Supplementary Data for

Cdk5 is a new rapid synaptic homeostasis regulator capable of initiating the early Alzheimer-like pathology

Yanghui Sheng[1,2,4], Lei Zhang[1,4], Susan C. Su[3], Li-Huei Tsai[3], and J. Julius Zhu[1]

[1]Department of Pharmacology, University of Virginia School of Medicine, Charlottesville, VA 22908
[2]Undergraduate Class of 2011, Yuanpei Honors College, Peking University, Beijing, 100871, China
[3]Picower Institute for Learning and Memory and Howard Hughes Medical Institute, Massachusetts Institute of Technology, Cambridge, MA 02139

[4]These authors contributed equally.

Figure S1

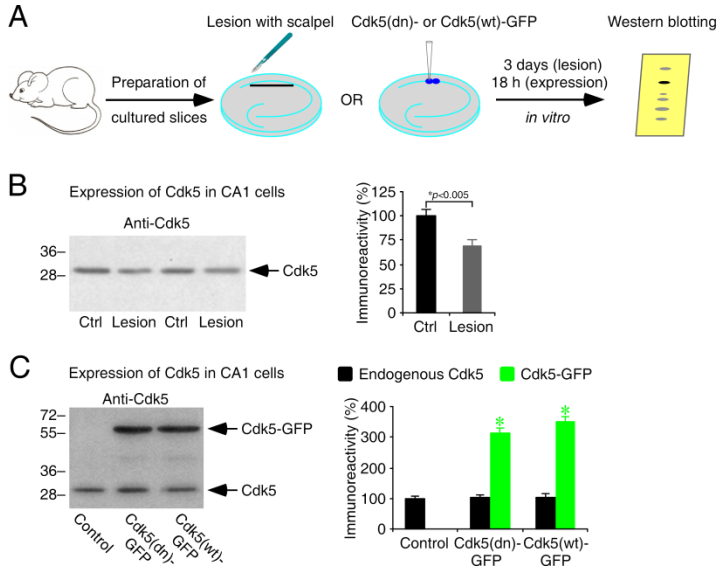

Figure S1. Expression of Cdk5 in hippocampal CA1 cells.
(A) Schematic drawing outlines *in vitro* experimental design. Note that in lesion experiments, a surgical incision was made at the base of the apical dendrite of CA1 pyramidal neurons for 3 days, which resulted in death and clear off of these neurons in cultured slices and thus revealed the significant expression of Cdk5 in non-pyramidal neurons.
(B) Left, expression of Cdk5 proteins in hippocampal CA1 regions and hippocampal CA1 regions with lesions of CA1 pyramidal neurons. Right, relative amounts of Cdk5 in hippocampal CA1 regions and hippocampal CA1 regions with lesions of CA1 pyramidal neurons (Ctrl: 100.0±7.0%; Lesion: 68.4±4.5%; n=12; p<0.005). Note the significant amount of Cdk5 in hippocampal CA1 regions with lesions, which was from the cells other than CA1 pyramidal neurons. Each lane was loaded with 45 µg hippocampal CA1 cell lysate protein. The relative values and standard errors were normalized to average amounts of endogenous Cdk5 from control hippocampal CA1 regions. Note the significant Cdk5 expression in non-pyramidal neurons, which was used to estimate the overexpression of Cdk5 in pyramidal neurons in (**C**).
(C) Left, expression of endogenous and recombinant Cdk5 proteins in hippocampal CA1 regions and hippocampal CA1 regions expressing Cdk5(dn)-GFP or Cdk5(wt)-GFP. Right, relative amounts of endogenous Cdk5 and recombinant Cdk5 in hippocampal CA1 pyramidal cells expressing Cdk5(dn)-GFP (Ctrl: 103.7±8.5%; Exp: 313.2±17.2%; n=12; p<0.005) or Cdk5(wt)-GFP (Ctrl: 103.9±10.1%; Exp: 352.4±16.1%; n=12; p<0.005). Each lane was loaded with 45 µg hippocampal CA1 cell lysate protein. The relative values and standard errors were normalized to average amounts of endogenous Cdk5 from control hippocampal CA1 pyramidal cells. Note that the expression was estimated with correction of the amount of Cdk5 expression in other cells in CA1 regions (**B**).

