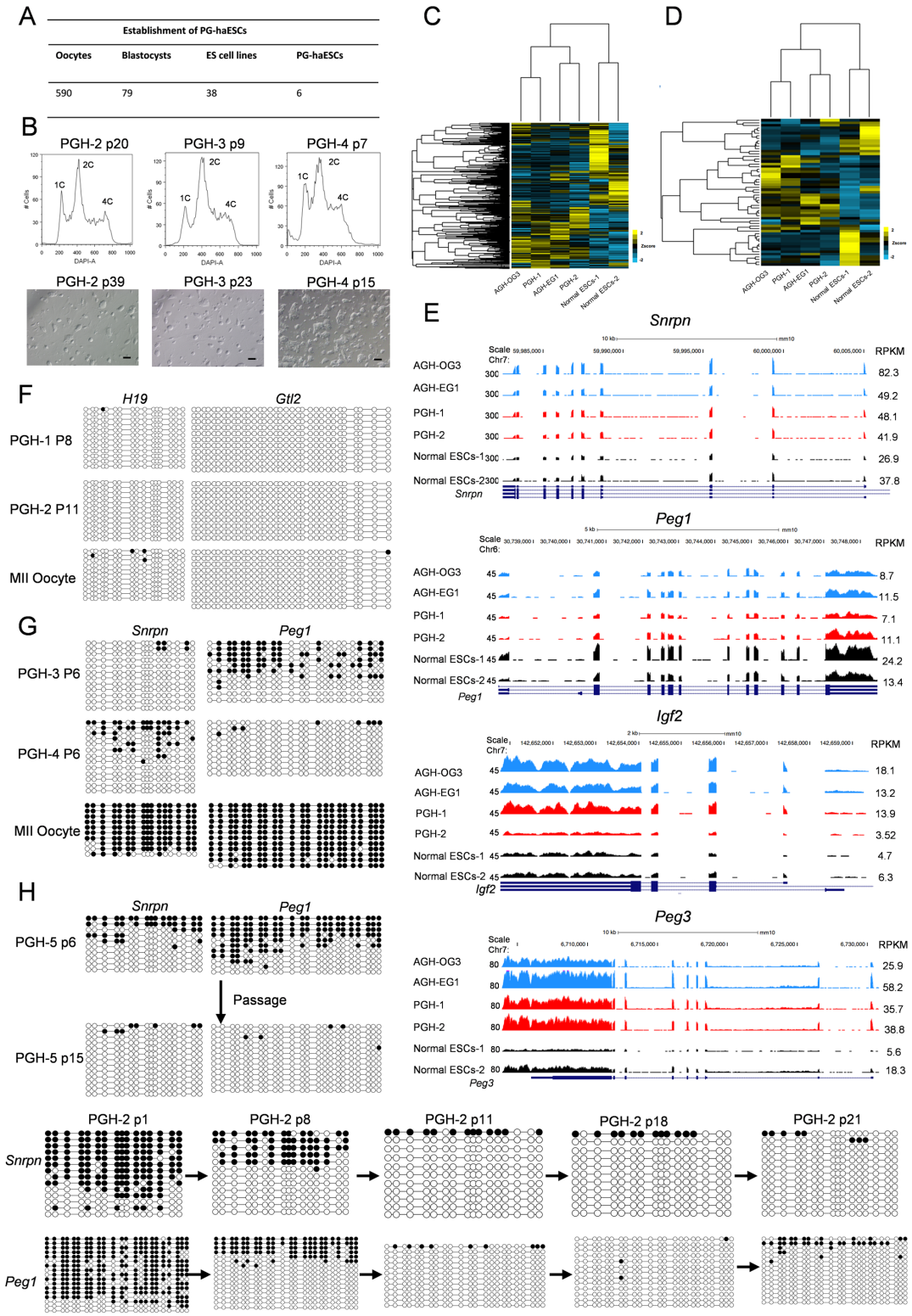
Supplementary Information



Supplementary information, Figure S1 Establishment of PG-haESCs.

