

Supplementary Information

Germline epigenome editing identifies H3K9me3 as a mediator of intergenerational DNA methylation recovery

Authors list

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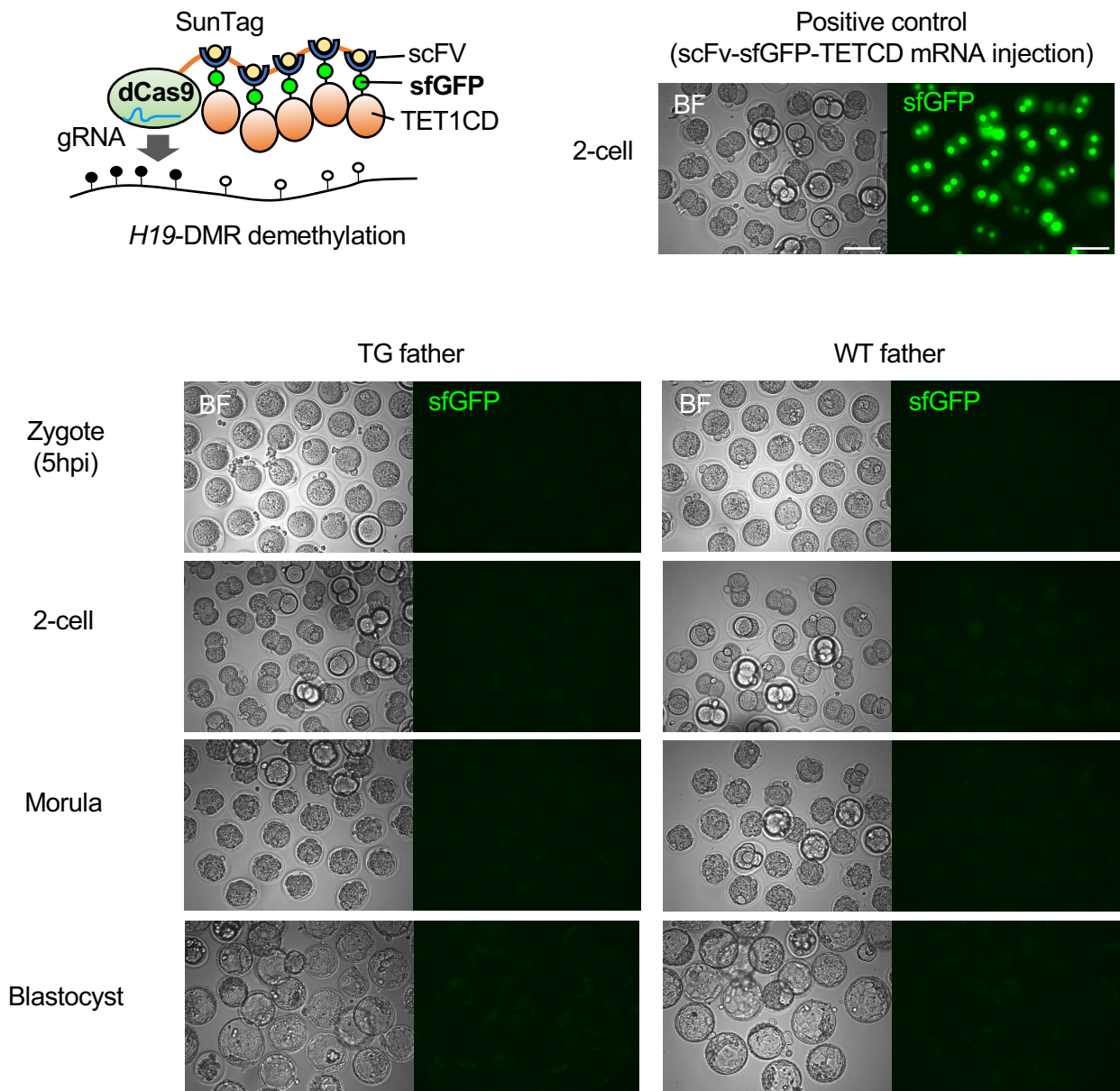
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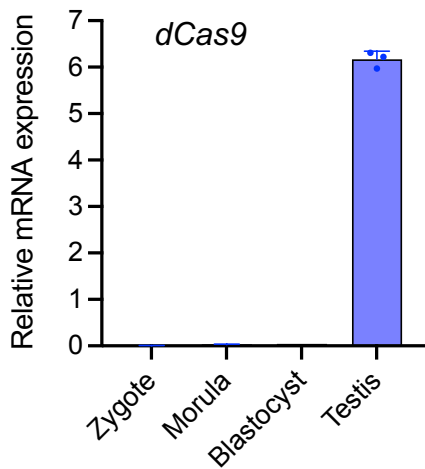
Table of contents

