PGC Worldwide Lab Call Details

DATE: **Friday, April 10th, 2015**

PRESENTER: Kevin Eggan, PhD, Professor in the Department of Stem Cell and Regenerative Biology, Harvard University.

TITLE: "Ruggedizing" Stem Cell Technologies for the Study of Psychiatric Disease

START: We will begin promptly on the hour.

1000 EDT - US East Coast
0700 PST - US West Coast
1500 BST - UK
1600 CEST - Central Europe
Midnight AEST – Australia (Fri., April 10th to 0100 Sat., April 11th, 2015)

DURATION: 1 hour

TELEPHONE:

- US Toll free: 1 877 703.6109
- International direct: +1 617 399.5126
- Toll-free number? See http://www.btconferencing.com/globalaccess/?bid=288_attended
- Operators will be on standby to assist with technical issues. "*0" will get you assistance.
- This conference line can handle up to 300 participants.

PASSCODE: 188 641 29

Lines are Muted **NOW**

Lines have been automatically muted by operators as it is possible for just one person to ruin the call for everyone due to background noise, electronic feedback, crying children, wind, typing, etc.

Operators announce callers one at a time during question and answer sessions.

Dial *1 if you would like to ask a question of the presenter. Presenter will respond to calls as time allows.

Dial *0 if you need operator assistance at any time during the duration of the call.

UPCOMING PGC Worldwide Lab

DATE: **Friday, May 8th, 2015**
PRESENTER: Benjamin Neale, PhD, from Harvard Medical School, ATGU MGH, and Broad Institute
TITLE: To Be Announced
START: We will begin promptly on the hour.
1000 EDT - US East Coast
0700 PST - US West Coast
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1600 CEST - Central Europe
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“Ruggedizing” Stem Cell Technologies for the Study of Psychiatric Disease

Kevin Eggan

Stem Cell Program Mission Statement

- **Our Mission** is to utilize human stem cell biology and reprogramming technologies to create model systems that allow us to study and understand the function of genetic variants that underlie psychiatric conditions.

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Outline

- A small human stem cell “genome project”.
- An automated approach to iPS cell generation.
- Generation and characterization of a prototype human pyramidal neuron.
- An Armamentarium for understanding the regulation of implicated genes.

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ESC Genome Project Goals

- What is the genetic make up of commonly used ES Cell lines?
- Given extensive cell culture, what is their mutational load?
- Catalog the haplotype structure of cell lines for study of regulatory variation.

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Cell Line Acquisition and Seq Plan

- Requested -150 lines from the NIH registry.
- Successfully obtained and banked >100 lines.
- Goal: 30X WGS on Illumina X10 platform and deeper exome sequence for each line.

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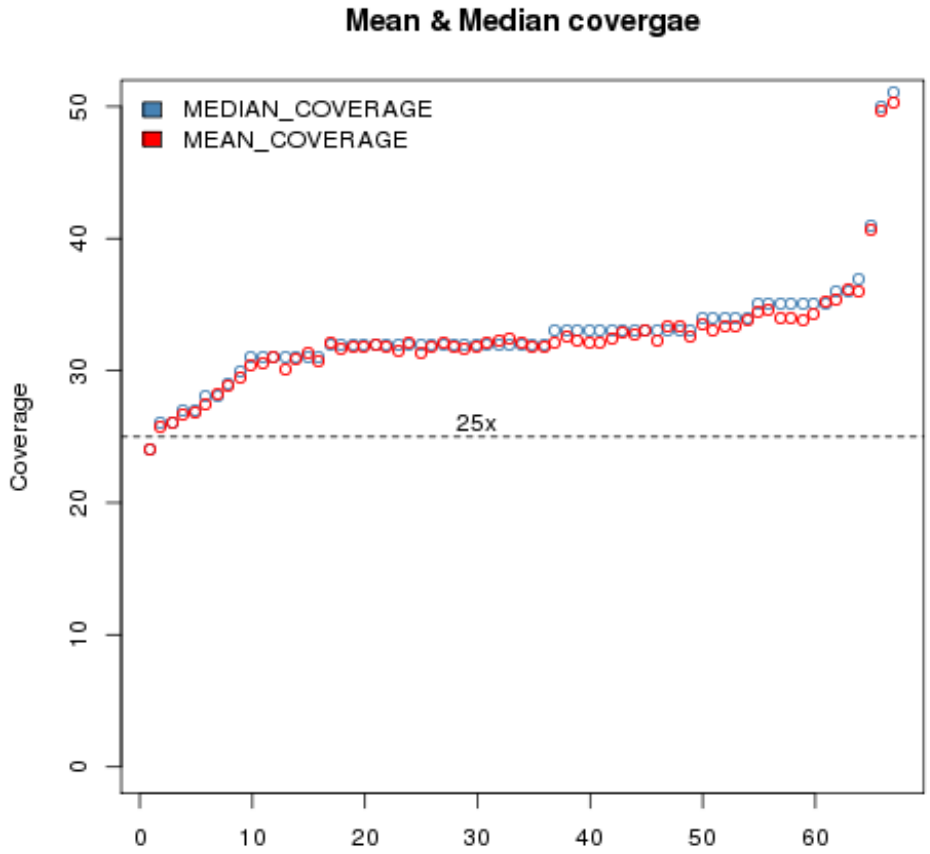
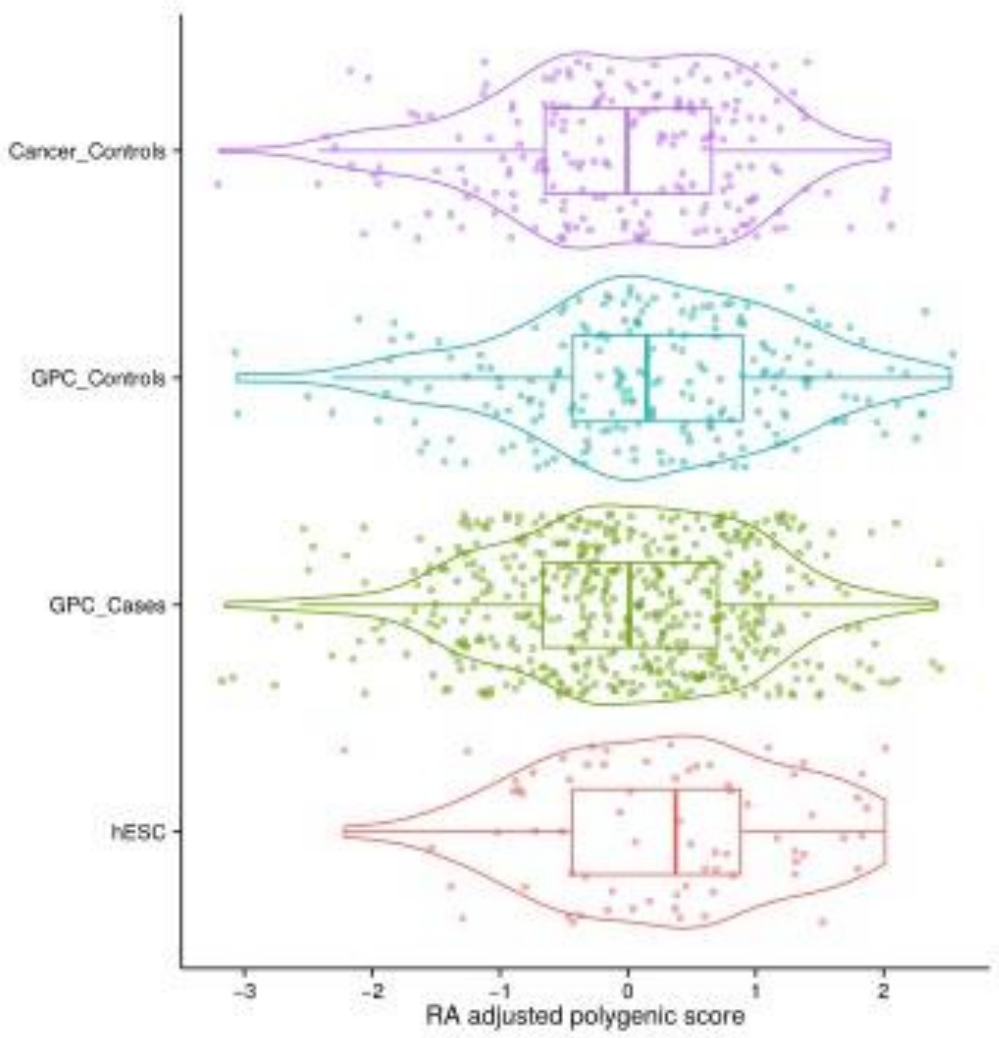
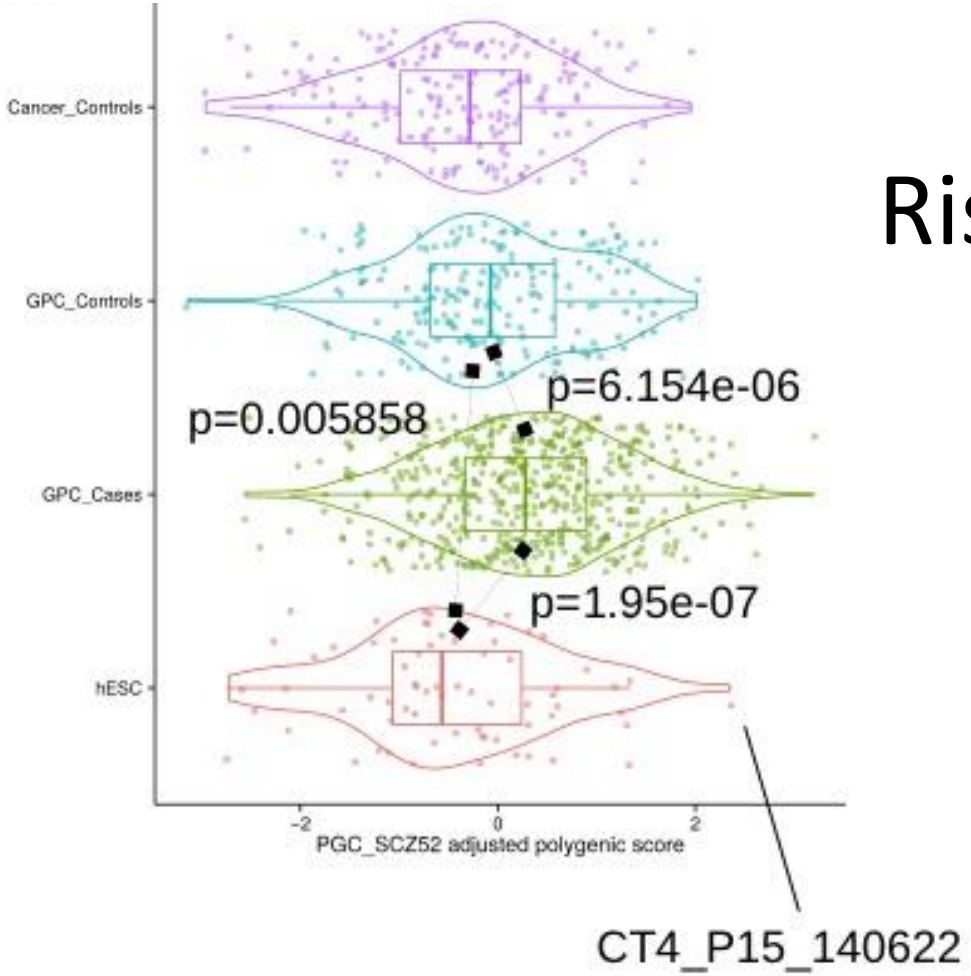


Human ES cell line analyzed



Polygenic Risk of Arthritis

Polygenic Risk of Schizophrenia



Initial impressions from sequences

- Lines are surprisingly intact.
- Small number of shared CNVs, mostly known.
- On average, 1-2 private mutations per line.
- While lines are on average depleted of risk, many cell lines harbor risk variants of interest for study.
- Ongoing deposition in dbGaP for data sharing.

