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# **Phosphorylation of HIC1 (Hypermethylated in Cancer 1) Ser694 by ATM is essential for DNA repair.**

Sonia PAGET<sup>1</sup>, Marion DUBUISSEZ<sup>1</sup>, Adeline PAGE<sup>2</sup>, Vanessa DEHENNAUT<sup>1</sup>, Ingrid LOISON<sup>1</sup>, Nathalie SPRUYT<sup>1</sup> and Dominique LEPRINCE<sup>1\*</sup>.

1- Univ. Lille, CNRS, INSERM, UMR9020-UMR-S1277 - Canther - Cancer heterogeneity, Plasticity and Resistance to Therapies, F-59000 Lille. France.

2- Proteomics Platform, Institut de Génétique et de Biologie Moléculaire et Cellulaire (IGBMC), INSERM U964, CNRS UMR7104, Strasbourg University, Illkirch, France.

Present Address: Marion Dubuissez, Maisonneuve-Rosemont Hospital Research Center, Montreal, QC H1T 3W5, Canada. Adeline Page, SFR Biosciences, CNRS UAR3444, INSERM US8, University of Lyon, ENS Lyon, 69007 Lyon, France.

**\*:Corresponding author.** Email: [dominique.leprince@ibl.cnrs.fr](mailto:dominique.leprince@ibl.cnrs.fr)

## **Highlights:**

- Upon induction of repairable DSBs, we identified 3 specific posttranslational modifications (PTMs) of HIC1: phosphorylation on Ser285 and Ser694 and acetylation of Lys623.

- ATM-dependent phosphorylation of HIC1 on Ser694 is required for DNA repair activity in functional Comet assays.
- The highly conserved C-terminus is an essential player in the DNA repair function exhibited by HIC1.
- Here, we identify HIC1 as a previously unrecognized substrate of ATM and demonstrate the role of Ser694 phosphorylation in the DNA damage response.

## **ABSTRACT**

The tumor suppressor gene *HIC1* (Hypermethylated in Cancer 1) encodes a transcriptional repressor involved in the DNA-damage response. A SUMOylation increase on HIC1 Lysine314 favors the direct transcriptional repression of *SIRT1* and thus the P53-dependent apoptotic response to irreparable DNA double strand breaks (DSBs). HIC1 is also essential for DSBs repair but in a SUMOylation-independent manner. Here, we show that repairable DSBs induced by a 1h Etoposide treatment results in three specific posttranslational modifications (PTMs) of HIC1. Two of these PTMs, phosphorylation of Serine 694 and Acetylation of Lysine 623 are located in the conserved HIC1 C-terminal region located downstream of the Zinc Finger DNA-binding domain. By contrast, phosphorylation of Serine 285 found in the poorly conserved central region is unique to the human protein. We showed that Ser694 phosphorylation is mediated mainly by the PIKK kinase ATM and is essential for the DNA repair activity of HIC1 as demonstrated by the lack of efficiency of the S694A point mutant in Comet assays.

Thus, our results provide the first evidence for a functional role of the conserved HIC1 C-terminal region as a novel ATM substrate that plays an essential role in the cellular HIC1-mediated cellular response to repairable DSBs.

**Keywords:** HIC1, DNA-damage Response, ATM, P53,

**Running Title:** HIC1 is an ATM substrate.

## 1. INTRODUCTION

Upon genotoxic stress, cells activate a complex signaling pathway called the DNA damage response (DDR) which results first in a transient cell cycle arrest followed by the activation of repair mechanisms such as HR or NHEJ or the induction of apoptotic cell death if the damages are too important to be repaired [1]. A key player in the DDR is the tumor suppressor gene P53 which determines cell fate. Indeed, upon stabilization by the DDR-activated apical kinases ATM or ATR and their effector kinases Chk2 or Chk1, P53 can transactivate both cell cycle regulating genes such as p21Waf1 or proapoptotic genes such as PUMA or Bax depending on the damage intensity [2].

The tumor suppressor gene *HIC1* (*Hypermethylated in cancer 1*) which is epigenetically silenced by promoter hypermethylation in many types of human cancers plays critical roles in the DDR [3, 4]. Indeed, *HIC1* which encodes a sequence-specific transcriptional repressor is a direct target-gene of P53. Upon induction of non-repairable DSBs, HIC1 regulates the p53-dependant apoptotic DNA-damage response [5]. HIC1 directly represses the transcription of the deacetylase *SIRT1* which inactivates P53 [5].

HIC1 interacts with the C-terminal region of MTA1, a core component of NuRD repressive complex, through a SUMOylation consensus motif in the HIC1 central region [6, 7]. SUMOylation is a reversible posttranslational modification (PTM) involved in the regulation of protein-protein interactions, notably in DDR [8]. We demonstrated that non-repairable

DSBs increases HIC1 SUMOylation [9] and the interaction of endogenous HIC1 and MTA1 proteins, thereby favoring NuRD recruitment onto HIC1 target genes, including *SIRT1* [10].

*HIC1* is also a key player in the response to repairable DNA-damages. Down-regulation of endogenous *HIC1* expression through RNA interference in normal human fibroblasts delays DNA repair [9]. However, HIC1 SUMOylation is dispensable for its role in DNA repair [10]. Multiple tightly coordinated PTMs including phosphorylation, ubiquitylation, SUMOylation, polyADP-ribosylation or acetylation are crucial for the repair pathways [11]. We hypothesized that HIC1 may be regulated by others PTMs in the context of repairable DSBs.

