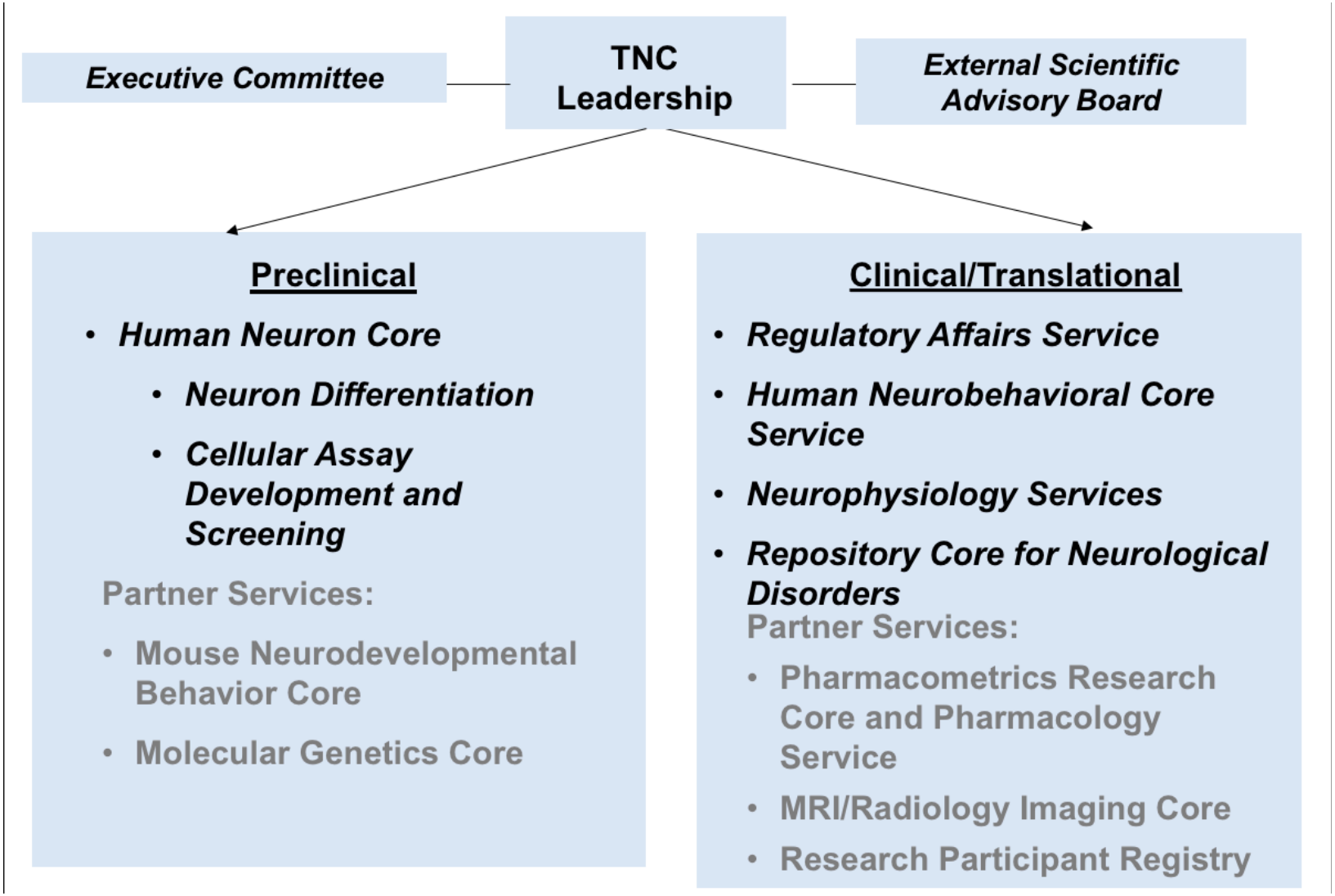
Translational Neuroscience Center @ Boston Children's Hospital

Mission of the Translational Neuroscience Center (TNC):

Translate, Collaborate, Educate, Communicate

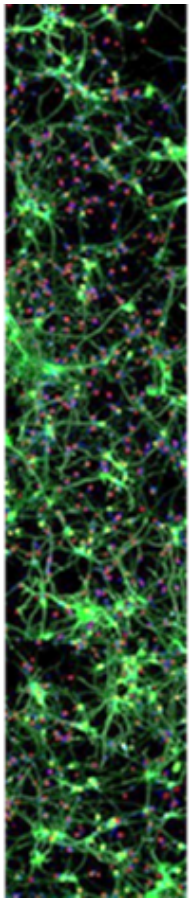
- Accelerate the **translation** of research discoveries into new cures for pediatric nervous system disorders.
- Develop effective new strategies for disease prevention and treatment through **collaborations** among Children's Hospital's world-renowned basic scientists in partnership with the external research community.
- **Educate** future leaders about critical components of pediatric translational neuroscience.
- **Communicate** new models of interdisciplinary translational medicine in pediatric neuroscience with local, national and international collaborators.

Translational Neuroscience Center



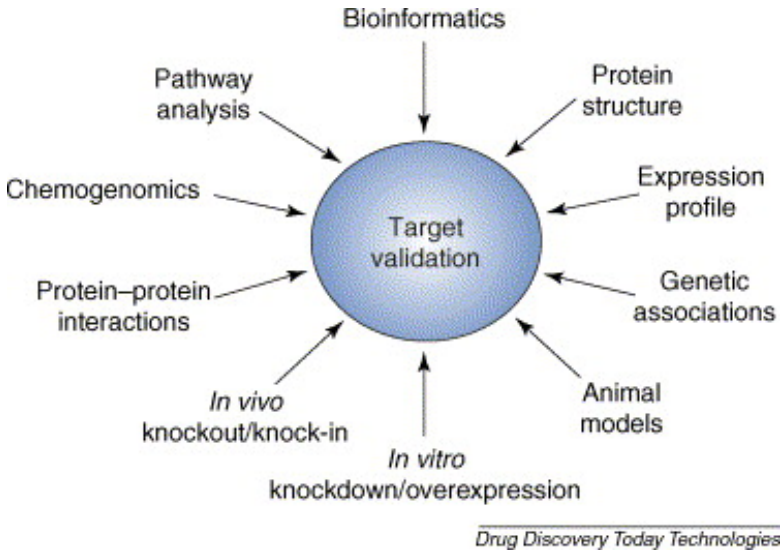
Challenges facing drug-discovery in neuroscience

