Neurotoxic astrocytic glypican-4 drives APOE4-dependent tau pathology

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ABSTRACT

Apolipoprotein E4 (APOE4) is the crucial genetic risk factor of late-onset Alzheimer's disease (AD). Aggregation of tau proteins into insoluble filaments and their spreading across the brain regions are major drivers of neurodegeneration in tauopathies, including in AD. However, the exact mechanisms through which APOE4 induces tau pathology remains unknown. Here, we report that the astrocyte-secreted protein glypican-4 (GPC-4), a novel binding partner of APOE4, drives tau pathology. GPC-4 preferentially interacts with APOE4 in comparison to other APOE isoforms and post-mortem APOE4-carrying AD brains highly express GPC-4 in neurotoxic astrocytes. The astrocyte-secreted GPC-4 induced both tau accumulation and propagation *in vitro*. CRISPR/dCas9 mediated activation of GPC-4 in a tauopathy animal model robustly induced tau pathology. Further, APOE4-induced tau pathology was greatly diminished in the absence of GPC-4. We found that GPC-4 promoted the stabilization of the APOE receptor low-density lipoprotein receptor-related protein 1 (LRP1) on the cellular surface, which effectively facilitates endocytosis of tau protein. Together, our data comprehensively demonstrate that one of the key APOE4-induced tau pathologies is directly mediated by GPC-4.

INTRODUCTION

AD is the most common type of dementia accompanied by neurodegeneration and cognitive decline. The total medical care costs for the treatment of AD in 2020 was estimated at $305 billion in the United States, with the cost expected to increase to more than $1 trillion as the population ages[1]. There is a critical need for therapeutic drugs that may prevent the disease or slow-down the rate of disease progression. Inherited autosomal-dominant forms of AD (familial AD) is caused by the presence of mutations in amyloid precursor protein gene (*APP*) or presenilin genes (*PSEN1* and *PSEN2)* and affects much younger patients[2].

Familial AD represents less than 1% of total AD cases. The remaining AD cases do not carry such autosomal mutations, and they are termed as late-onset or sporadic AD[3]. The exact causes of sporadic AD remain unclear. However, the apolipoprotein variant E4 (APOE4) is considered as the most crucial genetic risk factor for sporadic AD.

Apolipoprotein APOE plays a major role in the circulation of high-density and very-low-density lipoproteins and mediates the transport of lipids between the cells [4]. Human APOE is expressed in three genetic variants; APOE2, APOE3, and APOE4. These variants differ in the position of two amino acid residues; APOE3 has a cysteine at position 112 and arginine at position 158, APOE2 has a cysteine at both positions, and APOE4 has an arginine at both positions[5]. Among the three APOE isoforms, APOE4 is the most crucial genetic risk factor for late-onset Alzheimer's disease (AD). The increase in AD risk varies depending on ancestral background, sex and multiple genetic or environmental factors. But as a rough estimate, having a single APOE4 allele increases AD risk 2- to 4-fold and having two APOE4 alleles increases AD risk about 8- to 12-fold [6]. APOE4 carriers also develop AD pathologies earlier compared to non-carriers [6,7]. By contrast, APOE2 carriers have a lower likelihood of developing AD; therefore, APOE2 protects against AD [7–12]. Aβ plaques and neurofibrillary tau tangles are characteristic features of AD pathology [13,14]. AD patients with cognitive impairment often show a strong correlation with tau accumulation and spreading [15–21]. AD patients homozygous for APOE4 suffer from significant cerebral atrophy [22–24] and animal studies have demonstrated that pathological tau drives the cerebral atrophy [25,26]. However, the mechanisms that drive tau pathology in APOE4 carrying individuals/animals are not well understood.

In the brain, APOE is secreted by glial cells, primarily astrocytes [6]. Cholesterol and phospholipids produced by astrocytes in the form of APOE-containing high-density lipoprotein-like particles are vital for neuronal survival[27]. Astrocytes play a critical functional role in the central nervous system, and the loss of physiological astrocytic functions can be a main contributor to neurodegeneration[28]. Recent studies suggest that a gain of toxic functions leads to astrocyte-mediated neurodegeneration in AD [29,30,31,32]. Although the emerging studies suggest that neurotoxic astrocytes may be a major driver of AD's neurodegeneration, the molecular interconnection between astrocytes and APOE4-mediated tau pathology has yet to be resolved. Here, we report that neurotoxic astrocytes secreted Glypican 4(GPC-4) strongly

interacts with APOE4 and exacerbates APOE4-induced tau pathology. Using invitro and animal models, our study demonstrates that GPC-4 drives APOE4-mediated tau pathology.

RESULTS

APOE variants and tau pathology

We performed immunohistochemical (IHC) staining with phosphorylated tau antibody AT8 (Ser202 and Thr205) and acetylated tau antibody (Lys174) on postmortem tissues of APOE2/3 (control), APOE2/2 (AD), APOE3/3 (AD) and APOE4/4(AD) individuals. Sample details are given in the supplementary table 1. We observed presence of neurofibrillary tangles, neuropil threads and neuritic plaque in AD patients **(Fig. 1A)**. The neurofibrillary tangles, neuropil threads and neuritic plaque are different forms of tau-associated neurofibrillary changes observed in the AD brain [33]. This triad of tau pathologies is significantly increased in APOE4/4 compared to APOE3/3 and APOE2/2 AD patients **(Fig. 1B)**.

We next determined whether APOE2 and APOE4 differentially influence tau spreading. For this purpose, we cocultured neurons from tau KO mice that express GFP from PS19 mice (carrying human P301S mutation in MAPT gene). With the cocultured neurons from these two mice, we expect that since the GFP+ neurons (tau KO) do not express endogenous tau proteins, the tau+ signal in GFP+ neurons can be considered to be the tau protein from P301S neurons. As expected, we observed the tau+ signal from the GFP+ neurons (tau KO neurons), suggesting the tau proteins were released by P301S neurons and were taken up by GFP+ neurons **(Fig. S1A).** When the neuronal cultures were treated with APOE isoforms, we found that APOE4 robustly enhanced tau spreading **(Fig. 1C, D and S1B).** Upon adding both APOE2 and APOE4 to the neuronal cultures, we observed no additional increase in tau spreading.

Interlink between GPC-4 and APOE4

Alboleda-Velasquez *et al* recently reported that an autosomal mutation carrying AD patient did not develop mild cognitive impairments until her seventies. She also had an unusual mutation in APOE3 gene (Christchurch R136S mutation). Compared to purified human APOE2 or APOE3, APOE4 proteins, APOE3 protein with a mutation a weaker interaction with heparin[16]. Authors proposed that these distinct interactions of APOE R136S with heparin may be a reason for why that autosomal mutation carrying AD patient did not show a sign of dementia and display very minimal of levels of tau spreading compared her counterparts.

We hypothesized that Heparan Sulfate Proteoglycans (HSPGs) may be involved in APOE4-mediated tau pathology. To determine which HSPGs are crucial for tau pathology, we screened a list of HSPGs and their interactions with APOE2 or APOE4, and found that GPC-4 strongly binds with APOE4 compared to APOE2. We incubated purified human GPC-4 protein either with purified human APOE2 or APOE4 protein at room temperature for 1 h as described in Figure 2A and analyzed with a native gel. APOE2+GPC-4 combination did not show any major shifts, whereas APOE4+GPC-4 mix showed a robust shift both with GPC-4 and APOE antibodies. Further, treatment with 2-mercaptoethanol disturbed the shift of GPC-4_APOE4, suggesting that GPC-4 and APOE4 are in direct interaction (**Fig. 2A**). We next validated this finding in postmortem human brain tissues. We immunoprecipitated GPC-4 proteins from APOE2/2 and APOE4/4 human brains using APOE antibody (**Fig. 2B**). The levels of eluted GPC-4 proteins were normalized by corresponding eluted APOE proteins. **Figure 2C** validates that GPC-4 preferentially binds with APOE4.

We next investigated whether GPC-4 is differentially expressed in APOE variants. IHC staining showed that APOE4-carrying AD patients expressed more GPC-4 protein in astrocytes compared to APOE4-noncarrying AD patients **(Fig. 2D, E)**. Astrocytes represent a diverse population of cells with varying complex morphology and functions[34,35]. Astrocytes with less branches are classified as resting astrocytes while astrocytes with more branches are classified as disease associated astrocytes (neurotoxic)[36,32,29]. **Figures 4F and 4G** shows that astrocytes with more branches express significantly higher levels of GPC-4 protein, suggesting that neurotoxic astrocytes express GPC-4. A1 reactive astrocytes, which are induced by microglial factors TNF-α and IL-1β, are considered as neurotoxic astrocytes [37]. As expected, TNF-α and IL-1β-treated astrocytes expressed significantly higher levels of GPC-4 protein **(Fig. S2A-C)**. Further, GPC-4 expression is blocked in the presence of NF-κB inhibitor IMD-0354 **(Fig. S2D, E)** suggesting that NF-κB dependent pathway regulates expression of GPC-4. Our results on expression of GPC-4 by neurotoxic astrocytes are supported a previous invitro study[29]. To independently validate these results, we analyzed previously published Single-cell RNA-sequencing (ScRNAseq) studies from human and mouse brains. ScRNAseq revealed the presence of AD-associated genes and astrocytes in humans [38–40]. We generated a heatmap of AD-associated genes to investigate which subtype of astrocytes express GPC-4 (**Fig. S3**). AD-associated genes are mainly expressed in astrocyte subtype 2 and 3. Interestingly, GPC-4 is expressed within AD associated astrocyte subcluster 3 (**Fig. S3**).

