

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30

Supplementary Information

**Requirement of hippocampal DG nNOS-CAPON dissociation for the
anxiolytic and antidepressant effects of fluoxetine**

Hu-Jiang Shi^{1#}, Dan-Lian Wu^{2#}, Rong Chen^{1#}, Na Li¹, Li-Juan Zhu^{1, 3*}

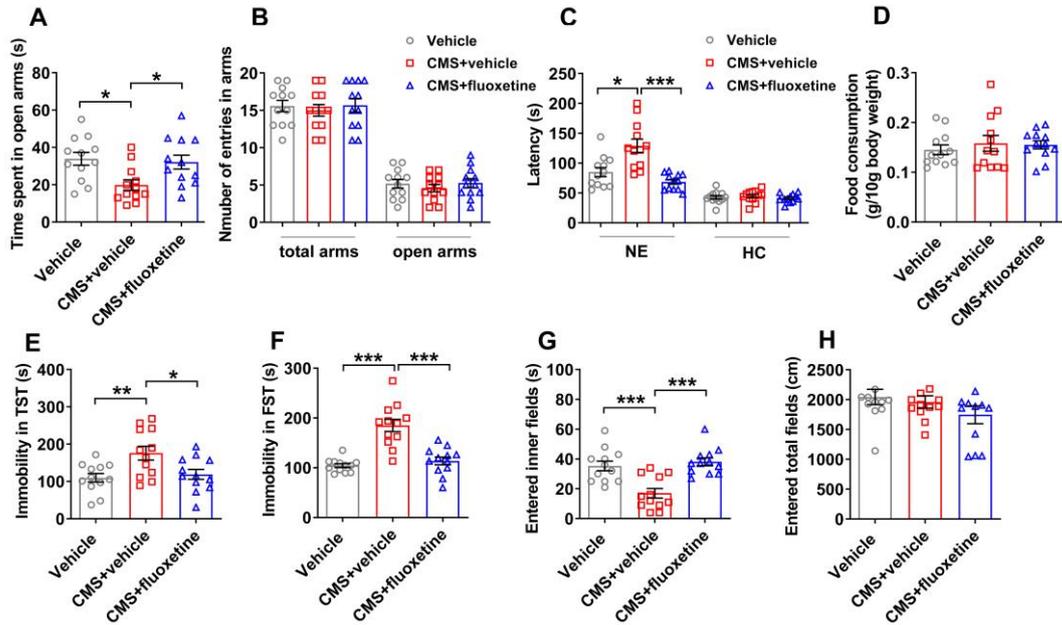
Correspondence to: (zhulj@seu.edu.cn).

This PDF file includes:

Supplementary Figures 1 to 6

31 **Supplementary Figures**

32



33

34 **Figure S1 Fluoxetine reversed chronic stress-induced behavioral modification.**

35 The adult male ICR mice were treated with fluoxetine (10 mg/kg/d) or its vehicle by

36 intraperitoneal administration for 28 consecutive d and exposed to CMS. (A) The time

37 spent in open arms ($F(2,33) = 5.233$, CMS + vehicle versus vehicle: $*p = 0.0149$;

38 CMS + fluoxetine versus CMS + vehicle: $*p = 0.0360$) and (B) number of entries in

39 the arms (for total arms: $F(2,33) = 0.1982$, $p = 0.8212$; for open arms: $F(2,33) =$

40 0.4254 , $p = 0.6571$) in the O-maze test. (C) The latency to feed in a novel

41 environment ($W(2,18.73) = 13.14$, CMS + vehicle versus vehicle: $*p = 0.0126$; CMS

42 + fluoxetine versus CMS + vehicle: $***p = 0.0005$) and in the home cage ($F(2,33) =$

