SUPPLEMENTARY INFORMATION

Title: Regulation of synaptic plasticity and cognition by SUMO in normal physiology and Alzheimer's disease

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Supplementary Figure S1

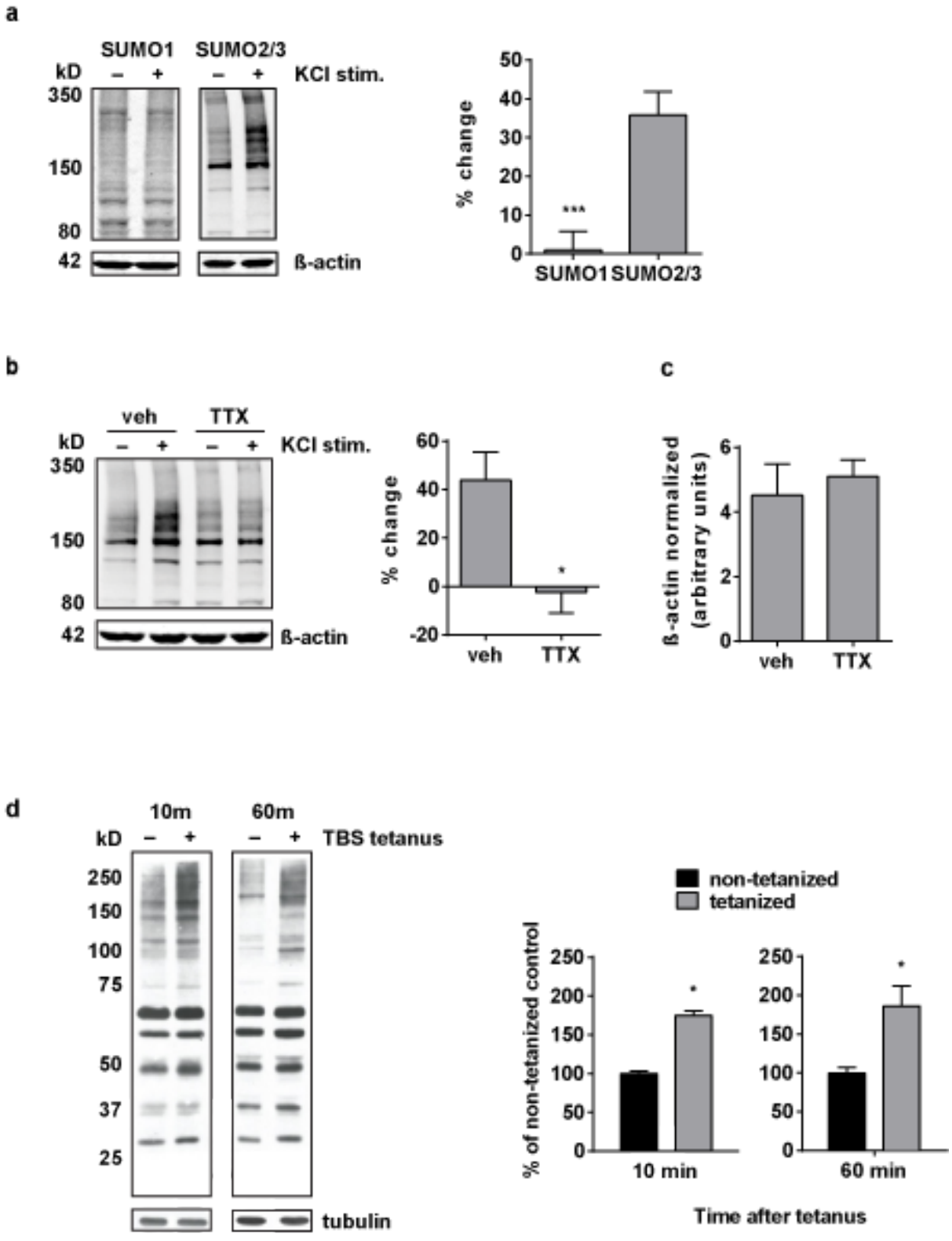

Figure S1. Characteristics of activity-induced SUMOylation

(A) SUMO1 conjugation is not significantly affected by high-KCl depolarization. Representative western blots show increase in SUMO2/3 high molecular weight conjugation that does not occur with SUMO1. SUMO1 n = 8, SUMO2/3 n = 14; ***p = 0.001.

(B) Tetrodotoxin (TTX) blocks the depolarization-induced increase in SUMOylation. Representative SUMO2/3 western blot. Vehicle n = 3, TTX n = 3; *p=0.03.

(C) TTX does not significantly affect basal high molecular weight SUMO2/3 conjugation. Quantified from western blots.

