LETTERS

SIRT1 regulates the histone methyl-transferase SUV39H1 during heterochromatin formation

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In contrast to stably repressive, constitutive heterochromatin and stably active, euchromatin, facultative heterochromatin has the capacity to alternate between repressive and activated states of transcription[1]. As such, it is an instructive source to understand the molecular basis for changes in chromatin structure that correlate with transcriptional status. Sirtuin 1 (SIRT1) and suppressor of variegation 3–9 homologue 1 (SUV39H1) are amongst the enzymes responsible for chromatin modulations associated with facultative heterochromatin formation. SUV39H1 is the principal enzyme responsible for the accumulation of histone H3 containing a tri-methyl group at its lysine 9 position (H3K9me3) in regions of heterochromatin[2]. SIRT1 is an NAD$^+$-dependent deacetylase that targets histone H4 at lysine 16 (refs 3 and 4), and through an unknown mechanism facilitates increased levels of H3K9me3 (ref. 3). Here we show that the mammalian histone methyltransferase SUV39H1 is itself targeted by the histone deacetylase SIRT1 and that SUV39H1 activity is regulated by acetylation at lysine residue 266 in its catalytic SET domain. SIRT1 interacts directly with, recruits and deacetylates SUV39H1, and these activities independently contribute to elevated levels of SUV39H1 activity resulting in increased levels of the H3K9me3 modification. Loss of SIRT1 greatly affects SUV39H1-dependent H3K9me3 and impairs localization of heterochromatin protein 1. These findings demonstrate a functional link between the heterochromatin-related histone methyltransferase SUV39H1 and the histone deacetylase SIRT1.

SIRT1 is a member of the Sir2 family of NAD$^+$-dependent histone deacetylases[3,4] and promotes heterochromatin formation through the coordination of several events[3]. SIRT1 deacetylates histones (H4K16 and H3K9), recruits histone H1b, and promotes the loss of a mark associated with transcriptionally active chromatin, H3K79me2, and the establishment of marks associated with repressed chromatin such as H3K9me3 and H4K20me1 (ref. 3). How SIRT1 affects the levels of histone modifications other than deacetylation remains unclear.

We first tested whether SIRT1 might recruit an enzyme responsible for H3K9me3, during purification of Flag-tagged SIRT1 expressed in human 293 cells. Indeed, immunoprecipitated SIRT1 contained histone H3 lysine methyltransferase (HKMT) activity (Fig. 1a, lanes 5–7), the levels of which were greatly decreased in a similarly purified but catalytically inactive form of SIRT1 (lanes 8–10)[3]. This HKMT activity was specific for H3K9 (Fig. 1b). Histone octamers reconstituted with recombinant histones containing either wild-type or mutant H3, in which Lys 27 was replaced with Ala (K27A), exhibited similar levels of methylation. In contrast, mutant H3, in which Lys 9 was replaced with Ala (K9A), was an inappropriate substrate.

Two candidates for this specific HKMT were SUV39H1 and the euchromatic histone-lysine N-methyltransferase 2 G9A (also known as EHMT2), given their specificity for H3K9me formation[5]. Interaction between SIRT1 and G9A was undetectable (not shown). However, when 293 cells were co-transfected with Flag–SIRT1 and a Myc-tagged version of the major cellular activity for H3K9me3 (SUV39H1), the presence of either resulted in the co-immunoprecipitation of the other in pull-down experiments (Fig. 1c, d). The interaction between SUV39H1 and SIRT1 was specific, because the related NAD$^+$-dependent deacetylase SIRT2 failed to pull-down SUV39H1 (Fig. 1c). This is consistent with the functional link found between the homologues of SUV39H1 and SIRT1 in Schizosaccharomyces pombe (Clr4 and Sir2, respectively)[6]. Chromatin immunoprecipitation experiments were then performed on cells containing an inducible SIRT1 fused to the yeast Gal4 DNA-binding protein domains[3] that were transfected with Myc-tagged SUV39H1 (Fig. 1e). Myc–SUV39H1 was present at the integrated thymidine kinase promoter containing Gal4 sites in these cells only on induction of Gal4–SIRT1 with which it colocalized, demonstrating their interaction in vivo. This correlated with a significant loss in H3K9ac levels and enrichment in H3K9me3 levels at the promoter.

Co-immunoprecipitation experiments were performed to identify the interaction domains. A point mutation that renders SIRT1 catalytically inactive caused deficiency in interaction (Fig. 1f). The amino terminus of SIRT1 that interacts with histone H1b (ref. 3) also interacts with SUV39H1 (see below). Removal of either the SET domain or the chromo domain of SUV39H1 decreased its interaction with SIRT1, whereas removal of the first 89 N-terminal residues including the chromo domain eliminated interaction (Supplementary Fig. 1a). The chromo domain alone exhibited detectable interaction (Supplementary Fig. 1b), indicating that the N-terminal domain (1–43) and the chromo domain (44–88) of SUV39H1 were both required for interaction with SIRT1. This is similar to the case reported for SUV39H1 interaction with the polycomb human protein PC2 (ref. 7).

To understand how SIRT1 affects global levels of H3K9me3, we tested for its affect on the levels of SUV39H1-specific activity using methyltransferase assays performed in vitro with histone octamers as substrates. Increasing amounts of recombinant SIRT1 led to elevated levels of SUV39H1-mediated methylation of histone H3 (Fig. 2a, compare lane 2 with lanes 4–6), whereas recombinant SIRT2 (Fig. 2a, lanes 8–10) and bovine serum albumin (not shown) were ineffectual. The increased SUV39H1 activity exhibited the expected substrate activity, being unable to methylate recombinant octamers containing mutant H3K9A or mononucleosomes (Supplementary Fig. 2). The SIRT1-mediated stimulation of SUV39H1 activity was

