Supplementary Notes

Additional methods

hESC Spontaneous Differentiation

Teratoma. Confluent hESCs grown on a MEF feeder layer were removed using a cell-scraper and 1 mg/mL Collagenase type IV (Life Technologies, 17104-019) treatment, and resuspended in PBS. One quarter of the cells from a confluent 100 mm dish was injected subcutaneously to the dorsal flank of a SCID mouse (NOD.Cg-Prkdc{scid} Il2rg{tm1Wjl}/SzJ (Stock #: 005557), Jackson Laboratory). Palpable tumors were typically observed 1-2 months after injection. Tumor samples were usually collected in 2-3 months, fixed in 4% paraformaldehyde and processed for paraffin embedding and hematoxylin and eosin staining following standard procedures. Mice injected with TKO hESCs were sacrificed 6 months after injection and dissected to confirm that teratomas had not formed. All mouse procedures were performed following NIH guidelines, and were approved by the local Institutional Animal Care and Use Committee (IACUC), the Institutional Biosafety Committee (IBC) as well as the Embryonic Stem Cell Research Committee (ESCRO).

Embryoid Body Formation and Spontaneous Differentiation. hESCs were expanded onto a 10 cm^2 dish on an iMEF feeder layer. Once the cells were confluent they were dissociated using 1 mg/mL Collagenase type IV (Life Technologies, 17104-019) and removed using a cell scraper. Cells were spun down at 200 g for 5 minutes and washed with phosphate buffered saline solution (PBS). This was repeated 2 times for a total of 3 washes. The cell clumps were then plated into 6-well low attachment plate in hESC media that lacks bFGF. One 10 cm dish gave enough cells for one 6 well plate. The cells were kept in the 6 well low attachment plate for a total of 5 days after which time they formed embryoid bodies. After 5 days the EBs were transferred to a 24 well

dish that has been coated with Geltrex (Life Technologies, A15696) for attachment and differentiation. The cells were kept in the 24 well dish for 7 days total with media change every 2 days. The media remained hESC media without bFGF.

Dot Blot

Genomic DNA was extracted using the DNeasy Blood & Tissue Kit (Qiagen, 69504) following manufacturer's guidelines and diluted to 200 ng/µl and 100 ng/µl in 20 µl total volume. 5 µl of 0.5 M NaOH was added to each sample and the samples were incubated at 99°C for 5 minutes. Samples were cooled on ice, spun down and neutralized with 2.5 µl of 6.6 M Ammonium Acetate. 2.75 µl of each mixture was spotted on a nitrocellulose membrane and allowed to air dry. The membrane was baked for 2 hrs at 80°C and then incubated in blocking buffer for 2 hrs at RT. 5hmC antibody (Active Motif, 39769) at a 1:10,000 dilution in blocking buffer was added to the membrane and incubated overnight at 4C. The membrane was washed in phosphate buffered saline (PBS) with 0.1% Tween 20 (PBSTw), 3 times for 15 minutes each and then incubated with HRP Goat anti-Rabbit IgG (Active Motif, 15015) in blocking buffer for 2 hrs at RT. The membrane was washed again 3 times in PBSTw, and then once in ddH2O for 5 minutes. Luminata Crescendo Western HRP Substrate (Millipore, WBLUR0100) was used for detection.

Mass Spectrometry Quantification of mC and 5hmC

4 µg of genomic DNA was denatured for 5 minutes at 95°C, then sequentially digested with nuclease P1 (Wako Chemicals, 0218055501), *Crotalus adamanteus* venom phosphodiesterase (Sigma, P3243), and *Escherichia coli* alkaline phosphatase (P5931, Sigma). Digested nucleosides were desalted, filtered and analyzed by UHPLC/MS/MS on an Agilent 1290 with UHPLC coupled to a 6460 Triple Quadrupole

LC/MS system using multiple reaction monitoring and the following ion transitions: dC, 228.1→112.1; mdC, 242.1→126.1; hmdC, 248.1→142.1. Molar quantities of dC, mdC, and hmdC were determined by fitting signal intensities to a standard curve of known concentrations. mdC and hmdC quantities were expressed as a molar ratio compared to total dC, mdC, and hmdC present in each sample.

Pluripotency and Lineage Marker Staining

Alkaline Phosphatase Staining. The VECTOR Red Alkaline Phosphatase (AP) Substrate Kit (Vector Labs, SK-5100) was used. Cultured hESCs were washed once with PBS and then incubated with substrate working solution for 30 minutes. Cells were washed 3 times with PBS and images were taken.