Glypican-4 induces tau pathology invitro and invivo

We next studied whether GPC-4 plays a role in tau pathology. We found that GPC-4 robustly induced AT8 levels in neuronal cultures (**Fig. 3A-C**). The IHC experiment yielded essentially the same result (**Fig. 3D, E**). To test the effect of GPC-4 on tau spreading, we cocultured neurons of PS19 and tau KO animals and found that GPC-4 increased tau spreading from P301S neurons to tau KO neurons (**Fig. 3F, G**). We next treated neuronal culture with astrocytes-conditioned medium (ACM) as described in **Figure 3H**. Like purified human GPC-4 protein (Fig. 2A-C), ACM-treated neurons expressed significantly higher levels of AT8 (**Fig. 3I-K**). We next treated the astrocytes with GPC-4 shRNA, and collected GPC-4 deprived ACM (**Fig. 3L**). Interestingly, GPC-4 deprived ACM failed to induce tau pathology **(Fig. 2M-O)**. To investigate the role of GPC-4 in tau pathology *in vivo*, we induced expression of GPC-4 in PS19 animals by a CRISPR/dCas9 system. After one week of incubation period, we observed a tremendous expression of GPC-4 proteins **(Fig. 4A).** Following 3 weeks of induction of GPC-4, we observed a robust phosphorylated tau protein in the CA1 region **(Fig. 4B, C)**. Additionally, induction of GPC-4 in cortical regions also induced tau accumulation **(Fig. 4D, E).**

Glypican-4 is necessary for APOE4-mediated tau propagation

We next reasoned that GPC-4 would play an important role in APOE4-mediated tau pathology. We treated the primary neuronal culture with ACM alone, APOE4+ACM or APOE4 with GPC-4 deprived ACM for 24 h and then incubated it with 1 µg/ml of human tau protein for 1 h. After washing, IHC with human tau antibody HT-7 showed that APOE4 increased tau uptake but this increase was reversed in the absence of GPC-4 (**Fig. 5A, B**). This result highlights the role of GPC-4 in regulating the APOE4-induced tau uptake. To investigate the role of GPC-4 in APOE4-induced tau pathology invivo, we isolated APOE2 or APOE4 containing particles from corresponding postmortem human brain. We injected these particles in CA1 regions on PS19 animals. APOE2 did not induce tau pathology **(Fig. 5C).** However, APOE4 robustly induced tau accumulation **(Fig. 5C, D)**. Interestingly, APOE4-mediated tau pathology was dramatically reduced in the absence of GPC-4 **(Fig. 5C, D and S3D)**.

Glypican -4 regulates APOE4-mediated membrane trafficking of LRP1 receptor

We finally wanted to understand the molecular interaction between APOE4 and GPC4 in inducing tau pathology. The LRP1 (low-density lipoprotein receptor-related protein 1) is a major APOE receptor and is suggested to be involved in tau uptake and spreading [41]. We therefore examined whether the APOE variants have differential effects on the LRP1 receptor. For this purpose, we monitored the effects of APOE2 and APOE4 on the total and surface LRP1 levels in neuronal culture. We found that the addition of APOE2 had no effect on both the total and surface LRP1 levels (**Fig. 6A-C**). By contrast, APOE4 enhanced the trafficking of surface LRP1 levels (**Fig. 6A-C**). Further, active exocytic and endocytic pathways are required for APOE4-induced surface LRP1 and APOE2 mediated downregulation of APOE4-induced surface LRP1, respectively (**Fig. S4**).

We next reasoned that GPC-4, being an astrocyte-secretory factor and APOE4 binding partner, would interact with neuronal LRP1. To test this notion, we first examined the likelihood of GPC-4–LRP1 interaction. An immunoprecipitation assay from human postmortem brain tissue revealed that GPC-4 directly interacts with LRP1**(Fig. 6D)**. In the neuronal culture, the addition of GPC-4 unaltered the total LRP1, whereas the surface LRP1 levels increased greatly **(Fig. 6E-G)**. We next investigated whether APOE4 is dependent on GPC-4 to induce surface LRP1 levels. We treated neurons with either APOE4 with astrocyte-conditioned medium (ACM) or APOE4 with GPC-4 shRNA treated ACM as shown in Figure 3H and 2L. APOE4 in the absence of GPC-4 showed a significant reduction in surface trafficking of LRP1 compared to APOE4 with ACM **(Fig. 6H-J)**. Further, in the absence of LRP1 (shRNA), GPC-4 induced tau pathology was significantly reduced in CA1 regions of PS19 mouse **(Fig. 6K, L).**

DISCUSSION

We have known for decades that APOE4-carrying individuals are at the highest risk for developing late-onset AD[7,8,42]. Nevertheless, the mechanism through which APOE4 induces AD pathology remains unknown. In addition to an earlier observation which suggested the presence of a strong correlation between tau protein accumulation and dementia[21], the recent studies have overwhelmingly demonstrated that tau accumulation/spreading is associated with neurodegeneration and dementia[16,20,25]. Human IPSCs-derived APOE4 neurons and APOE4 mouse models (PS19) displayed more tau accumulation [25,43], and the human tau PET studies agree with these observations [12,44,45]. Here, we report that GPC-4 protein drives APOE4-mediated tau uptake and propagation.

GPC-4 is one of the six members from the glypican family. GPC-4 is an astrocyte secreted protein which has been shown to regulate synaptic plasticity in the developing brain[46,47] We found that GPC-4 preferentially binds with APOE4 isoform over APOE2 isoform. A subtype of activated A1 astrocytes are considered as neurotoxic astrocytes, whose secretory molecules may be involved in worsening of AD pathology[37,48]. We showed that APOE4 carriers displayed a higher number of activated A1 astrocytes, which is consistent with severe tau pathology that we observed in APOE4 carrying AD patients. Our data also shows that GPC-4 is mainly expressed within a neurotoxic astrocytic population in APOE4 carrying AD patients. This result is consistent with snRNAseq analysis from AD patients and AD mouse models[39,49]. We also found that the expression of GPC-4 is regulated via the NF-kB pathway in the presence of microglial factors. In fact, a mouse study has shown that human APOE4 expressing animals bred with PS19 displayed an increased microglial activity[25].

Our invitro studies suggested that APOE2 induces a protective response by decreasing the AT8 levels and APOE4-induced tau spreading. This data is in agreement with a previous report where authors reported the presence of human APOE4 in PS19 animals enhanced tau pathology[25]. Although the AD field being aware that tau pathology correlates well with the onset and progression of AD, only recently the tau-based therapy has become attractive for clinical trials. At this juncture, it is critical to understand every step-in tau pathology. The role of HSPGs in Aβ pathology is well documented[50]. It is shown that HSPGs are involved in every stage of Aβ pathology in AD[51–53]. However, the role of HSPGs in tau pathology is poorly understood. An AD patient with autosomal dominant mutation who did not develop cognitive impairments for several decades had a mutation in APOE3 allele (R136S, Christchurch)[16]. While normal APOE3 strongly interacted with HSPGs invitro, APOE3 R136S showed a weak interaction[16]. This suggests that HSPGs can potentially have major impacts in tau pathology. After demonstrating that GPC-4 preferentially binds with APOE4 over APOE2, we showed that GPC-4 enhances phosphorylation of tau protein and their propagation invitro. Further activation of GPC-4 invivo using CRISPR/dCas9 system induced further tau pathology in PS19 animals. Given that mice do not express APOE isoforms, injection of APOE particles isolated from the human brain closely resembles human conditions to study the effect of their role AD pathologies. Interestingly, APOE4-induced tau pathology was greatly diminished in the absence of GPC-4, suggesting that GPC-4 plays a critical role in tau pathology. In invitro/invivo, it is shown that GPC-4 enhances neuronal excitability[46]. Similarly, it is known that APOE4-carrying AD patients/animals display hyperexcitability[54,55]. Given that neuronal activity is proposed to enhance tau

propagation[56], future studies are warranted to examine the interlink between neuronal activity and GPC-4/APOE4 in tau pathology.

It was recently proposed that APOE receptor LRP1 binds with tau protein and internalizes them[41]. We showed that APOE4 induces cellular surface trafficking of LRP1 and tau pathology. Notably, addition of APOE2 did not alter the surface levels of LRP1 and tau pathology, but attenuated APOE4-induced trafficking of LRP1 and tau pathology. These results suggest that APOE2 involves rescue mechanisms in the presence of APOE4. We further showed that GPC-4 is in the complex with LRP1 and APOE4, and APOE4-mediated surface trafficking of LRP1 is dependent on GPC-4. This differential action of APOE isoforms on surface trafficking of LRP1 thereby rely on the presence of associated factors such as GPC-4 which interacts more strongly with APOE4 than APOE2.

A limitation in our study, in fact in the AD field itself, is the lack of animal models for sporadic AD. As a result, researchers utilize either dominant mutations containing Aβ AD (such as 5xFAD) animal and frontotemporal dementia tau animal models (such as PS19) to understand AD amyloid and tau related pathology, respectively[25,57,58]. However, in sporadic AD, tau and amyloid-beta accumulate in the absence of mutations either in MAPT or APP/PSEN genes. Lack of genetically modifiable animal models to study sporadic AD has been a major setback to the field. Therefore, while other models including injection of AD brain -derived tau species have dramatically improved our understanding of tau pathology[59], modeling human sporadic AD related organisms or organoids will improve therapeutic options for human AD.

