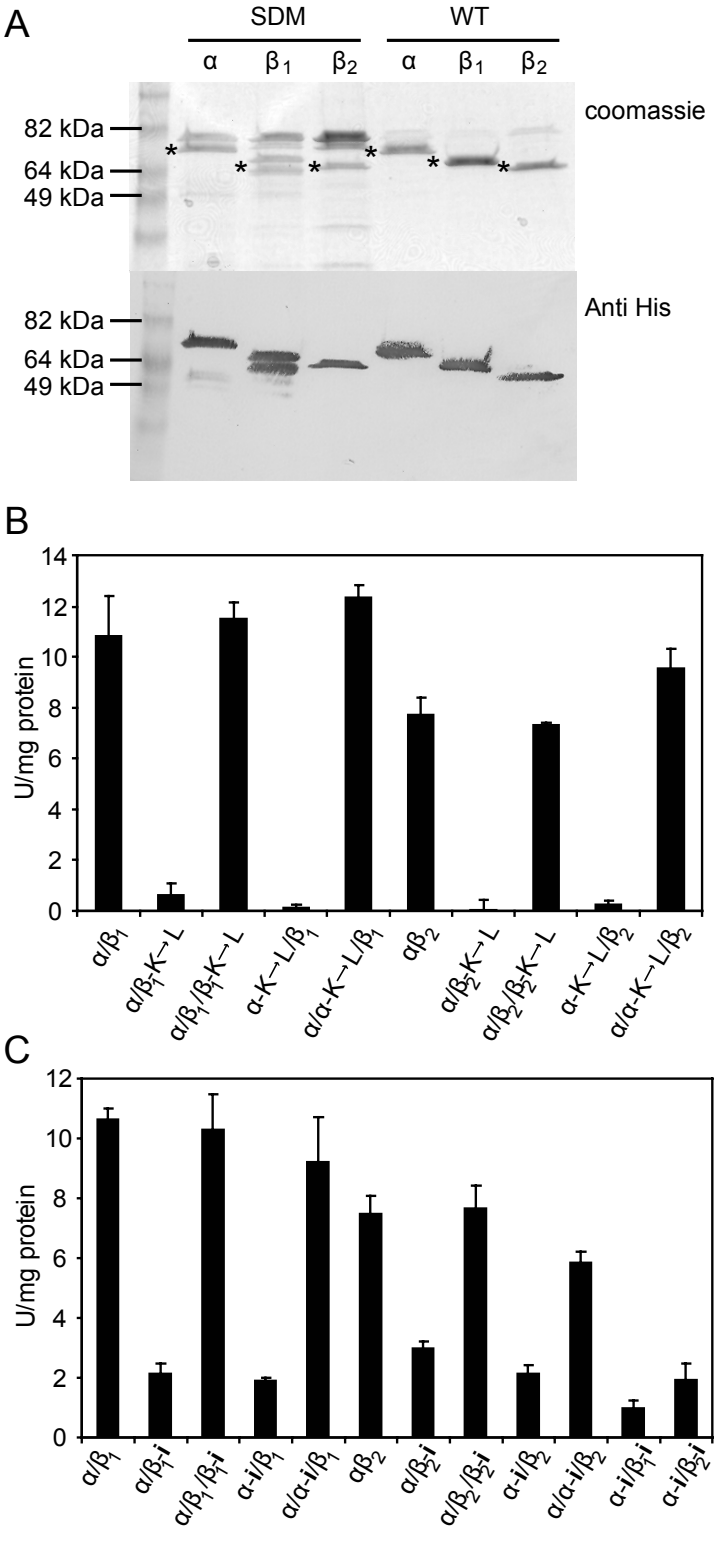

Supplemental Figure 3. PK subunit inactivation by site-directed mutagenesis and chemical modification.

(A) SDS-PAGE and anti-His immunoblot of affinity purified site-directed mutant (SDM) and wild-type (WT) PK_p subunits. SDM proteins were generated as described in materials and methods. Approximately 15 pmol of each subunit was loaded per well. Conditions for protein purification were the same for all six proteins, but contaminating protein bands can be seen in the mutant protein lanes. The anti-His immunblot reveals which bands are the actual his-tagged PK_p subunits (marked with * on the SDS-PAGE gel).
(B) PK activity assays with wild-type and site-directed mutant (denoted with K→L) proteins. Assays were conducted with 2 pmol per subunit at pH 8.0 with saturating substrates. PK activity is only observed when both a wild-type α and a wild-type β subunit are present (α/β_1, α/β_2). No activity is observed when only one subunit has a wild-type PK active site (α/β_1K→L, αK→L /β_1, α/β_2K→L, αK→L/β_2). The inclusion of a mutant protein extract does not inhibit the activity of otherwise wild-type complexes, indicating the contaminating bands seen in the SDM lanes of the SDS-PAGE gel of panel A do not interfere with PK activity ($\alpha/\ \beta_1/\beta_1$K→L, α/αK→L /β_1, $\alpha/\beta_2/\beta_2$K→L, α/αK→L/β_2). Thus, the loss of PK activity was due only to modification of the active site of either subunit of the PK_p complex, and both α and β_1 or β_2 subunits are required for PK activity.
(C) PK activity assays with wild-type and chemically inactivated (denoted with **i**) proteins. Assays were conducted with 2 pmol per subunit at pH 8.0 with saturating substrates. TNBS treatment conditions are described in the materials and methods section. The PK subunits were not completely inactivated as determined by mixing TNBS treated α and β subunits (α-i/β_1-i, α-i/β_2-i). Nonetheless, maximum PK activity was only observed with mixtures of mock-treated subunits (α/β_1, α/β_2). Activity was reduced 60-80% when one subunit was chemically inactivated (α/β_1-i, α-i/β_1, α/β_2-i, α-i/β_2). Addition of a TNBS-treated subunit to an active mixture did not affect PK activity, meaning carryover of a component of the TNBS treatment was not responsible for reductions in PK activity ($\alpha/\beta_1/\beta_1$-i, α/α-i/β_1, $\alpha/\beta_2/\beta_2$-i, α/α-i/β_2). Therefore, the observed reductions in PK activity when using one TNBS-treated subunit in combination with a mock-treated one are due only to modification of lysine residues on the protein. In accordance with the site-directed mutant studies, two wild-type subunits are required to make an active PK_p complex.