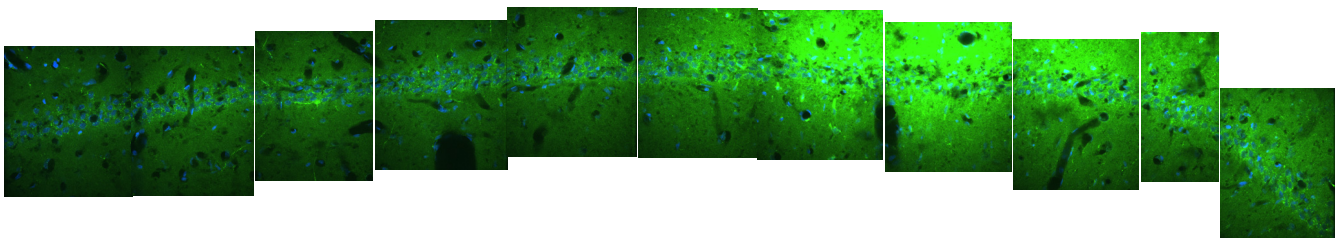
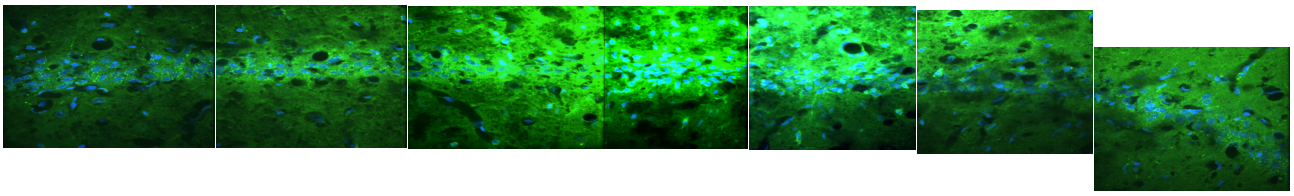
Supplemental Figure S1

