Supplementary Figures

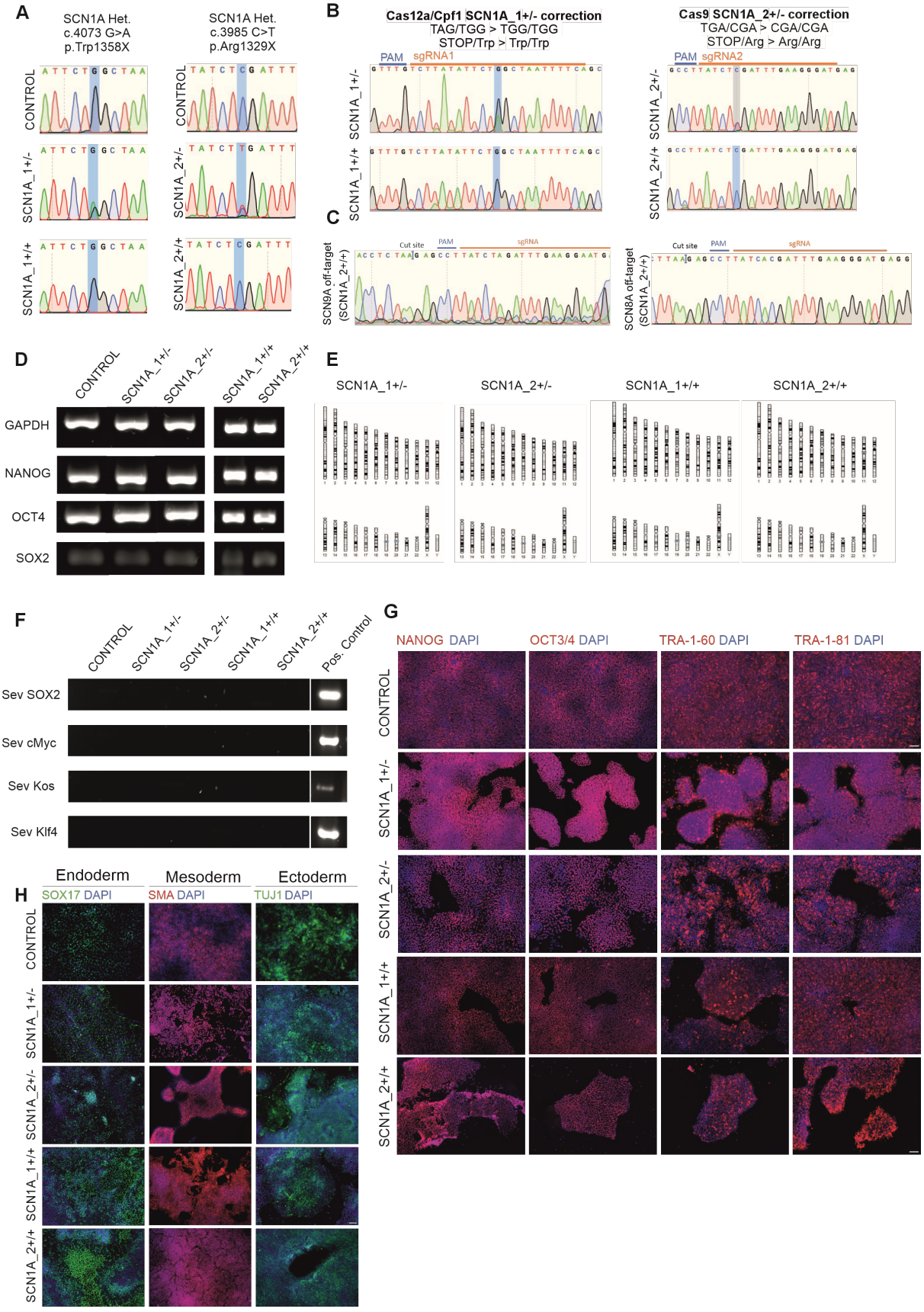


Figure S1 – Generation and characterisation of patient and isogenic iPSC lines

A. Sanger sequencing of patient mutations loci in patient and isogenic iPSC lines and a control iPSC line used as a reference. Mutated base is highlighted. B. Sanger sequencing results showing effective correction of the disease-causing mutation in *SCN1A* for Pat1 *SCN1A*$^{+/-}$ and Pat2 *SCN1A*$^{+/-}$ using CRISPR/Cas12a and CRISPR/Cas9 respectively, resulting in the generation of Pat1 *SCN1A*$^{+/+}$ and Pat2 *SCN1A*$^{+/+}$ isogenic control iPSC lines. A silent PAM mutation can be observed in Pat2 *SCN1A*$^{+/+}$. C. Predicted off-target sites in *SCN8A* and *SCN9A* were verified using Sanger sequencing, showing no off-target indels were introduced and no alterations in the original genetic sequence. D. The expression of the genes *SOX2*, *NANOG* and *OCT4* was evaluated using RT-PCR to assess pluripotency of the iPSC lines. Expression of the housekeeping gene *GAPDH* is also shown. E. Illumina CytoSNP-12-v2.1 BeadChip array analysis showing karyotype integrity for patient and isogenic lines used in the study. F. Sendai virus infected iPSC cell line at very early passage (P1) was used as positive control. RT-PCR for Sendai virus clearance is performed for SeV KOS, KLF4, C-MYC and SOX2 genome, for all iPSC lines used in the study. GAPDH is used as a reference housekeeping gene. G. Immunofluorescence staining for pluripotency markers OCT3/4, TRA-1-60, NANOG, and TRA-1-81 for all iPSC lines used in this study. Scale bar 100 µm. H. Immunofluorescence analysis of markers AFP (endoderm), TUJ1 (ectoderm) and SMA (mesoderm) following trilineage differentiation using the STEMdiff™ Trilineage Differentiation Kit (StemCell Technologies). Scale bar 100 µm.

