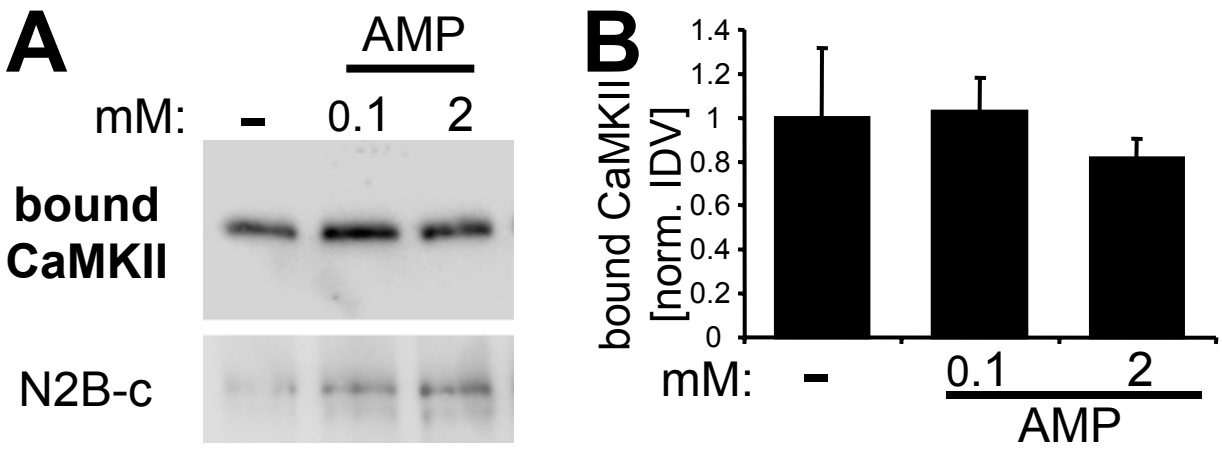
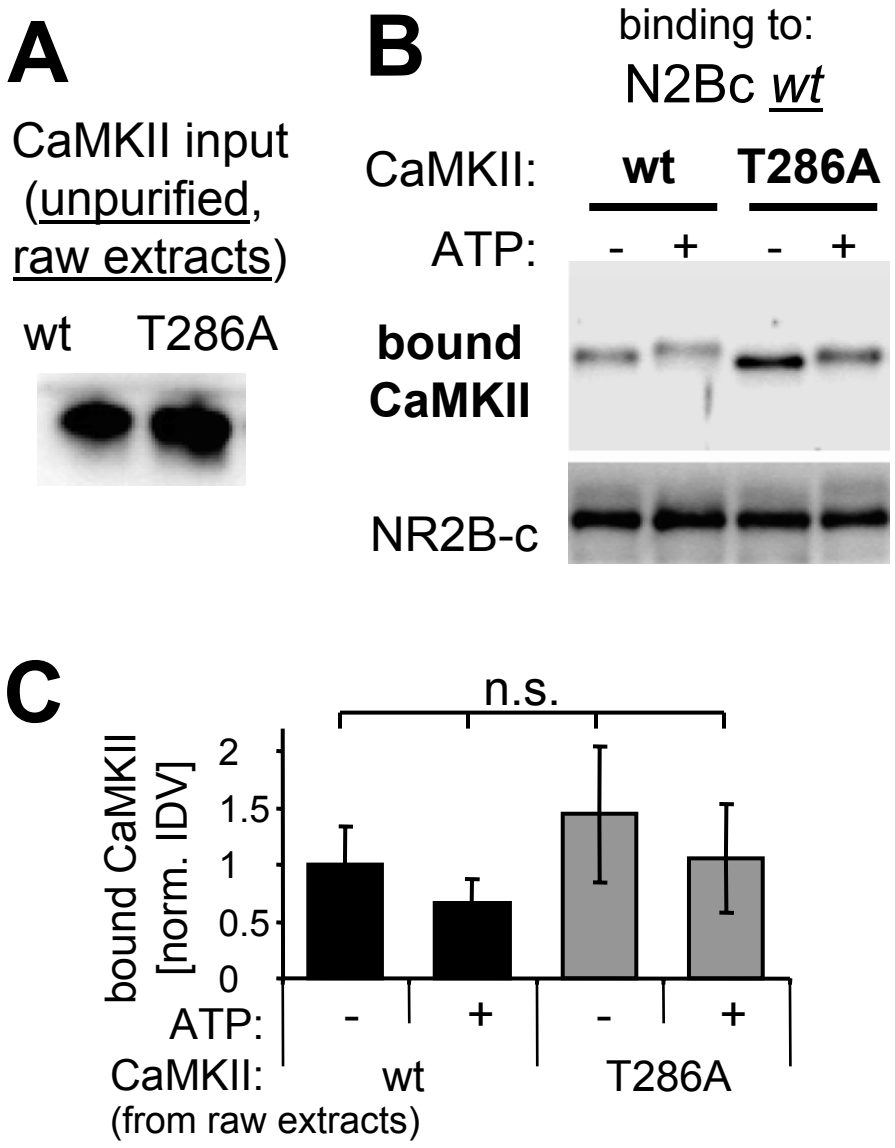