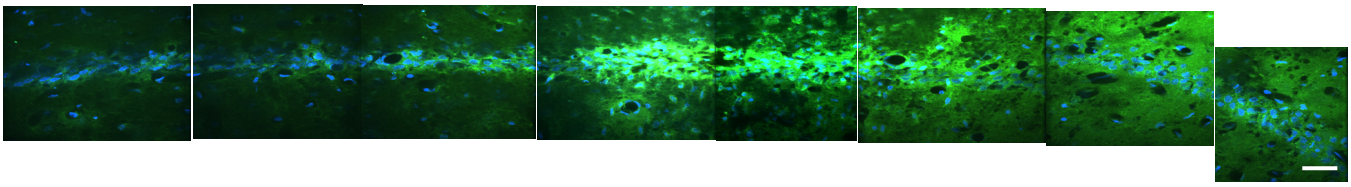
Rostral two session



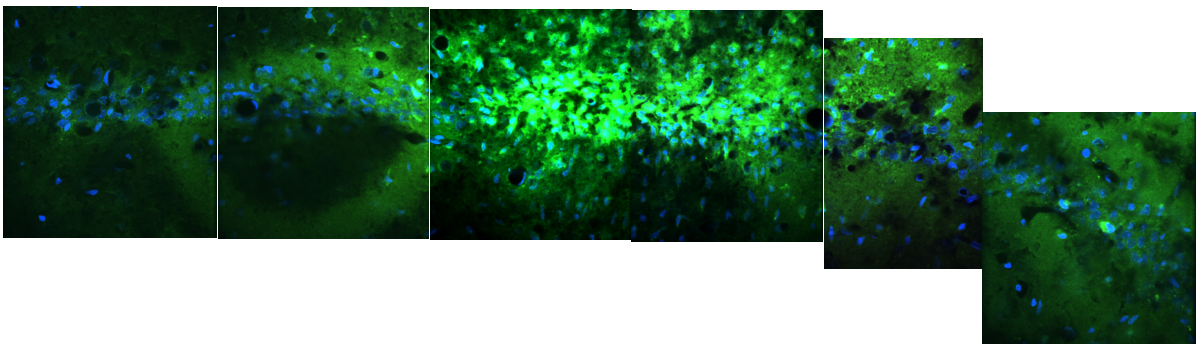
Rostral one session



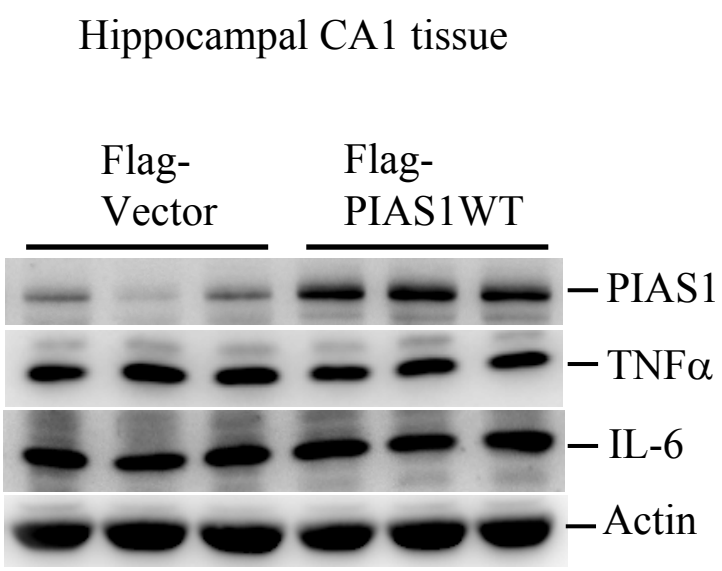
Caudal one session



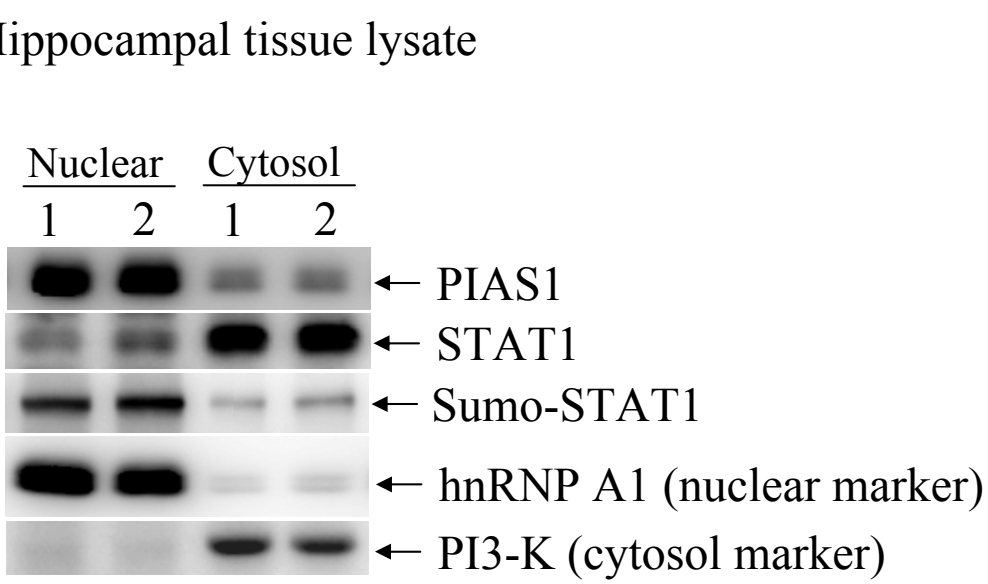
Caudal two session



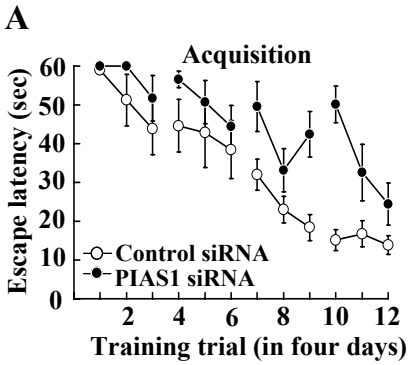
Supplemental Figure S2



Supplemental Figure S3

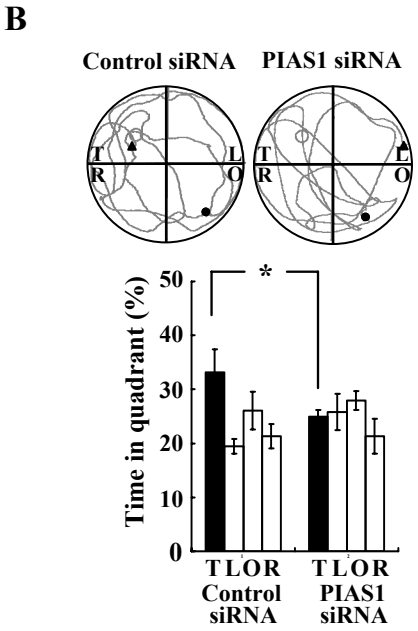


Supplemental Figure S4

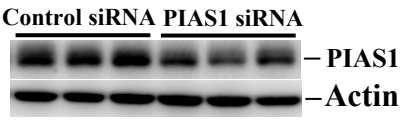


2nd PIAS1 siRNA
(N=8 each group)

