Expanded View Figures

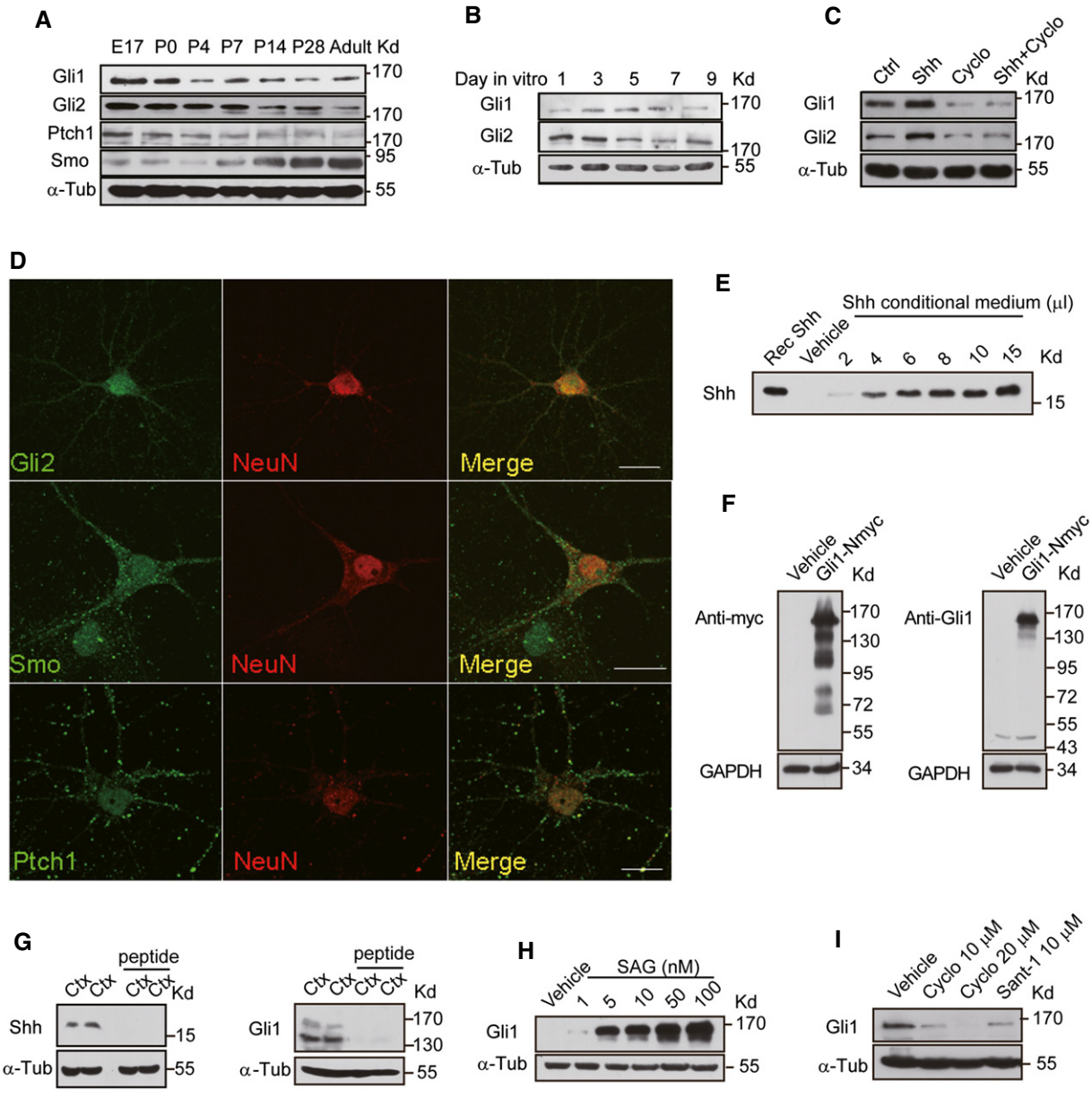

Figure EV1. Expression of molecules in Shh pathway and verification of antibodies against Shh or Gli1.

