RESULTS

1. SETDB1 UNDERGOES AUTO-METHYLATION *IN VITRO*

As many other proteins, SETDB1 is subject to a number of post-translational modifications that can play crucial roles in regulating protein-protein interactions, enzyme activity and stability of the protein. For instance, SETDB1 is modified by ubiquitination at K867 and that modification was shown to be required for its H3K9 methyltransferase activity and regulation of the expression of its target genes (2). Moreover, methylation of non-histone proteins at lysine residues by SETDB1 has been reported (6,8). In an attempt to determine whether SETDB1 is capable to methylate itself as a non-histone substrate, we produced different SETDB1 truncated mutants as GST-fusion proteins **(Figures 1A and 1B**). Then, we performed *in vitro* methylation assay using recombinant active full-length SETDB1 as enzyme and radioactive 3H-S-Adenosyl Methionine (SAM) as methyl donor group. Strikingly, the upper band detected in the reactions with the mutants revealed a full-length SETDB1 auto-methylation. Interestingly, when the substrate is histone 3, a preferential methylation pattern was observed towards H3. Moreover, the Bifurcated domain and the Carboxy terminal region of SETDB1 were also methylated by the full-length SETDB1 in contrast to the Pre-SET and Post-SET domains (**Figure 1C**).

We further explored the implication of targeted lysine residues in SETDB1 auto-methylation. A lysine methylome published by Guo A *et al.* reported that SETDB1 contains two possible sites of methylation on lysine residues inside its catalytic domain (K1770 and K1778) (199). Strikingly, these two residues are in the Carboxy terminal region that we found is subject to SETDB1 auto-methylation. We used the GST-SETDB1 fusion protein that encodes the Carboxy terminal region (amino acids 1101 – 1290) to generate three mutants harboring single amino acid substitutions in one or two methylation sites (K1770A and/or K1778A) (**Figure 1D**) and performed *in vitro* methylation assay.

Our results showed that methylation level decreases in the GST-SETDB1 double lysine mutant (**Figure 1E**), suggesting that K1770 and K1778 are involved in SETDB1 auto-methylation. Interestingly, the alignment of the sequence harboring both methylation sites resembled the motif ARKS in the N-tail of the histone 3 targeted by SETDB1 (**Figure 1F**).

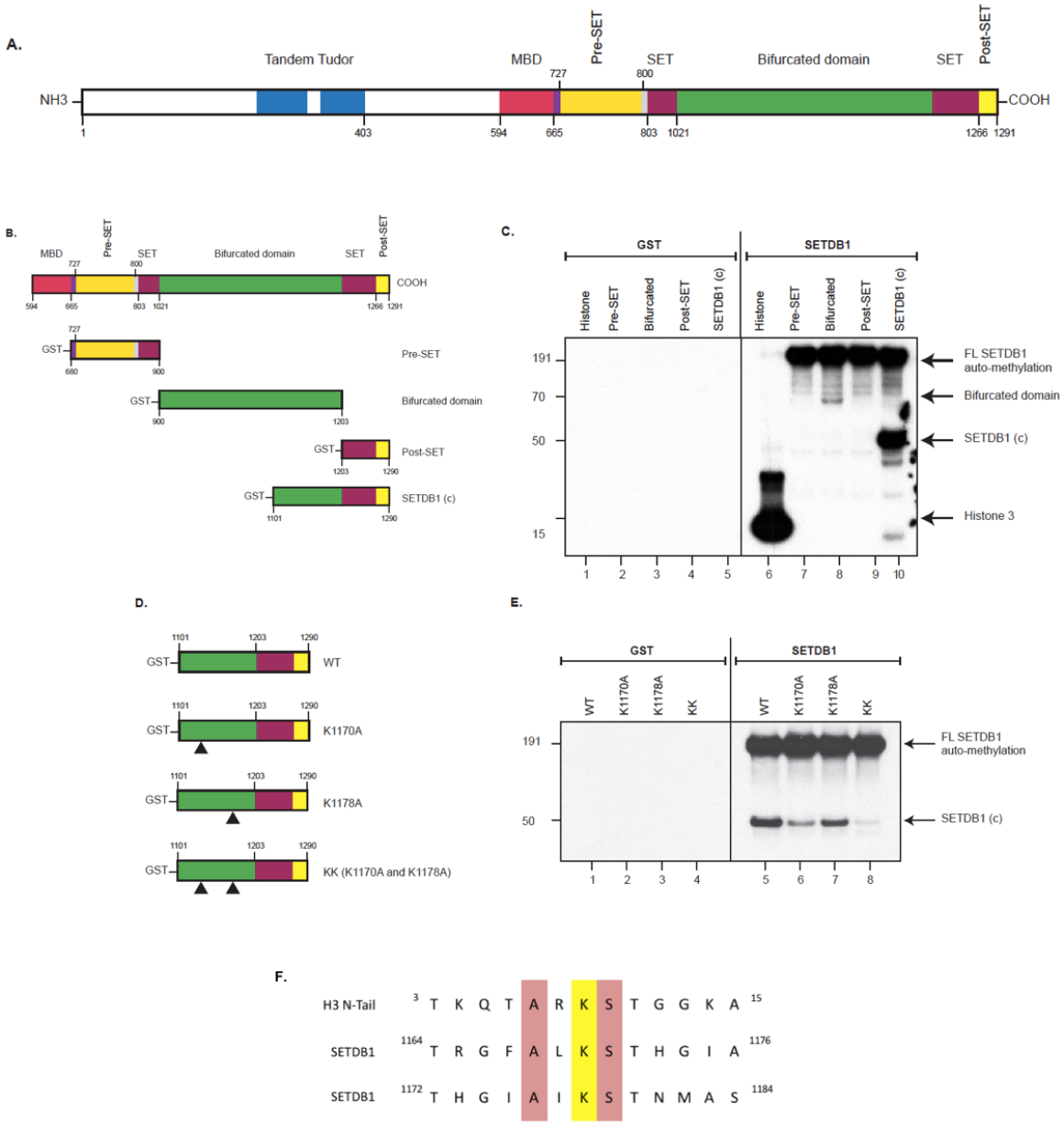


Figure 1. SETDB1 methylation *in vitro*. A) Schematic illustration of SETDB1 protein. **B)** Schematic illustration of recombinant GST-SETDB1 truncation mutants used in (C). **C)** Detection of protein methylation by transfer of radioactively labeled methyl groups. Purified GST-SETDB1 proteins were incubated with recombinant SETDB1 in the presence of radioactive SAM and separated on an SDS polyacrylamide gel, and the methylation of the target proteins was analyzed by autoradiography. Full-length SETDB1 auto-methylation is observed in the upper part of the gel (lines 7 to 10). The SETDB1 Pre-SET and Post-SET domains (which do not contain the auto-methylation sites) are shown in lanes 7 and 9 respectively. As a control, methylation of histone H3 (lane 6) is shown. **D)** Schematic illustration of recombinant GST-SETDB1 which encodes part of the catalytic domain (WT) and the relative position of single amino acid substitutions introduced. **E)** SETDB1 undergoes auto-methylation in K1170 and K1178 (lane 8). **F)** Sequence alignment of the region surrounding the human SETDB1 K1170 and K1178 methylation sites and the sequence surrounding mouse H3K9 N-tail. Residues identical in all sequences are shown in red and aligned lysine sites in yellow.

2. SETDB1 CATALYTIC ACTIVITY AND INTEGRITY OF K1770/K1778 ARE REQUIRED FOR ITS AUTO-METHYLATION

We next sought to confirm whether SETDB1 catalyzes its own methylation *in vivo*. *Setdb1* KO mouse embryonic fibroblasts (MEFs) were transfected with either pCS2 empty vector or expressing myc-tagged wild-type (WT), double lysine mutant (K1770A and K1778A, KK on the figures), or enzymatically inactive (C1243A, CA on the figures) SETDB1. Nuclear extracts were used for immunoprecipitation (IP) with Myc-trap technology and precipitates were then analyzed by western blot using anti-pan-tri-methyl-lysine (panKme) antibody. Interestingly, the double lysine mutant SETDB1 extract showed a reduction in the level of lysine methylation on SETDB1. Notably, for the SETDB1 catalytically-inactive mutant, the reduction was more dramatic (**Figure 2A**). These results suggest that SETDB1 catalytic activity is required for its auto-methylation, and the residues K1770 and K1778 are involved in this process.

We then examined whether the H3K9 methyltransferase activity of SETDB1 is affected by the presence of the double lysine mutations in the immunoprecipitated Myc-tagged products from the reconstituted MEFs nuclear extracts. We performed *in vitro* methylation assay using the IP products as enzyme, a cocktail of histones as substrate and radioactive S-Adenosyl Methionine (SAM) as methyl donor group. We found that both wild-type and non-methylatable forms of SETDB1 showed comparable catalytic activities toward histone 3 (**Figure 2B**).

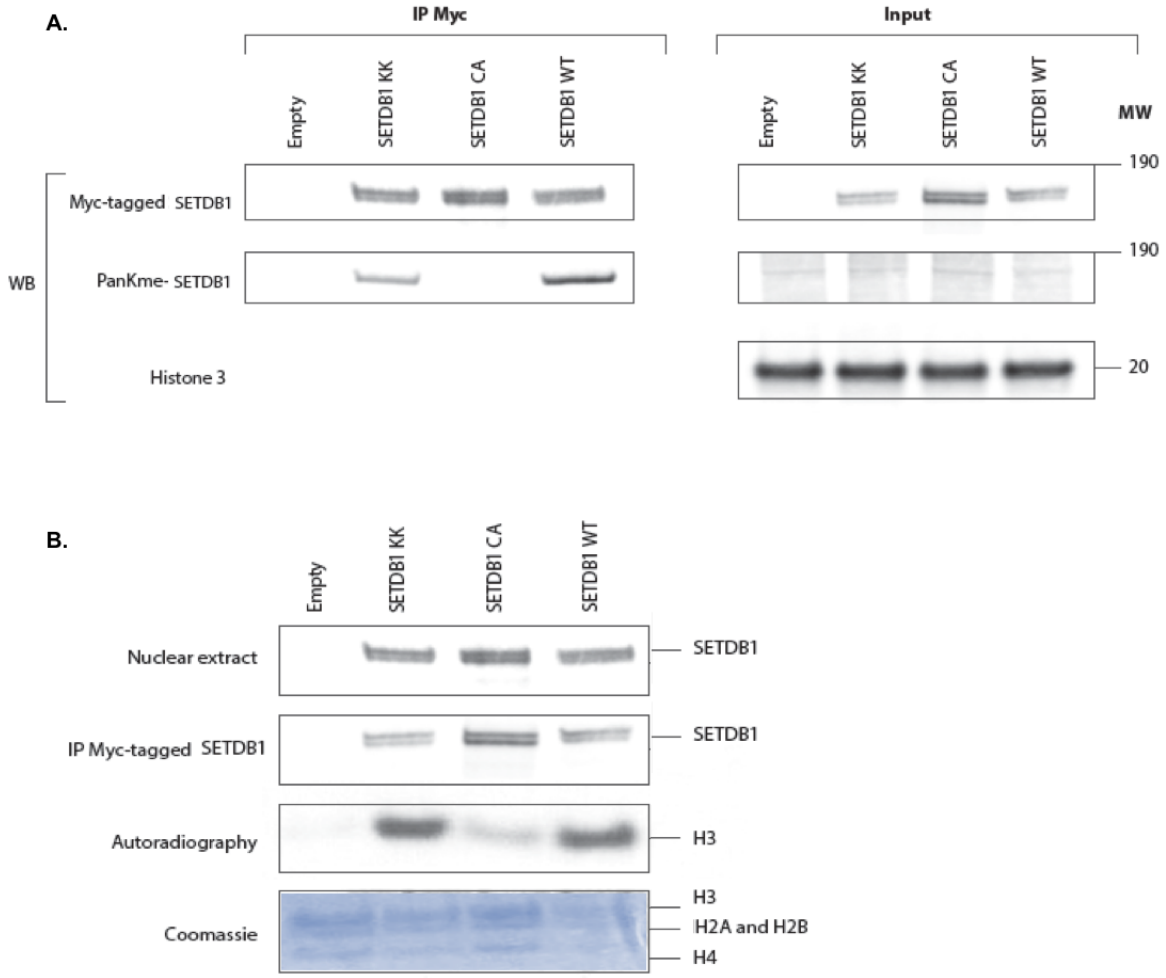


Figure 2. The catalytic activity of SETDB1 is required for its methylation. A) Four different myc-tagged SETDB1 cDNAs including full-length (WT), non-methylatable (KK) and catalytic-inactive (C1243A) forms were cloned and expressed in *Setdb1* KO MEFs. Myc-SETDB1 was immunoprecipitated by Myc-Trap technology and subjected to WB with anti-Pan-tri-methyl-lysine (PanKme) and anti-Myc antibodies. **B)** Detection of protein methylation by transfer of radioactively labeled methyl groups. Myc-SETDB1 precipitates were incubated with a recombinant cocktail of histones in the presence of radioactive SAM and separated on an SDS polyacrylamide gel. Complexes containing WT or the non-methylatable (KK) SETDB1 can catalyze the transfer of radiolabelled methyl groups to histone H3, as detected by autoradiography. A Coomassie-stained control gel of the proteins is also shown.

3. METHYLATION STATUS OF SETDB1 IMPACTS ITS PROTEIN-PROTEIN INTERACTIONS

Because the methyltransferase activity of SETDB1 is not directly affected by the double lysine mutation, we postulated that this modification event is involved in modulating other features, such as key protein–protein interactions. To address this, we have analyzed the immunoprecipitated Myc-tagged products from the MEFs nuclear extracts by western blot. Interestingly, both the integrity of K1770/K1778 and SETDB1 catalytic activity were required for its interaction with many chromodomain-containing proteins such as, Suv39h1, HP1γ (Heterochromatin Protein 1 gamma) and CDYL (Chromodomain Y-Like) (**Figures 3A and 3B**). Similar results were obtained using a TAP-tag approach from HeLa cell extracts (not shown). Since CDYL transcriptional repressor complex includes SETDB1 (122), we hypothesize that SETDB1/CDYL interaction could be mediated by methylation of SETDB1. Also, given that HP1 is a reader of methylated lysines and is crucial for heterochromatin formation and proviral silencing (11), methylation of SETDB1 could affect the assembly of repressor complexes recruited to establish and/or maintain heterochromatin.

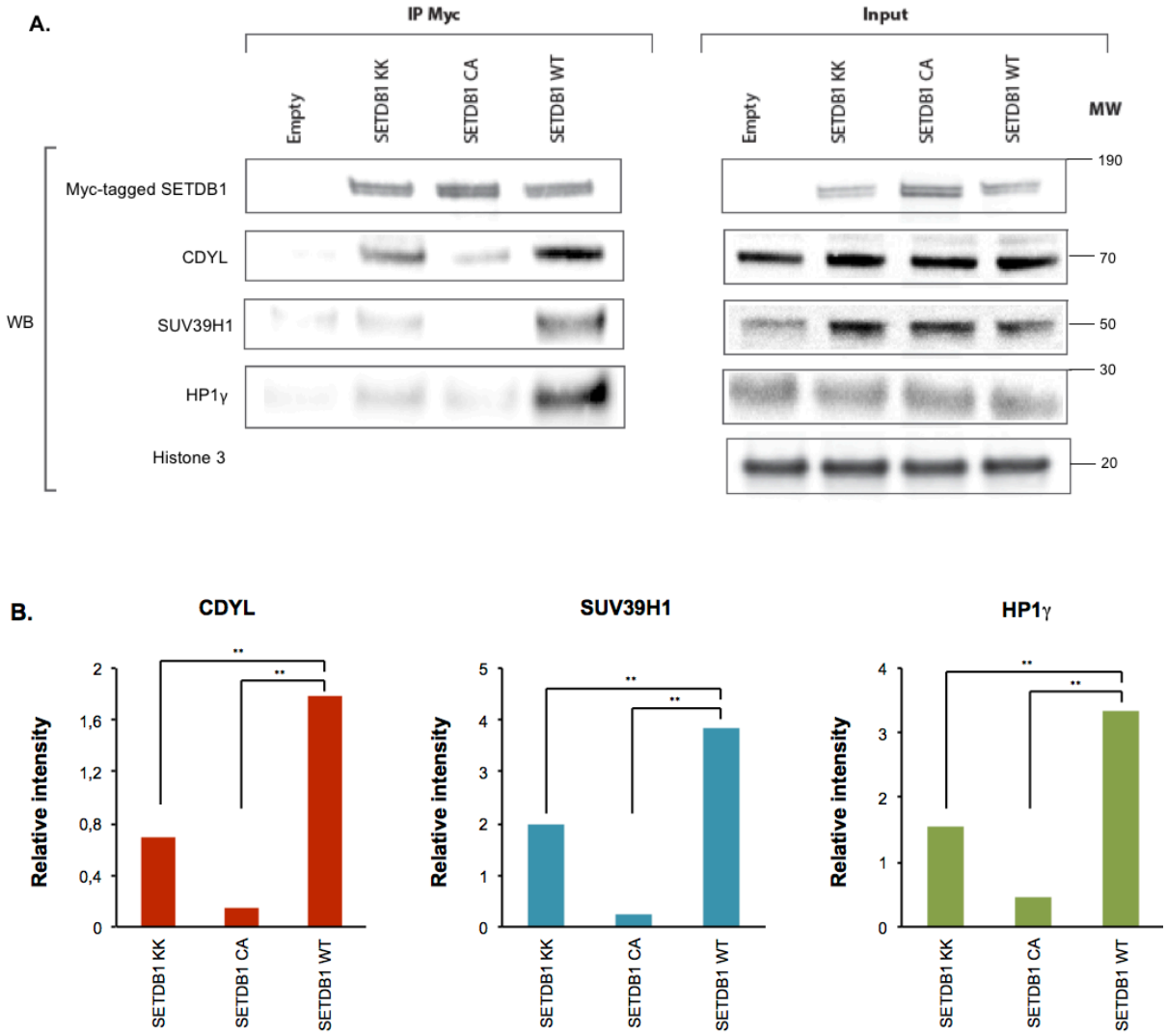


Figure 3. Methylation status and catalytic activity of SETDB1 have an impact on its protein-protein interactions. A) Decrease of interaction between SETDB1 catalytic mutant (C1243A) and double lysine mutants (KK) with the chromodomain-containing proteins SUV39H1, CDYL and HP1γ. Four different myc-tagged SETDB1 cDNAs including full-length (WT), non-methylable (KK) and catalytic-inactive (C1243A) forms were cloned and expressed in *Setdb1* KO MEFs. Nuclear extracts were used for immunoprecipitation (IP) with Myc-Trap technology. The resulting precipitates were then subjected to WB with indicated Abs. MW: molecular weight marker in KDa. **B)** WB signals were quantified and presented as the ratio between the protein analyzed over the Myc-tagged SETDB1. All values are expressed as the mean ± SEM (n = 5).

4. mESCS VIABILITY IS AFFECTED BY THE NON-METHYLATABLE AND THE CATALYTIC INACTIVE FORMS OF SETDB1

As discussed in the introductive section, SETDB1 is crucial during development and cellular differentiation. Moreover, SETDB1 is essential in mouse ESC pluripotency, self-renewal and viability (3,4) and *Setbd1* KO is lethal at the peri-implantation stage at 3.5 days postcoitum (dpc) (5).

We thus tested whether the integrity of K1770/K1778 and catalytic activity of SETDB1 can affect mESC viability. To address this, we used conditional *Setdb1* KO TT2 mESCs (cKO) re-expressing either wild-type, catalytic-dead (C1243A) mutant (87), or the double-lysine mutant (K1770A and K1778A) forms of SETDB1. Notably, in these TT2 mESCs, endogenous SETDB1 depletion starts at 48h and is achieved after 96h of treatment with 4-hydroxy-Tamoxifen (OHT) (**Figure 4D**). First, we verified the distribution of SETDB1 in mESCs after tamoxifen treatment and as it is shown in the **Figure 4A**, SETDB1 is localized in the nucleus as well as in the cytoplasm in all cell types expressing Flag-tagged SETDB1, a phenotype comparable with the expression of the endogenous SETDB1 in non-treated mESCs (3306 cells).

As reported previously, *Setdb1* cKO TT2 mESCs showed a growth defect after 6 days of tamoxifen treatment. Remarkably, this growth defect was rescued with the ectopic re-expression of wild-type SETDB1 in these *Setdb1* cKO TT2 mESCs, while re-expression of SETDB1 catalytic-inactive did not (87). This prompted us to determine whether the growth defect observed in *Setdb1* cKO TT2 mESCs can be rescued after re-expression of the non-methylatable form of SETDB1. We first analyzed the viability of the cells at 48h and 96h of treatment with tamoxifen and 2 days after without treatment (144h on the figure). Our results

showed a decrease in cell viability in *Setdb1* cKO mESCs as well as in mESCs expressing the double-lysine or the catalytic inactive SETDB1 mutants compared to the wild-type SETDB1 only by the day 2 post-tamoxifen treatment (144h of cell culture) but not before (**Figure 4B**). Furthermore, reduced proliferation was associated with induction of apoptosis at 144h of cell culture, as determined by the number of cells expressing early or late apoptosis markers (Annexin V and/or propidium iodide) (**Figure 4C**). Taken together, these results suggest that expression of the non-methylatable for of SETDB1 seems to compromise cell viability, after depletion of endogenous SETDB1, as has been observed and also reported for the catalytic inactive form of SETDB1.

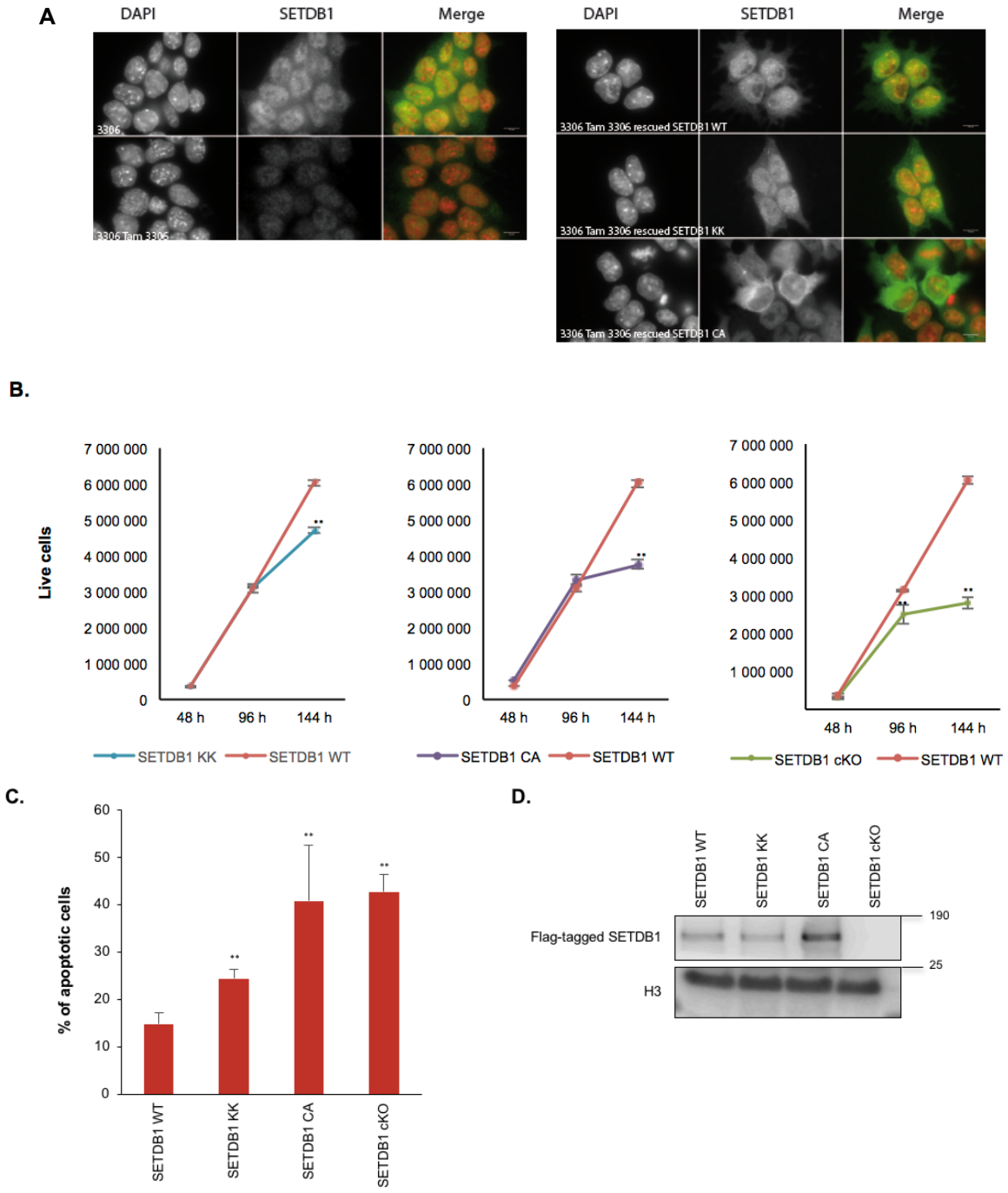


Figure 4. The catalytic inactive and the non-methylatable forms of SETDB1 show a growth defect after SETDB1 endogenous depletion. A) Expression of flag-tagged SETDB1 forms in TT2 mESCs. SETDB1 (green) was revealed by indirect immunofluorescence (IF). DNA was labelled with DAPI (red). Scale bar=10 µm. **B)** mESCs were cultured with tamoxifen until 96 h and without treatment during 2 days more. SETDB1 was depleted by the day four of treatment. Growth defect was observed at 144 h of culture for mESCs expressing the non-methylatable and catalytic inactive SETDB1 mutants. Cell viability was reduced at 96 h of tamoxifen treatment for cKO *Setdb1* mESCs. **C)** Percentage of cells marked positive for Annexin V and/or propidium iodide. **D)** Nuclear extracts were subjected to WB with indicated Abs. All values are expressed as the mean ± SEM (n = 3). For significance Student's paired *t*-test was applied. **p < 0.05.

