1 **Supplementary Materials for**

2 **Amyloid β oligomers suppress excitatory transmitter release via presynaptic**

3 **depletion of phosphatidylinositol-4,5-bisphosphate**

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9 **This PDF file includes:**

10 Figs. S1 to S15

11 Materials and Methods

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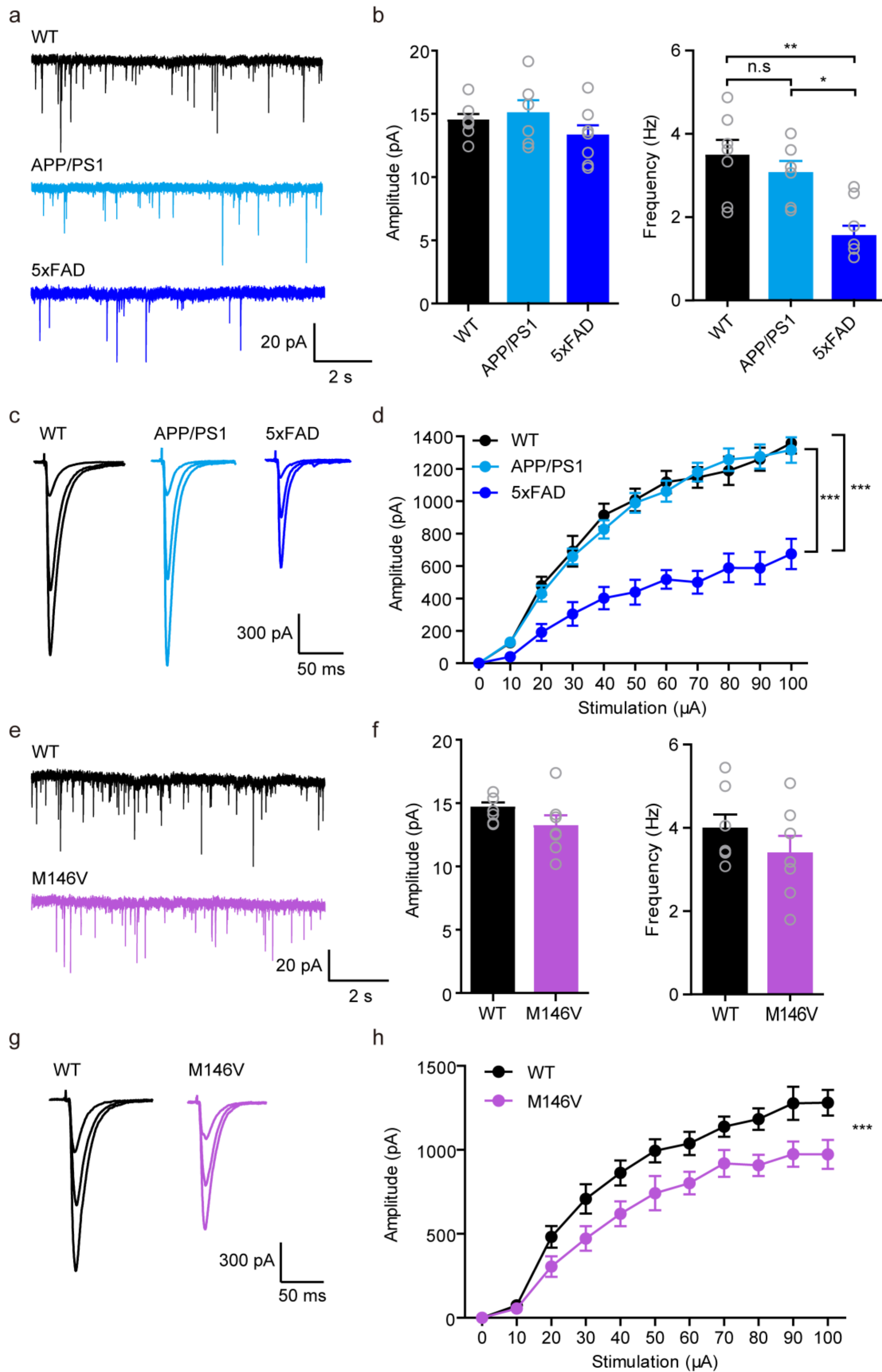

13

14 **Supplementary Figure 1** Excitatory synaptic transmission deficits in AD mouse

15 models. **a, b** Representative traces (**a**) of mEPSCs in CA1 pyramidal neurons and

16 quantification (**b**) of mEPSC amplitude (left) and frequency (right) in 4-month-old WT,

17 APP/PS1, and 5xFAD mice. One-way ANOVA with post hoc Dunnett's test; $F_{(2,17)}$ =

18 1.19 (amplitude); $F_{(2,17)}$ = 9.484 (frequency); *, $P < 0.05$; **, $P < 0.01$; N = 6-7 per

19 group. **c,d** Representative traces of SC-CA1 EPSCs evoked by stimulus intensities of

20 10, 30, and 100 μA (**c**) and quantification of EPSC amplitude to stimulus intensity (**d**)

21 in 4-month-old WT, APP/PS1, and 5xFAD mice. Two-way ANOVA with post hoc

22 Bonferroni test; animal, $F_{(2,198)}$ = 168.1, $P < 0.001$; stimulation, $F_{(10,198)}$ = 107.6, P <

23 0.001; ***, $P < 0.001$; N = 6-8 per group. **e, f** Representative traces (**e**) of mEPSCs in

24 CA1 pyramidal neurons and quantification (**f**) of mEPSC amplitude (left) and

25 frequency (right) in 6-month-old WT and M146V mice. *t* test; $P > 0.05$; N = 7 per group.

26 **g, h** Representative traces of SC-CA1 EPSCs evoked by stimulus intensities of 20, 40,

27 and 100 μA (**g**) and quantification of EPSC amplitude to stimulus intensity (**h**) in

28 6-month-old WT and M146V mice. Two-way ANOVA with post hoc Bonferroni test;

29 animal, $F_{(1,143)}$ = 49.34, $P < 0.001$; stimulation, $F_{(10,143)}$ = 70.41, $P < 0.001$; ***, P <

30 0.001; N = 7-8 per group. Data are mean ± SEM. Source data are provided as a

31 Source Data file.

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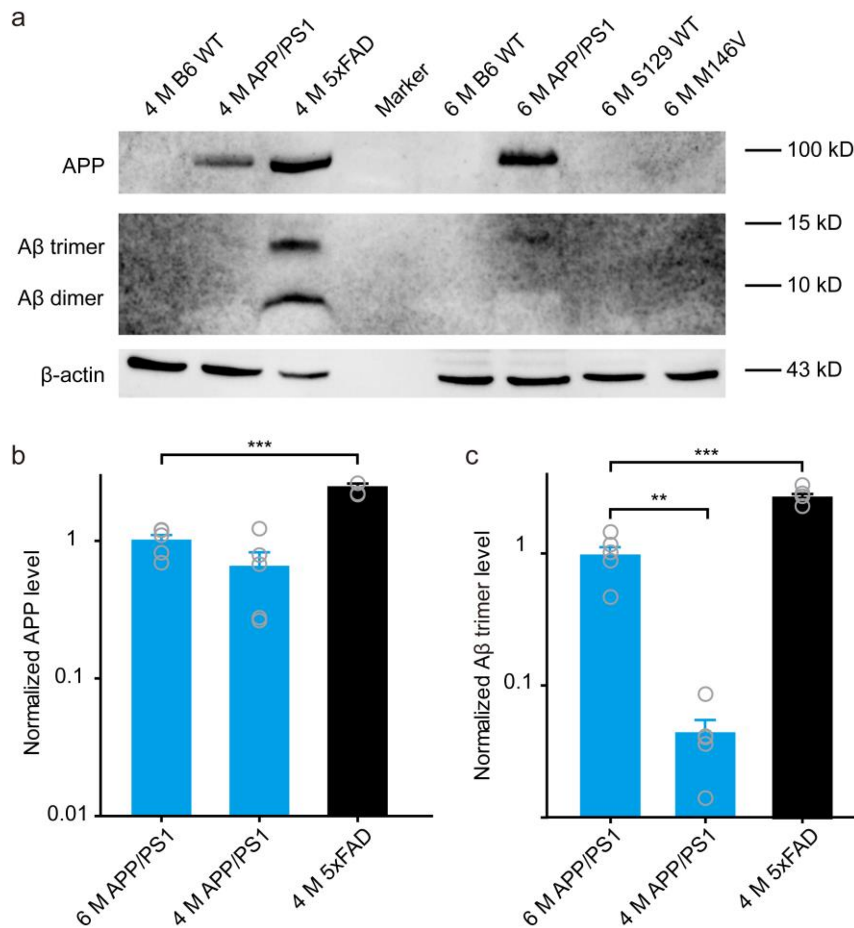

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35 **Supplementary Figure 2** Oligomeric Aβ levels are greatly enhanced in 4-month-old
36 5xFAD and 6-month-old APP/PS1 mice in comparison to 4-month-old APP/PS1 mice.
37 **a** Representative Western blots of APP and Aβ oligomers (trimer/dimer) in
38 4-month-old (4 M) WT, APP/PS1, and 5xFAD mice and 6-month-old (6 M) WT,
39 APP/PS1, S129 WT, and M146V mice. **b, c** Quantification of APP (**b**) and Aβ trimer (**c**)
40 in 4 M 5xFAD, 4 M APP/PS1, and 6 M APP/PS1 mice. One-way ANOVA with post hoc
41 Dunnett's test; $F_{(2,12)}$ = 39.025 in **b**; $F_{(2,12)}$ = 86.069 in **c**; **, $P < 0.01$; ***, $P < 0.001$; N
42 = 5 per group. Data are mean ± SEM. Source data are provided as a Source Data file.

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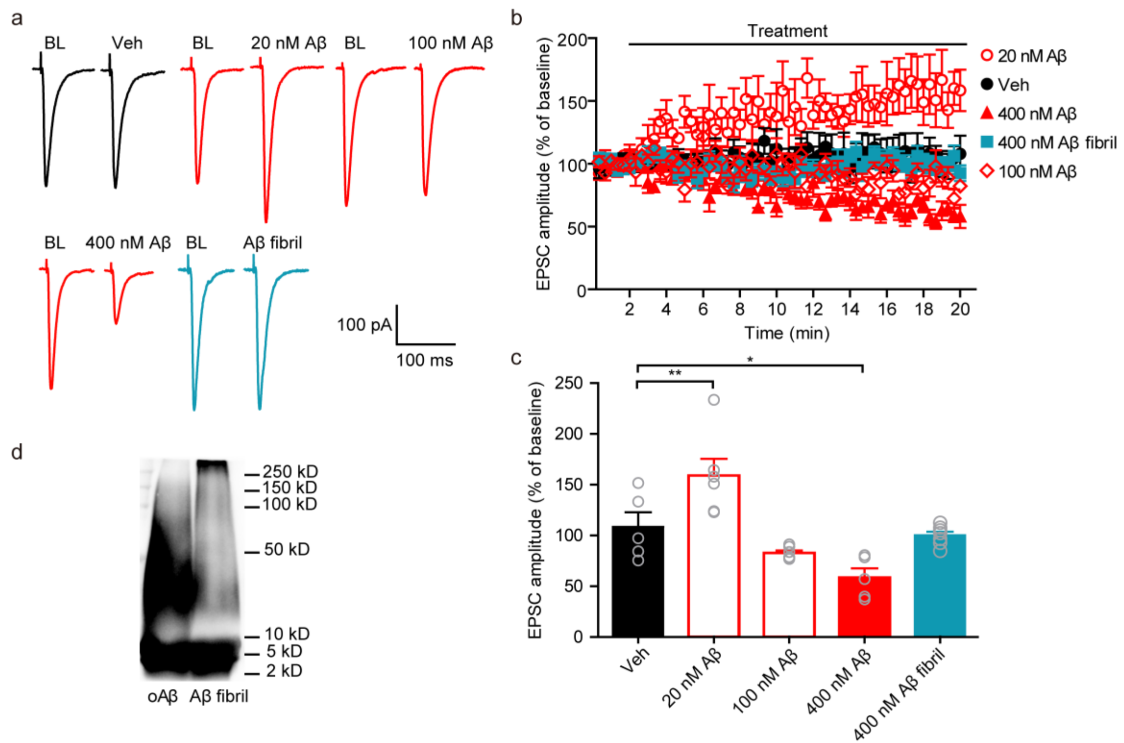

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46 **Supplementary Figure 3** Regulation of evoked responses at the SC-CA1 synapse by

47 Aβ fibrils and oligomers at various concentrations. **a, b** Representative traces (**a**) and

48 the time course (**b**) of evoked EPSCs at the SC-CA1 synapse before (baseline, BL)

49 and after DMSO (vehicle, Veh), oligomeric $A\beta_{42}$ (20, 100, or 400 nM), or fibrillar $A\beta_{42}$

50 (400 nM) treatment. **c** Quantification of EPSC amplitudes recorded in the last 1 min in

51 **b**. One-way ANOVA with post hoc Dunnett's test; $F_{(4,24)} = 12.64$; *, $P < 0.05$; **, $P <$

52 0.01; $N = 5$-7 per group. **d** Representative Western blots of synthetic oligomeric $A\beta_{42}$

53 (oAβ) and fibrillar $A\beta_{42}$ (Aβ fibril). Data are mean ± SEM. Source data are provided as

54 a Source Data file.

