

Supplementary information for

Metabolic control of DNA methylation in naive pluripotent cells

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Supplementary Methods

Generation of mutant cell lines

Dnmt3b ^{-/-} ESCs Knockout production was performed using TALEN technology as described in¹. In brief, cells were transfected with the two TALEN constructs targeting Exon 17 of murine Dnmt3b and after 16 hours were seeded as a single cell. After 1 week, clones were screened by western blot analyses. Positive clones were analyzed by genomic sequencing of the TALEN target.

Dnmt3a^{-/-} and Dnmt3a/b^{-/-} ESCs generation were performed by CRISP-R/ Cas9 method. E14 Wild-type cells and Dnmt3b^{-/-} were co-transfected with Cas9 construct and the two RNA guide construct targeting Exon 19 (for the sequence see Supplementary Table 6) of murine Dnmt3a. After 16 hours single cells sorting was carried out. Ten days later, clones were screened by western blot analysis. Positive clones were analyzed by genomic sequencing.

For DNA transfection, we used Lipofectamine 2000 (Life Technologies, cat. 11668-019) and performed reverse transfection. For one well of a 12-well plate, we used 3 µl of transfection reagent, 1 µg of plasmid DNA, and 150,000 cells in 1 ml of KSR medium. The medium was changed after overnight incubation.

Stable transgenic ESCs lines expressing sh-TET1/2, sh-Scramble or MLS-Stat3-NES were generated by transfecting cells with PiggyBac transposon plasmids CAG-sh-TET1/2, CAG-sh-Scramble (in S3^{+/+} cells) or CAG-MLS-Stat3-NES (in S3^{-/-} cells) derived from² (target sequences and Mitochondrial Localization Signal and Nuclear Export Signal sequence are in Supplementary Table 6); with piggyBac transposase expression vector pBase. Selection for transgenes was applied, and stable clones were selected in 2iLIF conditions.

STAT3ER plasmid transfection previously described in³ was performed with a 1 µg of linearized plasmid (enzyme PbuI). Plasmid encodes for the entire coding region of mouse STAT3 followed by the modified ligand-binding domain (G525R) of mouse estrogen receptor under control of CAG promoter (pCAGGS vector).

Dnmt3a1/2 constructs for Overexpression experiments were obtained by PCR amplification of the entire coding region (Dnmt3a1 or Dnmt3a2) and cloned into the XbaI–NotI site of pEF6/V5-His vector (Invitrogen). Dnmt3b construct was obtained by PCR amplification and cloned into pEF6/V5-His vector (Invitrogen) previously described in¹.

Alkaline Phosphatase Staining

Alkaline Phosphatase Staining as previously described in⁴.

Fixation solution: 65% Acetone, 25% Citrate (provided with kit), 8% Formaldehyde

Staining solution: Alkaline Phosphatase (AP) kit (Sigma-Aldrich, cat. 86R-1KT) according to the manufacturer's protocol.

Culture medium was removed from adherent cells and they were fixed with fixation solution. Plates were then washed with H₂O and the staining solution was added for 5 minutes in the dark. Then plates were washed again with H₂O and dried.

Colonies were scored manually using an optical microscope, discriminating between undifferentiated (AP-positive), mixed or differentiated (AP-negative).

Oxygen consumption assay (Seahorse Assay)

Oxygen consumption assay (Seahorse Assay) as previously described in⁴. Oxygen consumption was measured using the Seahorse XF24 (Seahorse Bioscience). For this, 20 hours before the analysis both S3^{+/+} and S3^{-/-} cells were seeded in a 24-well cell culture plate (Seahorse Bioscience) coated with laminin (Sigma-Aldrich, cat. L2020) at a density of 100,000 cells per well in KSR media supplemented with 2i + LIF. It is crucial to have an evenly plated monolayer of cells to obtain reliable measurements. Cells were maintained in a 5% CO₂ incubator at 37°C, and 1 hour before the experiment, the cells were washed and incubated in 600 µl of DMEM (Sigma-Aldrich, cat. D5030-10X1L) with 2mM Glutamine, 1mM NaPy, 25 mM glucose, 3 mg/L phenol red and 143 mM NaCl, with pH 7.4 at 37°C in a non-CO₂ incubator.

