



In Vitro Models Of Human Olfactory Neural Precursor Cells To Study Early Mechanisms Of Progressive Supranuclear Palsy

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Background

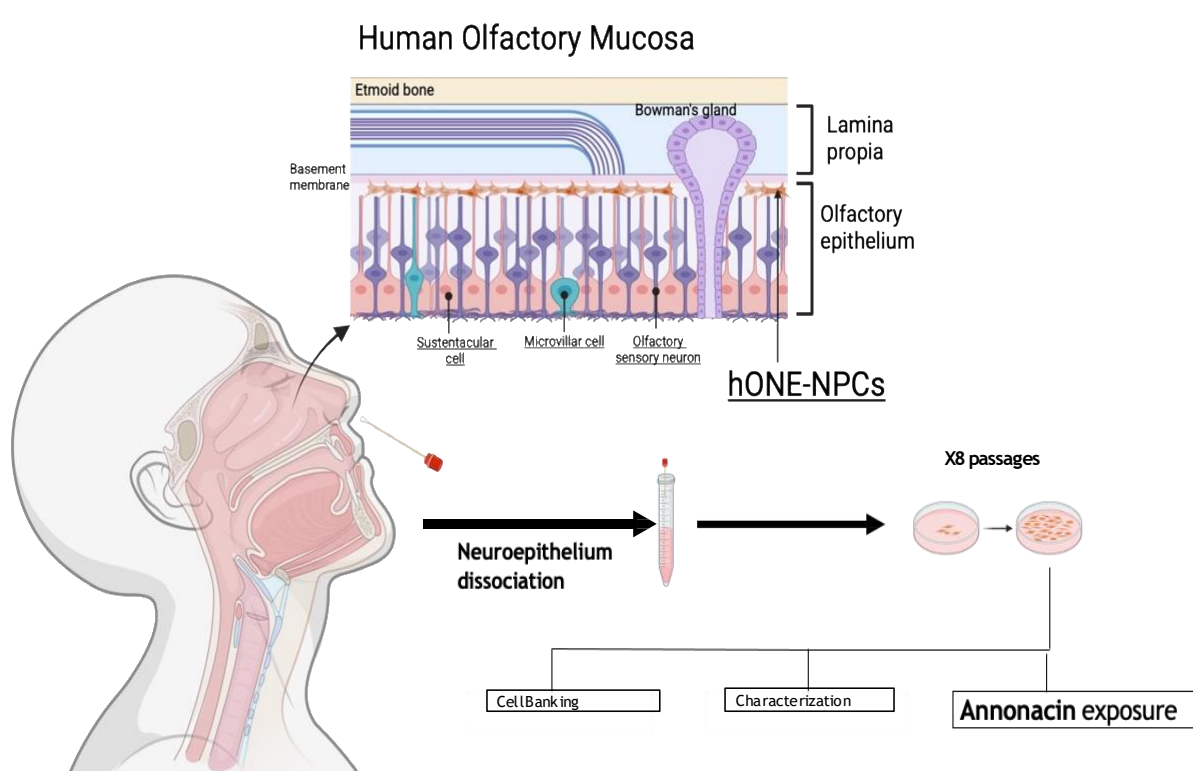
- PSP is a rare neurodegenerative tauopathy characterized by a predominance of **4R tau** and is mainly sporadic. The age of onset is in the 6th decade of life.
- It is commonly misdiagnosed as Parkinson's.
- The process of cellular reprogramming causes a reduction in the 4R tau expression in iPSCs-derived models.
- The treatment with Annonacin (ANN), a chemical compound present in the *Annonaceae* family of tropical fruit, has been used to increase 4R tau expression *in vitro*.
- The human olfactory neuroepithelium precursors cells (hONE-NPCs) are located near the olfactory bulb as a source of peripheral brain cells.
- The hONE-NPCs are undifferentiated clonogenic cells that possess the capacity of auto-renewal and have been used to model neurodegenerative and psychiatric diseases ^{1,3}.

Aim

- To establish *in vitro* models to study PSP disease through hONE-NPCs derived from patients affected with PSP and a model with pharmacologically induced tau 4R expression.

