Supplementary Figures.

Figure S1. Human H1 (a) and H9 (b) ES cells maintained a normal karyotypes after transduction by two lentiviral vectors and multiple rounds of Dox induction. (a) H1 human ES cells were transduced first by tTS/RFP vector, then by iDuet101A, induced by Dox and selected by hygromycin. At the end of re-induction experiments, karyotyping of the resultant H1 human ES cells were examined by staining cells in metaphase (G-banding, 400). An example of chromosome sets in one of 20 metaphase spreads examined is shown. 20 out of the 20 spreads are normal. (b) Similarly H9 human ES cells after the series treatment were karyotyped. An example of 15 metaphase examined (G-banding, 300) is shown. 15 out of the 15 spreads are normal.

Figure S2. Inducible and reversible regulated transgene expression in hES cells in a representative experiment. H1 hES cells (passage 42-62) were plated in Matrigel coated 6-well plates and transduced by the lentiviral vector tTS/RFP. The sorted cells were expanded on feeders for multiple passages. Then the tTS/RFP transduced hES cells were transduced with the second lentiviral vector iDuet101A expressing tet-regulated GFP, and analyzed after one passage on feeder cells (6 days). Levels of GFP expression in undifferentiated (SSEA-3+) hES cells expressing tTS (+tTS) or lacking tTS (-tTS) were shown in (a) as a dot plot against side scatter (SSC). Percentages of cells detected as positive (5% and 61%) were denoted. Then the double-transduced cells were passaged on feeder cells again and incubated with Dox (0.5 μg/ml) for one passage (b). The induced (33%) cells were sorted by FACS according to RFP and GFP expression (b). The sorted GFP+RFP+ cells (boxed) were washed and expanded in the absence
of Dox. The lack of GFP expression in the “off” state was confirmed (c). The re-induction by Dox was analyzed after 4 or 8 days, respectively (d). As in (a), undifferentiated hES cells cultured on feeder cells were first gated by SSEA-3 expression and levels of GFP expression is displayed as dot plots. Similar data were obtained with I-6 hES cells.

Figure S3. Induced expression of GFP transgene in I-6 hES cells expressing tTS. I-6 hES cells were first transduced by tTS/RFP regulator vector, sorted and expanded as described before for other hES cells. Then I-6 hES cells were transduced either by the iDuet101A vector that expresses GFP from the EF1α promoter controlled by the tetO/tTS system (a-b) or a control vector lacking the GFP transgene (c-d). After transduction, I-6 hES cells were treated with (a and c) or without (b and d) Dox for one passage (6 days) and then analyzed by flow cytometry. GFP and RFP expression in individual ES cells were plotted. Percentages in each quadrant were denoted. The double-transduced I-6 cells as well as the parental I-6 hES cells used in this experiment were found to be karyotypically abnormal, and then discontinued.