Figure 1
Supplement

A

Western

α−Myc

MycTlk1kd

GSTChk1

IP: α−Myc

MycTlk1

MycTlk1 / DM

MycTlk1 / GSTChk1 and UCN-0

Western

Asf1

[32P]

Specific activity

CB-stained

MycTlk1

[32P]

100%, 78%, 99%

B
Legends supplementary figure 1

Prephosphorylation of Tlk1 by Chk1 reduces Tlk1 activity against Asf1a *in vitro*.  

A) Recombinant GST-Chk1 was incubated alone or mixed with MycTlk1 kd in a kinase reaction. MycTlk1 kd was then immunoprecipitated from the reaction as indicated. The position of MycTlk1 kd was determined by immunoblotting (left panel).  

B) Immobilized recombinant kinase active MycTlk1 was allowed to autophosphorylate in the presence (lane 2 and 3) or absence (lane 1) of GST-Chk1, and non-labeled ATP. UCN-01 was added (200 nM) to one reaction (lane 3). Following extensive washing, to remove GST-Chk1, a second kinase reaction was performed using GST-Asf1a as substrate in the presence of \([\gamma^{–}\text{P}]\text{ATP}\). UCN-01 was included in all reactions at this stage to inhibit any remaining GST-Chk1 activity. After SDS-PAGE the lower part of the gel was stained with CB, dried and exposed for \([^{32}\text{P}]\) incorporation into GST-Asf1a, while the upper part of the gel was processed for immunoblotting of MycTlk1 (9E10) and exposed for \([^{32}\text{P}]\) labeled MycTlk. The specific activity of MycTlk1 was determined as the ratio between \([^{32}\text{P}]\) incorporation into GST-Asf1a and the amount of MycTlk1 protein in each reaction.