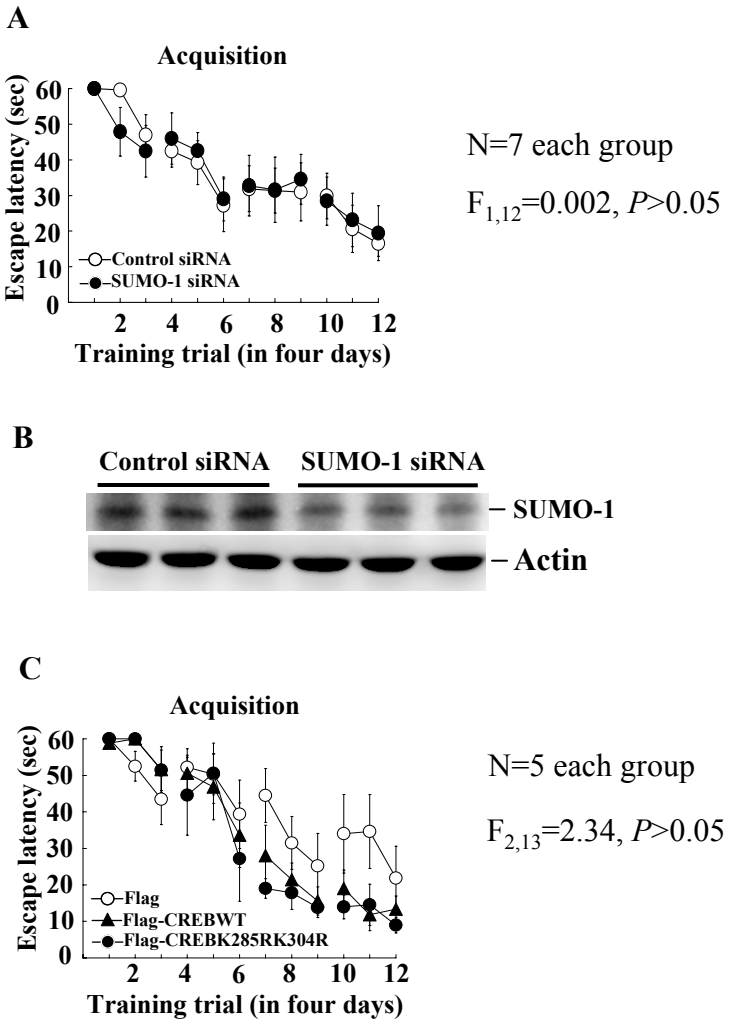
$F_{1,14}$=12.31, P<0.01



$t_{1,14}$=2.9, P<0.05 for target quadrant comparison

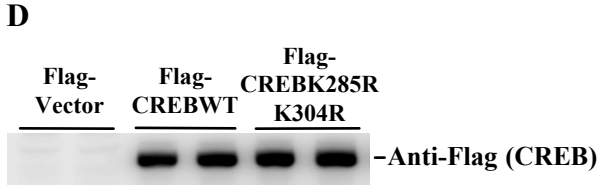


Supplemental Figure S5

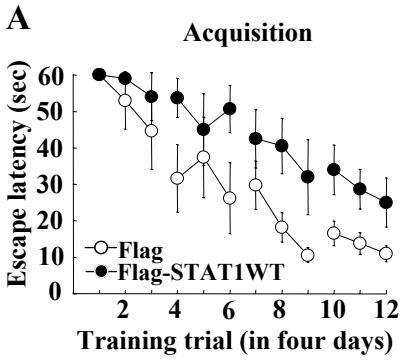


q=3.06, $P<0.05$ for comparison of CREBWT and Flag-vector groups

q=3.53, $P<0.05$ for comparison of CREBK285RK304R and Flag-vector groups