Methods

- The hONE-NPCs were obtained through nasal exfoliation of healthy donors without cognitive impairment and patients affected with PSP (Figure 1).



Exploratory Cohort

hONE-NPCs	n	Age mean ± SD	Sex (F/M)
Controls	5	64 ± 7.1	3/2
Patients	7	68.7 ± 7.7	4/3

Results

1. hONE-NPCs express pluripotency, neural stem and proliferation markers

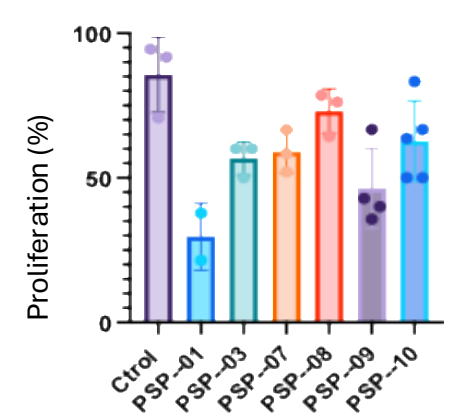


Fig. 2 Phenotypic characterization hONE-NPCs. Neural progenitors (GFAP, olig1, Nestin, 8III-tubulin and Sox2) proliferation (ki67) and pluripotency (Oct-3/4, Nanog) by immunofluorescence and/or Western blot. For the IF the nucleus are shown in blue (DAPI). Scale bar equals 20 µm. The graphics shown proliferation percentage of the different hONE-NPCs.

