LRP1 Triggers Pro-inflammatory Cell-signaling in Response to Extracellular Tau Independently of the NMDA Receptor

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ABSTRACT

In Alzheimer's Disease (AD) and other neurodegenerative diseases, microtubule-associated protein Tau forms abnormal intracellular aggregates. The mechanisms by which Tau may promote AD progression remain incompletely understood. Injured and dying neurons release Tau into the extracellular spaces in the CNS. The released Tau may be taken up by receptors in the LDL Receptor gene family, including Low Density Lipoprotein Receptor-related Protein-1 (LRP1), which is expressed by microglia, astrocytes, and neurons. This process may be important for clearing Tau from extracellular spaces but may also promote the seeding of Tau aggregates in new cells. Our laboratory has shown that endocytosis of LRP1 ligands is coupled to the activation of cell-signaling and regulation of inflammation. Notably, different LRP1 ligands can induce either pro-inflammatory or anti-inflammatory responses, depending on the co-receptors that function with LRP1. Here, we demonstrate that in cultured macrophages, microglia, and astrocytes, extracellular Tau induces an LRP1-dependent pro-inflammatory response, characterized by NFκB activation and expression of pro-inflammatory cytokines. Unlike other LRP1 ligands that elicit anti-inflammatory responses, the response to Tau occurs independently of the NMDA receptor. When LRP1 is deleted or silenced, macrophages, microglia, and astrocytes do not respond to Tau, whereas when *Grin1* is deleted or the NMDA-R is pharmacologically inhibited, the responses remain unchanged. Because we have evidence that LRP1 in microglia expresses anti-inflammatory activity in response to ligands other than Tau, understanding the role of LRP1 in microglia and astrocytes *in vivo* in Alzheimer's Disease and other neuroinflammatory processes is an important future goal.

Key words: Tau, LRP1, NMDA-R, neuro-inflammation, Alzheimer's Disease

45 ## SIGNIFICANCE STATEMENT

46 In Alzheimer's Disease and other neurodegenerative diseases, microtubule-associated protein
47 Tau forms abnormal intracellular aggregates that contribute to disease progression. When
48 released extracellularly, Tau binds to the transmembrane receptor LRP1, expressed by diverse
49 cells in the CNS. LRP1 has the unique ability to couple ligand uptake with activation of cell-
50 signaling. We demonstrated that Tau binding to LRP1 activates pro-inflammatory responses in
51 macrophages, microglia, and astrocytes, characterized by NFκB activation and cytokine release.
52 This signaling occurs independently of the NMDA receptor, which distinguishes Tau from other
53 LRP1 ligands. These results define a novel pathway by which extracellular Tau regulates
54 neuroinflammation in Alzheimer's Disease, providing new therapeutic opportunities that target
55 LRP1 without interfering with NMDA-R functions.

56 **INTRODUCTION**

57 Microtubule-associated protein Tau is an intracellular protein that forms abnormal oligomers and
58 fibrils in Alzheimer's Disease (AD) and in neurodegenerative diseases called Tauopathies (Lee
59 et al., 2001; Congdon and Sigurdsson, 2018; Gao et al., 2018). Tau aggregation is regulated by
60 gene polymorphisms, alternative splicing, phosphorylation, and other post-translational
61 modifications. While the formation of neurofibrillary tangles through Tau aggregation is a key
62 pathological hallmark, emerging evidence suggests that Tau's contribution to AD extends beyond
63 its aggregation state (Congdon and Sigurdsson, 2018; Gao et al., 2018).

64 Tau is released by neurons and other cells in the CNS, in extracellular vesicles (EVs) and
65 independently of EVs, allowing for transfer of Tau between cells (Saman et al., 2012; Pooler et
66 al., 2013; Asai et al., 2015; Evans et al., 2018; Merezhko et al., 2018; Brunello et al., 2020).
67 Because Tau transfer between cells in the CNS may play a role in AD progression, identification
68 of Low Density Lipoprotein Receptor-related Protein-1 (LRP1) as an endocytic receptor for
69 extracellular Tau is highly significant (Rauch et al., 2020; Cooper et al., 2021). SORL1, which is
70 structurally related to LRP1, also may mediate Tau uptake (Cooper et al., 2024).

71 LRP1 is a 600-kDa, type 1 transmembrane receptor for over 100 structurally diverse ligands,
72 including apolipoprotein E, α_2-macroglobulin (α_2M), shed non-pathogenic cellular prion protein (S-
73 PrP^C), and proteins released from injured and dying cells (Strickland et al., 2002; Fernandez-
74 Castaneda et al., 2013; Gonias and Campana, 2014; Mantuano et al., 2020). LRP1 ligands are
75 internalized in clathrin-coated pits and usually transferred to lysosomes for degradation. A subset
76 of LRP1 ligands trigger cell-signaling via mechanisms that require co-receptors, such as the
77 NMDA Receptor (NMDA-R), Trk, $p75^{NTR}$, and/or PrP^C (Bacskai et al., 2000; May et al., 2004;
78 Martin et al., 2008; Shi et al., 2009; Mantuano et al., 2013; Stiles et al., 2013; Gonias and
79 Campana, 2014; Krogh et al., 2014; Mattei et al., 2020). Assembly of different LRP1 co-receptors
80 in response to different ligands may explain why the cell-signaling responses elicited by various

81 LRP1 ligands are non-uniform (Gonias and Campana, 2014). LRP1 is expressed by neurons,
82 astrocytes, oligodendrocytes, and microglia in the CNS and by macrophages throughout the body
83 (Gonias and Campana, 2014; Auderset et al., 2016).

84 Binding of Tau to LRP1 is mediated by the Tau microtubule-binding domain (Rauch et al.,
85 2020; Cooper et al., 2021). The Tau alternative splicing variant with four microtubule-binding
86 repeats (2N4R) binds to LRP1 with higher affinity than the variant with three. Tau phosphorylation
87 events, which exacerbate Tau aggregation (Alonso et al., 2001; Augustinack et al., 2002),
88 apparently decrease LRP1-binding affinity (Cooper et al., 2021). Thus, LRP1-mediated uptake of
89 extracellular Tau may be a physiologic process; however, Tau in extracts from Alzheimer's
90 Disease brains partially escapes lysosomal degradation, seeding new intracellular aggregates
91 (Rauch et al., 2020; Cooper et al., 2021).

92 Specific LRP1 ligands, including tissue-type plasminogen activator (tPA), α_2M, and S-PrP^C,
93 activate anti-inflammatory cell-signaling responses in macrophages by a pathway that requires
94 the NMDA-R (Mantuano et al., 2017, 2022). Determining whether Tau initiates LRP1-activated
95 cell-signaling is an important question. There is considerable evidence that Tau accumulation in
96 the CNS is pro-inflammatory (Yoshiyama et al., 2007; Nilson et al., 2017). In P301S-Tau
97 transgenic mice, which express a mutated form of human Tau that forms intracellular filamentous
98 lesions, microglial activation and neuro-inflammation precede formation of detectable Tau
99 aggregates (Yoshiyama et al., 2007). Anti-inflammatory treatment blocks disease progression in
100 P301S-Tau transgenic mice. Although activated microglia demonstrate some activities that are
101 beneficial in AD, excessive microglial activation and chronic neuro-inflammation may promote AD
102 progression (Akiyama et al., 2000; Heneka et al., 2015; Hong et al., 2016; Ransohoff, 2016).

