Gamma-H2AX upregulation caused by Wip1 deficiency increases depression-related cellular senescence in hippocampus

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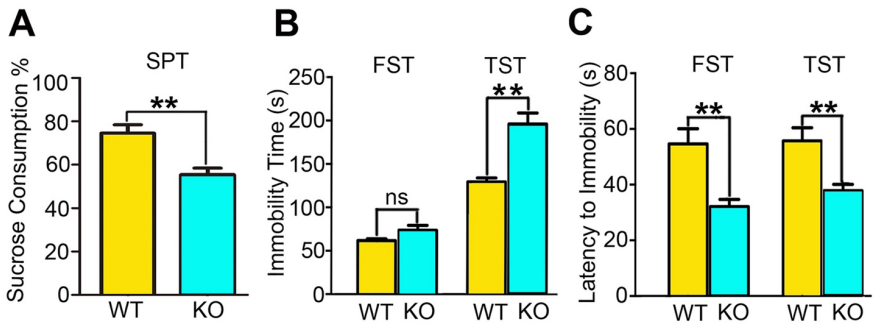
Supplemental Figure legends

Figure S1. Wip1 gene knockout increased depression-like behaviors in adult mice

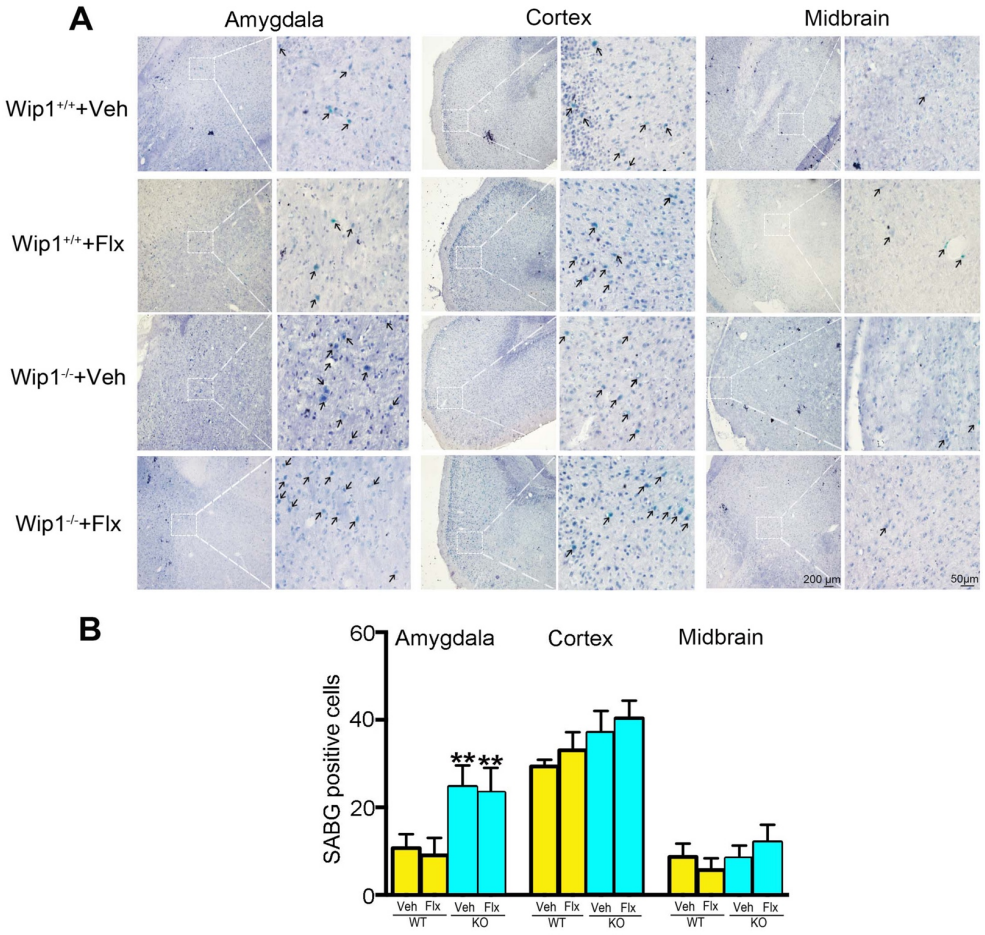


(A) Wip1 KO mice showed declined response to the incentive stimulus of sucrose in SPT compared to their wildtype (WT) littermates (WT: 74.6 ± 6.4%; KO: 55.5 ± 2.9%, $P < 0.01$).

