

## 1 **Supplementary data**

2

## 3 **Materials and Methods**

### 4 **Compound preparation**

5 Oligomeric A $\beta$  were prepared as previously described (1, 2). Briefly, lyophilized human A $\beta$ 1-42 (rPeptide,  
6 A-1002-1) was dissolved in 1,1,1,3,3,3- hexafluoro-2-propanol (HFIP) and subsequently evaporated in  
7 nitrogen flow. Afterwards, DMSO and cold PBS were added to dissolve A $\beta$ , followed by sonication for 15  
8 minutes. Neurobasal medium or PBS was added to make stock concentration to 100  $\mu$ M. Before use, the  
9 A $\beta$  solution was incubated at 4  $^{\circ}$ C for 6 hours.

10

11 Sp-8-BnT-cAMPS, "S220" (Biolog, #B046), 8-pCPT-2'-O-Me-cAMP, "8-pCPT" (Biolog, #C041) and Rp-8-  
12 CPT-cAMPS, "Rp-cAMP" (Biolog, #C011) were dissolved in water. ESI-05 (Biolog, #M092) was dissolved  
13 in DMSO.

14

### 15 **Slice preparation**

16 Experiments were performed using brain slices from 4- to 5-weeks-old wild-type C57Bl6 mice. Animals  
17 were killed by decapitation and their brain was rapidly removed and placed in ice-cold modified artificial  
18 cerebrospinal fluid (mACSF, in mM: choline chloride 120, KCl 3.5, NaHCO<sub>3</sub> 25, D-glucose 10, CaCl<sub>2</sub> 0.5,  
19 MgSO<sub>4</sub> 6, NaH<sub>2</sub>PO<sub>4</sub> 1.25, continuously bubbled with 95% O<sub>2</sub>-5% CO<sub>2</sub> (pH=7.4)). 300  $\mu$ m-thick coronal  
20 slices of the hippocampus were cut with a vibroslicer (VT1200S, Leica) in ice-cold mACSF and  
21 subsequently incubated at 32 $^{\circ}$ C for 30 min in ACSF (in mM: NaCl 118.1, KCl 2.5, NaHCO<sub>3</sub> 26.2, D-glucose  
22 22.2, CaCl<sub>2</sub> 2.00, MgCl<sub>2</sub> 1.00, and NaH<sub>2</sub>PO<sub>4</sub> 1.00, continuously bubbled with 95% O<sub>2</sub>-5% CO<sub>2</sub> (pH=7.4)).  
23 Slices were then kept at least 30 min at room temperature before recordings.

24

25 **Animals**

26 Seven to eight months-old male J20 AD mice or C57BL/6J mice were used for Morris water maze. The J20  
27 mice carried Swedish and Indiana mutations in APP and exhibited cognitive decline and senile plaques at  
28 the age of 5-7 months (3). The mice were maintained on a 12-hour dark/light cycle with access to food and  
29 water ad libitum. In this study, the use of animals was approved by ethical committee of university of  
30 Groningen (AVD10500202010985).

31

32 **IP injection.**

33 The Epac2 activator Sp-8-BnT-cAMPS, "S220", was dissolved in PBS. S220 (10 mg/kg), or PBS as vehicle,  
34 was daily administered to the mice for 14 consecutive days via intraperitoneal (IP) injection.

35

36 **Habituation**

37 To minimize the stress from handling and behavioral paradigms, the animals were individually handled for  
38 2 minutes by the researcher in the experimental room for 5 consecutive days prior to the behavioral tests.

39

40 **Elevated plus maze**

41 Elevated plus maze (EPM) was used to assess the anxiety level of surgery mice prior to drug injection. This  
42 plus-shape maze consisted of two open arms and two closed arms (30 cm length × 5.5 cm width) in opposite  
43 directions with 60 cm height from the ground. The light intensity was set to 10 lux and 12 lux in the maze  
44 center and the open arms respectively. During EPM, the mice were placed towards closed arms in the  
45 center of the maze, followed by 8 minutes free exploration. The time spent in open/closed/central areas  
46 were recorded by EthoVision XT software.