- Access: the blood-brain barrier
- Complex biology and circuitry still being defined
- There are few validated targets; decades old or ineffective
  - Antidepressants – SSRIs, Tetracyclics
  - Anxiolytics – GABAA receptors, 5-HT receptors
  - Antipsychotics – D1 & D2 dopamine receptors
  - Anti-epileptics – many ineffective
  - Pain relievers – ineffective; side effects
- Large Pharma is taking a step back in neuroscience



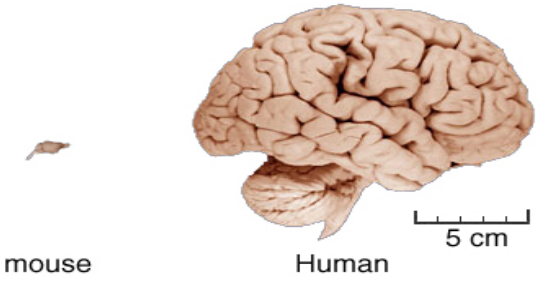
What do we need to validate a new target?

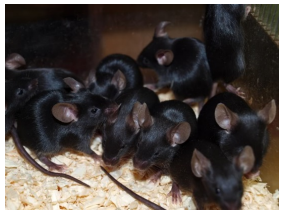
- Confidence that manipulating that pathway/circuit with:
  - Defined patient population
  - Specific stage of disease
  - Will provide a measurable therapeutic benefit
- Therapeutic benefit has a well-validated clinical endpoint



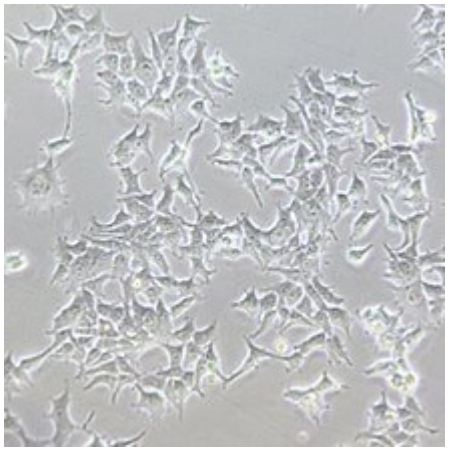
This is RARE!

Improved target validation requires better preclinical data





- Efficacy in rodent models hasn't translated
  - Genetic diversity not reflected
  - Ineffective behavioral models
- Human genetic data enhances confidence in target
  - When to treat?
  - How long to treat?
  - What exposure profile?
  - What outcome measures will demonstrate efficacy?



- Engineered cell lines
  - Don't recapitulate neuronal fundamental properties and signaling mechanisms

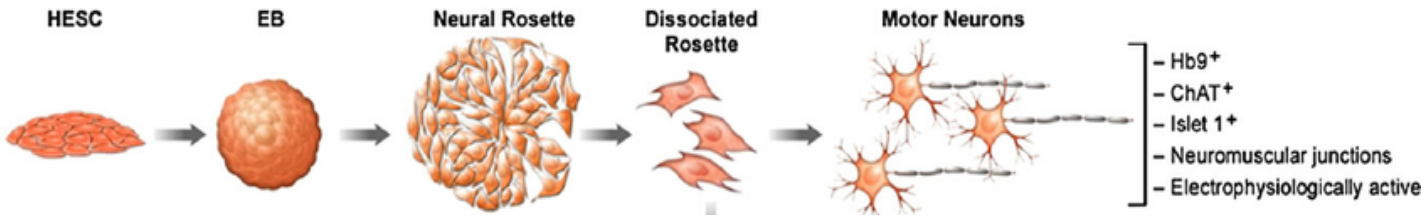
Human iPSC-derived neurons and glia for target validation in CNS disorders

Strengths

- Human
- Scalable
- Patient relevant genetic backgrounds
- Can be maintained in vitro for months, maybe years?
- Genetically modifiable
- Some well defined cell types can be differentiated
- 3D organoids

Limitations

- Embryonic, immature
- Variability from line to line and clone to clone and differentiation to differentiation
- Relatively expensive and labor intensive
- Exact identity of cell types produced is not precisely analogous to those in patients



Early success using human neurons to support drug discovery

ALS; MNs have reduced Kv7.2/3 current

Intrinsic Membrane Hyperexcitability of Amyotrophic Lateral Sclerosis Patient-Derived Motor Neurons

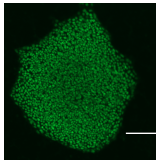
Brian J. Wainger,[1,2,8] Evangelos Kiskinis,[3,8] Cassidy Mellin,[1] Ole Wiskow,[3] Steve S.W. Han,[3,4] Jackson Sandoe,[3] Numa P. Perez,[1] Luis A. Williams,[3] Seungkyu Lee,[1] Gabriella Boulting,[3] James D. Berry,[4] Robert H. Brown, Jr.,[5] Merit E. Cudkowicz,[4] Bruce P. Bean,[6] Kevin Eggan,[3,4,7,*] and Clifford J. Woolf[1,6,*]

AD; human models of AB and tau pathology

doi:10.1038/nature13800

A three-dimensional human neural cell culture model of Alzheimer's disease

Se Hoon Choi[1]*, Young Hye Kim[1,2]*, Matthias Hebisch[1,3], Christopher Sliwinski[1], Seungkyu Lee[4], Carla D'Avanzo[1], Hechao Chen[1], Basavaraj Hooli[1], Caroline Asselin[1], Julien Muffat[5], Justin B. Klee[1], Can Zhang[1], Brian J. Wainger[4], Michael Peitz[3], Dora M. Kovacs[1], Clifford J. Woolf[4], Steven L. Wagner[6], Rudolph E. Tanzi[1] & Doo Yeon Kim[1]



