Fig. S1. Saneyoshi et al.

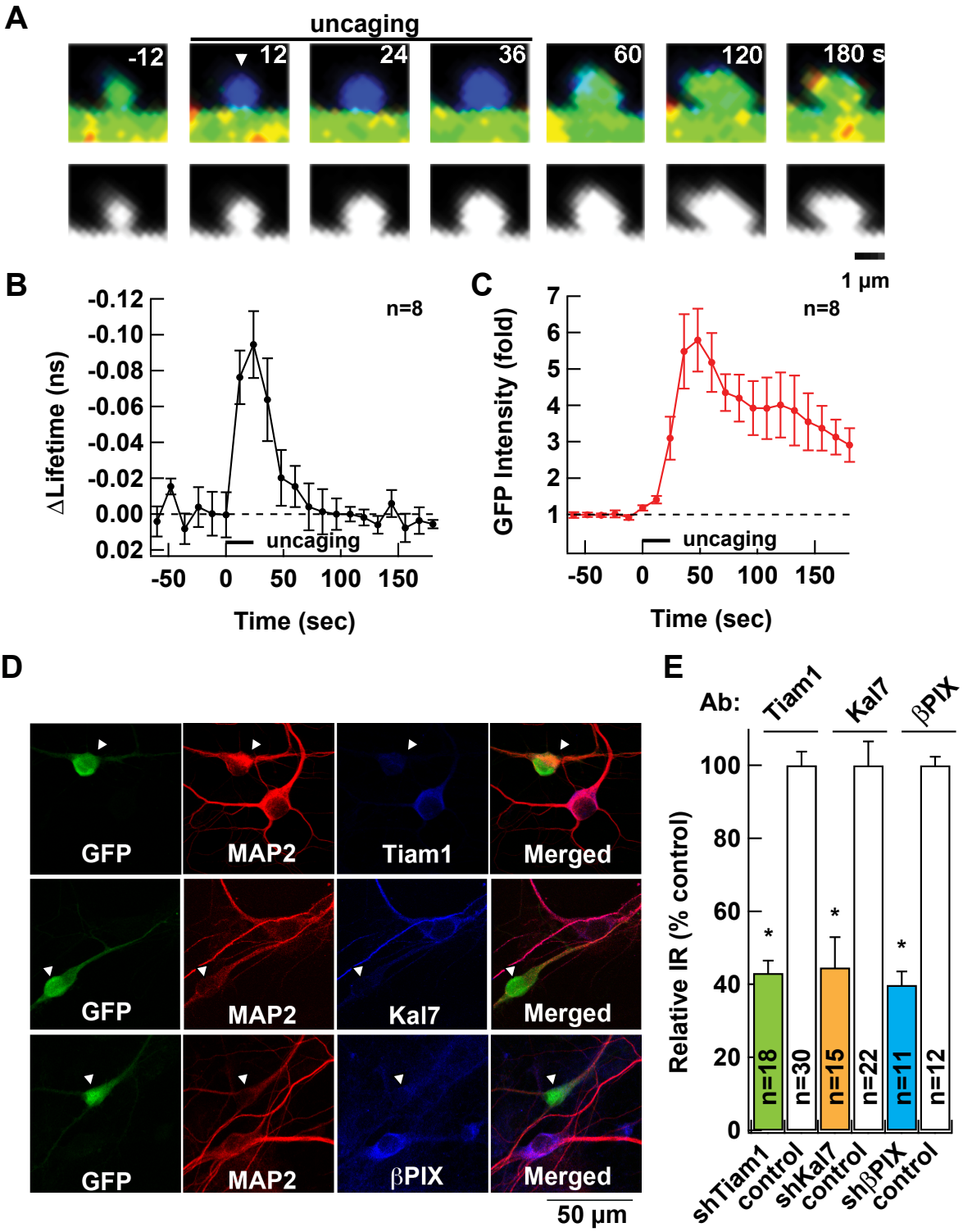

Figure S1, related to Figures 1 and 2. Activation of CaMKII during sLTP is transient in hippocampal organotypic slice culture and efficacy of shRNA against the RacGEF.

A. Activation of CaMKII as visualized by FRET-FLIM-based CaMKII activity sensor, Camui, during sLTP. CaMKII activity and distribution of Camui sensor, as a proxy of structure, are shown. Cooler colour hues in FLIM images indicates higher CaMKII activity.

