1. Supplementary Figures

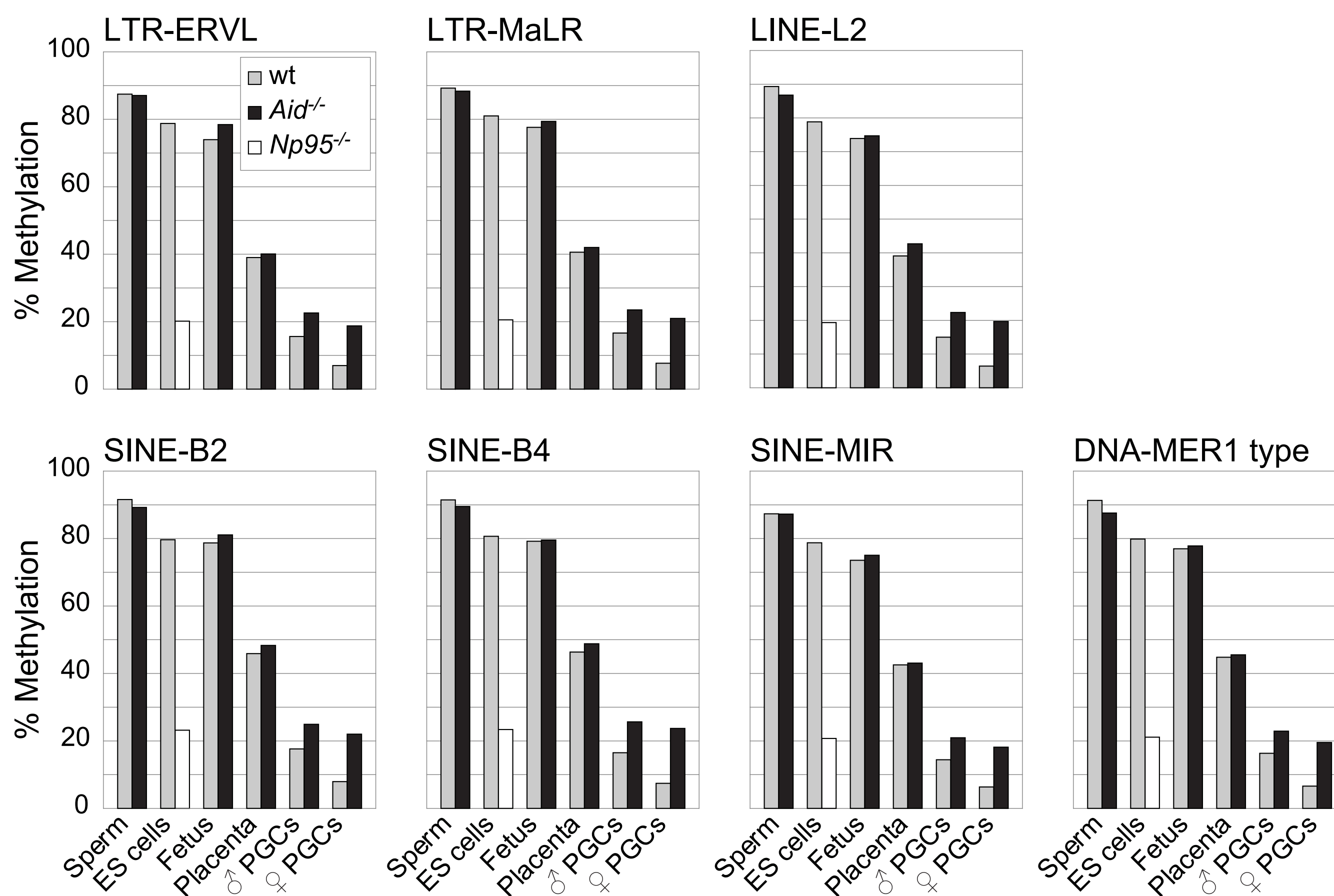


Figure S1 *DNA methylation levels in transposable elements*
Methylation levels of different classes of transposons in ES cells, *Np95*$^{-/-}$ ES cells, and various tissues of C57BL/6J and *Aid*$^{-/-}$ knockout mice are shown based on ratios of methylated to unmethylated BS-Seq reads. Placenta, fetal carcass and PGCs were all collected at day E13.5. Note that Aid deficient PGCs remain more methylated in all retrotransposon families analysed.

