SUPPLEMENTARY FIGURES

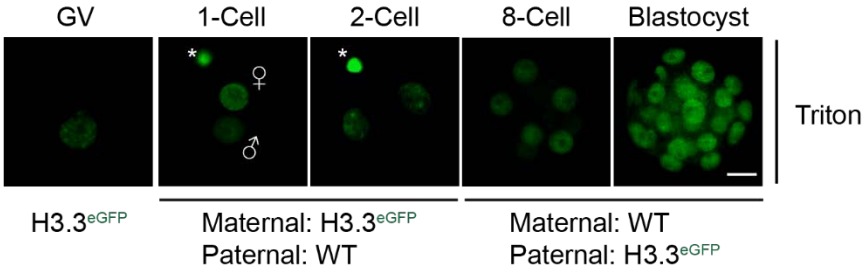

Fig. S1. H3.3 is consistently incorporated into parental chromatin in oocytes and embryos. Confocal imaging (full projection) of GV oocytes and early embryos (1C to blastocyst) expressing eGFP-tagged H3.3 with indicated genotypes. Oocytes and embryos were collected and treated with 0.5% Triton for 10 min before fixation for imaging, in order to remove any non-chromatin bound proteins. Asterisk, polar bodies; scale bar, 20 μm.

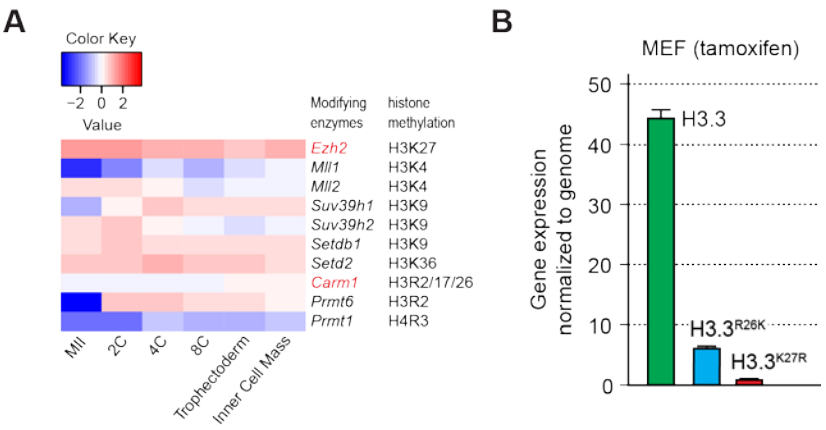

Fig. S2. Selection of R26 and K27 for mutation to construct Rainbow cassette. (A) Heatmap for transcript abundance of histone modifying enzymes during preimplantation development. Color code for expression level (Z-score normalized $\log_2$ RPM value). Enzymes modifying K27 or R26 are indicated in red. (B) Recombination efficiency was quantified in transgenic HISRainbow;CreER MEF cells (n =3 cell lines from 3 mice) 2 days after induction with 1 μM 4-hydroxytamoxifen. Gene expression was determined by realtime RT-PCR and expression of $H3.3^{K27R}$ was set as 1. Mean ± s.e.m.

