

ABSTRACT

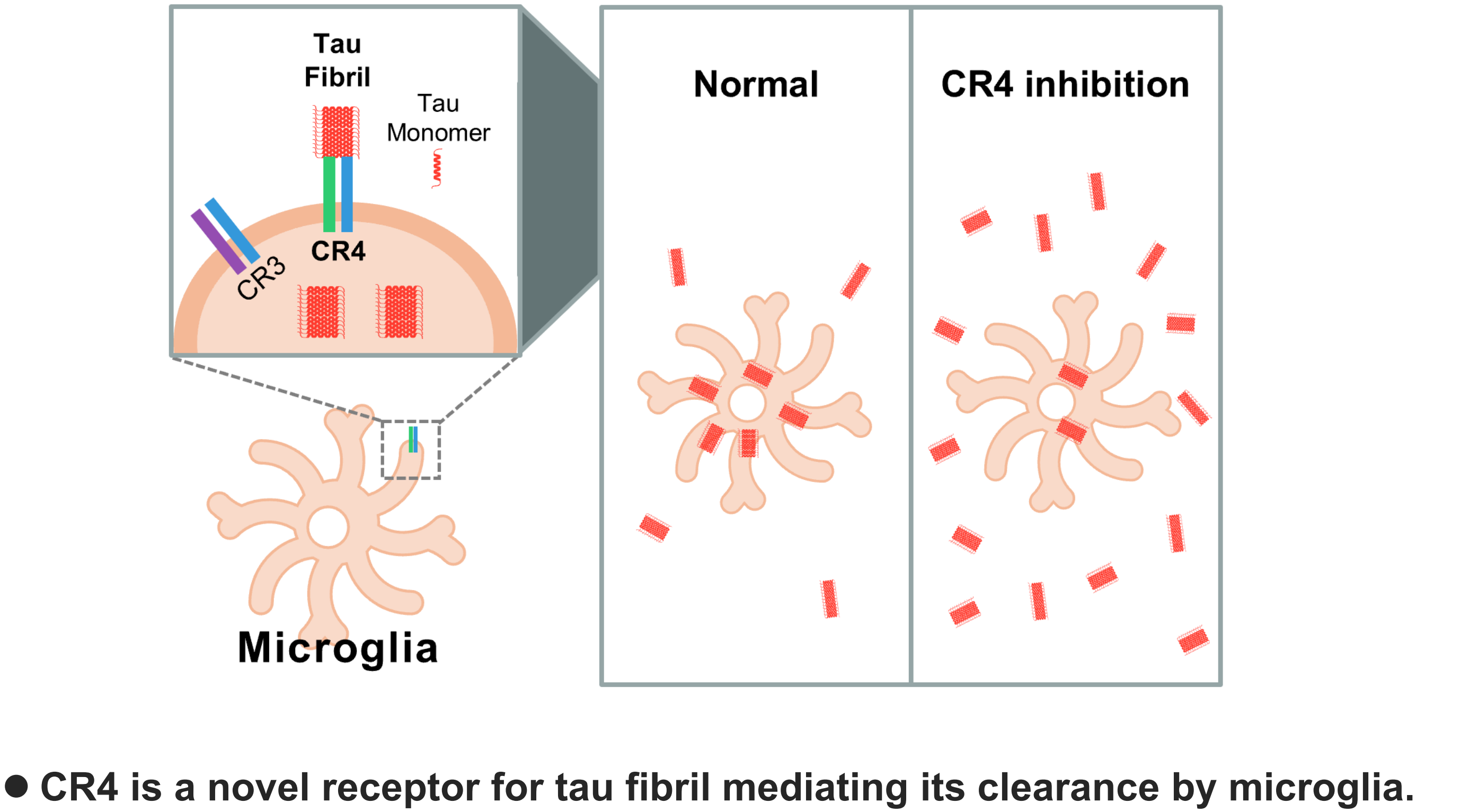
Background | Tauopathies exhibit characteristic accumulation of misfolded tau aggregates in the brain. Tau pathology shows disease-specific spatiotemporal propagation through intercellular transmission, which is closely correlated with the progression of clinical manifestations. Therefore, identifying molecular mechanisms that prevent tau propagation is critical for developing therapeutic strategies for tauopathies. The various innate immune receptors, such as complement receptor 3 (CR3) and complement receptor 4 (CR4), have been reported to play a critical role in the clearance of various extracellular toxic molecules by microglia. However, their role in tau clearance has not been studied yet.

