



(51) International Patent Classification:

A61K 38/44 (2006.01) C12N 15/877 (2010.01)

C12N 15/873 (2010.01) C12N 15/89 (2006.01)

(21) International Application Number:

PCT/US2018/042890

(22) International Filing Date:

19 July 2018 (19.07.2018)

(25) Filing Language:

English

(26) Publication Language:

English

(71) Applicant: **CHILDREN'S MEDICAL CENTER CORPORATION** [US/US]; 55 Shattuck Street, Boston, Massachusetts 02115 (US).

(72) Inventors: **ZHANG, Yi**; c/o Children's Medical Center Corporation, 55 Shattuck Street, Boston, Massachusetts 02115 (US). **INOUE, Azusa**; c/o Children's Medical Center Corporation, 55 Shattuck Street, Boston, Massachusetts 02115 (US).

(74) Agent: **HUNTER-ENSOR, PH.D., Melissa**; GREENBERG TRAURIG, LLP, One International Place, Boston, Massachusetts 02110 (US).

(81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JO, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ,

OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Declarations under Rule 4.17:

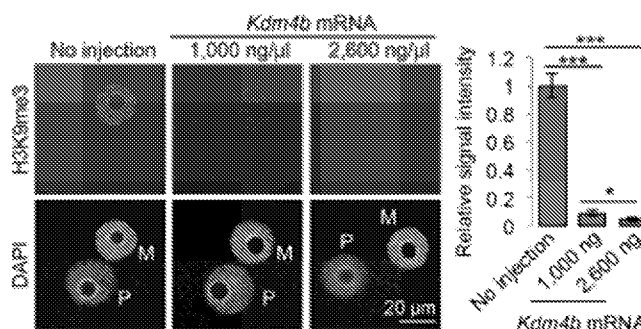
- of inventorship (Rule 4.17(iv))
- as to non-prejudicial disclosures or exceptions to lack of novelty (Rule 4.17(v))

Published:

- with international search report (Art. 21(3))

(54) Title: COMPOSITIONS AND METHODS FOR GENERATING PHYSIOLOGICAL X CHROMOSOME INACTIVATION

FIG. 1A



(57) Abstract: The present invention features compositions and methods for recapitulating physiological X-chromosome inactivation (XCI) in a cell, including a cell of any embryo generated by Somatic Cell Nuclear Transfer (SCNT). In one aspect, the invention features a method for generating physiological X chromosome inactivation in an embryo generated by SCNT, the method comprising injecting the embryo generated via SCNT with an H3K27me3-specific demethylase polypeptide or a polynucleotide encoding said demethylase. Disclosed herein are methods, compositions, and kits comprising an agent which increases the expression of genes encoding an H3K27me3-specific demethylase, or increases the activity of human H3K27me3-specific demethylase.

COMPOSITIONS AND METHODS FOR GENERATING PHYSIOLOGICAL X CHROMOSOME INACTIVATION

BACKGROUND OF THE INVENTION

5 In females of certain therian mammals, including rodents, one of the two X chromosomes is inactivated to achieve gene dosage compensation. During development, X-chromosome inactivation (XCI) can take place in either an imprinted or a random manner. For imprinted XCI, the paternal X chromosome (Xp) is selectively inactivated during preimplantation development. Although imprinted XCI is maintained in the extraembryonic
10 cell lineage, it is lost in the pre-epiblast cell lineage of late blastocysts. At the peri-implantation stage, epiblast cells undergo random XCI, resulting in the silencing of either the Xp or maternal X chromosome (Xm). Previous studies have demonstrated an important role of *Xist*, an X-linked long noncoding RNA, in both imprinted and random XCI. The *Xist* RNA participates in XCI by coating and inactivating the X chromosome in *cis*.

15 To selectively silence the Xp during preimplantation development, *Xist* is imprinted in the Xm with a long sought-after but yet-to-be-identified mechanism. Previous studies using nuclear transfer approaches have suggested that genomic imprinting of *Xist* is established during oogenesis. However, analyses of DNA methyltransferase maternal knockout embryos revealed that oocyte DNA methylation is dispensable for *Xist* imprinting. A recent study
20 demonstrated that overexpression of an H3K9me3 demethylase, Kdm4b, in parthenogenetic (PG) embryos partially derepresses *Xist*, suggesting the involvement of H3K9me3 in imprinted *Xist* silencing. However, the fact that PG embryos undergo nonphysiological *Xist* derepression of a single maternal allele raises the question of whether the derepression effect observed in the H3K9me3-depleted PG embryos is physiologically relevant. Recently, it was
25 discovered that maternal H3K27me3 serves as an imprinting mark for DNA methylation-independent autosomal gene imprinting.

Such observations are relevant to therapeutic and reproductive cloning, where a donor nucleus from a somatic cell is transferred into an enucleated oocyte. This process is termed Somatic Cell Nuclear Transfer (SCNT) and may be accomplished by fusion of the somatic
30 cell with the enucleated oocyte, injection of the nucleus into the enucleated oocyte, or by any other method. At present, only about 1 percent of SCNT result in the successful generation of a viable embryo. The low success rate of SCNT is associated with defects in imprinting.

Accordingly, novel compositions and methods for increasing the success of SCNT and correcting defects in imprinting are required.

SUMMARY OF THE INVENTION

5 As described below, the present invention features compositions and methods for recapitulating physiological X-chromosome inactivation (XCI) in a cell, including a cell of any embryo generated by SCNT.

In one aspect, the invention features a method for generating physiological X chromosome inactivation in an embryo generated by Somatic Cell Nuclear Transfer (SCNT),
10 the method comprising injecting the embryo generated via SCNT with an H3K27me3-specific demethylase polypeptide or a polynucleotide encoding said demethylase. In one embodiment, the embryo is injected with an mRNA encoding a H3K27me3-specific demethylase. In another embodiment, the polynucleotide encodes a Kdm6a, Kdm6b, or Kdm6c polypeptide. In another embodiment, the embryo is injected with between about
15 1000 and 2000 ng/ μ L of the mRNA. In another embodiment, the embryo is injected with 1800 ng/ μ L of mRNA. In another embodiment, the X chromosome is present in a donor nucleus derived from a somatic cell. In another embodiment, the donor nucleus is transferred into an oocyte or embryonic stem cell. In another embodiment, the polynucleotide encodes an enzymatically active fragment of an H3K27me3-specific demethylase. In another
20 embodiment, the polynucleotide is present in a mammalian expression vector. In another embodiment, the mammalian expression vector comprises a promoter directing constitutive or inducible expression of the H3K27me3-specific demethylase. In another embodiment, the injected polypeptide is Kdm6a, Kdm6b, or Kdm6c. In another embodiment, the method reduces the expression of X-linked genes. In another embodiment, the method does not
25 significantly change the expression of genes that escape X-chromosome imprinting. In another embodiment, the method does not significantly change autosomal gene expression. In another embodiment, the maternal allele expression bias of X-linked genes is greater than about 35-60%. In another embodiment, maternal allele expression bias of X-linked genes is greater than about 50%. In other embodiments, the embryo is an early blastocyst stage
30 embryo or is derived from an adult somatic cell. In another embodiment, the somatic cell is obtained from a human subject. In another embodiment, the method further comprises

culturing a cell from the embryo to obtain a tissue suitable for transplantation into the human subject.

In another aspect, the invention features a blastocyst produced according to the method of a previous claim.

5 In another aspect, the invention features a cell or tissue produced according to the method of a previous aspect.

In another aspect, the invention features a cloned organism produced by implanting the blastocyst of a previous aspect into a host uterus.

10 Other features and advantages of the invention will be apparent from the detailed description, and from the claims.

Definitions

Unless defined otherwise, all technical and scientific terms used herein have the meaning commonly understood by a person skilled in the art to which this invention belongs.

15 The following references provide one of skill with a general definition of many of the terms used in this invention: Singleton et al., Dictionary of Microbiology and Molecular Biology (2nd ed. 1994); The Cambridge Dictionary of Science and Technology (Walker ed., 1988); The Glossary of Genetics, 5th Ed., R. Rieger et al. (eds.), Springer Verlag (1991); and Hale & Marham, The Harper Collins Dictionary of Biology (1991). As used herein, the following
20 terms have the meanings ascribed to them below, unless specified otherwise.

By “H3K27me3-specific demethylase” is meant a protein that specifically demethylates trimethylated H3 'Lys-27. Exemplary demethylases include Kdm6a, Kdm6b, and Kdm6c.

25 By “KDM6A polypeptide” (lysine-specific demethylase 6A, also referred to as histone demethylase UTX) is meant a protein having at least about 85% amino acid identity to the sequence provided at NCBI Reference Sequence: O15550.2, or a fragment thereof, and having demethylase activity. An exemplary KDM6A amino acid sequence is provided below:

30 1 mkscgvslat aaaaaaafgd eekkmaagka sgeeseeasps ltaeereal gldsrifgfv
61 rfhedgartk allgkavrcy eslilkaegk vesdfffcqlg hfnllledyp kalsayqryy
121 slqsdyywka aflyglglvy fhynafqwai kafqevlyvd psfcrakeih lrlglmfkvn
181 tdyesslkhf qlalvdcnpc tlnsaeiqfh iahlyetqrk yhsakeaye q llqtenlsaq
241 vkatvllqqlg wnhhtvdllg dkatkesyai qylqkslead pnsqgswyfl grcyssigkv

301 qdafisyrrqs idkseasadt wcsigvlyqq qnqpmadalqa yicavqldhg haaawmdlgt
 361 lyescnqpqd aikcylnatr skscntsals aarikylqaq lcnlpqgslq nktklpsie
 421 eawslpipae ltsrqgamnt aqntsdsnws gghavshppv qqqahswclt pqklqhleql
 481 ranrnnlnpa qklmleqles qfvlmqqhqm rptgvaqvrs tgipngptad sslptnsvsq
 5 541 qqpqlaltrv psvsqpgvrp acpgqplang pfsaghvpcs tsrtlgstdt ilignnnhitg
 601 sgsngnvpvl qrnaltlphn rtnltssae pwknqlsnst qglhkgqssh sagpnngerpl
 661 sstgpsqhlq aagsgiqnqn ghptlpsnsv tqgaalnhls shtatsggqq gitltkeskp
 721 sgniltvpst srhtgetpns tasveglpnh vhmqtadavc spshgdsksq gllssdnpql
 781 sallmgkann nvgtgtcdkv nnihpavhtk tdnsvassps saistatpss ksteqtttss
 10 841 vtslnsphsg lhtingegme esqspmktld llvnhkpspq iipsmsvsiy pssaevlkac
 901 rnlgknglsn ssilldkcpp prppsspypp lpkdklnppt psiylenkrd affpplhqfc
 961 tnpnnpvtvi rglagalkld lglfstktlv eannehmvev rtqllqpade nwdptgtkki
 1021 whcesnrst tiakyaqyqa ssfqueslree nekrshhkdh sdsestssdn sgrrrkgpfc
 1081 tikfgtnidl sddkkwklql heltklpafv rvvsagnlls hvghtilgm tvqlymkvpq
 15 1141 srtpghqenn nfcsvninig pgdcewfvvp egywgvlnf cekknlflm gswwpnledl
 1201 yeavpvyrf iqrpgdlvwi nagtvhwvqa igwcnniawn vgpltacqyk laveryewnk
 1261 lqsvksivpm vhlswnmarn ikvsdpklfe mikycllrtl kqcqtlreal iaagkeiiwh
 1321 grtkeepahy csicevevfd llfvtnesns rktyivhcqd carktsqnle nfvvlegqkm
 1381 edlmqvdydqf tlappplsas s

By “KDM6A polynucleotide” is meant a nucleic acid molecule encoding a KDM6A polypeptide. An exemplary KDM6A polynucleotide sequence is provided at NM_001291415.1.

By “KDM6B polypeptide” (lysine-specific demethylase 6, also referred to as JmjC domain-containing protein 3) is meant a protein having at least about 85% amino acid identity to the sequence provided at NCBI Reference Sequence: O15054.4, or a fragment thereof, and having demethylase activity. An exemplary KDM6B amino acid sequence is provided below:

1 mhravdppga raareafalg glscagawss cpshppprsa wlpggrcsas igqpplpapl
 30 61 ppsghsssggh psypyapga ptprrplhgkl eslhgcqvql lrepaqpglw eqlgqlyese
 121 hdseeatrcy hsalryggsf aelgprigr l qqaqlwnfht gscqhrakvl ppleqvwnll
 181 hlehkrnyga krggppvkra aeppvvqpvp paalsgpsge eglspggkrr rgcnseqtql
 241 ppglplpppp lppppppppp pppplpglat sppfqltkpg lwstlhgdaw gperkgsapp
 301 ergeqrhslp hpypypapay tahppghrlv paappgpgpr ppgaeshgcl patrppgsdl
 35 361 resrvqrsm dssvspaatt acvpyapsrp pglpgtttss sssssntgl rgvepnpgip
 421 gadhyqtpal evshhgrlqp sahssrkpfl gapaatphls lppgpssppp ppcprllrpp
 481 pppawlkgpa craaredgei leelffgteg pprpappplp hregflgppa srfsvgtqds
 541 htpptpptpt tsssnssnsgs hssspagpvs fppppylars idplprppsp aqnpqdpplv

601 pltlalppap psschqntsg sfrpesprp rvsfpktpev gpgpppgpls kapqvpvpgv
 661 gelpargprl fdfpptpled qfeepaefki lpdganimk mldesirkee eqqqheagva
 721 pqpplkepfa slqspfpdt aptttapava vttttttttt ttatqeeekk pppalppppp
 781 lakfpppsqp qpppppppsp asllkslasv legqkycyrg tgaavstrpg plpttqyspg
 5 841 ppsgatalpp tsaapsaqgs pppsassssq fstsggpwar errageepvp gpmtptqppp
 901 plslpparse sevleeisra cetlvervgr satdpadpvd taepadsge rllppaqake
 961 eaggvaavsg sckrrqkehq kehrrhrrac kdsvgrrpre grakakakvp keksrrvlgn
 1021 ldlqseeiqg reksrpdlgg askakpptap appsapapsa qptppsasvp gkkareeapg
 1081 ppgvsradml klrsllseggp kelkirlikv esgdketfia severeerrlm adltishcaa
 10 1141 dvvrasrnak vkgkfresyl spaqsvkpi nteeklprek lnpptpsiyl eskrdafspv
 1201 llqfctdprn pitvirglag slrlnlglfs tktlveasge htvevrtqvq qpsdenwdlt
 1261 gtrqiwpces srshttiaky aqyqassfge slqeekesed eeseepdstt gtpssapdp
 1321 knhhiikfgt nidlsdakrw kpqlqellkl pafmrvtstg nmlshvghti lgmntvqlym
 1381 kvpgsrtprgh qennnfcsvn inigpgdcew favhehywet isafcdrhgv dyltgswwpi
 15 1441 lddlyasnip vyrfvqrpqd lvwinagtvd wvqatgwcn iawnvgplta yqyqlalery
 1501 ewnevknvks ivpmihvswv vartvkisdp dlfdmikfcl lqsmkhcqvq reslvragkk
 1561 iayqgrvnde payycnecdv evfnilfvts engsrntylv hcegcarrs aglqgvvle
 1621 qyrteelaqa ydaftlapas tsr

20 By “KDM6B polynucleotide” is meant a nucleic acid molecule encoding a KDM6B polypeptide. An exemplary KDM6B polynucleotide sequence is provided at NM_001080424.2 and reproduced below:

1 ggcaacatgc cagccccgta gcactgcca cccacccac tgtggtctgt tgtacccac
 61 tgctgggggtg gtggttccaa tgagacaggg cacaccaaac tccatctggc tgttactgag
 25 121 gcgagagacac gggatgatgat tggctttctg gggagagagg aagtcctgtg attggccaga
 181 tctctggagc ttgccgacgc ggtgtgagga cgctccacac gagggccgaa ttggctgtga
 241 aaggactgag gcagccatct gggggtagcg ggcactctta tcagagcggc tggagccgga
 301 ccatcgctccc agagagctgg ggcagggggc cgtgcccaat ctccagggct cctggggcca
 361 ctgctgacct ggctggatgc atcgggcagt ggaccctcca gggggccgcg ctgcacggga
 30 421 agcctttgcc cttggggggc tgagctgtgc tggggcctgg agctcctgcc cgcctcatcc
 481 ccctcctcgt agcgcatggc tgcctggagg cagatgtctc gccagcattg ggcagccccc
 541 gcttcctgct ccctacccc cttcacatgg cagtagttct gggcacccca gcaaaccata
 601 ttatgtctca ggggcgcca ctccaagacc cctccatggg aagctggaat ccctgcatgg
 661 ctgtgtgcag gcattgctcc gggagccagc ccagccaggg ctttggaac agcttgaggc
 35 721 actgtacgag tcagagcacg atagtgagga ggccacacgc tgctaccaca gcgcccttcg
 781 atacggagga agcttcgctg agctggggcc ccgcatggc cgactgcagc agggccagct
 841 ctggaacttt catactggct cctgccagca ccgagccaag gtctgcccc cactggagca
 901 agtgtggaac ttgctacacc ttgagcacia acggaactat ggagccaagc ggggaggtcc
 961 cccgggtgaag cgagctgctg aacccccagt ggtgcagcct gtgcctcctg cagcactctc

1021 agggccctca ggggaggagg gcctcagccc tggaggcaag cgaaggagag gctgcaactc
 1081 tgaacagact ggccttcccc cagggtgcc actgcctcca ccaccattac caccaccacc
 1141 accaccacca ccaccaccac caccaccctt gcctggcctg gctaccagcc cccatttca
 1201 gctaaccaag ccagggtgtt ggagtaccct gcatggagat gcctggggcc cagagcgcaa
 5 1261 gggttcagca ccccagagc gccaggagca gcggcactcg ctgcctcacc catatccata
 1321 ccagctcca gcgtacaccg cgcaccccc tggccaccgg ctggtcccg ctgctcccc
 1381 agggccaggc ccccgcccc caggagcaga gagccatggc tgctgctg ctaccctgc
 1441 ccccggaagt gaccttagag agagcagagt tcagaggctg cggatggact ccagcgtttc
 1501 accagcagca accaccgctt gcgtgcctta cggcccttcc cggcccccctg gcctccccgg
 10 1561 caccaccacc agcagcagca gtagcagcag cagcaaacact ggtctccggg gcgtggagcc
 1621 gaaccaggc attcccggcg ctgaccatta ccaaactccc gcgtggagg tctctacca
 1681 tggccgctg gggccctcg cacacagcag tcggaaaccg ttcttggggg ctcctgctgc
 1741 cactccccac ctatccctgc cacctggacc ttctcacc cctccacccc cctgtccccg
 1801 cctcttacgc cccccaccac cccctgcctg gttgaagggt ccggcctgccc gggcagcccg
 15 1861 agaggatgga gagatcttag aagagctctt ctttgggact gagggacccc cccgcccctgc
 1921 cccaccacc ccccccatc gcgagggtt cttggggcct ccggcctccc gcttttctgt
 1981 gggcactcag gattctcaca cccctccac tcccccaacc ccaaccacca gcagtagcaa
 2041 cagcaacagt ggcagccaca gcagcagccc tgctgggcct gtgtcctttc cccaccacc
 2101 ctatctggcc agaagtatag acccccttcc ccggcctccc agcccagcac agaaccacca
 20 2161 ggaccacact cttgtacccc tgactcttgc cctgcctcca gcccctcctt cctcctgcca
 2221 ccaaaatacc tcaggaagct tcaggcgcgc ggagagcccc cggcccaggg tctccttccc
 2281 aaagaccccc gaggtggggc cggggccacc ccaggcccc ctgagtaaag cccccagcc
 2341 tgtgcccgc ggggttgggg agctgcctgc ccgaggccct cgactctttg attttcccc
 2401 cactccgctg gaggaccagt ttgaggagcc agccgaattc aagatcctac ctgatgggct
 25 2461 ggccaacatc atgaagatgc tggacgaatc cattcgcaag gaagaggaac agcaacaaca
 2521 cgaagcaggc gtggcccccc aacccccgct gaaggagccc tttgcatctc tgcagtctcc
 2581 tttccccacc gacacagccc ccaccactac tgctcctgct gtcgccgtca ccaccaccac
 2641 caccaccacc accaccacca cggccaccca ggaagaggag aagaagccac caccagccct
 2701 accaccacca ccgctcttag ccaagttccc tccaccctct cagccacagc caccaccacc
 30 2761 cccaccccc agcccggcca gcctgctcaa atccttggcc tccgtgctgg agggacaaaa
 2821 gtactgttat cgggggactg gagcagctgt ttccaccgg cctgggccc tggccaccac
 2881 tcagtattcc cctggcccc catcaggtgc taccgcccct cggcccacct cagcggcccc
 2941 tagcgcaccag ggctccccac agccctctgc ttctcgtca tctcagttct ctacctcagg
 3001 cgggcccctg gcccgggagc gcaggcggg cgaagagcca gtcccgggccc ccatgacccc
 35 3061 caccacaacc cccccacccc tatctctgcc ccctgctcgc tctgagctg aggtgctaga
 3121 agagatcagc cgggcttgcg agacccttgt ggagcgggtg ggccggagt cactgaccc
 3181 agccgaccca gtggacacag cagagccagc ggacagtggg actgagcgac tgctgcccc
 3241 cgcacaggcc aaggaggagg ctggcggggt ggcggcagt tcaggcagct gtaagcggcg
 3301 acagaaggag catcagaagg agcatcggcg gcacaggcgg gcctgtaagg acagtgtggg

