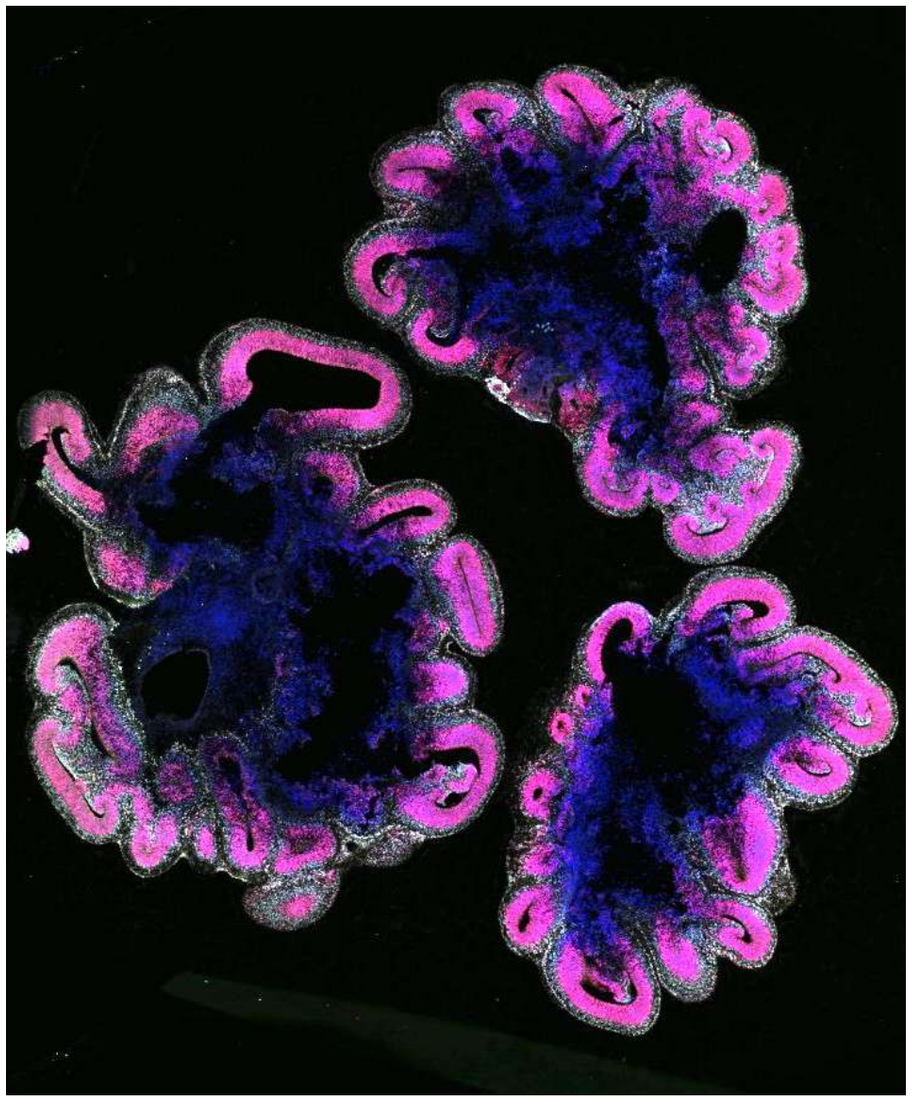
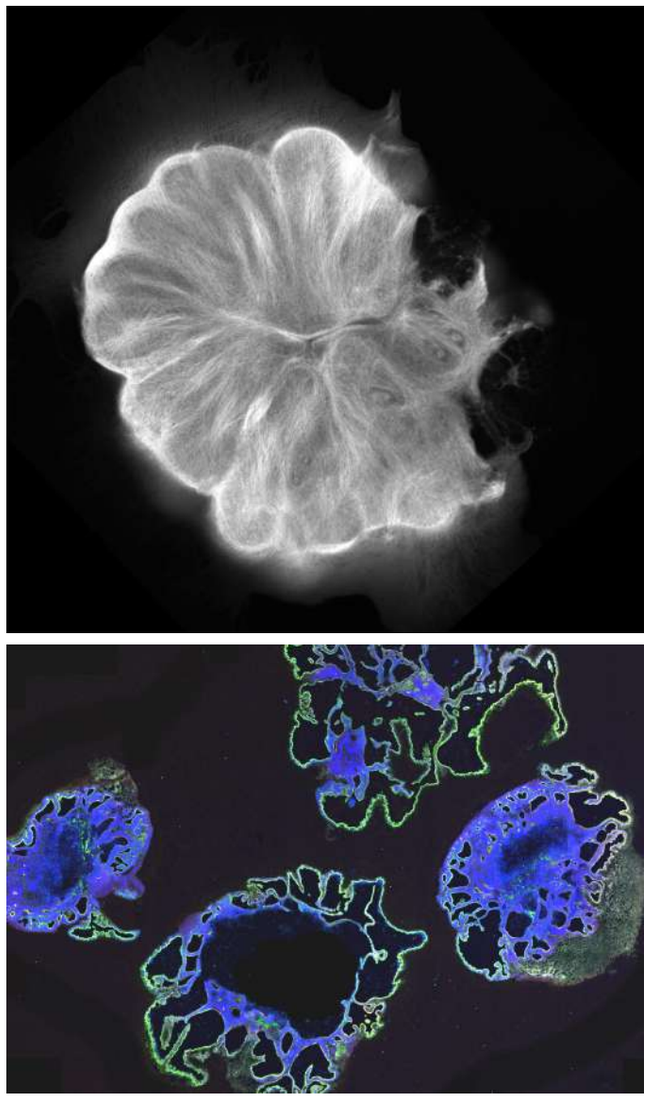
Redefining key features of neurodevelopment with brain organoids





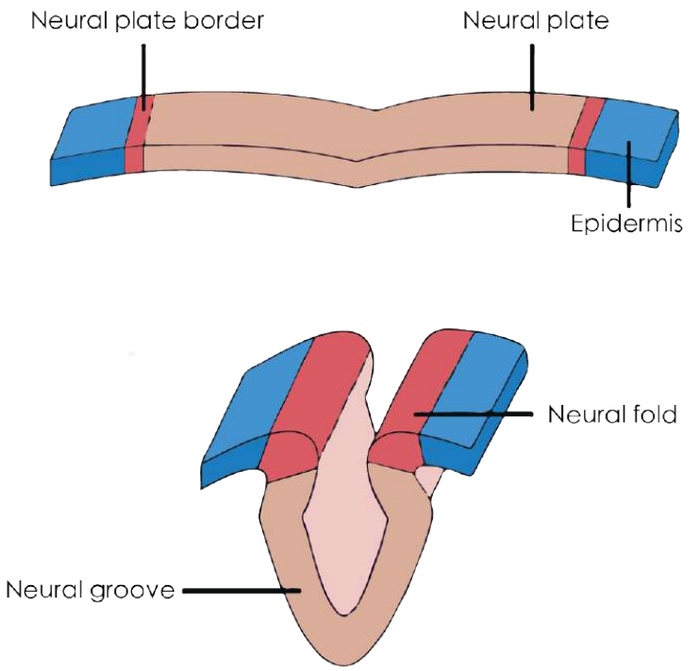


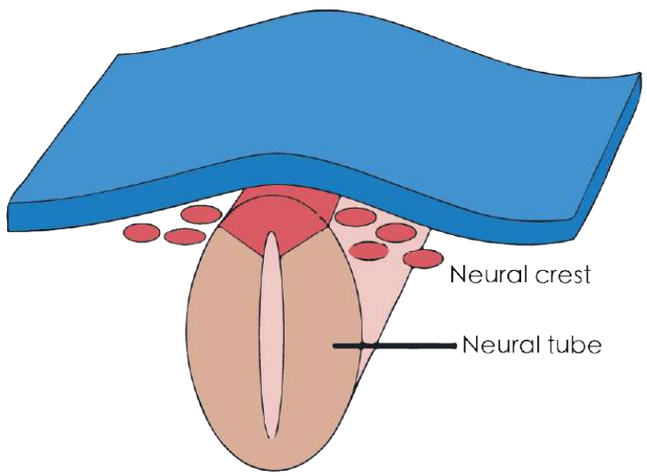
Ilaria Chiaradia

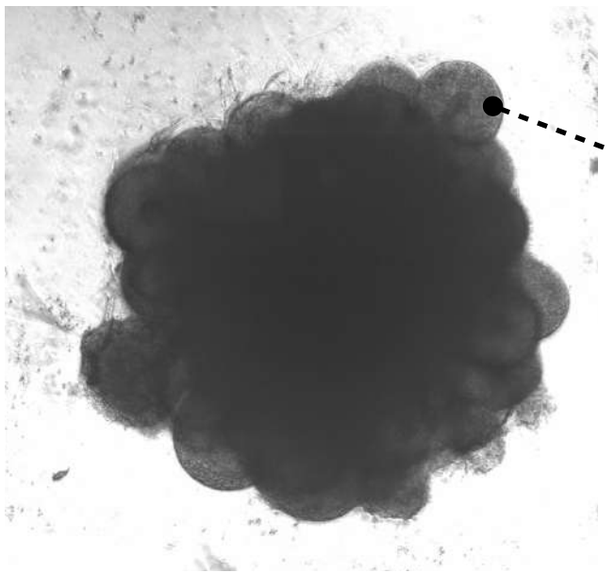
17th Nov 2026

Contact: ilariachiaradia.bio@gmail.com

Human developing brain

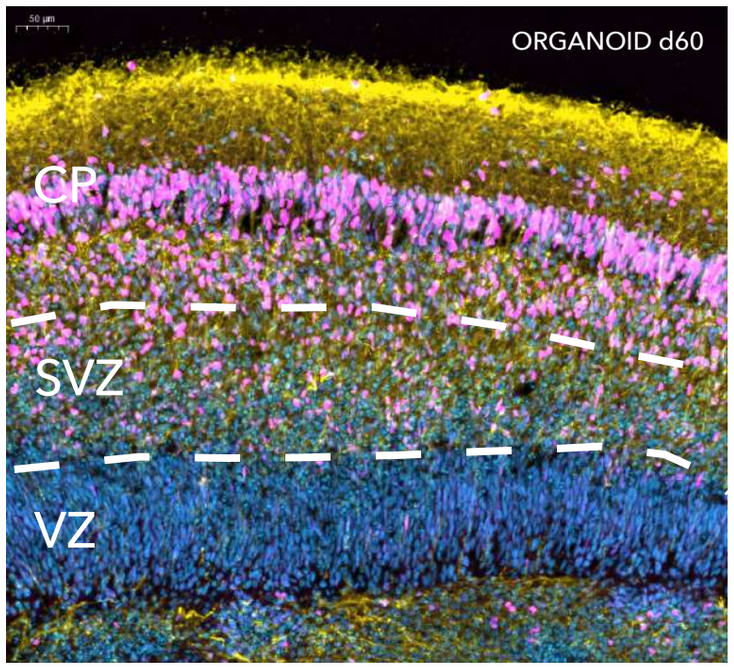


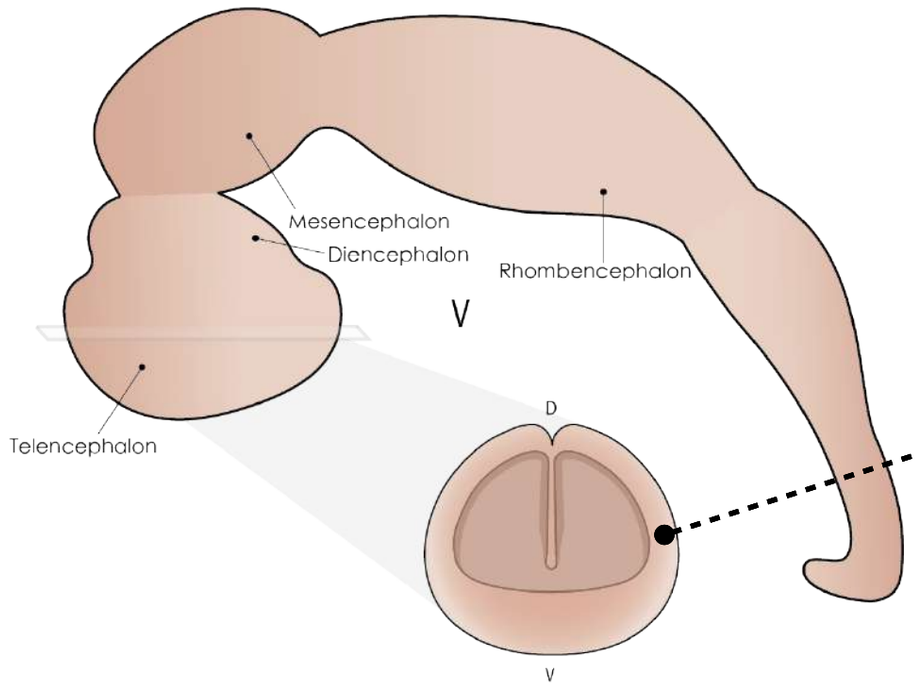


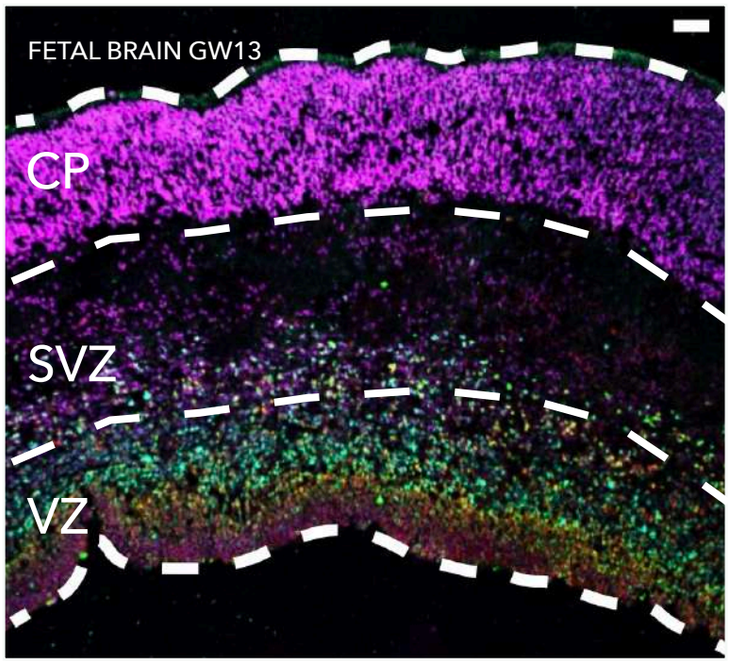


D

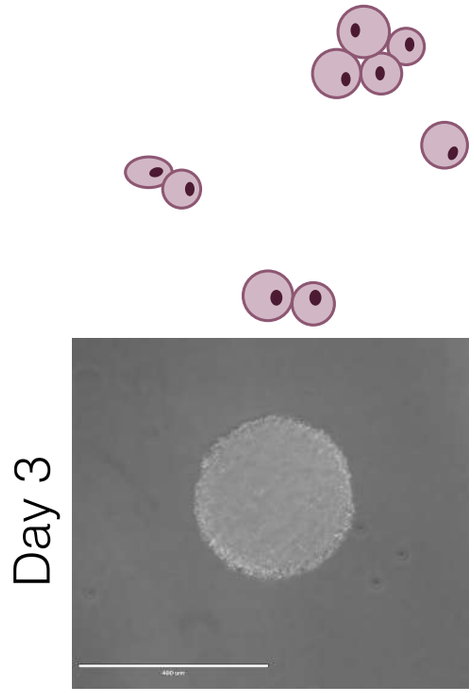
Neural tube







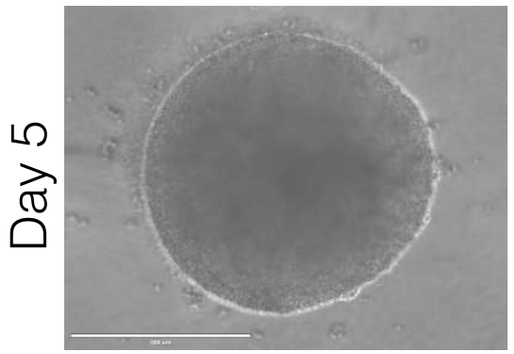
Brain organoid protocol



Pluripotent stem cells

Day 3

Embryoid bodies

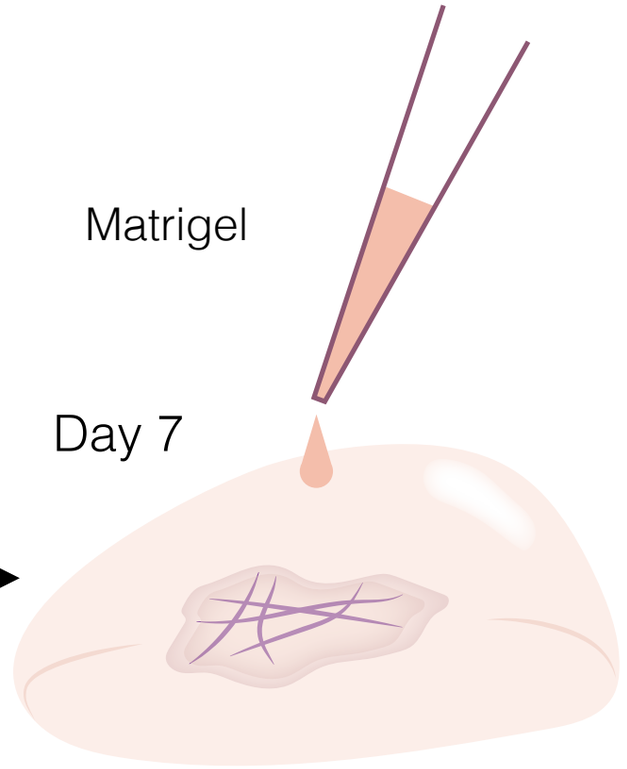


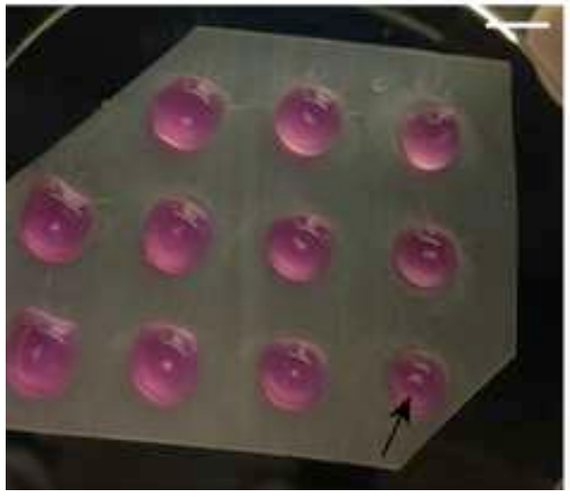
Day 5

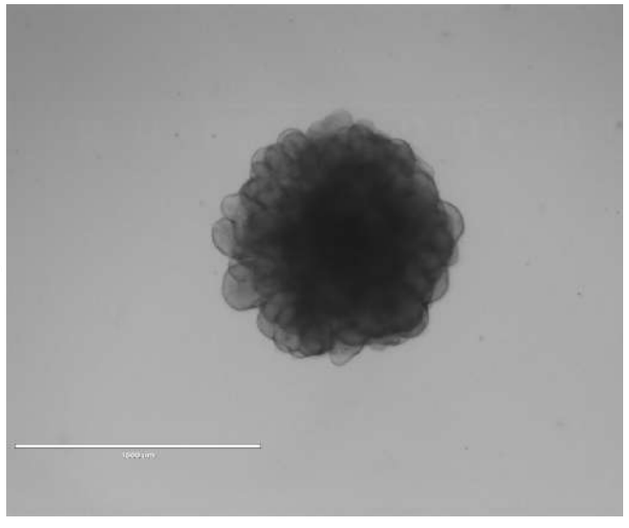
Neural ectoderm

Matrigel

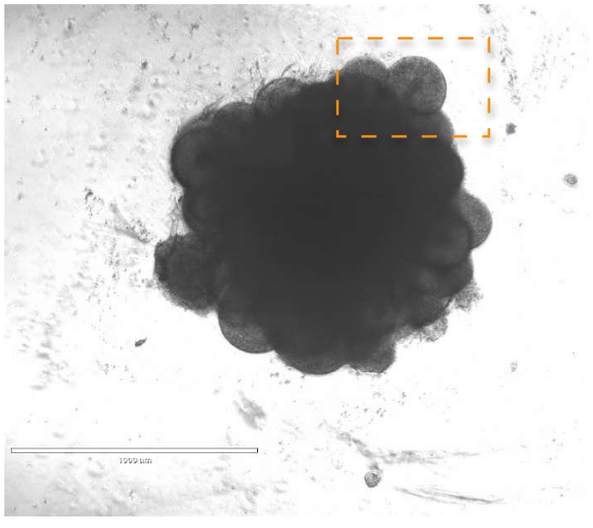
Day 7







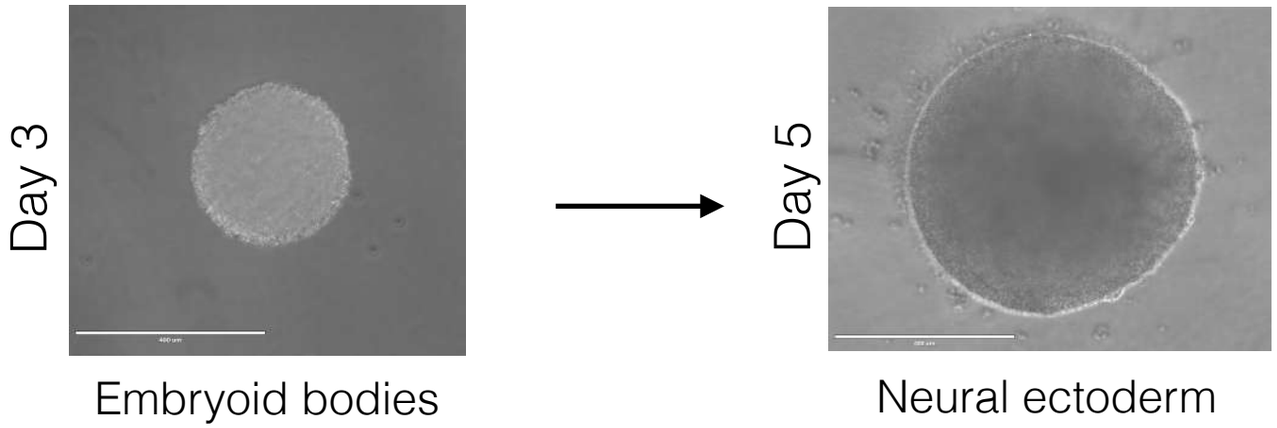
Day 10



Day 20

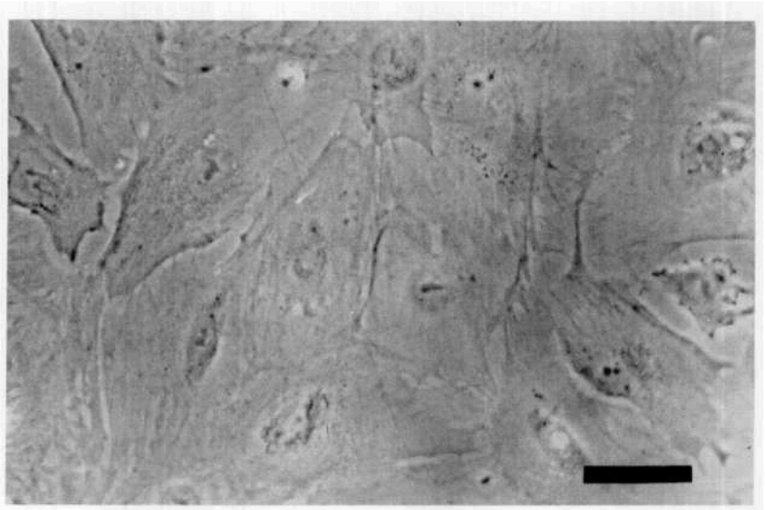
Lancaster et al. Nature, 2013

Default theory of neural induction



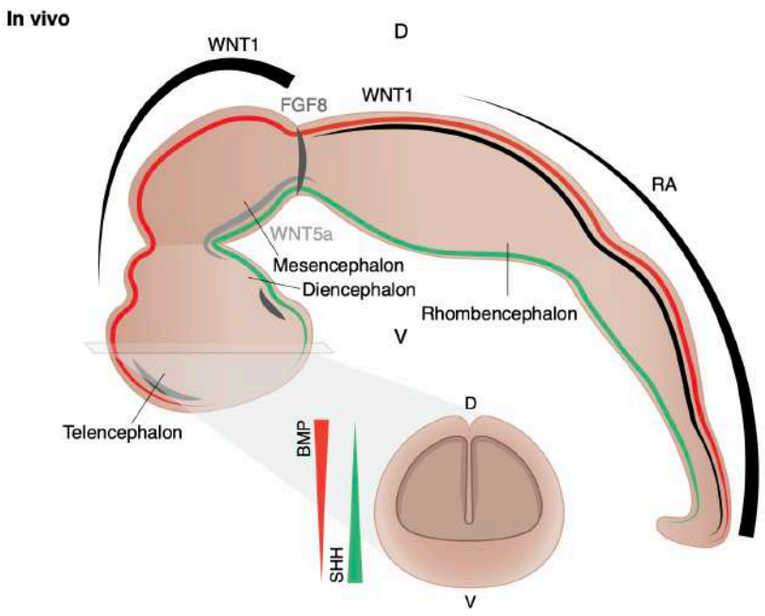


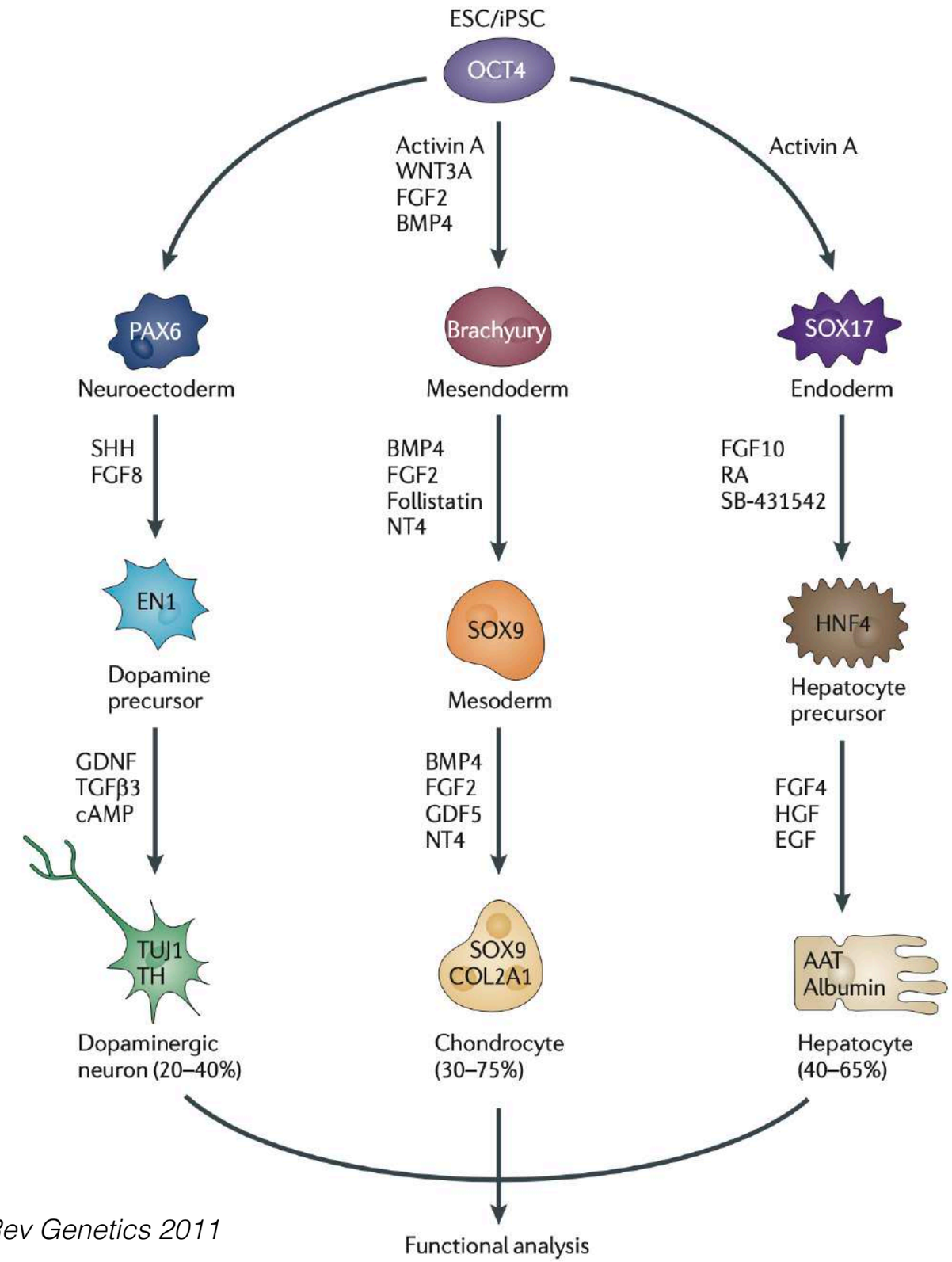
Hemmati-Brivanlou, A. & Melton, D. A. Cell 77, 273–281 (1994).



Bain et al., Dev. Biol. 168, 342–357 (1995).

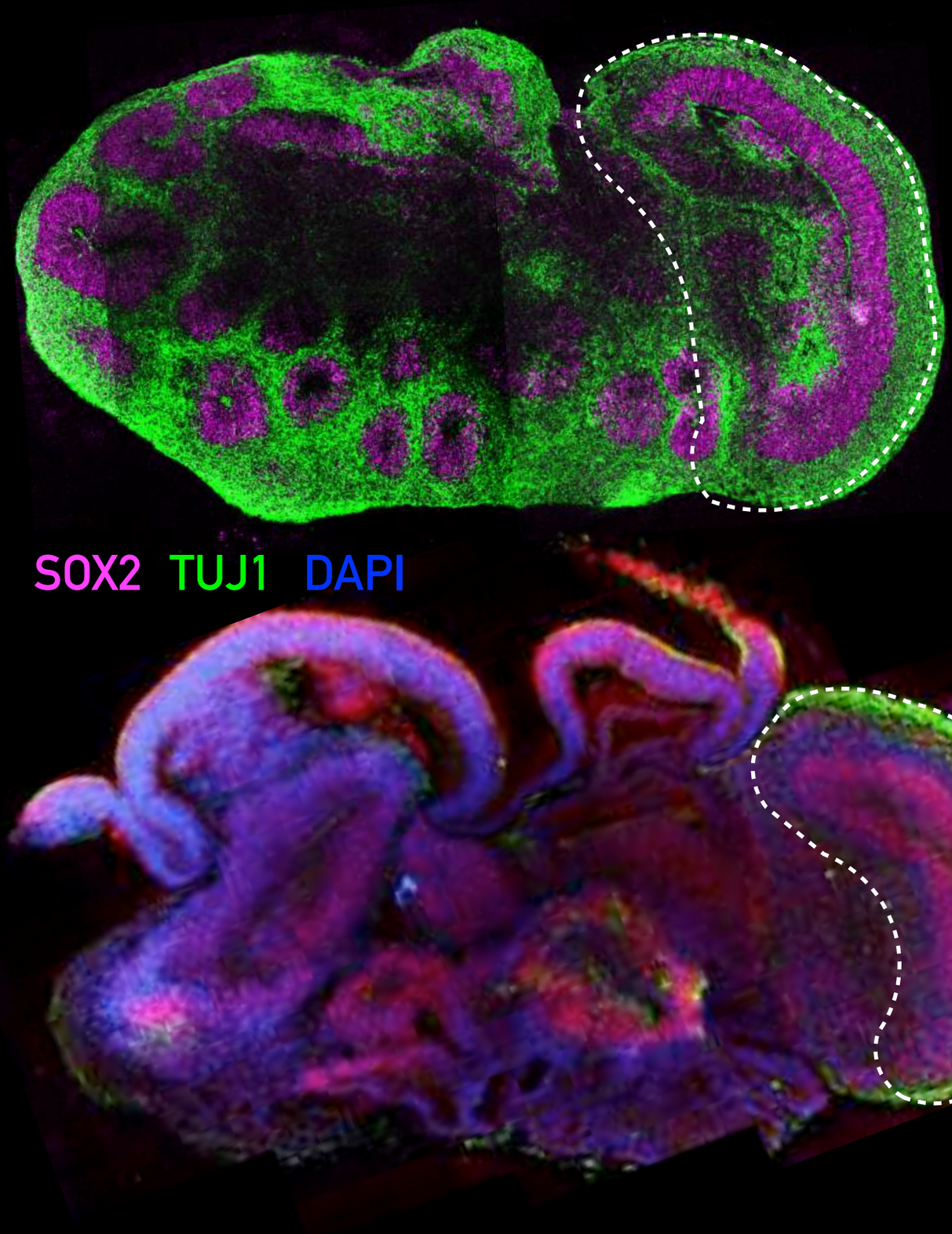
Default theory of neural induction

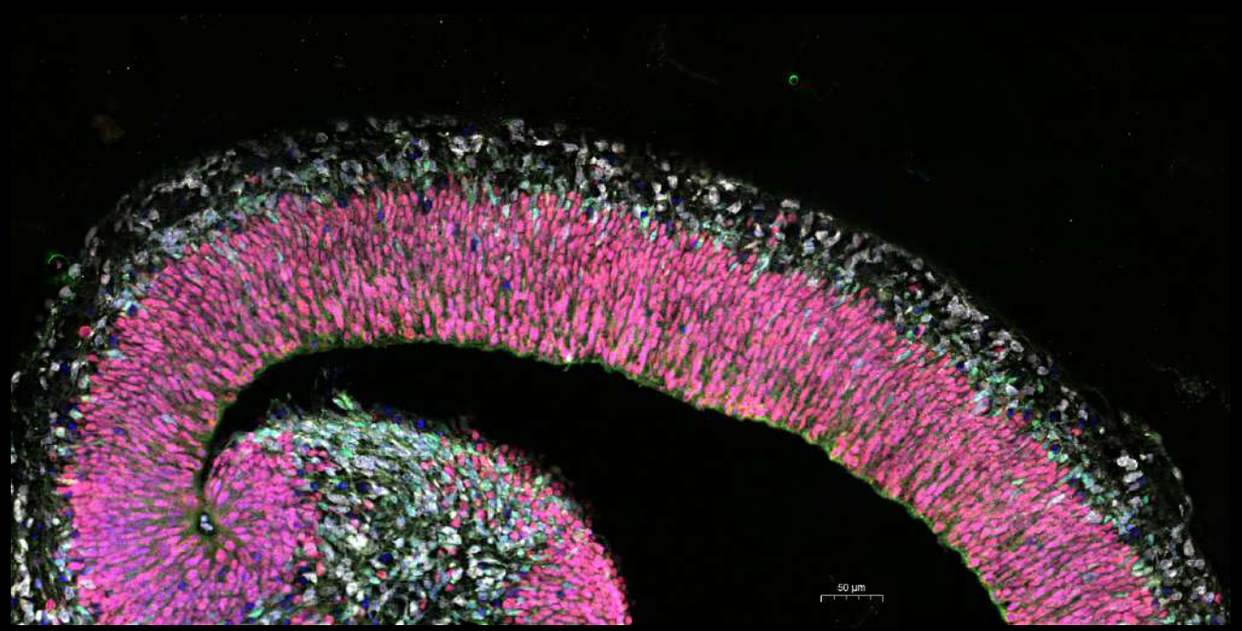


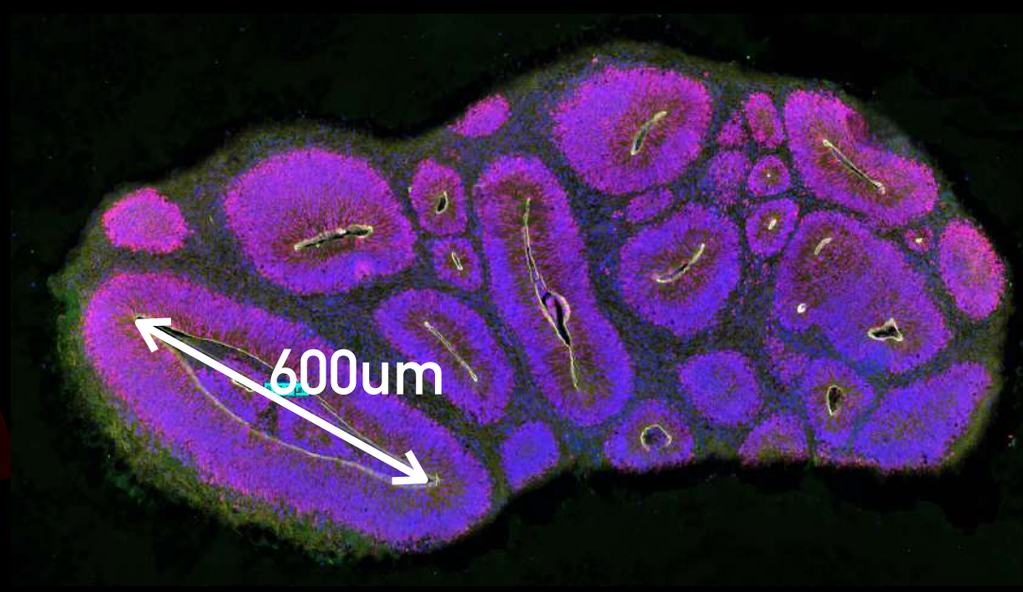


Cohen and Melton Nat Rev Genetics 2011

Organoids form long ventricles

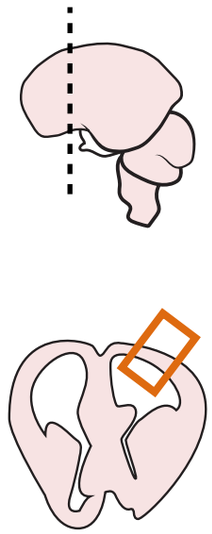




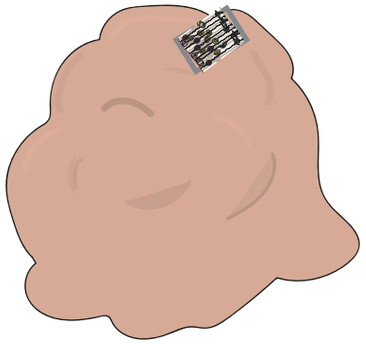


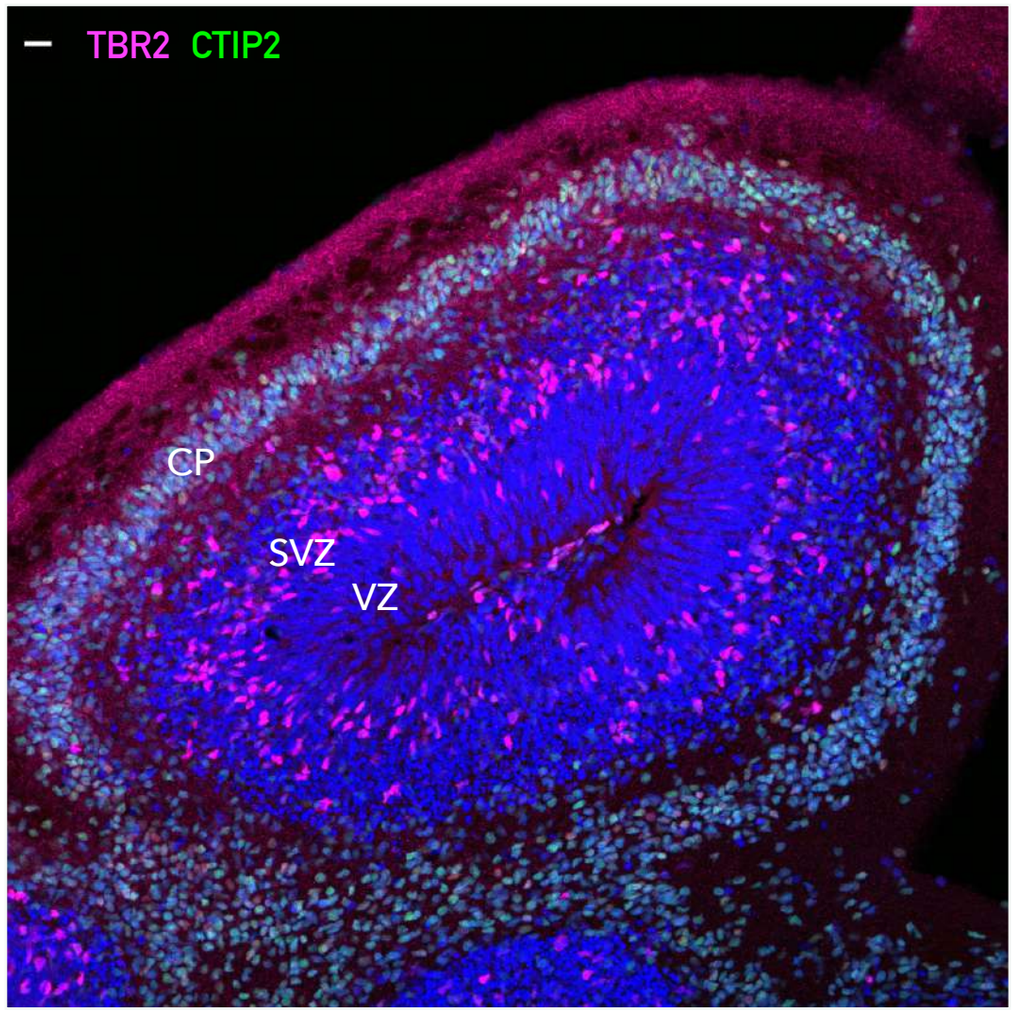
Lancaster et al. Nature, 2013.