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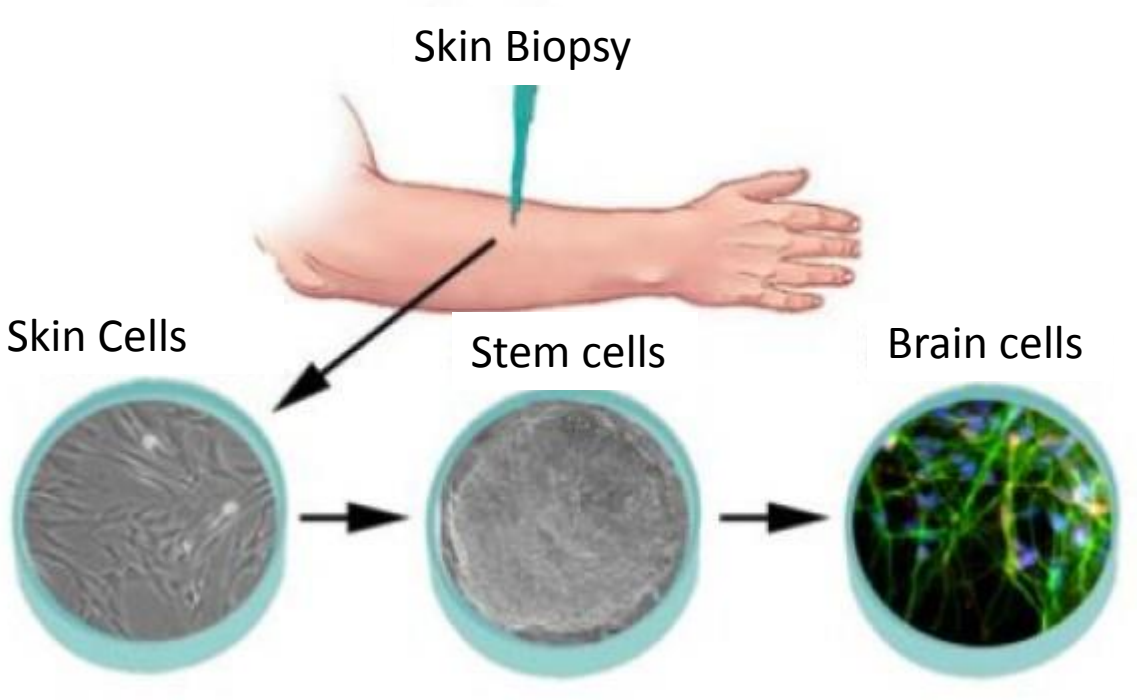
Reprogramming Breakthrough



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Takahashi, Yamanaka and many others

The Challenge: Artisanal Production

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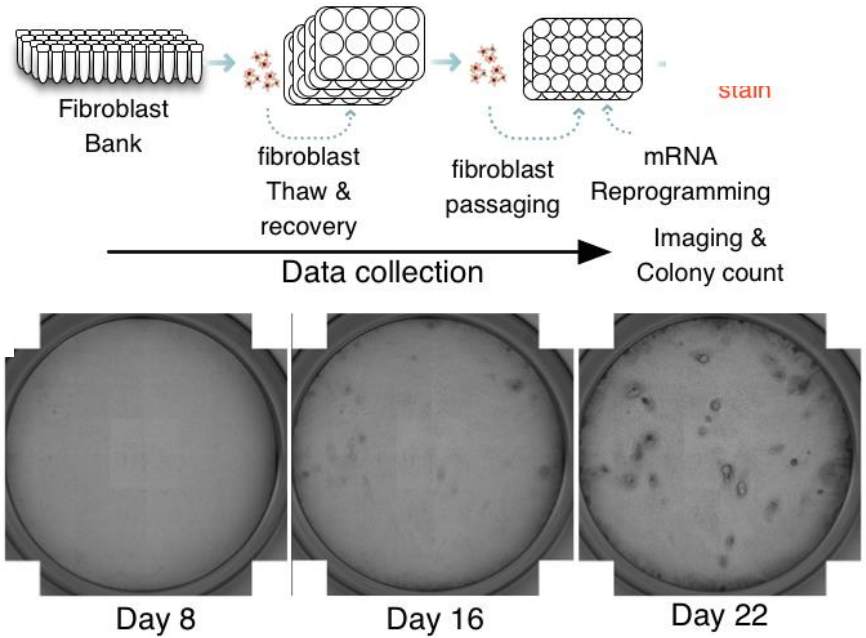
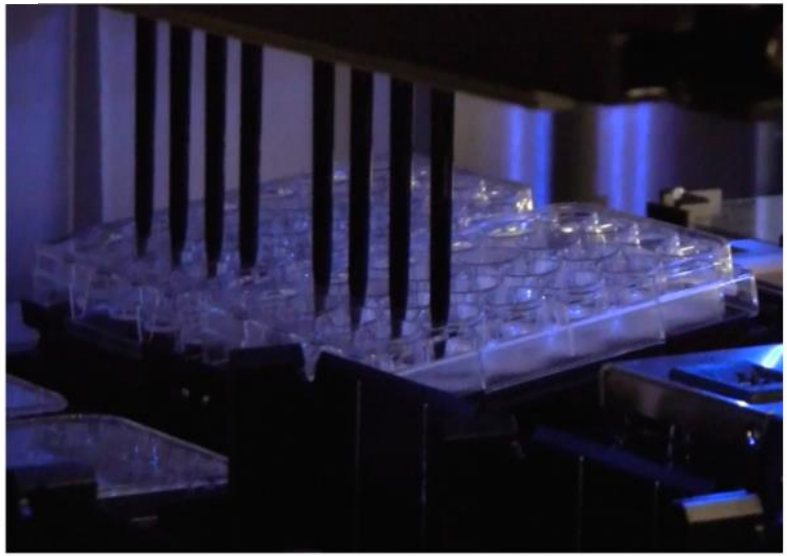
Robotic Production of iPS Cells





NYSCF The New York Stem Cell Foundation

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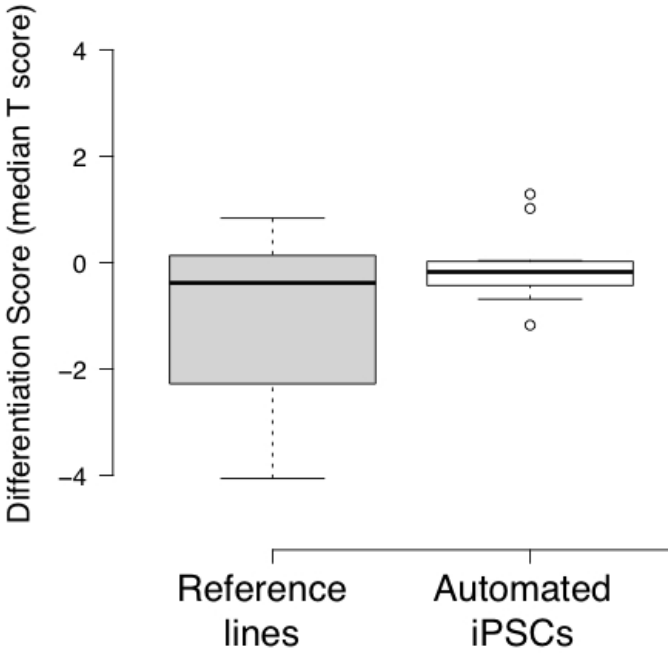
Daniel Paul, Scott Noggle, Alex Meissner

Automation Reduces Stem Cell Performance



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Daniel Paul, Scott Noggle, Alex Meissner

A Growing Collection of Somatic Samples

Diagnosis	Subjects Collected
Bipolar Disorder:	101
Schizophrenia:	81
Schizoaffective Disorder:	43
Healthy Controls:	58

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Reprogramming Progress Report

- 100 individuals to be reprogrammed in 2015.
- Initial focus on high/low levels of polygenic risk and calcium channel genotypes.
- Group conversation ongoing concerning which Swedish high-risk individuals to collect/study.
- Intent to distribute these iPS cells broadly.

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Bruce Cohen, Christina Hultman

A prototype pyramidal neuron

- Sudhof and Wernig labs reported that forced expression of *NGN2* drives neuronal specification of human pluripotent stem cells (Zhang *et al* 2013).

- What are the reproducible qualities of these neurons?
- Can we use these as a stable baseline for evaluation of variants implicated in psychiatric disease?

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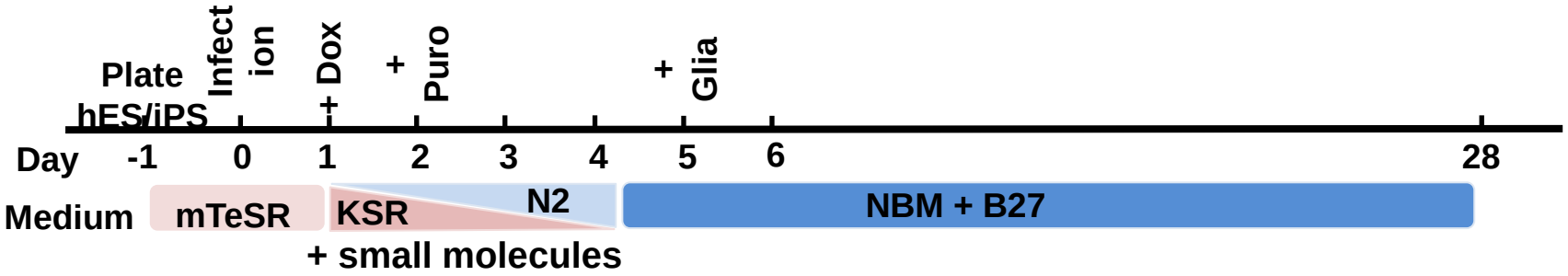
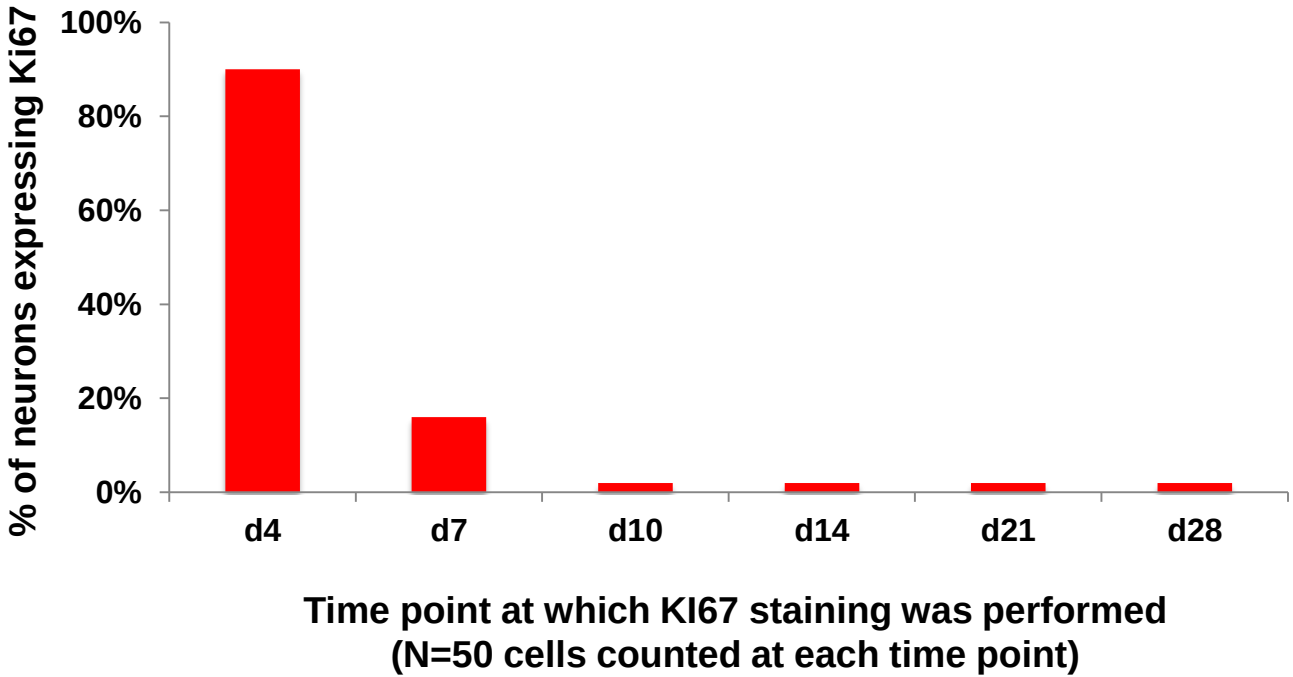
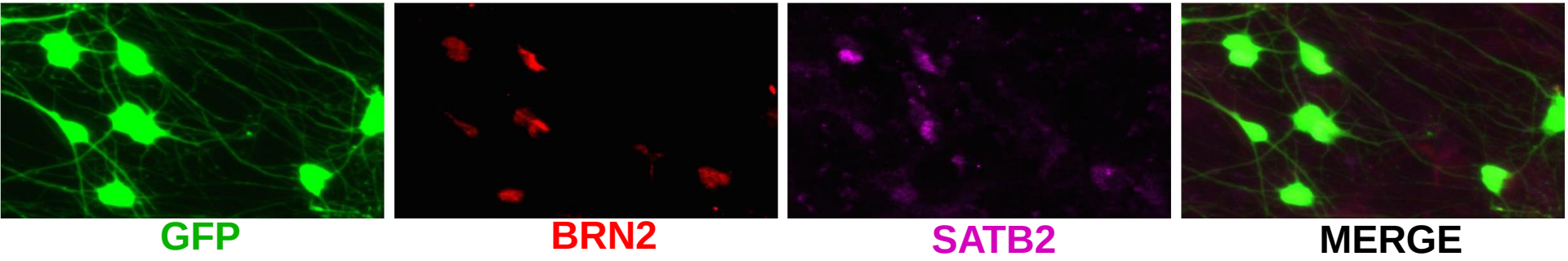
The NGN2 rapid differentiation protocol

