Highly efficient induction of non-human primate iPS cells by combining RNA transfection and chemical compounds

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Figure S1. Characteristics of iPS cells generated by RNA transfection

A. Proportion of mRNA_iPS cells exhibiting normal karyotype. A representative karyogram of the mRNA_iPS cells is shown.
B. Immunofluorescence analyses of ES cell marker genes. Images of immunofluorescence and DAPI staining are shown.
C. qPCR analyses of differentiation marker genes in EBs and iPS cells. Cq values are shown. N.D. = Not detected

Figure S2. iPS cells induced in the presence of chemical compounds exhibit the pluripotent ability.
qPCR analyses of ES cell marker genes (left) and differentiation marker genes (right). The expression levels in iPS cells and EBs are shown. The number of passages of iPS cells examined are shown in parentheses. Error bars represent S.E. (N = 3).

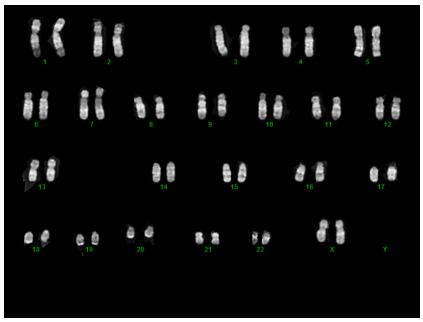
Figure S3. iPS cells induced from six cell lines
Conditions used for induction and the number of passages are indicated on the left of the pictures.

Figure S4. qPCR analysis of ES cell marker genes. Two or more iPS cell lines were examined for each cell line. The results of the original cells and mRNA_iPS cells are also shown. The number of passages of iPS cells examined are shown in parentheses. For fetus skin-derived cells, subcloning was performed at P6. Error bars represent S.E. (N = 3).

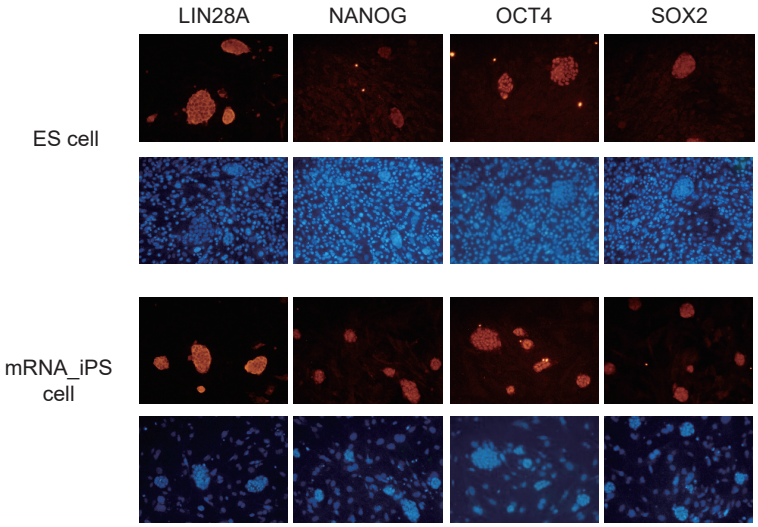
Figure S1

A

| Cell line | Proportion of 46, XX |
|---|---|
| mRNA iPS cell | 7/7 |



B




C

| | Cq value | | | |
|---|---|---|---|---|
| | GAPDH | HNF1a | PDX1 | AFP |
| mRNA_iPS rep1 | 22.4 | N.D. | N.D. | N.D. |
| mRNA_iPS rep2 | 22.1 | 37.5 | N.D. | N.D. |
| mRNA_iPS rep3 | 22.6 | N.D. | N.D. | N.D. |
| EB rep1 | 24.0 | 35.4 | 34.3 | 36.3 |
| EB rep2 | 23.5 | 33.4 | 36.1 | 30.8 |
| EB rep3 | 23.4 | 32.5 | 34.9 | 30.4 |