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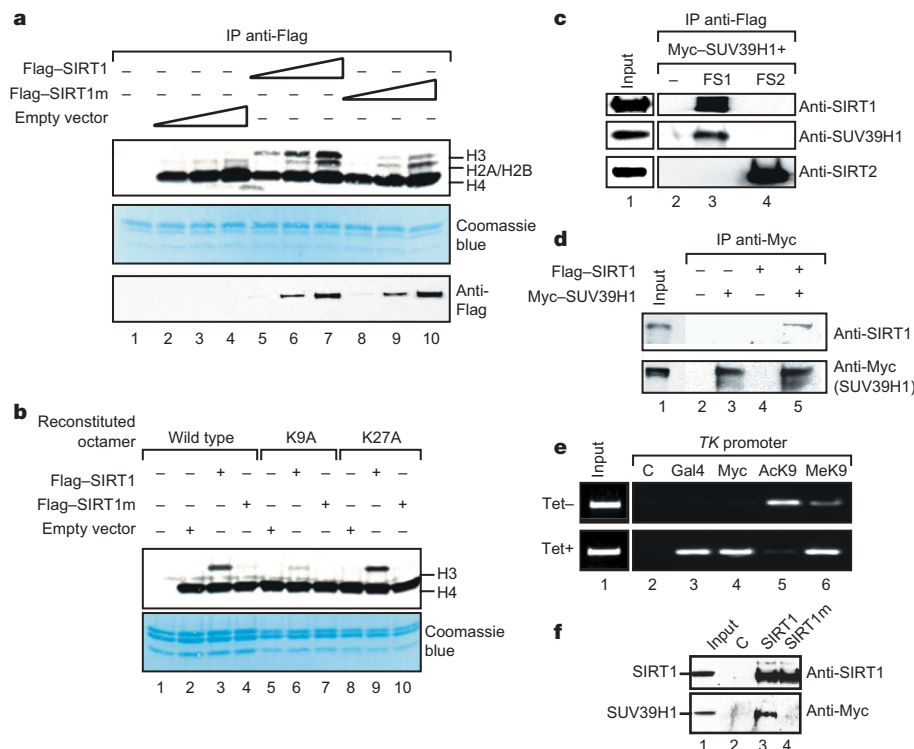
Figure 1 | The histone lysine methyltransferase SUV39H1 interacts with catalytically active SIRT1 *in vitro* and *in vivo*. a, Affinity-purified Flag-tagged SIRT1 was assayed for the presence of histone methyltransferase activity (top). Recombinant histone octamers used as substrates were stained with Coomassie blue (middle). Flag-tagged wild-type and catalytically inactive SIRT1(H363Y)[3] were analysed in western blots (bottom). SIRT1m, SIRT1 mutant. **b,** The HKMT is specific for histone H3 lysine 9 in HKMT assays. Assays were performed as in **a**, but using octamers reconstituted with histones as indicated. **c, d,** SIRT1 and SUV39H1 exhibit reciprocal co-immunoprecipitation *in vitro*, as shown in western blots. FS1, Flag-tagged SIRT1; FS2, Flag-tagged SIRT2. **e,** Chromatin immunoprecipitation using cells carrying tetracycline (Tet)-inducible Gal4–SIRT1 and an integrated luciferase reporter, transfected with Myc-tagged SUV39H1. The generation of these cells was described earlier[3]. The probe is specific for the promoter of the integrated thymidine kinase (TK) reporter containing Gal4 sites. C, control. **f,** Western blot of anti-Flag antibody co-immunoprecipitations performed *in vitro* with Myc–SUV39H1 and control (C), Flag–SIRT1 (SIRT1) or Flag–SIRT1 mutant (SIRT1m)[3].

NAD^{+}-independent (Fig. 2b), but the N terminus of SIRT1 (Fig. 2c) was required, as it was for SUV39H1 interaction (Fig. 2e). Titration of recombinant protein consisting of only the N-terminal 250 residues of SIRT1 was sufficient to stimulate the methyltransferase activity (Fig. 2c), and a baculovirus-expressed catalytically inactive

mutant SIRT1, which contains an intact N-terminal domain, was similarly effective (Fig. 2d). In addition, overexpression of the SIRT1 N terminus in 293 cells gave rise to elevated levels of H3K9me3 *in vivo* without affecting the levels of SUV39H1, histone H3, or di- or tri-methylated lysine residue 27 of histone H3 (Fig. 2f).
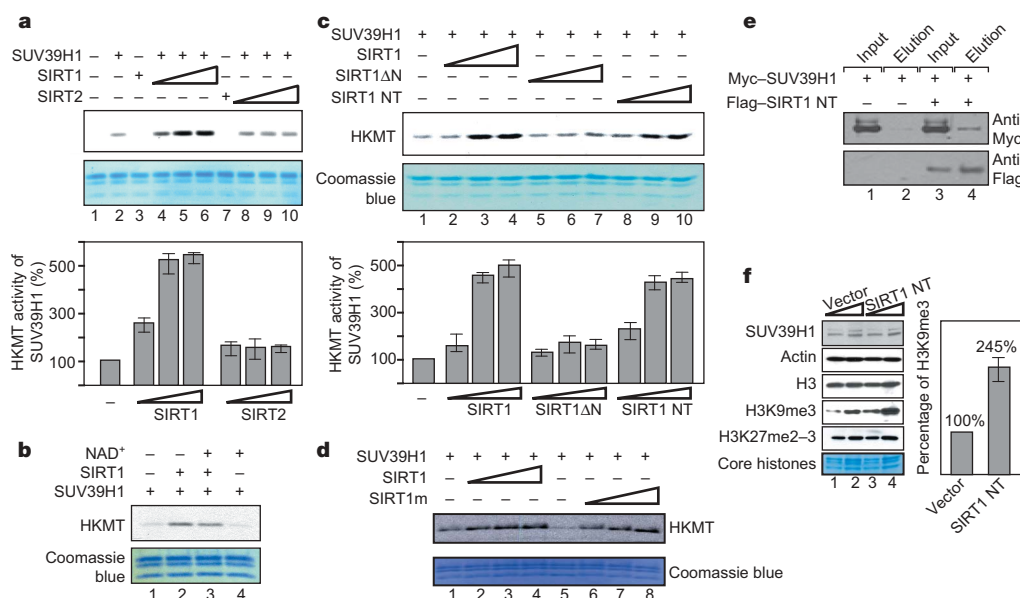

