Chantalat_Supplemental Methods

Supplemental Methods

Antibodies used in ChIP and Immunofluorescence experiments

Table S3

Histone modification	Reference	Batch#	In the present study: Applications	In the present study: Validated by Western Blot
H3K36me3	Abcam, ab9050	136352	ChIP	
		453638	ChIP	
		467477	ChIP	
		475787	ChIP, IF	yes
		653080	ChIP, IF	yes
		712985	ChIP, IF	yes
		GR3639-1	ChIP	
H4K20me3	Abcam, ab9053	232666	ChIP	
		351833	ChIP	
		361931	ChIP	
H3K9me3	Abcam, ab8898	422483	ChIP	
		472044	ChIP, IF	
		733954	ChIP	
H3K79me3	Abcam, ab2621	435039	ChIP	
		456837	ChIP	
H3K4me2	Abcam, ab32356 (monoclonal Ab Y47)	506639	CHIP, IF	
	Upstate , 07-030 (polyclonal Ab)	26335	ChIP	
H4Ac	Upstate, 06-599	29532	ChIP	
H3Ac	Upstate, 06-866	25233	ChIP	
H3K27me2/3	Monoclonal 7B11, from D Reinberg		ChIP	
Others Ab used in this study				
HP1α	Euromedex, 2HP-1H5-As	992709	IF	
RNAPII CTD repeat	Abcam, ab5408 monoclonal 4H8	646081	ChIP	

Table S3 : References and batches of the antibodies used in this study. The preferential enrichment of H3K36me3 onto the repressed, maternally contributed *Snurf-Snrpn* region was observed for all seven batches of abcam 9050 antibodies that we tested in this study. Upstate (Upstate Biotechnology/Millipore, Billerica MA, USA) ; Abcam (Abcam, Cambridge, UK).

Chromatin Immunoprecipitation using high stringency washing buffers

We confirmed the specificity of H3K9me3 and H3K36me3 distribution pattern by performing ChIP experiments with high stringency washes. After binding to Protein G Sepharose, immunoprecipitated chromatin was sequentially washed at RT with low salt buffer (150mM NaCl, 20mM Tris pH 8.0, 2mM EDTA, 1% Triton X-100, 0.1% SDS), high salt buffer (500mM NaCl, 20mM Tris pH 8.0, 2mM EDTA, 1% Triton X-100, 0.1% SDS), lithium chloride buffer (250mM LiCL, 20mM Tris pH 8.0, 1mM EDTA, 1% deoxycholic acid, 1% IGEPAL), and ChIP buffer. Bound chromatin was eluted as described in the "Methods" section of the manuscript. ChIP experiments presented in Supplemental S7 were performed using this alternative protocol.

Design of allele-specific primers for ChIP-qPCR

To measure allele-specific chromatin differences in the *Snurf-Snrpn* 3Mb region, we developed a quantitative real-time PCR assay that allows the discrimination of maternal and paternal alleles within the region spanning position 66527171 to 70251665 on chromosome 7 (NCBI build 37). We initially chose to analyze histone modifications at the level of 67 different loci, which were selected following two criteria: i) probe one locus every 50 kb (in average) in this region, to follow the distribution of histone marks across the 3 Mb region ii) probe genes and other loci of interest (e.g. the Imprinting Center, located upstream of *Snrpn*). Using PCR amplification of 700-900 bp fragments and sequencing, we identified around 400 sequence polymorphisms (either SNPs or small deletion/insertion) within the mouse *Snurf-Snrpn* imprinted region of C57BL/6 and JF1/Ms strains. Sequenced regions and associated polymorphisms were next used to design PCR primers that could distinguish the JF1/Ms and C57BL/6 genomes at each locus, as previously described (Gupta et al. 2005). Except for one primer set, polymorphisms were systematically incorporated into the primer at the 3' terminus. The primer having a mismatch at the 3' end when hybridized to the template is amplified with a reduced efficiency, allowing discrimination between matched and mismatched templates. Each allele-specific primer was used in combination with a second primer that hybridizes equally the C57BL/6 and JF1/Ms genomes. Out of the 67 selected regions, 35 regions could be efficiently amplified with pairs of oligonucleotides specific for each strain. For ten primer sets, we incorporated an additional nucleotide mismatch located two or three bases from the 3' end of the allele-specific primers, to improve mismatch detection (Gupta et al. 2005). Examples of allele-specific PCR amplification are shown in Supplementary Figure S1. Primer sequences are available in Table S1 (columns 3 to 5). Our primer sets can discriminate the two parental alleles of all *Snurf-Snrpn* coding

genes, four positions within the Imprinting Center, and a series of randomly chosen non-coding regions (Fig. 1A).

