

**Supplementary Figure S1. Isolation of 2C and 4C neuronal nuclei.** Freshly prepared cell nuclear populations from P15 cerebral cortices labeled with a NeuN-specific antibody and propidium iodide are shown in the upper plot (propidium iodide-area vs. propidium iodide height). Neuronal nuclei included in the box can be sorted out giving rise to two nuclear populations highly enriched in 2C (lower left plot) and 4C (lower right plot) neuronal nuclei.



Supplementary Figure S2. Sanger sequencing methylation analysis of the *Peg3-* and *Snrpn-*imprinting control regions. DNA methylation patterns of individual bisulfite-sequenced clones of the *Peg3-* ( $\mathbf{A}$ ) and *Snrpn-* ( $\mathbf{B}$ ) controlling DMR are shown. Unmethylated CpG sites are indicated by open circles and methylated CpG sites are indicated by solid circles.

А



Supplementary Figure S3. CGH array analysis comparing genomic DNA isolated from 2C vs. 4C neuronal nuclei. CGH array analysis was performed with genomic DNA obtained from 2C and 4C neuronal cell nuclei. No deviation of the ratio plot from the modal value of 0 was found for all chromosomal domains, thus indicating that the genome is fully duplicated in tetraploid neurons.



**Supplementary Figure S4.** Methylation levels within the *Peg3* gene region (positions  $6,705 \times 10^6$ - $6,730 \times 10^6$  on mouse chromosome 7) as evidenced by illumina sequencing. PCD location is indicated as a light green rectangle. Green oval labels the PCD subdomain analyzed in this study. CpGs are illustrated as +.



**Supplementary Figure S5.** Analysis of representative libraries from sodium borohidride (BH)- or O-hydroxyethylamine (EA)-treated control 5fC-containing ds-DNA converted with bisulfite. The percentage of modified 5fC in the control DNA that reads as a cytosine after mock [(-) BH/EA], sodium borohydride [(+) BH] or O-hydroxyethylamine [(+) EA] treatment was measured after illumine sequencing. Mean  $\pm$  s.e.m. are shown.



Supplementary Figure S6. PCD methylformylation can maintain the monoallelic expression pattern of the Peg3 imprinted gene. (A) Scheme of the complementary breeding as that described in Figure 7. (B,C) Representative pyrograms from the cDNA sequence of 2C cortical neurons using the primer indicated with a dashed (B) or dotted (C) arrow in Figure 7A. The SNP (T/C) is shown in yellow. T from position 6 (T6) in (B), and G4 and T7 in (C) are negative controls. Means of the proportion of C5 (B) and C6 (C)  $\pm$  s.e.m are shown.