Figure S2

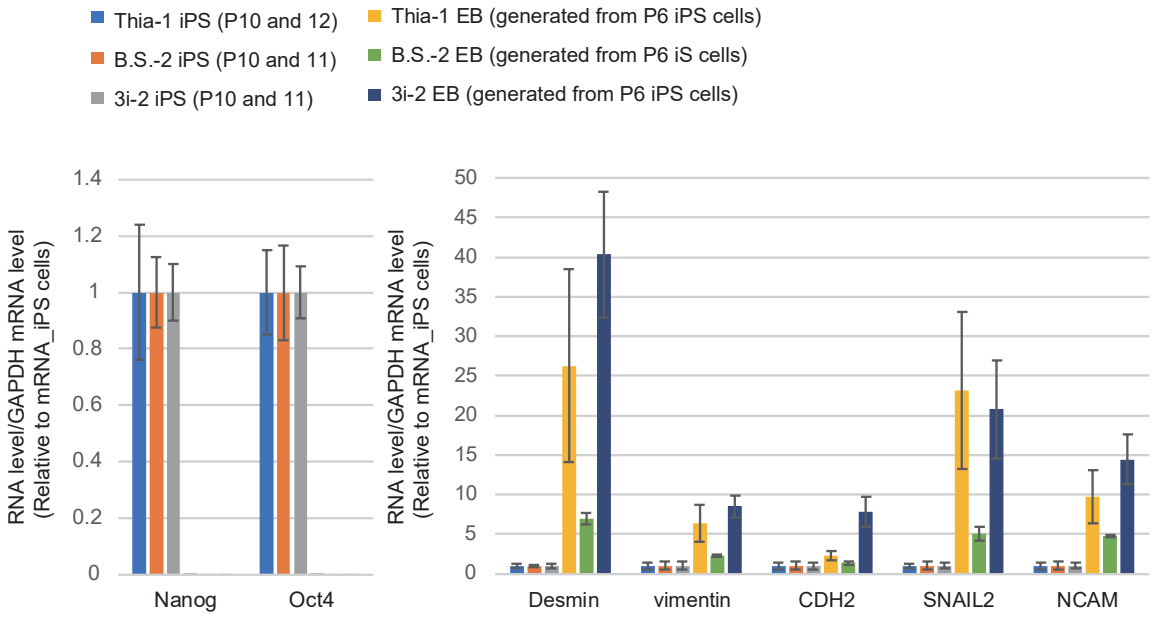
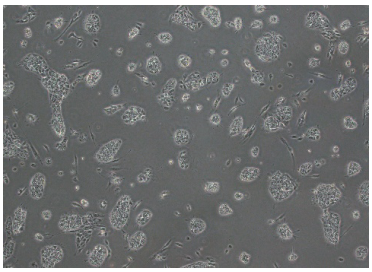

Thia-1 iPS (P10 and 12)
B.S.-2 iPS (P10 and 11)
3i-2 iPS (P10 and 11)
Thia-1 EB (generated from P6 iPS cells)
B.S.-2 EB (generated from P6 iS cells)
3i-2 EB (generated from P6 iPS cells)
RNA level/GAPDH mRNA level (Relative to mRNA_iPS cells)
1.4
1.2
1
0.8
0.6
0.4
0.2
0
Nanog
Oct4
RNA level/GAPDH mRNA level (Relative to mRNA_iPS cells)
50
45
40
35
30
25
20
15
10
5
0
Desmin
vimentin
CDH2
SNAIL2
NCAM

