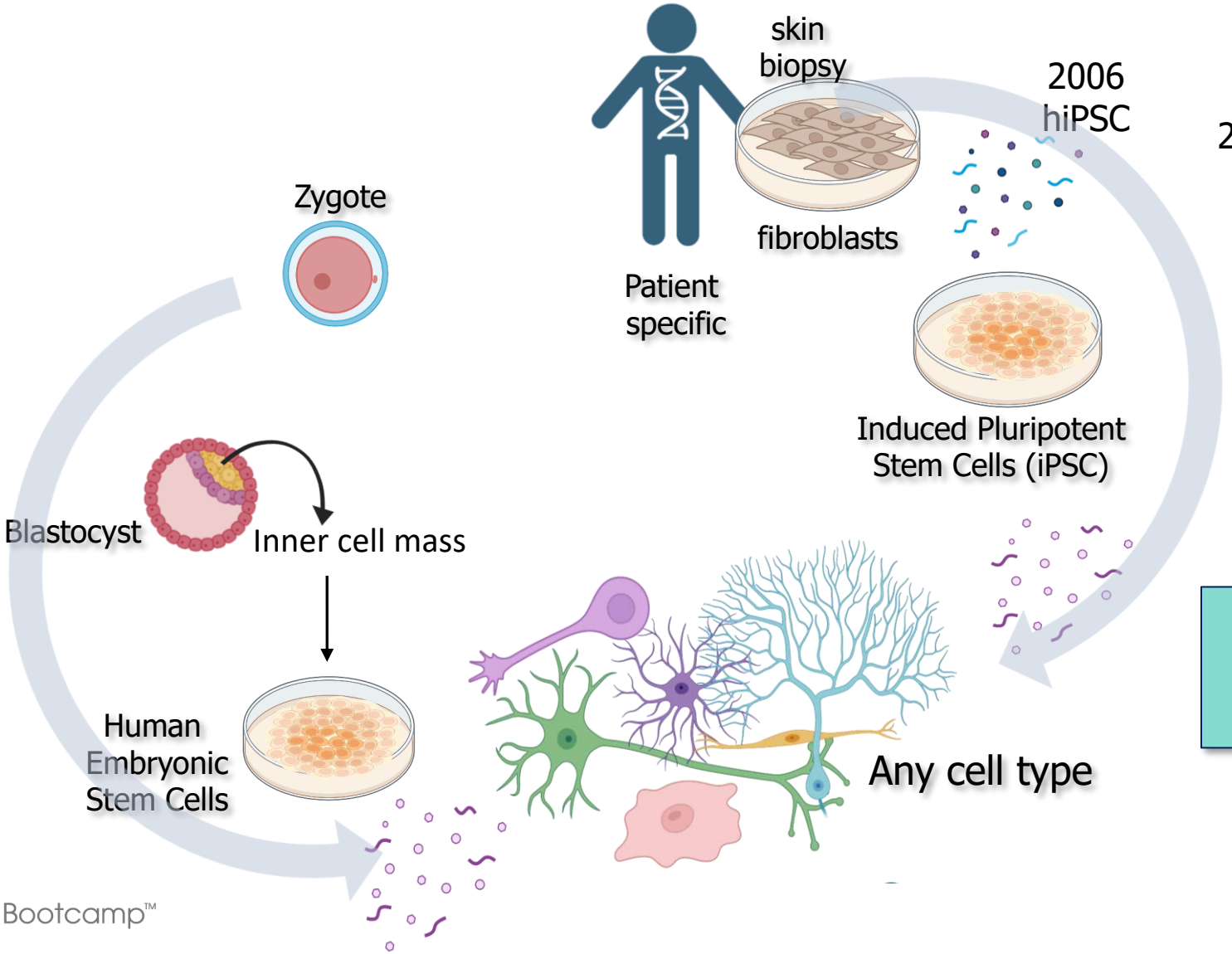
Ex: HeLa cells

## Primary Cultures

Limited lifespan



# Human Pluripotent Stem Cells – A Revolution




2012 Nobel Prize for Medicine

**Shinya Yamanaka**

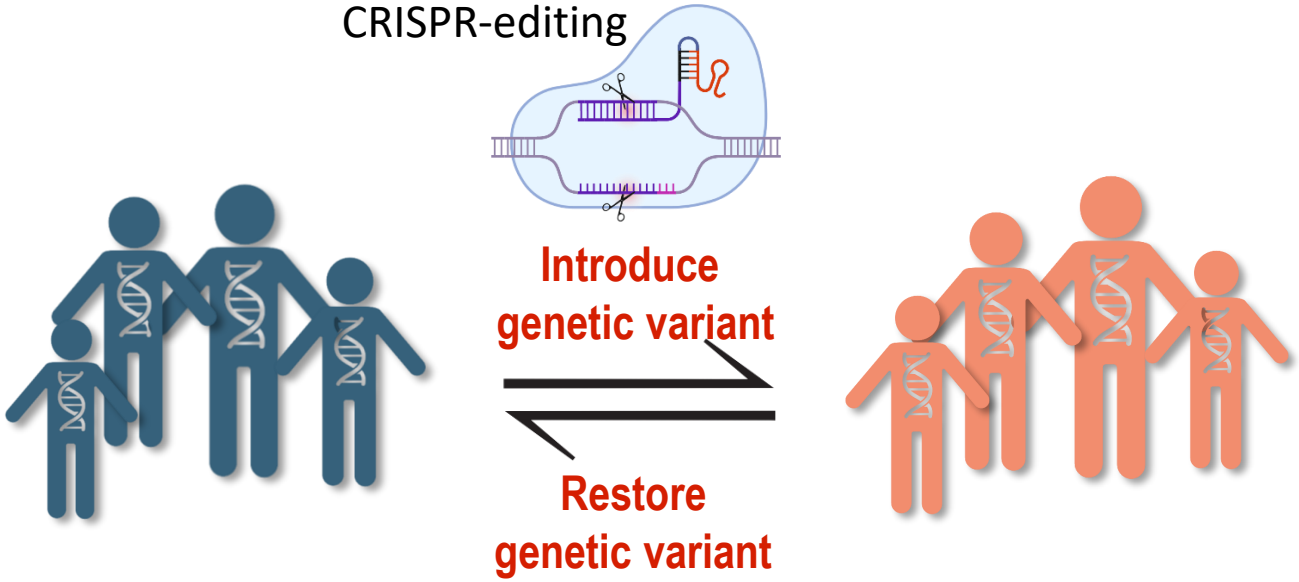
The more patients the better
Must generate
2-3 hiPSC Lines/patient

# CRISPR – A Revolutionary Tool to Edit Genomes

2020- Nobel Prize in Chemistry

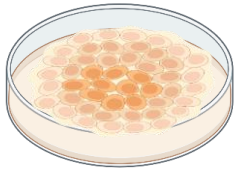
Emmanuel Charpentier
&
Jennifer Doudna

CRISPR-editing

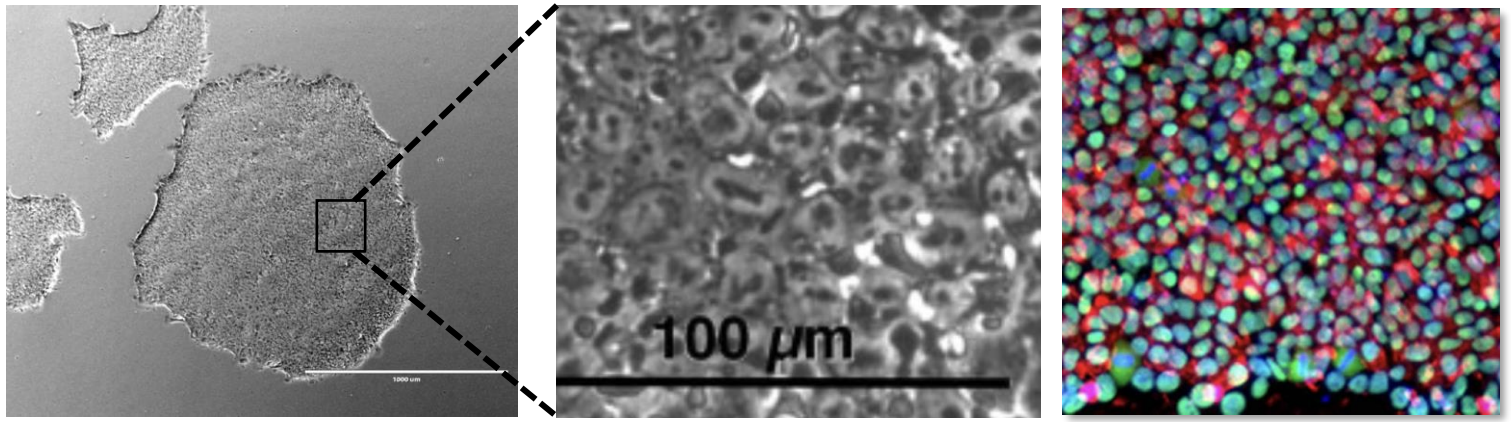



Must correct mutation in patient lines &
Must create mutation in more than healthy donor line

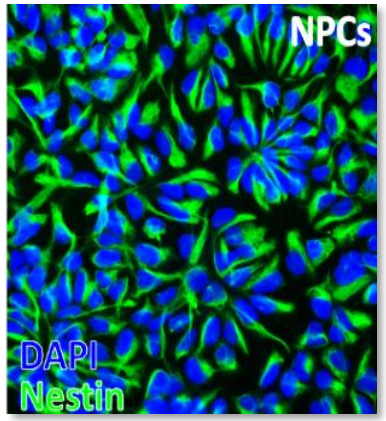
# Two-Dimensional (2D) hPSC-Derived Cultures



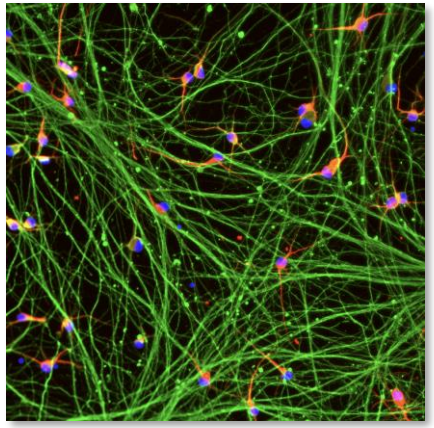
Induced Pluripotent Stem Cells (iPSC)



