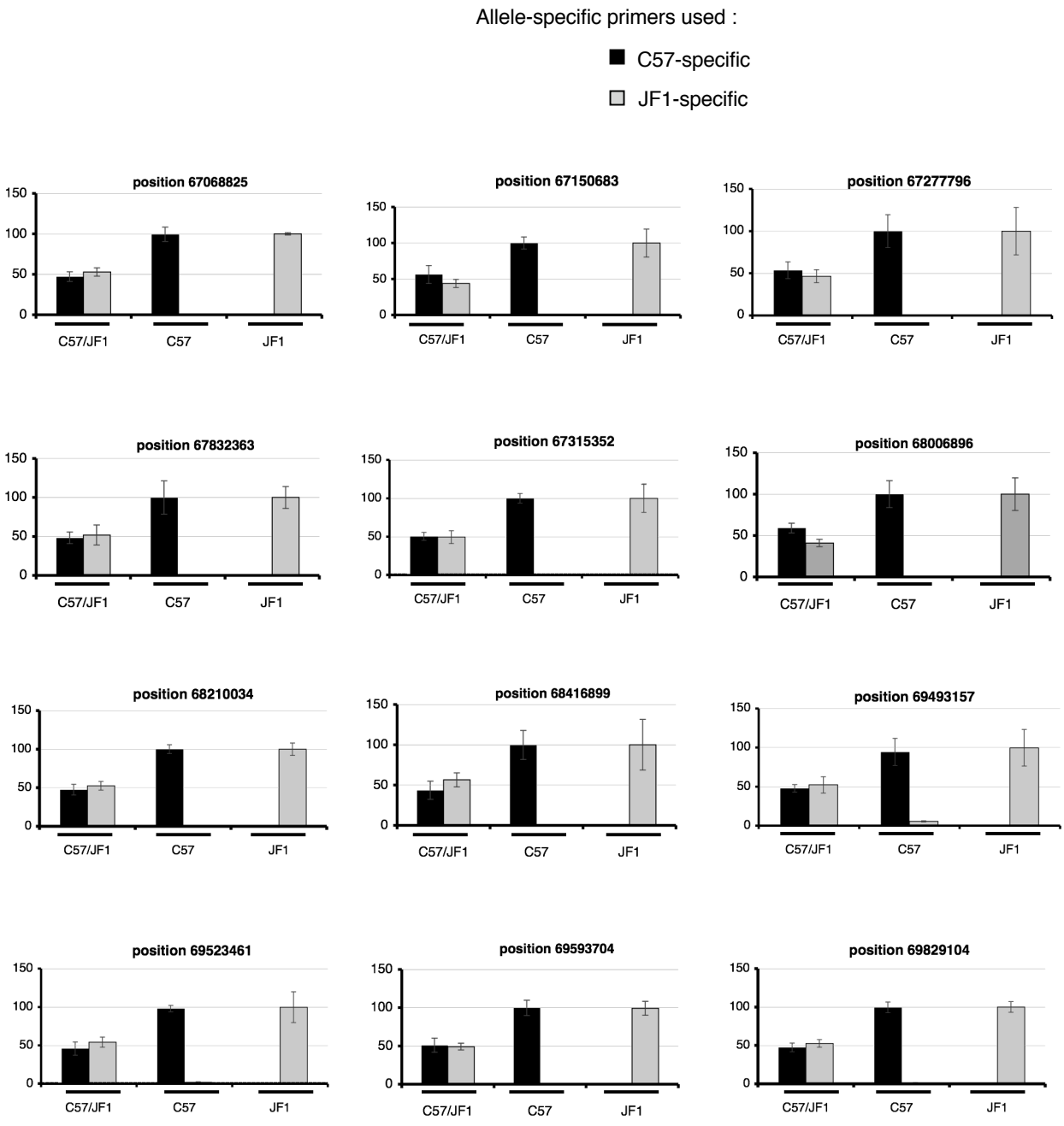
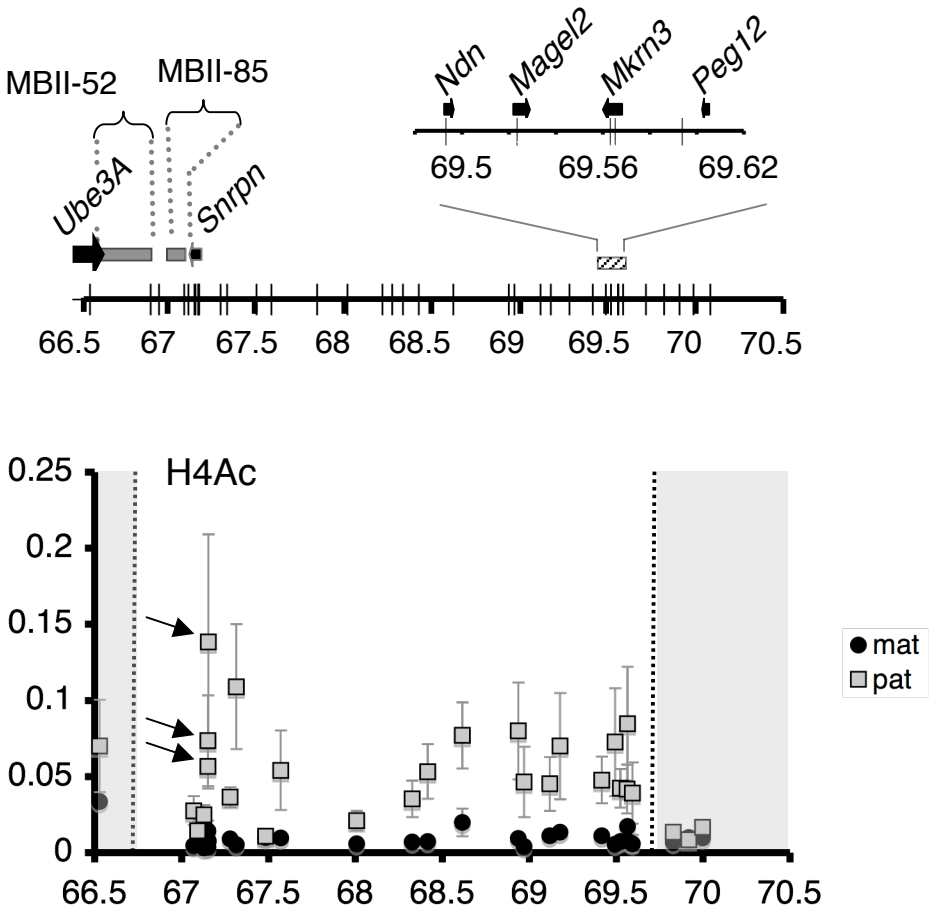
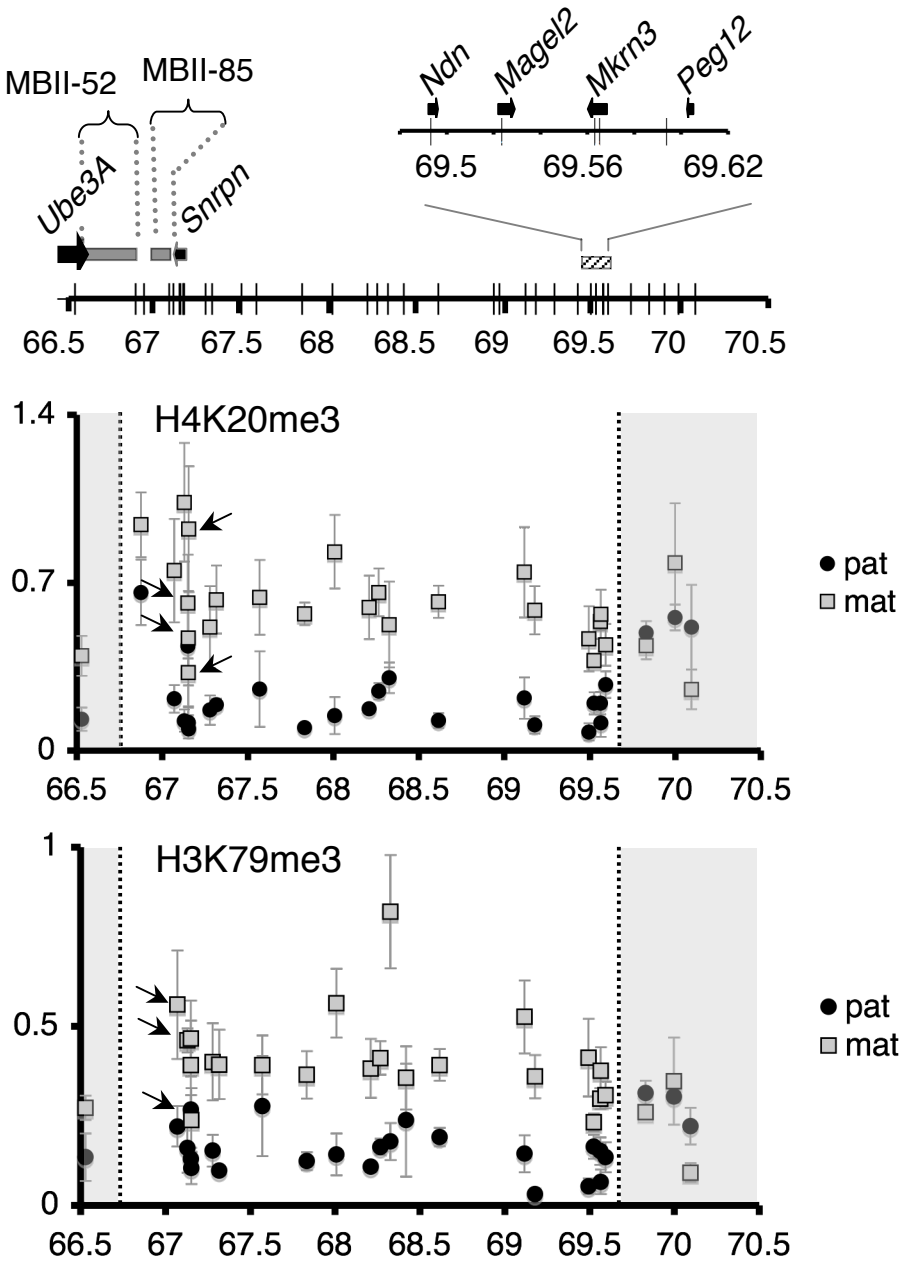


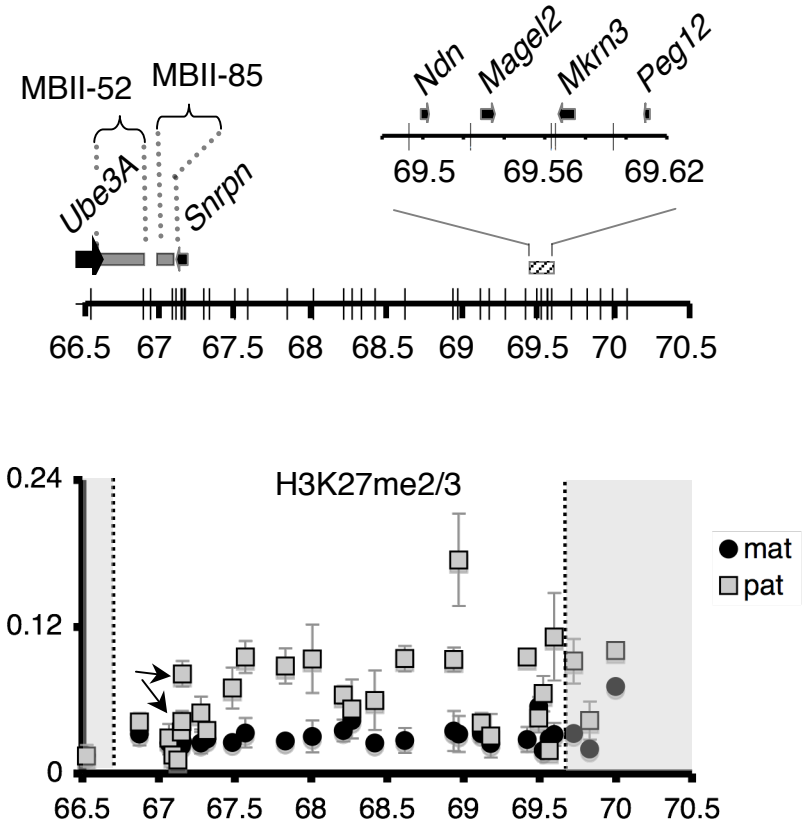
Supplemental Figure S1. Allele-specific qPCR amplification of heterozygous F1 DNA and homozygous parental DNA samples . Twelve representative primer sets were tested for their allele-specificity using genomic DNA samples from heterozygous C57BL/6 / JF1/Ms hybrid and from the parental homozygous strains (C57BL/6 and JF1/Ms) as templates for PCR amplification. The origin of the DNA templates is indicated on the x-axis. Positions correspond to the coordinates of the polymorphisms (NCBI build 37). Primers are described for each position in table S1. Black and grey bars indicate the amount of qPCR products amplified using C57BL/6- or JF1/Ms- specific primers, respectively. The y-axis indicates the percent of C57BL/6- or JF1/Ms-specific allele in the total genomic DNA amplified. All experiments were carried out in triplicates. Values are mean ± SD. The two parental alleles were amplified at a similar level in the heterozygous strain, whereas only one specific allele could be amplified in each homozygous strain.