(B) There was a tendency to be significant when comparing the immobility time of FST between the two groups (WT: 61.7 ± 2.1 s; KO: 73.8 ± 5.8 s, $P = 0.068$), while Wip1 KO mice showed obviously increased immobility time of despaired behaviors in TST (WT: 129.7 ± 4.5 s; KO: 196.6 ± 12.7 s, $P < 0.01$).

(C) Wip1 KO mice showed shorter latency to be immobile in both FST (WT: 54.6 ± 6.5 s; KO: 32.1 ± 2.6 s, $P < 0.01$) and TST (WT: 55.7 ± 4.8 s; KO: 37.8 ± 2.3 s, $P < 0.01$) than wildtype mice did. n = 9 for each group. **: $P < 0.01$.

Figure S2. Effects of Wip1 knockout and fluoxetine treatment on cellular senescence of amygdala, cerebral cortex, and midbrain

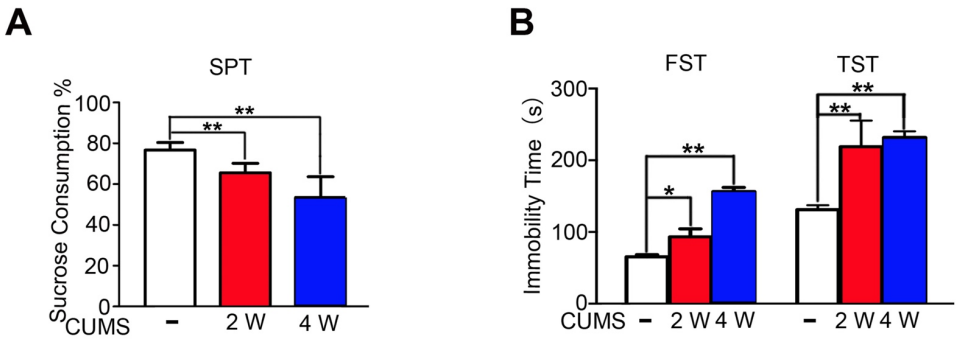


(A) Representative images showing SABG-staining positive cells located in the amygdala, cerebral cortex, and midbrain areas of different groups. Wip1 KO mice and their wildtype (WT) littermates were treated with vehicle (veh.) or fluoxetine (flx.) for 2 weeks.

(B) Statistical analyses for the number of SABG staining positive cells in amygdala, cerebral cortex, and midbrain (n = 6 mice for each group). Cellular senescence in amygdala was significantly increased in the Wip1 KO plus vehicle or fluoxetine groups when compared to the WT groups (Genotype: $F_{(1, 20)} = 49.2$, $P < 0.01$;Two-way ANOVA). The effects of fluoxetine treatment were not significant in amygdala (Treatment: $F_{(1, 20)} = 0.3$; Treatment × genotype: $F_{(1,}$

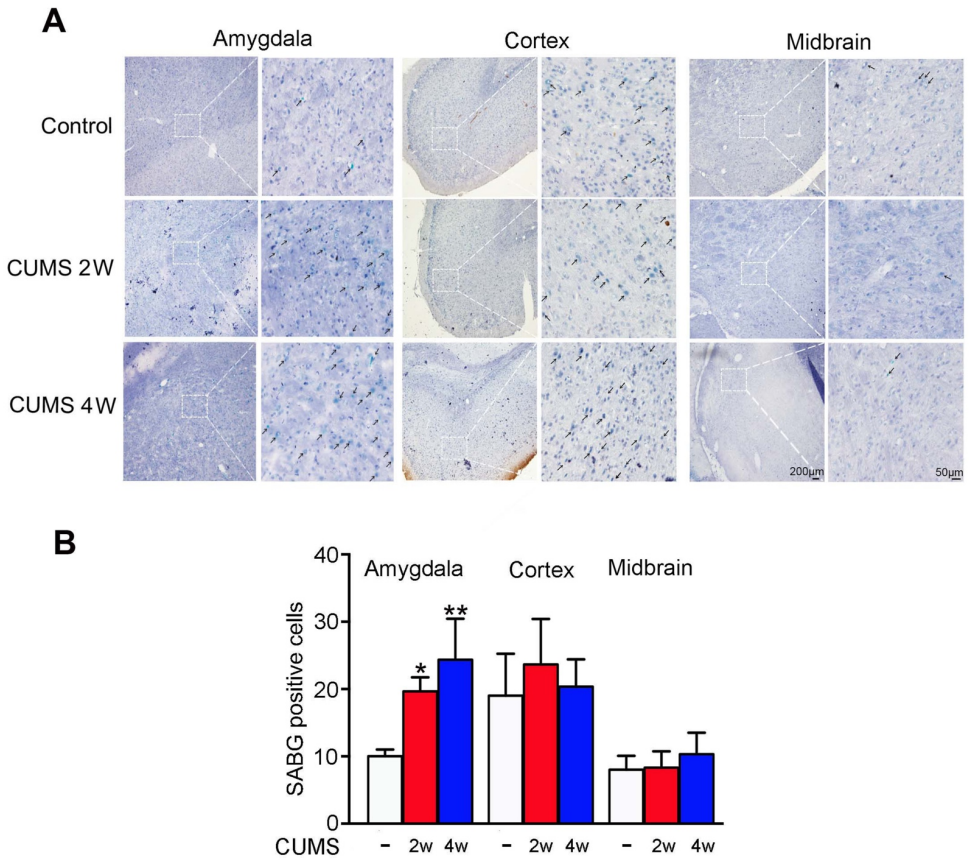
$_{20)}$ = 1.1, both P > 0.3). There were no significant effects of genotype and treatment on cellular senescence in cerebral cortex (Genotype: $F_{(1, 20)}$ = 3.8; Treatment: $F_{(1, 20)}$ = 1; Treatment × genotype: $F_{(1, 20)}$ = 1.3, all P > 0.05) and midbrain (Genotype: $F_{(1, 20)}$ = 2.9; Treatment: $F_{(1, 20)}$ = 0.07; Treatment × genotype: $F_{(1, 20)}$ = 0.07, all P > 0.05) . **: P < 0.01 when compared to the WT + vehicle group.

