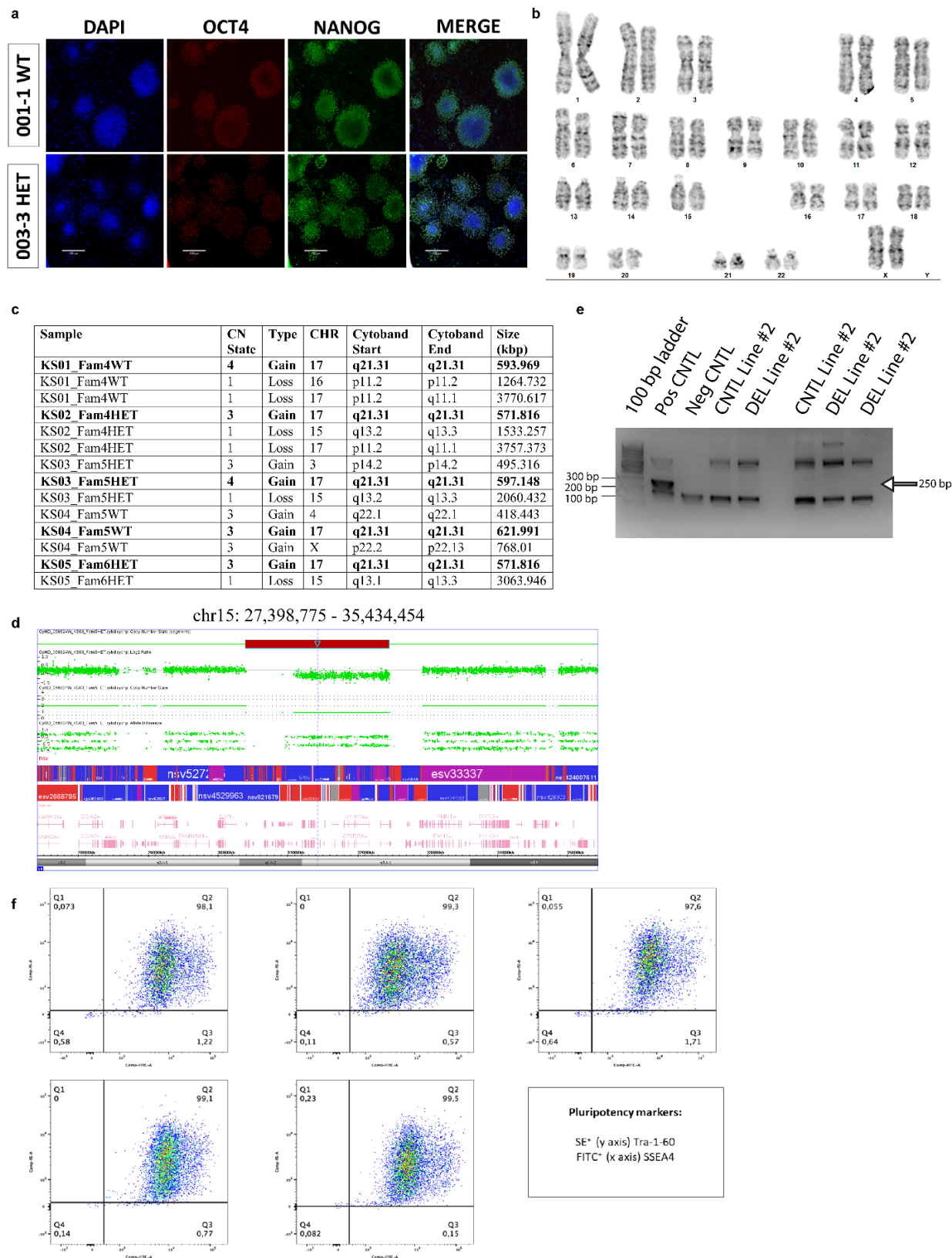
