

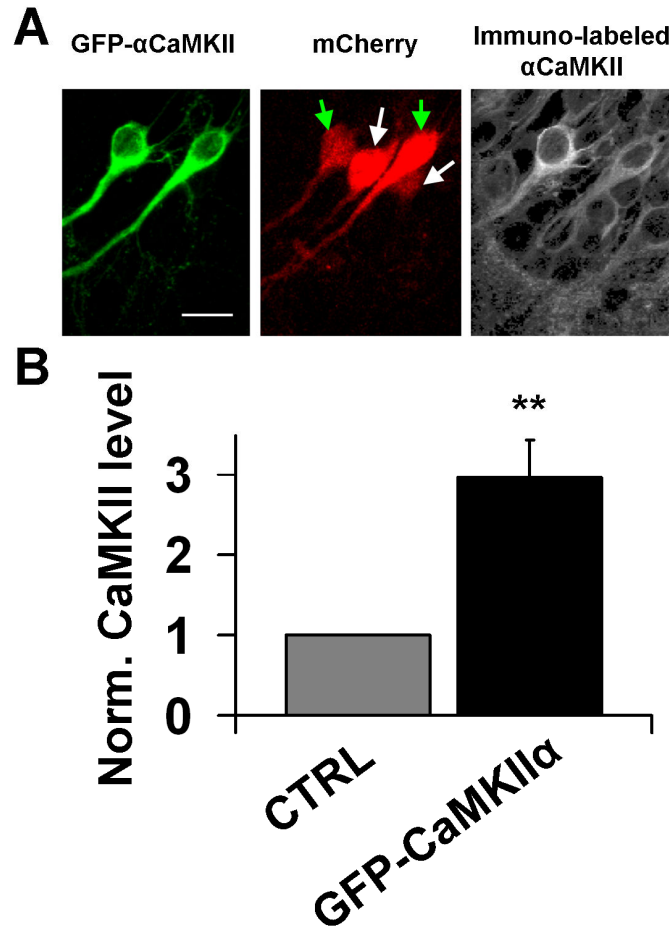


Fig. S1. The overexpression level of GFP-CaMKII alpha at the soma is about 3-fold. Two neighboring neurons were transfected with GFP-CaMKII alpha (WT)/mCherry and mCherry, respectively, in advance, and only these neurons were analyzed after immunostaining. After about 24 hr, slices were fixed with 4% paraformaldehyde in PBS, 0.5% Triton X-100, preblocked with 10% goat serum at 4°C, incubated with anti-CaMKII alpha (mouse-anti-rat, 1:100; ABR) overnight at 4°C and followed by incubation with Alexa405-conjugated goat-anti-mouse IgG (Molecular Probes Inc.) for overnight at 4°C. Immunolabeled neurons were imaged with Leica TCS RS. For analysis, the regions below the nucleus were selected, and projections of three stacked sections were analyzed using custom-built program in IGOR Pro 6 (WaveMetrics). **A.** The level of CaMKII alpha was quantified by immunostaining with antibodies specific for CaMKII alpha. To minimize experimental variations, two neighboring neurons were selected; and one neuron was transfected with mCherry (morphological marker), and the other was transfected with mCherry/GFP-CaMKII alpha (WT) for 24 hours. Scale bar = 20μm. **B.** The overexpression level of GFP-CaMKII alpha = 2.97 +/-0.480, n = 14 pairs, **p<0.01.

