

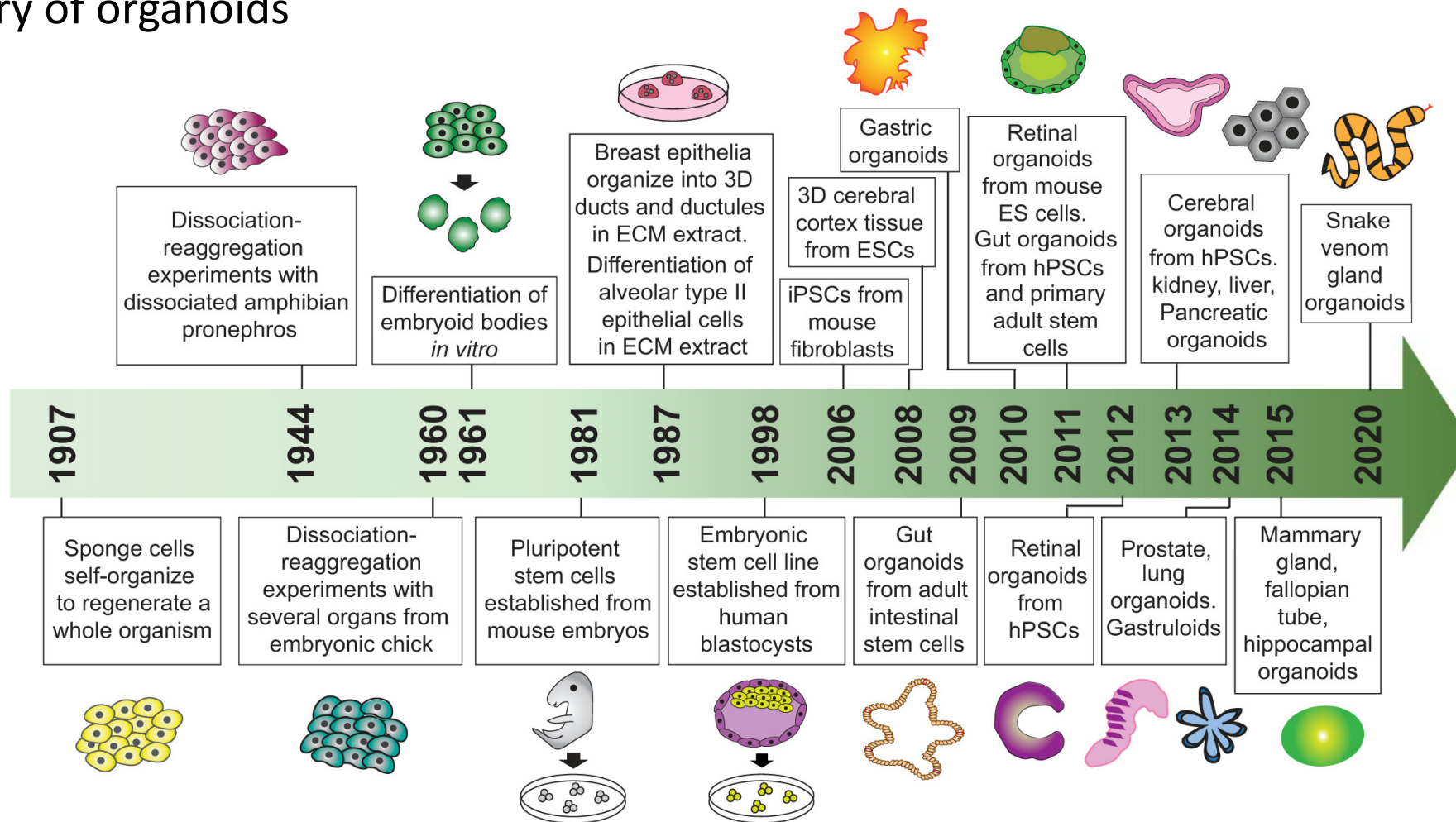
# Using organoids to study human brain evolution

