SUPPLEMENTARY FIGURES

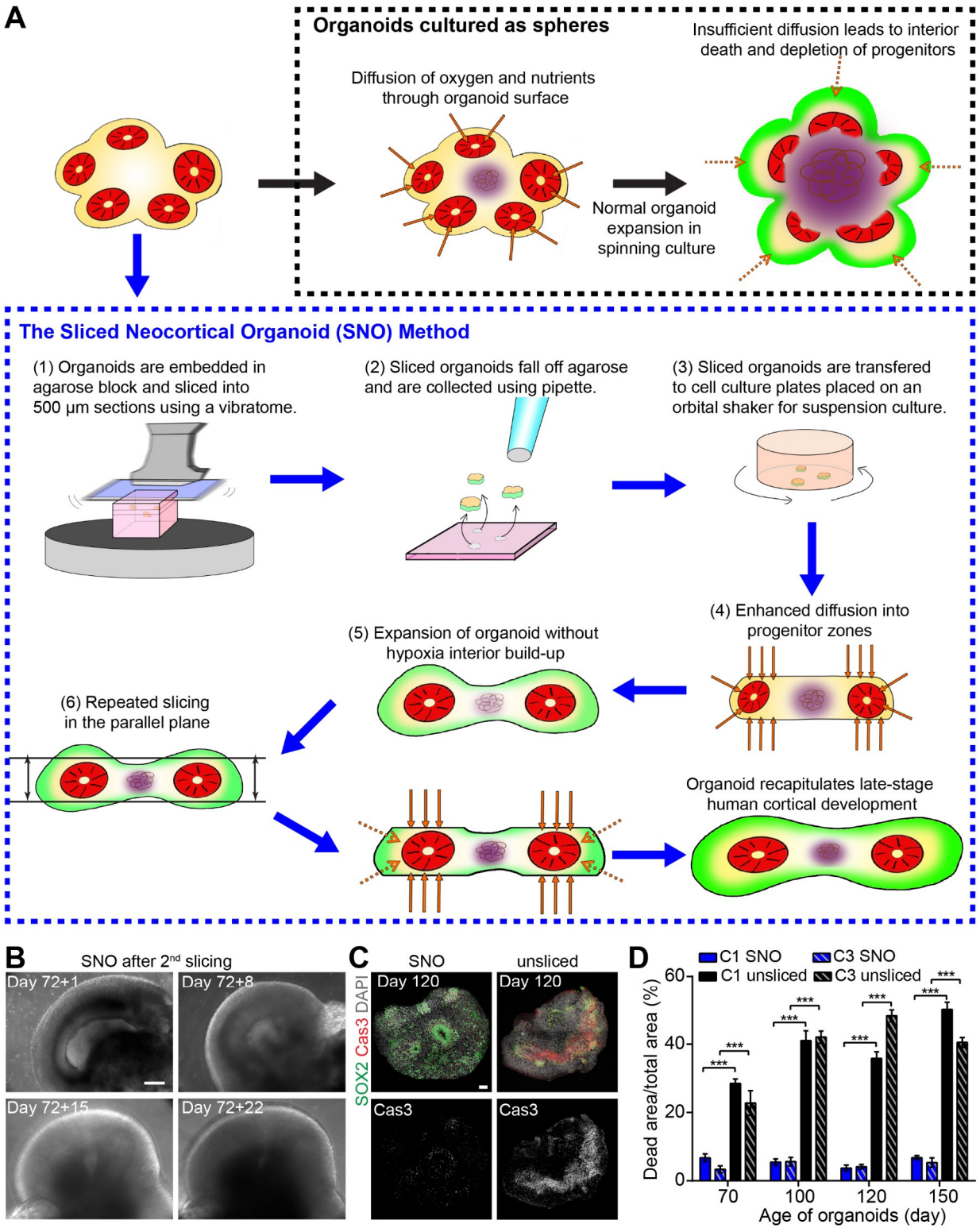

Figure S1. Schematic Illustration of the SNO Method and Additional Characterizations of Cell Viability, related to Figure 1.

(A) Schematics highlighting the difference between spherical organoid protocols (unsliced, black box) to the SNO protocol (blue box). The drawings represent a side-view (y-z plane in Figure 1A) of a disk-shaped SNO.
(B) Sample bright-field images captured at 10X magnification, showing that the ventricular structure was maintained and continued to grow after the second slicing at Day 72. Scale bar, 200 μm.
(C-D) Analyses of the dead area in unsliced organoids and SNOs. Shown in (C) are sample tiling confocal images (Scale bar, 100 μm) and shown in (D) is the quantification for the proportion of the dead area over the total area. Values represent mean ± S.E.M. (n = 5 organoids for each iPSC lines; ***$p < 0.0005$, Student's t-test).

