1 **Fibrillar Aβ triggers microglial proteome alterations and dysfunction in Alzheimer mouse**
2 **models**
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Abstract

Microglial dysfunction is a key pathological feature of Alzheimer´s disease (AD), but little is known about proteome-wide changes in microglia during the course of AD pathogenesis and their functional consequences. Here, we performed an in-depth and time-resolved proteomic characterization of microglia in two mouse models of amyloid β (Aβ) pathology, the overexpression APPPS1 and the knock-in APP-NL-G-F (APP-KI) model. We identified a large panel of Microglial Aβ Response Proteins (MARPs) that reflect a heterogeneity of microglial alterations during early, middle and advanced stages of Aβ deposition. Although both mouse models display severe microglial alterations at late stages of amyloid pathology, MARP signatures occur earlier in the APPPS1 mice. Strikingly, the kinetic differences in proteomic profiles correlated with the presence of fibrillar Aβ, rather than dystrophic neurites, suggesting that fibrillar Aβ aggregates are the main drivers of the AD-associated microglial phenotype and the observed functional decline. The identified microglial proteomic fingerprints of AD provide a valuable resource for functional studies of novel molecular targets and potential biomarkers for monitoring AD progression or therapeutic efficacy.

Introduction

Microglia play fundamental roles in a variety of neurodegenerative diseases, including AD (McQuade and Blurton-Jones, 2019). Changes in brain immunity, together with extracellular Aβ deposition and neurofibrillary tangles, are major pathological culprits in AD (Gjoneska et al., 2015; Guillot-Sestier and Town, 2013; Holtzman et al., 2011; Shi and Holtzman, 2018). The importance of microglia in AD pathogenesis is well illustrated by the increasing number of identified AD risk genes which are expressed in microglia and have functions in brain immunity (Cuyvers and Sleegers, 2016; Guerreiro et al., 2013; Jansen et al., 2019; Jonsson et al., 2013; Karch and Goate, 2015; Lambert et al., 2009; Naj et al., 2011; Sims et al., 2017). For example, the triggering receptor expressed on myeloid cells 2 (*Trem2*) and apolipoprotein E (*ApoE*) are major genetic risk factors for sporadic AD that are expressed by plaque-associated microglia and involved in Aβ clearance (Bradshaw et al., 2013; Castellano et al., 2011; Kleinberger et al., 2014; Parhizkar et al., 2019; Reddy et al., 2009; Wang et al., 2015). It has also been shown that microglial phagocytosis decays over the course of AD (Hickman et al., 2008; Koellhoffer et al., 2017; Orre et al., 2014a; Solito and Sastre, 2012; Zuroff et al., 2017). Along these lines, Aβ clearance was found reduced in sporadic AD and it is assumed to be a key factor in the pathogenesis (Mawuenyega et al., 2010; Saido, 1998; Wildsmith et al., 2013). Importantly, Aβ clearance defects in AD microglia are reversible (Daria et al., 2017) and enhancing microglial phagocytic function has been explored as a therapeutic approach since substantial reduction of Aβ burden appears to correlate with cognitive benefits (Bacskai et al., 2001; Bard et al., 2000; Bohrmann et al., 2012; Janus et al., 2000; Lathuiliere et al., 2016; Morgan et al., 2000; Nicoll et al., 2006; Nicoll et al., 2003; Schenk et al., 1999; Schilling et al., 2018; Sevigny et al., 2016; Wilcock et al., 2004). However, when and how microglia change along AD progression is still not clear. Thus, understanding molecular alterations of microglia at different stages of AD is crucial and a pre-requisite for developing safe and efficacious therapy.

Transcriptional expression profiles for microglia were previously revealed under physiological, neurodegenerative or neuroinflammatory conditions (Butovsky et al., 2014; Galatro et al., 2017; Gosselin et al., 2017; Gotzl et al., 2019; Grabert et al., 2016; Holtman et al., 2015; Kamphuis et al., 2016; Krasemann et al., 2017; Mazaheri et al., 2017; Orre et al., 2014a; Orre et al., 2014b; Wang et al., 2015; Yin et al., 2017). Transcriptional signatures were also recently reported at single-cell resolution, demonstrating regional and functional heterogeneity of brain myeloid cells (Hammond et al., 2019; Jordao et al., 2019; Keren-Shaul et al., 2017; Mathys et al., 2017; Sala Frigerio et al., 2019). In neurodegenerative mouse models, two major profiles have been proposed along the spectrum of

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80   microglial alterations. One is the homeostatic microglial signature that occurs under physiological

81   conditions and is characterized by the expression of several genes, including *P2ry12*, *Tmem119* and

82   *Cx3cr1*. The other key signatures, referred to as disease-associated microglia (DAM), microglial

83   neurodegenerative phenotype (MGnD) or activated response microglia (ARM) are observed under

84   neurodegenerative conditions (Keren-Shaul et al., 2017; Krasemann et al., 2017; Sala Frigerio et al.,

85   2019) and characterized by increased expression of *ApoE*, *Trem2*, *Cd68*, *Clec7a* and *Itgax* (*Cd11c*),

86   among others. These changes were quantified using RNA transcripts, but transcript levels do not

87   necessarily reflect protein levels which ultimately control cell function (Bottcher et al., 2019; Mrdjen

88   et al., 2018; Sharma et al., 2015). Importantly, a recent study postulated that transcriptomic profiles

89   of microglia from another AD mouse model (5xFAD) do not correlate well with proteomic changes

90   (Rangaraju et al., 2018), suggesting the existence of additional translational or post-translational

91   regulation mechanisms in AD microglia. Additionally, little is known about Aβ-associated changes

92   in the microglial proteome in a time-resolved manner, or which proteome alterations underscore

93   microglial dysfunction. Accordingly, we analyzed the microglial proteome at distinct stages of

94   Aβ pathology in two commonly used mouse models of amyloidosis; the APPPS1 (Radde et al., 2006),

95   and the APP-KI mice (Saito et al., 2014). In contrast to the APPPS1 mouse model that overexpresses

96   mutated human amyloid precursor protein (APP) and presenilin-1 (PS1), the APP-KI model bears

97   endogenous levels of APP with a humanized Aβ sequence containing three AD mutations (NL-G-F),

98   and has no alterations of PS1 (Radde et al., 2006; Saito et al., 2014).

99   Our study determines the proteome of microglia from APPPS1 and APP-KI mice in a time

100   resolved manner, starting from pre-deposition to early, middle and advanced stages of amyloid

101   deposition and reveals a panel of MARPs that progressively change throughout Aβ accumulation.

102   Although both mouse models display severe microglial alterations at late stages of Aβ pathology, the

103   occurrence of MARP signatures differs and appears earlier in the APPPS1 mice. Strikingly, the kinetic

104   differences in proteomic profiles correlated with the presence of fibrillar Aβ, rather than dystrophic

105   neurites, suggesting that fibrillar Aβ aggregates are the main drivers of the AD-associated microglial

106   phenotype and corresponding functional decline. The time-resolved microglial profiles may serve as

107   benchmark proteomic signatures for investigating novel microglial targets or monitoring the efficacy

108   of future pre-clinical studies aiming at microglial repair.

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111   **Results**

112   *APPPS1 microglia develop an AD-associated proteomic signatures earlier compared to the APP-*

113   *KI microglia*

114   To facilitate proteomic analysis, we first optimized the microglial isolation procedure. CD11b positive

115   microglia were isolated from mouse cerebrum using MACS technology. The purity of the CD11b-

116   enriched fraction was controlled by fluorescence activated cell sorting (FACS), revealing that a 97%

117   of isolated cells were CD11b positive (Suppl. Fig 1A). Of note, only 0.49% of CD11b positive cells

118   were detected in the CD11b-depleted fraction (Suppl. Fig 1B), demonstrating high isolation

119   efficiency. Next, we optimized the data acquisition method for microglial proteome analysis (Suppl.

120   Fig 2A; Suppl. Table 1). Recently, it was shown that Data Independent Acquisition (DIA) for label-

121   free quantification (LFQ) of proteins identifies and quantifies consistently more peptides and proteins

122   across multiple samples, compared to Data Dependent Acquisition (DDA) (Bruderer et al., 2015).

123   Thus, we first evaluated the performance of DDA *vs.* DIA (Suppl. Table 2) using microglial lysates

124   from WT and APPPS1 mice. DDA identified 53912 peptides on average compared to 74281 peptides

125   identified by DIA, representing a 37.8% increase in detection by DIA method (Suppl. Table 2).

126   Overall, the main advantage of DIA was the improved consistency of protein quantifications among

127   the replicates and the identification of proteins with lower abundance, leading to 29% increase of

128   relatively quantified proteins from 4412 with DDA to 5699 with DIA (Suppl. Table 2; Suppl. Fig 2B

129   and C). We therefore selected DIA for further proteomic characterization of APPPS1 and APP-KI

130   microglia. Notably, we also detected a consistent relative quantification of proteins with an overlap

131   of 93.5% between the two mouse models (Suppl. Fig 2D), supporting our selection of DIA as a robust

132   method for microglial proteomic analysis.

133   Amyloid plaque deposits appear at similar ages (between 6-8 weeks) in APPPS1 and APP-KI

134   mouse models (Radde et al., 2006; Saito et al., 2014). To reveal the dynamics of microglial proteomic

135   alterations across different amyloid stages, we analyzed microglia from 1, 3, 6 and 12 month old

136   APPPS1 and APP-KI mice and their corresponding age-matched wild-type (WT) mice (Suppl. Fig

137   2A). For our proteomic analysis, we have set as a threshold a log2 fold change larger than 0.5 or

138   smaller than -0.5 compared to the WT with a p-value less than 0.05, and significance after False

139   Discovery Rate (FDR) correction. No data imputation was performed.

140   According to Aβ burden in both mouse models, we refer to one month of age as a pre-

141   deposition stage, and to 3, 6 and 12 months of age as early, middle and advanced stages of amyloid

142   pathology, respectively (Suppl. Fig 3). At the pre-deposition stage (1 month), microglial proteomes

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143   of APPPS1 and APP-KI mice did not show significant alterations compared to WT (Fig 1A and B),

144   demonstrating that microglia are not affected prior to development of Aβ pathology. At 3 months of

145   age, microglia in APPPS1 mice already displayed a significant up-regulation of 332 proteins and

146   down-regulation of 678 proteins, compared to WT microglia (Fig 1C; Suppl. Table 3A). In contrast,

147   APP-KI microglia were hardly affected at 3 months of age (Fig 1D; Suppl. Table 3B), which is

148   particularly surprising because both mouse models show comparable amyloid burden at this stage

149   (Suppl. Fig 3). At 6 months of age, microglia in APPPS1 mice displayed 309 up-regulated and 261

150   down-regulated proteins, compared to WT microglia (Fig 1E; Suppl. Table 3A). In contrast to 3

151   months of age (Fig 1D), APP-KI mice at 6 months of age displayed a substantial alteration of their

152   microglial proteome, illustrated by 140 up-regulated and 151 down-regulated proteins (Fig 1F; Suppl.

153   Table 3B). Still, microglial alterations in 6 month old APP-KI mice were less pronounced compared

154   to the proteome of APPPS1 mice (Fig 1E and F). Noteworthy, by 12 months of age, APPPS1 microglia

155   revealed a significant up-regulation of 776 proteins and down-regulation of 633 proteins, while APP-

156   KI microglia displayed 704 up-regulated and 666 down-regulated proteins (Fig 1G and H; Suppl.

157   Table 3A and B). This indicates comparable changes in APPPS1 and APP-KI mice at advanced stages

158   of Aβ pathology. Overall, our data show that amyloid plaque accumulation triggers microglial

159   progression towards an AD-associated phenotype in both mouse models, but that response dynamics

160   are different in APPPS1 and APP-KI microglia.

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Identification of MARPs as signatures of early, middle and advanced amyloid stages

163   Next, we determined protein alterations that first appear in early, middle or advanced stages of

164   Aβ deposition and remain altered thought all analyzed stages, thus following amyloid accumulation.

165   To this end, we selected the APPPS1 mouse model as a reference since it displays earlier changes and

166   thus provides a better time resolution of protein alterations to amyloid response, compared to the APP-

167   KI model (Fig 2A). Correspondingly, we defined early (proteins changed at 3, 6, and 12 months),

168   middle (proteins changed only at 6 and 12 months) and advanced (proteins changed only at 12 months)

169   MARPs. Only proteins with a consistent quantification in all samples of an age group were used for

170   relative quantification. Furthermore, in order to determine robust and model-independent Aβ-

171   triggered microglial alterations, we only selected MARPs that were altered with a significantly

172   changed abundance in both mouse models (even if in APP-KI microglia changes appear later). This

173   analysis identified 90 early, 176 middle, and 435 advanced MARPs (Suppl. Fig 4A). The most

174   strongly regulated MARPs with early, middle and advanced response are displayed in corresponding

175 heatmaps (Fig 2B-D). In addition, we compared MARP signatures with the previously delineated

176 RNA signatures of 5xFAD mice (Keren-Shaul et al., 2017) to visualize the overlap, as well as

177 differences, between proteomic and transcriptomic microglial profiles.

178 Early MARPs included several of the previously identified transcriptional DAM markers

179 (Keren-Shaul et al., 2017) such as ITGAX (CD11c), APOE, CLEC7a, LGALS3 (Galectin-3) and

180 CD68, which were found with an increased abundance (Fig 2B). Moreover, proteins involved in

181 antigen presentation such as CD74, H2-D1, TAP2, TAPBP and H2-K1 were revealed as up-regulated

182 early MARPs. In addition, we discovered prominent changes in interferon signaling represented by

183 the up-regulation of early MARPs, including MNDA, OAS1A, IFIT3, ISG15, GVIN1, STAT1 and 2

184 (Fig 2B). Even though early MARPs were mainly up-regulated, we also identified early MARPs with

185 a decreased abundance, including KRAS, a protein involved in cell proliferation and the endocytosis

186 regulator EHD2 among others (Fig 2B). A gene ontology (GO) cluster enrichment analysis of early

187 MARPs revealed that up-regulated proteins were enriched for immune and viral response, interferon

188 beta and cytokine response, antigen processing and presentation as well as biotic and lipid response

189 (Fig 3A; Suppl. Fig 5A und D). Thus, these processes represent first molecular alterations that

190 progressively follow Aβ plaque pathology.

191 The middle MARPs included the up-regulated proteins FABP3, FABP5, CD63, TREM2, MIF

192 and GUSB (Fig 2C), demonstrating a progressive conversion of the microglial proteome towards a

193 disease state that accompanies Aβ accumulation. Importantly, middle MARPs also reveal down-

194 regulation of the proposed homeostatic markers such as CX3CR1, TMEM119 and P2RY12 (Fig 2C).

195 Among the down-regulated middle MARPs, we identified additional chemotaxis and cell migration

196 related proteins like SYK, FER, CX3CL1, and BIN2 (Fig 2C; Suppl. Table 4), underscoring a loss of

197 key homeostatic functions of microglia throughout AD progression.

198 Advanced MARPs represent proteins that were only altered upon extensive amyloid pathology

199 and show a high correlation between the two models (Suppl. Fig 4B). This group included up-

200 regulation of proteins involved in calcium ion binding such as NCAN, MYO5A, HPCAL4, TTYH1

201 and GCA and down-regulation of proteins that play a role in the endocytosis/lysosomal system such

202 as TFEB, TFE3 and BIN1 (Fig 2D; Suppl. Table 4). In addition, different G protein-coupled receptor

203 signaling proteins, including GNG2, GNG5 and GNG10, also displayed a decreased abundance (Fig

204 2D).

205 A GO cluster enrichment analysis of middle and advanced MARPs identified down-regulation

206 of biological processes including cell motility, migration and chemotaxis, as well as cell development

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207   and proliferation (Fig 3B and C; Suppl. Fig 5B, C, E and F). Conversely, we found an up-regulation

208   of protein glycosylation and carbohydrate metabolism (Fig 3C; Suppl. Fig 5E and F). Additionally,

209   alterations in ion transport processes involving ion homeostasis and pH regulation were also detected

210   (Fig 3C). These findings indicate that after an initial inflammatory response, several cellular processes

211   related to chemotaxis and phagocytosis are progressively dysregulated upon increased Aβ deposition.

212   Importantly, our proteomic analysis also detected alterations in proteins related to different

213   genetic risk factors of AD (Karch and Goate, 2015), including significantly increased levels of APOE,

214   TREM2, and INPP5D, and decreased levels of PLCG2, ABI3, and BIN1 in both mouse models

215   (Suppl. Table 5A and B).

216   The overlap of consistently quantified proteins and a previously published transcriptome study

217   (Keren-Shaul et al., 2017) was 38.4%, whereas 2152 and 2841 gene products were only quantified on

218   protein and transcript level, respectively (Suppl. Fig 4C). Single cell transcriptomics (Keren-Shaul et

219   al., 2017) has demonstrated a similar regulation of a number of early MARPs while we found less

220   overlap for middle and advanced MARPs (Fig 2B-D). We also identified proteins with an inverse

221   regulation compared to transcriptomic signatures such as the early MARP RPL38, middle MARPs

222   MCM3 and GFPT1 or advanced MARPs CDC88A, GALNT2, EIF4B and CHMP6 (Fig 2B-D; Suppl.

223   Table 4). Furthermore, the advanced MARP HEXB showed a consistent up-regulation in our

224   proteomic analysis, despite being previously anticipated as a homeostatic gene (Suppl. Table 4).

225   Overall, our study presents a robust and reliable method to track microglial proteome and

226   provides a resource that maps changes in brain immunity during different phases of Aβ accumulation.

