Histone H1 eviction by the histone chaperone SET reduces cell survival following DNA damage

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Summary statement:

SET removes histone H1 from chromatin and its absence results in enhanced resistance to various types of DNA lesions.

Abstract

Many chromatin remodeling and modifying proteins are involved in the DNA damage response by stimulating repair or inducing DNA damage signaling. Interestingly, here we identified that down regulation of the H1-interacting protein SET results in increased resistance to a wide variety of DNA damaging agents. We found that this increased resistance is not the result of an inhibitory effect of SET on DNA repair, but rather the consequence of a suppressed apoptotic response to DNA damage. We further provide evidence that the histone chaperone SET is responsible for the eviction of H1 from chromatin. Knock down of H1 in SET-depleted cells resulted in re-sensitization of cells to DNA damage, suggesting that the increased DNA damage resistance in SET-depleted cells is the result of enhanced retention of H1 on chromatin. Finally, clonogenic survival assays show that SET and p53 are epistatic in attenuating DNA damage-induced cell death. Altogether, our data show a role for SET in the DNA damage response as a regulator of cell survival following genotoxic stress.
Introduction

The integrity of DNA is constantly challenged by exogenous and endogenous DNA damaging agents that can cause a wide variety of DNA lesions. Cells have evolved a sophisticated network of different pathways called the DNA damage response (DDR) (Jackson and Bartek 2009) to properly respond to genomic insults. This DDR includes activation of different DNA repair mechanisms, each endowed to detect and remove specific subsets of DNA lesions (Hoeijmakers 2009), and a complex damage signaling network to control cell cycle progression, transcriptional reprogramming and apoptosis.

The DDR is active in different cell types, genomic environments and cell cycle phases, but always acts in the context of chromatin. Chromatin is considered to impose an important constraint for repair proteins to access DNA lesions. However, it has also been shown to serve as a crucial regulatory platform for many DNA damage signaling events (Smerdon 1991; Riley et al. 2008; Soria et al. 2012; Mandemaker et al. 2014). In accordance with the important role of chromatin in the DDR, many proteins have been implicated in regulating chromatin changes and plasticity in response to DNA damage, including ATP-dependent chromatin remodelers, histone modifying enzymes and histone chaperones (Escargueil et al. 2008; Lans et al. 2012; Soria et al. 2012; Dinant et al. 2013; Mandemaker et al. 2014). For example, the DDR-kinases ATM and ATR are important for phosphorylation of histone H2AX (γH2AX) during the DDR, which stimulates the assembly of repair complexes and is involved in the regulation of cell cycle checkpoints and transcription (Yuan et al. 2010). Deletion of the BRG1 and BRM catalytic ATPase subunits of the SWI/SNF chromatin remodeling complex also interferes with the induction of γH2AX and impairs repair (Park et al. 2006). Furthermore, histone chaperones are involved in the DDR by assembling and disassembling nucleosomes during the repair processes (Mandemaker et al. 2014). The histone chaperone FACT has been shown to stimulate exchange of histones H2A and H2B at sites of UV-induced DNA damage thereby promoting transcriptional restart after UV irradiation (Dinant et al. 2013). Alternatively, histone chaperones may also replace histones by specific histone variants. For example, HIRA deposits newly synthesized histone H3.3 onto the chromatin at damaged sites (Adam et al. 2013).

Thus far most research has focused on the remodeling, modifications and exchange of core histones and their variants. However, increasing evidence shows that also the linker histone H1 plays an important role in the DDR, even though its exact role remains elusive. While one study shows that a 50% reduction in H1 levels results in enhanced H2AX and Chk1 phosphorylation and increased survival after DNA damage in mouse embryonic stem cells (Murga et al. 2007), another study found that H1-depleted cells were more sensitive to genotoxic agents (Nishiyama et al. 2009). More recently, a more specific DDR-related role for H1, as an ubiquitin substrate in the RNF8/RNF168 pathway, was described (Thorslund et al. 2015; Mandemaker et al. 2017). Ubiquitylation of H1 leads...
to the recruitment RNF168 that triggers ubiquitylation of core histones and eventually leads to the binding of downstream DDR factors such as 53BP1 and BRCA1 (Thorslund et al. 2015; Mandemaker et al. 2017). Together, these data indicate that histone H1 is an important regulator of the DDR.

In this study, we set out to further investigate the role, and underlying regulatory mechanism, of histone H1 in the DDR. Among many other chromatin-associated factors, we identified the proto-oncogenic protein SET/TAF-1β as a H1 interacting protein. Since SET was previously identified as a histone H1 chaperone (Kato et al. 2011), we here focused on the role of SET in DDR. SET was first identified as an inhibitor of protein phosphatase 2A (PP2A) (Li et al. 1995) and is a negative regulator of c-Myc and other pro-survival pathways (Arnold and Sears 2008). In addition, SET inhibits the pro-apoptotic NM23-H1 complex, involved in the suppression of tumor metastasis (Fan et al. 2003). In line with these functions, overexpression of SET is found to be involved in the initiation of various types of cancer (Christensen et al. 2011; Hung and Chen 2017). SET also has diverse functions in transcription; as part of the INHAT complex it represses transcription by inhibiting the activity of the CBP/p300 transcriptional co-activators (Seo et al. 2001; Loven et al. 2003), but it also stimulates transcription by remodeling the chromatin (Okuwaki and Nagata 1998; Gamble et al. 2005). In addition, SET directly binds to p53 in unstressed conditions, thereby repressing its activity (Kim et al. 2012; Wang et al. 2016).

Here we show that down regulation of SET results enhances resistance to a wide variety of genotoxic agents, without stimulating DNA repair. Using different imaging techniques, we show that SET is involved in the dissociation of H1 from chromatin which suggests that depletion of SET will result in increased H1 binding to chromatin. In line, knockdown of H1 reversed the increased DNA damage resistance induced by SET depletion, indicating that this effect is directly caused by its H1 chaperone activity. Furthermore, survival experiments indicated that p53 and SET are epistatic for the increased resistance to genotoxic stress. Together, our data suggests that both histone H1 and SET, like p53, have an important role in regulating cellular survival after DNA damage.
Results

SET depletion leads to DNA damage resistance

To address the role of H1, and more specifically its chromatin deposition, during the DDR (Murga et al. 2007; Nishiyama et al. 2009), we set out to identify factors involved in H1 regulation using quantitative proteomics on pulldowns for GFP-tagged histone H1.2. In addition to core histones and chromatin involved factors, we identified the oncoprotein SET, a suggested H1 chaperone (Kato et al. 2011; Zhang et al. 2015), as H1 interactor (Table S1). (Kato et al. 2011; Zhang et al. 2015)

