

Supplementary Materials

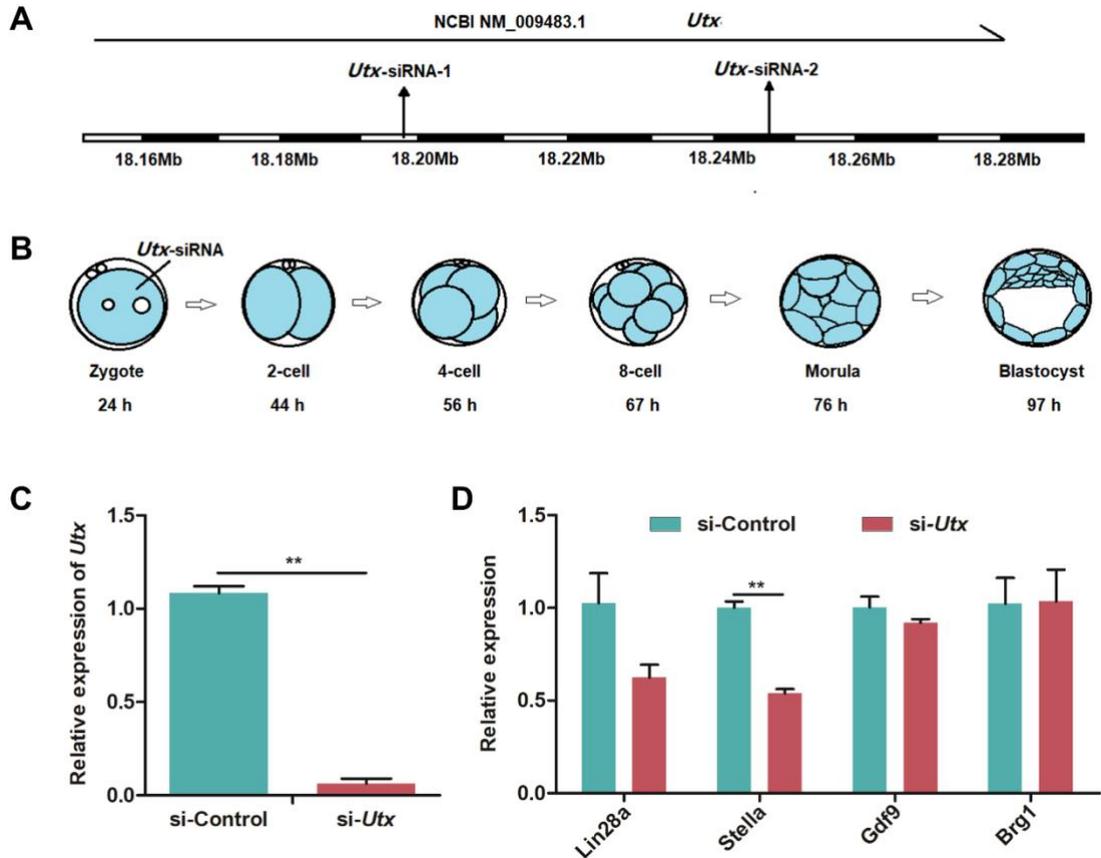


Figure S1. Functional verification of *Utx*-siRNA. (A) Schematic representation of *Utx*-siRNA. Two siRNAs were designed to target different regions of *Utx*. (B) Schematic illustration of *Utx*-siRNA injected into zygotes and *in vitro* culture. (C) qPCR results showing mRNA level of *Utx* in si-*Utx*-injected 2-cell embryos. Error bars indicate SEM. Values were normalized to *Gapdh*. ** $P < 0.01$ by the two-tailed Student's *t*-test. (D) qPCR results showing mRNA levels of maternal effector genes in si-*Utx*-injected 2-cell embryos. Error bars indicate SEM. All values were normalized to *Gapdh*. ** $P < 0.01$ by the two-tailed Student's *t*-test.