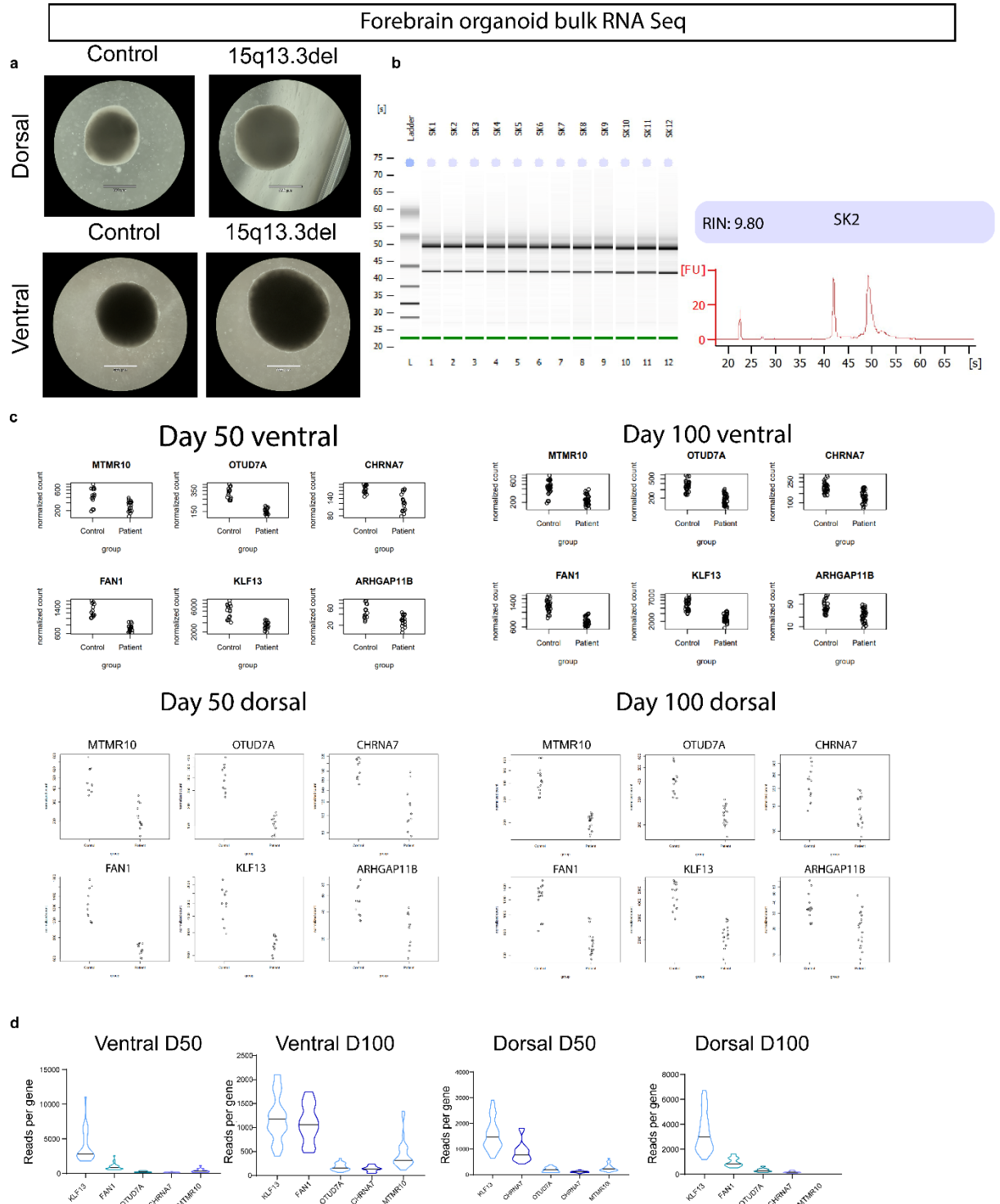