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228   ***Proteomic changes are detected in plaque-associated microglia***

229   Next, we validated proteomic changes by western blot analysis using isolated microglia from 12

230   month old APPPS1 and APP-KI mice. This analysis confirmed the pronounced increase of the early

231   MARPs APOE and CD68, the middle MARPs TREM2 and FABP5, as well as reduced levels of the

232   middle MARP CSF1R (Suppl. Fig 4D) in both transgenic mouse models compared to WT mice.

233   Furthermore, proteomic changes were also validated by immunohistochemistry in order to visualize

234   spatial distribution of altered microglial proteins in APPPS1 and APP-KI mice. Immunohistological

235   analysis of 3 month old APPPS1 mice already revealed increased immunoreactivity of selected

236   MARPs such as CLEC7a (Fig 4), TREM2 (Suppl. Fig 6) and APOE (Suppl. Fig 7) that mark initial

237   stages of microglial activation in AD. This increase was detected in IBA1 positive microglia that were

238   clustering around amyloid plaques, but not in microglia further away from plaques and was – in

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239   agreement with our proteomic data – less pronounced in 3 month old APP-KI mice. Accordingly, at

240   12 months, both APPPS1 and APP-KI mice showed a similar increase in the levels of selected MARPs

241   such as CLEC7a (Fig 5) and decreased levels of TMEM119 (Fig 6) compared to the WT mice, once

242   again in microglia surrounding amyloid plaques. Taken together, we validated selected microglial

243   proteomic alterations from our dataset by applying biochemical and immunohistochemical methods.

244   In addition, we confirmed the kinetic differences in AD-associated proteomic signatures of APPPS1

245   and APP-KI microglia. Our data suggest that interaction between microglia and Aβ is likely triggering

246   the proteomic changes as they could be observed in plaque-associated microglial population.

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248   *APPPS1 and APP-KI mice show similar dynamics of amyloid plaque deposition, but differ in*

249   *plaque fibrillization*

250   The magnitude of proteomic microglial changes was found to correlate with Aβ plaque accumulation

251   throughout disease progression. However, the appearance of MARP signatures differed between the

252   models and occurred earlier in the APPPS1 mice (Fig 1C and D; Fig 2A) despite the comparable

253   plaque load observed in both mouse models (Suppl. Fig 3). Thus, it appears possible that the nature

254   of amyloid plaques is different between the APPPS1 and APP-KI mice. To examine this, we analyzed

255   amyloid plaques in 3, 6 and 12 month old APPPS1 and APP-KI mice by immunohistochemistry. We

256   used the anti-Aβ antibody NAB228 (Abner et al., 2018) to detect amyloid plaques, and Thiazine red

257   to visualize fibrillar amyloid plaque cores (Daria et al., 2017) (Fig 7A). In agreement with amyloid

258   plaque pathology reported in this model (Radde et al., 2006), APPPS1 mice contained fibrillar amyloid

259   plaque cores already at 3 months of age. In contrast, fibrillar Aβ was barely detectable in APP-KI

260   mice at 3 months of age (Fig 7A). The amount of fibrillar Aβ in APP-KI mice increased at 6 and 12

261   months, but overall still remained lower compared to the APPPS1 mice. This result was also

262   confirmed by biochemical analysis in which fibrillar Aβ was specifically detected via immunoblot of

263   the insoluble brain fraction (Fig 7B). Therefore, we excluded differences in the detection and binding

264   properties of Thiazine red to be the underlying cause for the observed reduction in the levels of fibrillar

265   Aβ in APP-KI mice. Taken together, although immunohistochemistry revealed comparable

266   Aβ plaque coverage in APPPS1 and APP-KI mice, the amount of fibrillar Aβ was significantly lower

267   in APP-KI mice.

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270 *Microglial recruitment is triggered by fibrillar Aβ and not by dystrophic neurites*

271 To determine what triggers microglial reactivity in AD, we first quantified microglial recruitment to

272 Aβ plaques in both mouse models. This analysis was done at the early pathological stage (3 months),

273 where we identified prominent differences in the proteome regulation (Fig 1C and D; Fig 2A) as well

274 as in the amount of fibrillar Aβ (Fig 7A and B) between the two AD mouse models.

275 Immunohistochemical analysis revealed IBA1 positive, amoeboid microglia recruited to large,

276 Thiazine red positive, fibrillar Aβ plaque cores in APPPS1 mice. Of note, we observed intracellular

277 fibrillar Aβ in APPPS1 microglia in close contact to the plaque core (Fig 8A) as previously reported

278 (Bolmont et al., 2008). Despite the significantly smaller fibrillar Aβ plaque core in APP-KI mice, we

279 could observe IBA1 positive microglia polarized towards the fibrillar Aβ, rather than to the

280 surrounding non-fibrillar Aβ positive material (Fig 8A). Quantification analysis revealed increased

281 clustering of IBA1 positive microglia around Aβ plaques in APPPS1 compared to the APP-KI mice

282 (Fig 8B), which display overall larger Aβ plaque size (Fig 8C). Likewise, we observed increased

283 CD68 immunoreactivity around Aβ plaques in the APPPS1 compared to the APP-KI mice (Fig 8D

284 and E). However, CD68 signal per individual microglial cell in the plaque vicinity was similar in both

285 models (Fig 8F), suggesting that differences in AD-associated microglial proteins are due to the

286 number of recruited microglia rather than differences in their individual CD68 protein levels.

287 Besides Aβ, microglial recruitment has also been associated with neuritic damage (dystrophic

288 neurites) (Hemonnot et al., 2019). Accordingly, we analyzed dystrophic neurite pathology in 3 month

289 old APPPS1 and APP-KI mice, using an antibody against APP that accumulates in these structures

290 (Cummings et al., 1992; Sadleir et al., 2016). As previously reported (Radde et al., 2006), amyloid

291 plaques in the APPPS1 mice were surrounded by prominent dystrophic neurites (Fig 8G).

292 Interestingly, despite the reduced load of fibrillar Aβ, we readily detected dystrophic neurites in the

293 APP-KI mice (Fig 8G). Moreover, our quantification analysis revealed a trend towards an increased

294 dystrophic neurite area in the APP-KI compared to the APPPS1 mice (Fig 8H). Therefore, the

295 differences in early microglial recruitment to APPPS1 plaques and the consecutive proteomic changes

296 are less likely to be triggered by dystrophic neurites.

297 Altogether, we hypothesize that microglial recruitment is primarily triggered by the fibrillar

298 Aβ content of amyloid plaques which drives the acquisition of MARP signatures.

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301 *Phagocytic impairments correlate with the occurrence of MARP signatures*

302 The differences observed in the dynamics of microglial response to amyloid in the APPPS1 and the

303 APP-KI mice prompted us to examine the association between microglial phagocytic function and the

304 appearance of MARP signatures. To this end, we assessed the phagocytic capacity of microglia from

305 3 and 6 month old APPPS1 and APP-KI mice compared to the corresponding age-matched WT

306 microglia using the *E.coli*-pHrodo uptake assay (Gotzl et al., 2019; Kleinberger et al., 2014). We

307 already detected phagocytic dysfunction in 3 month old APPPS1 microglia, which was reflected by a

308 prominent decrease in the amount of intracellular *E.coli* particles (Fig 8I; Suppl. Fig 8A) and a reduced

309 number of CD11b positive cells that were capable of *E.coli* uptake (Fig 8J; Suppl. Fig 8B). Notably,

310 APPPS1 phagocytic impairment did not change further in 6 month old microglia, suggesting that

311 microglial functional deficits, as measured by the *E.coli* uptake assay, were fully established already

312 at 3 months of age and characterized by early MARPs. In contrast, APP-KI microglia remained

313 functional at 3 months, but at 6 months displayed similar impairments as seen in APPPS1 microglia

314 (Fig 8I and J). Overall, we observed different kinetics of microglial dysfunction among mouse models

315 which correlate with the appearance of MARPs and, in turn, with the presence fibrillar Aβ.

316
317 **Discussion**
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319 This study presents an in-depth and time-resolved proteome of microglia isolated across different

320 stages of Aβ accumulation in the APPPS1 and APP-KI mouse models, resulting in the identification

321 of early, middle, and advanced MARPs. Importantly, we demonstrated that the structure of amyloid

322 plaques (fibrillar *versus* non-fibrillar) is a major determinant driving the molecular alterations of

323 microglia. Key microglial signatures encompass proteins with a central function in microglial biology

324 and AD pathogenesis. Moreover, our functional analysis shows that early MARP signatures already

325 reflect microglial phagocytic dysfunction.

326 To achieve robust and reproducible relative quantification of microglial proteins from single

327 mice, we improved the yield of acutely isolated microglia to an average of $2x10^6$ cells per mouse

328 brain, compared to recently published protocols (Flowers et al., 2017; Rangaraju et al., 2018). Next,

329 by establishing the more sensitive DIA method for protein quantification, we improved the number of

330 consistently identified proteins by 29.3% and obtained on average 5699 (APPPS1) and 5698 (APP-

331 KI) relatively quantified proteins. Notably, our analysis enhanced the detection of low abundance

332 proteins and does not require data imputation. The advancement to previous studies (Rangaraju et al.,

333 2018; Sharma et al., 2015) is also exemplified by quantification of membrane proteins, including well

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334 known microglial homeostatic markers TMEM119 or P2RY12. We also measured alterations in

335 proteins that were postulated to be only altered at the transcriptional level in AD microglia (Rangaraju

336 et al., 2018), including up-regulation of middle MARPs FABP3, FABP5, MIF and PLP2. In summary,

337 our study achieved a major improvement in quantitative proteomic analysis of rodent microglia

338 (Flowers et al., 2017; Rangaraju et al., 2018; Thygesen et al., 2018). This methodological advance

339 enabled us to map microglial changes across diverse stages of Aβ pathology in two widely explored

340 pre-clinical models of amyloidosis. Generated proteomic profiles characterize microglia under

341 diseased conditions and can be used as a resource to track changes upon microglial therapeutic

342 modification, such as Aβ immunotherapy. Such studies would facilitate discovery of clinically

343 relevant molecular alterations that are necessary for microglial functional repair, monitoring disease

344 progression and therapeutic efficacy.

345 The TREM2/APOE axis plays a key role in the regulation of the microglial transcriptional

346 program and guides the homeostatic/DAM signature switch (Jay et al., 2017; Keren-Shaul et al., 2017;

347 Krasemann et al., 2017). Our time-resolved proteomic analysis observed major rearrangements of the

348 microglial proteomic landscape in both APPPS1 and APP-KI mice and revealed a partial overlap

349 between MARPs and transcriptional profiles of DAM and homeostatic microglia (Keren-Shaul et al.,

350 2017), but also identified additional microglial marker proteins throughout different stages of

351 Aβ deposition.

352 Early MARPs include proteins of the interferon response, which is consistent with the recently

353 identified interferon-responsive microglial sub-population in AD mice (Sala Frigerio et al., 2019).

354 Numerous up-regulated early MARPs, including CD74, CTSZ, HEXA, CTSH, GLB1, CD68, NPC2,

355 CLN3 and PI4K2A, reflect alterations in endo-lysosomal homeostasis as an early pathological insult

356 in AD microglia (Van Acker et al., 2019). Additionally, factors of the fatty acid and cholesterol

357 metabolism are altered throughout all pathological phases. Up-regulated are the early (APOE,

358 ACACA, and SOAT1) middle (FABP3, FABP5, NCEH1, APOD, AACS, ACOX3, HACD2) and

359 advanced MARPs (ACOT11, ACSBG1, ECHS1, ELOVL1, and FASN) and down-regulated are

360 several middle and advanced MARPs (NAAA, FAM213B, HPGD, HPGDS, and PRKAB1), linking

361 microglial lipid dyshomeostasis and AD pathology.

362 An inflammatory response in AD is suggested by the significant up-regulation of early MARPs

363 LGALS3 and its binding protein (LGALS3BP). Recent findings suggested that the LGALS3/TREM2

364 signalling pathway, that acts as an inflammatory regulator of amyloid plaque formation, may also be

365 of relevance for AD pathology in humans (Boza-Serrano et al., 2019). Further evidence that some of

12

366 the presented proteomic alterations of rodent microglia may be relevant for human disease is given by

367 the detection of up-regulated early/middle microglial MARPs, including CD68, TREM2 and ITGAX

368 in microglia surrounding amyloid plaques in postmortem AD brains (Hopperton et al., 2018). As

369 microglia emerge as a promising therapeutic target in AD, additional MARP signatures should be

370 validated in human tissue. In particular, early MARPs that are strongly increased in both AD mouse

371 models may serve as a resource to identify novel AD biomarkers and more specific microglial positron

372 emission tomography (PET) tracers that are urgently needed to monitor microglial reactivity *in vivo*

373 (Edison et al., 2018; Hemonnot et al., 2019). Middle and late MARPs reveal a decrease of microglial

374 homeostatic functions affecting chemotaxis, cell migration and phagocytosis (e.g., CX3CR1, SYK,

375 P2RY12, BIN2, TFEB and TFE3) and thus mark AD progression.

376   It is still being discussed which is the main trigger for microglial recruitment to amyloid

377 plaques and their molecular switch from a homeostatic to a neurodegenerative phenotype (Hemonnot

378 et al., 2019; Jung et al., 2015; Krasemann et al., 2017). Our study proposes that microglial recruitment

379 to Aβ deposits and their corresponding disease-associated proteomic alterations are triggered by

380 fibrillar Aβ, rather than by dystrophic neurites. We observed more diffuse amyloid plaque

381 morphology with less fibrillar Aβ and prominent neuritic dystrophies in the APP-KI mice. Similar

382 plaque morphology, with less fibrillar Aβ, is also observed in AD mice deficient for TREM2 or APOE

383 that also have less microglial cells recruited to amyloid plaques and display prominent neuritic

384 dystrophies (Parhizkar et al., 2019; Sala Frigerio et al., 2019; Ulrich et al., 2018; Wang et al., 2015;

385 Yuan et al., 2016). APOE may have a dual role and control the transcriptional/translational response

386 of microglia to amyloid as well as amyloid plaque compactness that directs microglial recruitment

387 and thus creates a regulatory feedback-loop. These findings are also strengthened by the relevance of

388 *ApoE* and *Trem2* as genetic risk factors of AD (Karch and Goate, 2015). Fibrillar Aβ as the trigger

389 for microglial recruitment is also supported by the human pathology where neuritic plaques in AD

390 brains were found surrounded by microglia. In contrast, microglial clustering was not detected at

391 diffuse plaques lacking fibrillar Aβ core (D'Andrea et al., 2004).

392   Although DAM signatures have been suggested as a protective response, there is still a lack of

393 direct experimental evidence linking specific transcriptomic or proteomic profiles to improved

394 microglial function. Importantly, our study demonstrates a functional link between proteomic changes

395 and reduced phagocytosis by AD microglia. APPPS1 microglia start acquiring early MARPs at the

396 age of 3 months, which is already accompanied by reduced phagocytic function. In contrast, less

397 altered proteomic signatures of 3 month old APP-KI microglia correlated with preserved phagocytic

398   function. Pronounced MARP signatures that appeared later in APP-KI microglia (6 months) were

399   subsequently in accordance with phagocytic impairments. Therefore, differences in plaque

400   fibrillization in both mouse models did not only affect microglial recruitment and activation, but also

401   the phagocytic function of microglia.

402   Reduced phagocytosis of AD microglia might be related to observed proteomic alterations in

403   lysosomal proteins or cell receptors. TREM2, which was found to be increased in both mouse models,

404   plays an important role in phagocytosis as mutations of TREM2 related to AD and FTLD impair

405   phagocytic activity of microglia (Kleinberger et al., 2014). However, up-regulation of the

406   TREM2/APOE axis involves up-regulation of many lysosomal proteins (e.g., cathepsins or CD68)

407   that are part of MARPs and altered in APPPS1 and APP-KI microglia. This may reflect a

408   compensatory mechanism initiated as a response of microglia to Aβ accumulation in order to enhance

409   phagocytic function. Eventually this frustrated microglial response fails to translate into improved Aβ

410   clearance capability.

411   Phagocytosis might also be altered through differential regulation of toll like receptors (TLR).

412   Among the TLRs, TLR2, an Aβ binding receptor (Liu et al., 2012; McDonald et al., 2016), showed

413   the strongest increase with age while TLR9 was significantly reduced in APPPS1 and APP-KI mice.

414   Along these lines, TLR2 deficiency reduced the inflammatory response of microglia to Aβ42, but

415   increased Aβ phagocytosis in cultured microglia (Liu et al., 2012) while TLR9 is associated with

416   improved Aβ clearance (Scholtzova et al., 2009). Thus, differential regulation of TLRs might

417   contribute to the reduced phagocytic activity of aged APPPS1 microglia (Daria et al., 2017).

418   Additionally, many purinergic receptors (e.g., P2RX7, P2RY12 or P2RY13), which are

419   important regulators of chemotaxis, phagocytosis, membrane polarization, and inflammatory

420   signaling and thus emerged as possible microglial targets in AD (Calovi et al., 2019; Hemonnot et al.,

421   2019), were found to be down-regulated in both AD mouse models. P2RY12 is regarded as a marker

422   for ramified non-inflammatory microglia (Mildner et al., 2017) that is reduced in response to Aβ

423   plaques and therefore represents a homeostatic microglial marker (Keren-Shaul et al., 2017;

424   Krasemann et al., 2017). In contrast, P2RX4, a purinergic receptor that is likely to be involved in

425   shifting microglia towards a pro-inflammatory phenotype (Calovi et al., 2019) or myelin phagocytosis

426   (Zabala et al., 2018) had an increased abundance in both AD models. Taken together, our data

427   emphasize alterations of purinergic receptor signaling in AD microglia that may regulate a

428   morphological change towards amoeboid microglia with reduced motility and increased pro-

429   inflammatory activity.

14

430   Our study confirms that both mouse models are valuable tools for studying Aβ−induced

431   pathological changes of microglia that are remarkably comparable at advanced stages of amyloidosis.

