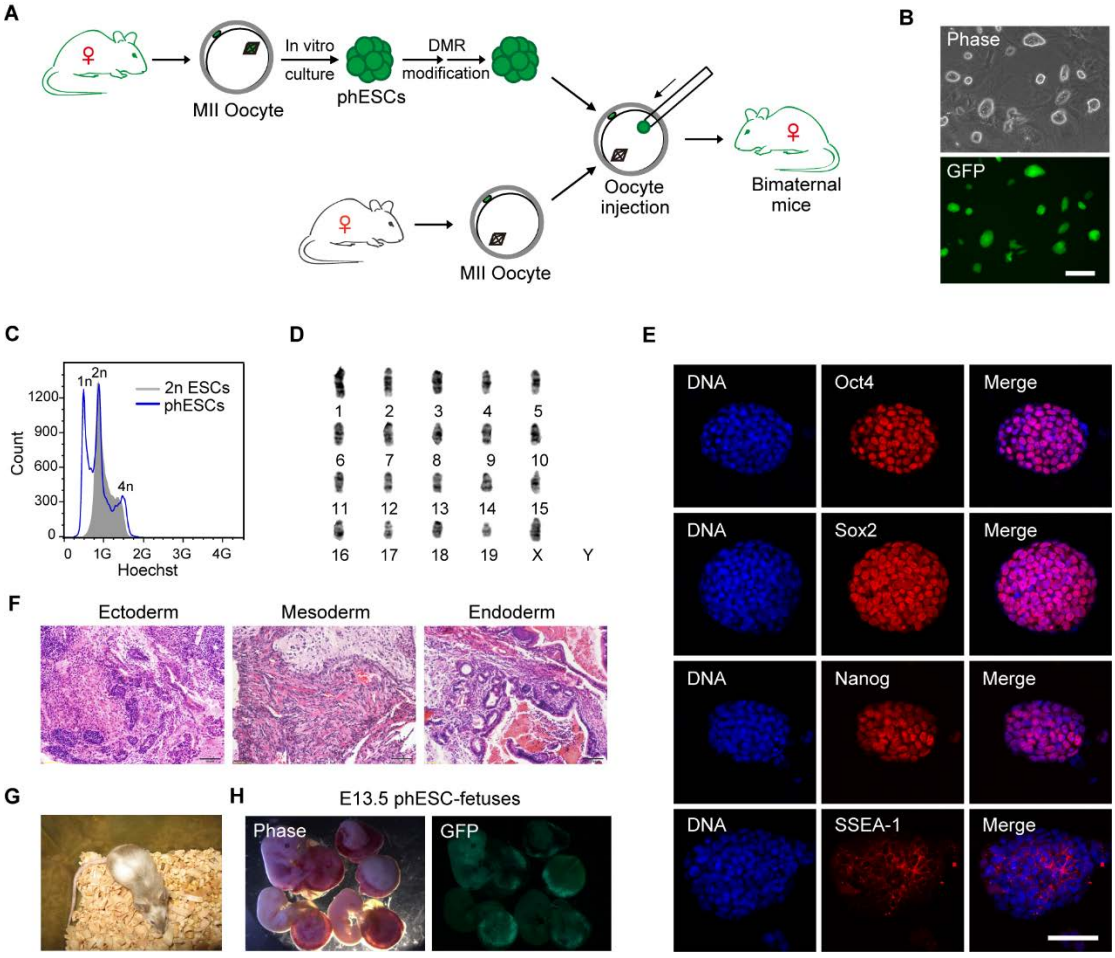


Supplementary information, Figure S1 Generation and characterization of parthenogenetic haploid ESCs.

(A) The scheme for the generation of bimaternal mice from mature MII oocyte including two steps: derivation of phESCs from MII oocytes carrying Gfp transgene and subsequent DMR modification; injection of genetically modified phESCs into unlabeled oocytes to produce diploid offspring.

(B) Morphology and fluorescence detection of phESCs carrying a Gfp transgene. Scale bar, 100 μm.

(C) FACS analysis of DNA content of phESC line phESC-1 (passage 11, blue), and the control diploid ESCs (gray).