Supplementary Figures 1-9

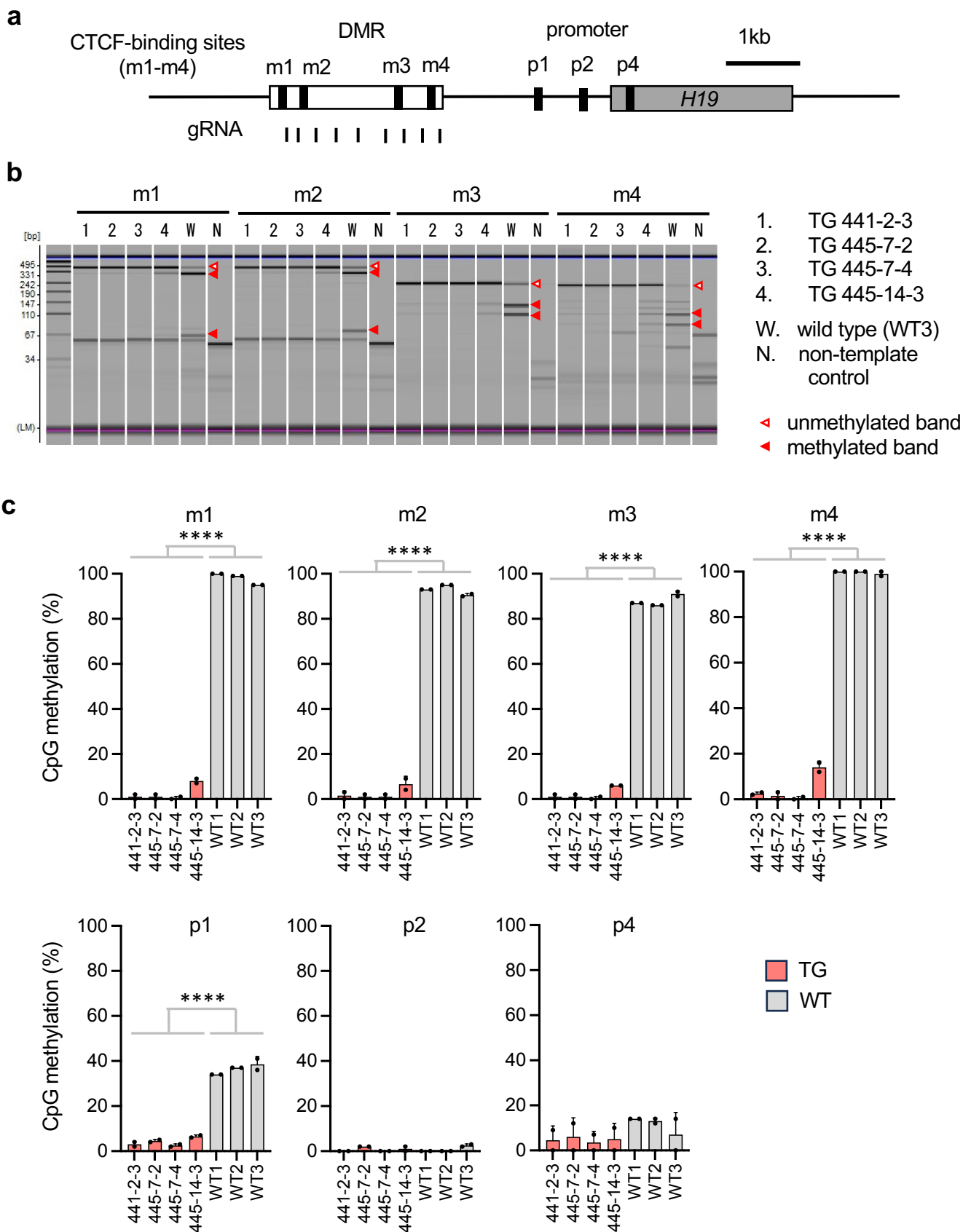
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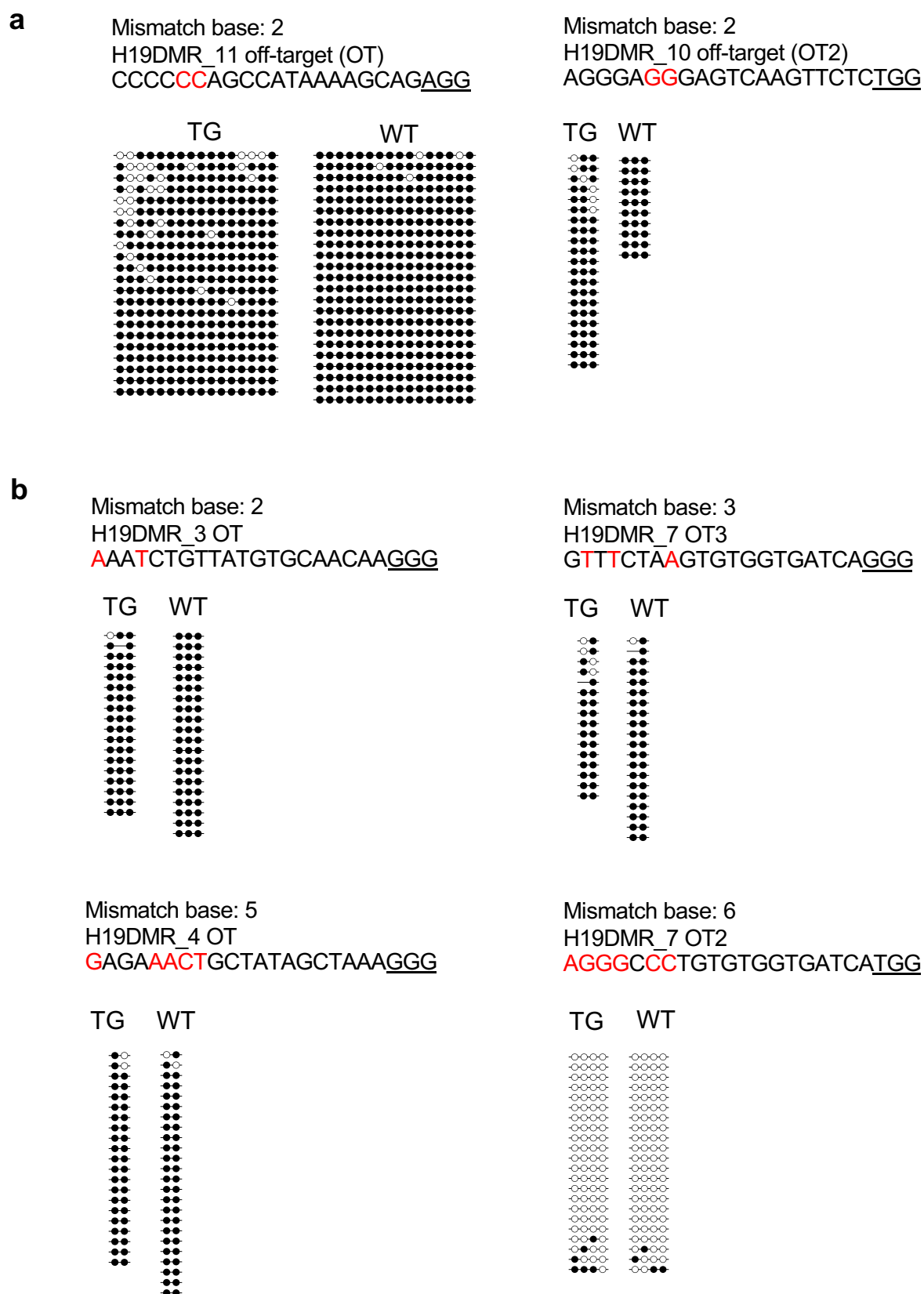
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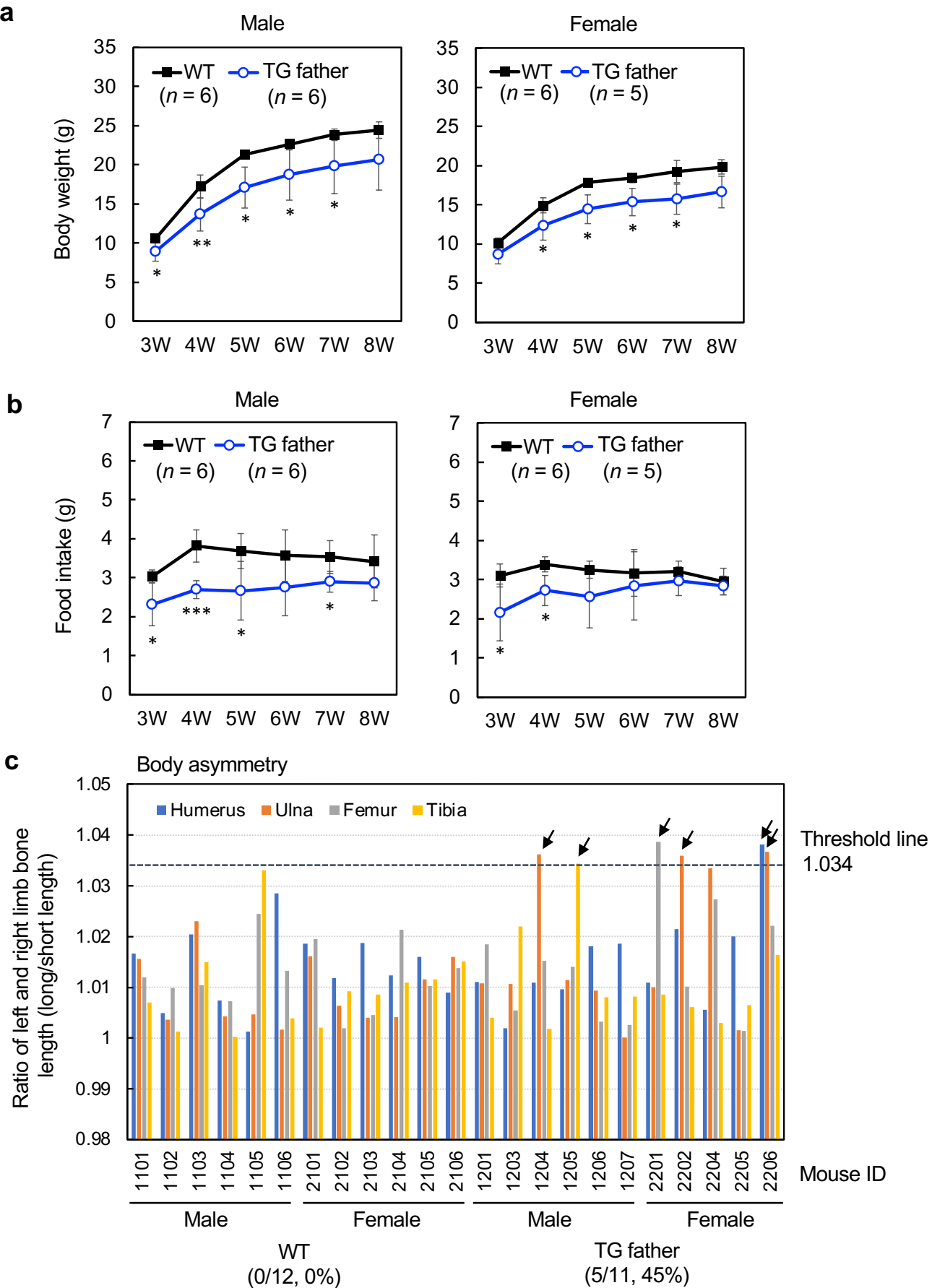
Supplementary Fig. 1: No expression of epigenome editing factors was observed in TG embryos. a, sfGFP expression in the TG father-derived preimplantation embryos. Scale bars: 100 μ m. **b,** The epigenome editing factor dCas9 expression in TG-father derived preimplantation embryos and testis. Values represent mean $\pm$ s.d. ($n = 3$ technical replicates)



