Supplemental figures

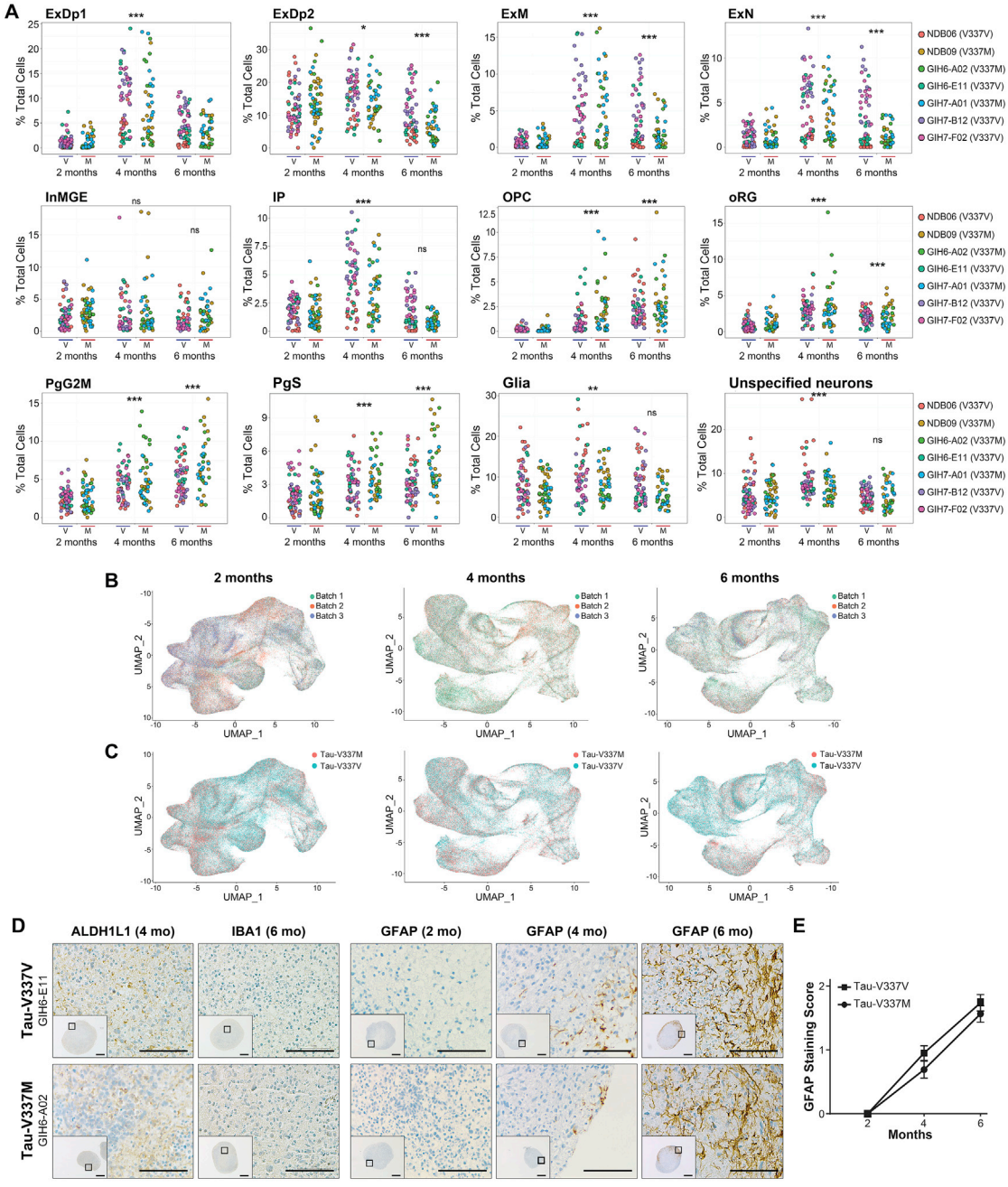

Figure S1. Cerebral organoid differentiation recapitulates key features of human brain patterning, related to Figure 1

(A) Cell type proportions and variability for individual organoids over time for all cell types identified. Points are colored by donor line. Differences in cell type proportion are calculated compared to proportion of cells at 2 months using a linear model, *p < 0.05, **p < 0.01, ***p < 0.001.

(B-C) UMAP reduction of cell hashing single cell sequencing data from cerebral organoids at 2, 4 and 6 months of age, colored by batch (*B*) and mutation (*C*).

