Fig. S1

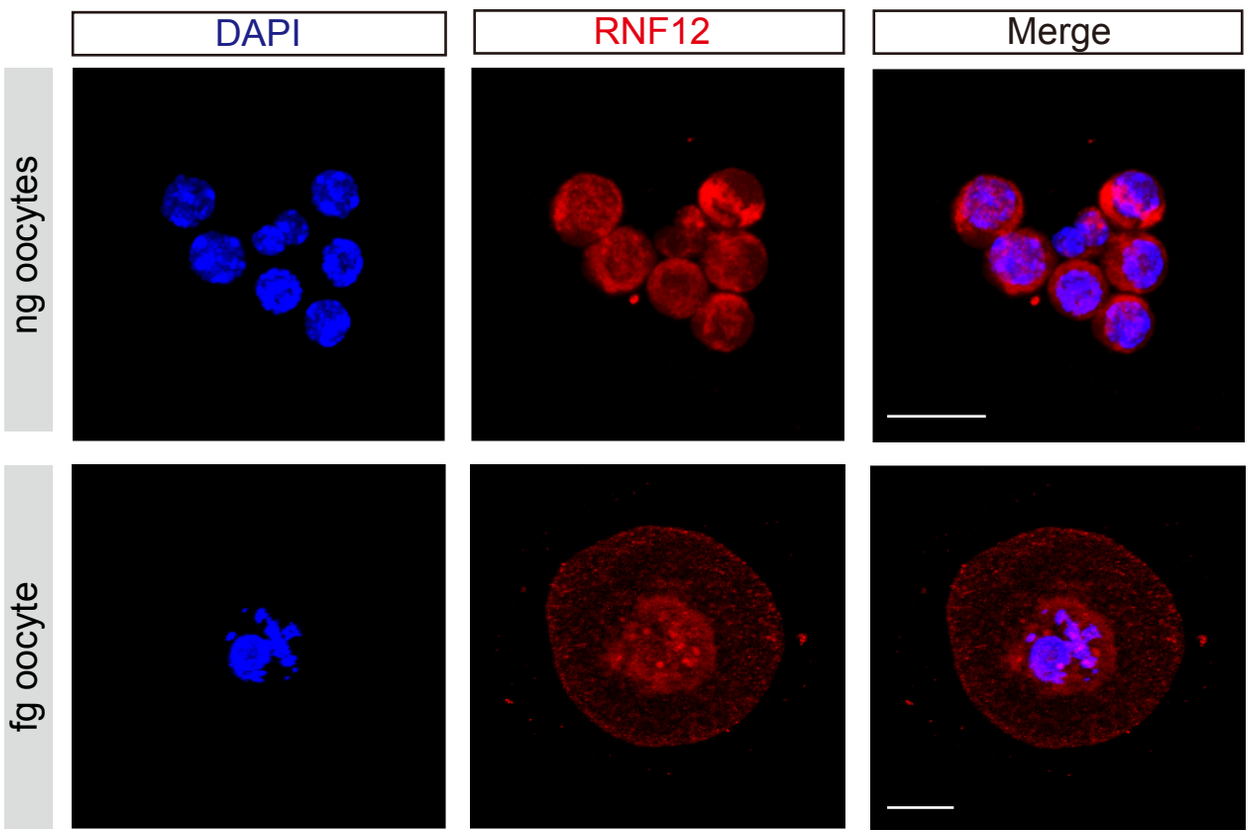