Figure S2

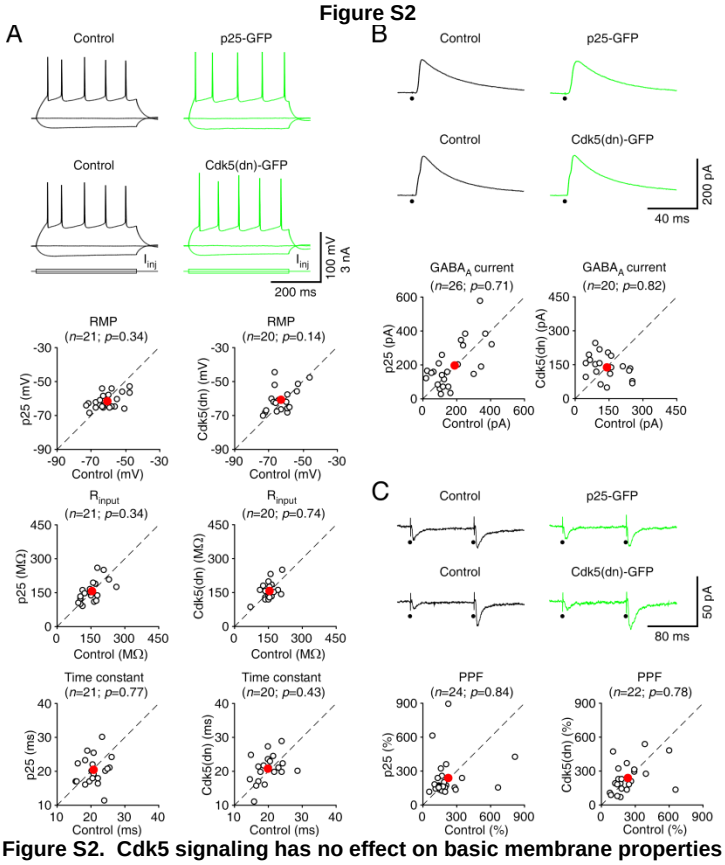

Figure S2. Cdk5 signaling has no effect on basic membrane properties and paired-pulse facilitation.
(A) Evoked responses to step depolarizing and hyperpolarizing pulses recorded from neighboring non-expressing (Ctrl) and p25-GFP or Cdk5(dn)-GFP expressing cells. Lower, resting membrane potentials (Ctrl: -59.3±1.6 mV; Exp: -61.0±1.0 mV; n=21; p=0.34 for p25-GFP; Ctrl: -62.1±1.5 mV; Exp: -59.4±1.6 mV; n=20; p=0.14 for Cdk5(dn)-GFP), input resistances (Ctrl: 162.8±9.6 MΩ; Exp: 158.6±9.9 MΩ; n=21; p=0.34 for p25-GFP; Ctrl: 162.6±7.0 MΩ; Exp: 159.4±8.7 MΩ; n=20; p=0.74 for Cdk5(dn)-GFP) and time constants (Ctrl: 21.3±0.7 ms; Exp: 20.7±0.9 ms; n=21; p=0.77 for p25-GFP; Ctrl: 20.3±0.8 ms; Exp: 21.0±0.9 ms; n=20; p=0.43 for Cdk5(dn)-GFP) in expressing cells are plotted against those obtained from control non-expressing cells.
(B) Evoked $GABA_A$-R- (0 mV) mediated responses recorded from neighboring non-expressing (Ctrl) and p25-GFP or Cdk5(dn)-GFP expressing cells. Lower, amplitudes of synaptic GABA responses in p25-GFP (Ctrl: 199.1±27.1 pA; Exp: 200.0±25.5 pA; n=26; p=0.71) and Cdk5(dn)-GFP (Ctrl: 148.4±15.4 pA; Exp: 142.8±11.9 pA; n=20; p=0.82) expressing cells are plotted against those obtained from control non-expressing cells.
(C) Evoked AMPA-R- (-60 mV) mediated responses recorded from neighboring non-expressing (Ctrl) and p25-GFP or Cdk5(dn)-GFP expressing cells. Lower, ratios of paired-pulse facilitation in p25-GFP (Ctrl: 242.1±35.3%; Exp: 250.0±39.2%; n=24; p=0.84) and Cdk5(dn)-GFP (Ctrl: 257.0±32.7%; Exp: 243.4±28.2%; n=22; p=0.78) expressing cells are plotted against those obtained from control non-expressing cells. Statistical significance was determined with Wilcoxon tests.