Immunofluorescence staining. Cells were fixed with 4% paraformaldehyde for 10 minutes, washed once with PBS and permeabilized in PBST (PBS + 0.15% Triton-X) for 15 minutes. Blocking was done for 5 minutes at RT with blocking solution (5% donkey serum in PBST). Primary and secondary antibodies were diluted in blocking solution. Primary antibodies were incubated at RT for 1 hr. The following primary antibodies were used at a 1:100 dilution: OCT4 (Santa Cruz, sc-5279), NANOG (CosmobioJapan, RCAB0004P-F), and SOX2 (Santa Cruz, sc-17320). The following antibodies were used at a 1:500 dilution: PAX6 (Covance, PRB-278P) and SOX1 (R&D Systems, AF3369). After primary antibody staining the cells were washed three times with PBST and then incubated with the appropriate Molecular Probes Alexa Fluor dye conjugated secondary antibodies (Life Technologies, AF3369) and DAPI for 1 hr at RT. After secondary antibody staining cells were washed 3 times with PBS and images were taken.

FACS (Fluorescence Activated Cell Sorting) Analysis

Intracellular marker. NE differentiated cells at the desired time point were disaggregated with TrypLE for 5 minutes and washed with cold FACS buffer (5% Fetal Bovine Serum, Life Technologies, 261400799, in PBS). Cells were pelleted by centrifugation and washed again with FACS buffer. Each sample was resuspended in FACS buffer and incubated with a Live/Dead Fixable stain (Life Technologies L34964, 1:1,000) for 30 minutes at RT. Afterwards cells were washed with FACS buffer and resuspended in fixation solution (eBioscience, 00-5523-00) for 1 hr at RT. Cells were washed with permeabilization buffer (eBioscience, 00-5523-00) and then resuspended in permeabilization buffer and the appropriate antibody: OCT4-APC (eBioscience 50-5841-82, 1:25) or PAX6 (Covance PRB-278P, 1:400) for 1 hr at RT. For OCT4 flow analysis the cells were washed with FACS buffer and analyzed by FACS. For PAX6 flow analysis, cells were washed with FACS buffer and then incubated with the appropriate Molecular Probes Alexa Fluor dye conjugated secondary antibody (Life Technologies A21206, 1:500) for 1 hr at RT. Cells were then washed with FACS buffer and analyzed by FACS.

Surface marker. Confluent hESCs were disaggregated with TrypLE for 5 minutes and washed with cold FACS buffer. Cells were pelleted by centrifugation and washed again with FACS buffer. Each sample was resuspended in FACS buffer with the appropriate conjugated antibody: Tra1-60-FITC (BD Biosciences, 560380) or Tra1-81-FITC (BD Biosciences, 560194). Cells were incubated in FACS buffer with the antibody for 30 minutes on ice. After staining cells were washed two times with FACS buffer and resuspended in FACS buffer with DAPI and analyzed by FACS.

Quantitative qRT-PCR

Total RNA was isolated with the RNeasy Mini Kit (Qiagen, 74136). DNA was removed from RNA samples using genomic DNA eliminator spin columns. cDNA was produced from RNA using SuperScript III Reverse Transcriptase kit (Life Technologies, 18080051) or High Capacity cDNA Reverse Transcriptase kit (Life Technologies, 4368813). Quantitative real-time PCR was performed in triplicate using ABsolute QPCR SYBR Green Low ROX Mix (Thermo Scientific, AB4322B). Primers used for qRT-PCR are in Supplementary Table 11. Please note that the primer pair we used for *PAX6* binds to Exon 6, as a result it downstream of the *PAX6* promoters (P0 and P1) and will not differentiate between P0 and P1 transcripts.

Epimark

In addition to base level genome-wide 5hmC profiling, 5hmC at the *PAX6* P0 promoter was validated using the Epimark 5hmC and 5-mC Analysis kit (NEB, E3317S) according to the manufacturer's protocol. Briefly genomic DNA was extracted using the DNeasy Blood & Tissue Kit (Qiagen, 69504) following manufacturer's guidelines. 10 ug of DNA for each sample was divided into 6 tubes. The first 3 tubes were treated with beta-gluocosyltransferase (BGT) overnight. 2 of the 6 total tubes were digested with MspI (one tube had been treated with BGT whereas the other had not), 2 of the 6 tubes were digested with HpaI (one tube had been treated with BGT whereas the other had not) and 2 of the 6 original tubes were left undigested. After overnight digestion we performed qRT-PCR analysis. The primers used for analysis (Epimark: PAX6 P0 promoter) are found in Supplementary Table 11.

Supplementary Tables

Supplementary Table 1. CRISPR gRNA sequences used for inactivation of *TET*, *DNMT3B* and *Cas9* genes, for HDR-mediated repair of *TET1* and for targeted demethylation of *PAX6* and *SOX10* promoters and *LEFTY2* enhancer.

