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Substrate-selective and Ca^{2+}-independent activation of CaMKII by α-actinin

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Supplementary Figures 1-5

Figure S1: Primary sequence similarity between CaM and α-actinin
Alignment of C-terminal domain (CTD) residues 819-894 from human α-actinin-2 (A2) with corresponding domains in α-actinin-1, 3 and 4 (A1, A3, A4) and full-length human CaM (CM) using Expresso 3D-Coffee (Armougom et al., 2006), with relative alignment strength indicated on a color coded scale (lower right). Asterisks above the sequences indicate residues targeted for mutagenesis in α-actinin-2.

Fig S1

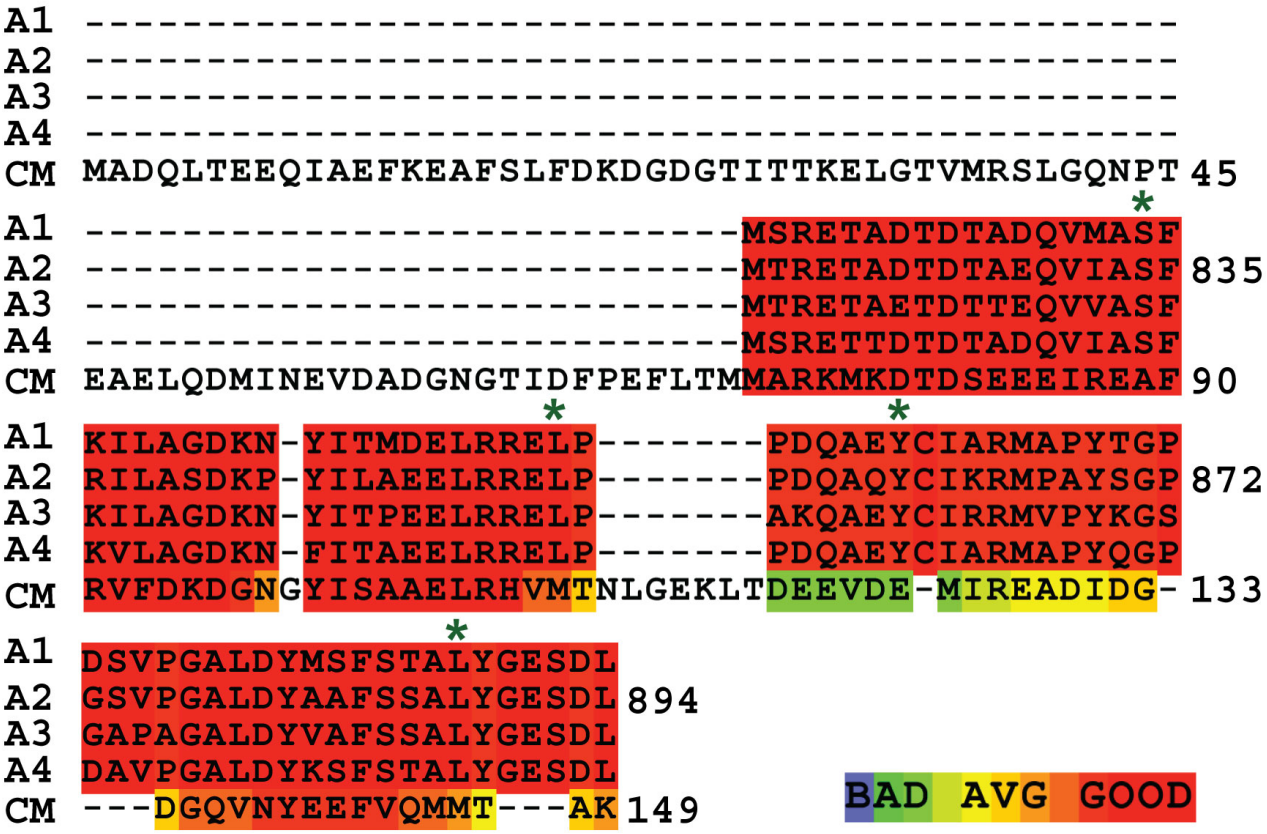

Figure S2: Structural similarity between CaM and α-actinin
A) Structure of Ca^{2+}/CaM bound to the CaMKII regulatory domain peptide (290-314) (PDB:1CM1). The N- and C-terminal domains (lobes) of CaM (residues 1-72 and 73-149) are shown in light and dark blue, respectively. The CaMKII peptide is in orange, with Thr306 in cyan. **B)** Structure of the α-actinin-2 CTD (residues 818-894) (red) bound to a z-repeat regulatory domain peptide from titin kinase (green) (PDB:1H8B). **C)** Alignment showing structural similarity between the α-actinin CTD (red) and the C-terminal lobe of Ca^{2+}/CaM in the CaM-CaMKII structure (dark blue), made using PyMol.