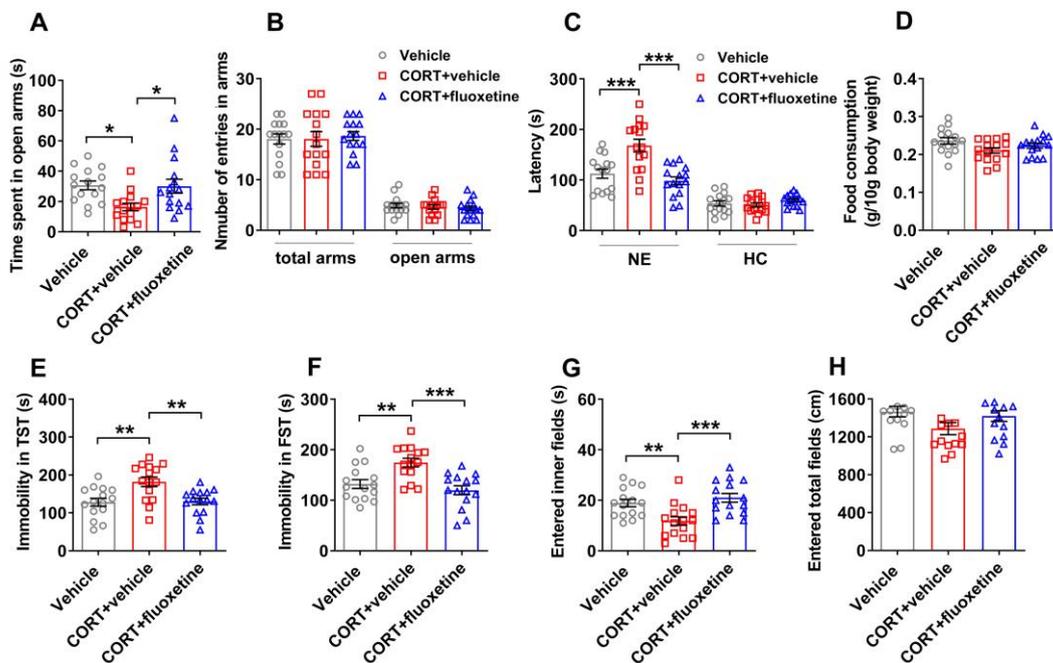
43 0.4669 , $p = 0.6310$) and (D) food consumption in the home cage ($F(2,33) = 0.3342$, p

44 $= 0.7183$) in the novelty-suppressed feeding test in adult mice. (E) The immobility

45 time in the TST ($F(2,33) = 5.925$, CMS + vehicle versus vehicle: $**p = 0.0088$; CMS

46 + fluoxetine versus CMS + vehicle: $*p = 0.0262$) and (F) FST ($W(2,18.35) = 18.45$,
 47 CMS + vehicle versus vehicle: $***p < 0.0001$; CMS + fluoxetine versus CMS +
 48 vehicle: $***p = 0.0004$) of the adult mice. (G) The time of entered inner fields
 49 ($F(2,33) = 14.42$, CMS + vehicle versus vehicle: $***p = 0.0004$; CMS + fluoxetine
 50 versus CMS + vehicle: $***p < 0.0001$) and (H) the total distance traveled ($F(2,33) =$
 51 1.418 , $p = 0.2566$) in the OF test. The behaviors in (A-H) ($n = 12$ mice) were assessed
 52 1 day after the last treatment. Data were normally distributed with Shapiro-Wilk test
 53 and one-way ANOVA followed by Tukey's multiple comparisons test (A-B, C-right,
 54 D-E, G-H), or Welch ANOVA with Dunnett's T3 multiple comparisons test (C-left
 55 and F) were carried out to test significance, $*P < 0.05$, $**P < 0.01$, $***P < 0.001$.
 56 Graphs show Mean \pm SEM. NE: novel environment, HC: home cage.