Journal Club

05.10.2021

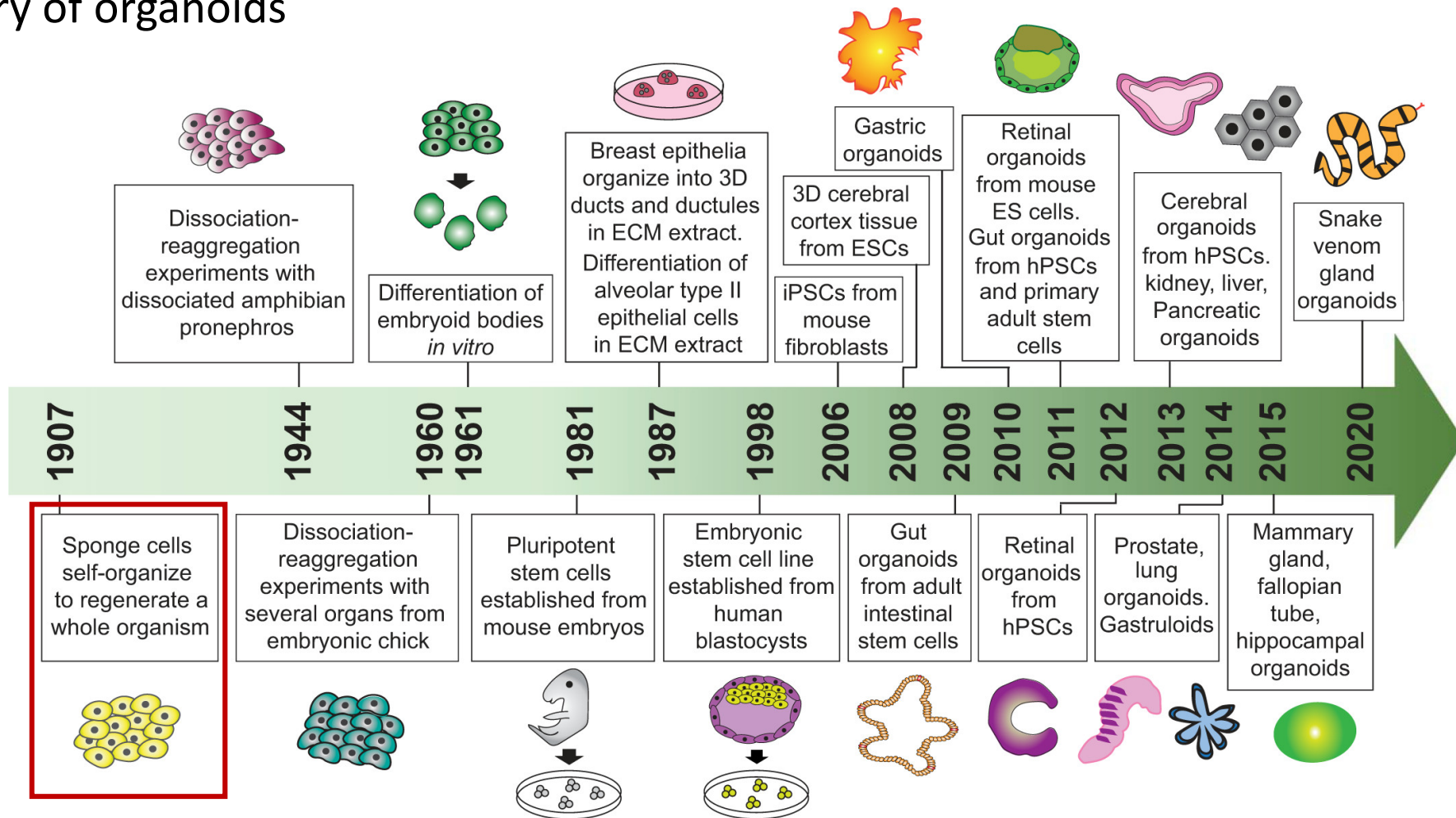
# Organoids

## Short history of organoids



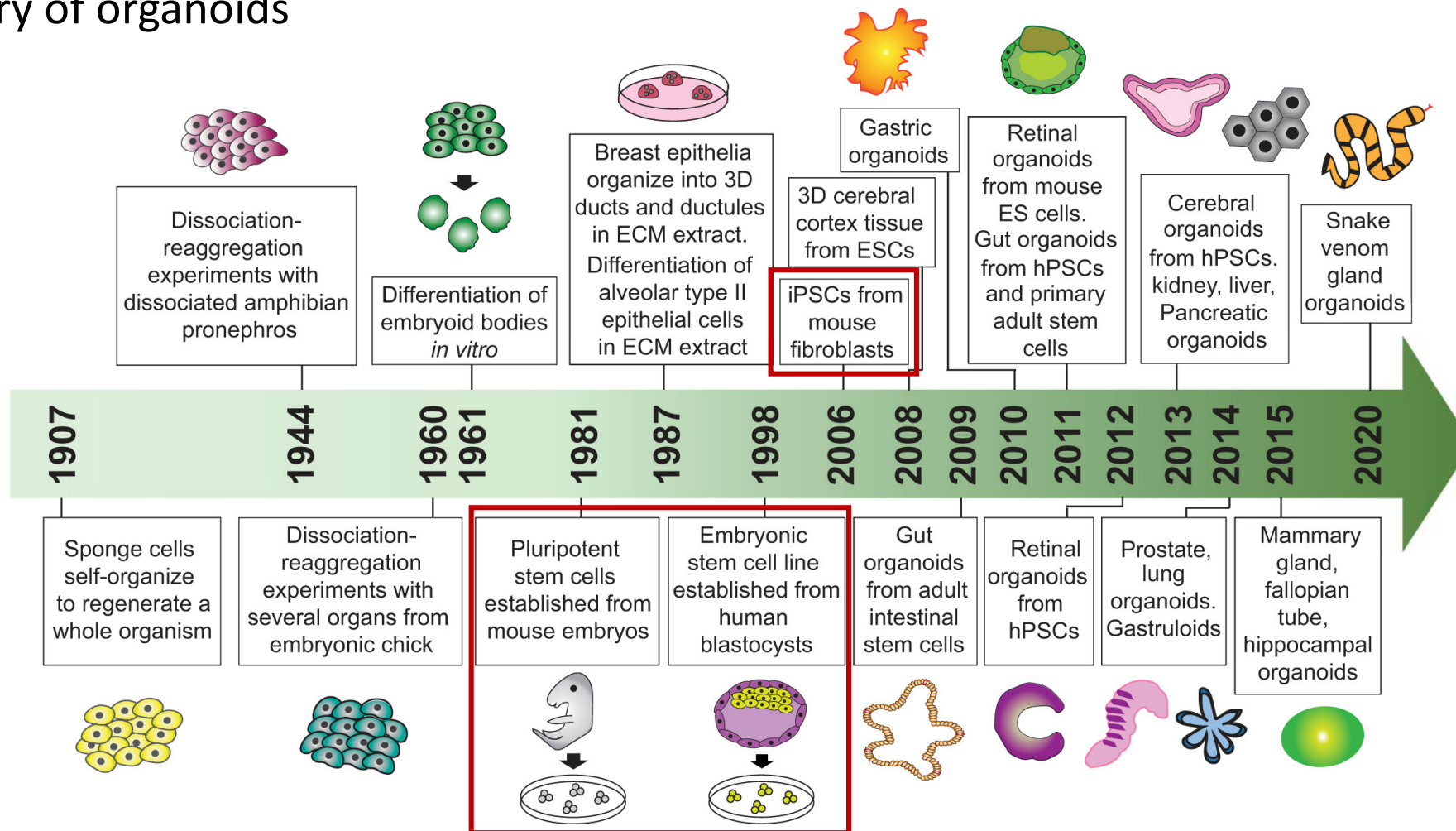
# Organoids

## Short history of organoids



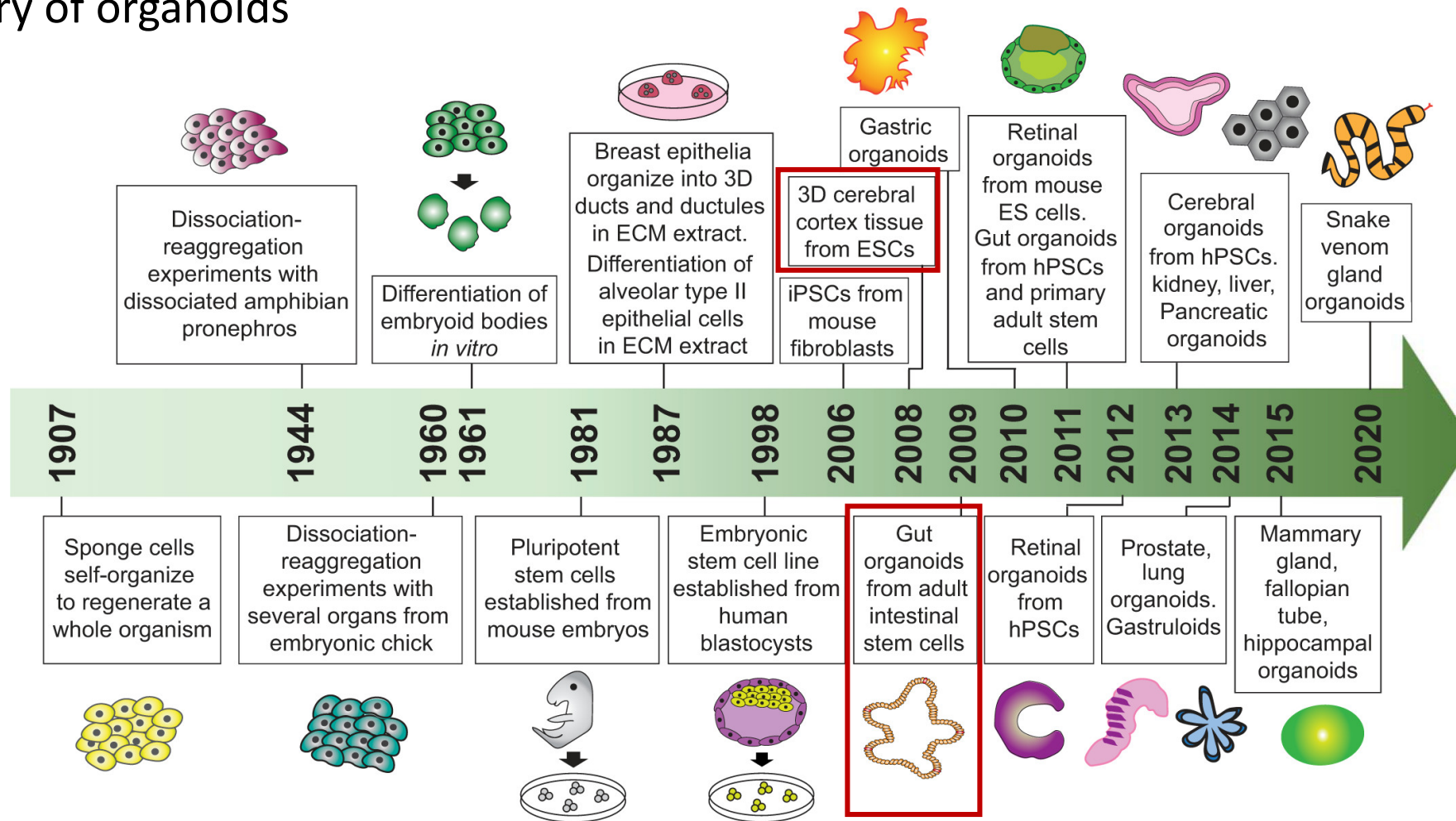
# Organoids

## Short history of organoids



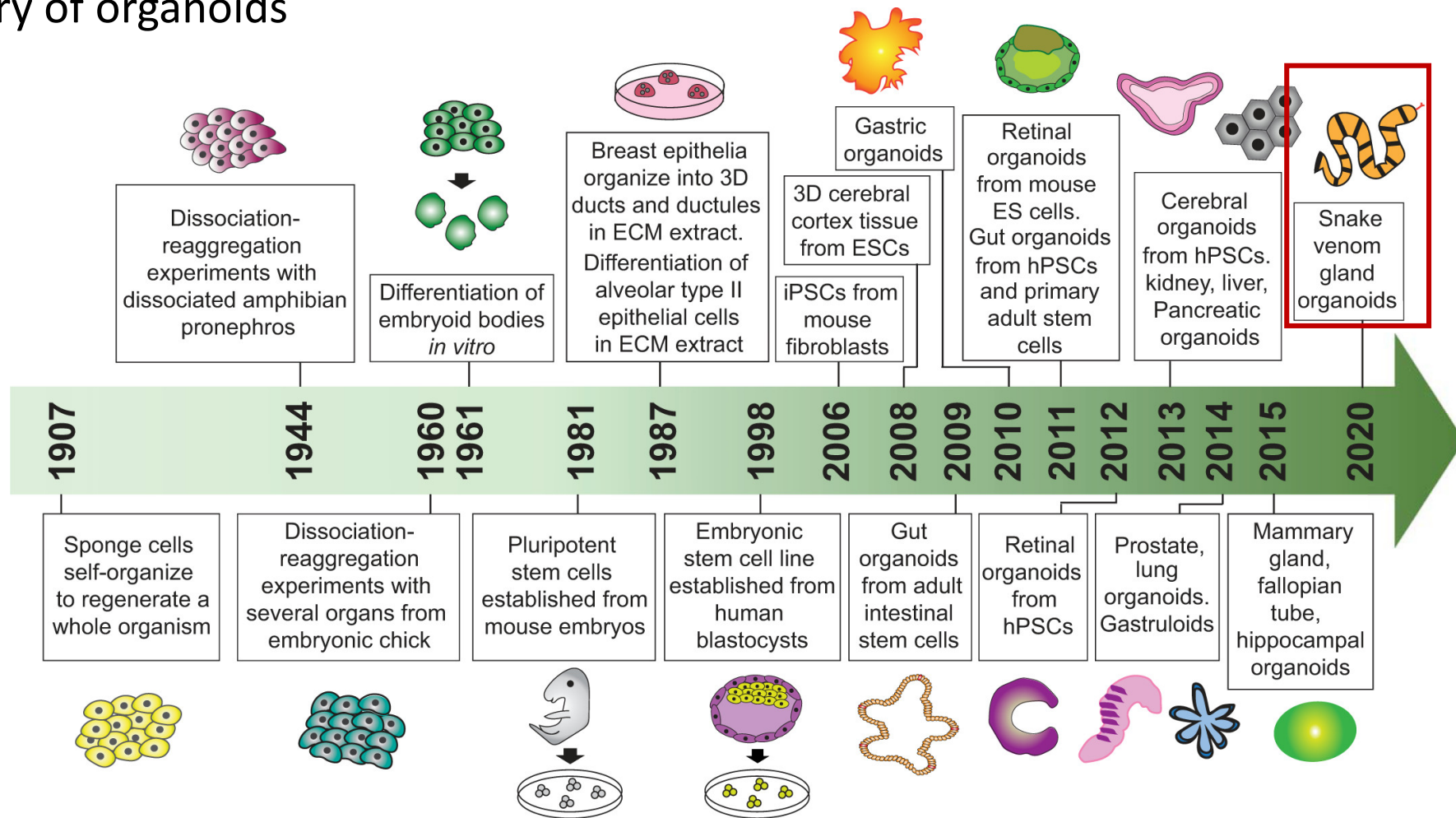
# Organoids

## Short history of organoids



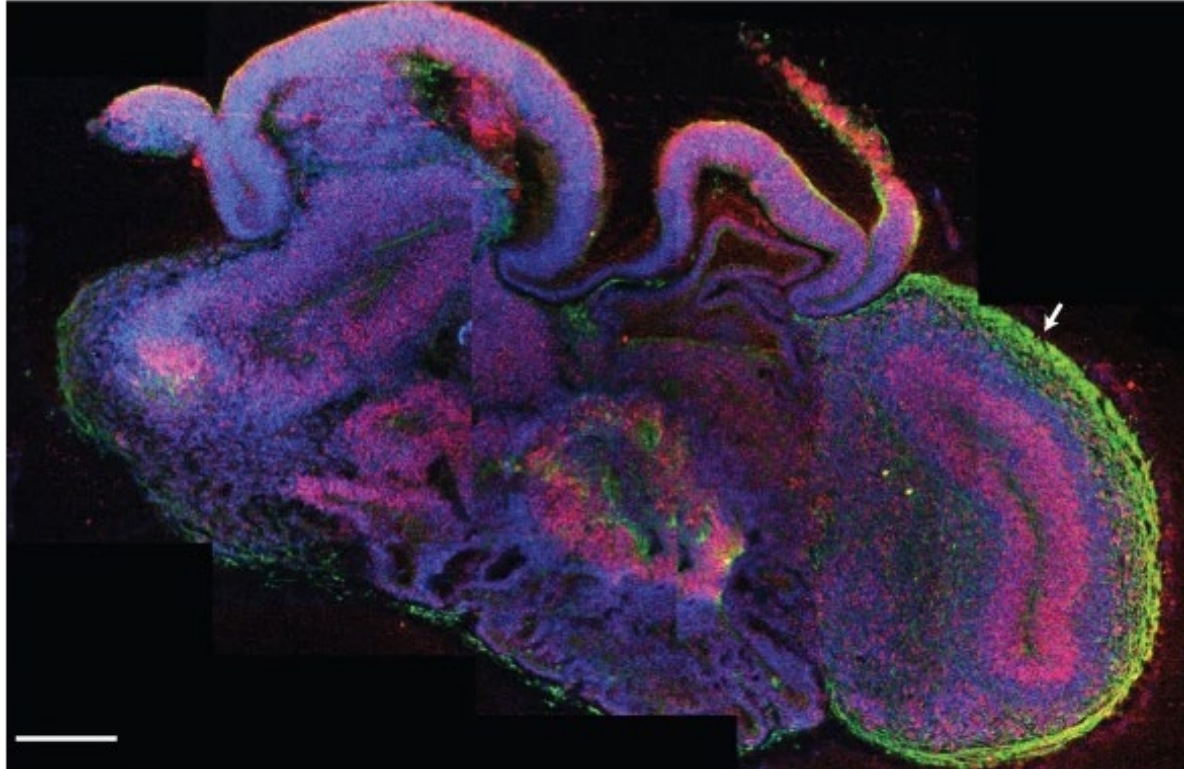
# Organoids

## Short history of organoids



# Brain organoids

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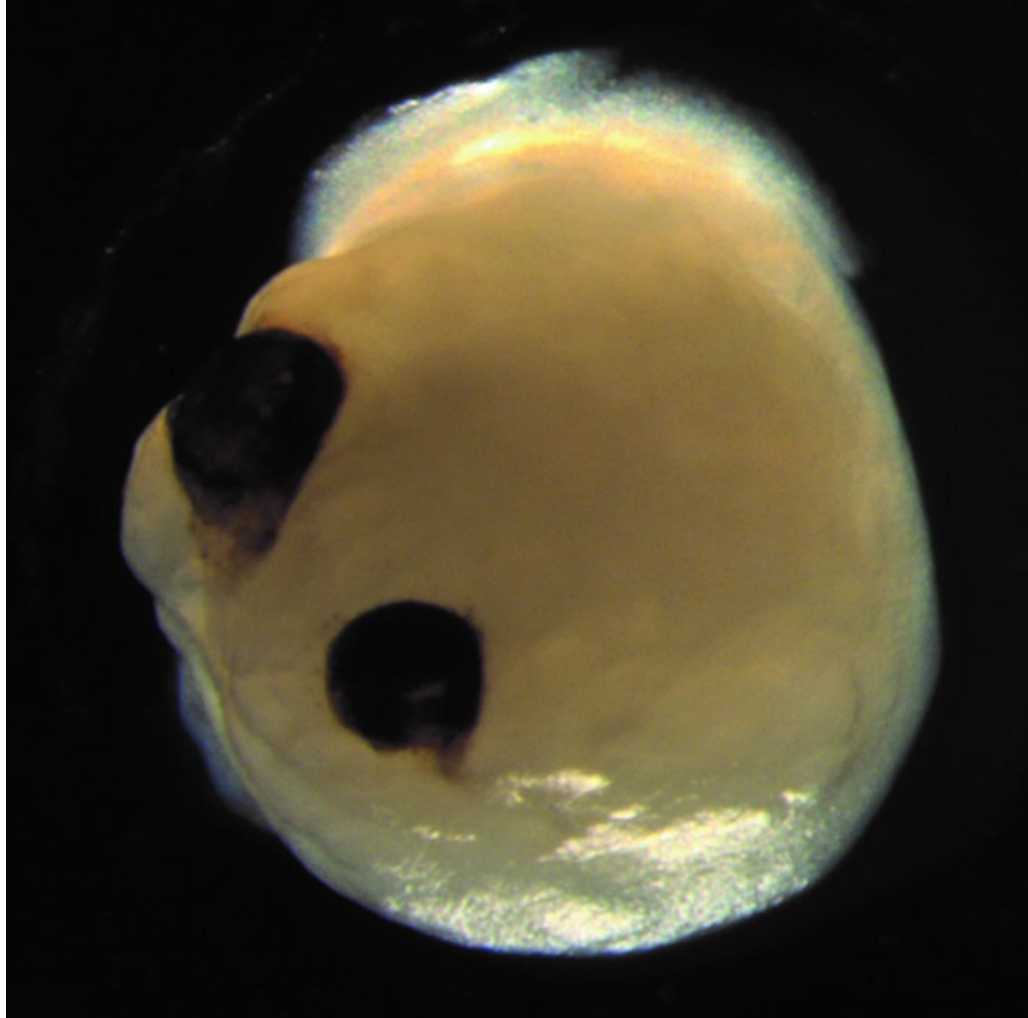
Lancaster et al, Nature, 2013

2013: First description of a human cerebral organoid derived from human pluripotent stem cells

Since then, technology developed rapidly, and a big variety of approaches can be used to derive organoids, recapitulating different brain regions

# One of the newest family members

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Human brain organoids assemble functionally integrated bilateral optic vesicles

This optic vesicle-containing brain organoids were sensitive to light!

What does the future hold?



# Brain organoids to investigate human brain-specific evolution

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Besides brain development and disease modeling, brain organoids are now being investigated as a model for human brain evolution

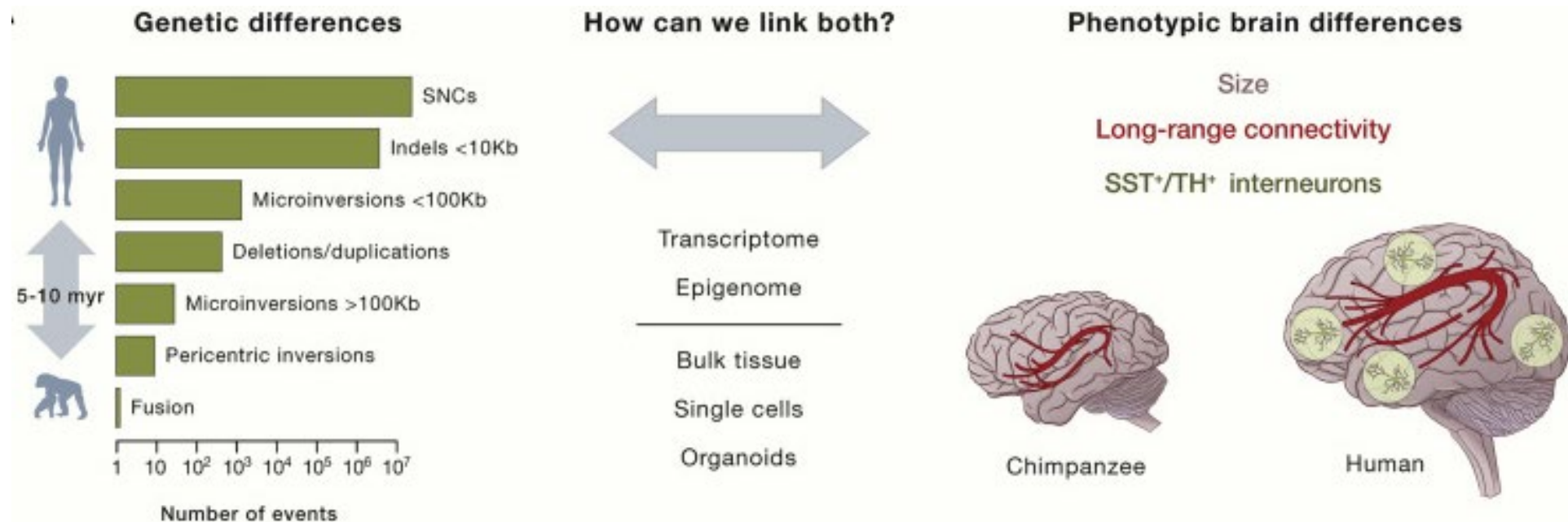
The human brain:

