

Supplemental Figure 1

(A) FLAG-CaMKK β was transiently over-expressed in HEK 293a cells and labeled for 4 h with 0.77 mCi/ml of ^{32}P -orthophosphate. CaMKK β was then partially purified using anti-FLAG M2-agarose. The sample was split and half was treated with λ -phosphatase for 1 h while the other half was left untreated. After phosphatase treatment, CaMKK β attached to anti-FLAG M2-agarose was washed extensively before CaMKK β was eluted using the FLAG peptide. Samples were run on a SDS-PAGE gel, silver stained, and then visualized on autoradiograph film.

(B) Bands containing CaMKK β were cut from the gel in (A) and levels of ^{32}P incorporation were measured using scintillation counting.

Supplemental Figure 2

Phosphopeptide sequence of human CaMKK β (121-142). Human FLAG-CaMKK β was isolated by immunoprecipitation, digested with trypsin and subjected to targeted MS/MS as described in Experimental Procedures. The parent triply charged ion is marked * (909.05); annotated a, b and y ions of the MS/MS spectrum are shown, the specific ions identifying pSer137, pSer133 and pSer129 are highlighted on the sequence.

Supplemental Figure 3

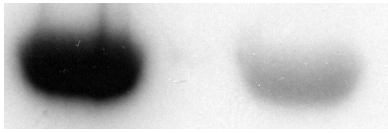
FLAG-CaMKK α and FLAG-CaMKK β were transiently over-expressed in HEK 293a cells. The cells were then treated as indicated with 100mM dibutryl-cAMP for 10 min before being harvested in lysis buffer. FLAG-CaMKK constructs were immunoprecipitated with anti-FLAG M2 agarose and the immunoprecipitates were blotted with a pan 14-3-3 antibody as well as a FLAG antibody as a loading control.

Supplemental Table 1

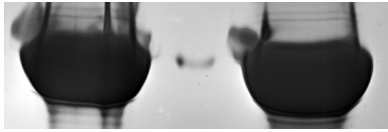
Primers used for site-directed mutagenesis.