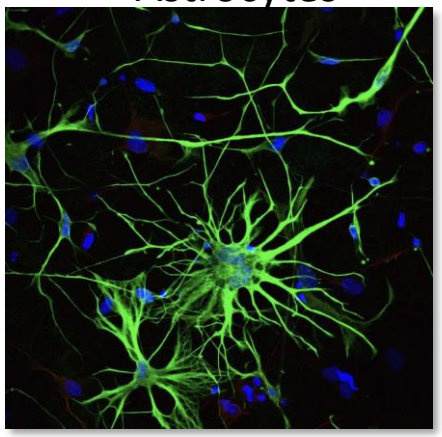

Neuronal Progenitors



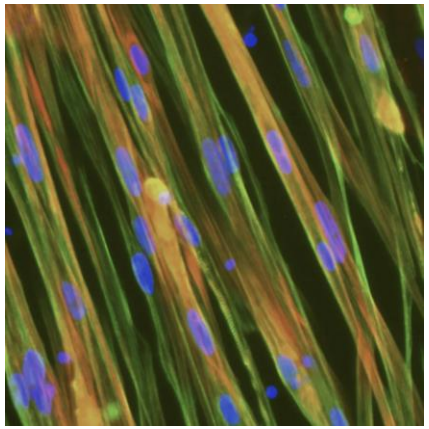

Neurons



Astrocytes



Muscle cells

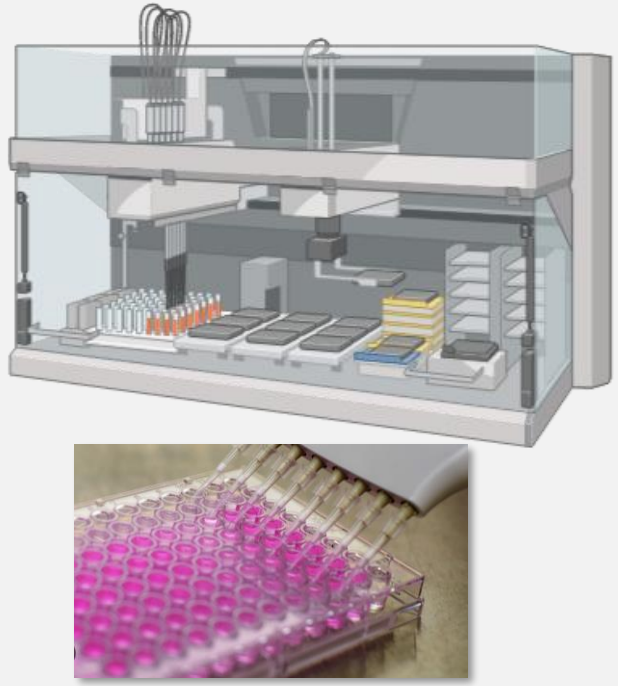


Which cell type? Disease-Dependent

# 2D Cells as Platforms for Phenotypic Screenings

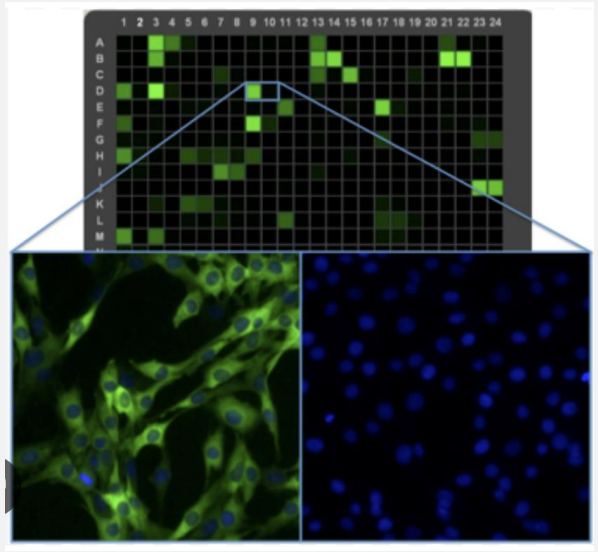
## Biomarkers

e.g., Inflammation



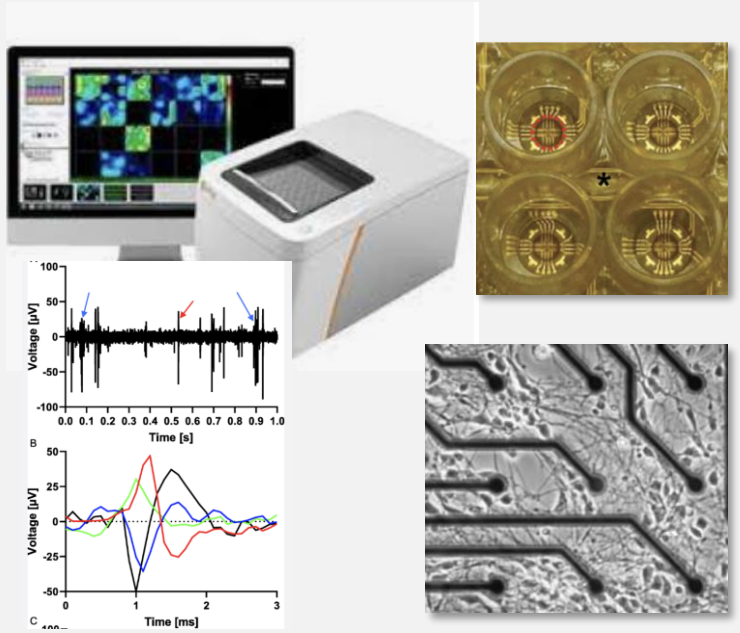
## Morphology

High content imaging (e.g., number of synapses)



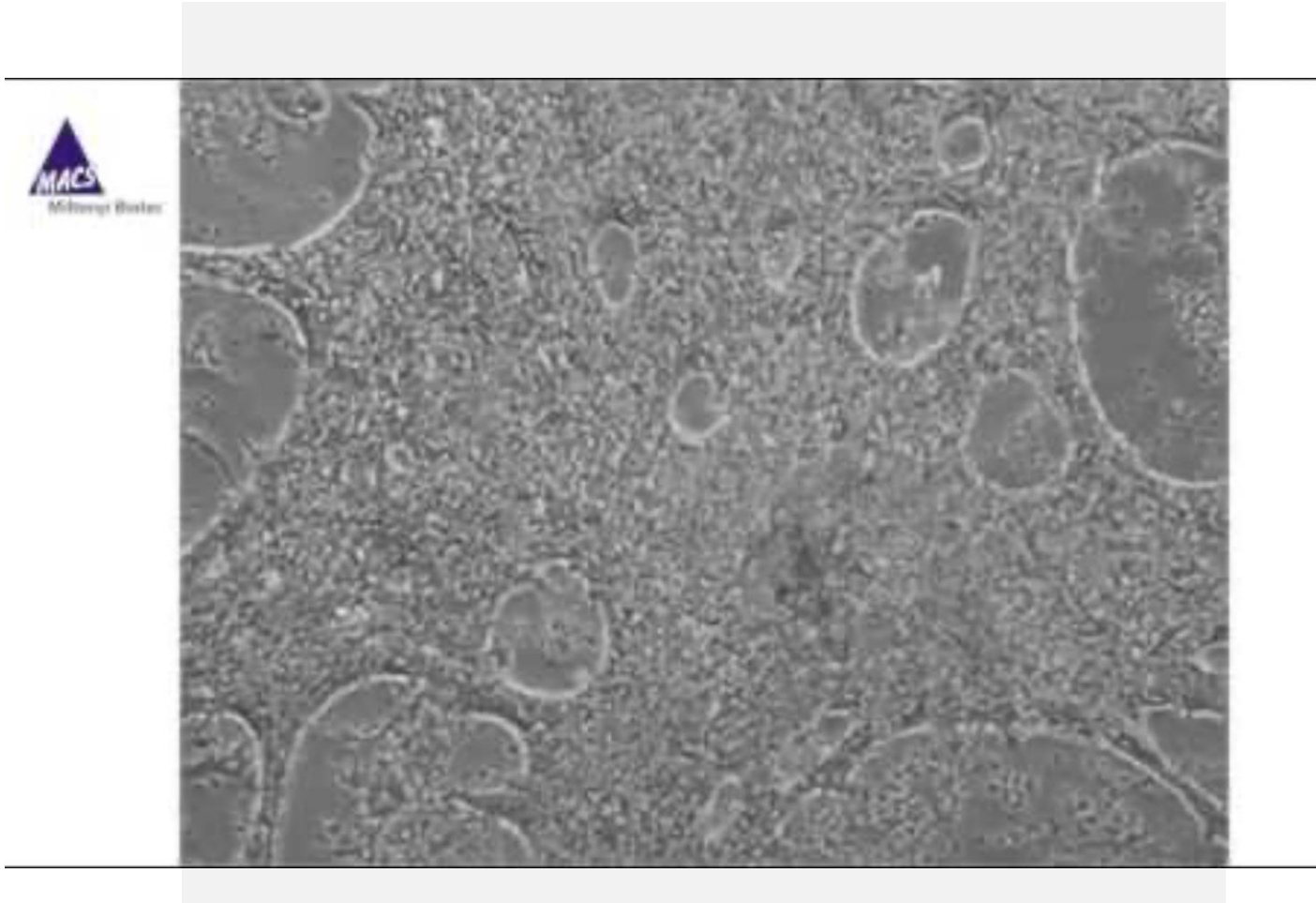
## Functionality

Multi-electrode arrays (e.g.,Neuronal Activity)

