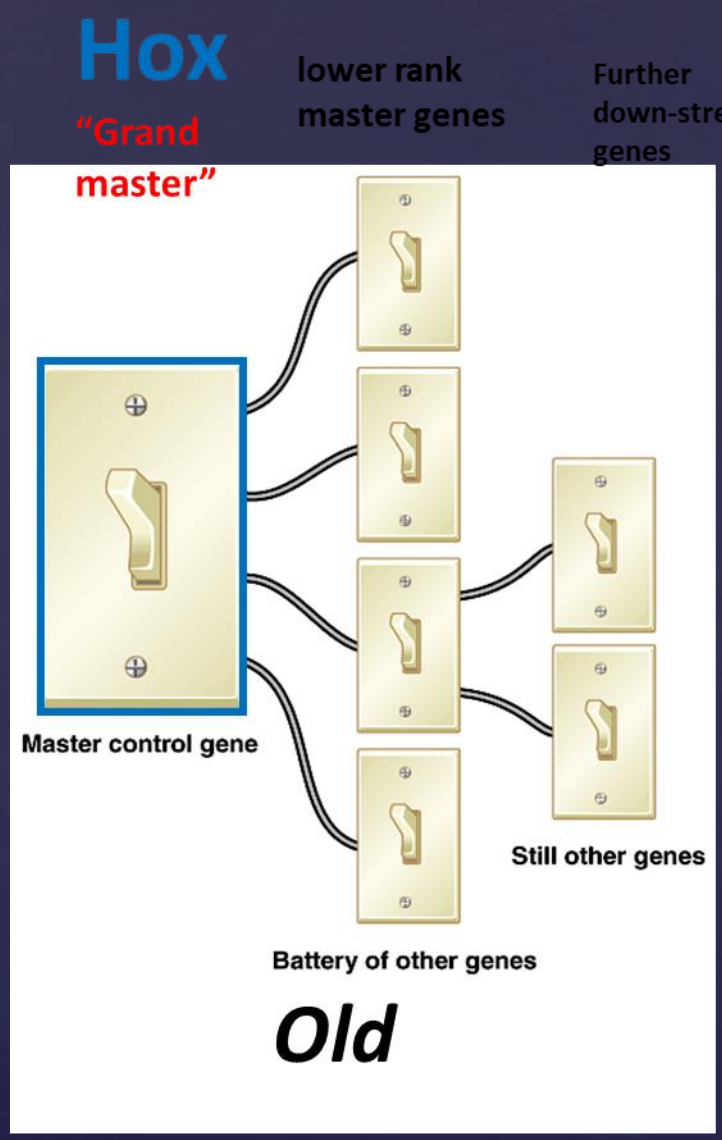
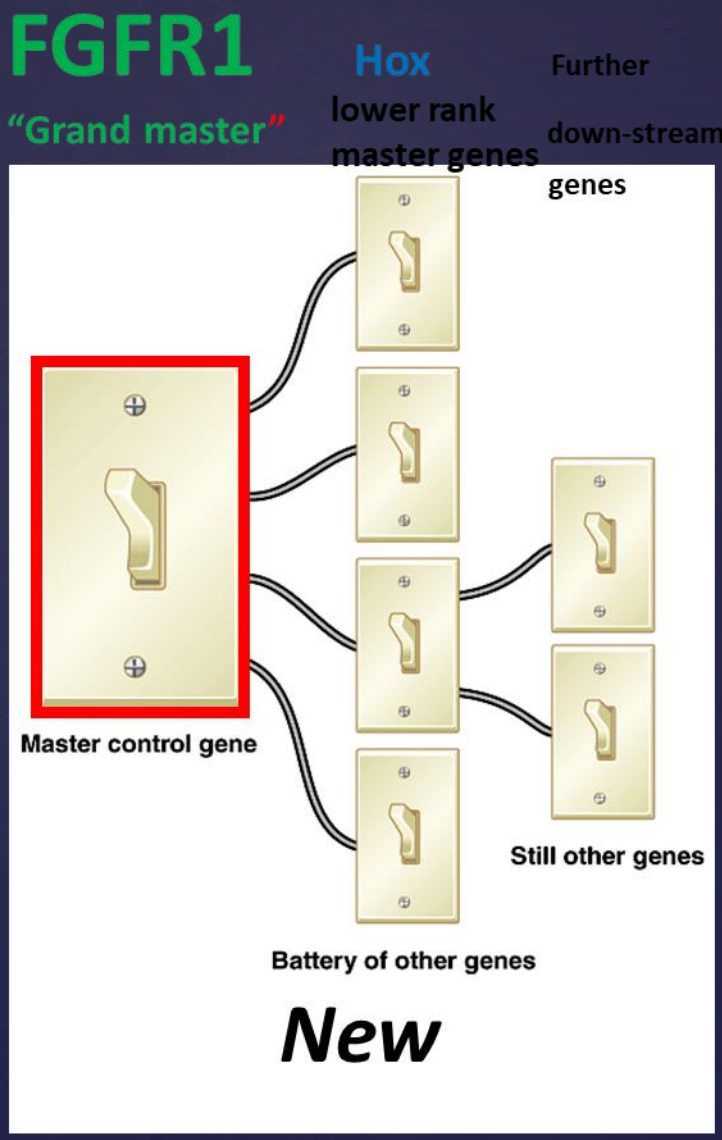
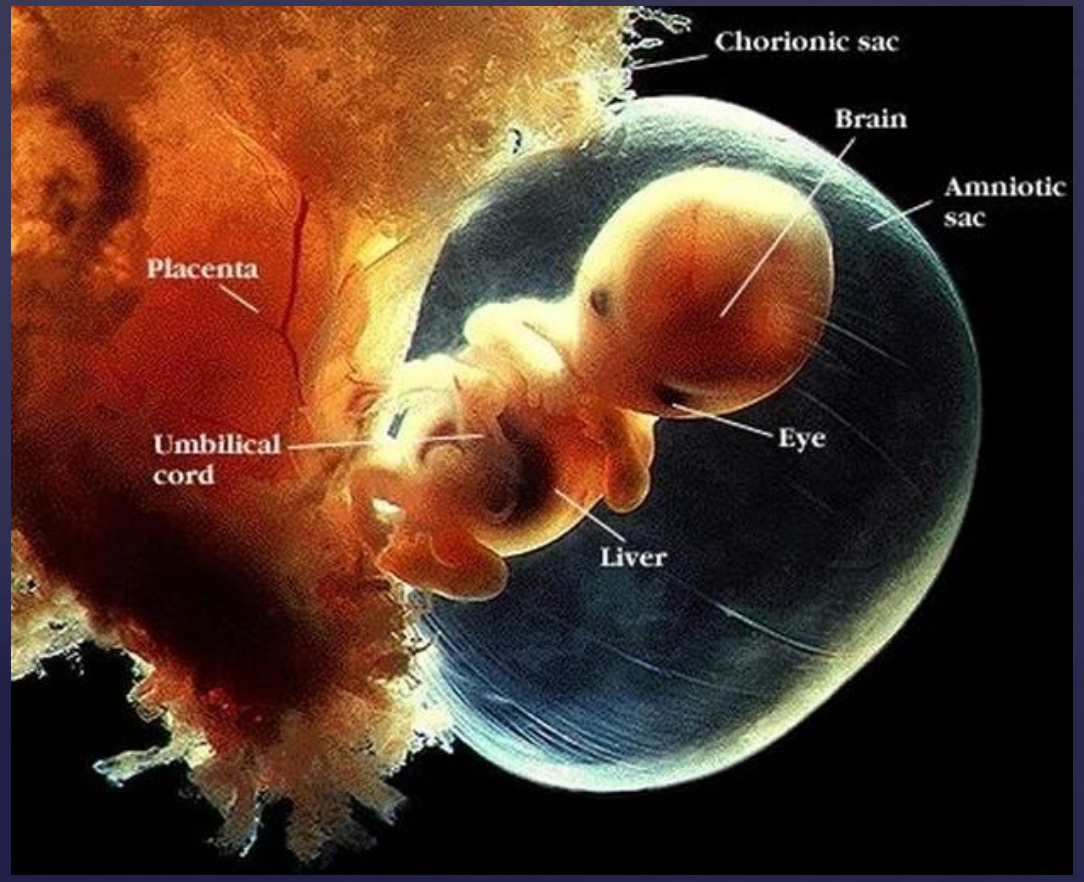
Change in the Hierarchy of Master Genes





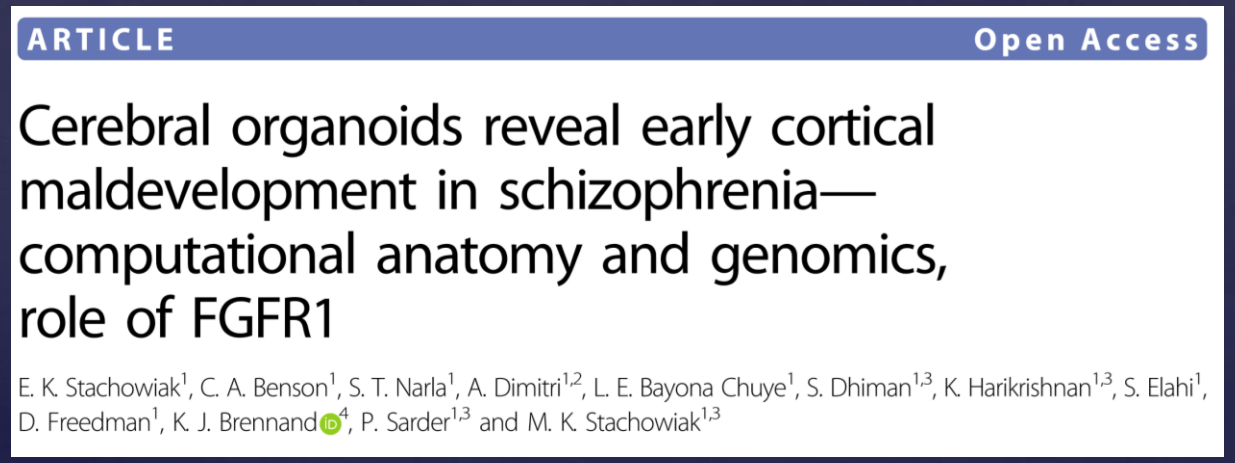
Preneuronal genome deprograming suggest that disease begins in utero during early brain development - how can we look back in time?



Growing 3D cerebral organoids

1. *1.Model early human brain development*
2.
3. *Embrionic Stem Cells -ESC*
4. *Induced Pluripotent Stem Cells - iPSC*

5. 2. Identification of early brain neurodevelopmental-anatomical parts


ARTICLE Open Access

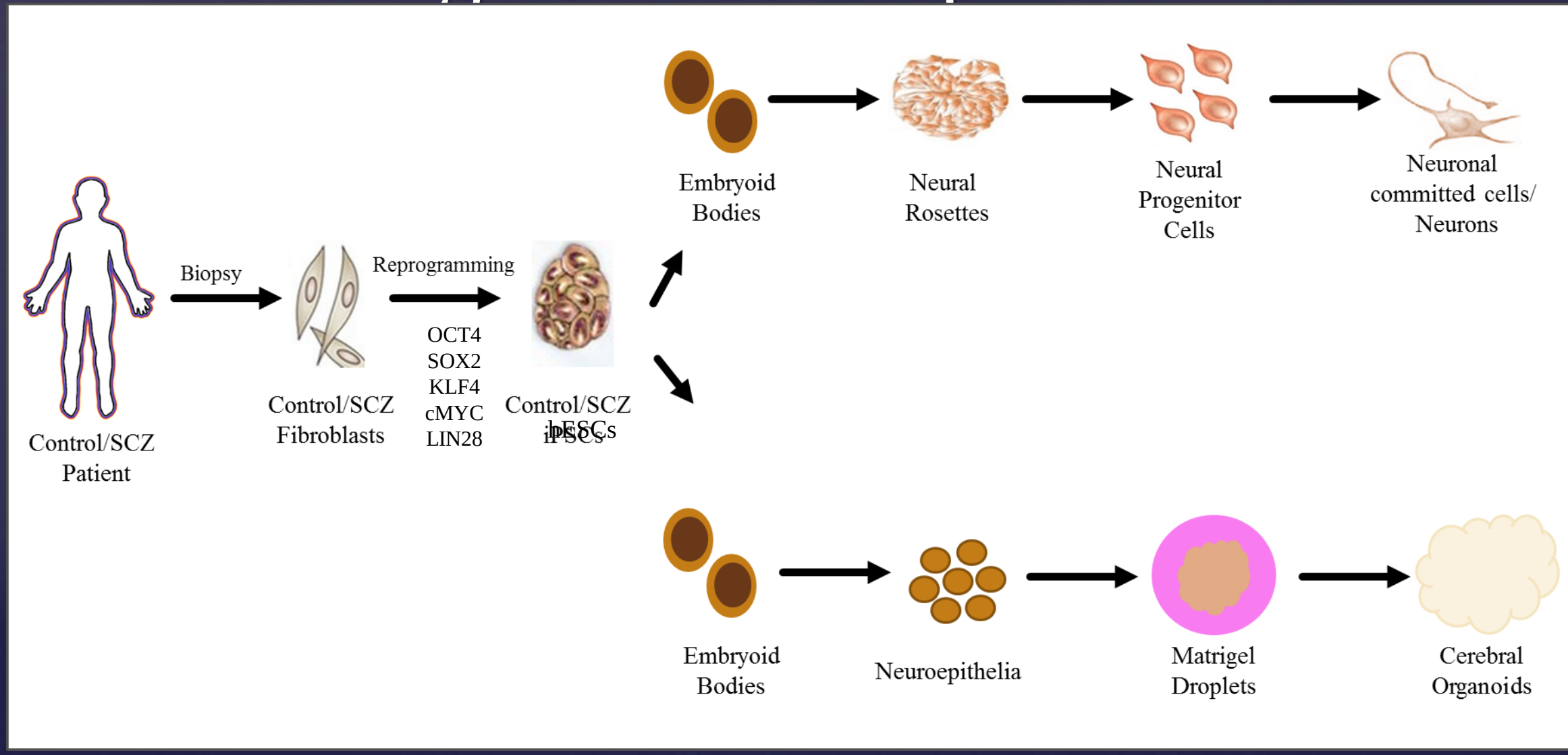
Cerebral organoids reveal early cortical maldevelopment in schizophrenia—computational anatomy and genomics, role of FGFR1

E. K. Stachowiak[1], C. A. Benson[1], S. T. Narla[1], A. Dimitri[1,2], L. E. Bayona Chuye[1], S. Dhiman[1,3], K. Harikrishnan[1,3], S. Elahi[1], D. Freedman[1], K. J. Brennand[4], P. Sarder[1,3] and M. K. Stachowiak[1,3]

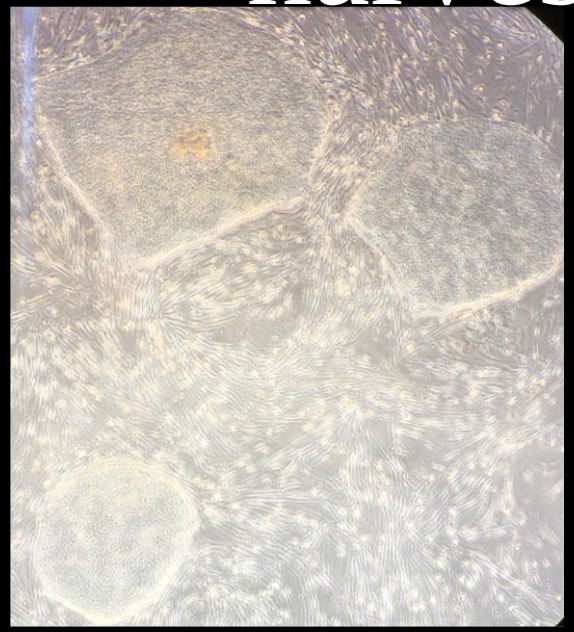
Translational Psychiatry - Nature

https://www.nature.com/tp/

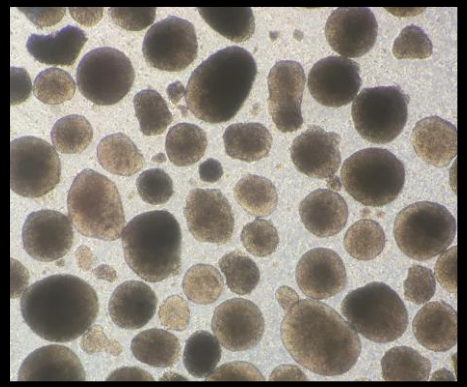
Modeling neurodevelopment



Organoid development through harvest



iPSCs on MEFs
Weeks to grow



Embryoid Bodies
4-5 days

Neuroepthelia
4 days

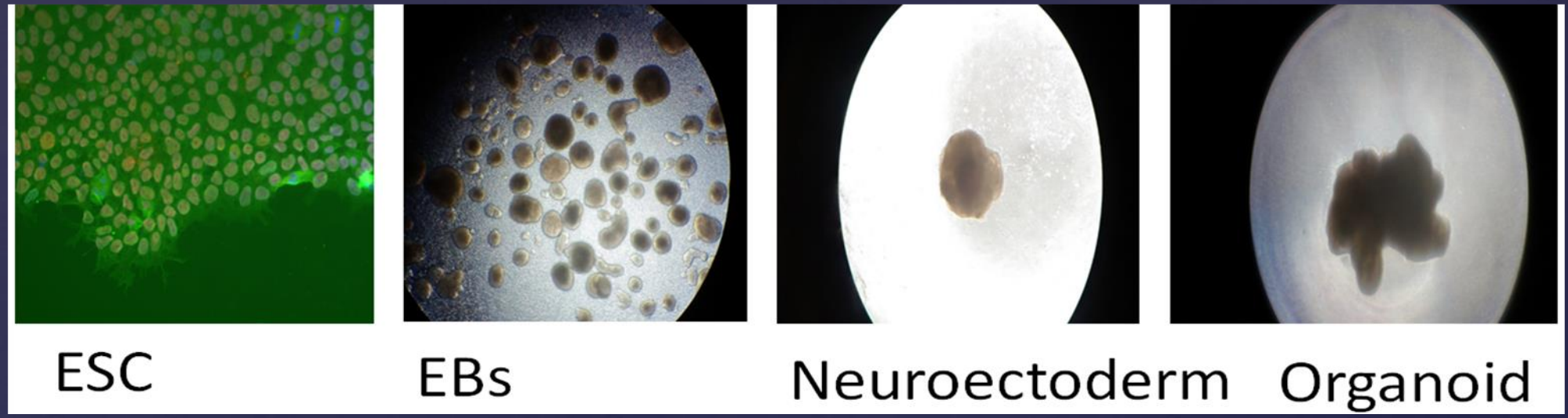
Neuroepthelia in matrigel droplets 4 days (media without RA. Stationary

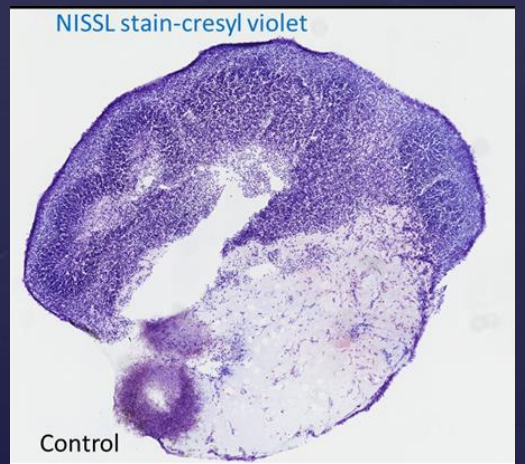
Cerebral Organoids on Shaker

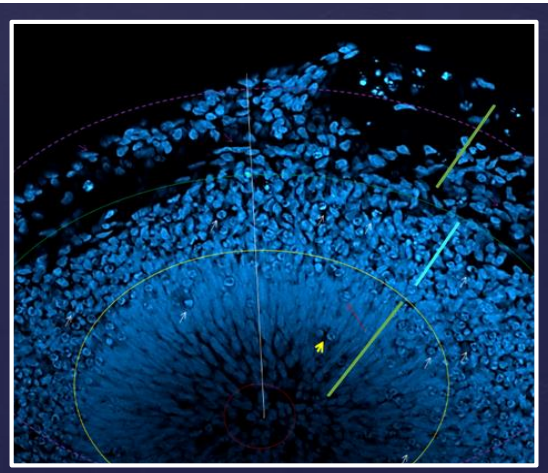
Freezing Organoids:
Sucrose gradient: 7.5%, 15%, 30%
Freeze on 4th day in Sucrose/Gelatin in liquid nitrogen. Store in -80C

