Supplementary information

Supplementary figure 1. Validation of the *KMT1F* tools for depletion and detection.
A. In order to investigate the distribution of KMT1F during the cell cycle, including mitosis, a clone derived from HeLa cells overexpressing a flagged form of KMT1F was analyzed by indirect immunofluorescence and confocal microscopy. The untransfected HeLa cells were used as a control and show no staining when immunofloresence is performed with an anti-Flag. However, in the HeLa 17N clone overexpressing a flagged KMT1F form, an intense staining is observed in intephase nuclei that overlaps at least partially with TOTO-3 intense regions while the signal does not overlap with mitotic chromosomes. **B.** The specificity of our rabbit polyclonal anti-KMT1F antibody was tested on extracts from 293T cells transfected with a plasmid containing a KMT1F-FLAG sequence (p KMT1F) and/or siRNA against *KMT1F*. The anti-KMT1F reveals a band of approximatively 84 kDA whose intensity is decreased after transfection with siRNA against *KMT1F*. **C.** Structure of the *KMT1F* gene (Genbank Accession number NM_031915.1). The position of the different exons is indicated and the three small interfering RNAs designed to knock down *KMT1F* expression are shown (SiRKMT1F-1, -2 and -3). **D.** The histogram shows the ratio of *KMT1F* mRNA depletion after transfection of control siRNA or the three different siRNA against *KMT1F*. Two days after their transfection using Lipofectamin 2000®, expression of *KMT1F* was measured by real time RT-PCR. Values were normalized to the *RPL19* internal standard.

Supplementary figure 2. KMT1F depletion affects the trimethylation of histone H3-K9 but not mono- and di- methylation.
A. Validation of the SUV39H1 siRNA. The histogram shows the ratio of *SUV39H1 or KMT1F* mRNA depletion after transfection of control siRNA (mock) or siRNA against *SUV39H1* (SiRSUV) or depletion of both SUV39H1 and KMT1F (SiRSUV- SiRKMT1F-3). Four days after their transfection using Lipofectamin 2000®, expression of *SUV39H1* or *KMT1F* were measured by real time RT-PCR. Values were normalized to the *RPL19* internal standard. **B.** Post-translational modifications of H3K9 were detected from whole cell extracts of 293T cells transfected either with a control SiRNA (left panel) or with a SiRNA designed against KMT1F (SiRKMT1F-3, right panel) as described earlier. Western blot analysis of tri, di and monomethylated H3K9 in control cells (mock), or cells depleted in KMT1F (SiRKMT1F-1 and SiRKMT1F-3), SUV39H1 (SiRSuv) or both (SiRSuv- SiRKMT1F-3 or SiRSuv- SiRKMT1F-1). **C**. A form of KMT1F resistant to SiR421 (SiRKMT1F-1) and containing 6 point mutations: AAGGCGTCACGCTAAAACTAA was generated (pN-KMT1F-R, referred thereafter as R) and transfected into 293T cells together with the corresponding SiRNA. Two days after transfection, nuclear extracts were prepared as described and the level of KMT1F protein was determined by western blot. Lane 1: R-KMT1F-Control SiRNA, lane 2: R-KMT1F- SiRKMT1F-1. Lane 3: N-KMT1F-Control, lane 4: N- SiRKMT1F-1. Depletion in KMT1F after siRNA transfection is compensated by overexpression of a form *KMT1F* resistant to the transient knock-down (lane 4).

Supplementary figure 3. KMT1F staining colocalizes with H3-K9 trimethylation and HP1 at pericentric regions. A. The distribution of KMT1F and HP1α were analyzed by indirect immunofluorescence and confocal microscopy in 293T cells transfected with control SiRNA, a *KMT1F* expression vector (pKMT1F) or siRNA against *KMT1F* (SiRKMT1F) or *SUV39H1* (SiRSUV39H). Cells counterstained with TOTO-3 are on the left and merged images are on the right of each panel. The scale bar represents 10 μm. The first line (control) highlights the similar dotty pattern of KMT1F and HP1α signals at a lower magnification. **B.** The distribution of H3K9me3 and CREST (CENP-A, B, C) signals were analyzed by indirect immunofluorescence during metaphase in controls, cells transfected with SiRKMT1F, SiRSUV39H or both (SiRKMT1F + SiRSUV39H). Counterstaining with TOTO-3 is displayed on the left and merged is on the right of each panel.