During the experiment, oxygen concentration was measured over time periods of 2 min at 5 minutes intervals, consisting of a 3-min mixing period and 2 minutes waiting period. Oxygen consumption rate (OCR) is measured before and after the addition of inhibitors to derive several parameters of mitochondrial respiration. Initially, cellular OCR is measured in basal conditions to derive the basal mitochondrial respiration; next, 200 nM mitochondrial uncoupler FCCP (carbonyl cyanide-p-trifluoromethoxyphenyl-hydrazone) is automatically added to the medium to maximize Electron Transport Chain (ETC) function, in order to derive maximal respiratory capacity. Next, Antimycin A and Rotenone - inhibitors of complex III and I - are released into the medium to block ETC, revealing the non- mitochondrial respiration

Gene expression analysis by quantitative PCR with reverse transcription

Total RNA was isolated using a Total RNA Purification kit (Norgen Biotek, cat. 37500), and complementary DNA (cDNA) was made from 500 ng using M-MLV Reverse Transcriptase

(Invitrogen, cat. 28025-013) and dN6 primers (Invitrogen). For real-time PCR, SYBR Green Master mix (Bioline, cat. BIO-94020) was used. Three technical replicates were carried out for all quantitative PCR. An endogenous control (beta-actin) was used to normalize expression as previously described in⁴. Primers are detailed in Supplementary Table 7.

Western blot

Western blot performed as previously described in⁴. Cells were washed in PBS and harvested with lysis F-buffer (10 mM TrisHCl pH7, 50 mM NaCl, 30 mM Sodium pyrophosphate tetrabasic, 50 mM NaF, 1% Triton X-100 Buffer). In order to obtain protein lysates, extracts were exposed to ultrasound in a sonicator (Diagenode Bioruptor) for 3 pulses. Cellular extracts were centrifuged for 10 minutes at 4°C (max speed) to remove the insoluble fraction. Extracts were quantified using bicinchoninic acid (BCA) assay (BCA protein assay kit; catalog no. 23225; Pierce). Samples were boiled at 95°C for 5 minutes in 1X Sample Buffer (50mM Tris HCl pH 6.8, 2% SDS, 0.1% Bromophenol Blue, 10% glycerol, 2% 2-mercaptoethanol).

Each sample was loaded in a commercial 4-12% MOPS acrylamide gel (Life Technologies; BG04125BOX/BG00105BOX) and electrophoretically transferred on a PVDF membrane (Millipore; IPFL00010) in a Transfer solution (50mM Tris, 40mM glycine, 20% methanol, 0.04% SDS). Membranes were then saturated with 5% Non-Fat Dry Milk powder (BioRad; 170-6405-MSDS) in TBST (8g NaCl, 2.4g Tris, 0.1% Tween20/liter, pH 7.5) for 1 hour at room temperature and incubated overnight at 4 °C with the primary antibody (Supplementary Table 5) diluted in a range of 0,5-1% milk powder (depending on antibody) in TBST. Membranes were then incubated with secondary antibodies conjugated with a peroxidase, diluted in 0,1% or 0,5% milk in TBST. Pico SuperSignal West chemiluminescent reagent (Thermo Scientific, cat. 34078) was used to incubate membranes and chemiluminescence from the interaction between peroxidase and substrate present in the commercial reagent was digitally acquired by ImageQuant LAS 4000. Full scan are provided as Source Data unprocessed.

