

How Heteroamyloids Between Tau And RNA Binding Proteins Can Influence Toxicity And Disease Progression In Alzheimer's Disease

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Alzheimer's disease (AD) is the most common age-related neurodegenerative disorder, characterized by the accumulation of amyloid- β plaques and neurofibrillary tangles composed of hyperphosphorylated Tau. Among Tau species, the smaller, **soluble oligomers** are now recognized as the most neurotoxic, with the capacity to propagate in a prion-like manner across cells and brain regions. Beyond **homotypic aggregation**, **Tau interacts with other proteins to form aggregates—referred to as hetero-amyloids—which are increasingly implicated in disease pathogenesis** due to their enhanced toxicity and complex structural diversity. This study explores the formation of **hetero-amyloids between Tau and the RNA-binding protein Musashi1 (MSI1)**, a regulator of mRNA translation and a protein associated with multiple neurodegenerative conditions. MSI1 has been observed, by our group, to aggregate and mis-localize in AD, exhibiting functional impairment in the presence of pathological Tau species. Here, the presence of MSI1:Tau hetero-amyloids is examined in human brain tissue, while recombinant approaches are used to reconstitute and biochemically characterize these aggregates as distinct molecular species. The detection of MSI1:Tau hetero-amyloids in Alzheimer's disease brain tissue introduces a novel and promising area for therapeutic and diagnostic development. Given the reported enhanced toxicity of hetero-amyloids in neurodegenerative disease, further investigation into their structure and function may uncover critical mechanisms driving disease progression.

Methodologies

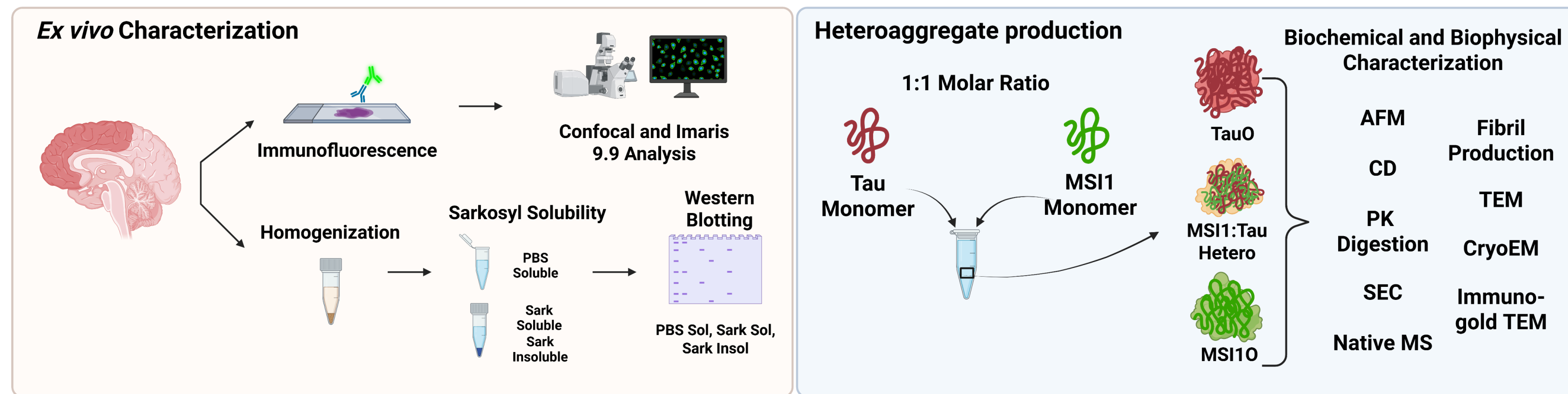


Figure 1. Methodologies. Made using biorender.com.