Gene Targeted	Purpose	CRISPR Target Sequence
TET1	Gene knockout (CrT1A)	GCCCCAAGGTGTGTATAATA
	Gene knockout (CrT1B)	GGCCCATATTATACACACCT
TET2	Gene knockout (CrT2A)	CTTATGGTCAAATAACGACT
TET3	Gene knockout (CrT3A)	GTCATCTACACGGGGAAGGA
	Gene knockout (CrT3B)	GATCGAGAAGGTCATCTACA
***TET1* (mutated)**	HDR-mediated correction of the *TET1* mutation	CCCAAGGTGTGTATAAATAT
DNMT3B	Gene knockout	TCATTCTTTGATGCTATCAC
Cas9	Gene knockout	TTTCGCCGCTGTCGAACAGC
***PAX6 P0* Promoter**	Targeted demethylation (Cr6)	CCTAGCTCCCCTCCAGGACC
	Targeted demethylation (Cr7)	TTACCAAGAAACTAGGTCAC
	Targeted demethylation (Cr9)	CTAGCTGGCCAGTTTCTGTC
***SOX10* Promoter**	Targeted demethylation	CACAGTAAGAGAGACTTCTC
***LEFTY2* Distal Enhancer**	Targeted demethylation	GAGAAGCAGAGCAGGAAGTC
HBB	Targeted demethylation, non-targeting Control	TTTGCATATTCTGGAGACGC

Supplementary Table 2. Sequences at *TET1*, *TET2* and *TET3* for the *TET* knockout lines.

hESC Line	*TET1* genotype	*TET2* genotype	*TET3* genotype	CRISPR gRNAs
HUES8 WT	WT	WT	WT	CrT1B, CrT2A, CrT3B
HUES8 *TET1* KO	-8bp/-8bp	WT	WT	CrT1B
HUES8 *TET2* KO	WT	-16bp/-16bp	WT	CrT2A
HUES8 *TET3* KO	WT	WT	-4bp/-4bp	CrT3B
HUES8 *TET1/2* DKO	-13bp/-1bp	-4bp/-11bp	WT	CrT1B, CrT2A
HUES8 *TET1/3* DKO	+1bp/+1bp	WT	-4bp/+1bp	CrT1B, CrT3B
HUES8 *TET2/3* DKO	WT	-8bp/+1bp	-4bp/-4bp	CrT2A, Cr3B
HUES8 TKO	+1bp/+1bp	+1bp/+1bp	-4bp/+1bp	CrT1B, CrT2A, CrT3B
MEL1 WT	WT	WT	WT	CrT1A, CrT2A, CrT3A
MEL1 TKO	+1bp/+1bp	-5bp/-1bp	-1bp/+1bp	CrT1A, CrT2A, CrT3A

Supplementary Table 3. qPCR primers used for mRNA expression analysis, Epimark 5hmC analysis and ChIP-qPCR (for TET1, DNMT3B, H3K4me3 and H3K27me3).

Genomic Region	Forward Primer	Reverse Primer
T	ACCCAGTTCATAGCGGTGAC	CCATTGGGAGTACCCAGGTT
DNMT1	AGGCGGCTCAAAGATTTGGAA	GCAGAAATTCGTGCAAGAGATTC
DNMT3A	AGTACGACGACGACGGCTA	CACACTCCACGCAAAAGCAC
DNMT3B	GACTGCTTGGAATACAATAGGA	GCACCACAAAACATCTTCTTT
FOXA2	GGGAGCGGTGAAGATGGA	TCATGTTGCTCACGGAGGAGTA
FOXG1	CCGCACCCGTCAATGACTT	CCGTCGTAAAACTTGGCAAAG
GAPDH	GGAGCCAAACGGGTCATCATCTC	GAGGGGCCATCCACAGTCTTCT
GSC	AACGCGGAGAAGTGGAACAAG	CTGTCCGAGTCCAAATCGC
LEFTY2	CTCCAACGCCAGGAACC	GACATGGAGGAGCTGGTCA
LHX2	CCAAGGACTTGAAGCAGCTC	GTAAGAGGTTGCGCCTGAAC
NANOG	GCCTGGTTGCCTCATGTTATTATGC	CCATGGAGGAAGGAAGAGGAGAGA
OCT4	TGGTCCGAGTGTGGTTCTGTAA	TGTGCATAGTCGCTGCTTGAT
OTX2	CATGCAGAGGTCCTATCCCAT	AAGCTGGGGACTGATTGAGAT
PAX6	TGGGCAGGTATTACGAGACTG	ACTCCCGCTTATACTGGGCTA
PAX6 3'UTR (Endo)	ATTGTGTTAATTCAGTCAGTG	CCCAGTGGTACAATACAGGAC
SOX1	AACACTTGAAGCCCAGATGGA	GCAGGCTGAATTCGGTTCTC
SOX10	CTTTCTTGTGCTGCATACGG	AGCTCAGCAAGACGCTGG
SOX17	GGCGCAGCAGAATCCAGA	CCACGACTTGCCCAGCAT
Epimark: *PAX6 P0 Promoter*	GGCAGGAATCATTTTTAGGAGGA	CCTGGAGAGACCTTTGGCCTA
ChIP qPCR: *PAX6 P0 Promoter 1*	CCTCCCACTGGCCACTCTAGT	TAGGGGCTTACCAAGAAACTA
ChIP qPCR: *PAX6 P0 Promoter 2*	CCCCGCCTGCTTATCTGCCTG	CTTCTCCCTCGGCGACCCCAG
ChIP qPCR: *PAX6 P0 Promoter 3*	CGGGAAGAAGGGCACCGCGGG	AGTCTGTGCTACCCCGGGCTG
ChIP qPCR: *PAX6 P1 Promoter 1*	CCTAAGCTGGACTCGGGACTC	CCGCCGCTGTGCTCTGTGTCT
ChIP qPCR: *PAX6 P1 Promoter 2*	GTCTGCTCAGTCCACGGAGGC	GGAGTGTACTGAGGTGTGTCC
ChIP qPCR: *PAX6 P1 Promoter 3*	CTCGCCTCCACCGCTCCTCAC	GAGAGCGAGCGGTGCATTTGC
ChIP qPCR: E *-6k Enhancer*	CATCCTTTCCTTCTTTCTGCT	CGTCCCCGCCGTGCAAAGAGA
ChIP qPCR: E *156k Enhancer*	ACTTAAATGCCTCAAACTTTT	TCTTGTAAATGAGTAGCCTAT
ChIP qPCR: *NANOG Promoter*	GATGGGGGAATTCAGCTCAGG	GTCTCTCTTAATCAGCACAGT
ChIP qPCR: *HERVK*	AGAGGAAGGAATGCCTCTTGCAGT	TTACAAAGCAGTATTGCTGCCCGC
ChIP qPCR: *GATA3*	GCTCCAGTCAAAGGCATCTC	TAGGCACGCATGGATCATTA
ChIP qPCR: *HAND1*	GGCAAGGCTGAAAATGAGAC	GATAGCCACTCCCCCTTTTC
ChIP qPCR: *GATA6*	GGATGAGAACGGTTTCTGGA	TTGTGAACTTGTGGCTCCTG
ChIP qPCR: *SOX1*	CAAGTGGTTTGTGCATCAGG	GACGGAGAGGAATTCAGACG