432   However, the observed differences in the dynamics of early, middle and late MARPs in APPPS1 and

433   APP-KI mice should be considered for the design of pre-clinical studies of microglial repair and will

434   require different time windows for microglial modulation.

435   In conclusion, we tracked pathological alterations of microglia in two AD mouse models using

436   a proteomic approach. Our work demonstrates that microglial alterations are triggered as a response

437   to Aβ deposition as pre-deposition stages do not reveal proteomic alterations. The conversion to

438   MARPs is supported by changes in TREM2-APOE regulation mechanism. AD microglia display

439   pronounced interferon stimulation, increased antigen presentation, alterations in cell surface receptors,

440   lipid homeostasis and metabolism. Those proteomic changes in microglia occur as a response to

441   fibrillar Aβ and are reflected in amoeboid microglial morphology and impaired phagocytic capacity.

442   Finally, our proteomic dataset serves a valuable research resource providing information on microglial

443   alterations over different stages of Aβ deposition that can be used to monitor therapeutic efficacy of

444   microglial repair strategies.

445

446   **Materials and Methods**

447   *Animals*

448   Male and female mice of the hemizygous APPPS1 mouse line overexpressing human APP$_{KM670/671NL}$

449   and PS1$_{L166P}$ under the control of the Thy-1 promoter (Radde et al., 2006), homozygous *App^{NL-G-F}*

450   mouse line (Saito et al., 2014) and the C57BL/6J (WT) line were used in this study. Mice were group

451   housed under specific pathogen-free conditions. Mice had access to water and standard mouse chow

452   (Ssniff® Ms-H, Ssniff Spezialdiäten GmbH, Soest, Germany) *ad libitum* and were kept under a 12/12

453   h light-dark cycle in IVC System Typ II L-cages (528 cm^2) equipped with solid floors and a layer of

454   bedding. All animal experiments were performed in compliance with the German animal welfare law

455   and have been approved by the government of Upper Bavaria.

456

457   *Isolation of primary microglia*

458   Primary microglia were isolated from mouse brains (cerebrum) using MACS Technology (Miltenyi

459   Biotec) according to manufacturer´s instructions and as previously described (Daria et al., 2017).

460   Briefly, olfactory bulb, brain stem and cerebellum were removed and the remaining tissue (cerebrum)

461   was freed from meninges and dissociated by enzymatic digestion using a Neural Tissue Dissociation

15

462   Kit P (Miltenyi Biotec). Subsequently, mechanical dissociation was performed by using 3 fire-

463   polished glass Pasteur pipettes of decreasing diameter. CD11b positive microglia were magnetically

464   labelled using CD11b MicroBeads, loaded onto a MACS LS Column (Miltenyi Biotec) and subjected

465   to magnetic separation, resulting in CD11b-enriched (microglia-enriched) and CD11b-depleted

466   (microglia-depleted) fractions. Obtained microglia-enriched pellets were either washed twice with

467   HBSS (Gibco) supplemented with 7 mM HEPES, frozen in liquid nitrogen and stored at -80°C for

468   biochemical or mass spectrometry analysis or resuspended in microglial culturing media and used for

469   phagocytosis assay as described below.

470

471   *Sample preparation for mass spectrometry*

472   Microglia-enriched pellets were lysed in 200 µL of STET lysis buffer (50 mM Tris, 150 mM NaCl, 2

473   mM EDTA, 1% Triton, pH 7.5) at 4°C with intermediate vortexing. The samples were centrifuged for

474   5 min at 16000 x g at 4°C to remove cell debris and undissolved material. The supernatant was

475   transferred to a LoBind tube (Eppendorf) and the protein concentration estimated using the Pierce 660

476   nm protein assay (ThermoFisher Scientific). A protein amount of 15 µg was subjected to tryptic

477   protein digestion applying the the filter aided sample preparation protocol (FASP) (Wisniewski et al.,

478   2009) using Vivacon spin filters with a 30 kDa cut-off (Sartorius). Briefly, proteins were reduced with

479   20 mM dithiothreitol and free cystein residues were alkylated with 50 mM iodoacetamide (Sigma

480   Aldrich). After the urea washing steps, proteins were digested with 0.3 µg LysC (Promega) for 16 h

481   at 37°C followed by a second digestion step with 0.15 µg trypsin (Promega) for 4 h at 37°C. The

482   peptides were eluted into collection tubes and acidified with formic acid (Sigma Aldrich). Afterwards,

483   proteolytic peptides were desalted by stop and go extraction (STAGE) with self-packed C18 tips

484   (Empore C18 SPE, 3M) (Rappsilber et al., 2003). After vacuum centrifugation, peptides were

485   dissolved in 20 µL 0.1% formic acid (Biosolve) and indexed retention time peptides were added (iRT

486   Kit, Biognosys).

487

488   *Liquid chromatography – tandem mass spectrometry analysis*

489   For LFQ of proteins, peptides were analyzed on an Easy nLC 1000 or 1200 nanoHPLC (Thermo

490   Scientific) which was coupled online via a Nanospray Flex Ion Source (Thermo Sientific) equipped

491   with a PRSO-V1 column oven (Sonation) to a Q-Exactive HF mass spectrometer (Thermo Scientific).

492   An amount of 1.3 µg of peptides was separated on in-house packed C18 columns (30 cm x 75 µm ID,

493   ReproSil-Pur 120 C18-AQ, 1.9 µm, Dr. Maisch GmbH) using a binary gradient of water (A) and

494 acetonitrile (B) supplemented with 0.1% formic acid (0 min., 2% B; 3:30 min., 5% B; 137:30 min.,

495 25% B; 168:30 min., 35% B; 182:30 min., 60% B) at 50°C column temperature.

496 For DDA, full MS scans were acquired at a resolution of 120000 (m/z range: 300-1400; AGC target:

497 3E+6). The 15 most intense peptide ions per full MS scan were selected for peptide fragmentation

498 (resolution: 15000; isolation width: 1.6 m/z; AGC target: 1E+5; NCE: 26%). A dynamic exclusion of

499 120 s was used for peptide fragmentation.

500 For DIA, one scan cycle included a full MS scan (m/z range: 300-1400; resolution: 120000; AGC

501 target: 5E+6 ions) and 25 MS/MS scans covering a range of 300-1400 m/z with consecutive m/z

502 windows (resolution: 30000; AGC target: 3E+6 ions; Suppl. Table 1). The maximum ion trapping

503 time was set to "auto". A stepped normalized collision energy of 26% ± 2.6% was used for

504 fragmentation.

505 Microglia from APPPS1 mice were analyzed using DDA and DIA for method establishment.

506 Microglia from APPPS1 and APP-KI mice were compared using DIA as it outperformed DDA.

507

508 ***Mass spectrometric LFQ and data analysis***

509 For data acquired with DDA, the data was analyzed with the software Maxquant (maxquant.org, Max-

510 Planck Institute Munich) version 1.6.1.0 (Cox et al., 2014). The MS data was searched against a

511 reviewed canonical fasta database of *Mus musculus* from UniProt (download: November the 1st 2017,

512 16843 entries) supplemented with the sequence of human APP with the Swedish mutant and the iRT

513 peptides. Trypsin was defined as a protease. Two missed cleavages were allowed for the database

514 search. The option first search was used to recalibrate the peptide masses within a window of 20 ppm.

515 For the main search peptide and peptide fragment mass tolerances were set to 4.5 and 20 ppm,

516 respectively. Carbamidomethylation of cysteine was defined as static modification. Acetylation of the

517 protein N-term as well as oxidation of methionine was set as variable modification. The FDR for both

518 peptides and proteins was set to 1%. The "match between runs" option was enabled with a matching

519 window of 1.5 min. LFQ of proteins required at least one ratio count of unique peptides. Only unique

520 peptides were used for quantification. Normalization of LFQ intensities was performed separately for

521 the age groups because LC-MS/MS data was acquired in different batches.

522 A spectral library was generated in Spectronaut (version 12.0.20491.11, Biognosys) (Bruderer et al.,

523 2015) using the search results of Maxquant of the APPPS1 dataset. The library includes 122542

524 precursor ions from 91349 peptides, which represent 6223 protein groups. The DIA datasets of both

525 mouse models were analyzed with this spectral library (version 12.0.20491.14.21367) with standard

526 settings. Briefly, the FDR of protein and peptide identifications was set to 1%. LFQ of proteins was

527 performed on peptide fragment ions and required at least one quantified peptide per protein. Protein

528 quantification was performed on maximum three peptides per protein group. The data of APPPS1

529 microglia was organized in age dependent fractions to enable separate normalization of the data. All

530 LC-MS/MS runs of the APP-KI dataset were normalized against each other because all samples were

531 analyzed in randomized order in one batch.

532 The protein LFQ reports of Maxquant and Spectronaut were further processed in Perseus (Tyanova et

533 al., 2016). The protein LFQ intensities were log2 transformed and log2 fold changes were calculated

534 between transgenic and wild type samples separately for the different age groups and mouse models.

535 Only proteins with a consistent quantification in all samples of an age group were considered for

536 statistical testing. A two-sided Student's t-test was applied to evaluate the significance of proteins

537 with changed abundance. Additionally, a permutation based FDR estimation was used (Tusher et al.,

538 2001). A log2 fold change larger than 0.5, or smaller than -0.5, a p-value less than 0.05, and significant

539 regulation after FDR filtering were defined as regulation thresholds. The same thresholds were used

540 for the comparison with transcriptomics data.

541 Gene ontology enrichment analysis was performed with the web-tool DAVID (version 6.8) (Huang

542 da et al., 2009a, b) using GO_FAT terms. Up- and down-regulated early, middle and advanced

543 MARPs were clustered separately for biological process, cellular component, and molecular function

544 with all 5500 proteins, consistently quantified in APPPS1 and APP-KI microglia, as a customized

545 background. A medium classification stringency was applied. An enrichment score of 1.3 was defined

546 as threshold for cluster enrichment.

547

548 *Biochemical characterization of brain tissue and isolated microglia*

549 RIPA lysates were prepared from brain hemispheres, centrifuged at 100000 x g (60 min at 4°C) and

550 the remaining pellet was homogenized in 0.5 mL 70% formic acid. The formic acid fraction was

551 neutralized with 20 x 1 M Tris-HCl buffer at pH 9.5 and used for Aβ analysis. For Aβ detection,

552 proteins were separated on Tris-Tricine (10-20%, Thermo Fisher Scientific) gels, transferred to

553 nitrocellulose membranes (0.1 µm, GE Healthcare) which were boiled for 5 min in PBS and

554 subsequently incubated with the blocking solution containing 0.2% I-Block (Thermo Fisher

555 Scientific) and 0.1% Tween 20 (Merck) in PBS for 1 hour, followed by overnight incubation with

556 rabbit polyclonal 3552 antibody (1:2000, (Yamasaki et al., 2006)). Antibody detection was performed

18

557 using the corresponding anti-HRP conjugated secondary antibody (Santa Cruz) and

558 chemiluminescence detection reagent ECL (Thermo Fisher Scientific).

559 Microglia-enriched pellets were resuspended in 100 µL of STET lysis buffer (composition as

560 described above for mass spectrometry, supplemented with proteinase and phosphatase inhibitors),

561 kept on ice for 20 min and then sonicated for 4 cycles of 30 seconds. Cell lysates were then

562 centrifugated at 9600 x g (5 min. at 4°C) and pellets discarded. Protein concentration was quantified

563 using Bradford assay (Biorad) according to manufacturer instructions. 10 µg per sample using two

564 independent microglial lysates per genotype were loaded on a bis-tris acrylamide gel (8% or 12%)

565 and subsequently blotted onto either a PVDF or nitrocellulose membrane (Millipore) using the

566 following antibodies: TREM2 (1:10, clone 5F4,(Xiang et al., 2016)); APOE (1:1000, AB947

567 Millipore); CD68 (1:1000, MCA1957GA, AbDserotec); CSF1R (1:1000, 3152, Cell Signaling) and

568 FABP5 (1:400, AF1476, R&DSystems). Blots were developed using horseradish peroxidase-

569 conjugated secondary antibodies (Promega) and the ECL chemiluminescence system (Amersham) or

570 SuperSignal™ West Pico PLUS (Thermo Scientific). An antibody against GAPDH (1:2000, ab8245,

571 Abcam) was used as loading control.

572

573 ***Immunohistochemistry***

574 3 and 12 month old mice from the APPPS1 and APP-KI transgenic lines were anesthetized i.p. with

575 a mixture of Ketamine (400 mg/kg) and Xylazine (27 mg/kg) and transcardially perfused with cold

576 0.1M PBS for 5 minutes followed by 4% Paraformaldehyde (PFA) in 0.1 M PBS for 15 minutes.

577 Brains were isolated and postfixed for 20 minutes in 4% PFA in 0.1 M PBS and transferred to 30%

578 sucrose in 0.1 M PBS for cryopreservation. Brains were embedded in optimal cutting temperature

579 compound (O.C.T./ Tissue-Tek, Sakura), frozen on dry ice and kept at -80°C until sectioning. 30 µm

580 coronal brain sections were cut using a cryostat (CryoSTAR NX70, Thermo Scientific) and placed in

581 0.1 M PBS until staining. Alternatively, sections were kept in anti-freezing solution (30% Glycerol,

582 30% Ethylenglycol, 10% 0.25 M PO_4 buffer, pH 7.2-7.4 and 30% dH_2O) at -20°C and briefly washed

583 in 0.1M PBS before staining. Briefly, free-floating sections were permeabilized with 0.5% Triton-

584 PBS (PBS-T) for 30 min, blocked either in 5% normal Goat Serum or 5% Donkey Serum in PBS-T

585 for 1 hour and incubated overnight at 4°C in blocking solution with the following primary antibodies:

586 IBA1 (1:500, 019-19741,Wako), IBA1 (1:500, ab5076, Abcam) NAB228 (1:2000, sc-32277, Santa

587 Cruz), CD68 (1:500, MCA1957GA, AbDserotec), TREM2 (1:50, AF1729, R&DSystems), APP-

588 Y188 (1:2000, ab32136, Abcam), CLEC7a (1:50, mabg-mdect, Invivogen), TMEM119 (1:200,

19

589   ab209064, Abcam), APOE-biotinilated (HJ6.3, 1:100, (Kim et al., 2012)) and 3552 (1:5000,

590   (Yamasaki et al., 2006)). After primary antibody incubation, brain sections were washed 3 times with

591   PBS-T and incubated with appropriate fluorophore-conjugated or streptavidine-fluorophore

592   conjugated (for APOE biotinylated antibody) secondary antibodies (1:500, Life Technologies)

593   together with nuclear stain Hoechst 33342 (1:2000, H3570,ThermoFisher), for two hours at room

594   temperature (RT). Fibrillar dense core plaques were stained with Thiazine red (Sigma Aldrich, 2 µM

595   solution in PBS) for 20 min in the dark at RT (after secondary antibody staining). Sections were

596   subsequently washed three times with PBS-T mounted onto glass slides (Thermo Scientific), dried in

597   the dark for at least 30 min, mounted using Gel Aqua Mount media (Sigma Aldrich) and analyzed by

598   confocal microscopy.

599   *Image acquisition, analysis and quantifications*

600   3 month old APPPS1 and APP-KI mice were used for the analysis of dystrophic neurites, microglial

601   recruitment to amyloid plaques and CD68 coverage area. All quantification analysis included 3 mice

602   per genotype. 30 z-stack images (~10 µm thick) of single cortical plaques were acquired per

603   experiment using a confocal microscope (63X water objective with 2x digital zoom, 600 Hz, Leica

604   TCS SP5 II) from 6 brain slices (5 plaques per slice) for the microglial recruitment and dystrophic

605   neurite analysis, or from 3 brain slices (10 plaques per slice) for CD68 coverage area analysis.

606   Microscopy acquisition settings were kept constant within the same experiment. Maximal intensity

607   projection pictures from every z-stack were created using ImageJ software and for every image, a

608   defined region of interest (ROI) was manually drawn around every plaque (including microglia

609   recruited -in contact- to the plaque). APP (Y188 antibody) and CD68 coverage area were quantified

610   using the "Threshold" and "Analyze Particles" (inclusion size of 1-Infinity) functions from ImageJ

611   software (NIH) within the ROI. The area covered by CD68 was normalized to the total Aβ plaque

612   area (NAB228 antibody) or was divided by the number of microglia (IBA1 positive cells) recruited

613   to the plaque within the ROI. The absolute values of area covered by neuritic dystrophies or Aβ

614   plaques are represented in square micrometers (µm²). Microglial recruitment to plaques was quantified

615   by counting the number of microglia (IBA1 positive cells) around amyloid plaques through the z-

616   stack images within the defined ROI using the cell counter function of ImageJ software. Number of

617   microglial cells at amyloid plaques was normalized to the area covered by Aβ (NAB228 antibody)

618   and expressed as number of microglial cells per µm² of Aβ plaque.

20

619   Representative images from microglial recruitment analysis (IBA1 positive cells and CD68 coverage)

620   were taken using the confocal microscope (63X water objective with 2x digital zoom, 400 Hz, Leica

621   TCS SP5 II). Representative picture of microglia polarized towards amyloid cores was taken using a

622   63X confocal water objective with 3x digital zoom.

