

Supplementary Materials

Supplementary Figure 1. The activity of other GTPases in the hippocampus of APP/PS1 mice and the elevation of Rac1 activity in the whole brain of AD flies

A Representative Western blot results for RhoA-GTP, Cdc42-GTP, RhoA, and Cdc42 in the hippocampus of 8-month-old APP/PS1 mice and age-matched WT mice.

B No difference in RhoA activity was observed in the hippocampus between APP/PS1 and WT mice (n.s, $P > 0.05$, t -test, two-tailed; $n = 4$ per group).

C No difference in the amount of RhoA was observed in the hippocampus between APP/PS1 and WT mice (n.s, $P > 0.05$, t -test, two-tailed; $n = 4$ per group, same mice as B).

D No difference in Cdc42 activity was observed in the hippocampus between APP/PS1 and WT mice (n.s, $P > 0.05$; t -test, two-tailed; $n = 4$ per group, same mice as B).

E No difference in the amount of Cdc42 was observed in the hippocampus between APP/PS1 and WT mice (n.s, $P > 0.05$; t -test, two-tailed; $n = 4$ per group, same mice as B).

F Exacerbated activation of Rac1 activity in the elder Tg-AD fly model ($**P < 0.01$, t -test, two-tailed; $n = 6$ for *elav/Y; +*; $n = 10$ for *elav/Y; UAS-A β 42/+*).

G The activity of PAK was significantly decreased in the human prefrontal cortex. Representative Western blot of phosphorylated PAK and total PAK ($*P < 0.05$, t -test, two-tailed; $n = 4$ for each group).

Data information: All values are expressed as mean $\pm$ SEM.

Supplementary Figure 2. The location of injection in the hippocampus and the escape latency during the MWM

A To verify that the hippocampus is the location where the rAAV was injected and that Rac1 DN is expressed in excitatory neurons. Representative confocal data show that the green fluorescent signal under the control of CaMKII α was strictly expressed in the hippocampus, mainly in the dentate gyrus (DG), scale bar: 1 mm.

B Two-hour memory and four-hour memory was retained in 3-month-old APP/PS1 mice after 6 training sessions in one day ($P > 0.05$, two-way ANOVA followed by Tukey's multiple comparison test; $n = 8$ per group)

Data information: All values are expressed as mean $\pm$ SEM.

Supplementary Figure 3. Structure of CS7171, CS7170, and JKF-034

A Chemical structure of CS7171.

B Chemical structure of CS7170, which is an analogue of CS7171.

C Chemical structure of JKF-034.

Supplementary Figure 4. Effects of CS7171 and JKF-034 on A β 42 aggregation and clearance and on other RhoGTPase activities

A Representative Western blot of A β 42 detected by A β 42 monoclonal antibody (6E10) after two weeks of administration (P.O) of CS7171 and JKF-034 in 7-month-old APP/PS1 mice.

B The level of A β 42 monomer was decreased by JKF-034. CS7171 did not affect the level of A β 42 monomer (* $P < 0.05$; n.s, $P > 0.05$, one-way ANOVA followed by Tukey's multiple comparison test; n = 4 for APP/PS1, n = 6 for CS7171, n = 5 for JKF-034).

C The levels of the oligomer form of A β 42 were not changed by CS7171 and JKF-034 (n.s, $P > 0.05$, one-way ANOVA followed by Tukey's multiple comparison test; n = 4 for APP/PS1, and n = 6 for CS7171 and JKF-034, same mice as B).

D Representative Western blot of total RhoA, total Cdc42, RhoA-GTP, and Cdc42-GTP after two weeks of drug administration (P.O) of CS7171 and JKF-034 in 7-month-old APP/PS1 mice.

E Cdc42 activity was not changed by the administration of CS7171 and JKF-034 (n.s, $P > 0.05$, one-way ANOVA followed by Tukey's multiple comparison test; n = 3 for each group).

F RhoA activity was not altered by the administration of CS7171 and JKF-034 (n.s, $P > 0.05$, one-way ANOVA followed by Tukey's multiple comparison test; n = 3 for each group, same mice as E).

Data information: All values are expressed as mean $\pm$ SEM.

