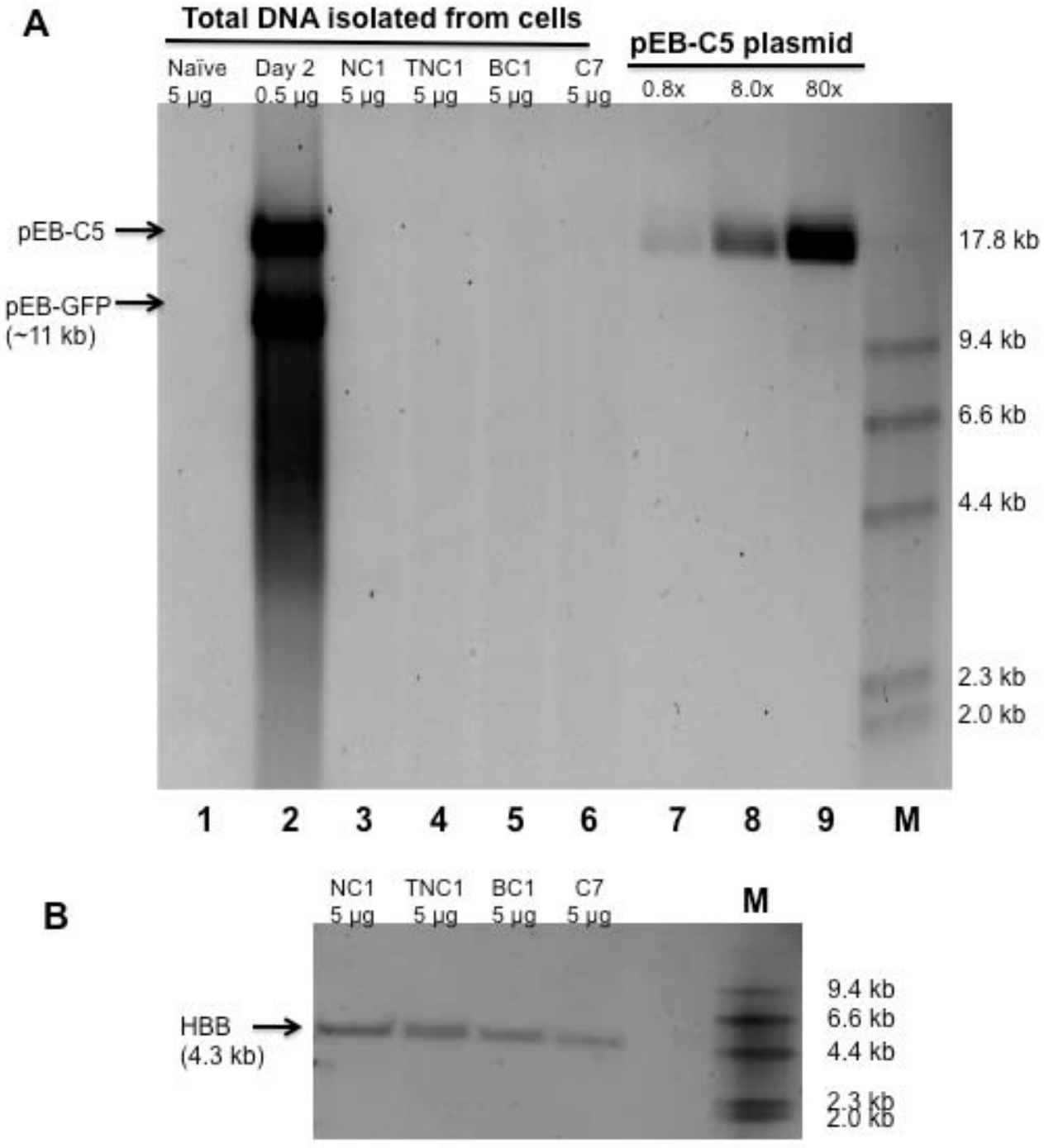
A
Total DNA isolated from cells
pEB-C5 plasmid
Naïve 5 µg
Day 2 0.5 µg
NC1 5 µg
TNC1 5 µg
BC1 5 µg
C7 5 µg
0.8x
8.0x
80x
pEB-C5
pEB-GFP (~11 kb)
17.8 kb
9.4 kb
6.6 kb
4.4 kb
2.3 kb
2.0 kb
1 2 3 4 5 6 7 8 9 M
B
NC1 5 µg
TNC1 5 µg
BC1 5 µg
C7 5 µg
M
HBB (4.3 kb)
9.4 kb
6.6 kb
4.4 kb
2.3 kb
2.0 kb

Supplementary information, Figure S11 Southern blot analyses for the lack of vector DNA in expanded iPSCs that are derived by episomal vectors.

(**A**). Detection of vector DNA sequences using EBNA1 DNA as a probe. Total DNA isolated from various cell extracts (lane 1-6) was digested by BamH I and separated by 0.7% agarose gel. Peripheral blood MNCs before (naïve, lane 1) and after nucleofection by two plasmids pEB-C5 and pEB-GFP at day 2 (lane 2) were included. Total DNA isolated from 4 iPSC lines was analyzed here: NC1 (passage 16, lane 3), TNC1 (passage 20, lane 4), BC1 (passage 28, lane 5) and C7 (passage 31, lane 6). Five μg total DNA isolated from various cell types was used, except that only 0.5 μg DNA (10%) from the day 2 transfected cells was used in lane 2. Plasmid DNA of pEB-C5 was also cut (once) by BamH I, and used as a positive control in detection (lane 7-9). Plasmid DNA was loaded in limiting dilutions (12 pg in lane 7, 120 pg in lane 8 and 1.2 ng in lane 9). The numbers of loaded vector DNA molecules per lane, relative to the copy numbers of nuclear genome of naïve cells and iPSCs, would be 0.8x, 8.0x and 80x, respectively. M: DNA size markers. (**B**). Quality controls of genomic DNA isolated from 4 iPSC lines. The same genomic DNA samples were digested and analyzed by Southern blot. The probe used is a cellular DNA fragment 3' to the *HBB* gene. As expected, the method detected the 4.3-kb DNA fragment in iPSCs as a single-copy gene.