623   For immunohistological validation of the proteome analysis and amyloid pathology, representative

624   pictures in similar regions were taken by confocal microscopy using the same settings for all three

625   different genotypes (WT, APPPS1 and APP-KI). Low magnification pictures were taken with 20X

626   dry confocal objective with 2x digital zoom and higher magnification ones with 63X confocal water

627   objective with 3x digital zoom. Images of Aβ pathology (NAB228 antibody) were taken with a tile

628   scan system covering similar brain regions (10X confocal dry objective). Representative images of

629   Aβ composition (NAB228, ThR and IBA1) were taken with a confocal 20X dry objective.

630

631   *Microglial phagocytosis of E.coli particles*

632   Microglial phagocytosis was performed similarly as previously described (Kleinberger et al., 2014).

633   Microglia isolated from 3 or 6 month old APPPS1, APP-KI and WT mice were plated onto 24 well

634   plate at a density of $2x10^5$ cells per well and cultured for 24 hours in a humidified 5% CO_2 incubator

635   at 36.5°C in DMEM/F12 media (Invitrogen) supplemented with 10% heat inactivated FCS (Sigma),

636   1% Penicillin-Streptomycin (Invitrogen) and 10 ng/mL GM-CSF (R&DSystems). After 24 hours,

637   plating media were replaced with fresh media. After 5 days in culture, microglia were incubated with

638   50 µL of *E.coli* particle suspension (pHrodo™ Green *E.coli* BioParticles™, P35366, Invitrogen™)

639   for 60 min. Cytochalasin D (CytoD, 10 µM, from 10 mM stock in DMSO) was used as phagocytosis

640   inhibitor and added 30 min prior to addition of bacterial particles. Bacteria suspension excess was

641   washed 4 times with PBS (on ice) and microglia that were attached to the plate were incubated with

642   CD11b-APC-Cy7 antibody (1:200, clone M1/70, 557657, BD) in FACS buffer (PBS supplemented

643   with 2mM EDTA and 1% FBS) for 30 min at 4°C. Microglia were then washed twice with PBS,

644   scraped off the wells in FACS buffer and analyzed by flow cytometry. For the analysis of 3 month

645   old mice, 3 independent experiments were performed per genotype, and each experiment included a

646   minimum of 3 technical replicates with the exception of CytoD condition (2 technical replicates). For

647   the analysis of 6 month old mice, 2 independent experiments were performed per genotype, and each

648   experiment included a minimum of 4 technical replicates with the exception of CytoD condition (2

649   technical replicates).

650

651 *FACS analysis*

652 For the microglial isolation quality control, around 12000 cells from a CD11b-enriched and CD11b-

653 depleted fractions were stained in suspension with CD11b-APC-Cy7 antibody (1:200, clone M1/70,

654 557657, BD) in FACS buffer for 30 minutes at 4°C. After several washes with PBS, microglia were

655 resuspended in FACS buffer for analysis. Propidium Iodide (PI) staining was done 10 minutes prior

656 FACS analysis. Flow cytometric data was acquired on a BD FACSverse flow cytometer by gating

657 according to single stained and unstained samples and analyzed using FlowJo software (Treestar).

658 Mean fluorescent intensity (MFI) is represented as the geometric mean of the according fluorochrome.

659

660 *Statistical analysis*

661 The data are presented as mean ± standard deviation of the mean (± SD) from 3 independent

662 experiments with the exception of the phagocytic assay in 6 month old mice (Fig 8) were 2

663 independent experiments were performed. For the microglial recruitment and analysis of dystrophic

664 neurites, statistical significance (P value) was calculated using the unpaired two-tailed Student's *t*-

665 test. Phagocytic assay was analyzed by the Dunnett's multiple comparison test of the Two-way

666 ANOVA. Both statistical analysis were performed in GraphPad Prism. P value of <0.05 was

667 considered to be statistically significant (*; $P < 0.05$, **; $P < 0.01$ and ***; $P < 0.001$, n.s. = not

668 significant).

669

670 *Data Availability*

671 The mass spectrometry proteomics data have been deposited to the ProteomeXchange Consortium via

672 the PRIDE partner repository (Perez-Riverol et al., 2019) with the dataset identifier PXD016075.

673

674 **Acknowledgements**

685

686
687   **Author Contributions**

688   S.T. and S.F.L. designed and supervised the study. S.T., S.F.L., S.A.M., L.S.M. wrote the manuscript

689   with input of all co-authors. L.S.M. performed animal experiments including microglial isolation,

690   target validation and functional studies. A.C. assisted in isolation of primary microglia. S.A.M., J.K.,

691   and A.B. performed the proteomic analysis. S.R., L.S.M. and A.L. performed FACS analysis. T.S.

692   and T.C.S. provided the APP-KI mouse model. J.H. contributed to amyloid plaque analysis. L.S.M.,

693   M.W., and C.H. contributed to biochemical analysis. Correspondence and requests for materials

694   should be addressed to S.T. or S.F. L.

695

696   **Competing Interests**

697   C.H. collaborates with Denali Therapeutics, participated on one advisory board meeting of Biogen,

698   and received a speaker honorarium from Novartis and Roche. C.H. is chief advisor of ISAR

699   Bioscience. All other authors declare that they have no competing interests.

700

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1030 **Figure Legends**

1031

1032 **Figure 1**. **Quantitative proteomics of acutely isolated microglia.** Volcano plots of APPPS1 and

1033 APP-KI *versus* WT microglia at 1 (**A** and **B**), 3 (**C** and **D**), 6 (**E** and **F**) and 12 (**G** and **H**) months of

1034 age. The minus log10 transformed p-value is plotted against the log2 transformed LFQ ratios. A

1035 permutation based FDR estimation was applied which is visualized as hyperbolic curves. Proteins

1036 with log2 LFQ ratio lower than -0.5 or higher than +0.5 with a p-value less than 0.05 which remain

1037 significantly changed after FDR correction are indicated as red circles. Non-significantly changed

1038 proteins are indicated as blue circles. Individual proteins are marked with their gene names.

1039

1040 **Figure 2. APPPS1 microglia display earlier proteomic changes compared to APP-KI microglia**.

1041 **A**. Profile plots of APPPS1 and APP-KI *versus* WT microglia at 1, 3, 6, and 12 months of age. Lines

1042 connect the average log2 fold changes of each protein at the different time points. Regulated proteins

1043 were grouped according to three profiles: significantly increased or decreased after FDR correction

1044 (log2 FC > 0.5 or < -0.5; p < 0.05; FDR significant) at 3, 6, and 12 months, only at 3 and 6 months,

1045 or only at 12 months. Proteins that fulfill these criteria are indicated as red and blue lines for increased

1046 and decreased abundance, respectively. Selected up- or down-regulated proteins are indicated with

1047 magenta and cyan. Proteins that were regulated at 3, 6, and 12 months in APPPS1 mice as well as at

1048 6 and 12, or only at 12 months in APP-KI mice were annotated as early MARPs. Proteins that were

1049 regulated at 6 and 12 months in APPPS1 as well as at 6 and 12, or only at 12 months in APP-KI mice

1050 were annotated as middle MARPs. Proteins that were regulated at 12 months in APPPS1 and APP-KI

1051 mice were annotated as advanced MARPs. Heatmaps show the log2 fold changes of the top 74 up- or

1052 down-regulated proteins for early (**B**), middle (**C**) and advanced (**D**) MARPs and are compared to the

1053 log2 fold changes of related transcripts of DAM and homeostatic microglia (Keren-Shaul et al., 2017),

1054 indicated with K-S. Crosses indicate missing values.

1055

1056 **Figure 3. Gene ontology enrichment cluster analysis for biological process (BP) of MARPs.** Bar

1057 graphs show the clustering of early MARPs (**A**), middle MARPs (**B**) and advanced MARPs (**C**) for

1058 BP. Up- and down-regulated proteins were analyzed separately using the web-based software tool

1059 DAVID 6.8 with all consistently quantified proteins (5500) as an individual background. Significantly

1060 enriched clusters (Enrichment Score > 1.301) for up-and down-regulated proteins are indicated in blue

1061 and red, respectively.

31

1062 **Figure 4. Microglial CLEC7a is prominently up-regulated in 3 month old APPPS1 mice.**

1063 Immunohistochemical analysis of CLEC7a (red) revealed an increased abundance of this early MARP

1064 in IBA1 positive (green) APPPS1 microglia surrounding Aβ plaques (magenta) that is less prominent

1065 in APP-KI microglia. CLEC7a was barely detected in WT microglia. Hoechst was used for nuclear

1066 staining (blue). Boxed regions in upper panels (scale bar: 50 µm) are shown with a higher

1067 magnification in lower panels (scale bar: 10 µm).

1068

1069 **Figure 5. Microglial CLEC7a is increased in both AD mouse models at 12 months of age.**

1070 Immunohistochemical analysis showed an up-regulation of CLEC7a (red) in IBA1 positive (green)

1071 APPPS1 and APP-KI microglia surrounding Aβ plaques (magenta), compared to WT where CLEC7a

1072 was barely detected. Hoechst was used for nuclear staining (blue). Boxed regions in upper panels

1073 (scale bar: 50 µm) are shown with a higher magnification in lower panels (scale bar: 10 µm).

1074

1075 **Figure 6. Microglial TMEM119 is down-regulated in both AD mouse models at 12 months of**

1076 **age.** Immunohistochemical analysis of TMEM119 (red) shows a broad coverage signal of this

1077 homeostatic marker in IBA1 positive (green) WT microglia. In the APPPS1 and APP-KI mice,

1078 TMEM119 signal is reduced in IBA1 positive microglia surrounding Aβ plaques (magenta). Of note,

1079 increased TMEM119 signal was often found in the core of amyloid plaques in APPPS1 and APP-KI

1080 mice. Hoechst was used for nuclear staining (blue). Boxed regions in upper panels (scale bar: 50 µm)

1081 are shown with a higher magnification in lower panels (scale bar: 10 µm) and illustrate reduced levels

1082 of TMEM119 in plaque associated microglia.

1083

1084 **Figure 7**. **APPPS1 Aβ plaques display a higher content of fibrillar Aβ compared to APP-KI**

1085 **plaques**. **A**. Immunohistochemical analysis showing total Aβ (green), fibrillar Aβ (ThR, red) and

1086 microglia (IBA1, magenta) in both mouse models at 3, 6 and 12 months of age. Hoechst was used for

1087 nuclear staining (blue). Scale bar: 100 µm **B**. Western blot analysis of insoluble Aβ at 3, 6 and 12

1088 months of age confirms increased levels of fibrillar Aβ in APPPS1 compared to APP-KI mice.

1089

1090 **Figure 8**. **Fibrillar Aβ triggers higher microglial recruitment in 3 month old APPPS1 mice and**

1091 **correlates with a phagocytic impairment. A**. Representative picture of IBA1 positive (green)

1092 microglia polarized towards the fibrillar Aβ core (ThR, red, white arrow), rather than the surrounding

1093   plaque halo (magenta) in 3 month old APPPS1 and APP-KI mice. Hoechst (HOE) was used for nuclear

1094   staining (blue). Yellow arrows indicate intracellular fibrillar Aβ within APPPS1 microglia. Scale bar:

1095   10 µm. **B**. Quantification of IBA1 positive cells recruited to amyloid plaques in 3 month old APPPS1

1096   and APP-KI mice. Microglial numbers are normalized to the plaque area. **C**. Quantification of plaque

1097   size in 3 month old APPPS1 and APP-KI mice. **D**. Imunohistochemical analysis of IBA1 (green) and

1098   CD68 (red) positive microglial cells recruited to Aβ plaques (magenta) in 3 month old APPPS1 and

1099   APP-KI mice. Hoechst was used for nuclear staining (blue). Scale bar: 20 µm. **E**. Quantification of

1100   CD68 coverage in 3 month old APPPS1 and APP-KI mice. CD68 coverage is normalized to the plaque

1101   area. **F**. Quantification of CD68 coverage per microglia in 3 month old APPPS1 and APP-KI mice.

1102   CD68 coverage is normalized to the number of IBA1 positive cells recruited to amyloid plaque. **G**.

1103   Immunohistochemical analysis of dystrophic neurites detected using an antibody against APP (green)

1104   in 3 month old APPPS1 and APP-KI mice. ThR was used to detect fibrillar Aβ plaque core (red) and

1105   the whole Aβ plaque was visualized using an antibody against Aβ (magenta). Hoechst was used for

1106   nuclear staining (blue). Scale bar: 20 µm. **H**. Quantification of area covered by dystrophic neurites in

1107   3 month old APPPS1 and APP-KI mice. In **B**, **C**, **E**, **F** and **H**, represented values are expressed as the

1108   mean of n=3 mice per genotype ± SD (*P < 0.05; **P < 0.01, unpaired two-tailed Student's T-test;

1109   n.s: not significant). **I.** Mean Fluorescence Intensity (MFI) of *E.coli*-pHrodo signal within the CD11b

1110   positive cells in 3 and 6 month old APPPS1, APP-KI and WT mice. CytoD was used to inhibit

1111   phagocytosis and serves as a negative control. a.u.: arbitrary units. **J**. Percentage of CD11b and *E.coli*-

1112   pHrodo double positive cells from the total CD11b positive population. In **I** and **J**, values for the 3

1113   month old group are expressed as the mean of n=3 mice per genotype ± SD from three independent

1114   experiments and for the 6 month old group as the mean of n=2 mice per genotype ± SD from two

1115   independent experiments (***P < 0.001, Two-way ANOVA, Dunnett's multiple comparison test; n.s:

1116   not significant).

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1122 **Supplementary Figure Legends**

1123 **Suppl. Figure 1. Quality control of microglial isolation using MACS.** FACS analysis of the

1124 CD11b-enriched (**A**) and CD11b-depleted fraction (**B**). Propidium Iodide (PI) was used to analyze

1125 cell viability (PI negative fraction).

1126

1127 **Suppl. Figure 2**. **Improvement of the data acquisition method for quantitative proteomics of**

1128 **microglia. A**. Workflow of the proteomic analysis. Microglia were acutely isolated using the CD11b

1129 MACS system. Protein lysates were subjected to tryptic digestion followed by quantitative LC-

1130 MS/MS analysis. APPPS1 microglia samples were first measured using DDA to generate a spectral

1131 library required for DIA analysis. Afterwards, both APPPS1 and APPKI microglia were measured

1132 using DIA and data was subjected to further bioinformatics analysis. **B**. Distribution of quantified

1133 proteins with DDA and DIA. The number of quantified proteins is plotted against the log10

1134 transformed iBAQ (intensity based absolute quantification) values with a bin size of 0.25. The iBAQ

1135 values are an estimate of the relative molar abundance of a protein within the sample. The number of

1136 consistently quantified proteins per bin for DDA and DIA are indicated in blue and red, respectively.

1137 Proteins that were only quantified with DDA or DIA are colored in light blue and orange, respectively.

1138 The single values are connected with dashed lines for better visibility. Please note that DIA provides

1139 additional quantifications for low abundant proteins. Hereby, DIA extends the dynamic range for

1140 protein quantification by almost one order of magnitude. **C**. Number of relatively quantified proteins

1141 between APPPS1 and WT microglia for 1, 3, 6, and 12 months with DDA (blue) and DIA (red). Only

1142 proteins that were consistently quantified in all APPPS1 and WT samples per age group were counted.

1143 Please note that DIA provides between 24 and 35% additional quantifications per age group. **D**. The

1144 comparison of relatively quantified proteins of APPPS1 and APP-KI *versus* WT at 12 months shows

1145 a large overlap of 93.5% indicating a very consistent quantification.

1146

1147 **Suppl. Figure 3. Aβ pathology in 3, 6 and 12 month old APPPS1 and APP-KI mice**.

1148 Immunohistochemical analysis of Aβ load (NAB228, green) in APPPS1 and APP-KI mice. Hoechst

1149 was used for nuclear staining (blue). Scale bar: 400 µm.

1150

1151 **Suppl. Figure 4. Comparison of the early, middle and advanced MARPs in APPPS1 and APP-**

1152 **KI mice. A**. A Venn diagram illustrates the classification of regulated proteins into early, middle and

1153 advanced MARPs. Proteins that were regulated at 3, 6, and 12 months in APPPS1 mice as well as at

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1154   6 and 12, or only at 12 months in APP-KI mice were annotated as early MARPs. Proteins that were

1155   regulated at 6 and 12 months in APPPS1 as well as at 6 and 12, or only at 12 months in APP-KI mice

1156   were annotated as middle MARPs. Proteins that were regulated at 12 months in APPPS1 and APP-KI

1157   mice were annotated as advanced MARPs. **B**. Correlation plot of log2 protein fold changes detected

1158   at 12 months for APPPS1 and APP-KI *versus* WT. The plot shows a significant correlation of

1159   proteomic changes at 12 months. Early, middle, and advanced MARPs are indicated in red, orange,

1160   and black circles with yellow filling, respectively. Selected proteins are annotated with their gene

1161   names. **C**. Comparison of our proteomic study with the single cell transcriptomics study (Keren-Shaul

1162   et al., 2017) reveals a modest overlap. A Venn diagram illustrates the comparison of quantified

1163   proteins in APPPS1 and APP-KI *versus* WT at 12 months and DAM and homeostatic microglial

1164   transcripts. Overall, 3348 common proteins and related transcripts were quantified. **D**. Biochemical

1165   validation of proteomic data was performed using Western blot analysis of microglial lysates from 12

1166   month old mice. This analysis revealed an increased abundance of up-regulated MARPs APOE,

1167   TREM2, CD68 and FABP5 as well as a decreased abundance of the down-regulated MARP CSF1R

1168   in APPPS1 and APP-KI compared to WT microglia. Arrows indicate antibody-detected specific bands

1169   and asterisk indicates unspecific bands.

1170

1171   **Suppl. Figure 5. Gene ontology enrichment cluster analysis for cellular component (CC) and**

1172   **molecular function (MF) of MARPs.** The bar graphs show the clustering of early MARPs (**A**, **D**),

1173   middle MARPs (**B**, **E**) and advanced MARPs (**C**, **F**) for CC and MF. Up- and down-regulated proteins

1174   were analyzed separately using the web-based software tool DAVID 6.8 using all consistently

1175   quantified proteins (5500) as an individual background. Significantly enriched clusters (Enrichment

1176   Score > 1.301) for up- and down-regulated proteins are indicated in blue and red, respectively.

1177

1178   **Suppl. Figure 6. Microglial TREM2 is prominently up-regulated in 3 month old APPPS1 mice.**

1179   Immunohistochemical analysis of TREM2 (red) revealed an increased abundance in IBA1 positive

1180   (green) APPPS1 microglia surrounding Aβ plaques (magenta) that is less prominent in APP-KI

1181   microglia. Hoechst was used for nuclear staining (blue). Boxed regions in upper panels (scale bar: 50

1182   µm) are shown with a higher magnification in lower panels (scale bar: 10 µm).

