Supplementary Information

Kinetics of endogenous CaMKII required for synaptic plasticity revealed by optogenetic kinase inhibitor

Hideji Murakoshi, Myung Eun Shin, Paula Parra-Bueno, Erzsebet Szatmari, Akihiro C. E. Shibata, and Ryohei Yasuda

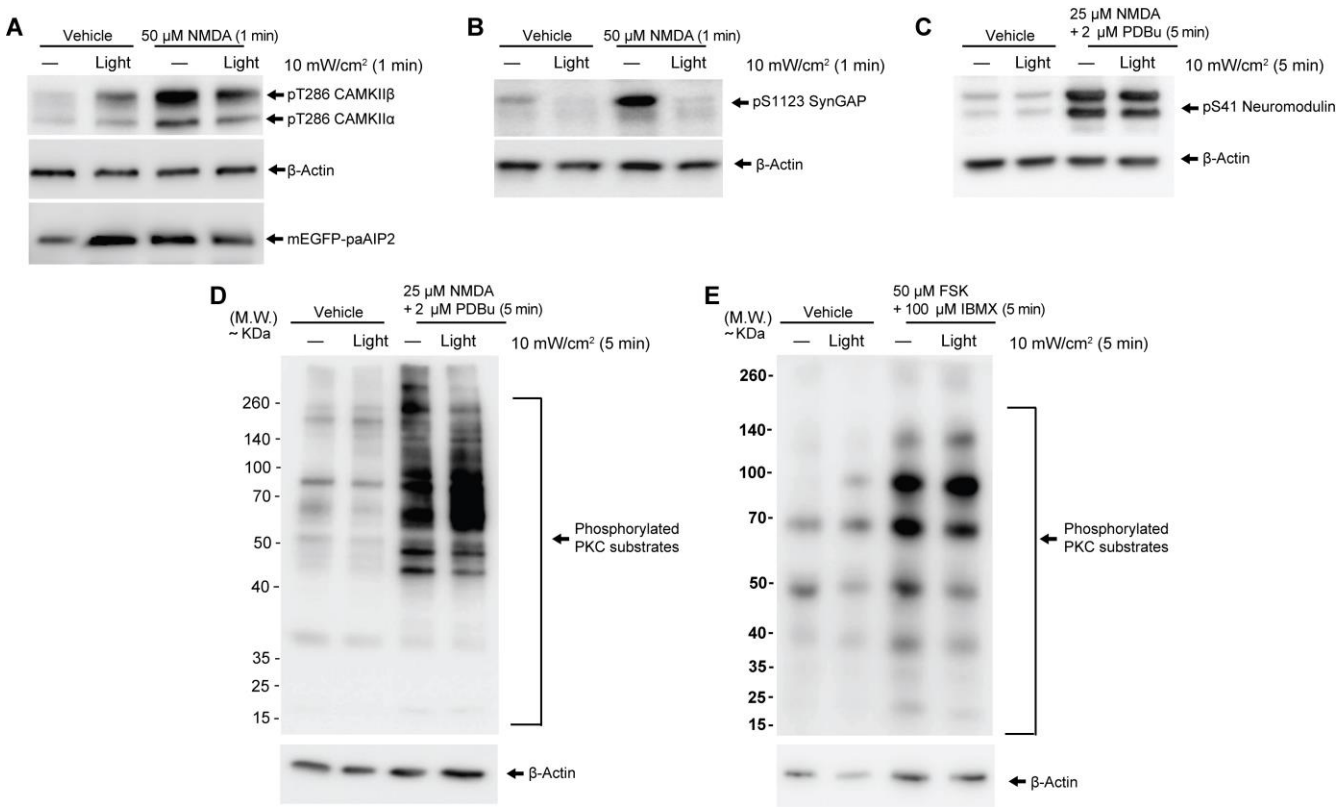

Figure S1. paAIP2 specifically inhibits CaMKIIα signaling in dissociated hippocampal neurons in a light-dependent manner, Related to Figure 2.

Neurons were infected with AAV-mEGFP-paAIP2 and pharmacologically simulated under the absence (-) or the presence (Light) of blue light illumination for the indicated time. The lysates were analyzed by immunoblotting.

A, CaMKIIα/β autophosphorylation at Thr-286 (pT286) following NMDA stimulation.

B, CaMKIIα-dependent phosphorylation of SynGAP following NMDA stimulation.

