

Supporting Information

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Exon	Wildtype	OCT4KO ^{tm1}
1	MAGHLASDFAFSPPPGGGGDGPGGPEPGWVD PRTWMSFQGPAGGGSGIGPGVVPGAEVWGLPPC PPPYDLCGGMAYCAQVGVGVPPGGLETQPQ EGEAGAGVESNSEGASPDPCAAAGAPAKLDKEK LEPNPEE	MAGHLASDFAFSPPPGGGGDGPGGPEPGWVD PRTWMSFQGPAGGGSGIGPGVVPGAEVWGLPPC PPPYDLCGGMAYCAQVGVGVPPGGLETQPQ EGEAGAGVESNSEGASPDPCAAAGAPAKLDKEK LEPNPEE
2	SQDIKALQKDL <u>E</u> QFAKLLQKRITLGYTQADVGLT LGVLFG	SQDIKALQKDL <u>E</u> QFAKLLQKRITLGY <u>P</u> RPWMWGS <i>PWGFSL</i>
3	KVFSQTTCRFEALQLSFKNMCKLRPLLQKWVEE ADNNENLQE	<i>ERCSAKRLSAVLRLCSSVSRTCVSCGPCCRSGWRK</i> LTTRICRR
4	ICKAETLVQARKRKRTSIE <u>N</u> RVRGNLES <u>M</u> FQCPK PTLQQISHIAQQGLEKD	<i>YARQRPLCRPERESGRVSRT*</i>
5	VVRWFWCNRRQKGKRSSSDYSQREDFEAAGSPF TGGPVSSPLAPGPHFGTPGYGGPHFTTLYSSVPF PEGEVFPSVSVTALGSPMHAN*	

Fig. S1. Predicted amino acid sequence of OCT4 protein in wild-type and OCT4KO^{tm1}. Frameshift mutation in exon 2 of OCT4KO^{tm1} is indicated by italics and underlined proline; asterisks represent termination codons. In exon 4, a premature termination codon 91 bp upstream the following exon-exon junction was introduced by homozygous deletion of 1 bp in exon 2, which is expected to result in nonsense-mediated decay of the mRNA (20).

Off target mutations at *ETF1*

Wildtype sequence

GAT CAC ACT TAG G AT AT ACC CAG G CCG AT GT G GGG CT C ACC CT

OCT4KO^{tm1}

Forward primer

GAT CAC ACT TAG G AT AT M T T A S C C T G A C T G A T G G G T C A
0 430 440 450

Reverse primer

T A G S T A G T A T A T C C C A G G C T G A T G T G G G G C T C A C C C T C
580 590 600 610*ETF1mut*^{tm1}

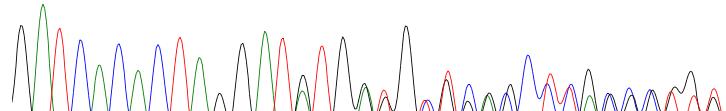
Forward primer

C A C A C T A G G G A T A T C C C M R G S Y K R W K K K G G G S T T C M C C C T G G G G G ·
230 240 250 260 270

Reverse primer

G R A G R R G R T Y M C M C T W R G R W W T W C C C A G G C T G A T G T G G G G C T C A C C C
510 520 530 540 550*OCT4KO*^{tm2}

Forward primer

G A T C A C A C T A G G A T G T G R K G Y T C R G C T C G C C C T G T
130 440 450 460

Reverse primer

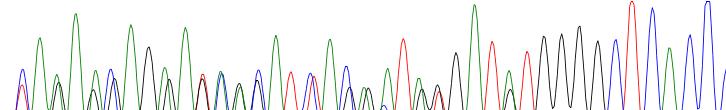
C A A A A C A G A A T A G C A T C A C R A T R K G A T R T G G G G C T C A C C C
0 590 600 610 62

Fig. S2. Sanger sequences on the off-target mutations at *ETF1*. *OCT4KO*^{tm1} and *ETF1mut*^{tm1} carry a monoallelic deletion of "A" 3 bp upstream of the PAM sequence. In *OCT4KO*^{tm2}, deletions of 5'-ATACCAGGCCATG-3' and 5'-ACCCAGGCC-3' occurred on the respective alleles.