47

48 **Y-maze**

49 Y-maze was used to assess the short-term working memory of surgery mice prior to drug injection. The Y-  
50 maze was in a shape of an equilateral triangle which consisted of 3 closed arms (40 cm length × 8 cm  
51 width). The light intensity was set to 10 lux in the maze center. During Y-maze, the mice were placed in the  
52 maze center, followed by free exploration for 10 minutes. The total entries and spontaneous alternation  
53 were calculated manually based on the following equation: Alternation% = numbers of triads/ (total entries  
54 – 2) × 100. The triads were defined as three straight entries in any order of all three different arms.

55

### 56 **Morris water maze**

57 Morris water maze (MWM) was used to evaluate the spatial memory in hippocampus. The procedures were  
58 describes as previous (10). Briefly, the mice were put in a circular pool (135 cm in diameter) filled with water  
59 based non-toxic white paint until the water level was 1-1.5 cm above the escape platform (15 cm in  
60 diameter). The water temperature was maintained in 22 °C. Various visual cues were placed around the  
61 walls of the experimental room and light intensity was set to approximately 40 lux in the pool center. The  
62 MWM was comprised of a training phase in a 8 straight days, followed by a probe trial phase in a 2 straight  
63 days. The training block of each day included 4 trials, with a maximum swimming duration of 120 seconds  
64 per trial for each mouse. The platform was removed during probe trials. Escape latency, the crossing  
65 frequency and the time spent in each quadrants were recorded using the EthoVision XT system. The mice  
66 showing thigmotaxis were excluded from the analysis.

67

### 68 **Western blot**

69 PCNs were homogenized in RIPA buffer supplemented with phosphatase inhibitor (PhosSTOP tablet,  
70 Roche, #4906837001) and protease inhibitor (cOmplete ULTRA tablet, Roche, #5892791001). The  
71 supernatant was collected from cell lysates after centrifugation at 12000 rcf for 15 minutes at 4°C. 20µg  
72 proteins were applied in 10% SDS-PAGE gels and transferred to nitrocellulose or PVDF membranes. The  
73 membranes were blocked with 5% milk or BSA for 60 minutes at room temperature (RT). Then the  
74 membranes were incubated with primary antibodies at 4°C overnight. Primary antibodies included rabbit

75 anti-p-PKA substrate (1:1000 dilution, Cell Signalling, #9624S) and mouse anti- $\beta$ -actin (1:3000 dilution,  
76 Santa Cruz, #sc-47778). On the following day, the membranes were incubated with secondary antibodies  
77 (#A9044, rabbit anti-mouse, 1:3000, Sigma–Aldrich; GTX2131, goat anti-rabbit, 1:3000, GeneTex) for 1  
78 hour at RT. ECL reagent (PerkinElmer, 203-21141) was used to develop blots.

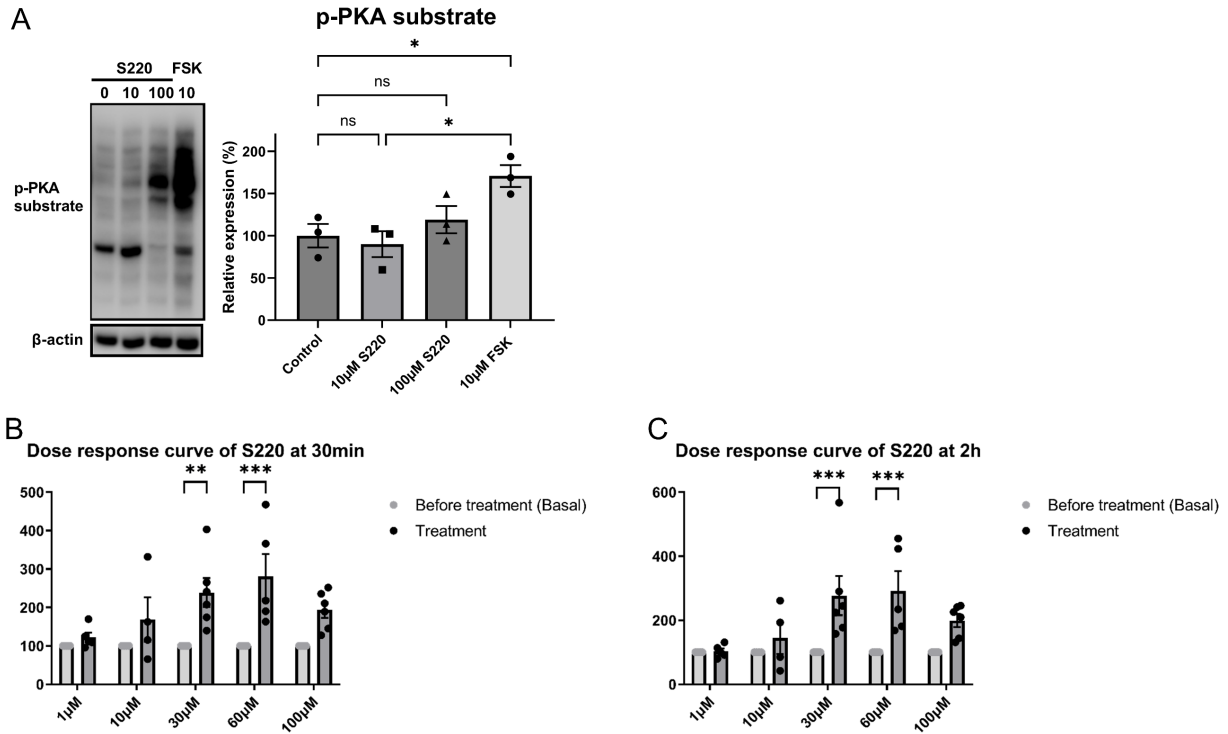
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## 80 **Immunohistochemistry**