C, Phosphorylation of Neuromodulin (GAP43), a specific PKC target, at Ser-41 following stimulation with 25 μM NMDA and 2 μM Phorbol 12,13-dibutyrate (PDBu).

D, Phosphorylation of PKC substrates detected with an antibody specific to classical PKC substrates containing phospho-Ser following stimulation with 25 μM NMDA and 2 μM PDBu.

E, Phosphorylation of PKA substrates detected with an antibody against phospho-Ser/Thr residue with Arginine at the -3 position following stimulation with 25 μM Forskolin (FSK) and 100 μM IBMX.

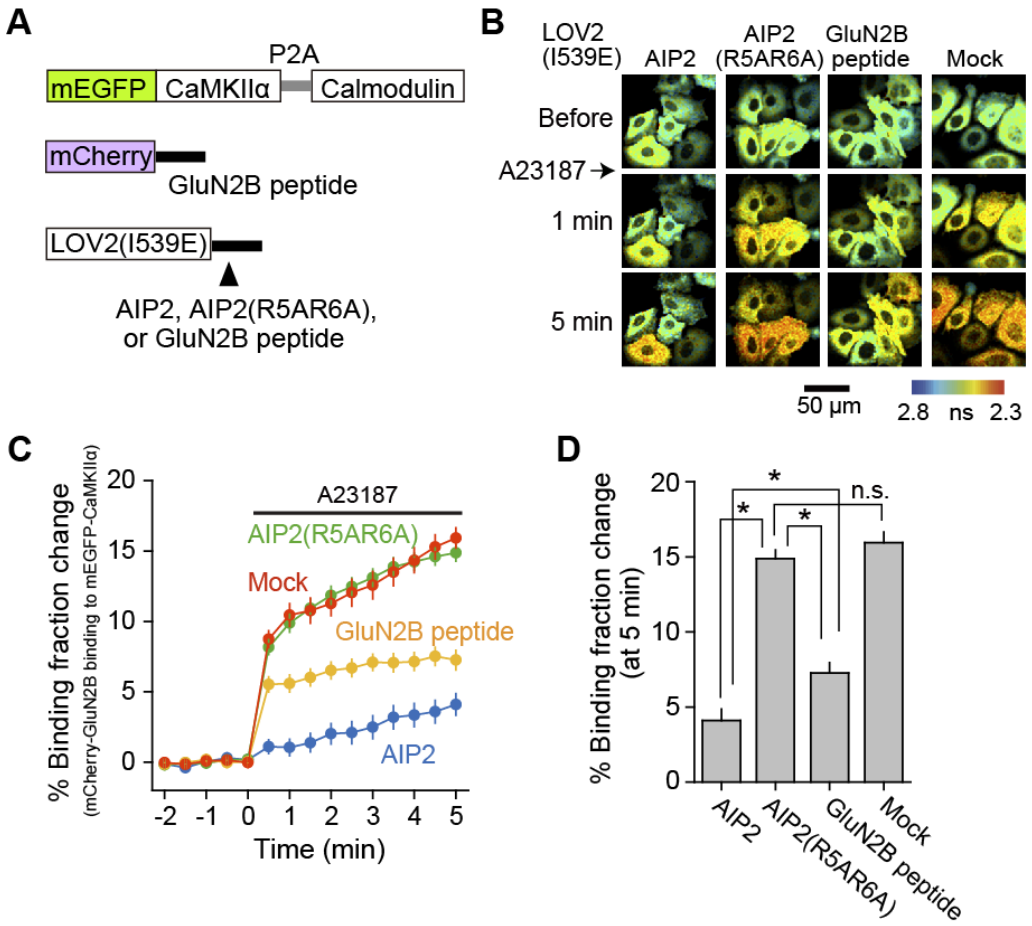

Figure S2. CaMKIIα and GluN2B interaction is competitively inhibited by AIP2, Related to Figure 3.

A, A schematic of DNA constructs used in the binding assay. mEGFP-CaMKIIα and mCherry-GluN2B$_{1277–1328}$ were co-expressed in HeLa cells, and their binding was monitored by 2-photon fluorescence lifetime imaging microscopy (2pFLIM). Respective peptides (AIP2, low affinity AIP2 $_{R5AR6A}$, or GluN2B$_{1277–1328}$) fused to the constitutively open form of LOV2$_{I539E}$ mutant were also co-expressed.

B, Representative fluorescence lifetime images of the mEGFP-CaMKIIα in HeLa cells before and after the application of 10 μM 4-Bromo-calcium ionophore (A23187). Warmer color indicates higher binding between mEGFP-CaMKIIα and mCherry-GluN2B peptide.

