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# Methylation of histone H4 at aspartate 24 by Protein L-isoaspartate O-methyltransferase (PCMT1) links histone modifications with protein homeostasis

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**Histone modifications play crucial roles in modulating chromatin function and transcriptional activity. Due to their long half-life, histones can, in addition to post-translational modifications, also accumulate spontaneous chemical alterations, which can affect their functionality and require either protein repair or degradation. One of the major sources of such protein damage or ageing is the conversion of aspartate into isoaspartate residues that can then be methylated. Here, we characterize a novel histone modification, the methylation of histone H4 at aspartate 24 (H4D24me). We generated H4D24me specific antibodies and showed that H4D24me is ubiquitously present in different mouse and human cells. Our *in vitro* and *in vivo* data identified PCMT1 (Protein L-isoaspartate O-methyltransferase), an enzyme involved in protein repair, as a novel H4D24 specific histone methyltransferase. Furthermore, we demonstrated that VprBP (HIV-1 viral protein R (Vpr)-binding protein), a chromo domain-containing protein, specifically recognizes H4D24me potentially implicating H4D24me in H4 degradation. Thus, this work links for the first time a histone modification with histone protein aging and histone homeostasis, suggesting novel functions for histone modifications beyond transcriptional regulation.**

Eukaryotic DNA is packaged into chromatin, resulting in a high degree of DNA compaction. Formation of higher order chromatin structures affects the functionality of DNA since it can regulate its accessibility for e.g. effector proteins. The first step of compaction is achieved by packaging the DNA into nucleosomes, which are the repeat unit of chromatin. The nucleosomal core particle is formed by wrapping 147 base pairs of DNA around a histone octamer containing two copies of each core histone H2A, H2B, H3 and H4<sup>1</sup>. Histones are tripartite proteins that are composed of a globular domain and unstructured N- or C-terminal tails that are subjected to several post-translational modifications (PTMs) such as methylation, acetylation, phosphorylation as well as addition of larger groups like ubiquitin and ADP-ribose (for a review see:<sup>2</sup>). Recently, many new types of histone PTMs have been identified such as crotonylation, proline isomerization, propionylation, butyrylation, formylation etc.<sup>3</sup>. It is currently a major challenge to understand how histone PTMs modulate chromatin function. As suggested by the “histone code hypothesis”, histone PTMs can be recognized and bound by specific reader proteins that than regulate downstream events such as transcription, replication or DNA repair<sup>4,5</sup>.

In addition to enzymatic modifications, proteins may also undergo spontaneous non-enzymatic chemical modifications due to exposure to e.g. oxidative reagents. Cells can cope with the accumulation of such damaged proteins by proteosomal degradation<sup>6</sup>. However, aged or damaged proteins can also be repaired. For example, in erythrocytes the methylation of aspartate residues was described as a possible step in the repair of aged membrane proteins<sup>7</sup>. Protein L-isoaspartate O-methyltransferase (PCMT1, or alternatively called PIMT) catalyzes the methylation of isoaspartate (isoasp) residues and facilitates their restoration to aspartate residues<sup>8–12</sup>.

During the process of protein aging, L-aspartyl residues are spontaneously converted to L-isoaspartyl residues, constituting a major source of spontaneous protein damage<sup>13–16</sup>. This occurs via the unstable intermediate L-



succinimide (Fig. 1a, step 1) that undergoes a spontaneous hydrolysis, generating a mixture of the normal L-aspartate (15–30%) and L-isoaspartate (70–85%) (steps 2 and 3)<sup>12</sup>. It has been previously shown that PCMT1 can rapidly methylate these L-isoaspartyl sites to  $\alpha$ -carboxyl-O-methyl esters (step 4), which can undergo demethylation and give rise to the L-succinimide intermediate (step 5). One cycle of repair is completed with the conversion of an L-succinimidyl to L-aspartatyl residue (step 2), while the remaining L-succinimidyl enters into another cycle (step 3).

Readers of histone PTMs typically contain evolutionarily conserved domains that specifically recognize the modified residue, such as chromo, Tudor and PHD domains in the case of lysine or arginine methylation<sup>17</sup>. VprBP (HIV-1 viral protein R (Vpr)-binding protein), also known as DCAF1 (DDB1- and CUL4-associated factor 1), is a chromo domain-containing protein which is ubiquitously expressed and localized both to the cytoplasm and the nucleus<sup>18,19</sup>. Interestingly, VprBP has been shown to be the substrate recognition component of the DCX (DDB1-CUL4-X-box) E3 ubiquitin-ligase complex and has been implicated in regulation of several cellular processes such as proliferation, DNA replication, telomere maintenance and DNA damage response (reviewed in 20). Similarly, the other component of the DCX complex, DDB1 (DNA damage-binding protein 1), is involved in nucleotide excision repair (NER) of UV-induced DNA damage lesions. Previous studies indicated that the interaction of DDB1 with CUL4 E3 ligase could facilitate protein ubiquitylation in response to DNA damage<sup>21,22</sup>.

Here we characterized a novel type of histone methylation, the methylation of histone H4 at aspartic acid 24 (H4D24me), and showed that this methylation is catalyzed *in vitro* and *in vivo* by PCMT1 potentially implicating H4D24me in protein repair. To gain insights in the function of H4D24me, we searched for H4D24me specific readers. We identified the chromo domain-containing protein VprBP as an H4D24me binder that could link H4D24me to protein degradation under stress conditions such as DNA damage.

## Results

**H4D24me is ubiquitously present in mouse and human.** Methylation of aspartate residues has been reported on multiple proteins<sup>8,11,12,16</sup>. Aspartate is an acidic amino acid, which is not frequently found in histones that are basic proteins. Only two aspartate residues occur in the histone tails. Since so far modifications of histone tails are best studied (reviewed in<sup>23</sup>) we focused our studies on one of these two residues, on aspartate 24 of histone H4. As a tool to study a potential H4D24 methylation, we raised polyclonal rabbit antibodies using a synthetic H4 tail peptide (amino acids 21–29) methylated at aspartic acid 24 as immunogen. The affinity purified antibodies showed high specificity towards the immunizing methylated H4 tail peptide compared to the unmodified peptide in immune dot blot analysis (Fig. 1.b). Next, we ask whether our antibody recognizes histone H4 purified from human cells. As shown in Fig. 1.c, the antibody specifically detected histone H4 in nuclear extract from a human HeLa cervical cancer cell line. Notably, peptide competition experiments performed using antibodies pre-incubated with free H4D24 unmodified or methylated peptides showed that only the methylated peptide resulted in blocking the recognition of native H4 (Fig. 1.d), strongly suggesting that our antibody preparation is highly specific towards histone H4 methylated at aspartic acid 24 and that H4D24 is indeed methylated *in vivo*.

