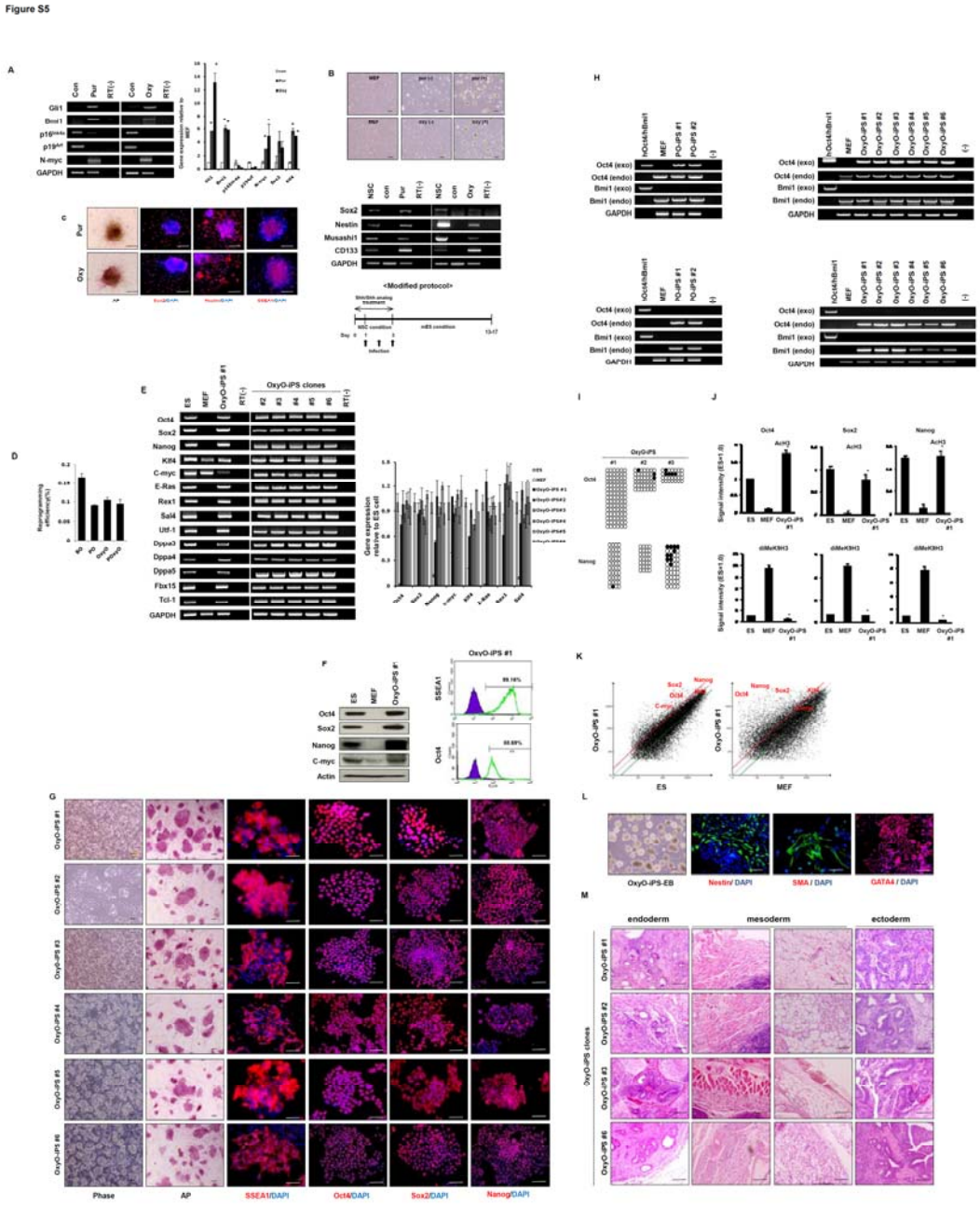

Supplementary information, Figure S5. Generation and characterization of PO-iPS cells and OxyO-iPS cells. **(A)** Transdifferentiation of MEFs into neural stem cell-like cells by purmorphamine or oxysterol. MEFs were cultured in DMEM containing 1 μM purmorphamine or 0.5 μM 25-hydroxycholesterol (oxysterol) for 3 days. mRNA levels of Gli1, Bmi1, $p16^{Ink4a}$, $p19^{Arf}$, and N-Myc were analyzed by RT-PCR (left panel) and real-time PCR (right panel). GAPDH was used as a control. Scale bars, 200 μm. **(B)** Phase contrast images of vehicle- (middle) and purmorphamine- or oxysterol-treated (right) MEFs cultured in proliferation medium (left) and in NSC medium (right). Purmorphamine- or oxysterol-treated MEFs cultured in NSC medium for 7 days rapidly changed morphology, resulting in bipolar cells (right) and expanded neurosphere-like cells (right). Scale bars, 200 μm. **(C)** Characterization of purmorphamine- or oxysterol-treated MEFs was performed by AP staining,

immunocytochemistry and RT-PCR for Sox2, Nestin, SSEA1 (left, panel), Musashi1 and CD133 (right, panel). Timeline for PO- and OxyO-iPS cell induction using purmorphamine or oxysterols treatment with retroviral transduction of Oct4 (right, panel). **(D)** The efficiency of BO-, PO-, OxyO-, and POxyO-iPS cells in reprogramming MEFs. Reprogramming efficiency was calculated by quantifying the percentage of Nanog positive colonies. **(E)** RT-PCR and qPCR analysis of ES cell marker genes in mES cells, MEFs, and OxyO-iPS clones. **(F)** Western blot (left) and FACS (right) analyses of OxyO-iPS-1 cells. Protein levels of Oct4, Sox2, Nanog, c-Myc, and SSEA1 were similar to those in mES cells. Antibody control, blue line; SSEA1 and