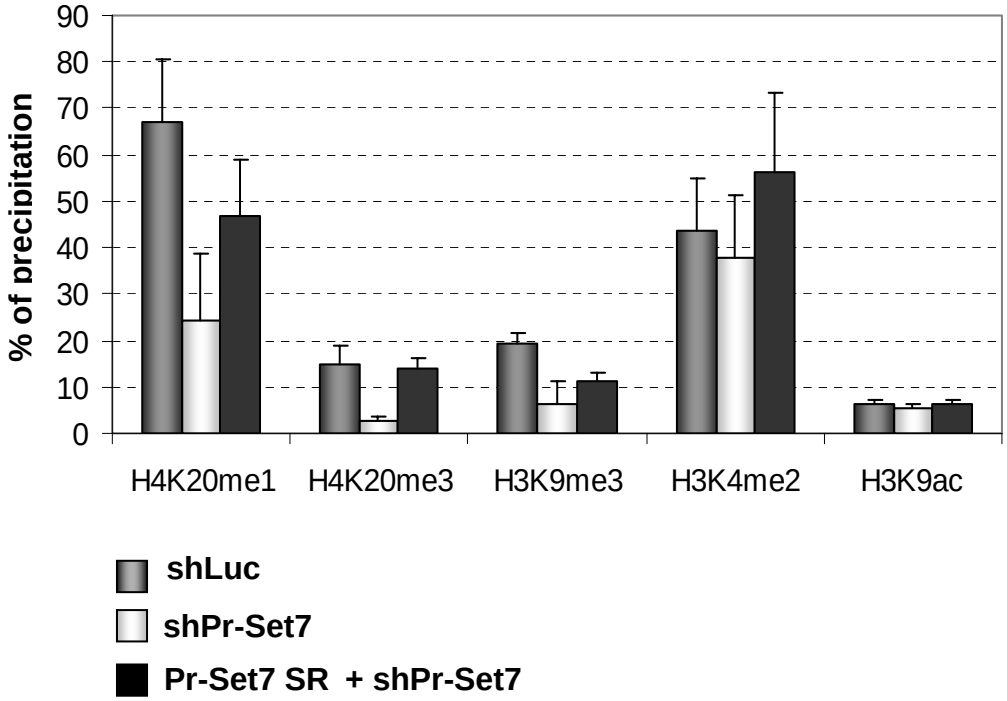
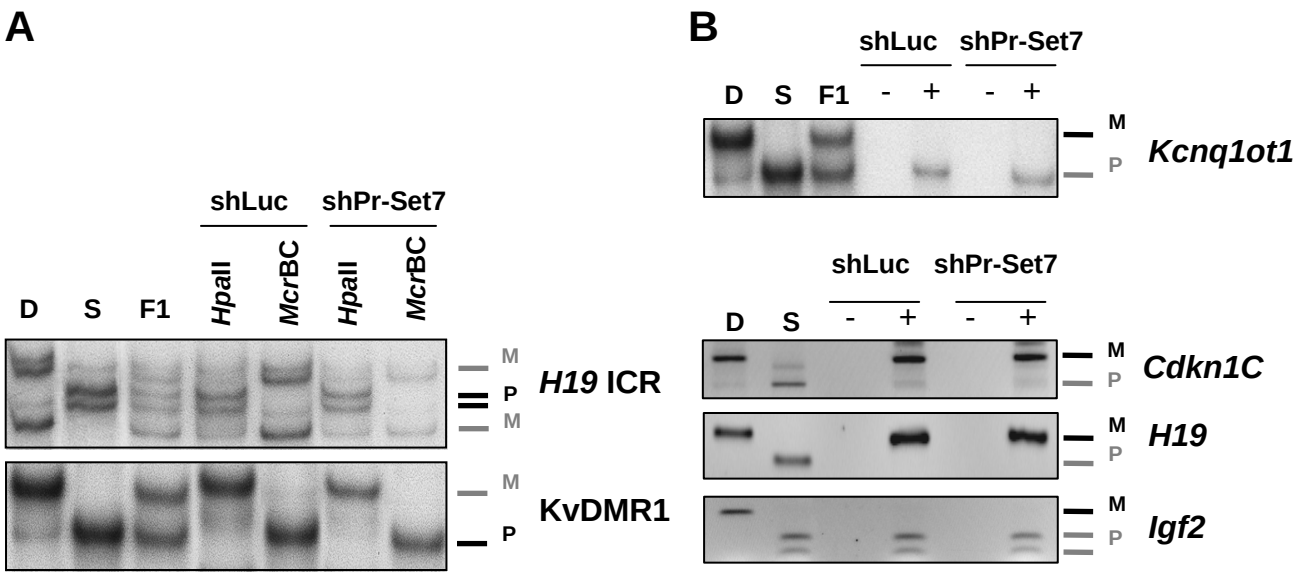
Pr-Set7 and Suv4-20h regulate H4 lysine-20 methylation at imprinting control regions in the mouse