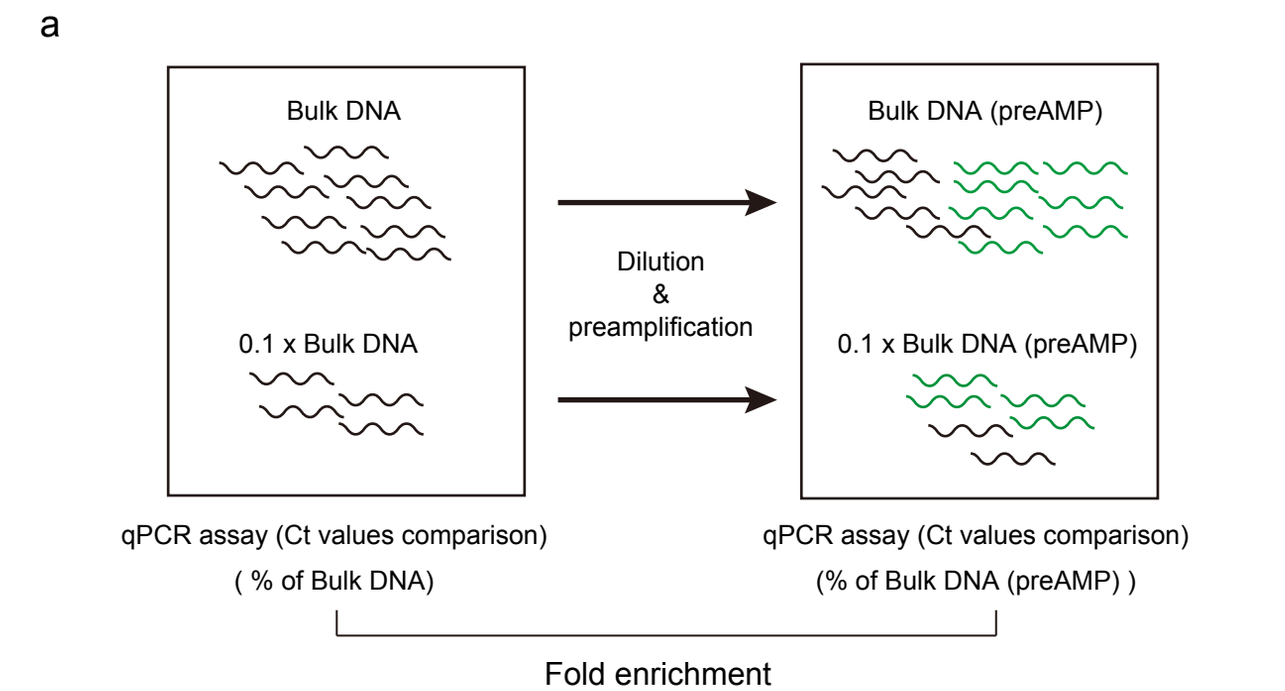
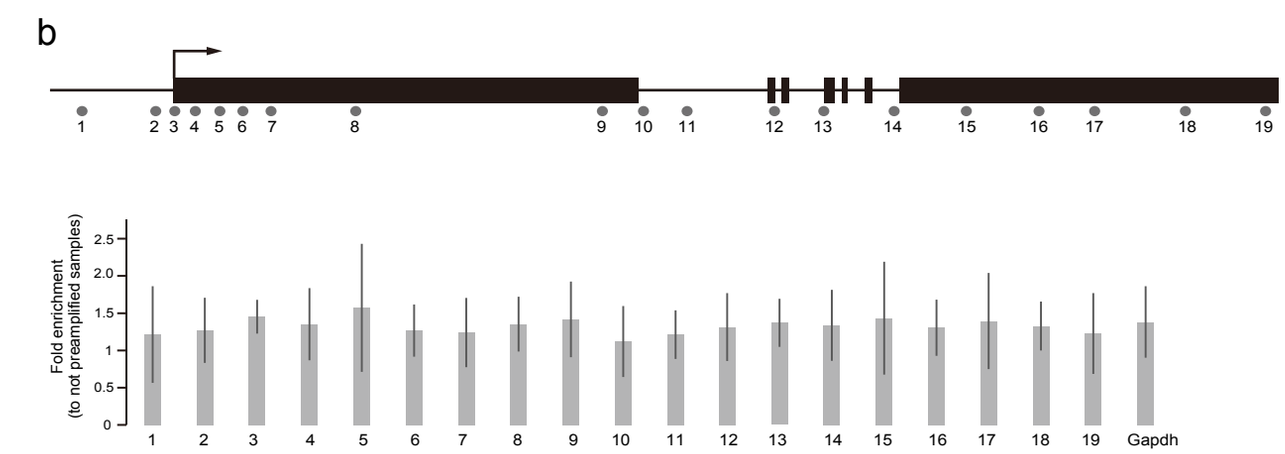