3361 tcgtcggccc cgtgagggca gggcaaaggc caaggccaag gtccccaag aaaagagccg
 3421 cggggtgctg gggaacctgg acctgcagag cgaggagatc cagggtcgtg agaagtcccc
 3481 gcccgatctt ggcggggcct ccaaggccaa gccaccaca gctccagccc ctccatcagc
 3541 tcctgcacct tctgcccagc ccacaccccc gtcagcctct gtccctggaa agaaggctcg
 5 3601 ggaggaagcc ccagggccac cgggtgtcag ccggggccgac atgctgaagc tgcgtcact
 3661 tagtgagggg ccccccaagg agctgaagat ccggtcatc aaggtagaga gtggtgacaa
 3721 ggagaccttt atcgctctg aggtggaaga gcggcggtg cgcatggcag acctcaccat
 3781 cagccactgt gctgctgacg tcgtgcgcgc cagcaggaat gccaaggta aagggaagtt
 3841 tcgagagtcc tacctttccc ctgcccagtc tgtgaaaccg aagatcaaca ctgaggagaa
 10 3901 gctgccccgg gaaaaactca acccccctac acccagcatc tatctggaga gcaaacggga
 3961 tgctttctca cctgtcctgc tgcagttctg tacagaccct cgaaatccca tcacagtgat
 4021 ccggggcctg gcgggctccc tgcggctcaa cttgggcctc ttctccacca agaccctggg
 4081 ggaagcgagt ggcgaacaca ccgtggaagt tcgcaccag gtgcagcagc cctcagatga
 4141 gaactgggat ctgacaggca ctcggcagat ctggccttgt gagagctccc gttcccacac
 15 4201 caccattgcc aagtacgcac agtaccaggc ctcatccttc caggagtctc tgcaggagga
 4261 gaaggagagt gaggatgagg agtcagagga gccagacagc accactggaa cccctcctag
 4321 cagcgcacca gaccgaaga accatcacat catcaagttt ggaccaaca tcgacttgtc
 4381 tgatgctaag cggtggaagc cccagctgca ggagctgctg aagctgcccg cttcatgcg
 4441 ggtaacatcc acgggcaaca tgctgagcca cgtgggccac accatcctgg gcatgaacac
 20 4501 ggtgcagctg tacatgaagg tgcccggcag ccgaacgcca ggccaccagg agaataacaa
 4561 cttctgctcc gtcaacatca acattggccc aggcgactgc gagtggttcg cgggtgcacga
 4621 gcactactgg gagaccatca gcgctttctg tgatcggcac ggcgtggact acttgacggg
 4681 ttcttggtgg ccaatcctgg atgatctcta tgcattcaat attcctgtgt accgcttcgt
 4741 gcagcgaccc ggagacctcg tgtggattaa tgcggggact gtgcactggg tgcaggccac
 25 4801 cggctggtgc aacaacattg cctggaacgt ggggccctc accgcctatc agtaccagct
 4861 ggccctggaa cgatacgagt ggaatgaggt gaagaacgctc aaatccatcg tgcccatgat
 4921 tcacgtgtca tggaacgtgg ctgcacgggt caaaatcagc gaccccgact tgttcaagat
 4981 gatcaagttc tgctgctgc agtccatgaa gcaactgccag gtgcaacgcg agagcctggg
 5041 gcgggcaggg aagaaaatcg cttaccaggg ccgtgtcaag gacgagccag cctactactg
 30 5101 caacgagtgc gatgtggagg tgtttaacat cctgttcgtg acaagtgaga atggcagccg
 5161 caacacgtac ctggtacact gcgagggctg tgcccggcgc cgcagcgag gcctgcaggg
 5221 cgtggtggtg ctggagcagt accgcactga ggagctggct caggcctacg acgccttcac
 5281 gctggtgagg gcccggcggg cgcgcgggca gcggaggagg gcaactgggc aggtgcagg
 5341 gacgggcttc gggagcccg cgcgccttt cctgagccc ccgcccgtt tctccccca
 35 5401 ggccccagcc agcacgtgc gatgaggccg gacgccccgc ccgcctgcct gccgcgcaa
 5461 ggcgcgcgg ggccaccagc acatgcctgg gctggaccta ggtccgcct gtggccgaga
 5521 agggggctcg gccagccct tccaccccat tggcagctcc cctcacttaa tttattaaga
 5581 aaaacttttt tttttttttt agcaaatatg aggaaaaaag gaaaaaaaat gggagacggg
 5641 ggagggggct ggcagccct cgcaccag cgcctcccct caccgacttt ggccttttta

5701 gcaacagaca caaggaccag gctccggcgg cggcgggggt cacatacggg ttccctcacc
5761 ctgccagccg cccgcccgcg cggcgagat gcacgcggct cgtgtatgta catagacgtt
5821 acggcagccg aggtttttta tgagattctt tctatgggct ttaccctcc cccggaacct
5881 cctttttttac ttccaatgct agctgtgacc cctgtacatg tctctttatt cacttggtta
5 5941 tgatttgtat tttttgttct tttcttgttt ttttgttttt aatttataac agtcccactc
6001 acctctattt attcatTTTT gggaaaaccc gacctccac accccaagc catcctgccc
6061 gcccctccag ggaccgcccg tcgcccggct ctccccgcgc ccagtggtgt gtccggggccc
6121 ggcccagaccg tctccacccg tccgcccgcg gctccagccg ggttctcatg gtgctcaaac
6181 ccgctccccct cccctacgtc ctgcactttc tcggaccagt cccccactc ccgaccgcac
10 6241 ccagagcccca cctgaggggtg agcaactcct gtactgtagg ggaagaagtg ggaactgaaa
6301 tggatattttg taaaaaaaaa aaataaaata aaaaaattaa aggttttaaa gaaagaacta
6361 tgaggaaaaag gaaccccgtc cttcccagcc ccggccaact taaaaaaca cagaccttca
6421 cccccacccc cttttctttt taagtgtgaa acaaccagc gccagggcct cactggggca
6481 gggacacccc ggggtgagtt tctctggggc tttattttcg ttttggttgt tgttttttct
15 6541 ccacgctggg gctgcggagg ggtggggggg ttacagtccc gcaccctcg actgcactgt
6601 ctctctgccc caggggcaga ggggtcttcc caaccctacc cctattttcg gtgatttttg
6661 tgtgagaata ttaatatata aaataaacgg agaaaaaaaa aaaaaaaaaa aaaaaaaaaa
6721 aaaaaaaaaa a

20 By “KDM6C polypeptide” (histone demethylase UTY, also referred to as ubiquitously-transcribed TPR protein on the Y chromosome) is meant a protein having at least about 85% amino acid identity to the sequence provided at NCBI Reference Sequence: O14607.2, or a fragment thereof, and having demethylase activity. An exemplary KDM6C amino acid sequence is provided below:

25 1 mkscavsltt aavafgdeak kmaegkasre seeesvsltv eerealggmd srlfgfvrlh
61 edgartktll gkavrcyesl ilkaegkves dffcqlghfn lledyskal sayqryyslq
121 adywknafll yglglvyfyy nafhwaikaf qdvlyvdpsf crakeihlrl glmfkvntdy
181 ksslkhfqla lidcnpctls naeiqfhiah lyetqrkyhs akeayeqlq tenlpaqvka
241 tvlqqglgwmh hnmdlvgdka tkesyaiqyl qksleadpns gqswyflgrc yssigkvqda
30 301 fisyrqsidk seasadtwcs igvlyqqqnq pmdalqayic avqldhghaa awmdlgtlye
361 scnqpqdaik cylnaarskr csntstlaar ikflqngsdn wnggqslshh pvqqvyslcl
421 tpqklqhleq lranrdnlnp aqkhqleqle sqfvlmqqmr hkevaqvrtd gihngaitds
481 slptnsvsnr qphgaltrvs svsqpgvrpa cveklssga fsagcipcgt skilgstdti
541 llgsnciags esngnvpylq qnthtlphnh tdlnssteep wrkqlnsaq glhksqsscl
35 601 sgpneeqplf stgsaqyhqa tstgikkane hltlpsnsvp qgdadshlsc htatsggqqg
661 imftkeskps knrslvpets rhtgdtsgc advkglshv hqliadavss pnhgdspnll
721 iadnpqlsal ligkangnvgtgtcdkvnni hpavhtktdh svasspsai statpspkst
781 eqrsinsvts lnsphsglht vnegelgksq sstkvdplla shrstsqilp smsvsicpss

841 tevlkacrnpg gknslsncsci lldkcprrp ptspypplpk dklnpptpsi ylenkrdaff
 901 pplhqfctnp knpvtvirgl agalkldlgl fstktlvean nehmvevrtq llqpadenwd
 961 ptgtkkiwrc esnrshhtia kyaqyqassf qeslreenek rtqhkdhdsn estssensgr
 1021 rrkpgpfktik fgtnidlsdn kkwklqlhel tklpafarvv sagnllthvg htilgmntvg
 5 1081 lymkvpgrsrt pghqennnfc svninigpgd cewfvvpedy wgvlndfcek nlnflmssw
 1141 wpnledlyea nvpvyrfiqr pgdlvwinag tvhwvqavgw cnniawnvgp ltacqyklav
 1201 eryewnkllks vkspvpmvhl swnmarnikv sdpklfemik ycllkilkqy qtlrealvaa
 1261 gkeviwhgrt ndepahycsi cevevfnnlf vtneantqkt yivhchdcarr ktskslenfv
 1321 vleqykmedl iqvydqftla lslssss

10

By “KDM6C polynucleotide is meant a nucleic acid molecule encoding a KDM6C polypeptide. An exemplary KDM6A polynucleotide sequence is provided at NM_001258249.1, which sequence is reproduced below:

1 gctcatcggtt tgttggttag ataatatcat gaactgataa atgcagttgc cacgttgatt
 15 61 ccctaggggcc tggcttaccg actgaggtca taagatatta tgccttctct ttagacttgg
 121 tcagtggaga ggaaatgggc aaagaaccag cctatggagg tgacaaggcc ttagggccaa
 181 aagtcttgag ggtgaagggt tagggcctgc gcagcttccc tgccatgccc cgcaagggtct
 241 cgcattcgca aggcttgtga cagtgggagc ctcatctacg actctcctaa agtccatggg
 301 gtcctctttt cgcatttgcg ccccggtggg gatgcccgat gccgcccttc ccatcgctct
 20 361 cttcccccttc aagcgtatcg caactgcaaa aacacccagc acagacactc ctttttctat
 421 cttaatgcat ttaactagca caacctacag gttgttccat cccagagact acccttttct
 481 ccatagacgt gaccatcaac caaccagcgg tcagaatcag tcagcctctg tcatgttcct
 541 aggtccttgg cgaactggct gggcgggggt ccagcagcct aggagtacag tggagcaatg
 601 cctgacgtaa gtcaacaaag atcacgtgag acgaatcagt cgcctagatt ggctacaact
 25 661 aagtgggttg gagcggggag gtcgcggcgg ctgctgtggg ttcgcccgtg acacaattac
 721 aactttgtgc tgggtgctgg aaagtttgtg attttaagaa attctgctgt gctctccagc
 781 actgcgagct tctgccttcc ctgtagtttc ccagatgtga tccaggtagc cgagttccgc
 841 tgcccgtgct tcggtagctt aagtctttgc ctgagctttt ttccttgtag ccgctgagga
 901 ggcgataaaa ttggcgtcac agtctcaagc agcgattgaa ggcgtctttt caactactcg
 30 961 attaagggttg ggtatcgctg tgggacttgg aaatttgttg tttccatgaa atcctgcgca
 1021 gtgtcgctca ctaccgccgc tgttgccctc ggtgatgagg caaagaaaat ggcggaagga
 1081 aaagcgagcc gcgagagtga agaggagtct gttagcctga cagtcgagga aaggaggcgc
 1141 cttgggtggca tggacagccg tctcttcggg ttcgtgagga ttcattgaaga tggcgccaga
 1201 acgaagaccc tactaggcaa ggctgttcgc tgctacgaat ctttaattctt aaaagctgaa
 35 1261 ggaaaagtgg agtctgactt cttttgccaa ttaggtcact tcaacctctt gttggaagat
 1321 tattcaaaaag cattatctgc atatcagaga tattacagtt tacaggctga ctactggaag
 1381 aatgctgcgt ttttatatgg ccttggtttg gtctacttct actacaatgc atttcattgg
 1441 gcaattaaag catttcaaga tgtcctttat gttgacccca gcttttgtcg agccaaggaa
 1501 attcattttac gacttgggct catgttcaaa gtgaacacag actacaagtc tagttttaaag
 40 1561 ctttttcagt tagccttgat tgactgtaat ccatgtactt tgtccaatgc tgaaattcaa
 1621 tttcatattg cccatttgta tgaaaccag aggaagtatc attctgcaaa ggaggcatat

1681 gaacaacttt tgcagacaga aaaccttcct gcacaagtaa aagcaactgt attgcaacag
 1741 ttaggttggg tgcatacata tatggatcta gtaggagaca aagccacaaa ggaaagctat
 1801 gctattcagt atctccaaaa gtctttggag gcagatccta attctggcca atcgtggtat
 1861 tttcttggaa ggtgttattc aagtattggg aaagttcagg atgcctttat atcttacagg
 5 1921 caatctattg ataaatcaga agcaagtgcg gatacatggt gttcaatagg tgtgttgtat
 1981 cagcagcaaa atcagcctat ggatgcttta caggcatata tttgtgctgt acaattggac
 2041 catgggcatg ccgcagcctg gatggaccta ggtactctct atgaatcctg caatcaacct
 2101 caagatgccg ttaaagtcta cctaaatgca gctagaagca aacgtttagg taataacctct
 2161 acgcttgctg caagaattaa atttctacag gctcagttgt gtaaccttcc acaaagtagt
 10 2221 ctacagaata aaactaaatt acttcctagt attgaggagg catggagcct accaatcccc
 2281 gcagagctta cctccaggca ggggtccatg aacacagcac agcaggctta tagagctcat
 2341 gatccaaata ctgaacatgt attaaaccac agtcaaacac caattttaca gcaatccttg
 2401 tcaactacaca tgattacttc tagccaagta gaaggcctgt ccagtcctgc caagaagaaa
 2461 agaacatcta gtccaacaaa gaatggttct gataactgga atggtggcca gagtctttca
 15 2521 catcatccag tacagcaagt ttattcgttg tgtttgacac cacagaaaatt acagcacttg
 2581 gaacaactgc gagcaaatag agataattta aatccagcac agaagcatca gctggaacag
 2641 ttagaaaagtc agtttgtctt aatgcagcaa atgagacaca aagaagttgc tcaggtagca
 2701 actactggaa ttcataacgg ggccataact gattcatcac tgcctacaaa ctctgtctct
 2761 aatcgacaac cacatggtgc tctgaccaga gtatctagcg tctctcagcc tggagttcgc
 20 2821 cctgcttggtg ttgaaaaact tttgtccagt ggagcttttt ctgcaggctg tattccttgt
 2881 ggcacatcaa aaattctagg aagtacagac actatcttgc taggcagtaa ttgtatagca
 2941 ggaagtgaag gtaatggaaa tgtgccttac ctgcagcaaa atacacacac tctacctcat
 3001 aatcatacag acctgaacag cagcacagaa gagccatgga gaaaacagct atctaactcc
 3061 gctcaggggc ttcataaaag tcagagttca tgtttgtcag gacctaataga agaacaacct
 25 3121 ctgtttttcca ctgggtcagc ccagtatcac caggcaacta gcactggtat taagaaggcg
 3181 aatgaacatc tcaactctgcc tagtaattca gtaccacagg gggatgctga cagtcacctc
 3241 tcctgtcata ctgtacctc aggtggacaa caaggcatta tgtttacaa agagagcaag
 3301 ccttcaaaaa atagatcctt ggtgcctgaa acaagcaggc atactggaga cacatctaatt
 3361 ggctgtgctg atgtcaaggg acttttctaatt catgttcatt agttgatagc agatgctgtt
 30 3421 tccagtccta accatggaga ttcaccaaatt ttattaattg cagacaattc tcagctctct
 3481 gctttgttga ttggaaaagc caatggcaat gtgggtactg gaacctgtga caaagtgaat
 3541 aatattcacc cagctgttca tacaaagact gatcattctg ttgcctcttc accctcttca
 3601 gccattttcca cagcaacacc ttctcctaaa tccactgagc agagaagcat aaacagtgtt
 3661 accagcctta acagtcctca cagtggatta cacacagtca atggagaggg gctggggaag
 35 3721 tcacagagct ctacaaaagt agacctgcct ttagctagcc acagatctac ttctcagatc
 3781 ttaccatcaa tgtcagtgtc tatatgcccc agttcaacag aagttctgaa agcatgcagg
 3841 aatccaggta aaaatggctt gtctaataagc tgcattttgt tagataaatg tccacctcca
 3901 agaccaccaa cttcaccata cccacccttg ccaaaggaca agttgaatcc acccacacct
 3961 agtatattact tggaaaataa acgtgatgct ttctttcctc cattacatca attttgtaca
 40 4021 aatccaaaaa accctgttac agtaatacgt ggccttgctg gagctcttaa attagatctt
 4081 ggacttttct ctacaaaaac tttggtagaa gctaacaatg aacatatggt agaagtgagg
 4141 acacagttgc tgcaaccagc agatgaaaac tgggatccca ctggaacaaa gaaaatctgg

4201 cgttgtgaaa gcaatagatc tcatactaca attgccaaat acgcacaata ccaggcttcc
 4261 tccttccagg aatcattgag agaagaaaat gagaaaaagaa cacaacacaa agatcattca
 4321 gataacgaat ccacatcttc agagaattct ggaaggagaa ggaaaggacc ttttaaaacc
 4381 ataaaaatttg ggaccaacat tgacctctct gataacaaaa agtggaagtt gcagttacat
 5 4441 gaactgacta aacttcctgc ttttgcgctg gtggtgtcag caggaaatct tctaaccat
 4501 gttgggcata ccattctggg catgaatata gtacaactgt atatgaaagt tccagggagt
 4561 cggacaccag gtcaccaaga aaataacaac ttctgctctg ttaacataaa tattggtcca
 4621 ggagattgtg aatggtttgt tgtacctgaa gattattggg gtgttctgaa tgacttctgt
 4681 gaaaaaaata atttgaattt tttaatgagt tcttggtggc ccaacctga agatctttat
 10 4741 gaagcaaagtg tccctgtgta tagatttatt cagcgacctg gagatttggg ctggataaat
 4801 gcaggcactg tgcattgggt tcaagctgtt ggctgggtgca ataacattgc ctggaatgtt
 4861 ggtccactta cagcctgcca gtataaattg gcagtggaaac ggtatgaatg gaacaaattg
 4921 aaaagtgtga agtcaccagt acccatgggt catctttcct ggaatatggc acgaaatatc
 4981 aaagtctcag atccaaagct ttttgaaatg attaatgatt gtcttttgaa aattctgaag
 15 5041 caatatcaga cattgagaga agctcttgtt gcagcaggaa aagaggttat atggcatggg
 5101 cggacaaatg atgaaccagc tcattactgt agcatttgtg aggtggagggt ttttaactctg
 5161 ctttttgtca ctaatgaaag caatactcaa aaaacctaca tagtacattg ccattgattgt
 5221 gcacgaaaaa caagcaaaag tttggaaaat tttgtggtgc tcgaacagta caaatggag
 5281 gacctaatcc aagtttatga tcaatttaca ctagctcttt cattatcatc ctcatcttga
 20 5341 tatagttcca tgaatattaa atgagattat ttctgctctt caggaaattt ctgcaccact
 5401 ggtttttagt ctgtttcata aaactgttga ctaaaagcta tgtctatgca acctccaag
 5461 aatagtatgt caagcaactg gacacagtgc tgcctctgct tcaggactta acatgctgat
 5521 ccagctgtac ttcagaaaaa taatattaat catatgtttt gtgtacgtat gacaaactgt
 5581 caaagtgaca cagaatactg atttgaagat agcctttttt atgtttctct atttctgggc
 25 5641 tgatgaatta atattcattt gtattttaac cctgcagaat tttccttagt taaaaacact
 5701 ttcctagctg gtcatttctt cataagatag caaatttaaa tctctcctcg atcagctttt
 5761 aaaaaatgtg tactattatc tgaggaagtt ttttactgct ttatgtttt gtgtgtttt
 5821 aggccatgat gattacattt gtggttccaa aataattttt ttaaataatta atagcccata
 5881 tacaagata atggattgca catagacaaa gaaataaact tcagatttgt gatttttgtt
 30 5941 tctaaacttg atacagattt acactattta taaatacgta tttattgcct gaaaatattt
 6001 gtgaatggaa tgttgtttt ttccagacgt aactgccatt aaatactaag gagttctgta
 6061 gtttttaaca ctactcctat tacattttat atgtgtagat aaaactgctt agtattatac
 6121 agaaattttt attaaaattg ttaaattgtt aaagggtttc ccaatgtttg agtttaaaaa
 6181 agactttctg aaaaaatcca ctttttgttc attttcaaac ctaatgatta tatgtattt
 35 6241 atatgttgtg gtatgtgtac acacatgtat aatatataca gaaacctcga tatataattg
 6301 tatagatttt aaaagtttta ttttttacat ctatggtagt ttttgagggt cctattataa
 6361 agtattacgg aagtttgctg tttttaagt aaatgtcttt tagtgtgatt tattaagttg
 6421 tagtcaccat agtgatagcc cataaataat tgctggaaaa ttgtatttta taacagtaga
 6481 aaacatatag tcagtgaagt aaatatttta aaggaaacat tatatagatt tgataaatgt
 40 6541 tgtttataat taagagtttc ttatggaaaa gagattcaga atgataacct cttttagaga
 6601 acaataaagt gacttatttt tttaaagcta gatgactttg aaatgctata ctgtcctgct
 6661 tgtacaacat ggtttgggt gaaggggagg aaagtattaa aaaatctata tcgctagtaa

```

6721 attgtaataa gttctattaa aacttggtatt tcatatgaaa aatttgctaa tttaatatta
6781 actcatttga taataatact tgtcttttct acctctc

```

By “tri-methylated histone H3 at lysine 27 (H3K27me3)” is meant the trimethylation of lysine 27 on histone H3 protein subunit. The H3K27me3 modification is generally associated with gene repression.

