Supplementary Data

Expression Constructs

In all the procedures described below, polymerase chain reaction (PCR) was conducted using LA AccuTaq DNA Polymerase (Sigma-Aldrich). PCR products were first subcloned into pGEM-T Easy plasmid (Promega). After the accuracy of expected PCR products was verified by sequencing, DNA fragments were excised by specific restriction enzymes, and were ligated into other plasmids. All the final constructs were also verified by sequencing. Sequencing reactions were conducted at the University of Hawaii's Advanced Studies in Genomics, Proteomics and Bioinformatics Facility.

Oct4

The full-length cDNA for mouse *Oct4 (Pou5f1)* was obtained from P19 EC cell cDNA by reverse transcription (RT)-PCR using the primers (Fw: CCA TGG CTG GAC ACC TGG CTT CAG ACT TCG CCT TC [*Nco*I site underlined]; Rv: GCG GCC GCG TTT GAA TGC ATG GGA GAG CCC AG [*Not*I site underlined]), which was then subcloned into the pEF/myc/cyto plasmid at *Nco*I/*Not*I restriction enzyme sites.

Oct4-GR

A DNA fragment encoding the hormone-binding domain of mouse glucocorticoid receptor (Nr3c1) was amplified with the primers (Fw: GCG GCC GCT TCT GAA AAC GCT AAC AAA ACA ATA GTT CC [*Not*I site underlined]; Rv: TCT AGA TCA TTT CTG ATG AAA CAG AAG CTT TTT G [*Xba*I site underlined]) from mouse embryo cDNA, and inserted into the *Not*I/*Xba*I sites of the Oct4 expression construct.

Oct4[V267P]

A DNA fragment was amplified with the primers (Fw: CCA TGG CTG GAC ACC TGG CTT CAG ACT TCG CCT TC [*Nco*I site underlined]; Rv: GCG GCC GCG TTT GAA TGC ATG GGA GAG CCC AG [*Not*I site underlined]) using pCAG-IP-HA267V/P [44] as a template, and subcloned into the pEF/myc/cyto plasmid at *Nco*I/*Not*I restriction enzyme sites.

Oct4ΔPOU

Two DNA fragments were separately amplified with the primers (Fw: CCA TGG CTG GAC ACC TGG CTT CAG ACT TCG CCT TC [*Nco*I site underlined]; Rv: CTG AAG GTT CTC ATT GTT CTG GGA CTC CTC GGG AGT), and the primers (Fw: ACT CCC GAG GAG TCC CAG AAC AAT GAG AAC CTT CAG; Rv: GCG GCC GCG TTT GAA TGC ATG GGA GAG CCC AG [*Not*I site underlined]). These two fragments, which overlap in sequence at the ends, were mixed, denatured, and annealed. Using the annealed product as a template, a new DNA fragment was amplified with the primers (Fw: CCA TGG CTG GAC ACC TGG CTT CAG ACT TCG CCT TC [*Nco*I site underlined]; Rv: GCG GCC GCG TTT GAA TGC ATG GGA GAG CCC AG [*Not*I site underlined]), and subcloned into the pEF/myc/cyto plasmid at *Nco*I/*Not*I restriction enzyme sites.

Oct4ΔHOM

Two DNA fragments were separately amplified with the primers (Fw: CCA TGG CTG GAC ACC TGG CTT CAG ACT TCG CCT TC [*Nco*I site underlined]; Rv: TCG TTG GGA ATA CTC AAT CCG GGC CTG CAC CAG GGT), and the primers (Fw: ACC CTG GTG CAG GCC CGG ATT GAG TAT TCC CAA CGA; Rv: GCG GCC GCG TTT GAA TGC ATG GGA GAG CCC AG [*Not*I site underlined]). These two fragments, which overlap in sequence at the ends, were mixed, denatured, and annealed. Using the annealed product as a template, a new DNA fragment was amplified with the primers (Fw: CCA TGG CTG GAC ACC TGG CTT CAG ACT TCG CCT TC [*Nco*I site underlined]; Rv: GCG GCC GCG TTT GAA TGC ATG GGA GAG CCC AG [*Not*I site underlined]), and subcloned into the pEF/myc/cyto plasmid at *Nco*I/*Not*I restriction enzyme sites.

EnR-Oct4

A DNA fragment encoding the EnR domain was excised from the pCS2+-EnR plasmid by digesting with *Xho*I (blunted) and *Nco*I, and ligated between the *Bam*HI (blunted) and *Nco*I sites of the Oct4 expression construct.

VP16-Oct4

A DNA fragment encoding the VP16 domain was amplified with the primers (Fw: GCC CAT GGC CAA GCT ACT GTC TTC TAT C [*Nco*I site underlined]; Rv: CGG GAT CCC CTG GCG ATC CCG GAC CCG G [*Bam*HI site underlined]) from pACT plasmid (Promega), and used to replace the sequence between the *Nco*I and *Bam*HI sites in the Oct4 expression construct.

Dkk1

The full-length cDNA for mouse *Dkk1* was amplified from mouse embryo cDNA using the primers (Fw: CCA TGG TTG TGT GTG CAG CGG CAG CTG TCC GG [*Nco*I site underlined]; Rv: ATT TGC GGC CGC GTG TCT CTG GCA GGT GTG GAG CCT AGA A [*Not*I site underlined]), which was then subcloned into the pEF/myc/cyto plasmid at *Nco*I/*Not*I restriction enzyme sites.