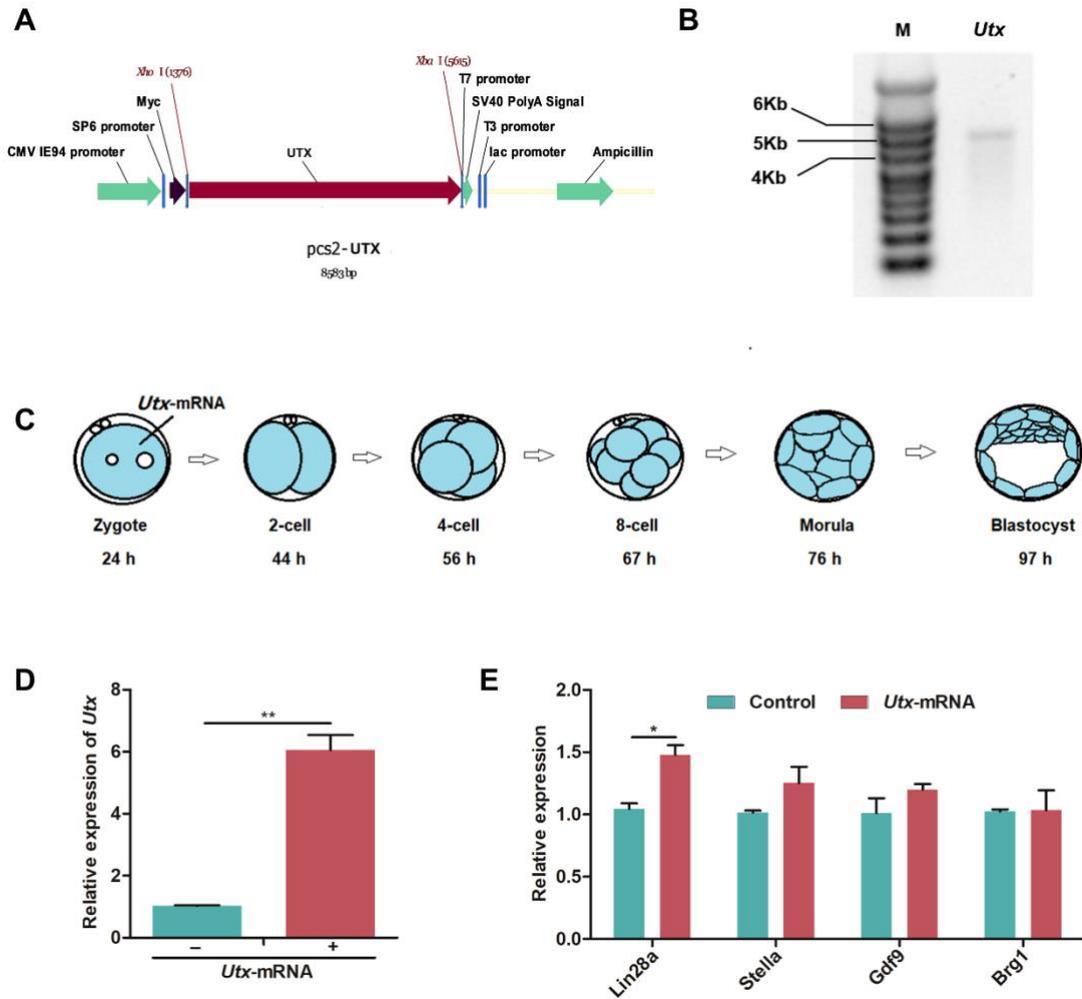


Figure S2. Functional verification of *Utx*-mRNA. (A) Schematic representation of pcs2-UTX plasmid. (B) The sketch of *in vitro* transcribed *Utx*-mRNA integrity was confirmed by electrophoresis with formaldehyde gels. M, marker. (C) Schematic illustration of *Utx*-mRNA injected into zygotes and *in vitro* culture. (D) qPCR results showing mRNA level of *Utx* in *Utx*-mRNA-injected 2-cell embryos. Error bars indicate SEM. Values were normalized to *Gapdh*. ** $P < 0.01$ by the two-tailed Student's *t*-test. (E) qPCR results showing mRNA levels of maternal effector genes in *Utx*-mRNA-injected 2-cell embryos. Error bars indicate SEM. All values were normalized to *Gapdh*. * $P < 0.05$ by the two-tailed Student's *t*-test.