By “agent” is meant a peptide, nucleic acid molecule, or small compound.

By “allele” is meant one of two or more alternative forms of a gene that are found at the same place on a chromosome.

By "alteration" is meant a change (increase or decrease) in the expression levels or activity of a gene or polypeptide as detected by standard art known methods such as those described herein. As used herein, an alteration includes a 10% change in expression levels, preferably a 25% change, more preferably a 40% change, and most preferably a 50% or greater change in expression levels.

By “ameliorate” is meant decrease, suppress, attenuate, diminish, arrest, or stabilize the development or progression of a disease.

The term “blastula” as used herein refers to an early stage in the development of an embryo consisting of a hollow sphere of cells enclosing a fluid-filled cavity called the blastocoel. The term blastula sometimes is used interchangeably with blastocyst.

The term "blastomere" is used throughout to refer to at least one blastomere (e.g., 1, 2, 3, 4, etc.) obtained from a preimplantation embryo. The term "cluster of two or more blastomeres" is used interchangeably with "blastomere-derived outgrowths" to refer to the cells generated during the *in vitro* culture of a blastomere. For example, after a blastomere is obtained from a SCNT embryo and initially cultured, it generally divides at least once to produce a cluster of two or more blastomeres (also known as a blastomere-derived outgrowth). The cluster can be further cultured with embryonic or fetal cells. Ultimately, the blastomere-derived outgrowths will continue to divide. From these structures, ES cells, totipotent stem (TS) cells, and partially differentiated cell types will develop over the course of the culture method.

The term "cloned (or cloning)" as used herein refers to a gene manipulation technique for preparing a new individual unit to have a gene set identical to another individual unit. In the present invention, the term "cloned" as used herein refers to a cell, embryonic cell, fetal cell, and/or animal cell has a nuclear DNA sequence that is substantially similar or identical

to the nuclear DNA sequence of another cell, embryonic cell, fetal cell, differentiated cell, and/or animal cell. The terms "substantially similar" and "identical" are described herein. The cloned SCNT embryo can arise from one nuclear transfer, or alternatively, the cloned SCNT embryo can arise from a cloning process that includes at least one re-cloning step.

5 In this disclosure, "comprises," "comprising," "containing" and "having" and the like can have the meaning ascribed to them in U.S. Patent law and can mean "includes," "including," and the like; "consisting essentially of" or "consists essentially" likewise has the meaning ascribed in U.S. Patent law and the term is open-ended, allowing for the presence of more than that which is recited so long as basic or novel characteristics of that which is
10 recited is not changed by the presence of more than that which is recited, but excludes prior art embodiments.

"Detect" refers to identifying the presence, absence or amount of the analyte to be detected.

By "disease" is meant any condition or disorder that damages, or interferes with the
15 normal function or development of a cell, tissue, or organ. Examples of disorders include defects (e.g., developmental defects, loss of viability) associated with the disruption of X chromosome imprinting in an embryo.

By "DNA" is meant deoxyribonucleic acid. In various embodiments, the term DNA refers to genomic DNA, recombinant DNA, or cDNA. In particular embodiments, the DNA
20 comprises a "target region." DNA libraries contemplated herein include genomic DNA libraries, and cDNA libraries constructed from RNA, e.g., an RNA expression library. In various embodiments, the DNA libraries comprise one or more additional DNA sequences and/or tags.

By "effective amount" is meant the amount of an agent required to restore a cell to a
25 physiologically normal condition. In one embodiment, an effective amount of the agent is the amount required to correct an imprinting defect and to impart a physiologically normal imprinting phenotype to the cell. In one embodiment, the injection of *Kdm6b* mRNA is sufficient to correct an imprinting defect associated with abnormal and/or undesirable X chromosome inactivation. The effective amount of active compound(s) used to practice the
30 present invention for therapeutic treatment of a disease varies depending upon the manner of administration, the age, body weight, and general health of the subject. Ultimately, the

attending physician or veterinarian will decide the appropriate amount and dosage regimen. Such amount is referred to as an "effective" amount.

By "embryo" is meant

By "fragment" is meant a portion of a polypeptide or nucleic acid molecule. This
5 portion contains, preferably, at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90% of the entire length of the reference nucleic acid molecule or polypeptide. A fragment may contain 10, 20, 30, 40, 50, 60, 70, 80, 90, or 100, 200, 300, 400, 500, 600, 700, 800, 900, or 1000 nucleotides or amino acids.

The terms "isolated," "purified," or "biologically pure" refer to material that is free to
10 varying degrees from components which normally accompany it as found in its native state. "Isolate" denotes a degree of separation from original source or surroundings. "Purify" denotes a degree of separation that is higher than isolation. A "purified" or "biologically pure" protein is sufficiently free of other materials such that any impurities do not materially affect the biological properties of the protein or cause other adverse consequences. That is, a
15 nucleic acid or peptide of this invention is purified if it is substantially free of cellular material, viral material, or culture medium when produced by recombinant DNA techniques, or chemical precursors or other chemicals when chemically synthesized. Purity and homogeneity are typically determined using analytical chemistry techniques, for example, polyacrylamide gel electrophoresis or high performance liquid chromatography. The term
20 "purified" can denote that a nucleic acid or protein gives rise to essentially one band in an electrophoretic gel. For a protein that can be subjected to modifications, for example, phosphorylation or glycosylation, different modifications may give rise to different isolated proteins, which can be separately purified.

By "isolated polynucleotide" is meant a nucleic acid (e.g., a DNA) that is free of the
25 genes which, in the naturally-occurring genome of the organism from which the nucleic acid molecule of the invention is derived, flank the gene. The term therefore includes, for example, a recombinant DNA that is incorporated into a vector; into an autonomously replicating plasmid or virus; or into the genomic DNA of a prokaryote or eukaryote; or that exists as a separate molecule (for example, a cDNA or a genomic or cDNA fragment
30 produced by PCR or restriction endonuclease digestion) independent of other sequences. In addition, the term includes an RNA molecule that is transcribed from a DNA molecule, as

well as a recombinant DNA that is part of a hybrid gene encoding additional polypeptide sequence.

By an "isolated polypeptide" is meant a polypeptide of the invention that has been separated from components that naturally accompany it. Typically, the polypeptide is isolated when it is at least 60%, by weight, free from the proteins and naturally-occurring organic molecules with which it is naturally associated. Preferably, the preparation is at least 75%, more preferably at least 90%, and most preferably at least 99%, by weight, a polypeptide of the invention. An isolated polypeptide of the invention may be obtained, for example, by extraction from a natural source, by expression of a recombinant nucleic acid encoding such a polypeptide; or by chemically synthesizing the protein. Purity can be measured by any appropriate method, for example, column chromatography, polyacrylamide gel electrophoresis, or by HPLC analysis.

Ranges provided herein are understood to be shorthand for all of the values within the range. For example, a range of 1 to 50 is understood to include any number, combination of numbers, or sub-range from the group consisting 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, or 50.

By "reduces" is meant a negative alteration of at least 10%, 25%, 50%, 75%, or 100%.

By "reference" is meant a standard or control condition.

A "reference sequence" is a defined sequence used as a basis for sequence comparison. A reference sequence may be a subset of or the entirety of a specified sequence; for example, a segment of a full-length cDNA or gene sequence, or the complete cDNA or gene sequence. For polypeptides, the length of the reference polypeptide sequence will generally be at least about 16 amino acids, preferably at least about 20 amino acids, more preferably at least about 25 amino acids, and even more preferably about 35 amino acids, about 50 amino acids, or about 100 amino acids. For nucleic acids, the length of the reference nucleic acid sequence will generally be at least about 50 nucleotides, preferably at least about 60 nucleotides, more preferably at least about 75 nucleotides, and even more preferably about 100 nucleotides or about 300 nucleotides or any integer thereabout or therebetween.

Nucleic acid molecules useful in the methods of the invention include any nucleic acid molecule that encodes a polypeptide of the invention or a fragment thereof. Such nucleic acid molecules need not be 100% identical with an endogenous nucleic acid sequence, but will typically exhibit substantial identity. Polynucleotides having “substantial identity” to an endogenous sequence are typically capable of hybridizing with at least one strand of a double-stranded nucleic acid molecule. Nucleic acid molecules useful in the methods of the invention include any nucleic acid molecule that encodes a polypeptide of the invention or a fragment thereof. Such nucleic acid molecules need not be 100% identical with an endogenous nucleic acid sequence, but will typically exhibit substantial identity. Polynucleotides having “substantial identity” to an endogenous sequence are typically capable of hybridizing with at least one strand of a double-stranded nucleic acid molecule. By “hybridize” is meant pair to form a double-stranded molecule between complementary polynucleotide sequences (e.g., a gene described herein), or portions thereof, under various conditions of stringency. (See, e.g., Wahl, G. M. and S. L. Berger (1987) *Methods Enzymol.* 152:399; Kimmel, A. R. (1987) *Methods Enzymol.* 152:507).

For example, stringent salt concentration will ordinarily be less than about 750 mM NaCl and 75 mM trisodium citrate, preferably less than about 500 mM NaCl and 50 mM trisodium citrate, and more preferably less than about 250 mM NaCl and 25 mM trisodium citrate. Low stringency hybridization can be obtained in the absence of organic solvent, e.g., formamide, while high stringency hybridization can be obtained in the presence of at least about 35% formamide, and more preferably at least about 50% formamide. Stringent temperature conditions will ordinarily include temperatures of at least about 30° C, more preferably of at least about 37° C, and most preferably of at least about 42° C. Varying additional parameters, such as hybridization time, the concentration of detergent, e.g., sodium dodecyl sulfate (SDS), and the inclusion or exclusion of carrier DNA, are well known to those skilled in the art. Various levels of stringency are accomplished by combining these various conditions as needed. In a preferred embodiment, hybridization will occur at 30° C in 750 mM NaCl, 75 mM trisodium citrate, and 1% SDS. In a more preferred embodiment, hybridization will occur at 37° C in 500 mM NaCl, 50 mM trisodium citrate, 1% SDS, 35% formamide, and 100 .mu.g/ml denatured salmon sperm DNA (ssDNA). In a most preferred embodiment, hybridization will occur at 42° C in 250 mM NaCl, 25 mM trisodium citrate,

1% SDS, 50% formamide, and 200 µg/ml ssDNA. Useful variations on these conditions will be readily apparent to those skilled in the art.

For most applications, washing steps that follow hybridization will also vary in stringency. Wash stringency conditions can be defined by salt concentration and by temperature. As above, wash stringency can be increased by decreasing salt concentration or by increasing temperature. For example, stringent salt concentration for the wash steps will preferably be less than about 30 mM NaCl and 3 mM trisodium citrate, and most preferably less than about 15 mM NaCl and 1.5 mM trisodium citrate. Stringent temperature conditions for the wash steps will ordinarily include a temperature of at least about 25° C, more preferably of at least about 42° C, and even more preferably of at least about 68° C. In a preferred embodiment, wash steps will occur at 25° C in 30 mM NaCl, 3 mM trisodium citrate, and 0.1% SDS. In a more preferred embodiment, wash steps will occur at 42° C in 15 mM NaCl, 1.5 mM trisodium citrate, and 0.1% SDS. In a more preferred embodiment, wash steps will occur at 68° C in 15 mM NaCl, 1.5 mM trisodium citrate, and 0.1% SDS.

Additional variations on these conditions will be readily apparent to those skilled in the art. Hybridization techniques are well known to those skilled in the art and are described, for example, in Benton and Davis (*Science* 196:180, 1977); Grunstein and Hogness (*Proc. Natl. Acad. Sci., USA* 72:3961, 1975); Ausubel et al. (*Current Protocols in Molecular Biology*, Wiley Interscience, New York, 2001); Berger and Kimmel (*Guide to Molecular Cloning Techniques*, 1987, Academic Press, New York); and Sambrook et al., *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory Press, New York.

By “Somatic Cell Nuclear Transfer” or “SCNT” is meant the transfer of a donor nucleus from a somatic cell into an enucleated oocyte. The process can be used in either reproductive or therapeutic cloning and may be accomplished by fusion of the somatic cell with the enucleated oocyte, injection of the nucleus into the enucleated oocyte, or by any other method.

The nucleus of the somatic cell provides the genetic information, while the oocyte provides the nutrients and other energy-producing materials that are necessary for development of an embryo. Once fusion has occurred, the cell is totipotent, and eventually develops into a blastocyst, at which point the inner cell mass is isolated.

The term “nuclear transfer” as used herein refers to a gene manipulation technique allowing an identical characteristics and qualities acquired by artificially combining an

enucleated oocytes with a cell nuclear genetic material or a nucleus of a somatic cell. In some embodiments, the nuclear transfer procedure is where a nucleus or nuclear genetic material from a donor somatic cell is transferred into an enucleated egg or oocyte (an egg or oocyte from which the nucleus/pronuclei have been removed). The donor nucleus can come from a somatic cell.

The term “nuclear genetic material” refers to structures and/or molecules found in the nucleus which comprise polynucleotides (e.g., DNA) which encode information about the individual. Nuclear genetic material includes the chromosomes and chromatin. The term also refers to nuclear genetic material (e.g., chromosomes) produced by cell division such as the division of a parental cell into daughter cells. Nuclear genetic material does not include mitochondrial DNA.

The term “SCNT embryo” refers to a cell, or the totipotent progeny thereof, of an enucleated oocyte which has been fused with the nucleus or nuclear genetic material of a somatic cell. The SCNT embryo can develop into a blastocyst and develop post-implantation into living offspring. The SCNT embryo can be a 1-cell embryo, 2-cell embryo, 4-cell embryo, or any stage embryo prior to becoming a blastocyst.

The term “donor human cell” or “donor human somatic cell” refers to a somatic cell or a nucleus of human cell which is transferred into a recipient oocyte as a nuclear acceptor or recipient.

The term "somatic cell" refers to a plant or animal cell which is not a reproductive cell or reproductive cell precursor. In some embodiments, a differentiated cell is not a germ cell. A somatic cell does not relate to pluripotent or totipotent cells. In some embodiments the somatic cell is a "non-embryonic somatic cell", by which is meant a somatic cell that is not present in or obtained from an embryo and does not result from proliferation of such a cell *in vitro*. In some embodiments the somatic cell is an "adult somatic cell", by which is meant a cell that is present in or obtained from an organism other than an embryo or a fetus or results from proliferation of such a cell *in vitro*.

The term "oocyte" as used herein refers to a mature oocyte which has reached metaphase II of meiosis. An oocyte is also used to describe a female gamete or germ cell involved in reproduction, and is commonly also called an egg. A mature egg has a single set of maternal chromosomes (23, X in a human primate) and is halted at metaphase II.

A “hybrid oocyte” refers to an enucleated oocyte that has the cytoplasm from a first human oocyte (termed a “recipient”) but does not have the nuclear genetic material of the recipient oocyte; it has the nuclear genetic material from another human cell, termed a “donor.” In some embodiments, the hybrid oocyte can also comprise mitochondrial DNA (mtDNA) that is not from the recipient oocyte, but is from a donor cell (which can be the same donor cell as the nuclear genetic material, or from a different donor, e.g., from a donor oocyte).

The term “enucleated oocyte” as used herein refers to an human oocyte which its nucleus has been removed.

The term “enucleation” as used herein refers to a process whereby the nuclear material of a cell is removed, leaving only the cytoplasm. When applied to an egg, enucleation refers to the removal of the maternal chromosomes, which are not surrounded by a nuclear membrane. The term “enucleated oocyte” refers to an oocyte where the nuclear material or nuclei is removed.

The “recipient human oocyte” as used herein refers to a human oocyte that receives a nucleus from a human nuclear donor cell after removing its original nucleus.

The term “fusion” as used herein refers to a combination of a nuclear donor cell and a lipid membrane of a recipient oocyte. For example, the lipid membrane may be the plasma membrane or nuclear membrane of a cell. Fusion may occur upon application of an electrical stimulus between a nuclear donor cell and a recipient oocyte when they are placed adjacent to each other or when a nuclear donor cell is placed in a perivitelline space of a recipient oocyte.

The term “living offspring” as used herein means an animal that can survive *ex utero*. Preferably, it is an animal that can survive for one second, one minute, one day, one week, one month, six months or more than one year. The animal may not require an in utero environment for survival.

The term “prenatal” refers to existing or occurring before birth. Similarly, the term “postnatal” is existing or occurring after birth.

The term “transgenic organism” as used herein refers to an organism into which genetic material from another organism has been experimentally transferred, so that the host acquires the genetic traits of the transferred genes in its chromosomal composition.

The term “implanting” as used herein in reference to SCNT embryos as disclosed herein refers to impregnating a surrogate female animal with a SCNT embryo described

herein. This technique is well known to a person of ordinary skill in the art. See, e.g., Seidel and Elsdon, 1997, Embryo Transfer in Dairy Cattle, W. D. Hoard & Sons, Co., Hoards Dairyman. The embryo may be allowed to develop in utero, or alternatively, the fetus may be removed from the uterine environment before parturition.

5 By "subject" is meant a mammal, including, but not limited to, a human or non-human mammal, such as an agriculturally significant mammal (e.g., bovine, equine, ovine, porcine), a pet (e.g., canine, feline), or a rare or endangered mammal (e.g., panda).

By "substantially identical" is meant a polypeptide or nucleic acid molecule exhibiting at least 50% identity to a reference amino acid sequence (for example, any one of
10 the amino acid sequences described herein) or nucleic acid sequence (for example, any one of the nucleic acid sequences described herein). Preferably, such a sequence is at least 60%, more preferably 80% or 85%, and more preferably 90%, 95% or even 99% identical at the amino acid level or nucleic acid to the sequence used for comparison.

Sequence identity is typically measured using sequence analysis software (for
15 example, Sequence Analysis Software Package of the Genetics Computer Group, University of Wisconsin Biotechnology Center, 1710 University Avenue, Madison, Wis. 53705, BLAST, BESTFIT, GAP, or PILEUP/PRETTYBOX programs). Such software matches identical or similar sequences by assigning degrees of homology to various substitutions, deletions, and/or other modifications. Conservative substitutions typically include
20 substitutions within the following groups: glycine, alanine; valine, isoleucine, leucine; aspartic acid, glutamic acid, asparagine, glutamine; serine, threonine; lysine, arginine; and phenylalanine, tyrosine. In an exemplary approach to determining the degree of identity, a BLAST program may be used, with a probability score between e^{-3} and e^{-100} indicating a closely related sequence.

25 By "Somatic Cell Nuclear Transfer" or "SCNT" is meant the transfer of a donor nucleus from a somatic cell into an enucleated oocyte. The process can be used in either reproductive or therapeutic cloning and may be accomplished by fusion of the somatic cell with the enucleated oocyte, injection of the nucleus into the enucleated oocyte, or by any other method.

30 The nucleus of the somatic cell provides the genetic information, while the oocyte provides the nutrients and other energy-producing materials that are necessary for

development of an embryo. Once fusion has occurred, the cell is totipotent, and eventually develops into a blastocyst, at which point the inner cell mass is isolated.

The term “nuclear transfer” as used herein refers to a gene manipulation technique allowing an identical characteristics and qualities acquired by artificially combining an enucleated oocytes with a cell nuclear genetic material or a nucleus of a somatic cell. In some embodiments, the nuclear transfer procedure is where a nucleus or nuclear genetic material from a donor somatic cell is transferred into an enucleated egg or oocyte (an egg or oocyte from which the nucleus/pronuclei have been removed). The donor nucleus can come from a somatic cell.