103 In this investigation, we studied the 2N4R variant of Tau. Our findings indicate that Tau elicits
104 a pro-inflammatory response in macrophages, microglia, and astrocytes in a manner entirely
105 dependent on LRP1. Unlike other LRP1 ligands that activate NMDA-R-dependent pathways, the
106 pro-inflammatory effects of Tau were NMDA-R-independent. These insights provide a new

107 understanding of Tau's role in AD pathology and open the door to therapeutic strategies that
108 target Tau-mediated inflammation independently of NMDA-R.
109
110

111 MATERIALS AND METHODS

112 *Proteins and reagents*

113 Recombinant Human 2N4R Tau, the largest Tau derivative with 441 amino acids, was from R&D
114 Systems. The NMDA-R inhibitors, dizocilpine (MK-801) and dextromethorphan hydrobromide
115 (DXM), were from Cayman Chemicals and Abcam, respectively.
116

117 *Experimental mice*

118 Wild-type (WT) C57BL/6J mice were obtained from Jackson Laboratory. Mice in which
119 macrophages are LRP1-deficient were obtained by breeding *Lrp1*$^{flox/flox}$ mice with mice express
120 Cre recombinase under the control of the lysozyme-M (LysM-Cre) promoter, in the C57BL/6J
121 background, as previously described (Overton et al., 2007; Staudt et al., 2013; Mantuano et al.,
122 2016). To generate mice in which macrophages are deficient in the essential NMDA-R GluN1
123 subunit, *Grin1*$^{flox/flox}$ mice were bred with mice that express Cre recombinase under the control of
124 the LysM-Cre promoter in the C57BL/6J background (Mantuano et al., 2023). All research
125 protocols involving mice were approved by the University of California San Diego Institutional
126 Animal Care and Use Committee.
127

128 *Cell culture model systems*

129 Bone-marrow-derived macrophages (BMDMs) were harvested from 16-week-old mice, as
130 previously described (Mantuano et al., 2016). Briefly, bone marrow cells were flushed from mouse
131 femurs, plated in non-tissue culture-treated dishes, and cultured in DMEM/F-12 medium

132 containing 10% FBS and 20 ng/mL mouse macrophage colony-stimulating factor (BioLegend) for
133 7 days. Non-adherent cells were removed by washing. Adherent cells included >95% BMDMs, as
134 determined by F4/80 and CD11b immunoreactivity.

135 Microglia were isolated from C57BL/6J mouse pups, as previously described (Ni and
136 Aschner, 2010; Pontecorvi et al., 2019). In brief, brains were harvested from postnatal day 1–6
137 mice. The cortices were dissected from the forebrain, and the surrounding meninges were
138 removed. Intact cortices were mechanically and enzymatically dissociated using the Neural
139 Tissue Dissociation Kit (Miltenyi Biotec). Mixed glial cell cultures were established in DMEM/F-12
140 supplemented with GlutaMAX, 10% FBS, and 1× Gibco Antibiotic-Antimycotic (Thermo Fisher
141 Scientific). After culturing for 10-14 days, microglia were dissociated by shaking the mixed
142 cultures at 200 rpm for 30 min at 37°C. The floating cells were collected by centrifugation (5 min,
143 600×*g*) and re-plated at 3×10^5 cells/well. Oligodendrocytes were removed by an additional 6 h of
144 shaking. The remaining cells, which included mainly astrocytes, were collected by trypsinization
145 and re-plated at 4×10^5 cells/well. Experiments were performed within 24 h of completing the
146 isolation procedure for microglia and within 48 h of completing the isolation procedure for
147 astrocytes. Microglial cell culture purity was >95% homogeneous as determined by immuno-
148 fluorescence (IF) microscopy for Iba1 (positive), glial fibrillary acidic protein (GFAP) (negative),
149 and OLIG1 (negative). Astrocyte cultures were >95% GFAP-immunopositive.

150

151 *mRNA expression studies*

152 BMDMs, microglia, and astrocytes were transferred to serum-free medium (SFM) for 1 h. Some
153 cultures were pre-treated with MK-801 (1 μM) or DXM (10 μM) for the final 30 min of the 1 h pre-
154 incubation. The cells then were treated for 6 h with Tau, at the indicated concentrations, or vehicle
155 (20 mM sodium phosphate, 150 mM NaCl, pH 7.4, PBS) in the presence of inhibitors, as indicated.
156 RNA was isolated using the NucleoSpin RNA kit (Macherey-Nagel) and reverse-transcribed using
157 the iScript cDNA synthesis kit (Bio-Rad). qPCR was performed using TaqMan gene expression

158 products (Thermo Fisher Scientific). Primer-probe sets were as follows: *Gapdh*
159 (Mm99999915_g1); *Tnf* (Mm00443258_m1); *Il6* (Mm00446190_m1); *Ccl3* (Mm00441259_g1);
160 and *Cxcl2* (Mm00436450_m1). The relative change in mRNA expression was calculated using
161 the $2^{\Delta\Delta Ct}$ method with *Gapdh* mRNA as a normalizer.
162
163 *Cell signaling/immunoblotting experiments*
164 BMDMs, microglia, and astrocytes were transferred to SFM for 1 h and then treated for 1 h with
165 Tau or vehicle as noted. Some cultures were pre-treated with MK-801 (1 μM) or DXM (10 μM) for
166 the final 30 min of the 1 h pre-incubation, as noted.
167 Extracts of BMDMs, microglia, and astrocytes, were prepared in RIPA buffer (Sigma-Aldrich)
168 supplemented with protease inhibitors and phosphatase inhibitors (Thermo Fisher Scientific).
169 Equal amounts of cellular protein were separated by SDS-PAGE and transferred to PVDF
170 membranes. The membranes were blocked with 5% nonfat dried milk and then incubated with
171 primary antibodies from Cell Signaling Technology that recognize: phospho-ERK1/2 (catalog
172 #9102); total ERK1/2 (catalog #4370); phospho-IκBα (catalog #2859); total IκBα (catalog #9242);
173 and β-actin (catalog #3700). The membranes were washed and incubated with horseradish
174 peroxidase-conjugated secondary antibody from Jackson ImmunoResearch (anti-rabbit: catalog
175 #111-035-003; anti-mouse: catalog #115-035-003). Immunoblots were developed using Thermo
176 Scientific SuperSignal West Pico PLUS Chemiluminescent Substrate (Thermo Fisher Scientific)
177 and imaged using the Azure Biosystems c300 digital system. When immunoblots were re-probed,
178 phospho-IκBα was detected first, followed by total IκBα, then p-ERK, total ERK1/2, and finally, β-
179 actin. Presented results are representative of at least three independent experiments.
180
181 *Proteome profiler mouse cytokine arrays*
182 BMDMs and microglia were transferred to SFM for 30 min and then treated with Tau or vehicle
183 for 6 h, as indicated. Conditioned medium (CM) was collected and clarified by centrifugation. An

184 equivalent amount of CM (1.0 ml for each condition) was incubated with each nitrocellulose
185 membrane provided in the Proteome Profiler Mouse XL Cytokine Array Kit (R&D Systems).
186 Membranes were developed following the instructions of the manufacturer.
187
188 *Statistical analysis*
189 Statistical analysis was performed using GraphPad Prism 9.5.1. All results are expressed as the
190 mean ± SEM. Each replicate was performed using a different BMDM, microglia, or astrocyte
191 preparation. Comparisons between two groups were performed using two-tailed paired t-tests. To
192 compare more than two groups, we performed a one-way ANOVA followed by post-hoc Dunnett's
193 multiple comparison test. When evaluating the effects of two independent factors, we performed
194 a two-way ANOVA followed by Šidák's multiple comparisons test. p-values of *p<0.05, **p<0.01,
195 ***p<0.001, and ****p<0.0001 were considered statistically significant.
196
197
198 ## RESULTS
199 **Tau elicits an LRP1-dependent pro-inflammatory response in macrophages**
200 BMDMs were treated with increasing concentrations of Tau for 6 h. RNA was harvested and RT-
201 qPCR was performed to examine mRNA levels for the genes encoding various cytokines. Tau, at
202 concentrations greater than or equal to 5 nM, significantly increased expression of the mRNAs
203 encoding TNFα (*Tnf*), interleukin-6/IL-6 (*Il6*), CCL3/MIP-1α (*Ccl3*), and CXCL2/ MIP-2α (*Cxcl2*)
204 (Fig. 1*A*).
205 IκBα phosphorylation is a key step in the activation of NFκB. Phosphorylated IκBα is
206 ubiquitinated and degraded, allowing NFκB to translocate to the nucleus and bind DNA (Karin
207 and Ben-Neriah, 2000; Viatour et al., 2005). NFκB activation promotes expression of pro-
208 inflammatory cytokines, such as TNFα and IL-6. When BMDMs were treated for 1 h with Tau, at

concentrations of 5 nM or greater, IκBα was phosphorylated and a decrease in the total abundance of IκBα was observed (Fig 1*B*). ERK1/2 also was phosphorylated in Tau-treated BMDMs. ERK1/2 phosphorylation is a commonly observed process in LRP1-initiated cell-signaling responses (Gonias and Campana, 2014).