Results | In this study, we investigated the role of CR3 and CR4 in regulating extracellular tau clearance. We found that CR4 selectively binds to tau fibrils but not to tau monomers, whereas CR3 does not bind to either of them. Inhibiting CR4, not CR3, significantly reduces the uptake of tau fibrils by BV2 cells and primary microglia. In contrast, inhibiting CR4 has no effect on the uptake of tau monomers by BV2 cells. Furthermore, inhibiting CR4 suppresses the clearance of extracellular tau fibrils, leading to more seed-competent tau fibrils remaining in the extracellular space relative to control samples. We also provide evidence that the expression of CR4 is upregulated in the brains of human Alzheimer's disease patients and the PS19 mouse model of tauopathy.

Conclusion | Taken together, our data strongly support that CR4 is a previously undescribed receptor for the clearance of tau fibrils in microglia and may represent a novel therapeutic target for tauopathy.

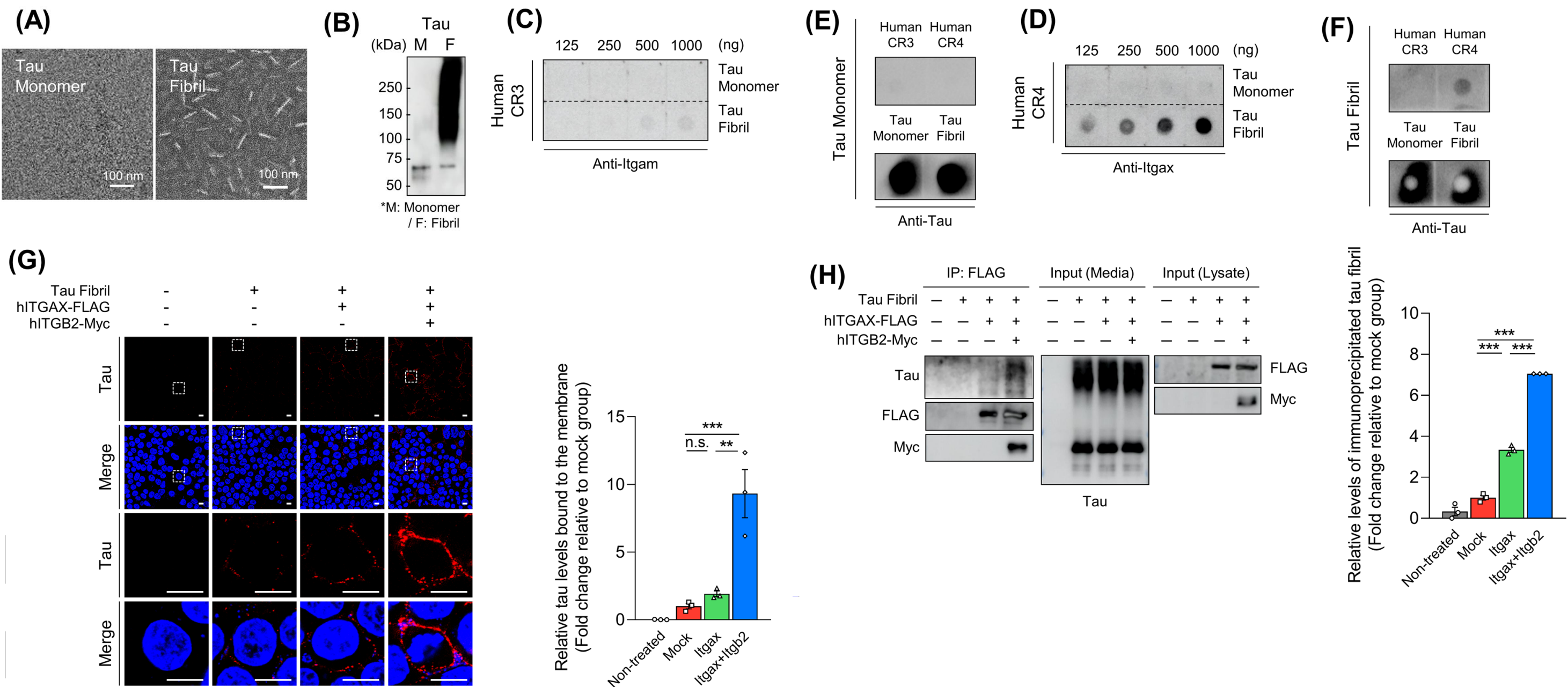
#Tauopathy #Uptake #Clearance #Alzheimer's disease #Microglia #Complement Receptors