(A) Summary of derivation of PG-haESCs.

(B) Upper, Flow analysis of DNA content of established PG-haES cell lines. Lower, Images of PG-haESCs derived from parthenogenetic blastocysts. Scale bars, 100 μM.

(C) Gene expression profiles of PG-haESCs using RNA-seq analysis. Gene expression profiles were clustered using all expressed genes. Two PG-haESC lines (PGH-1 and PGH-2) show similar expression profiles to the AG-haESCs (AGH-OG3 and AGH-EG1). To avoid the influence of diploidized cells on the expression profile, we collected samples after FACS of cells in the G1/G0 phase.

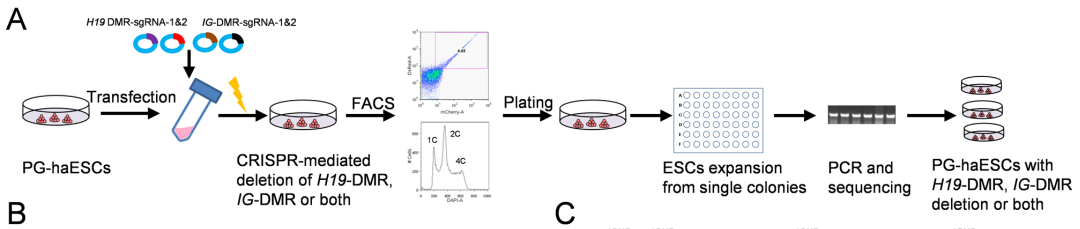
(D) Gene expression profiles of PG-haESCs based on imprinting genes. Two PG-haESC lines show similar expression profiles to the AG-haESCs.

(E) Bigwig track of maternal imprinted genes (*Snrpn, Peg1, Igf2* and *Peg3*). RNA-seq of PG-haESCs and AG-haESCs reveals similar expression levels of both maternal and paternal imprinted genes in PG-haESCs compared with AG-haESCs.

(F) Methylation state of paternally imprinted genes (*H19* and *Gtl2*) in PG-haESCs.

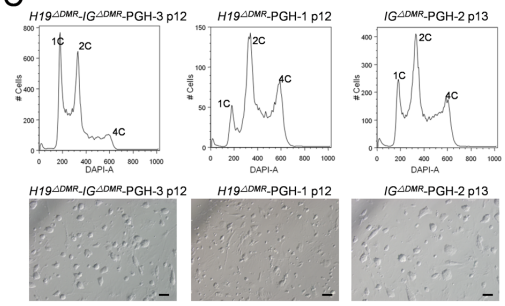
(G) Methylation state of maternally imprinted genes (*Snrpn* and *Peg1*) in PG-haESCs. PGH-3 and PGH-4 lost maternal imprints at early passages.

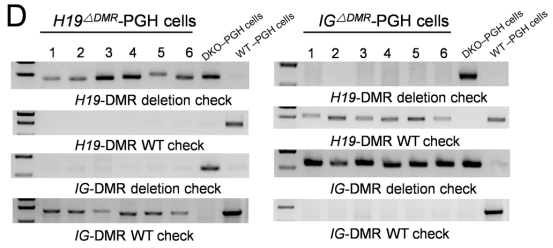
(H) Methylation profiles of *Snrpn* and *Peg1* in PGH-2 and PGH-5 ES cell lines. PGH-2 and PGH-5 gradually lost maternal imprints during cell passage.