Fig. S2





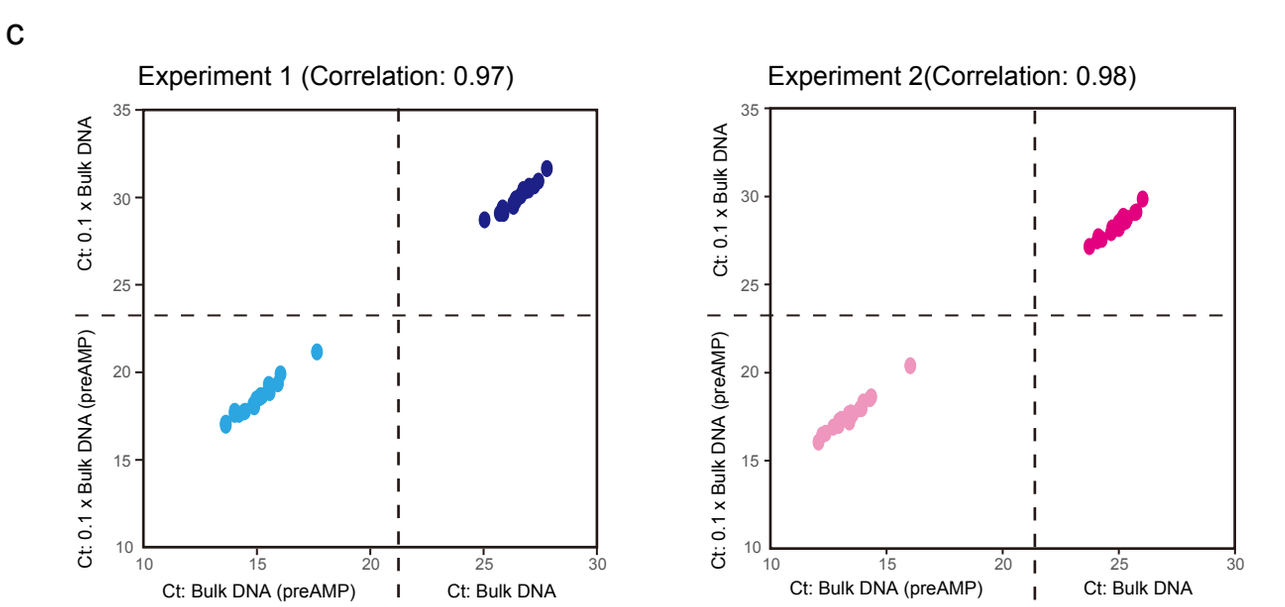
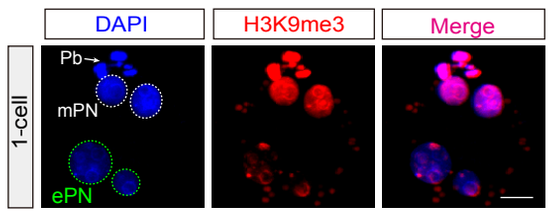


