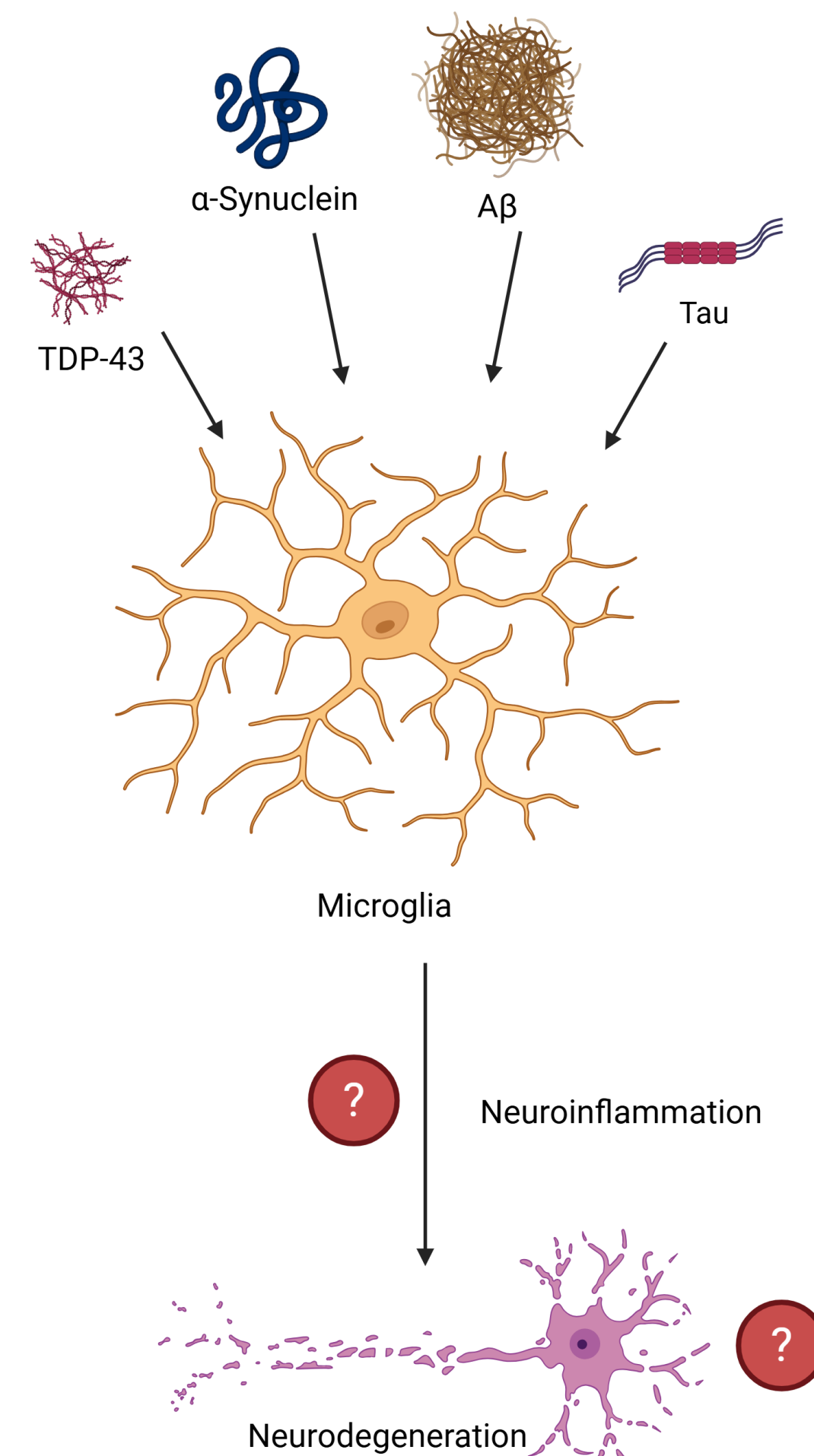


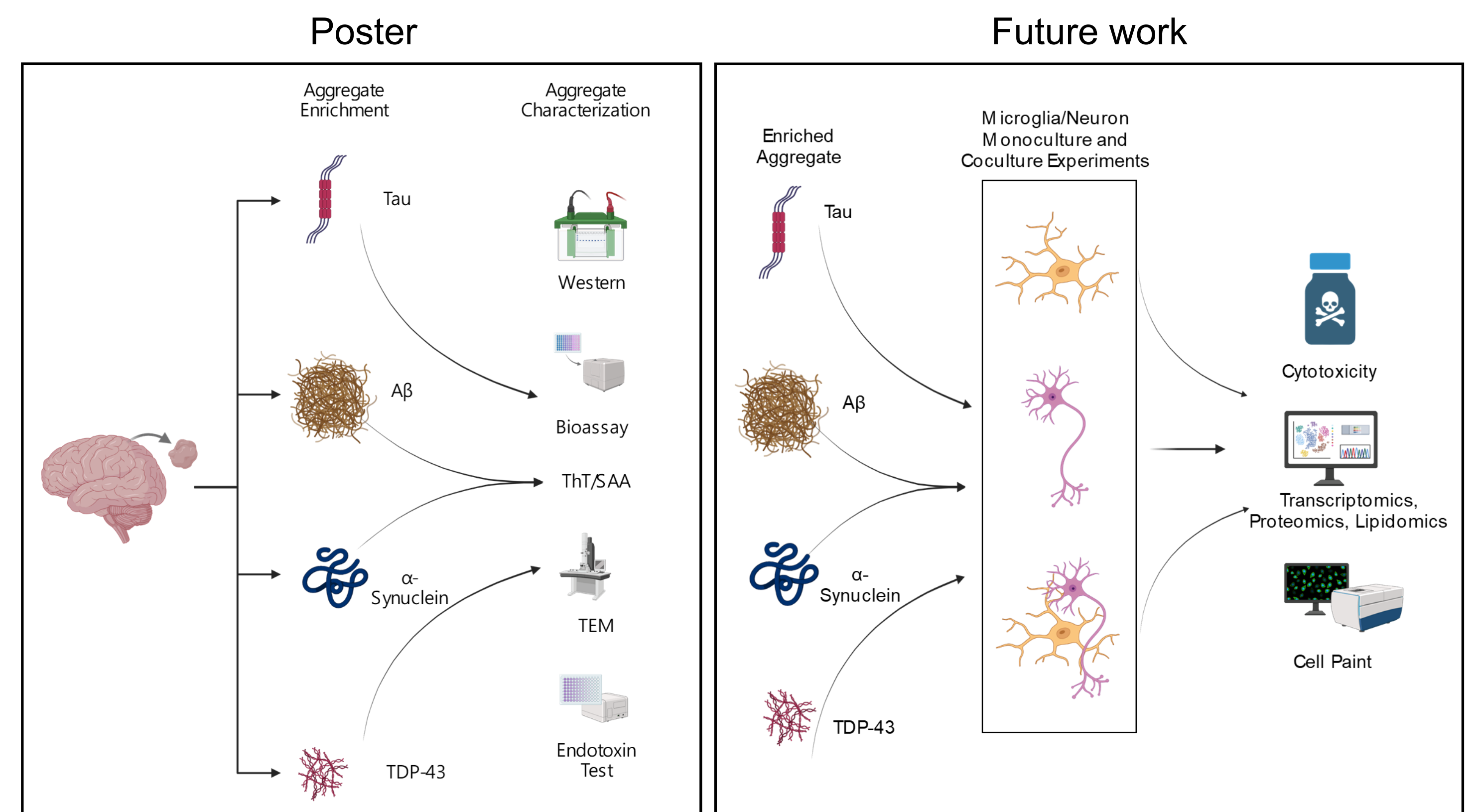
Introduction

- Neuroinflammation plays an important role in the development and progression of neurodegenerative disorders.
- Genome-wide association studies indicate that many inflammatory genes associated with Alzheimer's Disease are exclusively expressed in microglia.
- AD is characterized by the accumulation of extracellular amyloid- β (A β) plaques and intracellular neurofibrillary tau tangles.
- Clinical evidence suggests more than 50% of AD patients also show accumulation of TDP-43, while approximately 33% exhibit accumulation of α -synuclein.