A–C Western blots of the total lysates extracted from rat hippocampus at different developmental stages (A), from cultured hippocampal neurons at different days *in vitro* (B) and neurons treated with either vehicle (Ctrl), Shh, cyclopamine (Cyclo), or Shh plus Cyclo for 24 h (C) with the indicated antibodies.

D Representative immunostaining of cultured hippocampal neurons with the indicated antibodies. Scale bar: 10 μm.

E Western blots of the medium from HEK293 cells transfected with empty vectors (Vehicle) or Shh construct by the anti-Shh antibody. The numbers indicate different loading volume (μl) of conditional medium. Recombinant Shh (Rec Shh; Sigma) was used as a positive control.

F Total lysates of HEK293 cells transfected with empty vectors (Vehicle) or *myc*-tagged Gli1 construct (Gli1-Nmyc) were Western-blotted and analyzed with the anti-myc antibody (left) or the anti-Gli1 antibody (right).

G Western blots of cortical extracts from mice using Shh (left) or Gli1 (right) antibodies with or without pre-incubation of antigenic peptide.

H, I The expression level of Gli1 detected by Western blots of lysates from NIH3T3 cells incubated with SAG (Smoothened agonist) at the indicated concentrations (H) or from hippocampal neurons incubated with Cyclo or Sant-1 at the indicated concentrations (I).

Data information: α-Tubulin (α-Tub) and GAPDH were used as loading controls.

Figure EV2. Inhibition of endogenous Shh pathway reduces epileptiform activities without affecting physiological synaptic transmission.

A Shh levels determined by ELISA in the medium of hippocampal slices with or without (Ctrl) TBS stimuli. TBS: theta-burst stimuli. n = 4–8.

B, C Shh levels determined by ELISA in the medium of hippocampal neurons with or without 20-Hz electrical stimulation for 30 min (B, n = 6–7) or incubated with the indicated treatments (C, n = 11). KCl, 50 mM; TTX, 1 μM.

D Input–output curves recorded from CA1 stratum radiatum of hippocampal slices treated with Cyclo or vehicle (Ctrl). fEPSP: field excitatory postsynaptic potential. n = 8.

E Normalized amplitude of AMPA receptor-mediated current at −70 mV. Black line: perfusion of Cyclo. EPSC: excitatory postsynaptic current. n = 11.

F Quantification of the paired-pulse ratio (PPR) of fEPSP in CA1 of hippocampal slices treated with Cyclo or vehicle (Ctrl). n = 9.

G TBS-induced LTP in the presence of Cyclo or vehicle (Ctrl). The slope of fEPSP plotted as percent of baseline before TBS. n = 9.

H–J Effects of Sant-1 (H), robotnikinin (Robot, I), or 5E1 (J) on the spontaneous epileptiform activity from hippocampal neurons incubated with 0Mg. Left: representative traces of whole-cell recordings with the indicated treatments. Right: the expanded view of a single burst (arrow) from the left parallel panels.

K, L Representative Western blots and quantification of Gli1 and Gli2 expression levels from cultured hippocampal neurons under the indicated treatments for 30 min. n = 3.

M Representative Western blots and quantification of Gli1, Gli2, and Shh expression levels from hippocampal slices under the indicated treatments. n = 3.

Data information: Cyclo: 5–10 μM used in all experiments. In (D, F, G), Cyclo was pre-incubated for 30 min. α-Tubulin (α-Tub) was used as a loading control. Data are mean ± SEM. Student's t-test was used.

