

1 ***INPP5D* expression is associated with risk for Alzheimer's disease and induced by**
2 **plaque-associated microglia**

3 Andy P. Tsai^{1#}, Peter Bor-Chian Lin^{1#}, Chuanpeng Dong^{2#}, Miguel Moutinho¹, Brad T. Casali^{1,3},
4 Yunlong Liu², Bruce T. Lamb^{1,4}, Gary E. Landreth^{1,5}, Adrian L. Oblak^{1,6*}, Kwangsik Nho^{6*}

5 ¹. Stark Neurosciences Research Institute, IUSM, Indianapolis, IN, USA. tandy@iu.edu (A.P.T);
6 pblin@iu.edu (P.B.L); mmoutinh@iu.edu (M.M); glandret@iu.edu (G.E.L); btlamb@iu.edu
7 (B.T.L); aoblak@iupui.edu (A.L.O)

8 ². Department of Medical and Molecular Genetics, Center for Computational Biology and
9 Bioinformatics, IUSM, Indianapolis, IN, USA. cpdong@iu.edu (C.D); yunliu@iu.edu (Y.L)

10 ³. Department of Neurosciences, Case Western Reserve University, School of Medicine,
11 Cleveland, OH, USA. btc8@case.edu (B.T.C).

12 ⁴. Department of Medical and Molecular Genetics, IUSM, Indianapolis, IN, USA. btlamb@iu.edu
13 (B.T.L)

14 ⁵. Department of Anatomy and Cell Biology, IUSM, Indianapolis, IN, USA. glandret@iu.edu
15 (G.E.L)

16 ⁶. Department of Radiology & Imaging Sciences, IUSM, Indianapolis, IN, USA.
17 aoblak@iupui.edu (A.L.O); knho@iupui.edu (K.N)

18 **Corresponding Author:** *For correspondence contact

19 Adrian L. Oblak, Department of Radiology & Imaging Sciences, IUSM, Indianapolis, IN, USA,
20 Methodist GH4101, RADY, Indianapolis, IN, 46202, USA. Phone: +1-317-274-0107; Email:
21 aoblak@iupui.edu

22 Kwangsik Nho, Department of Radiology & Imaging Sciences, IUSM, Indianapolis, IN, USA,
23 Methodist GH4101, RADY, Indianapolis, IN, 46202, USA. Phone: +1-317-963-7503; Email:
24 knho@iupui.edu

25 *Corresponding author

26 #Contributed equally

27 **Abstract**

28 **Background**

29 Alzheimer's disease (AD) is a progressive neurodegenerative disorder characterized by
30 cognitive decline, robust microgliosis, neuroinflammation, and neuronal loss. Genome-wide
31 association studies recently highlighted a prominent role for microglia in late-onset AD (LOAD).
32 Specifically, inositol polyphosphate-5-phosphatase (*INPP5D*), also known as SHIP1, is
33 selectively expressed in brain microglia and has been reported to be associated with LOAD.
34 Although *INPP5D* is likely a crucial player in AD pathophysiology, its role in disease onset and
35 progression remains unclear.

36 **Methods**

37 We performed differential gene expression analysis to investigate *INPP5D* expression in
38 LOAD and its association with plaque density and microglial markers using transcriptomic
39 (RNA-Seq) data from the Accelerating Medicines Partnership for Alzheimer's Disease (AMP-
40 AD) cohort. We also performed quantitative real-time PCR, immunoblotting, and
41 immunofluorescence assays to assess *INPP5D* expression in the 5xFAD amyloid mouse model.

42 **Results**

43 Differential gene expression analysis found that *INPP5D* expression was upregulated in
44 LOAD and positively correlated with amyloid plaque density. In addition, in 5xFAD mice, *Inpp5d*
45 expression increased as the disease progressed, and selectively in plaque-associated
46 microglia. Increased *Inpp5d* expression levels in 5xFAD mice were abolished entirely by
47 depleting microglia with the colony-stimulating factor receptor-1 antagonist PLX5622.

48 **Conclusions**

49 Our findings show that *INPP5D* expression increases as AD progresses, predominantly
50 in plaque-associated microglia. Importantly, we provide the first evidence that increased
51 *INPP5D* expression might be a risk factor in AD, highlighting *INPP5D* as a potential therapeutic

52 target. Moreover, we have shown that the 5xFAD mouse model is appropriate for studying
53 *INPP5D* in AD.

54

55 **Keywords:** Alzheimer's disease (AD), Microglia, *INPP5D*, AD risk, Plaque

56

57 **Background**

58 Alzheimer's disease (AD) is the most common cause of dementia, with pathogenesis
59 arising from perturbed β -amyloid ($A\beta$) homeostasis in the brain [1]. The mechanisms underlying
60 the development of the most common form of AD, late-onset AD (LOAD), are still unknown.
61 Microglia, the primary immune cells in the brain play a crucial role in AD pathogenesis [2].
62 Recent large-scale genome-wide association studies (GWAS) reported that many genetic loci
63 associated with LOAD risk are related to inflammatory pathways, suggesting that microglia are
64 involved in modulating AD pathogenesis [3, 4]. Among the microglia-related genetic factors in
65 LOAD, a common variant in *INPP5D* (phosphatidylinositol 3,4,5-trisphosphate 5-phosphatase
66 1), rs35349669, confers an increase in LOAD risk (OR=1.08) [4, 5]. Conversely, the intronic
67 *INPP5D* variant rs61068452 is associated with a reduced CSF t-tau/ $A\beta$ 1-42 ratio, plays a
68 protective role in LOAD ($p=1.48E-07$) [6]. *INPP5D* encodes inositol polyphosphate-5-
69 phosphatase which participates in regulation of microglial gene expression [7]. Specifically,
70 *INPP5D* inhibits signal transduction initiated by activation of immune cell surface receptors,
71 including Triggering receptor expressed on myeloid cells 2 (TREM2), Fc gamma receptor
72 (Fc γ R) and Dectin-1 [8]. The conversion of PI(3,4,5)P3 to PI(3,4)P2 is catalyzed by *INPP5D*
73 following its translocation from the cytosol to the cytoplasmic membrane. The loss of
74 PI(3,4,5)P3 prevents the activation of the immune cell surface receptors [9]. Interestingly,
75 genetic variants of TREM2, Fc γ R, and Dectin-1 are also associated with increased AD risk [10-
76 12] and are potentially involved in regulating *INPP5D* activity. Inhibiting *INPP5D* promotes

77 microglial proliferation, phagocytosis, and increases lysosomal compartment size [13]. Although
78 *INPP5D* has been shown to play an important role in microglial function, its role in AD remains
79 unclear.

80 Here, we report that *INPP5D* is upregulated in LOAD, and elevated *INPP5D* expression
81 levels are associated with microglial markers and amyloid plaque density. Furthermore, in the
82 5xFAD mouse model, we found a disease-progression-dependent increase in *INPP5D*
83 expression in plaque-associated microglia. Our results suggest that *INPP5D* plays a role in
84 microglia phenotypes in AD and is a potential target for microglia-focused AD therapies.

85 **Methods**

86 **Human participants and RNA-Seq**

87 RNA-Seq data were obtained from the AMP-AD Consortium, including participants of the
88 Mayo Clinic Brain Bank cohort, the Mount Sinai Medical Center Brain Bank (MSBB) cohort, and
89 the Religious Orders Study and Memory and Aging Project (ROSMAP) cohort.

90 In the Mayo Clinic RNA-Seq dataset [14], the RNA-Seq-based whole transcriptome data
91 were generated from human samples of 151 temporal cortices (TCX) (71 cognitively normal
92 older adult controls (CN) and 80 LOAD) and 151 cerebella (CER) (72 CN and 79 LOAD). LOAD
93 participants met the neuropathological criteria for AD (Braak score ≥ 4.0), and cognitively normal
94 participants had no neurodegenerative diagnosis (Braak score ≤ 3.0).

95 In the MSBB dataset [15], data were generated from human samples from CN, mild
96 cognitive impairment (MCI), and LOAD participants' parahippocampal gyrus (PHG) and inferior
97 frontal gyrus (IFG), superior temporal gyrus (STG) and frontal pole (FP). The clinical dementia
98 rating scale (CDR) was used to assess dementia and cognitive status [16]. LOAD patients had a
99 CDR ≥ 0.5 , while MCI and CN participants had a CDR of 0.5 and 0, respectively. CN participants
100 had no significant memory concerns. This study included 108 participants (16 CN, 14 MCI, and

101 78 LOAD) for PHG, 137 participants (21 CN, 18 MCI, and 98 LOAD) for STG, 136 participants
102 (18 CN, 16 MCI, and 102 LOAD) for IFG, and 153 participants (22 CN, 20 MCI, and 111 LOAD)
103 for FP.

104 In the ROSMAP dataset [17], RNA-Seq data were generated from the dorsolateral
105 prefrontal cortices of 241 participants (86 CN and 155 LOAD).

