Brain deletion of Serf2 shifts amyloid conformation in a mouse model

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24 **Highlights**

25 - Loss of SERF2 slows embryonic development and causes perinatal lethality
26 - SERF2 affects proliferation in a cell-autonomous fashion
27 - Brain-specific *Serf2* knockout does not affect viability or Aβ production
28 - Brain deletion of *Serf2* shifts the amyloid conformation of Aβ

Abstract

Loss of protein homeostasis accelerates ageing and contributes to age-related brain diseases like Parkinson's and Alzheimer's diseases. Disease-specific proteins become aggregation-prone and form amyloid deposits that characterize disease, but the biological mechanisms driving their transition in cells and tissues are poorly understood. MOAG-4/SERF accelerates aggregation and toxicity of disease-related proteins in worm- and cell models. Whether MOAG-4/SERF affects amyloid pathology in brain, however, has remained unknown. Here we show that brain-specific knockout of SERF2 is viable and shifts aggregation in a mouse model for amyloid-beta aggregation. Without affecting amyloid-beta levels, knockout of SERF2 redirects its assembly into structurally and morphologically distinct amyloid fibrils and deposits. Our results show that SERF modifies the structural outcome of the aggregation process in mammalian brain. With structural polymorphisms recently being associated with disease severity and progression, our results may help to understand the molecular basis for structural diversity and find possibilities for structure-based interventions.

42 **Introduction**

43 Protein aggregation is a pathological hallmark shared by several age-related neurodegenerative
44 diseases, such as Alzheimer (AD), Parkinson and Huntington disease. The amyloid-like aggregates
45 that accumulate in each of these diseases are composed of disease-specific proteins, i.e., amyloid-beta
46 (Aβ) and tau in AD[1–4], alpha-synuclein (α-Syn) in Parkinson disease[5] and Huntingtin (HTT) in
47 Huntington disease[6]. While the exact molecular mechanisms underlying the disease pathology remain
48 to be elucidated, genetic evidence indicates that these aggregation-prone proteins play a key role in the
49 disease processes. In AD multiple causative mutations have been found in three genes: the amyloid
50 precursor protein (*APP*)[7–15], presenilin-1 (*PSEN1*)[16–20] and presenilin-2 (*PSEN2*)[20–23] (for a full list of
51 known AD-linked mutations see https://www.alzforum.org/mutations). The *APP* gene encodes the
52 precursor protein from which Aβ is produced through sequential cleavage by a β- and γ-secretase. The
53 *PSEN* genes encode the main catalytic subunit of the γ-secretase complex. The fact that all the
54 causative mutations affect the production, length and/or structure of the Aβ peptides and are sufficient
55 to cause the disease[7,8,17–23,9–16], is arguably the strongest piece of evidence for the involvement of Aβ in
56 the disease process. Causative mutations generally affect the Aβ peptide in one of three ways: (i) an
57 overall increase in Aβ production[9,24], (ii) a shift in the production of shorter variants, like $A\beta_{40,}$ to more
58 aggregation-prone, longer variants, like $A\beta_{42}$ (or $A\beta_{43}$)[11,18,25–27], (iii) Aβ peptides with a modified core
59 region, resulting in an increased propensity for aggregation[15,28–30]. Taken together, these mutations
60 do not necessarily increase the amount of Aβ, but rather increase the likelihood of Aβ
61 aggregation. Similarly, several mutations in the *SNCA* gene that have been linked to Parkinson
62 disease have been shown to alter α-Syn aggregation[31–36]. In Huntington disease, any expansion of a
63 polyglutamine stretch in the *HTT* gene that exceeds 36 repeats has been shown to facilitate HTT
64 aggregation and cause the disease[37]. Hence, changes in the folding and aggregation of these disease
65 proteins are believed to be at the basis of the observed pathology. In addition, there is increasing
66 evidence that distinct structural variants exist between aggregates of the same disease protein and that
67 these polymorphs are associated with varying degrees of toxicity[38–47] . For instance, recent studies with
68 brain samples from human AD patients have shown that variations in the disease type and disease

69 progression correlate with the presence of structurally different aggregates and fibrils[40,41,48,49]. The
70 knowledge that small changes in the structural conformation of these aggregates can have far-reaching
71 effects on the disease manifestation could provide a new avenue for the development of therapeutics.
72 Until now, most research into potential treatments of AD has focused on either reducing the overall
73 amount of aggregating proteins or preventing aggregation, not modifying the aggregation process.

74 In a previous study, our group performed a genetic screen in *Caenorhabditis elegans* (*C. elegans*) and
75 identified a small, intrinsically disordered protein of unknown function with the capacity to modify
76 protein aggregation and to enhance the resulting toxicity of multiple disease-related, aggregation-
77 prone proteins[50]. This peptide was dubbed *mo*difier of *ag*gregation-4 (MOAG-4) and was found to be
78 an ortholog of two human genes: *Serf1A* and *Serf2*. Both SERF1A and SERF2 were shown to
79 exacerbate protein aggregation and toxicity in human neuroblastoma and HEK293 cell lines as well[50].
80 *In vitro* assays with MOAG-4 and SERF1A have demonstrated that these proteins can specifically
81 accelerate the aggregation kinetics of amyloidogenic proteins, but do not affect aggregation of non-
82 amyloidogenic proteins[51,52]. For α-Syn, this amyloid-promoting effect has been shown to depend on
83 the transient interaction of these positively-charged peptides with negatively-charged regions and the
84 disruption intramolecular electrostatic interactions within the protein[51–53]. Removal of MOAG-4 or
85 SERF from a biological system did not change the overall levels of the aggregation-prone proteins or
86 activate chaperone pathways known to suppress aggregation[50]. Therefore, the reduced toxicity would
87 appear to rely solely on the structural shift in the aggregation process[50,51]. In this study, we investigate
88 whether the removal of SERF2 modifies the aggregation of Aβ in the biologically more complex
89 environment of the mammalian brain.

90 **Results**

91 *Full-body Serf2 KO results in developmental delay and perinatal lethality in mice*

92 To establish the role of SERF2 *in vivo*, full-body *Serf2* knockout (KO) mice were generated (Figure
93 S1A-C). During an initial cross, using the four homozygous *Serf2* KO animal (*Serf2*$^{-/-}$) and eight
94 heterozygous *Serf2* KO animals (*Serf2*$^{+/-}$) we obtained, only one *Serf2*$^{-/-}$ survived the day of birth
95 compared to 27 *Serf2*$^{+/-}$ and 96 *Serf2* wild-type (*Serf2*$^{+/+}$) mice (Supplemental Table 1). After
96 backcrossing into C57BL/6J background, interbreeding of *Serf2*$^{+/-}$ animals did not result in any viable
97 *Serf2*$^{-/-}$ mice at the time of weaning (Figure 1A,1B). To determine whether *Serf2*$^{-/-}$ mice died during
98 fetal development, we examined the offspring at multiple developmental stages: embryonic day 13.5,
99 15.5 and 17.5. In all of the examined embryonic stages we observed the expected Mendelian ratio of
100 genotypes, around 25 % *Serf2*$^{+/+}$ and *Serf2*$^{-/-}$ embryos and around 50 % *Serf2*$^{+/-}$ (Chi Square: E13.5
101 p=0.9672; E15.5 p= 0.4432; E17.5 p=0.7674) (Figure 1B). At birth (P0), all *Serf2*$^{-/-}$ displayed
102 respiratory distress and the majority (5/7) died within 30 minutes of being born. At the time of
103 weaning, the Mendelian ratio was maintained between the *Serf2*$^{+/+}$ and *Serf2*$^{+/-}$ animals, but no *Serf2*$^{-/-}$
104 mice survived until this stage (Chi Square: P22 p<0.0001) . Examination of *Serf2* mRNA expression at
105 E13.5 showed a 50 % reduction for *Serf2*$^{+/-}$ and complete KO for *Serf2*$^{-/-}$ compared to *Serf2*$^{+/+}$ (One-
106 way ANOVA: *Serf2*$^{+/-}$ and *Serf2*$^{-/-}$ vs *Serf2*$^{+/+}$ p_{Bon}<0.0001) (Figure 1C). A similar ratio in SERF2
107 expression was also observed on the protein level (Figure 1D). The *Serf1* mRNA levels were not
108 affected by the change in *Serf2* levels, indicating there is no compensatory mechanism between these
109 genes (One-way ANOVA: p=0.2403) (Figure 1C). Further analysis of the *Serf2*$^{-/-}$ embryonic
110 phenotype revealed a reduction in embryo size of *Serf2*$^{-/-}$ compared to *Serf2*$^{+/+}$ and *Serf2*$^{+/-}$ from E15.5
111 until birth (Two-way ANOVA: E17.5 WT vs KO p_{bon}<0.05; P0 WT vs ko p_{Bon}<0.0001) (Figure 1E,
112 S1D-E). No differences in size could be observed between *Serf2*$^{+/+}$ and *Serf2*$^{+/-}$ embryos during the
113 embryonic stage leading up to birth (P0). Histological examination of various tissues revealed a
114 developmental delay of approximately one to two days in the organs of E17.5 *Serf2*$^{-/-}$ embryos
115 compared to the previous time points and standard embryonic development[54,55]. This was most
116 prominently in the lungs, through reduced expansion of alveoli and augmented tissue density, and in

117 the kidneys, by the increased amount of mesenchyme between the tubules and glomeruli (Figure S1F).
118 At the time of birth, the lungs of *Serf2*$^{-/-}$ pups displayed a significantly reduced saccular space (t-test:
119 p= 0.0088) (Figure 1F), reflecting insufficient lung expansion and maturation. This partial fetal
120 atelectasis seems to explain the observed respiratory distress and a likely cause of the perinatal
121 lethality in *Serf2*$^{-/-}$ pups. Placental abnormalities have been shown to cause impaired transfer of
122 nutrients and oxygen to the embryo, resulting in growth delays[56]. To exclude this as a potential cause,
123 we examined the placenta at all three embryonic stages for microscopic lesions in the placental
124 labyrinth or other changes. No genotype-related pathology could be found, indicating that absence of
125 SERF2 in the embryo itself is responsible for the observed growth phenotype. This seems to be
126 supported by the fact that CRISPR-induced *Serf2*$^{-/-}$ clones of HEK293T cells display a delay in
127 proliferation (Figure S2A). Moreover, RNA sequencing analysis of mouse embryonic fibroblasts
128 (MEF) isolated from E13.5 embryos showed a clear enrichment of GO-terms clustered around three
129 closely related biological functions: cell cycle, cell proliferation and cell adhesion (Supplemental
130 Figure S2A-B). Interestingly, in the cell cycle cluster we observe an overall upregulation of factors
131 driving cell division and downregulation of growth arrest factors in *Serf2*$^{-/-}$ MEFs (Supplemental Table
132 2). This observation seems in line with a developmental delay phenotype in *Serf2*$^{-/-}$ mice, because it is
133 known that cell proliferation is favored over differentiation in earlier stages of embryonic development
134 compared to later stages[57]. Overall, the cell culture results point towards a cell-autonomous effect of
135 SERF2 and suggest that loss of *Serf2* results in delayed growth. While our previous study also
136 demonstrated a cell-autonomous function for moag-4, it did not show any effects of moag-4 deletion
137 on the viability, life span or size of *C. elegans*[50]. All together, our results suggest that *Serf2* KO causes
138 delayed maturation of certain organs at the time of birth resulting in death. Given the cell-autonomous
139 nature of the effects of SERF2, we aimed to avoid these adverse effects by creating an organ-specific
140 conditional KO model.

141 *Brain-specific KO of Serf2 does not affect viability or the Aβ production pathway*

142 To study the effect of *Serf2* KO on amyloid pathology in the brain, we circumvented the perinatal
143 lethality of full-body *Serf2* KO mice by generating a brain-specific *Serf2* KO mouse model (*Serf2*$^{br-/-}$).

