

Supplementary information to:

Original article:

A FACILE METHOD TO GENERATE CEREBRAL ORGANOID FROM HUMAN PLURIPOTENT STEM CELLS

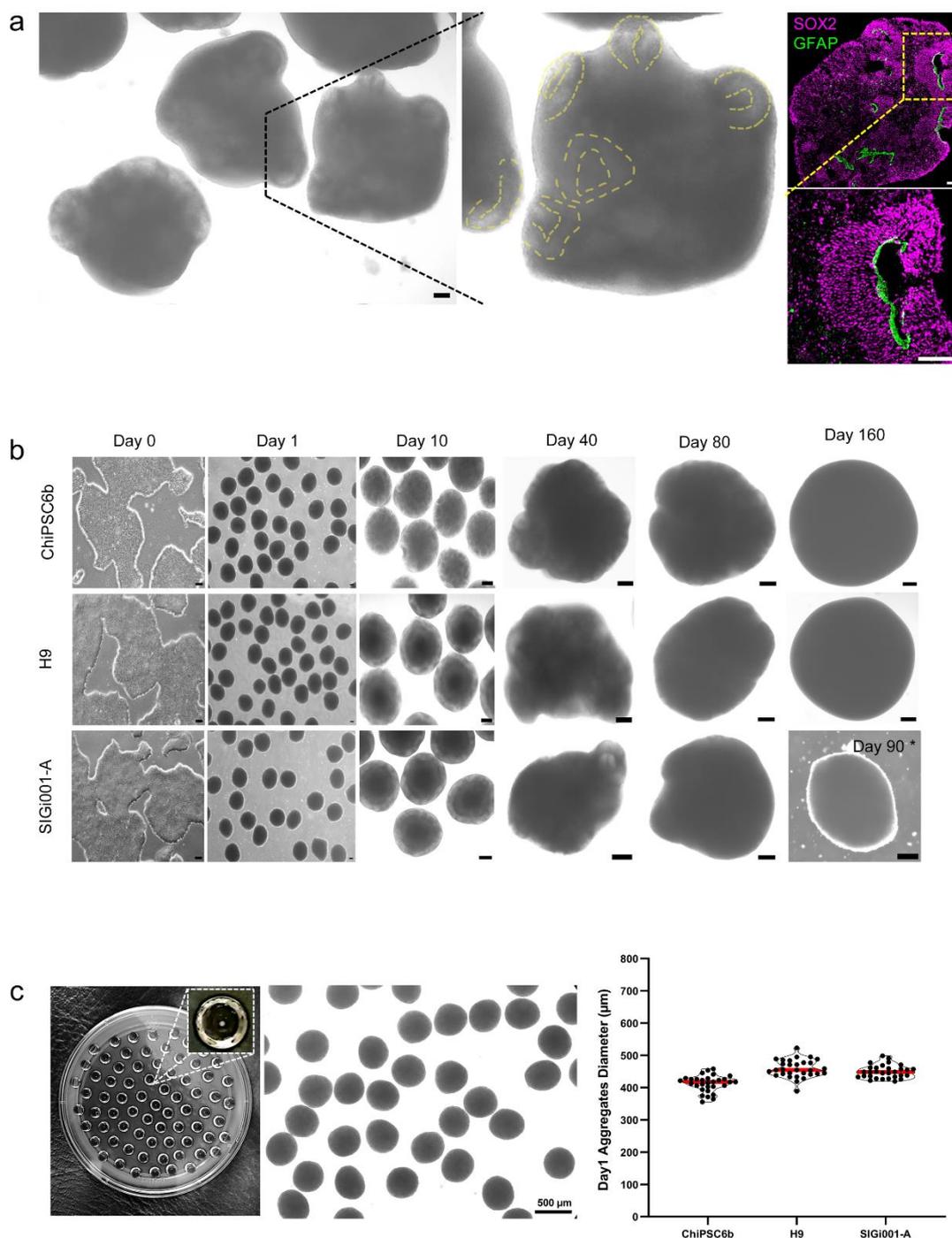
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(Supplementary) Figure S1: Generation of cerebral organoids from three human pluripotent cell lines (ChiPSC6b-hiPSC, H9-hESC, SIGi001-A-hiPSC). (a) Formation of multiple VZ-like structures within the COs between days 35 to 40. These structures encompassed a VZ-like cavity space that is surrounded by radially organized neural progenitors and depicted by SOX2/GFAP immunostaining. (b) Bright-field images showing morphological changes of COs derived from different human pluripotent cell (hPSC) lines during different days of the protocol. * COs derived from SIGi001-A-hiPSC showed extensive cell death around day 90 and did not survive until day 160. All the scale bars are 100 µm.