In this study, we identified Serine 694 in the conserved HIC1 C-terminal end as a phosphorylation site for the phosphatidylinositol 3-kinase-related kinases family (PIKK) which is a critical regulator of DDR. Mass spectrometry analyses conducted on HIC1-transfected HEK293T treated for 1h with Etoposide identified three specific HIC1 PTMs and notably its phosphorylation by ATM on Ser694. We validated this PTM on endogenous HIC1 proteins and demonstrated that this ATM-mediated Ser694 phosphorylation is essential for the repair activity of HIC1 in functional Comet assays. Therefore, our studies identified HIC1 as a new direct target of ATM.

## **2. MATERIAL AND METHODS**

### ***2.1 Cell lines and transfection***

Cos-7, HEK293T and BJ-hTERT cells were maintained in Dulbecco modified Eagle medium (Invitrogen) supplemented with 10% fetal calf serum, non-essential amino acids and gentamycin. Cells were cultured at 37°C in humidified 5% CO<sub>2</sub> atmosphere [10]. Etoposide (Sigma-Aldrich) and the ATM kinase inhibitor, KU-55933 were used as previously described [9]. The DNA-PKcs inhibitor, KU-57788 (Selleckchem) was used at a final concentration of 10 μM.

## ***2.2 LC-MS/MS analyses of HIC1***

HEK293T cells were transfected for 48h with expression vectors for wt FLAG-HIC1 and treated or not with 20  $\mu$ M Etoposide for 1h [9, 10]. After immunoprecipitation with anti-FLAG M2 antibodies, the HIC1 band was purified by SDS/PAGE and subjected to manual in-gel reduction, alkylation and digestion. Prior to the nanoLC-MS/MS analysis, samples were subjected to phospho-enrichments on Fe-NTA IMAC spin-column and TiO<sub>2</sub> spin-tips (Thermo Scientific) and processed according to manufacturer's protocol. For LC-MS/MS analyses, samples were analyzed using an Ultimate 3000 nano-RSLC (Thermo Scientific, San Jose California) coupled on line with a LTQ-Orbitrap ELITE mass spectrometer via a nano-electrospray ionization source (Thermo Scientific, San Jose California).

## ***2.3 Comet assays***

Cos7 cells were transfected for 48h with expression vectors for wt FLAG-HIC1 and for the non-phosphorylatable point mutant, FLAG-HIC1 S694A. For each condition, 2,000 cells were suspended in 80 $\mu$ l of 0.5% low melting point agarose at 42°C. The suspension was immediately laid onto a comet slide (TREVIGEN Inc.). Agarose was allowed to solidify at 4°C for 20 min. The comet slides were then immersed in prechilled lysis solution (1.2M NaCl, 100mM EDTA, 10mM Tris, 1% Triton, pH 10) at 4°C, for 90 min in the dark. Comet slides were next placed in a horizontal electrophoresis unit and let to equilibrate in electrophoresis buffer (Tris 89mM, Boric acid 89mM, EDTA 2mM, pH 8) to detect double strand breaks) for 10 min at 4°C, in the dark [10]. After migration (40V for 25 minutes), the slides were stained with SYBR green (Molecular Probes-1000X) according to manufacturer's recommendations.

## ***2.4 Western blotting and antibodies.***

For total proteins extraction, cells were lysed in RiPA buffer (10 mM Tris pH 7.4, 150 mM NaCl, 1 mM EDTA, 1% Triton X-100, 0.5% sodium deoxycholate, 0.1% SDS and proteases inhibitors added at the time of preparation). Protein concentration was determined using the Micro BCA Protein Assay Kit (Thermofisher Scientific). Western blots were performed as previously described [9]. The secondary antibodies were Anti-mouse IgG-HRP (NA931V) and donkey anti-rabbit IgG-HRP (AP182P), purchased from GE Healthcare and Millipore, respectively.

The home-made rabbit anti-HIC1 antibody has been described [9]. To generate polyclonal antibodies against phosphorylated-Ser694 of HIC1, control and phosphorylated peptides corresponding to AA 685-698 of human HIC1 (Q14526-2) [H2N-C-SPDKAAEVLS(PO3H2)QGAH-CONH2] were synthesized, coupled to KHL and used to immunize rabbits (Covalab, Villeurbanne, France). Control and phosphospecific antibodies were purified by affinity chromatography using standard protocols.

Commercial antibodies of the following specificity were used: Phospho ATM/ATR Substrate, S/TQG (6966) and phospho-Chk2Thr68 (2661) from Cell Signaling; Mouse monoclonal anti-gammaH2Ax (NB100-78256) from Interchim; normal rabbit IgG (sc-2027), rabbit anti-Actin (sc-1616-R), rabbit anti-ATM (sc-7230), rabbit anti-pATM (sc-47739) from Santa Cruz; monoclonal anti-FLAG (M2) and rabbit anti-p DNAPKcsS2056 (SAB4504) from SIGMA.

### **3. RESULTS**

***3.1 A HIC1 phylogenetically conserved C-terminal Serine, Ser694 is phosphorylated by PIKK kinases in response to repairable DNA damages.***

*In silico* analyses of the human HIC1 protein sequence identified Serine 694 embedded into a robust phosphorylation consensus motif for PIKK: -S/TQG with an hydrophobic amino-acid, generally Leucine at position -1 [12]. This LS<sup>694</sup>QG consensus motif is phylogenetically conserved in the carboxyl-terminal part of vertebrate HIC1 orthologs (Figure 1A, Supplementary Figure 1) [13]. To validate HIC1 as a PIKK substrate, we immunoprecipitated FLAG-HIC1 proteins from transfected HEK293T cells and analyzed its phosphorylation before and after induction of repairable DNA damage. For that, 48h after transfection, cells were treated for 1 hour with Etoposide before recovery in complete culture medium without Etoposide for various times (2 and 5h) to monitor the repair process. Immunoblotting analyses with an anti-phospho-S/TQG antibody demonstrated that HIC1 is phosphorylated in an Etoposide-inducible and time-dependent manner (Figure 1B). This signal decreased after 2h and disappeared after 5h, following the kinetics of  $\gamma$ H2AX, as a surrogate of DNA repair and Chk2 (the effector kinase of ATM) phosphorylation. Similarly, in Etoposide-treated BJ-hTERT fibroblasts, endogenous HIC1 proteins are phosphorylated by PIKK kinases (Figure 1C).

