Production of Retrovirus

293 cells, stably expressing the viral *gag* and *pol* genes (gp293), were kindly provided by Linda Wolff (NCI, NIH) and cultured in DMEM supplemented with 10% FBS, 2mM l-glutamine, β-ME, and NEAA. gp293 cells were plated at 75% confluency in a T-75 flask and allowed to attach overnight. The following day cells were transfected with plasmid DNAs using Lipofectamine (60μl). To produce virus, each T-75 was transfected with a plasmid containing the viral envelope gene VSV-g (12μg) and one of the following pMX-based retroviral vectors (provided by Shinya Yamanaka, Kyoto, Japan): *hOCT4, hSOX2, hKLF4*, and *hMYC* (12μg). The medium was changed after 24 hours (10ml per T-75). At 48 hours, the medium was collected from each flask and replaced with fresh medium (10ml per T-75). Collected medium was spun at 1,000rpm for 10 minutes to remove debris. The supernatant containing virus was collected and used immediately or stored at 4°C for up to 10 hours.

Human iPS cell derivation

Primary human foreskin fibroblasts, generously provided by Atsushi Terunama and Jonathan Vogel (NCI, NIH), were obtained under an IRB approved protocol and used to generate the human iPS cell line NIH-i1. Three additional human fibroblast cell cultures were obtained from the Coriell Institute for Medical Research (Campden, NJ). All three cell cultures were from patients with a clinical diagnosis of Parkinson's Disease. Sample AG20443 was derived from the skin of a 71-year-old male and was used to generate 8 human iPS cell lines of which 4 were used in this study, NIH-i2, NIH-4, NIH-i5 and NIH-i11. Sample AG08395 was derived from the skin of an 85-year-old female and was used to generate the human iPS cell line NIH-i7. Sample AG08396 was derived from the lung of the same 85-year-old female as AG08395 and was used to generate human iPS cell lines NIH-i12 and NIH-i13.

Human fibroblasts were cultured in DMEM supplemented with 10% FBS, l-glutamine, NEAA, βME, and Pen/Strep. Cells were plated at $1.33 \times 10^3/cm^2$ in a T-75 flask the night before infection. For infection, fibroblast culture medium was removed and 2ml of medium from each of the four viral productions (*hOCT4, hSOX2, hKLF4, hMYC*) was supplemented with 8μg/ml polybrene (Sigma) and added to each T-75 of

fibroblasts (for a total of 8ml). Control plates were also infected with each virus individually in order to monitor the proportion of cells infected by each virus by quantitative RT-PCR or immunocytochemistry. Fibroblasts were infected four times (time 0, +6 hours, +24 hours, and +30 hours). At 48 hours, virus containing medium was removed and replaced with fresh culture medium. On day 5, cells were trypsinized and plated at a density of 868 cells/cm^2 into 6-well plates containing feeders. Feeders were prepared the previous day as described for human ES cells. Infected human fibroblasts were allowed to attach overnight in fibroblast culture medium containing serum. The following morning, cells were fed with human ES cell medium and then fed every 48 hours until day 10. After day 10, cells were fed everyday with MEF-conditioned medium to account for the progressive loss of feeders during the extended culture period.

Individual human ES-like colonies were picked on days 24-42 and expanded. After the initial expansion these colonies were screened for expression of the endogenous pluripotency marker *NANOG* by real time PCR. The PCR showed that all four factors are integrated into the genome of all *NANOG*-positive lines and that the viral transgenes are repressed, but not completely silenced in all lines.