



Frontotemporal Dementia Causing P301S Mutation Alters Non-phosphorylated Tau Redistribution In The Nucleolus In SH-SY5Y Cells

Mohammad Nasser, Zaid Muhammad, Mahmoud Bukar Maina
Sussex Neuroscience, University of Sussex, United Kingdom

Introduction

- Tauopathies are defined by pathological tau aggregation; however, early subcellular events preceding overt aggregation remain poorly understood.
- The nucleolus regulates ribosomal biogenesis and is highly sensitive to cellular stress. Emerging evidence suggests tau can localise to the nucleus and nucleolus, potentially disrupting nucleolar homeostasis.
- The P301S MAPT mutation has been associated with altered protein dynamics, but its impact on early nuclear and nucleolar redistribution of non-phosphorylated tau (nP-Tau) remains unclear.
- We hypothesised that mutant tau drives early nucleolar accumulation of nP-Tau prior to overt structural nucleolar stress.

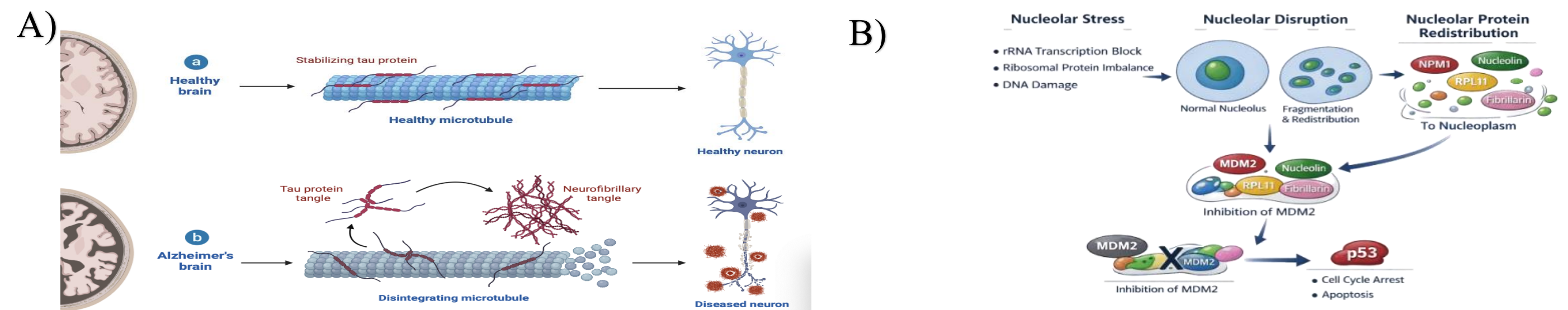


Figure 1. A) Tau Pathology and B) Nucleolar Stress Signalling Pathway

Methods

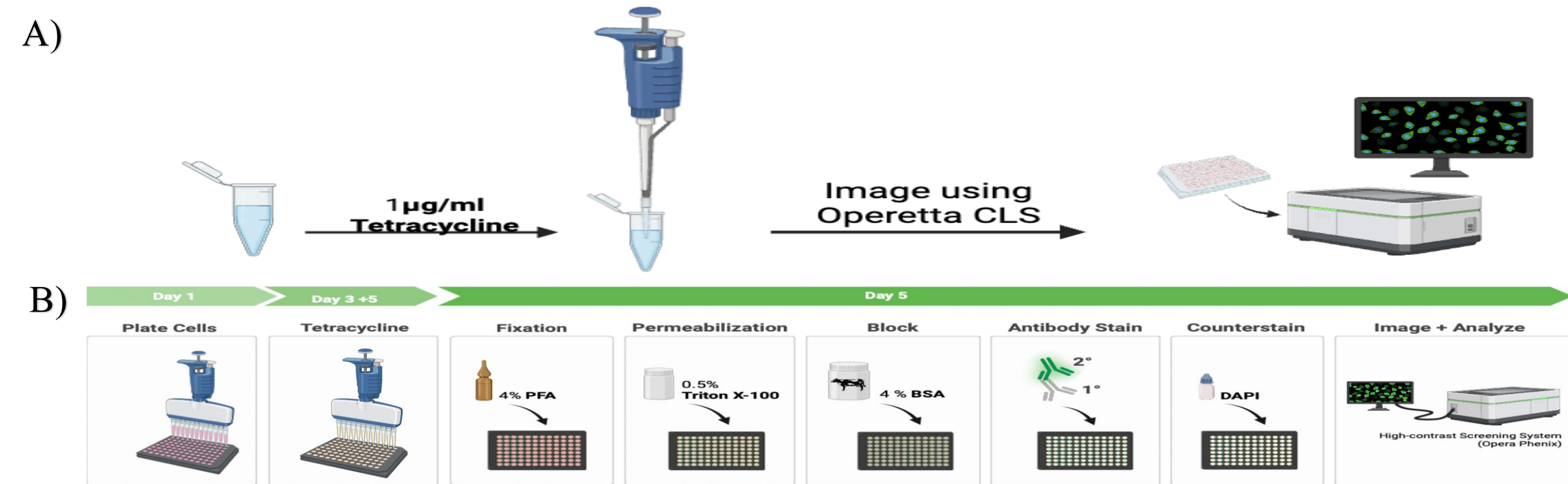


Figure 2. A) Tetracycline-Induced 4R Tau Activation and B) Immunofluorescence Imaging Workflow

- Tetracycline-inducible 4R-P301S SH-SY5Y model activated (1 µg/mL; 1 h & 48 h) to capture early tau-driven stress responses.
- High-content immunofluorescence imaging (Operetta CLS) following fixation and compartment-specific staining for 4R Tau, nP-Tau (Tau-1), and nuclei (DAPI).
- Automated 3D segmentation enabled quantitative analysis of cytoplasmic, nuclear, and nucleolar tau intensity, alongside nucleolar morphometry.