To test whether LRP1 is necessary for Tau-induced cytokine expression, we harvested BMDMs from mice in which *Lrp1* is deleted under the control of the LysM promoter (Overton et al., 2007; Staudt et al., 2013; Mantuano et al., 2016). LRP1 protein is decreased by greater than 95% in these cells (Staudt et al., 2013). In LRP1-expressing control cells, which carry two floxed *Lrp1* genes but not LysM-Cre, 20 nM Tau significantly increased expression of the mRNAs encoding TNFα, IL-6, CCL3, and CXCL2, as anticipated (Fig. 2A). In LRP1-deficient BMDMs, 20 nM Tau failed to induce expression of these cytokines.

To examine cytokine expression at the protein level, in an unbiased manner, we treated LRP1-expressing and -deficient BMDMs with 20 nM Tau for 6 h. Conditioned medium (CM) was harvested and subjected to array analysis to detect secreted cytokines. In CM from Tau-treated LRP1-expressing cells, we observed TNFα and IL-6, as anticipated based on our RT-qPCR results (Fig. 2*B*). Other cytokines that were increased in CM from Tau-treated LRP1-expressing cells included but were not limited to CXCL2/MIP2, CXCL1/KC, CXCL10/ IP10, CCL3/CCL4 (MIP1α/β), and IL-12. By contrast, CM from Tau-treated LRP1-deficient BMDMs was entirely lacking in the Tau-elicited cytokines detected by this array. In separate studies, Tau failed to induce phosphorylation of IκBα in LRP1-deficient BMDMs, even when the Tau concentration was increased to 20 nM (Fig. 2*C*). Collectively, these results demonstrate that LRP1 is necessary for Tau-induced pro-inflammatory responses in BMDMs.

The NMDA-R is not required for macrophages to respond to Tau

tPA, α_2M, and S-PrP^C are examples of LRP1 ligands that activate cell-signaling via pathways that require the NMDA-R (Bacskai et al., 2000; Martin et al., 2008; Mantuano et al., 2017, 2020). To

235 test whether the NMDA-R is necessary for Tau-elicited responses in BMDMs, first we treated cells
236 with two pharmacologic inhibitors of the NMDA-R. Neither MK-801 nor DXM inhibited the effects
237 of Tau on expression of the mRNAs encoding TNFα or IL-6. (Fig. 3*A*). Similarly, Tau-induced IκBα
238 phosphorylation was unchanged in the presence of MK-801 or DXM (Fig. 3*B*).

239 Next, we isolated NMDA-R-deficient BMDMs from mice in which the gene encoding the
240 essential GluN1 subunit (*Grin1*) is deleted under the control of the LysM promoter (Mantuano et
241 al., 2023). BMDMs from these mice fail to respond to S-PrP^{C} (Mantuano et al., 2023). However,
242 as shown in Fig. 3*C*, 20 nM Tau significantly increased expression of the mRNAs encoding TNFα,
243 IL-6, CCL3, and CXCL2 in NMDA-R-deficient BMDMs. Furthermore, Tau (10 nM and 20 nM)
244 caused IκBα phosphorylation in NMDA-R-deficient BMDMs, together with the concomitant
245 decrease in total abundance of IκBα (Fig. 3*D*). Taken together, these results demonstrate that
246 Tau-activated cell-signaling in BMDMs occurs independently of the NMDA-R.

247

248 **Tau induces LRP1-dependent pro-inflammatory responses in microglia and astrocytes**

249 Microglia isolated from mouse pups were treated with 20 nM Tau or vehicle for 6 h. CM was
250 harvested and subjected to cytokine array analysis (Fig. 4*A*). Multiple cytokines were detected, in
251 a pattern that overlapped with those detected in Tau-treated BMDMs, including TNFα, IL-6,
252 CCL3/CCL4, and CXCL2. To validate the results of the microarray analysis and test the role of
253 the NMDA-R in Tau-elicited cytokine expression in microglia, we performed RT-qPCR studies.
254 Tau (20 nM) increased expression of the mRNAs encoding TNFα, IL-6, CCL3, and CXCL2 in
255 microglia (Fig. 4*B*). The increases in mRNA expression were not altered by the NMDA-R
256 inhibitors, MK-801 and DXM.

257 ERK1/2 was activated in microglia treated with 20 nM Tau for 1 h (Fig. 4*C*). In the same cells,
258 IκBα was phosphorylated and this event was accompanied by a decrease in the total abundance
259 of IκBα. These cell-signaling events were not inhibited by MK-801 and DXM, confirming that the
260 NMDA-R does is not required for the response to Tau in microglia.

261 To test the role of LRP1 as a mediator of the Tau response in microglia, we silenced
262 expression of *Lrp1* using siRNA. The level of LRP1 was substantially decreased at the protein
263 level, compared with cells transfected with NTC siRNA (Fig. 4*D*). Tau (20 nM) failed to activate
264 ERK1/2 in microglia in which *Lrp1* was silenced.

265 In cultured mouse astrocytes, Tau (20 nM) induced expression of the mRNAs encoding
266 TNFα, IL-6, CCL3, and CXCL2 (Fig. 5*A*). The response to Tau in astrocytes was not inhibited by
267 MK-801 or DXM. Tau also caused IκBα phosphorylation in astrocytes (Fig. 5*B*). This response
268 was not inhibited by MK-801. Collectively, these results demonstrate that extracellular 2N4R Tau
269 elicits similar pro-inflammatory responses in macrophages, microglia and astrocytes by an LRP1-
270 dependent mechanism.

271

272

273 **DISCUSSION**

274 LRP1 functions in the clearance of proteins from extracellular spaces, in lipoprotein uptake, as a
275 regulator of the plasma membrane proteome, in myelin phagocytosis, in efferocytosis, and as a
276 direct activator of cell-signaling (Strickland et al., 2002; Gonias and Campana, 2014). In AD, LRP1
277 may regulate disease progression by functioning as a receptor for β-amyloid peptide (Aβ)
278 (Kanekiyo et al., 2013). LRP1 binds secreted forms of amyloid precursor protein (APP) and may
279 regulate processing of cellular APP so that the amount of Aβ generated and secreted by cells is
280 altered (Kounnas et al., 1995; Ulery et al., 2000). Isoforms of apolipoprotein E bind to LRP1 (Liu
281 et al., 2007). Furthermore, LRP1 has been implicated in transport of Aβ across the blood-brain
282 barrier (Deane et al., 2009).

283 The identification of LRP1 as a Tau receptor raises new questions regarding the activity of
284 LRP1 in AD and in Tauopathies (Rauch et al., 2020; Cooper et al., 2021). Because binding of Tau
285 to LRP1 does not require post-translational modification events such as phosphorylation or

286 aggregation (Cooper et al., 2021), LRP1 may play an important physiologic role, removing low
287 levels of Tau form the extracellular spaces in the CNS and delivering the internalized Tau to
288 lysosomes for degradation. However, because Tau may partially escape endosomal transport
289 and enter the cytoplasm, when the amount of Tau in the extracellular spaces in the CNS
290 increases, LRP1 may be involved in propagation of Tau lesions in new cells (Rauch et al., 2020;
291 Cooper et al., 2021).

292 Because LRP1 is known to couple ligand internalization with activation of cell-signaling
293 (Gonias and Campana, 2014), our goal was to test whether the Tau-LRP1 interaction affects cell
294 physiology in a manner that may play a role in progression of AD and other forms of
295 neurodegeneration. We have shown that, in diverse cells, including macrophages, microglia, and
296 astrocytes, the interaction of 2N4R Tau with LRP1 induces pro-inflammatory responses. Our
297 study shows that these responses occur independently of the NMDA-R, which has been
298 implicated in other LRP1 cell-signaling events (Bacskai et al., 2000; Martin et al., 2008; Mantuano
299 et al., 2017, 2020).

300 Given that the NMDA-R has been implicated in the pathology of AD through its role in
301 excitotoxicity and inflammatory signaling, the finding that Tau-LRP1 signaling is NMDA-R-
302 independent raises questions about the co-receptors or alternative signaling pathways that might
303 mediate Tau's pro-inflammatory effects. Recent studies have suggested that other receptor
304 systems, such as Toll-like receptor 4 (TLR4), may be involved in Tau-induced neuroinflammation,
305 providing a potential explanation for how Tau can trigger inflammation independently of NMDA-R
306 (Calvo-Rodriguez et al., 2020). Further studies are needed to explore whether TLR4 or other
307 receptors act in concert with LRP1 to propagate Tau-driven inflammation in AD.

