



Supplementary information, Figure S6 Generation and characterization of 1F PO-iPS cells. (A) RT-PCR and qPCR analysis of ES cell marker genes in mES cells, MEFs, and PO-iPS-1 clones. *P < 0.05 compared to MEFs. (B) Western blot (left) and FACS (right) analyses of PO-iPS-1 clones. 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. (C) Phase contrast images show mES cells (upper left) and the ESC-like morphology of PO-iPS-1 clones (lower left) on feeder cells. AP staining, as well as SSEA1, Oct4, Sox2, and Nanog immunoreactivity, was detected in mES cells (upper) and PO-iPS-1 clones (lower). Scale bars, 200 um. (D) Bisulfite genomic sequencing of Oct4 and Nanog promoters in PO-iPS clones. (E) ChIP analysis of Oct4, Sox2, and Nanog promoters for diMeK9H3 and AcH3 status in mES cells, MEFs, and PO-iPS cells. *P < 0.05 compared to MEFs. (F) Scatter plots of the global gene expression patterns comparing PO-iPS-1 clones with either mES cells or MEFs as described previously. (G) In vitro differentiation of PO-iPS-1 clones. Micrographs show EBs generated from PO-iPS-1 clones. In vitro differentiation into ectodermal, mesodermal, and endodermal cell types was revealed bv

immunoreactivity to the tissue-specific markers Nestin, SMA, and GATA4, respectively. Nuclei were counterstained with DAPI (blue). Scale bars, 200 µm. RT-PCR analysis shows that cDNAs from EBs exhibit mRNA expression of representative lineage markers in differentiating cells. (H) The *in-vivo* developmental potential of PO-iPS-1 clones. Teratomas generated by PO-iPS-1 clones differentiated into neural rosettes (ectoderm), muscle and fat (mesoderm), and epithelium (endoderm). Hematoxylin and eosin-stained sections of teratomas derived from PO-iPS-1 clones in a nude mouse after 8-10 weeks are shown. Scale bars, 200 µm. (I) Chimeric mouse and germline contribution of PO-iPS-1 clones in adult chimera gonads. Established iPS cells give rise to live chimeras (upper panels) after injection of PO-iPS-1 clones (CF1 genetic background) into a C57BL6 blastocyst and contribute to the germline (lower panels). Genomic DNA PCR to detect exogenous and endogenous transgene integration in chimeric (upper right) and germline transmission mice (lower right) produced with PO-iPS cells.