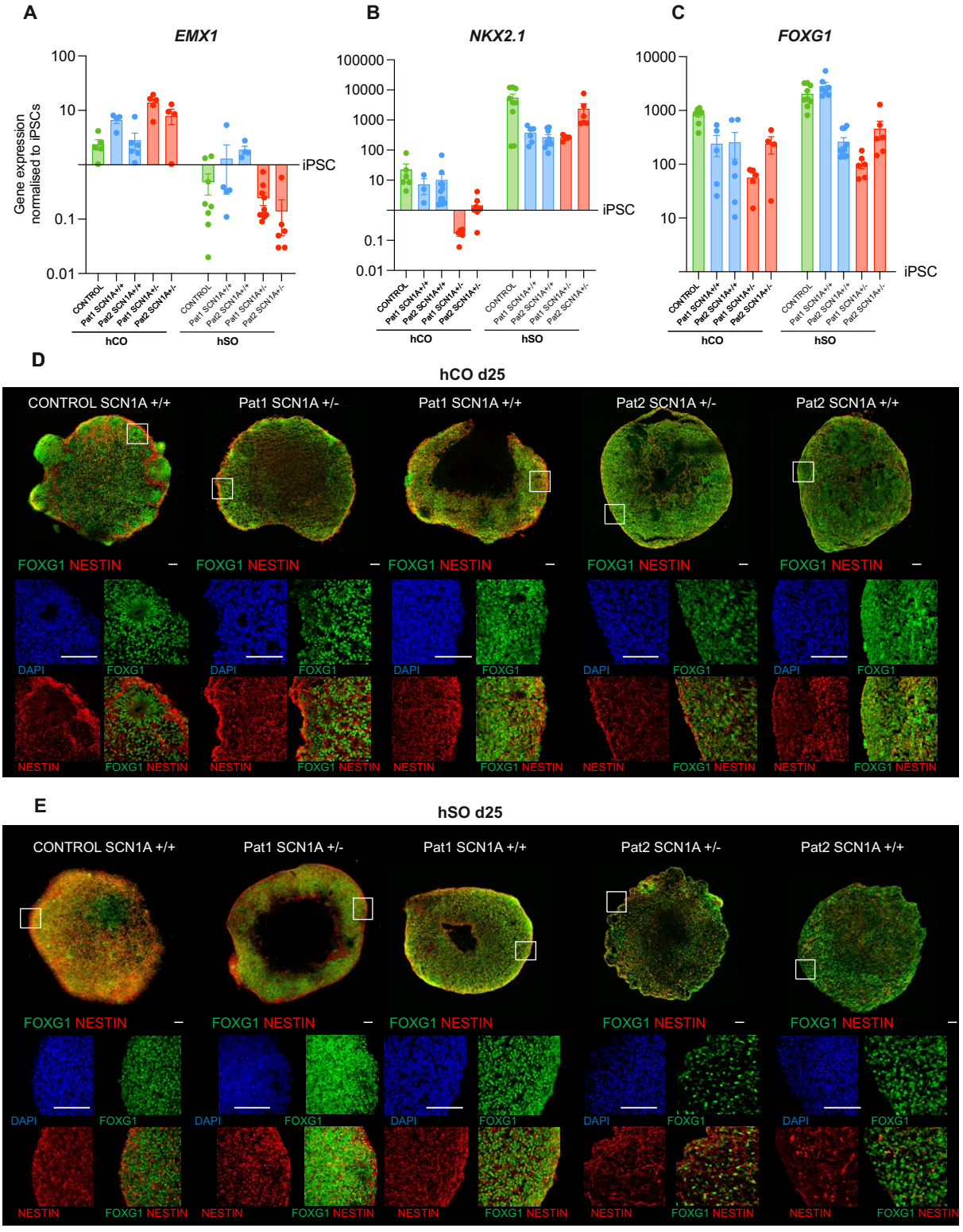
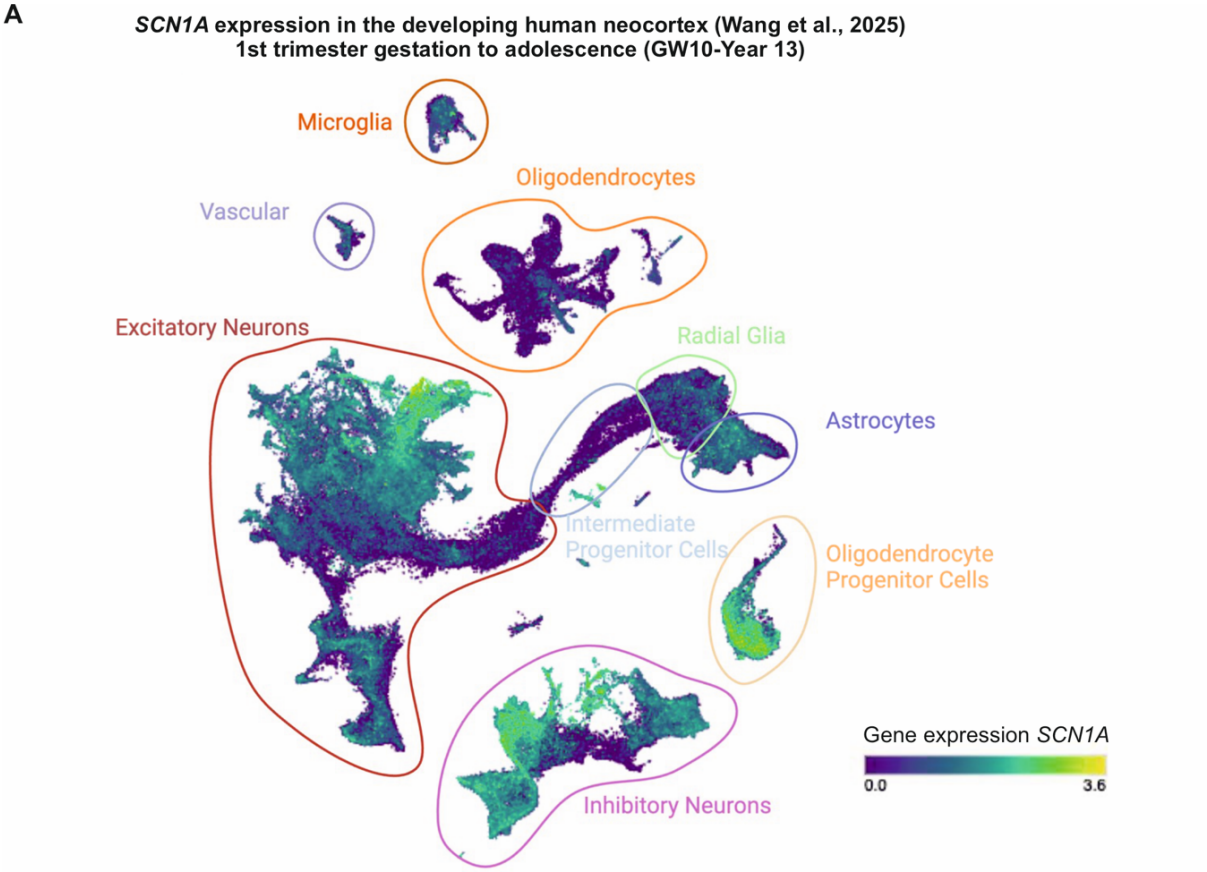


Figure S2: Specification of dorsal and ventral forebrain identity in d25 hCO and hSO derived from DS patient and isogenic iPSC lines.

