SUPPORTING MATERIAL FOR WORLD WIDE WEB EDITION

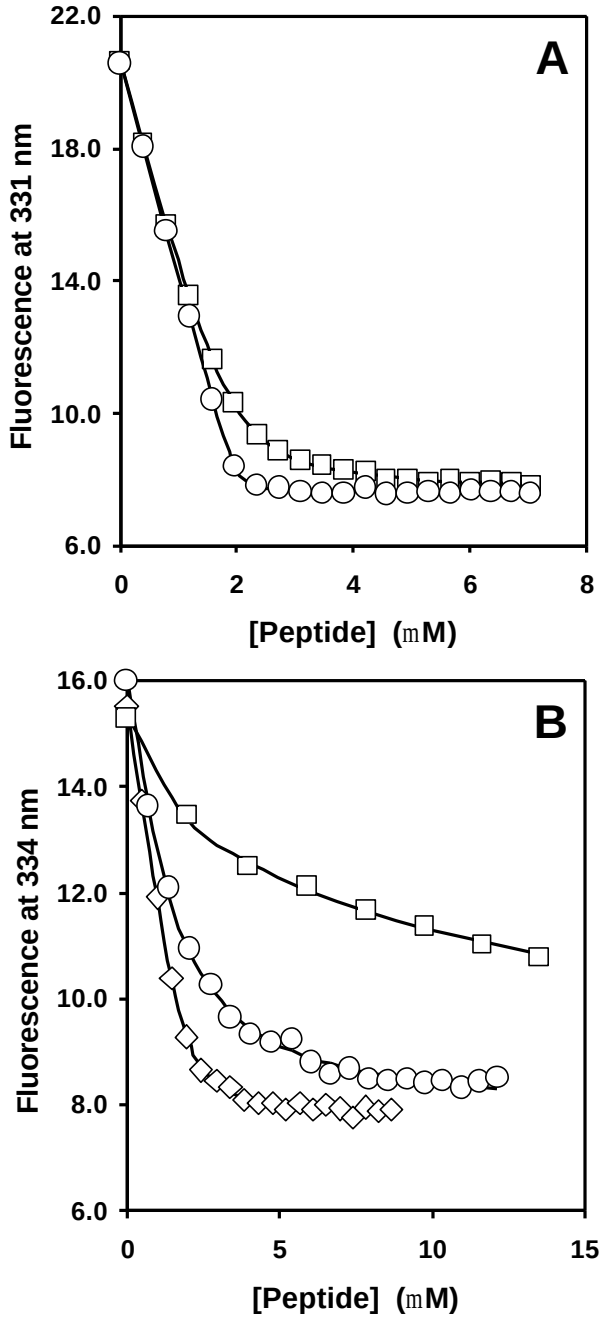

Figure S1: Determination of peptide affinities. (A) Displacement titration of CaM (2μM) + NM2 (4μM) with FFFu (□) and FFFp (○). (B) Displacement titration of CaM (2μM) + WFF (2.1μM) with FFFu (□), FFFp (○) and CBP1 (◇).

The 1:1 stoichiometry for all Trp-containing peptides was established by fluorescence titration at high concentration using solutions of CaM and peptide whose concentrations were established by spectroscopic methods. Dissociation constants for two peptides with moderate affinity for CaM (NM2 and FWF) were determined by direct fluorometric titrations using standard methods ($K_{d,NM2}$ = 31 ± 4nM, and $K_{d,FWF}$ = 6.2 ± 1.2nM; data not shown). Competition titrations in which a Trp-containing peptide is displaced from its complex with CaM by a spectroscopically "silent" peptide were used to determine dissociation constants for the silent peptides. Typical competition titrations of Ca_4-CaM-NM2 with the spectroscopically silent peptides FFFu and FFFp are shown in Figure S1A. Analysis with $K_{d,NM2}$ fixed at 31 nM gave $K_{d,FFFu}$ = 750 ± 200pM and $K_{d,FFFp}$ = 45 ± 5pM (These values agree well with values of 650 ± 120pM (FFFu) and 75 ± 15pM (FFFp) determined in competition assays using Ca_4-CaM-FWF; data not shown).

