References

Supplamental Methods

Chromatin Fractionation: 1x10^6 cells were plated onto 10 cm plates in duplicate and incubated overnight. Cells were scraped and washed twice with PBS. One replicate was lysed with 100 µl of SDS Loading Buffer and briefly sonicated. The other replicate was fractionated as described in Méndez et al (29). To extract KAP1 from the NP fraction, Nuclear Extraction Buffer containing 500mM was substituted in the final extraction step (28).

GST Binding Assay: KAP1 peptides were dissolved in PBS. 5 ug of KAP1 peptide was incubated with 5 ug of GST alone or GST-HP1 lysate. GST proteins were prepared as described in (31). The GST protein was immobilized on glutathione-Sepharose (Pharmacia) at room temperature for 1 hr in BB500 Buffer containing 20 mM Tris pH 7.9, 500 mM NaCl, 0.2 mM EDTA pH 8, 10% glycerol, 0.1% NP40, and 1 mM PMSF. The resin was then washed 3 times in BB750 Buffer containing 20 mM Tris pH 7.9, 750 mM NaCl, 0.2 mM EDTA pH 8, 10% glycerol, 0.1% NP40, and 1 mM PMSF, once in BB500 Buffer, and once in BB100 Buffer containing 20 mM Tris pH 7.9, 100 mM NaCl, 0.2 mM EDTA pH 8, 10% glycerol, 0.1% NP40, and 1 mM PMSF. The KAP1 peptides (either pHP1BD or HP1BD) was then added to the GST-bound resin and incubated at room temperature for 1 hr in BB100 Buffer. The resulting resin was then washed 3 times in

BB500 Buffer and once in BB100 Buffer. Retention of the KAP1 peptides was then resolved on a 4-12% SDS-PAGE gel (Invitrogen) and Coomassie stained. Following Coomassie staining, resolved proteins were electrotransferred to a nitrocellulose membrane and then immunoblotted with a HRP-Streptavidin antibody (Abcam).

Analysis of Cell Cycle Distribution: 7.5 x 10^5 cells were plated and allowed to grow overnight. At the appropriate times following treatment, cells were trypsinized and washed once in cold PBS. The resulting cell pellet was resuspended in 500 ul of PBS containing 0.1% Glucose. Five milliliters of ice cold 70% Ethanol were then added dropwise while gently vortexing. The resulting fixed cells were stored at 4 C until staining. Two to three hours prior to cell cycle analysis, the fixed cells were spun down at 1850 rpm for 5 minutes at 4 C. The resulting cell pellet was washed once in cold PBS and then resuspended in 300 ul PBS containing 69 uM Propidium Iodide and 38 mM Sodium Citrate pH 7.4. Twenty microliters of 10 mg/ml DNase free RNaseI was added to each sample and then incubated at 37 C for 30-45 min. The cell cycle profile was obtained by plotting the cell number (Y axis) verse the intensity of Propidium Iodide staining (X axis) for a population of 10,000 cells.

Supplamental Figure Legends

Figure S1. Immunohistochemical detection of HP1α (A, in green) or pKAP1-s824 (B, in green) and γH2AX-s139 (A and B, in red) in untreated parental MEFs or irradiated parental MEFs 6 hrs-post 9 Gy IR. Immunofluorescent Confocal microscopy of NIH3T3 2/4 cells transfected with plasmids expressing CFP-LacI (blue) using antibodies specific for KAP1 (C, in red) or HP1α (D, in red). The sites where two proteins (represented in red or green) overlap are shown in yellow. Line scans representing the colocalization of

proteins within each image are presented on the right. Scale bars (uM) are the white bars on the left.