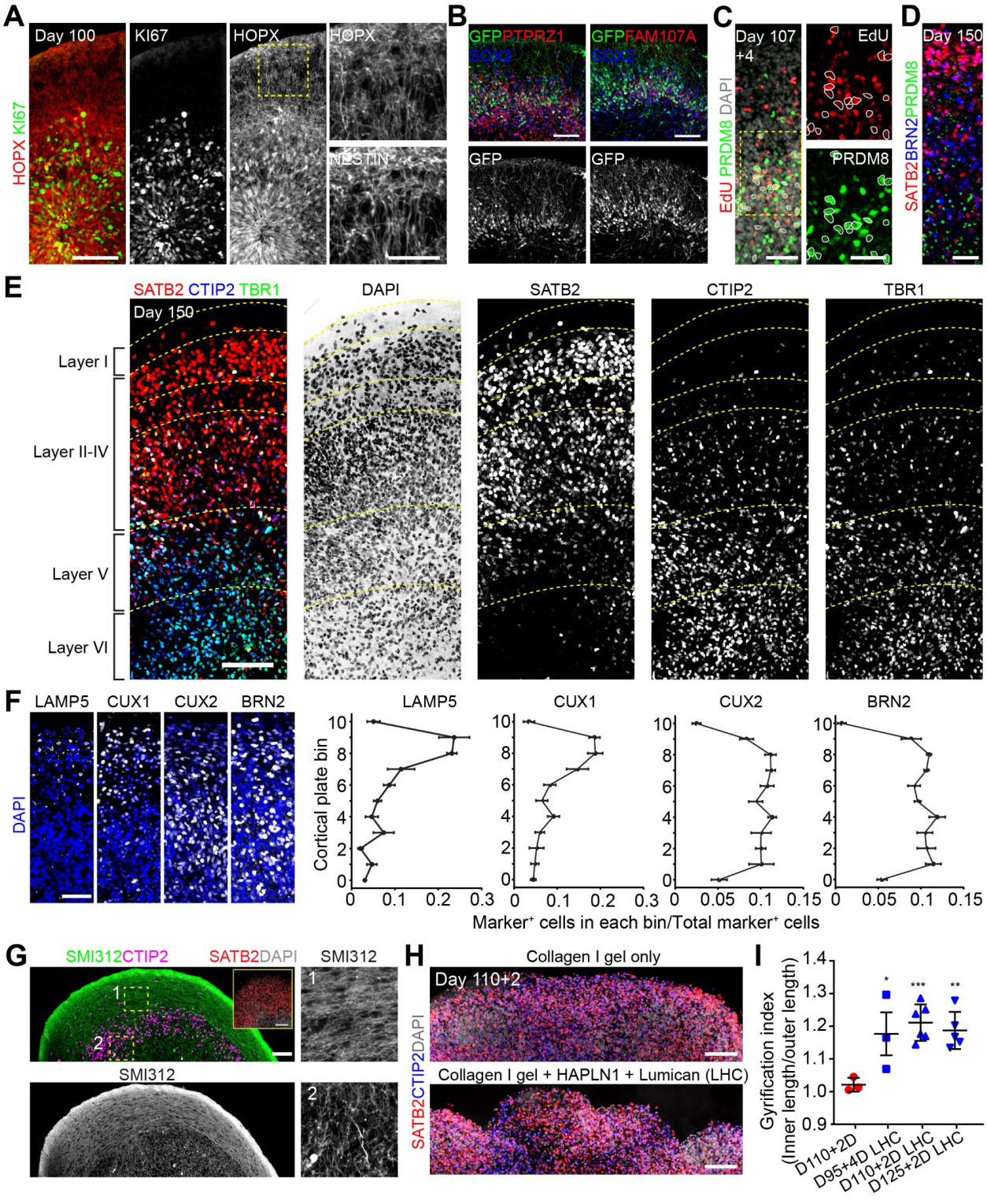

Figure S2. Additional Characterization of the Progenitor Zones and Cortical Plate in SNOs, related to Figures 2 and 4.

(A) The RGs in SNOs exhibited aligned basal processes and expressed radial glia markers HOPX and NESTIN, and cell proliferation marker KI67. Insets show a magnified view. Scale bars, 100 μm and 50 μm (inset).

