

Additional file 2. Legends and Supplemental Figures S1-S8.

Legends for Supplemental Figures

Supplemental Figure S1. Characterization of iPSCs. (A) Exome sequencing identified a heterozygous missense variant in the FAD domain-encoding region of the *GPD2* gene (chr2:157352686 (hg19) G>A, NM_001083112.2 c.233G>A, p.G78E) in individuals in the pedigree under study, including the unaffected mother (UM), intermediate phenotype sister (IS), and affected proband (AP), as well as in the trait-affected brother (TB) not studied here. (B) This variant was confirmed by Sanger sequencing and was not present in an unrelated, unaffected control (UC). (C-E) Expression of markers of pluripotency was assessed by (C) RT-qPCR and (D) immunocytochemistry (representative images shown), comparing the cell lines under study (n=4 biological replicates from one clonal line for each subject). *GPD2* protein level was also assessed in these iPSC lines by (D) immunocytochemistry or (E) Western blotting (n=2 biological replicates from one clonal line for each subject). (F) Both the UC line and clonal lines 1 and 2 derived from the UM, IS, and AP were karyotypically normal. (G-H) iPSCs were stained with propidium iodide and analyzed by FACS for DNA content analysis. (G) Representative FACS plots are shown. (H) Mean percentages of cells in each cell cycle stage, with no differences between cell lines observed (n=4 biological replicates from one clonal line for each subject). p-values: * $P < 0.05$ were determined by one-way ANOVA in (H) or by unpaired t-test for RT-qPCR in (C), and all other pairwise comparisons had a non-significant p-value ($P \geq 0.05$). In (D), scale bar=200 μ m.

Supplemental Figure S2. Characterization of iPSC-derived cExN NPCs. (A-C) After 12 days of differentiation, cExN NPCs were stained with propidium iodide and analyzed by FACS for DNA content. (A) Schematic of cExN NPC differentiation protocol. (B) Representative FACS plots. (C) Mean percentages of cells in each cell cycle stage (n=4 biological replicates from one clonal line for each subject). (D-E) cExN NPCs were plated in equal numbers for each sample

and were counted after four days of culture (n=6 biological replicates from one clonal line for each subject). Data are quantified in (D), and representative images are shown in (E) (scale bar = 300µm). (F) GPD2 protein levels were detected in cExN NPC samples by Western blotting (n=2 biological replicates from one clonal line for each subject). p-values: * $P < 0.05$ were determined by one-way ANOVA and all other pairwise comparisons had a non-significant p-value ($P \geq 0.05$).

Supplemental Figure S3. Maturation of cExN NPCs. (A) Differentiation scheme, including timeline and small molecules used. (B) Immunocytochemistry for proteins marking cExN specification, differentiation, maturation, and neuronal function (scale bar=50µm, n=1 biological replicate from one clonal line for each subject; representative images are shown).

Supplemental Figure S4. Characterization of iPSC lines during differentiation into cIN NPCs (A) iPSCs were differentiated for 12 days, as shown in the schematic. (B-C) Differences in neurosphere size at day 5 and 12 are shown in (B) and quantified at day 5 in (C) (mean±SEM; scale bar=250µm; n=7 total biological replicates from two clonal lines for each subject, and one clonal line for the UC). p-values: *** $P < 0.001$ were determined by one-way ANOVA and all other pairwise comparisons had a non-significant p-value ($P \geq 0.05$).