B, C. Summary of CaMKII activity (B) and volume of the spine (C).

D, E. Test of efficiency of shRNA against the RacGEFs. Hippocampal dissociated neurons were transfected with GFP and shRNA construct against Tiam1, Kalirin-7 (Kal7), or βPIX. Four days after transfection, neurons were fixed and stained with antibodies against RacGEFs (Tiam1, Kal7, or βPIX), MAP2, and Hoechst 33342 (D). Arrow heads indicate GFP positive cells. Quantification of RacGEF protein in shRNA expressing neuron (E). Immunoreactivities (IR) were compared with a GFP positive neuron and its neighbor cell: both cells were MAP2 positive. Relative IR were normalized to MAP2 positive/GFP negative cells as 100% (control). *, $p < 0.05$, compared to control; *t*-test.

Data are represented as mean ± SEM (B, C, E).

Fig. S2. Saneyoshi et al.

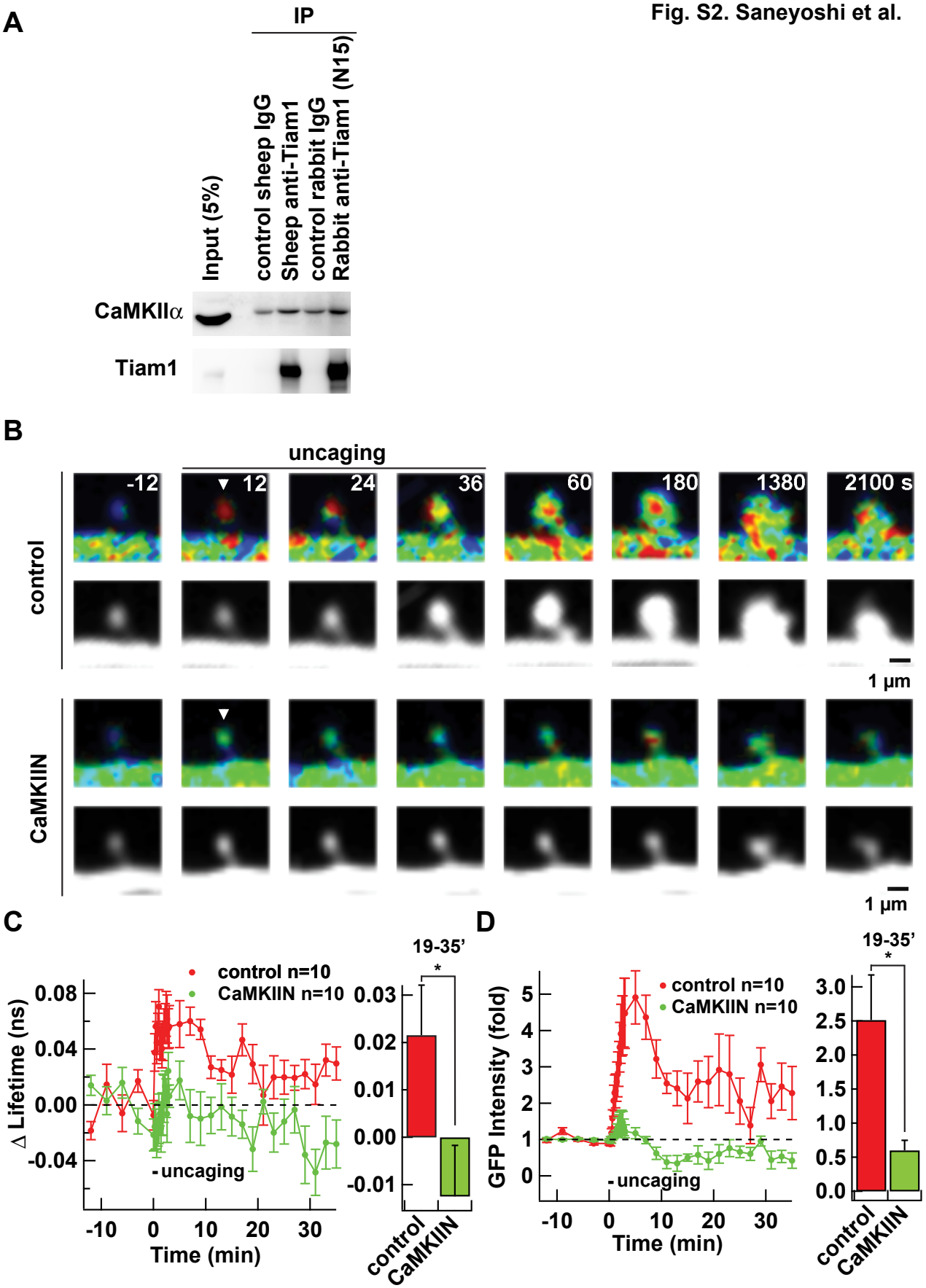

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Figure S2, related to Figure 3. Interaction between Tiam1 and CaMKII and effect of CaMKIIN overexpression