(D) Immunohistochemical staining of glial markers ALDH1L1 (4 months), IBA1 (6 months) (negative as expected), and GFAP (2, 4 and 6 months) in tau-V337M (GIH6-A02) and isogenic corrected (GIH6-E11) organoids. Scale bars 250 μm (inserts) and 50 μm.

(E) Quantification of GFAP+ cells at 2, 4 and 6 months of organoid differentiation in tau-V337M (GIH6-A02) and isogenic corrected (GIH6-E11) organoids.

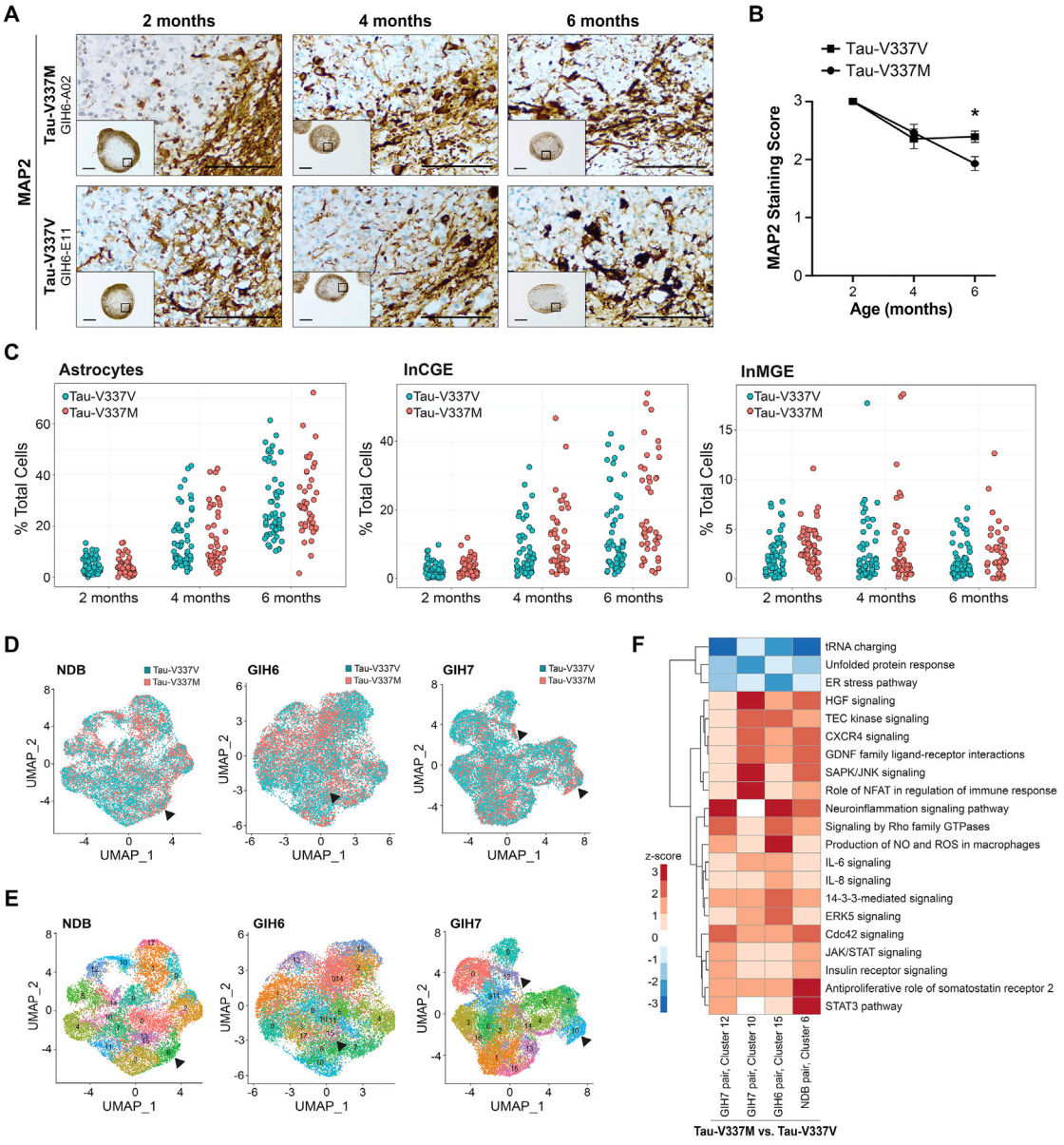

Figure S2. Tau-V337M organoids exhibit neuron-specific loss over time, related to Figure 2

(A-B) Imaging of MAP2+ neurons (*A*) and quantitative analysis (*B*) at 2, 4 and 6 months, in mutant (GIH6-A02) and isogenic corrected (GIH6-E11) organoids. Mann-Whitney: at 6 months *p = 0.006.