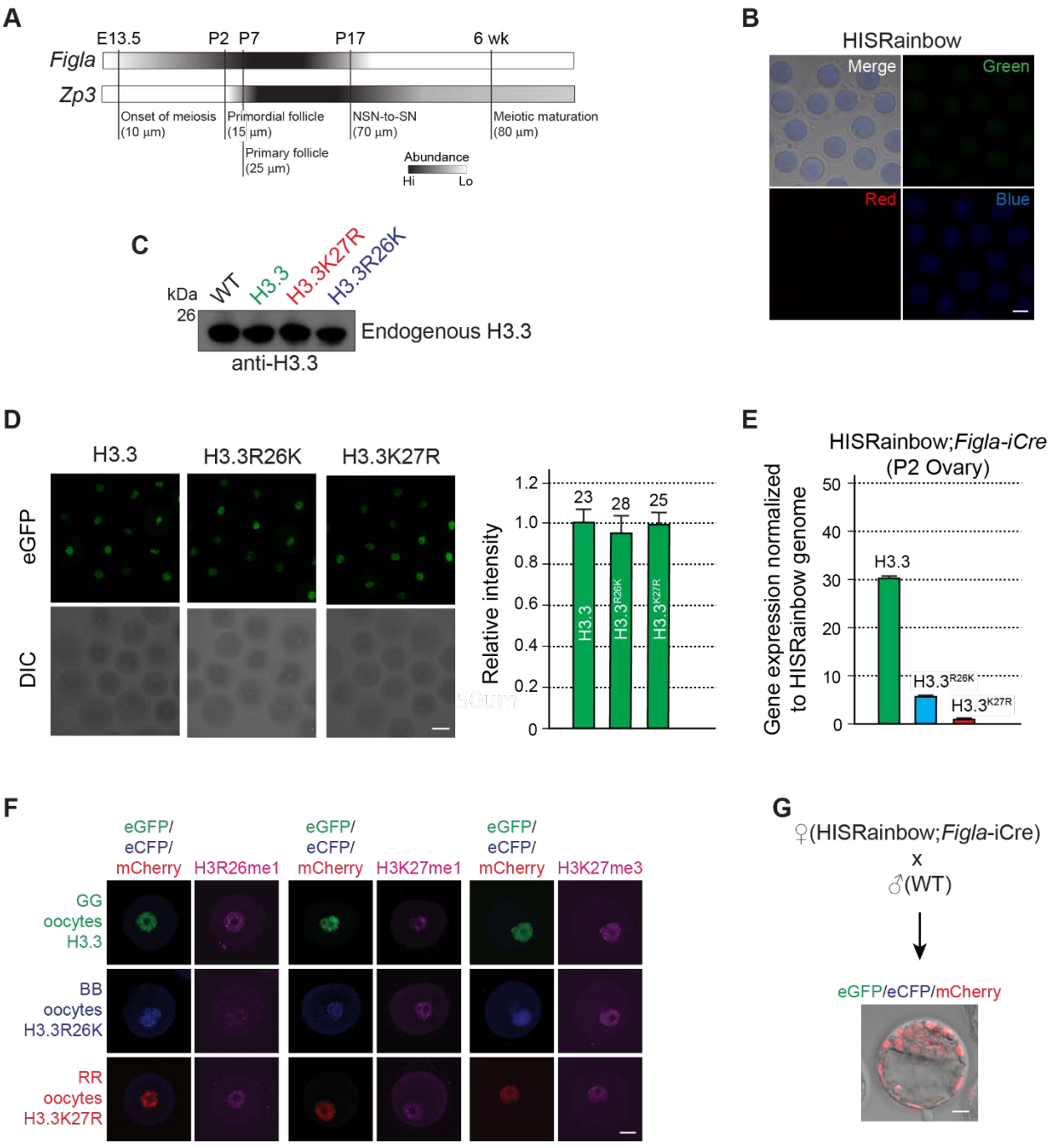

Fig. S3. Recombination of the HISRainbow cassette in oocytes. (A) Timelines of *Figla* and *Zp3* expression beginning with the onset of meiosis at E13.5 and continuing through folliculogenesis to fully grown, meiotically mature oocytes. Activation of the *Figla* promoter begins at meiosis and peaks in primordial follicles. Activation of the *Zp3* promoter begins in primary follicles and extends through the 2-week growth phase of