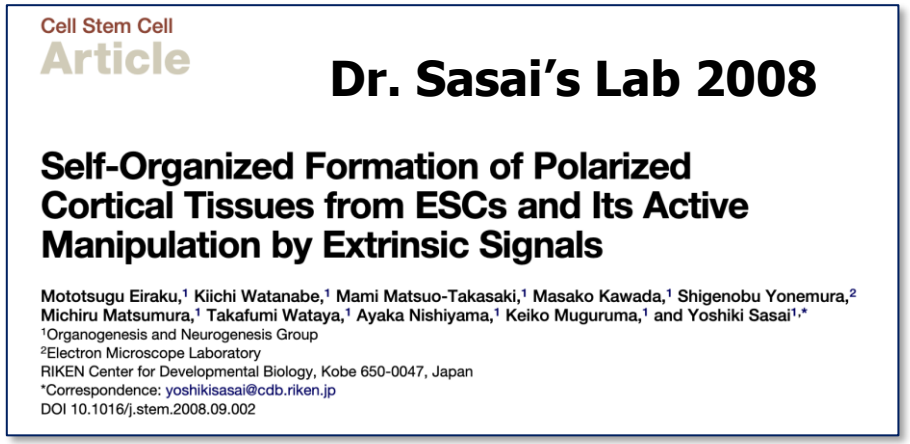


# Limitations of the 2D hPSC-Derived Cultures

- Lack of tissue complexity
- Incomplete cellular maturation
- Homogeneity of cell populations
- Inability to model tissue biomechanics
- Artifact-prone experimental conditions
- Limited lifespan (weeks)



# 3D-Organoids Derived From Human Stem Cells

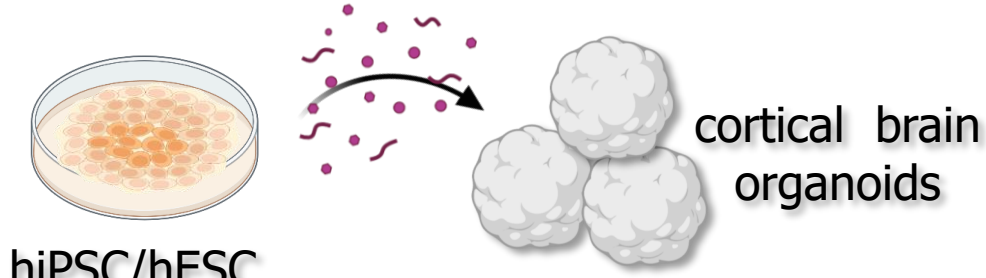


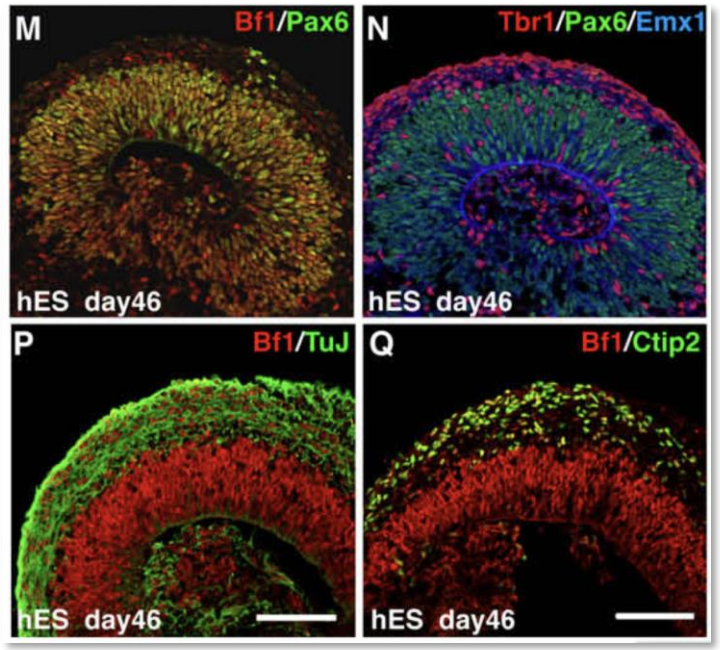
Cell Stem Cell
Article

**Dr. Sasai's Lab 2008**

**Self-Organized Formation of Polarized Cortical Tissues from ESCs and Its Active Manipulation by Extrinsic Signals**

Mototsugu Eiraku,[1] Kiichi Watanabe,[1] Mami Matsuo-Takasaki,[1] Masako Kawada,[1] Shigenobu Yonemura,[2] Michiru Matsumura,[1] Takafumi Wataya,[1] Ayaka Nishiyama,[1] Keiko Muguruma,[1] and Yoshiki Sasai[1,*]
[1]Organogenesis and Neurogenesis Group
[2]Electron Microscope Laboratory
RIKEN Center for Developmental Biology, Kobe 650-0047, Japan
*Correspondence: yoshikisasai@cdb.riken.jp
DOI 10.1016/j.stem.2008.09.002