Figure S3

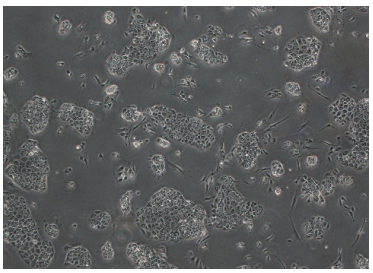
1. Fetus skin cell

iPS RNA (-P53DD) 1
Passage 12



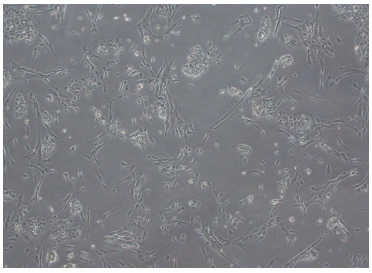
2. Adult ear cell #1

iPS Thiazovivin
Passage 6



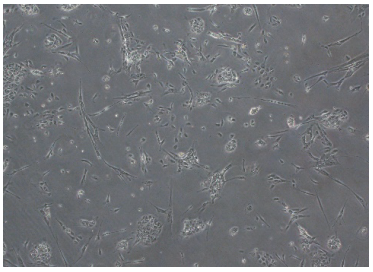
3. Adult ear cell #2

iPS Boost Supplement 1
Passage 8



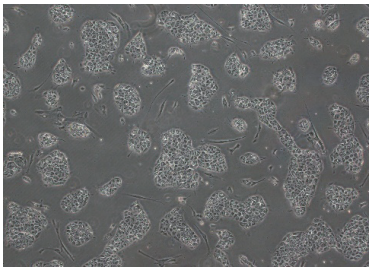
3. Adult ear cell #2

iPS Boost Supplement 2
Passage 7



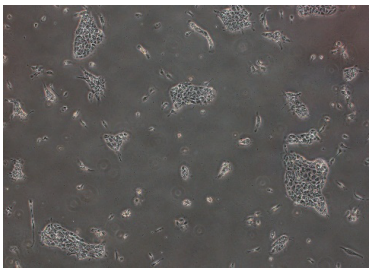
4. Adult ear cell #3

iPS Thiazovivin 1
Passage 5



5. Newborn skin cell

iPS Boost Supplement 1
Passage 6



6. Newborn liver cell

iPS Thiazovivin 1
Passage 8

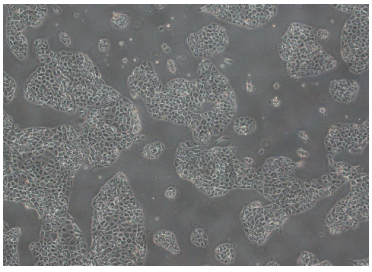
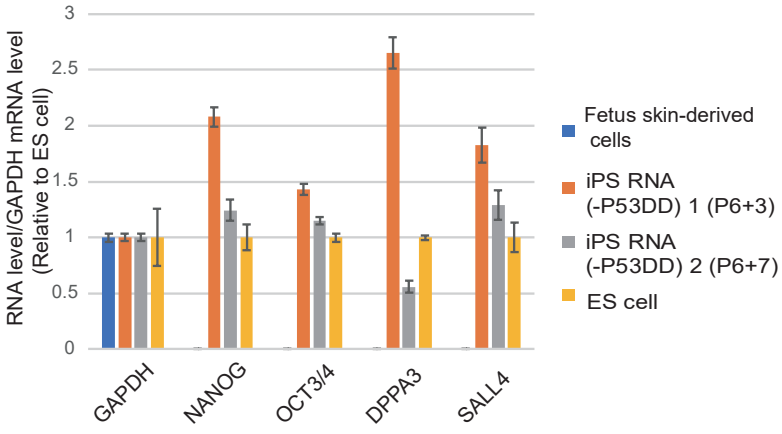
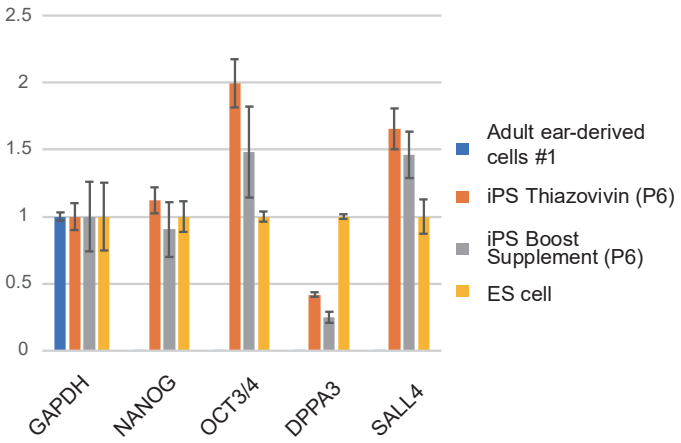