A-C. Expression of cortical and subpallial marker genes *EMX1*, *NKX2.1* and *FOXG1* in *SCN1A*+/- and *SCN1A*+/+ hCO and hSO at d25 relative to *GAPDH* and normalised to expression in iPSCs using the ΔΔCt method (n = 4-6). D-E. Immunostaining of hCO and hSO

at day 25 for Nestin and FOXG1 markers of neural stem cells and forebrain identities in all lines. Scale bar = 100 μm.

A


SCN1A expression in the developing human neocortex (Wang et al., 2025)
1st trimester gestation to adolescence (GW10-Year 13)
Microglia
Oligodendrocytes
Vascular
Excitatory Neurons
Radial Glia
Astrocytes
Intermediate
Progenitor Cells
Oligodendrocyte
Progenitor Cells
Gene expression SCN1A
0.0
3.6
Inhibitory Neurons

B

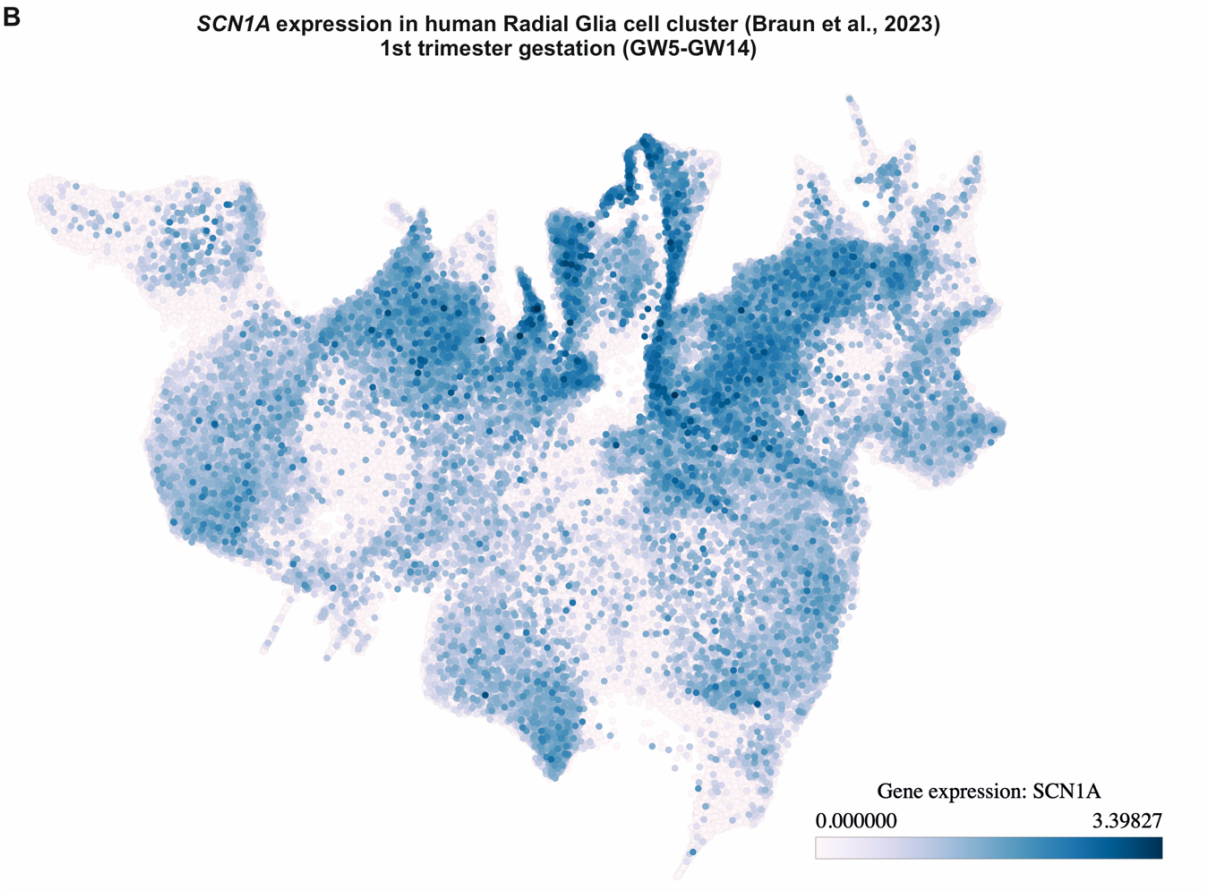

SCN1A expression in human Radial Glia cell cluster (Braun et al., 2023)
1st trimester gestation (GW5-GW14)
Gene expression: SCN1A
0.000000
3.39827

Figure S3 – *SCN1A* expression in the developing human neocortex and radial glia compartment

A. UMAP representation showing *SCN1A* expression levels in different cell clusters of the developing human neocortex (scRNAseq dataset from Wang et al.[44], data available at: https://cell.ucsf.edu/snMultiome/build1/). B. UMAP representation showing *SCN1A* expression levels in radial glia cell cluster during first trimester of gestation (scRNAseq dataset from Braun et al.[43], data available at: https://hdca-sweden.scilifelab.se/tissues-overview/brain/).