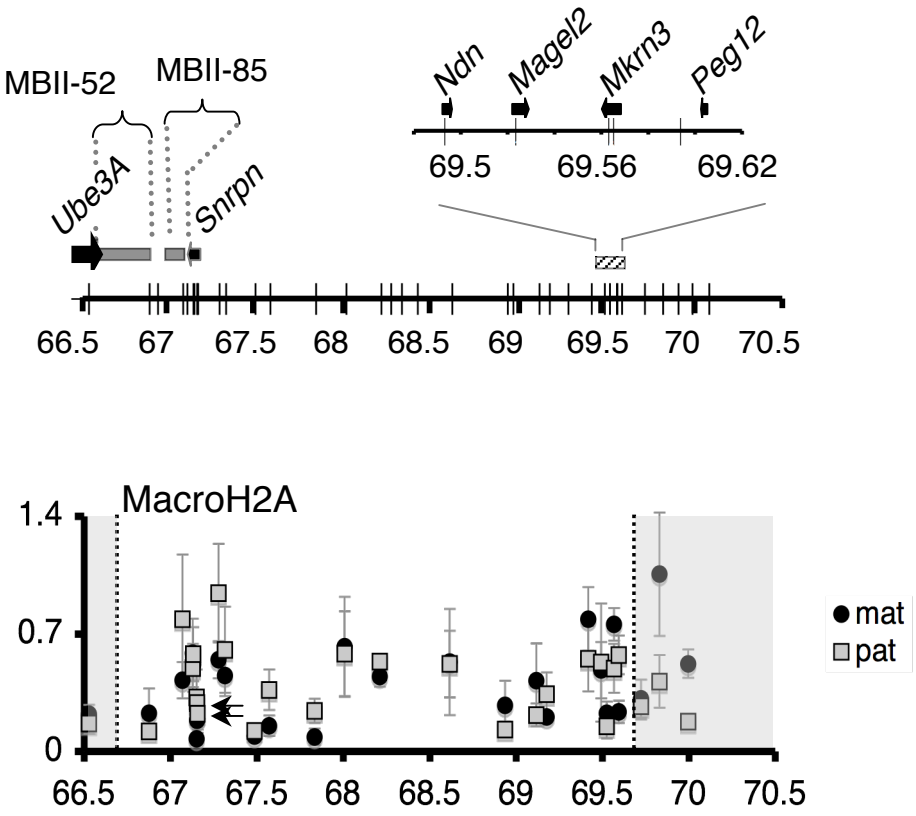
Supplemental Figure S2. Distribution of acetylated histone H4 (H4Ac) within the imprinted *Snurf-Snrpn* cluster. ChIP was performed using E17 brain chromatin prepared from fetuses conceived by crossing JF1/Ms males x C57BL/6 females. Detection of H4Ac onto the paternally and maternally contributed *Snurf-Snrpn* genomic regions is indicated by grey squares and black circles, respectively. Data are expressed as relative abundance and are normalized to input. qPCR experiments were performed in triplicates. Values are mean ± standard deviation. Arrows indicate positions in the *Snurf-Snrpn* IC.



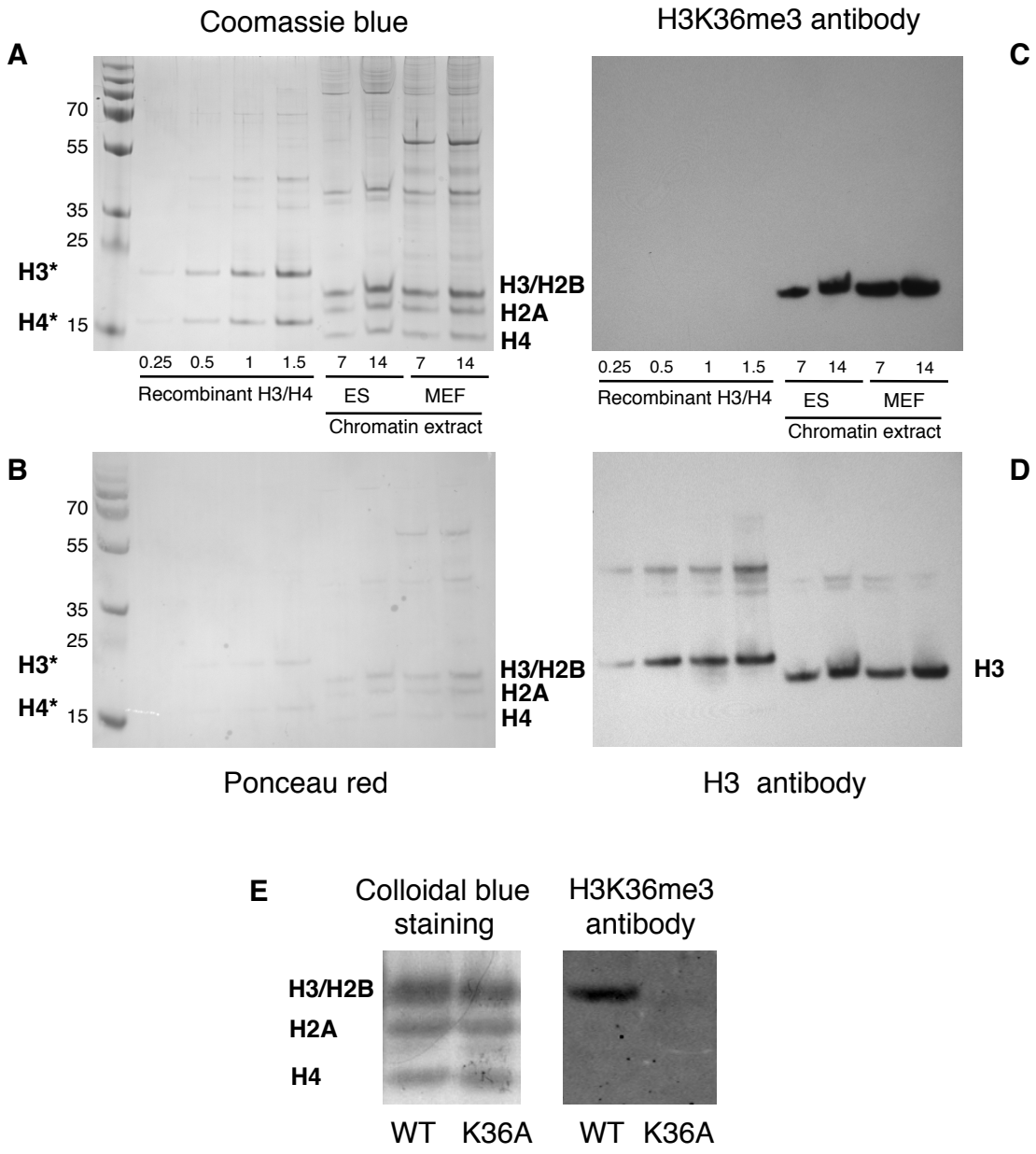
Supplemental Figure S3. Distribution of H4K20me3 and H3K79me3 in the *Snurf-Snrpn* region revealed by ChIP experiments performed with brain chromatin isolated from E17 fetuses, conceived by mating C57BL/6 males with JF1/Ms females. The 3Mb *Snurf-Snrpn* region is represented as in Figure 1A. Distribution of histone marks onto the paternally and maternally contributed *Snurf-Snrpn* genomic regions is indicated by black circles and grey squares, respectively. For each graph, data are expressed as relative abundance and are normalized to input. Values are the mean of two independent experiments ± standard deviation. Arrows indicate positions in the *Snurf-Snrpn* IC.