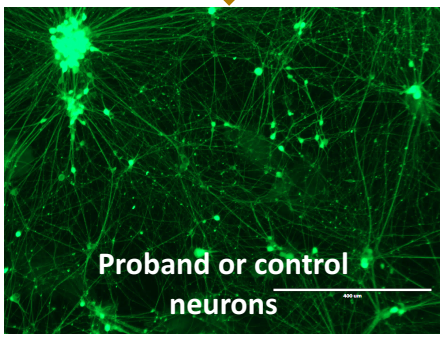
Patient-specific iPSCs

Reprogramming

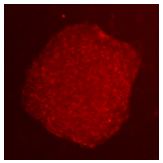
Differentiation

Kleefstra Syndrome

patient fibroblast or blood sample



Differentiation

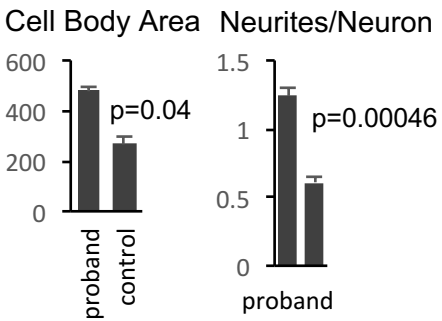


(Sample collected from parent to generate control iPSCs)

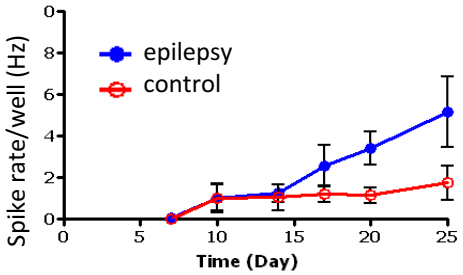
Control iPSCs

Disease modeling

Arrayscan XTI
High content analyses



Axion Maestro
Multi-electrode array analyses



Biochemistry &

Ion Torrent
AmpliSeq

Identification of pathways and targets with altered regulation in patient neurons

Drug repurposing screens

Identification of novel targets

Filling the gap for rare neurodevelopmental disorders

- We have blood and fibroblasts collected from over 230 patients
- We have iPSCs from 50 patients and controls
- Diseases include:
  - Kleefstra syndrome
  - TSC
  - SSADH deficiency
  - CDKL5 disorder
  - Other epilepsy indications – different genetic causes
  - Early-onset psychosis – rare and novel CNVs
  - Hereditary spastic paraplegia type 47
  - Spinal Muscular Atrophy, type 1, 2, and 3
  - Fragile X

Two case examples

- 1) Early-onset psychosis associated with a novel CNV
  - Using patient iPSC-derived neurons to uncover cellular mechanisms
- 2) Refractory epilepsy
  - What features of patient iPSC-derived neurons are different than healthy control neurons and can be used to screen for new therapeutics?

Uncovering mechanisms responsible for early-onset psychosis

- Strange or bizarre thinking, perceptions (sight, sound), behaviors, and emotions
- Psychiatrist Joseph Gonzalez-Heydrich, MD sees patients with early-onset psychosis
  - With Catherine Brownstein, novel potential risk variants are identified
- 16p13.11 Copy Number Variant (CNV) associated with neuropsychiatric/neurodevelopmental disorders
  - **Duplication** associated with schizophrenia, cognitive impairment, congenital heart defects, and ASD
  - **Deletion** associated with intellectual disability, microcephaly, and epilepsy, but has yet to be confirmed as associated with psychosis

A patient with a 16p13.11 microdeletion – clinical phenotype

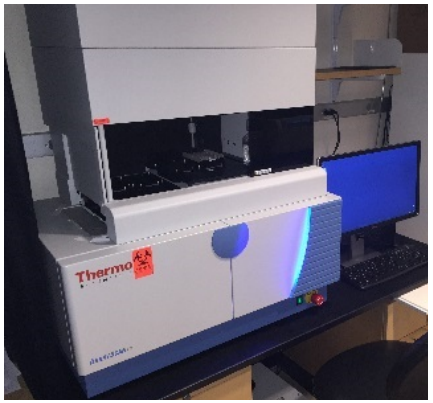
- Sat at 6mo, crawled at 8mo, walked at 10mo
- History of motor dyscoordination
- Delayed language development
- Intellectual disability
- Behavioral problems since early childhood: tantrums, physical aggression
- Age of psychosis onset: 6 years old
  - Auditory/visual/tactile hallucinations and delusions
- Probable schizophrenia in biological father (no longer in picture)
- History of anxiety and depression in biological mother
- Exposure to physical abuse by father during the first year of life

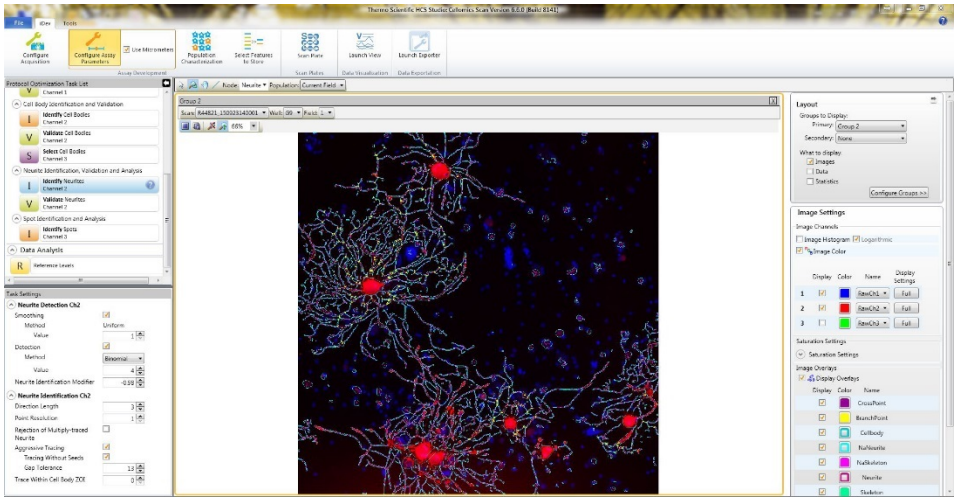
Genes affected by 16p13.11 microdeletion