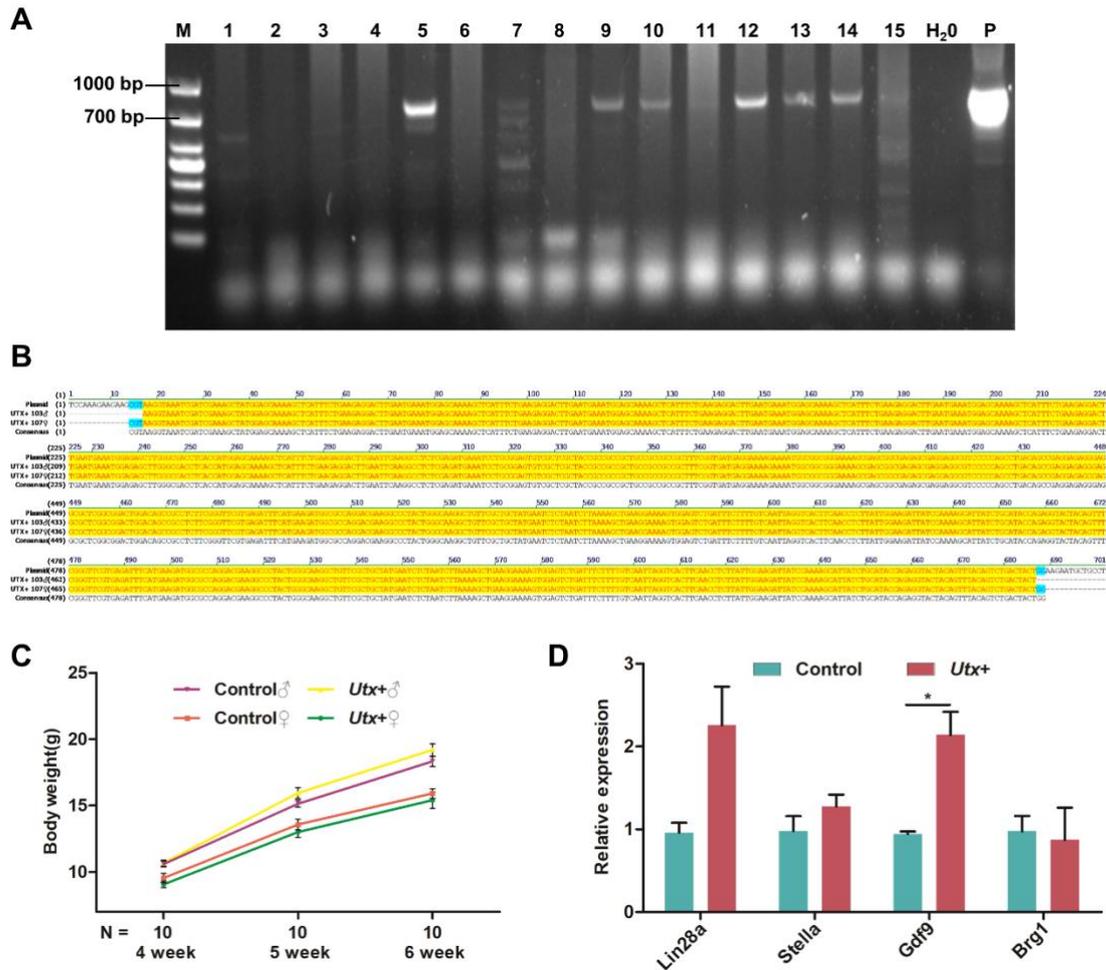


Figure S3. Identification of *Utx*⁺ mice. (A) PCR analysis of the *Utx*⁺ mice. Genomic DNA was amplified with specific primers (Table S2) detecting the *Utx*⁺ mice. M, marker. 1–15, PCR templates were *Utx*⁺ mice genomic DNA. H₂O, PCR template was H₂O. P, PCR template was pcs2-UTX plasmids. (B) Sanger sequencing alignment of *Utx*⁺ mice PCR products. (C) Body weight of *Utx*⁺ mice for 3 consecutive weeks. ♂ and ♀ indicate the male and female, respectively. N, total number of mice in each group. Error bars indicate SEM. (D) qPCR results showing mRNA levels of maternal effector genes in *Utx*⁺ mice 2-cell embryos. Error bars indicate SEM. All values were normalized to *Gapdh*. **P* < 0.05 by the two-tailed Student's *t*-test.