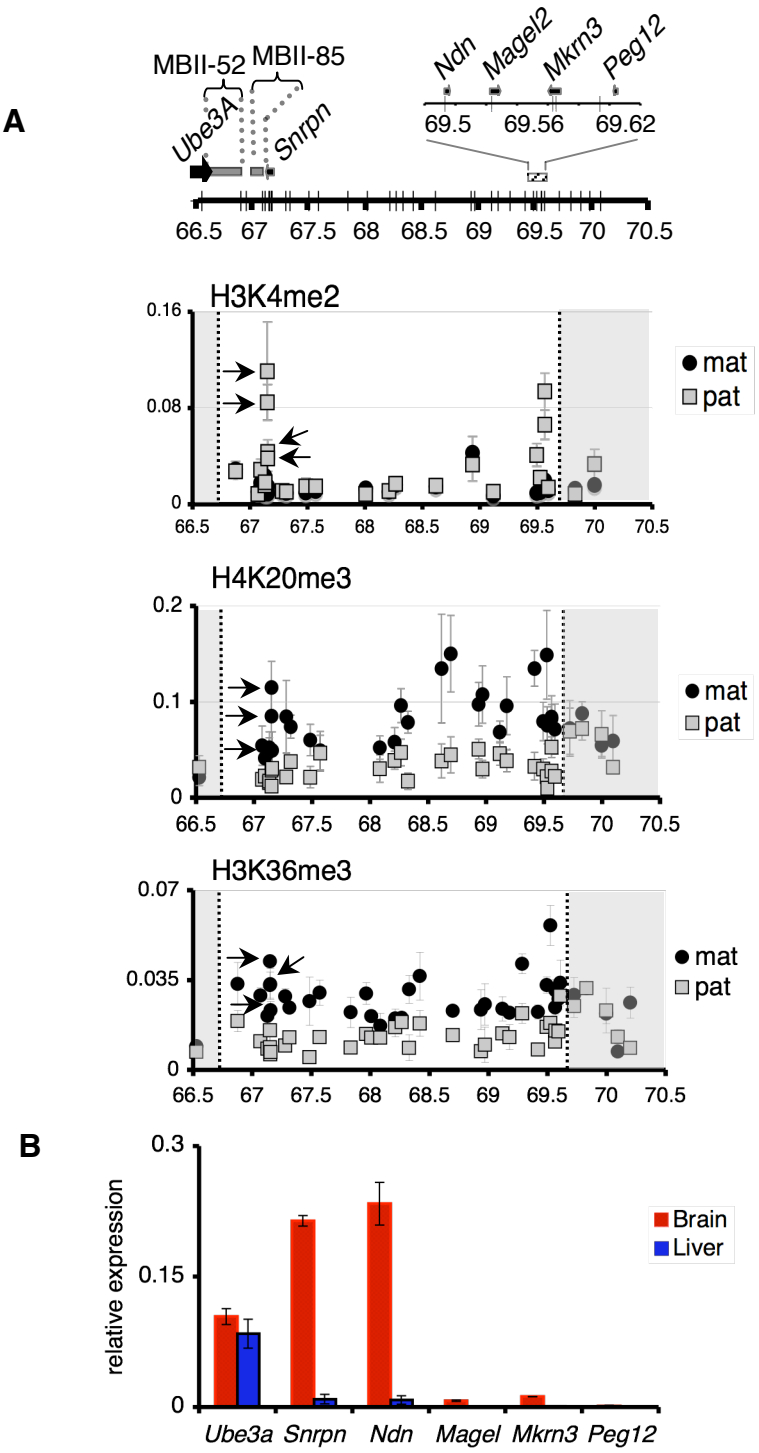
Supplemental Figure S4. Distribution of di/trimethylated histone H3 at lysine 27 within the imprinted *Snurf-Snrpn* cluster. ChIP was performed using E17 brain chromatin prepared from fetuses conceived by crossing JF1/Ms males x C57BL/6 females. Detection of H3K27me2/3 onto the paternally and maternally contributed *Snurf-Snrpn* genomic regions is indicated by grey squares and black circles, respectively. Arrows indicate positions in the *Snurf-Snrpn* IC. Data are expressed as relative abundance and are normalized to input. ChIP experiments were performed in duplicates. Values are mean ± standard deviation.



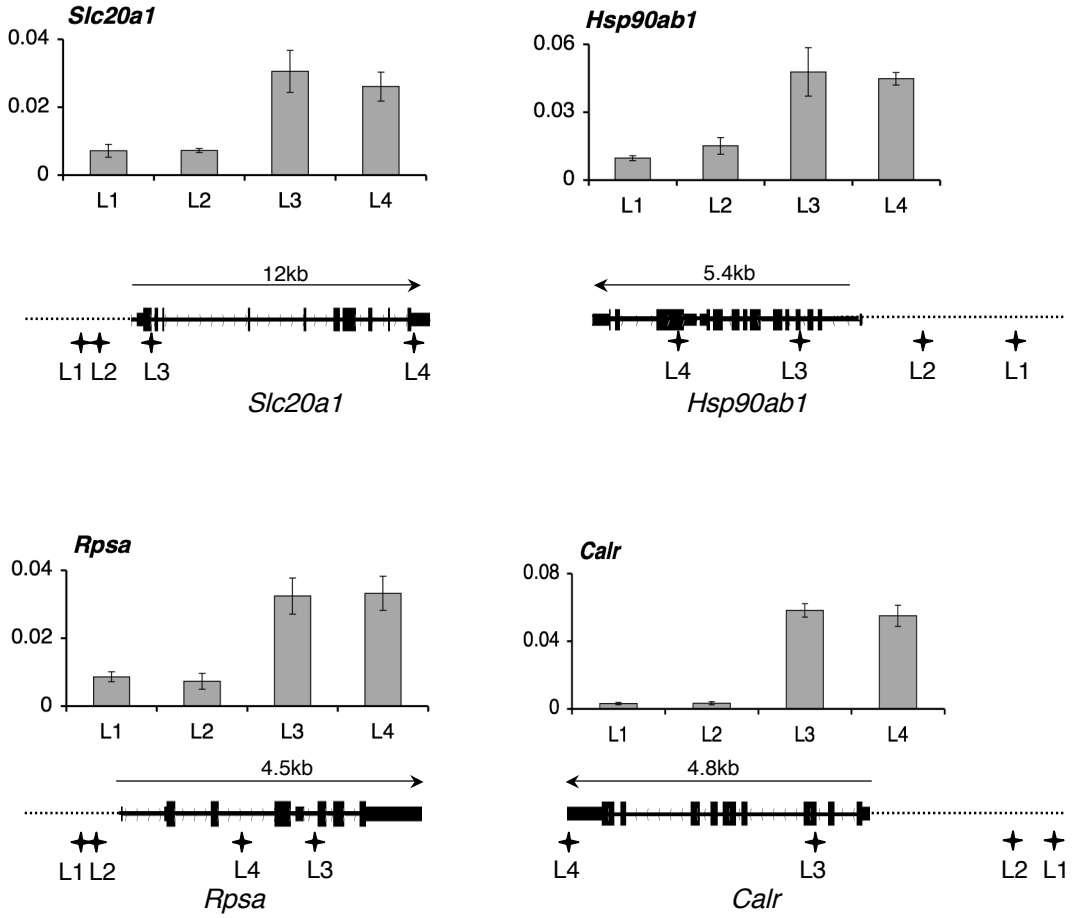
Supplemental Figure S5. Distribution of MacroH2A within the imprinted *Snurf-Snrpn* cluster. ChIP was performed using E17 brain chromatin prepared from fetuses conceived by crossing JF1/Ms males x C57BL/6 females. MacroH2A distribution onto the paternally and maternally contributed *Snurf-Snrpn* genomic regions is indicated by grey squares and black circles, respectively. Data are expressed as relative abundance and are normalized to input. Values are mean ± SD (n=3). Arrows indicate positions in the *Snurf-Snrpn* IC.