144 The conditional KO was obtained by combining our *Serf2*$^{flox/flox}$ mice with a *Sox1-Cre* model[58] (Figure
145 S3A). SOX1 is one of the earliest transcription factors expressed in neuronal precursors of the
146 ectoderm from embryonic day 7.5[59,60]. Consequently, the *Sox1* promotor was selected as a pan-
147 neuronal driver of Recombinase cre expression to restrict homologous recombination to the central
148 nervous system[58]. Analysis of *Serf2* expression on the mRNA and protein level in various organs
149 confirmed brain-specific ablation of *Serf2* by *Sox1*-mediated *Cre* expression in our *Serf2*$^{flox/flox}$ mice (t-
150 test WT vs *Serf2*$^{br-/-}$ brain: 1m p=0.0022; 3m p=0.00527) (Figure S3B-C). This conditional KO was
151 crossed with the *APPPS1-21* amyloid (AM) model[61], which contains human transgenes for both APP
152 with the Swedish KM670/671NL mutation and PSEN1 with the L66P mutation. From this cross we
153 obtained four experimental groups: WT, *Serf2*$^{br-/-}$, AM and AM;*Serf2*$^{br-/-}$ (Figure 2A). Upon crossing
154 of the *Serf2*$^{br-/-}$ with the amyloid model, we observed the expected Mendelian ratio for all the
155 genotypes at P22, indicating that the viability is not significantly affected by brain-specific KO of
156 *Serf2* (Chi Square: p= 0.37)(Supplemental Table 3). Based on the known progression of Aβ plaque
157 pathology in the AM mice, we selected two age groups for further analysis: before (1 month) and after
158 (3 months) Aβ plaque deposition (Figure 2B). Analysis of the *Serf2* mRNA levels in both age groups
159 showed that *Serf2* expression was not altered by the plaque pathology and confirmed the KO in the
160 *Serf2*$^{br-/-}$ and AM;*Serf2*$^{br-/-}$ mice. Some residual *Serf2* expression could be detected in the brains of the
161 KO groups likely due to the presence of cells from non-neuronal lineage in the samples, e.g. cells from
162 circulatory system (Two-way ANOVA: *Serf2*$^{br-/-}$ and AM;*Serf2*$^{br-/-}$ vs WT 1m p_{bon} <0.001; 3m p_{bon}
163 <0.0001) (Figure 2C). These results were also confirmed on the protein level using western blot
164 (Figure 2D). In accordance with the effects observed in the full-body *Serf2*$^{-/-}$ mice, the *Serf2*$^{br-/-}$ and the
165 AM;*Serf2*$^{br-/-}$ mice did show a reduction in brain weight compared to WT in both age groups (Two-
166 way ANOVA: *Serf2*$^{br-/-}$ vs WT both ages p_{bon}<0.01; AM;*Serf2*$^{br-/-}$ vs WT 1m p_{bon}<0.0001; 3m
167 p_{bon}<0.001) (Figure 2E). While the weight reduction seems to be slightly more pronounced in
168 AM;*Serf2*$^{br-/-}$ compared to *Serf2*$^{br-/-}$, AM by itself displayed no significant change in brain weight. This
169 would indicate that *Serf2* ablation, *Cre* expression or a combination of both is most likely responsible
170 for the difference in weight. However, AM mice may be more susceptible to this effect (Figure 2E).
171 To distinguish between the effects of *Cre* expression and *Serf2* KO, we also examined the *Sox1-Cre*

172 mice[58] without floxed *Serf2* (*Cre*^{br}) and crossed them with *APPPS1-21*[61] without floxed *Serf2*. The
173 *Cre*^{br} mice also showed a reduction in brain weight compared to the WT controls, which was more
174 pronounced at 1 month (One-Way ANOVA: *Cre*^{br} vs WT 1m $p_{bon}<0.05$) (Figure S3D), but was overall
175 less extensive than the reduction in *Serf2*$^{br-/-}$ animals (One-way ANOVA: *Cre*^{br} vs WT $p_{bon}<0.01$;
176 AM;*Cre*^{br} vs WT $p_{bon}<0.001$) (Figure 2F). Together, this suggests that *Cre* expression is partially
177 responsible for the observed decrease in brain size, particularly at 1 month of age. Due to ongoing
178 brain maturation between 1 and 3 months, all groups show a significant increase in brain weight (Two-
179 way Anova: Age $p<0.0001$), but this maturation appeared unaffected by *Serf2* KO. Moreover, the
180 difference in brain weight between WT and *Serf2*$^{br-/-}$remained constant beyond the age of 3 months up
181 to 11 months of age (Figure S3E). This suggests that the reduction in brain size takes place during
182 specific stages of brain development, but that the continued development is not necessarily altered by
183 these changes. Additional histological examination of the brains with hematoxylin-eosin staining
184 revealed no difference in the cell density (Figure 2G), nor did we find any evidence of degeneration,
185 apoptosis or necrosis. Apart from the overall reduction in weight, the brains showed no structural
186 abnormalities and the tissue appeared healthy.

187 Next, we examined whether the brain KO of *Serf2* affected any of the key components in the Aβ
188 aggregation process, like altering the levels of APP or the production of its cleavage products, $A\beta_{40}$
189 and $A\beta_{42}$. We determined the expression of human *APP* in WT, *Serf2*$^{br-/-}$, AM and AM;*Serf2*$^{br-/-}$ mice
190 at both 1 and 3 months of age and observed no difference due to brain specific *Serf2* KO (t-test: 1m
191 p=0.9170; 3m p=0.9963) (Figure 2H, data from WT, *Serf2*$^{br-/-}$ not included due to lack of APP
192 construct and absence of signal). Western blot analysis confirmed there was also no difference in APP
193 protein levels between AM and AM;*Serf2*$^{br-/-}$ (t-test: 1m p= 0.1157;3m p=0.5908) (Figure 2I and J).
194 Further analysis of $A\beta_{40}$ (Two-way ANOVA: Genotype p=0.8841; Age $p<0.0001$;Interaction
195 p=0.8841) (Figure 2K) and $A\beta_{42}$ (Two-way ANOVA: Genotype p=0.7006; Age $p<0.0001$;Interaction
196 p=0.6522) (Figure 2L) showed an increase in concentration between 1 and 3 months, but this was the
197 same in both AM and AM;*Serf2*$^{br-/-}$. Given the variability in the Aβ concentrations at 3 months and the
198 fact that the ratio between these two peptides is known to affect the aggregation process, we also

199 investigated the correlation between these two Aβ levels (Figure 2M). This analysis showed that mice
200 with a high $A\beta_{42}$ concentration display a similarly high $A\beta_{40}$ level, maintaining a comparable $A\beta_{40}$
201 /$A\beta_{42}$ ratio for both AM and AM;*Serf2*$^{br-/-}$ animals. Taken together, this data suggests that SERF2 does
202 not affect the levels of APP and its cleavage products, $A\beta_{40}$ and $A\beta_{42}$. These findings are in accordance
203 with the previously observed effects of MOAG-4 on the aggregation of polyglutamine, Aβ and αSyn
204 in *C. elegans*, which modifies the aggregation process without changing the overall levels of these
205 aggregation-prone proteins[50]. While the difference in brain size is definitely a factor to be considered
206 during further analysis, given the fact that we observed no obvious changes in the overall health,
207 continued development and APP processing pathway of these brain-specific *Serf2* KO mice, we
208 proceeded with our analysis of Aβ pathology in these animals.

209 *Serf2 KO alters the amount of Aβ deposition in the brain*

210 We next investigated whether SERF2 affects the Aβ aggregation by performing immunohistological
211 analysis of the Aβ plaque pathology. Initially, we performed a general Aβ staining using the 6E10
212 antibody. In accordance with the known progression of Aβ plaque pathology in the amyloid model, we
213 only found extracellular deposits in the 3-month-old AM and AM;*Serf2*$^{br-/-}$ mice. As expected, most
214 plaques were found in the cortex, with some pathology beginning to spread to the hippocampus as
215 well (Figure S4). Microscopic inspection of the Aβ deposits revealed a broad range of sizes with a
216 wide variety in staining intensity and patterns, but we found no clear morphological differences
217 between the plaque population found in AM and AM;*Serf2*$^{br-/-}$ (Figure 3A, S4). Quantification of the
218 6E10-positive deposits showed a slight increase in AM;*Serf2*$^{br-/-}$ compared to AM, but this just failed
219 to reach statistical significance in the Bonferroni corrected post-hoc comparison (Two-way ANOVA:
220 all 3m non-AM vs AM groups p_{bon} <0.0001; AM vs AM;*Serf2*$^{br-/-}$ p_{bon} =0.063)(Figure 3B). Given the
221 high variability in plaque load between animals within the same experimental group, we examined
222 whether this variability reflected the previously observed variation in Aβ levels. Unsurprisingly,
223 animals with higher levels of $A\beta_{40}$ (Figure 3C) and $A\beta_{42}$ (Figure 3D) displayed a higher overall plaque
224 load in both groups. More interestingly, our linear regression analysis revealed that AM;*Serf2*$^{br-/-}$ mice
225 tend to have a significantly higher plaque load than AM mice with similar Aβ levels (Supplemental

226 Table 4). A second general Aβ stain with the W0-2 antibody displayed a similar slight increase in the
227 amount of plaque deposits in AM;*Serf2*$^{br-/-}$ at 3 months (Two-way ANOVA: all 3m non-AM vs AM
228 groups p_{bon} <0.0001; AM vs AM;*Serf2*$^{br-/-}$ p_{bon} =0.101) (Figure 3E). In fact, a comparison between the
229 6E10 and the W0-2 staining in individual animals confirmed the similarity between both staining
230 patterns at 3 months (Figure 3F, S5, Supplemental Table S3). Interestingly, our detection algorithm
231 also picked up a small increase in the counts of W0-2-positive foci in the 1 month AM;*Serf2*$^{br-/-}$ group
232 (Two-way ANOVA: 1m WT vs AM;*Serf2*$^{br-/-}$ p_{bon} =0.088 ; *Serf2*$^{br-/-}$ vs AM;*Serf2*$^{br-/-}$ p_{bon} =0.081; AM
233 vs AM;*Serf2*$^{br-/-}$ p_{bon} =0.127; all other comparisons p_{bon} >0.999) (Figure 3E). Closer examination of the
234 microscopic images revealed the identified spots were not extracellular plaques, but were the result of
235 increased levels of W0-2-positive intracellular staining in AM;*Serf2*$^{br-/-}$ (Figure S7A). Some low level
236 intracellular W0-2 and 6E10 staining could also be observed in the other 1-month-old AM mice, but
237 not to the extent that it was picked up by the detection algorithm (Figure S7A-B). This would suggest
238 that the deletion of *Serf2* already alters the intracellular accumulation of Aβ prior to plaque deposition.
239 Overall, these results indicate that the presence of SERF2 is indeed able to modify Aβ aggregation in
240 the mouse brain.

