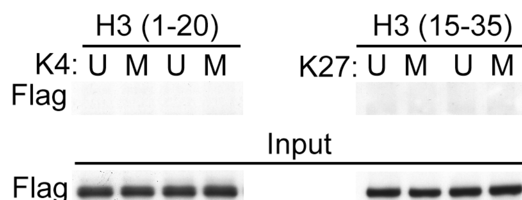
Online Supplementary Information

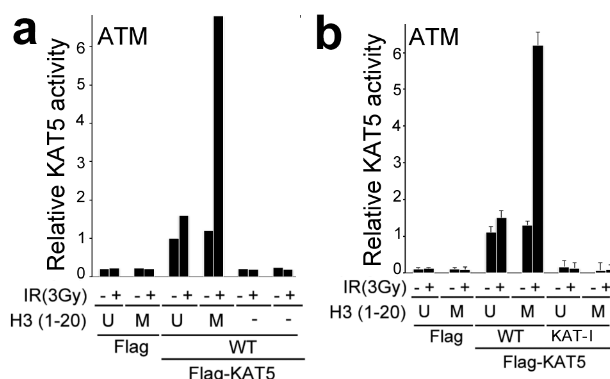
The online supplementary information include:

Supplementary figures and supplementary figure legends (1-15)
Supplementary methods
Supplementary tables (1-2)

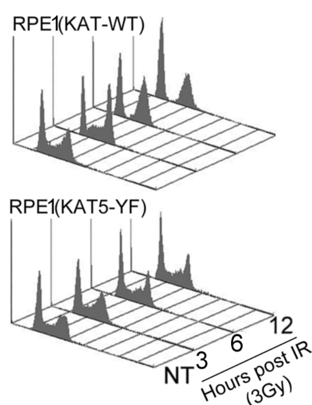
Supplementary Figues



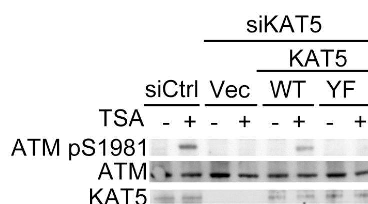
Supplementary Fig. 1. KAT binding to histone-derived methylated peptides. Peptide pull-down assays were used to examine binding of Flag-KAT5 to H3-derived peptides (residues 1-20) in which K4 is methylated (M) or unmethylated (U), or to H3-derived peptides (residues 15-35) in which K27 is methylated (M) or unmethylated (U). Eluates of immunopurified KAT5 were incubated with M or U peptides that were immobilized on agarose beads. After extensive washing, samples were resolved by SDS-PAGE and examined by western blotting.



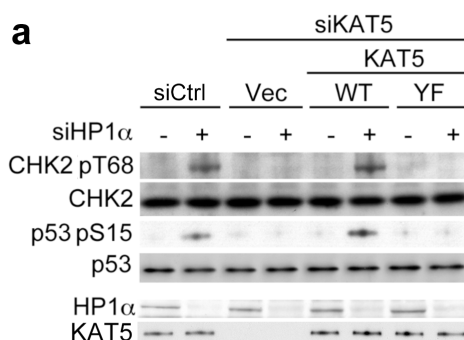
Supplementary Fig. 2. KAT5 activity towards ATM is stimulated by H3K9me3 peptide and enhanced by IR. a, Flag-KAT5 was purified from HeLa cells that had been IR treated or mock treated. Effects of H3-derived peptides – methylated (M) or unmethylated (U) on H3K9 (M) – on Flag-KAT activity towards ATM were measured by *in vitro* acetylation assays followed by western blotting with an anti-acetyl-lysine antibody and quantification. Results shown are from a representative experiment that was performed three times. **b,** Flag-KAT5 (WT or catalytically-dead KAT-I bearing mutations within its acetyltransferase active site: Gln-377 to Glu and Gly-380 to Glu) were purified from HeLa cells before or after IR treatment. Assays were as in **a**. Results shown are from three independent experiments presented as means ± SE.

