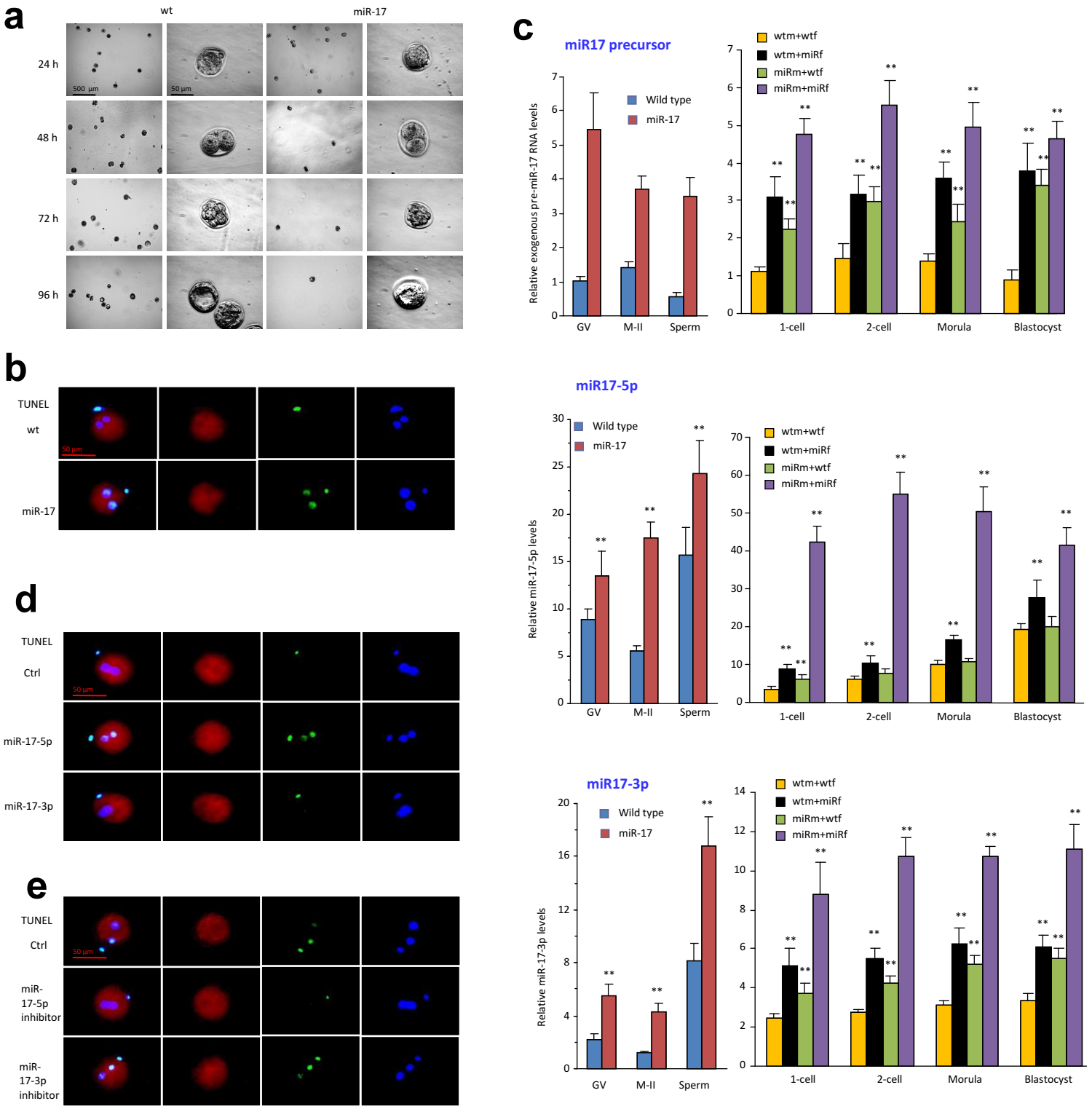


Fig S1. Expression of miR-17 inhibited early embryonic development

(a) Photos of embryos in various stages collected from oviduct or uterus in 24, 48, 72 and 96 hours after hCG treatment. Decreased numbers of embryos observed in miR-17 transgenic mice (miRm+miRf). **(b)** Increased TUNEL staining in the nuclei of miR-17 transgenic zygotes collected from oviduct 24 hours after hCG injection (miRm+miRf). **(c)** Upper, ectopic expression of miR-17 not only promoted pre-miR-17 expression in the embryos of various stages, but also enhanced pri-miR-17 expression in unfertilized oocytes (GV and M-II) and sperms. ** $p<0.01$. Error bars, SD ($n=4$).