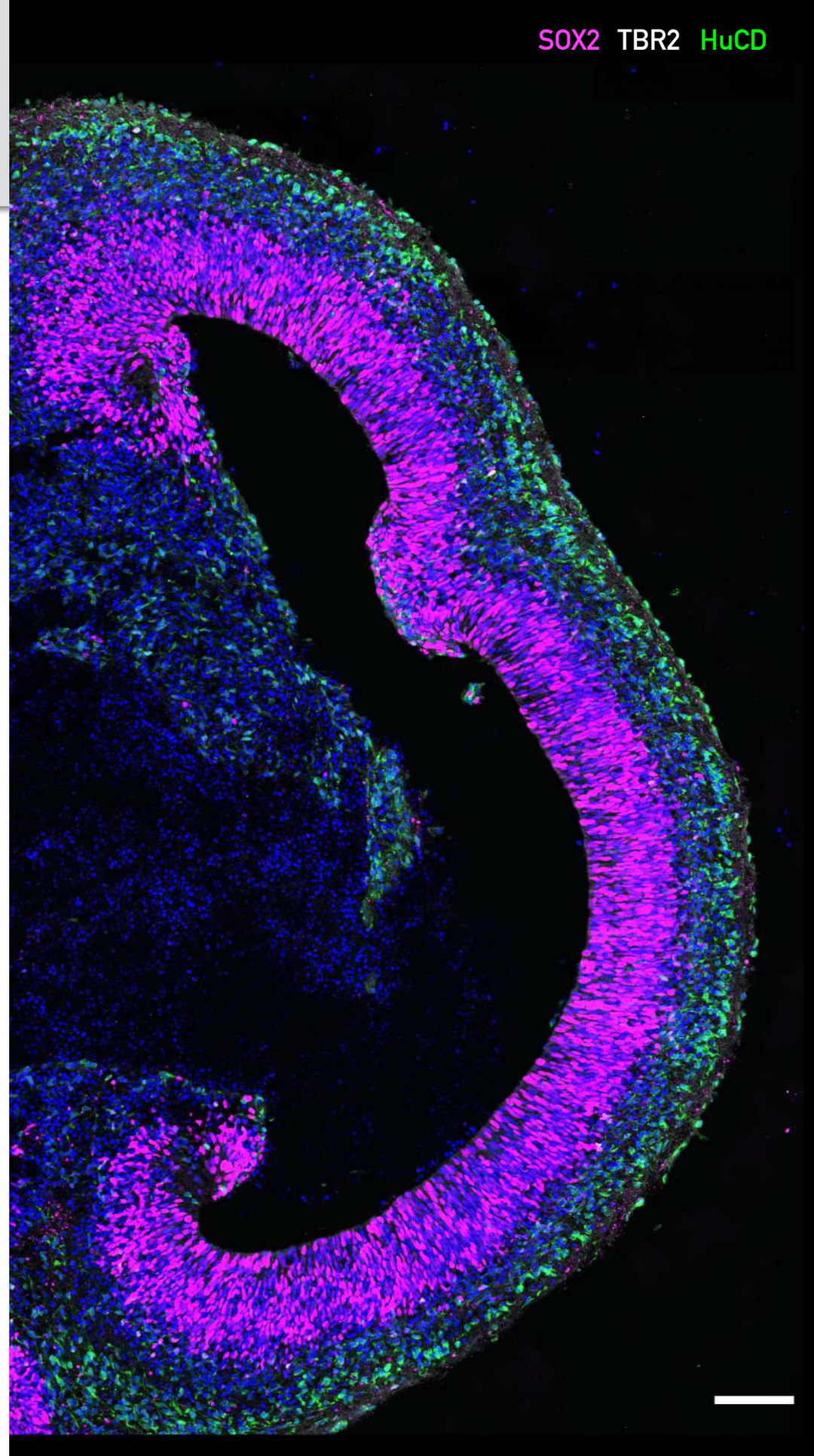
Human developing brain

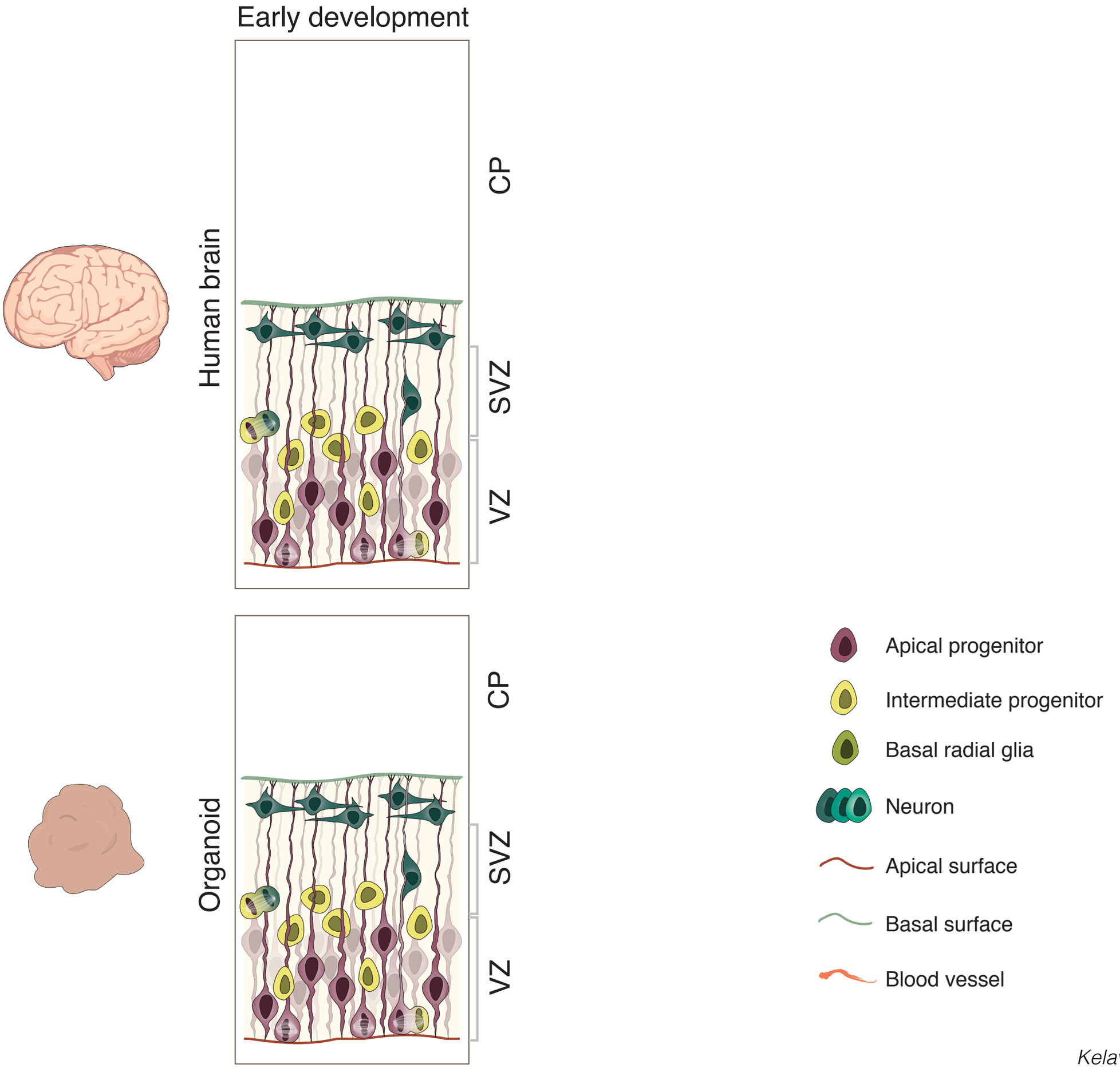


Into the tissue architecture of brain organoids





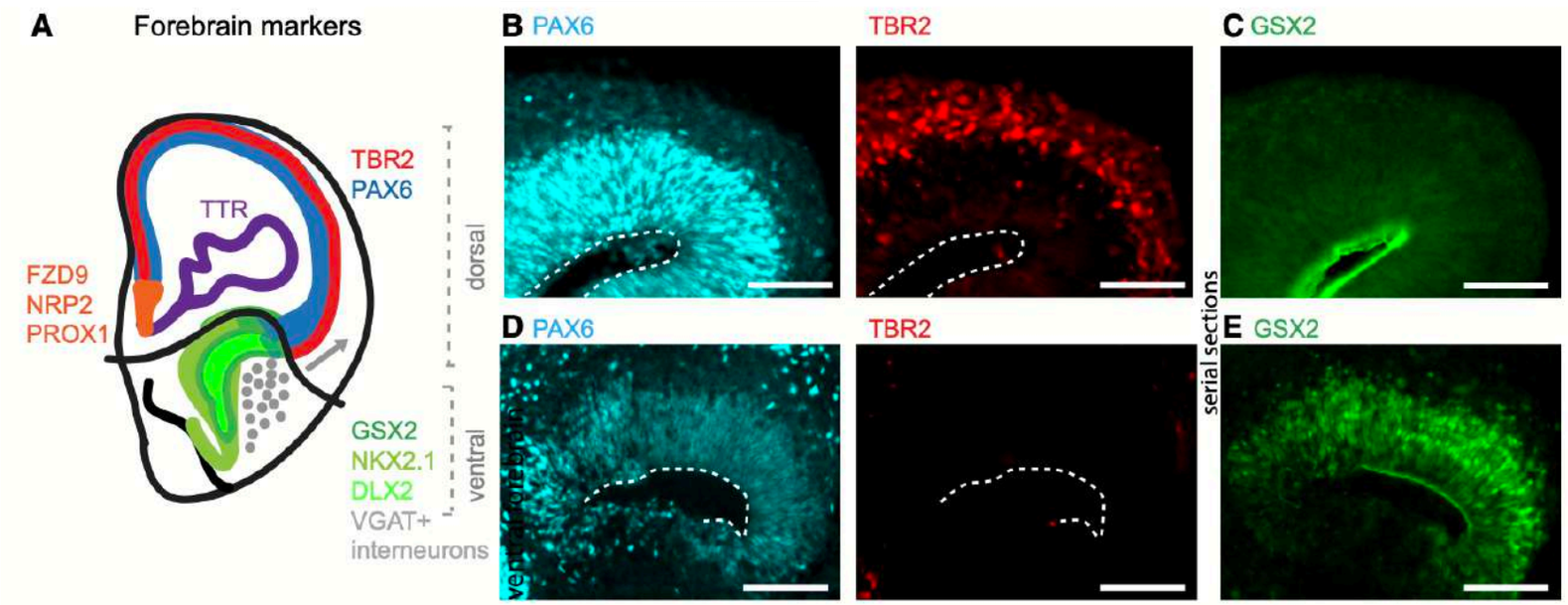


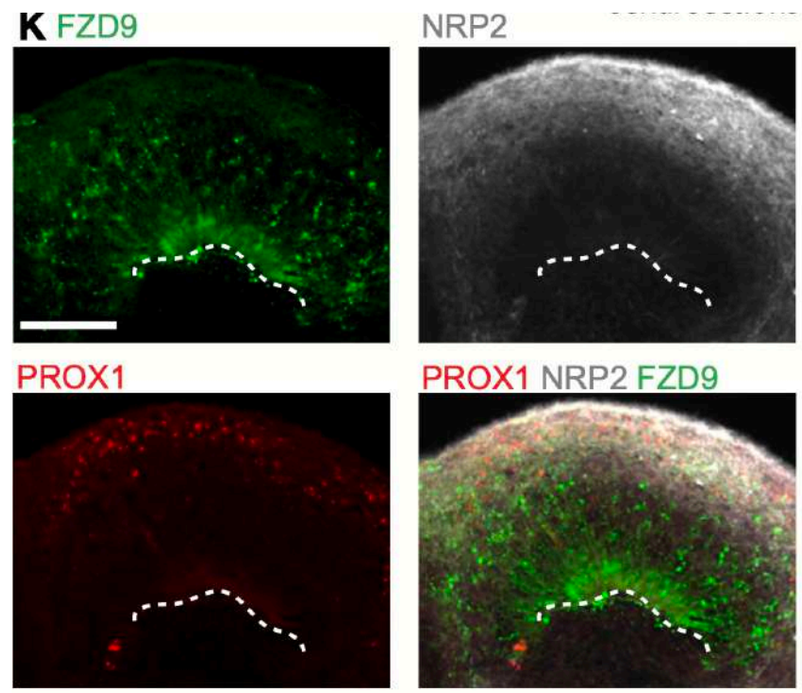


Kelava and Lancaster Dev Biol, 2016.

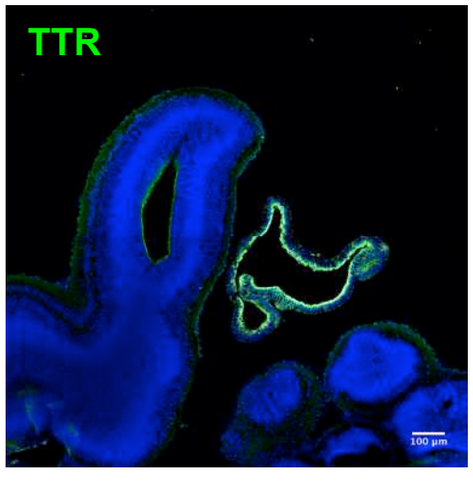
Brain organoids develop various brain regions

Dorsal forebrain

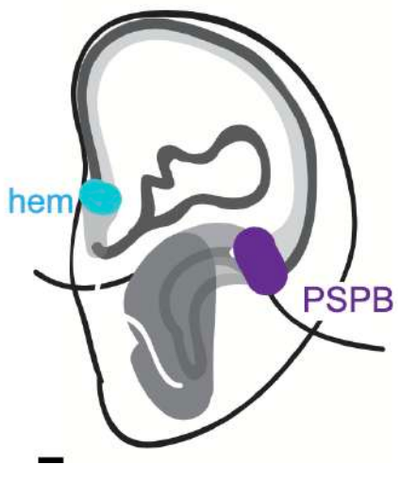




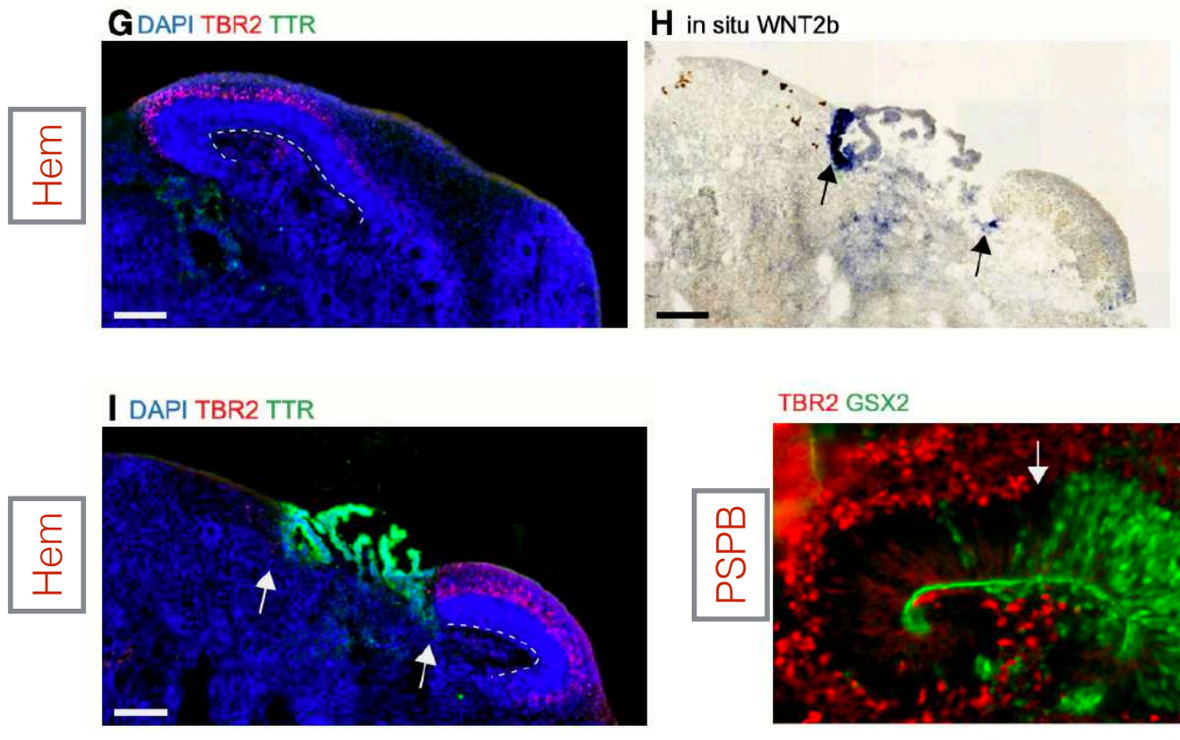
Ventral forebrain



Choroid plexus



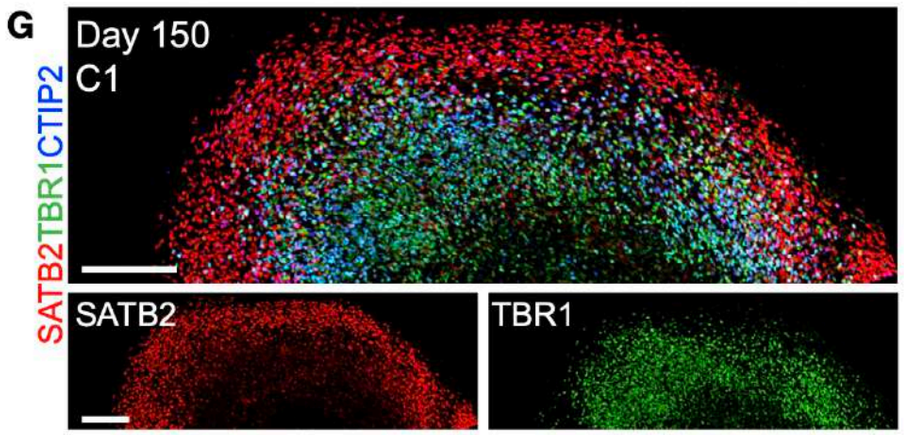
Hippocampus



Pellegrini et al., 2020

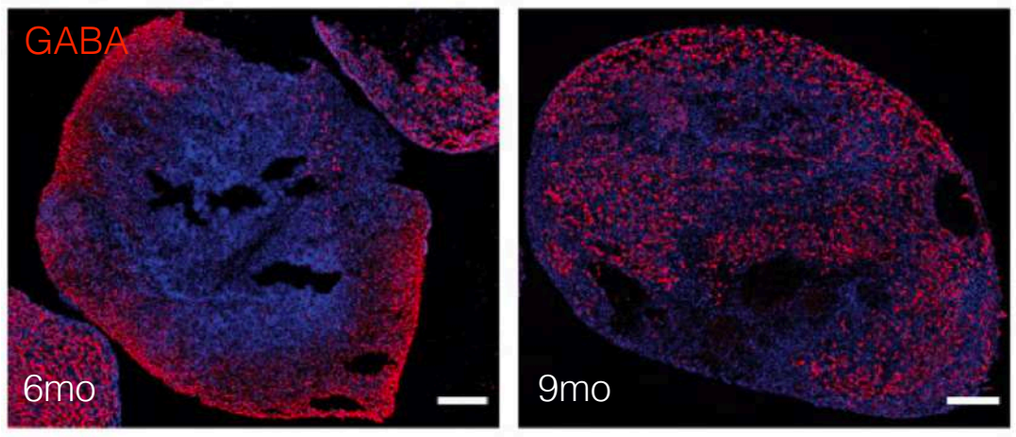
Renner et al., 2017

Brain organoids display variegate cell composition



Excitatory neurons

Qian et al., 2020

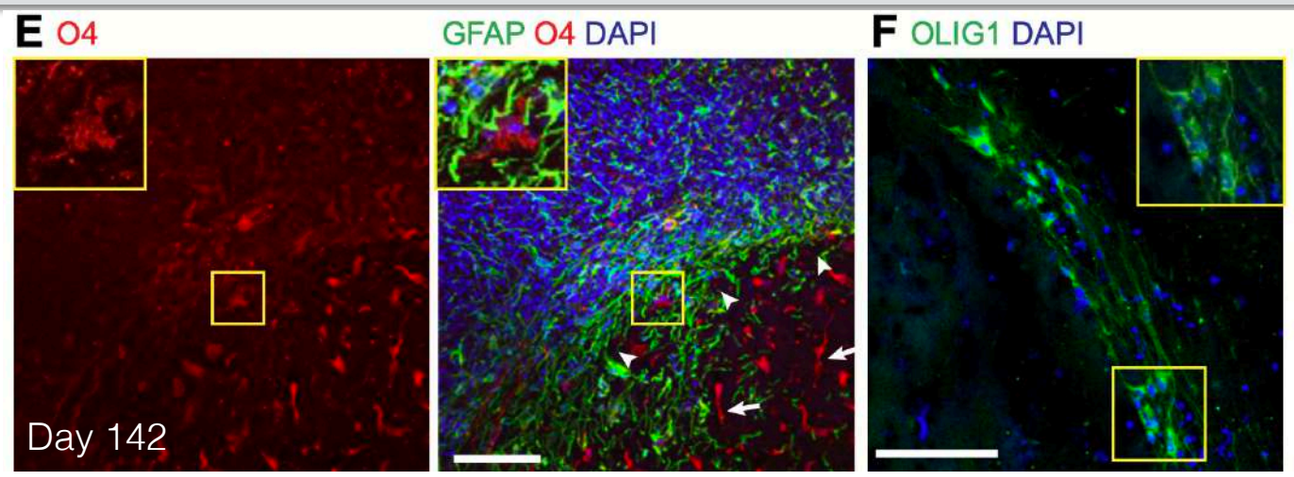


Inhibitory neurons

Quadrato et al., 2017

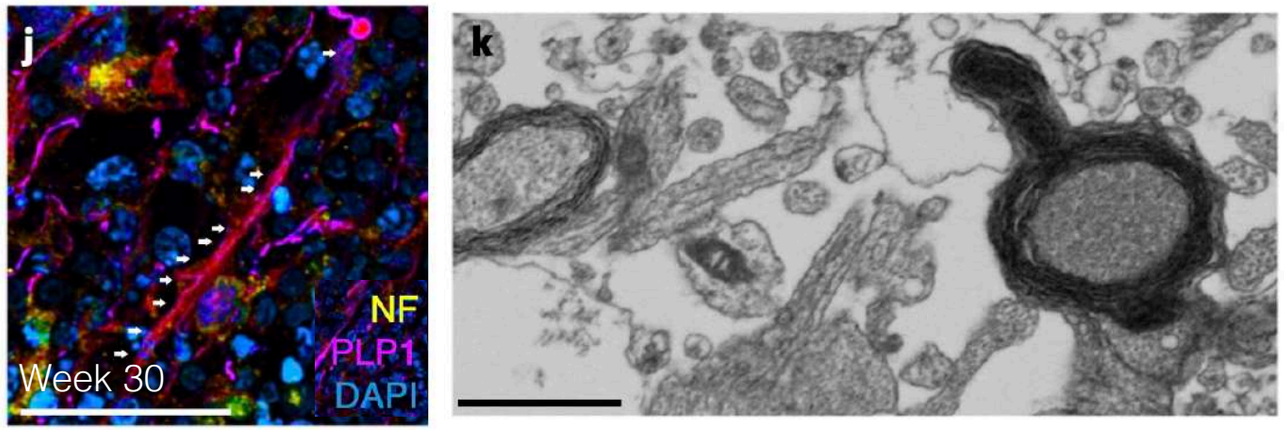
Astrocytes

Qian et al., 2020



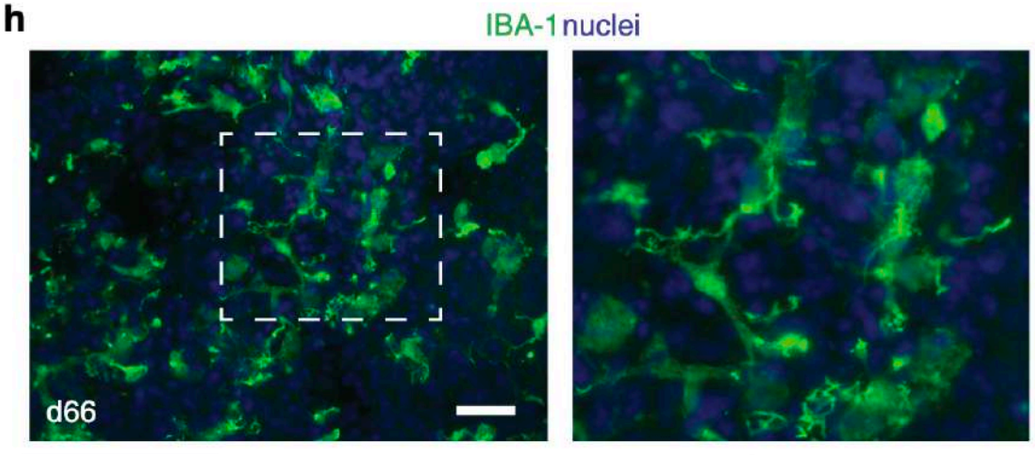
Oligodendrocytes

Renner et al., 2017



Myelin in oligocortical spheroids

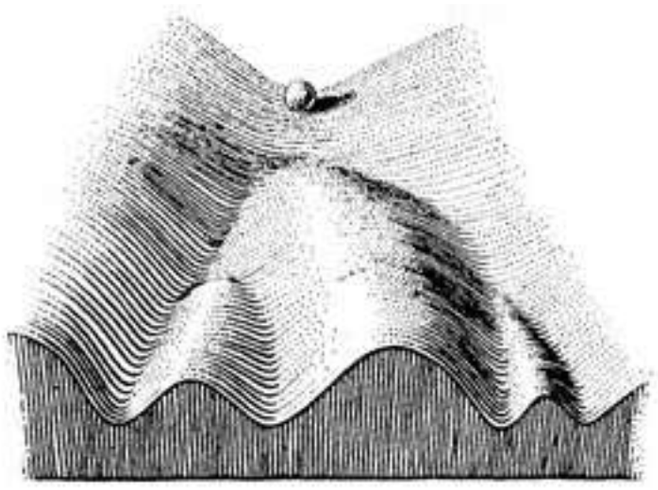
Madhavan et al., 2018



Microglia

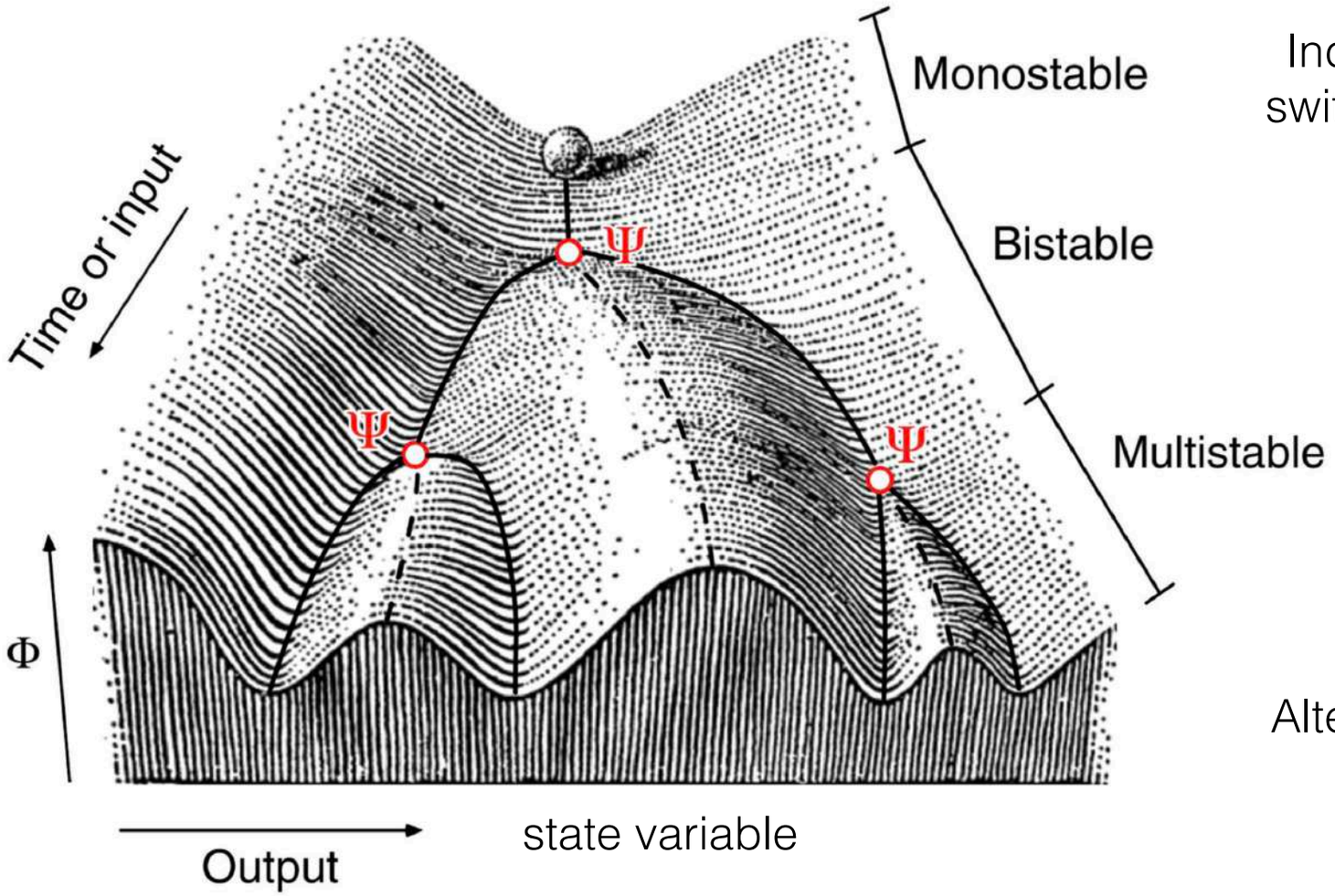
Ormel et al., 2018

Redefining the epigenetic landscape





Conrad Waddington postulated the concept of EPIGENETIC LANDSCAPE in 1956



Induction results in an all- or-none switch between qualitatively distinct cell fates

Alternative cell states as alternative steady states coexist

James E. Ferrel Jr., Curr Bio 2012

Redefining the epigenetic landscape

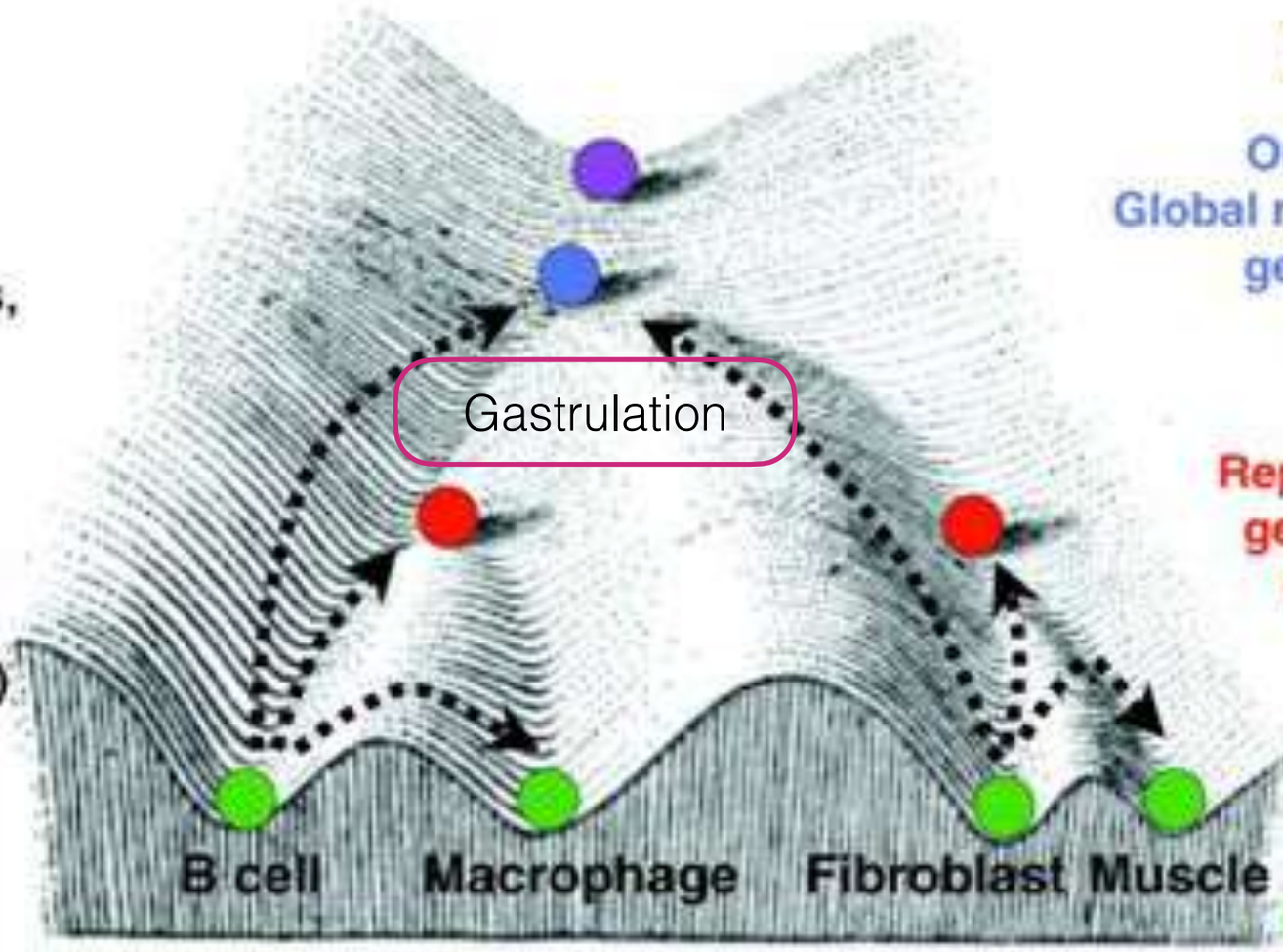
Developmental potential

Totipotent
Zygote

Pluripotent
ICM/ES cells, EG cells, EC cells, mGS cells iPS cells

Multipotent
Adult stem cells (partially reprogrammed cells?)