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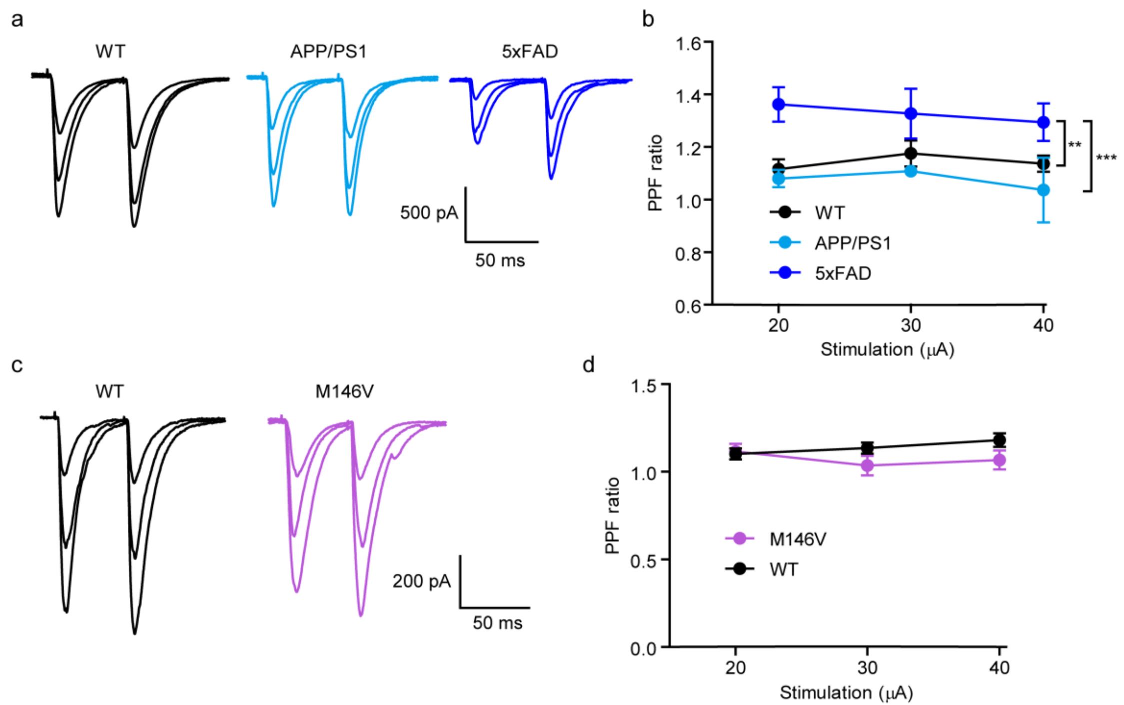
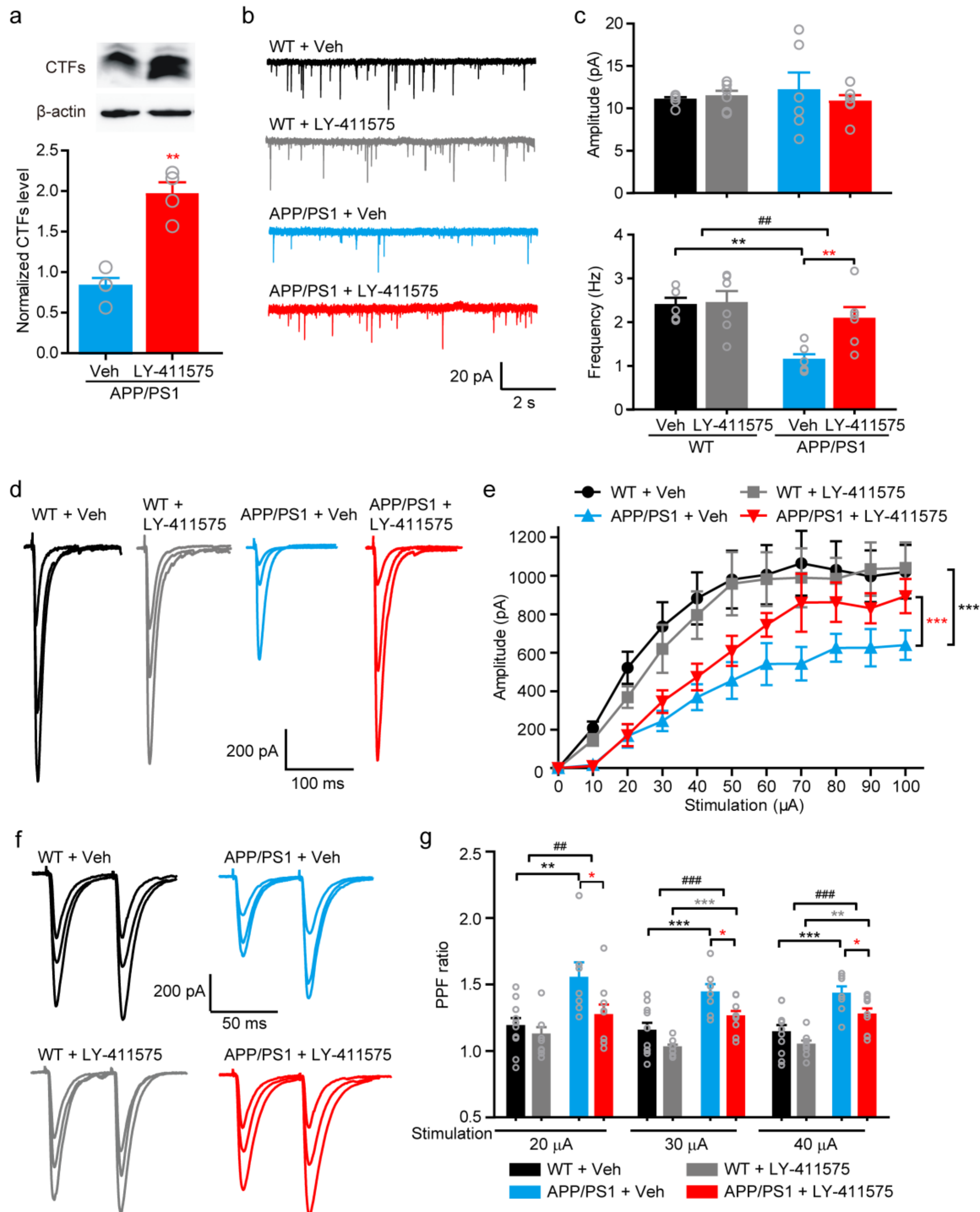


Supplementary Figure 4 PPF at the SC-CA1 synapse is strongly increased in 4-month-old 5xFAD mice, but not in 6-month-old M146V mice. **a, b** Representative traces (**a**) and quantification (**b**) of PPF in response to stimulus intensities of 20, 30, and 40 μA in 4-month-old WT, APP/PS1, and 5xFAD mice. Two-way ANOVA with post hoc Bonferroni test; animal, $F_{(2,51)} = 11.27$, $P < 0.001$; stimulation, $F_{(2,51)} = 0.3558$, $P < 0.001$; **, $P < 0.01$; ***, $P < 0.001$; $N = 6\text{-}8$ per group. **c, d** Representative traces (**c**) and quantification (**d**) of PPF in response to stimulus intensities of 20, 30, and 40 μA in 6-month-old WT and M146V mice. Two-way ANOVA with post hoc Bonferroni test; animal, $F_{(1,42)} = 3.745$, $P = 0.07$; stimulation, $F_{(2,42)} = 0.4371$, $P = 0.65$; $P > 0.05$; $N = 7\text{-}9$ per group. Data are mean ± SEM. Source data are provided as a Source Data file.



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70 **Supplementary Figure 5** Reducing Aβ production by γ-secretase inhibitor LY-411575
71 partially rescues excitatory synaptic deficits in 6-7-month-old APP/PS1 mice. **a**
72 Representative Western blots (upper) and quantification (bottom) of hippocampal
73 CTFs in APP/PS1 mice treated with vehicle (Veh) or LY-411575. *t* test; **, $P < 0.01$; N
74 = 4 per group. **b, c** Representative traces (**b**) of mEPSCs in CA1 pyramidal neurons

75 and quantification (**c**) of mEPSC amplitude (upper) and frequency (bottom) in WT and
76 APP/PS1 mice treated with vehicle (Veh) or LY-411575. Two-way ANOVA with post
77 hoc Bonferroni test; upper panel in **c**: animal, $F_{(1,29)} = 0.0391$, $P = 0.845$; treatment,
78 $F_{(1,29)} = 0.143$, $P = 0.709$; bottom panel in **c**: animal, $F_{(1,29)} = 12.814$, $P = 0.002$;
79 treatment, $F_{(1,29)} = 4.733$, $P = 0.040$; **, $P < 0.01$; $^{\#\#}$, $P < 0.01$ (APP/PS1 vs. WT); N =
80 5-6 per group. **d, e** Representative traces of SC-CA1 EPSCs evoked by stimulus
81 intensities of 20, 30, and 100 μA (**d**) and quantification of EPSC amplitude to stimulus
82 intensity (**e**) in WT + Veh, WT + LY-411575, APP/PS1 + Veh, and APP/PS1 +
83 LY-411575 groups. Two-way ANOVA with post hoc Bonferroni test; group, $F_{(3,209)}$ =
84 38.257, $P < 0.001$; stimulation, $F_{(10,209)} = 46.014$, $P < 0.001$; ***, $P < 0.001$; N = 5-7 per
85 group. **f, g** Representative traces (**f**) and quantification (**g**) of PPF of SC-CA1 EPSCs
86 evoked by stimulus intensities of 20, 30, and 40 μA in WT and APP/PS1 mice treated
87 with Veh, or LY-411575. Two-way ANOVA with post hoc Bonferroni test; 20 μA: animal,
88 $F_{(1,30)} = 11.471$, $P = 0.002$; treatment, $F_{(1,30)} = 5.540$, $P = 0.025$; 30 μA: animal, $F_{(1,30)}$ =
89 26.206, $P < 0.001$; treatment, $F_{(1,30)} = 9.045$, $P = 0.005$; 40 μA: animal, $F_{(1,30)} = 27.079$,
90 $P < 0.001$; treatment, $F_{(1,30)} = 6.278$, $P = 0.018$; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$;
91 $^{\#\#}$, $P < 0.01$; $^{\#\#\#}$, $P < 0.001$ (APP/PS1 vs. WT); N = 7-10 per group. Data are mean ±
92 SEM. Source data are provided as a Source Data file.
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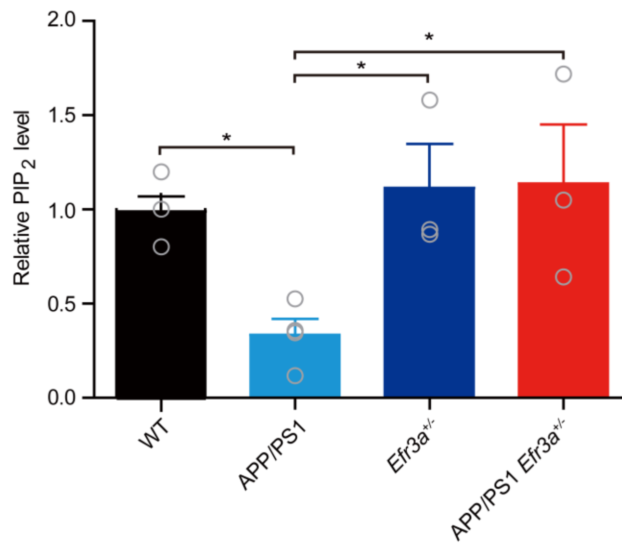

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96 **Supplementary Figure 6** Halving *Efr3a* copy number restores the decreased PIP_2