In conclusion, we found that astrocytic protein GPC-4 preferably interacts with APOE4, and poste-mortem APOE4 AD patients brains highly expressed GPC-4 in neurotoxic astrocytes. We showed that GPC-4 induced tau accumulation and propagation invitro. CRISPR/dCAS9-mediated activation of GPC-4 induced tau pathology invivo. In the absence of GPC-4, APOE4-mediated tau pathology was greatly diminished. We further demonstrated a molecular interlink between GPC-4, APOE4 and its receptor LRP1 which is also implicated in tau pathology. Together, we propose that GPC-4 works along with APOE4 and its receptor LRP1 to induce tau pathology (**Fig. S5**). Therefore, targeting GPC4/APOE4/LRP1 complexes can open therapeutic windows against tau pathology in AD.

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Figure legends

Figure 1. Post-mortem APOE4 AD patients brains accumulate more tau proteins and APOE4 enhance tau propagation. A) Representative IHC images of postmortem tissues from APOE2/3(control), APOE2/2(AD), APOE3/3 (AD) and APOE4/4 (AD) individuals stained with AT8 and aceTau (Lys174) antibodies (n=5-7). Note the presence of Neurofibrillary tangles (arrow), neuropil threads (asterisk) and neuritic plaque (arrowhead). **B**) Compared to APOE3/3 and APOE2/2, APOE4/4 shows presence of more neurofibrillary tangle-containing neurons. **C**) We treated the neurons (Tau P301S* Tau KO animals) with APOE2 or APOE4, and accessed tau pathology after 5 days. Representative IHC staining of APOE treated neuronal culture shows that APOE 4 treatments enhanced tau spreading whereas APOE2 significantly reduced APOE4-mediated tau spreading (**D**). n=4, one-way ANOVA, IHC scale bars=20 μm. **P<0.01 and ****P<0.0001.

Figure 2. APOE4 interacts with GPC-4 and APOE4 AD patients express more GPC-4 in neurotoxic astrocytes. A) GPC-4 protein was incubated either with APOE2 or APOE4 at room temperature for 1h,

and then separated by a native gel. Western blot analysis with GPC-4 and APOE antibodies reveal that combination of APOE4+GPC-4 shifted while no observable changes occurred in APOE2+GPC-4. **B, C)** Proteins isolated from APOE2/2 and APOE4/4 human brains were immunoprecipitated with APOE antibody (n=3) (B). The levels of immunoprecipitated GPC-4 proteins were normalized with corresponding APOE immunoreactive bands (C). **D, E)** Representative IHC staining of APOE2/3(control), APOE2/2(AD), APOE3/3 (AD) and APOE4/4 (AD) tissues with GFAP and GPC-4 antibodies. APOE4 carrying AD patients expressed significantly higher levels of GPC-4 in astrocytes compared to control and other APOE genotypes (E). **F, G)** Schematic IHC image of an astrocyte show that astrocytes were grouped into two categories based on the number of the branches at 15μm radius (F). Group 1: less than 10 branches. Group 2: more than 10 branches. Astrocytes with more branches expressed significantly elevated levels of GPC-4 (G). one-way ANOVA or unpaired t-test, ***P<0.001 and ****P<0.0001. IHC scale bars=20 μm.

Figure 3. GPC-4 induces tau pathology *invitro*. A-C) Western blot analysis of proteins isolated from neuronal culture which were treated with GPC-4 protein shows that GPC-4 significantly enhanced pTau levels (C) whereas no changes were observed in total tau protein (B). **D, E)** Representative IHC staining of GPC-4 treated neuronal culture with AT8 and MAP2 antibodies demonstrates that GPC-4 treatment enhanced pTau levels in neurons (E). **F, G)** Representative IHC staining of GPC-4 treated neuronal co-culture (Tau P301S* Tau KO animals) shows that GPC-4 treatment enhances tau spreading (G). **H)** Schematic diagram shows that astrocyte-conditioned media (ACM) from astrocyte culture was added to neuronal culture. **I-K)** Addition of ACM to neuronal culture increased pTau (AT8) levels whereas total tau was unaltered. **L)** Schematic diagram shows that astrocytes were treated with GPC-4 shRNA and the resulting GPC-4 deprived ACM was added to neuronal culture. **M-O)** Addition of GPC-4 deprived ACM failed to induce tau phosphorylation in neurons. n=4, unpaired t-test, IHC scale bars=20 μm. *P<0.05, **P<0.01 and ***P<0.001.

Figure 4. GPC-4 induces tau pathology *invivo*. A) In order to induce expression of GPC-4 proteins, we activated GPC-4 gene by injecting GPC-4 CRISPR/dCas9 lentivirus activation systems in cortex or hippocampus. Representative IHC with GPC-4 and GFAP antibodies show that, following 3 weeks of injection, GPC-4 CRISPR/dCas9 robustly induced GPC-4 expression compared to control lentiviral activation particles. **B, C)**. IHC images with AT8 antibody shows that GPC-4 induced significantly higher

levels of pTau in CA1 regions of hippocampus. **D, E)**. IHC images with AT8 antibody shows that GPC-4 induced significantly higher levels of pTau in the cortex. n=4-5, unpaired t-test, ***P<0.001. IHC scale bars=20 µm.

Figure 5. GPC-4 drives APOE4-mediated tau pathology. A, B) Cultured neurons (WT) were treated with ACM alone, ACM (scrambled) with APOE4 and GPC-4 deprived (shRNA treated) ACM with APOE4. After 24hrs, neurons were incubated with purified human tau proteins for 1hr, washed several times, and immunostained with human tau antibody (HT-7) to detect human tau proteins. Representative IHC staining of APOE4 + ACM treated neuronal culture shows that APOE4 treatment enhanced tau uptake, but in the absence of GPC-4 (GPC-4 shRNA treated ACM) APOE4-induced tau uptake was significantly reduced (B). **C, D)** We injected APOE2 or APOE4 particles isolated from corresponding human brains, in the absence or presence of GPC-4 shRNA. Following 3 weeks of injections, no tau pathology was detected with APOE2. APOE4 robustly induced tau pathology, but APOE4 failed to induce tau pathology in the absence of GPC-4 (D). n=4-5, one-way ANOVA, **P<0.01 and ***P<0.001. IHC scale bars=20 µm.

Figure 6. GPC-4 regulates trafficking of APOE receptor LRP1. A) Western blot analysis from neuronal culture treated with APOE (s) show that APOE4 significantly enhances surface LRP1 (S.LRP1) (C). There were no changes in total LRP1 (T.LRP1) (D). **D)** Immunoprecipitation of human postmortem brain protein samples with LRP1 antibody and subsequent western blotting revealed that LRP1 and GPC-4 are in the same complex. **E-G)** Western blot analysis from neuronal culture treated with GPC-4 protein shows that GPC-4 significantly enhanced trafficking of surface LRP1 levels (G), whereas total LRP1 levels were not affected (F). **H-J**) Western blot analysis from neuronal culture treated with ACM alone (control), APOE4 + ACM and with APOE4 + GPC-4 shRNA treated ACM shows that APOE4 induced surface expression of LRP1 is mediated by GPC-4. The GPC-4 shRNA treatment reduced the surface expression of LRP1 in the presence of APOE4 (J). **K, L)** Representative IHC image shows that GPC-4 induced tau pathology was reduced in the absence of LRP1 (L). n=5-7, one-way ANOVA or unpaired t-test, **P<0.01, ***P<0.001 and ****P<0.0001. IHC scale bars=20 µm.

