



## **Supplementary Information, Figure S6. Analysis of intramolecular interaction of Plk3 subdomains.**

(A) Schematic representation of full-length Plk3 (Plk3-WT), myc-tagged kinase domain (myc-KD) and V5-tagged Polo-box domain of Plk3 (V5-PBD) used in co-immunoprecipitation experiments (B and C). (B) HEK 293T cells were co-transfected with myc-Plk3-KD, V5-Plk3-PBD and increasing amounts of Flag-procaspase-8 for 24 h. The cell lysates were immunoblotted for V5, myc and Flag (upper left panel). Myc-KD or V5-PBD were immunoprecipitated from cell lysates with anti-V5 or anti-myc antibodies. Precipitates were immunoblotted for V5 and myc (upper right panel). The binding abilities of V5-PBD to myc-KD and vice versa in the presence of increased amounts of Flag-procaspase-8 were quantified by ImageJ (lower panels). (C) HeLa cells were co-transfected with myc-KD and V5-PBD for 24 h and subsequently treated with CD95L and CHX for different time periods. The cell lysates were immunoblotted for caspase-8, myc and V5 (upper left panel). Myc-KD or V5-PBD were immunoprecipitated from cell lysates with anti-V5 or anti-myc antibodies. Precipitates were immunoblotted for V5, myc and pT273 caspase-8 (upper right panels). Caspase-8 and -3/7 activities were determined using a Caspase-Glo 8 and 3/7 assays (lower left panels). The binding abilities of V5-PBD to myc-KD and pT273 procaspase-8 to V5-PBD were quantified by ImageJ (lower right panels). (D) Proposed model for the activation of Plk3 and its regulation by DISC formation. CD95 stimulation by its cognate ligand leads to the recruitment of FADD and procaspase-8 to the CD95 and the DISC formation. The interaction of FADD and caspase-8 with the PBD of Plk3 facilitates opening the conformation of Plk3 inducing its enzymatic activation.