(c) Aggregate formation using the modified hanging drop method led to generation of uniform spherical structures with a diameter size between 350 to 450 µm from hPSC lines on day 1. (The red median line in the graph indicates the average size of the aggregates.)

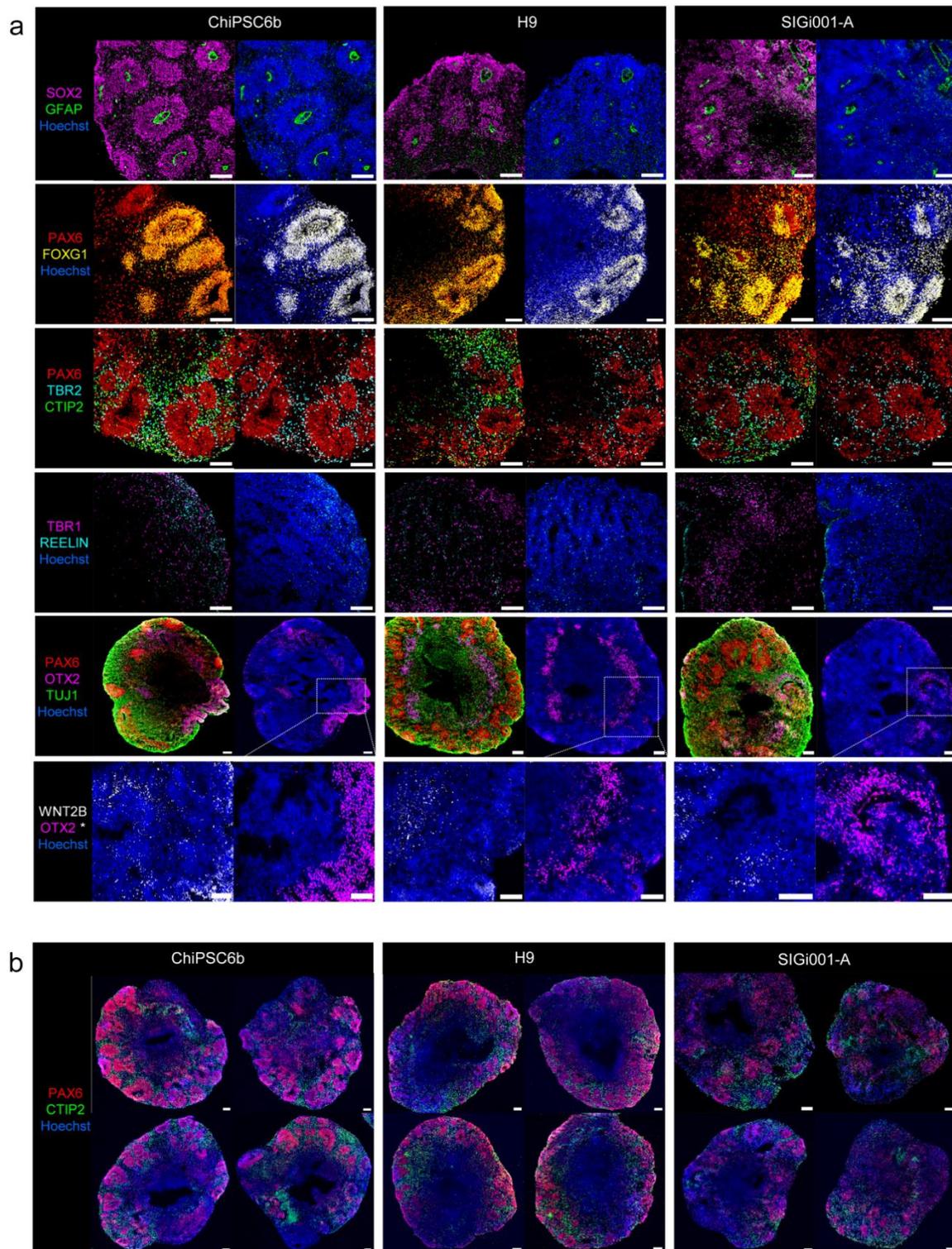


Figure S2: Immunostaining analysis to investigate different cell type identities and organization in day 40 cerebral organoids derived from three human pluripotent cell lines (ChiPSC6b-hiPSC, H9-hESC, SIGi001-A-hiPSC). (a) Representative immunostaining images indicating similar expression patterns of specific markers for positional neural cell fates (PAX6/FOXG1, OTX2, and WNT2B), ventricular-like zone (SOX2/GFAP), and early cortical neuron layering organization (TBR2/CTIP2 and TBR1/REELIN) on day 40, in different hPSC lines derived COs. (b) Sample immunostaining images from different hPSC lines derived COs on day 40, demonstrating cytoarchitecture of VZ-like regions (PAX6) and early cortical neurons layer (CTIP2). All the scale bars are 100 μ m.

