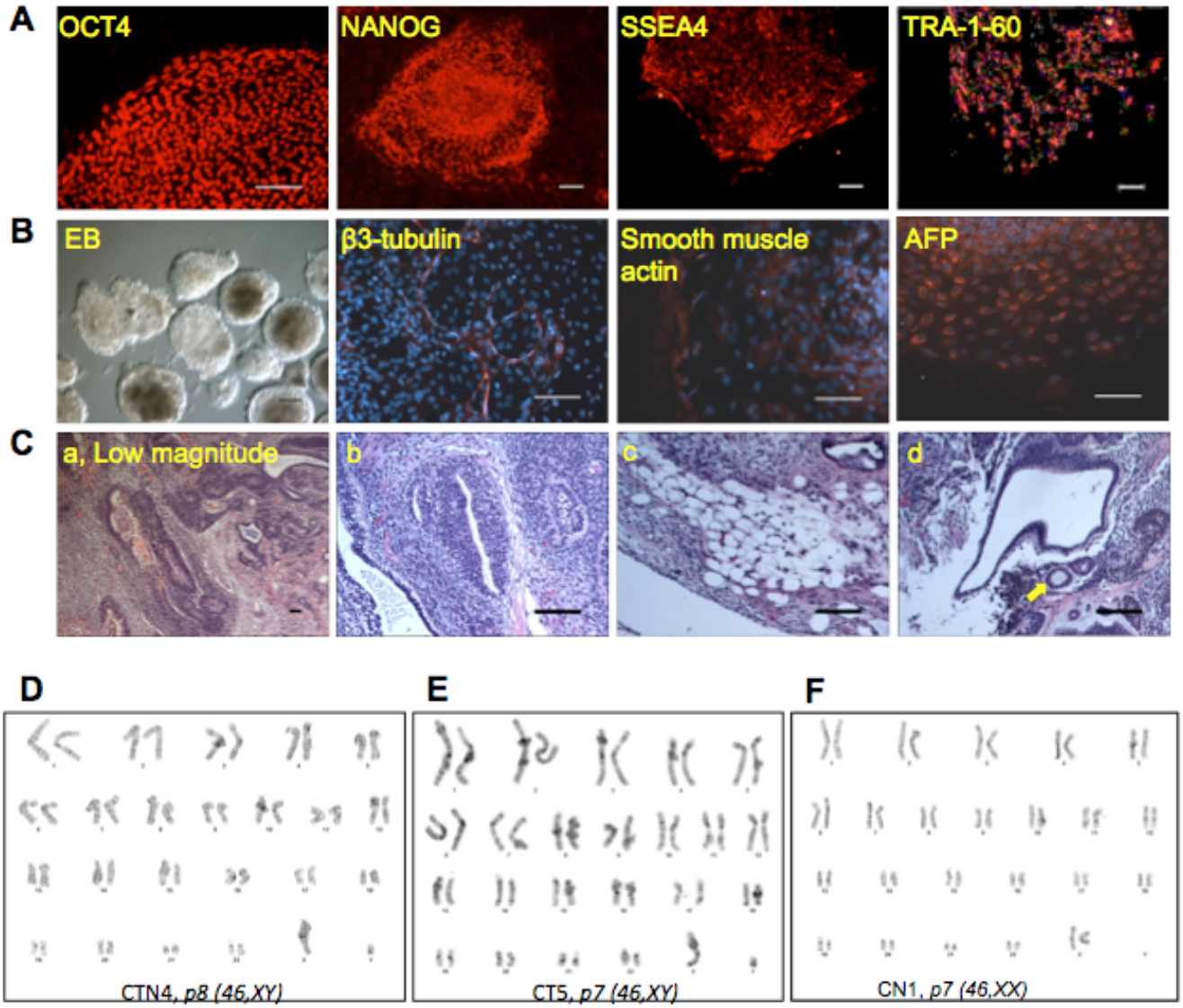
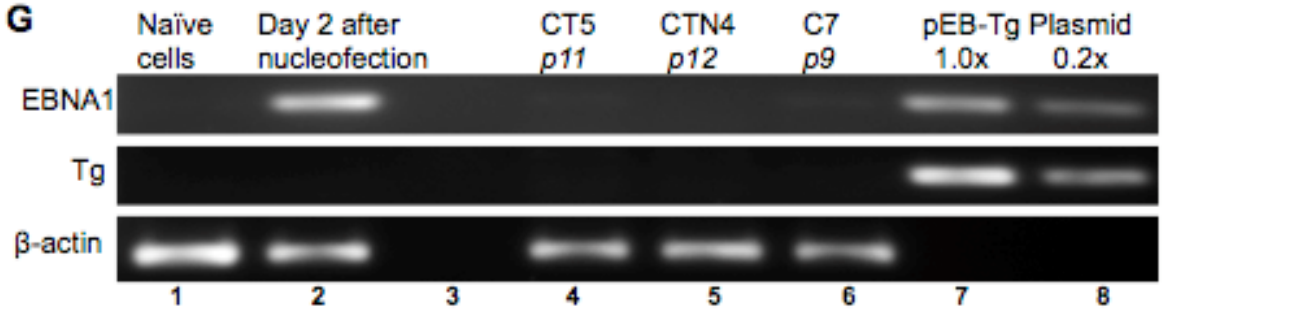