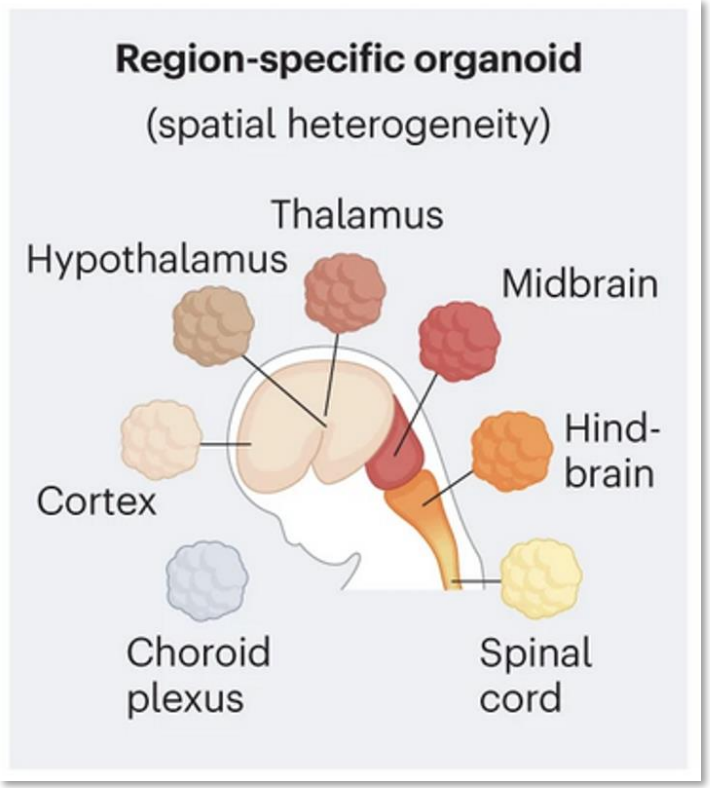


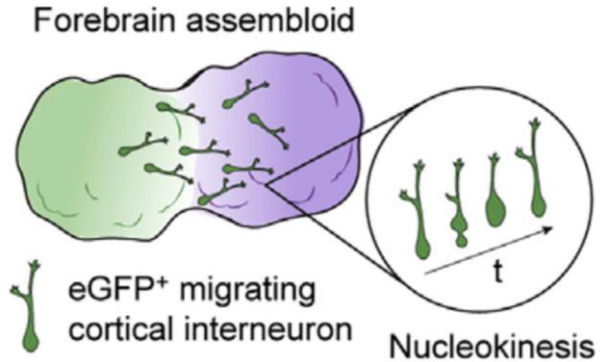

*Sassai Lab 2008*

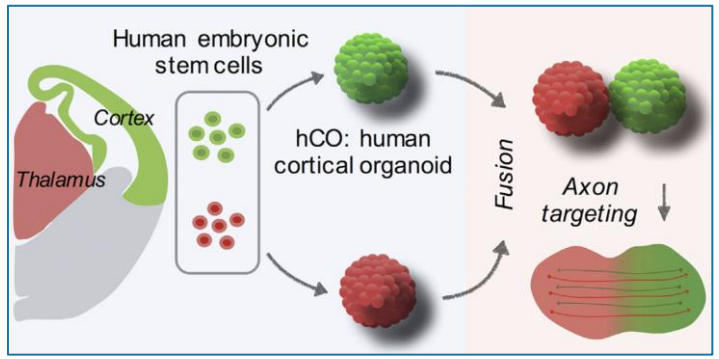
# Increasing Brain Complexity In Vitro

## DIFFERENT BRAIN REGIONS




## CONECTING DIFFERENT BRAIN REGIONS = ASSEMBLOIDS

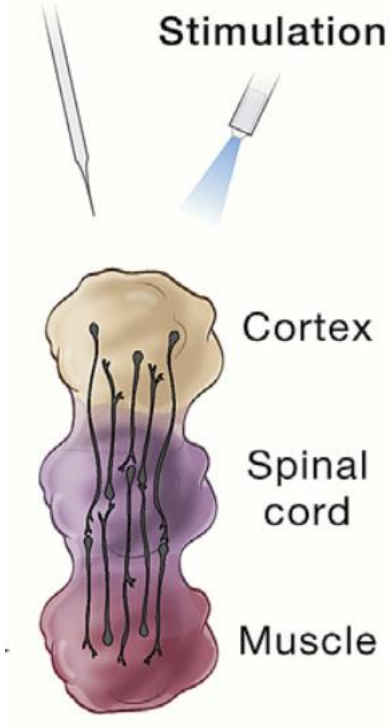


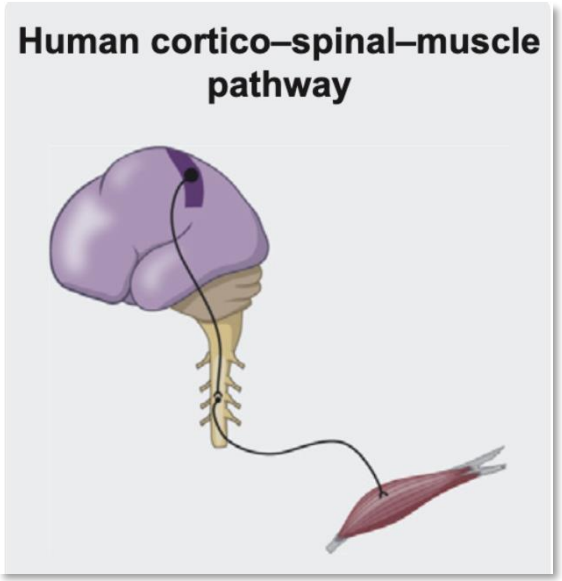

*Xiang et al., 2019; Cell Stem Cell*

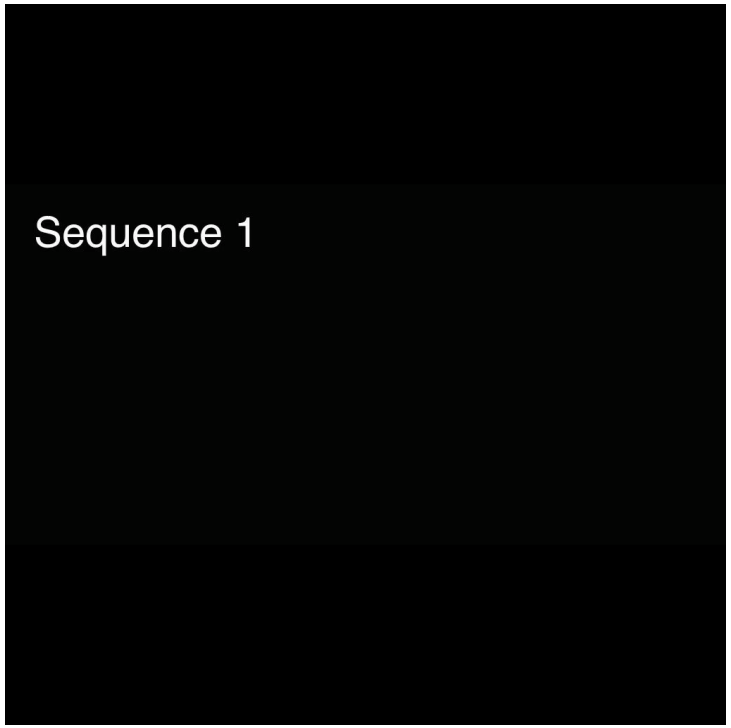
# Tri-Assembloids – Where is the limit?

