# Supplementary Figures and Tables

## Contrasting epigenetic states of heterochromatin in the different types of mouse pluripotent stem cells

Tosolini Matteo, Brochard Vincent, Adenot Pierre, Chebrout Martine, Grillo Giacomo, Navia Violette, Beaujean Nathalie, Francastel Claire, Bonnet-Garnier Amélie and Jouneau Alice

# Supplementary Figure legends

**Supplementary Fig. 1:**

(A) Magnification of nuclei of 2i-ESCs, serum-ESCs and EpiSCs, representative of the categories described in Fig. 1B. (B) Mean number of H3K9me3, H3K27me3 and DAPI clusters in each category and each type of pluripotent cells.

**Supplementary Fig.2**

RNA-FISH for Major satellites in ESCs: examples of nuclei showing localization of RNA foci near chromocenter (A), within peri-nuclear heterochromatin (B) or peri-nucleolar heterochromatin (C). DAPi is in red, major satellite probe is in green.

**Supplementary Fig. 3:**

(A) Pictures of EpiSCs converted from WT or Suv39hdn ESCs. (B) Relative expression of different common pluripotency (*Oct4, Sox2, Nanog*), naive-specific (*Klf4, Esrrb*) and epiblast specific (*Dnmt3b*, *Fgf5, Otx2*) transcripts by qRT-PCR analysis normalized to *Sdha* and *Pbgd* housekeeping genes. 2i-ESC expression was set to 1.Three independent conversions were made. (C) Western-blot analysis of EZH2, H3K27me3 and H3K9me3 and H3 as loading control, in WT and Suv39hdn cells. (D) Immunostaining images (single plan) for H3K9me3, H3K27me3 and DAPI in WT 2i-ESCs and WT cEpiSCs. (E) Representative nuclei of the categories as in Fig. 1B and percentages of each in the WT 2i-ESC (WT01 line) and cEpiSC populations. (F) ChIP-QPCR analysis of H3K9me3 and H3K27me3 at major satellites in WT and *Suv39hdn* 2i-ESCs and cEpiSCs (n=4; error bars are s.e.m). Values were normalized to input and shown relative to WT 2i-ESCs.

**Supplementary Fig. 4:**

(A) Pictures of ESCs and cEpiSCs obtained after 10 days of conversion. (B) Relative expression of different common pluripotency (*Oct4*, *Sox2*, *Nanog*), naive-specific (*Klf4*, *Esrrb*) and epiblast specific (*Fgf5*, *Otx2*) transcripts by qRT-PCR analysis normalized to *Sdha* and *Pbgd* housekeeping genes. 2i-ESC expression was set to 1. Two independent conversions were made. (C) Percentages of each category of nuclei for WT-ESCs and WT cEpisCs.