GRAPHICAL ABSTRACT



RESULTS

Fig. 1. CR4 interacts with tau fibrils



(A, B) Recombinant human monomeric (M) and fibrillar (F) forms of 2N4R full-length tau P301S were characterized by transmission electron microscopy (A) and western blot (B).
(C, D) Representative dot-blot images showing the interaction of tau with CR3 (C) or CR4 (D).
(E, F) Representative dot-blot images showing the interaction of CR3 and CR4 with tau monomer (E) or fibril (F).
(G, H) The levels of tau fibrils bound to HEK293T cells are increased upon CR4 overexpression. Tau fibrils bound to cells were assessed by immunocytochemistry using fluorescence confocal microscopy (G) and an immunoprecipitation assay (H).

Fig. 2. Inhibiting CR4 reduces the uptake of tau fibrils by microglia

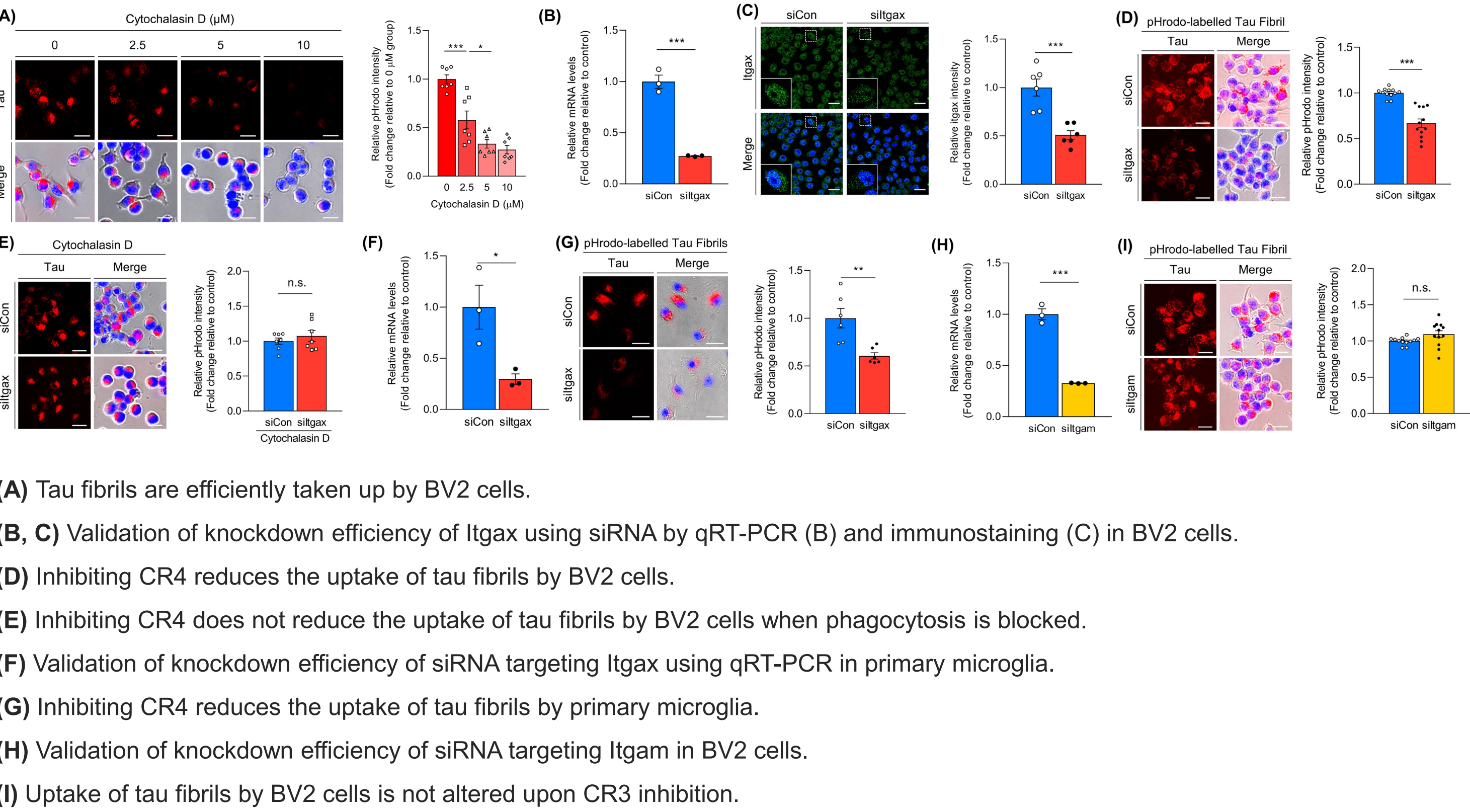
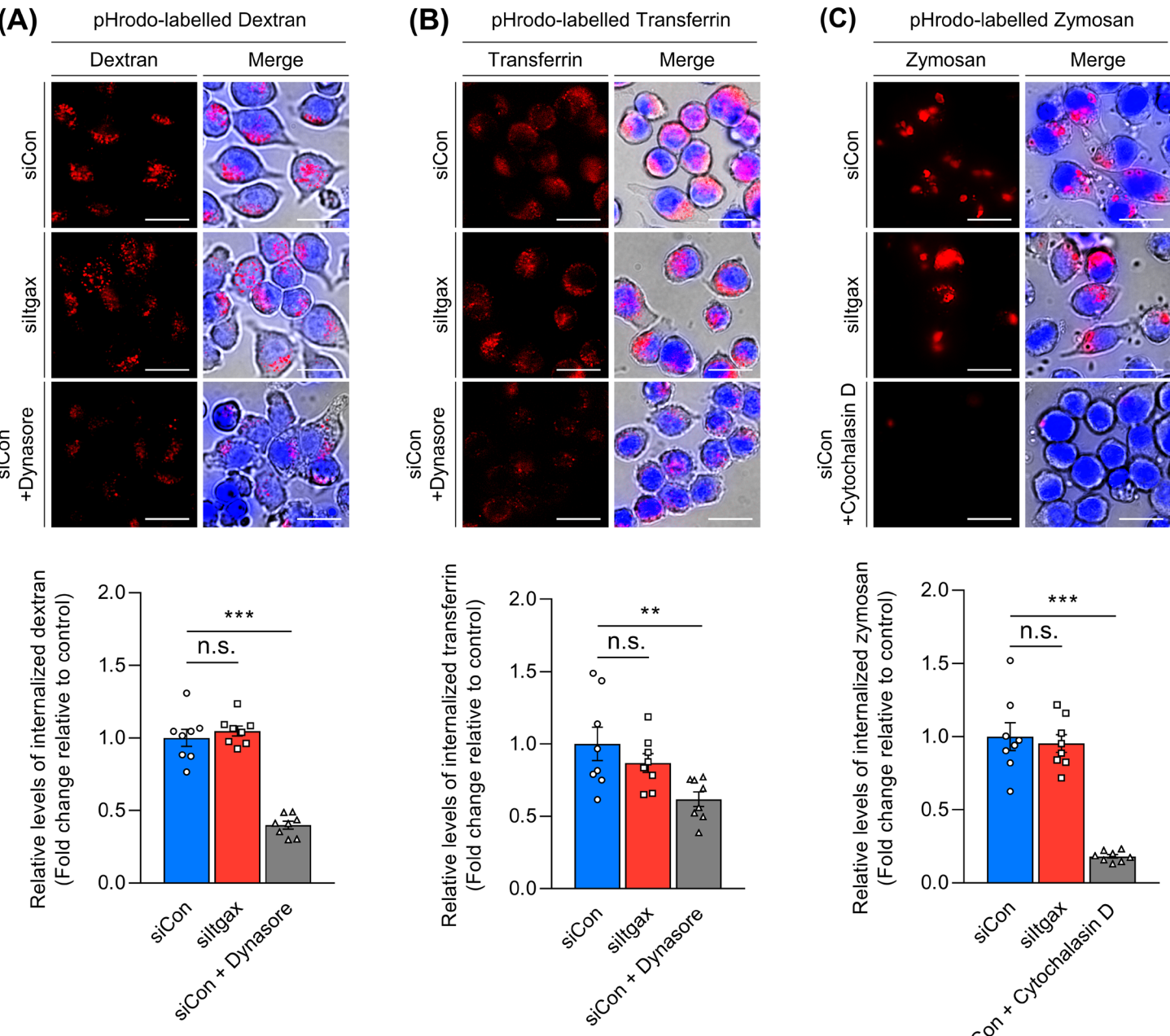