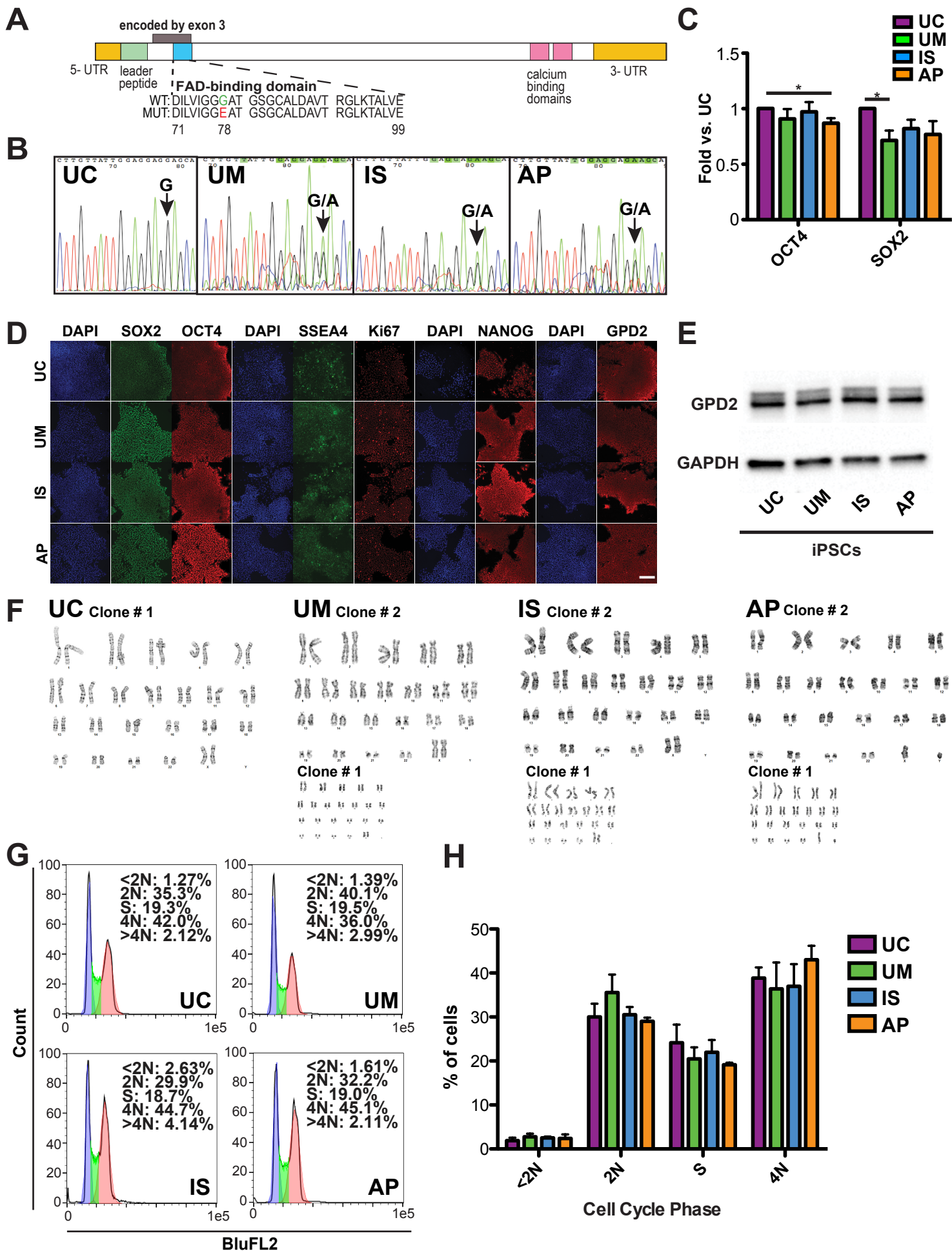
Supplemental Figure S5. Transcriptome analysis of cExN and cIN NPCs by RNA-seq. (A) Multidimensional scaling plot for the cExN and cIN NPC samples. (B-C) Numbers of differentially expressed genes obtained for each pairwise sample comparison, indicating up- and down-regulated genes for the (B) cExN NPCs and (C) cIN NPCs ($p < 0.05$, fold-change > 2).

Supplemental Figure S6. Networks of DEGs with expression that is similar in the AP/IS and different than the UM. Networks of DEGs are related to (A) cExN 'neurological disease' (B) cIN 'nervous system development and function', and (C) cIN 'neurological'. Within each

network, red symbols indicate upregulated genes and green symbols indicate downregulated genes, where the color intensity represents relative degree of differential expression.

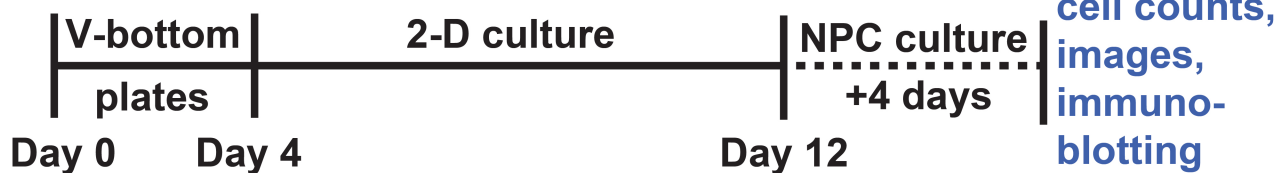
Supplemental Figure S7. Co-expressed DEGs in cExN and cIN NPCs were defined by hierarchical clustering and visualized on a heatmap. Log₂-CPMs of DEGs were scaled by z-score, as described in the methods, and were plotted on a heatmap for **(A)** cExN NPCs and **(B)** cIN NPCs.