The term “nuclear genetic material” refers to structures and/or molecules found in the nucleus which comprise polynucleotides (e.g., DNA) which encode information about the individual. Nuclear genetic material includes the chromosomes and chromatin. The term also refers to nuclear genetic material (e.g., chromosomes) produced by cell division such as the division of a parental cell into daughter cells. Nuclear genetic material does not include mitochondrial DNA.

The term “SCNT embryo” refers to a cell, or the totipotent progeny thereof, of an enucleated oocyte, which has been fused with the nucleus or nuclear genetic material of a somatic cell. The SCNT embryo can develop into a blastocyst and develop post-implantation into living offspring. The SCNT embryo can be a 1-cell embryo, 2-cell embryo, 4-cell embryo, or any stage embryo prior to becoming a blastocyst.

The term “donor human cell” or “donor human somatic cell” refers to a somatic cell or a nucleus of human cell that is transferred into a recipient oocyte as a nuclear acceptor or recipient.

The term "somatic cell" refers to an animal cell, which is not a reproductive cell or reproductive cell precursor. In some embodiments, a differentiated cell is not a germ cell. A somatic cell does not relate to pluripotent or totipotent cells. In some embodiments the somatic cell is a "non-embryonic somatic cell", by which is meant a somatic cell that is not present in or obtained from an embryo and does not result from proliferation of such a cell *in vitro*. In some embodiments the somatic cell is an "adult somatic cell", by which is meant a cell that is present in or obtained from an organism other than an embryo or a fetus or results from proliferation of such a cell *in vitro*.

The term "oocyte" as used herein refers to a mature oocyte which has reached metaphase II of meiosis. An oocyte is also used to describe a female gamete or germ cell involved in reproduction, and is commonly also called an egg. A mature egg has a single set of maternal chromosomes (23, X in a human primate) and is halted at metaphase II.

5 A "hybrid oocyte" refers to an enucleated oocyte that has the cytoplasm from a first human oocyte (termed a "recipient") but does not have the nuclear genetic material of the recipient oocyte; it has the nuclear genetic material from another human cell, termed a "donor." In some embodiments, the hybrid oocyte can also comprise mitochondrial DNA (mtDNA) that is not from the recipient oocyte, but is from a donor cell (which can be the
10 same donor cell as the nuclear genetic material, or from a different donor, e.g., from a donor oocyte).

The term "enucleated oocyte" as used herein refers to an human oocyte whose nucleus has been removed.

The term "enucleation" as used herein refers to a process whereby the nuclear
15 material of a cell is removed, leaving only the cytoplasm. When applied to an egg, enucleation refers to the removal of the maternal chromosomes, which are not surrounded by a nuclear membrane.

The "recipient human oocyte" as used herein refers to a human oocyte that receives a nucleus from a human nuclear donor cell after removing its original nucleus.

20 The term "fusion" as used herein refers to a combination of a nuclear donor cell and a lipid membrane of a recipient oocyte. For example, the lipid membrane may be the plasma membrane or nuclear membrane of a cell. Fusion may occur upon application of an electrical stimulus between a nuclear donor cell and a recipient oocyte when they are placed adjacent to each other or when a nuclear donor cell is placed in a perivitelline space of a recipient oocyte.

25 The term "living offspring" as used herein means an animal that can survive *ex utero*. Preferably, it is an animal that can survive for one or more hours, one day, one week, one month, six months or more than one year. The animal may not require an in utero environment for survival.

The term "prenatal" refers to existing or occurring before birth. Similarly, the term
30 "postnatal" is existing or occurring after birth.

The term "blastocyst" as used herein refers to a preimplantation embryo in placental mammals (about 3 days after fertilization in the mouse, about 5 days after fertilization in

humans) of about 30-150 cells. The blastocyst stage follows the morula stage, and can be distinguished by its unique morphology. The blastocyst consists of a sphere made up of a layer of cells (the trophectoderm), a fluid-filled cavity (the blastocoel or blastocyst cavity), and a cluster of cells on the interior (the inner cell mass, or ICM). The ICM, consisting of undifferentiated cells, gives rise to what will become the fetus if the blastocyst is implanted in a uterus. These same ICM cells, if grown in culture, can give rise to embryonic stem cell lines. At the time of implantation the mouse blastocyst is made up of about 70 trophoblast cells and 30 ICM cells.

The term “transgenic organism” as used herein refers to an organism into which genetic material from another organism has been experimentally transferred, so that the host acquires the genetic traits of the transferred genes in its chromosomal composition.

The term “implanting” as used herein in reference to SCNT embryos as disclosed herein refers to impregnating a surrogate female animal with a SCNT embryo described herein. This technique is well known to a person of ordinary skill in the art. See, e.g., Seidel and Elsden, 1997, Embryo Transfer in Dairy Cattle, W. D. Hoard & Sons, Co., Hoards Dairyman. The embryo may be allowed to develop in utero, or alternatively, the fetus may be removed from the uterine environment before parturition.

By “subject” is meant a mammal, including, but not limited to, a human or non-human mammal, such as an agriculturally significant mammal (e.g., bovine, equine, ovine, porcine), a pet (e.g., canine, feline), or a rare or endangered mammal (e.g., panda).

As used herein, the terms “treat,” “treating,” “treatment,” and the like refer to reducing or ameliorating a disorder and/or symptoms associated therewith. It will be appreciated that, although not precluded, treating a disorder or condition does not require that the disorder, condition or symptoms associated therewith be completely eliminated. In one embodiment, the term “treating” refers to the treatment of imprinting defects in SCNT embryos.

Unless specifically stated or obvious from context, as used herein, the term “or” is understood to be inclusive. Unless specifically stated or obvious from context, as used herein, the terms “a”, “an”, and “the” are understood to be singular or plural.

Unless specifically stated or obvious from context, as used herein, the term “or” is understood to be inclusive. Unless specifically stated or obvious from context, as used herein, the terms “a”, “an”, and “the” are understood to be singular or plural (*i.e.*, at least one). By way of example, “an element” means one element or more than one element.

Unless specifically stated or obvious from context, as used herein, the term “about” is understood as within a range of normal tolerance in the art, for example within 2 standard deviations of the mean. About can be understood as within 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, 1%, 0.5%, 0.1%, 0.05%, or 0.01% of the stated value. Unless otherwise clear from context, all numerical values provided herein are modified by the term about.

The recitation of a listing of chemical groups in any definition of a variable herein includes definitions of that variable as any single group or combination of listed groups. The recitation of an embodiment for a variable or aspect herein includes that embodiment as any single embodiment or in combination with any other embodiments or portions thereof.

Any compositions or methods provided herein can be combined with one or more of any of the other compositions and methods provided herein.

BRIEF DESCRIPTION OF THE DRAWINGS

FIGS. 1A-1D show that ectopic removal of H3K9me3 did not induce maternal *Xist* expression. FIG. 1A depicts representative images of zygotes stained with anti-H3K9me3 antibody. (M) Maternal pronucleus; (P) paternal pro- nucleus. The bar graph shows relative H3K9me3 signal intensity of the maternal pronuclei. The average signal intensity of noninjected zygotes was set as 1.0. The total numbers of embryos examined were eight (no injection), nine (1000 ng/μL *Kdm4b*), and nine (2600 ng/μL *Kdm4b*). Error bars indicate standard error (SE). (***) $P < 0.001$; (*) $P < 0.05$, two-tailed Student's *t*-test. FIG. 1B depicts representative images of *Xist* RNA FISH (magenta) in *Kdm4b*-injected four-cell embryos. The gender of each embryo was assessed by simultaneous DNA FISH against the *Rnf12* locus (gray). Arrows indicate the blastomeres, which are shown enlarged in the *bottom* panels. FIG. 1C depicts graphs showing the ratio of blastomeres with the indicated number of *Xist* RNA clouds and spots in male embryos. Each bar represents an individual embryo. The numbers of male embryos examined were six (no injection), five (1000 ng/μL *Kdm4b*), and 12 (2600 ng/μL *Kdm4b*). FIG. 1D depicts graphs showing the ratio of blastomeres with the indicated number of *Xist* RNA clouds and spots in female embryos. Each bar represents an individual embryo. The numbers of female embryos examined were six (no injection), six (1000 ng/μL *Kdm4b*), and eight (2600 ng/μL *Kdm4b*).

FIGS. 2A-2C show that maternal H3K27me3 coated *Xist* in oocytes and preimplantation embryos. FIG. 2A depicts a genome browser view of H3K27me3 ChIP-seq

(Zheng et al. 2016. Mol Cell 63: 1066–1079), DNase I-seq (Inoue et al. 2017. Nature 547: 419–424), and DNA methylation levels (Kobayashi et al. 2012. PLoS Genet 8: e1002440) at the *Xist* locus. (Oo) MII oocyte; (Sp) sperm; (7d) growing oocytes collected from 7-day-old females; (14d) growing oocytes collected from 14-day-old females; (GV) fully grown GV stage oocytes. FIG. 2B depicts a genome browser view of allelic H3K27me3 in one-cell, two-cell, and blastocyst embryos and E6.5 epiblast at the *Xist* locus. The highlighted square indicates a computationally determined region where the maternal allele bias of H3K27me3 enrichment is retained in blastocyst embryos. (Mat) Maternal allele; (Pat) paternal allele. The H3K27me3 ChIP-seq data sets were described in Zheng et al. (2016; Mol Cell 63: 1066–1079). FIG. 2C depicts a higher-resolution view of the *Xist* locus in blastocyst embryos. The maternal allele-biased H3K27me3 domain is shaded.

FIGS. 3A-3E show that loss of H3K27me3 induced maternal *Xist* expression. FIG. 3A depicts representative images of zygotes stained with anti-H3K27me3 antibody. (M) Maternal pronucleus, (P) paternal pronucleus. The bar graph indicates the relative H3K27me3 signal intensity of maternal pronuclei. The average signal of *Kdm6b*^{MUT}-injected zygotes was set as 1.0. The total numbers of embryos examined were 15 (*Kdm6b*^{MUT}) and 13 (*Kdm6b*^{WT}). Error bars indicate standard error (SE). (***) $P < 0.001$, two-tailed Student's *t*-test. FIG. 3B is a genome browser view of the *Xist* locus showing loss of maternal H3K27me3 ChIP-seq signal in *Kdm6b*^{WT}-injected morula embryos. (Mat) Maternal allele; (Pat) paternal allele. FIG. 3C depicts representative images of *Xist* RNA FISH (light gray) in *Kdm6b*-injected four-cell embryos. The gender of each embryo was assessed by simultaneous DNA FISH against the *Rnf12* locus (gray). FIG. 3D depicts graphs showing the ratio of blastomeres with the indicated number of *Xist* RNA clouds and spots in male four-cell embryos. Each bar represents an individual embryo. The numbers of male embryos examined were eight (*Kdm6b*^{MUT}) and 15 (*Kdm6b*^{WT}). FIG. 3E depicts graphs showing the ratio of blastomeres with the indicated number of *Xist* RNA clouds and spots in female four-cell embryos. Each bar represents an individual embryo. The numbers of female embryos examined were eight (*Kdm6b*^{MUT}) and 12 (*Kdm6b*^{WT}).

FIGS. 4A-4E show that loss of H3K27me3 induced maternal X-chromosome inactivation (XCI). FIG. 4A is an illustration depicting maternal XCI caused by *Kdm6b*^{WT}-mediated maternal *Xist* expression. FIG. 4B depicts representative images of *Xist* RNA FISH (light gray) in *Kdm6b*-injected morula embryos. The gender of each embryo was assessed by

simultaneous DNA FISH against the *Rnf12* locus (gray). FIG. 4C depicts graphs showing the ratio of blastomeres with the indicated number of *Xist* RNA clouds in male morula embryos. Each bar represents an individual embryo. The numbers of male embryos examined were 19 (*Kdm6b^{MUT}*) and 35 (*Kdm6b^{WT}*). FIG. 4D depicts graphs showing the ratio of blastomeres with the indicated number of *Xist* RNA clouds in female morula embryos. Each bar represents an individual embryo. The numbers of female embryos examined were 34 (*Kdm6b^{MUT}*) and 35 (*Kdm6b^{WT}*). FIG. 4E depicts a box plot showing the relative expression of genes on individual maternal chromosomes between *Kdm6b^{MUT}*- and *Kdm6b^{WT}*-injected blastocysts. Genes with enough SNP reads (SNP reads > 10, RPM [reads per million] > 0.5) were analyzed. The middle lines in the boxes represent the medians. Box edges and whiskers indicate the 25th/75th and 2.5th/97.5th percentiles, respectively. (***) $P < 0.001$, Mann-Whitney-Wilcoxon test.

FIGS. 5A and 5B show that ectopic *Kdm6b^{WT}* mRNA injection resulted in reduction of H3K27me3 in preimplantation embryos. These results are related to those shown in FIGS. 3A-3E. FIG. 5A depicts representative images of *Kdm6b*-injected 4-cell and morula embryos immunostained with H3K27me3 antibody. *Kdm6b* mRNA was injected into zygotes. The embryos were fixed at 46 (4-cell) and 78 (morula) hrs after fertilization. FIG. 5B is a graph depicting relative H3K27me3 signal intensity. The signal intensities of multiple blastomeres were measured and averaged to obtain the value of a single embryo. The average signals of *Kdm6b^{MUT}* embryos were set as 1.0. The total numbers of embryos examined were 9 (*Kdm6b^{MUT}*) and 9 (*Kdm6b^{WT}*) for 4-cell and 19 (*Kdm6b^{MUT}*) and 22 (*Kdm6b^{WT}*) for morulae. Error bars indicate standard error (SE). ***, $p < 0.001$, * $p < 0.05$ (two-tailed Student t-test).

FIGS. 6A-6F show validation of H3K27me3 ULI-NChIP in embryonic stem cells (ESCs) and *Kdm6b*-injected morula embryos. These results are related to those shown in FIGS. 3A-3E. FIG. 6A is a scatterplot showing a correlation between H3K27me3 peaks detected in mouse ESCs in the ENCODE project and in our hands using 500 or 2,000 ESCs. FIG. 6B depicts a scatterplot showing a correlation between H3K27me3 peaks of a published dataset (Liu et al. 2016. Nature 537: 558–562) and *Kdm6b^{MUT}*-injected morula embryos. FIG. 6C depicts a Venn diagram showing a correlation between H3K27me3 peaks of a published dataset (Liu et al. 2016. Nature 537: 558–562) and *Kdm6b^{MUT}*-injected morula embryos. FIG. 6D depicts genome browser views of representative loci showing almost identical H3K27me3 enrichment in the public dataset and *Kdm6b^{MUT}*-injected morula

embryos. FIG. 6E is a graph depicting the number of H3K27me3 peaks detected in *Kdm6b^{MUT}*- and *Kdm6b^{WT}*-injected morula embryos. FIG. 6F depicts a genome browser view of the *Xist* locus showing loss of H3K27me3 domain in *Kdm6b^{WT}*-injected embryos. The parental alleles were not distinguished in these tracks.

FIG. 7 is a graph depicting RT-qPCR analysis of *Rnf12* in *Kdm6b*-injected embryos. The data were normalized to *18S*, and then the values of *Kdm6b^{MUT}* embryos were set as 1.0. Error bars indicate standard error (SE) of three biological replicates. Each experiment used a pool of 18-24 embryos per group. Note that *Rnf12* is downregulated rather than upregulated in *Kdm6b^{WT}*-injected embryos. Without being bound by theory, this is likely due to maternal XCI occurring as early as the 4-cell stage in *Kdm6b^{WT}*-injected embryos, given that *Rnf12* is a non-escapee X-linked gene (Borensztein et al. 2017. Nat Struct Mol Biol 24: 226–233).

FIGS. 8A-8D show that maternal X chromosome inactivation in *Kdm6b^{WT}*-injected blastocyst embryos. These results are related to those shown in FIGS. 4A-4E. FIG. 8A is a scatter plot showing the correlation between biological duplicate of RNA-seq samples. FIG. 8B is a box plot showing the maternal allelic expression ratios [Mat/(Mat+Pat)] of individual chromosomes in *Kdm6b*-injected blastocysts. Middle lines in the boxes represent the medians. Box edges and whiskers indicate the 25th/75th and 2.5th/ 97.5th percentiles, respectively. *Kdm6b^{MUT}*: light gray, left bar; *Kdm6b^{WT}*: dark gray, right bar; p-value < 2.2e-16. FIG. 8C is a graph showing the relative expression levels of X-linked genes between *Kdm6b^{WT}*- and *Kdm6b^{MUT}*- injected blastocyst embryos. The expression levels of the maternal allele were analyzed. Each dot represents an individual gene showing enough SNP reads (RPM>0.5). FIG. 8C shows genes excluding known escapees, depicted in FIG. 8D. FIG. 8D is a graph showing the relative expression levels of X-linked genes between *Kdm6b^{WT}*- and *Kdm6b^{MUT}*- injected blastocyst embryos. The expression levels of the maternal allele were analyzed. Each dot represents an individual gene showing enough SNP reads (RPM>0.5). FIG. 8D shows genes that are known escapees. From left to right: *Rbm3*, *Suv39h1*, *Tbc1d25*, *Atp6ap2*, *Araf*, *Ndufb11*, *Nkap*, *Lamp2*, *Utp14a*, *Idh3g*, *Eif2s3x*, *Xist*, *Kdm5c*, *Sms*, *Pdha1*, *Syap1*, and *Msl3*.

DETAILED DESCRIPTION OF THE INVENTION

The present invention features compositions and methods for recapitulating physiological X chromosome inactivation in a cell, including a cell of any embryo generated by Somatic Cell Nuclear Transfer (SCNT).

5 *Kdm6b* is an H3K27me3-specific demethylase. The present invention is based at least in part on the discovery that *Kdm6b*-mediated maternal X-chromosome inactivation recapitulates physiological-chromosome inactivation.

X-chromosome inactivation

10 During development, X-chromosome inactivation (XCI) occurs in an imprinted or a random manner. For imprinted XCI, the paternal X chromosome (Xp) is selectively inactivated during preimplantation development. Random XCI occurs in epiblasts where it results in the silencing of either the Xp or maternal X chromosome (Xm). *Xist* is an X-linked long noncoding RNA that functions in both imprinted and random XCI. *Xist* is imprinted in
15 the Xm during oogenesis. *Xist* RNA participates in XCI by coating and inactivating the X chromosome in *cis*.

H3K27me3-specific demethylase

In one aspect, the invention provides a method of increasing the efficiency of human
20 SCNT comprising: contacting the nuclei or cytoplasm of a donor human somatic cell, a recipient human oocyte, a hybrid oocyte (e.g., human enucleated oocyte comprising donor genetic material prior to fusion or activation) or a human SCNT embryo (i.e., after fusion of the donor nuclei with the enucleated oocyte) with an H3K27me3-specific demethylase (Kdm6a, Kdm6b, Kdm6c, etc.) capable of recapitulating physiological X-chromosome
25 inactivation. As discussed herein, the inventors have discovered that H3K27me3-specific demethylase can be used to normalize maternal X chromosome inactivation relative to paternal X chromosome inactivation thereby increasing the efficiency of human SCNT.

H3K27me3-specific demethylase activators

30 In one aspect, the invention provides a method of increasing the efficiency of human SCNT comprising: contacting the nuclei or cytoplasm of donor human somatic cell, a recipient human oocyte, a hybrid oocyte (e.g., human enucleated oocyte comprising donor

genetic material prior to fusion or activation) or a human SCNT embryo (i.e., after fusion of the donor nuclei with the enucleated oocyte) with an agent that activates an H3K27me3-specific demethylase (Kdm6a, Kdm6b, Kdm6c, etc.).

In some embodiments, a H3K27me3-specific demethylase activator useful in the methods, compositions and kits as disclosed herein is an agent which increases the expression of genes encoding an H3K27me3-specific demethylase, or increases the activity of human H3K27me3-specific demethylase, for example, human Kdm6a, Kdm6b, Kdm6c, etc.

In some embodiments, a H3K27me3-specific demethylase activator useful in the methods, compositions and kits as disclosed herein is a nucleic acid agent which encodes a Kdm6a, Kdm6b, or Kdm6c polypeptide.

In some embodiment, the agent comprises a nucleic acid sequence encoding a human H3K27me3-specific demethylase activator, including but not limited to Kdm6a, Kdm6b, or Kdm6c, or a biologically active fragment or homologue or variant thereof having at least 80% sequence identity (or at least about 85%, or at least about 90%, or at least about 95%, or at least about 98%, or at least about 99% sequence identity) thereto which increases the efficiency of human SCNT to a similar or greater extent as compared to the corresponding wild-type sequence encoding human Kdm6a, Kdm6b, or Kdm6c,.

In some embodiments, a H3K27me3-specific demethylase activator for use in the methods as disclosed herein is selected from any human Kdm6a, Kdm6b, or Kdm6c polypeptide, or a variant or biological active fragment of such a human Kdm6a, Kdm6b, or Kdm6c polypeptide. It is encompassed in the present invention that one of ordinary skill in the art can identify an appropriate human homologue of human Kdm6a, Kdm6b, or Kdm6c polypeptides, and the nucleic acid encoding such a human homologue for use in the methods and composition as disclosed herein. In some embodiments, a H3K27me3-specific demethylase activator is a nucleic acid agent encoding a H3K27me3-specific demethylase polypeptide, which is expressed from a vector, e.g., a viral vector.