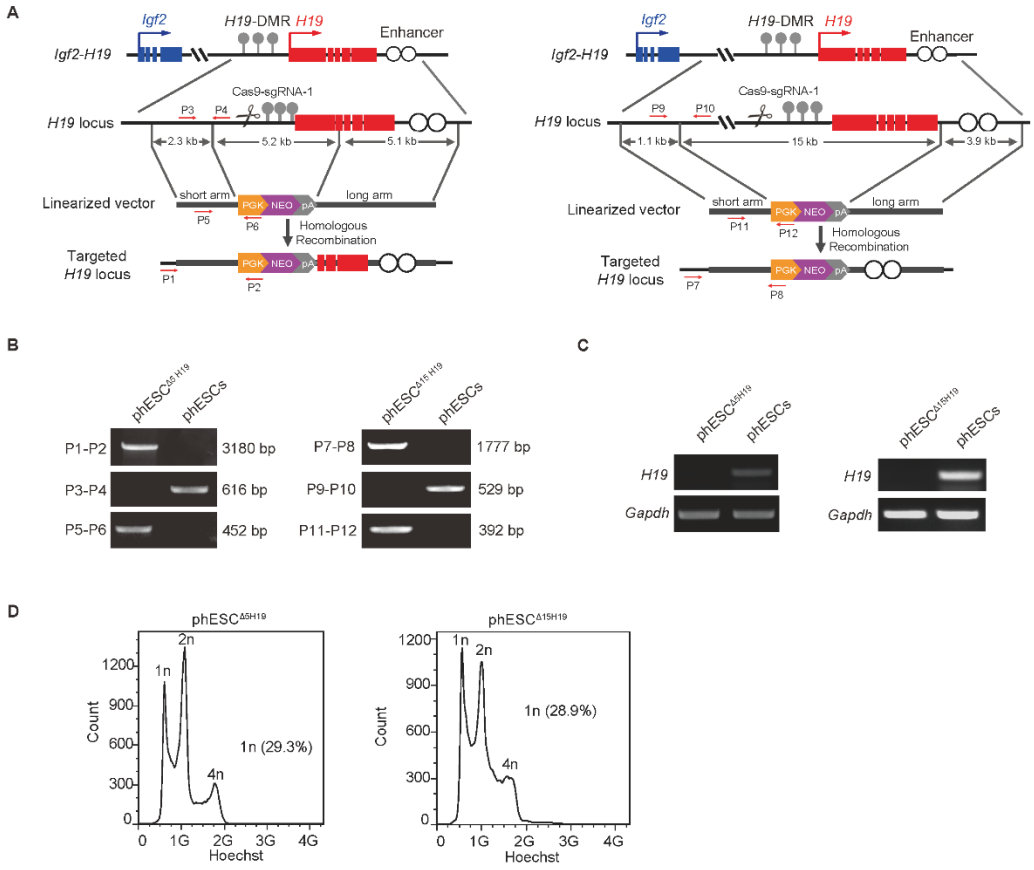
(D) Karyotype of an established phESC line.

(E) Immunostaining of pluripotent markers Oct3/4, Nanog, Sox2 and SSEA-1 in phESCs. Propidium Iodide (PI) is used to stain DNA. Scar Bar, 50 μm.

(F) Teratoma formation of phESCs. Shown are the teratoma dissection slices representing all three germ layers identified after staining with haematoxylin and eosin. Scale bar, 50 μm.

(G) Adult chimaeric mouse produced by microinjection of phESCs into diploid CD1 blastocysts.

(H) Bimaternal fetus recovered on E13.5.