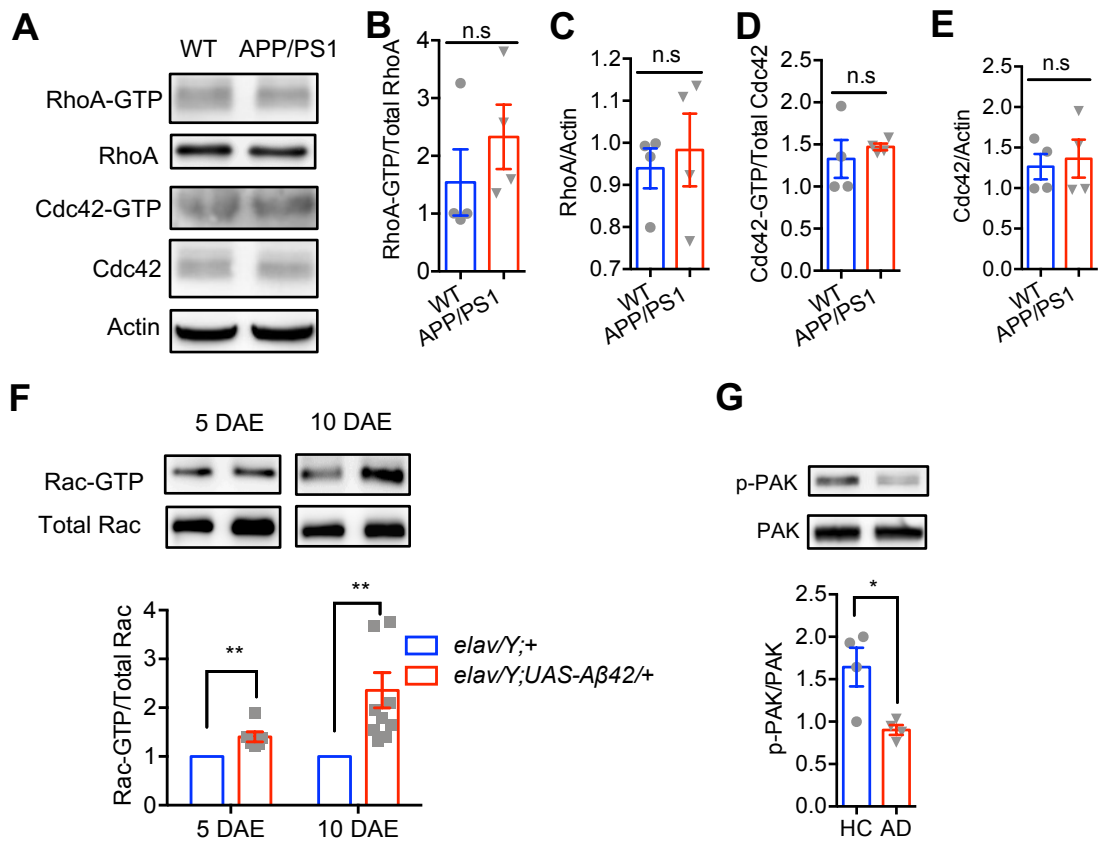


Figure S1

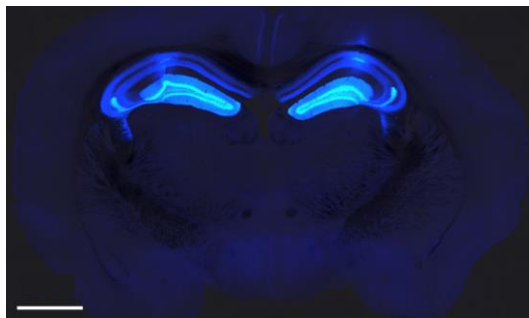
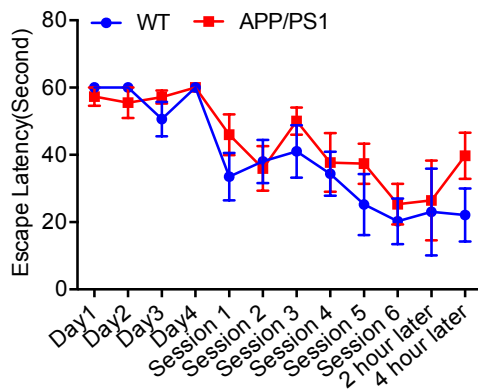
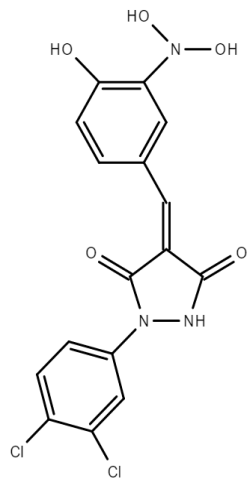
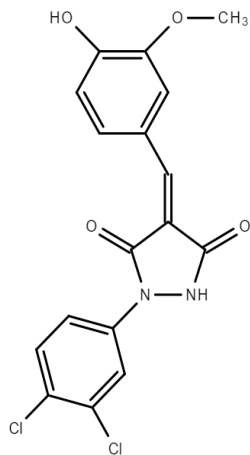
A**B**