- is bigger
- has a greater number of neurons and glia
- has evolved new neural circuits and functional regions, particularly in secondary associative areas of the cerebral cortex
- has distinct aspects of cognition, such as syntactical-grammatical language, working memory, decision making, and theory of mind

when compared to our closest relatives such as Chimpanzees

# Brain organoids to investigate human brain-specific evolution

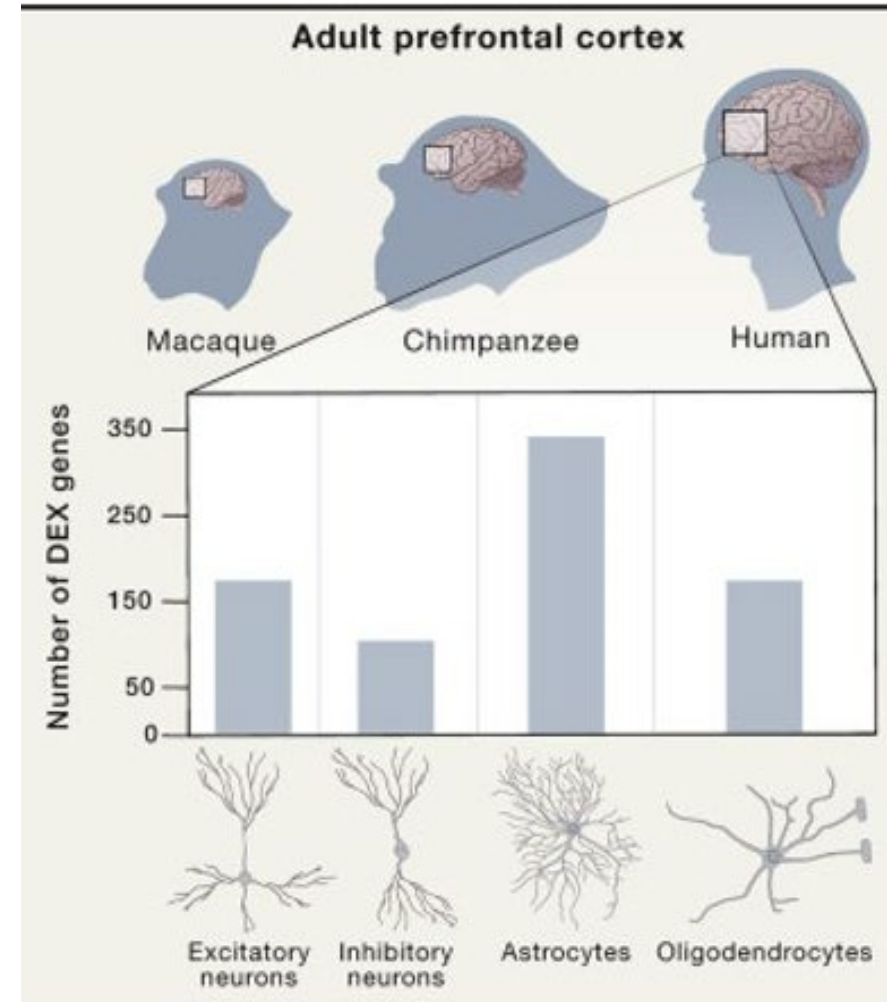
Understanding the evolution of these features requires to first dissect and catalog phenotypic changes using comparative analyses and then associate those changes with their underlying primary genetic causes.



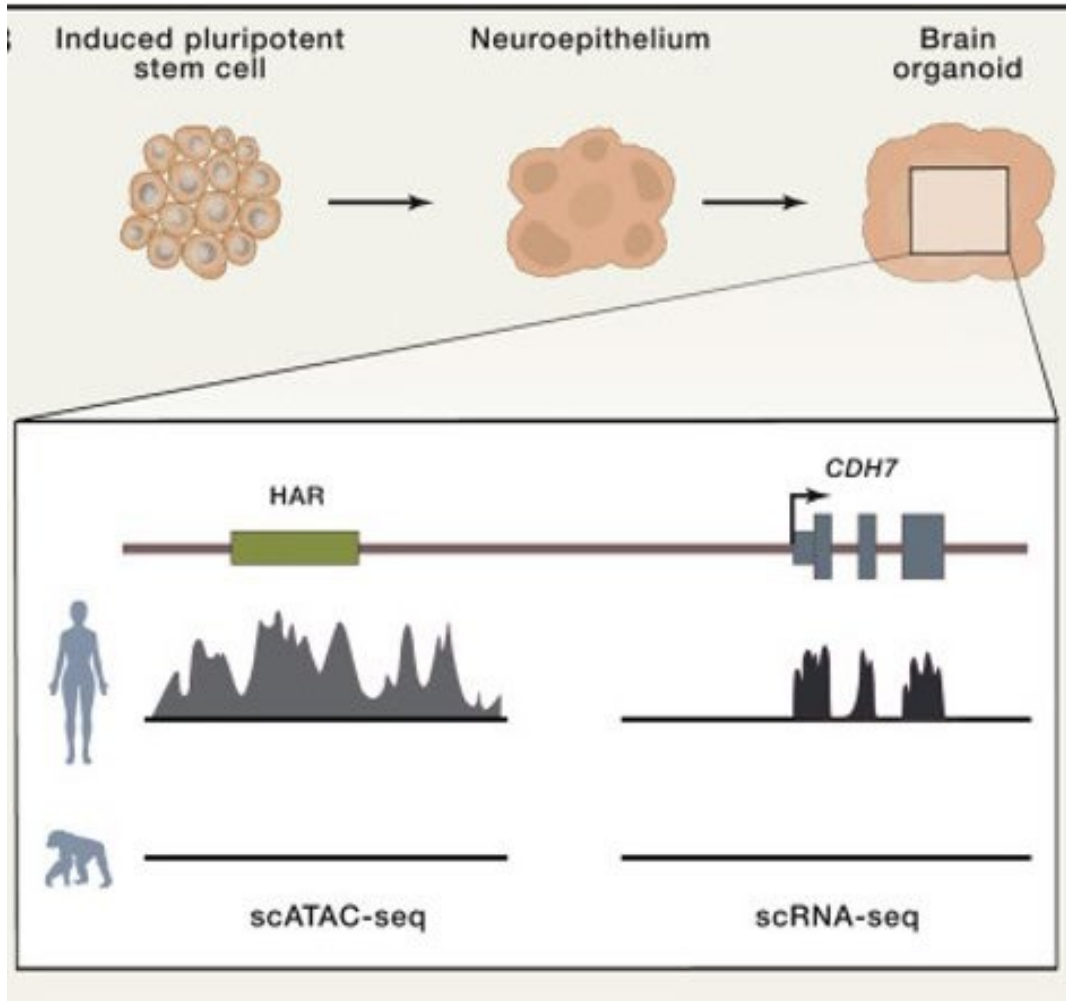
# Brain organoids to investigate human brain-specific evolution

Comparing tissue of adult specimen

e.g. single-cell RNA-seq in adult human, chimpanzee, and macaque prefrontal cortex



# Brain organoids to investigate human brain-specific evolution



However, big differences already occur early in the development, necessitating the investigation of early events

But investigation of brain development is a rather tricky business, as fetal tissue of Chimpanzee is not available in vast amounts

Therefore, comparing organoids, resembling the early brain development, of different species can be used as a model for specific differences

**Cell**

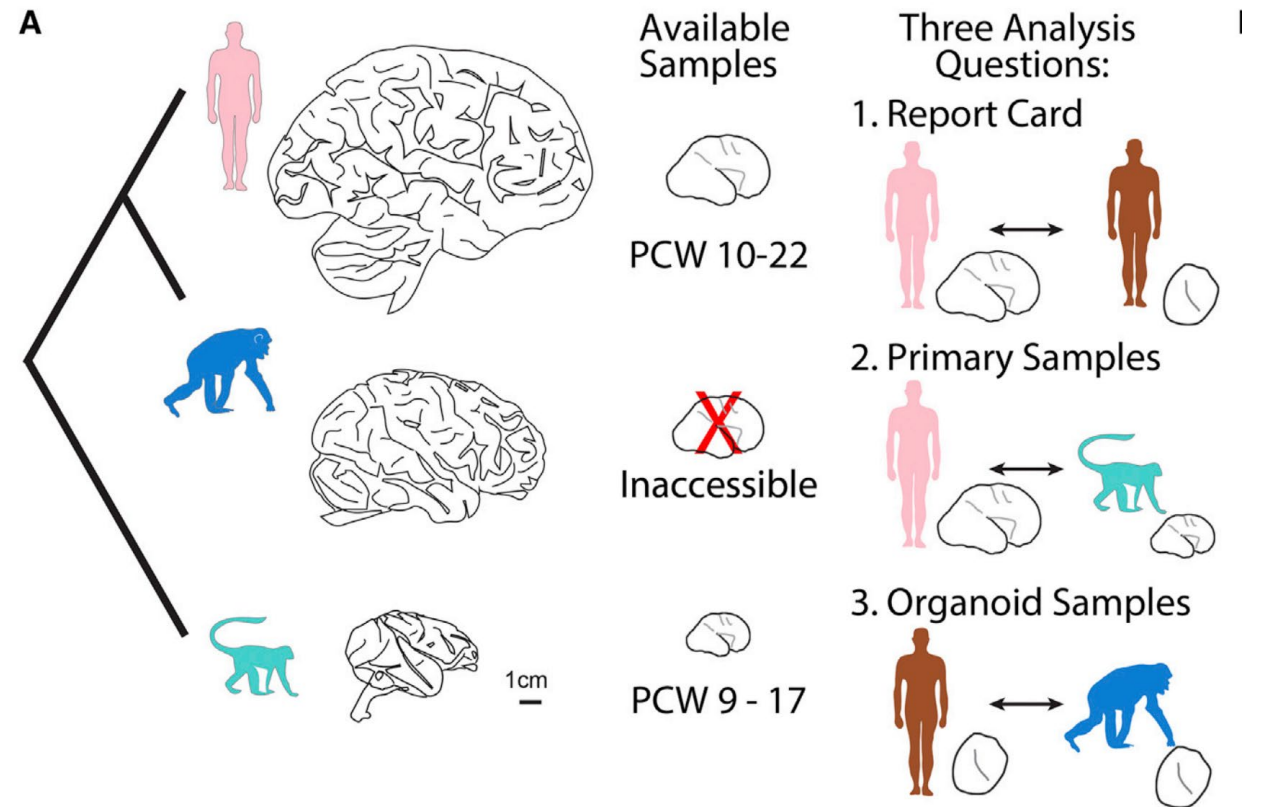
Article

**Establishing Cerebral Organoids as Models of Human-Specific Brain Evolution**

# Comparison of primary tissue and cerebral organoids

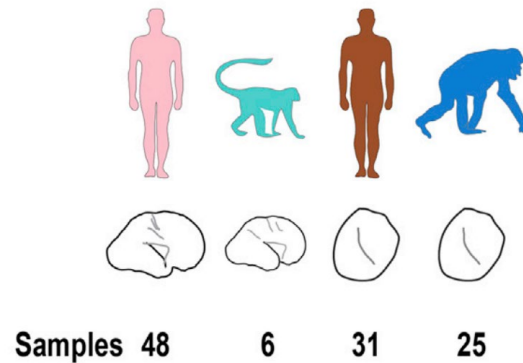
To assess evolutionary changes specific for human brain development, primary tissue from human and macaques as well as cerebral organoids from humans and more closely related chimpanzees are compared using scRNAseq

Developing primary macaque and human tissue is readily available and can be directly compared, whereas developing tissue from chimpanzees is largely inaccessible

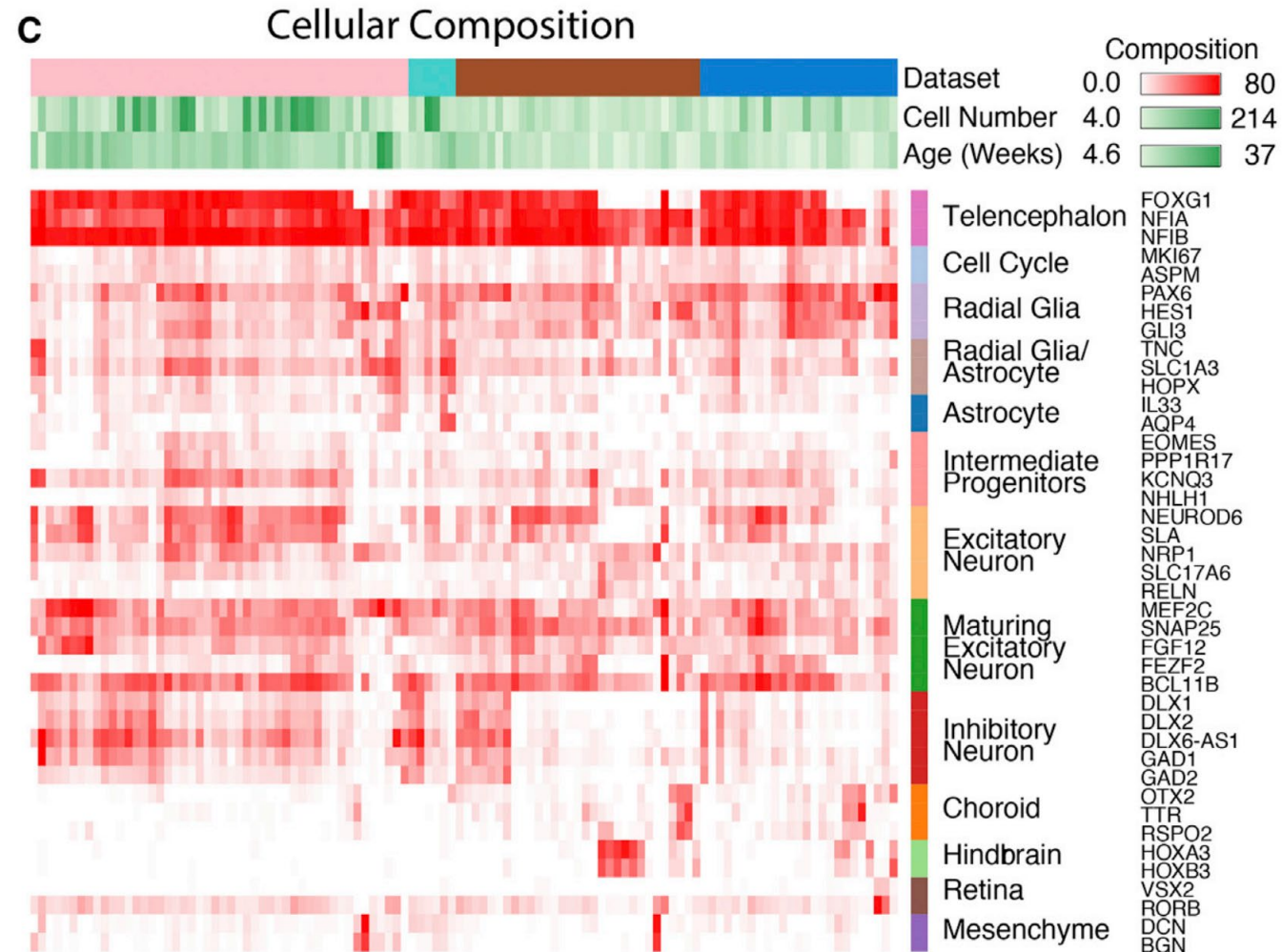


# 1. Cell Diversity in Primary Samples and Organoid Models

Evaluating the extent to which cell types, gene regulatory networks, and developmental trajectories are preserved in organoid models.



