Lemieux et al., http://www.jcb.org/cgi/content/full/jcb.201202058/DC1

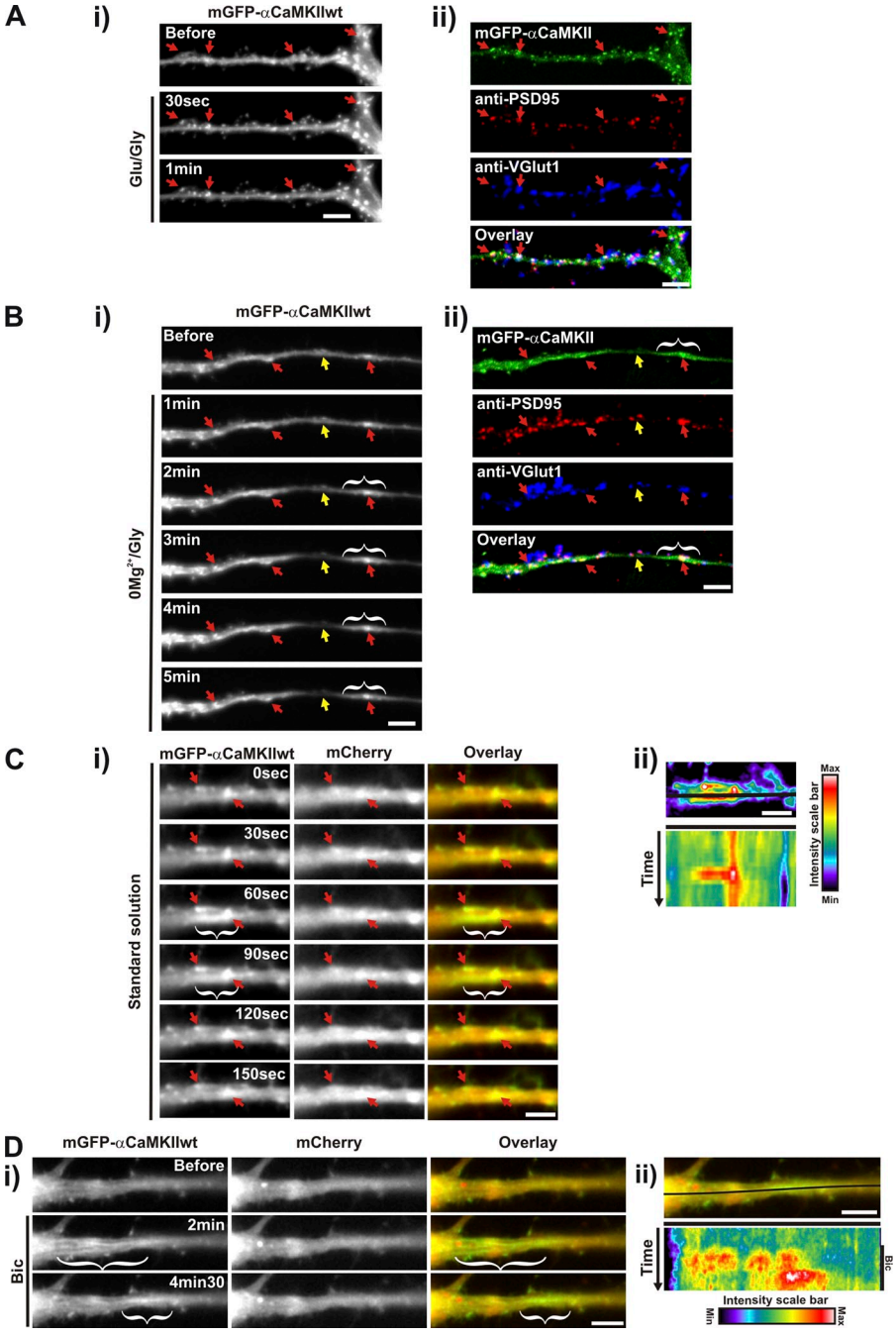


Figure S1. **Stimulated or spontaneous subdendritic translocation of CaMKII near excitatory synapses.** Ai and Bi) Time-lapse imaging of a dendrite expressing mGFP-αCaMKII WT during a 1-min stimulation with Glu/Gly (A) or a 5-min stimulation with $0Mg^{2+}$/Gly (B). (Aii and Bii) Same dendrite as in the i panels, fixed after the stimulation and immunolabeled for PSD95 and VGlut1. (Ci and Di) Time-lapse imaging of a dendrite expressing mGFP-αCaMKII WT and mCherry in standard solution (C), showing spontaneous dendritic translocation of the enzyme, and during application of 10 µM bicuculline (D). (Cii and Dii) Kymograph of the change in the ratio of fluorescence (mGFP-αCaMKII/mCherry) over time across the dendrite (black line). Stimulation period in Dii is indicated (Bic). Arrows point to synapses where CaMKII accumulated (red) or not (yellow). Brackets indicate regions of dendritic CaMKII hot spot. Bars: (A, B, and D) 5 µm; (C) 2 µm. Related to Fig. 1. See also Video 2.