(D) Theta-burst stimulation (TBS) induces SUMOylation increase in area CA1 of acute hippocampal slices, at 10 minutes and 60 minutes post-tetanus. Representative SUMO2/3 western blots (from separate experiments for the 10 minute and 60 minute time points). n = 5 slices per time point.

Data presented as means ± SEM.

Supplementary Figure S2

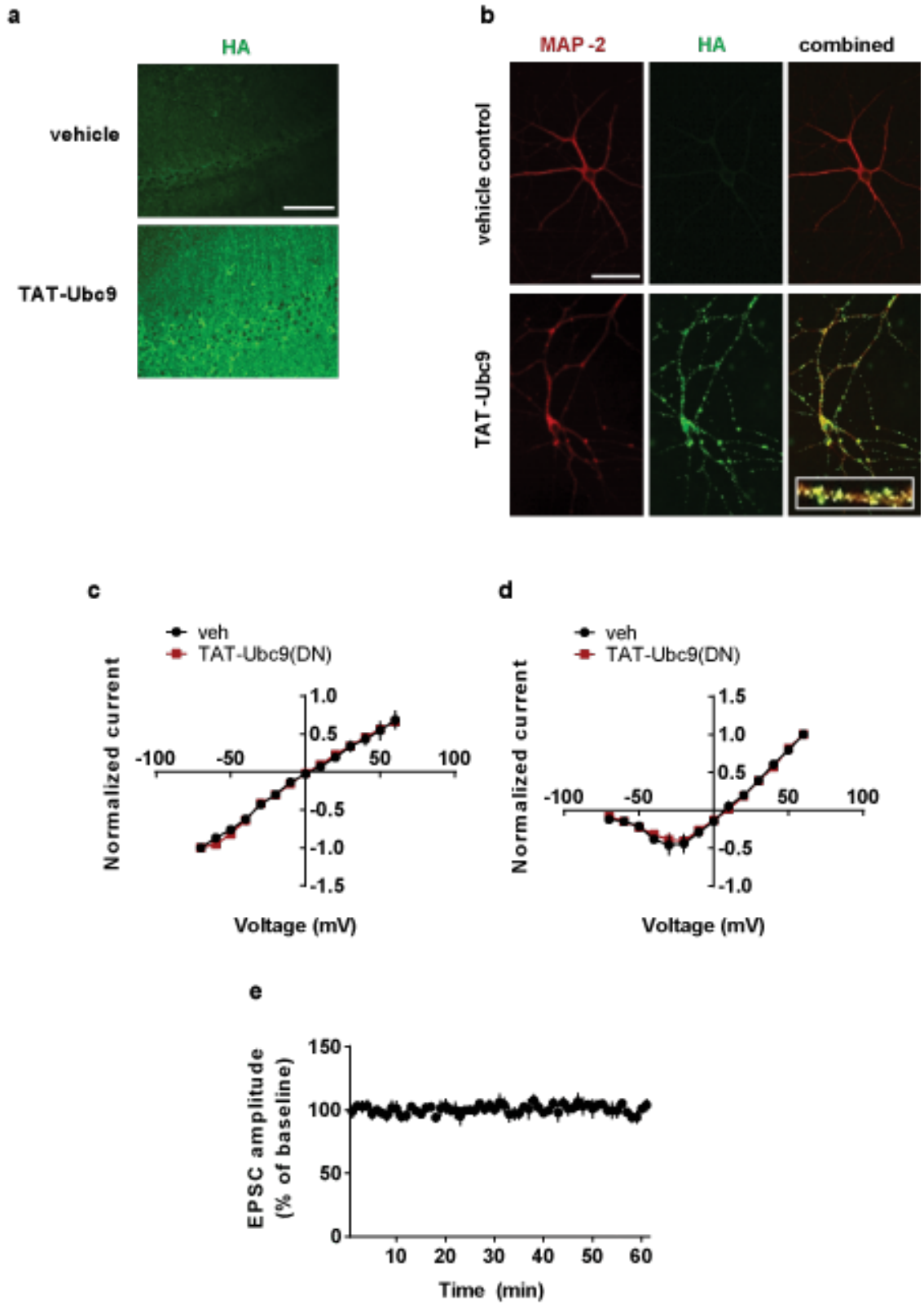

Figure S2. TAT-Ubc9 transduces neurons, does not impact basal synaptic transmission

(A) TAT-Ubc9 transduces neurons in the cell body layer of hippocampal area CA1 (1hr 100 nM incubation). Scale bar 50 μm.

(B) TAT-Ubc9 transduces primary hippocampal neuronal cultures (1hr 100 nM incubation). Scale bar 100 μm.

(C) AMPAR I-V curves. AMPA receptor currents are not affected by TAT-Ubc9(DN). Vehicle and TAT-Ubc9(DN) n = 3 cells each. Currents normalized to -70 mV.

