Journal Club

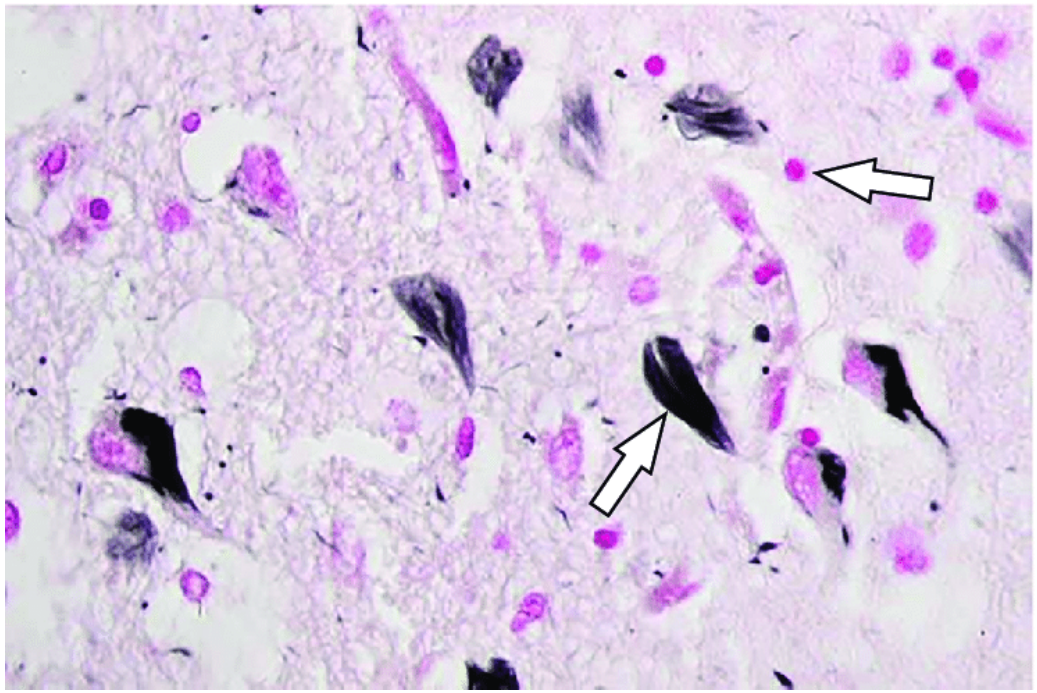
Microglia become hypofunctional and release metalloproteases and tau seeds after phagocytosing live neurons with P301S tau aggregates

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Apr 14, 2021

Components of Alzheimer's Pathology

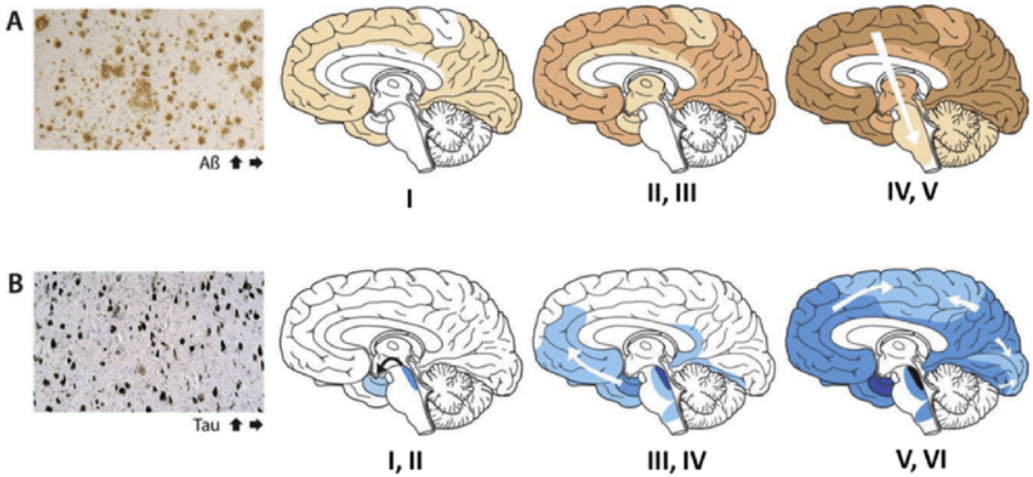
- Extracellular plaques Aβ $\rightarrow$ Pink
- Intracellular fibrils τ $\rightarrow$ Black



Microtubule-associated protein tau

- Highly expressed in neurons
- Role in maintaining the stability of microtubules in axons
- Major component of intracellular fibrils found in AD brain
- Tau fibrils in AD are found to be hyperphosphorylatated

Braak staging in AD



Tau is intracellular

How does it spread?