To validate the phosphorylation of HIC1 on Ser694 and to identify other potentially modified residues, we ectopically expressed FLAG-HIC1 in HEK293T cells. 48 hours after transfection the cells were treated with Etoposide or DMSO as mock control for 1 hour. The proteins immunoprecipitated by the anti-FLAG M2 monoclonal antibody were subjected to trypsin digestion and analyzed by Mass Spectrometry. The Etoposide-induced genotoxic stress results in 8 phosphorylation also found in control conditions and in 3 specific PTMs of HIC1. They included as expected its phosphorylation on Ser694 but also its phosphorylation on Ser285 in its poorly conserved central region and its acetylation on Lys623 in the conserved C-terminal part (Figure 2A) (Supplementary Figure 2).



First, we generated a phosphospecific antibody that would recognize HIC1 phosphorylated on Ser694. In HIC1-transfected HEK293T cells, this phosphospecific antibody detected a strong signal in Etoposide-treated cells (Figure 2B). Mutation of Ser694 to Alanine in the FLAG-HIC1 S694A non-phosphorylatable point mutant totally abolished this Etoposide-induced phosphorylation of HIC1. We also noticed that the phosphorylation of Ser694 or its absence in the S694A point mutant has no effect on its SUMOylation in agreement with our previous results (Figure 2B) [10]. Similar experiments using the anti p-S/TGQ antibodies confirmed these results and further demonstrated that Ser694 is the only site targeted by PIKK kinases in HIC1 (Figure 2C).

The phosphorylation of Ser285, embedded in a PSDPF motif, would result in a pSxxF phosphopeptide motif able to directly bind BRCT (BRCA1 C-terminal) domains which are found in numerous proteins, notably implicated in DNA repair [14]. However, this Ser285 is unique to the human HIC1 protein [15], suggesting a different regulation of function for HIC1 in primates and other species.

By contrast, Lys623 is, as Ser694, well-conserved in the vertebrate HIC1 proteins (Supplementary Figure 1). Lys623 is embedded in a GxGK motif which is preferentially acetylated by TIP60, an Histone AcetylTransferase involved in DNA repair [16, 17] (Supplementary Figure 1).

Collectively, these results define the phylogenetically conserved carboxy-terminal part of HIC1 as a target for posttranslational modifications by enzymes involved in DNA repair.

***3.2 HIC1 phosphorylation on Ser694 is required for efficient DNA damage repair in Comet assays.***

To determine if HIC1 phosphorylation on Ser694 is important for DSBs repair, Cos7 cells which as immortalized epithelial cell lines did not significantly express endogenous *HIC1* [18]. We first transfected Cos7 cells with wt HIC1 or with the non-phosphorylatable S694A mutant in the neutral were analyzed by Comet assays which specifically measured DSBs at the level of individual cells. As previously described, 48h after transfection, cells were treated for 1 hour with Etoposide before recovery in complete culture medium without Etoposide for various times (2, 4, 6 and 24h) to monitor the repair process [10]. Results showed that the same amount of DSBs were induced in Cos7 transfected with the two expression vectors. However, salient differences were observed during the time-course recovery in normal medium of cells expressing the wt or the S694A phospho-deficient HIC1 mutant (Figure 3). As early as 2h of repair, 30% of the S694A cells still exhibited Comets as compared to 15% in the wt cells. These differences were even more marked at 4h and 6h of recovery with an almost complete repair in wt HIC1 cells and more than 20% of the S694A cells still with unrepaired DNA. By contrast, at the end of the process (24h), no major differences were observed (Figures 3A and B). This kinetics is highly similar to the repair process of BJ-hTERT fibroblasts with total endogenous *HIC1* inactivation by siRNA as compared to BJ-hTERT siCtrl cells [9]. This strongly suggests that the lack of HIC1 phosphorylation on Ser694 could have the same effect than the total inactivation of *HIC1* in DNA repair. In addition, the lack of phosphorylation on Ser694 has no impact on its SUMOylation in repair conditions (Figure 3C) [10]. Finally, no salient differences were observed during the same Comet assays analyses of HEK293T cells transfected with the wt or the K623R acetylation-deficient point mutant (data not shown), suggesting that this modification does not play an important functional role.

Thus, these results strongly suggest that HIC1 phosphorylation on Ser694 is the main PTM sustaining HIC1 function in DSBs repair.

### ***3.3 HIC1 phosphorylation on Ser694 after DNA damage is dependent on ATM.***

The two major PIKK kinases recruited at DSBs are DNA-PKcs and ATM [19]. Whereas DNA-PKcs is the major effector of DNA repair through NHEJ (non-homologous end joining), ATM is involved in HR (homologous recombination) as well as in the repair of DSBs in heterochromatin. The LS<sup>694</sup>QG consensus is highly related to an ATM specific consensus [12, 20]. Upon induction of repairable damages in BJ-hTERT fibroblasts, the phosphorylation of endogenous HIC1 proteins on Ser694 detected by the anti-pS/TQG antibodies is almost completely abolished in presence of the ATM specific inhibitor, KU-55933 (Figure 4A). Similar results were obtained in a time-course experiment using HEK293T cells transiently transfected with a FLAG-HIC1 expression vector, treated with Etoposide with or without the ATM inhibitor and analyzed by immunoblotting with the phospho-specific anti-pSer694 HIC1 antibodies (Figure 4B). By contrast, this phosphorylation of HIC1 on Ser694 is not abolished by the DNAPK-cs inhibitor KU-57788 (Figure 4C).

