

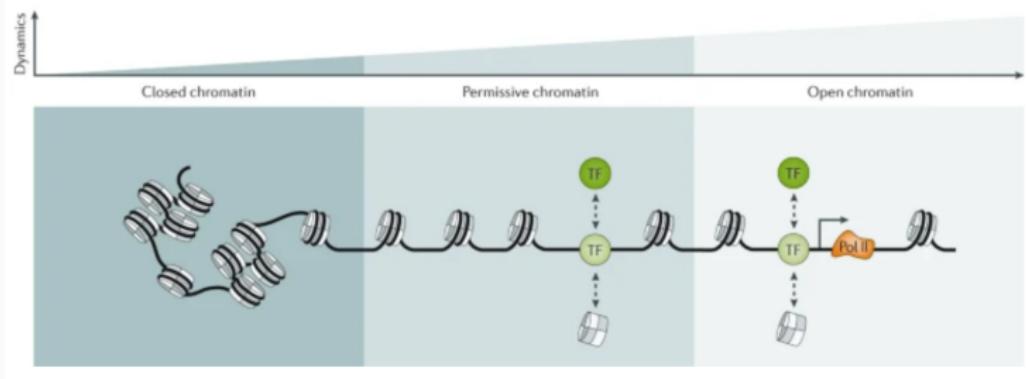
Single-cell epigenomics reveals mechanisms of human cortical development

Edmund Miller

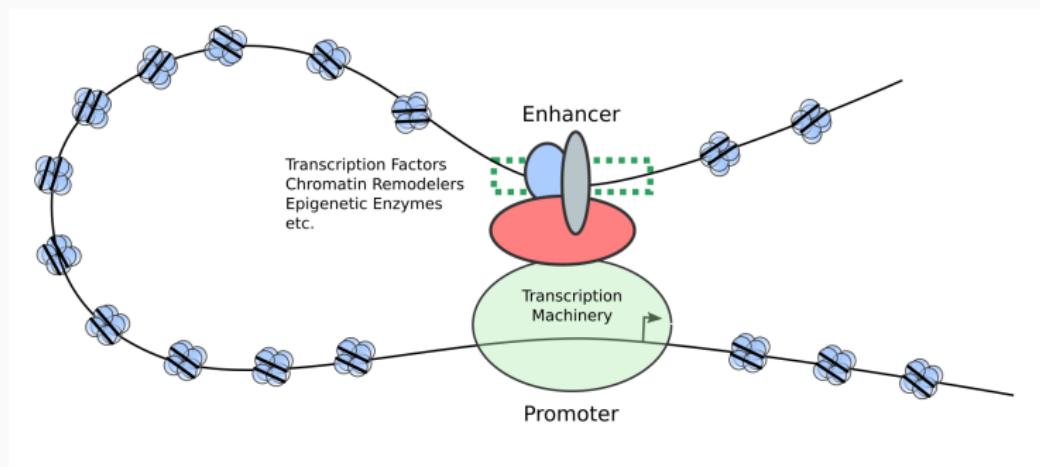
2021-10-25 Mon

Background

Chromatin Accessibility



Enhancers



Cerebral Organoids

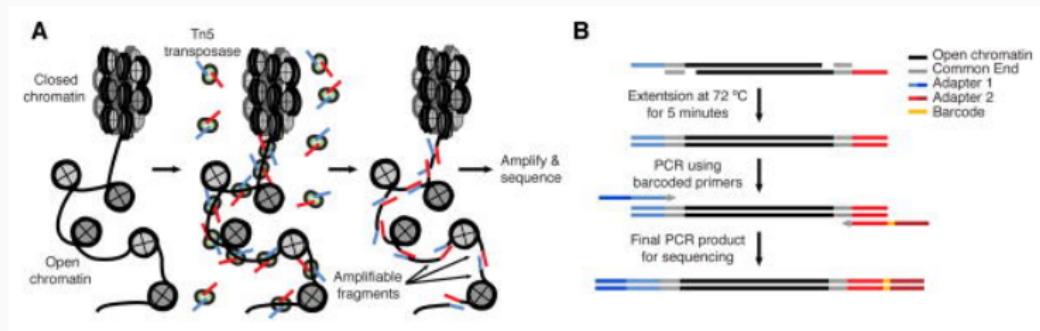
- Created *in vitro* miniature organ resembling a brain
 - Created by culturing pluripotent stem cells in a three-dimensional rotational bioreactor
 - Chaotically arranged, instead of structural organized
- Can be used to investigate early human brain development
- Need to ensure the organoids form in a reproducible way

Motivation

- Better understanding of signalling pathways that contribute to neurodevelopmental delay (Vitamin A)
- Better understanding of mammalian development
- Provide a blueprint for evaluating cerebral organoids as a model for cortical development

Methods

ATAC-seq



- A proxy to how easily a transcription factor can bind to the genome
- Uses TN5 transposase, which binds to open chromatin and inserts DNA sequencing adapters

