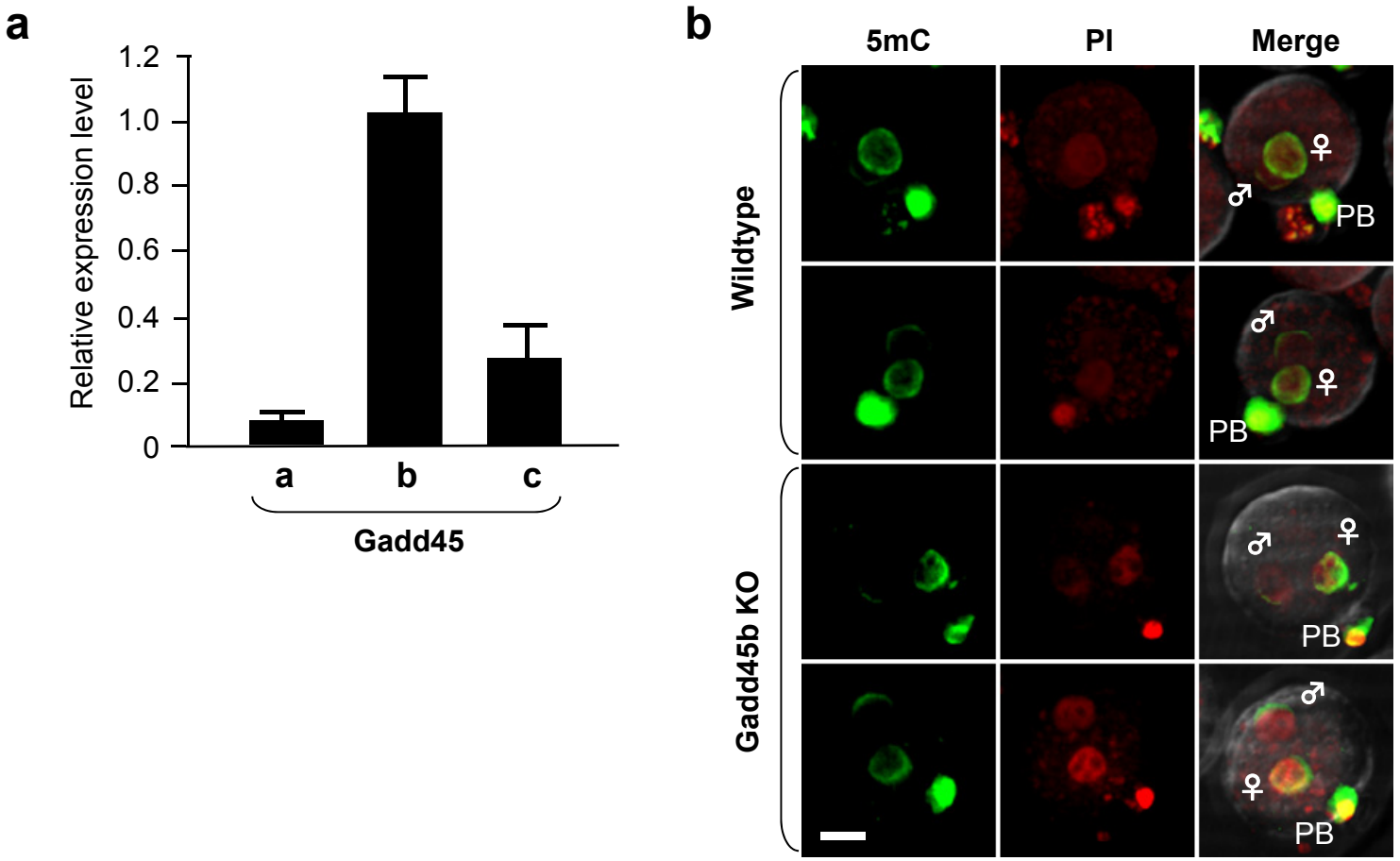

Figure S1. Gadd45b-deficiency does not affect paternal DNA demethylation (a) Relative expression level of Gadd45 family members in mouse zygotes. (b) 5mC staining of wildtype and Gadd45b-deficient zygotes at pronuclear (PN) stage 4-5. Pronuclear staging and genders were determined based on criteria defined previously (Santos et al. 2002, *Dev. Biol*. 241, 172-182). 5mC-positive signal was detected using FITC-labeled secondary antibody (green; left column). DNAs were stained with PI (red; middle column). ♂: male pronucleus, ♀: female pronucleus, PB: polar body. Bar = 25 μm.