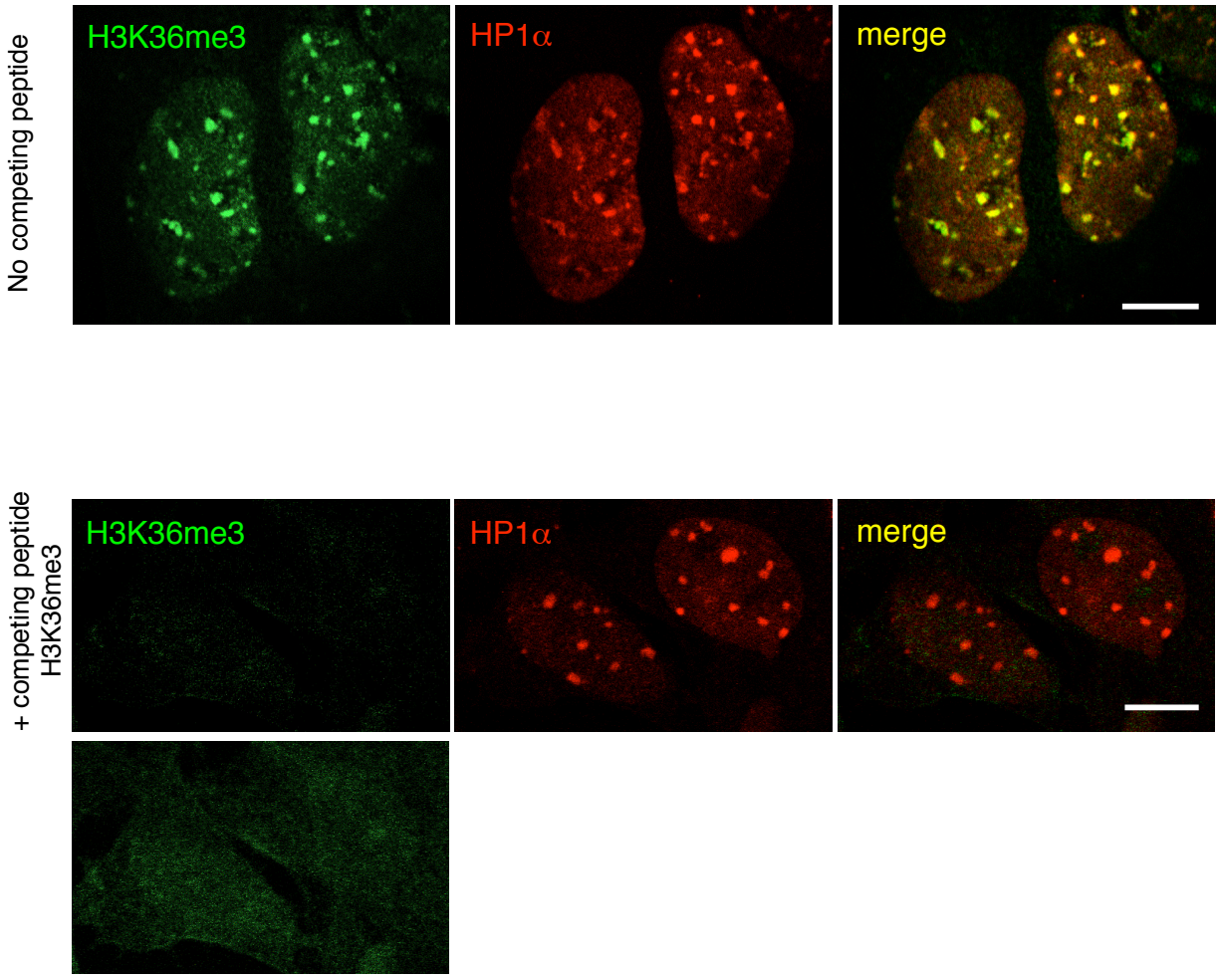
Supplemental Figure S6. Western blot analysis of anti-H3K36me3 antibodies quality.
(A) Coomassie blue-stained gel of recombinant histone H3/H4, and chromatin extracts from ES and MEF cells. Recombinant histones (*) H3 and H4 are His and Flag-tagged, respectively, and thus migrate slightly slower than native histones. Numbers indicate the amount (μg) of protein loaded on the gel. (B) A gel loaded as described above was migrated and transferred onto a nitrocellulose membrane, which was then stained with Ponceau S red. (C) The membrane was incubated with an antibody against H3K36me3 (Abcam 9050 lot 475787), which recognized a single band at the level of histone H3 in native chromatin from MEF and ES cells, but not in recombinant H3/H4. Identical results were obtained with a second lot of anti-H3K36me3 (Abcam 9050, lot 712985, data not shown). (D) The membrane was stripped and then incubated with an antibody against histone H3. (E) Histones were extracted from a wild-type (WT) *S. cerevisiae* strain, as well as from the K36A yeast strain that expresses a mutant histone H3, in which lysine 36 is substituted by an alanine residue. Histones were separated by SDS-PAGE, and transferred onto a nitrocellulose membrane, which was subsequently incubated with the antibody against H3K36me3 (Abcam 9050, lot 653080). The K36A substitution in histone H3 resulted in the loss of the band revealed by anti-H3K36me3.