5. INTEGRITY OF SETDB1 K1770/K1778 IS REQUIRED FOR PROVIRAL SILENCING

Since SEDTB1 is required for H3K9me3 and silencing of ERVs in mESCs (87), we investigated whether methylation of SETDB1 on K1770 and K1778 can regulate SETDB1-dependent transcriptional silencing of ERVs and LINE sequences in mESCs. Our results showed that expression of the double-lysine mutant SETDB1 in mESCs resulted in de-repression of MLV, MusD, IAP and LINE-1 elements (~1,5 fold). Surprisingly, MMERVK10c elements were strongly induced not only in the double-lysine SETDB1 mutant mESCs (~20 fold), but also in mESCs expressing the catalytic-dead SETDB1 mutant (~37 fold) (**Figure 5A**). Interestingly, we have validated the reactivation of MLV, MusD, IAP, and LINE-1 elements (~3 fold) and MMERVK10c (~170 fold) after SETDB1 depletion compared to previous reports (191,200). Taken together, these results reveal that the expression of the non-methylatable form of SETDB1 disrupts SETDB1/H3K9me3-mediated silencing of ERVs in mESCs and its effect is similar to that seen in mESCs expressing the catalytic inactive form of SETDB1.

6. H3K9me3 ENRICHMENT IS REDUCED ON PROVIRAL CHROMATIN WHEN INTEGRITY OF SETDB1 K1770/K1778 IS COMPROMISED

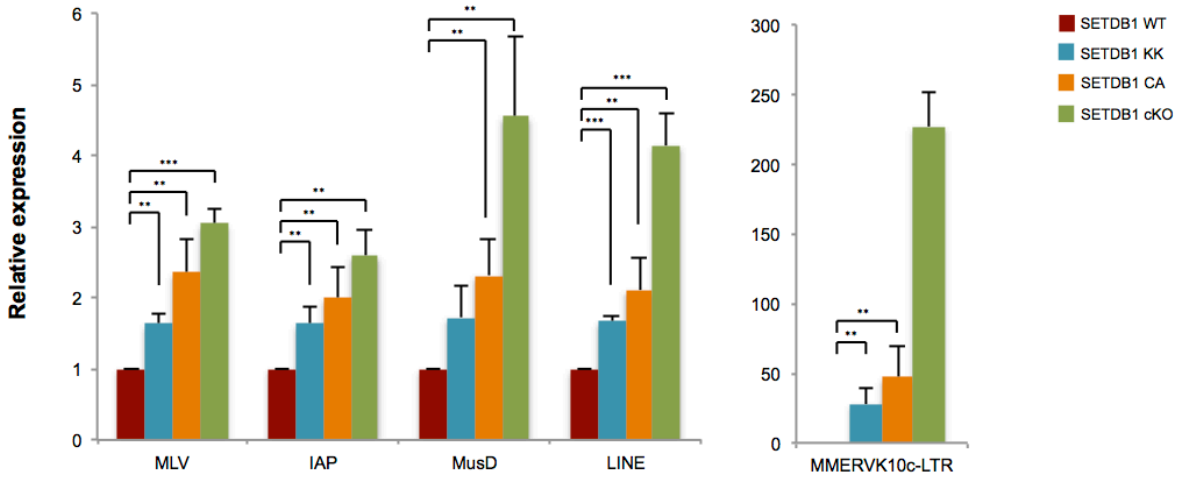
SETDB1-deposited H3K9me3 is necessary for silencing of several ERV subfamilies in ESCs (87,200). To test whether integrity of K1770/K1778 is required for SETDB1 recruitment to ERVs and LINE-1 sequences, we conducted ChIP-qPCR analysis of Flag-tagged SETDB1 in mESCs expressing either the wild-type SETDB1 or the non-methylatable SETDB1. A reduction of SETDB1 enrichment was apparent at all transposable elements in *Setdb1* cKO mESCs, confirming the specificity of our antibody (**Figure 5B**). Our results showed a low decrease in the recruitment of SETDB1 at MLV, IAP and MusD elements in mESCs

expressing the double-lysine mutant of SETDB1 (**Figure 5B**). We also tested whether expression of the catalytically-inactive SETDB1 affects its recruitment to ERVs and LINE-1 sequences. As it has been reported previously for the IAP elements (87), the inactivity of the enzyme did not affect SETDB1 enrichment levels at ERVs or LINE-1 sequences (**Figure 5B**). Importantly, SETDB1 protein levels were comparable in mESCs expressing SETDB1 wild-type and double-lysine mutant SETDB1 while SETDB1 protein levels were higher in the cells expressing the catalytic-inactive mutant SETDB1. (**Figure 4D**).

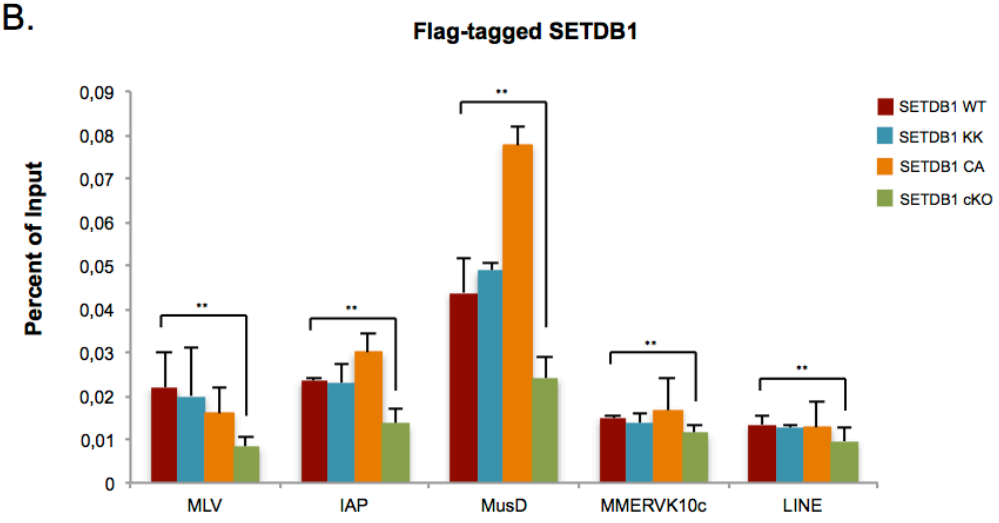
To verify whether reactivation of ERVs and LINE sequences is accompanied by loss of H3K9me3 in the mESCs expressing the non-methylatable form of SETDB1, we analyzed the H3K9me3 enrichment by ChIP-qPCR. Our results revealed a decrease in this repressive mark in MLV, MusD, IAP and MMERVK10c elements (**Figure 5C**), indicating that the integrity of K1770/K1778 in SETDB1 is required to maintain proviral silencing *via* deposition of H3K9me3. Interestingly, when SETDB1 is enzymatically inactive, reactivation of MLV, MusD, IAP and MMERVK10c elements and loss of H3K9me3 were also observed (**Figure 5C**), which is consistent with previously reported only for IAP (87).

Altogether these results suggest that although integrity of K1770/K1778 and catalytic activity of SETDB1 did not affect SETDB1 binding to chromatin, the methyltransferase activity and integrity of K1770/K1778 are critical for SETDB1/H3K9me3-mediated silencing of ERVs in mESCs.

A.


Relative expression
MLV
IAP
MusD
LINE
MMERVK10c-LTR
SETDB1 WT
SETDB1 KK
SETDB1 CA
SETDB1 cKO

B.


Flag-tagged SETDB1
Percent of Input
MLV
IAP
MusD
MMERVK10c
LINE
SETDB1 WT
SETDB1 KK
SETDB1 CA
SETDB1 cKO

C.

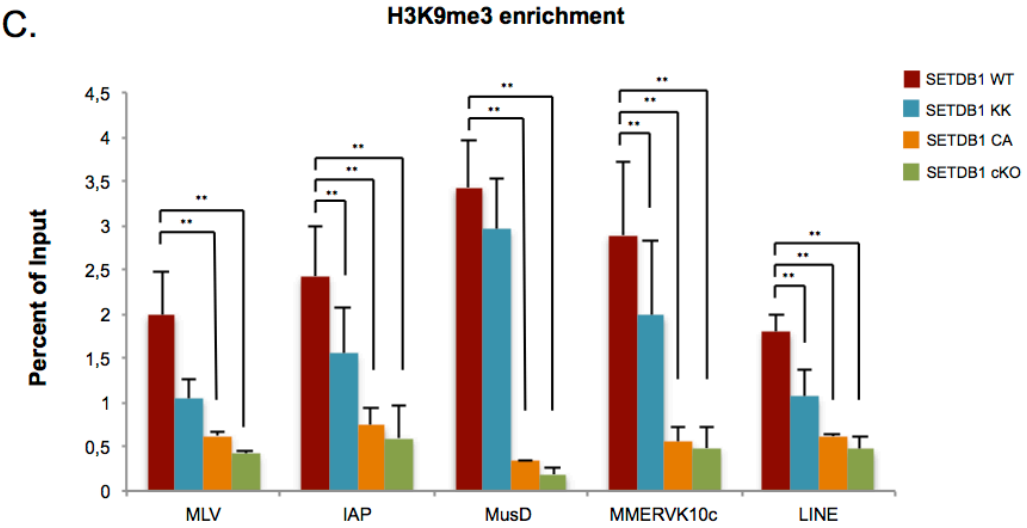

H3K9me3 enrichment
Percent of Input
MLV
IAP
MusD
MMERVK10c
LINE
SETDB1 WT
SETDB1 KK
SETDB1 CA
SETDB1 cKO

Figure 5. Integrity of SETDB1 K1770/K1778 and SETDB1 catalytic activity are required for gene silencing of class I and II ERVs in mESCs. A) ERVs silencing is regulated by SETDB1 methylation status and catalytic activity. mRNA levels of conditional *SETDB1* cKO mESCs and cells expressing wild-type (WT), double-lysine (KK) mutant and catalytic-dead (CA) SETDB1 were normalized to those of cyclophilin A. **B)** Binding of SETDB1 at proviral chromatin. ChIP-qPCR for SETDB1 as indicated ERVs 5'LTR and LINE-L1 in mESCs used in A. **C)** Deposition of H3K9me3 at proviral chromatin as indicated ERVs 5'LTR and LINE-L1 in mESCs used in A. All values are expressed as the mean ± SEM (n = 3). For significance Student's paired *t*-test was applied. **$p < 0.05$ or ***$p < 0.01$.

7. GENOME-WIDE PROFILING OF GENE EXPRESSION, SETDB1-BINDING AND H3K9me3 ENRICHMENT IN mESCS EXPRESSING SETDB1 WILD-TYPE, CATALYTIC-DEAD OR DOUBLE-LYSINE MUTANTS

To determine whether methylation of SETDB1 on K1770 and K1778 and/or SETDB1 catalytic activity can regulate SETDB1-dependent gene regulation, we performed RNA-seq (excluding ribosomal RNAs) from three biological replicates of *Setdb1* cKO TT2 mESCs re-expressing either wild-type SETDB1, catalytic-dead (C1243A) or the double-lysine (K1770A and K1778A) SETDB1 mutants. Our preliminary analysis revealed that the replicate samples have high similarity (**Figure 6A**). A first stringent analysis showed that a total of 818 genes were de-regulated in mESCs expressing the non-methylatable form of SETDB1 and 2646 genes were de-regulated in mESCs expressing the catalytic inactive form of SETDB1. Strikingly, 758 genes were commonly de-regulated in both cell types ($p<0,001$) (**Figure 6B and Figure 6C**). Gene ontology (GO) analysis revealed that de-regulated genes in mESCs expressing SETDB1 mutants were enriched for "Regulation of transcription-DNA binding" category (**Figure 6D and Figure 6E**). Notably, this category included transcription factors such as *Nanog* and *Klf4,* which were down-regulated. These results suggest that expression of SETDB1 mutants in mESCs could lead to a loss of pluripotency, an event that has been demonstrated in mESCs after loss of SETDB1 (3,5).

We next investigated whether expression of the non-methylatable and the catalytic inactive forms of SETDB1 in mESCs result in deregulation of germline genes, which are directly repressed by SETDB1-dependent H3K9me3 and DNA methylation in mESCs (200). Our results showed that 10 germline genes including *Dazl, Hormad1, Nlrp4c, Rex2, Rpl39l, Serpine1, Stk31, Tex101, Tuba3a,* and *Gm13212* were up-regulated in both cell lines, confirming that any disruption of SETDB1 is sufficient for transcriptional activation.

We next investigated whether the integrity of K1770/K1778 in SETDB1 and/or SETDB1 catalytic activity affect SETDB1 binding and SETDB1-dependent H3K9me3. To address this, we performed genome-wide analyses for enrichment of Flag-tagged SETDB1 and H3K9me3 by chromatin immunoprecipitation and subsequent DNA sequencing (ChIP-seq) in mESCs expressing either the wild-type SETDB1, the catalytic inactive and the non-methylatable forms of SETDB1. The number of sequenced reads for H3K9me3 ChIP-seq and inputs was ~50 - 60 million in all samples. Around 48 million reads were mapped for Flag-SETDB1 in the cells expressing the mutants, but unfortunately only ~25 million reads were detected in the wild-type cells (**Table 3**). This difference could create a bias in the analysis, however we will perform the ChIP-seq again in the wild-type cells and we will conduct a second independent ChIP-seq experiment to validate our current results in all cell types.

Using the software MACS2, and after filtering (FC ratio >5) we were able to detect around ~9.000 SETDB1-binding sites in the cells expressing wild-type SETDB1 and the SETDB1 catalytic inactive mutant, corresponding to 6.155 genes and 6.290 genes, respectively. In addition, ~3.300 SETDB1-binding sites, corresponding to 2.873 genes were detected in the cells expressing the non-methylatable form of SETDB1. On the other hand, we detected 6.200 H3K9me3-enriched regions, corresponding to 4.281 genes in the cells expressing wild-type SETDB1, which is more than twice compared to cells expressing SETDB1 double-lysine mutant (~2.300 H3K9me3-enriched regions, corresponding to 1.812 genes). Only 58 H3K9me3-enriched regions, corresponding to 39 genes were detected in the cells expressing the SETDB1 catalytic inactive mutant. However, the number of H3K9me3-enriched regions is underestimated, that is why, knowing that spread histone marks such as H3K9me3 need a particular attention in terms of peak calling, we will perform a less stringent peak calling strategy using SICER peak caller, adapted for this type of spread histone marks (201).

So far, the majority of H3K9me3 enrichment in mESCs expressing wild-type SETDB1 and the non-methylatable form of SETDB1 is located in intergenic (~60%) and intronic regions (~20%). The distribution in coding sequences (<1%) or regulatory elements, such as enhancers or promoters (~17%), showed less H3K9me3 enrichment. Strikingly, in mESCs expressing the catalytic inactive SETDB1, a difference in the distribution of H3K9me3 enrichment was observed (intergenic region (49%) and promoters (28%) (**Figure 6F** and **Table 4**). Regarding SETDB1 binding, all cell types showed approximately the same distribution, ~60% intergenic, ~20% intronic and ~18% promoters (**Table 4**).

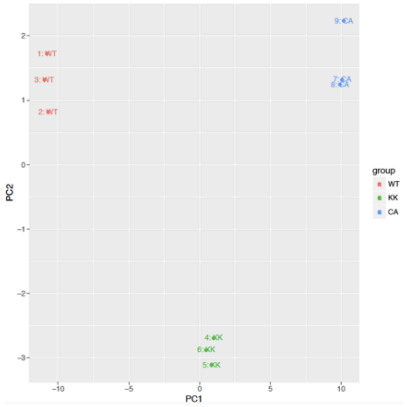
Among the 4.281 genes H3K9me3-marked in the cells expressing wild-type SETDB1, 1.515 genes harbor this mark in the cells expressing the non-methylatable form of SETDB1 as well (**Figure 6G**). Noteworthy, the H3K9me3 enrichment is almost lost in the cells expressing the catalytic-inactive form of SETDB1 (**Figure 6H**). Remarkably, 98% of the genes that lost the H3K9me3 mark in the cells expressing the SETDB1 double-lysine mutant also lost it in the cells expressing the SETDB1 catalytic-inactive mutant (**Figure 6I**). Moreover, among the genes that lost the H3K9me3 mark in the cells expressing the SETDB1 double-lysine mutant, we found 93 deregulated genes ($p<0,001$).

Among the 6.155 genes bound by SETDB1 in the cells expressing wild-type SETDB1, we found that 2.547 of these genes were also SETDB1-marked in the cells expressing the non-methylatable form of SETDB1 as well. Interestingly, SETDB1 binding in the cells expressing the SETDB1 catalytic-dead mutant was higher (4.564 genes). However, this result could change after the new sequencing of the wild-type SETDB1.

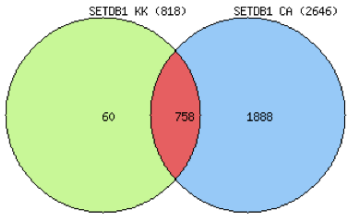
Our preliminary ChIP-seq results showed a decrease in the recruitment of SETDB1 and reduced levels of H3K9me3 levels at the promoter region of a set of imprinted genes including *H19, Nnat, Peg10* and *Peg3* in mESCs expressing the catalytic inactive and the double-lysine mutants of SETDB1 (**Figure 6J**). Furthermore, ChIP-qPCR analysis of Flag-tagged SETDB1 and H3K9me3 validated this reduction of SETDB1 binding as well as H3K9me3 on *H19*, *Nnat* and other imprinted gene *Mest* (**Figure 6K and Figure 6L**). Consistently, the RNA-seq analysis revealed that *H19, Peg3* and *Peg10* were de-regulated in mESCs expressing SETDB1 mutants. However, *Mest* and *Nnat* showed no significant changes in all cell types.

In conclusion, our results suggest that methyltransferase activity and integrity of K1770/K1778 of SETDB1 are critical for SETDB1/H3K9me3-mediated gene silencing. However, further analyses are needed to study the role of additional mechanisms in transcriptional repression in our cellular models.

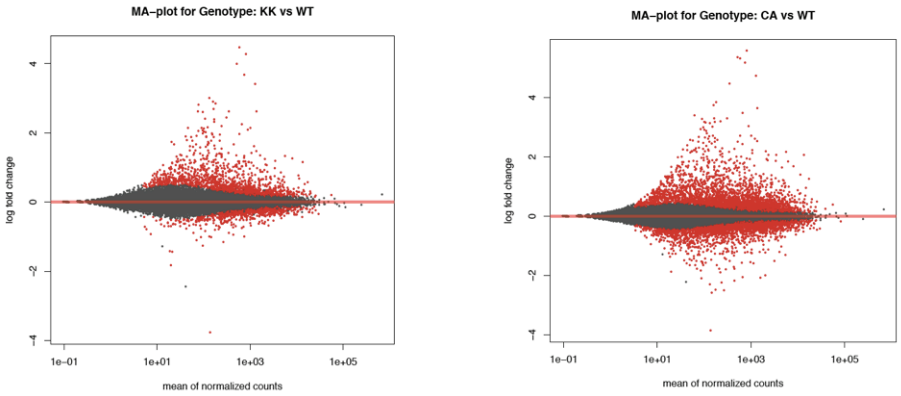
A.


PC2
PC1
group
WT
KK
CA

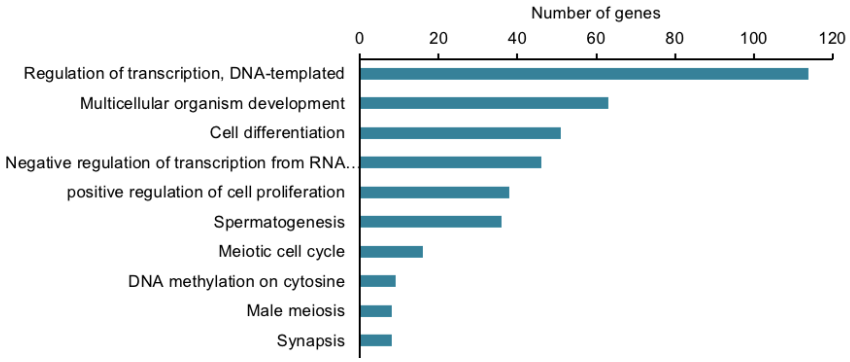
B.


SETDB1 KK (818)
SETDB1 CA (2646)
60
758
1888

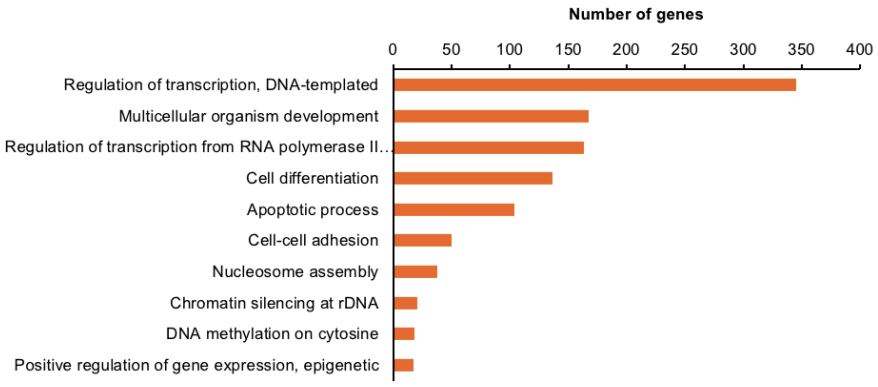
C.


MA–plot for Genotype: KK vs WT
MA–plot for Genotype: CA vs WT
log fold change
mean of normalized counts
1e−01
1e+01
1e+03
1e+05

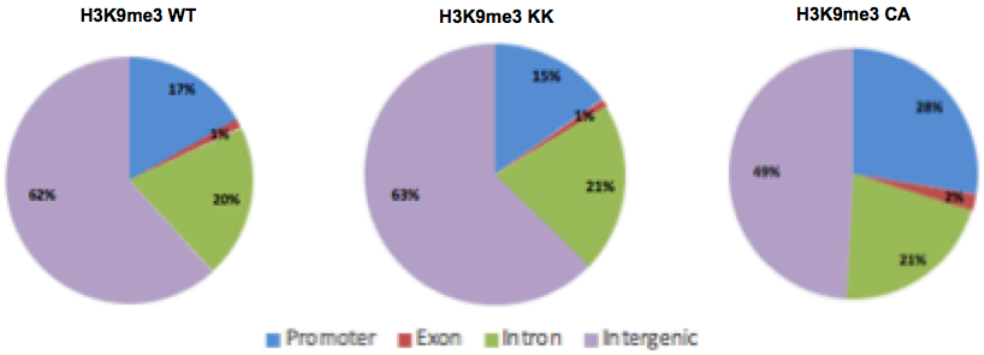
D.


Number of genes
0
20
40
60
80
100
120
Regulation of transcription, DNA-templated
Multicellular organism development
Cell differentiation
Negative regulation of transcription from RNA...
positive regulation of cell proliferation
Spermatogenesis
Meiotic cell cycle
DNA methylation on cytosine
Male meiosis
Synapsis

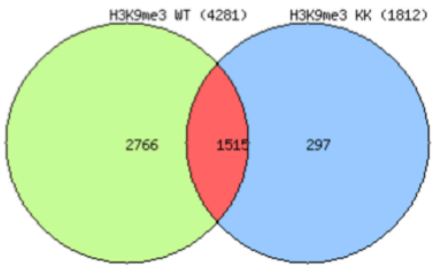
E.


Number of genes
0
50
100
150
200
250
300
350
400
Regulation of transcription, DNA-templated
Multicellular organism development
Regulation of transcription from RNA polymerase II...
Cell differentiation
Apoptotic process
Cell-cell adhesion
Nucleosome assembly
Chromatin silencing at rDNA
DNA methylation on cytosine
Positive regulation of gene expression, epigenetic

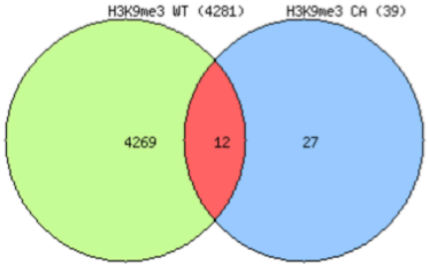
F.


H3K9me3 WT
H3K9me3 KK
H3K9me3 CA
17%
3%
20%
62%
15%
1%
21%
63%
28%
2%
21%
49%
Promoter
Exon
Intron
Intergenic

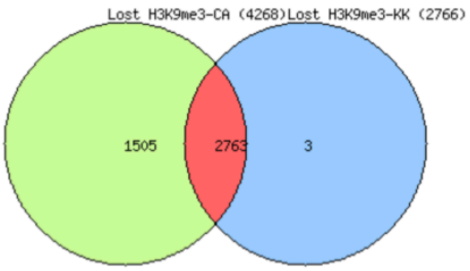
G.


H3K9me3 WT (4281)
H3K9me3 KK (1812)
2766
1515
297

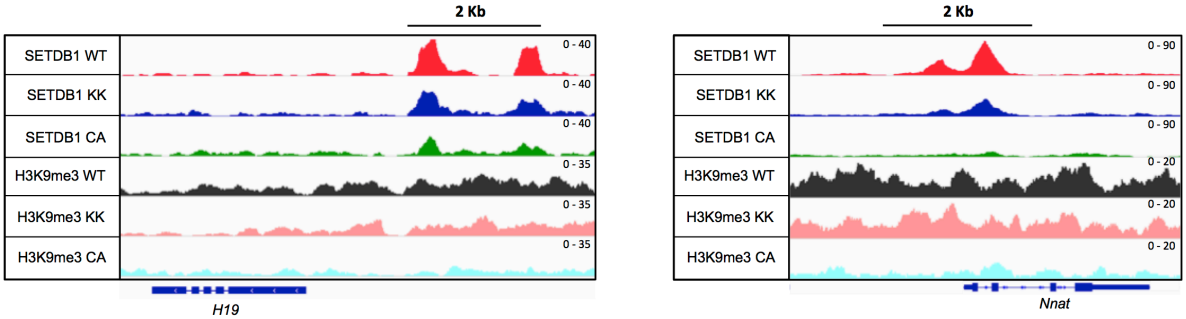
H.