57

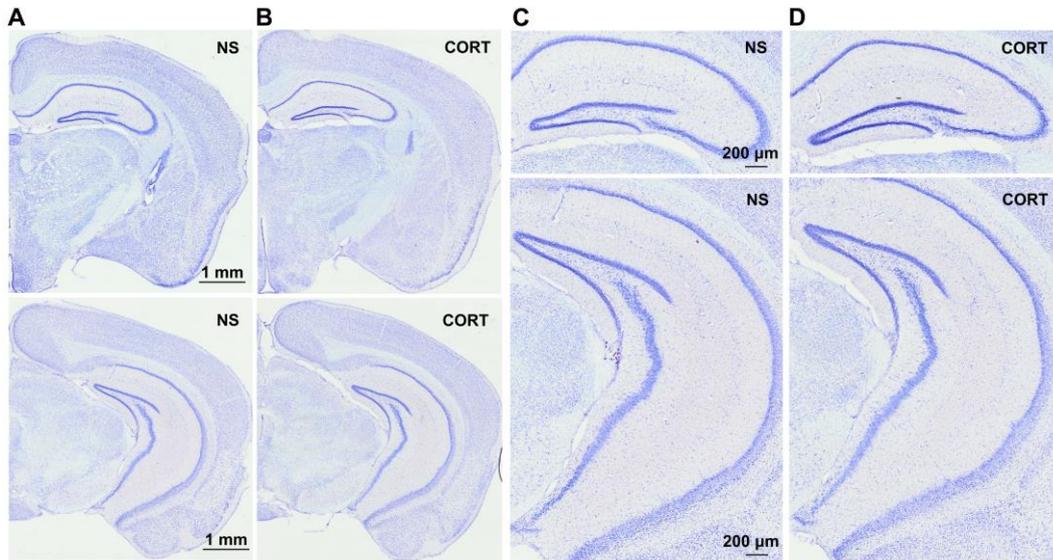


58

59 **Figure S2 Fluoxetine reversed chronic CORT-induced behavioral modification.**

60 The adult male ICR mice were treated with CORT (10 μ M) alone or in combination

61 with fluoxetine (10 mg/kg/d) or its vehicle by intraperitoneal administration for 28
62 consecutive d. **(A)** The time spent in open arms ($F(2,42) = 5.594$, CORT + vehicle
63 versus vehicle: $*p = 0.014$; CORT + fluoxetine versus CORT + vehicle: $*p = 0.0186$)
64 and **(B)** number of entries in the arms (for total arms: $F(2,42) = 0.09206$, $p = 0.9122$;
65 for open arms: $F(2,42) = 0.4439$, $p = 0.6445$) in the O-maze test. **(C)** The latency to
66 feed in a novel environment ($F(2,42) = 13.47$, CORT + vehicle versus vehicle: $***p =$
67 0.001 ; CORT + fluoxetine versus CORT + vehicle: $***p < 0.0001$) and in the home
68 cage ($F(2,42) = 1.273$, $p = 0.2906$) and **(D)** food consumption in the home cage
69 ($F(2,42) = 2.859$, $p = 0.0686$) in the novelty-suppressed feeding test in adult mice. **(E)**
70 The immobility time in the TST ($F(2,42) = 8.214$, CORT + vehicle versus vehicle:
71 $**p = 0.0025$; CORT + fluoxetine versus CORT + vehicle: $**p = 0.0038$) and **(F)** FST
72 ($F(2,42) = 10.9$, CORT + vehicle versus vehicle: $**p = 0.0036$; CORT + fluoxetine
73 versus CORT + vehicle: $***p = 0.0002$) of the adult mice. **(G)** The time of entered
74 inner fields ($F(2,42) = 8.741$, CORT + vehicle versus vehicle: $**p = 0.0097$; CORT +
75 fluoxetine versus CORT + vehicle: $***p = 0.0008$) and **(H)** the total distance traveled
76 ($F(2,42) = 2.54$, $p = 0.0909$) in the OF test. The behaviors in **(A-H)** ($n = 15$ mice)
77 were assessed 1 day after the last treatment. Data were normally distributed with
78 Shapiro-Wilk test and one-way ANOVA followed by Tukey's multiple comparisons
79 test **(A-H)**, was carried out to test significance, $*P < 0.05$, $**P < 0.01$, $***P < 0.001$,
80 $****P < 0.0001$. Graphs show Mean \pm SEM. NE: novel environment, HC: home
81 cage.