Sequencing with Index/barcodes

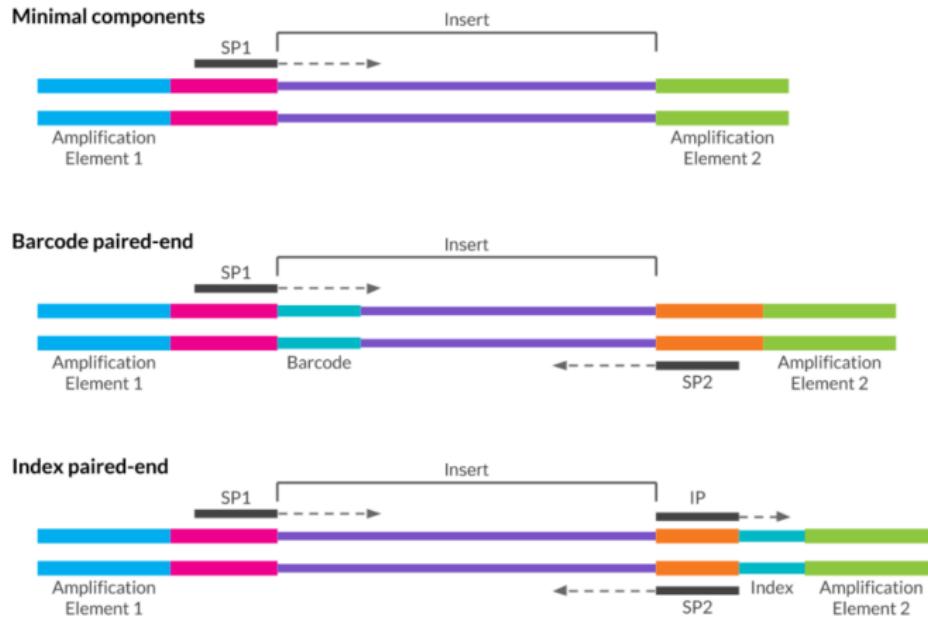
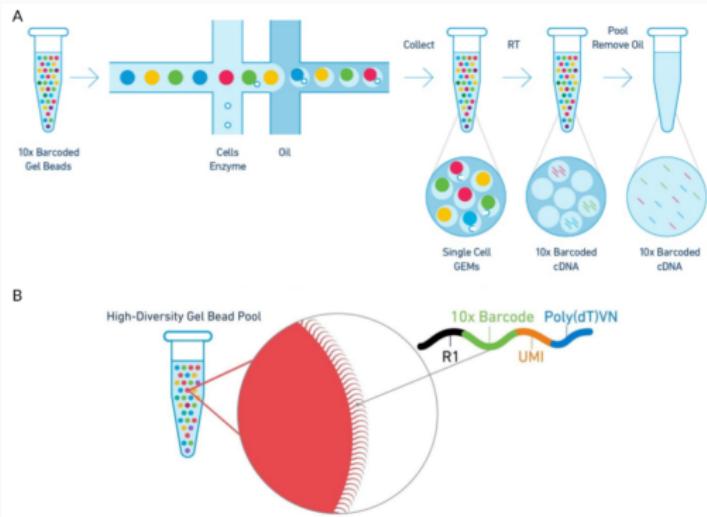


Figure 1.2: Illustration of adapter arrangements for the Illumina platform ((A) minimal adapter components, (B) "in-line" barcode configuration for paired-end sequencing, (C) index configuration for paired-end sequencing). Sequencing primers SP1 (primary) and SP2 (paired-end) allow initiation of synthesis of the insert sequence. Index or barcode sequences allow multiplexing of multiple samples with the index being sequenced from a separate index primer (IP). (Image adapted from RNA-seqlopedia, <http://rnaseq.uoregon.edu> [6]).

Droplet-based cell capture



- Cheaper per cell because of fewer doublets and ability to capture tens of thousands of cells
- Popularized by Drop-seq and InDrop DIY systems
- Commercially available platform is the 10x Genomics Chromium device

Single Cell Sequencing

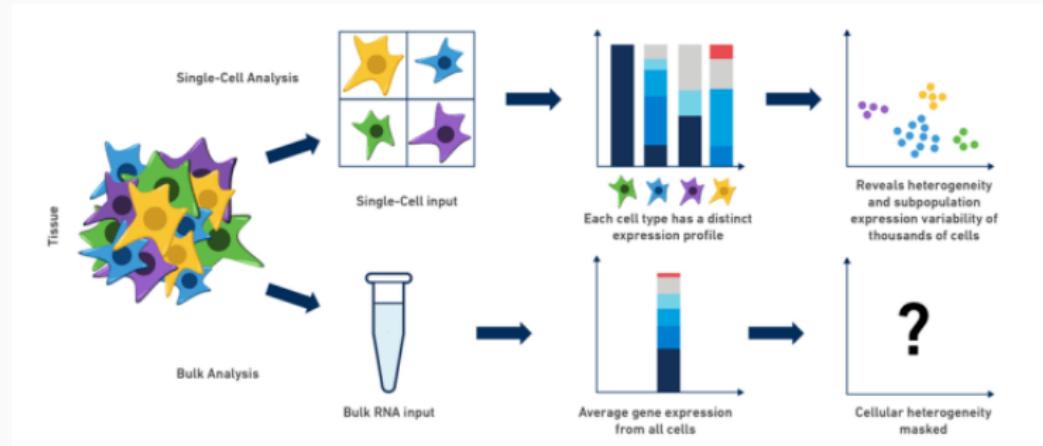
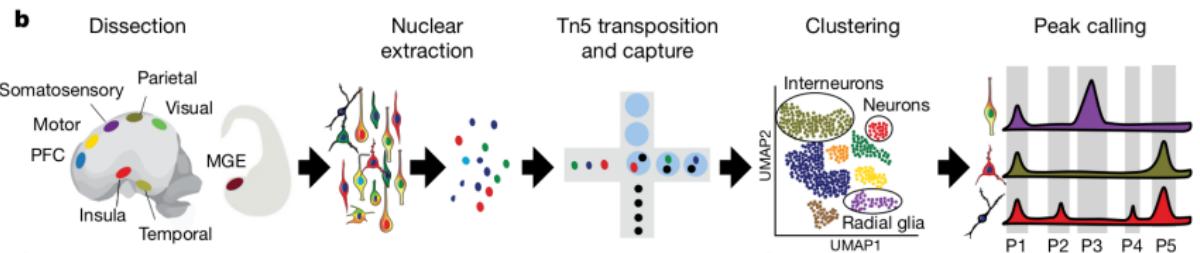
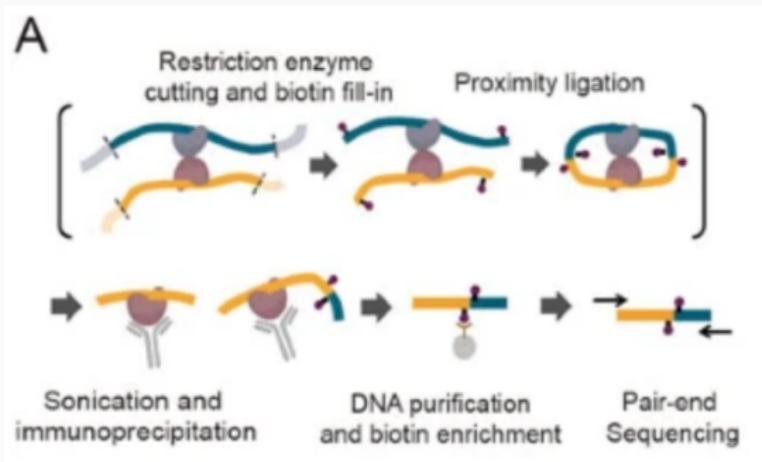


