Supplemental data

Generation of genome integration-free pluripotent stem cells from the fibroblasts of C57BL/6 mice without c-Myc transduction

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Fig. S1. iPS clones generated by 3F infection of C57BL/6 MEFs.
(A) RT-PCR analysis of ES marker genes. The relative expression levels compared with each transcript in the ES cells are presented. Error bars indicate the standard deviations (n = 3). (B) Methylation analysis of the Oct4 promoter by bisulfate genomic sequencing. Open and closed circles indicate unmethylated and methylated CpGs, respectively. (C) Immunocytochemical analysis of Oct3/4 expression using the h-134 antibody (Santa Cruz). DAPI was used as a nuclear counter stain. (D) Teratomas derived from the R-3FiPS clone 5-4. Scale bar, 100 μm. (E) Chimeric mice generated from R-3FiPS clones 5-7. iPSCs were aggregated with ICR embryos and transferred to pseudo-pregnant female mice. iPSC contributions are indicated by the black coat color. (F) Germline transmission of the R-3FiPS clone 5-7. (G) Genotype of the F1 mice derived from R-3FiPS clones 5-7. The primers used for the detection of integrated retroviral vectors were as follows: 5'-CAC TGC TCT GGG CTC TCC CA-3' for Oct4, 5'-TAA CGG CAC ACT GCC CCT GT-3' for Sox2, 5'-CGG ACC ACC TTG CCT TAC AC-3' for Klf4, 5'-GGA AAC GAC GAG AAC AGT TG-3' for c-Myc and 5'-TCT GTT CCT GAC CTT GAT CT-3' for 3'LTR.

Fig. S2 Genomic PCR analysis of 2A-3FiPS clones.
Clones 5, 7, 8 and 9 are positive for genome integration. Clone 2A-4F-25 was used as a c-Myc integration-positive control (PC).

Fig. S3 Expression analysis of 2A-3F-iPS clones generated from B6-EGFP TG mice.
RT-PCR analysis of ES-marker genes. Error bars indicate standard deviations (n = 3).

Fig. S4 Expression analysis of R-3F-iPS clones.
RT-PCR analysis of ES-marker genes. Error bars indicate standard deviations (n = 3).

Fig. S5 Frequency of iPS cell generation assayed using a 96-well plate.
Small numbers of MEFs (approximately 250) were seeded in each well and iPSC-positive wells were counted. Experiments were performed twice.

Fig. S6
(A) Endogenous c-Myc transcripts in C57BL/6 and Nanog-GFP fibroblasts.
Real-time PCR was performed and the relative quantity of endogenous c-Myc transcripts in C57BL/6 MEFs versus Nanog-GFP TG MEFs is presented. Error bars indicate standard deviations (n = 2). (B) Frequency of iPS cell generation for (Nanog-GFP x B6) F1 MEFs.

Fig. S7
(A) Frequency of iPS cell generation. (B) Molecules contributing to the effects of mouse strain differences upon iPS cell generation.