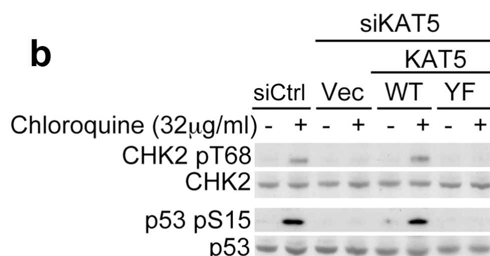


Supplementary Fig. 3. KAT tyrosine phosphorylation is required for checkpoint activation after IR. Cell cycle analyses of RPE1 cells, expressing siRNA resistant KAT5 derivatives (endogenous KAT5 was siRNA depleted), at the indicated times after exposure to IR (3Gy).

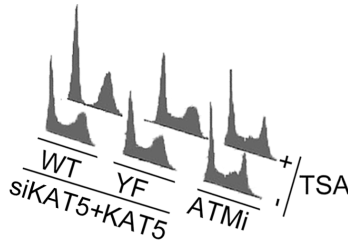


Supplementary Fig. 4. KAT5 tyrosine phosphorylation is required for ATM auto-phosphorylation after TSA treatment. RPE1 cells expressing siRNA-resistant KAT5 derivatives (endogenous KAT5 was siRNA depleted) were examined for ATM auto-phosphorylation after TSA treatment (5 h) by western blotting.

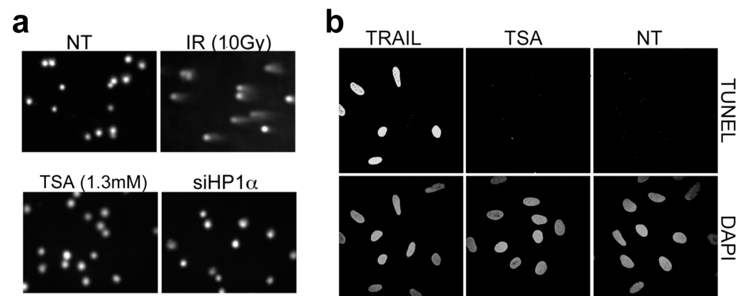




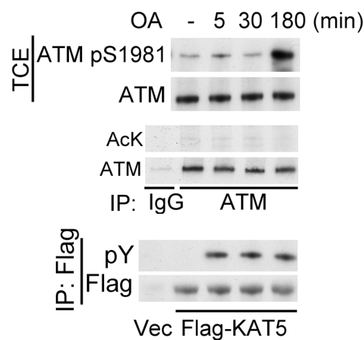
Supplementary Fig. 5. Links between KAT5 tyrosine phosphorylation and ATM-dependent signaling after chromatin alterations. a, RPE1 cells expressing siRNA-resistant KAT5 derivatives (endogenous KAT5 was siRNA depleted) were examined for ATM-mediated signaling after HP1α depletion. **b,** similar analysis to **a** but after chloroquine treatment (3 h).



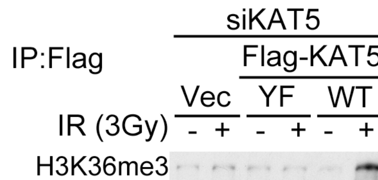
Supplementary Fig. 6. KAT5 tyrosine phosphorylation is required for G1/S checkpoint after TSA treatment. Flow cytometry analyses of RPE1 cells treated with TSA for 12 h. In the left and middle panels, cells were depleted of endogenous KAT5 and complemented with KAT5-WT or KAT5-YF; in the right panel, cells were pre-treated with ATMi (1h) and left for an extra 12 h after TSA treatment.