iPSC/ESC$^{SCZ\ and\ Control}$ → Embryoid Bodies → → Neuroectoderm → Organoids

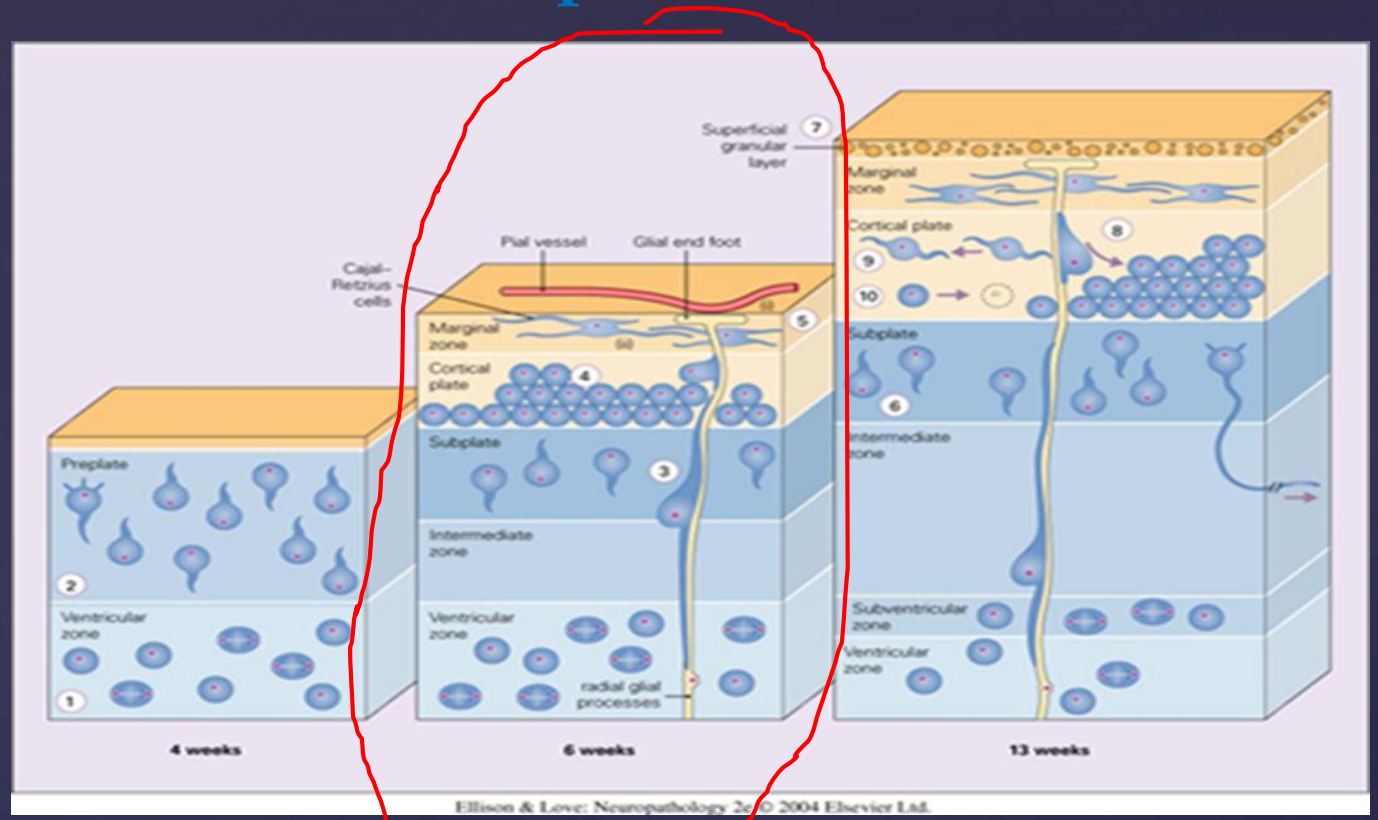
A

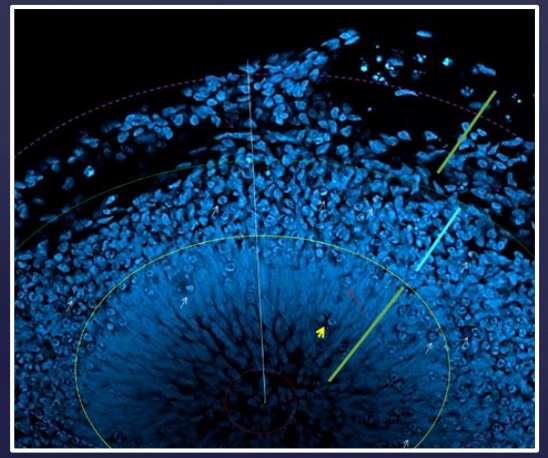






Inside out development of human brain cortex



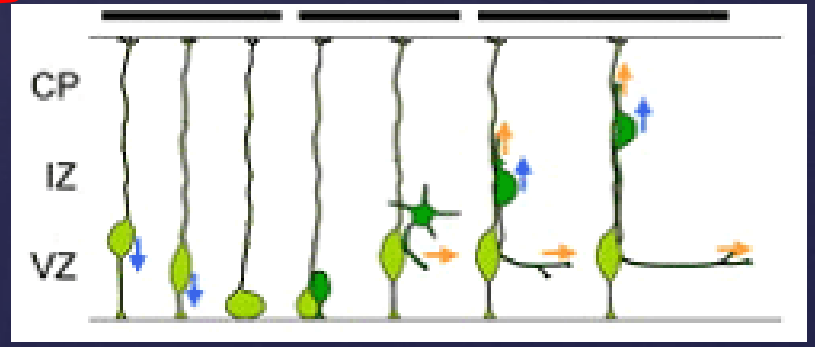


Marginal zone

Cortical Plate

Intermediate Zone

Ventricular Zone



Ventricle lumen

ESC – H8 H9

Type of Cell Line	Cell line	Gender	Description
hESC	HUES8	Male	Control
hESC	H9	Female	Control

iPSC

Type of Cell Line	Cell line ID	Gender	Age	Description	Type of SCZ
iPSC	2497	Male	23	SCZ	SCZ
iPSC	2038	Male	22	SCZ	SCZ
iPSC	1835	Female	27	SCZ	SCZ-affectiv
iPSC	BJ #1	Male		Control	---
iPSC	2937	Male	22	Control	---
iPSC	3440	Male	20	Control	---
iPSC	9319	Female	24	Control	---
iPSC	3651	Female	25	Control	---

Summary of Genomic Investigation:

Our results indicate:

(i) Early (pre-neuronal) genomic etiology of schizophrenia,

(ii) Dysregulated genes and the new coordinated gene networks are common to unrelated cases of schizophrenia,

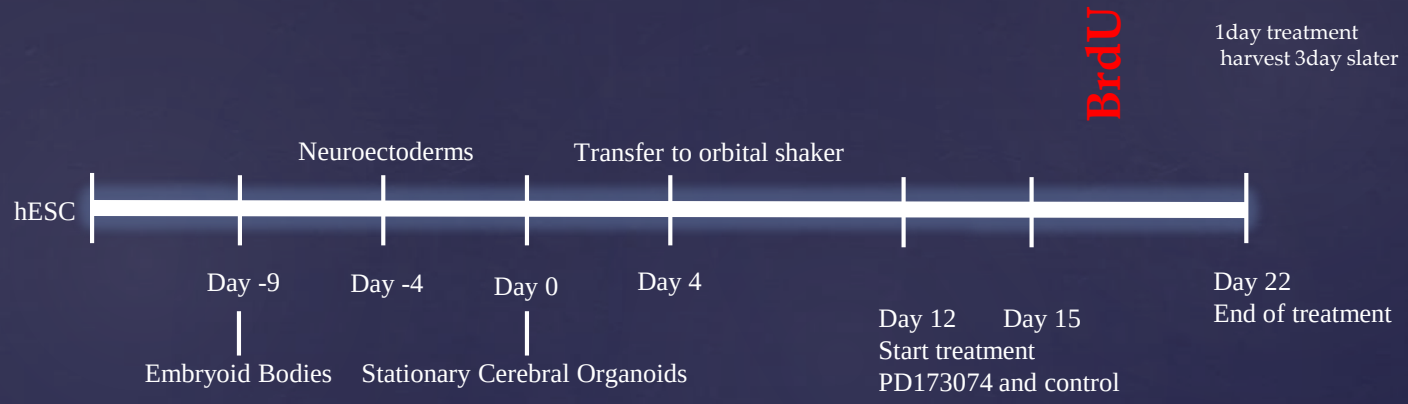
(iii) Gene dysregulations are accompanied by changes in nFGFR1-genome interactions

(iv) The results designate nFGFR1 signaling as a common dysregulated mechanism and potential therapeutic target in schizophrenia.

(v) Dysregulated genes belong to diverse neurodevelopmental pathways indicating that early brain development may be affected in schizophrenia

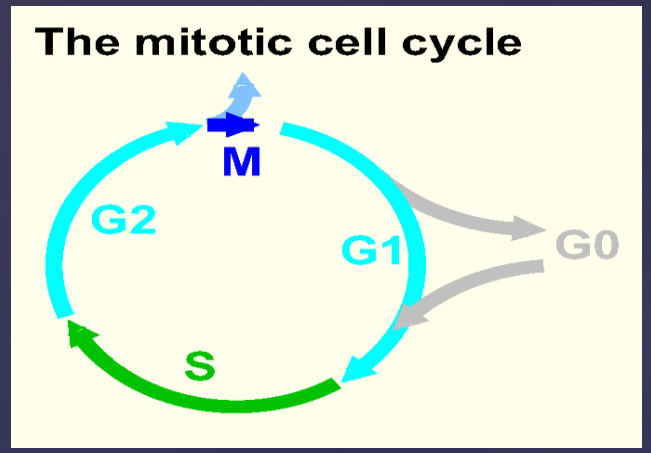
Does inhibition of FGFR1 affect organoid growth and cell differentiation?

PD173074



Pulse labeling developing cells with BrdU

Antibody used



FGFR1- Gene dysregulations

DAPI - stains chromatin

BrDU - proliferation

Ki67 – expressed in G1, S, G2 and M phases but not Go of the cell cycle

GFAP –radial glia (stem cells), astrocytes

TBR1 – early neuroblasts

Doublecortin (DCX) - neuroblasts and imm

Calretinin -interneurons

REELIN -Cajal –Retzius cells

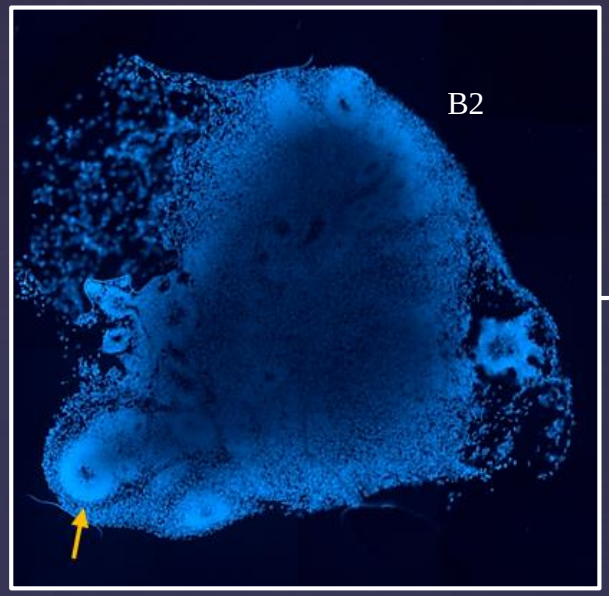
- βIII –Tubulin - young neurons

PAnNeuN - mature neurons

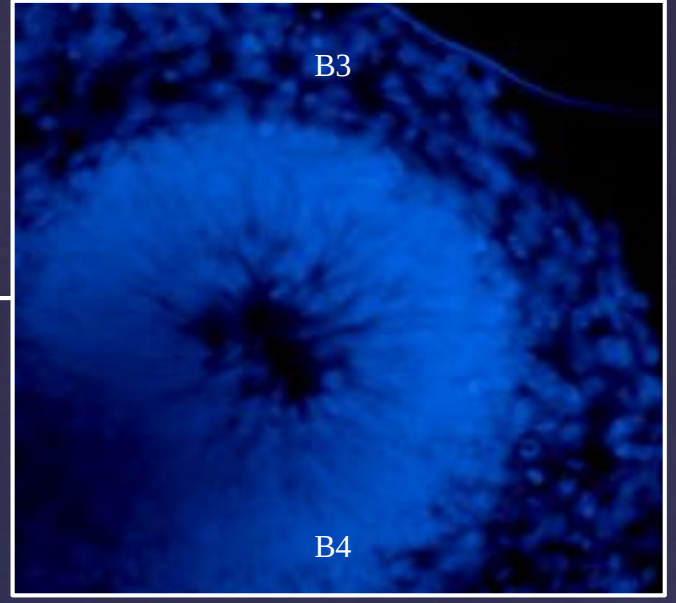
Cortical cerebral organoids at 12 and 22 days

12 days

B1



B2



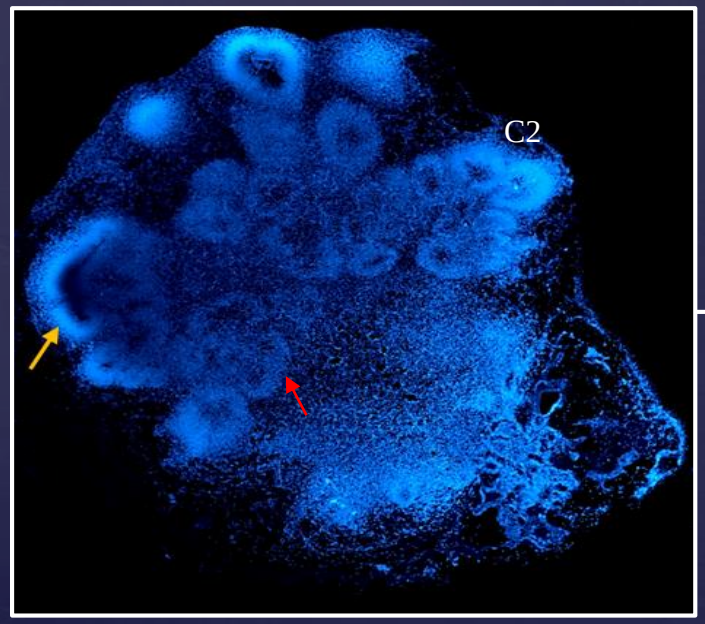
B3

B4

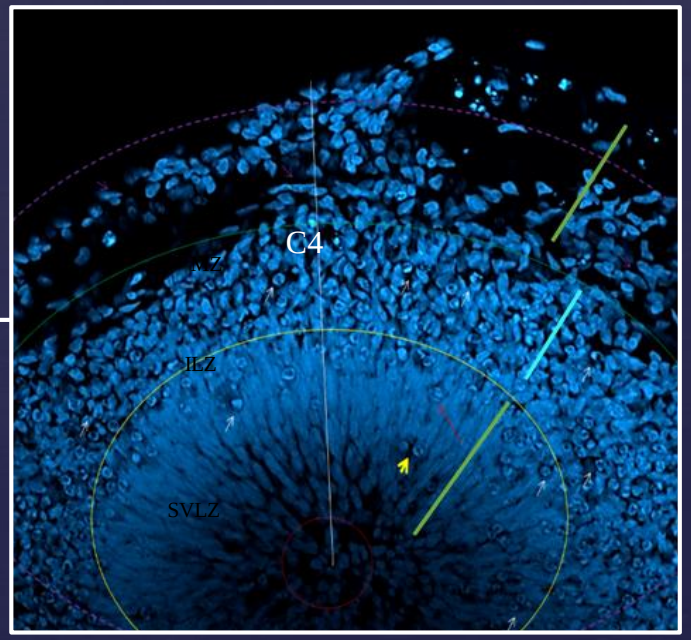
C3

22 days

C1



C2



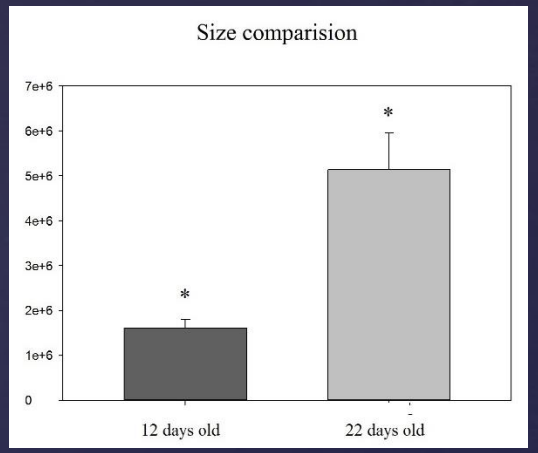
C4

ILZ

SVLZ

C5

D



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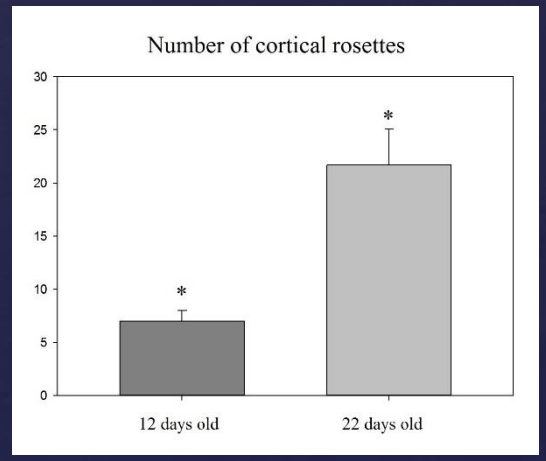
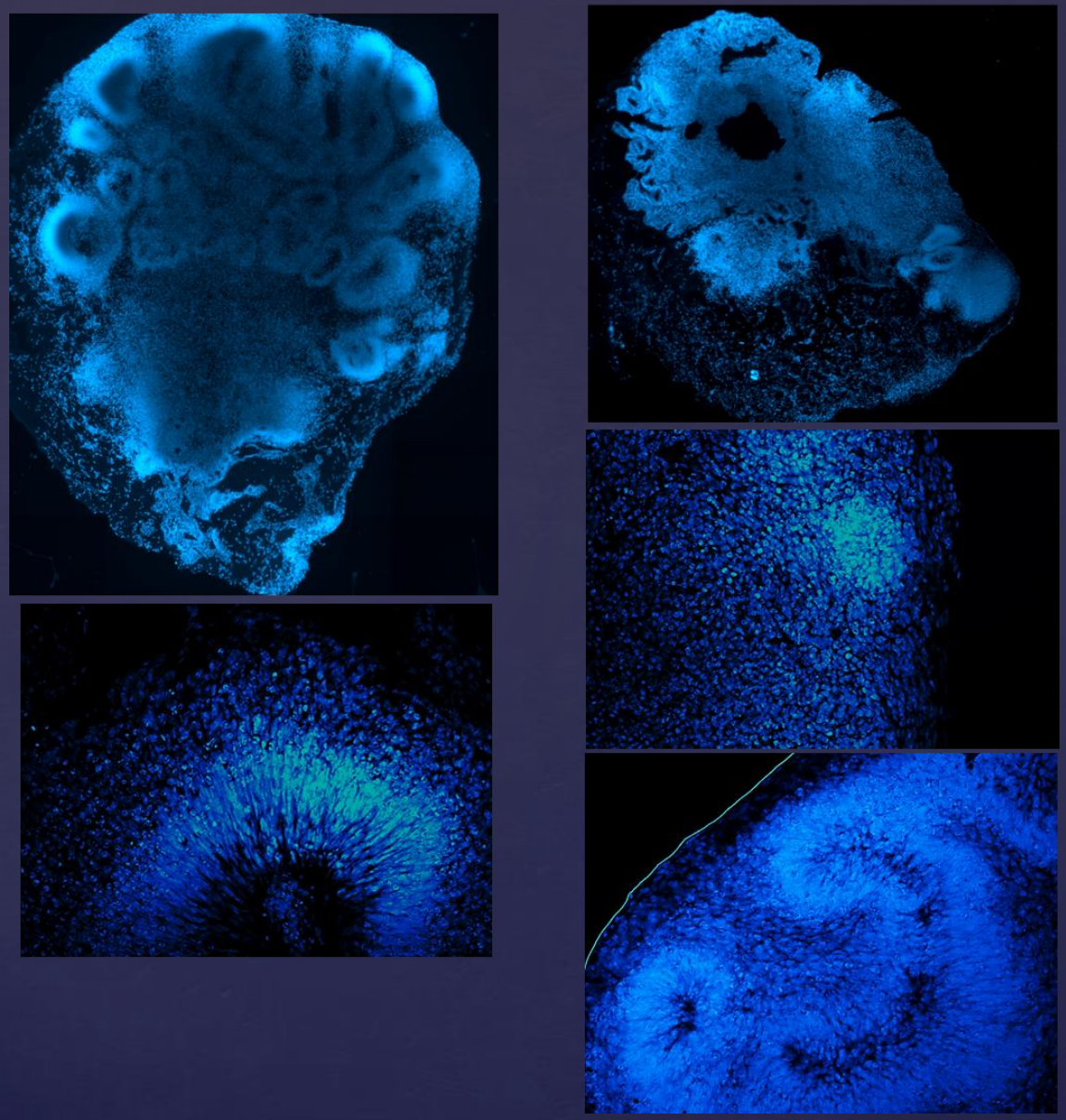


Inhibition of FGFR1 reduces size organoids and number of cortical rosettes

Control CONTROL

PD 173074



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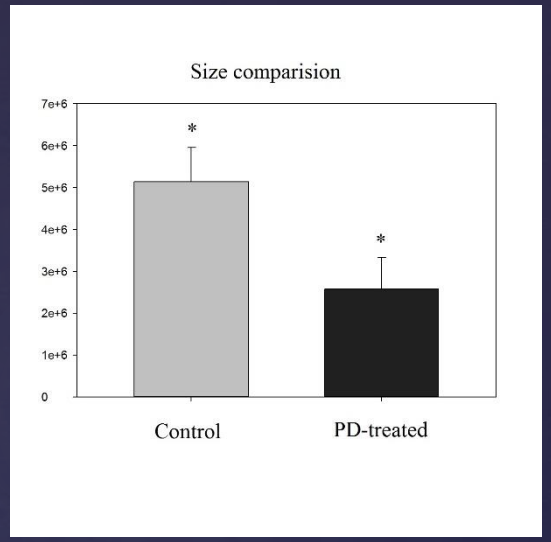
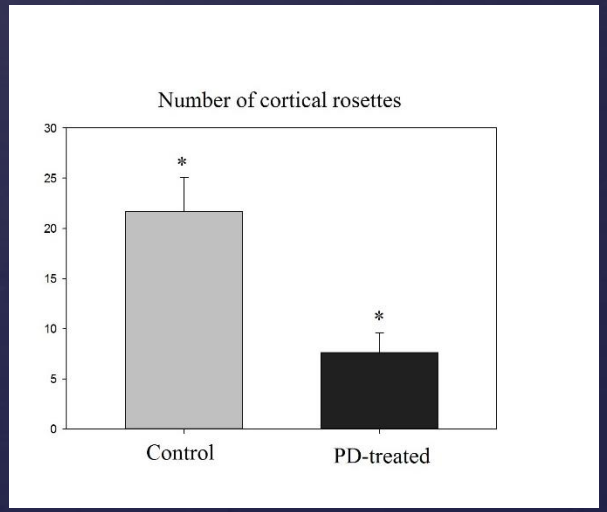
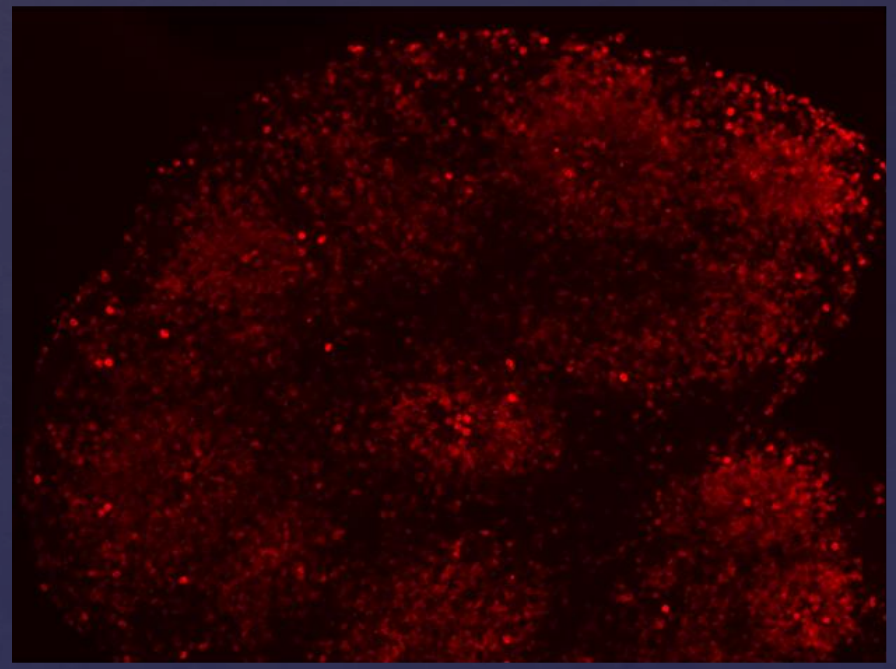


