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Distinct requirements for the maintenance and establishment of mouse embryonic stem cells
(マウスES細胞の維持と樹立における異なる要求性)

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ABSTRACT

Mouse embryonic stem cells (ESCs) are derived from the inner cell mass (ICM) of blastocysts. The culture condition with the cytokine leukemia inhibitory factor (LIF) and two inhibitors of glycogen synthase kinase-3-β (GSK3β) and mitogen-activated protein kinase (MEK) signaling (2iL) greatly enhances the establishment of ESC. Genomic imprinting is an epigenetic phenomenon that regulates allele-specific gene expression by DNA methylation or histone modifications, but it is lost in female ESCs under 2iL condition. This loss of imprinting causes the loss of full-term development potential of female ESCs. Therefore, a novel method to establish the ESCs maintaining normal imprinting is required. To this end, I analyzed the necessary culture condition for the maintenance and establishment of ESCs in detail. Even at low concentration of the GSK3β inhibitor and LIF (LowGiL), the expression levels of pluripotency markers and the chimera-producing ability of the cells were comparable with those of ESCs cultured in 2iL. However, blastocysts underwent spontaneous differentiation, and ESCs were not established under LowGiL condition. Time-course analysis showed that 2iL condition for three days from the initiation of culture was sufficient for the acquisition of permanent pluripotency. The female ESCs established using this LowGiL condition maintained DNA methylation at differentially methylated regions (DMRs) of imprinted genes. Gynogenetic and androgenetic ESCs established using LowGiL condition also displayed the parent-of-origin gene expression of all imprinted genes that were bi-allelic expressed in the ESCs established under 2iL condition. Taken together, the novel method proposed in this study would be the powerful tool to produce the ESCs keeping genomic imprinting.

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1. Introduction

1.1. Genomic imprinting

Genomic imprinting is monoallelic gene expression that is mainly regulated by parental allele-specific DNA methylation at differentially methylated regions (DMRs) or maternal tri-methylation at lysine 27 of histone H3 (H3K27me3) (Fig. 1A). Most of DMRs are established during oogenesis or spermatogenesis, and inherited to the next generations through the fertilization. Maternal allele-specific H3K27me3 was established during oogenesis and maintained in extraembryonic lineages. While imprinting is faithfully maintained in somatic lineages, it is erased in primordial germ cells (PGCs) prior to the re-establishment of oocyte- and sperm-specific imprinting patterns (Spahn and Barlow, 2003; Thorvaldsen and Bartolomei, 2007) (Fig. 1B). The mechanisms of imprinting in mammals were studied in the 1980s by the Solter and Surani laboratories using uniparental embryos (Barton et al., 1984; McGrath and Solter 1983, 1984; Surani and Barton 1983; Surani et al., 1984). Parthenogenetic and gynogenetic embryos have only maternal genome; androgenetic embryos harbor only paternal genome. Parthenogenetic embryos are generated by stimulation of unfertilized oocytes. On the other hand, gynogenetic and androgenetic embryos are produced by enucleation and transplantation of one of pronuclei at one-cell stage. Gynogenetic and parthenogenetic embryos developed into tissues predominantly of embryonic origin, with failed in the growth of extraembryonic lineages. Whereas, androgenetic embryos died shortly after implantation. The lethal phenotype of uniparental embryos demonstrated that both paternal and maternal genomes play an indispensable role in normal development. From these results and later studies, imprinted genes that were predominantly expressed from either paternal or maternal allele were identified (Plasschaert and Bartolomei, 2014).

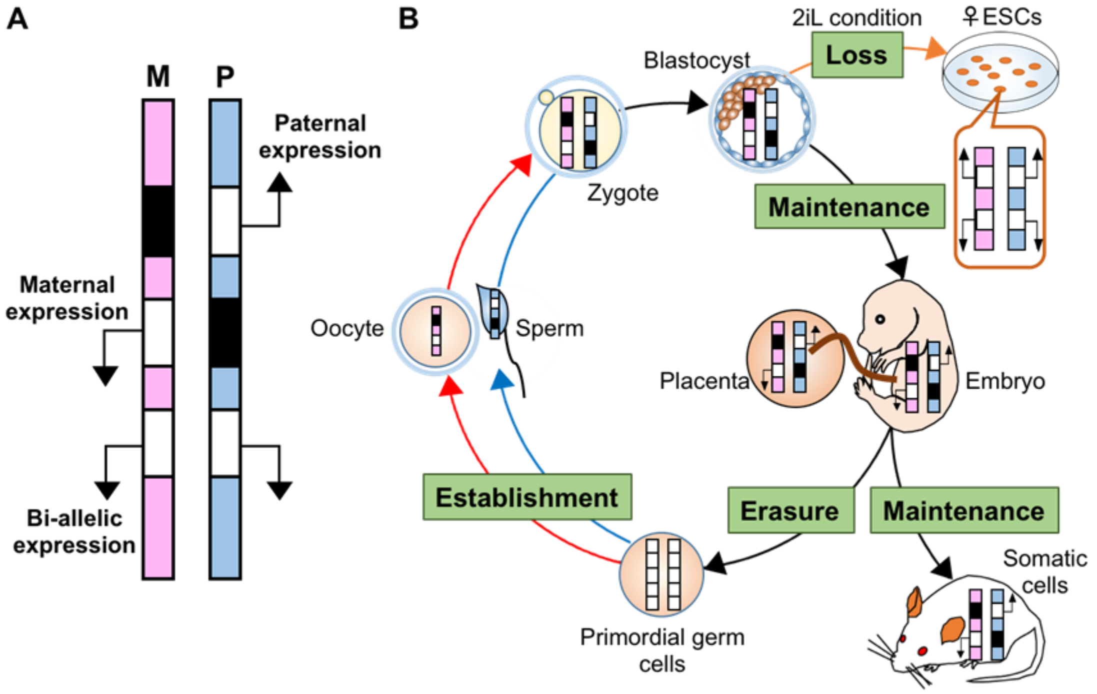


Figure 1. The regulation of genomic imprinting.
(A): The schematic diagram of allele-specific gene expression patterns. Black-filled boxes indicate methylated regions, open boxes indicate unmethylated regions. **(B):** The life cycle of genomic imprinting. M: maternal allele (pink), P: paternal allele (light blue).

1.2. Mouse embryonic stem cells (ESCs)

Mouse ESCs are derived from the inner cell mass (ICM) of embryonic day 3.5 (E3.5) or E4.5 blastocysts (Evans and Kaufman, 1981; Martin, 1981; Smith, 2001). ICMs retain the pluripotency that an ability to give rise to all three germ layers, including germ cells. In contrast to ESCs, which can maintain pluripotent state almost permanently, the pluripotent state of the ICM is transient and gradually lost after implantation (Fig. 2A). The ICM undergoes differentiation into the epiblast and primitive endoderm via distinct FGF signaling-pathways at the pre-implantation stage (Fig. 2B). FGF4/FGFR1 signaling plays a critical role in epiblast maturation, whereas both FGFR1 and FGFR2 are required for cell-fate decision of primitive endoderm (Kang et al., 2017; Molotkov et al., 2017).

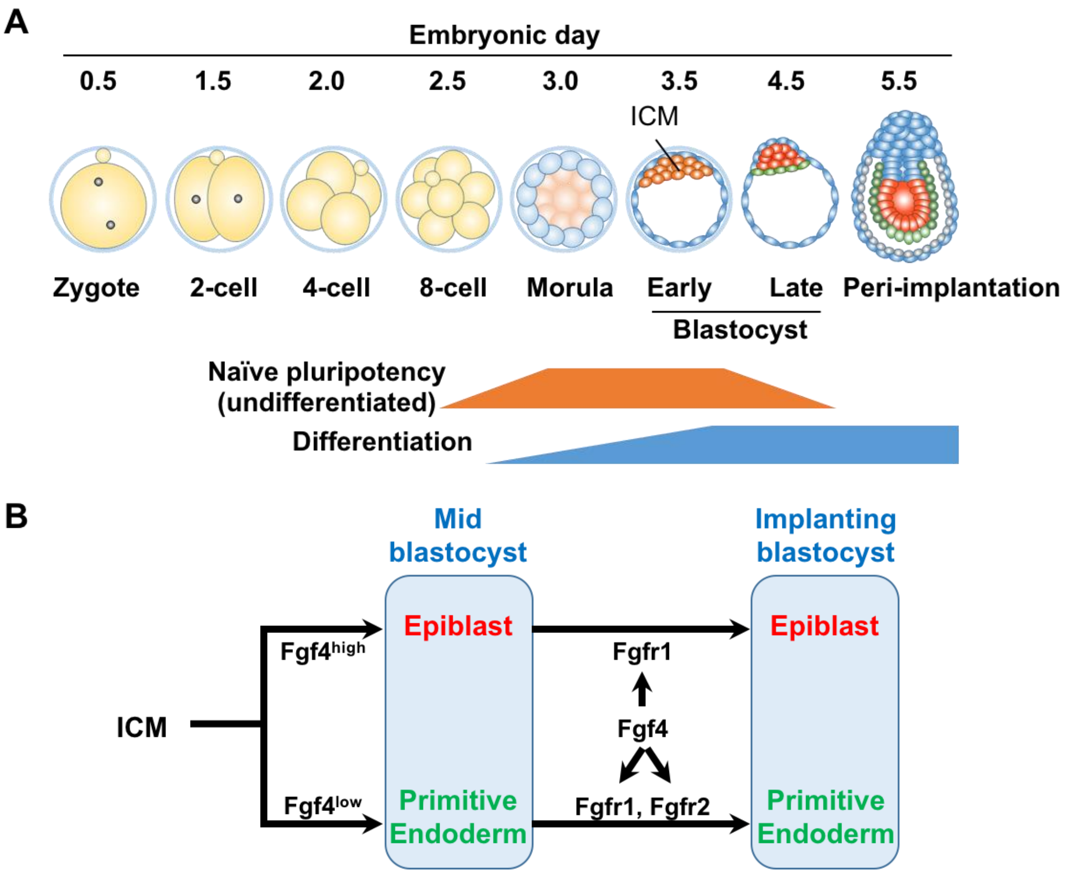
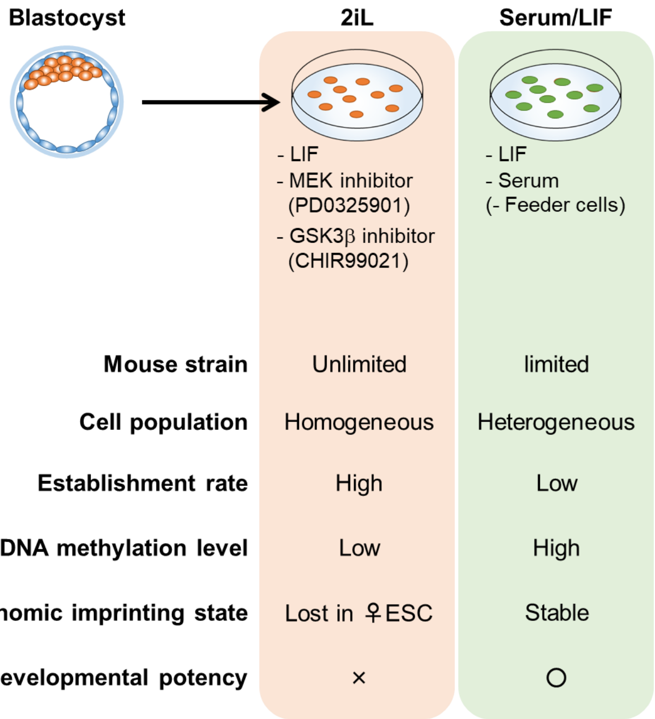


Figure 2. Pluripotency of cells in mouse preimplantation embryos.
(A): The schematic diagram of early mouse embryonic development. The colored bars show the spectral term of cell potencies. **(B):** The role of FGF signaling during cell fate decision.

Historically, the way for the establishment of ESC was founded on works with mouse teratocarcinomas, the specific type of germ cell tumor (Zwaka and Thomson, 2005). The embryonal carcinoma cells (ECC) lines that were derived from teratocarcinomas were able to differentiate into multiple cell types and maintain the undifferentiated state (Damjanov and Solter, 1974; Dixon and Moore, 1952; Kleinsmith and Pierce, 1964; Martin and Evans, 1975; Stevens and Little, 1954). In 1981, ECC culture condition, which included serum with feeder layers, was used to establish ESCs from preimplantation mouse embryos (Evans and Kaufman, 1981; Martin, 1981). In 1988, the cytokine leukemia inhibitory factor (LIF) was reported to support self-renewal of ESCs as a replacement of feeder layer (Smith et al., 1988). Serum and LIF condition (serum/LIF) has been used for both routine culture and derivation of mouse ESC (Hayashi et al., 2008; Niwa et al., 2009; Williams et al., 1988). In 2003, it was shown that serum could be replaced by the bone morphogenetic proteins (BMPs). However, the pluripotent states of ESCs maintained under both serum/LIF and BMPs/LIF were shown to be metastable and heterogeneous (Smith et al., 1988; Hayashi et al., 2008; Niwa et al., 2009; Williams et al., 1988, Ying et al., 2003) (Fig. 3). In addition, the efficiency of ESC derivation under serum/LIF condition was noticeably low and only applicable to a few permissive strains, including 129/Sv and C57BL/6 (Kawase et al., 1994; Czechanski et al., 2014). These problems were resolved by the use of two inhibitors of glycogen synthase kinase-3-β (GSK3β) and MEK signaling, and LIF (2iL) (Ying et al., 2008; Nichols and Smith, 2009; Dunn et al., 2014). This 2iL culture condition enabled us to maintain ESCs in a naïve and homogeneous pluripotent state (Fig. 3). Moreover, 2iL culture condition was found not only improving the efficiency of ESC derivation, but also establishing ESC from multiple non-permissive strains, including BALB/c and Nonobese diabetic (NOD) (Hanna et al., 2009; Nichols et al., 2009; Czechanski et al., 2014). The culture of preimplantation embryos with an inhibitor of MEK and extracellular signal-related kinase 1/2 (ERK1/2), which functions downstream of FGF4/FGFR signaling, suppressed differentiation of the primitive endoderm (Yamanaka et al., 2010; Nichols et al., 2009). Given that the transcriptional profile of naïve ESCs was similar to that of E4.5 early epiblast cells, it is proposed that maturation of the ICM into an early epiblast-like state is a prerequisite for ESC establishment (Boroviak et al., 2014; Boroviak et al., 2015). However, it is unclear when and how the pluripotent state of the ICM becomes permanent during ESC derivation.



	2iL	Serum/LIF
Mouse strain	Unlimited	limited
Cell population	Homogeneous	Heterogeneous
Establishment rate	High	Low
Global DNA methylation level	Low	High
Genomic imprinting state	Lost in ♀ESC	Stable
Full-term developmental potency	×	○

Figure 3. The distinct features of ESCs under serum/LIF and 2iL conditions.

1.3. Loss of imprinting in female ESCs under 2iL condition

Female ESCs under 2iL condition failed to support full-term development of tetraploid blastocyst-complemented embryos because of DNA hypomethylation accompanied by a loss of imprinting (Choi et al., 2017; Yagi et al., 2017). The presence of two active X chromosomes gives rise to the reduction of DNA methylation at repetitive and unique sequences, including DMRs (Zvetkova et al., 2005). This is because X-linked MAPK phosphatase DUSP9, which has a dose dependent effect down-regulates expression of DNA methyltransferase and their cofactors (Choi et al., 2017). Although replacement of MEK inhibitor with a Src inhibitor preserves the developmental potential of ESCs, prolonged culture of female ESCs under modified 2iL condition results in a gradual loss of DNA methylation at DMRs (Choi et al., 2017; Yagi et al., 2017) (Fig. 4).

Therefore, there was still no method for the derivation and maintenance of ESCs keeping the normal genomic imprinting state of embryos.

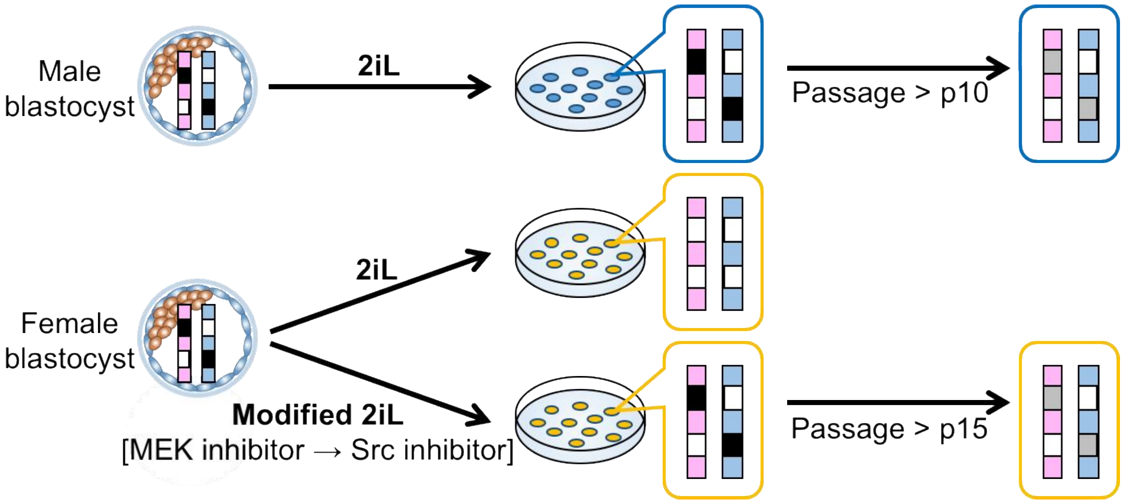


Figure 4. The inhibition of MAPK signaling causes the loss of imprinting in female ESCs. Black-filled boxes indicate methylated state, gray-boxes indicate hypomethylation state, and white unfilled boxes indicate unmethylated state.

In this study, I found that the low concentration of GSK3β inhibitor and LIF (LowGiL) condition without MEK inhibition stably maintained the pluripotency of ESCs. ESCs maintained under LowGiL condition expressed representative pluripotency factors at comparable levels with those in ESCs maintained under 2iL condition, and contributed to all three germ layers in chimeric mouse embryos. On the other hand, pluripotency factors were silenced, and ESCs could not be established from blastocysts under LowGiL condition. The time-course experiments of switching the culture conditions of blastocysts demonstrated that a pluripotent state was maintained under LowGiL condition after 3-day-culture under 2iL condition. I established female ESCs by a modified condition using LowGiL, and found that this ESC maintained genomic imprinting even after the prolonged culture. Thus, my study identified a critical period for ESC establishment and provides alternative methods for ESC derivation and maintenance.

2. Materials and methods

2.1. Cell culture and proliferation assay

ESCs were basically maintained under 2iL condition using DMEM/F-12, GlutaMAX, 0.5× N-2, 0.5× B-27, 100 U/mL mouse LIF, 3 μM CHIR99021, 1 μM PD0325901, 1 mM L-glutamine, and penicillin/streptomycin on 0.1 % gelatin-coated dish without a feeder layer. The ESC lines used in this study were V6.5 (Rideout et al., 2000), G4 (George et al., 2007), E14 (Hopper et al., 1987) and, hybrid ESCs (HyESCs) harboring CAG-GFP, which were established in our lab from C57BL/6 × CAST F1 blastocysts. To evaluate the proliferation rate of ESCs, the cells were counted and passaged into 1.0×10^5 six-well plates every 3 days.

2.2. Blastocyst collection and derivation of ESCs

Superovulated B6D2F1 (BDF1) females were mated with BDF1 or B6; B6C3-Tg (CAG/Acr-EGFP) CX-FM139Osb, the transgenic mice expressing GFP ubiquitously from CAG-EGFP on X chromosome (XGFP) males (Isotani et al., 2005). The appearance of the vaginal plug at noon was defined as embryonic day (E) 0.5. E3.5 embryos were collected by flashing of oviduct or uterus with M2 media + 4 mg/mL bovine serum album (BSA). Zona pellucida was removed using 0.5 % pronase. The embryos were individually plated into single wells of 96 well plates coated with matrigel, cultured for the first 3 days under 2iL or LowGiL with 10 % of fetal bovine serum (FBS), and then switched to serum-free conditions.

2.3. Reverse transcription quantitative PCR (RT-qPCR) analysis

RNA of ESCs was purified using TRIzol (Thermo Fisher Science) according to the manufacturer's instructions. Genomic DNA was removed using TURBO DNase (Thermo Fisher Science), and RNA was purified with the RNeasy Plus Kit (QIAGEN). cDNA was prepared using the Super Script First-Stand Synthesis System (Thermo Fisher Science). RNA of intact or cultured blastocysts was isolated with the PicoPureTM Isolation Kit (ABI) according to the manufacturer's instructions. Real-time qPCR was performed using THUNDERBIRD SYBR qPCR Mix (TOYOBO) and the Thermal Cycler CFX384 Real-Time System (BIO-RAD). A standard curve of each primer set was generated with 10-fold serial dilutions of samples. The primer sets used in this study are shown in Table 1.

Table 1. Primer Sequences for RT-qPCR.

Gene	Forward sequence (5’ to 3’)	Reverse sequence (5’ to 3’)
Nanog	AACCAAAGGATGAAGTGCAAGCGG	TCCAAGTTGGGTTGGTCCAAGTCT
Oct4	TGGCGTGGAGACTTTGCA	GAGGTTCCCTCTGAGTTGCTTTC
Sox2	GCACATGAACGGCTGGAGCAACG	TGCTGCGAGTAGGACATGCTGTAGG
Klf4	CGAACTCACACAGGCGAGAA	CGGAGCGGGCGAATTT
Esrrb	AGTACAAGCGACGGCTGGAT	CCTAGTAGATTCGAGACGATCTTAGTCA
Utf1	GGACCCTTCGATAACCAGATCC	TGCAGACTTCGTCGTGGAAG
Lin28b	AACGTGCGCATGGGATTCG	CCCGTATTGACTCAAGGCCT
GATA4	TCTCACTATGGGCACAGCAG	GCGATGTCTGAGTGACAGGA
Cdx2	GCAGTCCCTAGGAAGCCAAGTGA	CTCTCGGAGAGCCCAAGTGTG
Hand1	CACCAAGCTCTCCAAGATCA	GCGCCCTTTAATCCTCTTCT
Sox1	GGAAAACCCCAAGATGCACAAC	CGCAGTCTCTTGGCCTCGTC

T	CTGGGAGCTCAGTTCTTTCG	CCCCTTCATACATCGGAGAA
Nr0b1	TCCAGGCCATCAAGAGTTTC	ATCTGCTGGGTTCTCCACTG
Eras	ACTGCCCCTCATCAGACTGCTACT	CACTGCCTTGTACTCGGGTAGCTG
Dusp9	GAGGGAGGGAAAGATGAAGG	GGTGTGGACTGCAATGAATG
Bex1	TGGTGGTGAGCATCTCTAGAAAGAG	TAGAAGCTGGTAACAGGGAG
Egfr	ATTGGCTCCCAGTACCTCCT	ATTCCAAAGCCATCCACTTG
Fgfr2	TGCACGCAGGATGGACCTCTCT	TGCTCCTCGGGGACACGGTTAA
H19	AGGCCTCAAGCACACGGCCA	ACTGGTTTGGAGTCCCGGAGATAGC
Meg3	AGGATTCCCTAGGATTCGTGTGGG	GGAAGGCAGAAAGGAAGATGGAGC
Rtl1-as	GCTATGATTCAAACCCGGAGTT	CCATGCTATAATCGGATGCCTC
Impact	CTGAAAGGGCAAGAACGCGCA	ACAGGGGCCACATGAGCCTGA
Snrpn	TTGGTTCTGAGGAGTGATTTGC	CCTTGAATTCCACCACCTTG
Kcnq1ot1	CTTGCTGCACCCCACGAAACT	CTTACAGAAGCAGGGGTGGTCT
Peg10	TGCTTGCACAGAGCTACAGTC	AGTTTGGGATAGGGGCTGCT

2.4. Production of chimeric mice

ESCs were precultured under 2iL or LowGiL conditions more than 2 weeks, and aggregated with BDF2 morulae in KSOM (Lawitts et al., 1993) overnight. Chimeric embryos were transferred to the uteruses of pseudopregnant ICR females.

