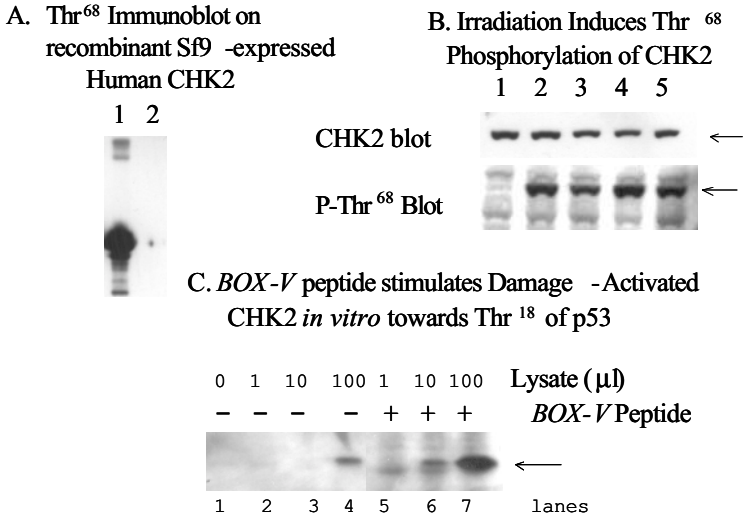
**Supplemental Figure 1. Activity of CHK2 expressed in human cells.** *(A) Recombinant CHK2 is phosphorylated at the activating site of Thr$^{68}$.* Recombinant CHK2 purified from insect cells (lane 1) or control with no CHK2 (lane 2) was immunobloted with the Thr$^{68}$ phospho-specific antibody to demonstrate that the protein is highly modified at this site. *(B) CHK2 is phosphorylated at Thr$^{68}$ in irradiated cells.* A375 cells were left untreated (lane 1) or irradiated with X-rays (5 Gy, lanes 2 and 3 or 10 Gy, lane 4 and 5). Cells were harvested 1 hour (lanes 2 and 3) or 3 hours (lanes 4 and 5) after irradiation. Lysates were immunoblotted with antibodies to CHK2 (top panel) or to Thr$^{68}$ modified CHK2 (bottom panel). *(C) Thr$^{68}$-activated CHK2 responds in vitro to the BOX-V peptide.* An increasing volume of lysate from irradiated cells (from 1 to 100 μl) containing Thr$^{68}$ modified CHK2 was immunoprecipitated with an anti-CHK2 antibody. After CHK2 capture, p53$^{N1\text{-}66}$ was added in kinase buffer and the incubations were continued at 30$^{o}$C for 20 minutes without the *BOX-V* peptide (lanes 2-4) or with the *BOX-V* peptide (lanes 5-7). The reaction products were immunoblotted with the Thr$^{18}$-phospho-specific antibody (arrow) since this is the predominant peptide-activated CHK2 phosphorylation site on p53.

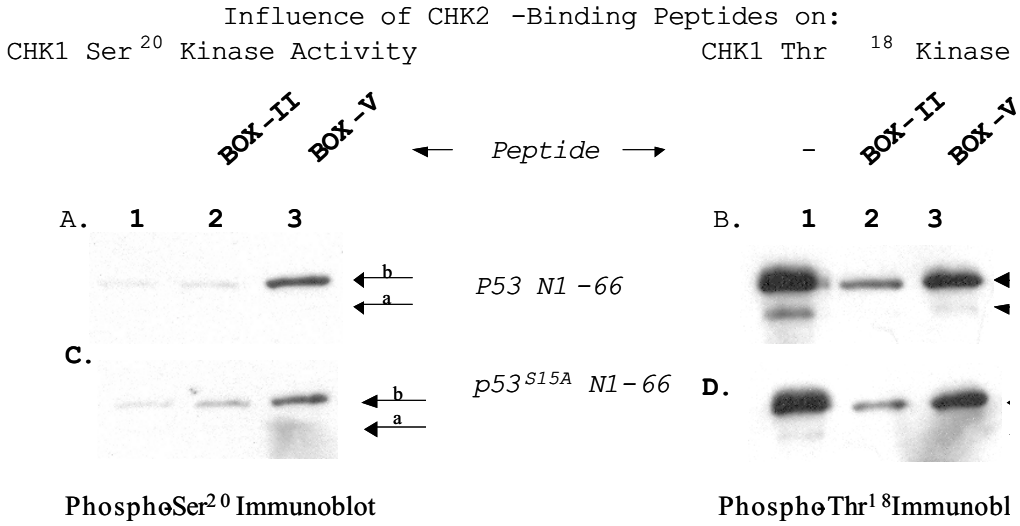
**Supplemental Figure 2. CHK1 is regulated by p53 DNA-binding domain peptides**. The CHK2 homologue, CHK1, was evaluated in the p53 kinase assay to determine whether the docking mechanism extends to a related kinase. CHK1 has not been characterized as well as CHK2 towards p53 (Shieh et al., 2000), however, CHK1 may replace CHK2 as a damage-induced kinase in vertebrates (Zachos et al., 2003). (A and C*). The BOX-II and BOX-V domain peptides activate CHK1 phosphorylation of p53 at Ser$^{20}$.* Kinase reactions containing p53$^{N1\text{-}66}$ and CHK1 were assembled without peptide (lanes 1) or with the indicated peptides (lanes 2 and 3). Reaction products were analysed for (A) p53$^{N1\text{-}66}$ or (C) p53[S15A]$^{N1\text{-}66}$ phosphorylation at Ser$^{20}$ (lanes 1-3). Protein band "a" represents migration of unphosphorylated or Ser$^{20}$-phosphorylated p53$^{N1\text{-}66}$, while protein band "b" is a kinase supershift that represents Thr$^{18}$ phosphorylation (as in Figure 2E and F). *(B and D) CHK1 exhibits constitutive activity towards Thr$^{18}$.* Kinase reactions containing p53$^{N1\text{-}66}$ and CHK1 were assembled without peptide (lanes 1) or with the indicated peptides (lanes 2 and 3). Reaction products were analysed for (B) p53$^{N1\text{-}66}$ or (D) p53[S15A]$^{N1\text{-}66}$ phosphorylation at Thr$^{18}$ (lanes 1-3). Protein band "a" is unphosphorylated or Ser$^{20}$-phosphorylated p53$^{N1\text{-}66}$, while protein band "b" is a kinase supershift that represents Thr$^{18}$ phosphorylation. CHK1 has high basal Thr$^{18}$ kinase activity (B and D, lane 1) and is attenuated allosterically as a Thr$^{18}$ kinase by the *BOX-II* and *BOX-V* domain peptides (B and D, lanes 2 and 3). However, *BOX-II* and/or *BOX-V* domain peptides stimulate Ser$^{20}$ phosphorylation (A and C, lanes 2-3). Further, these data indicate that CHK1 targeting of Thr$^{18}$ can occur independent of Ser$^{15}$ phosphorylation and are consistent with the possibility that CHK1 can be an alternate Ser$^{20}$/Thr$^{18}$ p53 transactivation domain kinase. The relatively complicated phenotypes of p53 missense mutants containing a Ser$^{20}$ phosphorylation site mutation to Ala$^{20}$ may be explained, in part, by adjacent Thr$^{18}$ phosphorylation by either CHK2, CHK1, or other homologous protein kinases.

References

Zachos, G., Rainey, M.D. & Gillespie, D.A. (2003) Chk1-deficient tumour cells are viable but exhibit multiple checkpoint and survival defects. EMBO J., 22, 713–723.

Supplemental Figure 1




Supplemental Figure 2.