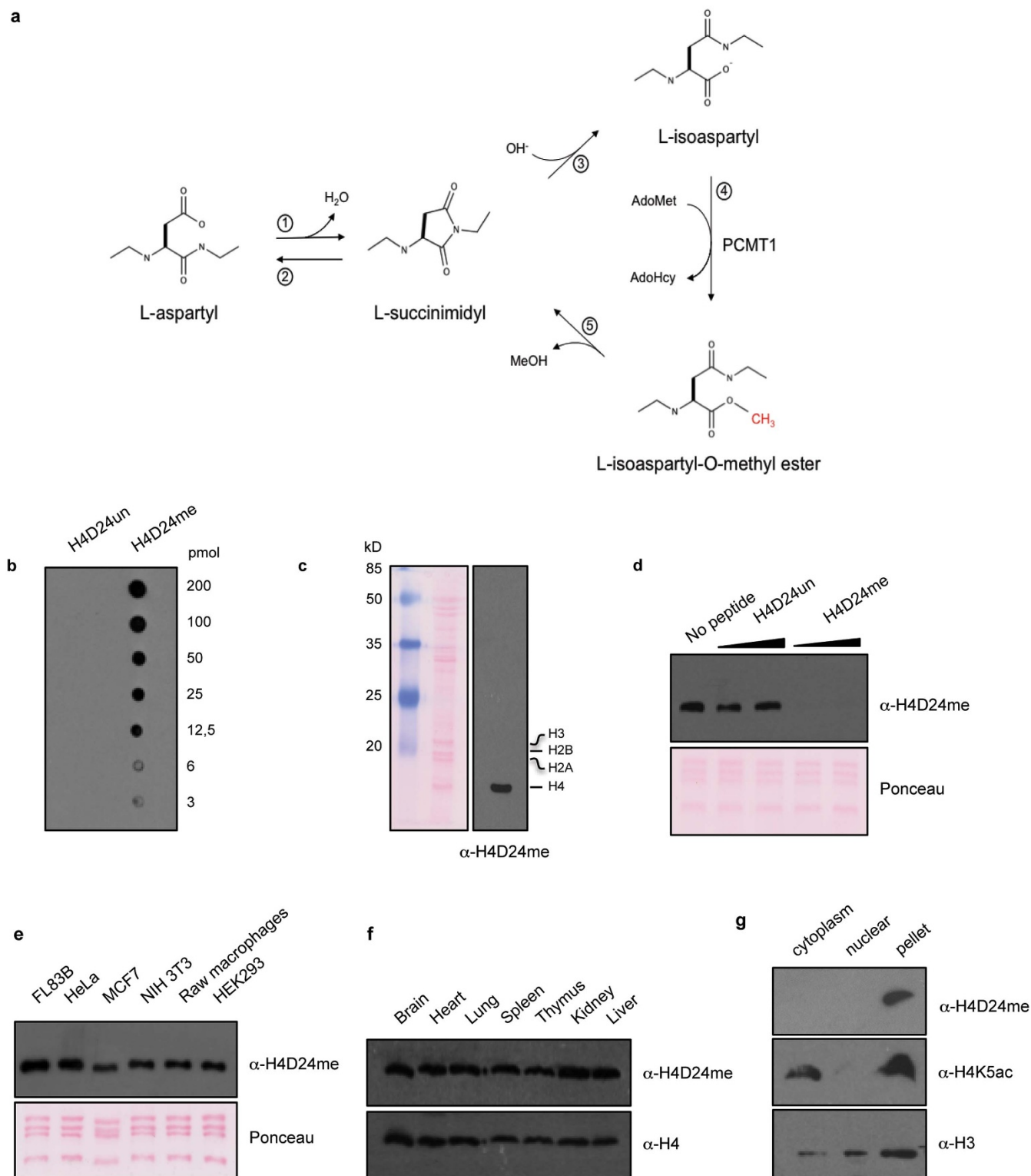
Immuno-blotting detected H4D24me in a variety of mouse and human cell lines as well as different mouse tissues tested (Fig. 1.e and f), suggesting a rather ubiquitous presence of H4D24me. In order to gain insight into the cellular distribution of H4D24me, we fractionated cytoplasmic, nucleoplasmic, and chromatin bound histones from HeLa cells and checked H4D24me levels in these fractions. As shown in Fig. 1g, we found H4D24me strongly enriched in the chromatin associated fraction (Fig. 1.g).

**H4D24 is methylated by PCMT1.** Next, we set out to identify the methyltransferase that is responsible for setting H4D24me. One potential candidate is Protein L-isoaspartate O-methyltransferase (PCMT1) that has been shown to have methyltransferase activity towards aspartic acid residues<sup>7–12</sup>. To test whether PCMT1 methylates indeed histone H4D24, we expressed a (human) PCMT1-(His)<sub>6</sub> fusion protein recombinantly (Supplementary Fig. 1) and performed *in vitro* histone methyltransferase (HMT) assays to assess for H4D24 specific activity. We used unmodified N-terminal H4 tail peptides and full-length recombinant H4, either wild type or a not methylatable D24 to A mutant, as substrates. Dot blot analysis with the H4D24me specific antibodies following the HMT assay showed that PCMT1 methylates the unmodified H4 N-terminal peptide (Fig. 2.a). We detected in immunoblot also methylation of wild type recombinant H4, but not the H4D24A mutant (Fig. 2.b). The lack of recognition of the H4D24A mutant is at the same time also an important additional control for the specificity of our antibody. The methyltransferase activity of PCMT1 on the H4 tail peptide was also validated by mass spectrometry (Fig. 2.c), supporting that PCMT1 can indeed methylate H4D24 *in vitro*.