(D) NMDAR I-V curves. NMDA receptor currents are not affected by TAT-Ubc9(DN). Vehicle and TAT-Ubc9(DN) n = 3 cells each. Currents normalized to +40 mV.

(E) Inclusion of GST-SENP1 in the patch pipette did not alter baseline synaptic transmission. Summary graph of EPSC recordings from CA1 pyramidal cells held at -70 mV (n=3 cells). Responses in the first 5 minutes were averaged and normalized to 100%. Each point of the graph represents the average of six successive recordings taken every 10 seconds.

Data presented as means ± SEM.

Supplementary Figure S3

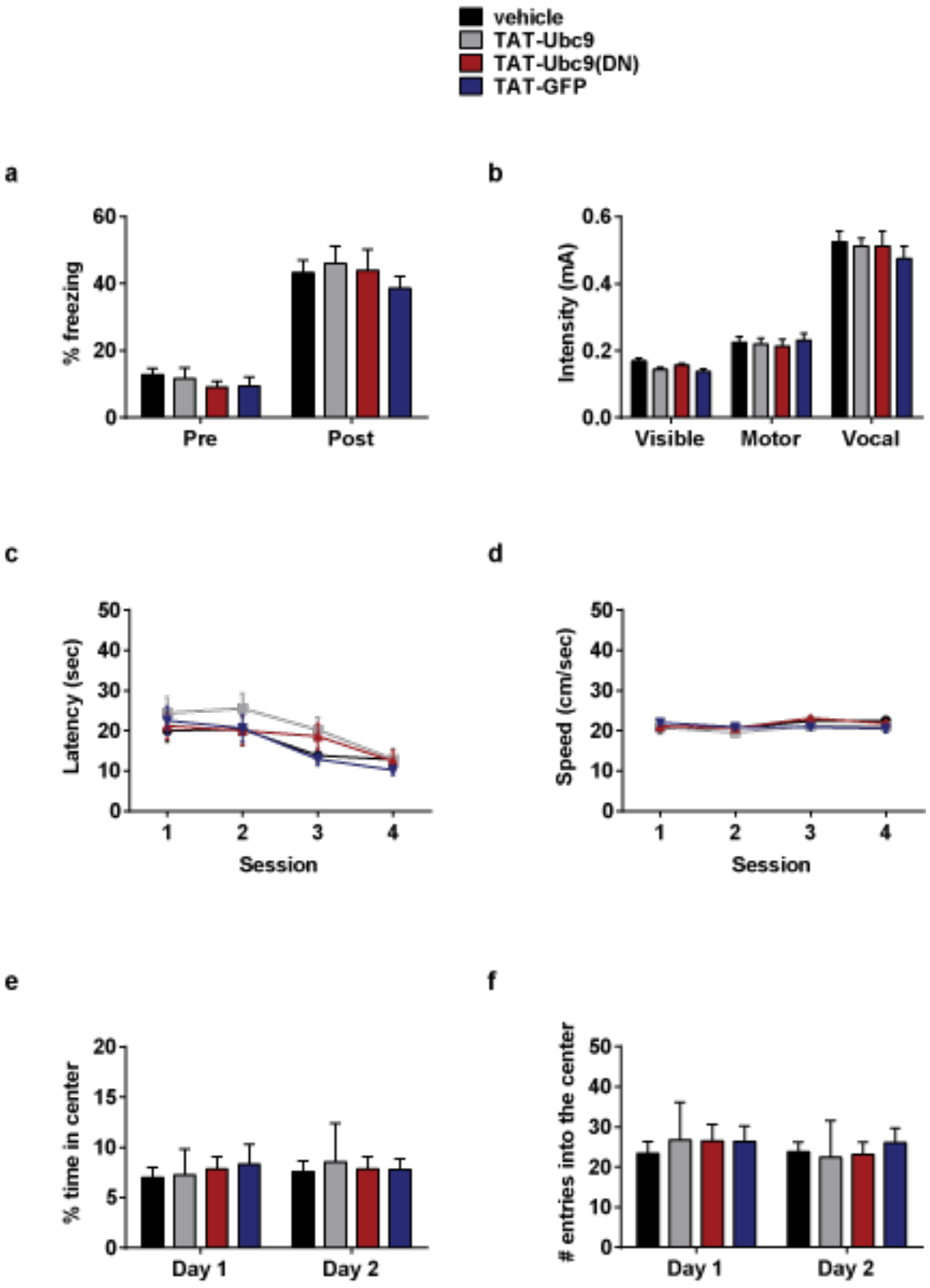

Figure S3. TAT-Ubc9(DN) effects on behavioral parameters (related to Figure 3)

(A) Cued conditioning was not significantly affected by TAT-Ubc9 or TAT-Ubc9(DN) infusions. Vehicle n = 16, TAT-Ubc9 n = 8, TAT-Ubc9(DN) n = 16, TAT-GFP n = 12.