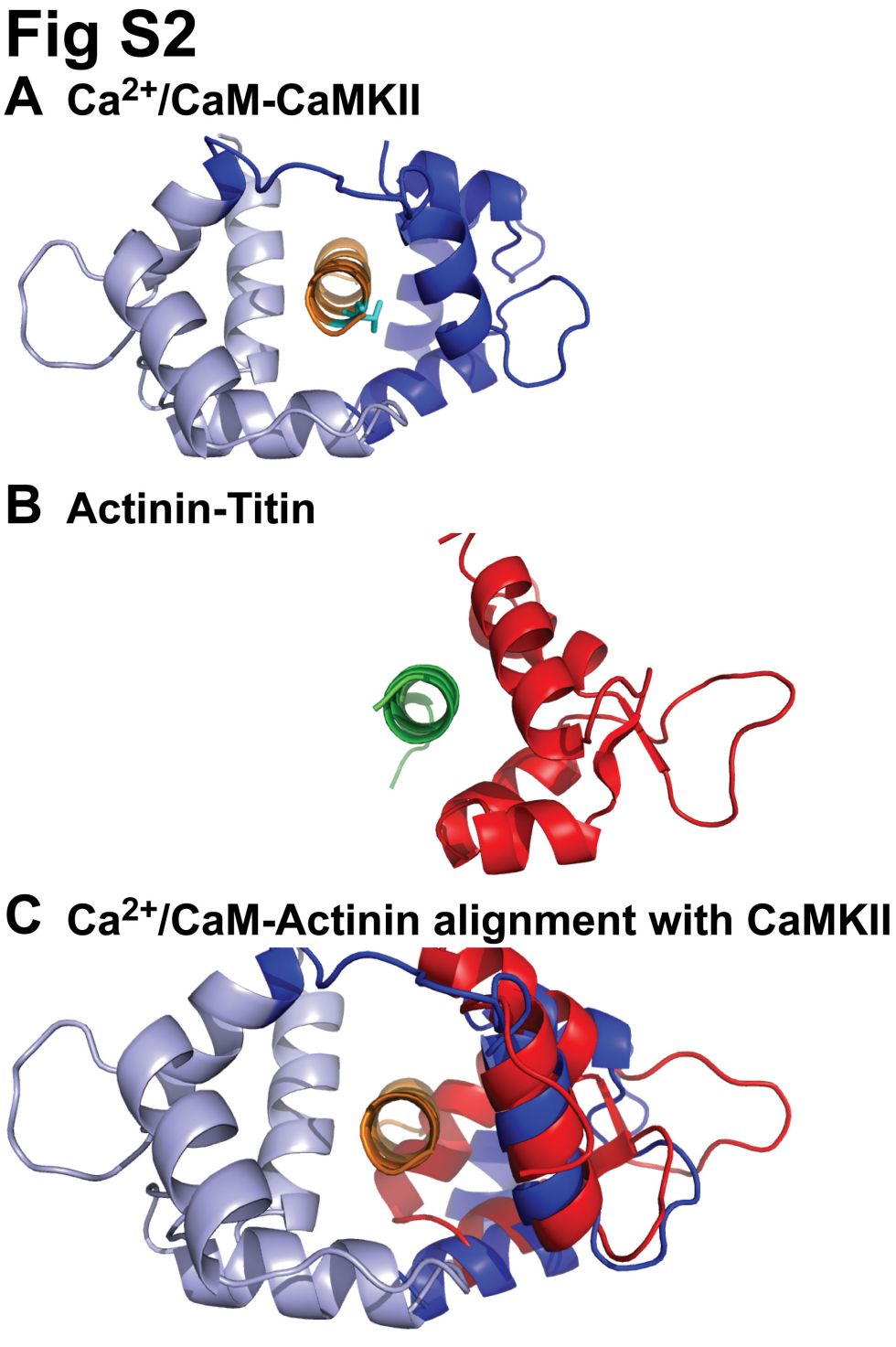

Figure S3: Additional analyses of mutated α-actinin-2 CTD proteins
GST-A2-CTD (WT or mutated) was incubated with His-densin (**A**) or CaMKII (**B**) and then isolated using glutathione-agarose. Complexes were then analyzed by protein staining for GST proteins and immunoblotting for bound His-densin or CaMKII. **A)** Mutations of Ser834, Leu854, Tyr861 or Leu888 to Arg in the CTD have no effect on interactions with densin, even though they disrupt CaMKII binding (see Fig. 2B of main manuscript). **B)** Mutations of CTD residues Leu854 or Tyr861, but not Ser834, to Ala also interfere with CaMKII binding.

