Figure S1

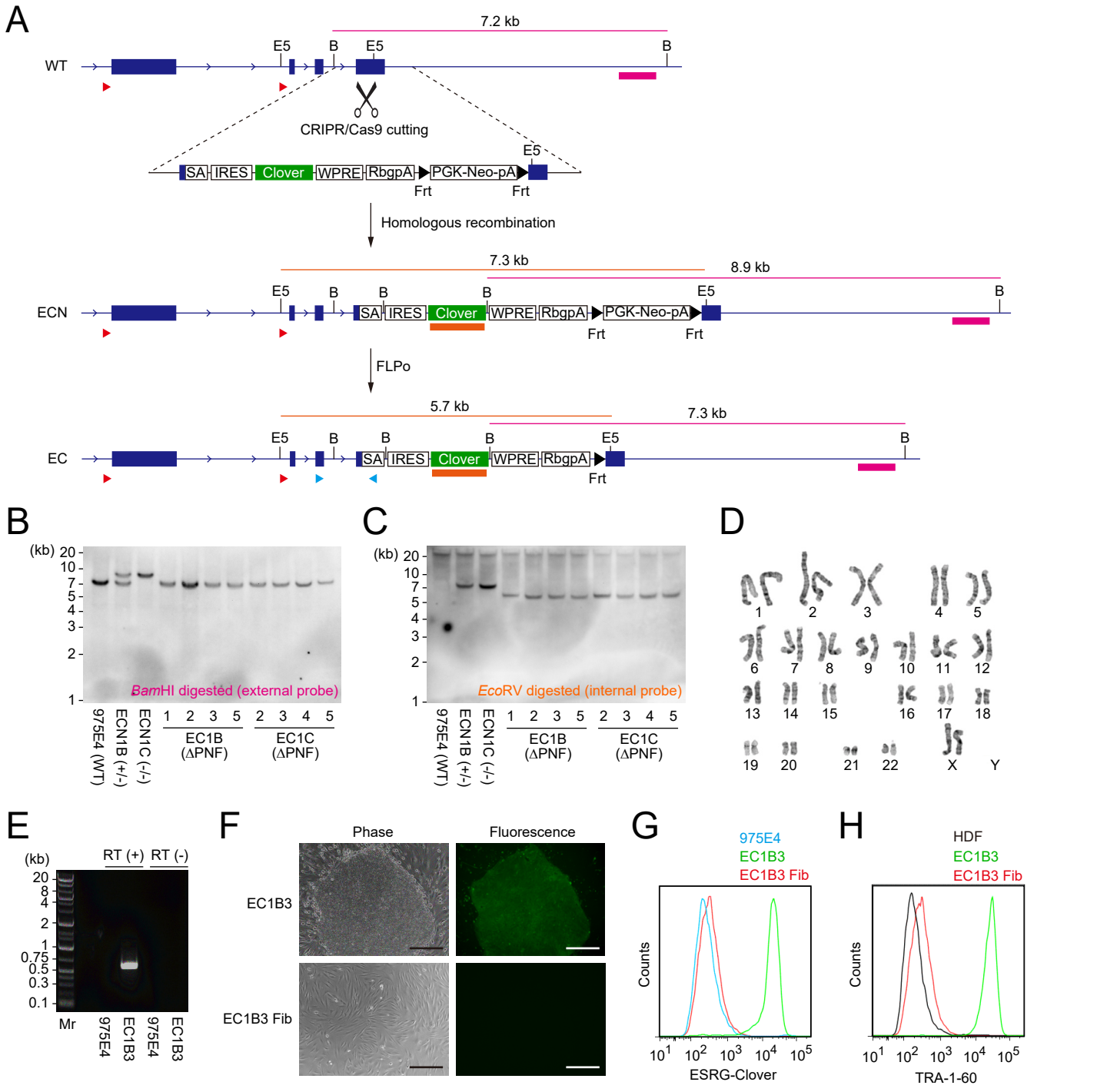

Figure S1: Generation of ESRG reporter iPSC line (related to Figure 1)
A, Schematic representation of ESRG-Clover reporter generation. The targeting vector containing a Clover reporter gene and an Frt-flanked PGK-Neo-pA (PNF) cassette was designed for knocking into the 4th exon of ESRG. SA, splicing acceptor; IRES, internal ribosome entry site; WPRE, woodchuck hepatitis virus post-transcriptional regulatory element; RbgpA, rabbit beta-globin poly A signal. Red arrowheads indicate the LTR7 position and direction. BamHI (B) and EcoRV (E5) were used to digest genomic DNA for Southern blotting. Magenta and orange boxes depict external and internal probes for Southern blotting, respectively. Light blue arrowheads indicate primers for RT-PCR used in Figure S1D. Expected band sizes in each case by Southern blotting are shown. B, Southern blotting with an external probe. We digested genomic DNA of wild-type iPSCs (975E4) and homologous recombinants (ECN1B & C) and their derivative clones after the FLPo-mediated excision of PNF cassettes (EC1B & EC1C series) with BamHI, and confirmed accurate targeting. C, Southern blotting with an internal probe. We digested genomic DNA of wild-type iPSCs (975E4) and homologous recombinants with EcoRV, and confirmed no random integration of targeting vector. D, Karyotype. Representative result of G-band staining revealing normal karyotype (46XX). E, Accuracy of reporter expression. Confirmation of ESRG-Clover chimeric transcripts by RT-PCR with the primers designed on exon3 (ESRG-Ex3-S) and inserted cassette (SA-RT-AS) shown as light blue arrowheads