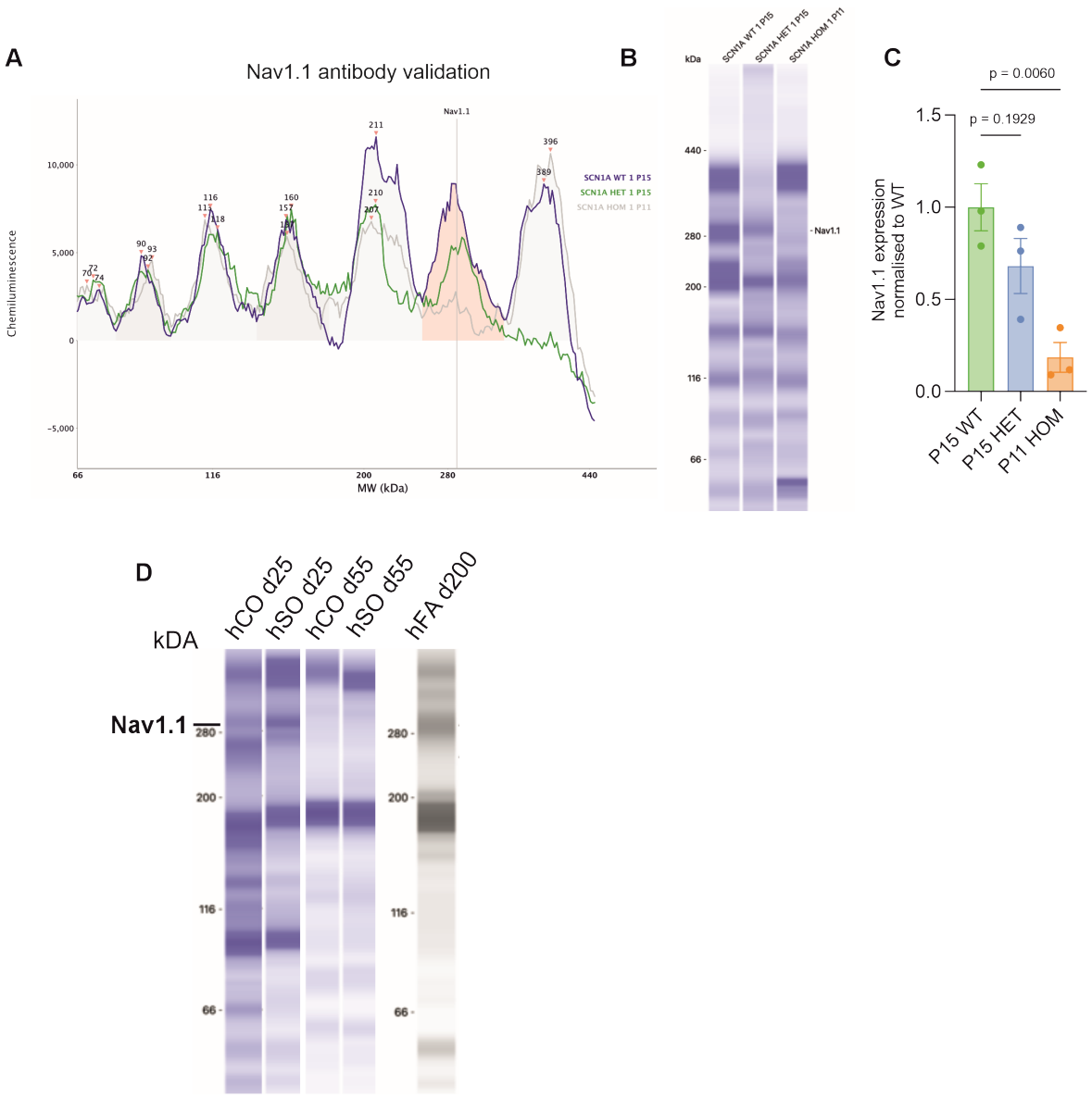


Figure S4: Validation of capillary-based assay for detection of Nav1.1 protein

A. Raw graphical results from Simple Western™ assay showing detected Nav1.1 signal peaks from protein samples isolated from cortex of WT *Scn1a*$^{+/+}$, HET *Scn1a*$^{+/-}$ and HOM *Scn1a* $^{-/-}$ mice. B. Lane representation of graphical data in panel A. Identified specific Nav1.1 band is shown at 285 kDA. C. Quantification of Nav1.1 peak area from WT, HET and HOM mouse cortex samples. D. Representative samples from Figure 2B, shown in lane format. Identified Nav1.1 band is shown at 285 kDA. In d25 hCO and hSO lanes, a smaller sized bands can be observed just below the 285 kDA Nav1.1 band.