Figure S3

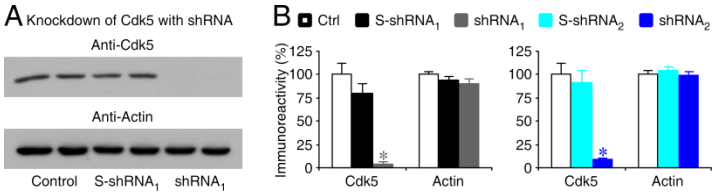

Figure S3. Expression of Cdk5-targeting shRNAs knockdowns endogenous Cdk5 neurons.
(A) Expression of Cdk5 and actin in normal control hippocampal neurons, hippocampal neurons expressing scrambled shRNA (S-shRNA$_1$) or Cdk5-targeting shRNA$_1$.
(B) Relative amounts of Cdk5 and actin in normal control hippocampal neurons and hippocampal neurons expressing S-shRNA$_1$, S-shRNA$_2$ and Cdk5-targeting shRNA$_1$ and shRNA$_2$. Values for the relative amounts of Cdk5 (S-shRNA$_1$: 79.7±10.2%; p=0.16; n=8; shRNA$_1$; 4.5±2.0%; p<0.05; n=8; Wilcoxon tests compared to Ctrl$_1$: 100.0±12.3%; n=8; S-shRNA$_2$: 91.5±13.3%; p=0.09; n=8; shRNA$_2$; 9.0±1.5%; p<0.05; n=8; Wilcoxon tests compared to Ctrl$_2$: 100.0±12.0%; n=8) and actin (S-shRNA$_1$: 93.9±4.1%; p=0.16; n=8; shRNA$_1$; 90.1±4.9%; p=0.05; n=8; Wilcoxon tests compared to Ctrl$_1$: 100.0±3.3%; n=8; S-shRNA$_1$: 104.3±4.3%; p=0.26; n=8; shRNA$_1$; 99.4±3.8%; p=0.40; n=8; Wilcoxon tests compared to Ctrl$_2$: 100.0±4.3%; n=8). The relative values and standard errors were normalized to average amounts of endogenous Cdk5 and actin.

Figure S4

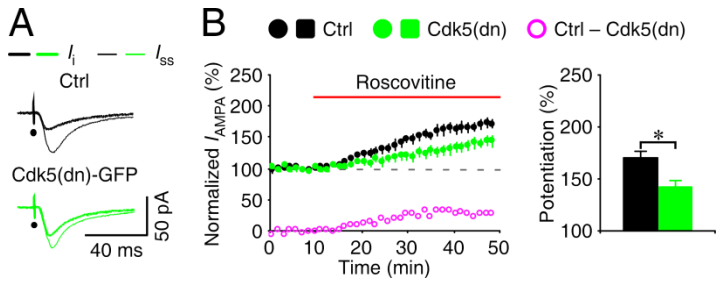

Figure S4. Rscovitine rapidly affect synaptic transmission.
(A) Evoked AMPA-R- (-60 mV) mediated responses recorded from neighboring control non-expressing and Cdk5(dn)-GFP expressing neurons before (I_i, initial 8-min responses in thick traces) and after (I_{ss}, steady-state 8-min responses in thin traces) the bath application of 25 μM roscovitine.
(B) Left, normalized simultaneously evoked responses recorded from control non-expressing and Cdk5(dn)-GFP expressing neurons against the time. Pink circles show the time course of difference of the roscovitine-induced potentiations between control non-expressing and Cdk5(dn)-GFP expressing neurons, reflecting the postsynaptic effect of roscovitine on Cdk5 signaling. Right, values for the average potentiation of AMPA control non-expressing and Cdk5(dn)-GFP expressing neurons (Ctrl: 170.0±6.9 %; Exp: 141.6±7.0 %; n=23; p<0.005). Asterisks indicate p<0.05 (Wilcoxon tests).