Fig 1b. Experimental Workflow



PLAC-seq



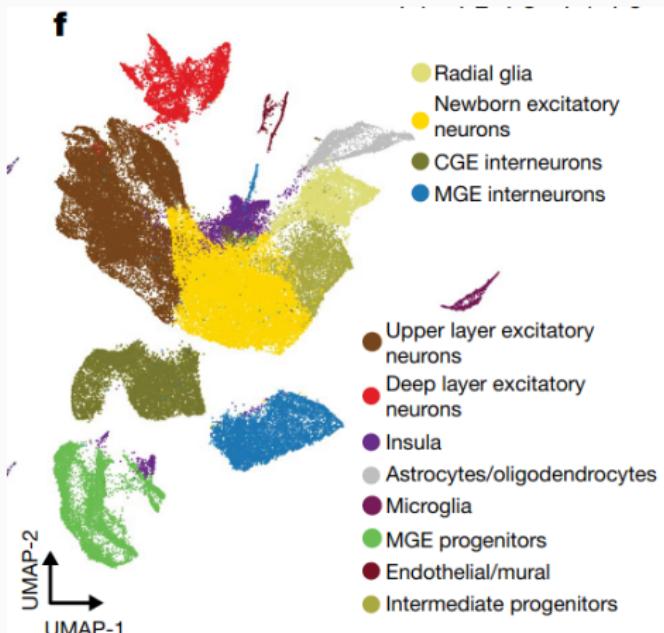
- Looking for long-range chromatin loops
- Rivaling Hi-C and ChIA-PET
- Proximity ligation is conducted in nuclei prior to chromatin shearing and immunoprecipitation (instead of proximity ligation are performed after chromatin shearing)

Results

Chromatin states of the developing brain

- Performed scATAC-seq on primary samples of human forebrain mid-gestation
- Confirmed that aggregate signal from the single-cell libraries matched up with bulk ATAC-seq libraries created in parallel
- Used CellWalker to assign cell-type labels based on previous scRNA-seq
- Able to identify differentially accessible peaks between **sub** types of cells

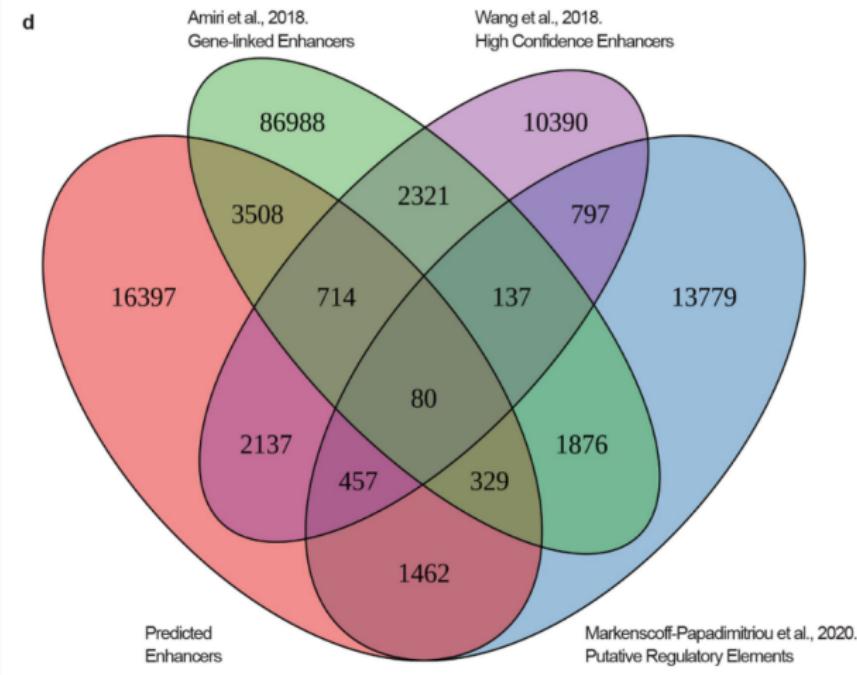
Fig 1f. UMAP projection of primary scATAC-seq cells coloured by broad cell type