(B) Sensory threshold assessments were not significantly affected by TAT-Ubc9 or TAT-Ubc9(DN) infusions.

(C) Time to reach a visible platform was not significantly affected by TAT-Ubc9 or TAT-Ubc9(DN) infusions. Vehicle n = 11, TAT-Ubc9 n = 11, TAT-Ubc9(DN) n = 12, TAT-GFP n = 11.

(D) Swimming speed was not significantly affected by TAT-Ubc9 or TAT-Ubc9(DN) infusions.

(E) Open field behavioral task. Percent time spent in the center was not significantly affected by TAT-Ubc9 or TAT-Ubc9(DN) infusions. All groups n = 8 each.

(F) Open field behavioral task. Number of entries into the center was not significantly affected by TAT-Ubc9 or TAT-Ubc9(DN) infusions.

Data presented as means ± SEM.

Supplementary Figure S4

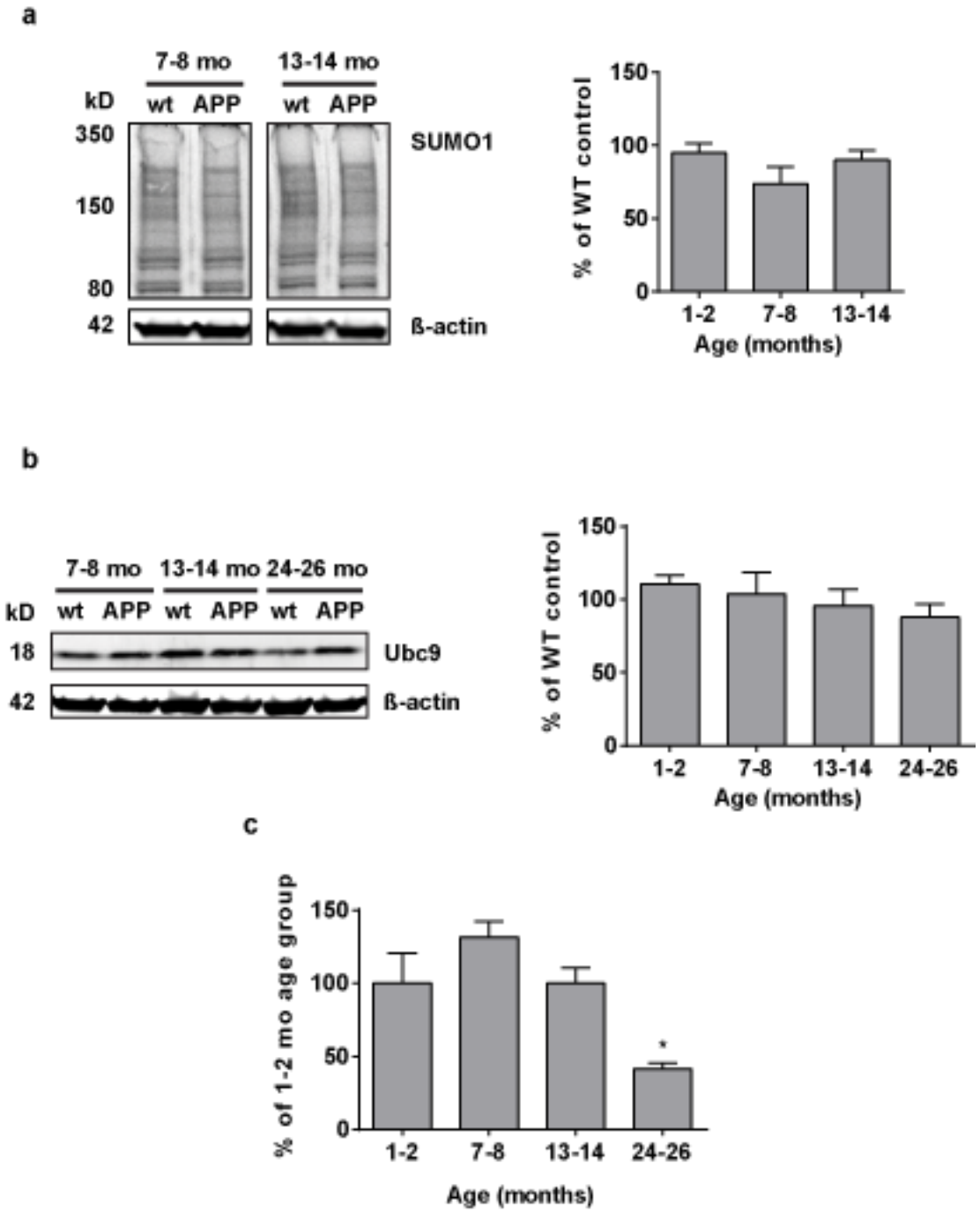

Figure S4. SUMO1 conjugation and Ubc9 levels are not affected in Tg2576 APP-overexpressing mice (related to Figure 4)

(A) Representative SUMO1 western blots with hippocampi from Tg2576 APP transgenic and wild-type littermate mice. 1-2 mo. n = 6 each WT and APP, 7-8 mo. n = 3 each, 13-14 mo. n = 4 each; no significant differences.