Middle, miR-17 transgenic mice expressed increased levels of miR-17-5p in unfertilized oocytes (GV and M-II), sperms, and embryos of zygote, 2-cell, morula and blastocyst stage. ** $p<0.01$. Error bars, SD ($n=4$). Lower, miR-17 transgenic mice expressed increased miR-17-3p in unfertilized oocytes (GV and M-II), sperms, and embryos of zygote, 2-cell, morula and blastocyst stage. ** $p<0.01$. Error bars, SD ($n=4$). **(d)** Wildtype zygotes were microinjected with control oligos, miR-17-5p and miR-17-3p, and incubated for 8 hours, followed by TUNEL staining. Expression of miR-17-5p increased TUNEL staining. **(e)** Transgenic zygotes were microinjected with 2 pl control oligos, miR-17-5p inhibitor (IN) and miR-17-3p IN, and incubated for 8 hours, followed by TUNEL staining. Expression of miR-17-5p IN decreased TUNEL staining.

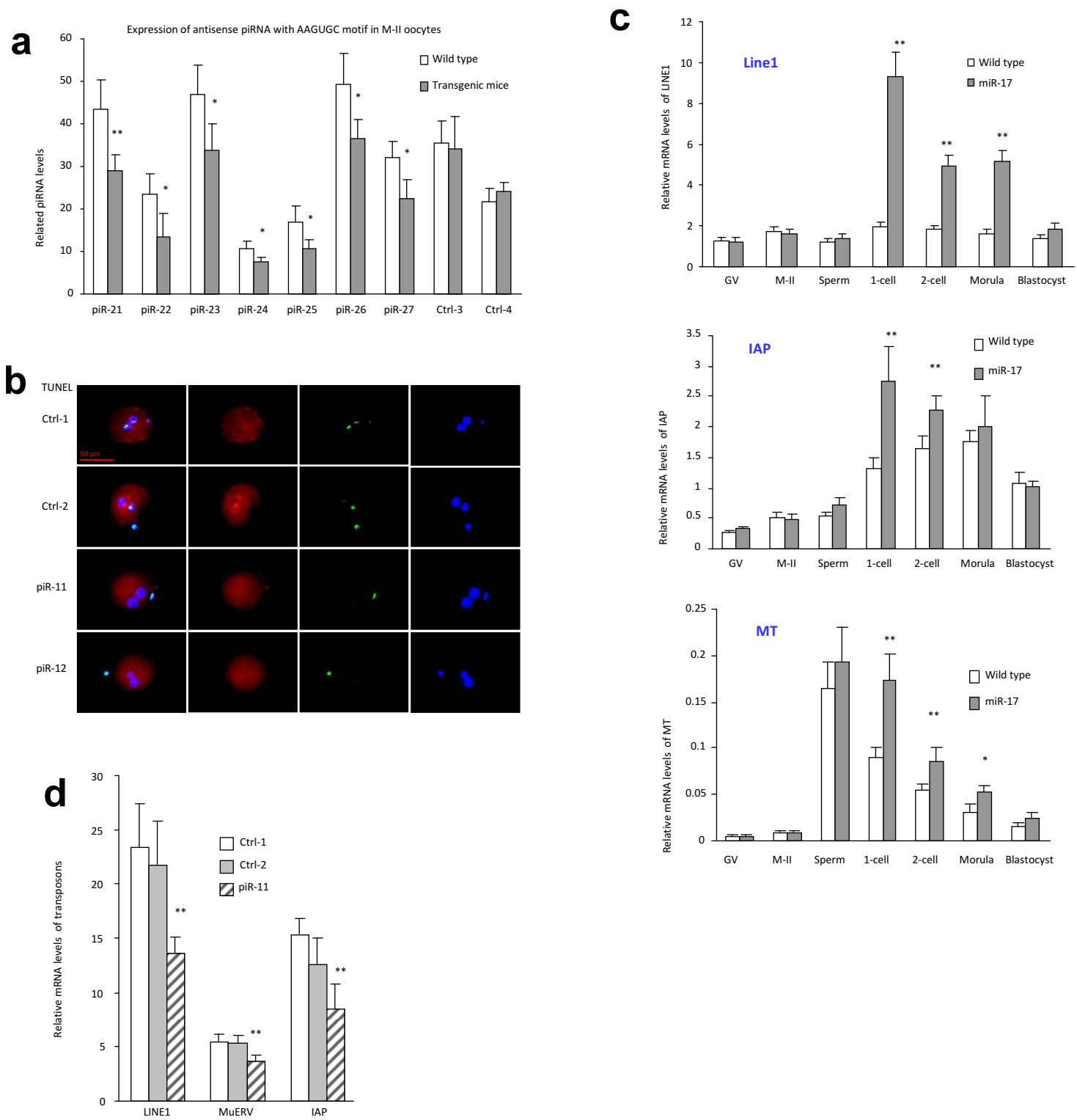


Fig S2. piRNA expression were repressed, while transposon was promoted in the early embryos of miR-17 transgenic mice