106 **Animal models**

107 Wild-type (WT) and 5xFAD mice were maintained on the C57BL/6J background (JAX MMRRC
108 Stock# 034848) for IHC and qPCR studies. Two-, four-, six-, eight-, and twelve-month-old mice
109 were used. In the PLX5622 study, we used WT and 5xFAD mice maintained on the mixed
110 C57BL/6J and SJL background [B6SJL-Tg (APP^{SwF1L}on, PSEN1^{*M146L*L286V}) 6799Vas,
111 Stock #34840-JAX] (**Fig. 3e and 3f**). The 5XFAD transgenic mice overexpress five FAD
112 mutations: the APP (695) transgene contains the Swedish (K670N, M671L), Florida (I716V),
113 and London (V717I) mutations and the PSEN1 transgene contains the M146L and L286V FAD
114 mutations. Up to five mice were housed per cage with SaniChip bedding and
115 LabDiet® 5K52/5K67 (6% fat) feed. The colony room was kept on a 12:12 hr. light/dark
116 schedule with the lights on from 7:00 am to 7:00 pm daily. They were bred and housed in
117 specific-pathogen-free conditions. Both male and female mice were used.

118 **PLX5622 animal treatment**

119 At four months of age, either normal rodent diet or PLX5622-containing chow was
120 administered to 5XFAD mice for 28 days. An additional cohort of four-month-old mice was
121 treated with PLX5622 or control diet for 28 days, then discontinued from PLX5622 feed and fed
122 a normal rodent diet for an additional 28 days. At six months of age, this cohort of mice was
123 euthanized. Plexikon Inc. provided PLX5622 formulated in AIN-7 diet at 1200 mg/kg [18].

124 **Statistical analysis**

125 In the human study, differential expression analysis was performed using *limma* software
126 [19] to investigate the diagnosis group difference of *INPP5D* between CN, MCI, and LOAD. Age,
127 sex, and *APOE ε4* carrier status were used as covariates. To investigate the association
128 between *INPP5D* expression levels and amyloid plaque density or expression levels of
129 microglia-specific markers (*AIF1* and *TMEM119*), we used a generalized linear regression
130 model with *INPP5D* expression levels as a dependent variable and plaque density or microglia-
131 specific markers along with age, sex, and *APOE ε4* carrier status as explanatory variables. The
132 regression was performed with the "glm" function from the stats package in R (version 3.6.1).

133 In the mouse study, GraphPad Prism (Version 8.4.3) was used to perform the statistical
134 analyses. Differential expression analysis of both gene and protein levels between WT and
135 5xFAD mice was performed using unpaired Student's t-test. The statistical comparisons
136 between mice with and without PLX5622 treatments were performed with one-way ANOVA
137 followed by Tukey's posthoc test. Graphs represent the mean and standard error of the mean.

138 **RNA extraction and quantitative real-time PCR**

139 Mice were anesthetized with Avertin and perfused with ice-cold phosphate-buffered
140 saline (PBS). The cortical and hippocampal regions from the hemisphere were micro-dissected
141 and stored at -80°C. Frozen brain tissue was homogenized in buffer containing 20 mM Tris-HCl
142 (pH=7.4), 250 mM sucrose, 0.5 mM EGTA, 0.5 mM EDTA, RNase-free water, and stored in an
143 equal volume of RNA-Bee (Amsbio, CS-104B) at -80°C until RNA extraction. RNA was isolated
144 by chloroform extraction and purified with the Purelink RNA Mini Kit (Life Technologies
145 #12183020) with an on-column DNase Purelink Lit (Life Technologies #12183025). 500 ng RNA
146 was converted to cDNA with the High-Capacity RNA-to-cDNA Kit (Applied Biosystems
147 #4388950), and qPCR was performed on a StepOne Plus Real-Time PCR system (Life
148 Technologies). Relative gene expression was determined with the $2^{-\Delta\Delta CT}$ method and assessed
149 relative to *Gapdh* (Mm99999915_g1). *Inpp5d* primer: Taqman Gene Expression Assay (*Inpp5d*:

150 Mm00494987_m1 from the Life Technologies). Student's *t*-test was performed for qPCR
151 assays, comparing WT with 5xFAD animals.

152 **Immunofluorescence**

153 Brains were fixed in 4% PFA overnight at 4°C. Following overnight fixation, brains were
154 cryoprotected in 30% sucrose at 4°C and embedded. Brains were processed on a microtome as
155 30 µm free-floating sections. For immunostaining, at least three matched brain sections were
156 used. Free-floating sections were washed and permeabilized in 0.1% Triton in PBS (PBST),
157 followed by antigen retrieval using 1x Reveal Decloaker (Biocare Medical) at 85°C for 10 mins.
158 Sections were blocked in 5% normal donkey serum in PBST for 1 hr. at room temperature (RT).
159 The following primary antibodies were incubated in 5% normal donkey serum in PBST overnight
160 at 4°C: IBA1 (Novus Biologicals #NB100-1028 in goat, 1:1000); 6E10 (BioLegend #803001 in
161 mouse, 1:1000; AB_2564653); and SHIP1/INPP5D (Cell Signaling Technology (CST) #4C8,
162 1:500, Rabbit mAb provided by CST in collaboration with Dr. Richard W. Cho). Sections were
163 washed and visualized using respective species-specific AlexaFluor fluorescent antibodies
164 (diluted 1:1000 in 5% normal donkey serum in PBST for 1 hr. at RT). Sections were
165 counterstained and mounted onto slides. For X-34 staining (Sigma, #SML1954), sections were
166 dried at RT, rehydrated in PBST, and stained for ten mins at RT. Sections were then washed
167 five times in double-distilled water and washed again in PBST for five mins. Images were
168 acquired on a fluorescent microscope with similar exposure and gains across stains and
169 animals. Images were merged using ImageJ (NIH).

170 **Immunoblotting**

171 Tissue was extracted and processed as described above, then centrifuged. Protein
172 concentration was measured with a BCA kit (Thermo Scientific). 50 µg of protein per sample
173 was boiled in SDS-PAGE protein sample buffer for 10 mins at 95°C, loaded into 4-12% Bis-Tris

174 gels (Life Technologies) and run at 100 V for 90 mins. The following primer antibodies were
175 used: SHIP1/INPP5D (CST #4C8 1:500, Rabbit mAb) and GAPDH (Santa Cruz #sc-32233).
176 Each sample was normalized to GAPDH, and the graphs represent the values normalized to the
177 mean of the WT mice group at each time point.

178 **Results**

179 ***INPP5D* expression levels are increased in LOAD.**

180 INPP5D is a member of the inositol polyphosphate-5-phosphatase (INPP5) family and
181 possesses a set of core domains, including an N-terminal SH2 domain (amino acids 5-101),
182 Pleckstrin homology-related (PH-R) domain (amino acids 292-401), lipid phosphatase region
183 (amino acids 401-866) with C2 domain (amino acids 725-863), and C-terminal proline-rich
184 region (amino acids 920-1148) with two SH3 domains (amino acids 969-974 and 1040-1051)
185 (**Fig. 1a**). Differential expression analysis was performed using RNA-Seq data from seven brain
186 regions from the AMP-AD cohort. Expression levels of *INPP5D* were increased in the temporal
187 cortex (logFC=0.35, p=1.12E-02; **Fig. 1b**), parahippocampal gyrus (logFC=0.54, p=7.17E-03;
188 **Fig. 1c**), and inferior frontal gyrus (logFC=0.44, p=2.33E-03; **Fig. 1d**) of LOAD patients with age
189 and sex as covariates (**Table 1**). Interestingly, *INPP5D* expression was also found to be
190 increased in the inferior frontal gyrus of LOAD patients compared with MCI subjects
191 (logFC=0.45, p=6.76E-03; **Fig. 1d**). Results were similar when *APOE* ϵ 4 carrier status was used
192 as an additional covariate. *INPP5D* remained overexpressed in the temporal cortex
193 (logFC=0.34, p=2.75E-02), parahippocampal gyrus (logFC=0.53, p=1.08E-02), and inferior
194 frontal gyrus (logFC=0.42, p=4.35E-03) of LOAD patients. However, we did not find any
195 differences between the diagnosis groups in the cerebellum, frontal pole, superior temporal
196 gyrus, or dorsolateral prefrontal cortex (**Table 1**). To examine whether *INPP5D* was associated
197 with microglia, we analyzed the association between *INPP5D* and microglia-specific marker
198 genes (*AIF1* and *TMEM119*). *AIF1* and *TMEM119* were significantly associated with *INPP5D*

199 expression levels in the parahippocampal gyrus (*AIF1*: $\beta=0.4386$, $p=4.10E-07$; *TMEM119*:
200 $\beta=0.7647$, $p<2E-16$), inferior frontal gyrus (*AIF1*: $\beta=0.2862$, $p=6.36E-08$; *TMEM119*: $\beta=0.6109$,
201 $p<2E-16$), frontal pole (*AIF1*: $\beta=0.2179$, $p=4.53E-04$; *TMEM119* $\beta=0.5062$, $p=4.00E-15$), and
202 superior temporal gyrus (*AIF1*: $\beta=0.3013$, $p=5.36E-07$; *TMEM119*: $\beta=0.6914$, $p<2E-16$) (**Table**
203 **2**).

204 ***INPP5D* expression levels are associated with amyloid plaque density in the human** 205 **brain.**

206 We investigated the association between *INPP5D* expression levels and mean amyloid
207 plaque densities in four brain regions (**Table 2**). Expression levels of *INPP5D* were associated
208 with amyloid plaques in the parahippocampal gyrus ($\beta=0.0212$, $p=3.02E-03$; **Fig. 2a**), inferior
209 frontal gyrus ($\beta=0.0163$, $p=1.95E-03$; **Fig. 2b**), frontal pole ($\beta=0.0151$, $p=1.22E-02$; **Fig. 2c**),
210 and superior temporal gyrus ($\beta=0.0220$, $p=5.05E-04$; **Fig. 2d**).