cortico-spinal-muscle circuit



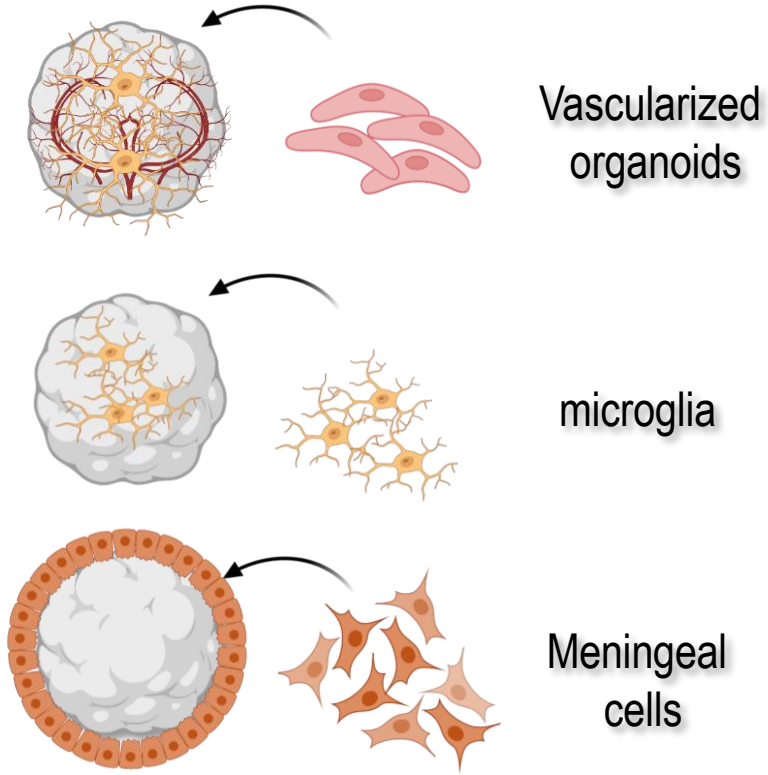

*Andersen 2020 Cell*



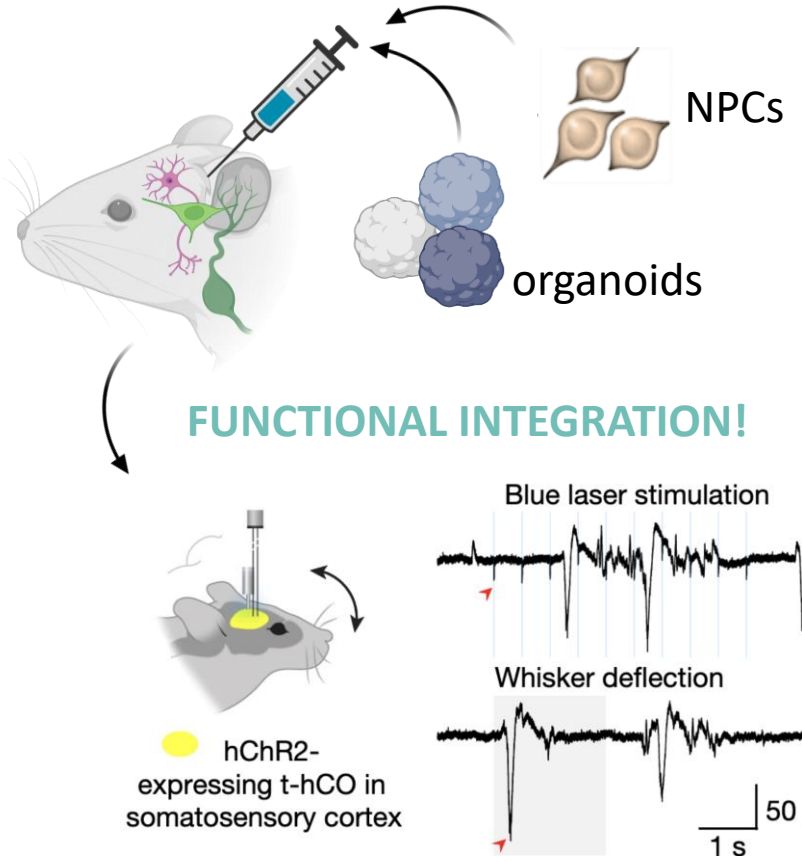



# Enhancing Maturity by Increasing Complexity

**Multi-lineage organoids**

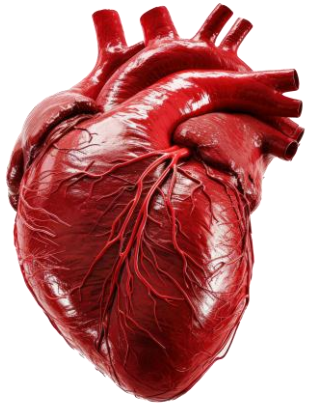


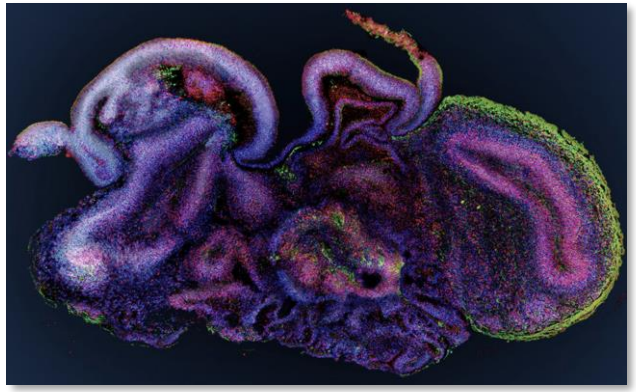

**In vivo transplantation**



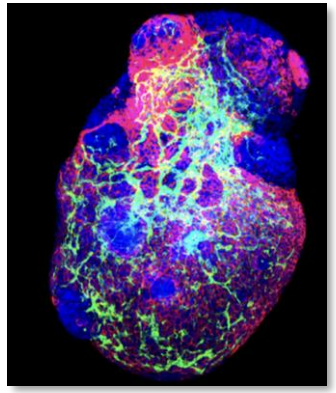

https://www.nature.com/articles/d41586-022-02073-4

# Miniaturizing Organs on a Dish

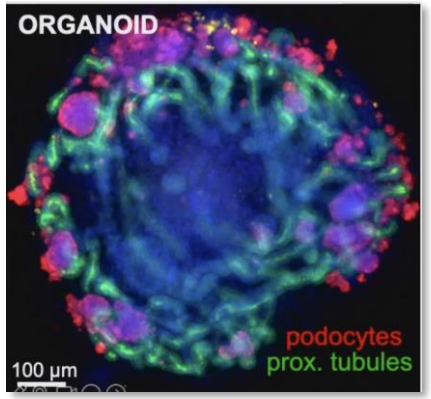




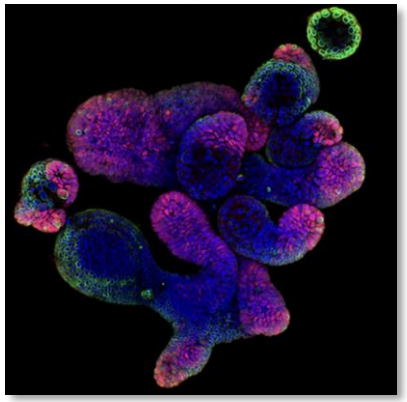
Knoblich Lab 2013



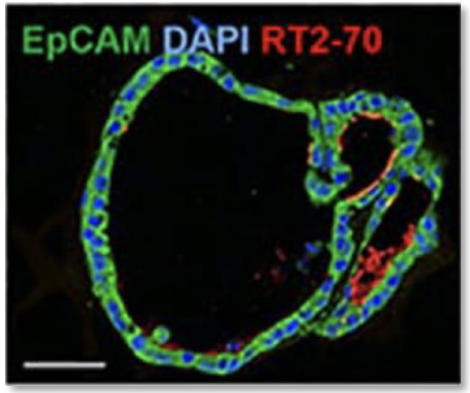
Aguirre Lab 2023




Freedman Lab 2024

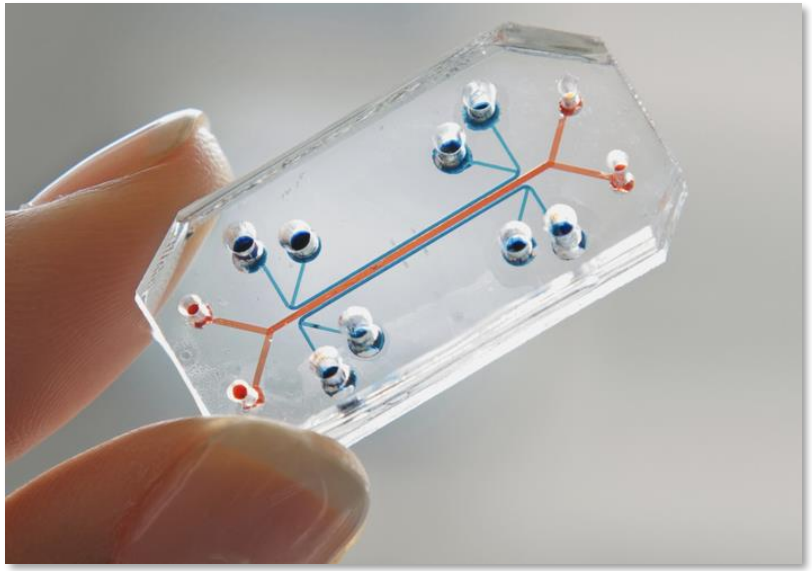


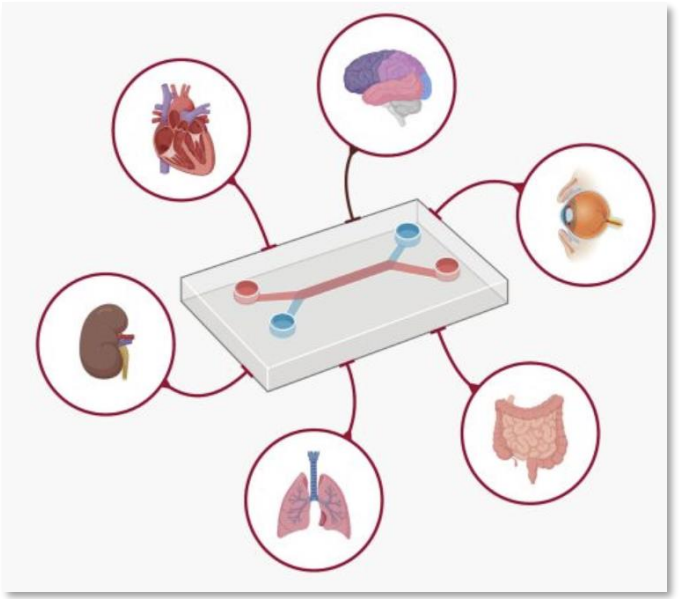
https://www.drugtargetreview.com




Fabian Lab 2021

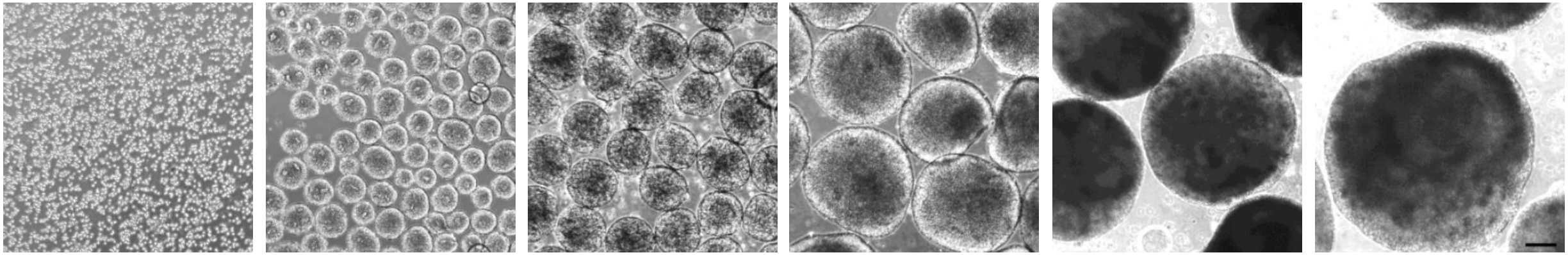
# Connecting Organs – Organoids-on-chip

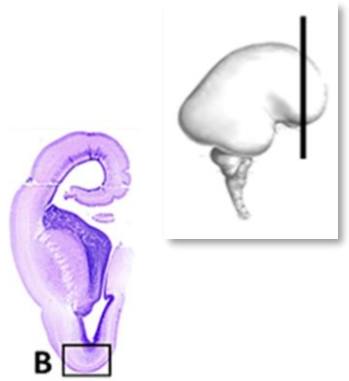


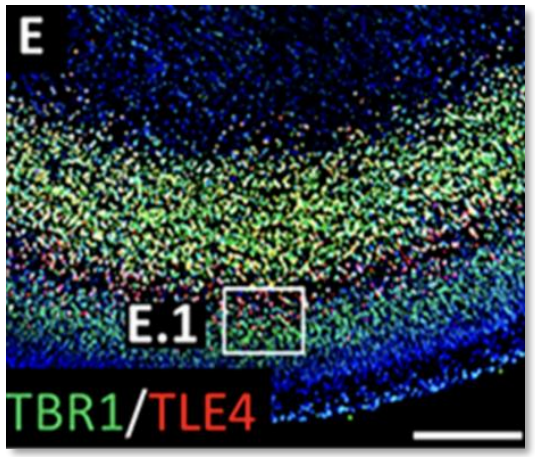


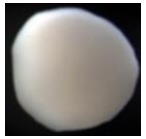
# The Muotri Lab Cortical Brain Organoids Recipe

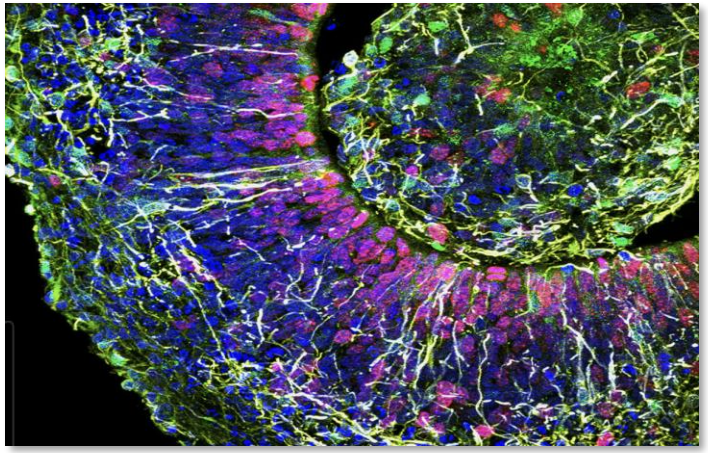
| Dissociated iPSC | Neural induction | NPC proliferation | | Neural differentiation | Neural maturation |
|---|---|---|---|---|---|
| 2 days | 7 days | 7 days | 7 days | 7 days | months |