Fig. S3

a



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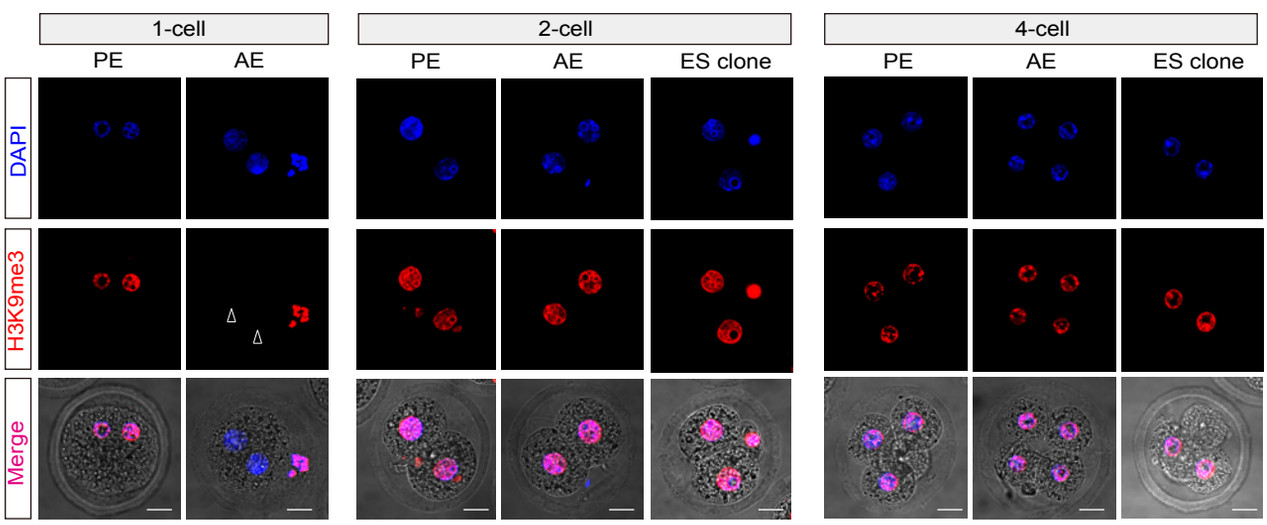

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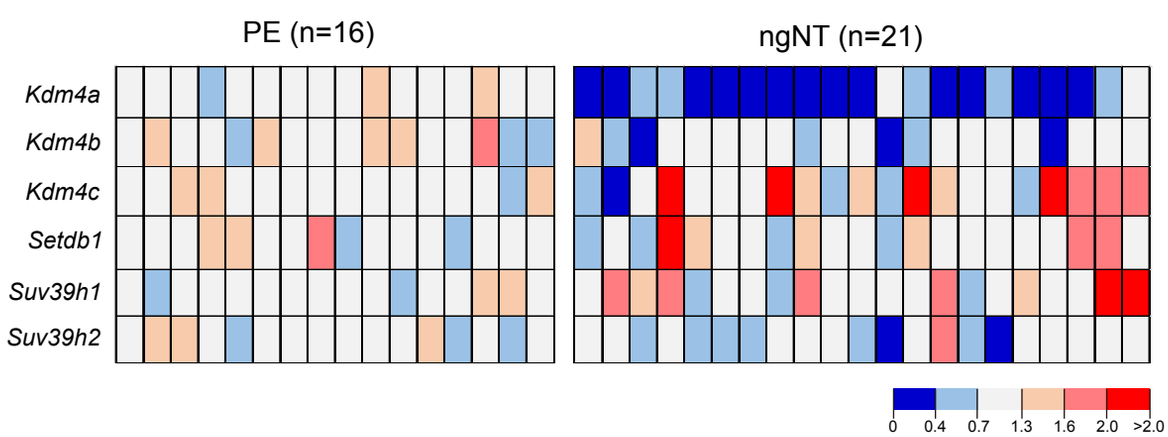
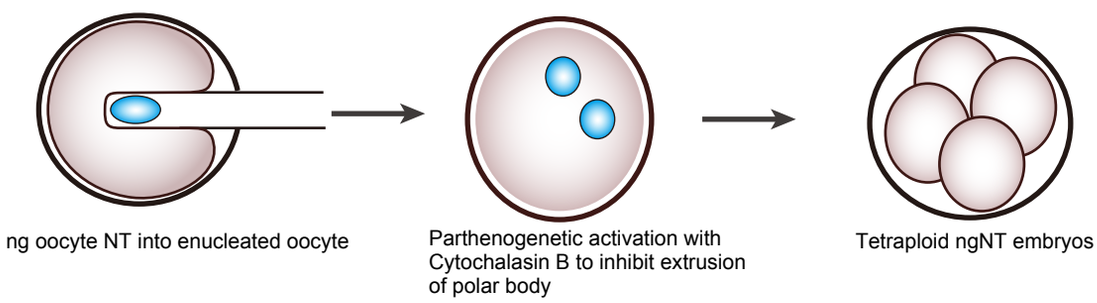


