

Table S1. Primer sequences for real-time PCR

Gene	5' Primer (5'-3')	3' Primer (5'-3')
<i>Actb</i>	GATCATTGCTCCTCCTGAGC	ACTCCTGCTTGCTGATCCA
<i>Cer1</i>	GGTTCTGGCATCGGTTCATG	GCAGGTCTCCCAGTGTACTT
<i>Sox17</i>	TGAACCTCTCAGACAGCCAC	CAAATGTCGGGGTAGTTGCA
<i>Snail</i>	CCACTGCAACCGTGCTTTT	CACATCCGAGTGGGTTTGG
<i>cdh11</i>	CATCACTGCCACCCAGAAGACTG	ATGCCAGTGAGCTTCCCGTTCAG
<i>Elf5</i>	GGTTCTGGCATCGGTTCATG	GCAGGTCTCCCAGTGTACTT

Table S2. Categories used in the present study

Category	Detailed Annotation in HOMER	Subfamilies included in the Detailed Annotation
promoter	promoter-TSS	
5'UTR	5'UTR	
exon	exon	
3'UTR	3'UTR	
TTS	TTS	
LTR	including LTR	IAPE, IAPLTR, RLTR, RMER, MME, etc.
LINE	including LINE	L1MdA, L1MdF, L1MdT, L1MdGf, L1Mus, etc.
SINE	including SINE	
Intron	intron	
Intergenic	Intergenic	
Others	not including annotations above (Other, RNA, DNA, miRNA, ncRNA, tRNA, rRNA, pseudo, CpG, Unknown, Satellite, etc.)	

Table S3. Average methylation levels of CpGs in the duplicate experiment (Exp. 2)

Genome	Methylation level (%)		
	Control	Arsenic	P-value*
Genome-unspecified	44.07	43.66	$<2.2 \times 10^{-16}$
Paternal	46.97	46.54	6.3×10^{-4}
Maternal	48.82	48.39	1.9×10^{-4}

*The difference between the methylation levels in the two groups was assessed by Welch's *t* test.

Supplement figures

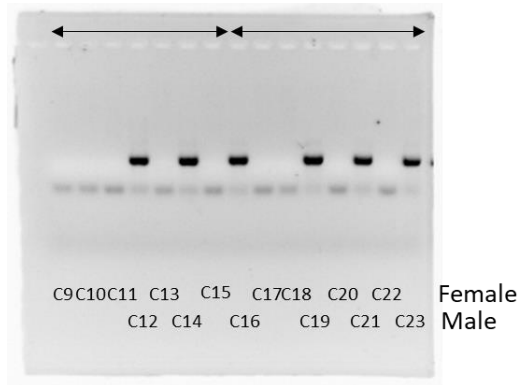


Fig. S1. Sex determination. DNA from each embryo was amplified by PCR with *Zfy* primers and examined by gel electrophoresis using 1.2% agarose gel. The gel image shows the PCR products from 15 embryos obtained from two litters.

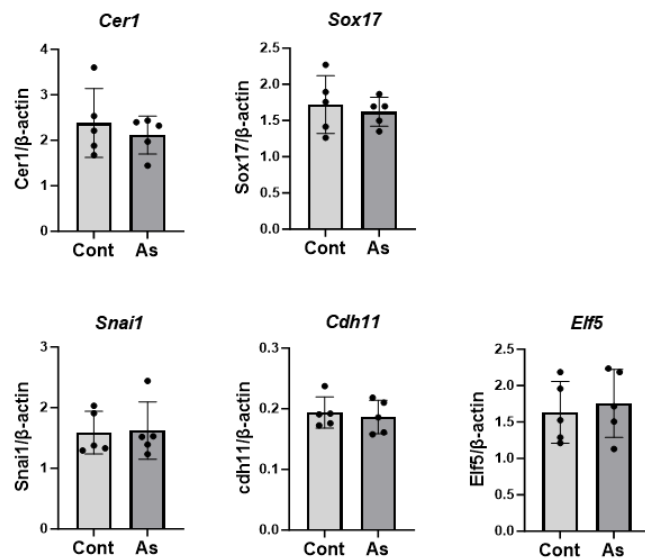


Fig. S2. The expression levels of development-associated genes. The genes whose expressions are characteristic for the developmental stage of mice embryos on E7.5 were investigated: *Cer1*

and *Sox17* for endoderm, *Snail* and *cdh11* for mesoderm, *Elf5* for extraembryonic ectoderm.