241 *SERF2 deficiency changes the proportion of ThS-positive cores, but not the immunoreactivity*
242 *of deposits*

243 To investigate if SERF2 also affected the amount of Thioflavin-positive fibrils, we performed
244 Thioflavin-S (ThS) staining. Here, we again observed a large variety in the amount of ThS-positive
245 plaques between individual animals, but we found no mean difference between AM and AM;*Serf2*$^{br-/-}$
246 mice (Two-way ANOVA: all 3m non-AM vs AM groups p_{bon} <0.001) (Figure 3G). However, further
247 analysis of the correlation between Aβ levels and the amount of ThS-positive plaques revealed that
248 AM mice showed a positive correlation similar to the general Aβ stainings, while AM;*Serf2*$^{br-/-}$ mice
249 displayed an inverse relationship (Figure 3H, 3I, Supplemental Table S3). Consequently, a general
250 increase in Aβ deposits does not equate to an increase in amyloid deposition in AM;*Serf2*$^{br-/-}$ mice, as
251 it does in AM mice (Figure 3J), further demonstrating a change in the amyloid formation in *Serf2* KO
252 mice. In addition, we investigated microglia activation and whether we could detect a change in the

253 gliosis associated with Aβ plaques. Staining for Iba1 showed a slight increase in the AM;*Serf2*$^{br-/-}$ mice
254 compared to AM (Two-way ANOVA: 3m non-AM groups vs AM p_{bon} <0.01; non-AM groups vs
255 AM;*Serf2*$^{br-/-}$ p_{bon} <0.0001; AM vs AM;*Serf2*$^{br-/-}$ p_{bon} <0.01) (Figure 3K), similar to what was observed
256 with the 6E10 staining (Figure 3B). We therefore examined if this increase was simply related to the
257 increase in Aβ deposits or if AM;*Serf2*$^{br-/-}$ also displayed a different immune response to plaques
258 compared to AM. Our analysis revealed a similar correlation between 6E10-positive deposits and
259 immune response for AM and AM;*Serf2*$^{br-/-}$ mice, suggesting the deposits evoked a similar immune
260 response in both groups(Figure S6A, Supplemental Table S3).

261 *Knockout of Serf2 changes the plaque composition and fibril structure*

262 Given the differences in the reactivity of the Aβ deposits between the various stains, we decided to
263 explore the composition within the individual plaques in greater detail. To this end, we made use of
264 two luminescent conjugated oligothiophenes (LCO), qFTAA (quadro-formylthiophene acetic acid)
265 and hFTAA (hepta-formylthiophene acetic acid). These LCOs are small hydrophobic ligands with a
266 high affinity for binding distinct types of amyloids and can easily be distinguished from each other
267 based on their spectral properties[62]. The smallest of the two molecules, qFTAA, is characterized by a
268 peak in its emission spectrum around 500 nm and has been shown to stain compact, multifilament
269 fibril structures, coinciding with ThT and Congo red staining[62–64]. Whereas hFTAA emits in the
270 yellow to red range with a peak around 600 nm and is capable of staining prefibrillar (non-
271 thioflavinophilic) and single fibril aggregates found during the lag-phase of in vitro ThT kinetics
272 (Figure 4A). The prefibrillar aggregates were shown to be stained with 6E10, but not Congo red, ThT
273 or qFTAA[62–64]. Previous studies have shown that by combining LCO dyes it is possible to uncover
274 structural differences in plaque composition[49,64,65]. We performed confocal spectral imaging of
275 randomly selected plaques in 3-month-old AM and AM;*Serf2*$^{br-/-}$ mice after double staining with both
276 LCOs and determined the ratio of the light intensity emitted at 502nm and 588nm as a measure for the
277 relative amounts of fibrillar and prefibrillar aggregates within the individual plaques. An exploratory
278 frequency analysis of the intensity ratios found in both groups revealed a three-peak pattern in which
279 the middle peak was similarly represented in both groups (Figure 4B, green area). The first peak,

280 however, was predominantly found in AM mice (Figure 4B, red area), while the third peak was mainly
281 detected in AM;*Serf2*$^{br-/-}$ (Figure 4B, blue area). This shift in ratio was also reflected by a significant
282 difference in the average intensity ratio between AM and AM;*Serf2*$^{br-/-}$ (t-test $p<0.001$) (Figure 4C).
283 This increase in intensity ratio could not be observed in AM;*Cre*^{br} mice (t-test $p=0.6047$) (Figure 4C,
284 insert), indicating that this effect is caused by the *Serf2* KO and unrelated to *Cre* expression. Given the
285 fact that AM;*Cre*^{br} animals also show a decrease in brain weight without a change in LCO staining,
286 this would also suggest that the weight reduction is not a factor in these results. Although, as the
287 change in brain weight is more pronounced in *Serf2*$^{br-/-}$ animals, we cannot completely exclude this as
288 a potential contributing factor. Next, we examined if the change in the intensity ratio correlated with
289 the slight increase in 6E10-positive deposits we observed in AM;*Serf2*$^{br-/-}$ (Figure 4D). However, the
290 higher average intensity ratio in AM;*Serf2*$^{br-/-}$ mice was not related to this general plaque load nor the
291 number of W0-2- or ThS-positive deposits (Figure S6B, S6C). Further microscopic analysis of low
292 intensity ratio deposits from AM mice showed a plaque with a qFTAA- and hFTAA-positive core and
293 a border that was only stained by hFTAA (Figure 4E, left panels). The high intensity ratio deposits
294 from AM;*Serf2*$^{br-/-}$ mice, on the other hand, revealed a qFTAA-positive core, but virtually no hFTAA-
295 staining (Figure 4E, right panels), demonstrating the difference in plaque composition reflected by the
296 shift in LCO intensity ratios. Additionally, we visualized the global fibrillar structure of the plaques on
297 a nanometer scale using high resolution, scanning transmission electron microscopy (STEM). Overall,
298 STEM images of plaques from AM;*Serf2*$^{br-/-}$ tended to show more condensed plaques composed of
299 short, thick and densely packed bundles of fibers with little space in between (Figure 4F, right panels).
300 In the AM mice, however, the plaques we observed displayed more loosely packed fibrils (Figure 4F,
301 left panels). Taken together, these findings suggest *Serf2* KO in mice leads to a shift in the Aβ
302 aggregation process, resulting in an altered structural composition of the plaques.

303 **Discussion**

304 Previous studies identified MOAG-4 and its human orthologs SERF1A and SERF2 and their potential
305 to modify the aggregation of amyloidogenic proteins. While the exact endogenous biological function
306 of these peptides remains unclear, a recent study has provided evidence for RNA-binding properties of

307 SERF1A through a region which is highly conserved in MOAG-4 and SERF2[66]. In this study, we have
308 demonstrated in cells and mice that SERF2 provides a growth advantage during development. The
309 absence of SERF2 or MOAG-4 in itself does not appear to be lethal in cells, worms or the brain-
310 specific KO mice. Therefore, we postulate that the observed perinatal lethality in the full-body KO
311 mice is a secondary effect of the delay in growth due to insufficient maturation of certain organs at
312 birth. This appears to be supported by the fact that some pups survived the initial minutes after birth
313 and incomplete penetrance of the lethality in the earliest generations with mixed genetic background.
314 The 129SV genetic background present in these mice is known to have a gestation period that is on
315 average around one day longer than C57/Bl6J mice[67], which could result in improved lung maturation.
316 While the exact function of SERF2 remains unknown, we propose its effects on growth and
317 development are most likely caused through one of two mechanisms: either SERF2 facilitates cell
318 proliferation or it protects cells from stressors that create an unfavorable environment for cell division.
319 In the latter case, we would expect to see an upregulation of stress pathways or cell cycle arrest
320 proteins inhibiting the cell cycle in *Serf2*$^{-/-}$ cells. Interestingly, the RNA seq analysis of the MEFs from
321 our *Serf2*$^{-/-}$ mice showed the exact opposite. Cell cycle driving factors were upregulated (e.g. cyclins).
322 Whereas, stress signaling and cell cycle inhibiting factors were downregulated (e.g. GADD45 and
323 GAS1). Thus, the *Serf2*$^{-/-}$ cells actually displayed an increased stimulation of cell proliferation
324 mechanisms, which would fit with cells from earlier stages of embryonic development. This would
325 suggest the first mechanism, with a beneficial role for SERF2 in the efficient execution of cell
326 proliferation, as the most likely explanation for the observed growth effect. Furthermore, an increase
327 in cellular stress would most likely result in decreased health and fitness. However, *moag-4* deletion
328 worms showed no changes in life span compared to controls[50] and, apart from the initial growth
329 deficit, the tissues of *Serf2* KO mice appeared healthy and continued to develop similarly to controls
330 after birth. A recent study showed SERF1a might play a role as an RNA-organizing protein, which
331 localizes to membraneless nuclear organelles, like the nucleolus, under normal physiological
332 conditions. However, under stress conditions, it was shown to migrate to the cytoplasm, where it could
333 drive amyloid toxicity.[66] While there was no compensatory upregulation of SERF1 in response to
334 SERF2 KO and a similar mode of action remains to be demonstrated for SERF2, the structural

335 homology of the two proteins would suggest a comparable function. Given the importance of
336 nucleolar disassembly and reassembly in cell cycle control[68,69], an RNA-organizing function could
337 explain how SERF2 facilitates cell proliferation. This developmental role would have limited
338 biological importance in non-proliferating, differentiated cells, like the brain's neurons or the cells of
339 adult *C. elegans*, and might also explain why we saw no adverse effects in the *moag-4* deletion worms
340 or our adult *Serf2*$^{br-/-}$ mice. While a reduced biological importance of SERF2 later in life could prove
341 interesting with regard to the treatment of age-related neurodegenerative disorders, further exploration
342 of SERF2's endogenous function and how it evolves with ageing will be needed to fully assess this
343 mechanism's therapeutic potential.

344 In this study, we confirmed the ability of SERF2 to modify the Aβ pathology *in vivo* in a mouse model
345 without changing the overall Aβ levels. We showed that mice lacking SERF2 were more prone to
346 form Aβ deposits and that the composition of these deposits was structurally different. In AM mice
347 higher levels of Aβ result in an increase in the numbers of Aβ deposits as well as an increase in the
348 number of ThS-positive deposits. AM;*Serf2*$^{br-/-}$ mice, on the other hand, also show an increase in Aβ
349 deposits, but this did not lead to higher numbers of ThS-positive plaques, indicating an altered
350 dynamic for amyloid formation. These findings were further corroborated by the LCO spectra, which
351 revealed that the plaques in AM;*Serf2*$^{br-/-}$ mice have a different conformation of pre-fibrillar and
352 fibrillar Aβ compared to AM mice. Finally, STEM imaging also confirmed a globally altered structure
353 of the amyloid fibrils in the plaques. Amyloid fibrils are formed through a nucleated self-assembly
354 process characterized by a structural conformation to a β-strand secondary structure and the formation
355 of a critical nucleus during the initial oligomerization. The nuclei act as seeds from which the fibrils
356 grow and have been shown to propagate a specific fibril structure[41,70]. In vitro kinetic assays with
357 SERF and MOAG-4 have shown that these peptides accelerate aggregation by acting on the nucleation
358 phase[51–53,71]. Recently, we have shown that SERF2 is equally capable to drive amyloid formation of α-
359 Syn and Aβ *in vitro* through a conserved, positively charged region[72]. Meanwhile, another study has
360 demonstrated that binding of the intrinsically disordered protein yeast SERF to α-Syn and Aβ results
361 in fuzzy complexes with heterogeneous structural conformations that are more extended in