A. Co-immunoprecipitation between Tiam1 and CaMKII from brain tissue. Whole brain lysate was immunoprecipitated with control IgG (Sheep or Rabbit) or anti-Tiam1 antibodies and protein-G sepharose. After extensive wash, immuprecipitated proteins (IP) were eluted with SDS-PAGE sample buffer, and then subjected to western blotting using Tiam1 and CaMKII antibodies.

B. Representative images of the interaction between Tiam1 and CaMKII with or without CaMKIIN overexpression. Other conventions are similar to Fig. 1.

C, D. Summary of FRET change (C) and volume (D) from multiple spines. *, $p < 0.05$, compared to control; *t*-test. Data are represented as mean ± SEM.

Fig. S3. Saneyoshi et al.

A

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Gallus gallus              DRSRPAMDTHASRMTQLKKQAALTGINGDVEGHG
Equus caballus             ERGRKTLDSHASRMTQLKKQAALSGINGGLESTS
Canis lupus familiaris     ERGRRALDSHASRMTQLKKQAALSGINGGLESPG
Mus musculus               ARGRRTLDSHASRMTQLKKQAALSGINGGLESAS
Homo sapiens               ERGRKTLDSHASRMAQLKKQAALSGINGGLESAS
Oryctolagus cuniculus      ERGRRTLDSHASRMAQLKKQAALSGGSGGLESAS
Anolis carolinensis        ERARYTGGTHVSRMAQLKKQAALPGINGGMEGNS
Xenopus tropicalis         TKVQNTMNTHASRMAQLKKQTAFSGMNGSIESNT
                            : : : .:*.***:*****:*: * .*.:*.
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B

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Tiam1      RTLDSHASRMAQLKKQAALSGIN
CaMKIIN1       KRPPKLGQIGRSKRVVIED
AIP                KKALRRQEAVDAL
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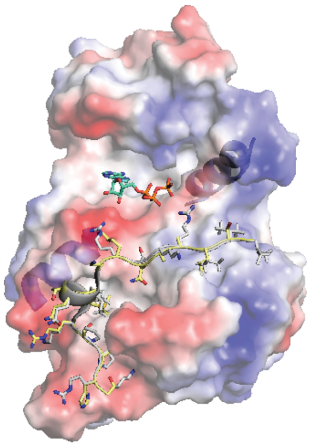
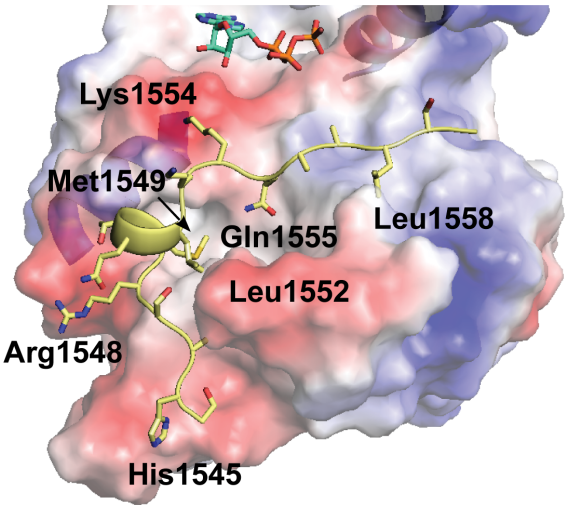
C