81 The procedures were described as previous (4) and performed on PFA-fixated free-floating brain slices in  
82 the thickness of 20  $\mu$ m. Briefly, the slices were blocked in 3% BSA in TBST (0.1% Triton X-100) for 1 hour  
83 at room temperature (RT). The slices were subsequently incubated with primary antibody (Anti-Epac2,  
84 1:800 dilution, Rockland, #600-401-BB2) at 4 °C overnight. On Day2, the slices were incubated in Alexa  
85 488 donkey Anti-rabbit secondary antibody (1:500 dilution, Invitrogen, #XJ357262) for 2 hours.  
86 Subsequently, the slices were mounted in Vectashield medium with DAPI (vector laboratories, #H-1200-  
87 10). Images were taken by Leica DM6000 microscope with Leica application Suite X software at 400x  
88 magnification. For image quantification, the thresholds of images were progressively decreased until the  
89 background noise was eliminated. Subsequently, the intensity coverage was calculated in the regions of  
90 interest by Image J.

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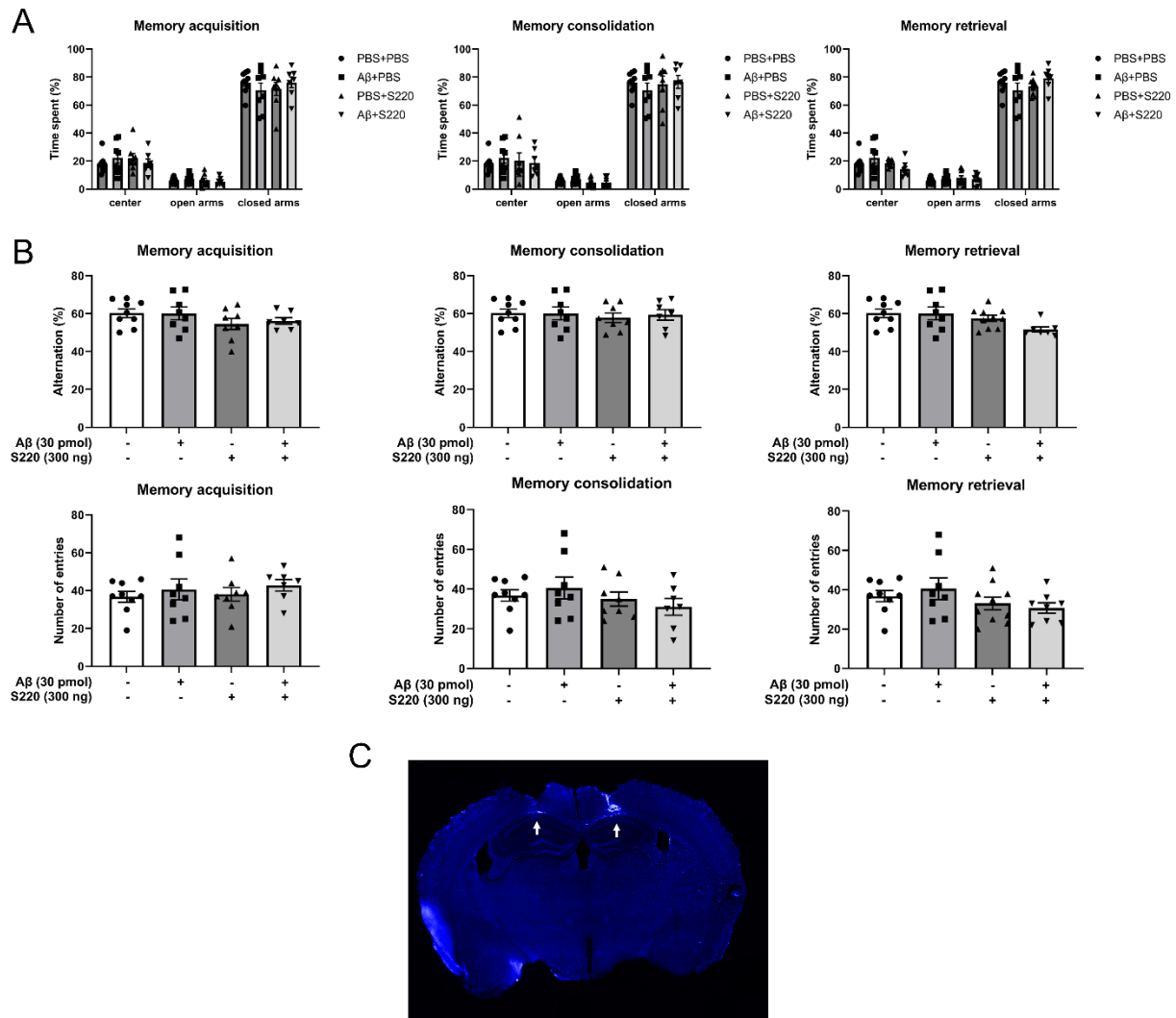
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94 **Figure S1.** S220 does not activate PKA in primary neurons. (A) S220 treatment for 20 minutes did not significantly alter  