Identifying cell-type-specific enhancers

- Intersected our peak set with the imputed 25-state chromatin model from Roadmap Epigenomics1
- Identified cell-type-specific differentially accessible peaks for each cell type
- Overlaid with H3K27ac (Didn't use H3K4me1 because they only wanted active enhancers?)
- Used PLAC-seq and H3K4me3(promoter)

Extended Data Fig. 5 - Venn diagram of overlap of all predicted enhancers



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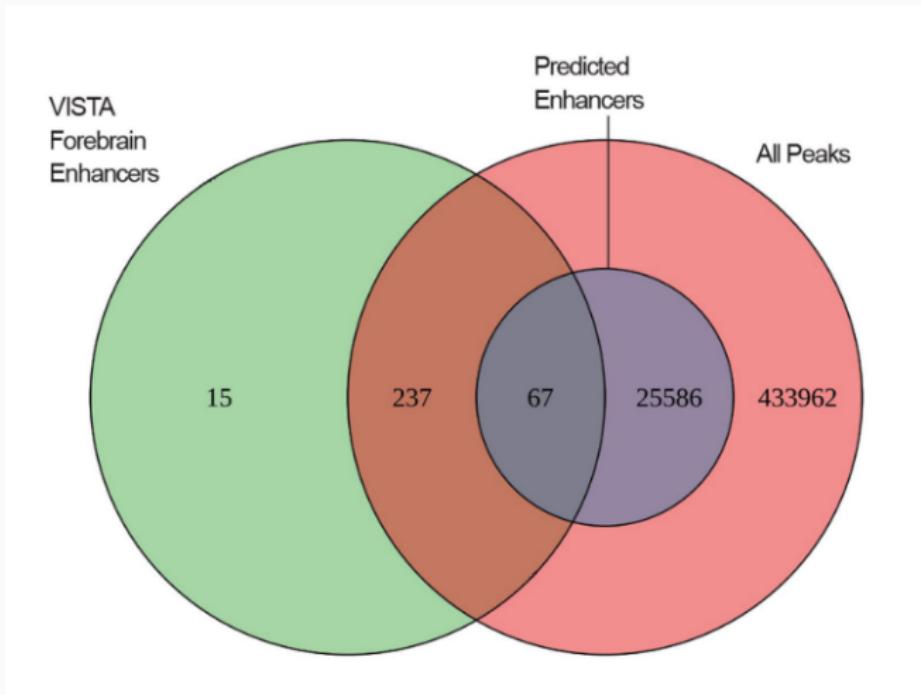
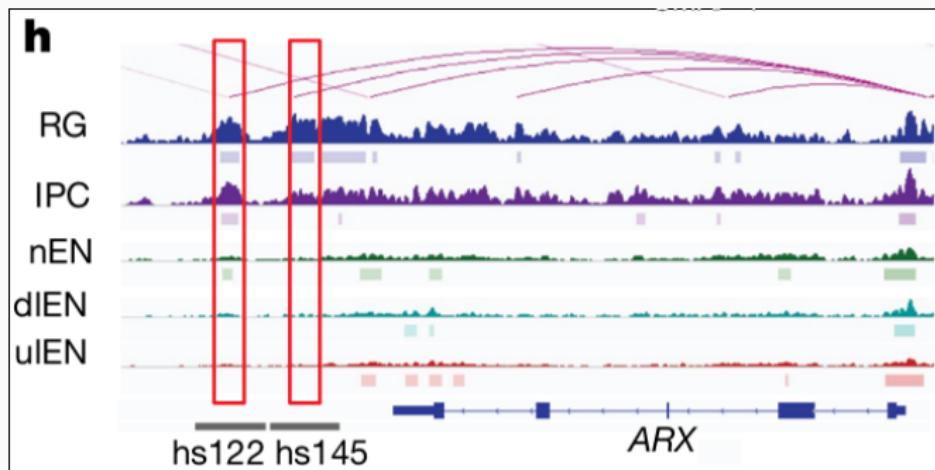


Fig 1h. predicted enhancer-gene interactions for RGs



- **Pink Curves** are predicted enhancer-gene interactions for RGs
- **Red Boxes** are predicted enhancers of ARX

Fig 1h. LacZ staining regions of enhancer activity for enhancer candidates

hs145



hs122

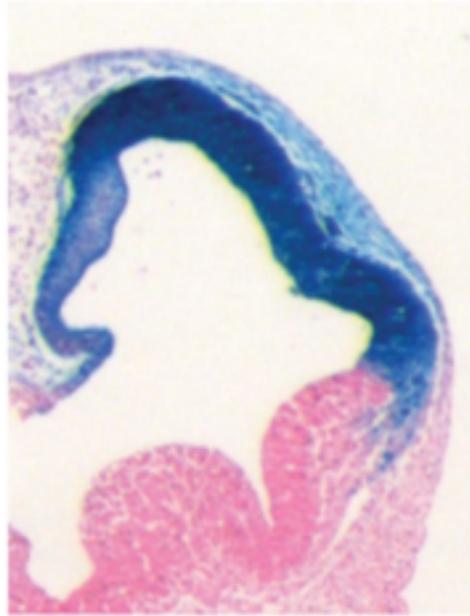
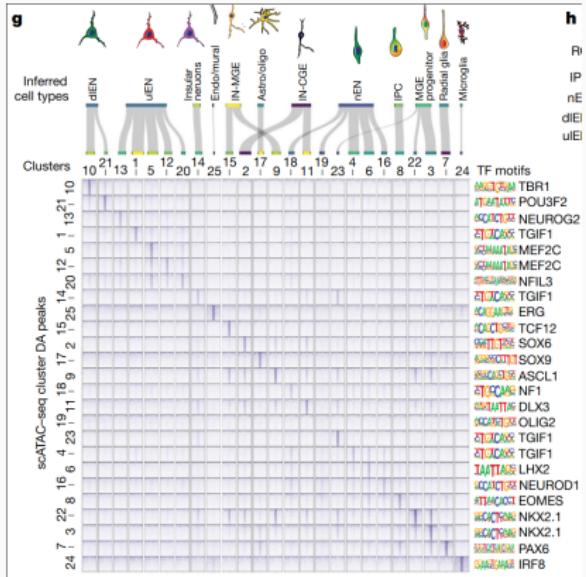


