Supplemental Figure 1. Validation of specificity of the affinity-purified rabbit polyclonal D52 pS^{136} antibody. HEK 293 cells were transiently transfected with pcDNA 3.1 plasmids containing cDNA inserts encoding for HA-tagged D52 using Effectene transfection reagent. Three days later, cells were exposed to indicated agents, fixed and analyzed by Western blotting as described in Methods.

A. Western blot of lysates from HEK 293 cells expressing HA-D52. Cb, 100 μM carbachol, 2 min; Ion, 3 μM ionomycin, 5 min.

B. Same samples on a replicate Western blot probed with affinity-purified D52 phosphoprotein antibody (pS^{136}) in the presence of either phospho- or non-phosphopeptide (0.2 μg peptide/μg antibody).

Supplemental Figure 2. Differences in patterns of D52 phosphorylation by recombinant CK2 and CAMK2.

A. Kinetics of ^{32}P incorporation from [$\gamma^{32}P$]-ATP into recombinant his-tagged D52 protein by CK2 and CAMK2. CK2 catalyzed linear ^{32}P incorporation into both D52 (CK2) and mutated D52 (S→A mutation at S^{136}; CAMK2/A^{136}). In contrast, CAMK2 catalyzed non-linear ^{32}P incorporation into D52 (CAMK2) and slow, linear incorporation into the mutated protein (CAMK2/A^{136}). Assays were performed and analyzed as described in Methods. Similar results were obtained with two different lots of CAMK2 and CK2. After two hours of incubation, CAMK2 and CK2 catalyzed the incorporation of 0.038 and 0.15 moles of ^{32}P/mole D52 and 0.043 moles and 0.07 moles of ^{32}P/mole of mutated D52, respectively. These later findings are in general agreement with those of Kaspar et al. (27) who assayed CK2 and CAMK2 activities after 2 hours of incubation. Quantitative differences between the two studies likely reflect different assay conditions and enzyme specific activities.

B. CAMK2, but not CK2, catalyzes S^{136} phosphorylation. Assays were performed as in *A* except that 10 mM cold ATP replaced [γ^{32}P]-ATP and reactions were stopped by the addition of 10X SDS stop solution. Similar specific activities of the two enzymes were used. S^{136} phosphorylation was detected by Western blotting with ECL detection using pS^{136} antibody (1:2,500 dilution) in conjunction with HRP-tagged donkey anti-rabbit secondary antibody (1:5,000) and quantified as described in Methods. No signal was obtained with mutated D52 (CAMK2/A^{136}). Inset shows Western blot of data depicted in the graph.