

Supplementary information

Nr0b1 is a negative regulator of *Zscan4c* in mouse embryonic stem cells

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Legends for supplemental data

Supplemental Figure 1. (related to Fig. 1) Western blot analysis of Nr0b1 expression in wild-type (+/Y), *Nr0b1^{fl/Y}* (fl/Y) and *Nr0b1^{KO/Y}* (KO/Y) ES cells. Data from two independent *Nr0b1^{fl/Y}* (fl/Y) ES cell lines were shown and left set was shown in Fig 1d.

Supplemental Figure 2. (related to Fig. 4) Immunostaining of *Nr0b1^{KO/Y}* ES cells by antibodies against Gata4 (C-20, Santa Cruz), Sox7 (R&D) and Sox17 (R&D); left: bright field, center: Hoechst staining, right: antibody staining detected by Alexa 594.

Supplemental Figure 3. (related to Fig. 4) Immunostaining by anti-Tcstv1 antibody.

(a) Immunostaining of mouse pre-implantation embryos; green: Tcstv1, red: Oct3/4. (b)

Immunostaining of OCRG9 ES cells; red: Tcstv1, green: Rex1Egfp, cyan: Oct3/4Ecfp, blue: Hoechst staining.

Supplemental Figure 4. (related to Fig. 7) Western blot analysis of Nr0b1 expression in wild-type (+/Y), *Nr0b1^{fl/Y}* (fl/Y) *Nr0b1^{KO/Y}* (KO/Y) ES cells, and Tg ES cells cultured with or without Dox. CDK2 acts as a control.

Supplemental Figure 5. Generation and analysis of inducible *Nr5a2*-null ES cells. (a)

Schematic representation of the strategy to generate the inducible *Nr5a2*-null ES cells.

(b) Colony morphologies of *Nr5a2^{fl/fl}* ES cells carrying hormone inducible Cre after the culture for 5 days without (upper panel) or with Tx (lower panel). Scale bar; 200 μ m.

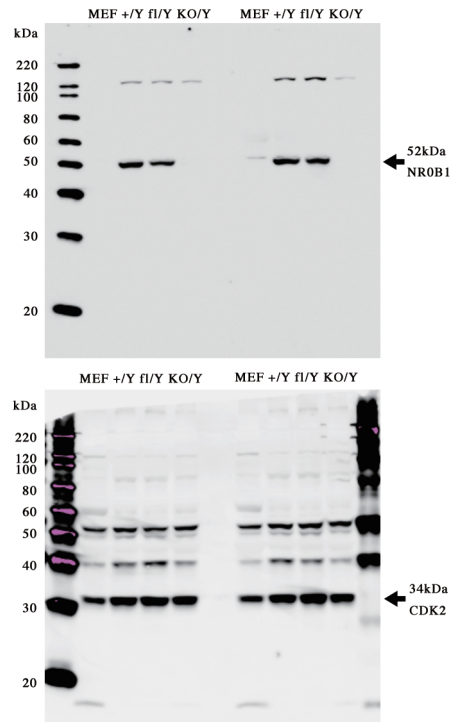
(c) Quantitative RT-PCR analysis of inducible *Nr5a2*-null ES cells cultured with or without Tx for 5 days. The expressions of pluripotency-associated genes (upper panel) and 2-cell specific transcript genes (lower panel) are indicated. The expression levels of each transcript in inducible *Nr5a2*-null ES cells without Tx was set at 1.0. Error bars indicate standard deviation (n=3).

