SUPPLEMENTAL FIGURES AND LEGENDS

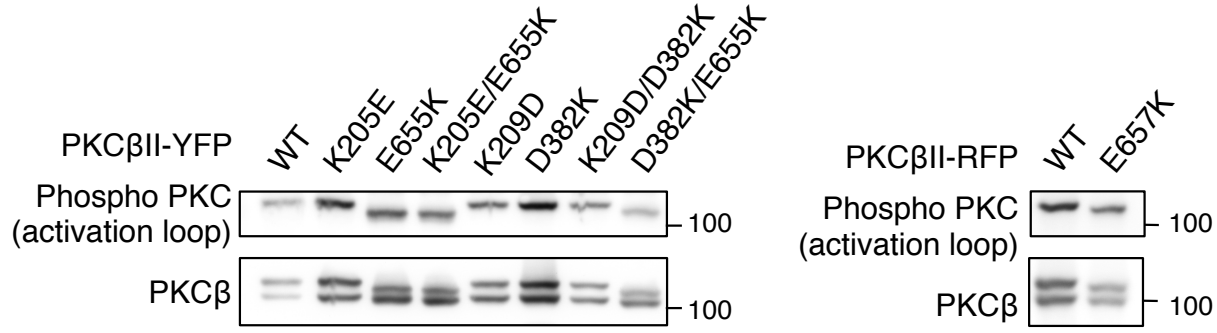

Figure S1. C2 and Kinase Domain Mutants Are Processed by Phosphorylation, Related to Figure 2

Immunoblot showing the phosphorylation state of the indicated YFP-tagged PKCβII proteins. The upper band in the PKCβ blot represents phosphorylated PKCβII, while the lower band represents unphosphorylated PKCβII. The band shift in the Glu/Lys mutants is induced by mutation of the Glu, and not by a difference in phosphorylation.

Figure S2. Modeling of PKCβII pseudosubstrate, Related to Figure 1

Sequence alignment between the PKCβII pseudosubstrate (aa.16-26) and PKIα (aa. 11-22).

SUPPLEMENTAL EXPERIMENTAL PROCEDURES

Structure Modeling and Molecular Docking

As the N-terminal pseudosubstrate region of PKCβII has a high level of sequence similarity with the cAMP-dependent protein kinase (PKA) inhibitor (PKI) (Figure S2), we used the structure of PKA:PKI complex (PDBID:1ATP) to model the eleven residues of PKCβII (aa. 16-26). We then used the C2 domain of PKCβII (PDBID:3PFQ aa. 161-292) for docking to the complex between the PKCβII catalytic core (PDBID:3PFQ aa.339-669) and the pseudosubstrate using ZDOCK server (v. 3.0.2) (Pierce et al., 2014), with no restrictions on the docking interface. Forty two complexes out of fifty contained the C2 domain positioned close to the active site of PKCβII between helices αG and αC similar to the position of the C2 domain from the symmetry mate of 3PFQ structure. Comparing the predicted docking complexes to the symmetry mate C2 domain, we selected the closest prediction model with RMSD from the position of the C2 domain in the 3PFQ structure 13Å (1095 atoms). The C1A domain or rat PKCβII was modeled based on the solution structure of the human PKCγ C1A domain (PDBID:2E73) using the PHYRE2 server (Kelley and Sternberg, 2009).

SUPPLEMENTAL REFERENCES

Kelley, L.A., and Sternberg, M.J. (2009). Protein structure prediction on the Web: a case study using the Phyre server. Nature protocols *4*, 363-371.

Pierce, B.G., Wiehe, K., Hwang, H., Kim, B.H., Vreven, T., and Weng, Z. (2014). ZDOCK server: interactive docking prediction of protein-protein complexes and symmetric multimers. Bioinformatics *30*, 1771-1773.