Supplementary Information: Materials and Methods

Recombinant protein expression and *in vitro* kinase assay.

GST and GST-p53 were purified according to standard protocol after induction with 0.5mM IPTG for 3h at 30ºC. 200ng of each purified protein was then used in the *in vitro* kinase assay. His-tagged Strap was expressed and purified from BL21 DE3 bacteria, using Ni^{2+} agarose beads and eluted into BC100 containing 100mM imidazole. Proteins were subsequently dialysed into BC100 buffer, and 200ng of protein was used in the *in vitro* kinase assay with 200ng of Chk2 kinase in 50mM Tris pH 7.4, 10 mM $MgCl_2$, 10mM $MnCl_2$, 1mM DTT, 50mM ATP and ^{32}P- γ-ATP (Demonacos et al., 2004), at 30ºC for 20min. The reaction was stopped by adding SDS loading buffer and samples were resolved by 10% SDS-PAGE.

Cell culture and transfection.

U2OS and SAOS2 cells were grown in DMEM with 5% FCS and transfected with 500ng of the appropriate expression vector and CMV β-galactosidase as an internal control, using GeneJuice (Merck) according to manufacturer's instructions. HCT15 cells were grown in DMEM with 20% FCS. HCT15 cells were transfected as above using Effectene (Qiagen) according to manufacturer's instructions. Inducible Flag-Strap cells were grown in 10% tet negative FCS with G418 and hygromycin B. Cells were lysed in TNN buffer and after normalising for transfection efficiency analysed by SDS-PAGE.

siRNA.

25nm of non targeting #2 siRNA (Dharmacon) and Strap siRNA (sequence CAGAGAAAGUUGACAGAAAUU) was transfected where indicated into U2OS

cells using Oligofectamine transfection reagent (InVitrogen) according to the manufacturer's protocol. After 72h of transfection, cells were harvested for further analysis.

Antibodies and immunostaining.

The following antibodies were used; mouse anti-HA HA11 (BAbCo), anti-GAPDH (V18), anti-PCNA (PC10) and mouse anti-p53 DO-1 (Santa Cruz), anti-phosphoserine and anti-PARP antibody (BD Biosciences), anti-Flag antibody M2 and anti-βactin (Sigma), and anti-Strap monoclonal antibody which was produced by standard procedures using full-length His-tagged Strap. For secondary antibodies, horse radish peroxidase-conjugated (Calbiochem, DAKO) anti-immunoglobulin was used.

Immunostaining was performed as previously described (Demonacos et al., 2004).

Mass spectrometry.

His-tagged wild-type Strap was incubated with recombinant Chk2 protein in an *in vitro* kinase assay as above. The reaction was resolved using SDS-PAGE and the band corresponding to Strap subjected to in-gel trypsin digestion. Individual tryptic peptides were analyzed by LC-MS/MS using a high capacity ion trap tandem mass spectrometer (HCTplusTM, Bruker Daltonics) coupled to a C18 reversed phase HPLC system (Dionex/LC Packings). MS/MS spectra were analysed using the Mascot Software package (Matrixscience).

Strap purification from conditionally-inducible cell line.

The Strap stable cell line was created in U2OS cells using BD Biosciences TET-ON gene expression system. Ectopic Flag-Strap expression was induced by the addition of doxycycline according to the manufacturer's instructions. The cells were then treated with etoposide (10μM) for 16h prior to harvesting. Immunoprecipitation was performed using anti-Flag M2 agarose (Sigma) and the extract was eluted using Flag peptide. The material was resolved on a 4-12% NuPage Bis Tris gel (Invitrogen) and then visualised by silver staining (Qiagen) according to manufacturer's instructions. The band corresponding to Strap was subjected to in-gel trypsin digest. The peptides were then analysed by LC-MS/MS.

Flow cytometry

U2OS cells were transfected with pBB14 (Us9-GFP; 500ng; Kalejta et al., 1999) and the appropriate expression vector. pBB14 (Us9-GFP) served as an internal marker for transfected cells. Cells were then treated with etoposide (10μm) before harvesting for flow cytometry analysis. Growth media was collected and adherent cells were harvested in Trypsin EDTA (Lonza), washed in PBS and fixed in 50% ethanol in PBS at 4º overnight. Fixed cells were washed in PBS, resuspended in 50mg/ml propidium iodide containing 125 U/Ml RNase A and then sorted on a FACScan cell sorter and analysed using the Cell Quest software package (Becton-Dickinson).