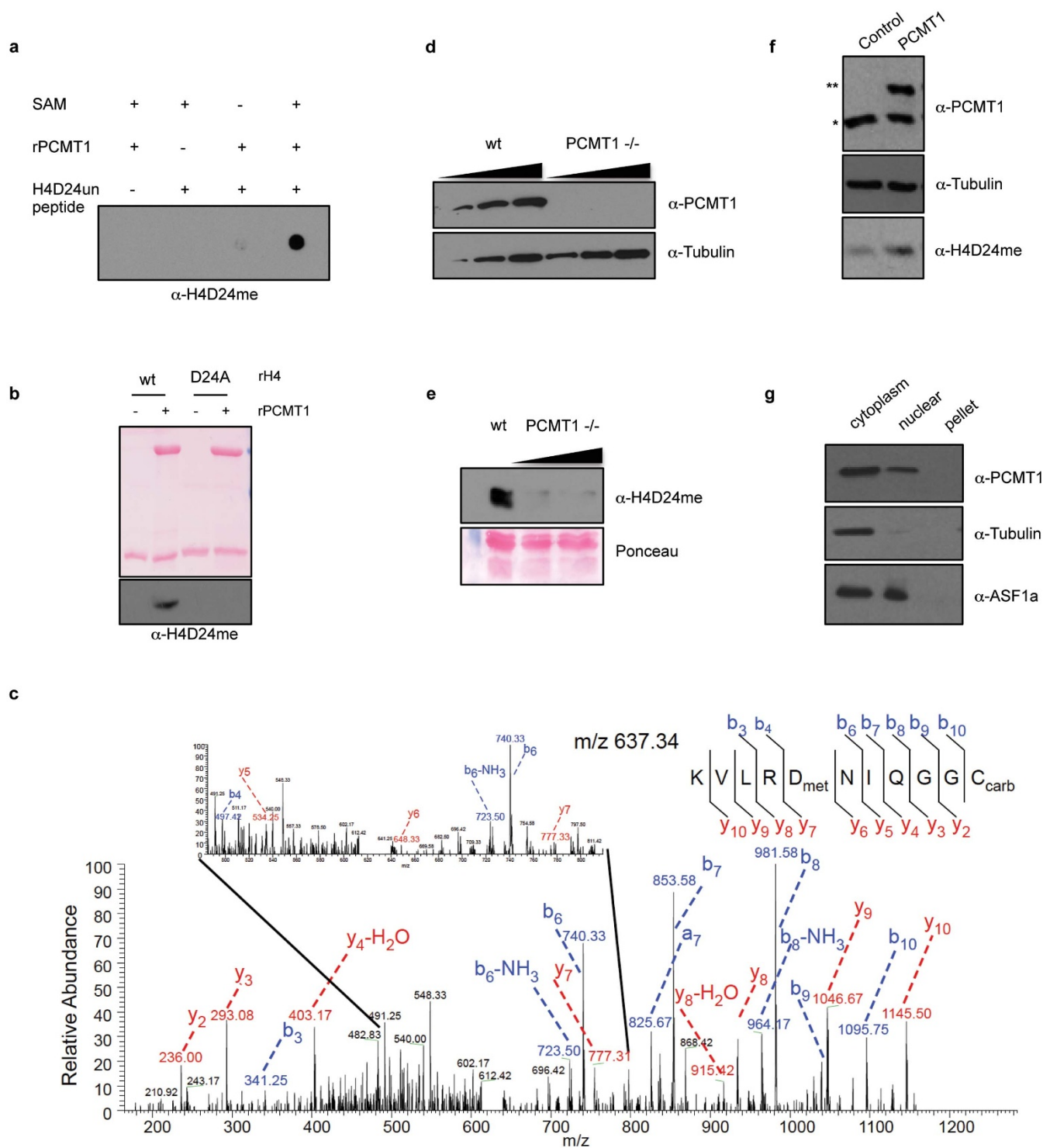
This data clearly established PCMT1 as an enzyme that can methylate H4D24 *in vitro*. To assess if PCMT1 also methylates H4D24me *in vivo*, we tried several siRNA and shRNA-mediated approaches to deplete PCMT1; however, although we achieved a dramatic reduction in the mRNA expression levels we were not able to significantly reduce PCMT1 protein levels (Supplementary Fig. 2). To circumvent this, we checked H4D24me levels on histone extracts prepared from PCMT1 wild type and knockout mouse livers. We found the H4D24me signal to be under detection limit in the tissues from the PCMT1 knockout mouse (Fig. 2.d and e). In line with this finding, transfecting HeLa cells with a construct over-expressing a PCMT1-FLAG-2xHA fusion protein resulted in increased levels of H4D24me (Fig. 2.f). Together, these *in vitro* and *in vivo* results clearly show that PCMT1 is a novel H4 histone methyltransferase that can methylate H4D24. In support of our findings that PCMT1 is a novel histone methyltransferase, and that H4D24me is found on nuclear H4, our immunoblot analysis on cytoplasmic, nucleoplasmic, and chromatin bound fractions revealed that PCMT1 localizes to both the nucleus and the cytoplasm (Fig. 2.g).

**VprBP is a specific H4D24me binder.** In order to gain insight into the function of H4D24me, we searched for potential H4D24me readers that could specifically recognize this novel histone modification and mediate downstream effects. For this, we performed peptide affinity purifications from HeLa nuclear extracts using unmodified and methylated H4 tail peptides, followed by separation of the associated proteins by SDS-PAGE (Supplementary Fig. 3). Mass spectrometry analysis of the bound proteins identified PCMT1 as a binder with preference for the unmodified peptide (Fig. 3.a), its substrate for methylation. Interestingly, we identified the chromo domain-containing protein VprBP as a potential H4D24me reader that preferentially binds to the methylated peptide (Fig. 3.a). We validated these interactions by immunoblots on independent peptide pull-downs, demonstrating again a preferential binding of VprBP to H4D24me peptides (Fig. 3.b).

