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Supplemental Results

To determine whether Chk1ΔκD reliably mimics the endogenous Chk1, we compared the serine 344 phosphorylation status of endogenous Chk1 to Chk1ΔkD after checkpoint activation by AT70. Chk1 protein is phosphorylated by ATR on serine 344 (Guo et al., 2000), and antibodies are available that specifically recognize the serine 344–phosphorylated and, hence, activated form of Chk1. As shown in Fig. S1, both endogenous Chk1 (top) and Chk1ΔkD (bottom) were phosphorylated on serine 344 in an AT70-dependent manner (compare lanes 3 and 4). Importantly, the ratio of serine 344–phosphorylated Chk1ΔkD to total Chk1ΔkD (bottom, lanes 4 and 2) was the same in AT70-containing extracts as the corresponding ratio for endogenous Chk1 (top, lanes 4 and 2). This demonstrates that Chk1ΔkD is phosphorylated to the same extent as endogenous Chk1 in checkpoint-activated extracts and, therefore, that Chk1ΔkD is a reliable surrogate for the endogenous protein.