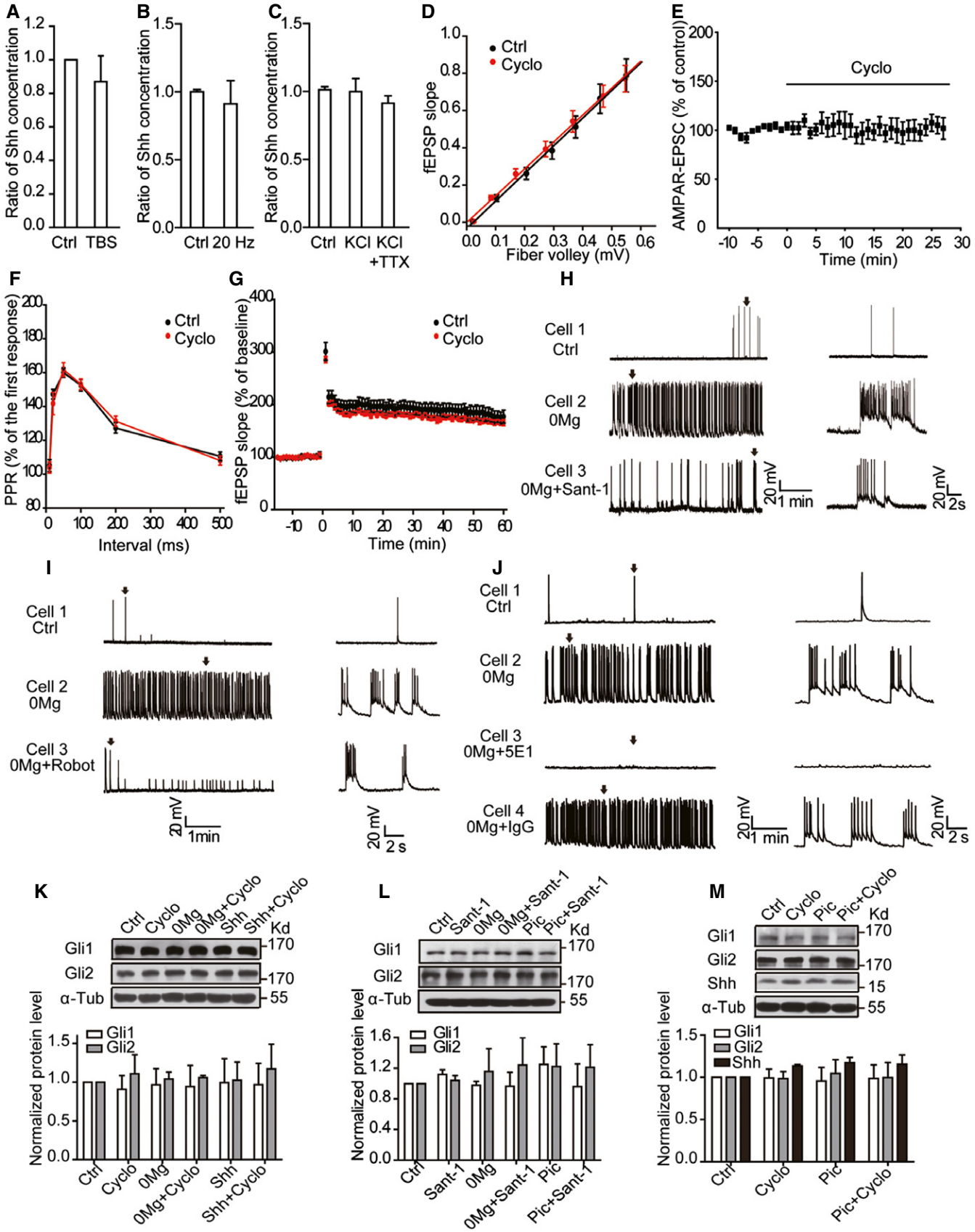

Figure EV2.

Figure EV3. Shh induces an APV-sensitive $[Ca^{2+}]_i$ elevation and Shh does not change the hippocampal neuron excitability.