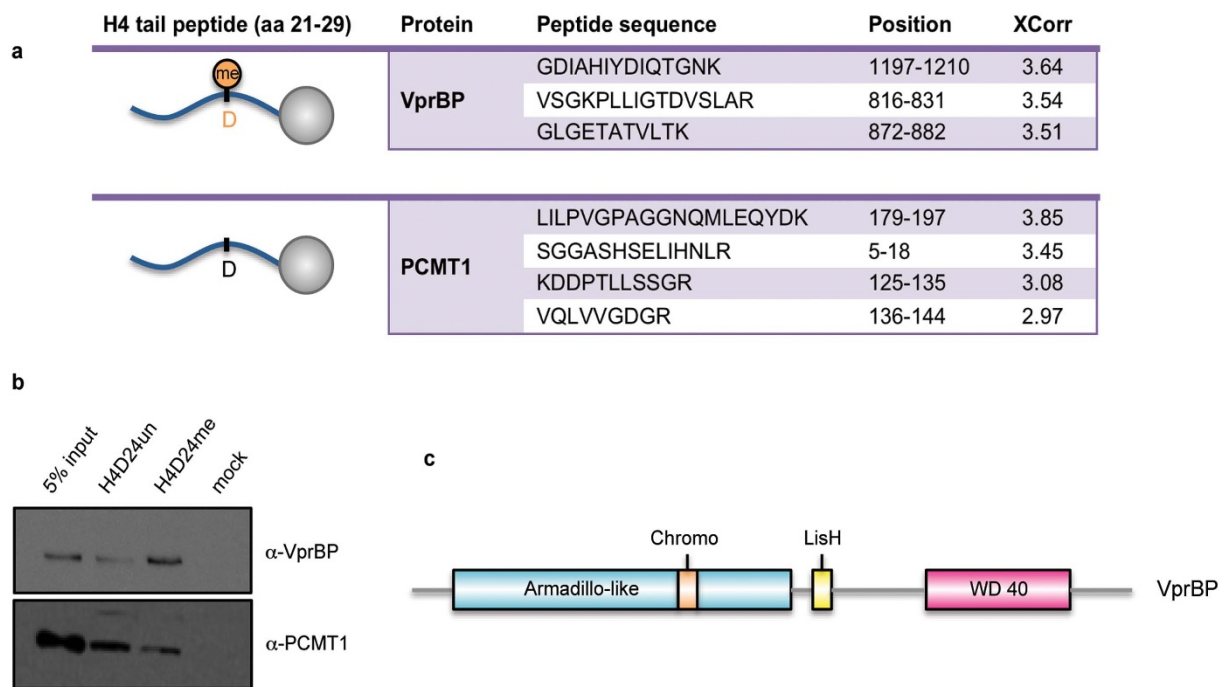
In addition to its chromo domain, VprBP also contains an Armadillo-like domain, a Lis homology motif (LisH) and tandem WD40 repeats that are suggested to have important functions in the dimerization of VprBP and interaction with other proteins including DDB1<sup>20</sup> (Fig. 3.c). DDB1 is another WD40 repeat-containing protein, which is part of the UV-damaged DNA-binding complex (UV-DDB) that is crucial for the nucleotide excision repair (NER) of DNA damage lesions induced by UV irradiation, as well as environmental mutagens including oxidative stress<sup>24</sup>. Because of this, we addressed whether there is a potential link between H4D24me and DNA



**Figure 1 | H4D24 methylation is present in multiple mammalian tissues.** (a) Methylation of isoaspartate residues during protein ageing can be part of protein repair (see text for details). (b) Immuno-dot-blot analysis with affinity purified H4D24me antibody on serial dilutions of unmodified (H4D24un) and methylated (H4D24me) histone H4 tail peptides. Note specific recognition of the immunizing (methylated) peptide. (c) The H4D24me antibody specifically recognizes histone H4 in HeLa nuclear extract suggesting the presence of H4D24me. (d) Pre-incubation of the H4D24me antibody with the H4D24me peptide, but not the unmodified peptide blocks recognition of native H4. Acid extracted histones from the indicated human and mouse cell lines (e) and mouse tissues (f) were immuno-blotted with the H4D24me antibody. Ponceau stainings or histone H4 immuno-blot are shown as loading control. (g) Fractionation of HeLa cells. H4D24me is enriched at the chromatin bound H4 fraction. Note that H4K5ac is enriched on cytoplasmic H4. Histone H3 immuno-blot is shown as loading control.



**Figure 2 | PCMT1 methylates H4D24 *in vitro* and *in vivo*.** (a) Recombinant PCMT1 methylates the unmodified H4 N-terminal tail peptide. *In vitro* HMT assay with S-Adenosyl-Methionine (SAM) as methyl group donor and detection of H4D24 methylation by immunoblot. (b) HMT assay on full-length recombinant wild type H4 (wt) and a H4 D24 to A mutant. Detection of methylation by immunoblot with H4D24me specific antibodies. Ponceau staining is shown as loading control. (c) Mass Spectrometry based verification of H4D24 methylation by PCMT1. PCMT1 assay on unmodified H4 peptide analyzed by nano-LC-MSMS on an Orbitrap XL. A doubly charged peptide with a nominal mass of  $m/z$ 637.34 was isolated. The figure shows the consecutive MSMS-spectrum. The c-terminal y-type series is indicated in red, the n-terminal b-type ion series is in blue. The critical area between  $y_5$  and  $y_7$  is magnified in the inlet. The modified y-series suggests methylation of D24. (d) PCMT1 protein levels detected by immunoblot on total cell extracts of PCMT1 wild type (wt) and knock out ( $-/-$ ) mouse livers.  $\alpha$ -tubulin was used a loading control. (e) Levels of H4D24me detected by immunoblot on histone extracts prepared from PCMT1 wild type (wt) and knockout ( $-/-$ ) mouse livers. Ponceau staining is shown as loading control. Note that H4D24me levels are under detection limit when PCMT1 is absent. (f) Immunoblots demonstrating the expression levels of the endogenous PCMT1 (\*) and the PCMT1-FLAG-2xHA fusion protein (\*\*). Overexpression of PCMT1 increases the levels of H4D24me. Ponceau stainings and tubulin blots are shown as loading control. (g) PCMT1 locates both to the cytoplasmic and the nuclear fractions. Tubulin and ASF1a blots serve as controls for the fractionation of HeLa cells.