Supplemental Figure S8. XIST mRNA expression levels and variancePartition analysis. **(A-B)** Comparison of XIST expression levels in cINPCs derived from the UC, UM, IS, and AP by (A) RNA-seq and (B) RT-qPCR analysis. For the UM, IS, and AP, the first clonal line from each subject was used for RNA-seq analysis (n=4 biological replicates), and the second clonal line from each subject was used for RT-qPCR (n=3 biological replicates), confirming findings for both clonal lines. The single UC clonal line was evaluated by both RNA-seq and RT-qPCR, which also yielded similar results. **(C)** VariancePartition analysis was conducted on RNA-seq data as described in the Methods, with violin plots indicating the percent of variance that was attributable to the cell type, the individual subject from which the model was derived, their age, or their sex.

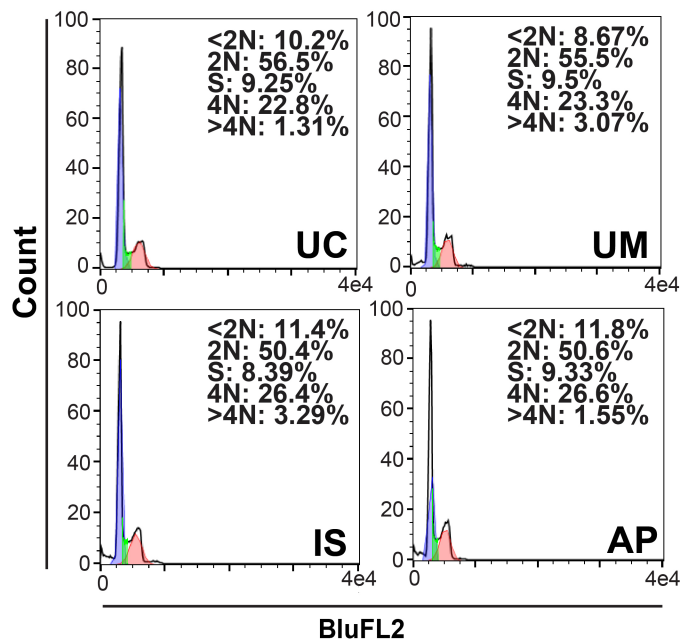


A cExN NPC derivation

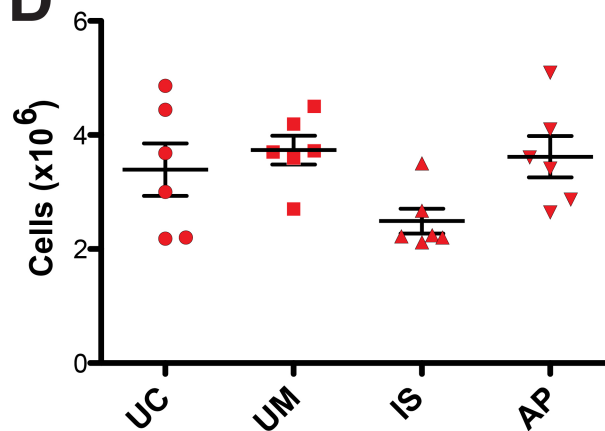
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+SB431542+LDN193189