Figure S1B shows competition titrations of Ca_4-CaM-WFF with (silent) FFFu, FFFp and CBP1. Analysis of the data for FFF_U with $K_{d,FFFu}$ fixed at 700pM (see above) gave $K_{d,WFF}$ = 120 ± 40pM, (cf ~200pM determined by direct fluorometric titration, (*35*)). Analysis of the other two curves with $K_{d,WFF}$ fixed at 120pM gave $K_{d,FFFp}$ = 55 ± 20pM and $K_{d,CBP1}$ = 6 ± 2pM.

Figure 7C (main text) shows competition titrations of Ca_4-CaM-CaMKIp with FFFu, FFFp, and CBP1. Analysis of these curves with dissociation constants for FFFu, FFFp and CBP1 fixed at 750, 50 and 6pM (see above) gave the K_d for the interaction of CaMKIp with Ca_4-CaM as ~1pM (using FFFu), 0.3pM (using FFFp) and 0.5pM (using CBP1). The linked assay involves

propagation of errors. However, titrations with peptides differing in affinity for CaM by a factor of 100 give consistent values for the binding of CaM to CaMKIp.

The numerical methods used in the analysis of these competition titrations are fully described elsewhere (S. R. Martin and P. M. Bayley, Regulatory implications of a novel mode of interaction of calmodulin with a double IQ motif target sequence from murine *dilute* myosin V. Protein Science, in the press 2002).

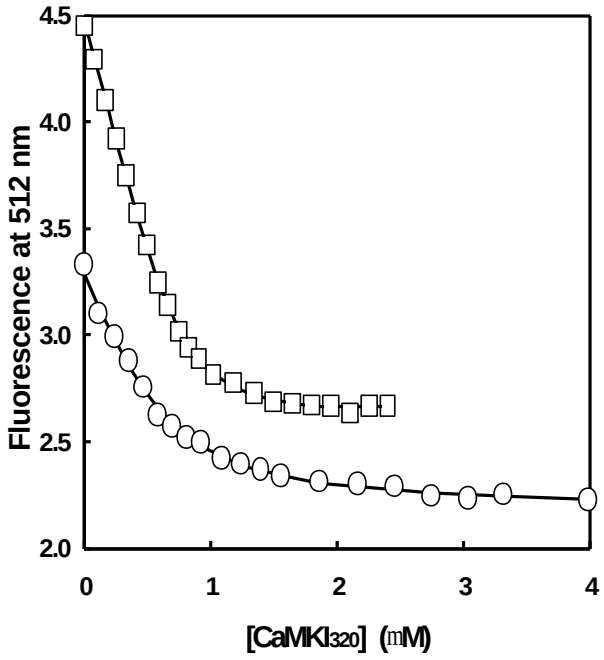

Figure S2: Interaction of $CaMKI_{320}$ with fluorescently labeled CaM. Titrations of CaMdm111 (0.77μM) (□) and CaMdm38 (0.59μM) (○) with $CaMKI_{320}$. Titrations were performed using two cysteine mutants of CaM (Ser-38→Cys and Asn-111→Cys) labeled with dansyl maleimide (CaMdm38 and CaMdm111), giving values of K_d = 44 ± 5nM (CaMdm111) and 250 ± 60nM (CaMdm38). The higher values compared to wt-CaM are probably due to some interference by the dansyl fluorophore.