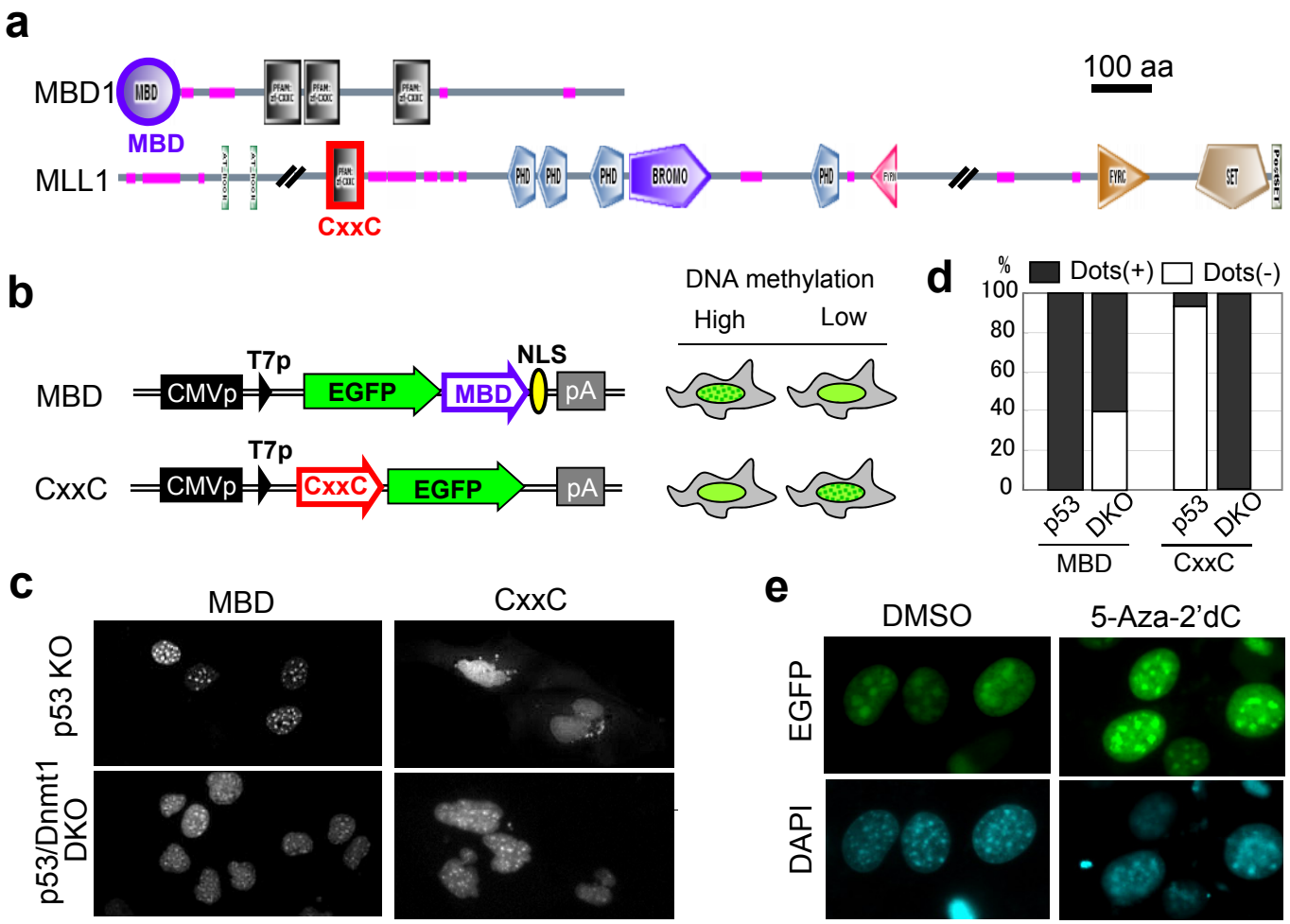

Figure S2. Construction and evaluation of a CxxC-EGFP reporter for monitoring DNA methylation state in real-time.
(a) Domain/motif structure of MBD1 and MLL1 proteins.
(b) Schematic representation of MBD-EGFP and CxxC-EGFP expression constructs and the expected subcellular distribution of the encoded proteins. The CMV promoter allows for expression in mammalian cells and the T7 promoter allows for *in vitro* generation of mRNA. An optimal polyA tail was engineered for efficient translation in zygotes.
(c) Subcellular distribution of EGFP-MBD (left) and CxxC-EGFP (right) reporters in p53 knockout (normal DNA methylation, top panels) and p53/Dnmt1 double knockout (low DNA methylation, bottom panels).
(d) Quantification of the results shown in (c). The data is presented as percentage of cells with nuclear dots over total transfected cells.
(e) Enhanced nuclear dot-formation of CxxC probe by 5-Aza-dC-mediated DNA demethylation. NIH3T3 cells that stably express CxxC-EGFP were selected in the presence of 1 mg/ml G418. 5-Aza-dC (Sigma-Aldrich) was applied at the concentration of 5 μM for 72 hours before DAPI staining and imaging.