Detection of some off-target lineages, including hindbrain, choroid plexus, retina, and mesenchymal cells as well as variation in the proportion of excitatory and inhibitory neurons in cerebral organoids



# 1. Cell Diversity in Primary Samples and Organoid Models

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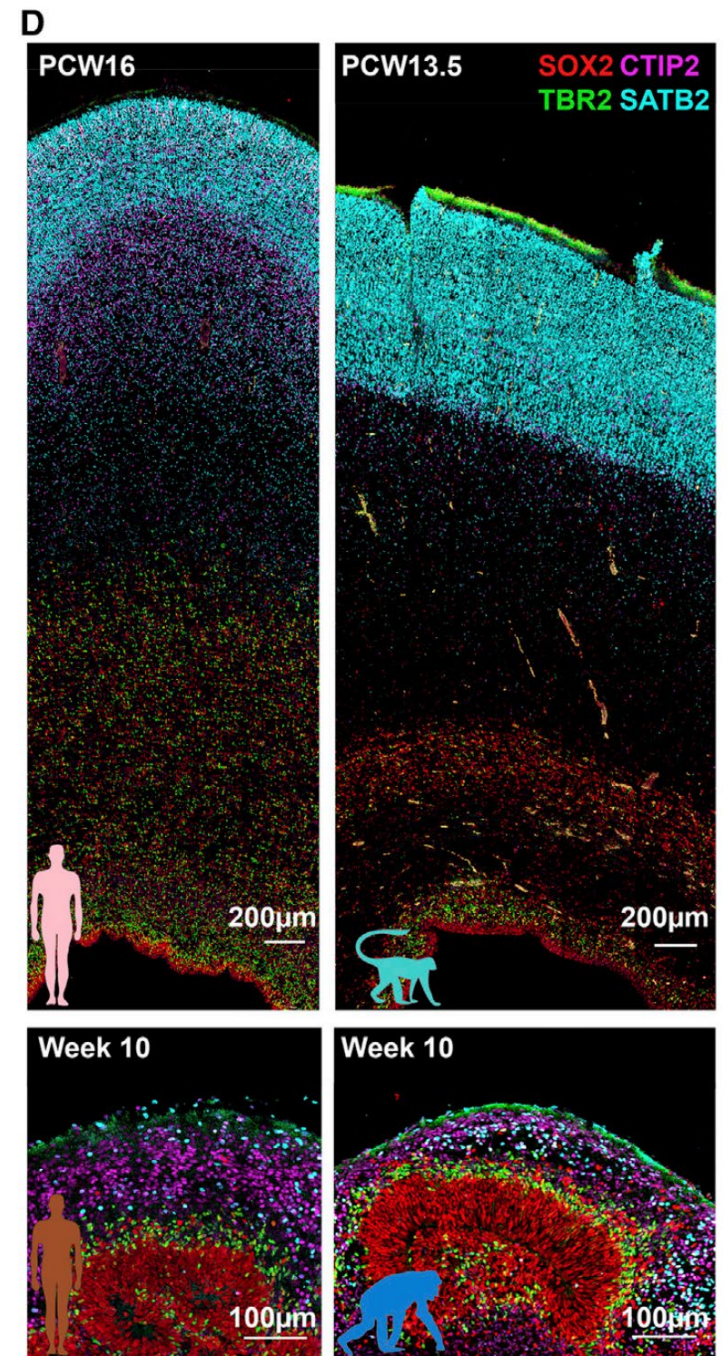
Primary cerebral cortices:

clear organization of radial glia and intermediate progenitors in the ventricular and subventricular zones and neurons migrating toward the cortical plate over an extensive intermediate zone

Cerebral organoids:

ventricular and subventricular zone-like structures in which cells expressed markers of radial glia and intermediate progenitors. Outside of these zones, cells expressing deep and upper layer markers, but the overall distance from the ventricle to the periphery was greatly compressed

Organoid models contained cell types and histological features reflective of cortical germinal zones but demonstrated restricted structural organization and varied in composition across experiments.





## 2. Define homologous cell types across model systems and species

For subsequent analysis, homologous cell types for the different models need to be defined

In each case, major telenphalic cell types were identified, including

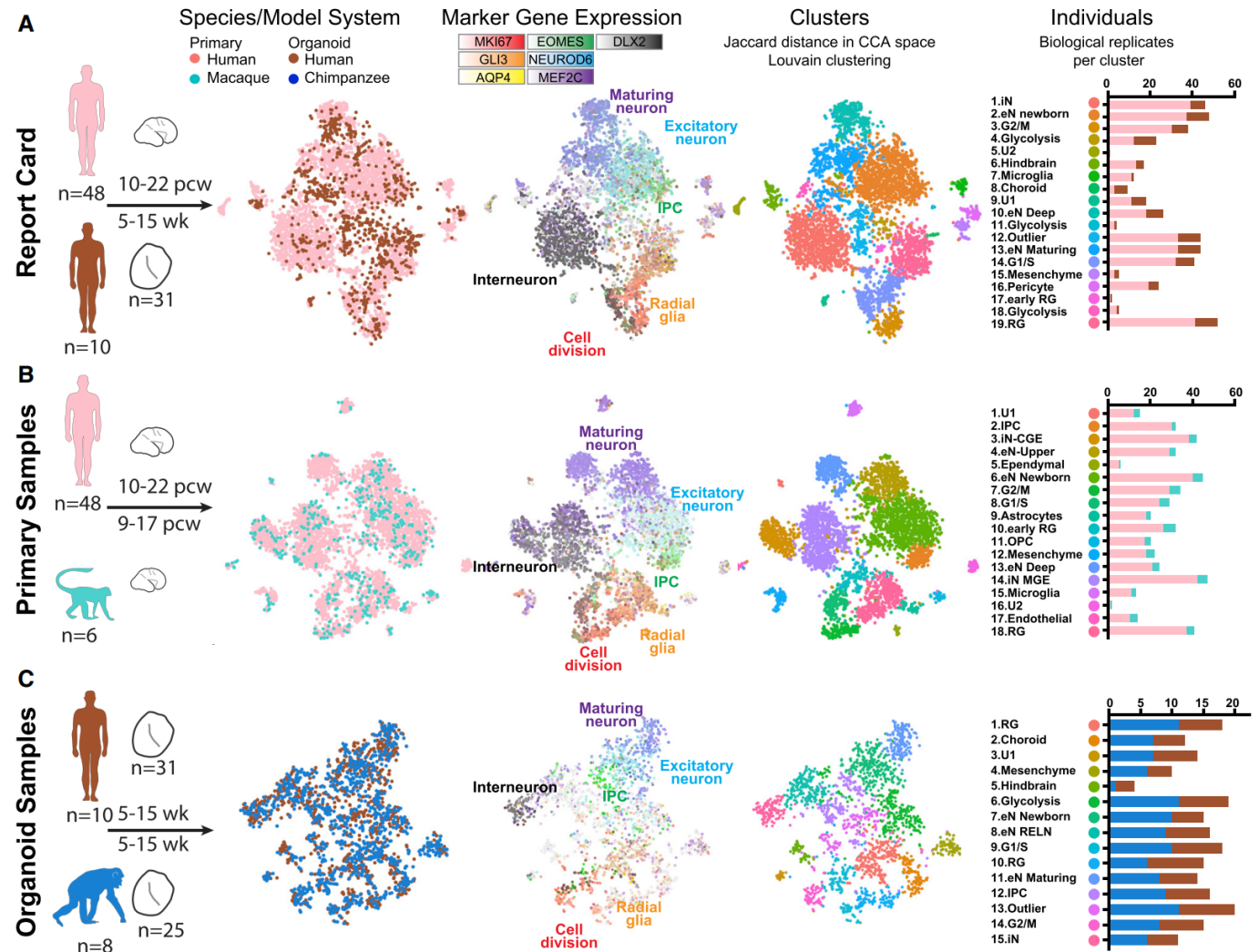
Radial glia

Intermediate progenitor cells

Excitatory neurons

Inhibitory neurons

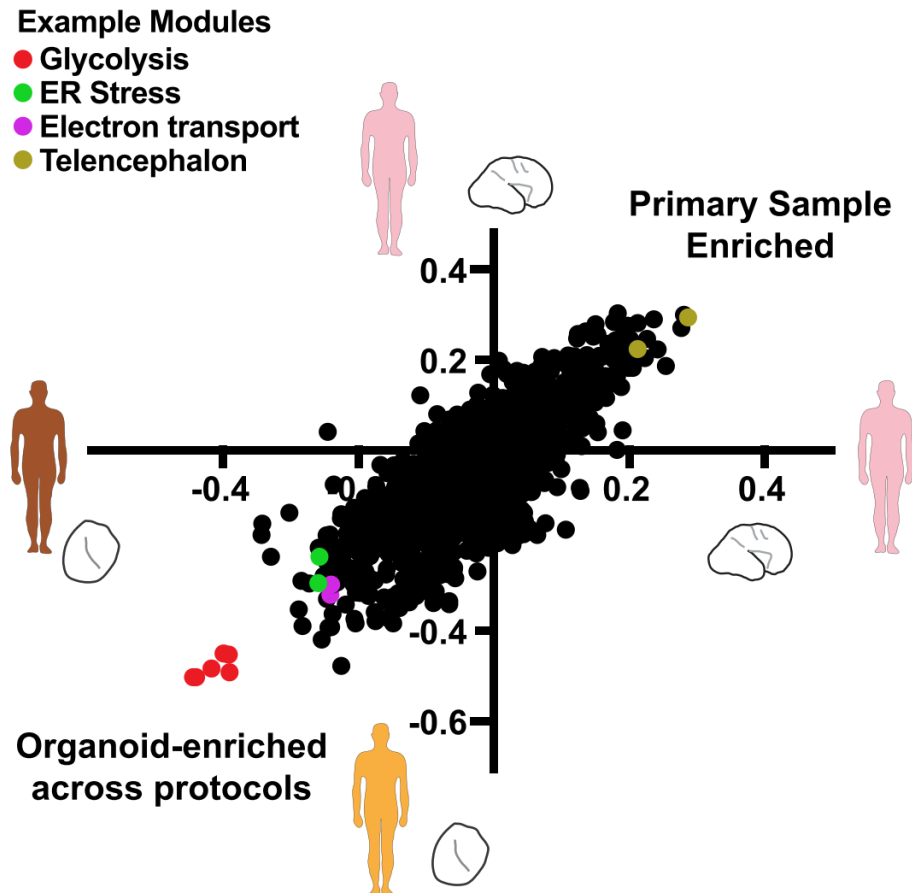
Overall cell composition was highly similar across species in both the primary sample comparison of human and macaque and the organoid comparison of human and chimpanzee



### 3. Assessing conserved processes based on gene co-expression networks

Examining the extent to which organoid models preserve expression of gene co-expression networks compared to primary tissue

#### Correlation of Gene Modules to Model System



Correlation of Gene modules to model systems:

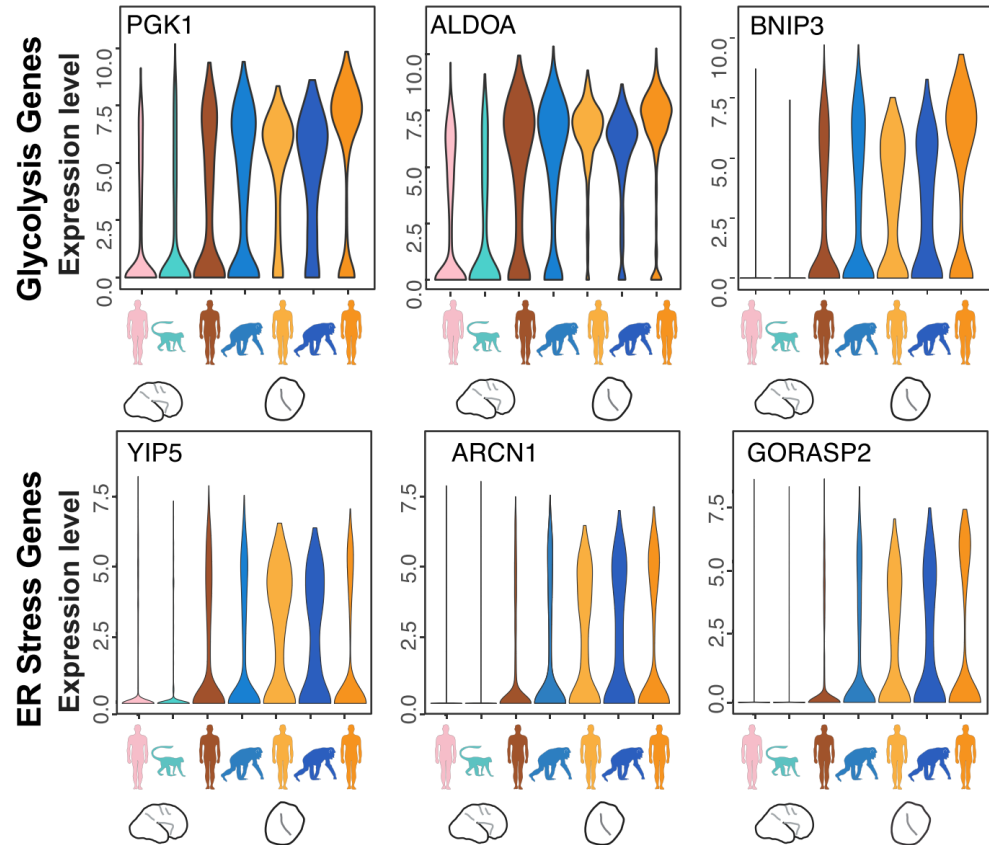
Independent organoid production protocols displayed organoid specific regulation of gene co-expression modules compared to primary samples.

**But overall, current organoid models preserve gene co-expression networks**

### 3. Assessing conserved processes based on gene co-expression networks

Examining the extent to which organoid models preserve expression of gene co-expression networks compared to primary tissue

#### Organoid Enriched Processes Across Protocols and Species



Correlation of Gene modules to model systems:

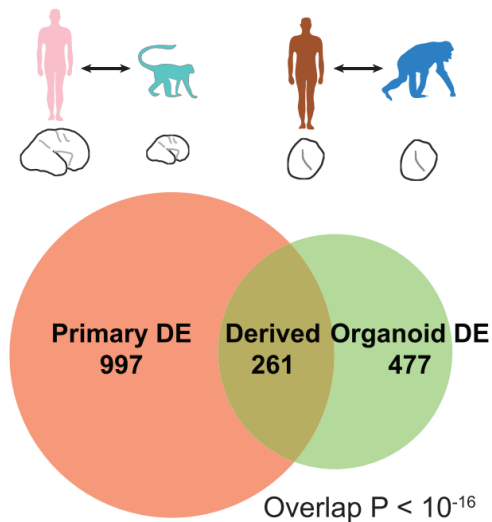
Enriched modules include glycolysis, implying a over-activation of metabolic pathway as well as ER-stress.