(B) GFP labeling using adenovirus revealed oRG-like unipolar NPCs in Day 120 SNOs, expressing human oRG markers, PTPRZ1 and FAM107A. Images were taken on consecutive sections at the same region as Figure 2E. Scale bars, 100 μm.
(C-D) Sample confocal images showing migratory neuron marker, PRDM8, enriched in the oSVZ but not CP. Shown in (C) is a sample image of 4 day EdU pulse-chase experiment, showing that newborn neurons born at Day 107 were located in the oSVZ and expressed PRDM8. EdU^+ $PRDM8^+$ nuclei are circled in white (C). Scale bars, 50 μm.
(E) Sample tiling confocal images of the CP in a Day 150 SNO, immunostaining for cortical neuron markers SATB2, CTIP2 and TBR1. The layer-dependent distribution of markers and cytoarchitecture resemble the Layers I-VI in the human GW29 frontal cortex (Saito et al., 2011). The fluorescent image for DAPI was inverted to a white background to help visualize the nuclei density in each layer. Dashed lines were drawn to distinguish morphologically distinct layers. Scale bar, 100 μm.
(F) Laminar distribution of additional cortical neuron markers, including LAMP5, CUX1, CUX2 and BRN2 in the CP of Day 150 SNOs. Shown on the left are 100 x 300 μm columns of Day 150 CP. Also shown on the right are quantifications of the distribution of markers across the CP, calculated as [# of $marker^+$ cell in a bin/# of total $marker^+$ cells in the CP]. Bins 0-10 follow apical-to-basal direction. Values represent mean ± S.E.M. (n = 5 SNOs).
(G) Aligned axons in Day 150 SNOs showed distinct patterns in the deep and upper cortical layers. Shown on top is a sample tiling confocal image of immunostaining for pan-axonal neurofilament SMI312. Top-right corner shows the $SATB2^+$ upper layer image in the same region from a consecutive section. Insets show magnified views. Axons in the upper layer (box 1) aligned tangentially, while axons in the deep layer (box 2) appeared vertical. Scale bar, 100 μm. Inset size, 100 x 100 μm.
(H-I) Two days after embedding into collagen I gel droplets containing HAPLN1 and Lumican (LHC), the CP in slices acutely sectioned from SNOs formed folds *in vitro* resembling that previously reported in organotypic human cortical slices (Long et al., 2018). Shown in (H) are sample tiling confocal images of the CP treated with LHC (bottom panel), over control treatment with Collagen I gel only (top panel), at Day 112, two days after the treatment. Scale bars, 100 μm. Shown in (I) is the quantification of the gyrification index after the treatment for two (2D) or four (4D) days. The gyrification index is analyzed by measuring the inner length and outer length of the pial surface and calculating their ratio. Values represent mean ± S.D. (n = 3 - 6 SNOs; $*p < 0.05$, $**p < 0.005$, $***p < 0.0005$, Student's t-test).