Figure 2 | SIRT1 upregulates SUV39H1 activity *in vitro* and *in vivo* through the SIRT1 N terminus. a, Methyltransferase (HKMT) activity of recombinant SUV39H1 as a function of increasing amounts of recombinant baculovirus-expressed SIRT1 or bacterially expressed SIRT2. Endogenous histone octamers (purified from HeLa cells) used as substrates were stained with Coomassie blue. Quantifications of multiple experiments are represented below and compared to the HKMT activity of SUV39H1 alone (lane 2), which is considered to be 100%. **b,** As in **a**, but in the absence or presence of NAD^{+}. **c,** As in **a**, but with increasing amounts of either wild type or SIRT1 deletion mutants (expressed in *Escherichia coli*) that either do not contain the N terminus (ΔN) or only contain the N terminus (NT). **d,** As in

a, but with increasing amounts of either wild-type SIRT1 or the catalytically inactive point mutant H363Y (expressed in baculovirus). **e,** Western blots from immunoprecipitations as in Fig. 1c, but using the Flag-tagged SIRT1 N-terminal region (SIRT1 NT)[3] and Myc–SUV39H1 as indicated. **f,** Western blots performed on extracts from 293 cells transfected with either empty vector or vector expressing the SIRT1 N terminus using the specific antibodies indicated. Core histones are stained with Coomassie blue. The right side shows quantification of the increased levels of H3K9me3 on overexpression of the N-terminal 250 residues of SIRT1 (SIRT1 NT) relative to the vector control. Error bars represent s.d. *n* = 4 for **a** and **c**; *n* = 3 for **f**.

Thus, the N terminus of SIRT1 is required both for interaction with SUV39H1 and for stimulation of its activity. The conformation of SUV39H1 is probably altered in a manner favourable to its activity on interaction with the N terminus of SIRT1.

SIRT1 is the principal NAD$^+$-dependent deacetylase in mammalian cells and, although SIRT1 targets histones, it also deacetylates a wide range of proteins[3,8]; therefore, we examined if SIRT1 could deacetylate SUV39H1. Human 293 cells overexpressing Myc-tagged SUV39H1 were exposed to the histone deacetylase (HDAC) inhibitor trichostatin A (TSA) (inhibitor of class I and II HDACs)[9] and to nicotinamide (inhibitor of the Sir2 family or class III HDACs)[10], together or independently. SUV39H1 was then affinity-purified, its protein levels normalized by western blot, and was assayed for

HKMT activity. A clear loss in Suv39h1 activity was observed with both inhibitors (Fig. 3a, c), and this affect was specific to nicotinamide treatment (Fig. 3b, c). The absence of the chromo domain of SUV39H1 was ineffectual (Fig. 3d, e). Of note, the absence of its N terminus rendered SUV39H1 nearly inactive (Fig. 3d, e). That nicotinamide treatment decreased SUV39H1 activity *in vivo* strongly implicated the deacetylase activity of SIRT1. We next tested if this HKMT exists in an acetylated form in the cell and whether or not such acetylation might modulate its activity.

SUV39H1 derived from nicotinamide-treated cells exhibited acetylation that mapped to Lys 266 (Fig. 3f and Supplementary Fig. 3), as compared to untreated cells. This residue is present within the catalytic SET (Su(var)3-9, Enhancer-of-zeste, Trithorax) domains of
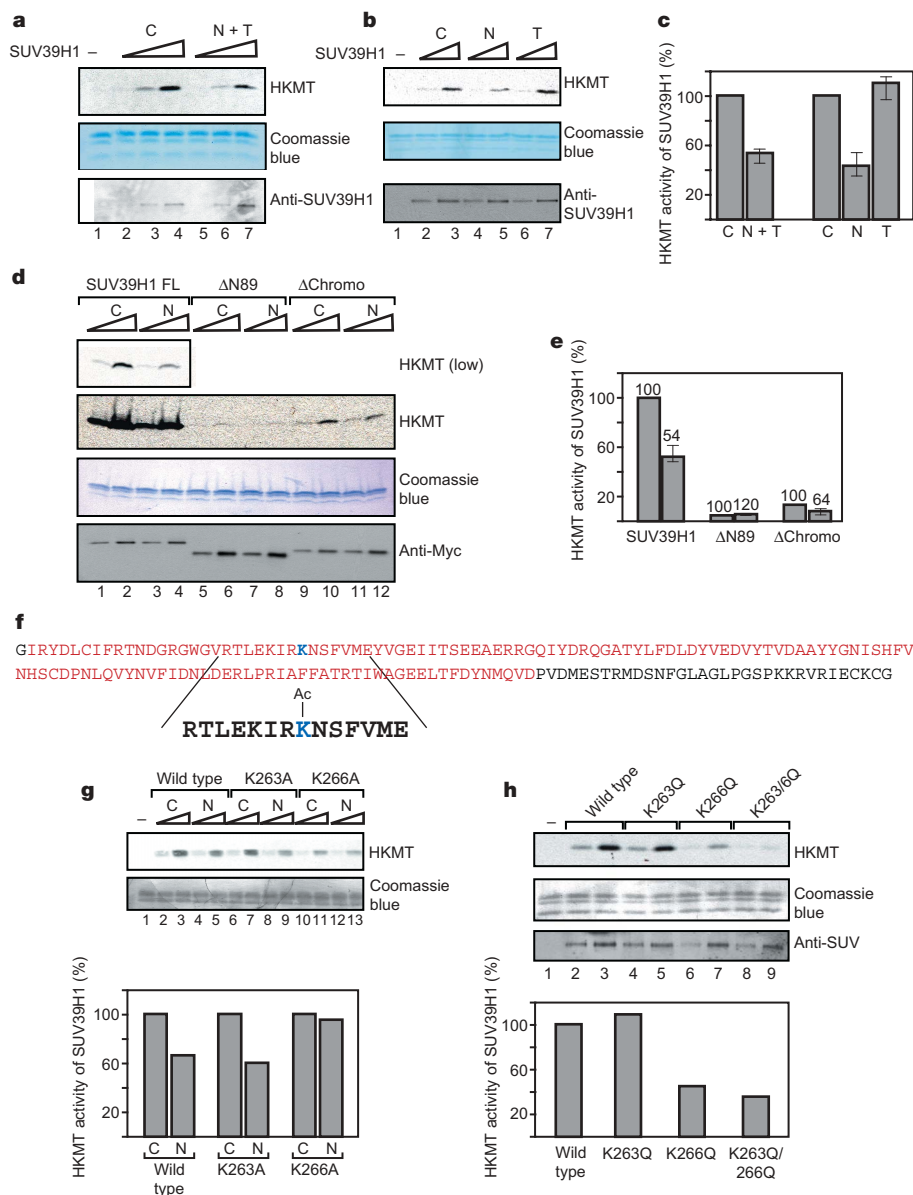