Fig. 3. Inhibiting CR4 has no effect on the uptake of dextran, transferrin, and zymosan by BV2 cells



(A) The uptake of dextran by BV2 cells is not altered upon CR4 inhibition. 48 h post-transfection with the indicated siRNAs, BV2 cells were pre-treated with/without 20 μ M Dynasore for 1 h and then incubated with 10 μ g/mL of pHrodo-labeled dextran for 6 h.
(B) The uptake of transferrin by BV2 cells is not altered upon CR4 inhibition. 48 h post-transfection with the indicated siRNAs, BV2 cells were pre-treated with/without 20 μ M Dynasore for 1 h and then incubated with 25 μ g/mL of pHrodo-labeled transferrin for 30 min.
(C) The uptake of zymosan by BV2 cells is not altered upon CR4 inhibition. 48 h post-transfection of siRNAs as indicated, BV2 cells were pre-treated with/without 2.5 μ M Cytochalasin D for 1 h and then incubated with 20 μ g/mL of pHrodo-labeled zymosan for 2 h.

Fig. 4. Inhibiting CR4 has no effect on the uptake of tau monomers by BV2 cells

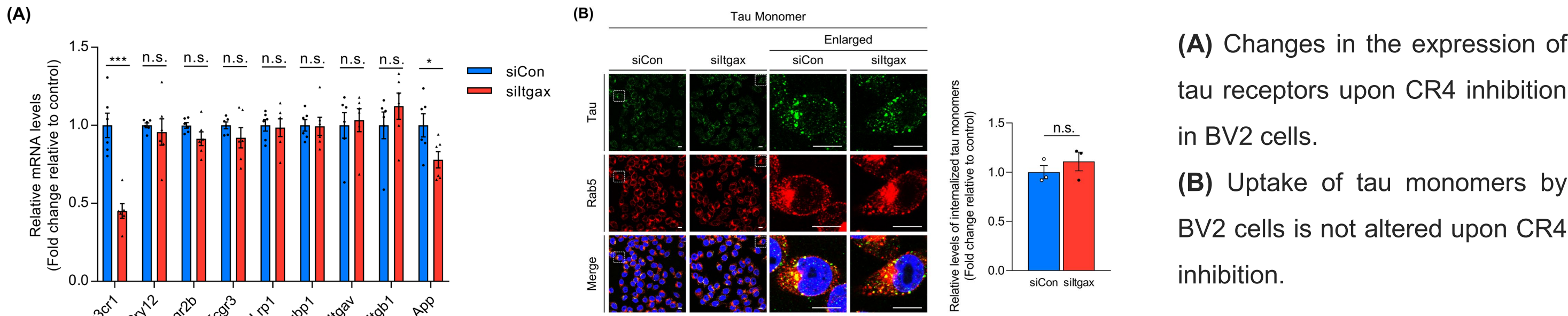


Fig. 5. Inhibiting CR4 reduces the clearance of extracellular tau fibrils by microglia

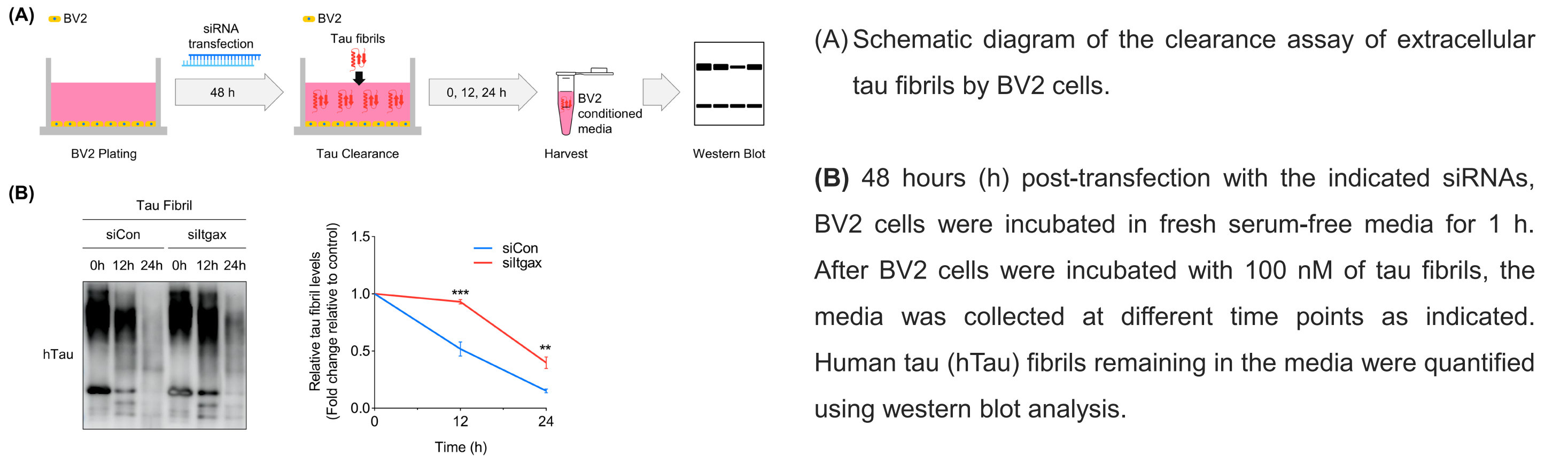


Fig. 6. Inhibiting CR4 leads to more seed-competent tau fibrils remaining in the culture media of BV2 cells than the control