Supplementary Table 4. 5mC MassArray bisulfite sequencing primers.

Genomic Region	Forward Primer	Reverse Primer
PAX6 P0 Promoter 1	AGGAAGAGAGAGTTTTTTTTAAGGTTGGTGGTAGG	CAGTAATACGACTCACTATAGGGAGAAGGCTCCTTCCTAACTAACCAATTTCTATCC
PAX6 P0 Promoter 2	AGGAAGAGAGAGTATTTTGTTGGGTTGTAGGGATT	CAGTAATACGACTCACTATAGGGAGAAGGCTACCTCCCACTAACCACTCTAATCTA
PAX6 P0 Promoter 3	AGGAAGAGAGATTTTAGGATATTTGAGGTTGGAGG	CAGTAATACGACTCACTATAGGGAGAAGGCTACCAAAAAACAACTCCAAACAAC
PAX6 P0 Promoter 4	AGGAAGAGAGTTTTAATTTGAAAGTGATAGTGGTGG	CAGTAATACGACTCACTATAGGGAGAAGGCTAAACAAAACAAAAAATCAAATCCAA
PAX6 P0 Promoter 5	AGGAAGAGAGGGTGTGAGGGAAAAATAGGTATAGAT	CAGTAATACGACTCACTATAGGGAGAAGGCTCTCTAAATAATCACCCTATCCCCTC
E -6k Enhancer 1	AGGAAGAGAGTTGTTGTTTGATTTTGAATATGAATAG	CAGTAATACGACTCACTATAGGGAGAAGGCTCCTAAAAACCCCTAAAACAAAAAAA
E -6k Enhancer 2	AGGAAGAGAGTTGTTTTTTTGTTTTAGGGGTTTTT	CAGTAATACGACTCACTATAGGGAGAAGGCTAAAACCTTAACCTAACTTACACCCC
E -6k Enhancer 3	AGGAAGAGAGGTAGGATTTGTTGGGAAAAAGGTAT	CAGTAATACGACTCACTATAGGGAGAAGGCTCTAAACAAAAAAACCCCTTCTAACC
E -6k Enhancer 4	AGGAAGAGAGGAGGTTTTGGTTTAGGTTTTTTTTG	CAGTAATACGACTCACTATAGGGAGAAGGCTACTACCTAACTTTAAATATAAACAATCACA
E -6k Enhancer 5	AGGAAGAGAGGTTTAGGTTTTTTTTGATTGGGATT	CAGTAATACGACTCACTATAGGGAGAAGGCTTAAAAAATAAATTTCTTCCCCTCCC
E -6k Enhancer 6	AGGAAGAGAGTGTAATTTGTAATTATTGTTTTTTATGTGG	CAGTAATACGACTCACTATAGGGAGAAGGCTTAATCTTCTCCCAACCTCCCTACTA
E -6k Enhancer 7	AGGAAGAGAGTTTTTTGTGATAAAGGTTTGTAGTTGTT	CAGTAATACGACTCACTATAGGGAGAAGGCTACCAAACCAAAACCTCCTCAATAC
E -6k Enhancer 8	AGGAAGAGAGGGAGTGGAGATTTTTTGGTTTTT	CAGTAATACGACTCACTATAGGGAGAAGGCTAAACCTTTAAACTCAACCCTTACTTTT
E 156k Enhancer 1	AGGAAGAGAGTGGGTTAAATTTAGTTTTATGTTGTTG	CAGTAATACGACTCACTATAGGGAGAAGGCTACTTTAAAACACTTTAAACTCCCTTCC
E 156k Enhancer 2	AGGAAGAGAGTGTTTTTTAGGTTGGAGTATAGTGA	CAGTAATACGACTCACTATAGGGAGAAGGCTCCACCTTAACCTCCCAAAATACTAA
E 156k Enhancer 3	AGGAAGAGAGATTATTTTGGTTGGTTAGGTTGGTT	CAGTAATACGACTCACTATAGGGAGAAGGCTACCTTCCAAAAATAAAATACCCAAAA
E 156k Enhancer 4	AGGAAGAGAGGTTTTTAATAGTTTTTGGAAATAAAGATAG	CAGTAATACGACTCACTATAGGGAGAAGGCTAACTCACACCTATAATCTCAACACTTT