D

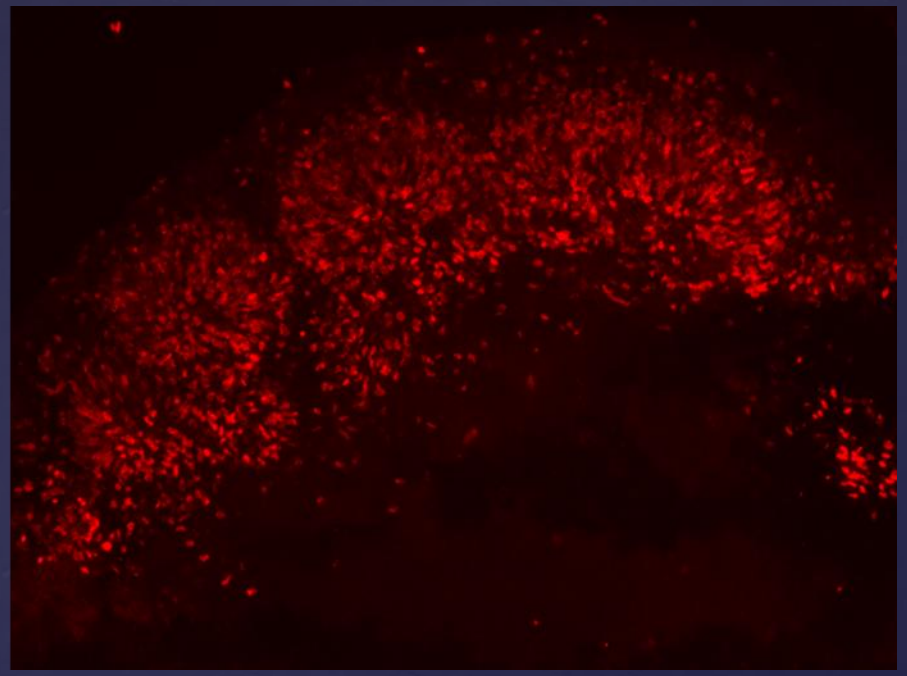


H9 22Days

BrdU



Control



PD173074

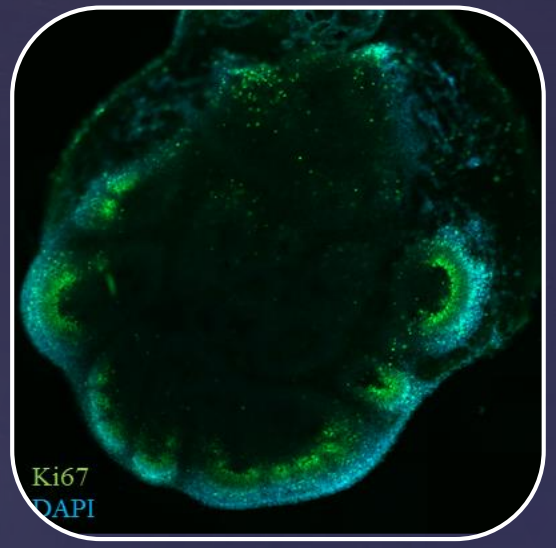
PD arrested BrDu lebelled cells in VZ
And prevented from migration to Cortex

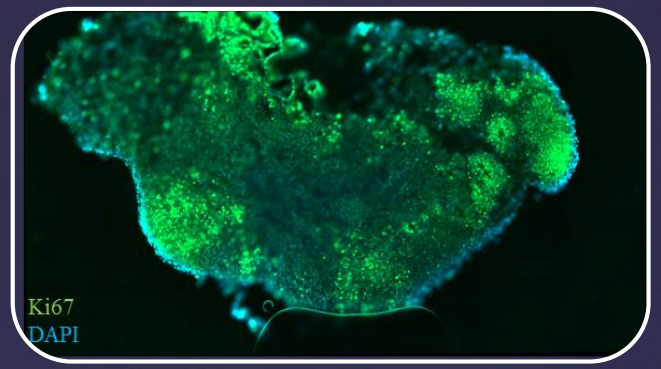
Inhibition of FGFR1 displaces proliferating cells

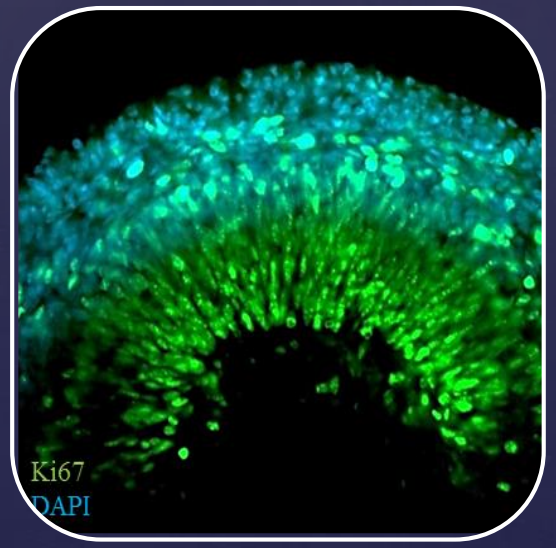
Ki67

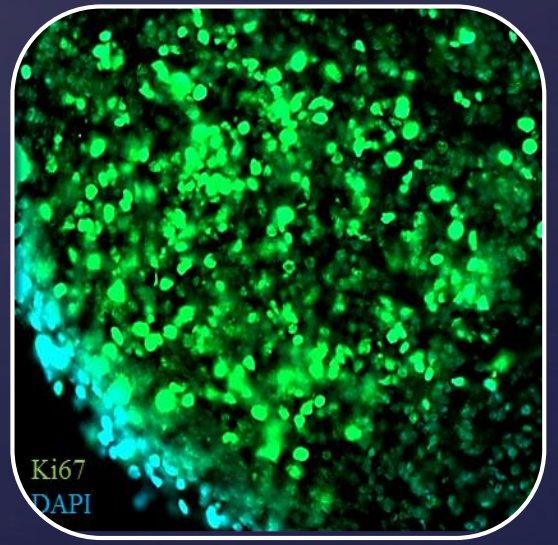
control

PD173074



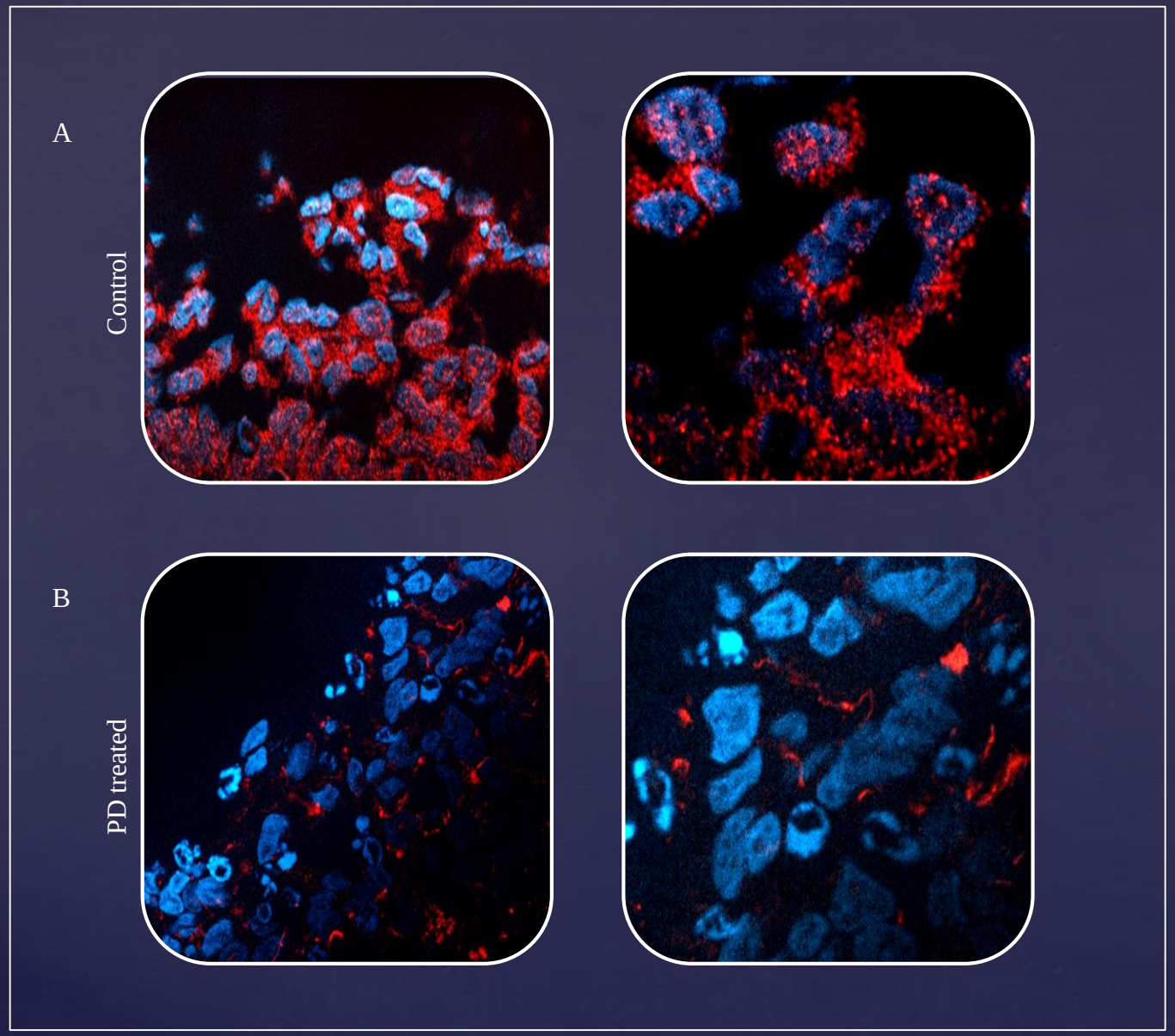


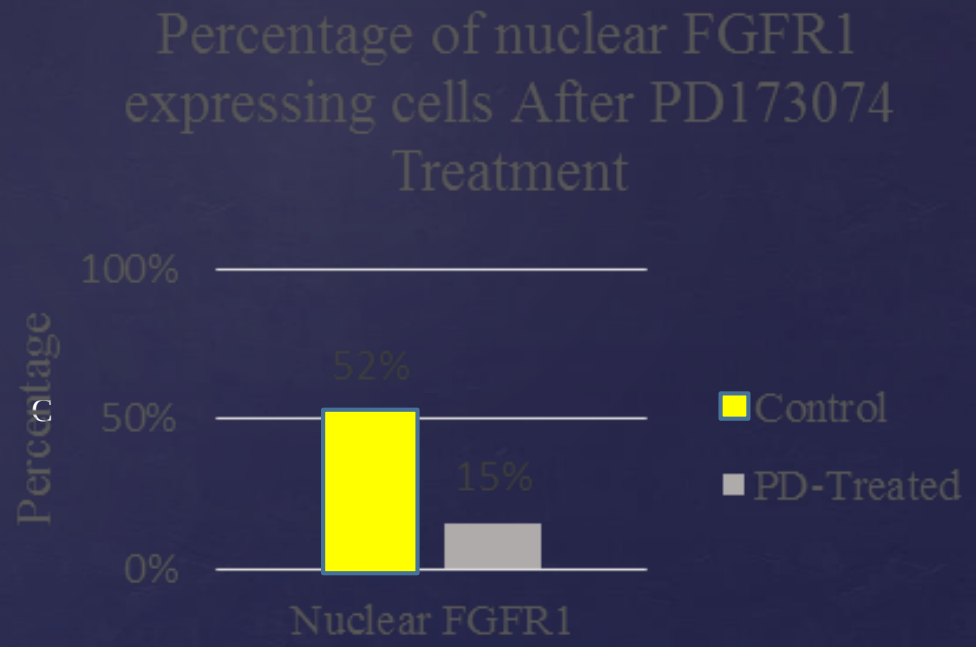




Tangential Pattern of Neuronal Development →

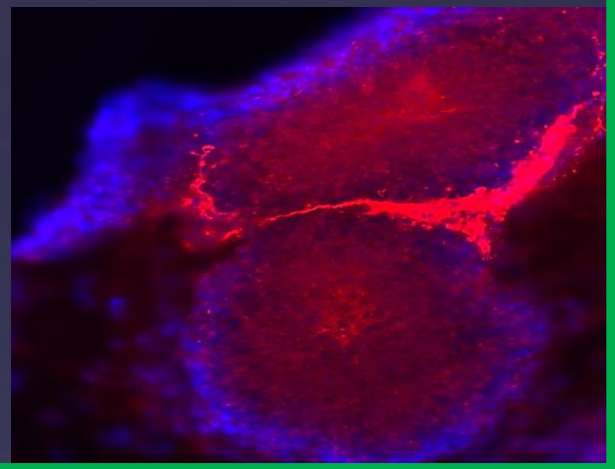
FGFR1 DAPI

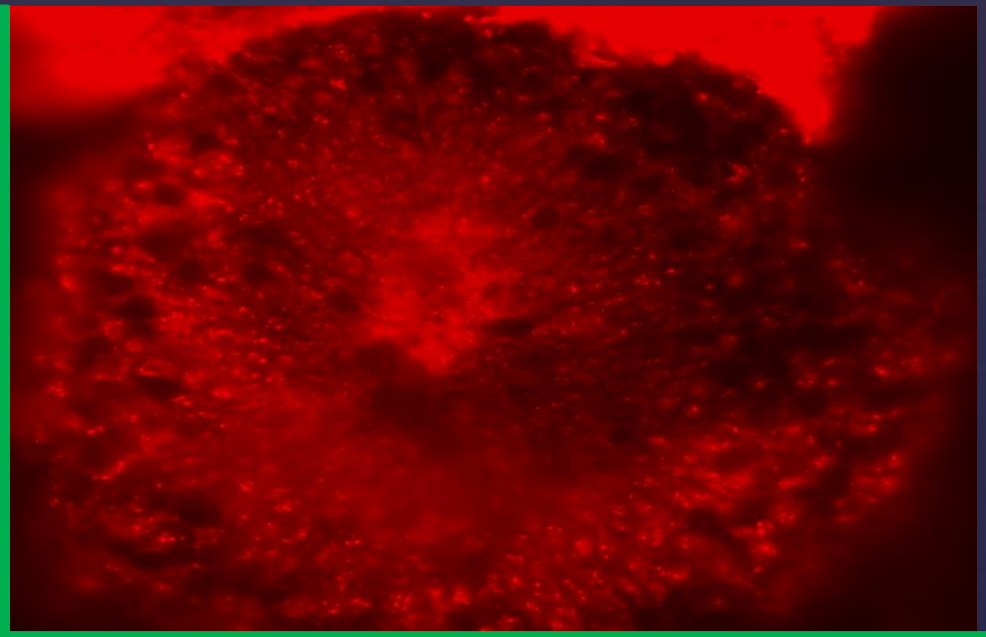

A
Control
B
PD treated


Percentage of nuclear FGFR1 expressing cells After PD173074 Treatment
Percentage
100%
50%
0%
52%
15%
Nuclear FGFR1
Control
PD-Treated