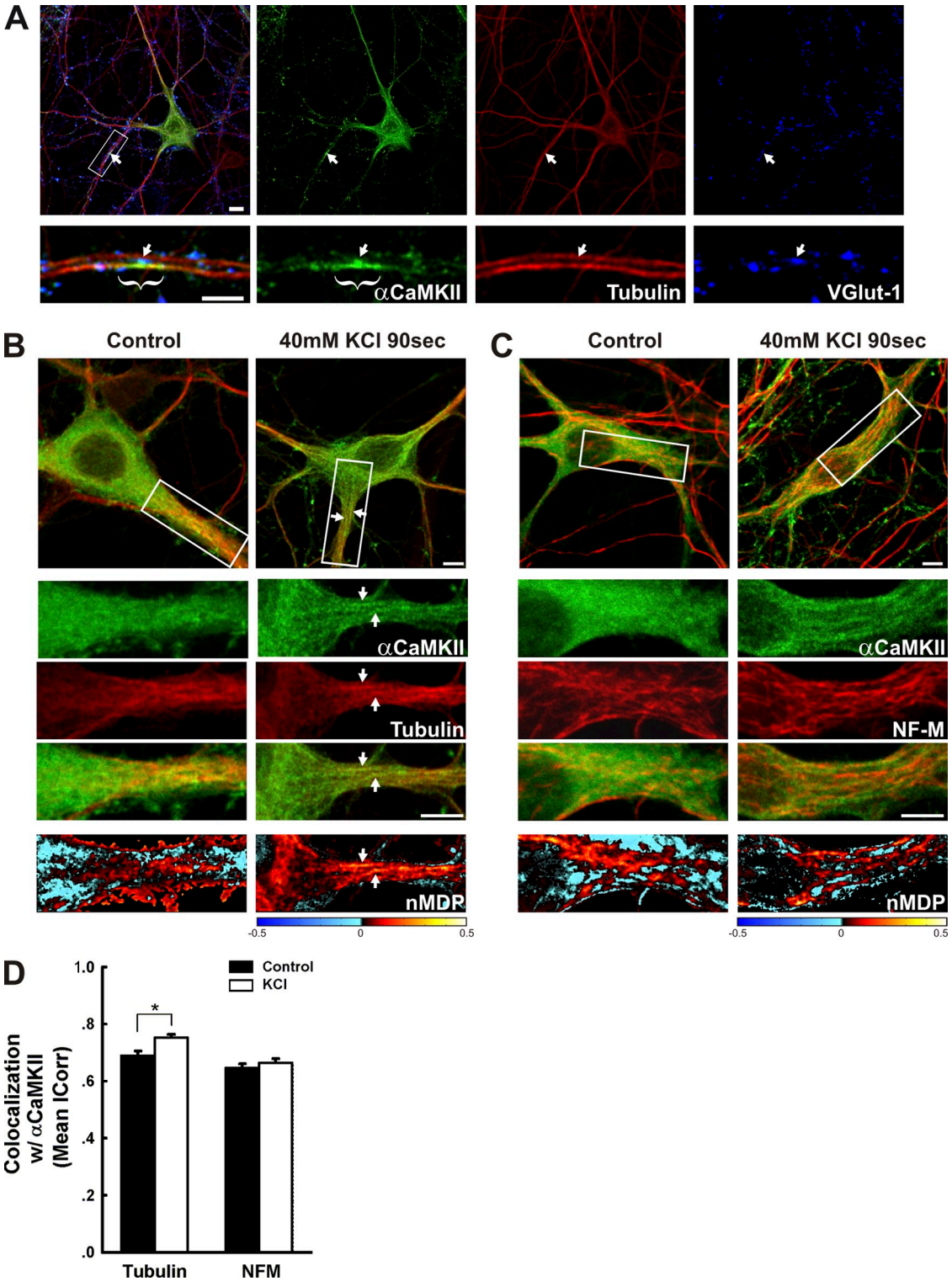


Figure S2. **Localization of endogenous αCaMKII to microtubules in stimulated neurons.** (A) Immunostaining for endogenous αCaMKII, tubulin, and VGlut1 in neurons stimulated with $0Mg^{2+}$/Gly for 5 min. Arrows point to a synapse where CaMKII accumulated. Brackets show a region of dendritic CaMKII hot spot. Bars: (neurons) 10 µm; (dendrites) 5 µm. (B) Immunostaining on neurons for αCaMKII and microtubules (tubulin). The bottom panels show a correlation-based colocalization analysis (see Materials and methods) of the above cropped images. (C) Control and stimulated neurons stained for endogenous αCaMKII and neurofilament-M (NF-M). Neurons were stimulated with 40 mM KCl for 90 s. nMDP images represent the correlation between the two signals in pseudocolor, hotter color (positive nMDP values) meaning more correlation. Bar, 5 µm. (D) Mean ± SEM I_{Corr} (image correlation) between αCaMKII and tubulin, and between αCaMKII and neurofilament-M, after KCl simulation. n = 40 neurons per condition, two experiments. *, $P < 0.05$ ANOVA followed by a Tukey's least-significant difference test. Related to Figs. 1 and 3.