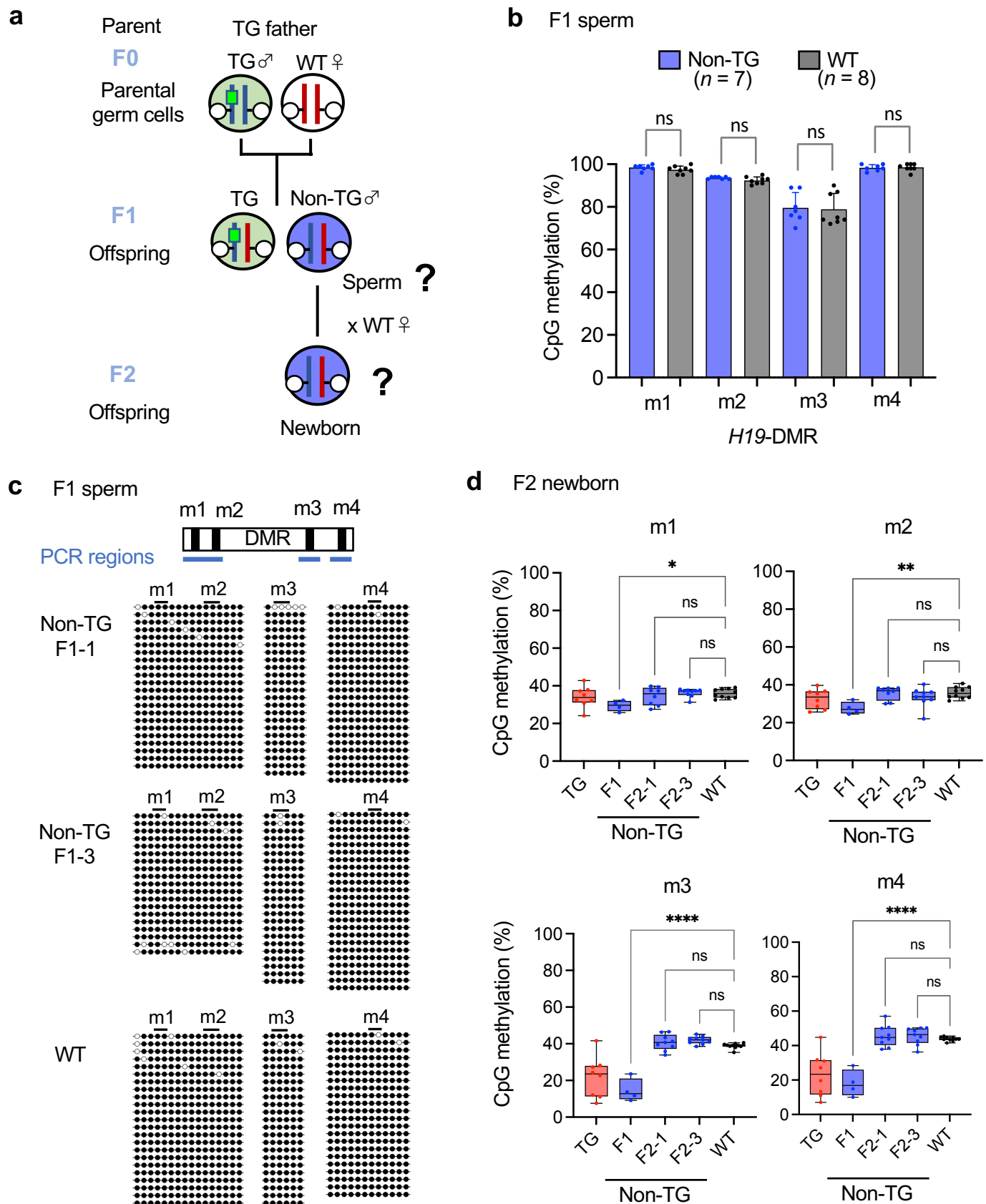
Supplementary Fig. 2: Target-specific demethylation of *H19*-DMR in sperm. **a**, Map of the *Igf2-H19* imprinted gene locus and positions of guide RNAs (gRNAs) used in this study. CpG methylation levels of *H19*-DMR (sites m1–m4) and promoter (p1, p2, and p4) were examined by COBRA. **b**, Representative image of capillary and microchip electrophoresis. When CpG sites were methylated, PCR products were digested by restriction enzymes. **c**, Methylation level was calculated as the percentage of cleaved DNA among total DNA. CpG demethylation in *H19*-DMR occurred in sperm of all TG male mice, particularly in the 445-7 strain. Values represent mean $\pm$ s.d. ($n = 2$ technical replicates). P values were generated using Welch's t test (two-tailed). **** $p < 0.0001$