Fig. S4



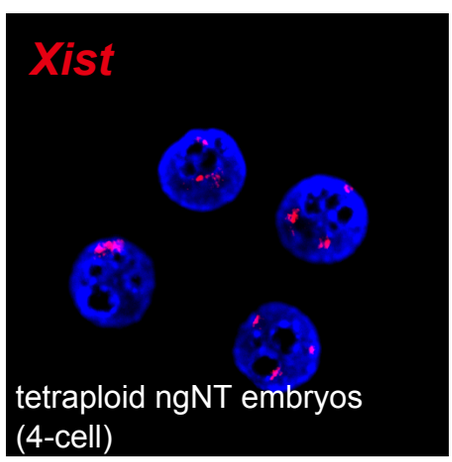
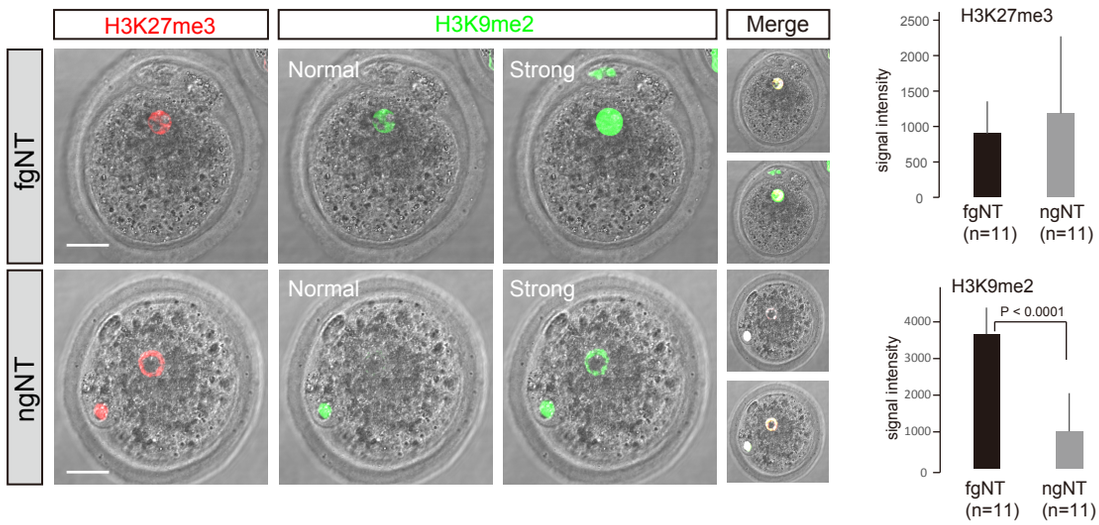