2.5. Immunostaining of the genital ridge

Genital ridges were dissected from chimeric embryos at E12.5, fixed with 4 % paraformaldehyde (PFA)/phosphate-buffered saline (PBS) at 4 °C overnight, and then washed with PBS three times at room temperature. Genital ridges were treated with 10 % sucrose/PBS and 20 % sucrose/PBS for 10 min at room temperature and soaked in a 1:1 dilution of 20 % sucrose/PBS in OCT at 4 °C overnight. They were embedded in OCT and sliced to a thickness of by 10 μm using cryostat sectioning. The sections were placed on slide glasses, rinsed with PBS, and permeabilized with 0.4 % Triton X-100/PBS for 20 min at room temperature. After a brief rinse with 0.1 % Triton X-100/PBS (PBS with Tween 20; PBST), they were incubated with 2% normal goat serum and 3 % BSA in PBST (blocking buffer) for 1 h at room temperature, followed by incubation with the first antibodies diluted in blocking buffer at 4 °C overnight. After washing three times with PBST, they were incubated with the second antibodies diluted in blocking buffer for 1 h at room temperature. Following washing three times with PBST, the slides were mounted with Slow Fade Gold anti fade reagent (Thermo Fisher Science). The primary antibodies used in this study were the following: MX-SSEA-1 monoclonal antibody (Mouse IgM) (1/200 dilution, Kyowa Medex, #TM13); Anti-green fluorescent protein, rabbit IgG fraction (1/500 dilution, Thermo Fisher Science, #A11122); anti-Oct3/4 rat monoclonal antibody (1/500 dilution, Arakawa, T., *et al.*, 2013). The secondary antibodies used in this study were as follows: Alexa Flour 488 goat anti rabbit IgG (H+L) (1/2000 dilution, Thermo Fisher Science, #A11008); Alexa Flour 568 goat anti mouse IgM (μ chain) (1/2000 dilution, Thermo Fisher Science, #A21043); Alexa Flour 647 goat anti rat IgG (H+L) (1/2000 dilution, Thermo Fisher Science, #A21247).

2.6. Immunosurgery

Zona pellucida-removed embryos were incubated with 11 % anti-mouse serum (SIGMA) in KSOM for 45 min at 38 °C, washed with M2 + 4 mg/mL BSA three times, and incubated in 23 % guinea pig serum in KSOM for 30 min at 38 °C. After washing with M2 + BSA, TE was removed by drawing the embryonic portion through a narrow glass pipette. The ICM-specific expression of GFP fluorescence in an OCT3/4-GFP transgenic mouse was used to monitor the efficiency of the procedure (Solter et al., 1975; Nishioka et al., 2009; Ohnishi et al., 2010).

2.7. RNA sequencing analysis

Five freshly harvested ICMs and 3-day-cultured ICMs were pooled to obtain one sample of each. Library preparation was performed using the SMARTer Ultra Low RNA Kit (Clontech, Mountain View, CA), to prepare amplified cDNA according to the manufacturer's instructions. Sequencing was performed on an Illumina HiSeq 2500 platform in 101-base single-end mode. Illumina Casava1 software (ver. 8.2) was used for base-calling. Sequenced reads were mapped to mouse reference genome sequences (mm10) using TopHat software (ver. 2.0.13) in combination with Bowtie2 (ver. 2.2.3) and SAM tools (ver. 0.1.19). The FPKM values were calculated using Cufflinks software (ver.2.2.1). Gene ontology enrichment was analyzed by DAVID functional annotation bioinformatics microarray analysis.

2.8. Bisulfite sequencing analysis

1 μg of genomic DNA was bisulfite-treated using EpiTect Plus DNA bisulfite Kit (QIAGEN) according to the manufacture's instruction. PCR was performed with Epi Taq HS (Takara). The condition used for PCR amplification was as follows: 1 min at 94 °C followed by 42 cycles of 10 s of 98 °C, 1 min at 60 °C, 2 min at 72 °C, and 5 min at 72°C. Nested PCR was performed to amplify the *Snrpn* DMR with the following condition: an initial round of 1 min at 94 °C followed by 30 cycles of 30 s at 94 °C, 30 s at 50 s, 1 min

at 72 °C, and a second round of 1 min at 94 °C followed by 30 cycles of 30 s 94 °C, 30 s at 50 °C, 30 s at 72 °C. PCR products were cloned into pGEM-T vector (Promega), and sequenced with an ABI PRISM 3100 Genetic Analyzer (Applied Biosystems). The primer sets used in this study are shown in Table 2.

Table 2. Primer Sequences for Bisulfite sequencing analysis.

DMR	Forward sequence (5' to 3')	Reverse sequence (5' to 3')
Peg10	GTATTTAATTTGGAAAGTTGTAGGAGAG	CTCCCAACCACCAAATCCCT
Meg3	AAATTTTGTAAGGAAAAGAATTTTTAGG	TTCAAAATTACTAATCAACATAAACCTC
Peg1/ Mest	GAGGTGGTGGTGAAGTAATTTAGG	CCCAACCATTCTCAACTTTAATTACCTTA
Nespas-Gnasxl	GAAGAATTAGATGGGGAGGGAGG	CTATCACCTTCCTAATTACACTTACCCC
H19	AATGGTTGAATTTTAGTTTTTGTTTTTATGGTT	ACCAATACAATCCCACATACTTTATCATAAAA
Lit1/ Kcnq1ot1	GTTGGGAAGGATTATGTAGAGAAAAGTATATT	CCAAAACCAAAAACATACTCATCTTTAACC
Snrpn 1st	TATGTAATATGATATAGTTTAGAAATTAG	AATAAACCCAAATCTAAAATATTTTAATC
Snrpn 2nd	AATTTGTGTGATGTTTGTAATTATTTGG	ATAAAATACACTTTCACTACTAAAATCC

2.9. Preparation of Gynogenetic (Gg) and Androgenetic (Ag) Embryos

Superovulated BDF1 females were mated with XGFP males. E0.5 embryos (zygote) were collected by flashing of oviduct with M2 media + 4 mg/mL BSA. Embryos were cultured under KSOM + Amino Acid (A.A.) with 4 mg/mL BSA. After incubation with M2 + BSA with 10 μg/mL cytochalasin B (CB) for 5 min, one of pronuclei was enucleated by a blunt Piezo-driven pipette in a droplet of M2 containing 4 mg/mL BSA and 10 μg/mL CB. Gg and Ag embryos were distinguished by the expression of paternally inherited XGFP at blastocyst stage.

2.10. Fluorescence-activated cell sorting (FACS) analysis of GgESCs and AgESCs

GgESCs and AgESCs were trypsinized, and stained with 10 µg/mL Hoechst 33342 (Nacalai) with culture media for 15 min at 37 °C, 5 % CO_2 incubator. DNA content was analyzed using BD FACS AriaII.

2.11. Chromosome counting

ESCs were trypsinized, and incubated in 0.075 M of KCl solution for 8 min at room temperature. Samples were mixed with a half volume of Carnoy's fixative. Treated cells were washed with Carnoy's fixative four times. The concentration of cell suspension was adjusted with Carnoy's fixative and dropped on the slide grass. The chromosomes were stained with 1 µg/mL DAPI (SIGMA). The chromosome numbers were counted by using ImageJ software.

3. Results and discussion

3.1. ESCs can be maintained but not established under LowGiL condition

First, I analyzed the essential conditions for ESC maintenance using various cell lines to elucidate the molecular mechanisms underlying ESC establishment and maintenance. Consistent with previous reports, undifferentiated dome-like colonies and their stable proliferation were observed in ESCs cultured under 2iL condition, while ESCs underwent immediate differentiation and stopped proliferating under LIF-only condition without any inhibitors (Fig. 5A and Fig. 6A). When G4, V6.5, and HyESC mouse ESC lines were used, partial differentiation, with a flattened colony morphology, and a reduced proliferation rate were observed in the absence of a GSK3β inhibitor (MiL condition) (Fig. 5A and Fig. 6A). In contrast, E14 ESCs were stably maintained under this condition. It is likely that sensitivity to the MiL condition varied among the cell lines; indeed, a previous study reported that the MiL condition stably maintained the pluripotency of ESCs (Dunn et al., 2014). Therefore, MiL is not an appropriate condition for ESC maintenance. Meanwhile, all of the ESC lines evaluated were in a pluripotent state when cultured in the presence of the GSK3β inhibitor and LIF but without the MEK inhibitor (GiL condition) (Fig. 5A and Fig. 6A). Even the low concentration of GSK3β inhibitor and LIF (LowGiL condition) produced essentially the same results as did the GiL and 2iL conditions using the same mouse ESC lines. These results show that culture under LowGiL condition is a prerequisite for ESC pluripotency.

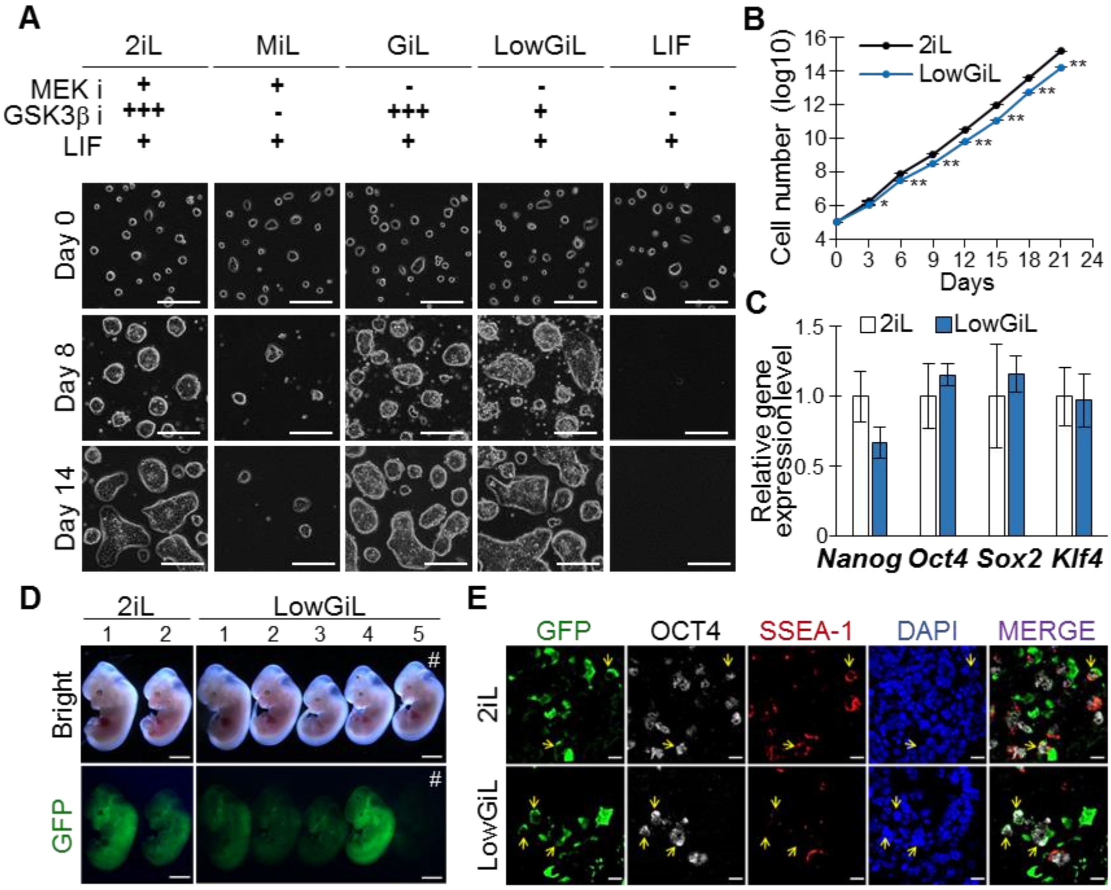


Figure 5. Maintenance of embryonic stem cells (ESCs) under LowGiL condition.
(A): Representative images of ESCs cultured under 2iL, MEK inhibitor + LIF (MiL), GSK3β inhibitor + LIF (GiL), low-concentration GSK3β inhibitor + LIF (LowGiL), and LIF conditions. HyESCs were maintained under 2iL condition without feeder until use. Culture condition was switched to each condition 24 h after the passage (day 0). Scale bar, 200 μm. **(B):** Growth of ESCs under 2iL and LowGiL conditions. **$P < 0.01$, *$P < 0.05$ by t-test. n = 3. Error bars, SEM. **(C):** Quantitative reverse transcription PCR (RT-qPCR) analyses of ESCs cultured under 2iL and LowGiL conditions. The relative gene expression level was normalized to *Gapdh*. n = 3. Error bars, SEM. **(D):** Chimeric embryos generated by the aggregation of wild-type morula and ESCs bearing the CAG-GFP transgene cultured under 2iL or LowGiL conditions. # the embryo with no ESC contribution, serving as the negative control. Scale bar, 2 mm. **(E):** Immunostaining of the genital ridges from chimeric embryos. Arrows indicate primordial germ cells derived from ESCs. A merge of GFP, OCT4, and SSEA-1 is shown to the right. Scale bar, 10μm.

ESCs cultured under LowGiL condition exhibited undifferentiated colonies and grew exponentially for more than 1 month. The proliferation rates of G4, V6.5 and E14 ESCs under LowGiL condition were comparable to those under 2iL condition. However, HyESC exhibited slightly but significantly lower proliferation rate under the LowGiL than 2iL conditions (Fig. 5B and Fig. 6B). The observed differences in sensitivity to LowGiL condition is possibly due to strain differences among the HyESC (F1 from CAST and C57BL/6), G4, V6.5 (F1 from 129/sv and C57BL/6), and E14 (129/Ola) cells. To evaluate whether the pluripotency of HyESCs was affected by LowGiL condition, gene expression and the differentiation potential of the cells were analyzed. Quantitative reverse transcription PCR (RT-qPCR) analysis revealed that the expression levels of *Nanog*, *Oct4*, *Sox2*, *Klf4, and Esrrb* were comparable between LowGiL and 2iL conditions, while *Lin28b, Utf1*, *Nr0b1* and *Eras* were up-regulated in ESCs cultured under LowGiL condition (Fig. 5C, Fig. 6C). Differentiation potency of ESCs cultured under LowGiL condition was confirmed by whole body contribution of ESCs in the aggregation chimeric embryos (Fig. 5D). Furthermore, immunohistochemical analysis with specific markers revealed the germ line contribution of each ESC (Fig. 5E). 2iL-ESCs and ESCs cultured under LowGiL condition exhibited similar contribution rates in chimerism (8/8 [100%] vs. 5/6 [83%]; *P*=0.43 by Fisher's exact test) (Table 3). Taken together, these results indicate that the pluripotency of ESCs can be stably maintained with LowGiL.

Table 3. Summary of chimeras generated with 2iL-ESCs and ESCs cultured under LowGiL condition (related to Fig. 5D).

Culture condition	# Transplanted	# Developed	GFP-positive	*P*-value
2iL	64	8	8 (100%)	0.43
LowGiL	40	6	5 (83%)	

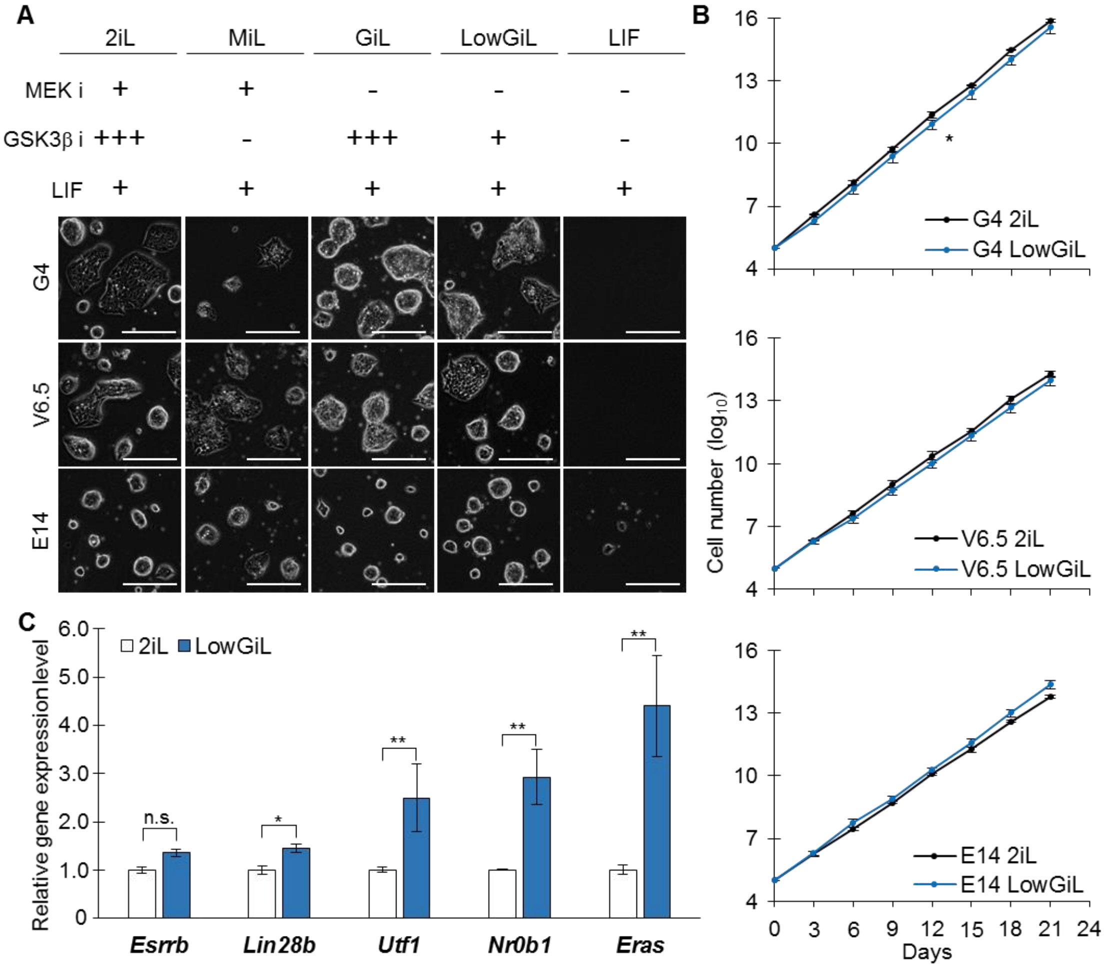


Figure 6. Maintenance of alternative ESC lines under various culture conditions. (A): Representative images of alternative ESC lines cultured for 14 days under 2iL, MEK inhibitor+LIF (MiL), GSK3β inhibitor+LIF (GiL), and LowGiL conditions. Each ESC line was maintained under 2iL condition prior to analysis, and the culture condition was switched 24h after passage (day 0). Scale bar, 200 μm. **(B):** Growth analysis of ESCs under 2iL and LowGiL conditions. ESCs were passaged and cells were counted every 3 days. *$P < 0.05$ by t-test. n = 3. Error bars, SEM. **(C):** Quantitative reverse transcription PCR (RT-qPCR) analyses of ESCs cultured under 2iL and LowGiL conditions. The relative gene expression level was normalized to *glyceraldehyde-3-phosphate dehydrogenase* (*Gapdh*). Error bars, SEM. **$P < 0.01$, *$P < 0.05$ by t-test. n = 3.

I investigated whether ESCs could be derived from E3.5 blastocysts under LowGiL condition. I added serum during first 3 days of blastocyst culture, because blastocyst cells hardly expanded in serum-free condition even with the supplementation of 2iL (data not shown). The 2iL condition resulted in very high derivation efficiency (10/11: 91%), but the LowGiL condition established virtually no ESCs (0/21: 0%). Consistent with the previous report, the colony morphology of the blastocyst outgrowth under LowGiL condition was indistinguishable from that under 2iL at day 7 (Boroviak et al., 2014). However, spontaneous differentiation took place under LowGiL condition after the first passage and no dome-like colony appeared even after a few passages (Fig. 7A, B). Blastocysts cultured under LowGiL condition silenced pluripotent factors, such as *Nanog*, *Oct4*, and *Sox2*, and up-regulated markers of differentiation (Fig. 7C). To identify the key factor of LowGiL condition that causes the failure in ESC establishment, I cultured blastocysts under GiL and Mi+LowGiL conditions. Blastocyst cells cultured in Mi+LowGiL expanded well and maintained OCT4-GFP expression at a comparable level to that cultured in 2iL, while most embryos cultured in GiL lost OCT4-GFP expression as early as day 3 (Fig. 8, Table 4). These results suggest that the presence of a MEK inhibitor during the early phase of ESC establishment is critical for the acquisition of pluripotency.

Table 4. The actual number of embryos classified by Oct4-GFP expression at day 7 of culture (related to Fig. 8). Classification of -, + and ++ colonies were based on Fig. 9A.

Embryonic Stage		-	+	++	Total
E3.5	GiL	20 (100%)	0 (0%)	0 (0%)	20
	Mi+LowGiL	2 (10%)	6 (30%)	12 (60%)	20
	2iL	1 (10%)	1 (10%)	8 (80%)	10

(Mi+LowGiL v.s. 2iL; *P*=0.61 by Fisher’s exact test.)

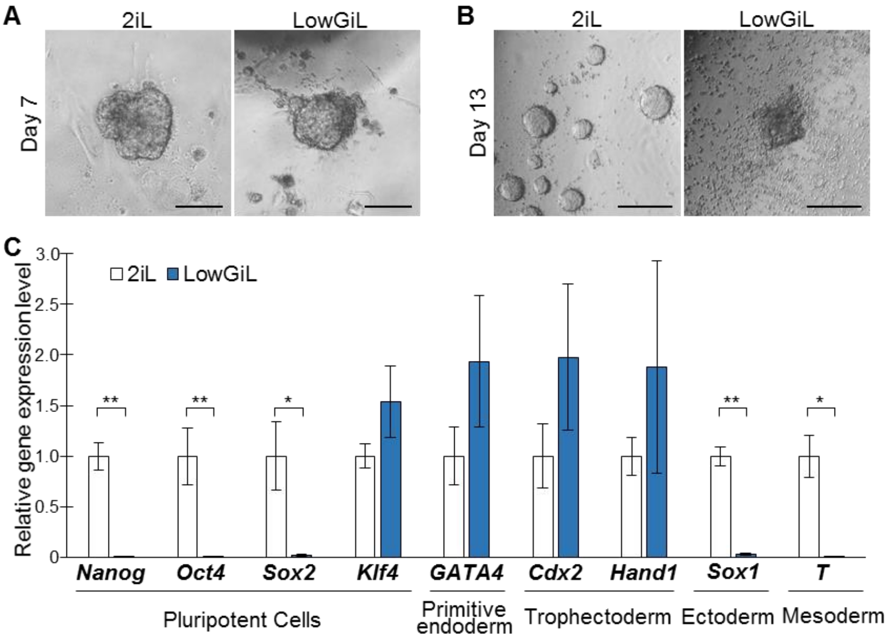


Figure 7. Establishment of ESCs under LowGiL condition.
(A, B): Blastocysts cultured under 2iL and LowGiL conditions for 7 days (**A**) and 13 days (**B**). ESCs were established with high efficiency under 2iL condition (10/11, 91%), whereas no ESCs were established under LowGiL condition (0/21, 0%). Scale bar, 200 μm **(C):** RT-qPCR analysis of blastocyst outgrowth cultured for 7 days under 2iL or LowGiL. Relative gene expression levels were normalized to *Gapdh*. **$P < 0.01$, *$P < 0.05$ by t-test. n = 3–7. Error bars, SEM.