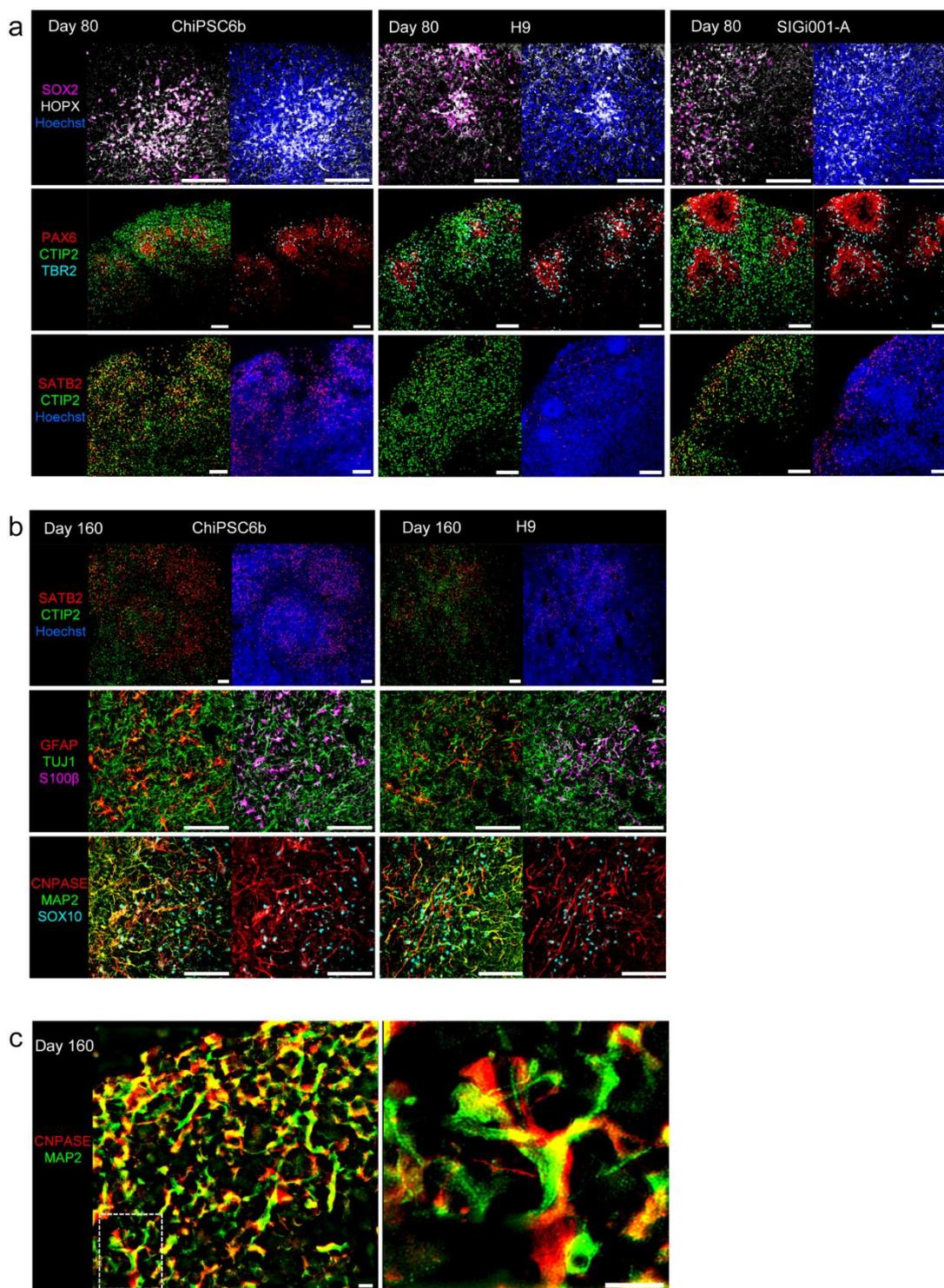


Figure S3: Overview of expression pattern for maturation indicating markers in cerebral organoids derived from different hPSC lines. (a) Immunostaining analysis for the presence of basal radial glial cells (SOX2/HOPX), intermediate progenitor cells (TBR2), and deep and superficial cortical layer neurons (CTIP2/SATB2) on day 80. (b) Day 160 COs stained for deep and superficial cortical layer neurons (CTIP2/SATB2), astrocytes (GFAP/S100 β), and oligodendrocytes (CNPASE/SOX10) markers indicating maturation progress in COs derived from different hPSC lines. (c) Higher magnifications of immunostaining for oligodendrocyte marker (CNPASE) illustrated these cells in close contact with neurite extensions (MAP2).

All the scale bars are 100 μ m.