1183

1184   **Suppl. Figure 7. Microglial APOE is prominently up-regulated in 3 month old APPPS1 mice.**

1185   Immunohistochemical analysis of APOE (red) revealed an up-regulation in IBA1 positive (green)

1186   APPPS1 microglia surrounding Aβ plaques (magenta) that is less prominent in APP-KI microglia.

1187   APOE could not be detected in WT microglia. Hoechst was used for nuclear staining (blue). Of note,

1188   astrocytic APOE immunoreactivity was also detected, particularly in the WT, reflecting its

1189   physiological expression. Yellow arrows indicate APOE positive microglia. Boxed regions in upper

1190   panels (scale bar: 50 µm) are shown with a higher magnification in lower panels (scale bar: 10 µm).

1191

1192   **Suppl. Figure 8. Phagocytosis is impaired earlier in APPPS1 compared to APP-KI microglia. A**.

1193   Histograms represent the fuorescence intensity of uptaken E.coli-pHrodo green particles within the

1194   CD11b positive population for every genotype and time point (3 and 6 months). Dashed lines indicate

1195   the mean fluorescence intensity (MFI) calculated with the geometric mean for each condition. **B**.

1196   FACS analysis representing the percentage of CD11b and E.coli-pHrodo positive cells out of the

1197   whole CD11b positive population for every genotype.

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Fig 1

Fig 2

Fig 3

Fig 4

Fig 5

Fig 6

A

B

Fig 7

Fig 8

A

CD11b-enriched fraction

B

CD11b-depleted fraction

Suppl. Fig 1

A

APPPS1/WT

1M Pre-plaque   **3M** Early   **6M** Middle   **12M** Advanced

APP-KI/WT

Brain isolation → CD11b+ Microglia (MACS) → Tryptic Digestion → LC-MS/MS → DDA / DIA → Spectral library → Data Analysis

B

C

D

APPPS1 vs WT 12M   APP-KI vs WT 12M

169 (2.9%)   **5500 (93.5%)**   215 (3.7%)

Suppl. Fig 2

Suppl. Fig 3

A

APPPS1 regulated 6+12

65

Middle MARP

176

Early MARP

30   90

669

Advanced MARP

435   527

APPPS1 regulated 3+6+12

Regulated in APP-KI

APPPS1 regulated only 12

B

○ Early MARP
○ Middle MARP
● Advanced MARP
○ Other

log2 LFQ ratio (APP-KI vs. WT) 12M

FABP5, CD74, ITGAX, HTRA1, FGF1, APOE, FABP3, ANKH, ACTR3B, CLEC7A, ADSSL1, CD63, LGALS3, ACACA, MNDA, IFI204, CNTNAP1, SOAT1, VTN, NCEH1, IFIT2

RPL38, HYAL1, NGP, GNG10, HMGA1, HIST2H2AC, SELK, EHD2, SP3, ZNF691, HIST1H2AB, CRYBB1, ASTN2, CAMP, TRP53I11, LRRC25, CX3CL1

pairs: 5500
r = 0.662
p < 0.0001

log2 LFQ ratio (APPPS1 vs. WT) 12M

C

APPPS1 vs WT 12M   APP-KI vs WT 12M

124 (1.4%)   2152 (24.7%)   124 (1.4%)

3348 (38.4%)

45 (0.5%)   91 (1.0%)

2841 (32.6%)

Keren-Shaul

D

kDa   WT   APPPS1   APP-KI

25   APOE

37   *
25   TREM2

100   CD68

15   FABP5

150   CSF1R

37   GAPDH

Suppl. Fig 4

Suppl. Fig 5

Suppl. Fig 6

Suppl. Fig 7

A

E.coli-pHrodo-Mean fluorescent intensity - 3 months

Sample (3M)	MFI (geom. mean)
WT	30298
WT + CytoD	1925
APPPS1	16365
APP + KI	24561

E.coli-pHrodo- Mean fluorescent intensity - 6 months

Sample (6M)	MFI (geom. mean)
WT	28836
WT + CytoD	1626
APPPS1	16930
APP + KI	19117

B

Suppl. Fig 8

Supplementary Tables

Suppl. Table 1: Optimized mass to charge (m/z) window distribution for Sequential Window Acquisition of all theoretical Mass Spectra (SWATH-MS) based on DIA.

Window	m/z start	m/z end	Center	Isolation width [m/z]
1	300	360	330	60
2	359	399	379	40
3	398	428	413	30
4	427	452	439.5	25
5	451	475	463	24
6	474	497	485.5	23
7	496	518	507	22
8	517	539	528	22
9	538	560	549	22
10	559	581	570	22
11	580	602	591	22
12	601	623	612	22
13	622	646	634	24
14	645	669	657	24
15	668	694	681	26
16	693	719	706	26
17	718	746	732	28
18	745	777	761	32
19	776	808	792	32
20	807	839	823	32
21	838	870	854	32
22	869	904	886.5	35
23	903	943	923	40
24	942	1,122	1,032	180
25	1,121	1,401	1,261	280

Suppl. Table 2: Comparison of proteomic results of APPPS1 microglia from DDA and DIA including the average peptide IDs, protein IDs, protein quantifications calculated for all samples**,** numbers of relatively quantified proteins from 1, 3, 6, and 12 months as well as their averages.

	DDA	DIA	DIA vs DDA
Overall Average Peptide IDs	53912	74281	137.8%
Overall Average Protein IDs	5502	5953	108.2%
Overall Average Protein Quantifications	5053	5952	117.8%
Relative quantifications (3 vs 3) APPPS1 vs WT 1M	4425	5491	124.1%
Relative quantifications (3 vs 3) APPPS1 vs WT 3M	4646	5789	124.6%
Relative quantifications (3 vs 3) APPPS1 vs WT 6M	4391	5848	133.2%
Relative quantifications (3 vs 3) APPPS1 vs WT 12M	4185	5669	135.5%
Average of relative quantifications	4412	5699	129.3%

Suppl. Table 3: Quantitative proteomic data analysis of APPPS1 (**A**) and APP-KI (**B**) *versus* WT microglia at 1, 3, 6, and 12 months using DIA. The table shows the number of consistently quantified proteins as well as proteins with a significant up- or down-regulation with and without FDR correction. A log2 fold change > 0.5 or < -0.5 and a p-value of less than 0.05 were applied as regulation thresholds. The amount of up-and down-regulated proteins with FDR correction is shown as percentage from the total number of quantified proteins.

A

	APPPS1 vs WT 1M	APPPS1 vs WT 3M	APPPS1 vs WT 6M	APPPS1 vs WT 12M
Quantifications (3 vs 3)	5491	5789	5848	5669
Regulated	98	1010	679	1409
Up-regulated	76	332	365	776
Down-regulated	22	678	314	633
Up-regulated FDR corrected	0	332	309	776
Down-regulated FDR corrected	0	678	261	633
Up-regulated FDR corrected (%)	0.0%	5.7%	5.3%	13.7%
Down-regulated FDR corrected (%)	0.0%	11.7%	4.5%	11.2%

B

	APP-KI vs WT 1M	APP-KI vs WT 3M	APP-KI vs WT 6M	APP-KI vs WT 12M
Quantifications (3 vs 3)	5713	5711	5653	5715
Regulated	41	700	559	1337
Up-regulated	19	22	245	704
Down-regulated	54	109	267	666
Up-regulated FDR corrected	0	1	140	704
Down-regulated FDR corrected	0	0	151	666
Up-regulated FDR corrected (%)	0.0%	0.0%	2.5%	12.3%
Down-regulated FDR corrected (%)	0.0%	0.0%	2.7%	11.7%

2

Suppl. Table 4: Identified early, middle, and advanced MARPs. Protein groups are represented by the major UniProt accession.

Gene Name	UniProt Accession	Protein Name	MARPs (up: +1; down: -1)		
			Early MARP	Middle MARP	Advanced MARP
Aacs	Q9D2R0	Acetoacetyl-CoA synthetase	0	1	0
Aagab	Q8R2R3	Alpha- and gamma-adaptin-binding protein p34	0	0	-1
Aak1	Q3UHJ0	AP2-associated protein kinase 1	0	0	1
Aarsd1	Q3THG9	Alanyl-tRNA editing protein Aarsd1	0	0	1
Abca9	Q8K449	ATP-binding cassette sub-family A member 9	0	-1	0
Abcb1b	P06795	Multidrug resistance protein 1B	0	1	0
Abcd2	Q61285	ATP-binding cassette sub-family D member 2	0	1	0
Abcg3	Q99P81	ATP-binding cassette sub-family G member 3	0	0	1
Abhd15	Q5F2F2	Protein ABHD15	0	-1	0
Abi1	Q8CBW3	Abl interactor 1	0	0	-1
Abi3	Q8BYZ1	ABI gene family member 3	0	0	-1
Acaca	Q5SWU9	Acetyl-CoA carboxylase 1	1	0	0
Acbd3	Q8BMP6	Golgi resident protein GCP60	0	0	1
Acot11	Q8VHQ9	Acyl-coenzyme A thioesterase 11	0	0	1
Acox3	Q9EPL9	Peroxisomal acyl-coenzyme A oxidase 3	0	1	0
Acsbg1	Q99PU5	Long-chain-fatty-acid--CoA ligase ACSBG1	0	0	1
Acss2	Q9QXG4	Acetyl-coenzyme A synthetase, cytoplasmic	0	0	1
Actn1	Q7TPR4	Alpha-actinin-1	0	-1	0
Actn4	P57780	Alpha-actinin-4	0	0	-1
Actr3b	Q641P0	Actin-related protein 3B	0	1	0
Ada	P03958	Adenosine deaminase	0	-1	0
Adam15	O88839	Disintegrin and metalloproteinase domain-containing protein 15	0	0	-1
Adam22	Q9R1V6	Disintegrin and metalloproteinase domain-containing protein 22	0	0	1
Adap2	Q8R2V5	Arf-GAP with dual PH domain-containing protein 2	0	-1	0
Add2	Q9QYB8	Beta-adducin	0	0	1
Adgre1	Q61549	Adhesion G protein-coupled receptor E1	0	-1	0
Adk	P55264	Adenosine kinase	0	0	1
Adss	P46664	Adenylosuccinate synthetase isozyme 2	0	1	0
Adssl1	P28650	Adenylosuccinate synthetase isozyme 1	1	0	0

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Ahcy	P50247	Adenosylhomocysteinase	0	0	1
Aif1	O70200	Allograft inflammatory factor 1	0	0	-1
Akap5	D3YVF0	A-kinase anchor protein 5	0	0	1
Akt3	Q9WUA6	RAC-gamma serine/threonine-protein kinase	0	0	1
Alb	P07724	Serum albumin	0	-1	0
Aldh1b1	Q9CZS1	Aldehyde dehydrogenase X, mitochondrial	0	0	1
Aldh1l1	Q8R0Y6	Cytosolic 10-formyltetrahydrofolate dehydrogenase	0	0	1
Aldh1l2	Q8K009	Mitochondrial 10-formyltetrahydrofolate dehydrogenase	0	0	1
Aldh2	P47738	Aldehyde dehydrogenase, mitochondrial	0	0	1
Aldoa	P05064	Fructose-bisphosphate aldolase A	0	1	0
Alg10b	Q3UGP8	Putative Dol-P-Glc:Glc(2)Man(9)GlcNAc(2)-PP-Dol alpha-1,2-glucosyltransferase	0	0	1
Ankh	Q9JHZ2	Progressive ankylosis protein	1	0	0
Anks1a	P59672	Ankyrin repeat and SAM domain-containing protein 1A	0	0	-1
Anxa1	P10107	Annexin A1	0	0	-1
Anxa2	P07356	Annexin A2	0	1	0
Anxa5	P48036	Annexin A5	0	1	0
Ap1s1	P61967	AP-1 complex subunit sigma-1A	0	0	1
Ap4b1	Q9WV76	AP-4 complex subunit beta-1	0	1	0
Apod	P51910	Apolipoprotein D	0	1	0
Apoe	P08226	Apolipoprotein E	1	0	0
Arhgap12	Q8C0D4	Rho GTPase-activating protein 12	0	-1	0
Arhgap22	Q8BL80	Rho GTPase-activating protein 22	0	0	-1
Arhgap30	Q640N3	Rho GTPase-activating protein 30	0	0	-1
Arhgap31	A6X8Z5	Rho GTPase-activating protein 31	0	0	-1
Arhgdib	Q61599	Rho GDP-dissociation inhibitor 2	0	0	-1
Arl6ip4	Q9JM93	ADP-ribosylation factor-like protein 6-interacting protein 4	0	-1	0
Arrb2	Q91YI4	Beta-arrestin-2	0	-1	0
Arsa	P50428	Arylsulfatase A	0	0	-1
Arsg	Q3TYD4	Arylsulfatase G	0	0	-1
Asah1	Q9WV54	Acid ceramidase	0	-1	0

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Asph	Q8BSY0	Aspartyl/asparaginyl beta-hydroxylase	0	0	1
Astn2	Q80Z10	Astrotactin-2	0	0	-1
Atg2a	Q6P4T0	Autophagy-related protein 2 homolog A	0	0	1
Atg7	Q9D906	Ubiquitin-like modifier-activating enzyme ATG7	0	0	1
Atp1b2	P14231	Sodium/potassium-transporting ATPase subunit beta-2	0	0	1
Atp1b3	P97370	Sodium/potassium-transporting ATPase subunit beta-3	0	0	1
Atp6v0a1	Q9Z1G4	V-type proton ATPase 116 kDa subunit a isoform 1	0	0	1
Atp6v1a	P50516	V-type proton ATPase catalytic subunit A	0	0	1
Atp6v1b2	P62814	V-type proton ATPase subunit B, brain isoform	0	0	1
Atp6v1c1	Q9Z1G3	V-type proton ATPase subunit C 1	0	0	1
Atp6v1d	P57746	V-type proton ATPase subunit D	0	0	1
Atp6v1e1	P50518	V-type proton ATPase subunit E 1	0	0	1
Atp6v1h	Q8BVE3	V-type proton ATPase subunit H	0	0	1
Atp8a2	P98200	Phospholipid-transporting ATPase IB	0	0	-1
Atxn10	P28658	Ataxin-10	0	0	1
B3galnt1	Q920V1	UDP-GalNAc:beta-1,3-N-acetylgalactosaminyltransferase 1	0	0	-1
B4galt5	Q9JMK0	Beta-1,4-galactosyltransferase 5	0	0	1
Baiap2	Q8BKX1	Brain-specific angiogenesis inhibitor 1-associated protein 2	0	0	1
Baz1b	Q9Z277	Tyrosine-protein kinase BAZ1B	0	0	-1
Bid	P70444	BH3-interacting domain death agonist	0	0	-1
Bin1	O08539	Myc box-dependent-interacting protein 1	0	0	-1
Bin2	D3Z6Q9	Bridging integrator 2	0	-1	0
Blvrb	Q923D2	Flavin reductase (NADPH)	0	0	-1
Bola2	Q8BGS2	BolA-like protein 2	1	0	0
Borcs5	Q9D920	BLOC-1-related complex subunit 5	0	0	-1
Borcs6	Q9D6W8	BLOC-1-related complex subunit 6	0	0	-1
Bri3bp	Q8BXV2	BRI3-binding protein	0	0	1
Brix1	Q9DCA5	Ribosome biogenesis protein BRX1 homolog	0	1	0
Brk1	Q91VR8	Protein BRICK1	0	0	-1
C5ar1	P30993	C5a anaphylatoxin chemotactic receptor 1	0	0	-1

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Calr	P14211	Calreticulin	0	0	1
Camp	P51437	Cathelicidin antimicrobial peptide	0	0	-1
Capg	P24452	Macrophage-capping protein	1	0	0
Capn1	O35350	Calpain-1 catalytic subunit	0	1	0
Casd1	Q7TN73	CAS1 domain-containing protein 1	0	0	1
Cask	O70589	Peripheral plasma membrane protein CASK	0	0	-1
Cbr2	P08074	Carbonyl reductase [NADPH] 2	0	-1	0
Cc2d1b	Q8BRN9	Coiled-coil and C2 domain-containing protein 1B	0	-1	0
Ccdc124	Q9D8X2	Coiled-coil domain-containing protein 124	0	0	-1
Ccdc30	Q8BVF4	Coiled-coil domain-containing protein 30	0	0	-1
Ccdc50	Q810U5	Coiled-coil domain-containing protein 50	0	0	-1
Ccdc88a	Q5SNZ0	Girdin	0	0	-1
Ccm2	Q8K2Y9	Cerebral cavernous malformations protein 2 homolog	0	0	-1
Ccnd1	P25322	G1/S-specific cyclin-D1	0	0	-1
Ccnk	O88874	Cyclin-K	0	0	1
Cd180	Q62192	CD180 antigen	1	0	0
Cd48	P18181	CD48 antigen	1	0	0
Cd63	P41731	CD63 antigen	0	1	0
Cd68	P31996	Macrosialin	1	0	0
Cd74	P04441	H-2 class II histocompatibility antigen gamma chain	1	0	0
Cdc42bpb	Q7TT50	Serine/threonine-protein kinase MRCK beta	0	-1	0
Cdk6	Q64261	Cyclin-dependent kinase 6	1	0	0
Cdk7	Q03147	Cyclin-dependent kinase 7	0	1	0
Cep170	Q6A065	Centrosomal protein of 170 kDa	0	0	-1
Cfl1	P18760	Cofilin-1	0	0	-1
Chmp3	Q9CQ10	Charged multivesicular body protein 3	0	0	-1
Chmp6	P0C0A3	Charged multivesicular body protein 6	0	0	-1
Chst12	Q99LL3	Carbohydrate sulfotransferase 12	0	1	0
Ckb	Q04447	Creatine kinase B-type	0	0	-1
Clec5a	Q9R007	C-type lectin domain family 5 member A	0	0	-1