Figure S5

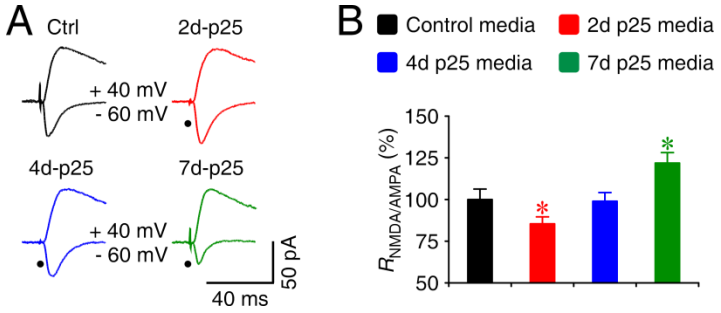

Figure S5. Chronic p25 overproduction releases molecules inducing Aβ-like effects on transmission.
(A) Evoked AMPA-R- (-60 mV) and NMDA-R- (+40 mV) mediated responses recorded from CA1 neurons cultured in daily collected media from the chambers incubating control non-expressing cultured slices, cultured slices expressing p25 for 2 days, 4 days and 7 days.
(B) Histograms show the ratio of NMDA and AMPA responses ($R_{NMDA/AMPA}$) in CA1 neurons cultured in media collected daily from the chambers incubating cultured slices expressing p25 for 2 days (1.58±0.06; n=39; p<0.05), 4 days (1.83±0.09; n=33; p=0.70), or 7 days (2.25±0.11; n=35; p<0.05) compared to that in CA1 neurons cultured in media collected daily from the chambers incubating control non-expressing cultured slices (1.84±0.11; n=31). The ratios of NMDA and AMPA responses and standard errors were normalized to average values from CA1 neurons cultured in media collected daily from the chambers incubating control non-expressing cultured slices. Asterisks indicate p<0.05 (Mann-Whitney Rank Sum tests).