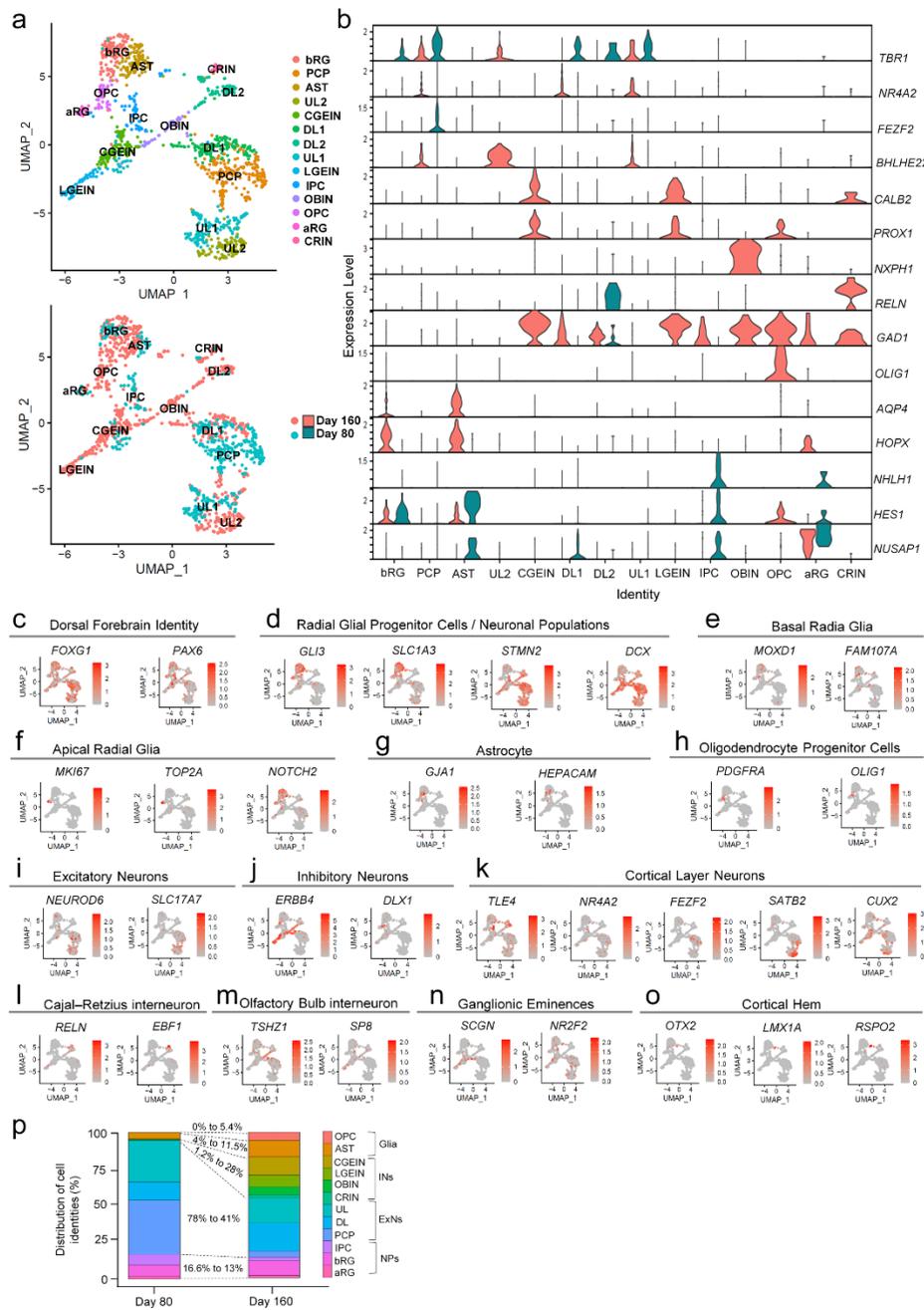


Figure S4: Determining the cell composition of H9 hPSC-COs by snRNA-seq analysis. (a) Uniform-manifold approximation map (UMAP) visualization of different cell-type clusters in COs derived from H9 cell line. UMAPs are represented as integrated data from day 80 and day 160 with n=447 and n=823 nuclei analyzed for each day, respectively. Each dot represents a single nucleus from a single cell. In the upper UMAP, the color of each dot entitles the defined cell identities, and in the lower UMAP colors illustrate the corresponding day. (b) Violin plots demonstrating the expression level of selected specific markers to ascertain different cell identities in each cluster. (c-o) Individual UMAP plots, indicating the expression pattern of marker genes used to annotate a defined cell identity for each cluster. The gradient color shows the relative expression pattern from dark orange (higher expression) to light gray (lower expression). (p) Bar graphs illustrating the percentage of cellular distribution in different clusters, on days 80 and 160 COs. The colors label the cell type identities in the graphs. (aRG: apical radial glial, bRG: basal radial glial, AST: astrocyte, OPC: oligodendrocyte progenitor cell, PCP: primary cortical plate, DL1,2: Deep layer cortical neuron 1,2, UL1,2: Upper layer cortical neuron 1,2, CGEIN: caudal ganglionic eminences interneuron, LGEIN: lateral ganglionic eminences interneuron, CRIN: Cajal–Retzius interneuron, OBIN: olfactory bulb interneuron, NPs: neural progenitor cells, IPC: intermediate progenitor cell, ExNs: excitatory neurons, INs: inhibitory neurons)