**Figure 3 | VprBP is an H4D24me binder.** (a) Schematic representation of peptide affinity purifications. Proteins interacting with the unmodified and methylated H4D24 tail peptides immobilized on agarose beads were identified by mass spectrometry. Unique PCMT1 peptides that bind to the unmodified H4D24 peptide, unique VprBP peptides that bind to the methylated H4D24 peptide and their XCorr values are shown. (b) Independent peptide affinity purifications followed by immunoblotting with VprBP and PCMT1 specific antibodies confirm preferential binding of PCMT1 to the unmodified H4 tail peptide and of VprBP to the H4D24me peptide. (c) Overview of domain structure of VprBP.

damage repair. For this purpose, we examined whether the levels of H4D24me are altered upon UV irradiation. We treated U2OS cells with UV irradiation (60 j/m<sup>2</sup>) followed by a recovery and monitored the H4D24me levels. We observed that global H4D24me levels were decreased upon UV irradiation compared to the non-irradiated (NI) cells (Fig. 4.a) and only slowly recovered. VprBP has also been shown to be a substrate recognition component of the DCX (DDB1-CUL4-X-box) E3 ubiquitin-ligase complex, which can specifically recognize mono-methylated lysines and can target them for proteosomal degradation<sup>25</sup>. This could suggest that the decrease we observed in H4D24me levels after UV irradiation is a result of the recognition of H4D24me by VprBP, which in turn could mediate H4 degradation. To investigate this possibility, we treated HeLa cells with a synthetic proteasome inhibitor (MG132) for up to 6 hours. As shown in Figure 4.b, this proteasome inhibition resulted in accumulation of H4D24me (Fig. 4.b) which suggests that histone H4 methylated at D24 could indeed be targeted for proteosomal degradation. In support of this, in the cells that were treated with the proteasome inhibitor, we detected elevated H4D24me levels in the cytoplasmic fraction, where the proteosomal degradation takes place, when compared to the untreated control cells (Fig. 4.c). Thus, our data suggest that H4D24me may potentially act in addition to protein repair also as a signal for histone degradation. Similar to our results, Kaur et al. reported the UV-induced degradation of replication factor Mcm10 to be mediated by VprBP-DDB1-CUL4<sup>26</sup>.

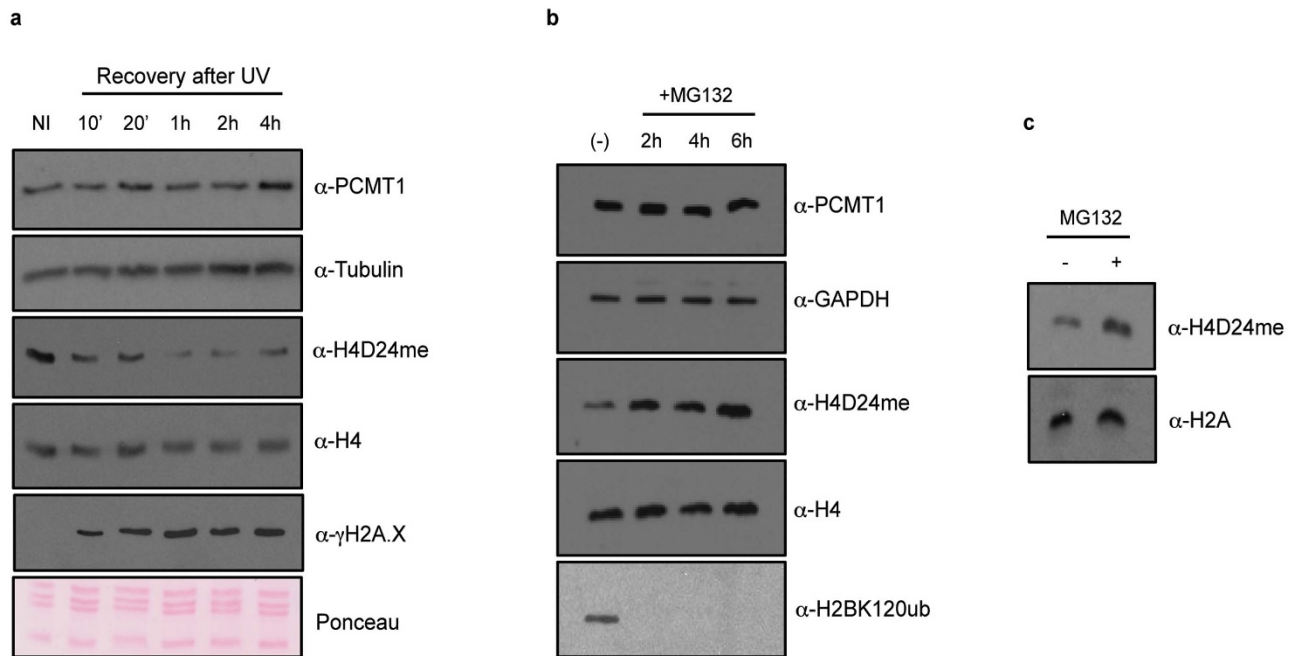
## Discussion

Here we identified PCMT1 as novel histone H4D24 methyltransferase and a novel histone modification. PCMT1 has been previously implicated in the repair of aged erythrocyte membrane proteins<sup>7</sup>, suggesting a functional link between H4D24 methylation and protein aging. Remarkably, we found that the chromo domain-containing protein VprBP is an H4D24 methylation specific binder that could link H4D24me additionally to protein degradation as well as DNA damage. Unfortunately, we have not been able to