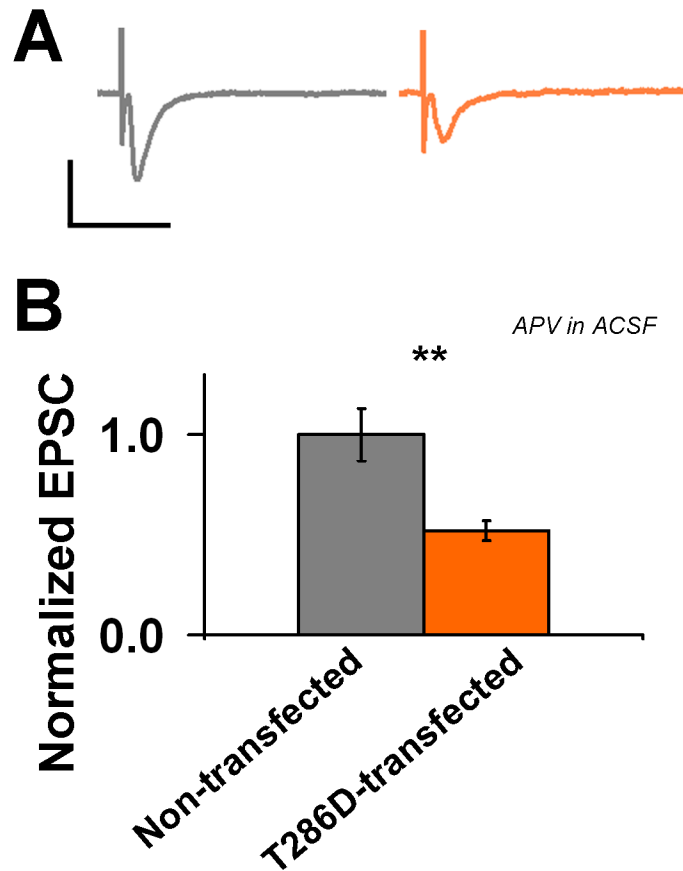


Fig. S2. T286D-dependent depression is not occurring during testing. APV (100 μ M) is added during test pulses, and AMPAR EPSC are measured from T286D-expressed and nontransfected neurons. T286D still causes the synaptic depression. **A.** Representative traces. Gray: nontransfected; orange: T286D. Scale bar: 50 pA, 50 msec. **B.** T286D causes the depression in the presence of APV in ACSF during testing. AMPAR EPSC: transfected = 33.9 $\pm$ 3.30 pA; nontransfected = 65.3 $\pm$ 8.63 pA, $n = 7$, $**p < 0.01$.