Mitochondria and nuclear isolation

Nuclear and mitochondrial isolation was performed as indicated in ^{5,6}, respectively, with some modifications. Briefly, cells 4×10^7 collected in PBS were centrifuged at 600 x g, 5 min, and the pellet, resuspended in 2 ml of Isolation buffer (IBc), was homogenized with a Dounce homogenizer and Teflon pestle by 20 strokes (x4) on ice. Then, the homogenate was centrifuged at 600 x g, 5 min, 4°C, and supernatant (SN) and pellet were collected to proceed separately with mitochondria and nuclei isolation. SN was centrifuged twice at 40 x g and pellets were conserved to proceed with

nuclei isolation; SNs were further centrifuged sequentially at 600 x g and 1200 x g for 5 min at 4°C, to further eliminate debris. Finally, the resulting SN was centrifuged at 7500 x g to obtain the mitochondrial pellet that was finally washed twice with IBC and centrifuged at 9000 x g. For nuclei isolation, the pellet obtained after the first centrifugation at 40 x g was resuspended in nuclear isolation buffer (20mM TRIS pH 7.5, 50 mM β -mercaptoethanol, 0.1mM EDTA, 2mM MgCl₂, 1mM PMSF) supplemented with protease inhibitor, and incubated in sequence 2 min at RT and 10 min on ice, to then proceed with centrifugation at 600 x g, 4°C. The obtained pellet was resuspended in 400 μ l of the same buffer with addition of 1% NP40. Nuclei were pelleted at 500 x g, 4°C, and washed 3 times with the same buffer. Finally, mitochondrial and nuclear pellets were lysed in RIPA buffer.

Nucleoside preparation for Mass Spectrometry

DNA was extracted using Puregene core kit A, then measured with a Nanodrop spectrophotometer. 50 μ g DNA were passed through the Microcon YM-10 centrifugal filtration cartridge (Millipore, cat. no. 42407, MRCPRT010) 10KDa columns two times. The first time 50 μ g of DNA were solubilized into 500 μ L of double-distilled water, then concentrated to about 30 μ L by spinning the columns at 13900g for 25 minutes. The second time, the 30 μ L of recovered DNA were solubilized into 500 μ L of 1X digestion buffer and then concentrated to about 15 μ L by spinning at 13900g for 35 minutes.

After the 2 steps, the DNA concentration was measured at the Nanodrop spectrophotometer. The DNA was then digested to nucleosides, at 37°C for 6-7 hours, using a mix containing 2U Antarctic Phosphatase (stock solution is 5U/ μ l) (New England Biolabs, M0289S), 3mU Snake venom phosphodiesterase I (stock solution is 1mU/ μ l) (Crotalus adamanteus venom, Sigma-Aldrich, P3243-1VL), 2.5U Benzonase (stock solution is 250U/ μ l) (Sigma-Aldrich E1014-5KU), in 3.4 μ l volume of enzyme mix + 1.6 μ l of double distilled water + 5 μ l 2X digestion buffer (20mM Tris Hcl pH 7.9 100mM NaCl, 20mM MgCl₂) + 5 μ l of DNA (7,5 – 10 μ g) in 1X digestion buffer. After the digestion 1 μ g of undigested genomic DNA and 1 μ g of digested DNA were loaded on a 2% gel, in order to confirm the complete digestion of the genomic DNA. 12 μ l of digested nucleosides were provided for MS analysis to CNRS at Gif-sur-Yvette⁷.

Mass Spectrometric Analysis of Total Nucleosides

Analysis of the nucleoside digests of DNA by HPLC was performed with a U-3000 HPLC system (Thermo-Fisher). An Accucore RP-MS (2.1 mm X 100 mm, 2.6 μ m particle, Thermo-Fisher) column was used at a flow rate of 200 μ L/min and a fixed temperature at 30°C. Mobile phases were

5 mM ammonium acetate, pH 5.3 (buffer A) and 40% aqueous acetonitrile (Buffer B). A multilinear gradient was used with only minor modifications from that described previously⁸. The injection volume was fixed at 6 μ L.

A LTQ orbitrap Mass Spectrometer (Thermo-Fisher) equipped with an electrospray ion source was used for the HPLC-MS identification and quantification of nucleosides. Mass Spectra were recorded in the positive ion mode over an m/z range of 100-1000 with a capillary temperature set at 300°C, spray voltage at 4.5 kV and sheath gas, auxiliary gas and sweep gas at 40, 12 and 7 arbitrary units, respectively.