in Figure S1A. Expected band size was 600 bp. Mr, size marker. F, ESRG expression in iPSCs but not fibroblasts. Microscopic images of undifferentiated EC1B3 iPSCs (upper) and EC1B3-derived fibroblasts (lower) under the phase contrast (left) and fluorescence (right). Bars indicate 200 μm. The fluorescence of Clover. Shown are representative phase contrast (left) and fluorescent (right) images of EC1B3 iPSCs (upper) and EC1B3-derived fibroblasts (lower). Bars indicate 100 μm. G, No ESRG-Clover expression after fibroblast differentiation. The expression of ESRG-Clover in 975E4 parental iPSCs (blue), EC1B3 iPSCs (green) and EC1B3-derived fibroblasts (red) analyzed by flow cytometry. H, No TRA-1-60 expression after fibroblast differentiation. Expression of TRA-1-60 in HDFs (black), EC1B3 iPSCs (green) and EC1B3-derived fibroblasts (red) analyzed by flow cytometry.

Figure S2

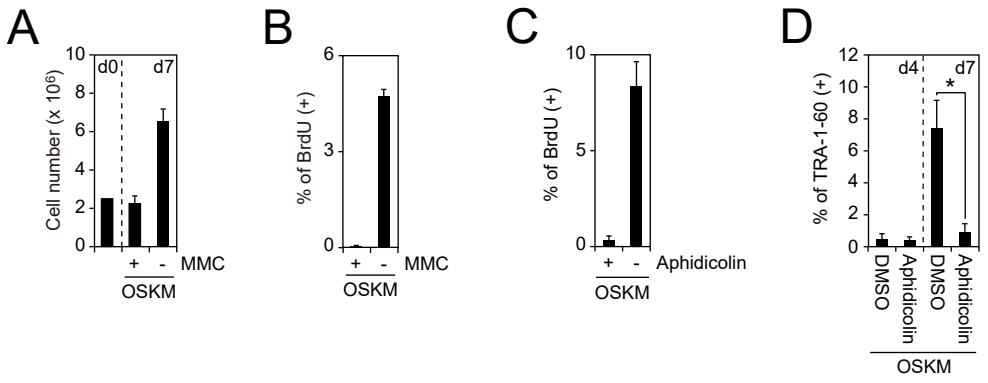

Figure S2: Effects of cell-cycle inhibitors (related to Figure 2).
A, No proliferation after MMC treatment. Cell numbers of OSKM-transduced HDFs with (+) or without (-) MMC treatment on day 7. n=3. B, No proliferation after MMC treatment. B, BrdU incorporation of OSKM-transduced HDFs with (+) or without (-) MMC treatment on day 7. n=3. C, Less proliferation after aphidicolin treatment. BrdU incorporation of OSKM transduced HDFs with (+) or without (-) Aphidicolin treatment on day 7. n=3. D, Aphidicolin decreases reprogramming efficiency. The graph shows the percentages of TRA-1-60 (+) cells on days 4 (d4) and 7 (d7) derived from OSKM-transduced HDFs treated with or without aphidicolin. *p<0.05 by unpaired t-test (n=3).