C, The time course of the binding between mEGFP-CaMKII and mCherry-GluN2B peptide in response to the application of ionophore (A23187). The numbers of cells analyzed are 38 (AIP2), 38 (AIP2$_{R5AR6A}$), 39 (GluN2B$_{1277–1328}$), and 39 (mock).

D, Quantification of the binding between mEGFP-CaMKII and mCherry-GluN2B peptide. The binding fractions at 5 min in **C** were statistically analyzed. The data are presented as mean ± SEM. Asterisks denote statistical significance (one-way ANOVA followed by Scheffé's *post hoc* test; $p < 0.05$).

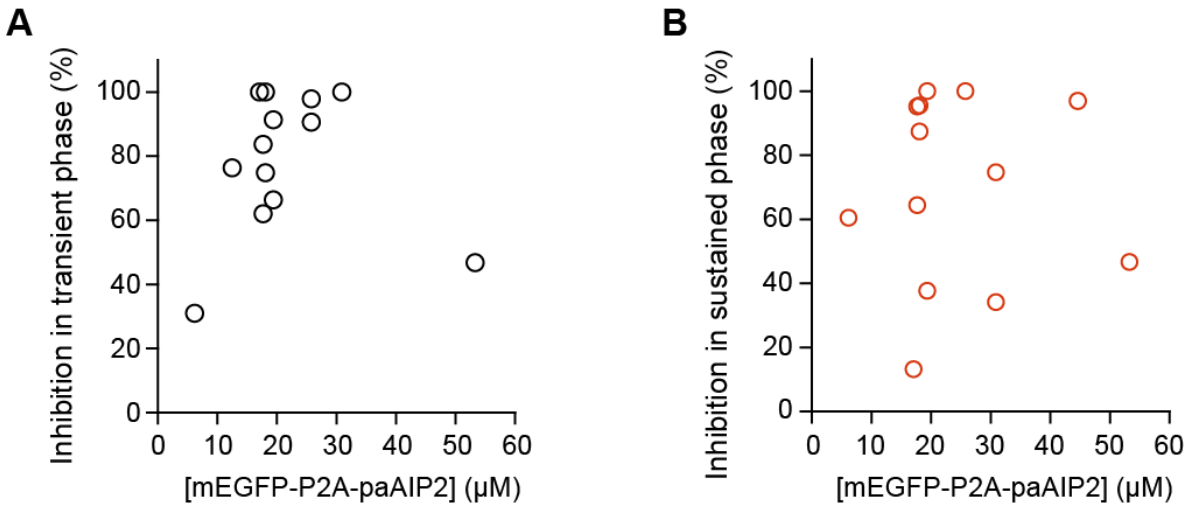

Figure S3. Effect of the expression level of paAIP2 on the blue-light-dependent inhibition of spine enlargement, Related to Figure 4.

The data in **Figure 4A, B** were re-analyzed. The degree of the inhibition of structural plasticity of each spine was plotted against the concentration of mEGFP-P2A-paAIP2 measured by comparing the fluorescence intensity of mEGFP in cells and purified mEGFP under a 2-photon excitation (920 nm). Each circle indicates individual cells. The degree of the inhibition of transient volume change (at 1min) (**A**) or sustained volume change (10–20 min) (**B**) was calculated as $(\Delta V_{\text{no light}} - \Delta V_{\text{light}})/ \Delta V_{\text{no light}}$, where $\Delta V_{\text{no light}}$ and ΔV_{light} are volume changes in the absence and presence of blue light, respectively. The respective $\Delta V_{\text{no light}}$ and ΔV_{light} were acquired from different spines in the same neuron.