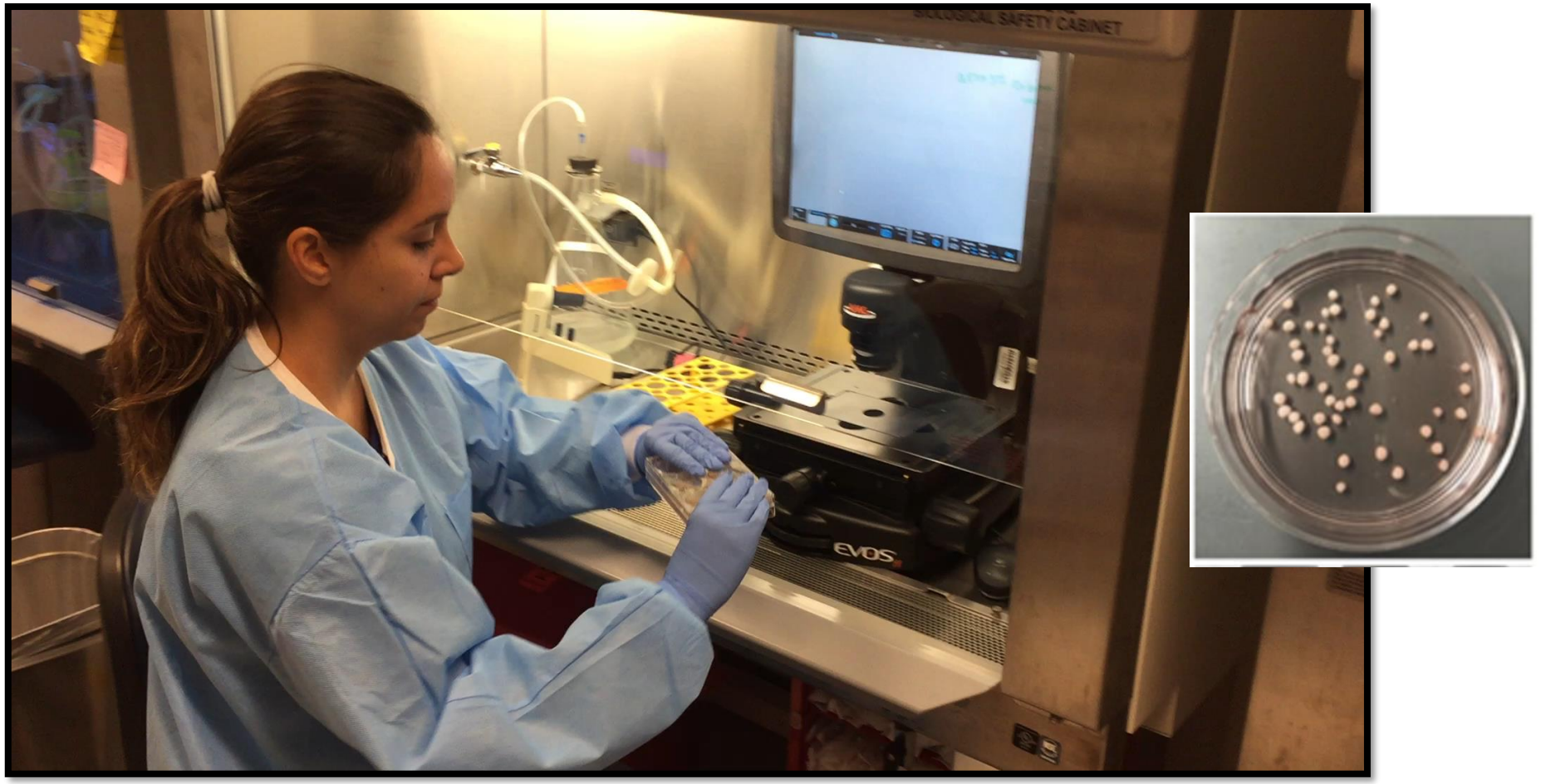



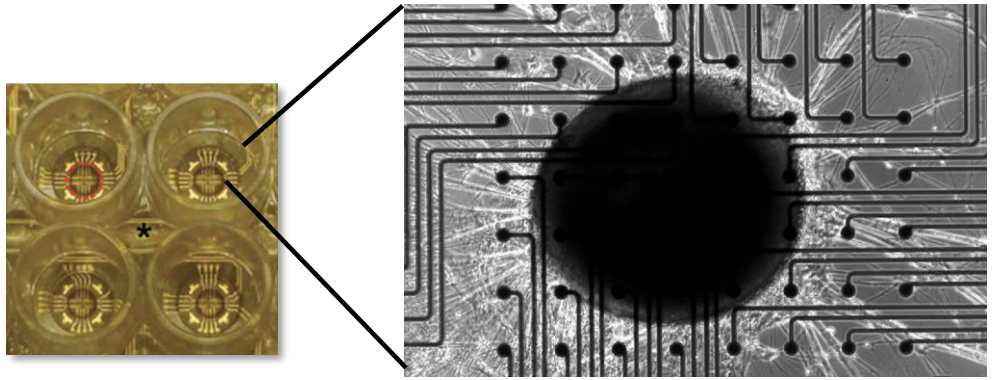


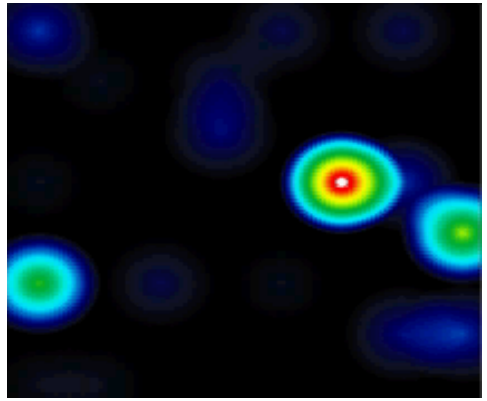
Rare Bootcamp™

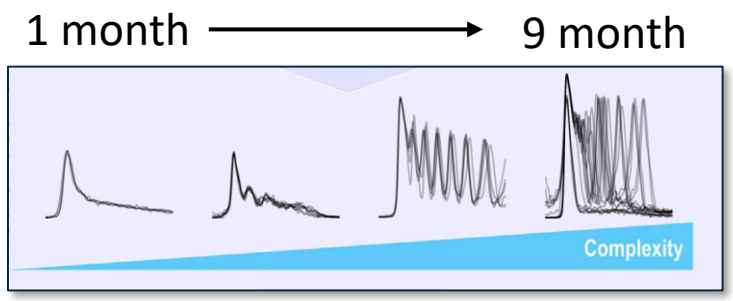
# Meet the Muotri Lab Cortical Brain Organoids