Unipotent
Differentiated cell types



Epigenetic status

Global DNA demethylation

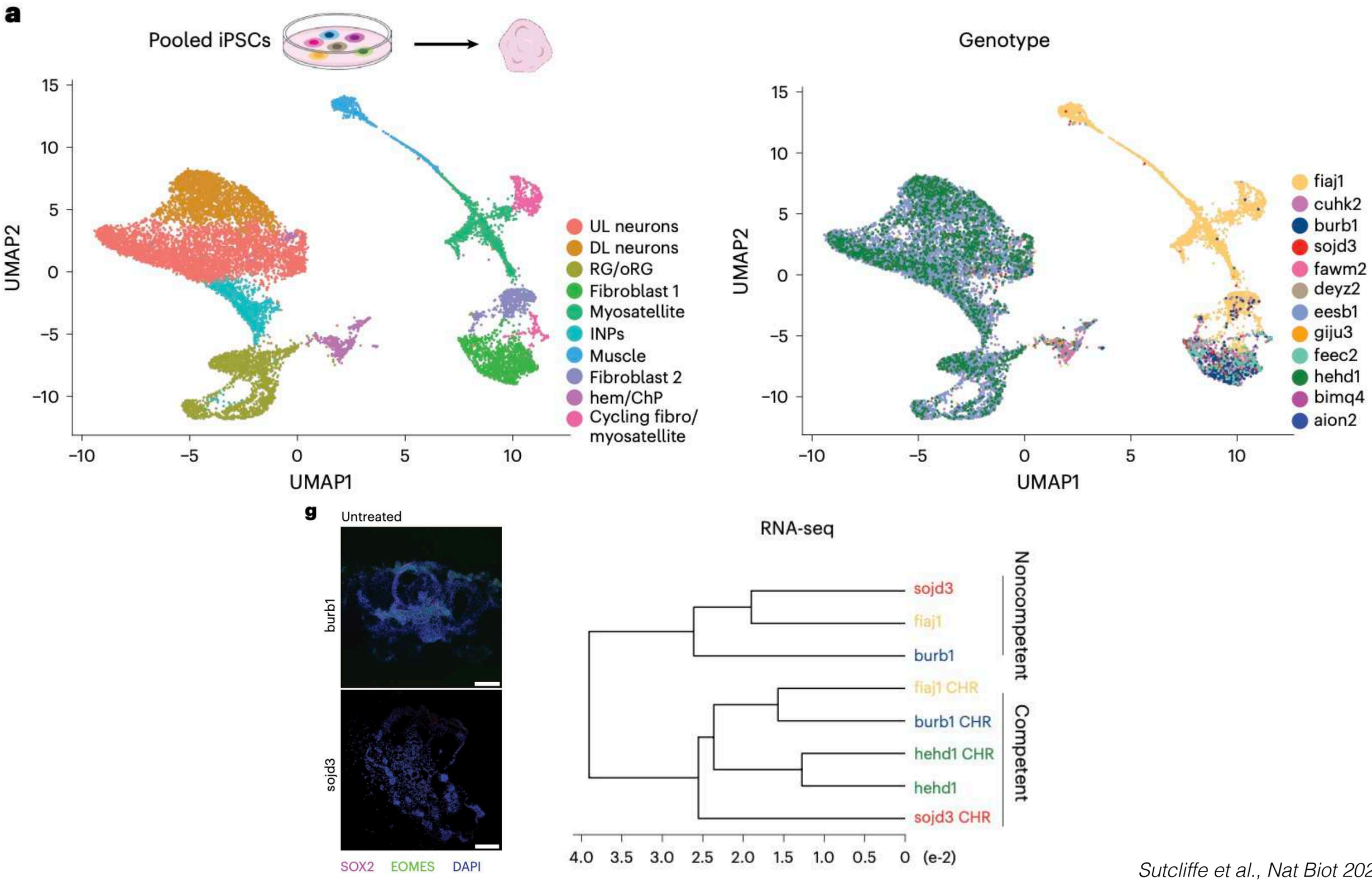
Only active X chromosomes; Global repression of differentiation genes by Polycomb proteins; Promoter hypomethylation

X inactivation; Repression of lineage-specific genes by Polycomb proteins; Promoter hypermethylation

X inactivation; Derepression of Polycomb silenced lineage genes; Promoter hypermethylation

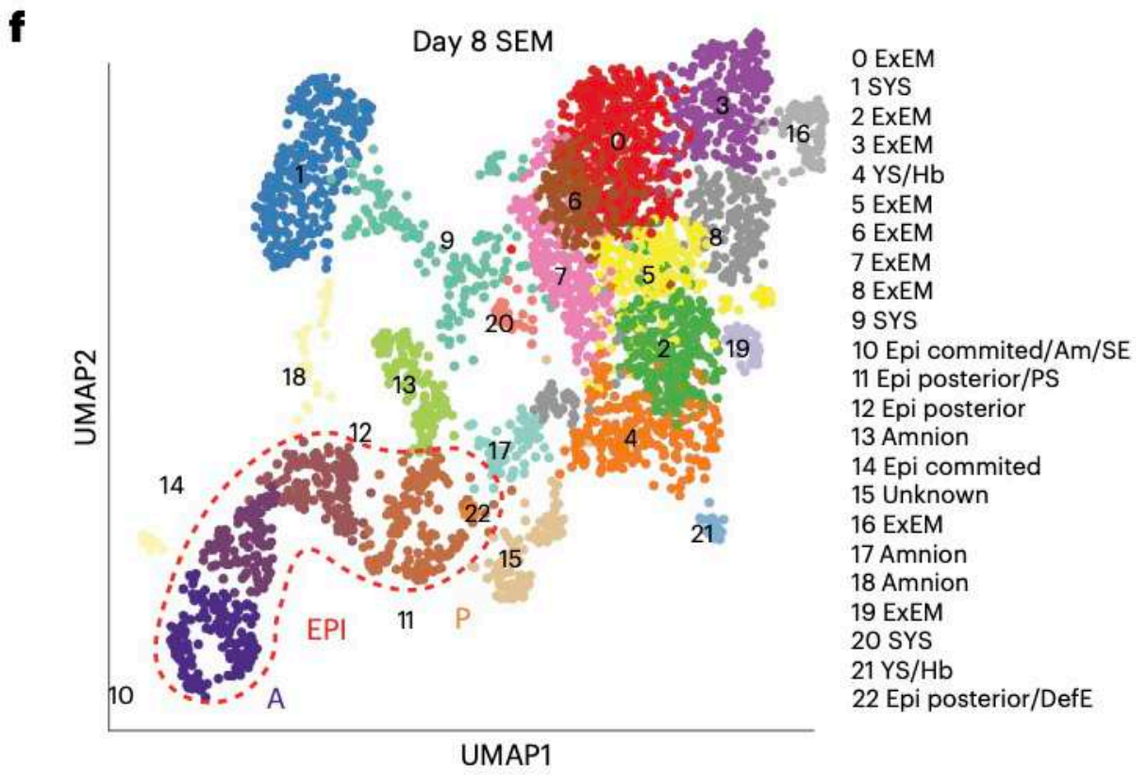
Hochedlinger and Plath, Development 2009

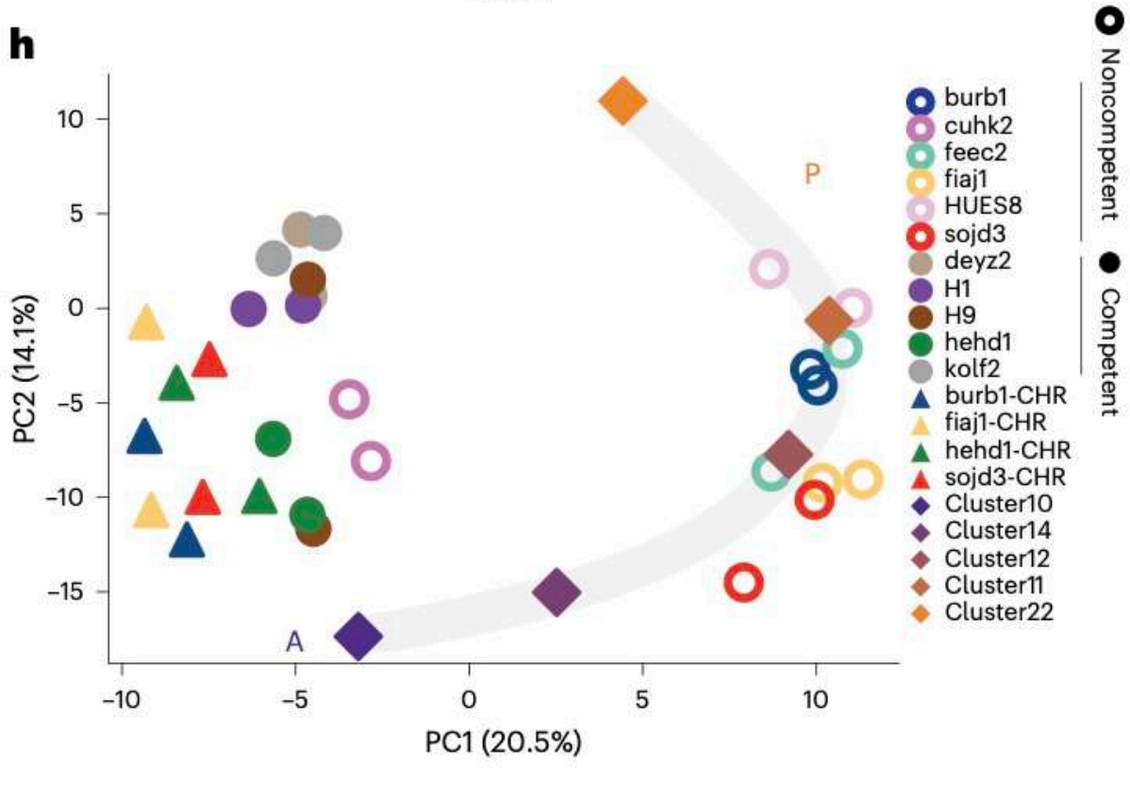
Redefining the epigenetic landscape

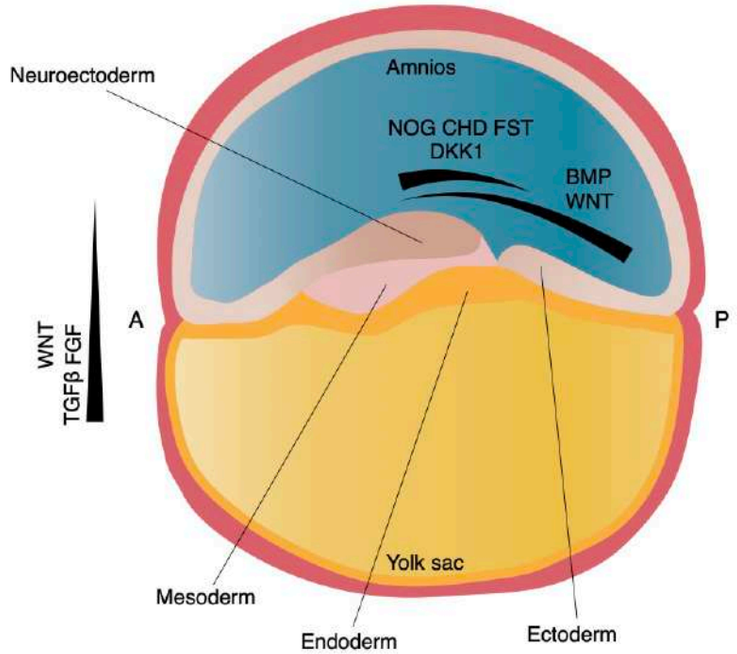


Sutcliffe et al., Nat Biot 2026

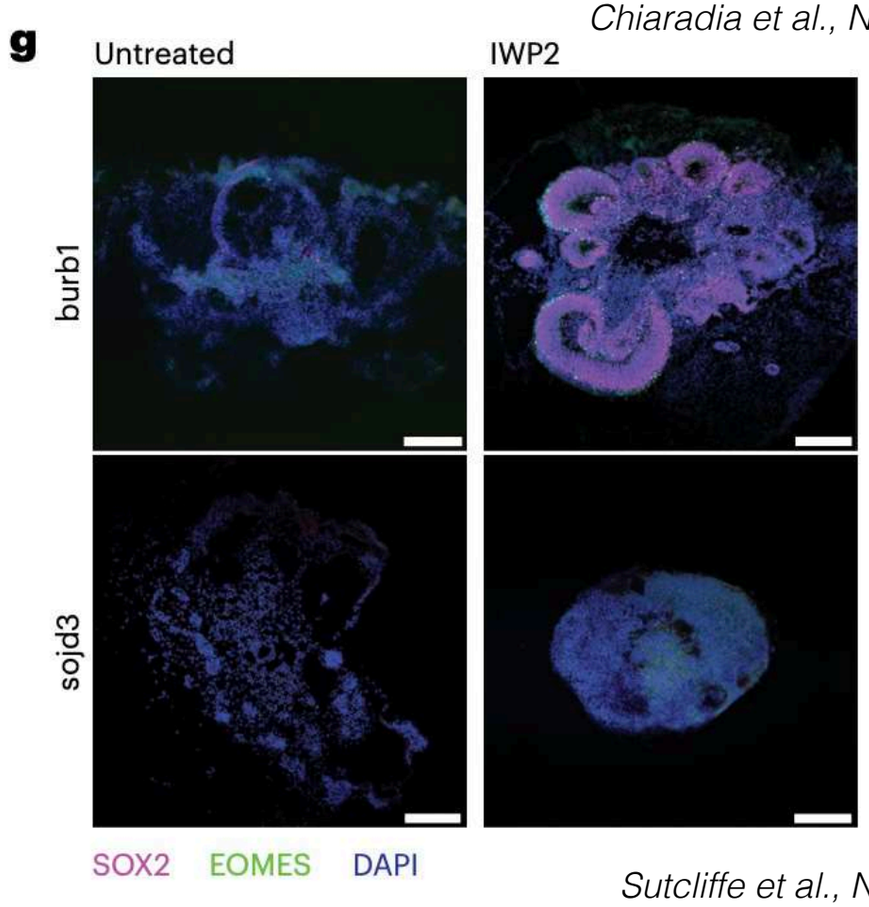
Redefining the epigenetic landscape







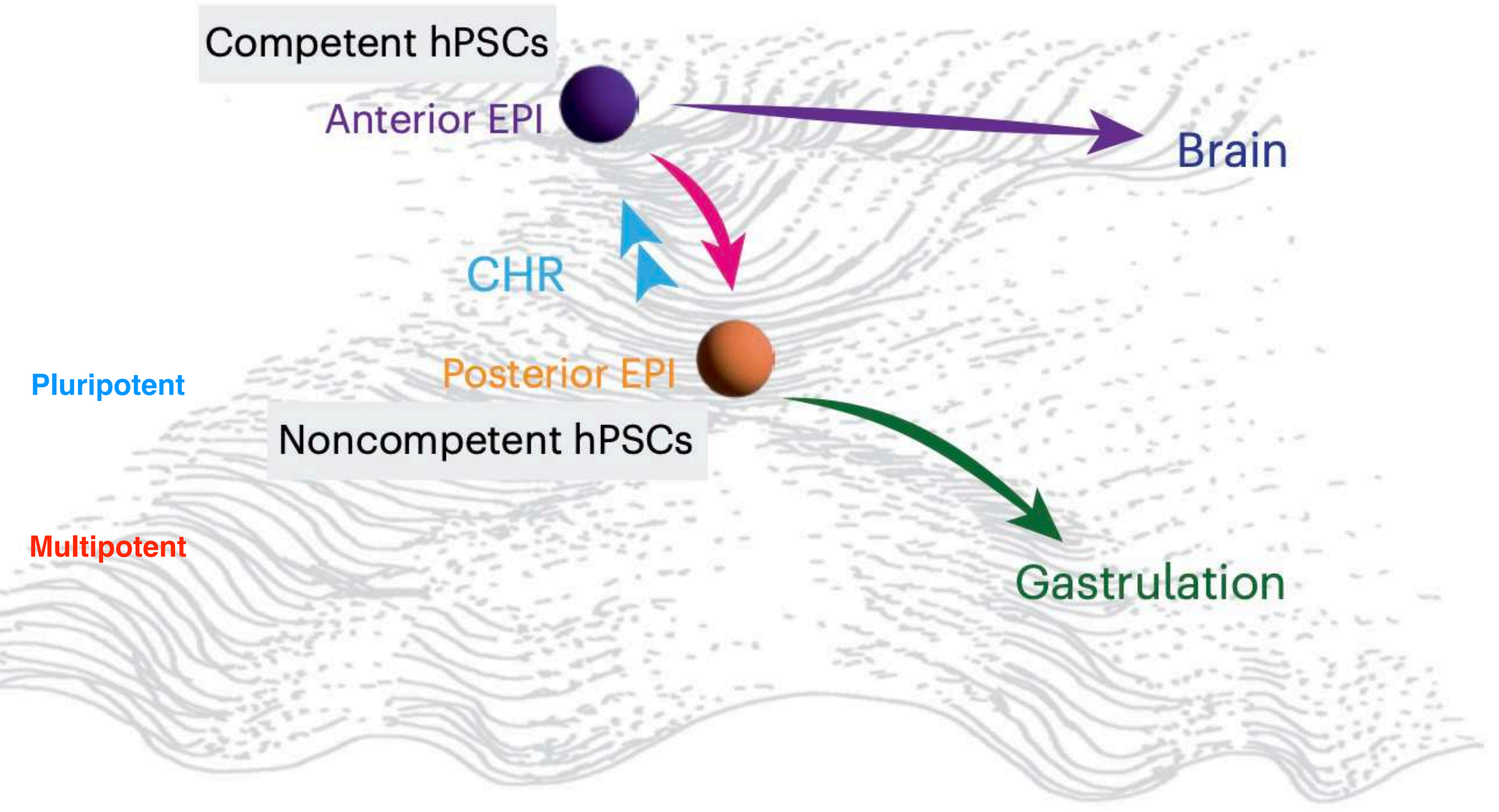
Chiaradia et al., Nat Neu 2020



Sutcliffe et al., Nat Biot 2026

Rewriting the epigenetic landscape

Totipotent

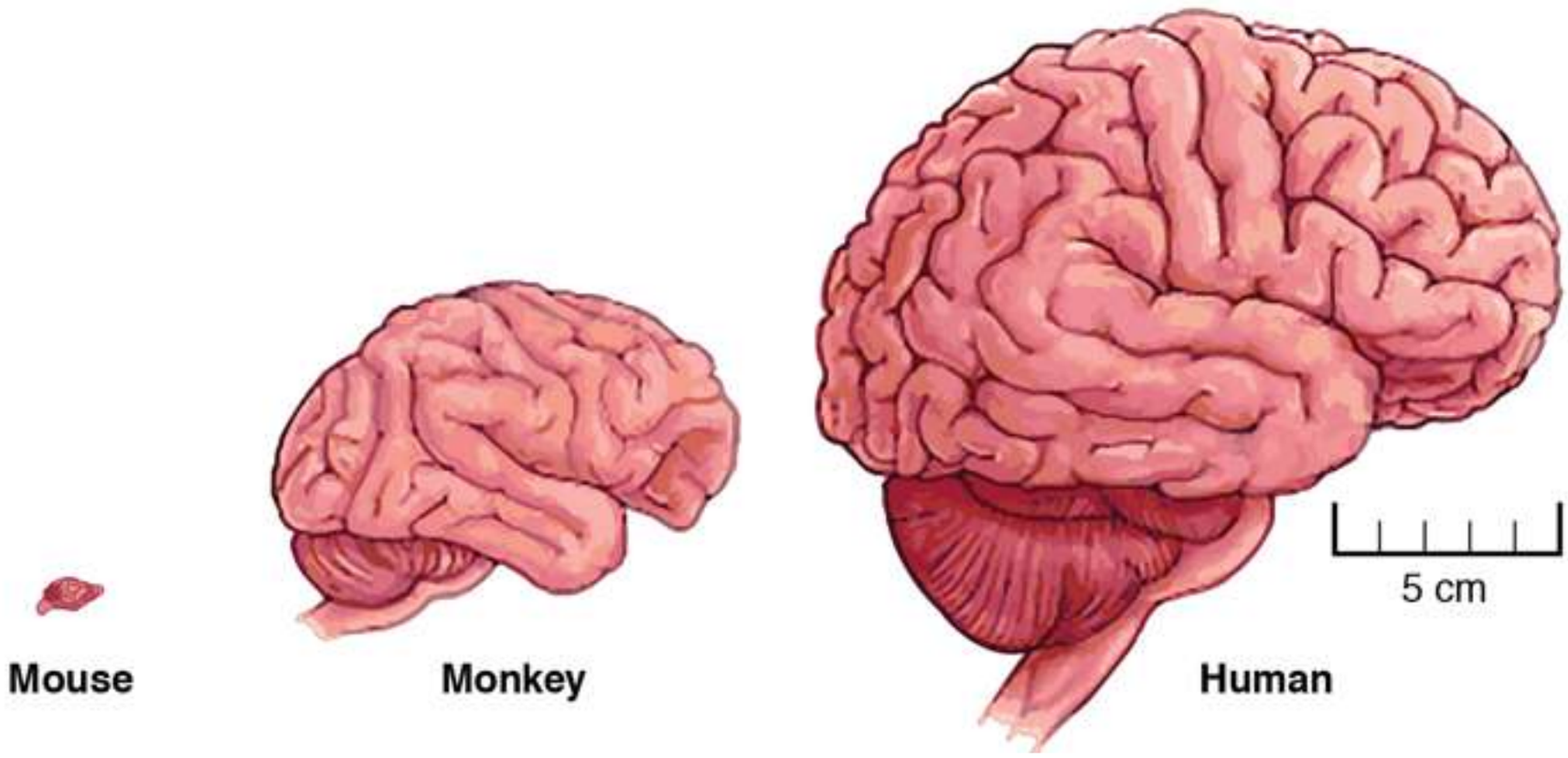


Pluripotent

Multipotent

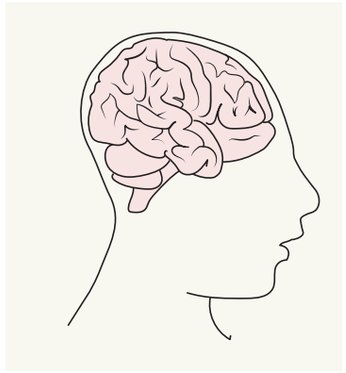
Sutcliffe et al., Nat Biot 2026

Why do we need organoids?

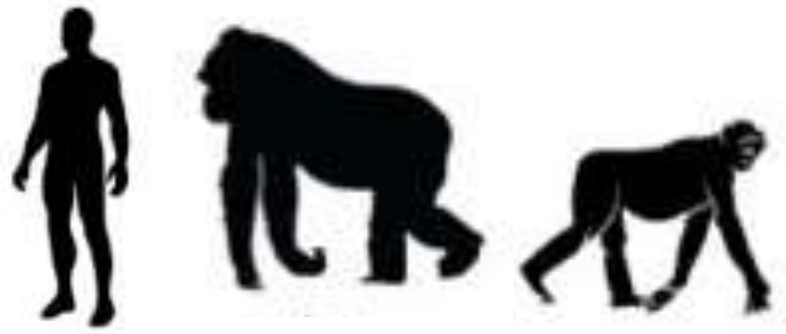


- Complex and enlarged compared to our closest relatives
- Very limited access to human brain tissue
- Complex genetic architecture of human brain disorders

Why do we need organoids?

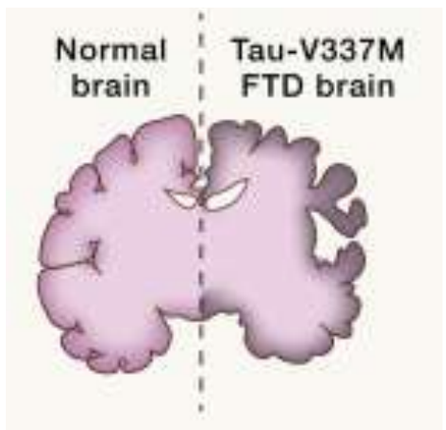


Neurodevelopmental disorders:
Microcephaly, lissencephaly, ASDs



Brain evolution

?



Neurodegenerative disease

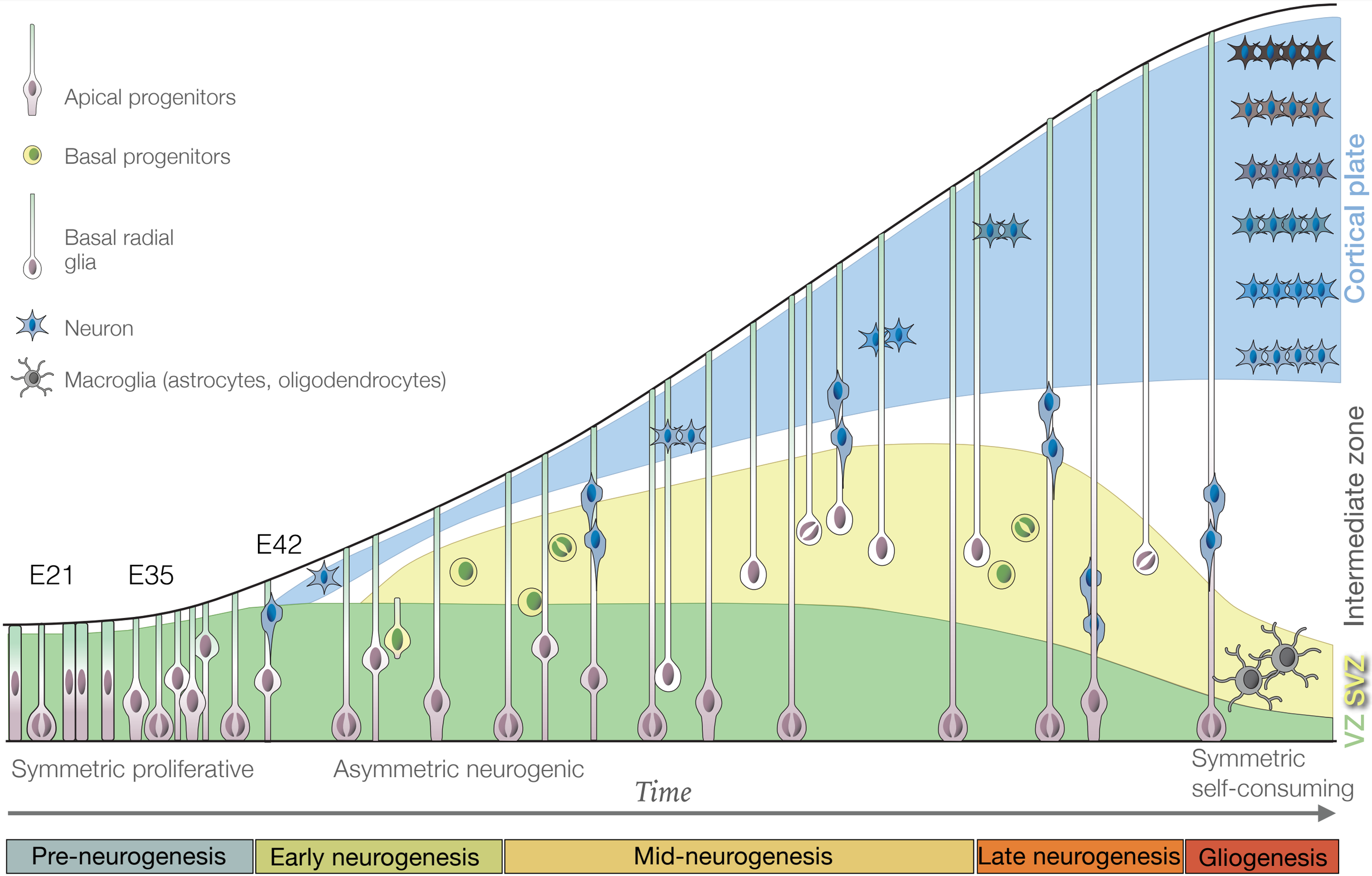


Personalised medicine

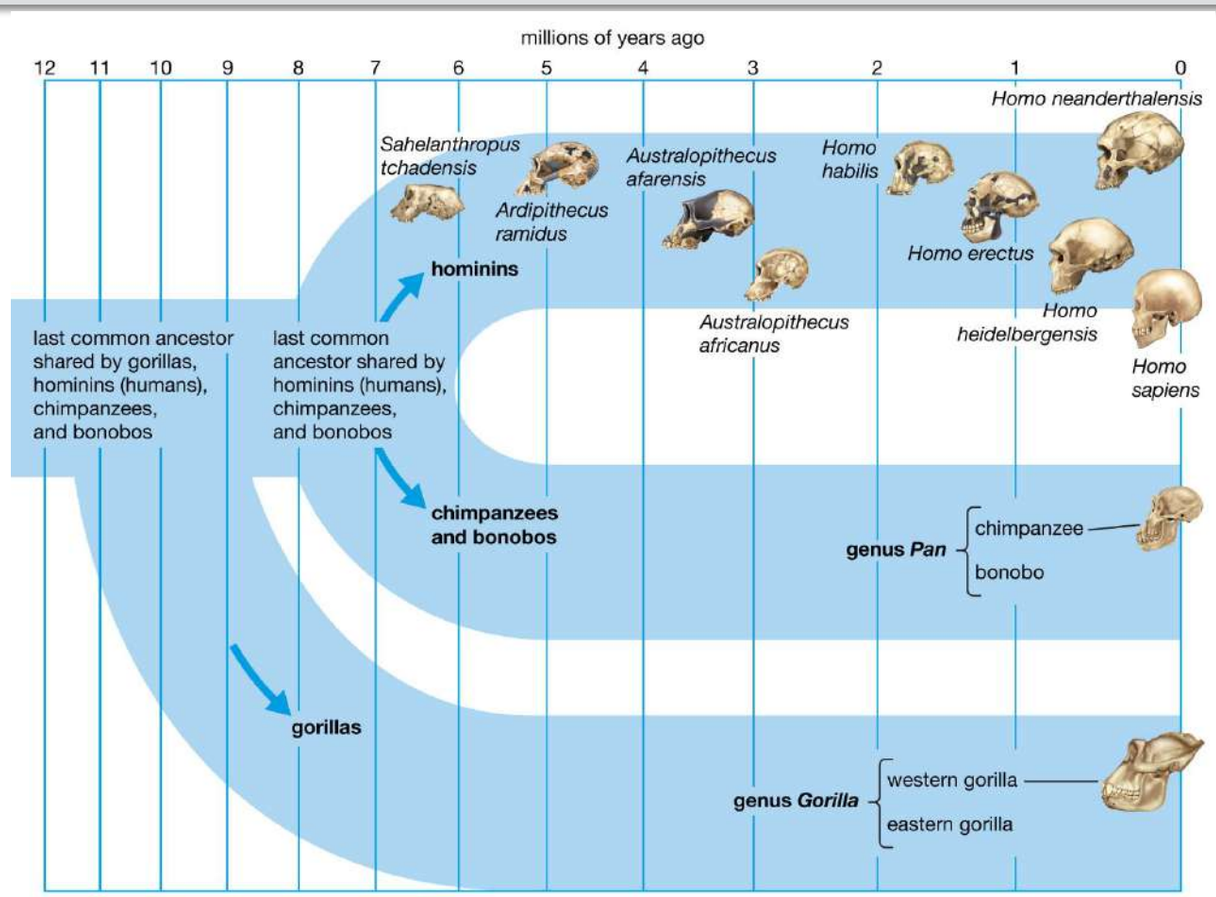


Diagnostics

Redefining the cell types in the developing neuroepithelium



Evolving larger brains



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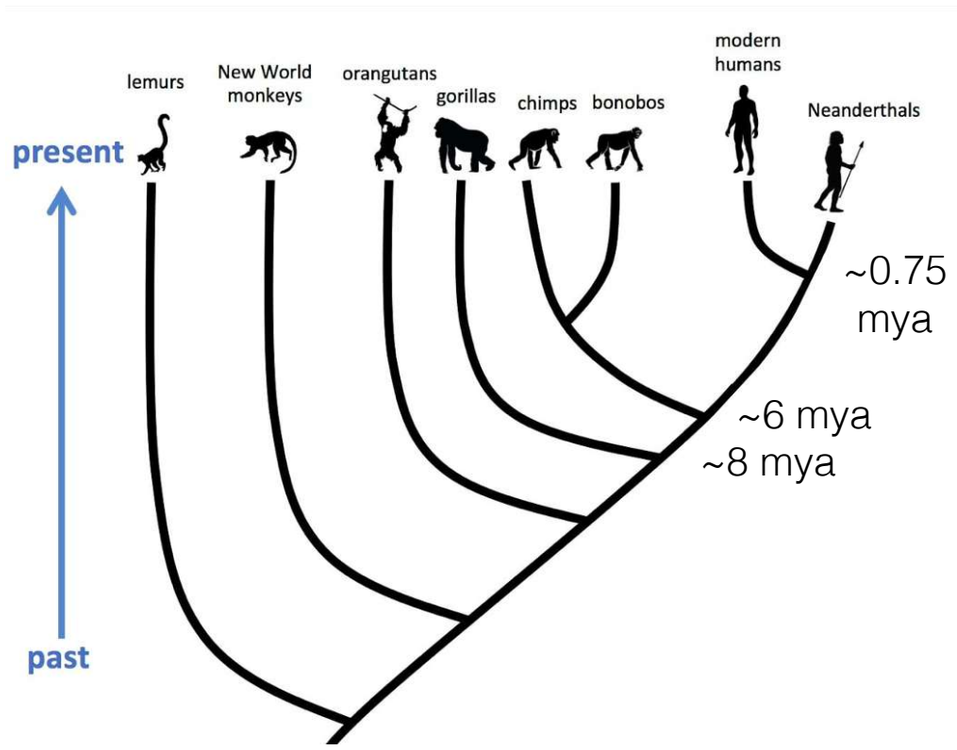
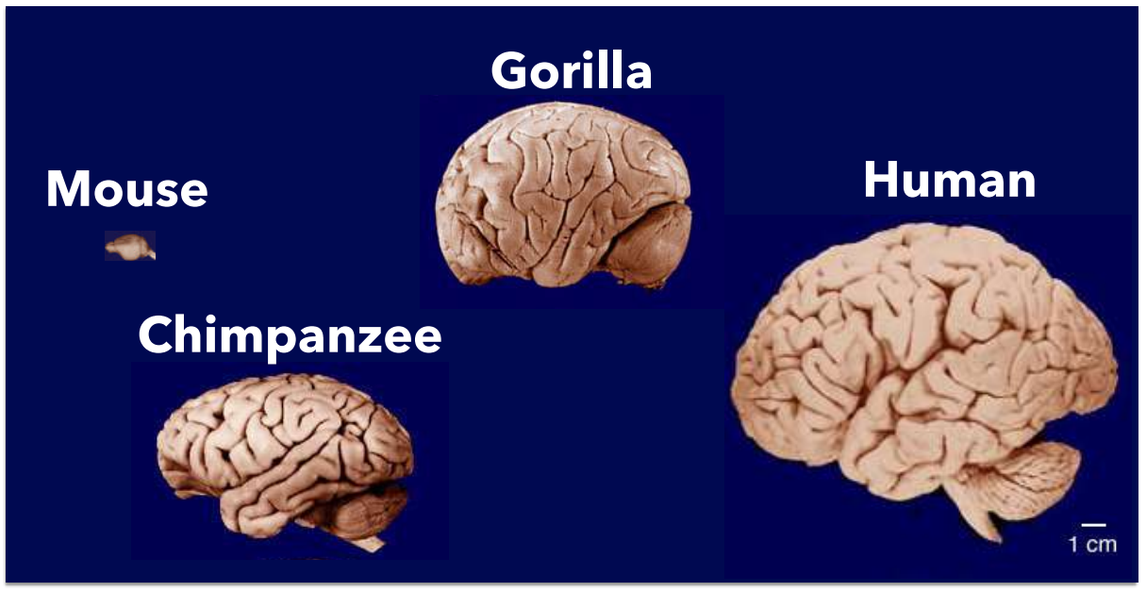


