



**Supporting Information Figure S19.** MS/MS spectrum of MBP-CDC25B3 trypsin peptide LKpTRpSWAGER, M (mono) = 1362.59, m/z = 682.29, z=2, phosphorylated in vitro by CHK1, showing phosphorylation of threonine 561 and serine 563.

#: loss of H<sub>3</sub>PO<sub>4</sub>; \*: loss of H<sub>2</sub>O; §: loss of NH<sub>3</sub>; !: loss of SOCH<sub>4</sub>; Mox: oxidized methionine; Cpr: cysteine, propionamide adduct; Cam: carbamidomethyl adduct. For the sake of clarity and unless otherwise required, only H<sub>2</sub>O and H<sub>3</sub>PO<sub>4</sub> losses are indicated in the sequence annotation.