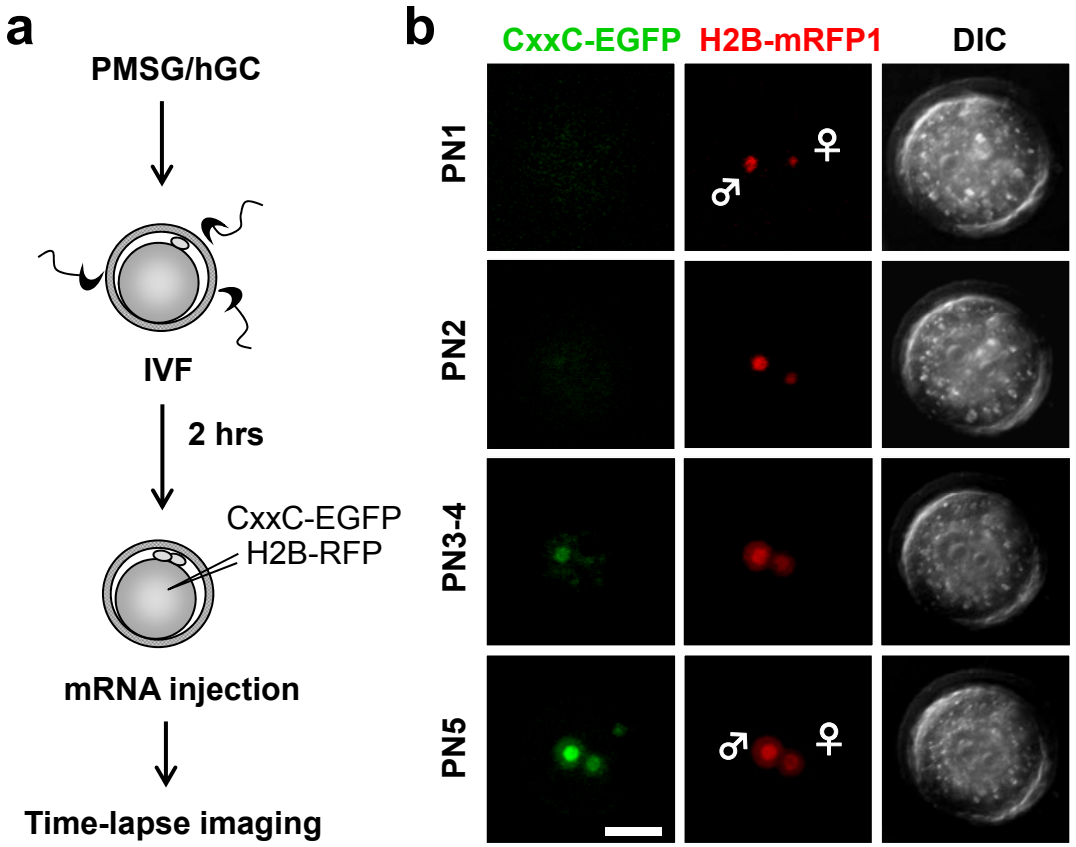

Figure S3. Evaluation of CxxC-EGFP reporter in zygote
(a) Scheme of the experimental design.
(b) Representative images to illustrate the dynamics of CxxC-EGFP distribution during zygotic development by time-lapse imaging. ♂: male pronucleus, ♀: female pronucleus. Bar = 25 μm.