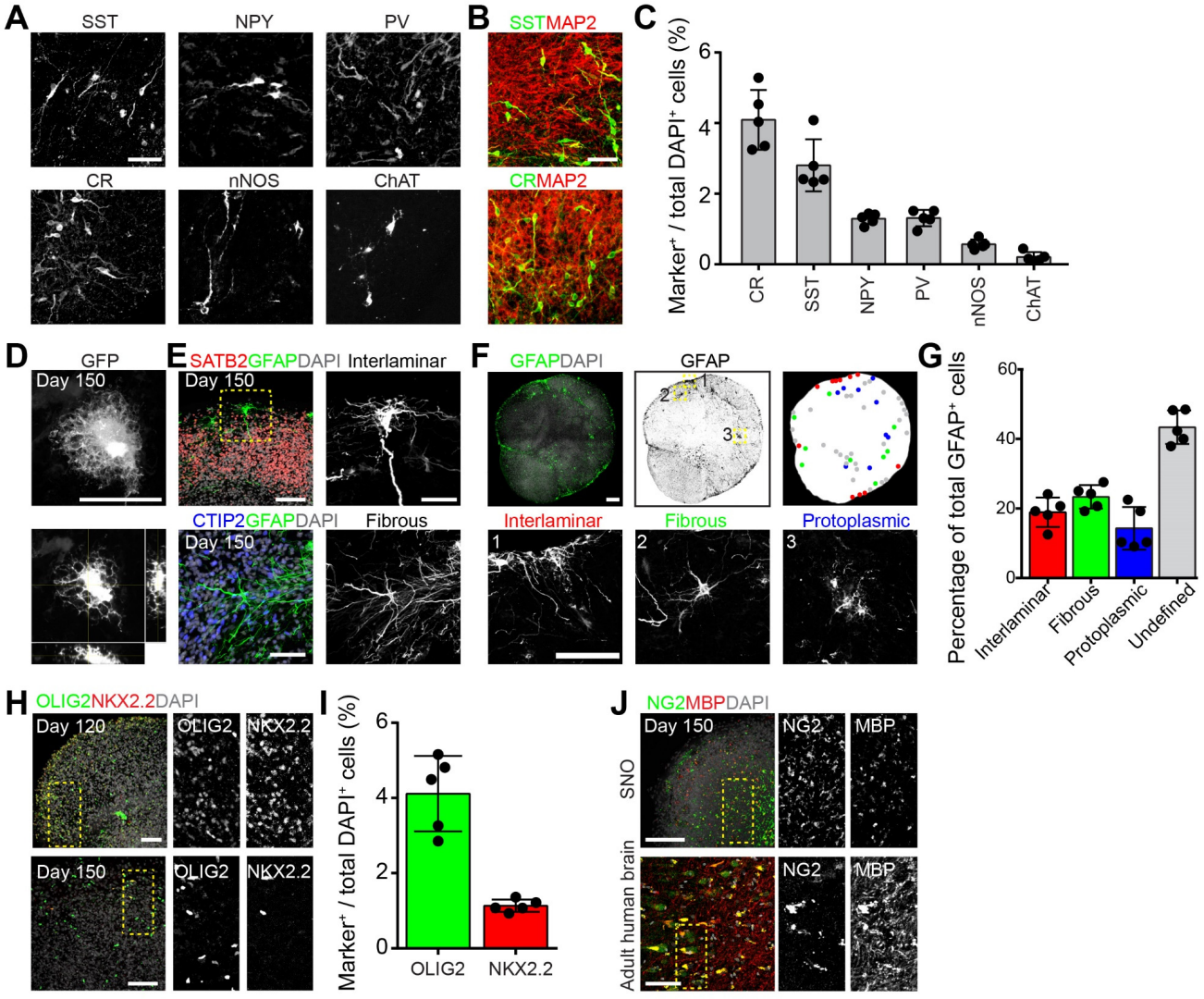

Figure S3. Interneurons and Glial Cells in SNOs, related to Figure 4.
(A-B) Sample confocal immunostaining images for various neuronal subtypes present in Day 150 SNOs. Shown in (B) are sample images co-immunostained with the pan-neuronal marker MAP2. Scale bars, 50 μm.
(C) Quantification of the percentages of cells expressing different interneuron markers over total cell number in Day 150 SNOs. Values represent mean ± S.D. (n = 5 SNOs).
(D) Sample confocal images of a protoplasmic astrocyte infected by adenovirus expressing GFP. Shown on the top panel is the maximal intensity projection of a 15-μm z-stack confocal image and shown on the bottom panel is the orthogonal view without projection. Scale bar, 50 μm.
(E) Additional sample confocal images for interlaminar and fibrous astrocytes. Images on the right panel show GFAP immunostaining. Scale bars, 100 μm and 50 μm (insets).
(F) Sample whole SNO image showing the distribution of astrocyte subtypes in a Day 150 SNO (top panel). Shown on the right is a schematic indicating the subtype identities of each astrocyte with colored dots: Red (interlaminar astrocytes), Green (fibrous astrocytes), Blue (protoplasmic astrocytes), and Grey (undefined astrocytes). The bottom panel shows magnified views of box 1-3. Scale bars, 200 μm and 100 μm (insets).

(G) Quantification of the proportions of each astrocyte subtypes among total GFAP$^+$ astrocytes in Day 150 SNOs. See **STAR Method** for how astrocyte subtype identity was determined based on morphology. Values represent mean ± S.D. (n = 5 SNOs).
(H) Sample confocal images showing oligodendrocyte precursor cells (OPC) expressing OLIG2 and NKX2.2 in Days 120 and 150 SNOs. Scale bars, 100 μm.
(I) Quantification of the percentages of OLIG2$^+$ and NKX2.2$^+$ cells over total cell number in Day 150 SNOs. Values represent mean ± S.D. (n = 5 SNOs).
(J) Sample confocal images showing NG2$^+$MBP$^+$ OPCs in Day 150 SNO (top panel) and in the adult human cortex (bottom panel). Scale bar, 100 μm (top panel) and 50 μm (bottom panel).