folliculogenesis. E, embryonic day; P, post-natal day. (B) Confocal (full projection) and DIC images of MII oocytes isolated from HISRainbow female mice. No fluorescent signals were observed. Scale bar, 50 μm. (C) Immunoblot analysis of HISRainbow;*Figla*-iCre GV oocytes with indicated genotypes for endogenous H3.3 expression. 25 oocytes were collected for each group: WT, H3.3^{eGFP} (GG), H3.3K27R^{mCherry} (RR) and H3.3R26K^{eCFP} (BB) and loaded into each lane. Representative gel of 2 experiments. (D) cRNAs encoding H3.3^{eGFP}, H3.3R26K^{eGFP} and H3.3K27R^{eGFP} were injected into GV oocytes. Oocytes were incubated at 37 °C in KSOM with 2.5 μM milrinone for 24 hr and fixed for imaging (left). Fluorescent signals were then quantified for 23-28 oocytes in each group and fluorescent intensity from H3.3^{eGFP} group was set as 1 (right). (E) Recombination efficiency was quantified in P2 HISRainbow;*Figla*-iCre ovaries (n =6 mice) by RT-PCR and expression of H3.3^{K27R} was set as 1. Mean ± s.e.m. (F) Immunofluorescence of histone modifications (H3R26me1, H3K27me1, H3K27me3) in HISRainbow;*Figla*-iCre oocytes. Scale bar, 20 μm. (G) HISRainbow;*Figla*-iCre female were mated with WT male mice. 1C zygotes were collected and cultured to the blastocyst stage. Confocal (full projection) image of a representative blastocyst expressing H3.3K27R^{mCherry}. Asterisk, polar body; scale bar, 20 μm.

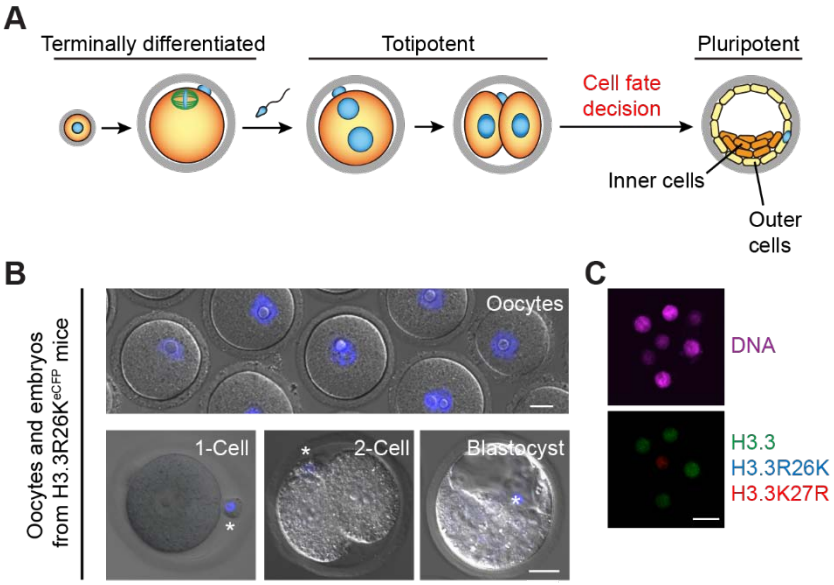

Fig. S4. Expression of H3.3 mutants in early mouse embryos. (A) Scheme of cell lineage specification in early mouse embryos. (B) GV oocytes were isolated from H3.3R26K^{eCFP} transgenic females. H3.3R26K transgenic females were mated with WT males, followed by zygote collection and culturing in KSOM until blastocyst stage. No eCFP signal was detectable in nuclei of embryos. Asterisk, polar body; scale bar, 20 μm. (C) Representative confocal (full projection) image of the mosaic embryo at 8-16-cell stage with individual blastomeres expressing either H3.3^{eGFP} or H3.3K27R^{mCherry}. DNA was stained by DRAQ5. Scale bar, 20 μm.