Exploring mechanisms that tau can spread in tauopathy

Basic Idea

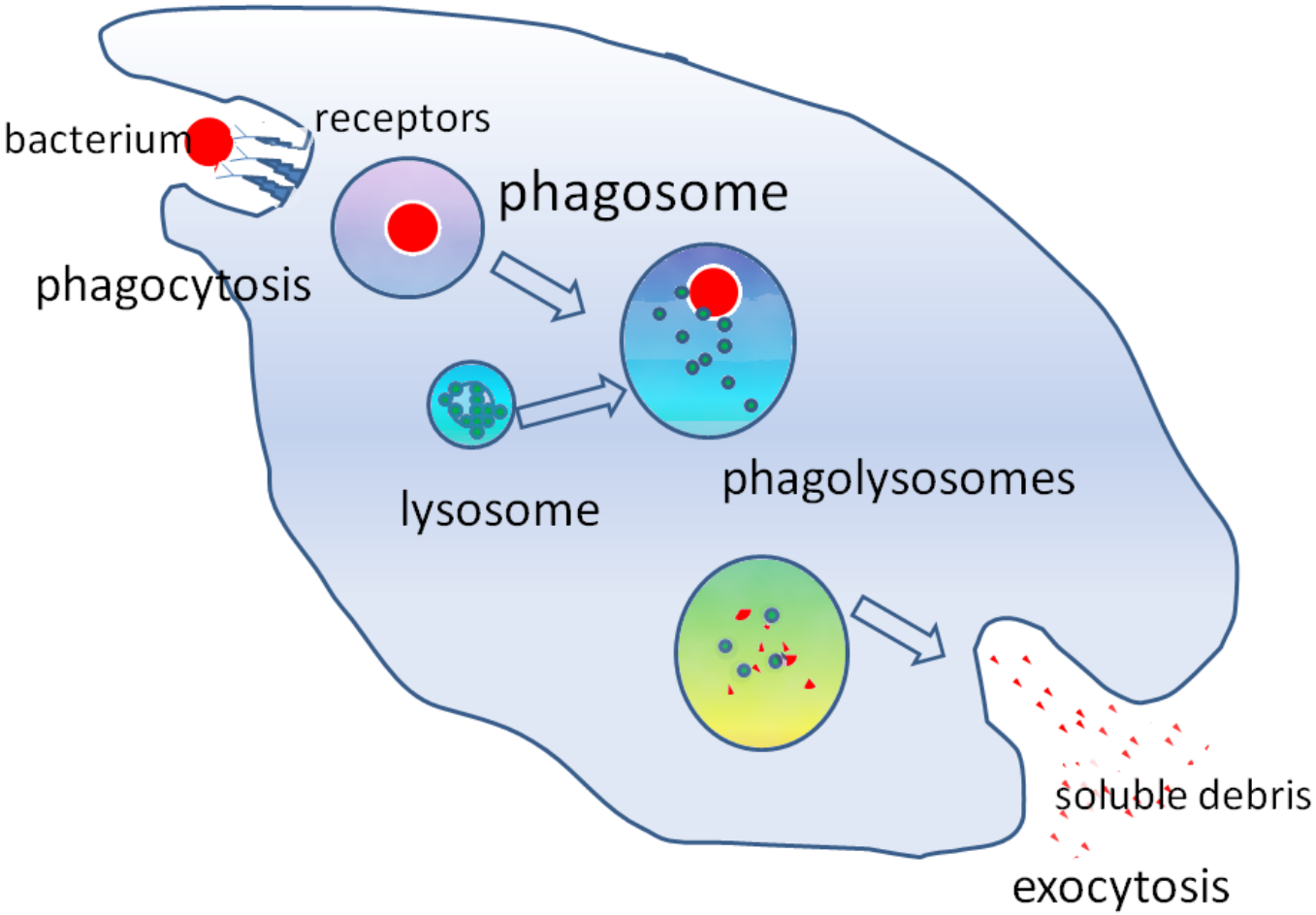
Microglia acts as a vector for tau spread in disease through phagocytosis of neurons

How to test this

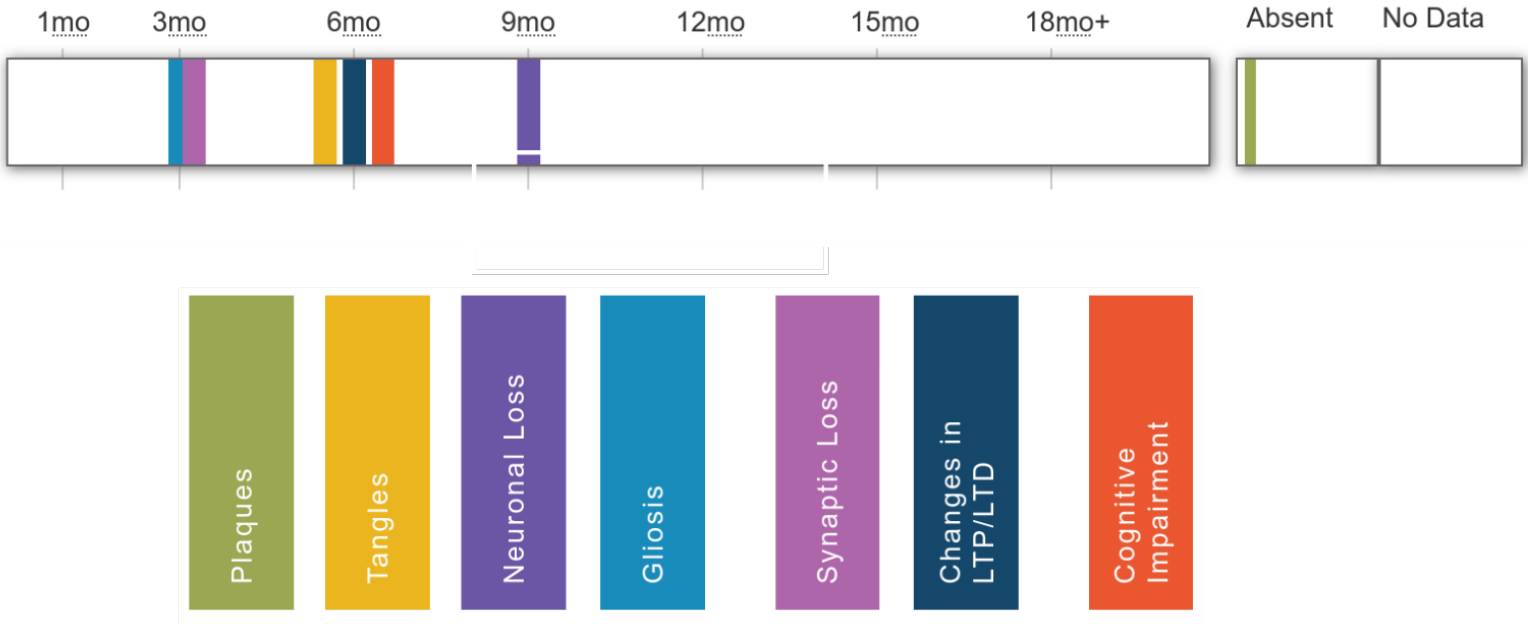
Collect neurons with the pathological tau feature and coculture with wild type Microglia