In alternative embodiments, a H3K27me3-specific demethylase activator encompassed for use herein is a synthetic modified RNA (modRNA) encoding Kdm6a, Kdm6b, or Kdm6c, or functional fragments thereof. Synthetic modified RNA (modRNA) are described in U.S. applications US2012/03228640; US2009/0286852 and US2013/0111615 and US Patents 8,278,036; 8,691,966; 8,748,089; 8,835,108, which are incorporated herein in their entirety by reference. In some embodiments, the synthetic, modified RNA molecule is

not expressed in a vector, and the synthetic, modified RNA molecule can be a naked synthetic, modified RNA molecule. In some embodiments, a composition can comprise at least one synthetic, modified RNA molecule present in a lipid complex.

In some embodiments, the synthetic, modified RNA molecule comprises at least two modified nucleosides, for example, at least two modified nucleosides are selected from the group consisting of 5-methylcytidine (5mC), N6-methyladenosine (m6A), 3,2'-O-dimethyluridine (m4U), 2-thiouridine (s2U), 2' fluorouridine, pseudouridine, 2'-O-methyluridine (Um), 2' deoxy uridine (2' dU), 4-thiouridine (s4U), 5-methyluridine (m5U), 2'-O-methyladenosine (m6A), N6,2'-O-dimethyladenosine (m6Am), N6,N6,2'-O-trimethyladenosine (m62Am), 2'-O-methylcytidine (Cm), 7-methylguanosine (m7G), 2'-O-methylguanosine (Gm), N2,7-dimethylguanosine (m2,7G), N2, N2, 7-trimethylguanosine (m2,2,7G), and inosine (I). In some embodiments, the synthetic, modified RNA molecule further comprises a 5' cap, such as a 5' cap analog, e.g., a 5' diguanosine cap. In some embodiments, a synthetic, modified RNA molecule for use in the methods and compositions as disclosed herein does not comprise a 5' triphosphate. In some embodiments, a synthetic, modified RNA molecule for use in the methods and compositions as disclosed herein further comprises a poly(A) tail, a Kozak sequence, a 3' untranslated region, a 5' untranslated region, or any combination thereof, and in some embodiments, the a synthetic, modified RNA molecule can optionally be treated with an alkaline phosphatase.

In introducing polynucleotides described herein, essentially any method for introducing a nucleic acid construct into cells can be employed. Physical methods of introducing nucleic acids include injection of a solution containing the construct, bombardment by particles covered by the construct, soaking a cell, tissue sample or organism in a solution of the nucleic acid, or electroporation of cell membranes in the presence of the construct. A viral construct packaged into a viral particle can be used to accomplish both efficient introduction of an expression construct into the cell and translation of the encoded protein. Other methods known in the art for introducing nucleic acids to cells can be used, such as lipid-mediated carrier transport, chemical mediated transport, such as calcium phosphate, and the like.

For expression within cells, DNA vectors, for example plasmid vectors comprising a promoter directing expression of a polynucleotide encoding the H3K27me3-specific demethylase polypeptide can be employed. In some embodiments, expression of the encoded

protein is controlled by an inducible promoter or a conditional expression system. Examples of useful promoters in the context of the invention are tetracycline-inducible promoters (including TRE-tight), IPTG-inducible promoters, tetracycline transactivator systems, and reverse tetracycline transactivator (rtTA) systems. Constitutive promoters can also be used, as
5 can cell- or tissue-specific promoters. Many promoters will be ubiquitous, such that they are expressed in all cell and tissue types. A certain embodiment uses tetracycline-responsive promoters, one of the most effective conditional gene expression systems in in vitro and in vivo studies.

10 Somatic cell Nuclear Transfer

Somatic cell nuclear transfer (SCNT) is a technique that may be used, for example, for the reproductive cloning of livestock (e.g., cows, horses, sheep, goats, pigs) or for therapeutic cloning, in which desired tissues are produced for cell replacement therapy. Unfortunately, cloned animals suffer from certain defects arising from improper X-
15 chromosome inactivation. The present invention addresses these defects by restoring physiologic X chromosome inactivation to embryos generated from in vitro fertilization, i.e., generating using SCNT.

Somatic cell nuclear transfer involves obtaining a nuclear donor cell, then fusing this nuclear donor cell into an enucleated recipient cell, most preferably an enucleated oocyte, to
20 form a nuclear transfer embryo, activating this embryo, and finally culturing the embryo or transferring this embryo into a maternal host. During nuclear transfer a full complement of nuclear DNA from one cell is introduced to an enucleated cell. Nuclear transfer methods are well known to a person of ordinary skill in the art. See, U.S. Pat. No. 4,994,384 to Prather et al., entitled "Multiplying Bovine Embryos," issued on Feb. 19, 1991; U.S. Pat. No. 5,057,420 to Massey, entitled "Bovine Nuclear Transplantation," issued on Oct. 15, 1991; U.S. Pat. No. 5,994,619, issued on Nov. 30, 1999 to Stice et al., entitled "Production of Chimeric Bovine or Porcine Animals Using Cultured Inner Cell Mass Cells; U.K. Patents Nos. GB 2,318,578 GB 2,331,751, issued on Jan. 19, 2000 to Campbell et al. and Wilmut et al., respectively, entitled "Quiescent Cell Populations For Nuclear Transfer"; U.S. Pat. No. 6,011,197 to Strelchenko et al., entitled "Method of Cloning Bovines Using Reprogrammed Non-Embryonic Bovine
30 Cells," issued on Jan. 4, 2000; and in U.S. patent application Ser. No. 09/753,323 entitled "Method of Cloning Porcine Animals (attorney docket number 030653.0026.CIP1, filed Dec.

28, 2000), each of which are hereby incorporated by reference in its entirety including all figures, tables and drawings. Nuclear transfer may be accomplished by using oocytes that are not surrounded by a zona pellucida.

In a nuclear transfer procedure, a nuclear donor cell, or the nucleus thereof, is introduced into a recipient cell. A recipient cell is preferably an oocyte and is preferably enucleated. However, the invention relates in part to nuclear transfer, where a nucleus of an oocyte is not physically extracted from the oocyte. It is possible to establish a nuclear transfer embryo where nuclear DNA from the donor cell is replicated during cellular divisions. See, e.g., Wagoner et al., 1996, "Functional enucleation of bovine oocytes: effects of centrifugation and ultraviolet light," *Theriogenology* 46: 279-284. In addition, nuclear transfer may be accomplished by combining one nuclear donor and more than one enucleated oocyte. Also, nuclear transfer may be accomplished by combining one nuclear donor, one or more enucleated oocytes, and the cytoplasm of one or more enucleated oocytes. The resulting combination of a nuclear donor cell and a recipient cell can be referred to as a "hybrid cell."

The term "nuclear donor" as used herein refers to any cell, or nucleus thereof, having nuclear DNA that can be translocated into an oocyte. A nuclear donor may be a nucleus that has been isolated from a cell. Multiple techniques are available to a person of ordinary skill in the art for isolating a nucleus from a cell and then utilizing the nucleus as a nuclear donor. See, e.g., U.S. Pat. Nos. 4,664,097, 6,011,197, and 6,107,543, each of which is hereby incorporated by reference in its entirety including all figures, tables and drawings. Any type of cell can serve as a nuclear donor. Examples of nuclear donor cells include, but are not limited to, cultured and non-cultured cells isolated from an embryo arising from the union of two gametes in vitro or in vivo; embryonic stem cells (ES cells) arising from cultured embryonic cells (e.g., pre-blastocyst cells and inner cell mass cells); cultured and non-cultured cells arising from inner cell mass cells isolated from embryos; cultured and non-cultured pre-blastocyst cells; cultured and non-cultured fetal cells; cultured and non-cultured adult cells; cultured and non-cultured primordial germ cells; cultured and non-cultured germ cells (e.g., embryonic germ cells); cultured and non-cultured somatic cells isolated from an animal; cultured and non-cultured cumulus cells; cultured and non-cultured amniotic cells; cultured and non-cultured fetal fibroblast cells; cultured and non-cultured genital ridge cells; cultured and non-cultured differentiated cells; cultured and non-cultured cells in a synchronous population; cultured and non-cultured cells in an asynchronous population;

cultured and non-cultured serum-starved cells; cultured and non-cultured permanent cells; and cultured and non-cultured totipotent cells. See, e.g., Piedrahita et al., 1998, Biol. Reprod. 58: 1321-1329; Shim et al., 1997, Biol. Reprod. 57: 1089-1095; Tsung et al., 1995, Shih Yen Sheng Wu Hsueh Pao 28: 173-189; and Wheeler, 1994, Reprod. Fertil. Dev. 6: 563-568, each of which is incorporated herein by reference in its entirety including all figures, drawings, and tables. In addition, a nuclear donor may be a cell that was previously frozen or cryopreserved.

Hybrid cells made by the process of nuclear transfer may be used, for example, in reproductive cloning or in regenerative cloning.

SCNT experiments showed that nuclei from adult differentiated somatic cells can be reprogrammed to a totipotent state. Accordingly, a SCNT embryo generated using the methods as disclosed herein can be cultured in a suitable in vitro culture medium for the generation of totipotent or embryonic stem cell or stem-like cells and cell colonies. Culture media suitable for culturing and maturation of embryos are well known in the art. Examples of known media, which may be used for bovine embryo culture and maintenance, include Ham's F-10+10% fetal calf serum (FCS), Tissue Culture Medium-199 (TCM-199)+10% fetal calf serum, Tyrodes-Albumin-Lactate-Pyruvate (TALP), Dulbecco's Phosphate Buffered Saline (PBS), Eagle's and Whitten's media. One of the most common media used for the collection and maturation of oocytes is TCM-199, and 1 to 20% serum supplement including fetal calf serum, newborn serum, estrual cow serum, lamb serum or steer serum. A preferred maintenance medium includes TCM-199 with Earl salts, 10% fetal calf serum, 0.2 M pyruvate and 50 ug/ml gentamicin sulphate. Any of the above may also involve co-culture with a variety of cell types such as granulosa cells, oviduct cells, BRL cells and uterine cells and STO cells.

In particular, epithelial cells of the endometrium secrete leukemia inhibitory factor (LIF) during the preimplantation and implantation period. Therefore, in some embodiments, the addition of LIF to the culture medium is encompassed to enhancing the in vitro development of the SCNT-derived embryos. The use of LIF for embryonic or stem-like cell cultures has been described in U.S. Pat. No. 5,712,156, which is herein incorporated by reference.

Another maintenance medium is described in U.S. Pat. No. 5,096,822 to Rosenkrans, Jr. et al., which is incorporated herein by reference. This embryo medium, named CR1,

contains the nutritional substances necessary to support an embryo. CR1 contains hemicalcium L-lactate in amounts ranging from 1.0 mM to 10 mM, preferably 1.0 mM to 5.0 mM. Hemicalcium L-lactate is L-lactate with a hemicalcium salt incorporated thereon. Also, suitable culture medium for maintaining human embryonic stem cells in culture as discussed
5 in Thomson et al., Science, 282:1145-1147 (1998) and Proc. Natl. Acad. Sci., USA, 92:7844-7848 (1995).

In some embodiments, the feeder cells will comprise mouse embryonic fibroblasts. Means for preparation of a suitable fibroblast feeder layer are described in the example which follows and is well within the skill of the ordinary artisan.

10 Methods of deriving ES cells (e.g., NT-ESCs or hNT-ESCs) from blastocyst-stage SCNT embryos (or the equivalent thereof) are well known in the art. Such techniques can be used to derive ES cells (e.g., hNT-ESCs) from SCNT embryos, where the SCNT embryos used to generate hNT-ESCs have a reduced level of H3K9me3 in the nuclear genetic material donated from the somatic donor cell, as compared to SCNTs which were not treated with a
15 member of the KDM4 demethylase family and/or an inhibitor of the histone methyltransferase SUV39h1/SUV39h2. hNT-ESCs can be derived from cloned SCNT embryos during earlier stages of development.

In certain embodiments, blastomeres generated from SCNT embryos generated using the methods, compositions and kits as disclosed herein can be dissociated using a glass
20 pipette to obtain totipotent cells. In some embodiments, dissociation may occur in the presence of 0.25% trypsin (Collas and Robl, 43 BIOL. REPROD. 877-84, 1992; Stice and Robl, 39 BIOL. REPROD. 657-664, 1988; Kanka et al., 43 MOL. REPROD. DEV. 135-44, 1996).

In certain embodiments, the resultant blastocysts, or blastocyst-like clusters from
25 the SCNT embryos can be used to obtain embryonic stem cell lines, eg., nuclear transfer ESC (ntESC) cell lines. Such lines can be obtained, for example, according to the culturing methods reported by Thomson et al., Science, 282:1145-1147 (1998) and Thomson et al., Proc. Natl. Acad. Sci., USA, 92:7544-7848 (1995), incorporated by reference in their entirety herein.

30 Pluripotent embryonic stem cells can also be generated from a single blastomere removed from a SCNT embryo without interfering with the embryo's normal development to birth. See U.S. application Nos. 60/624,827, filed Nov. 4, 2004; 60/662,489, filed Mar. 14,

2005; 60/687,158, filed Jun. 3, 2005; 60/723,066, filed Oct. 3, 2005; 60/726,775, filed Oct. 14, 2005; 11/267,555 filed Nov. 4, 2005; PCT application no. PCT/US05/39776, filed Nov. 4, 2005, the disclosures of which are incorporated by reference in their entirety; see also Chung et al., Nature, Oct. 16, 2005 (electronically published ahead of print) and Chung et al., Nature V. 439, pp. 216-219 (2006), the entire disclosure of each of which is incorporated by reference in its entirety). In such a case, an SCNT embryo is not destroyed for the generation of pluripotent stem cells.

In one aspect of the invention, the method comprises the utilization of cells derived from the SCNT embryo in research and in therapy. Such pluripotent stem cells (PSCs) or totipotent stem cells (TSC) can be differentiated into any of the cells in the body including, without limitation, skin, cartilage, bone, skeletal muscle, cardiac muscle, renal, hepatic, blood and blood forming, vascular precursor and vascular endothelial, pancreatic beta, neurons, glia, retinal, inner ear follicle, intestinal, lung, cells.

In another embodiment of the invention, the SCNT embryo, or blastocyst, or pluripotent or totipotent cells obtained from a SCNT embryo (e.g., NT-ESCs), can be exposed to one or more inducers of differentiation to yield other therapeutically-useful cells such as retinal pigment epithelium, hematopoietic precursors and hemangioblastic progenitors as well as many other useful cell types of the ectoderm, mesoderm, and endoderm. Such inducers include but are not limited to: cytokines such as interleukin-alpha A, interferon-alpha A/D, interferon-beta, interferon-gamma, interferon-gamma-inducible protein-10, interleukin-1-17, keratinocyte growth factor, leptin, leukemia inhibitory factor, macrophage colony-stimulating factor, and macrophage inflammatory protein-1 alpha, 1-beta, 2, 3 alpha, 3 beta, and monocyte chemotactic protein 1-3, 6kine, activin A, amphiregulin, angiogenin, B-endothelial cell growth factor, beta cellulin, brain-derived neurotrophic factor, C10, cardiotrophin-1, ciliary neurotrophic factor, cytokine-induced neutrophil chemoattractant-1, eotaxin, epidermal growth factor, epithelial neutrophil activating peptide-78, erythropoietin, estrogen receptor-alpha, estrogen receptor-beta, fibroblast growth factor (acidic and basic), heparin, FLT-3/FLK-2 ligand, glial cell line-derived neurotrophic factor, Gly-His-Lys, granulocyte colony stimulating factor, granulocytemacrophage colony stimulating factor, GRO-alpha/MGSA, GRO-beta, GRO-gamma, HCC-1, heparin-binding epidermal growth factor, hepatocyte growth factor, heregulin-alpha, insulin, insulin growth factor binding protein-1, insulin-like growth factor

binding protein-1, insulin-like growth factor, insulin-like growth factor II, nerve growth factor, neurotrophin-3,4, oncostatin M, placenta growth factor, pleiotrophin, rantes, stem cell factor, stromal cell-derived factor 1B, thromopoietin, transforming growth factor--(alpha, beta 1,2,3,4,5), tumor necrosis factor (alpha and beta), vascular endothelial growth factors, and bone morphogenic proteins, enzymes that alter the expression of hormones and hormone antagonists such as 17B-estradiol, adrenocorticotrophic hormone, adrenomedullin, alpha-melanocyte stimulating hormone, chorionic gonadotropin, corticosteroid-binding globulin, corticosterone, dexamethasone, estriol, follicle stimulating hormone, gastrin 1, glucagons, gonadotropin, L-3,3',5'-triiodothyronine, leutinizing hormone, L-thyroxine, melatonin, MZ-4, oxytocin, parathyroid hormone, PEC-60, pituitary growth hormone, progesterone, prolactin, secretin, sex hormone binding globulin, thyroid stimulating hormone, thyrotropin releasing factor, thyroxin-binding globulin, and vasopressin, extracellular matrix components such as fibronectin, proteolytic fragments of fibronectin, laminin, tenascin, thrombospondin, and proteoglycans such as aggrecan, heparan sulphate proteoglycan, chondroitin sulphate proteoglycan, and syndecan. Other inducers include cells or components derived from cells from defined tissues used to provide inductive signals to the differentiating cells derived from the reprogrammed cells of the present invention. Such inducer cells may derive from human, non-human mammal, or avian, such as specific pathogen-free (SPF) embryonic or adult cells.

Blastomere Culturing. In one embodiment, the SCNT embryos can be used to generate blastomeres and utilize in vitro techniques related to those currently used in pre-implantation genetic diagnosis (PGD) to isolate single blastomeres from a SCNT embryo, generated by the methods as disclosed herein, without destroying the SCNT embryos or otherwise significantly altering their viability. As demonstrated herein, pluripotent embryonic stem (hES) cells and cell lines can be generated from a single blastomere removed from a SCNT embryo as disclosed herein without interfering with the embryo's normal development to birth.

The discoveries of Wilmut et al. (Wilmut, et al, Nature 385, 810 (1997) in sheep cloning of "Dolly", together with those of Thomson et al. (Thomson et al., Science 282, 1145 (1998)) in deriving hESCs, have generated considerable enthusiasm for regenerative cell transplantation based on the establishment of patient-specific hESCs derived from SCNT-embryos or SCNT-engineered cell masses generated from a patient's own nuclei. This strategy, aimed at avoiding immune rejection through autologous transplantation, is perhaps

the strongest clinical rationale for SCNT. By the same token, derivations of complex disease-specific SCNT-hESCs may accelerate discoveries of disease mechanisms. For cell transplantations, innovative treatments of murine SCID and PD models with the individual mouse's own SCNT-derived mESCs are encouraging (Rideout et al, Cell 109, 17 (2002); Barberi, Nat. Biotechnol. 21, 1200 (2003)). Ultimately, the ability to create banks of SCNT-derived stem cells with broad tissue compatibility would reduce the need for an ongoing supply of new oocytes.

In certain embodiments of the invention, pluripotent or totipotent cells obtained from a SCNT embryo (e.g., hNT-ESCs) can be optionally differentiated, and introduced into the tissues in which they normally reside in order to exhibit therapeutic utility. For example, pluripotent or totipotent cells obtained from a SCNT embryo can be introduced into the tissues. In certain other embodiments, pluripotent or totipotent cells obtained from a SCNT embryo can be introduced systemically or at a distance from a site at which therapeutic utility is desired. In such embodiments, the pluripotent or totipotent cells obtained from a SCNT embryo can act at a distance or may home to the desired site.

In certain embodiments of the invention, cloned cells, pluripotent or totipotent cells obtained from a SCNT embryo can be utilized in inducing the differentiation of other pluripotent stem cells. The generation of single cell-derived populations of cells capable of being propagated in vitro while maintaining an embryonic pattern of gene expression is useful in inducing the differentiation of other pluripotent stem cells. Cell-cell induction is a common means of directing differentiation in the early embryo. Many potentially medically-useful cell types are influenced by inductive signals during normal embryonic development including spinal cord neurons, cardiac cells, pancreatic beta cells, and definitive hematopoietic cells. Single cell-derived populations of cells capable of being propagated in vitro while maintaining an embryonic pattern of gene expression can be cultured in a variety of in vitro, in ovo, or in vivo culture conditions to induce the differentiation of other pluripotent stem cells to become desired cell or tissue types.

The pluripotent or totipotent cells obtained from a SCNT embryo (e.g., ntESCs) can be used to obtain any desired differentiated cell type. Therapeutic usages of such differentiated cells are unparalleled. As discussed herein, the donor cell, or the recipient oocyte, the hybrid oocyte or SCNT embryo can be treated with an H3K27me3-specific demethylase according to the methods as disclosed herein.