308 LRP1's role in Tau clearance and pro-inflammatory signaling makes it a promising
309 therapeutic target for AD. Modulating LRP1 activity could have dual benefits: promoting the
310 clearance of extracellular Tau to reduce its propagation while inhibiting the pro-inflammatory
311 signaling that exacerbates neurodegeneration. Because LRP1-mediated Tau signaling occurs

312 independently of NMDA-R, targeting LRP1 may allow for more specific interventions that do not
313 interfere with NMDA-R's essential functions in synaptic transmission. This approach could avoid
314 the limitations of current NMDA-R-targeting drugs like memantine, which aim to reduce
315 excitotoxicity but may not address the full scope of Tau's neurotoxic effects (Kabir et al., 2019).
316 Another critical aspect of LRP1 biology is its ability to undergo proteolytic shedding. In response
317 to inflammatory stimuli, such as LPS or interferon-γ, LRP1 is cleaved by transmembrane
318 proteases in the ADAM family, releasing a soluble product that includes the entire α-chain and a
319 fragment of the β-chain ectodomain (Quinn et al., 1999; von Arnim et al., 2005; Gorovoy et al.,
320 2010). Shed LRP1 is found in plasma and its level is increased in patients with rheumatoid arthritis
321 or systemic lupus erythematosus (Gorovoy et al., 2010). Shed LRP1 that is purified from human
322 plasma is biologically active and independently pro-inflammatory against macrophages and
323 microglia (Gorovoy et al., 2010; Brifault et al., 2017). It is possible that LRP1 shedding is involved
324 in the pathway by which Tau induces pro-inflammatory responses in macrophages, microglia, and
325 astrocytes. If future studies confirm this, it would suggest that shed LRP1 acts as a soluble factor
326 that amplifies neuroinflammation by interacting with receptors on nearby cells. This could further
327 potentiate the effects of Tau, promoting widespread inflammation and neurodegeneration in the
328 CNS.

329 There are two possible models that could explain how LRP1 mediates Tau-induced
330 inflammation. In the first model, membrane-anchored LRP1 functions with co-receptors to initiate
331 Tau signaling at the cell surface. In the second model, shed LRP1 acts as a soluble factor,
332 triggering pro-inflammatory responses by interacting with receptors on neighboring cells. These
333 two models are not mutually exclusive, and both may contribute to the complex dynamics of Tau-
334 induced neuroinflammation.

335 The bimodal activity of LRP1 as a ligand-dependent inhibitor or activator of inflammation is
336 important to consider when reviewing complex animal model studies in which LRP1 may be
337 exposed to diverse ligands. For example, LRP1 deficiency in cultured microglia is mildly pro-

338 inflammatory in the absence of inflammation-regulating mediators (Yang et al., 2016; Brifault et
339 al., 2017). LRP1-deficient microglia in culture demonstrate more robust pro-inflammatory
340 responses to $A\beta_{1-42}$ (He et al., 2020). In these cases, the effects of LRP1 deficiency may be
341 explained by loss of NMDA-R-dependent anti-inflammatory cell-signaling pathways. By contrast,
342 in an *in vivo* model of peripheral nerve injury, microglial LRP1 exacerbates inflammation in the
343 spinal cord, in association with central sensitization of pain processing (Brifault et al., 2019). In
344 this case, the available LRP1 ligands *in vivo* may activate the LRP1 pathway and/or induce LRP1
345 shedding. Animal models of AD should be carefully examined to determine how LRP1's diverse
346 functions influence disease progression, particularly in response to its various ligands.

347 In summary, the studies presented here identify a novel LRP1-depedent mechanism by which
348 Tau may regulate inflammation, neuro-inflammation, and AD progression. The NMDA-R-
349 independent nature of Tau's pro-inflammatory effects opens new possibilities for targeting LRP1-
350 Tau interactions as a therapeutic strategy. The potential involvement of shed LRP1 in amplifying
351 Tau-induced inflammation highlights additional layers of complexity in LRP1 biology that warrant
352 further exploration. By developing therapeutic strategies that modulate LRP1 activity, we may be
353 able to slow AD progression by reducing Tau-induced neuroinflammation and preventing the
354 spread of Tau pathology within the brain.

355 **REFERENCES**

356 Akiyama H et al. (2000) Inflammation and Alzheimer's disease. Neurobiology of Aging 21:383–
357 421.

358 Alonso A, Zaidi T, Novak M, Grundke-Iqbal I, Iqbal K (2001) Hyperphosphorylation induces self-
359 assembly of tau into tangles of paired helical filaments/straight filaments. Proc Natl Acad Sci
360 U S A 98:6923–6928.

361 Asai H, Ikezu S, Tsunoda S, Medalla M, Luebke J, Haydar T, Wolozin B, Butovsky O, Kügler S,
362 Ikezu T (2015) Depletion of microglia and inhibition of exosome synthesis halt tau propagation.
363 Nat Neurosci 18:1584–1593.

364 Auderset L, Cullen CL, Young KM (2016) Low Density Lipoprotein-Receptor Related Protein 1 Is
365 Differentially Expressed by Neuronal and Glial Populations in the Developing and Mature
366 Mouse Central Nervous System. PLOS ONE 11:e0155878.

367 Augustinack JC, Schneider A, Mandelkow E-M, Hyman BT (2002) Specific tau phosphorylation
368 sites correlate with severity of neuronal cytopathology in Alzheimer's disease. Acta
369 Neuropathol 103:26–35.

370 Bacskai BJ, Xia MQ, Strickland DK, Rebeck GW, Hyman BT (2000) The endocytic receptor
371 protein LRP also mediates neuronal calcium signaling via N-methyl-D-aspartate receptors.
372 Proc Natl Acad Sci U S A 97:11551–11556.

373 Brifault C, Gilder AS, Laudati E, Banki MA, Gonias SL (2017) Shedding of Membrane-associated
374 LDL Receptor-related Protein-1 from microglia amplifies and sustains neuroinflammation.
375 Journal of Biological Chemistry 292:18699–18712.

376 Brifault C, Kwon H, Campana WM, Gonias SL (2019) LRP1 deficiency in microglia blocks neuro-
377 inflammation in the spinal dorsal horn and neuropathic pain processing. Glia 67:1210–1224.

378 Brunello CA, Merezhko M, Uronen R-L, Huttunen HJ (2020) Mechanisms of secretion and
379 spreading of pathological tau protein. Cell Mol Life Sci 77:1721–1744.

380 Calvo-Rodriguez M, García-Rodríguez C, Villalobos C, Núñez L (2020) Role of Toll Like Receptor
381 4 in Alzheimer's Disease. Front Immunol 11:1588.
382 Congdon EE, Sigurdsson EM (2018) Tau-targeting therapies for Alzheimer disease. Nat Rev
383 Neurol 14:399–415.
384 Cooper JM, Lathuiliere A, Migliorini M, Arai AL, Wani MM, Dujardin S, Muratoglu SC, Hyman BT,
385 Strickland DK (2021) Regulation of tau internalization, degradation, and seeding by LRP1
386 reveals multiple pathways for tau catabolism. Journal of Biological Chemistry 296:100715.
387 Cooper JM, Lathuiliere A, Su EJ, Song Y, Torrente D, Jo Y, Weinrich N, Sales JD, Migliorini M,
388 Sisson TH, Lawrence DA, Hyman BT, Strickland DK (2024) SORL1 is a receptor for tau that
389 promotes tau seeding. Journal of Biological Chemistry 300:107313.
390 Deane R, Bell R, Sagare A, Zlokovic B (2009) Clearance of amyloid-β peptide across the blood-
391 brain barrier: Implication for therapies in Alzheimer's disease. CNS Neurol Disord Drug
392 Targets 8:16–30.
393 Evans LD, Wassmer T, Fraser G, Smith J, Perkinton M, Billinton A, Livesey FJ (2018) Extracellular
394 Monomeric and Aggregated Tau Efficiently Enter Human Neurons through Overlapping but
395 Distinct Pathways. Cell Reports 22:3612–3624.
396 Fernandez-Castaneda A, Arandjelovic S, Stiles TL, Schlobach RK, Mowen KA, Gonias SL,
397 Gaultier A (2013) Identification of the Low Density Lipoprotein (LDL) Receptor-related Protein-
398 1 Interactome in Central Nervous System Myelin Suggests a Role in the Clearance of Necrotic
399 Cell Debris. J Biol Chem 288:4538–4548.
400 Gao Y-L, Wang N, Sun F-R, Cao X-P, Zhang W, Yu J-T (2018) Tau in neurodegenerative disease.
401 Ann Transl Med 6:175–175.
402 Gonias SL, Campana WM (2014) LDL receptor-related protein-1: A regulator of inflammation in
403 atherosclerosis, cancer, and injury to the nervous system. American Journal of Pathology
404 184:18–27.