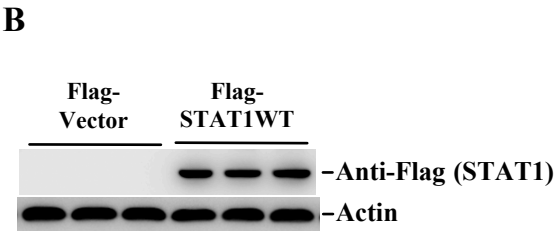


Supplemental Figure S6

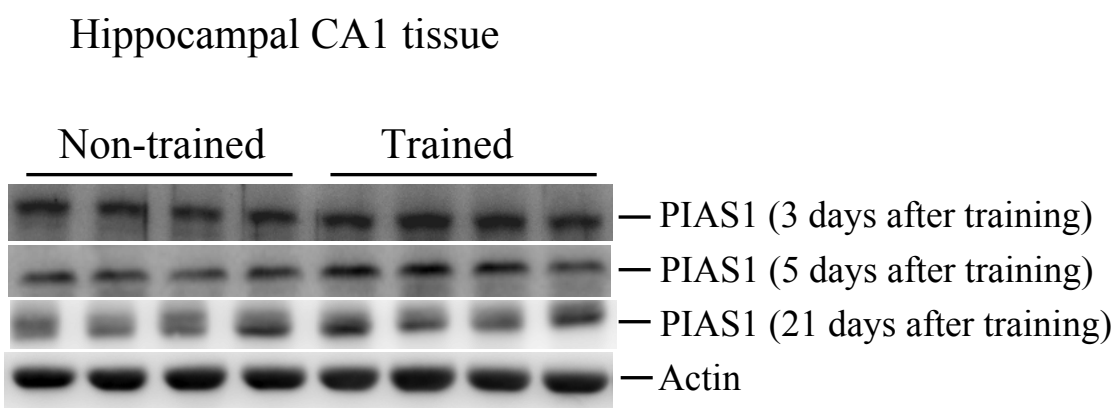


N=5 each group

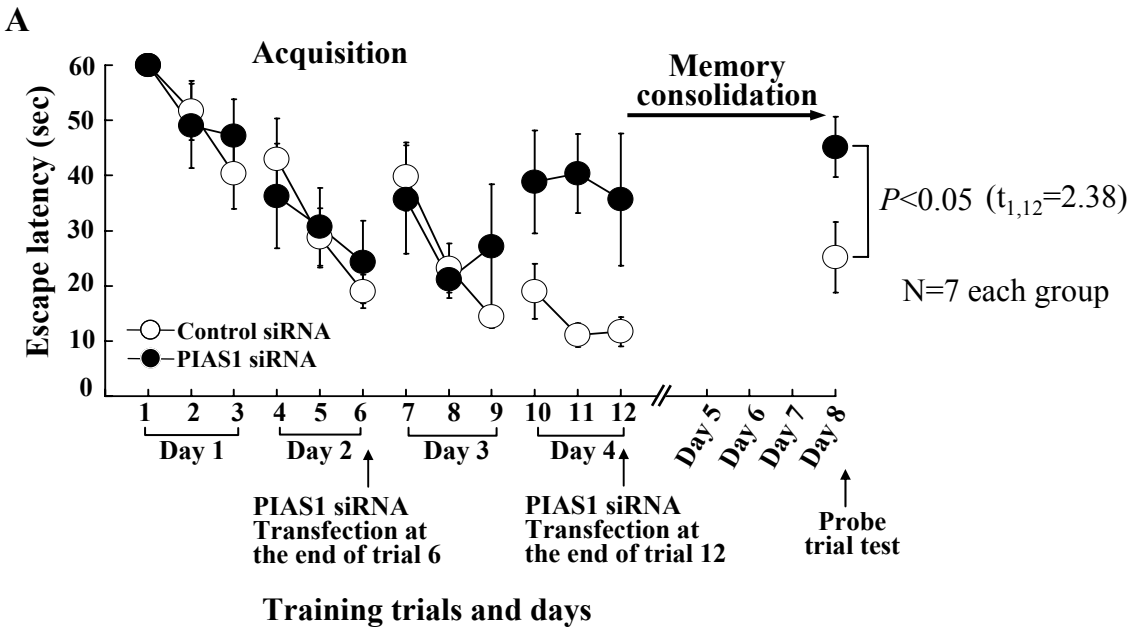
$F_{1,8}$=5.17, *P*=0.05

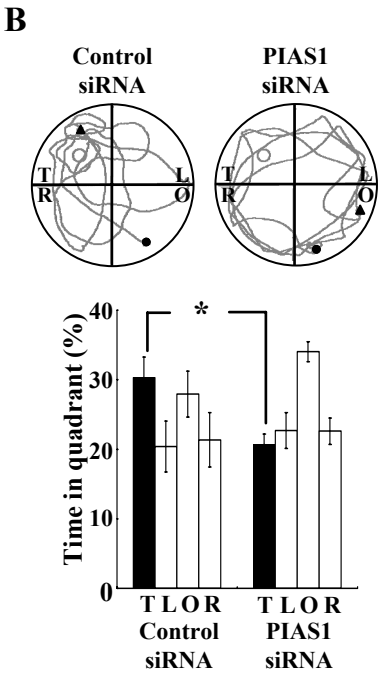


Supplemental Figure S7

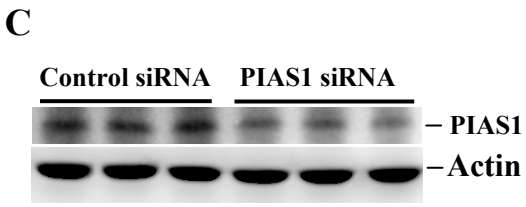


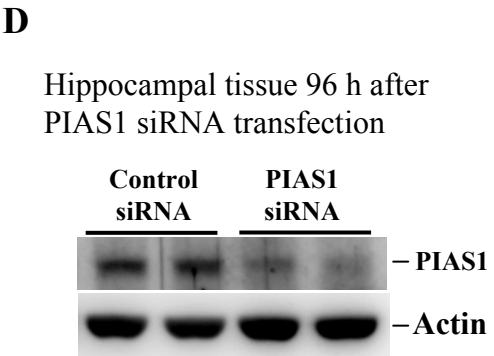
Supplemental Figure S8