(A) SUPPLEMENTARY FIGURES

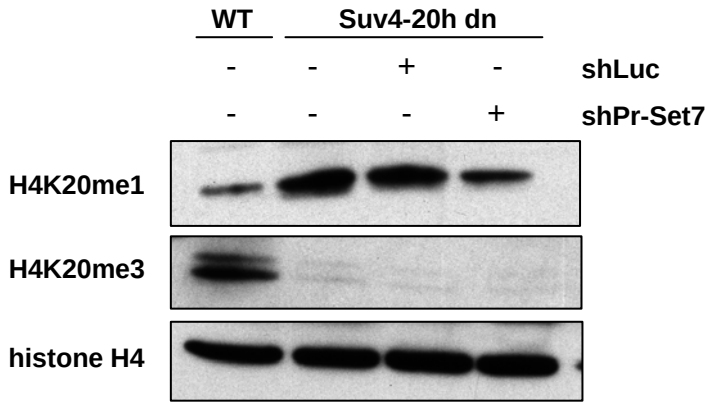


Supplementary Fig 1
Rescue of Pr-Set7 expression restores normal histone methylation levels in shPr-Set7 cells. ChIP was performed on chromatin extracted from WT (dark grey bars) and shPr-Set7 (light grey bars) MEFs. Pr-set7 protein levels were brought back to normal in shPrSet7 cells, by co-expressing a Pr-Set7-SR transgene (black) which is not regulated by the hairpin RNA (due to single nucleotide changes). Error bars represent standard deviation between at least three different ChIP experiments on independent chromatin preparations. Precipitation levels (% of input) at the *H19* ICR were determined by dividing the average value of the IPs by the average value of the corresponding input chromatin. For technical reasons, these experiments had to be performed on immortalised cells, as opposed to the ones shown in Fig 3C which were performed on primary cells.



