Supplemental Table 1. The primers used for qPCR in this study.

Gene name	Primer (5’-3’)
GAPDH	Forward :AACGACCCCTTCATTGACCT Reverse: TGGAAGATGGTGATGGGCTT
Oct4	Forward : CCTTGCAGCTCAGCCTTAAG Reverse: GCGATGTGATGATCTGCTG
Sox2	Forward :CCAAGATGCACAACTCGGAT Reverse: CTCCGGGAAGCGTGTACTTA
Klf4	Forward :AGAAGTGTGACAGGGCCTTT Reverse: TCGTGGGAAGACAGTGTGAA
Nanog	Forward :CGCCATCACACTGACATGAG Reverse: AGAAGAATCAGGGCTGCCTT
Eras	Forward :CTACTGGAAGGAAGTGGCCA Reverse: TATCTGCTGCAACTGGTCCA
Dax1	Forward :CTATGTGTGCGGTGAAGAGC Reverse: TGGAAGCAGGGCAAGTACTT
Zfp296	Forward :GTCAACTCCAAACGTCCTCG Reverse: TGGATTCTGAGATGGGGTCG
Esg1	Forward :GGCAACTGTTATGGCGTGAA Reverse: TCCTGACGAAGCTGTGTCAT
C-Myc	Forward :CACACAACGTCTTGGAACGT Reverse: CGTCTGCTTGAATGGACAGG

PCNA	Forward :TATGCCGAGACCTTAGCCAC Reverse: GGTTACCGCCTCCTCTTCTT
CDK1	Forward :TCCTGGGCAGTTCATGGATT Reverse: TCCTGGGCAGTTCATGGATT
CDK2	Forward :AATGCAGAGGGGTCCATCAA Reverse: CTCCAGATATCCACGGCTGT
CyclinD1	Forward :AGAAGTGCGAAGAGGAGGTC Reverse: CTTAGAGGCCACGAACATGC
P27	Forward :GACCAAATGCCTGACTCGTC Reverse: TCTTCTGTTCTGTTGGCCCT
Gata4	Forward :AAAACGGAAGCCCAAGAACC Reverse: ATAGTGAGATGACAGCCCGG
Sox17	Forward :GCCCTTTGTGTATAAGCCCG Reverse: GCAATAGTAGACCGCTGAGC
Foxa2	Forward :CTACACACACGCCAAACCTC Reverse: TTGAAGGAGAGAGAGTGGCG
Lamb1	Forward :AGCTTGCGTGTGTTTGTGAT Reverse: CAATGTTGTGGTGGCACTGA
BMP4	Forward :CTTCAACCTCAGCAGCATCC Reverse: GATGAGGTGTCCAGGAACCA
T	Forward :ACCCAGCTCTAAGGAACCAC Reverse: GCTGGCGTTATGACTCACAG

Eomes	Forward :TCGTGGAAGTGACAGAGGAC Reverse: TAGTTGTCCCGGAAGCCTTT
Actc1	Forward :TGCCGATCGTATGCAAAAGG Reverse: GGCCTGCCTCATCATACTCT
Nestin	Forward :AGGTGTCAAGGTCCAGGATG Reverse: AAGGAAGCAGACTCAGACCC
Neurod1	Forward :AGGAGGAGGATCAAAAGCCC Reverse: GGGTCTTGGAGTAGCAAGGT
Sox1	Forward :ACACAGTGGGGATAACAGCA Reverse: GCCACGCCCTCATGATATTG
Otx1	Forward :CGGAAGCTATGGTCAGGGAT Reverse: TGAAGATTGGCTCAGTGGGT
Bcl-2	Forward :AACTCTTCAGGGATGGGGTG Reverse: TACTCAGTCATCCACAGGGC
Bax	Forward :GAGACACCTGAGCTGACCTT Reverse: GTCCACGTCAGCAATCATCC
cleaved caspase-3	Forward :CAGCCAACCTCAGAGAGACA Reverse: ACAGGCCCATTTGTCCCATA
cleaved caspase-9	Forward: CCAAGATGCACAACTCGGAG Reverse: ATCCGGGTGTTCCTTCATGT
Bmal1	Forward: AGGCCCACAGTCAGATTGAA Reverse: GAACAGCCATCCTTAGCACG