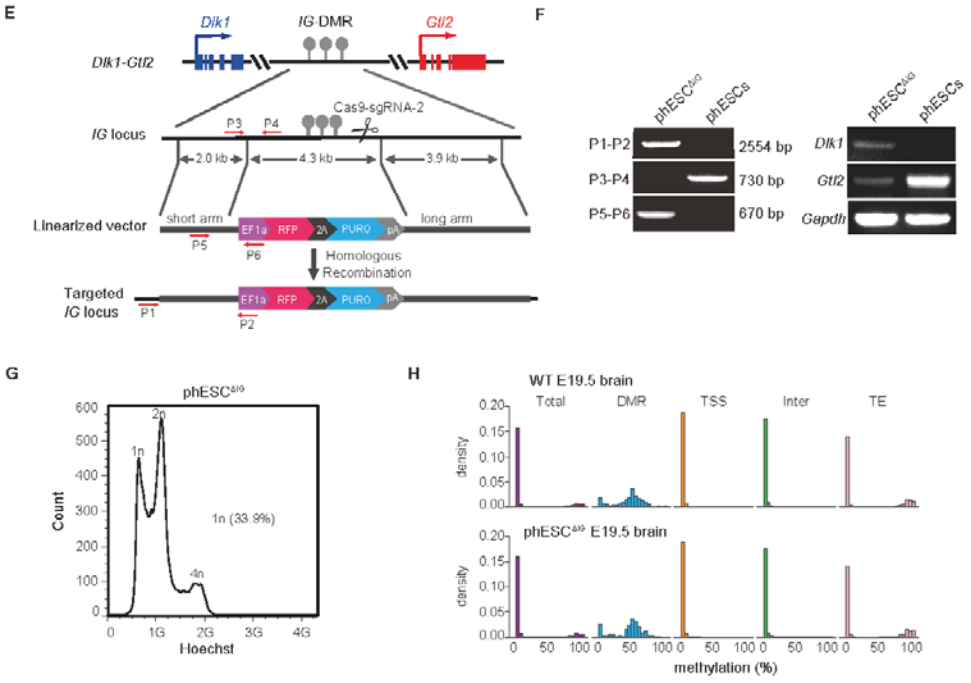
Supplementary information, Figure S2 Generation and characterization of imprinting-modified phESC lines and the bimaternal pups.

(A) Schematic overview of generating H19-DMR targeted allele in phESCs. The cutting site of Cas9-sgRNA-1 was indicated with a scissor. Primer sets P1-P2 (Δ5H19) and P7-P8 (Δ15H19) recognize targeted alleles at the H19-DMR locus. P3-P4 (Δ5H19) and P9-P10 (Δ15H19) recognize the wild-type allele. P5-P6 (Δ5H19) and P11-P12 (Δ15H19) recognize the vector sequences. Genomic DNA of wild-type phESCs was used as control.

(B) PCR analysis of gene targeting in phESCs with primers P1-P12.

(C) RT-PCR analysis of H19 and Gapdh in targeted and WT phESCs.

(D) fluorescence-activated cell sorting (FACS) analysis of DNA content of phESCΔ5H19 and phESCΔ15H19 lines.



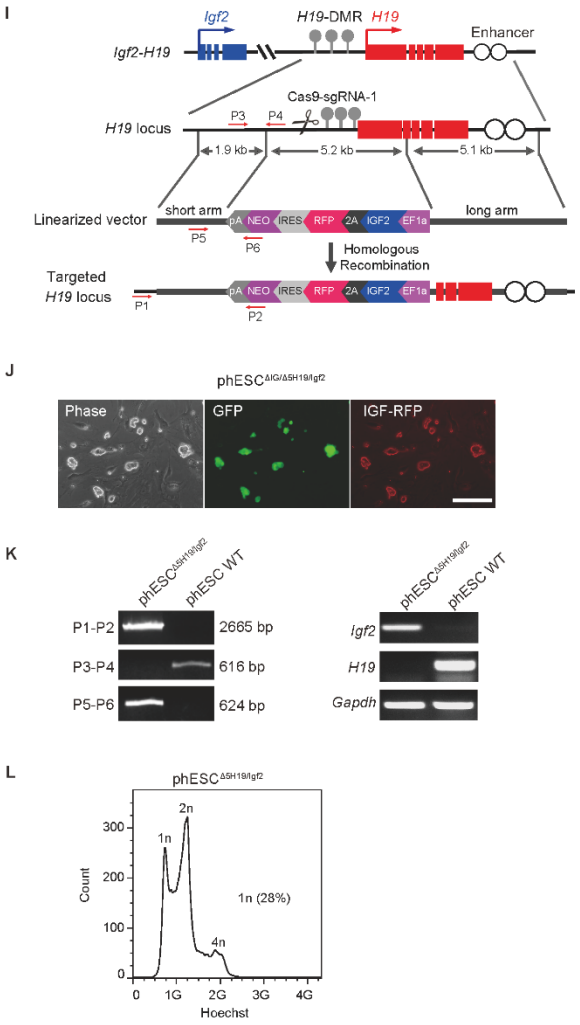
Supplementary information, Figure S2 Generation and characterization of imprinting-modified phESC lines and the bimaternal pups.

(E) Schematic overview of generating IG-DMR targeted allele in phESCs. The cutting site of Cas9-sgRNA-2 was indicated with a scissor. Primer set P1-P2 recognizes targeted allele at the IG-DMR locus. P3-P4 and P5-P6 recognize the wild-type allele and vector sequences, respectively. Genomic DNA of wild-type phESCs was used as control.