H3K9me3 WT (4281)
H3K9me3 CA (39)
4269
12
27

I.


Lost H3K9me3-CA (4268)Lost H3K9me3-KK (2766)
1505
2763
3

J.


2 Kb
SETDB1 WT
SETDB1 KK
SETDB1 CA
H3K9me3 WT
H3K9me3 KK
H3K9me3 CA
0 - 40
0 - 40
0 - 40
0 - 35
0 - 35
0 - 35
H19
2 Kb
SETDB1 WT
SETDB1 KK
SETDB1 CA
H3K9me3 WT
H3K9me3 KK
H3K9me3 CA
0 - 90
0 - 90
0 - 90
0 - 20
0 - 20
0 - 20
Nnat

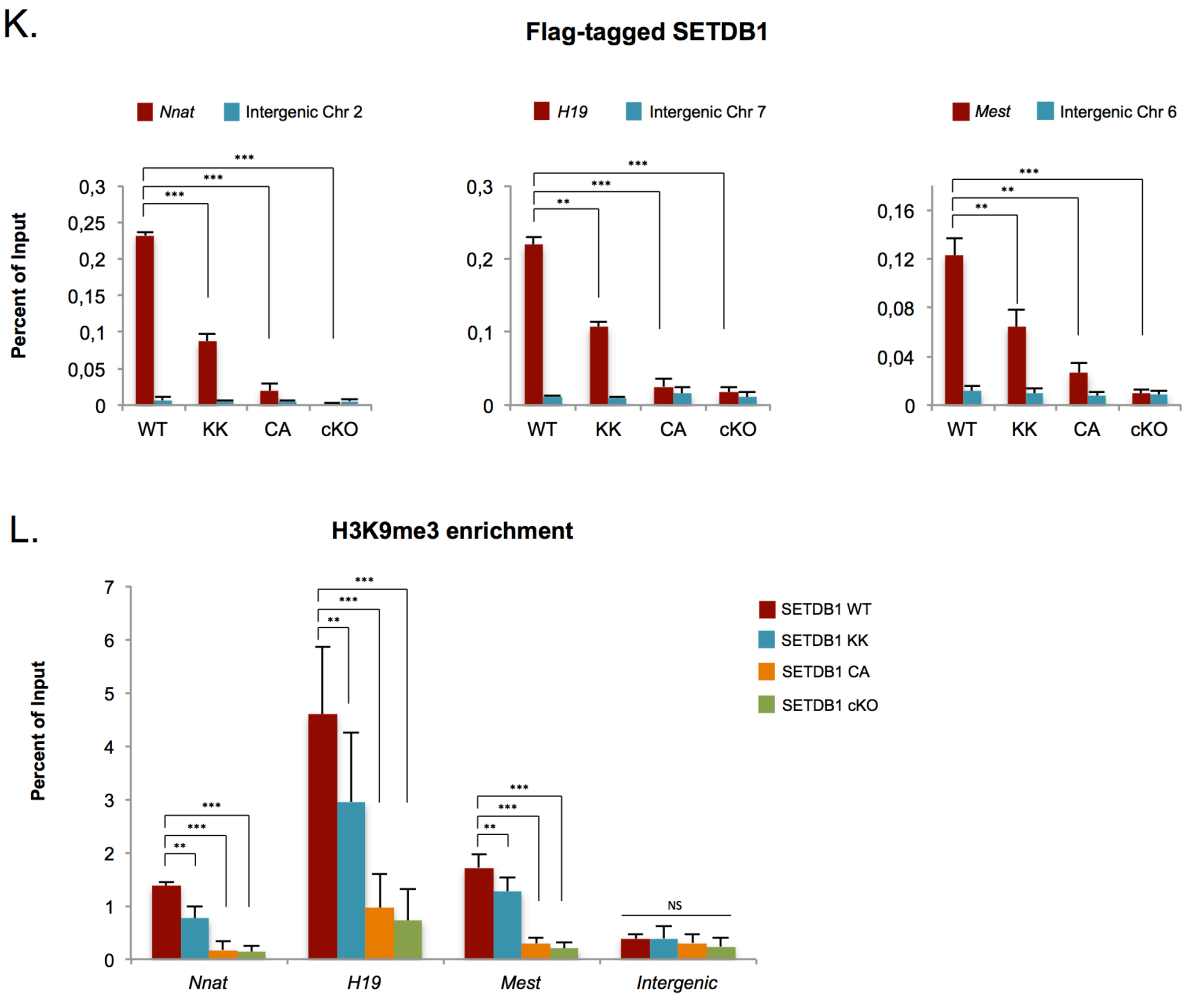


Figure 6. Integrity of SETDB1 K1770/K1778 and SETDB1 catalytic activity are required for silencing of different set of genes. RNA-seq and ChIP-seq were performed on *Setdb1* cKO TT2 mESCs re-expressing either wild-type SETDB1, catalytic-dead (C1243A), or the double-lysine (K1770A and K1778A) SETDB1 mutants. **A)** Principal component analysis using normalized RNA-seq data. Each cell type is represented in different color. Clustering of the biological replicates showed low variance within each group. **B)** Venn diagram showing overlap of genes de-regulated in mESCs expressing the SETDB1 double-lysine mutant and genes de-regulated in mESCs expressing the SETDB1 catalytic-dead mutant. **C)** RNA-seq 2D scatterplots of gene expression from mESCs expressing wild-type SETDB1 and double lysine SETDB1 mutant (left) or mESCs expressing wild-type SETDB1 and catalytic inactive SETDB1 mutant (right). Genes de-regulated are labelled in red. D) Gene ontology (GO) analysis of 816 deregulated genes in mESCs expressing the non-methylatable form of SETDB1. The top 10 biological processes with p-value < 0,001 are presented. **E)** GO analysis of 2645 deregulated genes in mESCs expressing the catalytic inactive form of SETDB1. The top 10 biological processes with p-value < 0,001 are presented. **F)** Genomic distribution of H3K9me3 enrichment in mESCs expressing wild-type SETDB1, catalytic-dead, or the double-lysine SETDB1 mutants. Enrichments were analyzed by ChIP-seq. We performed one SETDB1 ChIP-seq experiment. **G)** Venn diagram representation of genes marked by H3K9me3 in cells expressing wild-type SETDB1 and the double-lysine SETDB1 mutant. **H)** Venn diagram representation of genes marked by

H3K9me3 in cells expressing wild-type SETDB1 and catalytic-dead SETDB1 mutant. **I)** Venn diagram representation of genes in which the H3K9me3 mark is lost. **J)** Genome browser representation including tracks for Flag-tagged SETDB1 ChIP-seq and H3K9me3 ChIP-seq in mESCs expressing SETDB1 wild-type (WT), double-lysine mutant (KK) and catalytic-dead mutant (CA) at *H19* and *Nnat* genes. **K)** and **L)** ChIP-qPCR analyses at the promoter regions of the indicated genes on mESCs used in F). Intergenic regions where SETDB1 does not bind and there is not H3K9me3 enrichment were used as negative control. All values are expressed as the mean ± SEM (n = 3). For significance Student's paired *t*-test was applied. **$p < 0.05$ or ***$p < 0.01$.

Sample	Number of reads
Input_WT	69.534.084
Input_CA	63.239.907
Input_KK	60.598.630
H3K9me3_WT	54.624.539
H3K9me3_CA	56.290.479
H3K9me3_KK	55.585.006
Flag-SETDB1_WT	26.637.088
Flag-SETDB1_CA	48.402.614
Flag-SETDB1_KK	48.991.431

Table 3. Number of reads generated in each ChIP-seq Flag-SETDB1 and H3K9me3 in *Setdb1* cKO TT2 mESCs re-expressing either wild-type SETDB1 (WT), catalytic-dead (CA) or the double-lysine mutant (KK).

Region	ChIP-seq H3K9me3		
	WT (%)	KK (%)	CA (%)
Promoter (1−2kb)	3,12	2,62	3,83
Promoter (2−3kb)	3,2	2,89	2,22
Promoter (3−4kb)	3,26	3,01	3,33
Promoter (4−5kb)	3,32	3	1,73
Promoter (<=1kb)	3,91	3,54	16,17
1st Exon	0,03	0,01	
Other Exon	1,15	0,99	2,1
1st Intron	6,05	6,54	7,04
Other Intron	13,91	14,44	13,95
Downstream (<=3kb)	0,72	0,72	0,99
Distal Intergenic	61,34	62,25	48,64

Region	ChIP-seq Flag-SETDB1		
	WT (%)	KK (%)	CA (%)
Promoter (1−2kb)	2,78	2,94	3,07
Promoter (2−3kb)	2,76	3,02	2,64
Promoter (3−4kb)	2,77	2,91	2,64
Promoter (4−5kb)	2,64	2,51	2,82
Promoter (<=1kb)	5,45	6,93	7,59
1st Exon	0,01	0,02	
Other Exon	0,93	1,02	1,1
1st Intron	5,99	7,01	6,02
Other Intron	14,46	16,4	15,01
Downstream (<=3kb)	0,64	0,63	0,74
Distal Intergenic	61,58	56,61	58,37

Table 4. Genomic distribution of SETDB1 binding and H3K9me3 enrichment in mESCs. We performed one ChIP-seq experiment.

8. SETDB1 CAN BE (TRANS)-METHYLATED BY SUV39H1 *IN VITRO*

SETDB1 and SUV39H1 are part of a multimeric complex involved in transcriptional silencing (1), probably *via* the cooperative establishment of the different H3K9 methylation levels. However, up to now, the regulation of the KMT complexes is not fully understood. Given the ability of H3K9 KMTs to modify non-histone proteins, we tested whether SUV39H1 might methylate SETDB1. To this end, we performed *in vitro* methylation assay using the recombinant GST-SETDB1 fusion protein that encodes the Carboxy-terminal region (amino acids 1101 – 1290), and the three mutants harboring single amino acid substitutions in one or two methylation sites (K1770A and/or K1778A). We used a recombinant active GST-SUV39H1 as enzyme and radioactive SAM as methyl donor group. As a result, SUV39H1 was able to methylate SETDB1 and methylation level was lower in the double-mutant SETDB1 protein (**Figure 7A**), indicating that K1770 and K1778 are crucial for this process. Unexpectedly, the upper band detected in the reactions with the mutants revealed a full-length SUV39H1 auto-methylation.

Next, we evaluated whether the H3K9 methyltransferase activity of SETDB1 is affected by the presence of SUV39H1. To address this, we immunoprecipitated SETDB1 in wild-type or *Suv39h1/2* KO mESCs. Then, we performed *in vitro* methylation assay using the IP products as enzyme, a cocktail of histones as substrate and radioactive SAM as methyl donor group. Our results showed that methylation of SETDB1 toward histone H3 decreases in the absence of SUV39H1 (**Figure 7B and Figure 7C**). Altogether, our results suggest that methylation of SETDB1 by SUV39H1 could play a role in the SETDB1/SUV39H1 interaction and SETDB1 methyltransferase activity.

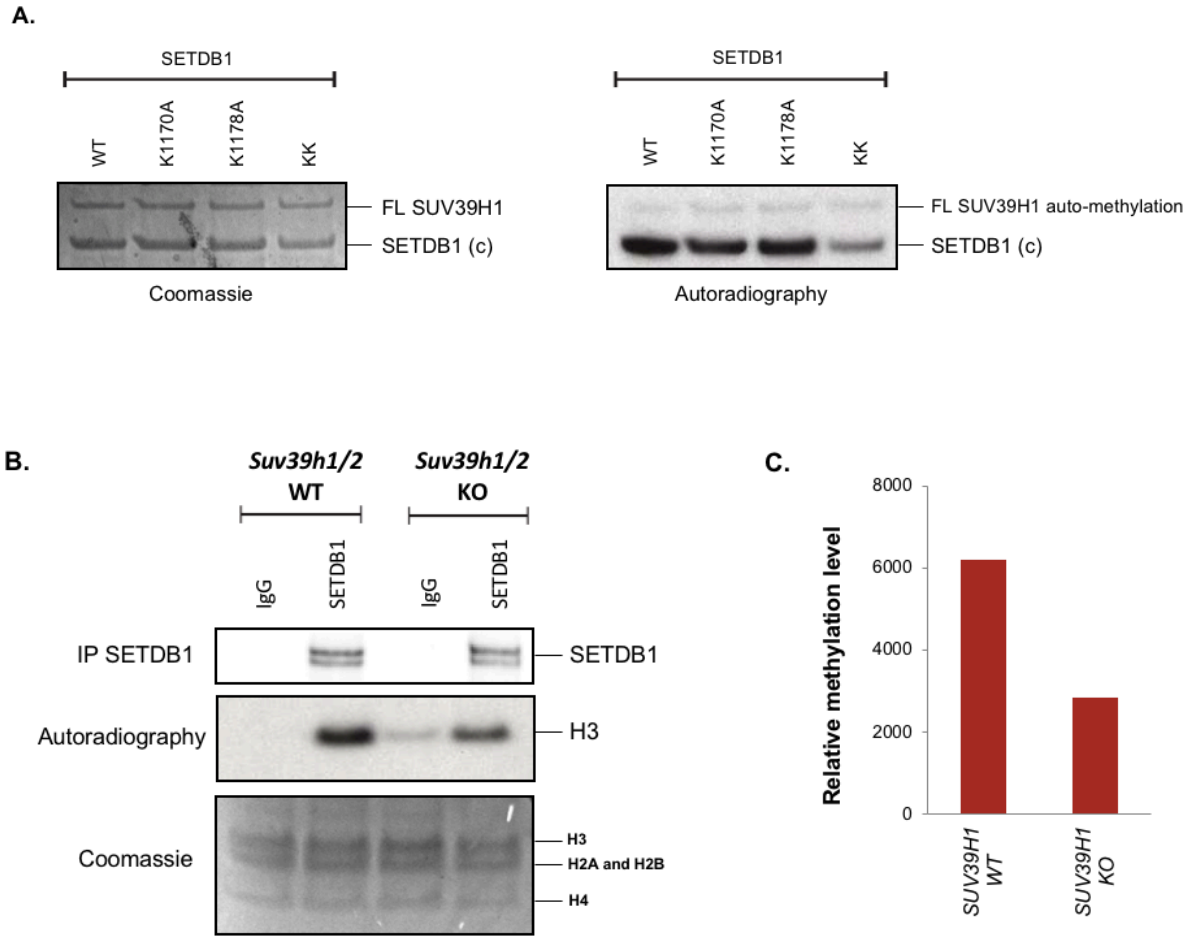


Figure 7. SETDB1 is methylated by SUV39H1. A) Recombinant GST-SETDB1 which encodes part of the catalytic domain (WT) and the relative position of single amino acid substitutions introduced (see Fig 1D) were incubated with active GST-SUV39H1 in presence of radioactive SAM and separated on an SDS polyacrylamide gel. A Coomassie-stained control gel of the proteins is also shown. **B)** SETDB1 precipitates from *Suv39h1/2* wild-type or *Suv39h1/2* double null mESCs were used for *in vitro* methylation assay in presence of a recombinant cocktail of histones and radioactive SAM. **C)** Quantification of the relative methylation level from the autoradiography was performed by subtracting the IgG signal. All values are expressed as the mean (n = 2).

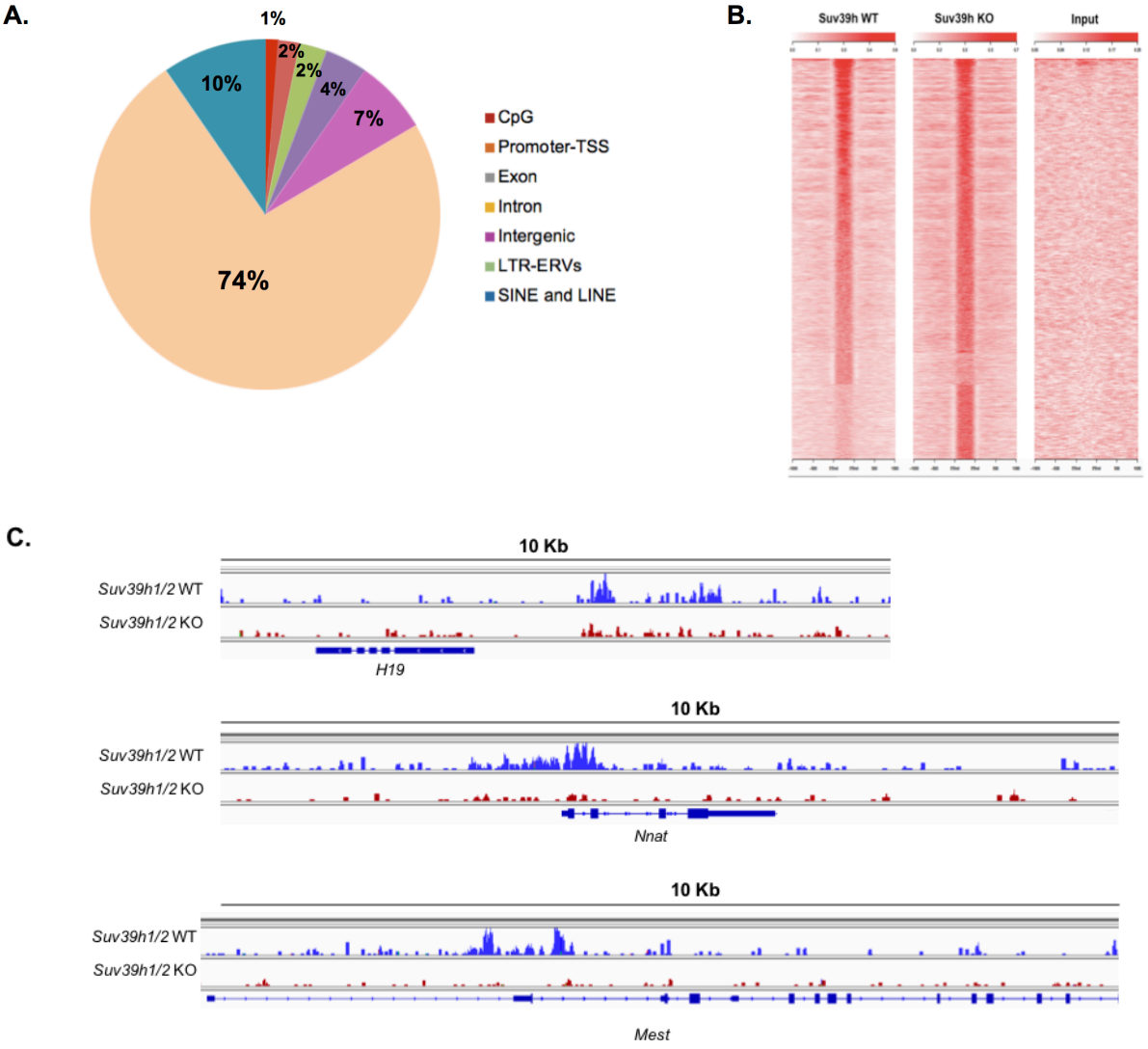
9. SETDB1 PAN-GENOMIC BINDING PROFILE IS DEREGULATED IN THE ABSENCE OF SUV39H1/2 IN mESCS

SETDB1 and SUV39H1 coordinate the establishment and maintenance of H3K9me3 at constitutive pericentromeric heterochromatin (9,10) and cooperate to regulate many genomic targets, including transposable elements, such as LINEs and ERVs (11). Since the methyltransferase activity of SETDB1 was found to be affected by the absence of SUV39H1, we hypothesized that the interaction between these H3K9 KMTs is crucial for recruitment of SETDB1 to chromatin and consequently in the expression of SETDB1 target genes. To address this, we performed genome-wide analyses for enrichment of SETDB1 by chromatin immunoprecipitation and subsequent DNA sequencing (ChIP-seq) in wild-type or *Suv39h1/2* KO mESCs. The results I presented here are preliminary.

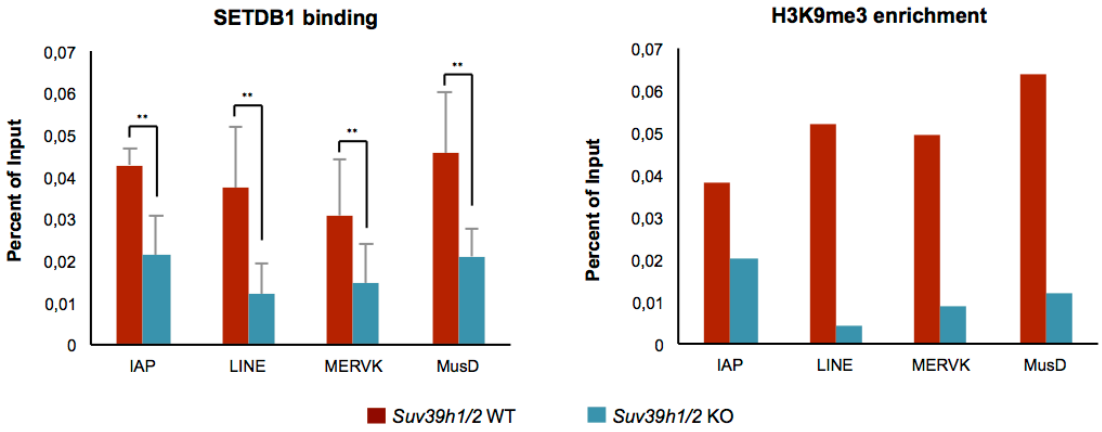
Our ChIP-seq preliminary results showed that a high proportion of SETDB1 binding occurred outside of genes, including approximately 80% transposable elements (ERVs, LINEs and SINEs elements) (**Figure 8A**). These observations are in agreement with previous results in mESCs (11). We detected regions where there is a loss of SETDB1 binding in absence of SUV39H1/2 that correspond to repetitive elements, mostly LTR-ERVs proximal to some imprinted genes such as *H19-Igf2, Mest, Nnat, Mest, Nespas Airn, Mcts2, Zrsr1, Nap1I5, Plagl1, Snurf and Grb10* (**Figure 8B and Figure 8C**). We have next validated that SETDB1 occupancy and H3K9me3 enrichment on these imprinted genes is impaired in absence of SUV39H1/2 (**Figure 8D**). Furthermore, we have evaluated mRNA expression by quantitative RT-PCR and we found that the absence in *Suv39h*-double null cells resulted in de-repression of *Igf2* (~5,7 fold), *Nnat* (~13 fold) and strikingly a strong induction of *H19* (~51 fold) was observed (**Figure 8F**). Together, these results suggest that in absence of SUV39H1/2, SETDB1 is not properly recruited to chromatin, resulting in impaired patterns of gene

expression. Further analyses are necessary to study DNA methylation levels and the imprinted expression pattern.

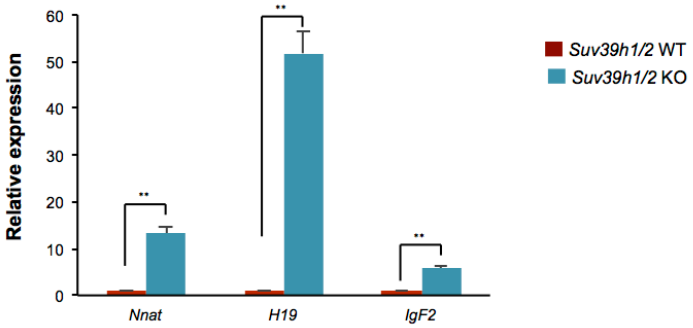
We next validated whether the SETDB1-dependent transcriptional silencing of ERV and LINE sequences is affected in absence of SUV39H1/2. We conducted ChIP-qPCR analysis of SETDB1 and H3K9me3 in wild-type or *Suv39h1/2* KO mESCs. A reduction in SETDB1 enrichment was apparent at ERVs (MusD, IAP, MMERVK10c) and LINE-L1 elements in *Suv39h1/2* KO mESCs (**Figure 8E**). Moreover, our results also suggest that there is a reduction of H3K9me3 (**Figure 8E**). Strikingly, the transcriptomic analysis revealed a decrease on expression of some ERVs and LINE-L1 elements in *Suv39h1/2* KO mESCs (**Figure 8G**) Altogether, these data suggest that although the SETDB1-deposited H3K9me3 mechanism for silencing ERVs and LINE elements is affected by the absence of SUV39H1, a compensatory mechanism seems to be responsible to prevent the induction of proviral expression, as has been proposed for the spreading of H3K27me3 at major satellite repeats in *Suv39h*-double null cells (202) or as has been reported co-occupancy of H3K9me3 and H3K27me3 in controlling distinct transposon families upon DNA demethylation in mESCs (203).


A.
1%
2%
2%
4%
7%
10%
74%
CpG
Promoter-TSS
Exon
Intron
Intergenic
LTR-ERVs
SINE and LINE
B.
Suv39h WT
Suv39h KO
Input
C.
10 Kb
Suv39h1/2 WT
Suv39h1/2 KO
H19
10 Kb
Suv39h1/2 WT
Suv39h1/2 KO
Nnat
10 Kb
Suv39h1/2 WT
Suv39h1/2 KO
Mest
D.
SETDB1 binding
Percent of Input
0,4
0,35
0,3
0,25
0,2
0,15
0,1
0,05
0
**
**
NS
Nnat
H19
Intergenic
H3K9me3 enrichment
Percent of Input
12
10
8
6
4
2
0
**
**
NS
Nnat
H19
Intergenic
Suv39h1/2 WT
Suv39h1/2 KO

E.


SETDB1 binding
H3K9me3 enrichment
Percent of Input
0,07
0,06
0,05
0,04
0,03
0,02
0,01
0
IAP
LINE
MERVK
MusD
**
Suv39h1/2 WT
Suv39h1/2 KO

F.


Relative expression
60
50
40
30
20
10
0
Nnat
H19
Igf2
**
Suv39h1/2 WT
Suv39h1/2 KO

G.