**But overall, current organoid models preserve gene co-expression networks**

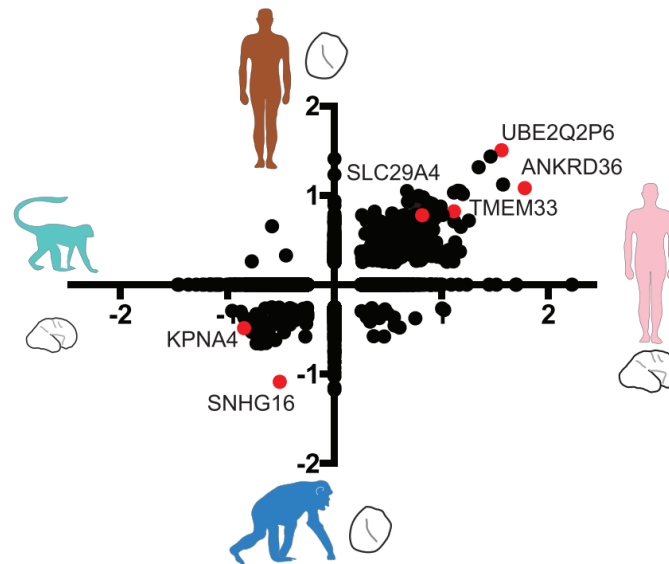
## 4. Assessing Human-Specific Gene Expression Changes

First, comparing primary human and primary macaque cortex to identify gene expression differences that occur during normal development across more distant primates. Next, identify which of these changes occurred in recent evolution by comparing gene expression in human and chimpanzee organoid models.

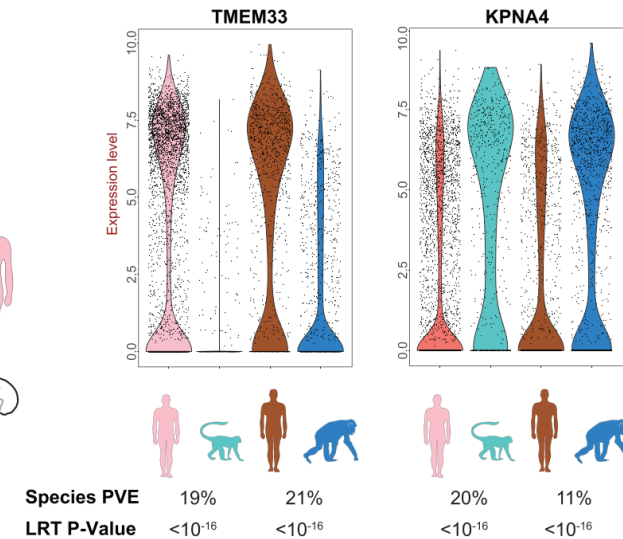
**A** Differential Expression



**B** Fold Change of Differentially Expressed Genes Across Species



**C** Example Derived Genes

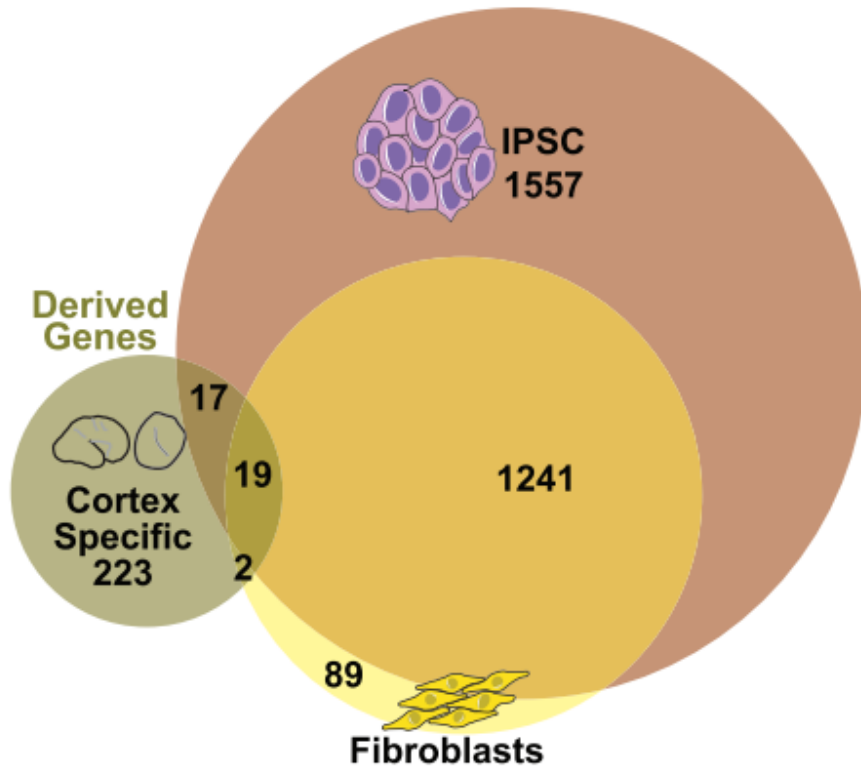


261 DEX genes overlapping in human – macaque and human organoid – chimpanzee organoid

## 4. Assessing Human-Specific Gene Expression Changes

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### D Differential Expression by Tissue

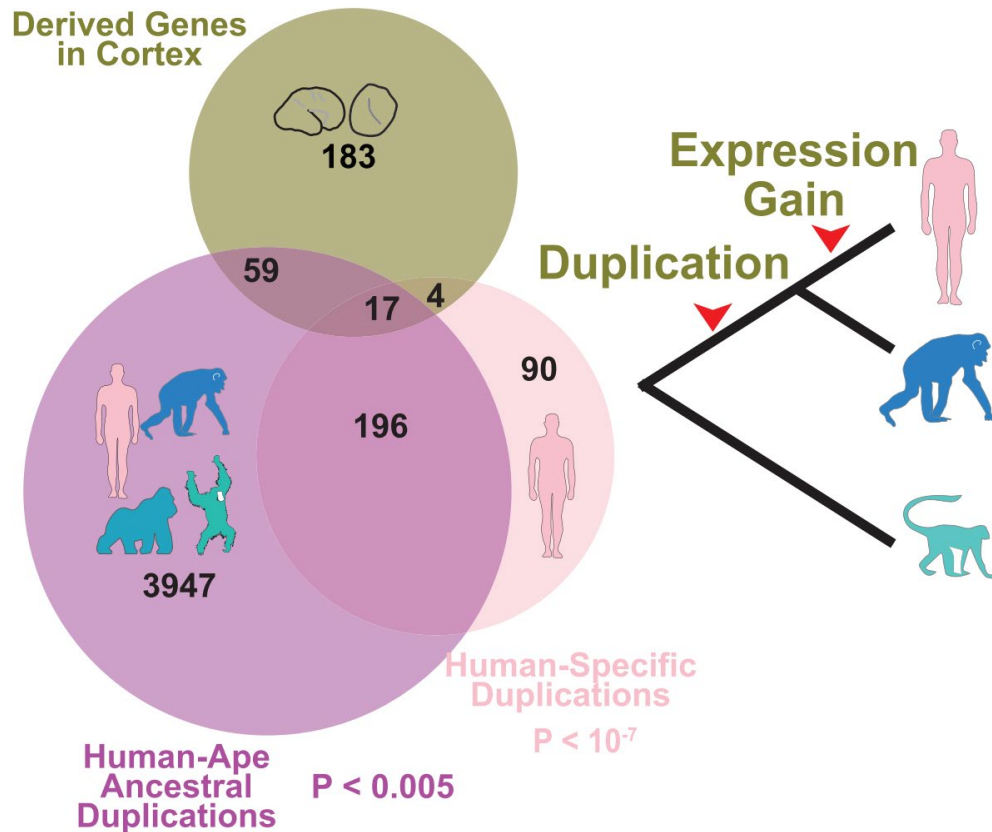


Comparison to a recent study using human and chimpanzee Fibroblasts and iPSCs to assess specificity of gene expression changes.

85% of the 261 genes were specific for cortical developments

# 4. Assessing Human-Specific Gene Expression Changes

## F Overlap with Gene Duplications



Are changes due to human –specific genetic changes?

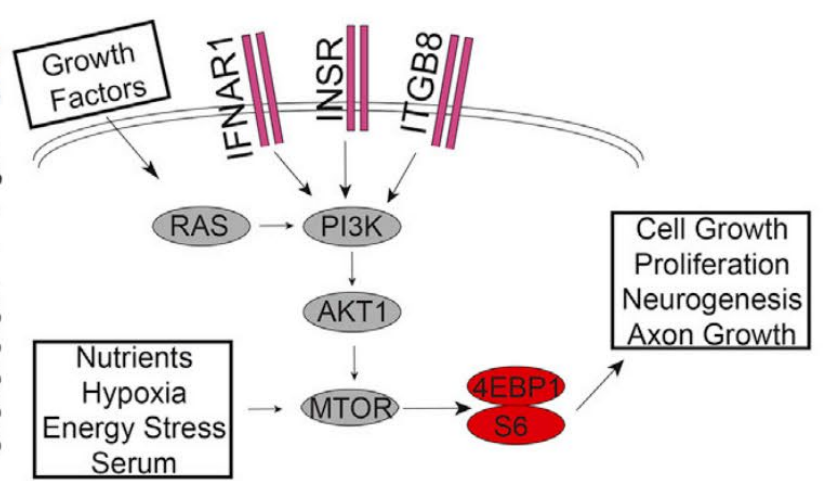
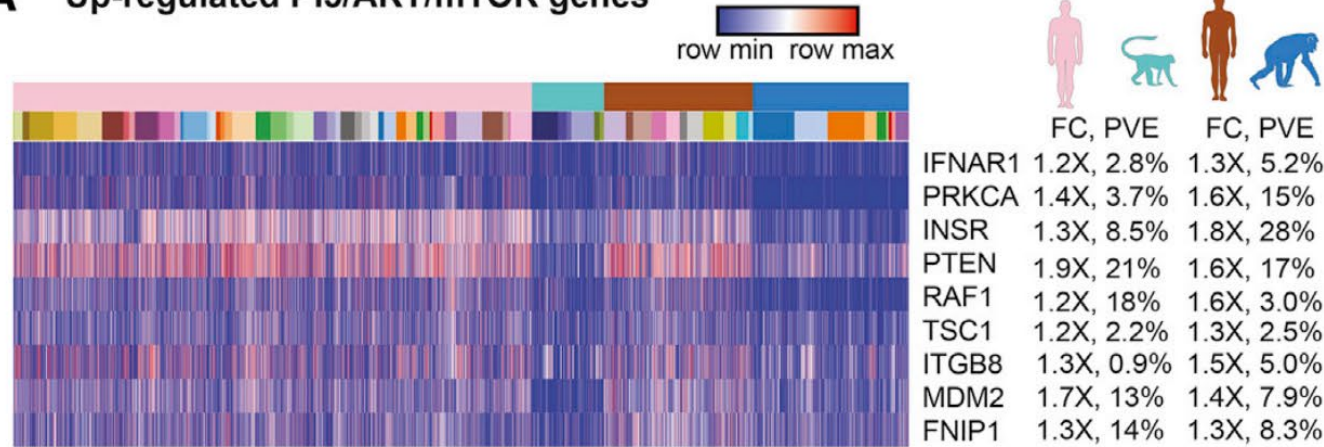
Overlapping major classes of structural genomic changes with human-specific gene expression differences

Recently duplicated genes were significantly over-represented among differentially expressed genes

Also some genes are higher expressed specifically in human tissue coming from duplication events prior to our divergence from chimpanzee

## 4. Assessing Human-Specific enriched Pathways

### A Up-regulated PI3/AKT/mTOR genes

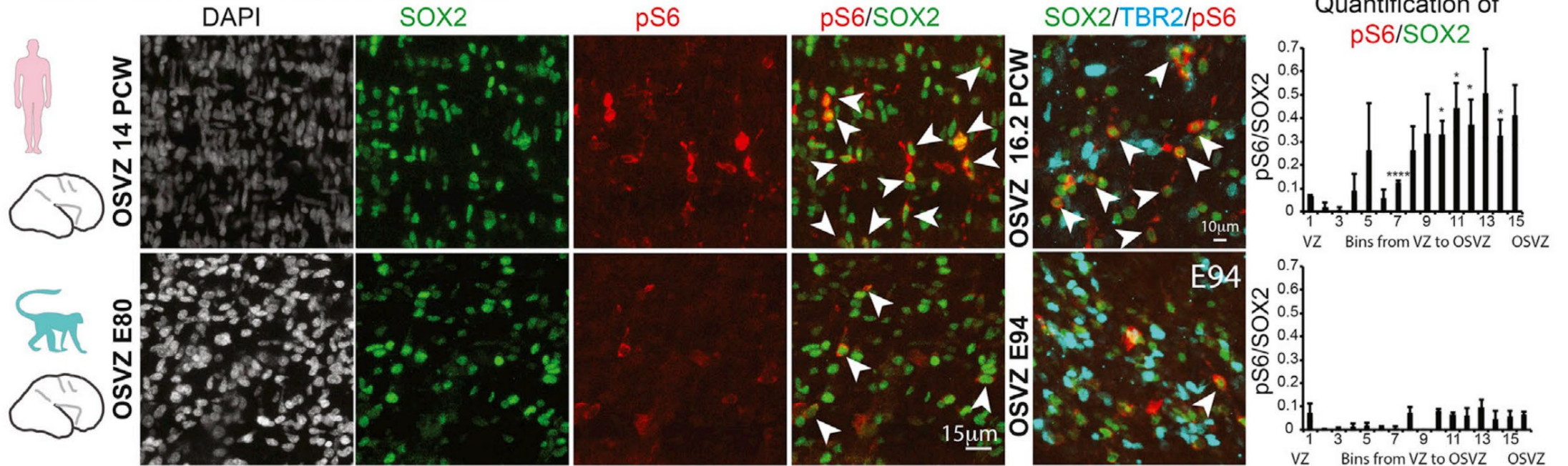


PI3K-AKT-mTOR pathway appeared in the top-ranked categories (e.g. FOXO3 signaling p=0.065)

Human specific upregulation of activators and repressors of mTOR pathway.