82

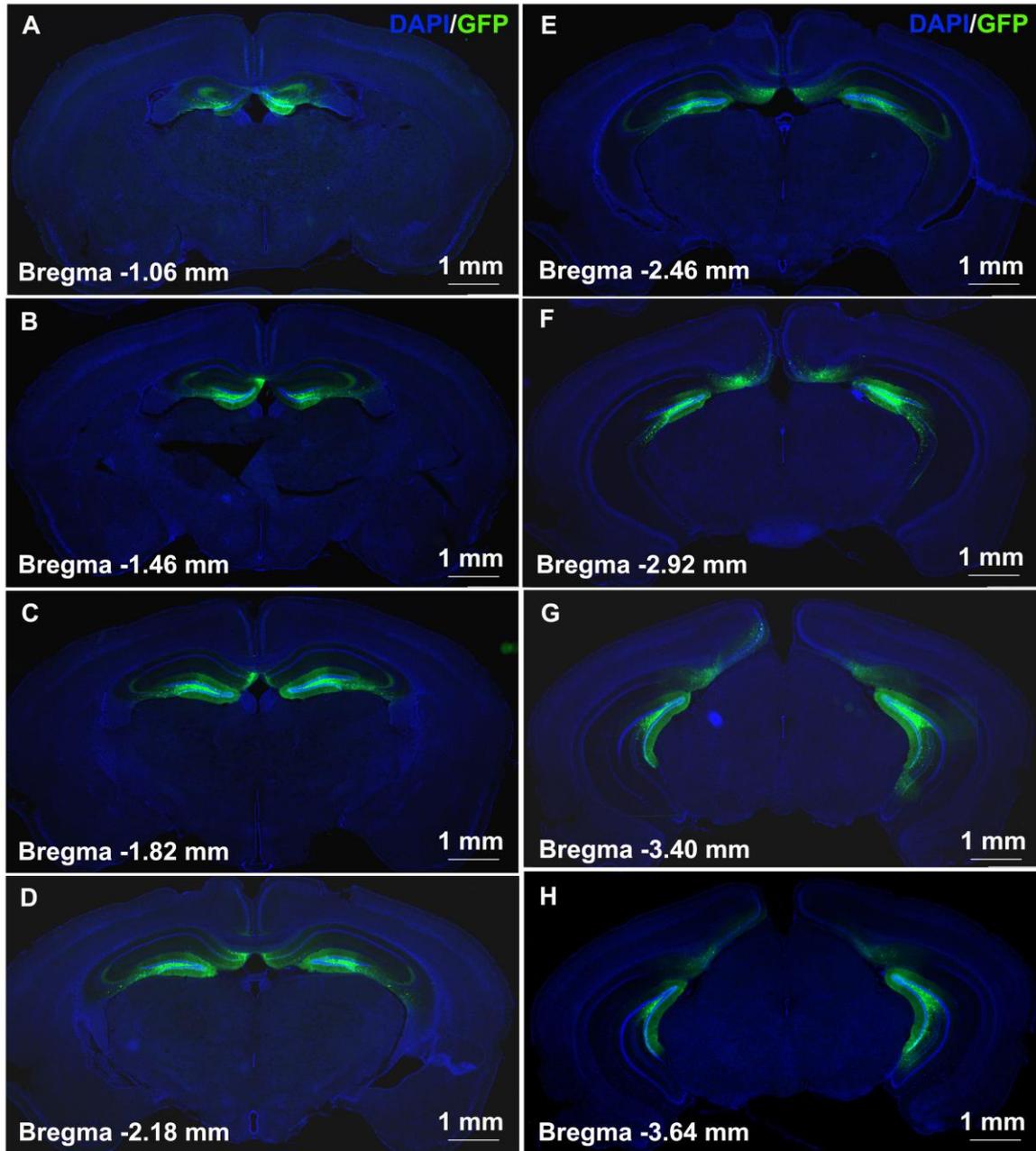
83 **Figure S3 Chronic CORT infusion did not alter the integrity of the infused**

84 **hippocampus.** Corticosterone (10 μM) was delivered into the DG of the hippocampus

85 by microinjection for 28 consecutive d. (A-D) Representative images showing chronic

86 CORT infusion did not alter the integrity of the infused hippocampus by using Nissl

87 staining. Scale bar: 1mm (A-B), 200μm (C-D).