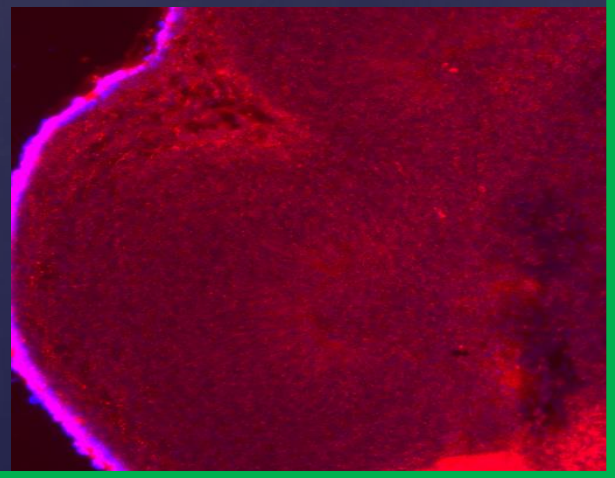
H9 22Days FGFR1

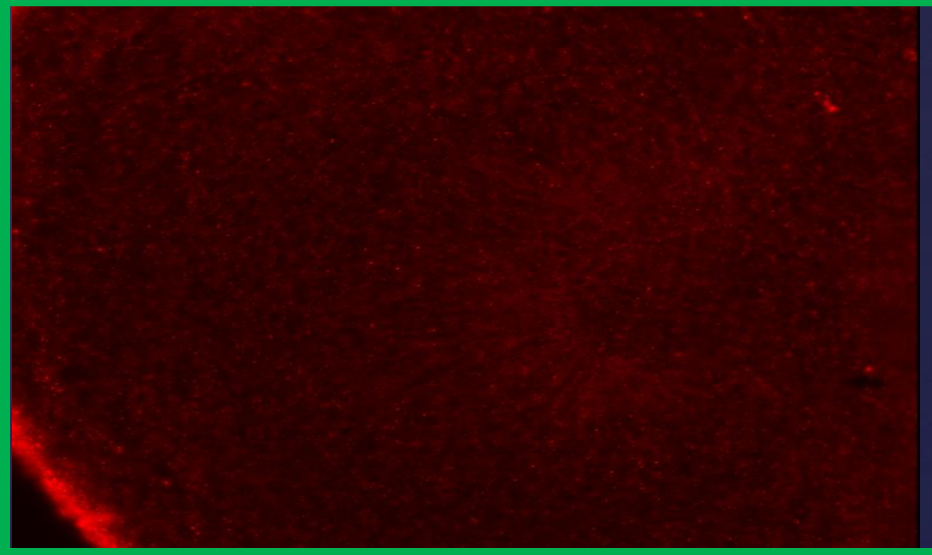
Control





PD





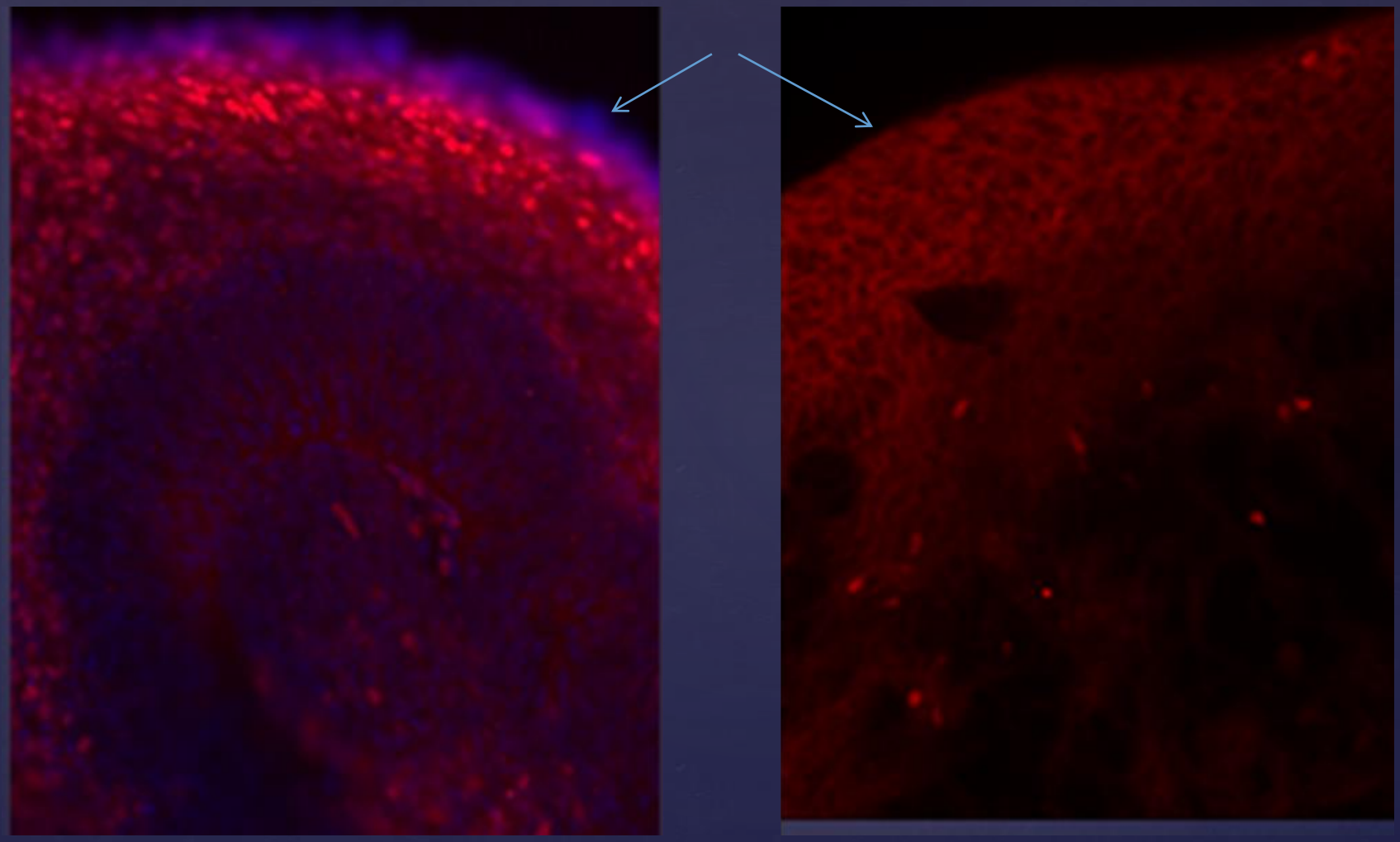
H9 22Days

TBR1

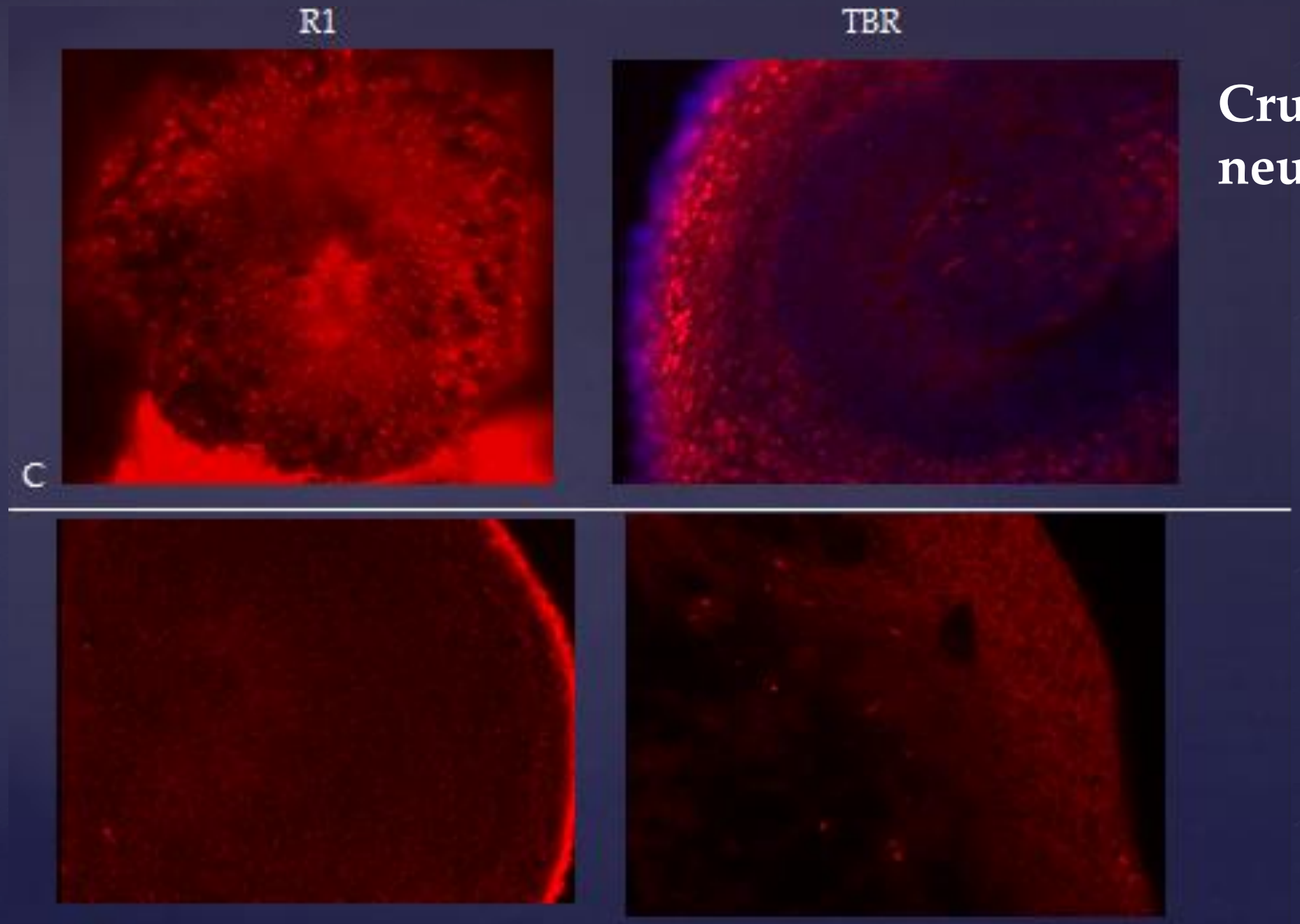
-early neuroblasts

Control

PD173074



Loss of T-box Brain 1 transcription factor expression in MZ



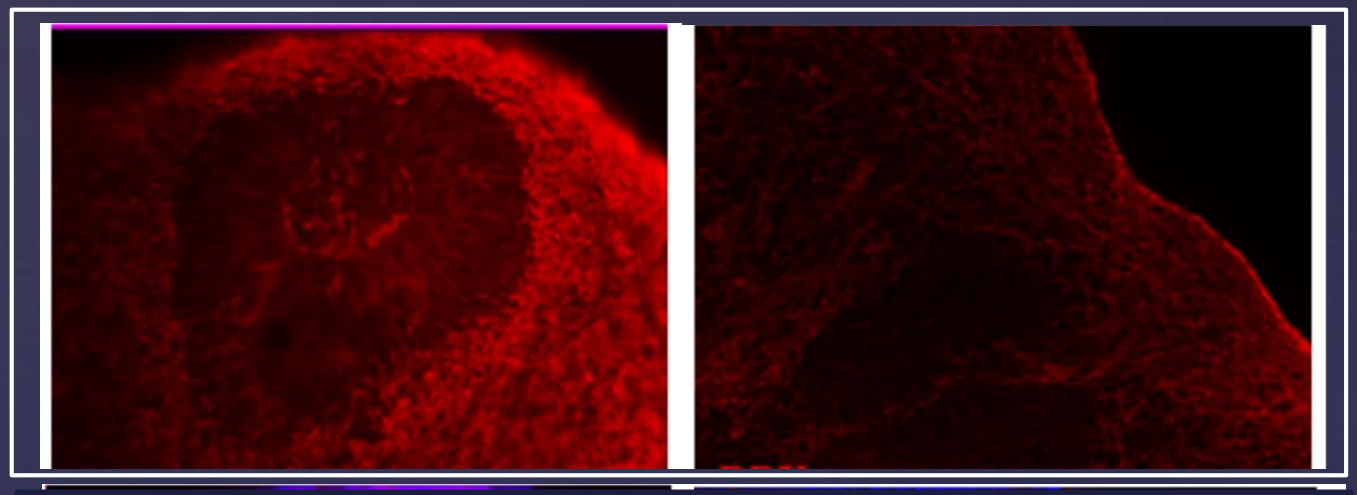
Crucial in neurodevelopment

!!!!!

DCX

Control

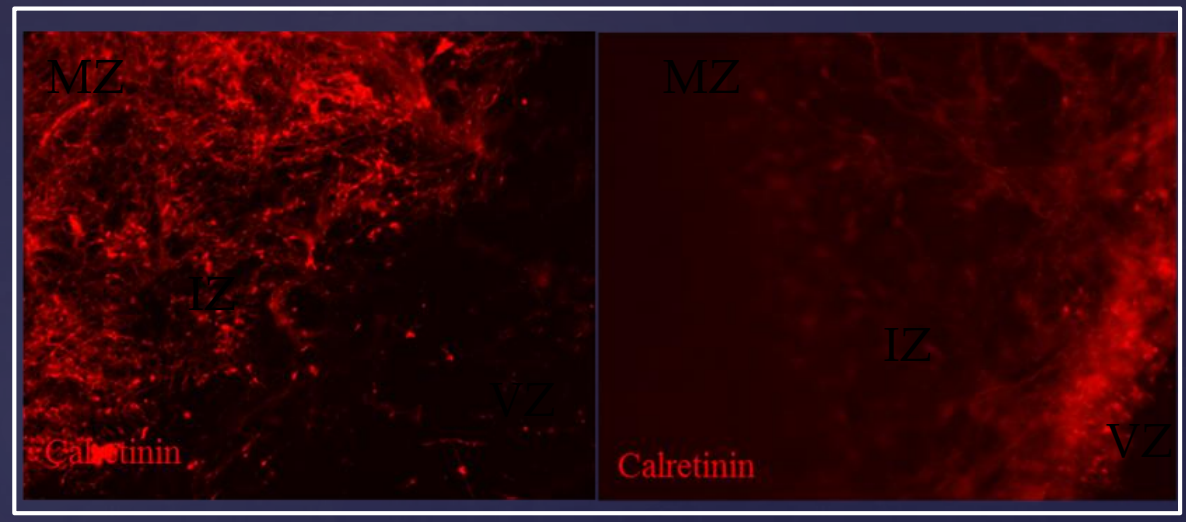
PD173074



Calretinin

Control

PD173074

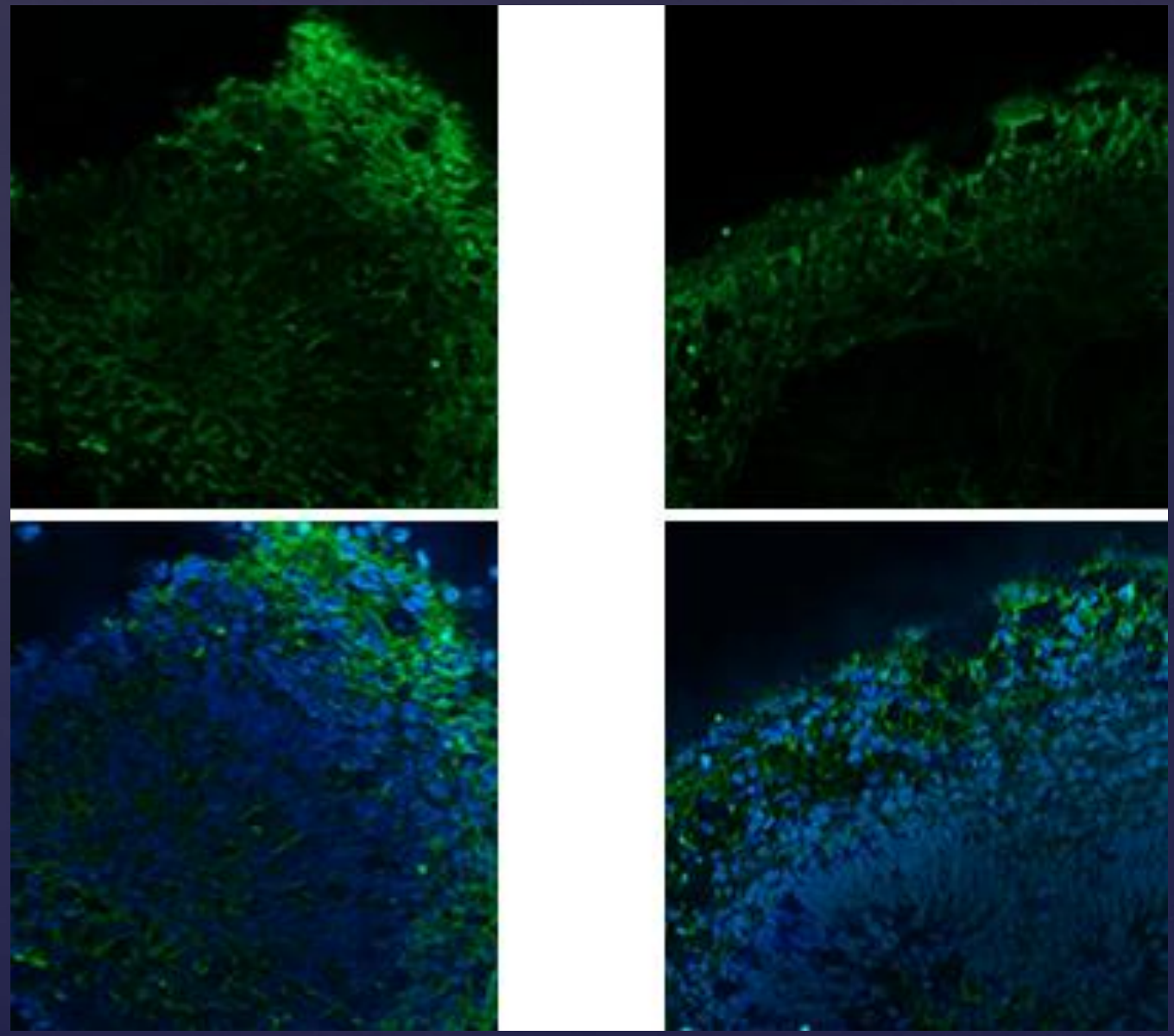


Depleted doublecortin^{+} neuroblasts/immature neurons and Calretinin^{+} interneurons

βIII-Tubulin

Control

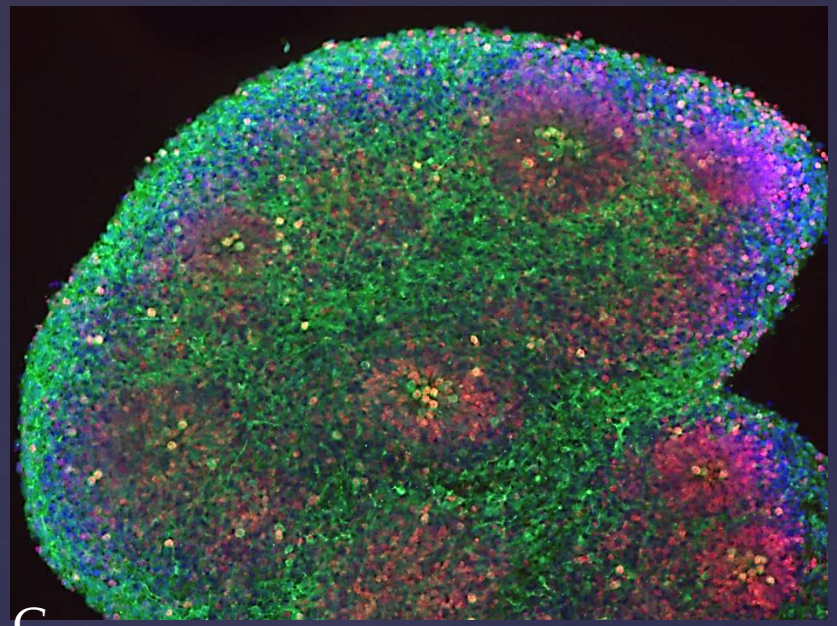
PD173074



Depleted
βIII Tubulin+ neurons

BrDu/PanNeu

10x



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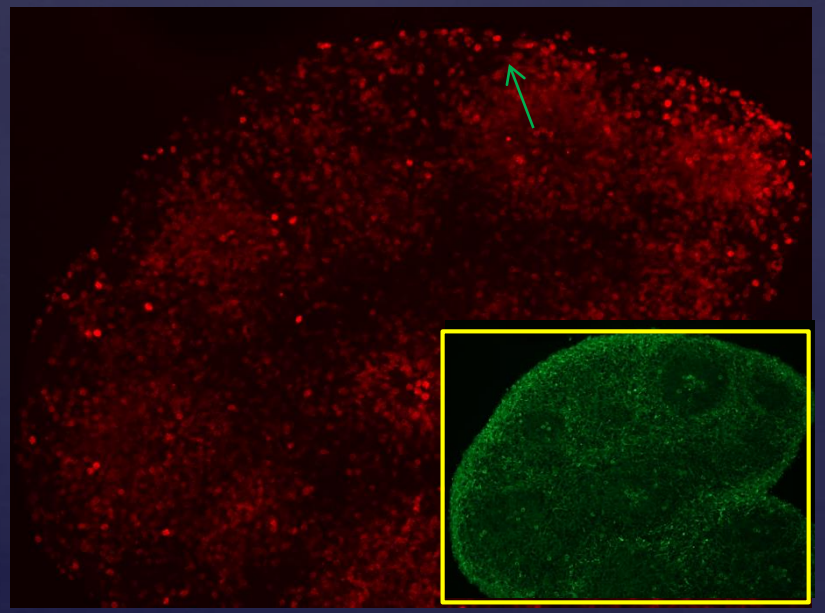
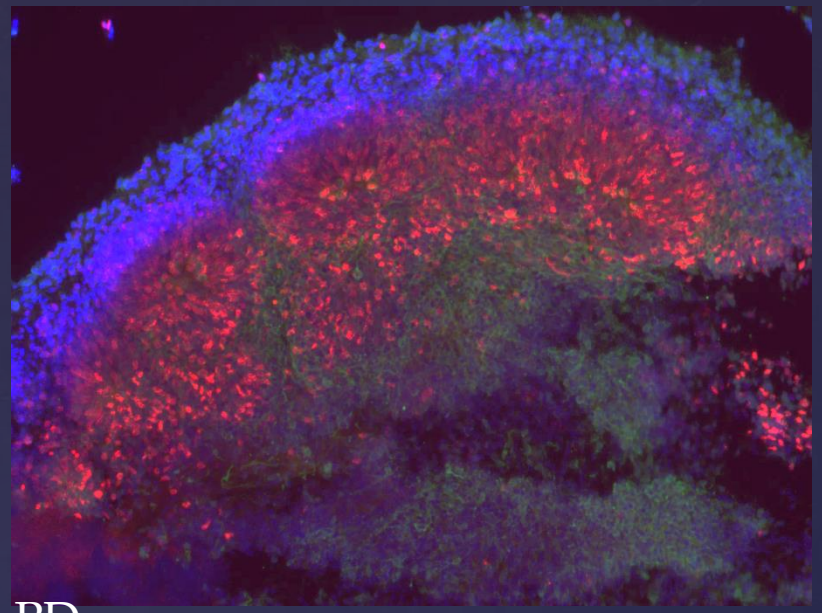
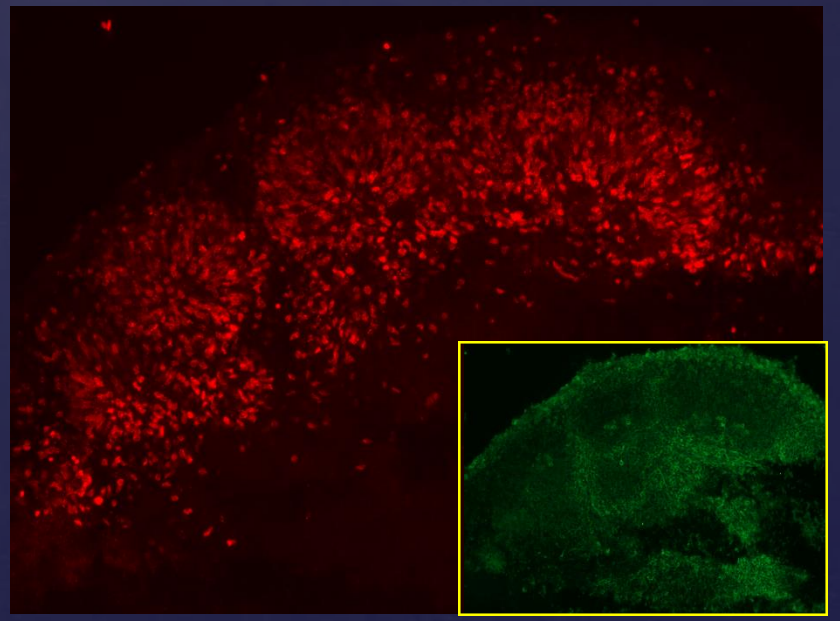


1178



PD



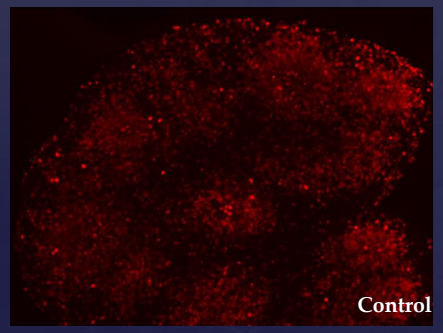
1183

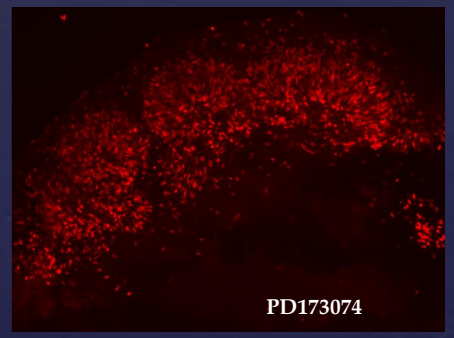
Depleted Pan NEU mature neurons

Conclusions:

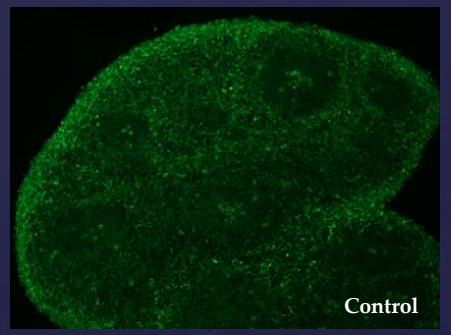
Delayed inactivation of FGFR1 with PD173074 in ESC CO influences neuronal development

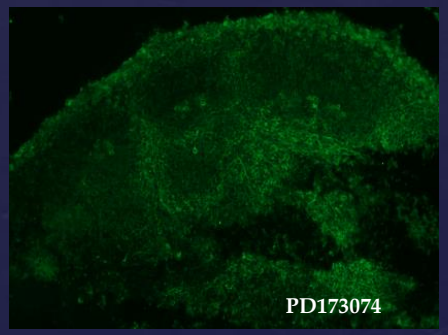
- *Inhibits nuclear accumulation of FGFR1, especially in cortical zone*
- displaces proliferating cells and arrests radial glia in VZ
- Turns of cortical TBR1 expression an early event essential for neuronal development
- Depletes neuroblasts (DCX+), cortical young neurons (βIII-Tubulin+), mature neurons (Pan Neu+) and interneurons (calretinin+)
- Affects developmental gene programs (CHiP analysis done)





PROLIFERATION - BrDU





Differentiation -Pan Neu

Schizophrenia as the effect
of gene dysregulation during
neuronal development.