Figure S4

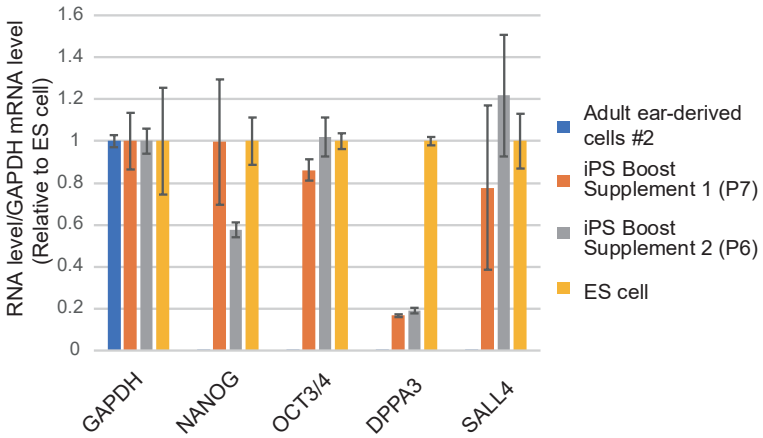
1. Fetus skin-derived cell



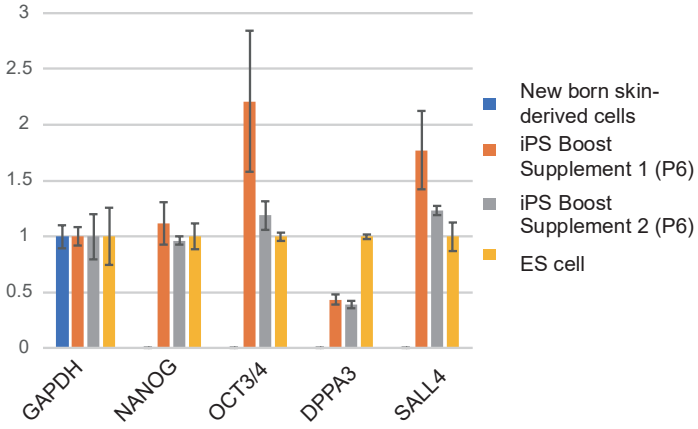

2. Adult ear-derived cell #1 (5-year)



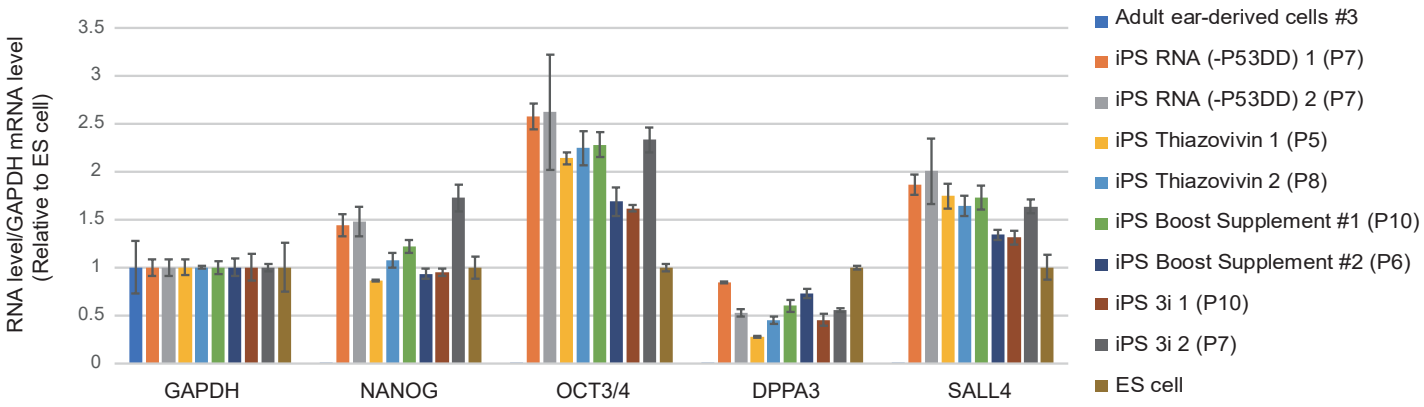

3. Adult ear-derived cell #2 (8-year)




5. Newborn skin-derived cell




4. Adult ear-derived cell #3 (6-year)




6. Newborn liver cell

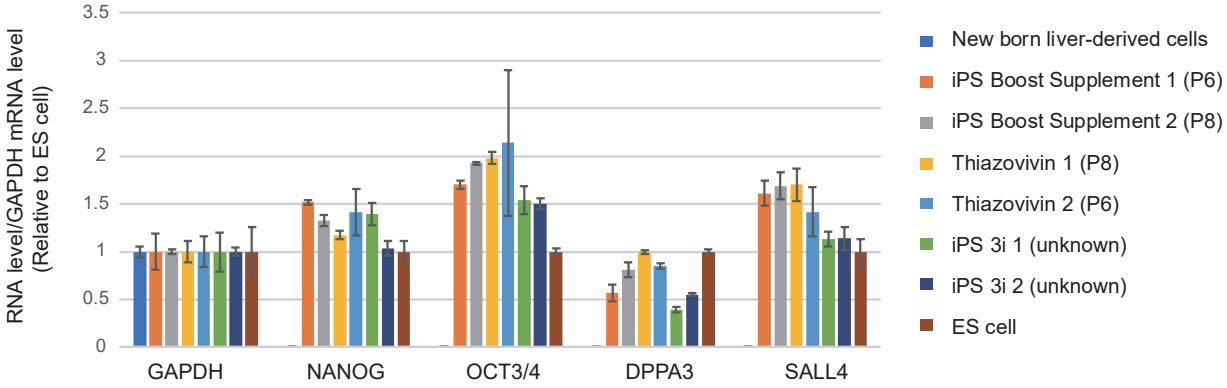


Table S1. Cq values of qPCR analyses in Figure 1B

| Gene name | Cq Values | | |
|---|---|---|---|
| | No chemical (-P53DD) | Boost supplement (+P53DD) | Thiazovivin (+P53DD) |
| GAPDH | 23.8 | 23.6 | 24.7 |
| NANOG | 25.2 | 25.5 | 37.6 |
| LIN28 | 27.8 | 28.6 | 34.6 |
| SALL4 | 27.5 | 27.5 | 37.4 |
| OCT4 | 24.3 | 24.5 | 33.1 |
| SOX2 | 28.2 | 28.9 | 37.1 |
| UTF1 | 28.7 | 28.5 | 38 |
| DPPA3 | 28.4 | 28.1 | 37.6 |
| GDF3 | 25.7 | 24.8 | 37.3 |

Table S2. Passage No. of iPS cells retaining undifferentiated state

| iPS cell | Passage No. |
|---|---|
| **iPS cell induced using RNAs (Figure 1)** | |
| mRNA_iPS cells | 27 |
| **iPS cell induced in the presence of chemichal compounds (Figure 2)** | |
| Thiazovivin-1 | 15 |
| Thiazovivin-2 | 15 |
| Boost Supplement-1 | 24 |
| Boost Supplement-2 | 9 |