In Vitro Biochemical Characterization

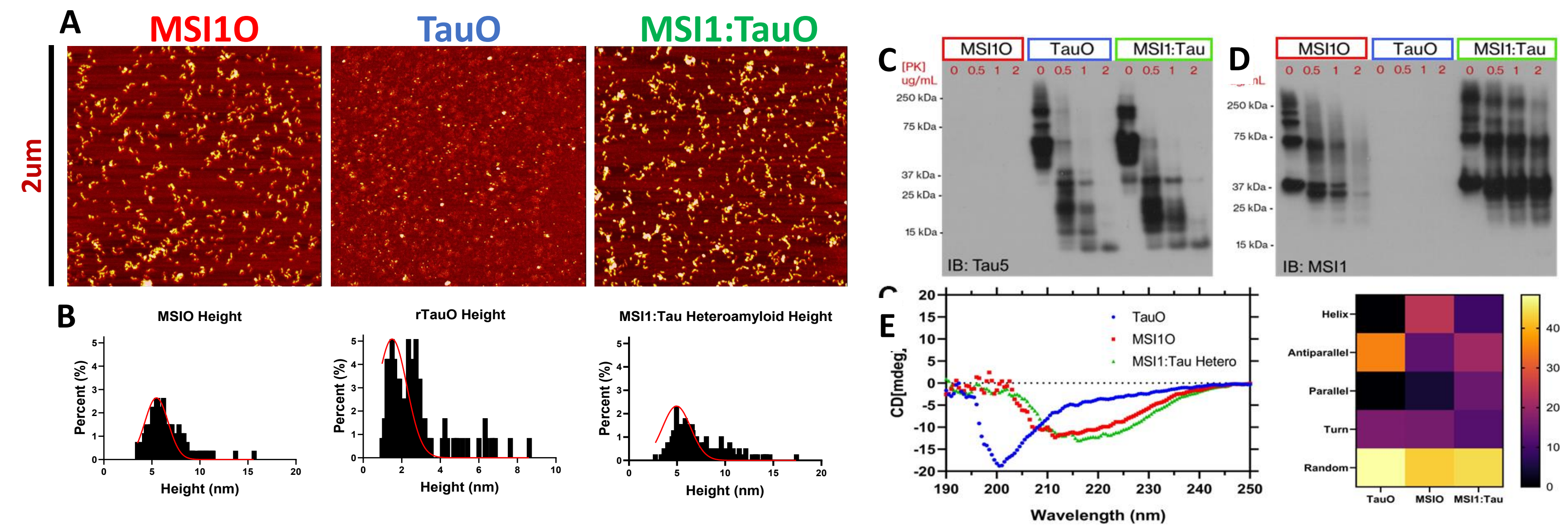


Figure 3. Biochemical Characterization. Human Recombinant MSI1O, TauO, and MSI1:Tau AFM (A-B). MSI1O, TauO, and MSI1:Tau hetero-amyloid AFM taken at 2nm after 48h incubation at 37C° with shaking (A). Height distribution from AFM showing gaussian distributions (red) and highlighting the distinct height profiles of each aggregate species (B). **Proteinase K Digestion of MSI1:Tau Hetero-amyloids (C-D).** MSI1O, TauO, and MSI1:Tau hetero-amyloids were all incubated with proteinase K for 30 mins at 37C at various concentrations (red). After PK digestion, samples were run on WB and immunolabeled with (C) Tau 5 or (D) MSI1 antibodies. **Circular Dichroism of MSI1:Tau Hetero-amyloids (E).** Representative CD spectra for TauO (blue), MSI1O (red), and MSI1:Tau hetero-amyloids (green). Deconvoluted analysis of CD spectra represented as a heat map for each secondary structure.

Tau Impact on Musashi1 Function

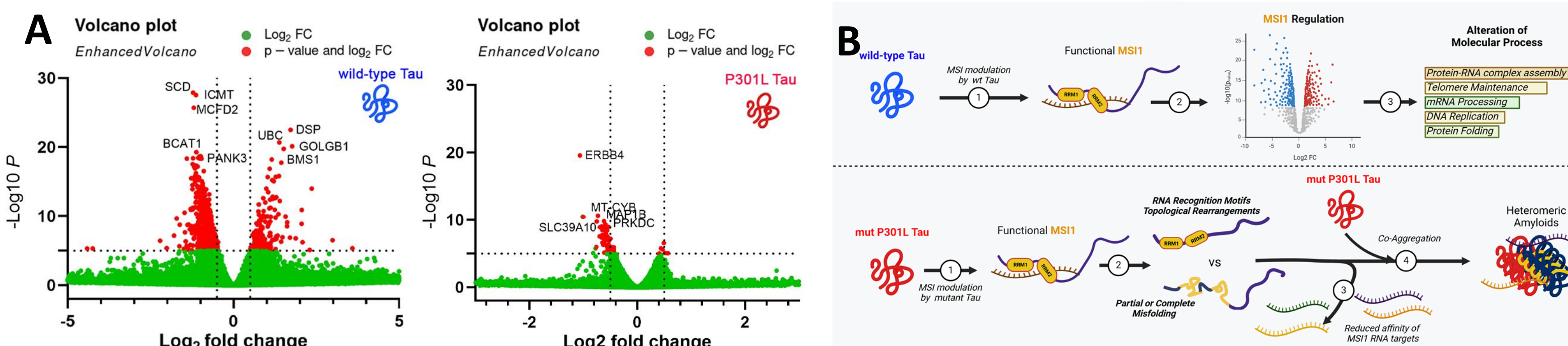


Figure 4. MSI1 and Tau oligomers and co-localization. (A) Volcano plots representative of RNA transcripts bound to MSI1 compared between treated and untreated iHEK WT or P301L cells. Functional alteration in the presence of WT Tau and loss of function of MSI1 in the presence of pathogenic Tau. (B) Proposed mechanism for the functional alteration of MSI1 in the presence of WT Tau or P301L Tau. The top diagram shows the impact of WT-Tau on functional MSI1, and the bottom diagram shows the impact of P301L Tau on functional MSI1.