Supplementary Fig. 3: Off-target DNA methylation analysis of sperm from germline-specific epigenome-edited mice. DNA methylation status around potential off-target regions that exactly match 12 bases at the 3' end and the NGG (PAM) were examined in sperm. **a**, Bisulfite sequencing was performed on two off-target regions where undesired DNA demethylation was observed in conventional TG mice stably expressing dCas9-SunTag-TET³². **b**, Bisulfite sequencing analysis of four other off-target regions. Closed circles, methylated CpGs; open circles, unmethylated CpGs. Mismatched base (highlighted), PAM site (underlined).

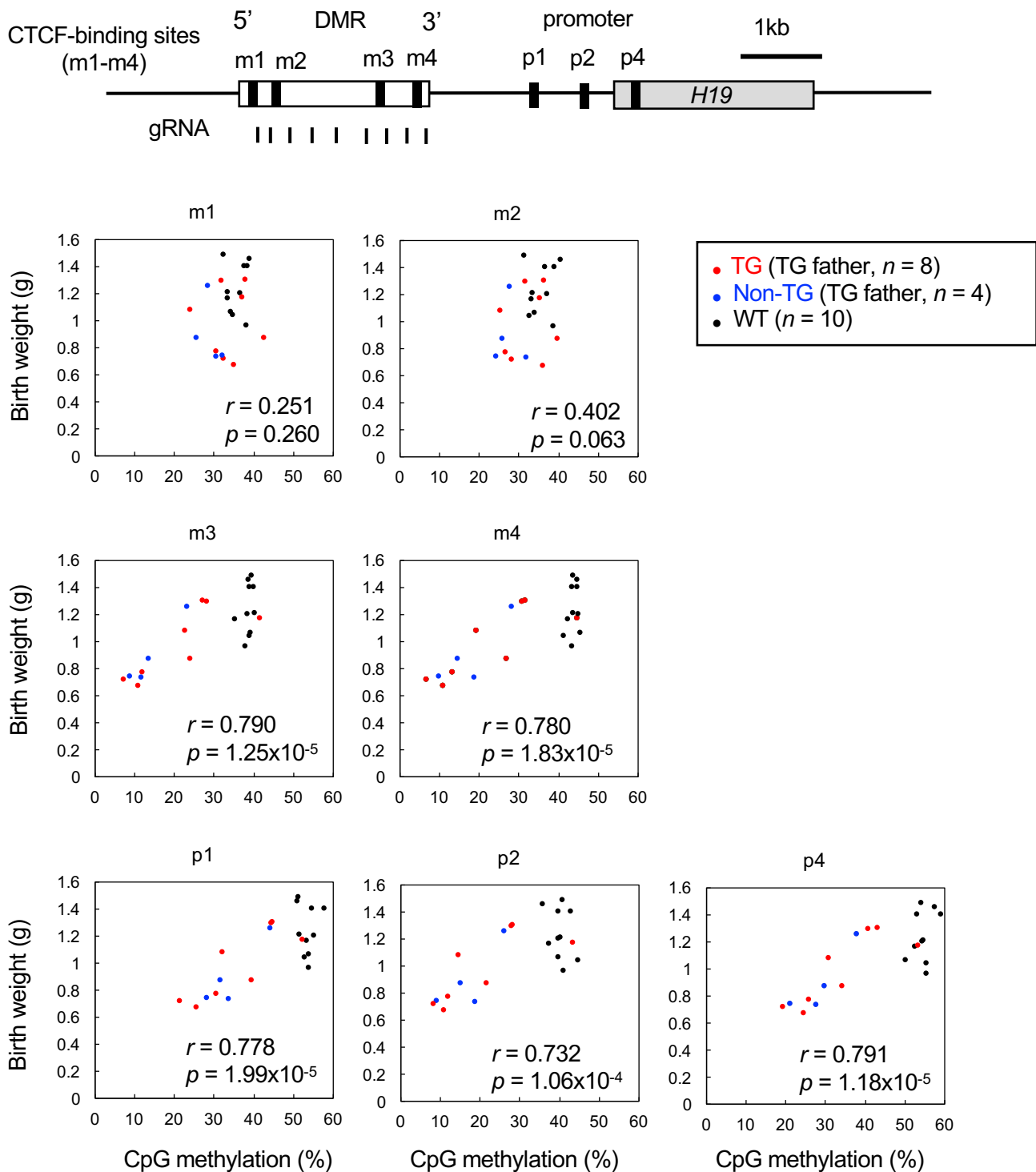


Supplementary Fig. 4: SRS-like phenotypes observed in offspring from TG fathers. Offspring from TG fathers showed: **a**, growth retardation after birth and **b**, lower food intake (3–8 weeks old). *P* values were generated using the Welch's *t*-test (two-tailed). **p* < 0.05, ***p* < 0.01, ****p* < 0.001 **c**, Body asymmetry analysis was performed by calculating the ratio of right and left limb bone lengths from 3D images of mice. Bar graphs illustrate the ratios of long lengths to short lengths. Body asymmetry was observed in the offspring of TG fathers (arrows; *p* = 0.0137). *P* values were generated using Fisher's exact test of data in a 2 × 2 table.