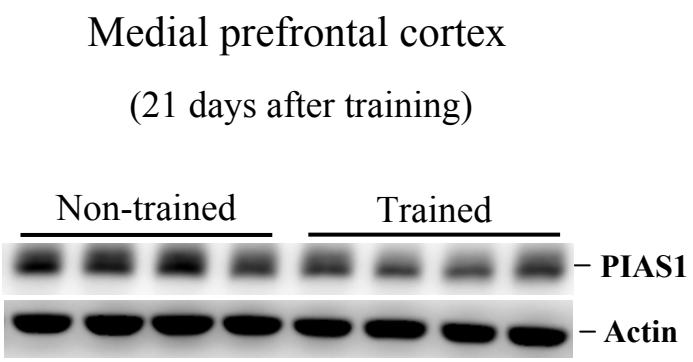


N=7 each group
$t_{1,12}$=2.95, P<0.05 for target quadrant comparison





Supplemental Figure S9



Supplemental Figure S10

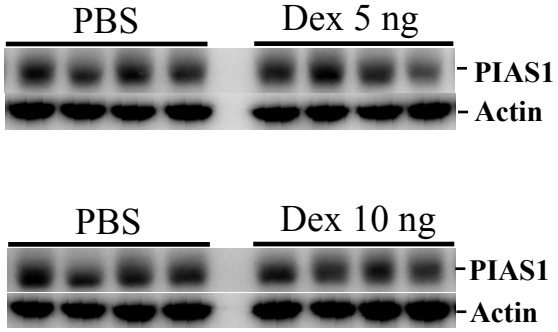
A

Corticosterone level in the blood (ng/ml)

Slow learners	**Fast learners**	N=7 each group
74.57 ± 8.53	62.51 ± 9.14	$t_{1,12}$=1.57, *P*>0.05