Figure S6

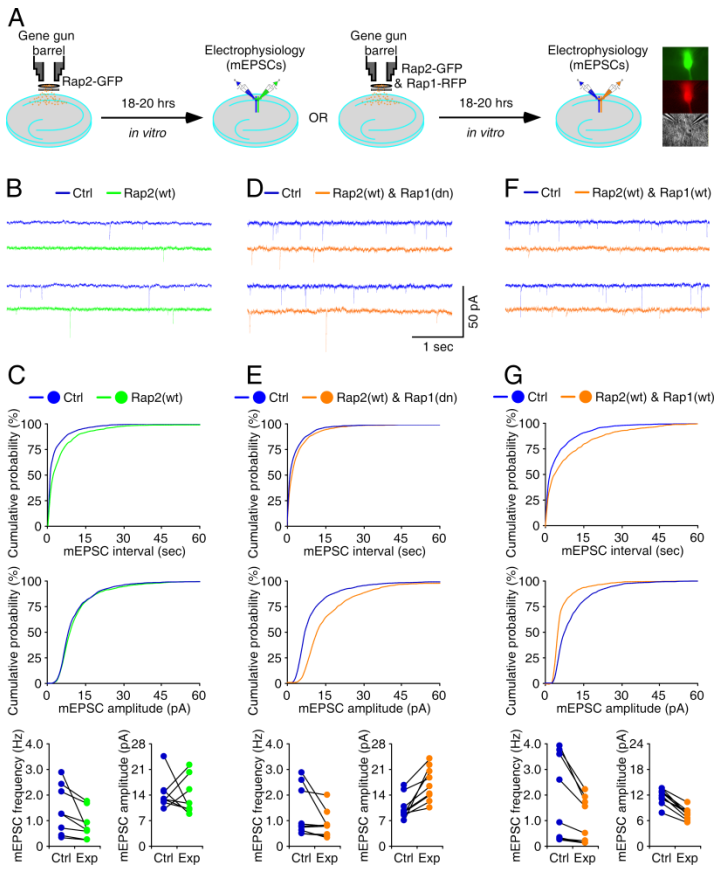

Figure S6. Rap1 and/or Rap2 signaling differentially alter synapse density and synaptic size.
(A) Schematic drawing outlines *in vitro* Rap1 and/or Rap2 expression experimental design. The right images show simultaneous whole-cell recordings from a pair of non-expressing (Ctrl) and co-expressing CA1 neurons under transmitted light (bottom) and fluorescence microscopy with GFP (top) or RFP (middle) filter.
(B) Miniature EPSCs recorded simultaneously from nearby control non-expressing neurons and neurons expressing Rap2(wt)-GFP.
(C) Left, cumulative distribution of mEPSC frequency and amplitude of control non-expressing neurons and neurons expressing Rap2(wt)-GFP. Right, paired comparisons of mEPSC frequency (Ctrl: 1.4±0.3 Hz; Rap2: 0.8±0.2 Hz, n=8; p<0.05) and amplitude (Ctrl: 12.4±1.3 pA; Rap2: 11.6±1.5 pA, n=8; p=0.89) of control non-expressing neurons and neurons expressing Rap2(wt)-GFP.

(D) Miniature EPSCs recorded simultaneously from nearby control non-expressing neurons and neurons co-expressing Rap2(wt)-GFP and Rap1(dn). Scale bars here apply to (**B**), (**D**) and (**F**).
(E) Left, cumulative distributions of mEPSC frequency and amplitudes of control non-expressing neurons and neurons co-expressing Rap2(wt)-GFP and Rap1(dn)-RFP. Right, paired comparisons of mEPSC frequency (Ctrl: 1.3±0.3 Hz; Rap1/2: 0.9±0.2 Hz, *n*=9; *p*<0.05) and amplitude (Ctrl: 10.6±1.1 pA; Rap1/2: 16.9±1.6 pA, *n*=9; *p*<0.01) of control non-expressing neurons and neurons expressing Rap2(wt)-GFP and Rap1(dn)-RFP.
(F) Miniature EPSCs recorded simultaneously from nearby control non-expressing neurons and neurons co-expressing Rap2(wt)-GFP and Rap1(wt).
(G) Left, cumulative distributions of mEPSC amplitudes and frequency of control non-expressing neurons and neurons co-expressing Rap2(wt)-GFP and Rap1(wt)-RFP. Right, paired comparisons of mEPSC frequency (Ctrl: 1.6±0.5 Hz; Rap1/2: 0.9±0.3 Hz, *n*=10; *p*<0.01) and amplitude (Ctrl: 11.7±0.6 pA; Rap1/2: 7.4±0.4 pA, *n*=10; *p*<0.01) of control non-expressing neurons and neurons expressing Rap2(wt)-GFP and Rap1(wt)-RFP.