211 ***INPP5D* expression levels are increased in an amyloid pathology mouse model**

212 We recapitulated our findings from the human data in the amyloidogenic mouse model,
213 5xFAD. We observed increased *Inpp5d* mRNA levels in 5xFAD mice throughout disease
214 progression compared with WT controls in the brain cortex (**Fig. 3a**) and hippocampus (**Fig. 3b**)
215 of four-, six-, eight-, and twelve-month-old mice (4-months: 1.57-fold in the cortex, 1.40-fold in
216 the hippocampus; 6-months: 1.86-fold in the cortex, 2.61-fold in the hippocampus; 8-months:
217 2.23-fold in the cortex and 2.53-fold in the hippocampus; and 12-months: 1.93-fold in the cortex
218 and 2.16-fold in the hippocampus). Similarly, *INPP5D* protein levels were increased in the
219 cortex of 5xFAD mice at four and eight months of age (1.79 and 3.31-fold, respectively; $p=0.06$)
220 (**Fig. 3c and 3d**). To assess *Inpp5d* induction was dependent on microglia, we depleted
221 microglia in four-month-old 5xFAD mice by treating the animals with the colony-stimulating
222 factor receptor-1 antagonist PLX5622 (PLX) for 28 days [18]. PLX treatment completely
223 abolished the increase of *Inpp5d* in 5xFAD mice (**Fig. 3e**). Furthermore, expression levels of

224 *Inpp5d* were restored after switching from the PLX diet to a normal diet for 28 further days (**Fig.**
225 **3f**).

226 ***INPP5D* expression levels are increased in plaque-associated microglia**

227 Immunohistochemistry of 5xFAD mice brain slices at eight months old revealed that
228 *Inpp5d* was mainly expressed in plaque-associated microglia (**Fig. 4**). *INPP5D*- and IBA1
229 (AIF1)-positive microglia cluster around 6E10-positive or X-34-positive plaques in the cortex
230 (**Fig. 4a**) and subiculum (**Fig. 4b**). We did not detect any *INPP5D* expression in WT control mice
231 (data not shown). Furthermore, analysis of transcriptomic data of sorted microglia from WT
232 mouse cortex-injected labeled apoptotic neurons [12] revealed a reduction of *Inpp5d* expression
233 levels in phagocytic microglia compared with non-phagocytic microglia (**Fig. 4c**), which is in
234 agreement with the report that *INPP5D* inhibition promotes microglial phagocytosis [13].

235 **Discussion**

236 Although genetic variants in *INPP5D* have been associated with LOAD risk [5, 6, 20, 21],
237 the role of *INPP5D* in AD remains unclear. We identified that *INPP5D* expression levels are
238 increased in the brain of LOAD patients. Furthermore, expression levels of *INPP5D* positively
239 correlate with brain amyloid plaque density and *AIF1* and *TMEM119* (microglial marker gene)
240 expression [22-24]. We observed similar findings in the 5xFAD amyloidogenic model, which
241 exhibited an increase in gene and protein expression levels of *Inpp5d* with disease progression,
242 predominately in plaque-associated microglia, suggesting induction of *Inpp5d* in plaque-
243 proximal microglia. Similarly, a recent study reported that *Inpp5d* is strongly correlated with
244 amyloid plaque deposition in the APPPS1 mouse model [25, 26]. These findings are consistent
245 with the observation of microgliosis in both AD and its mouse models.

246 *INPP5D* inhibition has been associated with microglial activation and increased
247 phagocytic activity, which is consistent with our transcriptomic data of sorted microglia from

248 murine brains injected with apoptotic neurons [12], showing a decrease in *Inpp5d* expression in
249 phagocytic microglia compared to non-phagocytic. These findings support the hypothesis that
250 an increase in *INPP5D* expression in AD is a part of an endogenous homeostatic microglial
251 response to negatively control their own activity. However, this “brake” might be excessive in
252 AD, as reflected in our findings that *INPP5D* expression is elevated in LOAD. *INPP5D*
253 overexpression might result in microglia with deficient phagocytic capacity, resulting in
254 increased A β deposition and neurodegeneration. Thus, the pharmacological targeting of
255 *INPP5D* might be a novel therapeutic strategy to shift microglia towards a beneficial phenotype
256 in AD. Future studies in genetic mouse models are necessary to further clarify the role of
257 *INPP5D* in microglial function and AD progression.

258 **Conclusions**

259 In conclusion, our results demonstrate that *INPP5D* plays a crucial role in AD
260 pathophysiology and is a potential therapeutic target. *INPP5D* expression is upregulated in
261 LOAD and positively correlated with amyloid plaque density. *Inpp5d* expression increases in the
262 microglia of 5xFAD mice as AD progresses, predominately in plaque-associated microglia.
263 Future studies investigating the effect of *INPP5D* loss-of-function on microglial phenotypes and
264 AD progression may allow for the development of microglial-targeted AD therapies.

265 **List of abbreviations**

266 AD: Alzheimer's disease, LOAD: late-onset AD, GWAS: genome-wide association
267 studies, INPP5D: phosphatidylinositol 3,4,5-trisphosphate 5-phosphatase 1, PI(3,4,5)P3:
268 phosphatidylinositol (3,4,5)-trisphosphate, PI(3,4)P2: phosphatidylinositol (3,4)-bisphosphate,
269 CSF: cerebrospinal fluid, OR: odds ratio, CI: confidence interval, β : β coefficient, WT: wild-type,
270 MCI: mild cognitive impairment, APOE ϵ 4: apolipoprotein ϵ 4 allele, PFA: paraformaldehyde,

271 PCR: polymerase chain reaction, Seq: sequencing, ANOVA: analysis of variance, qPCR:
272 quantitative real-time PCR, mAb: monoclonal antibody.

273 **Declarations**

274 **Ethics approval and consent to participate**

275 Animals used in the study were housed in the Stark Neurosciences Research Institute
276 Laboratory Animal Resource Center at Indiana University School of Medicine and all
277 experimental procedures were approved by the Institutional Animal Care and Use Committee.

278 **Consent for publication**

279 All participants were properly consented for this study.

280 **Availability of data and materials**

281 The datasets analyzed during the current study are available from the corresponding
282 author on reasonable request.

283 **Competing interests**

284 The authors declare that they have no competing interests.

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289 **Author contributions**

290 A.P.T, P.B.L, C.D, Y.L, B.T.L, G.E.L, A.L.O, and K.N designed the study. A.P.T, P.B.L,
291 C.D, M.M, B.T.C, and K.N performed the experiments and analyzed the data. A.P.T, M.M,

292 G.E.L, A.L.O, and K.N wrote the manuscript. All authors discussed the results and commented
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Fig. 1 **Relative quantification of *INPP5D* expression in the studied participants**

a

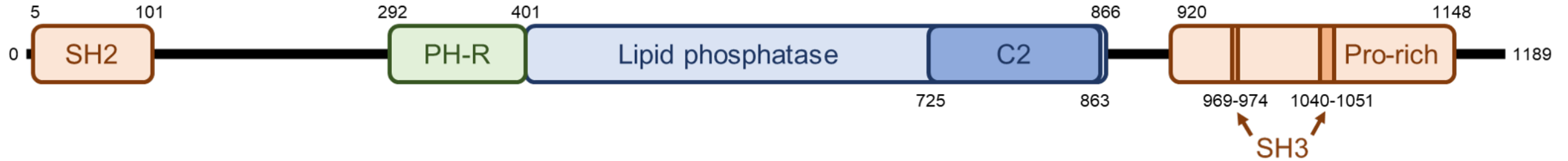


Fig 1. Relative quantification of *INPP5D* expression in the studied participants

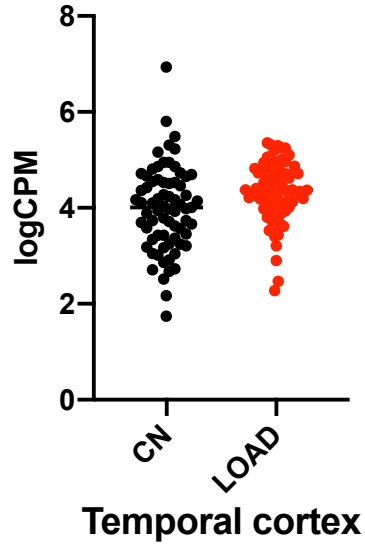
(a) Domain architecture of *INPP5D* drawn to scale. Gene expression of *INPP5D* is shown as logCPM values in (b) Temporal cortex (TCX)-Mayo, (c) Parahippocampal gyrus (PHG)-MSBB, (d) Inferior frontal gyrus (IFG)-MSBB, (e) Cerebellum (CER)-Mayo, (f) Frontal pole (FP)-MSBB, (g) Superior temporal gyrus (STG)-MSBB, (h) Dorsolateral prefrontal cortex (DLPFC)-ROSMAP.