362 nature[71]. For α-Syn, the disruption of intra-molecular electrostatic interactions by the positively
363 charged region of MOAG-4/SERF1 was found to expose an amyloid nucleation site[52,53]. Intra-
364 molecular electrostatic interactions have also been proven to play a part in the dynamics and structure
365 of the Aβ monomer folding, which is at the basis of nucleus formation. In fact, several of the familial
366 AD mutations located within the Aβ sequence and reported not to affect the Aβ levels, appear to exert
367 their effects by modifying the intra-molecular interactions and monomer folding[30,73–75]. These single
368 amino acid modifications in the Aβ sequences also result in alterations in the aggregation kinetics,
369 fibril structure and aggregate toxicity in *in vitro* studies[29,76,77]. Moreover, patients and mouse models
370 with these mutations show differences in the localization of Aβ accumulation and deposition, as well
371 as differences in its structure, giving rise to specific disease phenotypes. For instance, while some
372 mutations result in increased Aβ deposition and altered plaque structure, other mutations at the same
373 location result in cerebral amyloid angiopathy with Aβ deposition in blood vessels rather than the
374 brain parenchyma or even an absence of extracellular Aβ deposition[15,28,82,83,30,65,73,77–81]. In addition,
375 some of these mutants showed increased intracellular Aβ accumulation which differed in structure-
376 specific staining characteristics[83–85]. The differences in Aβ pathology between these mutants seem to
377 display some similarities to the changes we observed between our AM and AM;*Serf2*$^{br-/-}$ mice.
378 Together with the *in vitro* findings about the mechanism of SERF2 interaction with aggregation-prone
379 proteins, this would appear to suggest modulation of intra-molecular interactions and altered
380 nucleation as the mechanism for SERF2's effect on aggregation and amyloid formation in our mice.
381 Interestingly, there is increasing evidence that qualitative, structural properties may be more related to
382 toxicity than the quantity of aggregates and deposits. Recent studies have provided increasing
383 evidence that structural polymorphs are associated with differences in toxicity and different clinical
384 phenotypes in sporadic and familial cases[40,41,47–49]. One recent study demonstrated that these structural
385 conformations of distinct disease phenotypes could also be detected by differences in the LCO spectra
386 of the plaques and that these spectral properties could, at least partially, be transmitted to a mouse
387 model through seeding[49]. While the effect of familial mutations on the formation of distinct
388 polymorphs has already been explored by others[49,84–86], our study provides the first evidence of a
389 single endogenous factor, separate from Aβ and its production pathway, contributing to a structural

390 shift of amyloid pathology in a mammalian system. Further research will be needed to elucidate the
391 exact structural changes at an atomic level and if they affect toxicity and disease progression similarly
392 to what was previously observed in *C. elegans*. This will provide new insights into the structural
393 properties and diversity of disease-protein aggregation, contributing to a better understanding of the
394 variability in disease manifestation and open up previously unexplored avenues for therapeutic
395 research.

396 **Materials and methods**

397 *Animals*

398 *Serf2* knockout mice were generated by introducing *loxP* sites in the *Serf2* gene on either side of exon
399 2, in accordance with the 'knockout-first' allele design as described by Skarnes and colleagues[87]. Full-
400 body *Serf2* knockout mice were obtained by crossing these floxed mice with 129SV mice expressing
401 *Cre* under the *Hprt* promoter, resulting in the removal of exon 2 by *Cre*-mediated recombination
402 (Figure S1A). Subsequently, these mice with mixed background were backcrossed at least 6 times to a
403 C57Bl/6J background. Homozygous and heterozygous full-body *Serf2* knockout mice were examined
404 with their wild-type littermates at embryonic day 13.5, 15.5 and 17.5, as well as at the day of birth
405 (P0) and day of weaning (P22).

406 Heterozygous *Sox1-Cre* mice[58] were backcrossed at least 8 times to a C57BL/6J background. These
407 animals were also used as controls to differentiate between effects of *Cre* expression and Serf2
408 knockout. To generate the conditional *Serf2* knockout mice, the floxed mice were first crossed with
409 Tg(ACTFLPe)9205Dym (Jackson#003800) mice to flip the FRT site and remove the lacZ and neo
410 cassette. Subsequently, these mice were backcrossed at least 6 times to a C57BL/6J background.
411 Finally, the resulting homozygous *Serf2*$^{flox/flox}$ mice were crossed with the backcrossed *Sox1-Cre* mice,
412 to obtain the brain-specific *Serf2* knockout mice (Figure S3A). The conditional *Serf2* knockout mice
413 were crossed with the APPPS1-21 transgenic mice (APP (KM670/671NL)/PS1(L166P), Rottenburg,
414 Germany) [61]. A final cross between heterozygous APPPS1-21; *Serf2*$^{flox/flox}$ and heterozygous *Sox1-Cre*;
415 *Serf2*$^{flox/flox}$ resulted in four experimental groups of *Serf2*$^{flox/flox}$ mice: *Sox1-Cre*$^{-/-}$; APPPS1-21$^{-/-}$ (WT),

416 *Sox1-Cre*$^{+/-}$; APPPS1-21$^{-/-}$ (*Serf2*$^{br-/-}$), *Sox1-Cre*$^{-/-}$; APPPS1-21$^{+/-}$ (AM) and *Sox1-Cre*$^{+/-}$; APPPS1-21$^{+/-}$
417 (AM;*Serf2*$^{br-/-)}$ (Figure 2A). All experimental groups were tested at 1 month or 3 months of age.

418 All experiments were approved by the Institutional Animal Care and Use Committee of the University
419 of Groningen (Groningen, The Netherlands). All mice were maintained on a C57BL/6J background
420 and housed in a 12:12 hour light/dark cycle and the animals had ad libitum access to food and water.
421 Genotyping of embryos was performed using tail and yolk sac biopsies. Otherwise, ear biopsies were
422 used. DNA was purified using prepGEM® Tissue kit according to a protocol adapted from the
423 manufacturer (ZYGEPTI0500, ZyGEM, VWR International BV) and subjected to PCR using the
424 primers listed in Supplemental Table S5. Mice were terminated through CO_2-inhalation and cervical
425 dislocation for subsequent tissue collection.

426 *Embryo processing and histochemistry*

427 Embryos were fixed in 4 % formalin (Kinipath) for a minimum of 24 hours at room temperature. For
428 the pathological analysis, the embryos and their placenta were bisected longitudinally and embedded
429 in paraffin. Using the microm HM 340E (Thermo Scientific) 4 µm sections were cut for the
430 hematoxylin-eosin (HE) staining. The HE sections were incubated at 60 °C for 15 minutes. Next,
431 sections were deparaffinized and rehydrated in xylene (2x), 100 % alcohol (2x), 96 % alcohol, 70 %
432 alcohol and water. HE staining was performed by incubation with hematoxylin for 4 minutes, water
433 for 10 minutes, eosin for 1 minute and water for 10 seconds. After staining all sections were
434 dehydrated in 70 % alcohol, 96 % alcohol, 100 % alcohol (2x) and xylene (2x).

435 *Brain processing*

436 Upon termination, all brains were collected and weighed. From each experimental group three whole
437 brains were collected for HE staining according to the protocol described under embryo processing.
438 HE-stained sections were scanned with the TissueFAXs microscope using 20X objective. Images were
439 processed for nuclei counts using Histoquest software. The remaining brains were divided sagittally.
440 The left hemibrain was prepared for histochemistry and fixed in 4 % paraformaldehyde (PFA) for 48

441 hours at room temperature on a shaker. Next, the brains were placed in 30 % sucrose for
442 approximately 12 hours. Afterwards, the excess sucrose was removed and hemibrains were frozen in
443 molds with Tissue Tek O.C.T. compound (Sakura, The Netherlands) on dry ice. The right hemibrain
444 was snap frozen in liquid nitrogen and stored at -80°C. This tissue was homogenized using a liquid
445 nitrogen cooled mortar for subsequent protein and RNA analysis.

446 *Quantitative RT-PCR*

447 Total RNA was extracted from snap frozen tissue using TRIzol Reagent (Life Technologies) according
448 to the manufacturers' description. Total RNA quality and concentration were assessed using a
449 NanoDrop 2000 spectrophotometer (Thermo Scientific/Isogen Life Science). cDNA was made from
450 1,5 µg total RNA with a RevertAid H Minus First Strand cDNA Synthesis kit (Thermo Scientific)
451 using random hexamer primers. Quantitative real-time PCR was performed using a Roche LightCycler
452 480 Instrument II (Roche Diagnostics) with SYBR green dye (Bio-Rad Laboratories) to detect DNA
453 amplification. Relative transcript levels were quantitated using a standard curve of pooled cDNA
454 solutions. Expression levels were normalized to β-Actin or 18S mRNA levels. The primers for
455 Quantitative PCR used are listed in Supplemental Table S5.

456 *Western Blot analysis*

457 For SERF2 analysis tissues were homogenized in RIPA buffer (50 mM Tris pH 8, 150 mM NaCl,
458 5mM EDTA, 0.5 % SDS, 0.5 % SDO, 1 % NP-40) with protease inhibitor cocktail (Roche) and
459 incubated on ice for 1 hour, spun down at 13,300 rpm for 30 minutes at 4 °C and the supernatant was
460 collected. Protein measurements were performed using a BCA kit (Pierce) and 150 µg was loaded on a
461 10-20 % tris/tricine SDS-page gels (Bio-Rad Laboratories) and blotted onto 0,2 µm nitrocellulose
462 membrane (Bio-Rad Laboratories). Membranes were incubated overnight with SERF2 (1/1500,
463 Protein tech) or Actin (1/10 000, MP biomedicals) antibody. Next, the membranes were incubated
464 with anti-mouse or –rabbit secondary antibodies tagged with horseradish peroxidase (1/10 000, Bio-
465 Rad Laboratories) for one hour at room temperature and visualized by chemiluminescence (Amersham
466 ECL prime western blotting detection reagent, VWR).

467 *RNA sequencing*

468 RNA sequencing analysis was performed on three MEF cell lines from *Serf2*$^{-/-}$ mice and four *Serf2*$^{+/+}$
469 littermate controls. Total RNA was isolated from MEFs using the Qiagen RNeasy isolation kit.
470 Integrity of the RNA based on RIN scores as determined by a Bioanalyzer (Agilent). RNA-sequencing
471 libraries were prepared using TruSeq Stranded Total RNA with Ribo-Zero Human/Mouse/Rat (RS-
472 122-2201; Illumina, CA, USA) according to manufacturers' protocol. Pooled libraries were sequenced
473 on an Illumina HiSeq 2500 (single-end 50 bp). Reads were aligned to the mouse reference genome
474 (mm10) using a splicing-aware aligner (StarAligner). Aligned reads were normalized to fragments per
475 million (FPM), excluding low abundance genes (mean FPM>1 in at least two samples). The raw count
476 data were pre-processed using the programming language R (3.4.0) [R Core Team, 2016, available
477 online at: www.r-project.org], the program RStudio (1.0.143) [RStudio Team, 2016, available online
478 at: http://www.rstudio.com/] and the EdgeR package (3.18.0)(Robinson et al., 2010). Genes that
479 displayed FPM value >1 in at least two libraries were retained, resulting in a list of 12,808 genes for
480 differential analysis. Differentially expressed (DE) genes between the *Serf2*$^{+/+}$ and *Serf2*$^{-/-}$ MEFs were
481 identified using the EdgeR general linear model approach. After statistical correction for multiple
482 comparisons with the "false discovery rate" (FDR) method (FDR<0.05), a list of 738 DE genes was
483 obtained. DAVID (6.8) was used to perform functional annotation analyses on this gene list and to
484 identify significantly enriched gene ontology (GO) terms [using GOTERM_BP_DIRECT] [88,89]. The
485 connectivity between the enriched GO terms was further examined by determining the amount of
486 associated genes found (AGF) that were shared between two GO terms. The most significant
487 relationships (where a GO term shared > 25 % of the AGF) were mapped in a network using the
488 igraph package (1.0.1). The color of the edges reflects the major node, with the largest amount of
489 AGF, in each relationship. The weight of the edges was determined by the percentage of AGF shared
490 by the minor node in the relationship. We defined three clusters of GO-terms, where multiple minor
491 GO-terms connected to one or two central, major GO-terms . Minor GO-terms were always clustered
492 with the major GO-term with whom they shared the strongest connection. One GO term, positive

493 regulation of the apoptotic process, showed no direct relation with one of the major GO-terms and was
494 therefore assigned to the only cluster it had a significant connection with (Figure S2A-B).