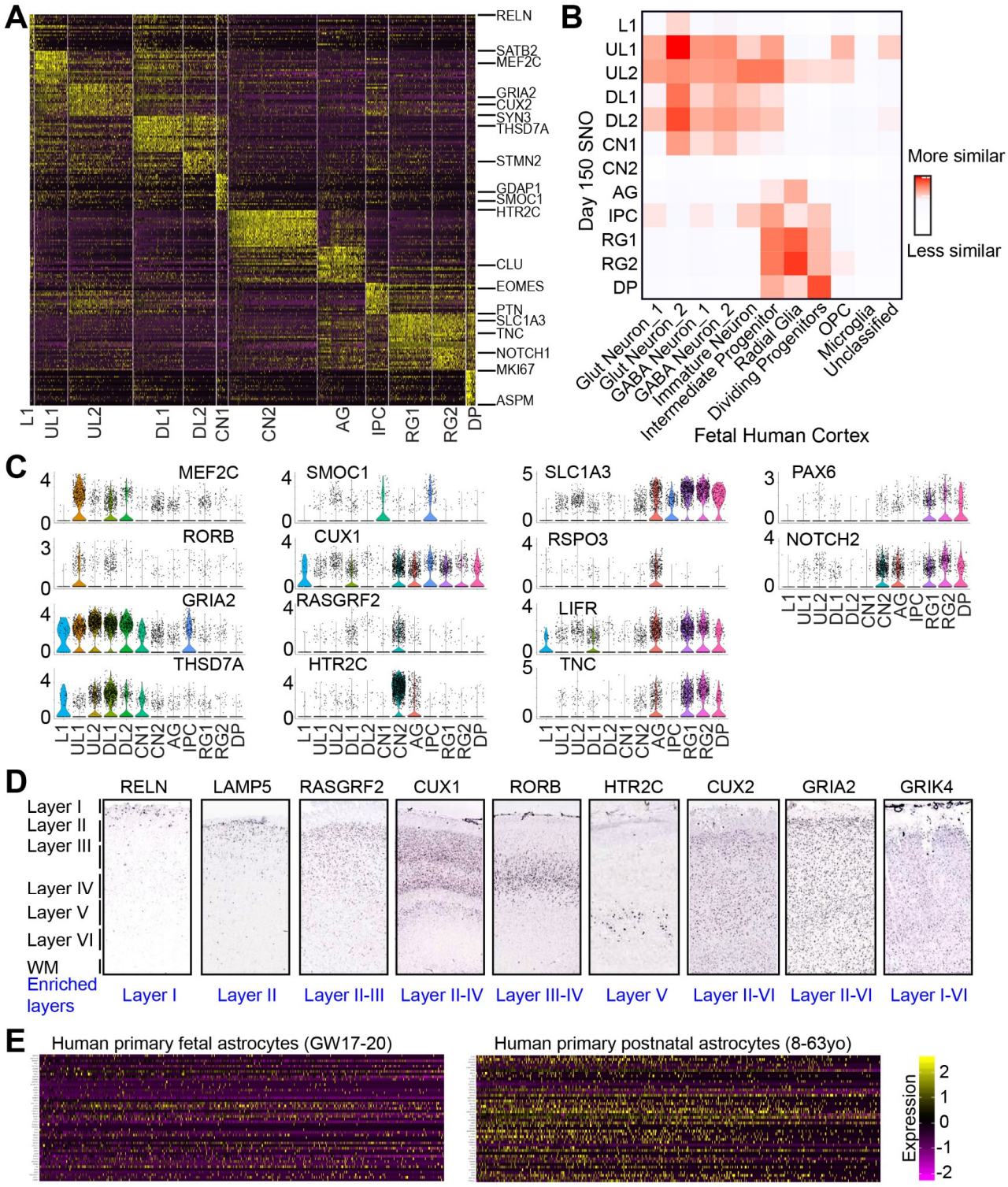

Figure S4. Single-nucleus RNA-seq Analyses of Day 150 SNOs, related to Figure 4.
(A) Heatmap demonstrating scaled levels of the top 20 differentially expressed genes in each of the clusters (1-12) with example genes listed on the right.

(B) Similarity matrix representing Pearson correlations between cell types of SNOs and the embryonic human prefrontal cortex. Embryonic human data are obtained from a published single-cell RNA-seq dataset (Zhong et al., 2018).
(C) Expression of additional well-known marker genes within the 12 clusters. Shown are violin plots overlaid on the scatter plots, where the proportion of cells expressing a given gene is the highest.
(D) *In situ* hybridization images showing the laminar distribution of cortical neuron markers in the adult human cerebral cortex, from control individuals taken from the Allen Brain Atlas (https://human.brain-map.org/ish/search). The grey matter of the cortex is cropped into 4 mm x 2 mm areas. The brightness and contrast of the original images were adjusted in Photoshop software to help visualization. The distinctions between Layers I- VI are determined based on the corresponding Nissl staining for each sample (not shown). Samples shown are from dorsolateral prefrontal cortex whenever available. See Table S1 for summary of the sample information and links to the original image.
(E) Heatmap plot showing the relative expression levels of top 50 differentially expressed genes between human primary fetal and postnatal astrocytes in the Day 150 SNO astroglia cluster. The human primary astrocyte bulk RNA-seq data is obtained from (Zhang et al., 2016).