- The project aims to investigate how this concomitant pathology affects microglia.
- The poster shows the enrichment and characterization of Amyloid- β , tau, TDP-43 and α -Synuclein from patient brain tissue.



Experimental Overview

- Enrichment and characterization of Amyloid- β , Tau, TDP-43 and α -synuclein from brain tissue of patients in the Oxford Project to Investigate Memory and Ageing (OPTIMA) cohort.
- Future work—Challenge microglia with all 4 enriched aggregates in different combinations and perform multiomics to study neuroinflammatory mechanisms and identify possible drug targets.



Results

Fig 1. Western blot confirms the enrichment of aggregates in the final preps. The protocol involves multiple washing steps in a sarkosyl buffer. The final pellet is the enriched aggregate as sarkosyl insoluble fraction. S1-S4 - supernatant from different steps of the protocol, Pellet - final prep resuspended in PBS.

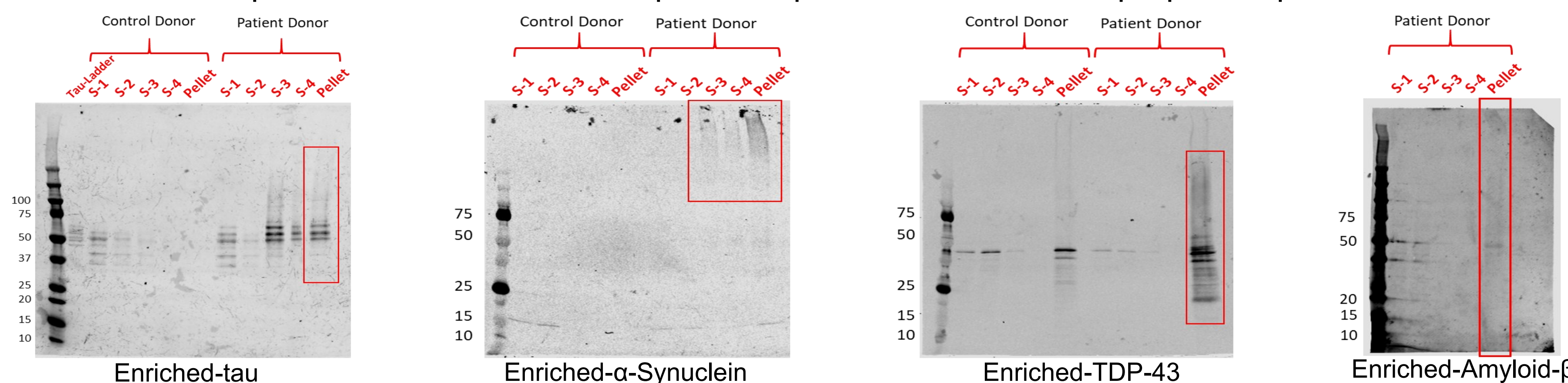


Fig 2. TEM confirms the presence of aggregates in the final prep. Amyloid- β and tau were enriched from AD, TDP-43 from FTLT and α -synuclein from DLB patient's brain tissue.