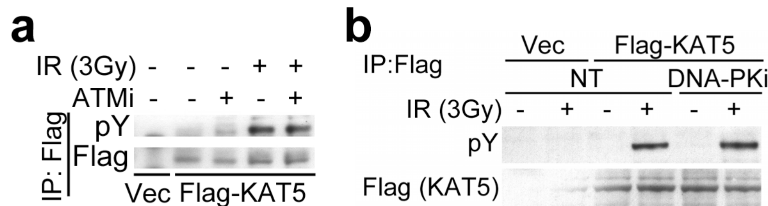
Supplementary Fig. 7. Chromatin alteration treatments (TSA, HP1α depletion) do not induce detectable DSBs. a, Neutral comet assay of RPE1 cells after IR exposure, TSA treatment or HP1α depletion. **f,** TUNEL (terminal deoxynucleotidyl transferase dUTP nick end-labeling) assays were performed on RPE1 cells after exposure to TRAIL (TNF-related apoptosis inducing ligand), TSA, or HP1α depletion.



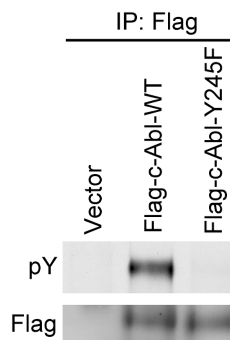
Supplementary Fig. 8. Okadaic acid treatment induces delayed ATM auto-phosphorylation but does not induces ATM acetylation. a, Top panel, RPE1 cells were treated with OA for the indicated times then analysed for ATM auto-phosphorylation. Middle panel, RPE1 cells were treated with OA for the indicated times then analysed for ATM acetylation after immunoprecipitation. Lower panel, analysis of KAT5 tyrosine phosphorylation in RPE1 cells after OA treatment. **b,** analysis of the effect of ATM inhibition on KAT5 tyrosine phosphorylation after IR. RPE1 cells were pre-treated with ATMi (10µM, for 1h) and exposed to IR. Cells were harvested 1h post IR and extracts were analyzed for KAT5 Tyr phosphorylation after immunoprecipitation.



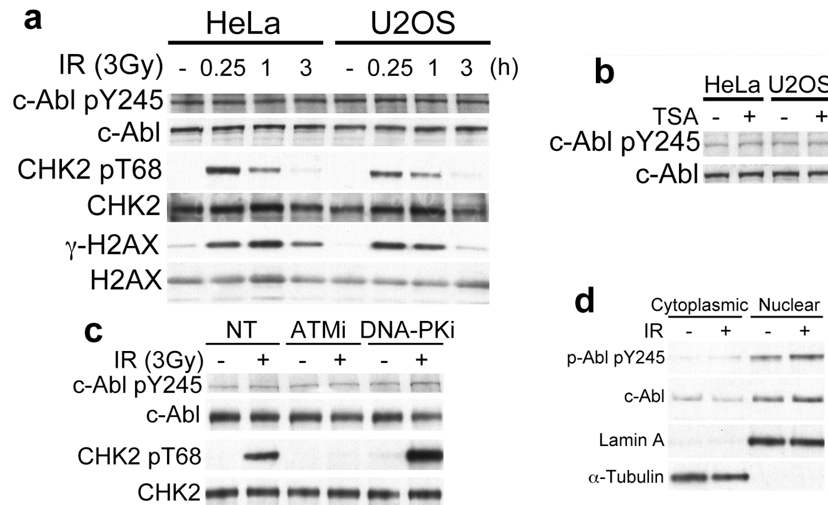
Supplementary Fig. 9. KAT5 interacts, in a Tyr-44 dependent manner, with H3 enriched for H3K36me3. Flag-based immunoprecipitations of KAT5 were performed on benzonase cell extracts – prepared from HeLa cells expressing siRNA-resistant KAT5 (WT or YF) in which endogenous KAT5 was siRNA depleted – before or after treating cells with IR. Elutes were analyzed by western blotting.