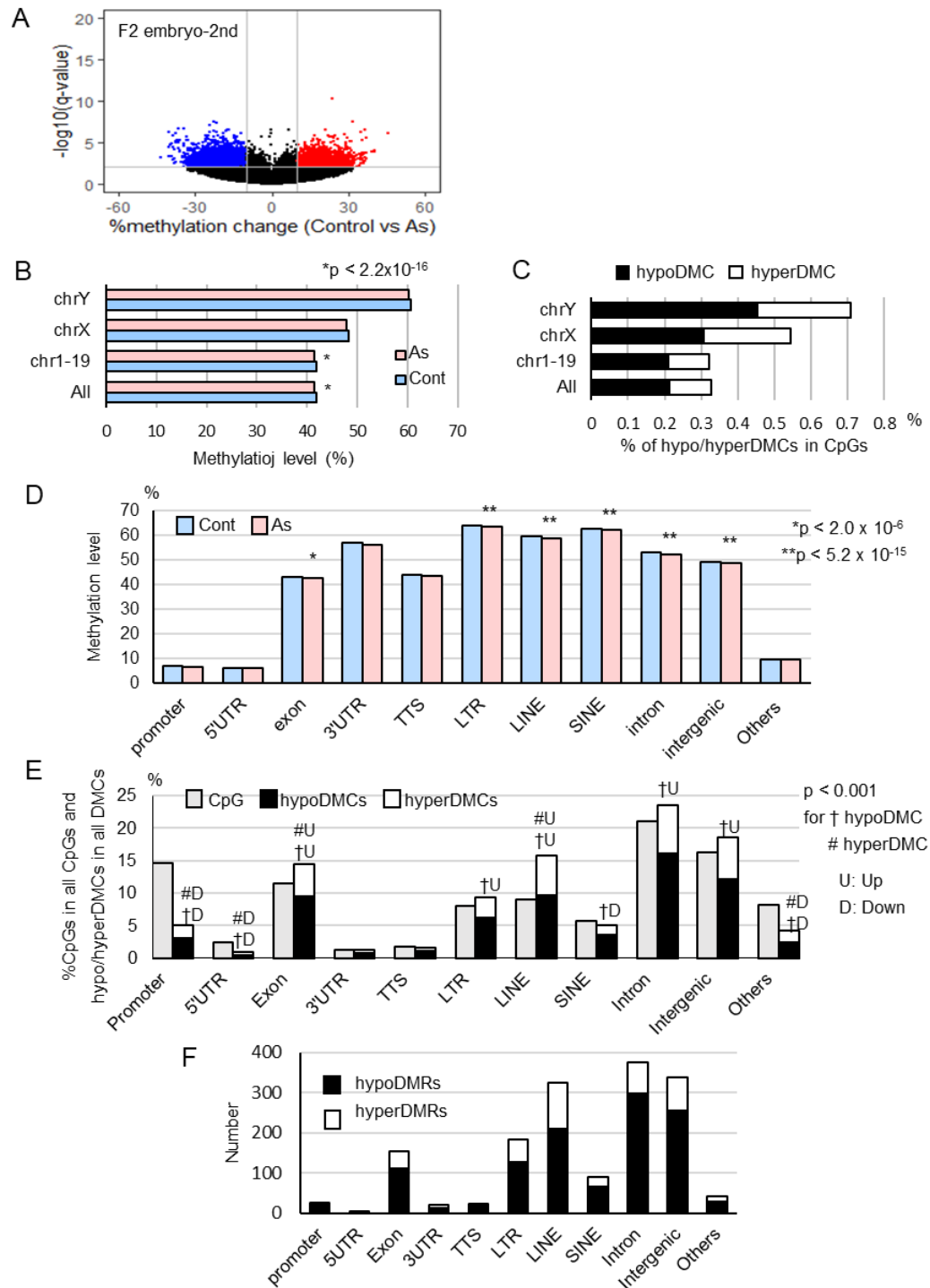


Fig. S3. Methylome profiles of F2 embryos in the control and arsenic groups in an independent second experiment. Descriptions for figures are the same as those for Fig. 2 and 3.

