Stem Cell Reports, Volume *16*

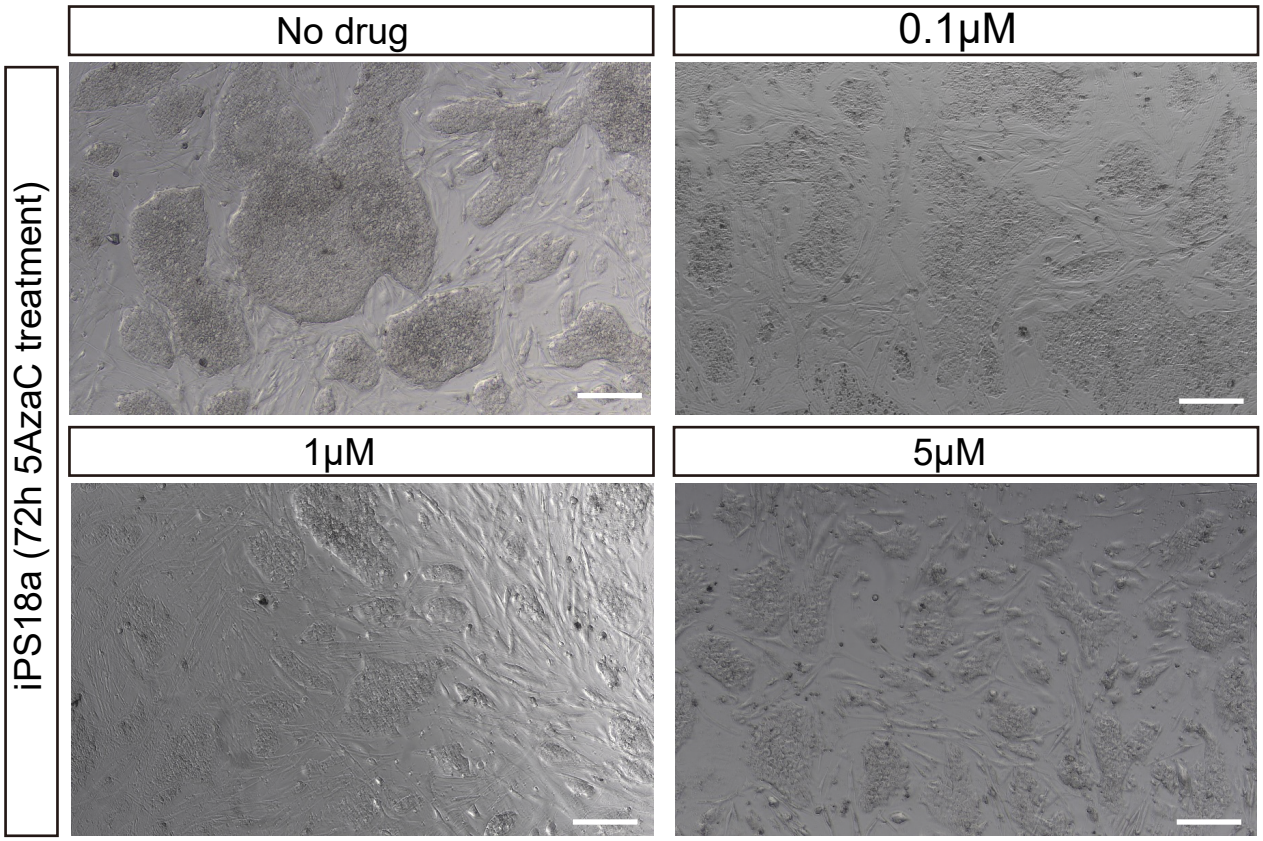
Supplemental Information

De novo DNA methyltransferases DNMT3A and DNMT3B are essential for *XIST* silencing for erosion of dosage compensation in pluripotent stem cells

Atsushi Fukuda, Dane Z. Hazelbaker, Nami Motosugi, Jin Hao, Francesco Limone, Amanda Beccard, Patrizia Mazzucato, Angelica Messana, Chisa Okada, Irune Guerra San Juan, Menglu Qian, Akihiro Umezawa, Hidenori Akutsu, Lindy E. Barrett, and Kevin Eggan