Supplementary figure 4. Validation of the experimental system in HeLa and 293T cells.
Since time lapse analysis was performed in HeLa cells, this experimental system was validated prior to analysis. **A.** Therefore, HeLa cells were mock-treated or transiently transfected with siRNA against KMT1F and the protein was analyzed by indirect immunofluorescence as described earlier. KMT1F signal can be detected in this cell line and disappears upon depletion of the KMT1F protein as

previously observed in 293T cells following transient transfection of SiRKMT1F-3. **B**. Using a polyclonal anti-KMT1F antibody raised in goat by Abcam, we were able to detect both H3K9me3 and KMT1F signal by indirect immunofluorescence. Forty eight hours after transfection, cells were fixed, permeabilized and incubated with goat anti-KMT1F (1/50, Abcam) and rabbit anti-trimethyl-H3K9 (1/600) for 1 hr. Secondary anti-goat and anti-rabbit antibodies were used for detection. Cells were counterstained using DAPI. Both signals overlap in control cells, disappear after siRNA transfection indicating that the observation previously made in 293T cells with rabbit anti- KMT1F are similar than those obtained here with the goat antibody.

Supplementary figure 5. Validation of the inducible KMT1F depletion system.
A. Different Zeocin-resistant T-Rex ™-293 clones containing either the pTER-SiRluc (control) or pTER-SiR*KMT1F* vector were selected and grown in the presence (+ Dox) or absence (- Dox) of doxycycline. Medium was replaced every day. In the different conditions, the expression of *KMT1F* was determined by quantitative Real-Time quantitative RT-PCR 72 hour after induction. The expression of *KMT1F* is significantly decreased in A9 and A7 clones upon induction of *KMT1F* shRNA in the presence of doxycyclin (60 to 80%, compared to non-induced cells) that were selected for further analysis. **B.** Time course depletion in *KMT1F* expression. The A9 clone was grown in the presence or absence of doxycyclin and the expression of *KMT1F* was measured at hour 1, 6, 12, 18, 24, 36 in induced *versus* non induced cells and normalized to the *RPL19* internal standard.

Supplementary movie 1. The upper panel correspond to progression through mitosis in control HeLa cells expressing histone *H2B-GFP* (corresponding to Figure 4). The lower panel corresponds to progression through mitosis in HeLa cells expressing histone *H2B-GFP* and depleted for KMT1F after transient transfection of the siR3 siRNA (corresponding to Figure 4). Z-stacks were taken every 90 sec using a spinning-disk confocal microscope and maximum intensity. Projections of the images of each stack were assembled in a movie.

Table 1. Using the ModFit software, The percentage of cells in G2/M after transfection of different siRNA against KMT1F, SUV39H or both was determined by flow cytometry after incorporation of propidium iodide, from two different experiments. Our results indicate that invalidation of both protein does not increase the percentage of cells arrested and does not result in a more severe phenotype suggesting that both factor might be involved in the same pathway or the same protein complex, although this hypothesis remain to be tested.

	% of G2/M cells
Control	16.21
SiRKMT1F-1	21.99
SiRKMT1F-3	21.87
SirSuv	22.63
SirSuv + SiRKMT1F-1	21.68
SirSuv + SiRKMT1F-3	23.78

Supplementary methods

Cell culture

Human embryonic kidney (293T) and epithelial cervix adenocarcinoma HeLa cell lines were maintained in Dulbecco's Modified Eagle's Medium (DMEM) with L-alanyl-L-glutamine (GlutaMAX^{TM}I), D-glucose and sodium pyruvate (Invitrogen, Paisley, UK), supplemented with 10% FBS and 1% Penicillin-Streptomycin, 10000 units/ml (Invitrogen) at 37°C, 5% CO_2 in a humidified atmosphere. T-Rex ^{TM}-293 cells (Invitrogen), stably expressing the Tet repressor from pcDNA6/TR©, were supplemented with blasticidin at 3µg/ml. For fluorescence analysis, cells were grown on Poly-L-lysine (Sigma) coated glass coverslips. Cells were transfected using Lipofectamine^{TM} 2000 procedure following manufacturer's instructions (Invitrogen).

SiRNA design and knock-down procedure

SiRKMT1F-1, SiRKMT1F-2 and SiRKMT1F-3 were designed for *KMT1F* mRNA silencing (Genbank Accession number AF334407) using SiRnadesigner software (Qiagen). The sequence of Suv39H1 SiRNA was kindly provided by M. Narita (unpublished results) and control SiRNA corresponds to the luciferase gene. SiRKMT1F-1: AAG ACG ACA TGC AAA GAC AAA, SiRKMT1F-2: AAG GCA ATT GAG GTT CAA ATT, SiRKMT1F-3: AAA TGT CGG CCG CTT CCT TAA and SiRSuv39H1: TCT AAG AGG AAC CTC TAT GAC. For life imaging, the medium was replaced with Opti-MEM medium containing 10% of FBS and 0.2 µg/ml vitamin C sodium salt two hours before recording.

