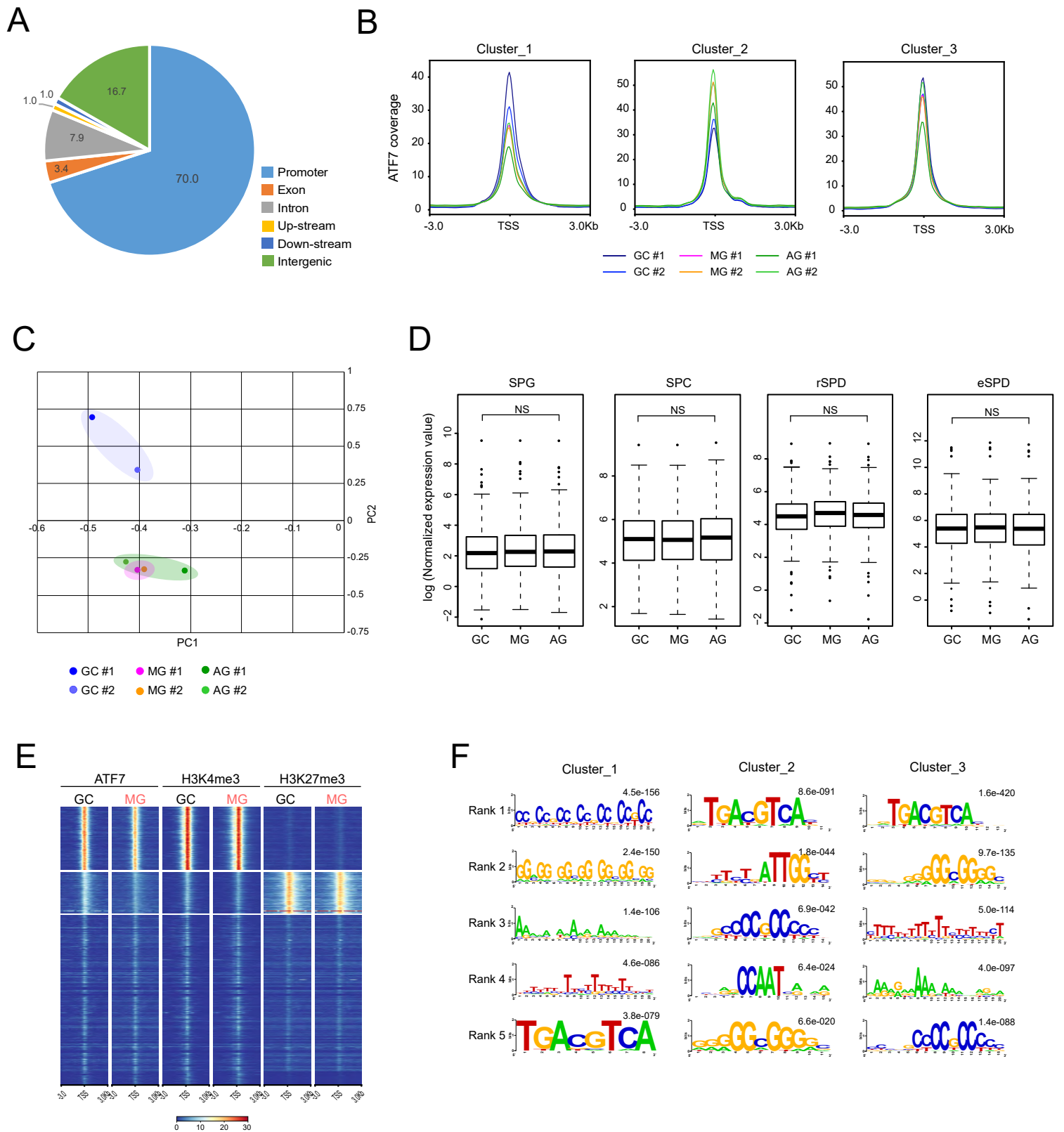


**Supplemental information**

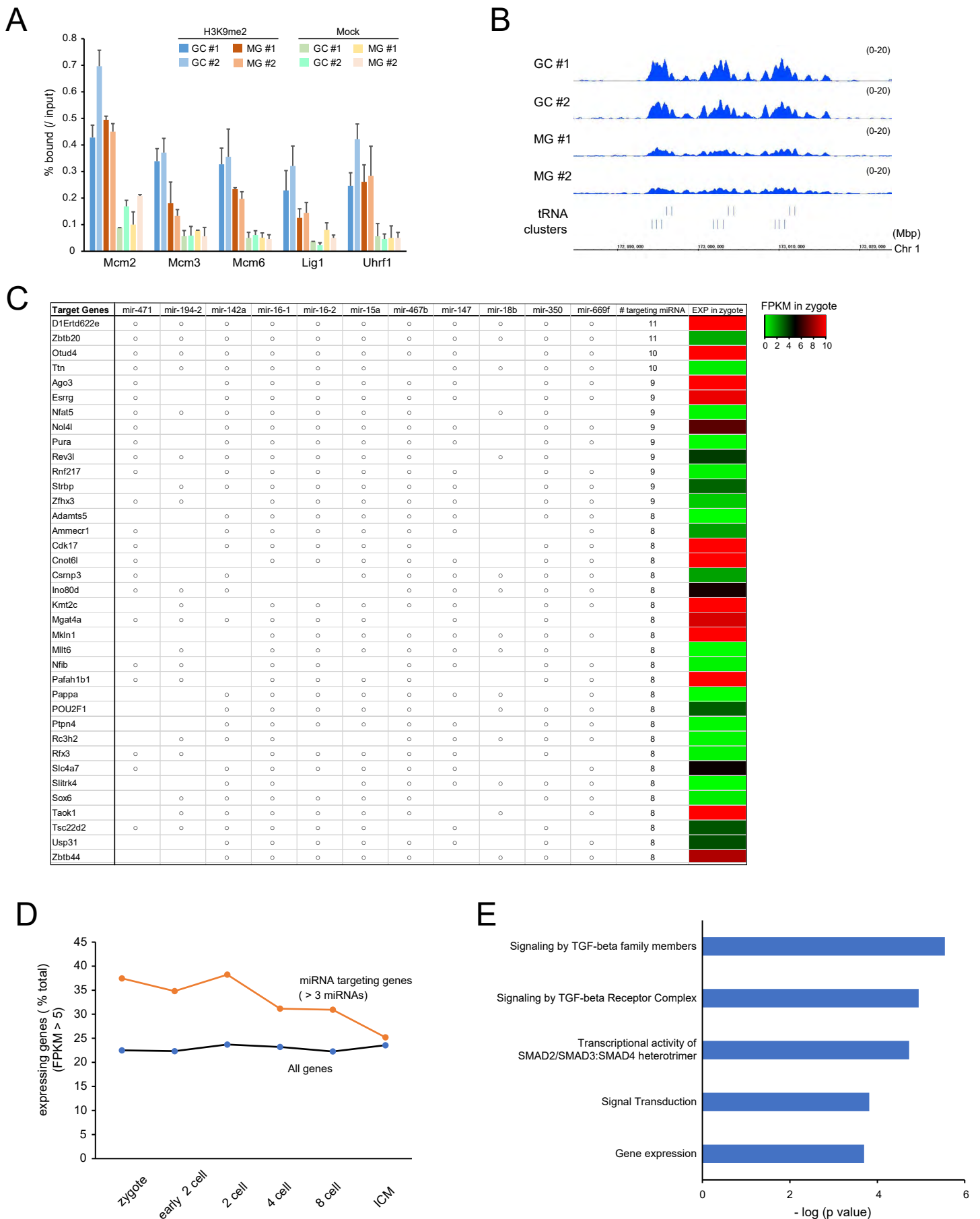
**Intergenerational effect of short-term  
spaceflight in mice**

**Keisuke Yoshida, Shin-ichiro Fujita, Ayako Isotani, Takashi Kudo, Satoru  
Takahashi, Masahito Ikawa, Dai Shiba, Masaki Shirakawa, Masafumi  
Muratani, and Shunsuke Ishii**



**Figure S1, Characteristics of ATF7-binding Genes in TGCs, Related to Figure 1.**

- (A) Genomic distribution of ATF7 binding sites in GC mice.
- (B) Profile plots for each cluster of signal intensity of ATF7 ChIP-seq using TGCs from GC, MG, and AG mice.
- (C) PCA result for signal intensity of ATF7 ChIP-seq on ATF7-binding sites including clusters 1, 2, and 3.
- (D) Box plot of expression level of marker genes of each spermatogenic cell in GC, MG, or AG testis. P-value was calculated using the Kruskal-Wallis one-way analysis of variance. N.S., not significant.
- (E) Heatmap of signal intensities from ATF7, H3K4me3, and H3K27me3 ChIP-seq data using GC/MG TGCs across all TSS. Raw reads were scaled to mapped regions in each sample (reads per million).
- (F) The most likely binding motifs of ATF7-binding sequences belonging to each cluster in TGCs, extracted by the MEME algorithm. E values for each motif are shown on the upper right of each plot.