| 3i-1 | 16 |
| 3i-2 | 10 |
| **iPS cell from six cell lines (Table S1)** | |
| *Fetus skin-derived cell (-P53DD) 1 | 6+4 |
| *Fetus skin-derived cell (-P53DD) 2 | 6+7 |
| Adult ear-derived cell #1 (Thiazovivin) | 6 |
| Adult ear-derived cell #1 (Boost Supplement) | 6 |
| Adult ear-derived cell #2 (Boost Supplement) 1 | 8 |
| Adult ear-derived cell #2 (Boost Supplement) 2 | 7 |
| Adult ear-derived cell #3 (-P53DD) 1 | 7 |
| Adult ear-derived cell #3 (-P53DD) 2 | 7 |
| Adult ear-derived cell #3 (Thiazovivn) 1 | 5 |
| Adult ear-derived cell #3 (Thiazovivn) 2 | 8 |
| Adult ear-derived cell #3 (Boost Supplement) 1 | 10 |
| Adult ear-derived cell #3 (Boost Supplement) 2 | 7 |
| Adult ear-derived cell #3 (3i) 1 | 8 |
| Adult ear-derived cell #3 (3i) 2 | 5 |
| New born skin-derived cell (Boost Supplement) 1 | 6 |
| New born skin-derived cell (Boost Supplement) 2 | 6 |
| New born liver-derived cell (Boost Supplement) 1 | 6 |
| New born liver-derived cell (Boost Supplement) 2 | 8 |
| New born liver-derived cell (Thiazovivin) 1 | 8 |
| New born liver-derived cell (Thiazovivin) 2 | 6 |
| New born liver-derived cell (3i) 1 | Unknown |
| New born liver-derived cell (3i) 2 | Unknown |