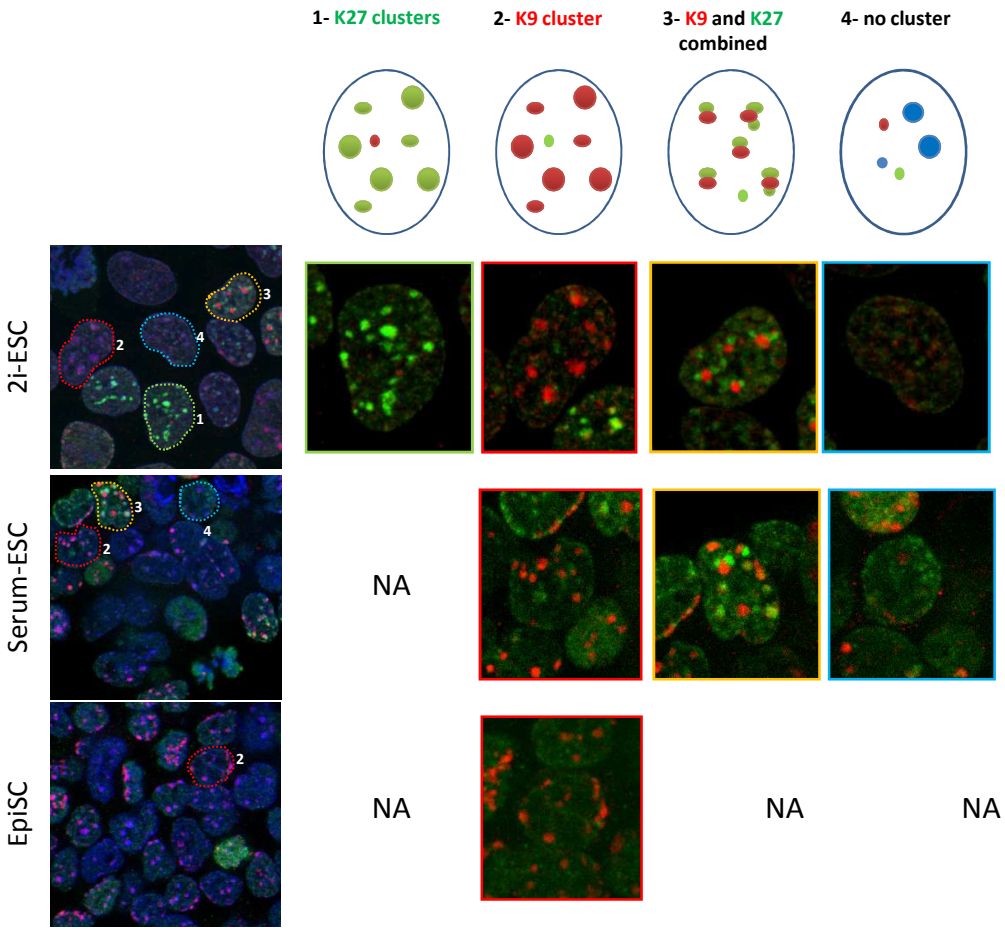
**Supplementary Fig. 5**

Immunostaining images of NANOG in the 3 pluripotent cell types.

**Supplementary Table S1: List of antibodies used for immunostaining and western-blot**

**Supplementary Table S2: list of primers used for qRT-PCR**

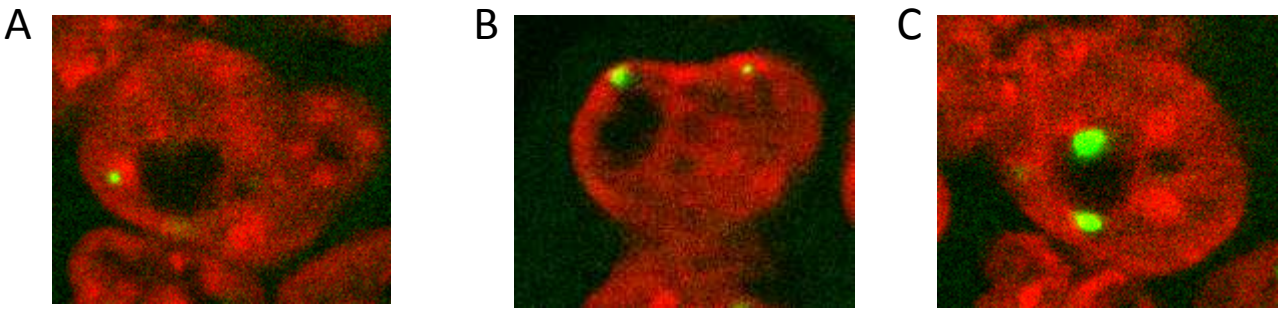
A




B

| Mean number of clusters/ categories | Cat. 1 | Cat. 2 | Cat. 3 | Cat. 4 |
|---|---|---|---|---|
| 2i-ESC (n=75) | | | | |
| **DAPI** | **17** | **14** | **11** | **15** |
| K9me3 | 2 | 11 | 9 | 2 |
| K27me3 | 17 | 2 | 9 | 1 |
| serum ESC (n=40) | | | | |
| **DAPI** | **NA** | **16** | **16** | **7** |
| K9me3 | NA | 18 | 13 | 2 |
| K27me3 | NA | 1 | 7 | 1 |
| EpiSC (n=30) | | | | |
| **DAPI** | **NA** | **15** | **NA** | **NA** |
| K9me3 | NA | 15 | NA | NA |
| K27me3 | NA | 0 | NA | NA |