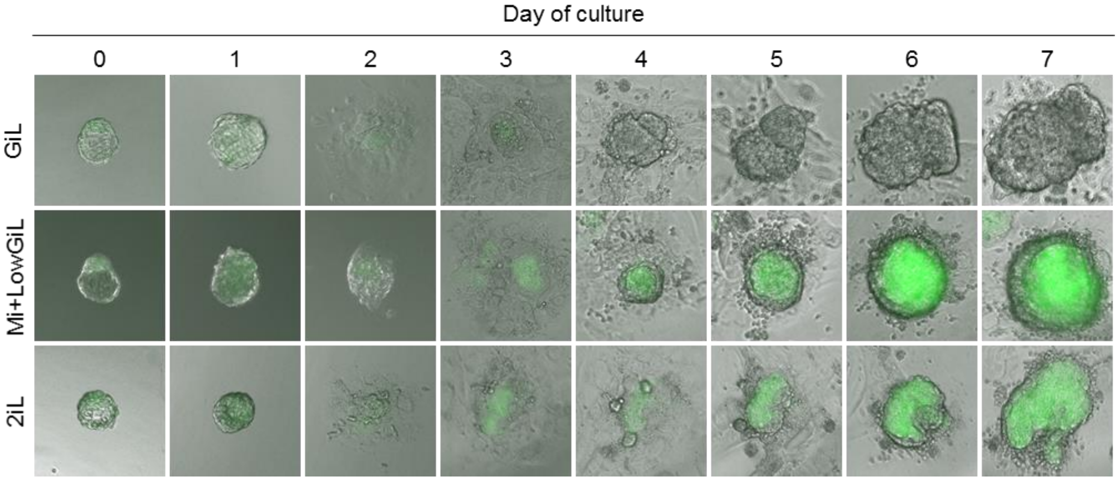


Figure 8. Representative images of E3.5 blastocyst outgrowth under GiL, Mi+LowGiL and 2iL conditions. The green signals indicate OCT4-GFP.

3.2. The Critical period for ESC establishment requires 2iL culture condition

To examine this in more detail, I performed a time course analysis regarding the requirement of MEK and GSK3β inhibitors for pluripotency. First, the culture condition was switched from 2iL to LowGiL at different time points, and pluripotency of clones was classified into three categories according to the ratio of OCT4-GFP-positive cells at day 7 (-: 0~25 %, +: 25~75 %, ++: 75~100 %) (Fig. 10A and Table 5). Clear difference in pluripotency between D0-2 and D0-3 demonstrated that the first 3 days were critical for obtaining permanent pluripotency (Fig. 9A and Fig. 5B). Second, the culture condition was changed from LowGiL to 2iL at different time points (Fig. 9B and Fig. 5C). When embryos were cultured under LowGiL condition for 24 h and then switched to 2iL condition (D1-), almost all of the embryos were classified as ++. This result is consistent with those of a previous study, showing that 24 h of culture under GiL condition improved ESC derivation from early blastocysts (Boroviak et al., 2014). However, the ratio of ++ cells was decreased to less than 40% when blastocysts were cultured under LowGiL condition for 2 days (D2-), and none of the colonies maintained OCT4-GFP expression when the LowGiL culture period exceeded 2 days (D3-). These results further support the notion that the initial 3-day culture period was critical for ESC establishment. Based on these results, I examined pluripotency acquisition of E3.5 blastocysts in an experiment with a variable time course (Fig. 9C and Fig. 10C). When blastocysts were cultured for 24 h under 2iL condition between days 1 and 2 (D1-2) or days 2 and 3 (D2-3), the percentage of ++ cells were less than 40% or 20%, respectively. Meanwhile, more than 60% of the blastocysts cultured for 48 h under 2iL condition between days 1 and 3 (D1-3) were classified as ++. Next, I cultured blastocysts under Mi+LowGiL between Day 1 and 3, to test whether higher dose of Gi at this period was required for the successful ESC derivation. The ratio of ++ clones in Mi+LowGiL: D1-3 were significantly lower than 2iL:D1-3, suggesting that not only Mi but also high dose of Gi at the critical period was important to gain sustainable pluripotent state (Fig. 9C, Fig. 10C-E and Table 5). It was reported that E4.5 embryos showed more efficient ESC derivation from a single cell than did E3.5 embryos (Boroviak et al., 2014). I evaluated ESC establishment from E4.5 blastocysts under various culture conditions, but E4.5 blastocysts showed similar results to E3.5 blastocysts (Fig. 10F, G and Table 5). These results revealed that there was 48 h of critical window for permanent pluripotency under 2iL condition, i.e., between days 1 and 3. Recently, epigenetic and genetic abnormalities of ESCs established under 2iL condition were reported (Choi et al., 2017; Yagi et al., 2017). My findings would help to minimize the culture period of the ICM in the presence of a MEK inhibitor, which plays a major role in the abnormalities (Choi et al., 2017; Yagi et al., 2017).

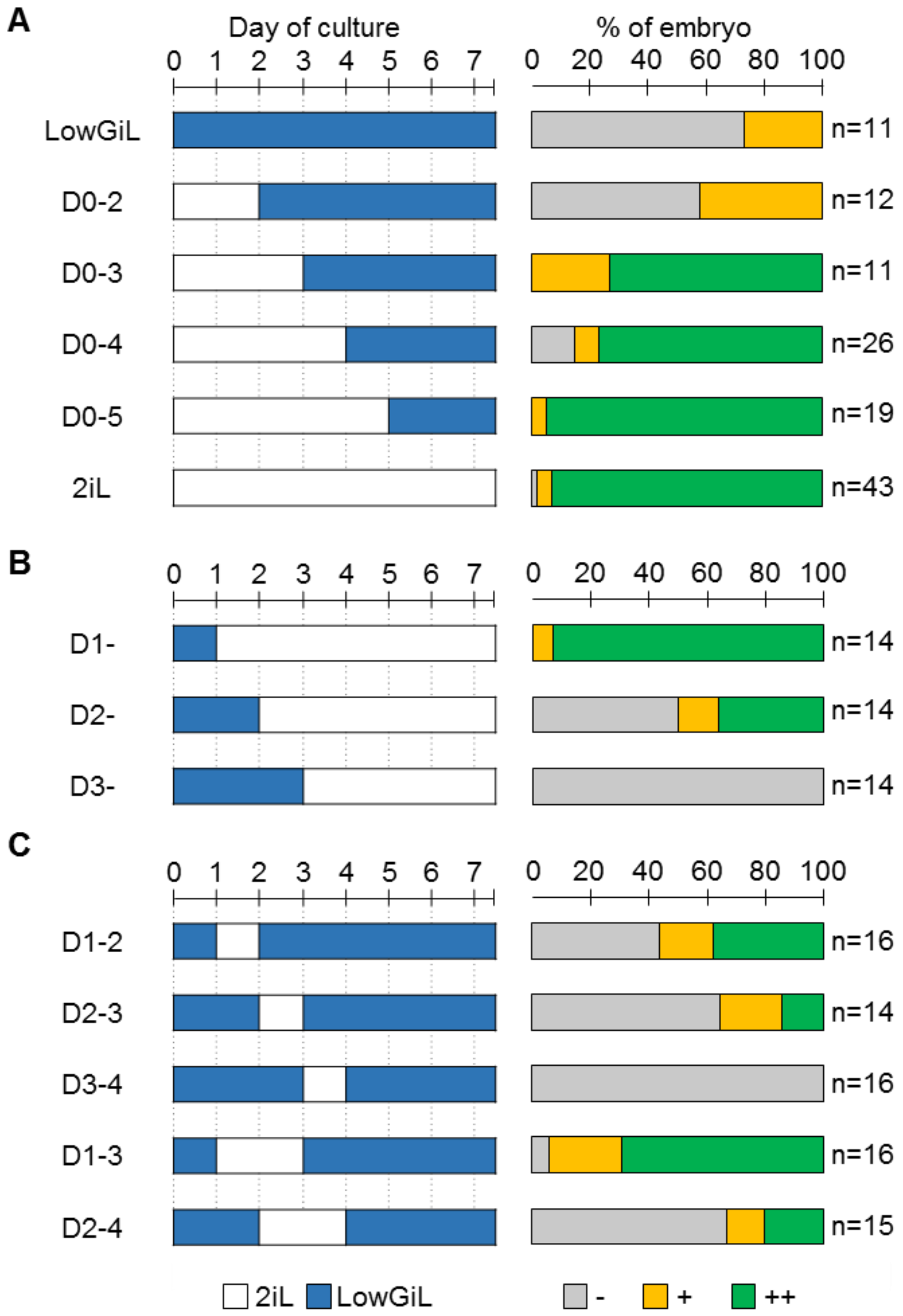


Figure 9. Time course experiments of ESC establishment.
(A-D): The figure shows the experimental design and results of changing the medium from 2iL to LowGiL **(A)**, and from LowGiL to 2iL **(B)**. Culture with 2iL at different periods for 1 or 2 days during ESC establishment **(C)**. Experimental design (left) and ratio of GFP-positive colonies (right) are shown. Embryos were classified according to the number of OCT4-GFP-positive cells at culture day 7 (see Fig. 9A-C).

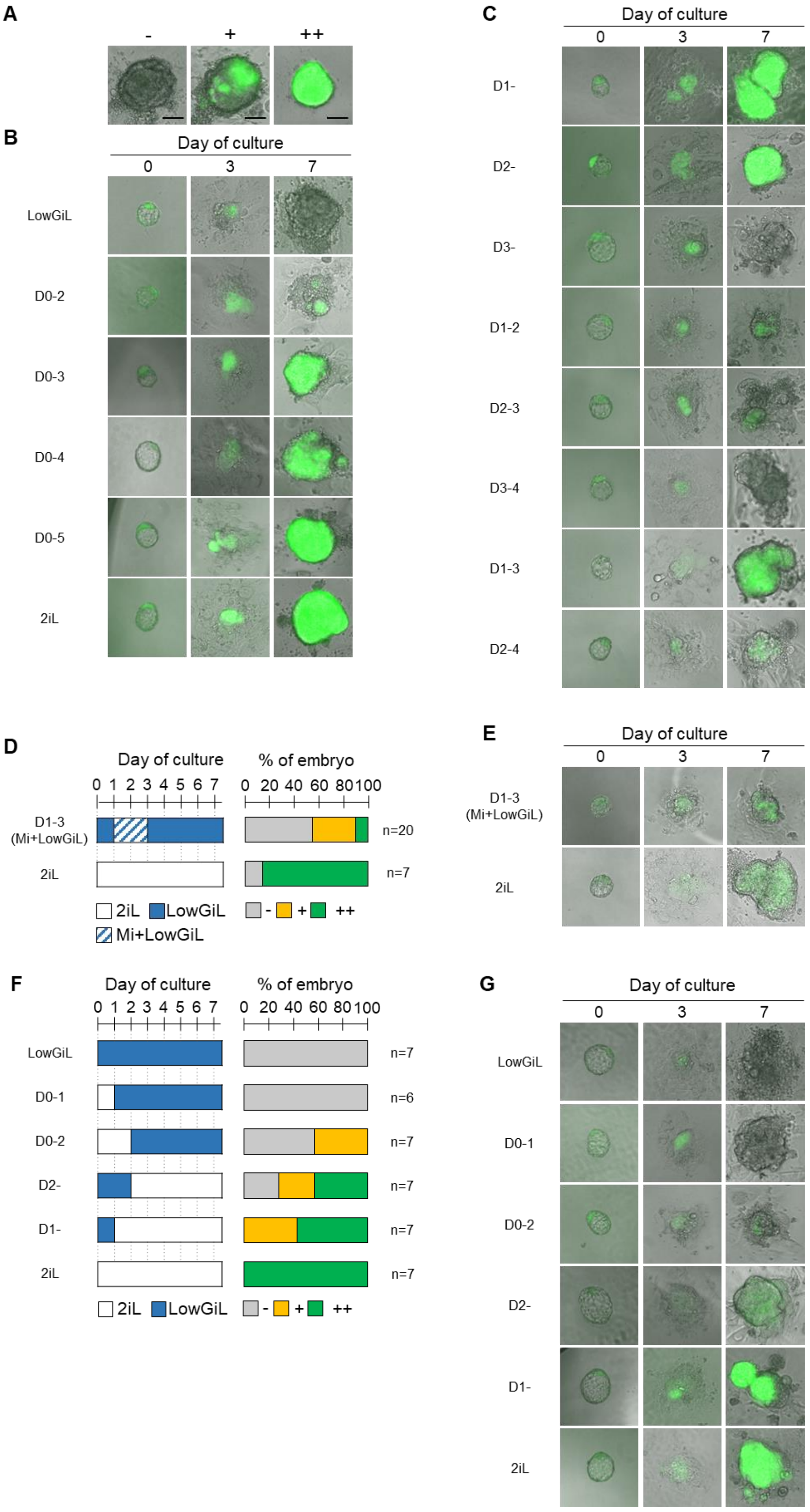

A
-
+
++
B
Day of culture
0
3
7
LowGiL
D0-2
D0-3
D0-4
D0-5
2iL
C
Day of culture
0
3
7
D1-
D2-
D3-
D1-2
D2-3
D3-4
D1-3
D2-4
D
Day of culture
0 1 2 3 4 5 6 7
% of embryo
0 20 40 60 80 100
D1-3
(Mi+LowGiL)
n=20
2iL
n=7
☐ 2iL ■ LowGiL
▨ Mi+LowGiL
■ - ■ + ■ ++
E
Day of culture
0
3
7
D1-3
(Mi+LowGiL)
2iL
F
Day of culture
0 1 2 3 4 5 6 7
% of embryo
0 20 40 60 80 100
LowGiL
n=7
D0-1
n=6
D0-2
n=7
D2-
n=7
D1-
n=7
2iL
n=7
☐ 2iL ■ LowGiL
■ - ■ + ■ ++
G
Day of culture
0
3
7
LowGiL
D0-1
D0-2
D2-
D1-
2iL

Figure 10. Time course experiments of ESC establishment from E3.5 or E4.5 blastocyst. **(A):** Classification of clones. Day 7 colonies were classified based on the ratio of OCT4-GFP-positive cells, as follows: less than 25%, -; 25~75%, +; and more than 75%, ++. Scale bar, 200 μm. **(B, C):** Representative images of E3.5 blastocyst outgrowth under the various conditions described in Figure 3. **(D):** The scheme and results of the experiments changing the culture conditions such as 2iL and Mi+LowGiL during ESC establishment from E3.5 blastocysts. **(E):** Representative images of E3.5 blastocyst outgrowth under the various conditions described in **(D)**. **(F):** The scheme and results of the experiments changing the culture conditions such as 2iL and LowGiL during ESC establishment from E4.5 blastocysts. **(G):** Representative images of E4.5 blastocyst outgrowth under the various experimental conditions.

Table 5. The actual number of embryos classified by Oct4-GFP expression at day 7 of culture (related to Fig. 9 and Fig. 10B, C, E, G). Classification of -, + and ++ colonies were based on Fig. 10A.

Embryonic Stage		-	+	++	Total
E3.5	LowGiL	8 (73%)	3 (27%)	0 (0%)	11
	D0-2	7 (58%)	5 (42%)	0 (0%)	12
	D0-3	0 (0%)	3 (27%)	8 (73%)	11
	D0-4	4 (15%)	2 (8%)	20 (77%)	26
	D0-5	0 (0%)	1 (5%)	18 (95%)	19
	D1-	0 (0%)	1 (7%)	13 (93%)	14
	D2-	7 (50%)	2 (14%)	5 (36%)	14
	D3-	14 (100%)	0 (0%)	0 (0%)	14
	D1-2	7 (44%)	3 (19%)	6 (38%)	16
	D2-3	9 (64%)	3 (21%)	2 (14%)	14
	D3-4	16 (100%)	0 (0%)	0 (0%)	16
	D1-3	1 (6%)	4(25%)	11(69%)	16
	D1-3 (Mi+LowGiL)	11 (55%)	7 (35%)	2 (10%)	20
	D2-4	10 (67%)	2 (13%)	3 (20%)	15
	2iL	2 (3%)	3 (5%)	57 (92%)	62
E4.5	LowGiL	7 (100%)	0 (0%)	0 (0%)	7
	D0-1	6 (100%)	0 (0%)	0 (0%)	6
	D0-2	4 (57%)	3 (43%)	0 (0%)	7
	D2-	2 (29%)	2 (29%)	3 (42%)	7
	D1-	0 (0%)	3 (43%)	4 (57%)	7
	2iL	0 (0%)	0 (0%)	7 (100%)	7

3.3. Gene expression changes during the early phase of ESC derivation

Evident differences in GFP expression at day 7, between the D0-2 and D0-3 conditions, suggested that 3-day culture of the ICM cells established a robust transcriptional network for pluripotency, which was maintained even under LowGiL condition (Fig. 9A and Fig. 10B). I analyzed the transcriptome of ICMs, before and after the culture under 2iL condition for 3 days, to gain insight into the molecular mechanisms of permanent pluripotency acquisition. In total, 529 and 321 genes were up- and down-regulated by the culture, respectively, among the 23,284 genes covered (Fold change [FC] > 4, $P < 0.05$) (Fig. 11A and Table 6). Up-regulated genes showed enrichment in terms of cell division and chromosome partitioning/cytoskeleton, negative regulation of cell proliferation, while the down-regulated genes showed mild enrichment in terms of amino acid transport, metabolism, and nucleosome assembly (Fig. 12A-D). The distribution of the histone H3 lysine 4 tri-methylation (H3K4me3) and histone H3 lysine 27 tri-methylation (H3K27me3) epigenetic markers changed remarkably during ESC derivation (Liu et al., 2016). Liu et al. (2016) reported that ESCs showed a loss of H3K27 methylation at H3K27me3-marked regions in the ICM. Thus, a global loss of repressive epigenetic marks such as H3K27me3 and DNA methylation is likely the molecular basis for gene activation in the ICM during culture. Although core pluripotent genes such as *Nanog*, *Oct4*, *Klf4* and *Sox2* were not significantly changed during the culture, some pluripotent genes, including *Dusp9*, *Eras*, and *Egfr*, were significantly up-regulated (Fig. 11B and Fig. 13) (Heo et al., 2006; Takahashi et al., 2003; Li et al., 2012). Primitive endoderm-related factors, such as *Fgfr2, Gata4, Gata6, Sox7* and *Sox17* were significantly down-regulated during the culture, whereas few of trophectoderm, ectoderm and mesoderm markers were changed (Fig. 11B and Fig. 13) (Molotkov et al., 2017).

Additionally, *Wnt11*, *Ctnnb1* (β-catenin), and Notch signal-related genes were significantly up-regulated, supporting the notion that Wnt/β-catenin and Notch signaling are activated during the derivation of ESCs (Fig. 13) (Umehara et al., 2007; Lee et al., 2009; Tang et al., 2010).

Significant up-regulation of *Dusp9,* a gene on the X chromosome which is a critical regulator of the ERK pathway (Li et al., 2012), suggests that *Dusp9* is critical for maintaining the pluripotency under MEK inhibitor-free condition. The average up-regulation of X chromosome-linked genes was greater than that of autosome-linked genes (Fig. 11A, C, D). These data suggest reactivation of the inactive X chromosome of female embryos (Okamoto et al., 2004; Minkovsky et al., 2012); to investigate this possibility, I harvested male and female blastocysts separately using X chromosome-linked GFP mice and analyzed the expression of several genes (Isotani et al., 2005). Up-regulation of X chromosome-linked genes, including *Dusp9*, *Eras*, *Nr0b1*, and *Bex1*, was observed in both female and male blastocysts, suggesting that a mechanism distinct from X chromosome reactivation regulates the expression of these genes (Fig. 11E) (Kelly et al., 2010). Among them, up-regulation of *Nr0b1* and *Eras* was not observed when blastocysts were cultured under LowGiL condition. These results suggest that X chromosome activation requires more stringent inhibition of the MEK and GSK3β pathways.

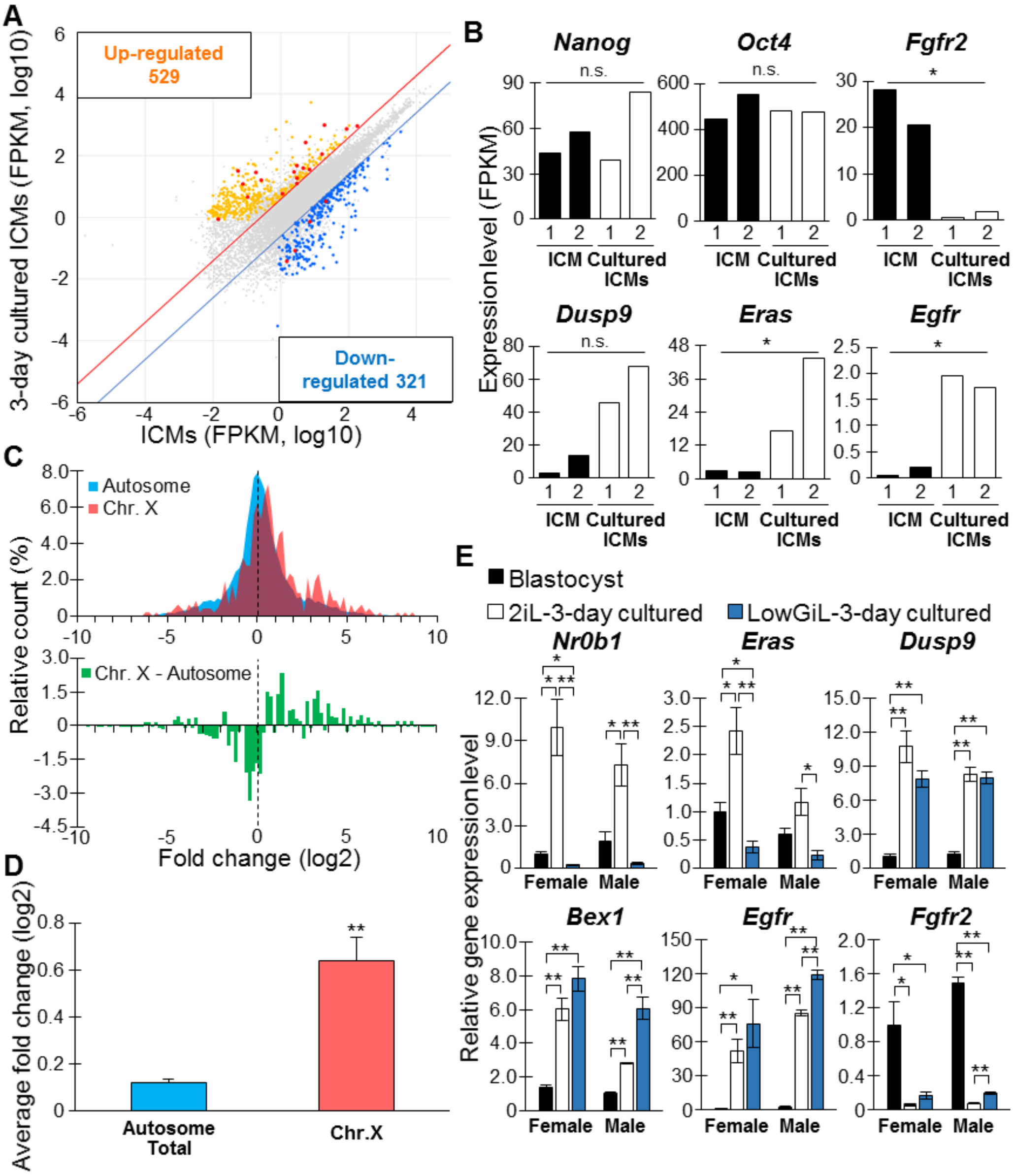


Figure 11. **Changes in gene expression in the inner cell mass (ICM) culture.**

(A): Scatter plot comparing E3.5 ICM and 3-day-cultured ICM under 2iL condition according to RNA sequencing. The total number of covered and expressed genes was 12,991 (fragments per kilobase of transcript per million fragments mapped [FPKM] > 1.0). Significantly up- and down-regulated genes are shown in yellow and blue, respectively (Fold change > 4.0; $P < 0.05$). X-linked genes are highlighted in red. **(B):** Example of differentially expressed genes in cultured ICM versus ICM. The data of two biological replicates were shown. **(C):** Histogram of fold-change fold change in gene expression on the autosome (n = 12,518) and X chromosome (n = 467) (top). The result of subtraction of the autosome fold change value from the X chromosome value is shown at the bottom of the histogram. **(D):** Comparison of the average fold change of autosomal and X-chromosome gene expression between the ICM and 3-day-cultured ICM. Only expressed genes were analyzed (FPKM > 1.0). The Y chromosome is not shown because only six genes were expressed, which was insufficient for statistical analysis. **(E):** RT-qPCR analysis of blastocysts and blastocyst outgrowth after 3-day culture under 2iL and LowGiL conditions. Relative gene expression levels were normalized to *Gapdh*. $^{**}P < 0.01$, $^{*}P < 0.05$ by t-test. n = 3. Error bars, SEM.