(C) Proportion of Astrocytes, InCGE (inhibitory neurons from the caudal ganglionic eminence), and InMGE (inhibitory neurons from the medial ganglionic eminence) per organoid at 2, 4 and 6 months of differentiation. Points are colored by mutation (V337M = red, V337V = blue). A linear model was conducted between glutamatergic cell type proportions in mutant versus isogenic-corrected organoids at each time point, p > 0.05, not significant.

(D-E) UMAP reduction plots of astrocytes from each isogenic cell line pair colored by mutation (*D*) and Seurat cluster (*E*). Black arrows indicate mutant cell-enriched clusters.

(F) Heatmap of enrichment z-scores of pathways significantly up- or downregulated in mutant-enriched astrocyte clusters in each isogenic pair using Ingenuity Pathway Analysis.

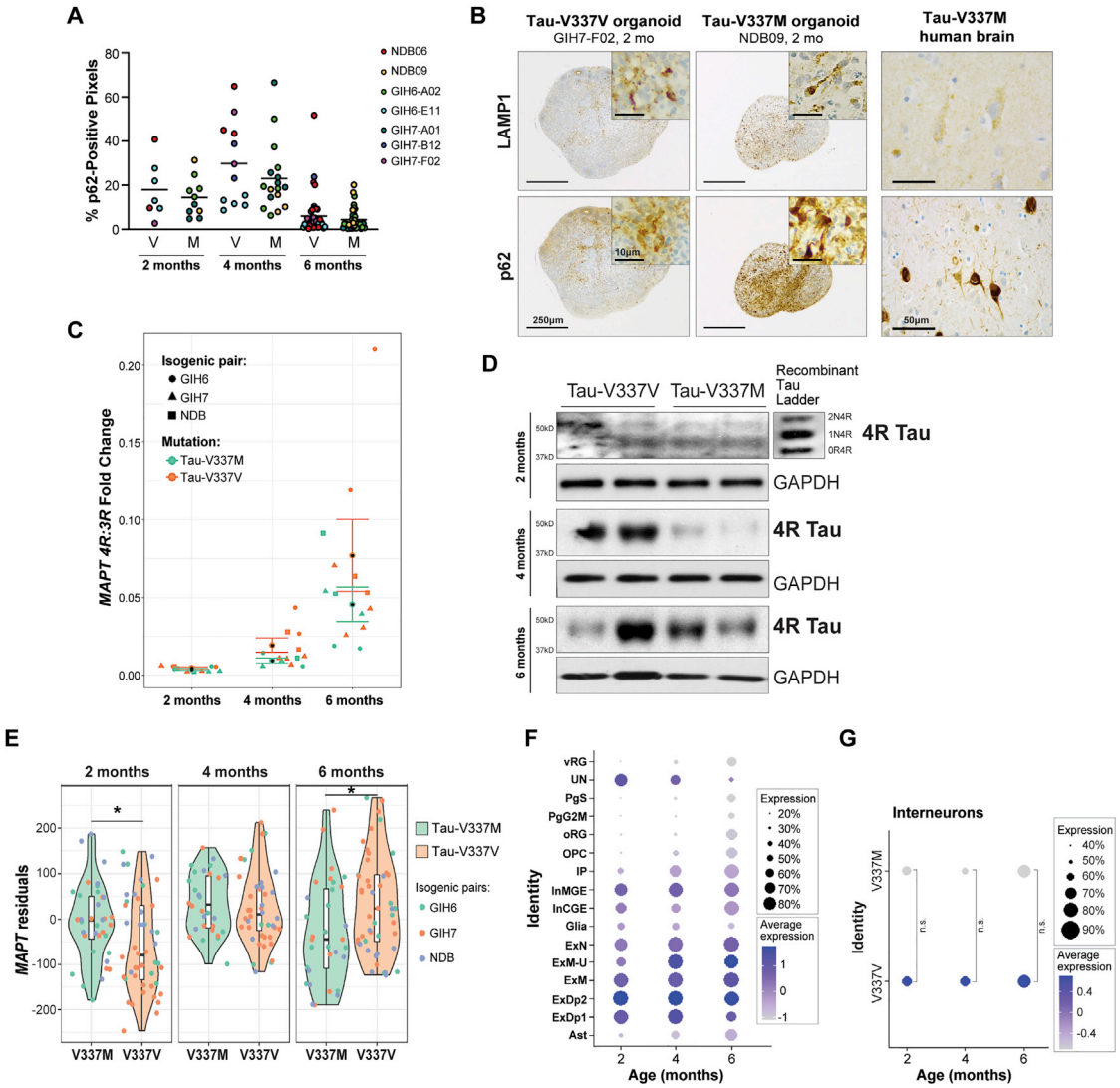

Figure S3. Characterization of autophagy markers and *MAPT* expression in organoids, related to Figures 2 and 3

(A) Quantitative analysis of IHC staining of p62 positive cells in tau-V337V and tau-V337M organoids over time.