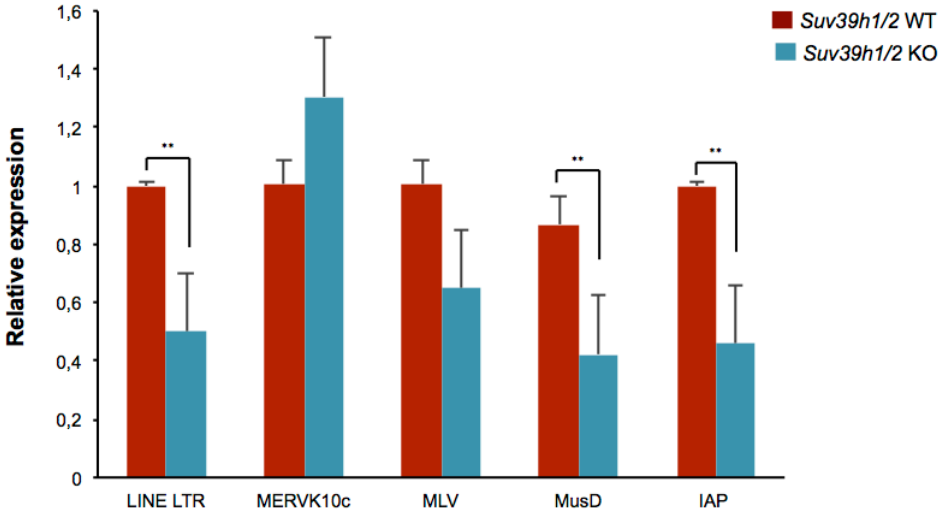

Relative expression
1,6
1,4
1,2
1
0,8
0,6
0,4
0,2
0
LINE LTR
MERVK10c
MLV
MusD
IAP
**
Suv39h1/2 WT
Suv39h1/2 KO

Figure 8. SETDB1 pan-genomic binding profile is deregulated in the absence of SUV39H in mESCs. A) Genomic distribution of SETDB1 binding in *Suv39h1/2* KO mESCs. Enrichments were analyzed by ChIP-seq. We performed one SETDB1 ChIP-seq experiment. **B)** Heatmap of ChIP-Seq data showing the binding profile of SETDB1 in wild-type mESCs or *Suv39h1/2* KO. Graphic presents average SETDB1 binding density of genomic regions surrounding (+/- 1 Kb) SETDB1 binding sites. **C)** Genome browser representation of SETDB1 binding profiles analyzed by ChIP-Seq, at the *H19, Nnat* and *Mest* genes in mESCs. **D) and E)** ChIP-qPCR analyses of SETDB1 and H3K9me3 enrichment wild-type mESCs or *Suv39h1/2* KO. Setdb1 occupancy and H3K9me3 in imprinted genes, LINE-L1 and ERVs (MMERVK10c, IAP and MusD) is impaired in the absence of SUV39H1/2. Intergenic region where SETDB1 does not bind and there is not enrichment of H3K9me3 enrichment were used as negative control. **F) and G)** Imprinted genes expression and proviral silencing are deregulated in absence of SUV39H1/2. mRNA levels were normalized to those of cyclophilin A. All values are expressed as the mean ± SEM (n = 3). For significance Student's paired *t*-test was applied. *p-values <0.05. Of note, H3K9me3 enrichment analysis in E) has been performed only once.

MATERIALS AND METHODS

1. CELL LINES AND CELL CULTURE

Setdb1 cKO mESCs were established by the group of Prof. Yoishi Shinkai via standard gene targeting procedures (87). To generate the *Setdb1* cKO mESC line, Cre recombinase and oestrogen receptor (Cre-ER) fusion gene was introduced into a clone containing targeted *Setdb1* cKO and KO alleles. We also used *Setdb1* cKO (re)-expressing 3XFLAG-SETDB1 Wild type and 3XFLAG-SETDB1 catalytic-dead (C1243A) mutant kindly donated by Prof. Yoishi Shinkai (87). *Suv39h1/h2* null HM1 mESCs were established by the group of Prof. Jeremy Brown (204). All mESCs were cultured in Dulbecco's modified Eagle's Medium DMEM (Sigma) supplemented with 15% fetal calf serum (Gibco), 1% penicillin/streptomycin (Sigma), 0.1 mM β-mercaptoethanol (Thermo), 1 mM nonessential amino acids (Sigma), and 1000 U/mL of Leukemia Inhibitory Factor (LIF) (Millipore). All mESC lines were cultured in standard feeder-free conditions with 0.2% gelatin, maintained at 37ºC and 8% CO_2. To induce deletion of the SETDB1 cKO allele, mESCs were cultured in 800nM 4-Hydroxy Tamoxifen (Sigma) for 4 days.

Setdb1 KO MEFs were cultured in DMEM (Sigma) supplemented with 15% fetal calf serum (Gibco) and 1% penicillin/streptomycin (Sigma). MEFs were maintained at 37ºC and 5% CO_2.

2. CLONING

Full-length SETDB1 cDNA was cloned into the *NotI* and *EcoRI* sites of the PCS2 vector given by Liu Yang, which contains a Myc epitope tag. In order to generate the catalytic-dead (C1243A) SETDB1 mutant, or the double-lysine SETDB1 mutant (K1770A and K1778A), we used the QuikChange II XL site-directed mutagenesis kit (Agilent) according to manufacturer's recommendations. Briefly, each reaction was prepared with 10X reaction

buffer, 10 ng dsDNA template, 125 ng of each oligonucleotide, 1 µL of dNTPs mix, 3 µL of QuikSolution and 2.5 U/µL PfuUltra HF DNA polymerase. PCR was used to incorporate each mutation inside the dsDNA. Finally, *DpnI* endonuclease which is an enzyme specific for methylated and hemimethylated DNA was used to digest the parental DNA template and to select for mutation-containing synthetized DNA. Successful mutagenesis was verified by Sanger sequencing.

SETDB1 fragment containing the mutations K1770A and K1778A was cloned into the *AspI* and *EcoRI* sites of the pCAG-3XFLAG-IRESbsd vector used by Prof. Yoishi Shinkai Lab. To generate ES cells stably expressing the non-methylatable form of SETDB1, the vector was introduced into *Setdb1* cKO mESCs via Lipofectamine 2000 (Invitrogen). Clones were selected in medium containing blasticidin (7 mg/mL).

GST-tagged SETDB1 truncated mutants were kindly donated by Prof. Or Gozani (6). The lysine mutations K1170A and/or K1178A were generated by site directed mutagenesis as previously described.

3. TRANSFORMATION

Competent cells (NEB Turbo Competent, New England BioLabs) were mixed with 50 ng of plasmid and incubated 30 min in ice. Then, cells were exposed to heat shock at 42°C for 45 sec and incubated 2 min in ice. 250 µL of SOC medium were added and transformed cells were incubated for 30 min at 37°C, followed by spreading onto LB agar with ampicillin. Plates were incubated overnight at 37°C.

4. PLASMID DNA PURIFICATION

A single colony picked from the agar plate was inoculated into LB medium and grow at 37°C for 12 h. Then, plasmid purification was carried out using Qiagen kits according to manufacturer's recommendations. Finally, DNAs were sent for sequencing (GATC Biotech).

5. TRANSFECTION

The SETDB1 expression constructs were transiently transfected into *Setdb1* KO MEFs using lipofectamine 2000 (Life Technologies) according to the product instructions. Cells were harvested at 48 h post-transfection for IP and western blot analysis.

6. EXPRESSION AND PURIFICATION OF GST-TAGGED PROTEINS FROM *E. coli*

GST fusion proteins were expressed in *E. coli* Bl21. Bacteria was induced until OD=0.7 by adding 1 mM IPTG at 37°C for 2h. After centrifugation, cells were resuspended in Lysis buffer (20 mM Tris pH 7.6, 150 mM NaCl, 0.5% Triton X-100, 0.01% NP40, 15 mM KCl, 10% Glycerol, 1 mM DTT, 0.5 mg/mL Lysosyme) and incubated for 30 min in ice. After, the solution was disrupted using a sonicator. Cell debris were removed by centrifugation at 12000 rpm for 30 min at 4°C. GST-tagged proteins were bound to Glutathione-S-Sepharose beads for 2 h at 4°C. After washing with wash buffer (50 mM Tris pH 7.6, 300 mM NaCl, 0.5% Triton-X-100 and 1 mM DTT), bound proteins were eluted with elution buffer (100 mM Tris (pH 8.0), 15 mM Glutathion, 1 mM DTT), collected by centrifugation and stored at -80°C.

7. IN VITRO HISTONE METHYLTRANSFERASE ASSAY

GST-purified proteins (1 µg) or products from immunoprecipitation were incubated in a solution of buffer 2X (100 mM Tris pH 8, 40 mM KCl, 20 mM MgCl2, 20 mM B-

Mercaptoethanol, 500 mM Sucrose), 30 mM S-adenosyl-[3Hmethyl]-L-methionine (SAM) and either 2 µg recombinant active SETDB1 (Active Motif) or 2 µg active GST-SUV39H1 or 5 µg Histone cocktail (H2A, H2B, H3 and H4). The reactions were incubated for 2 h at 37°C. Then, the samples separated by SDS-PAGE and the proteins were fixed with 50% methanol and 10% acetic acid and visualized by Coomassie blue stain. Finally, the gel was incubated with En3hance, dried and exposed to special film.

8. IMMUNOFLUORESCENCE (IF) AND MICROSCOPIC ANALYSIS

Cells were grown on glass coverslips, which had been coated with laminin 10 mg/ml in PBS for 1 h at 37 °C and rinsed with PBS. mESCs cells were fixed with 4% formaldehyde (Electron Microscopy Sciences) 20 min, incubated 50 mM NH4Cl for 10 min to quench formaldehyde, permeabilized with 0.2% Triton-X-100 10 min. Primary and secondary Abs were diluted in PBS containing 2% SVF serum and 0.1% Tween and incubated overnight at 4° C or 1 h at room temperature, respectively. DNA was stained with 1 µg ml^{-1} DAPI (Life Technologie). Coverslips were mounted with Vectashield mounting media (Clinisciences). Microscopy was performed using inverted microscope Leica DMI-6000. Images were taken with the HQ2 Coolsnap motorized by Metamorph software. All images were processed with ImageJ software.

9. FLOW CITOMETRY

Cells were trypsinized and 1*10^5 cells were incubated for 5 minutes at RT under lightless condition in 1X AnnexinV binding buffer containing FITC Annexin V and 0.25 mg/ul propidium iodide according to FITC AnnexinV Apoptosis Detection Kit (BD Biosciences). 10.000 cells were sampled for each replicate on BD FACSCalibur™ flow cytometer and cells

were gated on forward and side scatter. Fluorescence measurement was on the FL1 (green) and FL2 (red). Data were analyzed using FlowJo software.

10. WESTERN BLOT

Nuclear extracts or IP samples were prepared in NuPAGE 4X loading buffer and 10X reducing agent. Separation of proteins was performed using pre-cast polyacrylamide gel cassettes (NuPAGE® Novex® 4-12% Bis-Tris) (Life technologies) and 1X NuPAGE MES SDS Running Buffer and transferred into nitrocellulose membrane in 20 mM phosphate transfer buffer (pH 6.7). Membrane was blocked in 5% skim milk in PBST Buffer (1X PBS, 0.2% Tween 20) and incubated overnight at 4°C with the primary antibodies. Primary antibodies were detected with IRDYE-conjugated secondary antibodies and scanned on the LI-COR imaging system. Quantification of bands was performed with LI-COR software or Image J.

11. ANTIBODIES

Antibodies used for immunoprecipitation were: anti-SETDB1 (Santa Cruz), anti-Myc (Santa Cruz), anti-pan-tri-methyl-lysine (CST), anti-CDYL (Abcam), anti-SUV39H1 (CST), anti-HPg (Thermo), anti-Histone 3 (Santa-Cruz), anti-Flag (Sigma). Antibodies used for ChIP were: anti-FLAG (Sigma), H3K9me3 (Abcam and Diagenode), IgG (Diagenode)

12. COOMASSIE STAINING

Proteins were visualized in SDS-PAGE by incubating in staining SimplyBlue SafeStain (Invitrogen) for 1 h at room temperature with constant agitation.

13. RNA AND QUANTITATIVE REVERSE TRANSCRIPTION-PCR (qRT-PCR)

Total RNA was extracted using RNeasy mini-kit (Qiagen) following manufacturer's procedures. DNase (Qiagen) treatment was performed to remove residual DNA. With High Capacity cDNA Reverse Transcription Kit (Applied Biosystems), 1 µg of total RNA was reverse transcribed. Real-time quantitative PCR was performed to analyze relative gene expression levels using SYBR Green Master mix (Applied Biosystems) following manufacturer indications. Relative expression values were normalized to the housekeeping gene mRNA Cyclophillin A. Primer sequences are listed in **Table 3.**

14. IMMUNOPRECIPITATION

20 million cells were lysed in buffer A (20 mM HEPES pH 7, 0.15 mM EDTA, 0.15 mM EGTA, 10 mM KCl), 10% NP40 and SR buffer (50 mM HEPES pH 7, 0.25 mM EDTA, 10 mM KCl, 70% (m/v) sucrose) supplemented with 1 mM DTT, spermidine-spermine (0.15 mM, 0.5 mM, respectively) and protease inhibitor (Sigma) to limit nuclei leak. Cell lysates were centrifuged at 2000 g for 5 min. The nuclei pellets were suspended in sucrose buffer (20 mM Tris pH 7.65; 60 mM NaCl; 15 mM KCl; 0.34 M Sucrose) and then high salt buffer was added (20 mM Tris-HCl pH 7.65; 0.2 mM EDTA; 25% glycerol; 900 mM NaCl; 1.5 mM MgCl2) to a final NaCl concentration of 300 mM. The nuclear extracts were treated with Micrococcal nuclease (0.0125 U/ml) with 1 mM CaCl2 at 37 °C during 10 min, EDTA was added to 4 mM final concentration and finally sonication for 5 min (15 sec ON, 1 min OFF) at medium frequency (Bioruptor Diagenode). The lysates were ultracentrifuged at 40000 rpm for 30 min and pre-cleared with protein G-agarose beads (Sigma) during 2 h at 4°C. Immunoprecipitations were carried out overnight at 4 °C using 5 µg of each antibody. Ultralink A/G beads (Perbio) were blocked overnight at 4 °C with 0,3 % BSA and 0,5 µg/µl ssDNA and then incubated with the immunocomplexes for 2 h at room temperature. For immunoprecipitation using Myc-Trap technology, the Myc-Trap beads were equilibrated with

wash buffer (10 mM Tris pH 7.5, 150 mM NaCl, 0.15 mM EDTA). Immunoprecipitations were carried out 1 h at 4°C using 25 µL of Myc-Trap bead slurry. The immunocomplexes were washed four times in wash buffer and the proteins were eluted in NuPAGE® LDS Sample Buffer (Life Technologies) at 96 °C during 5 min. Finally, the immunoprecipitates were examined by western blot.

15. CHROMATIN IMMUNOPRECIPITATION (ChIP)

Formaldehyde (Sigma) was added to culture medium to a final concentration of 1%. Cross-linking was allowed to proceed for 10 min at RT and stopped by addition of glycine at a final concentration of 0.125 M. Fixed cells were washed and harvested with PBS. Chromatin was prepared by two subsequent extraction steps (10 min at 4 °C) with Buffer 1 [50 mM Hepes/KOH (pH 7.5); 140 mM NaCl; 1 mM EDTA; 10% Glycerol; 0.5% NP-40; 0.25% Triton X-100] and Buffer 2 [200 mM NaCl; 1 mM EDTA; 0.5 mM EGTA; 10 mM Tris (pH 8.0)]. Nuclei were then pelleted by centrifugation, resuspended in Buffer 3 [50 mM Tris (pH 8.0); 0.1% SDS; 1% NP-40; 0.1% Na-Deoxycholate; 10 mM EDTA; 150 mM NaCl], and sonicated with Bioruptor (Diagenode), yielding genomic DNA fragments with a bulk size of 150–300 bp. Reverse-crosslink chromatin was analyzed using D5000 screen tape on the Tape Station (Agilent). All buffers were supplemented with protease inhibitors prior to usage. Chromatin was pre-cleared with protein G-agarose beads (Sigma) during 2 h at 4°C and immunoprecipitation with the specific Abs was carried out overnight at 4°C. Ultralink A/G beads (Perbio) were blocked overnight at 4 °C with 0,3 % BSA. Immune complexes were recovered by adding pre-blocked protein A/G ultralink beads and incubated for 2 h at room temperature. Beads were washed twice with Low salt buffer [0.1% SDS; 1% Triton; 2 mM EDTA; 20 mM Tris (pH 8.0); 150 mM NaCl], twice with High salt buffer [0.1% SDS; 1% Triton; 2 mM EDTA; 20 mM Tris (pH 8.0); 500 mM NaCl], once with LiCl wash buffer [10

mM Tris (pH 8.0); 1% Na-deoxycholate; 1% NP-40, 250 mM LiCl; 1 mM EDTA], and once with TE supplemented with 50 mM NaCl. Beads were eluted in TE with addition of 1% SDS and 0.2 M NaCl at 65°C, and cross-link was reversed overnight at 65 °C. Eluted material was incubated with Proteinase K 2 h at 37°C and DNA was obtained using the kit Qiagen Mini Elute. DNA was resuspended in water, and q-PCR performed using PowerSYBR Green PCR Master mix (Applied Biosystems) and analyzed on a Via 7 System (Applied Biosystems). ChIP-qPCR results were represented as percentage (%) of IP/input signal (% input). Primer sequences are listed in **Table 3.**

16. ChIP-SEQUENCING

The IPed DNA was quantified using the Qubit™ dsDNA High Sensitivity HS assay (ThermoFisher Scientific) and fragment size distribution (between 100-600 bp) was analyzed by capillary electrophoresis (Agilent 2200 TapeStation Nucleic Acid System with the High sensitivity D5000 kit). Libraries were prepared using the MicroPlex V2 library preparation ™ kit (Diagenode) following the manufacturer's instructions: starting amount of fragmented DNA varying between sub-nanograms and 500 pg of DNA (from ChIP SETDB1), 1,5 ng of DNA (from ChIP H3K9me3) and 1,5 ng for inputs were used. After end repair of the double-stranded DNA templates, we ligated the cleavable stem-loop adaptors and amplify the adaptor enriched DNA with high fidelity amplification of 10 PCR cycles to add Illumina compatible indexes. The libraries were then purified with the Agencourt AMPure XP bead-based purification system. Final libraries were quantified with Qubit™ dsDNA HS assay (Thermofisher Scientific) and size distribution was monitored by capillary electrophoresis with Agilent 2100 Bioanalyzer using High Sensitivity HS DNA Chips (Agilent).

After pooling, libraries were paired-end sequenced (2X40 bp) on a Nextseq 500 instrument (Illumina).

Gene	Sequence	Tecnique
Cyclo A -RT Fw	GTCAACCCCACCGTGTTCTT	RT-qPCR
Cyclo A -RT Rev	CTGCTGTCTTTGGGACCTTGT	RT-qPCR
MLV-LTR-RT Fw	AGAGGTATGGTTGGAATAAGTA	RT-qPCR
MLV-LTR-RT Rev	TAGATGGAGCCTACCAAGCTCTCAA	RT-qPCR
MusD-LTR-RT Fw	TCTTAATAATAGGGAGGAGATGTAGT	RT-qPCR
MusD-LTR-RT Rev	GGTCCAGTAGAAAGGTCGACG	RT-qPCR
MMERVK10c-LTR	CAAATAGCCCTACCATATGTCAG	RT-qPCR and ChIP
MMERVK10c-LTR	GTATACTTTCTTCTTCAGGTCCAC	RT-qPCR and ChIP
IAP-POL RT Fw	CTTGCCCTTAAAGGTCTAAAAGCA	RT-qPCR
IAP-POL RT Rev	GCGGTATAAGGTACAATTAAAAGATATGG	RT-qPCR
LINE1-RT p2 Fw	TTTGGGACACAATGAAAGCA	RT-qPCR and ChIP
LINE1-RT p2 Rev	CTGCCGTCTACTCCTCTTGG	RT-qPCR and ChIP
H19 RT-Sybr p1 Fw	AGAGGACAGAAGGGCAGTCA	RT-qPCR
H19 RT-Sybr p1 Rev	TGGGTGGACAATTAGGTGGT	RT-qPCR
Nnat-RT-Ex 3 Fw	CCTGTCCCACTACCGGC	RT-qPCR
Nnat-RT-Ex 3 Rev	TATTGACCACAACTGCTGCG	RT-qPCR
Mest-RT-Ex 9-11 Fw	GGACCGTATACTCGACCCAC	RT-qPCR
Mest-RT-Ex 9-11 Rev	GGATGGACACAGAAGCAAGC	RT-qPCR
MLV-LTR ChIP Fw	TGGGCAGGGGTCTCCAAATCT	ChIP
MLV-LTR ChIP Rev	ATAAAGCCTCTTGCTGTTTGCATC	ChIP
IAP-LTR ChIP Fw	GCTCCTGAAGATGTAAGCAATAAAG	ChIP
IAP-LTR ChIP Rev	CTTCCTTGCGCCAGTCCCGAG	ChIP
MusD-LTR ChIP Fw	CCCTTCCTTCATAACTGGTGTCGCA	ChIP
MusD-LTR ChIP Rev	TAGCATCTCTCTGCCATTCTTCAGG	ChIP
Mest-ChIP-R1 Fw	CTTCGCTTGGCCTTCACTTTC	ChIP
Mest-ChIP-R1 Rev	GCCGCACCTTTGACTTTGGT	ChIP
Mest-ChIP-NEG-P2 Fw	GGCTGGTTCAGATGGTCCTT	ChIP
Mest-ChIP-NEG-P2 Rev	GGGGACTGCTGGTCAATAGG	ChIP
Nnat_ChIP_73_Fw	CCCTACCCAACCCATCCTATC	ChIP
Nnat_ChIP_74_Rev	CCACCGCGGCACTTTG	ChIP
Nnat-ChIP-NEG Fw	CAGCTTAGGAATCAAGGGGCA	ChIP
Nnat-ChIP-NEG Rev	GCTGACAGCAGTGTAGCGTA	ChIP
H19-ChIP-R2 Fw	TGCAGAGAGTAAGCCGACCT	ChIP
H19-ChIP-R2 Rev	GGGTCACAAATGCCACTAGG	ChIP
H19-ChIP-R2-P2 Fw	CCCCTGAGGTACTGAACTTGG	ChIP
H19-ChIP-R2-P2 Rev	TCGATTGCGCCAAACCTAAAG	ChIP

Table 3. List of primers used for RT-qPCR and ChIP-qPCR

17. STATISTICAL PARAMETERS FOR ChIP-SEQ ANALYSIS

Sequenced reads were demultiplexed to attribute each read to a DNA sample and then aligned to reference mouse genome mm10 with bowtie2. After PCR duplicates were removed, Flag-tagged SETDB1 and H3K9me3 enrichments were analyzed over their respective control (Input DNA) by the software MACS2. The P-value threshold was 0,001, and the shift size 50 pb.. For Flag-tagged SETDB1 and H3K9me3 ChIP-seq peaks only binding sites with P-values <10e-5, FDR<1% and five fold enrichment over the control were retained. For genome annotation we used Homer software v4.7. For bound genes, we used GREAT software (v3.0). Plots were generated using deep tools. DAVID software was used for Gene Ontology analyses.

18. RNA PURIFICATION AND RNA-SEQ

RNA was isolated as described above. After RNA extraction, RNA concentrations were obtained using a fluorometric Qubit RNA HS™ assay (ThermoFisher Scientific). The quality of the RNA (RNA integrity number 8.2) was determined on the Agilent 2100 Bioanalyzer (Agilent Technologies, Palo Alto, CA, USA) as per the manufacturer's instructions.

To construct libraries, 1 ug of high quality total RNA sample (RIN >8) was processed using Truseq® stranded total RNA kit (Illumina®) according to manufacturer instructions. Briefly, after removal of human ribosomal RNA (using Ribo-zero® rRNA) confirmed by QC control on pico chip™ on the Agilent 2100 Bioanalyzer (Agilent Technologies, Palo Alto, CA, USA), total RNA molecules are fragmented and reverse- transcribed using random primers. Replacement of dTTP by dUTP during the second strand synthesis will permit to achieve the strand specificity. Addition of a single A base to the cDNA is followed by ligation of adapters.

Libraries were quantified by qPCR using the KAPA Library Quantification Kit for Illumina Libraries (KapaBiosystems) and library profiles were assessed using the DNA High Sensitivity™HS kit on an Agilent Bioanalyzer 2100. Libraries were sequenced on an Illumina® Nextseq 500 instrument using 75 base-lengths read V2 chemistry in a paired-end mode.

19. BIOINFORMATIC ANALYSIS OF RNA-SEQ

After sequencing, a primary analysis based on AOZAN software (ENS, Paris) was applied to demultiplexe and controls the quality of the raw data (based of FastQC modules / version 0.11.5). Obtained fastq files were then aligned using Star algorithm (version 2.5.2b) and quality control of the alignment realized with Picard tools (version 2.8.1). Reads were then count using Feature count (version Rsubread 1.24.1) and the statistical analyses on the read counts were performed with the DESeq2 package version 1.14.1 to determine the proportion of differentially expressed genes between two samples.

20. STATISTICAL ANALYSIS

All values will be presented as mean +/- standard deviation. For statistical significance Student's paired t-test was applied. P-values less than 0.05 will be considered significant.

DISCUSSION AND PERSPECTIVES

1. DISCUSSION

Post-translational modifications (PTMs) are covalent chemical changes proteins may undergo after translation. PTMs play important roles in regulating almost every aspect of cell biology including gene expression, signal transduction, interaction with partners, cell-cell interaction and communication between the intracellular and extracellular environment (205). Protein methylation is one of the functionally stable yet versatile form of PTM and is the focus of the present study.

Protein lysine methylation was first reported by Richard Ambler and Maurice Rees in 1959, while analyzing bacterial flagellar proteins they observed an amino acid that has not been previously found to occur naturally, which lately they identified as a methylated lysine (206).

This work paved the way to discover many methylated proteins, characterize methyl-modifying enzymes including methyltransferases (writers), demethylases (erasers) and effectors of methylation (readers), and to better understand biological processes that are regulated by methylation. Moreover, at the turn of the 21st century, increased availability of genomic sequence information and the development of techniques to study chromatin biology including ChIP coupled with analysis of the precipitated DNA, provided information on the *in vivo* association of histones, their modifications and regulatory proteins with specific genomic regions (207).

