Supplemental Information

Loss of H3K27me3 imprinting in somatic cell nuclear transfer embryos disrupts post-implantation development

Shogo Matoba, Huihan Wang, Lan Jiang, Falong Lu, Kumiko A. Iwabuchi, Xiaoji Wu, Kimiko Inoue, Lin Yang, William Press, Jeannie T. Lee, Atsuo Ogura, Li Shen and Yi Zhang

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Supplemental Figures

Figure S1

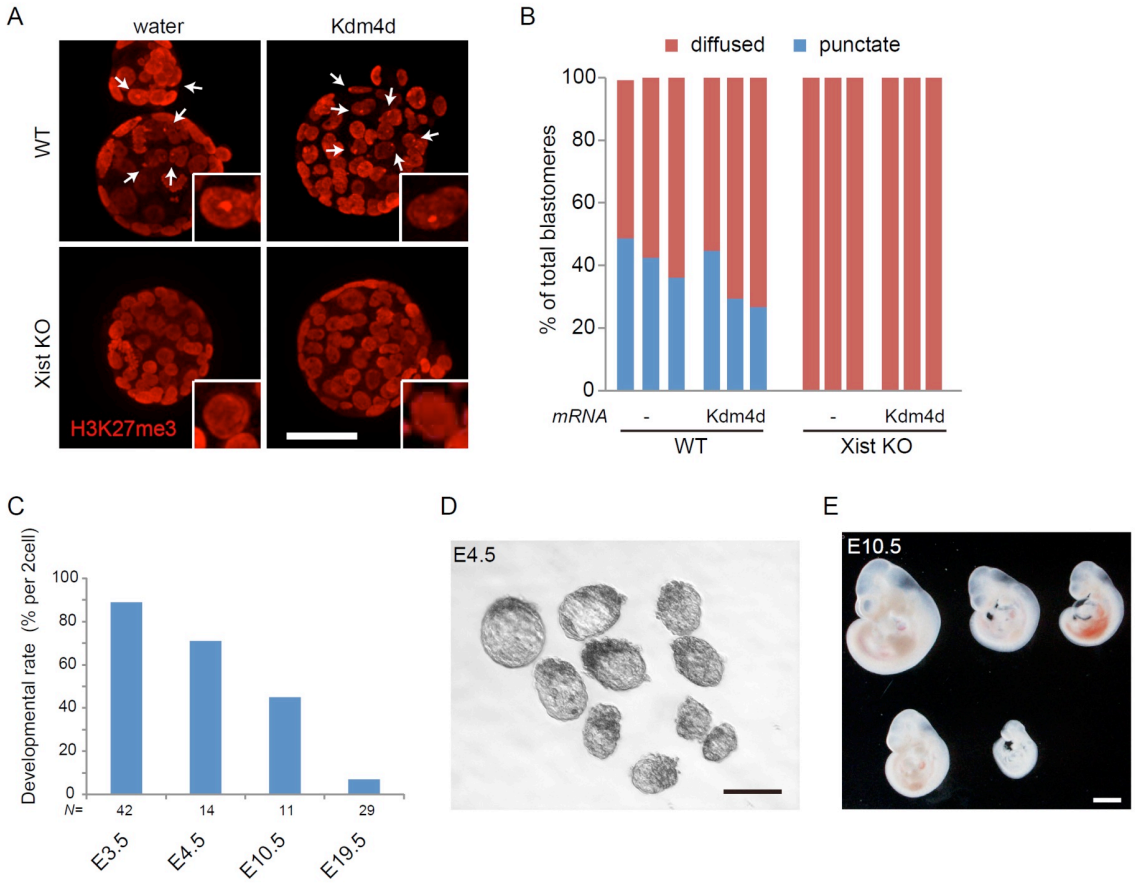

Figure S1. Postimplantation developmental arrest of SCNT embryos, Related to Figure 1.

