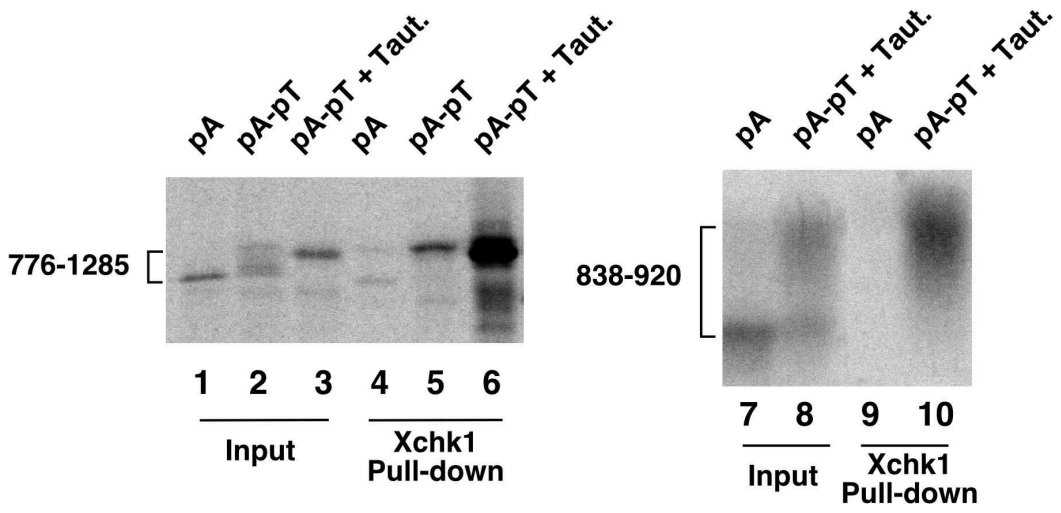

Figure S1 **Assay for identification of the CKBD.** Representative ^{35}S-labeled fragments of Claspin (amino acids 776-1285, left; and amino acids 838-920, right) were incubated in *Xenopus* egg extracts containing pA (lanes 1, 4, 7, 9), pA-pT (lanes 2, 5), or both pA-pT and tautomycin (lanes 3, 6, 8, 10) in the presence of Xchk1-GST-His6 bound to nickel agarose beads. After 100 min, aliquots of the extracts were subjected to SDS-PAGE (lanes 1-3, 7, 8). The beads were isolated, washed, and subjected to SDS-PAGE and autoradiography to assess the binding of truncated Claspin proteins to Xchk1 (lanes 4-6, 9, 10).

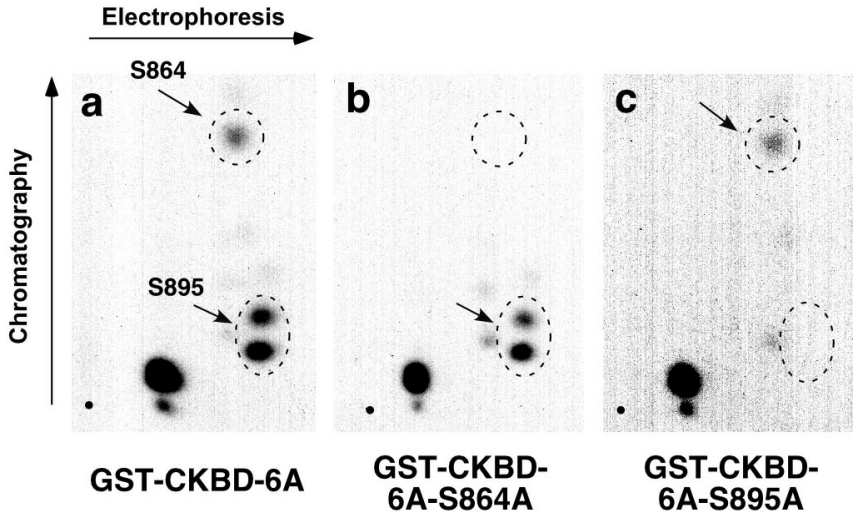

Figure S2 **Ser-864 and Ser-895 in the CKBD are phosphorylated in egg extracts.** GST-CKBD-6A **(a)**, GST-CKBD-6A-S864A **(b)**, and GST-CKBD-6A-S895A **(c)** were incubated in egg extracts containing pA-pT, tautomycin, and ^{32}P orthophosphate. The various GST-CKBD polypeptides were isolated with glutathione beads and subjected to SDS-PAGE and autoradiography. The ^{32}P-labeled GST-CKBD polypeptides were digested with trypsin and endoproteinase Glu-C. The resulting peptides were separated by two-dimensional electrophoresis and chromatography. The dots in each left lower corner indicate the origins where peptides were loaded before separation. In the case of the S864A mutant, a spot was missing in the upper right hand corner of the phosphopeptide map. Similarly, for the S895A mutant, two closely spaced spots were absent from the lower right hand region of the map. The two spots are most probably due to incomplete digestion with endoproteinase Glu-C, rather than incomplete alkylation, since digestion with trypsin alone yielded only one ^{32}P-labeled spot corresponding to the Ser-895-containing peptide.