(a) miR-17 transgenic mice expressed decreased piRNA (antisense) levels with AAGUGC motif, but not those without AAGUGC motif. * $p<0.05$. ** $p<0.01$. Error bars, SD ($n=4$). **(b)** Transgenic zygotes were microinjected with 2 pl control 1 (PBS), control 2, and piR-11, and incubated for 8 hours, followed by TUNEL staining. Expression of piR-11 decreased TUNEL levels. **(c)** Upper, 50 sperms, unfertilized and fertilized oocytes were collected for real-time PCR. miR-17 embryos expressed increased mRNA levels of retrotransposon LINE1 in zygote, 2-cell and morula stages. ** $p<0.01$. Error bars, SD ($n=4$). Middle, miR-17 embryos expressed increased mRNA levels of retrotransposon IAP in zygote and 2-cell stage. ** $p<0.01$. Error bars, SD ($n=4$). Lower, miR-17 embryos expressed increased mRNA levels of retrotransposon MT in zygote, 2-cell and morula stages. * $p<0.05$, ** $p<0.01$. Error bars, SD ($n=4$). **(d)** Transgenic zygotes were microinjected with 2 pl control 1 (PBS), control 2 and piR-11, and incubated for 8 hours. Real-time PCR analysis showed that piR-11 injection repressed mRNA levels of retrotransposon LINE1, MuERV, IAP and MT in zygotes. ** $p<0.01$. Error bars, SD ($n=4$).