97 level measured with PIP_2 ELISA in APP/PS1 mice. One-way ANOVA with post hoc

98 Dunnett's test; $F_{(3,10)} = 4.93$; *, $P < 0.05$; $N = 3\text{-}4$ per group. Data are mean ± SEM.

99 Source data are provided as a Source Data file.

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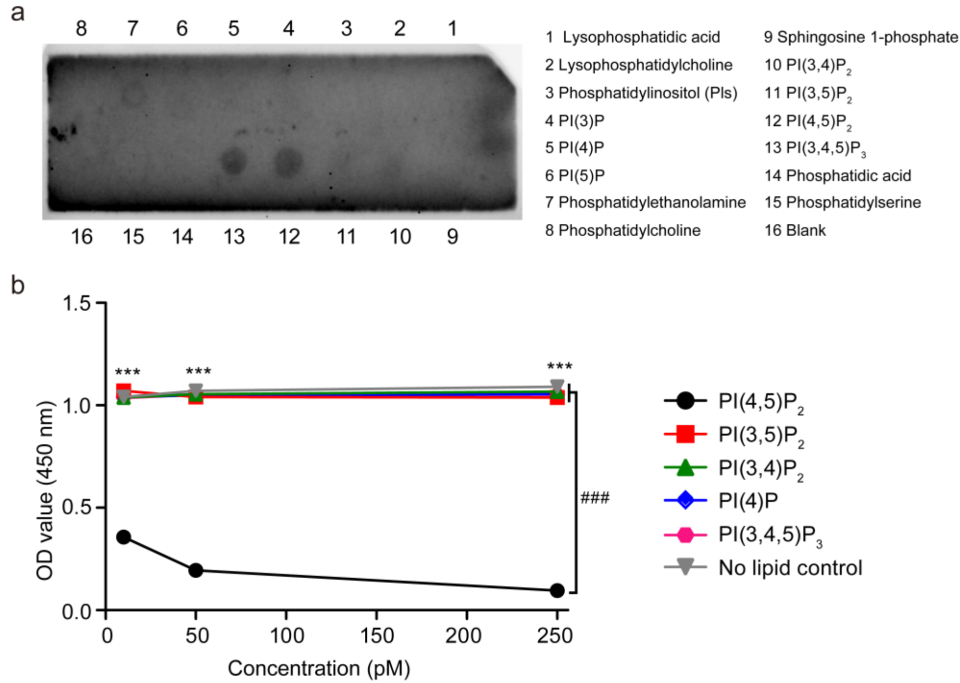

102

103 **Supplementary Figure 7** Anti-PI(4,5)P_2 antibody and ELISA kit specificities. **a**
104 Representative PIP strip showing the mouse anti-PI(4,5)P_2 antibody specifically
105 recognizes PI(4,5)P_2 and PI(3,4,5)P_3. **b** Plot of colorimetric signals for various
106 phosphatidylinositol phosphates at concentrations of 10, 50, and 250 pM. Two-way
107 ANOVA with post hoc Bonferroni test; PIP species, $F_{(5,45)}$ = 4634.6, P < 0.001; dose,
108 $F_{(2,45)}$ = 12.527, P < 0.001; ***, P < 0.001; $^{###}$, P < 0.001; N = 3-6 per group. Data are
109 mean ± SEM. Source data are provided as a Source Data file.

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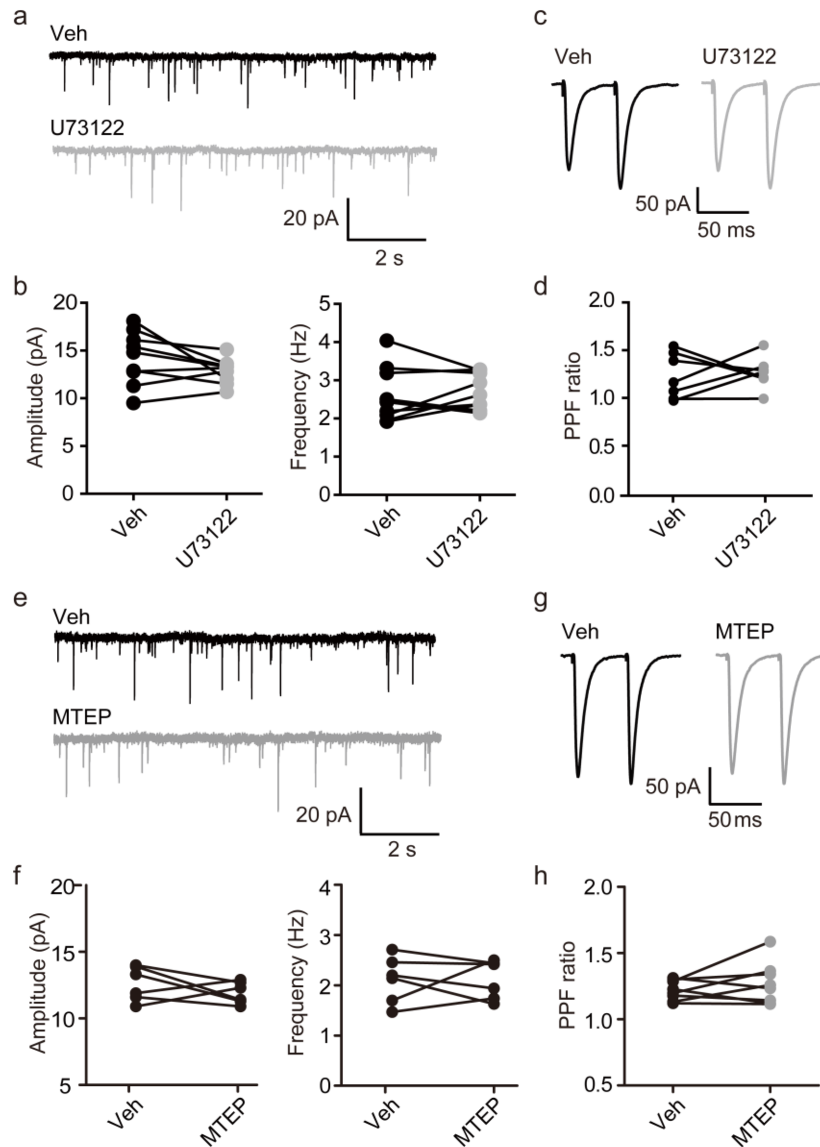

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113 **Supplementary Figure 8** Inhibiting PLC or mGluR5 has no influence on mEPSCs in
114 CA1 pyramidal neurons and PPF at the SC-CA1 synapse in WT animals. **a, b**
115 Representative traces (**a**) of mEPSCs in CA1 pyramidal neurons and quantification (**b**)
116 of mEPSC amplitude (left) and frequency (right) in WT hippocampal slices before (Veh)
117 and after U73122 treatment. *t* test; $P > 0.05$; $N = 9$ per group. **c, d** Representative
118 traces (**c**) and quantification (**d**) of PPF of SC-CA1 EPSCs in WT mice before (Veh)
119 and after U73122 treatment. *t* test; $P > 0.05$; $N = 7$ per group. **e, f** Representative

120 traces (**e**) of mEPSCs in CA1 pyramidal neurons and quantification (**f**) of mEPSC
121 amplitude (left) and frequency (right) in WT hippocampal slices before (Veh) and after
122 MTEP treatment. *t* test; $P > 0.05$; $N = 6$ per group. **g, h** Representative traces (**g**) and
123 quantification (**h**) of PPF of SC-CA1 EPSCs in WT mice before (Veh) and after MTEP
124 treatment. *t* test; $P > 0.05$; $N = 8$ per group. Data are mean ± SEM. Source data are
125 provided as a Source Data file.
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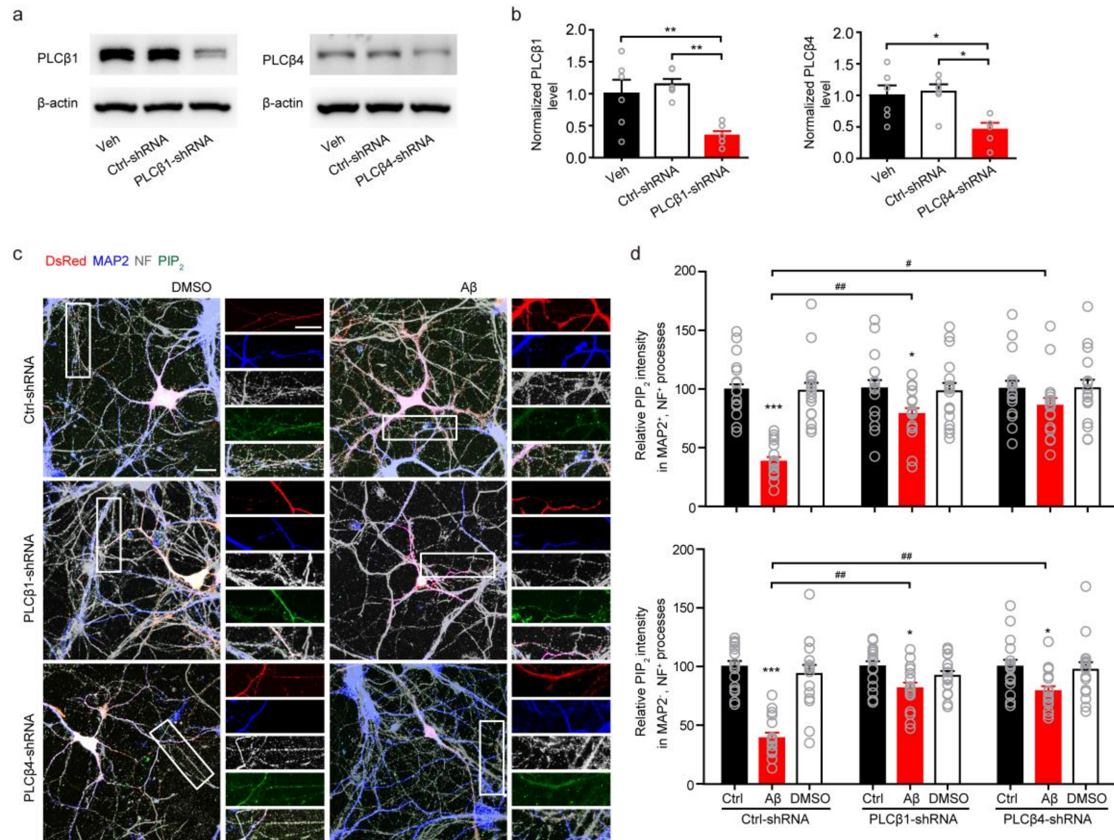

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129 **Supplementary Figure 9** Knocking down PLCβ1 or PLCβ4 prevents oligomeric

130 Aβ-induced PIP_2 reduction in cultured hippocampal neurons. **a, b** Representative

131 Western blots (**a**) and quantification (**b**) of PLCβ1 (left) and PLCβ4 (right) in primary

132 hippocampal neurons treated with vehicle (Veh) or transfected with lentivirus carrying

133 control (Ctrl)-shRNA, PLCβ1-shRNA, or PLCβ4-shRNA. One-way ANOVA with post

134 hoc Dunnett's test; $F_{(2,15)} = 9.190$ (**b**, left); $F_{(2,14)} = 5.886$ (**b**, right); *, $P < 0.05$; **, $P <$

135 0.01; $N = 5\text{-}6$ per group. **c** Confocal images of primary hippocampal neurons infected

136 with lentivirus carrying Ctrl-shRNA, PLCβ1-shRNA, or PLCβ4-shRNA showing the

137 effect of DMSO or Aβ treatment (blank Ctrl treatment not shown) on colocalization of

138 PIP_2, MAP2, and neurofilament (NF) along neuronal processes. Bars, 20 μm. **d**

139 Quantification of relative PIP_2 intensity in dendrites (upper panel) and axons (bottom

140 panel) of lentiviral-infected, DsRed positive neurons. Two-way ANOVA with post hoc

141 Bonferroni test; upper panel: cell type, $F_{(2,144)} = 5.693$, $P = 0.004$; treatment, $F_{(2,144)} =$

142 25.666, $P < 0.001$; bottom panel: cell type, $F_{(2,135)} = 6.999$, $P < 0.001$; treatment, $F_{(2,135)}$

143 $= 33.85$, $P < 0.001$; *, $P < 0.05$; ***, $P < 0.001$ (compared with Ctrl treatment); $^{\#}$, $P <$

144 0.05; $^{\#\#}$, $P < 0.01$; $N = 16\text{-}17$ per group. Data are mean ± SEM. Source data are

145 provided as a Source Data file.