Results - Validation of 4R Tau Inducible Model

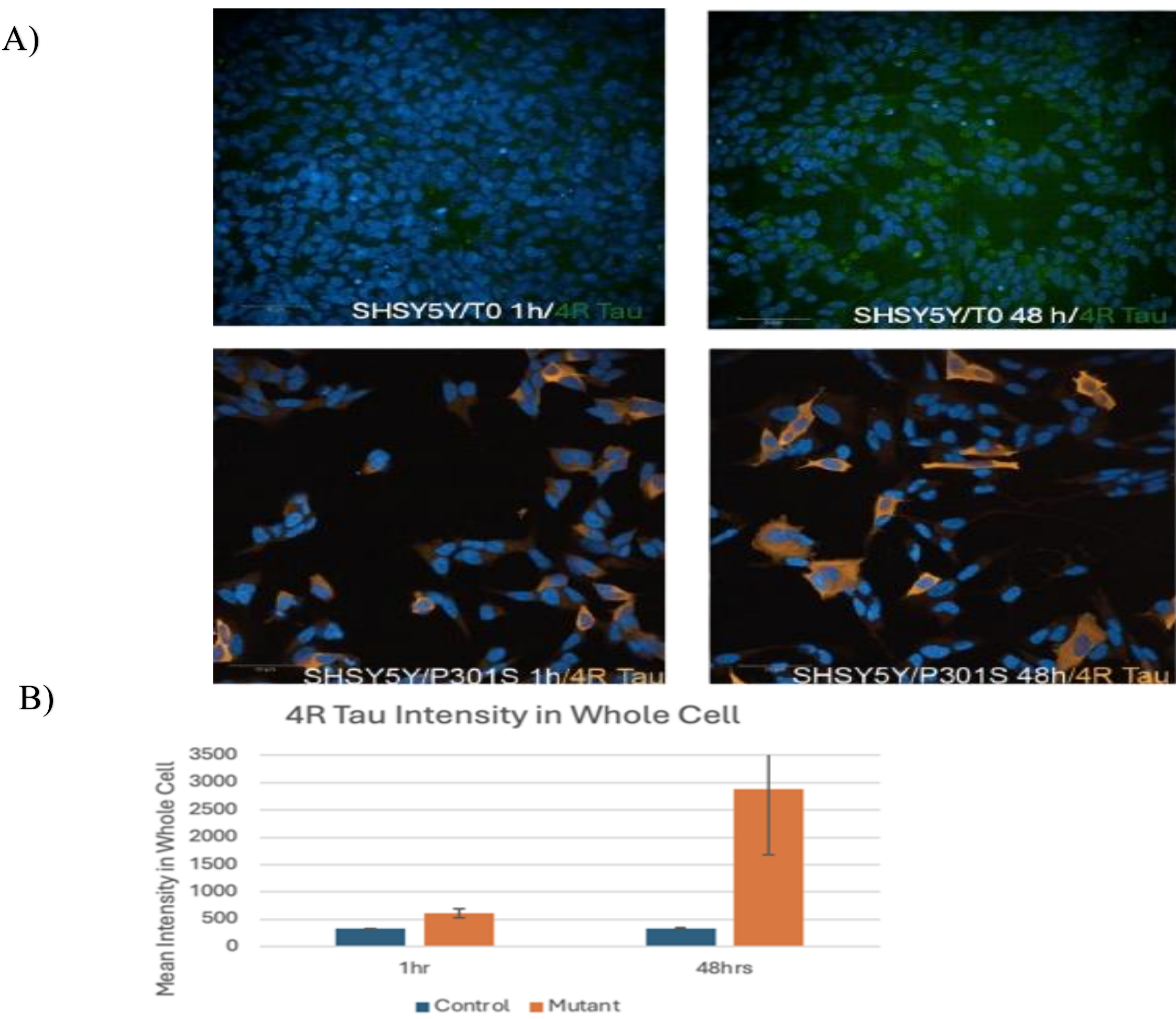


Figure 3. Validation of inducible 4R-P301S tau model.

(A) Representative immunofluorescence images of SH-SY5Y cells at 1h and 48h post-tetracycline induction (1 µg/mL), showing 4R Tau expression in control (T0) and P301S cells. (B) Quantification of whole-cell 4R Tau intensity confirms significant upregulation in P301S cells at 1 h and 48 h.

- Tetracycline induction significantly increased 4R Tau expression as early as 1 hour ($p < 0.001$).
- Expression levels remained elevated at 48 hours.
- Confirms robust and rapid activation of the inducible P301S model.