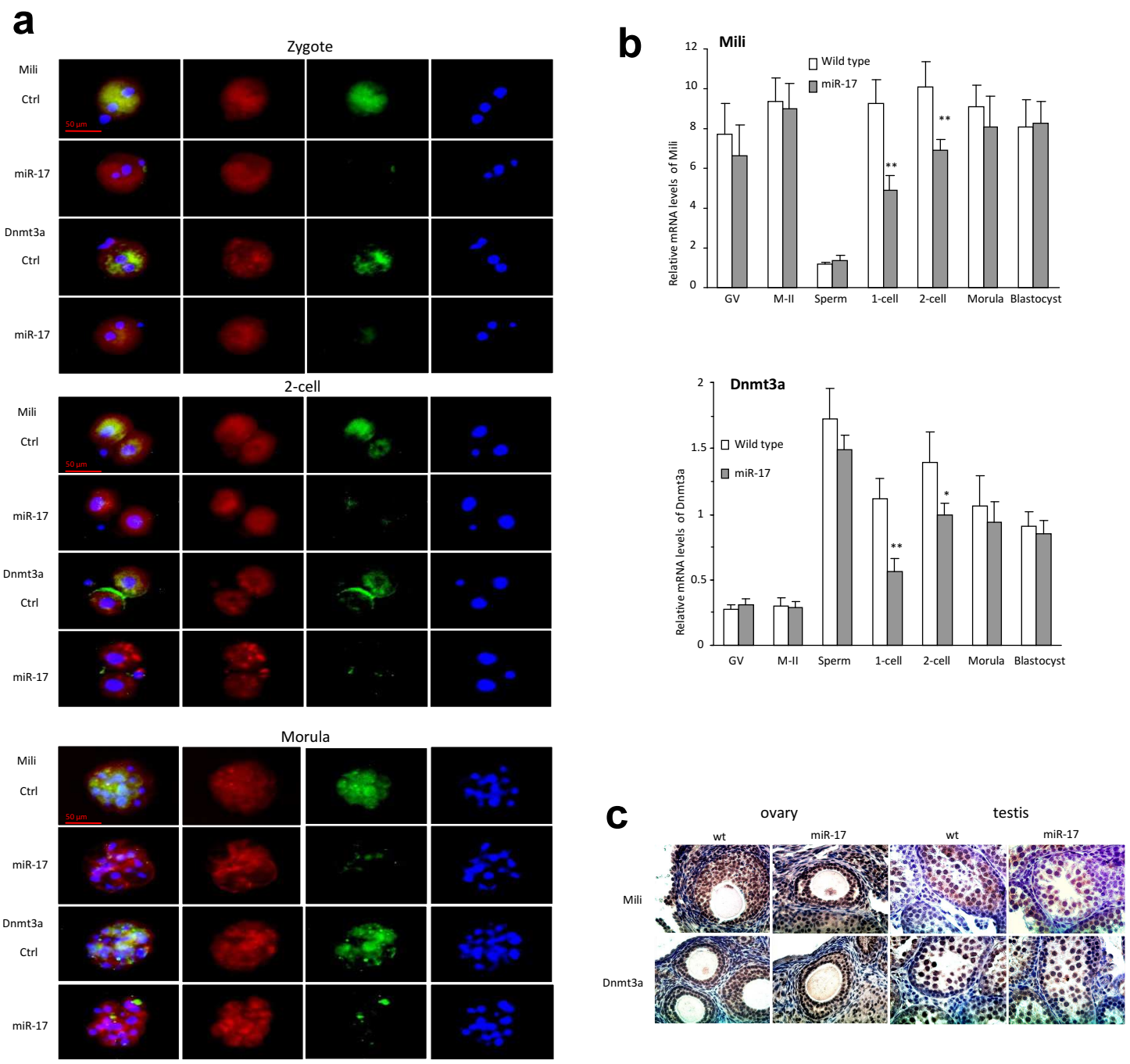


Fig S3. Effect of miR-17 on Mili and Dnmt3a expression.

(a) Embryos were stained with phalloidins (red), DAPI (blue), and green fluorescence showing expression of Mili or Dnmt3a. miR-17 transgenic embryos expressed decreased Mili and Dnmt3a protein in 2-cell, morula and blastocyst stages.

(b) 50 sperms, unfertilized and fertilized oocytes were collected for real-time PCR. miR-17 embryos expressed decreased mRNA levels of Mili (upper) and Dnmt3a (lower) in zygote and 2-cell stage. * $p<0.05$. ** $p<0.01$. Error bars, SD ($n=4$).

(c) IHC staining showing expression of miR-17 did not change Mili and Dnmt3a expression in ovary and testis tissues.