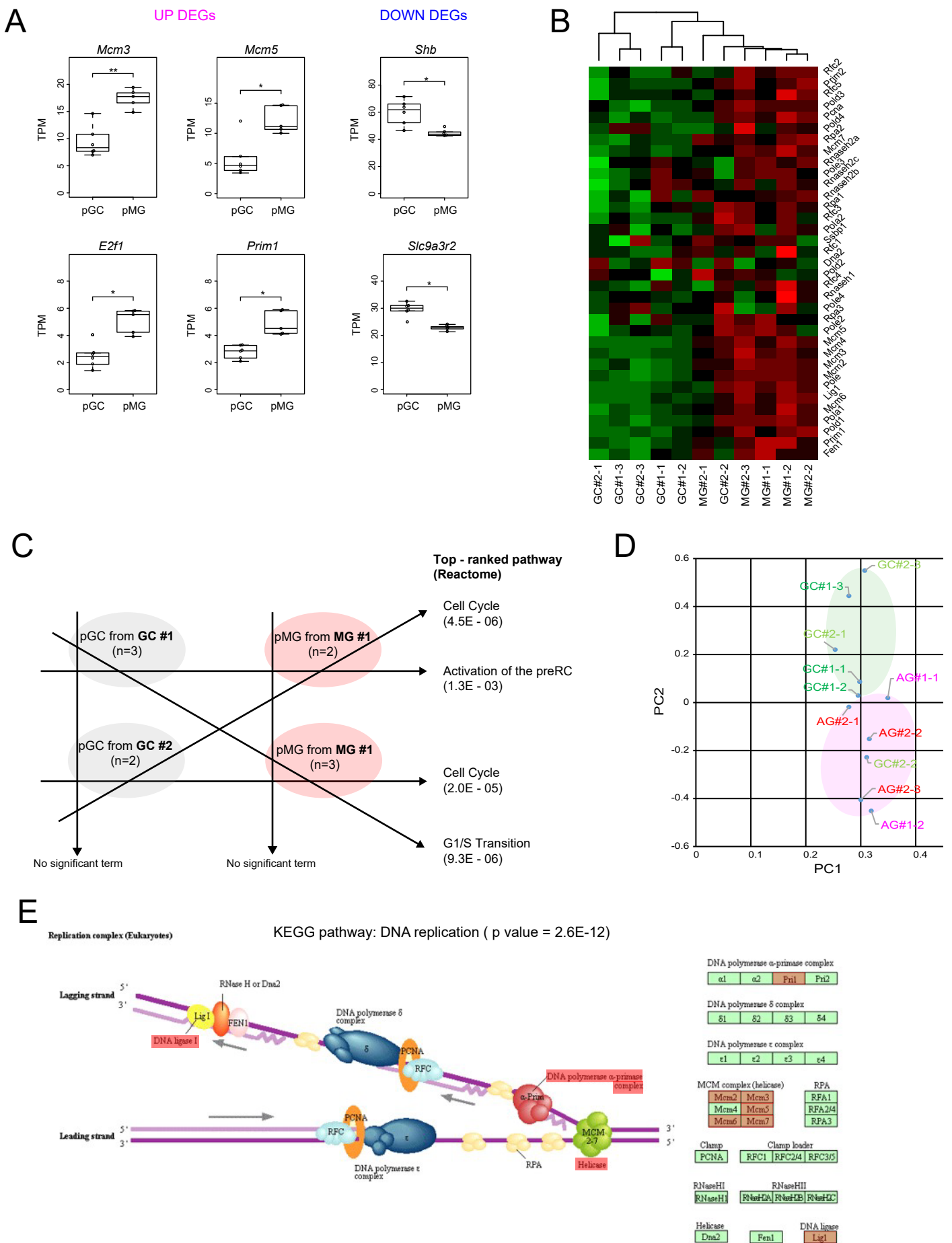


**Figure S2, MG-induced Alteration of Epigenetic Level and Small RNA Expression in Spermatozoa, Related to Figure 2.**

(A) H3K9me2 qChIP assay for ATF7 target genes in testis using HRCS collected from GC and MG mice ( $n = 2$ ). Values were obtained by calculating the amount of DNA after ChIP and PCR amplification relative to input DNA, and experimental errors of qPCR ( $n=3$ ) are indicated by error bars (mean  $\pm$  SD).

(B) ATF7 ChIP-seq data in TGCs from GC or MG mice for the tRNA cluster region of three tandem loci on chromosome 1 (horizontal axis). Blue signals indicate the number of raw reads, which is scaled to mapped regions in each sample.

- (C) Genes multiply targeted by 8 of 11 miRNAs. The targeting miRNA to each gene is indicated by “○”. The expression level of each gene in zygote is indicated by color scale.
- (D) Ratio of expressed genes (FPKM > 5) to total genes in embryogenesis for all genes and genes targeted by at least four miRNAs.
- (E) Reactome pathway analysis for genes targeted by at least four miRNAs.