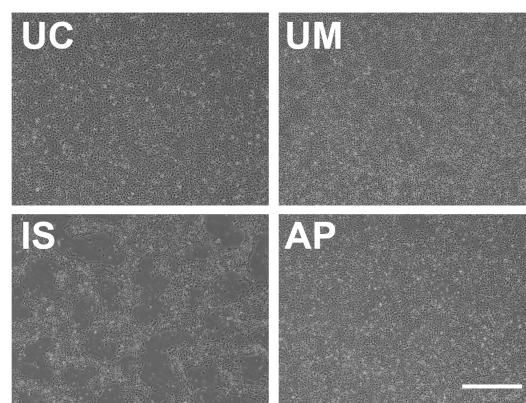
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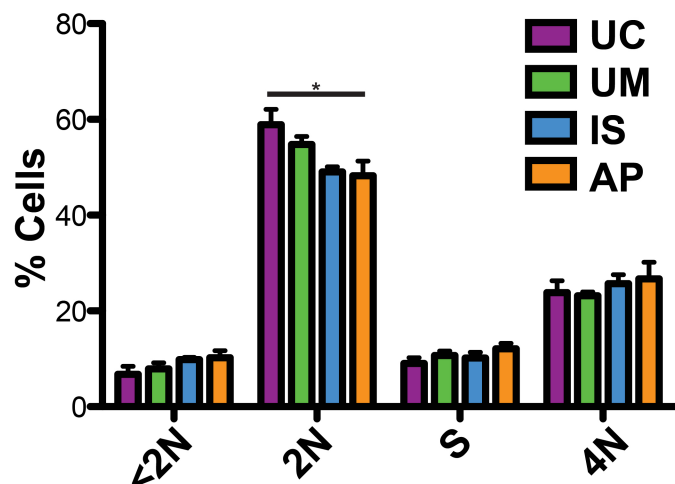
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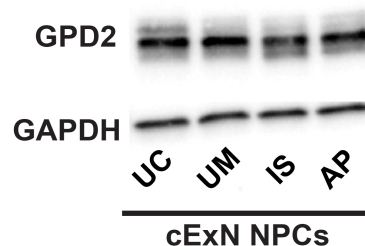
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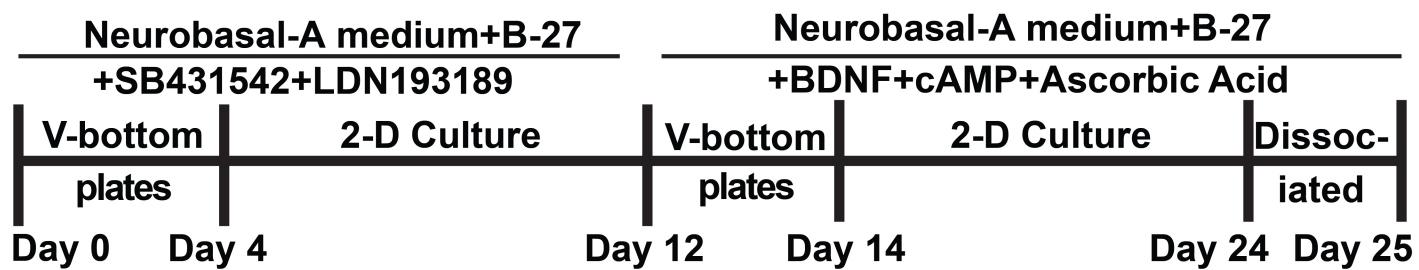