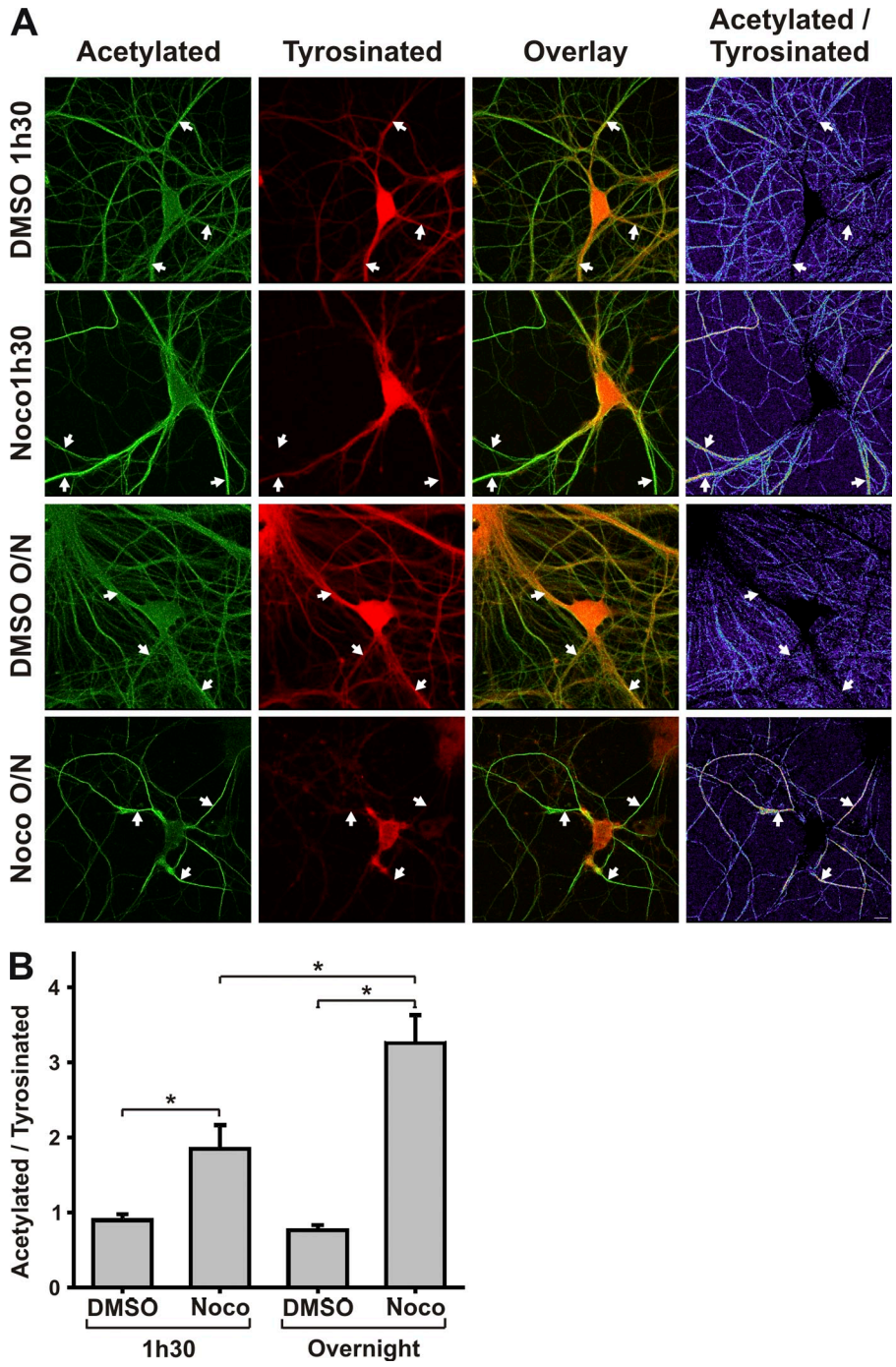


Figure S3. **Effect of nocodazole on microtubule destabilization assessed by measuring changes in ratio of acetylated/tyrosinated tubulin.** (A) Immunostaining for acetylated (more stable microtubules) and tyrosinated (more dynamic microtubules) tubulin in neurons treated for 90 min or overnight (16–24 h) with 10 µM nocodazole or 0.1% DMSO. Pseudocolored images of the right panels are the ratio of the images of acetylated tubulin over tyrosinated tubulin. Bar, 10 µm. (B) Mean ± SEM of the intensity ratio in dendritic regions of immunolabeled acetylated over tyrosinated tubulin. *n* = 10–20 neurons per condition. *, P < 0.05 Kruskal Wallis followed by a Tukey's least-significant difference test. These results demonstrate that incubation of nocodazole over 90 min causes only partial destabilization of microtubules. After overnight treatment, the morphology of the neurons was strongly affected. Related to Fig. 3. Based on Witte et al., 2008.