Thus, these results strongly suggest that ATM is the main PIKK kinase involved in HIC1 phosphorylation on Ser694 during DSBs repair.

## **4. DISCUSSION**

Multiple posttranslational modifications (PTMs) of DDR effectors are coordinately orchestrated to elicit an optimal response to DNA damages. Previously, we demonstrated that HIC1 SUMOylation is important for the P53-dependent apoptotic response to non-repairable DSBs but is dispensable for its roles in DNA repair. Here, by a combination of *in silico* analyses, mass spectrometry analyses and functional assays, we identified three specific

PTMs of HIC1 after induction of repairable DNA damages, notably the phosphorylation of Ser694 by the PIKK kinase, ATM.

This Ser694 residue is located in the phylogenetically conserved C-terminal part of HIC1 for which no functional motifs or interacting partner had been identified so far. This was in striking contrast with the poorly conserved HIC1 central region which contains a CtBP-interacting motif and a SUMOylation consensus site [4]. Our results provide thus the first evidence for a function of the highly conserved C-terminus, as an essential player regulated by PTMs of the DNA repair function exhibited by HIC1. This C-terminus is absent in the highly related HIC2/HRG22 homolog strongly suggesting that it does not play a role in DNA damage repair.

Another specific PTM of HIC1 in case of repairable DNA damage is the acetylation of the conserved Lysine 623 in the C-terminal end, probably by the histone acetyltransferase TIP60, as suggested by its GxGK<sup>623</sup> consensus and the use of TIP60 specific inhibitor (data not shown). The simultaneous phosphorylation of Ser694 and acetylation of Lys623 appears similar to the ATM activation through autophosphorylation by ATM and Acetylation by TIP60 also in response to DNA damage [21]. However, the similar activity observed for wild-type HIC1 and for a K623A point mutant strongly argued against an essential functional role for Lys623 acetylation in DNA damage repair (data not shown).

Finally, the phosphorylation of Ser285 has not been further studied since this residue found in the poorly conserved central region is unique to the human HIC1 protein in striking contrast with the phylogenetically conserved SUMOylation consensus site at Lys314.

This ATM-mediated phosphorylation could possibly regulate the interaction of HIC1 with one of its interacting partners either positively to favor DNA repair or negatively to open heterochromatin as shown for KAP1 [1]. However, a yeast two-hybrid screening experiment using the conserved HIC1 C-terminus as a bait failed to select potential candidates. It could be

worthwhile to reproduce this experiment with a S694D point mutant mimicking constitutive phosphorylation mutant S694D.

This Ser694 phosphorylation occurs in the C-terminal part adjacent to the Zinc Finger DNA-binding domain. Therefore, it might fine-tune the DNA-binding activity of HIC1 as demonstrated for the CREB transcription factor [22]. Our preliminary results indicate that in fractionation experiments HIC1 proteins phosphorylated on Ser694 are excluded from chromatin and found in the soluble nucleoplasmic fraction (data not shown)

Several ATM substrates are evicted from chromatin such as KAP1, the obligate co-repressor of the KRAB-Zinc fingers family or the histone demethylase, KD2MA [1]. Therefore, ChIP-Seq experiments comparing HIC1 binding sites in control conditions or upon induction of repairable damages together with RNA-Seq analyses would help to decipher the impact of this ATM phosphorylation on HIC1 transcriptional activity and/or its function in heterochromatinization through its interaction with NuRD, CtBP and Polycomb repressive complexes.

As a whole, our studies identify HIC1 as a novel ATM substrate playing an important role in the cellular response to DNA damage.

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## 7. LEGENDS TO FIGURES

### **Figure 1: HIC1 is phosphorylated by PIKK kinases in response to DNA damage.**

A) Schematic representation of the human HIC1 protein with the N-terminal BTB/POZ domain, the MK<sup>314</sup>HEP SUMOylation motif and the five C-terminal *Krüppel* C<sub>2</sub>H<sub>2</sub> Zinc fingers motifs. The sequences surrounding Ser694 in the C-terminal end are highly conserved



among species. A complete CLUSTAL alignment of the C-terminal region is shown as Supplementary Figure 1.

**B)** HIC1 is phosphorylated by PIKK kinases after DNA damage *in vivo*. HEK293T cells were transfected for 48h with the empty pcDNA3-FLAG expression vector or wt FLAG-HIC1. Cells were either mock-treated with DMSO (-) or treated with 20  $\mu$ M Etoposide (+) for 1h. After removal of the drug, cells were allowed to recover in normal medium for various times and subjected to immunoprecipitation (IP: FLAG) followed by immunoblotting analyses with the p-S/TQG antibodies specific for ATM/ATR substrates. The Input (1%) were analyzed with HIC1 antibodies as control and anti pChk2T68 and  $\gamma$ H2AX antibodies to monitor the DDR. Actin was used as a loading control.

**C)** Endogenous HIC1 proteins are phosphorylated by PIKK kinases after DNA damage *in vivo*. BJ-hTERT fibroblasts were treated with DMSO (-) or with 80  $\mu$ M Etoposide (+) for 1h. Total proteins extracts were analyzed as described above.

**Figure 2: Identification of HIC1 Ser694 as a phosphorylation site by PIKK kinases in response to DNA damage.**

**A)** Summary of HIC1 posttranslational modifications (PTMs) identified by tandem mass spectrometry both in control conditions and in Etoposide-treated cells (black) and specifically in Etoposide-treated cells (red).