Real-time PCR analysis of control regions after H3K36me3 chromatin immunoprecipitation

qPCR experiments were carried out directly onto immunoprecipitated chromatin, in a total volume of 10µl, in 384-well plates. The composition of the quantitative PCR assay included 2.5 µl DNA (immunoprecipitated DNA or corresponding pre-amplified input), 0.5 mM forward and reverse primers, and 1X SYBR Green PCR Master Mix (Applied Biosystems, Foster City CA, USA). Amplifications were performed as follows: 2 mn at 95°C, 40 cycles at 95°C for 15 sec and 60°C for 60 sec in the ABI/Prism 7900HT real-time PCR machine (Applied Biosystems). Primers are listed in table S4.

Table S4 : Primers for ChIP analysis

Gene		Forward primer	Reverse primer
Slc20a1	**L1**	TTCTGGCTGGAATTCTTAAGT	TTGCCTAAGCTCAGAACTAAC
	L2	GAGAGCAGAGAGCGTTTTAGA	GTTTTATTGCCTAAGCTCA
	L3	CGCTCCACCGAAGTATGACA	ACCGAACGAATTTGCTACATC
	L4	TCATCCTGCCAGTGTGACGCT	CCCACGGCTCCTTTAAGAC
Calr	**L1**	GATTCTTTGCCATCCTTGACT	GCCCTTTGTGCTGAACAC
	L2	GAGGTTTGCGTTTCATCACTA	TCTTCCTGACGGGTGTAA
	L3	GGCCAGACAACACCTATGAG	AGCATCAGGGTCCTTTATCTT
	L4	TTTTGTGTCTTCCCTATTGTG	GCTGAAACATACGTCACCC
Hsp90ab1	**L1**	CCCAATGGCTAGGTAATTTAT	GCCCTGGAACTCATGGT
	L2	AGCGCAGAGGGAGGGATA	CACTAGAAGGTGTCCGCAGTC
	L3	GGATTCTACTCGGCCTATCTA	GCCACCCGCAGACGACT
	L4	GAGGGCAGGGCTTCTTCCA	GCCCAGTGCTGCTGTTCCTGA
Rpsa	**L1**	CCTGAGTGCTGGCTAGGATTA	AACTTGCCACATCGTGAATG
	L2	TAGGCCACCACTACCTGA	ATTTAACTTGCCACATCGTGA
	L3	GTGTGCCCAGTGTATTCCTGT	CAGCCCGACTCATGTC
	L4	GACTCGGTGGCAAACAGA	CAAAGACTTAACTTGGCTATT

Quantitative RT-PCR

The following protocol was used to quantify RNA transcripts without distinguishing parental allelic origin. Random-primed RT-PCR was performed at 52°C using superscript III reverse transcriptase (Invitrogen) with 1 μg of total RNA isolated from cells or tissues. qPCR were carried out in 10 μl using the SYBR Green fluorescent dye. Relative expression was calculated after normalization with *Actb*. All reactions were performed in triplicate. Values are mean ± SD. Primers used in this study are listed in the Table S5.

Table S5

	Forward primer	**Reverse primer**
Ndn	GGGCACACTGATAGTTTCTGA	CTGCACTGTGGATTTGGGATA
Mkrn3	GATCAGTAAGTCCTGCCCACA	CCTGCACGCTTTCTGGCTCAT
Magel2	CTGCCACCTCAAGGGTCTTTC	AGTGACTTTGGGCTCTCTGA
Peg12	CTGCAGCGACCCTGGCGATT	CTGTGGCCACCGGGGTTATTA
Snrpn	AATCTTCATTGGCACCTTCA	TGCATTCTTTGGCTTGATCTT
Ube3a	CTTTCGTGACTTGGGAGACT	TAGATCATACATCATTGGGTT
Actb	AGATGACCCAGATCATGTTTGAGA	CACAGCCTGGATGGCTACGT
Hprt	CGTCGTGATTAGCGATGA	TCCAAATCCTCGGCATAATGA
Setd2	CGGCTCATGGTTAGAATTGAAAC	GGCAGGACTGTGAGTGTGTGTT
Whsc1	CAGCAGCCCAGTTTTTAGTCT	CCACTGGGACCCAAGCAATTC
Whsc1l1	GTCCGCCGAGGTTGTGC	CCCTTTCTGTAACCCGGTCTT
Nsd1	CCGAAATCATGAGCATGTTA	CGATATTTAGGCATTCTCGAT