Extended Data Figures



### Extended Data Fig. 1: Validation of iPSC reprogramming and cellular pluripotency.

**a**, Representative pluripotency immunocytochemistry of reprogrammed iPSCs cocultured with mouse embryonic fibroblasts. Images taken at 20x on an epifluorescent ECHO microscope. Scalebars represent 430  $\mu\text{m}$ . **b**, Representative G-Band analysis on reprogrammed iPSCs shows no karyotypic abnormalities. **c**, Microarray genotyping of three 15q13.3del families through Affymetrix Cytoscan HD array **d**, with representative 15q13.3del microdeletion plot below. *Track 1*: location of CNV segment, *track 2*: log2 ratio for each probe, *track 3*: estimated copy number state for each probe, *track 4*: allele difference per probe, *track 5*: gene names and locations. Copy number changes reported in DGV are color coded as follows: purple = copy number neutral LOH region; red = copy number losses; blue = copy number gains, grey = copy number changes reported, but of unknown type. **e**, Representative mycoplasma screen in 15q13.3 iPSCs (lanes 1-9: NEB 100bp ladder, mycoplasma positive control, negative control, Control #1, 15q13.3del #1, Control #2, 15q13.3del #2, 15q13.3del #3,). The arrow indicates 250 bp mycoplasma band in positive control lane only. **f**, Flow cytometry in P17 reprogrammed iPSCs in 5 unique lines for pluripotency markers TRA-160 (SE labeled, y axis) and SSEA4 (FITC labeled, x axis).

