



**FIGURE S1. MRM assay for PKC $\delta$  phosphorylation at Thr<sup>295</sup> and Ser<sup>359</sup> (a novel phosphorylation site in the Gly-rich ATP binding loop). **A and B:** Total Ion Chromatographs (XIC) are shown for 4 peptides, phosphorylated Thr<sup>295</sup> (LLAEALNQVT(phospho)QR), phosphorylated Ser<sup>359</sup> (VLGKGS(phospho)FGK) and their counterparts LLAELNQVTQR and VLGKGSFGK in WT-PKC $\delta$  and PKC $\delta$ - $\Delta$ C2, respectively. It is worth noting that VLGKGSFGK (unphosphorylated Ser<sup>359</sup> peptide) ionizes poorly in the MS instrument. While it is detected (elution time ~16 mins), levels are below the lower limit of quantification. **C and D:** MRM spectrum of the phosphorylated peptide LLAELNQVT(phospho)QR in WT-PKC $\delta$  (**C**) and PKC $\delta$ - $\Delta$ C2 (**D**). **E and F:** MRM spectrum of the phosphorylated peptide VLGKGS(phospho)FGK in WT-PKC $\delta$  (**E**). This peptide is undetectable in PKC $\delta$ - $\Delta$ C2 (**F**) zoomed chromatographs are depicted in inserts.**