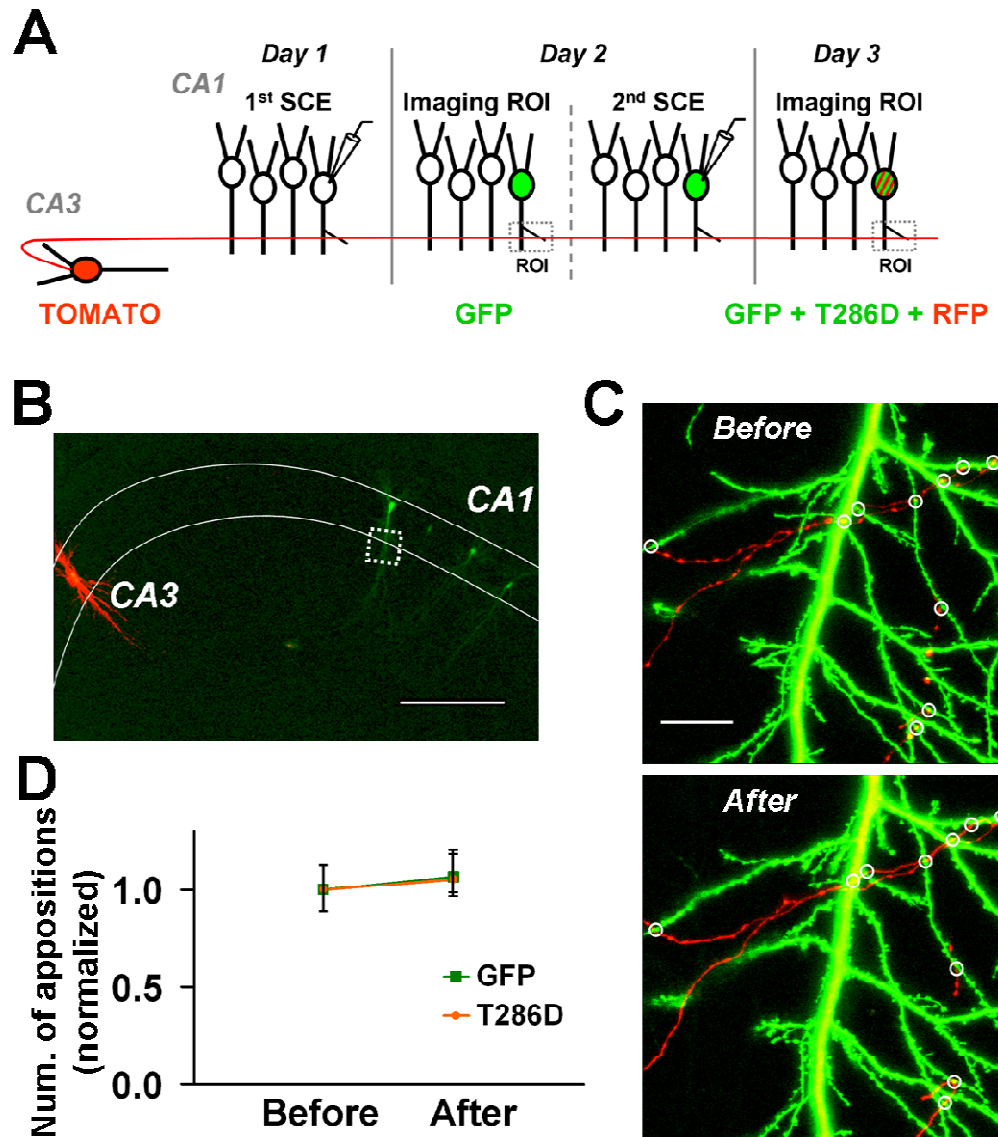


Fig. S3. T286D does not stimulate compensatory process in cultured slices. The T286D CaMKII mutant has been reported to remodel synapses of cortical neurons in primary cultures (Pratt et al., 2003); the number of putative synaptic contacts is reduced, but those remaining are strengthened. It was proposed that the synaptic potentiation by T286D CaMKII was compensated by decreasing the number of synapses, thereby maintaining the level of neuronal activities. However, we did not find evidence that T286D CaMKII produces significant changes in the number of axons approaching transfected cells. **A.** Dual sequential transfection (DST) allows monitoring the changes in the number of close appositions between CA3 and CA1 neurons at the identified locations over time. *Day1:* A single-cell electroporation (SCE) is performed on a single CA3 neuron and CA1 neurons to introduce morphological markers, tdTomato and GFP, respectively. *Day2:* After counting the number of appositions between CA3 and CA1 neurons, the

second transfection is performed in GFP-expressed CA1 neurons with GFP-T286D/mRFP or GFP/mRFP. mRFP, here, is a marker to check that the second transfection is successful but is not detected by two-photon microscope imaging because the infrared laser only can excite tdTomato, but not mRFP. *Day3*: The number of the appositions is counted in the same region. **B.** The exemplary slice has a CA3 neuron transfected with tomato and CA1 neurons transfected with GFP. Scale bar = 350 μm . **C.** The number of the appositions does not change by DST of T286D in the region boxed in B. Red denotes CA3 axons, and green denotes dendritic branches of the CA1 neuron. White open circles indicate the close appositions. Scale bar = 20 μm . **D.** Neither T286D (orange) nor control condition (GFP; green) change the number of the appositions. T286D: the number of appositions (before) = 6.5 ± 1.64 , the number of appositions (after) = 6.8 ± 1.40 , $n = 6$, $p = 0.47$; GFP: the number of appositions (before) = 21.3 ± 5.31 , the number of appositions (after) = 22.7 ± 4.71 , $n = 3$, $p = 0.27$).