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Clec7a	Q6QLQ4	C-type lectin domain family 7 member A	1	0	0
Cln3	Q61124	Battenin	1	0	0
Clstn1	Q9EPL2	Calsyntenin-1	0	0	-1
Cmklr1	P97468	Chemokine-like receptor 1	0	0	-1
Cmtm7	Q9ESD6	CKLF-like MARVEL transmembrane domain-containing protein 7	0	-1	0
Cnih4	Q9CX13	Protein cornichon homolog 4	0	0	1
Cnn2	Q08093	Calponin-2	0	-1	0
Cnpy2	Q9QXT0	Protein canopy homolog 2	0	0	1
Cntnap1	O54991	Contactin-associated protein 1	1	0	0
Cog2	Q921L5	Conserved oligomeric Golgi complex subunit 2	1	0	0
Cog5	Q8C0L8	Conserved oligomeric Golgi complex subunit 5	1	0	0
Cog7	Q3UM29	Conserved oligomeric Golgi complex subunit 7	0	1	0
Cog8	Q9JJA2	Conserved oligomeric Golgi complex subunit 8	0	0	1
Colgalt1	Q8K297	Procollagen galactosyltransferase 1	1	0	0
Commd1	Q8K4M5	COMM domain-containing protein 1	0	0	-1
Commd10	Q8JZY2	COMM domain-containing protein 10	0	0	-1
Commd5	Q8R395	COMM domain-containing protein 5	0	0	-1
Commd8	Q9CZG3	COMM domain-containing protein 8	0	0	-1
Coro1b	Q9WUM3	Coronin-1B	0	0	-1
Cpq	Q9WVJ3	Carboxypeptidase Q	0	-1	0
Cpsf3l	Q9CWS4	Integrator complex subunit 11	1	0	0
Crip1	P63254	Cysteine-rich protein 1	0	1	0
Crlf3	Q9Z2L7	Cytokine receptor-like factor 3	0	0	-1
Crybb1	Q9WVJ5	Beta-crystallin B1	0	0	-1
Csad	Q9DBE0	Cysteine sulfinic acid decarboxylase	0	0	-1
Csf1r	P09581	Macrophage colony-stimulating factor 1 receptor	0	0	-1
Csrp2	P97314	Cysteine and glycine-rich protein 2	0	-1	0
Cst3	P21460	Cystatin-C	0	0	-1
Cstb	Q62426	Cystatin-B	0	1	0
Cstf3	Q99LI7	Cleavage stimulation factor subunit 3	0	0	1

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Ctbs	Q8R242	Di-N-acetylchitobiase	0	-1	0
Ctnnb1	Q02248	Catenin beta-1	0	0	1
Ctsa	P16675	Lysosomal protective protein	0	0	1
Ctsd	P18242	Cathepsin D	1	0	0
Ctsh	P49935	Pro-cathepsin H	1	0	0
Ctsz	Q9WUU7	Cathepsin Z	1	0	0
Cttnbp2nl	Q99LJ0	CTTNBP2 N-terminal-like protein	0	0	-1
Cutc	Q9D8X1	Copper homeostasis protein cutC homolog	0	0	1
Cux1	P53564	Homeobox protein cut-like 1	0	0	1
Cx3cl1	O35188	Fractalkine	0	-1	0
Cx3cr1	Q9Z0D9	CX3C chemokine receptor 1	0	-1	0
Cybb	Q61093	Cytochrome b-245 heavy chain	0	0	1
Cyfip1	Q7TMB8	Cytoplasmic FMR1-interacting protein 1	0	0	-1
Cyhr1	Q9QXA1	Cysteine and histidine-rich protein 1	0	1	0
Cyp20a1	Q8BKE6	Cytochrome P450 20A1	0	0	1
Cyth4	Q80YW0	Cytohesin-4	0	0	-1
Dalrd3	Q6PJN8	DALR anticodon-binding domain-containing protein 3	0	0	-1
Dapp1	Q9QXT1	Dual adapter for phosphotyrosine and 3-phosphotyrosine and 3-phosphoinositide	0	-1	0
Dcxr	Q91X52	L-xylulose reductase	0	0	-1
Ddb2	Q99J79	DNA damage-binding protein 2	0	1	0
Ddx31	Q6NZQ2	Probable ATP-dependent RNA helicase DDX31	0	1	0
Ddx5	Q61656	Probable ATP-dependent RNA helicase DDX5	0	0	-1
Dennd1c	Q8CFK6	DENN domain-containing protein 1C	0	0	-1
Dffa	O54786	DNA fragmentation factor subunit alpha	0	0	-1
Dhcr7	O88455	7-dehydrocholesterol reductase	0	1	0
Dhrs3	O88876	Short-chain dehydrogenase/reductase 3	1	0	0
Dhx32	Q8BZS9	Putative pre-mRNA-splicing factor ATP-dependent RNA helicase DHX32	1	0	0
Dhx58	Q99J87	Probable ATP-dependent RNA helicase DHX58	1	0	0
Dip2b	Q3UH60	Disco-interacting protein 2 homolog B	0	0	-1
Dkc1	Q9ESX5	H/ACA ribonucleoprotein complex subunit 4	1	0	0

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			EarlyMARP	MiddleMARP	Advanced MARP
Dnaja2	Q9QYJ0	DnaJ homolog subfamily A member 2	0	0	-1
Dnajb14	Q149L6	DnaJ homolog subfamily B member 14	1	0	0
Dock10	Q8BZN6	Dedicator of cytokinesis protein 10	0	0	-1
Dock4	P59764	Dedicator of cytokinesis protein 4	0	0	-1
Dok1	P97465	Docking protein 1	0	0	-1
Dok3	Q9QZK7	Docking protein 3	0	0	-1
Dpagt1	P42867	UDP-N-acetylglucosamine--dolichyl-phosphate N-acetylglucosaminephosphotransferase	0	0	1
Dpy19l4	A2AJQ3	Probable C-mannosyltransferase DPY19L4	0	0	1
Dusp3	Q9D7X3	Dual specificity protein phosphatase 3	0	0	1
Dynlt3	P56387	Dynein light chain Tctex-type 3	0	0	1
Echs1	Q8BH95	Enoyl-CoA hydratase, mitochondrial	0	0	1
Eef2k	O08796	Eukaryotic elongation factor 2 kinase	0	0	-1
Ehd2	Q8BH64	EH domain-containing protein 2	-1	0	0
Eif4b	Q8BGD9	Eukaryotic translation initiation factor 4B	0	0	-1
Elmo2	Q8BHL5	Engulfment and cell motility protein 2	0	1	0
Elovl1	Q9JLJ5	Elongation of very long chain fatty acids protein 1	0	0	1
Eml2	Q7TNG5	Echinoderm microtubule-associated protein-like 2	0	0	1
Eno1	P17182	Alpha-enolase	0	0	1
Enpp1	P06802	Ectonucleotide pyrophosphatase/phosphodiesterase family member 1	0	1	0
Enpp4	Q8BTJ4	Bis(5'-adenosyl)-triphosphatase enpp4	0	-1	0
Entpd1	P55772	Ectonucleoside triphosphate diphosphohydrolase 1	0	0	-1
Epb41l2	O70318	Band 4.1-like protein 2	0	0	-1
Epdr1	Q99M71	Mammalian ependymin-related protein 1	0	0	-1
Epha2	Q03145	Ephrin type-A receptor 2	0	0	-1
Ephx1	Q9D379	Epoxide hydrolase 1	0	1	0
Epn1	Q80VP1	Epsin-1	0	0	-1
Ergic2	Q9CR89	Endoplasmic reticulum-Golgi intermediate compartment protein 2	0	0	1
Erlec1	Q8VEH8	Endoplasmic reticulum lectin 1	0	0	1
Ero1a	Q8R180	ERO1-like protein alpha	0	0	1
Erp29	P57759	Endoplasmic reticulum resident protein 29	0	1	0

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			Early MARP	Middle MARP	Advanced MARP
Erp44	Q9D1Q6	Endoplasmic reticulum resident protein 44	0	0	1
Evi5	P97366	Ecotropic viral integration site 5 protein	0	0	-1
Exoc1	Q8R3S6	Exocyst complex component 1	0	0	-1
F11r	O88792	Junctional adhesion molecule A	0	0	-1
F13a1	Q8BH61	Coagulation factor XIII A chain	0	-1	0
Fabp3	P11404	Fatty acid-binding protein, heart	0	1	0
Fabp5	Q05816	Fatty acid-binding protein, epidermal	0	1	0
Fam160b1	Q8CDM8	Protein FAM160B1	0	0	1
Fam213b	Q9DB60	Prostamide/prostaglandin F synthase	0	0	-1
Fam3c	Q91VU0	Protein FAM3C	0	0	1
Fam45a	Q9D8N2	Protein FAM45A	0	0	-1
Fam49b	Q921M7	Protein FAM49B	0	0	-1
Fasn	P19096	Fatty acid synthase	0	0	1
Fbxl14	Q8BID8	F-box/LRR-repeat protein 14	0	1	0
Fchsd2	Q3USJ8	F-BAR and double SH3 domains protein 2	0	0	-1
Fdps	Q920E5	Farnesyl pyrophosphate synthase	0	1	0
Fer	P70451	Tyrosine-protein kinase Fer	0	-1	0
Fez2	Q6TYB5	Fasciculation and elongation protein zeta-2	0	0	-1
Fgd2	Q8BY35	FYVE, RhoGEF and PH domain-containing protein 2	0	0	-1
Fgf1	P61148	Fibroblast growth factor 1	0	0	1
Fgf2	P15655	Fibroblast growth factor 2	0	1	0
Fhit	O89106	Bis(5'-adenosyl)-triphosphatase	0	-1	0
Filip1l	Q6P6L0	Filamin A-interacting protein 1-like	0	0	-1
Fkbp2	P45878	Peptidyl-prolyl cis-trans isomerase FKBP2	0	1	0
Fmnl1	Q9JL26	Formin-like protein 1	0	0	-1
Fmnl2	A2APV2	Formin-like protein 2	0	-1	0
Fmnl3	Q6ZPF4	Formin-like protein 3	0	-1	0
Fnbp1	Q80TY0	Formin-binding protein 1	0	0	-1
Frmd4a	Q8BIE6	FERM domain-containing protein 4A	0	0	-1
Fry	E9Q8I9	Protein furry homolog	0	0	1

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Fscn1	Q61553	Fascin	0	-1	0
Fth1	P09528	Ferritin heavy chain	0	0	1
Fuom	Q8R2K1	Fucose mutarotase	1	0	0
Fxyd1	Q9Z239	Phospholemman	0	0	1
Fyn	P39688	Tyrosine-protein kinase Fyn	0	0	1
Gab1	Q9QYY0	GRB2-associated-binding protein 1	0	-1	0
Gab2	Q9Z1S8	GRB2-associated-binding protein 2	0	0	-1
Galnt1	O08912	Polypeptide N-acetylgalactosaminyltransferase 1	0	0	1
Galnt2	Q6PB93	Polypeptide N-acetylgalactosaminyltransferase 2	0	0	1
Gatm	Q9D964	Glycine amidinotransferase, mitochondrial	0	0	-1
Gca	Q8VC88	Grancalcin	0	0	1
Gcat	O88986	2-amino-3-ketobutyrate coenzyme A ligase, mitochondrial	0	0	1
Gcc2	Q8CHG3	GRIP and coiled-coil domain-containing protein 2	0	0	1
Gde1	Q9JL56	Glycerophosphodiester phosphodiesterase 1	0	0	1
Get4	Q9D1H7	Golgi to ER traffic protein 4 homolog	0	0	1
Gfap	P03995	Glial fibrillary acidic protein	0	0	1
Gfpt1	P47856	Glutamine--fructose-6-phosphate aminotransferase [isomerizing] 1	0	1	0
Gja1	P23242	Gap junction alpha-1 protein	0	0	1
Glb1	P23780	Beta-galactosidase	1	0	0
Glg1	Q61543	Golgi apparatus protein 1	0	0	1
Glmn	Q8BZM1	Glomulin	0	0	1
Gmip	Q6PGG2	GEM-interacting protein	0	0	-1
Gna12	P27600	Guanine nucleotide-binding protein subunit alpha-12	0	-1	0
Gna15	P30678	Guanine nucleotide-binding protein subunit alpha-15	0	0	-1
Gnai1	B2RSH2	Guanine nucleotide-binding protein G(i) subunit alpha-1	0	0	-1
Gnai2	P08752	Guanine nucleotide-binding protein G(i) subunit alpha-2	0	0	-1
Gnb1	P62874	Guanine nucleotide-binding protein G(I)/G(S)/G(T) subunit beta-1	0	0	-1
Gnb2	P62880	Guanine nucleotide-binding protein G(I)/G(S)/G(T) subunit beta-2	0	0	-1
Gng10	Q9CXP8	Guanine nucleotide-binding protein G(I)/G(S)/G(O) subunit gamma-10	0	0	-1
Gng2	P63213	Guanine nucleotide-binding protein G(I)/G(S)/G(O) subunit gamma-2	0	0	-1

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Gng5	Q80SZ7	Guanine nucleotide-binding protein G(I)/G(S)/G(O) subunit gamma-5	0	0	-1
Gngt2	Q61017	Guanine nucleotide-binding protein G(I)/G(S)/G(O) subunit gamma-T2	0	0	-1
Gns	Q8BFR4	N-acetylglucosamine-6-sulfatase	0	0	1
Golga3	P55937	Golgin subfamily A member 3	0	0	1
Golga4	Q91VW5	Golgin subfamily A member 4	0	1	0
Golga5	Q9QYE6	Golgin subfamily A member 5	0	0	1
Golt1b	Q9CR60	Vesicle transport protein GOT1B	0	0	1
Got1	P05201	Aspartate aminotransferase, cytoplasmic	0	0	1
Gpd1	P13707	Glycerol-3-phosphate dehydrogenase [NAD(+)], cytoplasmic	-1	0	0
Gpi	P06745	Glucose-6-phosphate isomerase	0	0	1
Gpld1	O70362	Phosphatidylinositol-glycan-specific phospholipase D	0	-1	0
Gpm6a	P35802	Neuronal membrane glycoprotein M6-a	0	0	1
Gpr84	Q8CIM5	G-protein coupled receptor 84	1	0	0
Grap	Q9CX99	GRB2-related adapter protein	0	0	-1
Gsdmdc1	Q9D8T2	Gasdermin-D	0	0	-1
Gsn	P13020	Gelsolin	0	1	0
Gtf3c4	Q8BMQ2	General transcription factor 3C polypeptide 4	1	0	0
Gusb	P12265	Beta-glucuronidase	0	1	0
Gvin1	Q80SU7	Interferon-induced very large GTPase 1	1	0	0
Gyg1	Q9R062	Glycogenin-1	0	0	1
Gys1	Q9Z1E4	Glycogen [starch] synthase, muscle	0	0	1
H2afz	P0C0S6	Histone H2A.Z;Histone H2A.V	0	-1	0
H2-D1	P01899	H-2 class I histocompatibility antigen, D-B alpha chain	1	0	0
H2-K1	P01901	H-2 class I histocompatibility antigen, K-B alpha chain	1	0	0
H2-T23	P06339	H-2 class I histocompatibility antigen, D-37 alpha chain	0	1	0
H3f3c	P02301	Histone H3.3C;Histone H3.3	0	0	-1
Haao	Q78JT3	3-hydroxyanthranilate 3,4-dioxygenase	1	0	0
Hacd2	Q9D3B1	Very-long-chain (3R)-3-hydroxyacyl-CoA dehydratase 2	0	1	0
Hapln1	Q9QUP5	Hyaluronan and proteoglycan link protein 1	0	1	0
Hck	P08103	Tyrosine-protein kinase HCK	0	0	-1

Gene Name	UniProt Accession	Protein Name	MARPs (up: +1; down: -1)		
			Early MARP	Middle MARP	Advanced MARP
Hepacam	Q640R3	Hepatocyte cell adhesion molecule	0	0	1
Hexa	P29416	Beta-hexosaminidase subunit alpha	1	0	0
Hexb	P20060	Beta-hexosaminidase subunit beta	0	0	1
Hic2	Q9JLZ6	Hypermethylated in cancer 2 protein	0	0	-1
Hist1h1a	P43275	Histone H1.1	0	1	0
Hist1h1b	P43276	Histone H1.5	0	1	0
Hist1h2ab	P22752	Histone H2A type 1	0	0	-1
Hist1h2bf	P10853	Histone H2B type 1-F/J/L	0	0	-1
Hist2h2ac	Q64523	Histone H2A type 2-C	0	-1	0
Hmga1	P17095	High mobility group protein HMG-I/HMG-Y	0	-1	0
Hmgn2	P09602	Non-histone chromosomal protein HMG-17	0	-1	0
Hmha1	Q3TBD2	Minor histocompatibility protein HA-1	0	0	-1
Hmox1	P14901	Heme oxygenase 1	0	1	0
Hpca	P84075	Neuron-specific calcium-binding protein hippocalcin	0	0	1
Hpcal1	P62748	Hippocalcin-like protein 1	0	0	1
Hpcal4	Q8BGZ1	Hippocalcin-like protein 4	0	0	1
Hpf1	Q8CFE2	Histone PARylation factor 1	0	0	1
Hpgd	Q8VCC1	15-hydroxyprostaglandin dehydrogenase [NAD(+)]	0	0	-1
Hpgds	Q9JHF7	Hematopoietic prostaglandin D synthase	0	0	-1
Hras	Q61411	GTPase HRas	0	0	-1
Hsd17b12	O70503	Very-long-chain 3-oxoacyl-CoA reductase	0	1	0
Hspa5	P20029	78 kDa glucose-regulated protein	0	0	1
Htra1	Q9R118	Serine protease HTRA1	1	0	0
Hyal1	Q91ZJ9	Hyaluronidase-1	0	0	-1
Idh2	P54071	Isocitrate dehydrogenase [NADP], mitochondrial	0	-1	0
Ier3ip1	Q9CR20	Immediate early response 3-interacting protein 1	0	0	1
Ifi204	P0DOV2	Interferon-activable protein 204	1	0	0
Ifit2	Q64112	Interferon-induced protein with tetratricopeptide repeats 2	1	0	0
Ifit3	Q64345	Interferon-induced protein with tetratricopeptide repeats 3	1	0	0
Ifitm3	Q9CQW9	Interferon-induced transmembrane protein 3	0	1	0

