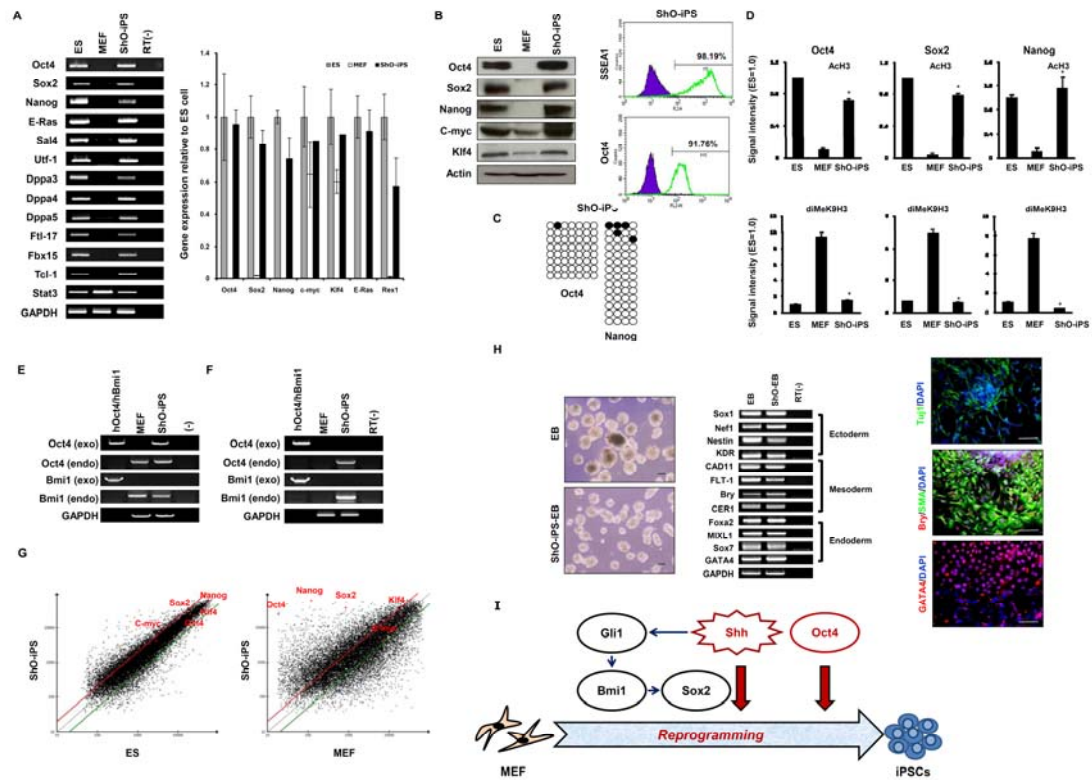


Figure S4



**Supplementary information, Figure S4.** Characterization of Sho-iPS generated from MEFs by treatment of Shh and retroviral transduction with Oct4. **(A)** RT-PCR analysis and qPCR analysis of ES cell marker genes in mES cells, MEFs, and Sho-iPS cells. **(B)** Western blot (left) and FACS (right) analyses of Sho-iPS 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. **(C)** Bisulfite genomic sequencing of the Oct4 and Nanog promoters from Sho-iPS cells. Open and filled circles indicate unmethylated and methylated CpG dinucleotides, respectively. **(D)** ChIP analysis of Oct4, Sox2, and Nanog promoters for diMeK9H3 and Ach3 in mES cells, MEFs and Sho-iPS cells. \*P < 0.05 compared to MEFs. **(E)** PCR of genomic DNA to detect integration of exogenous Oct4 and Bmi1 genes in MEFs and Sho-iPS cells. hBmi1/Oct4 was used as positive controls using expression plasmid DNA (pBabe hBmi1 and pBabe Oct4). **(F)** RT-PCR to detect expression of exogenous Oct4 and Bmi1 transcripts in MEFs and Sho-iPS cells. **(G)** Scatter plots of the global gene expression comparing Sho-iPS cells with either mES cells or MEFs as described above. **(H)** *In-vitro* differentiation of Sho-iPS cells. Micrographs show EBs generated from mES cells (left) and Sho-iPS cells (right). *In-vitro* differentiation of Sho-iPS cells into ectodermal, mesodermal, and endodermal cell types was confirmed by RT-PCR and immunoreactivity to typical markers  $\beta$ III Tubulin (Tuj1), Brachyury and SMA, and GATA4, respectively. Scale bars, 200  $\mu$ m. **(I)** Induction of Bmi1 by shh in the course of reprogramming.