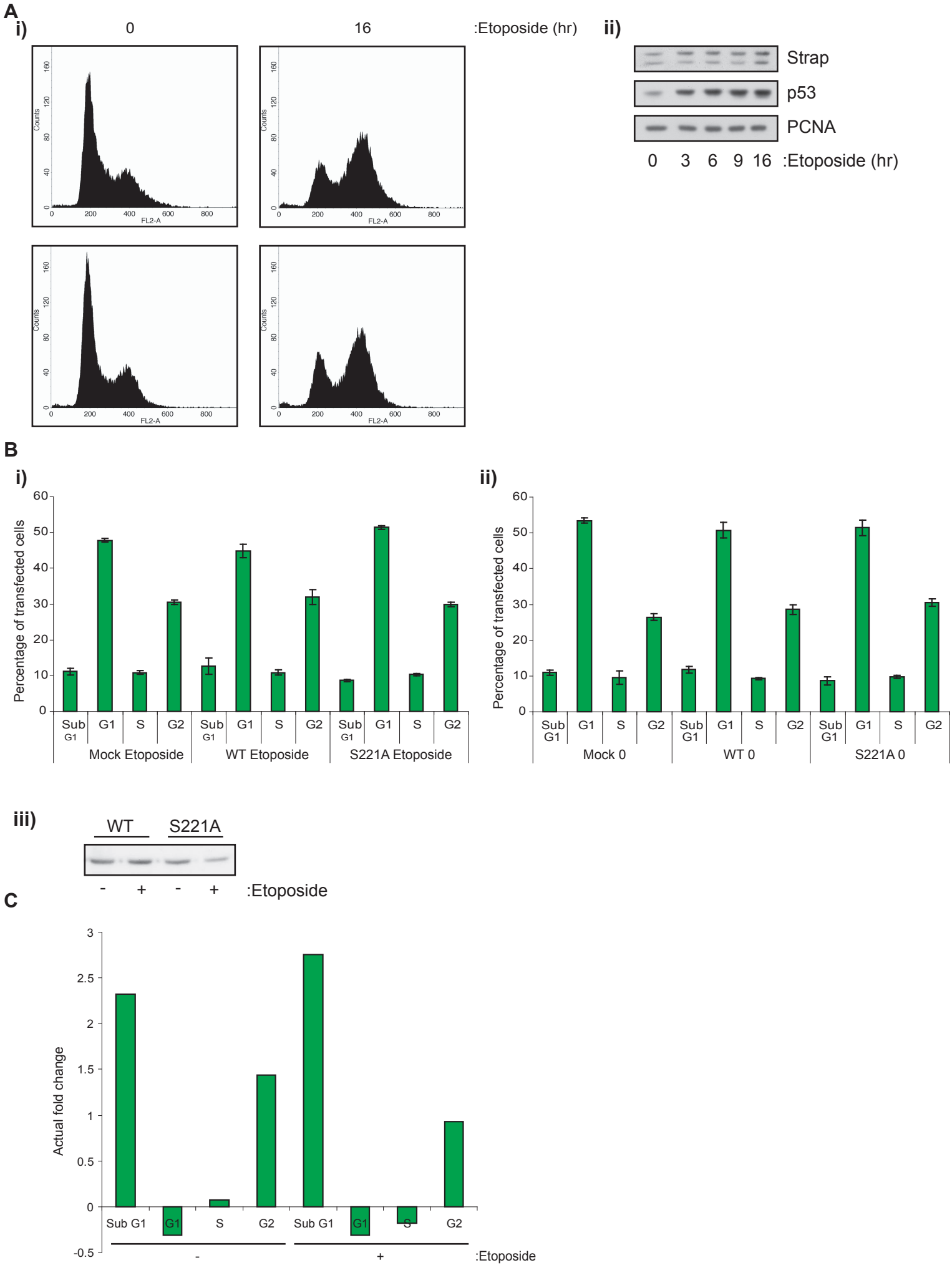
Supplementary Information: Figure 1

a) U2OS cells were treated with etoposide (10μm) for the times indicated. Cells were then harvested for FACS as previously described. The cell cycle profiles were determined by FACS analysis. (i) Immunoblot from the cells used in the FACS profiles shown (ii). Strap was detected using the monoclonal Strap antibody, DO1 for p53 and PCNA was used as a loading control.

b) SAOS2 cells were transfected with pBB14 (Us9-GFP; 500ng) as an internal transfection marker and the appropriate expression vector (500ng). Cells were treated with (i) or without (ii) etoposide (10μM for 16h) as indicated and then harvested for flow cytometry analysis as described. The graph represents cell cycle profiles of transfected cells only.

iii) Immunoblot from the cells used in the FACS profiles. Cells were loaded on the basis of β-galactosidase activity and Strap was detected using anti-HA antibody HA11.

c) U2OS cells were transfected with Strap or control non targeting (NT) siRNA (25nm) for 48h and then treated with etoposide (+; 10μM) for 16h before harvesting. Cells were then harvested for flow cytometry analysis as described. The graph represents the actual fold change relative to the appropriate controls.



Supplementary information Figure 1