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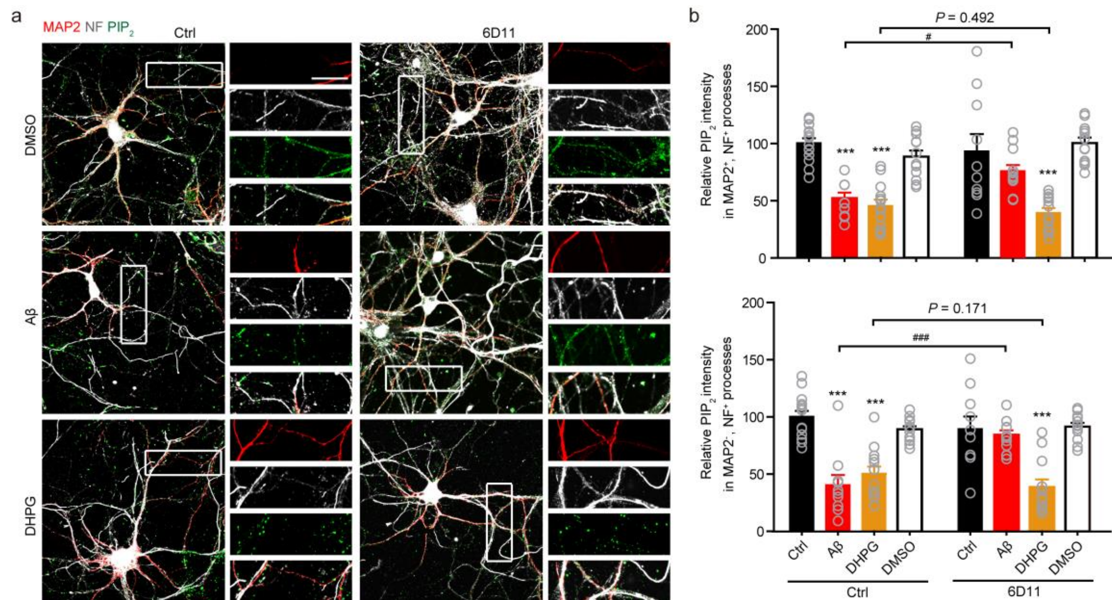
147 

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149 **Supplementary Figure 10** Blocking PrP^C prevents oligomeric Aβ-induced PIP_2

150 reduction in cultured hippocampal neurons. **a** Confocal images of cultured

151 hippocampal neurons blocked with medium containing anti-PrP^C antibody 6D11 or

152 control (Ctrl) medium showing the effect of DMSO, oligomeric Aβ, or DHPG treatment

153 (blank Ctrl treatment not shown) on colocalization of PIP_2, MAP2, and neurofilament

154 (NF) along neuronal processes. Bars, 20 μm. **b** Quantification of relative PIP_2 intensity

155 in dendrites (upper panel) and axons (bottom panel) showing 6D11 prevents Aβ-, but

156 not DHPG-induced suppression of PIP_2 in neurites. Two-way ANOVA with post hoc

157 Bonferroni test; upper panel: Ctrl/6D11, $F_{(1,87)}$ = 1.596, P = 0.21; treatment, $F_{(3,87)}$ =

158 32.585, $P < 0.001$; bottom panel: Ctrl/6D11, $F_{(1,88)}$ = 1.650, P = 0.202; treatment, $F_{(3,88)}$

159 = 30.481, $P < 0.001$; ***, $P < 0.001$ (compared with Ctrl-Ctrl or 6D11-Ctrl group); $^{\#}$, $P <$

160 0.05; $^{\#\#\#}$, $P < 0.001$; N = 10-14 per group. Data are mean ± SEM. Source data are

161 provided as a Source Data file.

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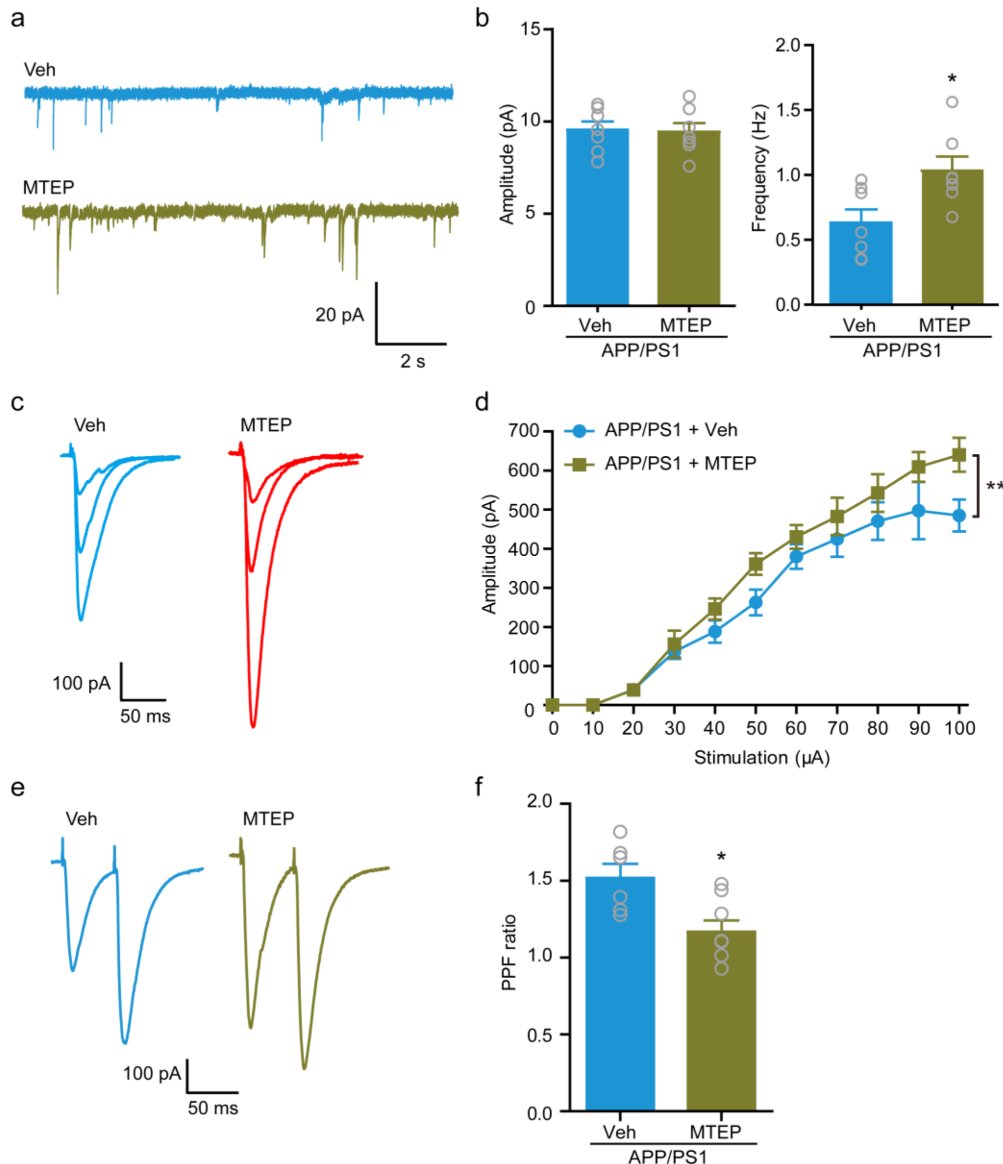

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164 **Supplementary Figure 11** Blocking mGluR5 increases excitatory synaptic
165 transmission and decreases PPF in the hippocampus of 6-7-month-old APP/PS1
166 mice. **a, b** Representative traces (**a**) of mEPSCs in CA1 pyramidal neurons and
167 quantification (**b**) of mEPSC amplitude (left) and frequency (right) in hippocampal
168 slices of APP/PS1 mice treated with vehicle (Veh) or MTEP. *t* test; *, $P < 0.05$; $N = 7$
169 per group. **c, d** Representative traces of SC-CA1 EPSCs evoked by stimulus
170 intensities of 30, 50, and 100 μA (**c**) and quantification of EPSC amplitude to stimulus
171 intensity (**d**) in APP/PS1 hippocampal slices treated with Veh or MTEP. Two-way

172 ANOVA with post hoc Bonferroni test; treatment, $F_{(1,88)}$ = 14.648, P < 0.001;

173 stimulation, $F_{(10,88)}$ =81.036, P < 0.001; **, P < 0.01; N = 5 per group. **e, f**

174 Representative traces (**e**) and quantification (**f**) of PPF of SC-CA1 EPSCs in APP/PS1

175 hippocampal slices treated with Veh or MTEP. *t* test; *, P < 0.05; N = 6-8 per groups.

176 Data are mean ± SEM. Source data are provided as a Source Data file.

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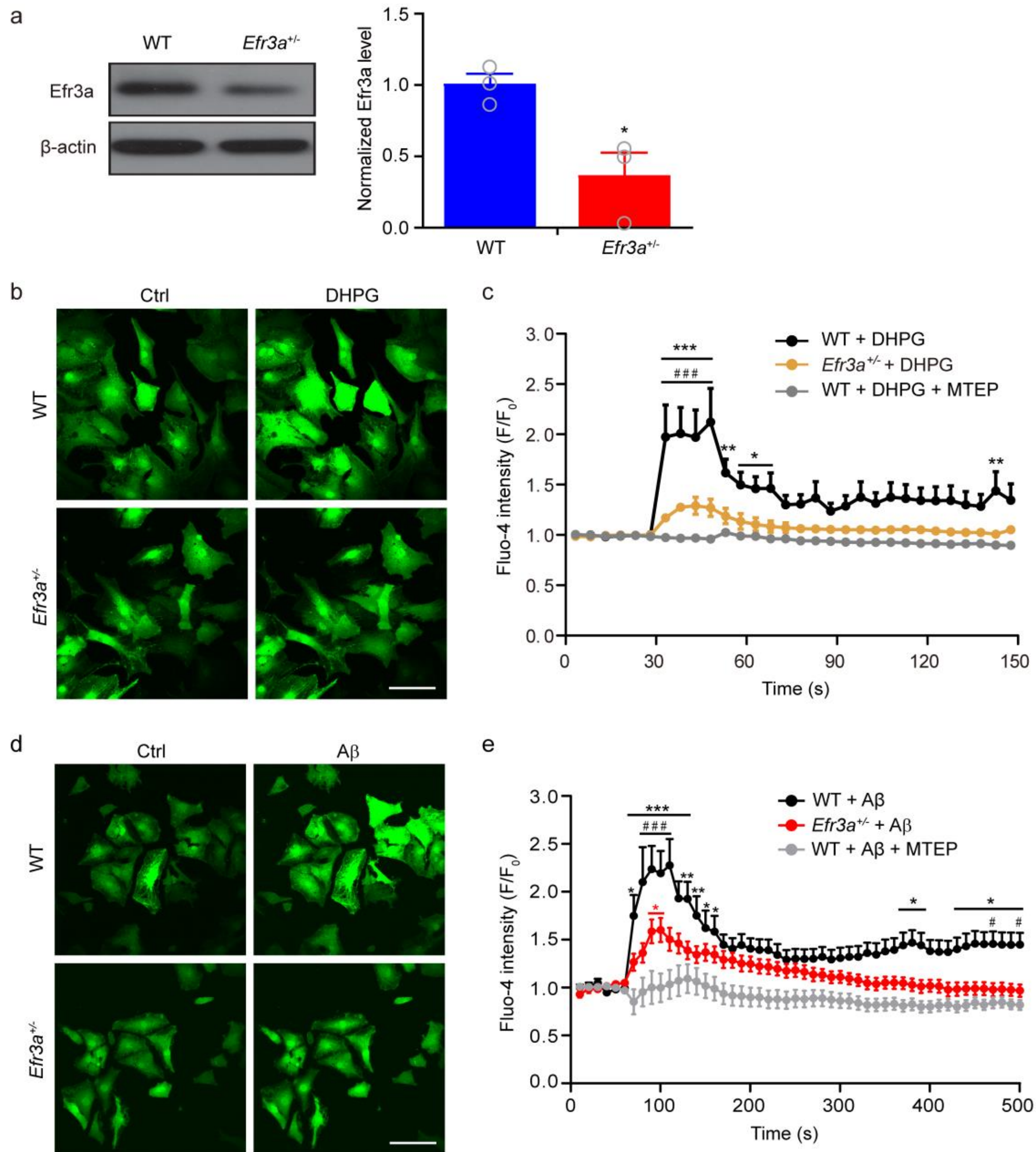

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180 **Supplementary Figure 12** DHPG- or oligomeric Aβ-induced, mGluR5-mediated

181 increase in $[Ca^{2+}]_i$ is downregulated by halving *Efr3a* copy number in astrocytes. **a**

