

Functional and Cytoarchitectural Characterization of a hiPSC-Derived Midbrain Organoid Model of Sporadic Tauopathy

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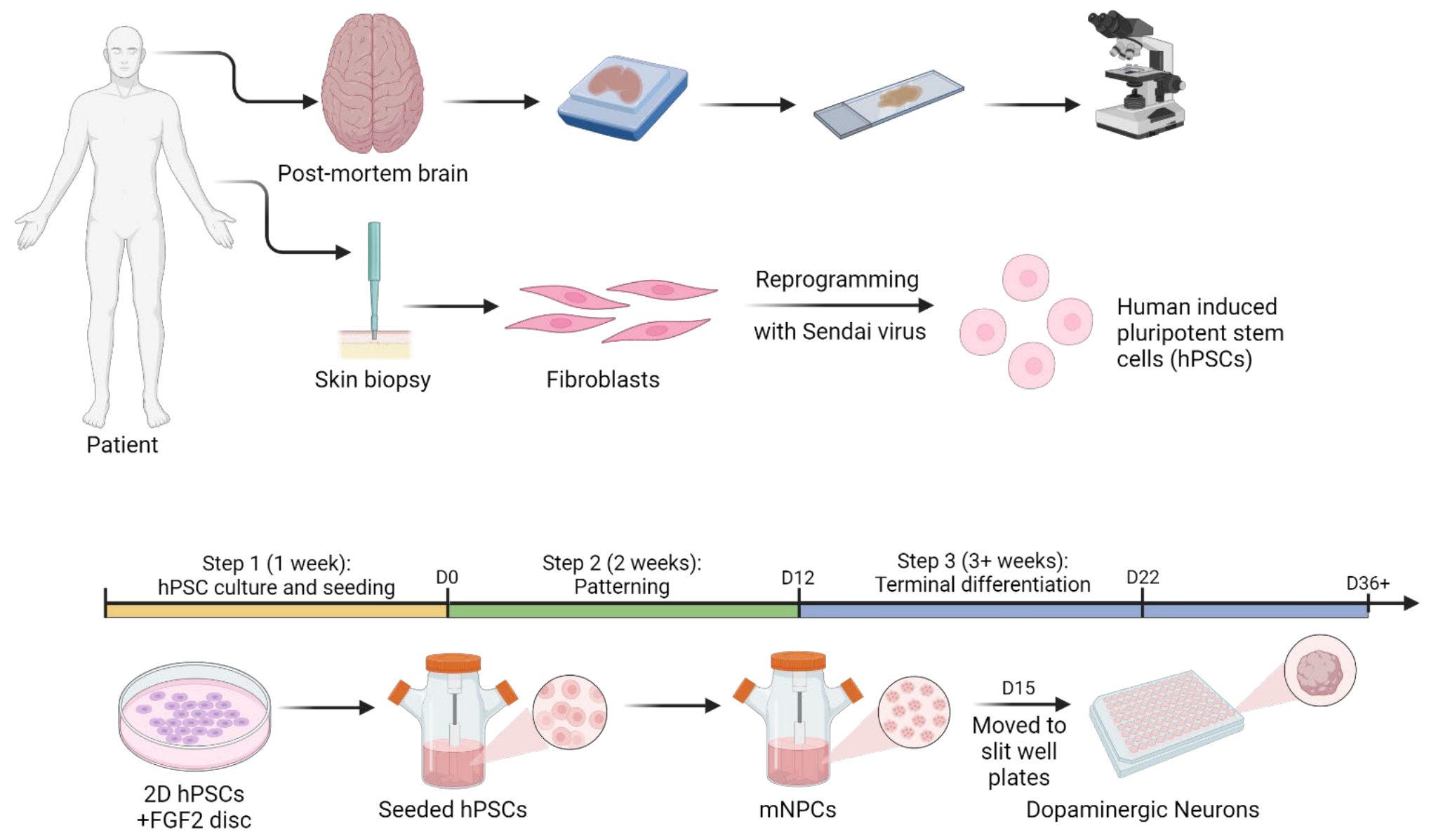
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BACKGROUND

Background: Progressive supranuclear palsy (PSP) is a primary tauopathy characterized by abnormal tau accumulation in neurons and glia. Although most PSP cases are sporadic, previous studies have identified several genetic risk associations. Human induced pluripotent stem cell (hiPSC)-derived organoids are powerful tools to model patient-specific genetic contributions and capture early changes preceding disease progression. Molecular mechanisms that drive PSP pathology remain poorly understood and organoids represent a patient-relevant model system to validate candidate disease mechanisms. Calcium imaging allows for rapid functional characterization, and 3D microscopy allows for complete visualization of organoid cytoarchitecture.

