Figure S3. Specificity of inhibition by PKCζinh.

(A) Specificity of inhibition of atypical PKCs versus conventional and novel PKCs by PKCζinh.
Cells treated with PKCζinh or with an equivalent amount of a scrambled peptide control, were probed with an antibody that specifically recognizes the autophosphorylated form of atypical PKCs (Thr660 on PKCζ, Thr655 on PKCα) (top). In parallel, treated cells were incubated with phospho-PKCζ (pan), an antibody that specifically recognizes the autophosphorylated form of conventional and novel PKCs (Ser660 on the hydrophobic site) (bottom). When at least 10 μM PKCζinh were used, a clear decrease in the intensity of the band corresponding to the atypical PKCs is observed while no difference is observed in the bands corresponding to the conventional and novel PKCs, showing that PKCζinh specifically inhibits atypical PKCs and has no effect on other PKCs.

(B) Specificity of inhibition between atypical PKCs (PKCζ versus PKCα) by PKCζinh.
To distinguish between PKCζ and PKCα, Huh7 cells were transfected with plasmids expressing either a tagged version of PKCζ or a tagged version of PKCα. The tag in either construct enables the overexpressed protein to be distinguished from the endogenous ones on SDS-PAGE, and the degree of autophosphorylation of each of the atypical PKCs to be analyzed independently. The expression level of PKCζ is approximately 2-fold higher than that of PKCα, as shown by quantification of signal intensity of the two proteins following propping with an antibody that recognizes both isoforms (left). Inhibition on those cells was only observed when 60 μM PKCζinh was used. Inhibition levels of PKCζ (middle) and PKCα (right) were assessed by probing with an antibody that specifically recognizes the autophosphorylated form of atypical PKCs. The relative specificities of PKCζinh for the two isoforms were determined by calculating the percentage of inhibition of autophosphorylation of each of them and correcting for the difference in their expression levels. Our results show that the inhibitory effect on PKCζ is approximately 2-fold that observed for PKCα.