Much attention has been directed toward elucidating the principles underlying histone lysine methylation and how this mechanism regulates gene expression through the specific

recruitment of effector proteins. However, the list of lysine-methylated non-histone proteins has increased and many of them are substrates of H3K9 KMTs (**Table 1**). For example, G9A methylates many transcription factors (e.g. C/EBPβ, Tp53, MEF2 and MyoD) (123,127–129), chromatin-binding proteins (e.g. WIZ, CDYL, ACINUS) and very interestingly, G9A is able to methylate itself as non-histone substrate (72,125,126). Like G9A, SETDB1 can also methylate proteins besides histones (e.g. UBF, ING2, p53 and Tat) (6–8,94). Here, for the first time, the capability of SETDB1 to undergo auto-methylation was demonstrated. Moreover, our results indicate that methylation site(s) of SETDB1 are located inside its catalytic SET domain, more specifically in the bifurcated domain. This is particularly important, bearing in mind that as many other proteins, PTMs of SETDB1 could play an essential role in regulating protein-protein interactions, catalytic activity and stability of the protein. For instance, SETDB1 is modified by ubiquitination at lysine 867 and that modification was showed to be required for its H3K9 methyltransferase activity and regulation of the expression of its target gene *Serpine1* (2). These remarkable facts lead me to investigate the lysine(s) located inside the SET domain of SETDB1 that potentially could be methylated.

- **The role of SETDB1 auto-methylation**

In order to identify methylated proteins, Guo and collaborators described an approach based on the use of different anti-methyl-lysine antibodies to immunoprecipitate methylated proteins and mass spectrometry (199). They found that lysine methylation appears to be restricted to a smaller group of proteins including translation elongation factors, chaperone proteins such as HSP70 and a subset of lysine methyltransferases including G9A, GLP, EZH1, EZH2 and SETDB1. Thus, SETDB1 could be methylated on the lysines 1170 (me2,

me3) and 1178 (me, me2, me3) (199). Remarkably, these two lysine residues are embedded in the Carboxy terminal region we found is subject for SETDB1 auto-methylation (aa 1101 to 1290 region). Moreover, our results showed that the GST-SETDB1 protein harboring single amino acid substitutions in both methylation sites (K1770A/K1778A) is less methylated by SETDB1.

Regarding K1770 and K1778, our protein-protein interaction data strongly suggest that these residues constitute binding sites for at least three chromodomain-containing proteins, namely HP1γ, SUV39H1 and CDYL. While the integrity of K1770 and K1778 is clearly required for this interaction, it remains unclear whether there is a preferential docking site (either K1170 and/or K1178), because the loss of interaction was observed with SETDB1 double-lysine mutant. It also remains to determine the degree of methylation mediating SETDB1 protein-protein interactions, since the co-IP experiments were performed using mainly an anti-trimethyl-lysine antibody and it is possible that SETDB1 can methylate K1770 and/or K1778 unmodified, mono- or di-methylated.

Since lysine residues can also be modified by other covalent modifications, we cannot exclude that K1170 and K1178 could be acetylated or ubiquitinated. However, with our cellular model we cannot conclude since substitution of lysine by alanine residues was performed.

Moreover, several studies have shown that acetylation of H3K9 synergizes with phosphorylation of histone H3 Serine 10, and the presence of these two modifications correlates with gene activation (208). Strikingly, adjacent to SETDB1 K1170 and K1178 there are serine residues (S1171 and S1179) and we cannot discard the possibility that

phosphorylation could play a role mediating the interaction of SETDB1 with HP1, CDYL and SUV39H1. Thus, adding further complexity to this situation, an operational binary 'methyl/phospho switch' has been reported for K9/S10 region of histone 3. HP1 associates with the H3K9me3 mark, recruiting HP1 to heterochromatin regions and mediating the formation and maintenance of heterochromatin (124). However, during mitosis, phosphorylation of H3S10 by Aurora B disrupts the HP1–H3K9me3 interaction and, as a consequence, HP1 is released from heterochromatin, even though the H3K9me3 mark persists (53). In addition, *in vitro* binding assays demonstrated a significant loss in the affinity of HP1 chromodomains for a dually modified H3K9me3/S10ph peptide, compared with a H3K9me3 peptide (53).

Chromodomain Y-like (CDYL) protein is a histone methyl-lysine reader and transcriptional corepressor involved in neural development (209). Chromodomains of CDY family (CDY, CDYL and CDYL2) have been shown to be sensitive to the presence of H3S10ph. Interaction of these chromodomains with H3K9me3S10ph peptide was ~76–100 fold weaker as compared with the binding to the H3K9me3 peptide (103).

Our results support the idea that SETDB1 contains two lysine methylation clusters (one around K1770 and the second around K1778) that mimic multiple features of histone lysine methylation. Alignment of the sequence harboring both methylation sites (K1770 and K1778) raises a clear analogy to the highly conserved motif ARKS in the N-terminal tail of histone 3 in which recognition of methylated H3K9 and H3K27 are mediated by the chromodomain-containing effectors (HP1 and Polycomb, respectively) (210). Notably, this effector protein recognition was also affected since a loss of interaction between the non-methylatable form of SETDB1 and HP1 and CDYL was detected as already described.

All in all, I propose that catalytic activity of SETDB1 is necessary to undergo its auto-methylation in K1170/K1178 and methylation of these two residues is crucial for interaction of SETDB1 with chromo-domain containing proteins such as SUV39H1, CDYL and HP1γ. Moreover, as I will detail it below, SETDB1 auto-methylation regulates not only the physical interaction with SUV39H1, CDYL and HP1γ, but also the assembly of repressor complexes recruited to establish and/or maintain heterochromatin.

- **Methylation status and catalytic activity of SETDB1 are required for proviral silencing and regulation of imprinting**

Retrotransposons, including LTR and non-LTR classes, are found in the genomes of all higher eukaryotes. These so-called "parasitic" sequences can be classified into two major groups. Non- LTR repeats, including long and short interspersed nuclear elements (LINEs and SINEs, respectively), representing ~30% – 35% of the genome, while those with LTRs, termed ERVs, representing ~8% and 10% of the human and mouse genomes, respectively (211). To minimize their impact on host fitness, numerous pathways have evolved to regulate each step of the retroviral life cycle, including transcription of the integrated ERVs. One of the best characterized transcriptional silencing mechanism involves the covalent transfer of a methyl group to the C-5 position of the cytosine ring of DNA by DNMT enzymes. Notably, DNA methylation has been shown to be required for repression of ERVs in epiblast-derived stem cells, MEFs and neuronal cells (212) cells. However, during early embryonic development, silencing of ERVs occurs without DNA methylation. ESCs naturally undergo almost total genome-wide loss of DNA methylation, likely as part of the acquisition of a pluripotent, flexible state (213). Moreover, genetic studies have demonstrated that mESCs can use DNA methylation-independent mechanisms to silence transposons: knocking-out the

three active DNA methyltransferases does not yield significant de-repression of transposable elements (87,200).

Multiple histone modifications, including H3K9 and H3K27 methylation, have been implicated in retrotransposons' transcriptional repression (87,200,203,214). In fact, H3K9me3 acts as the main transposon controller in pluripotent mESCs (87,183,200). Notably, KAP1 is targeted to retrotransposons by the Krüppel-associated box-containing zinc-finger proteins (KRAB-ZFPs). KAP1, in turn, recruits SETDB1, that deposits repressive H3K9me3 marks at the provirus (87,183,189,200,215). In this work, I demonstrated that the essential role of SETDB1 in retroviral repression in mESCs is compromised when the integrity of the lysines embedded in its catalytic SET domain (K1770 and K1778) is affected. In mESCs expressing the SETDB1 non-methylatable form, recruitment of SETDB1 is maintained but H3K9me3 is no longer deposited efficiently, leading to de-repression of MLV, MusD, IAP, MMERVK10c and LINE-1 elements. This phenotype is consistent with the observation that the catalytic activity of SETDB1 is crucial for high ERV repression, which is supported by previous reports (87,216). Of note, upregulation of ERV and LINE elements in cells expressing either double-lysine or catalytic-dead SETDB1 mutants was not as strong as in *Setdb1* KO mESCs. One possible explanation could be that cells expressing SETDB1 mutants acquired compensatory mechanisms that prevent a strong induction of proviral expression. Peters and colleagues showed the spreading of H3K27me3 at major satellite repeats in *Suv39h*-double null cells, suggesting that H3K27me3 could compensate the absence of H3K9me3 in mESCs (202). Furthermore, cohabitation of H3K9me3 and H3K27me2/3 was reported by Jamieson and collaborators. In *Neurospora crassa*, elimination of H3K9me3 methylation complex caused redistribution of H3K27me. Regions of facultative heterochromatin lost H3K27me3, while regions that are normally marked by H3K9me3 became methylated at H3K27 (217).

Interestingly, although all ERVs and LINE-1 elements showed a significant upregulation in cells expressing the non-methylatable form of SETDB1 compared to the wild-type SETDB1, different levels were observed. MLV, IAP, MusD and LINE-1 were upregulated ~1,5 fold. However, MMERVK10c elements were strongly induced (~20 fold). These results suggest the presence of different mechanisms to control expression transposon subfamilies. For instance, Walter and collaborators revealed overlapping roles of H3K9 and H3K27 trimethylation in controlling distinct transposon families upon DNA demethylation. They classified transposons based on the chromatin changes that take place upon DNA demethylation. In the category A, which includes LINE1 elements, they observed that the loss of DNA methylation was associated with a co-occupancy of H3K9me3 and H3K27me3. The category B, that includes IAPEz and MMERVK10C, showed loss of DNA methylation and maintenance of H3K9me3, without increase in H3K27me3. The category C, containing MERVL, the loss of DNA methylation was associated with a loss of H3K9me3 and replacement by H3K27me3 on the total length of DNA repeats (203). These findings have important implications for understanding the molecular basis of transposon control in the pluripotent cells of the early mammalian embryo.

Our preliminary genome-wide analyses revealed a total of 818 genes de-regulated in mESCs expressing the non-methylatable form of SETDB1 and 2646 genes de-regulated in mESCs expressing the catalytic inactive form of SETDB1. A cohort of germline genes including *Dazl, Hormad1, Nlrp4c, Rex2, Rpl39l, Serpine1, Stk31, Tex101, Tuba3a,* and *Gm13212* were up-regulated in both cell lines, indicating that any disruption of SETDB1 could be sufficient for transcriptional activation. Interestingly, these genes have been shown to be upregulated also after loss of SETDB1 in mESCs (200).

Our preliminary ChIP-seq results showed a decrease in the recruitment of SETDB1 and reduced levels of H3K9me3 levels at the promotor region of a cohort of imprinted genes including *H19, Nnat, Mest, Peg10* and *Peg3* in mESCs expressing the catalytic inactive and the double-lysine mutants of SETDB1. However, deregulation was observed at different levels. Upregulation was observed in *Peg10* and *Peg3*, downregulation was observed in *H19*, whereas *Nnat* and *Mest* showed no significant changes in all cell types. One possibility to explain these differences in transcriptional repression could be the significant increase of 5hmC enrichment in *Mest* and *H19* in absence of SETDB1 as reported by Leung and collaborators using mESCs with the same background that the present study (188). Further analyses will be necessary to study the status of DNMTs in the cells expressing the double-lysine mutant SETDB1, since a link between SETDB1 and DNMT enzymes has been reported. KAP1 is recruited to ICRs by the KRAB zinc-finger protein ZFP57. Moreover, the complex ZFP57/KAP1 recruits DNMT3A and DNMT1 at the ICRs to establish and maintain the methylation at imprinted genes (190,218).

However, we cannot exclude that another explanation for these transcriptional changes is the relatively high level of DNA methylation in mESCs cultured in serum relative to two-inhibitor (2i) media. Under the latter conditions, mESCs adopt a "naïve" hypomethylated state, more reflective of the inner cell mass of the E3.5 blastocyst (219).

Altogether, my results indicate that when integrity of SETDB1 K1170 and K1178 and SETDB1 catalytic activity are compromised, the SETDB1/H3K9me3-mediated silencing of ERVs and imprinting genes is affected. Notably, it has been proposed that mechanisms involved in both, the maintenance of genomic imprinting during development and the maintenance of ERVs silencing show mechanistic similarities (Reviewed in (220)). Regarding

establishment of repressive chromatin on ERVs by H3K9 KMTs, is known that KRAB-ZFPs interact with the corepressor KAP1, thereby the recruitment of SETDB1 which deposits repressive H3K9me3 marks at the provirus (Reviewed by (215)). Likewise, the DNA-methylated parental allele has a repressive chromatin organization similar to that found at repressed ERVs including the KRAB-ZFP Zfp57. Further studies will be needed to determine the imprinted expression pattern dependent on SETDB1. For example, SNP genotyping assays for allelic discrimination allow to study whether the effect on the de-regulated imprinted genes is associated with reactivation of the gene expression of the methylated allele.

- **The biochemical point of view: SETDB1 and SUV39H1 cooperate in the establishment of heterochromatin in SETDB1-methylation dependent way**

Our lab showed that the main H3K9 KMTs (SETDB1, G9A, GLP and SUV39H1) form a functional megacomplex involved in transcriptional silencing (1). However, how these KMTs interact and cooperate remains elusive. Our results showed that both the integrity of K1170 and K1178 and SETDB1 catalytic activity are required for its interaction with SUV39H1, CDYL and HP1γ, which are chromodomain-containing proteins.

Since CDYL transcriptional repressor complex includes SETDB1 (122), I hypothesize that SETDB1/CDYL interaction could be mediated by methylation of SETDB1. In fact, CDYL bridges the interaction between the tumor suppressor REST and G9A and functions as a REST corepressor that facilitates G9A recruitment to REST target genes. The repressor complex REST/CDYL/G9A inhibits transcription of the proto-oncogene *TrkC*, whose overexpression causes cellular transformation. In addition, not only an interaction between CDYL and EZH2 has been reported, but also, genome-wide analysis revealed that CDYL and

PRC2 share genomic targets in the breast cancer cell line MCF-7 (221). Recently, Liu and colleagues demonstrated that CDYL is associated with the chromatin assembly factor 1 (CAF-1) and the replicative helicase MCM complex during DNA replication and it seems that this complex is implicated in the recruitment of EZH2, G9A and SETDB1 to the chromatin, at least in U2OS cells (Osteosarcoma epithelial cells) (222).

SETDB1 and SUV39H1 coordinate the establishment and maintenance of H3K9me3 at constitutive pericentromeric heterochromatin (9,10) and co-regulate many genomic targets within heterochromatin, including transposable elements, such as long interspersed nuclear elements (LINEs) and endogenous retroviruses (ERVs) (11). Since SUV39H1 is not able to initiate H3K9 (mono)-methylation, SETDB1 could probably provide mono- or di-methylated H3K9. Then, HP1γ, which is a reader of H3K9me2 and H3K9me3, forms a stable complex with SUV39H1, thereby providing a mean for the spreading and maintenance of H3K9me3 at heterochromatin (9,10). Interestingly, my results point to a model in which SETDB1 auto-methylation paves the way to a subsequent trans-methylation by SUV39H1 at K1170 and K1178. This mechanism could regulate not only the SETDB1/SUV39H1 physical interaction (*via* the SUV39H1 chromodomain), but also cooperation in the establishment and maintenance of heterochromatin and proviral silencing, at least in mESCs (**Illustration 17**).

Our preliminary results indicate that the SETDB1 pan-genomic binding profile is deregulated in the absence of SUV39H1/2 in mESCs. In *Suv39h* double KO mESCs, SETDB1 binding is lost in regions where there are repetitive elements, mostly LTR-ERVs proximal to a cohort of imprinted genes including *H19-Igf2, Mest, Nnat, Mest, Nespas Airn, Mcts2, Zrsr1, Nap1I5, Plagl1, Snurf and Grb10*. Moreover, in absence of SUV39H1/2 upregulation of *H19, Igf2,* and *Nnat* was observed. These results support the hypothesis that recruitment of SETDB1 is

dependent of the presence of SUV39H1/2 in mESCs. Notably, in the absence of SETDB1, retroviral elements could be reactivated and play a role as enhancers or anti-sense transcripts. Importantly, imprinted genes are known to be involved in aging and cancer (223).

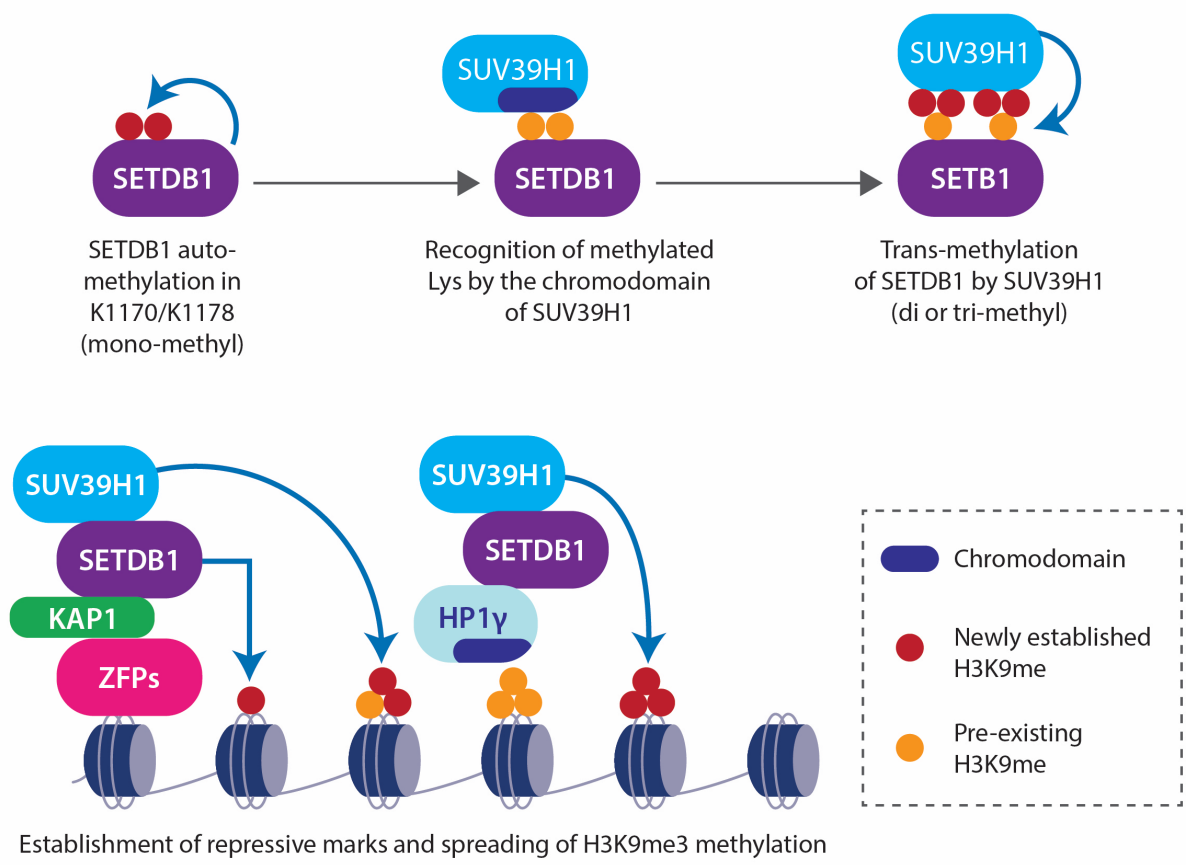


Illustration 17. SETDB1 and SUV39H1 cooperate in the establishment of heterochromatin in SETDB1-methylation dependent way. **A)** SETDB1 undergoes auto-methylation (probably mono-methylation), which is required for a subsequent trans-methylation by SUV39H1. **B)** SETDB1 monomethylates H3K9 and thereby provides the substrate for SUV39H1 to establish H3K9me3 either on gene regulatory regions and provirus. HP1γ, which is a reader of H3K9me2 and H3K9me3, forms a stable complex with SUV39H1, thereby providing a mean for the spreading and maintenance of H3K9me3 at heterochromatin.

1. PERSPECTIVES

- **Determine whether SETDB1 binding is dependent on the enzymatic activity of SUV39H1**

Our group have previously demonstrated that the main H3K9 KMTs (SETDB1, G9a, GLP and SUV39H1) form a functional megacomplex involved in transcriptional silencing (1). Interestingly, the stability of this complex was affected in *SUV39H* KO MEFs. Since, our current results showed that SETDB1 pan-genomic binding was deregulated in *SUV39H* KO mESCs, I wonder whether either the presence of SUV39H1 or its enzymatic activity affect SETDB1 binding. It would be interesting to use mESCs *SUV39H* KO cell lines to stably express either wild-type or catalytic-dead SUV39H1 and determine SETDB1 binding and H3K9me3 enrichment to answer the question.

- **Regulation of SETDB1/H3K9me3-mediated proviral silencing in differentiated cells**

It has been generally considered that SETDB1/H3K9me3-mediated proviral silencing pathway in mESCs is replaced by a more permanent silencing mechanism, i.e., KAP1-mediated *de novo* DNA methylation in differentiated embryonic cells (183). However, recent evidence showed that specific sets of ERVs are reactivated in different types of SETDB1-deficient somatic cells (88). Thereafter, it would be very interesting to study how the proviral silencing stablished by SETDB1 changes along a differentiation process, i.e., muscle differentiation. Interestingly, changes on the proviral silencing could be affected by the SETDB1 subcellular localization since it has been shown that SETDB1 has both nuclear and cytoplasmic localizations. Moreover, this relocalization is modulated by activation of biological pathways such as Wnt/β-catenin pathway during muscle differentiation (224).

Altogether, it could reveal unknown properties and functions of SETDB,1 which can be exploited in novel disease therapies.

- **Characterize SETDB1 behavior during carcinogenic process**

SETDB1 has been found to be amplified in different types of cancer (15,16). Moreover, SETDB1 can methylate P53, one of the most relevant proteins involved in cancer (7). Considering the possibility that SETDB1 could be a biomarker for early detection of cancer or a target for anti-tumor therapy is necessary to carry out studies along the carcinogenic process. For instance, there is an interesting cellular model established by Mercatelli and collaborators recapitullating all breast cancer stages including normal breast cells, preneoplastic, *in situ* and metastatic cells (225). In the future it would be important to verify whether SETDB1/H3K9me3-mediated gene silencing pathway changes and also to stablish its protein partners during the carcinogenic process. In addition, given the recent evidence showing SETDB1 involved in regulation of the three-dimensional (3D) genome organization (226) and that this 3D genomic architecture is often perturbed in cancer (227), it would be interesting to investigate the role of SETDB1 in the 3D genome organization during cancer progress. Altogether, this would bring new concepts bearing potential medical applications, including therapies targeting the epigenetic mechanisms.

ANNEX

RÉSUMÉ SUBSTANTIEL EN LANGUE FRANÇAISE

RESUME DE LA RECHERCHE

La méthylation de la lysine 9 de l'histone 3 (H3K9), établie par les lysine méthyltransférases (KMTs) SETDB1, SUV39H1, G9A et GLP, représente un mécanisme épigénétique central dans la régulation du destin cellulaire. En particulier, la méthylation d'H3K9 est directement impliquée dans la formation de l'hétérochromatine et l'extinction des gènes. Notre laboratoire a montré que les principales KMTs (SETDB1, G9A, GLP et SUV39H1) spécifiques de H3K9 forment un méga-complexe impliqué dans la répression transcriptionnelle (1), probablement *via* une coopération pour établir les différents niveaux de méthylation. Néanmoins, la régulation des complexes de H3K9 KMT n'est jusqu'à présent pas bien comprise. Il est à noter que des modifications post-traductionnelles (PTM) ont été impliquées dans la régulation des fonctions des KMTs (2). Dans ce contexte, mon projet visait à comprendre comment la méthylation de SETDB1 régulerait son activité (incorporation dans des complexes, interaction avec ses partenaires, recrutement à la chromatine). Le but étant d'établir quel impact auraient ces modifications de SETDB1 sur la formation de l'hétérochromatine, l'expression des gènes et la régulation du destin cellulaire.

SETDB1 est cruciale lors du développement et de la différenciation cellulaire. De plus, SETDB1 est essentielle pour la pluripotence et le renouvellement des cellules souches embryonnaires murines (mESC) (3,4). L'inactivation génique de ou KO de *Setdb1* est létal au stade préimplantatoire à 7,5 jours post-coïtum (dpc) (5). En plus des histones, SETDB1 méthyle d'autres protéines comme UBF, ING2 et p53 (6-8). Mes résultats montrent

notamment, que SETDB1 s'autométhyle sur les lysines K1170 et K1178 localisées dans le domaine catalytique SET.

SETDB1 et SUV39H1 coordonnent l'établissement et la maintenance de H3K9me3 dans l'hétérochromatine péricentromérique constitutive (9,10) et co-régulent de nombreuses cibles génomiques dans l'hétérochromatine, dont les éléments transposables comme les *Long Interspersed Nuclear Elements* (LINEs) et les rétrovirus endogènes (ERVs) (11).

Comme SUV39H1 est une triméthyltransférase qui utilise H3K9me1 ou H3K9me2 comme substrat primaire, SETDB1 pourrait probablement fournir les mono- ou di-méthyl H3K9 (9,10). Mes résultats suggèrent un modèle dans lequel l'auto-méthylation de SETDB1 est pré-requise à la trans-méthylation subséquente par SUV39H1. Ce mécanisme pourrait réguler non seulement l'interaction physique entre SETDB1 et SUV39H1, *via* le chromodomaine de SUV39H1, mais aussi leur coopération dans l'établissement et la maintenance des blocs (grands domaines) d'hétérochromatine et l'extinction des éléments transposables, au moins dans les cellules souches. Ainsi, nous souhaitons mieux comprendre comment le « dialogue » entre ces deux H3K9 KMT majeures, SETDB1 et SUV39H1, est impliqué dans leurs interactions et leurs recrutements aux *loci* cibles.