SH2 *Src Homology 2 domain*, SH3 *SRC Homology 3 domain*, C2 *C2 domain*

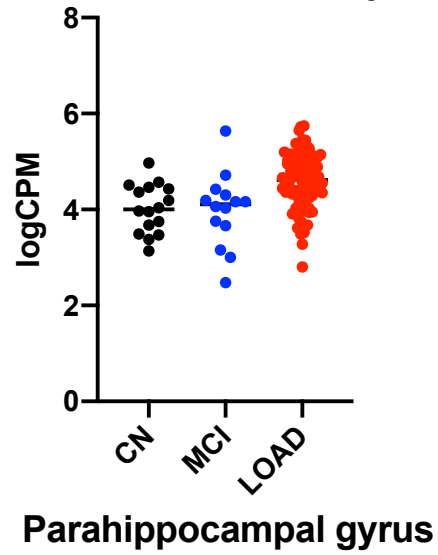
Fig. 1

Relative quantification of *INPP5D* expression in the studied participants

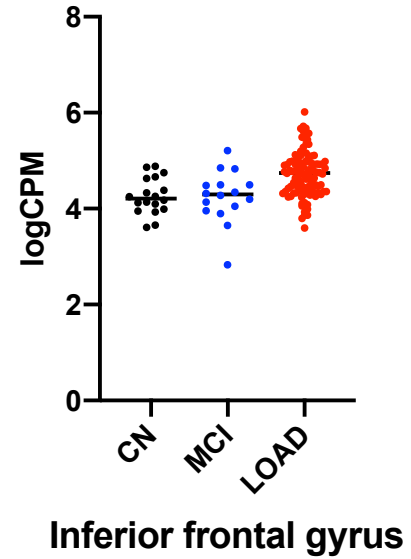
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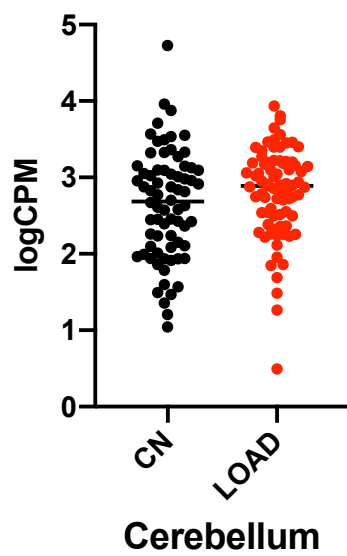
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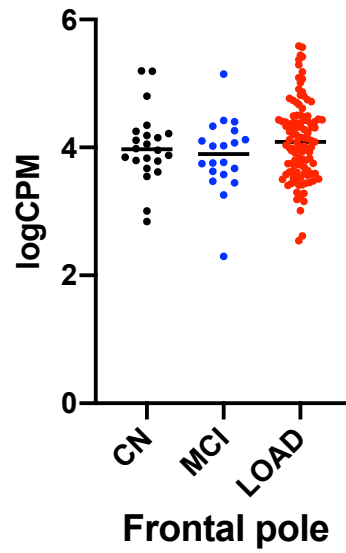
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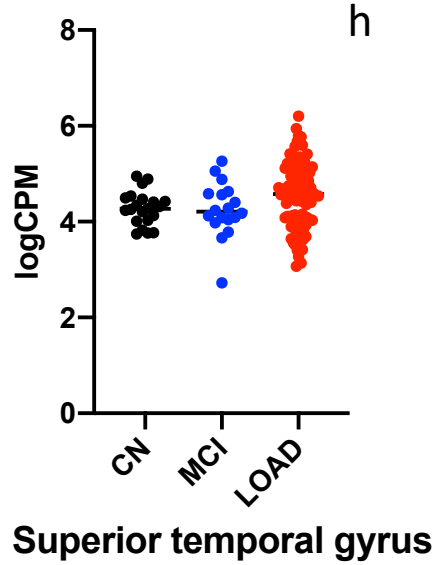
e



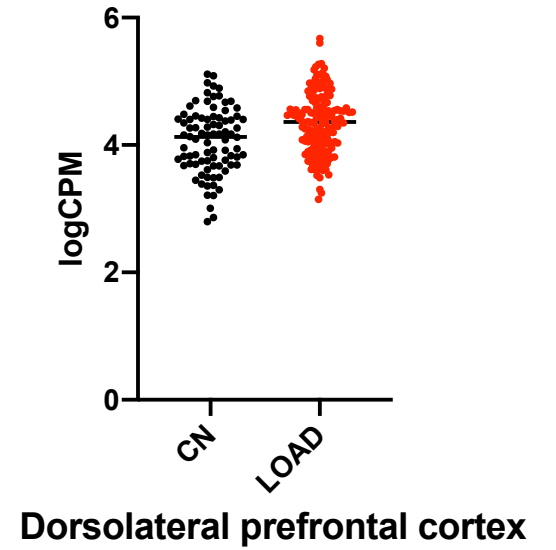
f



g



h



CN cognitively normal, MCI mild cognitive impairment, LOAD Late-Onset Alzheimer's disease

Table 1.

***INPP5D* expression levels were increased in LOAD**

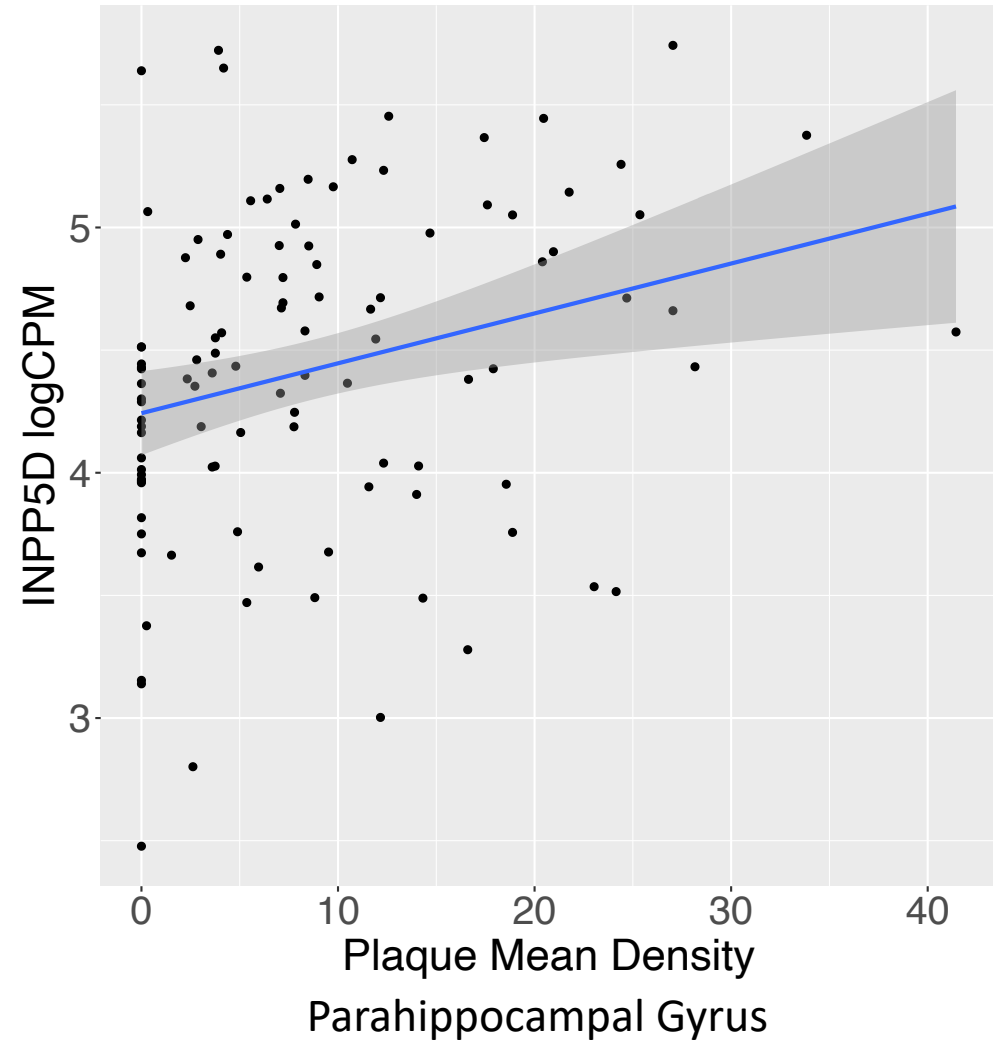
Brain Regions	Temporal Cortex	Parahippocampal Gyrus			Inferior Frontal Gyrus		
		Covariate: Age and Sex					
Contrast	CN vs. LOAD	CN vs. MCI	MCI vs. LOAD	CN vs. LOAD	CN vs. MCI	MCI vs. LOAD	CN vs. LOAD
logFC	0.34916854	0.012305567	0.565288103	0.536580956	-0.009944963	0.44545717	0.43695423
p-value	1.12E-02	9.99E-01	7.56E-02	7.17E-03	1.00E+00	6.76E-03	2.33E-03
		Covariate: Age, Sex, and APOE ε4 status					
Contrast	CN vs. LOAD	CN vs. MCI	MCI vs. LOAD	CN vs. LOAD	CN vs. MCI	MCI vs. LOAD	CN vs. LOAD
logFC	0.34072691	-0.034336626	0.57213088	0.52809965	-0.0546836	0.46476734	0.42180431
p-value	2.75E-02	1.00E+00	1.00E-01	1.08E-02	1.00E+00	8.00E-03	4.35E-03