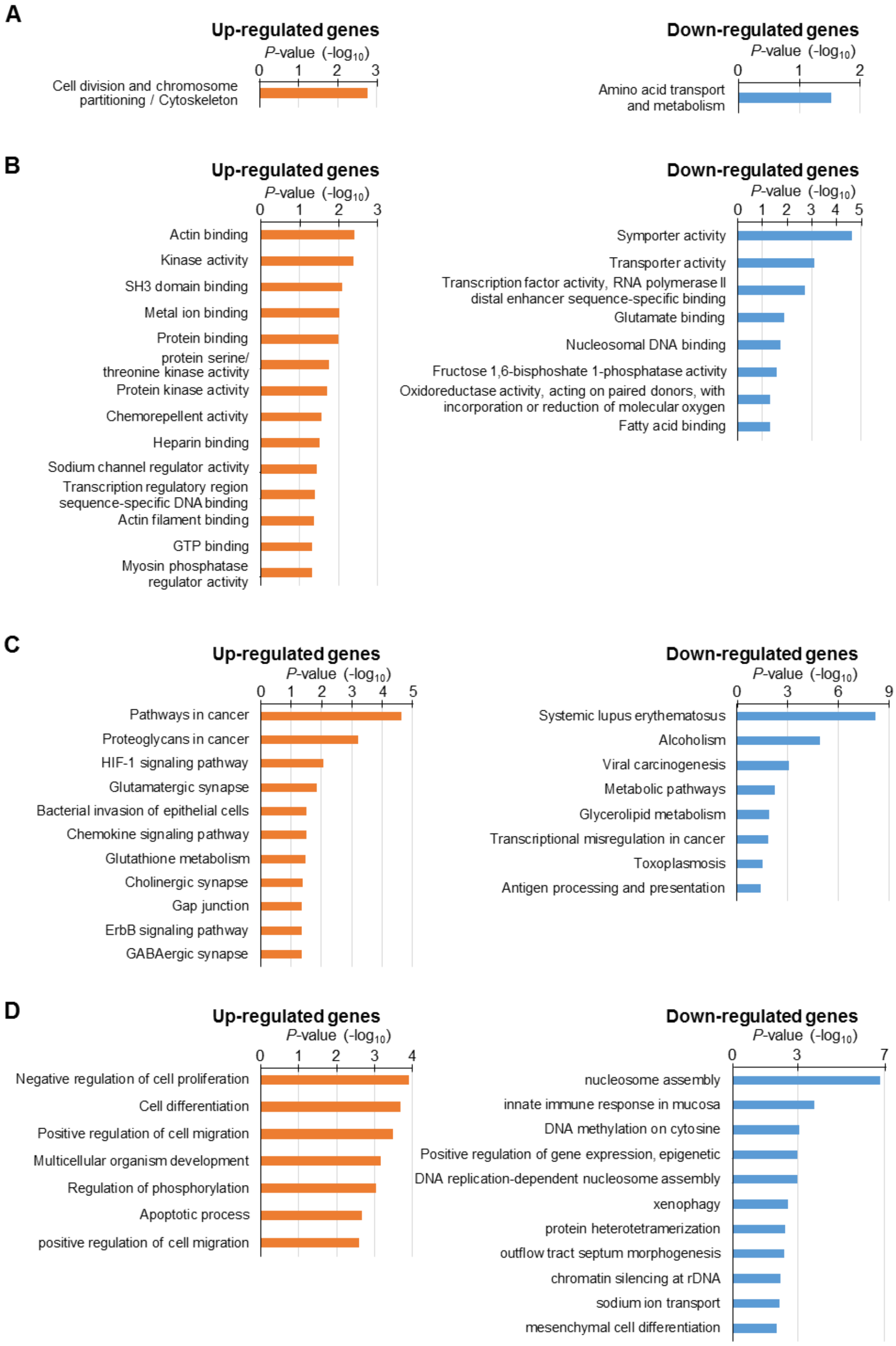


Figure 12. Gene ontology analyses of differentially expressed genes between E3.5 ICM and 3-day-cultured ICM under 2iL condition.

(A): Gene ontology enrichment. **(B)**: Molecular-function (MF) enrichment. **(C)**: Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways. **(D)**: Biological process (BP) enrichment.

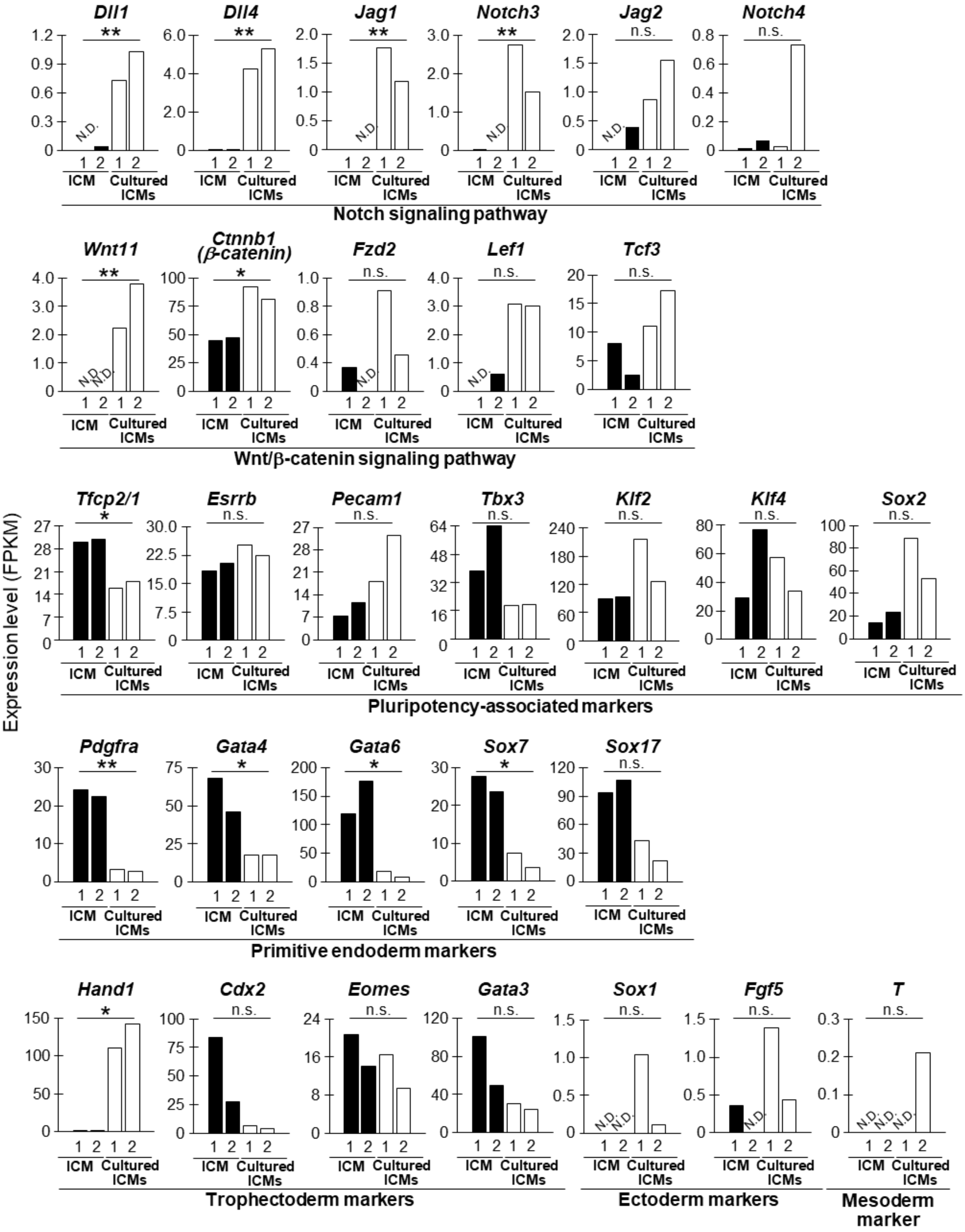


Figure 13. Examples of differentially expressed genes between ICM and the cultured ICM.

The data of two biological replicates were shown. $^{**}P < 0.01$, $^{*}P < 0.05$. n.s., no significance. N.D., not detected.

I identified the critical period of ESC derivation and found a requirement for strong differentiation inhibition during this period using two inhibitors. Since the FGF/MEK signaling pathway induces primitive endoderm differentiation in the ICM, inhibition of this pathway during the earliest stage of culture is likely to be critical for the maintenance of pluripotency in the ICM and subsequent successful ESC establishment. A recent study revealed that GATA6-positive primitive endoderm progenitors from E3.25-3.75 blastocysts were eliminated after 4-8 h of culture in the presence of FGF/MEK inhibitors (Bessonnard et al., 2017). In other words, the progenitor of primitive endoderm appeared a little later than the differentiation of pluripotent epiblasts in an FGF/MEK signaling-dependent manner. Consistent with this finding, RNA sequencing revealed that the ICM at E3.5 showed high-level expression of primitive endoderm markers, including *Gata4*, *Gata6*, and *Sox17,* and that these markers were significantly down-regulated under 2iL condition (Fig. 13). Taken together, the potential of the ICM to differentiate into primitive endoderm was presumably lost under 2iL condition. *Fgfr2* silencing could be the molecular basis for this phenomenon (Fig. 11B). A loss of differentiation potential in primitive endoderm would render FGF/MEK inhibition unnecessary to sustain pluripotency under LowGiL condition.

3.4. Genomic imprinting was maintained LowGiL condition

Genomic imprinting was reported to be lost in the female ESCs under the 2iL condition at the earliest stage of derivation (Choi et al., 2017; Yagi et al., 2017). I hypothesized that genomic imprinting could be maintained if female ESC was established by the modified condition I established in this study. To this end, female ESCs were established under LowGiL condition except for initial 3 days, which is cultured in 2iL condition (LowGiL-ESCs). Female LowGiL-ESCs exhibited undifferentiated dome-like colonies similar to the ESCs established under 2iL condition (2iL-ESCs) (Fig. 14A). Bisulfite sequencing analysis revealed that female LowGiL-ESCs maintained higher DNA methylation levels at *Peg10* and *Snrpn* DMRs compared to female 2iL-ESCs (Fig. 14B). Consistent with previous study, female 2iL-ESCs showed hypomethylation at all analyzed DMR, including *Snrpn*, *Peg10*, *Lit/Kcnq1ot1*, *Peg1*/*Mest*, and *Nespas-Gnasxl* (Fig. 14B). In contrast, female LowGiL-ESCs maintained relatively higher methylation levels that were comparable to MEF (Fig. 14B). These results suggested that genomic imprinting was well maintained in female ESCs under LowGiL condition.

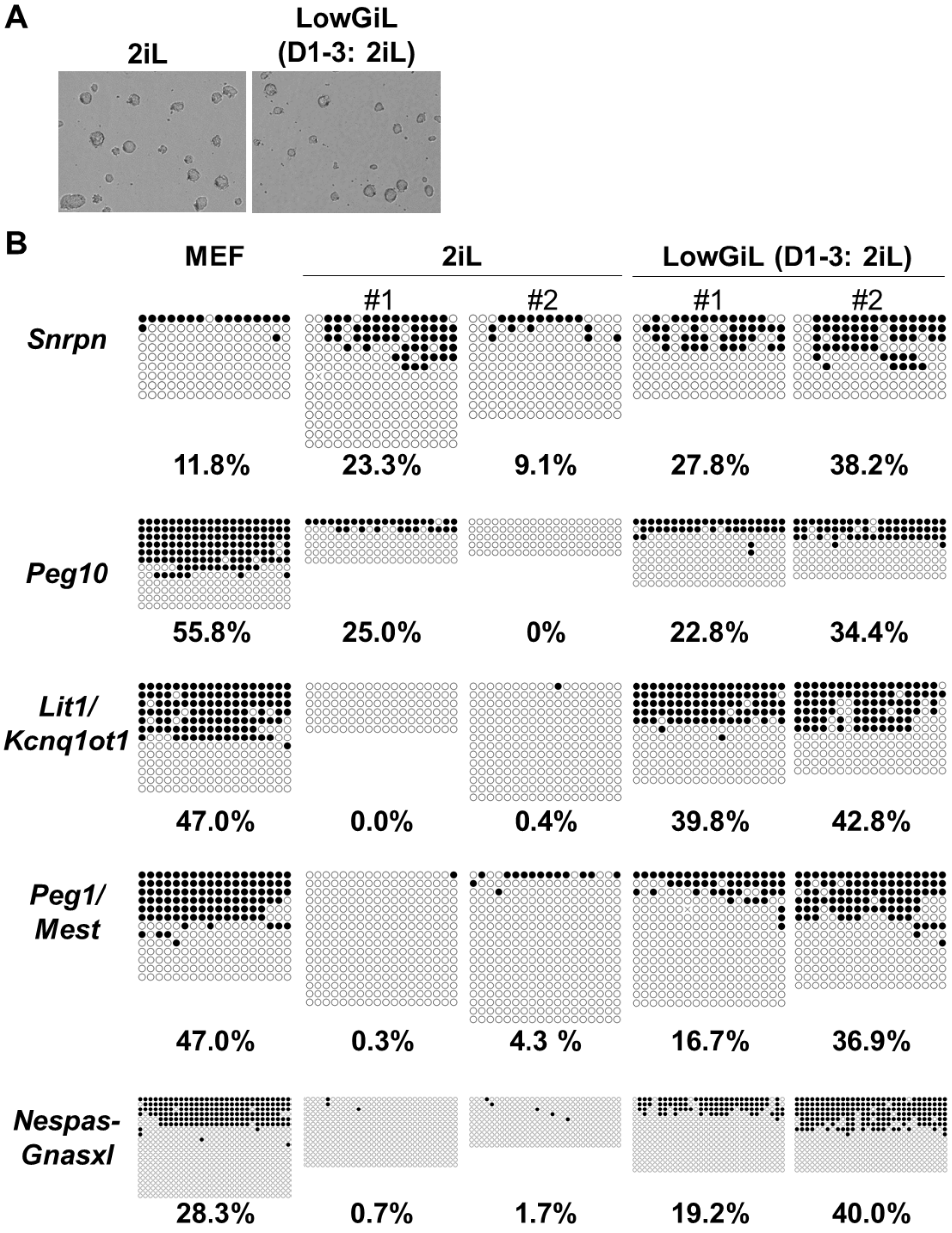


Figure 14. Maintenance of DNA methylation at DMRs in female LowGiL-ESCs.
(A): Representative images of female 2iL-ESC and LowGiL-ESCs. **(B):** Bisulfite sequencing analysis of DMRs within *Snrpn* and *Peg10*, *Lit1/Kcnq1ot1*, *Peg1/Mest* and *Nespas-Gnasxl*.

3.5. Allele-specific gene expression pattern is stably maintained under LowGiL condition

Conventional bisulfite sequencing analysis could not distinguish maternal and paternal alleles. To evaluate whether allele-specific gene expression pattern was maintained, I established gynogenetic (Gg) haploid ESC (hESC) and androgenetic (Ag) hESC, which retain only maternal and paternal genome respectively (Fig. 15A). The haploid blastocysts, which were derived by the enucleation of one pronuclear at 1-cell stage were cultured under LowGiL condition following 2iL treatment for the first 5 days (Fig. 15A). After the 7 time-passages, all ESC lines were confirmed to be diploid through the self-diploidization as reported in the previous studies (Fig. 15B) (Li et al., 2017). The chromosome number of single-cell derived sub-clones was counted, and the clones harboring normal karyotype was used for the following analysis. RT-qPCR analysis showed that maternally expressed *H19*, *Rtl1-as* and *Meg3* genes were completely repressed in AgESCs, while paternally expressed *Impact*, *Snrpn*, *Kcnq1ot1* and *Peg10* genes were repressed in two out of three GgESC lines compared with AgESCs (Fig. 15C). The paternally expressed genes were de-repressed in #3 GgESC line (Fig. 15C). This suggests that maternal imprinting is unstable and easily derepressed, whereas that of paternal imprinting is stable. Next, I performed RNA sequencing analysis of Gg and AgESC lines to analyze how allele-specific expression pattern was maintained (Fig. 16A, Table 7). Both GgESCs and AgESCs expressed pluripotent factors and epigenetic regulating factors at comparable level except for *Nanog* and *Tet2* (Fig. 16B). Among 24,346 covered genes, 212 were up-regulated in GgESCs compared with AgESCs (Fold change > 5); 101 were up-regulated in AgESC compared with GgESCs, and expression of 90 known imprinted genes were detected. Previous report showed that imprinted genes including *Meg3*, *Rian*, *Peg13*, *Peg3*, *Mest*, *Sgce*, *Impact* and *Peg10* were biallelically expressed in female 2iL-ESCs (Choi et al., 2017;Yagi et al., 2017) (Fig. 16D, E). GgESCs and AgESCs established in LowGiL condition maintained allele-specific expression of all these genes (Fig. 16D, E: highlighted). A set of paternally expressed genes, including *Gab*1, *Jade1* (as known as *Phf17*), *Slc38a4*, *Sfmbt2* and *Smoc1*, and maternally expressed genes, including *Gnas* and *Igfr2*, were derepressed (Fig. 16C, D). *Gab*1, *Jade1*, *Slc38a4*, *Sfmbt2* and *Smoc1* were non-canonical imprinted genes, which exhibited mono-allelic expression in ICM and extraembryonic cell lineage through maternally deposited H3K27me3 (Inoue et al., 2017). Since these non-canonical imprinted genes were biallelically expressed in embryonic lineage of late blastocyst, derepression of maternal allele in LowGiL GgESC were reasonable.

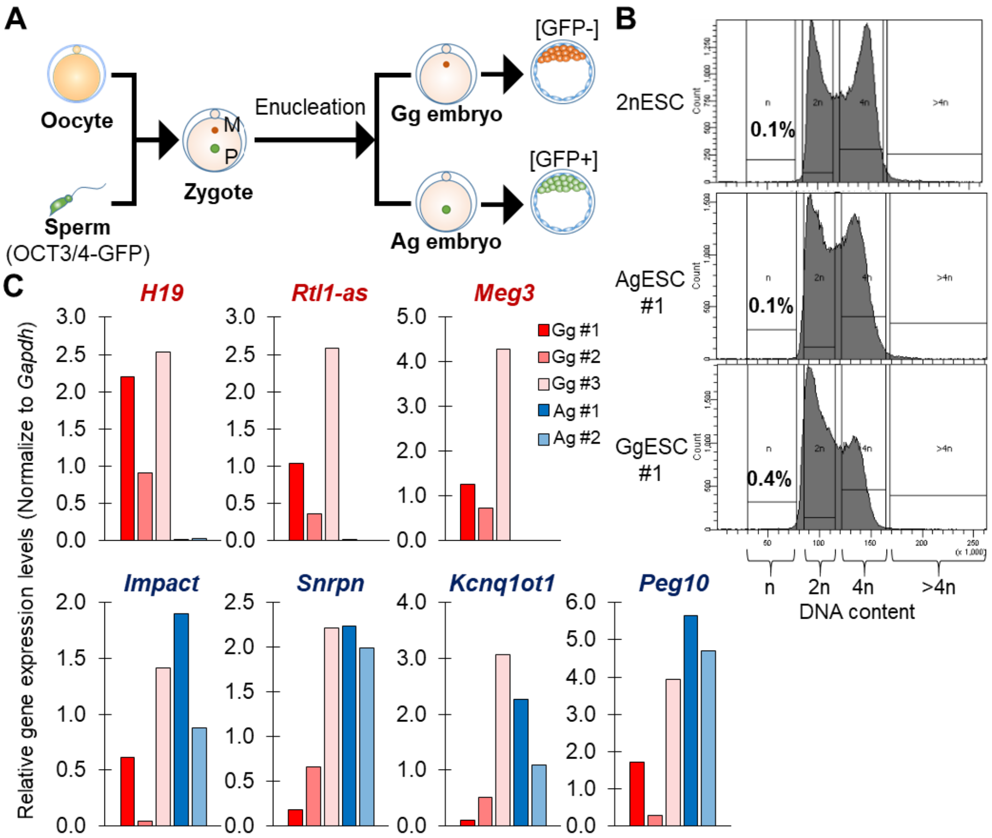


Figure 15. The self-diploidization of GgESCs and AgESCs under LowGiL condition. (A): The schematic diagram showed the process of construction of uniparental embryos. **(B):** FACS analysis of the DNA content of GgESC line and AgESC line. 2nESC line was analyzed as a control. **(C):** RT-qPCR analysis of 3 lines of GgESCs and 2 lines of AgESCs. Relative gene expression levels were normalized with *Gapdh*.