To address the involvement of SET in the DNA damage response, we performed clonogenic survival experiments following UV exposure with mouse embryonic stem (ES) cells in which SET is knocked down by stable shRNA expression (Fig. 1A) (Edupuganti et al. 2017). Surprisingly, upon SET knockdown (KD), cells were less sensitive to UV irradiation than cells expressing a non-targeting control shRNA (Fig. 1B). This effect is not specific for ES cells, as increased UV-resistance was also observed in U2OS cells following siRNA mediated SET knockdown (Fig. 1C, D). Depletion of the essential nucleotide excision repair (NER) gene XPC resulted in the expected increased UV-sensitivity. The SET knockdown-induced DNA damage resistance is for an important part caused by a reduced level of UV-damage induced apoptosis, as determined by cytochrome C release from mitochondria (Figs 1E, S1). As expected, an UV-induced increase in apoptotic cells was observed 32h after UV irradiation (Dunkern et al. 2001). Induction of apoptosis was enhanced in XPA-depleted cells, while a ~50% reduction damage-induced apoptosis was observed following siRNA mediated SET knockdown.

The increased survival in SET-depleted cells is independent of repair

The SET knockdown-mediated increase in UV resistance could be explained by differences in replication as UV-induced photoproducts can block replication, thereby causing replication stress and affecting the cellular survival. To test this, we studied whether SET influences the cell cycle; however, we found no difference in cell cycle distribution upon SET-depletion in either mock or UV treated cells (Figs 2A, S2), indicating that the observed higher UV-resistance in SET-depleted cells is not a consequence of changes in replication rate.

In addition, resistance to UV-irradiation could be caused by a change in the efficiency of the repair of the UV-lesions by NER. NER is initiated by recognition of the lesion by the global genome repair (GG-NER) proteins XPC and the DDB-complex or, when lesions are in actively transcribed genes, by the stalling of RNAP II (transcription coupled repair (TC-NER)) (Sugasawa 2016). After
damage recognition, the NER reaction proceeds with helix unwinding and the lesion verification step, which is similar for both GG-NER and TC-NER. Subsequently, a 25-30 nucleotide long piece of DNA containing the damaged nucleotide is removed (Oksenych and Coin 2010; Fagbemi et al. 2011; Li et al. 2015; Marteijn et al. 2015) and finally the resulting single stranded DNA gap is filled by DNA polymerases and the nicks are sealed (Ogi et al. 2010). We tested whether SET influences the efficiency of repair of UV-induced lesions by measuring NER activity. First, we quantified the removal of UV-induced 6-4 photoproducts (6-4PPs) by NER. No change in the induction or removal of UV-induced 6-4PP lesions was observed in SET-depleted cells compared to control cells (Figs 2B, S2B), while a clear decrease in 6-4PP removal was observed in cells in XPA-depleted cells. In line with this, no difference in unscheduled DNA synthesis (UDS) (Limsirichaikul et al. 2009), a measure for the final gap-filling step of NER, was observed following SET knockdown, while depletion of XPC resulted, as expected, in a decrease of UDS signal (Fig. 2C). Together, this shows that NER-activity is not affected by SET depletion and indicates that SET depletion-mediated resistance is not caused by an increased repair activity. To further corroborate this we performed clonogenic survival assays in XP-C cells, which are deficient in GG-NER. Although these XP-C cells are very sensitive to UV irradiation, we still observed a better survival after SET depletion (Fig. 2D), indicating that the enhanced survival is independent of repair. As the above assays mainly monitor the activity of GG-NER, we also tested whether SET influenced TC-NER. TC-NER activity was assessed by the recovery of RNA synthesis (RRS) after UV-induced transcription inhibition by quantifying transcription rates using pulse labeling with the uridine analog EU (Nakazawa et al. 2010). While 16 hours after UV irradiation, the RRS was clearly reduced in TC-NER deficient XPA-depleted cells, no difference in RRS between control or SET-depleted cells was observed (Fig. 2E), indicating that TC-NER efficiency is not affected by SET.

Previously, it was shown that SET affects cell survival after DNA double strand break induction (Kalouisi et al. 2015). In line with this, we observed that SET KD also leads to increased resistance to ionizing radiation in both U2OS (Fig. 3A) and ES cells (Fig. S3A). In response to double strand breaks SET densifies chromatin via KAP1-dependent recruitment of HP1, leading to a shift in the balance between homologous recombination (HR) and non-homologous end joining (NHEJ) (Kalouisi et al. 2015). However, NHEJ and HR are not the major repair pathways involved in UV-survival (Hoeijmakers 2001) and therefore it is not likely that the observed UV-resistance in SET-depleted cells is caused by this mechanism. In line with this, KAP1-depleted cells show a similar survival as control cells upon UV exposure, while SET-depleted cells are more resistant (Fig. 3B, C). This implies that enhanced survival of SET-depleted cells is not mediated by KAP1.

As SET affects survival after UV (Fig. 1B, D) and IR-induced (Fig. 3A) (Kalouisi et al. 2015) DNA damage, we also tested the response of SET-depleted cells to other structurally different types of
DNA lesions that are repaired by different repair pathways. To this end, we performed clonogenic survival assays following treatment with hydrogen peroxide and potassium bromate that mainly induce oxidative base damage which is repaired by base excision repair and with mitomycin C that creates DNA interstrand cross-links that are repaired by the Fanconi Anemia pathway. Interestingly, SET KD resulted in higher cellular resistance to all tested DNA damaging agents and to hydroxyurea-induced replication stress (Figs 3, S3).

Altered DNA damage signaling is not the cause of the enhanced survival of SET-depleted cells

As SET depletion resulted in resistance to a wide variety of DNA damages, it is highly unlikely that the enhanced resistance in SET-depleted cells is the result of enhanced repair. Instead SET most likely affects a cellular response that is common in the cellular response to different types of DNA damage. A likely candidate of such a mechanism is DNA damage signaling by phosphorylation of histone H2AX, as this common and abundant PTM plays an important role in the DDR and is induced by different types of DNA damage (Rogakou et al. 1998; Ward and Chen 2001; Mogi and Oh 2006; Hanasoge and Ljungman 2007; Marteijn et al. 2009; Zhang et al. 2016). Furthermore, previous studies have shown that SET depletion resulted in an increased γH2AX signaling following double-strand break induction (Kalousi et al. 2015). To test if SET also affects γH2AX signaling after other types of DNA damage, we studied H2AX phosphorylation following replication stress by inducing replication fork blocks at a defined and traceable single locus (Beuzer et al. 2014). For this purpose, we expressed Lac repressors (LacR) in U2OS cells that harbor an integrated array of 256 repeats of the Lac operon (LacO) (Fig. 4A), resulting in the tethering of the LacR to the LacO-locus thereby inducing replication stress. 36h after transfection of mCherry-LacR, γH2AX signal was quantified at the Lac operon. We observed a more than two-fold reduction in γH2AX signal when mCherry-SET-LacR was targeted to LacO compared to the mCherry-LacR (Fig. 4A, B). This reduction in γH2AX signaling is not caused by a reduction in histone H2AX levels at the Lac operon following SET tethering (Figs 4C, S4A).