Supplemental Figure 1

A

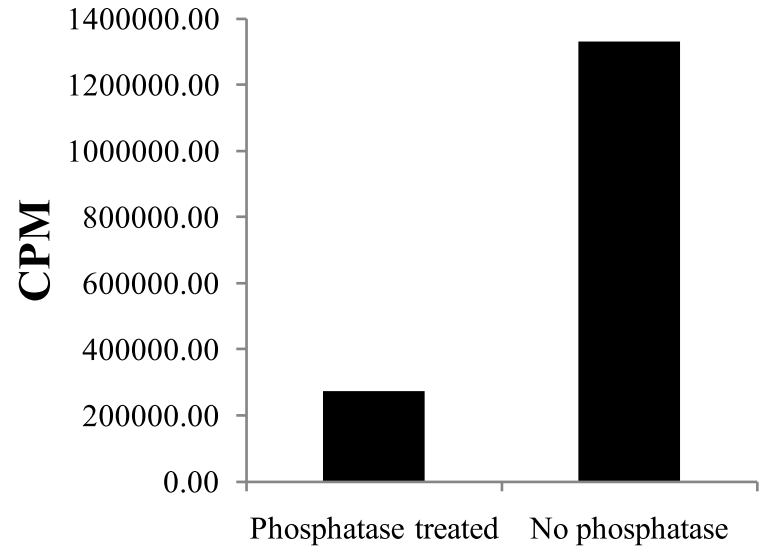


Autoradiograph

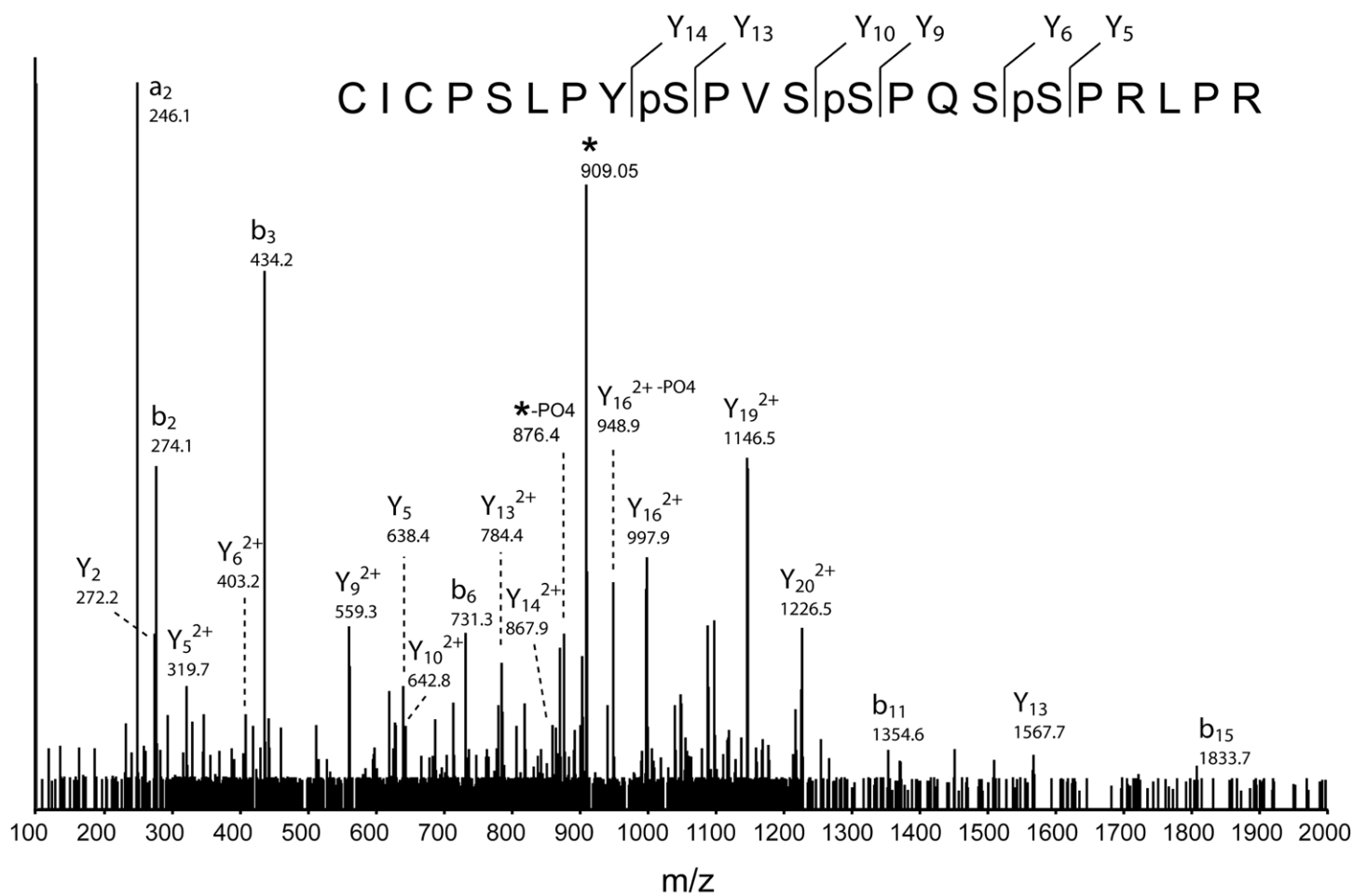


Silver Stain

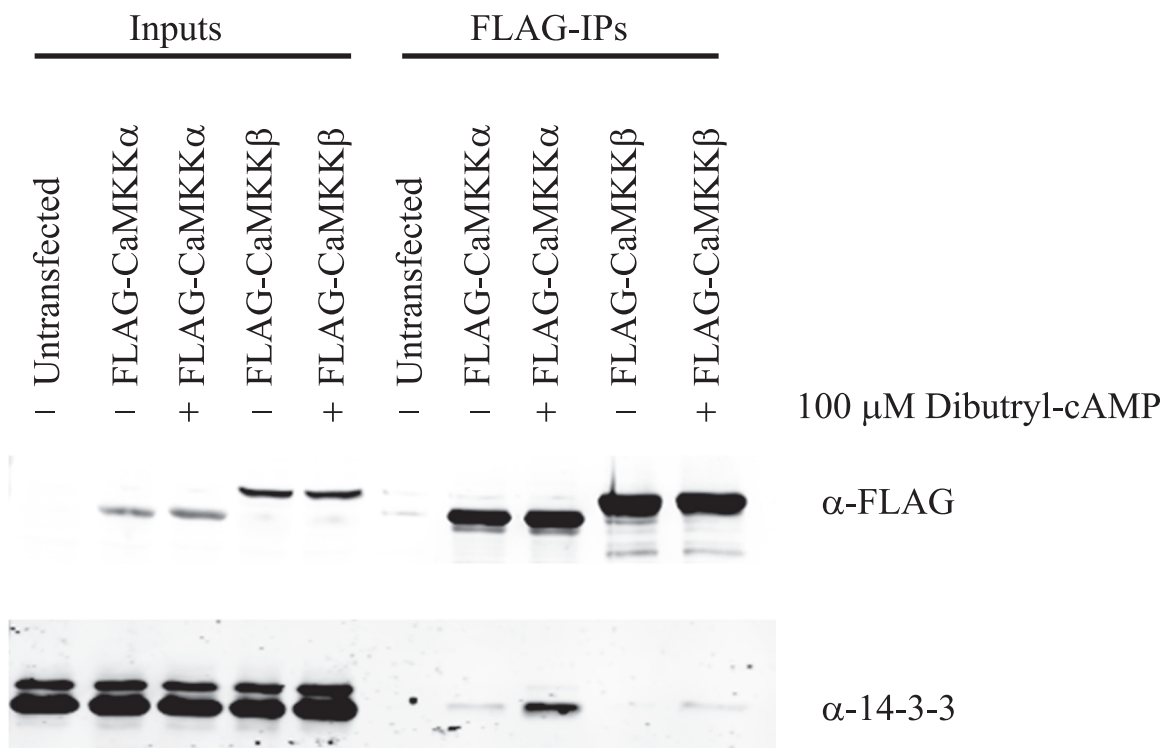
B



Supplemental Figure 2



Supplemental Figure 3



Supplemental Table 1

Mutation	5'-3' Forward Primer	5'-3' Reverse Primer
CaMKK β S129A	CCA TCC CTG TCC TAC GCA CCA GCC AGC TCC CC	GGG GAG CTG GCT GGT GCG TAG GAC AGG GAT GG
CaMKK β S133A	C TAC TCA CCA GCC AGC GCC CCA CAG TCC TCT C	GAG AGG ACT GTG GGG CGC TGG CTG GTG AGTAG
CaMKK β S137A	C AGC TCC CCA CAG TCC GCT CCC CGG ATG CCC CGG	CCG GGG CAT CCG GGG AGC GGA CTG TGG GGAGCTG
CaMKK β S129A, S133A, S137A	CCC TGT CCT ACG CAC CAG CCA GCG CCC CAC AGT CCG CTC CCC GGA TGC CC	GGG CAT CCG GGG AGC GGA CTG TGG GGC GCT GGC TGG TGC GTA GGA CAG GG
CaMKK β S129D, S133D, S137D	CCC TGT CCT ACG ATC CAG CCA GCG ACC CAC AGT CCG ATC CCC GGA TGC CC	GGG CAT CCG GGG ATC GGA CTG TGG GTC GCT GGC TGG ATC GTA GGA CAG GG
CaMKK β D311A	GAA GAT CAT TCA CCG GGC CAT CAA ACC CTC CAAC	GTT GGA GGG TTT GAT GGC CCG GTG AAT GAT CTT C
AMPK α 1 D139A	GGT GGT CCA CAG AGC TTT GAA ACC TGAAA CG	CGT TTT CAG GTT TCA AAG CTC TGT GGA CCA CC