495 *ELISA*

496 The ELISA experiments were performed as described previously[90]. Briefly, the frozen brain samples
497 were homogenized in tissue protein extraction reagent (Pierce) supplemented with complete protease
498 inhibitors (Roche), and centrifuged for 1 hour at 430 000 g at 4 °C. The supernatant was used for
499 ELISA. The $A\beta_{40}$ and $A\beta_{42}$ levels were determined by standard sandwich ELISAs using end-specific
500 antibodies (Janssen Pharmaceutical), and the monoclonal JRFcAβ_{40}/28 and JRFcAβ_{42}/26 antibodies as
501 the capture antibodies. Horseradish peroxidase conjugated JRFAβN/25 or JRFAβN/25 were used as
502 the detection antibodies for, respectively, human Aβ or murine Aβ. Samples were loaded in triplicate
503 for each ELISA. The ELISAs were performed in triplicate for the 3-month-old animals and in
504 duplicate for the 1-month-old animals.

505 *Brain immunohistochemistry*

506 To evaluate Aβ-related pathology, the brain was cut in 30 µm thick serial cryostat sections. A series of
507 sections was selected with 300 µm spacing between individual sections and blind-coded, 6 per mouse
508 for the 6E10 and Iba1 antibody and 3 per mouse for W02. Sections were fixed in 4 % PFA for 10
509 minutes, followed by 20 minutes antigen retrieval in citrate acid (pH 6.0) and 30 minutes in 1 % H_2O_2
510 in methanol. Next the sections were incubated for 1 hour in 10 % goat or donkey serum, depending on
511 the secondary antibody, and immunolabeled overnight with antibodies against Aβ, and Iba1
512 (Supplemental Table S6). The sections were washed and incubated with the complementary
513 biotinylated secondary antibody (1/500, Jackson Immunoresearch) for 2 hours. Next, sections were
514 incubated in ABC complex and reacted with diamino-benzidine (0.5 mg/ml H2O with 0.01 % H_2O_2).
515 The stained sections were scanned with the TissueFAXs microscope using 20X objective. All images
516 were analyzed using the Fiji platform of the ImageJ software[91]. For unbiased feature detection we used
517 a single automated script with optimized threshold method and parameters for the complete image set
518 of each staining method.

519 *Thioflavin-S Staining*

520 A 300 µm-spaced series of three blind-coded 30 µm sections were fixed in 4 % PFA for 10 min,
521 followed by incubation in 0.25 % potassium permanganate solution for 15 min, and a bleaching step
522 with 1 % potassium metabisulfite/1 % oxalic acid for 5 min, incubation with 0.02 % Thioflavin-S
523 solution in 50 % ethanol (Sigma T1892) for 8 minutes, rinsed with water between every step. Finally
524 the sections were incubated with 1 µl/ml DAPI. The sections were analyzed as described for the
525 immunostained sections. Stained sections were scanned with the TissueFAXs microscope using 20X
526 objective. Images were processed using Tissuequest software, selecting the cortex for automated
527 analyses.

528 *Luminescent conjugated oligothiophene (LCO) staining*

529 For the Luminescent conjugated oligothiophenes (LCO) stainings, two different LCO variants,
530 qFTAA (quadro-formyl thiophene acetic acid) and hFTAA (hepta-formyl thiophene acetic acid) were
531 used. Blind-coded 30 µm sections were double-stained with qFTAA and hFTAA (2.4 µM qFTAA and
532 0.77 µM hFTAA in PBS) similar to a previous description (Nyström et al., 2013). Sections were
533 incubated for 30 minutes in the dark at room temperature. Stainings were analyzed on the Zeiss LSM
534 510 META confocal microscope equipped with an argon 458 nm laser (Carl Zeiss MicroImaging
535 GmbH, Jena, Germany). An 40x objective (oil-immersion, 1.3 NA, Zeiss) was used for spectral
536 imaging of the Aβ deposits. Stacked images were acquired every 10 nm in the emission spectrum
537 between 470 to 695 nm. Locations were selected randomly from the plaque containing regions of the
538 temporal, frontal and occipital cortex. Images were analyzed using Fiji (Schindelin et al., 2012, 2015)
539 and standard computer algorithms to provide a reproducible, unbiased detection of the plaques. First,
540 the stacks of images across the different wavelengths of the spectrum were combined into one image
541 using the Z-projection sum. A threshold was applied to the resulting image using the "Triangle dark"
542 algorithm to automatically identify and delineate the plaques. Partial plaques on the edge of the images
543 and particles smaller than 25 μm^2 were excluded. These settings allowed us to detect over 98 % of all
544 the plaques in the images. The missed plaques were either too small or too low in intensity to be

545 accurately distinguished from the background. Incorrectly identified particles due to (lipofuscin) auto
546 fluorescence were readily identified based on their wavelength intensity profile and visual inspection
547 and were also excluded from the analysis. We identified between 17-25 plaques in each animal for the
548 spectral intensity measurements. The ratio of the intensity of emitted light at the blue-shifted portion
549 (502 nm) and red-shifted peak (588 nm) was used as a parameter for spectral distinction of different
550 Aβ deposits. These peaks of the spectra were selected to maximize the spectral distinction.

551 *Large-scale STEM (nanotomy)*

552 The scanning transmission electron microscopy (STEM) experiments were adapted from previously
553 described experiments (Kuipers et al., 2015). Briefly, paraffin embedded cortical sections of AM and
554 AM;*Serf2*$^{br-/-}$ were deparaffinized and postfixed with 1 % osmium tetroxide/1.5 % potassium
555 ferrocyanide in 0.1 M sodium cacodylate, dehydrated through ethanol and embedded in EPON (Serva)
556 using a tissue processor (Leica EM TP 709202). Ultrathin sections (80 nm) were cut using the Leica
557 uc7 ultramicrotome and collected on formvar coated cupper grids (electron microscopy sciences,
558 Hatfield, Pennsylvania). A large area scan using Scanning Transmission Detection was made using a
559 Zeiss supra55 SEM with Atlas. STEM detection with a four quadrant STEM detector was used in
560 inverted darkfield mode, at 28 kV with 30 µm aperture at 3.5 mm working distance. All images were
561 recorded at the same scan speed (cycle time 1.5 minute at 3072×2304 pixels). Contrast and brightness
562 were set based on a live histogram. High resolution large-scale STEM images at 2.5 nm pixel size
563 were generated with the external scan generator ATLAS (Fibics, Canada), individual tiles were
564 stitched in VE viewer (Fibics, Canada), exported as a html-file and uploaded to the website
565 www.nanotomy.org.

566 *Cell culture*

567 Mouse embryonic fibroblasts (MEFs) were isolated from *Serf2*$^{+/+}$, *Serf2*$^{+/-}$ and *Serf2*$^{-/-}$ E13.5 embryos.
568 MEFs were cultured in T75 culture flasks (Greiner Bio-One, 658175), high–glucose Dulbecco's
569 modified Eagle's medium (Gibco), supplemented with 10% fetal bovine serum (Sigma 12133C), 1 %

570 penicillin/streptomycin (Gibco), non-essential amino acids (Gibco) and β-mercaptoethanol at 37°C, 5
571 % CO_2 and 3 % O_2. E13.5 embryos. Wild-type HEK293T and two independent clonal lines of
572 CRISPR *Serf2* KO mutant HEK 293T cells were cultured in Dulbecco's Modified Eagle Medium
573 (DMEM, Gibco; high glucose, pyruvate, 41966052), supplemented with 10 % Bovine Cow Serum
574 (BCS; Sigma 12133C) and 1 % Penicillin/Streptomycin (Gibco; 10 000 U/mL, 15140122), at 37 °C, 5
575 % CO_2. For passaging of cells, 0.05 % Trypsin-EDTA (1X), Phenol Red (Invitrogen; 25300-054) was
576 used. Regular mycoplasma tests were performed.

577 *Cell proliferation assay*

578 For the proliferation assay, 200 000 cells of each cell line were plated in triplicate in a standard 12-
579 well plate and incubated inside the IncuCyte Zoom Live-Cell Imaging System (Essen Bioscience) for
580 over 100 hours. The Incucyte system captured phase contrast images with a 10X magnification every 2
581 hours at nine distinct locations in each well to determine average confluency as a measure of cell
582 growth. The quantification was performed using the IncuCyte Zoom software. The growth experiment
583 was repeated three times. The confluency data of each experiment was normalized by min-max scaling
584 and the average of the three experiments was plotted (solid line) with the standard deviation (dashed
585 lines).

586 *Statistical analysis*

587 Unless specified otherwise, statistical analysis was performed using Graphpad v7.02. The exact
588 statistical test used for each comparison is specified in the main body of the text, together with the
589 resulting p-values. In the case of multiple comparisons, a post-hoc analysis with Bonferroni correction
590 was performed. Any corrected p-values derived from this post-hoc analysis are indicated in the text as
591 p_{bon}.

592 **Data availability**

593 RNA-seq data have been deposited in the ArrayExpress database at EMBL-EBI
594 (www.ebi.ac.uk/arrayexpress) under accession number E-MTAB-10083.
595 (http://www.ebi.ac.uk/arrayexpress/experiments/E-MTAB-10083)

596 Nanotomy datasets are open access available via the repository website
597 www.nanotomy.org/PW/temp02/EllenNollen.

598 ***[to be deleted] Upon acceptance of this work the data will be open access available, without***
599 ***password. Pending acceptance, the website can only be accessed by collaborators and reviewers***

600

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609 **Author Contributions**

610 E.S., O.S., L.J. and E.A.A. conceptualized the project. E.S., L.J., O.S. and W.H. performed initial
611 sample collection, sample processing and validation experiments verifying the Serf2 KO models and
612 controls. M.K., L.H., S.A.Y. and A.d.B. provided the embryonic morphological and histological
613 assessment. N.B. and M.J. provided the LCO staining and confocal imaging. A.H.G.W. performed the
614 large-scale STEM imaging. B.B. and F.F. provided assistance in the isolation and RNA seq analysis of
615 the MEFs. A.T. performed western blotting and ELISA measurements for APP and Aβ content.

616 B.v.d.S. and J.v.D. generated the initial Serf2KO animals. L.J. performed the data analysis and wrote
617 the manuscript in collaboration with E.S. and E.A.A.

618

619 **Conflict of Interests**

620 The authors declare that they have no conflict of interest.

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831

832 **Figure legends**

833 **Figure 1. Full-body *Serf2*$^{-/-}$ mice display a developmental delay and perinatal lethality. (A)**
834 Schematic overview of the crossing scheme with full-body *Serf2*$^{+/-}$ mice and the resulting genotypic
835 composition of the offspring at embryonic and postnatal stage. **(B)** Absolute animal counts for the
836 three genotypes in the offspring at various days of embryonic development(E13.5, E15.5 and E17.5),
837 at birth (P0) and at time of weaning (P22). **(C)** Real time RT-PCR analyses of *Serf1* and *Serf2* RNA
838 levels in E13.5 heads normalized to housekeeping gene beta-actin (n=4/group, mean±SD, One-way
839 ANOVA for each gene, Bonferroni corrected post-hoc comparison between genotypes ***p<0.001).
840 **(D)** Western blot of SERF2 and actin in *Serf2*$^{+/+}$, *Serf2*$^{+/-}$, *Serf2*$^{-/-}$embryos at E13.5 (black arrow
841 indicates SERF2 band). **(E)** Length measurements of *Serf2*$^{+/+}$, *Serf2*$^{+/-}$, *Serf2*$^{-/-}$ embryos at E15.5, E17.5
842 and P0 (black line = mean, Two-way ANOVA with factors age and genotype, Bonferroni corrected
843 post-hoc comparison for *Serf2*$^{+/-}$ and *Serf2*$^{-/-}$ compared to *Serf2*$^{-/-}$ *p<0.05; ***p<0.001). **(F)**
844 Hematoxylin and eosin stained lung tissue from *Serf2*$^{+/+}$ and *Serf2*$^{-/-}$ pups at P0 (top: *Serf2*$^{-/-}$, bottom:
845 *Serf2*$^{+/+}$, scale bar=500µm, rectangle =5x magnification in right picture, **(G)** Quantification of the
846 saccolar space in lung tissue from *Serf2*$^{+/+}$ and *Serf2*$^{-/-}$ pups at P0 (black line = mean, t-test **p<0.01).
847 For all mouse data panels: *Serf2*$^{+/+}$= black, *Serf2*$^{+/-}$ = dark grey, *Serf2*$^{-/-}$ = light grey.