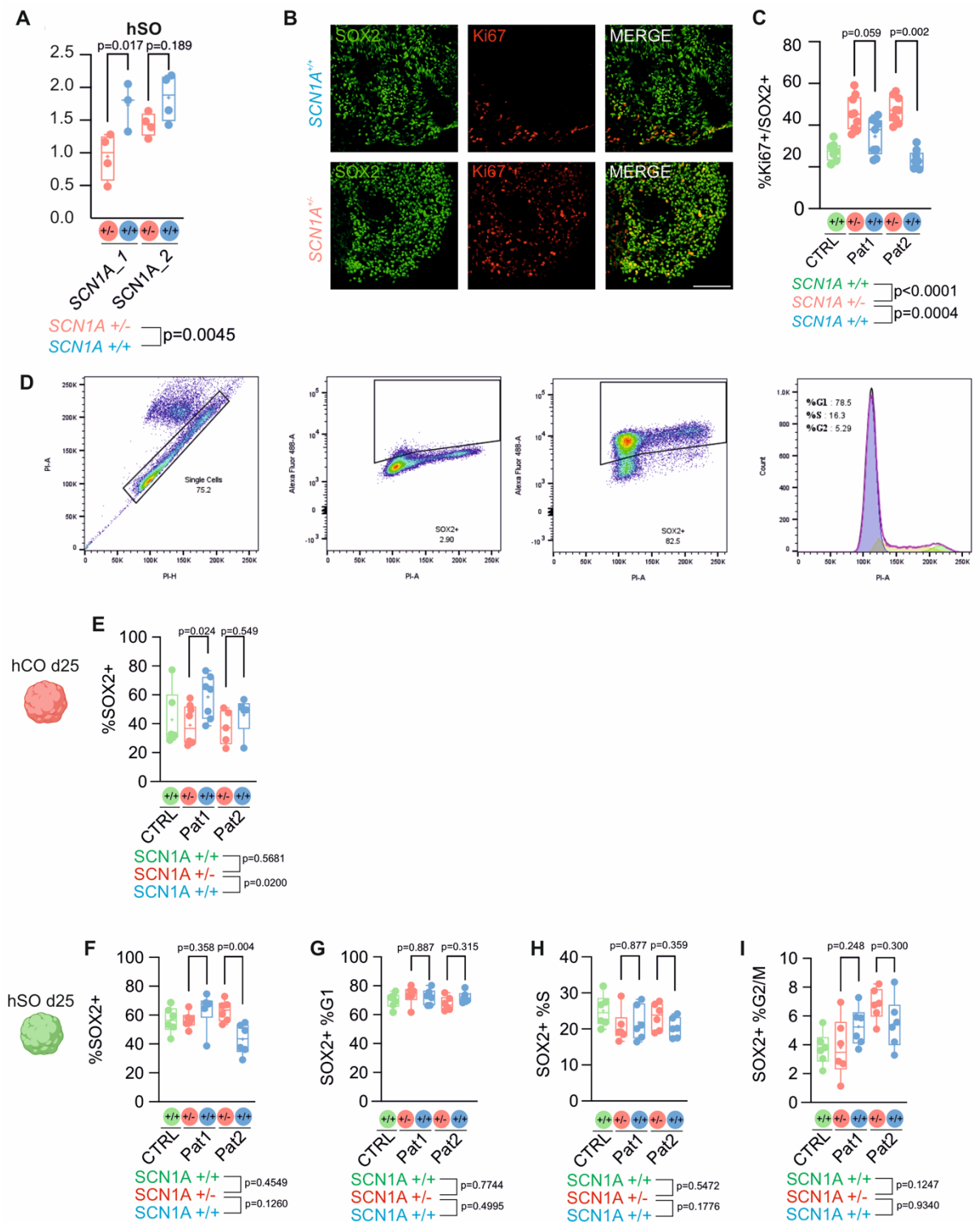


Figure S5: Cell cycle phenotypes in hCO and hSO at d25.

A. Nav1.1 protein levels normalised to total protein in hSO at d25 across lines. n = 3-4 organoid samples from N ≥ 2 differentiations. B. Representative 20X confocal images of SOX2 and Ki67 staining in SCN1A+/- and SCN1A+/+ corrected isogenic control line. C. Quantification of Ki67+ cells within the SOX2+ progenitor population in SCN1A+/- and SCN1A+/+ hCO at d25. n ≥ 5 organoids from N = 3 differentiations. Unpaired t-test for CONTROL *SCN1A*+/+ (green) vs

SCN1A_1 and SCN1A_2 *SCN1A*+/- (red). Two-way ANOVA followed by Šídák's multiple comparisons test for *SCN1A_1*+/+ and *SCN1A_2*+/+ (blue) vs *SCN1A_1*+/- and *SCN1A_2*+/- (red). D. Gating strategy used for flow cytometry experiment and representative graph of a cell cycle analysis performed on a single cell SOX2+ population based on frequencies of PI-A (left). E-F. Proportion of the neural stem cell SOX2+ population within total detected cells in hCO and hSO at d25. G-I. Percentage of SOX2+ cells in each phase of the cell cycle in hSO at d25. n = 6 samples from N = 3 differentiations. Unpaired t-test for CONTROL *SCN1A*+/+ (green) vs SCN1A_1 and SCN1A_2 *SCN1A*+/- (red). Two-way ANOVA followed by Šídák's multiple comparisons test for *SCN1A_1*+/+ and *SCN1A_2*+/+ (blue) vs *SCN1A_1*+/- and *SCN1A_2*+/- (red).

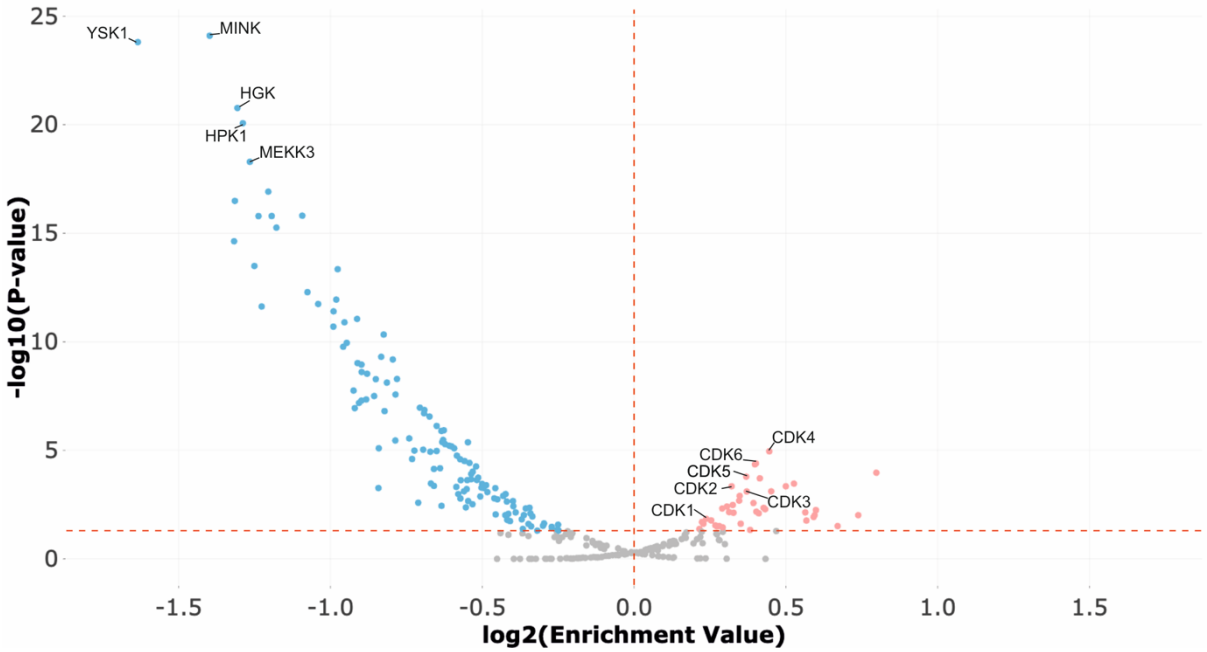


Figure S6: Volcano plot of kinase enrichment predictions from phosphoproteomics dataset.