Figure S3

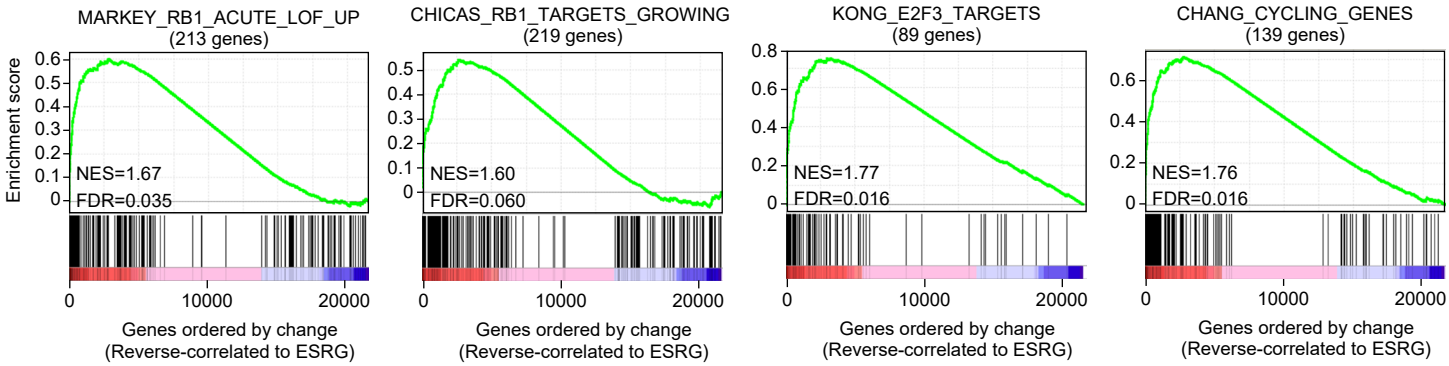

Figure S3: Bioinformatics of gene set showing reverse-correlation to ESRG (related to Figure 3).
GSEA Enrichment plots for selected gene sets from the “RB/E2F pathways” network and the “cell-cycle pathways” network obtained from ESRG (+) cells (n=14) and ESRG (-) cells (n=10) on day 3 post-transduction of OSKM. The NES (Normalized Enrichment Score) and the FDR are presented. See also Table S4.