## 4. Assessing Human-Specific enriched Pathways

### B mTOR effector pS6 in primary samples

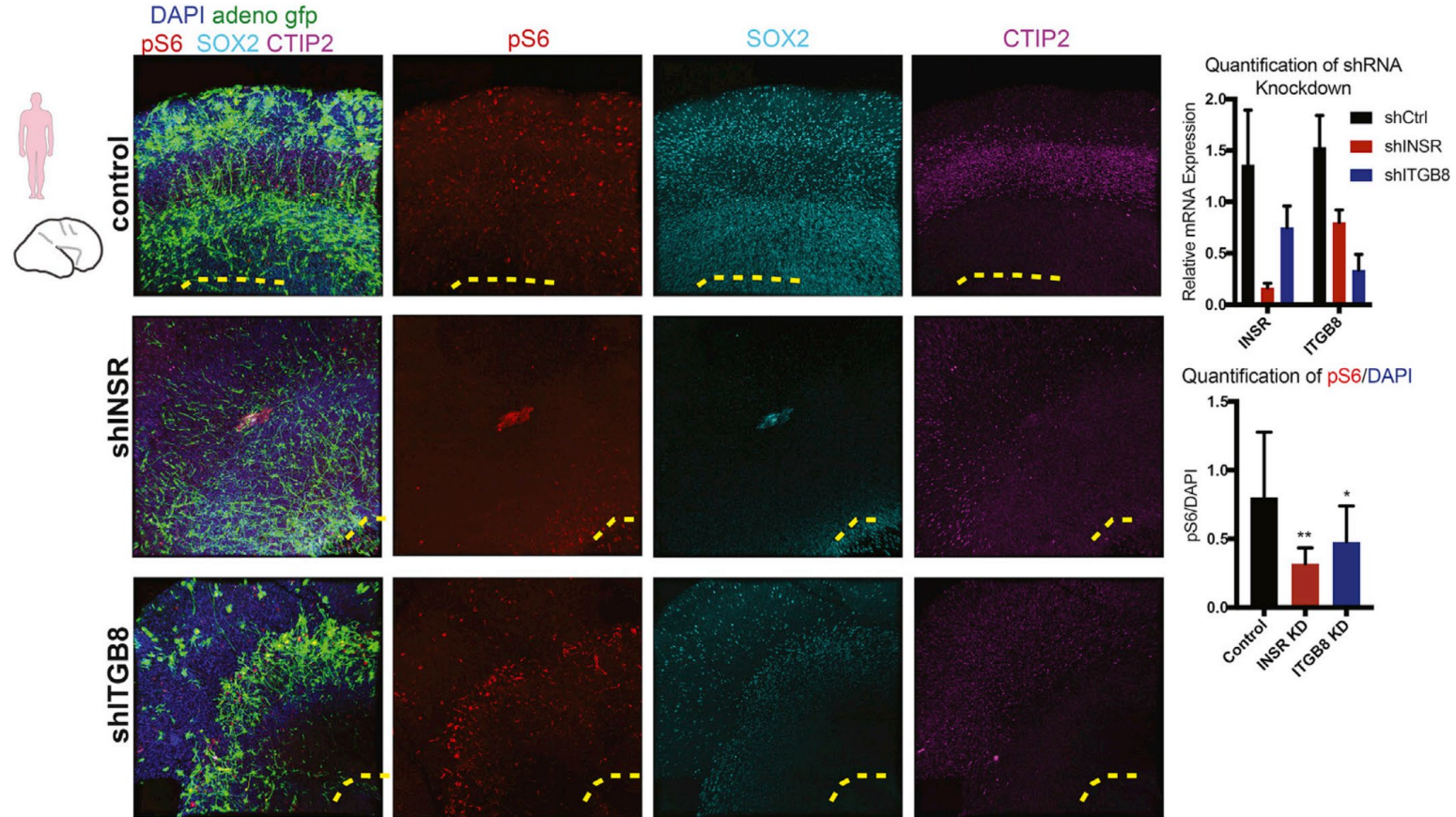


Stronger pS6 immunoreactivity, which is an effector of mTOR, in outer subventricular zone radial glia in human tissue when compared to primary macaque tissue.



# 4. Assessing Human-Specific enriched Pathways

## Necessity of INSR and ITGB8 for radial glia mTOR activation



Additionally upregulated genes were INSR and ITGB8, which are upstream regulators of pS6, with which they interfere in primary human slice culture. Downregulation of INSR and ITGB8 reduce overall levels of p6S.

# Summary & Conclusions I

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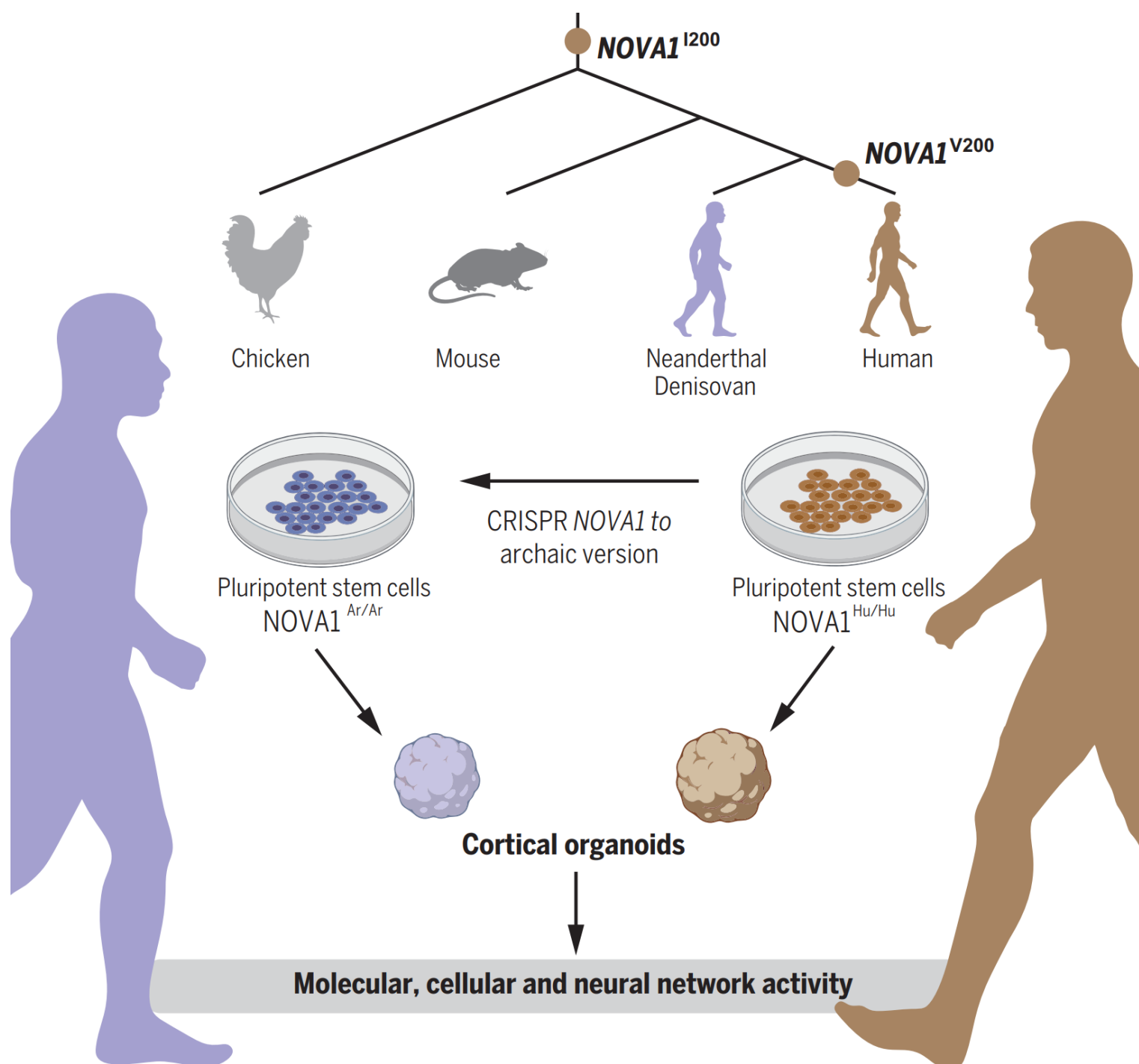
- Comparative studies of brain development are essential for understanding the molecular basis of human brain evolution.
- As primary tissue is lacking, they established great ape cerebral organoids as a model system for studying human-specific molecular changes that influence neurogenesis.
- They found that gene regulatory mechanisms observed during neurogenesis in primary cortex are mostly recapitulated in organoid models and identified human-specific gene expression patterns during cortical neurogenesis through independent comparisons of human and primate primary cells and organoid models.
- This comparative transcriptomic data among primates allowed to examine signaling pathways that may have changed in recent evolution. An increase in the activity of PI3K-AKT-mTOR distinguishes human outer subventricular zone radial glia from those in other primates.
- Future improvements to organoid protocols that demonstrate reproducibility of cell composition and neuronal connectivity relationships may enable evolutionary analysis of additional facets of human and great ape brain development beyond this initial focus on gene regulation.

Investigating evolutionary differences between homo sapiens and a more recent relative, homo neanderthalensis, by introducing a Neanderthal gene variant into a homo sapiens organoid model, to investigate homo sapiens specific brain developmental events.

## RESEARCH ARTICLE

### HUMAN EVOLUTION

# Reintroduction of the archaic variant of *NOVA1* in cortical organoids alters neurodevelopment



Genetic data from the 1000 Genomes Project and SGDP and from two high-coverage Neanderthal genomes and one high-coverage Denisovan genome were compared. A set of 61 positions in which all humans carry an autosomal fixed derived mutation was identified.

Among them:

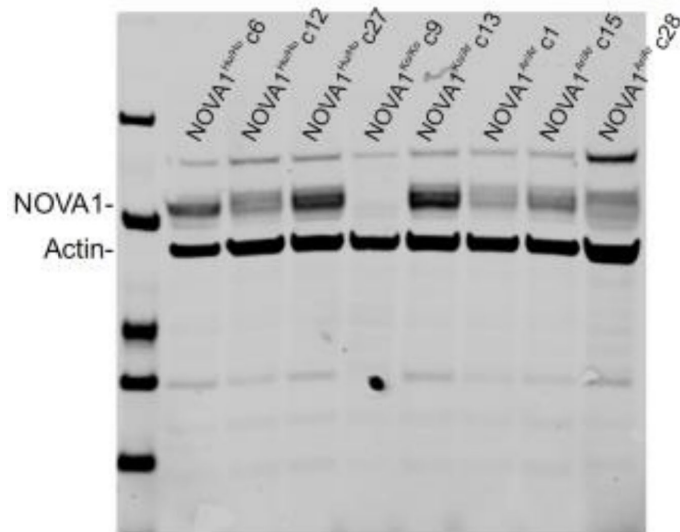
NOVA1<sup>V200</sup>, an RNA binding protein which regulates splicing in the developing nervous system.

# 1. Generating Organoids from human iPSC line harbouring the neanderthal NOVA1<sup>I200</sup> variant

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CRISPR-Cas9 to introduce the archaic variant of NOVA1 into the genome of induced pluripotent stem cells (iPSCs) derived from two neurotypical human individuals with distinct genetic backgrounds. Cells were used to generate Organoids