A) Volcano plot for the differences in the methylation levels of CpG sites between the control and arsenic groups. **B)** Average methylation levels of all CpGs. **C)** Percentages of hypo/hyperDMCs in all CpGs. **D)** Average methylation levels of all CpGs in the annotated regions. **E)** Distribution (%) of detailed annotated regions among all CpGs (gray bars) and among all DMCs (hypoDMCs (black bars) plus hyperDMCs (white bars)). Please see the legend for Fig. 3B. **F)** Number of hypo/hyperDMRs assigned in the individual annotated regions.

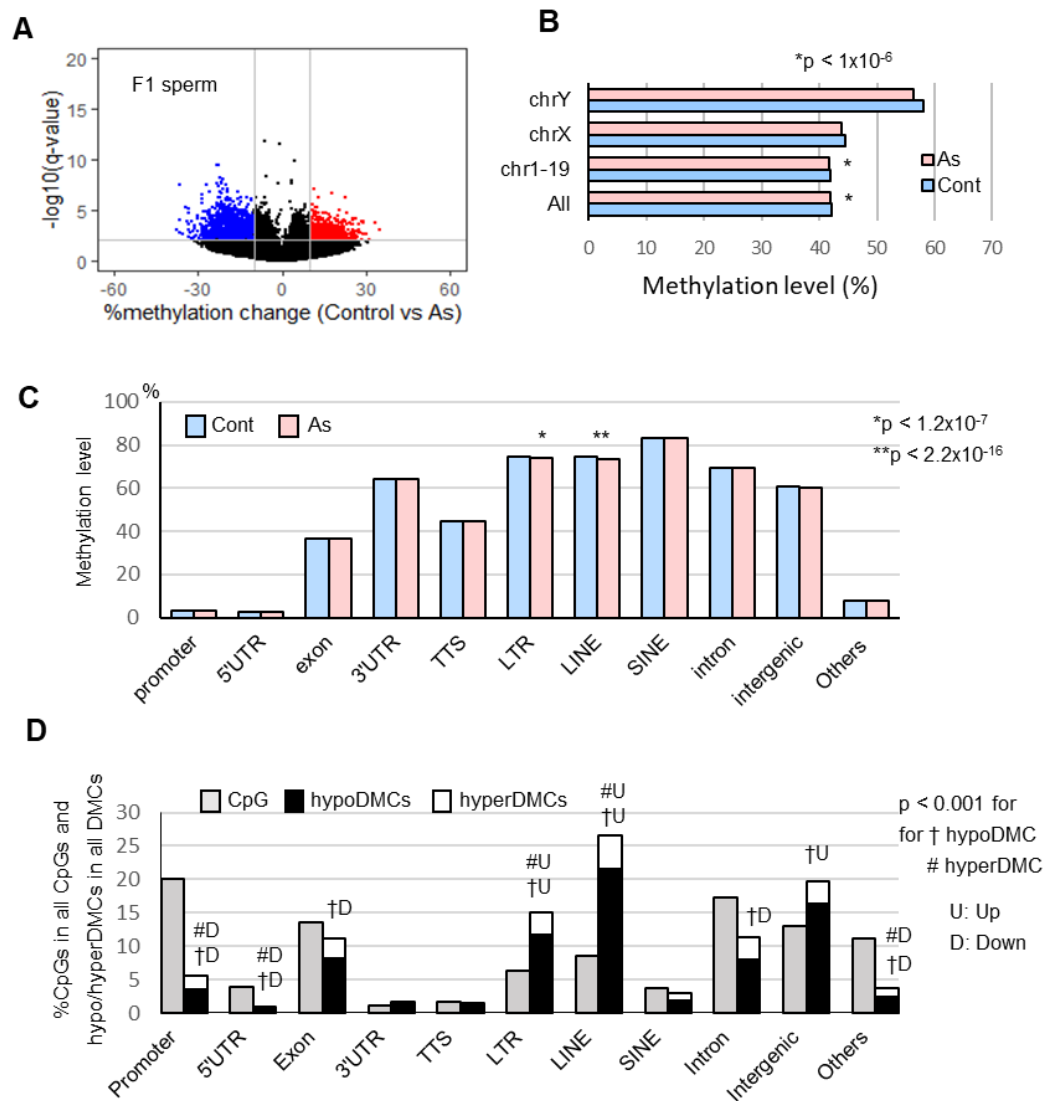


