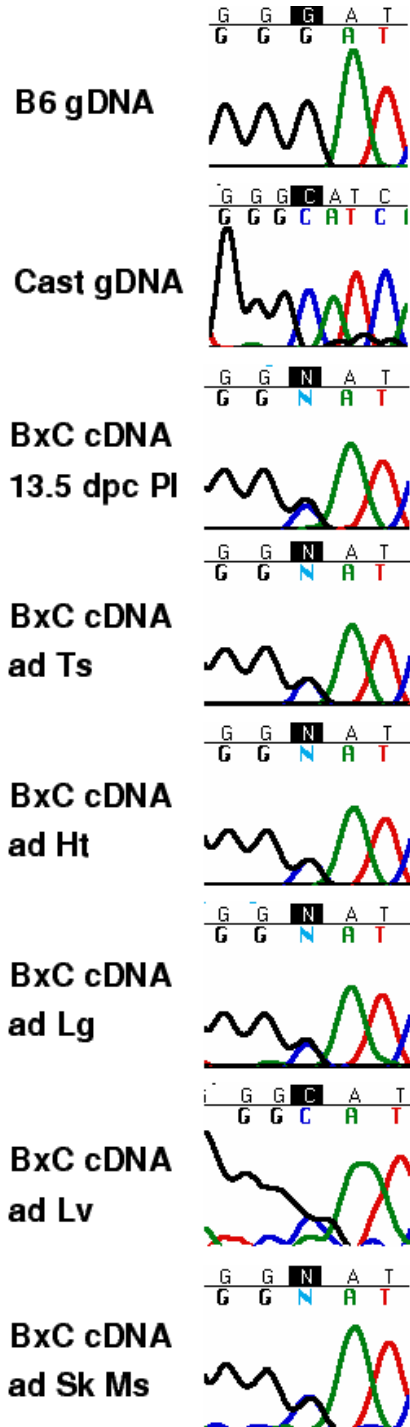
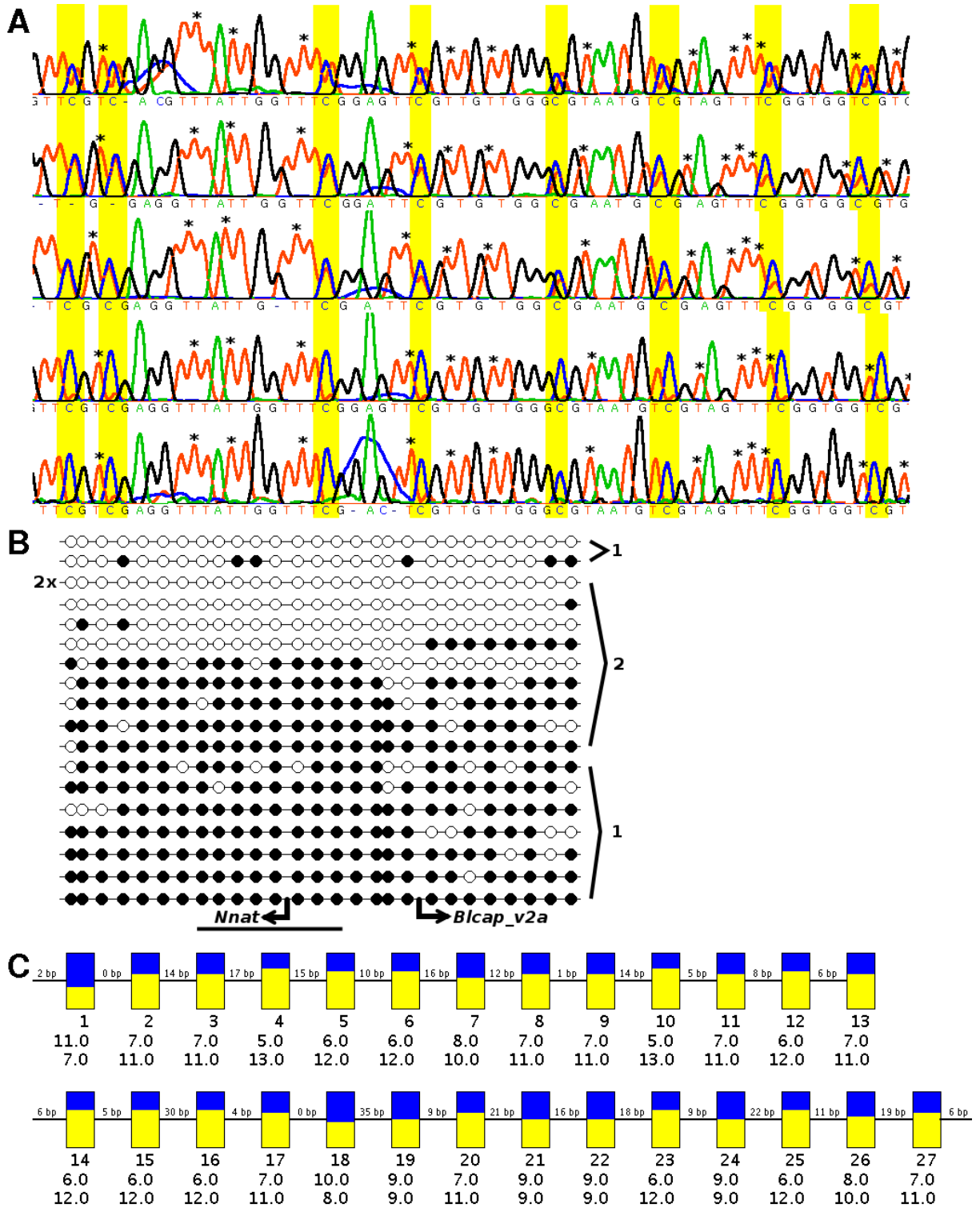