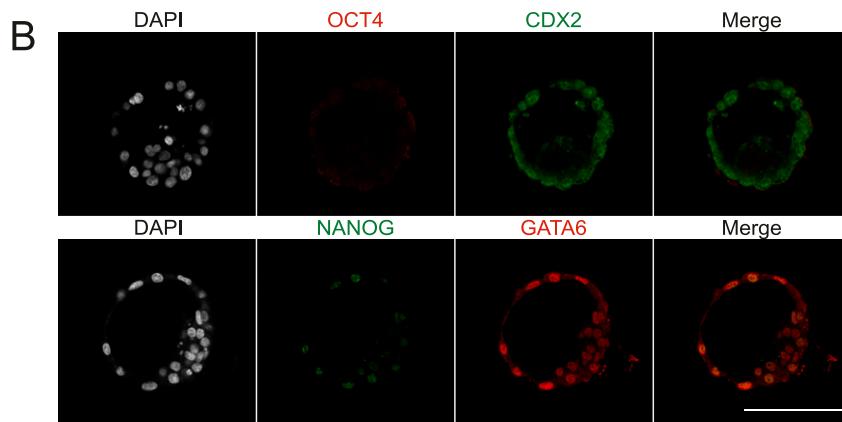
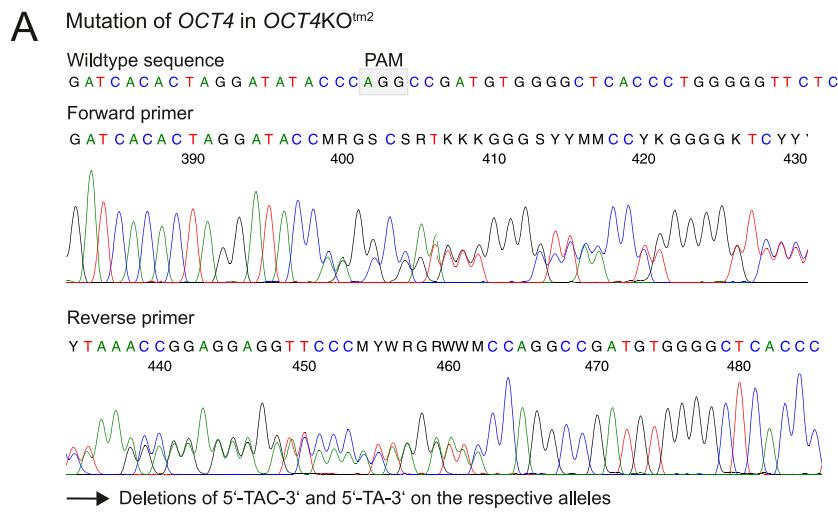


Fig. S3. (A) Sanger sequencing of mutation at OCT4 in OCT4KO^{tm2}. (B) Representative confocal plane of day 7 blastocysts stained against OCT4/CDX2 (Upper) and NANOG/GATA6 (Lower) from OCT4KO^{tm2}. (Scale bar, 100 μ m.)

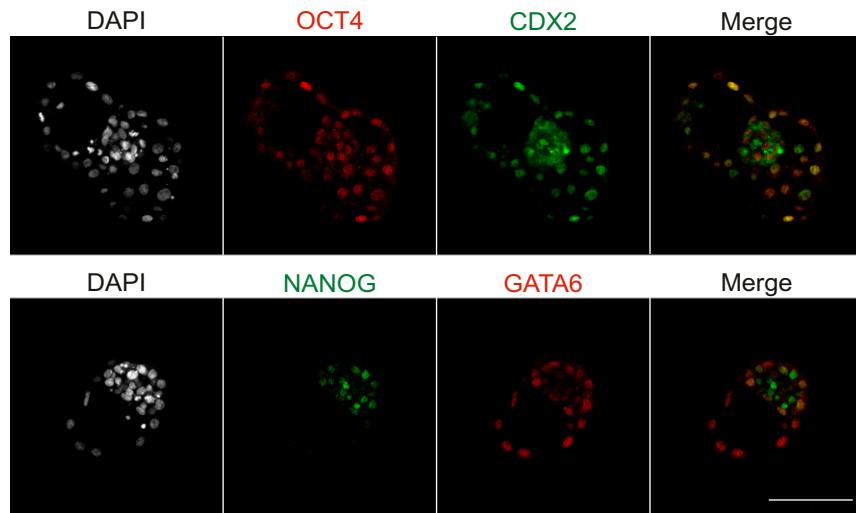


Fig. S4. Representative confocal plane of day 7 blastocysts stained against OCT4/CDX2 (Upper) and NANOG/GATA6 (Lower) from ET1mut^{tm1}. (Scale bar, 100 μ m.)

Table S1. Primers used for mutation screening

Primer	Sequence
OCT4 2f	5'-TTGTGGGACCTCAAAGTAATC-3'
OCT4 2r*	5'-CTGCAGATTCTCGTTGTTGT-3'
OCT4 12f*	5'-TATGTTCTTACATATCCTCTGC-3'
ETF1 2f	5'-TTGGGTGTGAAGTGGGTTG-3'
ETF1 2r*	5'-CTGGGCCATGTGGCTAATT-3'
ETF1 3f*	5'-CTATGACTTGTGTGGAGGGATG-3'

*Primer used for Sanger sequencing.

Other Supporting Information Files

[Dataset S1 \(XLXS\)](#)