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Gene Name	UniProt Accession	Protein Name	MARPs (up: +1; down: -1)		
			Early MARP	Middle MARP	Advanced MARP
Igfbp7	Q61581	Insulin-like growth factor-binding protein 7	0	-1	0
Il16	O54824	Pro-interleukin-16	0	0	-1
Il6st	Q00560	Interleukin-6 receptor subunit beta	0	0	-1
Ilf2	Q9CXY6	Interleukin enhancer-binding factor 2	0	0	1
Inf2	Q0GNC1	Inverted formin-2	0	0	1
Inpp4b	Q6P1Y8	Type II inositol 3,4-bisphosphate 4-phosphatase	0	0	-1
Ints1	Q6P4S8	Integrator complex subunit 1	0	0	1
Ints2	Q80UK8	Integrator complex subunit 2	1	0	0
Ints3	Q7TPD0	Integrator complex subunit 3	0	0	1
Ints5	Q8CHT3	Integrator complex subunit 5	0	0	1
Ints7	Q7TQK1	Integrator complex subunit 7	0	0	1
Ipo4	Q8VI75	Importin-4	0	1	0
Irak1	Q62406	Interleukin-1 receptor-associated kinase 1	0	0	-1
Isg15	Q64339	Ubiquitin-like protein ISG15	1	0	0
Ist1	Q9CX00	IST1 homolog	0	1	0
Itga9	B8JK39	Integrin alpha-9	0	-1	0
Itgav	P43406	Integrin alpha-V	0	0	-1
Itgax	Q9QXH4	Integrin alpha-X	1	0	0
Itgb6	Q9Z0T9	Integrin beta-6	0	0	-1
Itpr2	Q9Z329	Inositol 1,4,5-trisphosphate receptor type 2	0	-1	0
Itpr3	P70227	Inositol 1,4,5-trisphosphate receptor type 3	0	-1	0
Ivns1abp	Q920Q8	Influenza virus NS1A-binding protein homolog	0	0	-1
Jak1	P52332	Tyrosine-protein kinase JAK1	0	0	-1
Kif1a	P33173	Kinesin-like protein KIF1A	0	0	1
Kras	P32883	GTPase KRas	-1	0	0
Krtcap2	Q5RL79	Keratinocyte-associated protein 2	0	0	1
Ldhb	P16125	L-lactate dehydrogenase B chain	0	0	-1
Lgals3	P16110	Galectin-3	1	0	0
Lgals3bp	Q07797	Galectin-3-binding protein	1	0	0
Lgals9	O08573	Galectin-9	0	0	-1

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Limd2	Q8BGB5	LIM domain-containing protein 2	0	0	-1
Llgl1	Q80Y17	Lethal(2) giant larvae protein homolog 1	-1	0	0
Lman1	Q9D0F3	Protein ERGIC-53	0	1	0
Lman2	Q9DBH5	Vesicular integral-membrane protein VIP36	0	0	1
Lmna	P48678	Prelamin-A/C	0	0	1
Lmnb2	P21619	Lamin-B2	0	0	1
Lpcat2	Q8BYI6	Lysophosphatidylcholine acyltransferase 2	0	0	-1
Lrp1	Q91ZX7	Prolow-density lipoprotein receptor-related protein 1	0	0	-1
Lrpap1	P55302	Alpha-2-macroglobulin receptor-associated protein	0	1	0
Lrrc20	Q8CI70	Leucine-rich repeat-containing protein 20	0	0	1
Lrrc25	Q8K1T1	Leucine-rich repeat-containing protein 25	0	-1	0
Lrrc8d	Q8BGR2	Volume-regulated anion channel subunit LRRC8D	0	0	1
Ltc4s	Q60860	Leukotriene C4 synthase	0	0	-1
Luc7l3	Q5SUF2	Luc7-like protein 3	-1	0	0
Ly86	O88188	Lymphocyte antigen 86	1	0	0
Lyn	P25911	Tyrosine-protein kinase Lyn	0	0	-1
Lyplal1	Q3UFF7	Lysophospholipase-like protein 1	0	0	1
Lyz2	P08905	Lysozyme C-2	0	0	1
Magt1	Q9CQY5	Magnesium transporter protein 1	0	0	1
Manf	Q9CXI5	Mesencephalic astrocyte-derived neurotrophic factor	0	0	1
Map1s	Q8C052	Microtubule-associated protein 1S	0	0	-1
Map3k3	Q61084	Mitogen-activated protein kinase kinase kinase 3	0	0	-1
Map4	P27546	Microtubule-associated protein 4	0	0	-1
Map4k4	P97820	Mitogen-activated protein kinase kinase kinase kinase 4	0	-1	0
Map7d1	A2AJI0	MAP7 domain-containing protein 1	0	-1	0
Mapre1	Q61166	Microtubule-associated protein RP/EB family member 1	0	0	-1
Marcks	P26645	Myristoylated alanine-rich C-kinase substrate	0	0	-1
Marcksl1	P28667	MARCKS-related protein	0	0	-1
Mcm2	P97310	DNA replication licensing factor MCM2	0	1	0
Mcm3	P25206	DNA replication licensing factor MCM3	0	1	0

Gene Name	UniProt Accession	Protein Name	MARPs (up: +1; down: -1)		
			Early MARP	Middle MARP	Advanced MARP
Mcm5	P49718	DNA replication licensing factor MCM5	1	0	0
Med15	Q924H2	Mediator of RNA polymerase II transcription subunit 15	0	0	1
Mgat2	Q921V5	Alpha-1,6-mannosyl-glycoprotein 2-beta-N-acetylglucosaminyltransferase	0	0	1
Mgst3	Q9CPU4	Microsomal glutathione S-transferase 3	0	0	1
Mif	P34884	Macrophage migration inhibitory factor	0	1	0
Milr1	Q3TB92	Allergin-1	1	0	0
Mnda	P0DOV1	Interferon-activable protein 205-B	1	0	0
Mospd2	Q9CWP6	Motile sperm domain-containing protein 2	0	1	0
Mov10	P23249	Putative helicase MOV-10	1	0	0
Mpeg1	A1L314	Macrophage-expressed gene 1 protein	1	0	0
Mpi	Q924M7	Mannose-6-phosphate isomerase	0	0	-1
Mras	O08989	Ras-related protein M-Ras	0	0	-1
Mrc1	Q61830	Macrophage mannose receptor 1	0	-1	0
Mrpl40	Q9Z2Q5	39S ribosomal protein L40, mitochondrial	0	0	1
Msmo1	Q9CRA4	Methylsterol monooxygenase 1	0	0	1
Mthfs	Q9D110	5-formyltetrahydrofolate cyclo-ligase	0	0	1
Mto1	Q923Z3	Protein MTO1 homolog, mitochondrial	0	0	1
Mtr	A6H5Y3	Methionine synthase	0	0	-1
Mvb12b	Q6KAU4	Multivesicular body subunit 12B	0	0	-1
Myo1b	P46735	Unconventional myosin-Ib	0	0	-1
Myo1g	Q5SUA5	Unconventional myosin-Ig	0	0	-1
Myo5a	Q99104	Unconventional myosin-Va	0	0	1
Myo9a	Q8C170	Unconventional myosin-IXa	0	0	-1
Naa25	Q8BWZ3	N-alpha-acetyltransferase 25, NatB auxiliary subunit	0	0	1
Naaa	Q9D7V9	N-acylethanolamine-hydrolyzing acid amidase	0	-1	0
Nampt	Q99KQ4	Nicotinamide phosphoribosyltransferase	0	1	0
Ncan	P55066	Neurocan core protein	0	0	1
Nceh1	Q8BLF1	Neutral cholesterol ester hydrolase 1	0	1	0
Ncl	P09405	Nucleolin	0	0	1
Ndrg2	Q9QYG0	Protein NDRG2	0	0	1

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Nenf	Q9CQ45	Neudesin	0	0	1
Neu4	Q8BZL1	Sialidase-4	0	0	-1
Nfatc1	O88942	Nuclear factor of activated T-cells, cytoplasmic 1	0	0	-1
Nfatc2	Q60591	Nuclear factor of activated T-cells, cytoplasmic 2	0	0	-1
Nfkbib	Q60778	NF-kappa-B inhibitor beta	0	0	-1
Ngp	O08692	Neutrophilic granule protein	0	0	-1
Nipsnap3b	Q9CQE1	Protein NipSnap homolog 3B	0	0	1
Nit1	Q8VDK1	Nitrilase homolog 1	0	0	-1
Nkiras2	Q9CR56	NF-kappa-B inhibitor-interacting Ras-like protein 2	0	0	1
No66	Q9JJF3	Bifunctional lysine-specific demethylase and histidyl-hydroxylase NO66	0	0	1
Nop10	Q9CQS2	H/ACA ribonucleoprotein complex subunit 3	1	0	0
Npc2	Q9Z0J0	Epididymal secretory protein E1	1	0	0
Nras	P08556	GTPase NRas	0	0	-1
Nrbf2	Q8VCQ3	Nuclear receptor-binding factor 2	1	0	0
Nsdhl	Q9R1J0	Sterol-4-alpha-carboxylate 3-dehydrogenase, decarboxylating	0	0	1
Nucb1	Q02819	Nucleobindin-1	0	0	1
Nucb2	P81117	Nucleobindin-2	1	0	0
Numb	Q9QZS3	Protein numb homolog	0	-1	0
Numbl	O08919	Numb-like protein	0	-1	0
Nup155	Q99P88	Nuclear pore complex protein Nup155	0	0	1
Oas1a	P11928	2'-5'-oligoadenylate synthase 1A	1	0	0
Olfml3	Q8BK62	Olfactomedin-like protein 3	0	0	-1
Omg	Q63912	Oligodendrocyte-myelin glycoprotein	-1	0	0
Ophn1	Q99J31	Oligophrenin-1	0	0	-1
ORF11	Q9ERY9	Probable ergosterol biosynthetic protein 28	0	0	1
Os9	Q8K2C7	Protein OS-9	0	1	0
Osbpl3	Q9DBS9	Oxysterol-binding protein-related protein 3	0	0	1
Osbpl8	B9EJ86	Oxysterol-binding protein-related protein 8	0	0	1
P2rx4	Q9JJX6	P2X purinoceptor 4	0	0	1
P2rx7	Q9Z1M0	P2X purinoceptor 7	0	0	-1

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			Early MARP	Middle MARP	Advanced MARP
P2ry12	Q9CPV9	P2Y purinoceptor 12	0	-1	0
P2yr13	Q9D8I2	P2Y purinoceptor 13	0	0	-1
Pacsin1	Q61644	Protein kinase C and casein kinase substrate in neurons protein 1	0	0	1
Pacsin2	Q9WVE8	Protein kinase C and casein kinase substrate in neurons protein 2	0	0	-1
Paf1	Q8K2T8	RNA polymerase II-associated factor 1 homolog	0	0	1
Pclo	Q9QYX7	Protein piccolo	0	0	-1
Pcna	P17918	Proliferating cell nuclear antigen	1	0	0
Pde3b	Q61409	cGMP-inhibited 3',5'-cyclic phosphodiesterase B	0	-1	0
Pdia4	P08003	Protein disulfide-isomerase A4	0	0	1
Pdlim4	P70271	PDZ and LIM domain protein 4	0	-1	0
Pdlim5	Q8CI51	PDZ and LIM domain protein 5	0	-1	0
Pdxdc1	Q99K01	Pyridoxal-dependent decarboxylase domain-containing protein 1	0	1	0
Pfkl	P12382	ATP-dependent 6-phosphofructokinase, liver type	0	1	0
Pgam1	Q9DBJ1	Phosphoglycerate mutase 1	0	0	1
Pgam2	O70250	Phosphoglycerate mutase 2	0	1	0
Pgap1	Q3UUQ7	GPI inositol-deacylase	0	0	1
Pgk1	P09411	Phosphoglycerate kinase 1	0	1	0
Pgm1	Q9D0F9	Phosphoglucomutase-1	0	0	1
Phf11	A6H5X4	PHD finger protein 11	1	0	0
Phyhd1	Q9DB26	Phytanoyl-CoA dioxygenase domain-containing protein 1	0	0	-1
Pi4k2a	Q2TBE6	Phosphatidylinositol 4-kinase type 2-alpha	1	0	0
Pid1	Q3UBG2	PTB-containing, cubilin and LRP1-interacting protein	0	0	-1
Pik3ap1	Q9EQ32	Phosphoinositide 3-kinase adapter protein 1	0	0	-1
Pik3r5	Q5SW28	Phosphoinositide 3-kinase regulatory subunit 5	0	0	-1
Pik3r6	Q3U6Q4	Phosphoinositide 3-kinase regulatory subunit 6	0	0	-1
Pikfyve	Q9Z1T6	1-phosphatidylinositol 3-phosphate 5-kinase	0	0	1
Pip4k2a	O70172	Phosphatidylinositol 5-phosphate 4-kinase type-2 alpha	-1	0	0
Pip4k2b	Q80XI4	Phosphatidylinositol 5-phosphate 4-kinase type-2 beta	0	-1	0
Pkm	P52480	Pyruvate kinase PKM	0	1	0
Pkn1	P70268	Serine/threonine-protein kinase N1	0	0	-1

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Plcb3	P51432	1-phosphatidylinositol 4,5-bisphosphate phosphodiesterase beta-3	0	0	-1
Plcl2	Q8K394	Inactive phospholipase C-like protein 2	0	0	-1
Pld4	Q8BG07	Phospholipase D4	0	0	-1
Plekha1	Q8BUL6	Pleckstrin homology domain-containing family A member 1	0	0	1
Plekho1	Q9JIY0	Pleckstrin homology domain-containing family O member 1	0	0	-1
Plgrkt	Q9D3P8	Plasminogen receptor (KT)	0	1	0
Plp2	Q9R1Q7	Proteolipid protein 2	0	1	0
Plpp3	Q99JY8	Phospholipid phosphatase 3	0	0	1
Plrg1	Q922V4	Pleiotropic regulator 1	0	0	1
Plscr3	Q9JIZ9	Phospholipid scramblase 3	0	-1	0
Plxnb2	B2RXS4	Plexin-B2	0	0	-1
Pml	Q60953	Protein PML	1	0	0
Pnp	P23492	Purine nucleoside phosphorylase	0	-1	0
Pofut2	Q8VHI3	GDP-fucose protein O-fucosyltransferase 2	0	1	0
Ppig	A2AR02	Peptidyl-prolyl cis-trans isomerase G	0	-1	0
Ppp1r18	Q8BQ30	Phostensin	0	0	-1
Praf2	Q9JIG8	PRA1 family protein 2	0	0	1
Prdx1	P35700	Peroxiredoxin-1	0	0	1
Prdx4	O08807	Peroxiredoxin-4	1	0	0
Prdx6	O08709	Peroxiredoxin-6	0	0	1
Prkab1	Q9R078	5'-AMP-activated protein kinase subunit beta-1	0	0	-1
Prkcd	P28867	Protein kinase C delta type	0	-1	0
Prpf40a	Q9R1C7	Pre-mRNA-processing factor 40 homolog A	0	0	-1
Prpf8	Q99PV0	Pre-mRNA-processing-splicing factor 8	0	0	-1
Psat1	Q99K85	Phosphoserine aminotransferase	1	0	0
Ptp4a2	O70274	Protein tyrosine phosphatase type IVA 2	0	0	-1
Ptp4a3	Q9D658	Protein tyrosine phosphatase type IVA 3	0	0	-1
Ptprj	Q64455	Receptor-type tyrosine-protein phosphatase eta	0	0	-1
Ptrf	O54724	Polymerase I and transcript release factor	0	-1	0
Pts	Q9R1Z7	6-pyruvoyl tetrahydrobiopterin synthase	0	0	-1