 95 the expression of p-PKA substrates in primary neurons (n=3, one-way ANOVA:  $F(3, 8)=6.045$ ,  $DF=3$ ). (B) Dose  
 96 response curve of S220 after 30-minute treatment (n=4-6, two-way ANOVA, Bonferroni post hoc analysis:  $F(1, 42) =$   
 97  $32.59$ ,  $DF=1$ ). (C) Dose response curve of S220 after 2-hour treatment (n=4-6, two-way ANOVA, Bonferroni post hoc  
 98 analysis:  $F(1, 42) = 24.74$ ,  $DF=1$ ). Data are expressed as mean  $\pm$  SEM. \*  $p \leq 0.05$ , \*\*  $p \leq 0.01$ , \*\*\*  $p \leq 0.001$ .

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100

101 **Figure S2.** The stereotactic surgery does not affect the anxiety and working memory. (A) The anxiety level measured

102 via EPM was not affected after recovery from surgery (Acquisition: PBS+PBS, n=9; Aβ+PBS, n=8; PBS+S220, n=8;

103 Aβ+S220, n=7; one-way ANOVA:  $F(3, 84)=4.854e-014$ ,  $DF=3$ ; Consolidation: PBS+PBS, n=9; Aβ+PBS, n=8;

104 PBS+S220, n=8; Aβ+S220, n=7, one-way ANOVA:  $F(3, 84)=6.153e-014$ ,  $DF=3$ ; Retrieval: PBS+PBS, n=9; Aβ+PBS,

105 n=8; PBS+S220, n=10; Aβ+S220, n=7; one-way ANOVA:  $F(3, 90) = 1.808e-013$ ,  $DF=3$ ). (B) The working memory

106 measured by alternation percentage surgery (Acquisition: PBS+PBS, n=9; Aβ+PBS, n=8; PBS+S220, n=8; Aβ+S220,