1,000-fold more neurons than mouse

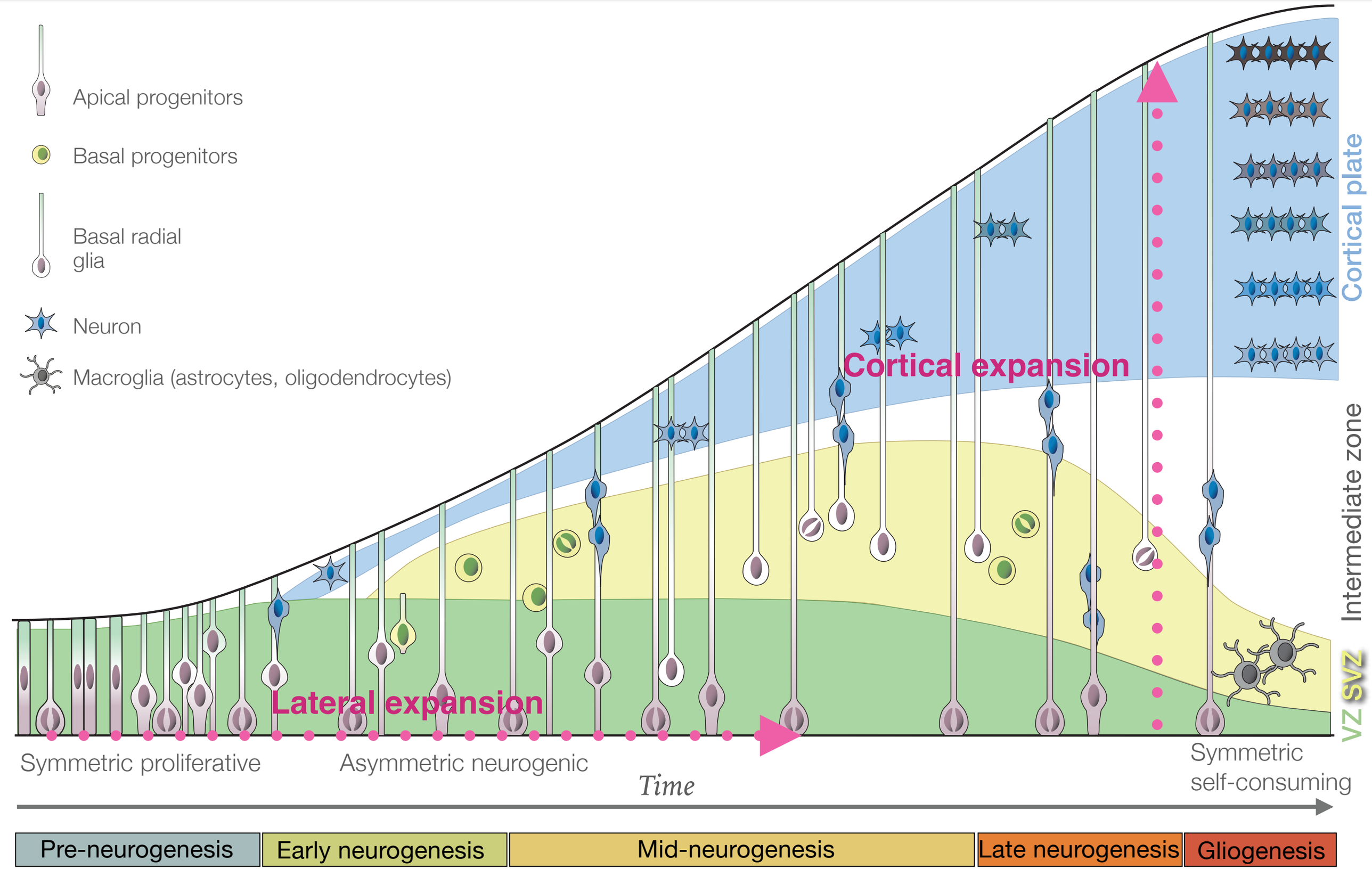
3-fold more neurons than chimpanzee and gorilla



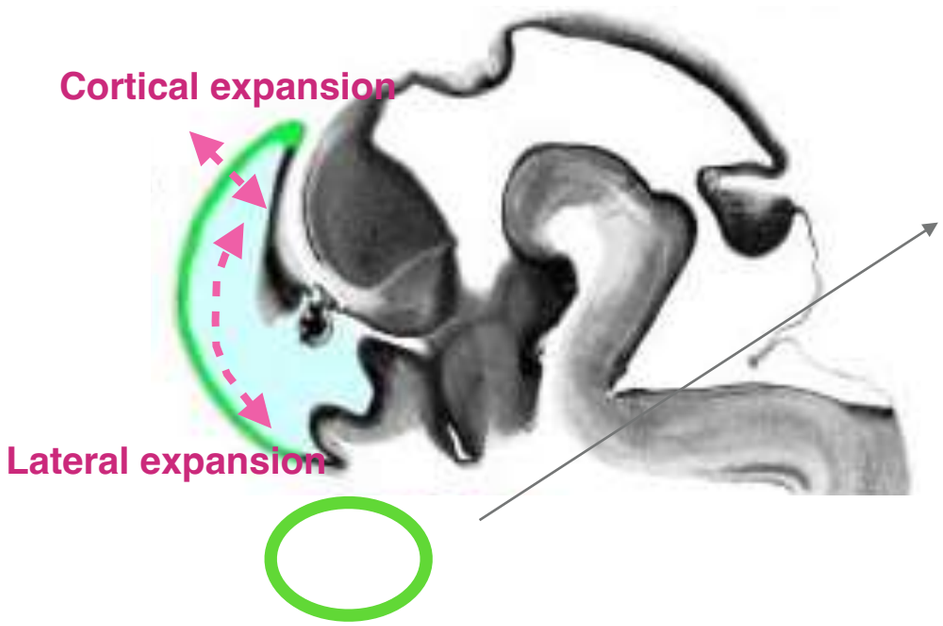
Comparative Brain Atlas (http://www.brainmuseum.org)

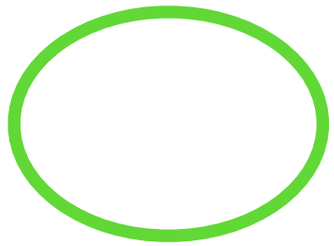
	Mouse	Chimpanzee	Gorilla	Human
Neuron number (billions)	0.071	28	33.4	86
EQ	0.5	2.63	1.75	6.56
Gestation period (days)	20	243	257	280

Evolving larger brains



Evolving larger brains



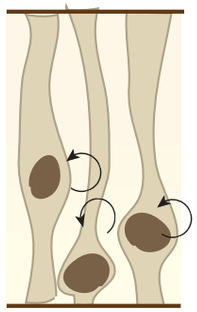


Increase cortical **surface area**



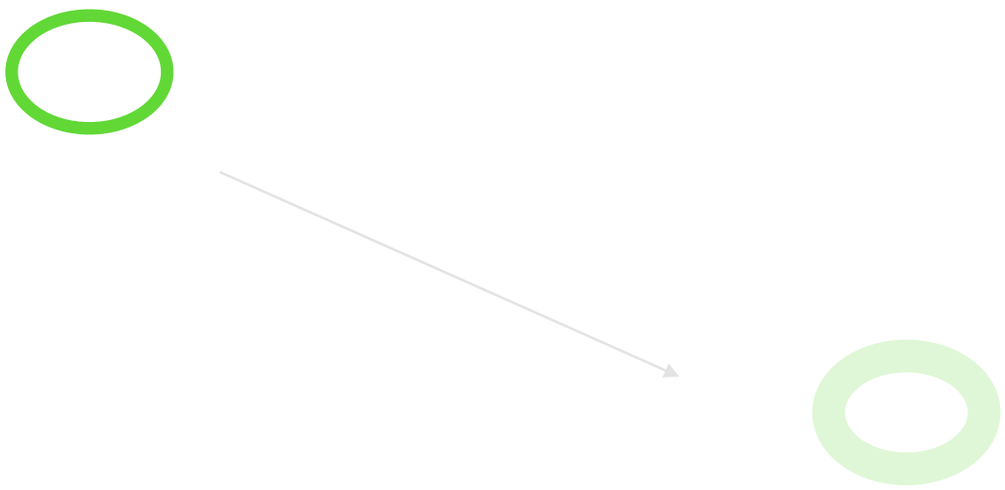
Increase cortical **thickness**

Evolving larger brains



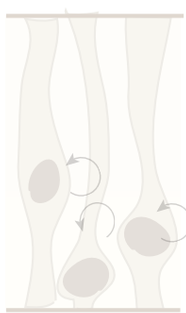
NECs

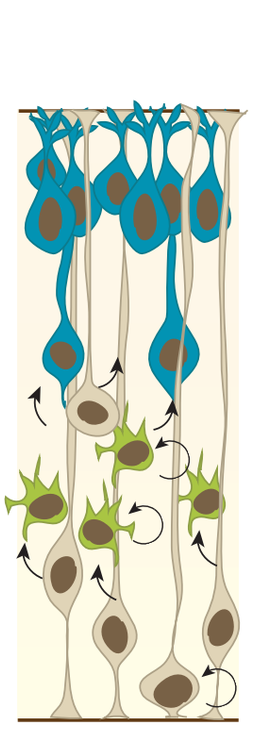
Expansion of neural progenitor cells prior to neurogenesis



Increase cortical **surface area**

Evolving larger brains



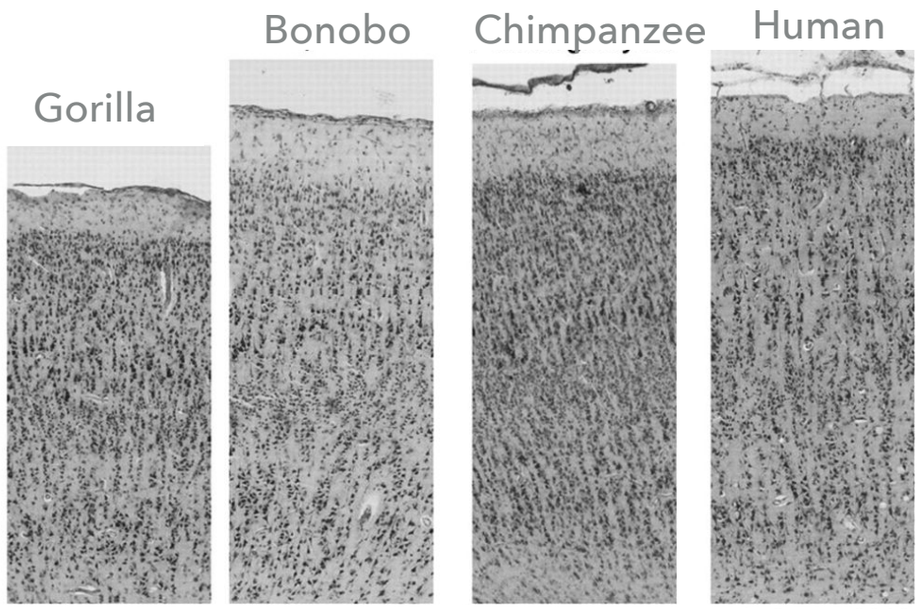


RGCs

Increase progenitor output during neurogenesis

Increase cortical **thickness**

Evolving larger brains



de Sousa, 2010

	Mean cortical thickness	Mean cortical surface area
Human	2.7 ± 0.1 mm	1,843 ± 196 cm2
Chimpanzee	2.6 ± 0.1 mm	599 ± 53 cm2

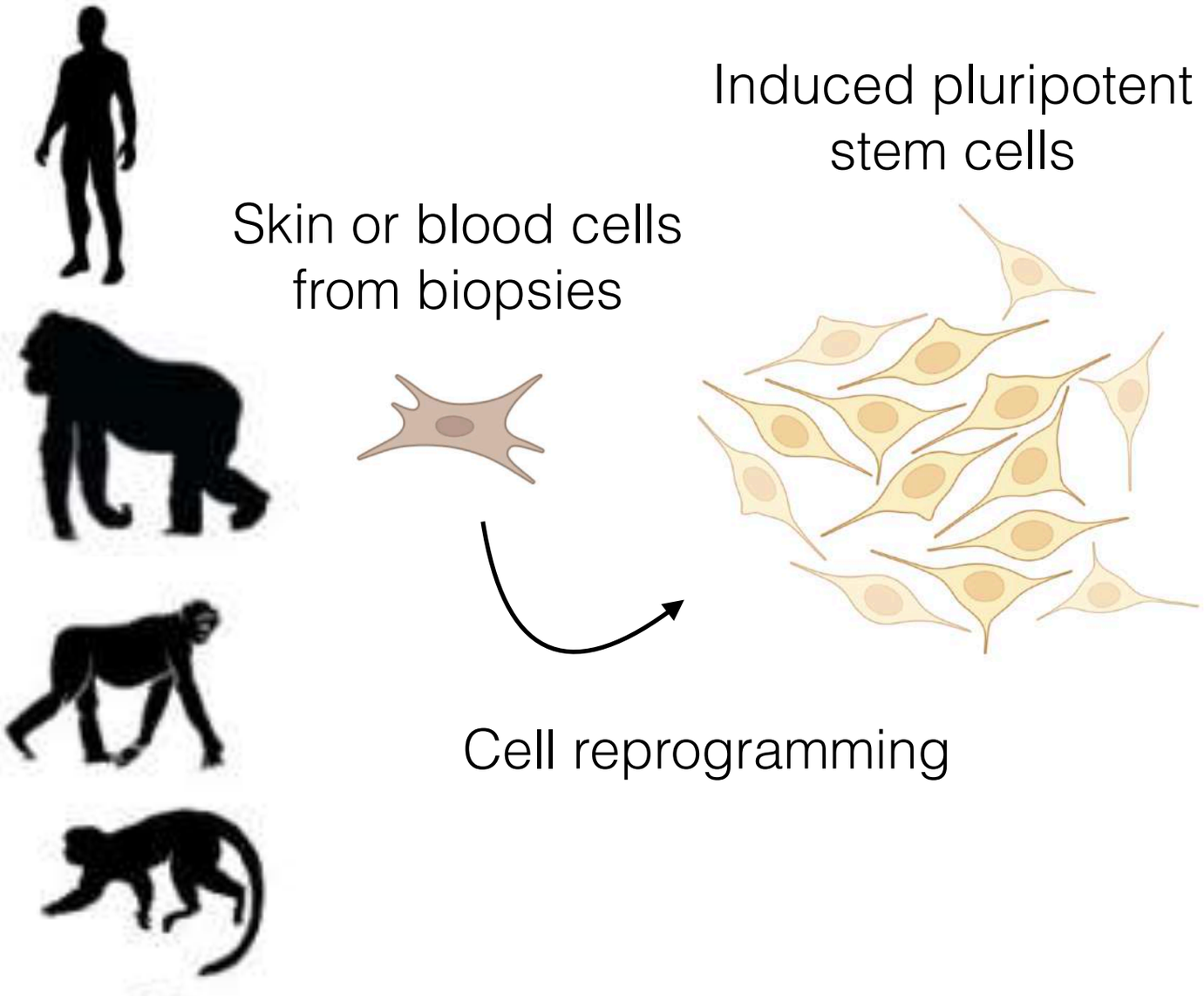
Donahue et al., 2018

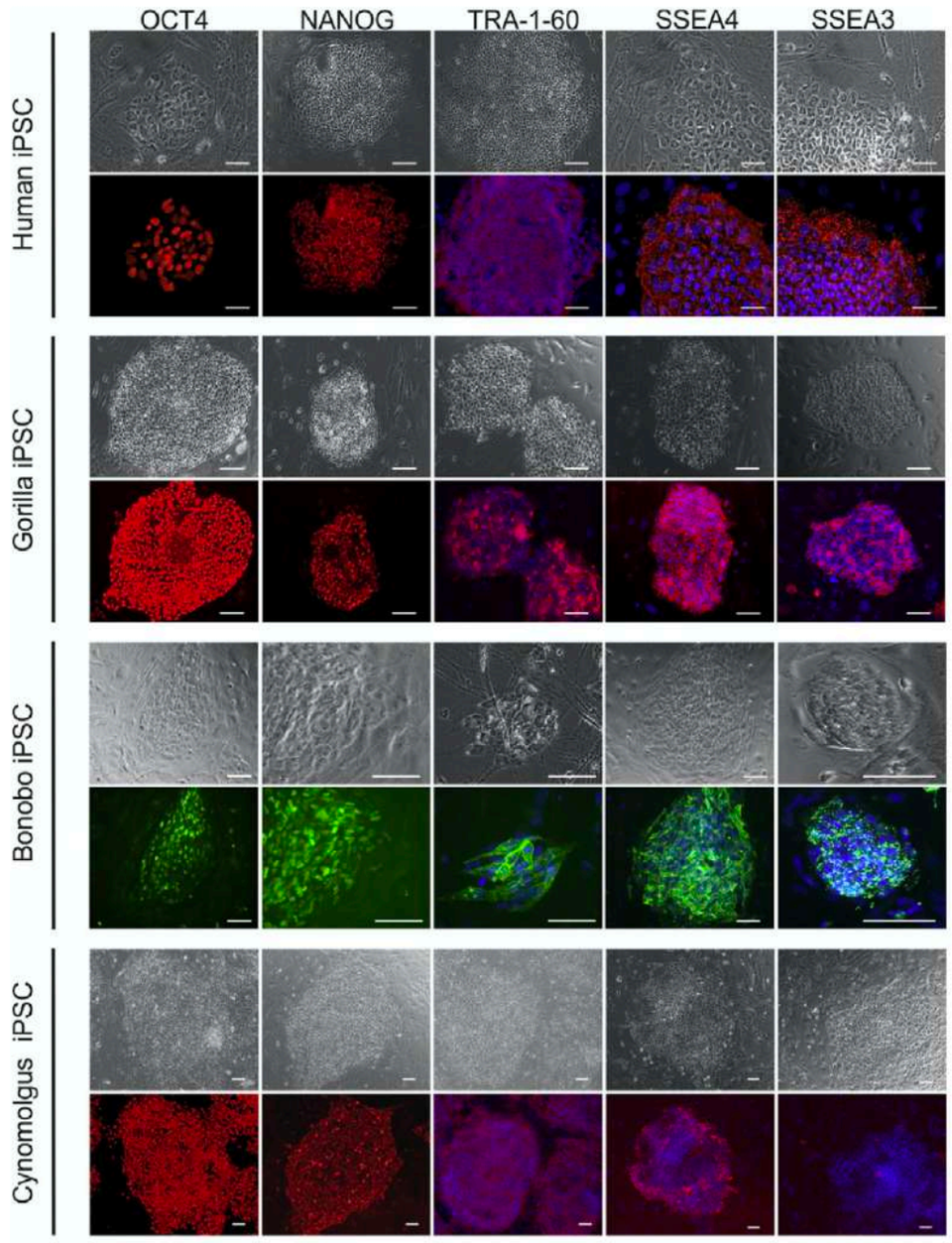
Cortical thickness is similar across apes

Humans and chimpanzees show a 3-fold difference in cortical surface area

Human brain is 3 times bigger than the chimpanzee brain

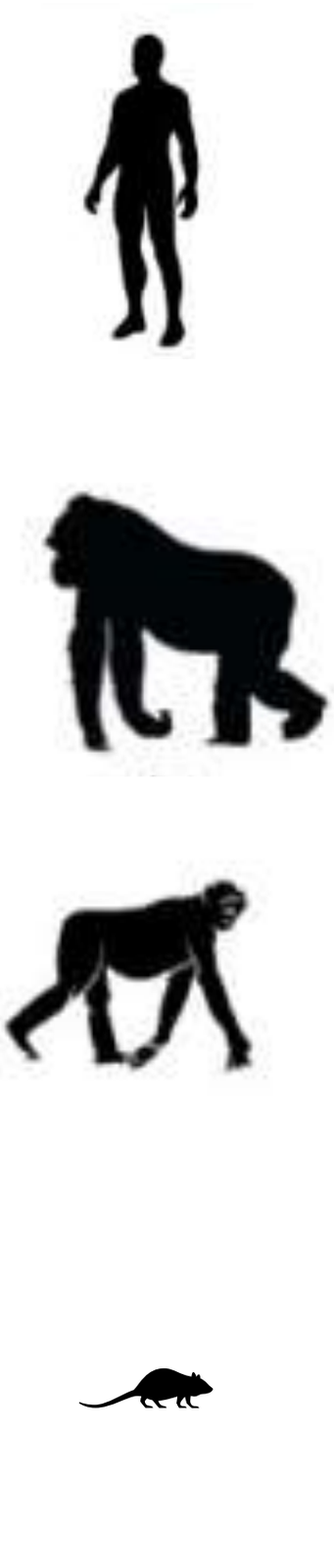
Studying brain evolution in vitro: making IPS cells from different species

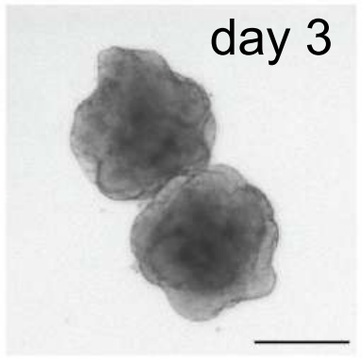


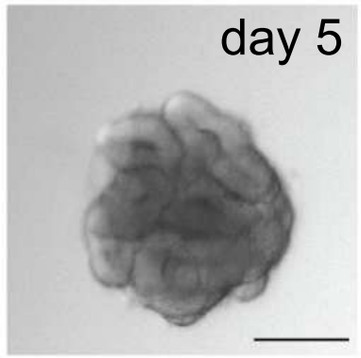


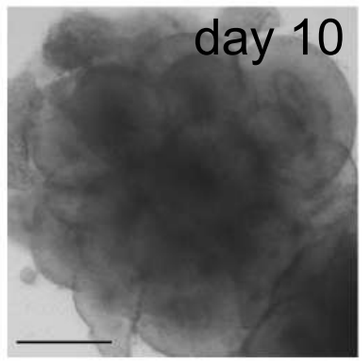
Wunderlich et al., 2014

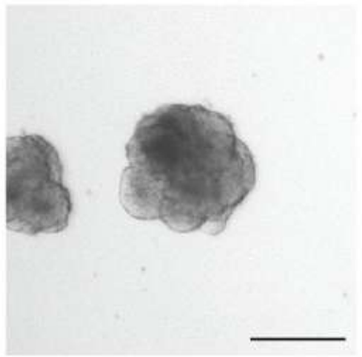
Studying brain evolution in vitro: making brain organoids from different species

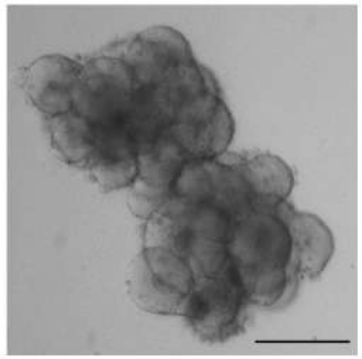


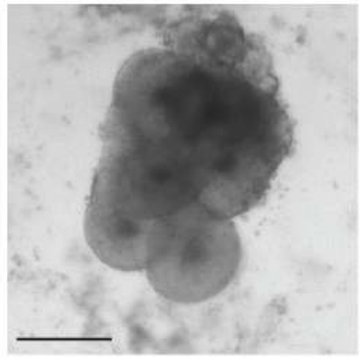


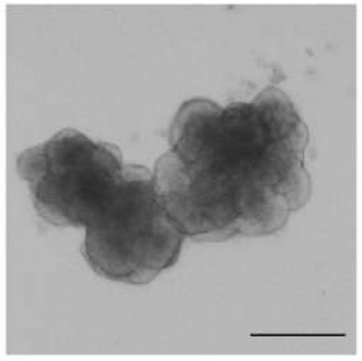


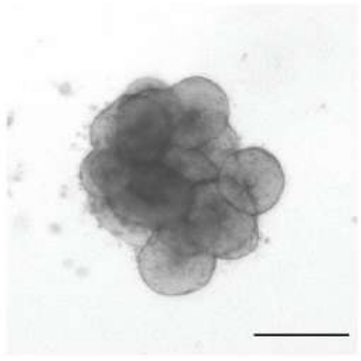


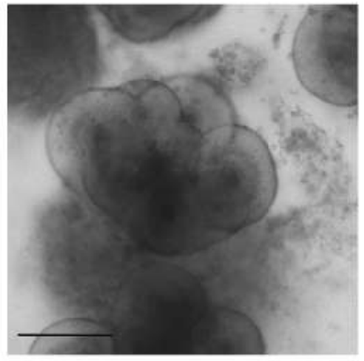


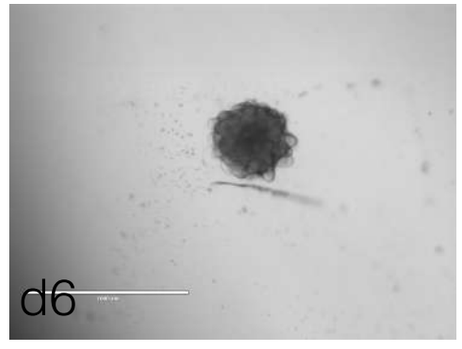


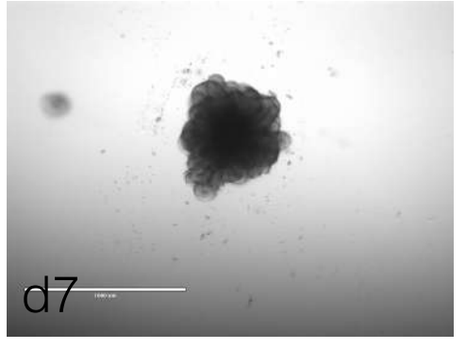


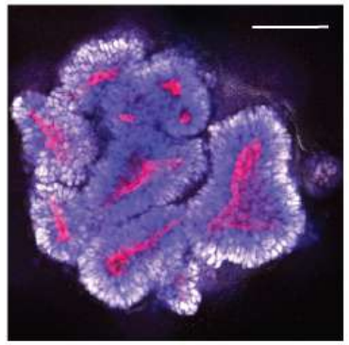


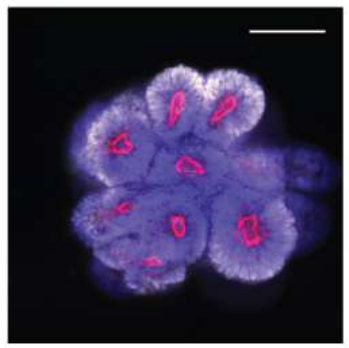


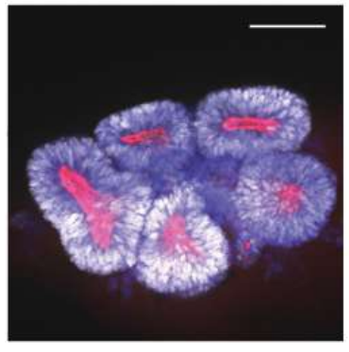


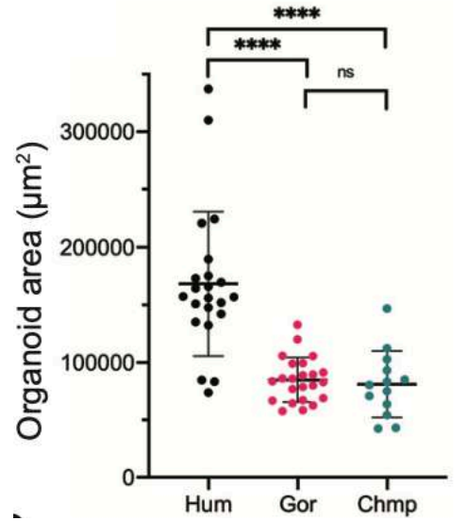


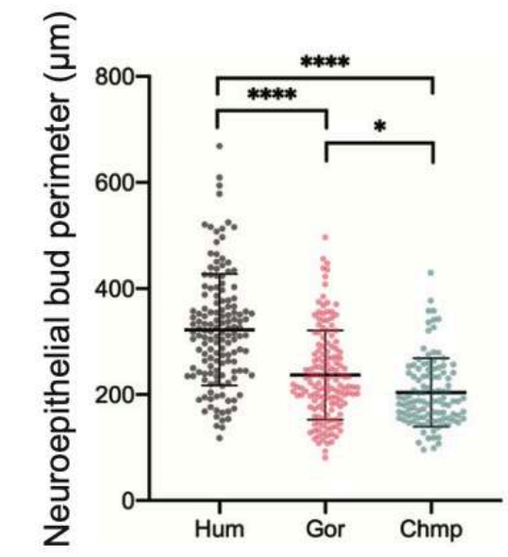






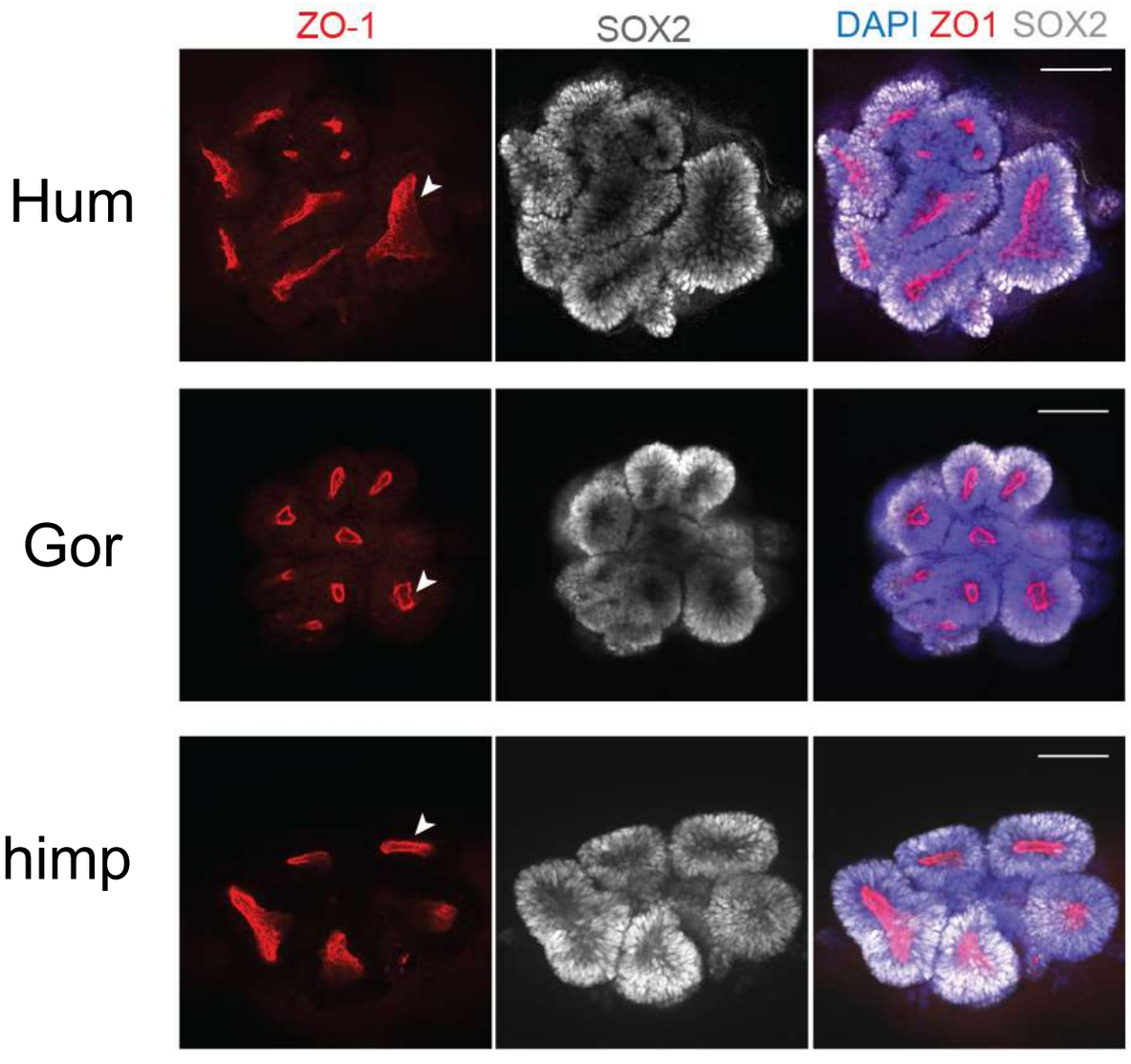


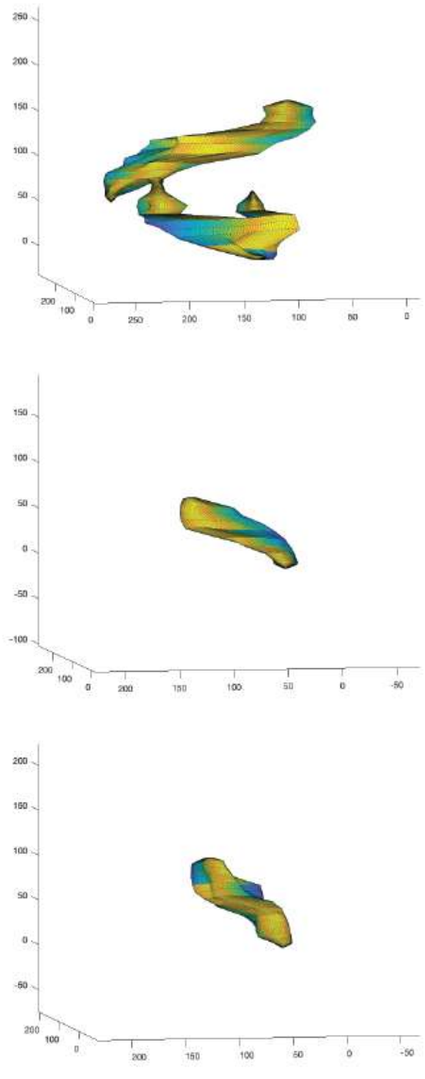


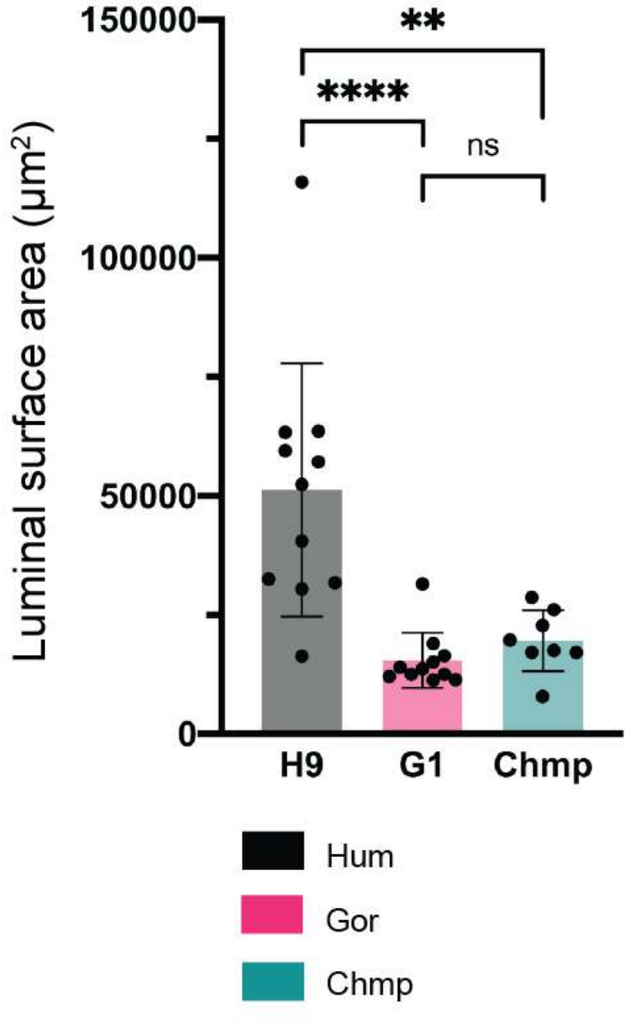


Benito-Kwiecinski et al. Cell, 2021

Human organoids show expanded apical lumen



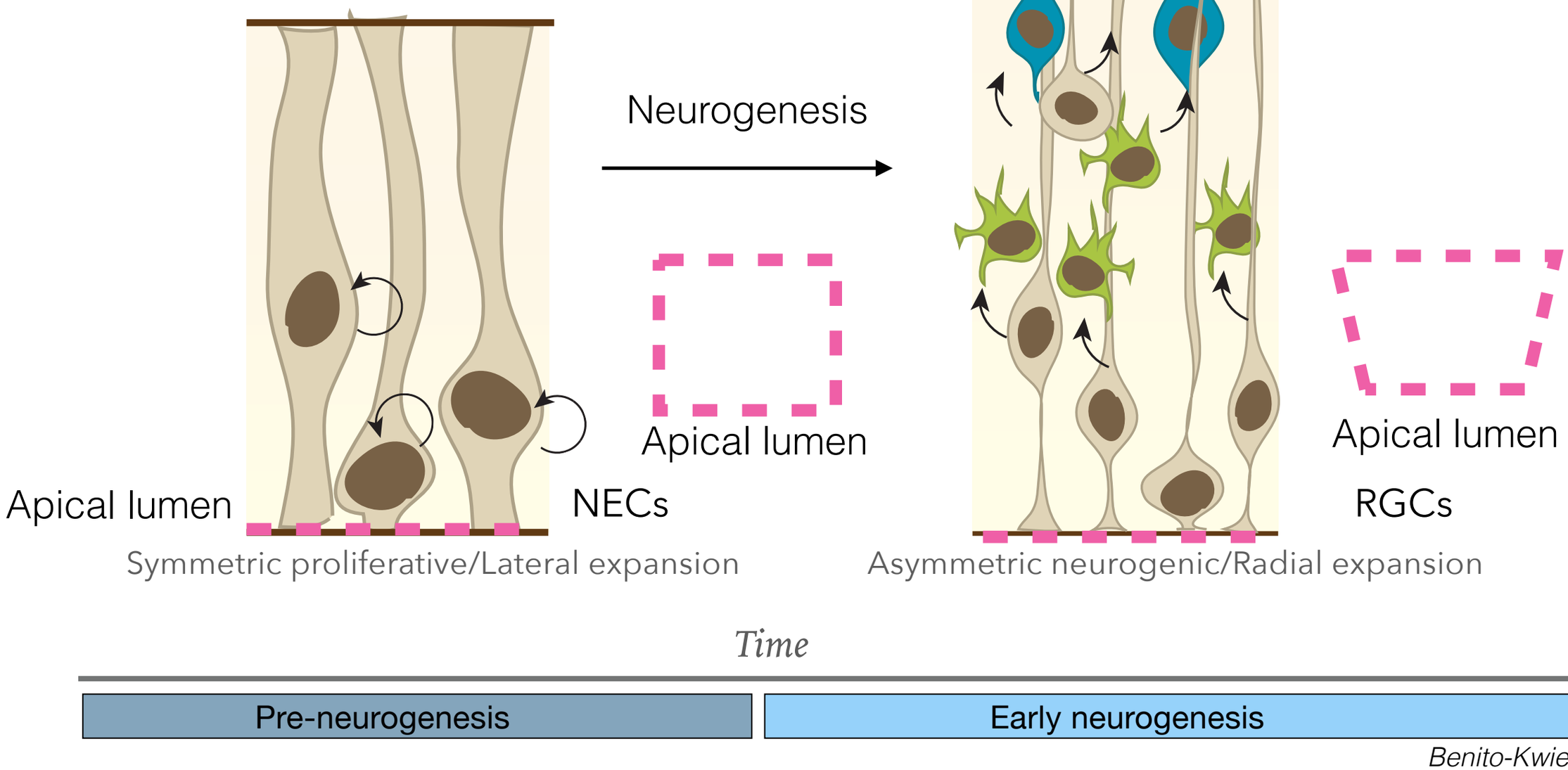




Benito-Kwiecinski et al., 2021

Neural progenitor differentiation

Onset of neurogenesis: neural progenitor cells transition from proliferative neuroepithelial cells (NECs) to neurogenic radial glial cells (RGCs)