405 Gorovoy M, Gaultier A, Campana WM, Firestein GS, Gonias SL (2010) Inflammatory mediators
406 promote production of shed LRP1/CD91, which regulates cell signaling and cytokine
407 expression by macrophages. J Leukoc Biol 88:769–778.
408 He Y, Ruganzu JB, Jin H, Peng X, Ji S, Ma Y, Zheng L, Yang W (2020) LRP1 knockdown
409 aggravates Aβ1–42-stimulated microglial and astrocytic neuroinflammatory responses by
410 modulating TLR4/NF-κB/MAPKs signaling pathways. Experimental Cell Research
411 394:112166.
412 Heneka MT et al. (2015) Neuroinflammation in Alzheimer's disease. The Lancet Neurology
413 14:388–405.
414 Hong S, Beja-Glasser VF, Nfonoyim BM, Frouin A, Li S, Ramakrishnan S, Merry KM, Shi Q,
415 Rosenthal A, Barres BA, Lemere CA, Selkoe DJ, Stevens B (2016) Complement and microglia
416 mediate early synapse loss in Alzheimer mouse models. Science 352:712–716.
417 Kabir MT, Sufian MA, Uddin MS, Begum MM, Akhter S, Islam A, Mathew B, Islam MS, Amran
418 MS, Ashraf GM (2019) NMDA Receptor Antagonists: Repositioning of Memantine as a
419 Multitargeting Agent for Alzheimer's Therapy. Current Pharmaceutical Design 25:3506–3518.
420 Kanekiyo T, Cirrito JR, Liu C-C, Shinohara M, Li J, Schuler DR, Shinohara M, Holtzman DM, Bu
421 G (2013) Neuronal Clearance of Amyloid-β by Endocytic Receptor LRP1. J Neurosci
422 33:19276–19283.
423 Karin M, Ben-Neriah Y (2000) Phosphorylation Meets Ubiquitination: The Control of NF-κB
424 Activity. Annual Review of Immunology 18:621–663.
425 Kounnas MZ, Moir RD, Rebeck GW, Bush AI, Argraves WS, Tanzi RE, Hyman BT, Strickland DK
426 (1995) LDL receptor-related protein, a multifunctional ApoE receptor, binds secreted beta-
427 amyloid precursor protein and mediates its degradation. Cell 82:331–340.
428 Krogh KA, Wydeven N, Wickman K, Thayer SA (2014) HIV-1 protein Tat produces biphasic
429 changes in NMDA-evoked increases in intracellular Ca2+ concentration via activation of Src
430 kinase and nitric oxide signaling pathways. J Neurochem 130:642–656.

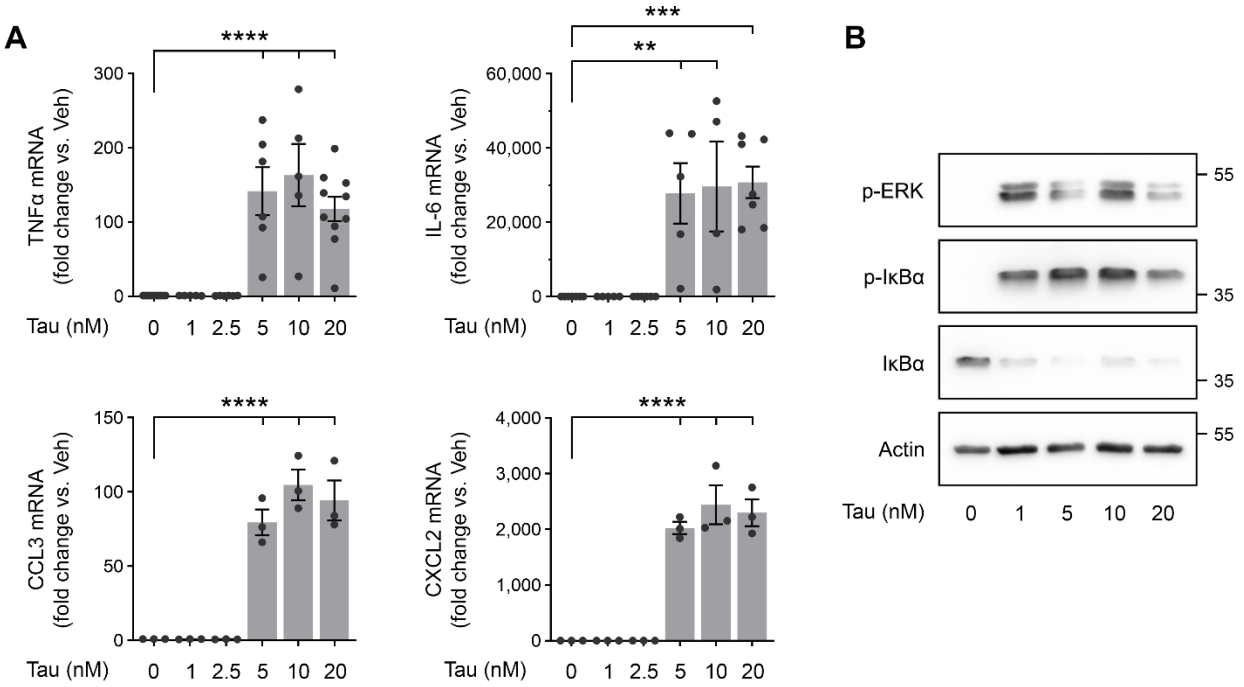
431 Lee VM, Goedert M, Trojanowski JQ (2001) Neurodegenerative tauopathies. Annu Rev Neurosci
432 24:1121–1159.
433 Liu Q, Zerbinatti CV, Zhang J, Hoe H-S, Wang B, Cole SL, Herz J, Muglia L, Bu G (2007) Amyloid
434 Precursor Protein Regulates Brain Apolipoprotein E and Cholesterol Metabolism through
435 Lipoprotein Receptor LRP1. Neuron 56:66–78.
436 Mantuano E, Azmoon P, Banki MA, Gunner CB, Gonias SL (2022) The LRP1/CD91 ligands,
437 tissue-type plasminogen activator, α2-macroglobulin, and soluble cellular prion protein have
438 distinct co-receptor requirements for activation of cell-signaling. Sci Rep 12:17594.
439 Mantuano E, Azmoon P, Banki MA, Lam MS, Sigurdson CJ, Gonias SL (2020) A soluble derivative
440 of PrPC activates cell-signaling and regulates cell physiology through LRP1 and the NMDA
441 receptor. J Biol Chem 295:14178–14188.
442 Mantuano E, Azmoon P, Brifault C, Banki MA, Gilder AS, Campana WM, Gonias SL (2017)
443 Tissue-type Plasminogen Activator Regulates Macrophage Activation and Innate Immunity.
444 Blood 130:1364–1374.
445 Mantuano E, Brifault C, Lam MS, Azmoon P, Gilder AS, Gonias SL (2016) LDL receptor-related
446 protein-1 regulates NFκB and microRNA-155 in macrophages to control the inflammatory
447 response. Proc Natl Acad Sci U S A 113:1369–1374.
448 Mantuano E, Lam MS, Gonias SL (2013) LRP1 assembles unique co-receptor systems to initiate
449 cell signaling in response to tissue-type plasminogen activator and Myelin-associated
450 glycoprotein. Journal of Biological Chemistry 288:34009–34018.
451 Mantuano E, Zampieri C, Azmoon P, Gunner CB, Heye KR, Gonias SL (2023) An LRP1-binding
452 motif in cellular prion protein replicates cell-signaling activities of the full-length protein. JCI
453 Insight 8:e170121.
454 Martin AM, Kuhlmann C, Trossbach S, Jaeger S, Waldron E, Roebroek A, Luhmann HJ, Laatsch
455 A, Weggen S, Lessmann V, Pietrzik CU (2008) The functional role of the second NPXY motif