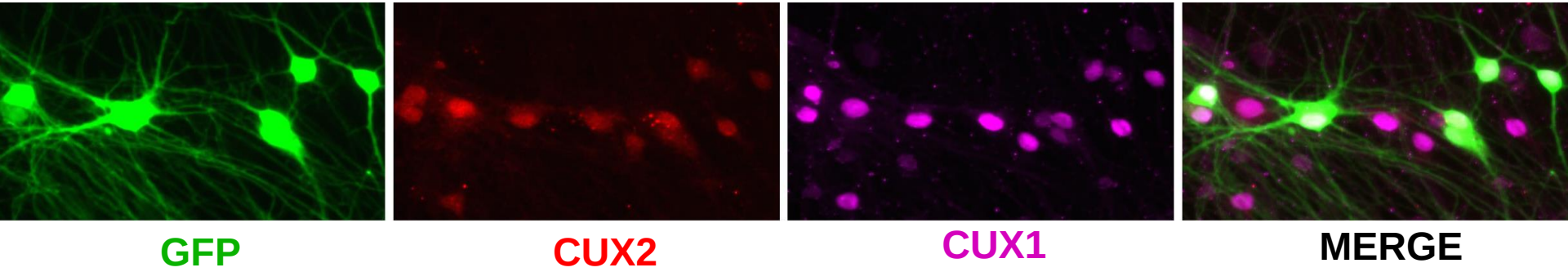


Ngn2 neurons exit cell cycle by day 10

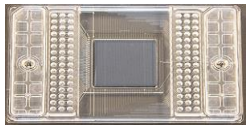


Ngn2-derived neurons express markers of superficial cortical layers



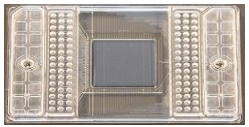


Analysis of gene expression in single cells using the Fluidigm Biomark



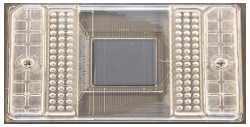
CHIP 1
Cell Identity

- ✓ Pluripotency
- ✓ Neural Crest
- ✓ Forebrain progenitors
- ✓ Other neuronal progenitors
- ✓ Astro-Glial markers
- ✓ Pan Neuronal
- ✓ Cortical Projection Neurons
- ✓ Corticospinal/Corticofugal PN



CHIP 2
Neuronal Maturity

- ✓ Forebrain progenitors
- ✓ Astro-Glial markers
- ✓ Pan Neuronal
- ✓ Cortical Projection Neurons
- ✓ Corticospinal/Corticofugal PN
- ✓ Interneuron
- ✓ Migratory
- ✓ Synaptic/Neurochemical



CHIP 3
Emerging GWAS genes

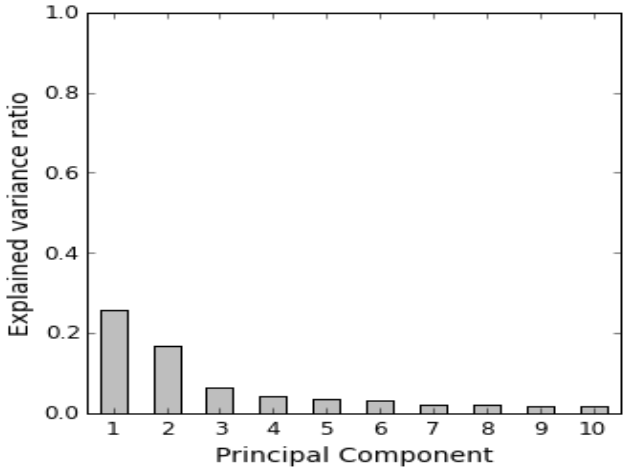
- ✓ “Single” gene haplotypes
- ✓ “Two” gene haplotypes
- ✓ “Autism” genes
- ✓ Controls

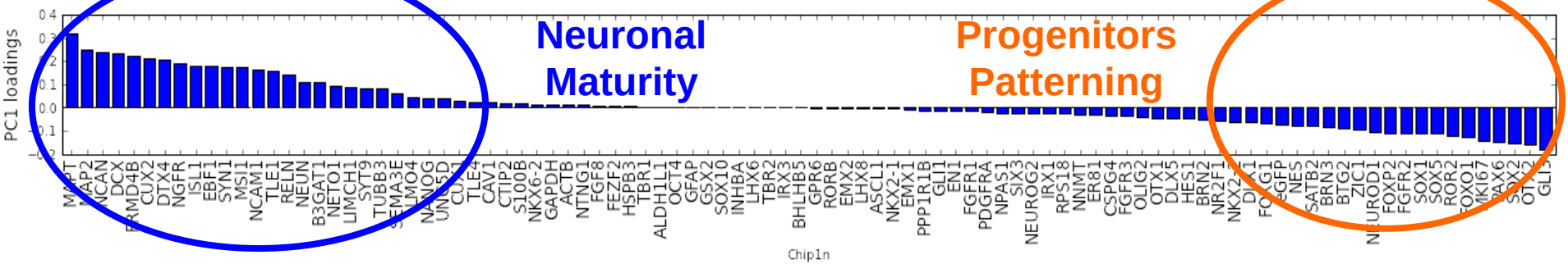
Designed with: Paola Arlotta, Ralda Nehme, Ema Zuccaro, Asif Maroof, Lindy Barrett

Cells show a strong transcriptional signal of maturation over time in culture (chip 1)

Number of cells assayed

Novartis d4	**90**
Novartis d7	**88**
Novartis d14	**84**
Novartis d21	**89**

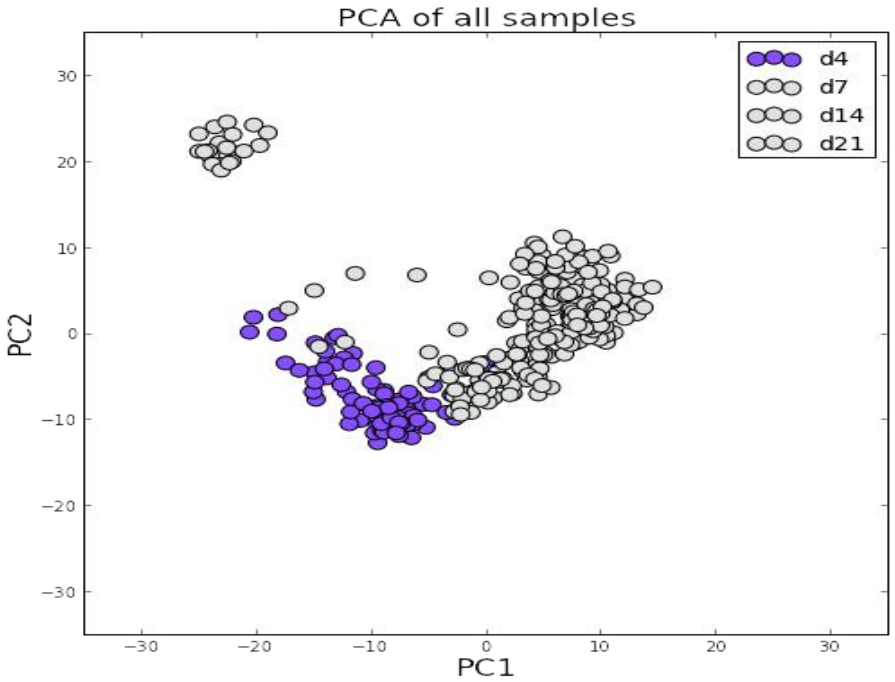




PCA analysis of NGN2 cells over 21 days of culture

d4 cells:

Express progenitor markers (including Ki67), some neuronal markers, some CPN markers (such as Brn2 and Satb2).