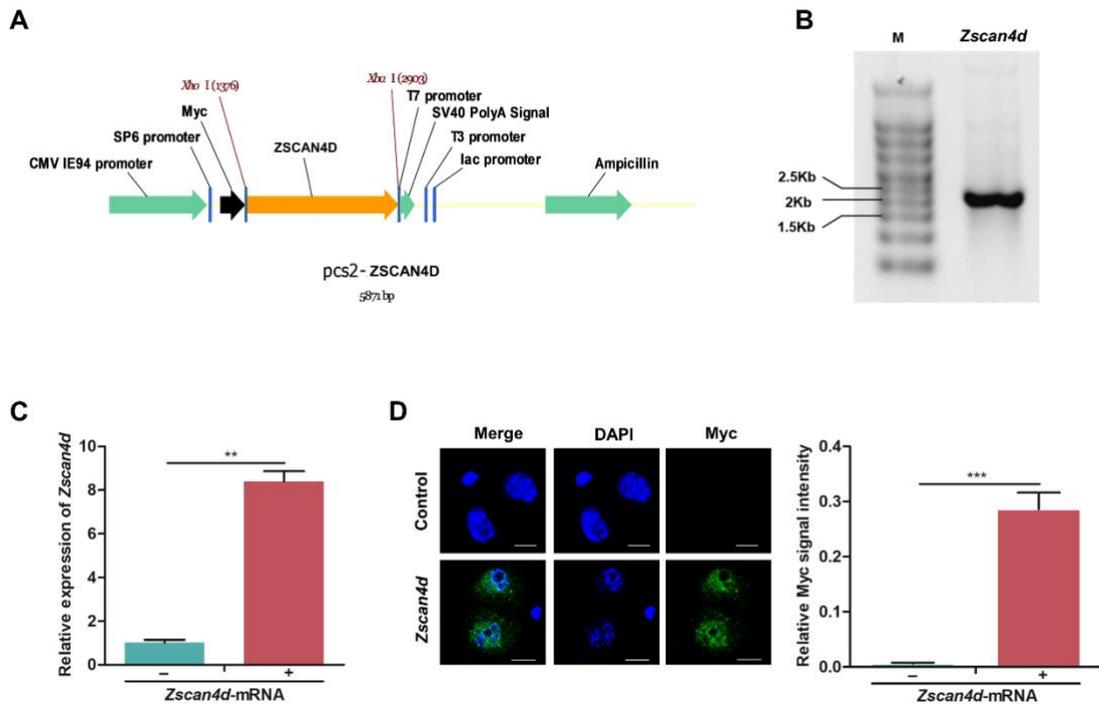


Figure S4. Functional verification of *Zscan4d* mRNA. (A) Schematic representation of pcs2-ZSCAN4D plasmid. (B) The sketch of *in vitro* transcribed *Zscan4d* mRNA integrity was confirmed by electrophoresis with formaldehyde gels. M, marker. (C) qPCR results showing mRNA levels of *Zscan4d* in *Zscan4d*-mRNA-injected 2-cell embryos. Error bars indicate SEM. Values were normalized to *Gapdh*. ** $P < 0.01$ by the two-tailed Student's *t*-test. (D) Immunofluorescence of Myc in *Zscan4d*-mRNA-injected embryos at the 2-cell stage (left), and quantification of Myc signal intensity (right). For the immunofluorescence images, bar graphs show the relative intensities of Myc/DAPI signal ratios. Error bars indicate SEM. Representative images from ≥ 20 embryos analyzed using Image J; independent micromanipulations for each condition are shown. Scale bar, 20 μm . *** $P < 0.001$ by the two-tailed Student's *t*-test.