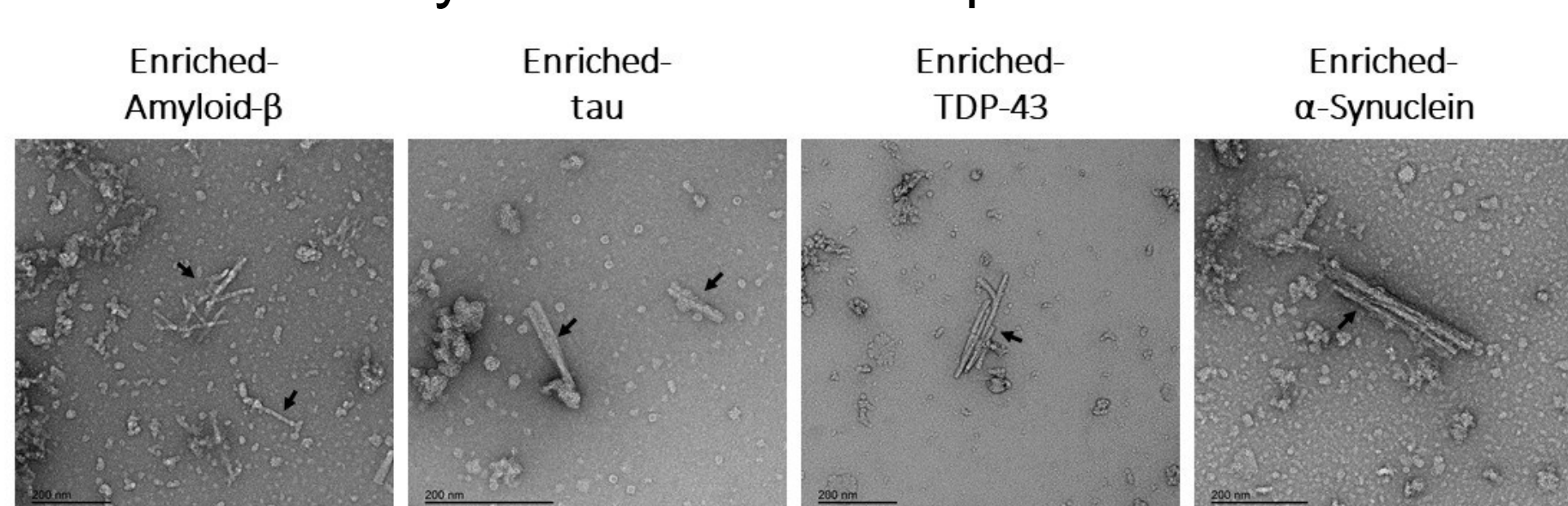


Fig 3. MSD assay reliably quantifies enriched-A β and tau in final preps.