(B) IHC imaging of autophagy-lysosomal pathway markers p62 and LAMP1 in tau-V337M organoids (2 months) and in human brain tissue; morphology characteristic of neurons. Organoid scale bar = 250 μm, insets = 10 μm. Human brain scale bar = 50 μm.

(C) qRT-PCR analysis of *4R:3R MAPT* ratio in tau-V337V and V337M organoids at 2, 4 and 6 months. Each isogenic pair is denoted by a different shape, tau-V337M lines = green, V337V = orange. N = 6-8.

(D) Western blot analysis of 4R tau in tau-V337V and V337M organoids at 2, 4 and 6 months (n = 3 per group). 2-month-old samples and the recombinant tau ladder were run in the same gel and the image was cropped for the sole purpose of excluding samples not included in this analysis.

(E) Violin plots show residuals of *MAPT* expression in organoids derived from bulk RNA-seq data following correction for covariates (V337M = green, V337V = orange). Each isogenic cell line is denoted by different color data points (GIH6 = green, GIH7 = orange, NDB = purple). Statistical comparisons (linear mixed model for repeated-measures) were carried out between V337M and V337V organoids at each time point, *$p < 0.05$.

(F) Proportion of *MAPT*-expressing cell-types in 2, 4 and 6-month organoids, with expression scaled within each time point. Dot size represents the proportion of cells expressing detectable *MAPT*, and depth of color denotes level of *MAPT* expression.

(G) Proportion of *MAPT* expressing interneurons in tau-V337V and V337M organoids at 2, 4 and 6 months. Dot size represents the proportion of cells expressing *MAPT*, and the depth of color denotes the level of *MAPT* expression. n.s./not significant.

