

Investigating the responses of human iPSC-derived microglia to fibrillar tau

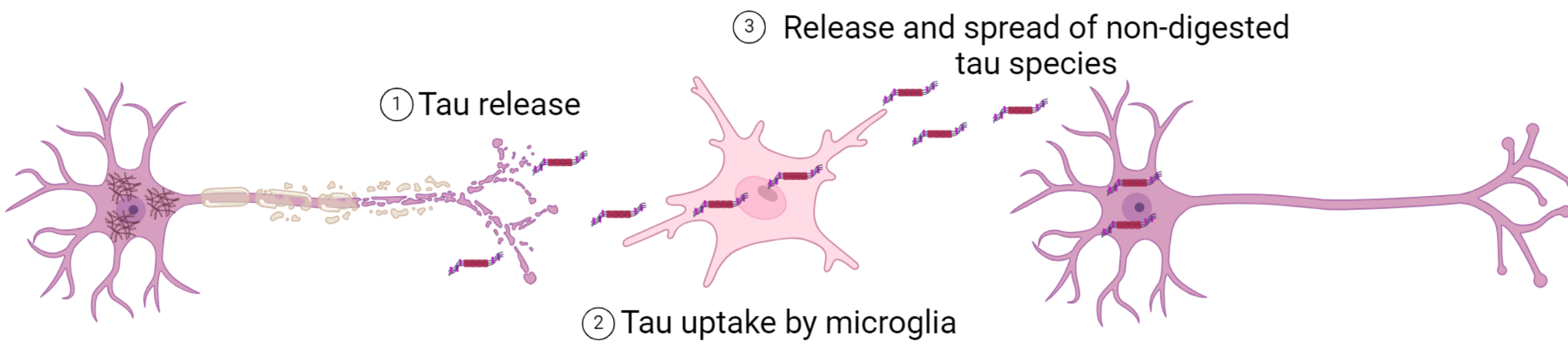
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1. Background and aims



In tauopathies, tau protein becomes hyperphosphorylated and loses its ability to interact with microtubules, leading to tau mislocalisation and accumulation in neurons. These tau aggregates can be transferred to synaptically connected neurons, triggering seeded tau aggregation in neighbouring neurons. Microglia, the brain-resident macrophages, are responsible for surveying the brain parenchyma to remove dying neurons and tau protein aggregates. However, the precise mechanisms of tau uptake, processing, and spread of tau by human microglia remain largely unexplored.