Phagocytosis

When cells uses its plasma membrane to engulf a large particle >0.5um $\rightarrow$ phagosome



P301S mouse



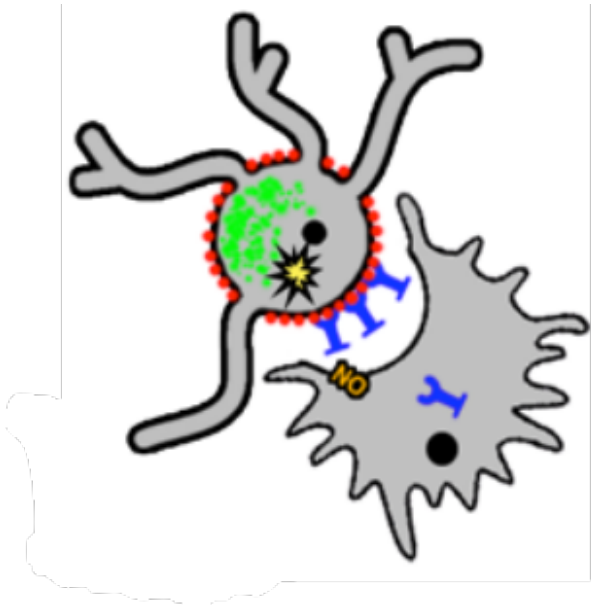
P301S Transgene

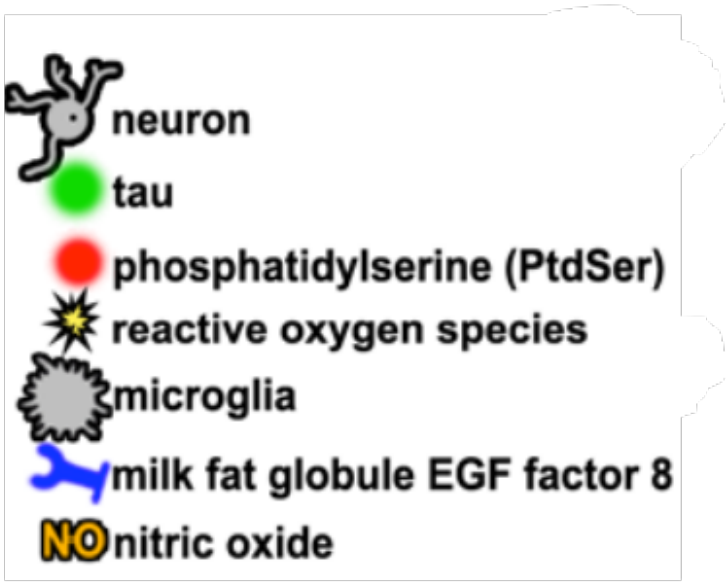
- Human MAPT with Frontotemporal dementia causing mutation
- Mouse prion promoter

Dorsal Root Ganglion neurons (DRGn)