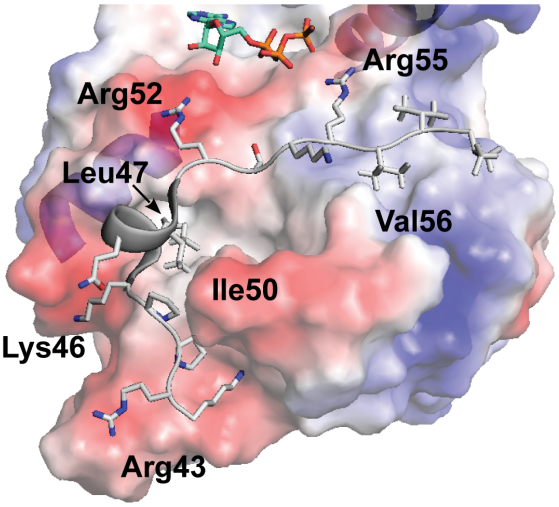
D

Tiam1 (predicted)

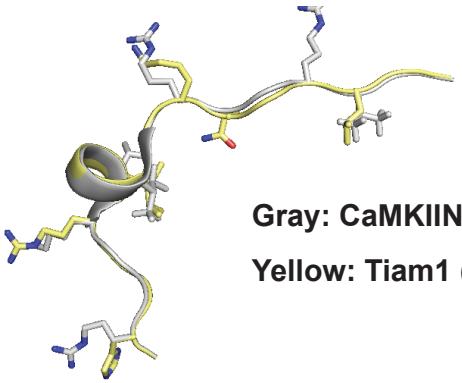


E

CaMKIIN1



F



Gray: CaMKIIN1

Yellow: Tiam1 (predicted)

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Figure S3, related to Figure 4. CBD of Tiam1 is conserved among vertebrate species, and a structural model of RAKEC formed between CaMKII and Tiam1.

A. Cross-species homology comparison of the CBD of Tiam1. Alignment was made by ClustalW program. The following information were used: *Gallus gallus*, XP_416699; *Equus cabllus*, XP_001498883, *Canis lupus familiaris*, XP_544855; *Mus musculus*, NM_009384.3; *Homo sapiens*, NM_003253.2; *Oryctolagus cuniculus*, XP_002716851; *Anolis carolinensis*, XP_003219107; *Xenopus Tropicalis*, XP_002938487.

B. A comparison of the CBD of Tiam1 and CaMKIIN1. The sequence in CaMKIIN1 important for binding, as determined by alanine scanning, is underlined (Coultrap and Bayer, 2011). Those residues that are particularly important are double-underlined. Consensus sequence (L/I)(K/R/H)(K/R)QXXΦ is in red and basic residues outside of the consensus sequence are in blue.

C-F. Prediction of binding between Tiam1 and CaMKII based on the X-ray crystallographic data of interaction between CaMKIIN1 and CaMKII (C) (Chao et al., 2010). Higher magnification of Tiam1/CaMKII interaction and the residues implicated in the binding are in D. CaMKIIN1/CaMKII interaction based on the original crystallographic data (E). Overlay of Tiam1 and CaMKIIN1 are shown (F).