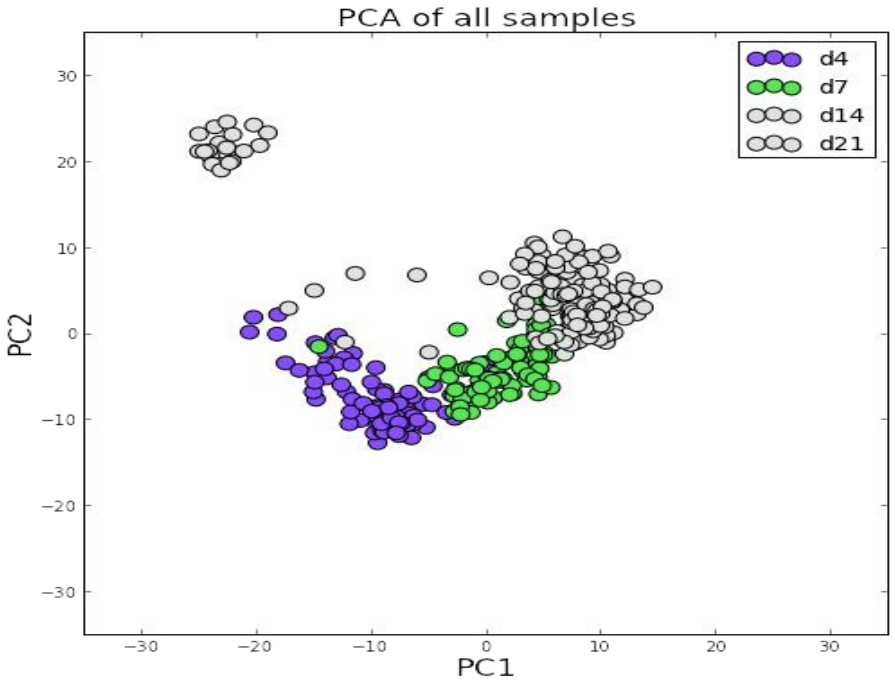


Less mature

More mature

PCA analysis of NGN2 cells over 21 days of culture

d7 cells:
cells become more neuronal

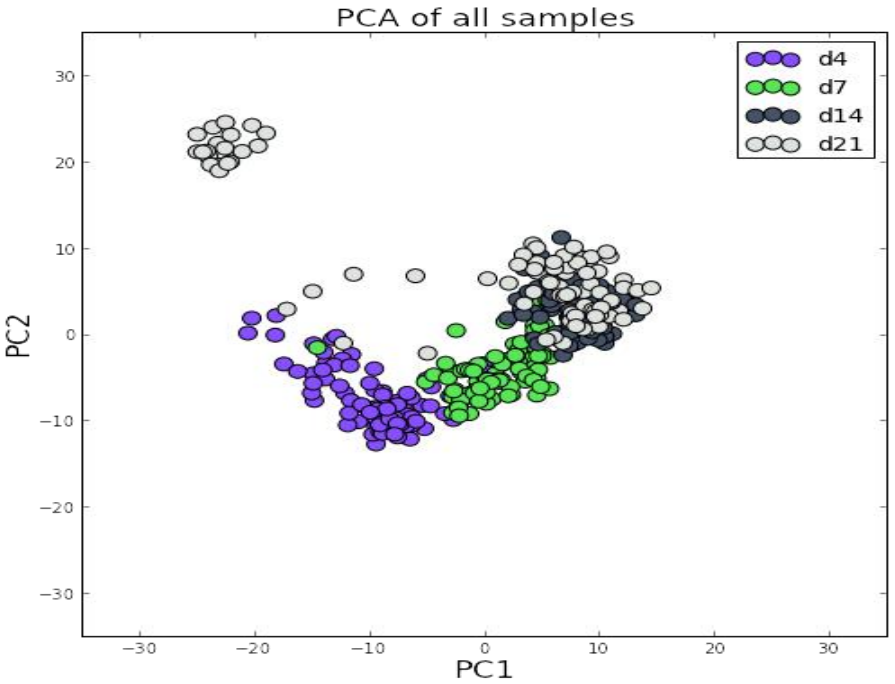


Less mature

More mature

PCA analysis of NGN2 cells over 21 days of culture

d14: even more neuronal. Cells express CPN, and some deep layer markers



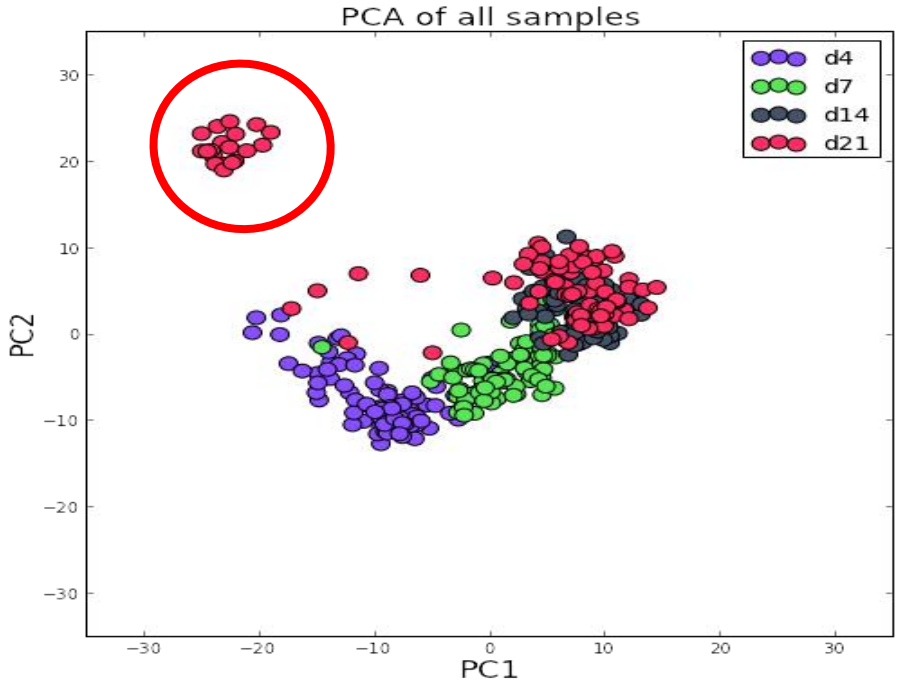
Less mature

More mature

PCA analysis of NGN2 cells over 21 days of culture

d21: most cells are neuronal

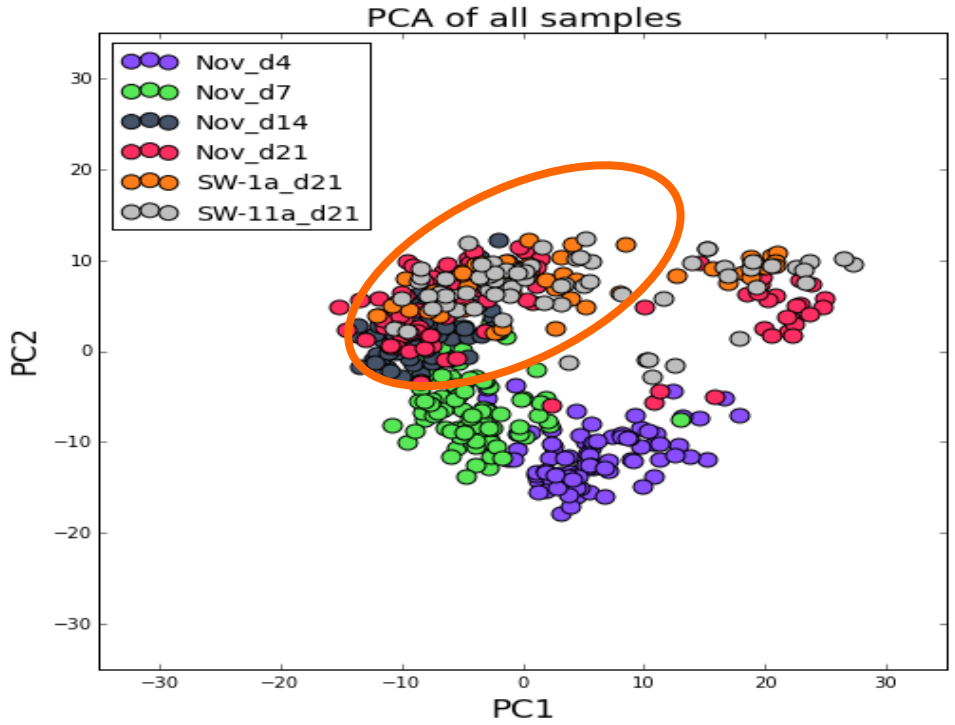
However, a progenitor population appears (20% of cells)



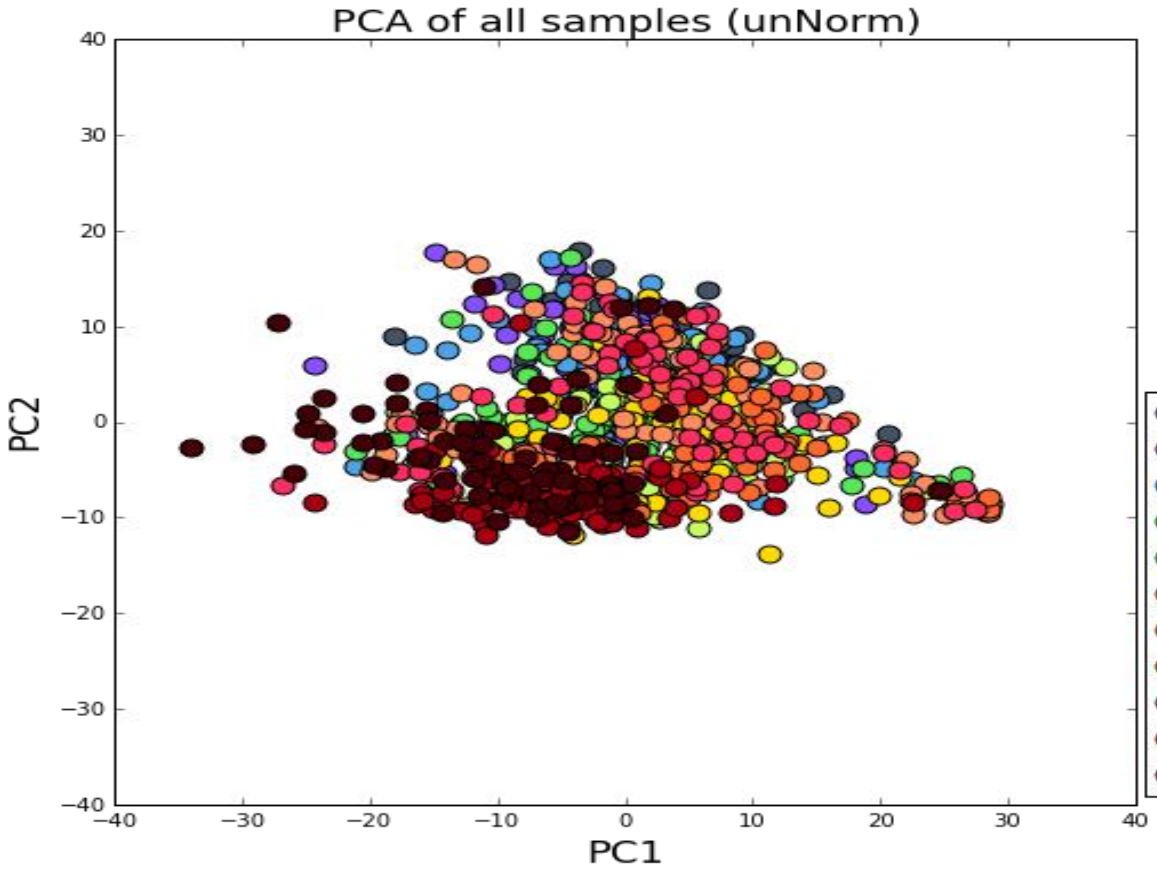
Less mature

More mature

Reproducible gene expression across line, site



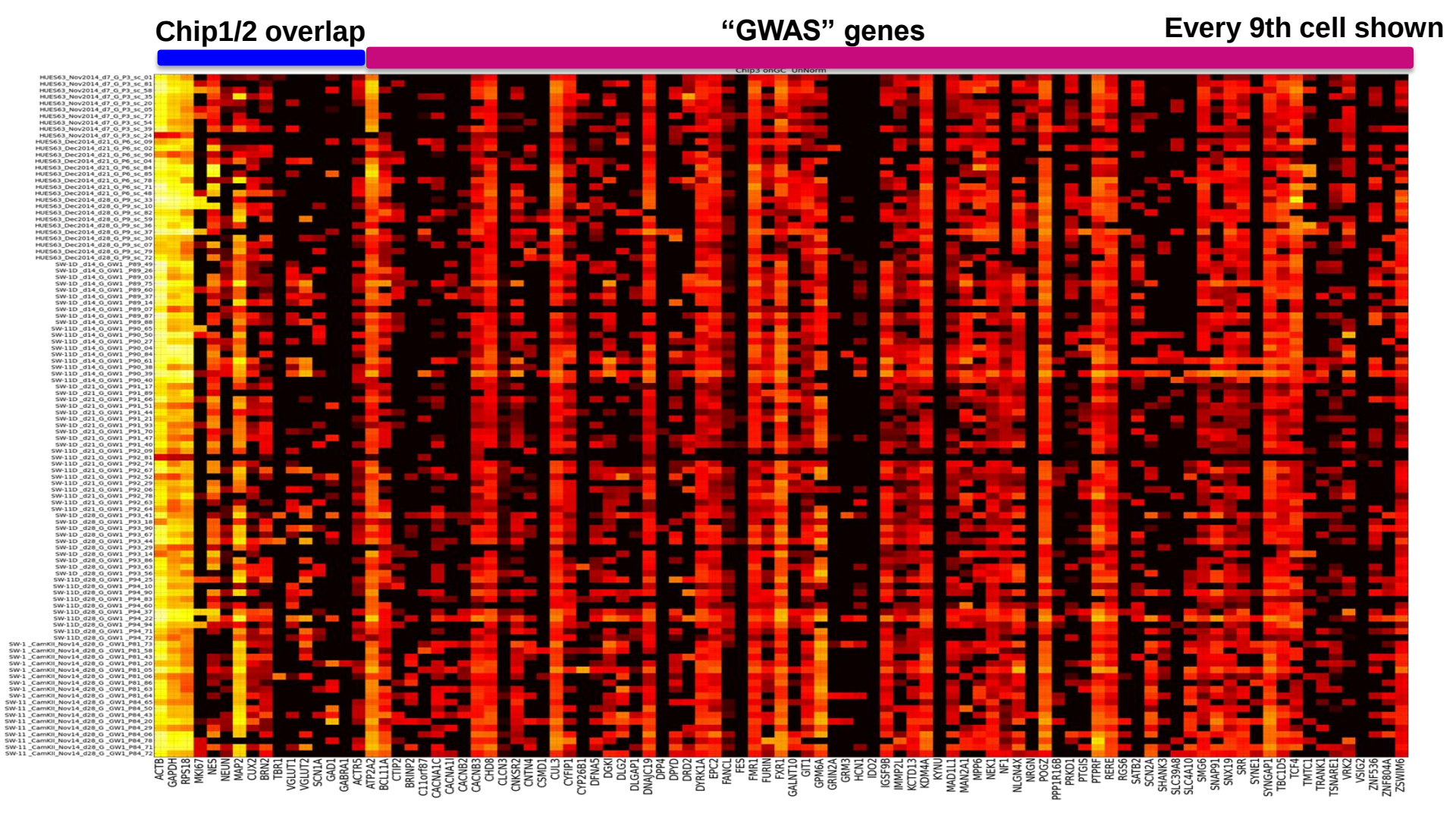
Chip3 (SCZ GWAS) comparisons



Samples:

- **SW-1 (d14, d21, d28)**
- **SW-11 (d14, d21, d28)**
- **Hues63 (d7, d21, d28)**
- **CamKII-gfp sorted:**
  - **SW-1 d28**
  - **SW-11 d28**

	Cell #
HUES63_d7_G	78
HUES63_d21_G	88
HUES63_d28_G	90
SW-1D _d14_G	82
SW-1D _d21_G	80
SW-1D _d28_G	86
SW-11D _d14_G	86
SW-11D _d21_G	87
SW-11D _d28_G	88
SW-1 CamKII d28_G	91
SW-11 CamKII d28_G	92


Chip1/2 overlap
“GWAS” genes
Every 9th cell shown
Chip3 onGC UnNorm
HUES63_Nov2014_d7_G_P3_sc_01
HUES63_Nov2014_d7_G_P3_sc_81
HUES63_Nov2014_d7_G_P3_sc_58
HUES63_Nov2014_d7_G_P3_sc_35
HUES63_Nov2014_d7_G_P3_sc_20
HUES63_Nov2014_d7_G_P3_sc_05
HUES63_Nov2014_d7_G_P3_sc_77
HUES63_Nov2014_d7_G_P3_sc_54
HUES63_Nov2014_d7_G_P3_sc_39
HUES63_Nov2014_d7_G_P3_sc_24
HUES63_Dec2014_d21_G_P6_sc_09
HUES63_Dec2014_d21_G_P6_sc_02
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HUES63_Dec2014_d28_G_P9_sc_79
HUES63_Dec2014_d28_G_P9_sc_72
SW-1D_d14_G_GW1_P89_49
SW-1D_d14_G_GW1_P89_26
SW-1D_d14_G_GW1_P89_03
SW-1D_d14_G_GW1_P89_75
SW-1D_d14_G_GW1_P89_60
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SW-11D_d28_G_GW1_P94_94
SW-11D_d28_G_GW1_P94_71
SW-11D_d28_G_GW1_P94_72
SW-1_CamKII_Nov14_d28_G_GW1_P81_73
SW-1_CamKII_Nov14_d28_G_GW1_P81_58
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SW-11_CamKII_Nov14_d28_G_GW1_P84_73
ACTB
GAPDH
RPS18
MKI67
NES
NEUN
MAP2
CUX2
BRN2
TBR1
VGLUT1
VGLUT2
SCN1A
GAD1
GABRA1
ACTR5
ATP2A2
BCL11A
CTIP2
BRINP2
C11orf87
CACNA1C
CACNA1I
CACNB2
CACNB3
CHD8
CLCN3
CNKSR2
CNTN4
CSMD1
CUL3
CYFIP1
CYP26B1
DFNA5
DGKI
DLG2
DLGAP1
DNAJC19
DPP4
DPYD
DRD2
DYRK1A
EPC2
FANCL
FES
FMR1
FURIN
FXR1
GALNT10
GIT1
GPM6A
GRIN2A
GRM3
HCN1
IDO2
IGSF9B
IMMP2L
KCTD13
KDM4A
KYNU
MAD1L1
MAN2A1
MPP6
NEK1
NF1
NLGN4X
NRGN
POGZ
PPP1R16B
PRKD1
PTGIS
PTPRF
RERE
RGS6
SATB2
SCN2A
SHANK3
SLC39A8
SLC4A10
SMG6
SNAP91
SNX19
SRR
SYNE1
SYNGAP1
TBC1D5
TCF4
TMTC1
TRANK1
TSNARE1
VRK2
VSIG2
ZNF536
ZNF804A
ZSWIM6