(B) Representative Ubc9 western blot with Tg2576 APP and wild-type littermate hippocampi. No significant differences.

(C) High-molecular weight SUMO2/3 conjugation is decreased in aged wild-type mice. 1-2 mo. n = 3, 7-8 mo. n = 3, 13-14 mo. n = 4; 24-26 mo. n = 3; no significant difference. Relates to Figure 1B.

Data presented as means ± SEM.

Supplementary Figure S5

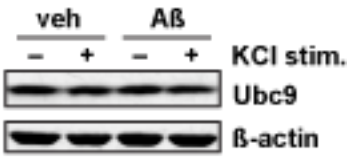

Figure S5. KCl stimulation and Aβ exposure do not affect Ubc9 protein levels (related to Figure 5)

Ubc9 protein levels are not significantly affected by high KCl depolarization or $A\beta_{42}$ treatment (200 nM, 20 minutes).

Supplementary Figure S6

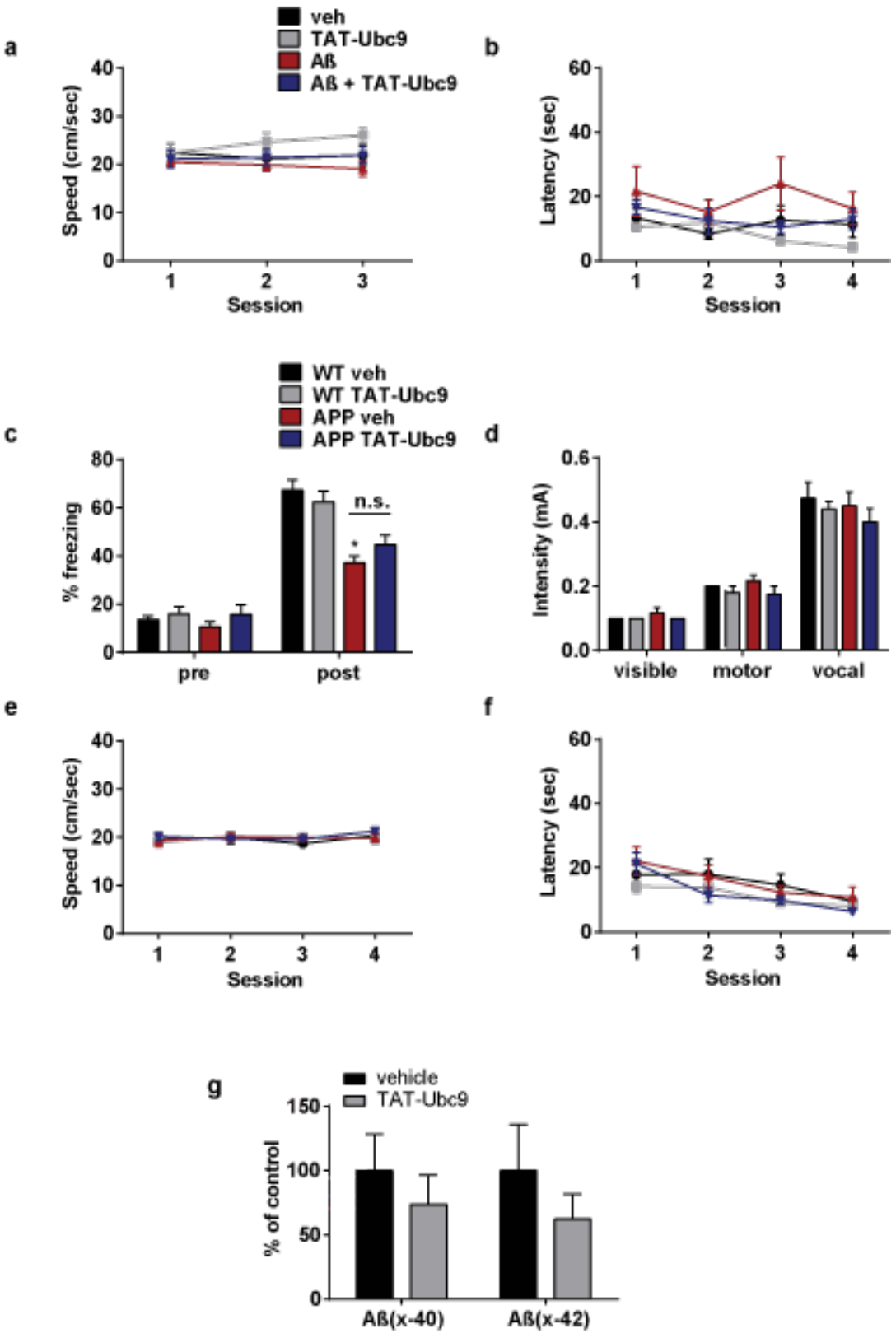

Figure S6. TAT-Ubc9 effects on Aβ levels and behavioral parameters (related to Figure 6)

(A) Swimming speed was not significantly affected by TAT-Ubc9 infusions.