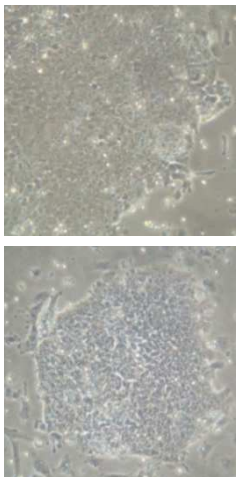
**Supp Figure 1**



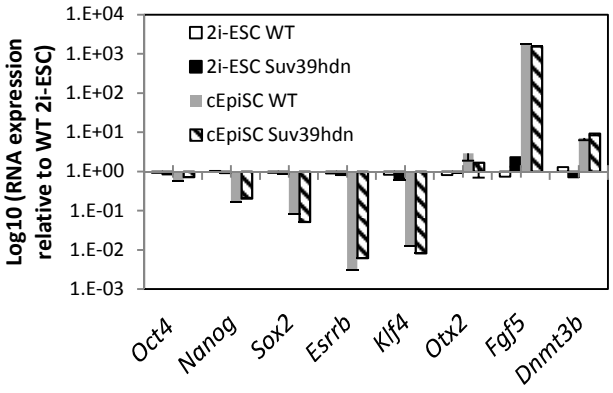

**Supplementary Fig.2**
RNA-FISH for Major satellites in ESCs: examples of nuclei showing localization of RNA foci near chromocenter (A), within peri-nuclear heterochromatin (B) or peri-nucleolar heterochromatin (C).