Gsk3b

The full-length cDNA for mouse *Gsk3b* was obtained from P19 EC cell cDNA by RT-PCR using the primers (Fw: GTC GAC ATG TCG GGG CGA CCG AGA ACC ACC TCC TTT [*Sal*I site underlined]; Rv: GCG GCC GCG GTG GAG TTG GAA GCT GAT GCA GAA GCG GC [*Not*I site underlined]), which was then subcloned into the pEF/myc/cyto plasmid at *Sal*I/*Not*I restriction enzyme sites.

Supplementary Table S1. Primer Sequences for Quantitative Reverse Transcription-Polymerase Chain Reaction

Gene name	*Forward primer sequence*	*Reverse primer sequence*
Actb	GAGAGGGAAATCGTGCGTGACATC	CAGCTCAGTAACAGTCCGCCTAGA
Aes	TCCTGAGCTGAACTCCATCATCCG	TCGTGTCCGTTCTTGTCCTCCTTG
Apc	TGGCATTTCTGGTTGGCACCCTCA	TTGTGTCTCTGCTTACTCCGGTGA
Apc2	ACACAGGACACAGTGTGACATCCA	GGTCAAGCACCCAGTGGTTAGTCT
Axin1	GACAGGCTCAGAGAGTCCGAAGGT	GGACAGAAGGCAGCTTGTGACTTG
Axin2	CACCACCACCATCAGCAGTGTCAT	GACTTGCTCTGCCGCTCACTCTCT
Brachyury	CCTCGGATTCACATCGTGAGAGTT	AGTAGGTGGGCTGGCGTTATGACT
Btrc	ACACTGGAATGCAAGCGGATTCTC	CAAAGCAGCCATAAGATCCCACAC
Cdx2	CCTCCCGACTTCCCTTCACCATAC	CCTCCCGACTTCCCTTCACCATAC
Ctnnbip1	CCCTGGTGATGGTGTGTGCTCTGT	TGATGCTGCCCTCCCTCCTCGAAT
Cby1	TAGGGAGGAGACACAGGATGGAAG	TGCCAGCTGTCTGAATATGGTGAT
Eef1a1	CTGGCATGGTGGTTACCTTTGCTC	GGTAGTCAGAGAAGCTCTCAACAC
Eomes	ATCTCCTAACACTGGCTCCCACTG	CGTTGGTCTGTGGCACGGTTCTCT
Fgf4	TACTGCAACGTGGGCATCGGA	GTGGGTTACCTTCATGGTAGG
Fgf8	GTTGCACTTGCTGGTTCTCTGCCT	AGTCCTTGCCTTTGCCGTTGCTCT
Foxd3	CTCTGATCCTGGTCCATCTGTCCT	GGTGCATTTTTGGAAATTCGGTTA
Gapdh	GCATGGCCTTCCGTGTTCCT	CCCTGTTGCTGTAGCCGTATTCAT
Gsk3a	AATCCGAGAGATGAACCCTAACTA	TTCATTTACCTCTGCCCTCTAGTC
Gsk3b	AGCCAAGCAGACACTCCCTGTGAT	GTCTCGCCCATTTGGTAGTTTGAC
Hoxb1	TCGTCAGAACCCAGCACTCTCACT	TTCCTGGGCAGCTCTAAACTGGTG
Lhx1	TGCGGCTCACTGTGCTAGTATGTA	AACACTTTCTCAGGTTGCTGGTGC
Nanog	CAGAAGCAGAAGTACCTCAGCCTC	CACCTGGTGGAGTCACAGAGTAGT
Oct4	AGGCAGGAGCACGAGTGGAAAGCA	GGAGGGCTTCGGGCACTTCAGAAA
Snai1	CCGTCCAGCTGTAACCATGCCTCA	TGGGAGACACATTGGCCAGGCTGA
Sp5	CAGGACAGGAAACTGGGTCGTAGT	GGCCTAGCAAAAACTTAGGCCTTG
Tax1bp3	CCAGAGACAGTATGAAGCCCACCT	TGGCTTGGTCACATGCTACAAGTT
Tbx6	GGCCTCTCTTCCACCCTTTAGTTC	CACTAGTAACAAGGCCCCCAGGAG
Tle1	TCCTCCTCCTCACATGAGAGTACC	GCAGCATGAGAAGCAGACCTTGGA
Tle2	AACCGAGACAACTACATCCGCTCC	CAGGAAGCGAACTTGAGTGACAGC
Tle3	GACAACTACATCCGCTCGTGCAAG	CGTCCTCCAGGCATTGAGAAGGTT
Tle4	TCCAAGGTCTGCTTCTCATGCTGC	TGTCACAGCTAAGCACCGATGAGG
Tle6	GTGGATACAACTTACCTGGCGTGA	TGTCCTTGGTGTCCACAGTGAGCA
Wnt3	CAGATGCCCGCTCAGCTATGAACA	AGCAGCACCAGTGGAAGACGCAAT
Wnt3a	GCCACAAGAGCTTCCTGATTGGTA	CCAGGCAGAAGACAGTCAGTCACC