T Rex Inducible system

pTER-SiRluc (control) and pTER-SiR*KMT1F* vectors were constructed by ligation SiRLuc and SiRKMT1F into pTER plasmids (kindly provided by H. Clevers) as described elsewhere (1). After doxycyclin (Tetracyclin) induction at a concentration of 0.5µg/ml, Zeocin-resistant cells were selected and characterized.

Flow cytometry.

DNA content was analyzed by Flow Cytometry in cells stained with Propidium Iodide (PI, 20 mg/ml) using a FACScan flow cytometer (Becton-Dickinson). Data were processed using CellQuest and Modfit T V3.1 (Vertity Software House, Topsham, Me) sofwares.

RNA extraction and RT-PCR

Total RNA extraction was performed using RNA Plus^{TM} (QBiogene) according to the manufacturer's instructions. Total RNA was quantified using the NanoDrop ND-1000 spectrophotometer (NanoDrop technologies), treated with *DNase* I (Invitrogen) and quantified a second time. Primers used for RT-PCR are: for *KMT1F* (Genbank accession number AF334407), *KMT1F*-Forward: CCA AGG GAG CAC AAA AGG AC; *KMT1F*-Reverse: TGG TCG CCT GGT TAC ATC TG; for *SUV39H1* (Genbank accession number NM_024670): *SUV39H1*-Forward: CTT GGA ATC AGC TGC AGG AC; *SUV39H1*-Reverse: CGC GGA TCT TCT TGT AAT CG; for *RPL19*: *RPL19*-Forward: ATC GAT CGC CAC ATG TAT CA; *RPL19*-R: GCG TGC TTC CTT GGT CTT AG. The quantification of the target genes was always normalized to the amount of *RPL19* cDNA in the samples.

Plasmids

For construction of the pKMT1F vector, *KMT1F* sequence was amplified by PCR from IMAGE BC047434 clone using the following primers: TCC T**GG TCT C**GG ATC CGG AGA AAA AAA TGG CGA TG and TCC T**GG TCT C**CT CGA GGC CTG ATA AGC TAA TTTCAC, both containing a *Bsa*I restriction site (in bold).

Plasmids containing sequences of SET domains of histones methyltransferase G9a (682-1000), and SUV39H1 (82-412) were generous gifts from T. Jenuwein (Vienna, Austria). These sequences were PCR amplified and subcloned in frame into a pOZ-FHH-N plasmid (V. Ogryzko and U. Mechold, unpublished data) allowing expression of proteins fused to the C-terminus of a triple tags : Flag-HA-6His under the control of the MMLV 5'LTR as a promoter. The sequences corresponding to full-length KMT1F and to the SET domain of KMT1F were also PCR amplified and cloned into a pOZ-FHH-N plasmid (pN-CLLD8, pN-CLLD8-SET). A form of CLLD8 resistant to SiRKMT1F-1 was

generated (pN-CLLD8- SiRKMT1F-1-R) that contained 6 point mutations in the region corresponding to SiR421 (SiRKMT1F-1): AAGGCGTCACGCTAAAACTAA.The sequences were checked by sequencing.

Western blot.
Western blot for histone modifications were performed as described in the main text using a 18 % polyacrylamide gel. The membrane was incubated with primary antibody, me-H3K9 (1/500, Upstate Ref 07-441), me2-H3K9 (1/250, Upstate Ref 07-450), me3-H3K9 (1/600) or anti-H3 (Upstate ref 06-755) in 2% non-fat dried milk in PBS-T for two hours at room temperature and was washed for several times in PBS-T. Then, a one hour incubation was performed with peroxydase-conjugated goat anti-rabbit IgG. After washing, Western blot were visualized with the ECL chemiluminescent detection kit (Amersham Life Science).