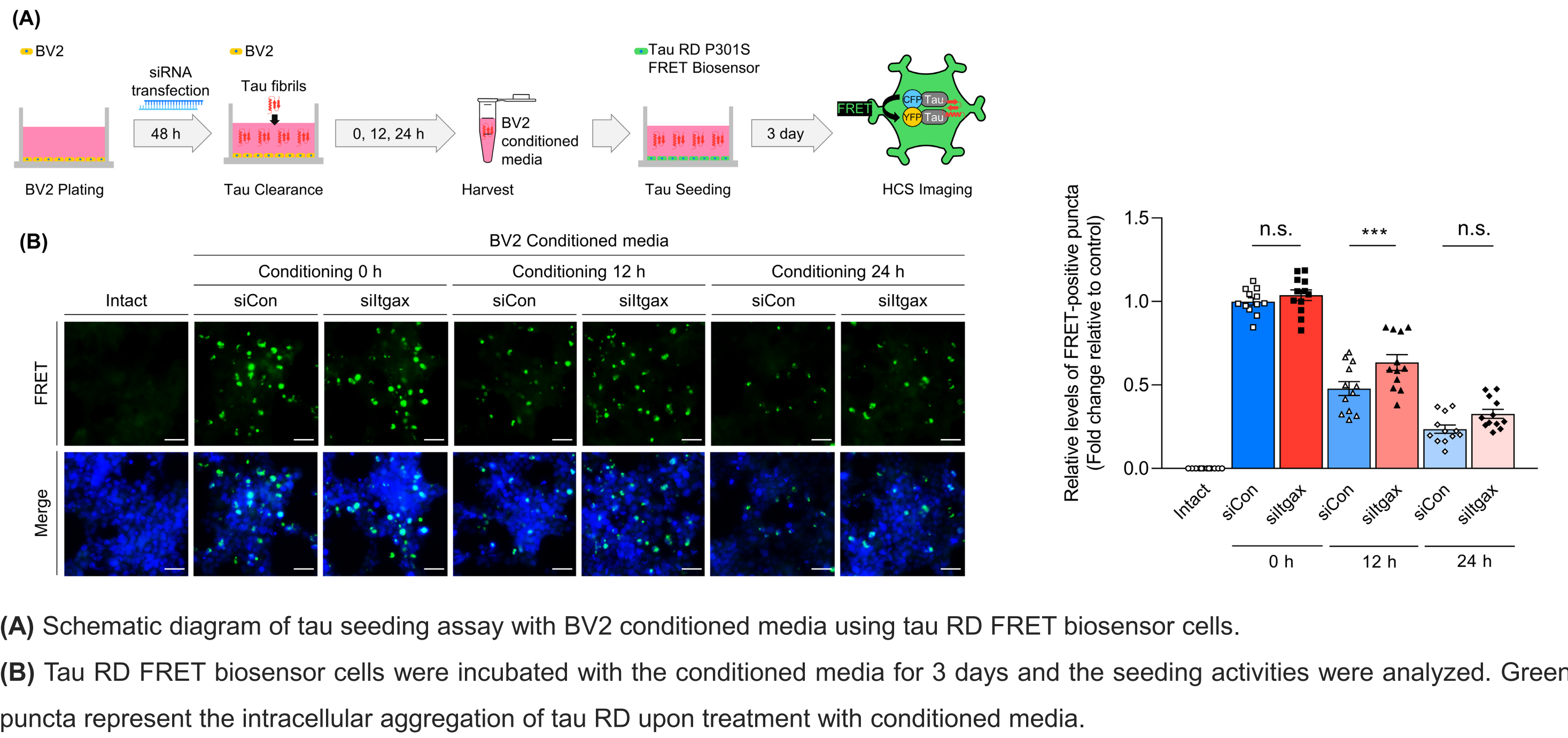
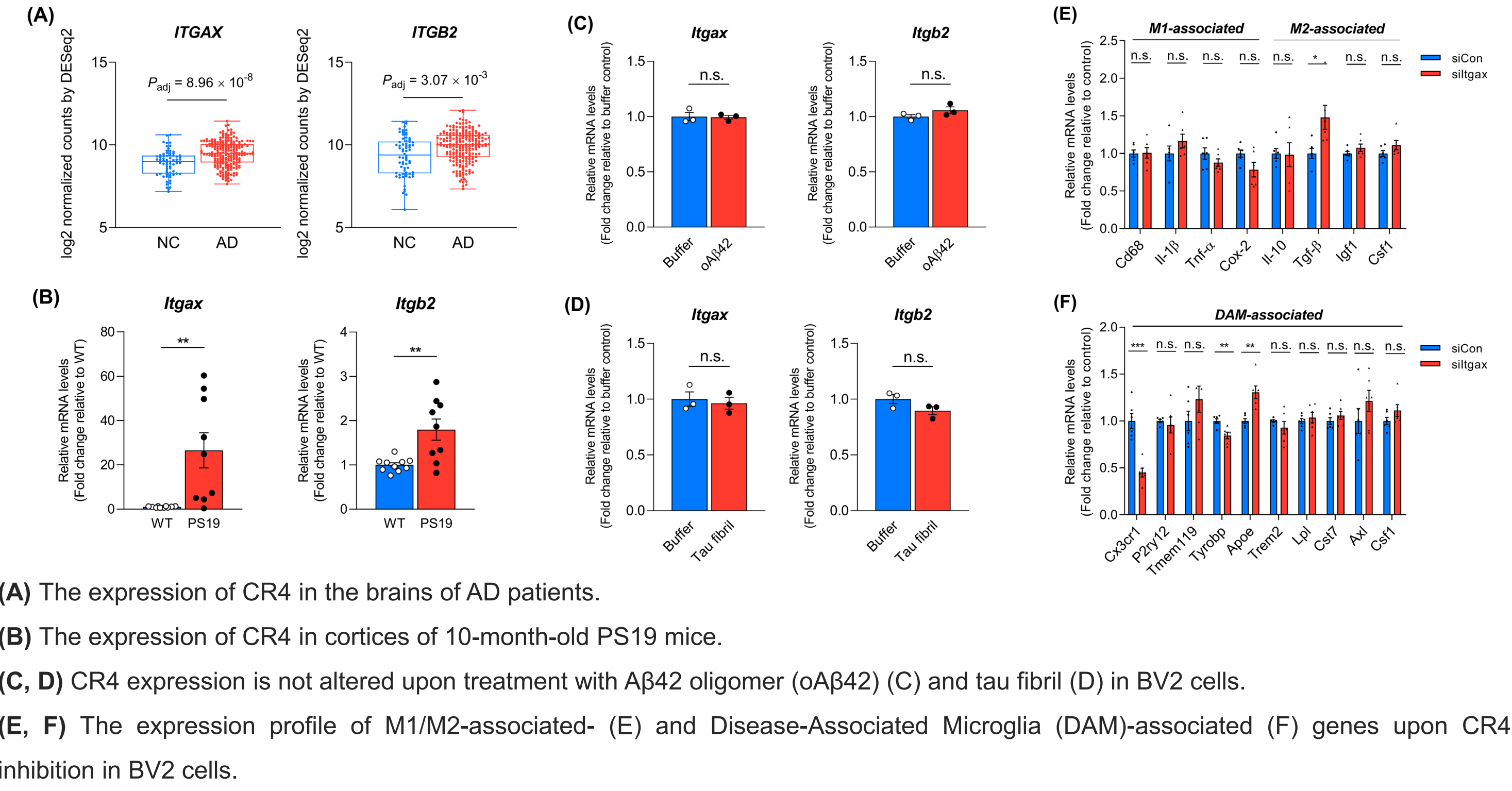


Fig. 7. The expression of CR4 is upregulated in the brains of human Alzheimer's disease (AD) patients and mouse model of tauopathy



(A) The expression of CR4 in the brains of AD patients.
(B) The expression of CR4 in cortices of 10-month-old PS19 mice.
(C, D) CR4 expression is not altered upon treatment with A β 42 oligomer (oA β 42) (C) and tau fibril (D) in BV2 cells.
(E, F) The expression profile of M1/M2-associated- (E) and Disease-Associated Microglia (DAM)-associated (F) genes upon CR4 inhibition in BV2 cells.

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