***Mots clés**: SETDB1, SUV39H1, modifications post-traductionnelles, méthylation de lysines*

OBJECTIFS GÉNÉRAUX DE LA THÈSE

1- Comprendre comment l'auto-méthylation de SETDB1, puis sa trans-méthylation par SUV39H1 régulent son activité, les interactions avec ses partenaires et son recrutement à la chromatine, ce qui, *in fine*, pourrait modifier l'expression de gènes.

2- Identifier les cibles génomiques co-régulées par SETDB1 et SUV39H1 et étudier le rôle de la méthylation de SETDB1 dans cette co-régulation.

PRINCIPAUX RÉSULATS

SETDB1 s'auto-méthyle est peut être trans-méthylée par SUV39H1: Mes résultats montrent que SETDB1 se méthyle sur deux lysines localisées dans son domaine catalytique SET. Ces méthylations permettent ensuite la trans-méthylation par SUV39H1.

L'état de méthylation et l'activité catalytique de SETDB1 influencent ses interactions protéines – protéines : L'intégrité des deux lysines méthylées et l'activité catalytique de SETDB1 sont nécessaires pour son interaction avec les protéines SUV39H1, CDYL (Chromodomain Y-Like) and HP1g (Heterochromatin Protein 1), qui toutes contiennent un domaine de liaison aux lysines méthylées, de type chromodomaine. Comme le complexe répresseur transcriptionnel CDYL contient SETDB1 (11), nous proposons que l'interaction SETDB1/CDYL pourrait être dépendre de la méthylation de SETDB1. De plus, comme HP1 est un "lecteur" de lysines méthylées et est crucial pour la formation de l'hétérochromatine, la méthylation de SETDB1 pourrait affecter l'assemblage des complexes répresseurs recrutés pour établir et/ou maintenir l'hétérochromatine.

L'état de méthylation et l'activité catalytique de SETDB1 régulent l'empreinte génétique parentale et est nécessaire à l'extinction des séquences provirales : Nous proposons que la méthylation de SETDB1 pourrait réguler son recrutement à ses gènes cibles et ensuite la répression transcriptionnelle dépendante de H3K9me3. Pour tester cela, nous avons utilisé des lignées de cellules souches embryonnaires murines (mESC) inductibles pour la délétion du gène *Setdb1* dans lesquelles nous avons ré-exprimé de façon stable soit la forme normale (WT), soit le double mutant sur les lysines méthylées (KK) qui est catalytiquement actif, ou un mutant catalytiquement inactif (CA). Puis nous avons réalisé des ChIP-qPCR et trouvé une diminution du recrutement de SETDB1 KK et CA non seulement sur les gènes soumis à l'empreinte parentale *Meg3, Nnat*, *Mest* et H19, mais également sur les éléments transposables comme ERVs (MLV, MusD, IAP, MERVK10C) et LINEs. Nous avons ensuite réalisé des ChIP-seq pour SETDB1 et H3K9me3, combinées à du RNA-seq et nous sommes actuellement en train d'analyser ces résultats. Ainsi, en plus de la régulation de l'expression des gènes, nous allons tester si la méthylation de SETDB1 peut influer la formation des blocs de H3K9me3, la marque de l'hétérochromatine co-régulée par SETDB1 et SUV39H1.

Le profil pan-génomique de la localisation de SETDB1 est dérégulé en absence de SUV39H dans les cellules mESC : Notre laboratoire a montré que SETDB1 intéragit avec SUV39H1 (1). SETDB1 et SUV39H1 non seulement partagent des cibles génomiques communes, mais coordonnent également la propagation de H3K9me3 au niveau de l'hétérochromatine péri-centromérique constitutive (9–11). En fait, SETDB1 peut mono-, di- or tri-méthyler H3K9, procurant ainsi un substrat pour SUV39H1 pour établir H3K9me3, puisque SUV39H1 n'est pas capable d'initier la méthylation de H3K9 (9,10). Malgré tout, la façon dont ces KMTs interagissent et coopèrent reste mal connue. Nos résultats montrent que SUV39H1 méthyle SETDB1 et les deux lysines précédemment mentionnées, localisées dans

le domaine catalytique, sont nécessaires dans ce mécanisme.

Ainsi, nous avons testé si SUV39H1 pouvait avoir un impact sur le recrutement global de SETDB1 à la chromatine. Dans ce but, nous avons réalisé des ChIP-seq pour SETDB1 dans les mESC HM-1 normales ou double KO pour les gènes *SUV39H* (*SUV39H1* and *SUV39H2*). Nous avons trouvé que le profil de fixation pan-génomique de SETDB1 était dérégulé en absence de SUV39H. Dans les mESC KO pour *SUV39H*, la fixation de SETDB1 est perdue au niveau des éléments répétés ERVs (MLV, MusD, IAP) ainsi qu'aux régions proches des gènes d'empreinte. En absence de SETDB1 ces éléments rétroviraux peuvent être réactivés et peuvent jouer un rôle « d'activateurs » ou transcrits « anti-sens ». Nous avons ensuite validé par ChIP-qPCR que le recrutement de SETDB1 à des gènes soumis à l'empreinte était bien altéré (*Meg3, Nnat*, *Mest* and H19) dans les mESC déficientes pour *SUV39H1* et *SUV39H2*. De plus, les analyses par RT-qPCR ont confirmé la dé-répression de ces gènes dans les mESC déficientes pour *SUV39H1* et *SUV39H2*. Ensemble, ces résultats suggèrent qu'en l'absence de SUV39H, SETDB1 n'est pas correctement recrutée à la chromatine (au moins pour certains des loci cibles) conduisant à un profil altéré de l'expression de ces gènes.

CONTEXTE DE LA RECHERCHE SUR LE CANCER

La transformation de cellules normales en cellules cancéreuses implique des changements dans l'expression de nombreux gènes. En effet, l'altération de l'expression de gènes clés du cycle cellulaire, de la réparation de l'ADN ou de la différenciation entraîne une prolifération anarchique de cellules qui ont perdu une partie de leur identité. Ces dérégulations peuvent être induites par des mécanismes génétiques de type amplifications, délétions, mutations ou

translocations. Cependant, les altérations de la séquence d'ADN ne permettent pas d'expliquer à elles seules l'ensemble des dérégulations d'expression géniques associées aux cancers et ces deux dernières décennies ont vu une accumulation d'évidences reliant une dérégulation des mécanismes dits « épigénétiques » avec la tumorigenèse. Depuis la découverte de la dérégulation de la méthylation de l'ADN dans le cancer en 1983, il a été montré que d'autres mécanismes épigénétiques aberrants peuvent réprimer des gènes essentiels à la régulation de la cellule aussi bien qu'activer l'expression de gènes favorisant l'hyperprolifération par exemple. Ces mécanismes épigénétiques impliquent essentiellement des enzymes qui régulent l'accessibilité de l'ADN aux facteurs de transcription en modifiant les histones, l'ADN ou les facteurs de transcription eux-mêmes. En particulier, la méthylation de la lysine 9 de l'histone H3 (H3K9) est associée à une répression transcriptionnelle stable et elle est d'ailleurs étroitement liée à la méthylation de l'ADN, qui est souvent dérégulée dans les cancers. Plusieurs protéines méthylant spécifiquement H3K9 sont surexprimées dans grand nombre de cancers. A l'avenir, il sera possible de mieux comprendre comment fonctionnent les méthyltransférases de H3K9, et comment leurs dérégulations amèneraient à la tumorigenèse.

REFERENCES

1. Fritsch L, Robin P, Mathieu JRR, Souidi M, Hinaux H, Rougeulle C, et al. A subset of the histone H3 lysine 9 methyltransferases Suv39h1, G9a, GLP, and SETDB1 participate in a multimeric complex. Mol Cell [Internet]. 2010 Jan 15;37(1):46–56. Available from: http://www.ncbi.nlm.nih.gov/pubmed/20129054
2. Ishimoto K, Kawamata N, Uchihara Y, Okubo M, Fujimoto R, Gotoh E, et al. Ubiquitination of Lysine 867 of the Human SETDB1 Protein Upregulates Its Histone H3 Lysine 9 (H3K9) Methyltransferase Activity. PLoS One [Internet]. 2016;11(10):e0165766. Available from: http://www.ncbi.nlm.nih.gov/pubmed/27798683
3. Yeap L-S, Hayashi K, Surani MA. ERG-associated protein with SET domain (ESET)-Oct4 interaction regulates pluripotency and represses the trophectoderm lineage. Epigenetics Chromatin [Internet]. 2009;2(1):12. Available from: http://www.ncbi.nlm.nih.gov/pubmed/19811652
4. Bilodeau S, Kagey MH, Frampton GM, Rahl PB, Young RA. SetDB1 contributes to repression of genes encoding developmental regulators and maintenance of ES cell state. Genes Dev [Internet]. 2009 Nov 1;23(21):2484–9. Available from: http://www.ncbi.nlm.nih.gov/pubmed/19884255
5. Dodge JE, Kang Y-K, Beppu H, Lei H, Li E. Histone H3-K9 methyltransferase ESET is essential for early development. Mol Cell Biol [Internet]. 2004 Mar;24(6):2478–86. Available from: http://www.ncbi.nlm.nih.gov/pubmed/14993285
6. Binda O, LeRoy G, Bua DJ, Garcia BA, Gozani O, Richard S. Trimethylation of histone H3 lysine 4 impairs methylation of histone H3 lysine 9: regulation of lysine methyltransferases by physical interaction with their substrates. Epigenetics [Internet]. 5(8):767–75. Available from: http://www.ncbi.nlm.nih.gov/pubmed/21124070
7. Fei Q, Shang K, Zhang J, Chuai S, Kong D, Zhou T, et al. Histone methyltransferase SETDB1 regulates liver cancer cell growth through methylation of p53. Nat Commun [Internet]. 2015;6:8651. Available from: http://www.ncbi.nlm.nih.gov/pubmed/26471002
8. Hwang YJ, Han D, Kim KY, Min S-J, Kowall NW, Yang L, et al. ESET methylates UBF at K232/254 and regulates nucleolar heterochromatin plasticity and rDNA transcription. Nucleic Acids Res [Internet]. 2014 Feb;42(3):1628–43. Available from: http://www.ncbi.nlm.nih.gov/pubmed/24234436
9. Loyola A, Tagami H, Bonaldi T, Roche D, Quivy JP, Imhof A, et al. The HP1alpha-CAF1-SetDB1-containing complex provides H3K9me1 for Suv39-mediated K9me3 in pericentric heterochromatin. EMBO Rep [Internet]. 2009 Jul;10(7):769–75. Available from: http://www.ncbi.nlm.nih.gov/pubmed/19498464
10. Towbin BD, González-Aguilera C, Sack R, Gaidatzis D, Kalck V, Meister P, et al. Step-wise methylation of histone H3K9 positions heterochromatin at the nuclear periphery. Cell [Internet]. 2012 Aug 31;150(5):934–47. Available from: http://www.ncbi.nlm.nih.gov/pubmed/22939621
11. Bulut-Karslioglu A, De La Rosa-Velázquez IA, Ramirez F, Barenboim M, Onishi-Seebacher M, Arand J, et al. Suv39h-dependent H3K9me3 marks intact retrotransposons and silences LINE elements in mouse embryonic stem cells. Mol Cell [Internet]. 2014 Jul 17;55(2):277–90. Available from: http://www.ncbi.nlm.nih.gov/pubmed/24981170
12. Baylin SB, Jones PA. A decade of exploring the cancer epigenome - biological and translational implications. Nat Rev Cancer [Internet]. 2011 Oct;11(10):726–34. Available from: http://www.ncbi.nlm.nih.gov/pubmed/21941284
13. Greer EL, Shi Y. Histone methylation: a dynamic mark in health, disease and inheritance. Nat Rev Genet [Internet]. 2012 Apr 3;13(5):343–57. Available from:

http://www.ncbi.nlm.nih.gov/pubmed/22473383
14. Ding J, Li T, Wang X, Zhao E, Choi J-H, Yang L, et al. The histone H3 methyltransferase G9A epigenetically activates the serine-glycine synthesis pathway to sustain cancer cell survival and proliferation. Cell Metab [Internet]. 2013 Dec 3;18(6):896–907. Available from: http://www.ncbi.nlm.nih.gov/pubmed/24315373
15. Ceol CJ, Houvras Y, Jane-Valbuena J, Bilodeau S, Orlando DA, Battisti V, et al. The histone methyltransferase SETDB1 is recurrently amplified in melanoma and accelerates its onset. Nature [Internet]. 2011 Mar 24;471(7339):513–7. Available from: http://www.ncbi.nlm.nih.gov/pubmed/21430779
16. Rodriguez-Paredes M, Martinez de Paz A, Simó-Riudalbas L, Sayols S, Moutinho C, Moran S, et al. Gene amplification of the histone methyltransferase SETDB1 contributes to human lung tumorigenesis. Oncogene [Internet]. 2014 May 22;33(21):2807–13. Available from: http://www.ncbi.nlm.nih.gov/pubmed/23770855
17. Liu L, Kimball S, Liu H, Holowatyj A, Yang Z-Q. Genetic alterations of histone lysine methyltransferases and their significance in breast cancer. Oncotarget [Internet]. 2015 Feb 10;6(4):2466–82. Available from: http://www.ncbi.nlm.nih.gov/pubmed/25537518
18. Burns KH, Boeke JD. Human transposon tectonics. Cell [Internet]. 2012 May 11;149(4):740–52. Available from: http://www.ncbi.nlm.nih.gov/pubmed/22579280
19. Waddington CH. The epigenotype. Endeavour. 1942;1:18.
20. Baxter E, Windloch K, Gannon F, Lee JS. Epigenetic regulation in cancer progression. Cell Biosci [Internet]. 2014;4:45. Available from: http://www.ncbi.nlm.nih.gov/pubmed/25949794
21. Egger G, Liang G, Aparicio A, Jones PA. Epigenetics in human disease and prospects for epigenetic therapy. Nature [Internet]. 2004 May 27;429(6990):457–63. Available from: http://www.ncbi.nlm.nih.gov/pubmed/15164071
22. Bannister AJ, Kouzarides T. Regulation of chromatin by histone modifications. Cell Res [Internet]. 2011 Mar;21(3):381–95. Available from: http://www.ncbi.nlm.nih.gov/pubmed/21321607
23. Saksouk N, Simboeck E, Déjardin J. Constitutive heterochromatin formation and transcription in mammals. Epigenetics Chromatin [Internet]. 2015;8:3. Available from: http://www.ncbi.nlm.nih.gov/pubmed/25788984
24. Grewal SIS, Jia S. Heterochromatin revisited. Nat Rev Genet [Internet]. 2007 Jan;8(1):35–46. Available from: http://www.ncbi.nlm.nih.gov/pubmed/17173056
25. Nishibuchi G, Déjardin J. The molecular basis of the organization of repetitive DNA-containing constitutive heterochromatin in mammals. Chromosome Res [Internet]. 2017 Mar;25(1):77–87. Available from: http://www.ncbi.nlm.nih.gov/pubmed/28078514
26. Wang J, Jia ST, Jia S. New Insights into the Regulation of Heterochromatin. Trends Genet [Internet]. 2016;32(5):284–94. Available from: http://www.ncbi.nlm.nih.gov/pubmed/27005444
27. Martin C, Zhang Y. The diverse functions of histone lysine methylation. Nat Rev Mol Cell Biol [Internet]. 2005 Nov;6(11):838–49. Available from: http://www.ncbi.nlm.nih.gov/pubmed/16261189
28. Jenuwein T, Allis CD. Translating the histone code. Science [Internet]. 2001 Aug 10;293(5532):1074–80. Available from: http://www.ncbi.nlm.nih.gov/pubmed/11498575
29. Ronner P. Netter's Essential Biochemistry. First edit. Elsevier Health Sciences; 2016. 43 p.
30. Felsenfeld G, Groudine M. Controlling the double helix. Nature [Internet]. 2003 Jan 23;421(6921):448–53. Available from:

http://www.ncbi.nlm.nih.gov/pubmed/12540921
31. Bhaumik SR, Smith E, Shilatifard A. Covalent modifications of histones during development and disease pathogenesis. Nat Struct Mol Biol [Internet]. 2007 Nov;14(11):1008–16. Available from: http://www.ncbi.nlm.nih.gov/pubmed/17984963
32. Huynh JL, Casaccia P. Epigenetic mechanisms in multiple sclerosis: implications for pathogenesis and treatment. Lancet Neurol [Internet]. 2013 Feb;12(2):195–206. Available from: http://www.ncbi.nlm.nih.gov/pubmed/23332363
33. Mozzetta C, Boyarchuk E, Pontis J, Ait-Si-Ali S. Sound of silence: the properties and functions of repressive Lys methyltransferases. Nat Rev Mol Cell Biol [Internet]. 2015 Aug;16(8):499–513. Available from: http://www.ncbi.nlm.nih.gov/pubmed/26204160
34. Shilatifard A. Chromatin modifications by methylation and ubiquitination: implications in the regulation of gene expression. Annu Rev Biochem [Internet]. 2006;75:243–69. Available from: http://www.ncbi.nlm.nih.gov/pubmed/16756492
35. Hou H, Yu H. Structural insights into histone lysine demethylation. Curr Opin Struct Biol [Internet]. 2010 Dec;20(6):739–48. Available from: http://www.ncbi.nlm.nih.gov/pubmed/20970991
36. Luco RF, Pan Q, Tominaga K, Blencowe BJ, Pereira-Smith OM, Misteli T. Regulation of alternative splicing by histone modifications. Science [Internet]. 2010 Feb 19;327(5968):996–1000. Available from: http://www.ncbi.nlm.nih.gov/pubmed/20133523
37. Kolasinska-Zwierz P, Down T, Latorre I, Liu T, Liu XS, Ahringer J. Differential chromatin marking of introns and expressed exons by H3K36me3. Nat Genet [Internet]. 2009 Mar;41(3):376–81. Available from: http://www.ncbi.nlm.nih.gov/pubmed/19182803
38. Bieberstein NI, Kozáková E, Huranová M, Thakur PK, Krchňáková Z, Krausová M, et al. TALE-directed local modulation of H3K9 methylation shapes exon recognition. Sci Rep [Internet]. 2016;6:29961. Available from: http://www.ncbi.nlm.nih.gov/pubmed/27439481
39. Di Lorenzo A, Bedford MT. Histone arginine methylation. FEBS Lett [Internet]. 2011 Jul 7;585(13):2024–31. Available from: http://www.ncbi.nlm.nih.gov/pubmed/21074527
40. Wesche J, Kühn S, Kessler BM, Salton M, Wolf A. Protein arginine methylation: a prominent modification and its demethylation. Cell Mol Life Sci [Internet]. 2017;74(18):3305–15. Available from: http://www.ncbi.nlm.nih.gov/pubmed/28364192
41. Carrozza MJ, Utley RT, Workman JL, Côté J. The diverse functions of histone acetyltransferase complexes. Trends Genet [Internet]. 2003 Jun;19(6):321–9. Available from: http://www.ncbi.nlm.nih.gov/pubmed/12801725
42. Rossetto D, Avvakumov N, Côté J. Histone phosphorylation: a chromatin modification involved in diverse nuclear events. Epigenetics [Internet]. 2012 Oct;7(10):1098–108. Available from: http://www.ncbi.nlm.nih.gov/pubmed/22948226
43. Cao J, Yan Q. Histone ubiquitination and deubiquitination in transcription, DNA damage response, and cancer. Front Oncol [Internet]. 2012;2:26. Available from: http://www.ncbi.nlm.nih.gov/pubmed/22649782
44. Cao R, Tsukada Y-I, Zhang Y. Role of Bmi-1 and Ring1A in H2A ubiquitylation and Hox gene silencing. Mol Cell [Internet]. 2005 Dec 22;20(6):845–54. Available from: http://www.ncbi.nlm.nih.gov/pubmed/16359901
45. Minsky N, Shema E, Field Y, Schuster M, Segal E, Oren M. Monoubiquitinated H2B is associated with the transcribed region of highly expressed genes in human cells. Nat Cell Biol [Internet]. 2008 Apr;10(4):483–8. Available from:

http://www.ncbi.nlm.nih.gov/pubmed/18344985
46. Zhu B, Zheng Y, Pham A-D, Mandal SS, Erdjument-Bromage H, Tempst P, et al. Monoubiquitination of human histone H2B: the factors involved and their roles in HOX gene regulation. Mol Cell [Internet]. 2005 Nov 23;20(4):601–11. Available from: http://www.ncbi.nlm.nih.gov/pubmed/16307923
47. Cubeñas-Potts C, Matunis MJ. SUMO: a multifaceted modifier of chromatin structure and function. Dev Cell [Internet]. 2013 Jan 14;24(1):1–12. Available from: http://www.ncbi.nlm.nih.gov/pubmed/23328396
48. Drag M, Salvesen GS. DeSUMOylating enzymes--SENPs. IUBMB Life [Internet]. 2008 Nov;60(11):734–42. Available from: http://www.ncbi.nlm.nih.gov/pubmed/18666185
49. Shin JA, Choi ES, Kim HS, Ho JCY, Watts FZ, Park SD, et al. SUMO modification is involved in the maintenance of heterochromatin stability in fission yeast. Mol Cell [Internet]. 2005 Sep 16;19(6):817–28. Available from: http://www.ncbi.nlm.nih.gov/pubmed/16168376
50. Uchimura Y, Ichimura T, Uwada J, Tachibana T, Sugahara S, Nakao M, et al. Involvement of SUMO modification in MBD1- and MCAF1-mediated heterochromatin formation. J Biol Chem [Internet]. 2006 Aug 11;281(32):23180–90. Available from: http://www.ncbi.nlm.nih.gov/pubmed/16757475
51. Wotton D, Pemberton LF, Merrill-Schools J. SUMO and Chromatin Remodeling. Adv Exp Med Biol [Internet]. 2017;963:35–50. Available from: http://www.ncbi.nlm.nih.gov/pubmed/28197905
52. Maison C, Almouzni G. HP1 and the dynamics of heterochromatin maintenance. Nat Rev Mol Cell Biol [Internet]. 2004 Apr;5(4):296–304. Available from: http://www.ncbi.nlm.nih.gov/pubmed/15071554
53. Fischle W, Tseng BS, Dormann HL, Ueberheide BM, Garcia BA, Shabanowitz J, et al. Regulation of HP1-chromatin binding by histone H3 methylation and phosphorylation. Nature [Internet]. 2005 Dec 22;438(7071):1116–22. Available from: http://www.ncbi.nlm.nih.gov/pubmed/16222246
54. Falkenberg KJ, Johnstone RW. Histone deacetylases and their inhibitors in cancer, neurological diseases and immune disorders. Nat Rev Drug Discov [Internet]. 2014 Sep;13(9):673–91. Available from: http://www.ncbi.nlm.nih.gov/pubmed/25131830
55. Hyun K, Jeon J, Park K, Kim J. Writing, erasing and reading histone lysine methylations. Exp Mol Med [Internet]. 2017;49(4):e324. Available from: http://www.ncbi.nlm.nih.gov/pubmed/28450737
56. Boyer LA, Plath K, Zeitlinger J, Brambrink T, Medeiros LA, Lee TI, et al. Polycomb complexes repress developmental regulators in murine embryonic stem cells. Nature [Internet]. 2006 May 18;441(7091):349–53. Available from: http://www.ncbi.nlm.nih.gov/pubmed/16625203
57. Mohn F, Weber M, Rebhan M, Roloff TC, Richter J, Stadler MB, et al. Lineage-specific polycomb targets and de novo DNA methylation define restriction and potential of neuronal progenitors. Mol Cell [Internet]. 2008 Jun 20;30(6):755–66. Available from: http://www.ncbi.nlm.nih.gov/pubmed/18514006
58. Brookes E, de Santiago I, Hebenstreit D, Morris KJ, Carroll T, Xie SQ, et al. Polycomb associates genome-wide with a specific RNA polymerase II variant, and regulates metabolic genes in ESCs. Cell Stem Cell [Internet]. 2012 Feb 3;10(2):157–70. Available from: http://www.ncbi.nlm.nih.gov/pubmed/22305566
59. Allis D. Epigenetics. Second. Cold Spring Harbor Laboratory Press; 2015.
60. Dillon SC, Zhang X, Trievel RC, Cheng X. The SET-domain protein superfamily: protein lysine methyltransferases. Genome Biol [Internet]. 2005;6(8):227. Available