456 of the LRP1 β-chain in tissue-type plasminogen activator-mediated activation of N-methyl-D-
457 aspartate receptors. J Biol Chem 283:12004–12013.
458 Mattei V, Manganelli V, Martellucci S, Capozzi A, Mantuano E, Longo A, Ferri A, Garofalo T,
459 Sorice M, Misasi R (2020) A multimolecular signaling complex including PrPC and LRP1 is
460 strictly dependent on lipid rafts and is essential for the function of tissue plasminogen activator.
461 J Neurochem 152:468–481.
462 May P, Rohlmann A, Bock HH, Zurhove K, Marth JD, Schomburg ED, Noebels JL, Beffert U,
463 Sweatt JD, Weeber EJ, Herz J (2004) Neuronal LRP1 Functionally Associates with
464 Postsynaptic Proteins and Is Required for Normal Motor Function in Mice. Mol Cell Biol
465 24:8872–8883.
466 Merezhko M, Brunello CA, Yan X, Vihinen H, Jokitalo E, Uronen R-L, Huttunen HJ (2018)
467 Secretion of Tau via an Unconventional Non-vesicular Mechanism. Cell Rep 25:2027-
468 2035.e4.
469 Ni M, Aschner M (2010) Neonatal rat primary microglia: isolation, culturing, and selected
470 applications. Curr Protoc Toxicol 43:12.17.1-12.17.16.
471 Nilson AN, English KC, Gerson JE, Barton Whittle T, Nicolas Crain C, Xue J, Sengupta U, Castillo-
472 Carranza DL, Zhang W, Gupta P, Kayed R (2017) Tau Oligomers Associate with Inflammation
473 in the Brain and Retina of Tauopathy Mice and in Neurodegenerative Diseases. J Alzheimers
474 Dis 55:1083–1099.
475 Overton CD, Yancey PG, Major AS, Linton MF, Fazio S (2007) Deletion of macrophage LDL
476 receptor-related protein increases atherogenesis in the mouse. Circ Res 100:670–677.
477 Pontecorvi P, Banki MA, Zampieri C, Zalfa C, Azmoon P, Kounnas MZ, Marchese C, Gonias SL,
478 Mantuano E (2019) Fibrinolysis protease receptors promote activation of astrocytes to
479 express pro-inflammatory cytokines. Journal of Neuroinflammation 16.
480 Pooler AM, Phillips EC, Lau DHW, Noble W, Hanger DP (2013) Physiological release of
481 endogenous tau is stimulated by neuronal activity. EMBO Rep 14:389–394.

482 Quinn KA, Pye VJ, Dai YP, Chesterman CN, Owensby DA (1999) Characterization of the soluble
483 form of the low density lipoprotein receptor-related protein (LRP). Exp Cell Res 251:433–441.
484 Ransohoff RM (2016) How neuroinflammation contributes to neurodegeneration. Science
485 353:777–783.
486 Rauch JN, Luna G, Guzman E, Audouard M, Challis C, Sibih YE, Leshuk C, Hernandez I,
487 Wegmann S, Hyman BT, Gradinaru V, Kampmann M, Kosik KS (2020) LRP1 is a master
488 regulator of tau uptake and spread. Nature 580:381–385.
489 Saman S, Kim W, Raya M, Visnick Y, Miro S, Saman S, Jackson B, McKee AC, Alvarez VE, Lee
490 NCY, Hall GF (2012) Exosome-associated tau is secreted in tauopathy models and is
491 selectively phosphorylated in cerebrospinal fluid in early Alzheimer disease. J Biol Chem
492 287:3842–3849.
493 Shi Y, Mantuano E, Inoue G, Campana WM, Gonias SL (2009) Ligand Binding to LRP1
494 Transactivates Trk Receptors by a Src Family Kinase–Dependent Pathway. Sci Signal 2:ra18.
495 Staudt ND, Jo M, Hu J, Bristow JM, Pizzo DP, Gaultier A, VandenBerg SR, Gonias SL (2013)
496 Myeloid cell receptor LRP1/CD91 regulates monocyte recruitment and angiogenesis in
497 tumors. Cancer Res 73:3902–3912.
498 Stiles TL, Dickendesher TL, Gaultier A, Fernandez-Castaneda A, Mantuano E, Giger RJ, Gonias
499 SL (2013) LDL receptor-related protein-1 is a sialic-acid-independent receptor for myelin-
500 associated glycoprotein that functions in neurite outgrowth inhibition by MAG and CNS myelin.
501 J Cell Sci 126:209–220.
502 Strickland DK, Gonias SL, Argraves WS (2002) Diverse roles for the LDL receptor family. Trends
503 in Endocrinology and Metabolism 13:66–74.
504 Ulery PG, Beers J, Mikhailenko I, Tanzi RE, Rebeck GW, Hyman BT, Strickland DK (2000)
505 Modulation of beta-amyloid precursor protein processing by the low density lipoprotein
506 receptor-related protein (LRP). Evidence that LRP contributes to the pathogenesis of
507 Alzheimer's disease. J Biol Chem 275:7410–7415.

508 Viatour P, Merville M-P, Bours V, Chariot A (2005) Phosphorylation of NF-kappaB and IkappaB
509 proteins: implications in cancer and inflammation. Trends Biochem Sci 30:43–52.
510 von Arnim CAF, Kinoshita A, Peltan ID, Tangredi MM, Herl L, Lee BM, Spoelgen R, Hshieh TT,
511 Ranganathan S, Battey FD, Liu C-X, Bacskai BJ, Sever S, Irizarry MC, Strickland DK, Hyman
512 BT (2005) The low density lipoprotein receptor-related protein (LRP) is a novel beta-secretase
513 (BACE1) substrate. J Biol Chem 280:17777–17785.
514 Yang L, Liu C-C, Zheng H, Kanekiyo T, Atagi Y, Jia L, Wang D, N'songo A, Can D, Xu H, Chen
515 X-F, Bu G (2016) LRP1 modulates the microglial immune response via regulation of JNK and
516 NF-κB signaling pathways. J Neuroinflammation 13:304.
517 Yoshiyama Y, Higuchi M, Zhang B, Huang S-M, Iwata N, Saido TC, Maeda J, Suhara T,
518 Trojanowski JQ, Lee VM-Y (2007) Synapse Loss and Microglial Activation Precede Tangles
519 in a P301S Tauopathy Mouse Model. Neuron 53:337–351.
520

521 **FIGURES AND FIGURE LEGENDS**



522

523 **Figure 1.** Tau induces an inflammatory response in macrophages. ***A***, BMDMs from wild-type mice
524 were treated for 6 h with increasing concentrations of Tau, as indicated. RT-qPCR was performed
525 to determine mRNA levels for TNFα, IL-6, CCL3, and CXCL2. Data are represented as mean ±
526 SEM (n = 3-8; one-way ANOVA: **p<0.01; ***p<0.001; ****p<0.0001). ***B***, BMDMs from wild-type
527 mice were treated for 1 h with increasing concentrations of Tau, as indicated. Immunoblot analysis
528 was performed to detect expression of p-ERK, p-IκBα, IκBα, and β-actin.