Alternatively, the donor cells can be adult somatic cells from a subject with a disorder, and the generated SCNT embryos can be used to produce animal models of disease or disease-specific pluripotent or totipotent cells which can be cultured under differentiation conditions to produce cell models of disease. The great advantage of the present invention is that by increasing the efficiency of SCNT, it provides an essentially limitless supply of isogenic or syngeneic ES cells, particularly pluripotent that are not induced pluripotent stem cells (e.g., not iPSCs). Such NT-ESCs have advantages over iPSCs and are suitable for transplantation, as they do not partially pluripotent, and do not have viral transgenes or forced expression of reprogramming factors to direct their reprogramming.

In some embodiments, the NT-ESCs generated from the SCNTs are patient-specific pluripotent obtained from SCNT embryos, where the donor cell was obtained from a subject to be treated with the pluripotent stem cells or differentiated progeny thereof. Therefore, it will obviate the significant problem associated with current transplantation methods, i.e., rejection of the transplanted tissue which may occur because of host-vs-graft or graft-vs-host rejection. Conventionally, rejection is prevented or reduced by the administration of anti-rejection drugs such as cyclosporin. However, such drugs have significant adverse side-effects, e.g., immunosuppression, carcinogenic properties, as well as being very expensive. The present invention should eliminate, or at least greatly reduce, the need for anti-rejection drugs, such as cyclosporine, imulan, FK-506, glucocorticoids, and rapamycin, and derivatives thereof.

Kits

The invention provides kits comprising an H3K27me3-specific demethylase activators (e.g., polynucleotides encoding Kdm6a, Kdm6b, or Kdm6c, a Kdm6a, Kdm6b, or Kdm6c polypeptide, or an enzymatically active fragment thereof).

In some embodiments, the kit comprises a sterile container which contains a therapeutic or prophylactic cellular composition; such containers can be boxes, ampoules, bottles, vials, tubes, bags, pouches, blister-packs, or other suitable container forms known in the art. Such containers can be made of plastic, glass, laminated paper, metal foil, or other materials suitable for holding medicaments.

If desired an agent of the invention is provided together with instructions for administering the agent to enhance the efficiency of SCNT. The instructions will generally include information about the use of the composition enhance physiologic X chromosome inactivation. In other embodiments, the instructions include at least one of the following:

5 description of the therapeutic agent; dosage schedule and administration for treatment or prevention of a neurological disease or symptoms thereof; precautions; warnings; indications; counter-indications; overdosage information; adverse reactions; animal pharmacology; clinical studies; and/or references. The instructions may be printed directly on the container (when present), or as a label applied to the container, or as a separate sheet, pamphlet, card,

10 or folder supplied in or with the container.

The practice of the present invention employs, unless otherwise indicated, conventional techniques of molecular biology (including recombinant techniques), microbiology, cell biology, biochemistry and immunology, which are well within the purview of the skilled artisan. Such techniques are explained fully in the literature, such as,

15 “Molecular Cloning: A Laboratory Manual”, second edition (Sambrook, 1989); “Oligonucleotide Synthesis” (Gait, 1984); “Animal Cell Culture” (Freshney, 1987); “Methods in Enzymology” “Handbook of Experimental Immunology” (Weir, 1996); “Gene Transfer Vectors for Mammalian Cells” (Miller and Calos, 1987); “Current Protocols in Molecular Biology” (Ausubel, 1987); “PCR: The Polymerase Chain Reaction”, (Mullis,

20 1994); “Current Protocols in Immunology” (Coligan, 1991). These techniques are applicable to the production of the polynucleotides and polypeptides of the invention, and, as such, may be considered in making and practicing the invention. Particularly useful techniques for particular embodiments will be discussed in the sections that follow.

The following examples are put forth so as to provide those of ordinary skill in the art with a complete disclosure and description of how to make and use the assay, screening, and

25 therapeutic methods of the invention, and are not intended to limit the scope of what the inventors regard as their invention.

EXAMPLES

30 **Example 1: H3K9me3 is dispensable for *Xist* silencing in biparental embryos**

To determine whether *Kdm4b*-mediated loss of H3K9me3 can induce *Xist* derepression in biparental embryos, *Kdm4b* mRNA was injected into *in vitro* fertilization-

derived embryos. Immunostaining analysis confirmed that *Kdm4b* mRNA injection effectively depleted H3K9me3 in zygotes in a concentration-dependent manner (Fig. 1A). To assess *Xist* RNA expression, RNA fluorescent in situ hybridization (FISH) analysis was performed in four-cell embryos. To distinguish between male and female embryos, X chromosomes were simultaneously labeled by DNA FISH using a probe specific for the *Rnf12* locus. As such, each blastomere of the male or female embryos should have one or two DNA FISH signals, respectively. The “no injection” control male embryos showed no *Xist* RNA signal, and the majority of female embryos showed one RNA cloud or spot signal (FIGS. 1B-1D). Similarly, *Kdm4b*-injected embryos did not induce maternal *Xist* expression in either male or female embryos (FIGS. 1B-1D). Without being bound by theory, this indicates that H3K9me3 does not play a major role in maternal *Xist* silencing under physiological biparental conditions.

Example 2: Maternal H3K27me3 coats *Xist* in oocytes and preimplantation embryos

Since maternal H3K27me3 can function as an imprinting mark (Inoue et al. 2017. Nature 547: 419–424), its potential involvement in *Xist* imprinting was examined. Analysis of H3K27me3 ChIP-seq (chromatin immunoprecipitation [ChIP] combined with high-throughput sequencing) data sets (Zheng et al. 2016. Mol Cell 63: 1066–1079) revealed that *Xist* is coated with a broad H3K27me3 domain, which spans ~450 kb in mature oocytes and is established during oocyte growth (FIG. 2A). Analyses of the oocyte DNase I sequencing (DNase I seq) (Inoue et al. 2017. Nature 547: 419–424) and DNA methylome (Kobayashi et al. 2012. PLoS Genet 8: e1002440) data sets revealed that this entire H3K27me3 domain exhibits low chromatin accessibility and low DNA methylation (FIG. 2A). Without being bound by theory, this suggests formation of a heterochromatin domain independent of DNA methylation. Analyses of the ChIP-seq data sets of post-fertilization embryos (Zheng et al. 2016. Mol Cell 63: 1066–1079) revealed that the maternal H3K27me3 domain is maintained throughout preimplantation development but is lost in the embryonic day 6.5 (E6.5) epiblast (FIG. 2B). Notably, the upstream ~200-kb region, which spans *Xist* to *Zcchc13*, but does not include the *Tsix* promoter, maintains the maternal allele bias of H3K27me3 enrichment in blastocyst embryos (FIG. 2C). These data support a potential role for maternal H3K27me3 in maternal *Xist* silencing.

Example 3. Maternal H3K27me3 is responsible for maternal *Xist* silencing

To examine whether H3K27me3 is responsible for maternal *Xist* silencing, H3K27me3 was depleted in zygotes by injecting mRNA coding an H3K27me3-specific demethylase, *Kdm6b* (FIG. 3A). As a negative control, zygotes were prepared by injection with the catalytic mutant, *Kdm6bMUT*, harboring a point mutation at the catalytic domain (FIG. 3A; Inoue et al. 2017. Nature 547: 419–424). Despite the transient expression of the exogenous Kdm6b, the H3K27me3 level in *Kdm6bWT* embryos was significantly lower than that of *Kdm6bMUT* embryos at the four-cell and morula stages (FIGS. 5A-5B).

To confirm that H3K27me3 is lost at the *Xist* locus in *Kdm6bWT*-injected embryos, ultralow input native ChIP-seq (ULI-NChIP) analysis was performed (Brind'Amour et al. 2015. Nat Commun 6: 6033), which worked efficiently using 500–2000 mouse embryonic stem cells (FIG. 6A). H3K27me3 ULI-NChIP was performed using $\square$ 2000 blastomeres from *Kdm6bWT*- or *Kdm6bMUT*-injected morula embryos. Data quality was validated by comparing *Kdm6bMUT*-injected embryos with a public morula embryo data set (FIG. 6B-6D; Liu et al. 2016. Nature 537: 558–562). The number of H3K27me3 peaks in *Kdm6bWT*-injected embryos was much smaller than that of *Kdm6bMUT*-injected embryos (FIG. 6E). Importantly, the *Xist* locus exhibits a marked decrease of H3K27me3 enrichment throughout the entire domain in *Kdm6bWT* embryos (FIG. 6F). Furthermore, analysis of single-nucleotide polymorphism (SNP) information revealed that the maternal H3K27me3 domain was lost in *Kdm6bWT* embryos (FIG. 3B).

To examine whether maternal *Xist* is depressed in *Kdm6bWT*-injected four-cell embryos, RNA/DNA FISH analysis was performed. RNA/DNA FISH analysis revealed that the majority of *Kdm6bWT*-injected males showed one *Xist* RNA cloud or spot, while all of the *Kdm6bMUT*-injected males showed no signal (FIGS. 3C-3D). Furthermore, the majority of *Kdm6bWT*-injected females showed two *Xist* RNA clouds or/and spots, while most of the *Kdm6bMUT*-injected females showed one cloud (FIGS. 3C, 3E). These results demonstrate that loss of maternal H3K27me3 at the *Xist* locus induced maternal *Xist* derepression at the four-cell stage.

Example 4. Loss of H3K27me3 induces maternal X-chromosome inactivation (XCI)

To examine whether maternal *Xist* expression continues until the morula stage (FIG. 4A), RNA/ DNA FISH analysis was performed. Strikingly, the majority of *Kdm6bWT*-

injected male and female embryos showed one and two RNA clouds, respectively, while most of the *Kdm6b*^{MUT}-injected male and female embryos showed none and one RNA cloud, respectively (FIG. 4B–4D), indicating that the reactivated *Xist* is persistent. Although *Xist* can be up-regulated by *Rnf12* overexpression (Tan et al. 2016. Proc Natl Acad Sci 113: 3197–3202), RT-qPCR analysis found no evidence of *Rnf12* up-regulation in *Kdm6b*^{WT}-injected embryos (FIG. 7), excluding the possibility that *Kdm6b*^{WT}-mediated *Xist* depression is due to *Rnf12* overexpression.

To determine whether maternal *Xist* expression leads to maternal XCI (FIG. 4A), RNA sequencing (RNA-seq) analysis was performed on early blastocyst stage hybrid mouse embryos with biological duplicates (FIG. 8A). Analysis of SNP information allowed examination of X_m-linked genes, which revealed that the expression level of X_m-linked genes, but not those of autosomal genes was significantly down-regulated in *Kdm6b*^{WT}-injected embryos (FIG. 4E). Consistently, the maternal allele expression bias [Mat/ (Mat + Pat)] of X-linked genes was significantly compromised in *Kdm6b*^{WT}-injected embryos, although it was still >50% (FIG. 8B). These data demonstrate that maternal XCI occurs at a level milder than paternal XCI in *Kdm6b*^{WT}-injected embryos. A closer examination of individual X-linked genes confirmed that most genes were down-regulated in *Kdm6b*^{WT}-injected embryos (FIG. 8C), while genes known to escape imprinted XCI (Borensztein et al. 2017. Nat Struct Mol Biol 24: 226–233) were unchanged (FIG. 8D). Without being bound by theory, this suggests that *Kdm6b*-mediated maternal XCI could recapitulate physiological XCI.

Taken together, the results described herein provide evidence that H3K27me₃ serves as the imprinting mark of *Xist*. The domain-based regulation of maternal H3K27me₃ at the *Xist* locus is an attractive observation and raises important questions. For example, how are the boundaries of the H3K27me₃ domain defined during oogenesis? How does the first half (≈200 kb) of the domain, spanning *Xist* and *Zcchc13*, maintain the maternal allele-specific H3K27me₃ enrichment in blastocyst embryos? Notably, this ≈200-kb region appears to form a topological association domain (Giorgetti et al. 2014. Cell 157: 950–963), implicating potential involvement of a protein such as CTCF in regulating chromatin boundaries. Interestingly, this domain highly overlaps transgenes whose insertion into an autosome recapitulated imprinted XCI (Okamoto et al. 2005. Nature 438: 369–373). Without being bound by theory, this raises a possibility that a core element attracting Polycomb group

complexes might exist in the region and contribute to imprinting establishment during oogenesis.

In conclusion, the present study thus not only identifies *Xist* as a new member of H3K27me3-dependent imprinted genes (Inoue et al. 2017), but also demonstrates the biological significance of H3K27me3-dependent genomic imprinting in safeguarding the X_m from XCI.

The results described above were obtained with the following materials and methods

Collection of mouse oocytes

All animal studies were performed in accordance with guidelines of the Institutional Animal Care and Use Committee at Harvard Medical School. The procedures of oocyte collection and in vitro fertilization were described previously (Inoue et al. 2017. Nature 547: 419–424). The hybrid embryos used for SNP analysis in this study were obtained by in vitro fertilization of B6D2F1/J((BDF1) oocytes and PWK sperm (Jackson Laboratory, 003715)

mRNA preparation and injection

The construction and preparation of *Kdm6b* mRNA and microinjection into fertilized oocytes were described previously (Inoue et al. 2017. Nature 547: 419–424). The *Kdm4b* construct was generated by cloning its cDNA amplicon into the pcDNA3.1-Flag-poly(A)₈₃ plasmid. mRNA was synthesized with mMES- SAGE mMACHINE T7 Ultra kit (Life technologies), purified by lithium chloride precipitation, and dissolved with nuclease-free water. The concentrations of injected mRNA of *Kdm6b*^{WT} and *Kdm6b*^{MUT} were 1800 ng/μL, and those of *Kdm4b* were 1000 or 2600 ng/μL.

Probe for FISH

A probe for *Xist* RNA was prepared by using Nick translation reagent kit (Abbott Molecular, 07J00-001) with Cy3-dCTP (GE Healthcare, PA53021). The template DNA was a plasmid coding the full-length mouse *Xist* gene (Addgene, 26760) (Wutz and Jaenisch. 2000. Mol Cell 5: 695–705). A probe for DNA FISH was prepared using the same kit with Green-dUTP (Abbott Molecular, 02N32-050). The template DNA was a BAC clone containing the *Rnf12* locus (RP23-36C20) (Fukuda et al. 2015. Development 142: 4049–

4055). The fluorescent probes were ethanol-precipitated with 5 µg of Cot-1 DNA (Life technologies), 5 µg of herring sperm DNA (Thermo Fisher Scientific), and 2.5 µg of yeast tRNA (Thermo Fisher Scientific, AM7119) and then dissolved with 20 µL of formamide (Thermo Fisher Scientific, 17899). The probes were stored at 4°C. Before being used, the probes (0.75 µL each) were mixed with 0.75 µL of Cot-1 DNA/formamide and 2.25 µL of 4× SSC/20% dextran (Millipore S4030). The probe mixtures were heated for 30 min at 80°C and then transferred to a 37°C incubator (“preannealed probes”).

Whole-mount RNA/DNA FISH

Four-cell or morula embryos were fixed at 46 or 78 hr. post-fertilization (hpf) in 2% paraformaldehyde (PFA) in PBS containing 0.5% Triton X-100 for 20 min at room temperature. After three washes with 0.1% BSA/PBS, embryos were treated with 0.1 N HCl containing 0.02% Triton X-100 for 15 min at 4°C. After three washes with 0.1% BSA/2× SSC, embryos were incubated in a series of 10%, 20%, and 50% formamide/2× SSC in a glass dish (Electron Microscopy Science, 705430-30) and incubated for 30 min. The samples were covered with mineral oil, heated for 30 min at 80°C, and then incubated for >30 min at 37°C. The embryos were then transferred into 4.5 µL of “preannealed probes,” covered with mineral oil on another glass dish, and incubated for >24 hr. at 37°C. Embryos were washed with 42°C prewarmed 0.1% BSA/2× SSC, left in the last drop for 30 min, and mounted on a glass slide in VectaShield with DAPI (Vector Laboratories). Fluorescence was detected under a laser-scanning confocal microscope (Zeiss, LSM800).

Whole-mount immunostaining

The procedure of immunostaining and quantification was described previously (Inoue et al. 2017. Nature 547: 419–424).

Identification of the maternal allele-biased H3K27me3 domain

The BED files, including RPKM (reads per kilobase per million mapped reads) values in 100-base-pair (bp) bins for H3K27me3 ChIP-seq in the inner cell mass (ICM), were from GSE76687 (Zheng et al. 2016. Mol Cell 63: 1066–1079). BED files labeled maternal- or paternal-containing RPKM values for two parental alleles, and allelic reads were normalized to total read number. “bedtools makewindows” was used to generate 1-kb bins for the entire

mm9 genome, and the RPKM value for each bin was calculated by “bedtools map.” All of the bins were classified to three categories of “no signal,” “biallelic,” and “maternal-biased” using a signal cutoff of 1 and a fold change cutoff of 4. A sliding window approach was used to identify domains that were enriched for “maternal-biased” H3K27me3 bins. The criteria used were as follows: Within a window of 20 kb, the minimum number of “maternal-biased” bins was three, and the percentage of “maternal-biased” bins was larger than “biallelic” bins. Overlapped windows were merged by “bedtools merge.” A total of 5986 domains was identified in the genome.

10 *ULI-NChIP*

At 78 hpf, □110 morula embryos per group were briefly treated with acid Tyrode’s solution (Sigma-Aldrich) to remove zona pellucida, washed with 0.2% BSA/PBS, and transferred to 1.5-mL tubes. The ULI-NChIP was performed using H3K27me3 antibody (Diagenode, C15410069) as described previously (Brind’Amour et al. 2015. Nat Commun 6: 6033) with the following modifications. First, we used Beckman SPRIselect beads (Beckman Coulter) instead of Agencourt Ampure XP beads. Second, the sequencing library was prepared using NEBNext Ultra II DNA library preparation kit for Illumina (New England Biolabs). Third, PCR amplification was performed using Kapa Hifi hot start ready mix (Kapa Biosystems). Lastly, no size selection was performed. For input samples, 10% volume of the chromatin lysate was taken and used for library construction and sequencing. The quantification and sequencing of the libraries were described previously (Inoue et al. 2017. Nature 547: 419–424).

Data analysis of ULI-NChIP

For normalization between *Kdm6bMUT* and *Kdm6bWT* samples, a strategy similar to MAnorm (Shao et al. 2012. Genome Biol 13: R16) was used. First, the common peaks were identified between the *Kdm6bMUT* and *Kdm6bWT* samples. Next, all samples were normalized to the highest coverage samples based on the RPKM value for each sample within the common peaks. SNP-specific reads were normalized to total uniquely mapped reads per library and further normalized to the highest-coverage allele based on the sum of SNP-specific reads per allele.

Reverse transcription and real-time PCR analysis

Kdm6b-injected embryos were collected at 46 hpf (four-cell), 60 hpf (eight-cell), and 78 hpf (morula). The procedures of reverse transcription and real-time PCR analysis were described previously (Inoue and Zhang, 2014. Nat Struct Mol Biol 21: 609–616) except for the use of random primers for reverse transcription in this study. Primer sequences used for real-time PCR were as follows: *I8S*-F (5'-TTG ACGGAAGGGCACCACCAG-3'), *I8S*-R (5'-GCACCACCACCCACGGA ATCG-3'), *Rnf12*-F (5' -TTTGTGCGCAGGGCAGTCTTA-3'), and *Rnf12*-R (5'-GTTTGCCCATCACTATTCCAGC-3

RNA-seq and data analysis

Blastocyst embryos at 96 hpf were treated briefly with acid Tyrode's solution, washed by 0.2% BSA/PBS, and stored in PCR tubes at –80°C. Forty to 46 embryos per group were pooled and used for RNA-seq. RNA-seq libraries were prepared as described previously [(Inoue et al. 2017) with the exception of using Nextera XT DNA library preparation kit (Illumina) following cDNA amplification.

Statistical analyses

Statistical analyses were implemented with R (<http://www.r-project.org>). Pearson's *R* coefficient was calculated using the “cor” function with default parameters. For FIG. 8B, Mann-Whitney *U*-Test was performed with the R function “wilcox.test.”

Code availability

A customized pipeline was used to split the alignment of sequencing data from hybrid embryos to their parental origin based on SNP information. The code is available at <https://github.com/lanjiangboston/UniversalSNPsplit>.

Data availability

ChIP-seq and RNA-seq data sets generated herein are deposited at the Gene Expression Omnibus database under accession number GSE103714. The WGBS data set for GV oocytes was downloaded from <http://www.nodai-genome.org/mouse.html?lang=en> (Kobayashi et al. 2012. PLoS Genet 8: e1002440). H3K27me3 ChIP-seq data sets were downloaded from GSE76687 (Zheng et al. 2016. Mol Cell 63: 1066–1079). The oocyte

DNase I-seq data set was from GSE92605 (Inoue et al. 2017. Nature 547: 419–424). The BAM file and peak file of ENCODE data were downloaded from <https://www.encodeproject.org/files/ENCFF001KDT>. Morula embryo H3K27me3 ChIP-seq data sets were from GSE73952 (Liu et al. 2016. Nature 537: 558–562).

5

Other Embodiments

From the foregoing description, it will be apparent that variations and modifications may be made to the invention described herein to adopt it to various usages and conditions. Such embodiments are also within the scope of the following claims.

10

The recitation of a listing of elements in any definition of a variable herein includes definitions of that variable as any single element or combination (or subcombination) of listed elements. The recitation of an embodiment herein includes that embodiment as any single embodiment or in combination with any other embodiments or portions thereof.

