



Supplementary Information, Figure S4. *In vitro* validation of the new phospho-caspase-8 antibody (pT273).

(A) Increasing amounts of purified, recombinant GST-tagged procaspase-8 or p18 were incubated with recombinant Plk3 in a kinase assay. Samples were immunoblotted against the novel affinity-purified phospho-antibody targeting the caspase-8-peptide containing phospho-T273 (C-GALTT(pT)FEELHFE-amide), GST and Plk3. (B) Recombinant GST-tagged procaspase-8 and p18 (WT) and a mutated form (T273A) were incubated with or without active Plk3 and immunoblotted against phospho-T273 caspase-8 (pT273 Casp8), Casp8 and Plk3. (C) Recombinant GST-tagged procaspase-8 and p18 were incubated with or without active Plk3 in the presence of BI2536 (0-1000 nM). Samples were immunoblotted against pT273 Casp8, Casp8 and Plk3. (D) Recombinant GST-tagged procaspase-8 and p18 (WT) and a mutated form (T273A) were incubated with active Plk3 followed by heat inactivation of Plk3. Samples were incubated with or without 800 U λ -phosphatase for 30 min at 30°C and immunoblotted against pT273 Casp8 and Casp8. (E) Increasing amounts of recombinant GST-tagged procaspase-8 or p18 were incubated with active Plk1. Samples were immunoblotted against pT273 Casp8, Plk1 and GST. (F) Recombinant GST-tagged procaspase-8, p18 and p10 fragments were incubated with active Plk3 or active Cdk1 and immunoblotted against pT273 Casp8, pS387 Casp8 and GST.