**Figure S3, Effect of Paternal Spaceflight on Hepatic Expression in F1 Mouse, Related to Figure 3.**

(A) Box plot of expression levels of typical DEGs (four genes of upregulated DEGs and two genes of downregulated DEGs).

(B) Heatmap for expressions of DNA replication-related genes (KEGG; mmu03030) in individual F1 data.

(C) Cross-comparison of expression profiles between each group of data-set (only GC#2-2 was removed as outlier). Reactome pathway analysis was performed in each comparison.

(D) PCA result of expression of genes up-regulated in proliferating cells (Gene set; M5198) for individual pGC and pMG F1 sample.

(E) KEGG pathway; “replication complex (eukaryote) belonging to DNA replication (mmu03030)”, with upregulated DEGs shown in red.

**Table S3. List of KEGG pathways in miRNA-targeting genes, related to Figure 2**

KEGG pathway	p-value	#genes	#miRNAs
TGF-beta signaling pathway	1.41E-06	34	16
Pathways in cancer	2.23E-06	111	18
Signaling pathways regulating pluripotency of stem cells	3.88E-06	46	19
Endocytosis	1.83E-05	67	16
Axon guidance	1.84E-05	46	17
Proteoglycans in cancer	6.60E-05	61	18
Ras signaling pathway	0.0002394	65	16
Hippo signaling pathway	0.0002394	53	17
MAPK signaling pathway	0.0002394	72	19
PI3K-Akt signaling pathway	0.0003098	92	18
Rap1 signaling pathway	0.0007591	59	17
Hedgehog signaling pathway	0.0010769	21	13
cAMP signaling pathway	0.0012308	57	18
FoxO signaling pathway	0.0012826	41	19
Glutamatergic synapse	0.002025	31	13
Thyroid hormone synthesis	0.00316	18	12
Neurotrophin signaling pathway	0.0033429	39	16
Lysine degradation	0.0036192	13	10
Endocrine and other factor-regulated calcium reabsorption	0.0036192	17	12
Renal cell carcinoma	0.0036192	22	13
Phosphatidylinositol signaling system	0.0036192	26	14
Regulation of actin cytoskeleton	0.0036192	61	17
Amphetamine addiction	0.0050716	19	12
Adherens junction	0.008575	22	12

**Table S5. GSEA result of F1 transcriptome (top 20), related to Figure 3**

NAME_1	NAME_2	ES	FDR q-val
DUTERTRE	ESTRADIOL RESPONSE 24HR UP	0.71797	< 1.0E-06
GNF2	CCNA2	0.830044	< 1.0E-06
ROSTY	CERVICAL CANCER PROLIFERATION CLUSTER	0.75977	< 1.0E-06
BLANCO_MELO_BRONCHIAL	EPITHELIAL CELLS INFLUENZA ADEL_NS1 INFECTION (DN)	0.747079	< 1.0E-06
SOTIRIOU	BREAST CANCER GRADE 1VS3 UP	0.739854	< 1.0E-06
GNF2	PCNA	0.817506	< 1.0E-06
GRAHAM	NORMAL QUIESCENT VS NORMAL DIVIDING (DN)	0.783831	< 1.0E-06
KOBAYASHI	EGFR SIGNALING 24HR(DN)	0.702698	< 1.0E-06
ZHOU	CELL CYCLE GENES IN_R_RESPONSE 6HR	0.796277	< 1.0E-06
GNF2	CENPF	0.819451	< 1.0E-06
GNF2	CDC2	0.824043	< 1.0E-06
GNF2	CDC20	0.832537	< 1.0E-06
GNF2	MCM4	0.819881	< 1.0E-06
KONG	E2F3 TARGETS	0.758044	< 1.0E-06
GNF2	RFC4	0.81371	< 1.0E-06
	MODULE_54	0.6878	< 1.0E-06
GNF2	CCNB2	0.81184	< 1.0E-06
ZHOU	CELL CYCLE GENES IN_R_RESPONSE 24HR	0.729285	< 1.0E-06
GNF2	FEN1	0.805418	< 1.0E-06
GNF2	RRM1	0.76176	< 1.0E-06



**Table S6. List of primers for qChIP experiment, related to STAR Methods**

Primer Name	Forward	Reverse
Mcm2	CGCGCGAAAAGTAGACCAC	TCCTTCCGCTCTAGATTGACG
Mcm3	CGTCATCCAGGAAGTCCAAG	CGTTTCCGACTGTTTGGTG
Mcm6	GTTCCACGCGTAGCTCCTTC	CCCTGTTATTGGCTGAGGTG
Uhrf1	GGGAAGGGCGGAACTTAAAC	CTAACTCCCCTCGGGACAC
Lig1	AGTGCGCGCGAAATCTG	CGCCACACTCGTCACTC