Fig. S1

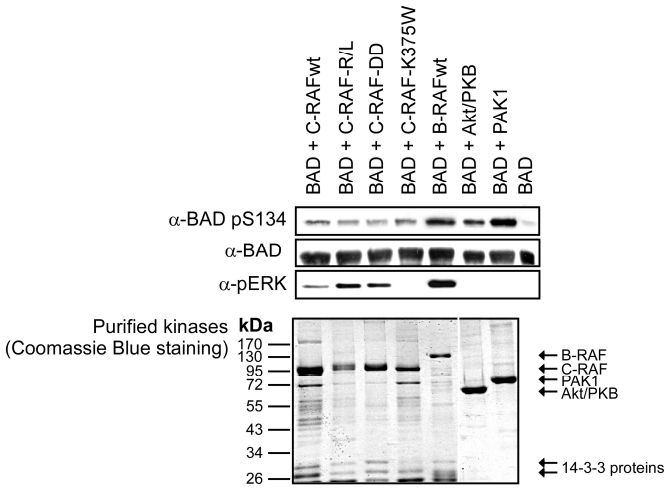

Fig. S1. *In vitro* phosphorylation of recombinant BAD by purified PAK1, Akt/PKB, and RAF kinases. Purified GST-BAD (20 pmol) was incubated in the presence of RAF kinases, constitutively active PAK1 (T423E) and Akt/PKB (T308D/S473D) (2 pmol of each). The highly active C-RAF-R/L was co-expressed with Ras12V (R) and Lck (L). C-RAF-DD represents the active C-RAF mutant C-RAF-Y340D/Y341D and C-RAF (K375W) is a kinase inactive form. Following SDS-PAGE and immunoblotting, BAD phosphorylation was visualized by a phosphospecific antibody directed against BAD phosphoserine 134. The activity of RAF kinases was analyzed by an antibody directed against phosphorylated ERK. The kinases were expressed in Sf9 insect cells and purified either by glutathione-Sepharose (GST-tagged RAF kinases) or nickel chelate affinity chromatography (PAK1 and Akt/PKB) as described in Experimental Procedures. The purified proteins were visualized by Coomassie blue staining (below). The RAF kinases shown are constitutively associated with 14-3-3 proteins (see arrows at approx. 30 kDa). The other co-purified proteins were recently identified as EF1, Cdc37, HSP40, HSP70 and HSP90 (not indicated in this illustration) (28).