(A) Representative images of SCNT blastocysts derived from MEF cells stained with anti-H3K27me3 antibody. Arrows indicate punctate H3K27me3 signals representing ectopically inactivated X chromosomes. Note that the ectopic XCI is not observed in SCNT embryos derived from *Xist* KO donor MEF cells. Scale bar, 50 μ m.

(B) Bar graphs showing the ratio of cells with or without punctate H3K27me3 signals (represent inactivated X chromosomes) in SCNT blastocysts. Each column represents a single blastocyst.

(C) Bar graphs showing developmental rate of SCNT embryos generated using *Xist* KO MEF cells combined with *Kdm4d* mRNA injection at the indicated time points. *N* represents the number of embryos at the 2 cell stage.

(D and E) Representative images of SCNT embryos collected at E4.5 (D) and E10.5 (E). Note that SCNT embryos exhibit big variation in embryo/body size at each stage. Scale bars, 100 μm in (D) and 1mm in (E).

Figure S2

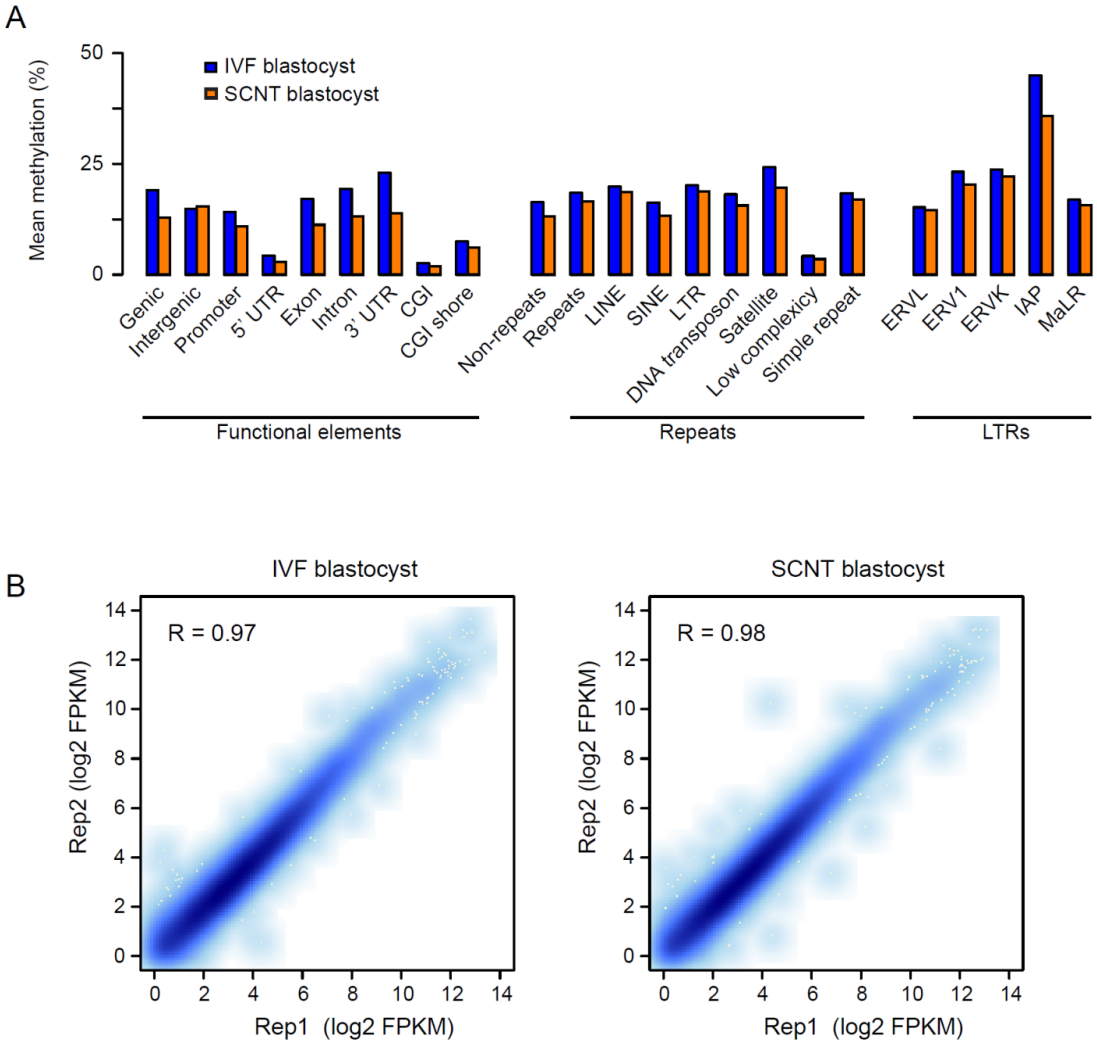

Figure S2. SCNT and IVF blastocysts have similar DNA methylome and transcriptome Related to Figure 2

(A) Box plots comparing mean methylation levels at various genomic features including repeats in IVF and SCNT blastocysts.

(B) Scatter plots comparing transcriptomes of biological replicates of IVF and SCNT blastocysts.