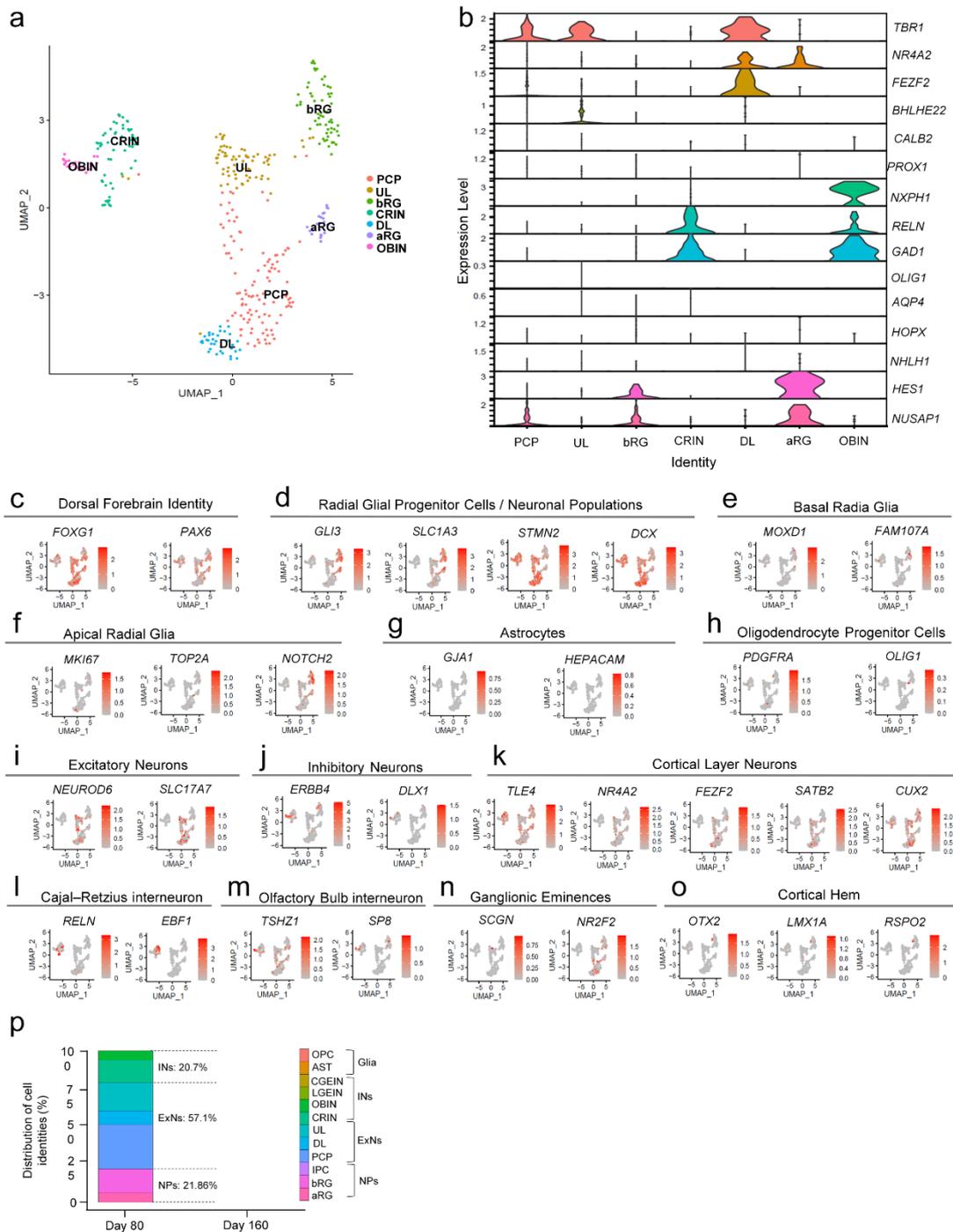


Figure S5: Determining the cell composition of SIGi001-A hPSC-COs by snRNA-seq analysis. (a) UMAP illustration of snRNA-seq data analysis of $n=379$ nuclei from day 80 COs derived from SIGi001-A hPSC line. The color of each dot entitles the defined cell identities of each analyzed nucleus. (b) Violin plots demonstrating the expression level of selected specific markers to ascertain different cell identities in each cluster. (c–o) Individual UMAP plots, indicating the expression pattern of marker genes used to annotate a defined cell identity for each cluster. The gradient color shows the relative expression pattern from dark orange (higher expression) to light gray (lower expression). (aRG: apical radial glial, bRG: basal radial glial, AST: astrocyte, OPC: oligodendrocyte progenitor cell, PCP: primary cortical plate, DL1,2: Deep layer cortical neuron 1,2, UL1,2: Upper layer cortical neuron 1,2, CGEIN: caudal ganglionic eminences interneuron, LGEIN: lateral ganglionic eminences interneuron, CRIN: Cajal–Retzius interneuron, OBIN: olfactory bulb interneuron, NPs: neural progenitor cells, IPC: intermediate progenitor cell, ExNs: excitatory neurons, INs: inhibitory neurons)