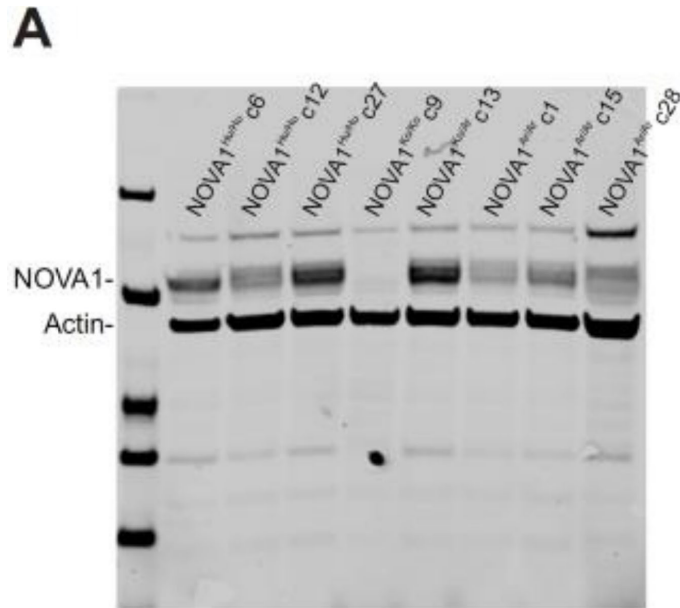
**A**



cells are expressing  
NOVA1 during organoid  
development

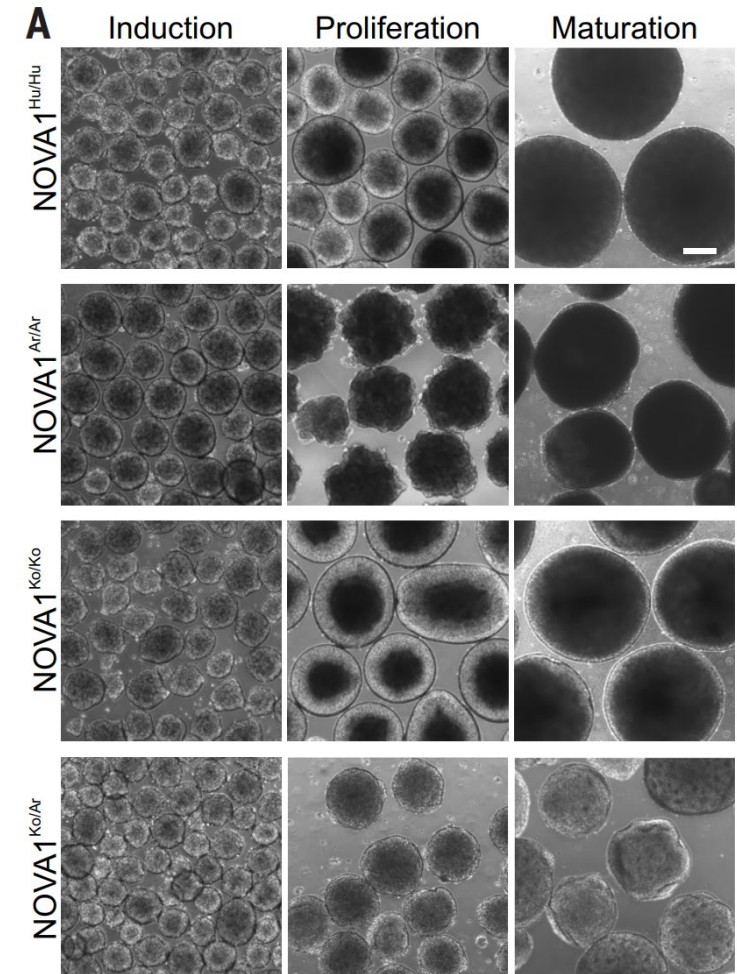
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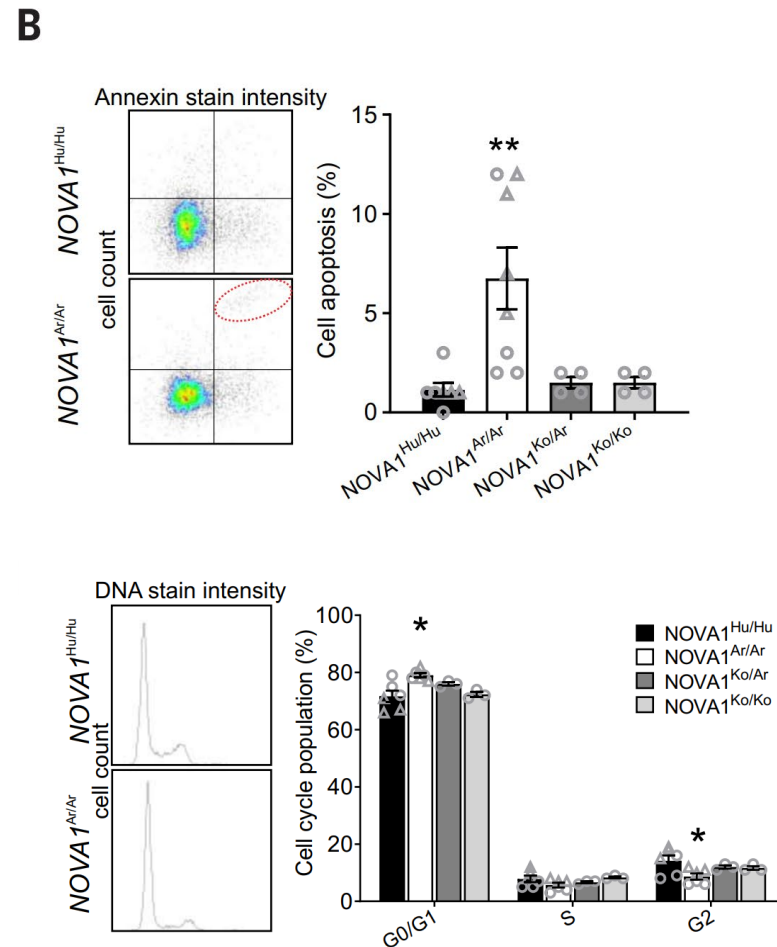
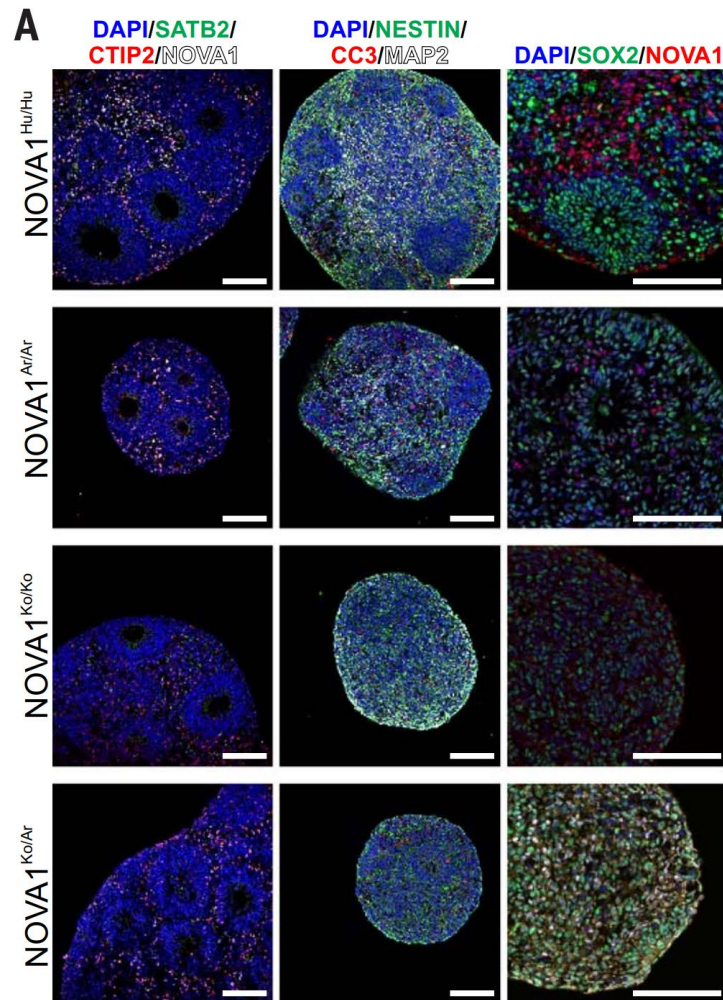
cells are expressing  
NOVA1 during organoid  
development

The derived organoids harbouring the archaic variant were smaller during proliferation and maturation stage



## 2. Analysis of developmental differences

Investigating molecular and cellular changes underlying the reduced size by assessing cell type composition, proliferation, cell cycle and apoptosis

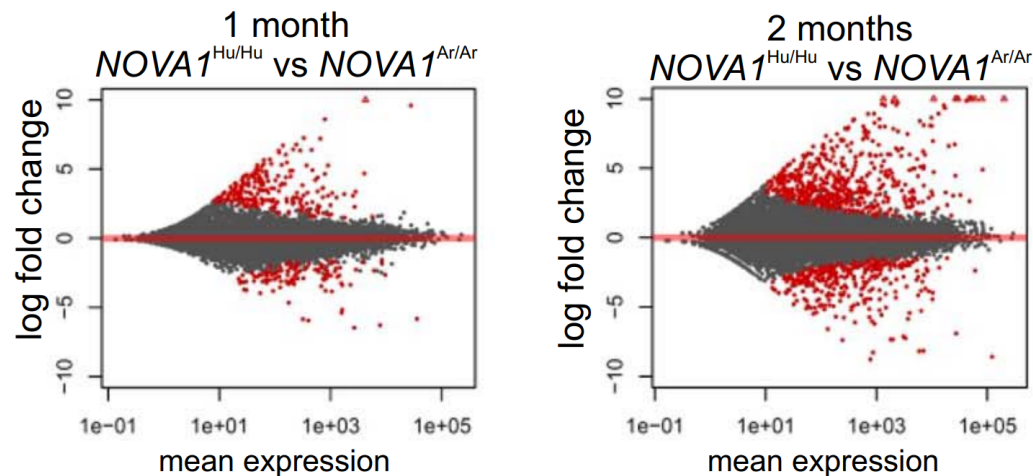


NOVA1<sup>Ar/Ar</sup> cortical organoids show the same cell type composition, however, have a higher number of apoptotic cells and a slower proliferation rate than NOVA1<sup>Hu/Hu</sup> organoids

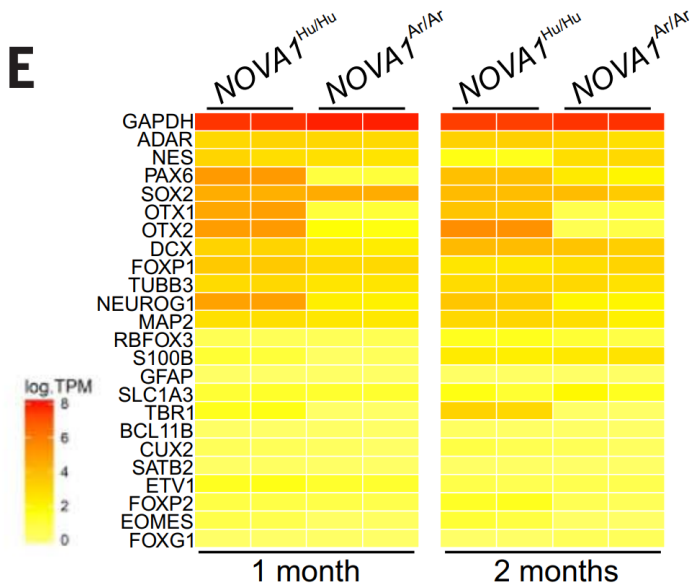
## 2. Analysis of developmental differences

RNAseq from cortical organoids at two developmental time points (1 and 2 months) to capture potential alterations in gene expression and alternative splicing.

**D**



**E**

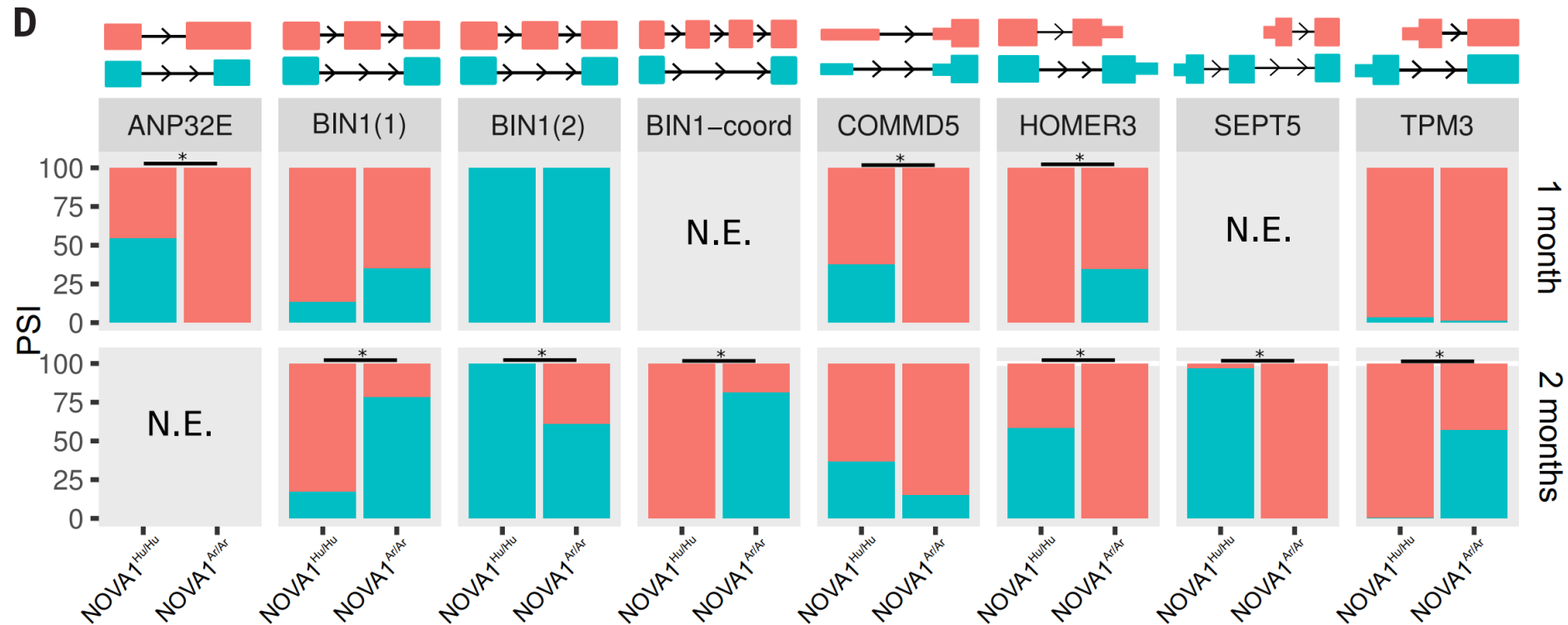


277 differentially expressed genes between  $NOVA1^{Ar/Ar}$  and  $NOVA1^{Hu/Hu}$  organoids at different stages of maturation, many of which are involved in neural developmental processes, were identified



## 2. Analysis of developmental differences

Gene splicing analysis between  $NOVA1^{Ar/Ar}$  and  $NOVA1^{Hu/Hu}$  organoids at the two time points

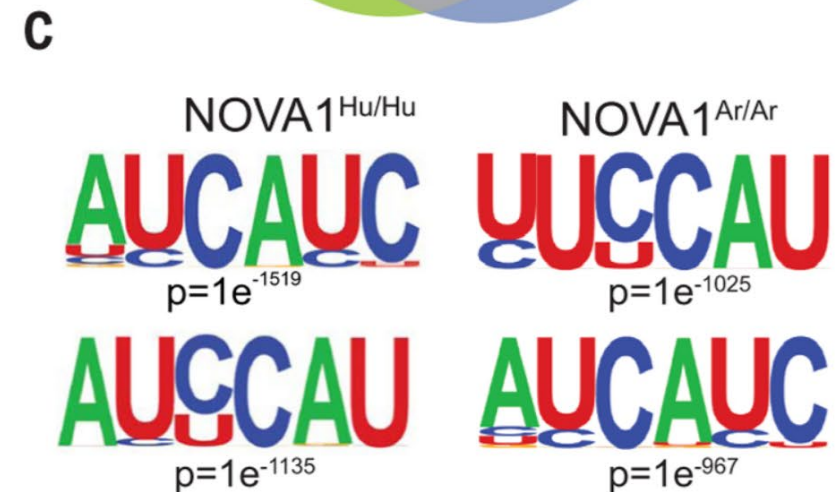
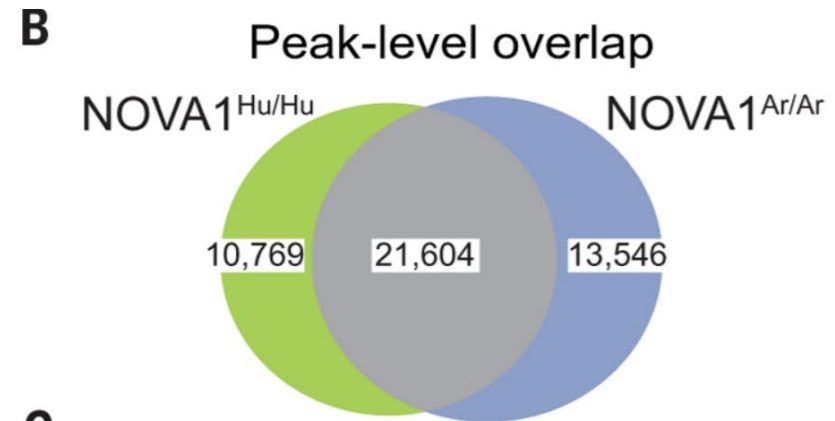
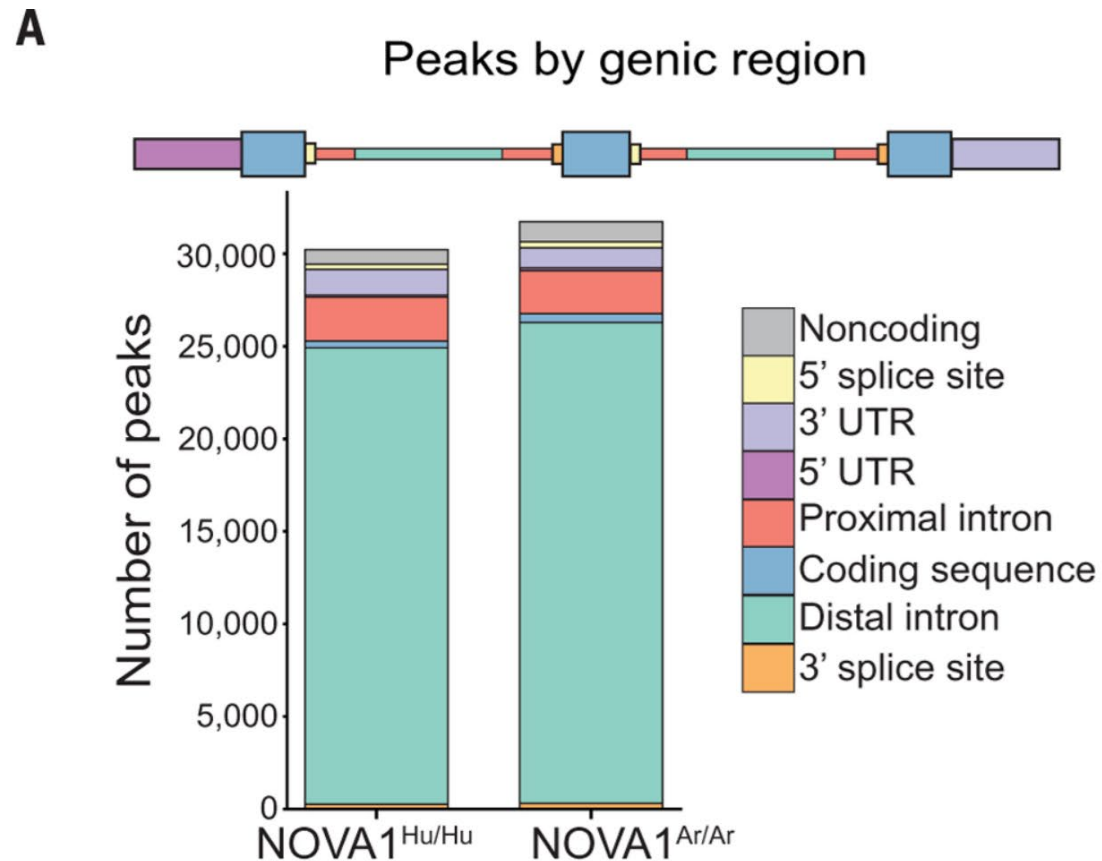


