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 in \textsuperscript{1}. 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 from \textsuperscript{2} (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 in \textsuperscript{3} was performed with a 1µg of linearized plasmid (enzyme Pbul). Plasmid encondes 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 costructs 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 in \textsuperscript{1}.
**Alkaline Phosphatase Staining**

Alkaline Phosphatase Staining as previously described in\(^4\).

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 H\(_2\)O and the staining solution was added for 5 minutes in the dark. Then plates were washed again with H\(_2\)O 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 in\(^4\). 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% CO\(_2\) 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-CO\(_2\) 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 in\textsuperscript{4}. Primers are detailed in Supplementary Table 7.

**Western blot**

Western blot performed as previously described in\textsuperscript{4}. 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\textsuperscript{5,6}, respectively, with some modifications. Briefly, cells 4 × 10\textsuperscript{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 µl 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. 50µg DNA were passed through the Microcon YM-10 centrifugal filtration cartridge (Millipore, cat. no. 42407, MRCPT010) 10KDa columns two times. The first time 50µg of DNA were solubilized into 500µL of double-distilled water, then concentrated to about 30 µL by spinning the columns at 13900g for 25 minutes. The second time, the 30µL of recovered DNA were solubilized into 500µL of 1X digestion buffer and then concentrated to about 15µL 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/µl) (New England Biolabs, M0289S), 3mU Snake venom phosphodiesterase I (stock solution is 1mU/µl) (Crotalus adamanteus venom, Sigma-Aldrich, P3243-1VL), 2.5U Benzonase (stock solution is 250U/µl) (Sigma-Aldrich E1014-5KU), in 3.4µl volume of enzyme mix + 1.6µl of double distilled water + 5µl 2X digestion buffer (20mM Tris HCl pH 7.9 100mM NaCl, 20mM MgCl2) + 5µl of DNA (7.5 – 10µg) in 1X digestion buffer. After the digestion 1µg of undigested genomic DNA and 1µg of digested DNA were loaded on a 2% gel, in order to confirm the complete digestion of the genomic DNA. 12µl 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 µm particle, Thermo-Fisher) column was used at a flow rate of 200 µL/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 previously. The injection volume was fixed at 6 µL.

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 (R²) 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

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

40µg of DNA were resuspended in 130µl 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).

10µg 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. 450µl (= 4µg tot) of denatured DNA were distributed in two low binding tubes with 51µl of 10X IP buffer (100 mM Na-Phosphate pH 7.0, 1.4 M NaCl, 0.5 % Triton X-100), plus 10µl 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 Ultrotome 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<sup>10</sup> Results obtained were confirmed by Mass Spectrometry for 5mC |
| Tet2     | Rabbit polyclonal | ABCAM Ab94580 | WB: 1:500 | Previously validated in Neri 2015 Cell Reports<sup>11</sup> |
| Dnmt3a   | Mouse monoclonal | Novus Biological NB120-13888 | WB: 1:500 | Previously validated in Neri 2015 Cell Reports<sup>11</sup> |
| Dnmt3b   | Mouse monoclonal | Novus Biological NB100-56514 | WB: 1:1000 | Previously validated in Neri 2015 Cell Reports<sup>11</sup> |
| Dnmt3b   | Rabbit polyclonal | ABCAM ab122932 | IF: 1:500 | Previously validated in Nakanishi 2019 Cell<sup>12</sup> |
| Lamin B  | Goat polyclonal | Santa Cruz Biotechnologies cat. 6216 | WB: 1:1,000 | Previously validated in Su 2013 Molecular Cell Biology<sup>13</sup> |
| Stat3    | Mouse monoclonal | Cell Signalling cat. 9139 | WB: 1:1,000 IF: 1:100 | Previously validated in Carbognin 2016 EMBOJ<sup>4</sup>. Signal is absent in Stat3-/- cells. |
| Atad3    | Rabbit monoclonal | AB-Biotechnologies | IF 1:100 | Previously validated in He 2007 Journal of cell |
| TIM23   | Mouse monoclonal | BD Biosciences cat. 611223 | WB 1:1000 | Previously validated in Kang 2016 eLife\textsuperscript{15} | biology\textsuperscript{14} |
|---------|------------------|-----------------------------|----------|----------------------------------------------------------|-----------------------------|
|         |                  |                              |          |                                                          |                              |

