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Supplementary Figures

PLK1 is a binding partner and a negative regulator of FOXO3 tumor suppressor

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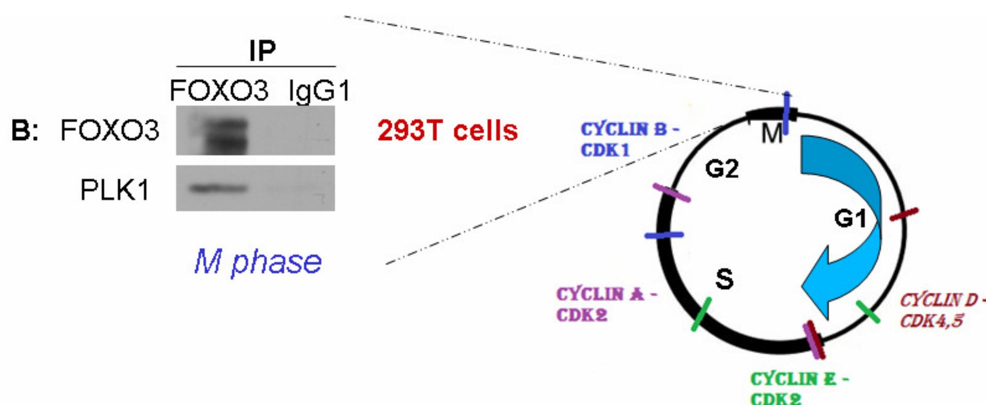
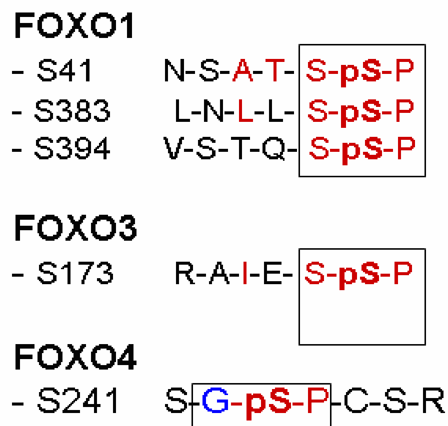
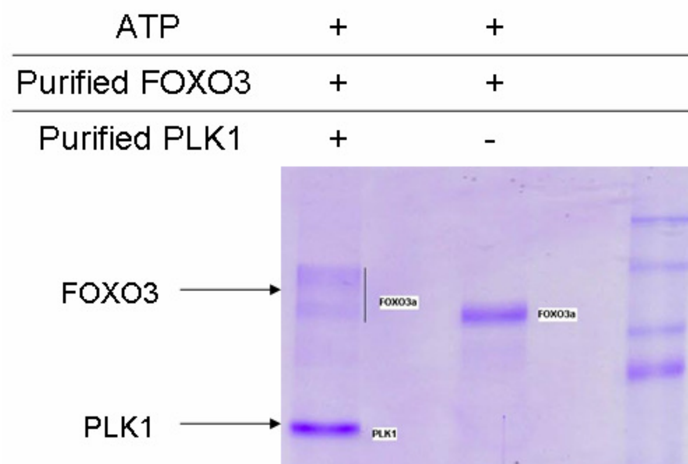


Supplementary Figure 1. Screening validation: endogenous FOXO3 – endogenous PLK1 interaction in 293T cells (in Support of Figure 2)
Synchronized 293T proliferating cells in M phase of the cell cycle were used to evaluate the endogenous-endogenous interaction between FOXO3 and PLK1. Cells were synchronized with Nocodazole (see Materials and Methods). An anti-FOXO3 antibody was used to immunoprecipitated FOXO3 and FOXO3-bound proteins. Endogenous PLK1 co-immunoprecipitate with endogenous FOXO3.



Supplementary Figure 2. This SSP region (the core consensus sequence for PLK1 binding) is found in FOXO1 and FOXO3, but not in FOXO4 (is not conserved between all FOXO family members) **(in support of Figure 3)**



Supplementary Figure 3. FOXO is *in vitro* phosphorylated by PLK1 at multiple Ser/Thr sites. Purified full length FOXO3 (Abnova) was incubated for 20 minutes in a Kinase Buffer solution (Cell Signaling, #9802) containing ATP (Cell Signaling, #9804) with or without purified PLK1 kinase (Cell Signaling, #7728), according to the manufacturer's recommendations. Separation of proteins was performed by SDS-PAGE and the gel was subsequently silver stained. FOXO3 bands were excised and analyzed by Mass Spectrometry for the identification of the phosphorylation sites. Mass Spectrometry analysis confirmed that FOXO3 is phosphorylated at multiple phosphorylation sites in the presence of PLK1, in vitro (data not shown).