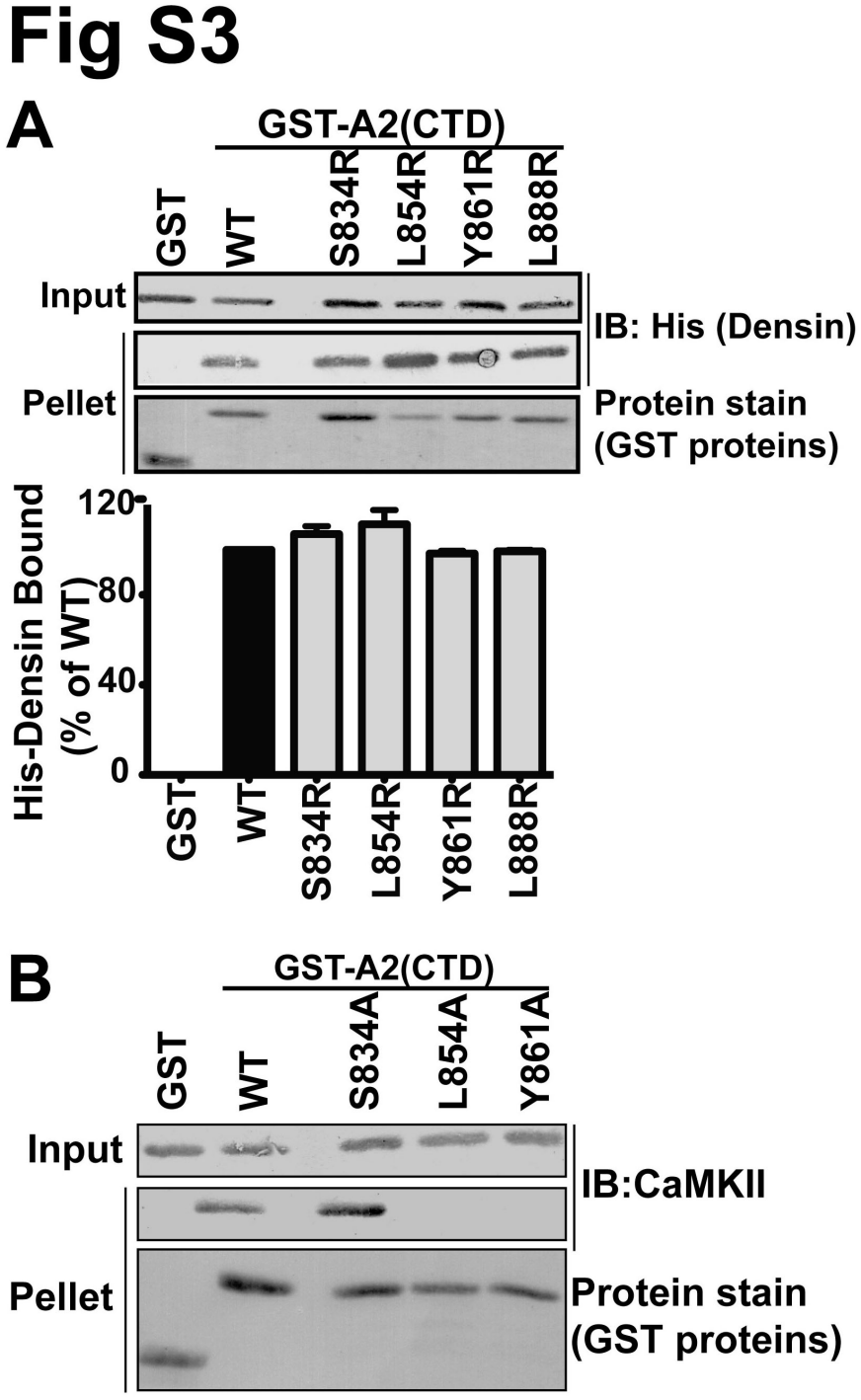

Figure S4: Binding of α-actinin or Ca^{2+}/CaM to CaMKII is modulated by phosphorylation or mutation within the CaMKII regulatory domain.
A) Basal autophosphorylation of WT CaMKII at Thr305/6 in HEK293 cells reduces binding to α-actinin-2 *in situ*. CaMKII was immunoprecipitated from extracts of HEK293 cells expressing WT or TT305/6AA-CaMKIIα with HA-actinin-2. Immune complexes were analyzed by protein staining to detect total CaMKII, and by immunoblotting for HA, phospho-Thr305 (P-T305) and phosphor-Thr286 (P-T286). WT CaMKII is significantly and specifically phosphorylated at Thr305/6. TT305/6AA mutation increases binding of HA-actinin-2 and enhances Thr286 autophosphorylation under basal conditions. **B-D)** Effects of mutations in the regulatory domain on CaMKII binding to GST-A2-CTD *in vitro*. Lysates of HEK293 cells expressing CaMKIIα (WT or with indicated mutations) were incubated with GST or GST-A2-CTD and glutathione-agarose, or with Ca^{2+}/CaM-agarose. Complexes were isolated and analyzed by immunoblotting. (**B**) Specific CaMKII