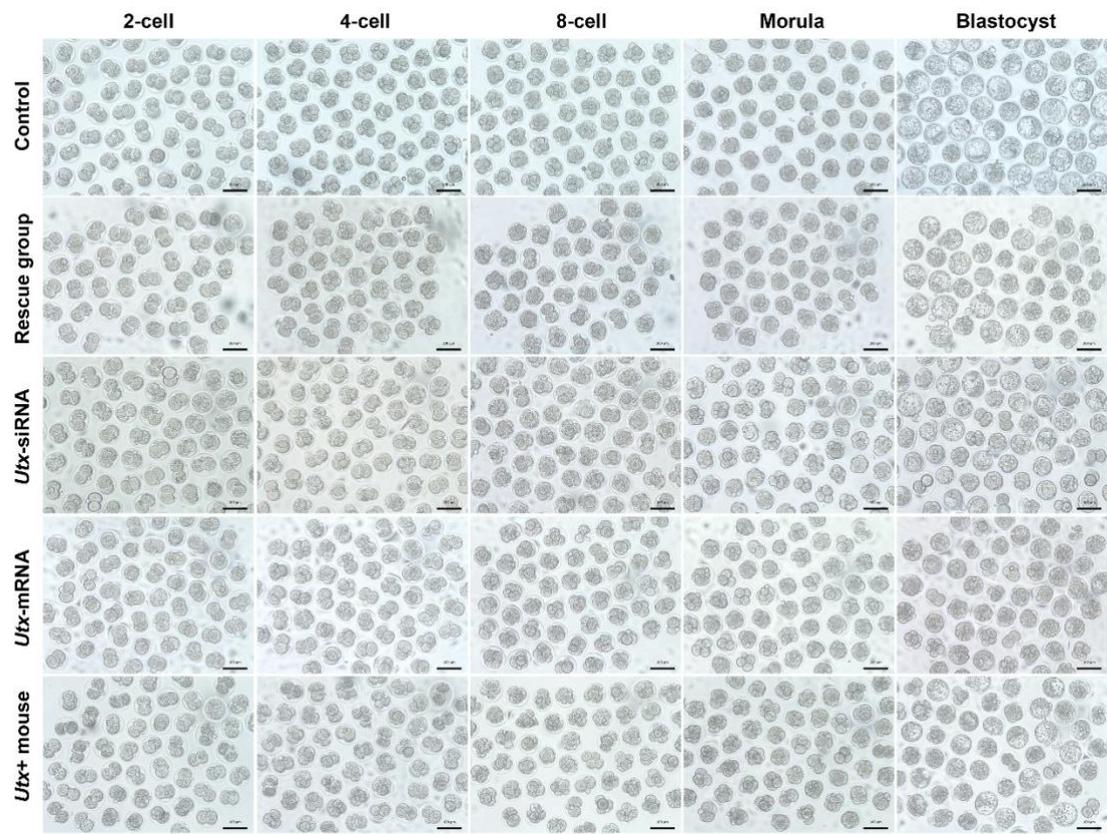


Figure S5. Bright field representative images of the embryos of the rescue (*Utx*-siRNA+*Zscan4d*-mRNA), and the control, *Utx*-siRNA, *Utx*-mRNA, and *Utx*+ mice, respectively at different culture times. Scale bar, 100 μ m.