182 Representative Western blots (left) and quantification (right) of Efr3a in the

183 hippocampus from WT and *Efr3a*$^{+/-}$ mice. *t* test; *, $P < 0.05$; $N = 3$ per group. **b-e**

184 Confocal images of cultured astrocytes from WT and *Efr3a*$^{+/-}$ mice stained with fluo-4

185 (**b, d**) and the time course (**c, e**) of the relative astrocytic fluo-4 intensity (F/F_0) in

186 response to control (Ctrl)/DHPG (**b, c**) or Ctrl/Aβ (**d, e**) treatment. MTEP blocks

187 DHPG- (**c**) or Aβ-induced (**e**) Ca^{2+} increase in astrocytes. Bars, 50 μm. Two-way RM

188 ANOVA with post hoc Bonferroni test; in **c**: group, $F_{(2,1769)} = 13.509$, $P < 0.001$; time,

189 $F_{(58,1769)} = 5.354$, $P < 0.001$; in **e**: group, $F_{(2,2744)} = 11.457$, $P < 0.001$; time, $F_{(98,2744)} =$

190 2.833, $P < 0.001$; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$ (compared with MTEP

191 groups in **c** and **e**); $^{\#}$, $P < 0.05$; $^{\#\#\#}$, $P < 0.001$ (WT + DHPG vs. *Efr3a*$^{+/-}$ + DHPG group

192 in **c**, WT + Aβ vs. *Efr3a*$^{+/-}$ + Aβ group in **d**); N = 12-25 per group. Data are mean ±

193 SEM. Source data are provided as a Source Data file.

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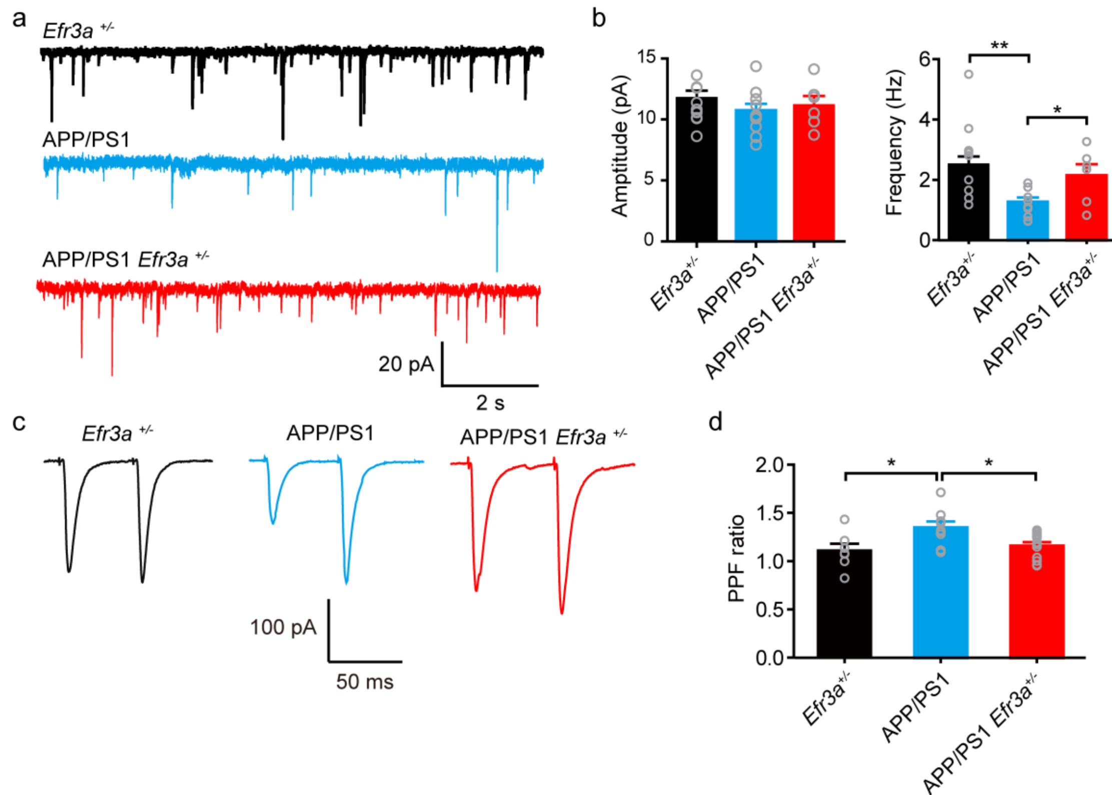

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196 **Supplementary Figure 13** Halving *Efr3a* copy number ameliorates early excitatory
197 synaptic deficits in APP/PS1 mice. **a, b** Representative traces (**a**) of mEPSCs in CA1
198 pyramidal neurons and quantification (**b**) of mEPSC amplitude (left) and frequency
199 (right) in 6-7-month-old WT, APP/PS1, and APP/PS1 *Efr3a*$^{+/-}$ mice. One-way ANOVA
200 with post hoc Dunnett's test; $F_{(2,24)}$ = 0.17 (amplitude); $F_{(2,24)}$ = 5.78 (frequency); *, P <
201 0.05, **, P < 0.01; N = 6-11 per group. **c, d** Representative traces (**c**) and
202 quantification (**d**) of PPF of SC-CA1 EPSCs in 6-7-month-old WT, APP/PS1, and
203 APP/PS1 *Efr3a*$^{+/-}$ mice. One-way ANOVA with post hoc Dunnett's test; $F_{(2,25)}$ = 4.9; *,
204 P < 0.05; N = 7-12 per group. Data are mean ± SEM. Source data are provided as a
205 Source Data file.

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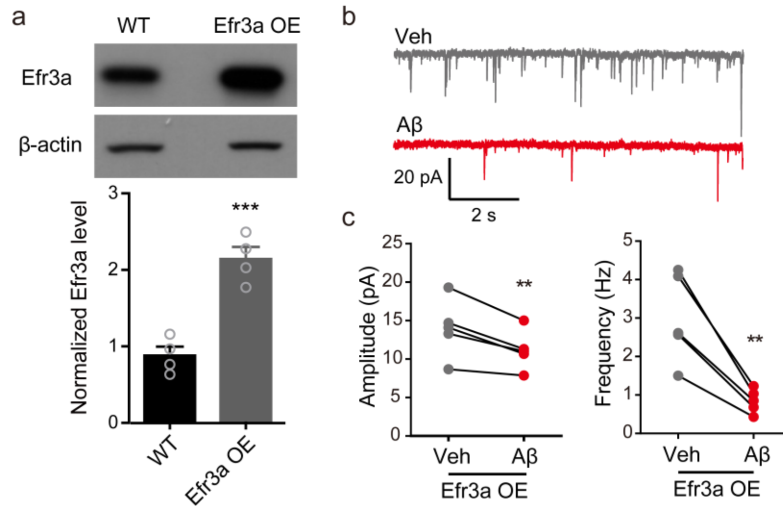

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209 **Supplementary Figure 14** Overexpression of *Efr3a* causes a more robust inhibition

210 of mEPSC frequency in CA1 pyramidal neurons treated with oligomeric Aβ. **a**

211 Representative Western blots of Efr3a (top) and quantification of Efr3a expression

212 (bottom) in the hippocampus from WT and Efr3a overexpression (OE) mice. *t* test; ***,

213 $P < 0.001$; $N = 4$ per group. **b, c** Representative traces (**b**) of mEPSCs in CA1

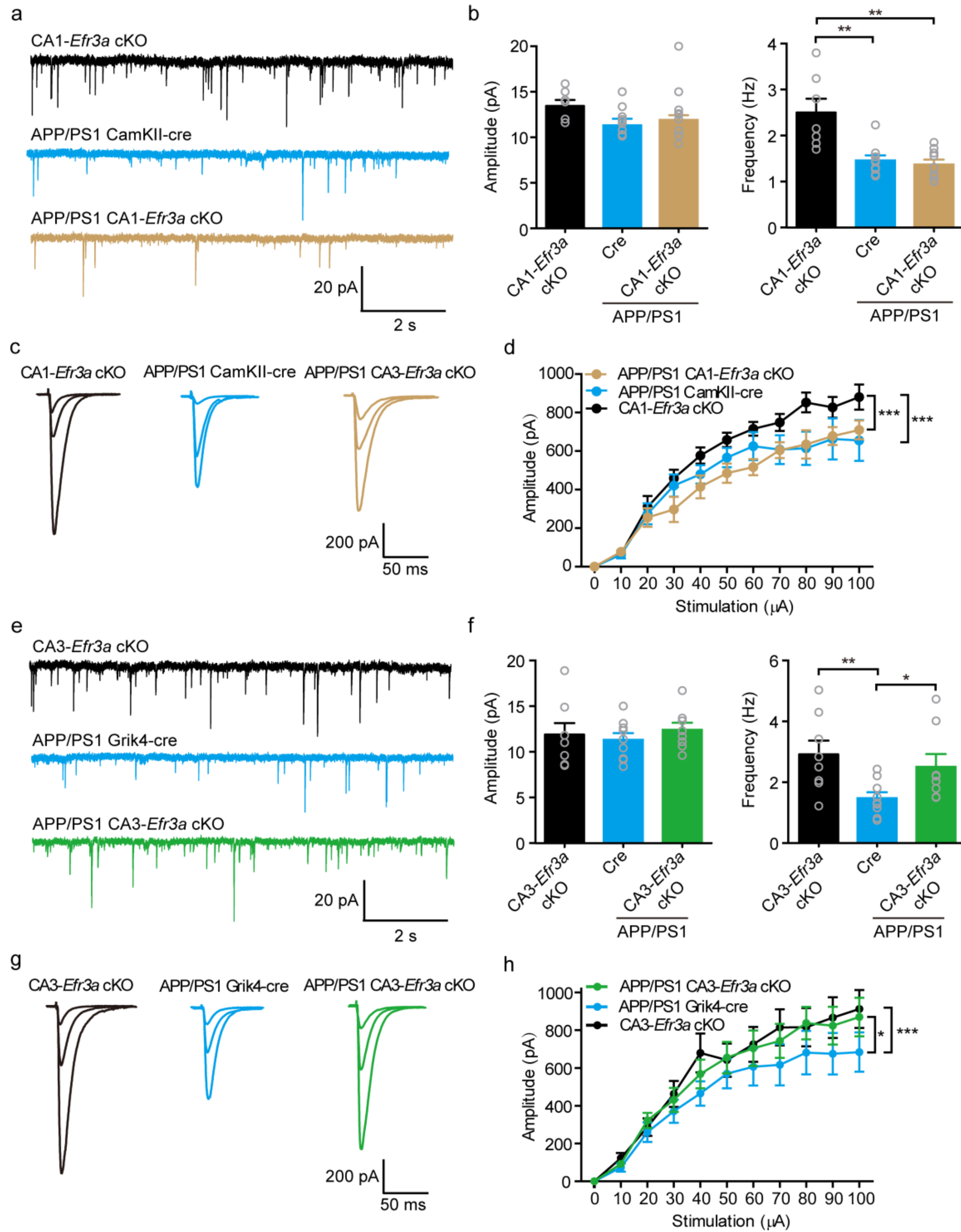
214 pyramidal neurons and quantification (**c**) of mEPSC amplitude (left) and frequency

215 (right) in hippocampal slices from Efr3a OE mice before (Veh) and after oligomeric

216 $A\beta_{42}$ (400 nM) treatment. *t* test; **, $P < 0.01$; $N = 5$ per group. Data are mean ± SEM.