SUPPLEMENTAL FIGURE S1. **AMP did not enhance CaMKII binding to GluN2B** (in contrast to ATP or ADP; compare Fig. 2), consistent with >500fold lower binding affinity of AMP compared to ATP or ADP described for other kinases (44-46).
A, Addition of AMP (0.1 or 2 mM) did not enhance Ca^{2+}/CaM-induced CaMKII binding to GluN2B *in vitro*, as determined by Western-blot analysis of the protein complex.
B, Quantification of experiments as shown in panels A (n=3) revealed no significant difference between the conditions ($p>0.7$; ANOVA). Error bars show s.e.m.

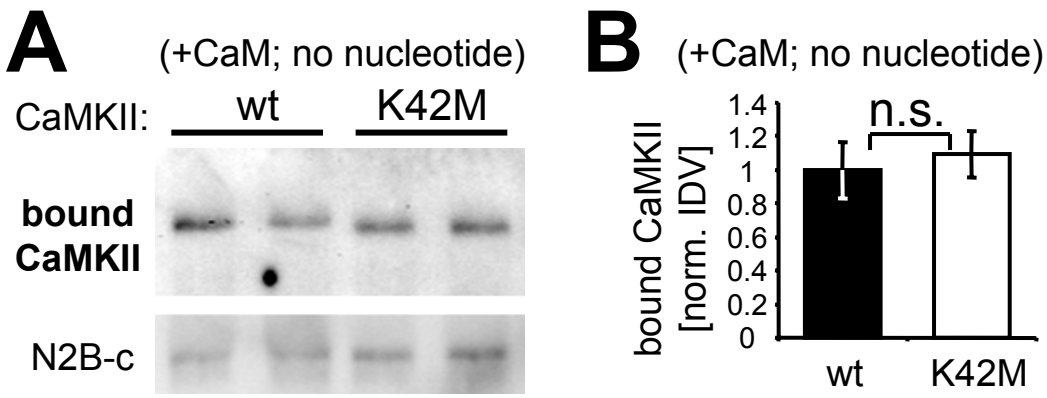


SUPPLEMENTAL FIGURE S2. **ATP did not enhance GluN2B binding of CaMKII wild type or T286A in raw extracts** (in contrast to purified enzyme; see Figs. 2-4), likely because the raw extracts already contain maximally stimulating concentration of nucleotide (compare Fig. 2D). Indeed, nucleotide concentration in the binding reactions with kinase from raw extracts are estimated to be ~40-80 μM, based on extract dilution and ~4 mM cellular nucleotide concentration. Much of the nucleotide in the extracts is expected to be ADP, generated by ATP conversion during and after cell lysis.

A, Similar amounts of GFP-CaMKII wild type and T286A mutant (in raw extracts from HEK 293 cells) were used in the binding experiments, as determined by Western-blot analysis of the input mix of the binding reaction.

B, In contrast to purified GFP-CaMKII (see Fig. 4), binding of unpurified GFP-CaMKII to GluN2B in vitro was not enhanced by the addition of ATP (100 μM), as determined by Western-blot analysis of the protein complex.

C, Quantification of binding experiments as shown in panel B (n=3) showed that addition of ATP did not significantly affect GluN2B binding of unpurified GFP-CaMKII ($p>0.05$; ANOVA).



SUPPLEMENTAL FIGURE S3. **In absence of nucleotide, CaMKII wild type and K42M show the same level of binding to GluN2B *in vitro*.**
A, Ca^{2+}/CaM-induced binding of purified GFP-CaMKII wild type and K42M to GluN2B was compared in biochemical assays. Western-blot analysis of GluN2B bound CaMKII is shown.
B, Quantification of experiments as in panel A showed no difference between CaMKII wild type and K42M ($p>0.6$, t-test; $n=7$). Error bars show s.e.m.