These findings prompted us to test whether the effect of SET on γH2AX signaling is the cause of the observed DNA damage resistance following SET depletion. Therefore, we performed colony survival assays in which H2AX phosphorylation was suppressed by inhibition of the PI3K-like protein kinases ATR, ATM and DNA-PK involved in H2AX phosphorylation (Sirbu and Cortez 2013; Guleria and Chandna 2016). As expected, inhibition of these kinases by caffeine (Sarkaria et al. 1999; Block et al. 2004) resulted in sensitization to UV (Rommelaere and Errera 1972) (Fig. 4D). Importantly, caffeine treated cells still presented an increased resistance to DNA damage upon SET depletion. Furthermore, similar effects of SET depletion on cell survival were observed when ATM and DNA-PK
were inhibited with kinase-specific inhibitors (Fig. S4B). In line with these results, siRNA-mediated depletion of histone H2AX, resulting in the absence of H2AX phosphorylation upon DNA damage (Fig. 4E), did not affect the SET knockdown-induced UV-resistance (Fig. 4F). Together our data suggest that, even though γH2AX signaling is affected by SET, it is not the cause of the increased survival of SET-depleted cells upon DNA damage.

**The enhanced survival in SET-depleted cells is dependent on histone H1**

Remodeling of the chromatin environment is important for a proper DDR (Lans et al. 2012; Polo and Almouzni 2015) and interestingly SET was previously implicated in nucleosome assembly and histone H1 chaperoning (Kato et al. 2011). More specifically, in vitro data indicate that SET plays mainly a role in the eviction of H1 from the chromatin (Zhang et al. 2015). To study whether SET has a similar H1 removal function in living cells, we first tested the effect of SET on H1 chromatin binding using fluorescent recovery after photo bleaching (FRAP) studies in cells stably expressing GFP-H1.2. In accordance with previous data (Kato et al. 2011; Edupuganti et al. 2017), we observed a slower recovery of the fluorescent intensity of GFP-H1.2 upon SET KD (Fig. 5A). The increased immobilized H1 fraction denotes a lower exchange rate of chromatin-bound GFP-H1.2 molecules, which is most likely the result of a reduced removal of chromatin-bound H1 molecules. To confirm that SET is mainly involved in unloading H1 from chromatin, we tethered mCherry-SET-LacR in LacO-array containing U2OS cells expressing GFP-H1.2, thereby inducing an increase in the local concentration of SET (Fig. 5B). In contrast to mCherry-LacR, tethering mCherry-SET-LacR at the Lac operon resulted in reduced GFP-H1.2 levels (Fig. 5C). A similar experiment with GFP-Histone H3, showed that SET tethering had no effect on H3 levels, indicating the preference of SET for histone H1 (Fig. 5D). In line with previous in vitro data (Zhang et al. 2015), these in vivo imaging experiments show that SET promotes histone H1 eviction from chromatin (Fig. 5A, B, C).

This H1 eviction function of SET suggests that SET depletion will result in enhanced retention of histone H1 on chromatin, which subsequently might affect chromatin structure and compaction or disturb specific transcriptional programs resulting in the observed increase in cellular survival. This hypothesis would suggest that reduction of H1 levels would overcome the increased resistance to DNA damage upon SET depletion. To test this, we depleted H1 in the presence or absence of SET. A combination of three different, previously published, siRNAs was used to target all six canonical histone H1 variants (Thorslund et al. 2015) and KD of H1.2 was confirmed by western blot (Fig. 5E). While histone H1 depletion alone did not affect cellular survival in response to UV-induced DNA damage, it reduced the resistance to UV damage induced by SET KD (Figs 5F, S5A). Upon oxidative damage, H1 depletion leads to higher sensitivity and also completely suppressed the increased...
survival in SET-depleted cells (Fig. S5B). Together this suggests that the increased DNA damage survival observed in SET-depleted cells is substantially caused by enhanced levels of chromatin bound H1, due to a loss of the histone H1 chromatin eviction function of SET.

Previously, it has been shown that enhanced H1 binding at p53-regulated promoters hampered gene expression of P53 regulated genes, thereby inhibiting apoptosis (Nishiyama et al. 2009). As SET-depleted cells displayed enhanced levels of chromatin bound H1 (Fig. 5A, B, C) and reduced apoptosis in response to DNA damage (Fig. 1E), we tested if SET is involved in the DNA damage-induced p53-response. Clonogenic survival experiments show that knock out of p53 resulted in a similar resistance to DNA damage as SET depletion (Fig 5G, H). Importantly, SET KD had no effect on the cell survival in p53 KO cells, indicating that SET and p53 function in the same pathway. Next, we tested if SET acted upstream of p53 by stimulating p53-induced gene expression by histone H1 unloading at p53-regulated promoters. To do so, we performed RT-qPCR to analyze the levels of the p53-regulated and DNA damage induced genes p21 and Noxa. We did not observe any significant differences in the induction of p21 and Noxa upon etoposide treatment after SET depletion (Fig. S5C). This data shows that SET KD does not hamper the expression p53-regulated genes, but rather suggests that SET acts downstream of p53 in preventing apoptosis in response to structurally different types of DNA damage (Figs 1E, 3).

Discussion

In this study, we identified SET as an H1 interactor and showed that SET stimulates chromatin eviction of H1 in living cells, in line with previously proposed H1-chaperoning functions of SET (Kato et al. 2011; Zhang et al. 2015). Interestingly, depletion of SET resulted in enhanced cellular resistance to structurally diverse DNA lesions (Figs 3, S3), which are targeted by different repair pathways. No effect of SET depletion was found on repair efficiency of either UV-induced lesions (Fig. 2B, C) or DSBs (Kalousi et al. 2015), suggesting that SET is not a general suppressor of DNA repair, but more likely functions in a response common to many types of DNA damages, like DNA damage signaling, chromatin remodeling or the induction of apoptosis.