217 Source data are provided as a Source Data file.

218



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221 **Supplementary Figure 15** Selectively knocking out *Efr3a* in CA3 area recues
222 excitatory transmission deficits in APP/PS1 mice. **a, b** Representative traces (**a**) of
223 mEPSCs in CA1 pyramidal neurons and quantification (**b**) of mEPSC amplitude (left)
224 and frequency (right) in 6-7-month-old CA1-*Efr3a* cKO, APP/PS1 CamKII-cre, and

225 APP/PS1 CA1-*Efr3a* cKO mice. One-way ANOVA with post hoc Dunnett's test; $F_{(2,22)}$ =
226 0.9 (amplitude); $F_{(2,22)}$ = 11.8 (frequency); **, $P < 0.01$; N = 7-9 per group. **c, d**
227 Representative traces of SC-CA1 EPSCs evoked by stimulus intensities of 10, 20,
228 and 100 μA (**c**) and quantification of EPSC amplitude to stimulus intensity (**d**) in
229 6-7-month-old CA1-*Efr3a* cKO, APP/PS1 CamKII-cre, and APP/PS1 CA1-*Efr3a* cKO
230 mice. Two-way ANOVA with post hoc Bonferroni test; animal, $F_{(2,176)}$ = 16.957, P <
231 0.001; stimulation, $F_{(10,176)}$ = 68.471, $P < 0.001$; ***, $P < 0.001$; N = 6-7 per group. **e, f**
232 Representative traces (**e**) of mEPSCs in CA1 pyramidal neurons and quantification (**f**)
233 of mEPSC amplitude (left) and frequency (right) in 6-7-month-old CA3-*Efr3a* cKO,
234 APP/PS1 Grik4-cre, and APP/PS1 CA3-*Efr3a* cKO mice. One-way ANOVA with post
235 hoc Dunnett's test; $F_{(2,22)}$ = 0.35 (amplitude); $F_{(2,22)}$ = 4.32 (frequency); *, $P < 0.05$; **,
236 $P < 0.01$; N = 8-9 per group. **g, h** Representative traces of SC-CA1 EPSCs evoked by
237 stimulus intensities of 10, 30, and 100 μA (**g**) and quantification of EPSC amplitude to
238 stimulus intensity (**h**) in 6-7-month-old CA3-*Efr3a* cKO, APP/PS1 Grik4-cre, and
239 APP/PS1 CA3-*Efr3a* cKO mice. Two-way ANOVA with post hoc Bonferroni test;
240 animal, $F_{(2,176)}$ = 7.037, P = 0.001; stimulation, $F_{(10,176)}$ = 32.706, $P < 0.001$; *, $P < 0.05$;
241 ***, $P < 0.001$; N = 6-7 per group. Data are mean ± SEM. Source data are provided as
242 a Source Data file.
243

244 **Supplementary Table 1.** The primers used in the study. TA, the optimal Annealing

245 Temperature.

Primer name		**Sequence**	**TA**
Efr3a flox	Efr3a_LoxP1-1-F	GCAGGACCACTGTTTTGGCTGC	64°C
	Efr3a_LoxP1-1-R	AGCCAGGGATGCGACTCATGA	
Efr3a KO	Efr3a_KO-1-F	TTATTTAGTATGTTGGACGAG	56°C
	Efr3a_KO-1-R	AACATGGAGGTTAAGTTTGT	
Efr3a KI	Efr3a_OE-1-F	CACTTGTAAGGAGTGGTGAAGGACCA	64°C
	Efr3a_OE-1-R	ATTGTGCAAGGCCCTGGGCTTAAT	
Grik4-Cre	Grik4-Cre-F	GCGGTCTGGCAGTAAAAACTATC	59°C
	Grik4-Cre-R	GTGAAACAGCATTGCTGTCACTT	
CamK2a-Cre	CamK2a-Cre-F	GACAGGCAGGCCTTCTCTGAA	61°C
	CamK2a-Cre-R	CTTCTCCACACCAGCTGTGGA	
APP/PS1	PSEN1dE9-F	GTGGATAACCCCTCCCCCAGCCTAGACC	64°C
	PSEN1dE9-R	AATAGAGAACGGCAGGAGCA	
	APP-F	GACTGACCACTCGACCAGGTTCTG	64°C
	APP-R	CTTGTAAGTTGGATTCTCATATCCG	
5xFAD	oIMR 3610-F	AGGACTGACCACTCGACCAG	54°C
	oIMR 3611-R	CGGGGGTCTAGTTCTGCAT	
	oIMR 1644-F	AATAGAGAACGGCAGGAGCA	54°C

	oIMR 1645-R	GCCATGAGGGCACTAATCAT	
M146V	oIMR 1586-F	AGGCAGGAAGATCACGTGTTCAAGTAC	69°C
	oIMR 1587-R	CACACGCACACTCTGACATGCACAGGC	

246

247

248 **Supplementary Table 2.** The drugs used in the study.

Reagents	**Company**	**Catalogue Number**
Tetrodotoxin	Abcam	ab120054
Bicuculline	Abcam	ab120108
β-amyloid$_{42}$	Thermo fisher scientific	1764958A
Mg-ATP	Sigma-Aldrich	P9187
Tris-GTP	Sigma-Aldrich	G9002
QX-314	Abcam	ab120117
Phosphocreatine di(tris) salt	Sigma-Aldrich	P1937
Tamoxifen	Sigma-Aldrich	T5648
PI(4,5)P$_2$ MASS ELISA KIT	Echelon Bioscience	K-4500
HFIP	Sigma-Aldrich	52517
DMSO	Sigma-Aldrich	D8418
DNQX	Abcam	ab120018
D-AP5	Abcam	ab120003
FM 1–43	Biotium	70030
ADVASEP-7	Biotium	70029
Fluo-4	Thermo fisher scientific	14201
DHPG	Abcam	ab120007
MTEP	Abcam	ab144307
U73122	Abcam	ab120998

$PI(4,5)P_2$ diC8	Echelon Bioscience	P-4508
PI4P diC16	Echelon Bioscience	P-4016
$PI(3,4)P_2$ diC16	Echelon Bioscience	P-3416
$PI(3,5)P_2$ diC16	Echelon Bioscience	P-3516
$PI(3,4,5)P_3$ diC16	Echelon Bioscience	P-3916
Sunflower oil	Sigma-Aldrich	S5007
Acrylamide	Shenggong (China)	A1032
N,N'-MethyleneBisacrylamide	Sigma-Aldrich	M7279
West Pico	Pierce	34078
West Femto	Pierce	34095
Ammonium Persulfate	Sigma-Aldrich	A3678
TEMED	Sigma-Aldrich	T9281
Glycine	Biosharp (China)	56-40-6
SDS	Sigma-Aldrich	L4390
Tris-Base	Sigma-Aldrich	T1503
Albumin bovine V (BSA)	BIOSHARP	Amresco0332
Skim milk powder	BIOFROX	1172GR100
Albumin from chicken egg white powder (Ovalbumin)	Sigma-Aldrich	A5253
Gelatin	Shenggong (China)	G9764
Neurobasal medium	Thermo fisher scientific	21103-049

Fetal bovine serum (FBS)	Thermo fisher scientific	10099-141
GlutaMax^{TM}	Thermo fisher scientific	35050-061
LY-411575	Sigma-Aldrich	SML0506

249

250 **Supplementary methods**

251 **Animals**

252 All procedures were carried out in accordance with the National Institutes of Health
253 Guidelines for the Care and Use of Laboratory Animals and were approved by the
254 Animal Advisory Committee at Zhejiang University. *Efr3a* double-flox (*Efr3a$^{f/f}$*) mice
255 were generated as reported by Qian et al.[1] , and *Efr3a$^{+/-}$* heterozygotes were
256 generated by breeding *Efr3a$^{f/f}$* mice to EIIa-cre mice as described in a previous report[2].
257 B6 (stock number 000664), APP/PS1 double-transgenic [B6.Cg-Tg
258 (APPswe,PSEN1dE9)85Dbo/Mmjax, stock number 34832], 5xFAD
259 [B6.Cg-Tg(APPSwFlLon,PSEN1*M146L*L286V)6799Vas/Mmjax, stock number
260 34848], PS1M146VKI [B6.129-Psen1^{tm1Mpm}/J stock number 004193], Grik4-cre
261 [C57BL/6-Tg(Grik4-cre)G32-4Stl/J, stock number 006474], and Camk2a-creERT2
262 [B6;129S6-Tg(Camk2a-cre/ERT2)1Aibs/J, stock number 012362] mice were
263 purchased from The Jackson Laboratory (Bar Harbor, ME). *Efr3a$^{f/f}$*, *Efr3a$^{f/f}$*-Grik4-cre,
264 *Efr3a$^{f/f}$*-Camk2a-creERT2, APP/PS1-*Efr3a$^{f/f}$*-Grik4-cre, APP/PS1-Grik4-cre,
265 APP/PS1-*Efr3a$^{f/f}$*-Camk2a-creERT2, and APP/PS1-Camk2a-creERT2 animals were
266 obtained by heterozygous mating. The transgenic strain overexpressing Efr3a was
267 generated with a BAC transgenic construct containing the genomic DNA of *Efr3a*

268 carrying the miss sense mutation of S356A. Tamoxifen was intraperitoneally (i.p.)
269 injected once a day for 5 consecutive days at a dose of 100 mg per kg to induce cre
270 recombinase expression in the creER lines. *Efr3a*$^{+/-}$ mice embryos for primary neuron
271 and astrocyte cultures were obtained from mating *Efr3a*$^{+/-}$ to B6 WT mice. All mice
272 were housed at the Animal Facility of Zhejiang University under a 12-h light/dark cycle
273 and had access to food and water ad libitum. For behavioral experiments, only male
274 mice were used. The mouse genotypes were identified by PCR using genomic DNA
275 from mouse tails and embryo tissues. The primers information can be found in
276 Supplementary Table 1.

277 To inhibit γ-secretase, we treated mice with LY-411575 (SML0506, Sigma-Aldrich).
278 LY-411575 was dissolved in DMSO at 100 mg per ml, and was then emulsified in
279 sunflower oil (S5007, Sigma-Aldrich) at 1 mg per ml. γ-secretase inhibitor treatment
280 was carried out as previously described[3]. Briefly, mice were injected subcutaneously
281 once daily for 2 days with 3 mg per kg LY-411575 or vehicle (sunflower oil). 8 to 10 h
282 after the injection, mice were sacrificed for electrophysiology recording.

283 **Antibodies and drugs**

284 The following commercially available antibodies were used: rabbit anti-amyloid
285 precursor protein/C-terminal fragments (anti-CTFs, A8717, Sigma-Aldrich, 1:6000),
286 purified mouse anti-β-Amyloid, 1-16 (6E10, 803003, Biolegend; 1:1000 for Western
287 blot; 2 μg per ml for slice recordings as previously reported[4, 5]), rabbit anti-Efr3a
288 (HPA023402, Sigma-Aldrich, 1:1000), mouse anti-PLCβ1, D-8 (sc-5291, Santa Cruz,
289 1:200), mouse anti-PLCβ4, A-8 (sc-16613, Santa Cruz, 1:100), mouse anti-β-actin

290 (TA-09, ZSGB-BIO, 1:20000), and HRP-conjugated secondary antibodies [(Goat
291 anti-rabbit IgG (H+L), 31460, 1:20000; goat anti-mouse IgG (H+L), 31430, 1:20000;
292 Thermo fisher scientific); goat anti-mouse IgG (H+L), 70-GAM007, Multi Sciences,
293 1:5000] were used in Western blotting; mouse anti-PIP_2 antibody (ab11039, Abcam,
294 1:200), chicken anti-neurofilament-L (anti-NF, CH22105, Neuromics, 1:250), rabbit
295 anti-MAP2 antibody (ab32454, Abcam, 1:8000), rabbit anti-mGluR5 (extracellular)
296 (AGC-007, Alomone labs, 1:50), mouse anti-MAP2 (M4403, Sigma-Aldrich, 1:1000),
297 purified anti-CD230 (Prion) antibody (6D11, 808001, Biolegend, 5 μg per ml), and
298 Alexa Fluor-conjugated secondary antibodies [488 donkey anti-rabbit (A-21206), 546
299 donkey anti-mouse (A-10036), 546 donkey anti-rabbit (A-10040) (all from Thermo
300 fisher scientific, 1:1000), 405 goat anti-rabbit IgG H&L (ab175654, Abcam, 1:500),
301 488 goat anti-mouse IgM mu chain (ab150121, Abcam, 1:500), and 647 goat
302 anti-chicken IgY H&L (ab150175, Abcam, 1:1000)] were used in
303 immunocytochemistry.

304 To prepare oligomeric $A\beta_{42}$[6], the $A\beta_{42}$ lyophilized powder (03112, Thermo Fisher
305 Scientific) was first suspended in 100% 1,1,1,3,3,3-hexafluoro-2-propanol (HFIP,
306 52517, Sigma-Aldrich) at a concentration of 1 mM. The $A\beta_{42}$-HFIP solution was then
307 incubated in polypropylene vials for complete solubilization at room temperature (RT)
308 for 2 h. HFIP was allowed to evaporate under a slight stream of nitrogen until a clear
309 peptide film was observed at the bottom of the vials. The vials were stored at -80°C
310 until use. Twelve hours before experiments, the film was re-suspended by adding
311 DMSO (D8418, Sigma-Aldrich) at a concentration of 5 mM and sonicated at RT for 10

312 min. The $A\beta_{42}$-DMSO solution was diluted 12 times in sterile PBS or culture medium
313 and incubated at 4°C for 12 h. Following a 5 min centrifugation at 14,000 g, the
314 concentration of the supernatant (~ 100 μg per ml) was determined by a microplate
315 reader (SpectraMax 190, Molecular Devices) and the oligomeric $A\beta_{42}$ solution was
316 diluted to 400 nM (based on monomeric $A\beta_{42}$) accordingly. To prepare fibrillar $A\beta_{42}$, the
317 $A\beta_{42}$-DMSO solution was diluted 12 times in 10 mM HCl and incubated for 24 h at
318 37°C.