Figure S4

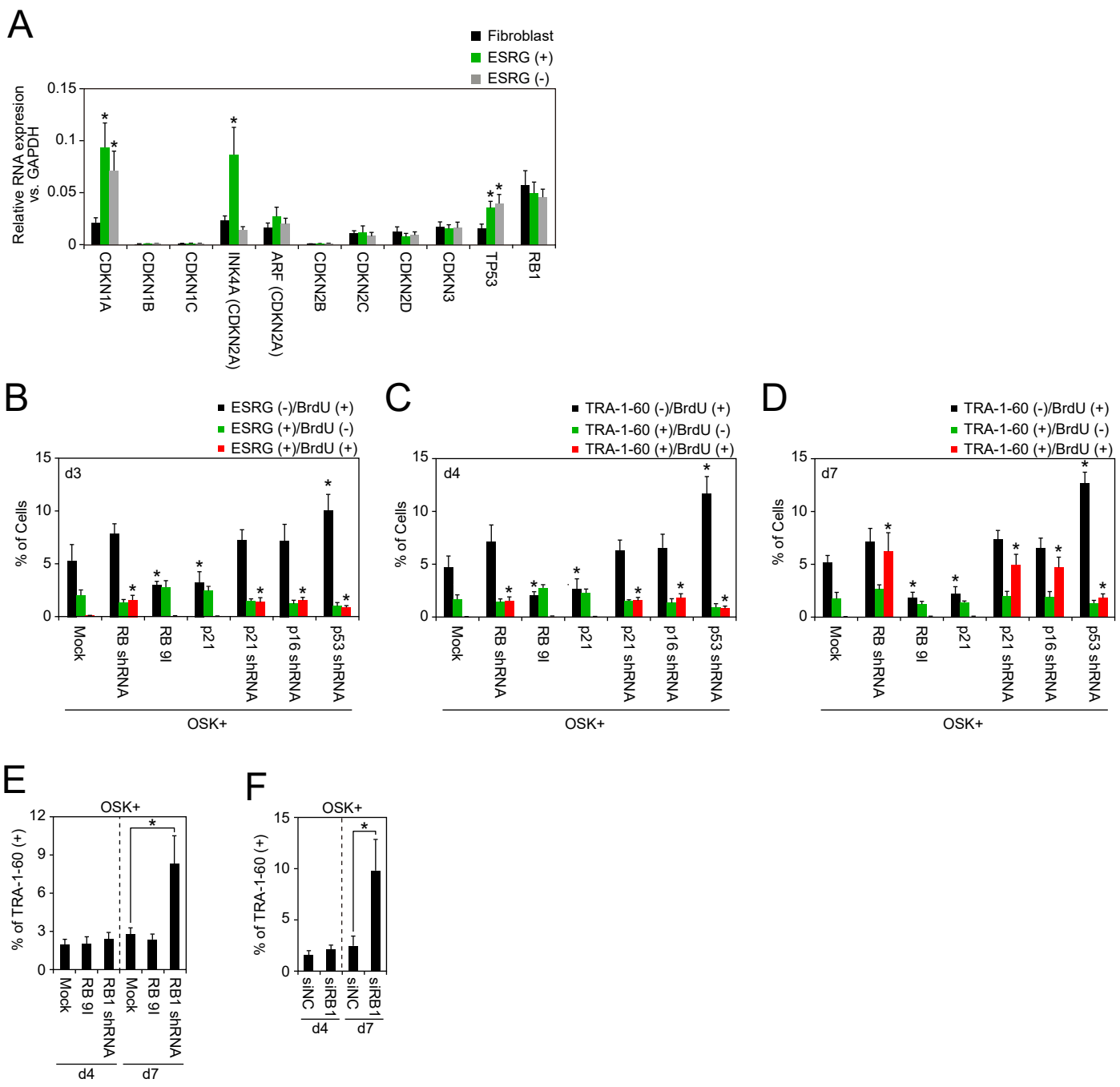

Figure S4: The roles of cell cycle-related genes in OSK-induced reprogramming (related to Figure 3).
A, Expression of CDK inhibitors in early stage of reprogramming. Shown are relative expressions of genes encoding CDK inhibitor proteins in ESRG-Clover fibroblast, ESRG (+) or (-) cells on day 3 post-transduction of OSK analyzed by qRT-PCR. *p<0.05 vs. Fibroblast by unpaired t-test (n=3). B, RB pathway affects proliferation of ESRG (+) cells. Shown are the percentages of ESRG (-)/BrdU (+) cells (black), ESRG (+)/BrdU (-) cells (green) and ESRG (+)/BrdU (+) cells (red) on day 3 post-transduction of OSK along with each indicated factor to ESRG-Clover fibroblasts. *p<0.05 Mock by unpaired t-test (n=3). C, RB pathway affects proliferation of new TRA-1-60 (+) cells. Shown are the percentages of TRA-1-60 (-)/BrdU (+) cells (black), TRA-1-60 (+)/BrdU (-) cells (green) and TRA-1-60 (+)/BrdU (+) cells (red) on day 4 post-transduction of OSK along with each indicated factor to HDFs. *p<0.05 vs.Mock by unpaired t-test (n=3). D, RB pathway affects proliferation of TRA-1-60 (+) cells. Shown are the percentages of TRA-1-60 (-)/ BrdU (+) cells (black), TRA-1-60 (+)/BrdU (-) cells (green) and TRA-1-60 (+)/BrdU (+) cells (red) on day 7 post-transduction of OSK along with each indicated factor to HDFs.*p<0.05 vs. Mock by unpaired t-test (n=3). E, Effects of RB on early stage of reprogramming. The graph shows the relative proportion of TRA-1-60 (+) cells on 7 days after transduction of OSK along with empty vector (Mock), RB 9I or RB1 shRNA to HDFs. RB inactivation enhances the proportion of TRA-1-60 (+) cells. *p<0.05 vs. Mock by Dunnett's test (n=3). F, Inactivation of RB facilitates expansion of early TRA-1-60 (+) cells. Shown are the effects of RB1 knockdown by siRNA transfection on OSK-induced TRA-1-60 (+) cell proportion on days 4 (d4) and 7 (d7). *p<0.05 by unpaired t-test (n=3).