2. LRP1- and HSPG-mediated tau internalisation by iMGL

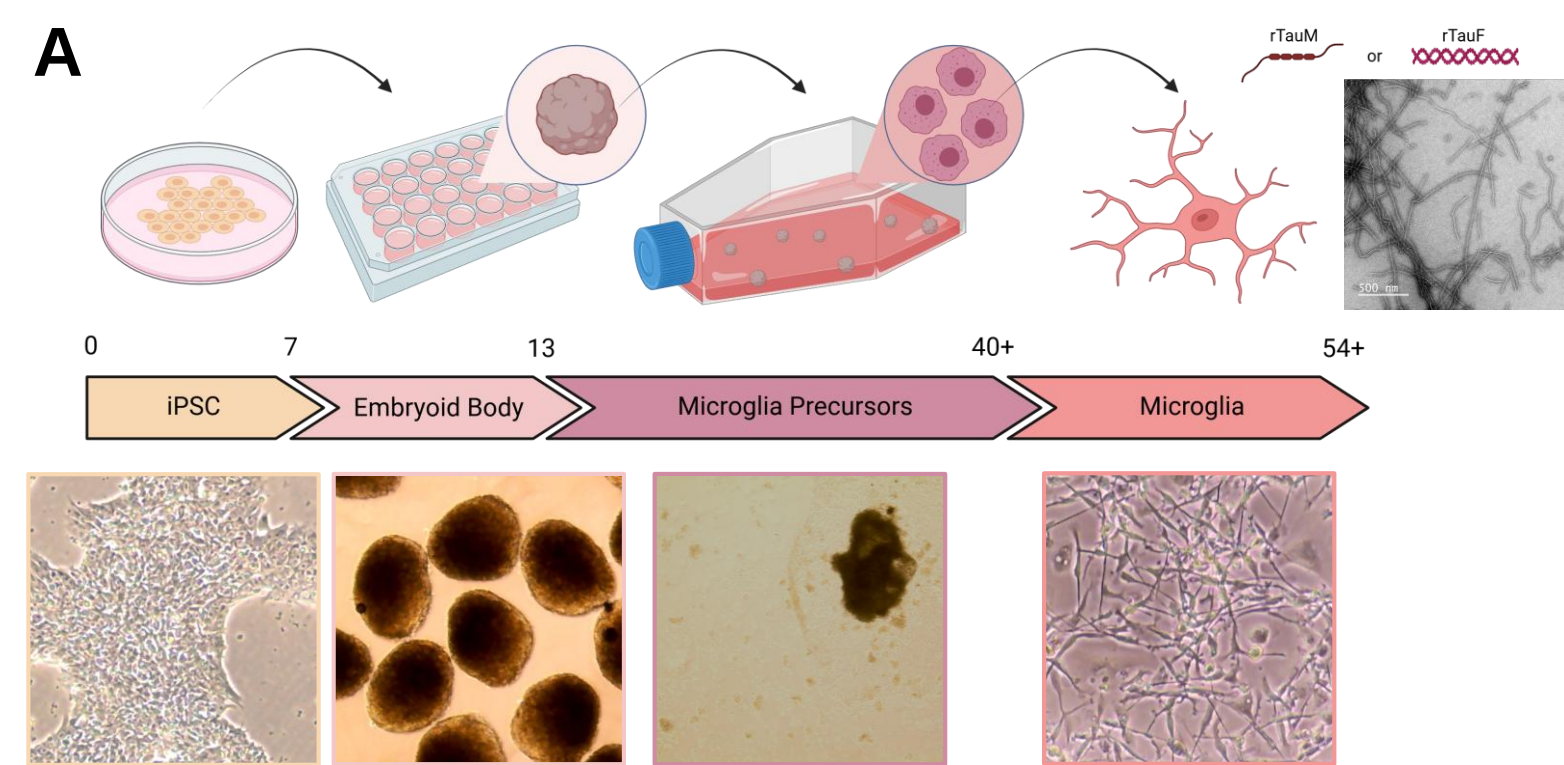
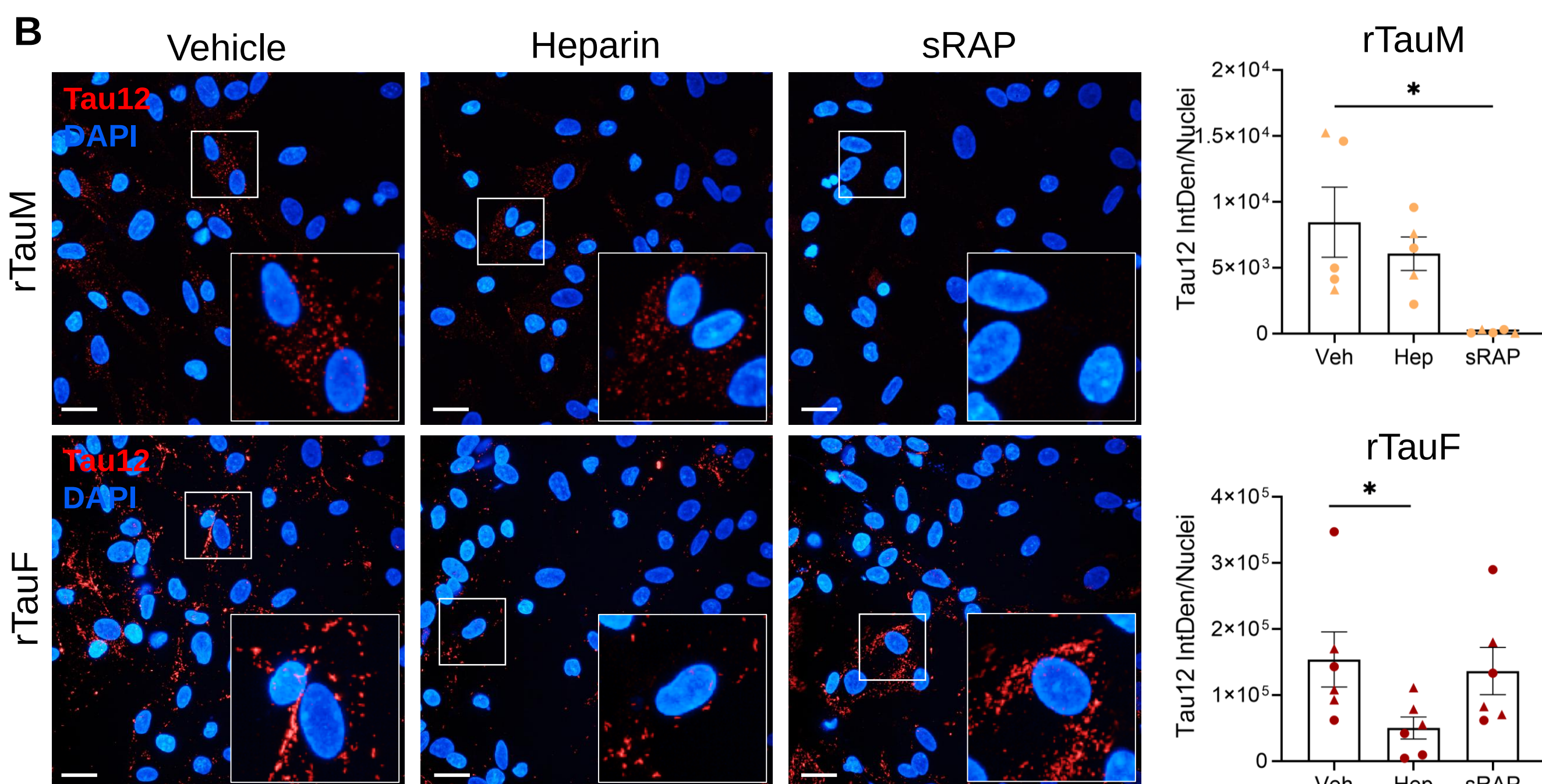