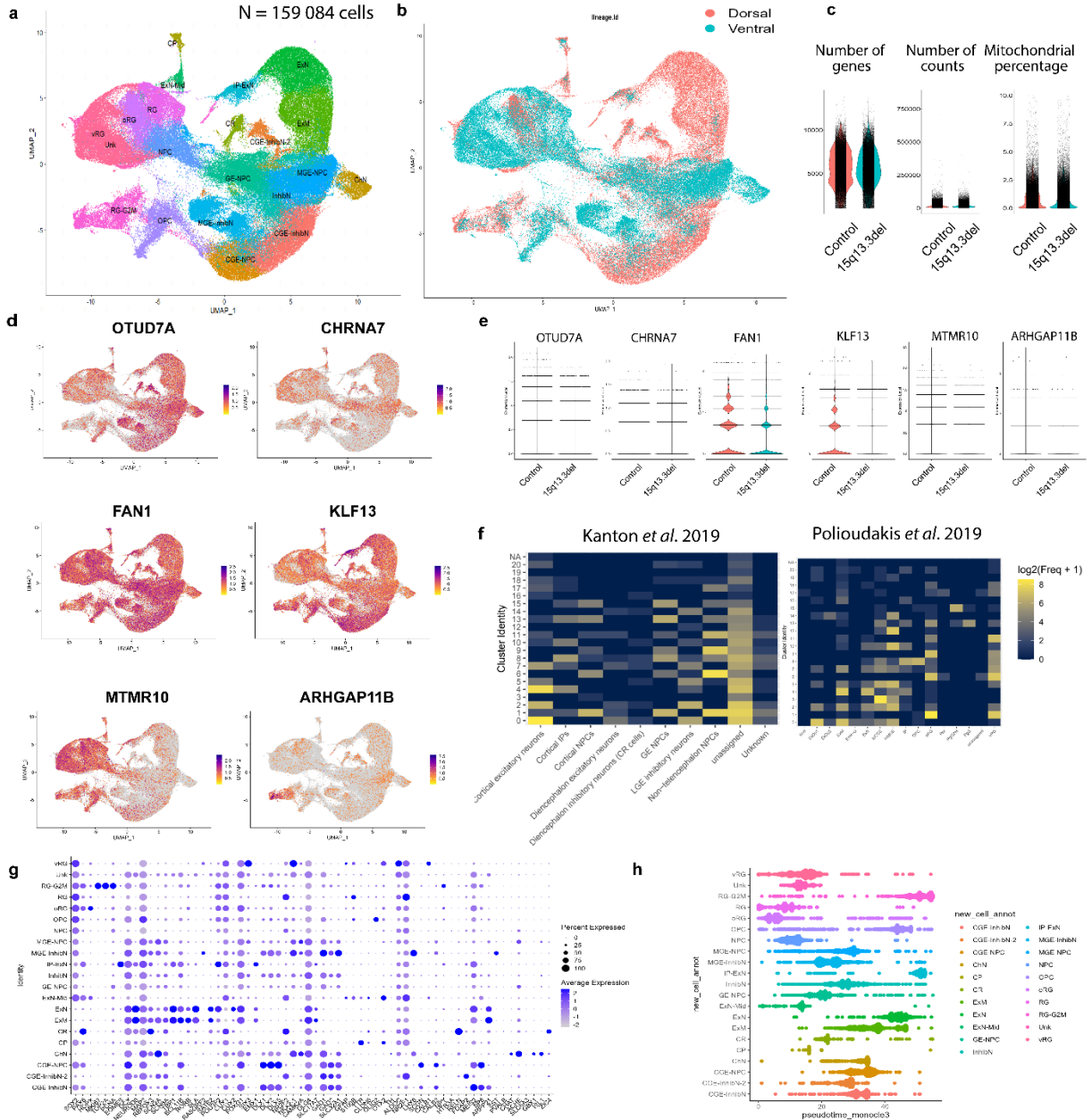


**Extended Data Fig. 2: Bulk RNA sequencing identifies *FAN1* and *KLF13* as the prominent genes expressed during human fetal and forebrain organoid development.**

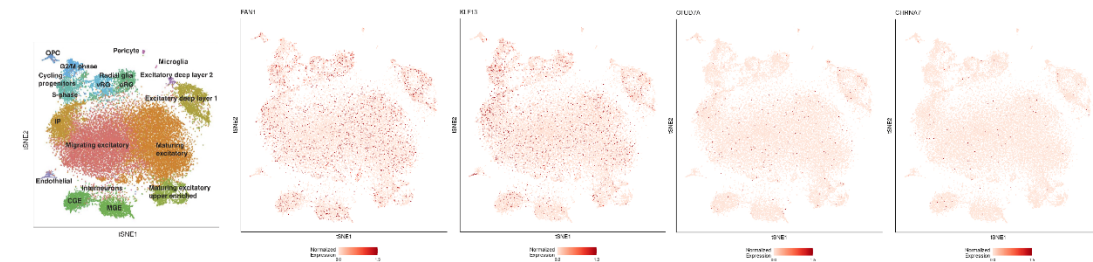
**a**, Brightfield images of unguided neural organoids throughout development. Images taken on an epifluorescent ECHO microscope; scale bars represent 890  $\mu$ m. **b**, RNA integrity assessment for bulk

transcriptomics. **c**, 15q13.3 genes are heterozygous in patient-derived day 50 and 100 forebrain organoid bulk RNA Seq. **d**, KLF13 and FAN1 have the highest expression throughout forebrain organoid development.