**Supp Figure 2**

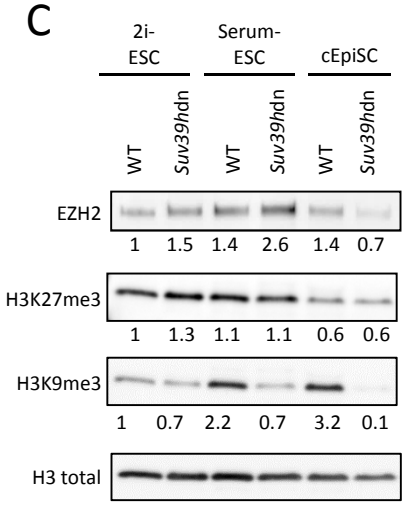
A



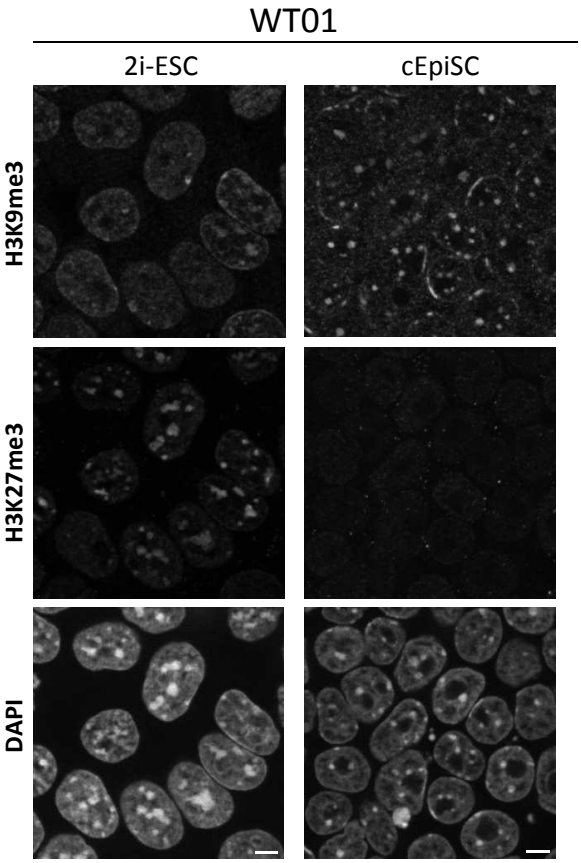

B



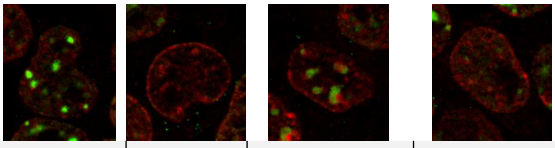

C




D

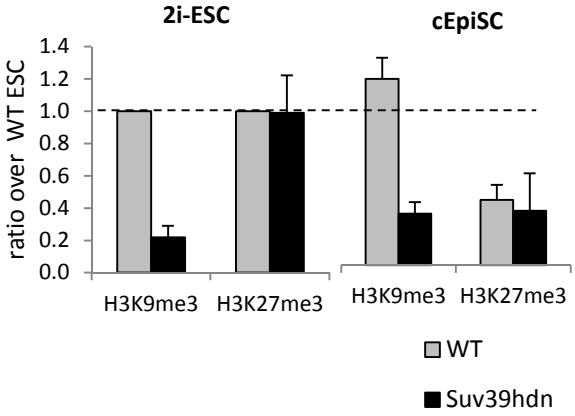



E



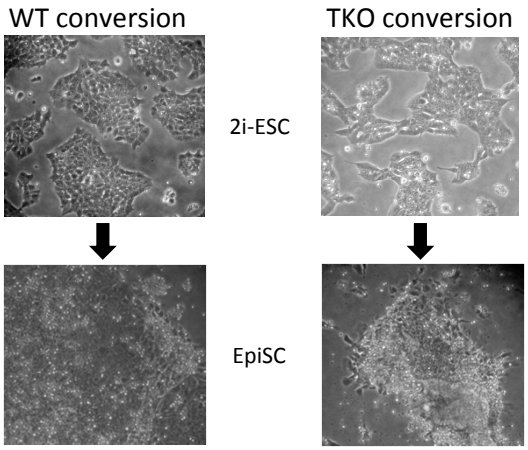
| Category of nucleus | 1- K27 cluster | 2- K9 cluster | 3- K9 and K27 combined | 4- No cluster |
|---|---|---|---|---|
| WT01 2i-ESCs (n=49) | 67% | 4% | 14% | 14% |
| WT01 cEpiSC (n=20) | 0% | 100% | 0% | 0% |

F

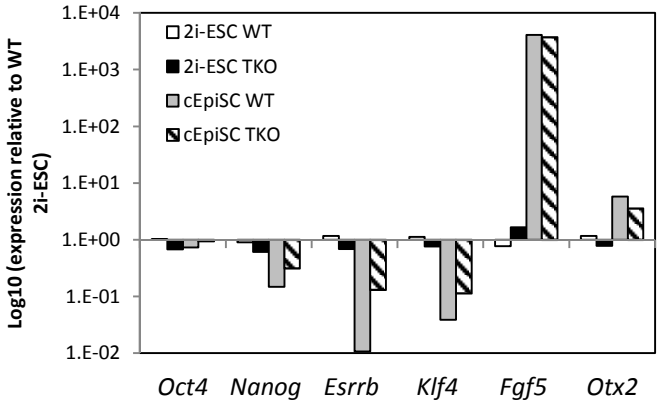



**Supp Figure 3**

A



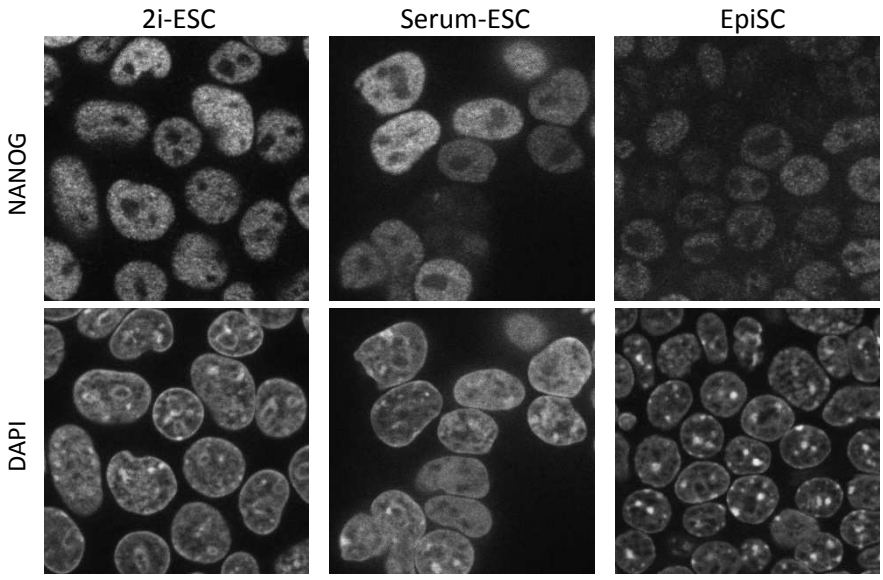

B




C

| Category of nucleus | 1- K27 cluster | 2- K9 cluster | 3- K9 and K27 combined | 4- No cluster |
|---|---|---|---|---|
| 159-WT 2i-ESCs (n=30) | 40% | 21% | 40% | 0% |
| 159-WT EpiSC (n=17) | 0% | 100% | 0% | 0% |