Supplementary Fig. 1

a



b

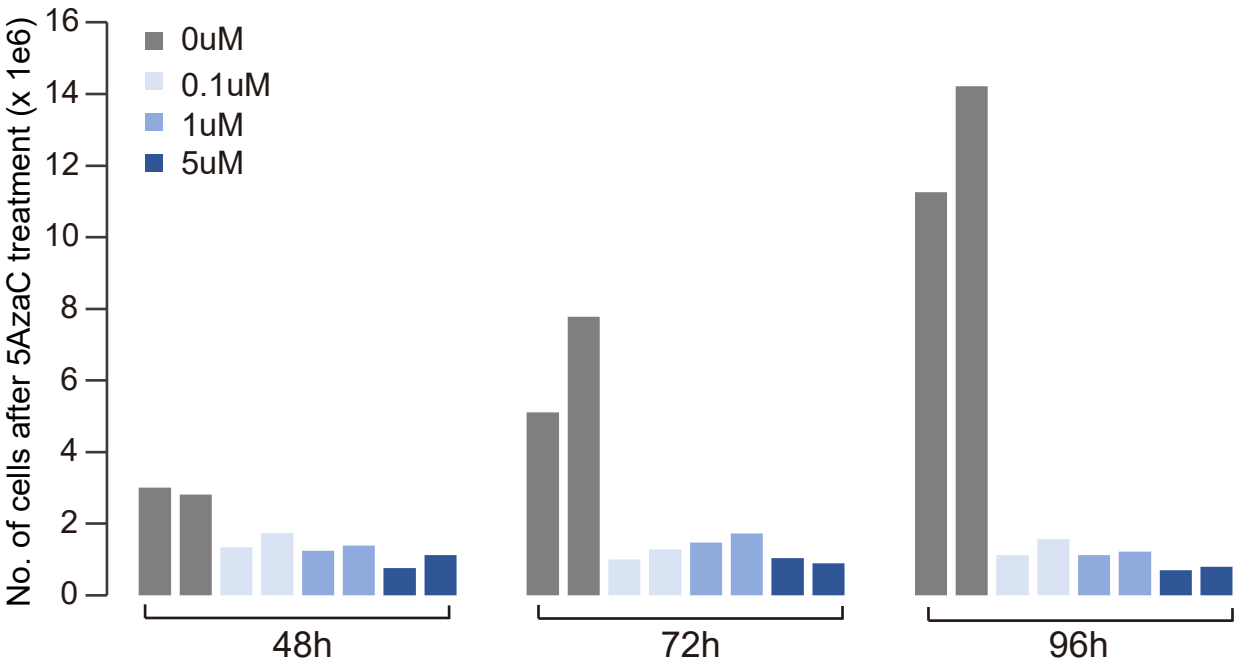


Figure S1. Effect of 5az treatment on hPSCs culture. (a) cultures 72 hours after 5azaC treatment with various concentrations. The scale bar indicates 250 μm. (b) Cell number analysis in each concentration.

