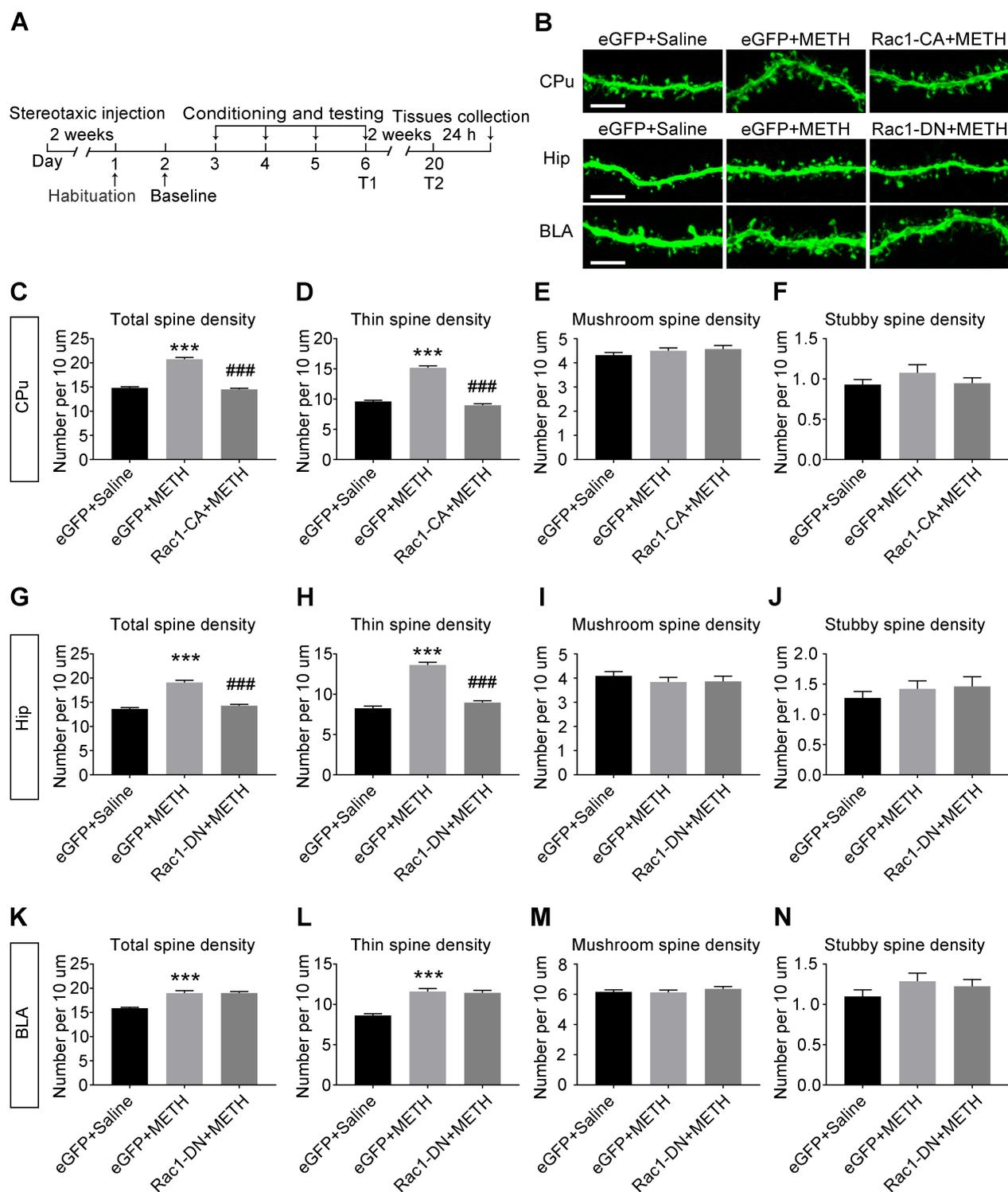


**Figure S1. Region-specific role of Rac1 signalling in the METH-associated contextual memory**

(A) Experimental paradigm for METH-induced conditioned place preference (CPP) and tissues collection. Mice were conditioned with METH or saline for 3 d, and each section of their brain tissues was dissected immediately after the final conditioning