Methods: Fibroblasts grown from sporadic PSP patient or control skin cells (n = 10 independent cell lines) were reprogrammed into hiPSCs. HiPSC-derived midbrain organoids were generated in spinner flasks through pharmacological directed differentiation and grown for up to ten months. For calcium imaging, organoids were incubated with 10uM cell-permeant calcium dye Fluo-4-AM for 3 hours and imaged in antibiotic-free base media. FIJI was used for manual ROI extraction, and a custom code was used for event detection and analysis. For tissue clearing, fixed organoids were delipidated with aqueous detergents and immunolabeled with TH, MAP2 and syto-24. Refractive index matching was achieved with EasyIndex. Images were acquired with a confocal microscope and Imaris 11.0 was used for 3D analysis. EM analysis was conducted using FIJI.



ORGANOID DEVELOPMENT

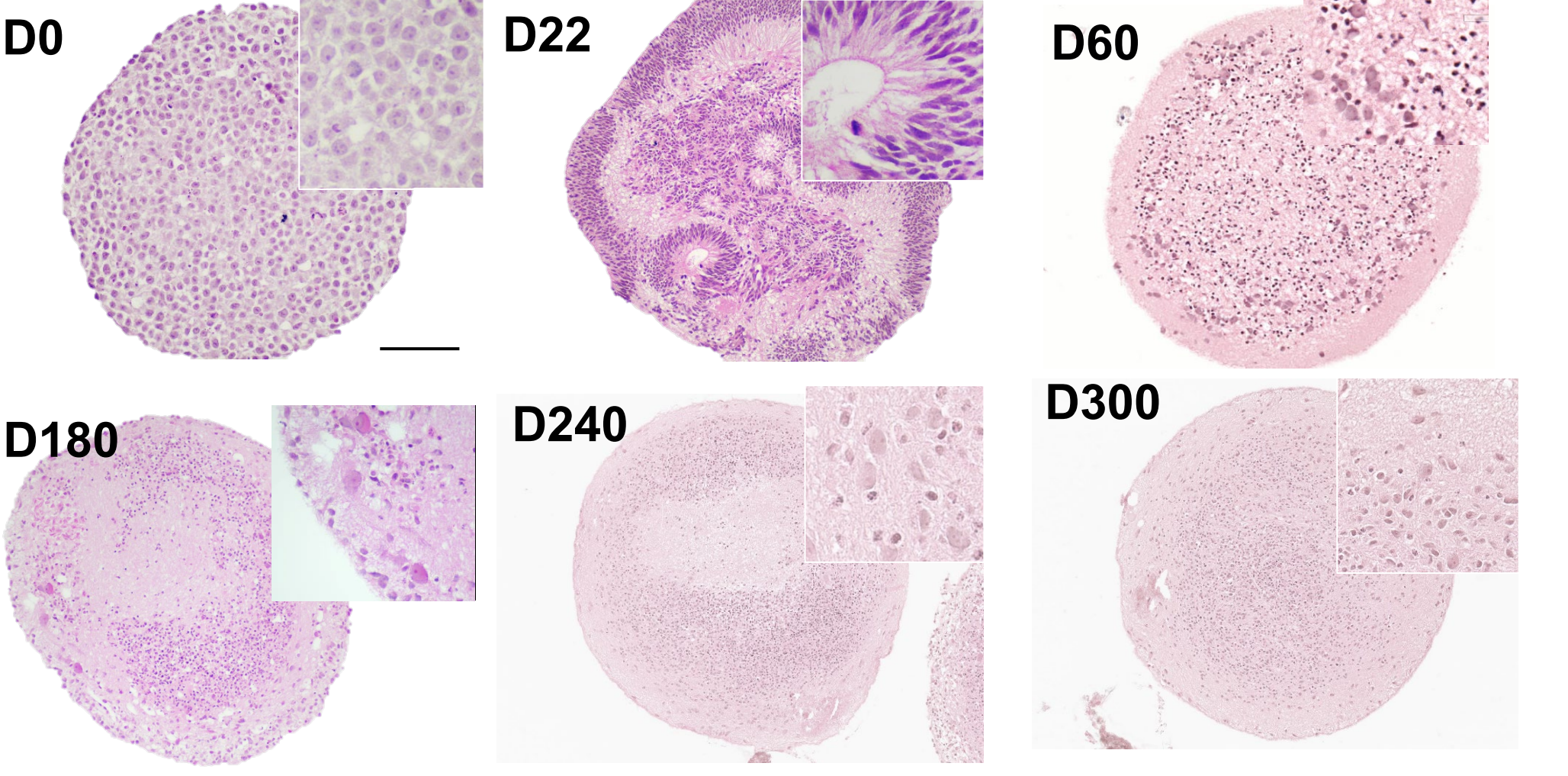


Figure 1: Organoid size, layering and cytoarchitecture. (C) Hematoxylin and eosin (H&E) sections show pluripotent spheres at D0 and expected cytoarchitectural features at D22 as the organoids mature, including the emergence of rosettes and layering. After D22, layering continues and different cell morphologies appear. Scale bars are 100 μ m.