A
OCT4
NANOG
SSEA4
TRA-1-60
B
EB
β3-tubulin
Smooth muscle actin
AFP
C
a, Low magnitude
b
c
d
D
CTN4, p8 (46,XY)
E
CT5, p7 (46,XY)
F
CN1, p7 (46,XX)


G
Naïve cells
Day 2 after nucleofection
CT5 p11
CTN4 p12
C7 p9
pEB-Tg Plasmid 1.0x
0.2x
EBNA1
Tg
β-actin
1
2
3
4
5
6
7
8

Supplementary information, Figure S4 Characterization of iPSC lines derived from CB CD34+ cells reprogrammed by pEB-C5 and pEB-Tg plasmids or by pEB-C5 alone.

(**A**) Staining of undifferentiated CTN4 iPSCs after expansion (>5 passages) for marker associated with pluripotency. Scale bar: 100 μm for all the micrographic images in A, B and C. CTN4 was derived by the two plasmids pEB-C5 (C5) + pEB-Tg (thus called CT) in the presence of sodium butyrate (NaB). (**B**) In vitro pluripotency assay by embryoid body (EB) formation and differentiation. Eight days after culture in suspension (left), EBs were allowed to adhere and further differentiate. Then the whole culture was stained by monoclonal antibodies recognizing various cells types including those from ectoderm (β3-tubulin), mesoderm (smooth muscle actin) and endoderm (alpha-fetal protein or AFP). DAPI was used to stain cellular DNA. (**C**) In vivo pluripotency assay by teratoma formation. The cystic teratoma-like tumors were excised from animals and sectioned. H & E stained sections were examined. Both low (a, 4x) and high (b-d, 20x) magnification images of multiple sections are shown. Various cell types such as neural rosettes (b, ectoderm), adipose (c, mesoderm) and glandular structures (arrowed in d, endoderm) are found. **(D)** The CTN4 iPSC line has a normal karyotype as examined by a certified cyto-geneticist after 8 passages. **(E)** Similarly the CT5 line that was derived from the same CD34+ cell donor by the two vectors (C5+Tg) but without NaB is also pluripotent by the same assays and karyotypically normal. **(F)** The CN1 iPSC line that are derived by C5 vector alone + NaB are similarly pluripotent and karyotypically normal. Characterization of C7, derived from the same CD34+ cell donor is shown in Figure 3. **(G)** PCR detection of (episomal) vector DNA in reprogrammed cells using specific primers for viral EBNA1 and Tg gene or the beta-actin cellular gene. Un-transfected (naïve, lane 1) cells or cells harvested at day 2 after the pEB-C5 transfection (lane 2) were used as negative or positive controls, respectively. The pEB-Tg plasmid (containing both EBNA1 and Tg DNA sequences) is used as a common DNA control (lane 7 & 8), in an amount equivalent to 1 or 0.2 copies per genome of cellular DNA in naïve cells. Although minute amounts of episomal DNA (< 0.2 copies per cell on average) may exist in C7 iPSCs at passage 9 (lane 6), it is undetectable by passage 12 (<0.04 copies per cell, shown in Figure 4G). No vector DNA is detected in CT5 (by passage 11) or in CTN4 (by passage 12).