a

Gene	Motif/Domain	Proposed function(s)	Knockdown efficiency at PN5
Cyp11a1	P450 family	Fat metabolism	90%
Smc6-like	smc	Unknown	86%(#1+#2)
Brm	HAT	Transcriptional coactivator	88%
Alkbh5	Alk	3'MeC (N-C bond) demethylase	85%
Nfu I	Fe-S clustering	Hira-binding protein	80%
Elp3	HAT Fe-S clustering	Elongation, tRNA modification, Fe-S clustering	98% (#1) 95% (#2)

b

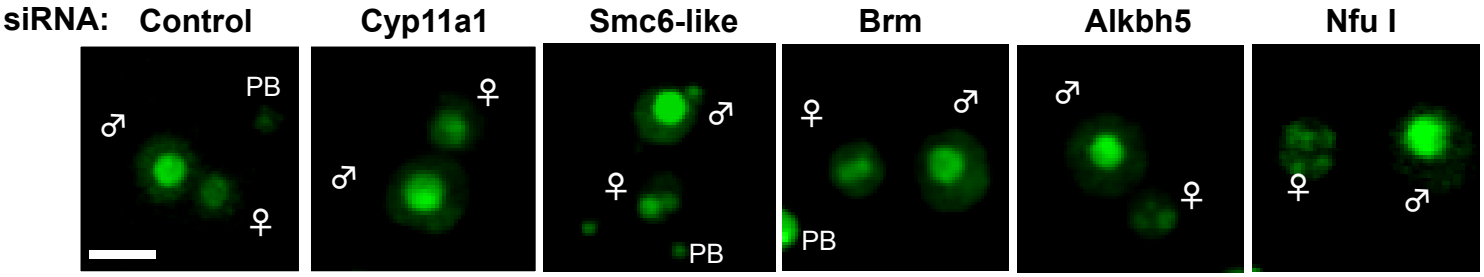

Figure S4. List of candidates with over 80% of knockdown achieved in zygotes and the distribution of the CxxC-EGFP at PN4-5 stage
(a) A list of tested candidates with over 80% of knockdown by RNAi. Knockdown efficiency was determined by RT-qPCR.
(b) Representative images of CxxC-EGFP distribution at PN4-5 after RNAi. ♂: male pronucleus, ♀: female pronucleus, PB: polar body. Bar = 25 μm.