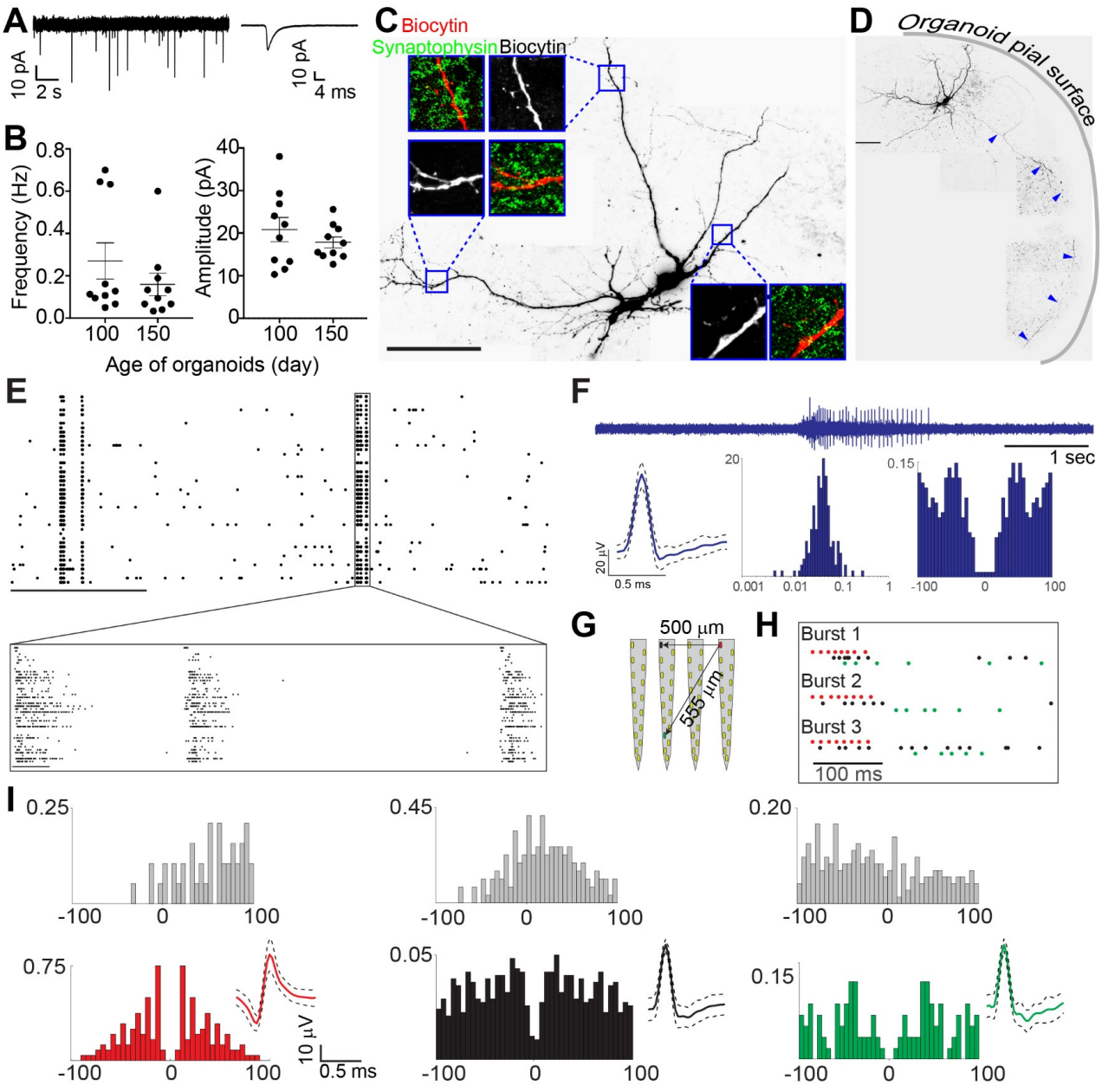

Figure S5. Functional Analyses of Cortical Neurons in SNOs, related to Figure 4.
(A) Sample recording traces of sEPSCs and the average sEPSC trace from whole-cell recording in slices acutely sectioned from SNOs.
(B) Summary of the frequencies (left panel) and amplitudes (right panel) of sEPSCs from recorded neurons in Day 100 (± 5) and 150 (± 5) SNOs. Values represent mean ± S.E.M. (n = 10 neurons from 5 SNOs).
(C-D) Sample images of a neuron filled with biocytin from a Day 150 SNO and immunostained after whole-cell recording. Dendritic spine structures can be visualized adjacent to Synaptophysin^{+} pre-synaptic terminals (insets in C). The image is inverted to a white background to help visualize neuron morphology. Its axon (arrowheads) extended for approximated 2 mm and projected to multiple targets in the CP region. Scale bars, 100 µm and 5 µm (insets, C).

(E) Raster plots of all isolated single-units from an intact Day 170 SNO using the four-shank multi-electrode laminar probe (n = 45 single-units). Top panel: Single units exhibit periods of coordinated bursting activity with some units displaying spontaneous activity between bursts. Bottom panel: Zooming in on the highlighted burst reveals complex and varied activity of single units within coordinated bursts. Scale bar, 200 sec (top panel) and 1 sec (bottom panel).
(F) Spontaneous single unit activity recorded from Day 170 SNOs. Top panel: Example LFP trace (bandpass filtered 0.6-6 kHz) from one electrode displaying clearly identifiable spiking activity during a burst. Scale bar, 1 sec. Bottom panel: Average waveform and SEM (left), ISI (middle), and auto-correlogram (right) of an isolated single unit from this trace. Same sample as (E). ISI, spike counts over log(sec); auto-correlogram, probability of spiking from -100 ms to 100 ms, bin size = 5 ms.
(G-I) Spatiotemporal activity of populations of single units reveals network activity over long distances. Shown in (G) is the spatial arrangement of the multi-electrode probe. Colored electrode sites indicate the location of the max spike height of the red, green, and black neurons, respectively (red-black distance = 500 μm, black-green distance = 240 μm, and red-green distance = 555 μm, respectively). Shown in (H) are the raster plots of the three neurons at the onset of each of the three bursts highlighted in the callout box from (E). Shown in (I) are cross-correlograms between units (grey) with a repetitive pattern of temporal activation with the red neuron always firing before the black and green neurons. Also shown in (I) are the average waveforms and SEMs with their associated auto-correlograms (red, black, and green).