Previously, it was found that SET functions in the response to DSBs by recruiting KAP1 and HP1 to lesions, thereby affecting DNA damage signaling by phosphorylation of H2AX and shifting the balance between HR and NHEJ (Kalousi et al. 2015). In line with the cellular response of SET-depleted cells to a wide variety of different types of DNA damages, in which NHEJ or HR are not expected to play a role, our data shows that the increased survival rates upon DNA damage are independent of KAP1 (Fig. 3B) or γH2AX signaling (Fig. 4D, E, F). Our data therefore suggests that the damage resistance following SET depletion is likely caused by other functions of SET.
Our data shows that SET is involved in the chromatin eviction of H1 and suggests that its histone chaperone activity is needed to reduce cellular survival in response to DNA damage. Hence, our data are consistent with a model whereby enhanced levels of chromatin-bound histone H1, caused by SET depletion, prevent efficient induction of cell death in response to DNA damage (Figs 5F, S5). Thus far it is not clear if the effect of SET KD on survival is due to enrichment of H1 throughout the entire chromatin or that SET acts on specific genomic regions. One scenario would be that upon DNA damage H1 unloading is necessary for the transcriptional activation of specific genes involved in apoptosis, which upon SET KD are no longer activated. Another possibility is that SET changes the chromatin conformation at DNA damage sites. Several labs have described a loss of H1 around DSBs (Sellou et al. 2016; Strickfaden et al. 2016; Clouaire et al. 2018). It is possible that SET is involved in actively removing H1 from the regions around the lesions. However, as it is generally assumed that an open chromatin conformation stimulates repair and thereby survival and that the presence of SET decreases cellular survival and does not affect repair, this seems a more unlikely scenario. Interestingly, since our FRAP studies show a substantial immobilization of H1 upon SET depletion (Fig. 5A), this rather suggests a genome-wide effect of SET on the chromatin loading of histone H1.

Our data shows no obvious effect on cell survival following SET depletion in the absence of p53 (Fig. 5G, H), indicating that SET is epistatic with the p53 pathway in reducing cell survival following genotoxic stress. In line with the increased chromatin binding of histone H1 in a genome-wide manner upon SET KD, SET does not seem to majorly affect specific p53-regulated transcription upon DNA damage (Fig. S5C) (Nishiyama et al. 2009; Lieberman et al. 2017). This suggests that SET may play a role downstream of p53, for example in stimulating apoptosis upon genotoxic stress. Of note, it was previously shown that in unstressed conditions SET can inhibit p53 acetylation, thereby reducing p53 transcriptional activity (Kim et al. 2012; Wang et al. 2016). This indicates that SET may play a multifaceted role in the intricate regulation of p53-mediated transcriptional programs. Moreover, SET is previously described as an oncogene and is shown to have an inhibitory effect on PP2A activity (Li et al. 1995), an important phosphatase implicated in DDR, which may further confound the dissection of the molecular mechanism of SET in the DDR.(Li et al. 1995)

It is intriguing to note that SET sensitizes cells to DNA damage, as thus far most proteins found to be involved in the DDR, like DNA repair and damage signaling proteins, protect cells against genomic insults. Possibly, our findings represent a mechanism which prevents the formation of mutations following DNA damage, in which SET actively stimulates cell death in cells that cannot reliably repair all DNA lesions, similarly to the established pro-apoptotic role of p53 in the DDR (Lieberman et al. 2017). In summary, we identified a new role for the histone H1 chaperone SET in
the DDR, in which unloading of H1 from chromatin is necessary for efficient reduction of cellular survival in response to DNA damage.

Methods

Cell lines and cell culture

U2OS, HeLa, Hct116 and XP4PA (sv40) cells were cultured in DMEM/F10 medium (Lonza) supplemented with 10% fetal calf serum (FCS) and 1% penicillin-streptomycin (PS, P0781 Sigma). CSRO (hTERT) cells were cultured in F10 supplemented with 15% FCS and 1% PS. Mouse embryonic stem cells (mES) were cultured in DMEM/BRL-conditioned medium containing 10% FCS, 1% PS, 1% non-essential amino acids (Lonza), 0.2% β-mercaptoethanol (Invitrogen) and 1000 U/ml leukemia inhibitory factor on gelatin (0.1%) pre-coated dishes. For stable isotope labeling by amino acids in cell culture (SILAC) experiments, cells were cultured for at least 10 cell doublings in lysine and arginine deficient DMEM (Thermo Scientific) with 10% dialyzed FCS (Invitrogen), 1% PS, 1% non-essential amino acids and 1% ultraglutamine (200 mM Lonza), supplemented with either light [12C6]lysine (73 μg/mL, Sigma) and [12C6, 14N4]arginine (42 μg/mL, Sigma) or similar concentrations of heavy [13C6]lysine and [13C6, 15N4]arginine (Cambridge Isotope Laboratories). All cells were cultured at 37°C and 5% CO2 in a humidified incubator. For UV treatments, cells were washed with phosphate-buffered saline (PBS) and irradiated with using a 254 nm Philips TUV UV-C lamp. Transfections with RNAi were performed with RNAiMax (Invitrogen) according to the manufacturer’s protocol 3 days before treatments; siControl: UGGUUUACAUGUCAGAUAA; siSETa: UCUCAAAGAUUUCAUGUAA; siSETb: smart pool Dharmacon (L-019586-00-0005); siH1: CCUUUAAACUCAGCAAGAA, CCUCUCAACUCAACACAGAA, CAGUGAAACCCCAAAGCAA (Thorslund et al. 2015); siXPC: CUGGAGUUUGAGACAUUAUC; siXPA: CUGAUGUAACACAAGCUUA; siH2AX a: GUCUCCAGAAGACAGUA; siH2AX b: CAACAGAGACGCAGAU; siKAP1: GCAUGAAACCUUGUGCU. Transfections with mCherry-LacR, mCherry-SET-LacR and GFP-H2AX vectors (2 µg) are performed with Fugene (Promega) according to manufacturer’s protocol 16h before fixation, unless stated otherwise. For these experiments a U2OS (2-6-3) cell line containing a chromosomal array of 256 lac operator repeats and a CFP reporter gene harboring 24 repeats of the MS2 bacteriophage RNA hairpins was used (Janicki et al. 2004). The mCherry-SET-LacR vector was made by ligating a PCR product containing the SET cDNA into the mCherry-LacR construct digested with Ascl. GFP-H1.2 construct was made by cloning a PCR product from a H1.2-Flag (kind gift from
Kyosuke Nagata) in a pENTR4-eGFP-C1 vector, followed by an LR clonase reaction to a pLenti-CMV vector (Campeau et al. 2009). Stably expressing GFP-H1.2 cell lines were made by lentiviral transduction. Medium containing lentivirus was harvested 2 days after transient transfection of HEK293 cells with pLenti-CMV-GFP-H1.2, pMDLg/pRRE, pRSV-REV and pMD2.G constructs. Control (scrambled, GCTCTATGGGACGAAGGTGAT) and SET (ATCTCCGTTTCTGTCTTAAT) shRNA oligos, used in mES cells (Edupuganti et al. 2017), had a forward MluI site overhang and a reverse ClaI site overhang. In addition, an NdeI site was inserted after the terminating signal of 5 “T” nucleotides to allow oligo insertion verification. Single stranded oligonucleotides were purchased (IDT) and hybridized to make double stranded oligos using standard hybridization procedures. Hybridized oligonucleotides were inserted into pLVTHM vector between MluI and ClaI sites. Lentiviral particles were prepared by transfecting pLVTHM-shSET, LV-VSVG and CMV-dr8.9-dvpr packaging plasmids into HEK 293T cells using Lipofectamine2000 (Invitrogen). Low passage R1 ESCs were infected with low titer of lentiviral particles to prevent multiple integrations. Several GFP positive colonies were picked, clonally expanded and checked for highest knockdown efficiency. All cells were routinely checked for mycoplasm contamination.