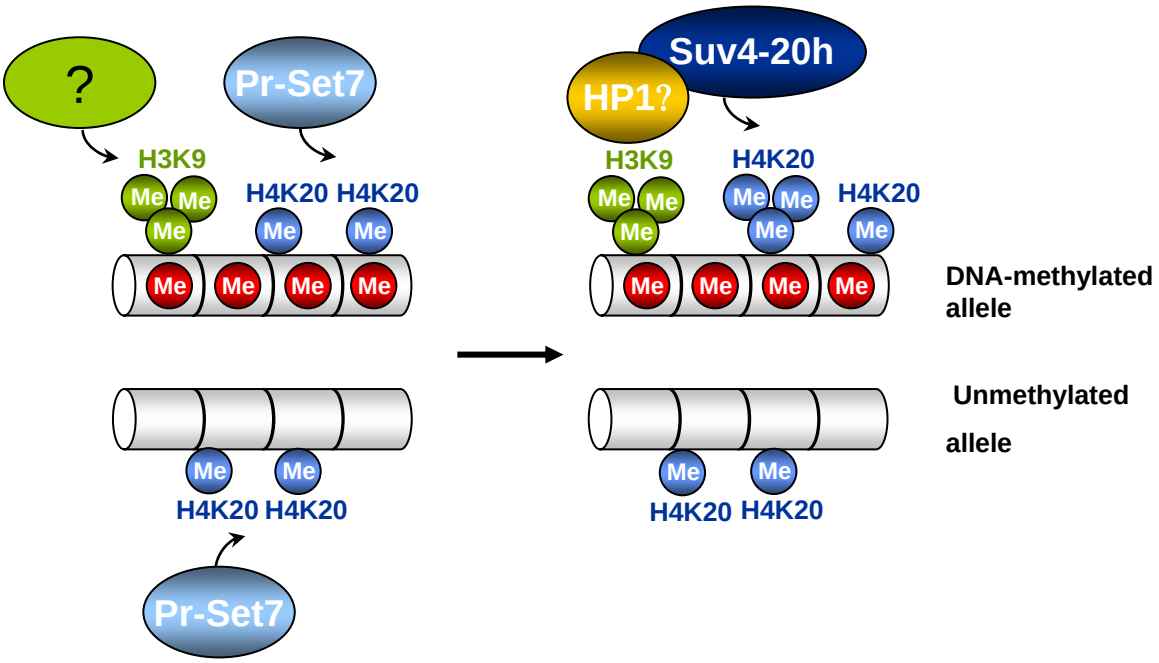
Supplementary Fig 2

Unaltered DNA methylation and gene expression in Pr-Set7 knock-down MEFs at the *Kcnq1* and *Igf2-H19* imprinted domains. **(A)** Allelic analysis of DNA methylation by PCR-SSCP at the *H19* ICR and KvDMR1, in Pr-Set7 knock-down cells (shPr-Set7) and control cells (shLuc). DNA digestion was with *Hpa*II (cuts unmethylated DNA) or *Mcr*BC (cuts methylated DNA). D, C57BL/6 control DNA; S, SDP711 congenic control with distal 7 of *M. spretus*; F1, (C57BL/6 x SDP711) F1 control; M, maternal allele; P, paternal allele. **(B)** Analysis of allelic expression of *Kcnq1ot1*, *Cdkn1*C, *H19* and *Igf2* in shPr-Set7 cells. Allelic *Kcnq1ot1* expression was studied by discrimination of SSCP polymorphisms by gel-electrophoresis; *Cdkn1c*, *H19* and *Igf2* were analysed using polymorphic *Ava*I (*Cdkn1c*), *Bgl*I (for *H19*) and *Bsa*AI (for *Igf2*) restriction sites (Supplementary Table 2).



Supplementary Fig 3

Immunoblot analysis of the levels of histone H4, H4K20me1 and H4K20me3 in wild-type (WT) and Suv4-20h dn MEFs, and in Suv4-20h dn MEFs 4 days after shLuc or shPr-Set7 infection. Note the increase in H4K20me1 and the absence of H4K20me3 in Suv4-20h dn MEFs compared to wild-type MEFs. Pr-Set7 depletion in Suv4-20h dn MEFs leads to a significant reduction in H4K20me1.



Supplementary Fig 4
Model for allelic H4K20me3 and H3K9me3 regulation at ICR. In somatic cells, Pr-Set7 mediates H4K20me1 at ICRs. On the DNA-methylated allele ('Me'), H4K20me1 is converted into H4K20me3 by Suv4-20h, which is recruited to this parental allele. Recruitment of Suv4-20h seems downstream to the maintenance of DNA methylation, since this is unaltered in Suv4-20h-deficient and Pr-Set7-depleted cells. Suv4-20h recruitment could be linked to the presence of H3K9me3, which is controlled by a HMT(s) other than Suv39h and could be recognized by HP1g proteins. The levels of H4K20me3 and H3K9me3 at the methylated alleles of ICRs appear to be linked in somatic cells, via a yet-unknown mechanism.

(B) SUPPLEMENTARY MATERIALS AND METHODS

ChIP on native and cross-linked chromatin ChIP on non-fixed chromatin was performed as previously described (Umlauf *et al*., 2004) using 5µg of antibody per 3µg of chromatin. We used antisera against H4K20me3 (Upstate, 07-463), H3K9me3 (Upstate, 07-442), H3K4me2 (Upstate, 07-030), H3K9ac (Upstate, 06-942), H4K20me1 (Abcam, ab9051), and H3K27me3 (Abcam ab6002). As a control, mock precipitation, we used a rabbit-antiserum against chicken IgG (Sigma, C2288). In each study, experiments were performed with at least three independently prepared chromatin samples. Input chromatin and immunoprecipitations (IP) were quantified by real-time PCR amplification (in triplicate) with a SYBR Green mix using an MX3000 apparatus (Stratagene).

The percentage of precipitation was calculated by dividing the average value of the IP (priorly corrected for the average value of the corresponding mock precipitations) by the average value of the corresponding input chromatin. When possible, parental alleles were distinguished by electrophoretic detection of SSCPs in PCR products (see Supplementary Table 1).

ChIP on cross-linked chromatin was performed as described before (Umlauf *et al,* 2004). Semi-confluent cells were cross-linked with 0.5% (for Suv4-20h, H3K27me3 and H3K9ac) or 1% (for HP1g) formaldehyde, for 10 min at room temp, and for subsequent ChIP we used antisera directed against Suv4-20h1/h2 (Abcam, ab18186), Hp1g (Upstate, 05-690), H3K27me3 (Abcam ab6002) and H3K9ac (Upstate, 06-942). Quantitative PCR was carried out as for ChIP on unfixed chromatin.

Western blot analysis Western blotting (Tardat *et al,* 2007) was performed using antisera directed against Pr-Set7 (1:1000; Upstate 07-316), b-actin (1:20000; Sigma), H4K20me1 (1:1000; Abcam, ab9051), H4K20me3 (1:1000; Upstate, 07-463) and histone H4 (1:2000; Upstate, 07-108).