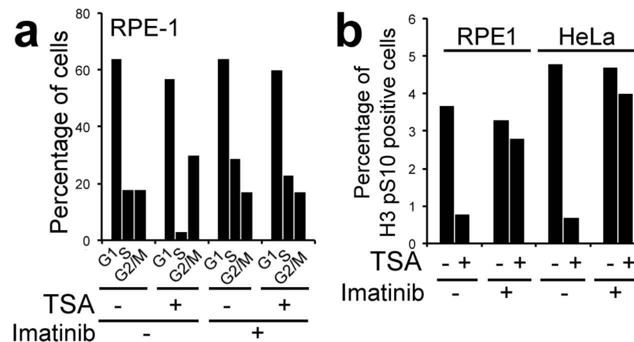
Supplementary Fig. 10. Inhibition of ATM or DNA-PK does not affect KAT5 Tyr phosphorylation. a, RPE1 cells were pretreated with ATMi (10µM) for 1 h followed by IR exposure; cell extracts were prepared 1 h after IR and were analysed by immunoprecipitation and western blotting as indicated. **b,** RPE1 cells were pretreated with DNA-PKi (2µM) for 1 h followed by IR exposure; cell extracts were prepared 1 h after IR and were analysed by immunoprecipitation and western blotting as indicated.



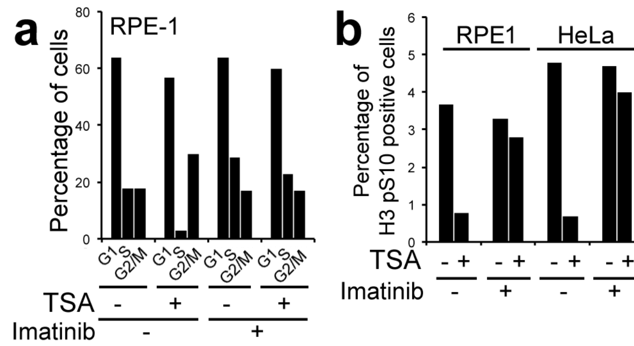
Supplementary Fig. 11. Validation of the specificity of c-Abl Tyr-245 phospho-specific antibody. RPE1 cell were transfected with c-Abl (WT or Y245F mutant), and the specificity of c-Abl Tyr-245 phospho-antibody was assessed by western blotting after Flag-based immunoprecipitation.



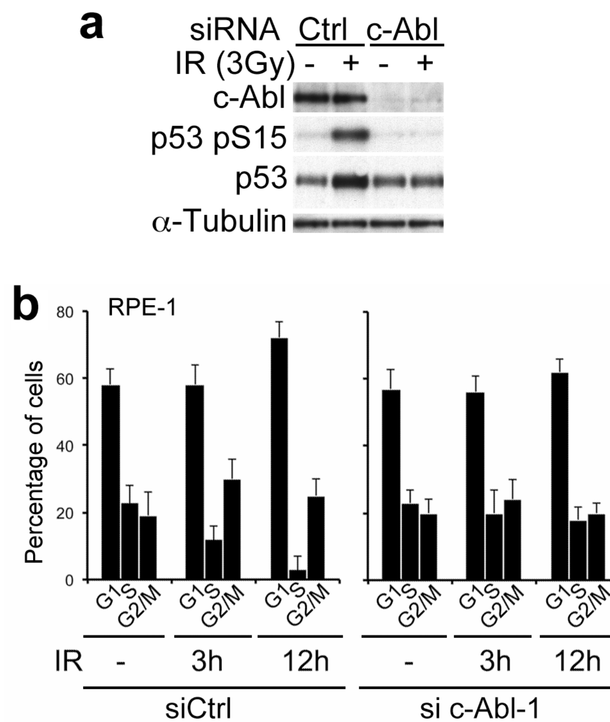
Supplementary Fig. 12. c-Abl phosphorylation on Tyr-245 is not markedly affected by IR or TSA treatment nor by ATM or DNA-PK inhibitors. a, analysis of c-Abl pY245 in U2OS and HeLa cells after IR. Cells were IR treated , extracts generated after the indicated times and analysed by western blotting. **b,** analysis of c-Abl pTyr-245 in U2OS and HeLa cells after TSA. Cells were treated with TSA, extracts generated after 5 h and analysed by western blotting. **c,** RPE1 cells were pretreated with ATMi or DNA-PKi for 1 h followed by IR exposure. Extracts were prepared after 1 h and were analysed by western blotting. **d,** cellular fractionation of RPE1 cell after exposure to IR followed by western blot analysis.