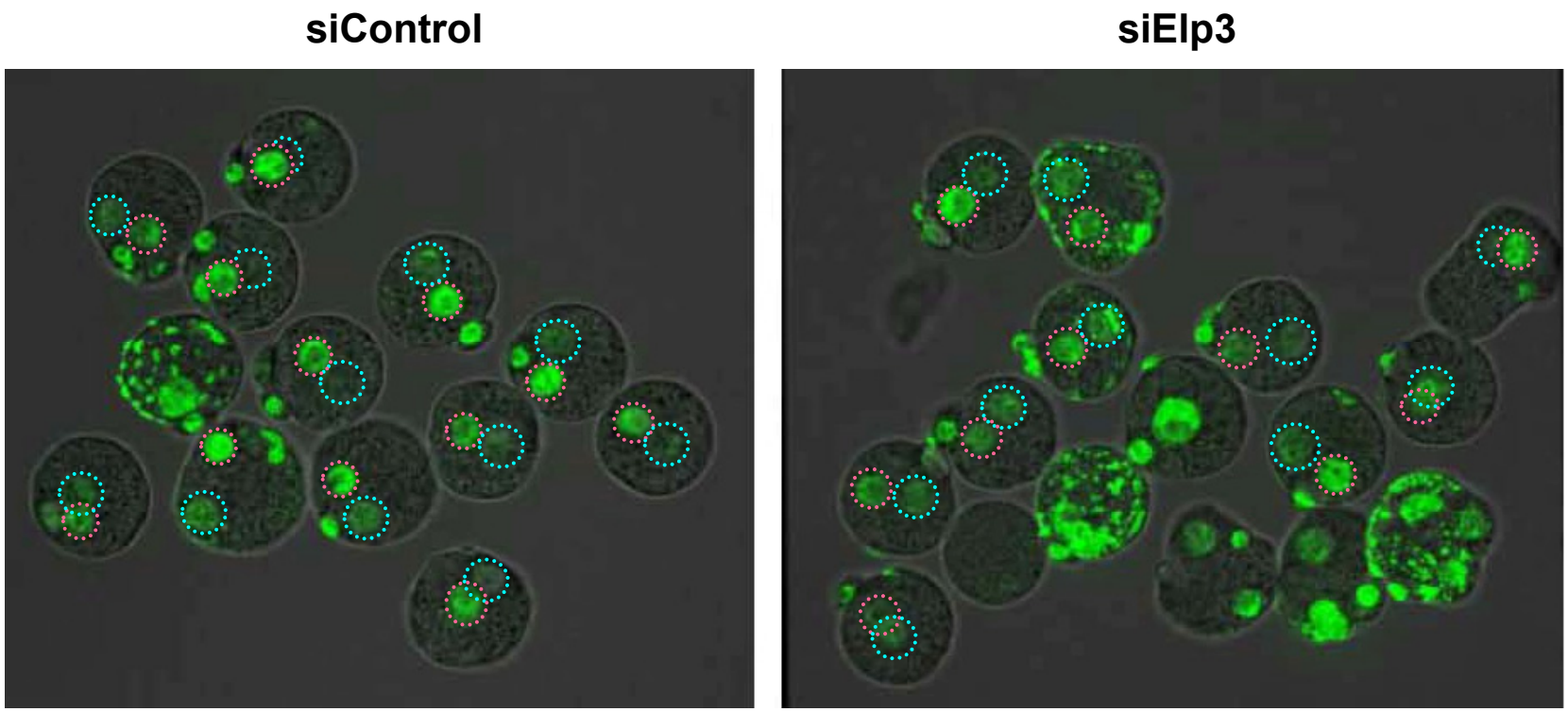

Figure S5. Representative images of 5mC staining in PN4-5 zygotes with (right panel) or without (left panel) Elp3 siRNA. Paternal and maternal pronuclei are indicated by blue and pink dotted circles, respectively.