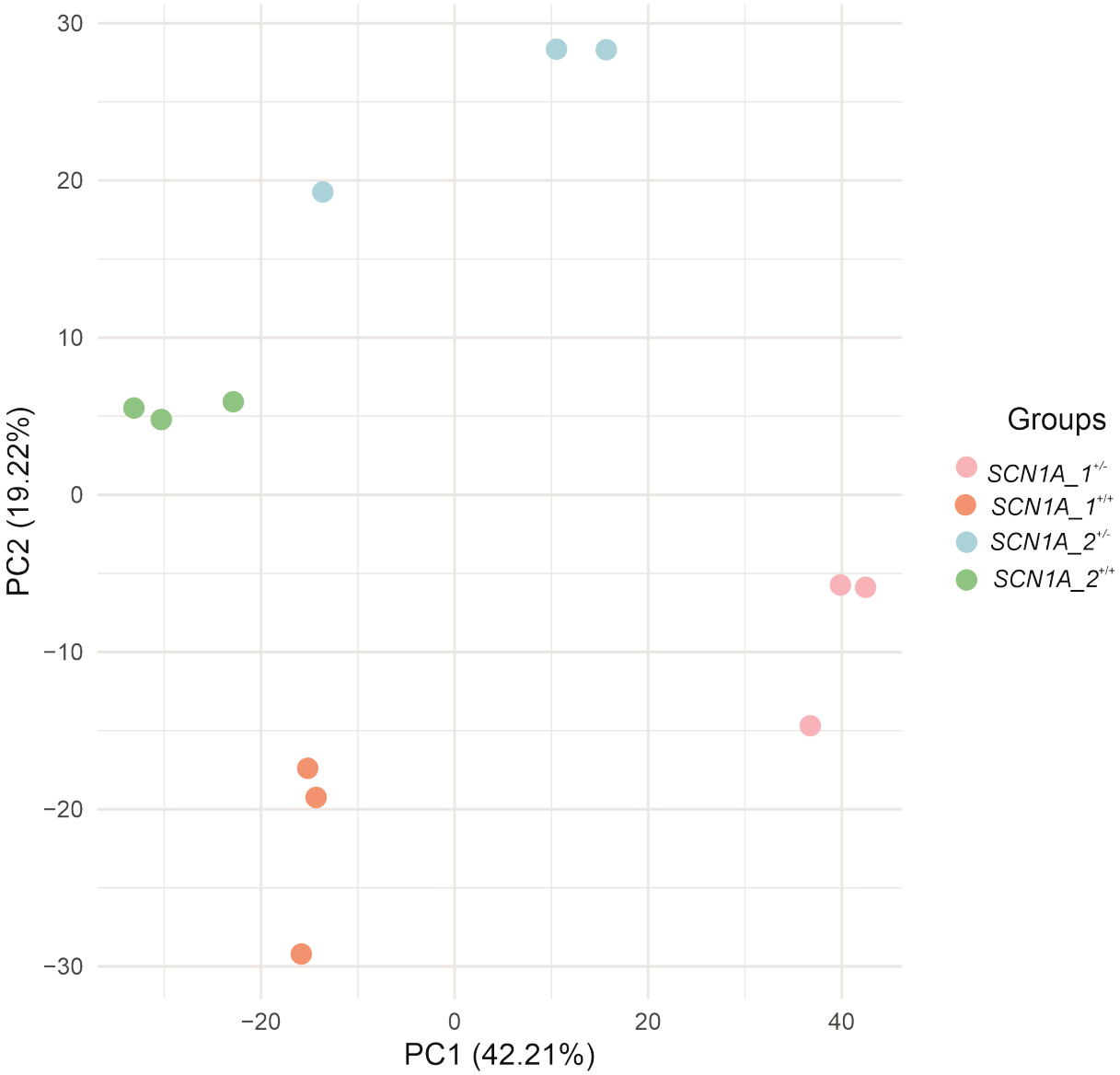


Figure S7: Principal Component Analysis (PCA) of d200 RNAseq samples.

PCA using the top 50% most variably expressed genes across all samples. The first two principal components are shown, with PC1 accounting for 42.21% of the total variance and PC2 accounting for 19.22%. PC1 separates the samples by condition, while PC2 distinguishes them by donor. Together, these components capture the majority of the variance in gene expression among samples, enabling visualisation of the primary sources of transcriptional variation.

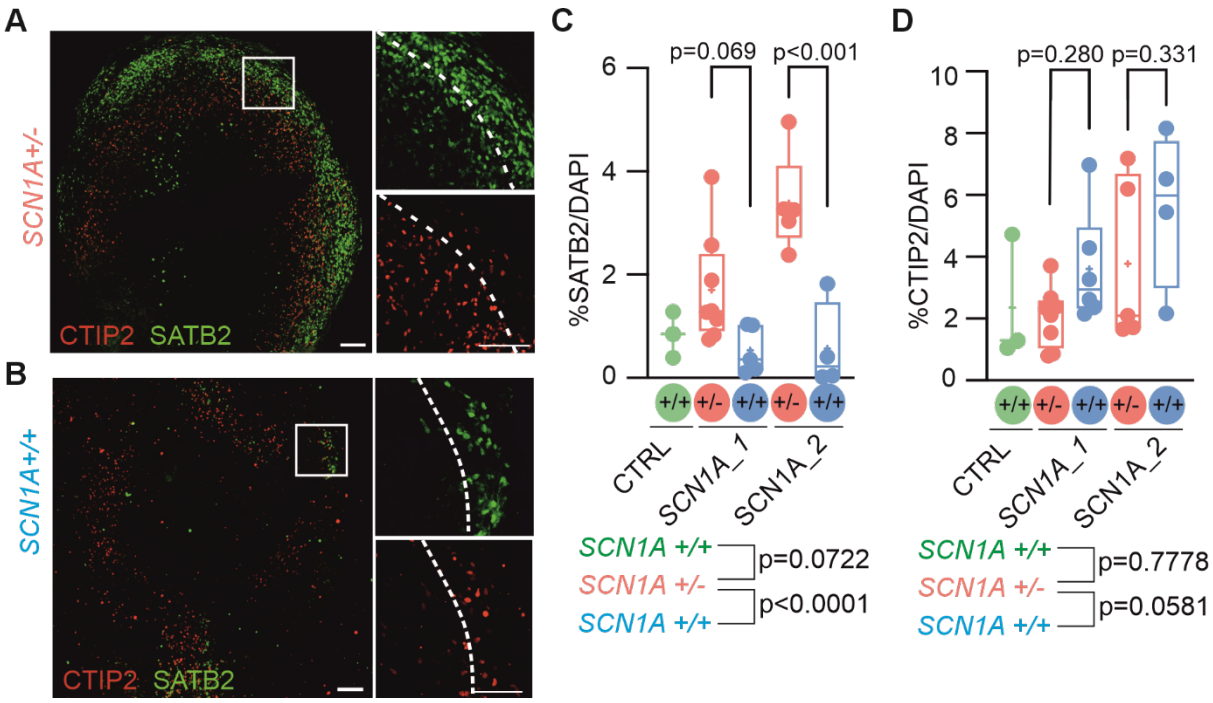


Figure S8: Quantification of SATB2+ and CTIP2+ neurons in d200 hFAs

A and B. Representative image showing CTIP2+ and SATB2+ neurons in *SCN1A*+/+ and *SCN1A*+/- hFA slices. Dotted line shows separation between upper and deeper cortical layers. Scale bars = 100 μm. C and D. Percentage of SATB2+ and CTIP2+ neurons normalised to DAPI. n ≥ 3 organoids from N ≥ 2 differentiations. Unpaired t-test for CONTROL *SCN1A*+/+ (green) vs *SCN1A_1*+/- and *SCN1A_2*+/- (red). Two-way ANOVA followed by Šídák's multiple comparisons test for *SCN1A_1*+/+ and *SCN1A_2*+/+ (blue) vs *SCN1A_1*+/- and *SCN1A_2*+/- (red).