Calibration curves were generated using a mixture of synthetic standards of 2'-Deoxycytidine (2dC)(Sigma-Aldrich), 5-Methyl-2'-deoxycytidine(5-mdC) and 5-hydroxymethyl-2'-deoxycytidine (5-hmdC) (Bertin-Pharma) in the ranges of 10-100 injected pmol for 2dC, 0.4-4 injected pmol for 5-mdC and 0.5-10 injected pmol for 5-hmdC. Each calibration point was injected in triplicate.

Extracted Ion Chromatograms (EIC) of base peaks of the following signals: 2dC (m/z 228.08-228.12), 5-mdC (m/z 242.10-242.13), and 5-hmdC (m/z 258.08-258.12), were used for quantification. In all cases, coefficients of variations for peak areas were always below 15%.

Experimental data (peak area *versus* injected quantity) were fitted with a linear regression model for each compound leading to coefficient of determination (R^2) values better than 0.97. Accuracies were calculated for each calibration point and were always better than 15%.

MEDIP

MEDIP was performed as previously described in ⁹

Genomic DNA was extracted with phenol-chloroform, resuspended in TE buffer containing 20 μ g/ml RNase A (Thermo-Fisher, cat. EN0531) and passed through a needle 10 times to reduce its viscosity, then measured at the Nanodrop spectrophotometer.

40 μ g of DNA were resuspended in 130 μ l of TE, transferred to a microtube (microtubes AFA fiber pre-slit snap cap 6x16mm, Covaris) and sonicated with the Covaris S2 (Duty cycle 10%, Intensity 5, Cycles burst 200, 45 seconds per cycle; 3 cycles to have a distribution of size between 100 and 600, 4 cycles to have a distribution of size between 100 and 400 and 5 cycles to have a distribution of size between 100 and 300).

10 μ g of sonicated DNA were diluted in 1,125ml TE and denatured for 10 minutes at 100°C in a thermoblock, then quickly cooled on ice for additional 10 minutes. 450 μ l (= 4 μ g tot) of denatured DNA were distributed in two low binding tubes with 51 μ l of 10X IP buffer (100 mM Na-Phosphate pH 7.0, 1.4 M NaCl, 0.5 % Triton X-100), plus 10 μ l of antibody anti-5mC (Supplementary Table 5) (IP sample) or IgG (mock control) were added. The tubes were left rotating with overhead shaking

for 2 hours at 4°C. The leftover (= 225µl) is the Input material (50% of Input), to be used in the quantitative PCR.

Dynabeads Protein G (Thermo-Fisher, cat. 10003D) were prepared by taking 40µl per each sample, then washed twice for 5 minutes in 800µl of 0.1% BSA in PBS and finally resuspended in 40µl of 1X IP buffer.

After 2 hours, Dynabeads Protein G were added to the IP and mock samples. Samples were left rotating at 4°C with overhead shaking for additional 2 hours.

The beads were then separated using the magnetic stand and washed 3 times for 10 minutes in 1X IP buffer; the supernatant was removed and trashed (unbound fraction). Finally the beads were resuspended in 250µl of proteinase K digestion buffer (50 mM Tris pH 8.0, 10 mM EDTA, 0.5 % SDS) containing 3.5µl of proteinase K (20mg/µl). The samples were incubated overnight at 50°C in a shaking thermoblock (500rpm). The day after the beads were separated with a magnetic rack and the supernatant was saved.

The DNA contained in the supernatant fraction was purified using Qiaquick PCR Purification kit (Qiagen, cat. 2816) and eluted in 30 µl. The saved Input material (50% Input) was re-purified and concentrated using Qiaquick kit; elution was done in a volume of 30µl. Primers are detailed in Supplementary Table 8.