(F) Left: PCR analysis of gene targeting in phESCs with primers P1-P6; Right: RT-PCR analysis of Dlk1, Gtl2 and Gapdh in targeted and WT phESCs.

(G) FACS analysis of DNA content of phESCΔIG line.

(H) Density distribution of cytosine methylation level of WT and phESCΔIG-fetus brain. The CpG sites with enough RRBS read coverage were used for total analysis (Total); the cytosine site in ICRs with methylation level differences > 0.5 between oocyte and sperm was considered as differential methylation region (DMR) to analyse; The cytosine sites in the regions +/- 2 kb of transcription start site (TSS) were used for the methylation level analysis of TSS. Transposon elements (TE) were annotated by RepeatMasker; CpG sites excluded from the above annotated regions (DMR, TSS and TE) and located between genes were used to analyse the methylation level of intergenic region (Inter).



Supplementary information, Figure S2 Generation and characterization of imprinting-modified phESC lines and the bimaternal pups.

(I) Schematic overview of generating H19-DMR knockout and Igf2 knockin allele in phESCs. The cutting site of Cas9-sgRNA-1 was indicated with a scissor. Primer set P1-P2 recognizes the targeted allele at the H19-DMR locus. P3-P4 and P5-P6 recognize the wild-type allele and vector sequences, respectively. Genomic DNA of wild-type phESCs was used as control.

(J) Morphology and fluorescence detection of phESCs$^{\Delta IG/\Delta 5H19/Igf2}$ that carry a *Gfp* transgene and an *Igf2-Rfp* targeted integration at the *H19* locus. Scale bar, 100 μm.

(K) Left: PCR analysis of gene targeting in phESCs with primers P1-P6; Right: RT-PCR analysis of Igf2 and Gapdh in WT and targeted phESC lines.

(L) FACS analysis of DNA content of phESCΔ5H19/Igf2 line.

Supplementary information, Table S1A Development of bimaternal mice produced from imprinting-modified phESCs.

Cell line	No. of Embryos injected	No. of 2-cell embryos[#]	No. of implantation sites (% ET)	No. of arrested 10.5-13.5 (% ET)	No. of arrested 14.5-17.5 (% ET)	No. of full-term pups (% ET)	No. of survival pups (% ET)
Round spermatid	115	109	-	-	-	15(13.8)	14(12.8)
phESC-1	224	210	38 (18.1)	5 (2.4)	0	0	0
phESC-2[*]	174	158	25 (15.8)	2 (1.3)	0	-	-
phESC-2[**]	168	155	26 (16.8)	3 (1.9)	-	-	-
phESC$^{\Delta 5H19}$-1	280	261	38 (14.6)	5 (1.9)	1 (0.4)	0	0
phESC$^{\Delta 5H19}$-2	377	363	49 (13.5)	6 (1.7)	3 (0.8)	0	0
phESC$^{\Delta 15H19}$-1	360	326	43 (13.2)	4 (1.2)	5 (1.5)	0	0
phESC$^{\Delta 15H19}$-2	310	285	42 (14.7)	4 (1.4)	2 (0.7)	0	0
phESC$^{\Delta IG}$-1	275	243	28 (11.5)	1 (0.4)	0	7 (2.9)	0
phESC$^{\Delta IG}$-2	361	325	45 (13.8)	1 (0.3)	0	6 (1.8)	0
phESC$^{\Delta IG/\Delta 5H19}$-1	318	284	40 (14.1)	3 (1.1)	1 (0.4)	9 (3.2)	0
phESC$^{\Delta IG/\Delta 5H19}$-2	120	102	22 (21.6)	0	1 (1.0)	4 (3.9)	0
phESC$^{\Delta IG/\Delta 5H19}$-3	226	182	22 (12.1)	4 (2.2)	2 (1.1)	8 (4.4)	0
phESC$^{\Delta IG/\Delta 15H19}$-1	220	210	54 (25.7)	0	0	20 (9.5)	15 (7.1)
phESC$^{\Delta IG/\Delta 15H19}$-2	128	120	19 (15.8)	0	0	13 (10.8)	10 (8.3)
phESC$^{\Delta IG/\Delta 15H19}$-3	165	145	22 (15.2)	0	1 (0.7)	10 (6.9)	6 (4.1)
phESC$^{\Delta IG/\Delta 5H19/Igf2}$-1	165	145	22 (15.2)	0	1 (0.7)	10 (6.9)	7 (4.8)
phESC$^{\Delta IG/\Delta 5H19/Igf2}$-2	208	175	28 (16.0)	0	0	15 (8.6)	12 (6.9)
phESC$^{\Delta IG/\Delta 5H19/Igf2}$-3	146	130	19 (14.6)	2 (1.5)	0	7 (5.4)	4 (3.1)