Supplementary Fig. 2

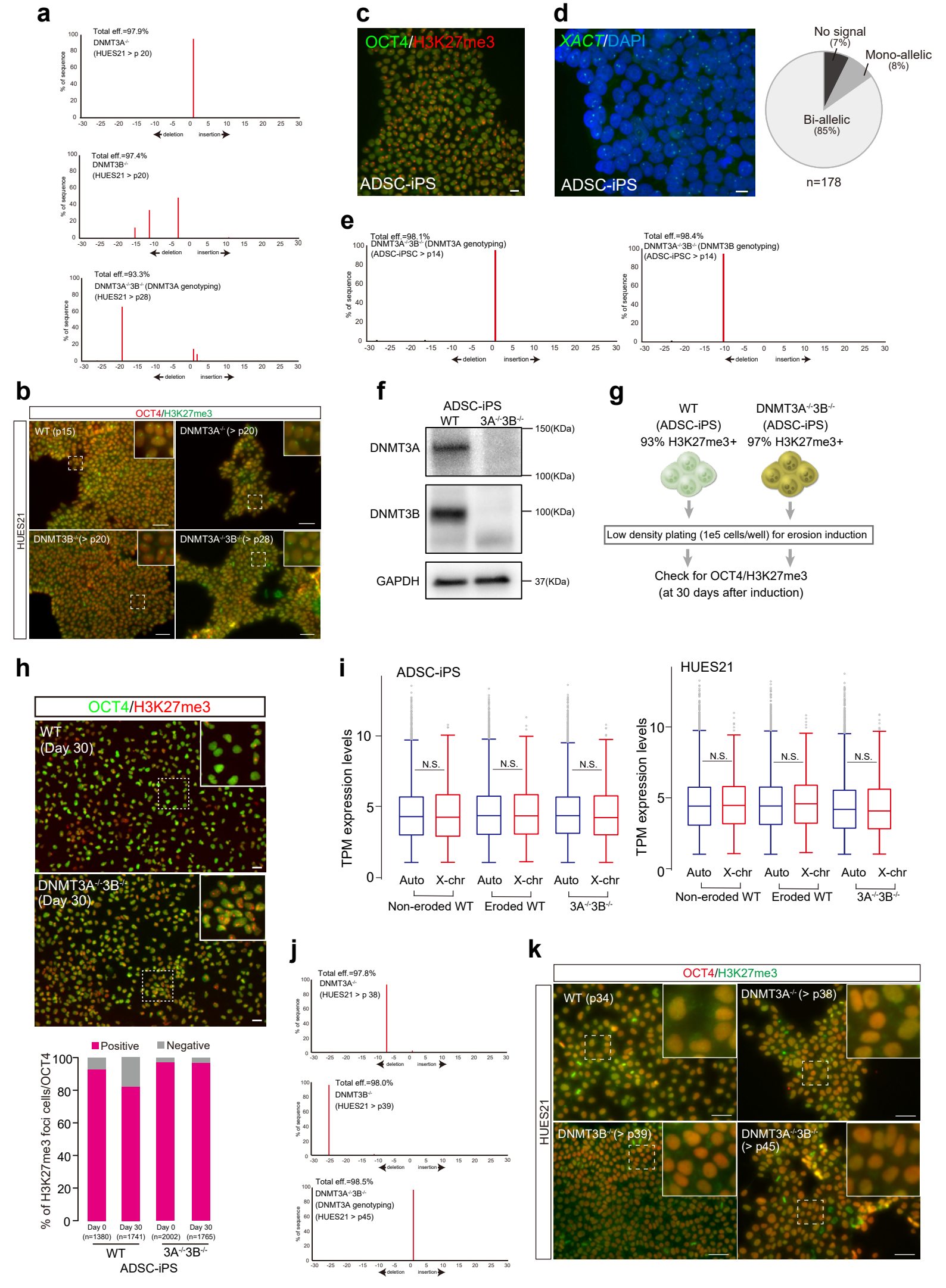


Figure S2. Generation of DNMT3A, 3B, and double knockout liens and RNA-sequencing analysis. (a) Genotyping results by TIDE analysis in HUES21 p14-16 line. (b) Immunofluorescence against OCT4/H3K27me3 in each knockout line derived from HUES21 p14-16. The scale bar indicates 50 μm. (c) H3K27me3/OCT4 staining in ADSC-iPS at p14. The scale bar indicates 20 μm. (d) *XACT* RNA-FISH in ADSC-iPS at p41. n = the number of cells analyzed. The scale bar indicates 10 μm. (e) Genotyping results by TIDE analysis in mutant lines derived from ADSC-iPS at p14. (f) Western blotting for DNMT3A and DNMT3B. GAPDH was used as loading control in mutant lines derived from ADSC-iPS at p14. (g) Experimental scheme for induction of erosion in ADSC-iPS lines. (h) H3K27me3/OCT4 staining 30 days after low-density plating in ADSC-iPS lines. The scale bar indicates 20 μm. Quantification of H3K27me3 foci cells was shown as bar graph. n = the number of cells analyzed. (i) Global gene expression status in each cell line by RNA-sequencing. Boxplots show TPM expression status in autosomal and X-linked genes. The genes used for the bootstrap analysis were measured in ADSC-iPS (left) and HUES21 (right) cell lines. The Wilcoxon rank sum test was used for statistical analysis. N.S. means no significances. (j) Genotyping results by TIDE analysis in DNMT3A, 3B, and double knockout liens derived from HUES21 p34. (k) Immunofluorescence against OCT4/H3K27me3 in each knockout line derived from HUES21 p34. The scale bar indicates 50 μm.