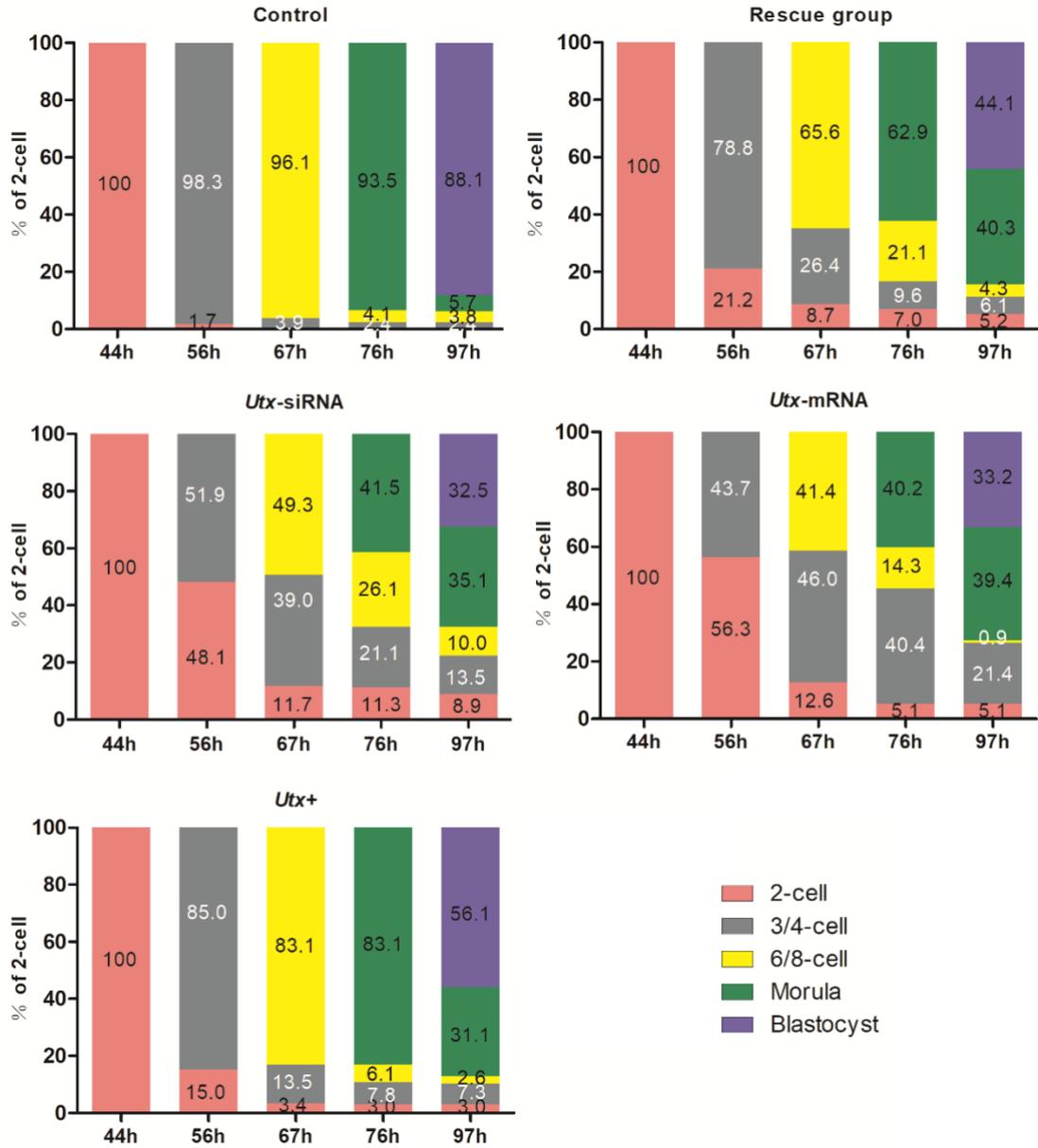


Figure S6. Development rates in the control, rescue, *Utx*-mRNA-injected, *Utx*-siRNA-injected and *Utx*+ mice groups, respectively. The efficiency was calculated based on the number of 2-cell embryos. $n \geq 3$.

Table S1. Developmental rate of embryos

Groups	No. of replicates	No. of 2-cell embryos	%4-cell per 2-cell±S.D.	%8-cell per 2-cell±S.D.	%morula per 2-cell±S.D.	%blastocyst per 2-cell±S.D.
Control	3	228	98.33 ± 0.7	96.06 ± 2.68	93.53 ± 2.33	88.1 ± 3.02
Rescue	3	209	78.8 ± 8.92 ^{n.s.}	65.63 ± 8.59 ^{n.s.}	62.93 ± 6.27*	44.1 ± 9.34*
<i>Utx</i> -mRNA injection	3	284	43.66 ± 11.59*	41.43 ± 13.24*	40.33 ± 14.24*	33.2 ± 13.59*
<i>Utx</i> -siRNA injection	3	278	51.93 ± 15.59*	49.3 ± 14.85*	41.53 ± 10.54*	32.5 ± 4.3**
<i>Utx</i> + mice	3	176	84.99 ± 0.09*	83.1 ± 1.98 ^{n.s.}	83.1 ± 1.98 ^{n.s.}	55.98 ± 3.12**

* $P < 0.05$, ** $P < 0.01$ as compared with the control group, by two-tailed Student's *t*-test. *n.s.*, not significant.