15

All patents, publications, and accession numbers mentioned in this specification are herein incorporated by reference to the same extent as if each independent patent, publication, and accession number was specifically and individually indicated to be incorporated by reference.

What is claimed is:

1. A method for generating physiological X chromosome inactivation in an embryo generated by Somatic Cell Nuclear Transfer (SCNT), the method comprising injecting the embryo generated via SCNT with an H3K27me3-specific demethylase polypeptide or a polynucleotide encoding said demethylase.

2. The method of claim 1, wherein the embryo is injected with an mRNA encoding a H3K27me3-specific demethylase.

3. The method of claim 1, wherein the polynucleotide encodes a Kdm6a, Kdm6b, or Kdm6c polypeptide.

4. The method of claim 2, wherein the embryo is injected with between about 1000 and 2000 ng/ μ L of the mRNA.

5. The method of claim 3, wherein the embryo is injected with 1800 ng/ μ L of mRNA.

6. The method of claim 1, wherein the X chromosome is present in a donor nucleus derived from a somatic cell.

7. The method of claim 5, wherein the donor nucleus is transferred into an oocyte or embryonic stem cell.

8. The method of claim 1, wherein the polynucleotide encodes an enzymatically active fragment of an H3K27me3-specific demethylase.

9. The method of claim 8, wherein the polynucleotide is present in a mammalian expression vector.

10. The method of claim 9, wherein the mammalian expression vector comprises a promoter directing constitutive or inducible expression of the H3K27me3-specific demethylase.

5 11. The method of claim 1, wherein the injected polypeptide is Kdm6a, Kdm6b, or Kdm6c.

12. The method of claim 1, wherein the method reduces the expression of X-linked genes.

10 13. The method of claim 1, wherein the method does not significantly change the expression of genes that escape X-chromosome imprinting.

14. The method of claim 1, wherein the method does not significantly change autosomal gene expression.

15

15. The method of claim 1, wherein maternal allele expression bias of X-linked genes is greater than about 35-60%.

16. The method of claim 1, wherein maternal allele expression bias of X-linked genes is
20 greater than about 50%.

17. The method of claim 1, wherein the embryo is an early blastocyst stage embryo.

18. The method of claim 1, wherein the embryo is derived from an adult somatic cell.

25

19. The method of claim 18, wherein the somatic cell is obtained from a human subject.

20. The method of claim 18, further comprising culturing a cell from the embryo to obtain a tissue suitable for transplantation into the human subject.

30

21. A blastocyst produced according to the method of claim 1.

22. A cell or tissue produced according to the method of claim 20.
23. A cloned organism produced by implanting the blastocyst of claim 1 into a host uterus.