Supplemental Table 1. List of the primer sequence

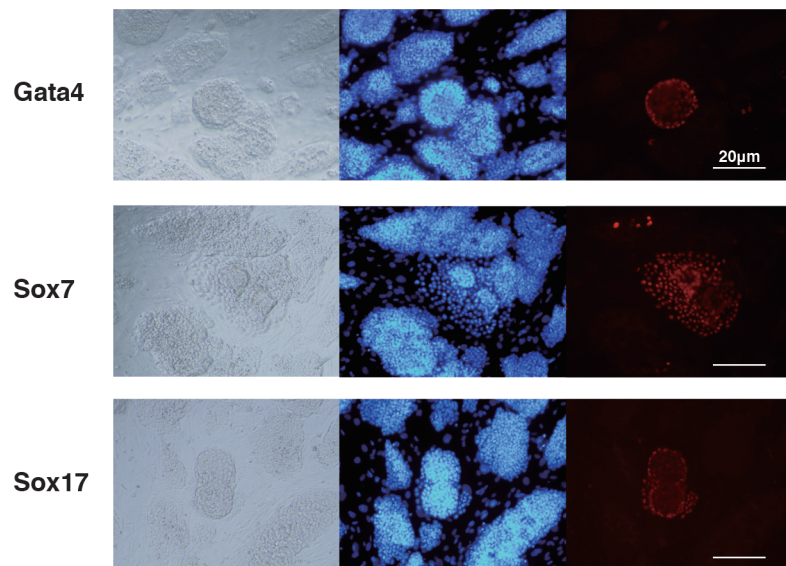
Supplemental Table 2. Efficiency of the production of chimeric embryos from *Nr0b1^{fl/Y}* and *Nr0b1^{KO/Y}* ES cells carrying *pPBCAG-Egfp-IP*. Embryos carrying GFP-positive cells were designated as chimeras.

Supplemental Movie 1. Live-imaging of the fluorescent signal of *Nr0b1^{fl/Y}* and *Nr0b1^{KO/Y}* ES cells carrying *pPB-Zscan4c-mCherry*.

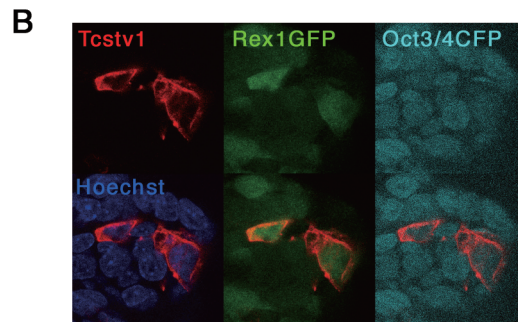
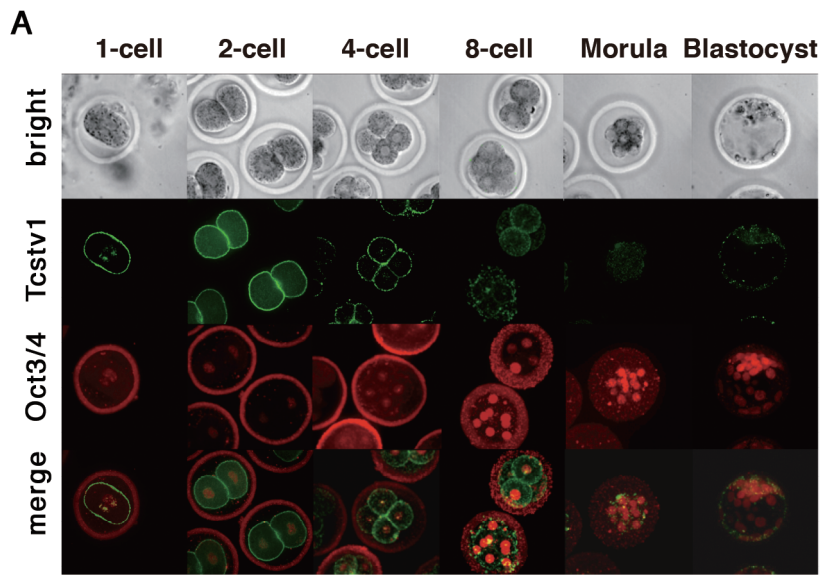
Supplemental Fig 1