Taken from 2 and 5 month old mouse

PtdSer phagocytosis mechanism





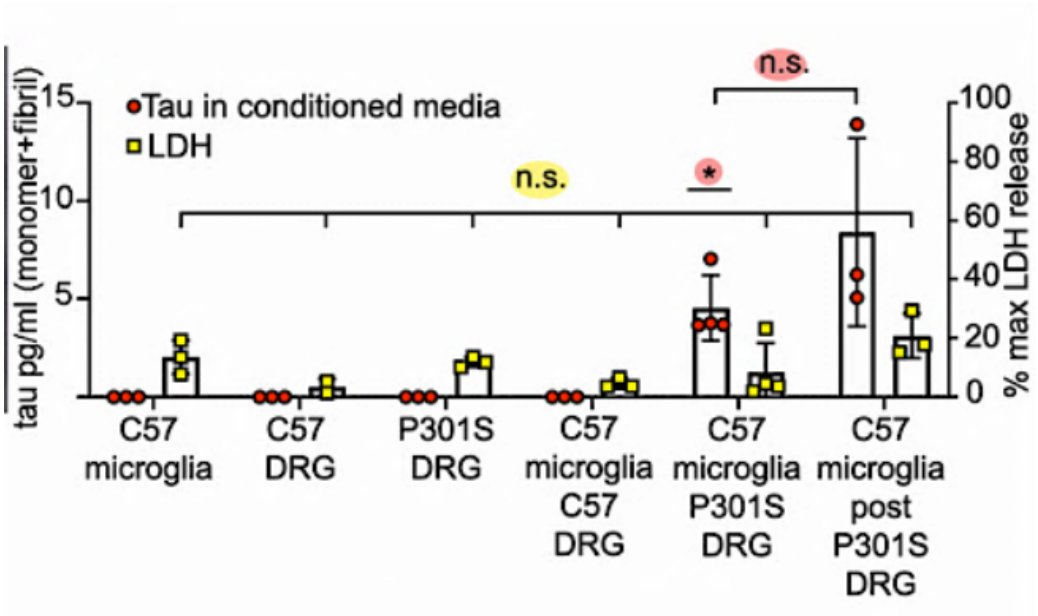
Jargon

- Phosphatidylserine - Phospholipid that plays a role in signalling apoptosis
- Milk Globule EGF factor 8 - Binds to PtdSer
- AnnV Annexin A5 - Binds to PtDSer/inhibits PtDSer Milk Globule EFG 8 activity

Experimental design

1. Extract Dorsal Root Ganglion neurons from P301S mouse
2. Coculture for 4 days with wild type microglia
3. Measure tau on coculture conditioned media (CM)
4. Isolate microglia and culture independently
5. Measure tau on post-coculture microglia conditioned media (CM)

P301S Neuronal phagocytosis by microglia release tau



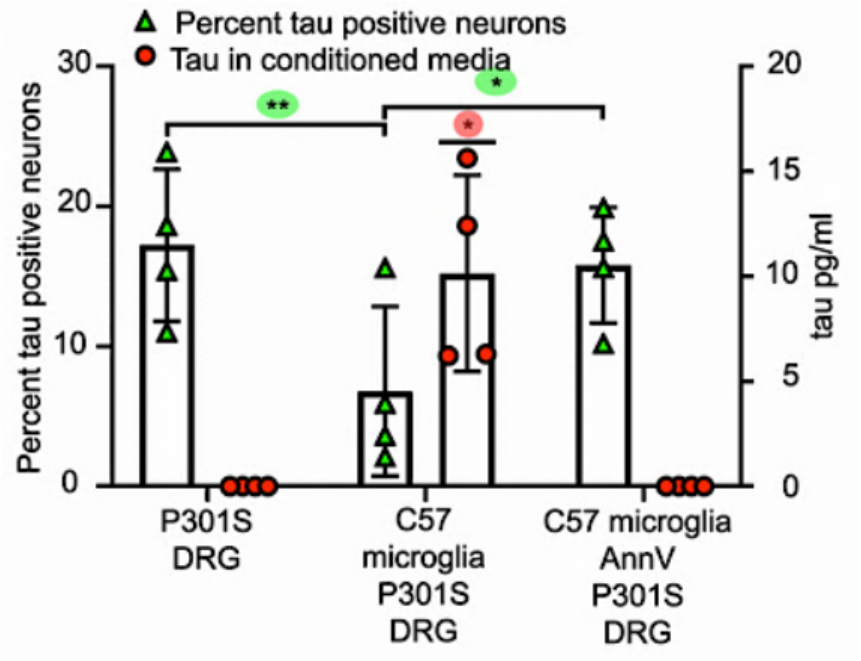
Methodology

- Coculturing microglia for 4 days causes increase of tau in CM
- LDH (Lactate dehydrogenase) Used as marker of tissue damage

Observations

- No cell death is evident by LDH (no positive control)
- Tau detectable in the conditioned media

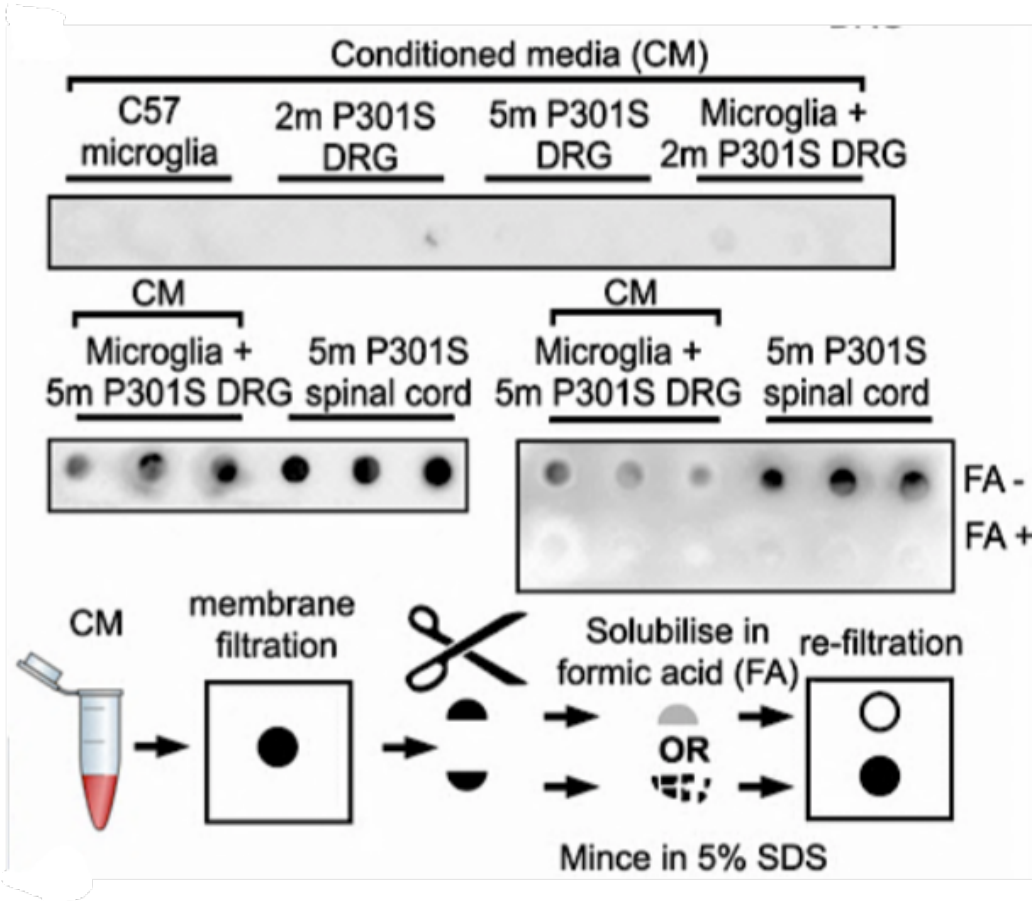
PtdSer mediated phagocytosis in P301S DRGn