FIG. 1A

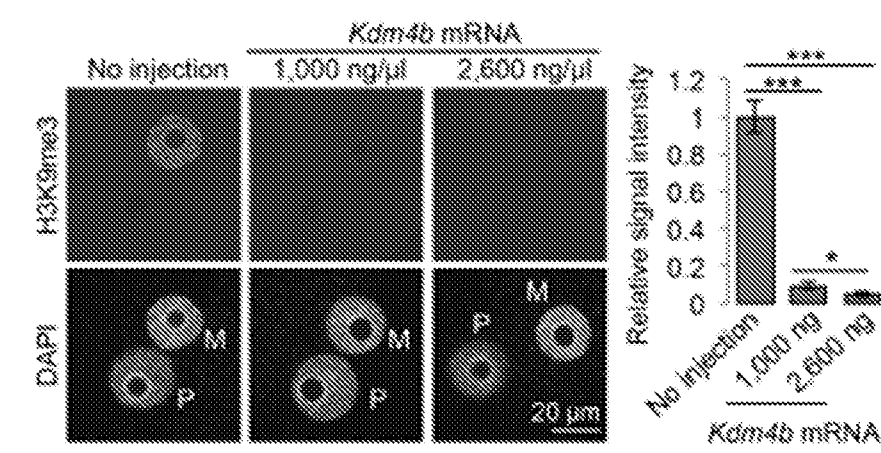


FIG. 1B

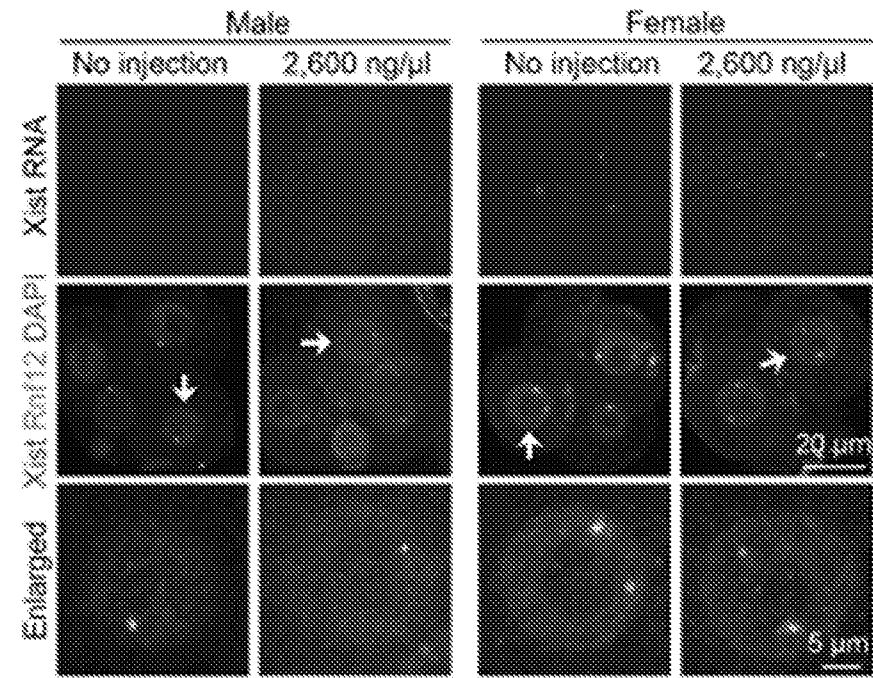


FIG. 1C

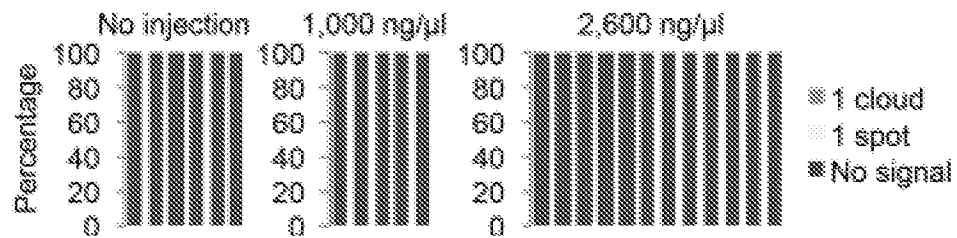


FIG. 1D

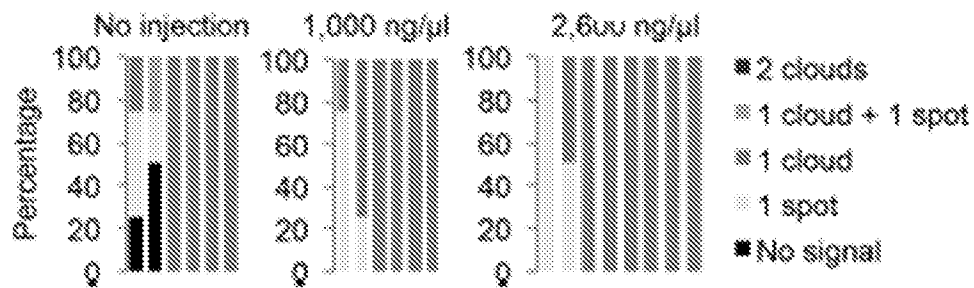


FIG. 2A

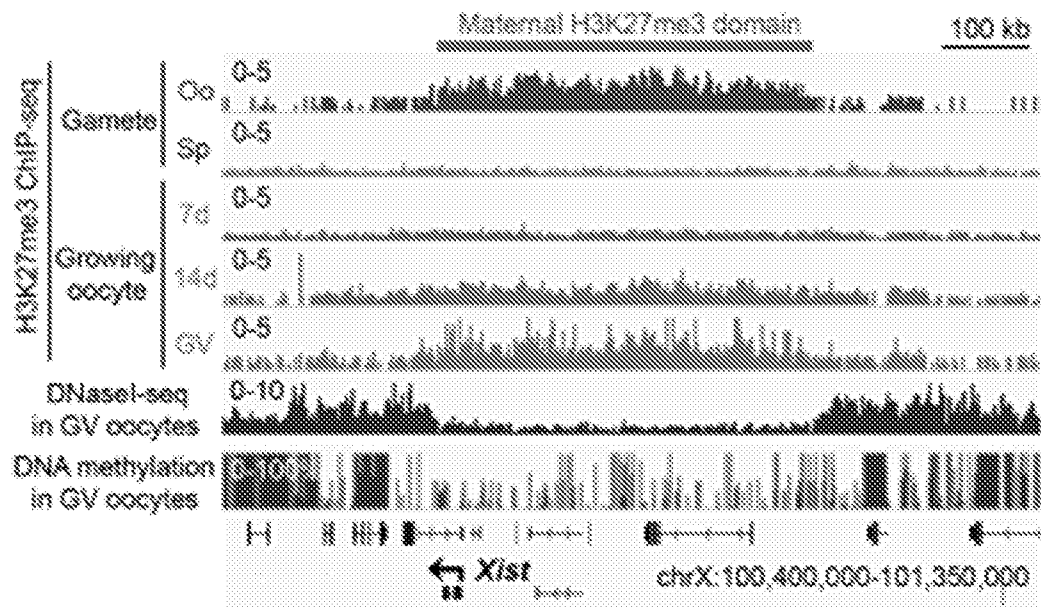


FIG. 2B

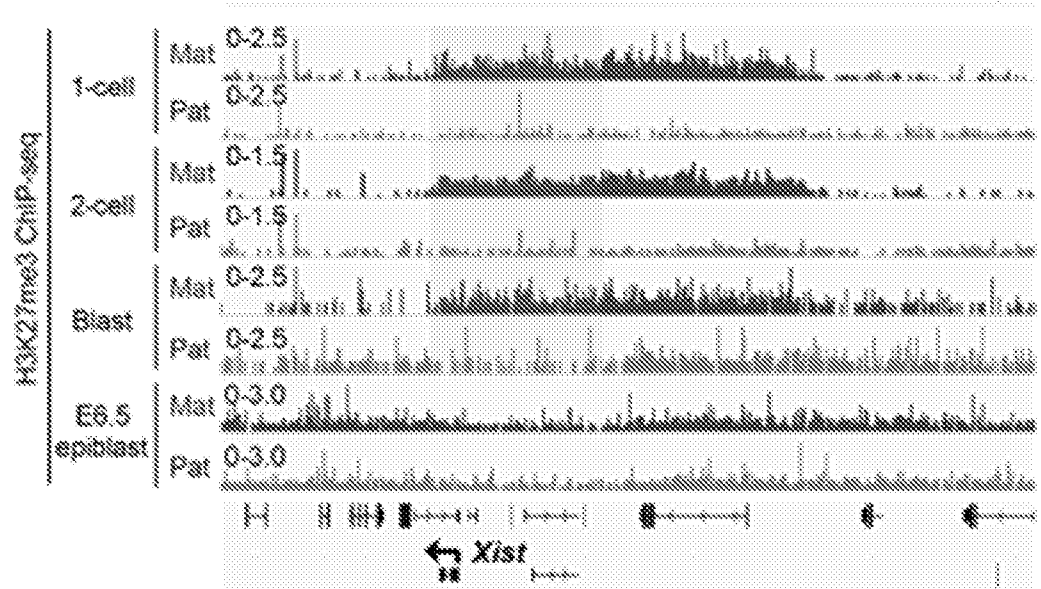


FIG. 2C

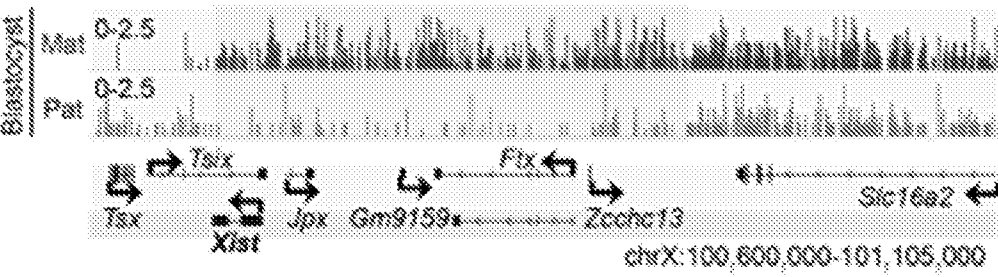


FIG. 3A

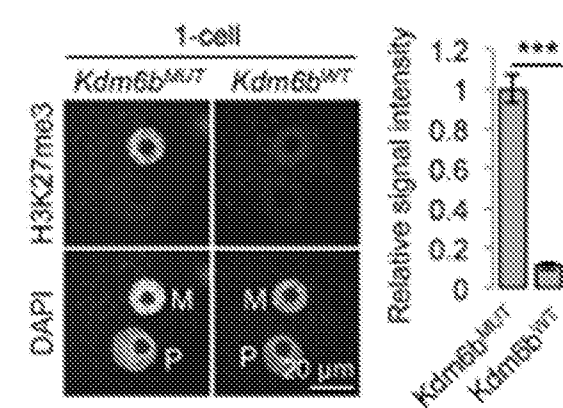


FIG. 3B

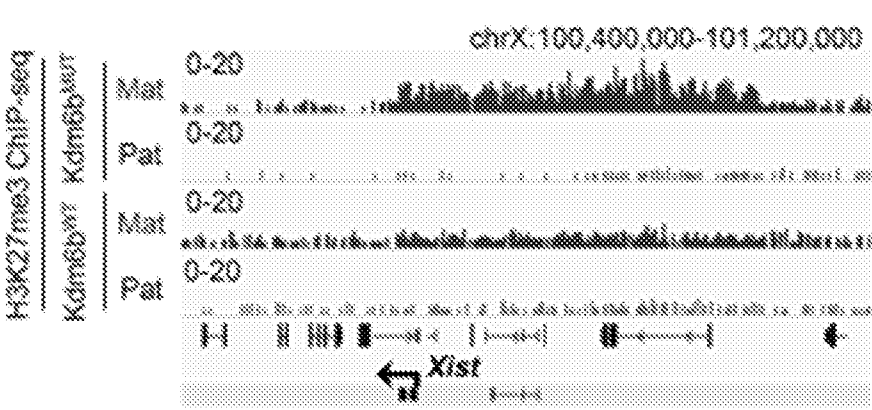


FIG. 3C

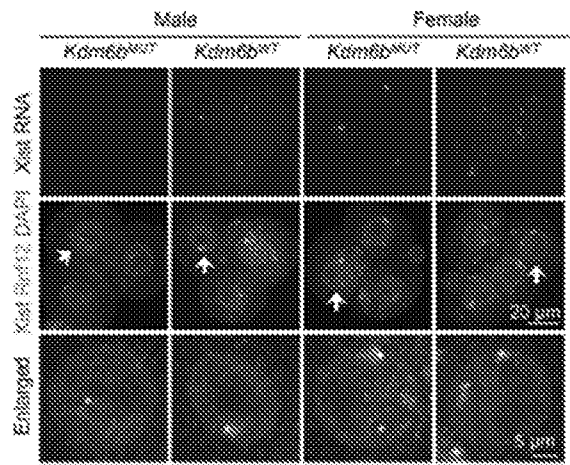


FIG. 3D

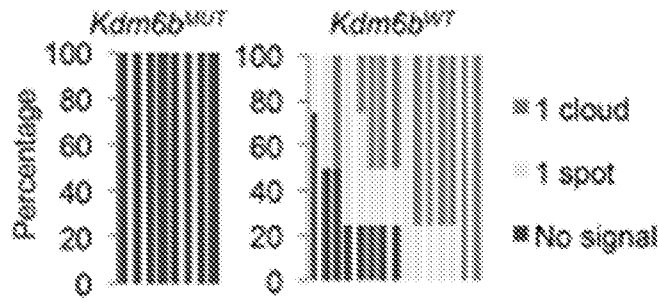


FIG. 3E

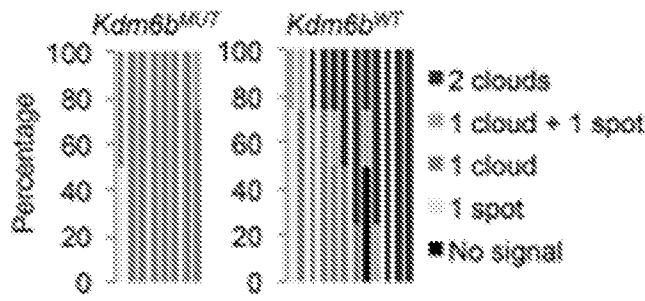


FIG. 4A

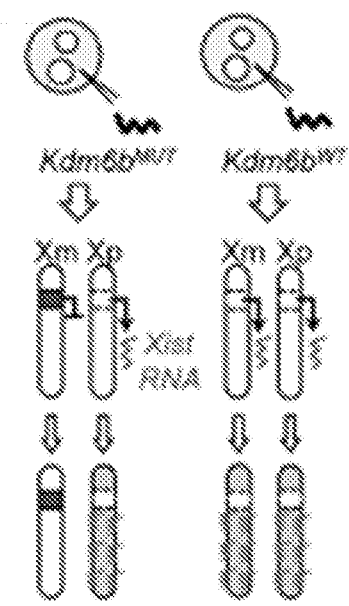


FIG. 4B

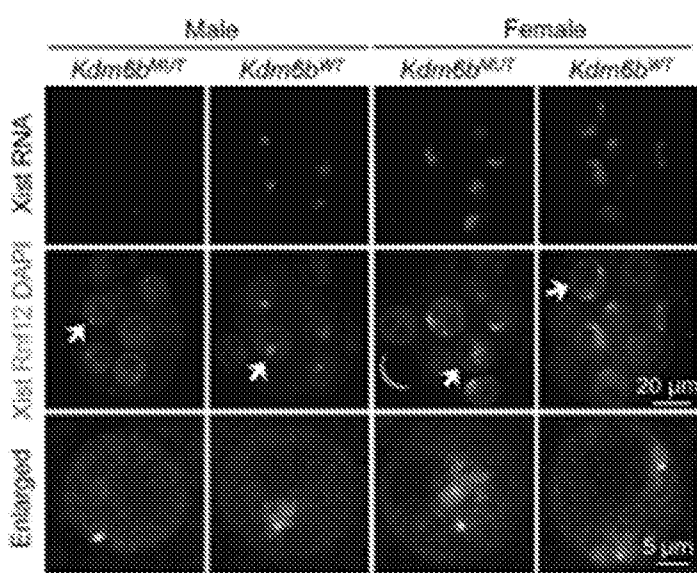


FIG. 4C

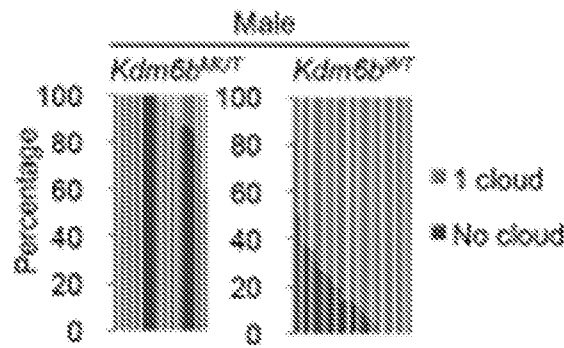


FIG. 4D

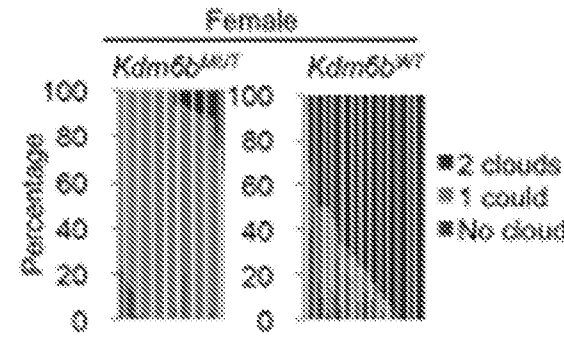


FIG. 4E

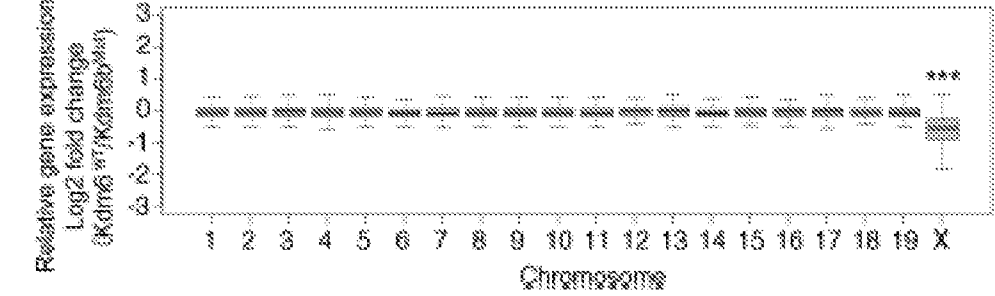


FIG. 5A

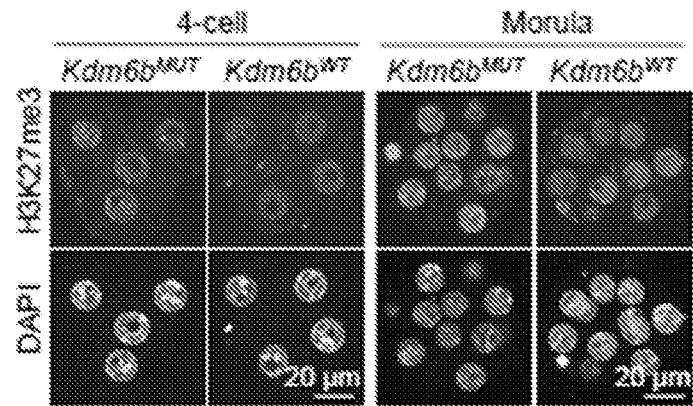
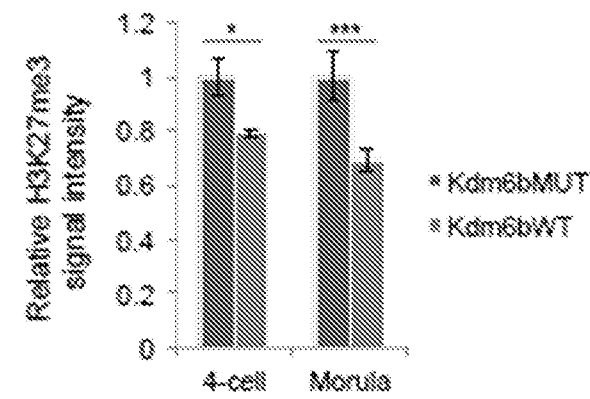
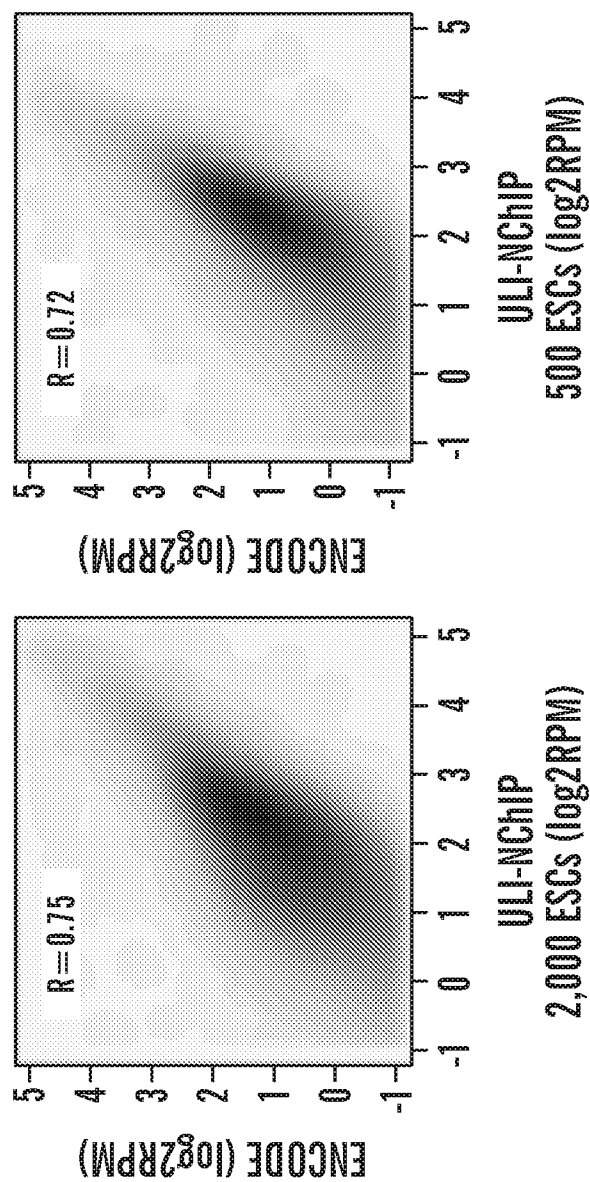


FIG. 5B



**FIG. 6A**

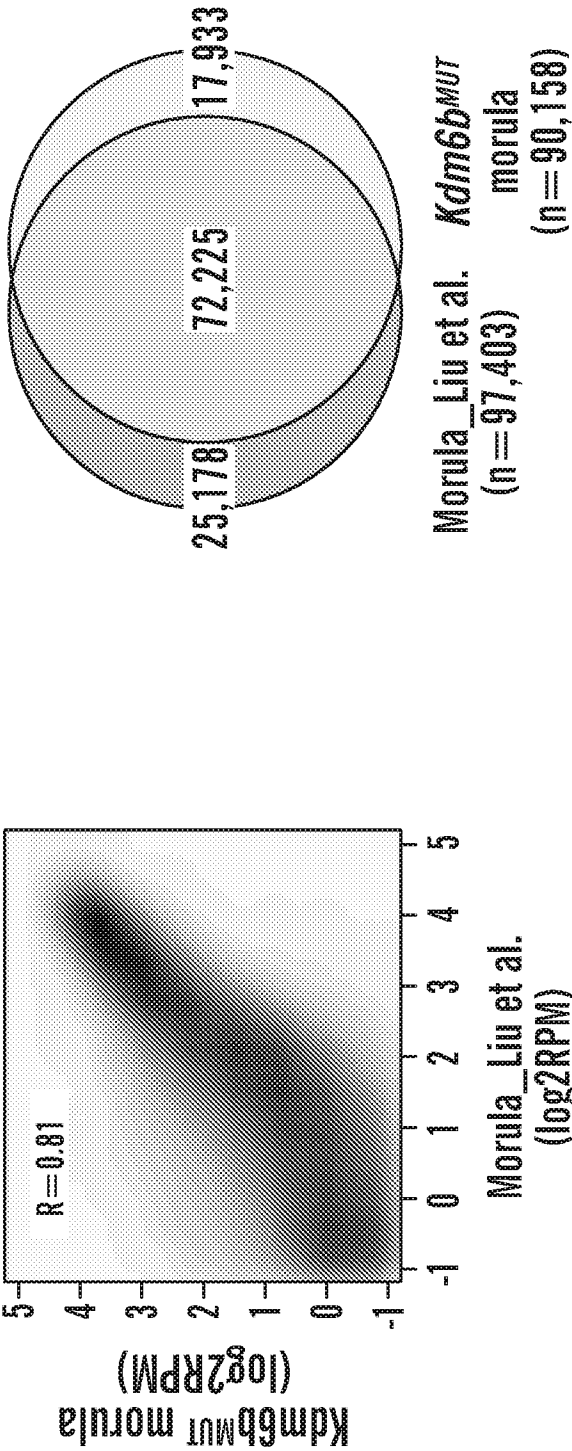


FIG. 6B

FIG. 6C

FIG. 6D

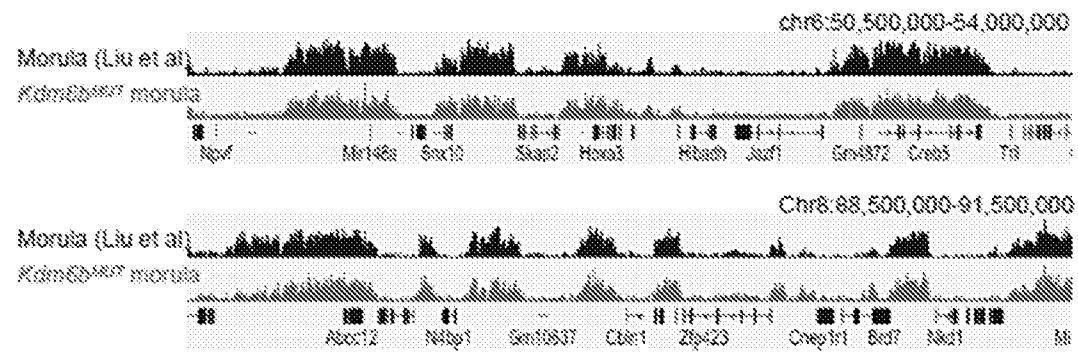


FIG. 6E

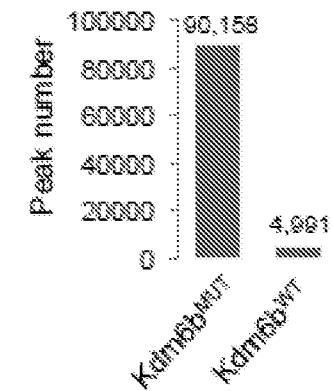


FIG. 6F

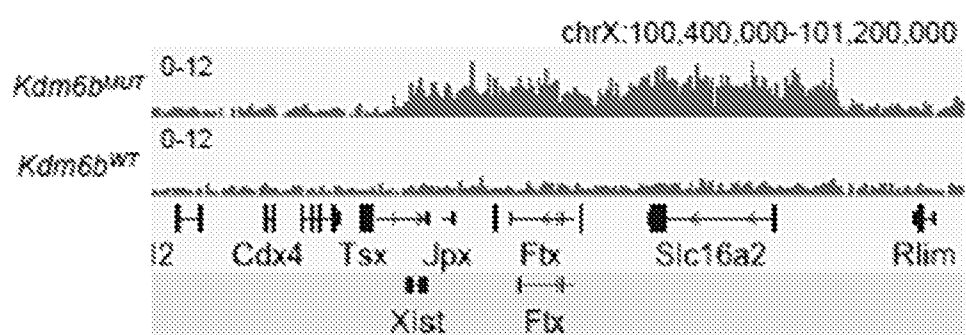


FIG. 7

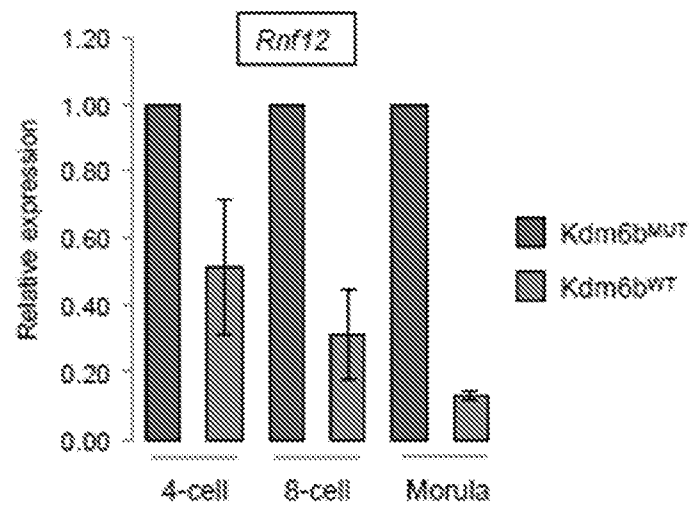


FIG. 8A

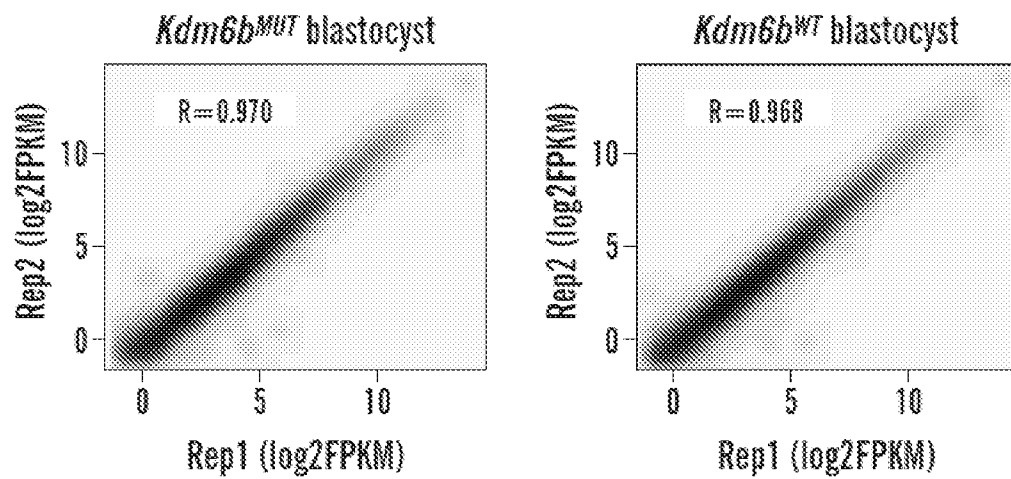


FIG. 8B

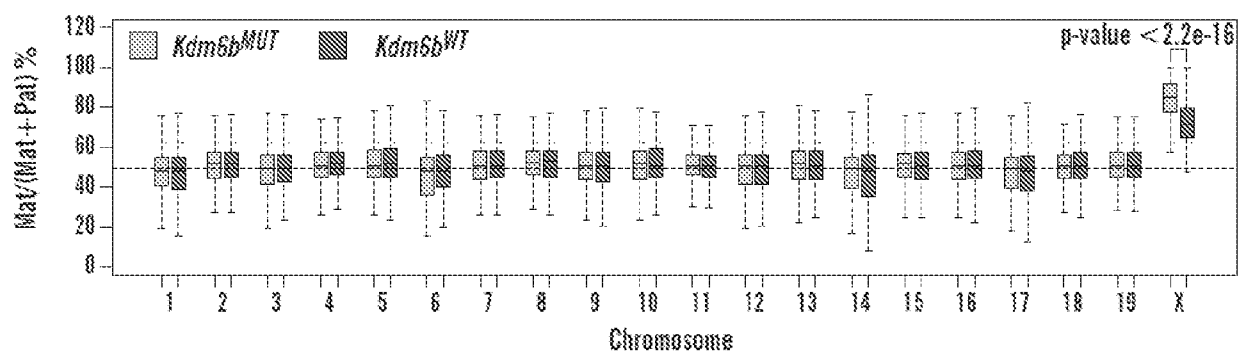
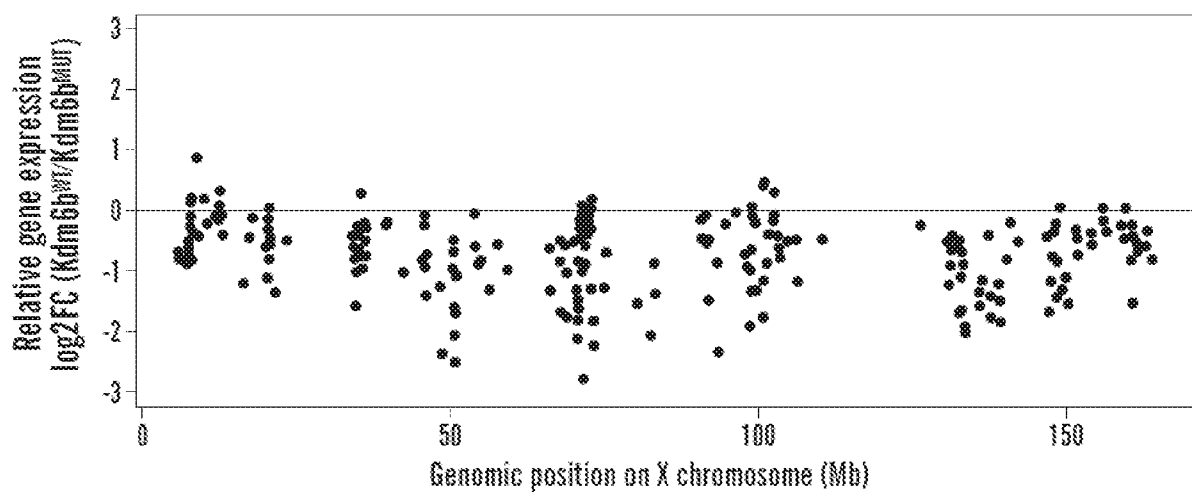


FIG. 8C



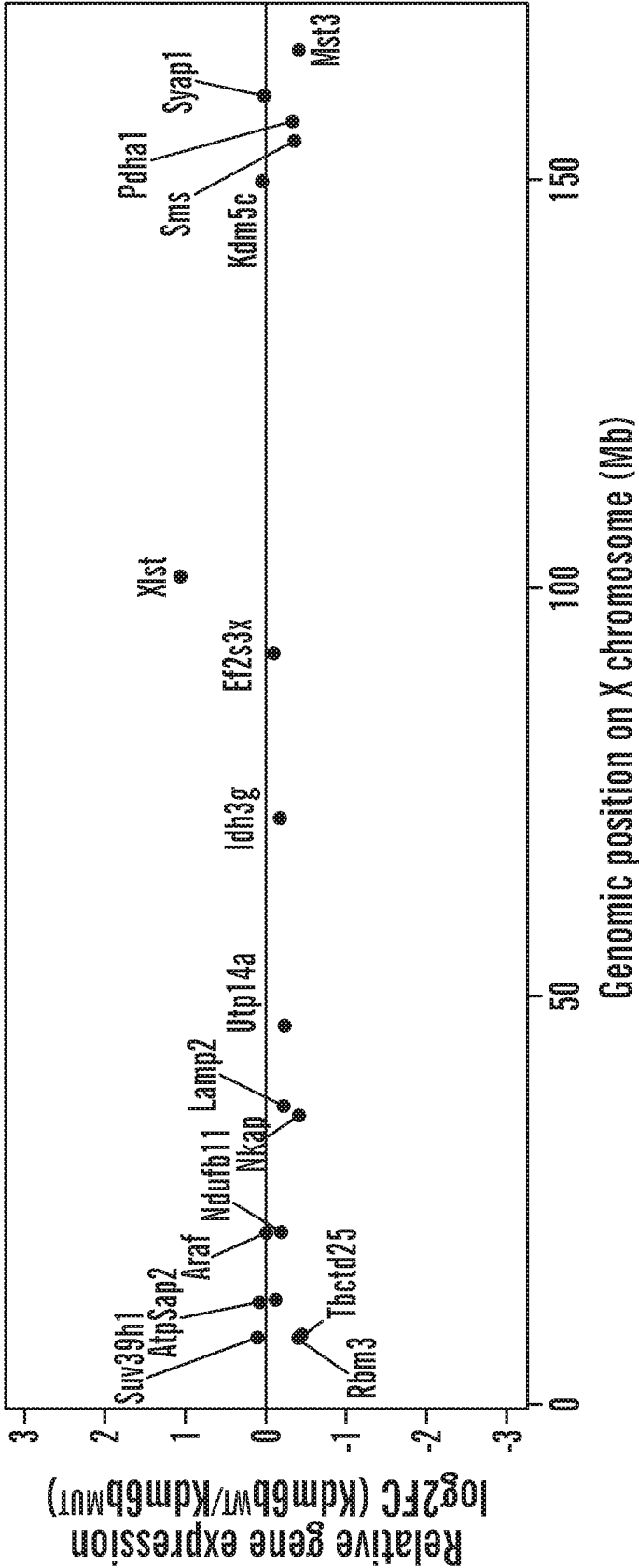


FIG. 8D

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2018/042890

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - A61K 38/44; C12N 15/873; C12N 15/877; C12N 15/89 (2018.01)

CPC - A01K 2217/054; A61K 38/44; C12N 15/873; C12N 15/877; C12N 15/89 (2018.08)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

See Search History document

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

USPC - 424/94.4; 435/455; 800/24 (keyword delimited)

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

See Search History document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	→ CN 107299113 A (INNER MONGOLIA UNIVERSITY) 27 October 2017 (27.10.2017) entire document; see machine translation	1, 11, 21
Y		2-10, 12-20, 22, 23
Y	WO 2017/062706 A1 (CHILDREN'S MEDICAL CENTER CORPORATION et al) 13 April 2017 (13.04.2017) entire document	2-10, 18-20, 22, 23
Y	→ INOUE et al. "Genomic imprinting of Xist by maternal H3K27me3," Genes & Development, 01 October 2017 (01.10.2017), Vol. 31, No. 19, Pgs. 1927-1932. entire document	12-17
A	WO 2018/073787 A2 (FRED HUTCHINSON CANCER RESEARCH CENTER et al) 26 April 2018 (26.04.2018) entire document	1-23
A	WO 2012/029957 A1 (OGURA ATSUO et al) 08 March 2012 (08.03.2012) entire document	1-23
A	→ WELSTEAD et al. "X-linked H3K27me3 demethylase Utx is required for embryonic development in a sex-specific manner," Proceedings of the National Academy of Sciences USA, 23 July 2012 (23.07.2012), Vol. 109, No. 32, Pgs. 13004-13009. entire document	1-23

☐ Further documents are listed in the continuation of Box C.☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

16 October 2018

Date of mailing of the international search report

16 NOV 2018

Name and mailing address of the ISA/US

Mail Stop PCT, Attn: ISA/US, Commissioner for Patents

P.O. Box 1450, Alexandria, VA 22313-1450

Facsimile No. 571-273-8300

Authorized officer

Blaine R. Copenheaver

PCT Helpdesk: 571-272-4300

PCT OSP: 571-272-7774