Figure S5

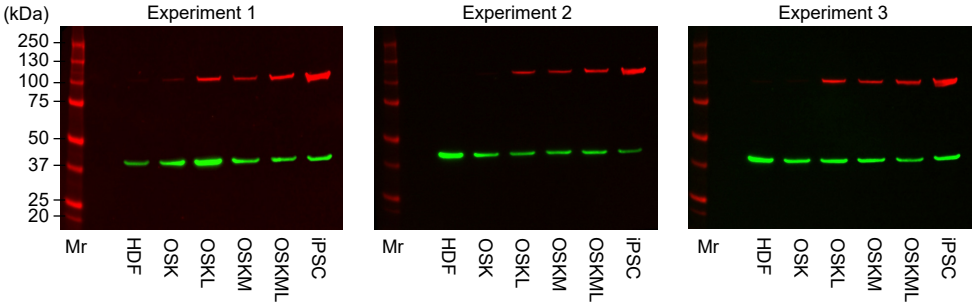

Figure S5: LIN41 increases the phosphorylation status of RB (related to Figure 4J)
Western blot images show the expression levels of phosphorylated RB (red) and β-actin (green) in HDFs, HDFs expressing OSK, OSKL, OSKM or OSKML on day 4 post-transduction, and iPSCs. Mr, size marker. n=3.