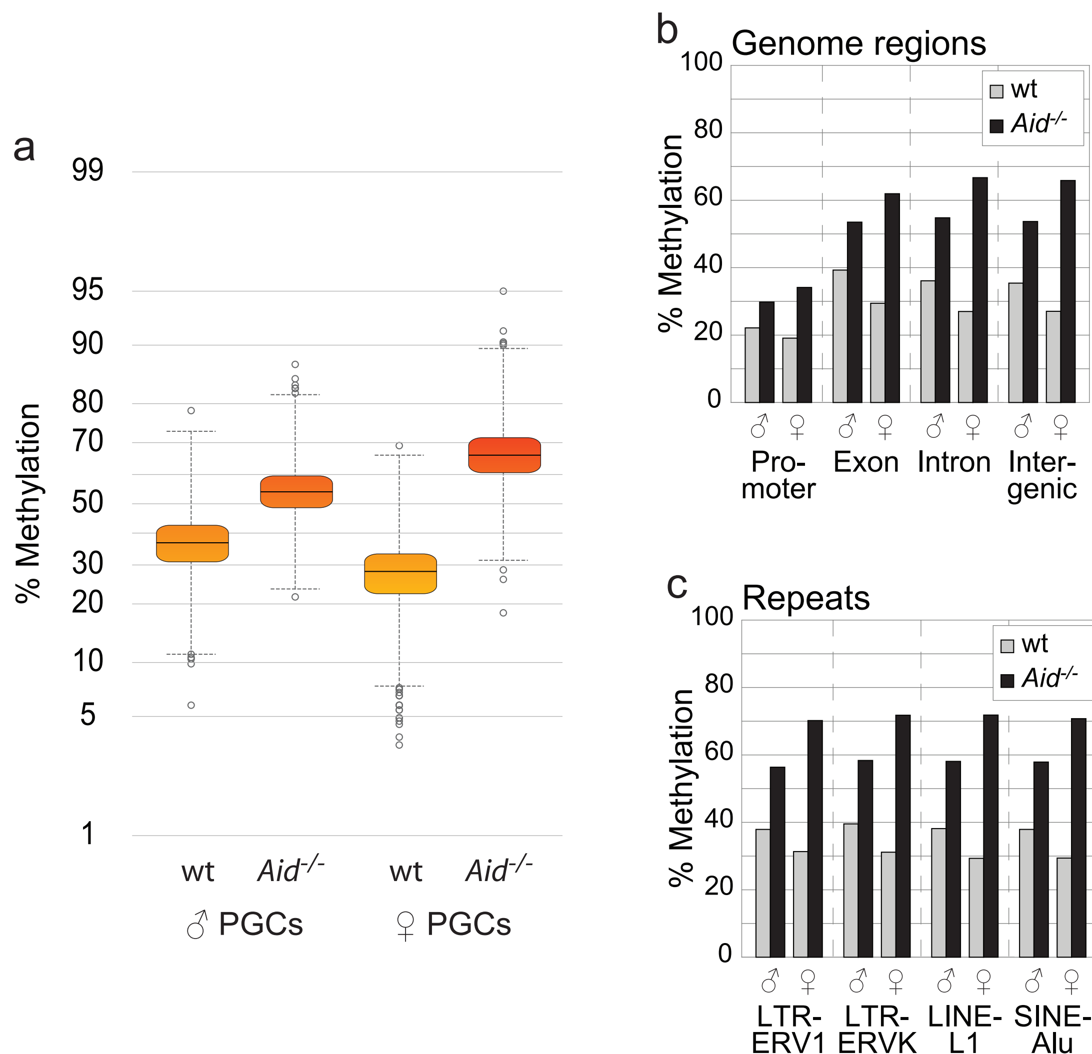