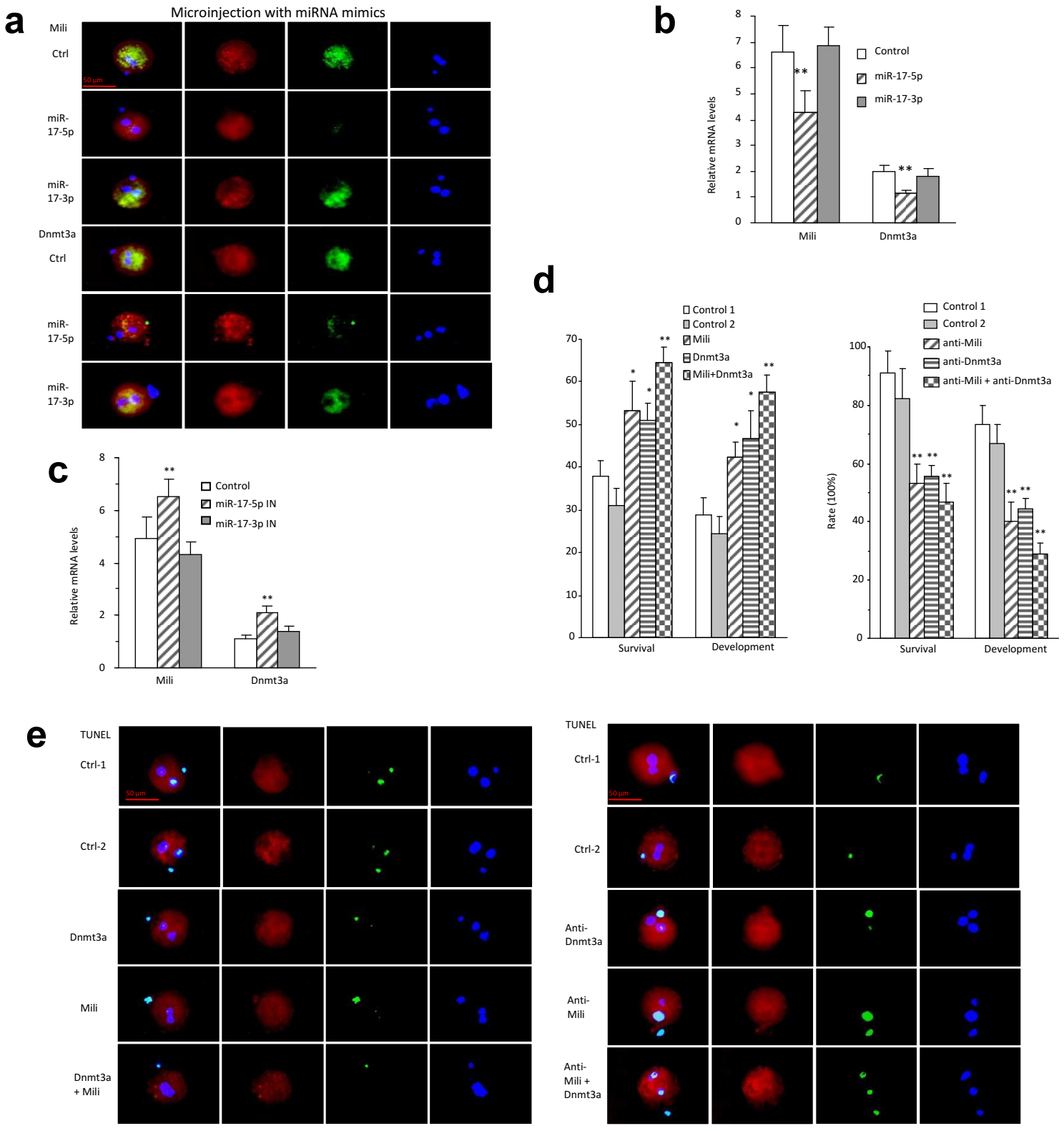


Fig S4. miR-17 targeting Mili and Dnmt3a **(a)** Embryos were stained with phalloidins (red), DAPI (blue), and green fluorescence showing expression of Mili and Dnmt3a. miR-17 embryos expressed decreased levels of Mili and Dnmt3a. **(b)** Wildtype zygotes were microinjected with miR-17-5p, miR-17-3p, and control oligos, and incubated for 8 hours, followed by real-time PCR. miR-17-5p injection repressed Mili and Dnmt3a expression. ** $p<0.01$. Error bars, SD ($n=4$). **(c)** Wildtype zygotes were microinjected with miR-17-5p-IN and miR-17-3p-IN and control oligos, and incubated for 8 hours, followed by real-time PCR. Injection with miR-17-5p-IN increased mRNA levels of Mili and Dnmt3a. ** $p<0.01$. Error bars, SD ($n=4$). **(d)** Left, miR-17 zygotes were microinjected with 2 pl control 1 (PBS), control 2 (mouse Ig), Mili, Dnmt3a, and Mili+Dnmt3a, and incubated for 96 hours. Injection with Mili, Dnmt3a, and Mili+Dnmt3a increased rates of survival and development. Right, Injection with antibodies against Mili and/or Dnmt3a decreased rates of survival and development in wildtype zygotes. * $p<0.05$, ** $p<0.01$. Error bars, SD ($n=4$). **(e)** miR-17 zygotes performed as in (e) were subject to TUNEL staining. Injection with Mili and/or Dnmt3a decreased (left), while injection with anti-Mili and/or anti-Dnmt3a increased (right) TUNEL staining.