Observations

- Treating with AnnV inhibitis microglia phagocytosis
- Tau+β III tubulin % was reduced 2fold when cocultured with microglia

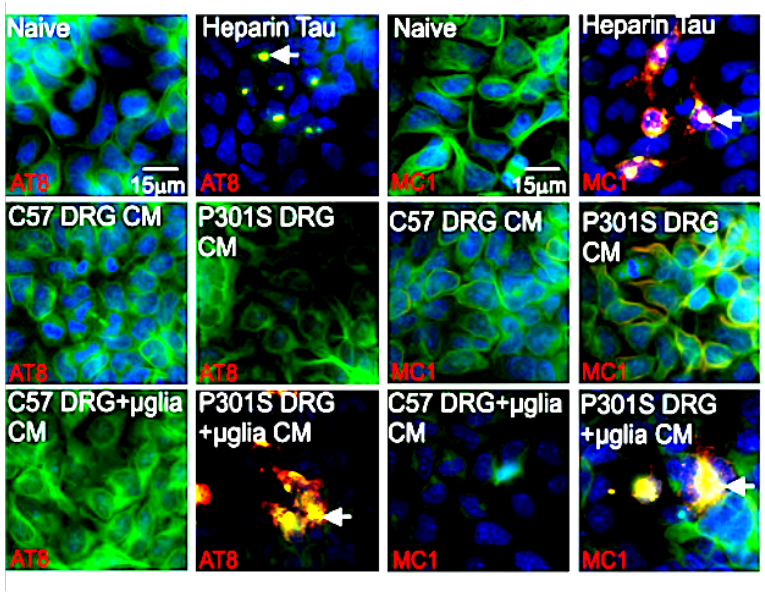
Tau in Condiotioned Media is insoluble



Observations

- 2 month old mice do not show insoluble tau

Tau microglia conditioned media has aggregation properties



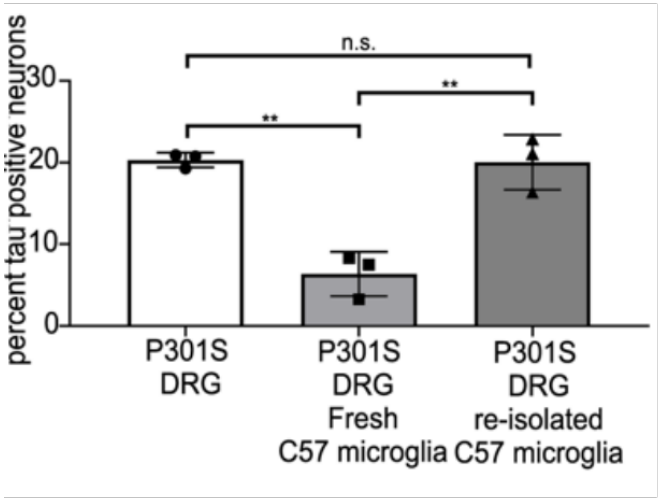
Components

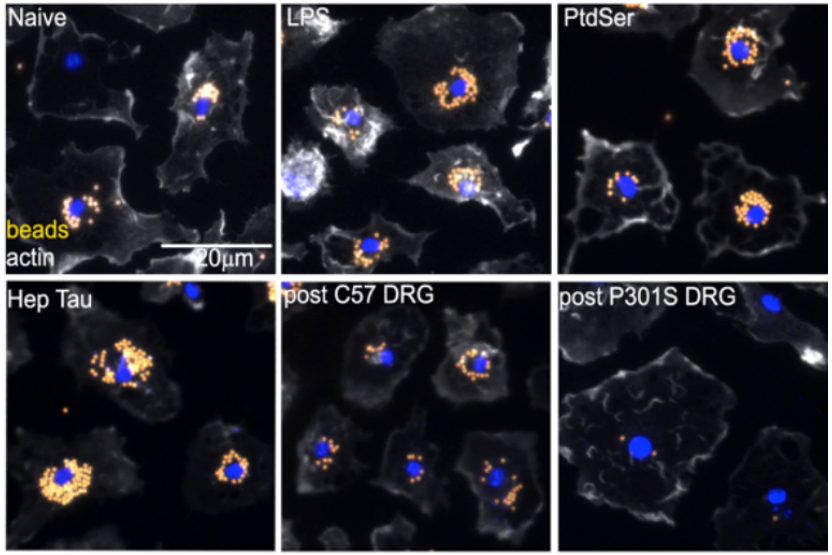
- AT8 is a tau monoclonal antibody to detect hyperphosphorylation
- MC1 detects misfolded tau
- HEK P301S-venus