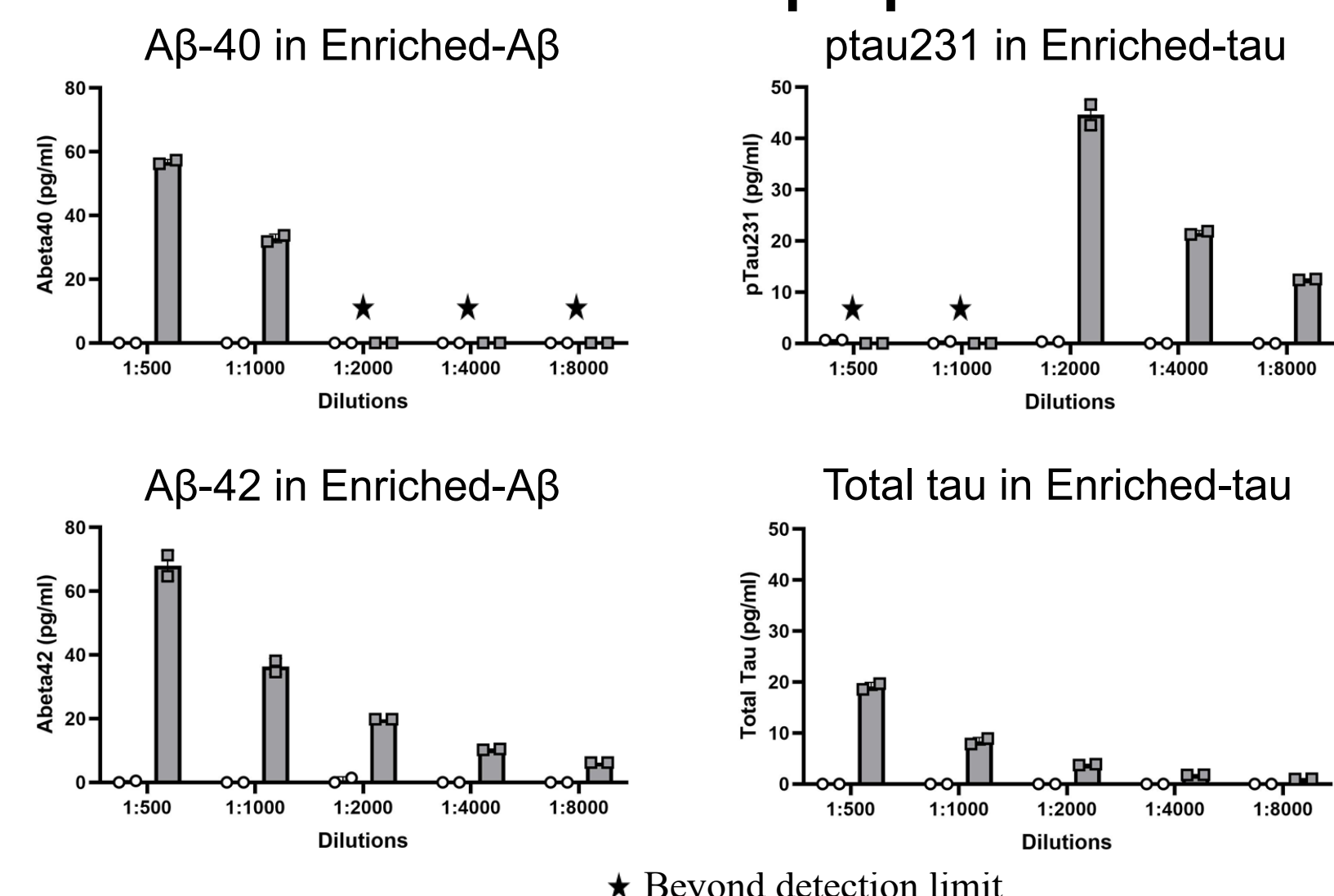


Fig 4. Enriched aggregates are not cytotoxic to microglia at up to 1.2ug/ml of total protein. Microglia were treated with 0.3ug/ml of total protein and LDH assay was performed to assess cytotoxicity