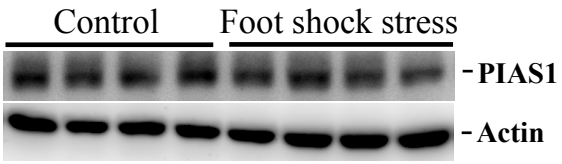
B

Hippocampal CA1 tissue



C

Hippocampal CA1 tissue



Supplemental figure legends

Figure S1. Confocal images showing immunohistochemistry of GFP (green) and DAPI (blue) for PIAS1 expression after GFP-PIAS1WT plasmid transfection to CA1 area. The expression pattern of PIAS1 from rostral to caudal CA1 tissue sessions is shown. These are adjacent sessions with 30 μm thickness each session. "Rostral" and "caudal" is in reference to the tissue session shown in Figure 2E. Scale bar equals 25 μm.

Figure S2. Over-expression of PIAS1 does not affect the expression of TNFα and IL-6. Flag-vector or Flag-PIAS1WT plasmid was transfected to rat CA1 area and animals were subjected to water maze learning and probe trial test. They were sacrificed after the probe trial test and their CA1 tissues were punched out for determination of PIAS1 expression, TNFα expression and IL-6 expression by western blot. N=5 each group.

Figure S3. PIAS1 sumoylation of STAT1 takes place in the nucleus. Hippocampal tissue lysate was fractionated into the nuclear fraction and cytosol fraction and is verified by using the specific markers. Tissue lysate was then subjected to western blot analysis of PIAS1 and STAT1 expression. STAT1 sumoylation by PIAS1 was carried out as that described in the Method Section in the manuscript.

Figure S4. Transfection of a different siRNA sequence against PIAS1 also impairs spatial learning. To avoid the possible off target effect of the PIAS1 siRNA used in the present study, a different set of PIAS1 siRNA (sequences are listed in

"Supplemental Information") was transfected to rat CA1 area and animals were subjected to (A) water maze learning and (B) probe trial test. T: target quadrant, L: left quadrant, O: opposite quadrant, R: right quadrant. •: start point, ▲: end point. (C) Representative gel pattern showing the level of PIAS1 expression by western blot after PIAS1 siRNA transfection. Data are mean SEM. N=8 each group. * $P<0.05$.

Figure S5. Transfection of SUMO-1 siRNA does not affect spatial learning whereas transfection of the CREB sumoylation mutant facilitates spatial learning. (A) SUMO-1 siRNA or control siRNA was transfected to CA1 area and rats were subjected to water maze learning. (B) Representative gel pattern showing the level of SUMO-1 expression by western blot. N=7 each group. (C) CREB wild-type (WT) plasmid or the CREB sumoylation double mutant plasmid (CREBK285RK304R) was transfected to CA1 area and rats were subjected to water maze learning. (D) Representative gel pattern of the anti-Flag band confirming the transfection and expression of the CREB plasmids in CA1 area. N=5 each group. Data are expressed as in Figure S4.

Figure S6. Transfection of STAT1WT plasmid impairs spatial acquisition. (A) Flag-STAT1WT plasmid or Flag-vector plasmid was transfected to CA1 area and rats were subjected to water maze learning. (B) Representative gel pattern of the anti-Flag band confirming the transfection and expression of the STAT1 plasmid in CA1 area. N=5 each group.

Figure S7. Spatial training does not apparently increase PIAS1 expression at three days and five days later. Representative gel pattern showing PIAS1 protein

expression in CA1 area in trained and non-trained animals at three days, five days and 21 days after training. N=6 each group.

Figure S8. Transfection of PIAS1 siRNA impairs memory consolidation. (A) Animals were subjected to water maze training for four days and were divided into two equal groups based on their escape latencies by the end of training day 2. One group of animals received PIAS1 siRNA transfection at the end of day 2 and day 4, (two injections in all), and probe trial test was conducted on day 8. The rationale for adopting this transfection schedule is to maintain the knockdown of PIAS1 expression during the memory consolidation process and to measure memory retention at a longer time interval after training (four days). Control animals received control siRNA transfection. (B) Probe trial test showing memory retention of PIAS1 siRNA-treated animals and control siRNA-treated animals on day 8. (C) Representative gel pattern showing the level of PIAS1 expression after PIAS1 siRNA transfection and probe trial test. N=7 each group. (D) Representative gel pattern showing the knockdown effect of PIAS1 siRNA transfection at 96 h later. N=4 each group. Data are expressed as in Figure S4.

