

Supplementary information, Figure S7 Generation and characterization of 1F OxyO-iPS-TTF cells. (A) Phase contrast images showing the ESC-like morphology of OxyO-iPS-TTF-1 (upper) and OxyO-iPS-TTF-2 clones (lower) on feeder cells and characterization of OxyO-iPS-TTF clones. AP staining, as well as SSEA1, Oct4, Sox2, and Nanog immunoreactivity, was detected in OyxO-iPS-TTF clones. Scale bars, 200 μ m. (B) qPCR analysis of ES cell marker genes in mES cells, MEFs, TTF, and OxyO-iPS-TTF clones (1 and 2). **P* < 0.05 compared to MEFs. (C) Western blot (left) and FACS (right) analyses of OxyO-iPS-TTF clones. Protein levels of Oct4, Sox2, Nanog, and SSEA1 were similar to those in mES cells. Antibody control, blue line; SSEA1 and Oct4, green line. (D) Bisulfite genomic sequencing of Oct4 and Nanog promoters in OxyO-iPS-TTF clones (1 and 2). Open and filled circles indicate unmethylated and methylated CpG dinucleotides, respectively. (E) ChIP analysis of Oct4, Sox2, and Oxt4, Sox2, and Nanog promoters for diMeK9H3 and AcH3 status in mES cells, TTFs, and OxyO-iPS-TTF clones. **P* < 0.05 compared to MEFs. (F) Scatter plots of

the global gene expression comparing OxyO-iPS-TTF clones with either mES cells or TTFs as described previously. (G) *In vitro* differentiation of OxyO-iPS-TTF clones. Micrographs show EBs generated from OyxO-iPS-TTF-1 clones (upper) and OxyO-iPS-TTF-2 clones (lower). *In vitro* differentiation of OxyO-iPS-TTF clones into ectodermal, mesodermal, and endodermal cell types was revealed by the immunoreactivity of the tissue-specific markers Nestin, SMA, and GATA4, respectively. Scale bars, 200 μ m. (H) The *in vivo* developmental potential of OxyO-iPS-TTF clones. Teratomas generated from OxyO-iPS-TTF 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-TTF clones in a nude mouse host after 4 weeks are shown. Scale bars, 200 μ m.