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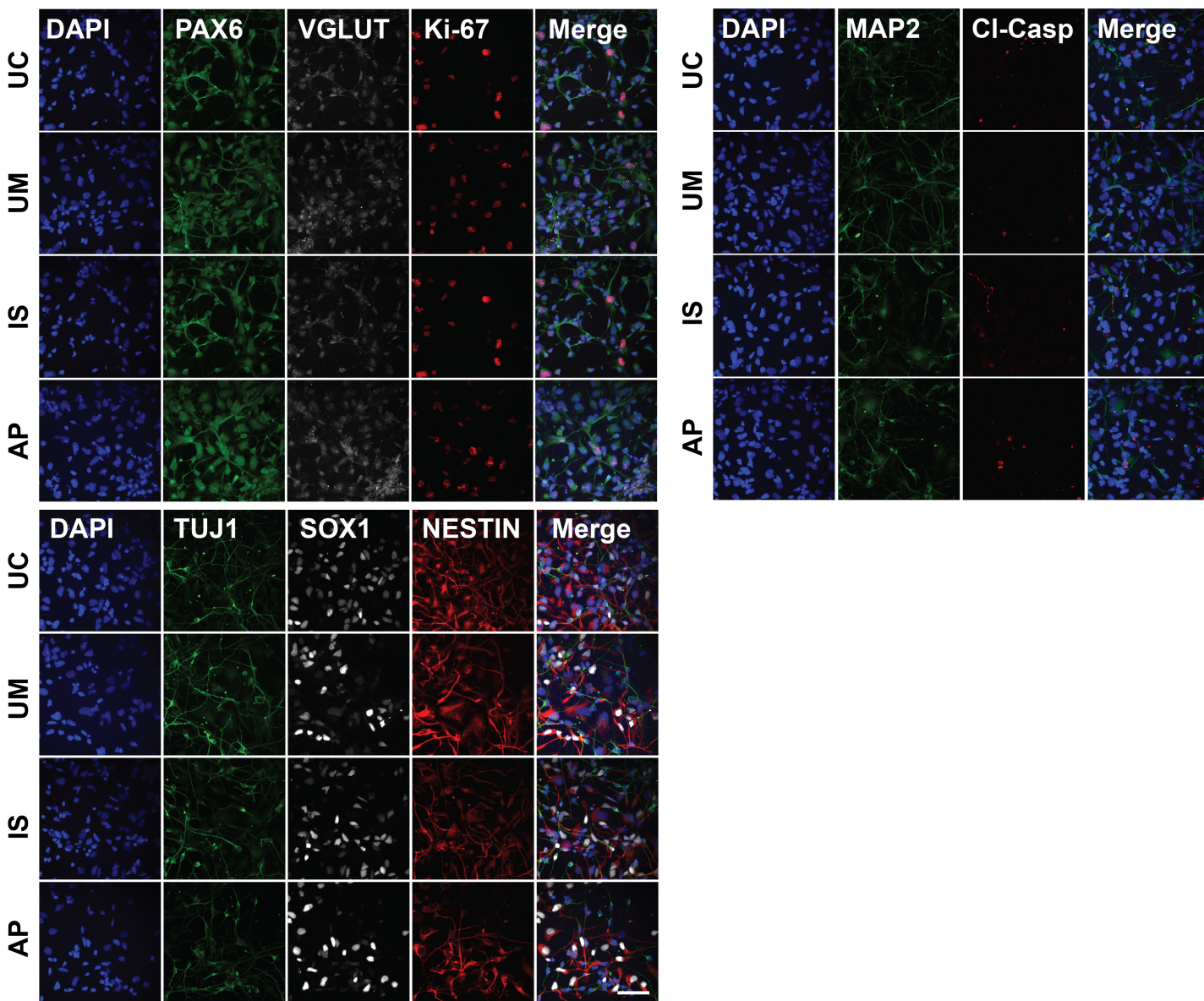
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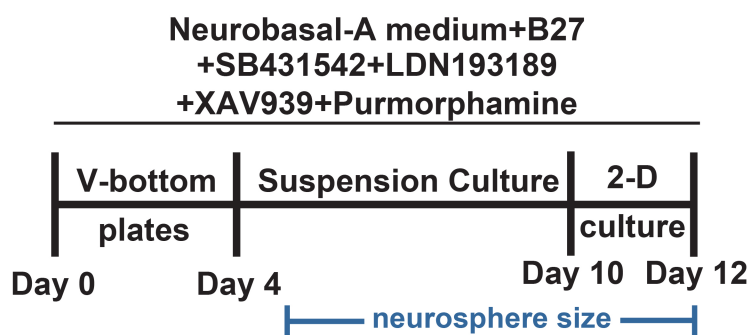
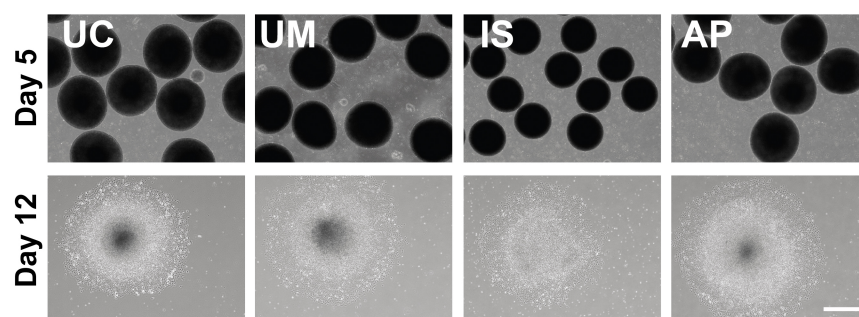
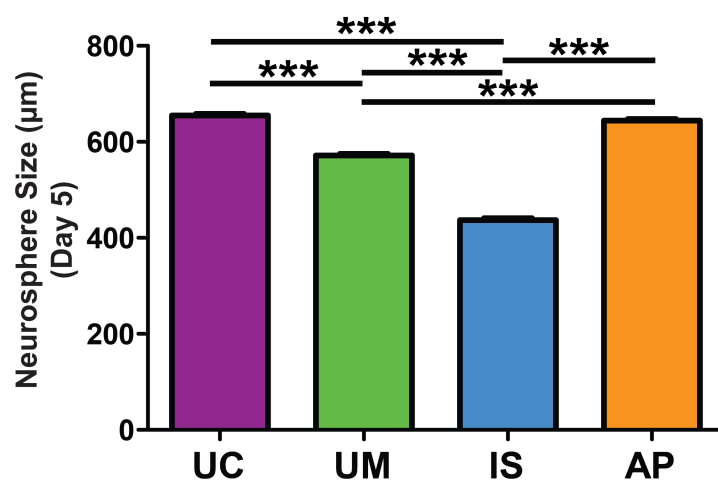