Fig. S4. Methylome profiles of F1 sperm in the control and arsenic group. The RRBS data (GSE150650) obtained in our previous study (Nohara et al. 2020) was edited by excluding those assigned in chromosomes other than chr1-19, chrX and chrY, and reanalyzed. Descriptions for figures are the same as those for Fig. 2 and 3.

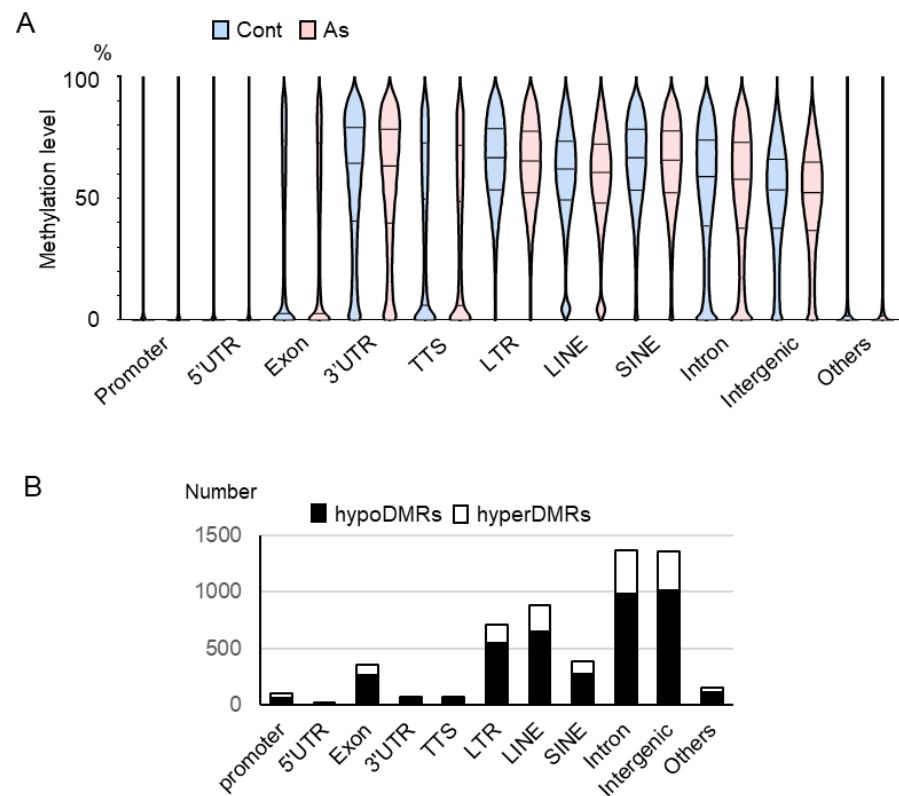


Fig. S5. Additional methylome profiles of F2 embryos in the control and arsenic groups. A)

Violin plots of average methylation levels of all CpGs in each subfamily in LINE and LTR. Bold lines represent medians and thin lines represent quartiles. **B)** Number of hypo/hyperDMRs in the individual annotated regions detected using methylKit.