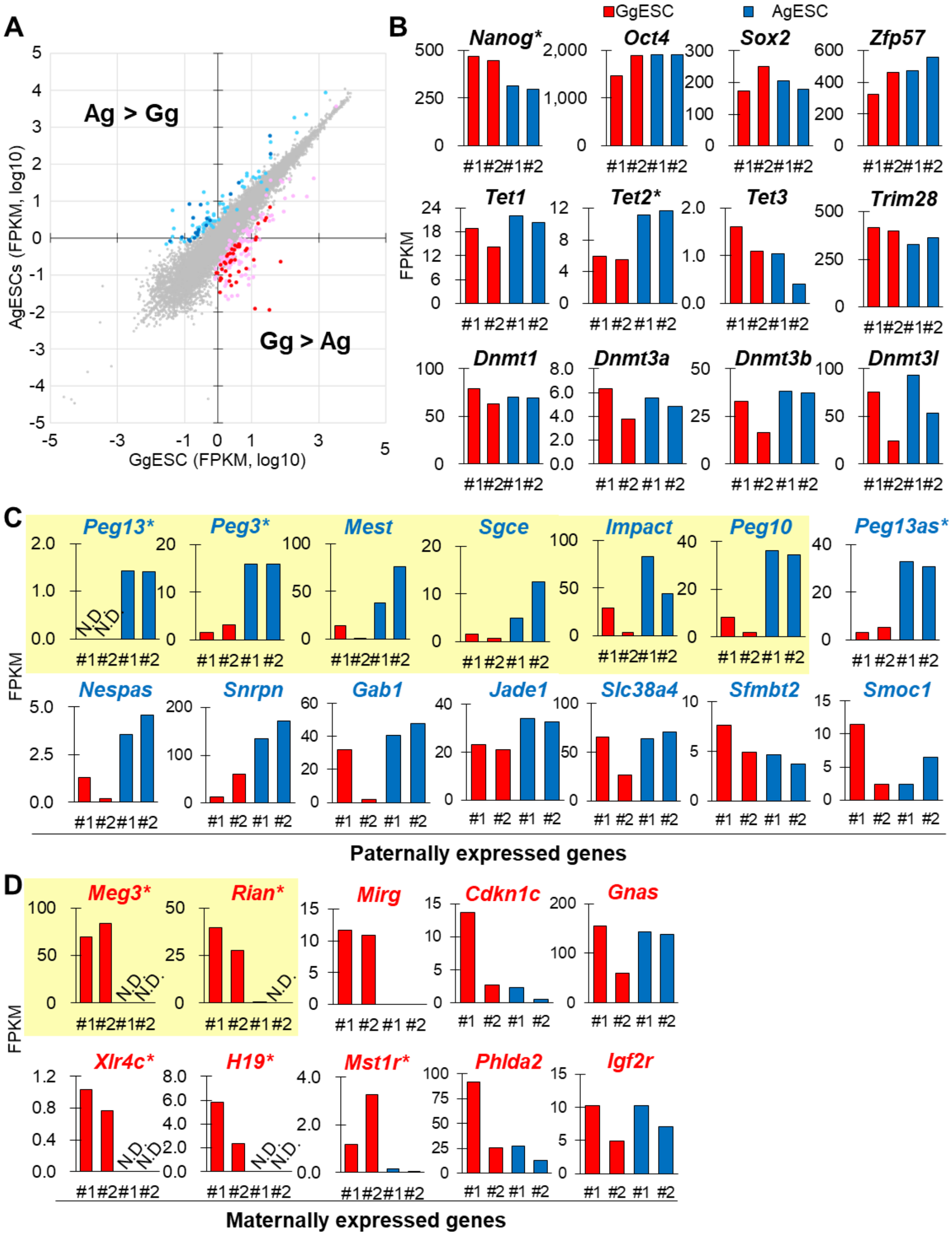


Figure 16. Stability of genomic imprinting in AgESC lines compared with in GgESC lines.

(A): Scatter plot comparing AgESCs and GgESCs according to RNA sequencing. The total number of covered and expressed genes was 12,474 (FPKM > 1.0). Differentially expressed genes between GgESCs and AgESCs (Fold change > 5.0) are shown in pink (Gg > Ag) or light blue (Ag > Gg). If gene expression levels show significantly difference between GgESCs and AgESCs, these genes are highlighted in red (Gg > Ag, $P < 0.05$) or blue (Ag > Gg, $P < 0.05$) (R=0.997). **(B):** The expression of pluripotency and epigenetics regulating factors. *$P < 0.05$. n.s., no significance. **(C, D):** The expression of representative imprinted genes, which are paternally expressed genes (C) and maternally expressed genes (D). Highlighted genes indicates reported genes that were lost imprinted markers in female ESCs under 2iL condition (Yagi et al., 2017). *$P < 0.05$. n.s., no significance. N.D., not detected.

DNA methylation at imprinted genes were well-maintained in female LowGiL-ESCs (Fig. 16B). It would be important to test whether LowGiL-ESCs retained the potency of full-term development by tetraploid complementation assay (Yagi et al., 2017). It has remained unclear why allele-specific expression patterns of *Meg3*, *Peg3*, *Peg13* and *Peg10* were more stable than other imprinted genes (Fig. 16C, D). *Meg3* in *Meg3/Dlk1* locus is regulated by two DMRs, *Meg3*-DMR and IG-DMR (Sato et al., 2011). *Meg3*-DMR is unmethylated in sperm and acquired methylation after the implantation, while IG-DMR is typical germ-line DMR (Sato et al., 2011). At the blastocyst stage, *Meg3* is exclusively expressed from maternal allele even though *Meg3*-DMR was unmethylated yet. Therefore, it is suggested that imprinting of *Meg3/Dlk1* locus is tolerant to hypomethyalted state. This should be confirmed by the detailed analysis of methylation state of both IG-DMR and *Meg3*-DMR during ESC derivation. DNA methylation of imprinted loci was reported to be maintained by the specific binding proteins. *Zfp57* and *Zfp445* specifically bind to imprinted genes and KO mice of these genes showed hypomethylation and de-repression of imprinted genes, including *Peg3* and *Peg13*. In addition, *Peg3*-DMR is known to be controlled by the binding of the transcription factor YY1 (Kim et al., 2007). It is likely that these factors maintain imprinting in a context-dependent manner. Retrotransposon-derived *Peg10* gene is one of the exceptional imprinting genes because its methylation was maintained even in *Zfp57*/*Zfp445*-double KO mice (Takahashi et al., 2018). Therefore, other unknown maintenance factor should be involved in the maintenance of *Peg10* imprinting. Importantly, many of imprinted genes including *Meg3, Peg3,* and *Peg10* lost imprinted expression pattern in 2iL condition (Yagi et al., 2017). The LowGiL-ESC I established in this study will be powerful tool to explore the molecular mechanism of the maintenance of genomic imprinting.

CONCLUSION

This study revealed that a MEK inhibitor is not necessary for maintenance, but is critical for establishment, of sustainable pluripotent ESCs. I demonstrated that the initial 3 days of blastocyst culture is a critical period for ESC derivation. Transcriptional activation of a subset of genes, especially X chromosome-linked genes, and suppression of primitive endoderm genes were observed when blastocysts were cultured under 2iL condition during this period. This may be one of the molecular bases for permanent pluripotency acquisition occurring during ESC derivation. The female ESC lines established by minimizing the usage of MEK inhibitor maintained genomic imprinting even after prolonged culture.

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Table 6. Differentially expressed genes between ICM and 3-day-cultured ICM. (FC>4, *P* <0.05)

Symbol	Day0_#1 (FPKM)	Day0_#2 (FPKM)	Day3_#1 (FPKM)	Day3_#2 (FPKM)	Category
Mir3061	0.000	0.000	242.258	171.530	Up-regulated gene
Snora44	0.000	0.000	226.451	80.169	Up-regulated gene
Arhgdib	0.000	0.078	14.375	66.187	Up-regulated gene
Prl3d3	0.319	1.113	386.520	1045.500	Up-regulated gene
Prl3d2	1.912	0.989	927.786	1572.920	Up-regulated gene
Ascl2	0.000	0.100	30.883	41.852	Up-regulated gene
Snora43	0.000	0.000	120.626	21.352	Up-regulated gene
Prl3d1	5.543	9.756	2532.400	8293.990	Up-regulated gene
Fasl	0.000	0.042	14.451	14.950	Up-regulated gene
Efs	0.054	0.021	23.359	25.373	Up-regulated gene
Gm648	0.108	0.000	39.789	24.138	Up-regulated gene
LOC545261	0.000	0.000	75.832	28.788	Up-regulated gene
Grem2	0.000	0.020	4.943	5.242	Up-regulated gene
Prl5a1	0.215	0.000	12.720	96.503	Up-regulated gene
Tgm1	0.035	0.000	8.427	8.085	Up-regulated gene
Serpinb9c	0.000	0.042	2.687	15.210	Up-regulated gene
Calca	0.115	0.000	18.686	25.806	Up-regulated gene
Trf	0.000	0.033	8.341	3.626	Up-regulated gene
Psg29	0.103	0.201	29.874	78.291	Up-regulated gene
A230065H16Rik	0.000	0.000	52.102	18.629	Up-regulated gene
1600025M17Rik	0.000	0.000	24.304	44.970	Up-regulated gene
Fkbp10	0.000	0.029	5.094	4.870	Up-regulated gene
Dpysl3	0.051	0.000	5.692	11.301	Up-regulated gene
Peg3as	0.000	0.320	66.476	39.531	Up-regulated gene
Lzts2	0.035	0.000	5.732	5.898	Up-regulated gene
AI467606	0.000	0.041	9.588	3.604	Up-regulated gene
Krt14	0.000	0.000	33.394	24.402	Up-regulated gene
Il24	0.000	0.154	15.662	27.571	Up-regulated gene
Rtkn	0.045	0.141	26.279	25.058	Up-regulated gene
Slc38a3	0.040	0.000	1.774	9.311	Up-regulated gene
Anxa8	0.109	0.000	24.060	5.303	Up-regulated gene
Mlana	0.357	0.000	43.939	50.692	Up-regulated gene
Aplp1	0.041	0.032	6.226	12.041	Up-regulated gene
Mup6	0.000	0.211	29.968	22.213	Up-regulated gene
Serpinb9f	0.053	0.000	2.148	10.874	Up-regulated gene
Sema3f	0.057	0.000	2.684	10.934	Up-regulated gene
C3ar1	0.022	0.068	6.818	14.053	Up-regulated gene
Gramd3	0.000	0.029	4.311	2.226	Up-regulated gene
Radil	0.000	0.020	2.486	1.935	Up-regulated gene
Als2cl	0.000	0.014	2.023	1.064	Up-regulated gene
Tbck	0.000	0.022	3.217	1.398	Up-regulated gene
Cyp27a1	0.054	0.000	8.454	2.682	Up-regulated gene
Ihh	0.040	0.000	3.857	4.380	Up-regulated gene
Bpgm	0.048	0.000	5.264	4.585	Up-regulated gene
Cox7b2	0.000	0.000	12.529	27.271	Up-regulated gene
Elmo1	0.017	0.000	2.017	1.300	Up-regulated gene
Dll4	0.028	0.022	4.217	5.276	Up-regulated gene
Tdrd7	0.027	0.000	3.247	1.949	Up-regulated gene
Sema4c	0.050	0.000	2.571	6.959	Up-regulated gene

Cyb561d1	0.000	0.018	2.478	0.830	Up-regulated gene
Myof	0.039	0.030	9.038	4.067	Up-regulated gene
Ms4a10	0.000	0.000	26.680	10.510	Up-regulated gene
Camkk2	0.019	0.000	2.446	1.151	Up-regulated gene
Notch3	0.023	0.000	2.742	1.522	Up-regulated gene
Cd81	3.648	0.694	492.273	297.524	Up-regulated gene
Jag1	0.017	0.000	1.779	1.197	Up-regulated gene
Fgd3	0.000	0.024	2.945	1.081	Up-regulated gene
Gpr56	0.027	0.000	1.853	2.630	Up-regulated gene
Hand1	0.160	1.366	110.394	141.875	Up-regulated gene
Il1rap	0.021	0.000	1.992	1.351	Up-regulated gene
Gm9112	0.362	0.000	12.488	45.759	Up-regulated gene
Hsd17b2	0.000	0.131	6.017	14.833	Up-regulated gene
Zmat4	0.000	0.018	1.487	1.316	Up-regulated gene
Msi2	0.043	0.022	6.413	3.840	Up-regulated gene
Rnf113a1	0.000	0.153	3.397	20.566	Up-regulated gene
Uchl1	0.192	0.820	70.840	85.081	Up-regulated gene
Bphl	0.000	0.210	24.531	7.585	Up-regulated gene
Olr1	0.027	0.021	5.548	1.424	Up-regulated gene
Col18a1	0.000	0.000	13.657	15.290	Up-regulated gene
Sgsm2	0.000	0.031	3.226	1.160	Up-regulated gene
Mybl1	0.000	0.021	1.785	1.182	Up-regulated gene
Plau	0.042	0.033	5.969	4.587	Up-regulated gene
6530418L21Rik	0.041	0.000	4.646	1.139	Up-regulated gene
Zfp3	0.056	0.000	6.207	1.499	Up-regulated gene
Rab3a	0.073	0.000	4.564	5.400	Up-regulated gene
Slc25a23	0.000	0.022	1.995	1.029	Up-regulated gene
Pcolce	0.135	0.000	5.721	12.732	Up-regulated gene
Fst	0.085	0.000	4.854	6.383	Up-regulated gene
Gbx2	0.000	0.146	14.017	5.106	Up-regulated gene
Cdh5	0.263	0.000	15.158	19.237	Up-regulated gene
Prkd2	0.029	0.000	1.662	2.073	Up-regulated gene
H6pd	0.020	0.000	1.646	0.923	Up-regulated gene
Psma8	0.182	0.000	14.756	8.077	Up-regulated gene
Slc22a17	0.086	0.000	5.848	4.879	Up-regulated gene
1700084E18Rik	0.000	0.000	20.640	3.897	Up-regulated gene
Dyrk2	0.000	0.036	2.941	1.455	Up-regulated gene
Cpt1c	0.000	0.325	27.224	11.851	Up-regulated gene
Nckap5l	0.000	0.015	0.652	1.137	Up-regulated gene
1700066M21Rik	0.000	0.025	1.693	1.258	Up-regulated gene
Nos2	0.000	0.000	19.497	3.680	Up-regulated gene
Eif2ak2	0.022	0.000	1.302	1.229	Up-regulated gene
G0s2	0.142	0.000	10.892	5.026	Up-regulated gene
2010002N04Rik	0.000	0.000	17.743	4.425	Up-regulated gene
Coro1a	0.000	0.050	4.028	1.545	Up-regulated gene
	0.020	0.000	1.580	0.620	Up-regulated gene
Sgce	0.000	0.000	14.861	7.025	Up-regulated gene
4930461G14Rik	0.000	0.000	6.114	15.392	Up-regulated gene
Tnfaip2	0.000	0.083	5.778	3.142	Up-regulated gene
Cbx2	0.000	0.059	3.058	3.224	Up-regulated gene
Kbtbd8	0.000	0.033	1.270	2.146	Up-regulated gene
8430419L09Rik	0.023	0.000	1.243	1.045	Up-regulated gene
Cdkn2b	0.000	0.000	15.935	3.993	Up-regulated gene

Tas1r1	0.000	0.027	1.628	1.067	Up-regulated gene
Tbcel	0.038	0.000	1.567	2.116	Up-regulated gene
Vopp1	0.000	0.026	0.841	1.685	Up-regulated gene
Klk7	0.000	0.000	4.601	13.889	Up-regulated gene
Irx1	0.049	0.000	1.425	3.027	Up-regulated gene
Ccl17	0.000	0.000	8.383	9.892	Up-regulated gene
Nkx2-9	0.000	0.000	11.419	6.773	Up-regulated gene
Batf3	0.179	0.000	4.780	11.246	Up-regulated gene
Ranbp6	0.000	0.060	4.269	1.075	Up-regulated gene
Timp3	0.060	0.031	5.489	2.620	Up-regulated gene
Spata6	0.040	0.063	5.366	3.479	Up-regulated gene
Birc7	0.000	0.000	12.578	4.481	Up-regulated gene
Celf5	0.043	0.000	2.806	0.855	Up-regulated gene
BC031353	0.000	0.053	3.335	1.151	Up-regulated gene
Plekhd1	0.027	0.000	0.679	1.591	Up-regulated gene
Rtn2	0.000	0.167	5.571	8.370	Up-regulated gene
Gm3336	0.000	0.074	4.445	1.632	Up-regulated gene
Tef	0.046	0.018	2.542	2.631	Up-regulated gene
Itgb3	0.210	0.013	5.188	12.818	Up-regulated gene
Casq1	0.000	0.084	1.772	4.954	Up-regulated gene
Cda	0.000	0.000	10.603	5.402	Up-regulated gene
Tdrd12	0.064	0.297	7.919	20.793	Up-regulated gene
Spn	0.071	0.040	1.827	6.866	Up-regulated gene
Scrn1	0.000	0.000	10.499	4.909	Up-regulated gene
Egln3	0.037	0.028	1.732	3.232	Up-regulated gene
Exoc3l4	0.000	0.000	4.264	10.861	Up-regulated gene
1300018J18Rik	0.000	0.033	1.119	1.304	Up-regulated gene
Peg3	0.042	0.098	3.827	6.620	Up-regulated gene
Leprel1	0.000	0.034	0.952	1.591	Up-regulated gene
Cmtm5	0.224	0.000	5.583	10.408	Up-regulated gene
C330046G13Rik	0.000	0.000	8.212	5.815	Up-regulated gene
Nlrp4f	0.000	0.000	8.311	5.686	Up-regulated gene
Creb3l1	0.000	0.068	2.976	1.707	Up-regulated gene
P2ry2	0.065	0.000	1.920	2.503	Up-regulated gene
Olig2	0.121	0.000	5.334	2.838	Up-regulated gene
Tmc4	0.211	0.033	8.904	7.488	Up-regulated gene
Tenm4	0.000	0.027	1.043	0.744	Up-regulated gene
Shroom1	0.000	0.000	5.376	7.898	Up-regulated gene
Hyal1	0.138	0.178	15.209	5.678	Up-regulated gene
Morc4	0.000	0.000	5.459	7.689	Up-regulated gene
Phactr1	0.025	0.347	10.114	14.345	Up-regulated gene
Fbxw17	0.000	0.000	9.756	3.384	Up-regulated gene
Ccno	0.054	0.000	2.609	0.891	Up-regulated gene
2410012M07Rik	0.000	0.000	9.773	3.083	Up-regulated gene
Mfsd2a	0.000	0.000	3.081	9.774	Up-regulated gene
Lrch3	0.000	0.049	2.082	1.041	Up-regulated gene
Zfp46	0.000	0.032	0.982	1.006	Up-regulated gene
B4galt4	0.000	0.100	4.606	1.709	Up-regulated gene
Zbtb38	0.023	0.036	1.661	2.054	Up-regulated gene
Rcbtb2	0.000	0.154	5.203	4.291	Up-regulated gene
4933402E13Rik	0.523	0.000	16.416	15.681	Up-regulated gene
Def6	0.000	0.000	5.596	6.568	Up-regulated gene
4933407K13Rik	0.028	0.000	1.006	0.712	Up-regulated gene

Clps	0.000	0.000	9.513	2.309	Up-regulated gene
Trim35	0.000	0.000	6.728	5.065	Up-regulated gene
Atp6v0e2	0.000	0.043	0.812	1.692	Up-regulated gene
2310008H04Rik	0.177	0.023	6.108	5.424	Up-regulated gene
Fam49a	0.000	0.061	0.868	2.657	Up-regulated gene
Phlda2	7.330	7.143	564.442	261.094	Up-regulated gene
Igsf9	0.000	0.143	6.233	1.863	Up-regulated gene
Acbd7	0.000	0.000	6.418	4.881	Up-regulated gene
Flnc	0.060	0.023	2.593	2.088	Up-regulated gene
Homer3	0.000	0.377	12.609	8.313	Up-regulated gene
Lypd2	0.000	0.000	8.759	2.326	Up-regulated gene
Nrk	0.000	0.000	4.387	6.651	Up-regulated gene
Hcls1	0.050	0.000	1.073	1.672	Up-regulated gene
Enho	0.000	0.000	2.992	7.943	Up-regulated gene
Rab43	0.044	0.000	0.990	1.389	Up-regulated gene
Slc4a8	0.054	0.000	1.476	1.439	Up-regulated gene
Ssbp2	0.028	0.011	1.362	0.722	Up-regulated gene
Ano3	0.000	0.000	7.933	2.651	Up-regulated gene
Sdc2	0.000	0.000	6.556	3.969	Up-regulated gene
Rhbdd1	0.000	0.106	4.306	1.200	Up-regulated gene
C1qtnf6	0.000	0.000	3.815	6.534	Up-regulated gene
Bex1	26.723	11.910	1336.720	661.886	Up-regulated gene
Zfp365	0.000	0.000	5.930	4.375	Up-regulated gene
Lsp1	0.302	0.000	9.104	6.446	Up-regulated gene
Fosl1	0.000	0.234	4.879	7.093	Up-regulated gene
Slc25a12	0.166	0.386	8.869	19.061	Up-regulated gene
Rusc2	0.072	0.098	3.654	4.899	Up-regulated gene
Golt1a	0.000	0.000	3.804	6.195	Up-regulated gene
Sp5	0.000	0.000	6.252	3.641	Up-regulated gene
Bex4	2.199	8.655	256.029	280.309	Up-regulated gene
Crabp1	0.000	0.000	6.270	3.510	Up-regulated gene
Itpripl1	0.027	0.021	1.481	0.866	Up-regulated gene
Bivm	0.000	0.125	3.535	2.503	Up-regulated gene
Cald1	1.154	0.329	35.795	34.559	Up-regulated gene
Pparg	0.000	0.000	5.129	4.302	Up-regulated gene
Aldh1a2	0.044	0.000	1.285	0.696	Up-regulated gene
Gnb4	0.000	0.165	3.911	3.505	Up-regulated gene
Pitx2	0.000	0.000	4.461	4.468	Up-regulated gene
Tmem59l	0.000	0.000	2.968	5.832	Up-regulated gene
Nmnat1	0.000	0.188	3.438	4.794	Up-regulated gene
Mcam	0.638	0.130	17.891	15.824	Up-regulated gene
Ltb4r2	0.000	0.000	2.146	6.615	Up-regulated gene
Rpgr	0.209	0.000	6.367	2.743	Up-regulated gene
Narf	0.000	0.067	1.349	1.531	Up-regulated gene
Gal3st1	0.064	0.000	1.321	1.442	Up-regulated gene
Ptger3	0.000	0.155	3.520	3.040	Up-regulated gene
	0.000	0.000	5.278	3.082	Up-regulated gene
Zfp36l3	0.000	0.000	4.039	4.209	Up-regulated gene
Nrn1l	0.000	0.000	5.273	2.904	Up-regulated gene
Krt17	0.000	0.000	2.147	5.996	Up-regulated gene
Dll1	0.000	0.043	0.724	1.025	Up-regulated gene
Laptm5	2.594	1.853	96.720	81.722	Up-regulated gene
Pcp4l1	0.066	0.051	3.695	0.966	Up-regulated gene