Whole-cell patch clamp of the Ngn2 neurons

Plate hES/iPS infected with TetO-Ngn2

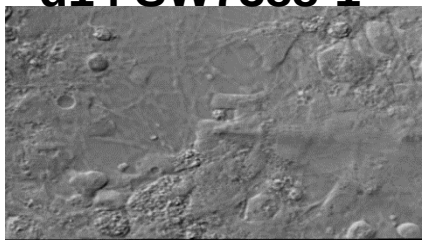
Replate on coverslips with glia

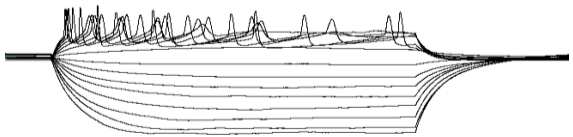
Whole-cell patch clamp recordings

Day 0 4 14 21 28 35

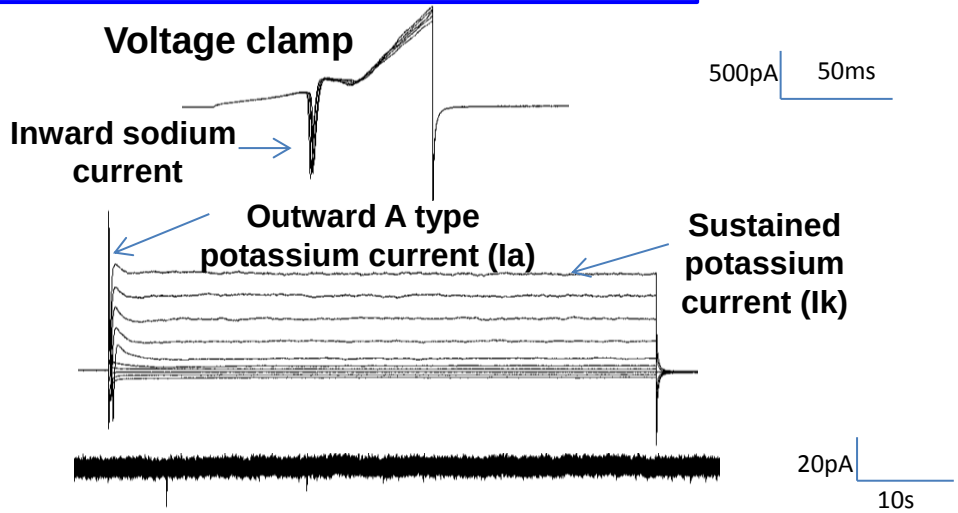
d14: Repetitive action potential firing and sodium currents

d14 SW7388-1





Current clamp



Zhanyan Fu and Chenchen Li

Whole-cell patch clamp of the Ngn2 neurons

Plate hES/iPS infected with TetO-Ngn2

Replate on coverslips with glia

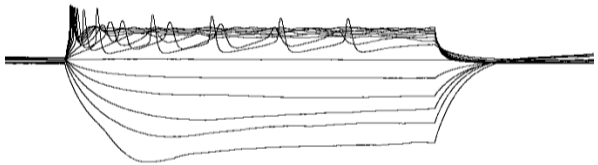
Whole-cell patch clamp recordings

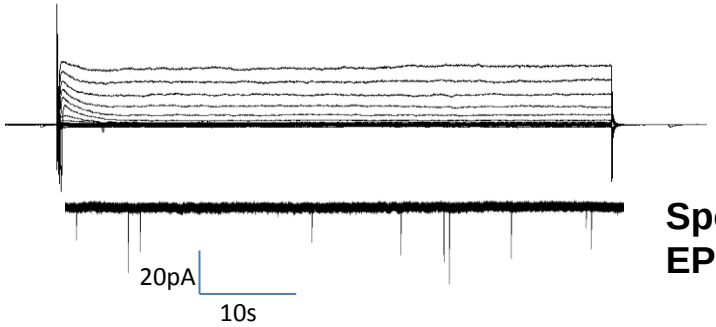
Day 0 4 14 21 28 35

Cells appear to be mature at **d28** and **d35**

- "Large" **synaptic transmission events**

d28 SW7388-1

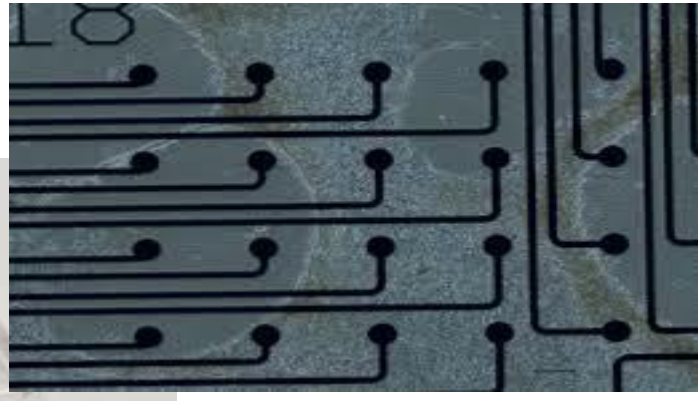




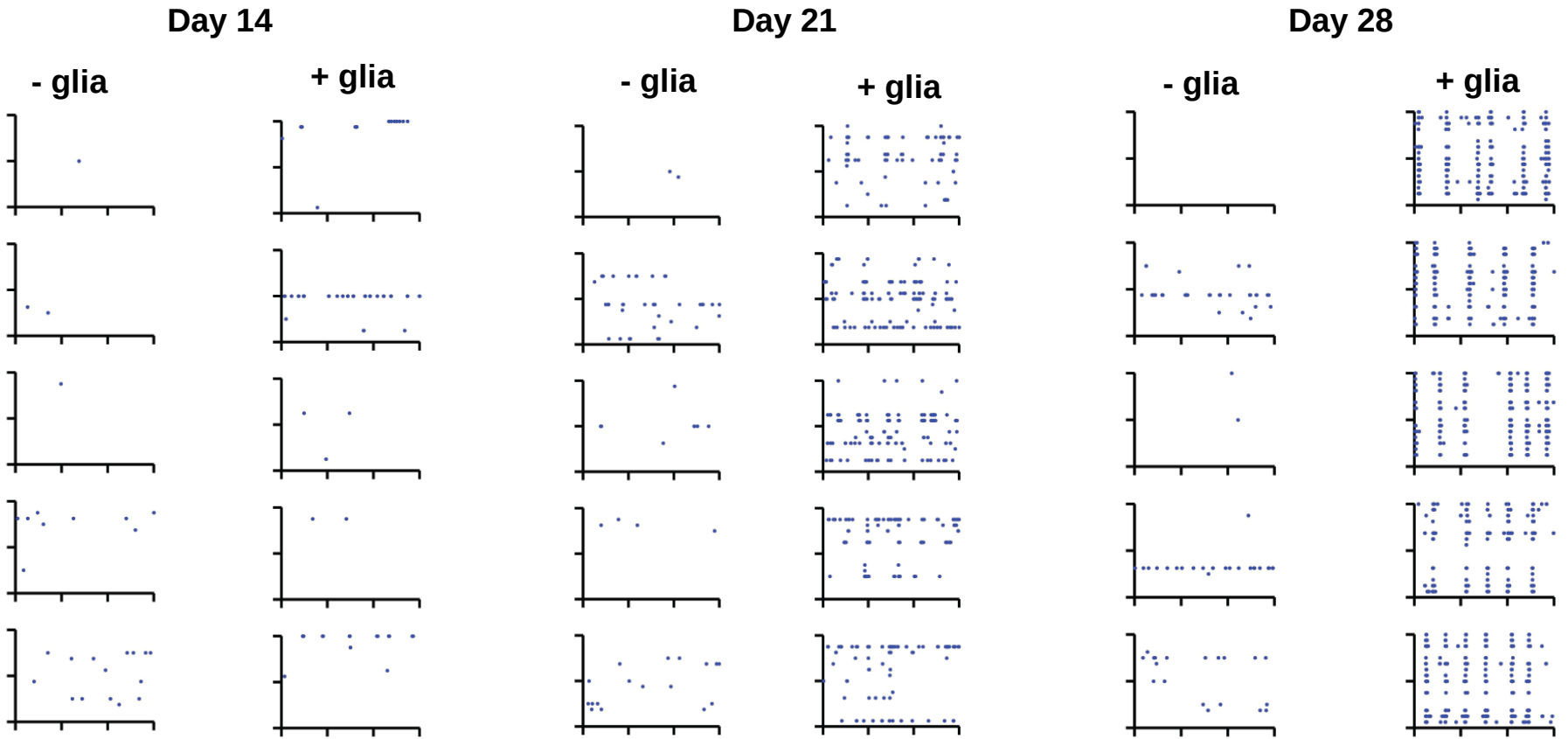
Spontaneous EPSCs

Zhanyan Fu and Chenchen Li

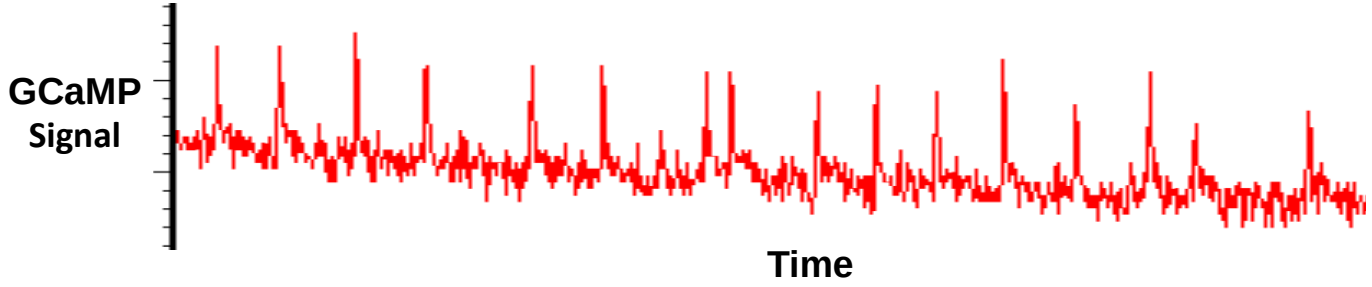
Monitoring activity in electrode array



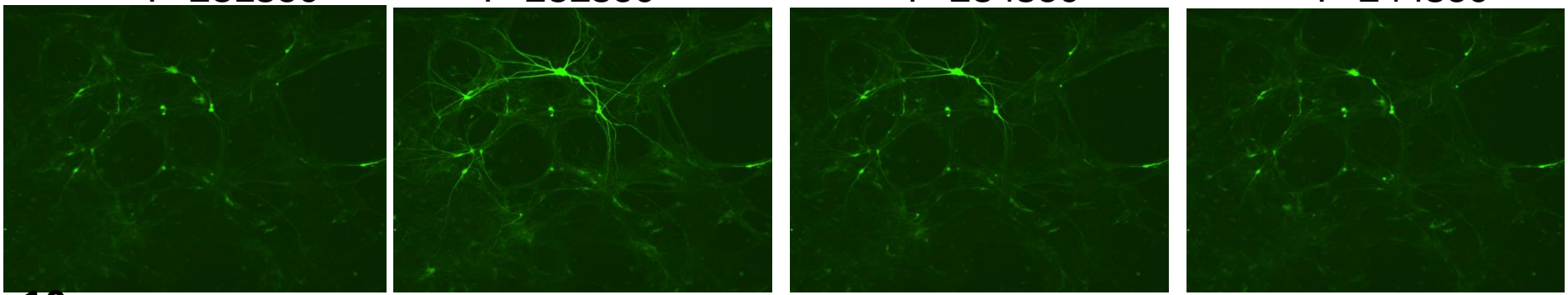
Astrocytes Induce Synchronized Network Activity