* Subcloning was performed at passage 6

Table S3. iPS cell stability depends on the original cells

| Cell line (chemical condition) | No of undifferentiated lines*/ No. of total lines |
|---|---|
| Fetus skin-derived cell (-P53DD) | 5/11 |
| Adult ear-derived cell #1 (Thiazovivin) | 1/8 |
| Adult ear-derived cell #1 (Boost Supplement) | 1/10 |
| Adult ear-derived cell #2 (Boost Supplement) | 6/42 |
| Adult ear-derived cell #3 (-P53DD) | 4/4 |
| Adult ear-derived cell #3 (Thiazovivn) | 6/10 |
| Adult ear-derived cell #3 (Boost Supplement) | 2/9 |
| Adult ear-derived cell #3 (3i) | 2/4 |
| New born skin-derived cell (Boost Supplement) | 7/10 |
| New born liver-derived cell (Boost Supplement) | 2/6 |
| New born liver-derived cell (Thiazovivin) | 4/6 |
| New born liver-derived cell (3i) | 6/9 |

*No. of cell lines that retain undifferentiated state when passaging for P4 culture

Table S4. Primers used in this study

| | |
|---|---|
| **Primers to cunstruct a plasmid for transcription of P53DD** | |
| Multiple cloning site F | GGCCGCATCGGATCCGTAGGCGCGCCAACGTCGACATAAAGCTTCACG |
| Multiple cloning site R | AATTCGTGAAGCTTTATGTCGACGTTGGCGCGCCTACGGATCCGATGC |
| bglobinUTR F Asc1 | GGTAG GGCGCGCCAGCTCGCTTTCTTGCTGTCCAATTTCT |
| bglobinUTR F Sal1 | CAGGTGTCGACAGCTCGCTTTCTTGCTGTCCAATTTCT |
| bglobinUTR R Sal1 | GCATAGTCGACGCAGCAATGAAAATAAATGTTTTTTATTAGGCA |
| bglobinUTR R HindIII | GTGAAAAGCTTGCAGCAATGAAAATAAATGTTTTTTATTAGGCA |
| 5UTR HBA Not1 | GATCGTAC GCGGCCGCTCTTCTGGTCCCCACAGACTCAGAGAGAACCCAC |
| P53 F | CTCAGAGAGAACCCACCACC ATGACTGCCATGGAGGAGTC |
| P53 R ASC1 | TTAACGGCGCGCC GTGTCTCAGCCCTGAAGTCATAA |
| **Primers for qPCR** | |
| CDH2 F | TGGAGCCTGATGCCATCAAG |
| CDH2 R | TGGAGCCACTGCCTTCATAG |
| SNAIL2 F | CTGAAGATGCACATTCGGAC |
| SNAIL2 R | GATTCCTCATGTTTGTGCAG |
| GATA4 F | GGTCACTATCTGTGCAACGC |
| GATA4 R | CGTGGAGCTTCATGTAGAGG |
| CXCR4 F | GAGGGCATCAGTATATACAC |
| CXCR4 R | GTGTAGATGACATGGACTGC |
| PDX1 F | GGATGAAGTCTACCAAAGCTCACTC |
| PDX1 R | TCCTTCTCCAGCTCCAGCAG |
| HNF1a F | CTCATCATGGCCTCACTTCCTG |
| HNF1a R | GATGACTGGCACACTCTGTG |
| AFP F | GCCAACTCAGTGAGGACGAA |
| AFP R | GGCCAACACCAGGGTTTACT |
| NANOG F | TGCTGAGATGCCTCACACAG |
| NANOG R | TCACTGCAGGGCTACTCTCT |
| VIMENTIN F | TCCCTGAACCTGAGGGAAACT |
| VIMENTIN R | CGTCTTAATCAGAAGTGTCCTTTTTG |
| DESMIN F | AGCTGCAGGAGCTCAACGAC |
| DESMIN R | TTGGTGAGCACCTCCACCTG |
| GAPDH F | TGCTGGCGCTGAGTATGTG |
| GAPDH R | AGCCCCAGCCTTCTCCAT |
| TUBB3 F | GGATCAGCGTCTACTACAATG |
| TUBB3 R | CTCCGTGTAGTGGCCCTTG |
| ACTA2 F | CGTGAGAAGATGACGCAGATCA |
| ACTA2 R | CAGCCTGGATGGCCACAT |
| NCAM F | ACCTGATCAAGCAGGATGA |