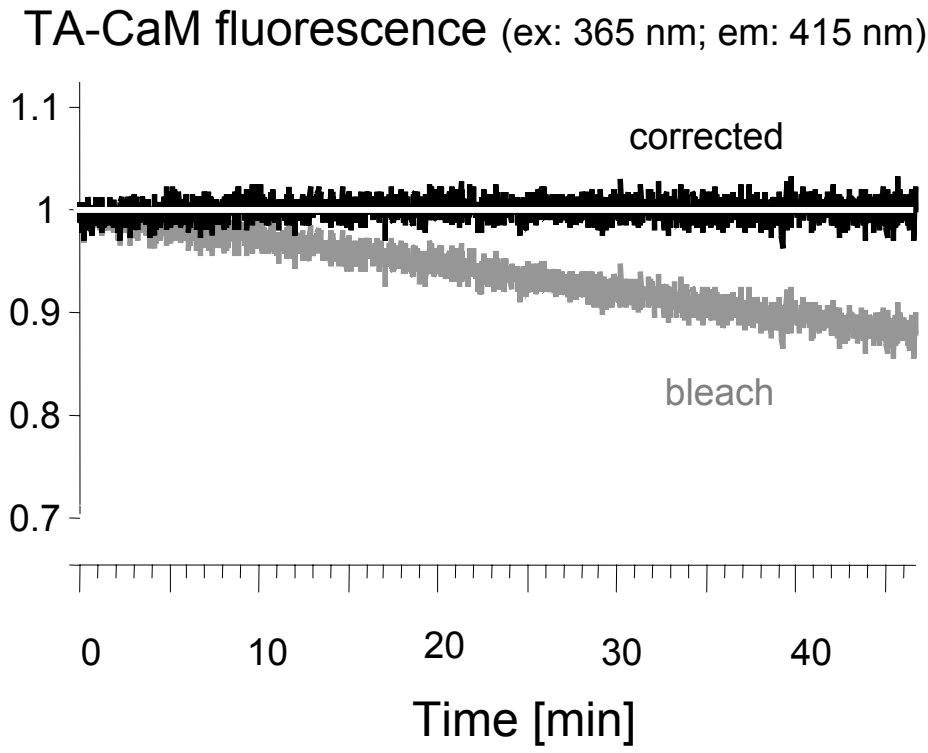
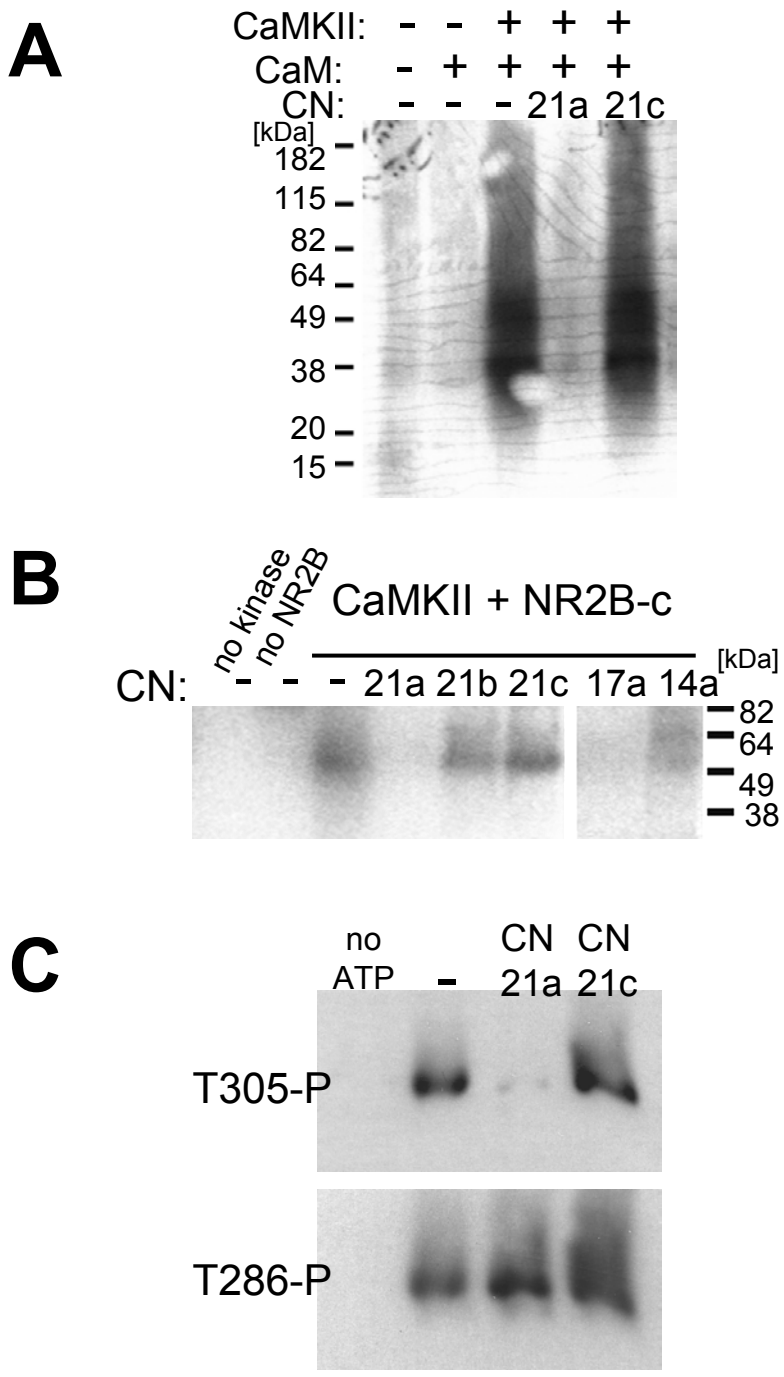
SI Fig. 9