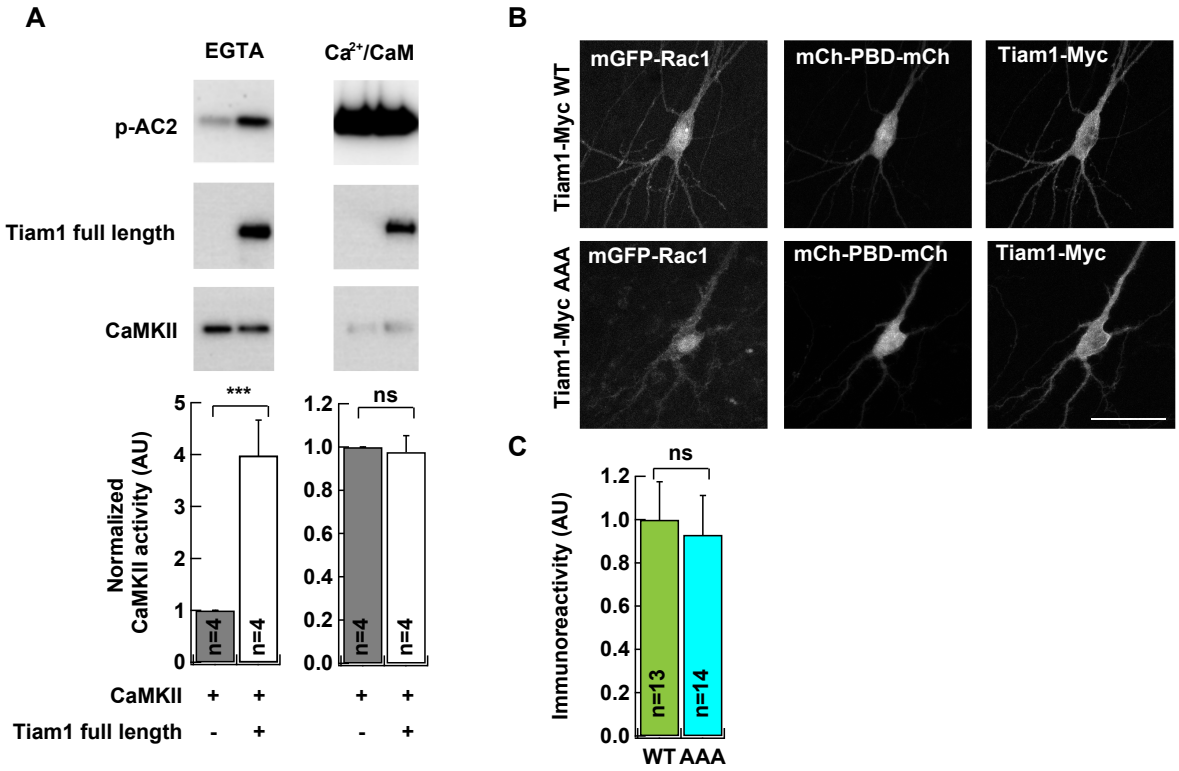

Figure S4, related to Figures 5 and 6. Binding with full length Tiam1 protein generates autonomous kinase activity of CaMKII and expression of WT and AAA Tiam1 rescue construct in neurons of hippocampal slice culture.

A. *In vitro* kinase assay of full length Tiam1-bound CaMKII using GST-AC2 as a substrate. CaMKII and Tiam1 were affinity-purified with a Flag antibody from HEK293T cells expressing Flag-tagged CaMKII or Tiam1. CaMKII was incubated with Tiam1 in the presence of Ca^{2+}/calmodulin to form a complex. Then EGTA was added to chelate Ca^{2+}. ATP was omitted in these steps to avoid autophosphorylation. Subsequently, the *in vitro* phosphorylation reaction was initiated by adding ATP in the absence (EGTA) or presence of Ca^{2+}/calmodulin (Ca^{2+}/CaM) using purified GST-AC2 as a substrate. The reaction products were subjected to western blotting with antibodies against phosphorylated-AKT substrate to detect phosphorylated AC2, Tiam1, and CaMKII. CaMKII bands in Ca^{2+}/CaM were shifted due to auto-

phosphorylation. Band intensities were measured and normalized to CaMKII without Tiam1 as one. ns, not significant; ***, $p < 0.01$, compared to CaMKII without Tiam1; *t*-test.

B. Representative images of neurons expressing shRNA against Tiam1, GFP-Rac1, mCherry-Pak1-mCherry, and shRNA resistant Tiam1 WT or AAA. Neurons in hippocampal organotypic slice culture were transfected with shRNA against Tiam1, GFP-Rac1, mCherry-Pak1-mCherry, and Myc-tagged shRNA resistant Tiam1 WT or AAA. Four days after transfection, slices were fixed, and stained with Myc antibody. Scale, 50 μm.

C. Quantification of Tiam1 rescue proteins in hippocampal organotypic slice culture. Immunoreactivities (IR) of Myc antibody were quantified in GFP and mCherry positive neurons. Relative IR was normalized to WT as one. ns, not significant; *t*-test.

Data are represented as mean ± SEM (A, C).

Fig. S5. Saneyoshi et al.