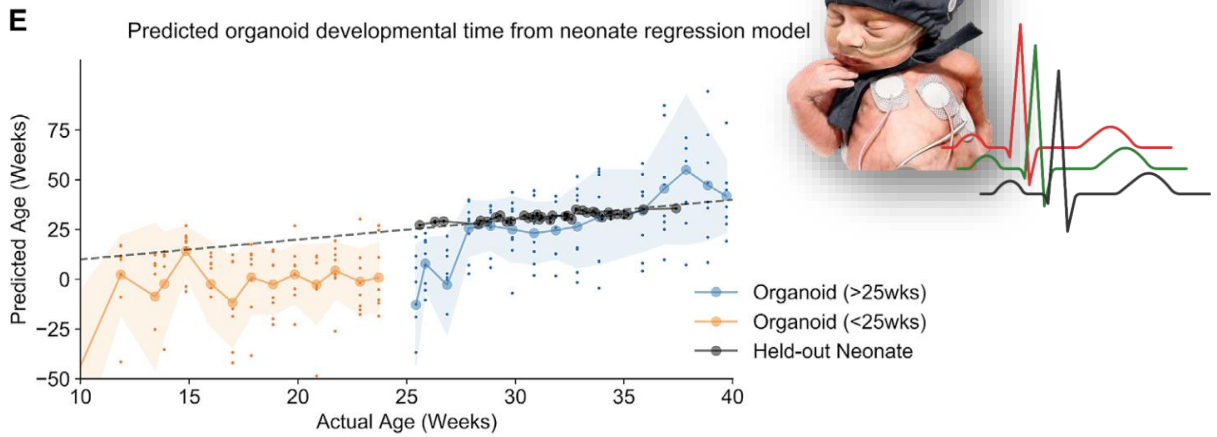


# Complex Network Connectivity

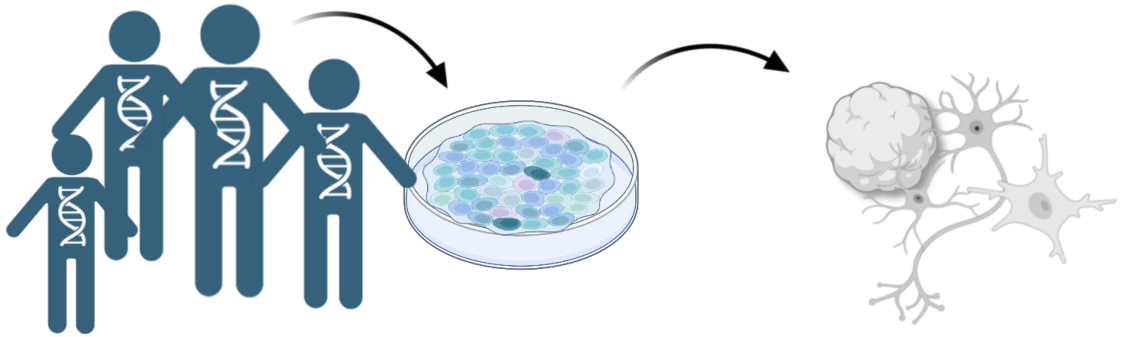


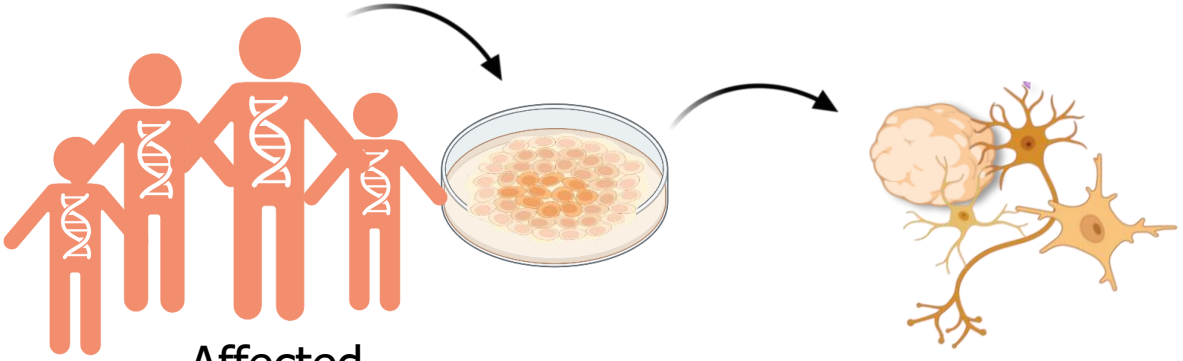


# Identifying Disease Phenotypes

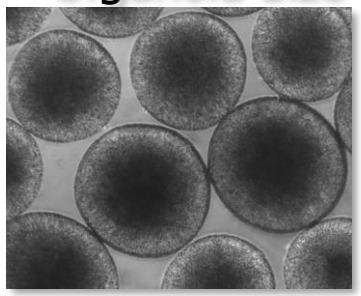


Neurotypical

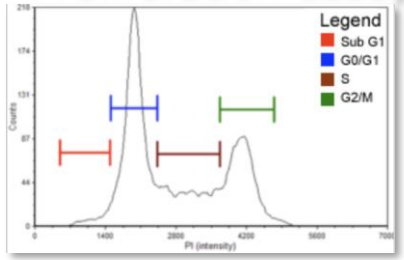


Affected

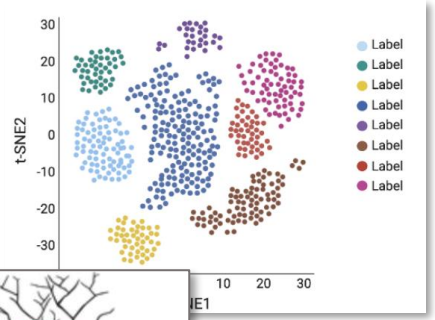
Organoid Size

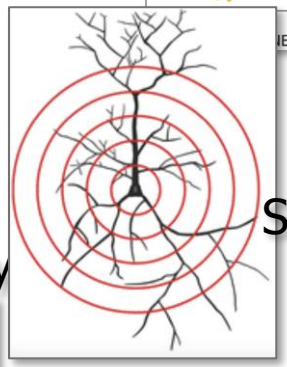


Proliferation assay

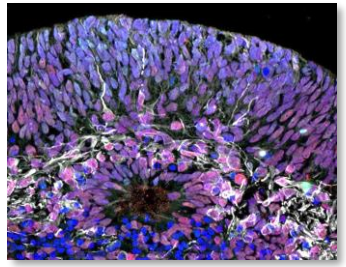



Single Cell -omics



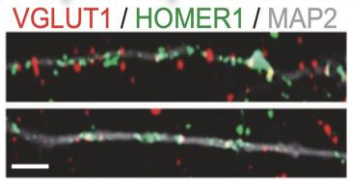



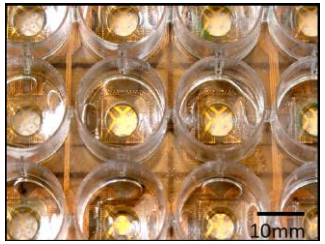
Morphology

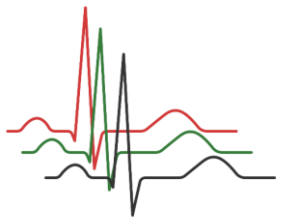


Cytoarchitecture

Synaptic quantification

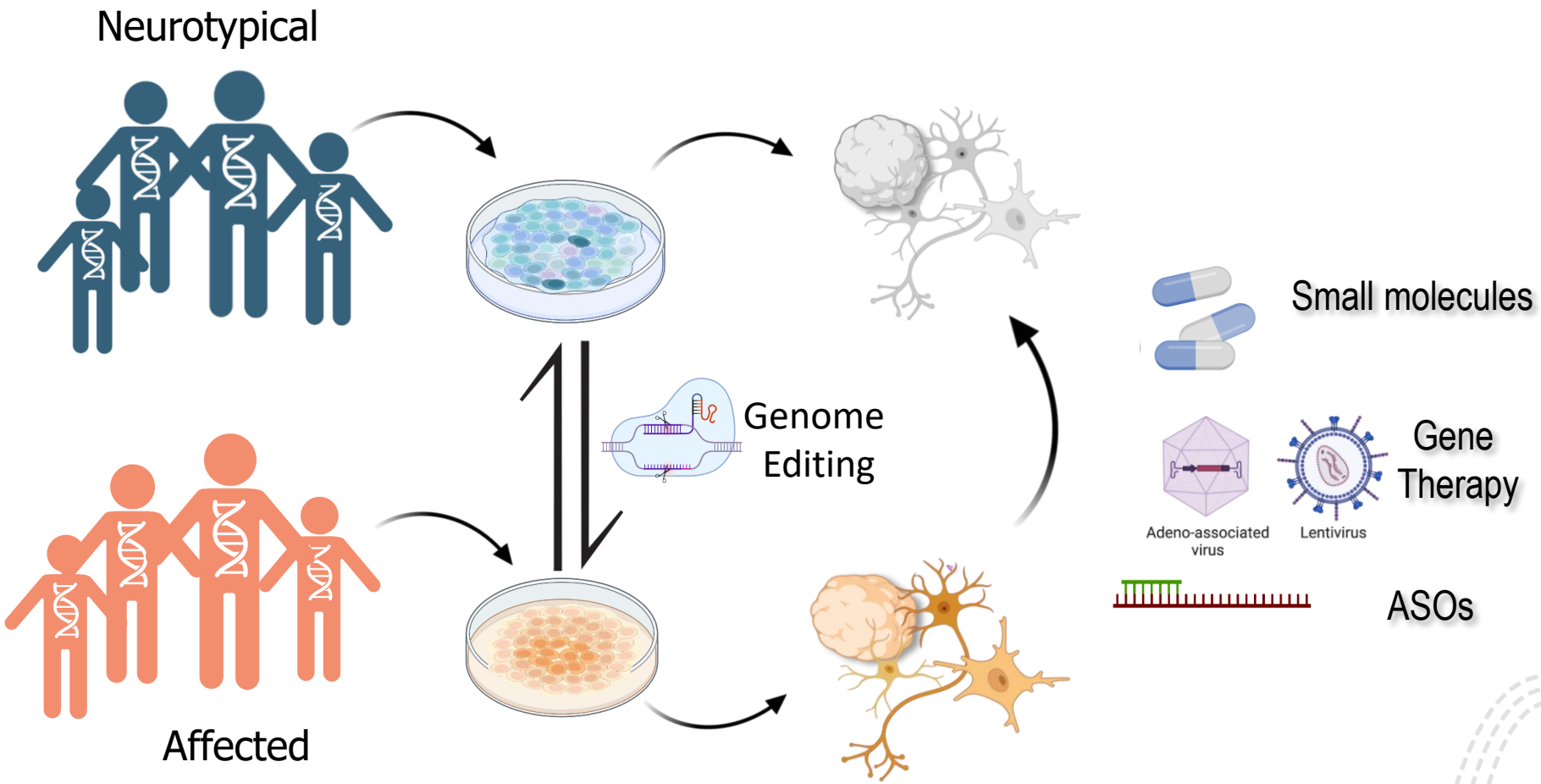


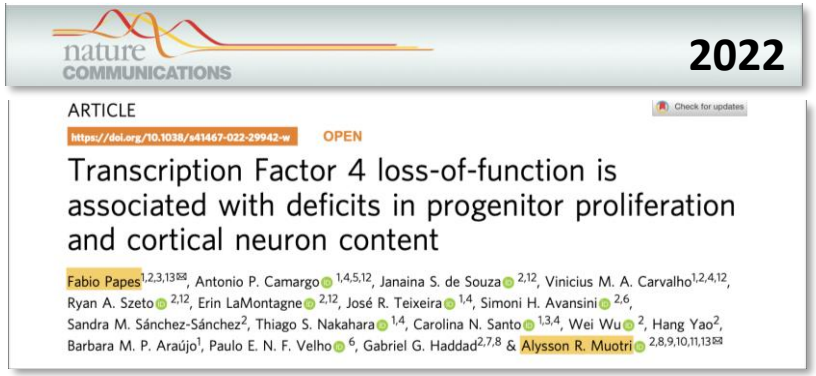



Neuronal Activity

# Personalizing Therapies



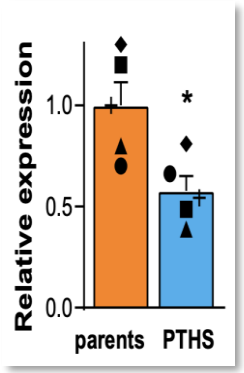
# Pitt-Hopkins Syndrome→ TCF4 Deficiency



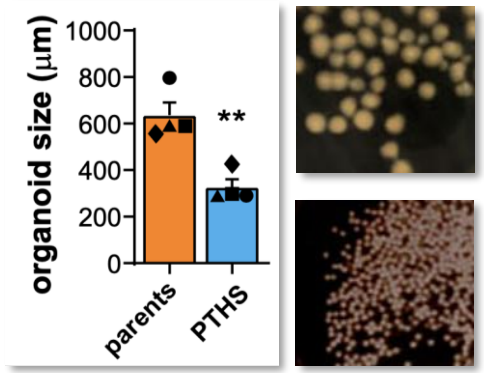

Rare genetic condition
Intellectual disability
Impaired speech
Breathing difficulty

5 patients
5 unaffected parents

**Lower TCF4**

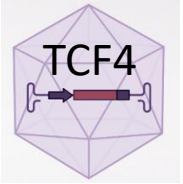


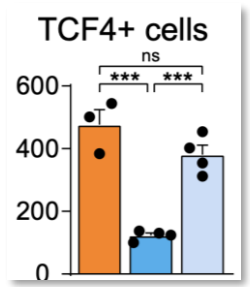

**Smaller Organoids**

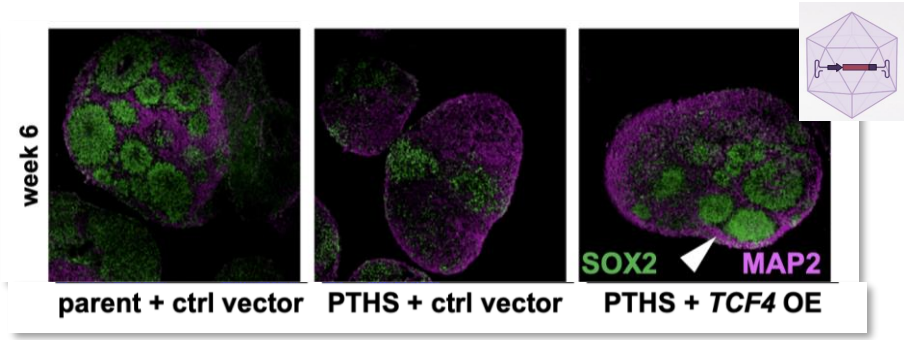




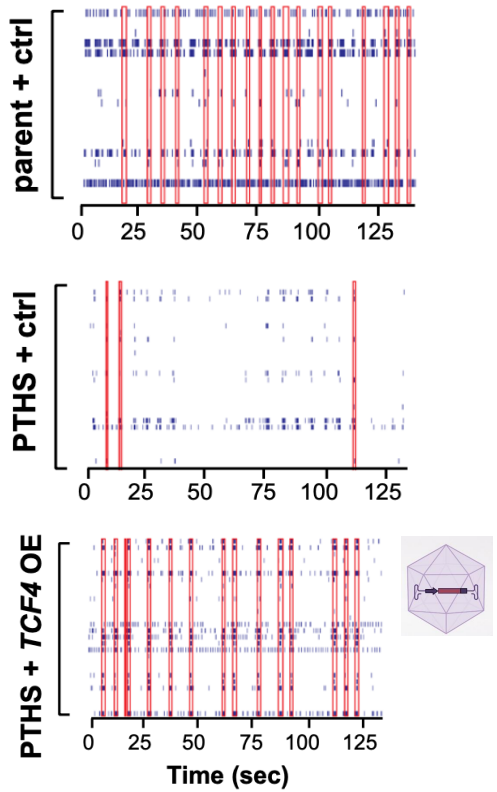

Adenovirus




Phenotypes Rescued




Phenotypes Rescued

**CIRM**
**$4 Million**

AAV9-base TCF4 replacement approach

MAHZI therapeutics

Only a few years

Rare Bootcamp™

# CDKL5 Deficiency Disorder (CDC)

Molecular Psychiatry
https://doi.org/10.1038/s41380-021-01104-2

2021

ARTICLE

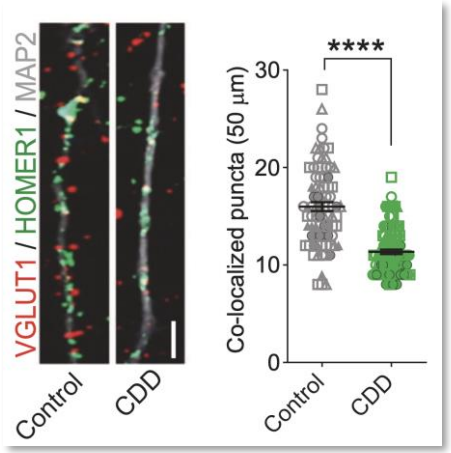
**Altered network and rescue of human neurons derived from individuals with early-onset genetic epilepsy**