Krt6a	0.000	0.000	6.065	1.825	Up-regulated gene
Sgk2	0.000	0.000	4.628	3.111	Up-regulated gene
Slco4a1	0.000	0.073	1.187	1.624	Up-regulated gene
Kit	0.000	0.099	2.469	1.321	Up-regulated gene
Vsig2	0.000	0.000	4.039	3.432	Up-regulated gene
Sirt4	0.000	0.000	4.737	2.700	Up-regulated gene
Ptpre	0.000	0.000	2.882	4.536	Up-regulated gene
Limk1	0.116	0.202	3.816	7.877	Up-regulated gene
Sh3bp2	0.033	0.051	2.271	0.826	Up-regulated gene
2900041M22Rik	0.000	0.000	3.899	3.439	Up-regulated gene
Rhbg	0.000	0.000	3.557	3.778	Up-regulated gene
Klk10	0.000	0.000	2.170	5.089	Up-regulated gene
Ctsw	0.000	0.000	5.009	2.096	Up-regulated gene
C2	0.000	0.000	5.072	1.987	Up-regulated gene
Rps6kl1	0.000	0.090	1.036	2.129	Up-regulated gene
Plk5	0.000	0.000	4.326	2.630	Up-regulated gene
Igsf9b	0.000	0.000	4.253	2.702	Up-regulated gene
Cpne2	0.000	0.000	3.006	3.915	Up-regulated gene
Tomm20l	0.000	0.000	4.607	2.269	Up-regulated gene
Mapkapk3	0.519	0.376	13.199	17.193	Up-regulated gene
Nrgn	0.000	0.000	4.440	2.282	Up-regulated gene
Ttc9	0.197	0.000	3.432	3.150	Up-regulated gene
Hdhd3	0.000	0.000	3.819	2.830	Up-regulated gene
Kcnh3	0.080	0.000	1.450	1.206	Up-regulated gene
Oas1c	0.000	0.000	2.655	3.865	Up-regulated gene
Cxx1b	0.000	0.000	4.022	2.418	Up-regulated gene
Kcnq2	0.000	0.000	3.936	2.481	Up-regulated gene
Chst14	0.000	0.000	3.887	2.518	Up-regulated gene
Gm11744	0.000	0.000	4.063	2.320	Up-regulated gene
Casp8	0.000	0.000	4.972	1.273	Up-regulated gene
Syce1	0.000	0.335	5.639	4.695	Up-regulated gene
Sall3	0.283	0.010	3.280	5.773	Up-regulated gene
Habp2	0.000	0.339	3.471	6.899	Up-regulated gene
Gtpbp2	0.265	0.000	5.908	2.102	Up-regulated gene
Wnt11	0.000	0.000	2.229	3.796	Up-regulated gene
1700001O22Rik	0.000	0.093	1.764	1.027	Up-regulated gene
Zfp750	0.000	0.000	3.712	2.175	Up-regulated gene
Asb3	0.030	0.162	1.442	4.212	Up-regulated gene
Olfr985	0.000	0.000	2.140	3.636	Up-regulated gene
Rhoq	0.046	0.161	3.633	2.333	Up-regulated gene
Susd4	0.000	0.000	4.381	1.355	Up-regulated gene
4930550C14Rik	0.000	0.000	4.380	1.337	Up-regulated gene
Ric8b	0.367	0.078	7.911	4.769	Up-regulated gene
Sult6b1	0.000	0.000	2.601	3.090	Up-regulated gene
Tmem67	0.083	0.000	1.029	1.339	Up-regulated gene
2310028H24Rik	0.000	0.000	4.292	1.389	Up-regulated gene
Mgst1	0.000	0.000	4.546	1.123	Up-regulated gene
Chac2	0.000	0.375	4.024	6.534	Up-regulated gene
Sox4	0.000	0.000	2.463	3.167	Up-regulated gene
Fsd1	0.000	0.000	2.018	3.537	Up-regulated gene
Slco3a1	0.000	0.000	2.925	2.628	Up-regulated gene
Tab1	0.132	0.000	1.227	2.343	Up-regulated gene
Mettl20	0.000	0.000	2.470	2.858	Up-regulated gene

Zfp41	0.000	0.000	3.486	1.824	Up-regulated gene
Rasl11b	0.000	0.000	3.885	1.411	Up-regulated gene
Sfxn3	0.103	0.000	1.268	1.436	Up-regulated gene
Ntn1	0.065	0.354	5.901	5.084	Up-regulated gene
Asb14	0.056	0.044	0.774	1.819	Up-regulated gene
Dnase1l1	0.000	0.000	2.450	2.751	Up-regulated gene
Dnajb5	0.086	0.067	2.663	1.283	Up-regulated gene
Nat6	0.151	0.664	13.153	7.899	Up-regulated gene
Plscr2	0.000	0.124	1.566	1.630	Up-regulated gene
Syn1	0.000	0.000	2.514	2.582	Up-regulated gene
Rnase4	0.137	0.532	9.758	7.273	Up-regulated gene
Crygn	0.000	0.000	3.140	1.946	Up-regulated gene
Fgfbp3	0.000	0.000	3.966	1.066	Up-regulated gene
Plcg2	0.000	0.205	3.724	1.426	Up-regulated gene
Ldhb	0.833	3.104	56.665	41.342	Up-regulated gene
Poli	0.317	0.000	4.340	3.532	Up-regulated gene
Fstl1	0.053	0.411	6.214	5.144	Up-regulated gene
Bnc2	0.014	0.067	0.896	1.092	Up-regulated gene
Ccdc136	0.000	0.000	1.217	3.621	Up-regulated gene
Gap43	0.000	0.000	2.915	1.913	Up-regulated gene
Ccl25	0.000	0.000	1.463	3.347	Up-regulated gene
Rln3	0.000	0.000	2.911	1.855	Up-regulated gene
Igfbp2	9.992	3.564	121.873	199.036	Up-regulated gene
Lrig3	0.000	0.000	2.932	1.790	Up-regulated gene
Abcb1b	0.283	0.913	18.486	9.723	Up-regulated gene
Lrrc49	0.000	0.000	1.626	3.070	Up-regulated gene
Gan	0.000	0.200	2.639	2.048	Up-regulated gene
Cnp	0.424	0.066	6.180	5.282	Up-regulated gene
Btbd3	0.285	0.016	4.402	2.587	Up-regulated gene
Cry2	0.363	0.037	6.002	3.273	Up-regulated gene
Zfp358	0.000	0.000	1.128	3.348	Up-regulated gene
Pcp2	0.000	0.000	2.347	2.071	Up-regulated gene
Cox6b2	16.400	13.842	450.664	215.208	Up-regulated gene
Xkr5	0.000	0.000	1.390	2.994	Up-regulated gene
Casp14	0.000	0.000	3.257	1.114	Up-regulated gene
Tspan32	0.000	0.000	1.850	2.420	Up-regulated gene
Ahr	0.000	0.146	2.131	0.968	Up-regulated gene
Adcy7	0.000	0.000	1.031	3.225	Up-regulated gene
Marveld3	0.000	0.000	3.187	1.046	Up-regulated gene
Tff3	0.000	0.000	3.110	1.101	Up-regulated gene
Trim32	0.030	0.234	3.149	2.396	Up-regulated gene
Gne	0.366	0.448	5.299	11.756	Up-regulated gene
Serpinb1b	0.000	0.000	1.492	2.696	Up-regulated gene
Bcam	1.968	3.086	70.141	35.577	Up-regulated gene
Apoa1	0.000	0.180	1.900	1.841	Up-regulated gene
Qprt	0.000	0.000	2.200	1.947	Up-regulated gene
Cercam	0.108	0.140	1.395	3.643	Up-regulated gene
Actr3b	0.180	0.279	3.826	5.454	Up-regulated gene
Zkscan6	0.388	0.000	3.822	3.862	Up-regulated gene
LOC106740	0.000	0.000	0.883	3.064	Up-regulated gene
Sbk1	0.186	0.324	4.841	5.170	Up-regulated gene
Park2	0.000	0.000	2.518	1.397	Up-regulated gene
Plcb4	0.026	0.341	4.505	2.653	Up-regulated gene

Trerf1	0.000	0.000	2.110	1.763	Up-regulated gene
Tmem63a	0.000	0.000	2.435	1.434	Up-regulated gene
Sdsl	0.000	0.000	1.553	2.309	Up-regulated gene
Mst1	0.000	0.202	1.907	1.959	Up-regulated gene
Pllp	0.000	0.000	1.202	2.618	Up-regulated gene
Galc	0.000	0.000	2.823	0.960	Up-regulated gene
Zhx1	0.000	0.000	2.405	1.376	Up-regulated gene
Zfp260	0.000	0.339	3.544	2.839	Up-regulated gene
Adamtsl5	0.116	0.032	1.351	1.426	Up-regulated gene
	0.353	0.078	4.081	3.965	Up-regulated gene
Zfp940	0.000	0.000	2.584	1.141	Up-regulated gene
Nanos3	0.000	0.000	1.191	2.530	Up-regulated gene
Tmem104	0.062	0.337	3.084	4.330	Up-regulated gene
Gstt3	0.000	0.126	0.933	1.388	Up-regulated gene
D630039A03Rik	0.000	0.000	2.139	1.535	Up-regulated gene
Snta1	0.284	0.367	7.212	4.674	Up-regulated gene
Crim1	0.327	0.701	10.580	7.881	Up-regulated gene
Dnajb3	0.000	0.000	2.497	1.088	Up-regulated gene
Gdpd1	0.000	0.000	1.980	1.595	Up-regulated gene
Foxp1	0.000	0.163	1.028	1.886	Up-regulated gene
Afap1	0.042	0.207	2.291	2.129	Up-regulated gene
Rdh12	0.000	0.000	2.597	0.938	Up-regulated gene
Gmpr	0.268	0.052	2.944	2.698	Up-regulated gene
Coro2a	0.000	0.000	1.521	2.004	Up-regulated gene
Extl2	0.132	0.054	1.488	1.768	Up-regulated gene
2900056M20Rik	0.000	0.000	2.497	0.993	Up-regulated gene
Pde10a	0.000	0.000	2.307	1.180	Up-regulated gene
Pvrl4	0.000	0.000	2.240	1.228	Up-regulated gene
Rgnef	0.134	0.012	1.220	1.300	Up-regulated gene
Zfp229	0.501	0.356	8.511	6.167	Up-regulated gene
Iqcd	0.000	0.000	2.555	0.867	Up-regulated gene
Fam120b	0.021	0.236	1.923	2.467	Up-regulated gene
Efcab7	0.000	0.000	1.833	1.537	Up-regulated gene
Prss41	0.000	0.000	1.719	1.582	Up-regulated gene
Pir	1.877	4.018	40.275	54.545	Up-regulated gene
Fam110c	0.000	0.000	2.115	1.095	Up-regulated gene
Bmp7	0.000	0.000	2.032	1.157	Up-regulated gene
Tmem132a	0.000	0.000	2.321	0.838	Up-regulated gene
Rprm	0.000	0.000	1.924	1.230	Up-regulated gene
Cmklr1	0.000	0.000	1.562	1.586	Up-regulated gene
Gstm1	7.306	6.507	125.362	90.839	Up-regulated gene
Egfr	0.037	0.198	1.950	1.722	Up-regulated gene
Myzap	0.043	0.067	1.011	0.716	Up-regulated gene
Wipf3	0.000	0.000	0.798	2.313	Up-regulated gene
Camk2n1	0.238	0.000	2.099	1.551	Up-regulated gene
4933416I08Rik	0.000	0.000	2.286	0.731	Up-regulated gene
Dtna	0.000	0.000	1.322	1.681	Up-regulated gene
Cep128	0.000	0.243	2.230	1.424	Up-regulated gene
Mtap1b	0.457	0.475	7.620	6.322	Up-regulated gene
Espn	0.000	0.316	2.622	2.113	Up-regulated gene
Usp46	0.568	0.464	9.581	5.762	Up-regulated gene
Upk1b	0.000	0.000	2.091	0.881	Up-regulated gene
Spats2l	0.000	0.000	1.940	0.942	Up-regulated gene

Wnt3	0.000	0.000	2.005	0.871	Up-regulated gene
Gm5	0.000	0.000	1.787	1.054	Up-regulated gene
Shpk	0.000	0.000	2.144	0.684	Up-regulated gene
Gprasp2	0.000	0.000	0.853	1.953	Up-regulated gene
Qser1	0.267	0.200	2.749	3.585	Up-regulated gene
Ror2	0.000	0.000	1.611	1.068	Up-regulated gene
Slc35a5	0.271	0.140	3.834	1.626	Up-regulated gene
Gm5124	0.000	0.000	1.233	1.404	Up-regulated gene
Zfp646	0.000	0.287	1.744	2.029	Up-regulated gene
Epg5	0.038	0.103	1.149	0.680	Up-regulated gene
Matn1	0.000	0.000	1.689	0.889	Up-regulated gene
Cbr3	13.633	10.798	179.483	134.828	Up-regulated gene
Zbtb33	0.000	0.000	1.164	1.393	Up-regulated gene
Sh2d5	0.000	0.143	1.246	0.582	Up-regulated gene
Zfp324	0.000	0.000	0.857	1.685	Up-regulated gene
Tmcc2	0.000	0.000	1.869	0.670	Up-regulated gene
Pam	0.116	0.144	1.795	1.497	Up-regulated gene
Gnb5	0.000	0.000	1.758	0.761	Up-regulated gene
Itga2	0.000	0.000	1.143	1.372	Up-regulated gene
Eras	2.594	2.254	17.169	43.245	Up-regulated gene
Gng3	1.814	0.938	20.273	13.727	Up-regulated gene
Ankrd23	0.103	0.240	2.492	1.702	Up-regulated gene
Dnajc18	0.073	0.282	1.912	2.407	Up-regulated gene
Dusp23	0.000	0.000	1.414	1.001	Up-regulated gene
Syt13	0.000	0.000	1.711	0.683	Up-regulated gene
Sema6b	0.000	0.000	1.299	1.092	Up-regulated gene
Jrkl	0.000	0.000	1.444	0.942	Up-regulated gene
Car11	0.000	0.000	1.640	0.715	Up-regulated gene
S100a6	56.119	19.582	541.896	336.521	Up-regulated gene
Fignl2	0.000	0.000	1.205	1.115	Up-regulated gene
Ikbkb	0.320	0.248	4.432	2.131	Up-regulated gene
Nfatc2	0.000	0.000	1.270	1.031	Up-regulated gene
Rara	0.401	0.345	5.456	3.101	Up-regulated gene
Lrmp	0.000	0.000	1.403	0.889	Up-regulated gene
Disp1	0.000	0.000	0.596	1.677	Up-regulated gene
5330426P16Rik	0.000	0.000	1.160	1.096	Up-regulated gene
Ptprv	0.000	0.000	0.688	1.555	Up-regulated gene
Smtnl1	1.161	2.568	20.514	21.150	Up-regulated gene
Zfp691	0.000	0.000	0.874	1.359	Up-regulated gene
1700066B19Rik	0.000	0.000	1.489	0.695	Up-regulated gene
Cyb5rl	0.000	0.000	0.834	1.339	Up-regulated gene
B230206H07Rik	0.000	0.000	0.570	1.594	Up-regulated gene
Rfx5	0.000	0.264	1.386	1.396	Up-regulated gene
Cep152	0.049	0.164	0.838	1.385	Up-regulated gene
Tcea3	9.893	4.040	73.819	70.113	Up-regulated gene
S100a1	6.204	4.127	71.994	34.209	Up-regulated gene
Zfp759	0.000	0.000	1.495	0.555	Up-regulated gene
Stmn2	6.273	10.808	75.688	98.974	Up-regulated gene
Fgf13	0.000	0.000	0.850	1.179	Up-regulated gene
Hpn	0.000	0.000	1.244	0.780	Up-regulated gene
Parp11	0.000	0.000	1.459	0.563	Up-regulated gene
Dusp18	0.183	0.032	1.247	0.908	Up-regulated gene
Tbc1d4	0.000	0.000	1.341	0.659	Up-regulated gene

Abcc4	0.375	0.396	4.372	3.220	Up-regulated gene
Msmp	0.000	0.000	1.307	0.514	Up-regulated gene
Nppb	135.423	70.586	1188.700	685.906	Up-regulated gene
Enpp5	0.000	0.000	1.002	0.810	Up-regulated gene
Tet3	0.059	0.170	0.886	1.183	Up-regulated gene
Cdca7l	0.000	0.000	0.679	1.122	Up-regulated gene
Kif3b	0.166	0.194	1.390	1.846	Up-regulated gene
Ak1	8.282	6.364	67.517	60.635	Up-regulated gene
Plcg1	0.320	0.166	2.353	1.887	Up-regulated gene
Xkr6	0.000	0.000	1.168	0.567	Up-regulated gene
Itm2a	3.926	5.257	47.147	31.721	Up-regulated gene
Nap1l5	0.000	0.000	1.060	0.653	Up-regulated gene
Adck1	0.390	1.010	6.354	5.531	Up-regulated gene
Plxdc1	0.000	0.000	0.668	1.028	Up-regulated gene
Scube3	0.000	0.000	1.176	0.516	Up-regulated gene
Wbp5	105.533	68.501	923.411	538.678	Up-regulated gene
Slc34a3	0.000	0.000	0.547	1.103	Up-regulated gene
Scoc	1.829	3.397	23.113	19.543	Up-regulated gene
Spag1	0.120	0.093	1.032	0.676	Up-regulated gene
Snhg4	5.068	2.078	28.430	28.824	Up-regulated gene
Tpm2	5.057	7.403	46.704	52.805	Up-regulated gene
Dzip1	0.824	0.492	5.917	4.576	Up-regulated gene
Unkl	0.647	0.318	4.725	2.820	Up-regulated gene
Kif21a	0.000	0.000	0.512	1.035	Up-regulated gene
Mup4	0.000	0.000	1.049	0.495	Up-regulated gene
Gm19705	5.775	11.723	85.809	47.912	Up-regulated gene
Phlda3	25.352	36.066	305.886	162.873	Up-regulated gene
Phldb2	1.211	0.966	7.216	9.261	Up-regulated gene
Dmpk	0.182	0.203	1.481	1.382	Up-regulated gene
Vps18	0.399	0.764	4.505	4.077	Up-regulated gene
Map3k11	0.878	1.148	8.753	6.045	Up-regulated gene
Pacs2	0.154	0.398	1.990	1.983	Up-regulated gene
Phc1	4.803	7.350	51.286	35.353	Up-regulated gene
Wdr8	3.692	2.061	15.407	24.861	Up-regulated gene
Chpf2	0.488	0.624	4.761	3.019	Up-regulated gene
Serpinb6b	1.529	0.890	6.589	9.931	Up-regulated gene
Bex2	17.801	15.325	109.850	115.915	Up-regulated gene
Sh3glb2	5.646	5.627	37.905	37.936	Up-regulated gene
Sap25	3.361	3.481	26.884	18.469	Up-regulated gene
Abhd6	5.479	3.208	33.829	22.159	Up-regulated gene
	8.546	19.332	80.702	98.107	Up-regulated gene
Csrp2	50.714	32.366	305.146	226.077	Up-regulated gene
Cisd3	26.851	20.573	178.720	124.275	Up-regulated gene
8430410A17Rik	29.554	27.193	176.605	184.791	Up-regulated gene
Exoc7	1.171	1.397	7.863	8.388	Up-regulated gene
Gdi1	3.608	2.343	13.453	24.195	Up-regulated gene
Exoc4	3.656	2.697	25.560	14.610	Up-regulated gene
1190005I06Rik	15.087	10.776	88.947	72.812	Up-regulated gene
Slc52a3	0.421	0.725	2.616	4.475	Up-regulated gene
	2.338	5.315	23.440	23.625	Up-regulated gene
Rmi1	1.548	1.084	8.517	7.576	Up-regulated gene
Sergef	0.792	0.838	4.096	5.845	Up-regulated gene
Zfp740	1.610	1.329	9.649	8.189	Up-regulated gene

Cog1	1.758	0.942	8.676	7.664	Up-regulated gene
BC048355	17.170	12.700	99.430	80.143	Up-regulated gene
Ercc5	1.020	1.656	7.950	7.930	Up-regulated gene
Ddx11	0.983	0.515	4.632	4.005	Up-regulated gene
Tubb6	9.275	11.888	49.071	72.462	Up-regulated gene
Pisd-ps1	1.702	2.140	13.586	8.041	Up-regulated gene
1110008L16Rik	1.612	2.381	11.112	11.064	Up-regulated gene
Prr12	0.500	0.629	3.972	2.227	Up-regulated gene
Ccdc14	0.439	0.431	2.073	2.681	Up-regulated gene
Taf1	1.068	1.132	5.809	6.173	Up-regulated gene
App	2.868	4.953	23.521	17.815	Up-regulated gene
Tuba1a	38.856	33.490	182.323	199.269	Up-regulated gene
Slc38a1	3.737	2.686	13.650	20.202	Up-regulated gene
Ift27	11.747	7.292	51.196	46.626	Up-regulated gene
Hmox1	84.496	72.419	381.255	420.042	Up-regulated gene
Kdsr	0.973	0.768	5.263	3.624	Up-regulated gene
Ngfrap1	195.780	161.605	1121.990	699.473	Up-regulated gene
Trim62	0.422	0.257	1.975	1.472	Up-regulated gene
Pard6g	3.072	4.052	17.261	18.764	Up-regulated gene
Ppp1r13l	0.463	0.623	3.005	2.467	Up-regulated gene
Dhx16	23.291	43.753	190.687	146.842	Up-regulated gene
Cltb	13.907	16.008	68.560	81.603	Up-regulated gene
Bbs5	1.489	2.889	12.962	8.950	Up-regulated gene
G6pdx	6.552	7.583	25.072	45.558	Up-regulated gene
Cdkn1a	46.480	45.102	299.874	157.520	Up-regulated gene
Zfp213	0.841	1.248	5.483	4.908	Up-regulated gene
Tfap2c	8.745	13.786	43.755	66.435	Up-regulated gene
Pycr1	1.366	1.319	5.370	7.645	Up-regulated gene
Nck1	4.090	2.795	20.939	12.255	Up-regulated gene
Mest	3.972	7.573	26.626	28.526	Up-regulated gene
Prkcd	1.967	1.962	6.599	12.152	Up-regulated gene
Ddit4l	5.597	4.761	31.044	17.930	Up-regulated gene
Cdc42bpg	1.091	0.955	6.419	3.252	Up-regulated gene
Tubb2b	7.470	3.674	26.748	25.005	Up-regulated gene
Mocs2	3.065	5.621	23.854	16.356	Up-regulated gene
Zfp955a	0.839	0.493	3.308	2.714	Up-regulated gene
B3galnt2	0.571	0.846	3.848	2.535	Up-regulated gene
S100a13	30.060	20.151	140.567	85.201	Up-regulated gene
Nme4	30.287	44.013	193.157	140.796	Up-regulated gene
Fam57a	2.123	2.132	9.257	9.831	Up-regulated gene
Rad51l3	0.776	0.408	2.324	2.986	Up-regulated gene
Tctn1	0.930	0.874	4.598	3.405	Up-regulated gene
Grb10	2.157	2.641	11.419	9.849	Up-regulated gene
Fkrp	0.937	1.212	3.739	5.781	Up-regulated gene
Copz2	4.252	6.890	19.401	29.458	Up-regulated gene
Gclm	21.220	18.129	70.686	100.622	Up-regulated gene
Eno1	68.907	39.291	212.211	257.626	Up-regulated gene
Fundc2	3.662	2.269	11.529	14.056	Up-regulated gene
Cul7	0.966	1.499	4.366	6.214	Up-regulated gene
Cd24a	4.934	5.439	23.534	20.974	Up-regulated gene
Dscr3	3.817	5.222	21.822	16.903	Up-regulated gene
Noxo1	0.799	0.448	3.139	2.195	Up-regulated gene
D5Ertd579e	0.777	0.463	2.464	2.828	Up-regulated gene