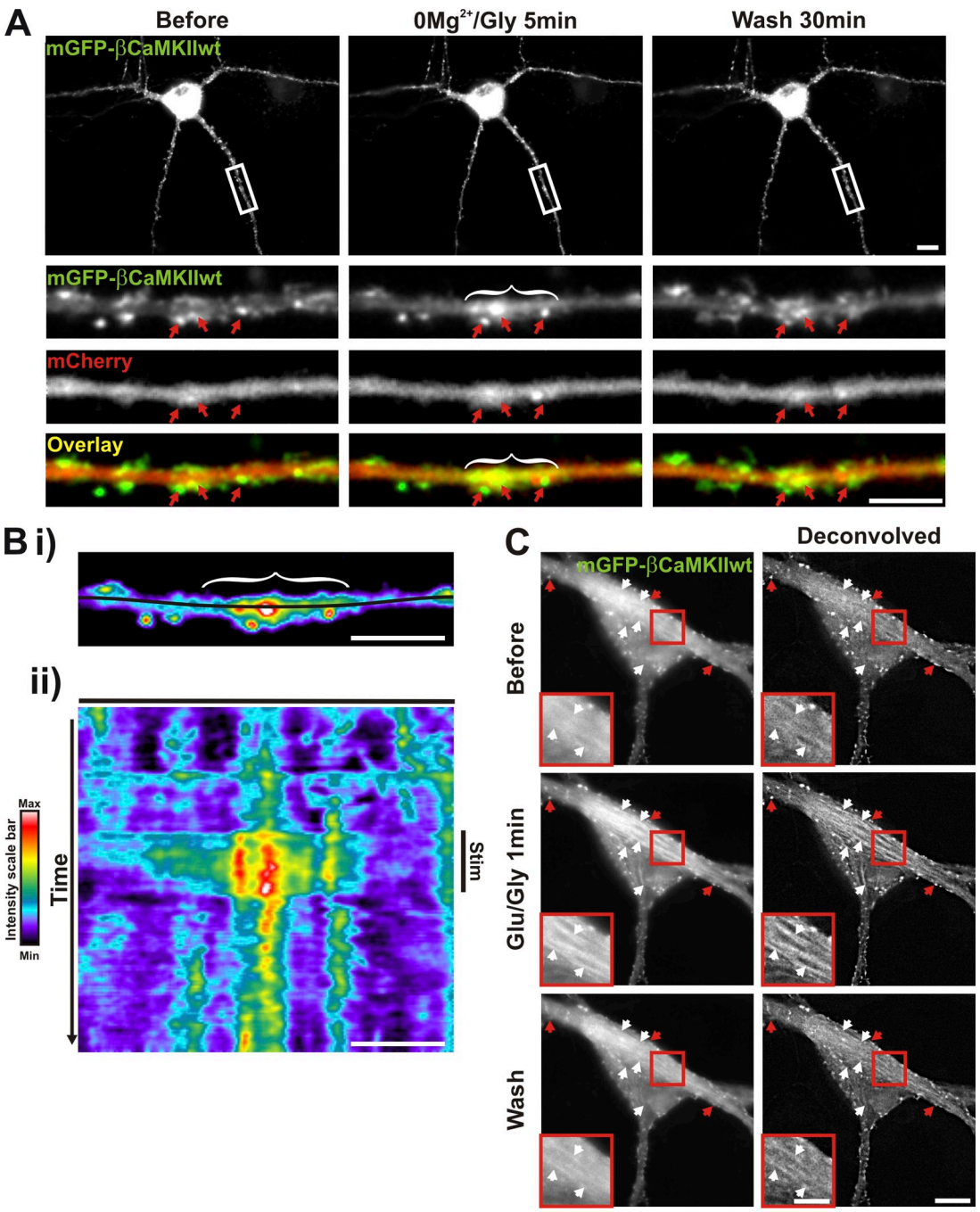


Figure S4. **Activity-dependent translocation of βCaMKII to microtubules.** (A) Neuron expressing mGFP-βCaMKII and mCherry stimulated with $0Mg^{2+}$/Gly for 5 min. Bars: (neuron) 10 µm; (dendrite) 5 µm. (B) Kymograph of the change in the ratio of fluorescence (mGFP-βCaMKII/mCherry) over time across the dendrite (black line). Stimulation is indicated (Stim). Bars, 5 µm. (C) Time-lapse imaging of mGFP-βCaMKII during a 1-min glutamate/glycine (100/10 µM) stimulation. Arrows point to synaptic (red) or microtubule-like sites (white) of CaMKII translocation. White brackets indicate a CaMKII dendritic hot spot. Bars: (neuron) 10 µm; (inset) 5 µm. Related to Figs. 1 and 3.