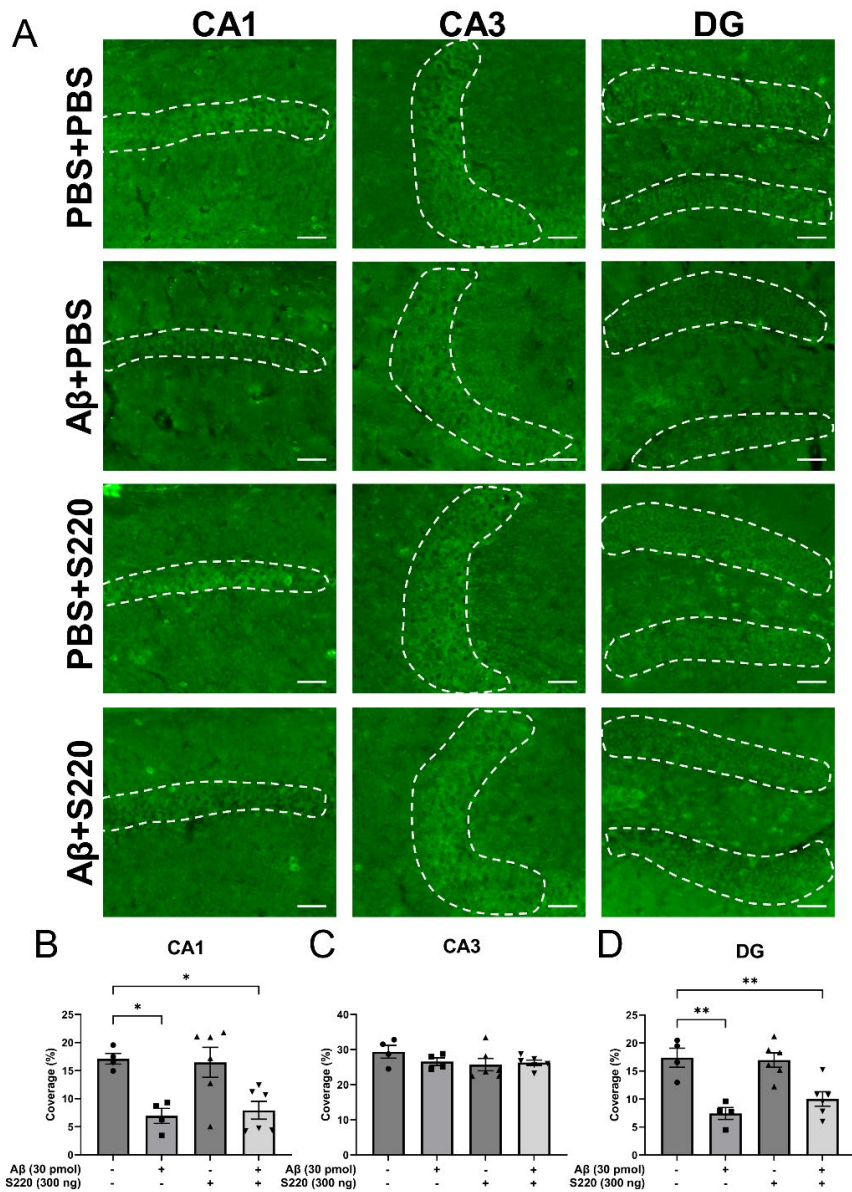
107 n=7; one-way ANOVA:  $F(3, 28)=1.185$ ,  $DF=3$ ; Consolidation: PBS+PBS, n=9; Aβ+PBS, n=8; PBS+S220, n=8;

108 Aβ+S220, n=7; one-way ANOVA:  $F(3, 28)=0.171$ ,  $DF=3$ ; Retrieval: PBS+PBS, n=9; Aβ+PBS, n=8; PBS+S220, n=10;

109 Aβ+S220, n=7; one-way ANOVA,  $F(3, 30)=2.769$ ,  $DF=3$ ) and total entry (Acquisition: PBS+PBS, n=9; Aβ+PBS, n=8;

110 PBS+S220, n=8; Aβ+S220, n=7; one-way ANOVA:  $F(3, 28)=0.469$ ,  $DF=3$ ; Consolidation: PBS+PBS, n=9; Aβ+PBS,

111 n=8; PBS+S220, n=8; A $\beta$ +S220, n=7; one-way ANOVA: F(3, 28)=0.8974, DF=3; Retrieval: PBS+PBS, n=9; A $\beta$ +PBS,  
112 n=8; PBS+S220, n=10; A $\beta$ +S220, n=7; one-way ANOVA, F(3, 31)=1.333, DF=3) in Y-maze was not affected after  
113 recovery from surgery. (C) Representative DAPI staining of injection point in CA1 regions. Data are expressed as mean  
114  $\pm$  SEM.  
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116