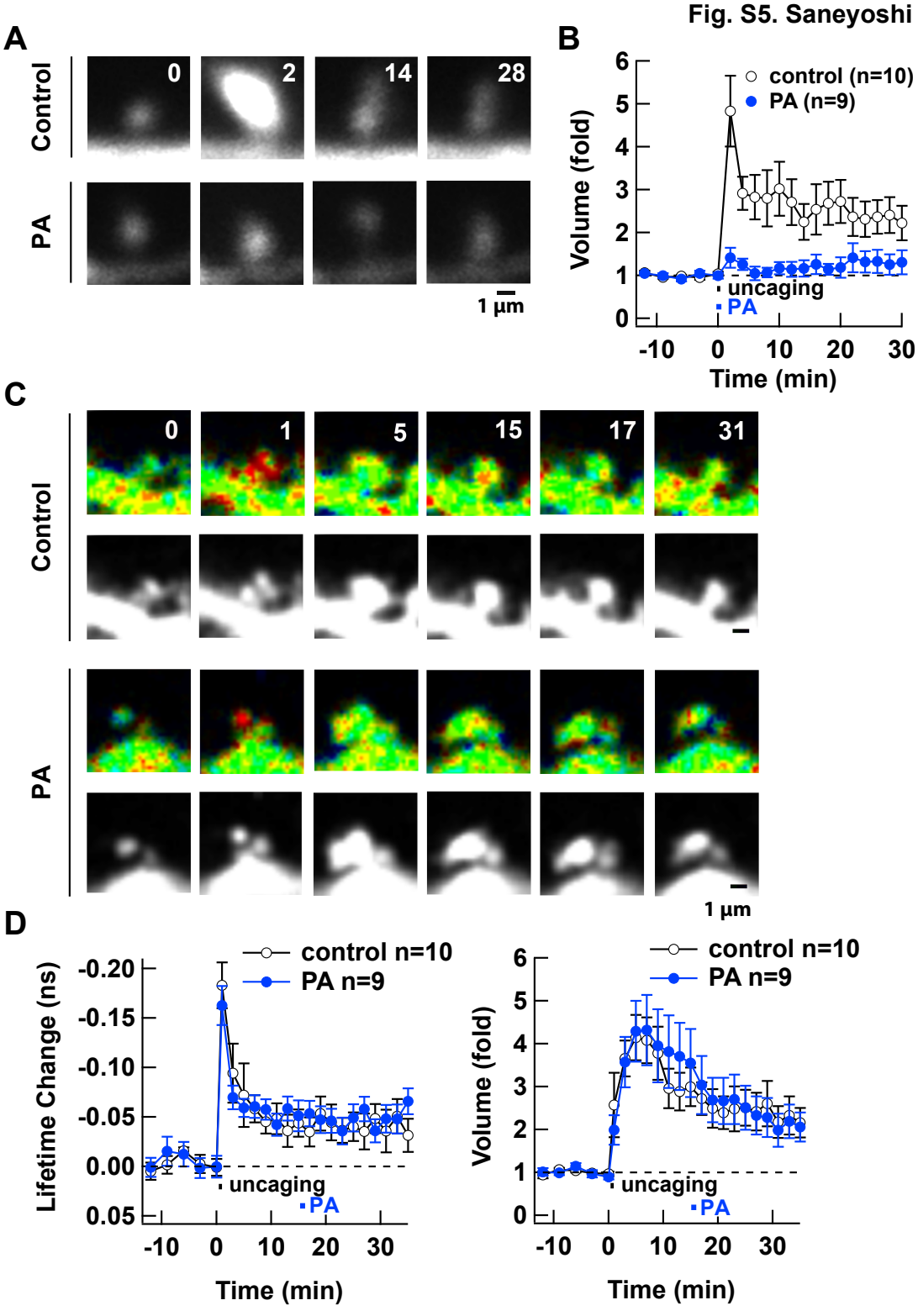

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Figure S5, related to Figures 6. paAIP2 was not able to disrupt the Tiam1/CaMKII complex in stimulated spines

A. Sample images of sLTP in neurons in hippocampal organotypic slice culture coexpressing GFP and mCherry-paAIP2. Neurons were stimulated by uncaging of caged-glutamate using a 720 nm two-photon laser (uncaging, 2 ms pulses at 1 Hz for 30 sec) without or with simultaneous photoactivation (PA) of paAIP2 using 473 nm laser (200 ms pulses at 1 Hz for 1 min), which by itself does not cause uncaging of caged-glutamate (Matsuzaki et al., 2001). Control, without photoactivation; PA, with photoactivation.

B. Summary of the effect of photoactivation of paAIP2 at the same time with uncaging of glutamate. Spine volume was measured by fluorescent intensity of GFP.

C. Representative images of the interaction between Tiam1 and CaMKII with or without photoactivation of paAIP2 at 15 min after induction of sLTP. Warmer hues indicate more interaction. The distribution of Tiam1-GFP, as a proxy of structure, is shown (bottom).

D. Summary of FRET change (left) and volume (right) from multiple spines.

Data are represented as mean ± SEM (B, D).