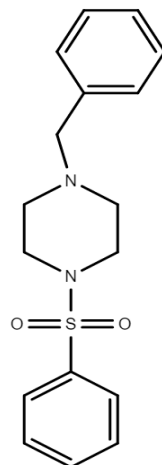
Figure S2

A

CS7171

B

CS7170

C

JKF-034

Figure S3

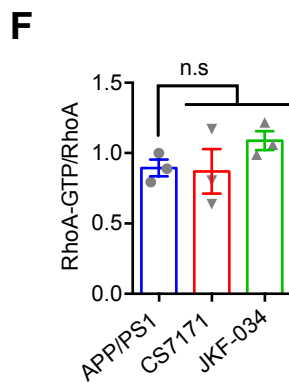
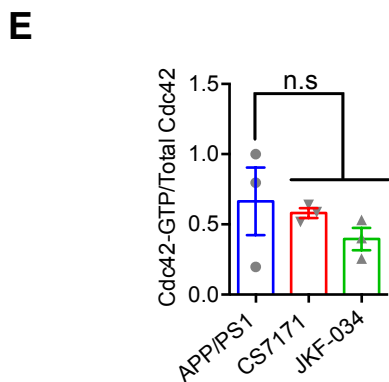
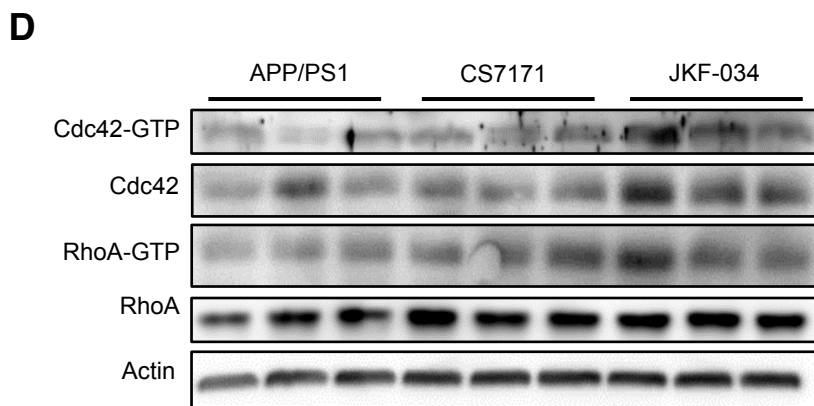
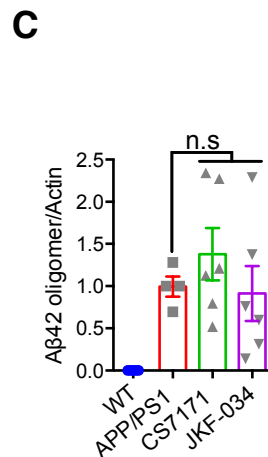
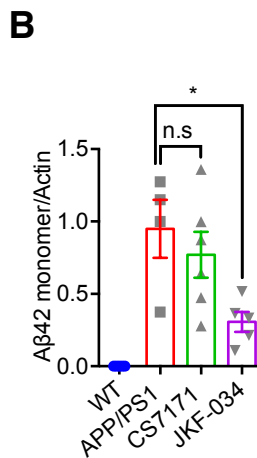
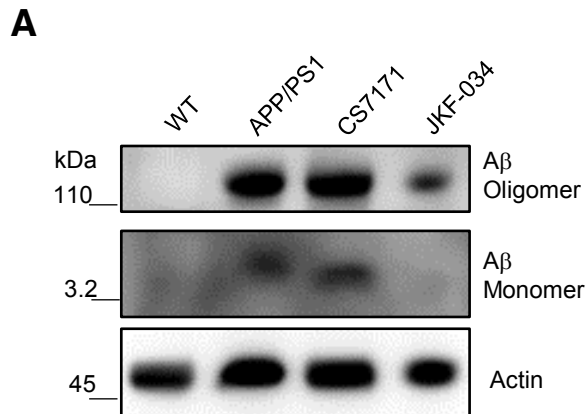


Figure S4