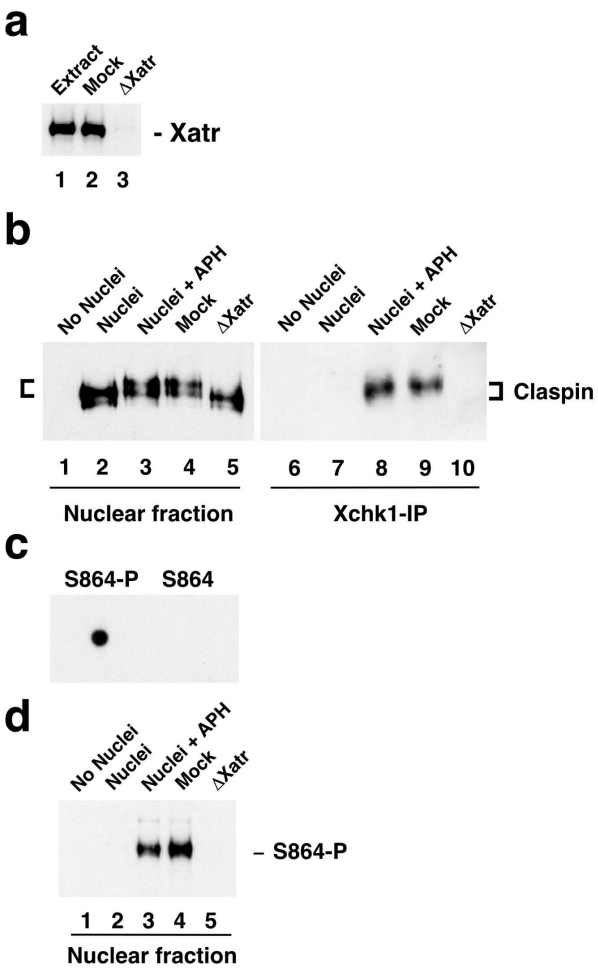

Figure S3 **Xatr regulates phosphorylation of Claspin and binding of Claspin to Xchk1 in egg extracts. a,** Immunodepletion of Xatr. Extracts (lane 1) were treated with either control IgG (lane 2) or anti-Xatr antibodies (lane 3). The samples were analyzed by SDS-PAGE and immunoblotting with anti-Xatr antibodies. **b,** Untreated extracts (lanes 1-3, 6-8) and extracts treated with control IgG (lanes 4, 9) or anti-Xatr antibodies (lanes 5, 10) were incubated at room temperature for 100 min in the presence of no sperm nuclei (lanes 1, 6), 3000 sperm nuclei μl^{-1} (lanes 2, 7), or both 3000 sperm nuclei μl^{-1} and 100 $\mu g\ ml^{-1}$ aphidicolin (lanes 3-5, 8-10). Nuclear fractions were collected from aliquots of the extracts by centrifugation through a 1 M sucrose cushion (lanes 1-5). Other aliquots of the extracts were immunoprecipitated with anti-Xchk1 antibodies (lanes 6-10). All samples were subjected to SDS-PAGE and immunoblotted with anti-Claspin antibodies. **c,** Specificity of antibodies against phosphorylated Ser-864 of Claspin. One hundred nanograms each of phosphorylated (S864-P) and unphosphorylated (S864) forms of the peptide ELLDLCSGQFK were spotted onto a PVDF membrane and immunoblotted with the purified anti-phosphopeptide antibodies. **d,** Ser-864 of Claspin is not phosphorylated in the absence of Xatr. Untreated extracts (lanes 1-3) and extracts treated with control IgG (lane 4) or anti-Xatr antibodies (lane 5) were incubated at room temperature for 100 min in the presence of no sperm nuclei (lane 1), 3000 sperm nuclei μl^{-1} (lane 2), or both 3000 sperm nuclei μl^{-1} and 100 $\mu g\ ml^{-1}$ aphidicolin (lanes 3-5). Nuclear fractions were collected from the extracts, subjected to SDS-PAGE, and immunoblotted with anti-phosphopeptide antibodies that detect Ser-864 of Claspin. The amount of Claspin in samples 2-5 was similar, as determined by immunoblotting with anti-Claspin antibodies (not shown).