Figure S3. CUMS evoked depression-like behaviors in adult wildtype mice



(A) When compared to the control group, CUMS-exposed mice consumed significantly less amount of sucrose in SPT ($F_{(2, 24)}$ = 8.3, P = 0.002, one-way ANOVA; CUMS-2 or 4 weeks *vs* the control group: both P < 0.05, Tukey's tests). (B) Mice of CUMS-exposed groups spent more immobility time in FST and TST than the control group (FST: $F_{(2, 24)}$ = 46.2; TST: $F_{(2, 24)}$ = 34.1, both P < 0.0001; CUMS-2 or 4 weeks *vs* the control group: P < 0.05 or 0.01). n = 9 mice for each group. *: P < 0.05; **: P < 0.01.

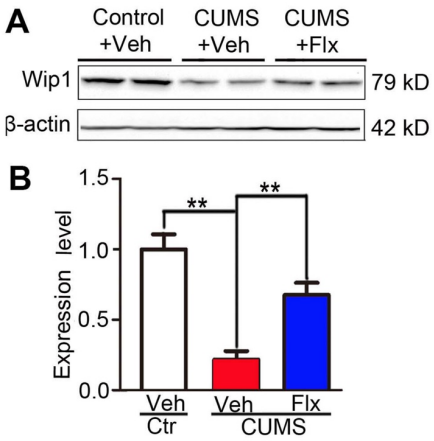
Figure S4. The effects of CUMS on cellular senescence of amygdala, cerebral cortex, and midbrain in wildtype mice



(A) Representative images showing SABG staining positive cells located in the amygdala, cerebral cortex, and midbrain areas. Wildtype mice of the control group were left undisturbed. The experimental groups were treated with CUMS for 2 or 4 weeks.

(B) Statistical analyses for the number of SABG staining positive cells in amygdala, cerebral cortex, and midbrain of different groups (n = 6 mice for each group). CUMS experience significantly increased cellular senescence in the amygdala ($F_{(2,15)}$ = 37.6, P < 0.01; CUMS 2 or 4 weeks *vs* Control, P < 0.05 or 0.01; Tukey's tests). However, CUMS treatment did not cause obvious increase of SABG-staining positive cells in the cerebral cortex ($F_{(2, 15)}$ = 0.3, P = 0.75) and the midbrain area ($F_{(2, 15)}$ = 0.7, P = 0.51). *: P < 0.05; **: P < 0.01 when compared to the control group.

Figure S5. Fluoxetine treatment prevented CUMS-induced reduction of hippocampal Wip1 expression in wildtype mice



(A) Illustration of Western blotting assays for the detection of Wip1 expression in hippocampus.

(B) One-way ANOVA revealed that fluoxetine treatment effectively prevented the suppressed expression of hippocampal Wip1 protein in CUMS-exposed wildtype mice ($F_{(2, 15)} = 20.6$, $P < 0.01$; CUMS + fluoxetine *vs* CUMS + vehicle: $P < 0.01$). n = 6 samples for each group. Wip1 expression was normalized to β-actin. **: $P < 0.01$.