E 156k Enhancer 5	AGGAAGAGAGGGTTGGATGAGAAAATATGTGTATATAATG	CAGTAATACGACTCACTATAGGGAGAAGGCTCATAAACCAAATCCAACTCCATATT
E 156k Enhancer 6	AGGAAGAGAGTTGGTTTTTTATGTTGGTTGTAAAAA	CAGTAATACGACTCACTATAGGGAGAAGGCTAATAAAACCCTATCTCTACTAAAAATTCAA
E 156k Enhancer 7	AGGAAGAGAGAAGAAATAGTTTTATTTTAGGTTAGGAAA	CAGTAATACGACTCACTATAGGGAGAAGGCTAAACTCAAACAATCCTCCTACCTC
E Ele4h Enhancer 1	AGGAAGAGAGTTAATTTTAGAGAATAGGGAGAGGGA	CAGTAATACGACTCACTATAGGGAGAAGGCTTCACAAAATAACAAAACTACCACAAA
E Ele4h Enhancer 2	AGGAAGAGAGTTTGTGGTAGTTTTGTTATTTTGTGA	CAGTAATACGACTCACTATAGGGAGAAGGCTCTTTCTAAAACAACCTTTCTTTTTCC
E Ele4h Enhancer 3	AGGAAGAGAGGTTTTGGTGAGGGTTTTTTTT	CAGTAATACGACTCACTATAGGGAGAAGGCTTAACTCCCTATTCAACTCTCCTCTC
E Ele4h Enhancer 4	AGGAAGAGAGTTTGGTATTAGTAGATTGGGAATTG	CAGTAATACGACTCACTATAGGGAGAAGGCTCTTCTCCAACAAAACAAAACCTAAC
E Ele4h Enhancer 5	AGGAAGAGAGTTTTGGTATTAGTAGATTGGGAATTGT	CAGTAATACGACTCACTATAGGGAGAAGGCTCCCACCTCTAAACACAAAAAACTT
E Ete1 Enhancer 1	AGGAAGAGAGGTTGTAGATTTGGGAATAGGTAGG	CAGTAATACGACTCACTATAGGGAGAAGGCTTAACCAAATTTAAAAATCACCCTCT
E Ete1 Enhancer 2	AGGAAGAGAGTTTATGATTGGAGGAGAAGGTTTAT	CAGTAATACGACTCACTATAGGGAGAAGGCTTATCCCCTTAATAAATCAAAAAAAA
E Ete1 Enhancer 3	AGGAAGAGAGTGGAAGTTTATTTTTAGGAGTGGAA	CAGTAATACGACTCACTATAGGGAGAAGGCTACCTAAACACCCTCTTTTCTTATCATTA
E Ete1 Enhancer 4	AGGAAGAGAGGGTAGGGGAAGTGGTAGATTTGAT	CAGTAATACGACTCACTATAGGGAGAAGGCTACCCAAAAAAATAACTAACCAACCT
E Ete1 Enhancer 5	AGGAAGAGAGATTTTTGTTTTGGTTTTTTTTGGTT	CAGTAATACGACTCACTATAGGGAGAAGGCTCCCTTTAAAACTTCCCTCTTAAAAA
E Ete1 Enhancer 6	AGGAAGAGAGTTTTTAAGAGGGAAGTTTTAAAGGG	CAGTAATACGACTCACTATAGGGAGAAGGCTAATCTCCATTCCAAAATAACCAAAC
E Ete1 Enhancer 7	AGGAAGAGAGGGTTTGTAGGATATTGATTTGTTGG	CAGTAATACGACTCACTATAGGGAGAAGGCTAAAAACTAACCCTAAAACCCTCCTC
E Ete1 Enhancer 8	AGGAAGAGAGGGTTGTTAGTTTGGGTGGTGTTAT	CAGTAATACGACTCACTATAGGGAGAAGGCTAATTAAAACAAACTATTCCCAACCC
E Ete1 Enhancer 9	AGGAAGAGAGTTTTTTTATAGAATTTGGATGATTGG	CAGTAATACGACTCACTATAGGGAGAAGGCTTCTCACAAAAAATAACTTCCAAACC