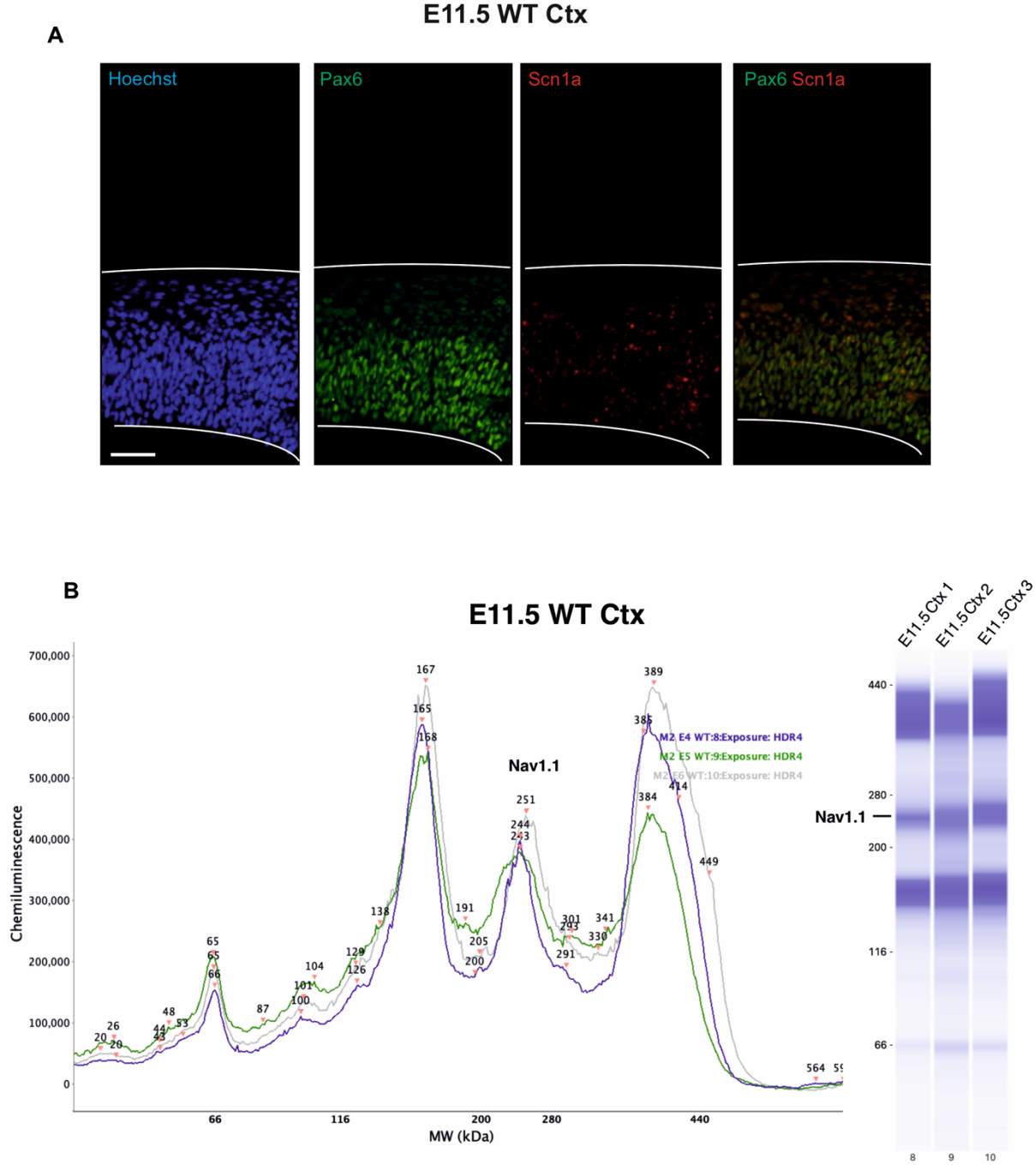


Figure S9: Detection of *Scn1a* mRNA and Nav1.1 protein in E11.5 embryonic mouse cortex.

A. RNAScope™ results showing *Scn1a* mRNA in ventricular zone containing Pax6-positive progenitor cells in WT mouse cortex at E11.5. B. Raw graphical results from Simple Western™ assay showing detected Nav1.1 signal peak from protein samples isolated from cortex of WT *Scn1a*$^{+/+}$ mice at E11.5 (left). Lane representation of graphical data. Identified Nav1.1 band is shown at 250 kDA (right).