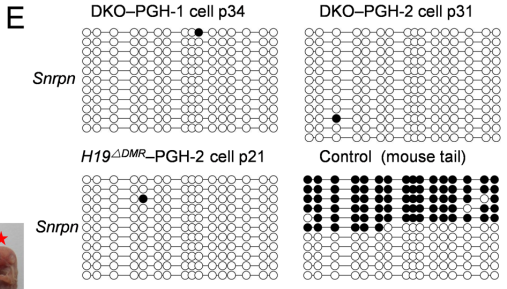


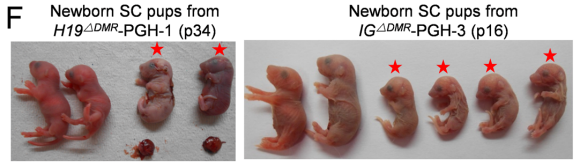
B

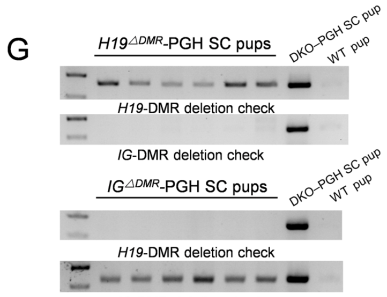
PG-haESCs	*H19*-DMR deletion/Total haESCs	*IG*-DMR deletion/Total haESCs	DKO ESCs /Total haESCs
PGH-1 p24	6/42	10/42	23/42
PGH-2 p12	2/12	4/12	5/12
PGH-4 p10	3/13	5/13	5/13
PGH-5 p10	6/24	5/24	11/24
Total	17/91	24/91	44/91

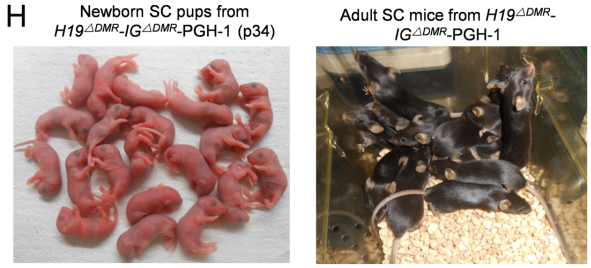


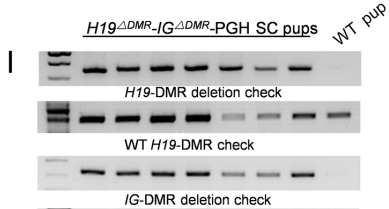












Supplementary information, Figure S2 Establishment of DKO-PG-haESCs.

(A) Diagram of generation of DKO-PG-haESCs using CRISPR-Cas9 technology.

(B) Summary of establishment of PG-haESCs carrying *H19*-DMR deletion, *IG*-DMR deletion or both.

(C) Establishment of PG-haESCs carrying *H19*-DMR deletion, *IG*-DMR deletion or both. Upper panel, flow analysis of DNA content of established haploid cell lines. Lower panel, represented images of PG-haESCs carrying different mutations. Scale bars, 100 μM.

(D) Genotyping analysis of PG-haESCs carrying *H19*-DMR or *IG*-DMR deletion.

(E) Methylation state of the *Snrpn* DMR in DKO-PG-haESCs.

(F) SC pups from ICPHCI using $H19^{\triangle DMR}$-PGH (left) and $IG^{\triangle DMR}$-PGH (right) cells. Pups and placentas obtained by C-section from pseudopregnant mice at E19.5 are shown. Asterisks indicate growth-retarded SC pups that died shortly after birth.

(G) Genotyping analysis of SC pups derived from PG-haESCs carrying *H19*-DMR or *IG*-DMR deletion.

(H) SC pups from ICPHCI using $H19^{\triangle DMR}$-$IG^{\triangle DMR}$-PGH cells. Left, newborn SC pups. Right, adult SC mice.

(I) Genotyping analysis of SC pups derived from DKO-PG-haESCs.

(J) Methylation state of the *Snrpn* and *Peg1* DMRs in adult SC mice.

Supplementary information, Table S1 Summary of *in vivo* development of SC mice from PG-haESCs carrying deletions of *H19*-DMR, *IG*-DMR or both.