Figure 3 | Acetylation of SUV39H1 negatively regulates its activity and is elevated in nicotinamide-treated cells. a, b, SUV39H1 purified from 293 cells untreated or treated with inhibitors for histone deacetylases TSA (T) and nicotinamide (N) either together (**a**) or independently (**b**) and assayed for HKMT activity (upper panel). Histone proteins were stained with Coomassie blue (middle panel) and the levels of purified SUV39H1 were normalized with western blots using antibody specific to the Myc-tag (bottom panel). **c,** Quantification of results shown in **a** and **b. d,** Full length (FL) or indicated deletion mutants of Myc-tagged SUV39H1 purified after transfection of untreated (C) or nicotinamide (N)-treated 293 cells and

assayed as in **a** and **b** but with anti-Myc-specific antibody. **e,** Quantification of results shown in **d. f,** Amino acid sequence of the SET domain of SUV39H1 with Lys 266, which is subject to acetylation as indicated. **g, h,** Human 293 cells transfected with either wild type or mutants of Myc–SUV39H1 containing alanine substitutions (**g**) or glutamine substitutions (**h**) at the residues indicated. Cells were untreated (C) or treated with nicotinamide (N) and the proteins were purified with Myc-resin, normalized as in **a**, **b** and **d**, and assayed for HKMT activity as in **a**, **b** and **d**. In both **g** and **h**, quantifications of the HKMT assays are shown below. Error bars represent s.d. *n* = 4 for **c** and **e**.

EZH2, SUV420H1, SUV420H2 and SUV39H1, but not within that of yeast Clr4 (Supplementary Fig. 5a). SUV39H1 in which Lys 266 was replaced with Ala was unresponsive to nicotinamide treatment, whereas a control Ala substitution at Lys 263 behaved similarly to the wild type (Fig. 3g). In addition, a glutamine substitution that mimics an acetylated lysine residue was ineffectual at the Lys 263 position, but resulted in significant loss in SUV39H1 activity when at Lys 266 (Fig. 3h). This loss in activity reflected that observed in the case of Myc-tagged SUV39H1 derived from nicotinamide-treated cells (Fig. 3b). Additionally, SIRT1 can partially deacetylate purified SUV39H1 with lysine residues radiolabelled with acetic anhydride (Supplementary Fig. 4). This was specific to SIRT1, because neither SIRT2 nor SIRT3 deacetylated SUV39H1 although both were active in deacetylating H4K16ac and in the nicotinamide exchange reaction (data not shown and Supplementary Fig. 4). These results established that the enzymatic activity of SUV39H1 is negatively regulated by acetylation at residue 266, and that SIRT1-mediated deacetylation relieves this inhibition. Interestingly, comparison between the SET domain of SUV39H1 and the published structure of the SET

domains of two homologues, *Schizosaccharomyces pombe* Clr4 and *Neurospora crassa* Dim-5, indicates that Lys 266 is located in an exposed loop that participates in mediating the interaction between the SET domain and the cysteine-rich post-SET domain (Supplementary Fig. 5b). Acetylation of Lys 266 could alter this inter-domain interaction and affect the specific activity of the enzyme, which would explain the stimulatory effect of Lys 266 deacetylation on SUV39H1 activity[11].

To corroborate this functional relationship, 293 cells were tested for colocalization of SIRT1 and green-fluorescent-protein-tagged SUV39H1. Consistent with published results[2,12], SUV39H1 was found in constitutive heterochromatin foci, although it was also spread throughout the nucleus at distinctive regions (data not shown, Fig. 4A). Because the loss of SIRT1 results in decreased levels of H3K9me3 (ref. 3) and SIRT1 interacts with and activates SUV39H1 (as shown in this report), SIRT1 might also be affected when SUV39H1 is deficient. Immunofluorescence studies performed on mouse embryonic fibroblasts (MEFs, wild type or $Suv^{-/-}$) demonstrated that SIRT1 localization was similar, although its
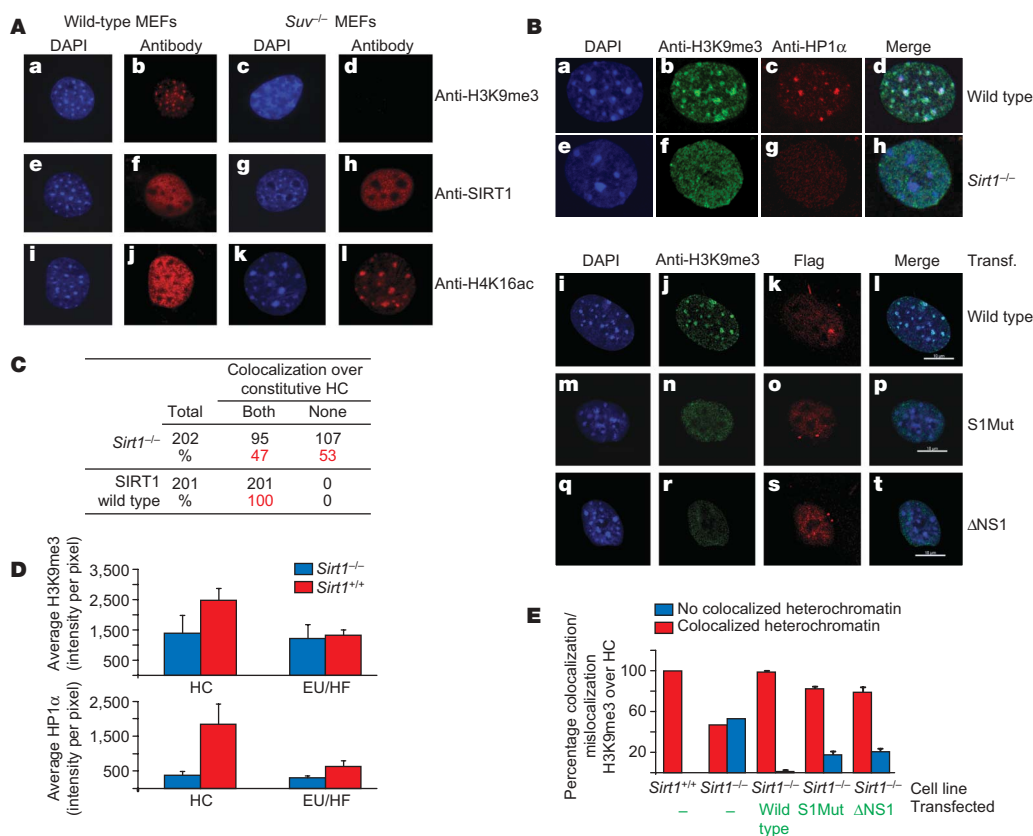