117 **Figure S3.** Epac2 expression is decreased in neuronal cell layers of CA1 and DG in Aβ-treated mice. (A)

118 Representative images of Epac2 expressions in CA1, CA3 and DG. Scale bar, 50 μm. (B) In pyramidal cell layer of

119 CA1, Epac2 expression was significantly decreased by Aβ, but not altered by S220 (PBS+PBS, n=4; Aβ+PBS, n=4;

120 PBS+S220, n=6; Aβ+S220, n=6; one-way ANOVA: F(3, 16)=7.905, DF=3). (C) In pyramidal cell layer of CA3, Epac2

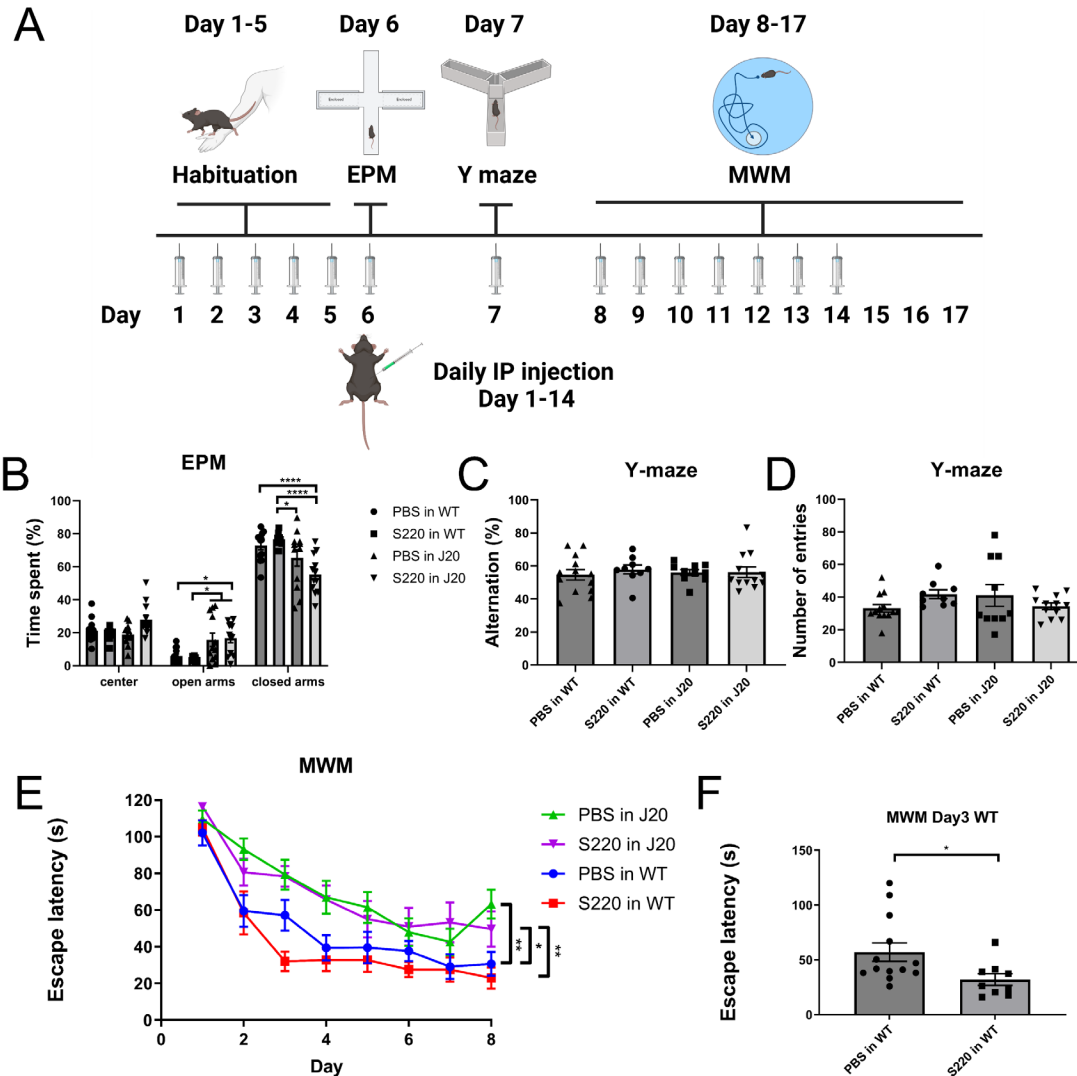
121 expression was not altered by both Aβ and S220 (PBS+PBS, n=4; Aβ+PBS, n=4; PBS+S220, n=6; Aβ+S220, n=6; one-