\textsuperscript{14} biology

\textsuperscript{15} Previously validated in Kang 2016 eLife
**Supplementary Table 6. Oligonucleotides**

| Oligonucleotides                          | Sequence                                      |
|------------------------------------------|-----------------------------------------------|
| Dnmt3b FW gRNA exn19                      | CACCgACCGCCTCCTGCATGATGCGCGG                 |
| Dnmt3b REV gRNA exn19                     | aaacCCGGCATCATGCAGGAGGCGGTc                 |
| sh-TET1/2 targets seq TET1'               | CTCATCTACTTCTCACCTAGTG                      |
| sh-TET1/2 targets seq TET1''              | AAGAGAACCTGGTGTCATCAGA                      |
| sh-TET1/2 targets seq TET2'               | AGCTCTGAACAGTATTCAAAAGC                     |
| sh-TET1/2 targets seq TET2''              | ATAGGACTATAATGTATAGATA                      |
| Scramble_miR30-shRNA                     | ACCTAAGGTTAAGTCGCCCTCG                      |
| seq MLS (Mitochondrial localization signal) | CTAGCAAGCTTGTGACCATGTCCGTCTGACGCCGCT     |
|                                          | GCTGCTGCGGGGCTTGACAGGCTCGGCCGCCGGGC       |
|                                          | TCCCAGTGCCGGCGCGACAAAGATCCATTCGGTT        |
| seq NES (Nuclear export signal)           | GTGGACGAGATGACCAAGAAGTTCCGGCACC            |
|                                          | CTGACCATCCACGACACCAGGAG                    |
**Supplementary Table 7.** Primers used for Real-time RT q-PCR.

| Gene RT-qPCR | Forward primer sequence | Reverse primer sequence |
|--------------|--------------------------|-------------------------|
| Socs3        | ATTTCGCTTCGGGACTAGC      | AACTTGCTGTTGGGTGACCAT   |
| Uhrf1        | GCTCCAGTGCGTTAAGACC      | CACGAGCACGGACATTCTTG    |
| Dnmt3l       | ATGGACAATCTCGTGCTGACTG   | CGCATAGCATTCTGTTAGTCTCTG|
| Tet1         | GAGCCTTTTCTCGATGTGG      | CAAACCCACCTGAGCGTGGTT  |
| Tet2         | TGGTTTTGTCAAGGGTGAGAATC  | TCTTGCTTTCTGGAACACTTACA|
| Dnmt1        | CATGGCTGACACTAAGCTG      | ACCAAACCAACCAACAAAAA    |
| Dnmt3b 3’UTR | CTCGCAAGGGTGGGCTTTGTAAAC | CTGGGCACTGCTGATCTTTTGACC|
| Dnmt3a 3’UTR | GACTCGCGTGCAATAACCTTAG  | GGTCACTTTCCCTCAGTCTGG  |
| Idh1         | ATGGGCGTTTCAAAGACATC     | CCTCGGACTCCATAGCTTTG    |
| Peg10        | ACGATGATGACCTGGAGCTT     | ATGAAAGGACCCACGATGTC    |
| Phlda2       | TCAGCGCTCAGGTCTGAGA      | CTCTGGGCTCCTGCTGAT      |
| Lin28a       | GTCTTTGTGCACCAGAGCAA     | CGCTCACTCCCAATCACAGAA   |
| Lin28b       | ACGGCAGGATTACTGATGG      | GCACTTTTTGGCGTGAAGAG    |
| Oct6/Pou3f1  | ACCACCACCACCAACT        | AAATCCAAAGCCACACAGAAT   |
| Cdkn1c       | GGAGCAGGACGGAATCAAG      | GGTCCTCTGCGAGTCTCTCCT   |
| Sfmbt2       | CATGTGGAGATCAGCATTCG     | TGTCACAGGTTGGTGATGAT    |
| Igf2         | GACGACTTTCCCAAGATACCC    | CTTTGAGCTTTTGGCAAGC     |
| Gene   | Forward Primer Sequence | Reverse Primer Sequence |
|--------|-------------------------|-------------------------|
| Brachyury | CTGGGAGCTCAGTTCTTTTCGA | GAGGAGCTGGCAGCTGAGA    |
| Hand1  | AGAGGAGACGCACAGAGAGC   | AGCACGTCATCAAGTAGGCC   |
| Snai   | TGAGAAGCCATTCTCCTGCT   | CTTCACATCCGAGTGGTGTTT  |
| Tubb3  | CATGGCAGTGTTCGGCTCTG  | CGCACGACATCTAGGACTGA   |
| Krt18  | CGAGGCACCTCAAGGAAGAAC  | GCTGAGGTTCCTGAGATTTGG  |
| Nes    | CTGCAGGGCCTGAAAAAGTT  | TTCCAGGATCTGAGCGATCT   |
| Tfcp2l1 | GGGGACTACTCGGAGCATCTC | TTCCGATCAGCTCCCTTG     |
| Tbx3   | TTGCAAAGGGTTTTCGAGAC  | TGCAATGTCAGCTGCTTTTC   |
| Dppa3  | TTTGTTTGTCGGTGCTGAAAG | TCATTCTCTTCGAGCCCTTTT  |
| Beta-actin | CTAAGGCCAACCCTGAAAAG | ACCAGAGGCACACAGGGACA   |
| Dnmt3a1 | GAAGGCCGTGGAGCCTCT    | CCATTTATGGATTCGATGTT   |
| Dnmt3a2 | AGAAGGTGTCTGCTGCACAC  | TGCTCCAGACACACTCTTGCAG |
| Dnmt3b INT | TGACGTCCGGAAAAATCAAC | TAAACCTTTGCGGGCAGGAT   |
| Otx2   | GGAAGAGGTGGCGACTGAAA  | CGGCACCTAGCTTCCGATT    |
| Sox1   | CACAAGCTCGAGATCAGCAA  | CTCGGACATGACCTCCACT    |
| Klf4   | CGACTAACCCTGGGCGTGA   | CGGGTTGTACTGCTGCAAG    |
| Tbx3   | TTGCAAAGGGTGTCTGAGAC  | TGCAAGGTGCAGCTGTTTCT   |
| Nanog  | TTCTTCTTACAAGGGTCTGCT | AGAGGAAGGGCGAGGAGA     |
| Prdm14 | GAAGGCACACAGGGACAAGCT | TCCAGTTCCAGAGCTTTTG    |
| Gene  | Sequence 1 | Sequence 2 |
|-------|------------|------------|
| Tcl1  | GATCTGGGAGAAGCAGTGTGA | TGACTGGGGGACATAGCTTC |
| Tceaa | GACAAGCTGGCCTCAGAAAT | CGGTTCCGGAATTCATGTC |
| Vegfa | CTGTAACGATGAAGCCCTGGAG | TGGTGAGGTTTGATCCGCAT |
| Phd3  | CTTCTCCTGTCCCTCATCG | ATACAGCGGCCATACCCATT |
### Supplementary Table 8. Primers used for MeDIP-PCR.