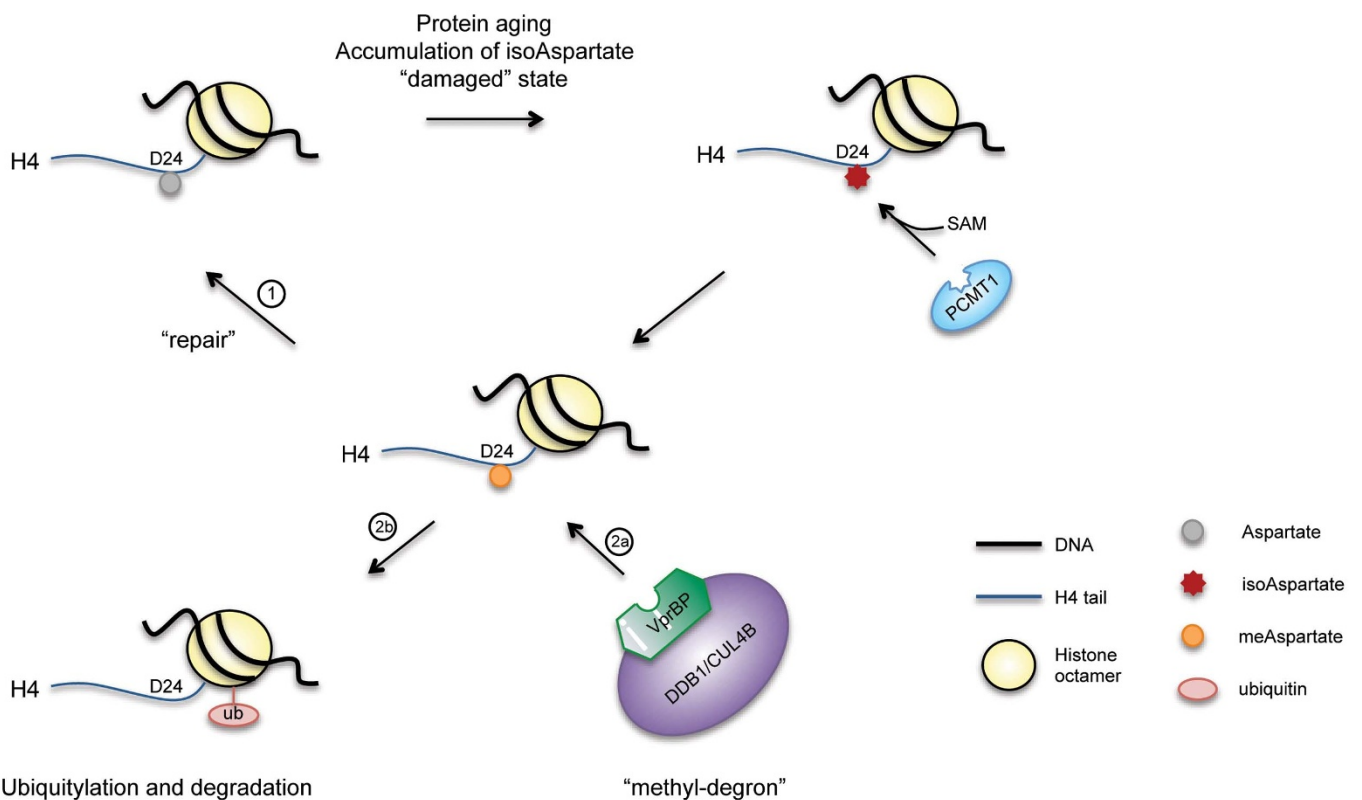
perform ChIP or immunofluorescence analysis for H4D24me to address the distribution of this novel histone modification that we describe. Indeed, despite our multiple attempts and different approaches, we have been unable to raise satisfactory ChIP grade antibodies for H4D24me. Nevertheless, our results allow us to put the following model for the function of H4D24me forward (Fig. 5): Histones are very stable proteins with a very long half-lifetime, with a reported half-life for histone H3 of up to 159 days<sup>27,28</sup>, and therefore prime candidates for protein aging. Moreover, contrary to the other three core histones, H4 does not have a replacement variant<sup>29</sup> and therefore its turnover might be even lower. During H4 protein aging, aspartate 24 of histone H4 can become spontaneously converted to isoaspartate. This isoAsp 24 on the H4 tail could then be recognized and methylated by the methyltransferase PCMT1. A fraction of the D24 methylated H4 could be subsequently repaired by spontaneous demethylation (Fig. 5 step 1). Alternatively, H4D24me can be recognized by the chromo domain protein VprBP, which is known to recruit the DDB1/CUL4 ubiquitin ligase complex, a “methyl-degron” (step 2a)<sup>25</sup>, eventually leading to the ubiquitylation and degradation of the damaged H4 (step 2b).

We find the interaction of H4D24me with VprBP very interesting given VprBP's role in regulating transcription in cancer cells and as a negative regulator of tumor suppressor genes<sup>30,31</sup>. While a specific interaction of VprBP with a histone modification has not been described prior to our report, its ability to recognize H4D24me provides a potential mechanism for specific recruitment to chromatin, which deserves further investigation.

Overall, our work links for the first time a histone modification with histone protein aging and potential protein repair and/or degradation. This is of particular importance considering the long half-lifetime of histones<sup>27,28</sup>. Our findings extend the significance of histone modifications beyond the classical functions in transcription and chromatin structure towards a novel role in histone protein homeostasis.



**Figure 4 | Stress-induced degradation of H4 methylated at D24.** (a) H4D24me levels decrease after UV treatment. Irradiation of U2OS cells with 60 j/m<sup>2</sup> followed by indicated recovery times. Non-irradiated cells (NI) serve as control. Ponceau staining, tubulin and H4 blot as loading controls.  $\gamma$ H2A.X as control for DNA damage induction. (b) H4D24me accumulates after treatment of HeLa cells with a synthetic proteasome inhibitor MG132 (20  $\mu$ M). H2BK120ub immunoblot serves as a control to monitor the proteasome inhibition. Histone H4 and GAPDH blots as loading controls. Note that PCMT1 levels are not changing. (c) Treatment with MG132 results in elevated levels of H4D24me in the cytoplasm, where proteosomal degradation takes place compared to the untreated cells. H4D24me immunoblot on cytoplasmic histones. H2A immunoblot is shown as loading control.



**Figure 5 | Proposed model for H4D24me function.** Histone H4D24 aspartate to isoaspartate conversion occurs during protein aging, which is then methylated by PCMT1 (H4D24me) and can get repaired by spontaneous demethylation (1). Alternatively, H4D24me can be recognized by VprBP (2a) and potentially targeted for ubiquitylation and degradation (2b). For details see text.