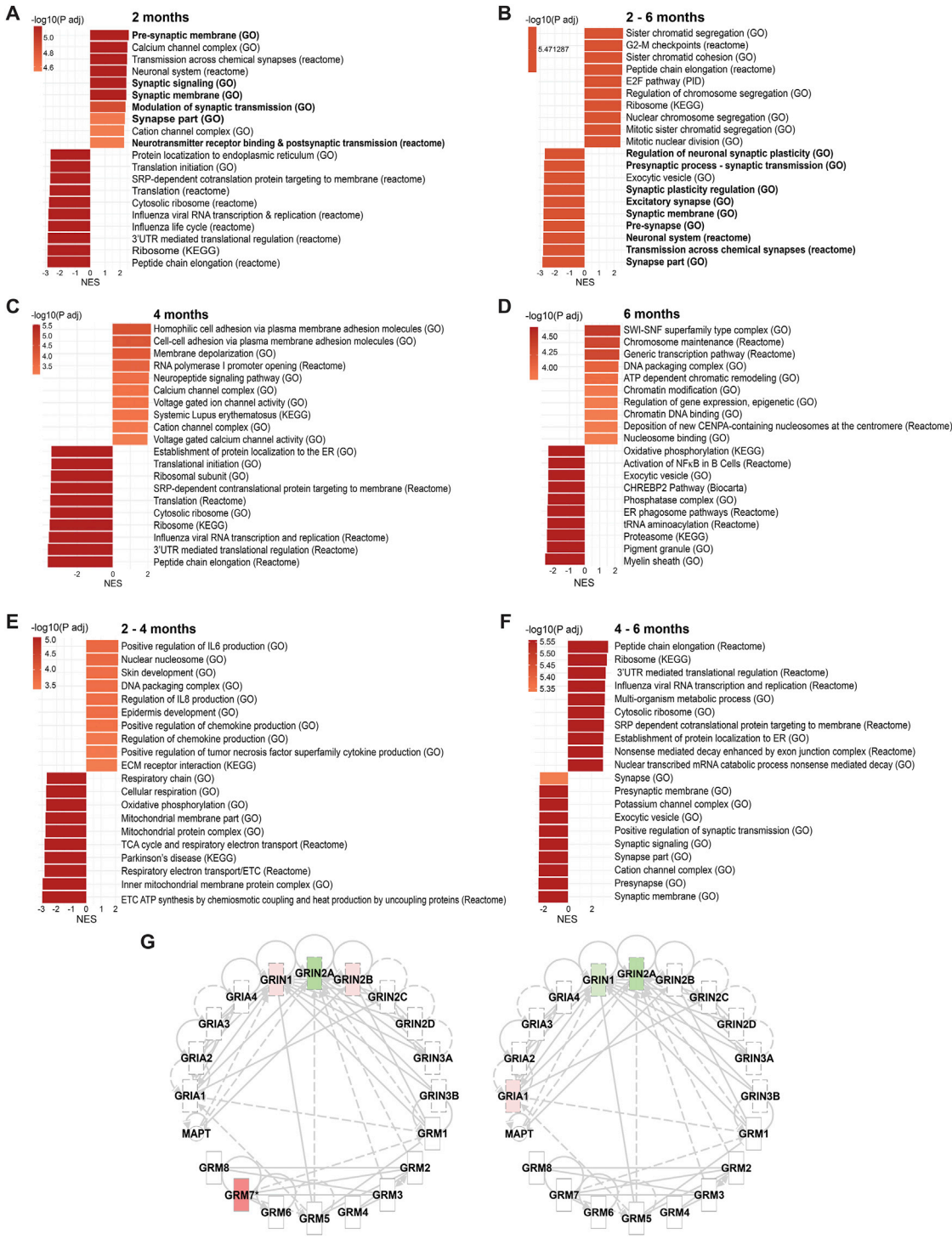

Figure S4. Changes in enriched gene expression pathways in tau-V337M organoids, related to Figure 4

(A-B) Gene set enrichment analysis of differentially expressed genes in the bulk RNA-seq data at 2 months of differentiation (*A*) and the interaction between mutation and age from 2-6 months (*B*). NES = normalized enrichment score. Adjusted -log10 *p-value* is indicated by depth of color on bars. Synaptic-related pathways are highlighted in bold.

(C-F) Gene set enrichment analysis of differentially expressed genes in the bulk RNA-seq data at 4 months (*C*), 6 months (*D*) and the interaction between mutation and age between 2-4 months (*E*) and 4-6 months (*F*). NES = normalized enrichment score. Adjusted -log10 *p-value* is indicated by depth of color on bars.

(G) Expression and connectivity of glutamatergic receptor genes and *MAPT* at 4 months (left) and 6 months (right) of organoid differentiation. Red indicates gene upregulation in V337M organoids compared to isogenic controls, and green indicates gene downregulation. Depth of color reflects the extent of fold-change expression.