Fig 1g. Characterizing the regulatory 'grammar' of cell types

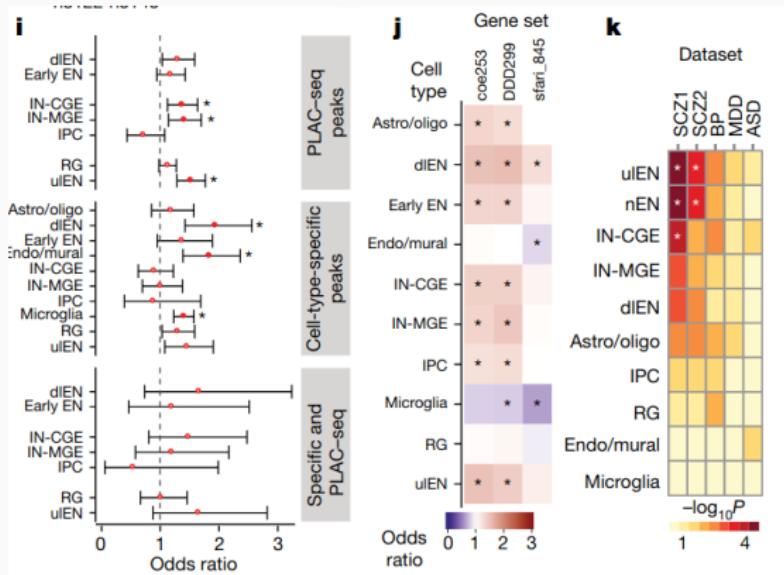


- Working towards discovering transcription factor code that controls cell lineage

Disease risk in the regulatory landscape

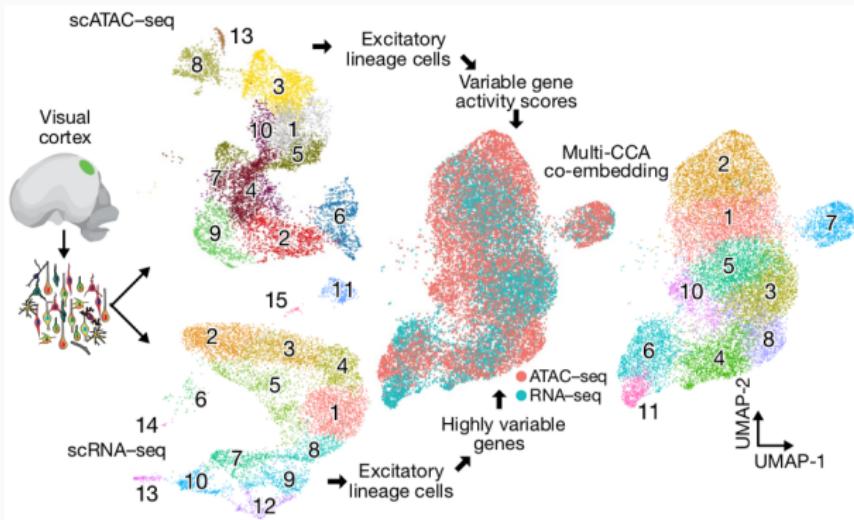
- Explored mutations in non-coding genomic regions, and loss-of-function in chromatin regulators
- Linked ATAC-seq peaks, and putative enhancers with variants

Fig 1ijk.



- Potential to identify specific regulatory programs during cortical development that confer the greatest risk for neurodevelopmental disorders

Fig 2a Workflow for co-embedding scATAC-seq and scRNA-seq data



- Why 3n in scATAC-seq and 2n in scRNA-seq?
- Right, UMAP projection of co-embedded scATAC-seq and scRNA-seq cells coloured by Leiden clusters

Fig 2bc.

