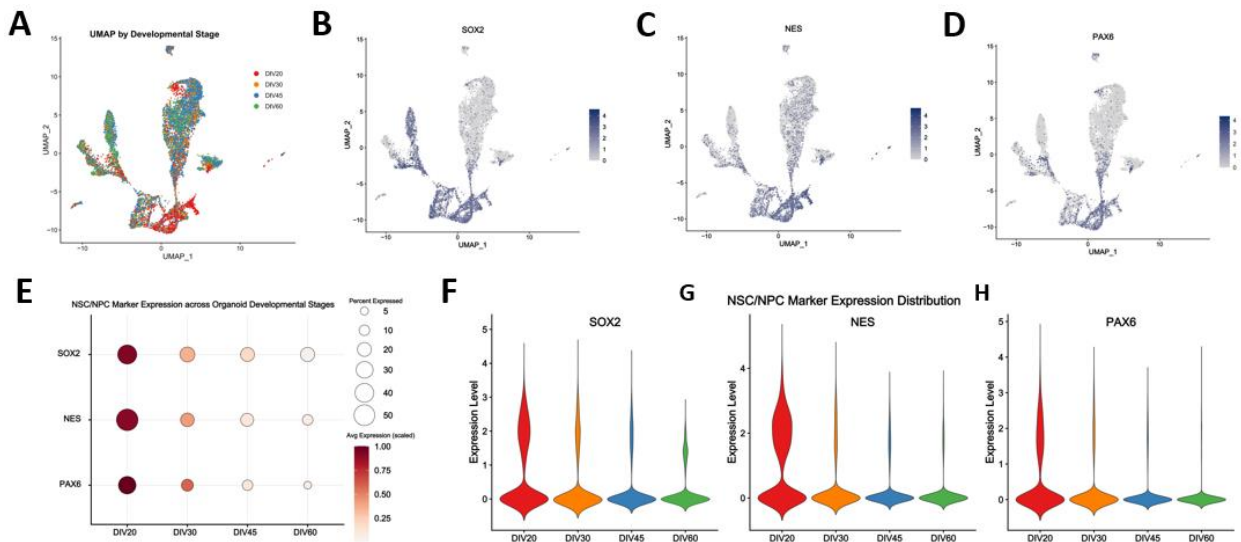


Supplementary Figure 1 Validation of REST-NOTCH1 transcriptional axis dynamics in hNSCs *via* pseudotime analysis. A: Dot plot showing the expression patterns of key marker genes across major cell types in the scRNA-seq dataset. Dot size represents the proportion of expressing cells, and color intensity indicates average expression levels; B: Pseudotime trajectories illustrating relative expression dynamics of key transcription factors (ASCL1, DCX, HES1, NEUROD2, NOTCH1, and REST); C: Correlation analysis of REST and NOTCH1 expression at the single-cell level.



Supplementary Figure 2 Single-cell transcriptomic characterization of neural stem/progenitor cell marker expression in human neural organoids at different developmental stages. A: UMAP plot showing the overall distribution of single cells derived from human neural organoids at DIV20, DIV30, DIV45, and DIV60. Different colors indicate different developmental stages; B-D: FeaturePlots showing the expression distributions of SOX2, NES/Nestin, and PAX6 in organoid-derived cells; E: Dot plot showing the normalized average expression levels and proportions of positive cells for SOX2, NES/Nestin, and PAX6 at DIV20, DIV30, DIV45, and DIV60. Dot size represents the proportion of cells expressing the corresponding gene, and color intensity indicates the normalized average expression level; F-H: Violin plots showing the expression distributions of SOX2, NES, and PAX6 across different developmental stages of the organoids. This figure is based on organoid scRNA-seq data and was used to descriptively characterize the presence and distribution of NSC/NPC-like cell populations at the transcriptomic level. No statistical comparison based on independent biological replicates was performed.

Supplementary Table 1 Sensitivity analysis of REST-based NPC stratification using alternative thresholds

Stratification method	REST-high/REST-low definition	Key-gene pattern	GO_NEUROGENESIS enrichment	Main conclusion
Original threshold	log2TPM > 2	REST high at early pseudotime; DCX, ASCL1, and NEUROD2 higher in REST-low cells	Enriched in REST-low cells	Reference pattern
Median split	Above <i>vs</i> below median REST expression	Same directional pattern as original threshold	Enriched in REST-low cells	Consistent with original threshold
Quartile comparison	Top 25% <i>vs</i> bottom 25% REST expression	Same directional pattern as original threshold	Enriched in REST-low cells	Consistent with original threshold

This sensitivity analysis was used to evaluate the directional robustness of REST-based NPC stratification. Key gene-expression patterns and GO_NEUROGENESIS enrichment remained directionally consistent across alternative REST-high/REST-low definitions.