Summary and Conclusions

- So far, our data is characterizing MSI1:Tau heteroamyloids as unique species and is pointing towards the existence of MSI1:Tau heteroamyloids in human tissue, which have never been shown so far
- Interactions with pathogenic Tau significantly interfere with Musashi1 function
- This provides insights into how Tau-mediated molecular mechanisms are involved in cellular toxicity *via* RBP dysfunction, opening new avenues for therapeutic advancement

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Tau and RBP Musashi1 in Aged and Alzheimer's Brains

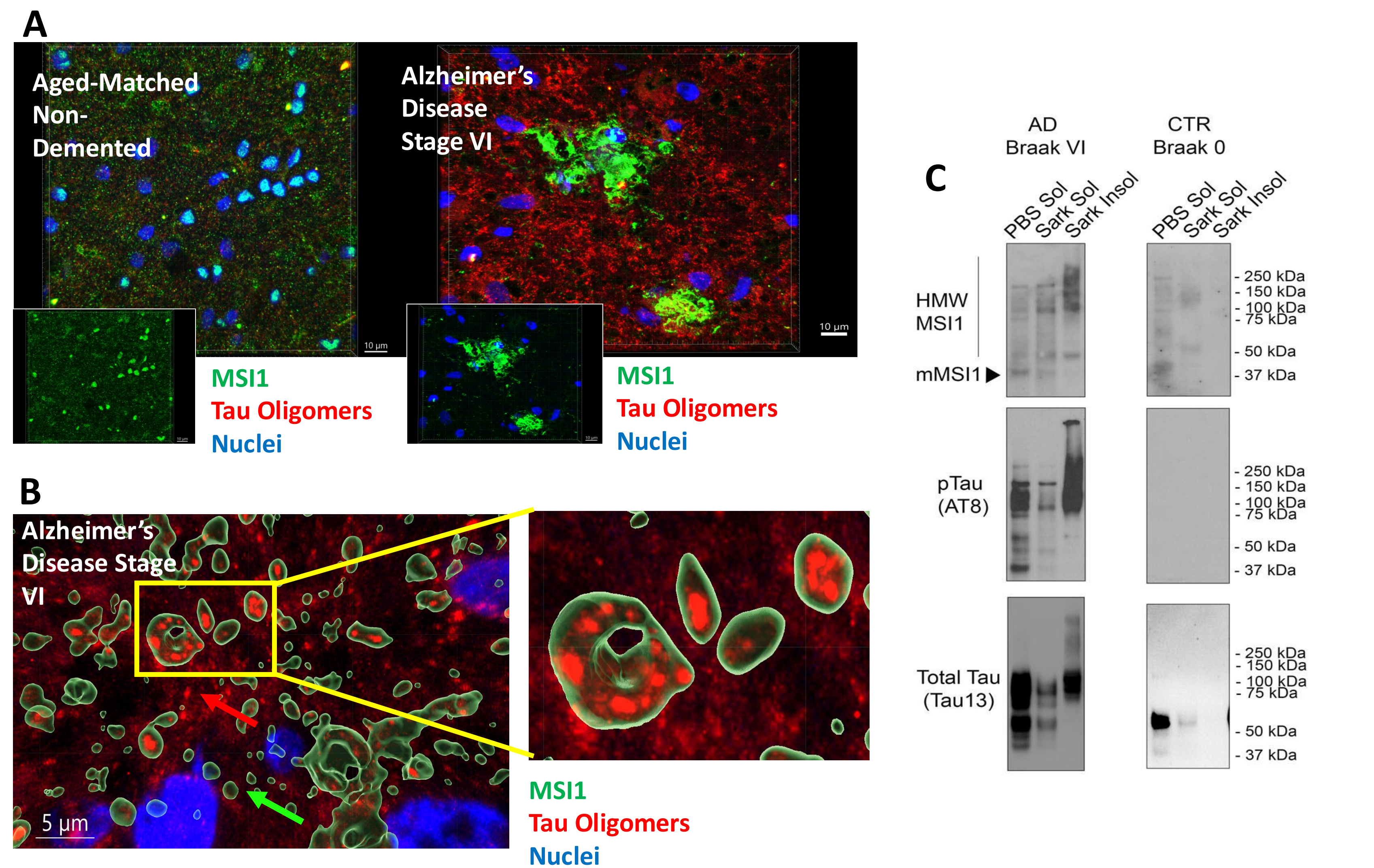


Figure 2. MSI1 and Tau oligomers in human brains. (A.) **MSI1** Co-Immunofluorescence staining and confocal microscopy in age-matched control (CTR) – left panel and Alzheimer's Disease human tissues – right panel. IF showed **MSI1** accumulation and co-aggregation with **Tau Oligomers** in AD Braak Stage VI brains. (B.) Imaris 9.9 3D rendering of IF AD slides showing MSI1 surrounding tau aggregates (yellow box). IF revealed non-hybrid tau aggregates (red arrow) and non-hybrid MSI1 aggregates (green arrow) as well. (C.) Representative AD (Braak VI) and CTRL (Braak 0) sarkosyl fractionations that were run on WBs and immunolabeled with MSI1, phospho-Tau (pTau), or Tau13 antibody. Here we show HMW banding of each protein species in the sarkosyl insoluble fraction of AD homogenates.