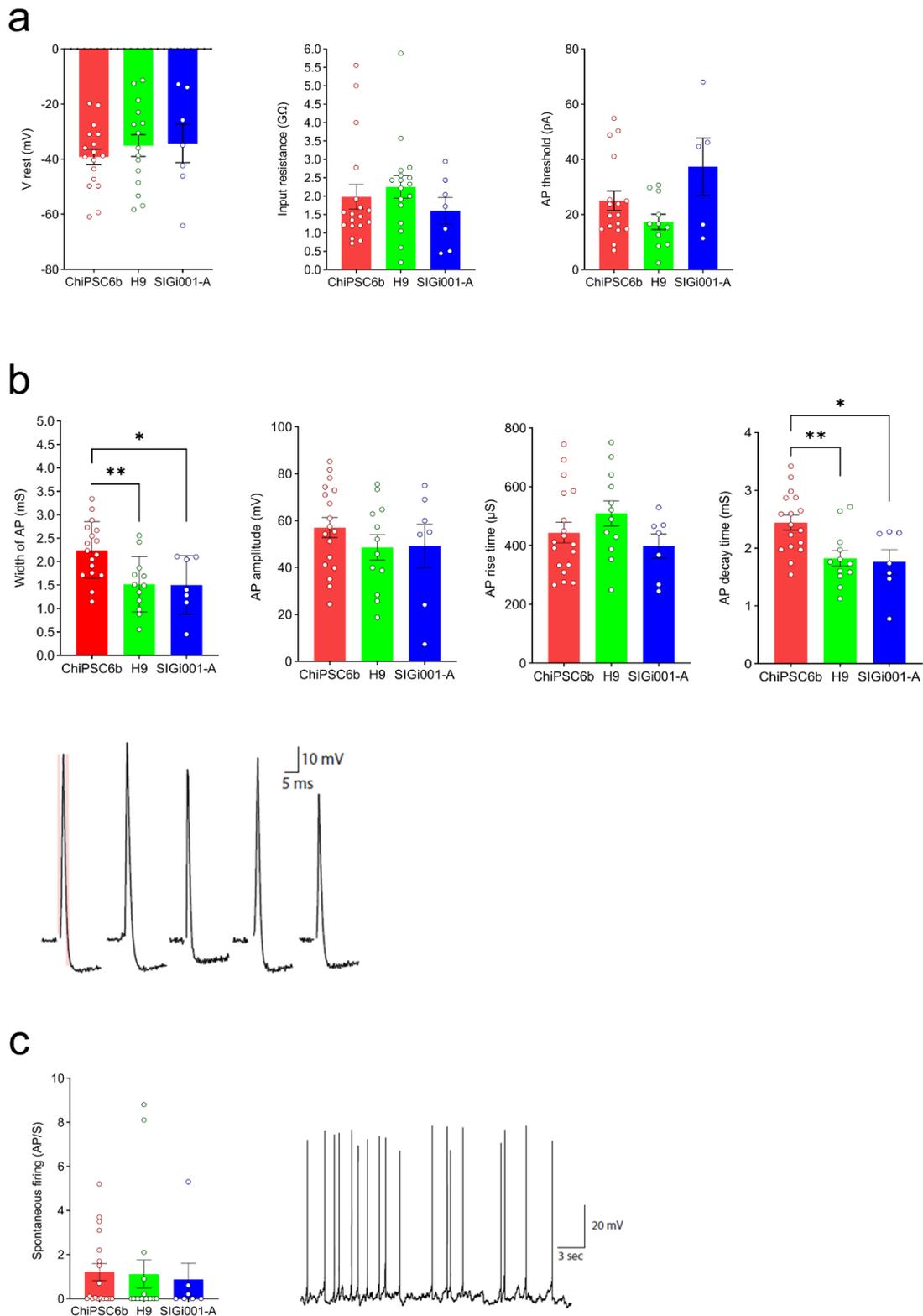


Figure S6: Electrophysiological analysis of cerebral organoids. (a) Measured intrinsic physiological properties of neurons including resting membrane potential, input resistance, and action potential threshold. (b) Single action potential parameters were measured for COs derived from different pluripotent cell lines. (c) Spontaneous action potential firing and related sample recorded trace. The number of neurons related to each pluripotent cell line-derived CO is depicted within each graph. All data represented as mean \pm SD; one-way ANOVA, * $p < 0.05$, ** $p < 0.01$).

Supplementary Video 1: KCl-induced depolarization followed by Ca²⁺ entry in Fluo-4 AM loaded cerebral organoid. See file https://doi.org/10.17179/excli2023-6299/supplementary_video

Supplementary Table 1: List of primary and secondary antibodies used for immunostaining

Antigen	Company	Cat. no.	Dilution
Rabbit anti-SOX2	Millipore	AB5603	1:100
Rat anti-GFAP	Thermo Fisher Scientific	0300-13	1:200
Mouse anti-PAX6	Abcam	ab78545	1:100