3. Patient-Derived hONE-NPCs Recapitulate Key Pathological Features of PSP

Enhanced Tau Oligomerization and Pathological Phosphorylation in PSP hONE-NPCs

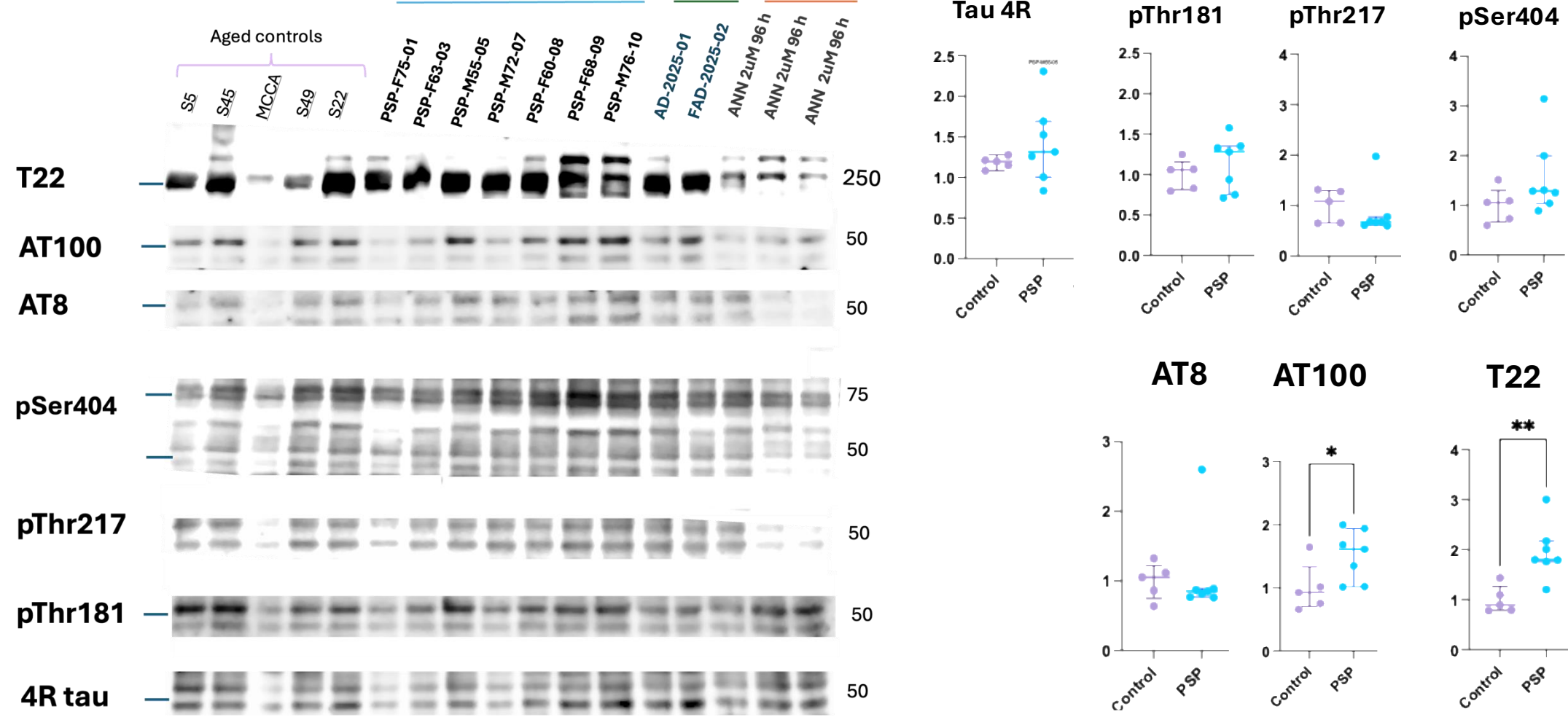


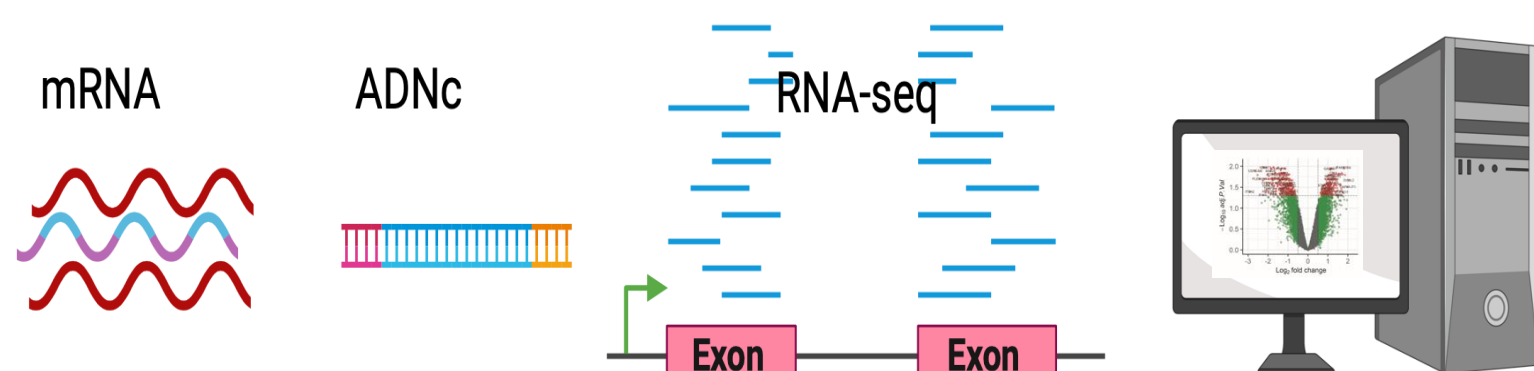
Fig. 5 Detection of 4R tau expression, phosphorylation, and oligomer formation in primary hONE-NPC cultures derived from PSP patients and densitometric quantification. AT8 (Ser202/Thr205); AT100 (Thr212/Ser214) and T22 recognize oligomeric formation. Data are presented as individual values, median, and interquartile range. Statistical analysis was performed using the Mann–Whitney test for PSP versus control comparisons.

Conclusions

- PSP-derived hONE-NPCs retained neural progenitor characteristics while displaying disease-associated alterations in morphology, proliferation, and marker localization.
- Both PSP-derived and ANN-treated hONE-NPCs recapitulated key molecular features of PSP and tauopathy, including alterations in PERK, Nrf2/pNrf2, SLCO1A2, 4R tau expression, tau hyperphosphorylation, and oligomerization, supporting their utility as minimally invasive patient-derived models for studying PSP pathophysiology.

Future work

Future studies will focus on identifying differentially expressed genes (DEGs) associated with pharmacologically induced 4R tau expression in the annonacin-based hONE-NPC model and in PSP-derived hONE-NPCs through transcriptomic and bioinformatic analyses, with the aim of elucidating molecular pathways involved in PSP pathogenesis.