Figure S9. Spatial training tends to decrease PIAS1 expression in the medial prefrontal cortex at 21 days later. Representative gel pattern showing PIAS1 protein expression in the medial prefrontal cortex at 21 days after spatial training. N=6 each group.

Figure S10. Dexamethasone and foot shock stress do not alter PIAS1 expression. (A) ELISA assay showing blood corticosterone level in fast learners and slow learners

after water maze learning. N=7 each group. (B) Representative gel pattern showing the level of PIAS1 expression after 5 ng and 10 ng dexamethasone administration in CA1 area. Dexamethasone was injected to rat CA1 area bilaterally and animals were sacrificed 60 min after injection. Their CA1 tissues were subjected to western blot analysis of PIAS1 expression. N=4 each group. (C) Electric foot shocks (for a total of 15 min) were delivered to rats to induce acute stress in animals. Animals were sacrificed 30 min after the end of the foot shocks and their CA1 tissues were punched out for western blot determination of PIAS1 expression. N=5 each group.

Supplemental Table S1

Sequences of PIAS1, GFP and HPRT primers used for real-time PCR

PIAS1 primer:
forward: 5’-TCCTGCTGTAGATACAAGCTAC-3’
reverse: 5’-TGCCAAAGATGGACGCTGTGTC-3’
GFP primer:
forward: 5’-CGACGGCAACTACAAGAC-3’
reverse: 5’-TAGTTGTACTCCAGCTTGTGC-3’
HPRT primer:
forward: 5’-GCCGACCGGTTCTGTCAT-3’
reverse: 5’-TCATAACCTGGTTCATCATCACTAATC-3’

Supplemental information

Materials and methods

Plasmid DNA construction and DNA/polyethyleneimine (PEI) complex preparation

The method and procedure used for construction of the CREB1a wild-type (WT) plasmid was the same as that described for PIAS1 plasmid construction. Briefly, for construction of the Flag-tagged CREB1a plasmid, full-length *creb* was cloned by amplifying the rat hippocampal *creb1a* cDNA with primers 5′-ATGACCATGGACTCTGGAGCAG-3′ amd 5′-TTAATCTGACTTGTGGCAGTAAAG-3′. The PCR product was sub-cloned between the *Eco*RI and *Xho*I sites of the mammalian expression vector pCMV-Tag2B (Strategene, La Jolla, CA). The CREB1a sumoylation site mutant, CREBK285RK304R (Comerford *et al.*, 2003), was generated by using the QuickChange Site-Directed Mutagenesis Kit (Strategene). The preparation and injection of the CREB plasmid and CREBK285RK304R was the same as that described for PIAS1WT plasmid in the Method Section.

RNA interference

To exclude the off target effect of PIAS1 siRNA transfection on spatial learning, we

have used another set of PIAS1 siRNA to knock down PIAS1 expression in rat CA1 area and examined its effect on spatial learning. The sense and antisense sequences were adopted from a previous study (Weber *et al.*, 2009). The sequence for the sense strand is: 5′-GAACUAAAGCAAAUGGUUATT-3′ and that for the antisense strand is: 5′-UAACCAUUUGCUUUAGUUCTT-3′. In addition, to assess the general effect of sumoylation on spatial learning, SUMO-1 siRNA was used to knock down SUMO-1 expression in rat CA1 area. The sense and antisense sequences used were adopted from a previous study (Meinecke *et al.*, 2007). The sequence for the sense strand is: 5′-CUGGGAAUGGAGGAAGAAGTT-3′ and that for the antisense strand is: 5′-CUUCUUCCUCCAUUCCCAGTT-3′. The PIAS1 siRNA and SUMO-1 siRNA were both synthesized from MWG Biotech (Ebersberg, Germany). The Silencer Negative Control number 1 siRNA (control siRNA) was synthesized from Ambion and was used as a control.