Observations

Aggregation of the endogenous tau (Red) and the one condititon Media (Not red)

Microglia becomes hypophagocytic after P301S coculture

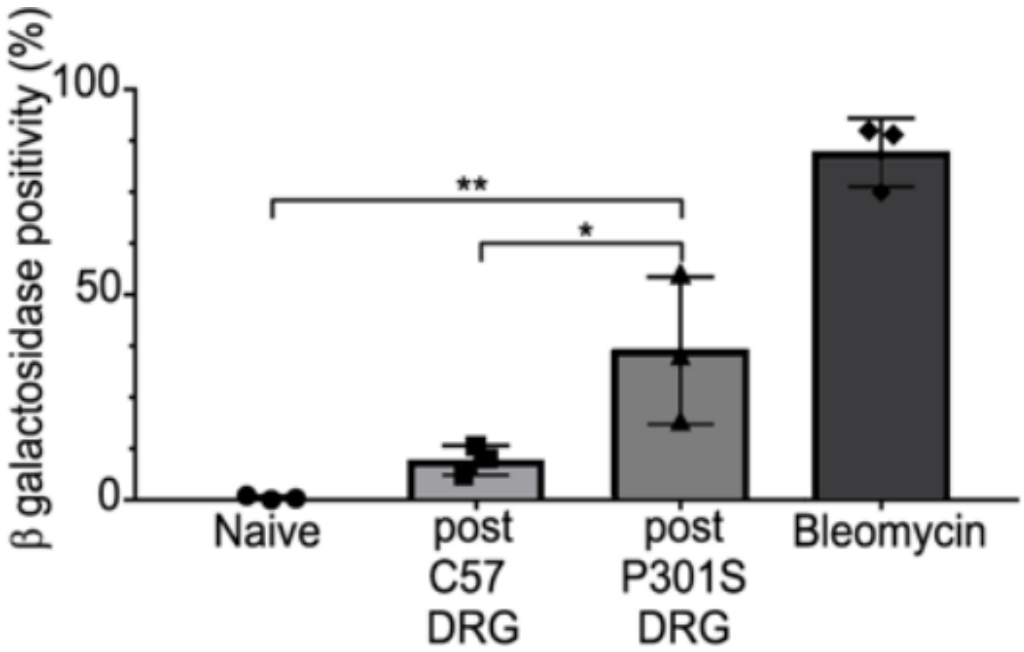




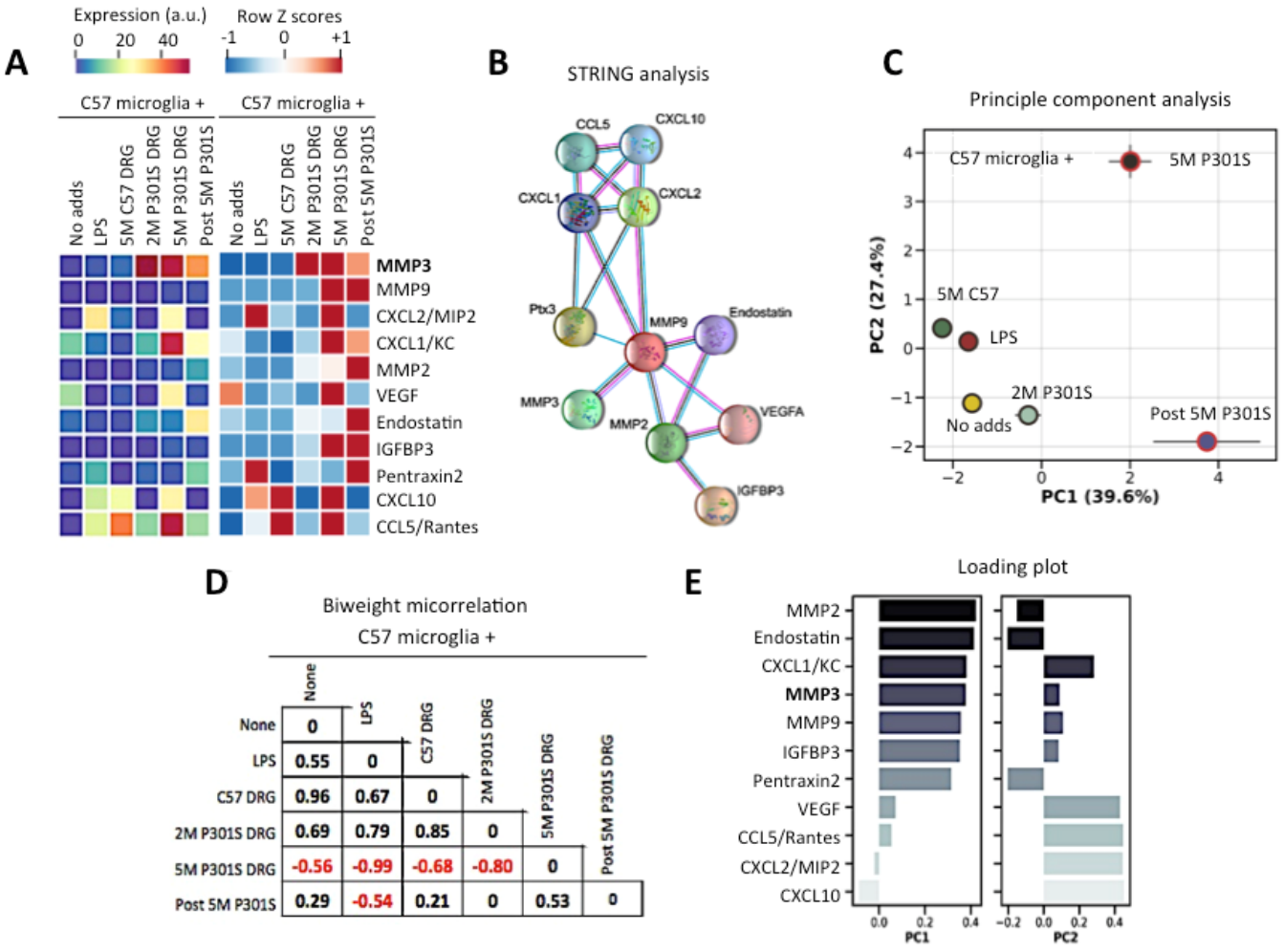
Observations

- During 2nd coculture microglia does not reduce tau positive neurons
- Carboxylate-modified latex beeds show microglia cultured with P301S DRGn is specifically hypophagocytic

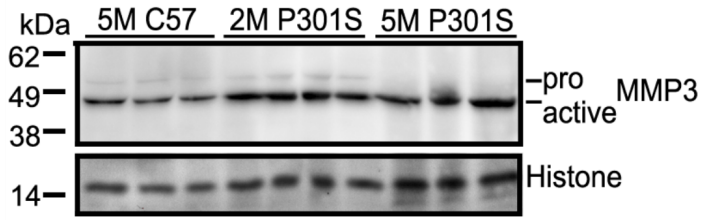
Activation of senescence associated β galactosidase