Figure S3

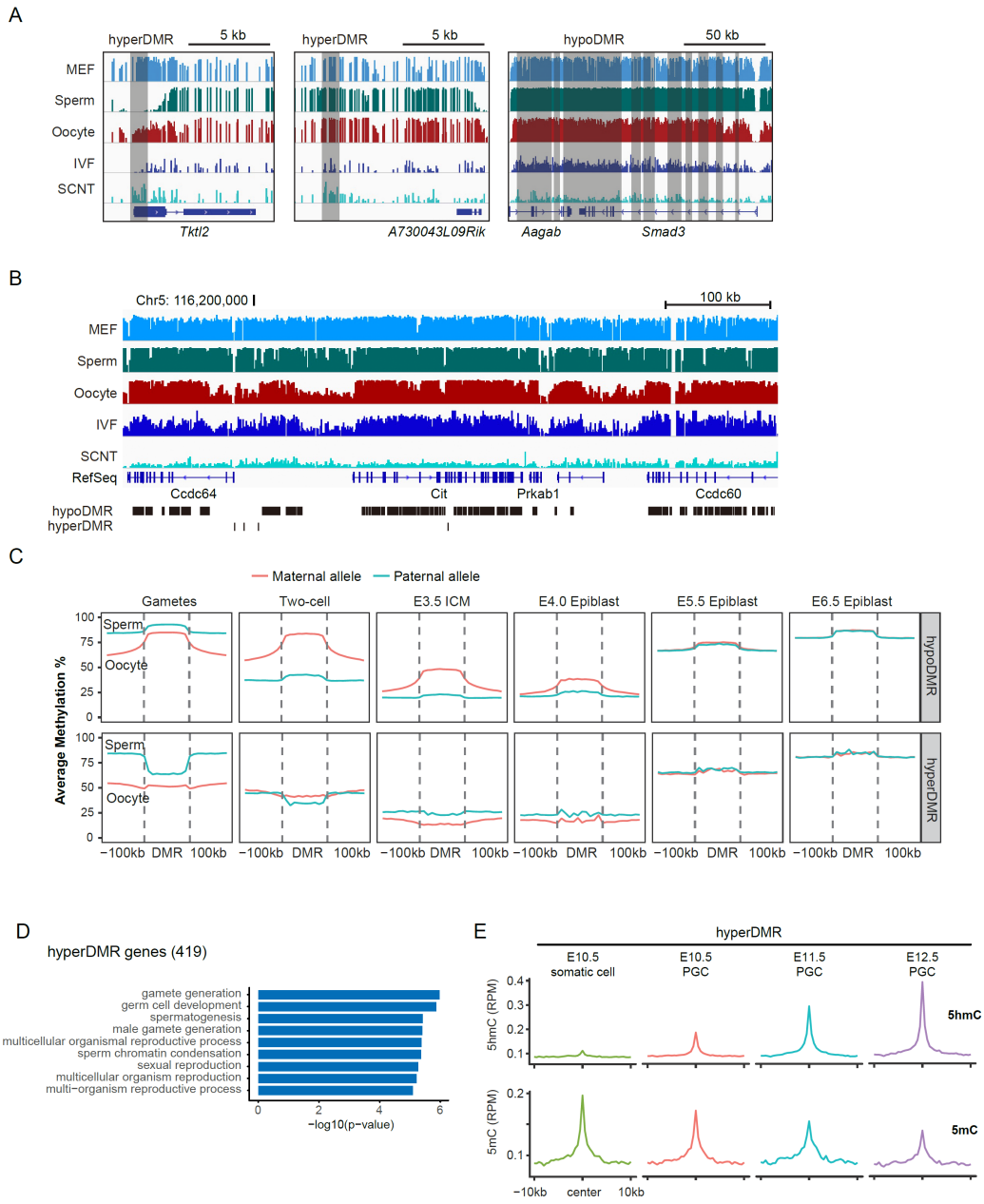

Figure S3. Features of hypo- and hyper-DMRs in SCNT blastocysts, Related to Figure 3

(A) Representative genome browser view of hyper- and hypo-DMRs.

(B) Representative genome browser view showing methylation peaks in oocytes overlap with those in IVF blastocysts.

(C) Peak plots showing dynamic changes of average methylation levels at hypo- and hyper-DMRs in different developmental stages from gametes to E6.5 epiblast. Datasets used were from GSE56697 and GSE76505.

(D) Gene ontology analysis of the hyperDMR-accociated genes.

(E) Peak plots showing mean methylation (5mC) and hydroxymethylation (5hmC) levels at hyperDMRs during PGC development. Datasets used are from SRP016940.