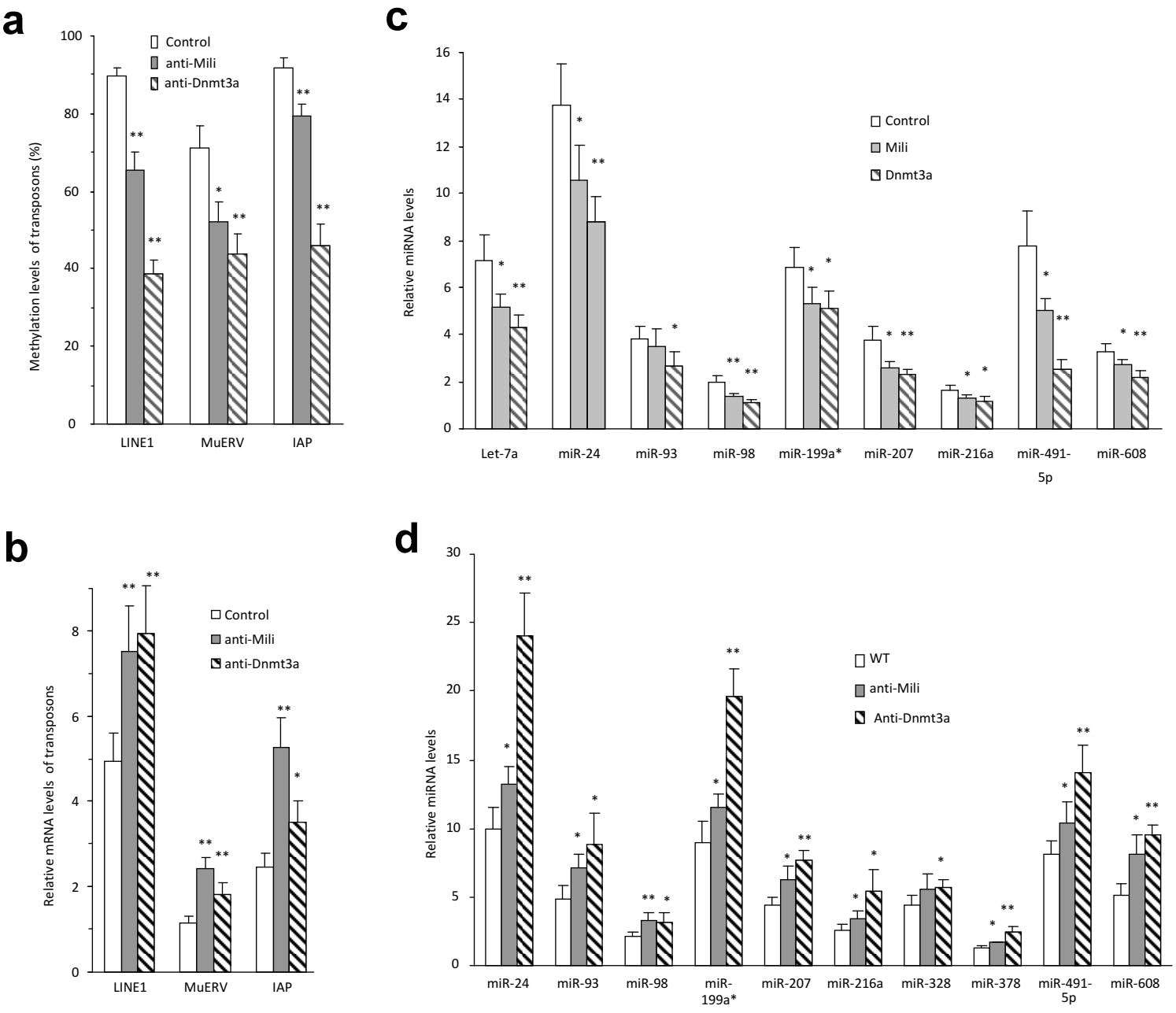


Fig S5. Methylation activities of Mili and Dnmt3a

(a) MSP analysis indicated that injection with anti-Mili or anti-Dnmt3a antibody decreased methylation levels of retrotransposon LINE1, MuERV and IAP. * $p<0.05$, ** $p<0.01$. Error bars, SD ($n=4$).

(b) miR-17 zygotes were microinjected with 2 pl control 1 (PBS), control 2 (mouse Ig), Mili, Dnmt3a, and Mili+Dnmt3a, incubated for 8 hours, followed by real-time PCR analysis. Injection with anti-Mili or anti-Dnmt3a antibody increased mRNA levels of LINE1, MuERV, IAP and MT. * $p<0.05$, ** $p<0.01$. Error bars, SD ($n=4$).

(c) Injection with Mili or Dnmt3a repressed miRNA levels in zygotes. * $p<0.05$, ** $p<0.01$. Error bars, SD ($n=4$).

(d) Injection with anti-Mili or anti-Dnmt3a antibody increased miRNA expression. * $p<0.05$. ** $p<0.01$. Error bars, SD ($n=4$).