MORPHOLOGICAL DIFFERENCES BASED ON DISEASE STATUS

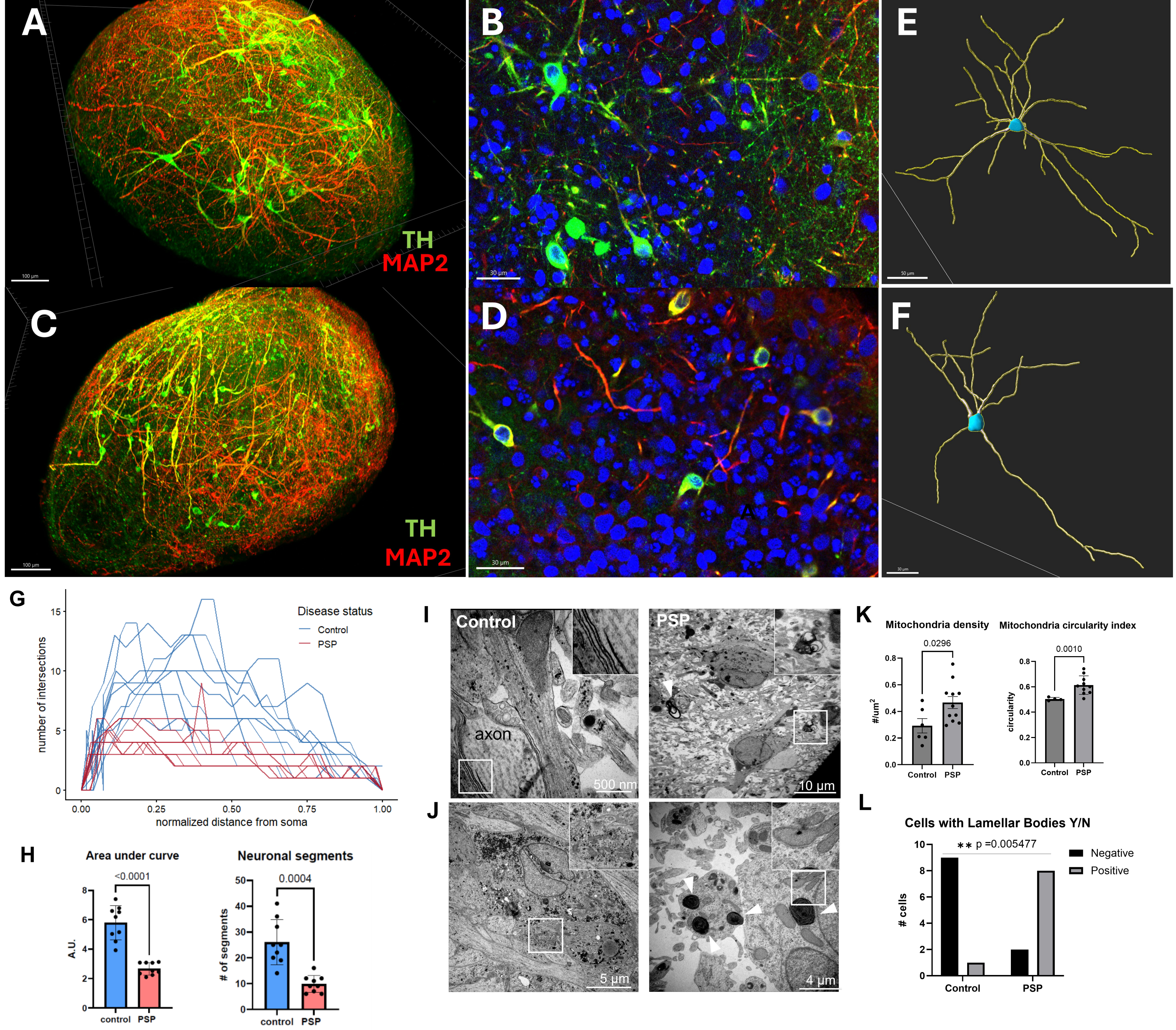


Figure 2: PSP organoids demonstrate morphological dystrophy. (A) and (C) 3D views of surface of D300 control and PSP organoid respectively. (B) and (D) Cross-sections of (A) and (C) respectively demonstrating rich network of TH and MAP2 positivity. Images were acquired with a 25X water immersion objective on a Leica Stellaris confocal microscope. (E) and (F) are representative neuronal reconstructions from PSP and control organoids respectively. (G) Sholl analysis on reconstructions demonstrates reduced morphological complexity in PSP organoids, as demonstrated as (H) reduced area under curve and fewer neuronal segments. Nine TH and MAP2 immunopositive neurons were manually reconstructed using Imaris Filament Tracer across 3 independent organoids. (I-J) Ultrastructure examination of 10 mo organoids by electron microscopy (EM). (I) Non-compact early myelin surrounds an axonal cross-section in a control organoid and dysplastic myelin in PSP organoid indicates myelin abnormalities. (J) Control organoid contains abundant organelles including mitochondria. PSP organoids show organelle abnormalities such as swollen mitochondria and lamellar bodies (arrowheads). (K-L) Quantification of EM showing abnormal mitochondrial morphology (K) and increased lamellar bodies (L) in PSP organoids.