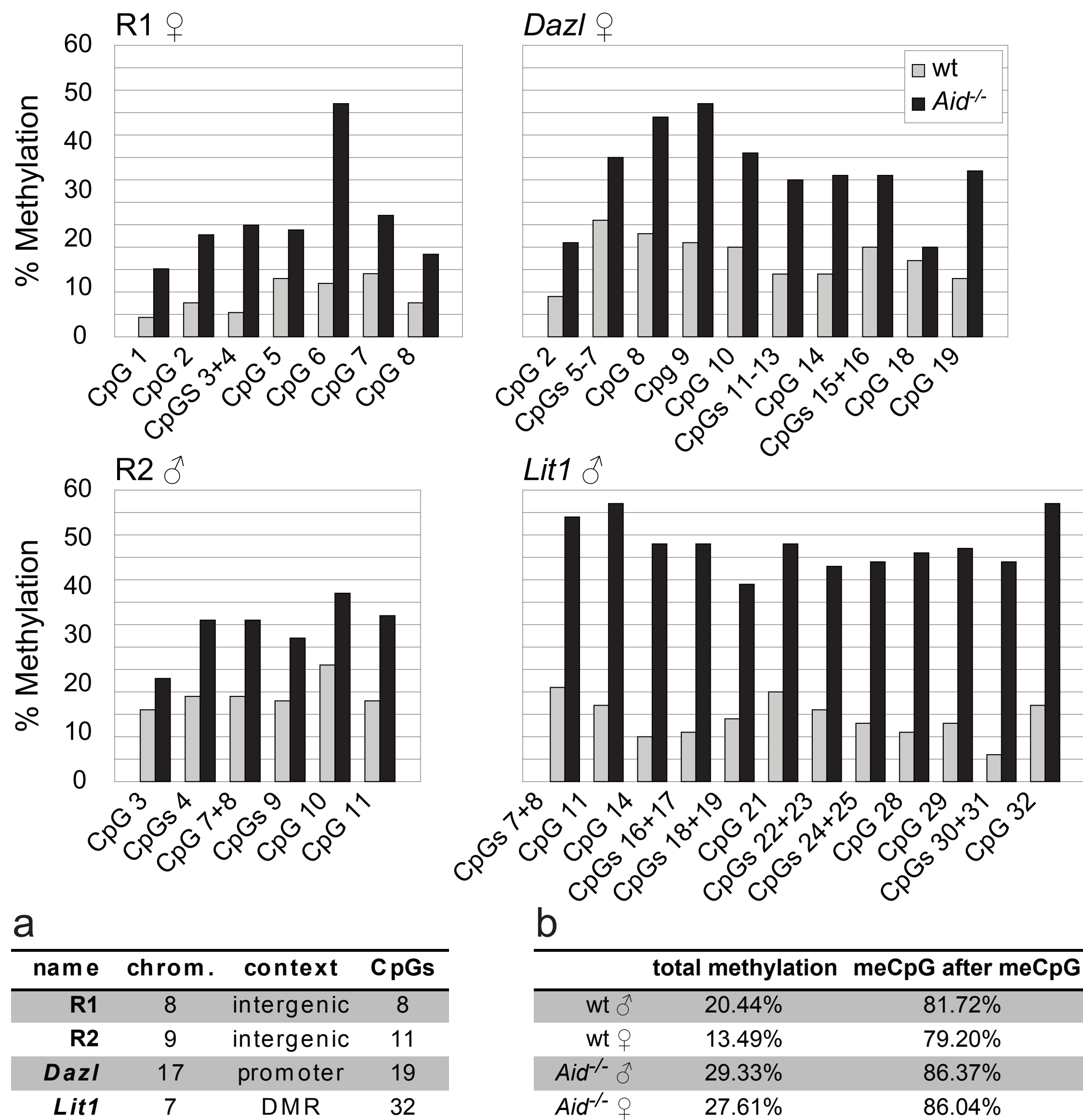