In the later timepoint, 156 genes were significantly affected by alternative splicing events. Many of the differentially spliced genes in  $NOVA1^{Ar/Ar}$  organoids are involved in synaptogenesis and neuronal connectivity.

## 2. Analysis of developmental differences

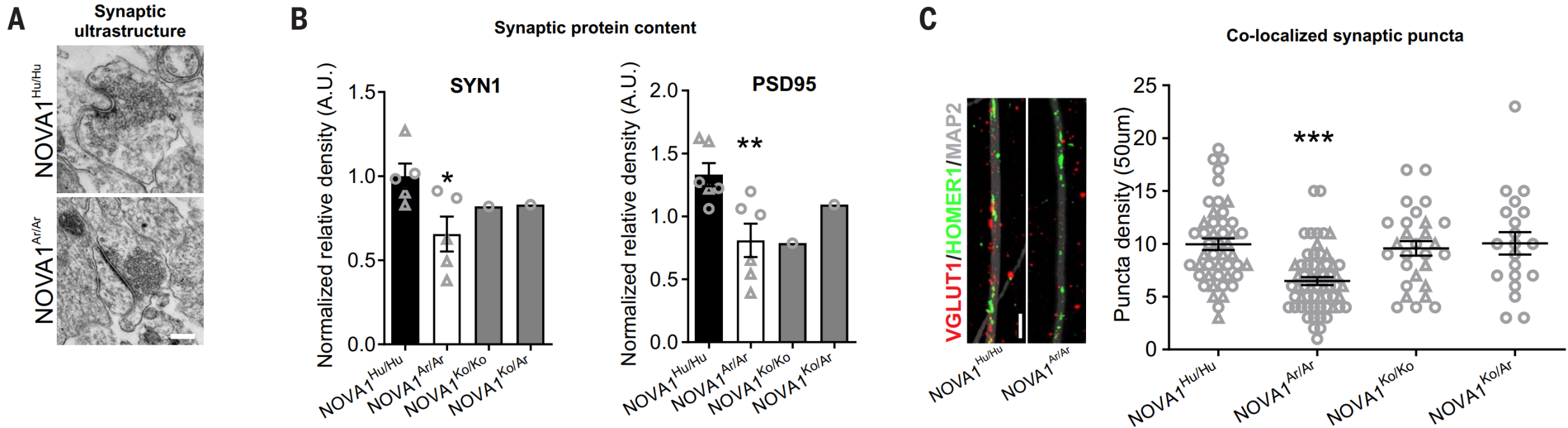
eCLIP analysis of the two different NOVA1 variants to determine binding preferences

About two-thirds of peak regions are overlapping between the genotypes, and the binding sites of both forms of NOVA1 are strongly enriched for the canonical YCA Y sequence motif



## 2. Analysis of developmental differences

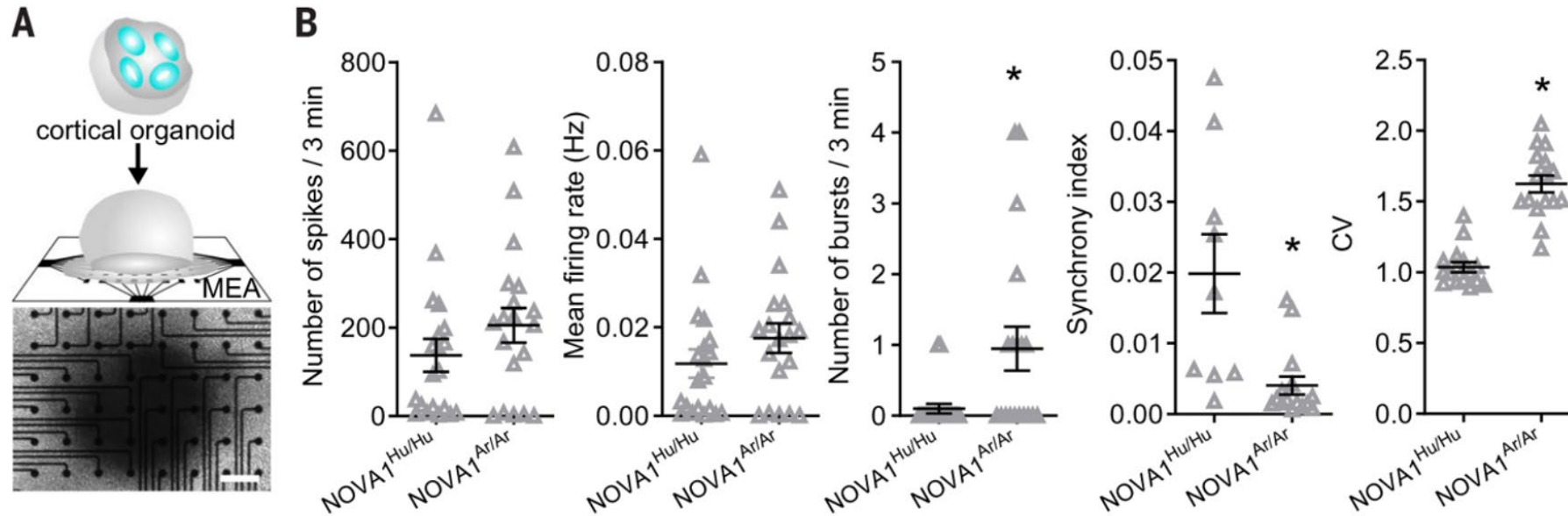
As many differentially expressed or spliced genes in  $\text{NOVA1}^{\text{Ar}/\text{Ar}}$  organoids are involved in synaptogenesis and neuronal connectivity, they verified whether synaptic protein levels showed an altered profile



$\text{NOVA1}^{\text{Ar}/\text{Ar}}$  cortical organoids expressed lower levels of pre- and postsynaptic proteins, resulting in reduced colocalized synaptic puncta compared with  $\text{NOVA1}^{\text{Hu}/\text{Hu}}$ .

## 2. Analysis of developmental differences

Investigation of functional consequences due to differences in synapses  
Multielectrode array to assess electrophysiological differences



Mature  $NOVA1^{Ar/Ar}$  cortical organoids displayed an increased number of bursts and a higher coefficient of variation (CV) while showing lower synchrony levels compared with  $NOVA1^{Hu/Hu}$  organoids.

Archaic *NOVA1* variant leads to modified synaptic protein interactions, affecting glutamatergic signaling, differences in neuronal connectivity, and higher heterogeneity of neurons regarding their electrophysiological profiles.

# Summary & Conclusions II

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- Comparison of genome sequences from modern humans with those from Neanderthals and Denisovans, our closest evolutionary relatives, reveals mutations exclusive to modern humans. A subset of these genetic changes may underly the phenotypic traits that separate our species from these extinct relatives.
- As Neanderthals and Denisovans are extinct, and no living cells could be retrieved as of yet, the reintroduction of archaic genes into human models has been established as a novel platform
- They observed differences in gene expression, organoid morphology, and cell proliferation when comparing cortical organoids carrying the  $NOVA1^{Ar/Ar}$  and the  $NOVA1^{Hu/Hu}$  genetic variants. Furthermore, the  $NOVA1^{Ar/Ar}$  cortical organoids displayed distinct excitatory synaptic changes, which may have led to the observed alterations in neural network development
- Limitation: specific human genetic background of  $NOVA1$ . It is likely that the genetic backgrounds between the archaic hominin and modern humans differed such that much of the genetic variation in these human cell lines did not coexist with the archaic version of  $NOVA1$ . Targets of the human-specific  $NOVA1$  that currently exist in humans may have undergone compensatory genetic changes to adapt to the derived version of  $NOVA1$  prevalent among humans today => observed phenotypes might be neither human nor Neanderthal.
- This genetic change might have been an important event in the evolution of modern human-specific neural phenotype

# Synopsis

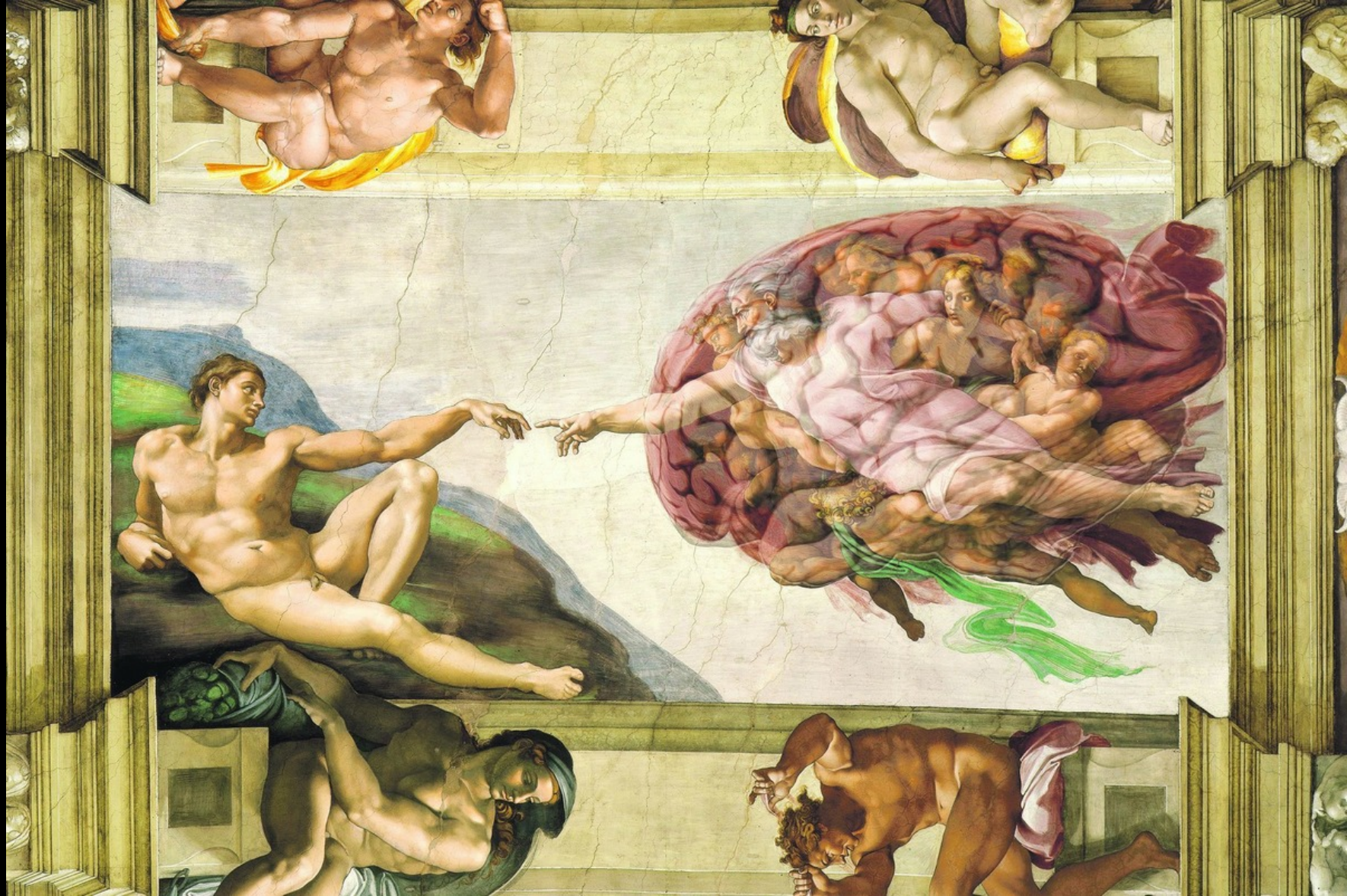
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The human brain has evolved new neural circuits and functional regions and has distinct aspects of cognition, which are unmatched by our evolutionarily closest relatives. Such a development must have an evolutionary basis.

Due to the lack of primary tissue, studying early brain development of our closest relatives was not possible. Novel chimpanzee organoids allow the comparative analysis of early brain developmental differences.

Also, the reintroduction of archaic gene variants, e.g. from Neanderthals, open up new avenues to use brain organoids to study the effect of evolutionary events on human brain development

Thank you for  
your attention!



The Creation of Adam?