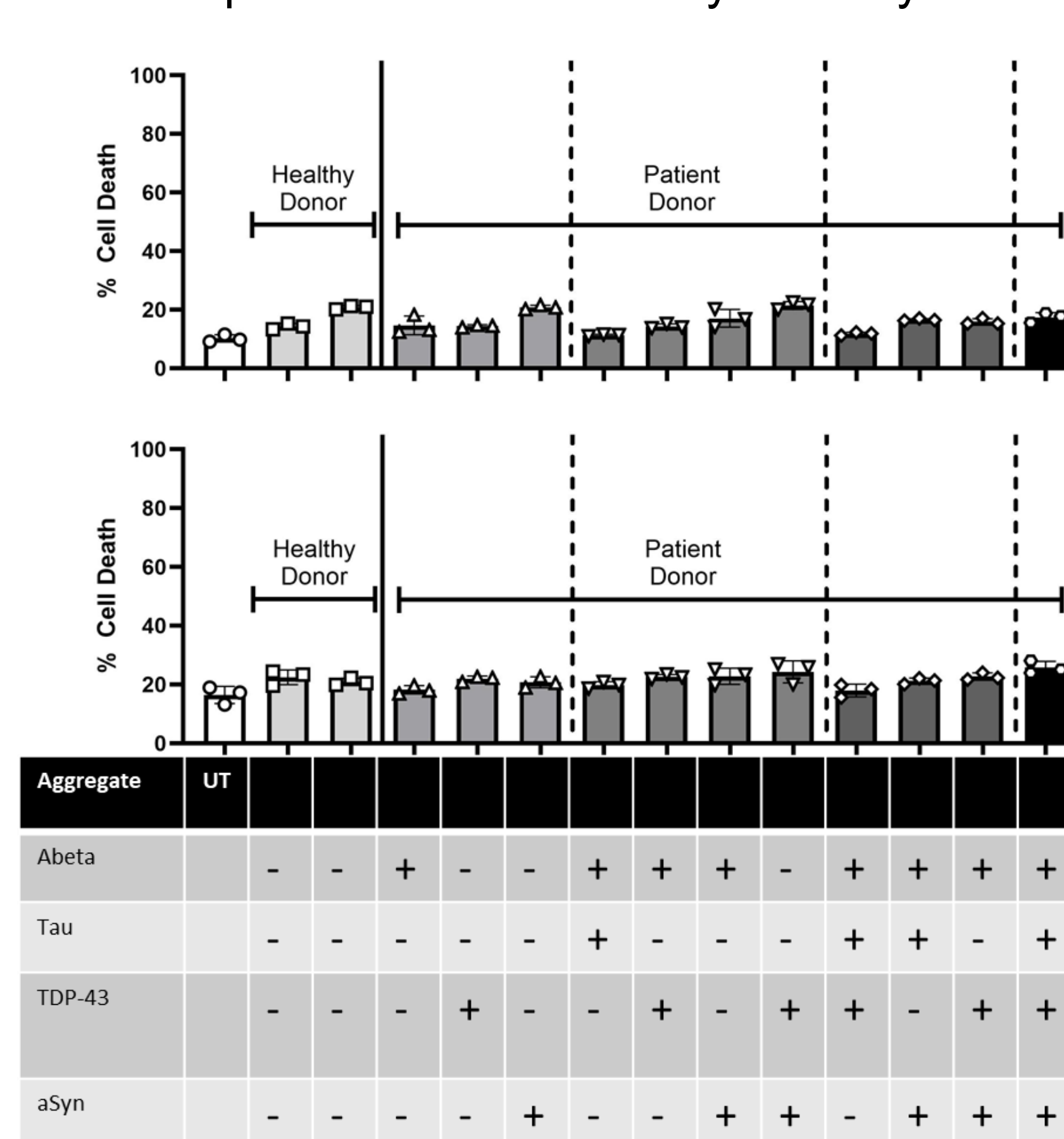
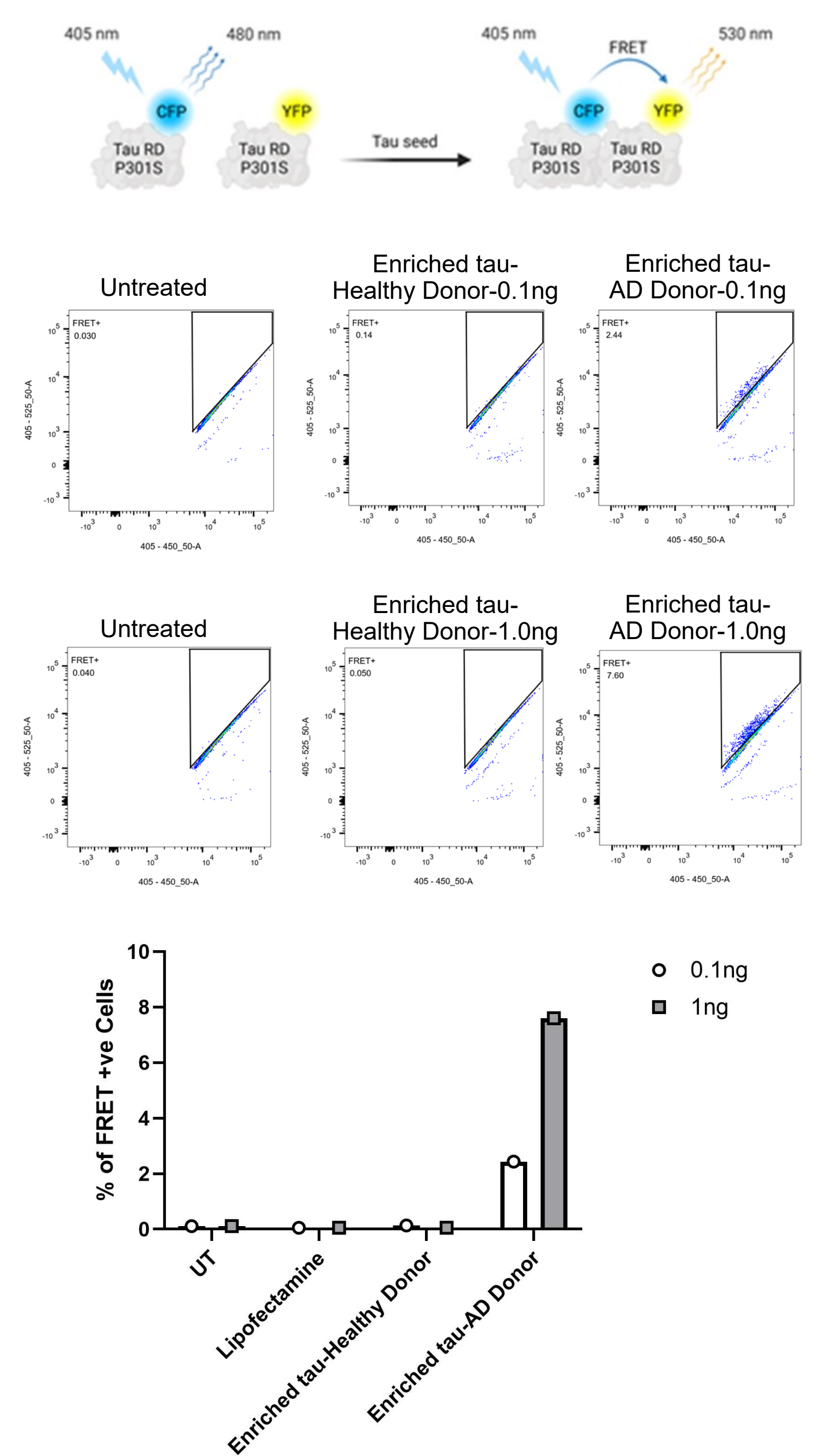


Fig 5. Seeding FRET Bioassay of enriched-tau. The assay shows dose dependent seeding by enriched-tau. (0.1, 1.0ng of total protein)



Conclusions

- Standardized enrichment of seed-competent aggregates from small scale tissue samples.
- pTau231 was enriched to a greater extent than total tau, demonstrating the robustness of the enrichment process, as hyperphosphorylated tau is more aggregation-prone and more relevant to disease pathology.
- MSD assay is most reliable and reproducible for quantifying Amyloid- β and Tau. (Comparison of several suppliers' ELISA kits shows them to give widely divergent results for the same samples).
- The enriched aggregates were not toxic to microglia at concentrations up to 1.2 μ g/ml of total protein in combination, or 0.3 μ g/mL of total protein when tested individually.

Future Directions

- Perform bioassays and ThT assays on all aggregates to assess their seeding capability.
- Challenge microglia with combinations of enriched aggregates for multi-omics analysis.
- Cell paint to investigate the localization of different aggregates and the organelles affected by them.