(B) Time to reach a visible platform was not significantly affected by TAT-Ubc9 infusions.

(C) Cued conditioning was not significantly affected by TAT-Ubc9 infusions in Tg2576 APP mice. WT vehicle (PBS) n = 11, WT TAT-Ubc9 n = 13, APP vehicle n = 13; APP TAT-Ubc9 n = 13.

(D) Sensory threshold assessments were not affected by TAT-Ubc9 infusions in APP mice.

(E) Swimming speed was not significantly affected by TAT-Ubc9 infusions in APP mice.

(F) Time to reach a visible platform was not significantly affected by TAT-Ubc9 infusions in APP mice.

(G) $A\beta_{(x-40)}$ and $A\beta_{(x-42)}$ ELISAs with the soluble homogenization fractions of hippocampal slices treated with TAT-Ubc9 (100 nM 1 hour). $A\beta_{(x-40)}$ n = 6 each vehicle and TAT-treated, $A\beta_{(x-42)}$ n = 5 each.

Data presented as means ± SEM.

Supplementary Figure S7

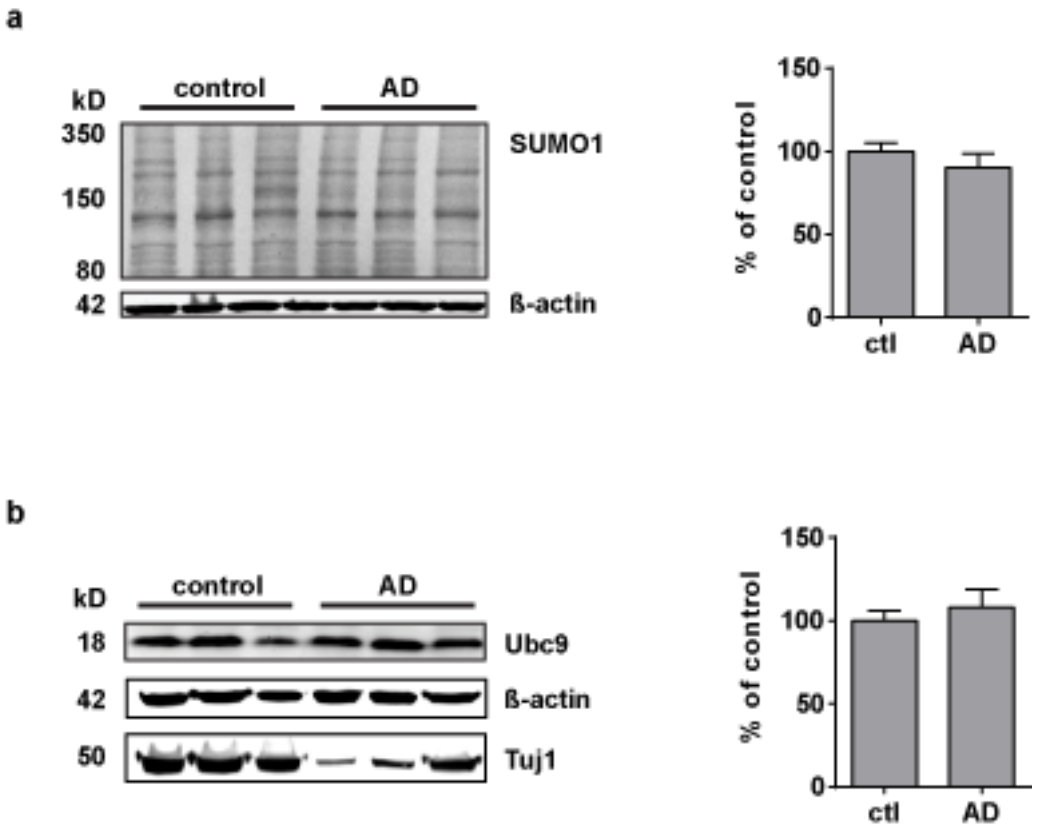

Figure S7. SUMO1 conjugation and Ubc9 levels in post-mortem Alzheimer's disease hippocampal tissue (related to Figure 7)

(A) Representative SUMO1 western blot with post-mortem hippocampal formation tissue from AD and control patients, showing high molecular conjugates. Control and AD n= 6 each; no significant difference.