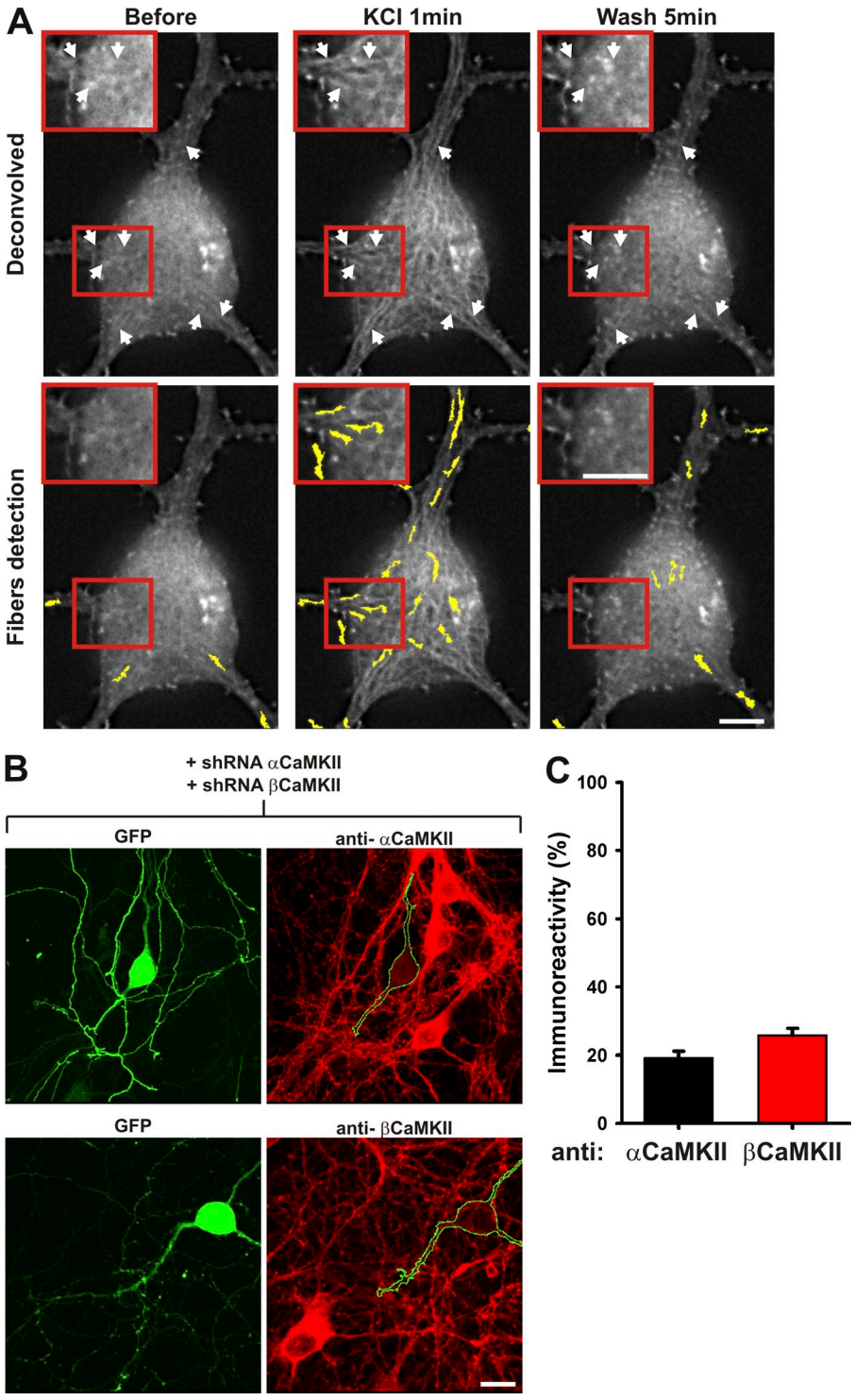
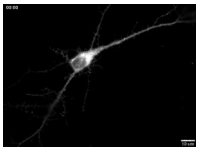
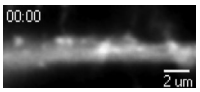


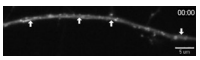
Figure S5. **Morphometric analysis of CaMKII translocation to microtubules and effect of shRNA treatment on endogenous level of α- and βCaMKII.** (A) Deconvolved images of a cell body from a neuron transfected with mGFP-αCaMKII WT on which fibers were detected (yellow) before, during, and after a stimulation with KCl for 1 min. Arrows point to regions where fibers appear during the stimulation. (B) Neurons transfected with GFP and shRNA against both α- and βCaMKII and immunolabeled for αCaMKII (top) or βCaMKII (bottom). (C) Quantification of the percentage of immunoreactivity for α- or βCaMKII in the transfected cell compared with neighbor neurons. *n* = 32–34 neurons per condition, 4 experiments. Bars: (A) 5 µm; (B) 20 µm. Related to Figs. 3 and 4.



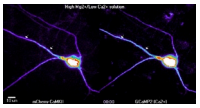
Video 1. **Translocation of αCaMKII to dendritic sites near synapses.** Time-lapse imaging of a neuron expressing mGFP-αCaMKII stimulated with $0Mg^{2+}$/Gly for 5 min showing αCaMKII translocation at some synaptic sites and the appearance of dendritic αCaMKII hot spots. Frames were taken every 10 s for the first 13 min and then every 30 s with a CoolSnap-HQ camera (Photometrics) on an Axioskop FS2 microscope (Achroplan 63x 0.95 NA objective; Carl Zeiss). Related to Fig. 1.