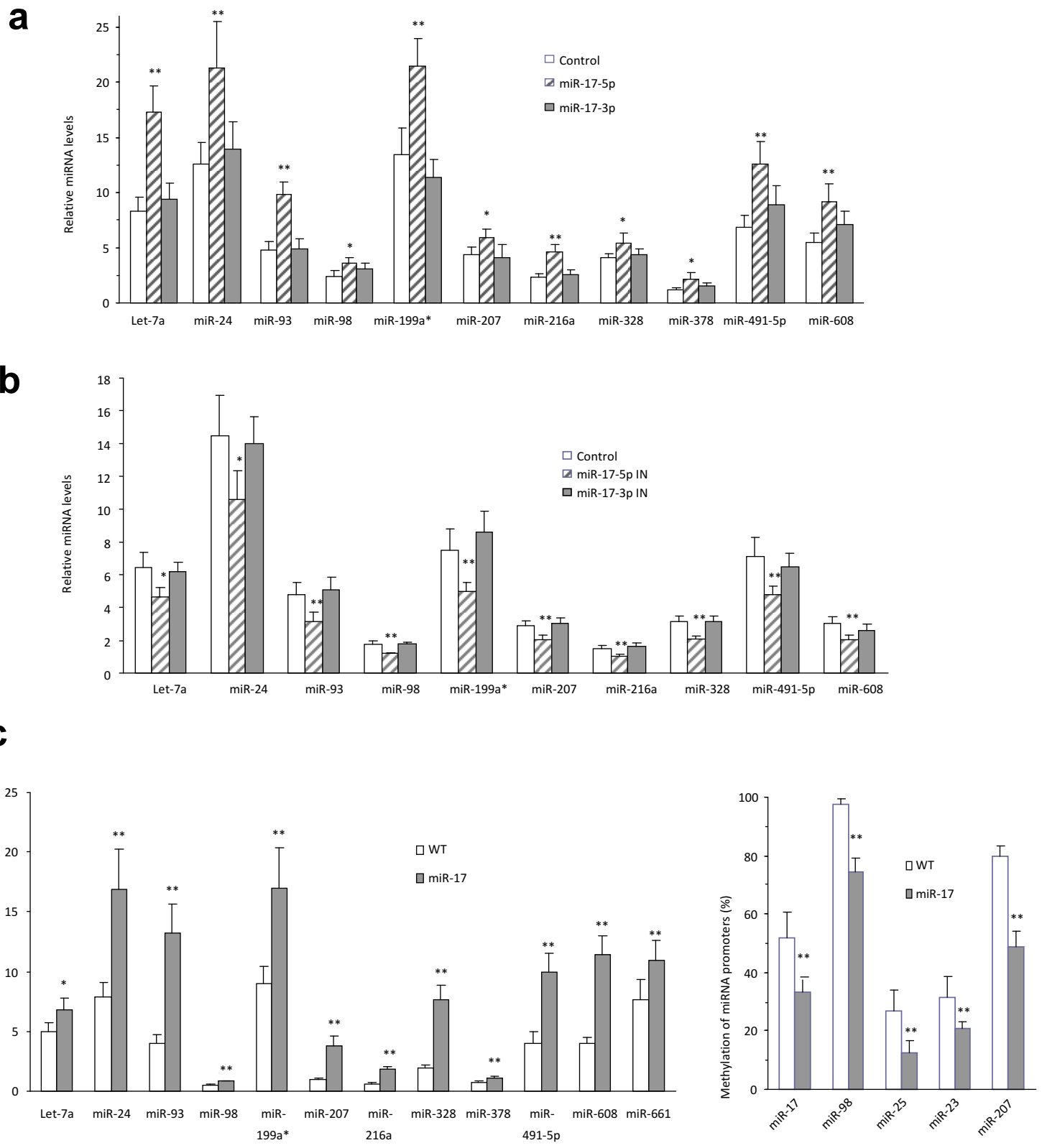
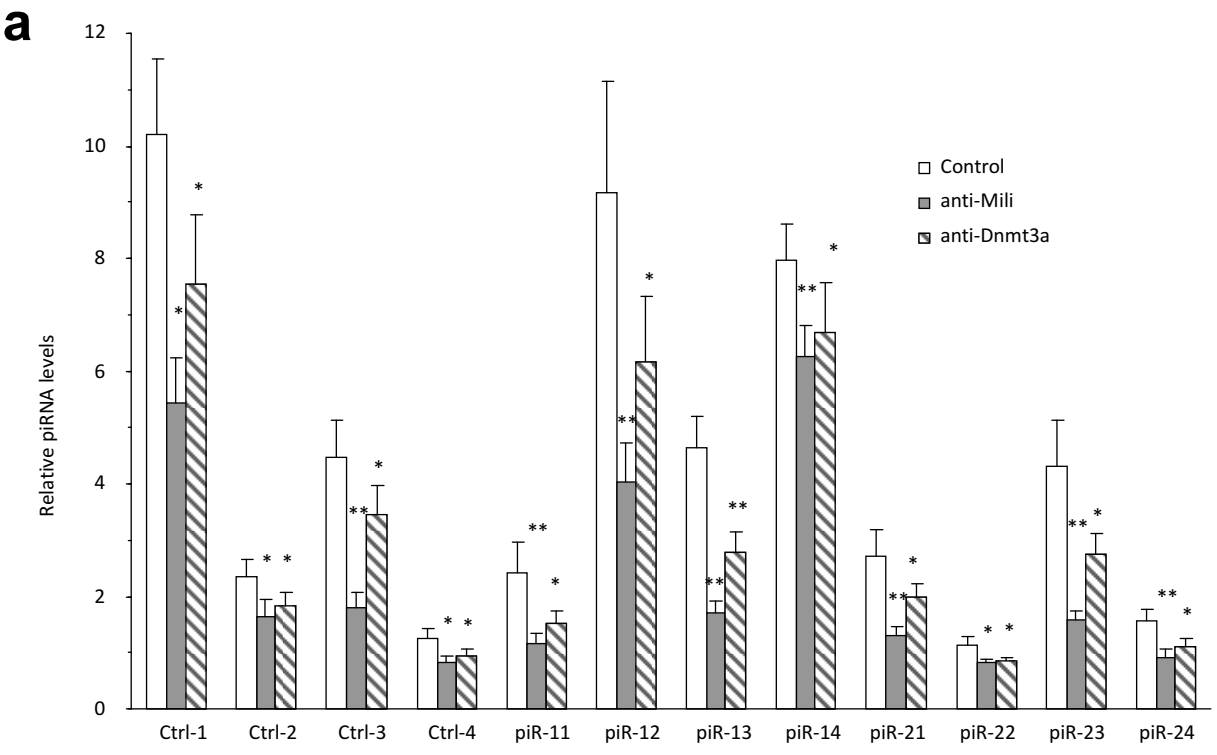