**B)** Identification of Ser694 as a HIC1 phosphorylation site by site-directed mutagenesis. HEK293T cells were transfected with the indicated empty FLAG, FLAG-HIC1 wt and FLAG-HIC1 S694A expression vectors. 48h after transfection cells were incubated for 1h with 20 $\mu$ M Etoposide (+) or DMSO (-) as control before direct lysis in denaturing conditions. Total cell extracts were analyzed by Immunoblotting (WB) with antibodies specific for

phosphorylated Ser694 (pHIC1Ser694) and FLAG as a control.  $\gamma$ H2AX and actin levels were used as control for DSBs induction and equal loading respectively.

C) Ser694 is the only site phosphorylated by PIKK kinases. As in panel B, HEK293T cells were transfected with the indicated expression vectors and treated or not with Etoposide. Whole-cell extracts immunoprecipitated with the anti-FLAG antibodies (IP FLAG) and 1% of each lysate (Input 1%) were immunoblotted with the p-S/TQG and anti-FLAG antibodies. In contrast with direct lysis conditions (panel B), HIC1-SUMOylated forms which are unstable are not detectable by immunoprecipitation.  $\gamma$ H2AX and actin antibodies were used as controls.

**Figure 3. HIC1 phosphorylation on Ser694 is required for efficient DSBs repair.**

A) Cos7 cells were transfected for 48h with wt FLAG-HIC1 or with the non-phosphorylatable S694A point mutant. Cells were either treated with DMSO (-) or with 20  $\mu$ M Etoposide (+) for 1h. After removal of the drug, cells were allowed to recover in normal medium for various times (2-24h) and subjected to neutral Comet assays monitoring DSBs. The percentage of Comet positive cells reflecting the unrepaired DNA breaks is depicted after counting at least 100 cells in each condition. The error bar indicates mean  $\pm$  standard deviation of three independent experiments (NS: not significant).

B) Representative Comet images of cells treated with DMSO or with Etoposide for 1h, with or without recovery in normal medium for 4 and 24h, respectively.

C) Western blot analyses. Samples of cells in each condition were taken before the Comet assays and immediately lysed in Laemmli loading buffer. These whole cell extracts were analyzed by Western blot with anti-FLAG antibodies to detect HIC1 and its SUMOylated

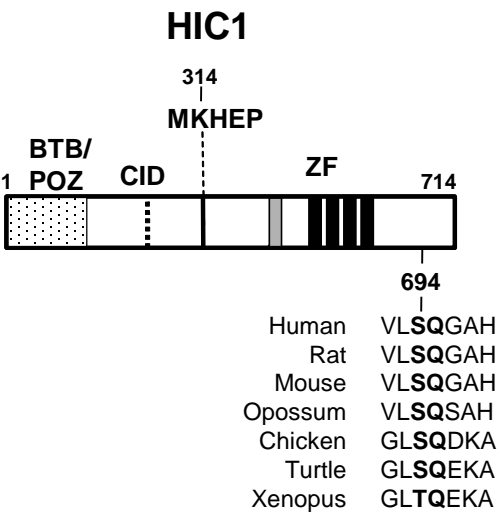
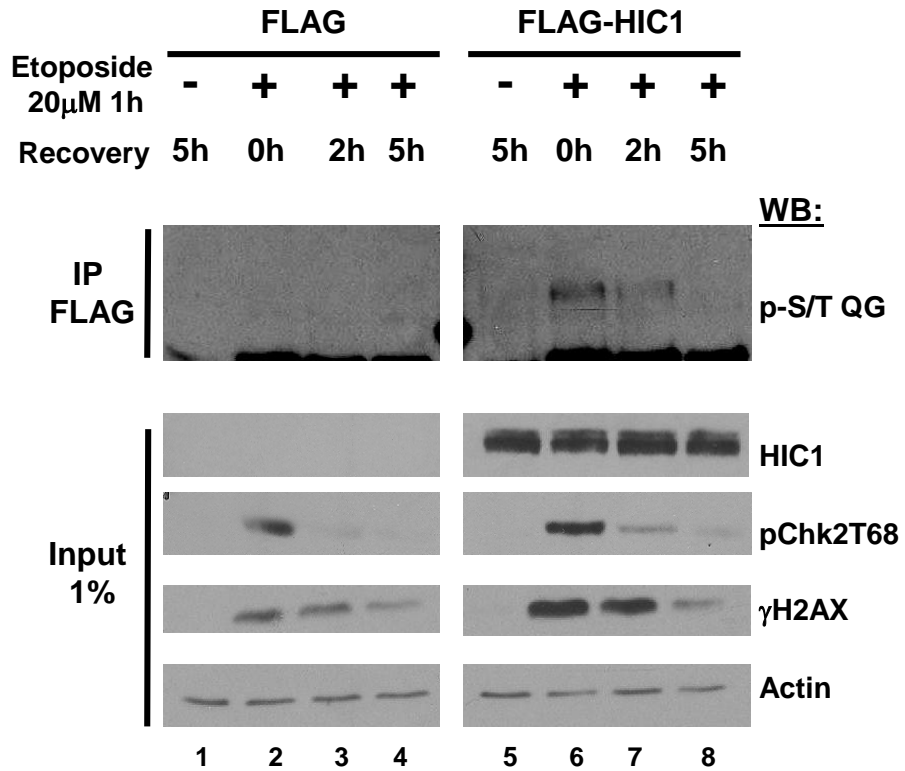
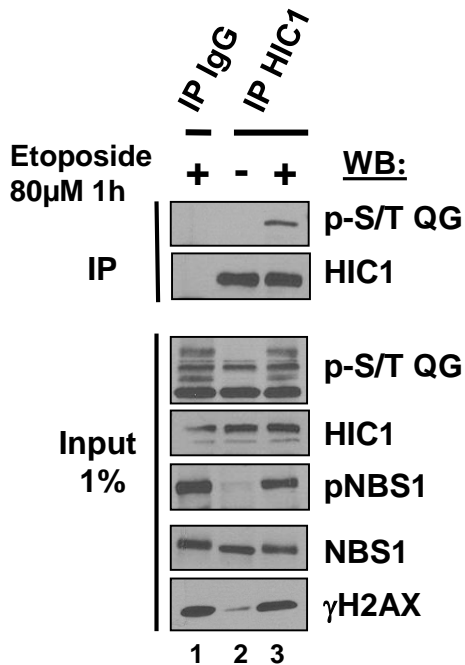
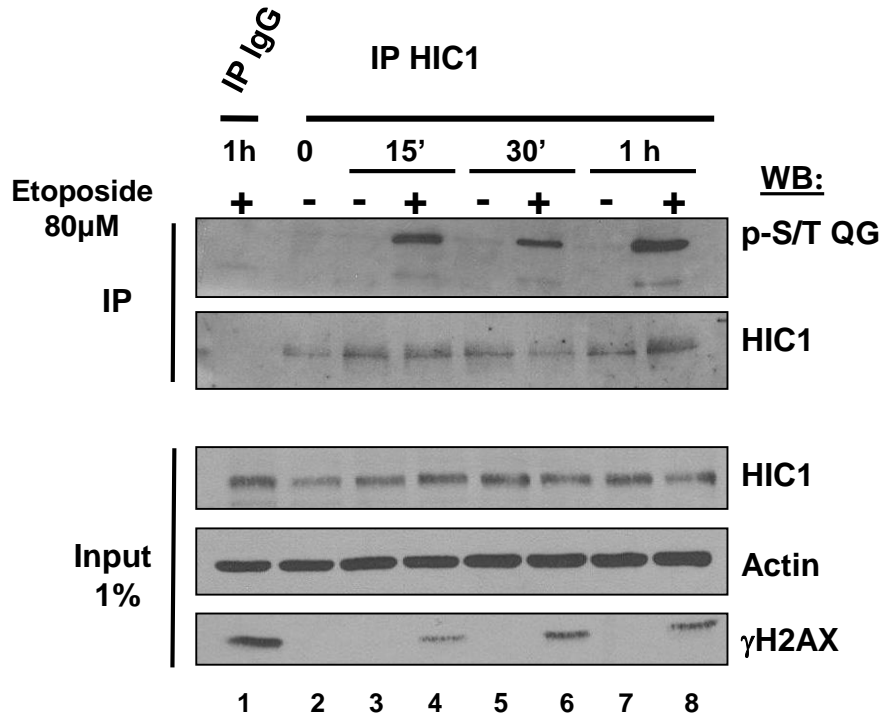
forms.  $\gamma$ H2AX and actin levels were used as controls for DSB induction and equal loading, respectively.