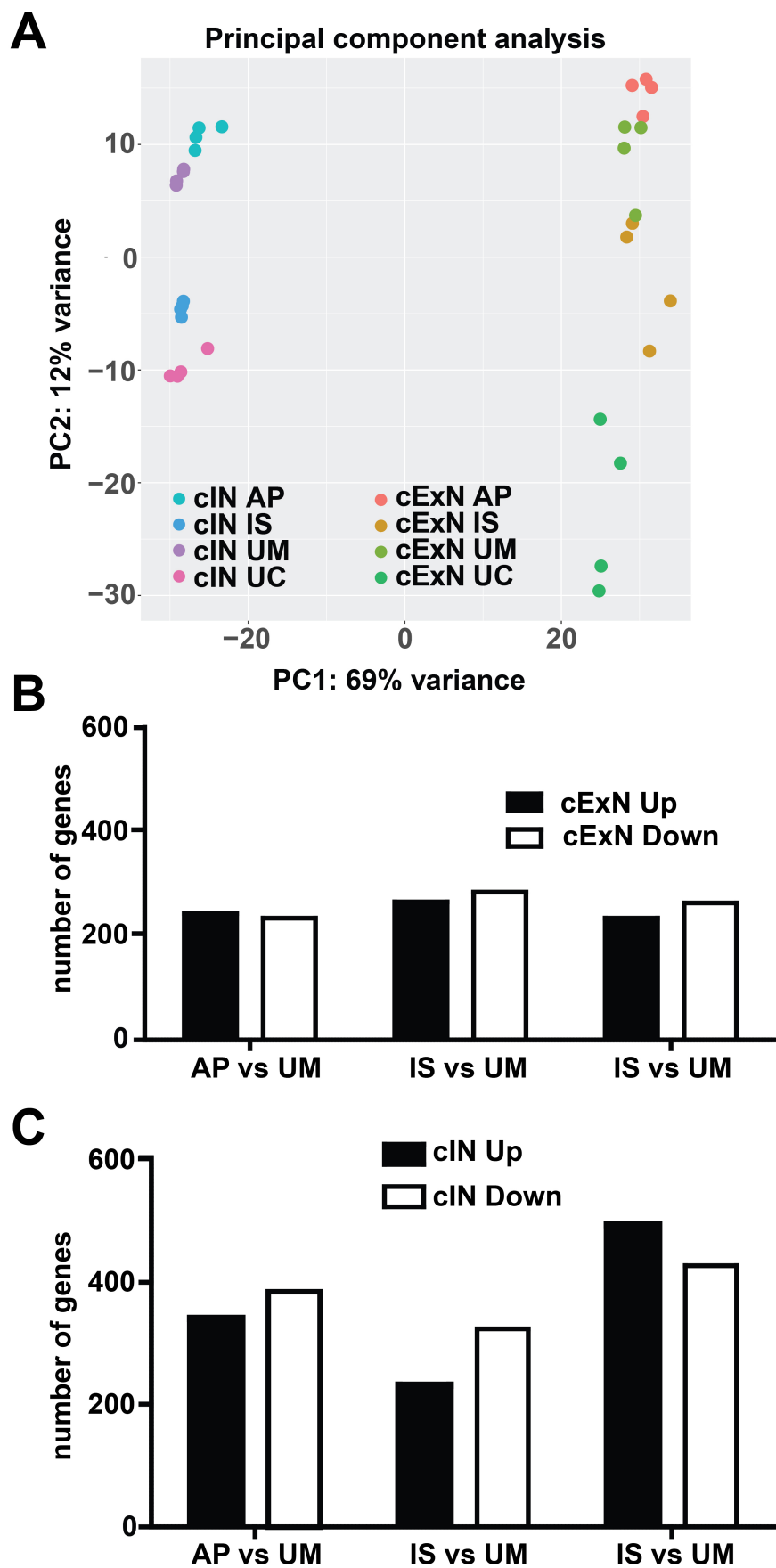
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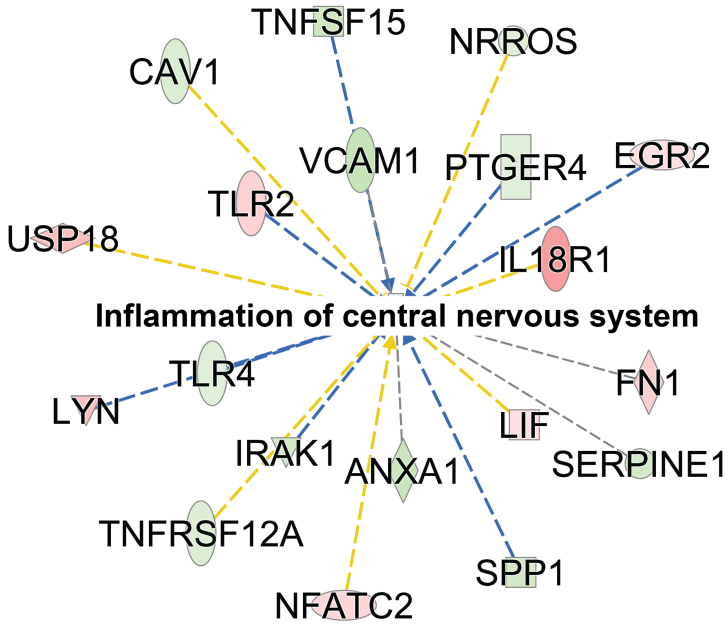