Figure S2 *Global methylation levels and methylation in different genomic elements of manually collected E13.5 PGCs analysed by BS-Seq*

a, BS-Seq reads of manually collected E13.5 PGCs from C57BL/6J and *Aid*$^{-/-}$ knock-out mice were analysed using windows of 250 kilobases across the whole genome. A total of 4140 such windows containing >10 Solexa reads and <5 exact duplicate reads in all tissues were measured. Boxes show the range of the 25-75th quartiles of the data and the line in the middle of each box is the median value. The upper and lower whiskers show either the absolute highest and lowest values in the data (if there are no outliers) or the upper and lower confidence intervals. Any outliers are shown as circles. Note the substantially higher levels of methylation in *Aid* deficient PGCs, reproducing qualitatively the results obtained from FACS-sorted PGCs. **b** Methylation levels in promoters, exons, introns and intergenic regions (**b**) and of different classes of repetitive elements (**c**) in E13.5 PGCs of C57BL/6J and *Aid*$^{-/-}$ knockout mice are shown based on ratios of methylated to unmethylated BS-Seq reads. Placenta, fetal carcass and PGCs were all collected at day E13.5.



a

name	chrom.	context	CpGs
R1	8	intergenic	8
R2	9	intergenic	11
Dazl	17	promoter	19
Lit1	7	DMR	32

b

	total methylation	meCpG after meCpG
wt ♂	20.44%	81.72%
wt ♀	13.49%	79.20%
Aid$^{-/-}$ ♂	29.33%	86.37%
Aid$^{-/-}$ ♀	27.61%	86.04%

Figure S3 *High resolution methylation profiles in wild type and Aid*$^{-/-}$ *E13.5 PGCs*
Methylation levels are shown for CpGs in individual fragments as detected by Sequenom EpiTYPER for amplicons R1, R2, *Dazl* and *Lit1* in male or female PGCs at E13.5. Most fragments contain one CpG while some contain multiple (up to three) CpGs. The total number of CpGs per amplicon is shown in Table **a** (note that not every CpG per amplicon can be analysed with this technique). Table **a** also indicates location and genomic context of each amplicon. Note that methylation of most CpGs increases uniformly in *Aid*$^{-/-}$ PGCs. In addition we have analysed BS-Seq reads with two or more CpGs from wild type and *Aid*$^{-/-}$ PGC libraries (more than 500K reads each) to determine the level of correlation between the methylation state of adjacent CpGs. This shows that the majority of methylated CpGs is followed by a methylated CpG (Table **b**) and only a small fraction of methylated CpGs is followed by an unmethylated CpG. Most importantly, the correlation between the methylation state of a CpG and its neighbour is very high in all samples, despite the difference in global methylation, supporting the notion of an 'all or none' change in methylation patterns rather than a difference in the density of methylation within molecules.