**Figure 4: HIC1 phosphorylation on Ser694 in response to repairable DNA damage is mainly dependent on ATM.**

**A)** A specific ATM inhibitor inhibits phosphorylation of endogenous HIC1 proteins by PIKK on Ser694. BJ-hTERT fibroblasts were pre-incubated or not with the ATM specific inhibitor KU-55933 (5  $\mu$ M) for 1h and immediately after were either mock-treated with DMSO (-) or treated with 80  $\mu$ M Etoposide (+) for 1h. Cells were subjected to immunoprecipitation with control rabbit IgG or with the anti-HIC1 antibodies followed by immunoblotting analyses with the p-S/TQG antibodies specific for ATM/ATR substrates. The Input (1%) were analyzed with HIC1 antibodies as control and with the p-S/TQG and  $\gamma$ H2AX antibodies to monitor the induction of damages and the inhibitory effect of KU-55933. Actin was used as a loading control.

**B)** FLAG-HIC1 proteins are phosphorylated by ATM on Ser694 after DNA damage. HEK293T cells were transfected with the FLAG-HIC1 expression vector, treated with 20 $\mu$ M Etoposide for 1h together with DMSO (6 left lanes) or the ATM inhibitor at 5 $\mu$ M (5 right lanes) for the indicated time. Total proteins extracts were directly analyzed by immunoblotting with the indicated antibodies.

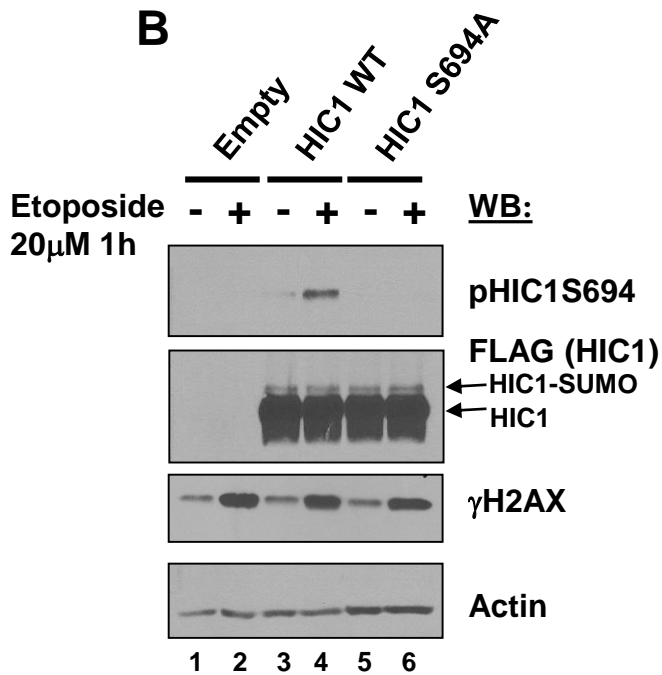
**C)** A DNAPK-cs inhibitor does not inhibit phosphorylation of FLAG-HIC1 on Ser694 after DNA damage. HEK293T cells were treated as described above in presence of the DNAPK-cs inhibitor KU-57788 at 10 $\mu$ M.