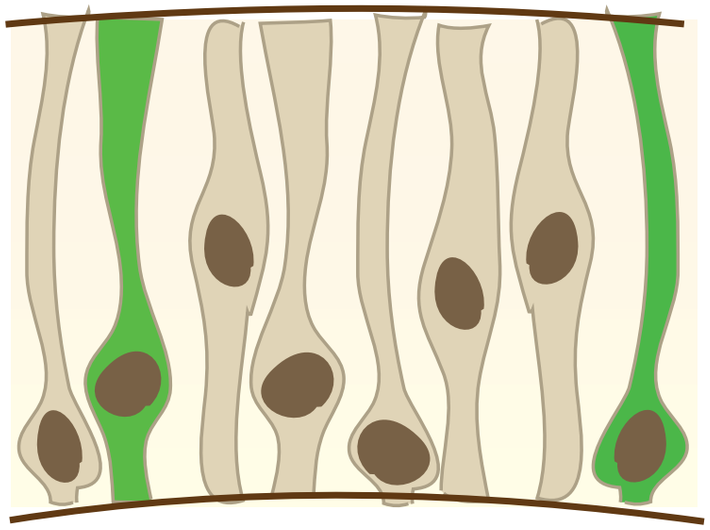


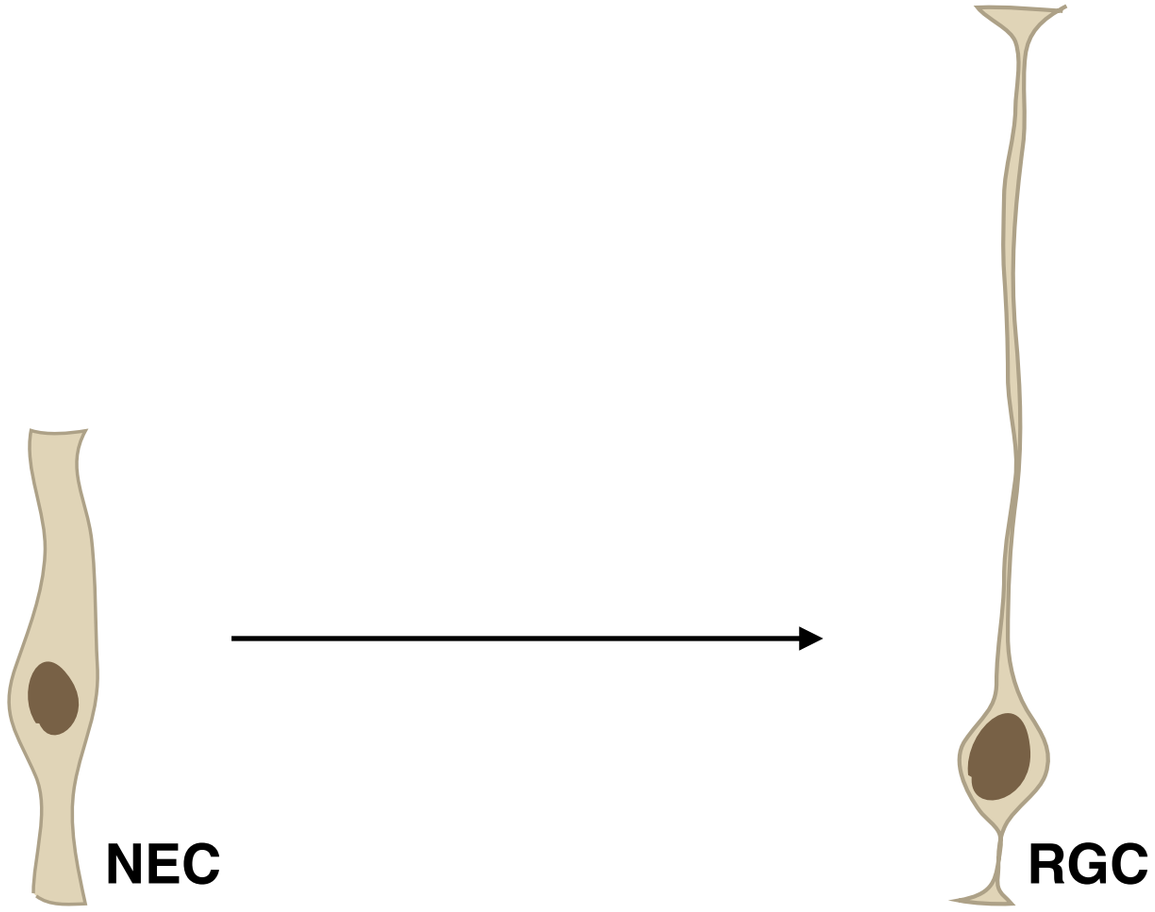
Time

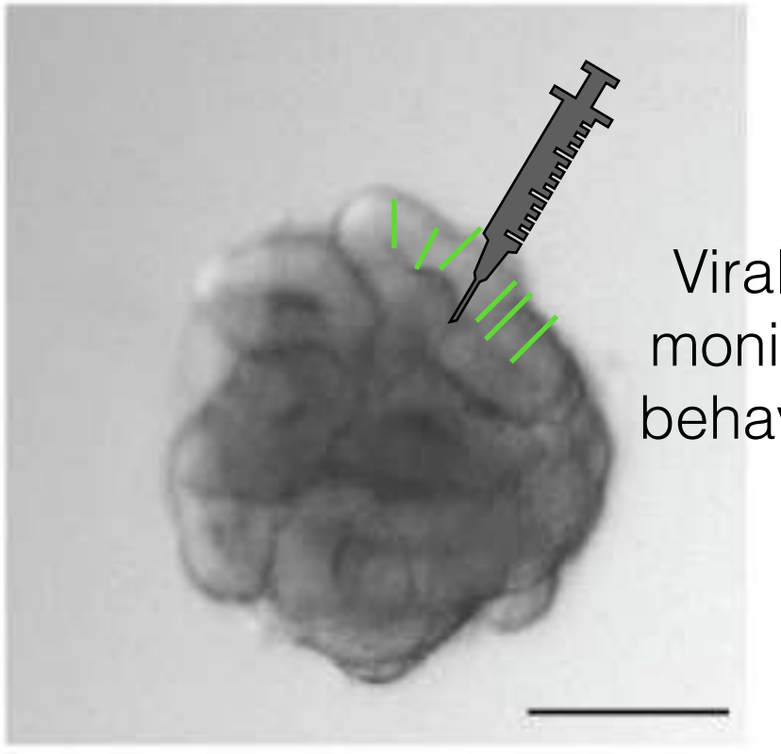
Pre-neurogenesis | Early neurogenesis

Benito-Kwiecinski et al., 2021

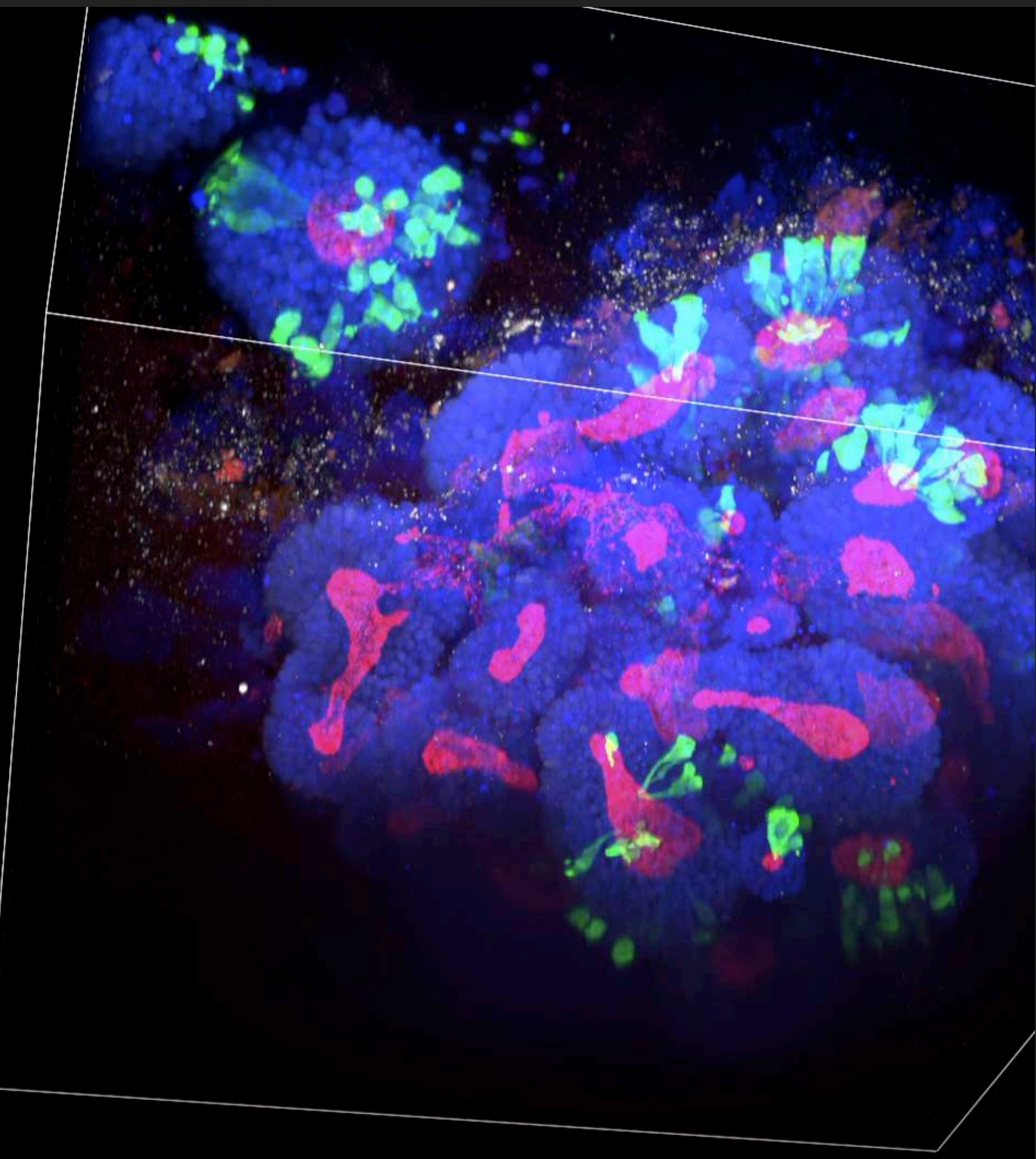
Do progenitors behave differently in human and gorilla?



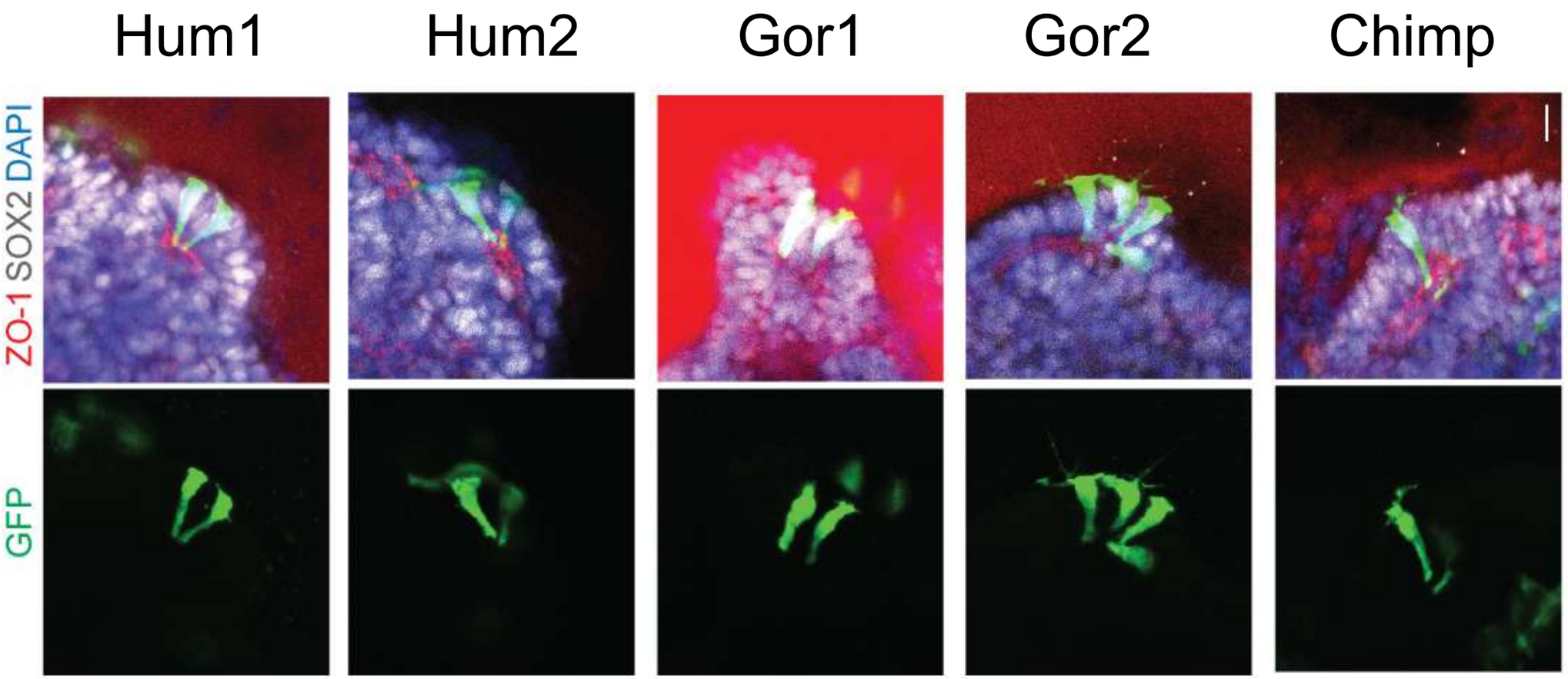


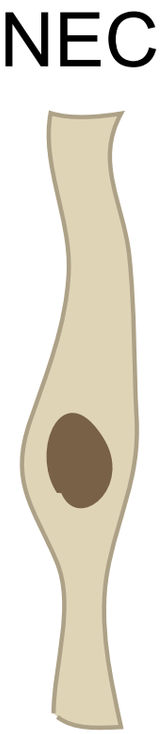


Viral GFP labelling to monitor the shape and behaviour of progenitor cells



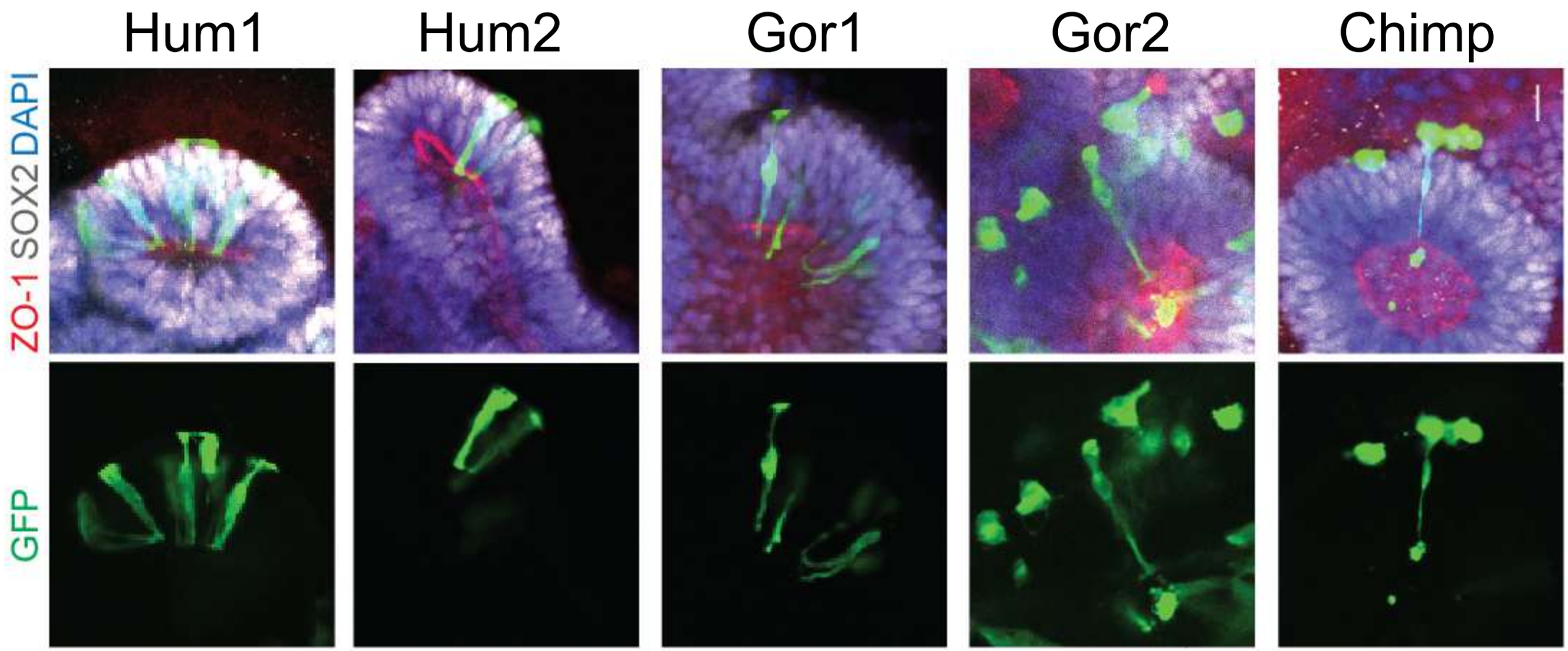
Day 3: Progenitors exhibit NEC-like morphology in all species





Benito-Kwiecinski et al., 2021

Day 5: nonhuman ape progenitors show transitioning morphology





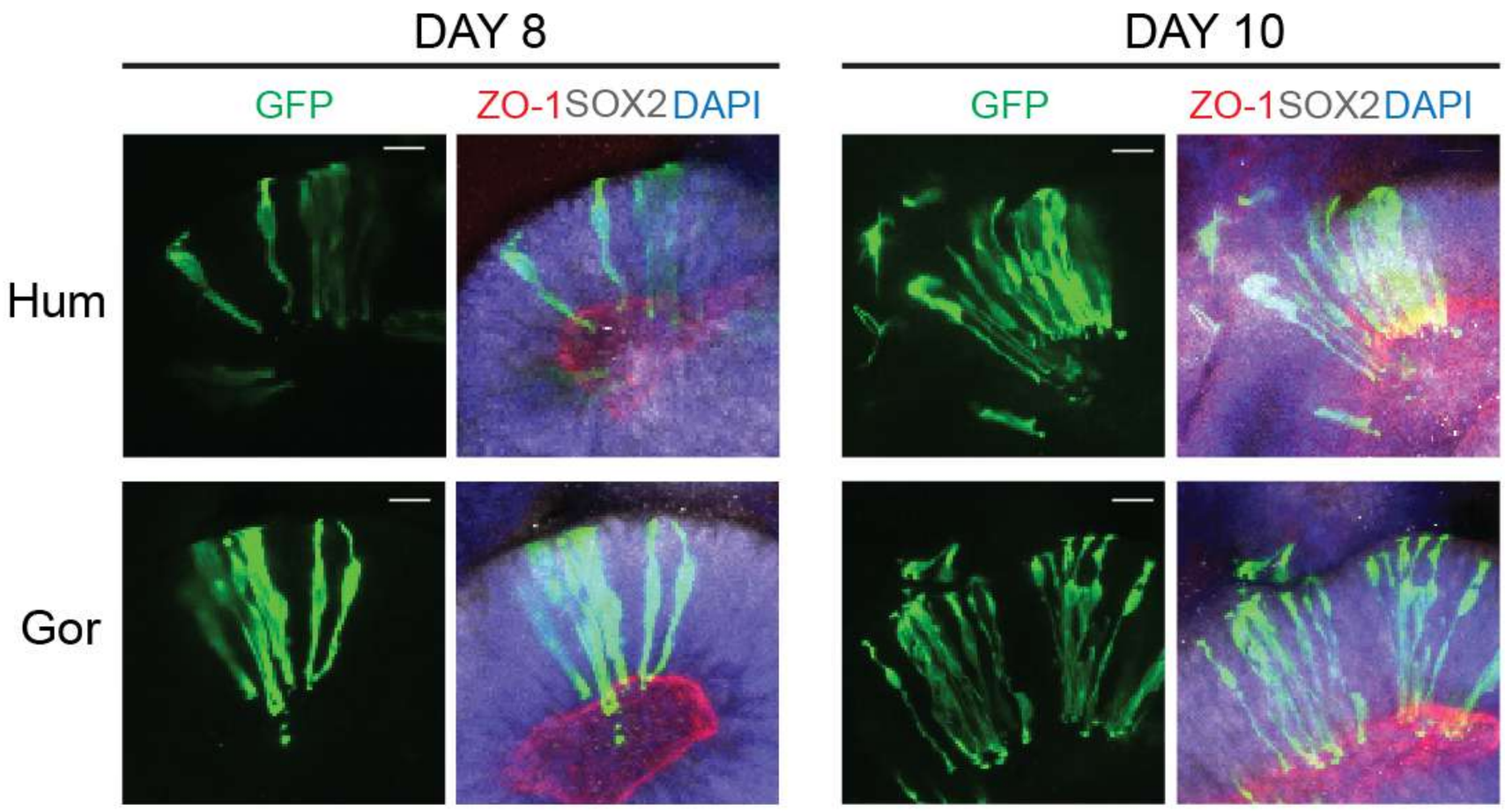
NEC

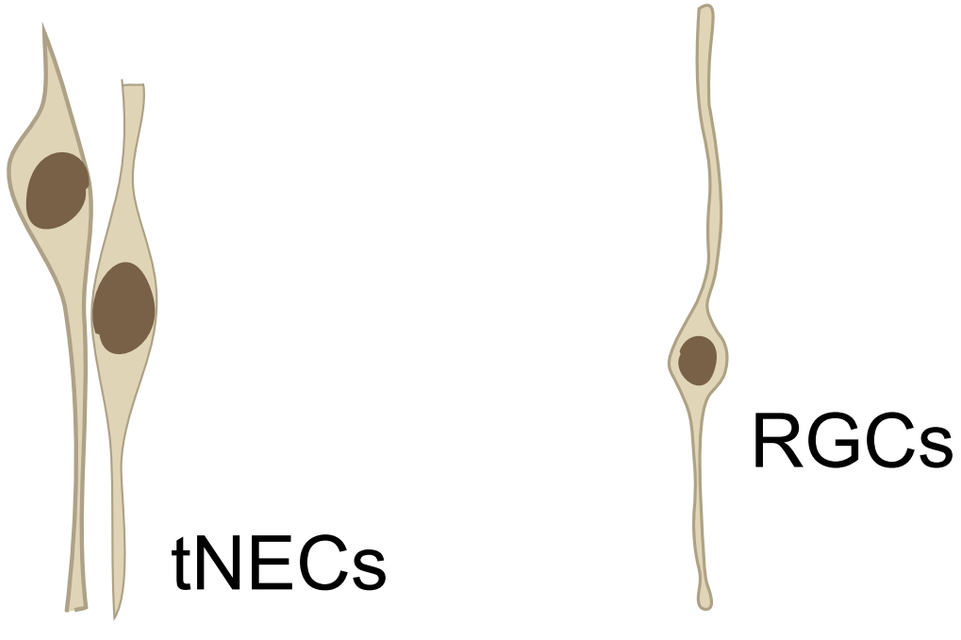


transitioning NEC (tNEC)

Benito-Kwiecinski et al., 2021

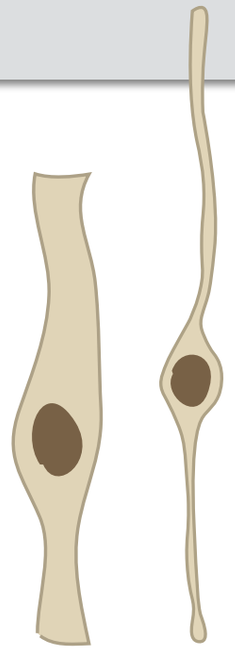
By day 10 progenitors have transitioned to neurogenic RGCs





Benito-Kwiecinski et al., 2021

Transition from NECs to RGCs implies a thinning of the apical process

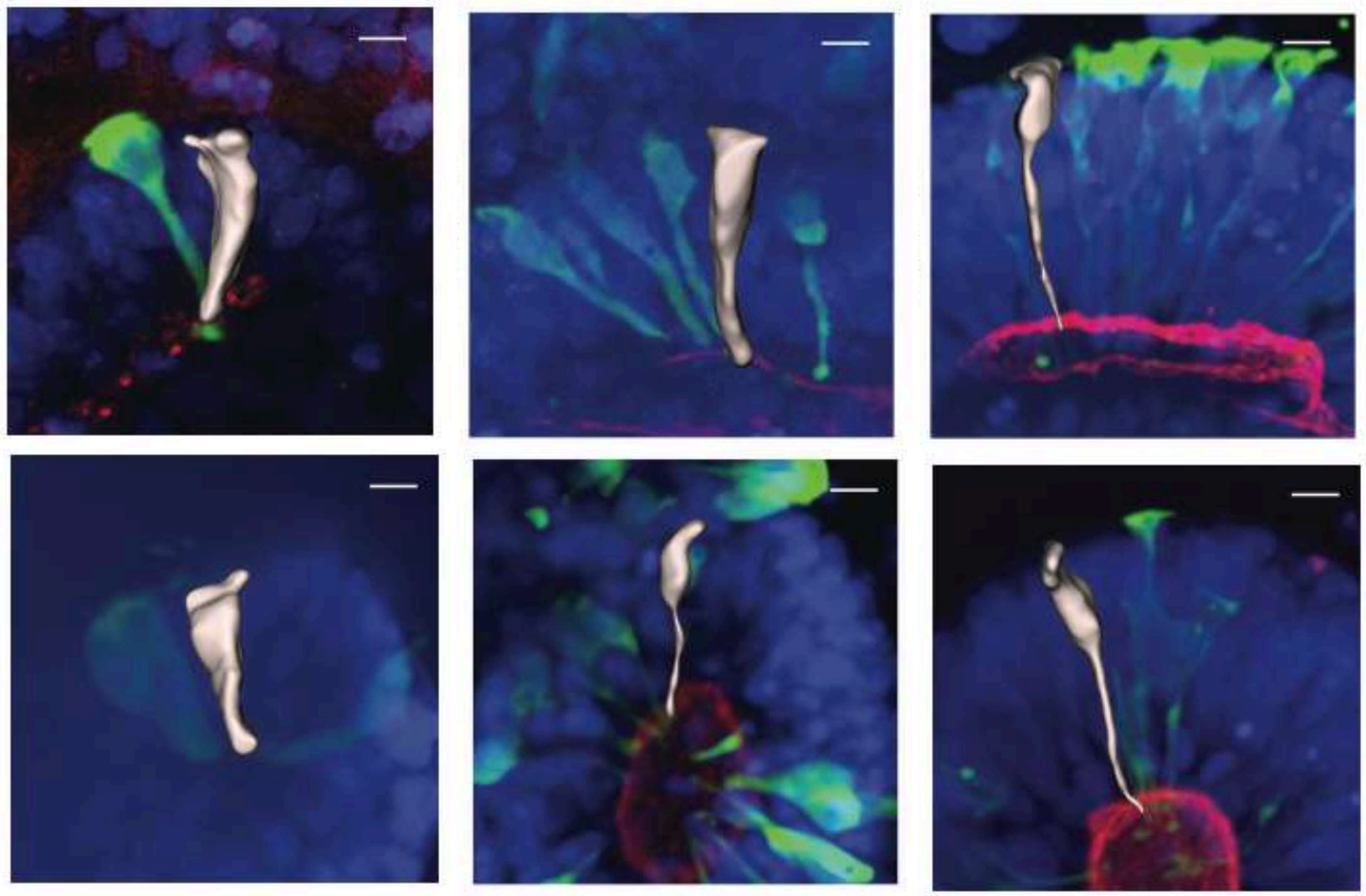


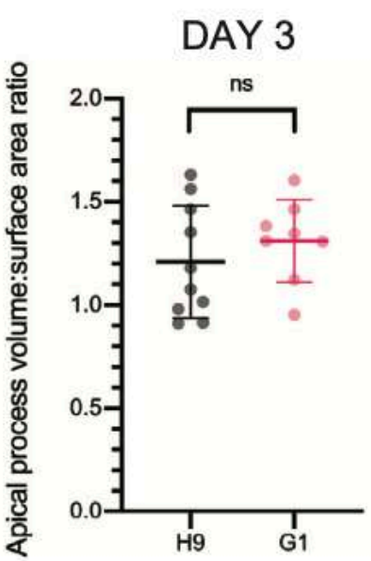
Apical

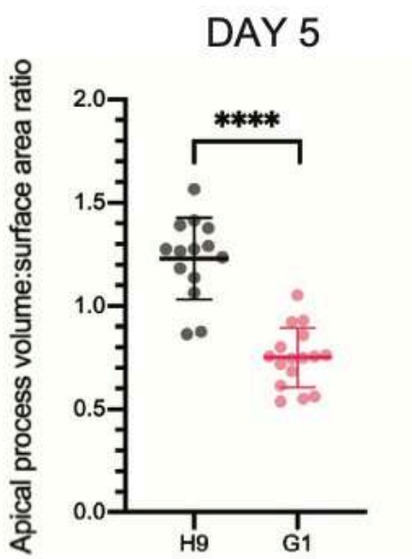
DAY 3 DAY 5 DAY 10

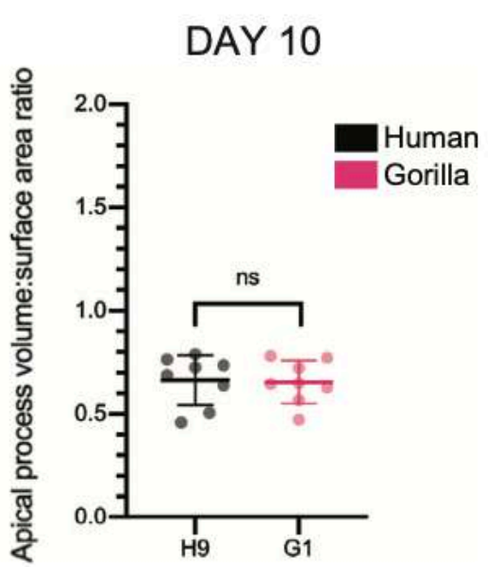
Hum

Gor





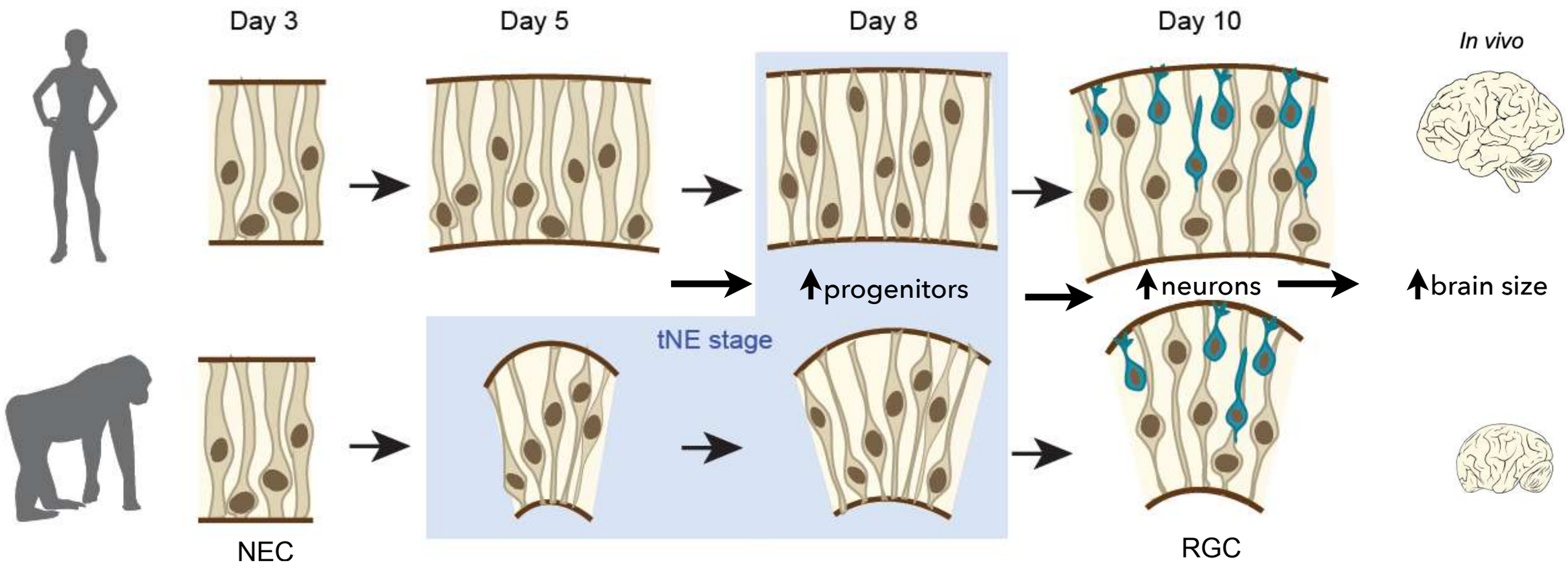




Benito-Kwiecinski et al., 2021

From NECs to RGCs: the emergence of tNECs

Working model:

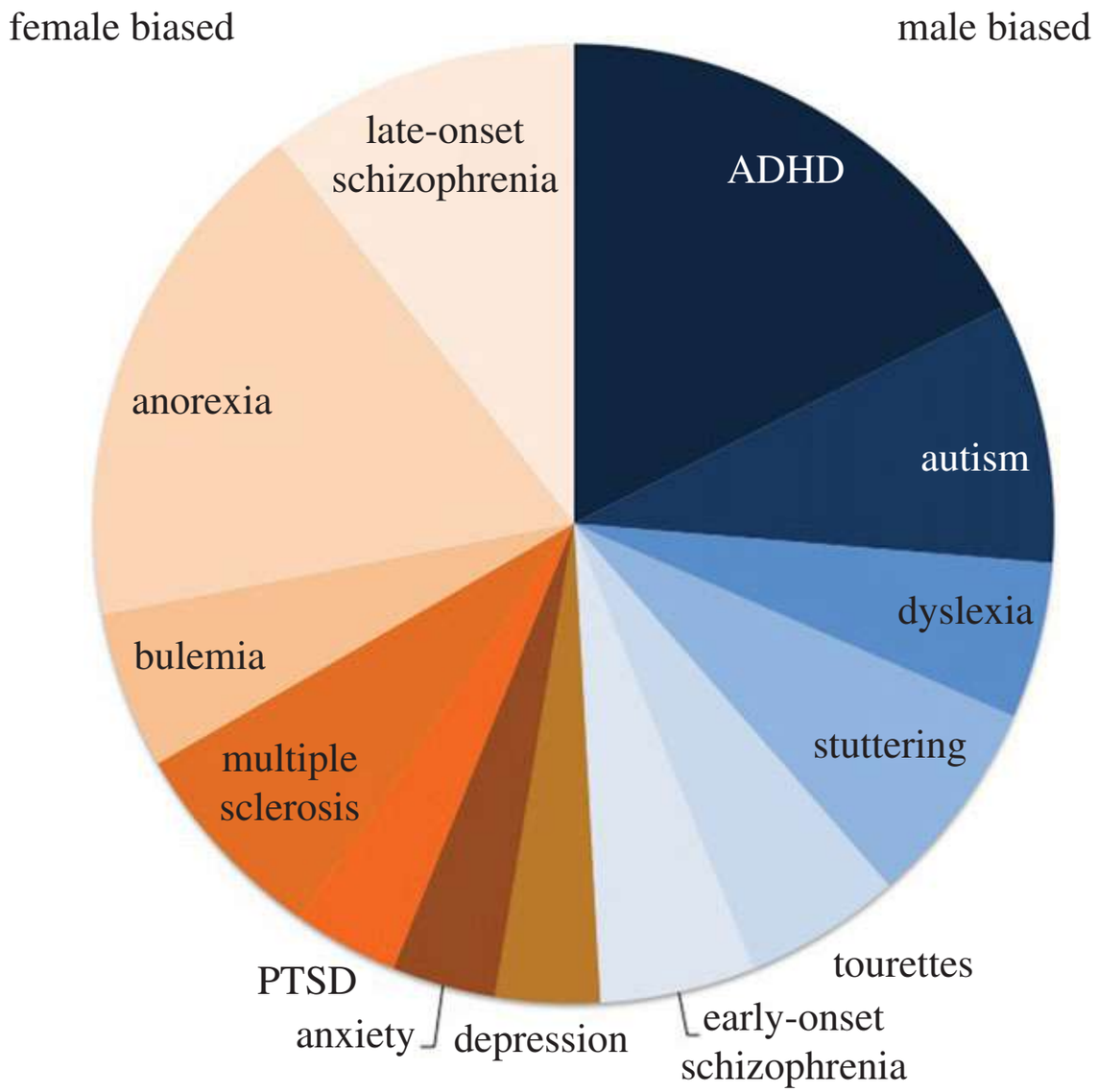


Benito-Kwiecinski et al., 2021

Brain organoids for studying sex-bias in brain development



ASD	1 : 4
Depression Anxiety	2 : 1
Schizophrenia	1 : 1.4
Parkinson's disease	1 : 1.5
Dementia	2 : 1



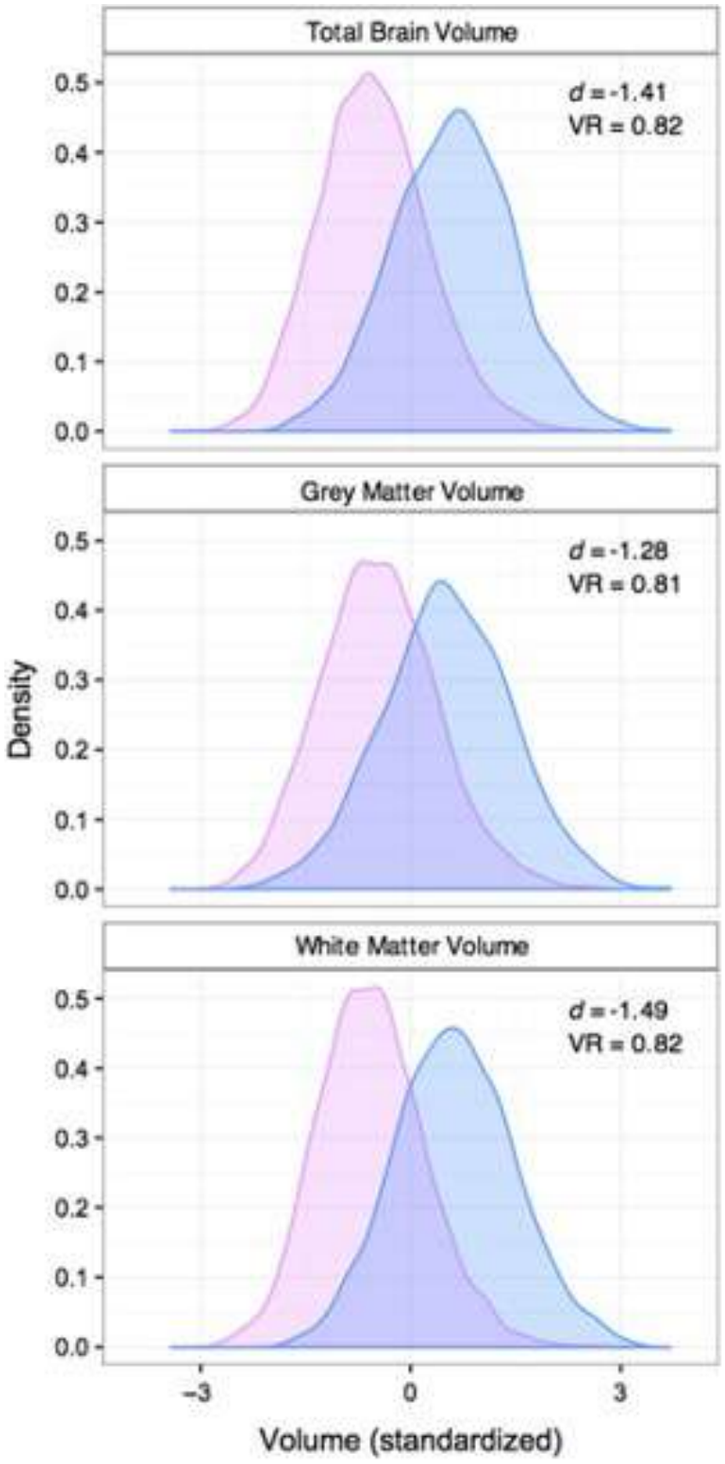
McCarthy, 2016.