from: http://www.ncbi.nlm.nih.gov/pubmed/16086857
61. Qian C, Zhou M-M. SET domain protein lysine methyltransferases: Structure, specificity and catalysis. Cell Mol Life Sci [Internet]. 2006 Dec;63(23):2755–63. Available from: http://www.ncbi.nlm.nih.gov/pubmed/17013555
62. Herz H-M, Garruss A, Shilatifard A. SET for life: biochemical activities and biological functions of SET domain-containing proteins. Trends Biochem Sci [Internet]. 2013 Dec;38(12):621–39. Available from: http://www.ncbi.nlm.nih.gov/pubmed/24148750
63. Matsumura Y, Nakaki R, Inagaki T, Yoshida A, Kano Y, Kimura H, et al. H3K4/H3K9me3 Bivalent Chromatin Domains Targeted by Lineage-Specific DNA Methylation Pauses Adipocyte Differentiation. Mol Cell [Internet]. 2015 Nov 19;60(4):584–96. Available from: http://www.ncbi.nlm.nih.gov/pubmed/26590716
64. Nekrasov M, Wild B, Müller J. Nucleosome binding and histone methyltransferase activity of Drosophila PRC2. EMBO Rep [Internet]. 2005 Apr;6(4):348–53. Available from: http://www.ncbi.nlm.nih.gov/pubmed/15776017
65. Margueron R, Reinberg D. The Polycomb complex PRC2 and its mark in life. Nature [Internet]. 2011 Jan 20;469(7330):343–9. Available from: http://www.ncbi.nlm.nih.gov/pubmed/21248841
66. Zhao J, Sun BK, Erwin JA, Song J-J, Lee JT. Polycomb proteins targeted by a short repeat RNA to the mouse X chromosome. Science [Internet]. 2008 Oct 31;322(5902):750–6. Available from: http://www.ncbi.nlm.nih.gov/pubmed/18974356
67. Rinn JL, Kertesz M, Wang JK, Squazzo SL, Xu X, Brugmann SA, et al. Functional demarcation of active and silent chromatin domains in human HOX loci by noncoding RNAs. Cell [Internet]. 2007 Jun 29;129(7):1311–23. Available from: http://www.ncbi.nlm.nih.gov/pubmed/17604720
68. Collins RE, Northrop JP, Horton JR, Lee DY, Zhang X, Stallcup MR, et al. The ankyrin repeats of G9a and GLP histone methyltransferases are mono- and dimethyllysine binding modules. Nat Struct Mol Biol [Internet]. 2008 Mar;15(3):245–50. Available from: http://www.ncbi.nlm.nih.gov/pubmed/18264113
69. Tachibana M, Sugimoto K, Nozaki M, Ueda J, Ohta T, Ohki M, et al. G9a histone methyltransferase plays a dominant role in euchromatic histone H3 lysine 9 methylation and is essential for early embryogenesis. Genes Dev [Internet]. 2002 Jul 15;16(14):1779–91. Available from: http://www.ncbi.nlm.nih.gov/pubmed/12130538
70. Rice JC, Briggs SD, Ueberheide B, Barber CM, Shabanowitz J, Hunt DF, et al. Histone methyltransferases direct different degrees of methylation to define distinct chromatin domains. Mol Cell [Internet]. 2003 Dec;12(6):1591–8. Available from: http://www.ncbi.nlm.nih.gov/pubmed/14690610
71. Happel N, Doenecke D. Histone H1 and its isoforms: contribution to chromatin structure and function. Gene [Internet]. 2009 Feb 15;431(1–2):1–12. Available from: http://www.ncbi.nlm.nih.gov/pubmed/19059319
72. Sampath SC, Marazzi I, Yap KL, Sampath SC, Krutchinsky AN, Mecklenbräuker I, et al. Methylation of a histone mimic within the histone methyltransferase G9a regulates protein complex assembly. Mol Cell [Internet]. 2007 Aug 17;27(4):596–608. Available from: http://www.ncbi.nlm.nih.gov/pubmed/17707231
73. Tachibana M, Ueda J, Fukuda M, Takeda N, Ohta T, Iwanari H, et al. Histone methyltransferases G9a and GLP form heteromeric complexes and are both crucial for methylation of euchromatin at H3-K9. Genes Dev [Internet]. 2005 Apr 1;19(7):815–26. Available from: http://www.ncbi.nlm.nih.gov/pubmed/15774718
74. Feldman N, Gerson A, Fang J, Li E, Zhang Y, Shinkai Y, et al. G9a-mediated irreversible epigenetic inactivation of Oct-3/4 during early embryogenesis. Nat Cell Biol [Internet]. 2006 Feb;8(2):188–94. Available from:

http://www.ncbi.nlm.nih.gov/pubmed/16415856
75. Tschiersch B, Hofmann A, Krauss V, Dorn R, Korge G, Reuter G. The protein encoded by the Drosophila position-effect variegation suppressor gene Su(var)3-9 combines domains of antagonistic regulators of homeotic gene complexes. EMBO J [Internet]. 1994 Aug 15;13(16):3822–31. Available from: http://www.ncbi.nlm.nih.gov/pubmed/7915232
76. Peters AH, O'Carroll D, Scherthan H, Mechtler K, Sauer S, Schöfer C, et al. Loss of the Suv39h histone methyltransferases impairs mammalian heterochromatin and genome stability. Cell [Internet]. 2001 Nov 2;107(3):323–37. Available from: http://www.ncbi.nlm.nih.gov/pubmed/11701123
77. Lehnertz B, Ueda Y, Derijck AAHA, Braunschweig U, Perez-Burgos L, Kubicek S, et al. Suv39h-mediated histone H3 lysine 9 methylation directs DNA methylation to major satellite repeats at pericentric heterochromatin. Curr Biol [Internet]. 2003 Jul 15;13(14):1192–200. Available from: http://www.ncbi.nlm.nih.gov/pubmed/12867029
78. Rea S, Eisenhaber F, O'Carroll D, Strahl BD, Sun ZW, Schmid M, et al. Regulation of chromatin structure by site-specific histone H3 methyltransferases. Nature [Internet]. 2000 Aug 10;406(6796):593–9. Available from: http://www.ncbi.nlm.nih.gov/pubmed/10949293
79. Nakayama J, Rice JC, Strahl BD, Allis CD, Grewal SI. Role of histone H3 lysine 9 methylation in epigenetic control of heterochromatin assembly. Science [Internet]. 2001 Apr 6;292(5514):110–3. Available from: http://www.ncbi.nlm.nih.gov/pubmed/11283354
80. Nielsen SJ, Schneider R, Bauer UM, Bannister AJ, Morrison A, O'Carroll D, et al. Rb targets histone H3 methylation and HP1 to promoters. Nature [Internet]. 2001 Aug 2;412(6846):561–5. Available from: http://www.ncbi.nlm.nih.gov/pubmed/11484059
81. Blackburn ML, Chansky HA, Zielinska-Kwiatkowska A, Matsui Y, Yang L. Genomic structure and expression of the mouse ESET gene encoding an ERG-associated histone methyltransferase with a SET domain. Biochim Biophys Acta [Internet]. 2003 Oct 1;1629(1–3):8–14. Available from: http://www.ncbi.nlm.nih.gov/pubmed/14522075
82. Du Q, Luu P-L, Stirzaker C, Clark SJ. Methyl-CpG-binding domain proteins: readers of the epigenome. Epigenomics [Internet]. 2015;7(6):1051–73. Available from: http://www.ncbi.nlm.nih.gov/pubmed/25927341
83. Lohmann F, Loureiro J, Su H, Fang Q, Lei H, Lewis T, et al. KMT1E mediated H3K9 methylation is required for the maintenance of embryonic stem cells by repressing trophectoderm differentiation. Stem Cells [Internet]. 2010 Feb;28(2):201–12. Available from: http://www.ncbi.nlm.nih.gov/pubmed/20014010
84. Yuan P, Han J, Guo G, Orlov YL, Huss M, Loh Y-H, et al. Eset partners with Oct4 to restrict extraembryonic trophoblast lineage potential in embryonic stem cells. Genes Dev [Internet]. 2009 Nov 1;23(21):2507–20. Available from: http://www.ncbi.nlm.nih.gov/pubmed/19884257
85. An J, Zhang X, Qin J, Wan Y, Hu Y, Liu T, et al. The histone methyltransferase ESET is required for the survival of spermatogonial stem/progenitor cells in mice. Cell Death Dis [Internet]. 2014;5:e1196. Available from: http://www.ncbi.nlm.nih.gov/pubmed/24763053
86. Tan S-L, Nishi M, Ohtsuka T, Matsui T, Takemoto K, Kamio-Miura A, et al. Essential roles of the histone methyltransferase ESET in the epigenetic control of neural progenitor cells during development. Development [Internet]. 2012 Oct;139(20):3806–16. Available from: http://www.ncbi.nlm.nih.gov/pubmed/22991445
87. Matsui T, Leung D, Miyashita H, Maksakova IA, Miyachi H, Kimura H, et al. Proviral silencing in embryonic stem cells requires the histone methyltransferase ESET. Nature

[Internet]. 2010 Apr 8;464(7290):927–31. Available from: http://www.ncbi.nlm.nih.gov/pubmed/20164836
88. Kato M, Takemoto K, Shinkai Y. A somatic role for the histone methyltransferase Setdb1 in endogenous retrovirus silencing. Nat Commun [Internet]. 2018 Apr 27;9(1):1683. Available from: http://www.ncbi.nlm.nih.gov/pubmed/29703894
89. Wu SC, Zhang Y. Active DNA demethylation: many roads lead to Rome. Nat Rev Mol Cell Biol [Internet]. 2010 Sep;11(9):607–20. Available from: http://www.ncbi.nlm.nih.gov/pubmed/20683471
90. Ryan RF, Schultz DC, Ayyanathan K, Singh PB, Friedman JR, Fredericks WJ, et al. KAP-1 corepressor protein interacts and colocalizes with heterochromatic and euchromatic HP1 proteins: a potential role for Krüppel-associated box-zinc finger proteins in heterochromatin-mediated gene silencing. Mol Cell Biol [Internet]. 1999 Jun;19(6):4366–78. Available from: http://www.ncbi.nlm.nih.gov/pubmed/10330177
91. Iyengar S, Farnham PJ. KAP1 protein: an enigmatic master regulator of the genome. J Biol Chem [Internet]. 2011 Jul 29;286(30):26267–76. Available from: http://www.ncbi.nlm.nih.gov/pubmed/21652716
92. Schultz DC, Ayyanathan K, Negorev D, Maul GG, Rauscher FJ. SETDB1: a novel KAP-1-associated histone H3, lysine 9-specific methyltransferase that contributes to HP1-mediated silencing of euchromatic genes by KRAB zinc-finger proteins. Genes Dev [Internet]. 2002 Apr 15;16(8):919–32. Available from: http://www.ncbi.nlm.nih.gov/pubmed/11959841
93. Schultz DC, Friedman JR, Rauscher FJ. Targeting histone deacetylase complexes via KRAB-zinc finger proteins: the PHD and bromodomains of KAP-1 form a cooperative unit that recruits a novel isoform of the Mi-2alpha subunit of NuRD. Genes Dev [Internet]. 2001 Feb 15;15(4):428–43. Available from: http://www.ncbi.nlm.nih.gov/pubmed/11230151
94. Van Duyne R, Easley R, Wu W, Berro R, Pedati C, Klase Z, et al. Lysine methylation of HIV-1 Tat regulates transcriptional activity of the viral LTR. Retrovirology [Internet]. 2008 May 22;5:40. Available from: http://www.ncbi.nlm.nih.gov/pubmed/18498648
95. Cho S, Park JS, Kang Y-K. Regulated nuclear entry of over-expressed Setdb1. Genes Cells [Internet]. 2013 Aug;18(8):694–703. Available from: http://www.ncbi.nlm.nih.gov/pubmed/23782009
96. Otto A, Schmidt C, Luke G, Allen S, Valasek P, Muntoni F, et al. Canonical Wnt signalling induces satellite-cell proliferation during adult skeletal muscle regeneration. J Cell Sci [Internet]. 2008 Sep 1;121(Pt 17):2939–50. Available from: http://www.ncbi.nlm.nih.gov/pubmed/18697834
97. Maier VK, Feeney CM, Taylor JE, Creech AL, Qiao JW, Szanto A, et al. Functional Proteomic Analysis of Repressive Histone Methyltransferase Complexes Reveals ZNF518B as a G9A Regulator. Mol Cell Proteomics [Internet]. 2015 Jun;14(6):1435–46. Available from: http://www.ncbi.nlm.nih.gov/pubmed/25680957
98. Bian C, Chen Q, Yu X. The zinc finger proteins ZNF644 and WIZ regulate the G9a/GLP complex for gene repression. Elife [Internet]. 2015 Mar 19;4. Available from: http://www.ncbi.nlm.nih.gov/pubmed/25789554
99. Frietze S, O'Geen H, Blahnik KR, Jin VX, Farnham PJ. ZNF274 recruits the histone methyltransferase SETDB1 to the 3' ends of ZNF genes. PLoS One [Internet]. 2010 Dec 8;5(12):e15082. Available from: http://www.ncbi.nlm.nih.gov/pubmed/21170338
100. Reed-Inderbitzin E, Moreno-Miralles I, Vanden-Eynden SK, Xie J, Lutterbach B, Durst-Goodwin KL, et al. RUNX1 associates with histone deacetylases and SUV39H1 to repress transcription. Oncogene [Internet]. 2006 Sep 21;25(42):5777–86. Available

from: http://www.ncbi.nlm.nih.gov/pubmed/16652147
101. Chakraborty S, Sinha KK, Senyuk V, Nucifora G. SUV39H1 interacts with AML1 and abrogates AML1 transactivity. AML1 is methylated in vivo. Oncogene [Internet]. 2003 Aug 14;22(34):5229–37. Available from: http://www.ncbi.nlm.nih.gov/pubmed/12917624
102. Mozzetta C, Pontis J, Fritsch L, Robin P, Portoso M, Proux C, et al. The histone H3 lysine 9 methyltransferases G9a and GLP regulate polycomb repressive complex 2-mediated gene silencing. Mol Cell [Internet]. 2014 Jan 23;53(2):277–89. Available from: http://www.ncbi.nlm.nih.gov/pubmed/24389103
103. Fischle W, Franz H, Jacobs SA, Allis CD, Khorasanizadeh S. Specificity of the chromodomain Y chromosome family of chromodomains for lysine-methylated ARK(S/T) motifs. J Biol Chem [Internet]. 2008 Jul 11;283(28):19626–35. Available from: http://www.ncbi.nlm.nih.gov/pubmed/18450745
104. Mozzetta C, Pontis J, Ait-Si-Ali S. Functional Crosstalk Between Lysine Methyltransferases on Histone Substrates: The Case of G9A/GLP and Polycomb Repressive Complex 2. Antioxid Redox Signal [Internet]. 2015 Jun 1;22(16):1365–81. Available from: http://www.ncbi.nlm.nih.gov/pubmed/25365549
105. Fujita N, Watanabe S, Ichimura T, Tsuruzoe S, Shinkai Y, Tachibana M, et al. Methyl-CpG binding domain 1 (MBD1) interacts with the Suv39h1-HP1 heterochromatic complex for DNA methylation-based transcriptional repression. J Biol Chem [Internet]. 2003 Jun 27;278(26):24132–8. Available from: http://www.ncbi.nlm.nih.gov/pubmed/12711603
106. Wang H, An W, Cao R, Xia L, Erdjument-Bromage H, Chatton B, et al. mAM facilitates conversion by ESET of dimethyl to trimethyl lysine 9 of histone H3 to cause transcriptional repression. Mol Cell [Internet]. 2003 Aug;12(2):475–87. Available from: http://www.ncbi.nlm.nih.gov/pubmed/14536086
107. Li H, Rauch T, Chen Z-X, Szabó PE, Riggs AD, Pfeifer GP. The histone methyltransferase SETDB1 and the DNA methyltransferase DNMT3A interact directly and localize to promoters silenced in cancer cells. J Biol Chem [Internet]. 2006 Jul 14;281(28):19489–500. Available from: http://www.ncbi.nlm.nih.gov/pubmed/16682412
108. Murayama A, Ohmori K, Fujimura A, Minami H, Yasuzawa-Tanaka K, Kuroda T, et al. Epigenetic control of rDNA loci in response to intracellular energy status. Cell [Internet]. 2008 May 16;133(4):627–39. Available from: http://www.ncbi.nlm.nih.gov/pubmed/18485871
109. Ameyar-Zazoua M, Rachez C, Souidi M, Robin P, Fritsch L, Young R, et al. Argonaute proteins couple chromatin silencing to alternative splicing. Nat Struct Mol Biol [Internet]. 2012 Oct;19(10):998–1004. Available from: http://www.ncbi.nlm.nih.gov/pubmed/22961379
110. Huang J, Perez-Burgos L, Placek BJ, Sengupta R, Richter M, Dorsey JA, et al. Repression of p53 activity by Smyd2-mediated methylation. Nature [Internet]. 2006 Nov 30;444(7119):629–32. Available from: http://www.ncbi.nlm.nih.gov/pubmed/17108971
111. Hamamoto R, Saloura V, Nakamura Y. Critical roles of non-histone protein lysine methylation in human tumorigenesis. Nat Rev Cancer [Internet]. 2015 Feb;15(2):110–24. Available from: http://www.ncbi.nlm.nih.gov/pubmed/25614009
112. Zhang X, Tanaka K, Yan J, Li J, Peng D, Jiang Y, et al. Regulation of estrogen receptor α by histone methyltransferase SMYD2-mediated protein methylation. Proc Natl Acad Sci U S A [Internet]. 2013 Oct 22;110(43):17284–9. Available from: http://www.ncbi.nlm.nih.gov/pubmed/24101509

113. Saddic LA, West LE, Aslanian A, Yates JR, Rubin SM, Gozani O, et al. Methylation of the retinoblastoma tumor suppressor by SMYD2. J Biol Chem [Internet]. 2010 Nov 26;285(48):37733–40. Available from: http://www.ncbi.nlm.nih.gov/pubmed/20870719
114. Hamamoto R, Toyokawa G, Nakakido M, Ueda K, Nakamura Y. SMYD2-dependent HSP90 methylation promotes cancer cell proliferation by regulating the chaperone complex formation. Cancer Lett [Internet]. 2014 Aug 28;351(1):126–33. Available from: http://www.ncbi.nlm.nih.gov/pubmed/24880080
115. Nishioka K, Chuikov S, Sarma K, Erdjument-Bromage H, Allis CD, Tempst P, et al. Set9, a novel histone H3 methyltransferase that facilitates transcription by precluding histone tail modifications required for heterochromatin formation. Genes Dev [Internet]. 2002 Feb 15;16(4):479–89. Available from: http://www.ncbi.nlm.nih.gov/pubmed/11850410
116. Ivanov GS, Ivanova T, Kurash J, Ivanov A, Chuikov S, Gizatullin F, et al. Methylation-acetylation interplay activates p53 in response to DNA damage. Mol Cell Biol [Internet]. 2007 Oct;27(19):6756–69. Available from: http://www.ncbi.nlm.nih.gov/pubmed/17646389
117. Kurash JK, Lei H, Shen Q, Marston WL, Granda BW, Fan H, et al. Methylation of p53 by Set7/9 mediates p53 acetylation and activity in vivo. Mol Cell [Internet]. 2008 Feb 15;29(3):392–400. Available from: http://www.ncbi.nlm.nih.gov/pubmed/18280244
118. Yang X-D, Huang B, Li M, Lamb A, Kelleher NL, Chen L-F. Negative regulation of NF-kappaB action by Set9-mediated lysine methylation of the RelA subunit. EMBO J [Internet]. 2009 Apr 22;28(8):1055–66. Available from: http://www.ncbi.nlm.nih.gov/pubmed/19262565
119. Ea C-K, Baltimore D. Regulation of NF-kappaB activity through lysine monomethylation of p65. Proc Natl Acad Sci U S A [Internet]. 2009 Nov 10;106(45):18972–7. Available from: http://www.ncbi.nlm.nih.gov/pubmed/19864627
120. Munro S, Khaire N, Inche A, Carr S, La Thangue NB. Lysine methylation regulates the pRb tumour suppressor protein. Oncogene [Internet]. 2010 Apr 22;29(16):2357–67. Available from: http://www.ncbi.nlm.nih.gov/pubmed/20140018
121. Wang D, Zhou J, Liu X, Lu D, Shen C, Du Y, et al. Methylation of SUV39H1 by SET7/9 results in heterochromatin relaxation and genome instability. Proc Natl Acad Sci U S A [Internet]. 2013 Apr 2;110(14):5516–21. Available from: http://www.ncbi.nlm.nih.gov/pubmed/23509280
122. Mulligan P, Westbrook TF, Ottinger M, Pavlova N, Chang B, Macia E, et al. CDYL bridges REST and histone methyltransferases for gene repression and suppression of cellular transformation. Mol Cell [Internet]. 2008 Dec 5;32(5):718–26. Available from: http://www.ncbi.nlm.nih.gov/pubmed/19061646
123. Huang J, Dorsey J, Chuikov S, Pérez-Burgos L, Zhang X, Jenuwein T, et al. G9a and Glp methylate lysine 373 in the tumor suppressor p53. J Biol Chem [Internet]. 2010 Mar 26;285(13):9636–41. Available from: http://www.ncbi.nlm.nih.gov/pubmed/20118233
124. Jacobs SA, Khorasanizadeh S. Structure of HP1 chromodomain bound to a lysine 9-methylated histone H3 tail. Science [Internet]. 2002 Mar 15;295(5562):2080–3. Available from: http://www.ncbi.nlm.nih.gov/pubmed/11859155
125. Chin HG, Estève P-O, Pradhan M, Benner J, Patnaik D, Carey MF, et al. Automethylation of G9a and its implication in wider substrate specificity and HP1 binding. Nucleic Acids Res [Internet]. 2007;35(21):7313–23. Available from: http://www.ncbi.nlm.nih.gov/pubmed/17962312
126. Rathert P, Dhayalan A, Murakami M, Zhang X, Tamas R, Jurkowska R, et al. Protein

lysine methyltransferase G9a acts on non-histone targets. Nat Chem Biol [Internet]. 2008 Jun;4(6):344–6. Available from: http://www.ncbi.nlm.nih.gov/pubmed/18438403
127. Ling BMT, Bharathy N, Chung T-K, Kok WK, Li S, Tan YH, et al. Lysine methyltransferase G9a methylates the transcription factor MyoD and regulates skeletal muscle differentiation. Proc Natl Acad Sci U S A [Internet]. 2012 Jan 17;109(3):841–6. Available from: http://www.ncbi.nlm.nih.gov/pubmed/22215600
128. Choi J, Jang H, Kim H, Lee J-H, Kim S-T, Cho E-J, et al. Modulation of lysine methylation in myocyte enhancer factor 2 during skeletal muscle cell differentiation. Nucleic Acids Res [Internet]. 2014 Jan;42(1):224–34. Available from: http://www.ncbi.nlm.nih.gov/pubmed/24078251
129. Leutz A, Pless O, Lappe M, Dittmar G, Kowenz-Leutz E. Crosstalk between phosphorylation and multi-site arginine/lysine methylation in C/EBPs. Transcription [Internet]. 2(1):3–8. Available from: http://www.ncbi.nlm.nih.gov/pubmed/21326902
130. Pless O, Kowenz-Leutz E, Knoblich M, Lausen J, Beyermann M, Walsh MJ, et al. G9a-mediated lysine methylation alters the function of CCAAT/enhancer-binding protein-beta. J Biol Chem [Internet]. 2008 Sep 26;283(39):26357–63. Available from: http://www.ncbi.nlm.nih.gov/pubmed/18647749
131. Yang L, Lin C, Liu W, Zhang J, Ohgi KA, Grinstein JD, et al. ncRNA- and Pc2 methylation-dependent gene relocation between nuclear structures mediates gene activation programs. Cell [Internet]. 2011 Nov 11;147(4):773–88. Available from: http://www.ncbi.nlm.nih.gov/pubmed/22078878
132. Iglesias N, Currie MA, Jih G, Paulo JA, Siuti N, Kalocsay M, et al. Automethylation-induced conformational switch in Clr4 (Suv39h) maintains epigenetic stability. Nature [Internet]. 2018 Jul 23; Available from: http://www.ncbi.nlm.nih.gov/pubmed/30051891
133. Piao L, Nakakido M, Suzuki T, Dohmae N, Nakamura Y, Hamamoto R. Automethylation of SUV39H2, an oncogenic histone lysine methyltransferase, regulates its binding affinity to substrate proteins. Oncotarget [Internet]. 2016 Apr 19;7(16):22846–56. Available from: http://www.ncbi.nlm.nih.gov/pubmed/26988914
134. Ryu H, Lee J, Hagerty SW, Soh BY, McAlpin SE, Cormier KA, et al. ESET/SETDB1 gene expression and histone H3 (K9) trimethylation in Huntington's disease. Proc Natl Acad Sci U S A [Internet]. 2006 Dec 12;103(50):19176–81. Available from: http://www.ncbi.nlm.nih.gov/pubmed/17142323
135. Lee J, Hwang YJ, Ryu H, Kowall NW, Ryu H. Nucleolar dysfunction in Huntington's disease. Biochim Biophys Acta [Internet]. 2014 Jun;1842(6):785–90. Available from: http://www.ncbi.nlm.nih.gov/pubmed/24184605
136. Chen T, Li E. Structure and function of eukaryotic DNA methyltransferases. Curr Top Dev Biol [Internet]. 2004;60:55–89. Available from: http://www.ncbi.nlm.nih.gov/pubmed/15094296
137. Tirado-Magallanes R, Rebbani K, Lim R, Pradhan S, Benoukraf T. Whole genome DNA methylation: beyond genes silencing. Oncotarget [Internet]. 2017 Jan 17;8(3):5629–37. Available from: http://www.ncbi.nlm.nih.gov/pubmed/27895318
138. Hirasawa R, Chiba H, Kaneda M, Tajima S, Li E, Jaenisch R, et al. Maternal and zygotic Dnmt1 are necessary and sufficient for the maintenance of DNA methylation imprints during preimplantation development. Genes Dev [Internet]. 2008 Jun 15;22(12):1607–16. Available from: http://www.ncbi.nlm.nih.gov/pubmed/18559477
139. Ishida M, Moore GE. The role of imprinted genes in humans. Mol Aspects Med [Internet]. 34(4):826–40. Available from: http://www.ncbi.nlm.nih.gov/pubmed/22771538
140. Ito S, D'Alessio AC, Taranova O V, Hong K, Sowers LC, Zhang Y. Role of Tet

proteins in 5mC to 5hmC conversion, ES-cell self-renewal and inner cell mass specification. Nature [Internet]. 2010 Aug 26;466(7310):1129–33. Available from: http://www.ncbi.nlm.nih.gov/pubmed/20639862
141. Ruzov A, Tsenkina Y, Serio A, Dudnakova T, Fletcher J, Bai Y, et al. Lineage-specific distribution of high levels of genomic 5-hydroxymethylcytosine in mammalian development. Cell Res [Internet]. 2011 Sep;21(9):1332–42. Available from: http://www.ncbi.nlm.nih.gov/pubmed/21747414
142. Ficz G, Branco MR, Seisenberger S, Santos F, Krueger F, Hore TA, et al. Dynamic regulation of 5-hydroxymethylcytosine in mouse ES cells and during differentiation. Nature [Internet]. 2011 May 19;473(7347):398–402. Available from: http://www.ncbi.nlm.nih.gov/pubmed/21460836
143. Leung DC, Dong KB, Maksakova IA, Goyal P, Appanah R, Lee S, et al. Lysine methyltransferase G9a is required for de novo DNA methylation and the establishment, but not the maintenance, of proviral silencing. Proc Natl Acad Sci U S A [Internet]. 2011 Apr 5;108(14):5718–23. Available from: http://www.ncbi.nlm.nih.gov/pubmed/21427230
144. Dong KB, Maksakova IA, Mohn F, Leung D, Appanah R, Lee S, et al. DNA methylation in ES cells requires the lysine methyltransferase G9a but not its catalytic activity. EMBO J [Internet]. 2008 Oct 22;27(20):2691–701. Available from: http://www.ncbi.nlm.nih.gov/pubmed/18818693
145. Epsztejn-Litman S, Feldman N, Abu-Remaileh M, Shufaro Y, Gerson A, Ueda J, et al. De novo DNA methylation promoted by G9a prevents reprogramming of embryonically silenced genes. Nat Struct Mol Biol [Internet]. 2008 Nov;15(11):1176–83. Available from: http://www.ncbi.nlm.nih.gov/pubmed/18953337
146. Arita K, Isogai S, Oda T, Unoki M, Sugita K, Sekiyama N, et al. Recognition of modification status on a histone H3 tail by linked histone reader modules of the epigenetic regulator UHRF1. Proc Natl Acad Sci U S A [Internet]. 2012 Aug 7;109(32):12950–5. Available from: http://www.ncbi.nlm.nih.gov/pubmed/22837395
147. Rothbart SB, Dickson BM, Ong MS, Krajewski K, Houliston S, Kireev DB, et al. Multivalent histone engagement by the linked tandem Tudor and PHD domains of UHRF1 is required for the epigenetic inheritance of DNA methylation. Genes Dev [Internet]. 2013 Jun 1;27(11):1288–98. Available from: http://www.ncbi.nlm.nih.gov/pubmed/23752590
148. Girardot M, Cavaillé J, Feil R. Small regulatory RNAs controlled by genomic imprinting and their contribution to human disease. Epigenetics [Internet]. 2012 Dec 1;7(12):1341–8. Available from: http://www.ncbi.nlm.nih.gov/pubmed/23154539
149. Barlow DP. Genomic imprinting: a mammalian epigenetic discovery model. Annu Rev Genet [Internet]. 2011;45:379–403. Available from: http://www.ncbi.nlm.nih.gov/pubmed/21942369
150. Sanli I, Feil R. Chromatin mechanisms in the developmental control of imprinted gene expression. Int J Biochem Cell Biol [Internet]. 2015 Oct;67:139–47. Available from: http://www.ncbi.nlm.nih.gov/pubmed/25908531
151. Barlow DP, Bartolomei MS. Genomic imprinting in mammals. Cold Spring Harb Perspect Biol [Internet]. 2014 Feb 1;6(2). Available from: http://www.ncbi.nlm.nih.gov/pubmed/24492710
152. Kota SK, Feil R. Epigenetic transitions in germ cell development and meiosis. Dev Cell [Internet]. 2010 Nov 16;19(5):675–86. Available from: http://www.ncbi.nlm.nih.gov/pubmed/21074718
153. Li X, Ito M, Zhou F, Youngson N, Zuo X, Leder P, et al. A maternal-zygotic effect gene, Zfp57, maintains both maternal and paternal imprints. Dev Cell [Internet]. 2008