binding to both GST-A2-CTD and Ca^{2+}/CaM is enhanced by TT305/6AA mutation and decreased by TT305/6DD mutation. (**C**) Interactions with GST-A2-CTD and Ca^{2+}/CaM are similarly affected by A302R and I303R mutations on a TT305/6AA background. (**D**) S414D mutation on a TT305/6AA background has little effect on interactions with GST-A2-CTD or Ca^{2+}/CaM, but T310D mutation disrupts binding to Ca^{2+}/CaM, with little effect on binding to GST-A2-CTD.

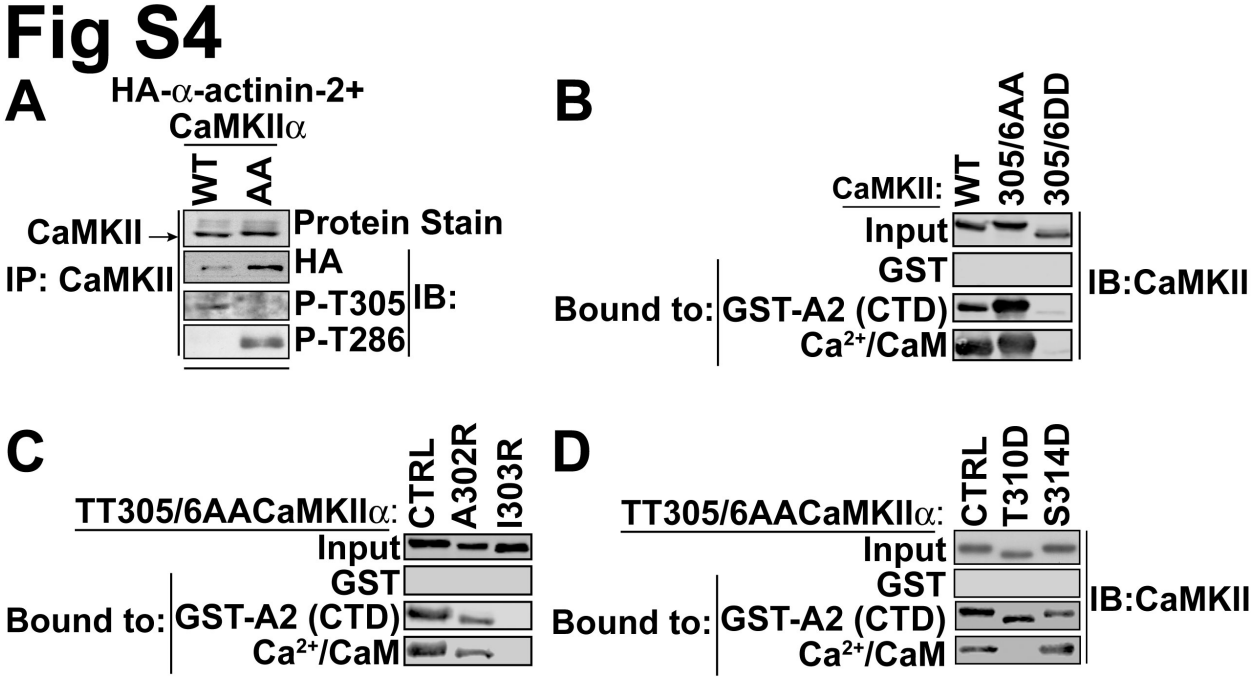

Figure S5: Thr305 and Thr306 in CaMKII are absolutely conserved through evolution

Primary sequence alignment of regulatory domains from human α, β, γ, and δ CaMKII isoforms and from CaMKII in various species. Yellow boxes highlight non-conserved residues. Human CaMKIIα :Q9UQM7, Human CaMKIIβ: Q13554, human CaMKIIδ: Q13557, Human CaMKIIγ: Q13555, macaque CaMKII: F7H4G9, mouse CaMKII: P11798, rat CaMKII: P11275, chicken CaMKII: Q9YHB8, drosophila CaMKII: Q00168, zebrafish CaMKII: Q32PV2, xenopus CaMKII: A4IIE5, C-elegans CaMKII: O62305-4, sea-urchin CaMKII: Q6UVK0, hydra CaMKII: 100200906

Fig S5