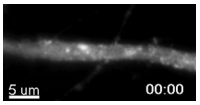
Video 2. **Spontaneous translocation of αCaMKII to dendritic sites near synapses.** Time-lapse imaging of a neuron expressing mGFP-αCaMKII in standard solution showing spontaneous αCaMKII translocation at two synaptic sites and the appearance of dendritic αCaMKII fiber-like structure nearby. Frames were taken every 30 s with a CoolSnap-HQ camera (Photometrics) on an Axioskop FS2 microscope (Achroplan 63x 0.95 NA objective; Carl Zeiss). Related to Fig. 1 and Fig. S1.



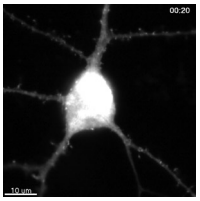
Video 3. **Repetitive translocation of αCaMKII to dendritic sites near synapses.** Time-lapse imaging of a neuron expressing mGFP-αCaMKII showing repetitive accumulation of CaMKII in dendritic hot spots near synapses during multiple $0Mg^{2+}$/Gly stimulations (3–5 min each). Frames were taken every 20 s with a CoolSnap-HQ camera (Photometrics) on an Axioskop FS2 microscope (Achroplan 63x 0.95 NA objective; Carl Zeiss). Related to Fig. 1.



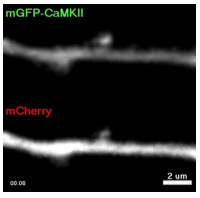
Video 4. **αCaMKII translocates to dendritic sites of higher Ca^{2+} concentration.** Time-lapse imaging of a neuron expressing both mCherry-αCaMKII (left) and GCaMP2 (right), a genetically encoded Ca^{2+} indicator, and stimulated with $0Mg^{2+}$/Gly for 5 min. Ca^{2+} oscillations during the stimulation lead to the gradual accumulation of mCherry-αCaMKII at sites where Ca^{2+} amplitude and/or frequency are higher (examples are pointed by the arrows). Frames were taken every 2 s with a QuantEM:512SC camera (Photometrics) on an Axioskop FS2 microscope (Achroplan 63x 0.95 NA objective; Carl Zeiss). Related to Fig. 2.



Video 5. **αCaMKII accumulates in dendritic segments by interacting with microtubular elements.** Time-lapse imaging of a dendrite from a neuron expressing mGFP-αCaMKII and stimulated with $0Mg^{2+}$/Gly for 5 min. During the stimulation, mGFP-αCaMKII translocates to a synapse and gradually accumulates in the dendritic segments near this synaptic site, revealing a microtubular-like pattern of fluorescence in the dendrite. This is accompanied by a reversible decrease in the amount of mGFP-αCaMKII on each side of this region. Frames were taken every 10 s for the first 8 min and then every 30 s with a CoolSnap-HQ camera (Photometrics) on an Axioskop FS2 microscope (Achroplan 63x 0.95 NA objective; Carl Zeiss). Related to Fig. 3.



Video 6. **Cell-wide depolarization induces αCaMKII translocation to microtubular elements throughout the neuron.** Time-lapse imaging of a neuron expressing mGFP-αCaMKII stimulated with 40 mM KCl for 1 min. During the stimulation, mGFP-αCaMKII translocates to synapses throughout the neuron, but also to microtubules in the somatodendritic area. Frames were taken every 10 s for the first 4 min and then every 30 s with a CoolSnap-HQ camera (Photometrics) on an Axioskop FS2 microscope (Achroplan 63x 0.95 NA objective; Carl Zeiss). Related to Fig. 3.



Video 7. **Localized dendritic translocation of αCaMKII correlates with spine growth.** Time-lapse imaging of mGFP-αCaMKII (top) and mCherry (down) in a spine and subjacent dendrite stimulated with $0Mg^{2+}$/Gly for 5 min. During the stimulation, mGFP-αCaMKII first accumulates into the spine and then in the dendritic segment at its base. The spine gradually grows and its increase in size persists 30 min after the stimulation. Frames were taken every 10 s for the first 8 min and then every 30 s with a CoolSnap-HQ camera (Photometrics) on an Axioskop FS2 microscope (Achroplan 63x 0.95 NA objective; Carl Zeiss). Related to Fig. 7.

Reference

Witte, H., D. Neukirchen, and F. Bradke. 2008. Microtubule stabilization specifies initial neuronal polarization. *J. Cell Biol.* 180:619–632. http://dx.doi.org/10.1083/jcb.200707042