Supplementary Fig. 3

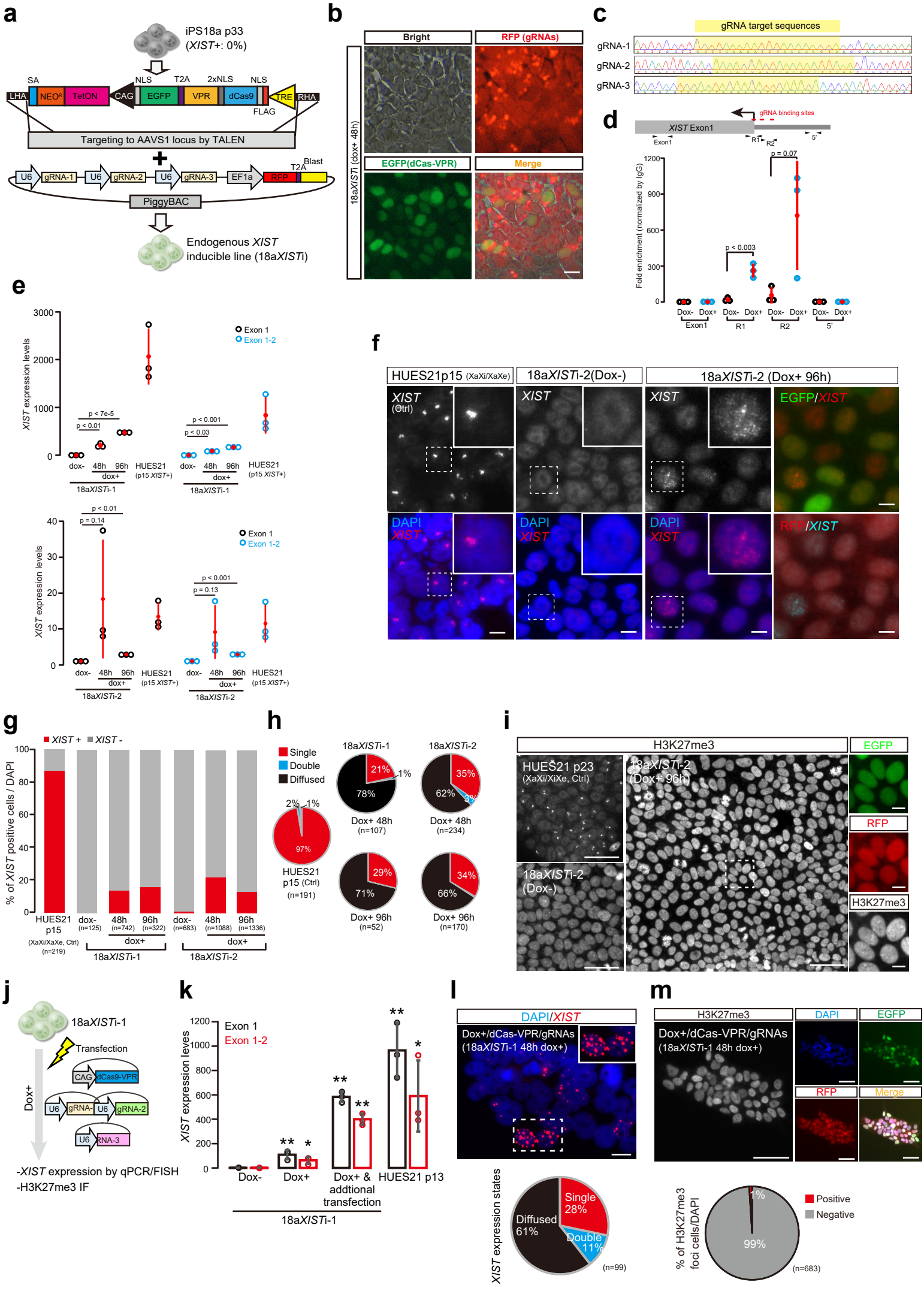


Figure S3. After erosion of dosage compensation CRSPRa can induce *XIST* expression but not restore XCI. (a) Generation of XIST inducible line (18aXISTi) for CRISPR activation studies. (b) Representative images of hPSCs with CRSPRa system after 48 hours of dox administration. The scale bar indicates 10 μm. See also Extended Data Figure 2b and 2c. (c) Sanger sequencing analysis for gRNA target sites. (d) ChIP-qPCR assay using Cas9 antibody. Three red lines indicate gRNA binding sites. The % of input data was normalized using an IgG only control. T-test was conducted for statistical analysis. Red bar indicates average ± SD. T-test was used for statistical calculation. (e) qPCR assay for XIST expression states in inducible lines and HUES21 with XIST positive. The left and right graphs show XIST expression levels of 18aXISTi-1 and 18aXISTi-2 lines, respectively. XIST expression levels were normalized to GAPDH levels. Red bar is average ± SD. T-test was used for statistical calculation. (f) XIST RNA-FISH images. HUES21 p15 (XaXi/XaXe) was used as positive control (Ctrl). The scale bar indicates 10 μm. (g) Quantification of XIST positive cells by RNA-FISH. n=number of cells analyzed. Xa: active X-chr, Xi: inactive X-chr, and Xe: eroded X-chr. (h) Quantification of XIST expression states based on the signals. Diffused state is shown as in (Figure 3e). n = number of cells analyzed. (i) H3K27me3 states in 18aXISTi lines. H3K27me3 immunofluorescence analysis at 96 hours after dox+. HUES21 p23 (XaXi/XaXe) was used as Ctrl. The scale bar indicates 50 μm. The scale bar of enlarged image of is 10 μm. (j) Experimental scheme. Additional dCas9-VPR and three gRNAs are transfected in 18aXISTi-1 line. The cells were cultured with or without dox. At 48 hours after transfection, the cells were analyzed by qPCR, XIST RNA-FISH and H3K27me3 staining. (k) XIST expression states by qPCR analysis. The expression level of dox – sample was set as 1. * and ** indicate $p < 0.05$ and $p < 0.01$, respectively. T-test was used for the statistical calculation. GAPDH was used as internal control for normalization. (l) XIST RNA-FISH in transfected cells with dox treatment for 48 hours. The expression states were based on the signal numbers in the nucleus. n = number of cells analyzed. Scale bar = 10 um. (m) Quantification of H3K27me3 foci in transfected cells with dox treatment for 48 hours. The representative picture is shown. n = number of cells analyzed. The scale bar indicates 50 μm.

Supplementary Fig. 4

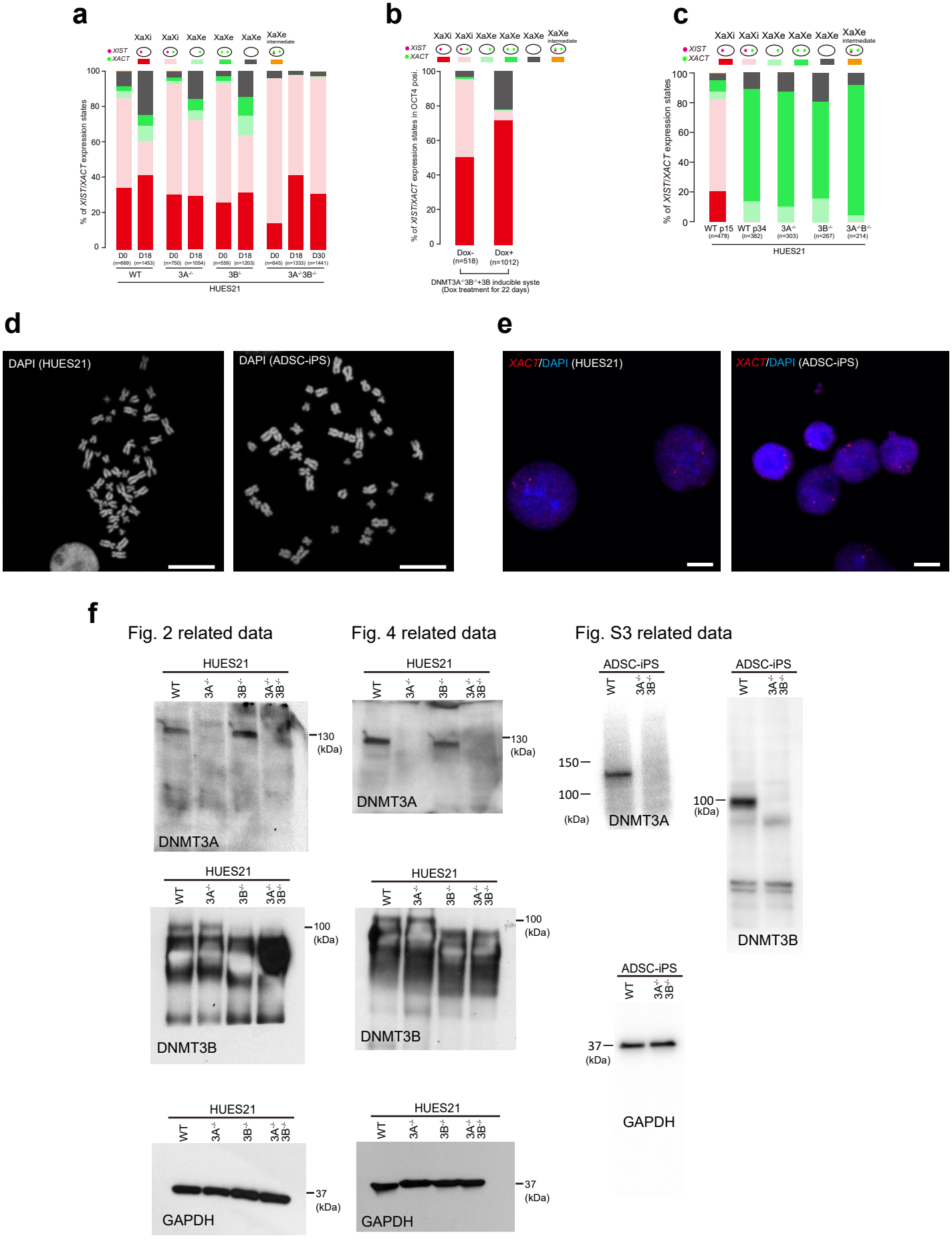


Figure S4. Detailed classification of RNA-FISH results (a-c), chromosome spreading/DNA-FISH (d and e), and uncropped images for western blotting (f). In chromosomal assays, HUES21 (p44) and ADSC-iPS (p56) were used for metaphase spreading (d) and HUES21 (p39) and ADSC-iPS (p52) were used for XACT DNA-FISH experiments, respectively. The scale bar indicates 10 μm. At least 50 cells were analyzed in both assays.