[#] means that all 2-cell embryos were transferred into the oviducts of CD1 surrogate females. ET, embryos transferred. *, Autopsy at 17.5 dpc. **, Autopsy at 13.5 dpc.

Supplementary information, Table S1B Genotype distribution of bimaternal mice offspring.

Genotype	H19$^{+/+}$IG$^{+/+}$	H19$^{\Delta 15/+}$ IG$^{+/+}$	H19$^{+/+}$IG$^{\Delta/+}$	H19$^{\Delta 15/+}$IG$^{\Delta/+}$
Survival ability	live	live	lethal	lethal
Expected ratio at fertilization (%)	25	25	25	25
Expected ratio survived (%)	25	25	0	0
Actual count survived *	14	11	0**	0

Note: * No. of litters =9.

** Four dead full term pups were born.

Supplementary information, Table S1C Primer used in the study.

Application	Primer	Sequence	Product size (bp)	Annealing (°C)
Recombination arms amplification	ΔIG-short-F	TCAGTGTCTGCCTTTCCTCAGAA	2037	67
	ΔIG-short-R	GTACGGGCGACATGAGCATGAC		
	ΔIG-long-F	TAAGCCTCACAGTTGAACCTCTAC	3858	65
	ΔIG-long-R	GGTTCATAGAGAGCTTGCTAGACAGG		
	Δ5H19-short-F	TAGTTGACCAAGGGCTGTAAGAA	2307	67
	Δ5H19-short-R	GTGGTGATTCGGGAACTGTAGGC		
	Δ5H19-long-F	GGGGAGTCAAGGGCACAGGAT	5108	65
	Δ5H19-long-R	GAGGGATGGAAAGGAAAGGAAAGAGG		
	Δ15H19-short-F	GAATTCTCCAAAGGGACAGGGA	1136	67
	Δ15H19-short-R	GGATCCGGGCAGAGGG		
	Δ15H19-long-F	ATGTTGGAGATTGGGGTAAGGGT	3930	67
	Δ15H19-long-R	AAACGTACCTACTCCTGGTGTTAGCC		
Cassette In-Fusion® cloning	*Igf2*-infusion-F	TATCGATAAAGCTAGTTTTATGAGGGACAGCC	4090	56
	Igf2-infusion-R	CCCACCAGCTGCTAGTGACCAGAGCAAGGGAT		
	Puro-infusion-F	ACCATATGTGCGGCCAAGGATCTGCGATCGCTCC	2165	66
	Puro-infuison-R	TAGTCGACGGTATCGTAAGATACATTGATGAGTTT GGACAAACC		
Cas9-sgRNA-1	F	CCGGGGCCTGGAAACTAACGTAGT	Annealed and ligated into sgRNA expression vectors according to ref. [9]	
	R	AAACACTACGTTAGTTTCCAGGCC		
Cas9-sgRNA-2	F	CCGGTCTTAGAGAGAAGAAAGAAG		
	R	AAACCTTCTTTCTTCTCTCTAAGA		
Igf2 CDS cloning	*Igf2*-CDS-F	TACCCTAAAGAAGCAGAAGA	773	56
	Igf2-CDS-R	CATTGCAGAATTACCACATA		
Igf2 real-time PCR	F	GTGCTGCATCGCTGCTTAC	222	65
	R	ACGTCCCTCTCGGACTTGG		
ΔIG genotyping	P1	GTTTCCAGATTGGGCTATTGG	2555	64
	P2	ATCACTTTCCCAGTTTACCCC		
	P3	ATCTTGTGATTCGTCTTGCC	730	56
	P4	ACCTTTTATTTATGCTGTGG		
	P5	TTCAAGTCGGCTACAGCACATAC	670	67
	P6	GCAGAACAGAAAACGAAACAAAG		
Δ5H19 genotyping	P1	TGGGCAAACAGAACCTAACTGAAGC	3180	68
	P2	CTGAGCCCAGAAAGCGAAGGA		
	P3	CTTCAGTTAGGTTCTGTTTGCCC	616	68
	P4	TTATGCCTCCTGGATGCTCGTGT		
	P5	CCTGAGTTAAAACCGAGAAAA	452	63
	P6	TGCACGAGACTAGTGAGACGT		
Δ15H19 genotyping	P7	TCCAGGCATTGGCTGCGTTT	1777	72
	P8	CTGAGCCCAGAAAGCGAAGGA		
	P9	ATGTGGGCTGAGTACTTGGACGC	529	71

Supplementary information,Table S1C Continued.