DIFFERENCES IN TAU PROTEOFORMS

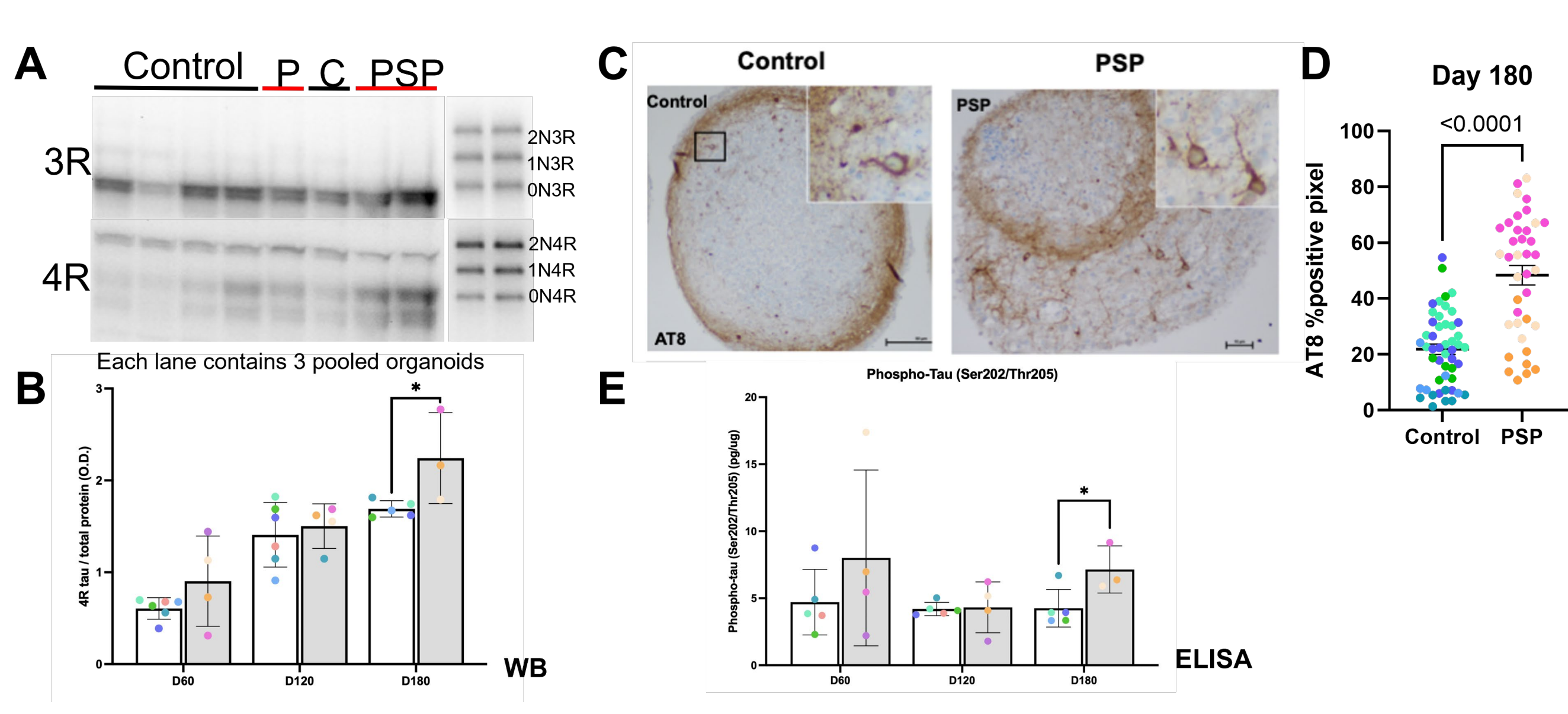


Figure 3. Toxic tau proteoforms in PSP organoids. Immunoblots (A) and quantification (B) for 3R tau (RD3) and 4R tau (RD4) showing increased 4R tau in 6 month PSP organoids. Samples were incubated with lambda phosphatase. (C) Immunohistochemistry for p-tau (AT8) in 6 mo PSP and control organoids. (D) Quantification for (C) showing increased AT8 positive pixels in 6 month PSP organoids. (E) Increased p-tau (AT8) in PSP organoids validated by ELISA.