88

89 **Figure S4 The expression of virus across the anterior posterior of hippocampus.**

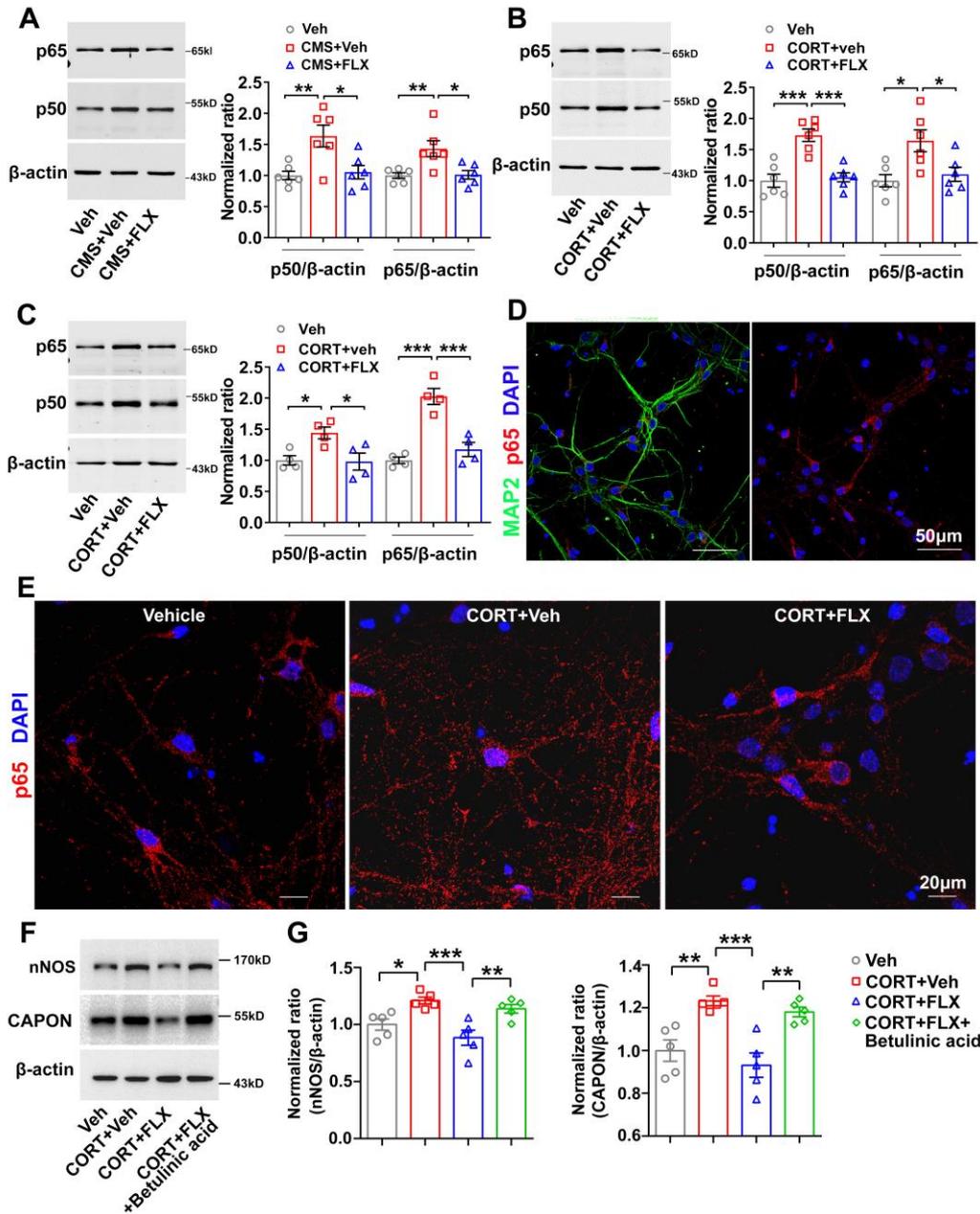
90 **(A-H)** The AAV-CAPON-L-GFP was injected into the DG of the hippocampus, and

91 effectively infected the hippocampus, produced considerable CAPON-L-GFP. Scale

92 bar: 1mm.