Supplementary Fig. 5: Epimutations occurring in *H19*-DMR are inherited intergenerationally, but not transgenerationally. **a**, Schematic representation of the experiment to validate transgenerational epigenetic inheritance of *H19*-DMR epimutations. **b**, COBRA and **c**, bisulfite sequencing analysis of sperm derived from F1 non-TG and WT mice. Values represent mean $\pm$ s.d. No CpG demethylation in *H19*-DMR was observed in F1 non-TG sperm. **d**, COBRA data from F2 newborn mice. CpG demethylation in F1 non-TG mice was recovered in F2 mice. F2-1 and F2-3 represent offspring derived from F1-1 and F1-3 non-TG male mice, respectively. Box plots show the median (center line), the 25th and 75th percentiles (box edges), and the minimum and maximum values (whiskers). *P* values were generated using Welch's *t* test (two-tailed) (**b**) and one-way ANOVA with Tukey's post-hoc HSD test (**d**).

p* < 0.05, *p* < 0.01, *****p* < 0.0001, ns: not significant.

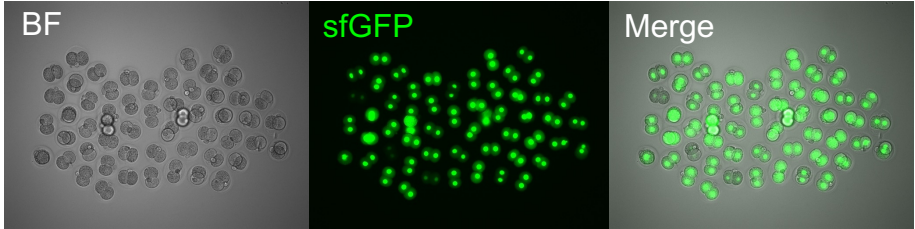


Supplementary Fig. 6: Strong correlation between birth weight and CpG methylation.

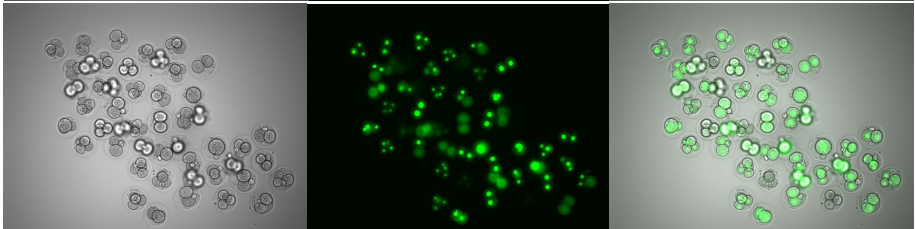
There were strong correlations between birth weight and CpG methylation in the 3' region of *H19*-DMR (m3 and m4) and promoter regions (p1, p2, and p4), but not in the 5' region of *H19*-DMR (m1 and m2). Data are presented as Pearson's correlation coefficient (r) with associated two-tailed p values.

dCas9-SunTag-Kdm4d

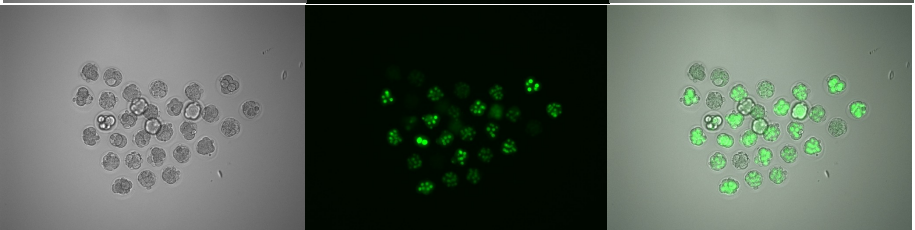
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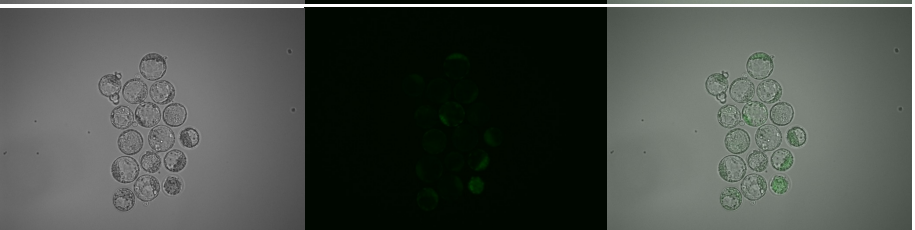
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(2 dpi)