Figure 4 | SIRT1 and SUV39H1 activities are linked *in vivo*. A, Comparison of wild-type and $Suv^{-/-}$ ($Suv39h1/2^{-/-}$) MEFs using immunofluoresence microscopy for the distribution of H3K9me3 (panels **b, d**), H4KA16ac (**j, l**) and SIRT1 (**f, h**). DAPI staining of the cells is shown as a control for DNA and chromatin distribution (**a, c, e, g, i** and **k**). **B**, Comparison of wild-type and $Sirt1^{-/-}$ MEFs using immunofluorescence microscopy for the distribution of DAPI (**a, e**), H3K9me3 (**b, f**) and HP1α (**c, g**). A merged image of them is shown (**d, h**). $Sirt1^{-/-}$ MEFs were transfected with Flag-tagged SIRT1 (wild type), the SIRT1 catalytically inactive point mutant H363Y (S1Mut) or ΔNSIRT1 (ΔNS1), and were analysed by immunofluorescence for the distribution of H3K9me3 (**j, n, r**), Flag tag (**k, o, s**) and DAPI staining (**i, m, q**). A merged image of DAPI and H3K9me3 is shown (**l, p, t**). The images shown are representative of the experiment. Full quantification of the data (percentage of cells that show H3K9me3 localization in heterochromatin on each condition) is included as a graph in **E. C**, Quantification of cells in **A**. Significant numbers of the cells (202 for $Sirt1^{-/-}$ and 201 for wild type) were checked for colocalization of HP1α and H3K9me3 over DAPI bright blue regions. Cells showing colocalization were

counted and expressed as a frequency of the total number of cells. Data were obtained from two different slides per group. **D**, Histograms show the average intensity per pixel of H3K9me3 (upper panel) and HP1α (lower panel) within constitutive heterochromatin (HC) and outside of constitutive heterochromatin, that is, euchromatin (EU) and facultative heterochromatin (HF), for $Sirt1^{-/-}$ and wild-type MEFs. The average HP1 and H3K9me3 intensity per pixel is higher in $Sirt1^{+/+}$ than $Sirt1^{-/-}$ within constitutive heterochromatin regions ($P < 0.001$). Error bars represent s.d. **E**, Quantification of the rescue experiments shown in **B**, panels **i–t**, represented in a graph showing the percentage of colocalization of H3K9me3 with heterochromatin (red bars), and the corresponding percentage of mislocalization (blue bars) for each experiment. Wild-type and $Sirt1^{-/-}$ percentages are shown on the left of the graph. The percentage of colocalization/mislocalization of the transfected cells are represented on the right of the graph. These experiments, which involved the quantification of 800 Flag-positive cells for each condition, were reproduced in triplicate and error bars (s.d.) are included. Representative immunofluorescence images of these experiments are shown in panel **B, i–t**.

distribution was slightly more punctuate in the mutant case (Fig. 4A, compare f and h). However, the distribution of the SIRT1 target, H4K16ac, was altered greatly. Although normally distributed to euchromatin (Fig. 4A, j), H4K16ac was now seen to invade constitutive heterochromatin, being almost exclusively located there in the case of $Suv^{-/-}$ cells (Fig. 4A, l). None of the other non-heterochromatin-associated modifications tested (H4K12ac, H3K9ac or H3K4me3) was found to invade heterochromatin in these cells (Supplementary Fig. 6a).

We next gauged SUV39H1 activity in the absence of SIRT1. $Sirt1^{-/-}$ MEFs showed a complete loss of SUV39H1-dependent H3K9me3 in heterochromatic regions (Fig. 4B, D) in more than 50% of the cells studied, in comparison to the wild-type case (Fig. 4C). This is in agreement with previous findings in which SIRT1 loss by RNA interference led to decreased levels of H3K9me3 (ref. 3) and in which Clr4-mediated methylation of H3K9me3 in constitutive heterochromatin required the presence of Sir2 in *S. pombe*[6]. Moreover, the localization of heterochromatin protein 1 (HP1) to the same loci was also abrogated in $Sirt1^{-/-}$ MEFs (Fig. 4B–D). However, in contrast to the case of *S. pombe*, the loss of SIRT1 or SUV39H1 in the mammalian system studied here did not result in an invasion of H3K9ac in those regions that exhibited a loss of H3K9me3 (Supplementary Fig. 6b). This indicates that mechanisms engendering heterochromatin formation may not be completely conserved from yeast to humans. Of note, the antibodies against H3K9me3 were derived using a branched methylated peptide, and the detection of H3K9me3 is SUV39H1-dependent (Fig. 4A, compare b with d). This effect is directly dependent on SIRT1, because expression of exogenous SIRT1 rescued H3K9me3 mislocalization in heterochromatic regions (Fig. 4B, i–l, E,). This SIRT1-mediated rescue depends on both its activity and the presence of its N-terminal domain (Fig. 4B, m–t, E), supporting a direct functional relationship between SIRT1 and SUV39H1. In addition, the high degree of identity between SUV39H1 and its close relative SUV39H2, together with our results obtained with the $Suv^{-/-}$ cells, may suggest also a SIRT1 link to SUV39H2, the function of which is not completely understood. Further work should shed a light on this point.