**A****B****HEK 293T****C****BJ-hTERT****D****BJ-hTERT**

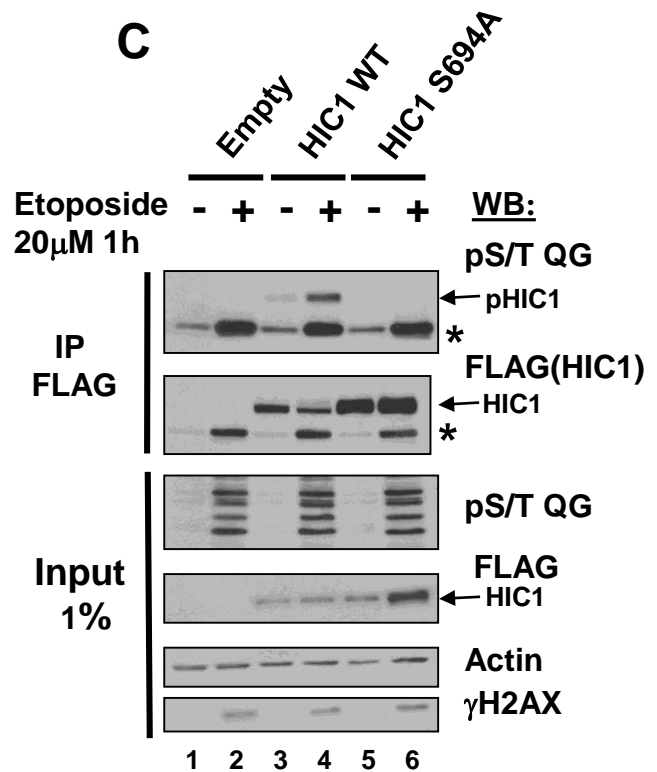
**A**

Peptides (Numbering from Q14526-2)	Modification Sites	DMSO	ETOP
216-RcsPLcGLDLSK-227	Sp218	X	X
244-ELPPRPDsPPsAGPAAYK-261	Sp251/Sp254	X	X
278-LEEAApPsDPFR-289	Sp285		X
315-HEPGLGsYGDELGR-328	Sp321	X	X
341-GGDAAVsPGGPPLGLAPPR-360	Sp347	X	X
361-YPGsLDGPGAGGDGDDYK-378	Sp364	X	X
565-IHsGEKPYECQVCGGK-580	Sp567	X	X
622-GkLDFPEGVFAVAR-635	K <sub>Ac</sub> 623		X
678-FTAELGLsPDK-688	Sp685	X	X
689-AAEVLsQGAHLAAGPDGR-706	Sp694		X
707-TIDRFsPT-714	Sp712	X	X

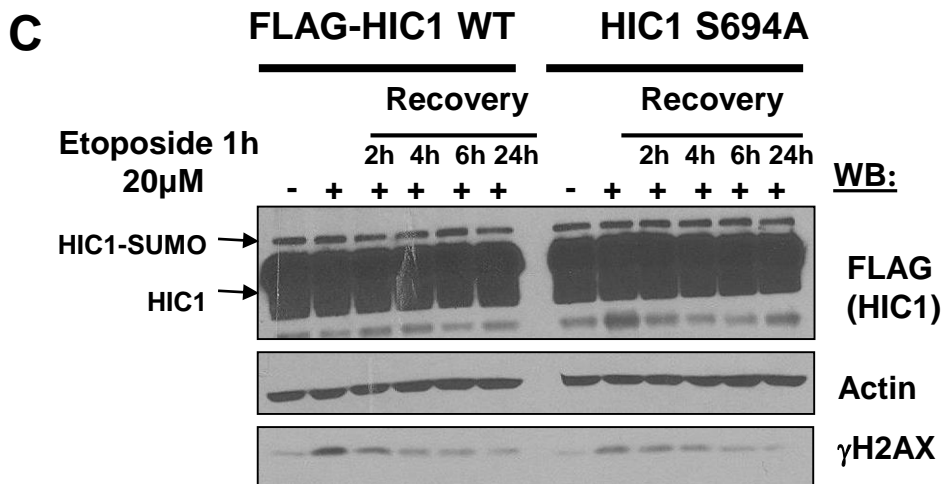
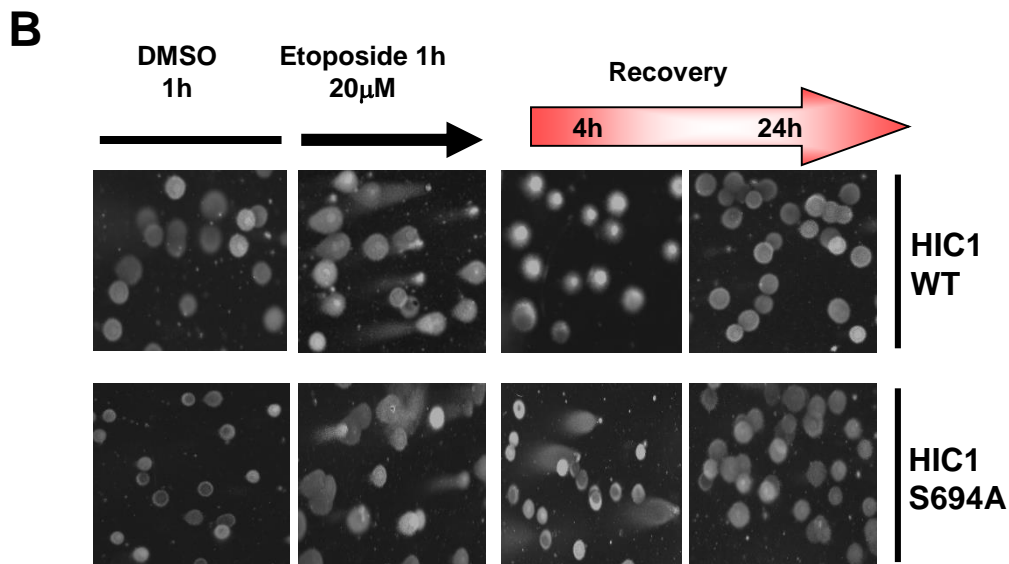
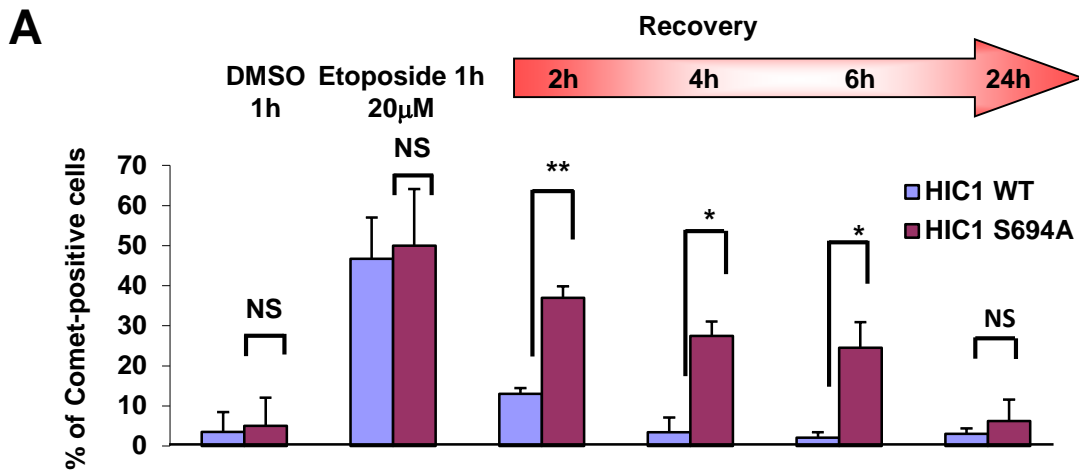
**B**