319 Tamoxifen (T5648, Sigma-Aldrich) was dissolved in 100% ethanol at 100 mg per
320 ml, and then was emulsified in sunflower oil (S5007, Sigma-Aldrich) at 10 mg per ml
321 and vortexed for 5-10 min until the solution was clear. The stock solution was
322 aliquoted and stored at -20°C. For slice recordings, $PI(4,5)P_2$ diC8 (PIP_2, P-4508,
323 Echelon Bioscience) was dissolved in the electrode solution at final concentration of
324 200 μM. For ELISA assay, $PI(4,5)P_2$ diC8, PI4P diC16 (P4016), $PI(3,4)P_2$ diC16
325 (P-3416), $PI(3,5)P_2$ diC16 (P-3516), and $PI(3,4,5)P_3$ diC16 (P3916; all from Echelon
326 Bioscience) were dissolved in PBS containing 0.25% Protein Stabilizer (PBS
327 0.25%PS) at final concentrations of 10, 50, and 250 pM. DHPG (ab120007, Abcam)
328 was dissolved in the bath solution or culture medium and applied at a final
329 concentration of 50 μM. MTEP (ab144307, Abcam) and U73122 (ab120998, Abcam)
330 were dissolved in DMSO and applied to the bath solution or culture medium at a final
331 concentration of 10 μM. In the Ca^{2+} imaging experiment, DHPG was applied at a final
332 concentration of 100 μM. Drugs were added in the ACSF perfusate in slice recording
333 experiments and in the culture medium 24 h before various experiments performed on

334 cell cultures. The final concentrations of DMSO did not exceed 0.1% throughout the
335 study. Drugs used in the study are described in Supplementary Table 2.

336 **Golgi staining**

337 Golgi stainings were carried out using an FD Rapid GolgiStain Kit (PK401, FD
338 NeuroTechnologies) according to the manufacturer's instructions[7]. In brief,
339 nonperfused mouse (WT and APP/PS1; 6-7-month-old) brains were immersed in
340 impregnation solution for 2 weeks, and then transferred to "Solution C" for 2 days.
341 Sections of 200 μm thickness were serially cut with a freezing microtome (CM30503,
342 Leica). Sections were mounted on 3% gelatin-coated slides and allowed to dry for 2
343 weeks before being stained with silver nitrate solution "Solution D and E", dehydrated
344 through descending alcohol series, and mounted with Permount. Images were
345 acquired with an Olympus BX53 microscope at RT, and neuronal morphology analysis
346 was performed using the NIH ImageJ software. At least 5 pyramidal neurons in the
347 hippocampal CA1 region per mouse were randomly selected. The density of dendritic
348 spines of a distinct branch was measured by a 100X oil-immersion objective
349 (numerical aperture 1.3). Spines visible along both sides of dendritic segments were
350 counted and expressed as mean number of spines per micrometer. Student t-test was
351 used to determine significant levels between the WT and APP/PS1 groups.

352 **Slice recording**

353 Hippocampal brain slices were prepared from 4- or 6-7-month-old mice. For
354 whole-cell recordings, mice were anesthetized with isoflurane and brains were
355 dissected rapidly and immersed in ice-cold and oxygenated (95% O_2 / 5% CO_2) ACSF

356 (in mM: 124 NaCl, 2 KCl, 2 $MgSO_4$, 1.25 KH_2PO_4, 2 $CaCl_2$, 26 $NaHCO_3$, 10 D-glucose,
357 pH 7.4, 300 mOsm). Transverse slices of hippocampus (300 μm) were cut with a
358 tissue slicer (VT 1200S, Leica) in oxygenated ACSF. Slices were allowed to recover
359 ~12 min in ACSF with low Na^+ and Ca^{2+} concentrations (in mM: 110
360 N-methyl-D-glucamine, 110 HCl, 2.5 KCl, 1.2 NH_2PO_4, 10 $MgSO_4$, 0.5 $CaCl_2$, 25
361 $NaHCO_3$, 25 D-glucose, pH 7.4, 300 mOsm) at 32°C, and subsequently in normal
362 ACSF for 1 h at RT. For fEPSP recordings, mouse brains were dissected rapidly and
363 immersed in ice-cold and oxygenated cutting solutions (in mM: 234 Sucrose, 5 KCl,
364 1.25 NaH_2PO_4, 5 $MgSO_4$, 26 $NaHCO_3$, 25 Dextrose, 1 $CaCl_2$, balanced with 95% O_2 /
365 5% O_2). Transverse brain slices (350 μm) containing the hippocampus were
366 incubated in oxygenated ACSF and were allowed to recover ~25 min in ACSF at 32°C,
367 and subsequently for ≧1 h at RT.

368 Whole-cell recordings were performed as previously described[7, 8]. Hippocampal
369 slices were transferred to the recording chamber at 32°C and perfused continuously
370 with ACSF bubbled with 95% O_2 / 5% CO_2 to ensure adequate oxygenation of slices.
371 CA1 pyramidal neurons were identified under infrared differential interference contrast
372 (IR-DIC) optics based on their location and morphology. Borosilicate glass (Sutter
373 instruments) pipettes (3 - 5 MΩ) were pulled with a horizontal pipette puller (P97,
374 Sutter instruments) and were filled with artificial intracellular fluid (in mM: 100
375 $CsCH_3SO_3$, 20 KCl, 10 HEPES, 4 Mg-ATP, 0.3 Tris-GTP, 7 $Tris_2$-Phosphocreatine, 3
376 QX-314; pH 7.3, 285-290 mOsm). Pipettes were connected to the headstage of a
377 Heka EPC 10 amplifier (Heka Elektronik), and fast and slow capacitances as well as

378 series resistance compensations were carefully adjusted. Liquid junction potentials
379 were not corrected. mEPSC signals were recorded at -70 mV in ACSF containing 0.5
380 μM tetrodotoxin (TTX; Abcam) and 10 μM bicuculline (Abcam). Series resistance was
381 normally less than 20 MΩ and recordings exceeding 20% change in series resistance
382 were terminated and discarded. Recordings were filtered at 2.0 kHz and digitized at
383 10 kHz.

384 Evoked EPSCs were elicited in the presence of 10 μM bicuculline using a bipolar
385 stimulating electrode (CE2C75, FHC Inc.) placed in stratum radiatum 300 μm away
386 from the recording site. The rectangle current pulses (duration: 180 μs, frequency: 0.1
387 Hz) were delivered via a constant-current stimulator (SIU91A, Cygnus Technology).
388 PPF experiments were carried out by delivering a pair of stimuli with an interval of 50
389 ms. PPF was assessed by the paired-pulse ratio (the second EPSC amplitude / the
390 first EPSC amplitude). To estimate the RRP size and release probability, a repeated
391 (10-20 repeats, 0.033 Hz) 20 Hz train stimulation (40 stimuli) protocol was used to
392 evoke 40 EPSCs. To effectively discharge the RRP, a slightly higher stimulation
393 intensity than the minimal stimulation was used to give about 5% failures. The RRP
394 size was calculated by linear interpolating the linear portion of the cumulative EPSC
395 amplitude plot to virtual stimulus 0. The release probability was calculated as
396 the mean amplitude of the 1st EPSC during the repeated train stimulations divided by
397 the RRP size.

398 Field EPSPs were elicited by stimulating the SC and recording with a borosilicate
399 glass electrode filled with ACSF placed in CA1 stratum radiatum. Baseline and tetanic

400 stimulations were delivered by a bipolar stimulating electrode (CE2C75, FHC Inc.)
401 placed 200 - 300 μm away from the recording electrode. To record baseline
402 responses before LTP induction, the intensity of each stimulus was adjusted to evoke
403 fEPSPs with an amplitude 30 - 50% of the maximum. We also adjusted the amplitude
404 of the baseline fEPSPs in control groups to match that in groups with reduced fEPSPs.
405 Baseline fEPSPs were evoked at 0.05 Hz and recorded for at least 20 min (response
406 variability < 10%). Three bursts of 20 pulses at 100 Hz separated by 1.5 s were
407 delivered to induce LTP as previously described[7]. We recorded LTP in at least 7 slices
408 from 3 - 4 mice for each group. Field EPSPs were recorded, filtered (1 kHz), and
409 sampled (20 kHz) by a Heka EPC 10 amplifier.

410 To investigate synthetic Aβ-induced alterations in mEPSCs, evoked EPSCs, and
411 short- and long-term plasticity, we perfused hippocampal slices with Aβ-containing
412 ACSF for at least 20 min before acquiring data, unless the time course data were
413 taken.

414 **Cell culture**

415 Primary hippocampal neuron cultures were prepared from embryonic day 18 (E18)
416 mice[7]. Briefly, embryos were removed from maternal mice anesthetized with
417 isoflurane and euthanized by decapitation. Hippocampi were dissected and placed in
418 Ca^{2+}- and Mg^{2+}-free HEPES-buffered Hank's balanced salt solution (HBSS; pH 7.45),
419 followed by a digestion with 0.25% w/v trypsin. After trituration through a Pasteur
420 pipette, neurons were centrifuged (1000 g for 5 min) and resuspended in Neurobasal
421 medium containing 2% B27 serum-free supplement, 1% v/v penicillin/streptomycin

422 (P/S), 0.5 mM glutamine, and 10 μM glutamate (Sigma-Aldrich). Dissociated cells
423 were then plated at a density of 0.03 - 0.05 x 10^6 cells per cm^2 onto 12 mm round
424 coverslips in 24-well plates (Corning Costar®, for lentivirus infection and
425 immunofluorescence staining), glass bottom confocal dishes (801002, NEST
426 Biotechnology, for vesicle detection) or 6-well plates (Corning Costar®, for Western
427 blotting and ELISA assay) pre-coated with poly-D-lysine (PDL, 50 μg per ml;
428 Sigma-Aldrich). Cultures were kept at 37°C in a 5% v/v CO_2 humidified incubator.
429 Thereafter, one third to half of the medium was replaced twice a week with
430 Neurobasal culture medium containing 2% B27 supplement and 0.5 mM glutamine.

431 Single-cell micro-island cultures of hippocampal neurons were prepared from
432 E18 mice[7]. Briefly, 6.5 mm Transwell® inserts (pore size 0.4 μm; BD Biosciences) in
433 24 well plates were coated with PDL (12 h before culture), and coverslips were
434 sprayed with island substrate solution containing 1 mg per ml PDL and 3 mg per ml rat
435 tail collagen (A1048301, Thermo Fisher Scientific) using a presterilized glass atomizer
436 (3 h before culture). Dissociated cells for micro-island cultures were similarly prepared
437 as dissociated cells for primary hippocampal neuron cultures. Dissociated cells were
438 then plated at a density of 2000 cells per cm^2 onto coverslips in 24-well plates (for
439 micro-island culture) or at a density of 50000 cells per cm^2 in Transwell® inserts in
440 24-well plates (as high density neuronal feeder layer). After an adherence time of 4 h,
441 the transwell inserts with neurons (high density) were placed into 24-well plates with
442 neurons on coverslips (low density). The low density system can facilitate the survival
443 of sparse individual neurons grown in islands of PDL substrates and thus the

444 formation of autaptic connections. In addition, co-culture with high density neurons
445 may allow low density neurons to receive trophic support that is sufficient to enable
446 long term survival. Cultures were kept at 37°C in a 5% v/v CO_2 humidified incubator.
447 Thereafter, one third to half of the medium was replaced every five days with
448 Neurobasal culture medium containing 2% B27 supplement and 0.5 mM glutamine.

449 Astrocyte cultures were prepared from 0-1-day-old (P0-1) mice[7]. Cortices were
450 dissected from 0-1-day-old mice and digested with 0.25% w/v trypsin in DMEM.
451 Tissue was triturated and resuspended after centrifugation in astrocyte culture
452 medium [DMEM containing 10% v/v fetal bovine serum (FBS), 1% v/v P/S]. Cells were
453 plated in T-75 flasks at a density of 2 cortices per flask (Corning Costar®) pre-coated
454 with PDL. Cells were grown for at least 7 days at 37°C with 5% v/v CO_2, and a
455 complete medium change was performed every other day. At confluence after
456 DIV8-10, cultures were shaken for 12-16 h at 250 g at 37°C on an orbital shaker
457 (KS4000i Control Incubating Shaker, IKA) followed by an incubation in culture
458 medium containing 20 μM cytosine-1-β-D-arabinofuranosid (Sigma-Aldrich) for 2-3
459 days to deplete the precursor cells and to achieve a confluent layer of astrocytes.
460 Astrocytes were then subcultured in PDL-coated glass bottom confocal dishes via
461 enzymatic digestion with 0.05% w/v trypsin with EDTA for Ca^{2+} imaging experiments.