Human brain is not sexually dimorphic



Male

Female

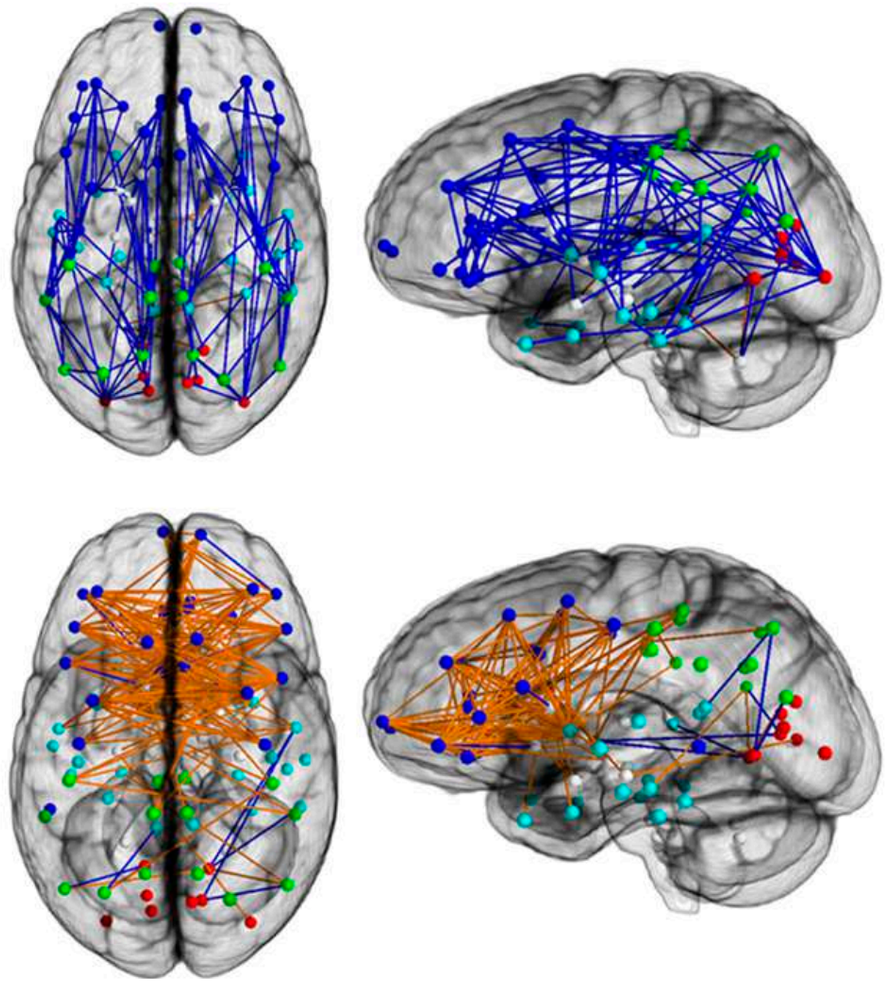


Ritchie et al., 2017.

♀ ♂

Higher raw cortical thickness. Higher white matter tract complexity.

Higher raw ctx surface area. Higher white matter fractional anisotropy.



Ingalhalikar et al., 2014.

Sex-related brain differences during fetal development



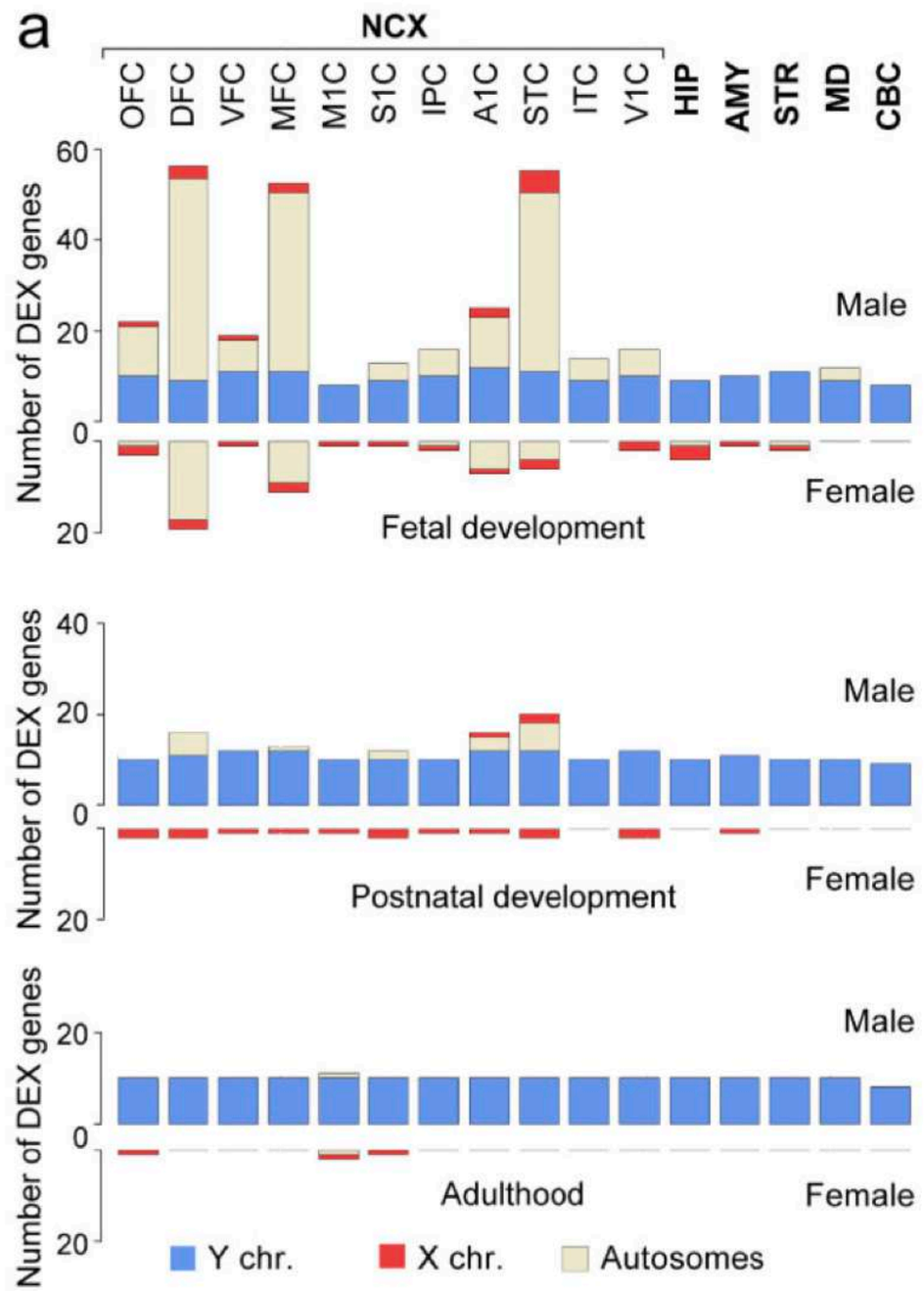
Sex differences in absolute brain volume (ROI volumetry)

Dependent variable	Male LS mean (SE)	Female LS mean (SE)	Percent difference (%)	*P*-value
Intracranial volume	493637 (5568)	466274 (5787)	5.87	<0.0001
Total GM	250831 (2727)	238119 (2836)	5.32	<0.0001
Total WM	170507 (2070)	161085 (2155)	5.85	<0.0001
Total CSF	61777 (1431)	56998 (1496)	8.38	0.0004
Cerebellar volume	25037 (407)	24288 (425)	3.08	0.0467
Prefrontal GM	29365 (592)	27621 (618)	6.31	0.0016
Prefrontal WM	26516 (466)	24725 (486)	7.24	<0.0001
Frontal GM	44900 (596)	42647 (623)	5.29	<0.0001
Frontal WM	40387 (559)	38409 (585)	5.51	0.0002
Parietal GM	61894 (839)	58985 (876)	4.93	0.0002
Parietal WM	50401 (728)	48044 (761)	4.91	0.0005
Occipital GM	72350 (980)	68701 (1021)	5.31	<0.0001
Occipital WM	44301 (642)	41513 (670)	6.72	<0.0001
Ventricle volume	4442 (289)	3926 (337)	13.14	0.1069

Note: Brain volumes are in mm^3, for all variables males had larger absolute volumes than females.

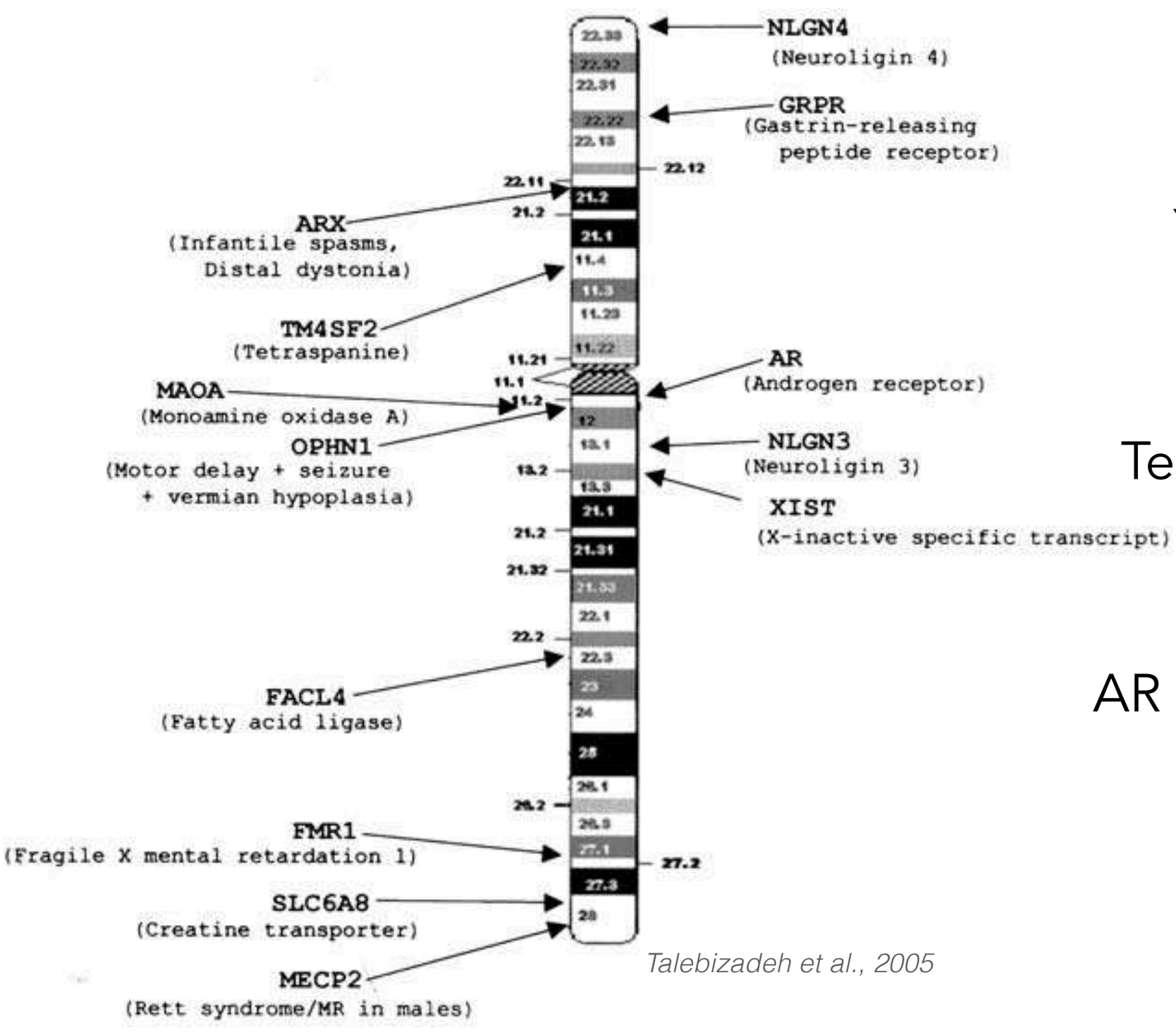
Knickmeyer et al., 2014.

Overall intracranial volume
5.87% higher in males



Kang et al., 2011.

Contribution of chromosomal complement to sex-based brain differences



Talebizadeh et al., 2005

Y chromosome

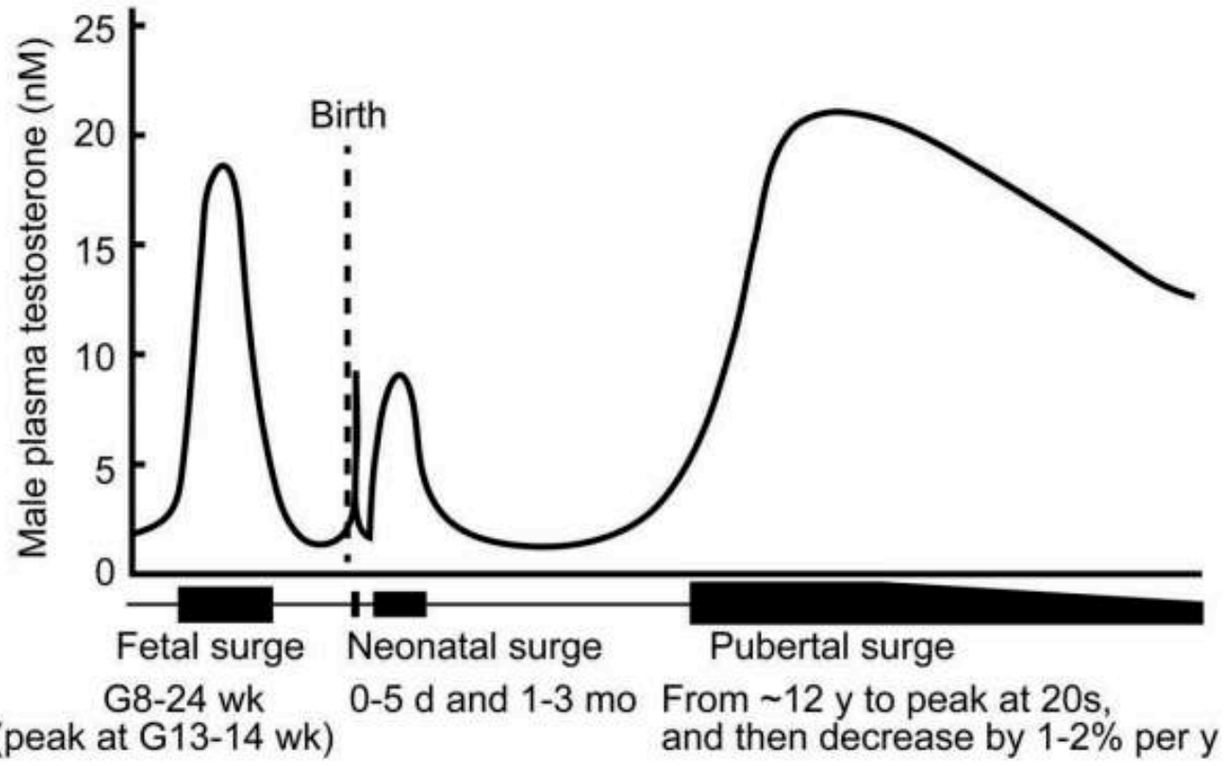
SRY

Testis development

Androgens

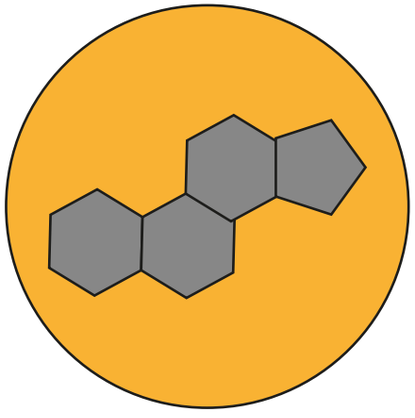
AR on X chromosome

Contribution of sex hormones to sex-based brain differences

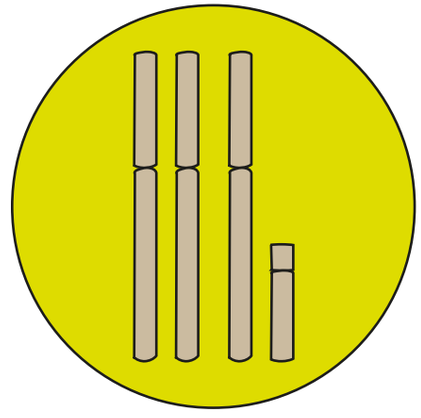


Harada, 2018.

sex-based brain differences



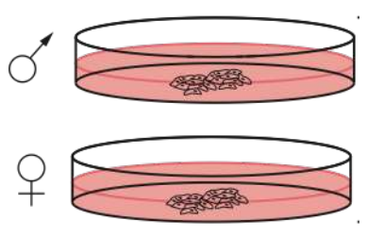
Hormones

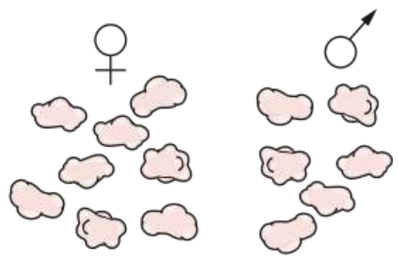


Chromosomes

Brain organoids for studying sex-bias in brain development







Ctrl

T

DHT

E

MECHANISM

- Receptors
- Signalling

CELL BIOLOGY

- Progenitors
- Proliferation
- Neuron production



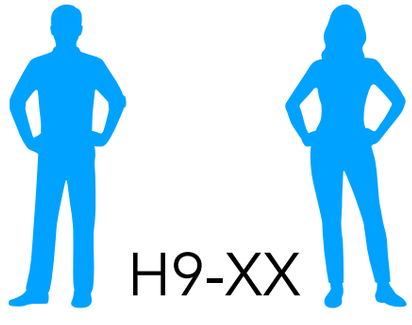
0

17

Hormone addition

35
collection

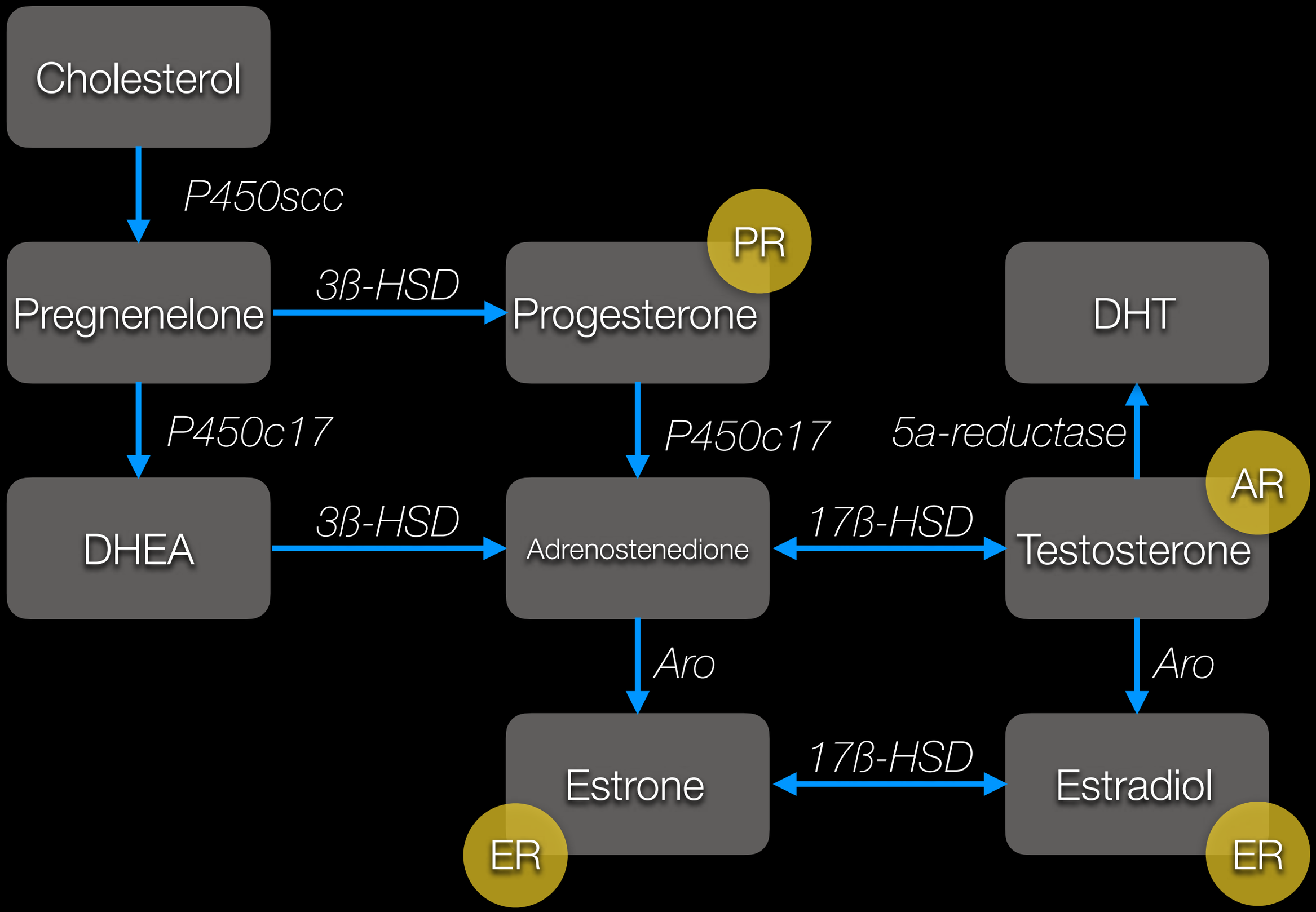
52
collection



H1-XY

H9-XX

Kelava et al., 2022 Nature

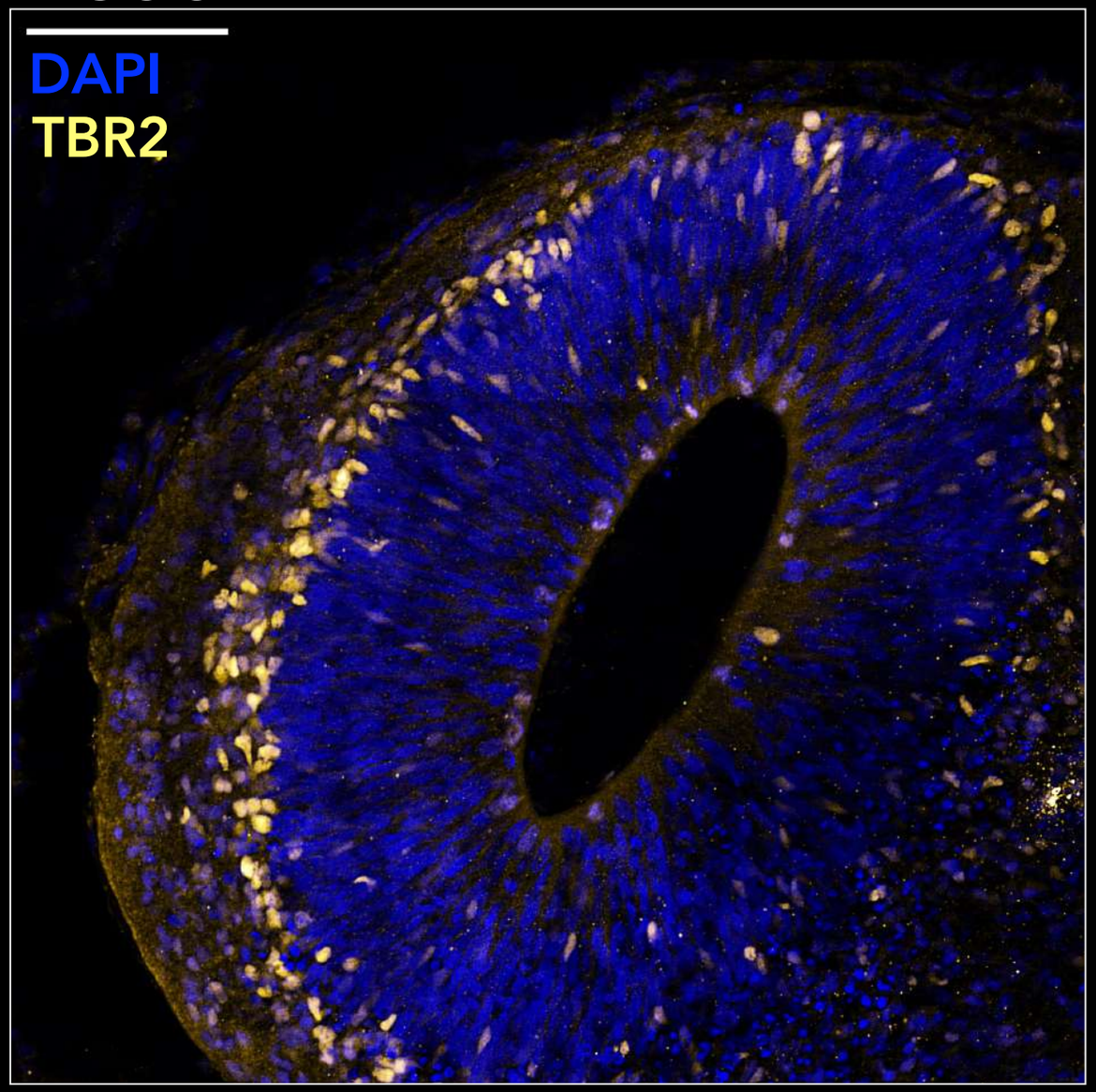


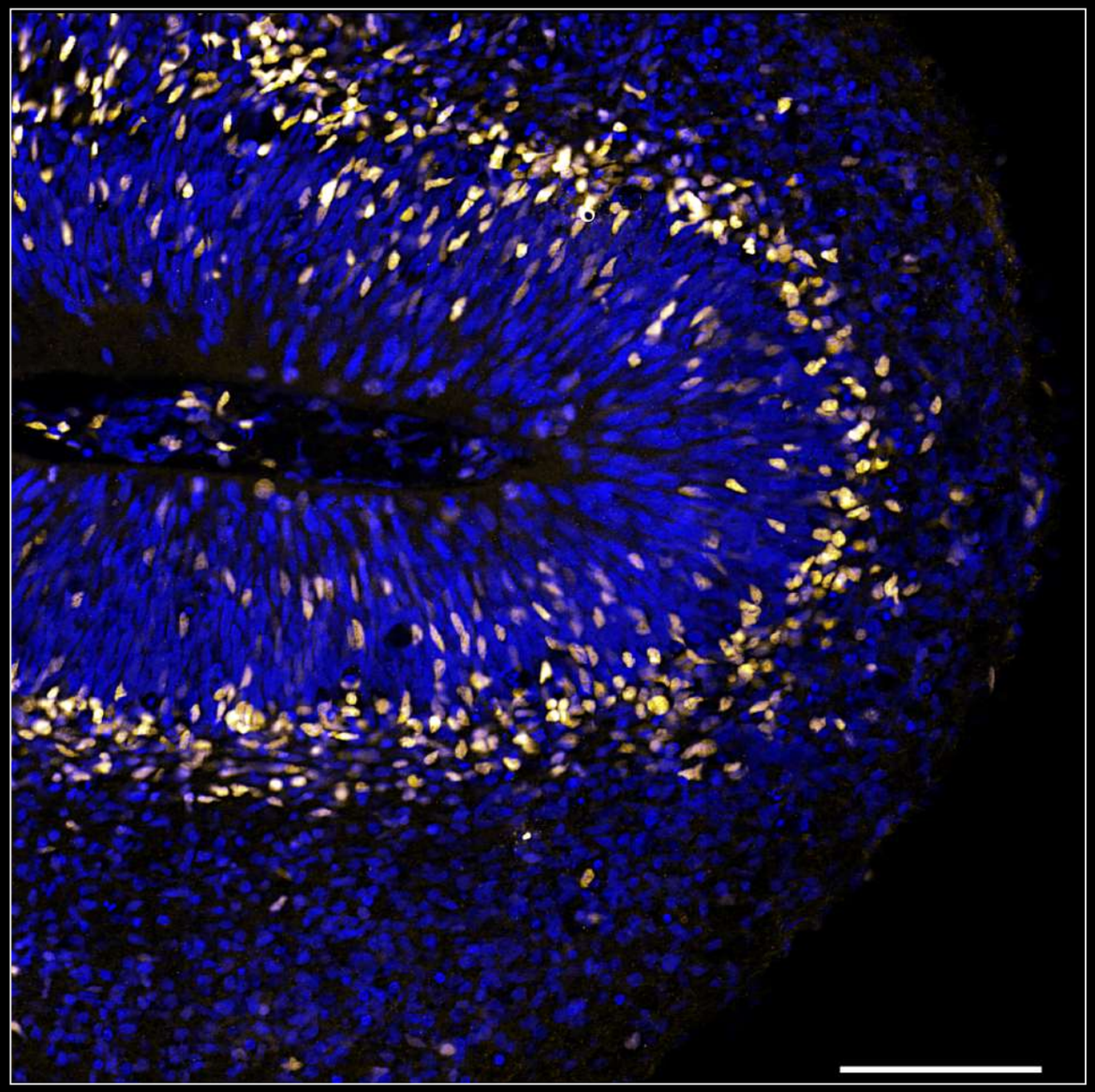
Sex steroid synthesis pathway

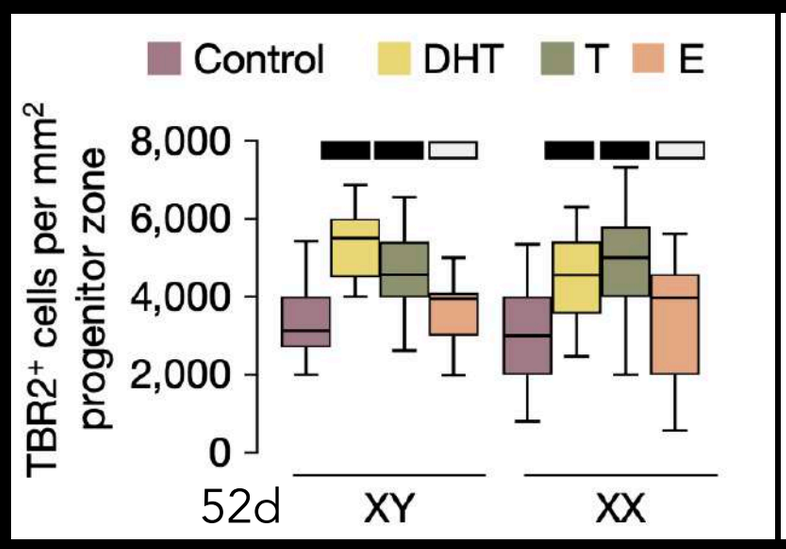
35d

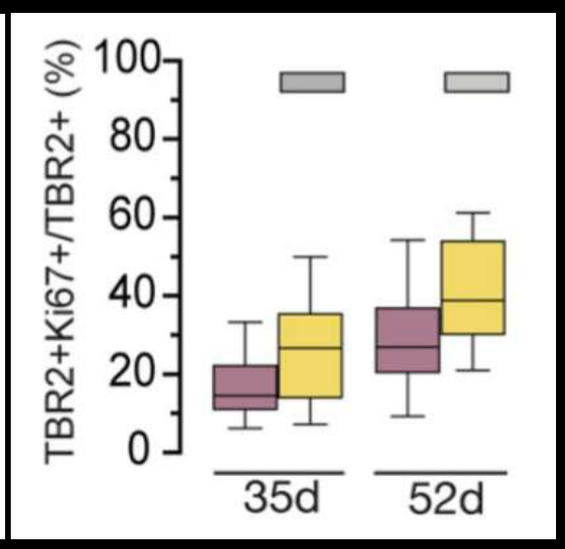
ctrl

DHT





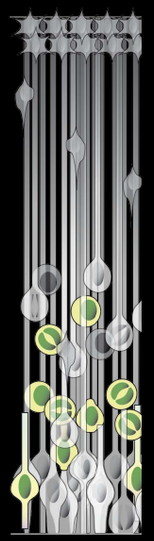






Androgens increase basal progenitor number

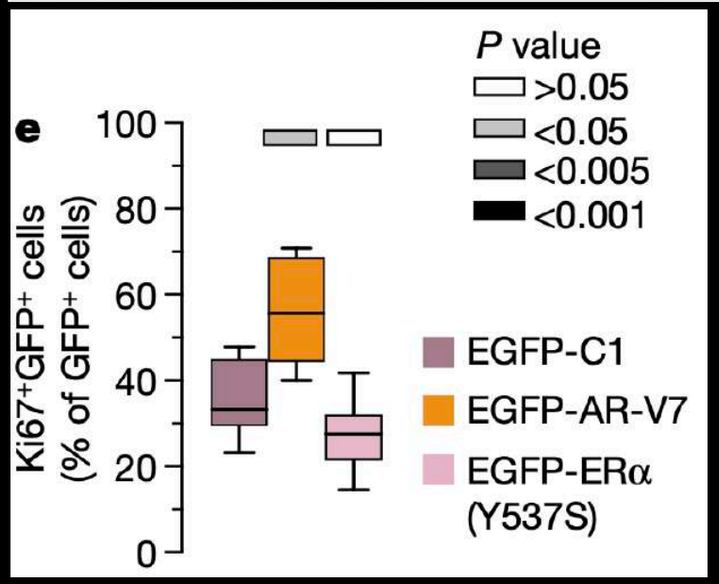
Kelava et al., 2022 Nature

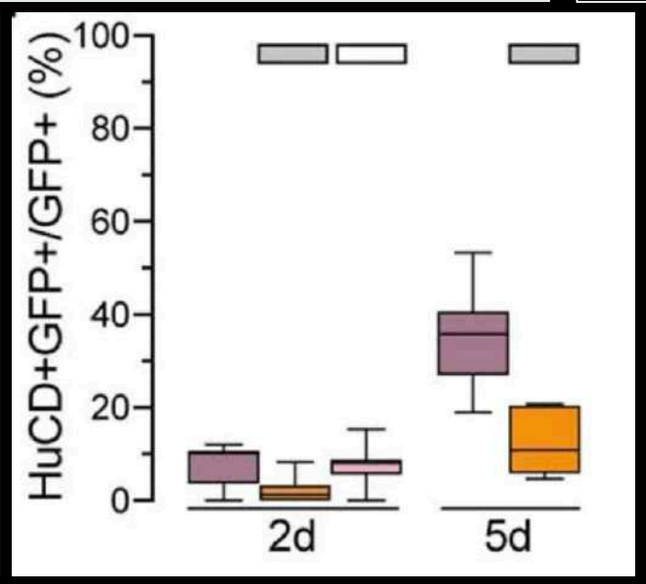


EGFP-Ctrl
TBR2

EGFP-AR-V7
TBR2

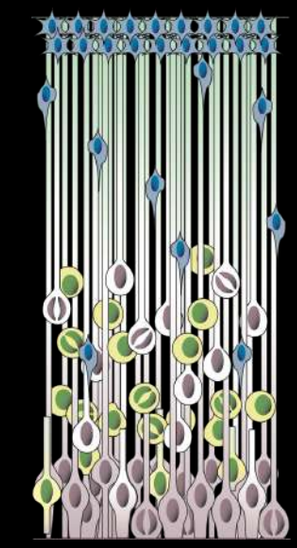
50d; EP@d45

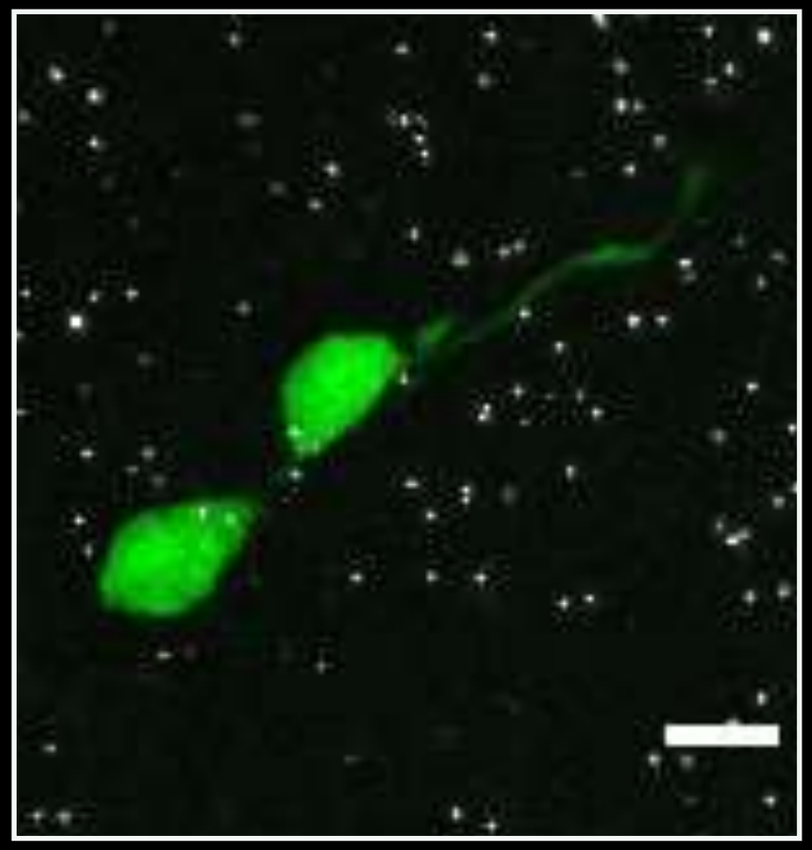




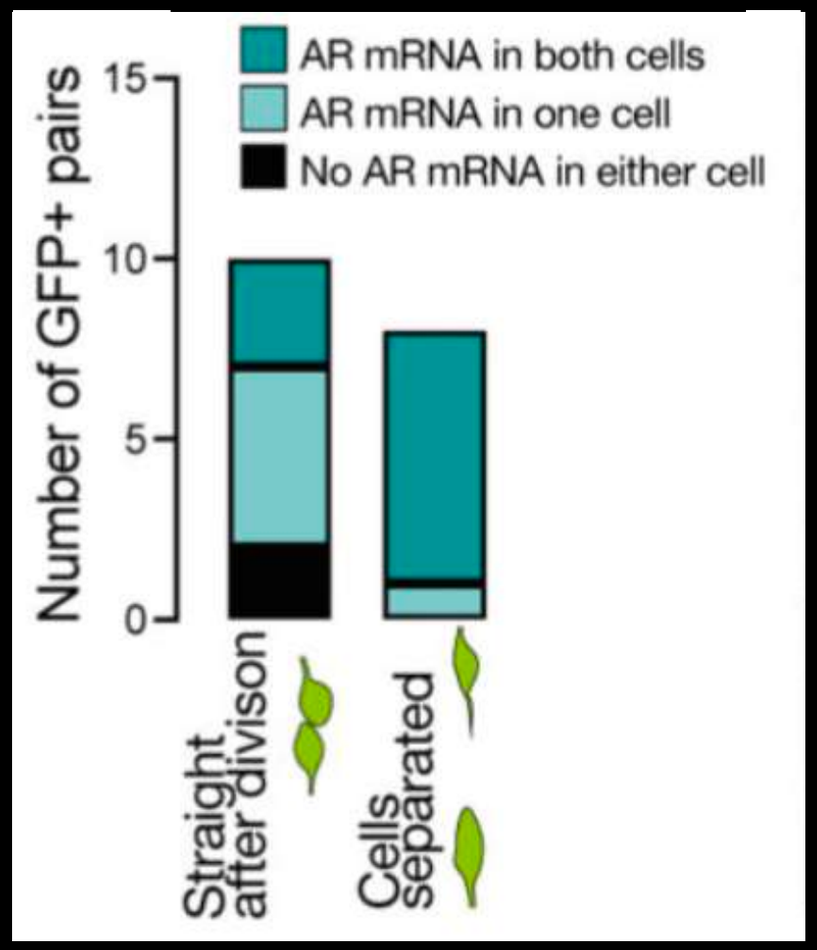
Androgens increase progenitor proliferation and delay differentiation