93

94



95

96 **Figure S5 Fluoxetine prevented chronic stress-induced NF-κB signaling**

97 **activation.** (A) The adult male mice were treated with fluoxetine (10 mg/kg/d) or its

98 vehicle by intraperitoneal administration for 28 consecutive d and exposed to CMS.

99 Representative immunoblots (left) and bar graph (right) showing hippocampal DG

100 p50 ($F(2,15) = 7.987$, CMS + vehicle versus vehicle: $**p = 0.0069$; CMS + FLX

101 versus CMS + vehicle: $*p = 0.0127$) and p65 ($F(2,15) = 7.82$, CMS + vehicle versus

102 vehicle: $**p = 0.009$; CMS + FLX versus CMS + vehicle: $*p = 0.0109$) levels in adult

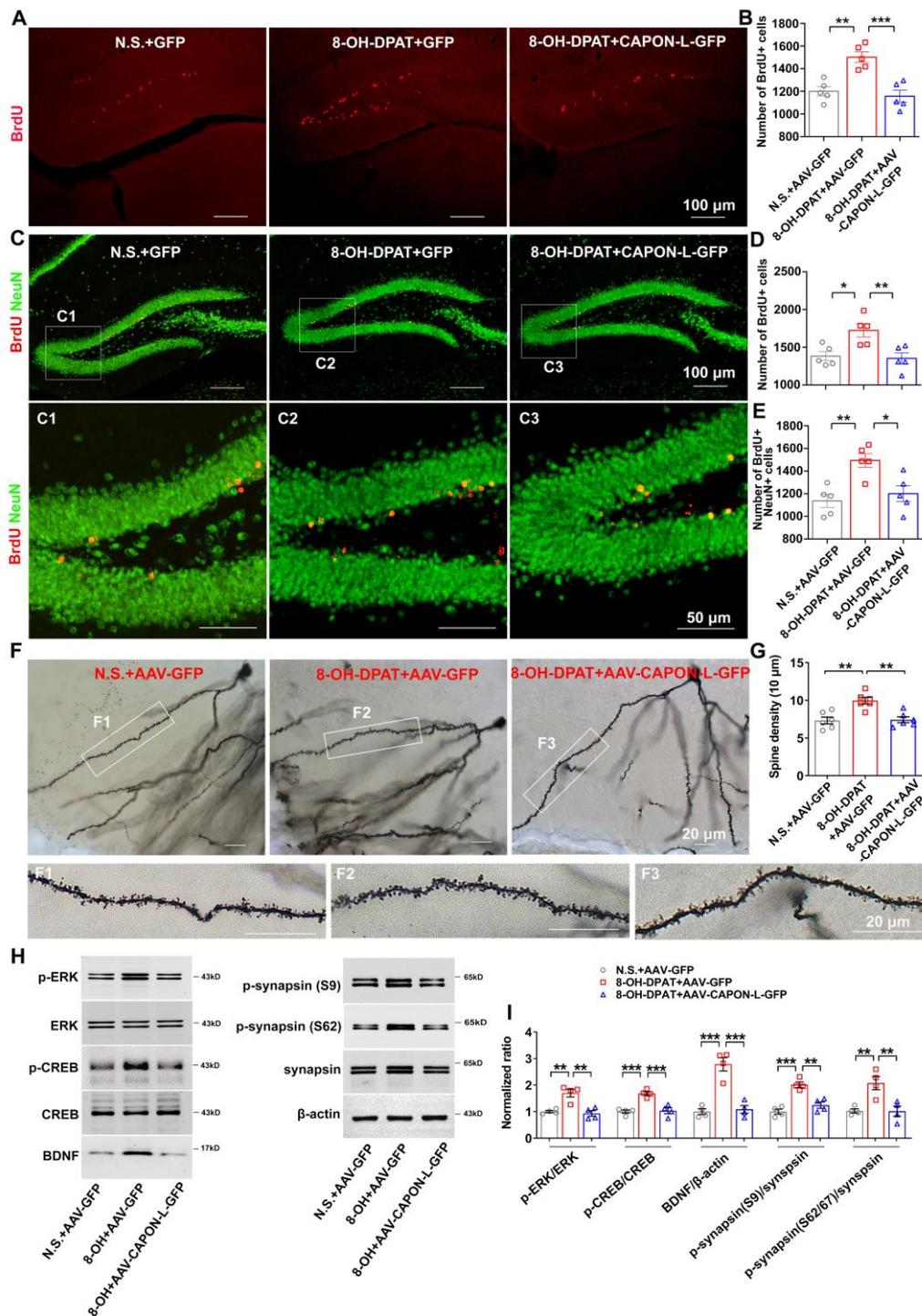
103 male ICR mice ($n = 6$). **(B)** The adult male mice were treated with CORT ($10 \mu\text{M}$)
104 alone or in combination with fluoxetine (10 mg/kg/d) or its vehicle by intraperitoneal
105 administration for 28 consecutive d. Representative immunoblots (left) and bar graph
106 (right) showing p50 ($F(2,15) = 18.95$, CORT + vehicle versus vehicle: $***p = 0.0002$;
107 CORT + FLX versus CORT + vehicle: $***p = 0.0004$) and p65 ($F(2,15) = 6.702$,
108 CORT + vehicle versus vehicle: $*p = 0.0102$; CORT + FLX versus CORT + vehicle:
109 $*p = 0.0302$) levels in hippocampal DG ($n = 6$). **(C)** Representative immunoblots (left)