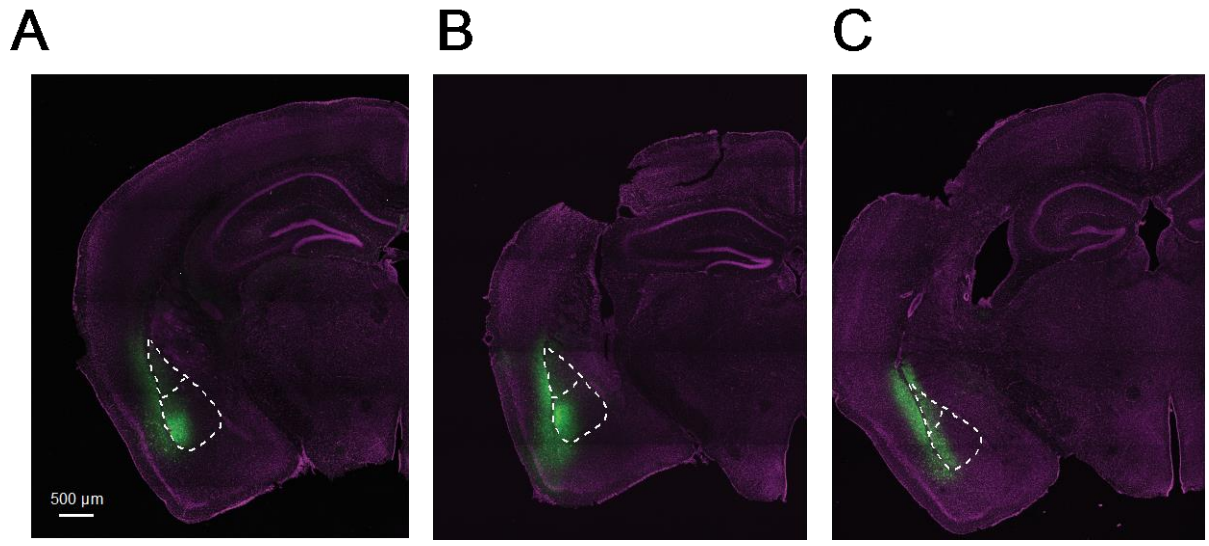

Figure S4. Expression of EGFP-paAIP2 in vivo, Related to Figure 7 and Table S1
Representative images of EGFP-paAIP2 expression in vivo following AAV injection. The upper region demarcated by white dotted line indicates lateral amygdala (LA). The lower region indicates basolateral amygdala (BLA). Scale bar = 500 µm. In some animals, the transgene expression was also found in the brain regions adjacent to the LA and BLA, such as entorhinal cortex, piriform cortex, endopiriform nucleus and central and medial amygdala.

Control	Mouse ID	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
Right	LA	2	0	1	3	2	4	3	0	1	2	1	1	4	0	2	1	3	3	3	1	4	1
	BLA	3	0	1	2	4	2	2	0	3	4	1	3	1	3	2	2	2	2	2	2	3	1
Left	LA	0	0	3	0	2	3	3	3	0	4	3	1	0	0	0	4	3	2	4	1	0	1
	BLA	0	2	2	0	2	2	1	2	0	4	2	4	2	1	1	2	2	1	2	3	0	1
Total Value		5	2	7	5	10	11	11	5	4	14	5	9	7	4	5	9	10	8	11	7	7	4

paAIP2	Mouse ID	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
Right	LA	0	1	0	0	4	0	1	0	2	2	1	0	2	1	1	2	2	1	2	1	1
	BLA	3	3	0	2	1	0	1	0	2	2	2	0	2	2	2	2	1	2	3	2	1
Left	LA	0	0	1	0	0	2	0	3	0	3	0	0	2	1	1	2	2	1	0	2	3
	BLA	3	0	2	0	0	0	0	2	0	2	0	3	2	2	2	2	2	1	2	2	3
Total Value		6	4	3	2	5	2	2	5	4	9	3	3	8	6	6	8	7	5	7	7	8

Time-Shift	Mouse ID	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
Right	LA	4	1	0	2	0	0	2	1	1	2	0	0	4	1	4	3	2	3	2	2	1
	BLA	2	1	0	2	0	1	3	3	4	3	3	1	3	2	2	2	2	3	2	2	2
Left	LA	4	0	2	0	1	0	1	2	0	0	1	0	0	1	2	1	4	4	4	1	1
	BLA	1	0	2	0	1	0	4	2	3	0	4	1	3	1	1	3	3	3	3	1	2
Total Value		11	2	4	4	2	1	10	8	8	5	8	2	10	5	9	9	11	13	11	6	6

Table S1. Quantification of EGFP-paAIP2 expression in LA and BLA, Related to Figure 7 and Figure S4.

We measured the percentage of the area expressing the transgene within LA and BLA in both hemispheres by eyes and assigned scores (0 = no expression, 1 = 0 ~ 25%, 2 = 25 ~ 50%, 3 = 50 ~ 75%, 4 = 75 ~ 100%). Individual as well as total values are shown for each animal. For example, expression values for brains in **Figure S4** is scored **A**: (1 LA, 2 BLA), **B**: (1 LA, 3 BLA), **C**: (4 LA, 2 BLA).