Rabbit anti-FOXP1	Abcam	ab196868	1:100
Rabbit anti-OTX2	Abcam	ab21990	1:100
Chicken anti-TUJ1	Millipore	AB9354	1:200
Rabbit anti-WNT2B	Abcam	ab178418	1:100
Rabbit anti-TBR2	Abcam	ab23345	1:100
Rat anti-CTIP2	Abcam	ab18465	1:100
Rabbit anti-TBR1	Abcam	ab31940	1:100
Mouse anti-REELIN	Abcam	ab78540	1:100
Mouse anti-HOPX	Santa Cruz Biotechnology	sc-398703	1:50
Rabbit anti-S100B	Abcam	ab52642	1:100
Rabbit anti-MAP2	Synaptic Systems	002188	1:200
Goat anti-SOX10	R&D Systems	AF2864	1:100
Mouse anti-CNPASE	Sigma	C5922	1:100
Mouse anti-SATB2	Abcam	ab51502	1:100
NorthernLights Streptavidin NL557	R&D Systems	NL999	1:200
Donkey anti-Rabbit, Alexa Fluor 647	Thermo Fisher Scientific	A-31573	1:500
Donkey anti-Rat, Alexa Fluor 488	Thermo Fisher Scientific	A-21208	1:500
Donkey anti-Mouse, Alexa Fluor 555	Thermo Fisher Scientific	A-31570	1:500
Goat anti-Chicken, Alexa Fluor 488	Thermo Fisher Scientific	A-11039	1:500
Donkey anti-Rabbit, Alexa Fluor 488	Thermo Fisher Scientific	A-21206	1:500
Donkey anti-Goat, Alexa Fluor 633	Thermo Fisher Scientific	A-21082	1:500