Supplementary Table S1.
Primer sequences

Name	Sequences	Assay
3A_Geno_F1 3A_Geno_R1	CTGTCCCCTCCACCTTCTC TAACCCTGCTTCCTCCCTTTCTA	Genotyping for DNMT3A KO
GF_2 GR_1	GGTAGTGTGACTCGCTCAAGCT GAAGCCCAACACCAGGAAGT	Genotyping for DNMT3B KO
R1_1-F R1_1-R	TTTAATAYGAAAAGTATATAGTAAAGATAAAGAGG TAACRAACTATACTTTATTAAATTATCCAAAATAAC	BS for BS-1 regions
R1_2-F R1_2-R	GTTGTGATTAATTTTATTTTTATTTTTAATTGGTTGGG CATAACRAACCTCTTTATCTTTACTATATAC	BS for BS-2 regions
XIST_E1-2-F XIST_E1-2-R	AGCTCCTCGGACAGCTGTAA GGACACATGCAGCGTGGTA	qPCR for *XIST* mature transcripts
XIST_E1-F XIST_E1-R	TAGCCAGTCAGGAGAAAGAAGTG GACAAATAAGAGGGGACAGAGGT	qPCR for *XIST* immature transcripts
GAPDH-F GAPDH-R	TTGTCAAGCTCATTTCCTGGTATG TCCTCTTGTGCTCTTGCTGG	qPCR for GAPDH
ChIP_e1-F ChIP_e1-R	GATGTAAGCAACGAGGAAGCA GTATTTGTGGACCTGTGTAGGAGAA	ChIP-qPCR for Exon1 regions
ChIP_gRNA-F ChIP_gRNA-R	AGGGCGGAGAGAGCATAAGAG ATAGATGAGAACTGGAAAACCCATT	ChIP-qPCR for R2 regions
ChIP_gRNA-P1-F ChIP_gRNA-P1-R	CAGCCCCGAGAGAGTAAGAAATA ACCAACCAAATCACAAAGATGTC	ChIP-qPCR for R1 regions
5'_XIST-F 5'_XIST-R	AGCAAGAGAGAAAAAGGAAAGAAAG TGGAGGAAATAGGAAAATAGTAAAGA	ChIP-qPCR for Exon4 regions

Experimental procedures

hPSC culture: HUES21 and iPS18a lines were cultured in mTeSer medium (Stem Cell Technology) without feeder cells or on irradiated CF1 mouse embryonic Fibroblasts (Thermofisher Scientific) with standard hPSCs medium containing KO-DMEM (Thermofisher Scientific) supplemented with 20% knockout serum replacement (Thermofisher Scientific), 1% nonesesential amino acids (Thermofisher Scientific), 2 mM glutamax-I (Thermofisher Scientific), 50 units/ml penicillin and 50 ug/ml streptomycin (Thermofisher Scientific), 0.055 mM 2-mercaptoethanol (Thermofisher Scientific), and 20 ng/ml bFGF (Millipore) (Mekhoubad et al., 2012). To establish female ADSC-iPS derived from adipose-derived stromal cells (ADSCs, Lonza Poietics, #PT-5006), mRNA Reprogramming kit (Reprocell, #00-0076) was used according to the manufacturer's instructions. ADSC-iPS line was cultured in StemFlex (Thermofisher Scientific). All cells were culture in 5% CO_2 at 37^{o}C. In the culture with mTeSer medium, Marigel (Corning) and gelatin were used as matrix for mTeSer and standard hPSCs, respectively. For hPSCs passage, 1 mM EDTA (Thermofisher Scientific) was used and 10 μM Y-27632 (Stemgent) was added for 24 hours. Chromosomal normality in high passaged lines was checked by chromosomal spreading and DNA-FISH (Figure S4d and S4e). The hESCs and iPSCs used in this study were previously approved by the institutional review boards of Harvard University, Massachusetts General Hospital, Columbia University, National Center for Child Health and Development, and Tokai University. Our lab regularly checks for mycoplasma contamination using the MycoAlert kit (Lonza) with no cell lines used in this study testing positive. The use of these cells at Harvard was further approved and determined not to constitute Human Subjects Research by the Committee on the Use of Human Subjects in Research at Harvard University.

Motor neuron differentiation: We performed motor neuron differentiation as previously reported

(Klim et al., 2019). In brief, differentiation began once cultured hPSCs became confluent (day 0). The medium was switched to motor neuron differentiation medium (½ Neurobasal (Life Technologies) ½ DMEM-F12 (Life Technologies) supplemented with ×1 B-27 supplement (Gibco), ×1 N-2 supplement (Gibco), ×1 Gibco GlutaMAX (Life Technologies) and 100 µM non-essential amino-acids: NEAA). Treatment with small molecules was conducted as follows: 10 µM SB431542 (Custom Synthesis), 100 nM LDN-193189 (Custom Synthesis), 1 µM retinoic acid (Sigma) and 1 µM Smoothend agonist (Custom Synthesis) on d0–d5; 5 µM DAPT (Custom Synthesis), 4 µM SU-5402 (Custom Synthesis), 1 µM retinoic acid (Sigma) and 1 µM Smoothend agonist (Custom Synthesis) on d6–d14. For the 5azacytidine (Sigma) experiment, 5 µM 5azacytidine was added to differentiation medium from day 3 to day 14.