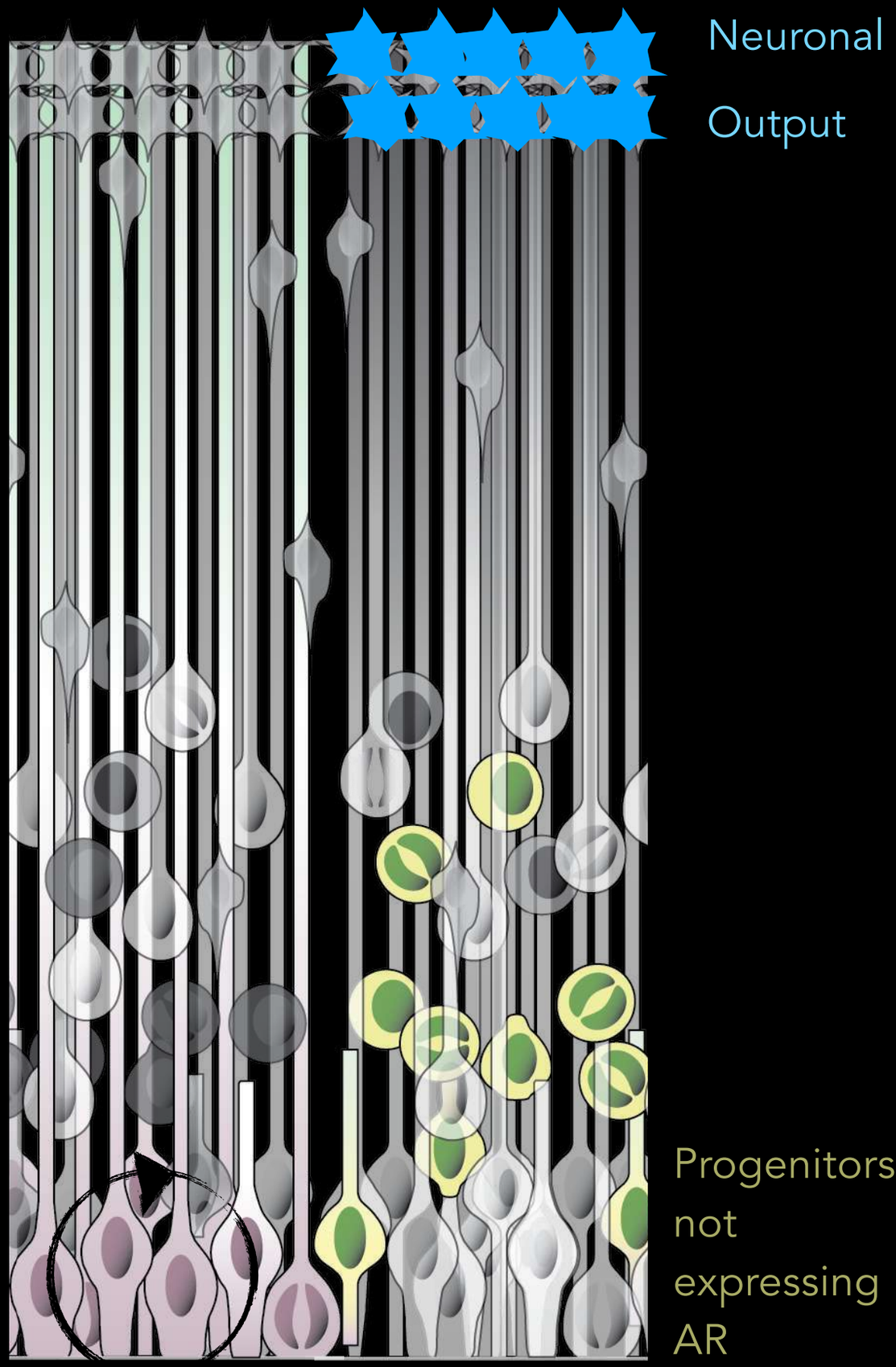
Kelava et al., 2022 Nature





AR mRNA in both dividing cells

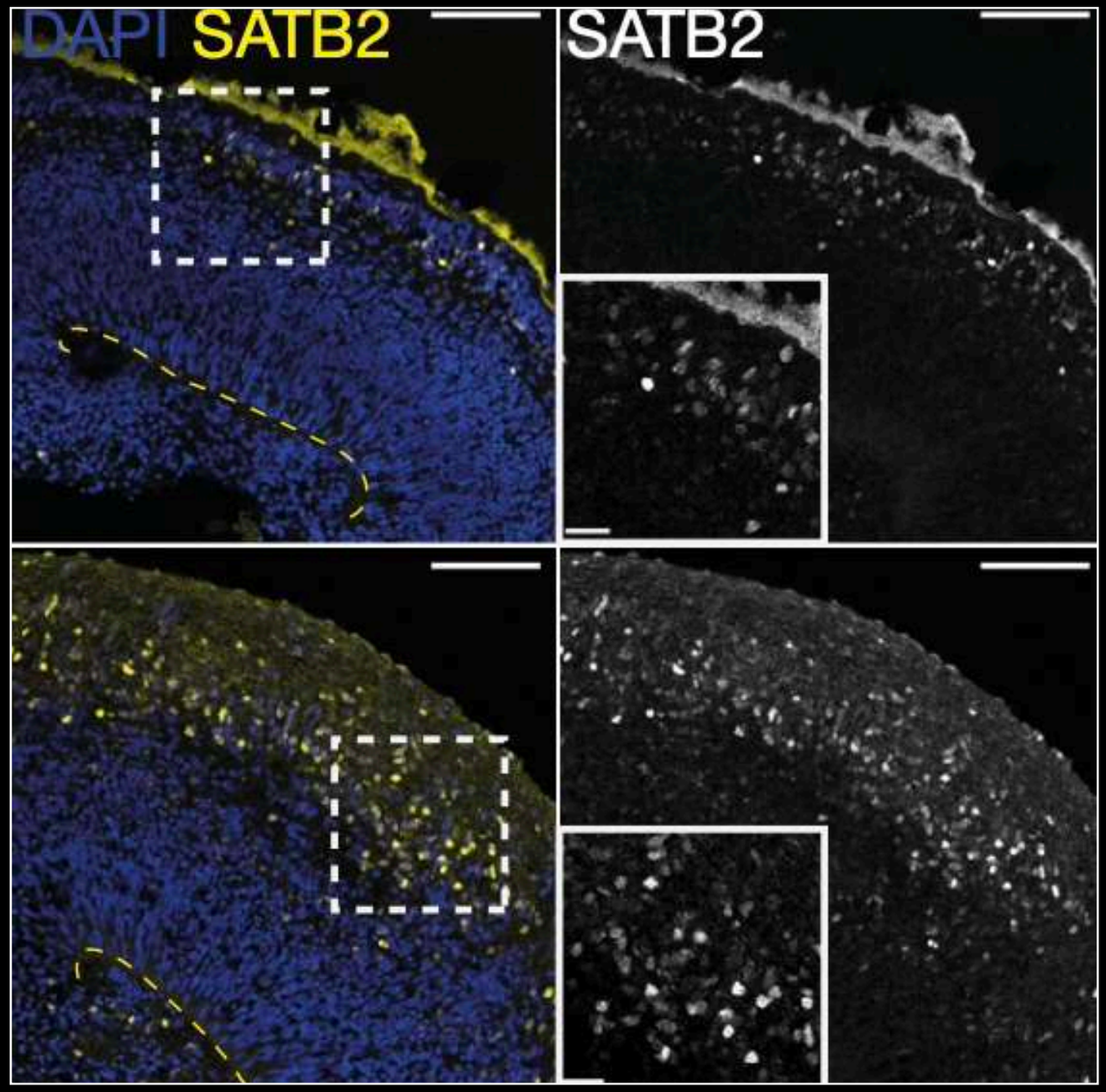


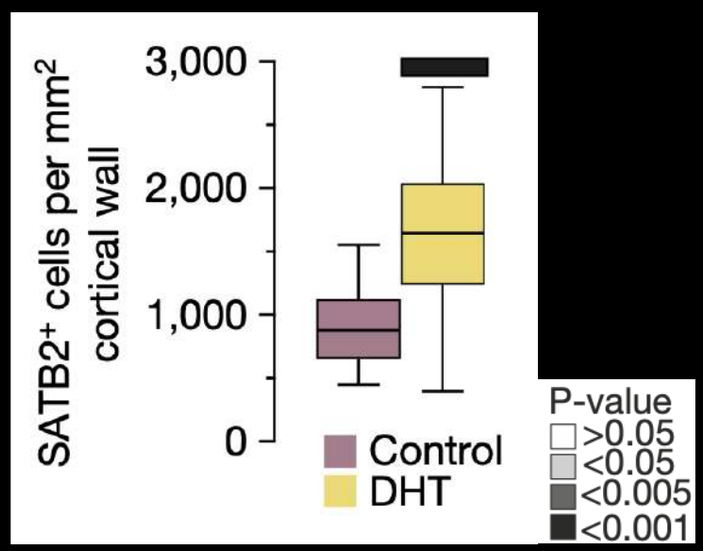


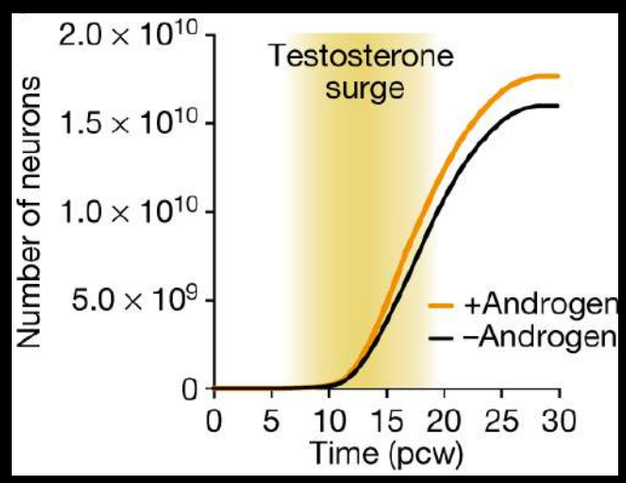
ctrl

DHT

52d

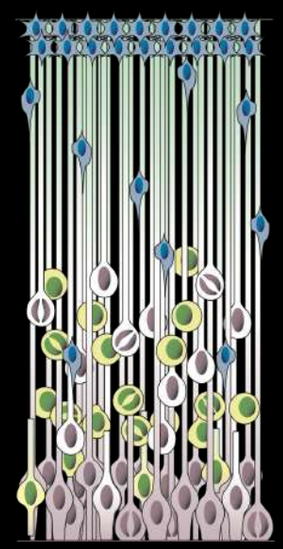


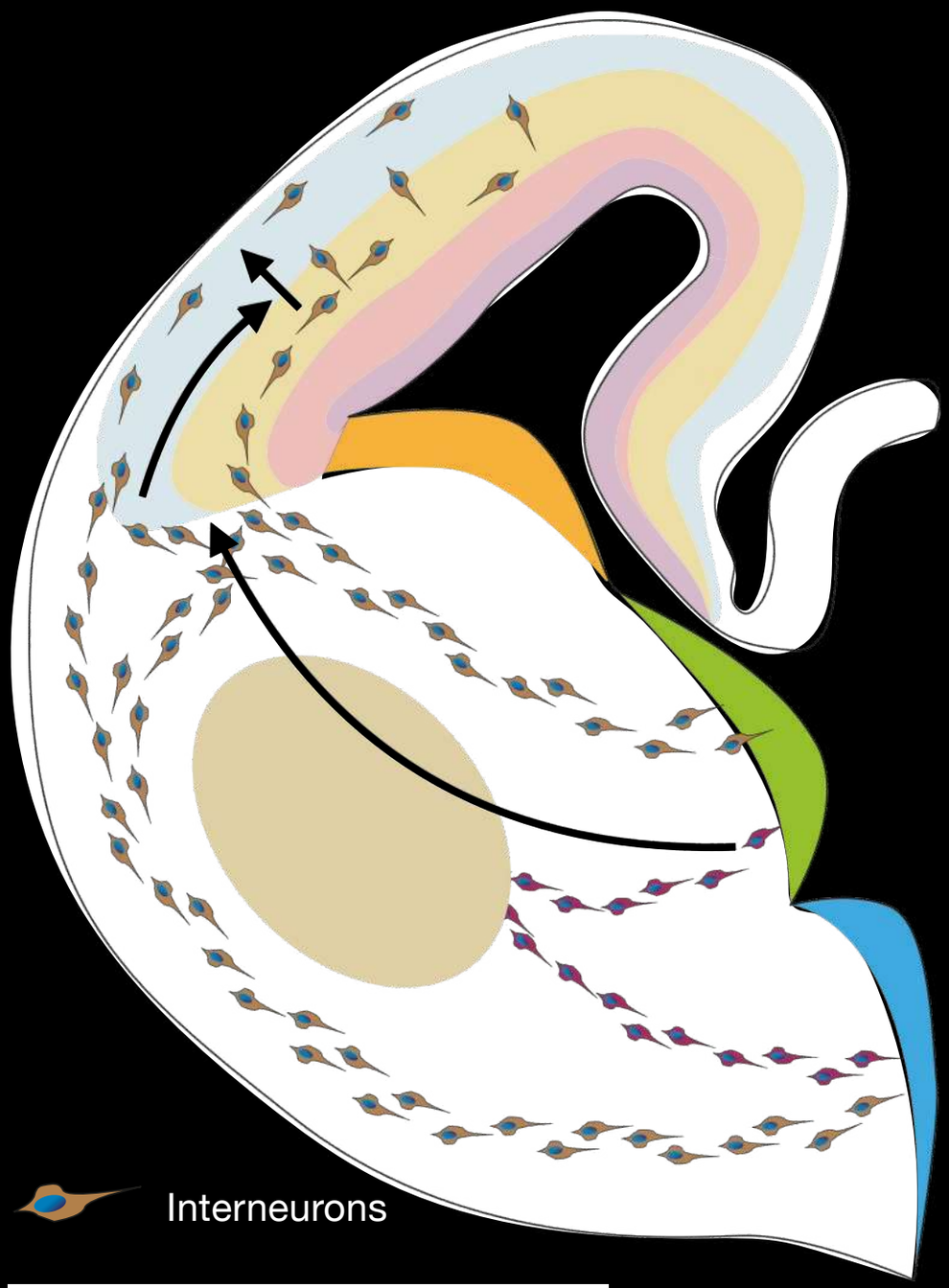




Androgens increase excitatory neurogenic output

Kelava et al., 2022 Nature





Interneurons

ctrl

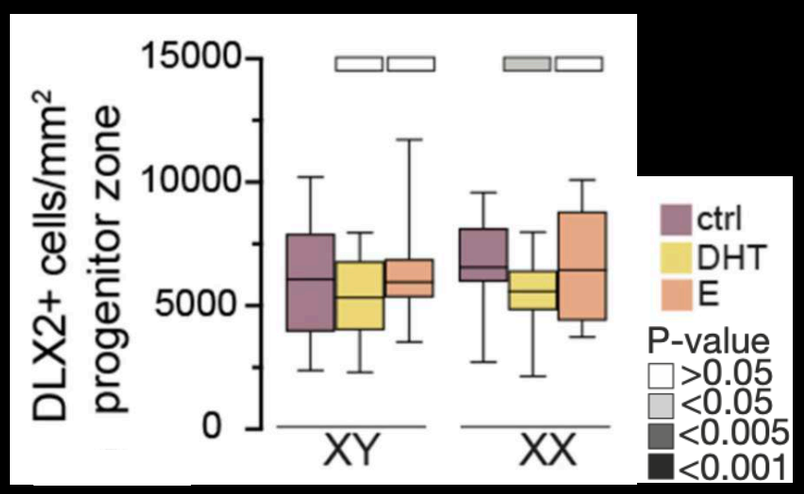
DHT



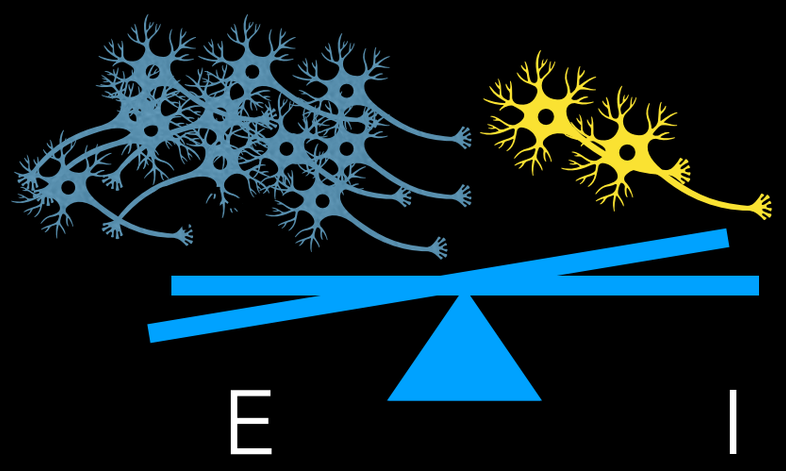


45d

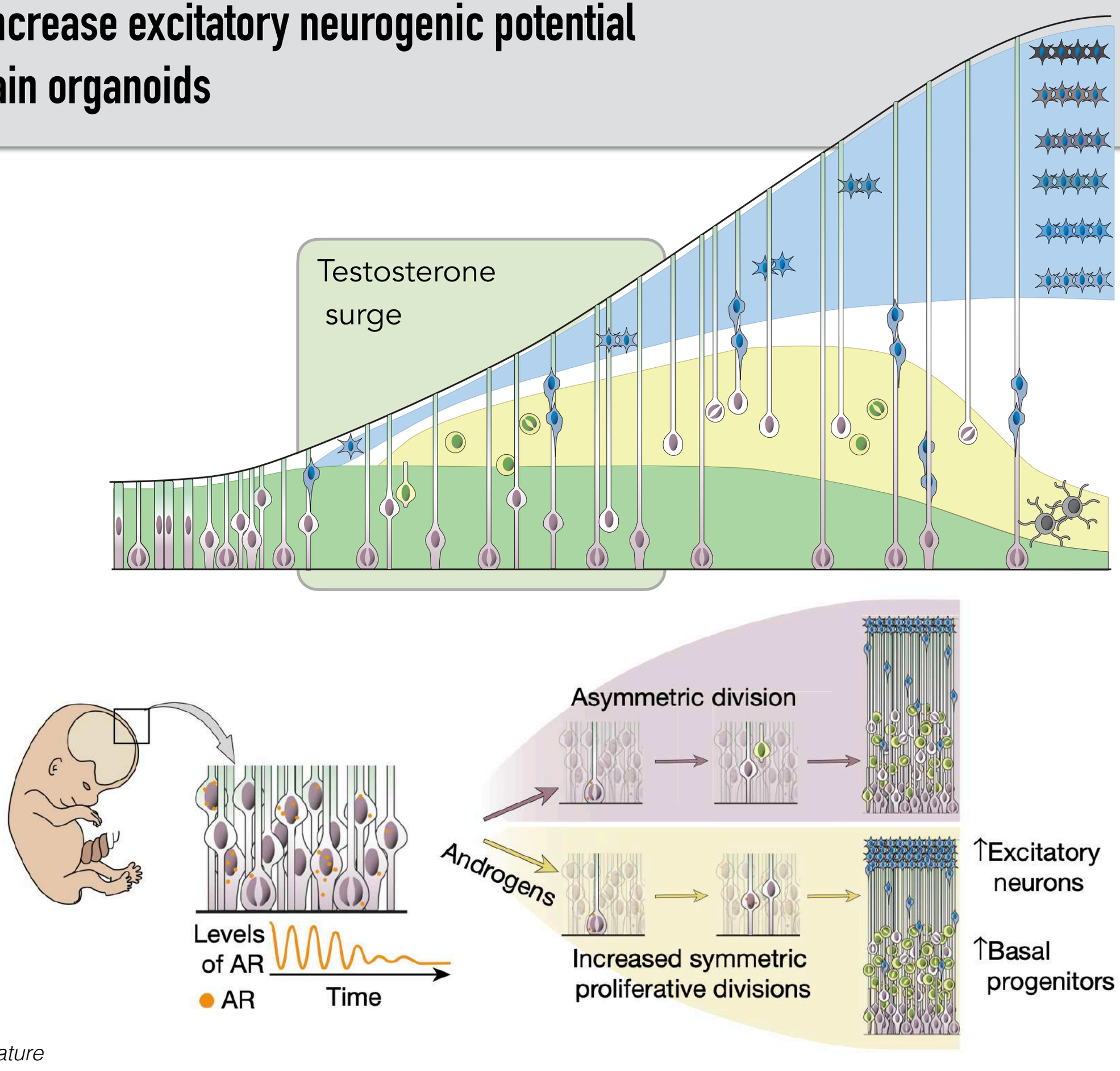
Kelava et al., 2022 Nature



Androgens do not increase inhibitory neurogenesis

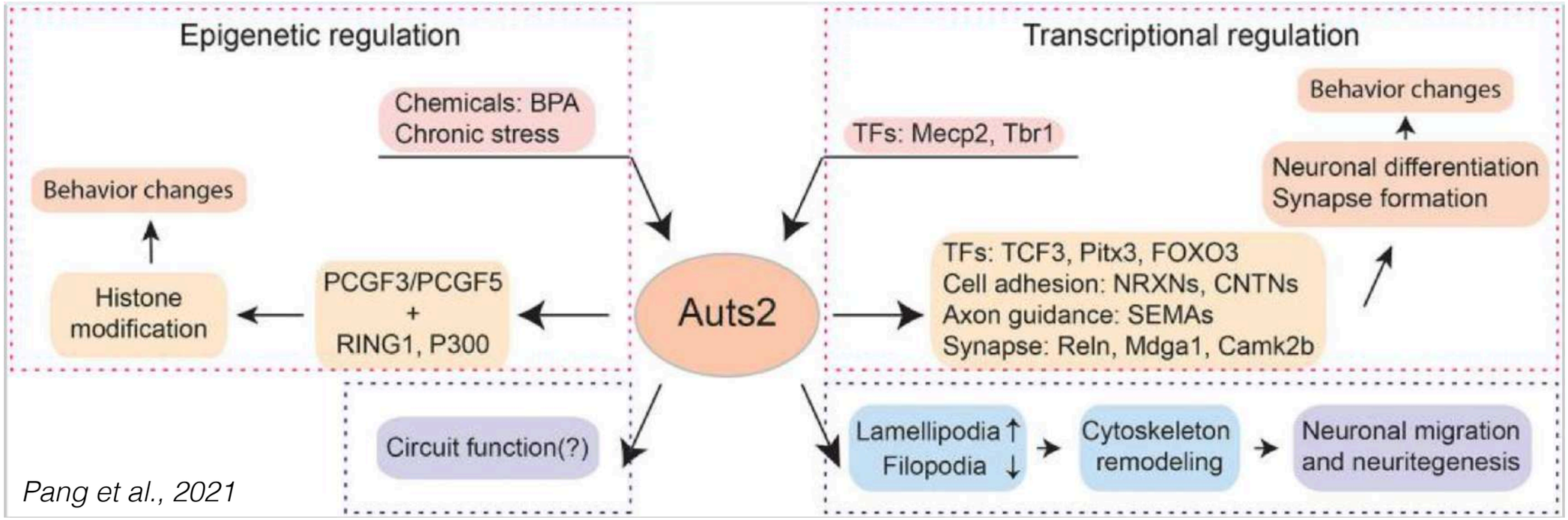


Androgens increase excitatory neurogenic potential in human brain organoids

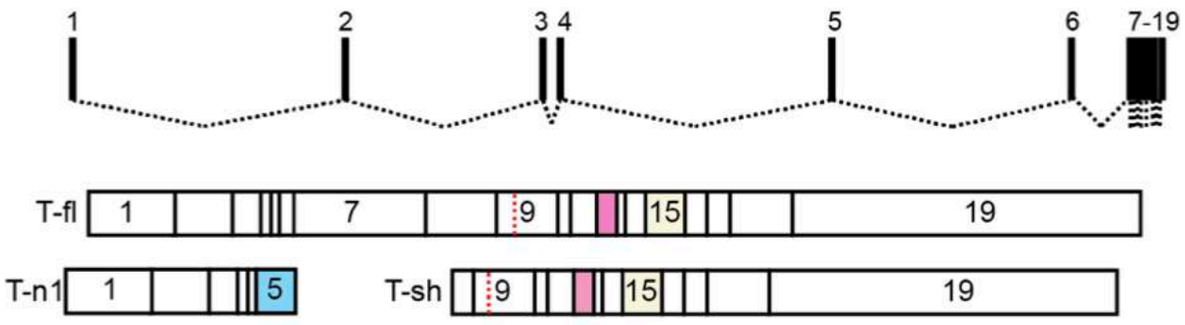


Kelava et al., 2022 Nature

Genetics of ASD – the AUTS2 case



Pang et al., 2021



FL-cytoplasmic and nuclear
cytoskeletal regulation
neuritogenesis
neuronal migration
Hori 2014

Sh-Nuclear
polycomb co-factor
neuronal differentiation
Gao 2014
Beunders 2013

AUTS2 syndrome

Macrocephaly *Talkowski 2012*

Microcephaly *Talkowski 2012, Nagamani 2013, Sultana 2002, Beunders 2013*

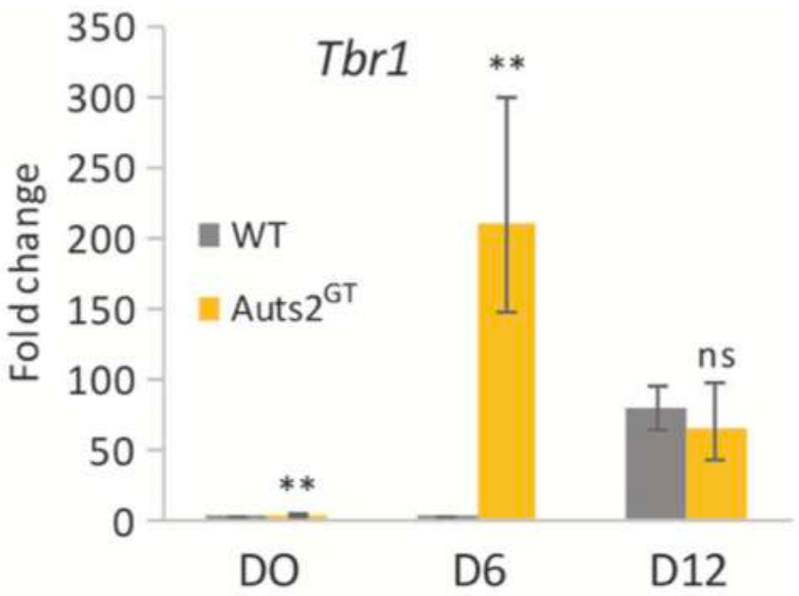
ASD *Sultana 2002, Nagamani 2013*

Epilepsy *Mefford 2010*

Different roles for different isoforms

C-terminally truncated *Auts2* accelerates neuronal differentiation in mouse 2D neurons

(Monderer-Rothkoff et al., 2019)



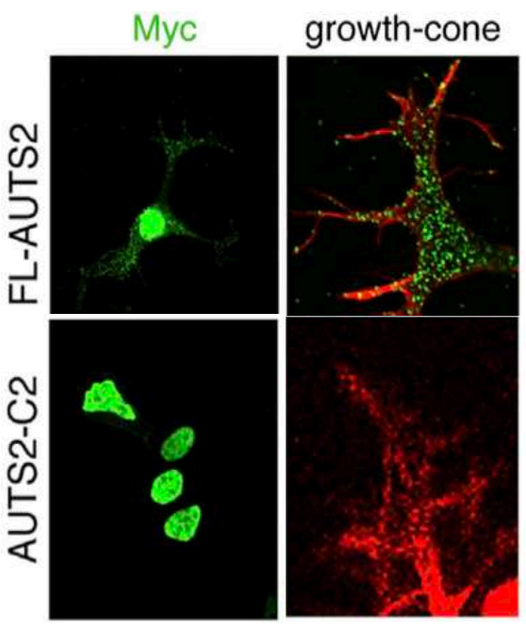
Auts2 KO in zebrafish results in microcephaly rescued by full-length and C-terminal human AUTS2

(Beunders et al., 2013; Oksenberg et al., 2013)

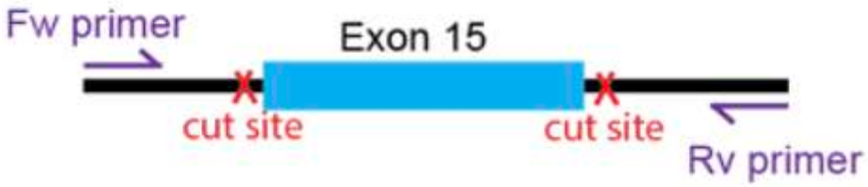


Full-length AUTS2 disruption in mice affected cortical neuronal migration

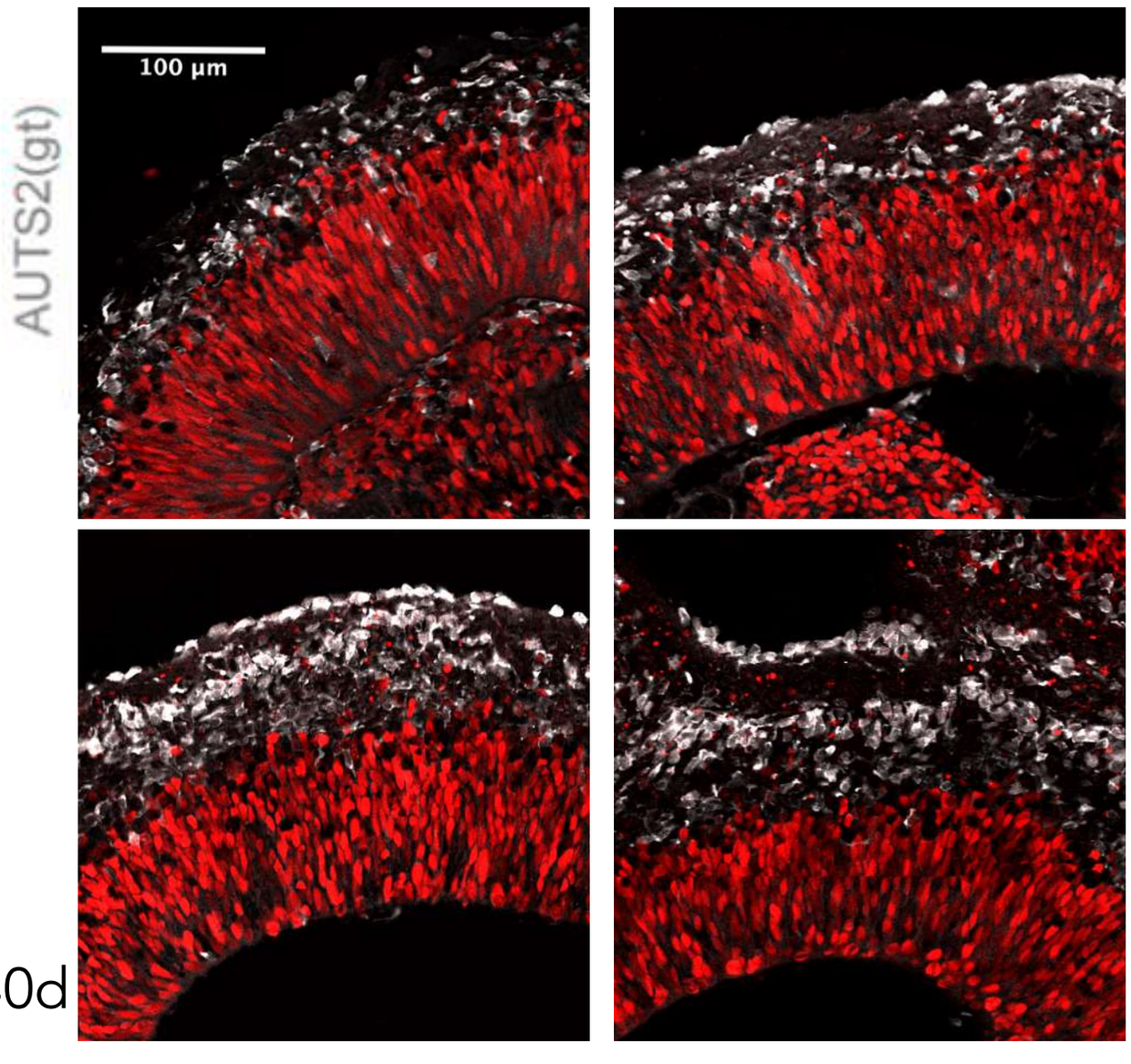
(Hori et al., 2014)