A An example of Fura-2 AM-loaded neurons. Red circles in differential interference contrast (DIC) panel indicate the regions from where the F340/380 ratios were obtained. The pseudo color panels show changes in F340/380 ratios in the cells before (Ctrl) and after Shh perfusion. Scale bar, 10 μm.

B–D Representative traces showing F340/380 ratio of neurons after Shh perfusion. Effects of Cyclo (C) and APV (D) on Shh-induced $[Ca^{2+}]_i$ elevation. Insets: quantification of the peak amplitudes of ΔR/R0 values. ES, extracellular solution. APV: 50 μM. n = 45–80 cells in each group of at least three independent experiments.

E, F Left: representative traces of NMDA or AMPA currents induced by 100 μM NMDA plus 1 μM glycine or 100 μM AMPA in the presence or absence of Shh. Shh was applied for 30 s and then applied with the indicated agonists. Membrane potential was clamped at +40 mV for NMDA-induced currents, or at −70 mV for AMPA-induced currents. Right: quantification of the effect of Shh on NMDA- or AMPA-induced current density from 7 to 8 cells in each group.

G Left: a representative trace showing the D-CPP-sensitive currents recorded from cultured hippocampal neurons incubated in 0Mg; right: the expanded view. Arrow: application of D-CPP. Black lines: expanded regions.

H Whole-cell recordings of spontaneous activities before and after application of D-CPP from the neurons in 0Mg.

I Left: representative traces of NMDA currents induced by 100 μM NMDA plus 1 μM glycine in the presence or absence of Cyclo. Cyclo was applied for 30 s and then applied with the indicated agonists. Membrane potential was clamped at +40 mV. Right: quantification of the effect of Cyclo on NMDA-induced current density from 7 to 8 cells in each group.

J Quantification of resting membrane potentials of neuron treated with Ctrl or Shh for 30 min.

K Averaged current threshold of neurons treated with Ctrl or Shh.

L Firing frequency of neurons treated with Ctrl or Shh in response to 70-, 100-, and 150-pA current injections for 500 ms.

Data information: n = 15–19 cells in each group (J–L). Data are mean ± SEM from at least three independent experiments. ***$P < 0.001$ with Student's t-test.