110 and bar graph (right) showing p50 ($F(2,9) = 6.072$, CORT + vehicle versus vehicle:
111 $*p = 0.0392$; CORT + FLX versus CORT + vehicle: $*p = 0.0318$) and p65 ($F(2,9) =$
112 28.03 , CORT + vehicle versus vehicle: $***p = 0.0002$; CORT + FLX versus CORT +
113 vehicle: $***p = 0.0007$) levels in the cultured neurons incubated with $10 \mu\text{M}$ CORT
114 alone or in combination with $0.1 \mu\text{M}$ fluoxetine or vehicle for 72 h ($n = 4$). **(D)**
115 Representative p65 immunofluorescence in cultured neurons (red, p65; green, MAP-2;
116 blue, DAPI). DAPI, 4',6-diamidino-2-phenylindole. Scale bar, $50 \mu\text{m}$. **(E)** Cultured
117 neurons were treated with $10 \mu\text{M}$ CORT alone or in combination with $0.1 \mu\text{M}$
118 fluoxetine or vehicle at 7 DIV for 72 h. Scale bar, $20 \mu\text{m}$. **(F-G)** Representative
119 immunoblots (left) and bar graph (right) showing nNOS ($F(3, 16) = 8.979$, CORT +
120 vehicle versus vehicle: $*p = 0.0213$; CORT + FLX versus CORT + vehicle: $***p <$
121 0.0001 ; CORT + FLX + Betulinic acid versus CORT + FLX: $**p = 0.0058$) and
122 CAPON ($F(3, 16) = 12.22$, CORT + vehicle versus vehicle: $**p = 0.003$; CORT +
123 FLX versus CORT + vehicle: $***p < 0.001$; CORT + FLX + Betulinic acid versus
124 CORT + FLX: $**p = 0.0017$) levels in the cultured neurons incubated with $10 \mu\text{M}$