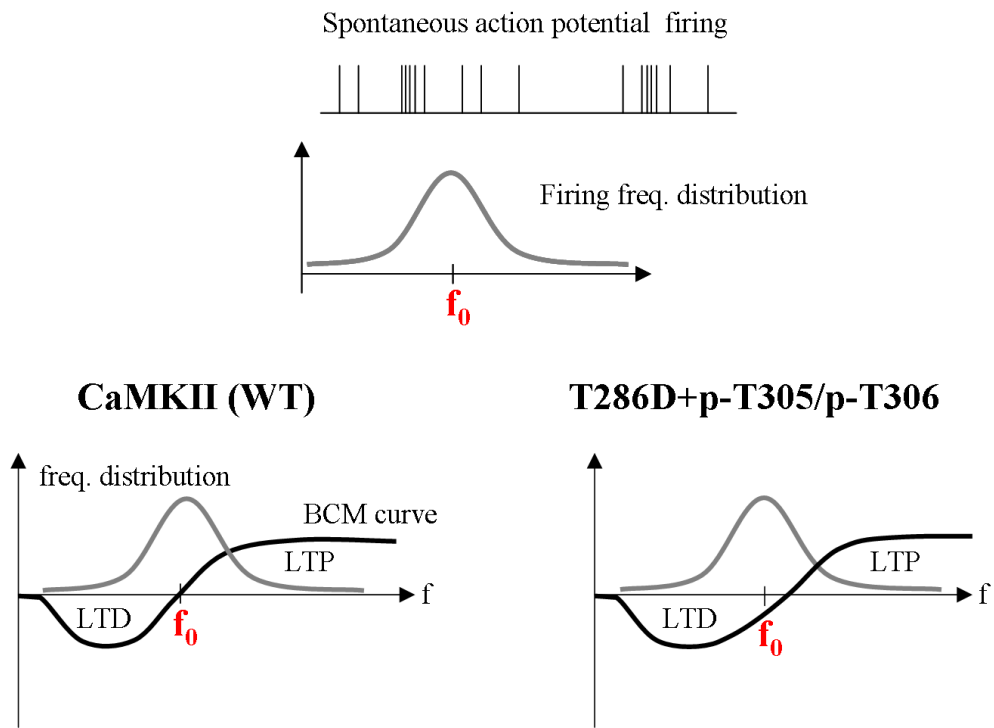


Fig. S4. Model: cultured slices have spontaneous neuronal activities that can cause LTP- and LTD-like processes. **Left panel:** In a normal condition, spontaneous action potential firings equally cause LTP and LTD. **Right panel:** T286D phosphorylates T305/T306. The phosphorylation at T305/T306 shifts the BCM curve such that spontaneous activities prefer inducing LTD. f_0 denotes the average frequency of spontaneous action potential firing. BCM curve denotes Bienenstock-Cooper-Munro function (Bienenstock et al., 1982).

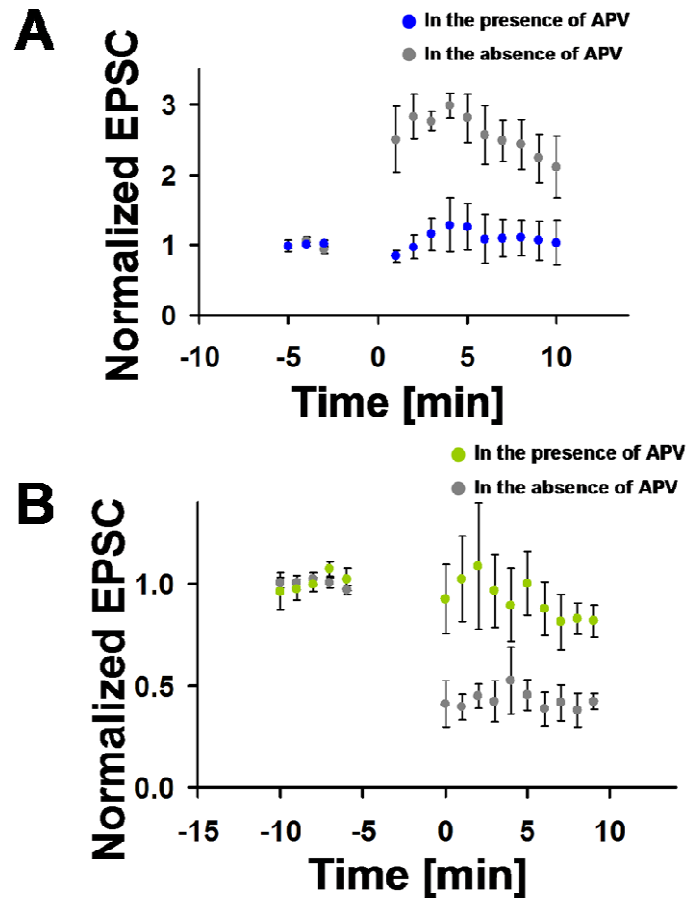


Fig. S5. LTP and LTD are NMDAR-dependent. **A.** LTP was induced by a pairing protocol using 200 pulses at 2 Hz at 0 mV. LTP induction was blocked by the addition of APV (100 μ M) in ACSF. The level of LTP: Gray circle = 2.25 ± 0.379 , $n = 4$; blue circle = 1.06 ± 0.289 , $n = 4$. $**p < 0.01$. **B.** LTD was induced by pairing 300 pulses at 1Hz at -45mV. LTD induction was blocked by the addition of APV (100 μ M) in ACSF. The level of LTD: Gray circle = 0.42 ± 0.038 , $n = 4$; green circle = 0.82 ± 0.078 , $n = 4$. $**p < 0.01$.