Nrg4	0.891	0.569	2.575	3.583	Up-regulated gene
Mto1	1.547	1.850	6.451	7.477	Up-regulated gene
B230120H23Rik	1.324	1.421	6.352	4.631	Up-regulated gene
1600010M07Rik	24.757	18.010	0.000	0.000	Down-regulated gene
1700018B08Rik	14.063	5.296	0.000	0.063	Down-regulated gene
1700030L20Rik	3.714	4.953	0.900	0.708	Down-regulated gene
1700057K13Rik	1.045	0.608	0.000	0.000	Down-regulated gene
1700084C01Rik	6.148	5.111	0.000	0.089	Down-regulated gene
2010107G23Rik	13.616	13.736	2.755	3.829	Down-regulated gene
2210409E12Rik	3643.370	3196.490	725.374	470.338	Down-regulated gene
2310039L15Rik	17.247	17.575	3.761	4.311	Down-regulated gene
2310042E22Rik	11.543	5.322	0.000	0.255	Down-regulated gene
Khdc3	1266.200	1472.650	20.557	14.629	Down-regulated gene
2610528J11Rik	178.177	120.742	30.997	15.410	Down-regulated gene
2700054A10Rik	4.215	2.134	0.447	0.633	Down-regulated gene
3100003L05Rik	48.453	21.485	0.000	0.121	Down-regulated gene
4930430J02Rik	6.390	3.791	0.000	0.229	Down-regulated gene
4930444P10Rik	3.543	16.910	0.000	0.000	Down-regulated gene
4930468A15Rik	2.042	1.584	0.000	0.000	Down-regulated gene
Ube2dnl2	7.754	8.371	0.000	0.000	Down-regulated gene
Mgarp	106.437	205.841	0.076	0.700	Down-regulated gene
4931440F15Rik	1.882	1.072	0.000	0.108	Down-regulated gene
4933430I17Rik	2.748	6.130	0.000	0.105	Down-regulated gene
5430416N02Rik	152.753	184.325	43.324	31.992	Down-regulated gene
5730460C07Rik	1.885	0.775	0.000	0.000	Down-regulated gene
6430548M08Rik	2.203	0.840	0.000	0.083	Down-regulated gene
9330159M07Rik	1.876	3.148	0.155	0.197	Down-regulated gene
AI317395	5.583	9.768	0.000	0.000	Down-regulated gene
AU019990	2.482	1.284	0.000	0.000	Down-regulated gene
AW551984	1.589	1.387	0.000	0.162	Down-regulated gene
Acaa1b	8.825	11.685	1.968	2.485	Down-regulated gene
Acer1	6.820	4.725	1.085	0.545	Down-regulated gene
Acrbp	28.054	37.185	5.841	8.857	Down-regulated gene
Acsf2	11.660	16.677	0.815	2.135	Down-regulated gene
Actn2	1.754	4.259	0.364	0.337	Down-regulated gene
Adad2	3.145	3.580	0.000	0.096	Down-regulated gene
Agpat9	43.273	46.868	4.891	6.452	Down-regulated gene
Ak4	47.155	51.771	5.439	1.796	Down-regulated gene
Akr1c12	3.878	10.806	0.000	0.054	Down-regulated gene
Akr1c13	50.279	72.905	0.076	0.376	Down-regulated gene
Aldh3a1	1.560	2.169	0.000	0.198	Down-regulated gene
Alppl2	19.841	10.803	0.000	0.000	Down-regulated gene
Amhr2	9.021	5.270	0.938	1.297	Down-regulated gene
Ankrd22	8.398	4.616	0.000	0.214	Down-regulated gene
Apoc1	1750.760	1447.770	229.703	194.295	Down-regulated gene
Aqp8	122.131	92.276	28.157	22.466	Down-regulated gene
Aqp9	9.327	3.906	0.000	0.000	Down-regulated gene
Arid5a	2.170	2.166	0.278	0.381	Down-regulated gene
Arrdc5	4.998	11.634	0.000	0.000	Down-regulated gene
Asgr2	2.324	3.186	0.000	0.331	Down-regulated gene
Asphd1	9.019	5.193	0.000	0.178	Down-regulated gene
Atg13	38.609	66.072	10.899	11.461	Down-regulated gene
Atn1	28.460	20.363	3.692	3.678	Down-regulated gene

Atp12a	2.770	2.804	0.851	0.441	Down-regulated gene
B3gnt6	1.169	0.518	0.000	0.000	Down-regulated gene
BC048679	41.206	32.286	2.741	5.689	Down-regulated gene
Bace2	5.315	3.054	0.000	0.047	Down-regulated gene
Baiap2l2	2.128	2.665	0.000	0.089	Down-regulated gene
Baz1a	47.970	50.088	11.165	5.703	Down-regulated gene
Bcl2l14	7.663	17.282	0.386	0.957	Down-regulated gene
Bhmt	14.664	48.219	0.000	0.000	Down-regulated gene
Bmp6	4.176	4.353	0.000	0.400	Down-regulated gene
Bnip3	112.005	77.963	25.701	16.349	Down-regulated gene
C1ql4	6.910	11.273	0.000	0.324	Down-regulated gene
C2cd4a	26.955	16.819	0.000	0.000	Down-regulated gene
Car7	5.288	5.313	0.058	0.165	Down-regulated gene
Caskin1	1.980	3.541	0.378	0.433	Down-regulated gene
Casp7	8.001	8.300	0.617	1.336	Down-regulated gene
Ccdc42	18.899	10.327	0.000	0.000	Down-regulated gene
Cd247	2.989	3.456	0.000	0.000	Down-regulated gene
Cd300ld	3.883	3.296	0.000	0.000	Down-regulated gene
Cd84	17.488	30.476	2.672	1.802	Down-regulated gene
Ceacam10	50.607	64.644	2.310	5.299	Down-regulated gene
Cebpa	3.301	2.043	0.607	0.724	Down-regulated gene
Cgnl1	13.580	6.252	1.187	1.362	Down-regulated gene
Chga	25.584	19.810	6.870	3.977	Down-regulated gene
Chi3l1	4.931	6.755	0.000	0.074	Down-regulated gene
Cilp	1.303	0.727	0.000	0.042	Down-regulated gene
Cish	10.480	5.123	0.438	1.014	Down-regulated gene
Ckm	11.429	7.246	1.545	1.504	Down-regulated gene
Clcnkb	8.542	4.036	0.000	0.206	Down-regulated gene
Cmbl	21.758	6.132	0.746	0.661	Down-regulated gene
Col7a1	3.199	1.720	0.000	0.104	Down-regulated gene
Cox18	101.924	112.201	23.692	28.852	Down-regulated gene
Cox7a1	260.273	272.230	39.266	29.192	Down-regulated gene
Cpn1	93.125	112.790	3.878	1.877	Down-regulated gene
Cpne7	1.559	1.115	0.000	0.000	Down-regulated gene
Cpxm2	1.504	1.676	0.000	0.000	Down-regulated gene
Crtam	4.706	2.199	0.000	0.098	Down-regulated gene
Cryge	54.443	113.676	3.940	1.141	Down-regulated gene
Csf3r	8.495	3.089	0.506	0.293	Down-regulated gene
Cst7	28.628	58.997	0.000	0.071	Down-regulated gene
Cyp2b23	8.587	9.357	1.811	1.867	Down-regulated gene
Cyp2s1	43.530	47.839	7.278	4.560	Down-regulated gene
Cyp4f14	23.503	13.295	0.000	0.572	Down-regulated gene
Dcaf12l1	21.301	21.922	3.481	3.187	Down-regulated gene
Dio3	3.639	1.897	0.000	0.232	Down-regulated gene
Dirc2	22.488	20.637	4.041	5.026	Down-regulated gene
Dkk1	113.813	105.909	24.675	27.319	Down-regulated gene
Dmgdh	0.878	1.338	0.000	0.040	Down-regulated gene
Dnahc2	1.296	1.762	0.000	0.257	Down-regulated gene
Dnaja4	3.127	3.527	0.111	0.177	Down-regulated gene
Dnajb1	170.894	318.062	25.359	46.401	Down-regulated gene
Dub1	2.253	1.232	0.032	0.023	Down-regulated gene
Dusp4	43.841	43.856	4.762	2.777	Down-regulated gene
Dynlrb2	6.463	3.192	0.000	0.000	Down-regulated gene

E330011O21Rik	5.605	1.986	0.000	0.000	Down-regulated gene
Efcab10	108.140	83.763	4.826	8.013	Down-regulated gene
Efhb	8.527	7.957	0.570	0.381	Down-regulated gene
Efnb2	25.747	16.014	4.045	2.114	Down-regulated gene
Egfl7	156.035	171.792	30.490	26.860	Down-regulated gene
Egln1	55.765	39.643	9.912	4.363	Down-regulated gene
Eif2c4	1.985	1.341	0.012	0.078	Down-regulated gene
Elf3	450.116	291.870	63.543	79.617	Down-regulated gene
Eno4	16.626	8.156	0.791	0.490	Down-regulated gene
Enpep	24.816	17.385	1.340	1.595	Down-regulated gene
Entpd3	2.585	2.890	0.000	0.153	Down-regulated gene
Erf	17.649	14.820	4.056	2.822	Down-regulated gene
Fabp5	1119.060	1606.810	19.965	52.592	Down-regulated gene
Fabp9	15.789	12.808	0.000	0.438	Down-regulated gene
Fam151a	35.446	24.771	2.714	0.977	Down-regulated gene
Emc9	53.169	36.569	10.935	7.742	Down-regulated gene
Fam159b	97.121	98.029	10.436	9.087	Down-regulated gene
Fam25c	1366.360	1777.030	464.874	284.943	Down-regulated gene
Fam46a	9.826	8.855	0.608	0.954	Down-regulated gene
Fbp1	22.891	13.365	0.000	0.045	Down-regulated gene
Fbp2	217.446	467.600	9.660	5.421	Down-regulated gene
Fbxo15	212.063	325.806	39.586	44.838	Down-regulated gene
Ffar2	4.846	1.647	0.000	0.065	Down-regulated gene
Fgfr2	28.022	20.647	0.451	1.718	Down-regulated gene
Flrt3	60.214	50.746	7.202	6.971	Down-regulated gene
Foxb2	1.449	2.516	0.000	0.000	Down-regulated gene
Foxc1	1.049	1.535	0.000	0.044	Down-regulated gene
G630016D24Rik	8.141	8.857	0.458	0.594	Down-regulated gene
Gad2	1.331	1.214	0.258	0.162	Down-regulated gene
Gata6	119.025	176.260	17.773	7.536	Down-regulated gene
Ggt1	20.130	37.565	1.328	1.911	Down-regulated gene
Glrx	221.830	312.564	49.513	63.281	Down-regulated gene
Gm11517	7.784	27.402	0.164	0.116	Down-regulated gene
Gm11545	23.329	28.519	2.627	1.284	Down-regulated gene
Gm13128	9.151	20.302	1.112	1.396	Down-regulated gene
Gm13152	14.737	18.540	3.607	2.873	Down-regulated gene
Spint5	57.579	98.890	13.894	6.793	Down-regulated gene
Gm14634	1.984	0.962	0.000	0.076	Down-regulated gene
Gm15698	125.169	309.483	26.678	20.988	Down-regulated gene
Gm1631	50.819	85.333	0.000	1.017	Down-regulated gene
Gm16702	256.603	193.231	60.991	41.237	Down-regulated gene
Gm2016	1.563	0.718	0.000	0.000	Down-regulated gene
Gm3604	28.568	30.555	8.283	4.140	Down-regulated gene
Gm5065	1.988	1.141	0.266	0.122	Down-regulated gene
Gm5480	133.285	212.179	36.021	28.887	Down-regulated gene
Gm5662	1.214	2.569	0.000	0.000	Down-regulated gene
Gml	24.318	4.080	0.000	0.000	Down-regulated gene
Gnrh1	2.011	1.560	0.000	0.175	Down-regulated gene
Gpcpd1	18.538	36.312	5.745	5.017	Down-regulated gene
Gpr161	3.222	14.477	0.000	0.000	Down-regulated gene
Gpr85	0.822	2.857	0.000	0.100	Down-regulated gene
Grb14	3.594	3.347	0.000	0.313	Down-regulated gene
Gsdma3	4.974	2.205	0.000	0.130	Down-regulated gene

Guca1a	28.747	103.326	0.000	0.662	Down-regulated gene
H2-K1	67.842	112.633	24.695	19.169	Down-regulated gene
H2-Oa	11.472	5.907	0.092	0.196	Down-regulated gene
H2-Q10	1.782	1.106	0.000	0.217	Down-regulated gene
Hemt1	1.970	3.293	0.000	0.000	Down-regulated gene
Hist1h2ai	8.285	5.295	0.840	1.487	Down-regulated gene
Hist1h2ap	0.487	1.135	0.001	0.000	Down-regulated gene
Hist1h2bc	282.155	460.358	60.530	31.429	Down-regulated gene
Hist1h2be	1.177	2.250	0.068	0.169	Down-regulated gene
Hist1h2bf	1.042	2.425	0.000	0.000	Down-regulated gene
Hist1h2bj	65.630	53.755	5.387	10.171	Down-regulated gene
Hist1h2bk	10.938	6.063	0.898	1.271	Down-regulated gene
Hist1h2bm	28.127	25.463	3.142	6.675	Down-regulated gene
Hist1h3c	125.683	68.191	18.577	11.332	Down-regulated gene
Hist1h3e	21.772	18.619	3.063	3.525	Down-regulated gene
Hist1h3g	10.798	12.815	3.285	2.132	Down-regulated gene
Hist1h4f	10.986	17.706	0.000	0.516	Down-regulated gene
Hist1h4m	21.060	23.802	3.157	3.352	Down-regulated gene
Hist2h2bb	29.433	34.381	3.546	5.794	Down-regulated gene
Hmga2	94.544	108.325	16.488	5.712	Down-regulated gene
Hnf4a	1.847	1.635	0.000	0.225	Down-regulated gene
Hoxd9	0.858	2.034	0.000	0.029	Down-regulated gene
Hsd17b6	5.334	8.277	0.000	0.088	Down-regulated gene
Hspa1a	144.187	100.809	1.500	0.552	Down-regulated gene
Hspa1b	147.403	170.197	1.374	0.846	Down-regulated gene
Icosl	22.860	26.662	3.885	1.949	Down-regulated gene
Il17f	8.366	1.803	0.000	0.000	Down-regulated gene
Itfg3	49.528	47.945	7.576	7.630	Down-regulated gene
Kcnk6	6.969	9.698	1.862	2.211	Down-regulated gene
Khdc1a	7.194	9.599	0.000	0.063	Down-regulated gene
Klf5	1119.820	1014.630	218.515	275.527	Down-regulated gene
LOC100302567	207.041	160.657	0.000	0.000	Down-regulated gene
LOC433944	2.286	3.792	0.000	0.240	Down-regulated gene
LOC639910	1.184	0.574	0.128	0.090	Down-regulated gene
Obp2a	48.393	6.651	0.000	0.218	Down-regulated gene
Lgals6	20.907	11.257	0.224	0.000	Down-regulated gene
Lpcat1	37.563	40.366	7.732	10.358	Down-regulated gene
Ly6a	381.353	200.438	12.159	5.591	Down-regulated gene
Ly6c1	3.998	10.060	0.376	0.716	Down-regulated gene
Ly96	74.206	67.501	18.693	8.638	Down-regulated gene
Map1lc3b	1275.900	1451.750	329.572	198.088	Down-regulated gene
Mboat2	13.218	15.900	1.829	2.183	Down-regulated gene
Mdfic	0.922	1.279	0.000	0.170	Down-regulated gene
Mfap4	3.593	6.380	0.000	0.042	Down-regulated gene
Mfsd2b	4.698	1.199	0.080	0.094	Down-regulated gene
Mir1901	2769.560	2149.080	0.000	0.000	Down-regulated gene
Mir719	70.505	22.858	0.000	0.000	Down-regulated gene
Mitf	2.907	3.744	0.581	0.764	Down-regulated gene
Mogat2	18.332	14.181	1.043	2.040	Down-regulated gene
Mras	39.138	30.919	3.048	5.597	Down-regulated gene
Mtl5	1.290	0.655	0.000	0.056	Down-regulated gene
Mybpc1	0.922	1.676	0.000	0.000	Down-regulated gene
Naalad2	3.415	3.777	0.000	0.288	Down-regulated gene

Necab2	9.768	3.929	0.573	0.749	Down-regulated gene
Nkx6-2	62.703	68.033	8.187	7.480	Down-regulated gene
Npr3	3.905	1.979	0.000	0.090	Down-regulated gene
Olfr836	7.605	4.215	0.000	0.221	Down-regulated gene
Optn	17.378	14.024	2.044	3.618	Down-regulated gene
Ostb	43.489	19.248	0.000	0.000	Down-regulated gene
Ovgp1	2.296	1.359	0.000	0.000	Down-regulated gene
Oxt	1.715	8.250	0.000	0.000	Down-regulated gene
Parp8	1.252	2.526	0.000	0.153	Down-regulated gene
Pcdh19	6.663	7.997	0.828	0.627	Down-regulated gene
Pde6g	6.003	3.342	0.000	0.159	Down-regulated gene
Pdgfra	24.269	22.460	3.147	2.714	Down-regulated gene
Pdp2	18.087	14.800	1.110	1.104	Down-regulated gene
Pemt	43.647	51.753	11.670	9.933	Down-regulated gene
Phlda1	185.957	120.280	39.809	22.449	Down-regulated gene
Pigz	2.875	2.478	0.315	0.028	Down-regulated gene
Plekhf1	129.607	141.223	17.888	26.023	Down-regulated gene
Plod1	17.682	20.156	3.625	5.259	Down-regulated gene
Pnlip	0.705	1.203	0.000	0.000	Down-regulated gene
Pnliprp2	35.403	35.960	1.086	0.202	Down-regulated gene
Popdc3	13.500	13.669	0.000	0.368	Down-regulated gene
Ppp1r14d	109.599	102.080	9.605	13.602	Down-regulated gene
Ppp1r2-ps3	6.355	5.518	0.000	0.369	Down-regulated gene
Pramef17	6.303	2.898	0.151	0.142	Down-regulated gene
Pramel5	10.230	22.948	0.000	0.397	Down-regulated gene
Pramel7	132.352	167.666	8.372	5.528	Down-regulated gene
Psmb9	21.413	14.978	3.119	1.748	Down-regulated gene
Ptcra	9.562	7.355	2.169	1.638	Down-regulated gene
Pyy	21.387	19.001	0.000	0.567	Down-regulated gene
Ramp2	24.988	38.042	4.447	7.541	Down-regulated gene
Rarb	3.865	4.008	0.000	0.107	Down-regulated gene
Reep1	7.658	17.553	1.363	0.935	Down-regulated gene
Rfk	118.827	99.035	15.411	22.258	Down-regulated gene
Rhpn2	22.714	11.303	1.323	2.571	Down-regulated gene
Rimkla	1.449	0.798	0.000	0.043	Down-regulated gene
Rnf130	76.825	151.581	10.594	15.811	Down-regulated gene
Rogdi	259.912	173.791	61.233	40.289	Down-regulated gene
Runx1t1	5.040	2.483	0.055	0.168	Down-regulated gene
Saa3	204.958	110.288	19.493	19.254	Down-regulated gene
Samd5	2.641	5.292	0.000	0.000	Down-regulated gene
Sema3b	1.923	1.058	0.000	0.227	Down-regulated gene
	1.561	1.529	0.000	0.000	Down-regulated gene
Serping1	24.834	26.171	5.399	4.710	Down-regulated gene
Slc10a1	0.903	1.399	0.299	0.206	Down-regulated gene
Slc13a2	5.411	8.362	0.000	0.000	Down-regulated gene
Slc13a5	17.790	12.136	0.635	1.402	Down-regulated gene
Slc15a2	248.810	68.857	2.558	0.623	Down-regulated gene
Slc1a1	24.523	13.692	3.419	4.574	Down-regulated gene
Slc1a3	11.749	12.967	0.875	1.417	Down-regulated gene
Slc22a13	1.155	0.623	0.000	0.061	Down-regulated gene
Slc22a13b-ps	2.603	3.759	0.561	0.750	Down-regulated gene
Slc22a16	2.127	4.504	0.000	0.054	Down-regulated gene
Slc24a5	5.328	16.738	0.045	0.063	Down-regulated gene

Slc25a41	5.049	6.323	0.000	0.000	Down-regulated gene
Slc2a8	27.938	31.716	3.108	6.015	Down-regulated gene
Slc30a3	10.158	7.336	0.000	0.460	Down-regulated gene
Slc30a4	8.814	7.835	1.431	1.869	Down-regulated gene
Slc34a2	15.072	11.184	0.627	0.555	Down-regulated gene
Slc35f4	2.048	5.060	0.000	0.099	Down-regulated gene
Slc45a3	3.913	2.301	0.174	0.334	Down-regulated gene
Slc6a13	2.507	1.945	0.000	0.028	Down-regulated gene
Smagp	271.033	191.618	43.340	51.891	Down-regulated gene
Smpd1	66.639	50.218	5.183	5.142	Down-regulated gene
Socs1	53.690	60.001	8.455	15.238	Down-regulated gene
Sox7	27.508	23.587	7.318	3.760	Down-regulated gene
Spic	55.192	93.862	4.852	2.759	Down-regulated gene
Spsb2	18.944	31.489	6.802	4.816	Down-regulated gene
St8sia5	2.422	3.602	0.000	0.031	Down-regulated gene
Stard10	396.647	196.897	25.196	15.562	Down-regulated gene
Stfa1	41.666	40.998	8.514	10.223	Down-regulated gene
Stra8	5.784	3.927	0.000	0.088	Down-regulated gene
Stx19	9.109	3.927	0.000	0.337	Down-regulated gene
Sult5a1	17.333	23.298	0.000	0.000	Down-regulated gene
Sycn	156.605	172.254	0.000	0.000	Down-regulated gene
Tcf23	3.358	2.624	0.000	0.113	Down-regulated gene
Tekt2	2.103	3.700	0.000	0.128	Down-regulated gene
Tes	57.632	96.236	20.556	14.259	Down-regulated gene
Tet2	16.790	33.666	4.927	5.034	Down-regulated gene
Tex19.2	36.791	29.196	1.649	2.993	Down-regulated gene
Tgfb2	1.198	0.728	0.017	0.073	Down-regulated gene
Timd2	154.598	113.741	1.458	3.724	Down-regulated gene
Tm6sf1	2.216	1.789	0.000	0.000	Down-regulated gene
Tmem106a	11.157	9.393	2.228	1.787	Down-regulated gene
Tmem191c	16.080	12.361	2.266	2.017	Down-regulated gene
Tmem231	19.486	19.664	1.972	2.772	Down-regulated gene
Tmem82	15.868	9.301	2.551	2.221	Down-regulated gene
Tmx4	5.860	3.626	0.676	0.735	Down-regulated gene
Tpo	1.027	0.614	0.000	0.000	Down-regulated gene
Tra2a	191.902	189.073	41.394	42.996	Down-regulated gene
Trim43a	39.045	54.578	0.000	0.000	Down-regulated gene
Trim43b	14.968	9.503	1.286	0.562	Down-regulated gene
Tsga10	1.299	1.556	0.000	0.207	Down-regulated gene
Ttyh2	9.882	5.837	0.766	0.847	Down-regulated gene
Tuft1	26.711	20.965	5.211	4.951	Down-regulated gene
Uap1	63.309	72.052	8.463	15.034	Down-regulated gene
Ube2l6	368.770	401.040	92.997	73.220	Down-regulated gene
Ulk1	47.176	75.317	14.471	13.588	Down-regulated gene
Upp1	1024.460	961.027	95.777	87.377	Down-regulated gene
Uros	33.917	29.984	7.465	7.791	Down-regulated gene
Vegfc	20.479	19.153	4.598	3.058	Down-regulated gene
Wdr65	1.349	0.573	0.000	0.062	Down-regulated gene
Wdr95	7.930	7.907	0.000	0.508	Down-regulated gene
Whamm	15.694	38.000	2.825	3.538	Down-regulated gene
Wsb1	184.727	127.829	37.864	35.271	Down-regulated gene
Xlr5c	1.630	3.584	0.000	0.166	Down-regulated gene
Ybx2	18.219	7.172	1.433	1.461	Down-regulated gene

Ypel2	12.389	15.187	2.185	1.793	Down-regulated gene
Zc3h12d	1.369	0.738	0.000	0.000	Down-regulated gene
Zfand2a	42.816	29.590	6.917	8.108	Down-regulated gene
Zfp810	2.351	3.533	0.000	0.309	Down-regulated gene
Zfpm1	8.369	11.513	2.616	1.714	Down-regulated gene
Zswim6	2.610	2.852	0.725	0.472	Down-regulated gene

Table 7. Differentially expressed genes between PgESC and AgESC lines. (Fold change > 5)