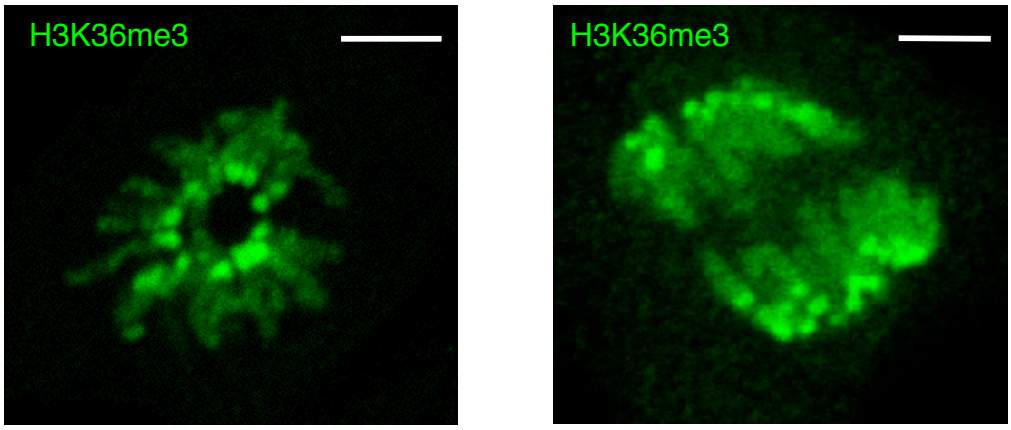
Supplemental Figure S7. Histone modifications and gene expression in liver tissue. (A) Distribution of H3K4me2, H4K20me3 and H3K36me3 within the imprinted *Snurf-Snrpn* cluster in the liver at post-natal day 1. ChIP was performed using P1 liver chromatin prepared from fetuses conceived by crossing JF1/Ms males x C57BL/6 females. Detection of the three histones marks onto the paternally and maternally contributed *Snurf-Snrpn* genomic region is indicated by grey squares and black circles, respectively. Data are expressed as relative abundance and are normalized to input. qPCR experiments were performed in triplicates. Arrows indicate positions in the *Snurf-Snrpn* IC. (B) Relative expression of the genes located into or in the vicinity of the *Snurf-Snrpn* cluster in the brain (in red) and in liver (in Blue) at post-natal day 1. Note that all genes within the *Snurf-Snrpn* region are expressed at much lower levels in the liver compared to the brain. Identical results were obtained from reciprocal crosses. Data are expressed as relative expression after normalization with *Actb* gene. Values are mean ±SD (n= three independant experiments).



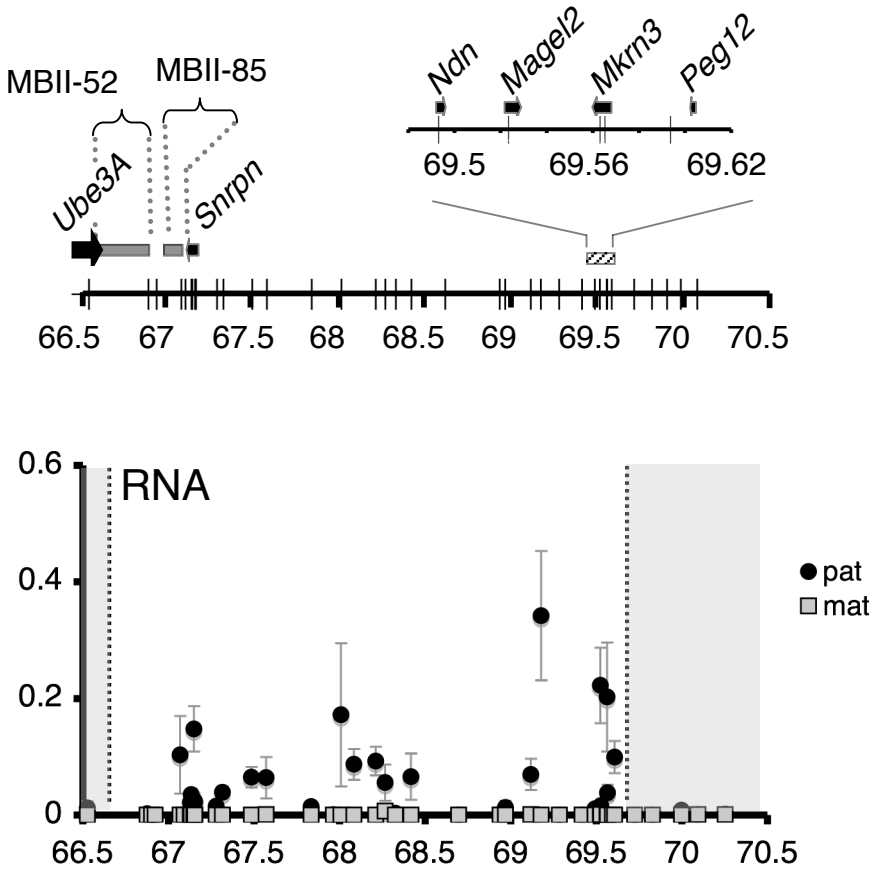
Supplemental Figure S8. Distribution of H3K36me3 in the transcribed regions of *Slc20a1*, *Hsp90ab1*, *Rpsa* and *Cair* genes and in their corresponding 5' upstream region. ChIP was performed using E17 brain chromatin prepared from fetuses conceived by crossing JF1/Ms males x C57BL/6 females. We used the same H3K36me3-immunoprecipitated DNA samples used in figure 2 for PCR amplifications, using primers specific for each of the indicated genes. Data are expressed as relative enrichment and are normalized to input. Values are mean ± standard deviation (n=3). The y-axis indicates the H3K36me3 relative enrichment for the four tested positions (L1 to L4). Genes are indicated as black lines and 5' upstream regions as dashed lines. Exons, 5' and 3' UTR are indicated as black boxes. A representative experiment is shown.