Figure 1

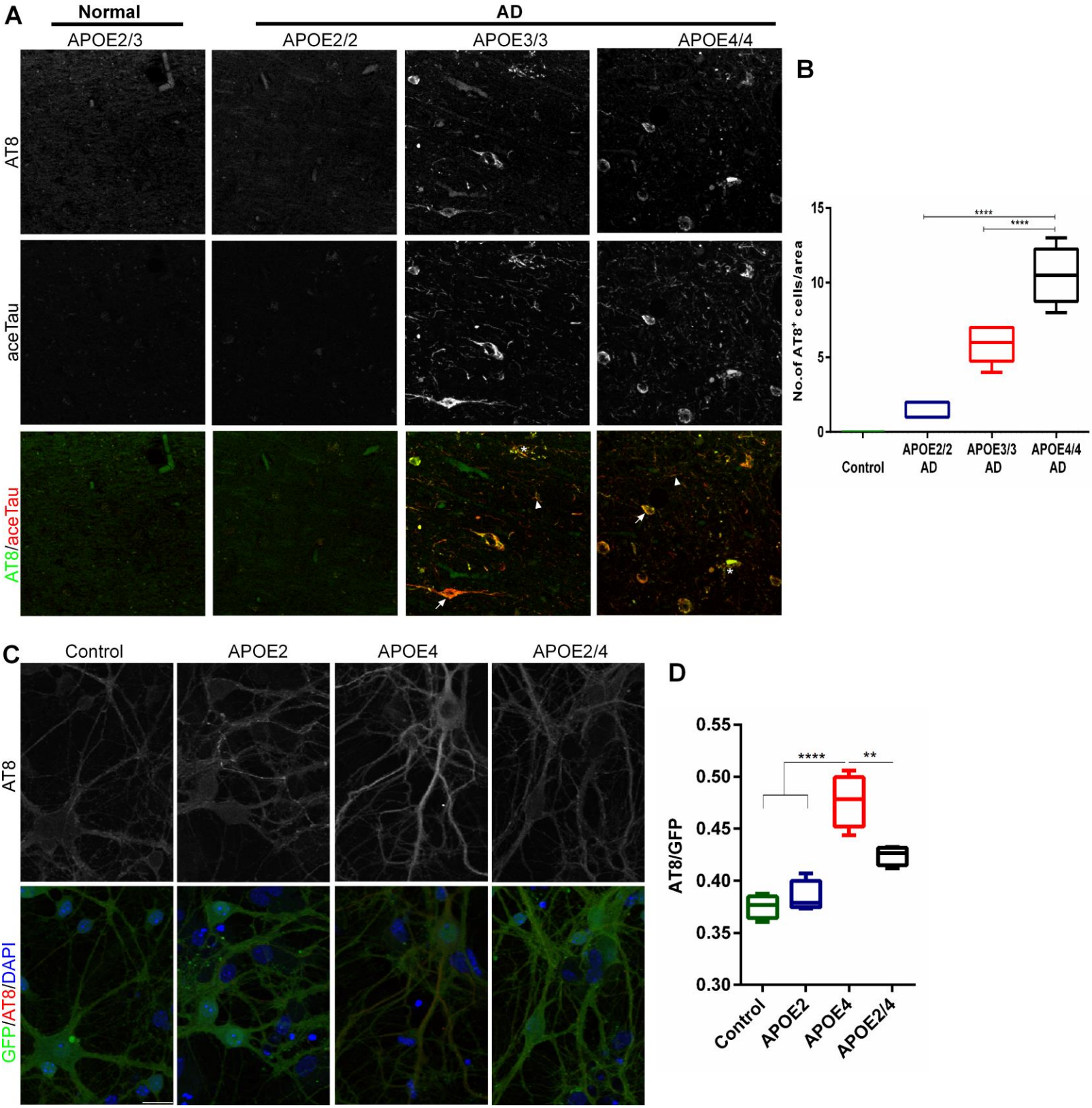
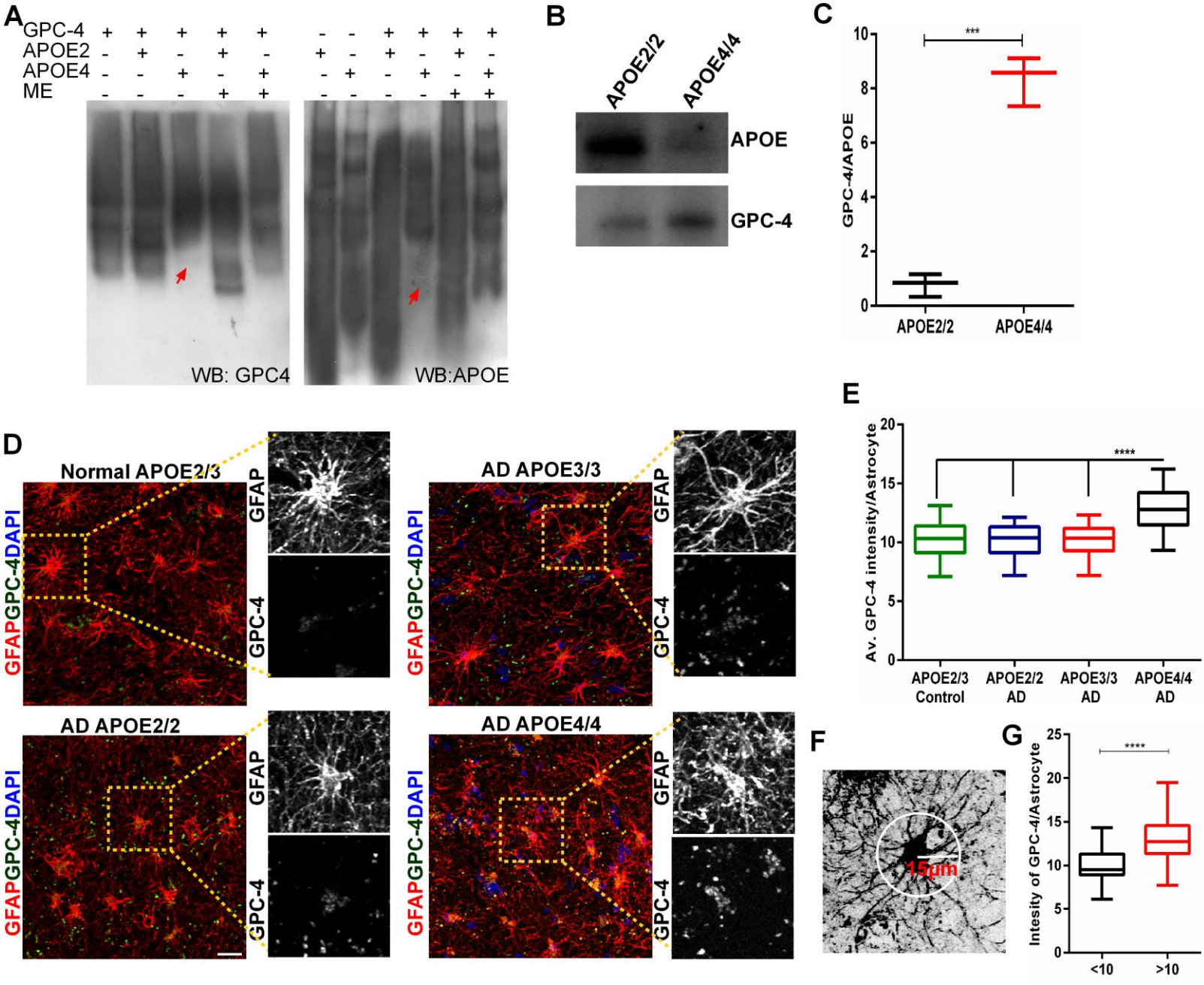


Figure 2



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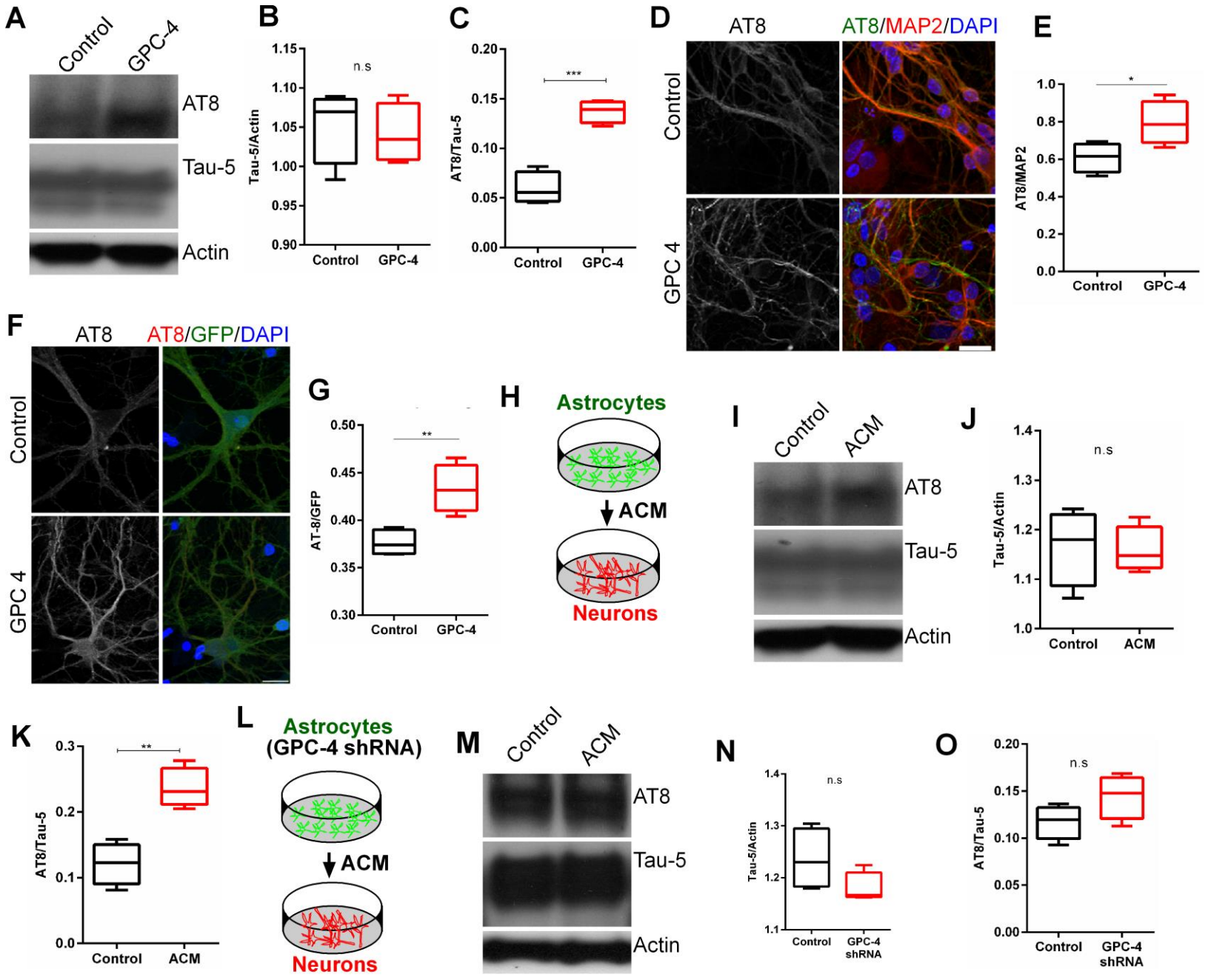