## Methods

**Antibody generation and characterization.** H4D24me-specific antibody was raised in rabbits against the H4D24-methylated peptide KVLRL(Dme)NIQGGC (Biosynthan) coupled to keyhole limpet hemocyanin (KLH). Immunoreactive serum was affinity purified using an H4D24me peptide immobilized on Sulfolink coupling resin (Pierce) according to manufacturer's instructions. For the characterization of the purified antibody, immuno-dot blots were performed as described previously<sup>32</sup>. For immunoblot analysis, proteins were separated on 8% to 18.7% SDS-PAGE according to the protein of interest, transferred to nitrocellulose membranes, blocked in 5% BSA-TBS, 0.1% Tween and incubated overnight with the H4D24me antibody diluted 1 : 1000 in the same buffer. The peptide competition assays were performed by pre-incubating the H4D24me antibody with the peptides (1 and 5 ng/ml) for 30 minutes before the membranes were probed.

The details of all the commercially available antibodies used in the study can be found in the Supplementary Table S1.

**Preparation of native histones and cell lysates.** Native histones were extracted as described by<sup>33</sup>. Extracts for peptide pull-downs were prepared following a protocol by<sup>34</sup>. Whole cell extracts were prepared by lysing the cells in RIPA buffer (150 mM NaCl, 50 mM Tris pH 8.0, 1% NP-40, 0.1% SDS and 0.5% Na-DCC), followed by sonication.

**Expression of recombinant proteins in E. coli.** Human PCMT1 containing pET30a vector (kind gift of Prof. Steven Clarke in UCLA) was expressed in E.coli BL21 strain. The proteins were purified using His-Select Nickel Affinity Gel (Sigma) according to the manufacturer's denaturing purification protocol, and dialysed against 50 mM Tris pH 8.0 with 0.5 mM EDTA. Recombinant full-length histone H4 was expressed and purified as described in<sup>35</sup>.

**In vitro histone methyltransferase (HMT) assays.** Unmodified H4-tail peptides or full-length recombinant H4 was mixed with S-adenosyl methionine (Biolabs) in 1X HMT buffer containing 50 mM Tris pH 8.0 and 50 mM  $\text{CH}_3\text{CO}_2\text{K}$  (potassium acetate) in a final volume of 50  $\mu\text{L}$  and incubated at 30°C for 1 hour.

**Peptide affinity purifications of specific binders.** 1 mg of HeLa nuclear extract was run on an H4D24un or -me peptide column in a buffer containing 150 mM NaCl, 50 mM Tris pH 8.0, 0.5% NP40 and protease inhibitors (Roche). Bound proteins were washed with the same buffer, eluted in 1X Laemmli and analyzed by silver staining after SDS-PAGE.

**Silver staining.** SDS-polyacrylamide gels were fixed overnight in 50% ethanol and 12% acetic acid and then washed 3 times with 35% ethanol and twice with water. The gels were sensitized for silver ion binding in 0.02% Sodium thiosulfate ( $\text{Na}_2\text{S}_2\text{O}_3$ ), washed 3 times in water and incubated in 118 mM  $\text{AgNO}_3$  and 0.03% formaldehyde containing silver stain solution for 20 minutes at room temperature. After briefly rinsing the gels with water, developing solution (0.57 M  $\text{Na}_2\text{CO}_3$ , 0.0185% formaldehyde, 0.0004%  $\text{Na}_2\text{S}_2\text{O}_3$ ) was added.

**Mass spectrometry analysis.** The detailed methodology of the analysis is provided in the supplementary information.

**Mammalian cell culture and treatment.** Cells were maintained at 37°C under 5%  $\text{CO}_2$  and 95% humidity in *Dulbecco's modified Eagle's medium* (DMEM) high glucose (4.5 g/L) supplemented with 10% Fetal Calf Serum (PAA or Perbio), 1% L-Glutamine (200 mM) and 1X Pen/Strep (100X) solution (PAA). The cells were washed in PBS and removed from the dish by incubation with trypsin-EDTA (PAA) solution. HeLa cells were treated with 20  $\mu\text{M}$  MG132 (CalBiochem) for up to 6 hours for proteasome inhibition. For the UV-induced DNA damage, U2OS cells were subjected to 60  $\text{J}/\text{m}^2$  UV irradiation followed by recovery.

**Generation of stable cell lines.** HeLa cells were transfected with 10  $\mu\text{g}$  of the pcDNA3 plasmid overexpressing PCMT1-FLAG-2HA fusion protein and 100  $\mu\text{L}$  of ExGen500 transfection reagent (Fermentas). Transfected cells were allowed to recover for 48 h before the medium was supplemented with G418 (Calbiochem) as selection agent. The selection process typically took 2–3 weeks during which the media was changed every 2–3 day. After the selection process was finished the cells were maintained in media supplemented with 1 mg/ml G418.

- Luger, K., Mäder, A. W., Richmond, R. K., Sargent, D. F. & Richmond, T. J. Crystal structure of the nucleosome core particle at 2.8 Å resolution. *Nature* **389**(6648), 251–60 (1997).
- Kouzarides, T. Chromatin modifications and their function. *Cell* **128**(4), 693–705 (2007).
- Tan, M. *et al.* Identification of 67 histone marks and histone lysine crotonylation as a new type of histone modification. *Cell* **146**(6), 1016–28 (2011).
- Turner, B. M. Decoding the nucleosome. *Cell* **75**(1), 5–8 (1993).
- Strahl, B. D. & Allis, C. D. The language of covalent histone modifications. *Nature* **403**(6765), 41–5 (2000).