Fig. S5

a



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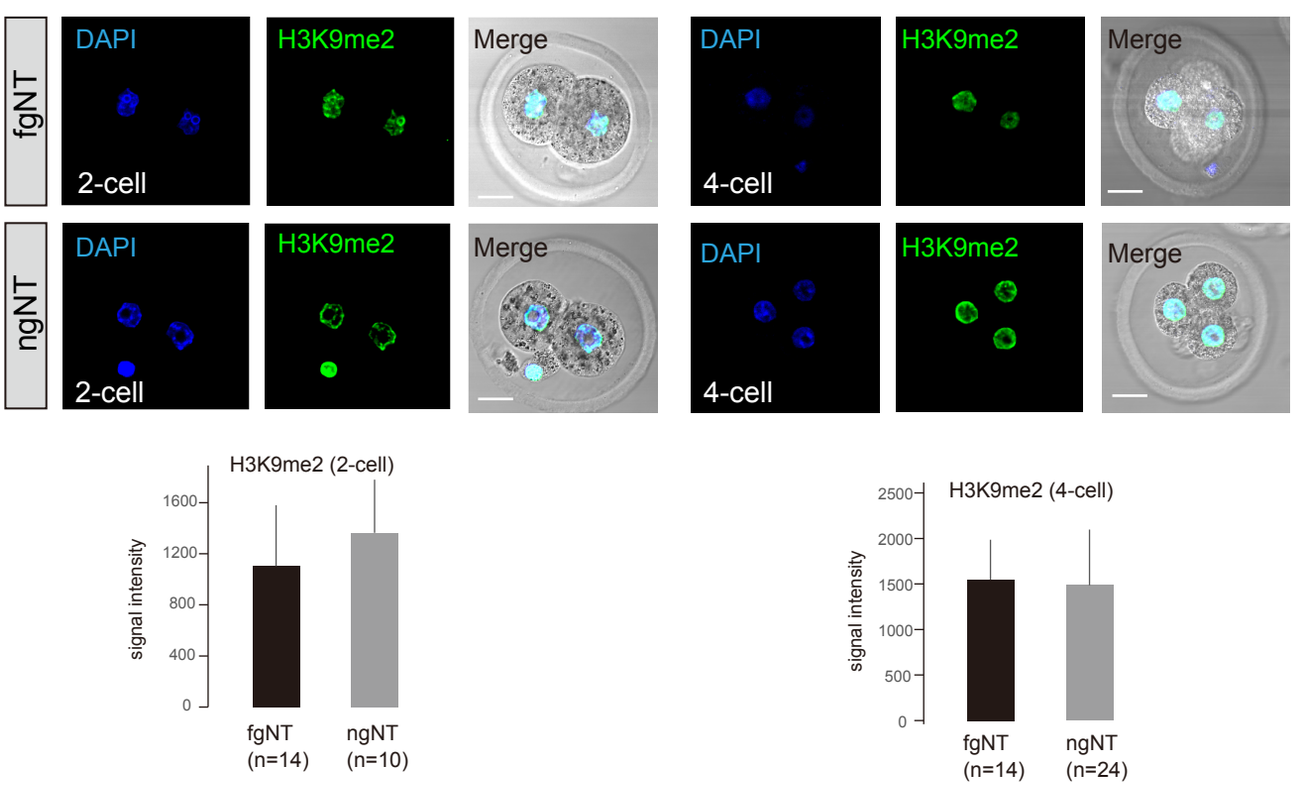

Fig. S6

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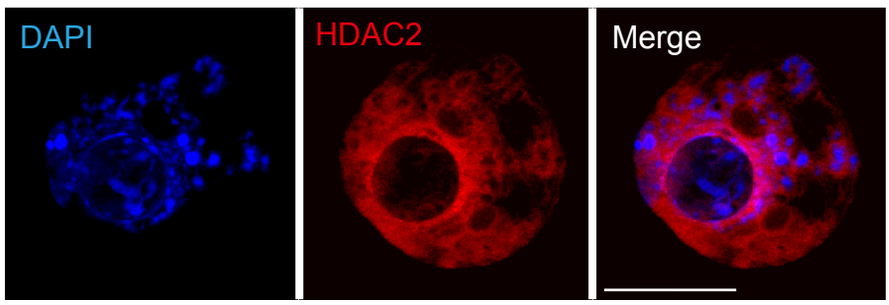

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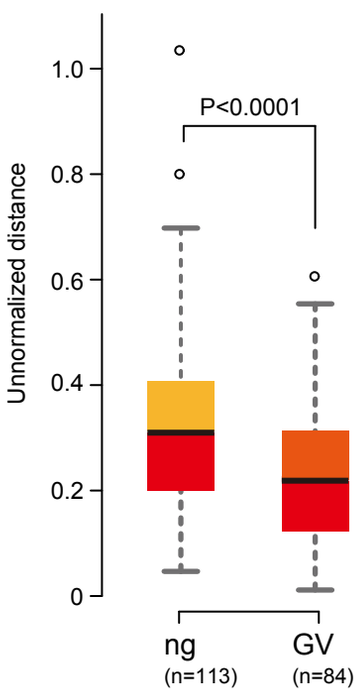

Supplementary Table S1. Primer and probe sequences for eChIP-qPCR assay.