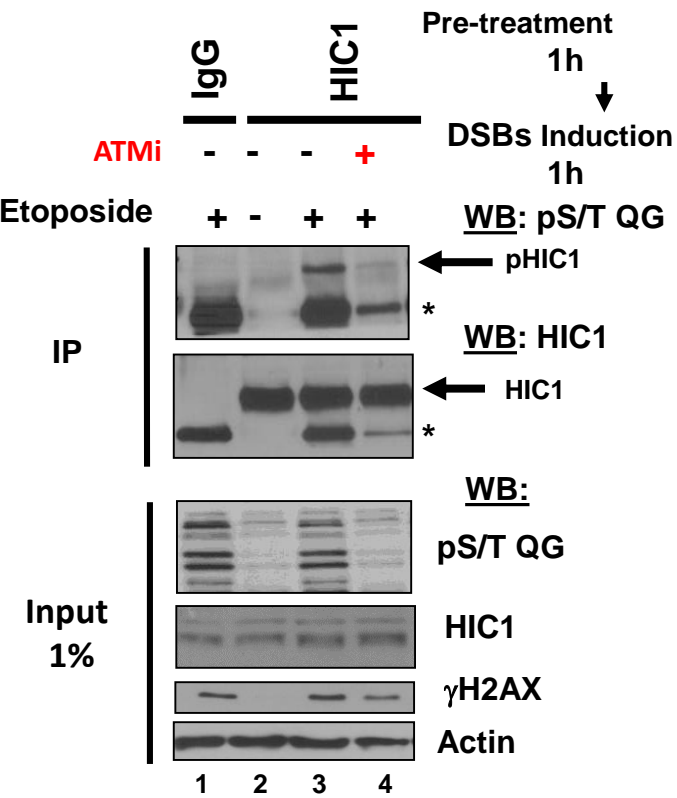
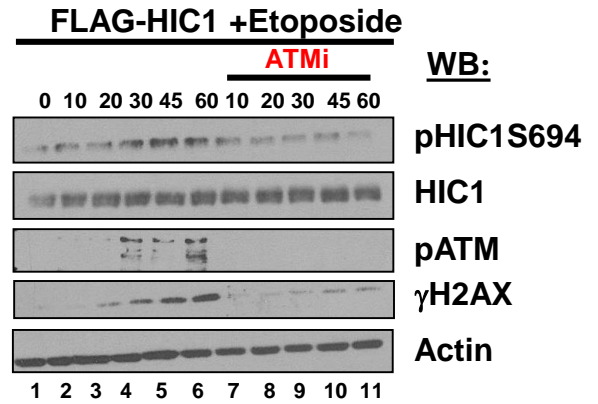
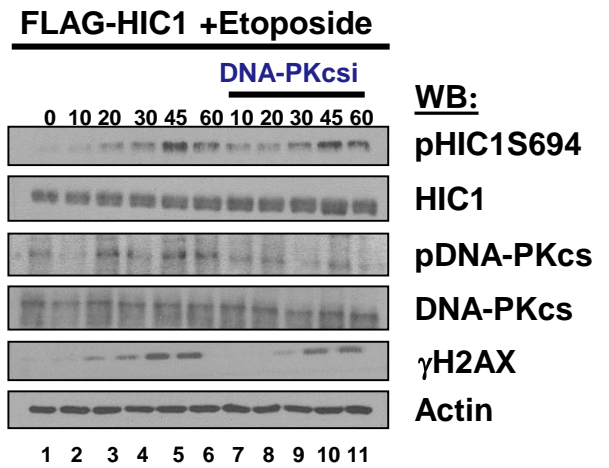


**C**



Paget et al., Figure 2



**A****B****C**

Paget et al., Figure 4