***E Ete1 Enhancer* 10**	AGGAAGAGAGTTTAGTTATTTGTGATAGGTGTTGGG	CAGTAATACGACTCACTATAGGGAGAAGGCTAAAATCTAAATAATAATTCCAATCATCCA
***E Ete1 Enhancer* 11**	AGGAAGAGAGTTTTTTAGGTTTTTTGGGAGTATTTT	CAGTAATACGACTCACTATAGGGAGAAGGCTAAAAAAAATCCACTTCCCACTAC
***E Ete1 Enhancer* 12**	AGGAAGAGAGTGTTTTTTTTAGTGAGTTAGGGAAGG	CAGTAATACGACTCACTATAGGGAGAAGGCTAAAATCCCAACACCTATCACAAAT
***E Ete1 Enhancer* 13**	AGGAAGAGAGGGTTGTAAAGTGTAGATGGTTGGAT	CAGTAATACGACTCACTATAGGGAGAAGGCTCCCTTCCCTAACTCACTAAAAAAAC
***E Ete1 Enhancer* 14**	AGGAAGAGAGGTTTTGAGTTGGGAGTAGGGG	CAGTAATACGACTCACTATAGGGAGAAGGCTATCCAACCATCTACACTTTACAACC
***E Ete1 Enhancer* 15**	AGGAAGAGAGGGATTATGGTAAGGTTTAGGTTTAGA	CAGTAATACGACTCACTATAGGGAGAAGGCTACCAAAAAACAACTCCAAACAAC
***E P1 Promoter E1 Enhancer* 1**	AGGAAGAGAGGGGTGTAAGTTTTTGTGTTGTTTTT	CAGTAATACGACTCACTATAGGGAGAAGGCTACCTCTCCAACCAAAACTATACCTC
***E P1 Promoter E1 Enhancer* 2**	AGGAAGAGAGGAGGTATAGTTTTGGTTGGAGAGGT	CAGTAATACGACTCACTATAGGGAGAAGGCTAATAAAACACACACACACACACACA
***E P1 Promoter E1 Enhancer* 3**	AGGAAGAGAGGGTAGGGAGGTTGAAATGAAGTAGT	CAGTAATACGACTCACTATAGGGAGAAGGCTACCCCACCTCTAACTAAAACC
***E P1 Promoter E1 Enhancer* 4**	AGGAAGAGAGTTGGAGGATGATGATAGAGGTTAGGT	CAGTAATACGACTCACTATAGGGAGAAGGCTAAAACATCCTTTCTAATTATCACAACTTC
***E P1 Promoter E1 Enhancer* 5**	AGGAAGAGAGTTGTTTTGTATAAAGTAATATTTTGTGTGA	CAGTAATACGACTCACTATAGGGAGAAGGCTAAAAATAACTACAACCAACACACCT
***E P1 Promoter E1 Enhancer* 6**	AGGAAGAGAGGGTGAGGATTTTTTAGGGTTTTTT	CAGTAATACGACTCACTATAGGGAGAAGGCTTATCCTTTCTTACCAACTCCAAAAC
***E P1 Promoter E1 Enhancer* 7**	AGGAAGAGAGGGTGAGGATTTTTTAGGGTTTTTTA	CAGTAATACGACTCACTATAGGGAGAAGGCTATCCTAACAACTCCATTCCAACTAC
***E P1 Promoter E1 Enhancer* 8**	AGGAAGAGAGGGATTGAGTAGATTTAGGAGAGGGA	CAGTAATACGACTCACTATAGGGAGAAGGCTAAAAACACAACTCTAACTAAAAAAACCC
***E P1 Promoter E1 Enhancer* 9**	AGGAAGAGAGTTTTGGTAATAGTTGAAGGGGAGTT	CAGTAATACGACTCACTATAGGGAGAAGGCTTAAAAAACCCTAAAAAATCCTCACC
***E P1 Promoter E1 Enhancer* 10**	AGGAAGAGAGGTTTTGTTTTGGGGTGTGTATAGTA	CAGTAATACGACTCACTATAGGGAGAAGGCTAAACAACTCCCCTTCAACTATTACC
***E P1 Promoter E1 Enhancer* 11**	AGGAAGAGAGGGTTGTTGGGTTTTAGGTAGGAA	CAGTAATACGACTCACTATAGGGAGAAGGCTAAACACACCTCAATACACTCCAAAA
***GAPDH* Promoter 1**	AGGAAGAGAGTTTAGAAAGGTAGGGTTAGGGATTG	CAGTAATACGACTCACTATAGGGAGAAGGCTCAATATACCTTTCATTCCATCCAAC
***GAPDH* Promoter 2**	AGGAAGAGAGGTTGGATGGAATGAAAGGTATATTG	CAGTAATACGACTCACTATAGGGAGAAGGCTAAACAAAAACCTAAAAAAAACCCAT
***GAPDH* Promoter 3**	AGGAAGAGAGGAGATGTTAGGAGTTAGGAGATGGG	CAGTAATACGACTCACTATAGGGAGAAGGCTTCCCAAAACTAAACTATAAACAACAAAA
***OCT4* Promoter 1**	AGGAAGAGAGGATTAGATTTTGGATTGATTGGG	CAGTAATACGACTCACTATAGGGAGAAGGCTAATTAAAAAACAAAAAACAATCCCC
***OCT4* Promoter 2**	AGGAAGAGAGTGTTTTTTAGGAATTTAGGTGTTTGA	CAGTAATACGACTCACTATAGGGAGAAGGCTAAAAAAAATAAAAACCAACCCCTT
***OCT4* Promoter 3**	AGGAAGAGAGGTTTAATGGTGGTGGTAATGGTGTT	CAGTAATACGACTCACTATAGGGAGAAGGCTACAAAACCTAAAAAAATACCAAAAA
***NANOG* Promoter 1**	AGGAAGAGAGTGTGGGAGTAAAGTTAGTTGTTTTG	CAGTAATACGACTCACTATAGGGAGAAGGCTTATCTATCCCTCCTCCCAAATAATC
***NANOG* Promoter 2**	AGGAAGAGAGTTTTTGTATTTTGTTTTTGGGTTTG	CAGTAATACGACTCACTATAGGGAGAAGGCTCTTAATAACCTTAACAACCCCCACT
***NANOG* Promoter 3**	AGGAAGAGAGAATTTTTTGAATTTGGGAGGTAGAG	CAGTAATACGACTCACTATAGGGAGAAGGCTAAAAAACATAACAAATCACCAAACC
***NANOG* Promoter 4**	AGGAAGAGAGGGGTATATTTTTTATTGATTTATTTTTGTG	CAGTAATACGACTCACTATAGGGAGAAGGCTTAACTAAAACTACAAACACCCACCA