Generation of *XIST* inducible cell lines (iPS18a*XIST*i) for CRISPR activation system. To create inducible iPS18a*XIST*i lines, 2.5 million iPS18a cells (Harvard University) were transfected with 10 µg TRE3G-dCas9-T2A-EGFP AAVS1 donor plasmid pT076 (Barrett Lab, Broad Institute) along with 1.5 µg AAVS1 TALEN L (Addgene #59025) and 1.5 µg AAVS1 TALEN R (Addgene #59026) via the Neon Electroporation System (ThermoFisher) at 1600 mV, 20 ms, 1 pulses. For the first round of clonal selection, we plated transfected cells at low-density (16,000 cells in a 10 cm dish) under selection with 50 ng/ul G418 (Gibco 10131035) to allow for single-cell colony formation. 10 µM Y-27632 was added to the culture for 14 days. Importantly, cells are kept under selection with 50 ng/ul G418 for the duration of experiments to protect against shutdown of the AAVS1 integrated cassette. In this strategy, colonies are picked and deposited into a 96-well plate and when sufficiently dense, the 96-well plate is triplicated to create 3 plates of identical clones. Plate 1 is frozen for storage, plate 2 is treated with doxycycline (Sigma, D9891-25g) at a final concentration of 2 µg/ml at 24 hours after duplication for visualization of EGFP+ colonies (with high levels of EGFP expression serving as a

proxy for high dCas9 expression), and plate 3 is maintained for expansion and banking of EGFP+ colonies (n=6) while the analysis of plate 2 is performed. Selected clones were genotyped by junction PCR to confirm the on target 5' and 3' integration of the dCas9-VPR cassettes into the AAVS1 locus. Primer sequences for AAVS1 integration are as follows: 5' junction primers GE381 (ACACTCTTTCCCTACACGACGCTCTTCCGATCTAGTCTTCTTCCTCCAACCCG) and GE332 TTCATCCTGCAGCTCGTTCA, 3' junction primers GE223 TCGACTTCCCCTCTTCCGAT and GE379 (GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTCTGCCTAACAGGAGGTGGG). Underline sequences represent target specific regions of the primers

For integration the multiplex PB vectors, 2.5 million iPS18a containing dCas9-VPR were transfected with 1 µg of pGEP150 piggyBac transposase plasmid (Barrett lab, Broad Institute) and 5 µg of pGEP163 piggyBac plasmid (Barrett lab, Broad Institute) containing 3 gRNAs targeting *XIST* via Neon electroporation under conditions described above. 24 hours after transfection, cells are treated with blasticidin at a final concentration of 2 µg/ml for 21-30 days to select for positive piggyBac integrands and allow for clearing of non-integrated plasmid. gRNA sequences for *XIST* activation were selected using CRISPR-ERA (http://crispr-era.stanford.edu/) and are as follows: gRNA1; ACCAGGAGTCACAACTTCAA. gRNA2; GGTTCAAAATTTACCCAGTA, gRNA3, TGGCCTAGAAGATTGAAAGC. We cross-referenced our selected gRNAs sequences to the FANTOM5 (http://fantom.gsc.riken.jp/) CAGE mapping database to ensure we were targeting the major predicted TSS for *XIST*. The three gRNAs driven by U6 promoter were cloned into pGEP163 via Golden Gate cloning as described (Sakuma et al., 2014). Homogeneous colonies were generated by single cell expansion with 2 µg/mL Blasticidin and gRNA expression states were confirmed by fluorescence microscopy.

Transient overexpression for *XIST* induction. The three pGEP001 (gifted from Feng Zhang) for

each gRNA (gRNA1-3) driven by the U6 promoter and SP-dCas9-VPR (Addgene, #63798) for dCas9-VPR expression driven by the EF1a promoter were co-transfected into iPS18a*XIST*i-1 via the Neon Electroporation System at 1050 mV, 30 ms, 2 pulses. After transfection, the cells were culture with 10 μM Y-27632 for 24 hours. For dox+ groups, the cells after transfection were cultured in the presence of dox for 48 hours.

Generation of single DNMT3A, single DNMT3B, and double knockout lines. HUES21 was used for the generation of all of DNMT3A/B knockout lines. Low passage (p14-16) cells were used for Figure 2 related experiments and high passage (>p34) cells were used for Figure 4 related experiments, respectively. The gRNAs previously validated were used for generation of knockout lines (Liao et al., 2015). The gRNA for DNMT3A or DNMT3B was cloned into pGEP001. The pSpCas9(BB)-2A-Puro (PX459) V2.0 (Addgene, #62988) and pGEP001 with gRNA were co-transfected via the Neon Electroporation System at 1050 mV, 30 ms, 2 pulses. At 24 hours after transfection, 1-2ug/mL puromycin was added to the culture medium for 24 hours. The Y-27632 was added to the medium after transfection until the colonies grown sufficiently for passage. The clones were subjected to H3K27me3 staining to select non-eroded clones in Figure 2.

Generation of DNMT3B inducible cell lines

The plasmids for AAVS locus targeting were obtained by Addgene (52341, 52342, and 52343). For DNMT3B coding site region with Myc-tag, which was amplified by PCR with MulI and SalI sites, was replaced into EGFP of original plasmid (52343). The sequences were confirmed by Sanger-sequence. After transfection of HUES21 DNMT3A$^{-/-}$3B$^{-/-}$ (non-eroded line) cells, the cells were grown until 80% of confluent and then subjected to puromycin (0.5-1.0 μg/mL) for 2 weeks. The efficiency of targeting and DNMT3B expression states were confirmed by immunofluorescence analysis using

anti-Myc (Cell Signaling Technology, 2276).