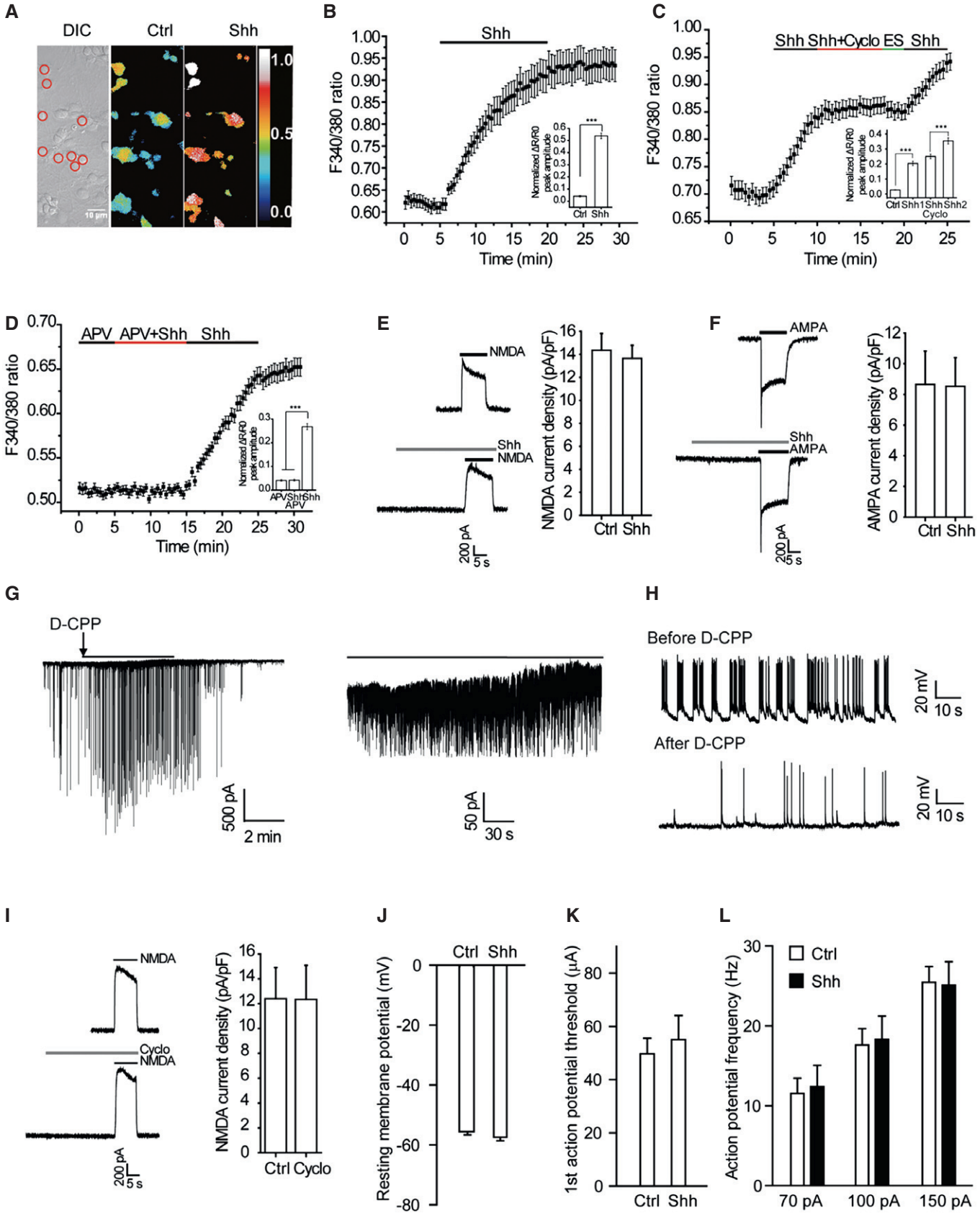

Figure EV3.

Figure EV4. Shh inhibits EAAC1 to enhance extracellular glutamate in a $G\alpha_{i/o}$-dependent manner.

A–C Whole-cell recordings of spontaneous epileptiform activity from cultured hippocampal neurons. (A) Left: representative traces showing the neuronal activity under the indicated treatments. Right: the expanded view of a single burst (arrow) from the left panel. (B) Quantification of the percentage of neurons showing epileptiform activity, n = 4–5. (C) The burst frequency shown in (A), n = 15–18.

D Quantification of ^{3}H-glutamate uptake by neurons in response to vehicle (Ctrl) or the indicated agents. n = 3–9.

E Upper panel: representative Western blots of the extracts from hippocampal neurons transfected with two lentivirus-based RNAi against GLAST (Gst3 or Gst4) or nonsense RNAi (Non). Lower panel: quantification of normalized GLAST protein levels, n = 3.

F Statistics of ^{3}H-glutamate uptake by cultured hippocampal neurons transfected with the indicated RNAi in response to Ctrl or Shh. n = 3.

G Quantification of EAAC1 levels in cultured hippocampal neurons transfected with two lentivirus-based RNAi against EAAC1 (R2 or R3) or nonsense RNAi (Non). n = 3.

H Quantification of the normalized current amplitude (13–22 cells in each group) of aspartate (Asp)-evoked current in neurons treated with Ctrl, TBOA, DHK, WAY213623 (WAY, a selective inhibitor of GLT-1), or UCPH-101 (UCPH, a selective inhibitor of GLAST).

I The effect of Shh on EAAC1 current density in the wash-in method (n = 9). ES: extracellular solution. ns: not significant.

J Quantification of EAAC1 current density from hippocampal neurons with the indicated treatments (n = 15–17).