***SOX2* Promoter 1**	AGGAAGAGAGAAAGATTTTAATAAGAGAGTGGAAGGAA	CAGTAATACGACTCACTATAGGGAGAAGGCTAACAAAACCAACCCTAACATTTTC
***SOX2* Promoter 2**	AGGAAGAGAGGTGGGATGTTAGGAAGTTGAAATTAT	CAGTAATACGACTCACTATAGGGAGAAGGCTTTAAAAAACCCAAACCTCTATCCTC
***SOX2* Promoter 3**	AGGAAGAGAGGGATAGAGGTTTGGGTTTTTTAATTT	CAGTAATACGACTCACTATAGGGAGAAGGCTAAACCAACCTACCAACCACTAAAA
***FOXA2* Promoter 1**	AGGAAGAGAGTGGTTTGGATATTTTATAAAGAGGGT	CAGTAATACGACTCACTATAGGGAGAAGGCTAAAATAAACACCCACATAAACTCACA
***FOXA2* Promoter 2**	AGGAAGAGAGTTTTTATTTTGTTTTTGGGTGGAA	CAGTAATACGACTCACTATAGGGAGAAGGCTCCTAAAAATTAAAACTCCAAAAAAACC
***FOXA2* Promoter 3**	AGGAAGAGAGTGTAGATTTGAGAGTTTTGGGGTTA	CAGTAATACGACTCACTATAGGGAGAAGGCTTCTCTATCCTCTCTATCTTCCAAAAAA
***FOXA2* Promoter 4**	AGGAAGAGAGTTTAATAAAATGGAAAGGGAAGGGT	CAGTAATACGACTCACTATAGGGAGAAGGCTTAAAACCCCAACCCCTAAATTCTAC
***SOX10* Promoter 1**	AGGAAGAGAGTTTGTTGAGGTTTATTTTGGGTTTA	CAGTAATACGACTCACTATAGGGAGAAGGCTCCACCTTAACCTCCTAACAACTTTA
***SOX10* Promoter 2**	AGGAAGAGAGTTTGGGTAGTAGGGTAGGGAG	CAGTAATACGACTCACTATAGGGAGAAGGCTAAAAACCCTAAACCTCAAACCAC
***SOX17* Promoter 1**	AGGAAGAGAGATATAGGTAAGTTGAGTTTTGGGGG	CAGTAATACGACTCACTATAGGGAGAAGGCTAAACCTTTTCTACACAAATATAACCAA
***SOX17* Promoter 2**	AGGAAGAGAGAAAGGATATTTAATTAGGGAAGGGG	CAGTAATACGACTCACTATAGGGAGAAGGCTCAACAAAATCCAAACCTACACAAC
***SOX17* Promoter 3**	AGGAAGAGAGTATTTGTAGTTGGGGTGGTTTTGTA	CAGTAATACGACTCACTATAGGGAGAAGGCTACCCCTTCCCTAATTAAATATCCTT
***SOX17* Promoter 4**	AGGAAGAGAGTAATAAATTTTTAGGAAATGGAGGAAGT	CAGTAATACGACTCACTATAGGGAGAAGGCTAAACCTAAAATAAACCTCCCCTACC
***GATA2* Promoter 1**	AGGAAGAGAGGAGTTTTAAAGGTAGGGGTTATAGGG	CAGTAATACGACTCACTATAGGGAGAAGGCTCCACATCCATCCTAACAAAACC
***GATA2* Promoter 2**	AGGAAGAGAGGGTTTTGTTAGGATGGATGTGG	CAGTAATACGACTCACTATAGGGAGAAGGCTCAAACTTTCTAAACAAACATCCC
***GATA2* Promoter 3**	AGGAAGAGAGTTGATTTGTTGGAATGGGAATTAG	CAGTAATACGACTCACTATAGGGAGAAGGCTCACATCCCTACACATATACACACAA
***LEFTY2* Enhancer 1**	AGGAAGAGAGGAGAAGTAGAGTAGGAAGTTAGGTTGG	CAGTAATACGACTCACTATAGGGAGAAGGCTATTACTCTAAAACCCTCTCCCCTTT
***LEFTY2* Enhancer 2**	AGGAAGAGAGTTTTTATATTTAGAAGTGGGGAGGG	CAGTAATACGACTCACTATAGGGAGAAGGCTCCAAAAACATTTCTATCTCTTAACACC
***LEFTY2* Enhancer 3**	AGGAAGAGAGAGGTTTTTTGTTGGGTATAGGGTAG	CAGTAATACGACTCACTATAGGGAGAAGGCTTCCTACCTACTTAAAAAACTCAAATAAAA