(C) Supplementary Tables

Supplementary Table I

Oligonucleotides used for SSCP and methyl-sensitive PCR				
ICR	Primer	Primer sequences	Location	Reference
H19	Forward	TCACTTAAGGAACCGCCAAC	3 kb upstream of *H19* start site	Delaval *et al*. 2007
	Reverse	ACATTGTCATGGGCAAACCT		
KvDMR1	Forward	CTCAGTTCCACGATACCCTTCC	6 kb downstream of *Kcnq1ot1* start site	Umlauf *et al*. 2004
	Reverse	CTTACAGAAGCAGGGGTGGTCT		

Oligonucleotides used for qPCR				
ICR	Primer	Primer sequences	Location	Reference
H19	Forward	TCACTTAAGGAACCGCCAAC	3 kb upstream of *H19* start site	Delaval *et al*. 2007
	Reverse	CAATTGATTTTGGGCTGACA		
KvDMR1	Forward	GATCGTCCTCTGCATGGTTT	6 kb downstream of *Kcnq1ot1* start site	Umlauf *et al*. 2004
	Reverse	CATGGAGGTCTAGGCTCAGG		
Gtl2	Forward	CTTTTGTTGACCACAACCCTTG	11 kb upstream of *Gtl2* start site	Delaval *et al*. 2007
	Reverse	AATCCCACCACAGCTTCTTAGC		
Snrpn	Forward	AGGTTGTGACTGGGATCTTG	200 bp downstream of *Snrpn* start site	This study
	Reverse	TGCAGCGGCAACAGAACTTCT		
Igf2r	Forward	TCGTGTAGAAATCCTCCGATCT	7 kb downstream of *Igf2r* start site	Delaval *et al*. 2007
	Reverse	TTTACGGGCGATTTAGAGCAC		
Major satellite	Forward	GACGACTTGAAAAATGACGAAATC		Peters *et al*. 2003
	Reverse	CATATTCCAGGTCCTTCAGTGTGC		

Supplementary Table II

Oligonucleotides used for PCR after bisulfite treatment			
ICR	Pimer	Primer sequences	Reference
KvDMR1	Forward	GGTGAGGTTATATTAGTTAGTTA	This study
	Reverse	CTAAACAAAAAAACTCTCCAAAT	

Oligonucleotides used for RT-PCR				
Gene	Pimer	Primer sequences	Polym.	Reference
Kcnq1ot1	Forward	CTCAGTTCCACGATACCCTTCC	SSCP	Umlauf *et al*. 2004
	Reverse	CTTACAGAAGCAGGGGTGGTCT		
H19	Forward	CATCCTGGAGCCAAGCCTCTAC	RFLP BglI	This study
	Reverse	CCTCAGACGGAGATGGACGACAG		
Igf2	Forward	ATCGTCCCCTGATCGTGTTAC	RFLP BsaI	Weber *et al*. 2003
	Reverse	GGAACTGTCCCTGCTCAAGA		
Cdkn1C	Forward	TTCAGATCTGACCTCAGACCC	RFLP AvaI	Caspary *et al*. 1998
	Reverse	AGTTCTCTTGCGCTTGGC		

Oligonucleotides used for shRNA		
Target	Oligonucleotides sequences	Reference
Luciferase	TGCGTTGCTAGTACCAAC	Tardat *et al*. 2007
Pr-Set7	GCTGCAGTCTGAAGAAAGG	This study

References cited in Supplementary Tables I and II:

Caspary T et al. (1998) Multiple mechanisms egulate imprinting of the mouse distal chromosome 7 gene cluster. *Mol Cell Biol* **18**:3466-3474

Delaval K *et al.* (2007) Differential histone modifications mark mouse imprinting control regions during spermatogenesis. *EMBO J* **26**:720-729

Peters AH *et al.* (2003) Partitioning and plasticity of repressive histone methylation states in mammalian chromatin. *Mol Cell* **12**:1577-1589

Tardat M *et al.* (2007) PR-Set7 dependent lysine methylation ensures genome replication and stability through S phase. *J Cell Biol* **179**:1413-1426

Umlauf D *et al.* 2004) Imprinting along the *Kcnq1* domain on mouse chromosome 7 involves repressive histone methylation and recruitment of Polycomb group complexes. *Nat Genet* **36**: 1296-1300

Weber M et al. (2003) Genomic mprinting controls matrix attachment regions in the Igf2 gene. *Mol Cell Biol* **23**:953-8959