Priscilla D. Negraes[1] · Cleber A. Trujillo[1] · Nam-Kyung Yu[2] · Wei Wu[1] · Hang Yao[1] · Nicholas Liang[1] · Jonathan D. Lautz[3,4] · Ellius Kwok[1] · Daniel McClatchy[2] · Jolene Diedrich[2] · Salvador Martinez de Bartolome[2] · Justin Truong[1] · Ryan Szeto[1] · Timothy Tran[1] · Roberto H. Herai[5] · Stephen E. P. Smith[3,4] · Gabriel G. Haddad[1,6] · John R. Yates 3rd[2] · Alysson R. Muotri[1,7,8]

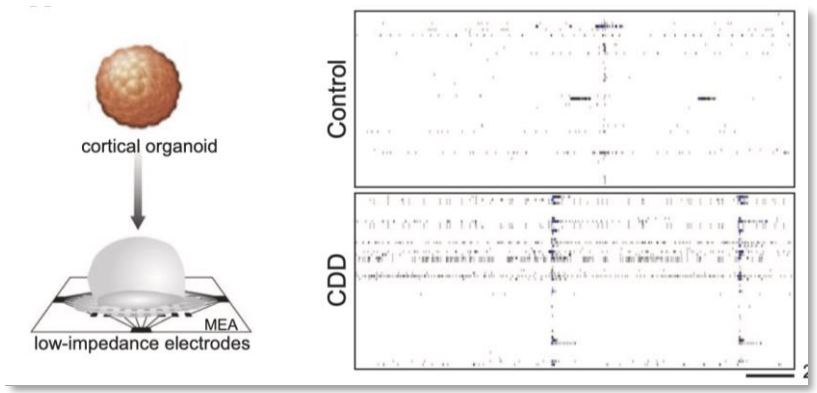
Rare (1:40,000)
Early onset seizures
Impaired cognition,
Speech, motor function

6 CDD patients
4 unaffected
2 control isogenics

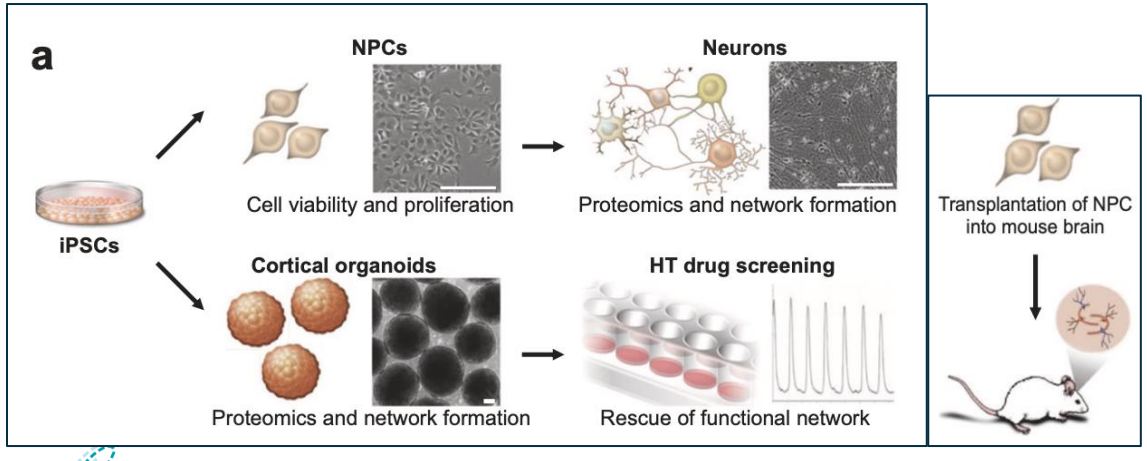
## Reduced synapses




## Hyperexcitability

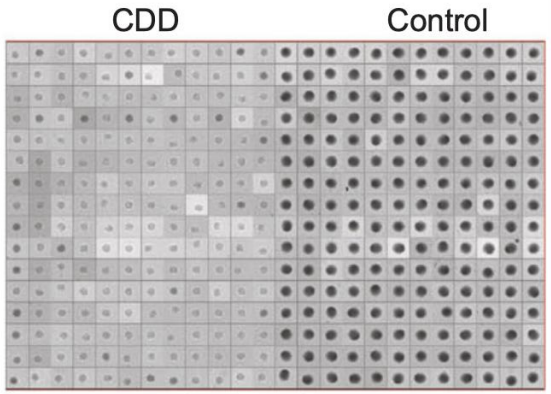


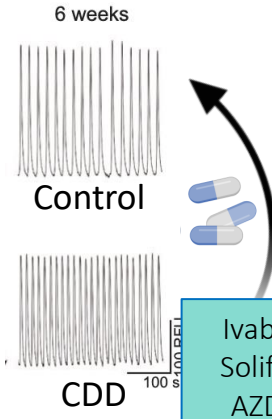

Human neurons










Ivabradine
Solifenacin
AZD1080
Crenigacestat

ultragenyx
Gene Therapy

Rare Bootcamp™

# Conclusions – Cellular Models

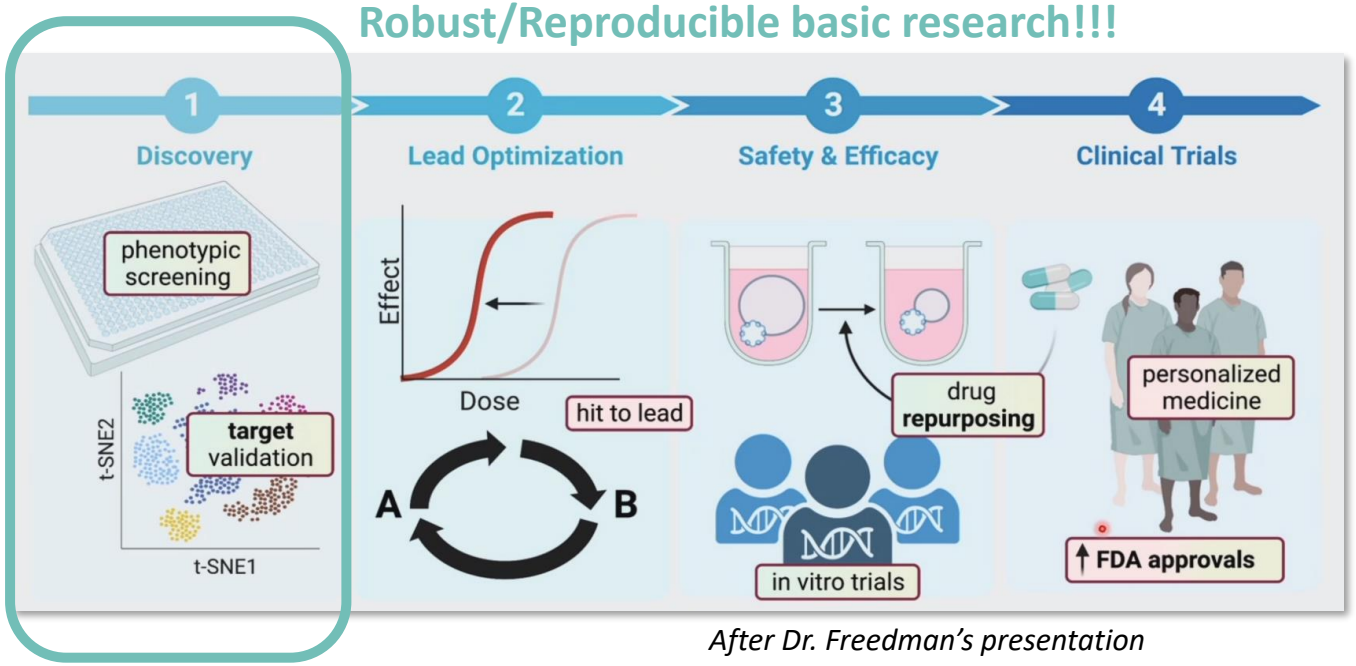
*"All models are wrong, but some are useful"* (George E.P. Box)

- Only recapitulate pre-natal features
- Reduced cellular complexity
- High variability
- No blood brain barrier
- Missing systemic contribution

>40 neurological disorders modeled using organoids

**FDA Modernization Act 2.0 (2022)**: This bill allows an application for market approval for a new drug to use alternatives to animal testing including cell-based assays.

**Robust/Reproducible basic research!!!**




*After Dr. Freedman's presentation*

# Funding & Timeline in a Human Stem Cell Lab

| YEAR 1 | YEAR 2 | YEAR 3 | YEAR 4 | YEAR 5 |
|---|---|---|---|---|
| $150K Foundation<br>$150K Muotri Lab | $150K Foundation<br>$150K Muotri Lab | $150K Muotri Lab | $1-1.5M R01/CIRM | >$4M<br>CIRM/Company |
| Generate iPSCs/QC<br>Expand/Bank<br>Organoids QC/phenotype | Complex experiments<br>Phenotype<br>Grant Application | Mechanism<br>Proof-of-concept<br>Grant Application | Confirmatory<br>Publication<br>Grant Application | Pre-clinical<br>Therapies<br>IND |

**$500** to culture 1 patient-skin fibroblasts
**$10-20K** to reprogram, QC, and establish one iPSC line
**$15K** to differentiate one iPSC line
**$20K** CRISPR-editing/line
**+ SALARIES (**25-50%) technician/(100%) postdoc or grad student

# The Muotri Lab


**THE SANFORD CONSORTIUM FOR REGENERATIVE MEDICINE, UC SAN DIEGO, CA**