Donor cells	Haploid ESC lines	Passage number	No. of embryos transferred	No. of growth-retarded pups (% of transferred embryos)	No. of normal pups (% of transferred embryos)
H19-DMR KO PG-haESCs	$H19^{\Delta DMR}$-PGH-1	p29	57	1 (1.8)	0
		p34	120	2 (1.7)	2 (1.7)
		p36	132	3 (2.3)	2 (1.5)
	$H19^{\Delta DMR}$-PGH-4	p21	116	2 (1.7)	7 (6.0)
	Subtotal	p21-p36	425	8 (1.9)	11 (2.6)
IG-DMR KO PG-haESCs	$IG^{\Delta DMR}$-PGH-1	p29	50	1 (2.0)	0
		p36	81	4 (4.9)	1 (1.2)
	$IG^{\Delta DMR}$-PGH-2	p16	90	4 (4.4)	2 (2.2)
	Subtotal	p16-36	221	9 (4.1)	3 (1.4)
DKO-PG-haESCs	$H19^{\Delta DMR}$-$IG^{\Delta DMR}$-PGH-1	p30	43	0	7 (16.3)
		p31	93	0	16 (17.2)
		p34	125	1 (0.8)	21 (16.8)
		p34	120	1 (0.8)	24 (20.0)
		p39	75	0	11 (14.7)
	$H19^{\Delta DMR}$-$IG^{\Delta DMR}$-PGH-2	p13	156	0	20 (12.8)
		p15	87	0	11 (12.6)
		p15	45	0	6 (13.3)
		p16	75	0	13 (17.3)
	$H19^{\Delta DMR}$-$IG^{\Delta DMR}$-PGH-3	p12	20	0	3 (15.0)
		p18	40	0	5 (12.5)
		p19	35	1 (2.9)	4 (11.4)
		p21	105	0	17 (16.2)
	Subtotal	p12-p39	1019	3 (0.3)	158 (15.5)

WT PG-haESCs	PGH-1	p30	132	0	0
	PGH-2	p32	116	0	0
	Subtotal	p30-32	248	0	0

Materials and Methods

Animal use and care

All animal procedures were performed under the ethical guidelines of the Institute of Biochemistry and Cell Biology (SIBCB), Shanghai Institutes for Biological Sciences (SIBS), Chinese Academy of Sciences (CAS).

Derivation of PG-haESCs

For generation of PG-haploid embryos, mature oocytes were obtained from superovulated female mice (C57BL/6 background) and cultured in CZB medium for 1 hr and then activated for 5–6 hr in activation medium containing 10 mM Sr^{2+} without CB. Following activation, all of the embryos were cultured in KSOM medium with amino acids at 37°C under 5% CO2 in air. The embryos that reached the morula or blastocyst stage by 3.5 days in culture were transferred into ESC medium for derivation of ESC lines. The zona pellucida was removed using acid Tyrode solution. Each embryo was transferred into one well of a 96-well plate seeded with ICR embryonic fibroblast feeders in ESC medium supplemented with 20% knockout serum replacement, 1,500 U/ml LIF, 3 uM CHIR99021, and 1 uM PD0325901. After 4–5 days in culture, the colonies were trypsinized and transferred to a 96-well plate with a fresh feeder layer in fresh medium. Clonal expansion of the ESCs proceeded from 48-well plates to 6-well plates with feeder cells and then in 6-well plates for routine culture. To sort haploid cells, ESCs were trypsinized, washed by DPBS (GIBCO), and then incubated with 15 ug/ml Hoechst33342 in a 37°C water bath. Subsequently, the haploid 1C peak was purified using BD FACS AriaII for further culturing.

ICPHCI and embryo transfer

To generate semi-cloned (SC) embryos, PG-haESCs arrested at M phase by culturing in medium containing 0.05 mg/ml demecolcine for 8 hr were used for intracytoplasmic injection (ICPHCI) as a previous report. Briefly, PG-haESCs were trypsinized and suspended in HEPES-CZB medium with or without 3% (w/v) polyvinylpyrrolidone. Each nucleus from M-phase haploid cells was injected into an

MII-arrested oocyte (B6D2F1 background) using a Piezo-drill micromanipulator. The SC embryos were activated and cultured in vitro for 24 hr to reach the two-cell stage. 15–20 two-cell SC embryos were transferred into each oviduct of pseudo-pregnant ICR females at 0.5 days postcoitum (dpc).