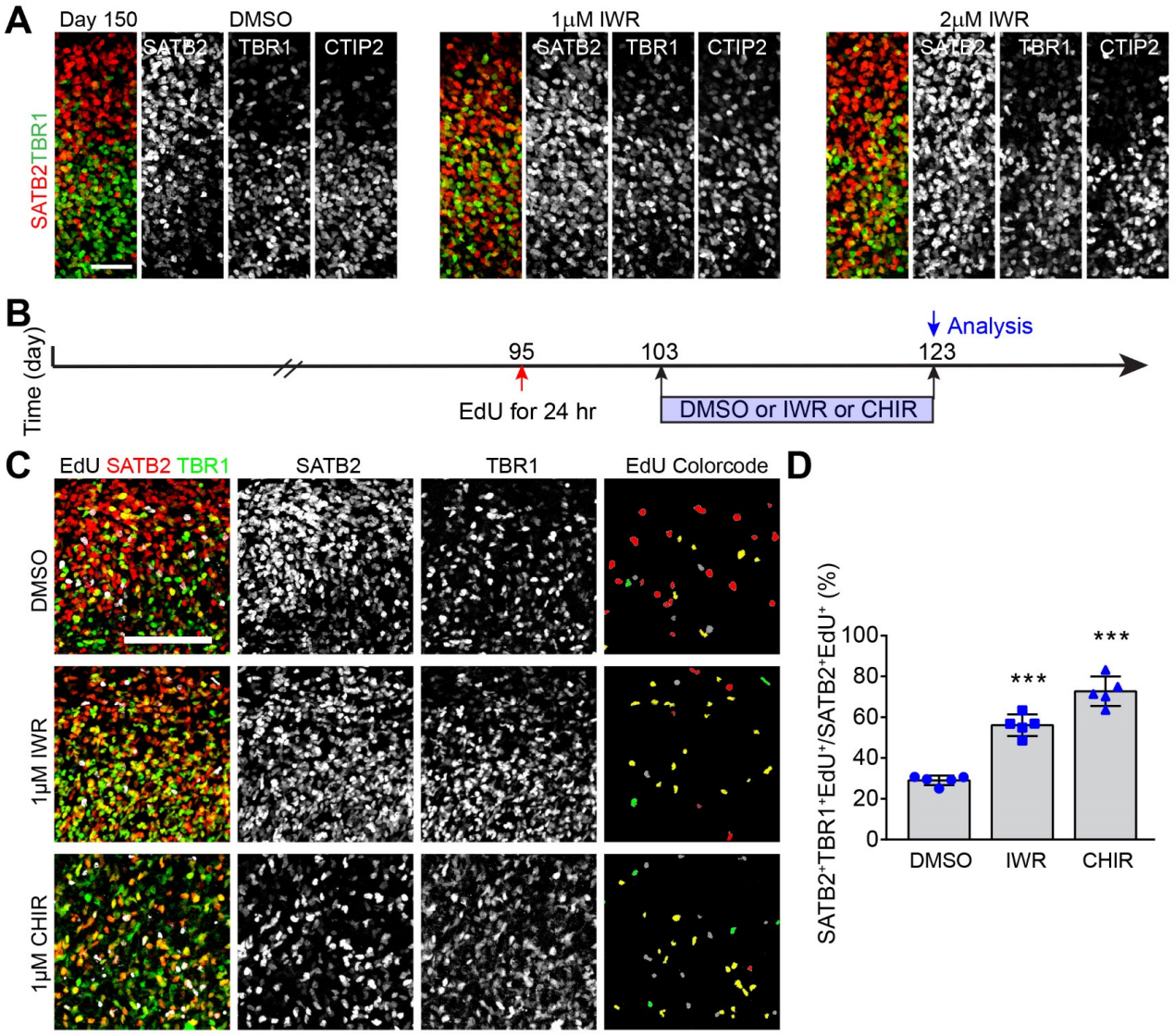

Figure S6. WNT/β-Catenin Signaling Regulates Cortical Neuron Fate Post-mitotically, related to Figure 5.

(A) The effect of IWR treatment persisted after withdrawal. SNOs were treated between Day 100 and Day 120, then drugs were withdrawn and SNOs were analyzed at Day 150. Shown are cropped 100 x 300 μm columns of sample confocal images of the CP of Day 150 SNOs, immunostaining for SATB2, TBR1 and CTIP2. Scale bar, 50 μm.

(B) Schematics showing the timeline of the EdU pulse-chase experiment. SNOs were pulsed with EdU for 24 hr at Day 95. Starting from Day 103, SNOs were treated with DMSO, IWR or CHIR for 20 days, and analyzed at Day 123.

(C) Sample confocal images showing the expression of SATB2 and TBR1 in EdU^+ cells in the CP at Day 123. The EdU^+ nuclei are pseudo-colored to represent their marker expression: Red ($SATB2^+$, $TBR1^-$); Green ($SATB2^-$, $TBR1^+$); Yellow ($SATB2^+$, $TBR1^+$); Grey ($SATB2^-$, $TBR1^-$). Scale bar, 100 μm.

(D) Quantitative analysis for the ratio of $EdU^+TBR1^+SATB2^+$ cells among EdU^+SATB2^+ nuclei. Values represent mean ± S.D. (n = 5 SNOs; ***$p < 0.0005$, Student's t-test).

