

Supplemental Experimental Procedures

Southern and western blots

10-15 μ g of genomic DNA was digested with restriction enzymes overnight and southern blot was performed as previously explained (Carey et al., 2010). For western blot, lysates were prepared from pre-plated feeder-free mESCs, analyzed on 4-12% Tris-Base polyacrylamide gels (Invitrogen) and transferred to PVDF membrane using BioRad mini transfer apparatus for 1.5hr at 50V. Membrane was blocked with 5% milk for one hour in room temperature and probed with Rabbit polyclonal Tet1 antibody (Millipore, 1:1000) or mouse monoclonal anti-actin (abcam, 1:40000) for 1 hour at room temperature. This was followed by washes in PBST (0.5% Tween 20) and incubation in HRP-conjugated secondary antibodies (Cal Biochem). Blots were developed using ECL reagent (GE Healthcare).

Generation of chimeric blastocysts and E10.5 embryos with GFP-labeled Tet1 knockout ES cells

ES cells were transduced with FUW-GFP lentivirus four times over the course of 48 hours and then FACs-sorted to enrich for GFP positive cells. 5 GFP positive cells were injected into B6D2F1 x B6D2F1 E2.5 embryos following standard procedures. Injected embryos were cultured in KSOM and 24hr later the developed E3.5 blastocysts were imaged using a fluorescence microscope. To generate chimeric embryos, 10-12 GFP positive cells were injected in to B6D2F1 x B6D2F1 E3.5 wild-type blastocysts and surgically implanted into 2.5 d.p.c. pseudo-pregnant Swiss Webster female mice

following standard procedures. E10.5 embryos were harvested, dissected and imaged under a fluorescence dissecting scope.

RNA extraction and Real time quantitative PCR

mESCs were trypsinized and pre-plated for 1hr to separate the feeders. Cells were washed and RNA was extracted using RNeasy Kit (Qiagen). 1.5 μ g of RNA was used to synthesize cDNA (Invitrogen Superscript III kit). Real time quantitative PCR was performed in an ABI 790000 cycler (Applied Biosystems). Gene expression was normalized to GAPDH. Tet1 (exon 11-exon 12), Tet2, Tet3, Brachyury, Pax6, Nanog, Oct4, Sox2 and GAPDH were detected using published RT primers (Carey et al., 2010; Ito et al., 2010). Tet1 RT primer sequences are as follows:

Tet1 (exon 1-exon 3)

Forward 5'TGCACCTACTGCAAGAATCG 3' (primer in exon 1)

Reverse 5'GGAAGCTGTGATGCCAATTT 3' (primer in exon 3)

Tet1 (exon 3-exon 4):

Forward 5'GCTGGATTGAAGGAACAGGA3' (primer in exon 3)

Reverse 5'GTCTCCATGAGCTCCCTGAC3' (primer in exon 4)

Tet1 (exon 4-exon 5):

Forward 5' GTCAGGGAGCTCATGGAGAC 3' (primer in exon 4)

Reverse 5'CCTGAGAGCTCTTCCCTTCC 3' (primer in exon 5)

Genotyping of mice

Mouse tail DNA was used for genotyping either by southern blot using AflII digestion and a 3' external probe or by PCR using primer pairs (Forward 5' aactgattcccttcgtgcag 3') and (Reverse 5' ttaaagcatgggtgggagtc 3'). Cycling conditions for PCR: 98C 5min, (98C 45sec, 58C 45sec, 72C 1min 30 sec) X 35, 72C 10min, 4C Hold. Products were analyzed on a 1.5% agarose gel.

In vitro differentiation to embryoid bodies (EBs) and neural progenitors (NPs)

ES cells were differentiated to EBs by hanging drop method in the absence of LIF for 4 days. Then the aggregates in drops were transferred to plastic plates and cultured for 2 days. Neural progenitors (NPs) were generated following the embryoid body formation protocol involving several steps: first, ES cells were cultured in suspension for 4 days in the absence of LIF to form EBs. In the second step, the EBs were cultured on tissue culture plates in ITSF media for 8 days. Finally, neural precursors were selected by expanding the culture on poly-D-ornithine and laminin coated plates in N2+bFGF media. Differentiation to NPs was confirmed by immunostaining for neural stem cell marker Nestin (Chemicon, 1:200).

Immunostaining

mESCs cultured on feeders were washed with PBS and fixed with 4% paraformaldehyde for 15mins in room temperature. Cells were then permeabilized and blocked in 0.2% TritonX 5% BSA in PBS for 30 min in room temperature. Primary and secondary incubations were performed as described previously (Hanna et al., 2009). Following

antibodies were used: Affinity purified Nanog Rabbit polyclonal antibody (Bethyl Laboratories, 1:250) and mouse monoclonal Oct4 clone C10 antibody (Santa Cruz, 1:100).

Immunohistochemistry of germ cells in gonads

Gonads were extracted from E13.5 embryos and fixed in 4% paraformaldehyde overnight and paraffin embedded. 5 micron longitudinal sections were prepared and stained with anti-Mvh (Abcam) as explained before (Gill et al., 2011).

Dot blot, gluc-MSqPCR and Quantification of mC by LC/MS

DNA was extracted from pre-plated mESCs following standard procedures. DNA was transferred to nylon membrane using BioRad slot blot vacuum manifold apparatus. Anti-5hmC (Active Motif 1:10000) was used to detect 5hmC following manufacturer's protocol. Signal of bands were quantified by Image J software. Locus specific quantification of 5hmC and 5mC was performed using EpiMark 5hmC and 5mC analysis kit (New England Biolabs) following manufacturer's protocol. Modified and digested DNA was analyzed by real time qPCR using previously published primer sequences for CpG islands in *Ctnna3*, *Bend3* and *Ecat1* (Ficz et al., 2011) which are briefly summarized below. Data were analyzed using ΔCT method and plotted as fold 5hmC or 5mC containing DNA.

Ctnna3 (CGI region B)

Forward CCTTTCTCATTAGCGCGTTT, Reverse CTCAGTACGGTCCTCCCAGA

Bend3 (CGI region E)

Forward TCTTCACCCACGAGAACCTG, Reverse AGAGGAGCTGCACGTAGTGG

Ecat1

Forward GGAGAGCACATCCCACATCT Reverse GTGAGCCAGATCAGTGAGCA

CpG Island in Chromosome 5: 5hmC A (Chr5:99466154-99466819)

Forward TGCACTTTATACCCCGAACC, Reverse GGCCCACACACTTGAAATAAA

Liquid chromatography/mass spectrometry (LC/MS) for quantification of total genomic mC levels was performed as previously explained (Friso et al., 2002). DNA from three independent passage 20 mESCs for each genotype was used in the analysis.

Tet2 knockdown by shRNA

Control and Tet2 shRNA sequences (Ito et al., 2010) were synthesized and cloned into pSicoR lentiviral vector, which co-expresses the hairpin and GFP from a U6 promoter (Ventura et al., 2004). Lentivirus was prepared as explained before using FuGENE 6 transfection reagent following manufacturer's protocol. Tet1 wild type and knockout ES cells were transduced 3 times (12hr each) with the viral supernatant. 48hrs later GFP positive cells were sorted and cultured on feeders. Tet2 knockdown was verified by Real time qPCR.