Δ15H19 genotyping	P10	ATGAAGGAAAATGGTGGTAGGGG		
	P11	CAGGGGAAGGAGGGAGCTGAATG	392	72
	P12	GGTGGATGTGGAATGTGTGCGAG		
Δ5H19/Igf2 genotyping	P1	TGGGCAAACAGAACCTAACTGAAGC	2665	68
	P2	TCGCAGCGCATCGCCTTCTAT		
	P3	CTTCAGTTAGGTTCTGTTTGCCC	616	68
	P4	TTATGCCTCCTGGATGCTCGTGT		
	P5	GGATGTTTCACTTCCACTCTCTCT	624	67
	P6	TCGCAGCGCATCGCCTTCTAT		
Bisulite sequencing of DMRs	Igf2r-F1	TAGAGGATTTTAGTATAATTTTAA	550	
	Igf2r-R1	CACTTTTAAACTTACCTCTCTTAC		
	Igf2r-F2	GAGGTTAAGGGTGAAAAGTTGTAT	490	
	Igf2r-R2	CACTTTTAAACTTACCTCTCTTAC		
	H19-DMR-F1	TATGAGTATTTAGGAGGTATAAGAATT	450	
	H19-DMR-R1	TTTTATCAAAAACTAACATAAACCCCT		
	H10-DMR-F2	TGTAAGGAGATTATGTTTTATTTTTGG	359	
	H19-DMR-R2	CCCTAACCTCATAAAACCCATAACTAT		
	Snrpn-F1	TATGTAATATGATATAGTTTAGAAATTAGT	703	
	Snrpn-R1	AATAAACCCAAATCTAAAATATTTTAATCA		
	Snrpn-F2	AATTTGTGTGATGTTTGTAATTATTTGG	312	
	Snrpn-R2	ATAAAAATACACTTTCACTACTACTAAAAT		

Supplementary information, Data S1 Materials and Methods

Mice

Specific pathogen-free (SPF)-grade mice were obtained from Beijing Vital River laboratory animal centre and housed in the animal facilities of the Institute of Zoology, Chinese Academy of Sciences. All studies were carried out in accordance with the guidelines for the Use of Animals in Research issued by the Institute of Zoology, Chinese Academy of Sciences. Female β-actin–*Gfp* transgenic mice (B6D2F1× C57BL/6) were used for phESCs derivation. Female mice of B6D2F1 (C57BL/6× DBA/2) and CD-1 backgrounds were used to provide oocytes and blastocysts for micromanipulation, respectively. Male mice (B6D2F1×C57BL/6) were used to provide round-spermatid for injection.

Collecting of MII oocytes

Eight-week old female mice were super-ovulated by consecutive injection of PMSG and hCG.

Oocytes were collected from the oviduct 13-15 h after injection. Cumulus cells were removed with hyaluronidase (300 IU/ml). Derived oocytes were washed with Hepes-CZB and cultured in M16 medium (Sigma) as described[1]. After 26-29 h of culturing, the two-cell embryos were transferred into KSOM (Millipore) and cultured at 37.5 °C under 5% of CO_2.

Derivation of phESCs

Oocytes were collected from female mice (B6D2F1× C57BL/6) carrying chicken β-actin–*Gfp* transgene and activated as previous report[2]. Collected oocytes were placed in Ca^{2+}-free CZB containing 10 mM Sr^{2+} without Cytochalasin B (CB) for 6 h. Embryos with one visible pronuclear and second polar body were considered as haploid. At the morula stage, haploid embryos were transferred and seeded in four-well dishes with medium of N2B27 plus 5% of knockout serum replacement (GIBCO). MEK inhibitor (1 μM) PD0325901 (Stemgent) and GSK3β inhibitor (3 μM) CHIR99021 (Stemgent) were added. After 5-7 days of culturing, the embryo outgrowth was digested with 0.25% of trypsin and passaged. The colonies of phESCs usually appeared 2 or 3 days after passaging. Derived phESC lines were cultured in 2i medium and 1:3 passaged every 2–3 days.