- 15kb-131kb deletion
- *NTAN1* (N-terminal asparagine amidase)
  - Converts N-terminal asparagine to aspartate
  - Targets proteins to proteasome degradation through specific E3 ubiquitin ligases
  - Schizophrenic-like behavior in *Ntan-/-* mice
- *PDXDC1* (pyridoxal-dependent decarboxylase domain-containing protein 1)
  - Associated with hearing loss
  - SNPs associated with changes in inflammatory markers
- *RRN3* (RNA polymerase 1 transcription factor)
  - Initiation factor for RNA polymerase 1-mediated transcription
  - Regulated by mTOR through phosphorylation

Using high-content imaging to uncover neurite phenotypes

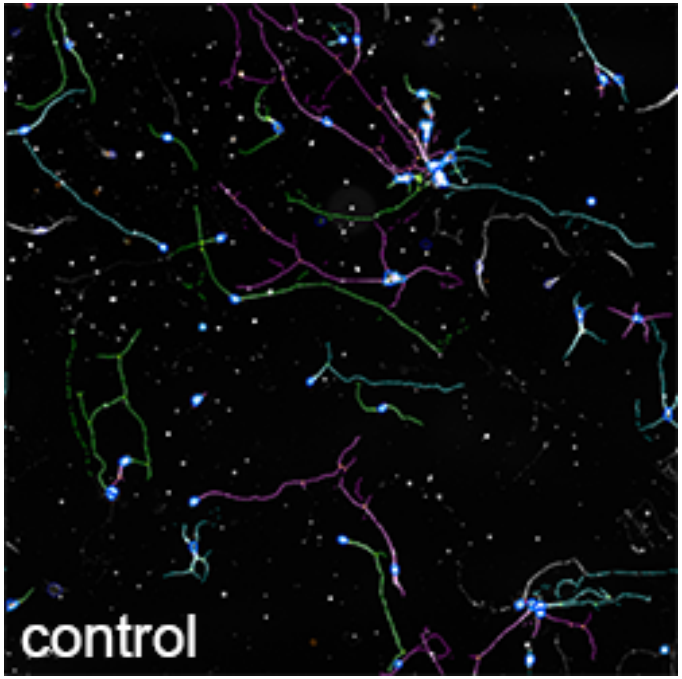
ArrayScanXTi High Content Platform
(ThermoFisher Scientific)

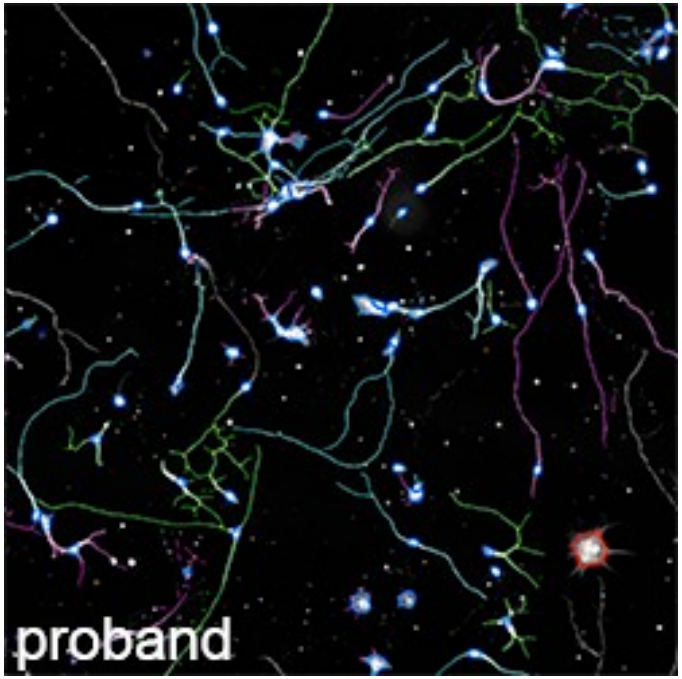


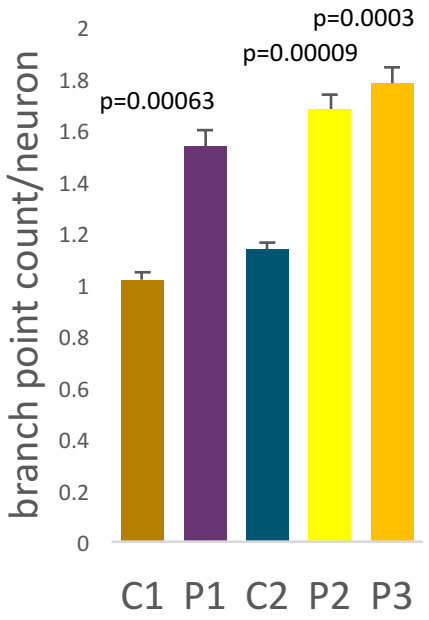


Color:	Green, Blue, Red-Orange, Yellow, Scarlet, Near-Infrared, Cyan, Far-Red
Format:	Slide(s), 96-well plate, 24-well plate, 384-well plate, 1536-well plate, 6-well plate, 8 wells
Optics:	CCD Camera, LED, solid-state 7-color light engine
Other:	Temperature and CO2 controlled live chamber & integrated liquid handler

16p13.11 deletion iPSC-derived neurons reveal increased branching, compared to healthy control neurons