Brain Regions	Cerebellum	Frontal Pole			Superior Temporal Gyrus		Dorsolateral Prefrontal Cortex	
		Covariate: Age and Sex						
Contrast	CN vs. LOAD	CN vs. MCI	MCI vs. LOAD	CN vs. LOAD	CN vs. MCI	MCI vs. LOAD	CN vs. LOAD	CN vs. LOAD
logFC	0.15322052	-0.1498388	0.20026554	0.09349678	-0.0115078	0.31887371	0.28945778	0.1761858
p-value	2.15E-01	9.85E-01	4.80E-01	8.65E-01	1.00E+00	2.09E-01	1.78E-01	5.76E-02
		Covariate: Age, Sex, and APOE ε4 status						
Contrast	CN vs. LOAD	CN vs. MCI	MCI vs. LOAD	CN vs. LOAD	CN vs. MCI	MCI vs. LOAD	CN vs. LOAD	CN vs. LOAD
logFC	0.207875239	-0.180168831	0.20013891	0.068420156	-0.020260737	0.282432909	0.22080636	0.16972131
p-value	1.23E-01	9.53E-01	5.13E-01	9.25E-01	1.00E+00	2.89E-01	3.26E-01	9.46E-02

Table 1 shows the p-values for the gene expression analyses performed with *limma* using RNA-Seq data from the AMP-AD Consortium. TCX *temporal cortex*, PHG *parahippocampal gyrus*, STG *superior temporal gyrus*, IFG *inferior frontal gyrus*, FP *frontal pole*, CER *cerebellum*, DLPFC *dorsolateral prefrontal cortex*, CN *cognitively normal*, AD *Alzheimer's disease*, MCI *mild cognitive impairment*, logFC *log fold-change*

Fig. 2 Association of *INPP5D* expression with amyloid plaque mean density

a



b

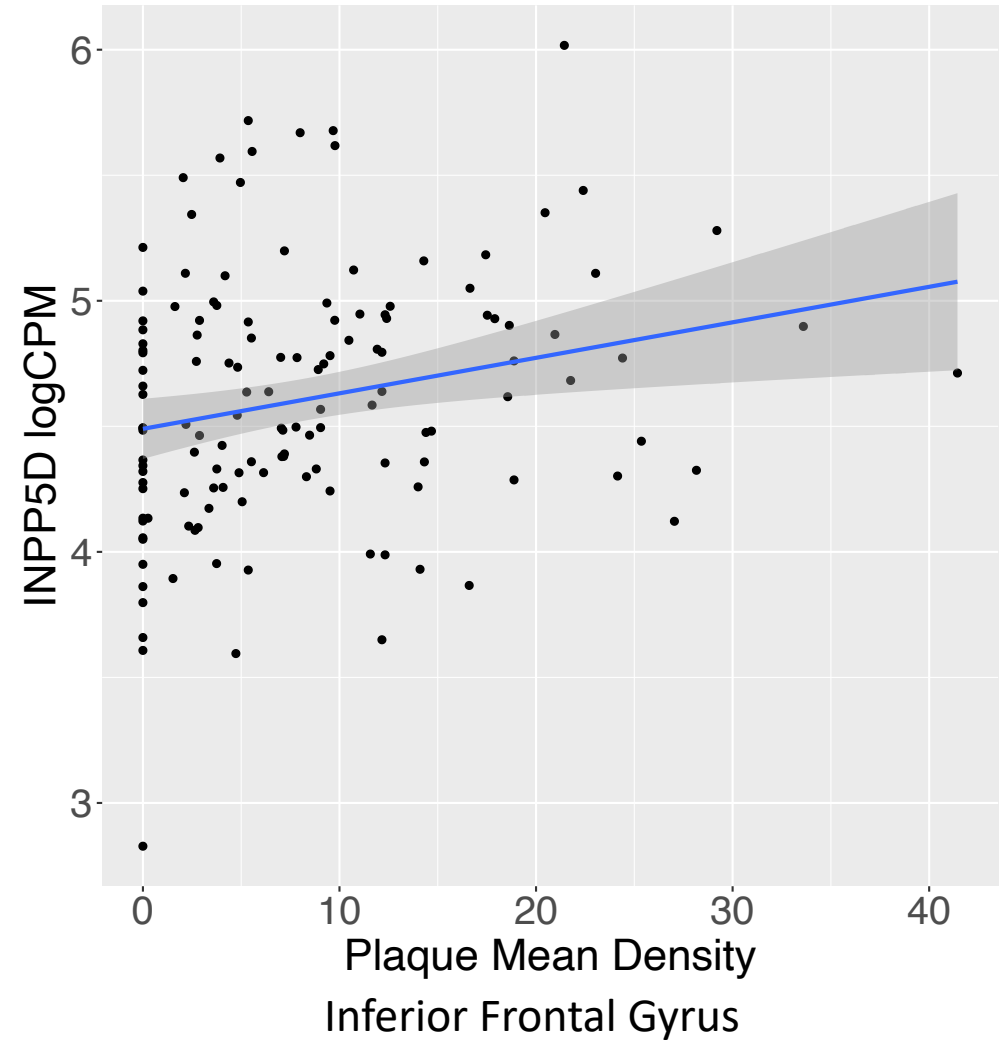


Fig. 2 Association of *INPP5D* expression with amyloid plaque mean density

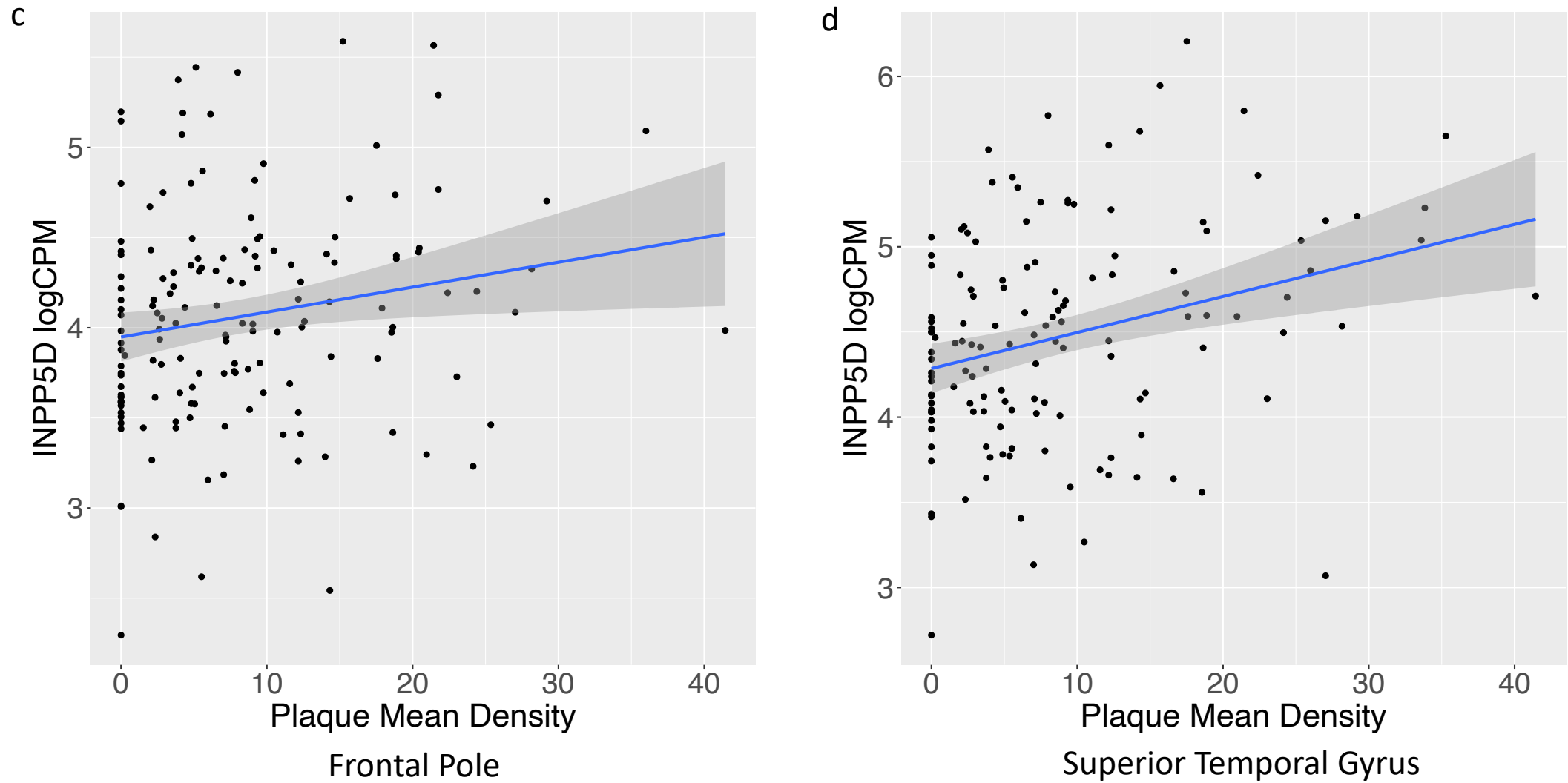


Fig 2. Association of *INPP5D* expression with amyloid plaque mean density.

The scatter plots show the positive association between *INPP5D* expression and plaque mean density in (a) parahippocampal gyrus, (b) inferior frontal gyrus, (c) frontal pole, and (d) superior temporal gyrus from the MSBB cohort.

Table 2.