Figure S7

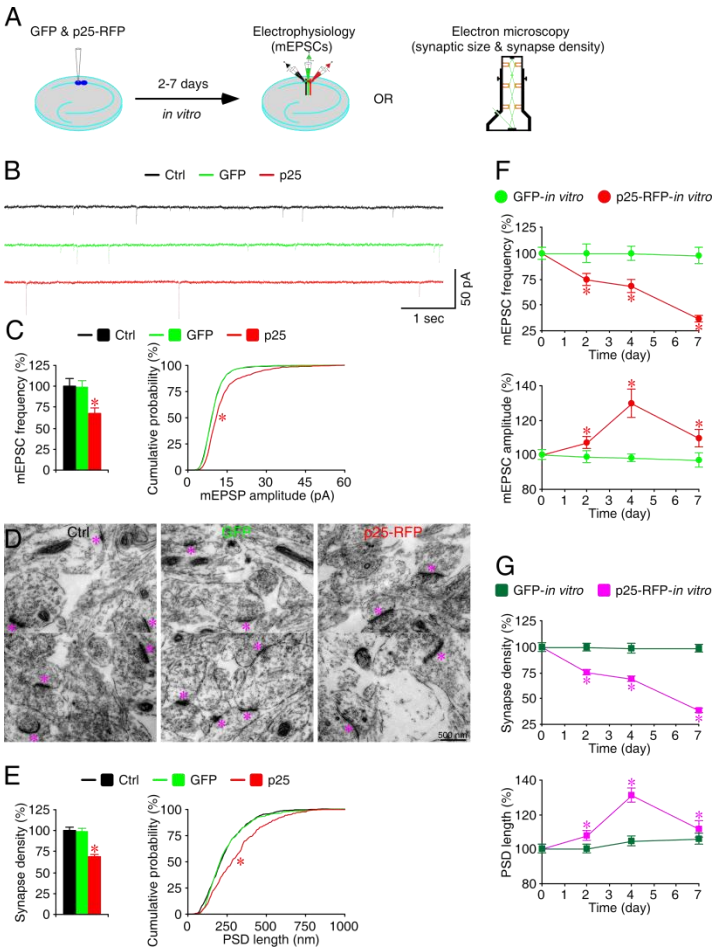

Figure S7. Chronic overproduction of p25 induces Alzheimer-like synaptic alterations *in vitro*.
(A) Schematic drawing outlines *in vitro* experimental design.
(B) Miniature EPSCs recorded simultaneously from nearby control non-expressing CA1 neurons, CA1 neurons overexpressing GFP or p25-RFP after 4 days of *in vitro* expression in cultured slices.
(C) Average mEPSC frequencies of control non-expressing CA1 neurons, CA1 neurons overexpressing GFP or p25-RFP (left), and cumulative distributions of mEPSC amplitudes of control non-expressing CA1 neurons, CA1 neurons overexpressing GFP or p25-RFP (right).
(D) Electron microscopic images from hippocampal CA1 stratum radiatum regions of control tissues, tissues overexpressing GFP or p25-RFP after 4 days of *in vitro* expression. Cyan asterisks indicate individual synapses.
(E) Averages synaptic densities of control non-expressing CA1 tissues, CA1 tissues overexpressing CFP or p25-RFP (left) and cumulative distributions of PSD lengths of control non-expressing CA1 tissues, CA1 tissues overexpressing CFP or p25-RFP (right).
(F) Left, relative mEPSC frequency in control non-expressing CA1 neurons, CA1 neurons overexpressing GFP, or p25-RFP in cultured slices at different expression time. Value for the average mEPSC frequency of each neuron group after 2-day (GFP: 1.51±0.13 Hz, *n*=10, *p*=0.72; p25: 1.12±0.09 Hz, *n*=10, *p*<0.01; Wilcoxon tests compared to Ctrl: 1.52±0.08 Hz, *n*=10), 4-day (GFP: 9.4±0.4, *n*=50 from 11 slices, *p*=0.57; p25: 6.6±0.3, *n*=50 from 11 slices, *p*<0.001; Wilcoxon tests compared to Ctrl: 9.6±0.4, *n*=50 from 11 slices), and 7-day (GFP: 1.62±0.12 Hz, *n*=9, *p*=0.86; p25: 0.62±0.05 Hz, *n*=9, *p*<0.01; Wilcoxon tests compared to Ctrl: 1.67±0.11 Hz, *n*=9) *in vitro* overexpression. Right, relative mEPSC amplitude in control non-expressing CA1 neurons, CA1 neurons overexpressing GFP, or p25-RFP in cultured slices at different expression time. Values for the average mEPSC amplitude of each neuron group after 2-day (GFP: 10.20±0.36 pA, *n*=10, *p*=0.965; p25: 11.14±0.36 pA, *n*=10, *p*<0.01; Wilcoxon tests compared to Ctrl: 10.41±0.31 pA, *n*=10), 4-day (GFP: 10.20±0.36 pA, *n*=10, *p*=0.965; p25: 11.14±0.36 pA, *n*=10, *p*<0.01; Wilcoxon tests compared to Ctrl: 10.41±0.31 pA, *n*=10), and 7-day (GFP: 10.15±0.53 pA, *n*=9, *p*=0.21; p25: 11.45±0.52 pA, *n*=9, *p*<0.05; Wilcoxon tests compared to Ctrl: 10.44±0.42 pA, *n*=9) *in vitro* overexpression.