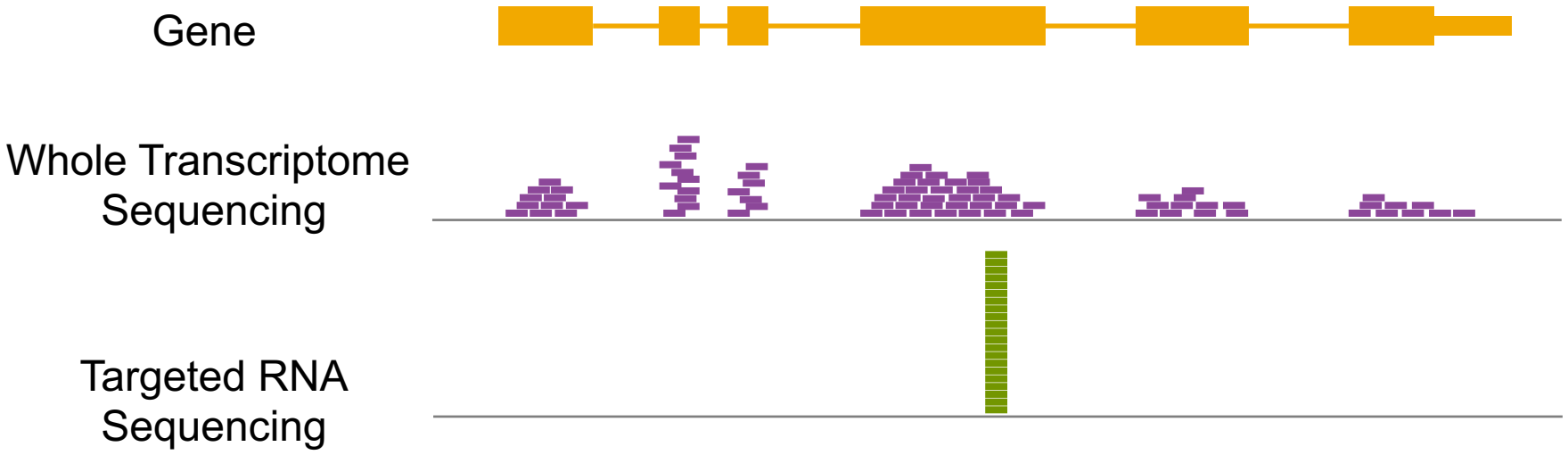




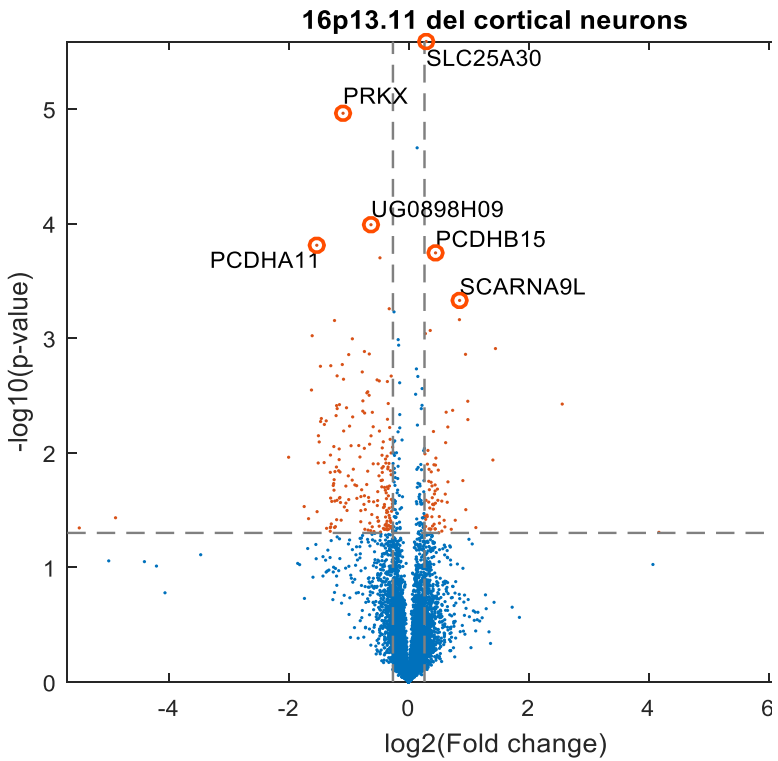
- No effect on neurite length per neuron
- Opposite of expected results based on published reports showing RRN3 disruption leads to decreased neurite outgrowth and branching

Targeted RNA Sequencing to identify novel mechanisms

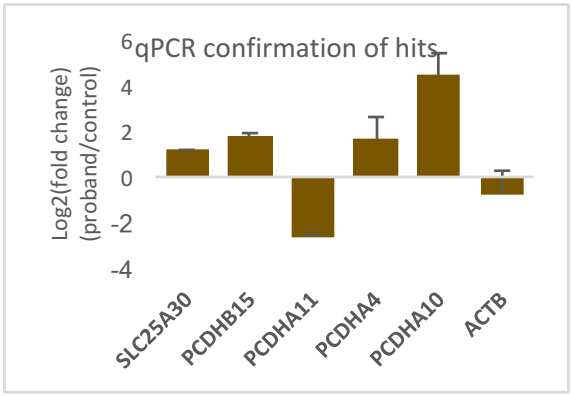
- Ion Torrent S5 Sequencer from ThermoFisher
- Ion AmpliSeq Technology



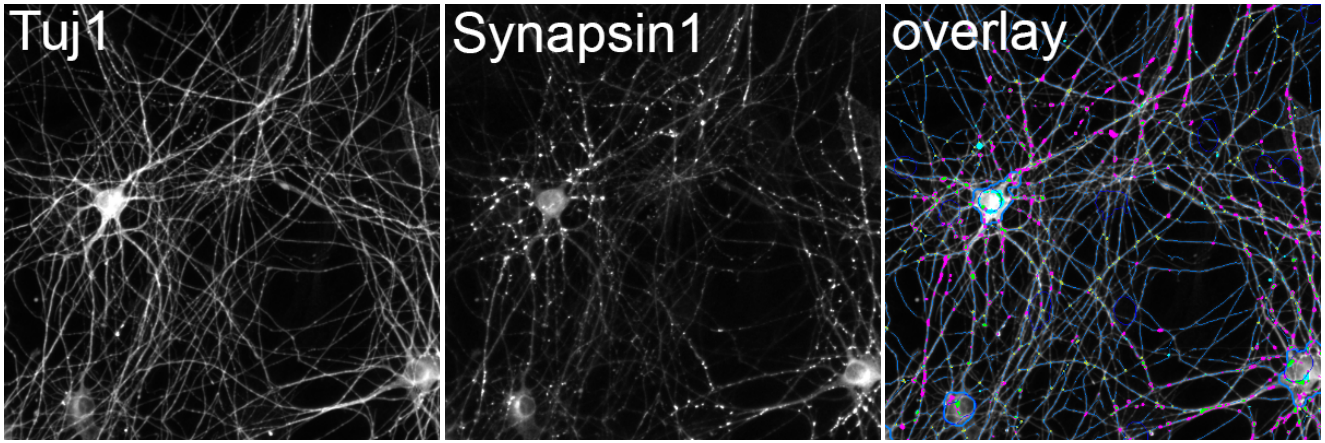
Targeted RNA sequencing reveals changes in protocadherin family