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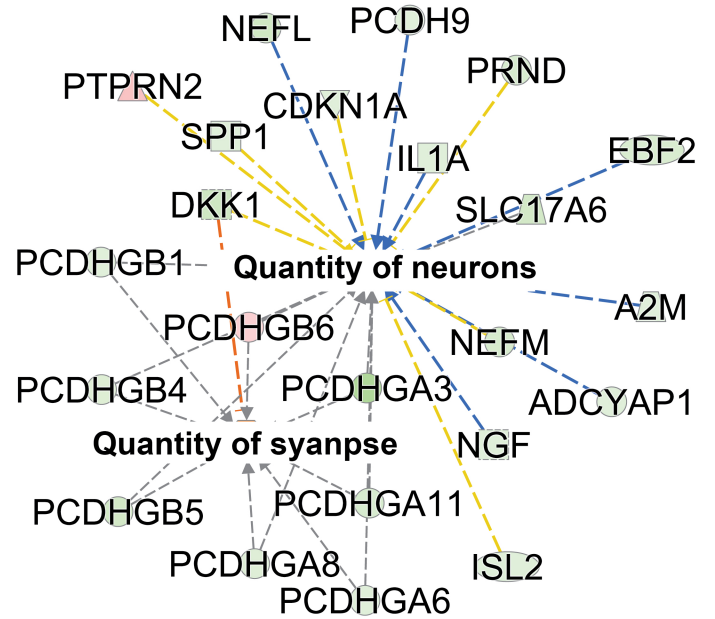
A cIN NPC derivation**B****C**