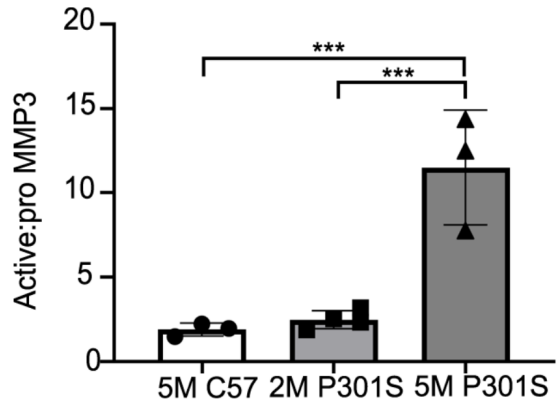


Screen for 86 mouse proteins in Conditioned Media



MMP3 protein levels in mouse brains

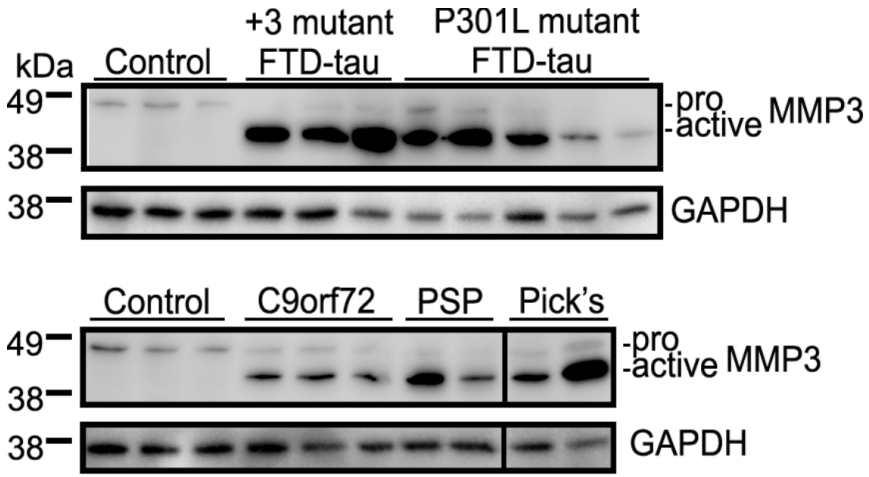




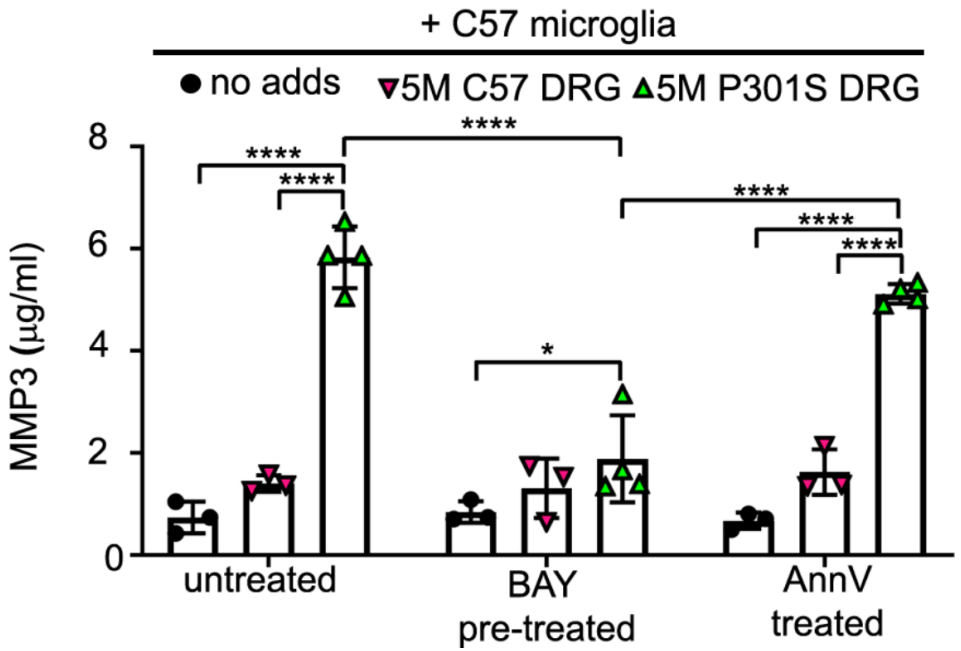
Observations

The pro to active ratio changes drastically

Active MMP3 as a marker in advanced tauopathy



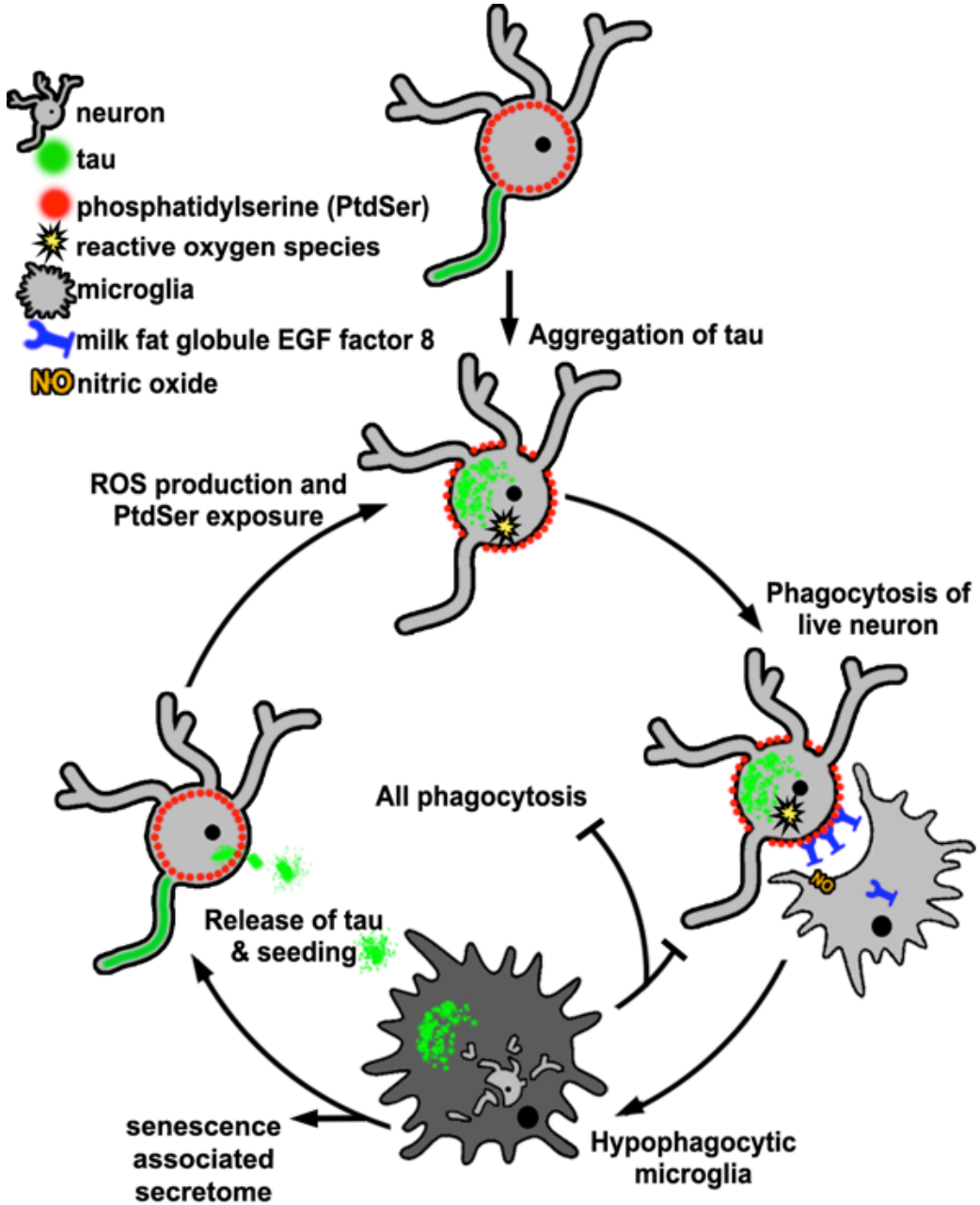
NFkβ modulates MMP3



Observations

- NFkβ was found upstream of MMP3 according to Enrichr
- Phagocytosis inhibition did not downregulate MMP3

Putting it all together



However. . .

- No senescencent signatures were recorded in AD
- Hypophagotic behaviour has been observed when microglia have phagocytosed Aβ

Why MMP3?

MMP3

- Matrix Metalloproteinase 3
- Enzyme that catalyses proteolysis

Pro form and active

- This ratio seems to be important
- It can serve as human biomarker of tauopathy

Inhibiting this protein

- This protein has proteolytic properties extracellularly
- Since it is a functional enzyme, why its inhibition would yield less hypophagotic microglia
- NFkβ seems has several downstream targets and triggers
- If NFkβ is involved who triggers it?