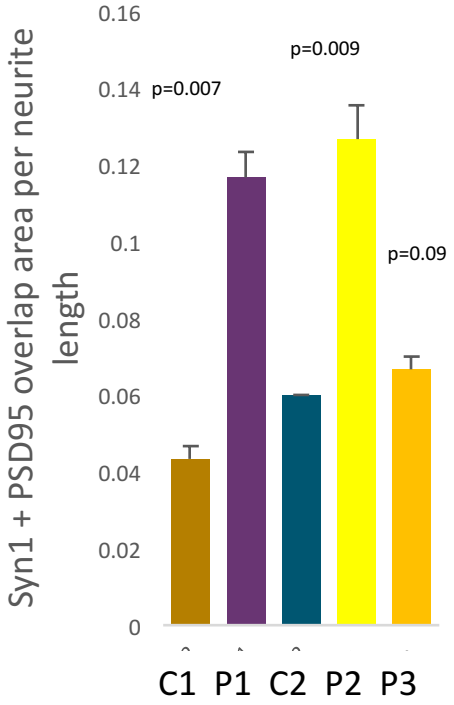


Gene	Fold change	Permutation p-values	FDR	q-values	FDR_Benjamini-Hochberg
SLC25A30	1.22	0.00000275	0.03	0.03	0.03
PRKX	-2.14	0.0000109	0.05	0.05	0.05
UG0898H0	-1.55	0.0000993	0.23	0.23	0.23
TRIB1	-1.39	0.000194102	0.26	0.26	0.26
PCDHB15	1.36	0.000174896	0.27	0.26	0.26
PCDHA11	-2.89	0.00015049	0.28	0.26	0.26
PCDHA10	5.895644	0.003707408	0.670778	0.652962	0.65389655
PCDHA4	1.980907	0.005062864	0.68692	0.68692	0.687903793
PCDH7	-1.66836	0.023554332	1	0.996342	0.997768504



High-content analysis reveals increase in synapse area in 16p13.11 deletion neurons



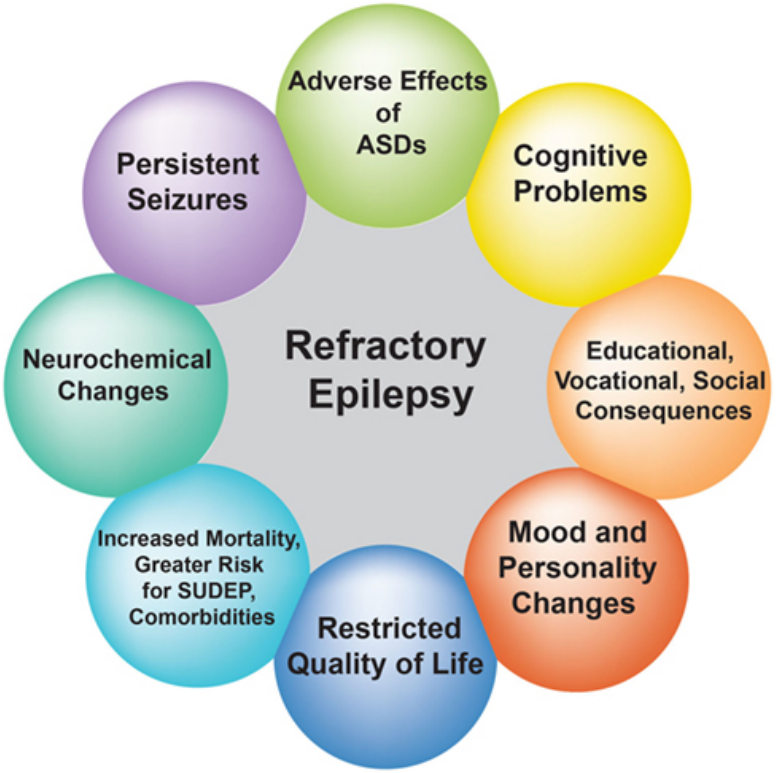


- N-end rule functions regulate synapse protein turnover
- Disrupted *NTAN1* would lead to increased protein accumulation at the synapse
- MEA studies will confirm functional deficits

Where do we go from here?

- CRISPR knockout each gene to determine phenotype specificity
- Compare 16p13.11 deletion and 16p13.11 duplication phenotypes for gene dosage effects
- Utilize novel phenotypes to run drug-repurposing screens