***INPP5D* expression levels are associated with amyloid plaque density and microglia-specific markers**

Brain Regions (MSBB)	Parahippocampal Gyrus			Inferior Frontal Gyrus			Frontal Pole			Superior Temporal Gyrus		
	β	SE	<i>p</i> -value	β	SE	<i>p</i> -value	β	SE	<i>p</i> -value	β	SE	<i>p</i> -value
Plaque Mean Density	0.0212	0.0070	3.02E-03	0.0163	0.0052	1.95E-03	0.0151	0.0059	1.22E-02	0.0220	0.0062	5.06E-04
<i>AIF1</i>	0.4386	0.0811	4.10E-07	0.2862	0.0499	6.36E-08	0.2179	0.0607	4.53E-04	0.3013	0.0572	5.36E-07
<i>TMEM119</i>	0.7647	0.0527	<2E-16	0.6109	0.0446	<2E-16	0.5062	0.0578	4.00E-15	0.6914	0.0496	<2E-16

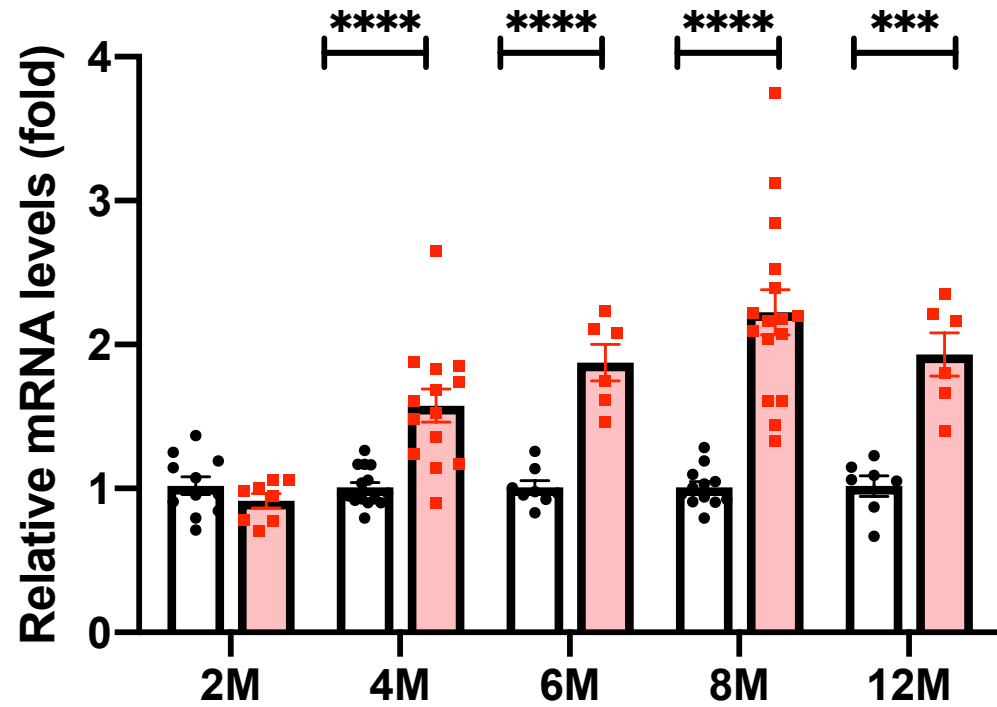
Table 2 shows the β coefficient (β), standard error (SE), and *p*-value for the association analysis between *INPP5D* expression levels and amyloid plaque density or expression levels of microglia-specific markers *AIF1* and *TMEM119* by general linear models.

Fig. 3

Inpp5d levels are increased in 5xFAD mice

a

Inpp5d expression (Cortex)



b

Inpp5d expression (Hippocampus)

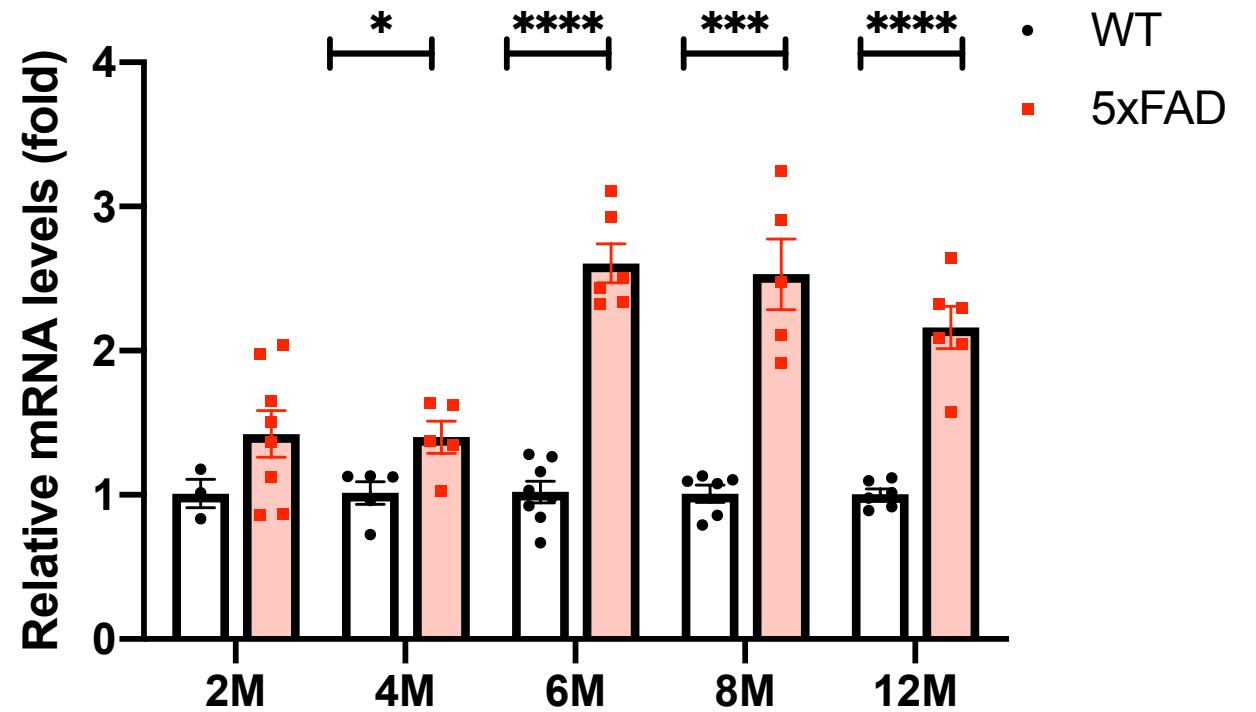
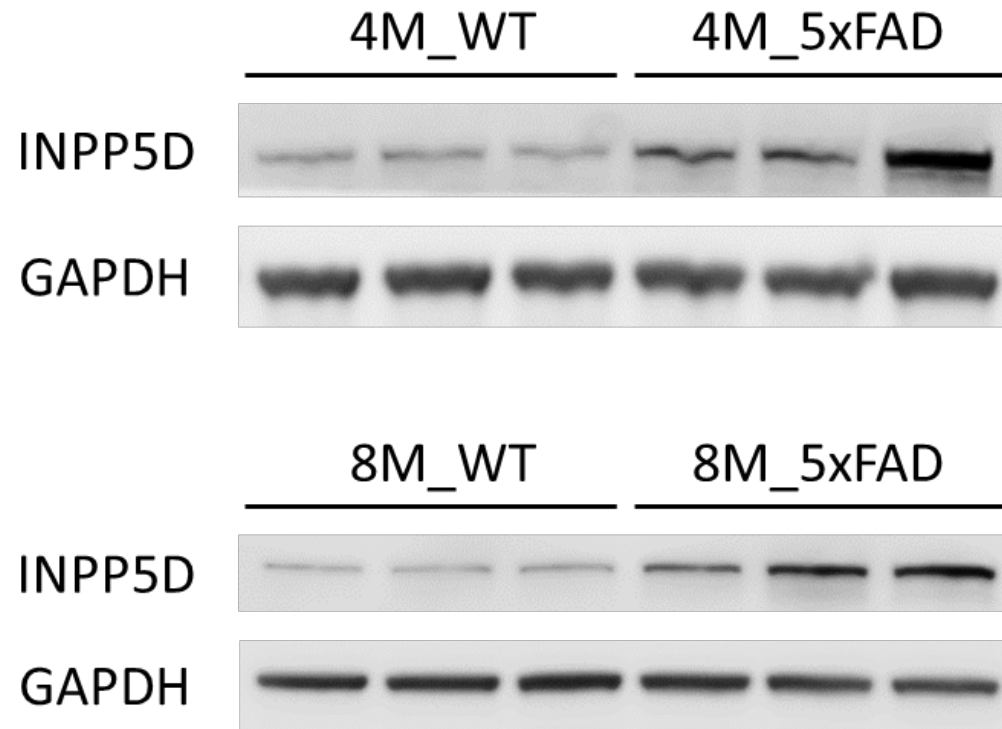