848 **Figure 2. Crossing a conditional *Serf2* KO with an AM background does not affect the overall**
849 **levels of APP and Aβ. (A)** Schematic overview of the cross between brain-specific *Serf2* KO mice
850 and the amyloid model resulting in four experimental groups: WT, *Serf2*$^{br-/-}$, AM and AM;*Serf2*$^{br-/-}$.
851 **(B)** Timeline for the Aβ pathology in the amyloid model and the selected time points for this study.
852 **(C)** Real time RT-PCR analyses of *Serf2* RNA levels in the brain tissue of all experimental groups at 1
853 and 3 months of age normalized to housekeeping gene beta-actin (n=6/group, mean±SD, Two-way
854 ANOVA with factors age and genotype, Bonferroni corrected post-hoc for individual groups
855 compared to WT). **(D)** Western blot of SERF2 and actin in brain tissue of all experimental groups at 1
856 and 3 months of age. **(E)** Brain weight of all experimental groups at 1 and 3 months of age (black line
857 = mean, Two-way ANOVA with factors age and genotype, Bonferroni corrected post-hoc for

858 individual groups compared to WT). **(F)** Brain weight of unfloxed control groups at 3 months of age
859 (black line = mean, One-way ANOVA, Bonferroni corrected post-hoc for individual groups compared
860 to WT). **(G)** Number of cortical nuclei per mm^2 in hematoxylin and eosin stained brain tissue of all
861 experimental groups at 3 months of age. (n=3/group, mean±SD, Kruskal-Wallis p=0.5566). **(H)** Real
862 time RT-PCR analyses of APP RNA levels in the brain tissue of AM and AM;*Serf2*$^{br-/-}$ mice at 1 and 3
863 months of age normalized to housekeeping gene beta-actin (n=6/group, mean±SD, t-test between
864 genotypes at both ages not significant). **(I)** Western blot of APP and beta-actin in brain tissue of AM
865 and AM;*Serf2*$^{br-/-}$ mice at 3 months of age versus 1-month-old non-AM controls. **(J)** Quantification of
866 total APP protein levels in brains of AM and AM;*Serf2*$^{br-/-}$ mice at 1 and 3 months of age normalized
867 to beta-actin levels. (black line = mean, t-test between genotypes at both ages not significant). **(K)**
868 $A\beta_{40}$ and **(L)** $A\beta_{42}$ levels in brain lysate from AM and AM;*Serf2*$^{br-/-}$ mice at 1 and 3 months of age as
869 determined by ELISA normalized to total protein content. (black line = mean, t-test between
870 genotypes at both ages not significant). **(M)** Correlation plot depicting the relationship between $A\beta_{40}$
871 and $A\beta_{42}$ levels in AM (black) and AM;*Serf2*$^{br-/-}$ (grey) mice at 3 months of age (detailed statistics in
872 Supplemental Table 3). In panels A-L: WT = black, *Serf2*$^{br-/-}$ = light grey , AM = dark grey and
873 AM;*Serf2*$^{br-/-}$ = white; ***p<0.001; **p<0.01.

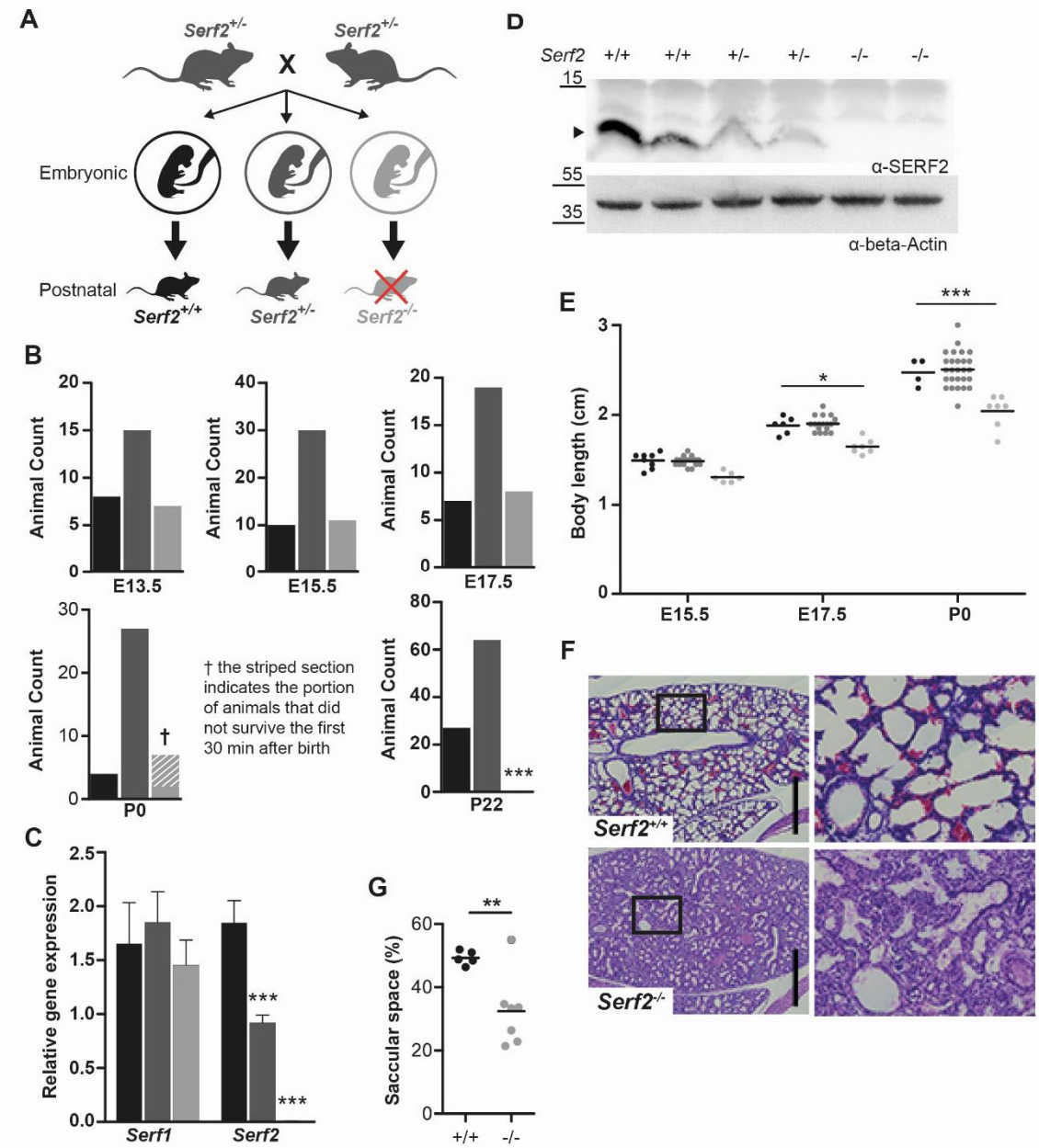
874 **Figure 3. Brain-specific *Serf2* KO display increased Aβ deposition and amyloid .(A)** Detailed light
875 microscope images of the 6E10-stained cortical region marked on the sagittal section in Supplemental
876 Figure 5 from 3-month-old AM and AM;*Serf2*$^{br-/-}$ mice (scale bar = 100μm). **(B)** Quantification of the
877 Aβ deposits in the region of interest (ROI) of 6E10 immunostained sagittal brain sections from 1- and
878 3-month-old AM and AM;*Serf2*$^{br-/-}$ mice (black line = mean, One-way ANOVA between genotypes at
879 both ages, Bonferroni corrected post-hoc between AM and AM;*Serf2*$^{br-/-}$ not significant). **(C)**
880 Correlation plot depicting the relationship between $A\beta_{40}$ levels and 6E10 plaque density in AM (black)
881 and AM;*Serf2*$^{br-/-}$ (grey) mice at 3 months of age. **(D)** Correlation plot depicting the relationship
882 between $A\beta_{42}$ levels and 6E10 plaque density in AM (black) and AM;*Serf2*$^{br-/-}$ (grey) mice at 3 months
883 of age. **(E)** Quantification of the Aβ deposits in the ROI of W0-2 immunostained sagittal brain
884 sections from 1- and 3-month-old AM and AM;*Serf2*$^{br-/-}$ mice (black line = mean, One-way ANOVA

885 between genotypes at both ages, Bonferroni corrected post-hoc between AM and AM;*Serf2*$^{br-/-}$ not
886 significant). **(F)** Correlation plot depicting the relationship between 6E10 deposits and W0-2 deposits
887 in AM (black) and AM;*Serf2*$^{br-/-}$ (grey) mice at 3 months of age. **(G)** Quantification of the plaque
888 density in the ROI of Thioflavin-S stained sagittal brain sections from 1- and 3-month-old AM and
889 AM;*Serf2*$^{br-/-}$ mice (black line = mean, One-way ANOVA between genotypes at both ages, Bonferroni
890 corrected post-hoc between AM and AM;*Serf2*$^{br-/-}$ not significant). **(H)** Correlation plot depicting the
891 relationship between Aβ_{40} levels and ThS plaque density in AM (black) and AM;*Serf2*$^{br-/-}$ (grey) mice
892 at 3 months of age. **(I)** Correlation plot depicting the relationship between Aβ_{42} levels and ThS plaque
893 density in AM (black) and AM;*Serf2*$^{br-/-}$ (grey) mice at 3 months of age. **(I)** Correlation plot depicting
894 the relationship between 6E10 deposits and ThS plaques in AM (black) and AM;*Serf2*$^{br-/-}$ (grey) mice
895 at 3 months of age. In all panels ROI = cortical and hippocampal area; In panels B,E and G: WT =
896 black, *Serf2*$^{br-/-}$ = light grey , AM = dark grey and AM;*Serf2*$^{br-/-}$ = white; for correlation plots: detailed
897 statistics in Supplemental Table 3; ***p<0.001; **p<0.01; *p <0.05.