2. ANN induce the overexpression of 4R tau and shown physiopathology characteristics of tauopathy in hONE-NPCs S49 at 2 µM for 96 h

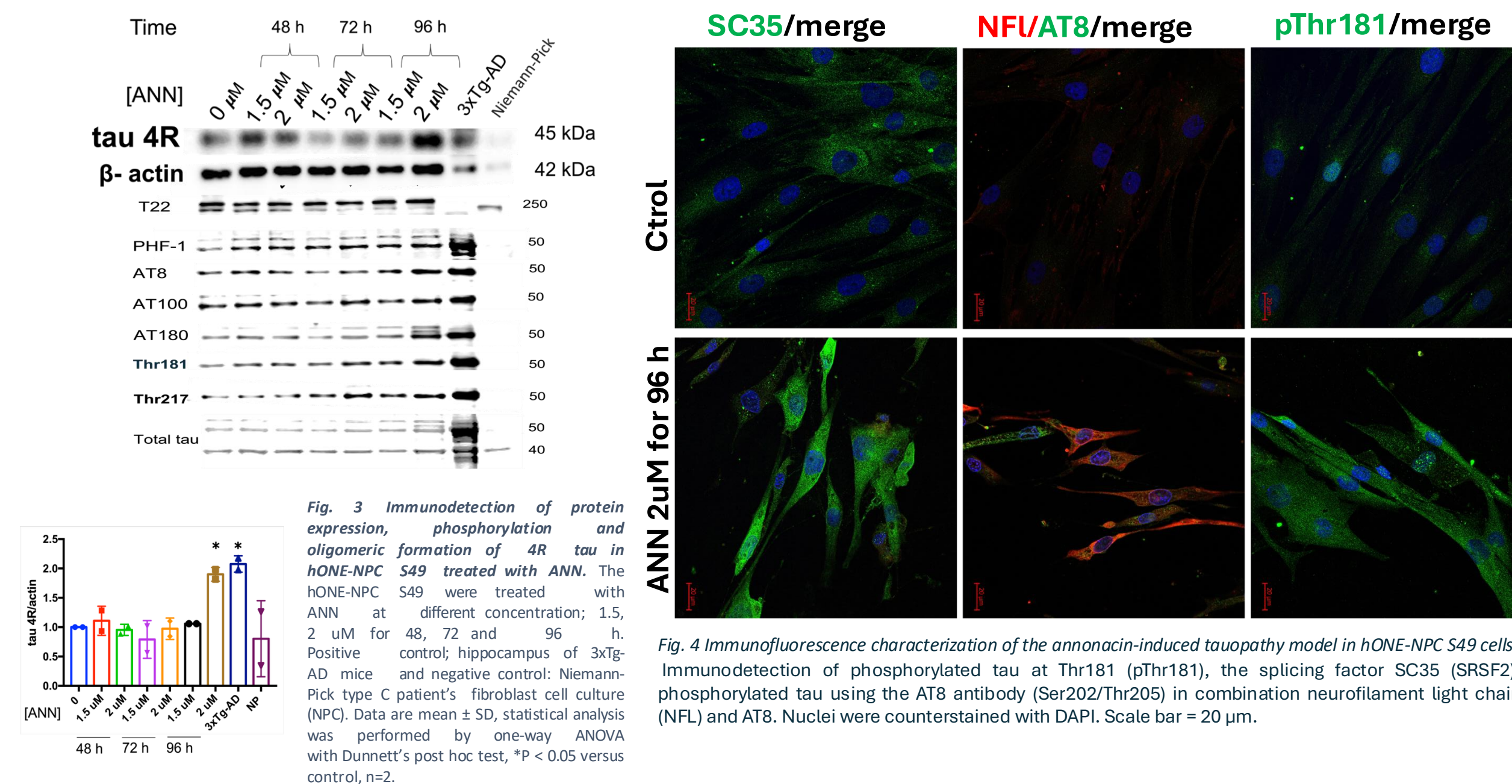


Fig. 3 Immunodetection of protein expression, phosphorylation and oligomeric formation of 4R tau in hONE-NPC S49 treated with ANN. The hONE-NPC S49 were treated with ANN at different concentration: 1.5, 2 µM for 48, 72 and 96 h. Positive control: hippocampus of 3xTg-AD mice and negative control: Niemann-Rick type C patient's fibroblast cell culture (NPC). Data are mean ± SD, statistical analysis was performed by one-way ANOVA with Dunnett's post hoc test, *P < 0.05 versus control, n=2.