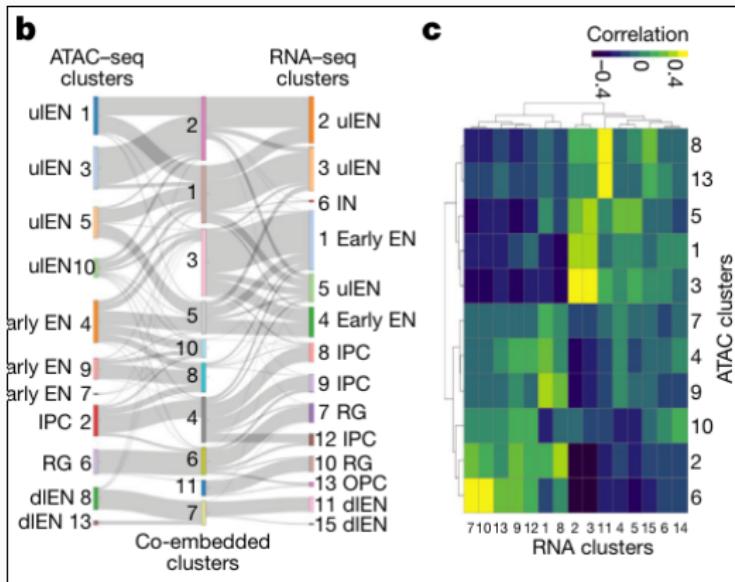


Fig 2e. Pseudotime

e Pseudotime

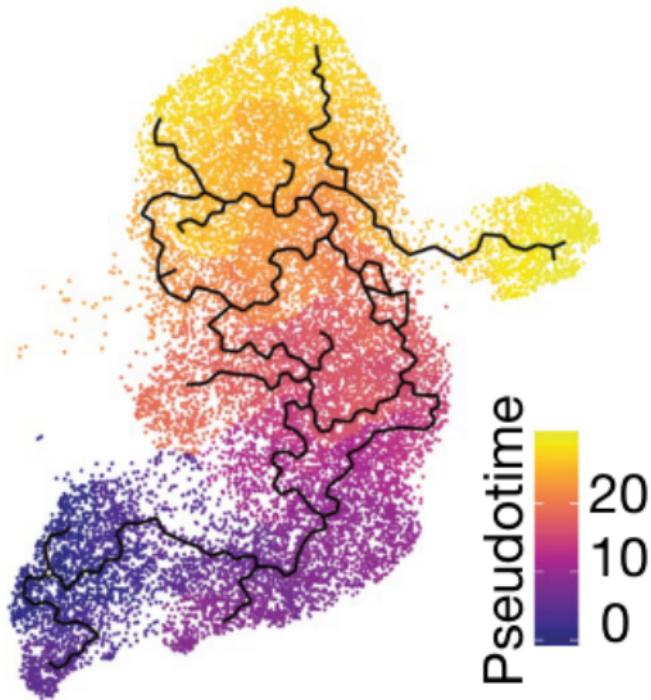
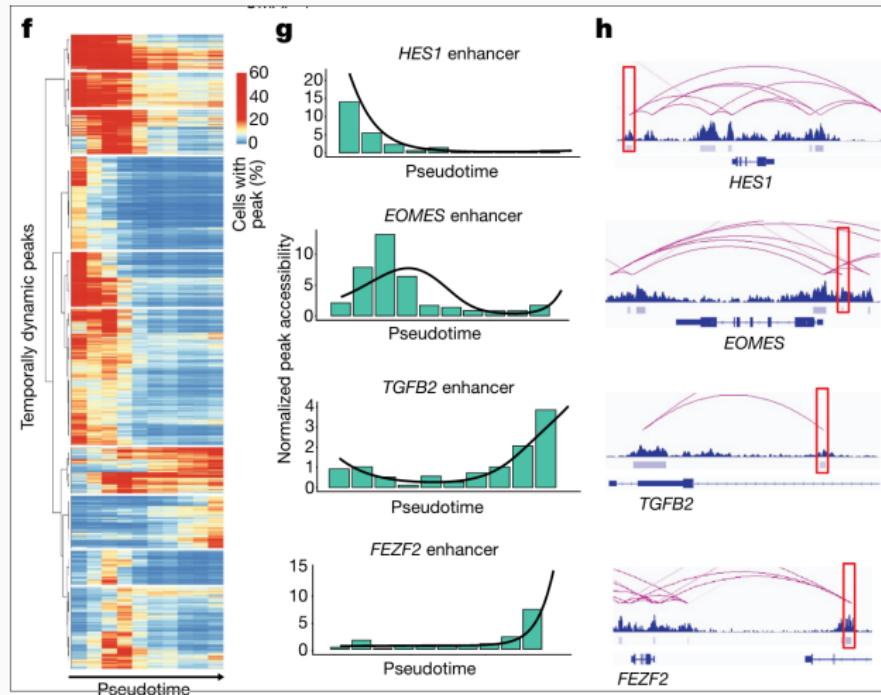


Fig 2fgh. Enhancers predicted to interact with genes linked to cell type identity



Gene activity score

- a proxy for gene expression
- ATACseq fragments in the gene body plus promoter (2 kb upstream from transcription start sites) of all protein-coding genes were summed for each cell