This report demonstrates the direct and functional interrelationship between two major enzymes that implement chromatin structural changes through histone modifications during the formation of heterochromatin. This underscores that the enzymes responsible for pivotal histone modifications do not function in isolation, but are co-regulatory. SIRT1 exhibits a multi-pronged role in promoting conditions favourable to transcriptional repression by deacetylating specific histone proteins, recruiting histone H1b and, as shown here, modulating SUV39H1 activity, resulting in the accumulation of another repressive chromatin mark. This streamlining of the participants that alter chromatin structure is just beginning to be appreciated and probably operates during other regulatory events that evolved to ensure this process.

METHODS SUMMARY

Immunofluorescence. The MEFs used in this work were fixed in 4% paraformaldehyde for 10 min at room temperature (22 °C). Membrane permeabilization was achieved by incubation in buffer A (0.1% sodium azide PBS, 0.5% Triton-X, 0.5–1% BSA) for 10 min at room temperature, as described previously[13]; this was followed by incubation with antibodies against H3K9me3, mouse SIRT1, HP1α and H4AK12 (Upstate); H3K9ac and H3K4me3 (Abcam); and H4K16ac (Serotec).

Mapping of acetylated residue in SUV39H1. Gel-resolved proteins from nicotinamide-treated and untreated (control) samples were digested with trypsin, batch-purified on a reversed-phase micro-tip, and analysed by matrix-assisted laser desorption/ionization reflectron time-of-flight (MALDI-reTOF) mass spectrometry (MS) (UltraFlex TOF/TOF, Bruker) for peptide mass fingerprinting, as described[14]. This served to confirm the identity of the proteins and to locate differences between the tryptic peptide maps of the modified (acetylated) and unmodified forms. Differential peak *m/z* values were matched to the protein sequence provided, allowing for the probable presence of acetyl groups (+42 Da). Mass-spectrometric sequencing of the putative acetylated peptides (with or without methionine oxidation) was then carried out by MALDI-TOF/TOF MS/MS analysis using the UltraFlex instrument in 'LIFT' mode. Fragment ion spectra, derived from averaging 2,000 laser shots, were inspected for a, b and y ions to compare with the computer-generated fragment ion series of the predicted tryptic peptides to locate the exact or approximate position of the acetylated amino acid.

HKMT assays and immunoprecipitations. The assays were performed as described previously[15]. Immunoprecipitations were performed using Flag agarose (Sigma) and agarose-crosslinked Myc antibody (Cell Signaling).

Cells and treatments. MEFs were generated as described[2,16,17]. Human 293 cells were treated with nicotinamide and/or TSA under conditions described previously[3]. Transfections were performed using Lipofectamin 2000 (Invitrogen) according to the manufacturer's instructions.

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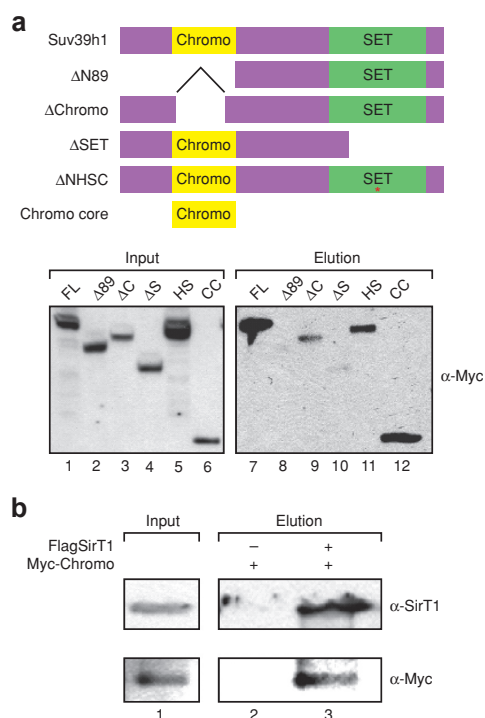
Figure S1

Figure S1. Suv39h1 interacts with SirT1 through its N-terminal region, including the chromodomain. a. Schematic of Myc-Suv39h1 deletion mutants and western blot of co-immunoprecipitations performed with FLAG-SirT1 and anti-FLAG antibody. **b.** Co-immunoprecipitation showing that the chromo-domain of Suv39h1 is sufficient for interaction with SirT1.
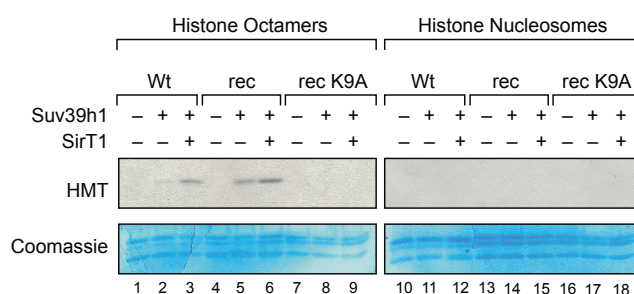
Figure S2