125 CORT alone or in combination with 0.1 μ M fluoxetine and 20 μ M Betulinic acid (n =
126 5 independent experiment). Data were normally distributed with Shapiro-Wilk test
127 and one-way ANOVA followed by Tukey's multiple comparisons test (**A-C, F**) was
128 carried out to test significance, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. Graphs show
129 Mean \pm SEM. Veh: Vehicle; FLX: fluoxetine.

130

131

132



133

134 **Figure S6 Augmenting nNOS-CAPON interaction reversed 5-HTAR agonist**

135 **induced neurogenic and synaptogenic effects. (A-I) The adult mice treated with**

136 **intra-hippocampal DG microinjection of AAV-CAPON-L-GFP or AAV-GFP in**

137 **combination with 8-OH-DPAT (0.1 mg/kg/d i.p.) for 28 days. (A-B) Representative**

138 **images (A) and bar graph (B) showing BrdU⁺ cells (red) in the DG of mice exposed to**

139 these treatments at 2 h after BrdU administration ($n=5$, $F(2,12) = 16.03$, AAV-GFP +
140 8-OH-DPAT versus AAV-GFP + vehicle: $**p = 0.0013$; AAV-CAPON-L-GFP +
141 8-OH-DPAT versus AAV-GFP + 8-OH-DPAT: $***p < 0.001$). Scale bar, 100 μm . **(C-E)**
142 Representative images **(C)** and bar graph showing BrdU⁺ cells **(D)** and BrdU⁺/ NeuU⁺
143 cells **(E)** in the DG of mice exposed to these treatments at 28 days after BrdU
144 administration ($n=5$, for BrdU⁺: $F(2,12) = 7.919$, AAV-GFP + 8-OH-DPAT versus
145 AAV-GFP + vehicle: $*p = 0.013$; AAV-CAPON-L-GFP + 8-OH-DPAT versus
146 AAV-GFP + 8-OH-DPAT: $**p = 0.0074$; for BrdU⁺/ NeuU⁺: $F(2,12) = 9.246$,
147 AAV-GFP + 8-OH-DPAT versus AAV-GFP + vehicle: $**p = 0.0033$;
148 AAV-CAPON-L-GFP + 8-OH-DPAT versus AAV-GFP + 8-OH-DPAT: $*p = 0.0123$).
149 Scale bar, 100 μm , 50 μm . BrdU⁺: red, NeuU⁺: green. **(F-G)** Representative images
150 with Golgi-Cox staining **(F)** and bar graph **(G)** showing dendrite spine density of
151 granular cells in the hippocampal DG of mice exposed to different treatments ($n = 6$,
152 10 neurons per sample, $F(2,15) = 12.43$, AAV-GFP + 8-OH-DPAT versus AAV-GFP +
153 vehicle: $**p = 0.0014$; AAV-CAPON-L-GFP + 8-OH-DPAT versus AAV-GFP +
154 8-OH-DPAT: $**p = 0.002$). Scale bar, 20 μm . **(H-I)** Representative immunoblots **(H)**
155 and bar graph **(I)** showing p-ERK, p-CREB, p-synapsin, BDNF in the DG of mice
156 with different treatments ($n = 4$, for p-ERK/ERK: $F(2,9) = 15.92$; AAV-GFP +
157 8-OH-DPAT versus AAV-GFP + vehicle: $**p = 0.0031$; AAV-CAPON-L-GFP +
158 8-OH-DPAT versus AAV-GFP + 8-OH-DPAT: $**p = 0.0017$; for p-CREB: $F(2,9) =$
159 21.57; AAV-GFP + 8-OH-DPAT versus AAV-GFP + vehicle: $***p = 0.0007$;
160 AAV-CAPON-L-GFP + 8-OH-DPAT versus AAV-GFP + 8-OH-DPAT: $***p = 0.0009$;

161 for BDNF: $F(2,9) = 32.17$; AAV-GFP + 8-OH-DPAT versus AAV-GFP + vehicle:
162 *** $p = 0.0001$; AAV-CAPON-L-GFP + 8-OH-DPAT versus AAV-GFP + 8-OH-DPAT:
163 *** $p = 0.0002$; for p-syn(S9)/syn: $F(2,9) = 24.17$; AAV-GFP + 8-OH-DPAT versus
164 AAV-GFP + vehicle: *** $p = 0.0002$; AAV-CAPON-L-GFP + 8-OH-DPAT versus
165 AAV-GFP + 8-OH-DPAT: ** $p = 0.0018$; for p-syn(S62/67)/syn: $F(2,9) = 10.85$;
166 AAV-GFP + 8-OH-DPAT versus AAV-GFP + vehicle: ** $p = 0.0077$;
167 AAV-CAPON-L-GFP + 8-OH-DPAT versus AAV-GFP + 8-OH-DPAT: ** $p = 0.0072$).
168 Graphs show Mean \pm SEM. Data were normally distributed with Shapiro-Wilk test
169 and one-way ANOVA followed by Tukey's multiple comparisons test (**B, D, E, G, I**)
170 was carried out to test significance, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

171

172

173

174

175

176