Supplementary Figure 1: Allele-specific sequencing of *Blcap_v1a* in 13.5 dpc placenta (PI) and adult (ad) tissues other than brain (Ts: testis, Ht: heart, Lg: lung, Lv: liver, Sk Ms: skeletal muscle) of BxC hybrids. The G/C SNP distinguishing the B6 and cast alleles is the same as in Figure 2B. For all of the tissues examined here, the double peaks in the sequencing traces show that *Blcap_v1a* was expressed biallelically.



Supplementary Figure 2: A Direct sequencing of products from independent PCR amplifications of the *Bicap_v2a-Nnat* promoter region from bisulfite-treated genomic DNA extracted from *Dnmt3l*^{+/+} 8.5 dpc embryos (first row) and *Dnmt3l*^{-/-} (second and third rows) as well as wildtype (last two rows) 7.75 dpc visceral yolk sac (VYS). Good quality sequence was obtained for a 60 bp region overlapping the *Nnat* promoter and containing eight CpG dinucleotides that have been highlighted in yellow (Chr2:157251532-157251592; Feb 2006 NCBI build 36). Asterisks mark the positions of all non-CpG cytosine residues prior to bisulfite conversion. The traces show only Ts at these positions, indicating a conversion reaction efficiency of close to 100%. For *Dnmt3l*^{+/+}, the traces show double C/T peaks at CpGs, while only Cs are present in the traces for wildtype. This is consistent with a loss of CpG methylation in *Dnmt3l*^{-/-} relative to wildtype samples and hence, *Dnmt3l*-dependent methylation of this region in oocytes. Complete maternal allele-specific methylation of the region has previously been demonstrated (9). We therefore expected to observe partial methylation in wildtype (C/T double peaks) and complete loss of methylation in *Dnmt3l*^{-/-} (Ts only). Our results however indicate a ~50% higher than expected methylation level in both wildtype and *Dnmt3l*^{-/-} samples, which likely reflects a systematic bias toward amplification of methylated template strands (41). **B** Methylation states of single DNA strands obtained by cloning and sequencing of the two PCR products generated from *Dnmt3l*^{-/-} 7.75 dpc VYS (second and third rows in **A**). Each strand is represented as a "beads-on-a-string" diagram where open and filled beads/circles correspond to unmethylated and methylated CpGs, respectively. The PCR amplicon covered the promoters of *Nnat* and *Bicap_v2a* (TSS's and direction of transcription are indicated at the bottom), and included 27 CpGs. The sets of strands recovered from each independent PCR are labelled as 1 or 2 on the right. All 9 strands in set 1 had a unique sequence; all but one of the 10 strands in set 2 were unique (third strand from the top occurred twice). On average, the conversion efficiency was >99%. Overall, 40% of CpGs were unmethylated, consistent with the direct sequencing results (horizontal line at the bottom indicates the CpGs included in **A**), and a single strand is typically either almost completely unmethylated or methylated. **C** Summary of the data in

B. Each CpG is shown as a box whose area is coloured blue and yellow in proportion to the number of times it was observed to be unmethylated and methylated (shown underneath each box, following the CpG number), respectively. The distance between neighboring CpGs is given in base pairs (bp).



References:

41. Warnecke, P.M., Stirzaker, C., Melki, J.R., Millar, D.S., Paul, C.L. and Clark, S.J. (1997) Detection and measurement of PCR bias in quantitative methylation analysis of bisulphite-treated DNA. *Nucleic Acids Res.*, **25**, 4422–4226.

Supplementary Figure 3: A Genomic organisation and features of the *Commd1/U2af1-rs1* locus on mouse proximal Chr 11. Shown are the exon-intron structures of relevant transcripts, CGIs, 430v2 microarray probe sets (regions interrogated by the constituent probes), SNP and primer positions. *U2af1-rs1* is a single exon gene transcribed antisense relative to *Commd1*. **B** Allele-specific sequencing of *Commd1_v2b* in BxC and CxB inter-subspecies hybrid newborn brain cDNA samples (right). A G/A SNP in the first exon of *Commd1_v2b* (left: genomic DNA traces) enabled the determination of the parental allele of origin of the transcripts. The presence of only the A allele in the BxC sample and only the G allele in the CxB sample indicates paternal allele-specific expression of *Commd1_v2b*.