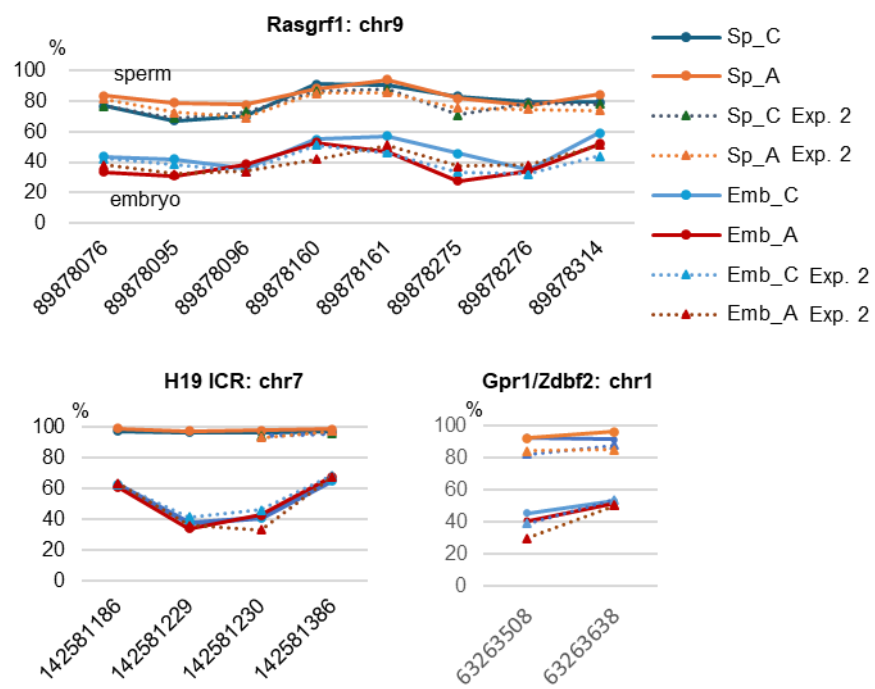


Fig. S6. DNA methylation levels of CpGs identified in the paternal imprinted regions. The results of independent two experiments for F1 sperm (Sp) and F2 embryos (Emb) were plotted.

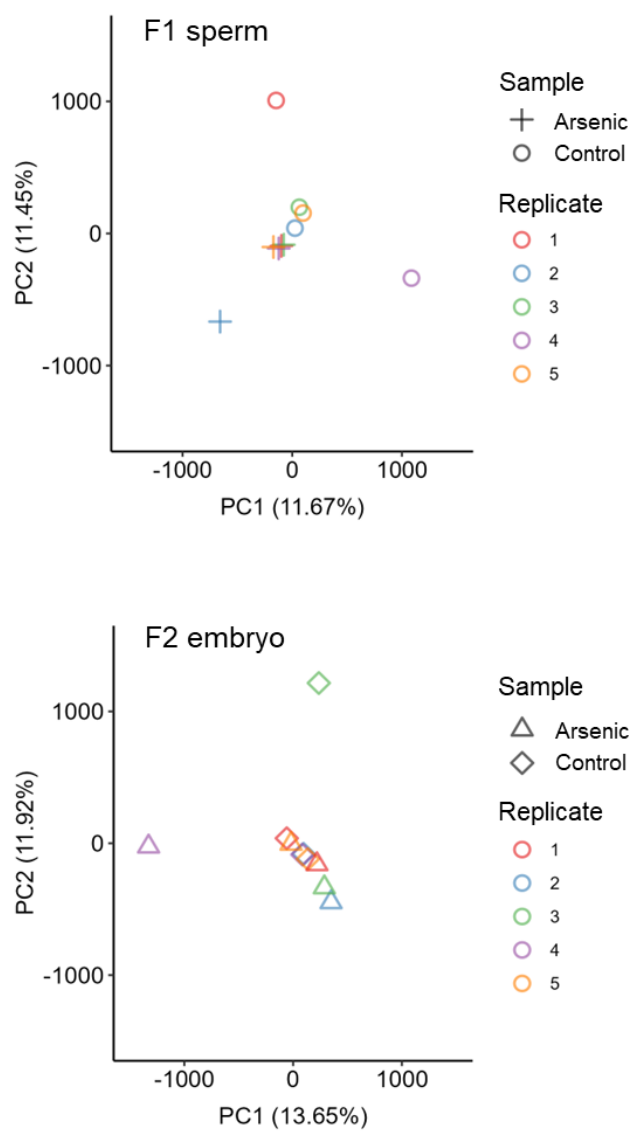


Fig. S7. Principal component analysis of methylomes in F1 sperm and F2 embryos.