Fig. 3

***Inpp5d* levels are increased in 5xFAD mice**

c



d

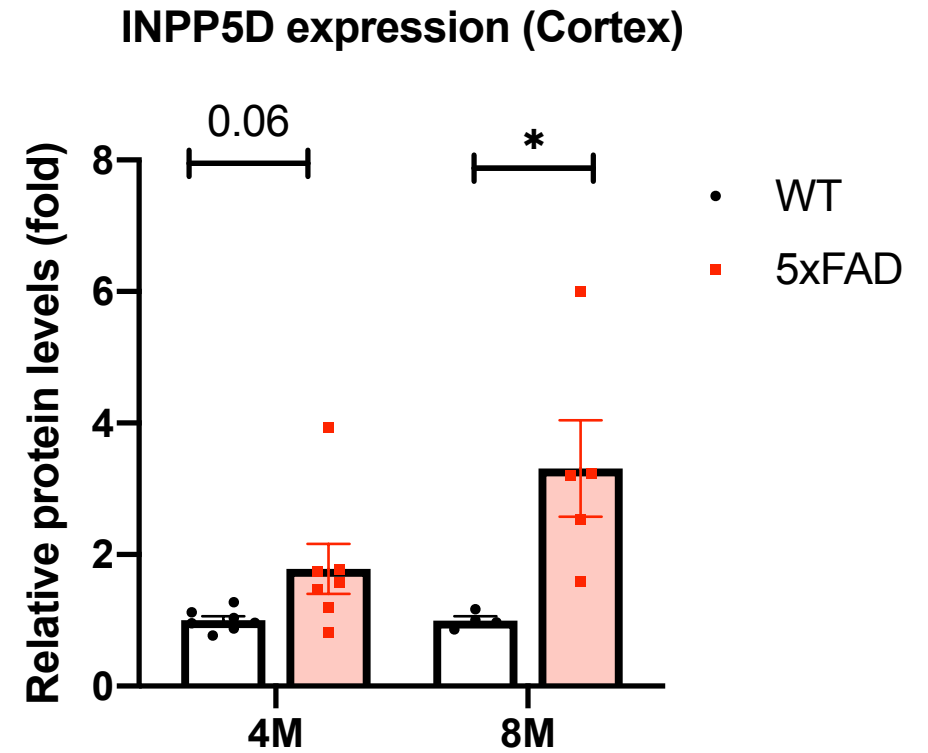


Fig.3

Inpp5d levels are increased in 5xFAD mice

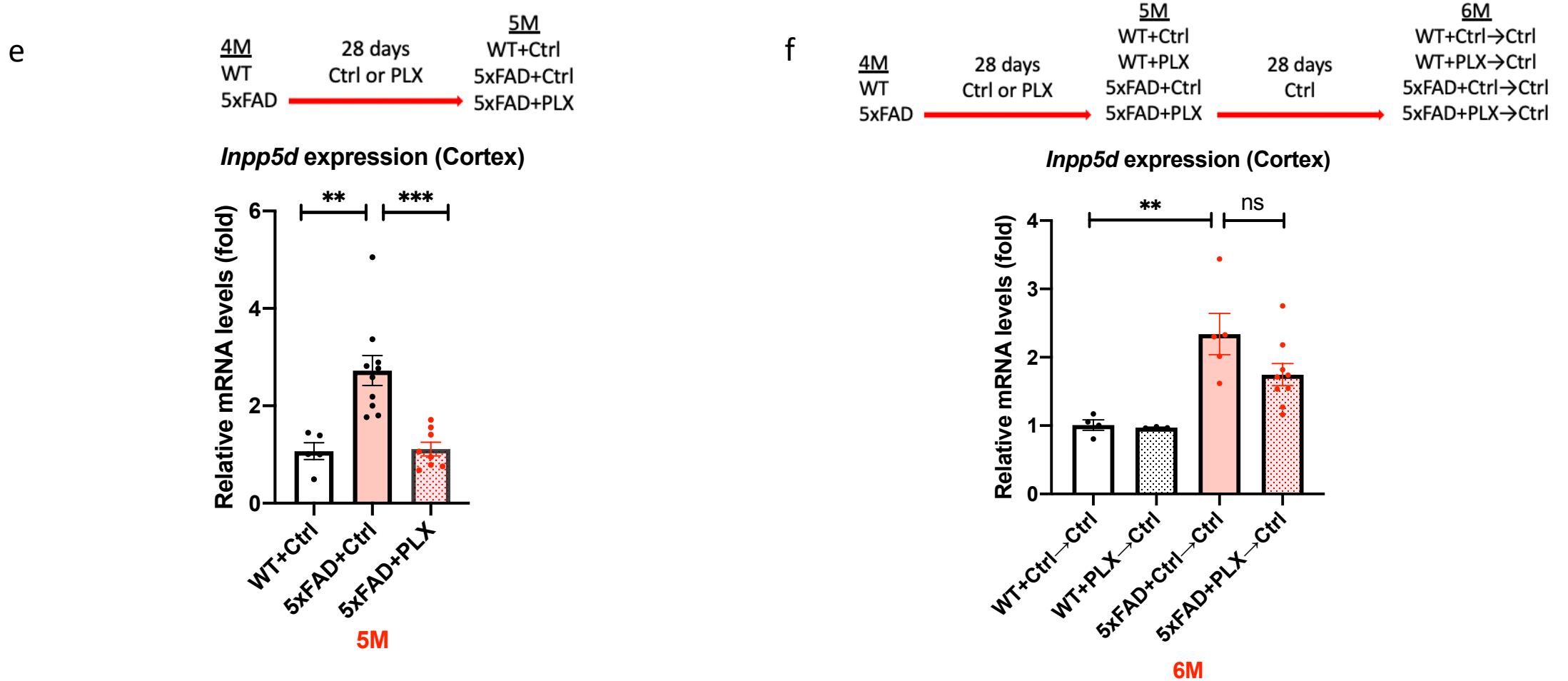


Fig 3. *Inpp5d* levels are increased in 5xFAD mice

Gene and protein levels of *Inpp5d* were assessed in cortical and hippocampal lysates from 5xFAD mice. Gene expression levels of *Inpp5d* were significantly increased in both cortex (a) and hippocampus (b) at 4, 6, 8, and 12 months of age (n=6-15 mice). There were significant changes in *Inpp5d* protein levels in the cortex at 8 months of age and an increased trend in the cortex at 4 months of age (n=4-7). Increased *Inpp5d* levels were abolished with PLX5622 treatment (e), and restored after switching PLX diet to normal diet (f) (n=3-10). *p<0.05; **p<0.01; ***p<0.001; ****p<0.0001, ns not significant.

Fig. 4

INPP5D expression levels are increased in plaque-associated microglia.

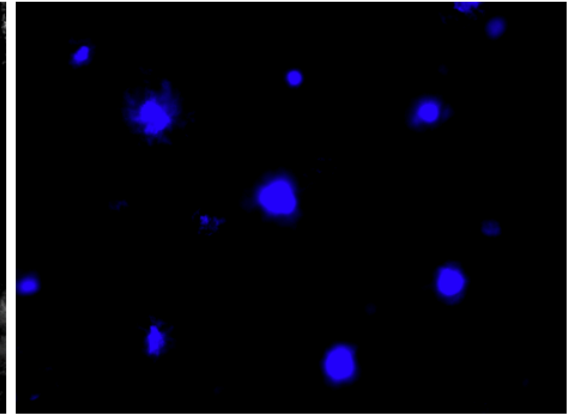
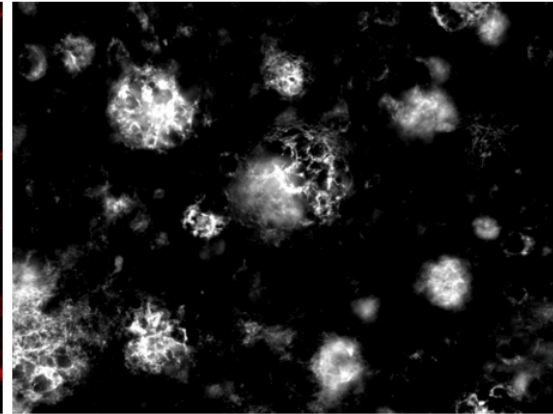
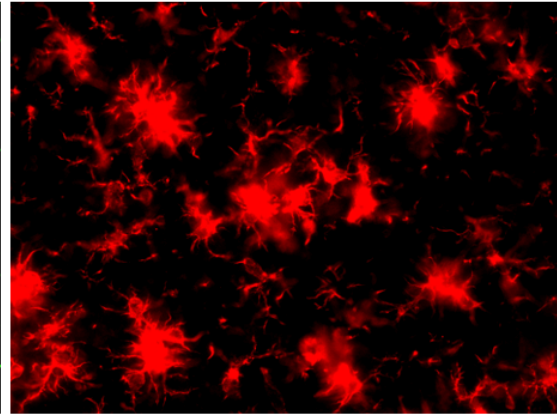
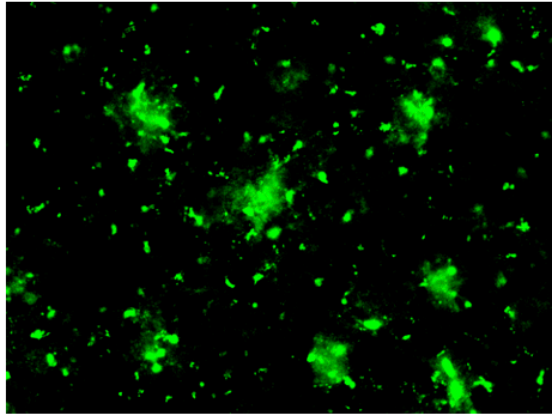
a **Cortex**