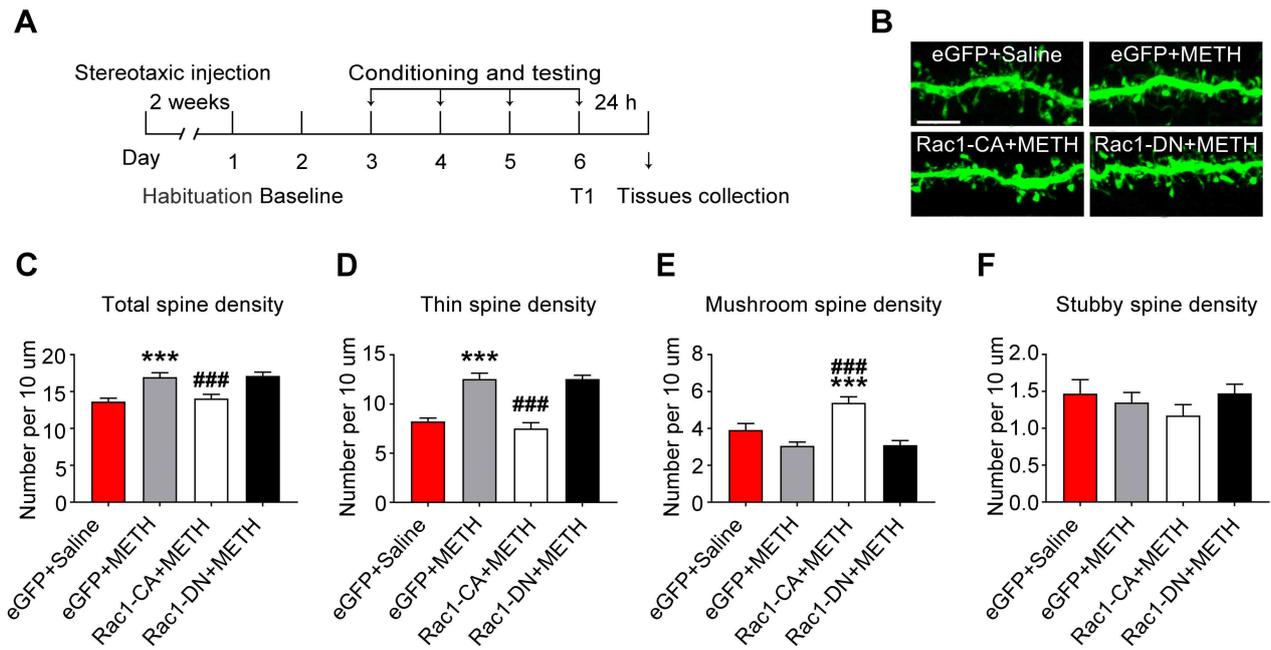
training session, and the levels of Rac1 and p-Pak were investigated. **(B-C)** The activity of the Rac1 and p-Pak was decreased in the CPu. **(D)** Anatomical location of the CPu in the mice injected with a lentivirus expressing eGFP. Scale bar=500  $\mu$ m. **(E)** Overexpression of Rac1-CA in the CPu attenuated METH-CPP acquisition. **(F-G)** The activity of the Rac1 and p-Pak was increased in the hippocampus. **(H)** Anatomical location of the hippocampus in the mice injected with a lentivirus expressing eGFP. Scale bar=500  $\mu$ m. **(I)** Overexpression of Rac1-DN in the hippocampus attenuated METH-CPP acquisition. **(J-K)** The activity of Rac1 and p-Pak was increased in the BLA. **(L)** Anatomical location of the BLA in the mice injected with a lentivirus expressing eGFP. Scale bar=200  $\mu$ m. **(M)** Overexpression of Rac1-DN in the BLA had no effect on METH-CPP acquisition. Data were analysed using a Student's t-test or one-way ANOVA followed by Bonferroni's post hoc test and presented as mean $\pm$ SEM. \*\*\* $p$ <0.001, \*\* $p$ <0.01, and \* $p$ <0.05 compared to the saline group; ### $p$ <0.001, ## $p$ <0.01, and # $p$ <0.05 compared to the eGFP METH group. BLA: basolateral amygdala; CPu: caudate putamen; Hip: hippocampus; T1: test 1; T2: test 2.



**Figure S2. Effect of Rac1 signalling on METH-induced spine remodelling in the CPu, Hip and BLA**

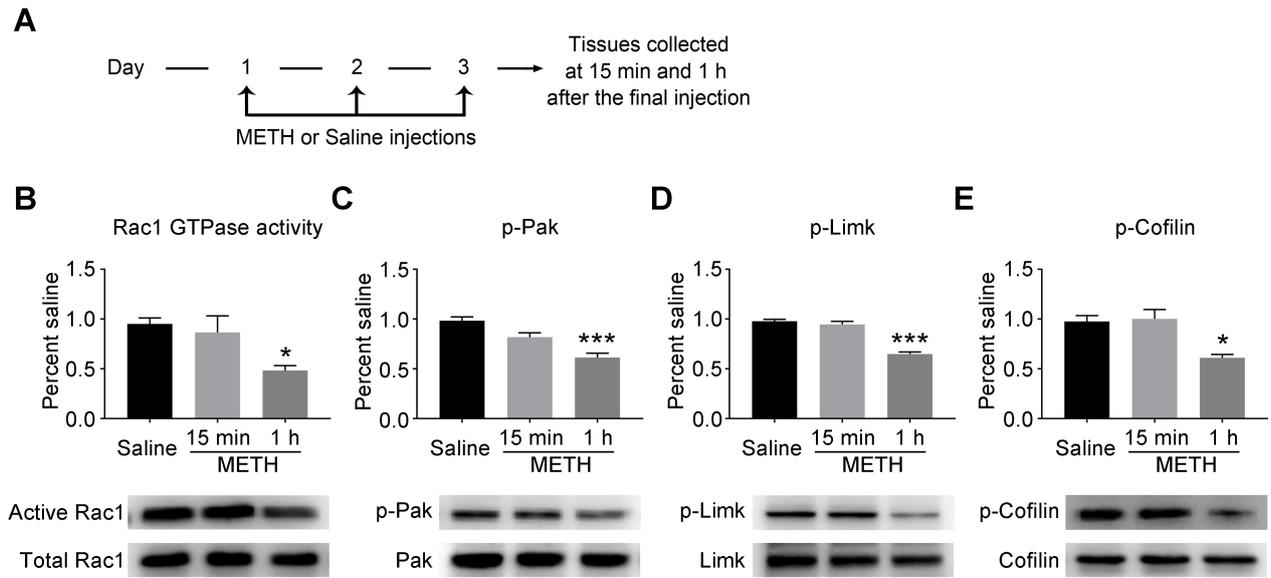
(A) Experimental design for virus injection and tissues collection. (B) Representative images of dendrites in all groups. Scale bar=5  $\mu$ m. (C-F) METH significantly increased the total and thin spine density in the CPu without affecting mushroom and