Clock	Forward: ATGCCACAGAACAGTACCCA Reverse: TTGTGTGGCGAAGGTAGGAT
Cry1	Forward: GGCAACTGTTATGGCGTGAA Reverse: TCCTGACGAAGCTGTGTCAT
Cry2	Forward: TTCTACACGGCAGCTACCAA Reverse: TGCCTCAGTTGGGTCATGAT
Per1	Forward: CACCCTGATGACCCACTCTT Reverse: CCTCCTCCTCCATAGCCAAG
Per2	Forward: AGCGTTACCTCTGAGCACAT Reverse: ATGGATGCAACCTGGTCAGA
Per3	Forward: CCCTGTCTGTCCTCTGTTGT Reverse: CTCTCTCCTTTGGCTGGTGA

Supplemental Table2. The clones used for detecting mutations in this study.

Total clones (number)	31
The sequences of bases have changed in clones	8 (25.8%)
CLOCK protein expression has changed in clones	6 (19.4%)
Homozygous clones	3 (9.68%)
Heterozygous clones	3 (9.68%)

We totally picked out 31 single mESC clones, and 8 (25.8%) of them (clone 5, clone 13,

clone 17, clone 19, clone 20, clone 23, clone 24, and clone 28) showed sequence modifications at the CRISPR/CAS9 cutting site. CLOCK protein expression has changed in 6 clones (19.4%) (clone 17, clone 19, clone 20, clone 23, clone 24, and clone 28) of the 8 positive clones with Western blotting. The results indicated that CLOCK protein expression was totally ablated in three clones (9.68%) (clone 17, clone 19, and clone 20), for homozygous clones; CLOCK protein expression was not totally ablated in three clones (9.68%) (clone 23, clone 24, and clone 28), for heterozygous clones.

Supplemental Table 3. The most probable ten off-target modification sites and the primers used for detecting off-target effects in this study.

Position	Sequence	Score	Ontarget	Gene	Primer
76694865	TCCATCTTTCTCGCGTTACCAGG	100	True	NM_007715	
125121193	TCTATTGTTCCACAGTACGCCAG	0.751669231	False	NM_146171	Fwd: TCTGGCTATCCGCTTCCCTAA
					Rev: CGCTGTCTTCCGCACTTGTT
102221420	TCAAGGCAACTCGAGAAAGAGAG	0.526289801	False	NM_011403	Fwd: CTTCCCACAGAGCAAACAGC
					Rev: CCAGCCAAGCCTGAAAGC
99910981	TCCCTGTAAAGCAAGAAAGATGG	0.509948268	False	NM_010659	Fwd: AGGACCAAGTGAGTTATTCAGCAT
					Rev: GTTCCTCTTGAGACACAGCAGC
172444809	GCTTCTTTTCTACAGTACGTGGG	0.485473295	False	NM_027528	Fwd: GAGCAAGATGAAAATGGAAGCA
					Rev: AAAGCCATCCATTCTGCCTCT

5179756	TCCTGGTCCTGG GAGAAAGAGGG	0.36666 4223	False	NM_0010809 24	Fwd: TTATCACACAGAGCCAAATCACC
					Rev: GGTTGAGGAGGGCTCTCTTTAT
66796510	CAAAGGTAGAGA GAGCCCAAGAG	0.33961 9976	False	NM_0011623 66	Fwd: TAGAGAGATGGTGGCGTTTGTTT
					Rev: TGACCCTCGCTGCTATCCC
136252045	AAAAAGTAGAAA GAGCCCAGGGG	0.25987 7595	False	NM_0010424 85	Fwd: GGCTGCTGCTGGCTTCCTA
					Rev: CAGCCATAGGAAAGACAATCACC
90993123	TCCTAGTAAAGA CAGAAAGACAG	0.24692 7836	False	NM_211138	Fwd: GACATTTTGGGTAAAGGGAAGG
					Rev: TTCAGGTGAGCAGGGAGGTAGT
90964219	CCCTGGAAACAC GAGACAGACAG	0.23525 4	False	NM_0010455 15	Fwd: TTAGAGCAGTCCTTAGAGACACCA
					Rev: CCCAGTTTTGTTGTCTTATGAGC
79182745	GAGAGGTAGAGA GAACGCAAGGG	0.22815 1896	False	NM_0010481 76	Fwd: GACATCAGCATCAGAACCCTAAT
					Rev: CACTGACCGTATGTGACCTGTAA