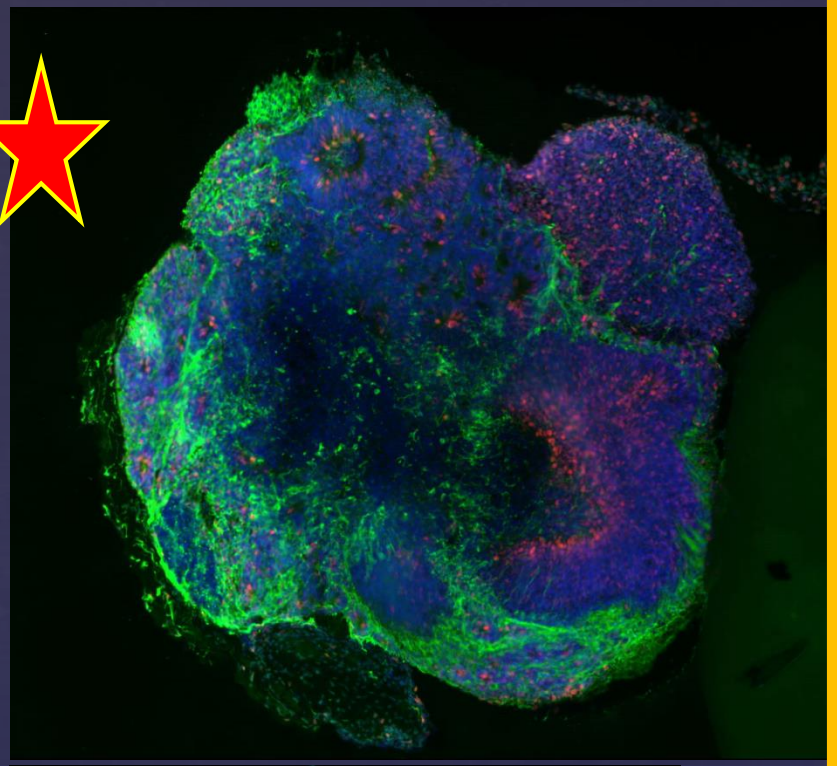
Role of FGFR1 in Schizophrenia

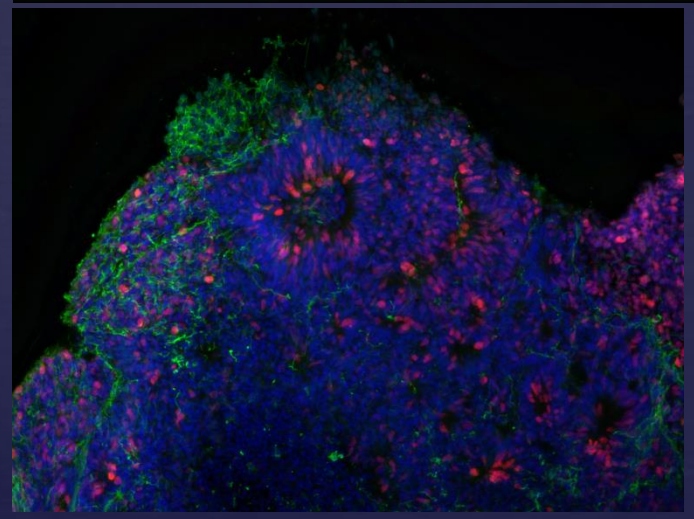
Ki67/PAN NEU

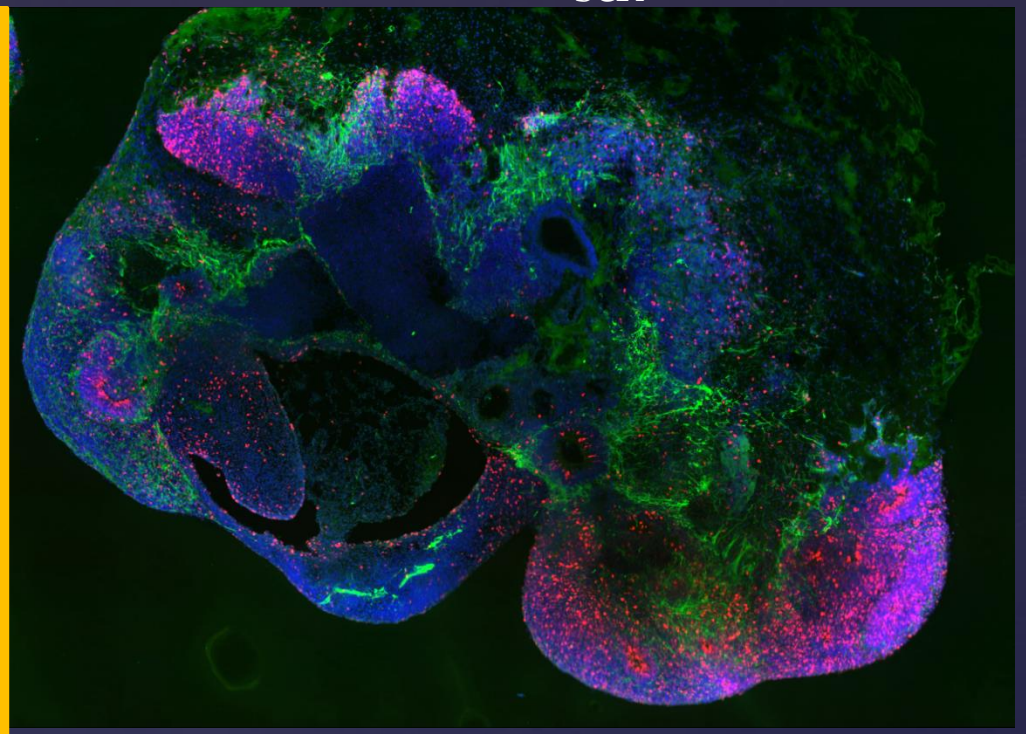
2wk

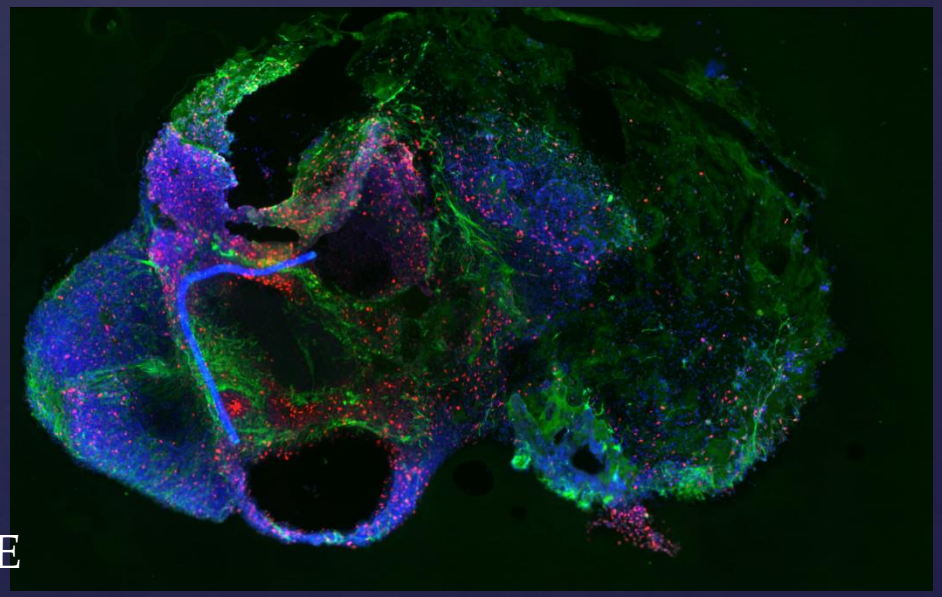
c

sch







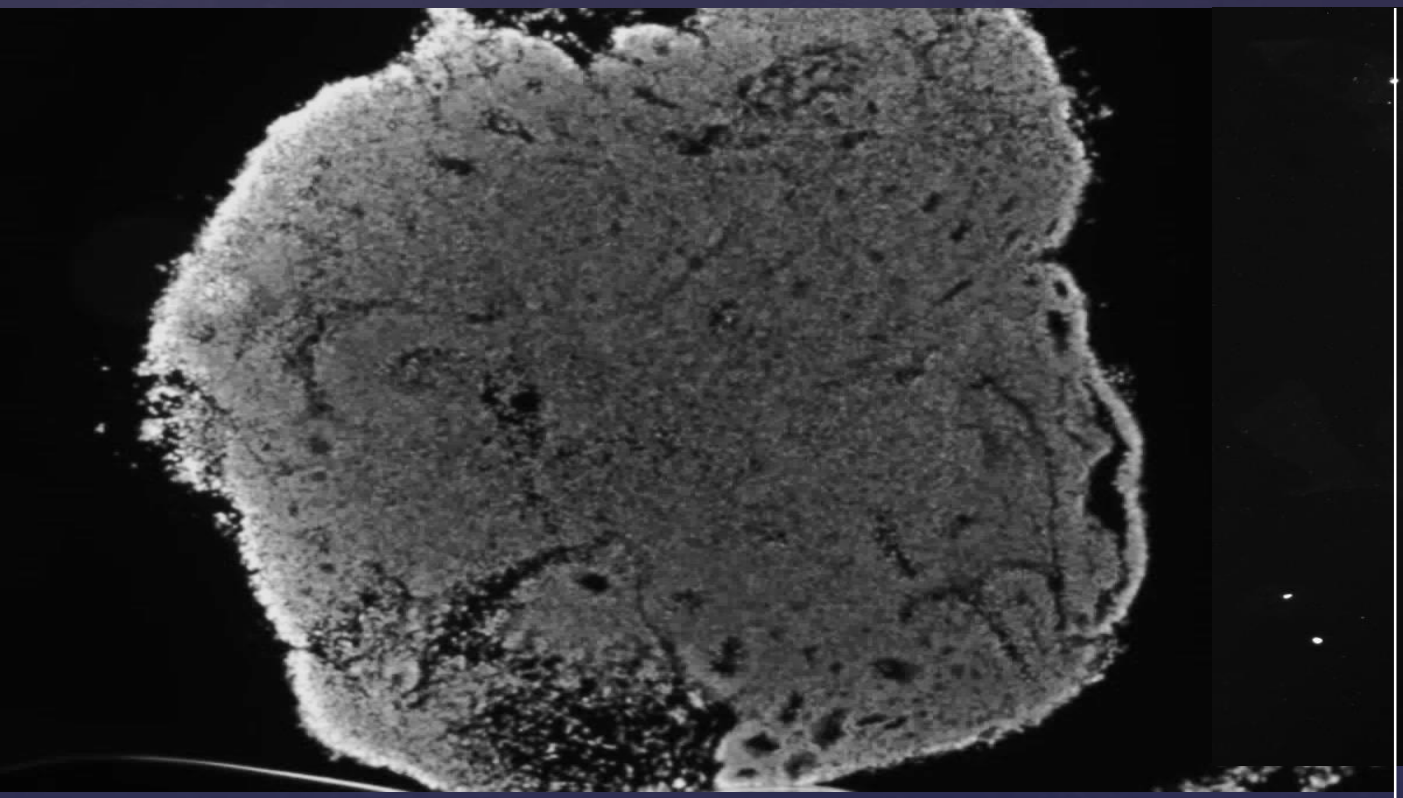


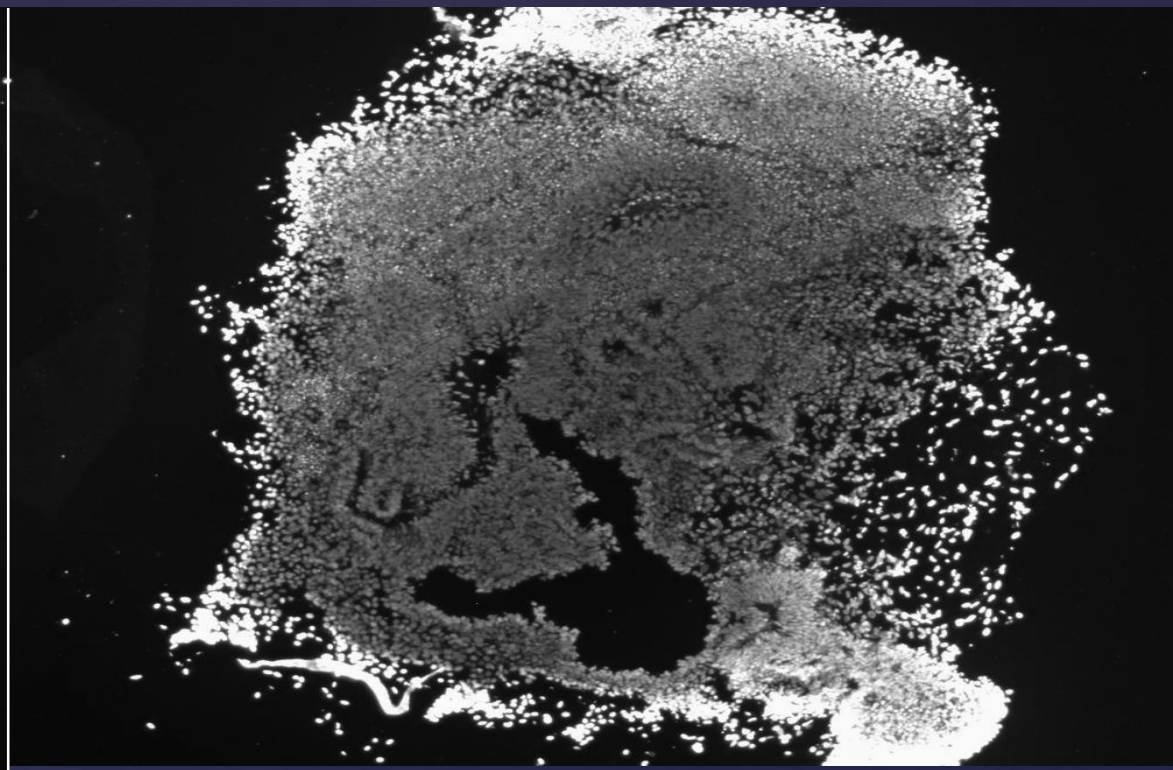
KEYENCE

DAPI 2 wk

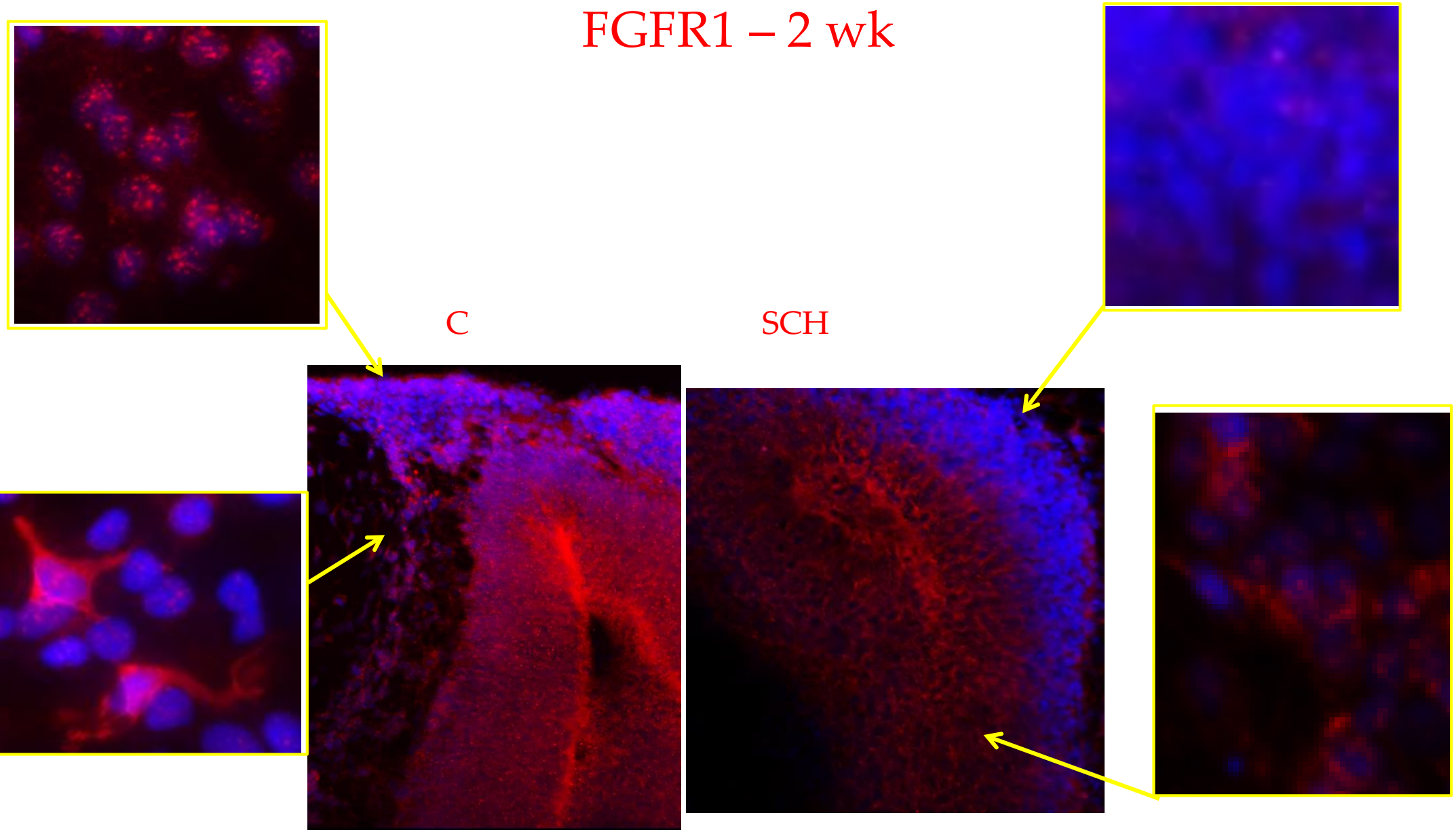
control

SCH

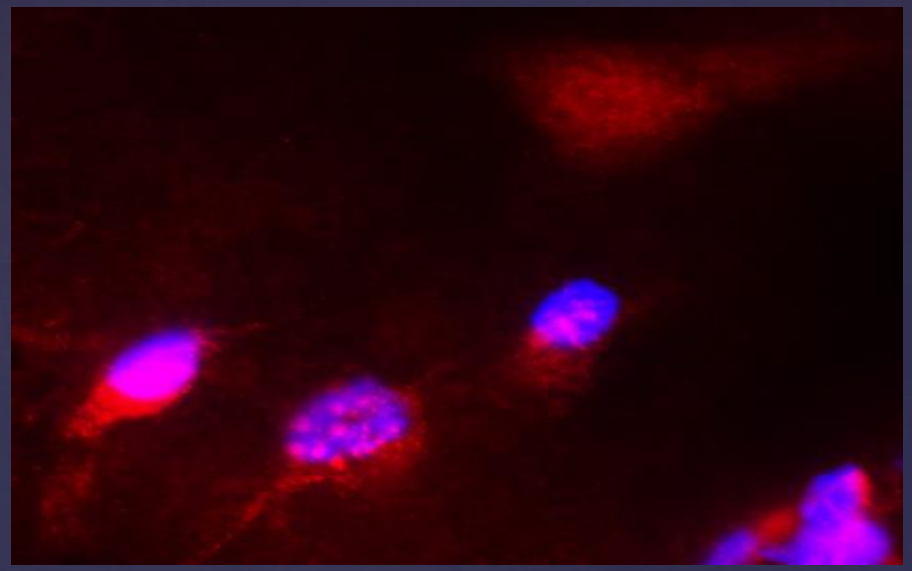


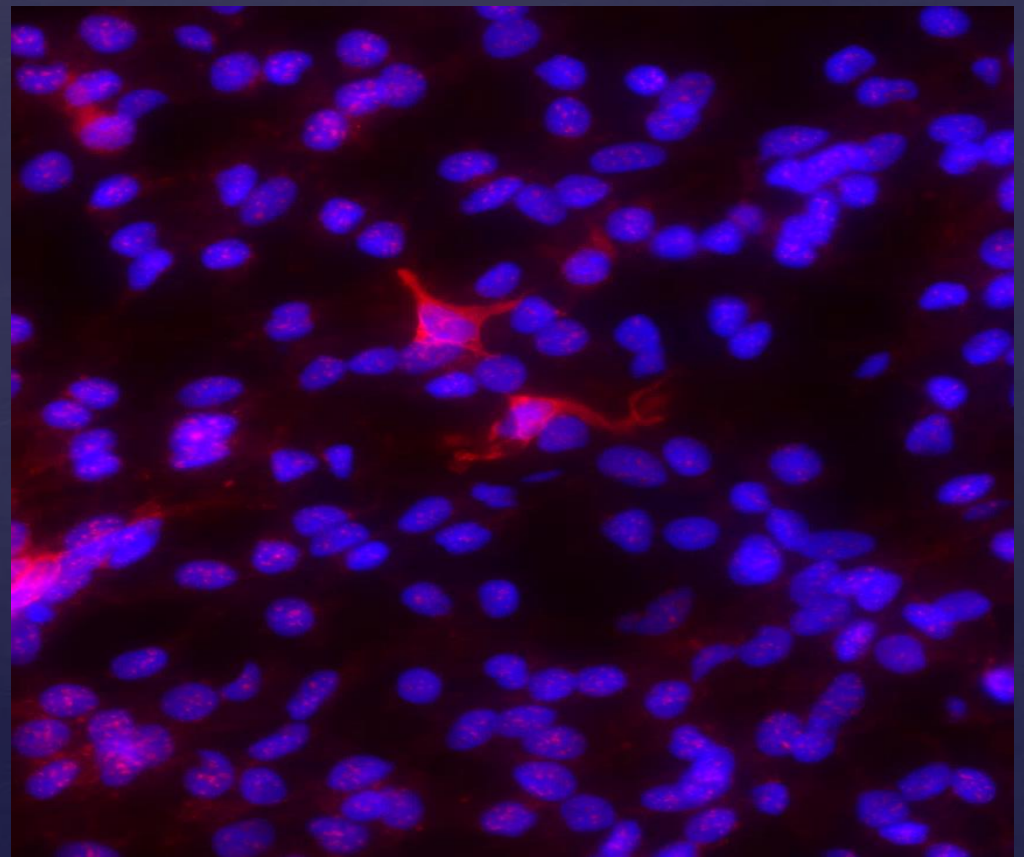


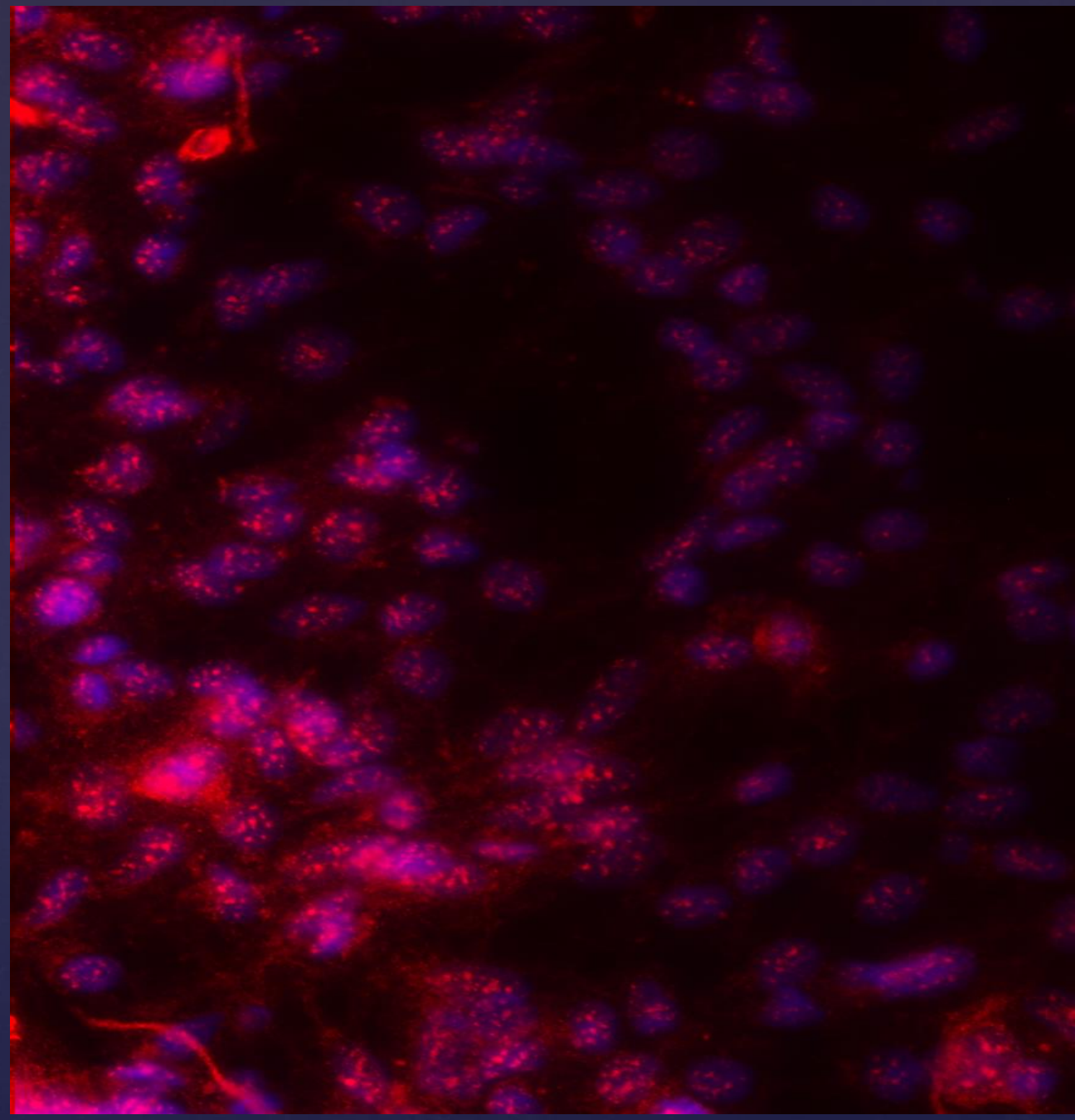
Loss of nFGFR1 expression in SCZ cortical cells