Morula
(3 dpi)



Blastocyst
(4 dpi)



Non-treatment

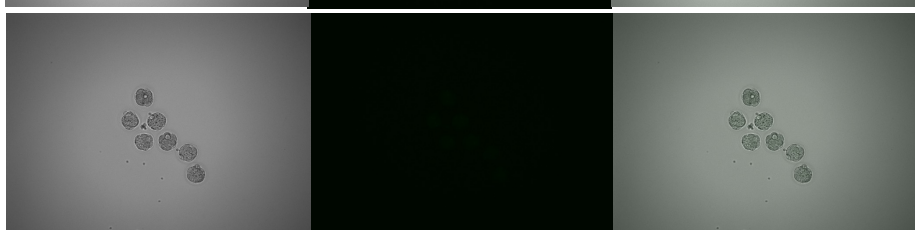
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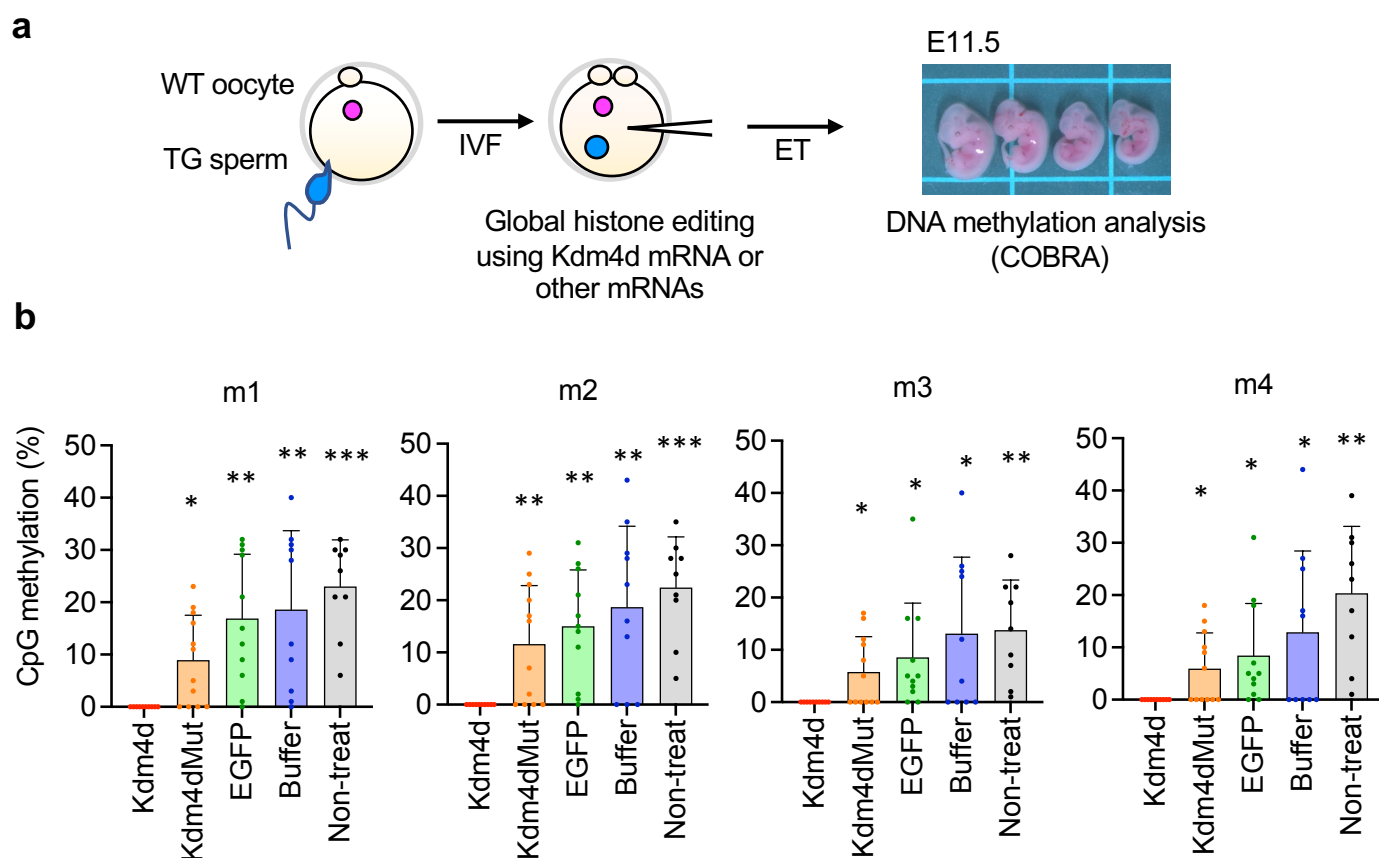
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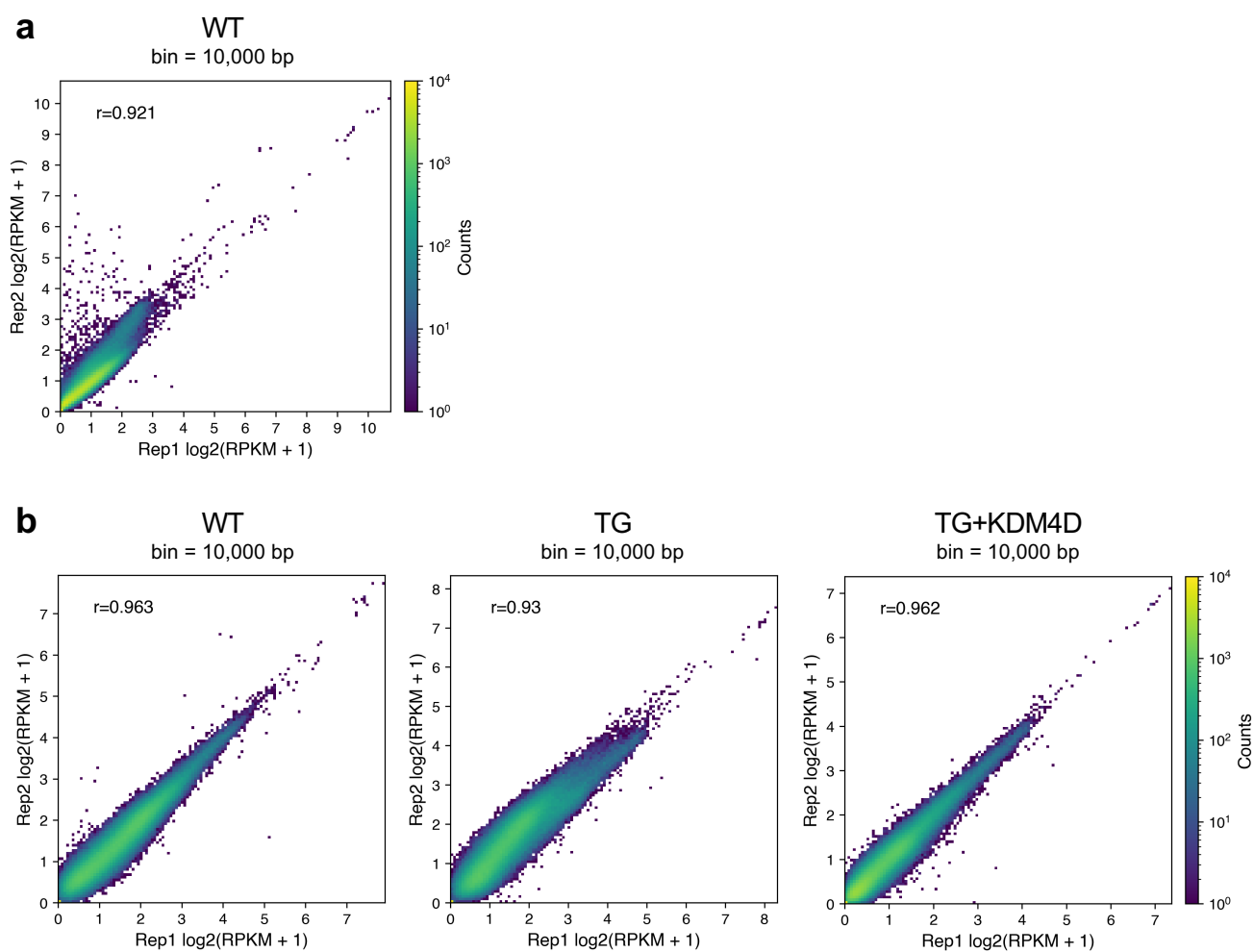
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Supplementary Fig. 7: Expression of epigenome editing factors during preimplantation development. The GFP-labeled epigenome editing factor (scFv-sfGFP-Kdm4d) was strongly expressed for at least 3 days until the morula stage. BF, bright field. dpi, days post injection.



Supplementary Fig. 8: H3K9me3 is crucial for DNA methylation recovery. **a.** H3K9 demethylation by global histone editing using *Kdm4d* mRNA. **b.** DNA methylation analysis (COBRA) of E11.5 embryos after global histone editing. *Kdm4d* ($n = 11$); *Kdm4dMut* ($n = 12$); EGFP ($n = 11$); Buffer (mRNA injection buffer (water); $n = 10$); Non-treated ($n = 9$). Values represent mean $\pm$ s.d. P values were generated using Welch's t test (two-tailed) to compare samples treated with *Kdm4d* to others. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.



Supplementary Fig. 9: Scatter plots showing the correlation between biological replicates of H3K9me3 CATCH-seq. The H3K9me3 enrichment of **a**, 1-cell zygotes and **b**, morula embryos was calculated as reads per kilobase per million reads (RPKM) by 10-kb bin. r indicates Pearson correlation coefficient.