Oct;15(4):547–57. Available from: http://www.ncbi.nlm.nih.gov/pubmed/18854139
154. Reese KJ, Lin S, Verona RI, Schultz RM, Bartolomei MS. Maintenance of paternal methylation and repression of the imprinted H19 gene requires MBD3. PLoS Genet [Internet]. 2007 Aug;3(8):e137. Available from: http://www.ncbi.nlm.nih.gov/pubmed/17708683
155. Ma P, Lin S, Bartolomei MS, Schultz RM. Metastasis tumor antigen 2 (MTA2) is involved in proper imprinted expression of H19 and Peg3 during mouse preimplantation development. Biol Reprod [Internet]. 2010 Dec;83(6):1027–35. Available from: http://www.ncbi.nlm.nih.gov/pubmed/20720167
156. Wu M-Y, Tsai T-F, Beaudet AL. Deficiency of Rbbp1/Arid4a and Rbbp1l1/Arid4b alters epigenetic modifications and suppresses an imprinting defect in the PWS/AS domain. Genes Dev [Internet]. 2006 Oct 15;20(20):2859–70. Available from: http://www.ncbi.nlm.nih.gov/pubmed/17043311
157. Pauler FM, Barlow DP, Hudson QJ. Mechanisms of long range silencing by imprinted macro non-coding RNAs. Curr Opin Genet Dev [Internet]. 2012 Jun;22(3):283–9. Available from: http://www.ncbi.nlm.nih.gov/pubmed/22386265
158. Pandey RR, Mondal T, Mohammad F, Enroth S, Redrup L, Komorowski J, et al. Kcnq1ot1 antisense noncoding RNA mediates lineage-specific transcriptional silencing through chromatin-level regulation. Mol Cell [Internet]. 2008 Oct 24;32(2):232–46. Available from: http://www.ncbi.nlm.nih.gov/pubmed/18951091
159. Li Q, Su Z, Xu X, Liu G, Song X, Wang R, et al. AS1DHRS4, a head-to-head natural antisense transcript, silences the DHRS4 gene cluster in cis and trans. Proc Natl Acad Sci U S A [Internet]. 2012 Aug 28;109(35):14110–5. Available from: http://www.ncbi.nlm.nih.gov/pubmed/22891334
160. Nagano T, Mitchell JA, Sanz LA, Pauler FM, Ferguson-Smith AC, Feil R, et al. The Air noncoding RNA epigenetically silences transcription by targeting G9a to chromatin. Science [Internet]. 2008 Dec 12;322(5908):1717–20. Available from: http://www.ncbi.nlm.nih.gov/pubmed/18988810
161. Kota SK, Llères D, Bouschet T, Hirasawa R, Marchand A, Begon-Pescia C, et al. ICR noncoding RNA expression controls imprinting and DNA replication at the Dlk1-Dio3 domain. Dev Cell [Internet]. 2014 Oct 13;31(1):19–33. Available from: http://www.ncbi.nlm.nih.gov/pubmed/25263792
162. Deininger PL, Batzer MA. Mammalian retroelements. Genome Res [Internet]. 2002 Oct;12(10):1455–65. Available from: http://www.ncbi.nlm.nih.gov/pubmed/12368238
163. Faulkner GJ, Carninci P. Altruistic functions for selfish DNA. Cell Cycle [Internet]. 2009 Sep 15;8(18):2895–900. Available from: http://www.ncbi.nlm.nih.gov/pubmed/19736519
164. Slotkin RK, Martienssen R. Transposable elements and the epigenetic regulation of the genome. Nat Rev Genet [Internet]. 2007 Apr;8(4):272–85. Available from: http://www.ncbi.nlm.nih.gov/pubmed/17363976
165. Böhne A, Brunet F, Galiana-Arnoux D, Schultheis C, Volff J-N. Transposable elements as drivers of genomic and biological diversity in vertebrates. Chromosome Res [Internet]. 2008;16(1):203–15. Available from: http://www.ncbi.nlm.nih.gov/pubmed/18293113
166. Cordaux R, Batzer MA. The impact of retrotransposons on human genome evolution. Nat Rev Genet [Internet]. 2009 Oct;10(10):691–703. Available from: http://www.ncbi.nlm.nih.gov/pubmed/19763152
167. Levin HL, Moran J V. Dynamic interactions between transposable elements and their hosts. Nat Rev Genet [Internet]. 2011 Aug 18;12(9):615–27. Available from: http://www.ncbi.nlm.nih.gov/pubmed/21850042

168. Garcia-Perez JL, Widmann TJ, Adams IR. The impact of transposable elements on mammalian development. Development [Internet]. 2016;143(22):4101–14. Available from: http://www.ncbi.nlm.nih.gov/pubmed/27875251
169. Mager DL, Stoye JP. Mammalian Endogenous Retroviruses. Microbiol Spectr [Internet]. 2015 Feb;3(1):MDNA3-0009-2014. Available from: http://www.ncbi.nlm.nih.gov/pubmed/26104559
170. Boeke J, Stoye J. Retrotransposons, Endogenous Retroviruses, and the Evolution of Retroelements [Internet]. Retroviruses. 1997. Available from: http://www.ncbi.nlm.nih.gov/pubmed/21433351
171. Ribet D, Harper F, Dupressoir A, Dewannieux M, Pierron G, Heidmann T. An infectious progenitor for the murine IAP retrotransposon: emergence of an intracellular genetic parasite from an ancient retrovirus. Genome Res [Internet]. 2008 Apr;18(4):597–609. Available from: http://www.ncbi.nlm.nih.gov/pubmed/18256233
172. Ribet D, Harper F, Dewannieux M, Pierron G, Heidmann T. Murine MusD retrotransposon: structure and molecular evolution of an "intracellularized" retrovirus. J Virol [Internet]. 2007 Feb;81(4):1888–98. Available from: http://www.ncbi.nlm.nih.gov/pubmed/17151128
173. Friedli M, Trono D. The developmental control of transposable elements and the evolution of higher species. Annu Rev Cell Dev Biol [Internet]. 2015;31:429–51. Available from: http://www.ncbi.nlm.nih.gov/pubmed/26393776
174. Marchi E, Kanapin A, Magiorkinis G, Belshaw R. Unfixed endogenous retroviral insertions in the human population. J Virol [Internet]. 2014 Sep 1;88(17):9529–37. Available from: http://www.ncbi.nlm.nih.gov/pubmed/24920817
175. Kuff EL, Lueders KK. The intracisternal A-particle gene family: structure and functional aspects. Adv Cancer Res [Internet]. 1988;51:183–276. Available from: http://www.ncbi.nlm.nih.gov/pubmed/3146900
176. Ribet D, Dewannieux M, Heidmann T. An active murine transposon family pair: retrotransposition of "master" MusD copies and ETn trans-mobilization. Genome Res [Internet]. 2004 Nov;14(11):2261–7. Available from: http://www.ncbi.nlm.nih.gov/pubmed/15479948
177. Peaston AE, Evsikov A V, Graber JH, de Vries WN, Holbrook AE, Solter D, et al. Retrotransposons regulate host genes in mouse oocytes and preimplantation embryos. Dev Cell [Internet]. 2004 Oct;7(4):597–606. Available from: http://www.ncbi.nlm.nih.gov/pubmed/15469847
178. Medstrand P, van de Lagemaat LN, Mager DL. Retroelement distributions in the human genome: variations associated with age and proximity to genes. Genome Res [Internet]. 2002 Oct;12(10):1483–95. Available from: http://www.ncbi.nlm.nih.gov/pubmed/12368240
179. Beck CR, Collier P, Macfarlane C, Malig M, Kidd JM, Eichler EE, et al. LINE-1 retrotransposition activity in human genomes. Cell [Internet]. 2010 Jun 25;141(7):1159–70. Available from: http://www.ncbi.nlm.nih.gov/pubmed/20602998
180. Elbarbary RA, Lucas BA, Maquat LE. Retrotransposons as regulators of gene expression. Science [Internet]. 2016 Feb 12;351(6274):aac7247. Available from: http://www.ncbi.nlm.nih.gov/pubmed/26912865
181. Luan DD, Eickbush TH. RNA template requirements for target DNA-primed reverse transcription by the R2 retrotransposable element. Mol Cell Biol [Internet]. 1995 Jul;15(7):3882–91. Available from: http://www.ncbi.nlm.nih.gov/pubmed/7540721
182. Leung DC, Lorincz MC. Silencing of endogenous retroviruses: when and why do histone marks predominate? Trends Biochem Sci [Internet]. 2012 Apr;37(4):127–33. Available from: http://www.ncbi.nlm.nih.gov/pubmed/22178137

183. Rowe HM, Trono D. Dynamic control of endogenous retroviruses during development. Virology [Internet]. 2011 Mar 15;411(2):273–87. Available from: http://www.ncbi.nlm.nih.gov/pubmed/21251689
184. Collins PL, Kyle KE, Egawa T, Shinkai Y, Oltz EM. The histone methyltransferase SETDB1 represses endogenous and exogenous retroviruses in B lymphocytes. Proc Natl Acad Sci U S A [Internet]. 2015 Jul 7;112(27):8367–72. Available from: http://www.ncbi.nlm.nih.gov/pubmed/26100872
185. Liu S, Brind'Amour J, Karimi MM, Shirane K, Bogutz A, Lefebvre L, et al. Setdb1 is required for germline development and silencing of H3K9me3-marked endogenous retroviruses in primordial germ cells. Genes Dev [Internet]. 2014 Sep 15;28(18):2041–55. Available from: http://www.ncbi.nlm.nih.gov/pubmed/25228647
186. Göke J, Ng HH. CTRL+INSERT: retrotransposons and their contribution to regulation and innovation of the transcriptome. EMBO Rep [Internet]. 2016;17(8):1131–44. Available from: http://www.ncbi.nlm.nih.gov/pubmed/27402545
187. Rowe HM, Friedli M, Offner S, Verp S, Mesnard D, Marquis J, et al. De novo DNA methylation of endogenous retroviruses is shaped by KRAB-ZFPs/KAP1 and ESET. Development [Internet]. 2013 Feb 1;140(3):519–29. Available from: http://www.ncbi.nlm.nih.gov/pubmed/23293284
188. Leung D, Du T, Wagner U, Xie W, Lee AY, Goyal P, et al. Regulation of DNA methylation turnover at LTR retrotransposons and imprinted loci by the histone methyltransferase Setdb1. Proc Natl Acad Sci U S A [Internet]. 2014 May 6;111(18):6690–5. Available from: http://www.ncbi.nlm.nih.gov/pubmed/24757056
189. Castro-Diaz N, Ecco G, Coluccio A, Kapopoulou A, Yazdanpanah B, Friedli M, et al. Evolutionally dynamic L1 regulation in embryonic stem cells. Genes Dev [Internet]. 2014 Jul 1;28(13):1397–409. Available from: http://www.ncbi.nlm.nih.gov/pubmed/24939876
190. Turelli P, Castro-Diaz N, Marzetta F, Kapopoulou A, Raclot C, Duc J, et al. Interplay of TRIM28 and DNA methylation in controlling human endogenous retroelements. Genome Res [Internet]. 2014 Aug;24(8):1260–70. Available from: http://www.ncbi.nlm.nih.gov/pubmed/24879559
191. Maksakova IA, Thompson PJ, Goyal P, Jones SJ, Singh PB, Karimi MM, et al. Distinct roles of KAP1, HP1 and G9a/GLP in silencing of the two-cell-specific retrotransposon MERVL in mouse ES cells. Epigenetics Chromatin [Internet]. 2013 Jun 4;6(1):15. Available from: http://www.ncbi.nlm.nih.gov/pubmed/23735015
192. Chiba T, Saito T, Yuki K, Zen Y, Koide S, Kanogawa N, et al. Histone lysine methyltransferase SUV39H1 is a potent target for epigenetic therapy of hepatocellular carcinoma. Int J cancer [Internet]. 2015 Jan 15;136(2):289–98. Available from: http://www.ncbi.nlm.nih.gov/pubmed/24844570
193. Khanal P, Kim G, Lim S-C, Yun H-J, Lee KY, Choi H-K, et al. Prolyl isomerase Pin1 negatively regulates the stability of SUV39H1 to promote tumorigenesis in breast cancer. FASEB J [Internet]. 2013 Nov;27(11):4606–18. Available from: http://www.ncbi.nlm.nih.gov/pubmed/23934277
194. Dworkin AM, Huang TH-M, Toland AE. Epigenetic alterations in the breast: Implications for breast cancer detection, prognosis and treatment. Semin Cancer Biol [Internet]. 2009 Jun;19(3):165–71. Available from: http://www.ncbi.nlm.nih.gov/pubmed/19429480
195. Tan J, Yang X, Zhuang L, Jiang X, Chen W, Lee PL, et al. Pharmacologic disruption of Polycomb-repressive complex 2-mediated gene repression selectively induces apoptosis in cancer cells. Genes Dev [Internet]. 2007 May 1;21(9):1050–63. Available from: http://www.ncbi.nlm.nih.gov/pubmed/17437993

196. Casciello F, Windloch K, Gannon F, Lee JS. Functional Role of G9a Histone Methyltransferase in Cancer. Front Immunol [Internet]. 2015;6:487. Available from: http://www.ncbi.nlm.nih.gov/pubmed/26441991
197. Zhang H, Cai K, Wang J, Wang X, Cheng K, Shi F, et al. MiR-7, inhibited indirectly by lincRNA HOTAIR, directly inhibits SETDB1 and reverses the EMT of breast cancer stem cells by downregulating the STAT3 pathway. Stem Cells [Internet]. 2014 Nov;32(11):2858–68. Available from: http://www.ncbi.nlm.nih.gov/pubmed/25070049
198. Regina C, Compagnone M, Peschiaroli A, Lena A, Annicchiarico-Petruzzelli M, Piro MC, et al. Setdb1, a novel interactor of ΔNp63, is involved in breast tumorigenesis. Oncotarget [Internet]. 2016 Jan 31; Available from: http://www.ncbi.nlm.nih.gov/pubmed/26840455
199. Guo A, Gu H, Zhou J, Mulhern D, Wang Y, Lee KA, et al. Immunoaffinity enrichment and mass spectrometry analysis of protein methylation. Mol Cell Proteomics [Internet]. 2014 Jan;13(1):372–87. Available from: http://www.ncbi.nlm.nih.gov/pubmed/24129315
200. Karimi MM, Goyal P, Maksakova IA, Bilenky M, Leung D, Tang JX, et al. DNA methylation and SETDB1/H3K9me3 regulate predominantly distinct sets of genes, retroelements, and chimeric transcripts in mESCs. Cell Stem Cell [Internet]. 2011 Jun 3;8(6):676–87. Available from: http://www.ncbi.nlm.nih.gov/pubmed/21624812
201. Xu S, Grullon S, Ge K, Peng W. Spatial clustering for identification of ChIP-enriched regions (SICER) to map regions of histone methylation patterns in embryonic stem cells. Methods Mol Biol [Internet]. 2014;1150:97–111. Available from: http://www.ncbi.nlm.nih.gov/pubmed/24743992
202. Peters AHFM, Kubicek S, Mechtler K, O'Sullivan RJ, Derijck AAHA, Perez-Burgos L, et al. Partitioning and plasticity of repressive histone methylation states in mammalian chromatin. Mol Cell [Internet]. 2003 Dec;12(6):1577–89. Available from: http://www.ncbi.nlm.nih.gov/pubmed/14690609
203. Walter M, Teissandier A, Pérez-Palacios R, Bourc'his D. An epigenetic switch ensures transposon repression upon dynamic loss of DNA methylation in embryonic stem cells. Elife [Internet]. 2016 Jan 27;5. Available from: http://www.ncbi.nlm.nih.gov/pubmed/26814573
204. Kourmouli N, Sun Y, van der Sar S, Singh PB, Brown JP. Epigenetic regulation of mammalian pericentric heterochromatin in vivo by HP1. Biochem Biophys Res Commun [Internet]. 2005 Nov 25;337(3):901–7. Available from: http://www.ncbi.nlm.nih.gov/pubmed/16213461
205. Deribe YL, Pawson T, Dikic I. Post-translational modifications in signal integration. Nat Struct Mol Biol [Internet]. 2010 Jun;17(6):666–72. Available from: http://www.ncbi.nlm.nih.gov/pubmed/20495563
206. AMBLER RP, REES MW. Epsilon-N-Methyl-lysine in bacterial flagellar protein. Nature [Internet]. 1959 Jul 4;184:56–7. Available from: http://www.ncbi.nlm.nih.gov/pubmed/13793118
207. Murn J, Shi Y. The winding path of protein methylation research: milestones and new frontiers. Nat Rev Mol Cell Biol [Internet]. 2017;18(8):517–27. Available from: http://www.ncbi.nlm.nih.gov/pubmed/28512349
208. Fischle W, Wang Y, Allis CD. Binary switches and modification cassettes in histone biology and beyond. Nature [Internet]. 2003 Oct 2;425(6957):475–9. Available from: http://www.ncbi.nlm.nih.gov/pubmed/14523437
209. Qin R, Cao S, Lyu T, Qi C, Zhang W, Wang Y. CDYL Deficiency Disrupts Neuronal Migration and Increases Susceptibility to Epilepsy. Cell Rep [Internet]. 2017;18(2):380–90. Available from: http://www.ncbi.nlm.nih.gov/pubmed/28076783

210. Fischle W, Wang Y, Jacobs SA, Kim Y, Allis CD, Khorasanizadeh S. Molecular basis for the discrimination of repressive methyl-lysine marks in histone H3 by Polycomb and HP1 chromodomains. Genes Dev [Internet]. 2003 Aug 1;17(15):1870–81. Available from: http://www.ncbi.nlm.nih.gov/pubmed/12897054
211. Thompson PJ, Macfarlan TS, Lorincz MC. Long Terminal Repeats: From Parasitic Elements to Building Blocks of the Transcriptional Regulatory Repertoire. Mol Cell [Internet]. 2016;62(5):766–76. Available from: http://www.ncbi.nlm.nih.gov/pubmed/27259207
212. Hutnick LK, Huang X, Loo T-C, Ma Z, Fan G. Repression of retrotransposal elements in mouse embryonic stem cells is primarily mediated by a DNA methylation-independent mechanism. J Biol Chem [Internet]. 2010 Jul 2;285(27):21082–91. Available from: http://www.ncbi.nlm.nih.gov/pubmed/20404320
213. Seisenberger S, Peat JR, Reik W. Conceptual links between DNA methylation reprogramming in the early embryo and primordial germ cells. Curr Opin Cell Biol [Internet]. 2013 Jun;25(3):281–8. Available from: http://www.ncbi.nlm.nih.gov/pubmed/23510682
214. Leeb M, Pasini D, Novatchkova M, Jaritz M, Helin K, Wutz A. Polycomb complexes act redundantly to repress genomic repeats and genes. Genes Dev [Internet]. 2010 Feb 1;24(3):265–76. Available from: http://www.ncbi.nlm.nih.gov/pubmed/20123906
215. Wolf G, Macfarlan TS. Revealing the complexity of retroviral repression. Cell [Internet]. 2015 Sep 24;163(1):30–2. Available from: http://www.ncbi.nlm.nih.gov/pubmed/26406368
216. Maksakova IA, Goyal P, Bullwinkel J, Brown JP, Bilenky M, Mager DL, et al. H3K9me3-binding proteins are dispensable for SETDB1/H3K9me3-dependent retroviral silencing. Epigenetics Chromatin [Internet]. 2011 Jul 20;4(1):12. Available from: http://www.ncbi.nlm.nih.gov/pubmed/21774827
217. Jamieson K, Wiles ET, McNaught KJ, Sidoli S, Leggett N, Shao Y, et al. Loss of HP1 causes depletion of H3K27me3 from facultative heterochromatin and gain of H3K27me2 at constitutive heterochromatin. Genome Res [Internet]. 2016 Jan;26(1):97–107. Available from: http://www.ncbi.nlm.nih.gov/pubmed/26537359
218. Zuo X, Sheng J, Lau H-T, McDonald CM, Andrade M, Cullen DE, et al. Zinc finger protein ZFP57 requires its co-factor to recruit DNA methyltransferases and maintains DNA methylation imprint in embryonic stem cells via its transcriptional repression domain. J Biol Chem [Internet]. 2012 Jan 13;287(3):2107–18. Available from: http://www.ncbi.nlm.nih.gov/pubmed/22144682
219. Marks H, Kalkan T, Menafra R, Denissov S, Jones K, Hofemeister H, et al. The transcriptional and epigenomic foundations of ground state pluripotency. Cell [Internet]. 2012 Apr 27;149(3):590–604. Available from: http://www.ncbi.nlm.nih.gov/pubmed/22541430
220. Pathak R, Feil R. Environmental effects on chromatin repression at imprinted genes and endogenous retroviruses. Curr Opin Chem Biol [Internet]. 2018 May 23;45:139–47. Available from: http://www.ncbi.nlm.nih.gov/pubmed/29800804
221. Zhang Y, Yang X, Gui B, Xie G, Zhang D, Shang Y, et al. Corepressor protein CDYL functions as a molecular bridge between polycomb repressor complex 2 and repressive chromatin mark trimethylated histone lysine 27. J Biol Chem [Internet]. 2011 Dec 9;286(49):42414–25. Available from: http://www.ncbi.nlm.nih.gov/pubmed/22009739
222. Liu Y, Liu S, Yuan S, Yu H, Zhang Y, Yang X, et al. Chromodomain protein CDYL is required for transmission/restoration of repressive histone marks. J Mol Cell Biol [Internet]. 2017 Jun 1;9(3):178–94. Available from: http://www.ncbi.nlm.nih.gov/pubmed/28402439

223. Matouk IJ, Halle D, Gilon M, Hochberg A. The non-coding RNAs of the H19-IGF2 imprinted loci: a focus on biological roles and therapeutic potential in Lung Cancer. J Transl Med [Internet]. 2015 Apr 9;13:113. Available from: http://www.ncbi.nlm.nih.gov/pubmed/25884481
224. Beyer S, Pontis J, Schirwis E, Battisti V, Rudolf A, Le Grand F, et al. Canonical Wnt signalling regulates nuclear export of Setdb1 during skeletal muscle terminal differentiation. Cell Discov [Internet]. 2016;2:16037. Available from: http://www.ncbi.nlm.nih.gov/pubmed/27790377
225. Mercatelli N, Beldiman C, Brouard T, Nairismägi M, Kratassiouk G, Largitte L, et al. Mir-205 modulates acinar size and morphology of transformed breast epithelial cells. OncomiRs. 2012;2–9.
226. Jiang Y, Loh Y-HE, Rajarajan P, Hirayama T, Liao W, Kassim BS, et al. The methyltransferase SETDB1 regulates a large neuron-specific topological chromatin domain. Nat Genet [Internet]. 2017 Aug;49(8):1239–50. Available from: http://www.ncbi.nlm.nih.gov/pubmed/28671686
227. Walker CJ, Miranda MA, O'Hern MJ, McElroy JP, Coombes KR, Bundschuh R, et al. Patterns of CTCF and ZFHX3 Mutation and Associated Outcomes in Endometrial Cancer. J Natl Cancer Inst [Internet]. 2015 Nov;107(11). Available from: http://www.ncbi.nlm.nih.gov/pubmed/26330387