Supplementary Fig. 13. Inhibition of c-Abl interferes with KAT5 chromatin accumulation. a, RPE1 cells expressing Flag-KAT5 were pretreated with imatinib for 3 h and CSK-based cell fractionation was performed. Samples were than analysed by western blotting. **b,** RPE1 cells expressing Flag-KAT5 were pretreated with imatinib for 3 h and benzonase extracts were subjected to Flag-based immunoprecipitation and western blot analysis.



Supplementary Fig. 14. Inhibition of c-Abl with Imatinib abrogates checkpoint activation. a, Flow cytometry analyses of RPE1 cells treated with TSA in the presence or absence of imatinib. Cells were collected 12 h after -treatment with TSA and analysed by flow cytometry. **b,** Analysis of the G2/M checkpoint in RPE1 and HeLa cells after TSA exposure in the presence or absence of imatinib. Cells were harvested 5 h and stained with H3pS10 antibody, then analysed by flow cytometry.



Supplementary Fig. 15. Depletion of c-Abl in U2OS cells inhibits p53 phosphorylation on Ser-15 and checkpoint activation. a, U2OS cells were depleted for c-Abl, exposed to IR and samples were prepared after 1 h and analysed by western blotting. **b,** Flow cytometry analyses of RPE1 cells treated with IR after depletion of c-Abl by siRNA. Cells were collected 3 or 12 h after treatment with IR and analysed by flow cytometry.

Table S1. List of antibodies used in the study

Antibody	**Provider**	**Use**
Phospho-tyrosine (pY)	Cell Signalling Technology (no: 9411)	WB (1:500)
Acetyl-lysine (AcK)	Upstate (no: 05-515)	WB (1:500)
Flag	Sigma (no: F7425)	WB (1:1000)
Flag-M2 beads	Sigma (no: A2220)	IP
KAT5	Upstate (no: 07-038)	WB (1:250) and IP
ATM	Gift from KuDOS Pharmaceuticals Ltd	WB (1:500) and IP
CHK2	Millipore (05-649)	WB (1:1000)
CHK2 pT68	Cell Signalling Technology (cat: 2665)	WB (1:1000)
p53	Cell Signalling Technology (no: 9282)	WB (1:1000)
p53 pS15	New England Biolabs (no: 9284L)	WB (1:1000)
HP1α	Millipore (no: 05-689)	WB (1:500)
H2AX	Abcam (ab11175)	WB (1:3000)
γH2AX	Millipore (no: 05-636)	WB (1:1000)
MRE11	Abcam (no: ab33125)	WB (1:1000)
H4K16ac	Millipore (no: 07-329)	ChIP
H4	Abcam (no: ab31827)	ChIP
c-Abl	Cell Signalling Technology (cat: 2862)	WB (1:1000)
c-Abl pY245	Cell Signalling Technology (cat: 2861)	WB (1:1000)
ATM pS1981	Epitomics (cat: 2151-1)	WB (1:1000)

Table S2. List of siRNA sequences used in the study

Target protein	Sequence
KAT5	GAGGGCUGGUGAUUGUAAATT
HP1α	AUGGAAAGACACAGAUGAATT
MRE11	GAGCAUAACUCCAUAAGUATT
Ctrl (control, lucifera)	CGUACGCGGAAUACUUCGATT
c-Abl-1	AUCAACAAACUGGAGAAUATT
c-Abl-2	CCUCCUUUGCUGAAAUCCATT