GCaMP6 reporter shows NGN2 neurons fire synchronously

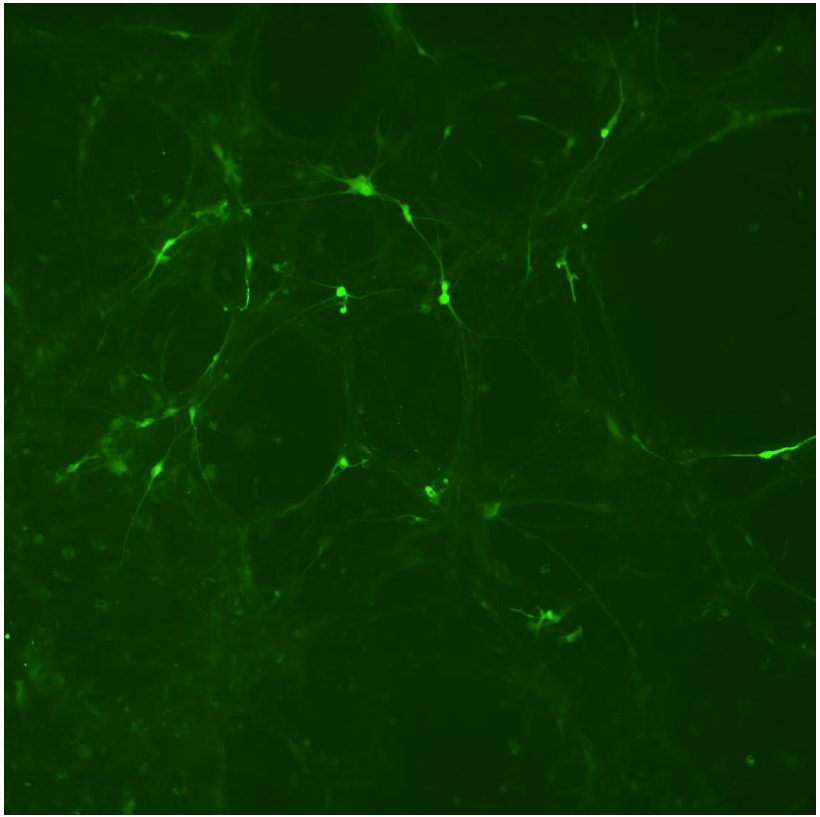


T=231sec T=232sec T=234sec T=244sec

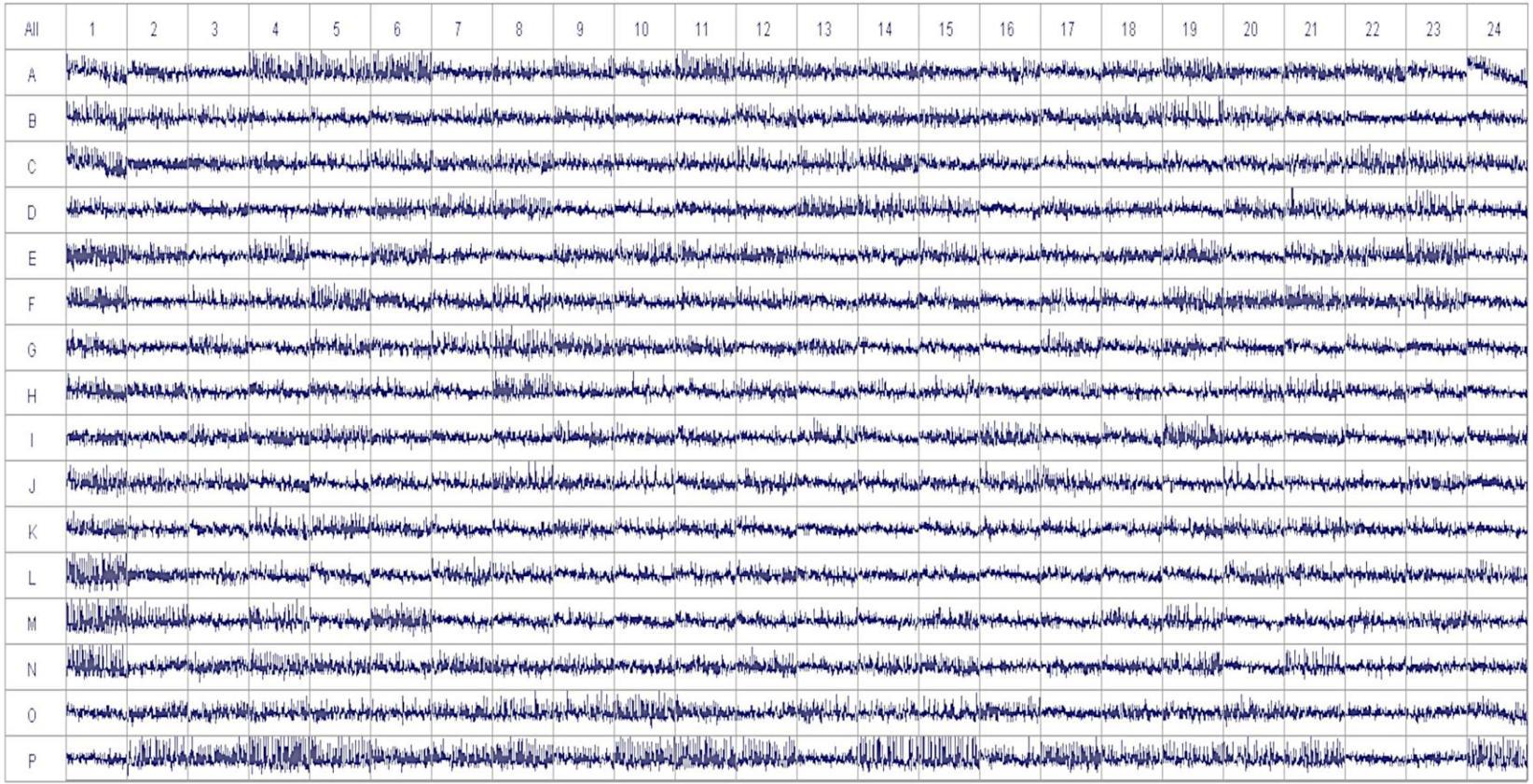


10x

GCaMP6 reporter shows NGN2 neurons fire synchronously



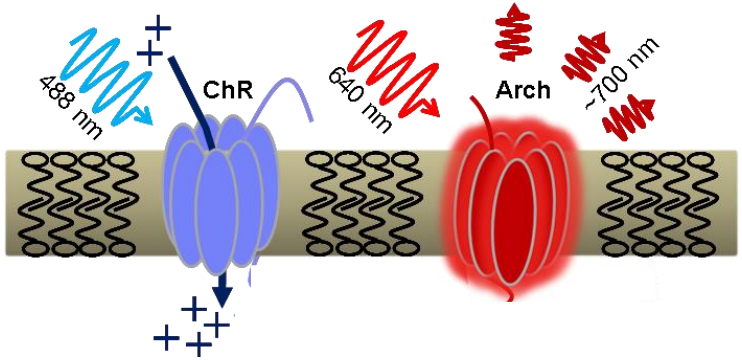
“Bursting” of NGN2 neurons in 384 plate format

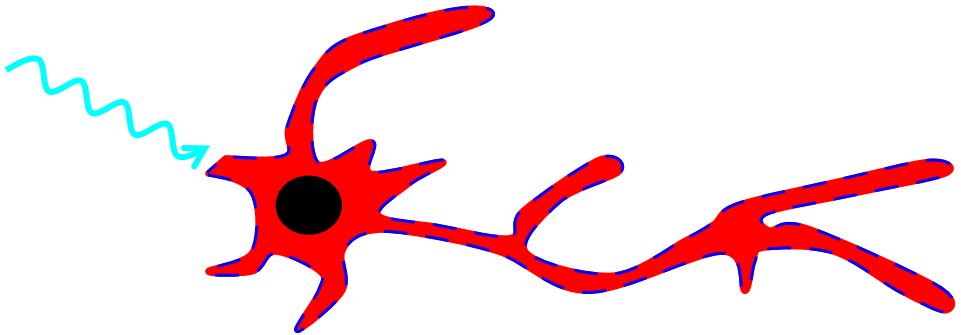


Yan-Ling Zhang and James Hawrot

Probing Activity with Light

Co-express **Actuator** and **Voltage indicator**





Blue light triggers neural activity.

Red light probes the response.

Dan Hochbaum, Joel Kralj, Adam Cohen

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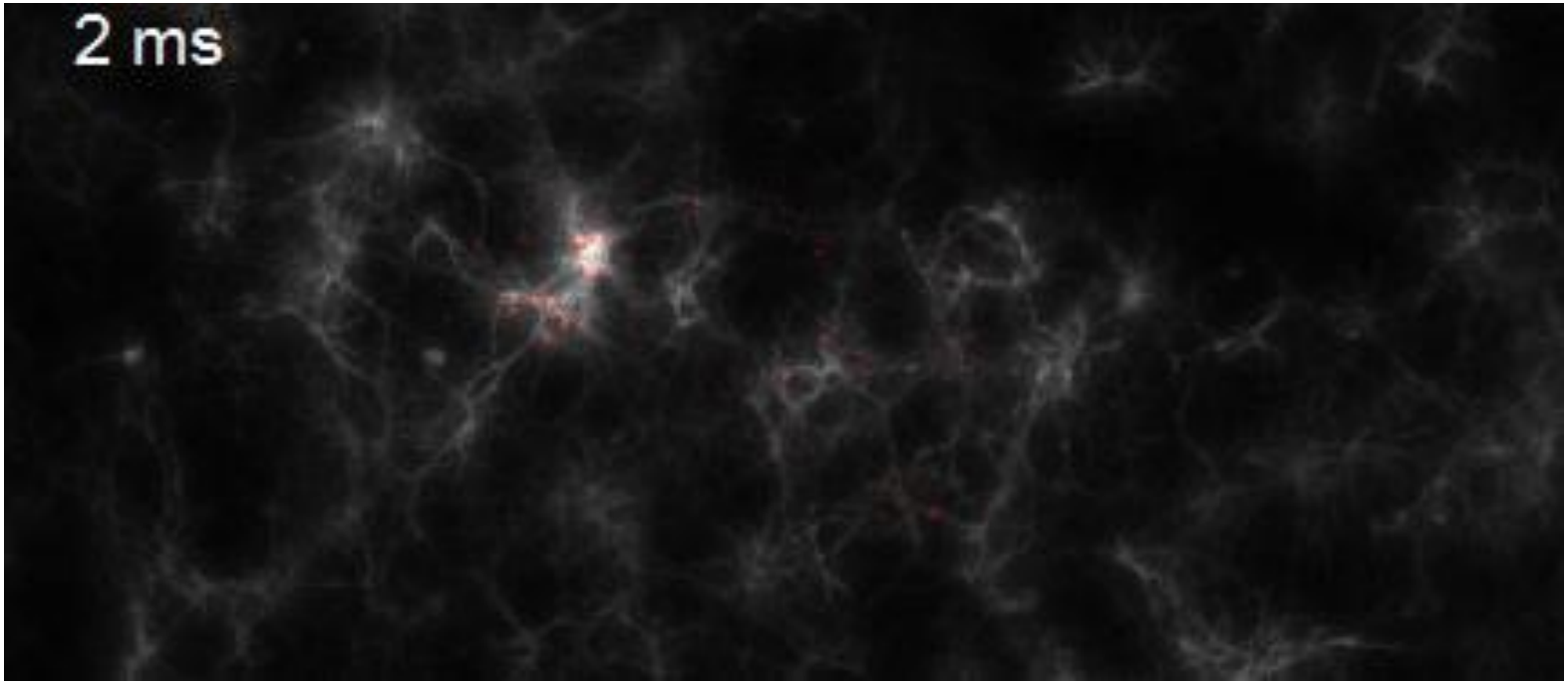
Visualizing Neuronal Activity



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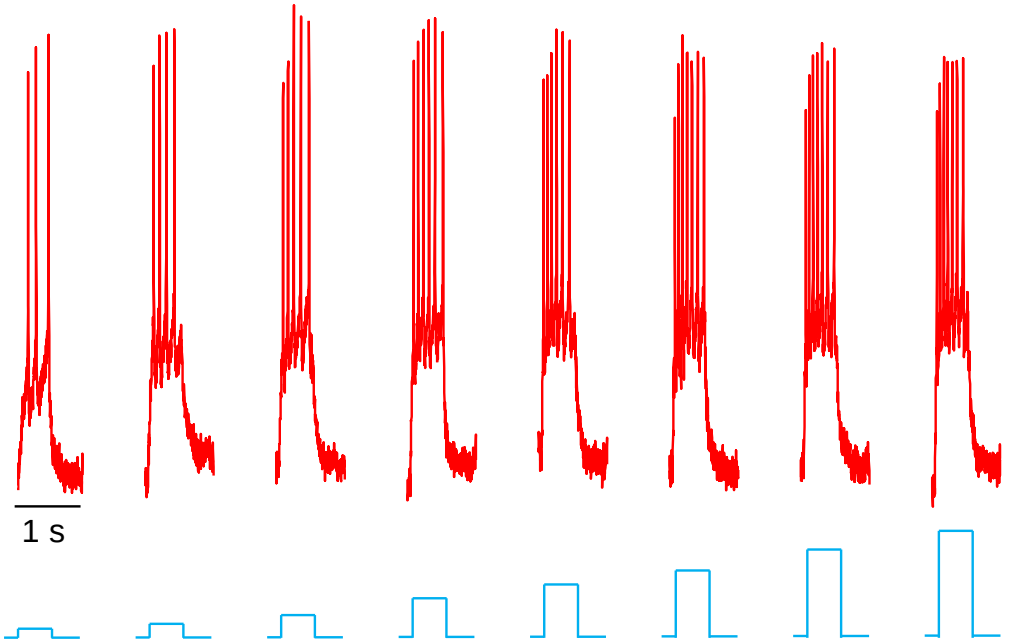
NGN2 neurons can be optically stimulated and recorded from

Optical response:



Blue light Stimulation:

Evangelos Kiskinis
Ralda Nehme
Dan Hochbaum

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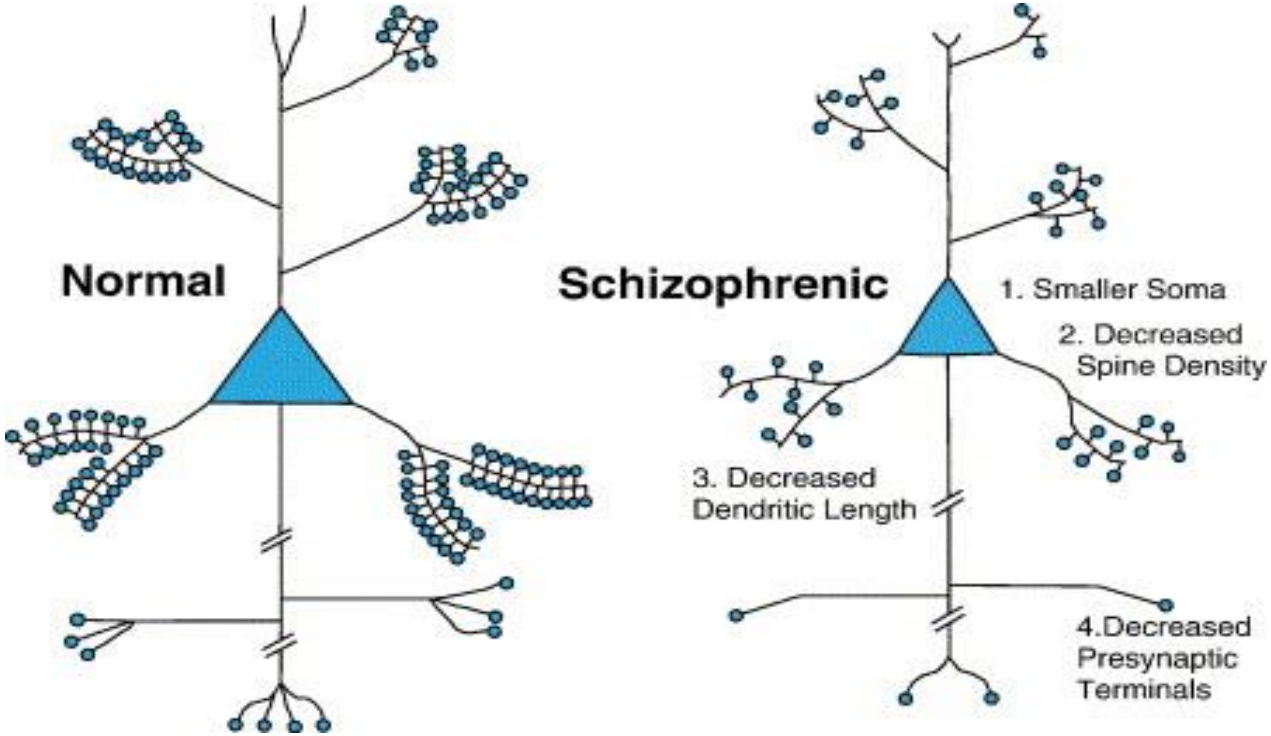
The Challenge: Modeling Patients



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Glantz et al 2006

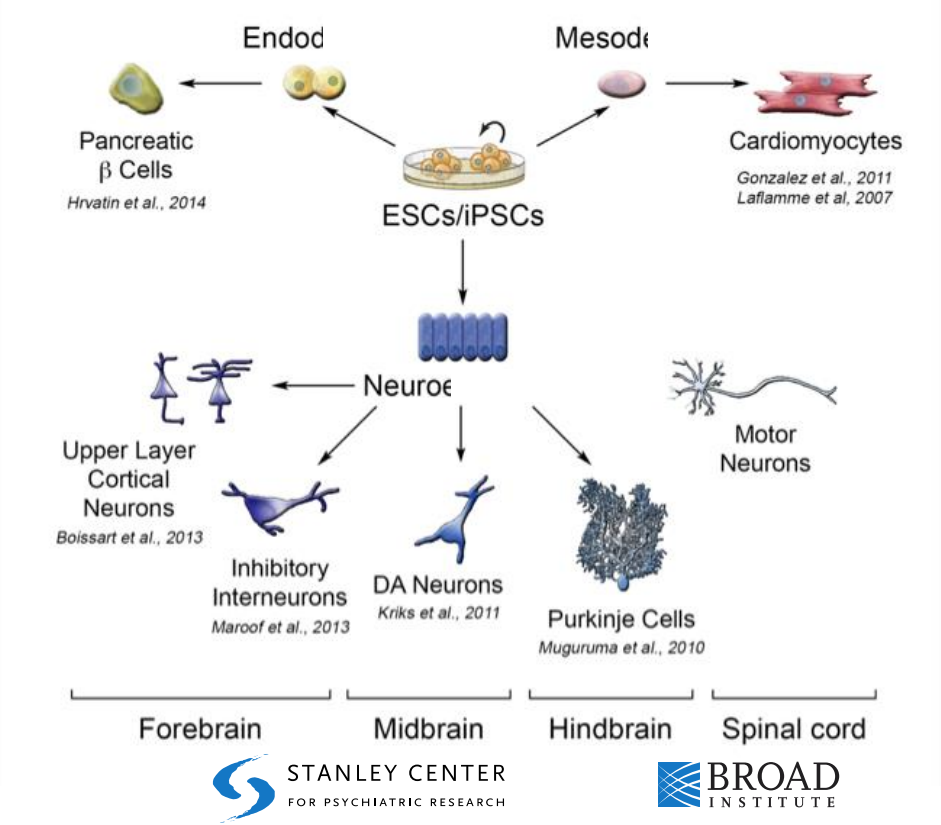
NGN2 Progress Report

- Show promising functionality, reproducibility.
- Good substrate for studying emerging genes.
- Goal: move from characterization to making rugged assays for interrogating gene function.
- Carry out similarly extensive characterization of “interneuron” preparations in 2015.

Hotter Colder/Up Down

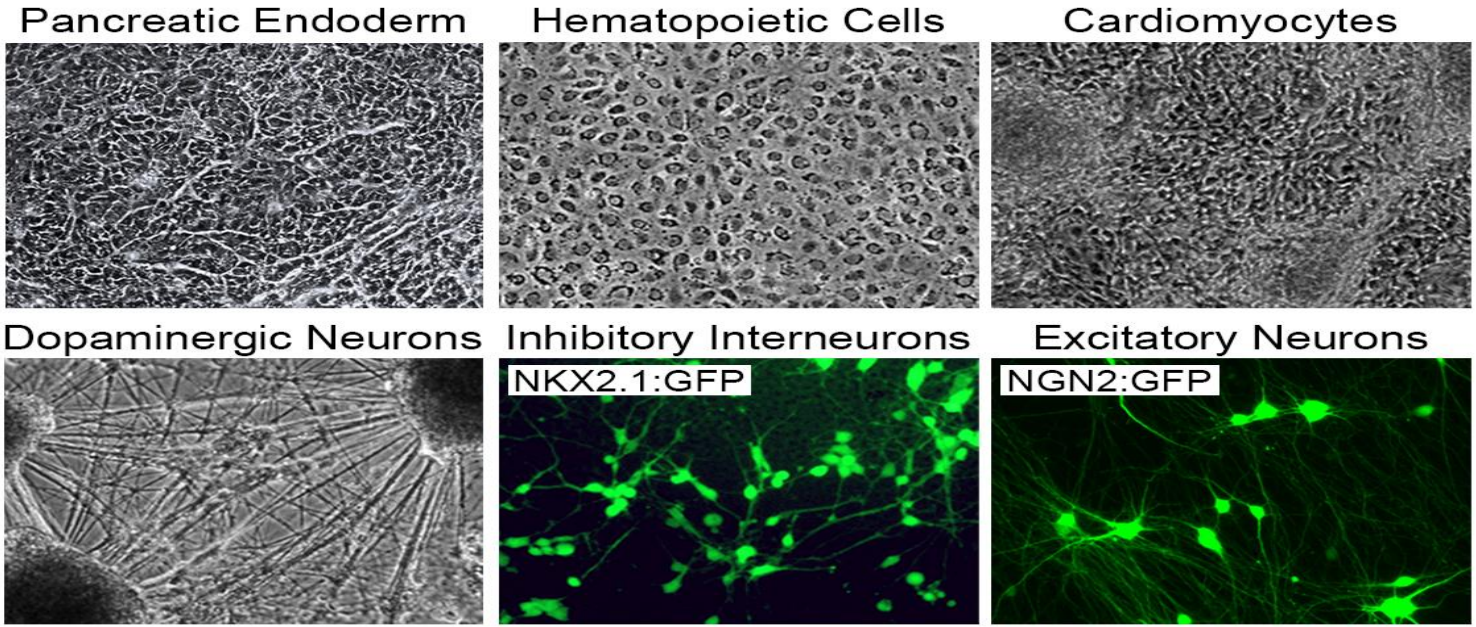
- Determine whether given cell types are particularly burdened by expression of emerging genes.
- Determine the magnitude and sign of effects of regulatory variants across cell types and states of maturation.

A Menagerie of Human Cell Types



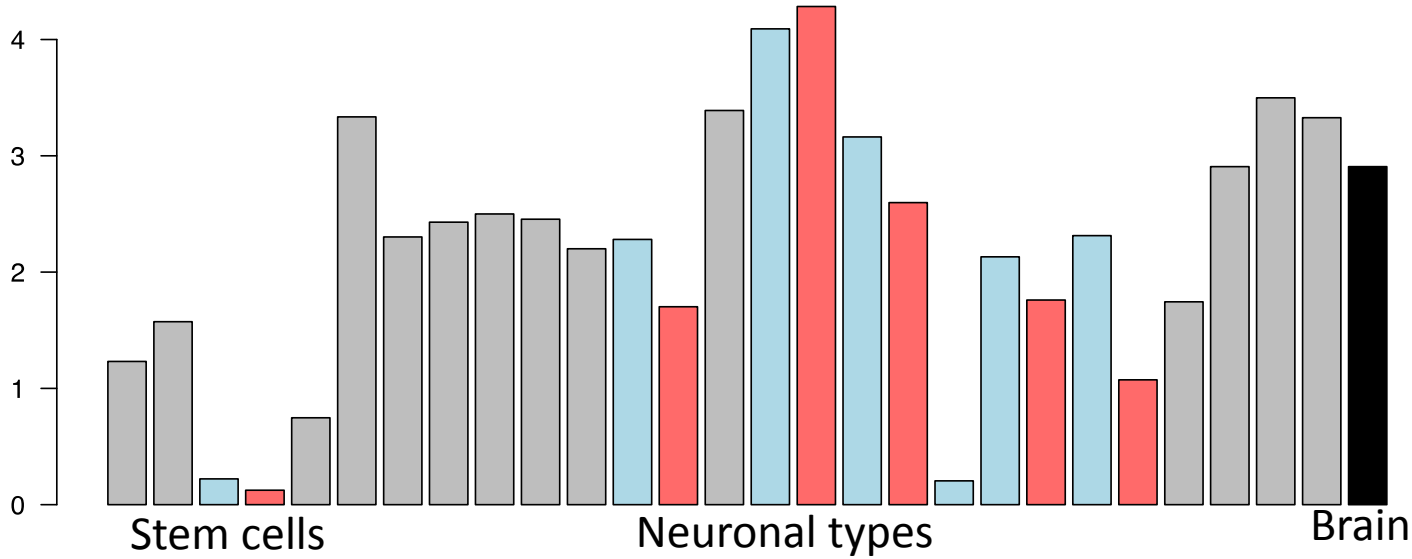
Jana Mitchel
Ralda Nehme
Nolan Kamataki
Dia Ghosh
Paola Arlotta
Steve McCarroll

Quantified outcomes



-RT-PCR, immunostaining quantification for key differentiation indicators

Mapping a Calcium Channel:



Jana Mitchel, Ralda Nehme, Nolan Kamataki, Dia Ghosh, Paola Arlotta, Steve McCarroll