Purification and G-banding chromosome analysis of phESCs

After being cultured for 4–5 passages, phESCs were purified by fluorescence-activated cell sorting as described (*13*). The phESCs were collected and single-cell suspensions were obtained by repetitive pipetting and transferred through a 40-mM cell strainer. Haploid and diploid ESCs were incubated with Hoechst 33342 (Invitrogen) and 50 mM Verapamil (Sigma) at 37°C for 30 min，then analysed and sorted on BD FACSAria II (BD Biosciences). Diploid (2n) ESCs were used as a control. Flow-cytometric data were analysed using the ModFit software (Verity Software House) following the manufacturer's instructions. Standard G-banding chromosome analysis was carried out to detect whether the phESCs had a normal karyotype. Approximately 30 separate metaphase spreads were examined for each cell line as previous (*13*).

Intracytoplasmic injection of phESCs and round spermatid

The phESCs injection procedure was modified from a previously reported procedure (*13*). In brief, matured MII oocytes were collected from the oviduct of super-ovulated 8-week-old female mice (B6D2F1, C57BL/6× DBA/2). G0- or G1-phase phESCs sorted with FACS were chosen as donors. Before the microinjection of phESC, oocytes were pre-activated by 10mM $SrCl_2$ in calcium-free CZB medium for 30 min. Sorted phESCs were injected into oocytes separately to construct bimaternal embryos. The constructed embryos were activated by 10mM $SrCl_2$ in calcium-free CZB medium at 37°C with 5% CO_2 for another 5 h. Completely activated embryos were washed and cultured in M16 medium (Sigma) at 37°C with 5% CO_2 as described[1]. In the next day, bimaternal embryos at 2-cell stage were transferred to the oviduct of psedudopregnant CD-1 mice at 0.5 dpc. To evaluate the development of bimaternal embryos, pregnant recipients were dissected on 13.5, 17.5 and 19.5 dpc, respectively. The round sperm injection (ROSI) experiment was carried out as control (B6D2F1×C57BL/6) with descrbed procedure[3].

Immunofluorescence microscopy analysis of phESCs

Cell samples were fixed with 4% paraformaldehyde (PFA) at 20–25 °C for 30 min, then washed 3 times with PBS, and penetrated with 0.5% Triton X-100 at 20–25 °C for 30 min. After which, samples were blocked with 500 μl 2% BSA at 20–25 °C for 1 hour, then incubated with primary antibody overnight at 4 °C. The antibody used included anti-Oct4 (Santa Cruz), anti-Sox2 (Santa Cruz), anti-SSEA1 (Millipore), and anti-Nanog (Abcam). On the second day, samples were washed with PBS, followed by incubating with AlexaFlur 488-conjugated secondary antibody (diluted with 2% BSA) at 20–25 °C for 1 h, then recorded with confocal microscope (ZEISS, LSM 780 META). Samples were observed with an inverted microscope (Leica DMI3000B, Leica Co.).

Teratoma formation

Teratoma formation analysis was carried out to evaluate the pluripotency of phESCs. Approximately 1×10^7 phESCs were injected subcutaneously into the hind limbs of a 6-week-old male severe-combined-immune-deficiency (SCID) beige mice. After 4 weeks, fully formed teratomas were dissected and fixed with PBS containing 4% paraformaldehyde,

then embedded in paraffin, sectioned and stained with haematoxylin and eosin for histological analysis.

Diploid blastocyst injection

Diploid blastocyst injection and chimaera generation assay was also carried out to evaluate the pluripotency of phESCs. Super-ovulated female CD-1 mice (3.5 dpc) were killed to collect recipient blastocysts. Twelve to fifteen phESCs were microinjected into a blastocyst to produce chimaeric embryo. After 1–4 h of culturing, these manipulated embryos were transferred into the oviduct of psedudopregnant CD-1 mice at 0.5 or 2.5 dpc. Chimaeras were identified by coat colors.