Results – Early Nuclear and Nucleolar Distribution of np-Tau

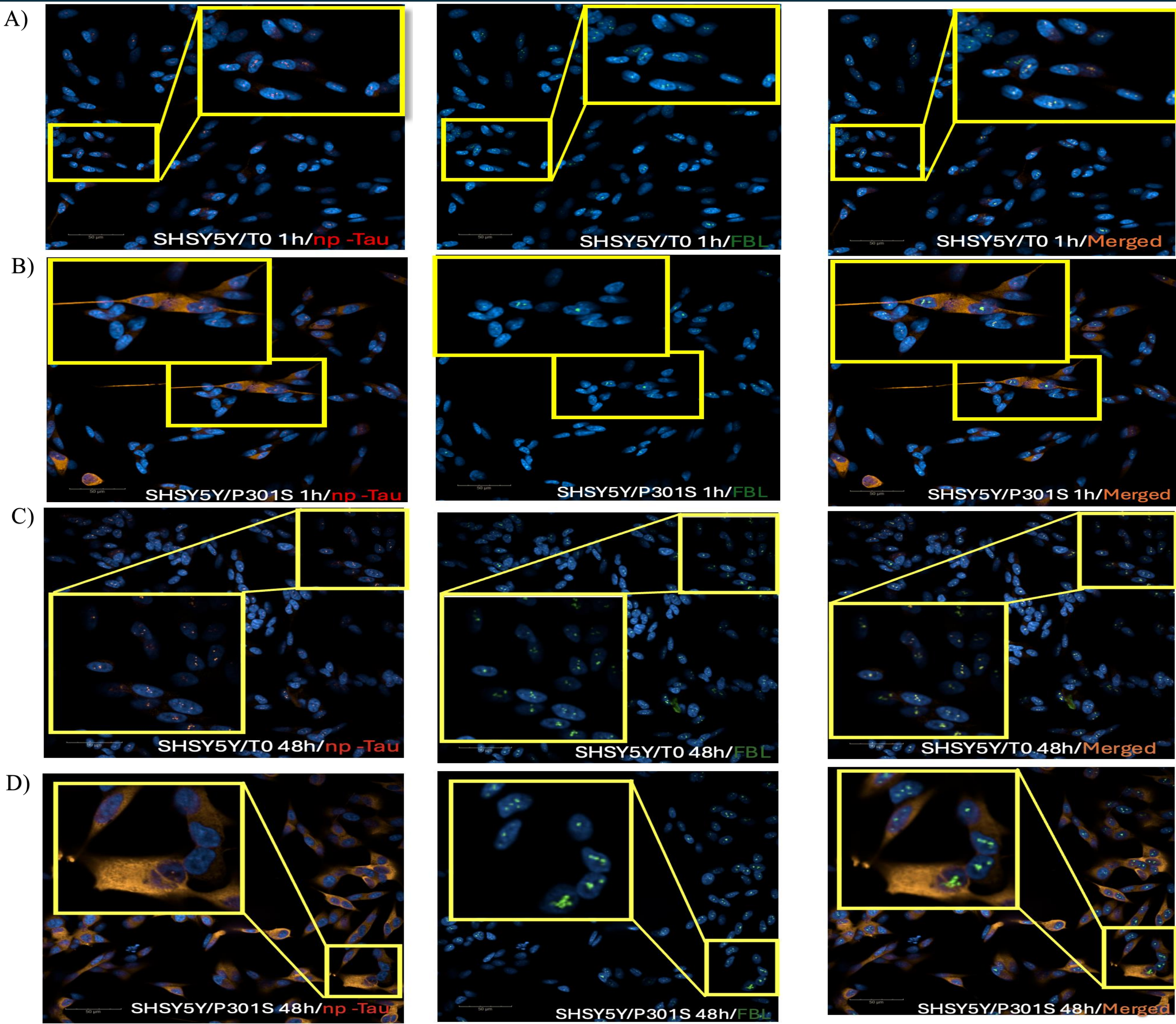


Figure 4. P301S tau promotes early nuclear and nucleolar redistribution of nP-Tau.

(A–D) Representative immunofluorescence images of SH-SY5Y cells: (A) T0 1 h, (B) P301S 1 h, (C) T0 48 h, (D) P301S 48 h following tetracycline induction (1 µg/mL). nP-Tau (red), fibrillarin (green), DAPI (blue). Insets highlight increased nuclear and nucleolar nP-Tau in P301S cells. Graphs (E–H) quantify nucleolar morphology and compartment-specific nP-Tau intensity

- nP-Tau intensity significantly increased in the nucleus at 1h and 48h ($p < 0.01$).
- nP-Tau accumulation was also elevated within nucleolar compartments at both time points.
- Nucleolar size remained unchanged.
- A non-significant trend toward reduced nucleolar number was observed.

Conclusion

- Mutant tau expression rapidly induces nuclear accumulation of nP-Tau.
- nP-Tau progressively enriches within nucleolar compartments.
- Redistribution occurs prior to overt morphological nucleolar stress.
- Nuclear and nucleolar nP-Tau accumulation may represent an early molecular event in tau-associated cellular stress

Acknowledgments