K Glutamate levels assayed by HPLC in the medium of neurons incubated with ARA-C and treated with the indicated agents. n = 6–11.

L Asp-evoked currents from hippocampal neurons incubated with ARA-C and treated with the indicated agents. n = 14–15.

M Glutamate levels in the medium of neurons pretreated with Ctrl or Shh in the absence or presence of pertussis toxin (PTX). n = 8–9.

N The current–voltage (I–V) relationship of Asp-induced currents recorded from hippocampal neurons in response to vehicle (Ctrl) or Shh, from 10 to 24 cells in each group.

O The dose–response curve of Asp-evoked EAAC1 currents, obtained from hippocampal neurons at −70 mV. There was no significant difference between Ctrl and Shh in all concentrations from 12–13 cells. The EC_{50} of Ctrl or Shh was 37.33 ± 2.56 or 43.61 ± 0.63 μM, respectively. The dose–response curve was fitted by logistic equation: $y = A_2 + (A_1 - A_2)/[1 + (x/x_0)^p]$, where y is the response; A_1 and A_2 are the maximum and minimum response, respectively; x is the drug concentration; and p is the Hill coefficient.

P Upper panel: representative Western blots of the extracts of hippocampal neurons under the indicated treatments using the indicated antibodies. Lower panel: quantification of the normalized expression level of EAAC1. n = 3.

Q Representative Western blots of fractionated lysates of rat hippocampus with antibodies against secretogranin II (SGII), synaptophysin (Syp), Smo, EAAC1, $G\alpha_i$-pan ($G\alpha_i$), $G\alpha_{i2}$, $G\alpha_{i3}$, or Gβ-pan (Gβ). SGII: a marker of large vesicles; Syp: a marker of small vesicles.

R Effect of SAG on extracellular glutamate in mouse hippocampus. n = 11.

Data information: The upper panels in (H, I, J, L) show representative traces of Asp-evoked current. α-Tubulin was used as a loading control. DHK: 300 μM; TBOA: 100 μM; WAY213623: 300 nM; UCPH-101: 10 μM. Data are mean ± SEM from at least three independent experiments. $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ vs. 0Mg, Ctrl, Non or Ctrl (Non); $^{\#\#\#}P < 0.001$ vs. Cyclo in (B) and (C); $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$ vs. Shh or Shh + Cyclo in (J) with Student's t-test.