(G) Left, relative synapse density in control non-expressing CA1 neurons, CA1 neurons overexpressing GFP, or p25-RFP in cultured slices at different expression time. Values for the average synapse density, counted as synapses per 100 μm^2, of each group after 2-day (GFP: 9.5±0.4, *n*=49 ultrathin sections from 10 slices, *p*=0.98; p25: 7.3±0.3, *n*=50 ultrathin sections from 10 slices, *p*<0.001, *p*<0.005; Mann-Whitney Rank Sum tests compared to Ctrl: 9.8±0.4, *n*=50 ultrathin sections from 10 slices), 4-day (GFP: 9.4±0.4, *n*=50 ultrathin sections from 11 slices, *p*=0.57; p25: 6.6±0.3, *n*=50 ultrathin sections from 11 slices, *p*<0.001; Mann-Whitney Rank Sum tests compared to Ctrl: 9.6±0.4, *n*=50 ultrathin sections from 11 slices), and 7-day (GFP: 9.5±0.4, *n*=50 ultrathin sections from 10 slices, *p*=0.86; p25: 3.7±0.2, *n*=50 ultrathin sections from 10 slices, *p*<0.001; Mann-Whitney Rank Sum tests compared to Ctrl: 9.6±0.4, *n*=50 ultrathin sections from 10 slices) *in vitro* overexpression. Right, relative PSD length in control non-expressing CA1 neurons, CA1 neurons overexpressing GFP, or p25-RFP in cultured slices at different expression time. Values for the average PSD length of each group after 2-day (GFP: 236.8±6.3 nm, *n*=466 synapses from 10 slices, *p*=0.34; p25: 259.4±7.4 nm, *n*=364 synapses from 10 slices, *p*<0.05; Mann-Whitney Rank Sum tests compared to Ctrl: 237.7±6.1 nm, *n*=488 synapses from 10 slices), 4-day (GFP: 248.4±6.6 nm, *n*=471 synapses from 11 slices, *p*=0.77; p25: 356.1±10.5 nm, *n*=329 synapses from 11 slices, *p*<0.001; Mann-Whitney Rank Sum tests compared to Ctrl: 241.3±6.0 nm, *n*=479 synapses from 11 slices), and 7-day (GFP: 250.7±7.2 nm, *n*=475 synapses from 10 slices, *p*=0.57; p25: 269.6±11.6 nm, *n*=185 synapses from 10 slices, *p*<0.05; Mann-Whitney Rank Sum tests compared to Ctrl: 241.5±6.1 nm, *n*=482 synapses from 10 slices;) *in vitro* overexpression. The relative values and standard errors were normalized to average mEPSC frequency, amplitude, synapse density and PSD length from control cells. Asterisks indicate *p*<0.05.

Table S1

Comparisons of LTP measured with whole-cell and perforated patch recording techniques

Cell type & expression time	Whole-cell-recorded potentiation (%)	Perforated patch-recorded potentiation (%)	*p* value (M-W Rank Sum)
Ctrl @ 18 hrs	193.1±28.7 (*n*=11)	206.4±4.5 (*n*=8)	0.894
Exp @ 18hrs	154.9±20.6 (*n*=11)	152.9±9.3 (*n*=8)	0.505
Ctrl @ 2 days	195.0±8.2 (*n*=9)	203.3±3.7 (*n*=8)	0.361
Exp @ 2 days	150.7±14.1 (*n*=9)	151.1±8.5 (*n*=8)	0.885
Ctrl @ 7 days	193.5±15.8 (*n*=10)	197.1±6.3 (*n*=8)	0.625
Exp @ 7 days	136.7±8.8 (*n*=10)	149.2±7.3 (*n*=8)	0.351