Electron microscopy and DAB staining

Cells were fixed in a 24 wells plate with 4% Paraformaldehyde in PBS (pH 7,4) for 30 min. at RT. After fixation cells were washed 5 times with PBS (5 min. each) blocked and permeabilized with 5% normal goat serum and 0,1% saponin in PBS for 30 min, and then incubated with primary antibody anti-Stat3 O.N at 4°C. in PBS 5% normal goat serum and 0,05% saponin. After 5 washing with PBS, (5 min each) cells were incubated with HRP-conjugated Fab fragments of the secondary antibody for 2h. RT. After 5 washing cells were incubated in the DAB solution (0.01gr DAB in 20ml TRIS-HCl buffer plus 30% H₂O₂ solution just before use). Subsequently the samples were postfixed with 1% osmium tetroxide plus potassium ferrocyanide 1% in 0.1M sodium cacodylate buffer for 1 hour at 4°. After three water washes, samples were dehydrated in a graded ethanol series and embedded in an epoxy resin (Sigma-Aldrich). Ultrathin sections (60-70 nm) were obtained with an Ultratome V (LKB) ultramicrotome, counterstained with uranyl acetate and lead citrate and viewed with a Tecnai G2 (FEI) transmission electron microscope operating at 100 kV. Images were captured with a Veleta (Olympus Soft Imaging System) digital camera.

Supplementary Table 5. Specifications of antibodies used for immunostaining.

Antibody	Species	Source	Dilution	Antibody validation
5mC	Mouse monoclonal	EUROGENTEC BI-MECY	IF: 1:250	Previously validated in M. Habib 1999 Experimental Cell Research ¹⁰ Results obtained were confirmed by Mass Spectrometry for 5mC
Tet2	Rabbit polyclonal	ABCAM Ab94580	WB: 1:500	Previously validated in Neri 2015 Cell Reports ¹¹
Dnmt3a	Mouse monoclonal	Novus Biological NB120-13888	WB: 1:500	Previously validated in Neri 2015 Cell Reports ¹¹
Dnmt3b	Mouse monoclonal	Novus Biological NB100-56514	WB: 1:1000	Previously validated in Neri 2015 Cell Reports ¹¹
Dnmt3b	Rabbit polyclonal	ABCAM ab122932	IF: 1:500	Previously validated in Nakanishi 2019 Cell ¹²
Lamin B	Goat polyclonal	Santa Cruz Biotechnologies cat. 6216	WB: 1:1,000	Previously validated in Su 2013 Molecular Cell Biology ¹³
Stat3	Mouse monoclonal	Cell Signalling cat. 9139	WB: 1:1,000 IF: 1:100	Previously validated in Carbognin 2016 EMBOJ ⁴ . Signal is absent in Stat3 ^{-/-} cells.
Atad3	Rabbit monoclonal	AB-Biotechnologies	IF 1:100	Previously validated in He 2007 Journal of cell

		cat. 224485		biology ¹⁴
TIM23	Mouse monoclonal	BD Biosciences cat. 611223	WB 1:1000	Previously validated in Kang 2016 eLife ¹⁵

Supplementary Table 6. Oligonucleotides

Dnmt3b FW gRNA exn19	CACCgACCGCCTCCTGCATGATGCGCGG
Dnmt3b REV gRNA exn19	aaacCCGCGCATCATGCAGGAGGCGGTc
sh-TET1/2 targets seq TET1'	<i>CTCATCTACTTCTCACCTAGTG</i>
sh-TET1/2 targets seq TET1''	<i>AAGAGAACCTGGTGCATCAGA</i>
sh-TET1/2 targets seq TET2'	<i>AGCTCTGAACAGTATTCAAAGC</i>
sh-TET1/2 targets seq TET2''	<i>ATAGGACTATAATGTATAGATA</i>
<i>Scramble_miR30-shRNA</i>	<i>ACCTAAGGTTAAGTCGCCCTCG</i>
seq MLS (Mitochondrial localization signal)	<i>CTAGCAAGCTTGTGACCATGTCCGTCCTGACGCCGCT GCTGCTGCGGGGCTTGACAGGCTCGGCCCGGCGGC TCCCAGTGCCGCGCGCCAAGATCCATTCGTTG</i>
seq NES (Nuclear export signal)	<i>GTGGACGAGATGACCAAGAAGTTCGGCACC CTGACCATCCACGACACCGAGAAG</i>

Supplementary Table 7. Primers used for Real-time RT q-PCR.