Figure S4

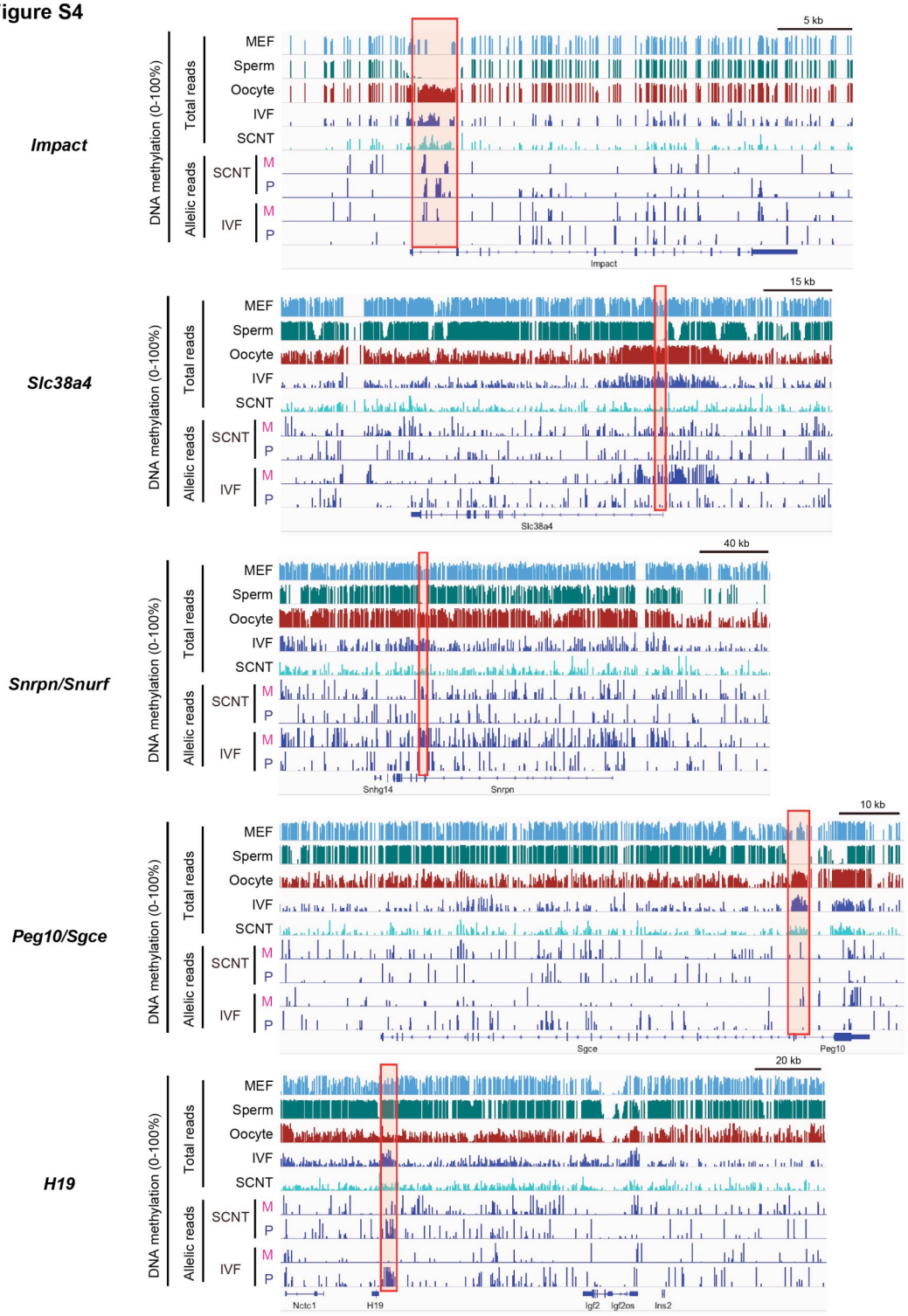

Figure S4. Imprinting status of representative ICRs. Related to Figure 4

Genome browser views showing DNA methylation levels at genomic regions of *Impact*-, *Slc38a4*-, *Snrpn/Snurf*-, *Peg10/Sgce*- and *H19*-ICR. Red boxes represent ICR regions (Tomizawa et al., 2011). M; Maternal allele, P; Paternal allele.