**Identification of GFP-H1.2 interactors with MS**

GFP-H1.2 containing protein complexes were enriched from nuclear extracts of SILAC labelled HeLa cells stably expressing GFP-H1.2 by immunopurification with GFP-trap beads (Chromotek) as described previously (Aydin et al. 2014). In short, GFP-H1.2 expressing or WT SILAC labeled HeLa cells from six 15 cm dishes were harvested by scraping in PBS. Nuclei were isolated by resuspending cells in 2x pellet volume Hepes buffer (10 mM Hepes pH 7.6, 1.5 mM MgCl$_2$, 10 mM KCl, 0.5 mM DTT and protease inhibitor cocktail (Roche)), douncing the cells using pestle A of a Dounce homogenizer and centrifugation for 10 min at 3000 rpm. Nuclei were lysed in Hepes buffer B (20 mM Hepes pH 7.6, 1.5 mM MgCl$_2$, 150 mM NaCl, 25% Glycerol, 0.5 mM DTT and protease inhibitor cocktail (Roche)) using pestle B from the dounce homogenizer. Chromatin was fragmented by MNase (25U, Sigma) digestion for 1h at 40°C. Lysates were cleared by centrifugation (15 min at 13.000 rpm) and incubated with GFP-trap beads (Chromotek) for 4h at 4°C. Beads were washed four times in Hepes buffer B and mixed together. Proteins were eluted with Laemmli sample buffer and loaded onto a 4-15% gradient SDS-PAGE gel (Biorad). After running, the gel was fixed and stained with Roti-blue (Carl Roth GmbH) according to manufacturer’s protocol. Gel lanes were cut into 2-mm slices using an automatic gel slicer and subjected to in-gel reduction with dithiothreitol, alkylation with iodoacetamide and digestion with trypsin (Promega, sequencing grade) (Schwertman et al. 2013). Nanoflow LC-MS/MS was performed on a quadrupole Orbitrap (Q-Exact, Thermo Fisher Scientific)
mass spectrometer equipped with an EASY-nLC 1000 (Thermo Fisher Scientific). Peptide samples were loaded onto ReproSil C18 reversed phase column (20 cm x 75 μm) and eluted with a linear gradient (70 min) from 5 to 80% acetonitrile containing 0.1% formic acid at a constant flow rate of 300 nl/min. Fragmentation of the peptides was performed in a data-dependent acquisition mode. MS1 spectra were collected at a resolution of 70,000, with an automated gain control target of 1E6 and a max injection time of 50 ms. The 10 most intense ions were selected for MS/MS. Precursors were filtered according to charge state (2-7z), and monoisotopic peak assignment. Previously interrogated precursors were dynamically excluded for 30 s. Peptide precursors were isolated with a quadrupole mass filter set to a width of 2.0 Th. MS experiments were performed in duplo with label swap to easily exclude contaminants and reduce false positive hits. Raw MS data was analyzed using MaxQuant software (version 1.3.0.5) (Cox et al. 2009; Cox et al. 2011) with false protein discovery rate set at 1% and minimum peptide length of 7. MS/MS spectra were searched against the human Uniprot fasta database (version 2013) using Andromeda search engine (Cox et al. 2011). Contaminants and reverse hits were removed.

Clonogenic survival assays
Cells were seeded in 6-well plates a day before treatment (U2OS and mES 400 cells/well, Hct116 250 cells/well). Cells were treated with a single doses of UV-C or IR, continuous exposure to KBrO₃ (Sigma) or H₂O₂ (Sigma) or treated for 1 hour with Mitomycin C (Kyowa) or 24 h with Hydroxyurea (Sigma) or Illudin S at the indicated concentrations. Each experiment was performed in triplicate. After 6-8 days the colonies were fixed and stained with 50% methanol, 43% H₂O, 7% acetic acid and 0.1% Brilliant blue R (Sigma). Number of colonies was counted using a GelCount™ (Oxford Optronix, version 1.1.2.0). The survival was plotted as the relative amount of colonies after treatment compared to the non-treated samples.

FRAP
GFP-H1.2 expressing HeLa cells were seeded on coverslips and kept at 37°C and 5% CO₂. A Leica sp5 confocal laser scanning microscope with a 63x oil immersion objective combined with the Leica LAS AF software was used for image acquisition. Imaging was performed at 1400 Hz with a line averaging of 2 and a 12x zoom. For FRAP analysis a strip of 32 pixels high, spanning the entire width of the cell nucleus was bleached using a 488 nm laser with high laser power (1 frame, 100%). Recovery of the fluorescent signal was measured every 0.2 seconds for 200 seconds. Fluorescent intensity was normalized to pre-bleach values.
**Immunofluorescence**