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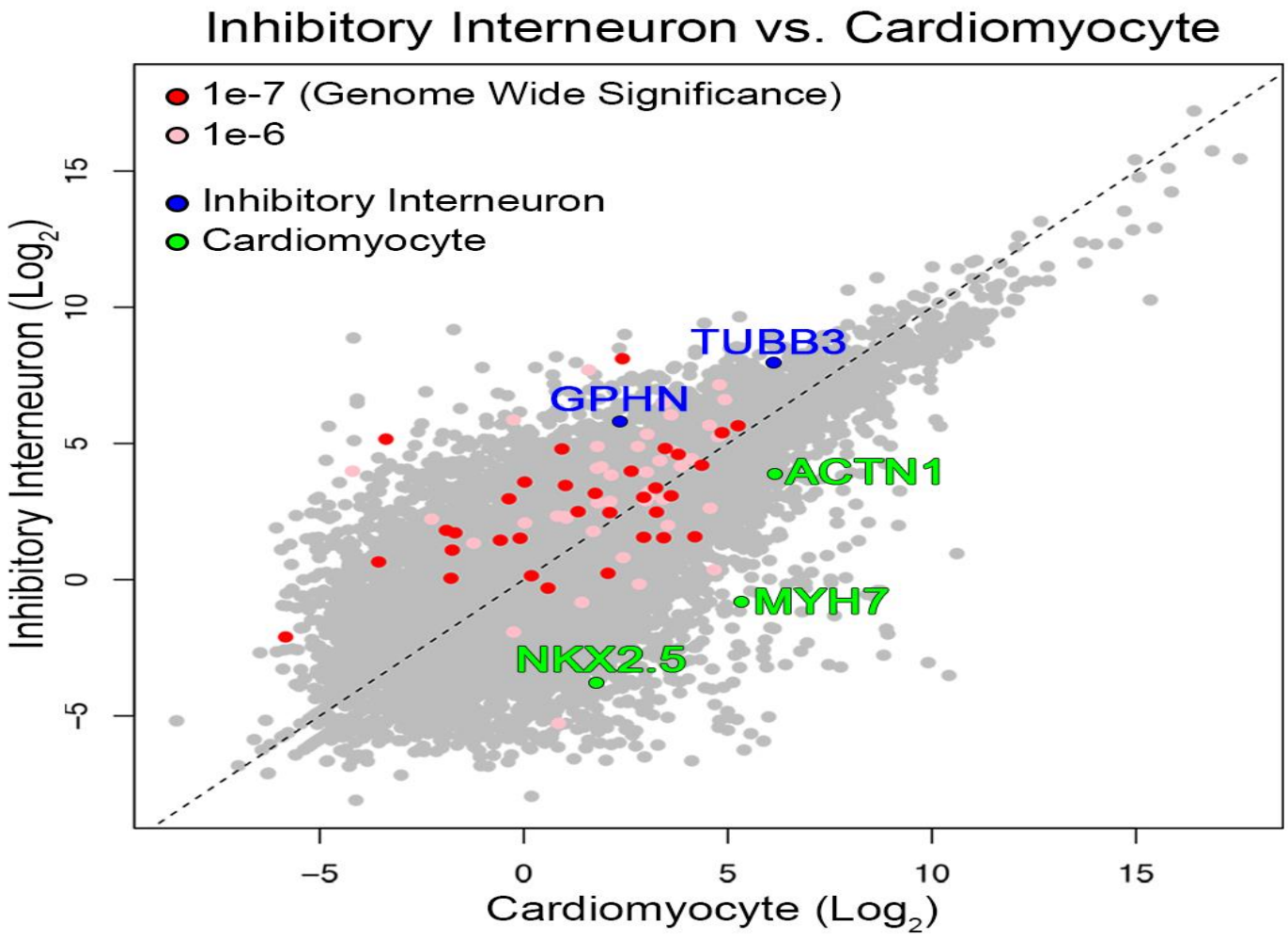
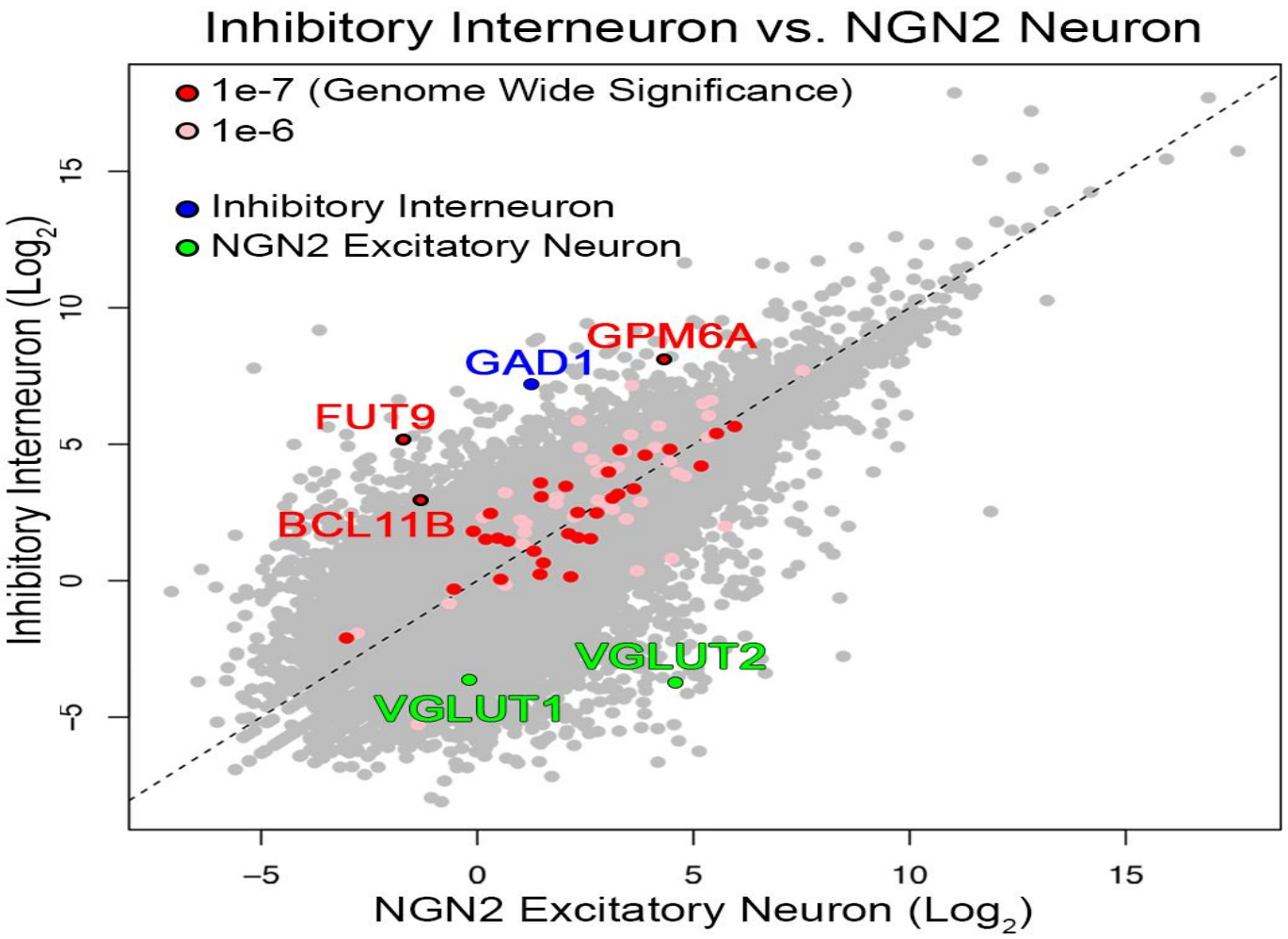

Inhibitory Interneuron vs. Cardiomyocyte
1e-7 (Genome Wide Significance)
1e-6
Inhibitory Interneuron
Cardiomyocyte
TUBB3
GPHN
ACTN1
MYH7
NKX2.5
Inhibitory Interneuron (Log2)
Cardiomyocyte (Log2)
−5
0
5
10
15

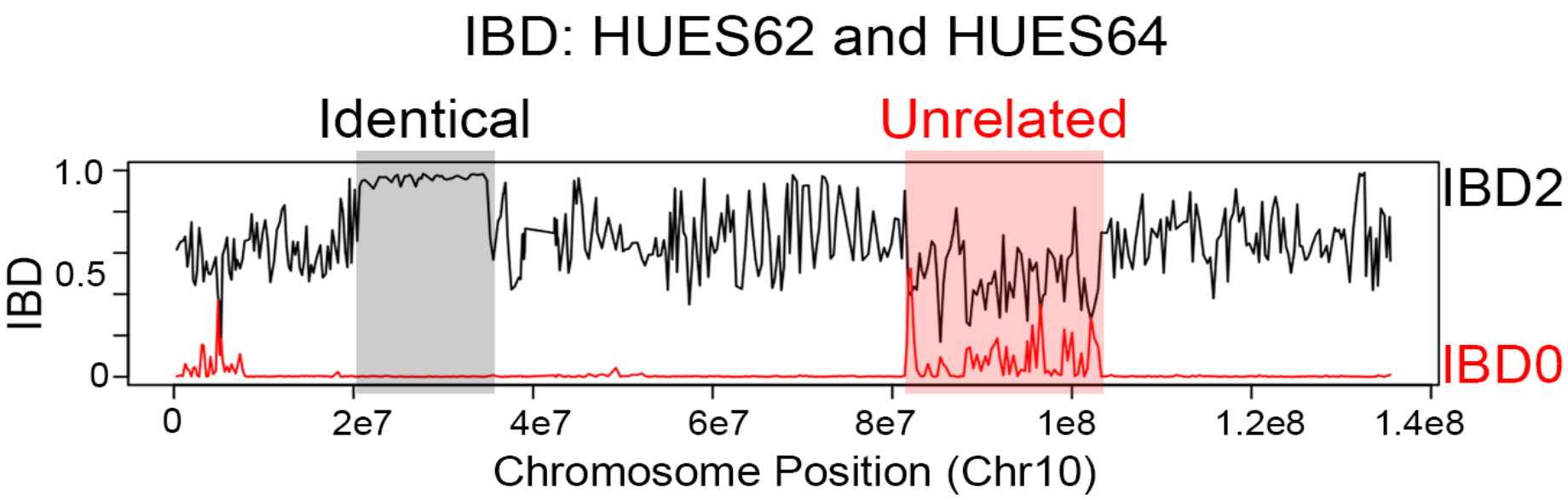

Inhibitory Interneuron vs. NGN2 Neuron
1e-7 (Genome Wide Significance)
1e-6
Inhibitory Interneuron
NGN2 Excitatory Neuron
GPM6A
GAD1
FUT9
BCL11B
VGLUT2
VGLUT1
Inhibitory Interneuron (Log2)
NGN2 Excitatory Neuron (Log2)
−5
0
5
10
15

Initial focus on 5 cell lines

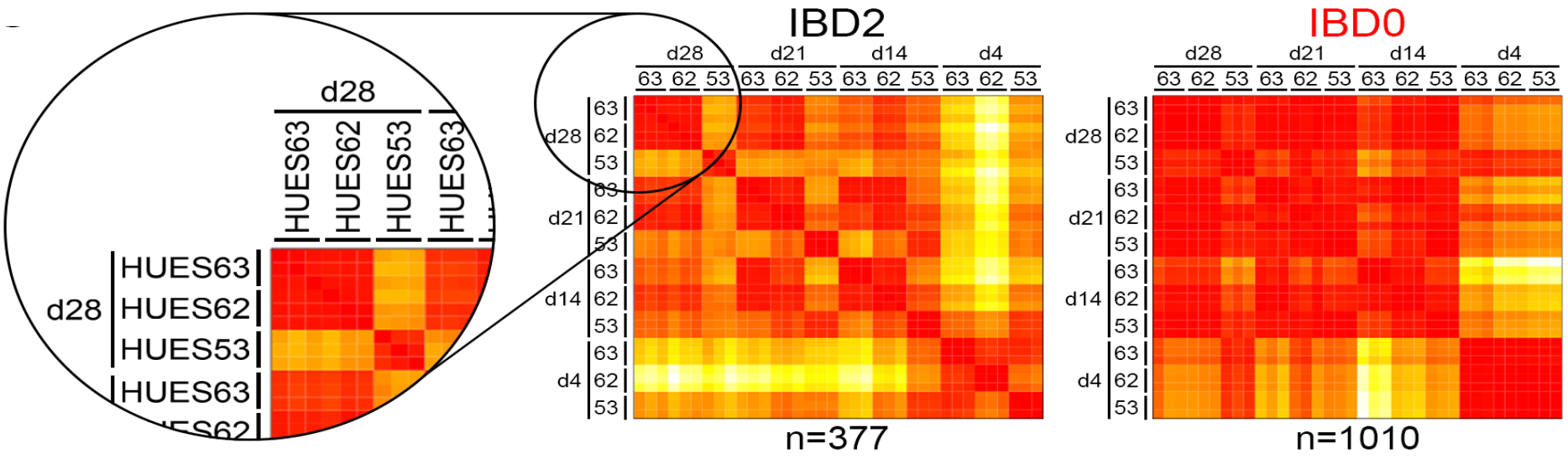
HUES62
HUES63
HUES64
} Sibling Trio

HUES53

HES3-NKX2.1:GFP

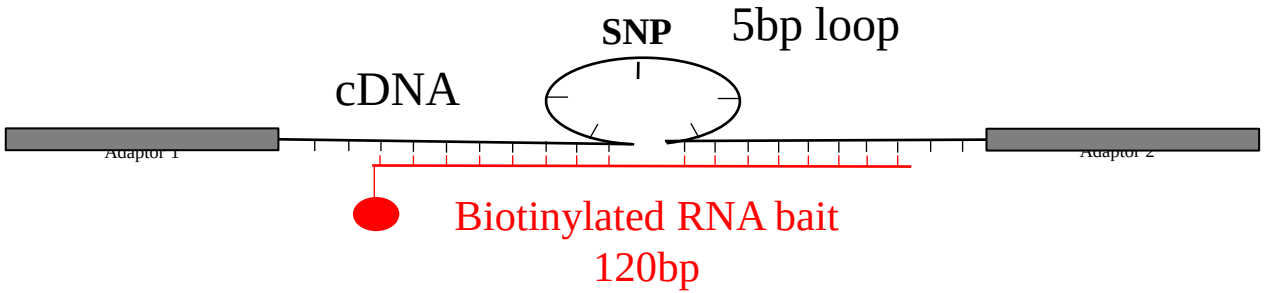

IBD: HUES62 and HUES64
Identical
Unrelated
IBD
1.0
0.5
0
IBD2
IBD0
0
2e7
4e7
6e7
8e7
1e8
1.2e8
1.4e8
Chromosome Position (Chr10)

Transcription More Correlated in Regions of Identity



Further directions

- Look at allelic skew to detect regulatory effects more sensitively with HetSeq enrichment



- Establish Direction of skew
- *in silico* phasing of sibling genome sequences
- *in vitro* phasing via single-haplotype limiting dilution in droplets
  (collaboration with X10)

Hotter Colder/Up Down Progress

- >400 RNA and het seq libraries in process.
- Initial focus has been on population seq to understand sample quality and composition.
- Het seq initiated.
- Need environment to share emerging data.

Acknowledgements

Ralda Nehme
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Lindy Barrett
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Dia Gosh
Nolan Kamataki
Steve McCarroll

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Katie Worringer
Bilada Bilican
Kraig Theriault

Zhanyan Fu
Chenchen Li
Yan-Ling Zhang
James Hawrot
John Sherwood
GAP Fluidigm platform

Eggan and McCarroll lab members
Stanley Center lab members
Bauer FACS facility


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HSCRB
harvard department of stem cell & regenerative biology


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