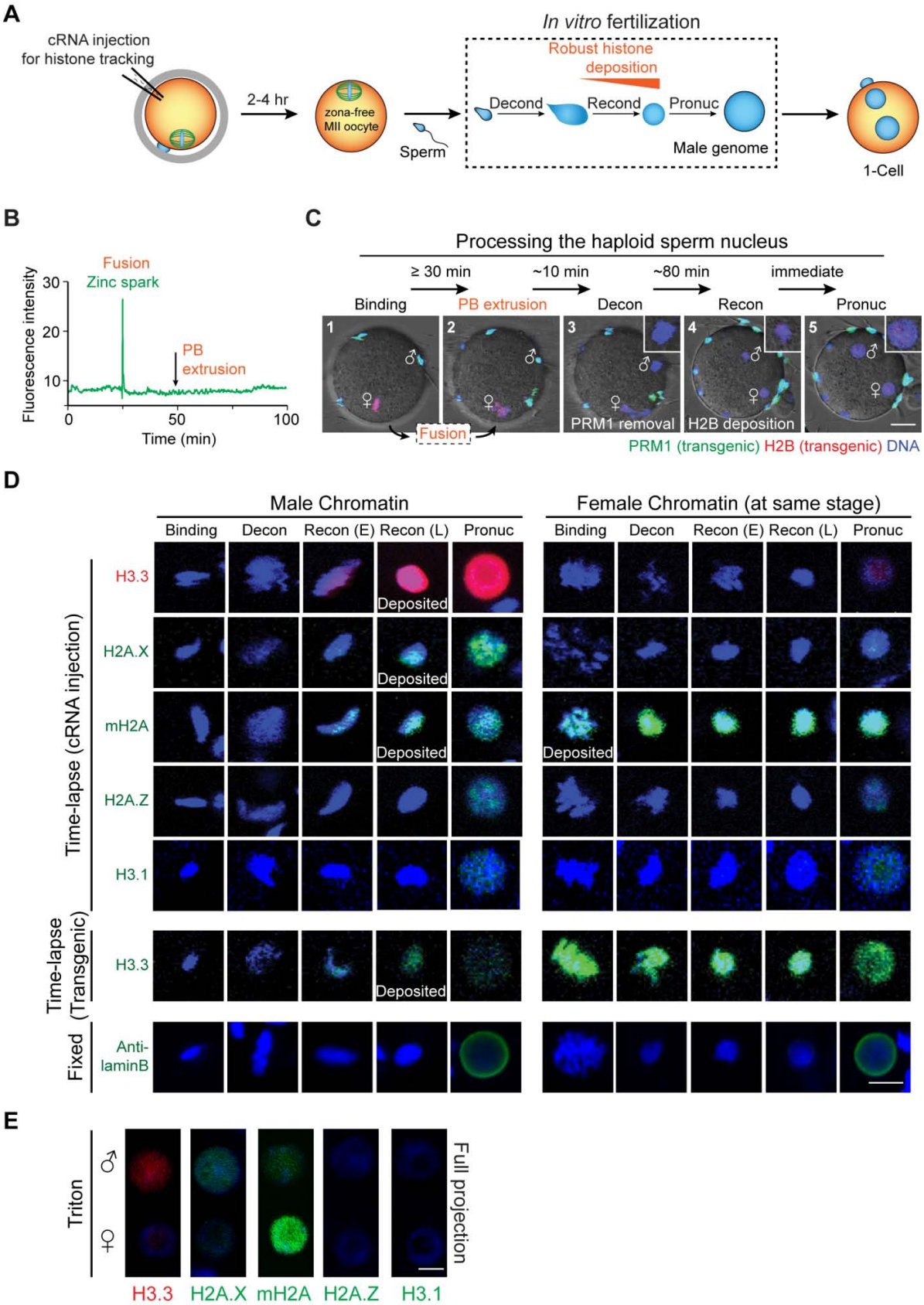

Fig. S5. Imaging parental genome reorganization during the protamine-to-histone exchange. (A) Histones with fluorescent tags can be imaged during fertilization to monitor their dynamics. (B) Sperm-oocyte fusion was determined by imaging zinc sparks with FluoZin-3. (C) Sperm expressing eGFP-tagged Protamine 1 (PRM1) and oocytes expressing mCherry-tagged H2B were imaged to track protamine removal and histone deposition, respectively, with representative images of 10 zygotes shown. 1, sperm-oocyte binding (Binding); 2, polar body (PB) extrusion; 3, male genome de-condensation (Decon); 4, re-condensation (Recon); 5, pronuclei formation (Pronuc). Insets in panels 3-5 are 2X magnification of the male genome. Approximate time between each step is indicated above. Scale bar, 20 μm. (D) For imaging during *in vitro* fertilization, either cRNAs encoding eGFP or mCherry-tagged histone variants (indicated by green or red color) were microinjected into MII oocytes, or MII oocytes were directly collected from hormonally stimulated transgenic female. Images of individual steps of male genome reorganization are shown. Early and late phases of re-condensation are indicated with E and L. To confirm nuclear membrane formation during this process, we collected zygotes every two hr until six hr, performed Triton treatment and paraformaldehyde fixation, stained with anti-lamin B antibody, and documented that pronuclei with nuclear membrane are formed after Recon (L). 