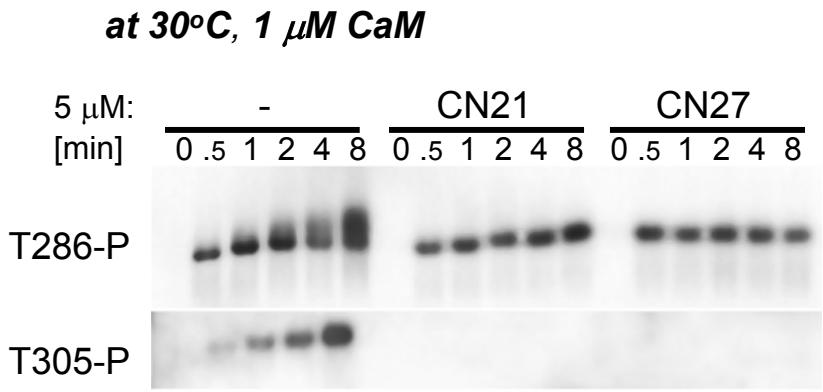


SI Fig. 9: Photobleach of TA-CaM in the experimental setup, and correction used.



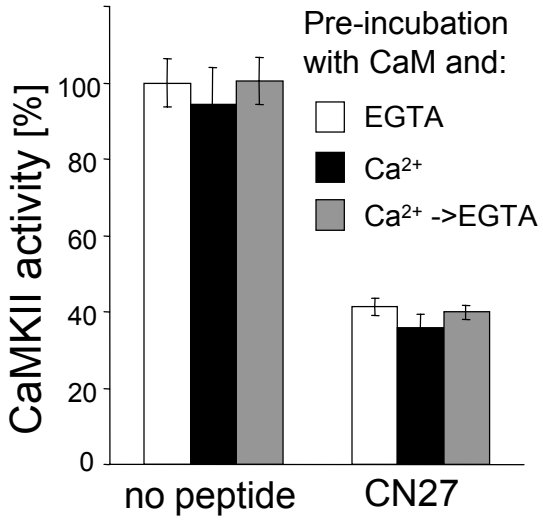
SI Fig. 10: CN21a (5 μM) blocks CaMKII phosphorylation of (A) crude liver protein extracts, (B) bacterially expressed GST-NR2B-c (containing C-terminal NR2B amino acids 1,120 to 1,482), and (C) T305 but not T286 autophosphorylation. Phosphorylation was detected by ^{32}P auto-radiography (A,B), or by phospho-specific antibodies (C). In A and B, CaMKII was auto-phosphorylated before the final reactions (3 min at 30°C) in order to reduce the T286 signal.

SI Fig. 11



SI Fig. 11: CN21 and CN27 (5 μM) did not detectably slow down T286 auto-phosphorylation, stimulated with 1 μM CaM at 30°C. By contrast, T305 and other auto-phosphorylation that result in a band-shift of CaMKII were completely blocked (resulting in tighter bands in presence of inhibitor, giving the false first impression of less T286-P signal intensity; compare Figure 5B,C). Auto-phosphorylation at T286 and T305 were detected by Western-analysis. The experiment was done essentially as in Figure 5B, but here stimulated with 1 μM instead of 0.1 μM CaM.

SI Fig. 12



SI Fig. 12: CN27 binding only to the activated form of CaMKII did not cause a measurable delay in inhibition of AC2 phosphorylation. Before the activity assay, CaMKII was preincubated with or without 2 μM inhibitor in presence of EGTA (2 mM; to prevent inhibitor pre-association), Ca^{2+}/CaM (0.5 mM/1 μM; to allow inhibitor pre-association) or first Ca^{2+}/CaM followed by addition of EGTA (to allow pre-association with the inhibitor and then dissociation of CaM). These pre-treatments did not affect kinase activity or inhibition.

Delayed inhibition was initially considered to explain that less inhibition was observed for AC2 compared to the study by Chan et al., 1998 (which used syntide2). We considered the possibility that in the previous study the inhibitor, CaMKII, and CaM might have been mixed before the kinase reactions, and that mixing at the initiation of the reactions would allow significant phosphorylation before inhibition is effective. The higher IC50 for AC2 was later explained by the competitive mode of AC2 not seen for other substrates (see Figs. 2,3).