(B) Representative Ubc9 western blot with AD and control tissue. No significant difference.

Data presented as means ± SEM.

Age	Sex	Diagnosis	Braak NFT	CERAD	NIA-R
54	F	Control / usual aging	0	n.a.	n.a.
89	M	Control / usual aging	III	n.a.	n.a.
60	M	Control / usual aging	II	n.a.	n.a.
74	M	Control / usual aging	III	n.a.	n.a.
89	F	Control / usual aging	IV	n.a.	n.a.
80	M	Control / usual aging	II	A	low
61	F	AD	VI	C	high
61	M	AD	VI	C	high
68	M	AD	VI	C	high
86	M	AD	V	B	int
71	M	AD	VI	C	high
60	M	AD	VI	C	high

Table S1. Brain bank sample characteristics

Frozen post-mortem hippocampal formation tissue samples. Diagnosis of either control / usual aging or Alzheimer's disease (AD). Scores include Braak neurofibrillary tangles (NFT), Consortium to Establish a Registry for Alzheimer's Disease (CERAD) Neuropsychologic Battery, National Institute of Aging – Reagan Institute rating. n.a. = not applicable.

SUPPLEMENTAL EXPERIMENTAL PROCEDURES

Extracellular field potential recordings. Transverse hippocampal slices (400 μm) were cut with a tissue chopper and maintained in an interface chamber at 29 C for at least 90 minutes prior to recording, as previously described [1]. The artificial cerebrospinal fluid (ACSF) bath buffer consisted of (in mM): 124 NaCl, 4.4 KCl, 1 Na_2HPO_4, 25 $NaHCO_3$, 2 $CaCl_2$, 2 $MgSO_4$, 10 glucose. The buffer was continuously aerated with 95% O_2 / 5% CO_2 to a final pH of 7.4. Field extracellular postsynaptic potentials (fEPSPs) were recorded using a stimulating electrode (bipolar tungsten) at the Schaffer collateral fibers and a recording electrode (ACSF-filled glass pipette) at the CA1 stratum radiatum. Basal synaptic transmission was assessed by plotting stimulus voltages (V) against the corresponding fEPSP slopes. Stimulus intensity was set so that baseline responses were approximately 1/3 of the maximum evoked response. LTP was induced using theta-burst stimulation (4 pulses at 100 Hz, with the bursts repeated at 5 Hz and each tetanus including three ten-burst trains separated by 15 sec). Responses were measured as fEPSP slopes expressed as percentages of the baseline average. Paired pulse facilitation (PPF) was elicited through two successive stimuli, separated by an inter-stimulus interval, and measured as a ratio of the two fEPSP slopes.

Whole cell patch clamp recordings. LTP recordings: P17-P28 male mice were used. Coronal hippocampal slices were cut using a Leica VT 1000S vibratome (Leica Microsystems) and maintained in a submerged chamber perfused with ACSF containing (in mM): 125 NaCl, 2.5 KCl, 1.25 Na_2HPO_4 , 25 $NaHCO_3$, 2 $CaCl_2$, 1.3 $MgCl_2$, 25 glucose, pH=7.4 (95% O_2 , 5% CO_2). Slices were permitted to recover for at least 90 min before use. Whole cell recordings were obtained from CA1 pyramidal cells that were voltage-clamped at -70 mV. The bath solution

contained ACSF with 50 μM picrotoxin. The internal electrode solution contained (in mM): 130 K-gluconate, 10 KCl, 4 NaCl, 10 HEPES, 1 $MgCl_2$ 0.06 $CaCl_2$, 0.1 EGTA, 4 MgATP, 0.3 Na_2GTP, 10 Phosphocreatine-Tris; pH was adjusted to 7.3 with KOH, and osmolarity was adjusted to 290 mOsm with sucrose. Synaptic responses were evoked by stimulating the Schaffer collateral pathway at a frequency of 0.1 Hz. LTP was induced with a theta-burst stimulation. Cells were held in current clamp mode during tetanus and then returned to the voltage clamp mode. To eliminate artifacts due to variation of the seal properties, the access resistance was monitored for constancy throughout all experiments. If changes of more than 10% were detected, the recordings were discarded. Responses were measured as EPSC amplitudes and expressed as percentage of baseline. Each point of the LTP graph represents the average of six consecutive recordings. AMPAR / NMDAR current recordings only: The internal electrode solution contained (in mM): 117.5 Cs2MeSO4, 17.5 CsCl, 10 Hepes, 4 NaCl, 1 EGTA, 4 MgATP, 0.3 Na2GTP, 10 phosphocreatine-Tris, and 10 QX-314-Cl, pH 7.3, 285mOsm. AMPAR currents were measured with 10 μM bicuculline and 40 μM D-APV in the bath solution. NMDAR currents were measured with 10 μM bicuculline and 10 μM CNQX in the bath solution.