Fig 2i. Gene activity scores compared to gene expression

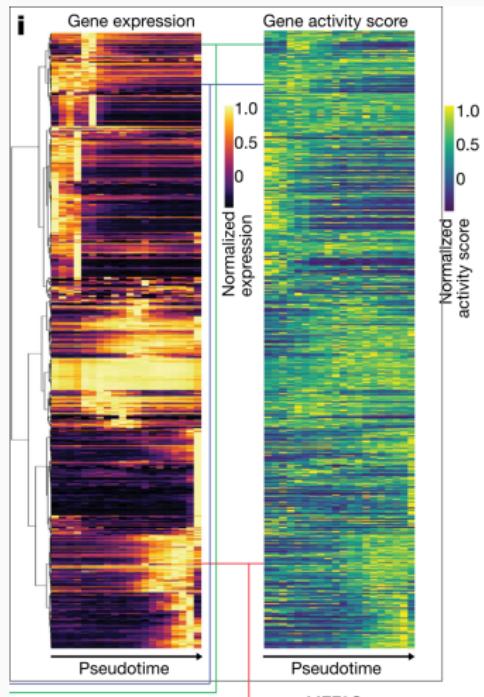


Fig 2j. Dynamic chromatin states in neurogenesis

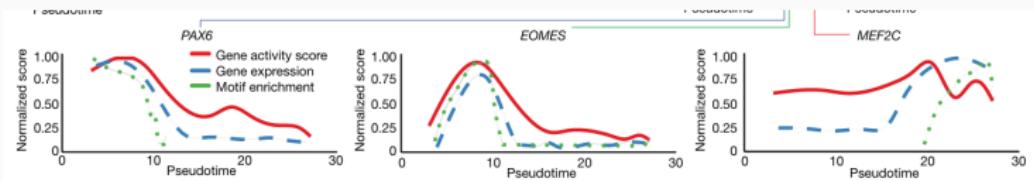


Fig 3a. Area-specific chromatin states

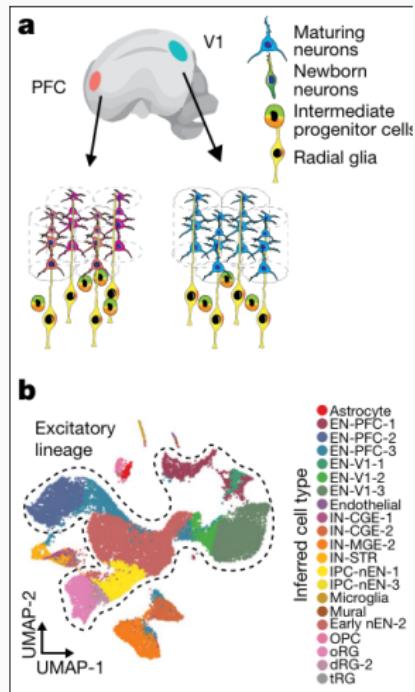


Fig 3. Area-specific chromatin states

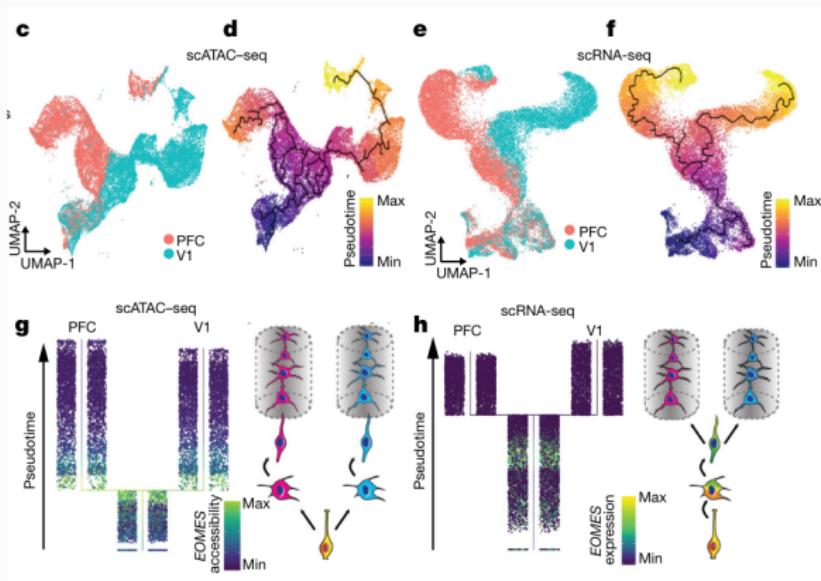
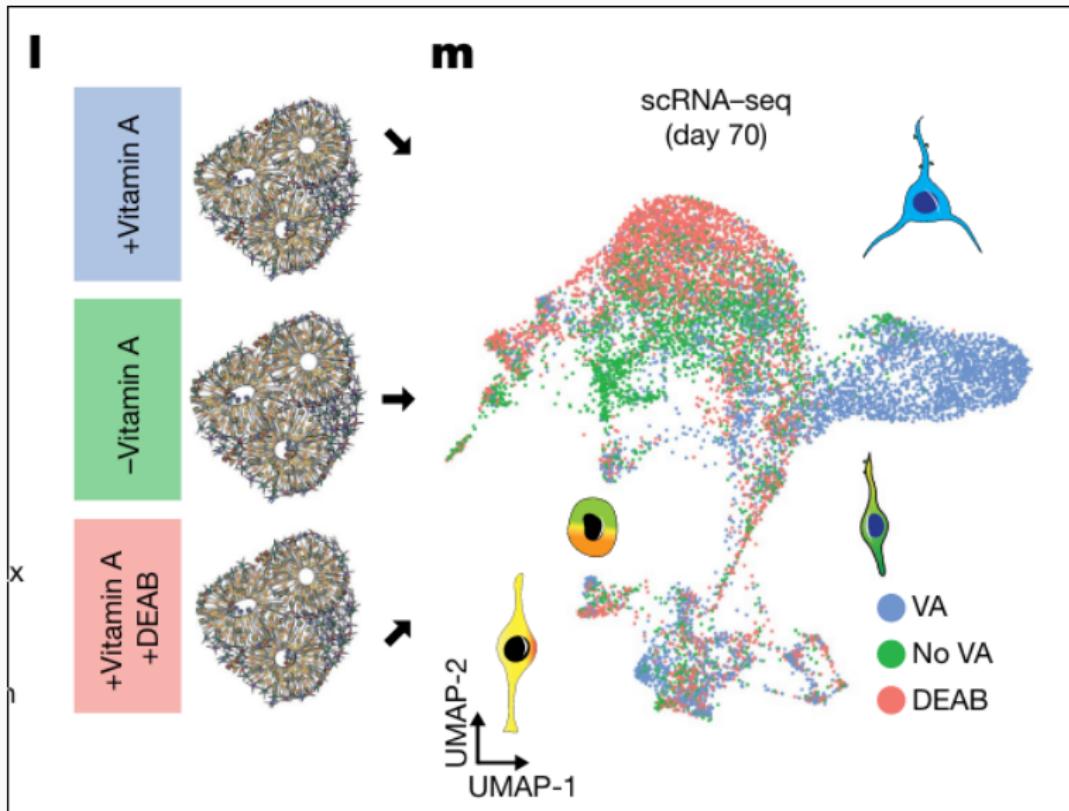