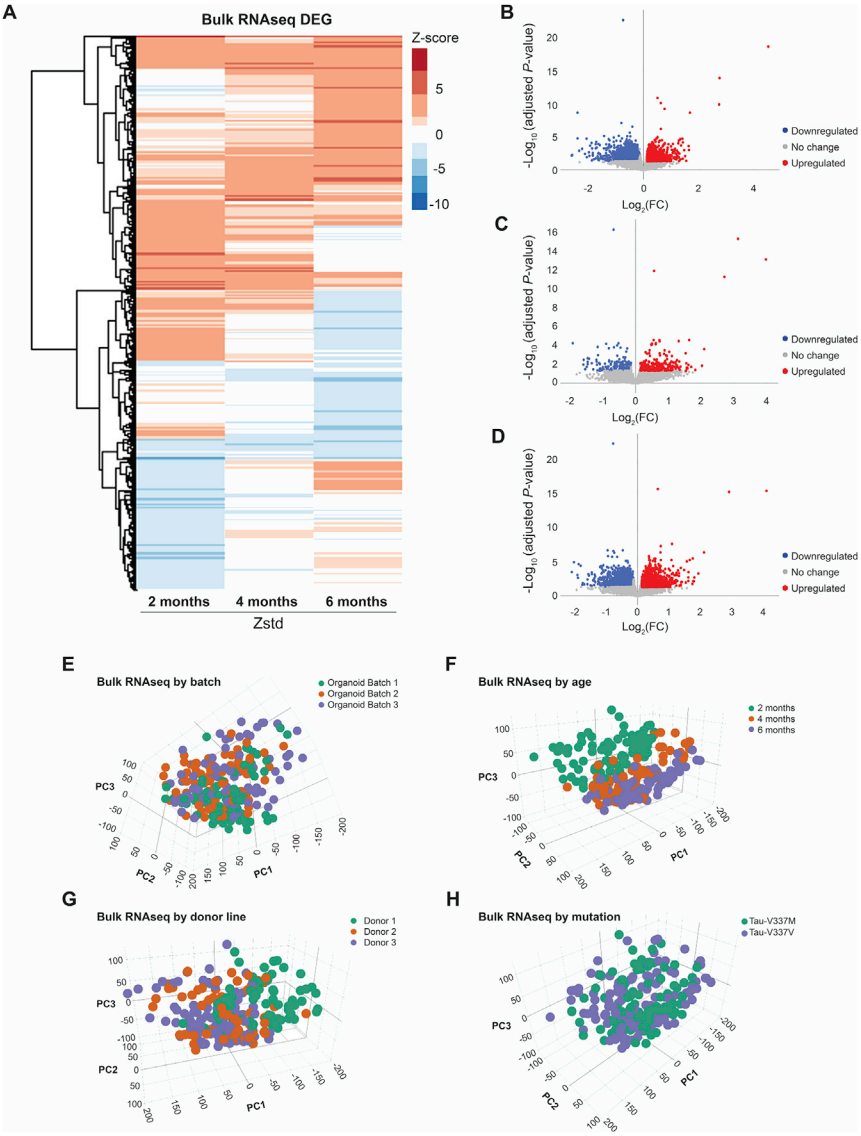

Figure S5. Summary of differential gene expression in tau-V337M organoids, related to Figure 4

(A) Standardized z-scores (Zstd) for all differentially expressed genes in the bulk RNaseq data between tau-V337M and V337V organoids at 2, 4 and 6 months, as determined by linear mixed model for repeated-measures.

(B-D) Volcano plots denoting number, fold change and significance of differentially expressed genes between tau-V337M and V337V organoids in the bulk RNA-seq data at 2 (*B*), 4 (*C*) and 6 (*D*) months.

(E-H) Principal components (PC) plots of bulk RNaseq samples, colored by batch (*E*), age (*F*), donor cell line (*G*) and mutation (*H*).