Forward	Reverse	Probe	Position
GGTTTGCTTATGGACGATCAAAGTG	TCCCTACCTGAACCACCTCAATAG	CCAGCAATTCACTAATCTT	1
AGATGAGGAGAGGAAAGGGTAGAAAT	AGAGAAAGACTAAGATGCCAATGACC	CCTCACAAAATGGC	2
GAGCAAGCCGTTGCACG	ACCTAAAGGTCCAATAAGATGTCAGAA	CTTTAACTGATCCGCGGCG	3
AAAAAAGAATGAAAAGGCAGGTAAGTAT	GGTGGACTTACCTTTCTTTCATTGTTT	ACACACAGGTATCCGTGGC	4
GCTCCTCGGTGTCCTAATTCTTG	CATTATGGCTTCTGCGTGATACG	ACTGGCTCGAGAATAG	5
CACTAGAGGGCAGGTCACATGA	TTGTGTGTCACATGTCAGCTTCA	TCTCCAGGCTAACTCA	6
GCGGATGAGTCAAATGTCCTTGAA	CGGGTTCTTGGTCGATGTAAATC	ACCTCACCTATTAAAAGAAAG	7
GGCACTGCATTTTAGCAATACGATT	CATCTATACCCCCTCCATACTGAGT	CAGAGTCATGGAATTTC	8
AGGCGACTTGACATGTTCTCAAA	TTGGTCACCAGTCCTTGCTTATTT	CCATCCAGTCACCTTTTAA	9
GATCTCAAGTGTGCTTATATTACATTAAGTCTTAAATAAC	TGTACCCAATCGCCATTATGCT	AAGCGATGATATTTTACAAAATT	10
TGCGAAGGAATTTACAGTGGACTT	CCTTGTCCATAACACTTAACATTTTTTTGC	ACGTGCAAATGACCCTC	11
AGGTATTTACTTAGGAGGTATAACTTGACACA	GCCCAAAGGTATTTCCGTTACTTG	TAGACCTTTAAGGGAAGATACT	12
CCACCTAGGGATCGTCAAAGG	GAATTGTGGGCTTGCTGCTTT	CAGTGCTGGCGACCTAT	13
ACTAAATTGCCAAAGGGCATACATCT	CGCTTATAGTGTTTGCTGCTTACAG	CTGTTACATGGATTAAAAGC	14
GCAAACACAAAGCAACAGAATGATG	GGGCCACTTTCTCTGTTTTCTTTT	TTGCTATTGGTGGATTTC	15
GAAAAGGATTTGCAACTAAGGAATATGCA	GAGCTTTTCCTATAAGGCATCAGACT	TTGTGGGTTCATGGTCTTAG	16
mm03952844_s1			17
ACTGCAGTCCCCTCAGAAACTA	ATGAAAGTGCAGTTCTAAGTACTAAAATTTCTAAGTA	CTTTGGGTTGCATCCTTT	18
CAGGTGTTTTCCATGTGTTTTTTCAC	CCTACCATCATCTGCTTTGATCACATA	ACTCCAGTCTTTCAACAACA	19
CATCCAGGGACGTGCTGACT	TGTGTTCTCCCCTCACTGATCTC	CTGCCCTCGTGGACA	Gapdh

Supplementary Table S2. TaqMan probes for gene expression analysis.

Kdm4a	Mm00805000_m1
Kdm4b	Mm01236310_m1
Kdm4c	Mm01263760_m1
Suv39h1	Mm00468956_m1
Suv39h2	Mm00469689_m1
Setdb1	Mm00450791_m1

SUPPLEMENTARY FIGURE LEGENDS

Fig. S1. IF analysis of Rnf12 in ng and fg oocytes.

The expression states of Rnf12 protein levels of ng- (upper) and fg- (lower) oocytes were examined by IF analysis. Scale bar shows 20 μm. More than 16 oocytes were analysed.