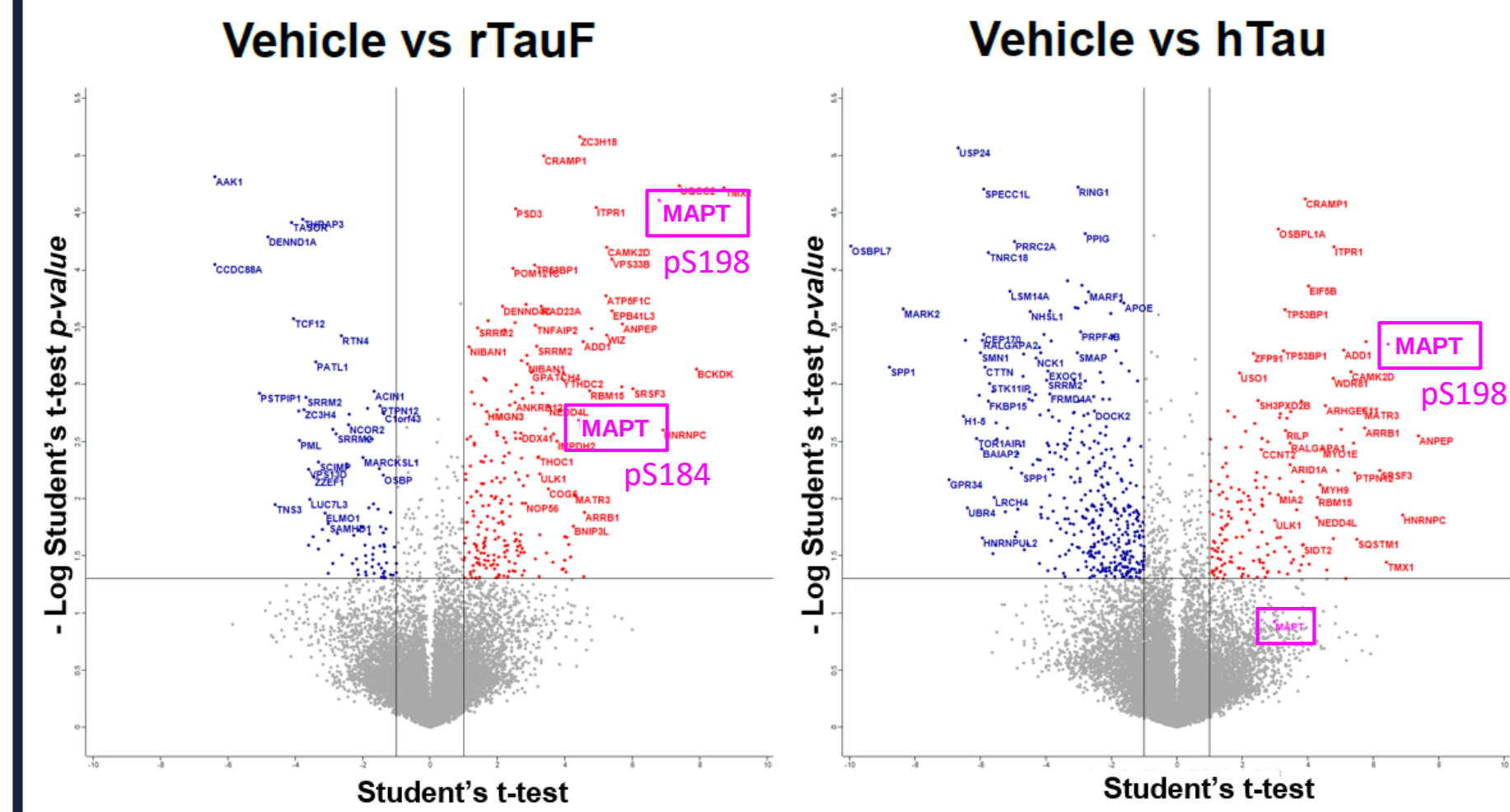
Image adapted from Washer et al., 2022. *Sci Rep*