Figure 4

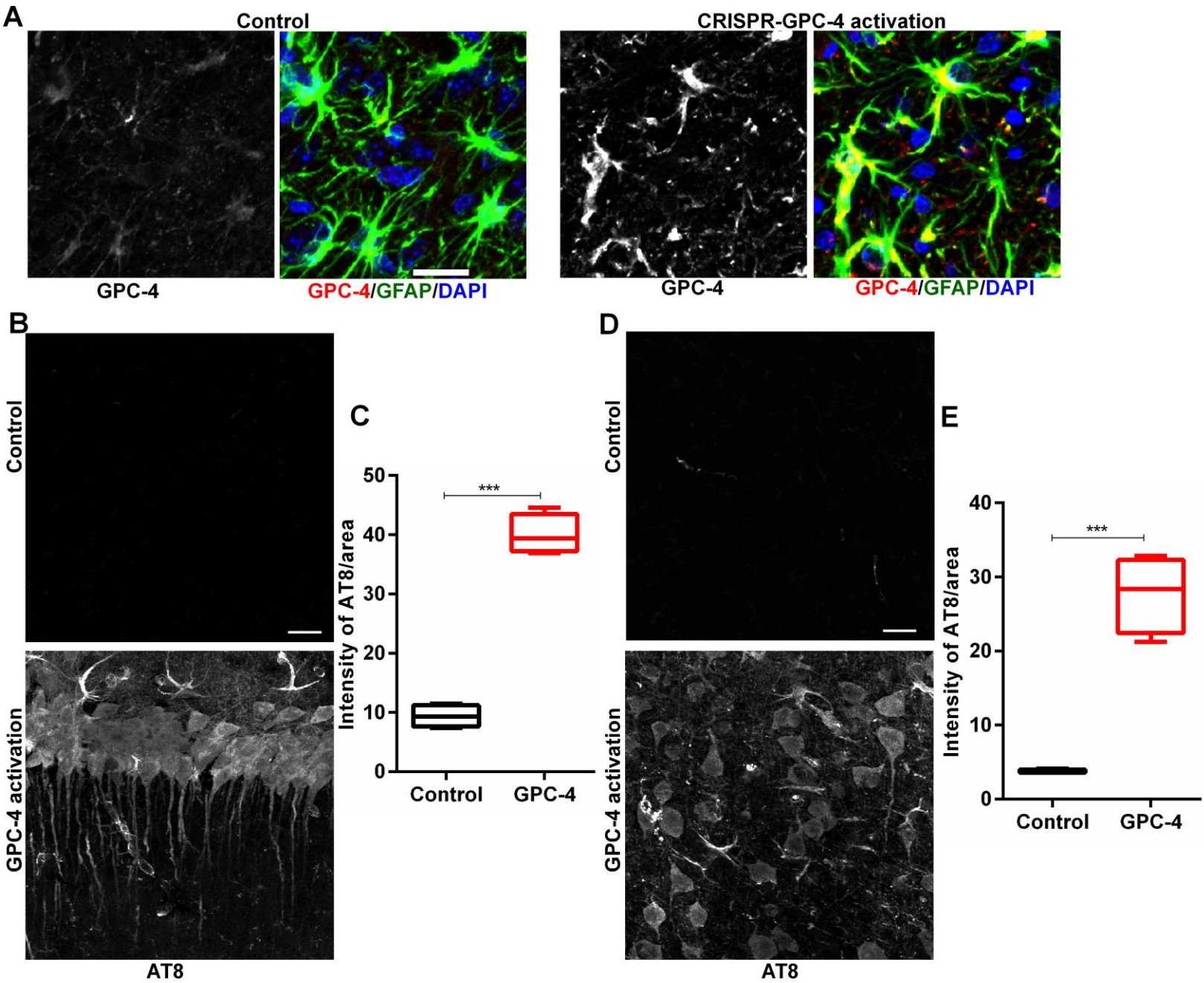
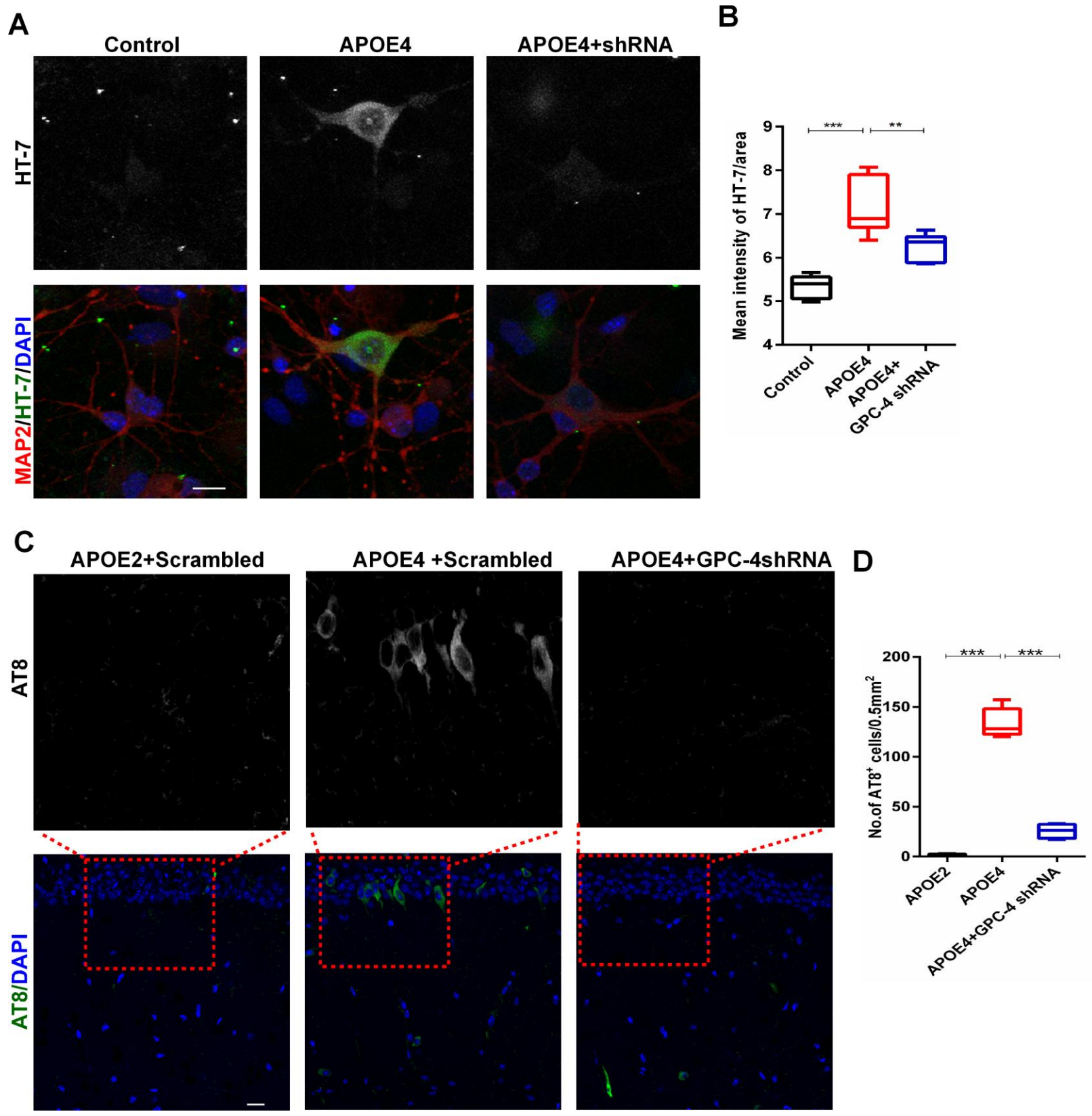
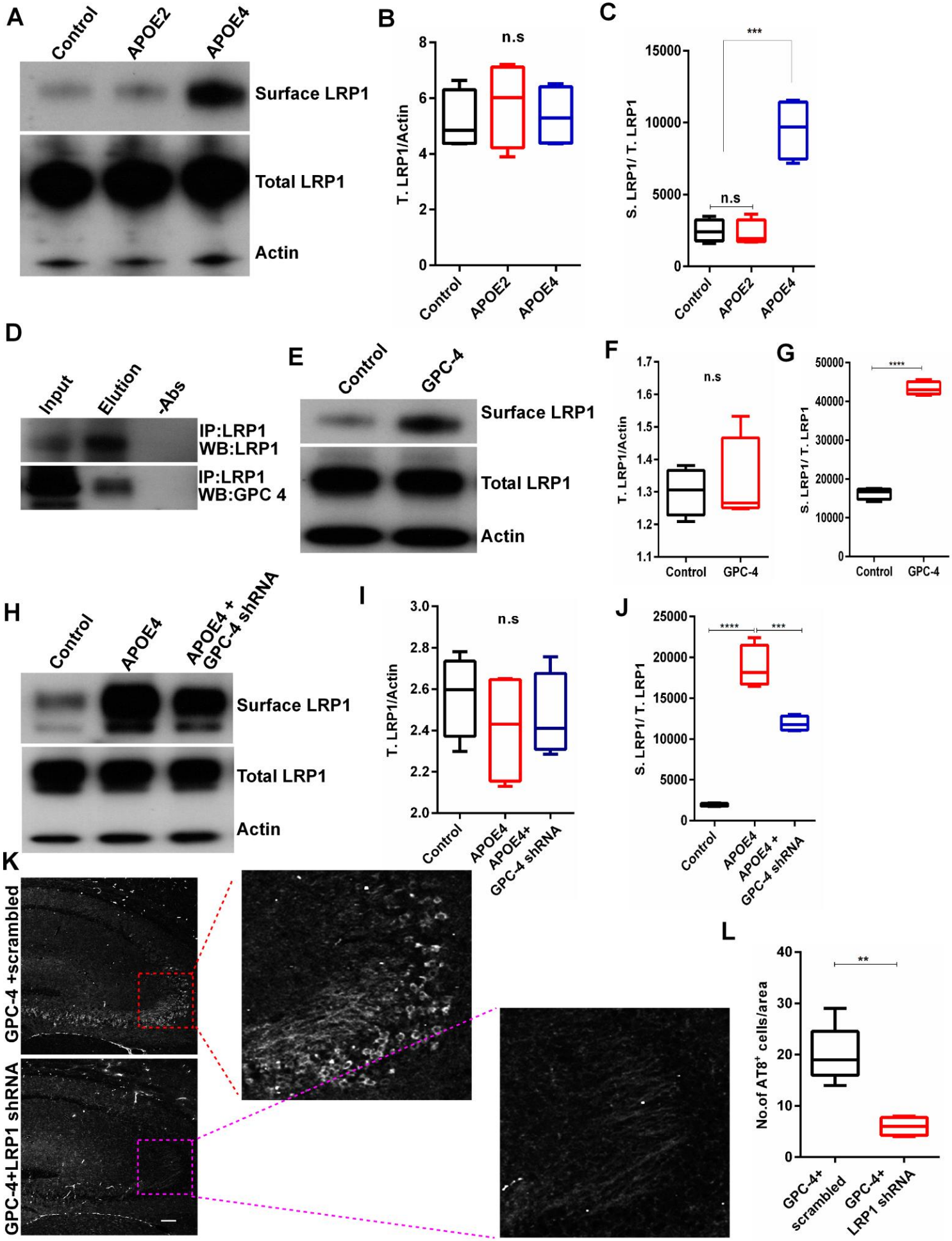
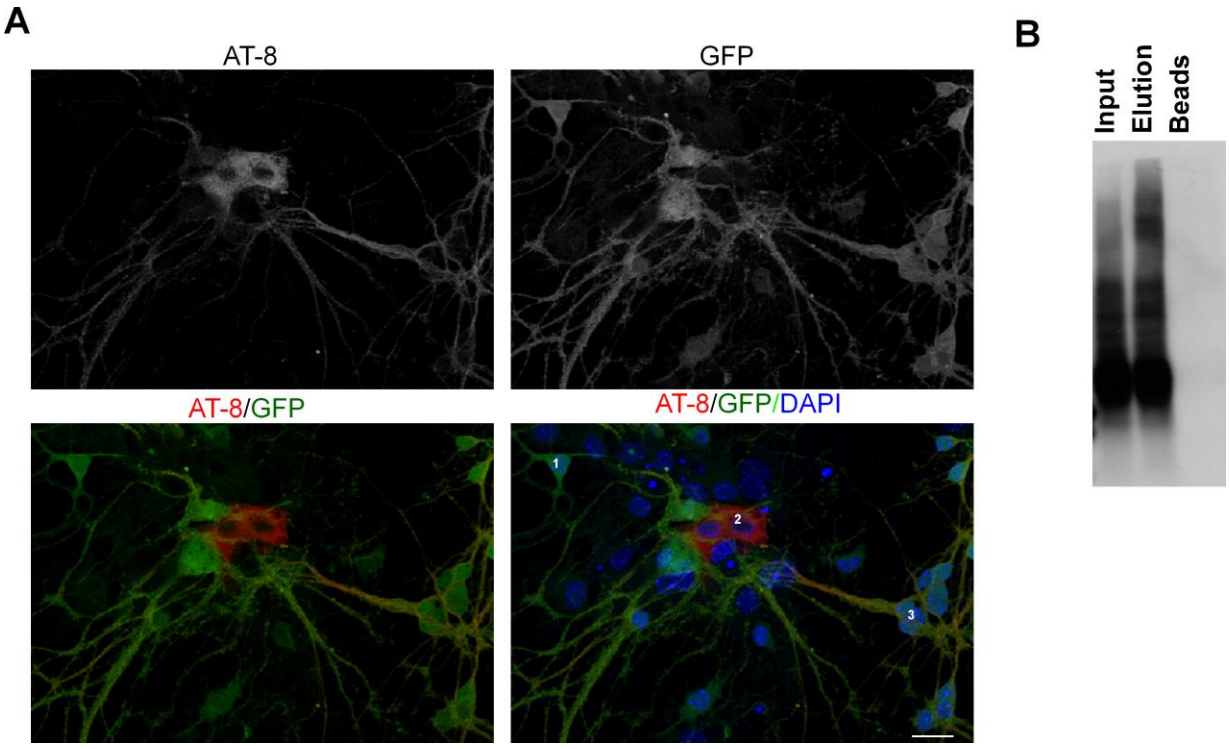