- Friguet, B., Bulteau, A. L., Chondrogianni, N., Conconi, M. & Petropoulos, I. Protein degradation by the proteasome and its implications in aging. *Ann. N. Y. Acad. Sci.* **908**, 143–54 (2000).
- Janson, C. A. & Clarke, S. Identification of Aspartic Acid as a Site of Methylation in Human Erythrocyte Membrane Proteins. *J. Biol. Chem.* **255**(24), 11640–3 (1980).
- McFadden, P. N. & Clarke, S. Methylation at D-aspartyl Residues in Erythrocytes: Possible Step in the Repair of Aged Membrane Proteins. *Proc. Natl. Acad. Sci. U. S. A.* **79**(8), 2460–4 (1982).
- Aswad, D. W. Stoichiometric methylation of porcine adrenocorticotropin by protein carboxyl methyltransferase requires deamidation of asparagine 25. Evidence for methylation at the alpha-carboxyl group of atypical L-isoaspartyl residues. *J. Biol. Chem.* **259**(17), 10714–21 (1984).
- Murray, E. D. Jr. & Clarke, S. Synthetic peptide substrates for the erythrocyte protein carboxyl methyltransferase. Detection of a new site of methylation at isomerized L-aspartyl residues. *J. Biol. Chem.* **259**(17), 10722–32 (1984).
- Young, G. W. *et al.* Protein L-isoaspartyl methyltransferase catalyzes in vivo racemization of Aspartate-25 in mammalian histone H2B. *J. Biol. Chem.* **80**(28), 26094–8 (2005).
- Carter, W. G. & Aswad, D. W. Formation, localization and repair of L-isoaspartyl sites in histones H2A and H2B in nucleosomes from rat liver and chicken erythrocytes. *Biochemistry* **47**(40), 10757–64 (2008).
- Volkin, D. B., Mach, H. & Middaugh, C. R. Degradative covalent reactions important to protein stability. *Mol. Biotechnol.* **8**(2), 105–22 (1997).
- Clarke, S. Aging as war between chemical and biochemical processes: protein methylation and the recognition of age-damaged proteins for repair. *Ageing Res. Rev.* **2**(3), 263–85 (2003).
- Reissner, K. J. & Aswad, D. W. Deamidation and isoaspartate formation in proteins: unwanted alterations or surreptitious signals? *Cell. Mol. Life Sci.* **60**(7), 1281–95 (2003).
- Vigneswara, V. *et al.* Proteomic Identification of Novel Substrates of a Protein Isoaspartyl Methyltransferase Repair Enzyme. *J. Biol. Chem.* **281**(43), 32619–29 (2006).
- Yun, M., Wu, J., Workman, J. L. & Li, B. Readers of histone modifications. *Cell Res.* **21**(4), 564–78 (2011).
- Zhang, S., Feng, Y., Narayan, O. & Zhao, L. J. Cytoplasmic retention of HIV-1 regulatory protein Vpr by protein-protein interaction with a novel human cytoplasmic protein VprBP. *Gene* **263**(1–2), 131–40 (2001).
- Li, W. *et al.* Merlin/NF2 suppresses tumorigenesis by inhibiting the E3 ubiquitin ligase CRL4(DCAF1) in the nucleus. *Cell* **140**(4), 477–90 (2010).
- Nakagawa, T., Mondal, K. & Swanson, P. C. VprBP (DCAF1): a promiscuous substrate recognition subunit that incorporates into both RING-family CRL4 and HECT-family EDD/UBR5 E3 ubiquitin ligases. *BMC Mol. Biol.* **14**, 22 (2013).
- Kapetanaki, M. G. *et al.* The DDB1-CUL4A/DDB2 ubiquitin ligase is deficient in xeroderma pigmentosum group E and targets histone H2A at UV-damaged DNA sites. *Proc. Natl. Acad. Sci. U. S. A.* **103**(8), 2588–93 (2006).
- Wang, H. *et al.* Histone H3 and H4 ubiquitylation by the CUL4-DDB-ROC1 ubiquitin ligase facilitates cellular response to DNA damage. *Mol. Cell.* **22**(3), 383–94 (2006).
- Grant, P. A. A tale of histone modifications. *Genome Biol.* **2**(4), REVIEWS0003 (2001).
- Tang, J. & Chu, G. Xeroderma pigmentosum complementation group E and UV-damaged DNA-binding protein. *DNA Repair (Amst)*. **1**(8), 601–16 (2002).
- Lee, J. M. *et al.* EZH2 generates a methyl degran that is recognized by the DCAF1/DDB1/CUL4 E3 ubiquitin ligase complex. *Mol. Cell.* **48**(4), 572–86 (2012).
- Kaur, M., Khan, M. M., Kar, A., Sharma, A. & Saxena, S. CRL4-DDB1-VPRBP ubiquitin ligase mediates the stress triggered proteolysis of Mcm10. *Nucleic Acids Res.* **40**(15), 7332–46 (2012).
- Piha, R. S., Cuénod, M. & Waelch, H. Metabolism of histones of brain and liver. *J. Biol. Chem.* **241**(10), 2397–404(1966).
- Commerford, S. L., Carsten, A. L. & Cronkite, E. P. Histone turnover within nonproliferating cells. *Proc. Natl. Acad. Sci. U. S. A.* **79**(4), 1163–5 (1982).
- Kamakaka, R. T. & Biggins, S. Histone variants: deviants? *Genes Dev.* **19**(3), 295–310 (2005).
- Kim, K. *et al.* Vpr-binding protein antagonizes p53-mediated transcription via direct interaction with H3 tail. *Mol. Cell. Biol.* **32**(4), 783–96 (2012).
- Kim, K. *et al.* VprBP has intrinsic kinase activity targeting histone H2A and represses gene transcription. *Mol. Cell.* **52**(3), 459–67 (2013).
- Kamieniarz, K. *et al.* A dual role of linker histone H1.4 Lys 34 acetylation in transcriptional activation. *Genes Dev.* **26**(8), 797–802 (2012).
- Di Cerbo, V. *et al.* Acetylation of histone H3 at lysine 64 regulates nucleosome dynamics and facilitates transcription. *Elife* **3**, e01632 (2014).
- Dignam, J. D., Martin, P. L., Shashtra, B. S. & Roeder, R. G. Eukaryotic gene transcription with purified components. *Methods Enzymol.* **101**, 582–98 (1983).
- Tropberger, P. *et al.* Regulation of transcription through acetylation of H3K122 on the lateral surface of the histone octamer. *Cell* **152**(4), 859–72 (2013).

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### Author's contributions

B.B. and R.S. designed the experiments, analyzed the results and wrote the manuscript. F.R. and G.M. carried out the mass spectrometry analysis of H4D24 peptides after in vitro HMT assay. All authors read and agreed on the manuscript.

### Additional information

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