898 **Figure 4. Brain-specific *Serf2* KO alters the structural composition of amyloid plaques. (A)**
899 Graph showing the theoretical LCO dye emission spectra for qFTAA, which binds mature Aβ fibrils
900 (blue), and hFTAA, which binds both mature fibrils and prefibrillar Aβ species (red). **(B)** Frequency
901 distribution of the ratio in fluorescence intensity at 502 nm and 588 nm for all amyloid plaques
902 identified by confocal microscopy in AM (blue) and AM;*Serf2*$^{br-/-}$ (red) mice at 3 months of age. Three
903 peak frequency categories were identified: low IR(red), medium IR (green) and high IR (blue) (n=6
904 mice/genotype). **(C)** Dot plot representing the intensity ratio of all amyloid plaques identified by
905 confocal microscopy for AM and AM;*Serf2*$^{br-/-}$ mice at 3 months of age (colors represent the IR
906 categories defined in panel B, black circles correspond to the plaques depicted in panel E, black line =
907 mean, t-test ***p<0.001) (n=6 mice/genotype). **(D)** Correlation plot depicting the relationship between
908 6E10 plaque density in the ROI and the LCO average intensity ratio in AM (black) and AM;*Serf2*$^{br-/-}$
909 (grey) mice at 3 months of age (ROI = cortical and hippocampal area, detailed statistics in
910 Supplemental Table 3). **(E)** Spectral confocal microscopy images of amyloid plaques in AM and
911 AM;*Serf2*$^{br-/-}$ mice at 3 months of age of double stained with qFTAA and hFTAA (top= fluorescence

912 at 502 nm, middle= fluorescence at 588 nm, bottom=merged image of 502 nm and 588 nm
913 fluorescence, scale bar = 5 μm). **(F)** Scanning transmission electron microscopy pictures of amyloid
914 plaques in the cortex of AM and AM;*Serf2*$^{br-/-}$ mice at 3 months of age (top scale bar 5μm, rectangle =
915 zoomed region in bottom picture, bottom scale bar = 1μm). High-resolution EM images are available
916 via http://www.nanotomy.org.

Figure 1


A
Serf2+/-
Serf2+/-
X
Embryonic
Postnatal
Serf2+/+
Serf2+/-
Serf2-/-
B
Animal Count
E13.5
E15.5
E17.5
P0
P22
† the striped section indicates the portion of animals that did not survive the first 30 min after birth
C
Relative gene expression
Serf1
Serf2
D
Serf2 +/+ +/+ +/- +/- -/- -/-
15
55
35
α-SERF2
α-beta-Actin
E
Body length (cm)
E15.5
E17.5
P0
F
Serf2+/+
Serf2-/-
G
Saccular space (%)
+/+
-/-

917

Figure 2

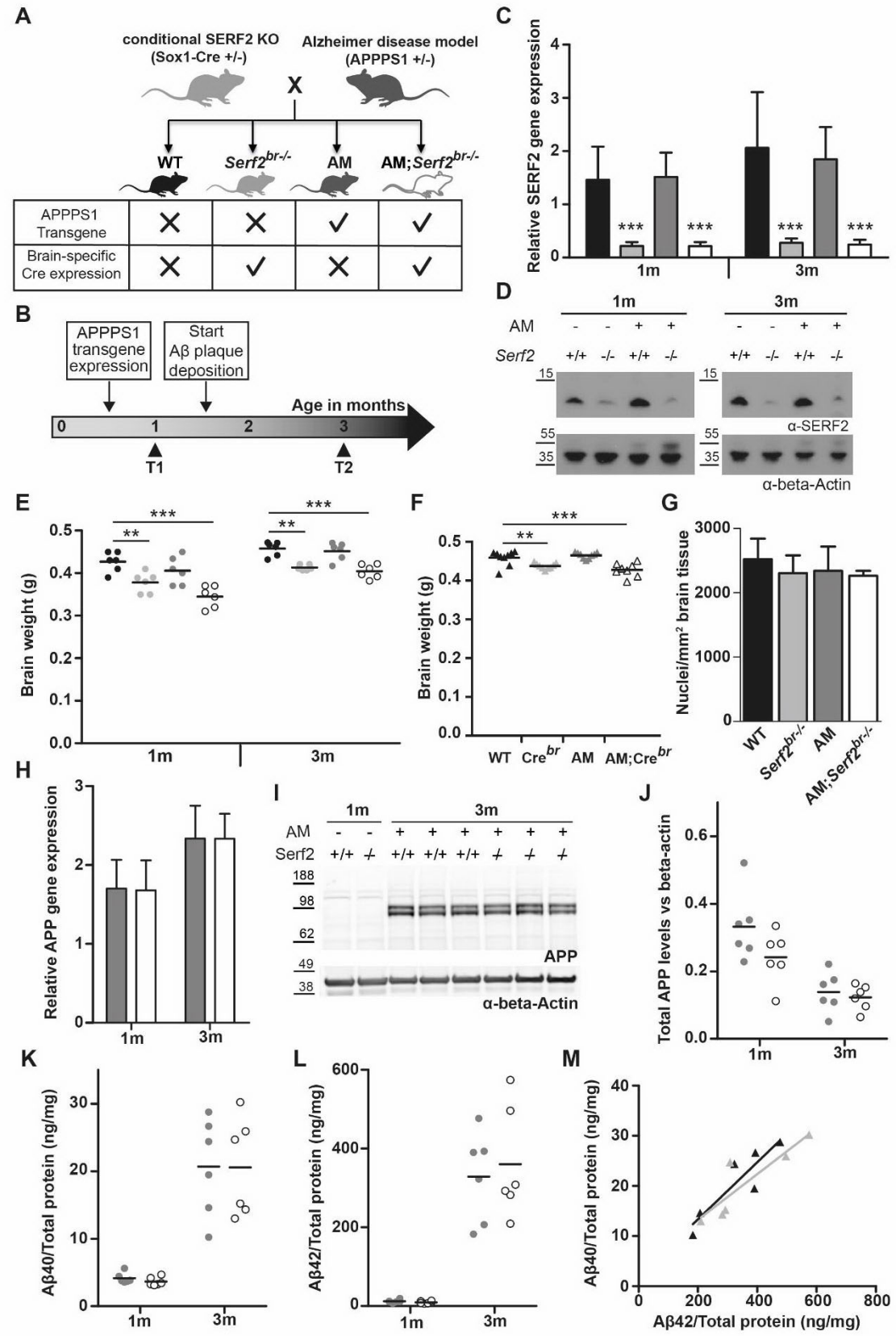

A
conditional SERF2 KO (Sox1-Cre +/-)
Alzheimer disease model (APPPS1 +/-)
X
WT
Serf2br-/-
AM
AM;Serf2br-/-
APPPS1 Transgene
Brain-specific Cre expression
B
APPPS1 transgene expression
Start Aβ plaque deposition
Age in months
T1
T2
C
Relative SERF2 gene expression
1m
3m
D
AM
Serf2
α-SERF2
α-beta-Actin
E
Brain weight (g)
F
Brain weight (g)
WT
Crebr
AM
AM;Crebr
G
Nuclei/mm² brain tissue
H
Relative APP gene expression
I
APP
α-beta-Actin
J
Total APP levels vs beta-actin
K
Aβ40/Total protein (ng/mg)
L
Aβ42/Total protein (ng/mg)
M
Aβ40/Total protein (ng/mg)
Aβ42/Total protein (ng/mg)

Figure 3

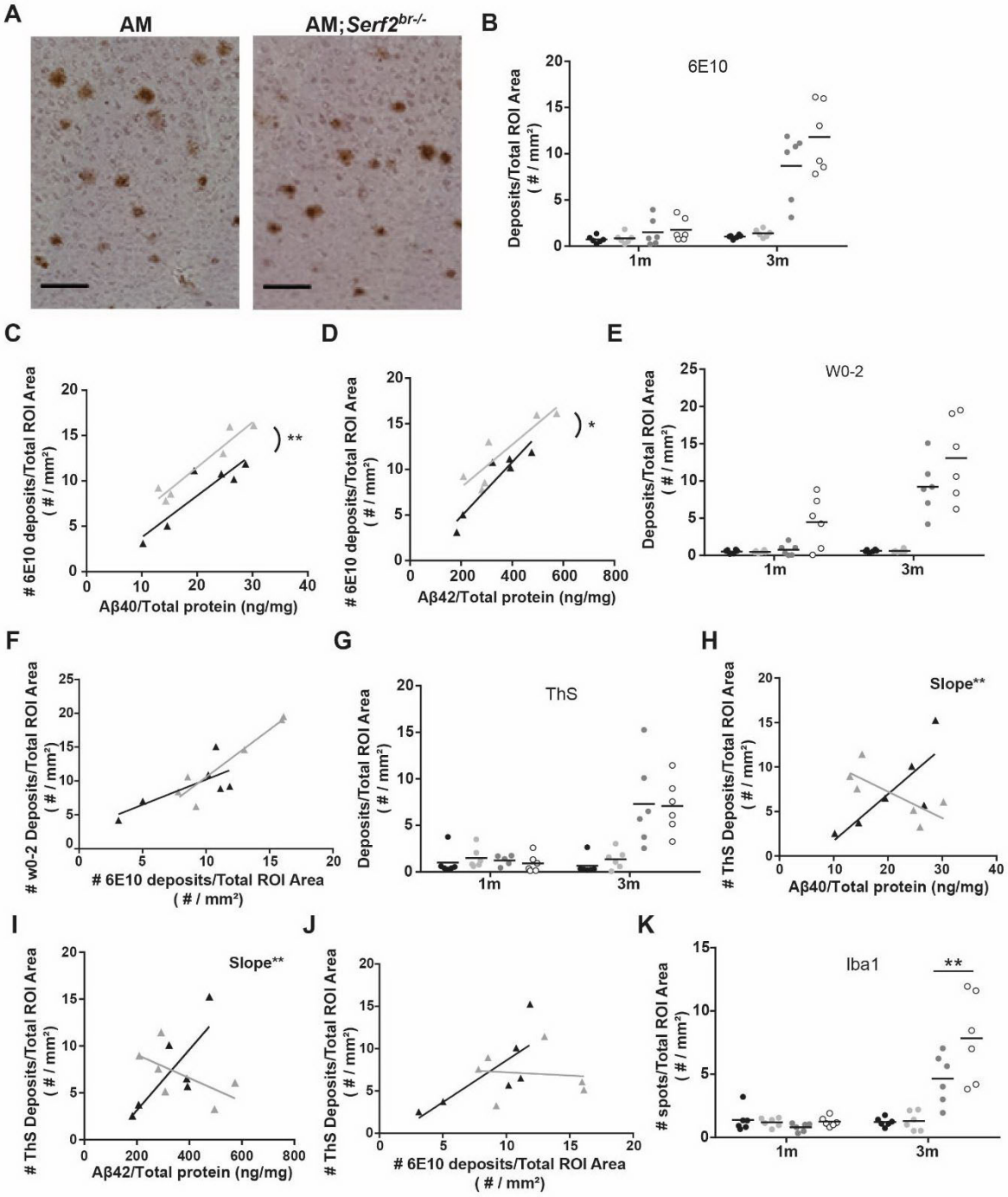
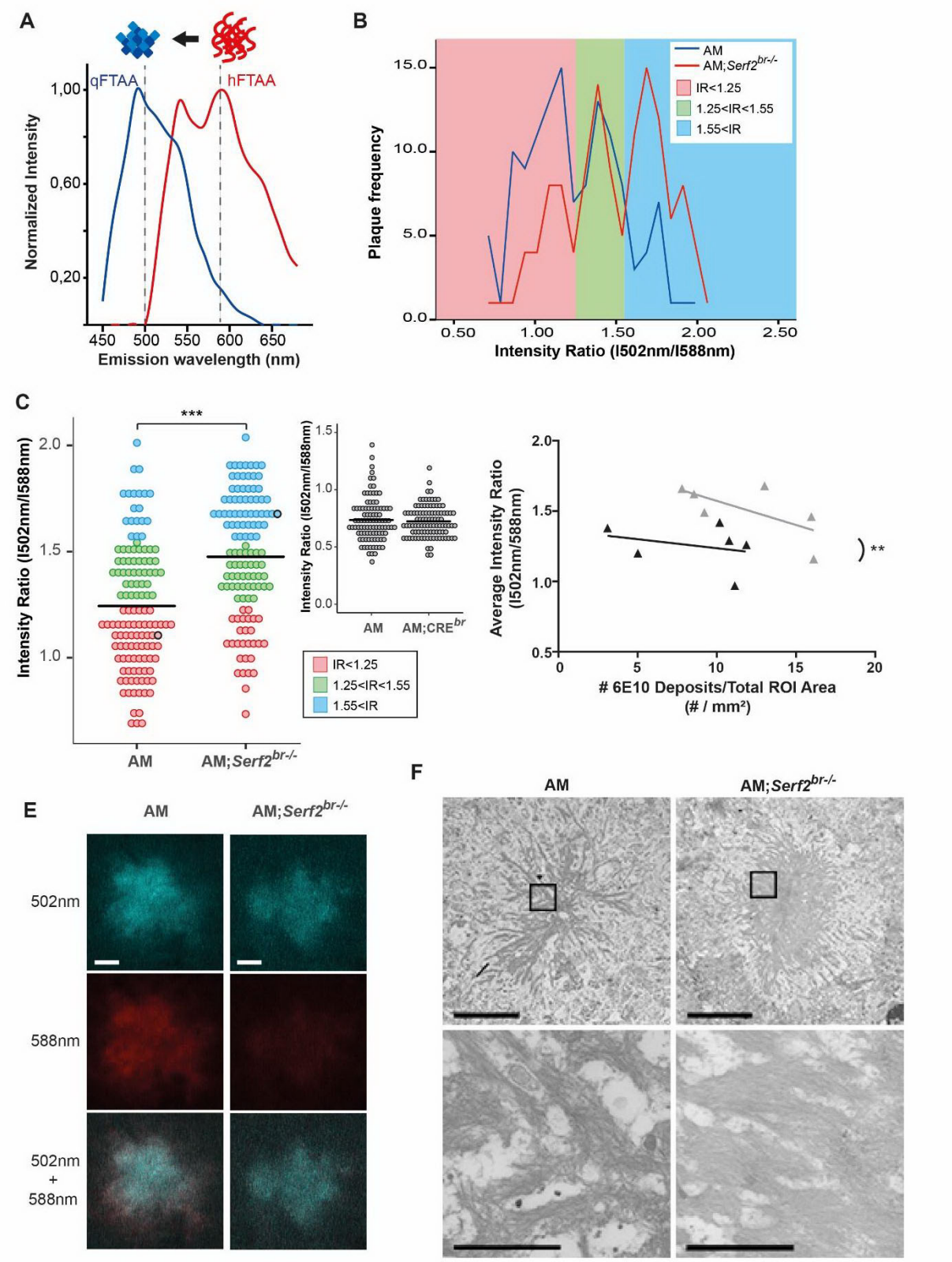