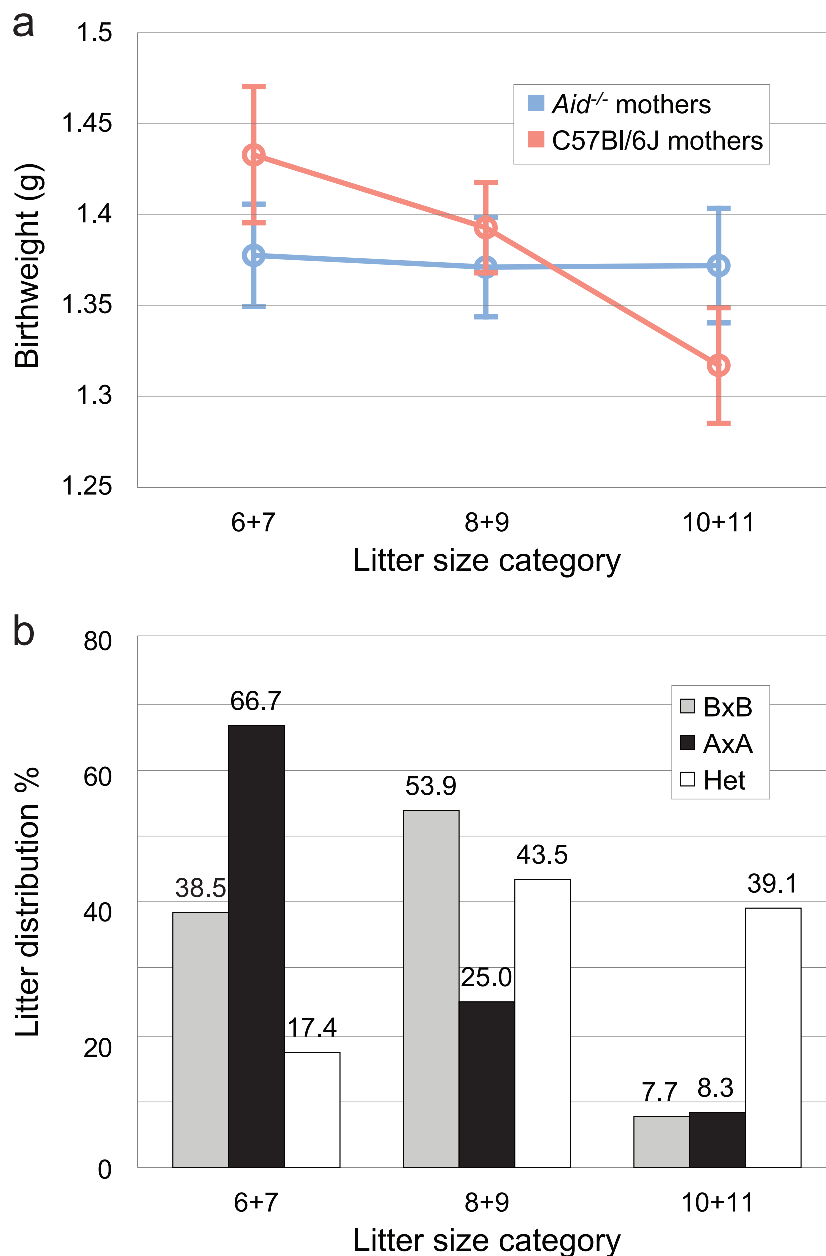


Figure S4 *Birth weight and Litter size*
Birth weights and litter sizes of crosses between *Aid*$^{-/-}$ females and males (AxA; 12 litters), wild type C57Bl/6J females and males (BxB; 13 litters), *Aid*$^{-/-}$ females and C57Bl/6J males (AxB; 12 litters) and the reciprocal cross (BxA; 11 litters) were recorded and split in categories of 6 + 7, 8 + 9 and 10 + 11 pups. **a,** Mean weights with confidence intervals of pups from combined litters of *Aid*$^{-/-}$ mothers (AxA and AxB) and C57Bl/6J mothers (BxA and BxB) are shown. Note that the birth weight of pups from wild type mothers is dependent on the litter size (ANOVA, p=0.001 for both BxB and BxA) while this is not true for pups from *Aid*$^{-/-}$ mothers (AxA and AxB; ANOVA, p=0.812 and p=0.868, respectively). **b,** The distribution of litters from different crosses (AxA, BxB and heterozygous (Het) = AxB and BxA combined) over all three litter groups is shown in percent, individual values are given above each bar. Note that AxA and BxB crosses produce smaller litters than heterozygous (Het) crosses (Chi2, p=0.019).

2. Supplementary Methods

Manual collection of PGCs. For a second, independent, PGC isolation and BS-Seq experiment, PGCs were collected from sexed E13.5 embryos as described[31]. Urogenital ridges were dissected and the mesonephros removed, leaving just the gonad. Gonads were sorted by sex and washed briefly in PBS (phosphate buffered saline; calcium and magnesium free) and then incubated in PBS + 0.6mM EDTA for 20-30 minutes. Next they were washed in M2 medium (Sigma) and were gently disrupted with a fine needle, taking care not to tear the tissue, in order to liberate the PGCs but leave somatic cells in place. After collection of PGCs from Oct4-Gfp transgenic mice samples were checked for green fluorescence. Samples of Aid-/- knockout PGCs were fixed and stained with alkaline phosphatase. On average, PGC preparations contained >90% germ cells as judged by morphological characteristics, green fluorescence and/or positive alkaline phosphatase staining.

Mouse weights and statistical analysis. For the collection of litter sizes and weights of pups at birth mice (*Aid-/-* x *Aid-/-*, *Aid-/-* x C57BL/6J, C57BL/6J x *Aid-/-*, C57BL/6J x C57BL/6J) were set up in pairs and males were removed before litters were born. Litters of 6-11 pups were used for analysis. Females were not mated to the same male more than once. For statistical analysis of litter size and birth weight the data were grouped into categories of 6 + 7, 8 + 9 and 10 + 11 pups, because they were not suitable for parametric analysis. To determine statistical significance of litter size distribution over all four crosses, a Chi-square test was applied. Birth and weaning weights were tested for significant changes by applying ANOVA tests. There was variability of pup birth weight across litters of the same parental cross and category, but this variability was ignored for further analysis as it was generally small.

31. Buehr, M. & McLaren, A. Isolation and culture of primordial germ cells. *Meth. Enzymol.*, **225**, 58–77 (1993).