Intra-hippocampal gene transfection and siRNA injection

Rats were anesthetized with pentobarbital (40 mg/kg, i.p.) and subjected to stereotaxic surgery. Cannulae were implanted bilaterally to the dorsal hippocampal CA1 area as described in the Method Section. After recovery from surgery, 0.7 μl CREBWT or CREB sumoylation mutant plasmid DNA complex (1.5 μg/μl) was injected into CA1

area at a rate of 0.1 μl/min. For siRNA injection, 0.7 μl of SUMO-1 siRNA (8 pmol/μl) or Silencer Negative Control number 1 siRNA (control siRNA) was transfected to the CA1 area by using the cationic polymer transfection reagent jetSI^{TM} 10 mM (Polyplus-Transfection). The injection needle used is the same as that described in the Method Section. The injection needle was left in place for 5 min to limit the diffusion of injected DNA and siRNA. Spatial training started 48 h after DNA injection or 72 h after siRNA injection. Plasmid DNA or siRNA was injected again at the beginning of the second training day. One hour was allowed between the second injection and spatial training.

Animals were sacrificed immediately after the probe trial test or after water maze training. Their brains were removed and the hippocampal tissue slices (2 mm thickness each slice, two slices in all) were dissected out by using a brain slicer. The CA1 tissue was further dissected out by using a punch with 2 mm in diameter for further western blot analysis.

Preparation of nuclear and cytosol fractions. The nuclear and cytosol fractions were prepared by using the kit purchased from Sigma (CelLytic Nuclear Extraction Kit). Hippocampal tissue was first washed with 1X PBS then lysed and scraped in 1X hypotonic lysis buffer containing 50 mM Tris-HCl (pH 7.5), 10 mM $MgCl_2$, 15 mM

$CaCl_2$ and 1.5 M sucrose with cocktail of protease inhibitor and 0.1 M DTT. The lysate was stroked for 20 times in a homogenizer. Next, tissue lysate was centrifuged at 11000 X g for 20 min to separate the nuclear fraction (pellet) and the cytosolic fraction (supernatant). The supernatant was then transfer to a fresh tube. The pellet was washed in hypotonic lysis buffer containing the protease inhibitor cocktail for 3 times. The pellet was then added with 1X extraction buffer containing 20 mM HEPES (PH7.9), 1.5 mM $MgCl_2$, 0.42 M NaCl, 0.2 mM EDTA TA and 25% glycerol with cocktail of protease inhibitor and 0.1 M DTT. The nuclei fraction was incubated with 1X extraction buffer at 4 °C for 1 h and then centrifuged at 21000 X g for 5 min to get the nuclear extract. Each fraction was subjected to 8% SDS-PAGE and the specific antibodies for nuclear protein, hnRNP A1 (Acris Antibodies GmbH, Herford, Germany), or cytosol marker, PI3-K (Millipore) were used in western blot.

Western blot

Hippocampal CA1 tissue lysate was lysed and prepared as that described in the Method Section. The lysate was resolved by 8% SDS-PAGE. The proteins resolved by SDS-PAGE were transferred to the polyvinylidene fluoride (PVDF) membrane (Millipore) and western blot was conducted by using the following antibodies: anti-SUMO-1 (Cell Signaling), anti-TNFα (Abcam), anti-IL6 (Abcam). The

secondary antibody used was HRP-conjugated goat-anti rabbit or mouse IgG antibody (Chemicon). Membrane was developed by reacting with chemiluminescence HRP substrate (Millipore) and exposed to the LAS-3000 image system (Fujifilm) for visualization of protein bands.

Foot shock stress

Foot shocks were delivered to the rats to induce acute stress according to that described previously (Lee *et al.*, 1986). Briefly, animals received acute foot shocks delivered at 2 mA (square wave current) and 250-msec duration for a total period of 15 min. The interval between two shocks was variable but with an average of 5 sec. These animals were then put back to their home cages. Control animals were also placed in the shock box for 15 min but no shock was delivered. Animals were sacrificed 30 min after the foot shocks and their hippocampal CA1 tissue and amygdala tissue were subjected to PIAS1 protein determination by western blot.

Corticosterone enzyme-linked immunosorbent assay (ELISA)

Rat serum corticosterone level was measured by corticosterone ELISA kit #402810 (Neogene Co., MI, USA). Serum corticosterone was extracted by ethyl ether from 100 μl rat serum, and ELISA was processed according to the manufacturer's instructions.