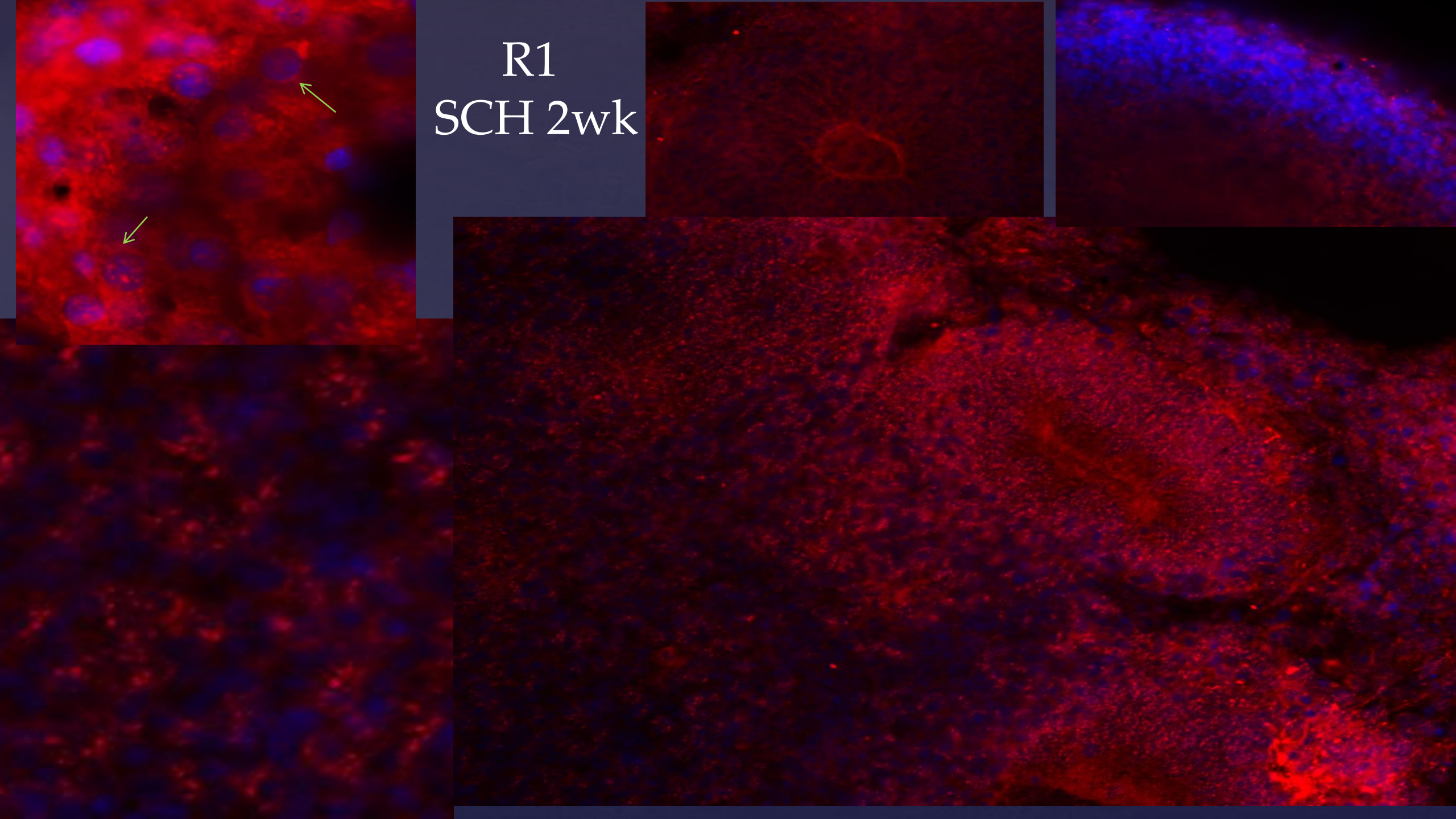


C 2wk
FGFR1



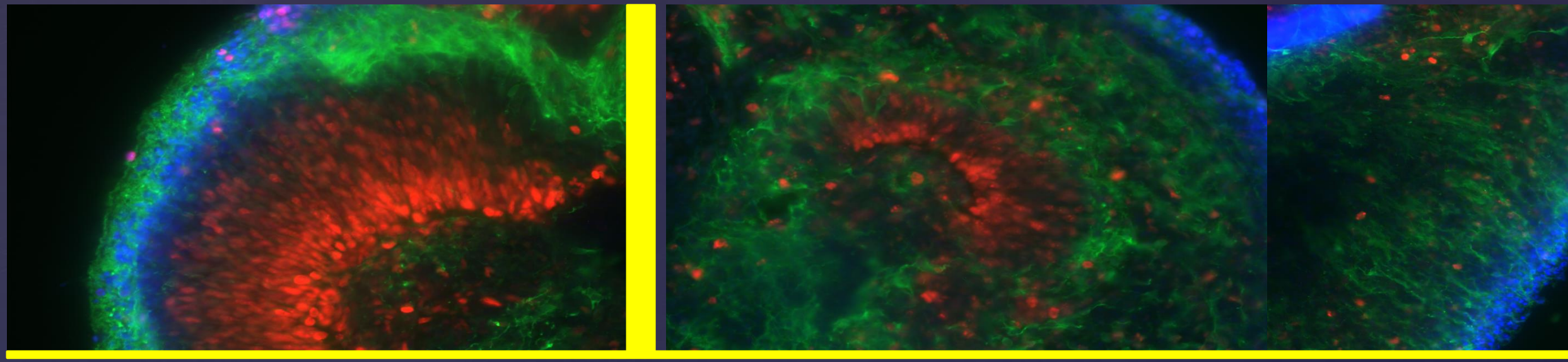





R1
SCH 2wk

Ki67/PAN NEU

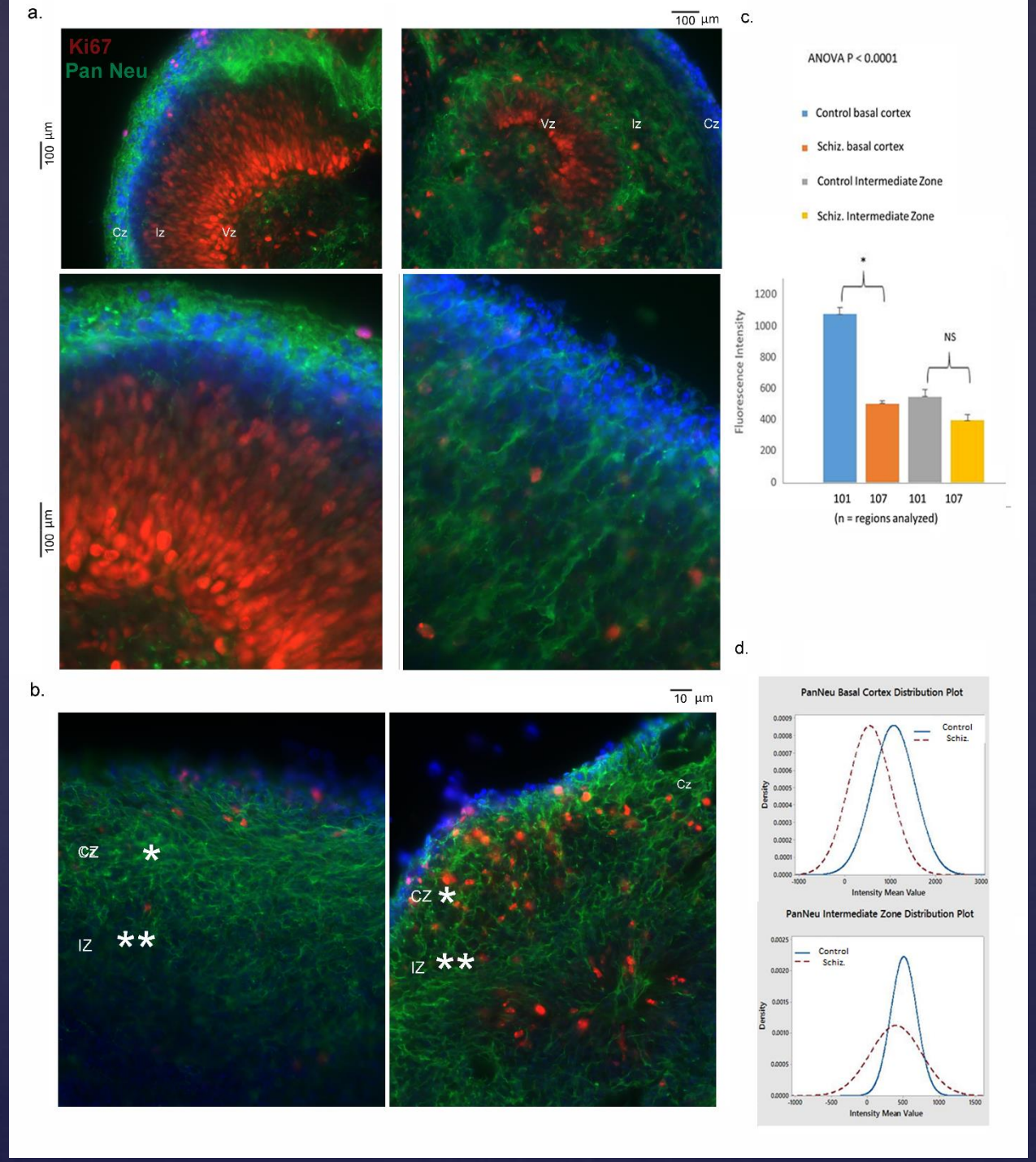
2wk C SCH



Maturation of Ki67 neuroblasts impaired in CZ

CZ

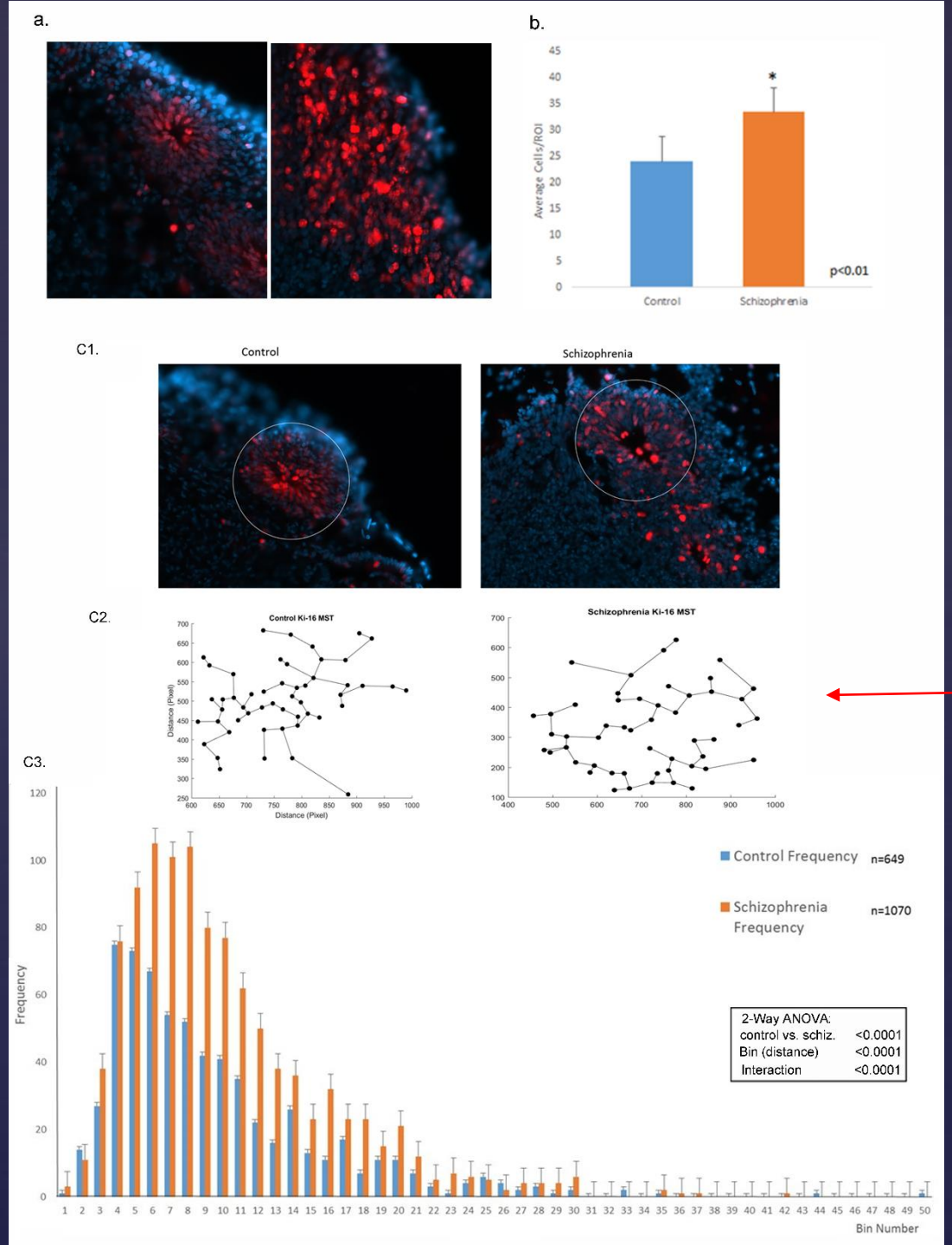
Figure 2.



Maturation of Ki67 neuroblasts n and differentiation to neuron are impaired in CZ

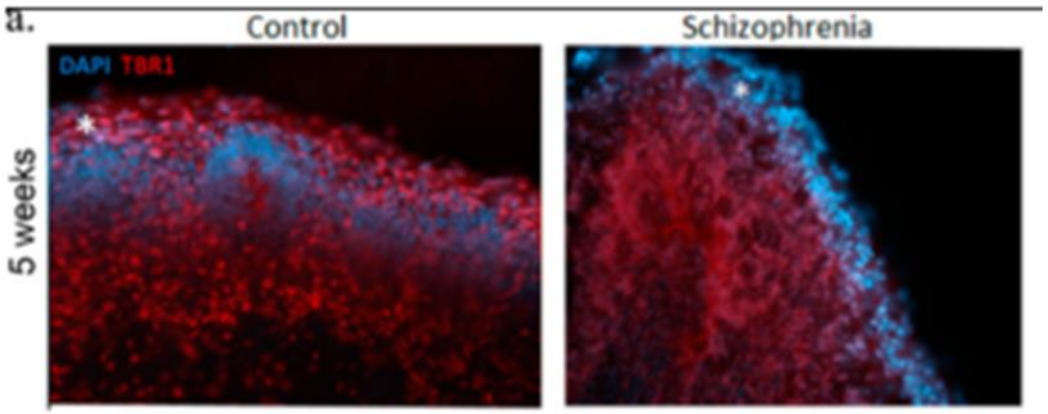
CZ

Figure 3.



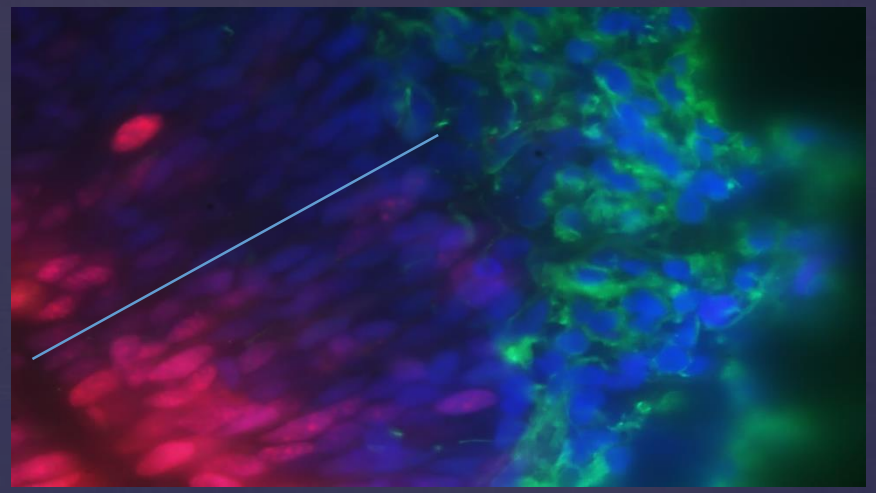
Global
Minimum
Spanning
Tree
analysis

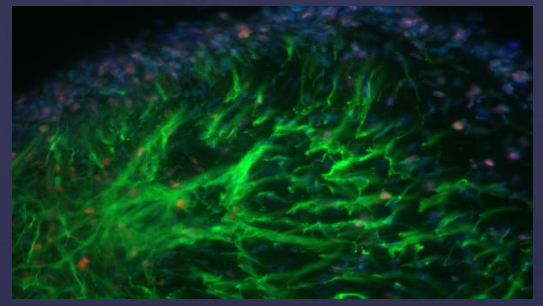
TBR1 5wk

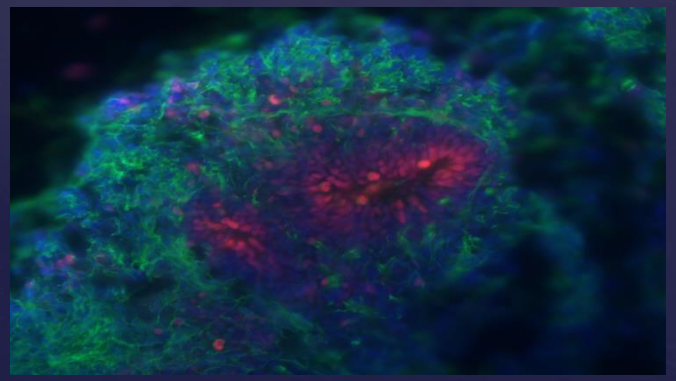


TBR1 – cortical in control, subcortical in schizophrenia

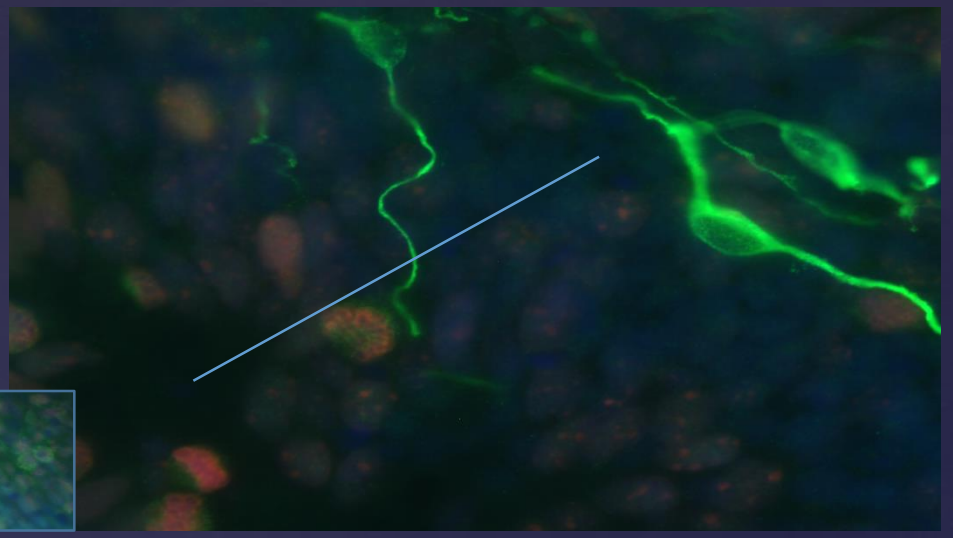
Ki67/PAN NEU 2wk

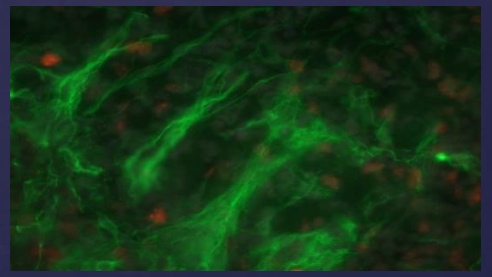


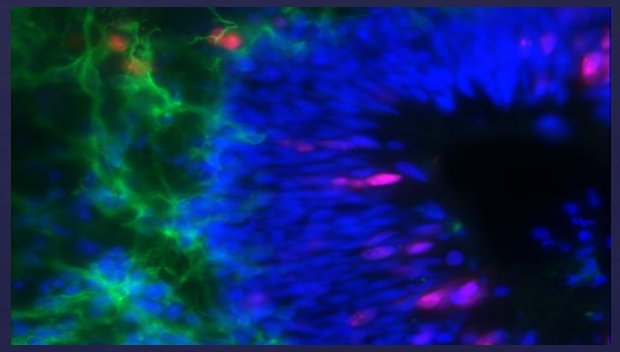




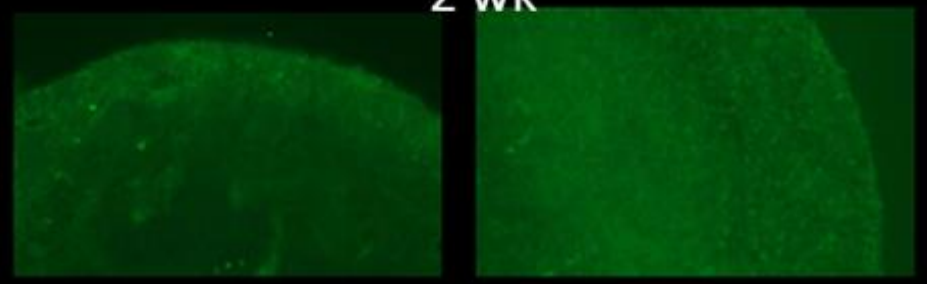
Tangential
Pattern of neuronal development