Cells were grown on glass 24 mm coverslips. Cells were fixed in 2% paraformaldehyde in PBS containing 0.1% Triton X-100 and washed 2 times for 10 min in PBS with 0.1% Triton X-100 and one time in PBS with 0.15% Glycine and 0.5% BSA. For 6-4PP and histone H3 staining, cells were incubated for 5 min in freshly made 0.07 M NaOH. Cells were incubated for 1-2h with the indicated primary antibodies in PBS with 0.15% Glycine and 0.5% BSA and subsequently washed 5 times with PBS with 0.1% Triton. After washing, the cells were incubated for 1-2h with secondary antibodies in PBS with 0.15% Glycine and 0.5% BSA and DAPI (0.1 ug/ml) or Sytox green (0.5 µM, Life technologies). Cells were washed 5 times with PBS containing 0.1% Triton X-100 and mounted using Aqua Poly/Mount (Polysciences). For cytochrome C release assays, cells were grown in glass bottom 96 well plates (Greiner). 32 h before fixation cells were UV-irradiated and the caspase inhibitor (Q-VD-OPH, 20 µM, MP Biomedicals) was added. Above described staining procedure was used and after secondary antibody staining cells were washed in PBS with Triton X-100, fixed with 2% paraformaldehyde and stored in PBS. Antibodies used: mouse-anti-6-4PP (1:1000, Cosmo, 64M2), goat-anti-H3 (1:250, Santa cruz), mouse-anti-Cytochrome C (1:100, BD Biosciences) and mouse-anti-phospho-H2AX (Ser139) (1:1000, Millipore, JWB301). Images were acquired using a Leica sp5 confocal scanning microscope (Cytochrome C) with a 20X HCX PL APO CS 0.7 NA objective or a Zeiss LSM700 confocal microscope equipped with a 40X oil Plan-apochromat 1.4 NA objective. Images were analyzed using ImageJ software (Schindelin et al. 2012).

**Western blotting**

Whole cell extracts were made by scraping cells in Laemmli buffer and boiling for 3 min. Lysates were separated on SDS-PAGE gels and transferred to PVDF membranes (0.45 µm, Millipore). Blots were blocked with 5% milk (Sigma) in PBS-Tween (PBS with 0.05% Tween) and incubated 1 h or o/n with primary antibodies. Blot were washed 5 times for 5 min with PBS-Tween and incubated with secondary fluorescent antibodies (Sigma) for 1 h, followed by another 5 washes in PBS-Tween. Antibodies are visualized using an Odyssey CLX Infrared Imaging System (LI-COR Biosciences). Primary antibodies used: mouse-anti-phospho-H2AX (Ser139) (1:1000, Millipore, JWB301), mouse-anti-Tubulin (1:5000, Sigma, B512), rabbit-anti-SET (1:1000, Abcam), rabbit-anti-XPA (1:250, Santa Cruz, SC-853), mouse-anti-KAP1 (1:1000, Abnova), rabbit-anti-H1.2 (1:1000, Abcam)

**Unscheduled DNA synthesis (UDS)**

CSR0 cells were seeded on 24 mm coverslips and cultured under low serum (1%) to accumulate cells in G0-phase cells. Cells were irradiated with 16 J/m² and incubated for 3 h in medium containing EdU
(5 μM, Invitrogen) and 5-fluorodeoxyuridine (1 μM, Sigma), followed by a 15 min chase of medium containing thymidine. Cells were fixed in 3.6% formaldehyde and permeabilized for 20 min in 0.5% Triton X-100 in PBS. Click-it reaction was performed according to manufacturer’s protocol (Invitrogen) and slides were mounted using DAPI vectashield (Vector Laboratories). Images were obtained with a Zeiss LSM700 equipped with a 40x 1.3 NA oil immersion Plan apochromat objective.

**Recovery of RNA synthesis (RRS)**

Cells were cultured on coverslips and irradiated with 6 J/m² UV-C or mock treated. Cells were incubated for 2 h in medium containing EU (20 μM, Base Click) at different time points after UV treatment. Cells were fixed in 3.6% formaldehyde and permeabilized in 0.5% Triton X-100 in PBS for 20 min. Click-it reaction was performed according to manufacturer’s protocol (Invitrogen) and slides were mounted using DAPI vectashield (Vector Laboratories). Images were obtained with a Zeiss LSM700 equipped with a 40x 1.3 NA oil immersion Plan apochromat objective and quantified using ImageJ software (Schindelin et al. 2012).

**Cell cycle analysis**

Cells were labelled with 10 μM EdU for 15 minutes at 37°C to identify S-phase cells. Subsequently, cells were harvested and fixed in 1% formaldehyde for 10 min at RT. Cells were permeabilized with 10% saponin in PBS for 10 min on ice and Click-it reaction was performed using the ClickIT EdU Alexa Fluor 594 Flow Cytometry Assay Kit (Invitrogen) according to manufacturer’s protocol. Cells were washed with 1% BSA in PBS and resuspended PBS containing 0.1 mg/mL RNase and 1 μg/mL DAPI. Cell cycle profiles were obtained by flow cytometry (LSR Fortessa BD Biosciences). Data was analyzed using FlowJo vX.0.7 (Tree Star Inc.).

**Reverse transcription quantitative polymerase chain reaction (RT-qPCR)**

When indicated, cells were treated with 20 μM Etoposide for 24h prior to RNA extraction. The RNeasy Plus Mini kit (Qiagen) was used to extract RNA from the cells according to the manufacturers protocol and cDNA was made from 500 ng RNA using Superscript III reverse transcriptase (Invitrogen) and random hexamers. For the qPCR PowerUp SYBR Green Master Mix (Applied Biosystems) was mixed with cDNA and the following primers: p21 fw: CTGAGACTCTCAGGGTCGAA; p21 rev: CGGCCTTTGGAGTGGTAGAA; Noxa fw: AGAGCTGGAAGTCGAGTGT; Noxa rev: GCACCTTCACATTCCTCCT; β-actin fw: AGAGCTCAGAGCTGACTGAC; β-actin rev: AGCCTCGTTGGCGGTACAG; GAPDH fw: AAATTCCATGGCCACCGTCA; GAPDH rev: CATCGCCCCACTTGATTTTG; HPRT fw: TATGGCGACCCGCAGCCT; HPRT rev:
CATCTGAGCAAGACGTTCA. Data was normalized to the average of the three housekeeping genes (β-actin, GAPDH and HPRT) and mock conditions.

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Competing interests

The authors declare that there are no competing interests.

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List of abbreviations:

DNA damage response (DDR), phosphorylated histone H2AX (γH2AX), protein phosphatase 2A (PP2A), embryonic stem (ES) cells, knockdown (KD), nucleotide excision repair (NER), global genome repair (GG-NER), transcription coupled repair (TC-NER), 6-4 photoproducts (6-4PPs), unscheduled DNA synthesis (UDS), recovery of RNA synthesis (RRS), homologous recombination (HR), non-homologous end joining (NHEJ), Lac repressors (LacR), Lac operon (LacO), fluorescent recovery after photo bleaching (FRAP), fetal calf serum (FCS), penicillin-streptomycin (PS), mouse embryonic stem (mES) cells, stable isotope labeling by amino acids in cell culture (SILAC), phosphate-buffered saline (PBS), reverse transcription quantitative polymerase chain reaction (RT-qPCR)
References