Allele-specific quantitative RT-PCR

The following protocol was used to distinguish the paternal and maternal transcripts within the *Snurf-Snrpn* region. Random-primed RT-PCR was performed at 52°C using superscript III reverse transcriptase (Invitrogen) with 8 μg of total RNA, isolated from E17 fetal brains with RNeasy lipid-rich kit (Qiagen), and treated with DNase I (Promega). Control reactions without enzyme were verified negative. For allele-specific assay, each quantitative real-time PCR was performed in 10 μl, using the SYBR Green fluorescent dye (Applied Biosystems) and the set of 35 allele-specific primer pairs, without prior amplification. All reactions were performed in two separate wells, with a common primer and either the C57BL/6-specific primer or the JF1/Ms-specific primer. qPCR were performed

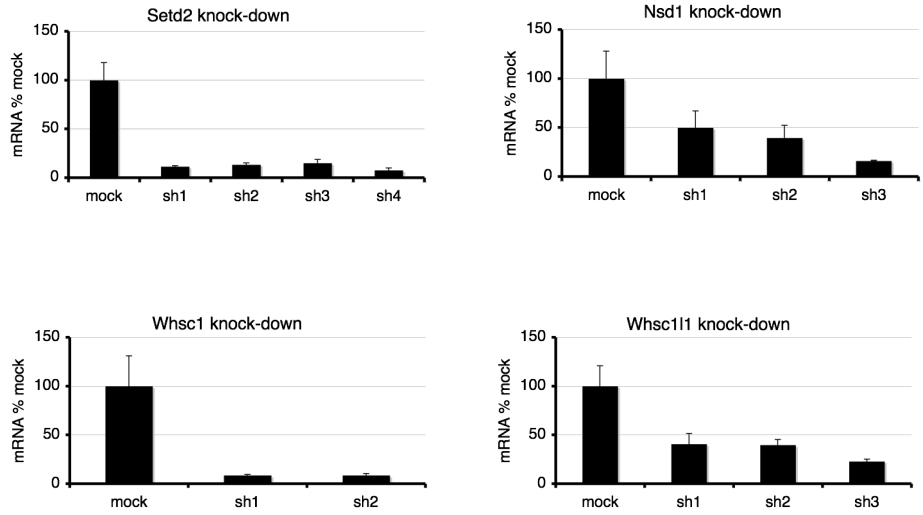
in triplicate, using three independent RNA preparations of post-natal day 1 brain and liver. Relative expression was calculated after normalization with *Hprt* gene.

Verification of *Setd2*, *Nsd1*, *Whsc1* and *Whsc1l1* knock-downs

shRNA vectors that target Setd2, Nsd1, Whsc1 and Whsc1l1 mRNAs were constructed using the pHYPER shRNA vector, as described (Berlivet et al. 2010). Sequences encoding the shRNAs, which were designed using the DSIR software (http://biodev.extra.cea.fr/DSIR/DSIR.html), are listed in Table S6.

Table S6		**Sense strand sequence**	**Anti-Sense sequence**
Setd2	**sh1**	GGTAGTGAATTAAGAACAATT	AATTGTTCTTAATTCACTACC
	sh2	GGTTGTAACAAATAATCTA	TAGATTATTTGTTACAACC
	sh3	AGACATGTTCAACTGTCTA	TAGACAGTTGAACATGTC
	sh4	AGGAGTACATCAAGAAGTA	TACTTCTTGATGTACTCC
Whsc1	**sh1**	TGGTCCAAAGTGTCAGGTTAC	GTAACCTGACACTTTGGACCA
	sh2	CCCAATACTACACCTATCAAA	TTTGATAGGTGTAGTATTGGG
Whsc1l1	**sh1**	CTCACCCGAGATTAAACTAAA	TTTAGTTTAATCTCGGGTGAG
	sh2	GTTAGAAACTTTAGCCCTACT	AGTAGGGCTAAAGTTTCTAAC
	sh3	CACAAAGTCTAAGCATGAAAG	CTTTCATGCTTAGACTTTGTG
Nsd1	**sh1**	GAGTAACAAAGACGACCAAGA	TCTTGGTCGTCTTTGTTACTC
	sh2	CGAACTCCAGTTAAGAGTAAC	GTTACTCTTAACTGGAGTTCG
	sh3	TGAATTTAGATGCCTCCGAAG	CTTCGGAGGCATCTAAATTCA

Analysis of knock-down efficiencies:



Efficient depletion of Setd2, Nsd1, Whsc1 and Whsc1l1 mRNAs in ES cells using specific shRNA vectors. ES cells were electroporated with plasmid vectors expressing shRNAs against *Setd2* (four independent shRNAs), *Nsd1* (three independent shRNAs), *Whsc1* (two independent shRNAs), *Whsc1l1* (three independent shRNAs), or a mock shRNA. Total RNAs were isolated 96h after transfection (including 72h puromycin selection), and relative levels of Setd2, Nsd1, Whsc1 and Whsc1l1 mRNA were quantified by RT-qPCR and normalized to Actb mRNA. Error bars represent the SD from triplicate PCRs.