Figure S2. A) Analysis of whole cell extracts isolated from M059K (DNA-PK +/+), M059J (DNA-PK-/-), and ATBDIVA (ATM-/-) cells at numerous timepoints post-9 Gy IR via immunoblotting using antibodies specific for pKAP1-s473, KAP1, and Actin. B) Immunohistochemical detection of pKAP1-s473 (green) and γH2AX-s139 (red) in U2OS cells 3 and 6 hrs post-9 Gy IR. The sites where two proteins (represented in red or green) overlap are shown in yellow. C) Immunofluorescent Confocal microscopy of NIH3T3 2/4 cells transfected with plasmids expressing CFP-LacI (blue) using antibodies specific for pKAP1-s473 (red). Line scans representing the colocalization of proteins within each image are presented on the right. Scale bars (uM) are the white bars on the left. D) Model depicting KAP1 HP1BD peptides. E) Coomassie Blue staining (top panel) and Western Blot analysis (bottom panel) of an *in vitro* binding assay using GST-tagged HP1 isoforms and Biotinylated unmodified HP1BD or phospho-s473 pHP1BD KAP1 peptides. Immunoblotting was carried out using a biotin-reactive HRP-Streptavidin antibody. E) Analysis of whole cell extracts isolated from transfected HEK293 cells at various timepoints post-9 Gy IR via immunoblotting using antibodies specific for pKAP1-s824, pKAP1-s473, KAP1, and Tubulin. Association of KAP1 with HP1β (F) and HP1γ (G) *in vivo* at various timepoints post-9 Gy IR was examined by a FLAG-directed immunoprecipitation of FLAG-HP1 co-expressed with a Myc-KAP1 in 293 cells. The levels of FLAG-HP1, Myc-KAP1, pKAP1-s824, and pKAP1-s473 were examined via immunoblotting.

Figure S3. A) Association of KAP1-WT or KAP1-Mut2 with HP1α *in vivo* 30 minutes and 3 hrs post 9 Gy IR was examined by a FLAG IP of FLAG-HP1α co-expressed with a Myc-KAP1 in 293 cells. The levels of Myc-KAP1 and FLAG-HP1α was examined via immunoblotting. B) Cell cycle distribution of U2OS KAP1 knockdown cells reconstituted with Vector, KAP1-WT, or KAP1-Mut2 at multiple timepoints post-9 Gy IR. The number of cells (Y axis) were plotted against the intensity of propidium iodide staining (X axis) for the 10,000 cells analyzed (on left). The average number of cells in each timepoint for each gated cell cycle phase was graphed as a histogram (on right). Cell cycle distribution of untreated parental U2OS cells and U2OS KAP1 knockdown cells reconstituted with Vector, KAP1-WT, or KAP1-Mut2 (C), or 72 hrs following treatment with 9 Gy IR (D), or 300 nM NCS (E). The number of cells (Y axis) were plotted against the intensity of propidium iodide staining (X axis) for the 10,000 cells analyzed (top). The average number of cells in each timepoint for each gated cell cycle phase was graphed as a histogram (bottom). F) Immunofluorescent Confocal microscopy of U2OS cells mixed with U2OS GFP-tagged shRNA vector cells or KAP1 knockdown cells (pseudo blue) 3 hrs-post 9 Gy IR using antibodies specific for pKAP1-s824 (green) and γH2AX-s139 (red). The sites where two proteins (represented in red or green) overlap are shown in yellow. G) Analysis of whole cell extracts isolated from NIH3T3 2/4 parental, KAP1 knockdown cells and KAP1 knockdown cells reconstituted with Vector, KAP1-WT or KAP1-Mut2 via immunoblotting using antibodies specific for KAP1, FLAG, and Actin. Immunofluorescent Confocal microscopy of NIH3T3 2/4 KAP1 knockdown cells reconstituted with KAP1-WT or KAP1-Mut2 transfected with plasmids expressing CFP-LacI (blue) using antibodies specific for FLAG (red). Line scans representing the colocalization of proteins within each image are presented on the right. Scale bars (uM) are the white bars on the left.

Figure S4. Chromatin fractionation analysis using 500 mM NaCl of vectors control and HP1 knockdown H1299 cells 30 minutes post-9 Gy IR via immunoblotting using antibodies specific for KAP1, HP1α, γH2AX-s139, and Actin.

Figure S5. Association of KAP1 with H2AX (A), H2B (B), H3.1 (C), 53BP1 (D) ZNF317 (E) and SETDB1 (F) *in vivo* at various timepoints post-9 Gy IR was examined by a FLAG-directed immunoprecipitation of FLAG-tagged constructs co-expressed with a Myc-KAP1 in 293 cells. The levels of these FLAG-tagged proteins, Myc-KAP1, pKAP1-s824, and pKAP1-s473 were examined via immunoblotting. The levels of γH2AX-s139 (A) were also examined for the FLAG-H2AX transfected samples via immunoblotting. G) Histogram depicting decreased Luciferase activity from a LexA DNA binding domain (LexA-DBD) is fused to the C-terminus of KAP1 (LexA-KAP1-CT) 8 hrs post 9 Gy IR. The LexA constructs were co-transfected into HEK293 cells with a LexA-Luciferase Reporter and a B-galactasidase expressing construct. Luciferase activity was normalized to B-galactasidase activity and protein concentration.