Figure S2. SirT1 stimulates Suv39h1 HKMT activity. HKMT assays performed with purified Suv39h1 in the absence or presence of purified SirT1 and with histone octamers or nucleosomes as indicated. Only histone octamers reconstituted with H3 containing wild type K9 were appropriate substrates for Suv39h1 activity and for SirT1-stimulated Suv39h1 activity.
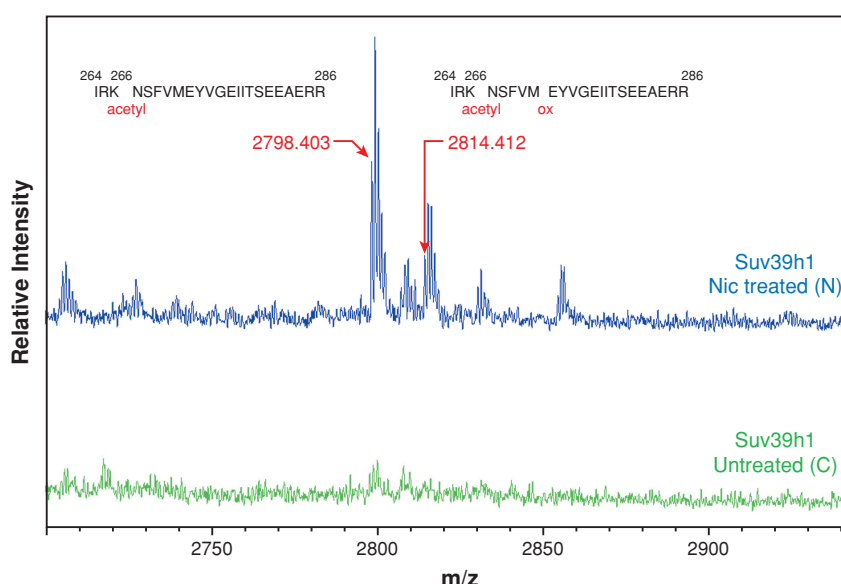
Figure S3

Figure S3. Identification of the Suv39h1 residue acetylated in vivo and deacetylated by SirT1. One m/z peak, at 2798.403 atomic mass units (amu), was observed in the spectra of "Nic treated" protein, but was absent from "untreated". The m/z value mapped to a predicted, mono-acetylated fragment of the Suv39h1 sequence (IRKNSFVMEYVGEIITSEEAERR) with a mass discrepancy of less than 1 ppm (0.002 Da) for the monoisotopic peak. This precursor ion was then selected for MALDI-TOF/TOF MS/MS analysis; a total of 2,000 fragment ion spectra were averaged. The presence of unique fragment ions confirmed the identity and the acetylation site of this peptide. The MS/MS analysis was also performed on a peptide peak with m/z=2814.412 which mapped to the methione-oxidized derivative of the same peptide with mass discrepancy of less than 3ppm (0.005 Da), yielding essentially the same result.
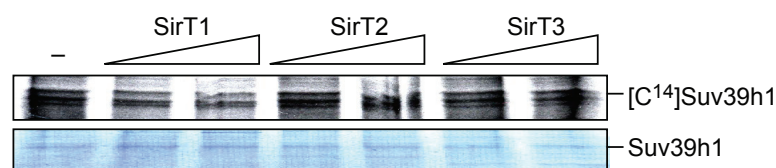
Figure S4

Figure S4. In vitro deacetylation of Suv39h1 by SirT1. Hyperacetylated Suv39h1 was prepared as follows. GST-Suv39h1 (40 ng) purified from E. coli was dialyzed into acetylation buffer (50 mM NaHCO3, pH 8/2 M NaCl) and then acetylated with 50 μCi [C14]-acetic anhydride on ice for 2 hr [18]. The sample was incubated with a titration of equimolar amounts of purified SirT1, SirT2 and SirT3 in the presence of NAD+. Samples were resolved by SDS-PAGE, transferred and exposed for radioactive signal.
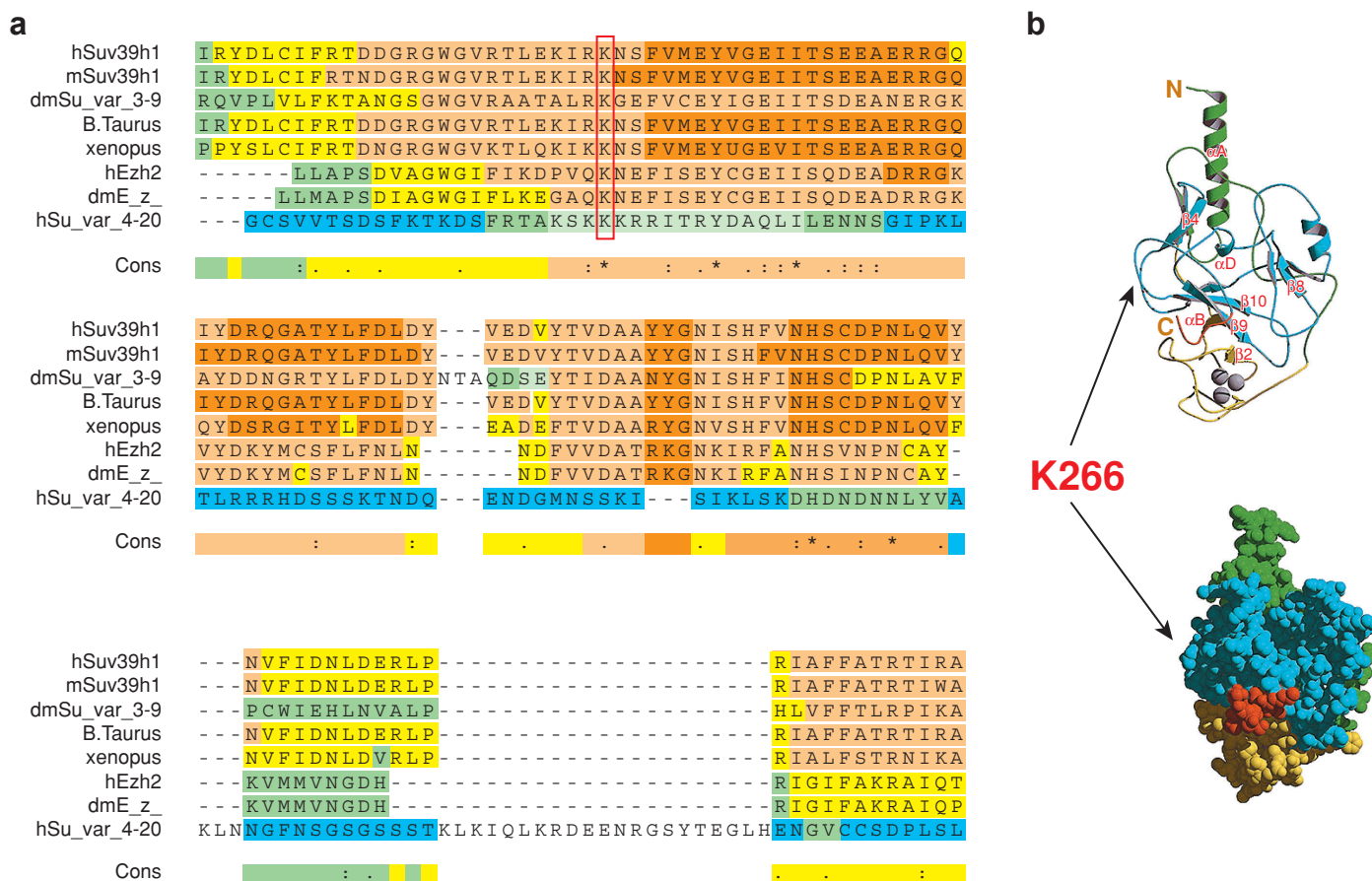
Figure S5