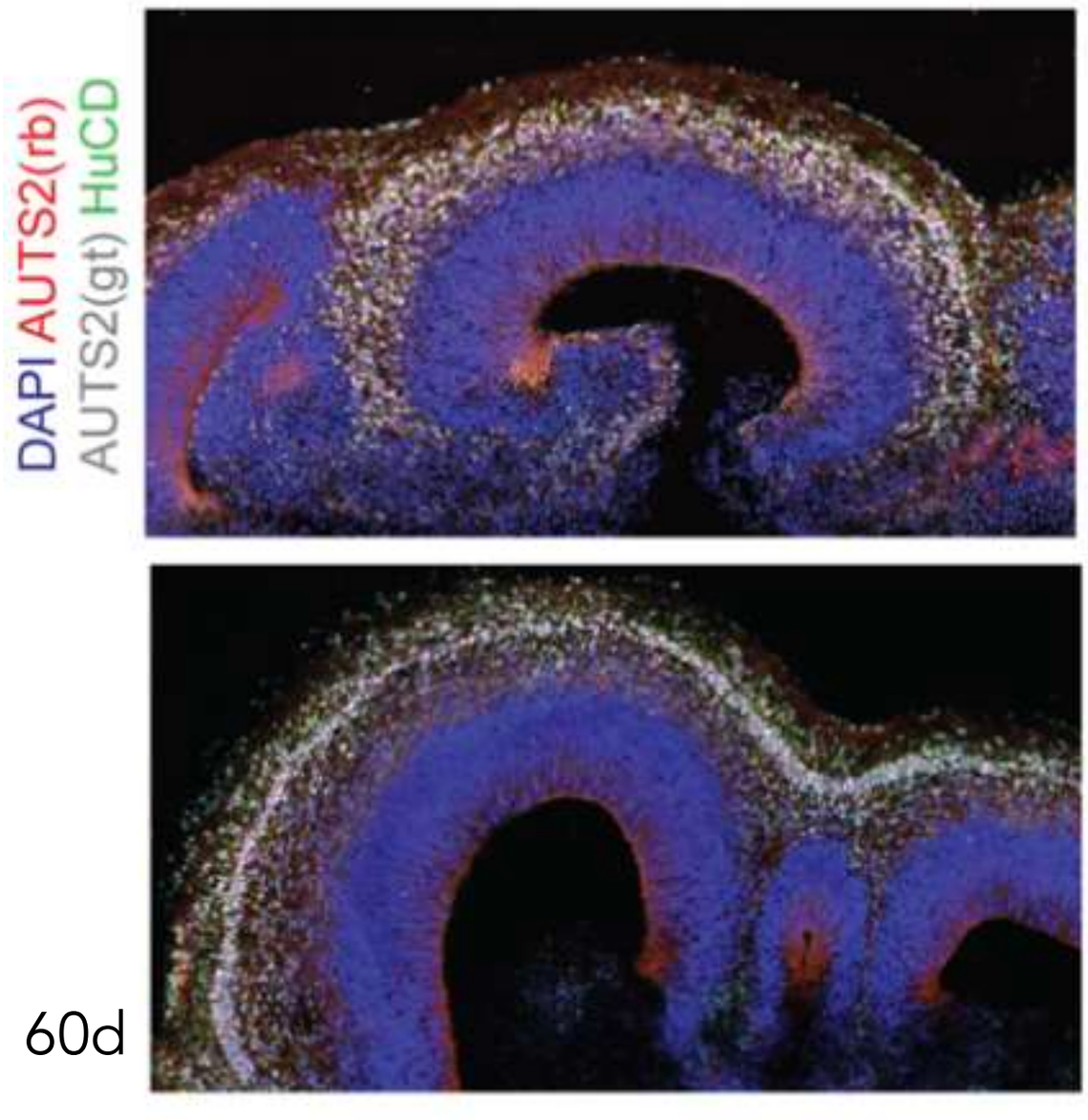


AUTS2del15 results in premature neurogenesis in organoids

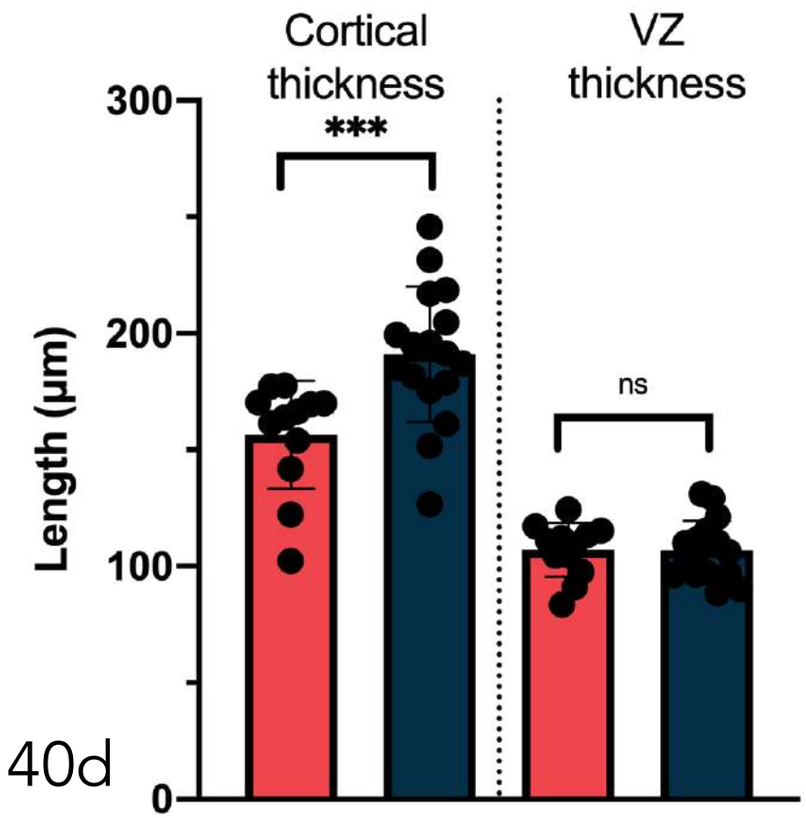


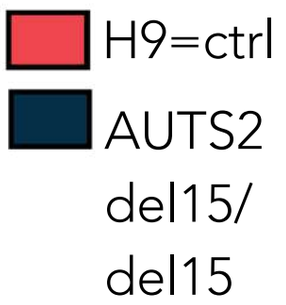
AUTS2del15 results in thicker CP

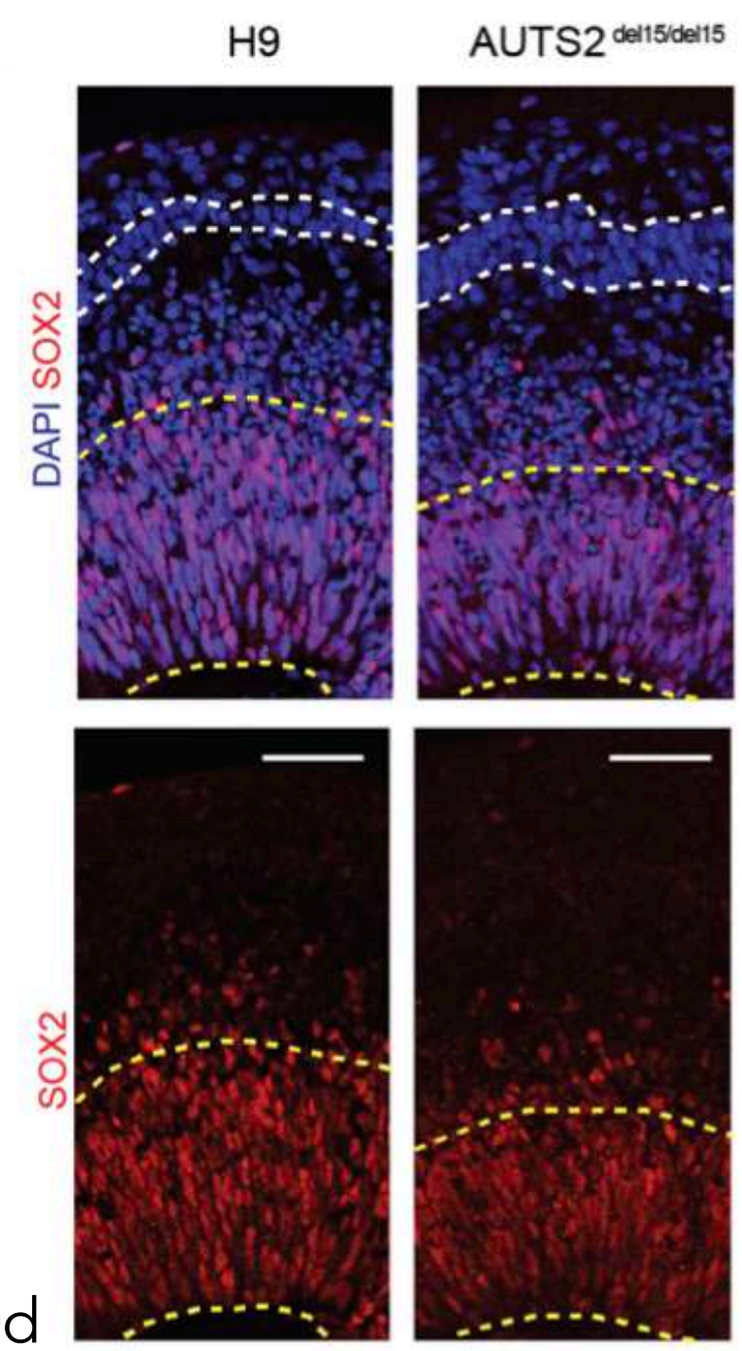


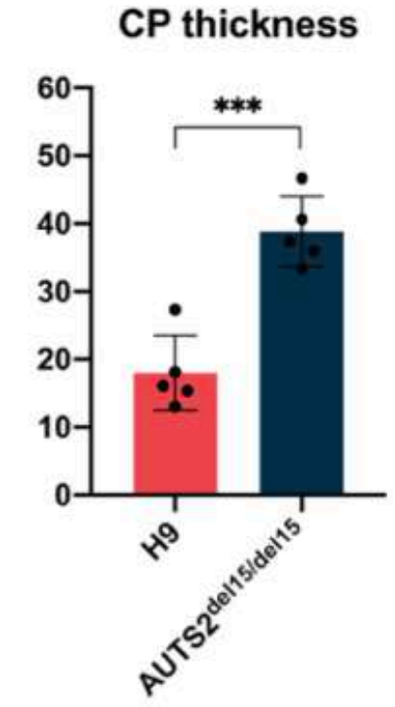


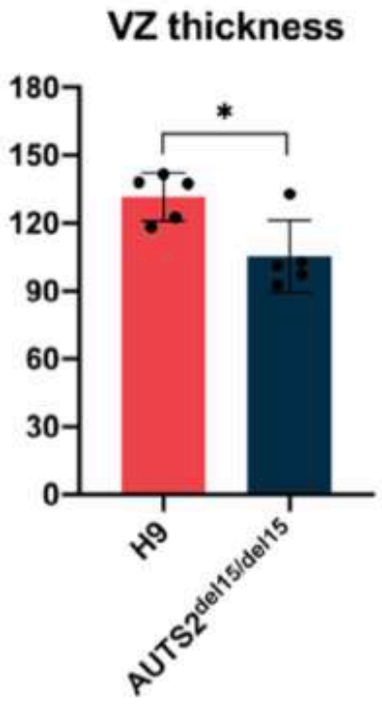
AUTS2del15 results in premature neurogenesis in organoids









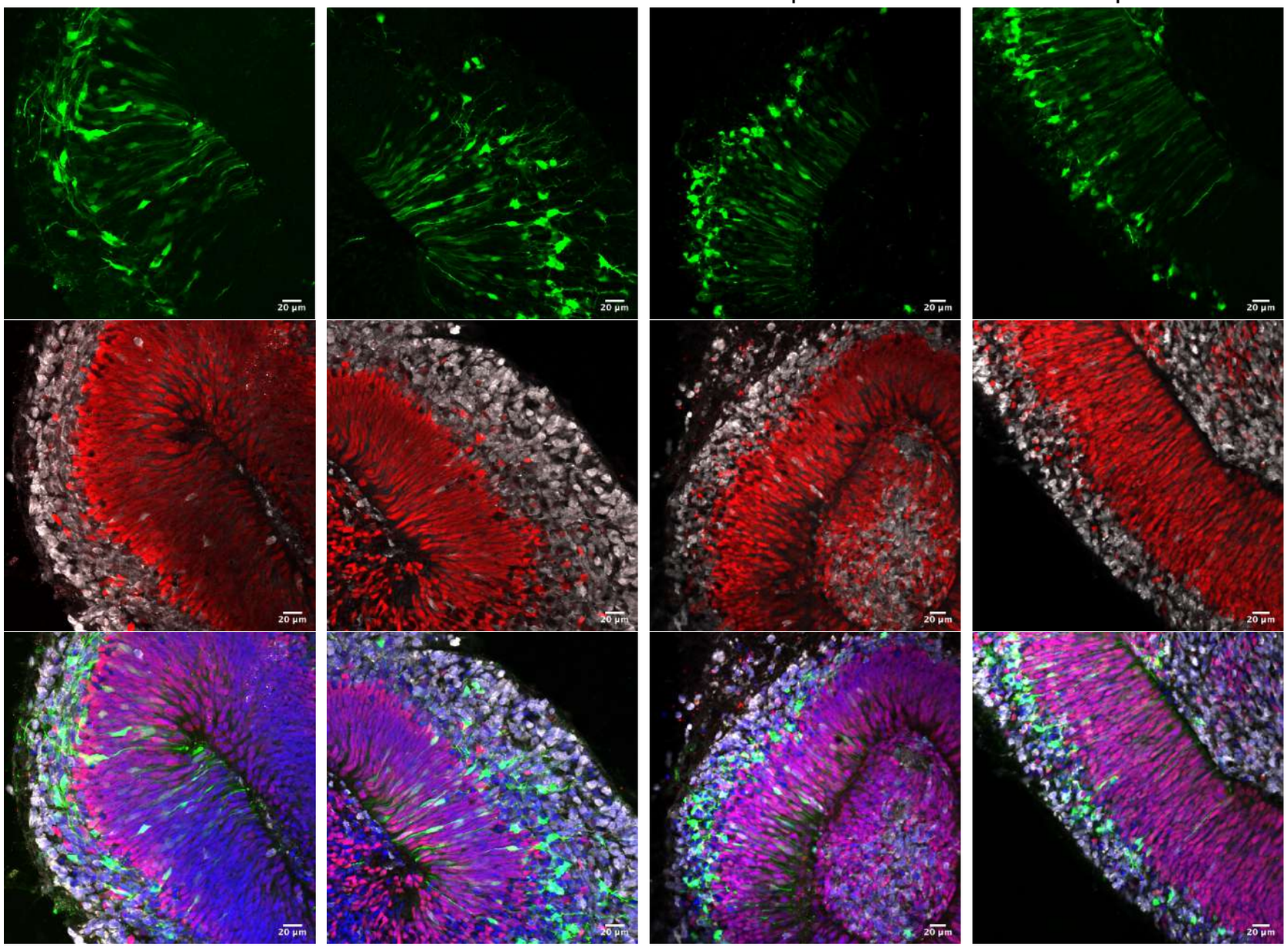


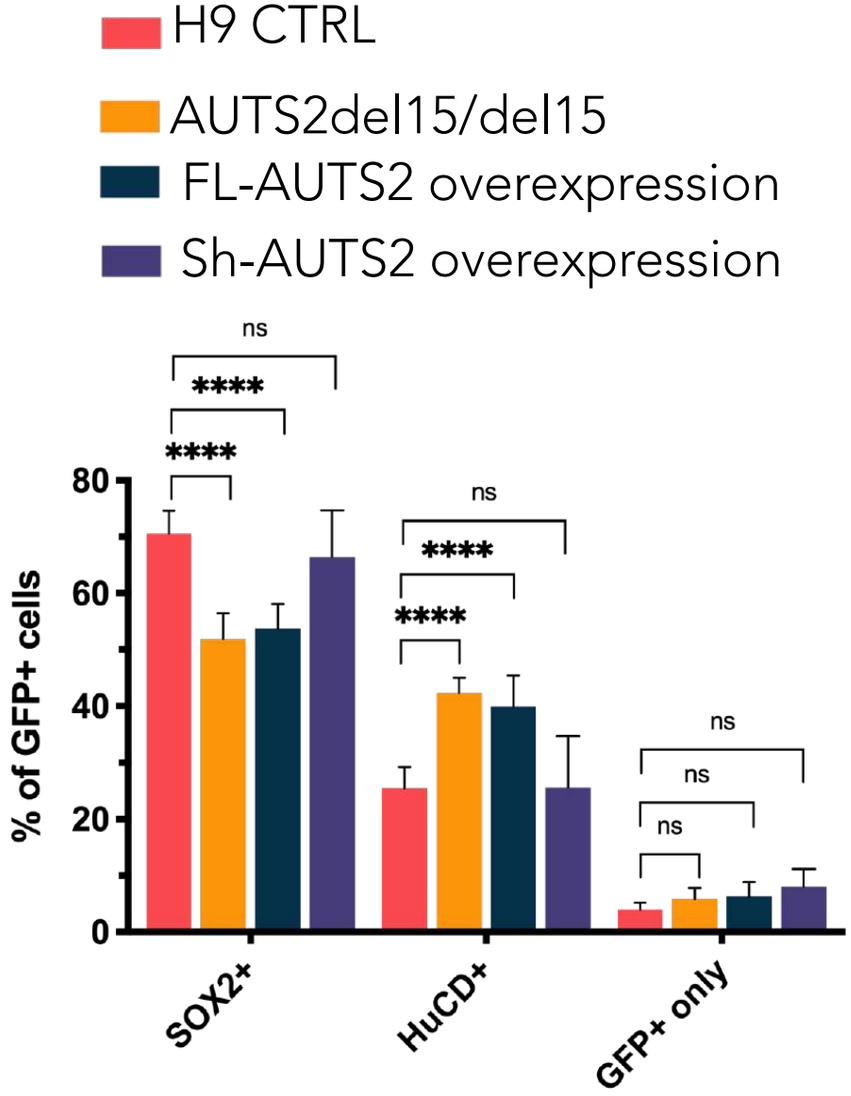
AUTS2del15 results in premature neurogenesis

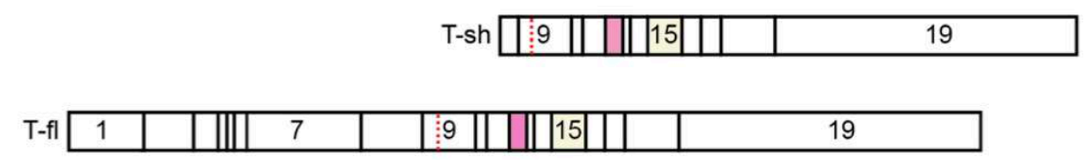
AUTS2 isoform unbalance results in premature neurogenesis

42d; EP@d38

H9 CTRL | AUTS2del15/del15 | FL-AUTS2 overexpression | Sh-AUTS2 overexpression







References

Chiaradia I, Lancaster MA. Brain organoids for the study of human neurobiology at the interface of in vitro and in vivo. Nat Neurosci. 2020 Dec;23(12):1496-1508. doi: 10.1038/s41593-020-00730-3. Epub 2020 Nov 2. PMID: 33139941.

Kelava I, Lancaster MA. Dishing out mini-brains: Current progress and future prospects in brain organoid research. Dev Biol. 2016 Dec 15;420(2):199-209. doi: 10.1016/j.ydbio.2016.06.037. Epub 2016 Jul 9. PMID: 27402594; PMCID: PMC5161139.

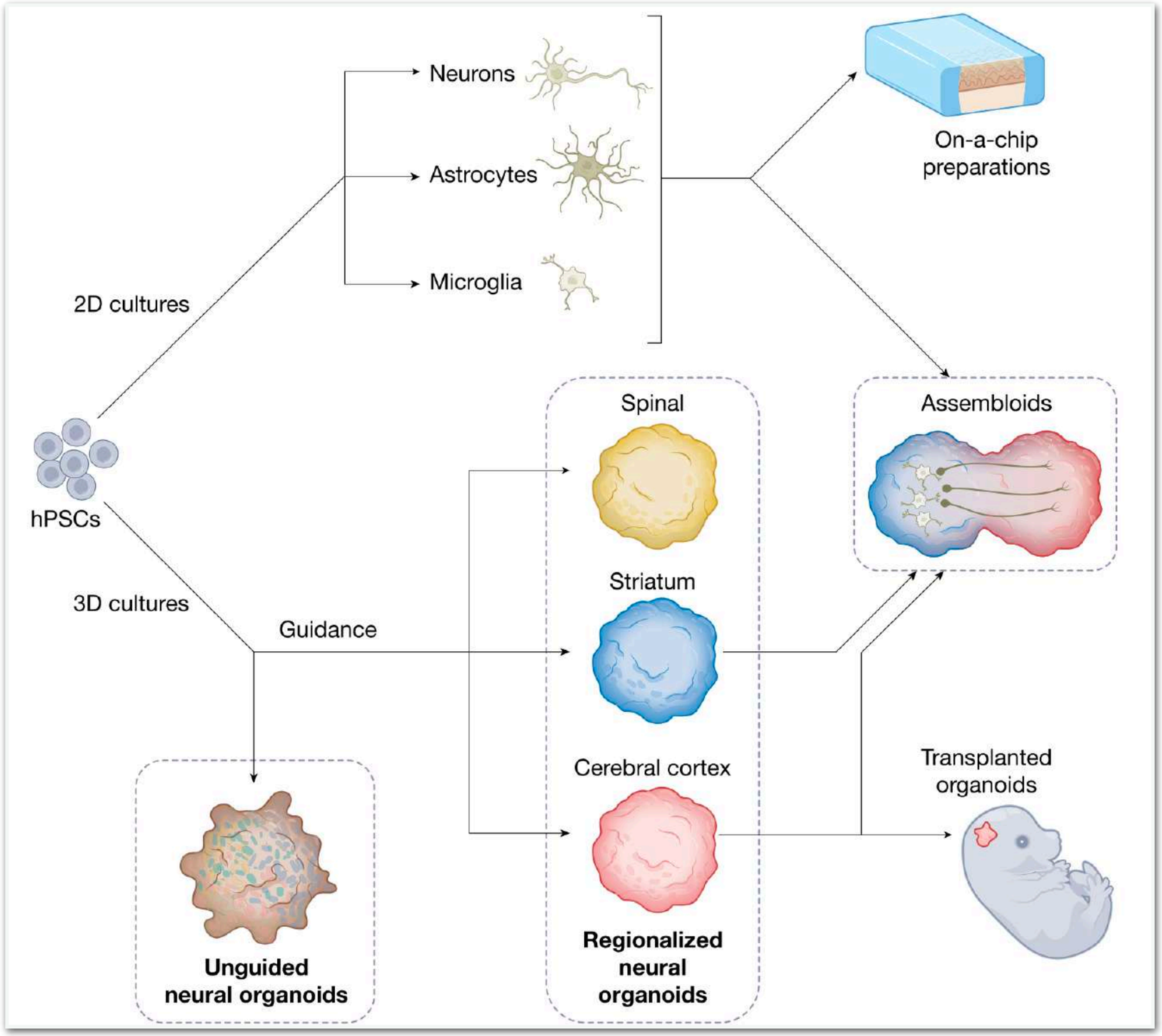
Lancaster MA, Renner M… Cerebral organoids model human brain development and microcephaly. Nature. 2013 Sep 19;501(7467):373-9. doi: 10.1038/nature12517. Epub 2013 Aug 28. PMID: 23995685; PMCID: PMC3817409.

Sutcliffe MA, Wong E, Wingett SW, Morris CAJ, Stachelscheid H, Schoenfelder S, Lancaster MA. Reversible epiblast regionalization determines differentiation potential of human pluripotent stem cells. Nat Biotechnol. 2026 Aug 7. doi: 10.1038/s41587-026-03254-6. Epub ahead of print. PMID: 42567931.

Benito-Kwiecinski S, Giandomenico SL… An early cell shape transition drives evolutionary expansion of the human forebrain. Cell. 2021 Apr 15;184(8):2084-2102.e19. doi: 10.1016/j.cell.2021.02.050. Epub 2021 Mar 24. PMID: 33765444; PMCID: PMC8054913.

Kelava I, Chiaradia I, Pellegrini L, Kalinka AT, Lancaster MA. Androgens increase excitatory neurogenic potential in human brain organoids. Nature. 2022 Feb;602(7895):112-116. doi: 10.1038/s41586-021-04330-4. Epub 2022 Jan 19. PMID: 35046577; PMCID: PMC7612328.

Different types of nervous system organoids



Pasca et al., 2022 doi.org/10.1038/s41586-022-05219-6.

Brain organoids, SFEBq, spheroids, forebrain organoids



Lancaster et al., 2013

Brain organoids

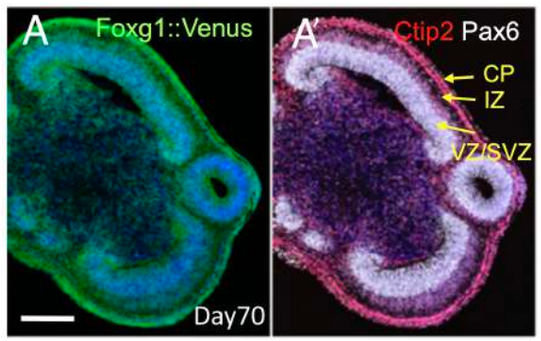


- Default neuralisation (different brain regional identities). Addition of FGF2 for the maintenance of stem cells' pluripotency and proliferation of neural progenitors
- Forebrain organising centers
- Large ventricles
- Discrete progenitor and neuronal layers
- High complexity

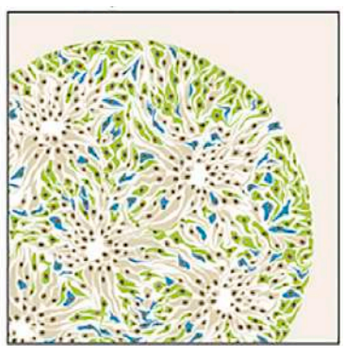


Kadoshima et al., 2013

SFEBq

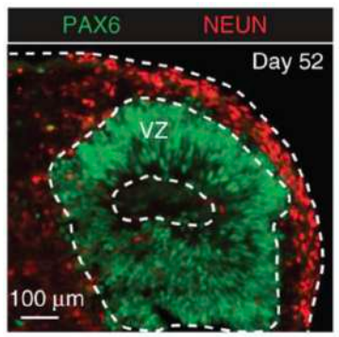


- Directed neuralisation through TFGβ and WNT inhibition (telencephalic identity)
- Large ventricles
- Discrete progenitor and neuronal layers
- High complexity

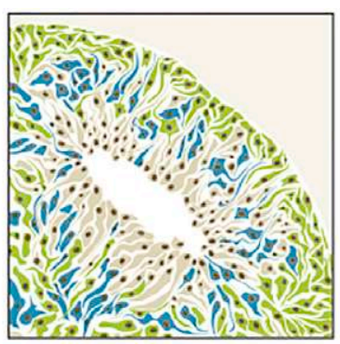


Pasca et al., 2015

Spheroids

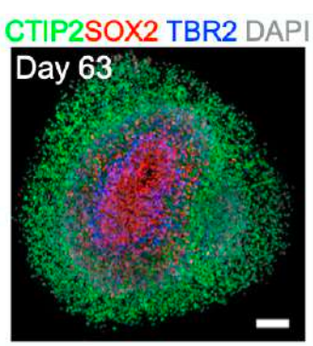


- Directed neuralisation through TFGβ and BMP inhibition (forebrain identity)
- Small ventricles
- Reproducibility
- Homogeneity



Qian et al., 2016

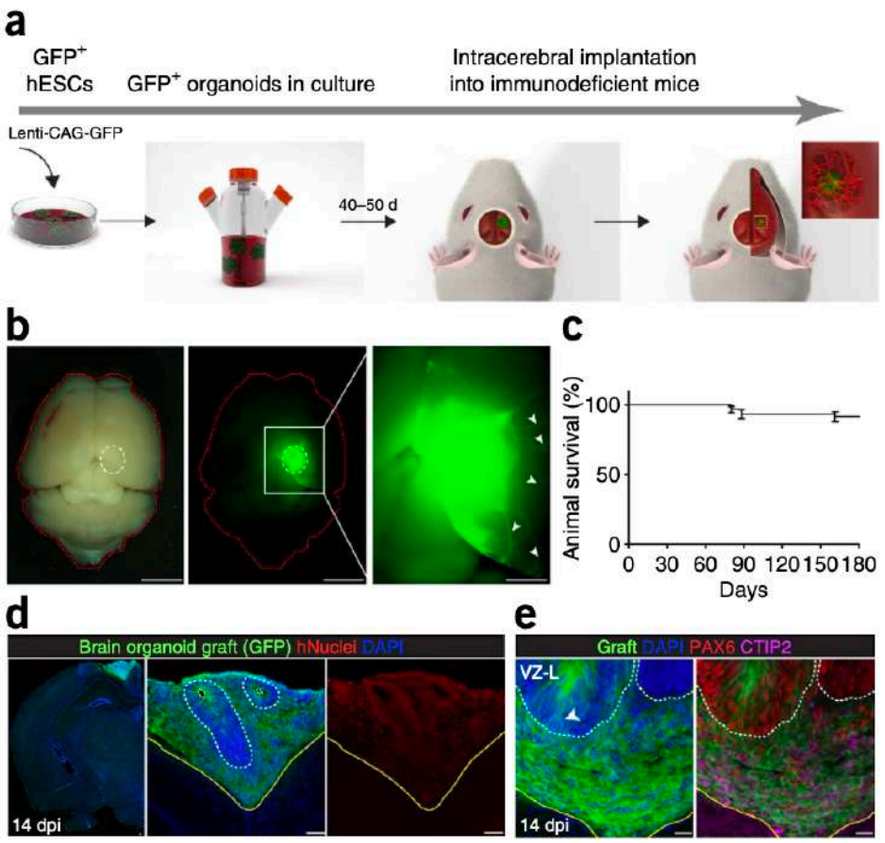
Forebrain organoids

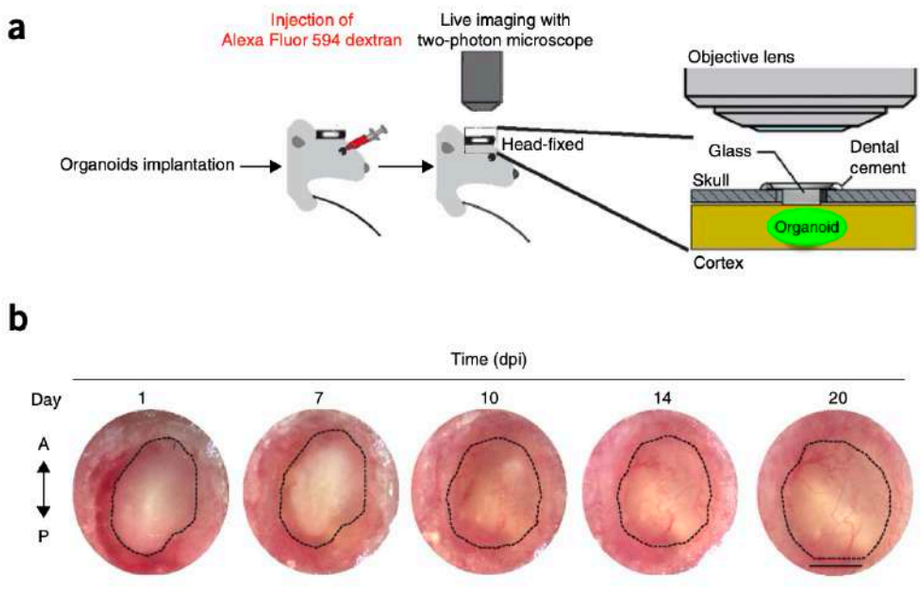


- Directed neuralisation through TFGβ and BMP inhibition (forebrain identity)
- Small size organoids
- Reproducibility

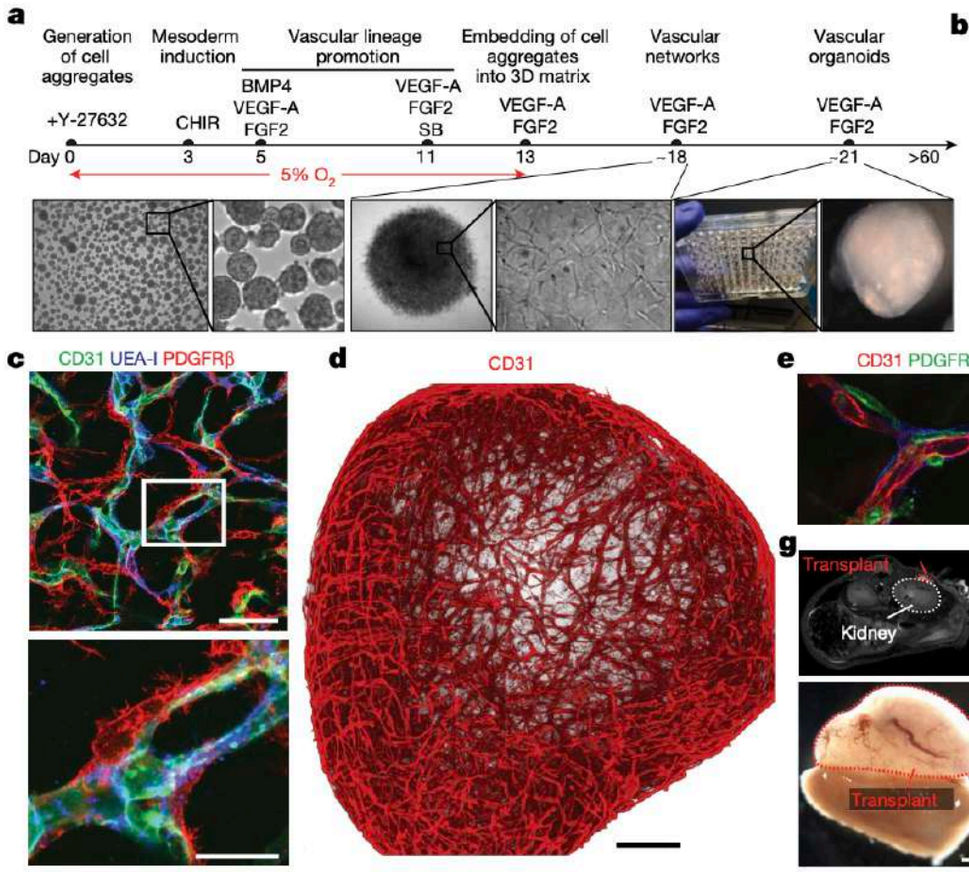
doi.org/10.1038/s41586-022-05219-6.

Brain organoids lack vasculature

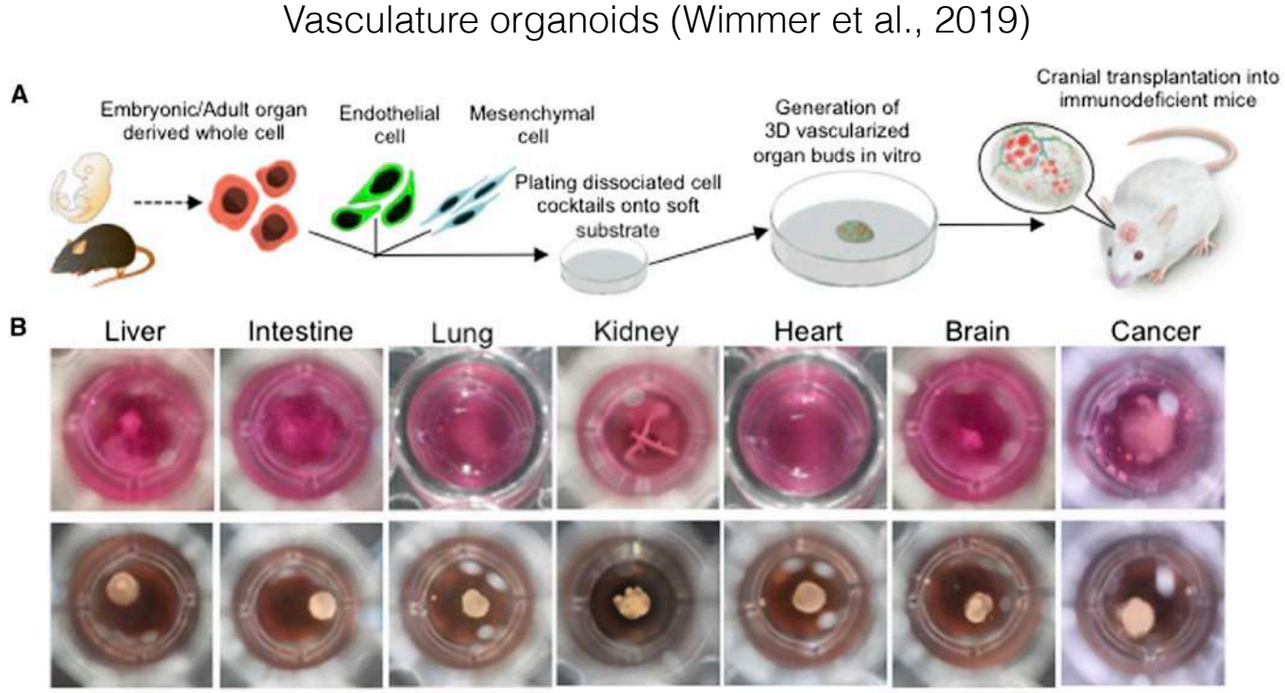




Transplantation in vivo (Mansour et al., 2018)



Vasculature organoids (Wimmer et al., 2019)



Co-culture with mesenchymal and endothelial cells (Takebe et al., 2015)