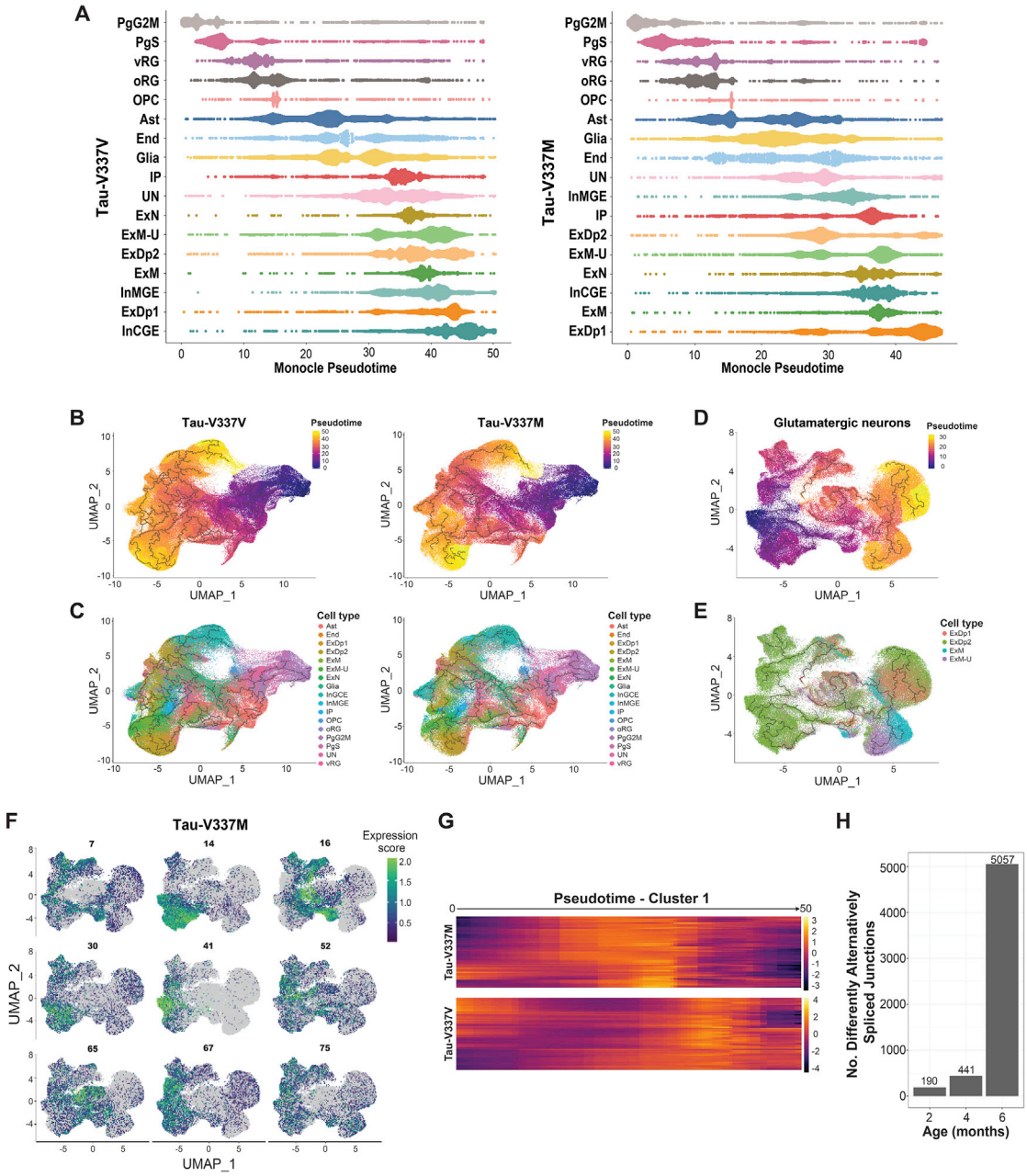

Figure S6. Ordering of organoid cell types and enrichment of tau-V337M and V337V gene expression modules in pseudotime, related to Figure 5

(A) Separation of cell types present in organoids by pseudotime in tau-V337V (left) and tau-V337M (right) organoids.

(B-C) UMAP reduction of all tau-V337V (left) and tau-V337M (right) cells colored by pseudotime (*B*) and cell type (*C*). Cells appearing earliest in pseudotime are denoted by dark purple, and those latest in pseudotime are in yellow.

(D-E) UMAP reduction of all excitatory neurons colored by pseudotime (*D*) and cell type (*E*). Cells earliest in pseudotime are denoted by dark purple, and those latest in pseudotime are in yellow.

(F) UMAPs of excitatory neurons indicating the location and expression score of the top 9 enriched gene clusters in tau-V337M organoids.

(G) Heatmaps of cluster 1 genes over pseudotime in tau-V337M (top) and V337V (bottom) glutamatergic neurons. Purple = low expression, orange = high expression.

(H) Number of significantly differentially spliced intron junctions between tau-V337M and tau-V337V organoids at each differentiation time-point (exact number shown above each bar) as determined by LeafCutter analysis.