Figure S5. Conservation of residue K266 through evolution and in different SET-containing proteins a. Comparison of the sequence of SET domains of Suv39h1 from human, mouse, Drosophila, bovine (B. taurus) and Xenopus; SET domains of Ezh2 from human and Drosophila (E(z)) and SET domain of human Suvar4-20. Conservation of K266 is indicated by a red rectangle. Sequences were analyzed withT-COFFEE (http://www.ch.embnet.org/software/TCoffee.html)[19]. The degree of conservation of the sequences is indicated by the color code. **b.** Structure and CPK model of the SET domain of Clr4 courtesy of Dr Rui-Ming Xu (NYU, NYC)[11]. Arrows indicate the hypothetical position of K266 residue in the structure by comparison to the Suv39h1 sequence.
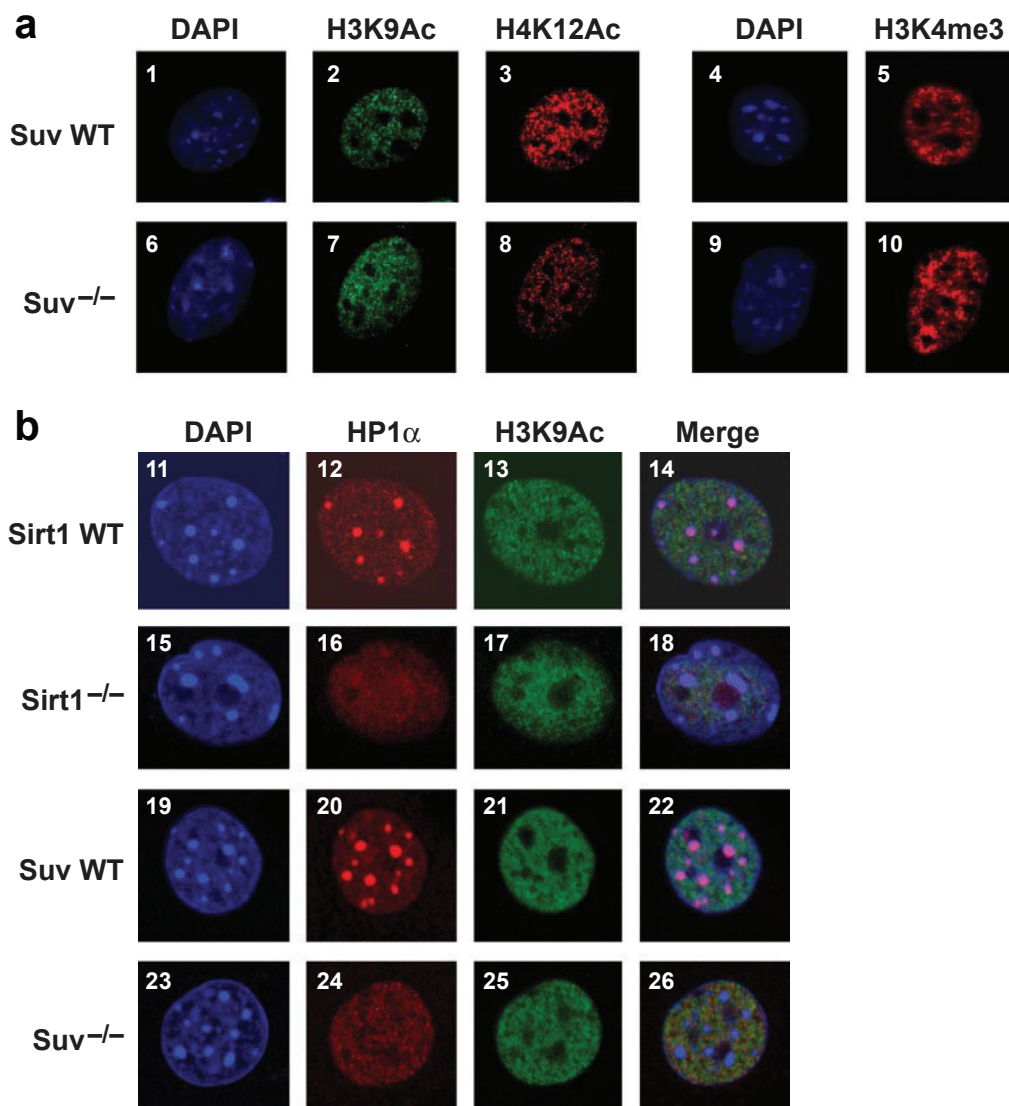
Figure S6

Figure S6. H4K16ac is the only modification of those tested that changes localization upon loss of Suv39h1.
a. Distribution of H3K9Ac (2, 7), H4K12Ac (3, 8) and H3K4me3 (5, 10) were compared in WT and Suv^{-/-} MEFs as in Fig 4C. **b.** Loss of either Suv39h1/2 or SirT1 in mammalian cells does not result in H3K9Ac invasion of heterochromatic regions. Immunofluorescence studies were performed as in a to study the distribution of HP1α (12, 16, 20, 24) and H3K9Ac (13, 17, 21, 25) in MEFs derived from WT SirT1, SirT1^{-/-}, WT Suv and Suv^{-/-} mice.

Suppl. References

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