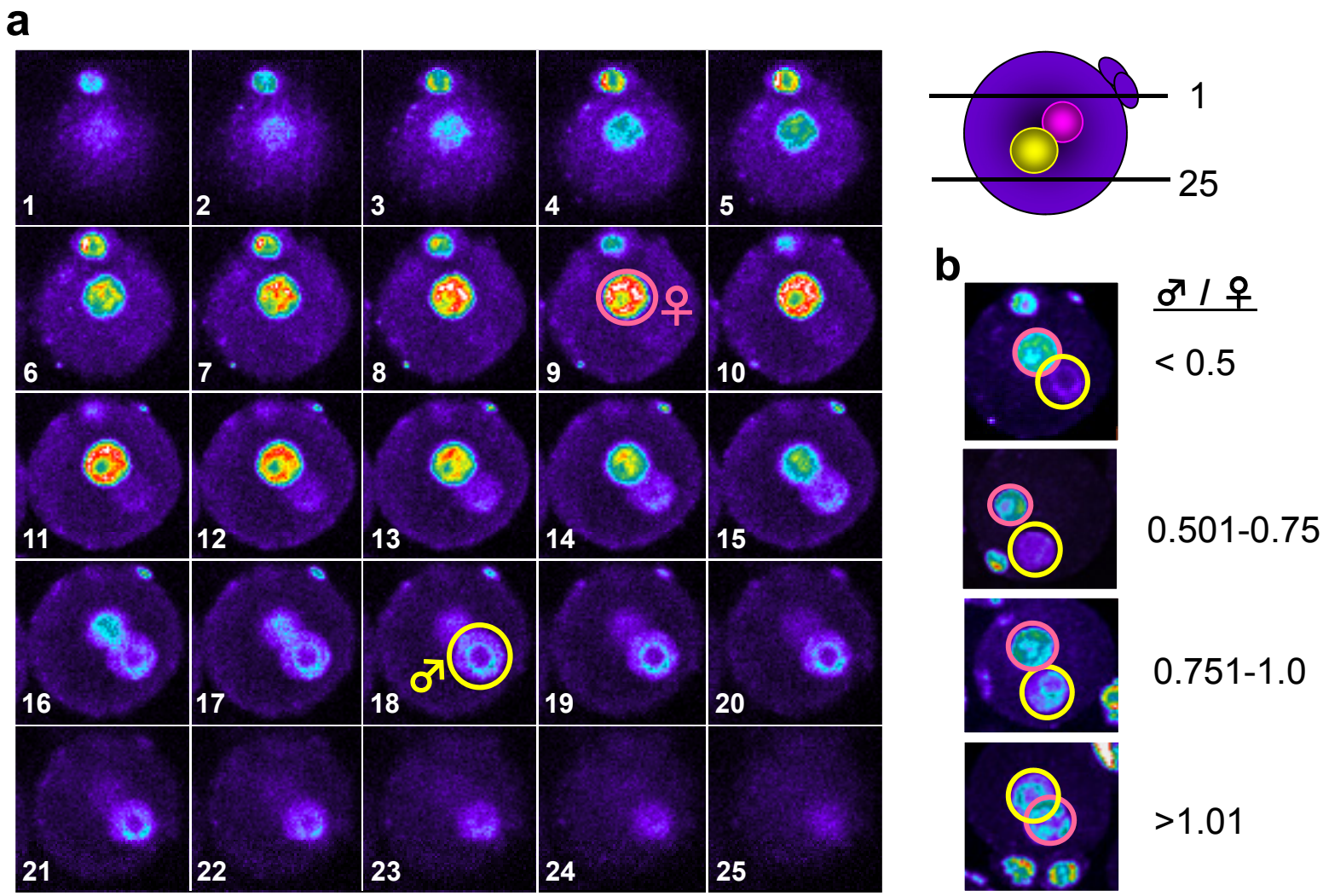

Figure S6. Quantification of 5mC intensity using MetaMorph. (a) Series of Z-sectioned images are pseudocolored to identify the section which contains either male or female pronuleus (PN) with the highest 5mC intensity. In this example, Section #9 contains the female PN with the highest intensity, whereas #18 contains male PN with the highest intensity. The value is calculated as a ratio (male/female) of 5mC intensity. (b) Representative Z-stacked images of 5mC staining in zygotes with different ♂/ ♀values. ♂: male PN, ♀: female PN.