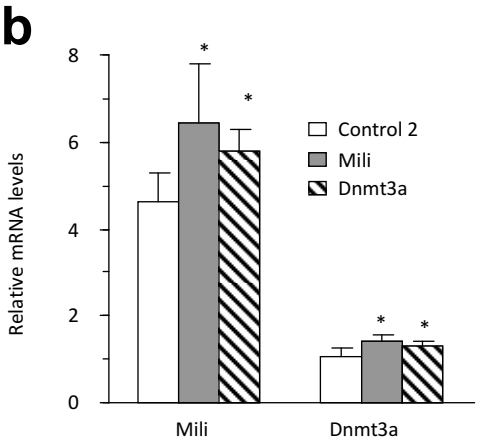
Fig S6. miRNAs were globally enhanced.

(a) Injection with miR-17-5p enhanced miRNA levels in zygotes. * $p<0.05$, ** $p<0.01$. Error bars, SD ($n=4$).

(b) Injection with miR-17-5p-IN repressed miRNA levels in zygotes. * $p<0.05$, ** $p<0.01$. Error bars, SD ($n=4$).

(c) Left, miR-17 zygotes (50 zygotes) expressed increased levels of miRNAs. Right, Decreased methylation of miRNA genes was found in miR-17 zygotes. * $p<0.05$, ** $p<0.01$. Error bars, SD ($n=4$).





c **Primers used in the study**

primer name	sequences
musMili-SacI	5' ccc **gagctc** **taa**ctgggaacttggacaccggct
musMili-MluI	5' cc **acgcgt** gata ctcct gcttc agt ctc cca
musMili-MluI-mut	5' cc **acgcgt** gata ctcct gcttc agt ctc cca tcac ct ggg
musDnmt3a-SacI	5' ccc **gagctc** acccaacacccttccctttaggtt
musDnmt3a-MluI	5' cc **acgcgt** cct gccc tgg ct gggga gcgg aac
musDnmt3a-MluI-mut	5' cc **acgcgt** cct gccc tgg ct gggga gcgg aac tttc tg cgag
musDnmt3a-SacI	5' ccc **gagctc** gggctgggatcagtggccaatagg
musDnmt3a-MluI	5' cc **acgcgt** acc ttg tcaaa gaaaaag caa gaa
musDnmt3a-MluI-mut	5' cc **acgcgt** acc ttg tcaaa gaaaaag caa gaa tttc tg ccca
EGFP-347F	5' gtc atc gct att acc atg gtg atg cgg
EGFP-668R	5' agc tct gct tat ata gac ctc cca ccg
EGFP981F	5' caa gga cga cgg caa cta caa gac
EGFP-CApaI	5' ccc **ggg ccc** ctt gta cag ctc gtc cat gcc
mo-Gapdh1F:	5' atg gtg aag gtc tgt gtg atc atc
mo-Gapdh250R:	5' tgg gtt ctc act cct gga aga agg

Fig S7. Inject with Mili and Dnmt3a

(a) Injection with anti-Mili or anti-Dnmt3a antibody decreased piRNA expression in zygotes. * *p<0.05.* Error bars, SD (*n*=4).

(b) Injection with Mili or Dnmt3a increased mRNA levels of both Mili and Dnmt3a. * *p<0.05.* ** *p*<0.01. Error bars, SD (*n*=4).

(c) Primers used in the study are listed.