Purification of recombinant proteins

Recombinant TAT transduction proteins were expressed in BL21-CodonPlus (DE3)-RIPL E. coli (Stratagene), with induction by 0.5 M IPTG. TAT proteins were purified by standard 6x His tag affinity chromatography, as previously described [2]. Briefly, bacterial pellets were lysed in HEPES-buffered 8 M urea buffer, sonicated and clarified by high-speed centrifugation. The supernatant fraction was purified using affinity chromatography with Ni-NTA agarose resin (Qiagen). After extensive washes, the TAT proteins were eluted using imidazole. The fractions

were subsequently desalted with PD-10 columns (GE Healthcare) into PBS + 15% glycerol. Aliquots were snap frozen and stored at -80C until use. Purified proteins were checked by western blot with antibodies against Ubc9 (BD) and HA tag (Covance).

Recombinant GST-SENP1 proteins were expressed and purified as previously described [3]. Briefly, proteins were expressed in E-coli BL21(DE3)pLysS E. coli (Novagen). Bacterial pellets were lysed in a buffer containing 300 mM NaCl, 50 mM Tris pH 8.0, 5 mM EDTA, 1× complete protease inhibitor (Roche), sonicated and clarified by centrifugation. The supernatant fraction was applied to glutathione sepharose beads for 2 hours at 4° C on a rotating shaker. The beads were extensively washed with a buffer containing 50 mM Tris, pH 7.4, 300 mM NaCl and eluted with 15 mM glutathione. Protein identity was confirmed by western blot with antibodies against SENP1 (Invitrogen) and GST (Sigma Aldrich).

Western blots

Western blots were performed according to standard protocols. Briefly, equal amounts of protein from RIPA-soluble homogenization fractions were separated by SDS-PAGE and transferred to nitrocellulose membranes. Blots were blocked with BSA or SeaBlock (Pierce) and incubated with primary antibodies overnight at 4C. The secondary antibodies used were conjugated to either horseradish peroxidase or DyLight fluorophores (Pierce). Blots were either developed with enhanced chemiluminescent substrate or scanned using the Licor Odyssey infrared imager. Quantification analyses were performed with ImageJ (NIH) software.

Behavioral studies

Fear conditioning (FC). FC was performed as previously described [1]. Briefly, mice were placed in a conditioning chamber for 2 minutes. Subsequently, a 30 second tone sound was played and a 2 second foot shock was administered at the end of the tone. The mice were left in the chamber for an additional 30 seconds. After 24 hours, the mice were placed back in the same chamber, and freezing behavior was scored using Freezeview software; freezing behavior was defined as the absence of movement except for breathing. Contextual fear memory was assessed over 5 minutes in the chamber. Cued fear learning was assessed 24 hours after the contextual assessment by placing the mice in a novel context for 2 minutes (pre-tone) and then playing the training tone sound for 3 minutes (post-tone). Sensory thresholds were assessed by measuring the minimal foot shock intensities at which a mouse manifested a behavioral response in three categories: visible response to shock (flinching), extreme motor response (jumping) and vocalized distress (screaming).

Radial arm water maze (RAWM). The two-day RAWM was performed as previously described [4]. Briefly, mice were trained to find an escape platform located in one of the six maze arms. On day 1, five blocks of 3 trials each are performed with alternation between visible and hidden platform. On day 2, the mice are trained for another five blocks of 3 trials each with only the hidden platform. Scoring is by the number of entries into incorrect maze arms (errors). Swimming ability and vision are assessed by measuring swim speed and the time it takes to reach a visible platform in an open pool.

Morris water maze (MWM). The MWM was performed as previously described [1]. Briefly, mice were trained to find a hidden platform in an open pool, with two sessions of three trials each repeated for three days. Scoring was by the time required to reach the platform. A probe trial was

performed after training to test memory retention; the platform was removed and the percent time spent in each quadrant of the pool was recorded with a video tracking system (HVS Image).

Primary cultures and immunofluorescence

Primary hippocampal neuron cultures were prepared from P0-P1 pups as previously described (Ninan and Arancio, 2004). Following TAT-Ubc9 incubation, 14 DIV cultures or transverse hippocampal slices were fixed in paraformaldehyde and processed for immunofluorescence according to standard procedures using antibodies for HA (Covance) and MAP-2 (Cell Signaling). Briefly, primary cultures/hippocampal sections were fixed in 4% paraformaldehyde and permeabilized with Triton X-100. Blocking was done in BSA plus normal goat serum. After primary antibody incubation, Alexa Fluor-conjugated secondary antibodies (Invitrogen) were applied. After mounting in Vectashield (Vector Labs), images were acquired using a Nikon D-Eclipse C1 confocal microscope. Images were acquired by confocal microscopy.

SUPPLEMENTAL REFERENCES

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