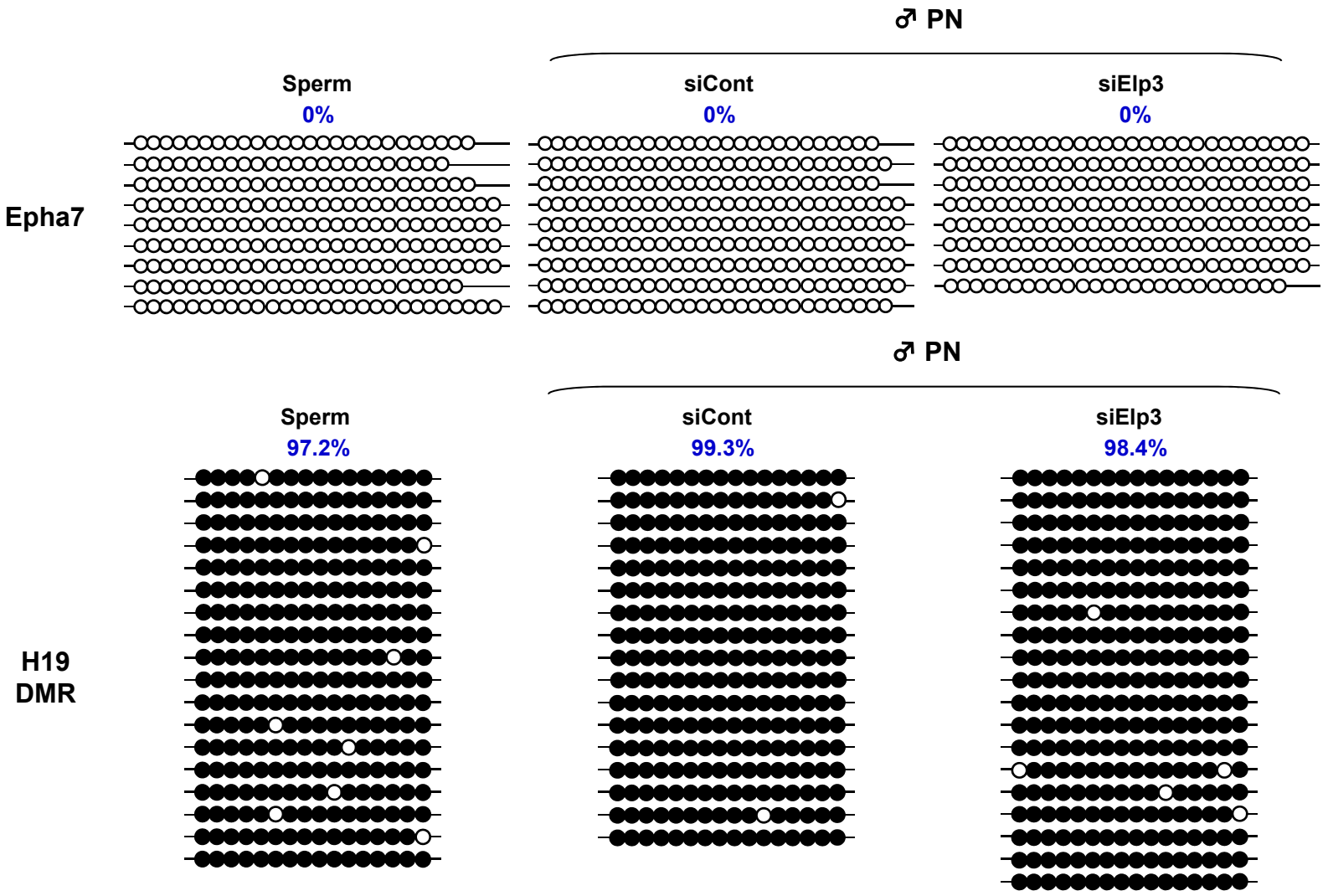

Figure S7. Bisulfite sequencing indicates that knockdown of Elp3 does not affect the methylation state of paternal H19 DMR or Epha7. Open circles and closed circles represent unmethylated and methylated CpG respectively. Each line represents an individual clone. 28 CpGs and 16 CpGs were analyzed for Epha7 and H19 respectively.

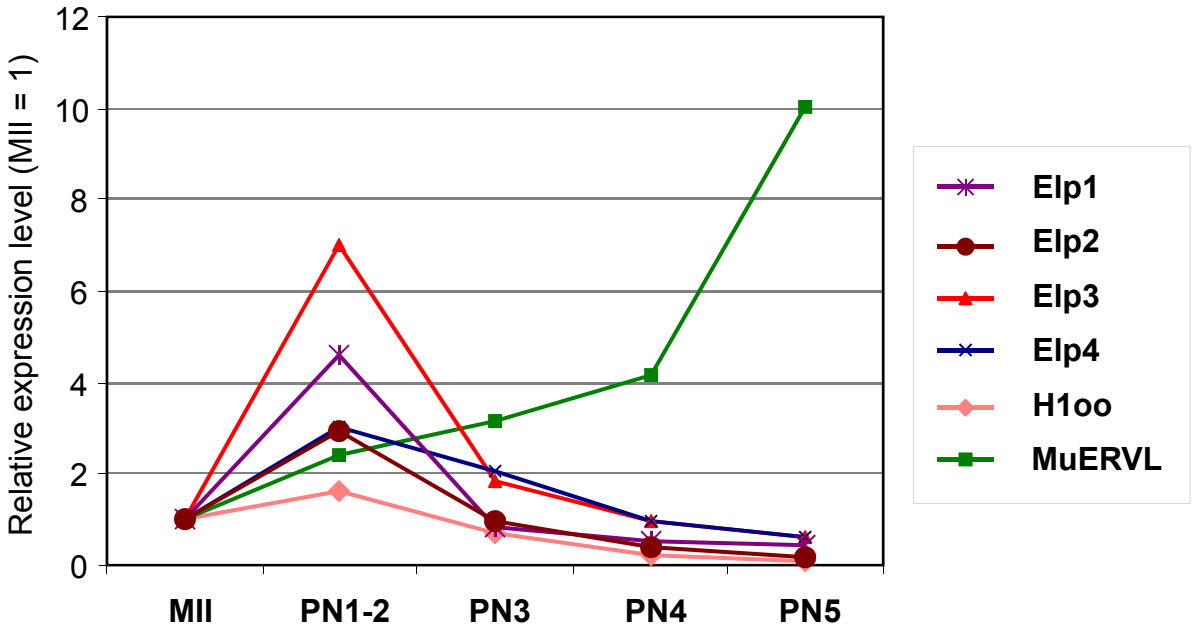

Figure S8. Relative expression levels of Elp family members at different zygotic stages determined by RT-qPCR. Results are normalized by 18S, and the MII expression level is set as 1.0. H1oo and MuERVL are served as controls whose expression patterns during the zygotic development are consistent with previous reports.

Table S1. siRNA sequences

Gene name	Ambion siRNA ID#	Sense sequence (5'->3')
Negative control	n/a	No information available (Cat# AM4611)
Elp1	s106425	GACUGACAGGUGUCGCUUUtt
Elp3 #1	s92451	CAUCCGAAGUUUACACGAUtt
Elp3 #2	s92453	GUGUUUCCAUAGUCCGAGAtt
Elp4	s211969	GCACCACUACUUGAUGAUAtt
Cyp11a1	s64660	GCUUCGUAAUUACAAGAUUtt
Smc6-like #1	s84719	GAUCUGCCCAGAACGGAUAtt
Smc6-like #2	s84718	CCGUGGUUUCUACUAGGAAtt
Brm	s84569	GAGCGAAUCCGUAAUCAUAtt
Alkbh5	s113995	ACCCUGCGCUGAAACCCAAtt
NfuI	s80958	GCAGUUAUUCAGAAUUGAAtt