Figure S6

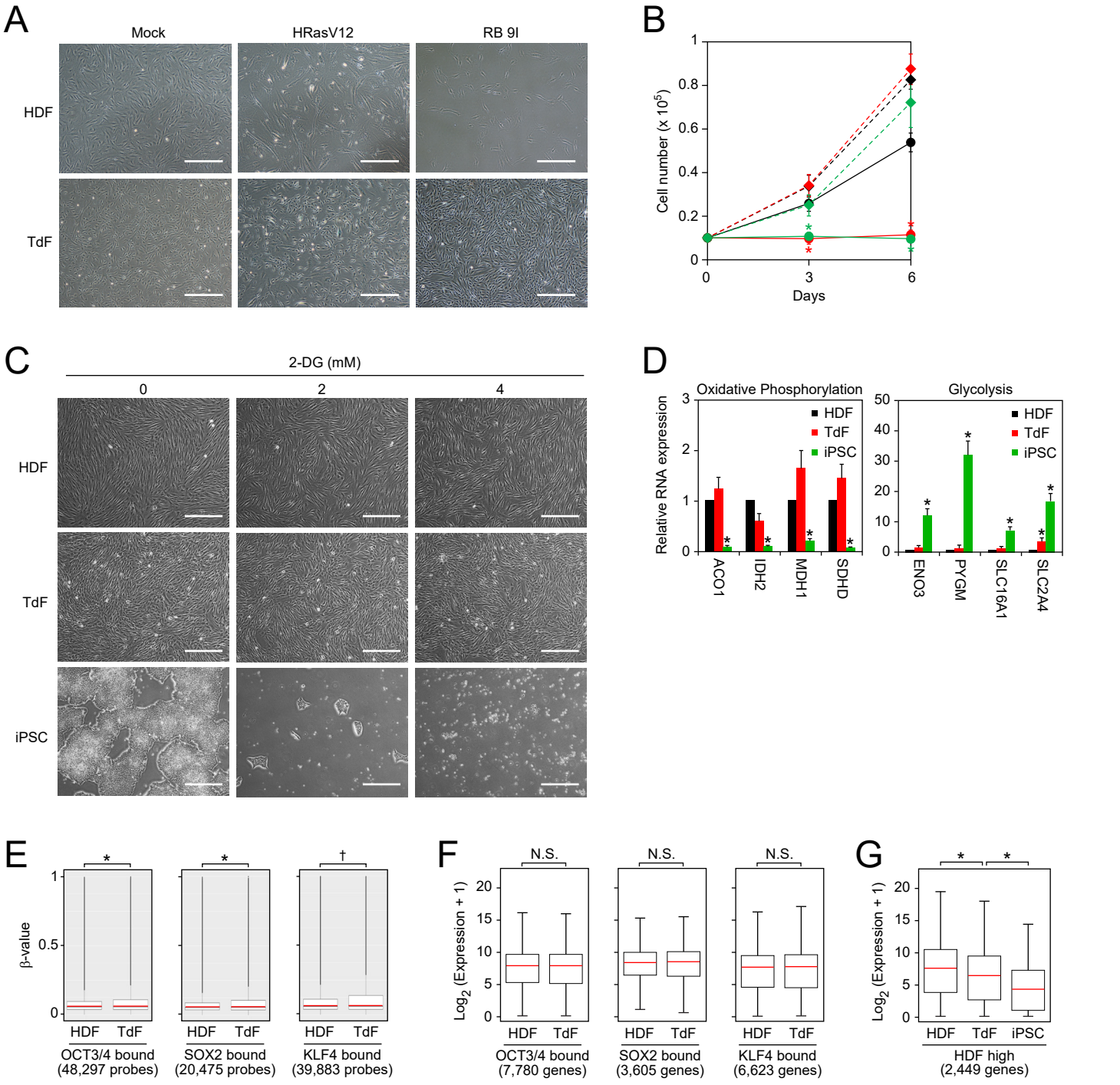

Figure S6: Characteristics of teratoma-derived fibroblasts (related to Figure 6).
A, Overexpression of oncogenes in HDFs and TdFs. Shown are representative images of Mock-, HRasV12- or RB9I-transfected HDFs (top) and TdFs (bottom) Bars indicate 200 μm. B, Proliferation of oncogene-overexpressed TdFs. The graph shows cell growth of HDFs (circles with normal lines) or TdFs (diamonds with broken lines) introduced with Mock (black), HRasV12 (red) or RB 9I (green). The cells were plated at 1 x 10^4 cells per well of 6-well plate, and cell numbers were counted on days 3 and 6. *p<0.05 (vs. Mock) by unpaired t-test (n=3). C, Survival with 2-DG treatment. Shown are representative images of HDFs, TdFs and iPSCs mainteined in the medium supplemented with 0, 2 or 4 mM of 2-deoxy-D-glucose (2-DG) for 6 days. Scale bar indicates 200 μm. D, The expression of metabolism-related genes. Shown are relative RNA expression of oxidative phosphorylation markers and glycolysis markers in HDF (black), TdF (red) and iPSC (green) analyzed by qRT-PCR. n=3. * p<0.05 vs. HDF by t-test. E, Distribution of CpG methylation on OSK-binding sites. The box plots show the distribution of methylation level at CpGs on OCT3/4 (left), SOX2 (center), and KLF4 (right) bound sites with overhang sequences (300 bp) in HDFs and TdFs by analyzing Infinium and ChIP-seq data. Red and black bars indicate the median and quartile, respectively. n=3. *p<2.2e-16, †p<8.7e-15 by the two-tailed nonparametric Mann-Whitney statistical significance test. F, Distribution of OSK target gene expression. The box plots show the distribution of gene expression with OCT3/4 (left), SOX2 (center), and KLF4 (right) binding around thier transcription start sites in HDFs and TdFs by analyzing RNA-seq and ChIP-seq data. Red and black bars indicate the median and quartile, respectively. n=3. N.S., not significant. G, Distribution of fibroblast-specific gene expression. The box plots show the distribution of fibroblast-specific gene (5-fold higher in HDFs than in iPSCs) expression in HDFs, TdFs and iPSCs by analyzing RNA-seq data. Red and black bars indicate the median and quartile, respectively. n=3. *p<0.0001 was culculated by the two-tailed nonparametric Mann-Whitney statistical significance test.