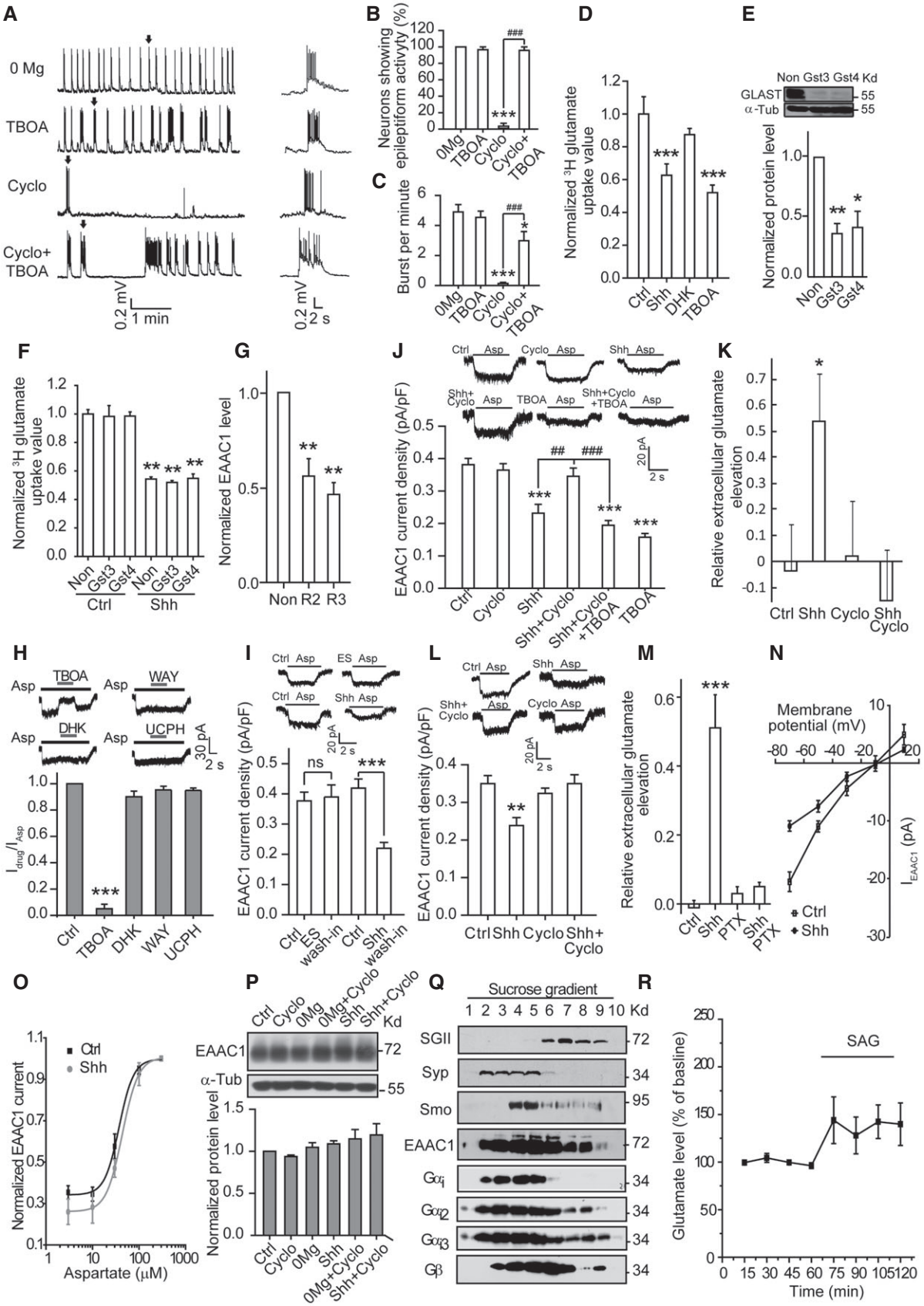

Figure EV4.