## D100 Forebrain organoid scRNA Seq

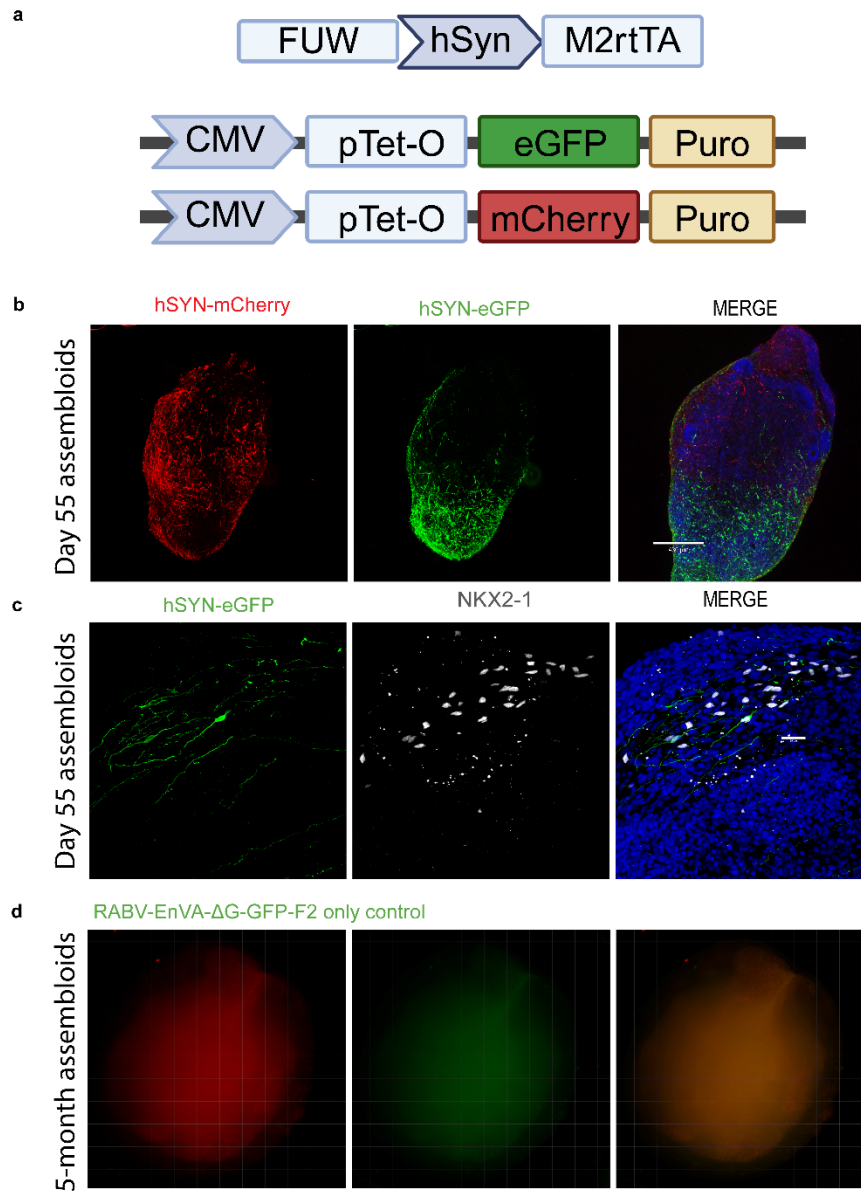


## GW17-18 Human fetal cortex



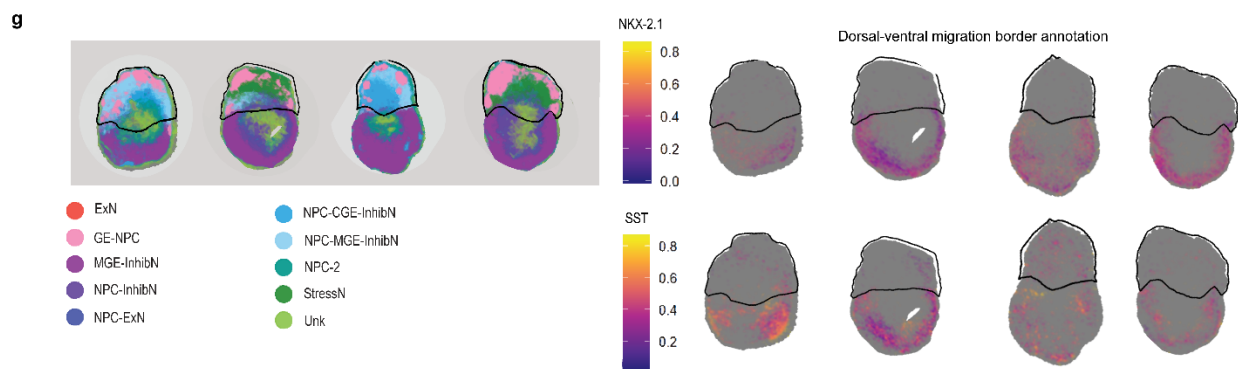
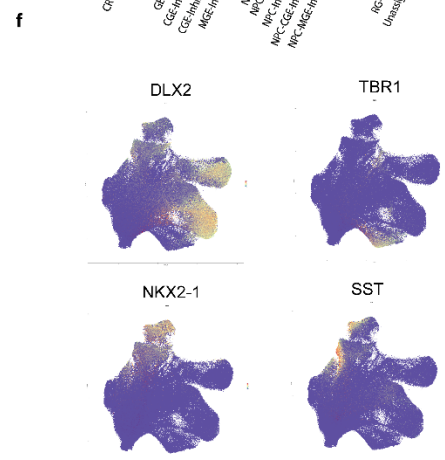
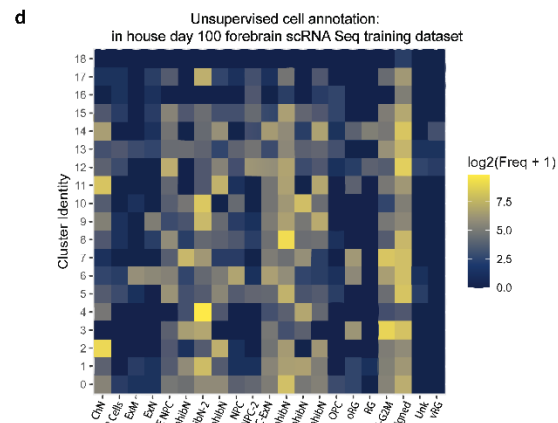
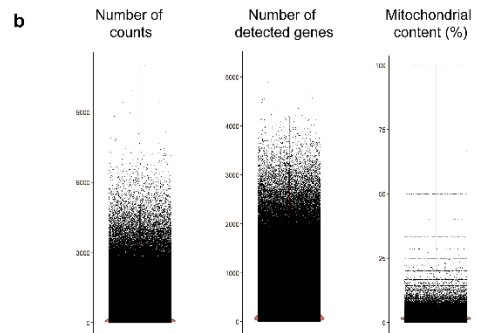
**Extended Data Fig. 3: Single cell RNA sequencing identifies *FAN1* and *KLF13* as the prominent genes expressed during human fetal and forebrain organoid development.**

**a**, UMAP plot of Day 100 forebrain scRNA Seq cell types (left) and **b**, forebrain lineage (right, pink dorsal, blue ventral), (N = 3 families, n = n = 68 455 cells). **c**, Quality control metrics for gene number, count, and mitochondrial content. **d**, UMAP plot of 15q13.3 genes shows *KLF13* and *FAN1* to have the highest expression and **e** heterozygosity of 15q13.3 genes. **f**, scClassify heatmaps UMAP plot of day 100 forebrain scRNA cell types. **g**, Canonical marker expression in annotated day 100 forebrain cell types. **h**, Emergence of cell populations throughout pseudotime. **i**, Expression of 15q13.3 genes in mid-gestation fetal cortex scRNA Seq data (published in Polioudakis *et al*<sup>109</sup>).



**Extended Data Fig. 4: Forebrain assembloid generation and circuit phenotyping quality control.**

**a**, Doxycycline-inducible constructs for sparse labeling. **b**, Endogenous immunofluorescence of mCherry and eGFP in sparsely labeled control assembloids. Scalebars represent 430  $\mu\text{m}$ . **c**, Colocalization of eGFP and MGE inhibitory marker NKX2-1. **d**, Negative control (rabies virus only) for CMV and Dlx5/6 monosynaptic tracing experiments.





**Extended Data Fig. 5: Quality control assessment of Visium spatial transcriptomic platforms.**

**a**, RNA integrity assessment of sectioned HD assembloid tissue. **b**, Gene counts and features in pooled pre-filtered Visium HD dataset and **c** per hiPSC line. **d**, Unsupervised cell annotation of Visium HD spots using scClassify and *in house* day 100 forebrain scRNA Seq training dataset. **e**, Top 5 variable genes per cell type cluster ranked by log2 fold change. **f**, Feature plots of canonical inhibitory (pan/MGE) and excitatory markers. **g**, Lineage spatial annotations using NKX2-1/SST/InhibN-MGEs as markers.