Genotyping. Genomic DNA was extracted using DNAeasy Blood and Tissue kit (Qiagen) and used for PCR. The amplified PCR products were sequenced and the sequence results were analyzed by TIDE (https://tide.nki.nl/) to select compound null lines (Brinkman et al., 2014). The primers used for genotyping are shown in Table S1.

RNA-FISH. The cells for RNA-FISH experiments were cultured onto coverslips. The cells were fixed with 4% paraformaldehyde in PBS- for 15 min at room temperature and then treated with 0.25% triton-X in PBS- for 20 min at room temperature. The treated samples were hybridized with gene specific probes at 37°C overnight. The hybridization buffer contains 4 mg/ml BSA, 4xSSC, 20% dextran-sulfate in nuclease free water. The gene specific DNA probes were prepared based on a previous study(Fukuda et al., 2014) by nick translation kit (Abbot) according to the manufacturer's instruction. Hybridization was conducted using a 1:1 ratio of DNA probe and hybridization buffer. After overnight hybridization, the coverslips were washed with 50% formamide containing 2x SSC at 45°C for 10 min at twice and then washed with 2xSSC containing 0.05% tween-20 at 45°C for 10 min twice. After washing, the coverslips were air-dried and mounted with VECTASHIELD with DAPI (Vectorlabs). Nikon ECIPSE Ti fluorescence microscopy and NIS-Elements AR software were used to obtain images.

For generation of *XIST* probes, plasmid containing 5-kb *XIST* exon 1 was purchased from OriGene (# SC312039). The CTD 3063K22 BAC clone was used for generating the *XACT* probe. For quantification, a minimum of 5 different colonies or fields were analyzed and the number of analyzed cells are described in each Figure. Only cells that did not overlap at interphase were used for analysis. The Fiji (https://fiji.sc/) software was used for quantification analysis and visualization.

Chromosome spreading and DNA-FISH

Sample preparation for the chromosome spreading assay was performed as previously described (Campos et al., 2009). Briefly, hPSCs were cultured in the presence of Colcemid (0.5 μg/mL) to induce metaphase. After treatment with KCl (75 mM), samples were fixed with methanol/glacial acetic acid (3:1). For the chromosome spreading assay, samples were mounted onto glass slides using VECTASHIELD (Vectorlabs) mounting medium with DAPI. For DNA-FISH, samples on the glass slides were incubated for 10 min at 85 °C with hybridization buffer containing the *XACT* probe, prepared as previously described (Fukuda et al., 2016).

Bisulfite DNA sequencing. Extracted genomic DNA was subject to bisulfite conversion using the EZ DNA methylation kit (Zymo Research). The converted DNA was used as template for PCR using EpiTaq HS (Takara). The amplified products were cloned into p-GEM t-easy vector (Promega) by TA cloning and sequenced. The results were analyzed using QUMA (http://quma.cdb.riken.jp/). The primer sequences are shown in Table S1.

qPCR. Total RNA was extracted using RNeasy plus micro kit (Qiagen) and the total RNA was converted to cDNA using iScript™ cDNA Synthesis kit (Bio-Rad). SYBR® Green Master Mix (Bio-Rad) was used for qPCR assay. The data was analyzed using $^{\Delta\Delta}$Ct methods. GAPDH was used as internal control and primers are described in Table S1.

Immunofluorescence stain. Cells were fixed with 4% paraformaldehyde in PBS- for 15 min at room temperature and then permeabilized with 0.25% triton-X in PBS- for 20 min at room temperature. The samples were incubated with 1.5% BSA in PBS- for 1 hour at room temperature. Primary antibodies

used: OCT4 (C-10, SantaCruz) and H3K27me3 (39055, Active Motif). The primary antibodies were incubated at 4°C for overnight. Secondary antibodies used: AlexaFluor 488, 555, and 647 conjugated (1:500, Invitrogen). The secondary antibodies were incubated for 1-2 hours at room temperature. After washing with PBS- at least three times, the samples were incubated with DAPI (Thermofisher Scientific) for those in plastic bottom plates. For samples cultured on glass coverslips, the samples were mounted with VECTASHIELD with DAPI. Nikon ECIPSE Ti fluorescence microscopy and NIS-Elements AR software were used to obtain images.

Immuno-FISH. Cells were fixed and permeabilized as described above. The cells were incubated with blocking buffer including RNaseOUT (1:100, Thermofisher) for 1 hour at room temperature. After wash with PBS-, the samples were incubated with ISL1 (ab109517, abcam) in blocking buffer with RNaseOUT for 1 hour. After wash with PBS-, the cells were treated with 2nd antibody in PBS- for 1 hour at room temperature. The samples were post-fixed with 4% paraformaldehyde for 10 min at room temperature. After wash with PBS-, the samples were subject to RNA-FISH.

Western Blotting. Protein was extracted using RIPA Lysis and Extraction Buffer (Thermofisher). The extracted protein was subjected to SDS-PAGE using 7.5% TGX gel (Bio-Rad) for DNMT3B (ab16049, Abcam) or 4-20% TGX Gel (Bio-Rad) for DNMT3A (2160S, Cell Signaling Technology) and GAPDH (2118, Cell Signaling Technology). Uncropped images are shown in Figure S4f.