Oct4, green line. **(G)** Phase contrast images showing ESC-like morphology of OxyO-iPS clones on feeder cells and characterization of OxyO-iPS clones. AP staining, as well as SSEA1, Oct4, Sox2 and Nanog immunoreactivity, was detected in OxyO-iPS clones. Scale bars, 200 μm. **(H)** Genomic DNA PCR and RT-PCR to detect expression and integration of exogenous Oct4 and Bmi1 genes (upper panels) and transcripts (lower panels), respectively, in MEFs, PO-iPS clones, and OxyO-iPS clones. hBmi1/Oct4 was used as positive controls using expression plasmid DNA (pBabe hBmi1 and hOct4) **(I)** Bisulfite genomic sequencing of Oct4 and Nanog promoters in mES cells, MEFs, and OxyO-iPS clones. Open and filled circles indicate unmethylated and methylated CpG dinucleotides, respectively. **(J)** ChIP analysis of Oct4 and Sox2 promoters for diMeK9H3 and AcH3 status in mES cells, MEFs, and OxyO-iPS-1 cells. $*P < 0.05$ compared to MEFs. **(K)** Scatter plots of the global gene expression comparing OxyO-iPS cells with either mES cells or MEFs as described previously. **(L)** *In vitro* differentiation of OxyO-iPS cells. Micrographs show EBs generated from OxyO-iPS cells (right). *In vitro* differentiation of OxyO-iPS cells into ectodermal, mesodermal, and endodermal cell types was revealed by immunoreactivity to the tissue-specific markers Nestin, SMA, and GATA4, respectively. Scale bars, 200 μm. **(M)** The *in vivo* developmental potential of OxyO-iPS clones. Teratomas of OxyO-iPS clones differentiated into epithelium (endoderm; left), muscle and fat (mesoderm; middle), and neural rosettes (ectoderm; right). Hematoxylin and eosin-stained sections of teratomas derived from OxyO-iPS cells in a nude mouse host after 8-10 weeks are shown. Scale bars, 200 μm.