462 **FM1-43 loading and synaptic vesicle detection**

463 Cultured neurons (DIV14) were transferred into a standard bath solution containing (in
464 mM): 145 NaCl, 5 KCl, 2 $CaCl_2$, 2 $MgCl_2$, 10 glucose and 10 HEPES. We also added
465 10 μM DNQX (Abcam) and 40 μM D-AP5 (Abcam) to the bath to inhibit AMPA and

466 NMDA receptors. Neurons were then incubated with 5 μM FM1-43 (70030, Biotium) in
467 a hyperkalemic bath solution (in mM: 31.5 NaCl, 90 KCl, 2 $CaCl_2$, 2 $MgCl_2$, 25 HEPES
468 and 30 glucose) for 90 s. Neurons were then perfused with the normal bath solution
469 for 10 min followed by adding ADVASEP-7 (1 mM, 70029, Biotium) for 60 s to reduce
470 background fluorescence. After an additional 10 min wash with the normal bath
471 solution, images were taken by a confocal laser-scanning microscope (Nikon A1).
472 FM1-43-loaded vesicles were viewed through a 40X oil-immersion objective
473 (numerical aperture 1.3) and images were acquired at a resolution of 1024 X 1024
474 pixel at RT.

475 **Western blotting**

476 Hippocampi were obtained and homogenized using a chilled Vibrahomogenizer (Vibra
477 cell, SONICS) in 2 ml of RIPA buffer [1% Triton X-100, 0.1% SDS, 150 mM NaCl, 2
478 mM EDTA, 50 mM NaF, 10 mM sodium pyrophosphate, 1.0 mM Na_3VO_4, 1.0 mM
479 PMSF, and complete protease inhibitor cocktail (Roche)]. The lysate was then
480 centrifuged at 20,000 g for 20 min at 4°C and the supernatant collected for Western
481 blot analysis. The protein concentration of the probes was determined using the
482 Pierce™ BCA Protein Assay Kit (Thermo Fisher Scientific) and the tubes were stored
483 at -20°C. Proteins were separated on SDS-PAGE under denaturing conditions (for
484 Efr3a, 10-15% Mini-PROTEAN TGX Gels, Bio-Rad; for Aβ and CTFs, 16.5%
485 Tris-Tricine Gels, WSHT Biotech Inc.) and transferred to polyvinylidene fluoride
486 (PVDF) microporous membrane (Millipore). The membranes were then blocked with
487 5% skim milk-TBS (for Aβ) or 0.35% gelatin-TBST (for other proteins) at RT for 1.5 h

488 and incubated with the primary antibodies over night at 4°C followed by
489 HRP-conjugated secondary antibodies (Thermo Fisher Scientific) at RT for 1.5 h.
490 Protein bands were then visualized using the ECL Western blotting detection
491 substrate (Thermo Fisher Scientific). Densitometric analyses were determined using
492 ImageJ software and normalized to β-actin.

493 **Cultured neuron recording**

494 Single-cell micro-island neuron cultures at DIV14 were transferred to a chamber
495 perfused with the standard bath solution containing (in mM): 145 NaCl, 5 KCl, 2 $CaCl_2$,
496 2 $MgCl_2$, 10 glucose and 10 HEPES (pH 7.40, 290 - 310 mOsm). Neurons were
497 recorded with patch pipettes (4 - 6 MΩ) filled with artificial intracellular fluid (in mM:
498 100 $CsCH_3SO_3$, 20 KCl, 10 HEPES, 4 Mg-ATP, 0.3 Tris-GTP, 7 $Tris_2$-Phosphocreatine,
499 3 QX-314; pH 7.3, 285-290 mOsm). Neurons were voltage clamped at -70 mV with a
500 Heka EPC 10 amplifier and mEACs were recorded at 32°C in bath solution containing
501 0.5 μM TTX and 10 μM bicuculline. Individual events were counted and analyzed with
502 MiniAnalysis software.

503 **Lipid strip assay**

504 PIP Strips ™ membranes (P23751, Thermo Fisher Scientific) were blocked with 0.1%
505 ovalbumin (albumin from chicken egg white powder, A5253, Sigma-Aldrich) in TBST
506 for 1 h at RT, and then incubated with 50 ng per ml anti-PIP_2 antibody in 0.1%
507 ovalbumin in TBST at RT for 1 h. After being washed with TBST for three times,
508 HRP-conjugated secondary antibody was added at RT for 1 h. Protein bands were
509 then visualized using the ECL western blotting detection substrate (Thermo Fisher

510 Scientific).

511 **ELISA PIP_2 assay**

512 Mass ELISA Kit K-4500 from Echelon Biosciences was used to determine PIP_2 levels
513 in hippocampi from WT and APP/PS1 mice (6-7-month-old) and in primary cultured
514 hippocampal neurons from WT and *Efr3a*$^{+/-}$ mice[9]. Briefly, hippocampi were dissected
515 and cut into small pieces using a pair of ophthalmic scissors, and were then
516 transferred into a 1.5 ml centrifuge tube containing 1 ml ice-cold 0.5 mM
517 trichloroacetic acid (TCA) immediately. Cultured neurons were incubated in TCA on
518 ice for 5 min after the medium was carefully aspirated, and then were collected and
519 transferred into a 1.5 ml centrifuge tube. The tubes were then centrifuged at 3000 g
520 for 7 min at 4°C. The pallet was resuspended in 1 ml 5% TCA/1 mM EDTA solution
521 and vortexed for 30 s. After centrifuging twice at 3000 g for 5min, the pallet was
522 resuspended in the MeOH: $CHCl_3$: 12 N HCl (80:40:1; 750 μl) solution and vortexed
523 for 25 min at RT to extract lipids. After centrifuging again at 3000 g for 5 min, the
524 supernatant was transferred to a 2 ml centrifuge tube and 250 μl $CHCl_3$ and 450 μl 0.1
525 mM HCl were added. After vortexing for 30 s, the organic and aqueous phases were
526 separated by centrifuging at 3000 g for 5min. The organic (lower) phase (0.5 ml) was
527 collected and transferred into a 1.5 ml centrifuge tube and dried under a slight stream
528 of nitrogen. PIP_2 and other PIPs [PI4P, $PI(3,4)P_2$, $PI(3,5)P_2$, and $PI(3,4,5)P_3$] were
529 then detected according to the manufacturer's instructions.

530 **Immunocytochemistry**

531 Immunofluorescence staining was carried out in cultured neurons at DIV14 (or DIV12

532 for lentivirus-infected neurons)[7]. Briefly, neurons were fixed by 4% paraformaldehyde
533 in PBS for 15 min and permeabilized by 0.2% Triton X-100 for 10 min. Neurons were
534 blocked with 10% BSA in PBS for 2.5 h at RT, and then incubated with mouse
535 anti-PIP_2 antibody in PBS containing 3% BSA for 1 h. After being washed with PBS for
536 three times, fluorescent secondary antibody (Alexa Fluor 488 goat anti-mouse IgM mu
537 chain) was added for 1 h. After being washed with PBS three times, neurons were
538 then blocked again with 10% BSA for 1.5 h and then were incubated with rabbit
539 anti-MAP2 and chicken anti-NF antibodies for 1 h. After being washed again with PBS
540 for three times, fluorescent secondary antibodies [Alexa Fluor 546 anti-rabbit IgG (or
541 Alexa Fluor 405 anti-rabbit IgG for infected neurons) and 647 anti-chicken IgY H&L]
542 were added for 1 h. Neurons were mounted with mounting reagent for subsequent
543 fluorescent image acquisition after being washed with PBS to remove unbound
544 secondary antibodies. For co-staining with mGluR5, neurons were permeabilized
545 before the second blocking step, and the following antibodies were used: mouse
546 anti-MAP2, chicken anti-NF, rabbit anti-mGluR5, Alexa Fluor 546 anti-mouse, 647
547 anti-chicken IgY H&L, and 488 anti-rabbit IgG. Fluorescent images were acquired
548 through a 60X oil-immersion objective (numerical aperture 1.4) using a Nikon A1
549 confocal laser-scanning microscope. Gain, threshold, and black levels were not
550 subjected to change during individual experiments. Neuronal images were analyzed
551 using MetaMorph with customized filter sets. All image analysis was done blind to the
552 experimental condition.

553 **Lentivirus-shRNA infection**

554 To knock down PLCβ1 or PLCβ4 in primary hippocampal neurons, we infected
555 hippocampal neuron cultures with lentiviruses carrying DsRed-PLCβ1-shRNA
556 (PLCβ1-shRNA), DsRed-PLCβ4-shRNA (PLCβ4-shRNA), or DsRed-scramble-shRNA
557 (Ctrl-shRNA) that were chemically synthesized by Obio Technology (Shanghai) Corp.,
558 Ltd. Target sequences for PLCβ1, PLCβ4 and Ctrl siRNAs are
559 GCTGTCTTTGTCTACATAGAA (GenBank accession number: NM_019677),
560 GCGACAAATGAGCCGCATT (GenBank accession number: NM_013829), and
561 TTCTCCGAACGTGTCACGT, respectively. Hippocampal neurons at DIV7 were
562 infected with lentiviruses according to the vendor's protocol. Neurons were then
563 treated with Aβ or DMSO 72 h after lentiviral infection, and were subsequently used
564 for Western blotting (interference efficiency detection) or immunofluorescence
565 staining.

566 **Ca^{2+} imaging**

567 Changes in $[Ca^{2+}]_i$ were measured in cultured astrocytes from WT and *Efr3a*$^{+/-}$ mice
568 using the calcium-sensitive fluorescent dye Fluo-4 (14201, Thermo Fisher Scientific).
569 Astrocyte cultures were washed with Krebs buffer (in mM: 118 NaCl, 4.7 KCl, 4
570 $NaHCO_3$, 1.2 $MgSO_4$, 1.2 KH_2PO_4, 8.5 HEPES, 1.3 $CaCl_2$, 11.7 glucose, pH 7.4) and
571 incubated with 4 μM Fluo-4 in Krebs buffer first at 37°C for 15 min, then at RT for 15
572 min. Astrocytes loaded with Fluo-4 were excited at 488 nm and fluorescence emission
573 was detected at 525 nm. The images were taken through a 60X oil-immersion
574 objective (numerical aperture 1.4) by a Nikon A1 confocal laser-scanning microscope.
575 After baseline data (F_0) were taken, we recorded fluorescent signals (F) in the

576 presence of 100 μM DHPG or 400 nM oligomeric Aβ. The relative Fluo-4 fluorescent
577 signals expressed in arbitrary units (F/F_0) were analyzed for individual cells using
578 MetaMorph with a fixed set of parameters.

579 **MWM test**

580 The MWM tests were performed in a circular tank (120 cm in diameter and 60 cm in
581 height) filled with opaque water at 25°C. The tank was divided into four quadrants with
582 different navigation landmarks for each quadrant. 24 h before the acquisition test, a
583 visible platform task was performed by measuring the time spent to find a colorful flag
584 placed on the top of a platform in a quadrant. The visible platform task was tested in
585 each quadrant to avoid habituation. In the hidden platform acquisition test, mice were
586 allowed to swim freely to search for the escape platform within 60 s. The platform
587 location remained constant throughout the test. The time taken to reach the platform
588 was recorded as the escape latency. The mouse was allowed to stay on the platform
589 for 10 s after the hidden platform was found. If a mouse failed to find the platform
590 within 60 s, the mouse was guided to the platform and stayed on the platform for 10 s,
591 and the escape latency was recorded as 60 s for this trial. The same animal was then
592 released from a new insertion point 4 min after the previous trial. The experiment was
593 repeated 4 times per mouse each day for 5 days. The four animal insertion points
594 were chosen to maintain a constant distance to the platform. The mean escape
595 latency was calculated to evaluate the spatial learning ability. 24 h after the hidden
596 platform acquisition test, probe trials were conducted by removing the platform. Mice
597 were placed in the diagonal quadrant of the hidden platform originally located and

598 were allowed to swim freely in the pool for 60 s. The numbers of entries into the area

599 where the original platform was located and crossings over the original platform were

600 recorded. The data were analyzed by the WaterMaze Software (Actimetrics, INC.).

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