Gene RT-qPCR	Forward primer sequence	Reverse primer sequence
Socs3	ATTTCGCTTCGGGACTAGC	AACTTGCTGTGGGTGACCAT
Uhrf1	GCTCCAGTGCCGTTAAGACC	CACGAGCACGGACATTCTTG
Dnmt3l	ATGGACAATCTGCTGCTGACTG	CGCATAGCATTCTGGTAGTCTCTG
Tet1	GAGCCTGTTCCCTCGATGTGG	CAAACCCACCTGAGGCTGTT
Tet2	TGTTGTTGTCAGGGTGAGAATC	TCTTGCTTCTGGCAAACCTTACA
Dnmt1	CCATGGCTGACACTAAGCTG	ACCAAACCAAACCAAACCAA
Dnmt3b 3'UTR	CTCGCAAGGTGTGGGCTTTTGTAAC	CTGGGCATCTGTCATCTTTGCACC
Dnmt3a 3'UTR	GACTCGCGTGCAATAACCTTAG	GGTCACTTTCCTCACTCTGG
Idh1	ATGGGCGTTTCAAAGACATC	CCTCGGACTTCATAGCTTGG
Peg10	ACGATGATGACCTGGAGCTT	ATGAAAGGACCCAGCATGTC
Phlda2	TCAGCGCTCTGAGTCTGAAA	CTCCTGGGCTCCTGTCTGAT
Lin28a	GTCTTTGTGCACCAGAGCAA	CGCTCACTCCCAATACAGAA
Lin28b	ACGGCAGGATTTACTGATGG	GCACTTCTTTGGCTGAGGAG
Oct6/Pou3f1	ACCACCACCACCACACT	AAATCCAAAGCAAAACCGAAT
Cdkn1c	GGAGCAGGACGAGAATCAAG	GTTCTCCTGCGAGTTCTCT
Sfmbt2	CATGTGGAGATCAGCATTCG	TGTCCACAGGTGGTGATGAT
Igf2	GACGACTCCCCAGATACCC	CTTTGAGCTCTTTGGCAAGC

Brachyury	CTGGGAGCTCAGTTCTTTCGA	GAGGACGTGGCAGCTGAGA
Hand1	AGAGGAGACGCACAGAGAGC	AGCACGTCCATCAAGTAGGC
Snai	TGAGAAGCCATTCTCCTGCT	CTTCACATCCGAGTGGGTTT
Tubb3	CATGGACAGTGTTCCGGTCTG	CGCACGACATCTAGGACTGA
Krt18	CGAGGCACTCAAGGAAGAAC	GCTGAGGTCCTGAGATTTGG
Nes	CTGCAGGCCACTGAAAAGTT	TTCCAGGATCTGAGCGATCT
Tfcp2l1	GGGGACTACTCGGAGCATCT	TTCCGATCAGCTCCCTTG
Tbx3	TTGCAAAGGGTTTTTCGAGAC	TGCAGTGTGAGCTGCTTTCT
Dppa3	TTTGTGTCCGGTCTGAAAG	TCATTTCCCTCGAGCCTTTT
Beta-actin	CTAAGGCCAACCGTGAAAAG	ACCAGAGGCATACAGGGACA
Dnmt3a1	GAAGGCCGTGGAGCCTCT	CCATTTTCATGGATTCGATGTT
Dnmt3a2	AGAAGTGTCTGCTGCACACA	TGCTCCAGACACTCTTGCAG
Dnmt3b INT	TGACGTCCGGAAAATCACCA	TAAACCTTTGCGGGCAGGAT
Otx2	GGAAGAGGTGGCACTGAAAA	CGGCACTTAGCTCTTCGATT
Sox1	CACAACTCGGAGATCAGCAA	CTCGGACATGACCTTCCACT
Klf4	CGACTAACCGTTGGCGTGA	CGGGTTGTTACTGCTGCAAG
Tbx3	TTGCAAAGGGTTTTTCGAGAC	TGCAGTGTGAGCTGCTTTCT
Nanog	TTCTTGCTTACAAGGGTCTGC	AGAGGAAGGGCGAGGAGA
Prdm14	GAAGGCACACAGGGACAACCT	TCCAGTCCCAGAACCTTTG