Immunoprecipitation of triple-tagged proteins.
The 293T cells were grown in 10 cm diameter dishes under the condition described above. Cells at about 40% of confluency were transfected with 2 μg of pOZ-FHH-N-based plasmids (N- G9a (682-1000), N- Suv39h1 (82-412), N-KMT1F or N-SET domain of KMT1F) and 18 μg of Bluescript plasmid by calcium phosphate co-precipitation. About 36 hours after transfection, the cells were harvested and lysed in the lysis buffer (50 Tris-HCl pH 7.4, 150 mM NaCl, 1 mM EDTA, 1% Triton X-100, protease inhibitor cocktail (Roche)). After sonication and centrifugation (13,000 rpm, 60 min), supernatants were incubated with an anti-Flag M2 affinity resin (Sigma) overnight at 4°C in a binding buffer (20 mM Tris-HCl pH 7.4, 150 mM NaCl, 0.05% Triton X-100, protease inhibitor cocktail). The resin was washed three times with the binding buffer, then twice in methyltransferase buffer (20 mM Tris-HCl pH 7.4, 200 mM NaCl, 4 mM EDTA, 1 mM PMSF, 0.5 mM DTT). The quantity of protein was verified by coomassie staining and a volume corresponding to 10% of the input was used in *in vitro* HMTase assay.

***In vitro* HMTase assay**
K9-dimethyled H3 peptide (NH2-ARTKQTARK (2Me) STGGKAPRKQLC-COOH) were generously provided by C. Caron (Institut Albert Bonniot, Grenoble, France). Methyltransferase assays were performed using proteins attached to anti-Flag M2 resin in the presence of S-adenosyl [*methyl*-^{14}C]-L-methyonine in methyltransferase buffer for 2 hours at 32°C. The proteins were eluted from the resin by a competition with 3X FLAG peptide under gentle shaking at 4°C. The mixture was then centrifuged. The supernatant (20μl) was spotted on P81 paper circles (Whatman). The circles were washed sequentially 3 times (5ml/circle) with cold 0.2M ammonium bicarbonate and dried. Then, 2.5 ml of SafeScint scintillation cocktail (American Bioanalytical) were added to each circle and C^{14} incorporation was measured using a scintillator. Alternatively, reaction mixtures were spotted onto a immobilon membrane and exposed to a PhosphorImager screen and the spots were quantified using the ImageQuant sofware.

Chromatin immunoprecipitation and quantitative PCR.
Primers used for the detection of immunoprecipitated sequences after ChIP are: Alpha satellite, F: TTC TCA GAA ACT TCT TTG TGA TGT G, R: TGA AGA TAT TCC GTT TCC A; Alu Y sequences *AluY*-F: ATC TCG GCT CAC TGC AAG C, *AluY*-R: CGA GAC CAT CCT GGC TAA CA; Line 1 retrotransposon, *L1*-F: CGA GAC CAT CCT GGC TAA CA, *L1*-R: ATC TCG GCT CAC TGC AAG C; Histone 4 Promoter, *H4*-F: AAA TGG TGG GAT CAC AGA CG, *H4*-R: CGA GCT TCT TGT TTC CGT GT; *GA3PDH-F*: GCT CCA ATT CCC CAT CTC A, *GA3PDH*-R : GAC TGA GAT TGG CCC GAT G; *SAT2* primers were previously described (2). Other primers have been previously validated (3).

Immunofluorescence.
After 2 to 6 days, cells grown on coverslips were washed and fixed with 4% paraformaldehyde for 10 minutes (4,5), permeabilized and incubated with rabbit anti-KMT1F (1/50), mouse anti-HP1α (2HP-1H5, 1/1000, kindly provided by Dr. R. Losson), rabbit anti-monomethyl H3K9 (1/50), anti-dimethyl-

H3K9 (1/100), rabbit anti-trimethyl-H3K9 (1/600, kindly provided by T. Jenuwein) or human CREST (1/1000) for 1 hour at 37°C. Secondary anti-rabbit conjugated with Alexa Fluor 488 or 555 (Molecular Probes) were used for detection. For antigen competition, anti-KMT1F was pre-incubated 2 hours either with a KMT1F or a control peptide at a final concentration of 50µg/ml. Nuclei were counterstained with DAPI (Sigma) for fluorescence analysis and TOTO-3 iodide (Molecular Probes) at 1-2 µM for confocal imaging, mounted in Vectashield (Vector Laboratories) and observed on an Axioplan-2 Imaging Zeiss fluorescent microscope (Carl Zeiss, Le Pecq, France) equipped with Apochromat 63x and 100x/1.4 Oil DIC objectives, Coolsnap HQ CCD camera and Metamorph 7.5 software (Molecular Devices) for conventional microscopy and on a confocal Axioplan 2 equipped with Neofluar 100x/1.4 Oil Ph3 objective using the LSM 510 v 3.2 software for confocal acquisitions. Z-Stacks were processed through Imaris 4.2 software (Bitplane AG, Zurich, Switzerland) for 3-D analyses.

References

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