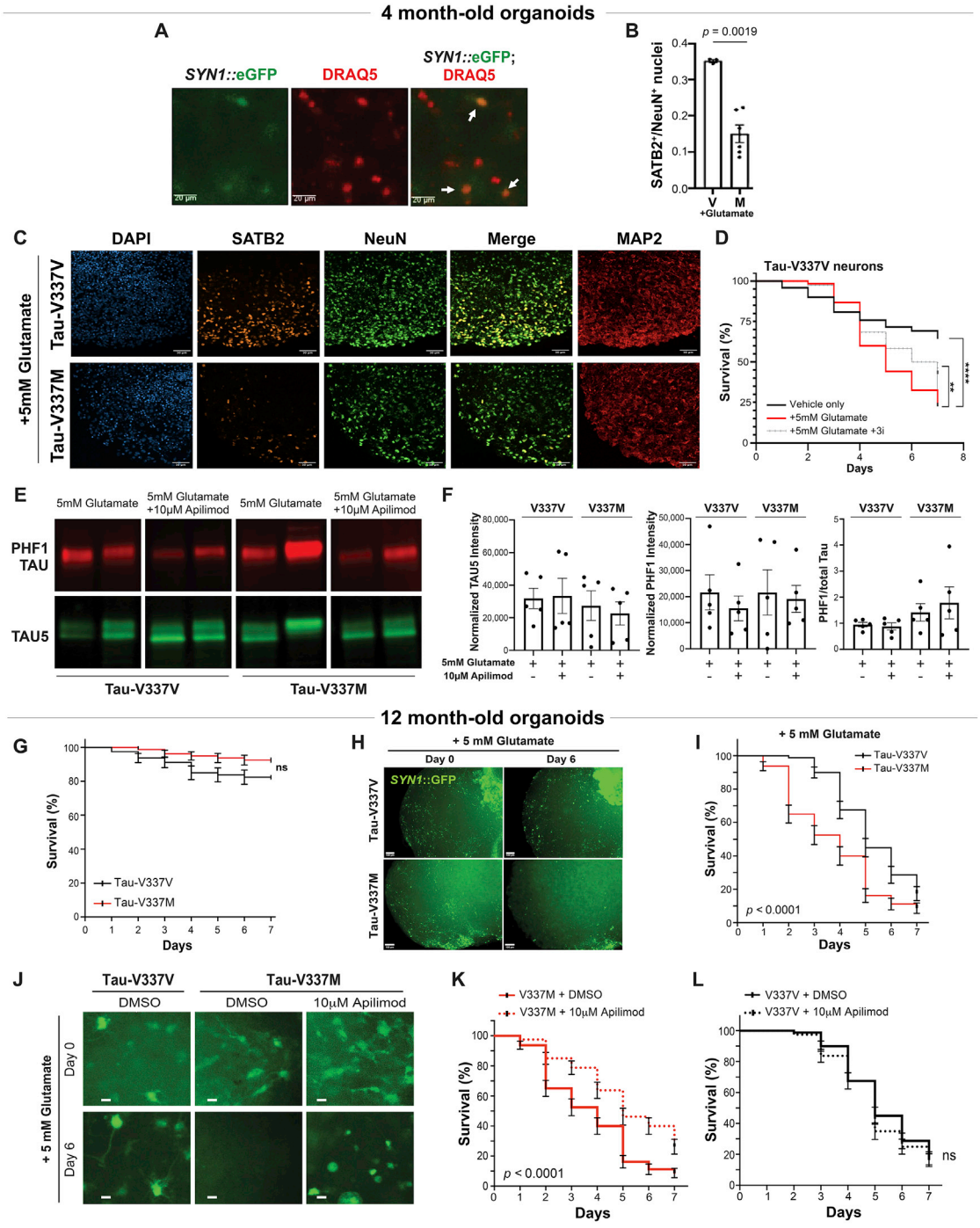

Figure S7. Susceptibility to glutamate excitotoxicity in tau-V337M organoids is reversed by apilimod, related to Figure 7

(A) Co-labeling of *SYN1*::eGFP transduced and non-transduced cells with the live cell marker DRAQ5 in 4 month old organoids.

(B-C) Quantification (*B*) and representative images (*C*) of the proportion of SATB2+/NeuN+ glutamatergic neurons in 4-month-old isogenic tau-V337V (GIH6-E11, n = 3) and tau-V337M (GIH6-A02, n = 6) organoids following 48h of 5 mM glutamate treatment. Line and error bars represent mean and SEM, respectively. Statistical analysis by unpaired Student's t test. Scale bar, 50 µm.

(D) Survival of SYN1::GFP+ neurons in tau-V337V (GIH6-E11) 4-month-old organoids with 5 mM glutamate treatment and DMSO, 3i (10 µM CNQX + 10 µM MK-801 + 2 µM Nimodipine), n = 120 neurons tracked from 5 individual organoids per group. Log-rank test: **p < 0.01, ****p < 0.0001. These organoids are not of the same isogenic pair as Figure 7G.

(E) Western blot of 4-month-old isogenic tau-V337V (GIH6-E11 and GIH7-B12) and tau-V337M (GIH6-A02 and GIH7-A01) organoids treated with 5 mM glutamate and DMSO ± 10 µM apilimod. Phosphorylated tau PHF1 (S396/S404) (red) and total tau (green).

(legend continued on next page)

(F) Quantification of (*E*), normalized to total protein levels. n = 5 organoids per group. Significance determined by One-way ANOVA with Tukey's multiple comparisons test.
(G) Survival curves for *SYN1*::GFP+ neurons in 12-month-old isogenic tau-V337V (NDB06) and tau-V337M (NDB09) organoids.
(H, I) IF imaging and survival curves for *SYN1*::GFP+ neurons in 12-month-old tau-V337M (NDB09) and isogenic V337V (NDB06) organoids with 5 mM glutamate treatment. n = 80 neurons tracked in total from 5 individual organoids per group. Significance determined by log-rank test, ****$p < 0.0001$.
(J) Images of 12-month-old isogenic control (NDB06) and tau-V337M (NDB09) organoids treated with 5 mM glutamate and DMSO or 10 μM apilimod. Neurons were labeled with a lentivirus encoding *SYN1*::GFP. Scale bars are 10 μm.
(K-L) Survival of 12-month-old *SYN1*::GFP$^+$ neurons in Tau-V337M (NDB09, *J*) and tau-V337V (NDB06, *K*) organoids with glutamate treatment and DMSO or apilimod. n = 80 neurons tracked in total from 5 individual organoids per group, log-rank test ****$p < 0.0001$ or ns/not significant.