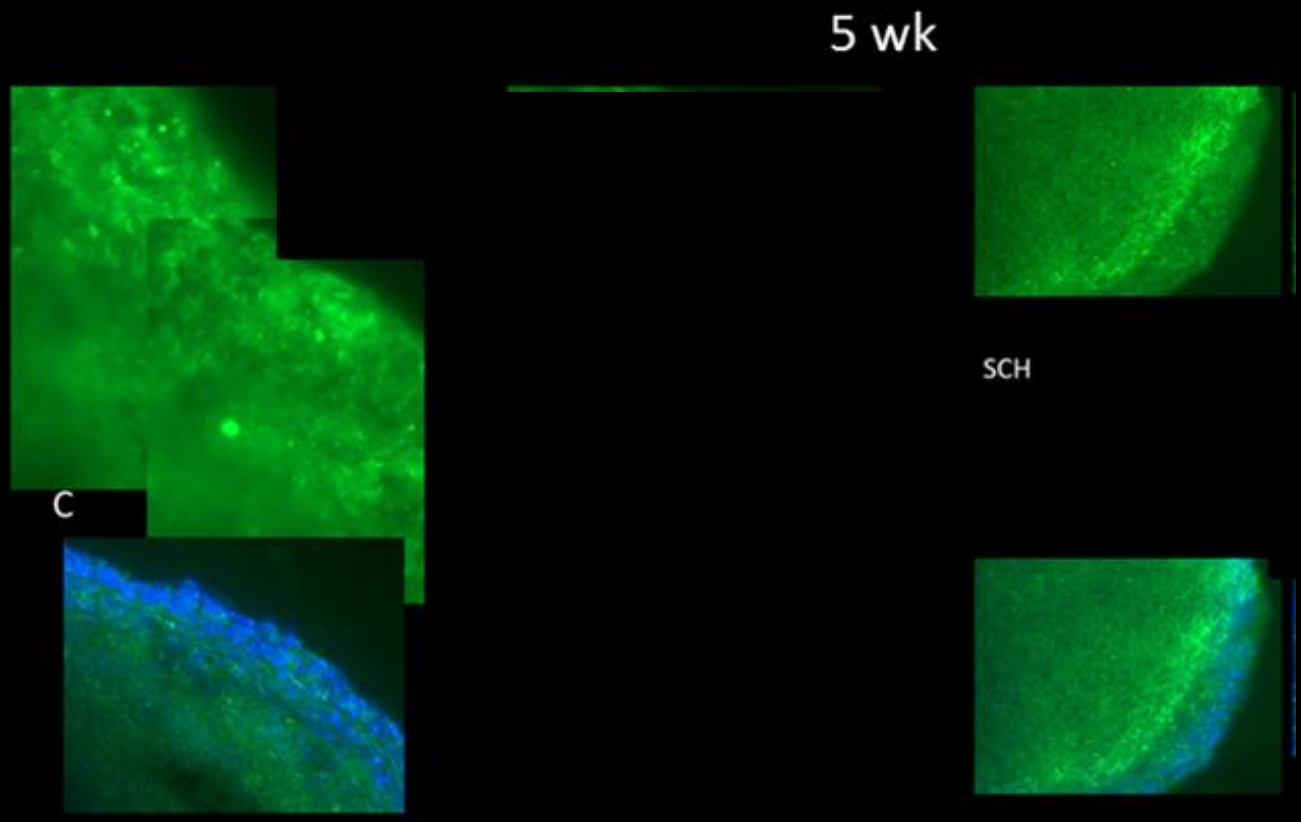






Reelin
secreted glycoprotein, controls neuronal migration and positioning in the developing brain.





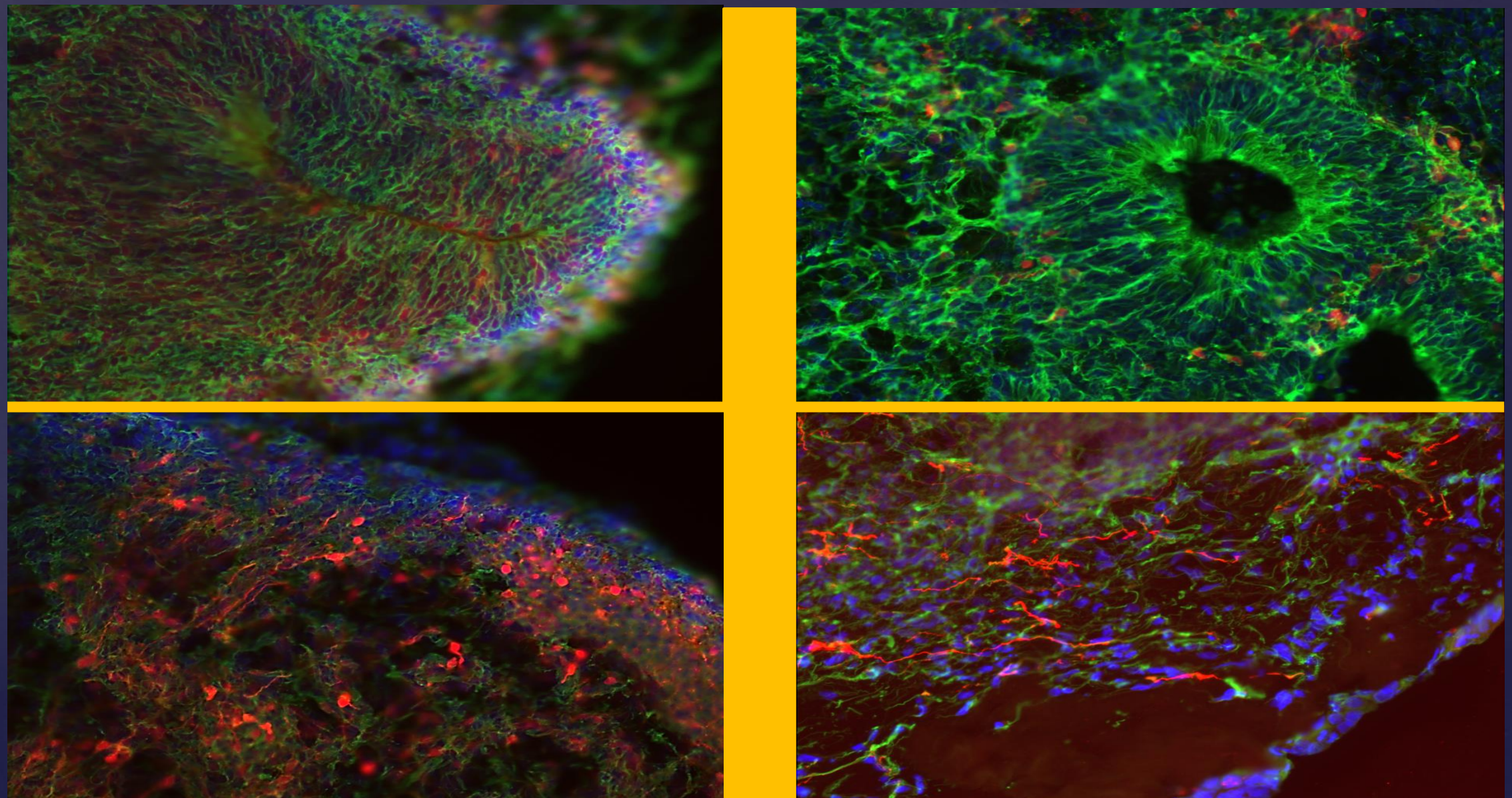
Reelin not expressed at 2 weeks. At 5 weeks, location of Reelin cells is different in control (cortical MZ) and schizophrenia (subcortical layer).

βIII Tubulin/CALRETININ

2wk

C

SCH

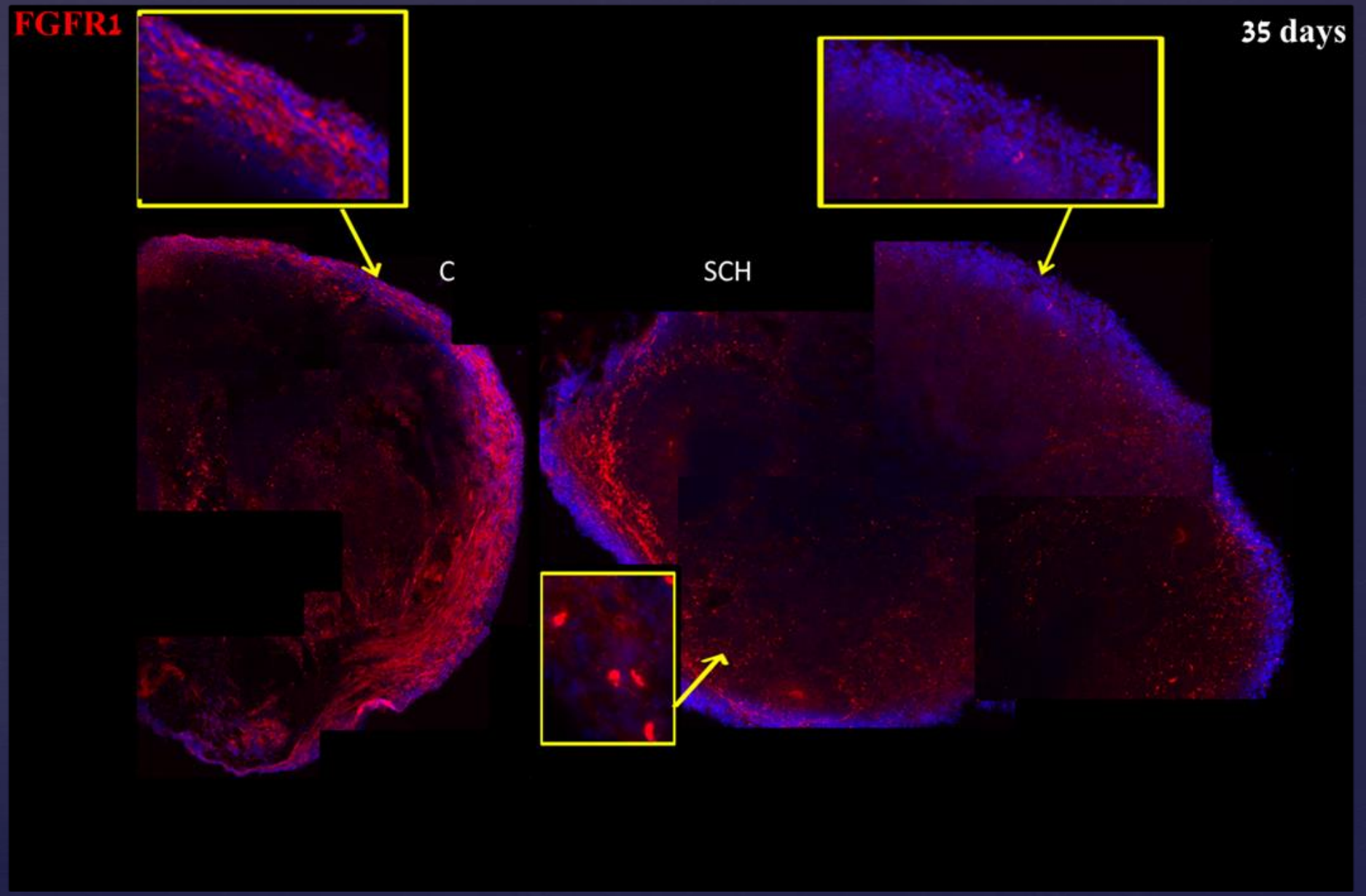


Summary of 2 wk iPSC organoids

	CONTROL	SCHIZOPHRENIA
FGFR1	in VZ and CORTEX nuclear	Depleted in in nuclei present in cytoplasm
	Proper layering = controlled maturation of Neurons	Disrupted layering= premature maturation of Neurons

5 wk organoids

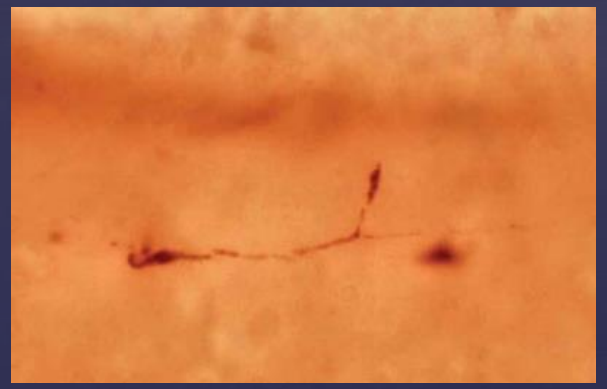
Loss of nFGFR1 expression in cortical cells



-

Reelin' in Clues to Schizophrenia

By Adam BostanciMar. 22, 2002 , 12:00 AM
People with schizophrenia have abnormally low levels of a protein called reelin in the brain. Now researchers have found that this protein seems to play an important role in guiding newborn neurons to their final homes.



Reelin

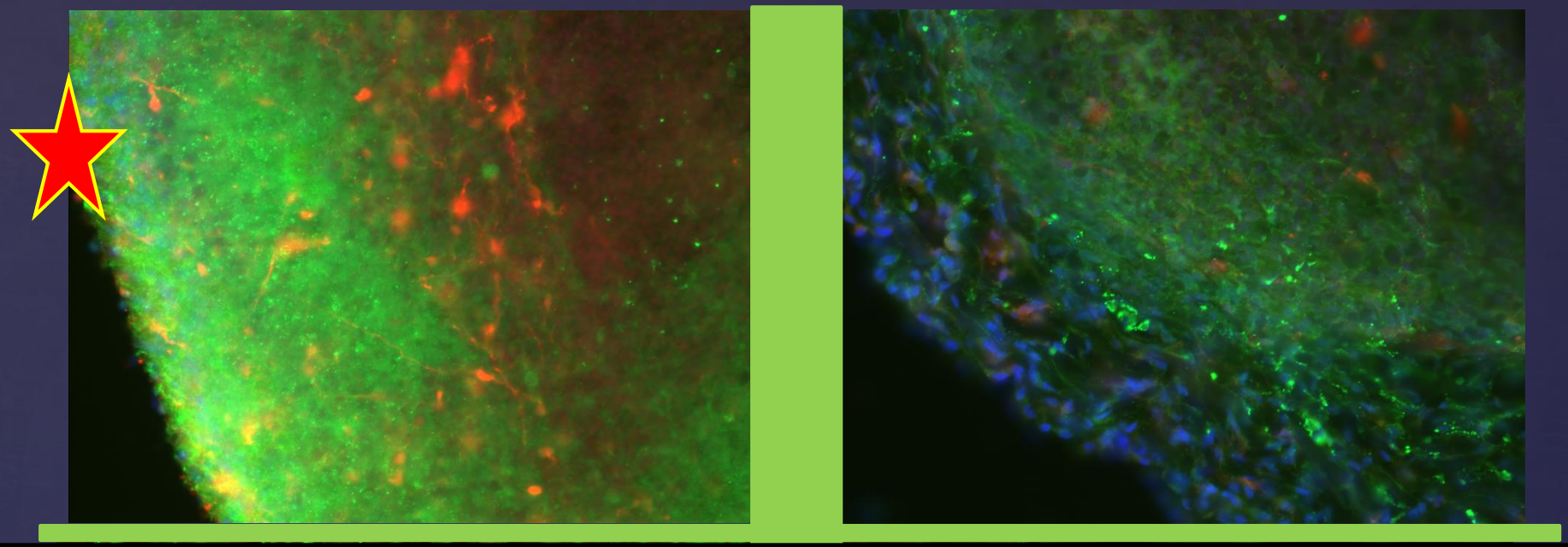
Glycoprotein secreted by Cajal Retzius cells that regulates processes of neuronal migration and positioning in the developing brain

Normal Reelin mutant

Reelin / CALRETININ

C

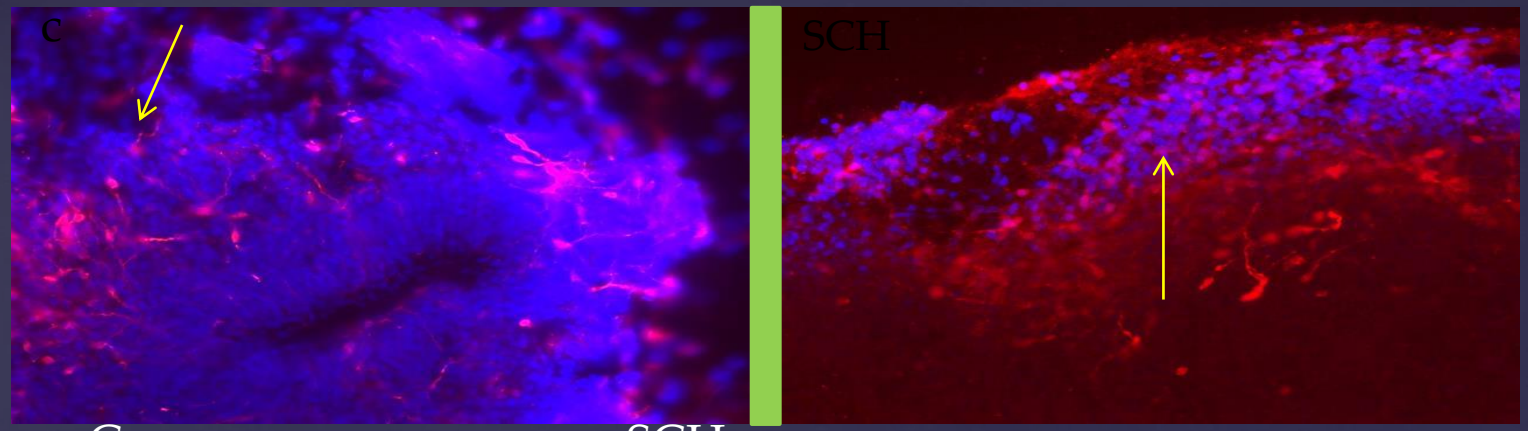
SCH



Reelin+ cells displaced in schizophrenia cortex

CALRETININ

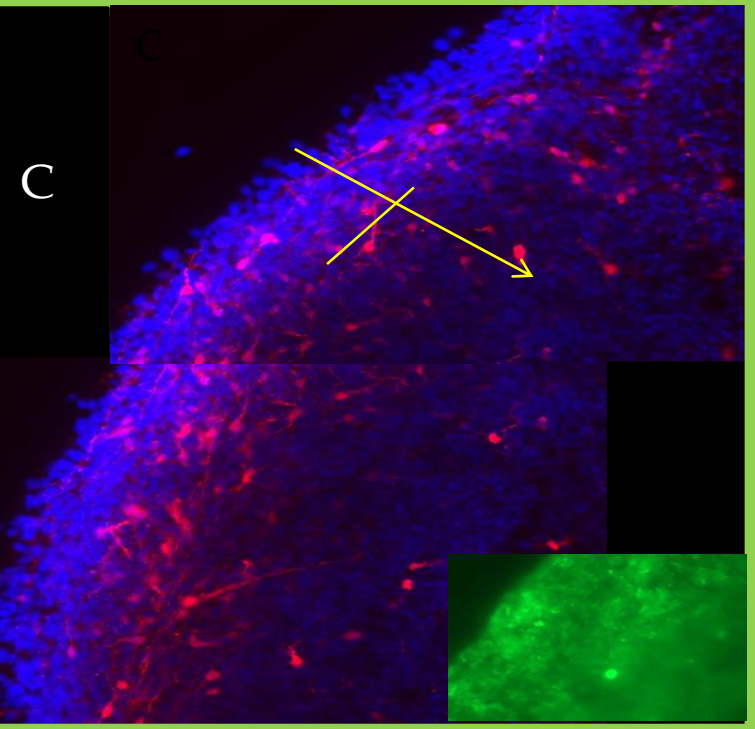
2wk

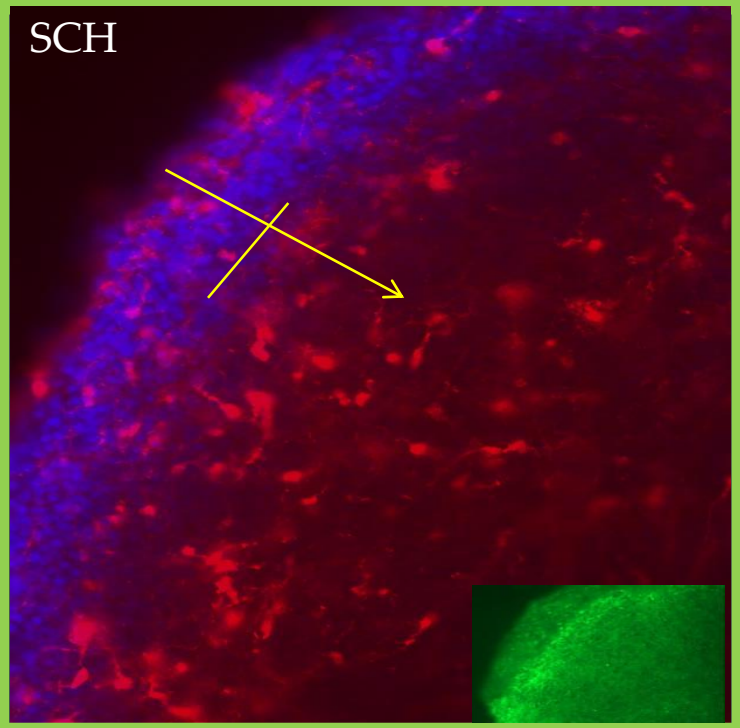


C SCH

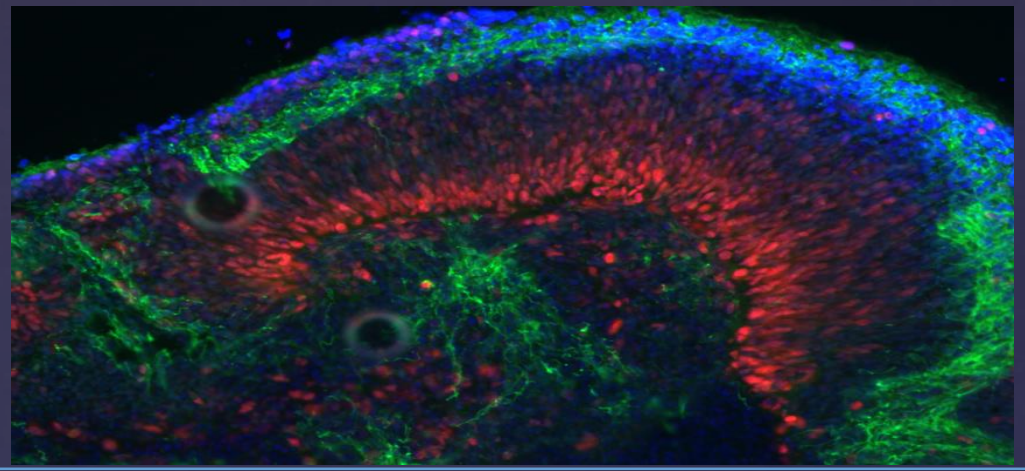
Dislocation of cortical interneurons and loss of processes in schizophrenia