6-10 zygotes have been imaged for each group with a representative image displayed. Scale bar, 10 μm. (E) To confirm histone variant association, MII oocytes were injected, fertilized by WT sperm and collected at the early 1-cell stage for treatment with 0.5% Triton (10 min), followed by fixation and imaging. 10-15 zygotes were imaged for each group with a representative image displayed. Scale bar, 10 μm.

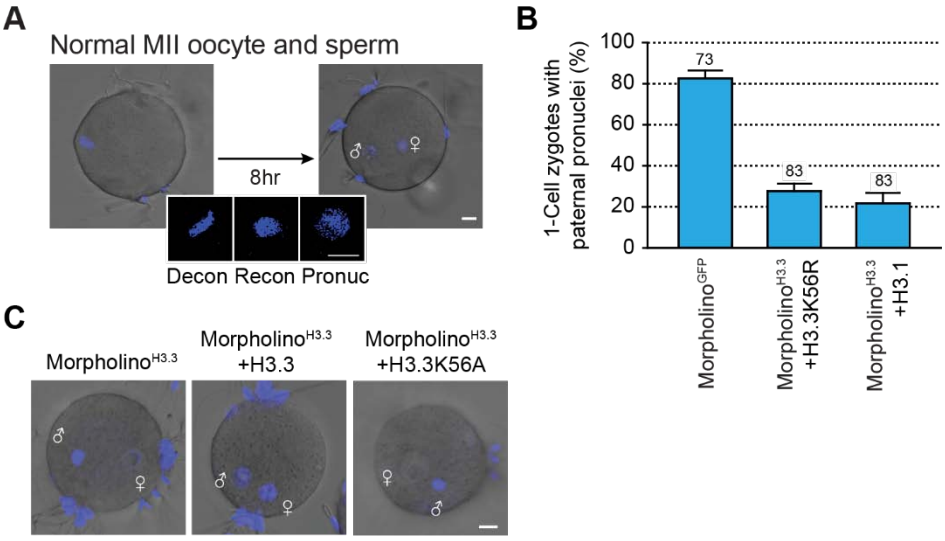

Fig. S6. H3.3K56 is essential for male pronuclei formation. (A) Representative merged DIC and fluorescent images of WT sperm progressing sequentially from de-condensation (Decond) to re-condensation (Recond) to pronucleus formation (Pronuc) after fusion with the *in vitro* matured MII oocyte (23 oocytes were imaged). (B) GV oocytes were injected with morpholinos and cRNA as indicated for *in vitro* maturation (18 hr) and *in vitro* fertilization. Oocytes, matured and fertilized *in vitro*, were fixed and observed 8 hr after insemination. Formation of paternal pronuclei was determined morphologically in fertilized oocytes treated with and without morpholinos (n =73-83). (C) Representative images of endogenous H3.3 deplete zygotes (left) rescued with cRNA encoding H3.3 WT (middle) or H3.3K56A (right). Scale bar, 10 μm.

Table S1. Primers.