**Supp Figure 4**




**Supp Figure 5**

| Antigen | dilution for immunostaining | dilution for western-blot | reference |
|---|---|---|---|
| H3K9me3 | 1/300 | 1/1000 | Active Motif 39161 |
| H3K27me2me3 | 1/300 | 1/1000 | Active Motif 39538 |
| H3K27me3 | | 1/1000 | Millipore DAM07-774 |
| EZH2 | 1/200 | 1/1000 | Novocastra NCL-L-EZH |
| EZH1 | | 1/500 | Active Motif 61583 |
| SUV39H1 | 1/100 | 1/1000 | Cell Signaling D11B6 #8729 |
| NANOG | 1/100 | | Abcam ab80892 |
| DNMT3B | 1/200 | 1/500 | Active Motif 39207 |
| DNMT3A | | 1/500 | Active Motif 39206 |
| H3total | | 1/20,000 | Abcam 1791 |
| | | | |
| Anti-Rabbit-Cy3 | 1/200 | | Jackson ImmunoResearch |
| Anti-Mouse-FITC | 1/200 | | Jackson ImmunoResearch |
| Anti-Goat-Cy3 | 1/200 | | Jackson ImmunoResearch |
| Anti-mouse-HPO | | 1/5000 | Jackson ImmunoResearch |
| anti-rabbit-HPO | | 1/5000 | Jackson ImmunoResearch |

# Supp Table S1

| Primers for qRT-PCR | | | | |
|---|---|---|---|---|
| **Gene** | **Primer Forward Sequence (5'-3')** | **Primer Revers Sequence (5'-3')** | **Reference or Primer Bank ID** | **Annealing** |
| ***Major satellite*** | GACGACTTGAAAAATGACGAAATC | CATATTCCAGGTCCTTCAGTGTGC | 1 | 60°C |
| ***Minor satellite*** | GAACATATTAGATGAGTGAGTTAC | GTTCTACAAATCCCGTTTCCAAC | 2 | 60°C |
| ***b-Actin promoter*** | CGTATTAGGTCCATCTTG | GCCATTGAGGCGTGATC | | 60°C |
| ***Sdha*** | GGAACACTCCAAAAACAGACCT | CCACCACTGGGTATTGAGTAGAA | | 60°C |
| ***Pbgd*** | CCTGGCATACAGTTTGAAATCAT | TTTTTCCAGGGCGTTTTCT | 3 | 60°C |
| ***Ezh2*** | AGTGACTTGGATTTTCCAGCAC | AATTCTGTTGTAAGGGCGACC | | 60°C |
| ***Suv39h1*** | GCAGTGTGTGCTGTAAATCTTCT | ATACCCACGCCACTTAACCAG | | 60°C |
| ***Dnmt3a*** | GAGGGAACTGAGACCCCAC | CTGGAAGGTGAGTCTTGGCA | 6681209a1 | 60°C |
| ***Dnmt3b*** | TCAGATGAGCAAGGTCAAGG | TGTACCAAAGCAAGGGGAAG | | 60°C |
| ***Oct4*** | CAGCCAGACCACCATCTGTC | GTCTCCGATTTGCATATC | 7305399a3 | 58°C |
| ***Nanog*** | CTTTCACCTATTAAGGTGCTTGC | TGGCATCGGTTCATCATGGTAC | 4 | 58°C |
| ***Sox2*** | GCGGAGTGGAAACTTTTGTCC | CGGGAAGCGTGTACTTATCCTT | | 60°C |
| ***Fgf5*** | TGTGTCTCAGGGGATTGTAGG | AGCTGTTTTCTTGGAATCTCTCC | 6753854a1 | 60 °C |
| ***Otx2*** | TATCTAAAGCAACCGCCTTACG | GCCCTAGTAAATGTCGTCCTCTC | 158518427c1 | 60°C |
| ***Esrrb*** | ATGCGAGTACATGCTTAACGC | CATCCCCACTTTGAGGCATTT | | 60°C |
| ***Klf4*** | GCAGTCACAAGTCCCCTCTC | GACCTTCTTCCCCTCTTTGG | 5 | 58°C |


1. Lehnertz, B. et al. Suv39h-mediated histone H3 lysine 9 methylation directs DNA methylation to major satellite repeats at pericentric heterochromatin. Curr. Biol. 13, 1192–1200 (2003).
2. Ferri, F., Bouzinba-Segard, H., Velasco, G., Hubé, F. & Francastel, C. Non-coding murine centromeric transcripts associate with and potentiate Aurora B kinase. Nucleic Acids Res. 37, 5071–5080 (2009).
3. Bernardo, A. S. et al. BRACHYURY and CDX2 Mediate BMP-Induced Differentiation of Human and Mouse Pluripotent Stem Cells into Embryonic and Extraembryonic Lineages. Cell Stem Cell 9, 144–155 (2011).
4. Hayashi, K. & Surani, M. A. Self-renewing epiblast stem cells exhibit continual delineation of germ cells with epigenetic reprogramming in vitro. Development 136, 3549–3556 (2009).
5. Jouneau, A. et al. Naive and primed murine pluripotent stem cells have distinct miRNA expression profiles. RNA 18, 253–64 (2012).


# Supp Table S2