Fig. S2. Validation of the unbiased eChIP-qPCR assay.

(a) Experimental scheme of the validation assay for eChIP-qPCR. Bulk DNA and 0.1× bulk DNA were diluted and preamplified. After qPCR analysis using TaqMan probes, the percentage of 0.1× bulk DNA and of 0.1× bulk DNA (preAMP) to that of undiluted samples were calculated, respectively, as well as the percentage of input in the normal ChIP-qPCR assay. Finally, for each *Xist* locus, the fold enrichments of preamplified samples were calculated. (b) Fold enrichments of preamplified samples in comparison with non-amplified samples in *Xist* and *Gapdh* promoter regions. Two independent experiments were conducted. Error bars show standard deviation (s.d.) (c) Correlation between preamplified and non-amplified samples based on Ct values.

Fig. S3. Genome-wide H3K9me3 modification in various types of embryos and H3K9me3-related gene expression in ngNT embryos. (a) H3K9me3 in ES genomes following NT. ePN and mPN show ES and maternal pronuclei, respectively. (b) H3K9me3 states in various type of embryos during early preimplantation phases. AE and PE represent androgenetic and parthenogenetic embryos, respectively. In (a) and (b), more than 10 embryos were analysed in each stage. (c) TaqMan qPCR analysis of single 4-cell embryos in ngNT and control parthenogenetic embryos. n represents the number

of analysed embryos. *Kdm4d* was not detectable. Heat map analysis shows the comparison of relative expression levels to the control average expression levels as calculated by Ct values. Note that all analysed samples were single 4-cell embryos, and thus, they were not normalized by an internal control. The scale bar shows 20 μm.

Fig. S4. Xm-*Xist* derepression in tetraploid ngNT embryos at the 4-cell stage. Representative image of FISH analysis in tetraploid ngNT embryos at the 4-cell stage. During activation, second polar body release was repressed in the presence of cytochalasin B. Some nuclei show more than 2 *Xist* signals. This result demonstrates that the Xm-*Xist* of tetraploid ngNT embryos is derepressed with the same timing as that of diploid ngNT embryos. More than 7 embryos were analysed.

Fig. S5. Global H3K27me3 and H3K9me2 in ngNT embryos. (a) IF analysis of H3K27me3 and H3K9me2 (double stain) at the 1-cell stage. The ngNT embryos were produced by serial NT. To evaluate the signal intensities, the lesser intensity for each observation was taken as being equivalent between the ngNT and control groups. Note that H3K9me2 modification was observed in ngNT embryos when the strong lesser was applied (right). (b) H3K9me2 in ngNT embryos at the 2- and 4-cell stages. There were no significant differences between ngNT and control embryos. The graph showed the signal intensity. n means the number of nuclei. P-value was calculated by student's t-test. Error bars showed standard deviation.

Fig. S6. IF analysis of HDAC2 for determination of the fg oocyte nuclear radius. (a) IF analysis of HDAC2. The nuclear radii were calculated using ImageJ software. Fourteen fg oocytes were analysed. The scale bar shows 20 μm. (b) The distance data in ng and fg oocytes without nuclear radius normalization.

SUPPLEMENTARY METHODS

Real-time PCR analysis of single 4-cell embryos

Zona pellucida-free single 4-cell embryos were lysed, and the remaining DNA was eliminated by DNase treatment. cDNA was synthesized from the lysate using the Single Cell-to-CT kit according to the manufacturer's instructions. The cDNA was used for qPCR analysis using TaqMan probes without preamplification. The used TaqMan probes were listed in Table S2.

ES cell culture

ES cells used for NT were cultured in knockout Dulbecco's modified Eagle medium (Life Technologies) containing recombinant human leukaemia inhibitory factor culture supernatant for mouse ES cell culture (Wako Pure Chemical Industries, Ltd), as well as GlutaMAX, 2-mercaptoethanol, non-essential amino acids, and 15% KSR (all from Life Technologies).