Immunofluorescence analysis of Setd2 and Whsc1 knock-down cells

ES and MEF cells were scored for a reduction of H3K36me3 immunofluorescence in the nucleoplasm. Cells transfected with the control pHYPER vector were used as a reference for the 'normal' level of H3K36me3 fluorescence. For each experiment, 400 nuclei were scored as 'normal H3K36me3 fluorescence' or 'low H3K36me3 fluorescence'. The following results are the average of two independent experiments:

Knock-down of *Setd2* in ES cells: 43 % 'normal' and 57 % 'low' (SD=5)

Knock-down of *Whsc1* in ES cells: 97 % 'normal' and 3 % 'low' (SD=2)

Knock-down of *Setd2* in MEF cells: 36 % 'normal' and 64 % 'low' (SD=8)

Knock-down of *Whsc1* in MEF cells: 100 % 'normal' and 0 % 'low' (SD=0)

Fetal brain nuclei preparation and nuclease digestion

E17 brains were dissected in PBS. After tissue homogenization, nuclei were obtained by treatment with 0.15% IGEPAL (Sigma) in the same buffers as for the preparation of chromatin for ChIP experiments. Nuclei were purified on sucrose cushions and resuspended in nuclease buffer: 50 mM Tris-HCl (PH 7.5), 20 mM NaCl, 0.32 M sucrose, 4 mM $MgCl_2$, 1 mM $CaCl_2$. About 6.10^6 nuclei were incubated with increasing quantities of DNase I (0.25 to 16 units) and digested during 2 mn. For restriction enzymes, nuclei were further centrifuged at 2000 g and resuspended in restriction enzyme buffers for *Kpn*I, *Sph*I or *Sac*I. 5.10^6 nuclei were incubated with 60 units of enzyme during one hour at 37°C. For all nucleases, digestions were stopped by adding SDS to 1% and EDTA to 5 mM. DNAs were prepared by proteinase K digestion followed by phenol-chloroform extraction and isopropanol precipitation.

Hybridization probes for Southern blot

Ndn exon probe was a 1 kb fragment or a 360 bp fragment derived from this sequence. *LacZ* probe was the full-length 3 kb sequence or a 240 bp fragment derived from this sequence. The mouse major satellite (240bp) probe has been described (Lewis et al. 1992). Probes for *Hoxd-11* were either *Hoxd-11* coding sequences or a DNA fragment including RVIII and RIX regulatory elements (Gerard et al. 1993). These probes gave identical results in nuclease sensitivity assays. Autoradiography exposure was 20 mn for major satellite and 10 days for single copy gene probes.

Western blot analysis

Mouse MEF and ES cell chromatin was prepared as described in (Umlauf et al. 2004). Three gels (12% SDS-PAGE) were migrated with 7 and 14 μg of chromatin, and 0.25, 0.5, 1 and 1.5 μg of recombinant histone H3/H4. One gel was stained with SimplyBlue SafeStain (Invitrogen), to reveal all histones. The two other gels were transfered onto PVDF 0.45 μm membranes (Millipore). Each membrane was incubated overnight with a batch of H3K36me3 antibodies (Abcam 9050, lot 475787 and lot 712985, 1:1000 dilutions). Membranes were washed and revealed with ECL (Amersham). The membranes were stripped and revealed with an antibody against histone H3 (Millipore, clone A3S, Ref 05-928, dilution 1/1000).

For the experiment with yeast chromatin, histones were acid-extracted from yeast as described in (Edmondson et al. 1996). 4μg of proteins were migrated in a 12% SDS-PAGE gel and transfered onto

nitrocellulose. The membrane was incubated overnight with the H3K36me3 antibody (Abcam 9050, lot 653080, 1:500 dilution). Yeast strains were YPH499 (wild type) and clone Boeke-EMH-H3-36|K36A of the non essential histone H3 and H4 mutant collection (OpenBiosystems, YSC5106).

Dot Blot analysis

DNA was prepared from fetal brain chromatin immunoprecipitated with antibodies against the indicated histone modifications, and spotted twice onto a nylon membrane. The membrane was then hybridized with a probe specific for major satellite repeats. Input DNA was used as a positive control.

Supplementary references

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Lewis, J.D., Meehan, R.R., Henzel, W.J., Maurer-Fogy, I., Jeppesen, P., Klein, F., and Bird, A. 1992. Purification, sequence, and cellular localization of a novel chromosomal protein that binds to methylated DNA. *Cell* **69**: 905-914.