Supplementary Table 5. Genomic coordinates for promoters and enhancers investigated for methylation analysis by 5mC MassArray and for targeted demethylation by dCas9-TET1CD.

Region	Genomic Coordinates (GRCh37/hg19)
***PAX6* P0 Promoter**	Chr11: 31839283-31840731
***PAX6* P1 Promoter**	Chr11: 31832705-31834156
***PAX6* 156k Enhancer**	Chr11: 31679370-31690371
***PAX6* Ele4h Enhancer**	Chr11: 31825691-31825906
***PAX6* E1E Enhancer**	Chr11: 31832708-31832765
***PAX6* Ete1 Enhancer**	Chr11: 31837446-31839211
***PAX6* -6k Enhancer**	Chr11: 31847524-31848122
***SOX10* Promoter**	Chr22: 38380465-38381233
***LEFTY2* Enhancer**	Chr1: 226138153-226139319
***GAPDH* Promoter**	Chr12: 6642271-6643589
***OCT4* Promoter**	Chr6: 31138570-31139891
***NANOG* Promoter**	Chr12: 7940446-7941959
***SOX2* Promoter**	Chr3: 181428186-181429721
***FOXA2* Promoter**	Chr20: 22566291-22567649
***SOX17* Promoter**	Chr8: 55369986-55370476
***GATA2* Promoter**	Chr3: 128211937-128212937

Supplementary Table 6. Primers for cloning *PAX6* overexpression vector, dCas9-TET1 catalytic domain fusion (dCas9-TET1CD) and mutagenesis of dCas9-TET1CD (dCas9-TET1CD/Mut).

Cloning Construct	Forward Primer	Reverse Primer
2A-GFP construct	GGATCCGCCACTAACTTCTCCCTGT	GGCGCGCCTTACTTGTACAGCTCGTCCAT
PAX6 cDNA construct	CACCGAATTCATGCAGAACAGTCACAGCGGA	GGATCCCTGTAATCTTGGCCAGTATTG
TET1 catalytic domain (for dCas9-TET1CD)	ACTGAGGCCGGCCAGCTGCCCACCTGCAGCTGTCTT	TTCTTGGCCGGCCTCAGACCCAATGGTTATAGG
TET1 catalytic domain mutagenesis	GGACTTCTGTGCTCATCCCTACAGGGCCATTCACAACAT	ATGTTGTGAATGGCCCTGTAGGGATGAGCACAGAAGTCC