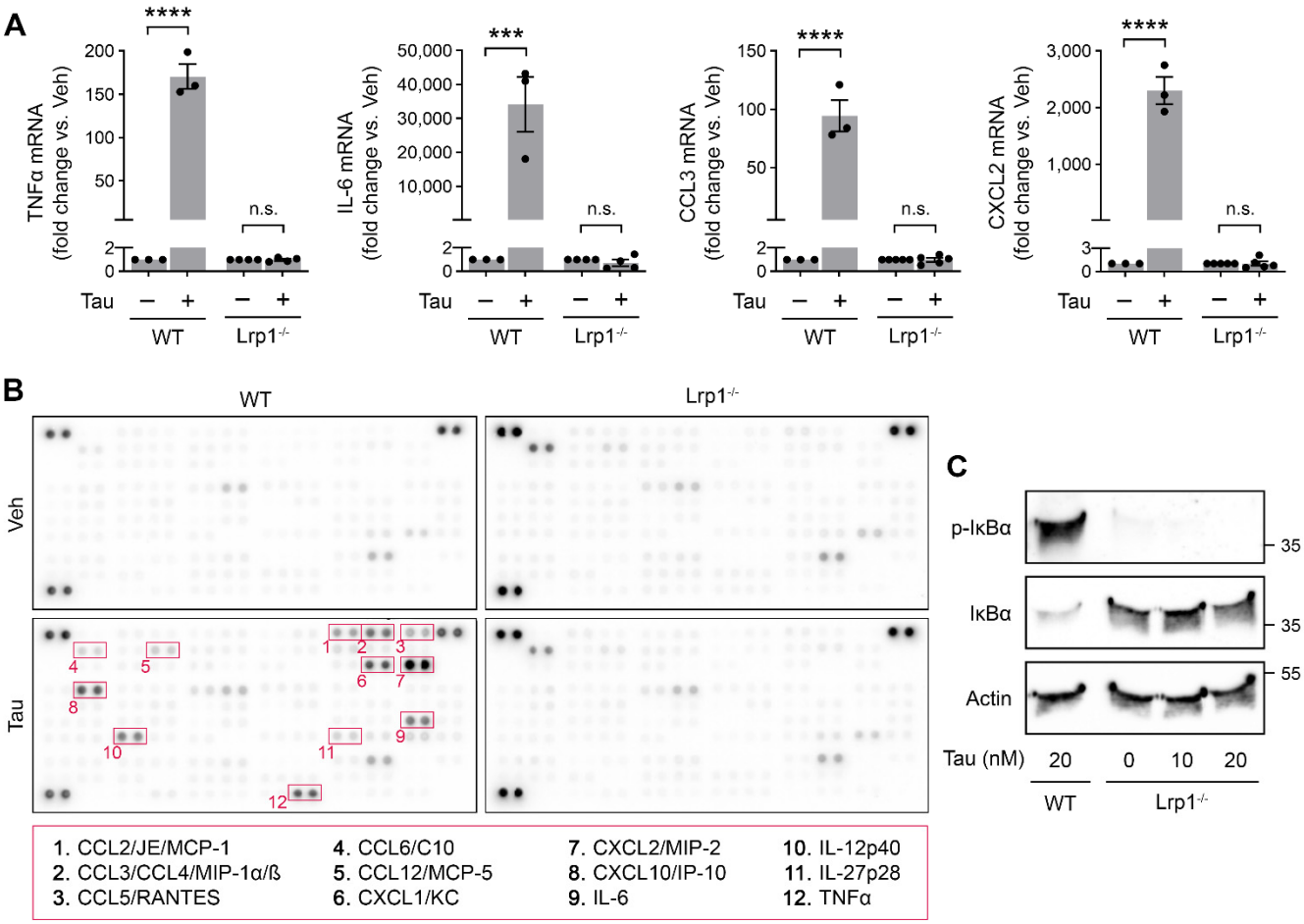


Figure 2. LRP1 mediates Tau-induced inflammation in macrophages. ***A***, BMDMs from wild-type (WT) and *Lrp1*$^{fl/fl}$-LysM-Cre-positive (*Lrp1*$^{-/-}$) mice were treated for 6 h with Tau (20 nM) or vehicle. mRNA levels for TNFα, IL-6, CCL3, and CXCL2 were determined by RT-qPCR. Data are represented as mean ± SEM (n = 3-5; two-way ANOVA: ***$p < 0.001$; ****$p < 0.0001$; n.s., not significant). ***B***, WT and *Lrp1*$^{-/-}$ BMDMs were treated for 6 h with Tau (20 nM) or vehicle. Conditioned medium (CM) was collected and analyzed using the Proteome Profiler Mouse XL Cytokine Array. Representative cytokines that were increased in Tau-treated WT BMDMs compared with *Lrp1*$^{-/-}$ cells are highlighted in red boxes and numbered. ***C***, WT and *Lrp1*$^{-/-}$ BMDMs were treated for 1 h with Tau, at the indicated concentrations. Protein extracts were analyzed by immunoblotting to detect p-IκBα, total IκBα, and β-actin.

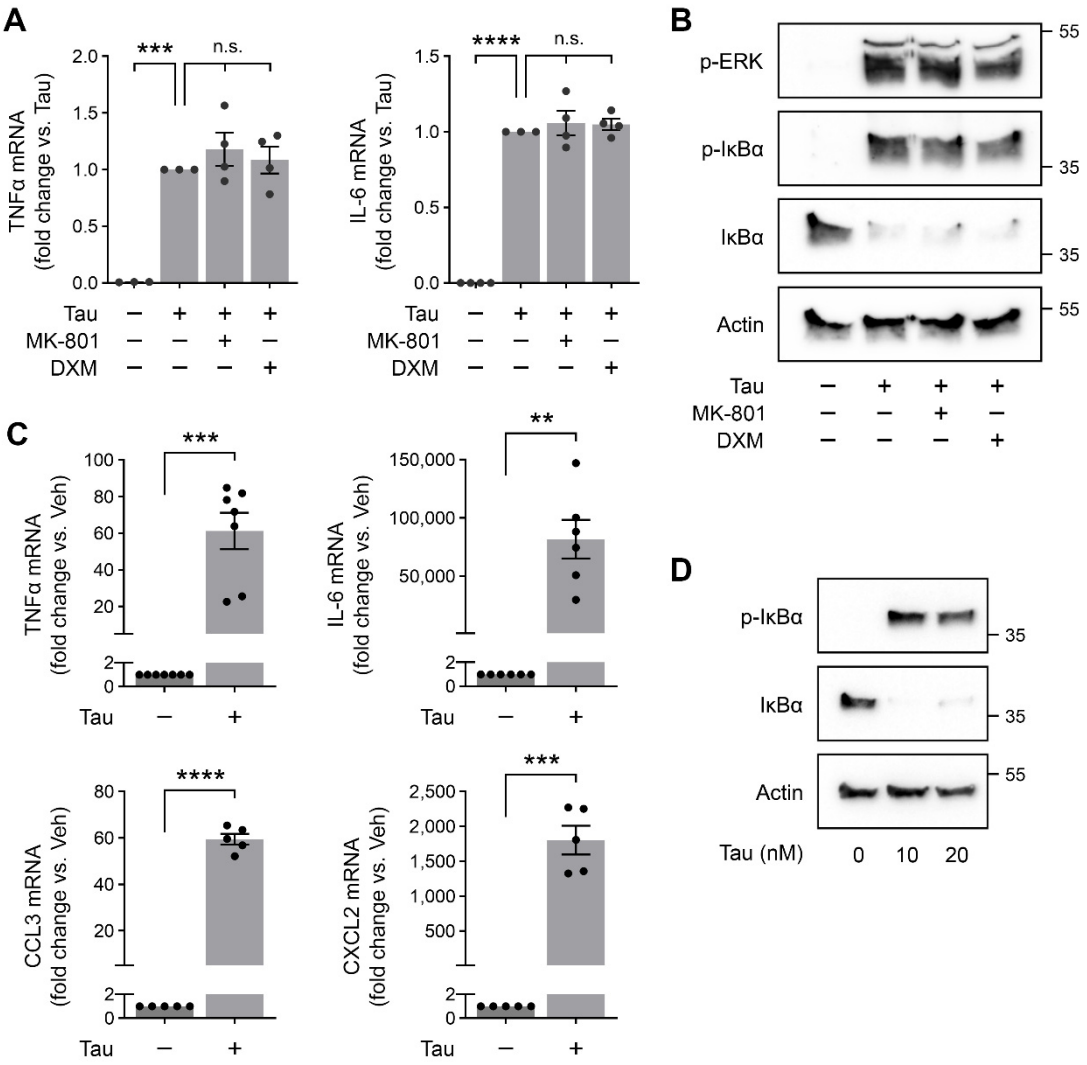
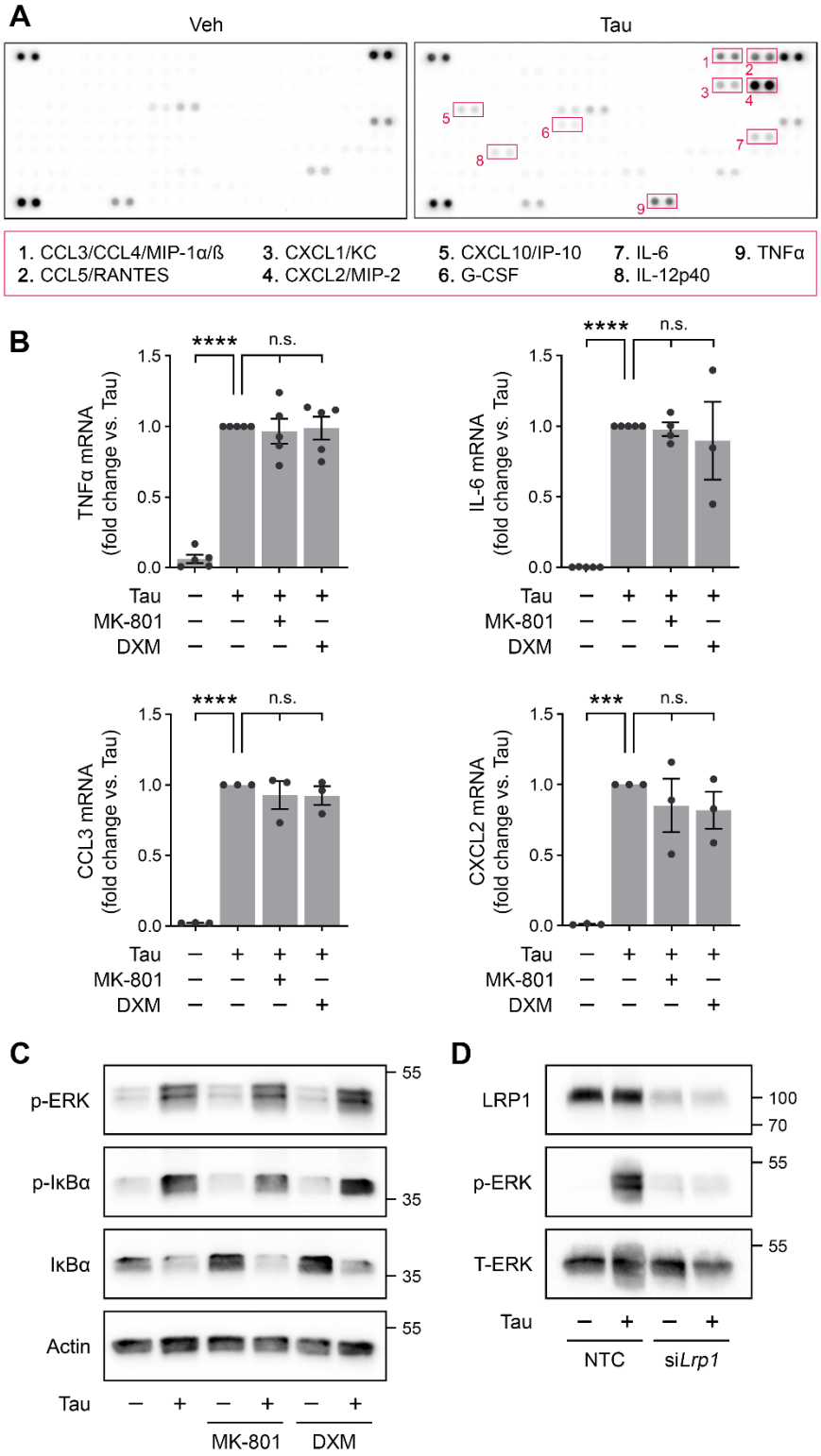