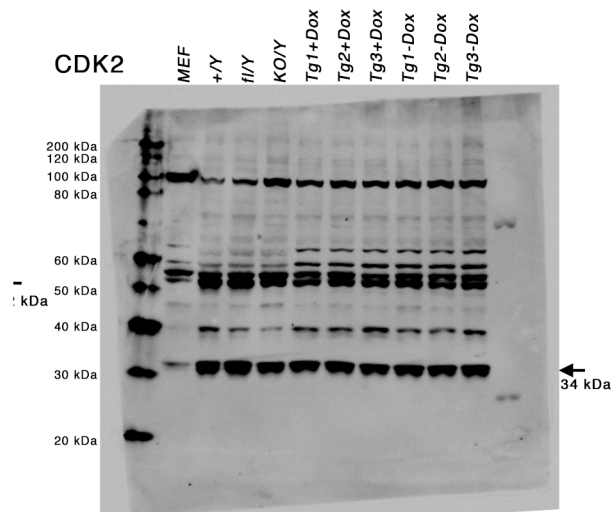
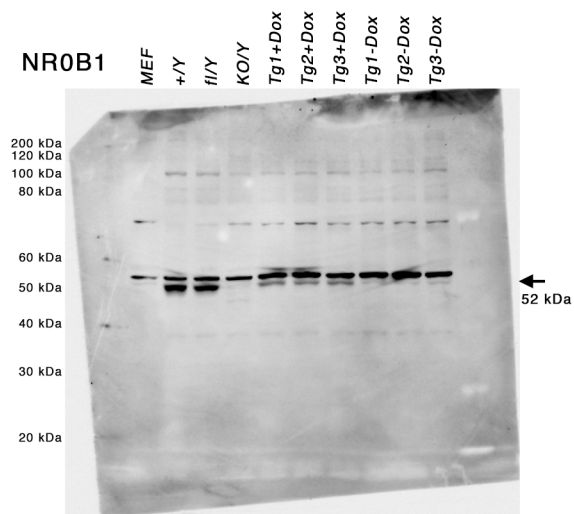
Supplemental Fig 2



Supplemental Fig 3



Supplemental Fig 4



Supplemental Fig 5

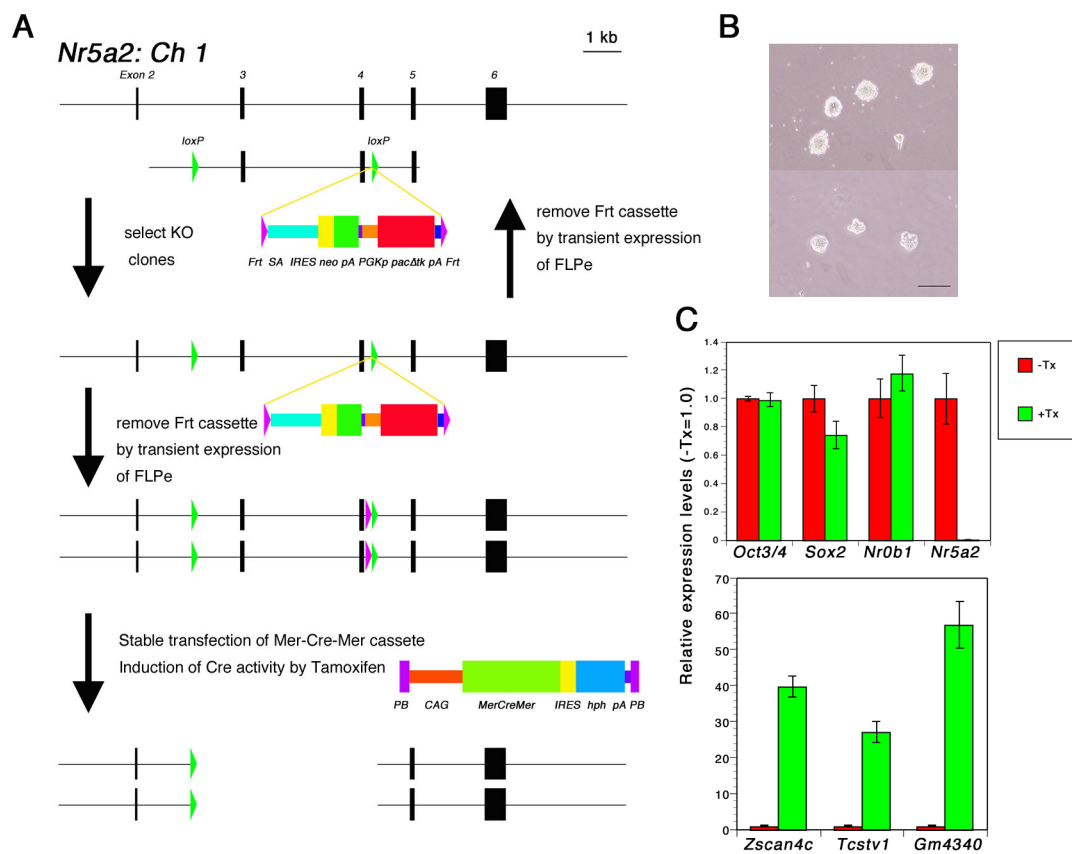


Table S1. List of primer sequence.