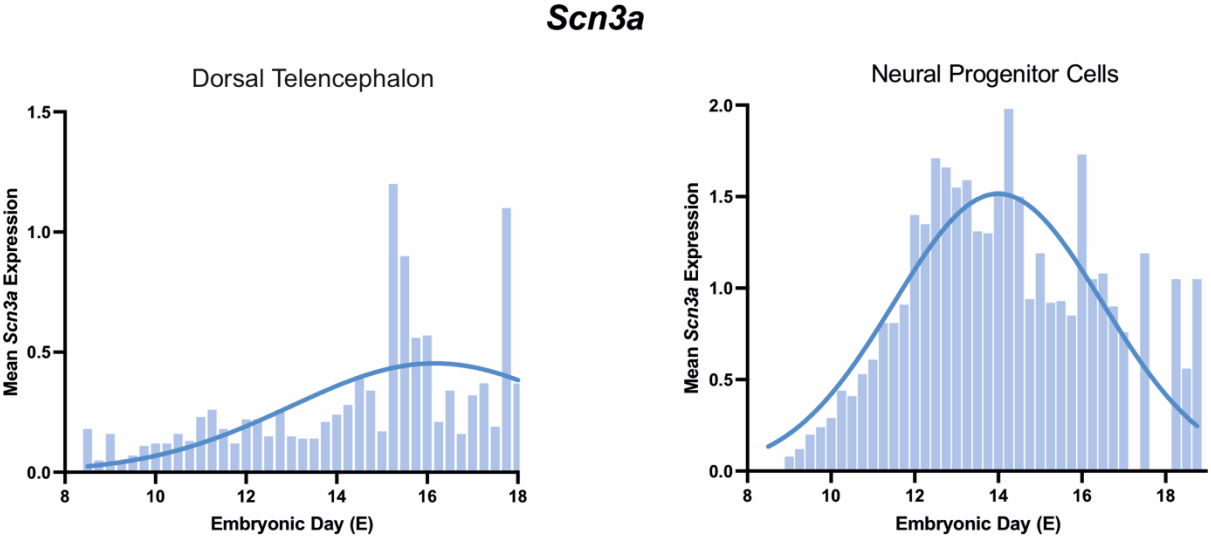


Figure S10: Developmental expression pattern of *Scn3a* in the embryonic mouse.
A-B. Temporal pattern of *Scn3a* expression in the developing mouse embryo from secondary analysis of data published in [63]. Gaussian curve fitted to the data is shown in blue.

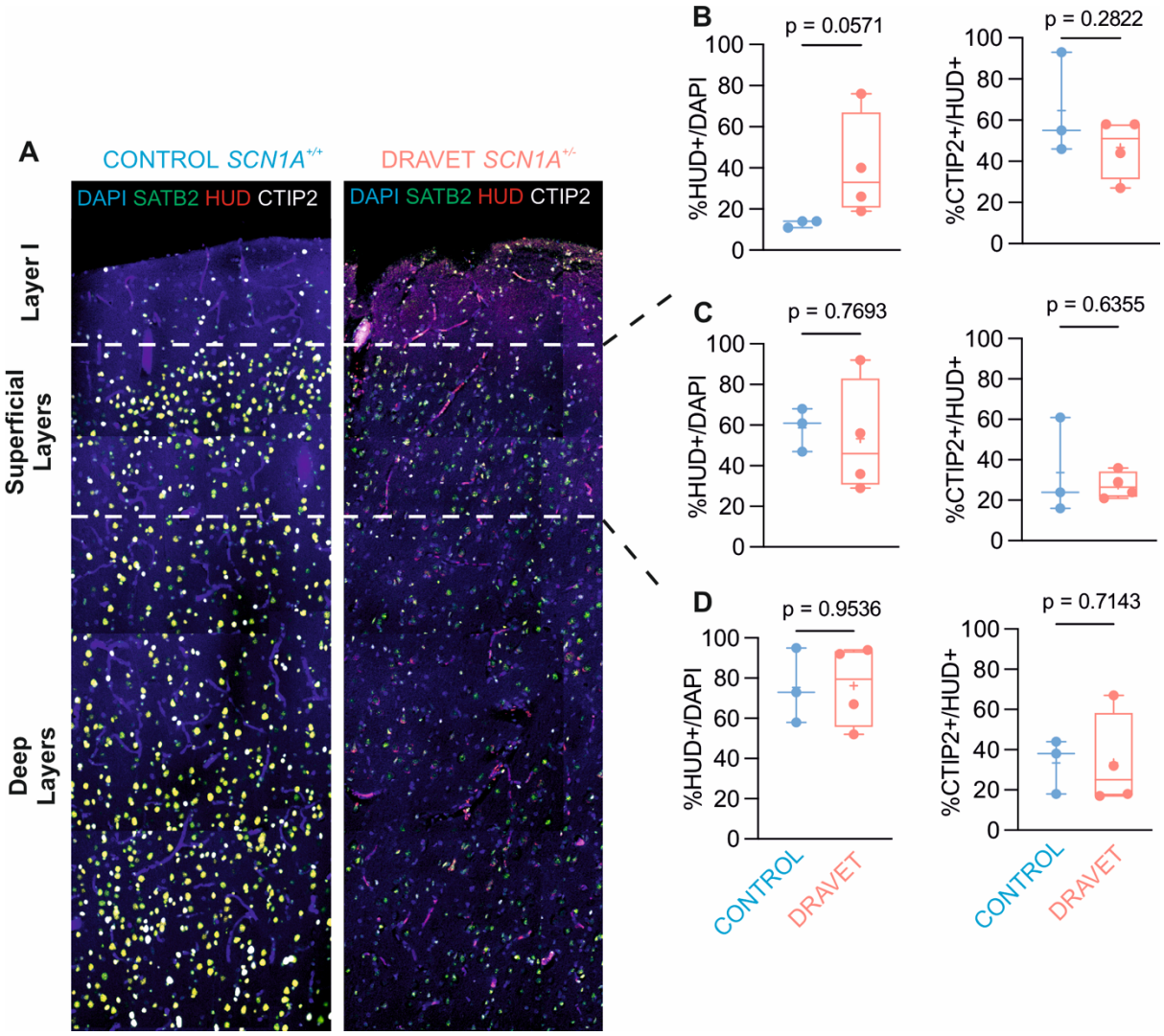


Figure S11: Analysis of HUD+ and CTIP2+ neurons in human post-mortem samples

A. Representative images of immunofluorescence staining for SATB2, HUD (pan-neuronal marker) and CTIP2 showing delineation of layer 1, superficial and deep layers. B-D. Quantification of total HUD, as well as proportion of CTIP2-positive neurons in layer 1 (B), superficial (C) and deep layers (D). Unpaired t-test.

Table S1: Fisher's exact test of overlap in differentially expressed genes between SCN1A_1 and SCN1A_2

Comparison	Overlap	Odds Ratio	p-value
Upregulated	213	39.06	$< 2.2 \times 10^{-16}$
Downregulated	175	15.1	$< 2.2 \times 10^{-16}$
All DEGs	388	11.02	$< 2.2 \times 10^{-16}$

Table S2: Post-mortem cases included in this study.

SAMPLE ID	SAMPLE GENOTYPE	AGE	SEX
NP16-25	*SCN1A*$^{+/+}$	25 yrs	F
N272P.19	*SCN1A*$^{+/+}$	1 yr	M
NP13-74	*SCN1A*$^{+/+}$	23 yrs	M
NU10-5	*SCN1A*$^{+/-}$	11 yrs	M
NP22P309	*SCN1A*$^{+/-}$	6 yr	F
NP10-33	*SCN1A*$^{+/-}$	20 months	M
17P153	*SCN1A*$^{+/-}$	1 yr	M