CALCIUM IMAGING CAPTURES SINGLE CELL EVENTS

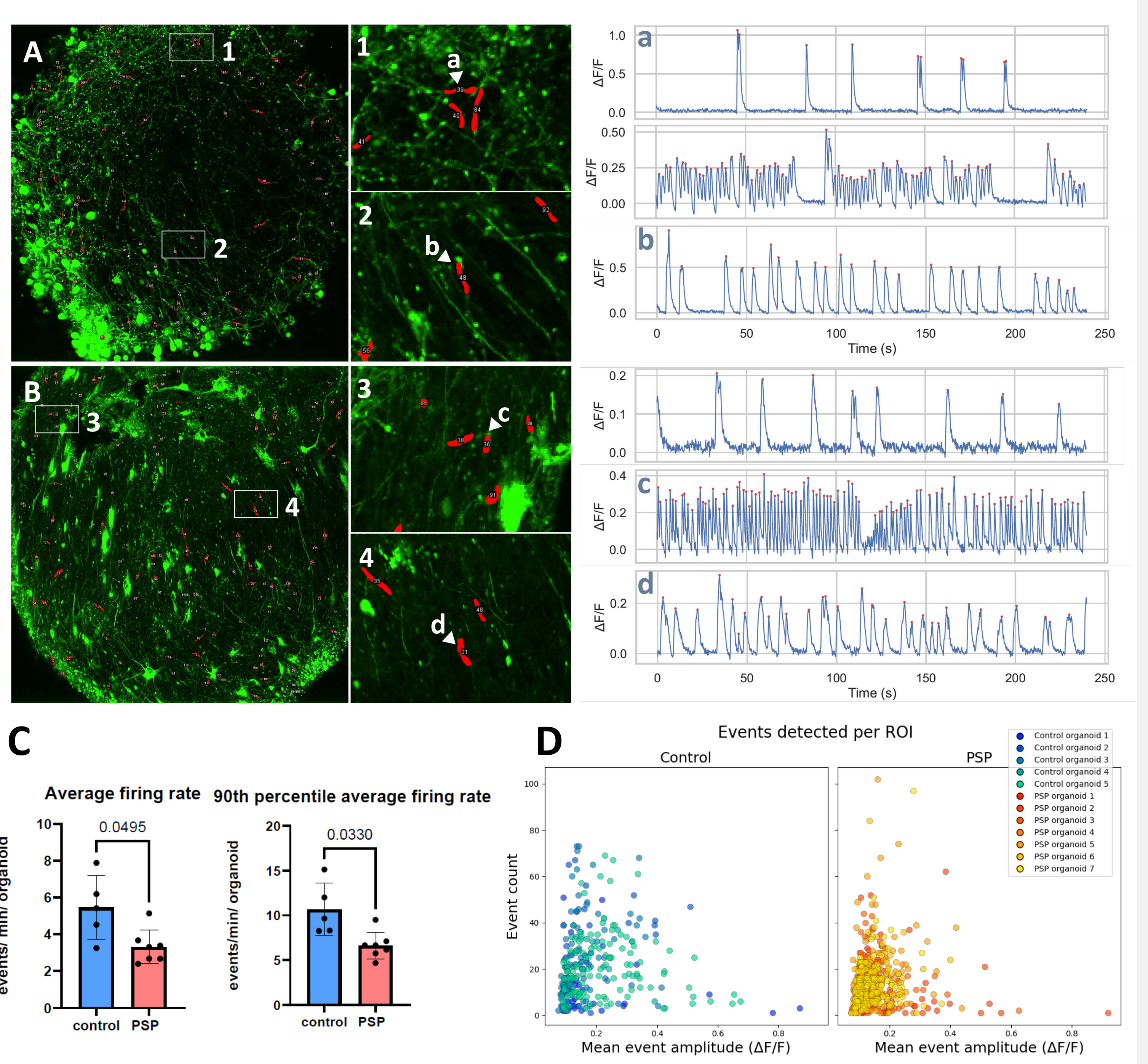


Figure 2: Organoids demonstrate functional difference based on donor disease status. (A) Maximum intensity projection (MIP) of a 4-minute time lapse image of one plane of a 327-day-old control organoid and (B) a 327-day-old PSP organoid. Red markings highlight manually selected ROIs for fluorescence extraction. Arrowheads represent representative ROIs selected for signal extraction with corresponding traces in right panel. Images were acquired on an Andor DragonFly spinning disk confocal microscope every 122.95 ms. Mean fluorescence at each slice was measured using FIJI. Following photobleaching correction, normalization, Savitzky-Golay smoothing and noise-adaptive event detection, events were identified and counted for further analysis. Red dots represent identified events. (C) Welch t-test demonstrates significant difference between PSP and control donor status at the organoid level in average firing rate per minute. This difference was stronger in the 90th percentile of all active ROIs. Duplicates were removed prior to analysis by filtering based on pairwise Pearson's correlation at 0.8 threshold and manual visualization of raw traces (D) Scatter plot of all ROIs used for analysis in (C) demonstrating that cells in PSP and control have a wide range of firing rates with comparable mean event amplitudes.

FUTURE DIRECTIONS

- Utilize collected single cell transcriptomics to obtain cell counts of neuronal types and identify populations differentially affected by donor status.
- Extend clearing and 3D microscopy to other donor lines to capture variability in disease phenotypes and track morphological changes over time.

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