Infant-onset and refractory epilepsy



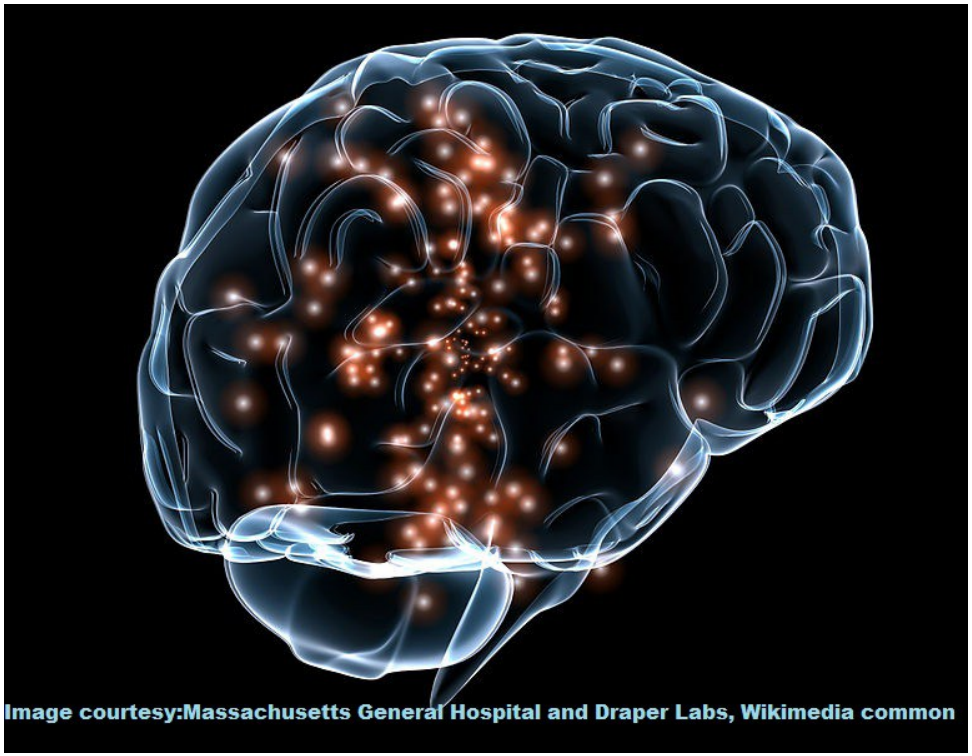


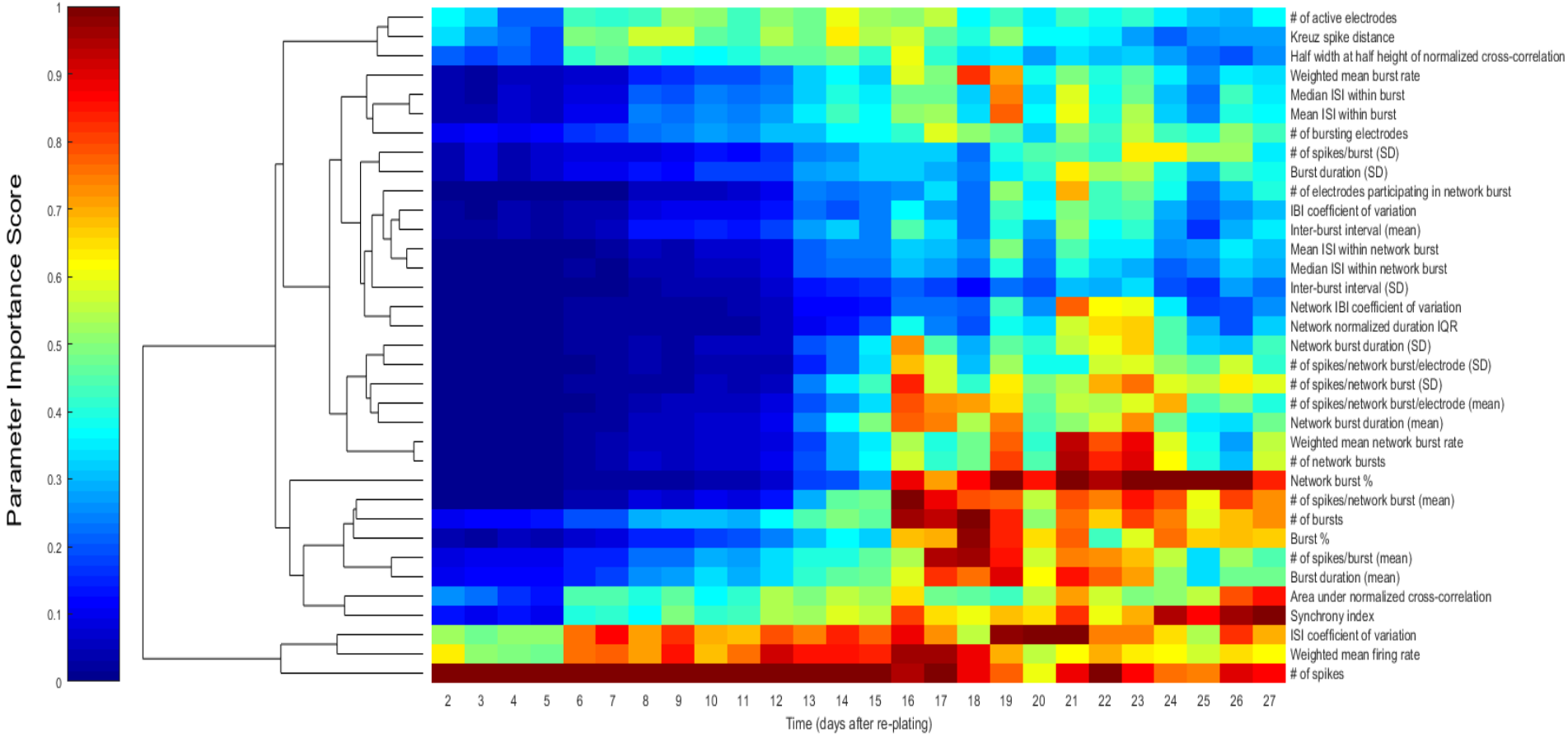
Image courtesy:Massachusetts General Hospital and Draper Labs, Wikimedia common