35 days

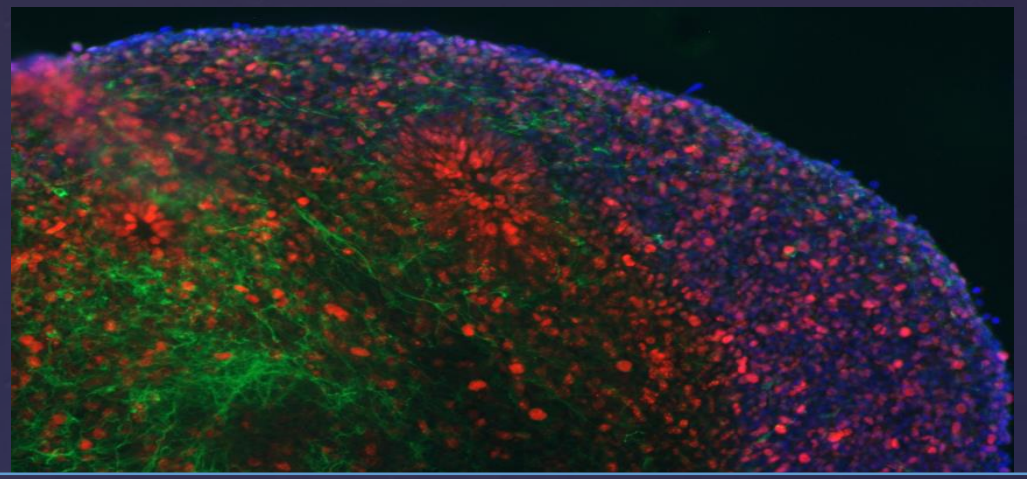




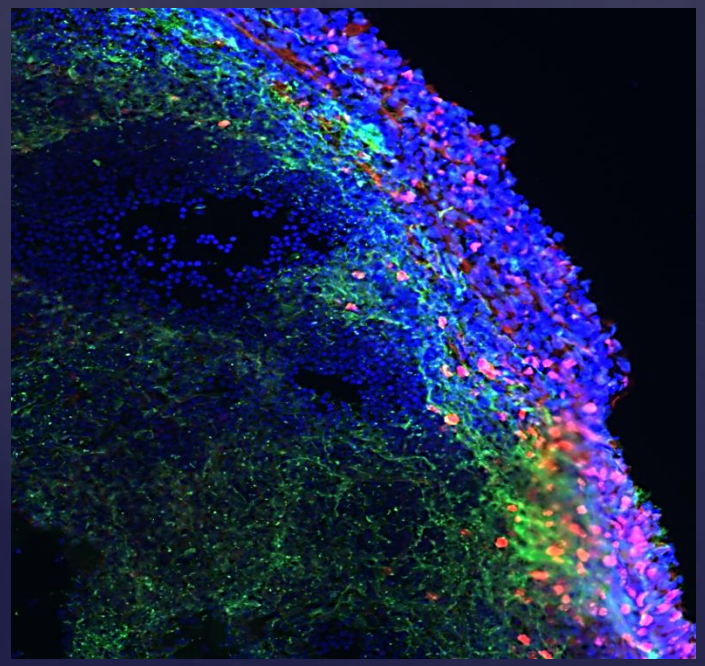
iPSC 2wk 10x c



sch



5wk c



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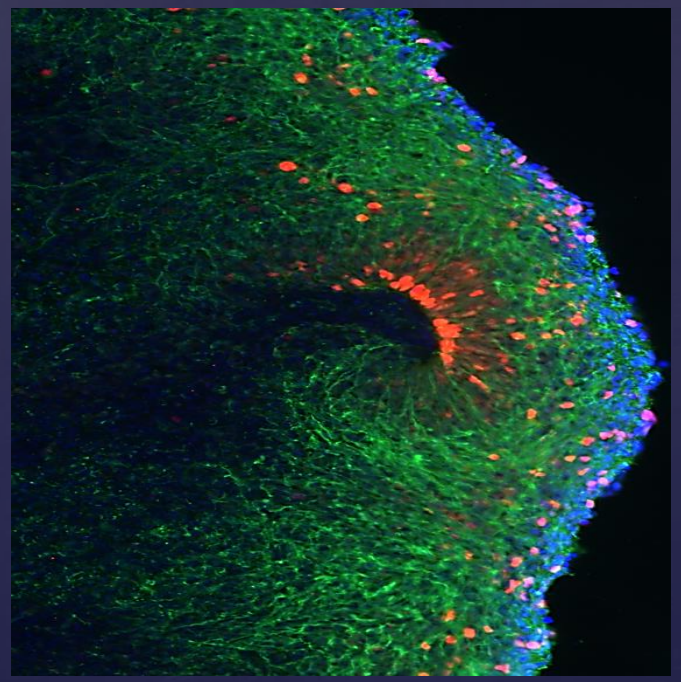
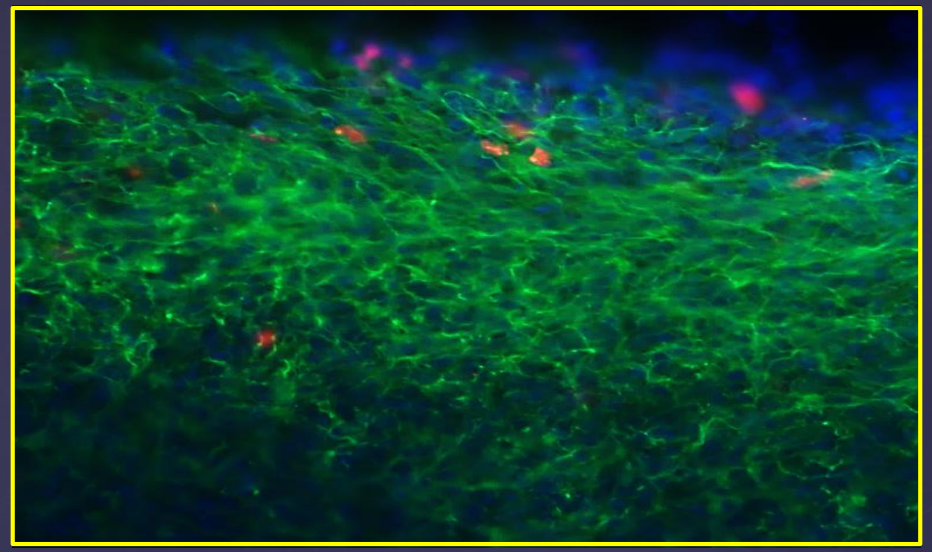


- Maturation of proliferative from the VZ is delayed

Ki67/PanNeu

35 d 20x

Ki67/PAN NEU



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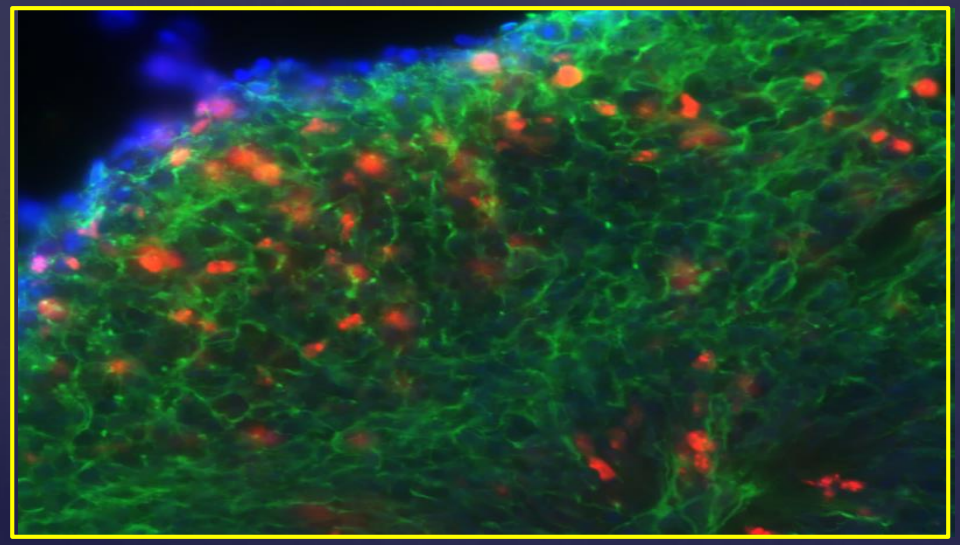


SCH

Conclusions:

Schizophrenia iPSC derived CO showed early developmental changes:

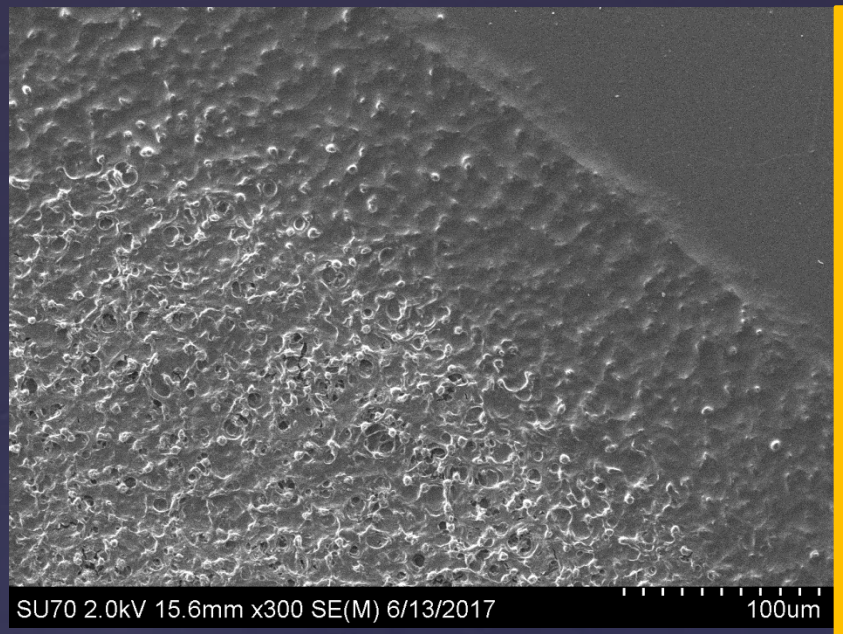
- Maturation of proliferative VZ is delayed
- Reelin+ cells are displaced in schizophrenia cortex
- cortical interneurons are displaced and have fewer processes (connections?) in schizophrenia
- Expression of cortical nFGFR1 is reduced in schizophrenia (potential negative impact on neuronal differentiation and maturation)

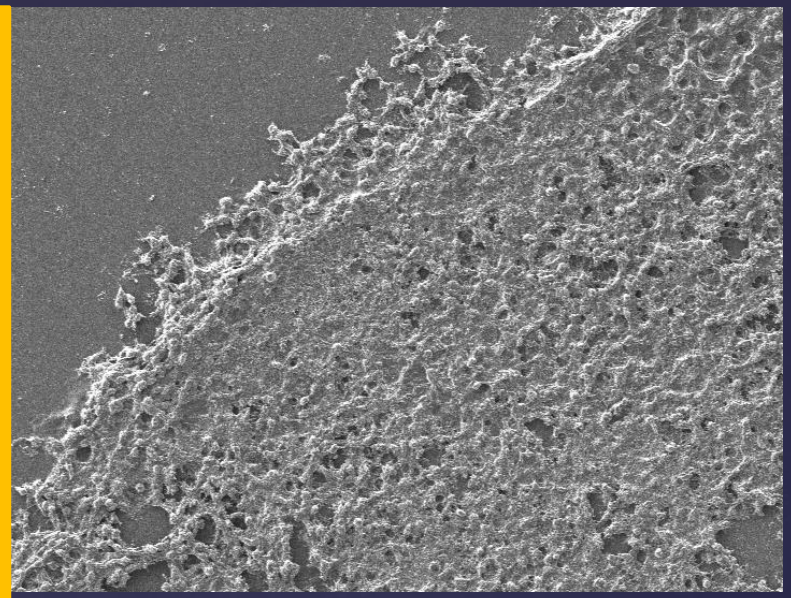
100um

CONTROL

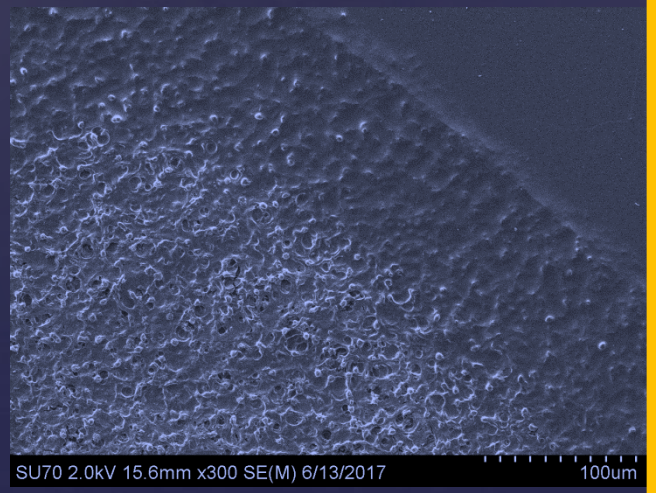
SCH

c

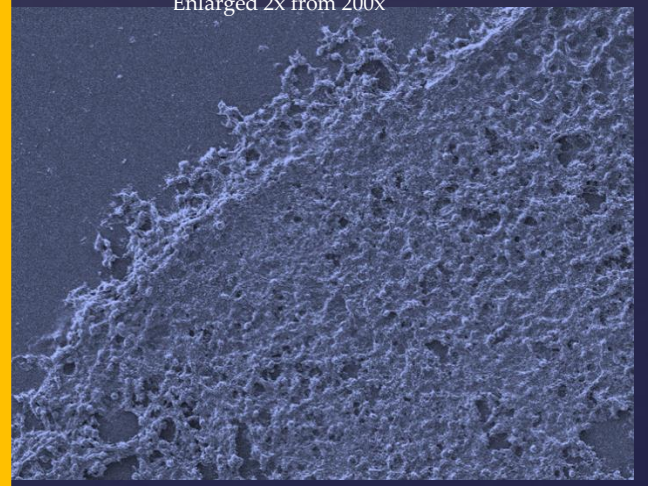




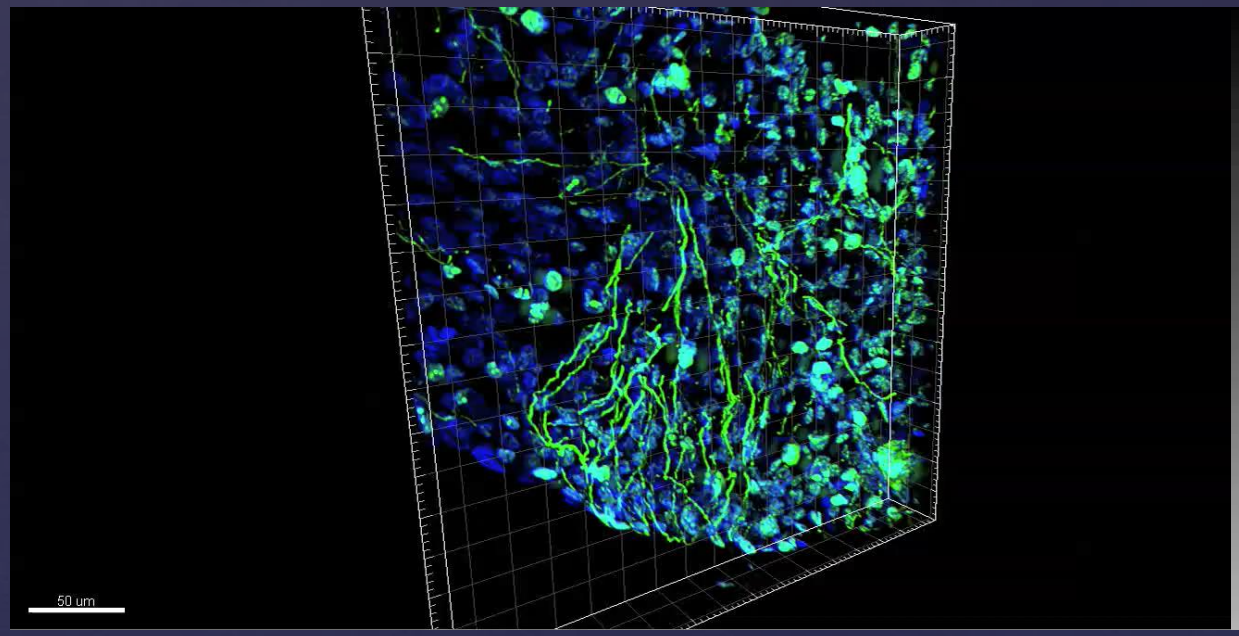
SEM images



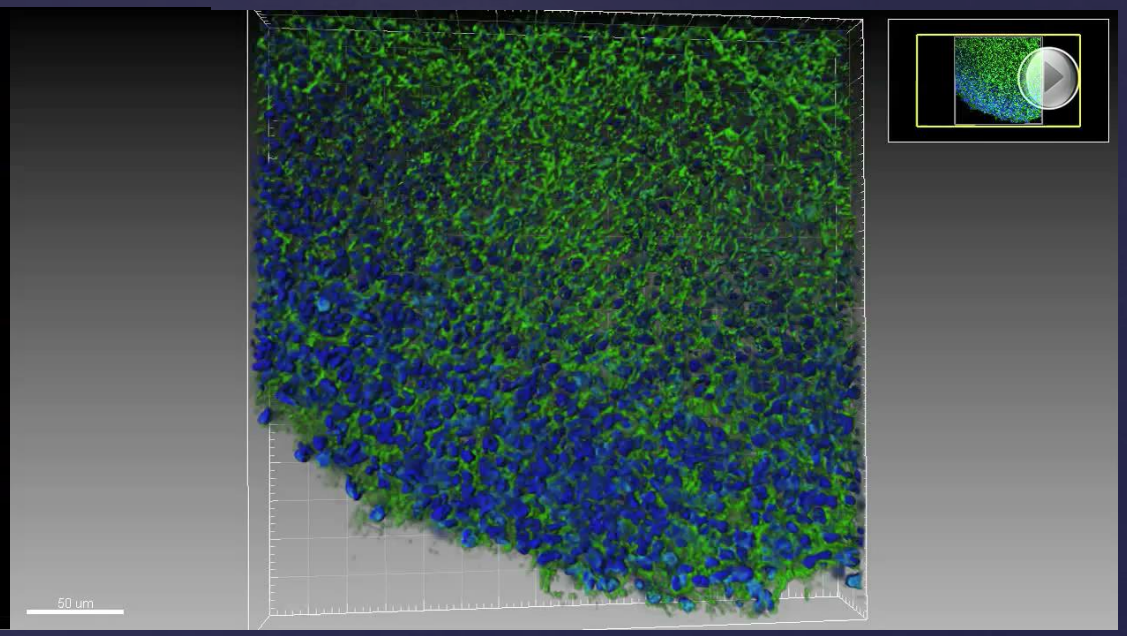
Enlarged 2x from 200x



Supplementary Video 1 legend: (3D confocal images). The single frame image illustrates (a) underdevelopment of the cortical Pan-Neu neurons with long process and the increased density of subcortical Pan-Neu neurons in schizophrenia organoids (iPSC line 3651) compared to (b) control (iSPC line 1835). CZ – cortical zone, IZ – intermediate zone



Control organoid (iSPC line 1835)



Schizophrenia organoids (iPSC line 3651)

Pan Neu / DAPI

Stachowiak et al. 2018

3 months ago we said Hello!

Now it is time to say Good Bye!