For plasmid construction

Target gene	Forward primer	Reverse primer
<i>Nr0b1</i> (5' arm)	5'-gctcgacggtatcgatactcacact cagatagcttgtagc -3'	5'-taatgtatgctatacgaagtatatggac tgaaataagttctaagtttcagaagca -3'
<i>Nr0b1</i> (flox)	5'-cgtatagcatacattatacgaagtta ttatttaggcccccagaaaacccttt -3'	5'-gtataatgtatgctatacgaagtatattc acataaagatgtttaaactctgttgag-3'
<i>Nr0b1</i> (3' arm)	5'-tgaagatctccttagaagcctggg aatc -3'	5'-aatgcgccgctgatgaattcatccac attctgcct -3'
<i>Nr0b1</i> (KO PCR 1)	5'-gcgacggtgacgaaggccagcaa ttagg -3'	5'-cggaataggaactcagatctcgatcta ta -3'
<i>Nr0b1</i> (KO PCR 2)	5'-aagtgttgcttctgaaaacttagaac ttattcagtcctataaactcgt -3'	5'-tgcactcacatgcacagacacacaca cacg -3'
<i>Zscan4c</i> (promoter)	5'-attgtcgacaccttctgttcatgcaag cctgg -3'	5'-aataccgggtctcgagtgggacaatgg gtgaaagattc -3'
<i>Zscan4c</i> (CDS)	5'-attctcgagtcaccacaatggcttca cagcaggca -3'	5'-aatgcgcccgcttcagtcagatctgtg gtaattcctc -3'
<i>Nr0b1</i> (CDS)	5'-ccctcgagcctcaggccatggcgg gtgaggaccaccgt -3'	5'-ccgcccggctcacagcttgcacaga acatc -3'

For QPCR

Target gene	Forward primer	Reverse primer
<i>Gapdh</i>	5'-tgccatcactgccaccagaagactg -3'	5'-tgaggccaccaccctgttgctgtag -3'
<i>Oct3/4</i>	5'- aagccctccctacagcagat -3'	5'- ctgggaaagggtgccctgta -3'
<i>Nr0b1</i>	5'- tccaggccatcaagagtttc -3'	5'- atctgctgggttctccactg -3'
<i>Sox2</i>	5'- gagtggaactttgtccgaga -3'	5'- gaagcgtgtacttatccttctcat -3'
<i>Nanog</i>	5'-acctgagctataagcaggtaagac -3'	5'- gtgctgagcccttctgaatcagac -3'
<i>Tbx3</i>	5'- tcccggaaacagaattcatc -3'	5'- gagacagcaggagaggatgc -3'

<i>Klf2</i>	5'- accaagagctcgcacctaaa -3'	5'- gtggcactgaaagggctctgt -3'
<i>Klf4</i>	5'- ccaaagaggggaagaaggtc -3'	5'- cctgtgtgtttgcggtagtg -3'
<i>Klf5</i>	5'-caagccgttccagtgcat-3'	5'-gtctgcggtttaaggatgg-3'
<i>Esrrb</i>	5'-ctcgccaactcagattcgat-3'	5'-agaagtgtgcacggcttg-3'
<i>Zscan4c</i>	5'-ccggagaaagcagtgaggtgga-3'	5'-cgaaaatgctaacagttgat-3'
<i>Tcstv1</i>	5'-tcccaggagatgtggaaa-3'	5'-cgtcacaggtcacaagatccaga-3'
<i>Gm4340</i>	5'-tggacttcattcacagcttc-3'	5'-catggaatttggccttgatc-3'
<i>Nr5a2</i>	5'-agatgccagaaaacatgcaa-3'	5'-tatcgccacacacaggacat-3'
<i>ClnE1</i>	5'-ttctgcagcgtcatcctct-3'	5'-tggagcttatagacttcgcaca-3'

Table S2. Efficiency of the production of chimeric embryos.

Cell line	Number of collected embryos	Number of chimeras
<i>Nr0b1^{Floxed/Y} 1</i>	7	0
<i>Nr0b1^{Floxed/Y} 2</i>	12	4
<i>Nr0b1^{Floxed/Y} 3</i>	4	0
<i>Nr0b1^{Null/Y} 1</i>	10	1
<i>Nr0b1^{Null/Y} 2</i>	6	0
<i>Nr0b1^{Null/Y} 3</i>	13	1
<i>Nr0b1^{Null/Y} 4</i>	6	2