Symbol	GgESCs_#1 (FPKM)	GgESCs_#2 (FPKM)	AgESCs_#1 (FPKM)	AgESCs_#2 (FPKM)	Category
Caly	3.036	2.124	0.587	0.434	Gg > Ag
Cd82	1.698	3.343	0.669	0.334	Gg > Ag
AF067061	2.555	0.000	0.000	0.000	Gg > Ag
Hormad2	4.743	0.759	0.934	0.148	Gg > Ag
Gm10696	1.137	0.230	0.000	0.000	Gg > Ag
Scara5	1.063	1.318	0.533	0.070	Gg > Ag
Asb14	1.656	0.607	0.273	0.139	Gg > Ag
Dmrtb1	2.497	1.826	1.622	0.106	Gg > Ag
Lgi2	2.827	3.690	0.617	0.635	Gg > Ag
Tppp3	10.812	11.422	6.404	0.720	Gg > Ag
Spata21	1.135	0.544	0.230	0.073	Gg > Ag
Pgpep1l	5.105	8.283	0.608	2.585	Gg > Ag
Rimkla	1.202	2.477	0.314	0.348	Gg > Ag
Ppef2	1.918	0.796	0.330	0.163	Gg > Ag
Tmem35	2.405	1.969	1.107	0.147	Gg > Ag
Wbp2nl	1.751	1.199	0.478	0.151	Gg > Ag
Snhg9	34.234	35.861	8.670	4.804	Gg > Ag
Gm8300	2.965	0.000	0.000	0.000	Gg > Ag
Ccdc74a	2.376	1.683	0.425	0.314	Gg > Ag
Gm6880	1.606	0.187	0.000	0.000	Gg > Ag
Tmem181a	10.563	4.646	1.696	0.960	Gg > Ag
Gsc2	0.289	1.061	0.000	0.000	Gg > Ag
Vstm2l	1.489	0.814	0.393	0.000	Gg > Ag
Rasgef1a	3.926	2.741	1.050	0.333	Gg > Ag
Scarna13	3.016	3.159	3.055	0.000	Gg > Ag
Lax1	1.448	2.200	0.037	1.016	Gg > Ag
Apom	2.950	6.334	0.896	0.662	Gg > Ag
Gm2022	3.150	0.000	0.000	0.000	Gg > Ag
Col2a1	5.415	3.649	1.411	0.444	Gg > Ag
Nlrp14	1.386	0.229	0.099	0.055	Gg > Ag
Ppp2r2c	14.549	5.797	1.545	1.691	Gg > Ag
Cdhr1	1.606	0.688	0.277	0.123	Gg > Ag
1700123I01Rik	2.820	0.892	0.431	0.179	Gg > Ag
Ms4a10	0.840	1.467	0.378	0.000	Gg > Ag
Cox7b2	5.135	9.354	2.940	0.501	Gg > Ag
Ankrd33b	3.466	4.523	4.787	0.023	Gg > Ag
Grm4	1.998	4.953	1.042	0.289	Gg > Ag
Fam159b	3.324	1.635	1.581	0.000	Gg > Ag
Hopx	9.685	8.033	12.074	0.187	Gg > Ag
Otx2	2.387	8.579	1.144	0.519	Gg > Ag
Lgals4	4.093	6.818	0.290	2.780	Gg > Ag
Mroh7	1.229	0.984	0.348	0.041	Gg > Ag
Aass	3.489	0.045	0.087	0.048	Gg > Ag
Grifin	1.130	0.710	0.229	0.000	Gg > Ag
Usp26	3.714	1.357	0.560	0.257	Gg > Ag
Cdh4	1.901	2.527	1.234	0.109	Gg > Ag
Gstp2	89.756	57.018	71.427	2.006	Gg > Ag
A2m	2.161	2.334	0.288	0.489	Gg > Ag
2410141K09Rik	177.139	19.633	5.196	18.545	Gg > Ag
Pramef25	3.672	0.000	0.000	0.000	Gg > Ag
Zscan4b	3.724	0.000	0.000	0.000	Gg > Ag
Asphd1	1.206	1.516	0.489	0.000	Gg > Ag
Stac2	14.394	16.098	2.719	2.245	Gg > Ag
Tcstv1	3.918	0.000	0.000	0.000	Gg > Ag
Maats1	3.041	5.421	0.711	0.591	Gg > Ag
Slco5a1	1.441	0.953	0.228	0.152	Gg > Ag
Gm6756	4.014	0.046	0.045	0.000	Gg > Ag

Lrcol1	3.449	4.926	0.476	0.880	Gg > Ag
Nfam1	2.705	3.422	0.400	0.561	Gg > Ag
Rps15a-ps6	83.997	4.323	2.213	3.952	Gg > Ag
Slit3	0.281	1.488	0.095	0.088	Gg > Ag
Ttll3	4.448	3.233	0.469	0.726	Gg > Ag
Slc44a3	4.482	0.333	0.161	0.214	Gg > Ag
Gm11544	4.360	0.000	0.000	0.000	Gg > Ag
Tmem92	4.360	0.000	0.000	0.000	Gg > Ag
4933427D06Rik	4.399	0.097	0.000	0.000	Gg > Ag
Lgals7	3.268	0.806	0.584	0.000	Gg > Ag
Fes	1.639	1.073	0.385	0.033	Gg > Ag
Gm12794	4.568	0.000	0.000	0.000	Gg > Ag
D17Ertd648e	21.157	1.317	1.273	0.470	Gg > Ag
4933438K21Rik	4.783	0.844	0.765	0.113	Gg > Ag
Zfp600	3.072	0.465	0.302	0.064	Gg > Ag
Kazald1	1.212	1.136	0.065	0.286	Gg > Ag
Kng2	0.479	1.004	0.000	0.000	Gg > Ag
H2-DMb1	1.320	1.820	0.211	0.234	Gg > Ag
Slc27a2	13.359	0.225	0.615	0.040	Gg > Ag
Tinagl1	2.141	2.602	0.781	0.144	Gg > Ag
Birc7	5.318	4.279	1.327	0.346	Gg > Ag
Ap3b2	4.904	3.722	0.610	0.583	Gg > Ag
9230105E05Rik	0.000	5.139	0.000	0.000	Gg > Ag
Gm20767	9.752	0.064	0.186	0.000	Gg > Ag
Kcnab2	3.284	2.280	0.406	0.350	Gg > Ag
Ndufs5	0.002	10.390	0.196	0.003	Gg > Ag
Il6ra	1.502	2.148	0.604	0.027	Gg > Ag
Sncg	20.406	23.618	7.950	1.121	Gg > Ag
Lipc	0.611	1.189	0.133	0.098	Gg > Ag
Mansc1	1.552	1.914	0.140	0.387	Gg > Ag
Gm8994	4.820	0.115	0.000	0.000	Gg > Ag
Aym1	1.169	0.490	0.000	0.000	Gg > Ag
Fgf15	2.946	0.294	0.142	0.105	Gg > Ag
AA792892	5.957	0.078	0.000	0.000	Gg > Ag
Tmem52	1.282	0.618	0.108	0.120	Gg > Ag
Smtnl1	28.692	25.466	2.975	4.000	Gg > Ag
AA467197	34.793	1.269	1.227	0.583	Gg > Ag
Dpp6	0.865	1.848	0.245	0.019	Gg > Ag
Col26a1	1.886	1.443	0.243	0.168	Gg > Ag
Fgf3	3.412	4.369	0.845	0.255	Gg > Ag
Masp2	23.388	26.944	3.436	2.624	Gg > Ag
Znf41-ps	14.838	12.237	3.215	0.790	Gg > Ag
Pdlim4	1.531	3.768	0.465	0.172	Gg > Ag
Ghrh	0.000	7.353	0.000	0.000	Gg > Ag
Cd84	2.087	7.168	1.229	0.163	Gg > Ag
Xlr3c	2.825	16.050	0.550	1.098	Gg > Ag
Aldh3a1	1.833	1.863	0.437	0.061	Gg > Ag
Cd7	1.707	0.847	0.182	0.101	Gg > Ag
Cdkn1c	13.703	2.665	2.347	0.594	Gg > Ag
Cfc1	2.883	0.283	0.000	0.101	Gg > Ag
Hnf4a	0.901	1.491	0.164	0.000	Gg > Ag
Ube2dnl2	0.839	1.464	0.142	0.000	Gg > Ag
A930004D18Rik	2.527	1.791	0.502	0.000	Gg > Ag
Tuba3a	66.316	15.170	6.578	1.677	Gg > Ag
G730013B05Rik	1.563	3.217	0.180	0.299	Gg > Ag
Rhox1	9.208	0.465	0.449	0.000	Gg > Ag
Gm3143	1.420	1.090	0.162	0.000	Gg > Ag
1700019A02Rik	1.785	3.926	0.723	0.000	Gg > Ag
Arrb1	6.768	3.040	1.382	0.146	Gg > Ag
Coro6	2.405	2.081	0.209	0.231	Gg > Ag

Tbx21	2.009	1.336	0.258	0.000	Gg > Ag
Col1a1	1.082	3.279	0.337	0.093	Gg > Ag
Gpat2	7.939	4.401	1.596	0.208	Gg > Ag
Kiss1	5.194	1.088	0.526	0.000	Gg > Ag
Ptgs1	0.844	8.898	0.684	0.063	Gg > Ag
Efhc2	3.187	1.769	0.355	0.143	Gg > Ag
Gm19784	0.830	1.338	0.000	0.000	Gg > Ag
H2-Ab1	7.439	10.098	2.614	0.256	Gg > Ag
Col5a3	3.815	0.613	0.206	0.100	Gg > Ag
Defb42	2.947	0.386	0.000	0.000	Gg > Ag
Map6	10.553	4.861	1.273	0.348	Gg > Ag
6430411K18Rik	1.351	0.920	0.068	0.000	Gg > Ag
Bglap3	5.700	3.072	1.192	0.117	Gg > Ag
Pnma5	71.200	96.959	65.421	0.807	Gg > Ag
Gm38509	7.223	20.453	1.126	0.980	Gg > Ag
Xlr4b	1.735	0.165	0.000	0.000	Gg > Ag
Xlr3b	3.396	0.000	0.054	0.000	Gg > Ag
Xlr4c	1.031	0.772	0.000	0.000	Gg > Ag
Serpinf1	7.325	9.866	2.061	0.261	Gg > Ag
Pnma3	8.044	13.277	7.106	0.105	Gg > Ag
Pga5	2.723	3.371	0.627	0.069	Gg > Ag
Tuba3b	2.861	0.519	0.000	0.000	Gg > Ag
Gm13251	19.438	13.997	2.826	0.646	Gg > Ag
Gm5779	0.000	16.247	0.108	0.000	Gg > Ag
Rapsn	3.283	3.051	0.215	0.297	Gg > Ag
1700019B21Rik	4.328	1.624	0.000	0.435	Gg > Ag
4930591A17Rik	15.750	0.297	0.287	0.000	Gg > Ag
B020031M17Rik	14.912	0.109	0.000	0.000	Gg > Ag
H2-Aa	1.216	1.364	0.000	0.000	Gg > Ag
Fkbp6	7.795	0.725	0.074	0.327	Gg > Ag
Snora44	36.313	38.039	73.567	0.000	Gg > Ag
Rpl14-ps1	0.154	22.081	0.000	0.173	Gg > Ag
Entpd3	0.737	2.776	0.093	0.026	Gg > Ag
BC021614	23.378	47.468	7.299	0.719	Gg > Ag
2300002M23Rik	7.692	5.573	0.291	0.646	Gg > Ag
Ptprtos	22.098	0.185	0.179	0.000	Gg > Ag
Krtdap	3.619	0.632	0.000	0.000	Gg > Ag
Smc1b	6.262	1.901	0.158	0.329	Gg > Ag
2210417A02Rik	1.749	1.374	0.000	0.000	Gg > Ag
Gm12238	26.149	27.392	0.000	29.358	Gg > Ag
Dnajc5g	15.449	1.098	0.056	0.681	Gg > Ag
Npy	3.551	11.856	0.225	0.747	Gg > Ag
Gm5039	4.591	0.547	0.053	0.000	Gg > Ag
Pramel7	3.620	0.711	0.000	0.000	Gg > Ag
C920006O11Rik	6.457	8.091	0.244	0.813	Gg > Ag
Fam183b	7.605	5.975	1.712	0.000	Gg > Ag
Mst1r	1.176	3.260	0.143	0.056	Gg > Ag
Magea4	1.212	2.223	0.000	0.000	Gg > Ag
Msln	9.605	6.447	2.264	0.088	Gg > Ag
Mmrn2	14.125	12.295	1.341	0.473	Gg > Ag
Gm13139	3.181	1.244	0.000	0.139	Gg > Ag
Lincenc1	8.180	29.047	8.301	0.054	Gg > Ag
Mir1199	29.550	0.000	0.000	0.000	Gg > Ag
1700030C10Rik	23.770	0.465	0.350	0.000	Gg > Ag
Snora75	38.196	0.000	0.000	0.000	Gg > Ag
Xlr3a	15.950	0.381	0.158	0.000	Gg > Ag
Ube2dnl1	16.632	23.718	6.513	0.157	Gg > Ag
Scarna6	2.790	1.461	0.000	0.000	Gg > Ag
Fmr1nb	142.617	192.809	24.852	2.353	Gg > Ag
Mir22	0.000	48.071	0.000	0.000	Gg > Ag

Spic	5.942	6.446	0.788	0.079	Gg > Ag
Olfml3	3.278	1.589	0.050	0.055	Gg > Ag
Fam25c	609.189	821.521	67.509	14.227	Gg > Ag
Myof	0.695	12.667	0.143	0.061	Gg > Ag
Rnu11	0.000	62.492	0.000	0.000	Gg > Ag
Pramel6	6.567	1.000	0.057	0.000	Gg > Ag
Smok4a	3.331	4.973	0.251	0.000	Gg > Ag
Mogat2	12.907	5.458	0.920	0.107	Gg > Ag
Rhox5	797.993	97.511	21.750	4.680	Gg > Ag
Mirlet7d	82.666	0.000	0.000	0.000	Gg > Ag
9630028I04Rik	2.775	4.195	0.128	0.000	Gg > Ag
1700057H21Rik	2.510	3.681	0.000	0.000	Gg > Ag
Mir6392	119.314	0.000	0.000	0.000	Gg > Ag
H19	5.823	2.334	0.000	0.000	Gg > Ag
Mlana	4.730	9.290	0.299	0.000	Gg > Ag
Rpl26	22.134	44.218	5.887	0.000	Gg > Ag
AF357399	0.000	179.834	0.000	0.000	Gg > Ag
Rpl39l	17.284	5.642	0.381	0.141	Gg > Ag
A230070E04Rik	3.413	5.577	0.000	0.000	Gg > Ag
Mir8097	82.319	187.460	80.503	0.000	Gg > Ag
Pla2g10os	4.205	5.035	0.000	0.000	Gg > Ag
1700013H16Rik	25.020	0.848	0.000	0.000	Gg > Ag
Mir92-1	615.692	0.000	0.000	0.000	Gg > Ag
Mir196a-1	0.000	650.691	0.000	0.000	Gg > Ag
H2-Eb1	76.656	100.239	10.315	0.057	Gg > Ag
Mirg	11.650	10.840	0.000	0.000	Gg > Ag
Nefm	8.634	17.000	0.025	0.000	Gg > Ag
Snord96a	3236.380	3390.190	0.000	7267.120	Gg > Ag
Mir1901	0.000	2405.610	0.000	0.000	Gg > Ag
Mir704	3236.380	0.000	0.000	0.000	Gg > Ag
Snora28	29.947	31.370	0.000	0.000	Gg > Ag
Rian	39.874	27.482	0.022	0.000	Gg > Ag
Gm21283	61.777	86.000	0.000	0.456	Gg > Ag
Meg3	69.592	83.503	0.000	0.000	Gg > Ag
Mir8116	131.085	137.315	0.000	0.000	Gg > Ag
Mir1902	0.000	0.000	643.022	2702.910	Ag > Gg
Gm23450	0.000	0.000	143.058	835.330	Ag > Gg
Snord83b	3236.380	0.000	9835.050	7267.120	Ag > Gg
Mir130c	854.011	0.000	3460.340	958.818	Ag > Gg
Mir678	0.000	0.000	1523.570	0.000	Ag > Gg
Snora33	72.170	0.000	146.212	648.215	Ag > Gg
Snord35b	435.749	0.000	441.400	978.451	Ag > Gg
Mir290a	0.000	0.000	0.000	793.778	Ag > Gg
Mir25	0.000	0.000	0.000	667.262	Ag > Gg
Mir92-2	0.000	0.000	472.214	0.000	Ag > Gg
Mir19b-1	0.000	0.000	0.000	425.282	Ag > Gg
Tmsb15b1	0.743	0.000	19.787	15.163	Ag > Gg
Mir93	0.000	0.000	0.000	372.806	Ag > Gg
Nod1	0.194	0.081	9.446	6.930	Ag > Gg
Mir29c	0.000	0.000	333.328	0.000	Ag > Gg
Mest	14.412	0.072	38.260	76.373	Ag > Gg
Scgb1b3	0.000	0.000	1.795	6.766	Ag > Gg
Snord7	0.000	0.000	115.317	0.000	Ag > Gg
Mir6363	0.000	0.000	85.884	0.000	Ag > Gg
Mir6385	0.000	0.000	0.000	81.027	Ag > Gg
Gm7120	0.373	0.222	7.706	8.185	Ag > Gg
Mir3062	0.000	0.000	73.106	0.000	Ag > Gg
Mir671	0.000	0.000	0.000	66.502	Ag > Gg
Sh3rf2	0.000	0.087	0.612	10.788	Ag > Gg
Mir1966	0.000	0.000	60.431	0.000	Ag > Gg

Snora52	52.217	0.000	52.895	58.626	Ag > Gg
Nkx6-3	0.774	0.193	4.742	12.458	Ag > Gg
Trim12a	0.000	0.000	2.358	1.593	Ag > Gg
Pigp	26.722	44.883	649.174	538.974	Ag > Gg
C330004P14Rik	0.178	0.187	1.623	5.397	Ag > Gg
Gm2083	0.000	0.000	0.750	3.323	Ag > Gg
Mthfd2l	0.918	0.038	6.915	3.132	Ag > Gg
Mir1892	39.924	339.227	2583.230	1075.540	Ag > Gg
Peg13	0.000	0.000	1.425	1.412	Ag > Gg
Egr1	2.140	0.574	13.750	14.389	Ag > Gg
Myo7a	0.321	0.022	2.221	2.162	Ag > Gg
Slc6a14	0.000	0.000	1.879	0.573	Ag > Gg
Slc15a2	0.000	3.814	3.130	10.627	Ag > Gg
Peg10	8.323	1.746	36.292	34.558	Ag > Gg
Snx24	0.849	0.187	2.895	4.112	Ag > Gg
Snora17	56.370	0.000	19.034	21.096	Ag > Gg
Neurod1	0.850	0.034	3.412	1.726	Ag > Gg
Foxi3	0.075	0.000	0.683	1.009	Ag > Gg
4933402E13Rik	0.347	0.136	2.461	1.315	Ag > Gg
Nespas	1.283	0.192	3.564	4.567	Ag > Gg
Snhg14	3.314	10.889	41.394	56.450	Ag > Gg
Peg3os	3.073	5.126	32.971	30.666	Ag > Gg
Tex21	1.223	0.000	1.927	4.013	Ag > Gg
Slc2a6	0.170	0.000	0.816	1.286	Ag > Gg
Sgce	1.558	0.684	4.835	12.548	Ag > Gg
Myl2	0.000	0.000	1.426	0.395	Ag > Gg
Hist1h4f	0.000	0.000	0.503	1.116	Ag > Gg
Trim34a	0.536	0.396	3.129	3.751	Ag > Gg
Bcl6b	0.418	0.146	1.200	2.713	Ag > Gg
Peg3	1.550	3.137	15.851	15.868	Ag > Gg
Rgs11	0.291	0.131	1.516	1.260	Ag > Gg
6720483E21Rik	0.164	0.000	0.581	1.381	Ag > Gg
Sepp1	1.077	0.390	3.902	5.162	Ag > Gg
Gc	7.224	0.098	5.335	6.227	Ag > Gg
Smarca1	0.686	0.205	2.859	2.214	Ag > Gg
Kcnj3	0.000	0.082	1.201	0.369	Ag > Gg
Mgst1	0.203	0.320	1.752	1.599	Ag > Gg
Fam109b	0.177	0.093	1.045	0.728	Ag > Gg
Fgl1	1.148	0.086	1.910	2.577	Ag > Gg
Impact	29.078	3.036	83.432	43.971	Ag > Gg
Fstl1	19.585	2.487	40.213	49.827	Ag > Gg
Tcf24	0.169	0.310	1.498	1.423	Ag > Gg
Cga	0.000	0.162	0.470	1.391	Ag > Gg
Spta1	0.912	0.172	2.688	2.263	Ag > Gg
Plagl1	1.391	10.941	21.775	26.145	Ag > Gg
Tbx19	0.000	0.096	0.031	3.591	Ag > Gg
Pla2g7	0.613	0.092	1.819	1.180	Ag > Gg
Gm5741	0.000	0.000	0.000	3.451	Ag > Gg
Ttll7	0.036	0.022	0.343	1.005	Ag > Gg
Gm10451	2.620	0.457	6.634	6.005	Ag > Gg
Gab1	31.890	1.919	40.852	47.897	Ag > Gg
1700065J11Rik	0.340	0.000	1.378	0.763	Ag > Gg
Zfp786	0.114	0.179	0.578	1.089	Ag > Gg
Lpar4	0.427	0.117	2.218	0.667	Ag > Gg
Zfp521	0.050	0.040	0.140	2.076	Ag > Gg
Rab38	0.437	0.343	1.106	3.924	Ag > Gg
Smr3a	1.479	0.172	3.995	1.845	Ag > Gg
Esrp1	3.001	0.250	2.859	7.589	Ag > Gg
Unc45b	0.246	0.141	1.019	0.979	Ag > Gg
Aqp11	1.074	0.281	2.855	3.014	Ag > Gg

Snrpn	13.501	60.775	134.002	170.917	Ag > Gg
Nckap1l	2.122	0.000	1.159	5.082	Ag > Gg
Hist1h2bj	0.000	1.418	1.714	2.280	Ag > Gg
Pcsk9	0.634	0.356	4.034	1.524	Ag > Gg
Napsa	1.059	1.787	4.231	12.152	Ag > Gg
Adamts9	0.616	0.537	2.352	3.802	Ag > Gg
Gprc5a	2.328	8.449	16.057	33.025	Ag > Gg
4930470H14Rik	2.744	22.940	47.891	35.203	Ag > Gg
Gm8580	1.113	36.932	37.217	29.375	Ag > Gg
Cadps2	0.117	0.085	0.226	1.356	Ag > Gg
Frem2	0.191	0.303	1.210	1.251	Ag > Gg
4930414N06Rik	0.558	0.195	1.131	2.506	Ag > Gg
Mir1291	36.316	38.039	220.709	163.090	Ag > Gg
Msc	0.396	0.311	3.814	0.834	Ag > Gg
C030039L03Rik	0.341	0.554	2.247	2.126	Ag > Gg
Gm15816	0.301	0.158	0.814	1.466	Ag > Gg

ACCOMPLISHMENTS

Publication

R. Konishi, T. Nakano, S. Yamaguchi. Distinct requirements for the maintenance and establishment of mouse embryonic stem cells, Stem Cell Research, 31, pp.55-61, 2018.

Presentation

2017 18th International Congress of Developmental Biology (#PS5.74)

2017 11th Young Researcher's Retreat, Poster

2016 39th Annual Meeting of the Molecular Biology Society of Japan (#2P-0486)

2016 10th Annual Meeting of the Japanese Society for Epigenetics (#P1-7)

2016 10th Young Researcher's Retreat, Poster

2015 9th Young Researcher's Retreat, Poster