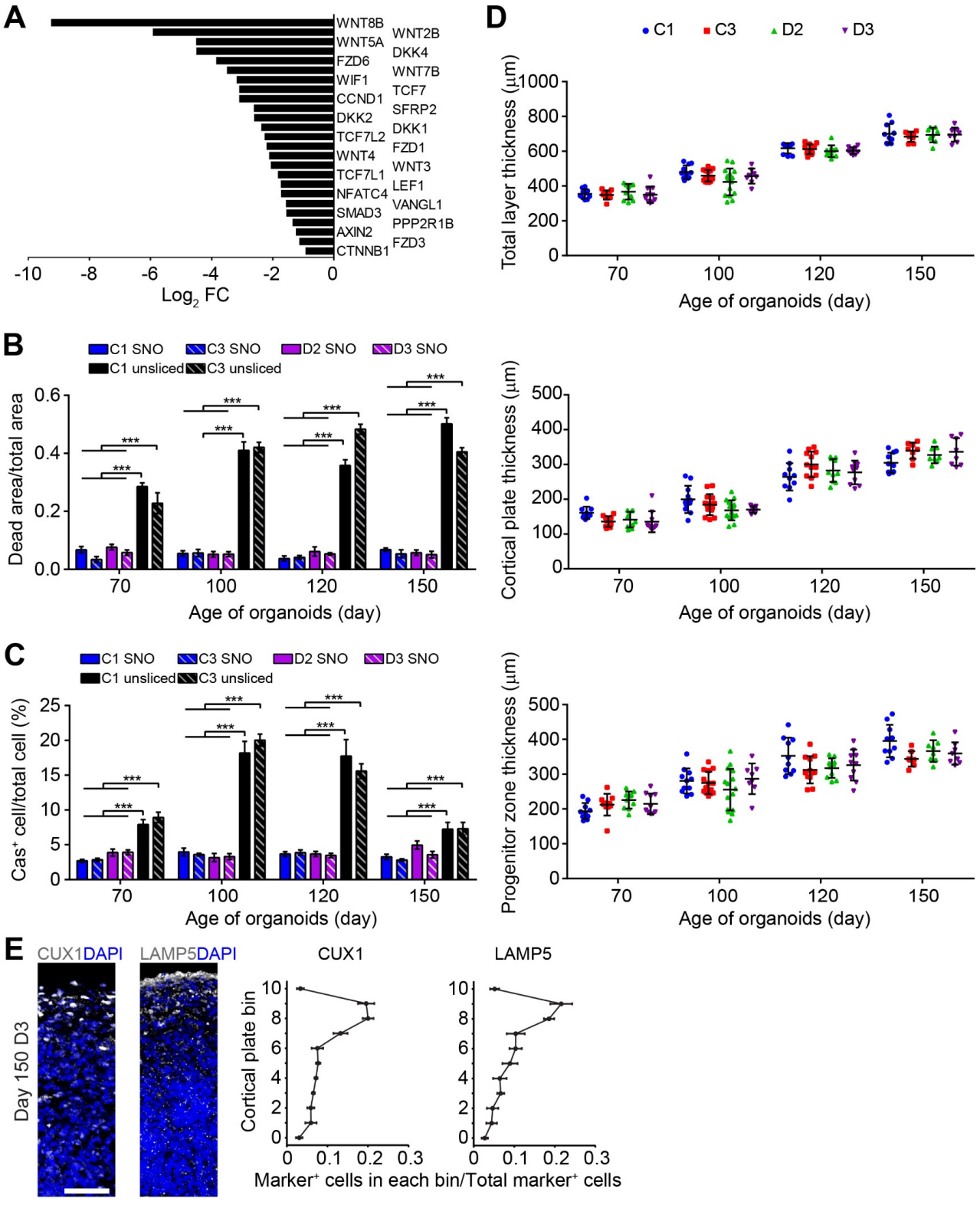

Figure S7. Characterizations of SNOs Derived from iPSCs with 4-bp Deletion of the *DISC1* gene, related to Figures 6 and 7.

(A) Differential gene expression analysis of bulk RNA-seq data on control and mDISC1 iPSC-derived cortical neurons from (Wen et al., 2014). Shown are significantly down-regulated genes in the WNT/β-Catenin signaling pathway.
(B-C) Quantitative analyses revealed that mDISC1 SNOs exhibited similar levels of necrotic and apoptotic cell death as control SNOs. Values represent mean ± S.E.M. (n = 5 organoids (B); n = 10 and 5 for SNOs and unsliced organoids, respectively (C); ***$p < 0.0005$, Student's t-test). The same data for C1 and C3 as in Figures S1D and 1G are replotted for comparison.
(D) mDISC1 SNOs exhibited normal growth and layer expansion. Shown are quantifications of the total thickness (top panel), CP thickness (middle panel) and progenitor zone thickness (bottom panel) of control and mDISC1 SNOs. Values represent mean ± S.D. (n = 10 SNOs for each cell line). The same data for C1 and C3 as in Figures 2D and 3B-C are replotted for comparison.
(E) Expression of LAMP5 and CUX 1 remained restricted to the upper layer in Day 150 mDISC1 SNOs. Laminar distribution of LAMP5 and CUX1 are shown as 100 x 300 μm columns of Day 150 CP. Scale bar, 50 μm. Also shown are quantitative analyses representing the marker distribution in the CP, calculated as [# of marker$^+$ cell in a bin/# of total marker$^+$ cells in the CP], to be compared with Figure S2F. Values represent mean ± S.E.M. (n = 5 SNOs).