Figure 1: Depletion of SET leads to enhanced cellular survival after UV-induced DNA damage. (A) Western blot showing the expression levels of SET in mES cells. Tubulin staining was used as loading control. (B) Clonogenic UV-survivals in mES cells expressing shControl or shSET. The relative colony survival, normalized to 100% at 0 J/m², is plotted against the UV-C dose. N=3 individual experiments, error bars represent s.e.m. Two-sided paired t-test of area under the curve (AUC) p=0.117. (C) Western blot using SET and XPA antibodies of whole cell extracts from U2OS cells showing the knock down efficiency of the indicated siRNAs. Tubulin is used as a loading control. (D) Clonogenic UV-survivals in U2OS cells transfected with either siControl, siXPC, siSET a or b. The colony survival is normalized to 100% at untreated conditions and plotted against the UV-C dose. N≥3 individual experiments, error bars represent s.e.m. One-way Anova with Dunnett's test of AUC siControl vs siSET a p=0.0037, vs siSET b p=0.0002, vs siXPC p=0.0036. (E) Quantification of apoptotic cells after UV irradiation (6 J/m²) by assessing cytochrome C release. Apoptosis assays were executed in the presence caspase inhibitor Q-VD-OPH (20 µM), which arrests the apoptotic process after release of cytochrome C, resulting in the accumulation of apoptotic cells. N>5, average ±s.e.m. One-way Anova with Dunnett’s test siControl vs siSET a p=0.0817, vs siSET b p=0.0020, vs siXPC p<0.0001.
Figure 2: Depletion of SET does not affect cell cycle and NER efficiency. (A) Cell cycle distribution analyzed by EdU (10 µM) incorporation and DAPI (1 µg/ml) staining of U2OS cell transfected with the indicated siRNAs followed by FACS analysis. Cells were analyzed 16h after mock or 2J/m² UV treatment. N=2 individual experiments and error bars represent s.e.m. (B) Quantification of relative amount of 6-4PP at different time points after UV irradiation (10 J/m²). Amount of 6-4PP was quantified by immunofluorescence intensity and data was normalized to the 0 hour time point. N=3 (≥100 cells per experiment) and error bars represent s.e.m. Two-sided paired t-test of siControl vs siSET b 2h p=0.0941, 5h p=0.9644 and 8h p= 0.6384. (C) Unscheduled DNA synthesis measured by the incorporation of EdU (20 µM) in non-S-phase C5RO cells after UV irradiation (161J/m²). UDS levels in siControl cells were set as 100%. N=2-3 experiments, with at least 100 cells analyzed per experiment, error bars represent s.e.m. One-way Anova with Dunnett’s test of siControl vs siSET b p=0.8057 and vs siXPC p=0.0150. (D) Clonogenic UV-survival in XP4PA (XPC-deficient) cells transfected with the indicated siRNAs. The relative colony survival, normalized to 0 J/m², is plotted against the UV-C dose. Average ±s.e.m. of 2 independent experiments. One-sided paired t-test of AUC p=0.0566 and of 6J/m² p=0.0308. (E) RNA synthesis determined by quantifying the amount of EU (100 µM) incorporation. Amount of EU incorporation 2 and 16 h after UV treatment is normalized to levels in undamaged cells. Mean ±s.e.m. is plotted of at least 2 independent experiments (N>75 cells per experiment). Two-sided t-test of siControl vs siSET b 2h p=0.5404 and 16h p=0.4081.
Figure 3: SET depletion leads to increased resistance to a wide variety of DNA damaging agents. (A) Colony survivals of U2OS cells transfected with siControl or siSETa treated with ionizing irradiation. Relative colony survival, normalized to 100% in untreated sample, is plotted against the dose. Average ±s.e.m. of 3 experiments is shown. One-sided paired t-test of AUC p=0.0365. (B) Clonogenic survival experiments of U2OS cells transfected with siControl, siSETa or siKAP1 treated with UV-C irradiation. Average ±s.e.m. of 3 experiments, normalized to 100% in untreated sample, is shown. One-way Anova with Sidak’s test of AUC siControl vs siSETa p=0.0568 and siControl vs siKAP1 p=0.8815. (C) Western blot of whole cell extract from U2OS cells showing knockdown efficiency of the depicted siRNAs. Blots are stained with antibodies against SET, KAP1 and tubulin. (D-E) Clonogenic survival assays of U2OS cells transfected with the indicated siRNAs treated with hydroxyurea (D) or hydrogen peroxide (E). Relative colony number was normalized to 100% at non-treated conditions. Average ±s.e.m. of 2 individual experiments. One-sided paired t-test of AUC p=0.0547 and of 1.5mM p=0.0359 (D) and AUC p=0.0984 and of 200mM p=0.0827 (E).
Figure 4: Altered DNA damage signaling is not the cause of the enhanced survival in SET-depleted cells. (A) Graphical representation and representative immunofluorescence images of U2OS LacO cells, containing 256 repeats of the Lac operon at a defined DNA locus. A Lac repressor specifically binds this operon, allowing the visualization of this locus by the indicated mCerry-LacR fusion proteins. yH2AX signal was determined by antibody staining. (B) Quantification of yH2AX signal at the lacO. Average ±s.e.m. of 8 experiments with 25 cells analyzed per experiment. Two-sided paired t-test p=0.0008. (C) Quantification of GFP-H2AX levels at the LacO of U2OS cells expressing the indicated LacR vectors. GFP-H2AX levels at the LacO are normalized to the average levels in the nucleus which was set at 100%. Average ±s.e.m. of at least 18 cells. Two-sided t-test p=0.8511. (D) Colony UV-survival experiments of cells treated with caffeine. Percentage of surviving colonies, normalized to 100% at 0 J/m², is plotted against UV-C dose. Average of 4 experiments is shown, error bars represent s.e.m. One-sided paired t-test of AUC siControl vs siSET a p=0.0014 and siControl caffeine vs siSET a caffeine p=0.0006. (E) Representative western blot from whole cell extracts from U2OS cells transfected with the indicated siRNAs showing the protein levels of SET, yH2AX and tubulin. One hour prior to lysis, cells were irradiated with 2 Gy to induce yH2AX signaling. (F)
Clonogenic UV-survival of cells transfected with siRNAs targeting histone H2AX. The relative colony survival, normalized to 0 J/m², is plotted against the UV-C dose. Average ± s.e.m. of 2 independent experiments. One-sided paired t-test of AUC siControl vs siSET a p=0.0432, siH2AX a vs siH2AX a + siSET a p=0.0023 and siH2AX b vs siH2AX b + siSET a p=0.0245.
Figure 5: Higher DNA damage resistance in SET-depleted cells is rescued by histone H1 down regulation.  