Figure 3. The Tau response in macrophages is independent of the NMDA-R. ***A***, BMDMs from wild-type (WT) mice were treated for 6 h with Tau (20 nM), MK-801 (1 μM), DXM (10 μM), or vehicle as indicated. RT-qPCR was performed to determine mRNA levels for TNFα and IL-6 (mean ± SEM; n=3-4; one-way ANOVA: ***p<0.001; ****p<0.0001; n.s., not significant). ***B***, WT BMDMs were treated for 1 h with Tau (20 nM), MK-801 (1 μM), DXM (10 μM), or vehicle as indicated. Cell extracts were subjected to immunoblot analysis to detect p-ERK, p-IκBα, total IκBα, and β-actin. ***C***, BMDMs from *Grin1*$^{fl/fl}$-LysM-Cre-positive (*Grin1*$^{-/-}$) mice were treated for 6 h with Tau (20 nM) or vehicle. mRNA levels for TNFα, IL-6, CCL3, and CXCL2 were determined by RT-qPCR (mean ± SEM; n = 5-7; paired t-test: **p<0.01; ***p<0.001; ****p<0.0001). ***D***, *Grin1*$^{-/-}$

550 BMDMs were treated for 1 h with increasing concentrations of Tau, as indicated. Immunoblot
551 analysis was performed to detect p-IκBα, IκBα, and β-actin.

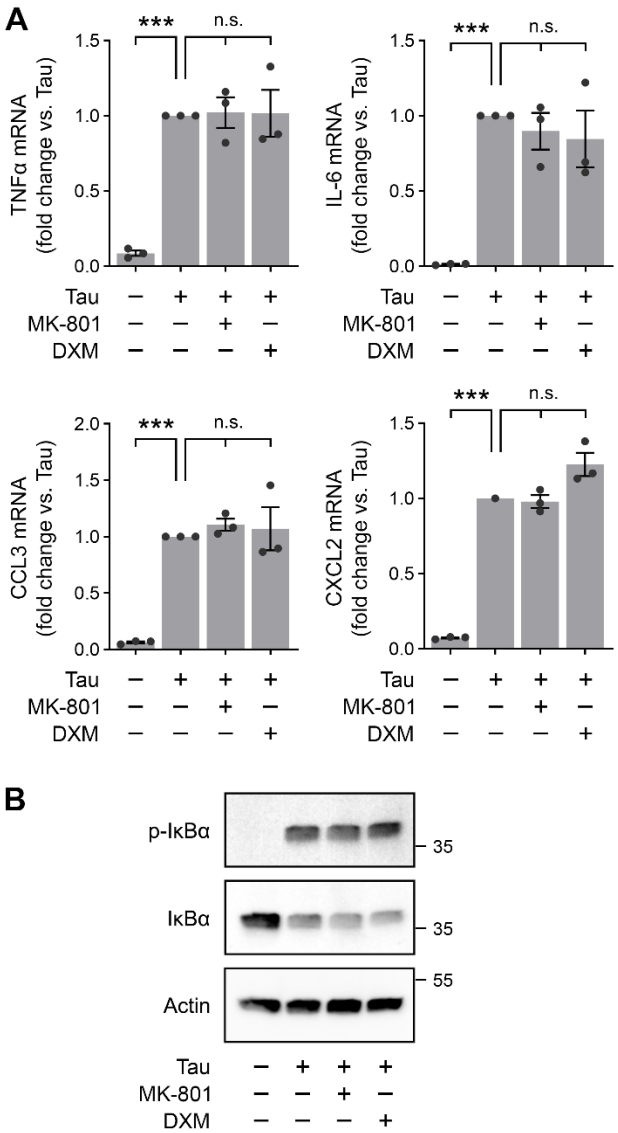


552

553 **Figure 4**. Tau induces a pro-inflammatory response in microglia that requires LRP1, but not the

554 NMDA-R. ***A***, Microglia from mouse pups were treated with Tau (20 nM) or vehicle for 6 h.

555 Conditioned medium (CM) was collected and analyzed using the Proteome Profiler Mouse XL
556 Cytokine Array. Representative cytokines that were increased compared with the control are
557 highlighted in red boxes and numbered. ***B***, Microglia from mouse pups were treated for 6 h with
558 Tau (20 nM), MK-801 (1 μM), DXM (10 μM), or vehicle as indicated. RT-qPCR was performed to
559 determine mRNA levels for TNFα, IL-6, CCL3, and CXCL2 (mean ± SEM; n = 3-5; one-way
560 ANOVA: ***p<0.001; ****p<0.0001; n.s., not significant). ***C***, Microglia from mouse pups were
561 treated for 1 h with Tau (20 nM), MK-801 (1 μM), DXM (10 μM), or vehicle as indicated. Cell
562 extracts were subjected to immunoblot analysis to detect p-ERK, p-IκBα, IκBα, and β-actin. ***D,***
563 Microglia from mouse pups were transfected with *Lrp1*-specific (siL*rp1*) or non-targeting control
564 (NTC) siRNA for 48 h. Cells were then treated with Tau (20 nM) or vehicle for 1 h and then
565 analyzed by immunoblotting to detect LRP1, p-ERK, and T-ERK.



566

567 **Figure 5.** Tau induces a pro-inflammatory response in astrocytes that does not require the NMDA-
568 R. ***A***, Astrocytes from mouse pups were treated for 6 h with Tau (20 nM), MK-801 (1 μM), DXM
569 (10 μM), or vehicle as indicated. RT-qPCR was performed to determine mRNA levels for TNFα,
570 IL-6, CCL3, and CXCL2 (mean ± SEM; n = 3; one-way ANOVA: ***$p < 0.001$; n.s., not significant).
571 ***B***, Astrocytes from mouse pups were treated for 1 h with Tau (20 nM), MK-801 (1 μM), DXM (10
572 μM), or vehicle as indicated. Protein extracts were analyzed by immunoblot to detect levels of p-
573 IκBα, IκBα, and β-actin.