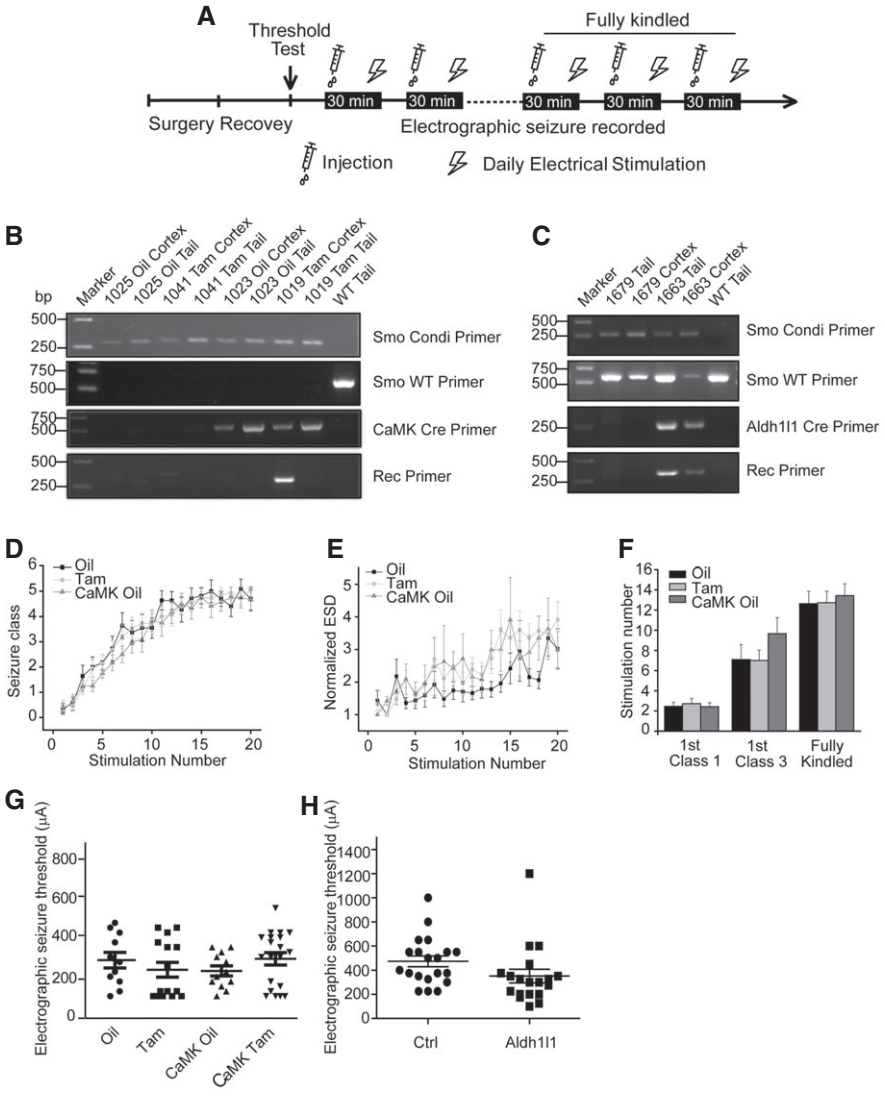

Figure EV5. Genetic inhibition of Smo suppresses epileptogenesis in mouse kindling model.

A Schematic of the kindling protocol.

B, C PCR analysis of lysates form the cortex or the tail tissue of wild-type mice (WT) and mice with conditional knockout of *Smo* in CaMKIIα-positive neurons (B) or Aldh1l1-positive cells (C). The presence of the $Smo^{+/fl}$ or $Smo^{fl/fl}$ was revealed by the fragments amplified by Smo Conditional Primer (Smo Condi Primer) and Smo WT Primer ($Smo^{fl/fl}$ without a fragment). The presence of the *Cre* was revealed by the fragments amplified by Cre Primer (CaMK Cre Primer in B or Aldh1l1 Cre Primer in C). The ablation of *Smo* was revealed by the fragment amplified by recombinant primer (Rec Primer). Oil: injection of vehicle oil; Tam: injection of tamoxifen. Primers for genotyping were as published [50,51].

D–F The intensification of behavioral seizure class (D), evoked electrographic seizure duration (ESD) (E), and the number of stimulations required to reach equivalent seizure intensity (F) in the indicated control mice.

G Mean electrographic seizure threshold in Oil, Tam, CaMK Oil, and CaMK Tam group littermates. Each data point represents a result from a single mouse.

H Mean electrographic seizure threshold in Ctrl or Aldh1l1 group littermates. Each data point represents a result from a single mouse. Ctrl: $Smo^{+/fl}$ (n = 20); Aldh1l1: $Smo^{+/fl}$*Aldh1l1-Cre* (n = 19).

Data information: For (D–G), Oil: $Smo^{fl/fl}$ induced by vehicle oil (n = 11); Tam: $Smo^{fl/fl}$ induced by tamoxifen (n = 15); CaMK Oil: $Smo^{fl/fl}$*CaMKIIα-*Cre^{ERT2} induced by vehicle oil (n = 12); CaMK Tam: $Smo^{fl/fl}$ *CaMKIIα-*Cre^{ERT2} induced by tamoxifen (n = 20). Oil, Tam, and CaMK oil groups were used as controls. Data are mean ± SEM with Student's t-test.