A
AM
AM;Serf2br-/-
B
6E10
Deposits/Total ROI Area (# / mm²)
1m
3m
C
6E10 deposits/Total ROI Area (# / mm²)
Aβ40/Total protein (ng/mg)
**
D
6E10 deposits/Total ROI Area (# / mm²)
Aβ42/Total protein (ng/mg)
*
E
W0-2
Deposits/Total ROI Area (# / mm²)
1m
3m
F
w0-2 Deposits/Total ROI Area (# / mm²)
6E10 deposits/Total ROI Area (# / mm²)
G
ThS
Deposits/Total ROI Area (# / mm²)
1m
3m
H
Slope**
ThS Deposits/Total ROI Area (# / mm²)
Aβ40/Total protein (ng/mg)
I
Slope**
ThS Deposits/Total ROI Area (# / mm²)
Aβ42/Total protein (ng/mg)
J
ThS Deposits/Total ROI Area (# / mm²)
6E10 deposits/Total ROI Area (# / mm²)
K
Iba1
**
spots/Total ROI Area (# / mm²)
1m
3m

Figure 4


A
qFTAA
hFTAA
Normalized Intensity
1,00
0,60
0,20
450 500 550 600 650
Emission wavelength (nm)
B
AM
AM;Serf2br-/-
IR<1.25
1.25<IR<1.55
1.55<IR
Plaque frequency
15.0
10.0
5.0
0.0
0.50 1.00 1.50 2.00 2.50
Intensity Ratio (I502nm/I588nm)
C
***
Intensity Ratio (I502nm/I588nm)
2.0
1.5
1.0
AM
AM;Serf2br-/-
Intensity Ratio (I502nm/I588nm)
1.5
1.0
0.5
0.0
AM
AM;CREbr
IR<1.25
1.25<IR<1.55
1.55<IR
Average Intensity Ratio (I502nm/I588nm)
2.0
1.5
1.0
0.5
0 5 10 15 20
6E10 Deposits/Total ROI Area
(# / mm²)
**
E
AM
AM;Serf2br-/-
502nm
588nm
502nm
+
588nm
F
AM
AM;Serf2br-/-

917 **Supplemental figure legends**

918 **Supplemental Figure 1. Additional full-body *Serf2*$^{-/-}$ mice validation and phenotype**
919 **confirmation. (A)** Targeting strategy used to delete exon 2 of *Serf2* by using the 'knockout-first'-
920 allele (adjusted from Skarnes et al. 2011). Top panel: exon/intron structure of *Serf2* including the
921 positions of the LacZ and Neo cassettes, as well as loxP sites. Bottom panel: the *Serf2* allele after
922 recombination events as a result of crosses with *Hprt-Cre* mice. **(B)** Western blot analysis of liver,
923 heart and brain tissue of a *Serf2*$^{+/+}$, *Serf2*$^{+/-}$ and *Serf2*$^{-/-}$ adult mice to detect Serf2 and actin. **(C)** Real
924 time RT-PCR analyses of *Serf2* in different organs of adult mice with the following genotypes *Serf2*$^{+/+}$
925 (n=3), *Serf2*$^{+/-}$ (n=8) and *Serf2*$^{-/-}$ (n=1). *Serf2* levels were normalized to the housekeeping gene 18S
926 (mean ± SEM). **(D)** Lateral view of a *Serf2*$^{-/-}$ and *Serf2*$^{+/-}$ embryo and placenta at embryonic day 15.5
927 and 17.5. **(E)** Weight measurements of *Serf2*$^{+/+}$, *Serf2*$^{+/-}$, *Serf2*$^{-/-}$ embryos at E17.5 and P0 (black line =
928 mean, Two-way ANOVA with factors age and genotype, Bonferroni corrected post-hoc comparison
929 for *Serf2*$^{+/-}$ and *Serf2*$^{-/-}$ compared to *Serf2*$^{-/-}$ *p<0.05; ***p<0.001). **(F)** Hematoxylin and Eosin staining
930 on *Serf2*$^{-/-}$ and *Serf2*$^{+/+}$ lung and kidney tissue at E17.5 (10 times magnification). For all panels:
931 *Serf2*$^{+/+}$= black, *Serf2*$^{+/-}$ = dark grey, *Serf2*$^{-/-}$ = light grey.

932 **Supplemental Figure 2. Cell culture data of *Serf2* KO and control HEK cells and RNA**
933 **sequencing analysis of mouse embryonic fibroblasts (MEFs) isolated from full-body *Serf2*$^{-/-}$ and**
934 **control mice. (A)** Growth curve of two CRISPR-induced *Serf2*$^{-/-}$ clones of HEK293T versus wild-type
935 HEK293T. (Three replicates measured,full line=replicate average, dashed lines=standard deviation on
936 average) **(B)** Network showing the interconnectivity of enriched GO-terms in RNA sequencing data
937 from *Serf2*$^{-/-}$ and control MEFs. Circle size indicates amount of differentially expressed genes found in
938 each GO-category. Edges were drawn when the minor category shared more than 25 % of its found
939 genes with the major category. Edge color indicates the major category in the relationship. Edge
940 thickness indicates the percentage of found genes shared by the minor category. **(C)** Bar chart
941 indicating the fold change for each GO-term as determined by DAVID. Brackets indicate the three
942 main GO-term clusters defined based on the network in (B).

943 **Supplemental Figure 3. Additional validation of the brain-specific knock-out of *Serf2*. (A)**
944 Modified targeting strategy used to delete the exon 2 of *Serf2* specifically in the central nervous
945 system. **(B)** Real time RT-PCR analyses of *Serf2* RNA expression in different organs $Serf2^{+/+}$ and
946 $Serf2^{br-/-}$ female mice at 1 month and 3 months of age. *Serf2* expression was normalized to
947 housekeeping gene beta-actin (all groups n=6, mean ± SEM). **(C)** Western blot analysis and
948 quantification of *Serf2* and beta-actin in brain lysates of WT and $Serf2^{br-/-}$ female mice at 1 month
949 (both groups n=6, mean ± SEM, t-test **p<0.01). **(D)** Brain weight of unfloxed WT and CRE^{br} mice at
950 1 and 3 months of age (black line = mean, Two-way ANOVA with factors age and genotype,
951 Bonferroni corrected post-hoc for CRE^{br} compared to WT $*p_{bon}<0.05$). **(E)** Evolution of brain weight
952 in WT and $Serf2^{br-/-}$ female mice between 1 and 11 months of age (black line = mean).

953 **Supplemental Figure 4. Overview of distribution and general morphological parameters of**
954 **6E10-stained Aβ deposits in AM and AM;$Serf2^{br-/-}$ mice at 3 months of age. (A)** Light microscope
955 images of the 6E10 immunostained sagittal section of 3-month-old AM and AM;$Serf2^{br-/-}$ female mice
956 (scale bar = 1mm, black rectangle = magnified region in main Figure 3). **(B)** Boxplots showing the
957 distribution of the surface area of the individual plaques measured in AM and AM;$Serf2^{br-/-}$ mice at 3
958 months of age. **(C)** Boxplots showing the distribution of the circularity of the individual plaques
959 measured in AM and AM;$Serf2^{br-/-}$ mice at 3 months of age. **(D)** Boxplots showing the distribution of
960 the solidity of the individual plaques measured in AM and AM;$Serf2^{br-/-}$ mice at 3 months of age. For
961 all boxplots: black line = median, box = 25th-75th percentile, whiskers = 1.5 x interquartile range
962 (IQR), circles = outlier value > 1.5x IQR, asterisks = extreme outlier value > 3x IQR.

963 **Supplemental Figure 5. Overview of distribution and detailed W0-2-stained Aβ deposits in AM**
964 **and AM;$Serf2^{br-/-}$ mice at 3 months of age.** Light microscope images of the W0-2 immunostained
965 sagittal section of 3-month-old AM and AM;$Serf2^{br-/-}$ female mice (scale bar = 1mm, black rectangle =
966 magnified in insert on the right, insert scale bar = 100µm).

967 **Supplemental Figure 6. Correlation plots illustrating the relationship of different stains in**
968 **individual mice. (A)** Correlation plot depicting the relationship between the density of 6E10 deposits

969 and Iba1-positive spots in AM (black) and AM;*Serf2*$^{br-/-}$ (grey) mice at 3 months of age. **(B)**
970 Correlation plot depicting the relationship between W0-2 plaque density and the LCO average
971 intensity ratio in AM (black) and AM;*Serf2*$^{br-/-}$ (grey) mice at 3 months of age. **(C)** Correlation plot
972 depicting the relationship between ThS plaque density and the LCO average intensity ratio in AM
973 (black) and AM;*Serf2*$^{br-/-}$ (grey) mice at 3 months of age. For all plots: detailed statistics in
974 Supplemental Table 3; *p <0.05.

975 **Supplemental Figure 7. Overview and detailed view of Aβ staining pattern after W0-2 and 6E10**
976 **staining in AM and AM;*Serf2*$^{br-/-}$ mice at 1 month of age. (A)** Light microscope images of the W0-2
977 immunostained sagittal section of 1-month-old AM and AM;*Serf2*$^{br-/-}$ female mice. **(B)** Light
978 microscope images of the 6E10 immunostained sagittal section of 1-month-old AM and AM;*Serf2*$^{br-/-}$
979 female mice. All pictures: main scale bar = 1mm, black rectangle = magnified in insert on the right,
980 insert scale bar = 100µm.