Using the MEA to interrogate circuitry differences in patient-derived cultures

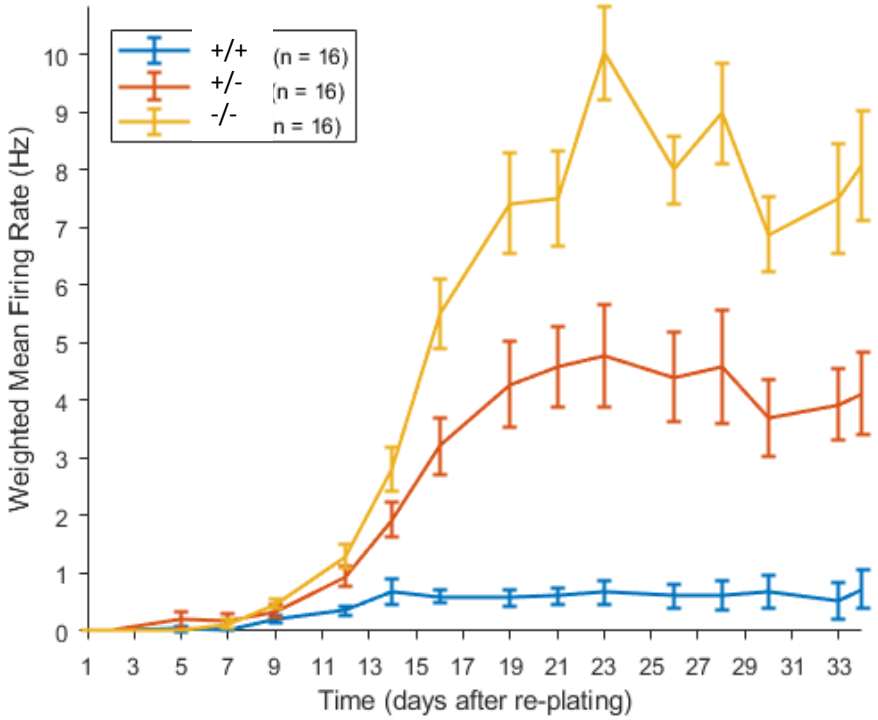
Axion Maestro MEA System

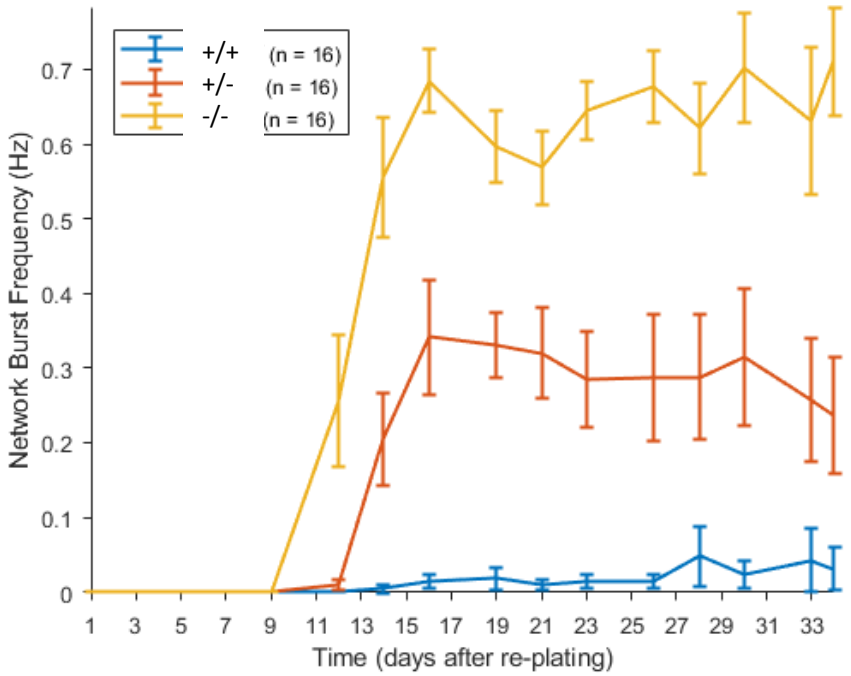
- 48-well and 96-well formats with 16 or 8 electrodes per well, respectively
- Monitor cultures over time to follow maturation

Using MEA to follow neuronal network development with non-biased methods



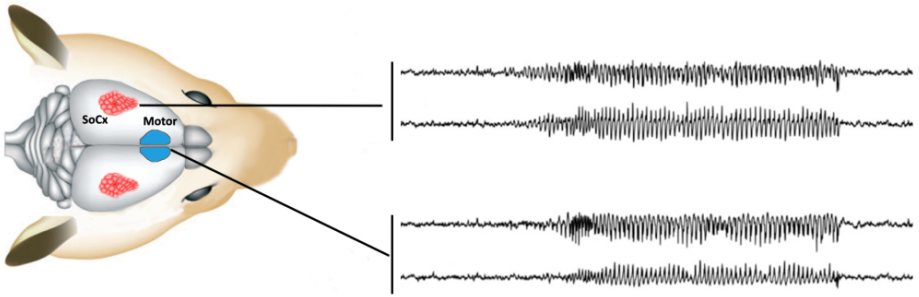
Understanding circuitry changes in epilepsy using iPSC-derived neurons on the MEA platform

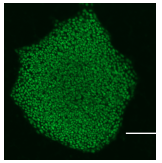




How can we use iPSC-derived neurons to develop new therapies for refractory epilepsy?

- Develop phenotypic screen to identify novel mechanisms that correct defects in synchrony and network activity
- Compare phenotypes across different genetic causes of refractory epilepsy and across diverse patient population
  - "clinical trial in a dish"
- Confirm drug effects in animal models of same genetic disorders – using novel EEG methods





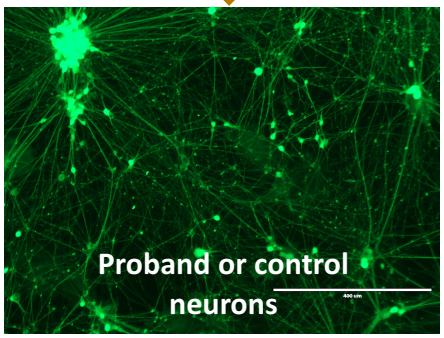
Patient-specific iPSCs

Reprogramming

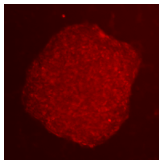
Differentiation

Kleefstra Syndrome

patient fibroblast or blood sample



Differentiation



(Sample collected from parent to generate control iPSCs)

Control iPSCs

Disease modeling

Arrayscan XTI

- *Deficits in neurite length and branching*

Axion Maestro

Multi-electrode array analyses

- *Changes in K+ channel expression may cause hyper-excitability*

Biochemistry &

Ion Torrent AmpliSeq

Identification of pathways and targets with altered regulation in patient neurons

Drug repurposing screens

Identification of novel targets

Thank you!

Boston Children's Hospital
Until every child is well™
Translational Neuroscience Center

- Mustafa Sahin
- HNC:
  - Ivy Chen
  - Lee Barrett
  - Sean Dwyer
  - Adil Wafa
  - Mary-Kate Dornon
- Manton Center:
  - Joseph Gonzalez-Heydrich
  - Catherine Brownstein

Funding:
Massachusetts Life Sciences Center, Tommy Fuss Fund, NIH-IDDRC, F.M. Kirby Neurobiology Center