Fig. 4 Immunofluorescence characterization of the annonacin-induced tauopathy model in hONE-NPC S49 cells. Immunodetection of phosphorylated tau at Thr181 (pThr181), the splicing factor SC35 (SRP2), phosphorylated tau using the AT8 antibody (Ser202/Thr205) in combination neurofilament light chain (NFL) and AT8. Nuclei were counterstained with DAPI. Scale bar = 20 µm.

PERK Pathway Activation and Increased SLCO1A2 Expression in PSP hONE-NPCs

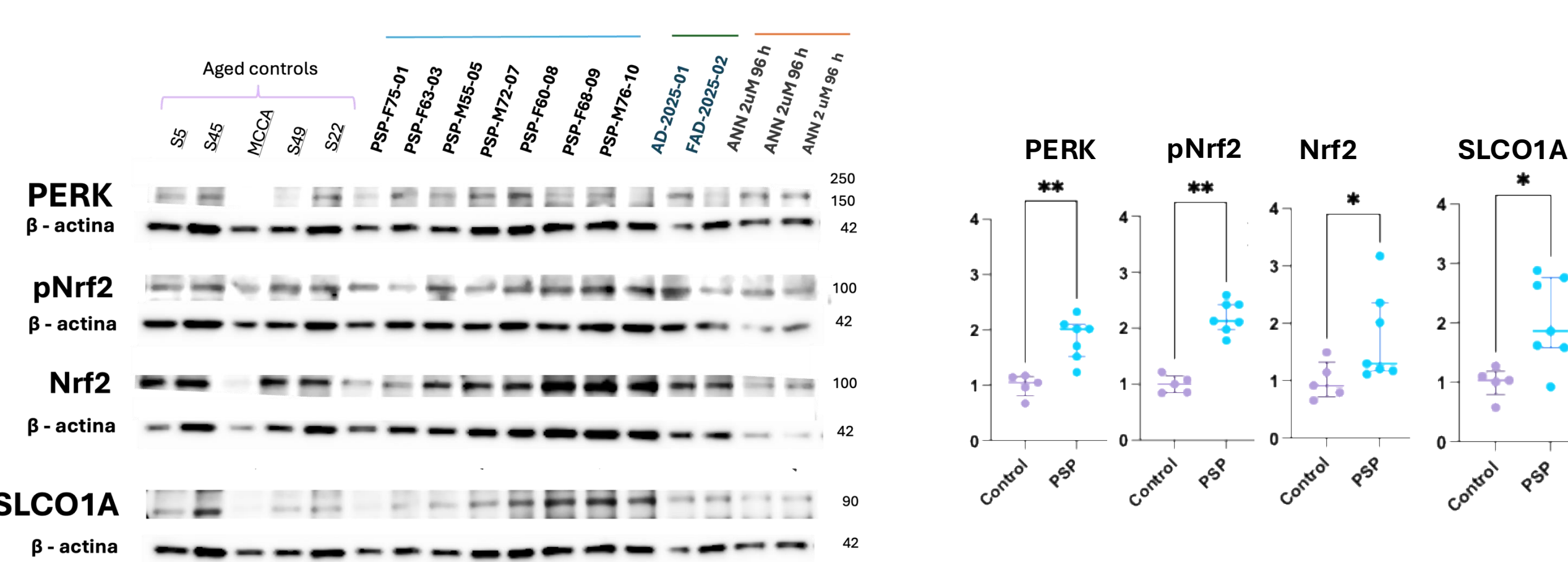


Fig. 6 Expression of proteins associated with the PERK–Nrf2 axis in hONE-NPCs and SLCO1A2 derived from PSP patients and densitometric quantification of proteins normalized to β-actin. Data are presented as individual values, median, and interquartile range. Statistical analysis was performed using the Mann–Whitney test for PSP versus control comparisons.

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