Table S2. All primers used in this study

Gene	Application	Sequence (5' - 3')
<i>Utx</i>	Real-time qPCR	Forward-TATTGGCCCAGGTGACTGTGAA Reverse-CAAATCTCCAGGTCGCTGAATAAAC
<i>MuERVL</i>	Real-time qPCR	Forward-CTCTACCACTTGGACCATATGAC Reverse-GAGGCTCCAAACAGCATCTCTA
<i>Zscan4d</i>	Real-time qPCR	Forward-TGCTTGAAGCCTCCTGTCAT Reverse-GTGTGGCCTTGTTCAGAT
<i>Tcstv1</i>	Real-time qPCR	Forward-GGATCCCTGAAGGTAAATCCTC Reverse-AACCATCCATCCTCAGGAAC
<i>Tcstv3</i>	Real-time qPCR	Forward-AGAAAGGGCTGGAAGTTGTGACCT Reverse-AAAGCTCTTTGAAGCCATGCCAG
<i>Cdc2</i>	Real-time qPCR	Forward-GGACTACAAGAACACCTTTC Reverse-CAGGAAGAGAGCCAACGGTA
<i>eIF-1a</i>	Real-time qPCR	Forward-TTTGGTCACTACTCAGGAGG Reverse-ATCAGAAGCAACTGGGACAC
<i>Rif1</i>	Real-time qPCR	Forward-GCAAGGATGTTGAGACTGAGC Reverse-TAGAGGCACTGGCAAGTATGTC
<i>Rpl23</i>	Real-time qPCR	Forward-CATGGTGATGGCCACAGTTA Reverse-GACCCCTGCGTTATCTTCAA
<i>U2afbp-rs</i>	Real-time qPCR	Forward-TAAGCTGCAACCTGGAACCT Reverse-CCTGCGTACCATCTTCCATT
<i>Ube2a</i>	Real-time qPCR	Forward-AATGGTTTGAATGCGGTCA Reverse-TGTTTGCTGGACTATTGGGA
<i>Mt1a</i>	Real-time qPCR	Forward-CACCAGATCTCGGAATGGAC Reverse-AGCAGCTCTTCTTGCAGGAG
<i>Wee1</i>	Real-time qPCR	Forward-AGCCATCTACCGAAAGCAGA Reverse-ATCTGTGAAGAGTGCCCGTT
<i>Lin28a</i>	Real-time qPCR	Forward-GCGAAGATCCAAAGGAGACA

		Reverse-TGTGGATCTCTTCCTCTTCC
<i>Stella</i>	Real-time qPCR	Forward-TGTTGTCGGTGCTGAAAGAC Reverse-CACTGTCCCGTTCAAACCTCA
<i>Gdf9</i>	Real-time qPCR	Forward-TTGGCAGTCTCTTCAGTCCA Reverse-GGGAGATCTTTCCACCTCAA
<i>Brg1</i>	Real-time qPCR	Forward-CGGCAGAAGATTGAGAAGGA Reverse-CCCAGCTTGATCTTCACCTT
<i>Gapdh</i>	Real-time qPCR	Forward-GTGGCAAAGTGGAGATTGTTG Reverse-CTCCTGGAAGATGGTGATGG
<i>Utx-M</i>	Genotyping of transgenic mice	Forward-TCCAAAGAAGAAGCGTAAGGTAA Reverse-AGGCAGCATTCTTCCAGTAGTCA
<i>Zscan4d-P</i>	ChIP-qPCR	Forward-AATCCAACCTTTCTCCCTCCAAT Reverse-ACAGAGCCATACATCCACCCAAT
<i>Zscan4d-E</i>	ChIP-qPCR	Forward-ATCAAGAAGATAGGGCAAGAAGA Reverse-ATTATGTCTAGGCATACAAGGGA