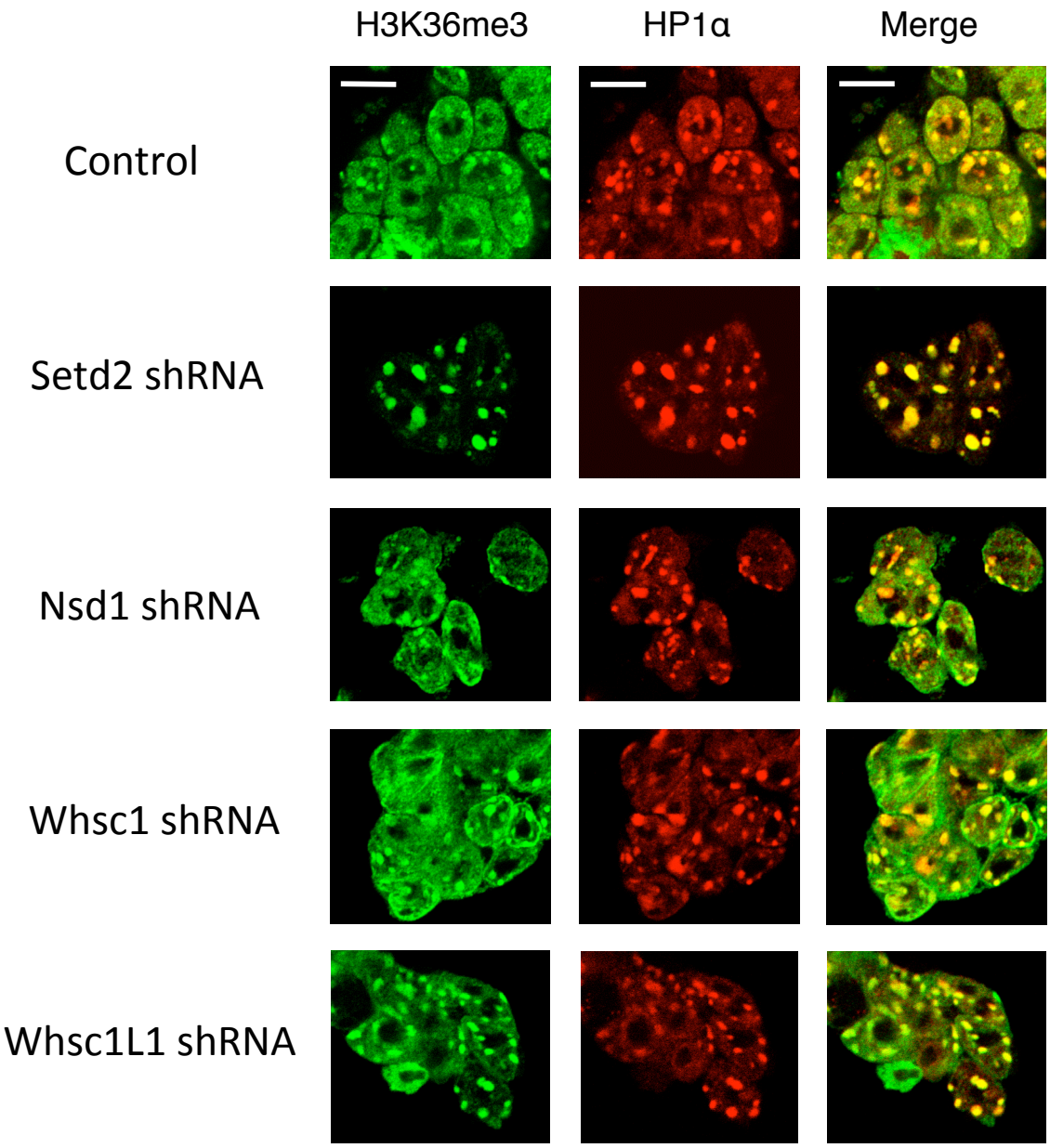
Supplemental Figure S9. Immunofluorescence confocal microscopy analysis showing the specificity of H3K36me3 association with pericentromeric heterochromatin in MEFs. Upper pannel: H3K36me3 is distributed throughout the nucleoplasm and co-localizes with HP1α at chromocenters. Bottom pannel: Immunofluorecence detection of H3K36me3 was performed in the presence of a competing H3 peptide bearing the K36me3 modification. H3K36me3 signal is lost both at chromocenters and in the nucleoplasm, even if we increase background levels (image at the bottom). Scale bar represents 10μm.



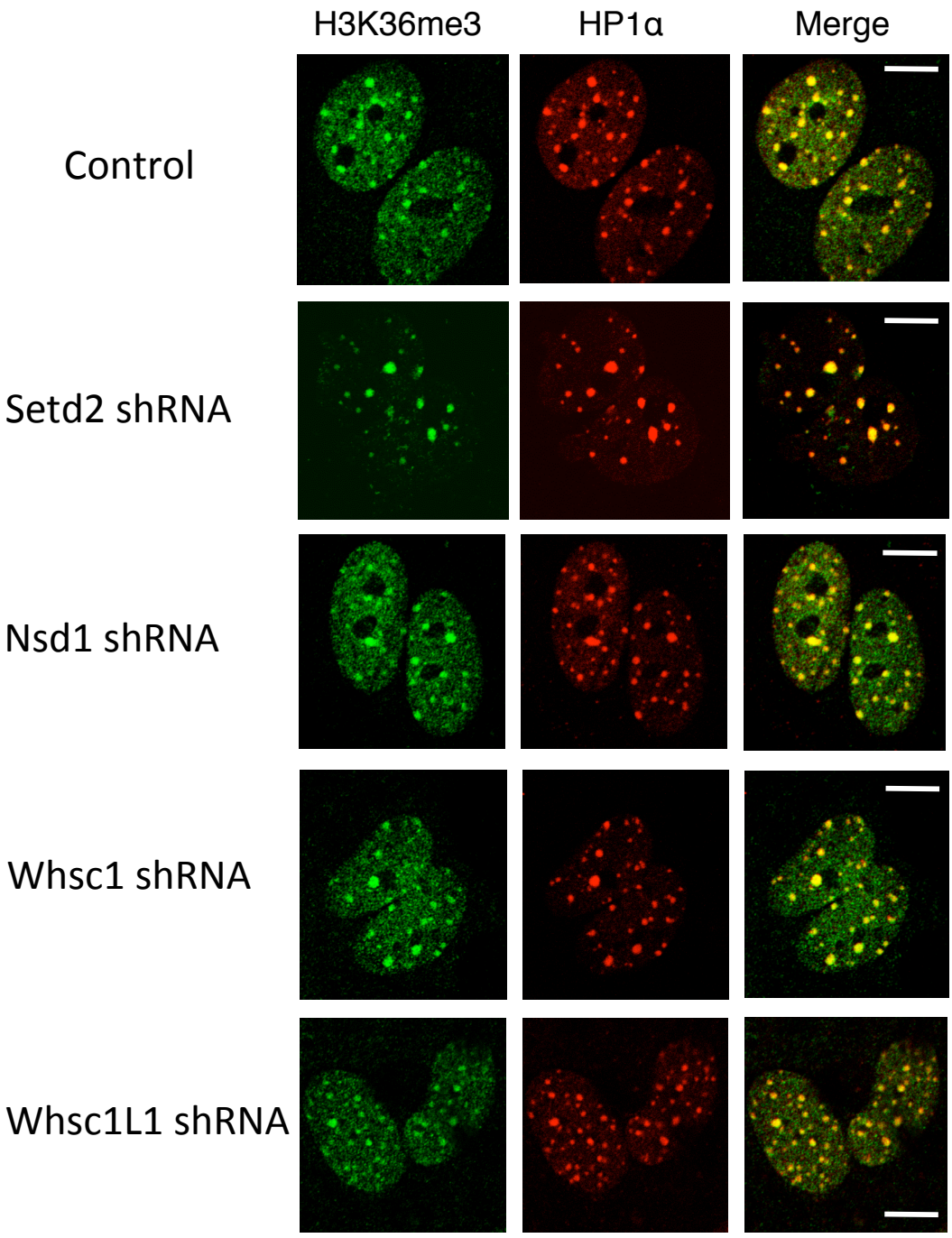
Supplemental Figure S10. Immunofluorescence confocal microscopy analysis of H3K36me3 distribution in MEFs during mitosis. Scale bar represents 5 μm.