A. iMGL were differentiated following the protocol by Washer et al., 2022, and treated with endotoxin-free recombinant 2N4R tau.

B. iMGL incubated with heparin (10 µg/mL) or sRAP (500 nM) for 1 h and challenged with 1 µg/mL rTauM or rTauF for 2 h were immunostained with anti-Tau12 antibody to measure tau internalisation. Scale bar: 20 µm.

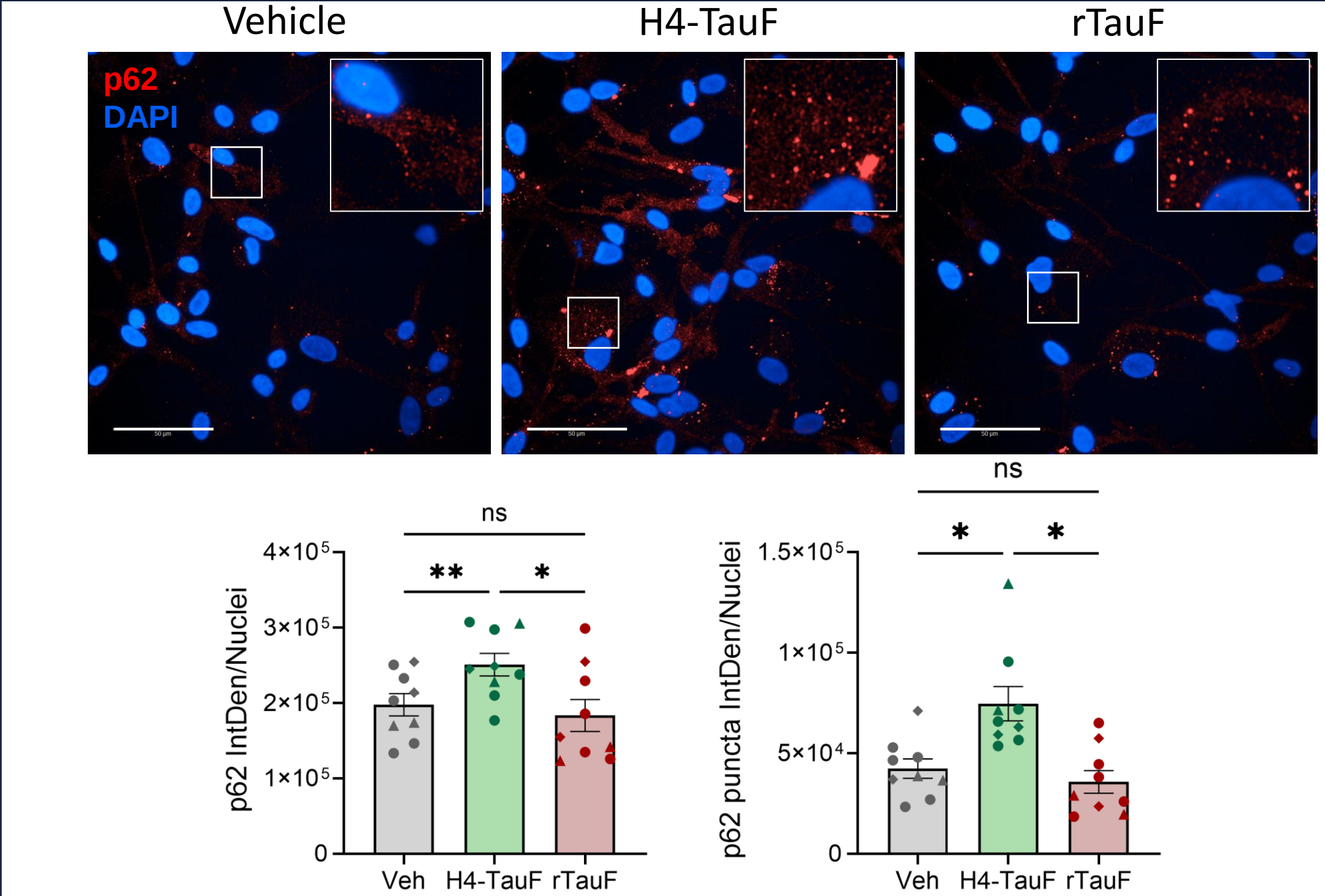


3. Tau-treated microglia undergo extensive phosphoproteome remodelling



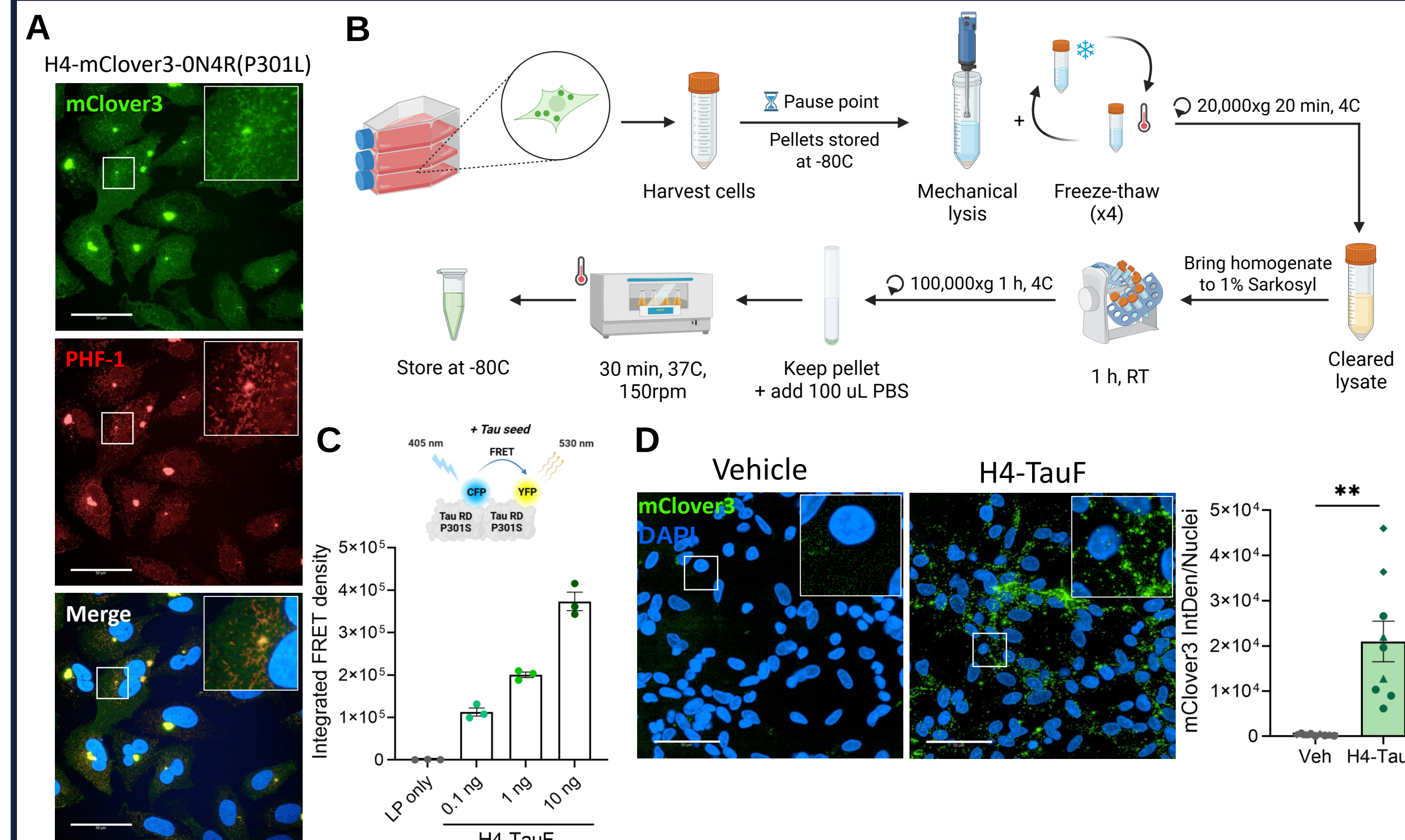
Differentially expressed phosphopeptides in iMGL lysates treated with rTauF or brain-derived tau (hTau) identified by LC-MS/MS.

5. H4-TauF promotes p62 accumulation in iMGL



p62 immunostaining of iMGL treated overnight with H4-TauF or rTauF (5 µg/mL). Cells were fixed after 24 h clearance. Scale bar: 50 µm.

4. Isolation of fibrillar tau from mammalian cells



A. H4 cells expressing permanent tau aggregates. Scale bar: 50 µm.

B. Workflow for purification of fibrillar tau from cultured H4 cells.

C. Isolated fibrils are seed-competent. Tau RD P301S FRET biosensor cells were used to assess the seeding competency.

D. iMGL internalised H4-derived tau fibrils (H4-TauF). 24 h tau treatment. Scale bar: 50 µm.

6. Conclusions

- **Monomeric tau** internalisation by iMGL is mediated by **LRP1**
- **Fibrillar tau** is partially internalised by **HSPG**.
- iMGL **phosphoproteome** undergoes extensive remodelling, including proteins associated with **cytoskeleton dynamics, autophagy** and **vesicle sorting**.
- iMGL directly phosphorylate tau at **S184** and **S198**.
- **H4 cells** expressing aggregated mClover3-ON4R P301L Tau are an alternative source of **endotoxin-free seed-competent tau**.
- **H4-TauF**, but not rTauF, promotes the **accumulation of p62** in iMGL, suggesting proteasomal or autophagy alterations.

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Karabova M.K., del Ser-Badia A., Hedegaard A., et al. Tracking tau and cellular responses in human iPSC-microglia: from uptake to seedable secretion, including in extracellular vesicles. *Alzheimer's Dement* (2026).

Washer, S.J., Perez-Alcantara, M., Chen, Y. et al. Single-cell transcriptomics defines an improved, validated monoculture protocol for differentiation of human iPSC to microglia. *Sci Rep* (2022).
Longhini, A.P., DuBose, A. et al. Precision proteoform design for 4R tau isoform selective templated aggregation. *Proc Natl Acad Sci* (2024).