(A) FRAP analysis of stable GFP-H1.2 expressing cells transfected with the indicated siRNAs. A small strip spanning the nucleus is bleached and fluorescent recovery was measured over time. The fluorescent intensity is normalized to pre-bleach levels. N=3 experiments (≥8 cells/experiment), error bars represent s.e.m. Two-sided paired t-test of AUC p=0.0005.  

(B) Representative immunofluorescence images of GFP-H1.2 expressing U2OS LacO cells transfected with either mCherry-LacR (top panel) or mCherry-SET-LacR (lower panel) vectors.  

(C) Quantification of the GFP-H1.2 levels at the LacO, normalized to the average GFP-H1.2 signal in the nucleus, which was set at 100%. N=10 individual experiments (25 cells/experiment). Error bars represent s.e.m. Two-sided paired t-test p=0.0002.  

(D) Quantification of relative GFP-histone H3 levels at the LacO in cells transfected with the indicated LacR vectors. Normalized to GFP-H3 signal in the nucleus, which was set at 100%. Average of 3 individual experiments (25 cells/experiment). Error bars represent s.e.m. Two-sided paired t-test p=0.7206.  

(E) Representative immune blot from WCE from U2OS cells showing the efficiency of the siRNAs targeting SET and histone H1. Tubulin is used as a loading control.  

(F) Colony survival of U2OS cells transfected with siControl, siSET a and siH1 and treated with UV-C. The relative colony survival, normalized to 100% at 0 J/m², is plotted against the UV-C
dose. Average ±s.e.m. of 5 independent experiments. One-way Anova with Sidak’s test of AUC siControl vs siSET a p=0.0169, siH1 vs siH1 + siSET a p=0.0291, siControl vs siH1 p=0.6839 and siControl vs siH1 +siSET a p=0.7348. (G-H) Colony survival of Hct116 WT and p53-/− cells transfected with siControl or siSET treated with UV (G) or IR (H) The relative colony survival, normalized to 100% at untreated condition, is plotted against the dose. N=2, error bars represent s.e.m. One-way Anova with Sidak’s test of AUC WT siControl vs WT siSET b p=0.4285, WT siControl vs p53-/− siControl p=0.2209 and p53-/− siControl vs p53-/− siSET b p=0.8248 (G). One-way Anova with Sidak’s test of AUC WT siControl vs WT siSET a p=0.3578, WT siControl vs p53-/− siControl p=0.1339 and p53-/− siControl vs p53-/− siSET a p=0.9417 (H).
Figure 1: Depletion of SET leads to enhanced cellular survival after UV irradiation. Representative immunofluorescence images of cells treated with UV (6 J/m²) and the caspase inhibitor Q-VD-OPH (20 µM). Asterisks (*) indicate apoptotic cells in which the cytochrome C is released from mitochondria.
Figure 2: Depletion of SET does not affect cell cycle and NER efficiency. (A) Plots of EdU (10 µM) and DAPI (1 µg/ml) labeled mock or UV treated U2OS cells transfected with either siControl, siSET a or siSET b as analyzed by FACS. Boxes indicate the G1, S and G2/M populations. (B) Quantification of relative amount of 6-4PP directly after UV irradiation (10 J/m²). Amount of 6-4PP was quantified by immunofluorescence intensity and data was normalized to the siControl condition. N=4 (≥100 cells per experiment) and error bars represent s.e.m. One-way Anova with Sidak’s test siControl vs siSET b p=0.9971 and siControl vs siXPA p=0.1705.
Figure S3: SET depletion leads to increased resistance to DNA damage. Clonogenic survival assays with mES cell expressing shControl or shSET treated with IR (A) potassium bromate (B), illudin S (C) or mitomycin C (D). The relative colony survival, normalized to untreated conditions, is plotted against the dose. Average ±s.e.m. of at least 2 individual experiments. One-sided paired t-test of AUC (A) p=0.0238, (B) p=0.0418, (C) p=0.0647 and (D) p=0.1349.
Figure S4: Altered DNA damage signaling is not the cause of the enhanced survival in SET-depleted cells. (A) Representative immunofluorescence images of GFP-H2AX expressing U2OS LacO cells transfected with either mCherry-LacR (top panel) or mCherry-SET-LacR (lower panel) vectors. (B) Colony survival experiments of cells treated with ATM and DNA-PK inhibitor. The number of colonies at 0 J/m² is normalized to 100%. Average ±s.e.m. of 2 independent experiments. One-sided paired t-test of AUC siControl vs siSET p=0.1339 and siControl +ATMi +DNAPKi vs siSET +ATMi +DNAPKi p=0.1414. One-sided paired t-test of 8J siControl vs siSET a p=0.0809 and siControl +ATMi +DNAPKi vs siSET a +ATMi +DNAPKi p=0.0455.
**Figure S5: Higher DNA damage resistance in SET-depleted cells is impeded by histone H1 down regulation.**

Colony UV-survivals of U2OS cells transfected with siControl, siH1 and siSET b treated with UV (N=5) (A), or potassium bromate (N=4) (B). Relative colony number, normalized to untreated conditions, is plotted against dose and error bars represent s.e.m. One-way Anova with Sidak’s test of AUC (A) siControl vs siSET b p=0.0933, siH1 vs siH1 + siSET b p=0.2668, siControl vs siH1 p = 0.3783 and siControl vs siH1 + siSET b p=0.2196. One-way Anova with Sidak’s test of 6J (A) siControl vs siSET b p=0.0116, siH1 vs siH1 + siSET b p=0.4825, siControl vs siH1 p=0.9979 and siControl vs siH1 + siSET b p=0.4556. One-way Anova with Sidak’s test of AUC (B) siControl vs siSET b p=0.0774, siH1 vs siH1 + siSET b p=0.9997 and siControl vs siH1 p=0.0308. (C) Relative p21 and Noxa RNA levels in U2OS cells transfected with siControl or siSET b upon etoposide treatment (20 µM, 24h), normalized to untreated RNA levels. N=3, error bars represent s.e.m. Two-sided paired t-test p21 p=0.0840 and Noxa p=0.1586.
**Table S1 GFP-H1.2 interacting proteins**

This table lists all the proteins identified by MS.

**Protein names:** Name of protein

**Gene names:** Name of gene

- **SILAC ratio GFP-H1.2/GFP Forward:** Normalized heavy(UV)/Light(Mock) SILAC ratio from experiment 1
- **SILAC ratio GFP-H1.2/GFP Reverse:** Normalized light(UV)/Heavy(Mock) SILAC ratio from experiment 2
- **Amount of identified peptides Forward:** Number of peptides identified for this protein in exp 1
- **Amount of identified peptides Reverse:** Number of peptides identified for this protein in exp 2

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