Bisulfite genomic sequencing

Genomic DNA was treated with CpGenome™ Fast DNA Modification Kit (Millipore) according to manufacturer's instructions. H19-, Igf2r-, Snrpn- DMRs were amplified with nested primers (Table S1). The first round of PCR was performed using 95 °C for 5 min; then 10 cycles of 94 °C for 30 s, 52 °C for 30 s and 72 °C for 60 s. One microliter of the first-round PCR product was used as template for the second round PCR, which was performed with 95 °C for 5 min; then 30 cycles of 94 °C for 30 s, 55 °C for 30 s and 72 °C for 30 s. The PCR products were cloned into pMD18-T vectors (Takara). At least 10 randomly selected clones were sequenced and analysed for unmethylated C to T conversion at each locus.

Reverse transcription, quantitative PCR and CDS cloning

RNA was extracted from mouse brain and phESCs using TRIzol (Invitrogen). The cDNA was synthesized with Oligo (dT) or Random primers (Promega) and the M-MLV Reverse Transcriptase kit (Promega) according to manufacturer's protocols and used as template to clone the *Igf2* CDS with primers listed in Table S1. Cloned CDS was sequenced and ligated into an *Rfp* containing piggyBac plasmid. PCR was performed with ExTaq (Takara), and qPCR was performed with SYBR Green (TOYOBO).

DMR modification in phESCs

We constructed targeting vectors deleting the H19-DMR or IG-DMR using PCR fragments amplified from the C57BL/6 genome as recombination arms, which were ligated into a vector containing a PGK-*neo*^{r} cassette successively. For the ΔIG targeting vector, the PGK-*neo*^{r} cassette was replaced by an *EF1a-Rfp-puro*^{r} cassette, which was cloned from HR100PA-1 vector (SBI) and ligated into the targeting vector. For the Δ5H19/Igf2 targeting vector, the PGK-*neo*^{r} cassette was replaced by an *EF1a-Igf2-Rfp-IRES-neo*^{r} cassette, which was cloned from a piggyBac-*Igf2-Rfp* vector and ligated into the targeting vector. The ligation reaction was performed with In-Fusion® HD Cloning Kit as manual (Clontech). The primer pairs used for recombination arms amplification and cassette ligation were listed in Table S1. For the modification of DMRs, we linearized each targeting vector, electroporated together with Cas9 and Cas9-sgRNA-1 or Cas9-sgRNA-2 expression vectors into phESCs (*Neon*) respectively, and selected ESC clones containing the integration of targeting vector with G418 (200 ug/ml) or puromycin (0.4 ug/ml). The DR4 drug resistant feeder cells was used (SIBCB). We confirmed the CRISPR/Cas9 helped homologous recombination events of putative targeted phESC clones by specific primer pairs listed in Table S1.

RNA-seq library preparation and data analysis

Total RNA was extracted from cells and brain tissues with TRIzol (Invitrogen), after which 1 μg of purified RNA was used for reverse transcription polymerase reaction each time (Invitrogen). For RNA-seq library construction, two rounds of PolyA+ tailed RNA purification were performed for each sample. Sequencing was performed on an Illumina HiSeq 2500 sequencer with 125 bp paired-end sequencing reaction. RNA-seq data analysis was performed with HISAT (version 0.1.6-β)[4] and Cufflinks (version 2.2.1) [5, 6]using the UCSC mm9 annotation. Reads with unique genome location were reserved for differential expressed gene analysis using Cuffdiff using default parameters.

RRBS library preparation and data analysis

The RRBS libraries were generated as previously described[7, 8]. Single-end or paired-end sequencing was performed on an Illumina HiSeq 2500 sequencer. Beside the RRBS data produced in this experiment, four RRBS datasets for wild type MII oocyte and sperm were

downloaded from GEO database (GSE61331) (*19*). All these data were analysed together. Briefly, the sequencing reads were mapped to the mouse genome (version mm9) by Bismark v0.13.1 (Babraham Bioinformatics) after trimming by Trim Galore (Babraham Bioinformatics) with the "-rrbs" option. The methylation levels of covered cytosine sites were calculated by dividing the number of reported C with the total number of reported C and T. CpG sites covered by more than ten reads were used for the analysis. All the heatmaps showed in the manuscript were plotted with the heatmap.2 function of R. Histograms of methylation level distribution were drawn by ggplot2.

Statistical analysis

Statistical analysis was carried out using SPSS 17.0 statistical software. The Student's t-test was used for statistical analysis. For all statistical analyses, a value of $P < 0.05$ was considered to be statistically significant.

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