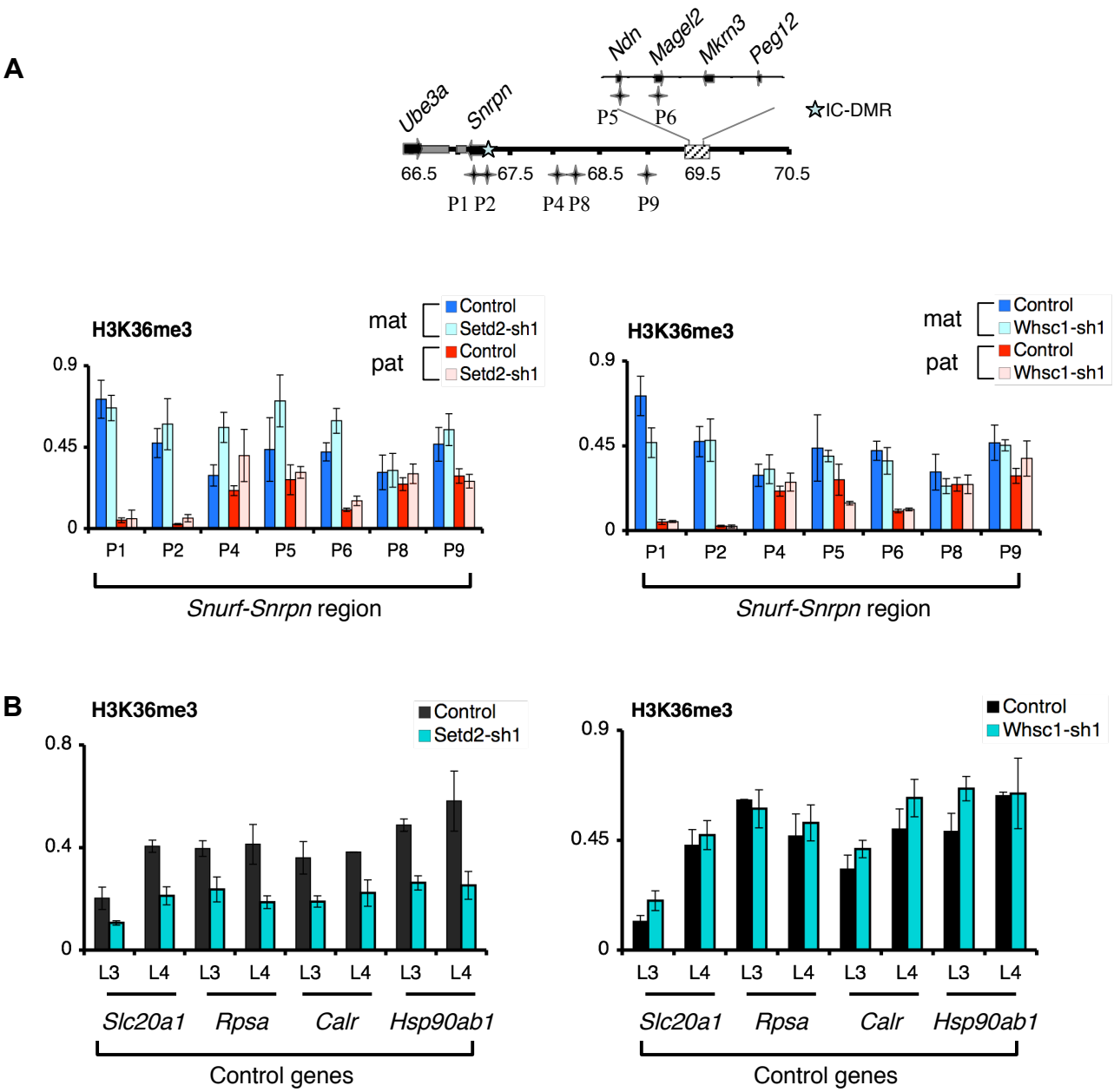
Supplemental Figure S11. Quantitative RT-PCR analysis of fetal brain RNAs expressed in the *Snurf-Snrpn* imprinted domain. Brains were dissected from fetuses conceived by mating C57BL/6 males with JF1/Ms females. Relative levels of RNAs expressed from the paternally (black circles) and maternally (grey squares) contributed *Snurf-Snrpn* genomic regions were quantified using our set of primer pairs that discriminates C57BL/6 and JF1/Ms genomes, and normalized to *Hprt* mRNA. Values are the mean of three independent experiments ± standard deviation.



Supplemental Figure S12. Immunofluorescence confocal microscopy analysis of H3K36me3 distribution in ES cells following shRNA-mediated knock-down of Setd2 family members. ES cells were transfected with shRNA vectors that target *Setd2*, *Nsd1*, *Whsc1*, and *Whsc1L1* mRNAs. H3K36me3 distribution (in green) was compared to the distribution of HP1α (in red) to reveal pericentromeric heterochromatin regions. Green and red signals were merged in the right panels. Loss of *Setd2* and *Nsd1* function severely inhibited ES cell growth, and resulted in smaller colonies. Knock-down of *Setd2* resulted in a reduction of H3K36me3 staining in the nucleoplasm of ES cells (58% of the cells displayed a reduction of H3K36me3 staining, see supplementary methods), but not at the level of chromocenters. Scale bars represent 10 μm.



Supplemental Figure S13. Immunofluorescence confocal microscopy analysis of H3K36me3 distribution in MEF cells following shRNA-mediated Knock-Down of Setd2 family members. MEF cells were transfected with shRNA vectors that target *Setd2*, *Nsd1*, *Whsc1*, and *Whsc1L1* mRNAs. H3K36me3 distribution (in green) was compared to the distribution of HP1α (in red) to reveal pericentromeric heterochromatin regions. Green and red signals were merged in the right panels. Knock-down of *Setd2* resulted in a reduction of H3K36me3 staining in the nucleoplasm of MEF cells (63% of the cells displayed a reduction of H3K36me3 staining, see supplementary methods), but not at the level of chromocenters. Scale bars represent 10 μm.



Supplemental figure S14. Knock-down of *Setd2* and *Whsc1* does not affect the distribution of H3K36me3 within the *Snurf-Snrpn* imprinted cluster in MEFs cells. (A) Distribution of H3K36me3 was analyzed by ChIP experiments using chromatin from MEFs obtained from embryos conceived by mating C57BL/6 females with JF1/Ms males. Cells were transfected at passage 3 with shRNA vectors against Setd2 (Setd2-sh1) or Whsc1 (Whsc1-sh1), or a control shRNA vector. Black stars indicate the locations of primers P1 to P9. P1 and P2 primer sets are amplifying DNA fragments within the IC-DMR. (B) H3K36me3 relative enrichment was analyzed in four control genes, using the same immunoprecipitated DNA samples as in (A). L3 and L4 positions, located in transcribed regions, are described in Supplemental figure S8. qPCR experiments were performed in triplicates. Values are mean ± SD.