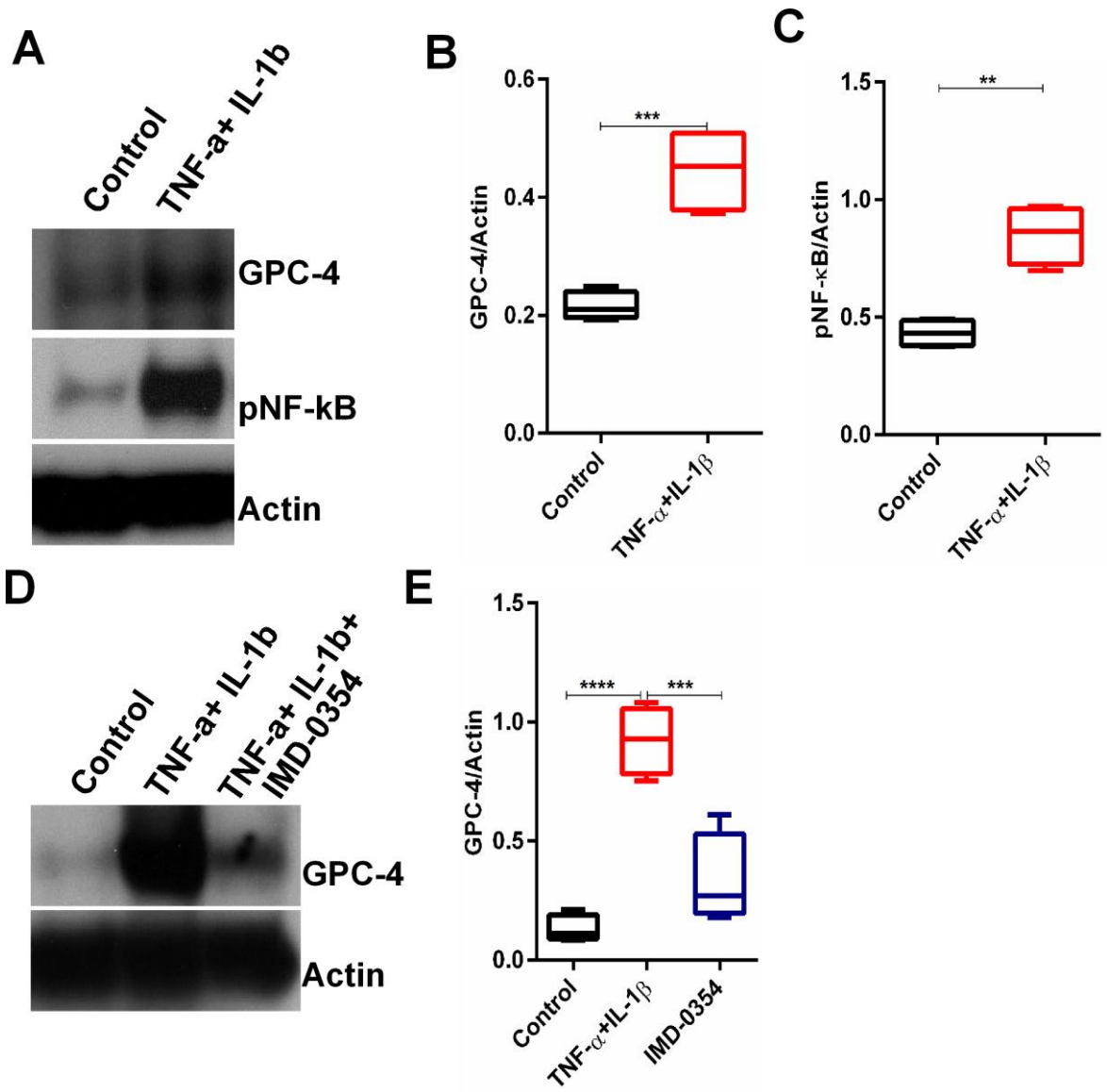
Figure 5



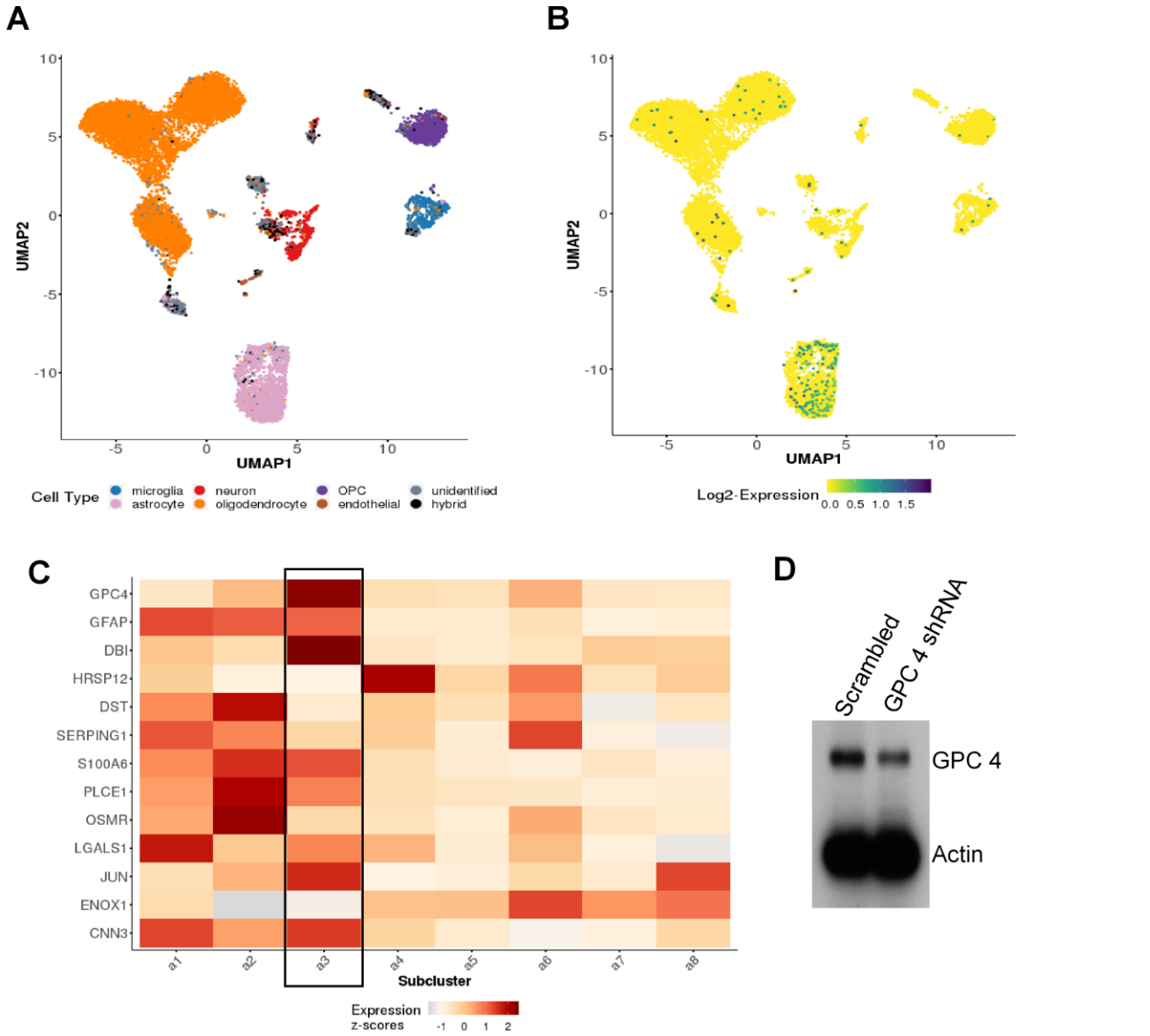




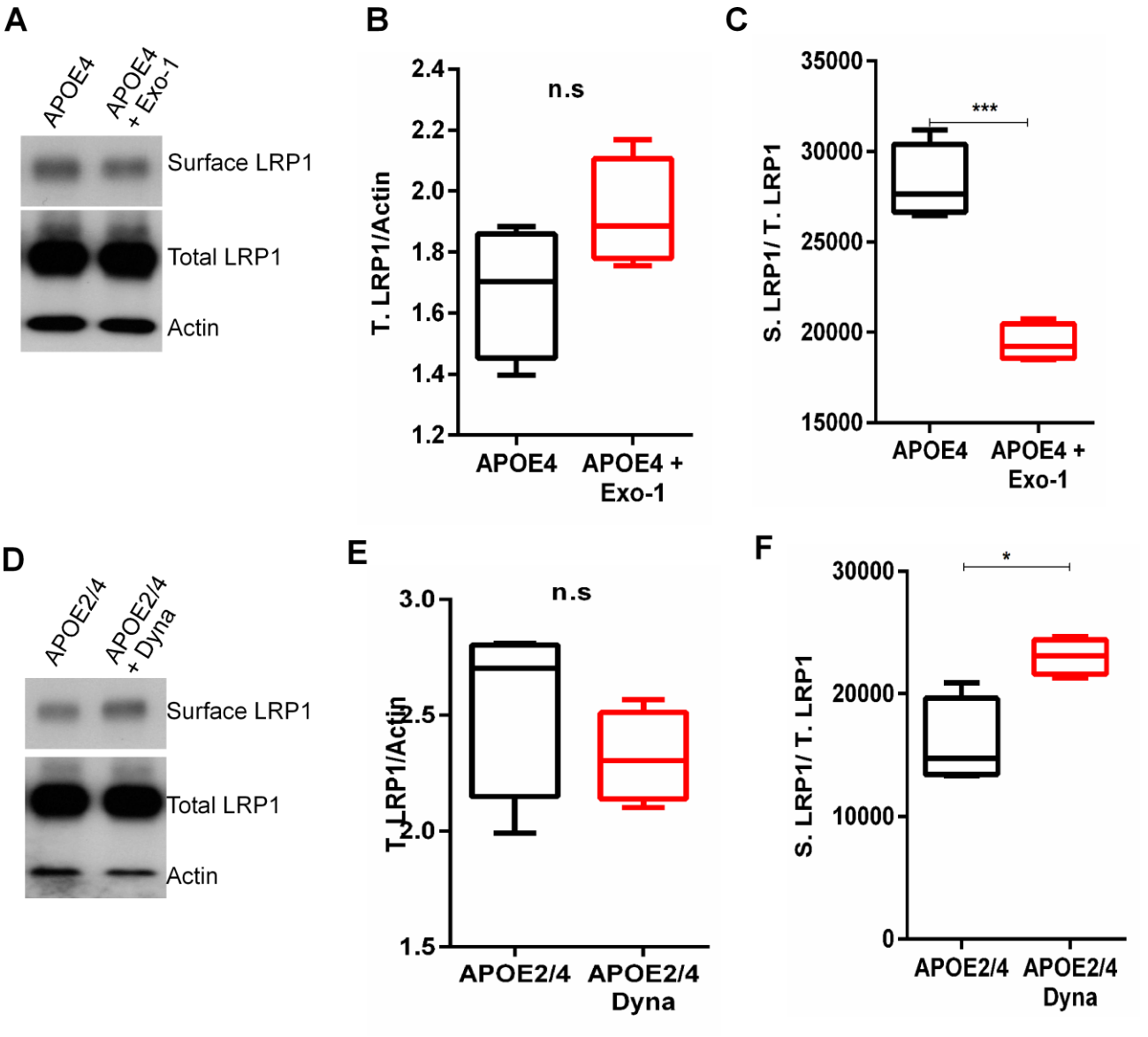
Supplementary Figure 1. A) Representative IHC staining of neuronal coculture from tau P301S* and Tau KO (GFP) animals show that tau protein (AT8) transferred from tau P301S neurons to Tau KO GFP neurons. **B)** APOE4 particles were isolated from human APOE4/4 postmortem brain. Western blot analysis with APOE antibody shows the eluted APOE particles.



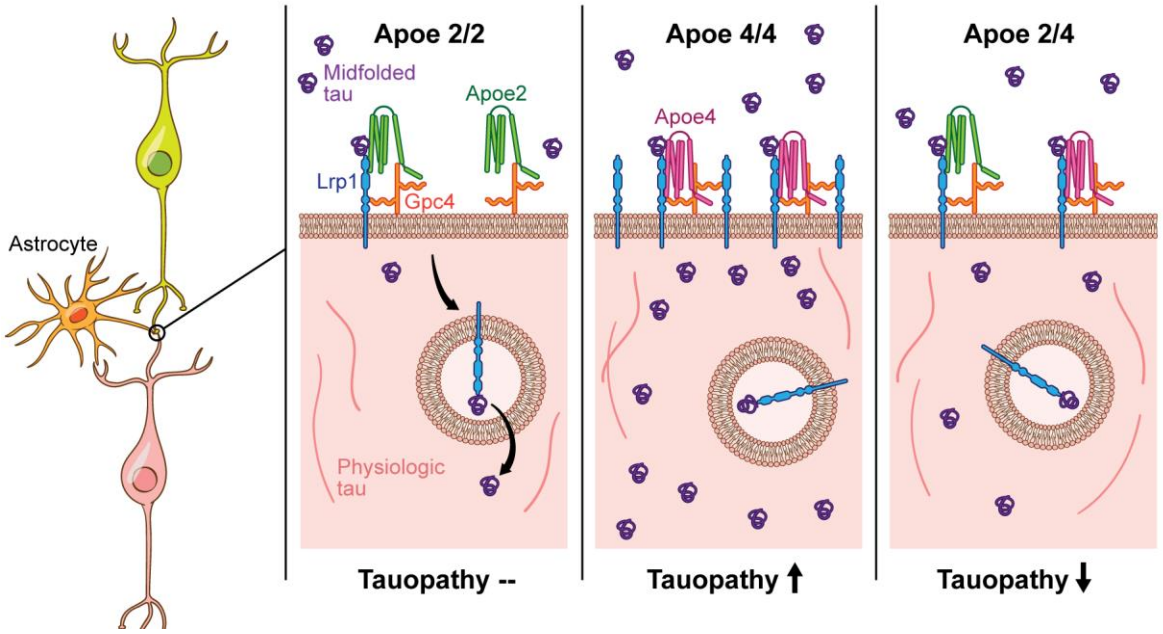
Supplementary Figure 2. A-C) Western blot analysis from astrocyte culture shows that treatment with TNF-α and IL-1β significantly increased expression of GPC-4 and activated NF-κB pathway. **D, E**) Western blot analysis from astrocyte culture treated NF-κB pathway blocker IMD-0354 reversed TNF-α and IL-1β induced expression of GPC-4. unpaired t-test, **P<0.01, ***P<0.001 and ****P<0.0001.



Supplementary Figure 3. The UMAP visualization of single nuclei shows cluster of brain cell types **(A)** and expression of GPC-4 in astrocytic cluster **(B). C)** The UMAP visualization of single nuclei shows that disease-associated genes are mainly expressed in astrocyte subtype 2 and 3. Note the enriched expression of GPC-4 in subtype 3. (Grubman et al, 2019). **D)** GPC-4 shRNA treatment reduced approximately 50% of GPC-4 expression.