ChIP-qPCR. Chromatin was extracted as described previously(Fukuda *et al.*, 2014). In brief, 2 million cells were collected by TryPL express (Thermofisher) and fixed with 1% formaldehyde. The cells were resuspended in SDS lysis buffer and the lysate was sonicated using S220 Focused-ultrasonicator (Covaris). The 10% of chromatin was used as input DNA collection. The chromatin was

immunoprecipitated with Protein A-beads (Veritas Life Sciences) conjugated to anti-Cas9 (Diagenode, C15310258) or rabbit IgG (Abcam, ab171870). DNA from immunoprecipitated chromatin was extracted using phenol chloroform and purified by ethanol precipitation. The DNA was analyzed by qPCR using SYBR green. The primer sequences are shown in Table S1.

RNA-sequence

Total RNA obtained from each sample was extracted using RNeasy Micro plus kit (Qiagen) and used to construct a sequencing library with the NEBNext Ultra II Directional RNA Library Prep Kit for Illumina (New England Biolabs, Ipswich, MA, USA) and NEBNext rRNA Depletion Kit (Human/Mouse/Rat) according to the manufacturer's protocols. Library quality was checked by Agilent 2200 TapeStation High Sensitivity D1000 (Agilent Technologies, Santa Clara, CA, USA). The pooled libraries of the samples were sequenced using the Illumina NextSeq 500 (Illumina, San Diego, CA) in 76-base-pair paired-end reads. Sequencing adaptors, low-quality reads, and bases were trimmed with the Trimmomatic-0.38 tool. The sequence reads were aligned to the human reference genome (hg38) using STAR 2.7.1a. Files of the gene model annotations and known transcripts were downloaded from Illumina's iGenomes website (http://support.illumina.com/sequencing/sequencing_software/igenome.html). The aligned reads were subjected to downstream analyses using the StrandNGS 3.2 software (Agilent Technologies).

Genes with ≧1 TPM in all three lines (average of biological replicates) were used for boot strap analysis. The bootstrap analysis was performed as described in previous reports (Fukuda et al., 2015; Sangrithi et al., 2017). Briefly, median X:A ratios were calculated by bootstrapping with 2000 replications and 100 expression values were randomly selected for each time from X-linked and autosomal genes using the 'sample' function of R 4.0.2 (https://www.r-project.org/). Ninety-five percent bootstrap intervals were also calculated using the 'quantile' function of R. For relative

expression analysis (Figure 3c-e), TMM normalization (Robinson and Oshlack, 2010) and genes with >1 TMM in one sample (average of biological replicates) were used. The statistics and the calculation of mean absolute deviation and hierarchical clustering analysis were conducted using R. All sequence data were deposited in GEO (GSE160454).

References

Brinkman, E.K., Chen, T., Amendola, M., and van Steensel, B. (2014). Easy quantitative assessment of genome editing by sequence trace decomposition. Nucleic Acids Res *42*, e168. 10.1093/nar/gku936.

Campos, P.B., Sartore, R.C., Abdalla, S.N., and Rehen, S.K. (2009). Chromosomal spread preparation of human embryonic stem cells for karyotyping. J Vis Exp. 10.3791/1512.

Fukuda, A., Mitani, A., Miyashita, T., Sado, T., Umezawa, A., and Akutsu, H. (2016). Maintenance of Xist Imprinting Depends on Chromatin Condensation State and Rnf12 Dosage in Mice. PLoS Genet *12*, e1006375. 10.1371/journal.pgen.1006375.

Fukuda, A., Tanino, M., Matoba, R., Umezawa, A., and Akutsu, H. (2015). Imbalance between the expression dosages of X-chromosome and autosomal genes in mammalian oocytes. Sci Rep *5*, 14101. 10.1038/srep14101.

Fukuda, A., Tomikawa, J., Miura, T., Hata, K., Nakabayashi, K., Eggan, K., Akutsu, H., and Umezawa, A. (2014). The role of maternal-specific H3K9me3 modification in establishing imprinted X-chromosome inactivation and embryogenesis in mice. Nat Commun *5*, 5464. 10.1038/ncomms6464.

Klim, J.R., Williams, L.A., Limone, F., Guerra San Juan, I., Davis-Dusenbery, B.N., Mordes, D.A., Burberry, A., Steinbaugh, M.J., Gamage, K.K., Kirchner, R., et al. (2019). ALS-implicated protein TDP-43 sustains levels of STMN2, a mediator of motor neuron growth and repair. Nat Neurosci *22*, 167-179. 10.1038/s41593-018-0300-4.

Liao, J., Karnik, R., Gu, H., Ziller, M.J., Clement, K., Tsankov, A.M., Akopian, V., Gifford, C.A., Donaghey, J., Galonska, C., et al. (2015). Targeted disruption of DNMT1, DNMT3A and DNMT3B in human embryonic stem cells. Nat Genet *47*, 469-478. 10.1038/ng.3258.

Mekhoubad, S., Bock, C., de Boer, A.S., Kiskinis, E., Meissner, A., and Eggan, K. (2012). Erosion of dosage compensation impacts human iPSC disease modeling. Cell Stem Cell *10*, 595-609. 10.1016/j.stem.2012.02.014.

Robinson, M.D., and Oshlack, A. (2010). A scaling normalization method for differential expression analysis of RNA-seq data. Genome Biol *11*, R25. 10.1186/gb-2010-11-3-r25.

Sakuma, T., Nishikawa, A., Kume, S., Chayama, K., and Yamamoto, T. (2014). Multiplex genome engineering in human cells using all-in-one CRISPR/Cas9 vector system. Sci Rep *4*, 5400. 10.1038/srep05400.

Sangrithi, M.N., Royo, H., Mahadevaiah, S.K., Ojarikre, O., Bhaw, L., Sesay, A., Peters, A.H., Stadler, M., and Turner, J.M. (2017). Non-Canonical and Sexually Dimorphic X Dosage Compensation States in the Mouse and Human Germline. Dev Cell *40*, 289-301 e283.

10.1016/j.devcel.2016.12.023.