stubby spine density. METH-induced increase in the spine density was reversed by Rac1-CA. **(G-J)** The increase in the total and thin spine density induced by METH in the hippocampus was reversed by Rac1-DN. **(K-N)** The total and thin spine density were increased in the BLA after METH treatment, and Rac1-DN had no effect on the spine density in the BLA. Data were analysed using one-way ANOVA followed by Bonferroni's post hoc test and presented as mean±SEM. \*\*\* $p < 0.001$ , \*\* $p < 0.01$ , and \* $p < 0.05$  compared to the eGFP saline group. ### $p < 0.001$ , ## $p < 0.01$ , and # $p < 0.05$  compared to the eGFP METH group. BLA: basolateral amygdala; CPu: caudate putamen; Hip: hippocampus; T1: test 1; T2: test 2.



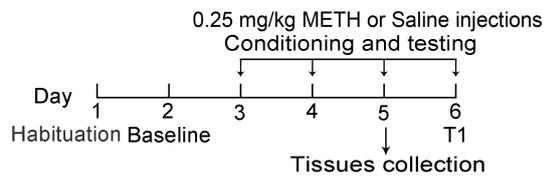
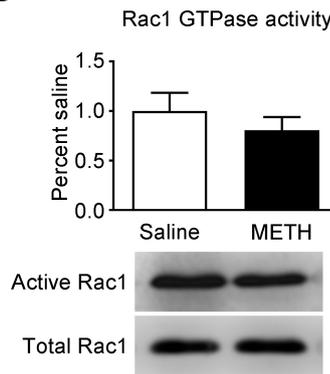
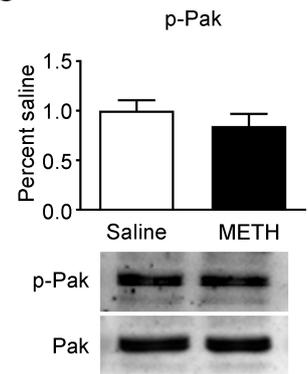
**Figure S3. Decreased Rac1 signalling mediates spine remodelling during the acquisition of METH-associated contextual memory**

(A) Experimental design used for the virus injection and tissues collection. (B) Representative images of dendritic segments in all group. Scale bar=5 μm. (C-F) METH increased the total and thin spine density 24 h after CPP test 1, and the METH-induced increases in the total and thin spine density were blocked by Rac1-CA. METH had no effect on mushroom and stubby spine density. Data were analysed using one-way ANOVA followed by Bonferroni's post hoc test and presented as mean±SEM. \*\*\*p<0.001, \*\*p<0.01, and \*p<0.05 compared to the eGFP saline group; ###p<0.001, ##p<0.01, and #p<0.05 compared to the eGFP METH group; T1: test 1.



**Figure S4. The Rac1 signalling was decreased after repeated METH treatment.**

(A) Timeline of the injections. Mice received three once-daily injections of either METH (2 mg/kg) or saline in their home cage, and then, the NAc tissues were collected 15 min or 1 h following the final injection. (B-E) The activity of Rac1, p-Pak, p-Limk, and p-Cofilin in the NAc was decreased 1 h after the final injection, without significant alterations 15 min after the final injection. Data were analysed using one-way ANOVA followed by Bonferroni's post hoc test and presented as mean±SEM. \*\*\* $p < 0.001$ , \*\* $p < 0.01$ , and \* $p < 0.05$  compared to the saline group.

**A****B****C**

**Figure S5. The Rac1 signalling was unchanged after repeated low-dose treatment with METH.**

**(A)** Experimental paradigm for METH-induced conditioned place preference (CPP). Mice were conditioned with METH (0.25 mg/kg) or saline, and then, the NAc tissues were collected immediately after the final METH conditioning training session. **(B-C)** Compared to the saline-treated mice, the activity of Rac1 and p-Pak in METH-treated (0.25 mg/kg) mice was unchanged. Data were analysed using a Student's t-test and presented as mean±SEM. T1: test 1.