

SUPPLEMENTARY FIG. S3. A GFP-positive cell obtained by forced expression of Cnot2 and Trim28 was able to be maintained as a clonal cell line. (A) A Nanog-GFP-positive colony was obtained from partial iPSCs (2B1) in which Cnot2 and Trim28 were expressed retrovirally by culturing at a subclonal density (10,000 cells per 10-cm dish) for 2 weeks. (B) The identified GFP-positive colony in A could expand robustly. After picking up, the GFP-positive colony was expanded briefly (7 days). Subsequently, 1.0×10^5 GFP-positive/DsRed-negative cells were sorted by fluorescence-activated cell sorting and plated on a culture dish with mitomycin C-treated feeder cells. These cells could be passaged 10 times without losing their strong GFP fluorescence or robust proliferation. (C) Quantitative PCR analyses of the expression of endogenous pluripotency marker genes in GFP-positive cells obtained by forced expression of Cnot2 and Trim28. RNAs from partial iPSCs (2B1) and those exposed to the 2i condition for 12 days were used as references for partial and genuine iPSCs, respectively. Sample Nos. 1, 2, and 3 correspond to partial iPSCs (*blue*), genuine iPSCs (*red*), and GFP-positive cells obtained by forced expression of Cnot2 and Trim28 (*green*), respectively. Expression data of genuine iPSCs were arbitrarily set to one. (D) Western blot analyses of Nanog pluripotency marker protein in GFP-positive cells obtained by forced expression of Cnot2 and Trim28. Sample numbers are as described in C.