Table S2. Primer sequences for qPCR

Gene	Forward (5'->3')	Reverse (5'->3')
18S	CGGCTACCACATCCAAGGAA	AGCTGGAATTACCGCGGC
Gadd45a	TGCGAGAACGACATCAACAT	TCCCGGCAAAAACAAATAAG
Gadd45b	GTTCTGCTGCGACAATGACA	TTGGCTTTTCCAGGAATCTG
Gadd45c	ATGACTCTGGAAGAAGTCCGT	CAGGGTCCACATTCAGGACT
Elp1	GAGTCAGACCTCTTCTCGGAAA	CGCACCTCATCTTTTAGCTTCT
Elp2	CTTTCGAAACCAAGGATGGTAG	CAGAGAATCATGGTTTTGTCCA
Elp3	TCCGTGCTAGATATGACCCTTT	CATCGTGTAAACTTCGGATGAA
Elp4	ACTCCCTGCACCACTACTTGAT	AATCCATGCCACTTTGAACTCT
Cyp11a1	CCAGTGTCCCCATGCTCAA	CAGCTGCATGGTCCTTCCA
Smc6-like	CGTACTGAAGGGGAATTGTGA	AGGAACAGCTGGCTTTCTAGG
Brm	GAGGAGGAGGAGGAAGAAGAAG	GCTGCTTTCATCTATTGGCTCT
Alkbh5	ACAGAGGCCTTCTAAGCAGC	CTGACCCCAAAGAGACTTCC
NfuI	ATGGGGAGCAGCGGTCGGTGTAGT	TGCGCGCAGCGGGAAAAGTGGTCT
H1oo	ACTGGAGATGGCACCTAAGAAA	TCGATTTCTCACCTTTGGTTTT
MuERVL	AAATGACTTGGAGATGCCTGAT	TGCGTCTTATAGAGCTGGTGAA

Table S3. Primer sequences and reaction conditions for bisulfite PCR

Gene		Forward (5'->3')	Reverse (5'->3')	Taq[a]	PCR[b]
Line1-5' (Ref. 27)	1st	GTTAGAGAATTTGATAGTTTTTGGAATAGG	CCAAAACAAAACCTTTCTCAAACACTATAT	Platinum	A
	2nd	TAGGAAATTAGTTTGAATAGGTGAGAGGT	TCAAACACTATATTACTTTAACAATTCCCA		
ETn (Ref. 26)	1st	CTTAACTACATTTCTTCTTTTACC	AGTTAGYGTTAGTATGTGTATTTGT	Platinum	A
	2nd	TCTAAATTCCTCTCTTACAACT			
H19 (Ref.)	1st	GAGTATTTAGGAGGTATAAGAATT	ATCAAAAACTAACATAAACCCCT	Ex HS	B
	2nd	GTAAGGAGATTATGTTTATTTTTGG	CCTCATTAATCCCATAACTAT		
Epha7 (Ref.)	1st	TGAGTAGTATTGTTATATATAGGAGTGTAAG	CTCCAATAATATCAATTAAAAAAAA	Platinum	C
	2nd	GTGAAGTTGTAGTAGTTAAAAGTTAAGTGT			

a) Platinum: Platinum Taq polymerase (Invitrogen), Ex HS: Ex Taq HS (TaKaRa)

b) A: Both first and second-round PCRs were performed under the following conditions: 2 min at 95°C, followed by 45 cycles of PCR consisting of 30 sec at 94°C, 30 sec at 50°C, 1 min at 72°C. B: First-round PCR was performed under the following conditions: 4 min at 94°C, 2 min at 55°C, and 2 min at 72°C for two cycles. Forty-eight cycles of PCR were then performed for 1 min at 94°C, 2 min at 55°C, and 2 min at 72°C. The condition for the second round was the same as the first round except that the first two cycles were omitted and total cycle number was 50. C: First-round PCR was performed under the following conditions: 5 min at 95°C, followed by 4 cycles of PCR consisting of 1 min at 94°C, 2 min at {56 → 55→ 54→ 53 (reduce 1°C at every cycle)} °C, 2 min at 72°C, followed by 41 cycles of 1 min at 94°C, 1 min at 53°C, and 1 min at 72°C. The second round PCR consists of 5 min at 95°C, followed by 45 cycles of PCR consisting of 1 min at 94°C, 1 min at 53°C, and 1 min at 72°C.