Fig 3IM. Retinoic acid in cortical arealization



Benchmarking cerebral organoids

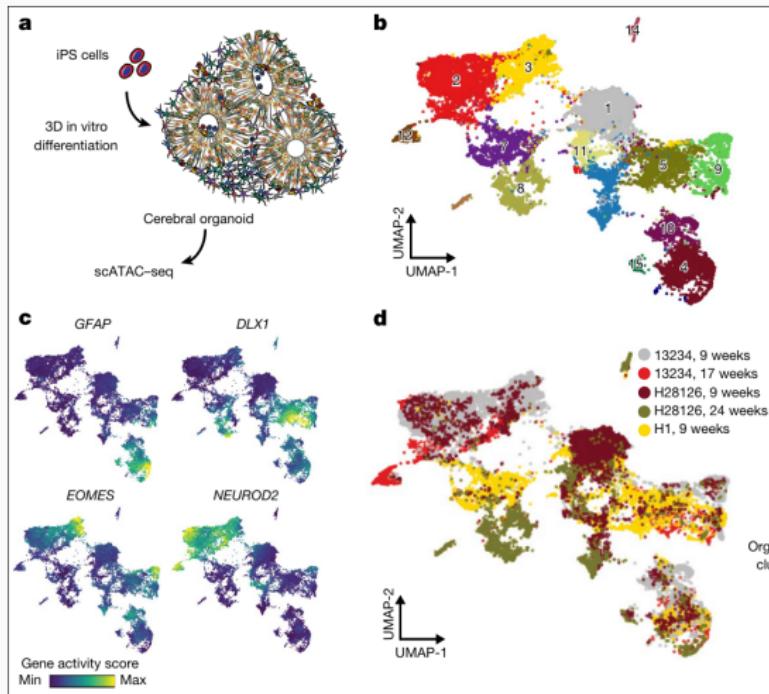
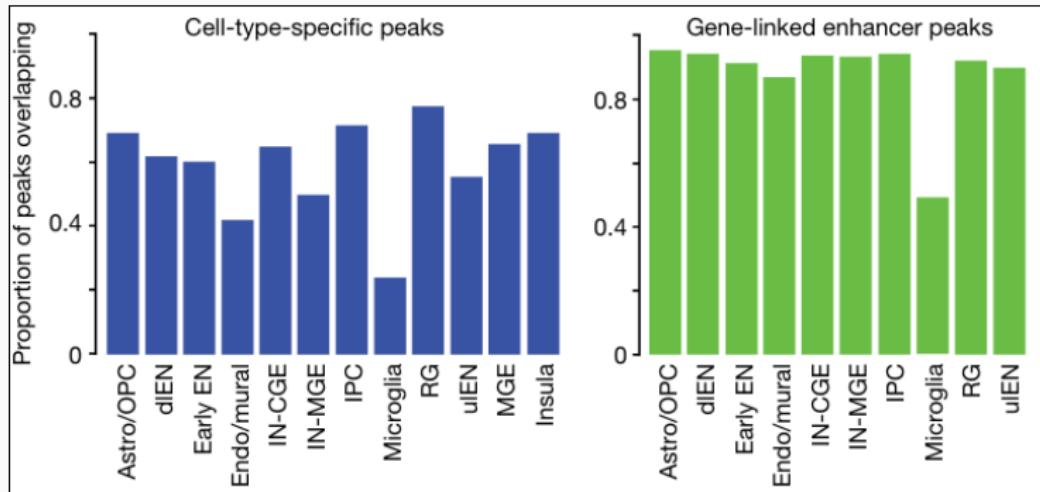
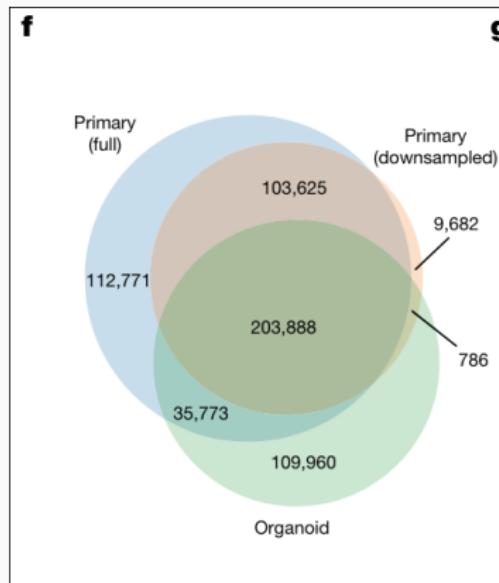


Fig 4i. 80% of predicted enhancers found in organoids



- Missing microglial enhancers

Fig 4f. Overlap of Peaks



Discussions

- Found thousands of transiently accessible loci that track with neuronal differentiation.
- Found states that may reveal mechanisms for cell fate
- Extend the established role of RA signaling in forebrain development
 - RA signaling plays a role in the specification of excitatory neurons

Article Critique

- Small number of n
- Difficulty reproducing enhancers

Future Directions

- Look for more disease-associated variants in regulatory regions that affect the developing cortex