Figure S5

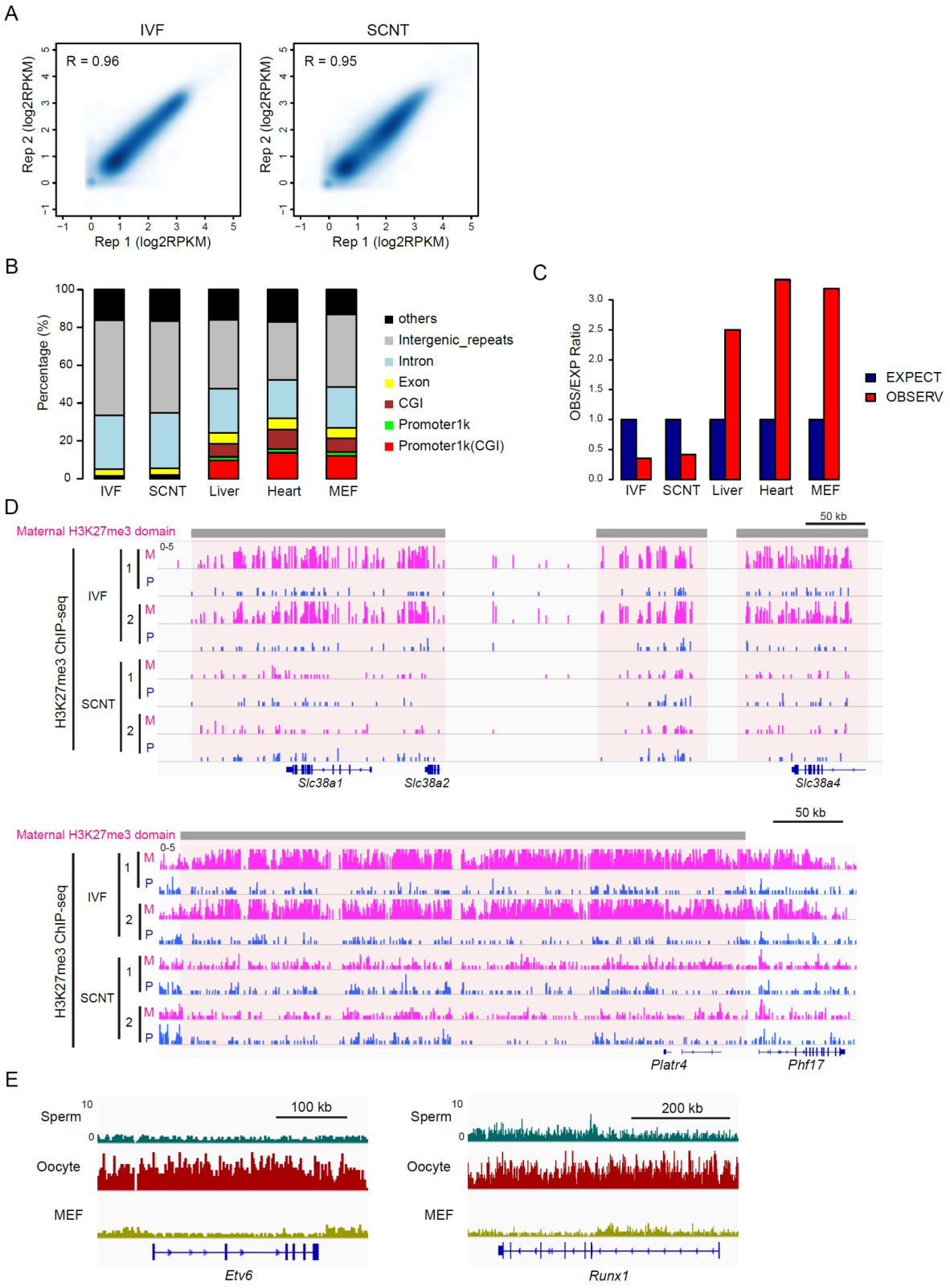
A
IVF
SCNT
R = 0.96
R = 0.95
Rep 2 (log2RPKM)
Rep 1 (log2RPKM)
B
Percentage (%)
IVF
SCNT
Liver
Heart
MEF
others
Intergenic_repeats
Intron
Exon
CGI
Promoter1k
Promoter1k(CGI)
C
OBS/EXP Ratio
EXPECT
OBSERV
D
Maternal H3K27me3 domain
50 kb
H3K27me3 ChIP-seq
Slc38a1
Slc38a2
Slc38a4
Platr4
Phf17
E
Sperm
Oocyte
MEF
100 kb
200 kb
Etv6
Runx1

Figure S5. H3K27me3 distribution patterns in SCNT embryos. Related to Figure 5

(A) Scatter plots comparing H3K27me3 signal levels of biological replicates of IVF and SCNT morulae.

(B) Bar graphs showing the genomic distribution of H3K27me3 peaks in IVF and SCNT morula embryo, as well as in liver, heart and MEF cells. Datasets used were from GSE49847 and GSM1624405.

(C) Bar graphs showing the observed relative to expected H3K27me3 peaks at promoters (TSS+/- 1 kb).

(D) Genome browser views of H3K27me3 signals at representative genomic regions containing H3K27me3-dependent imprinted genes, *Slc38a1, Slc38a2, Slc38a4*, *Platr4* and *Phf17*.

(E) Genome browser views of H3K27me3 ChIP-seq signals at representative H3K27me3-dependent imprinted genes in sperm, oocytes and MEF cells. Datasets used were from GSE49847 and GSE76687.