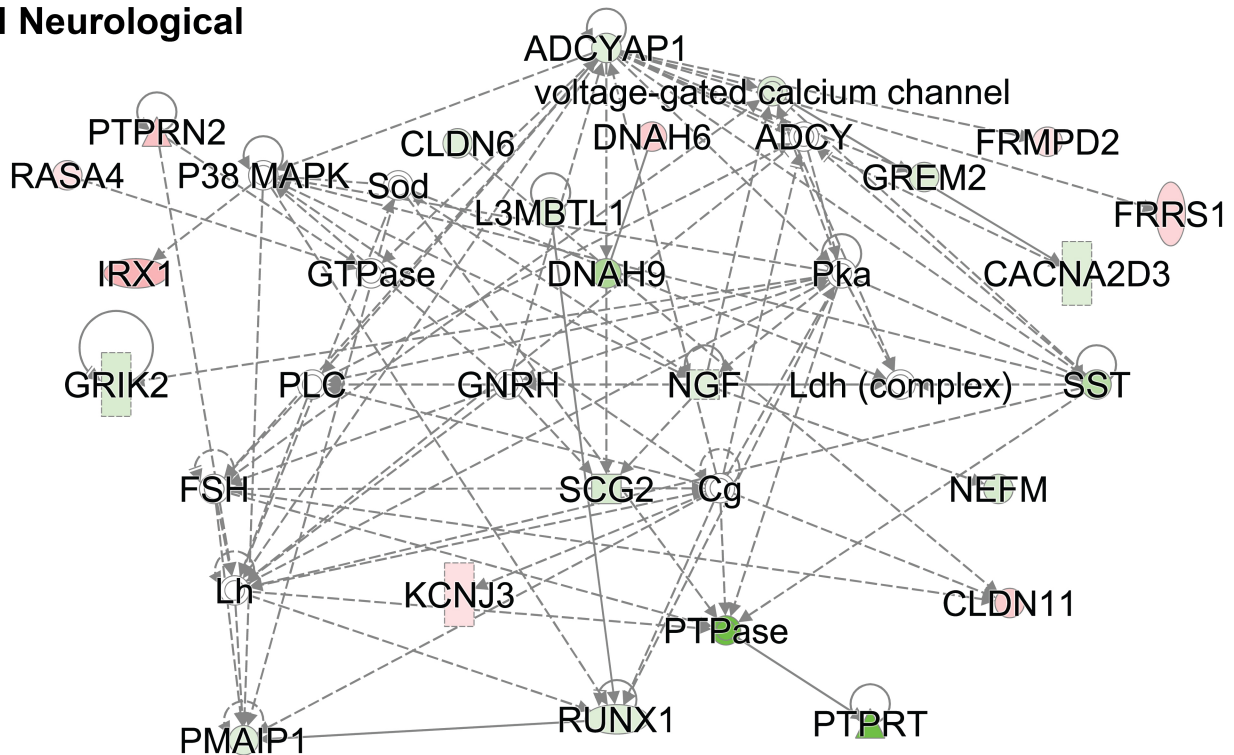
A cExN Neurological Disease

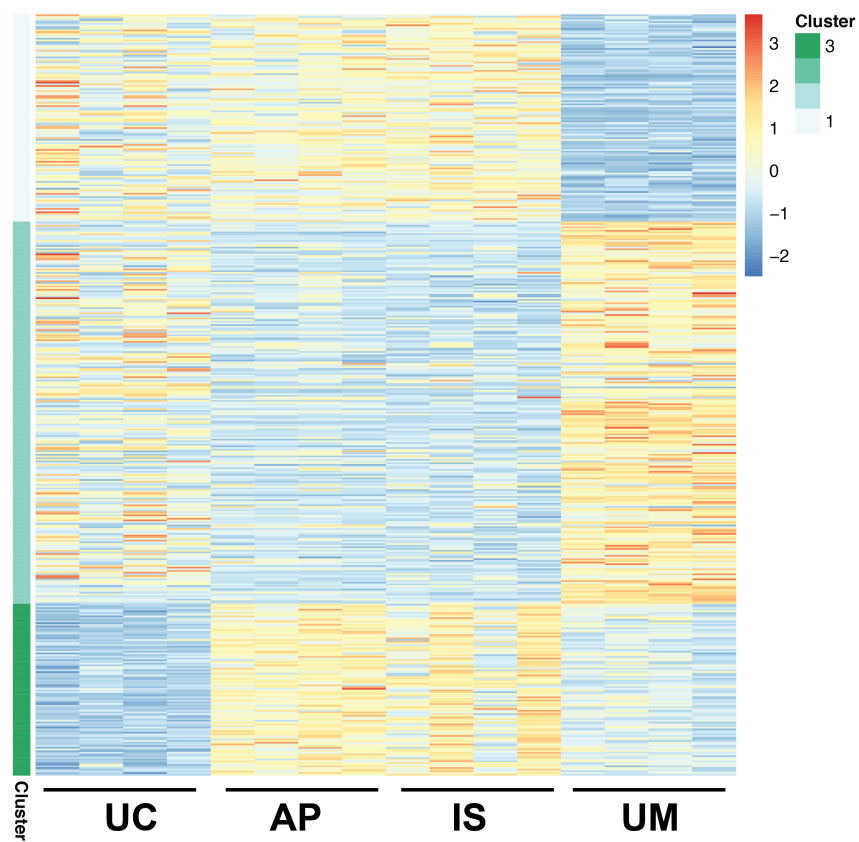


B cIN Nervous System Development and Function



C cIN Neurological



A cExN WGCNA hierarchical clustering**B** cIN WGCNA hierarchical clustering