	Mouse line/gene name	Primer sequence (forward, reverse)	PCR product (size)
Genotype	HISRainbow pCAG-H3.3^{eGFP}	CCAATCTGTGCGCCATTCAC ACATGAACTGAGGGGACAGG	327 bp
	ZP3-H2B^{mCherry}	CCGACTACTTGAAGCTGTCCTT CATGGTCTTCTTCTGCATTACG	185 bp
Tag discrimination	eGFP	TCGTGACCACCCTGACCTAC CTGCTTGTCGGCCATGATAT	293 bp
	eCFP	CGTGACCACCCTGACCTGG GTTCTTCTGCTTGTCGGCGG	298 bp
	mCherry	CCGACTACTTGAAGCTGTCCTT CATGGTCTTCTTCTGCATTACG	185 bp
	H33XFPUTR	CAATCTGTGCGCCATTCAC TGTTGCCAAACTCTAAACCAA	1116 bp

Table S2. Distribution of subpopulations in oocytes.

Genotype	GG[1]	BB	RR	GB	GR	BR	Total
HISRainbow; *Figla*-iCre[3] (P2 Ovary, n =6 mice)	67.83 ± 1.87[2]	2.29 ± 0.28	0.09 ± 0.03	24.54 ± 1.31	4.44 ± 0.74	0.82 ± 0.17	100
HISRainbow; *Figla*-iCre (n =7 mice)	9.18 ± 2.91	9.73 ± 2.84	22.43 ± 2.89	18.36 ± 1.86	16.69 ± 2.33	23.61 ± 3.35	100
HISRainbow; *ZP3*-Cre (n =8 mice)	8.15 ± 2.38	8.08 ± 1.95	19.95 ± 2.79	20.99 ± 4.16	21.78 ± 2.79	21.04 ± 3.48	100

[1]G indicates H3.3, B indicates $H3.3^{R26K}$, R indicates $H3.3^{K27R}$.

[2]Data is displayed in percentage ± s.e.m; n, number of mice analyzed.

[3]Subpopulation distributions in P2 ovary were calculated from the percentages of total H3.3 (G^{total}), total $H3.3^{R26K}$ (B^{total}), and total $H3.3^{K27R}$ (R^{total}) transcripts expressed from the HISRainbow cassette (Fig. S3C): GG =$(G^{total})^2$, BB =$(B^{total})^2$, RR =$(R^{total})^2$, GB =$2(G^{total})(B^{total})$, GR =$2(G^{total})(R^{total})$, BR =$2(B^{total})(R^{total})$.

Table S3. Distribution of blastomeres expressing H3.3^{eGFP} (G) and H3.3K27R^{mCherry} (R) in early blastocysts (HISRainbow;CreER).

H3.3 Isoform	Inner cells	Outer cells	Total
G	30.24 ± 1.17[1]	69.76 ± 1.17	100
R	19.23 ± 3.09	80.77 ± 3.09	100

[1]Data is displayed in percentage ± s.e.m of 30 blastocysts.

Blastocysts	Inner cells (G)	Outer cells (G)	Inner cells (R)	Outer cells (R)
1	4[2]	13	0	2
2	4	9	1	5
3	6	14	1	3
4	5	11	1	5
5	8	19	0	2
6	3	8	0	1
7	6	11	0	4
8	4	10	2	2
9	5	10	0	2
10	1	5	0	1
11	3	7	1	2
12	3	6	1	5
13	5	13	1	2
14	3	8	2	2
15	4	6	2	3
16	3	8	0	3
17	4	6	0	3
18	2	7	2	3
19	5	8	2	5
20	4	9	2	6
21	3	11	1	3
22	3	7	0	2
23	2	8	0	2
24	8	10	2	4
25	5	9	1	2
26	2	4	1	4
27	2	7	2	3
28	4	9	0	2
29	3	8	1	3
30	5	8	1	3
Total	119	269	27	89

[2]Data is displayed as cell number in blastocyst.

Table S4. Distribution of blastomeres expressing H3.3^{eGFP} (G) and H3.3K27R^{mCherry} (R) in 8-16-cell embryos (HISRainbow;CreER).

Embryo	G	R
1	4[1]	1
2	4	2
3	3	2
4	3	1
5	4	2
6	5	1
7	5	2
8	4	1
9	6	1
10	5	2
11	5	1
12	4	1
13	6	2
14	4	2
15	5	1
16	4	2
17	6	2
18	5	1
Total	82	27

[1]Data is displayed as cell number in embryos.