Tcl1	GATCTGGGAGAAGCACGTGTA	TGACTGGGGGACATAGCTTC
Tcea3	GACAAGCTGGCCTCAGAAAT	CGGTTCCGGTACTTCATGTC
Vegfa	CTGTAACGATGAAGCCCTGGAG	TGGTGAGGTTTGATCCGCAT
Phd3	CTTCCTCCTGTCCCTCATCG	ATACAGCGGCCATCACCATT

Supplementary Table 8. Primers used for MeDIP-PCR.

Gene MeDIP-qPCR	Forward primer sequence	Reverse primer sequence
Nnat	GAGTATGTACCCGGGCTTTG	ATAGGATGGGTTGGGTAGGG
Peg10	CCCCCTCCTAGGATCTCTCT	GGATTCTTCGACACACACCA
Kif27	CCAGCTGAGGGGATAACTCA	TCTGGGTCCTTTCAATACCAA
Iap	CTCCATGTGCTCTGCCTTCC	CCCCGTCCCTTTTTTAGGAGA
Iapey3	AGAGAGGAGGACAACCTGCTC	AACCTTACACAGGCAAAAGC
Line L1	CTGGCGAGGATGTGGAGAA	CCTGCAATCCCACCAACAAT
MajSat	GACGACTTGAAAAATGACGAAATC	CATATTCCAGGTCCTTCAGTGTGC
MinSat	CATGGAAAATGATAAAAACC	CATCTAATATGTTCTACAGTGTGG
Sine B1	GTGGCGCACGCCTTTAATC	GACAGGGTTTCTCTGTGTAG
Sine B2	GAGATGGCTCAGTGGTTAAG	CTGTCTTCAGACACTCCAG
Gapdh	TCCCTAGACCCGTACAGTGC	CTCTGCTCCTCCCTGTTCC
Zdbf2	CCAAACCCATCTCCTCTTCA	TGGCCTGGTCTAGTCGTCTC
H19	GCATGGTCCTCAAATTCTGCA	GCATCTGAACGCCCAATTA

Other supplementary Materials:

Supplementary Table 1. (separate file)

Table reports bulk RNA sequencing data, including differential expression analysis of following comparisons: S3^{-/-} cells vs S3^{+/+} cells (Figure 7a), MitoS3.A cells vs S3^{-/-} cells and MitoS3.B cells vs S3^{-/-} cells. Table also reports absolute expression data (TPM) of: S3^{+/+} cells in 2i; S3^{+/+}, S3^{-/-}, MitoS3.A and MitoS3.B cells in 2iLIF; S3^{+/+}, S3^{-/-}, MitoS3.A and MitoS3.B cells without 2iLIF for 48h.

Supplementary Table 2. (separate file)

Table reports the differential expression analysis (RNA-seq) and RRBS analysis of S3^{-/-} vs S3^{+/+} cells, for the following genomic features: promoters; enhancers; imprinted DMRs.

Supplementary Table 3. (separate file)

This table reports Mass spectrometry proteomics data of following samples: S3^{+/+} cells in 2i; S3^{+/+}, S3^{-/-}, MitoS3.A and MitoS3.B cells in 2iLIF.

ProteomeXchange Consortium via the PRIDE partner repository with the dataset identifier PXD020385.

Supplementary Table 4. (separate file)

This table reports the differential expression analysis (single cell RNA-seq) for the following samples: E3.5 S3^{-/-} vs E3.5 S3^{+/+} cells; E3.75 S3^{-/-} vs E3.75 S3^{+/+} cells. Table also reports absolute expression levels (FPKM) of selected genes.

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