| Gene MeDIP-qPCR | Forward primer sequence       | Reverse primer sequence       |
|-----------------|-------------------------------|-------------------------------|
| Nnat            | GAGTATGTACCCGGGCTTTG          | ATAGGATGGGTTGGTAGGG           |
| Peg10           | CCCCTCCTAGGATCCTCCTCT        | GGATTCTCGACACACACCA           |
| Kif27           | CCAGCTGAGGGGATAACTCA          | TCTGGGTCTTTCAATACCAA          |
| Iap             | CTCCATGTGCTCTGCTTCC          | CCCCCGTCCTTTTTTGGAGGA         |
| Iapey3          | AGAGAGGAGGACAACGTGCTC        | AACCTTACACAGGCAAAAGC          |
| Line L1         | CTGGCCGAGGATGTTGAGAA         | CCTGCAATCCCAACCAAAAT          |
| MajSat          | GACGACTTTGAAAAATGACGAAATC    | CATATTCCAGTTCTTACGTGTGC       |
| MinSat          | CATGGAAAAATGATAAAAAACC       | CATCTAATATGTCTACGTGTGG        |
| Sine B1         | GTGGGCACGCCTTTAATC           | GACAGGTTTCTCTGTGTA            |
| Sine B2         | GAGATGGCTCAGTGGTTAAG         | CTGTCTTCAGACACTCCAG           |
| Gapdh           | TCCCTAGACCCGTACAGTGC         | CTCTGCCTCTCCCTGTCC            |
| Zdbf2           | CCAAACCCATCCTCCTTCA          | TGGCCTGGTCTAGTCTGTCC          |
| 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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