Supplementary Figure 4. A) Western blot analysis from neuronal culture treated with APOE 4 or with exocytosis inhibitor Exo-1 shows that exocytosis is required for APOE4 to mediate surface expression of LRP1 (B, C). D) Western blot analysis from neuronal culture treated with APOE2/4 or with endocytosis inhibitor, Dynasore, shows that APOE2 down-regulated APOE4-mediated surface LRP1 via endocytosis (E, F). n=4, unpaired t-test, *P<0.05 and **P<0.01.



Supplementary Figure 5. This cartoon illustrates an interlink between GPC-4, APOE and LRP1 in tau pathology. GPC-4 strongly interacts with APOE4 compared to APOE2. GPC-4 mediates APOE4-induced cellular surface trafficking of LRP1 & tau uptake. We propose that GPC-4 is necessary for APOE4-induced tau pathology.

Materials and Methods

Astrocyte culture

The procedures were adapted from previous publication[46]. Briefly, P1-2 mouse cortices were microdissected, digested with papain followed by trituration in low and high ovomucoid solutions. Cells were passed through a 20 µm mesh filter, resuspended in astrocyte growth media (DMEM supplemented with 10% FBS, Glutamax, sodium pyruvate, insulin and Penicillin/Streptomycin). Plates containing cells were incubated at 37^{o}C in 5% CO_2. Once it reached the 80% of confluences, cells were washed with PBS, added conditioned medium, and incubated for three days. At the end of day 3, the medium was collected (ACM or astrocyte conditioned medium), concentrated/dialyzed using 10 kD Amicon® Ultra Centrifugal filter tubes. The concentrated ACM was added to neuronal culture. For shRNA experiments, either GPC-4 shRNA or scrambled shRNA was added to astrocyte culture when incubated with conditioned medium to knock-down GPC-4.

Neuronal culture

Cortical neuronal culture was performed from wild-type or P301S or Tau KO P1 pups as indicated throughout the manuscript. The procedures were performed as described previously with minor modifications [60]. Following dissection in Hybernate A solution, cortex was digested for 30 min in papain. Papain solution was inactivated with ovomucoid inhibitor, tissues were dissociated, and passed through a 20-µm mesh filter. To remove the cell debris and immune cells, we performed negative immunopanning in Lectin 1 coated dishes with goat-anti mouse IgG+IgM and goat-anti rat IgG antibodies. A positive immunopanning with neural cell adhesion molecule L1 antibody was performed to enrich the neurons. Neurons were cultured in Neurobasal™ Plus Medium supplemented with B27, Glutamax and Penicillin/Streptomycin. A half-of medium was replaced every 3 days. To study an effect of GPC-4 on tau pathology, neurons were treated on day 11 with 5 µg/ml of protein for 4 days. To investigate the role of APOE variants or GPC-4 on surface LRP1, neurons were treated for 24 h. GPC-4 protein was purified from frozen human tissues. We treated the neurons with 1 µg/ml APOE particles isolated from the human brain (see immunoprecipitation below). To study the role of ACM on tau pathology, neurons were treated with ACM for 4 days.

Isolation of surface proteins

To isolate cell surface proteins, Neurons were plated in 24-well plates. The cells from three wells/condition were pooled together. Cell surface proteins were biotinylated and isolated using Pierce Cell Surface Protein Isolation Method. All the steps were performed at 4^0C unless otherwise mentioned. The cells were washed briefly with PBS and incubated with Sulfo-SS-Biotin for 30 mins. After the reaction was quenched, the cells were centrifuged at 1,200rpm for 4mins, and the resulting pellet was lysed with a lysis buffer and incubated for 1 h. Some fraction of samples was collected at this stage to evaluate the total protein levels. After 1 h incubation with a lysis buffer, samples were centrifuged at 12,000 rpm for 5 mins. The supernatant was incubated with NeutrAvidin Agarose for 1 h in a rotating shaker. Unbound proteins were washed with a wash buffer 3 times. The slurry containing proteins were boiled for 5mins at 95^0C with 50 mM DTT containing SDS sample buffer. Finally, it was centrifuged at 1,200 rpm for 2 mins to collect the proteins.

Animals

All animal procedures were performed according to the Icahn School of Medicine at Mount Sinai Institutional Animal Care and Use Committee (IACUC) guidelines. 4 months old P301s animals were used for the following experiments. To induce expression of GPC-4, glypican-4 CRISPR/dCas9 lentiviral activation particles (sc-420640-LAC-2, Santa Cruz) were injected in the right CA1 region (AP-2, ML-1.3 and DV-1.5). And the same volume of control lentiviral activation particles (sc-437282, Santa Cruz) was injected in a separate cohort of animals for comparison. After one week of incubation period, animals were sacrificed and processed for IHC to access the expression of GPC-4. After 3 weeks of injection, the tau pathology was examined. In another study, 2μg of APOE4 or APOE2 particles were unilaterally injected in CA1 regions. After 24 h, one group received scrambled shRNA alone (sc-37007, Santa Cruz), one group of APOE4 injected animals received scrambled shRNA and another APOE4 injected group received GPC-4 shRNA (sc-145457-V, Santa Cruz). Animals were sacrificed after 3 weeks of injection for further analysis.

Protein binding assays

To investigate whether GPC-4 differentially interacts with APOE2 and APOE4, the protein binding assay was performed. The concentration of any indicated protein was at 1 μg/μl. The final volume was made to

five microliters with Tris buffer. The mixture was incubated at a shaker for one hour. The samples were boiled with or without 2-mercaptoethanol and separated by a native gel.

Immunoprecipitation

The total protein was isolated from frozen human brain samples using 1% Triton in Tris buffer. Briefly, Pierce Direct IP method was used to isolate proteins of interest. The AminoLink Plus Coupling Resin was washed with a coupling buffer, and the resin was incubated with 10μg of LRP1 or APOE antibodies for 2 h in a rotator. The unbound antibodies were washed away, and 1-3 μg of isolated proteins was added to resin and incubated overnight, 4^0C, in a rotator. It was washed three times to remove unbound proteins, and antibody-coupled proteins were eluted and verified with western blot. In addition, isolated APOE particles were also used for invitro/invivo studies. For this purpose, the isolated particles were dialyzed with sterile PBS to remove any contaminants.

Electrophoresis and western blot

Samples were lysed with SDS-lysis buffer (Laemmli Sample Buffer, Biorad), boiled at 95^0C for 5 mins with 50 mM DTT, and separated based on their molecular weights using TGX Stain-Free Gels (Biorad). The separated proteins were transferred to PVDF membrane, blocked with 2% BSA for 1 h, incubated with primary antibodies overnight at 4^0C. Membranes were washed with PBST 6 x10 mins, incubated with HRP-conjugated secondary antibodies for 1 h, again washed with PBST 6 x10 mins and then developed using ECL solution (Clarity Western ECL substrate, Biorad). The following primary antibodies were used: TAU-5 (Thermo Scientific), AT-8 (Thermo Scientific), Actin (Cell Signaling), LRP1 (Abcam), APOE (Abcam), Glypican-4 (Thermo Scientific and Sigma).

Tau uptake assay

On day 11 of neuronal culture, the cells were treated either with GPC-4 shRNA, LRP1 shRNA or scrambled shRNA. After 24 h of treatment, it was incubated with 5 μg/ml APOE4 protein for 24 h as described in the manuscript. Finally, the neurons were incubated with 1 μg/ml of human tau protein for 1h and washed with PBS 3 times before proceeding to immunohistochemistry.

Immunohistochemistry and imaging

The cultured cells containing coverslips were fixed for an hour with 4% paraformaldehyde. After a brief wash with PBS, incubated with 10% Donkey serum in PBST for 30 min. Primary antibodies were added and incubated for 48 h at 4^0C, washed 3 x 10 mins with PBST, probed with corresponding secondary antibodies for 2 h, then washed again with PBST before counterstaining with DAPI. The tissues were mounted with Aqua-Poly/Mount and imaged using Leica TCS SP5 confocal laser scanning microscope. The following primary antibodies were used: TAU-5 (Thermo Scientific), AT8 (Thermo Scientific), MAP2 (Millipore), LRP1(Abcam), HT-7 (Thermo Scientific) and GFP (Abcam).

Data and statistical analysis

Statistical analyses were performed with Graphpad prism software. Unpaired t-test or One-way ANOVA with Bonferroni correction were used. Quantification of western blots and immunocytochemical staining were performed by Image J software. a) For western blot quantification, the intensity of each immunoreactive band was measured and then normalized to the corresponding beta-actin immunoreactive band. Normalized values were grouped and compared for statistical analysis. b) To analyze tau uptake (HT-7 staining), we imaged the neurons with 40x objective. Before staining, the coverslips were washed thoroughly with PBS to remove any extracellular tau. Four different places/coverslip were imaged. The intensity of HT-7 staining in the imaged area was quantified. c) Number of AT8 positive cells/area were counted manually in a blinded manner. The intensity of AT8 staining/area was measured using Image J. 3-5 slices per animal were used for quantification purposes. d) Activated astrocytes were quantified as described before[32,36]. Briefly, astrocytes were imaged with 60x objective, z-series with 0.5µm distance. Number of cellular processes leaving the soma was assessed in the z-series. Based on the number of cellular processes at 15µm radius from the center of the cell body, the astrocytes were grouped into two categories as described in the main text. e) scRNAseq data was analyzed using a link provided by the authors[39]. The genes related to disease-associated astrocytes from a mouse scRNAseq study was retrieved[40]. These disease astrocytic genes were plotted with GPC-4 in human astrocytic sub- clusters[39] to examine whether disease associated human astrocytes express GPC-4.