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			Early MARP	Middle MARP	Advanced MARP
Pxk	Q8BX57	PX domain-containing protein kinase-like protein	0	-1	0
Pxn	Q8VI36	Paxillin	0	0	-1
Pyhin1	Q8BV49	Pyrin and HIN domain-containing protein 1	1	0	0
Rab39a	Q8BHD0	Ras-related protein Rab-39A	0	0	-1
Rab3d	P35276	Ras-related protein Rab-3D	0	0	1
Rab3il1	Q8VDV3	Guanine nucleotide exchange factor for Rab-3A	0	0	-1
Rac2	Q05144	Ras-related C3 botulinum toxin substrate 2	0	0	-1
Rala	P63321	Ras-related protein Ral-A	0	-1	0
Ralgapb	Q8BQZ4	Ral GTPase-activating protein subunit beta	0	0	1
Rap1b	Q99JI6	Ras-related protein Rap-1b	0	0	-1
Rap2b	P61226	Ras-related protein Rap-2b	0	0	1
Rap2c	Q8BU31	Ras-related protein Rap-2c	0	0	-1
Rassf2	Q8BMS9	Ras association domain-containing protein 2	0	0	-1
Rbbp9	O88851	Putative hydrolase RBBP9	0	0	-1
Rbm39	Q8VH51	RNA-binding protein 39	0	-1	0
Rcc1	Q8VE37	Regulator of chromosome condensation	0	-1	0
Rcsd1	Q3UZA1	CapZ-interacting protein	0	0	-1
Rer1	Q9CQU3	Protein RER1	0	1	0
Rgs10	Q9CQE5	Regulator of G-protein signaling 10	0	0	-1
Rgs19	Q9CX84	Regulator of G-protein signaling 19	0	0	-1
Rhoc	Q62159	Rho-related GTP-binding protein RhoC	0	1	0
Rhog	P84096	Rho-related GTP-binding protein RhoG	0	0	-1
Rhoq	Q8R527	Rho-related GTP-binding protein RhoQ	0	0	-1
Rnaseh2a	Q9CWY8	Ribonuclease H2 subunit A	0	0	1
Rnaseh2c	Q9CQ18	Ribonuclease H2 subunit C	0	1	0
Rnf130	Q8VEM1	E3 ubiquitin-protein ligase RNF130	0	0	-1
Rp2	Q9EPK2	Protein XRP2	0	0	-1
Rpl13a	P19253	60S ribosomal protein L13a	1	0	0
Rpl38	Q9JJI8	60S ribosomal protein L38	-1	0	0
Rpl7	P14148	60S ribosomal protein L7	1	0	0

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			Early MARP	Middle MARP	Advanced MARP
Rps15	P62843	40S ribosomal protein S15	1	0	0
Rps27a	P62983	Ubiquitin-40S ribosomal protein S27a	0	0	1
Rps6ka1	P18653	Ribosomal protein S6 kinase alpha-1	0	-1	0
Rps6ka3	P18654	Ribosomal protein S6 kinase alpha-3	0	0	-1
Rras2	P62071	Ras-related protein R-Ras2	0	0	-1
Rtn4	Q99P72	Reticulon-4	0	-1	0
Scly	Q9JLI6	Selenocysteine lyase	0	0	1
Scpep1	Q920A5	Retinoid-inducible serine carboxypeptidase	0	0	1
Sdcbp	O08992	Syntenin-1	1	0	0
Sec14l2	Q99J08	SEC14-like protein 2	0	0	1
Sec23ip	Q6NZC7	SEC23-interacting protein	0	1	0
Sec61a1	P61620	Protein transport protein Sec61 subunit alpha isoform 1	0	0	1
Sel1l	Q9Z2G6	Protein sel-1 homolog 1	0	0	1
Serpinc1	P32261	Antithrombin-III	0	-1	0
Serpini1	O35684	Neuroserpin	0	0	-1
Sf3b5	Q923D4	Splicing factor 3B subunit 5	0	0	-1
Sh3bgrl	Q9JJU8	SH3 domain-binding glutamic acid-rich-like protein	0	0	1
Sh3bp1	P55194	SH3 domain-binding protein 1	0	0	-1
Sil1	Q9EPK6	Nucleotide exchange factor SIL1	1	0	0
Sirpa	P97797	Tyrosine-protein phosphatase non-receptor type substrate 1	0	0	-1
Slc16a1	P53986	Monocarboxylate transporter 1	0	0	1
Slc17a7	Q3TXX4	Vesicular glutamate transporter 1	0	0	1
Slc23a2	Q9EPR4	Solute carrier family 23 member 2	0	1	0
Slc2a5	Q9WV38	Solute carrier family 2, facilitated glucose transporter member 5	0	-1	0
Slc30a3	P97441	Zinc transporter 3	0	0	1
Slc30a6	Q8BJM5	Zinc transporter 6	1	0	0
Slc35b1	P97858	Solute carrier family 35 member B1	0	0	1
Slc35b2	Q91ZN5	Adenosine 3'-phospho 5'-phosphosulfate transporter 1	0	1	0
Slc35e1	Q8CD26	Solute carrier family 35 member E1	0	1	0
Slc37a2	Q9WU81	Glucose-6-phosphate exchanger SLC37A2	0	0	1

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Slc38a10	Q5I012	Putative sodium-coupled neutral amino acid transporter 10	0	1	0
Slc4a4	O88343	Electrogenic sodium bicarbonate cotransporter 1	0	0	1
Slc9a1	Q61165	Sodium/hydrogen exchanger 1	0	0	-1
Slco2b1	Q8BXB6	Solute carrier organic anion transporter family member 2B1	0	0	-1
Slmap	Q3URD3	Sarcolemmal membrane-associated protein	0	0	-1
Smad1	P70340	Mothers against decapentaplegic homolog 1	0	-1	0
Smad2	Q62432	Mothers against decapentaplegic homolog 2	0	0	-1
Smad3	Q8BUN5	Mothers against decapentaplegic homolog 3	0	-1	0
Smim1	P0C8K7	Small integral membrane protein 1	0	-1	0
Smox	Q99K82	Spermine oxidase	0	0	-1
Smpdl3a	P70158	Acid sphingomyelinase-like phosphodiesterase 3a	0	-1	0
Smpdl3b	P58242	Acid sphingomyelinase-like phosphodiesterase 3b	0	-1	0
Snx17	Q8BVL3	Sorting nexin-17	0	0	-1
Snx18	Q91ZR2	Sorting nexin-18	0	0	-1
Snx9	Q91VH2	Sorting nexin-9	0	0	-1
Soat1	Q61263	Sterol O-acyltransferase 1	1	0	0
Soga1	E1U8D0	Protein SOGA1	0	0	-1
Sort1	Q6PHU5	Sortilin	0	0	-1
Sp100	O35892	Nuclear autoantigen Sp-100	1	0	0
Sp3	O70494	Transcription factor Sp3	0	0	-1
Sparc	P07214	SPARC	0	0	-1
Sparcl1	P70663	SPARC-like protein 1	0	-1	0
Spg20	Q8R1X6	Spartin	0	0	-1
Spg21	Q9CQC8	Maspardin	1	0	0
Spock2	Q9ER58	Testican-2	0	-1	0
Sqrdl	Q9R112	Sulfide:quinone oxidoreductase, mitochondrial	0	-1	0
Srbd1	Q497V5	S1 RNA-binding domain-containing protein 1	0	1	0
Srgap2	Q91Z67	SLIT-ROBO Rho GTPase-activating protein 2	0	-1	0
Srpk2	O54781	SRSF protein kinase 2	1	0	0
Ssr1	Q9CY50	Translocon-associated protein subunit alpha	0	1	0

Gene Name	UniProt Accession	Protein Name	MARPs (up: +1; down: -1)		
			Early MARP	Middle MARP	Advanced MARP
Ssr4	Q62186	Translocon-associated protein subunit delta	0	0	1
Stab1	Q8R4Y4	Stabilin-1	0	-1	0
Stard3nl	Q9DCI3	MLN64 N-terminal domain homolog	0	0	-1
Stat1	P42225	Signal transducer and activator of transcription 1	1	0	0
Stat2	Q9WVL2	Signal transducer and activator of transcription 2	1	0	0
Stk10	O55098	Serine/threonine-protein kinase 10	0	0	-1
Stk26	Q99JT2	Serine/threonine-protein kinase 26	0	0	-1
Sts	P50427	Steryl-sulfatase	0	0	1
Stt3b	Q3TDQ1	Dolichyl-diphosphooligosaccharide--protein glycosyltransferase subunit STT3B	0	0	1
Sult1a1	P52840	Sulfotransferase 1A1	0	0	-1
Susd3	Q9D176	Sushi domain-containing protein 3	0	-1	0
Syk	P48025	Tyrosine-protein kinase SYK	0	-1	0
Syngr1	O55100	Synaptogyrin-1	0	0	1
Tamm41	Q3TUH1	Phosphatidate cytidylyltransferase, mitochondrial	-1	0	0
Taok1	Q5F2E8	Serine/threonine-protein kinase TAO1	0	0	-1
Tap2	P36371	Antigen peptide transporter 2	1	0	0
Tapbp	Q9R233	Tapasin	1	0	0
Tbc1d10b	Q8BHL3	TBC1 domain family member 10B	-1	0	0
Tbc1d22a	Q8R5A6	TBC1 domain family member 22A	0	0	1
Tbc1d4	Q8BYJ6	TBC1 domain family member 4	0	0	1
Tfap2a	P34056	Transcription factor AP-2-alpha	0	0	-1
Tfe3	Q64092	Transcription factor E3	0	0	-1
Tfeb	Q9R210	Transcription factor EB	0	0	-1
Tgfbr1	Q64729	TGF-beta receptor type-1	0	0	-1
Tgm2	P21981	Protein-glutamine gamma-glutamyltransferase 2	0	-1	0
Them6	Q80ZW2	Protein THEM6	0	0	1
Tigar	Q8BZA9	Fructose-2,6-bisphosphatase TIGAR	0	0	1
Tjp1	P39447	Tight junction protein ZO-1	0	0	-1
Tkt	P40142	Transketolase	0	1	0
Tlk2	O55047	Serine/threonine-protein kinase tousled-like 2	0	0	1

Gene Name	UniProt Accession	Protein Name	MARPs (up: +1; down: -1)		
			Early MARP	Middle MARP	Advanced MARP
Tlr2	Q9QUN7	Toll-like receptor 2	1	0	0
Tlr3	Q99MB1	Toll-like receptor 3	0	0	-1
Tlr9	Q9EQU3	Toll-like receptor 9	0	-1	0
Tm6sf1	P58749	Transmembrane 6 superfamily member 1	0	-1	0
Tm9sf1	Q9DBU0	Transmembrane 9 superfamily member 1	0	1	0
Tm9sf3	Q9ET30	Transmembrane 9 superfamily member 3	0	1	0
Tm9sf4	Q8BH24	Transmembrane 9 superfamily member 4	0	0	1
Tmed10	Q9D1D4	Transmembrane emp24 domain-containing protein 10	0	1	0
Tmed3	Q78IS1	Transmembrane emp24 domain-containing protein 3	0	0	1
Tmed4	Q8R1V4	Transmembrane emp24 domain-containing protein 4	0	1	0
Tmed9	Q99KF1	Transmembrane emp24 domain-containing protein 9	0	1	0
Tmem100	Q9CQG9	Transmembrane protein 100	0	-1	0
Tmem119	Q8R138	Transmembrane protein 119	0	-1	0
Tmem163	Q8C996	Transmembrane protein 163	0	0	1
Tmem167a	Q9CR64	Protein kish-A	0	0	1
Tmem173	Q3TBT3	Stimulator of interferon genes protein	0	0	-1
Tmem205	Q91XE8	Transmembrane protein 205	0	0	1
Tmem214	Q8BM55	Transmembrane protein 214	0	0	1
Tmem38b	Q9DAV9	Trimeric intracellular cation channel type B	0	0	1
Tmsb10	Q6ZWY8	Thymosin beta-10	0	0	1
Tnfaip8l2	Q9D8Y7	Tumor necrosis factor alpha-induced protein 8-like protein 2	0	-1	0
Top2b	Q64511	DNA topoisomerase 2-beta	0	0	1
Tpcn1	Q9EQJ0	Two pore calcium channel protein 1	0	0	-1
Tpd52	Q62393	Tumor protein D52	0	1	0
Tpi1	P17751	Triosephosphate isomerase	0	1	0
Tpmt	O55060	Thiopurine S-methyltransferase	0	0	-1
Trem2	Q99NH8	Triggering receptor expressed on myeloid cells 2	0	1	0
Trim3	Q9R1R2	Tripartite motif-containing protein 3	0	0	-1
Trim47	Q8C0E3	Tripartite motif-containing protein 47	0	0	-1
Trim65	Q8BFW4	Tripartite motif-containing protein 65	0	0	-1

Gene Name	UniProt Accession	Protein Name	MARPs (up: +1; down: -1)		
			Early MARP	Middle MARP	Advanced MARP
Trio	Q0KL02	Triple functional domain protein	0	0	-1
Triobp	Q99KW3	TRIO and F-actin-binding protein	0	1	0
Trp53i11	Q4QQM4	Tumor protein p53-inducible protein 11	0	-1	0
Tsc22d1	P62500	TSC22 domain family protein 1	0	0	1
Ttc38	A3KMP2	Tetratricopeptide repeat protein 38	0	0	-1
Ttc39b	Q8BYY4	Tetratricopeptide repeat protein 39B	0	0	1
Ttyh1	Q9D3A9	Protein tweety homolog 1	0	0	1
Tubb5	P99024	Tubulin beta-5 chain	0	0	-1
Ube2d1	P61080	Ubiquitin-conjugating enzyme E2 D1	0	0	-1
Ubl4a	P21126	Ubiquitin-like protein 4A	0	0	1
Upk1b	Q9Z2C6	Uroplakin-1b	0	-1	0
Usp24	B1AY13	Ubiquitin carboxyl-terminal hydrolase 24	0	0	-1
Vamp4	O70480	Vesicle-associated membrane protein 4	0	0	1
Vasp	P70460	Vasodilator-stimulated phosphoprotein	0	0	-1
Vat1	Q62465	Synaptic vesicle membrane protein VAT-1 homolog	0	0	1
Vav2	Q60992	Guanine nucleotide exchange factor VAV2	0	0	-1
Vim	P20152	Vimentin	0	0	1
Vps13c	Q8BX70	Vacuolar protein sorting-associated protein 13C	0	1	0
Vrk1	Q80X41	Serine/threonine-protein kinase VRK1	0	-1	0
Vtn	P29788	Vitronectin	0	1	0
Was	P70315	Wiskott-Aldrich syndrome protein homolog	0	0	-1
Wasf2	Q8BH43	Wiskott-Aldrich syndrome protein family member 2	0	0	-1
Wdfy2	Q8BUB4	WD repeat and FYVE domain-containing protein 2	0	0	-1
Wdr37	Q8CBE3	WD repeat-containing protein 37	0	-1	0
Wls	Q6DID7	Protein wntless homolog	0	0	1
Wnk1	P83741	Serine/threonine-protein kinase WNK1	0	0	-1
Xdh	Q00519	Xanthine dehydrogenase/oxidase	0	0	1
Xpo5	Q924C1	Exportin-5	0	0	1
Yes1	Q04736	Tyrosine-protein kinase Yes	0	0	-1
Yif1a	Q91XB7	Protein YIF1A	0	0	1

Gene Name	UniProt Accession	Protein Name	MARPs (up: +1; down: -1)		
			Early MARP	Middle MARP	Advanced MARP
Yipf3	Q3UDR8	Protein YIPF3	1	0	0
Zadh2	Q8BGC4	Prostaglandin reductase-3	1	0	0
Zfpl1	Q9DB43	Zinc finger protein-like 1	0	0	1
Znf335	A2A5K6	Zinc finger protein 335	0	0	1
Znf652	Q5DU09	Zinc finger protein 652	0	0	-1
Znf691	Q3TDE8	Zinc finger protein 691	0	0	-1
Zyx	Q62523	Zyxin	0	0	-1
	Q91V76	Ester hydrolase C11orf54 homolog	0	0	-1
	Q9CRC3	UPF0235 protein C15orf40 homolog	0	0	-1
	Q99K99	Uncharacterized protein C4orf19 homolog	0	0	-1
	P01864	Ig gamma-2A chain C region secreted form	0	-1	0
	Q9CXL3	Uncharacterized protein C7orf50 homolog	1	0	0

Suppl. Table 5: Relative quantification of proteins from seven AD risk genes in APPPS1 (**A**) or APP-KI (**B**) *versus* WT microglia. The protein LFQ ratios and p-values of AD risk genes are presented. P-values less than 0.05 are indicated in red.

A

Gene Name	Ratio APPPS1 vs WT				p-value APPPS1 vs WT			
	1 month	3 months	6 months	12 months	1 month	3 months	6 months	12 months
APOE	1.01	2.44	4.20	9.25	8.59E-01	2.45E-02	4.47E-03	4.37E-05
TREM2	0.94	1.40	2.20	2.88	1.50E-01	1.09E-02	4.82E-03	7.36E-06
CLU	1.15	0.97	1.07	1.87	5.70E-03	6.81E-01	6.36E-01	1.11E-03
INPP5D	1.10	1.38	1.80	1.71	2.66E-02	4.91E-04	2.86E-03	9.29E-05
PLCG2	0.99	1.05	0.99	0.86	7.01E-01	2.27E-01	8.45E-01	1.39E-01
ABI3	1.00	1.05	0.90	0.52	9.56E-01	4.72E-01	4.39E-01	1.44E-03
BIN1	0.95	1.03	0.74	0.40	3.93E-01	7.08E-01	7.62E-02	2.85E-04

B

Gene Name	Ratio APP-KI vs WT				p-value APP-KI vs WT			
	1 month	3 months	6 months	12 months	1 month	3 months	6 months	12 months
APOE	1.06	1.22	2.88	15.03	3.29E-01	3.04E-02	7.66E-04	3.09E-05
TREM2	0.99	1.12	1.44	3.85	9.16E-01	5.55E-01	6.38E-03	6.40E-04
CLU	0.84	1.10	0.76	0.72	2.84E-01	3.95E-01	1.80E-01	8.13E-02
INPP5D	0.94	1.08	1.26	1.34	1.68E-01	4.30E-01	2.43E-02	5.14E-05
PLCG2	0.97	0.99	0.74	0.66	2.33E-01	9.27E-01	1.31E-01	1.12E-02
ABI3	0.95	0.95	0.67	0.53	6.55E-01	6.83E-01	1.08E-02	1.78E-03
BIN1	0.99	0.97	0.72	0.59	7.39E-01	7.90E-01	5.49E-03	1.05E-03