INPP5D

IBA1_Microglia

6E10_Plaques

X34_Plaques



INPP5D_Microglia

INPP5D_6E10

INPP5D_X34

Merge

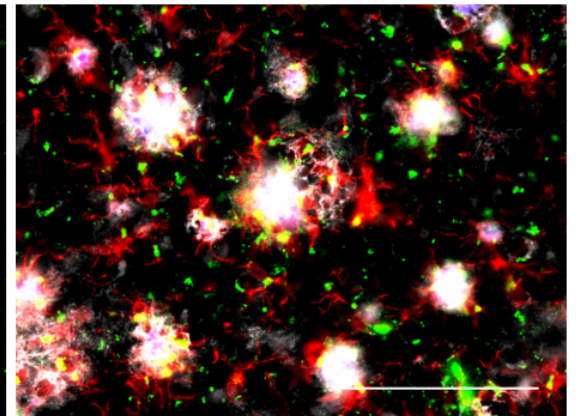
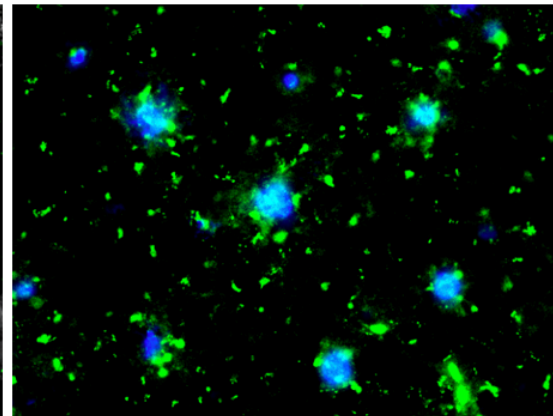
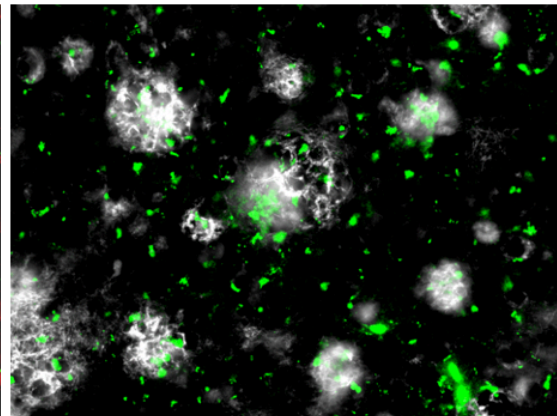
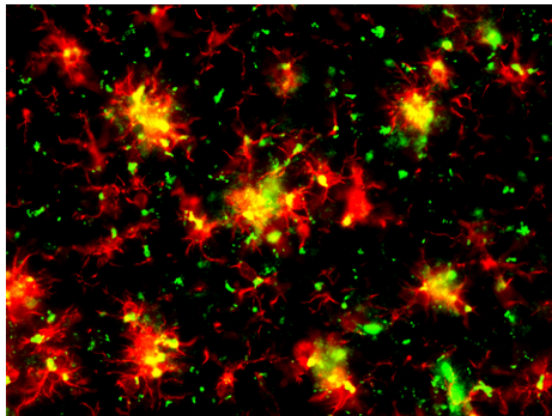


Fig. 4 **INPP5D expression levels are increased in plaque-associated microglia.**

b **Subiculum**

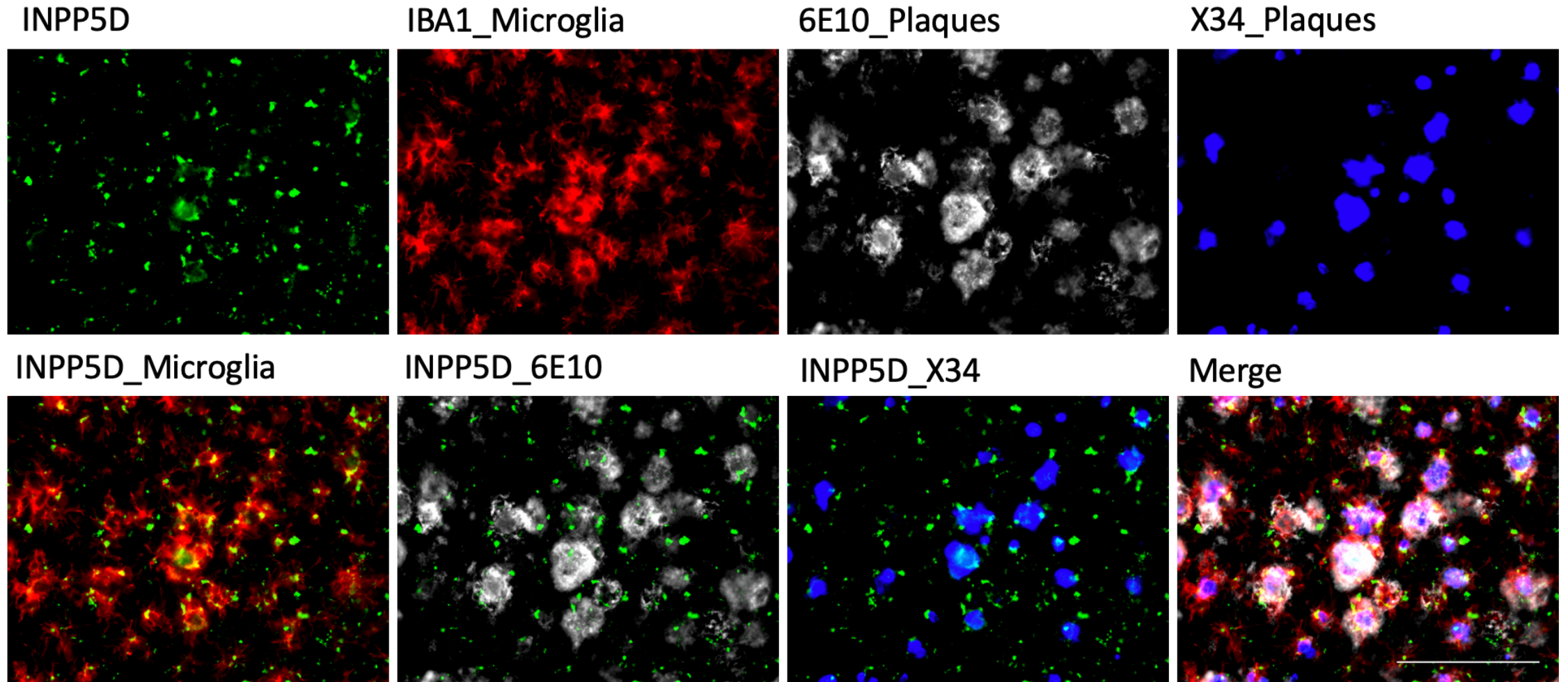


Fig. 4

INPP5D expression levels are increased in plaque-associated microglia.

C

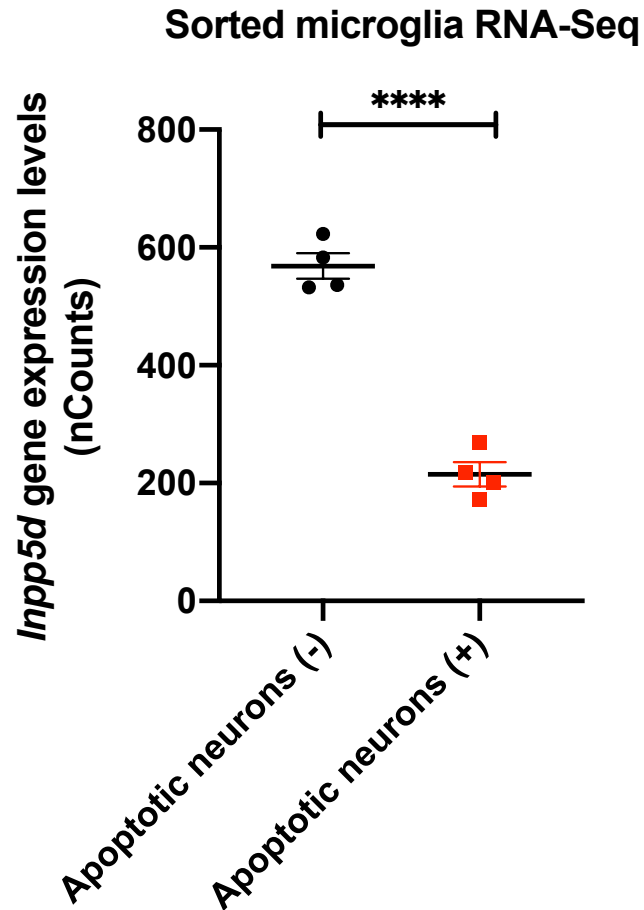


Fig 4. INPP5D expression levels were increased in plaque-associated microglia. INPP5D was mainly expressed in plaque-associated microglia. INPP5D- and IBA1 (AIF1)-positive microglia cluster around 6E10-positive or X-34-positive plaques in both cortex (a) and subiculum (b) of 8-month-old mice. Analysis of transcriptomic data of sorted microglia from wild-type mice cortex-injected labeled apoptotic neurons revealed that *Inpp5d* expression is increased in non-phagocytic microglia (Krasemann et.al) (c). Scale bar, 10 μ m. **** $p < 0.0001$