Supplemental Tables

Table S1. Postimplantation development of SCNT embryos, Related to Figure 1

Donor cell				mRNA injected	No. of recipients	No. of 2-cell embryos transferred	No. of implanted (% per ET)	No. of pups at birth (% per ET)	No. of pups survived to adults (% per birth)	Body weight at birth (g ± SD)	Placenta weight at birth (g ± SD)
cell type	background	Sex	*Xist* allele								
Cumulus	B6D2F1	Female	WT	-	6	171	52 (30.4)	2 (1.2)	2 (100)	1.56 ± 0.04	0.36 ± 0.06
			WT	*Kdm4d*	8	179	110 (61.5)	15 (8.4)	14 (93.3)	1.59 ± 0.14	0.34 ± 0.06
			KO	*Kdm4d*	4	75	46 (61.3)	14 (18.7)	13 (92.9)	1.50 ± 0.12	0.30 ± 0.04
Sertoli	B6D2F1	Male	WT	-	3	55	25 (45.5)	1 (1.8)	1 (100)	1.50	0.28
			WT	*Kdm4d*	4	77	47 (61.0)	7 (9.1)	6 (85.7)	1.45 ± 0.06	0.27 ± 0.06
			KO	*Kdm4d*	4	85	57 (67.1)	20 (23.5)	18 (90.0)	1.46 ± 0.16	0.27 ± 0.08
MEF	129CASTF1	Male	WT	-	5	40	15 (37.5)	0 (0.0)	N/A	N/A	N/A
			WT	*Kdm4d*	5	53	36 (67.9)	2 (3.8)	1 (50.0)	1.70 ± 0.18	0.38 ± 0.04
			KO	*Kdm4d*	5	29	23 (79.3)	2 (6.9)	1 (50.0)	1.89 ± 0.25	0.40 ± 0.13

Concentration of injected *Kdm4d* mRNA was 1500 ng/ul. N/A, not applicable. ET, embryo transfer.

Table S2. List of the differentially regulated genes in SCNT blastocysts, Related to Figure 2 (see excel file)

Table S3. List of the hypoDMRs and hyperDMRs of SCNT blastocysts, Related to Figure 3 (see excel file)

Table S4. DNA methylation levels at the 23 ICRs, Related to Figure 4

Name of ICR	Position			Average methylation level (%)	
	Chromosome	Start	End	IVF	SCNT
H13,Mcts2	chr2	152,512,421	152,513,169	34.8	31.6
Blcap,Nnat-1	chr2	157,385,609	157,386,430	41.8	25.4
Blcap,Nnat-2	chr2	157,387,096	157,387,535	21.0	10.6
Gnas-1	chr2	174,122,340	174,123,003	33.1	26.0
Gnas-2	chr2	174,123,635	174,126,630	30.1	23.9
Gnas-3	chr2	174,152,526	174,153,681	24.8	14.5
Peg10,Sgce	chr6	4,696,691	4,698,891	42.1	22.0
Mest	chr6	30,687,664	30,688,606	33.8	24.0
Herc3,Nap1l5	chr6	58,856,396	58,857,391	32.8	34.9
Peg3	chr7	6,681,989	6,683,417	37.6	32.5
Snrpn,Snurf	chr7	67,149,094	67,150,214	43.5	20.3
Inpp5f	chr7	135,830,870	135,832,249	39.4	31.0
H19	chr7	149,766,117	149,766,601	37.0	38.8
Kcnq1	chr7	150,480,921	150,482,670	37.1	24.0
Rasgrf1	chr9	89,774,382	89,774,897	31.9	28.5
Plagl1	chr10	12,810,051	12,811,257	36.6	29.7
Grb10	chr11	11,925,322	11,927,142	39.8	21.5
Commd1,Zrsr1	chr11	22,871,787	22,873,304	40.6	29.5
Dlk1,Meg3	chr12	110,766,361	110,766,887	37.2	22.8
Trappc9	chr15	72,639,707	72,641,342	44.0	25.8
Slc38a4	chr15	96,884,723	96,885,730	38.1	8.9
Igf2r	chr17	12,934,126	12,935,626	38.0	31.0
Impact	chr18	13,131,236	13,133,240	29.4	27.3

Table S5. List of the maternal H3K27me3-domains identified in IVF morula, Related to Figure 5 (see excel file)