122 way ANOVA: F(3, 16)=1.150, DF=3). (D) In granular cell layer of DG, Epac2 expression was significantly decreased by

123 Aβ, but not altered by S220 (PBS+PBS, n=4; Aβ+PBS, n=4; PBS+S220, n=6; Aβ+S220, n=6; one-way ANOVA: F(3,

124 16)=12.67, DF=3). Data are expressed as mean ± SEM. \* p≤0.05, \*\* p≤0.01.

125



146 **Figure S4.** S220 does not improve memory impairment in J20 mice. (A) A schematic timeline of experimental  
 147 procedures. (B) Time spent in center area, open arms and closed arms in EPM. J20 mice spent more time in open  
 148 arms than WT mice (PBS-treated WT, n=13; S220-treated WT, n=9; PBS-treated J20, n=12; S220-treated J20, n=12;  
 149 two-way ANOVA). (C-D) Alternation percentage and number of entries in Y-maze did not exhibit difference among  
 150 groups (PBS-treated WT, n=13; S220-treated WT, n=9; PBS-treated J20, n=12; S220-treated J20, n=12; one-way  
 151 ANOVA). (E) Escape latency during 8-day training in MWM. S220 treatment did not enhance the escape latency in J20  
 152 mice (PBS-treated WT, n=13; S220-treated WT, n=9; PBS-treated J20, n=12; S220-treated J20, n=12; two-way ANOVA).  
 153 (F) Escape latency on Day 3 in MWM. S220 treatment exhibited significantly reduced the escape latency compared  
 154 with PBS-treated mice (PBS-treated WT, n=13; S220-treated WT, n=9; t-test). Data are expressed as mean  $\pm$  SEM. \*P  
 155 < 0.05, \*\*P < 0.01, \*\*\*\*P < 0.0001.



157 **References**

- 158 1. Dekens, D. W., De Deyn, P. P., Sap, F., Eisel, U. L. M., and Naude, P. J. W. (2020) Iron chelators  
159 inhibit amyloid-beta-induced production of lipocalin 2 in cultured astrocytes *Neurochem Int* **132**,  
160 104607
- 161 2. Granic, I., Masman, M. F., Kees Mulder, C., Nijholt, I. M., Naude, P. J., de Haan, A. *et al.* (2010)  
162 LPYFDa neutralizes amyloid-beta-induced memory impairment and toxicity *J Alzheimers Dis* **19**,  
163 991-1005
- 164 3. Mucke, L., Masliah, E., Yu, G. Q., Mallory, M., Rockenstein, E. M., Tatsuno, G. *et al.* (2000) High-  
165 level neuronal expression of abeta 1-42 in wild-type human amyloid protein precursor transgenic  
166 mice: synaptotoxicity without plaque formation *J Neurosci* **20**, 4050-4058
- 167 4. Orti-Casan, N., Zuhorn, I. S., Naude, P. J. W., De Deyn, P. P., van Schaik, P. E. M., Wajant, H. *et*  
168 *al.* (2022) A TNF receptor 2 agonist ameliorates neuropathology and improves cognition in an  
169 Alzheimer's disease mouse model *Proc Natl Acad Sci U S A* **119**, e2201137119

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