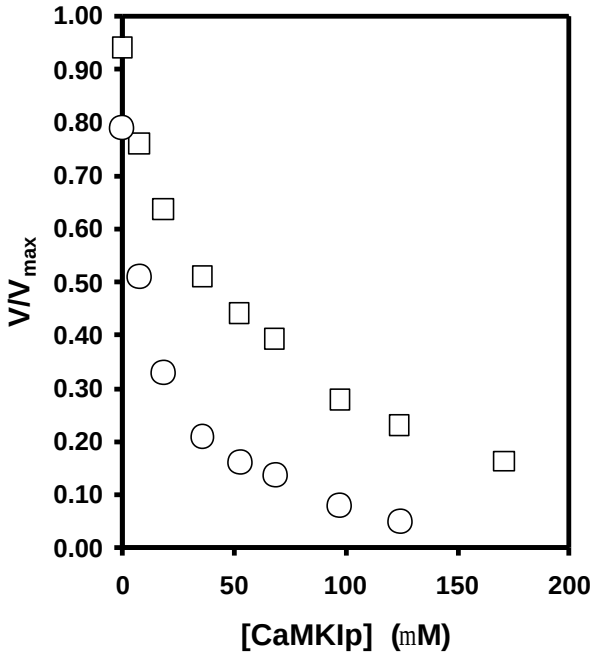

Figure S3: Inhibition of $CaMKI_{293}$ by CaMKIp. Normalized reaction velocity (plotted as V/V_{max}) as a function of CaMKIp for substrate (ADR1G) concentrations of 100μM (□) and 25μM (○). These curves can be simulated using K_d of 2μM for the interaction of CaMKIp with $CaMKI_{293}$.

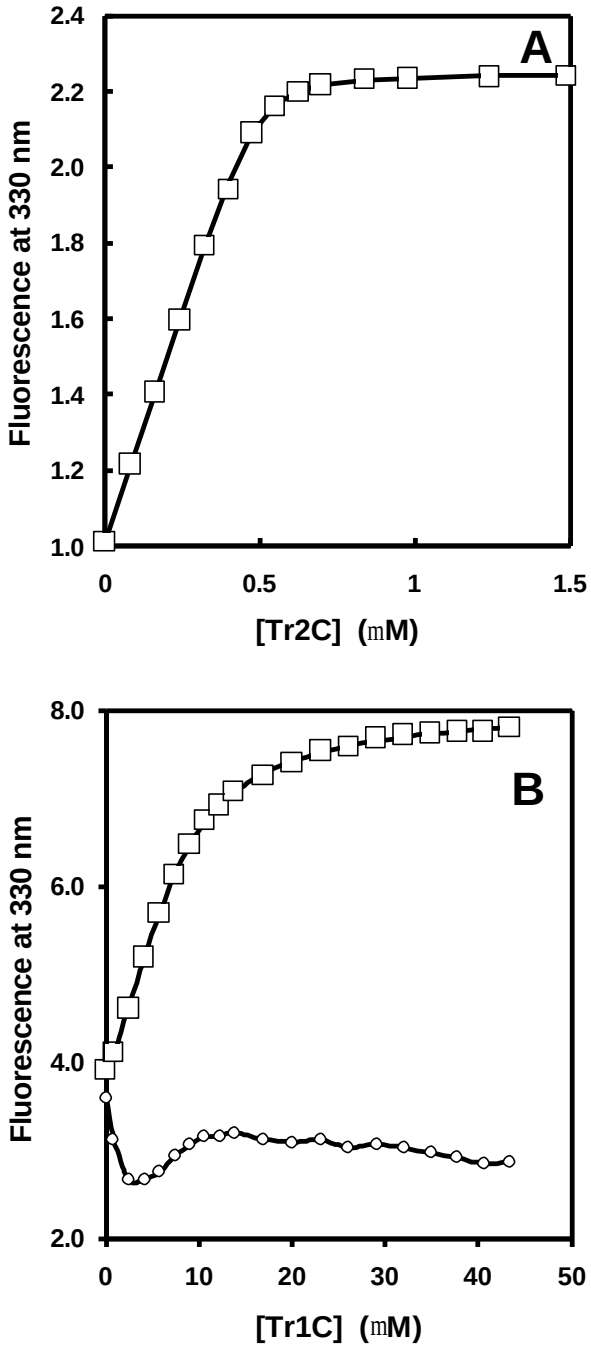

Figure S4: Interaction of CaM C-domain and CaM N-domain with CaMKIp. (A) Titration of CaMKIp (0.5μM) with CaM C-domain. (B) Titration of CaMKIp (3.0μM) with CaM N-domain. Solid lines are the computed best fits for the models described in the text. The small open circles in panel (B) show the residuals for the best fit for formation of a simple 1:1 complex.