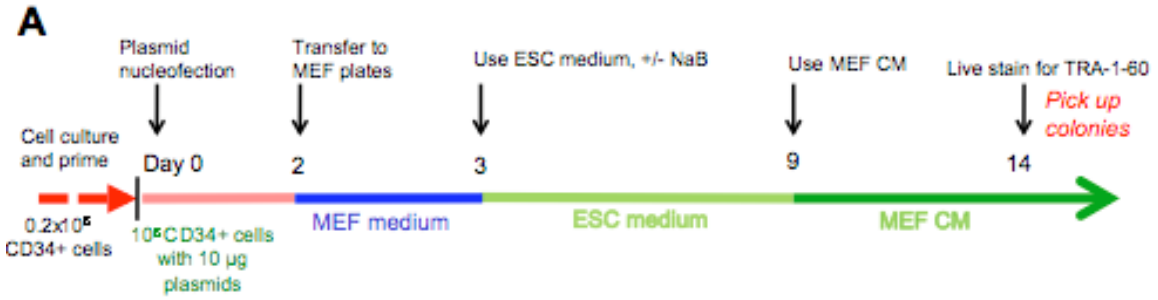
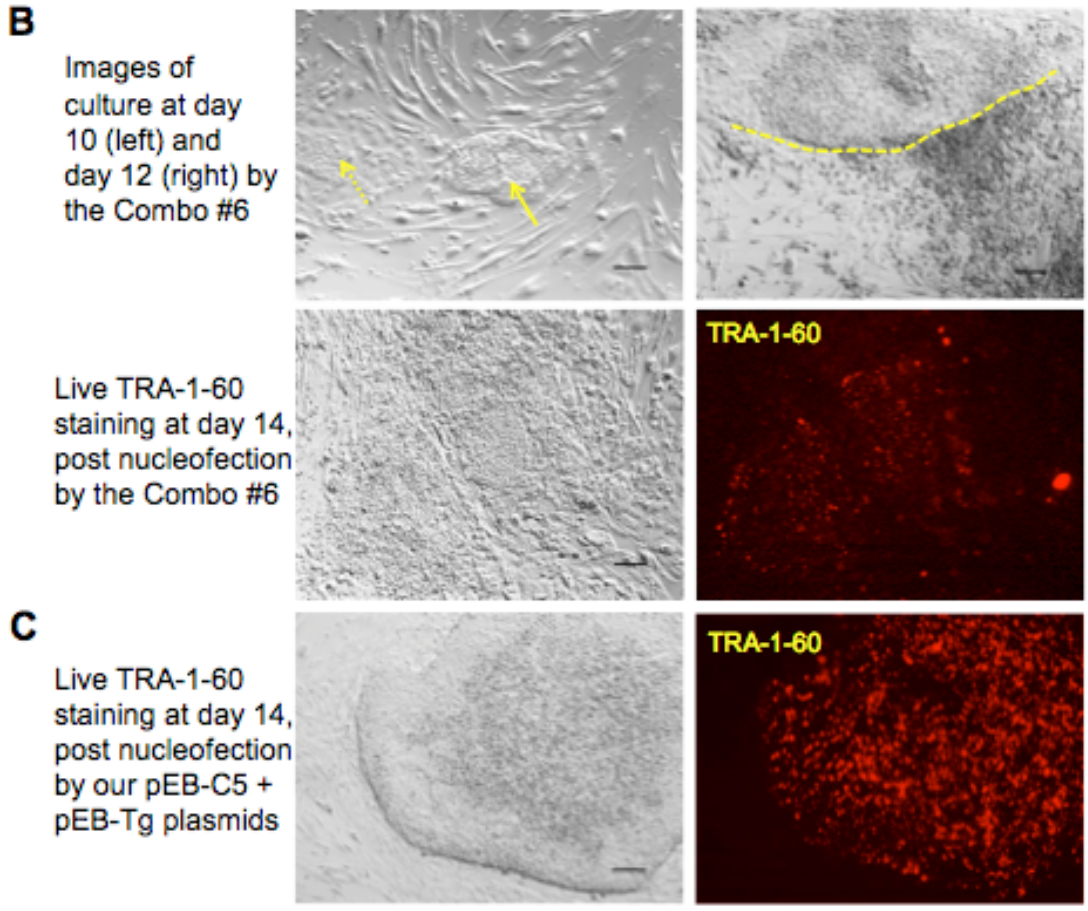
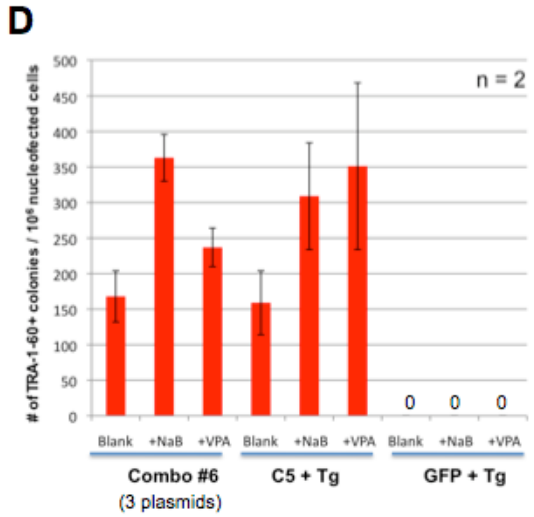
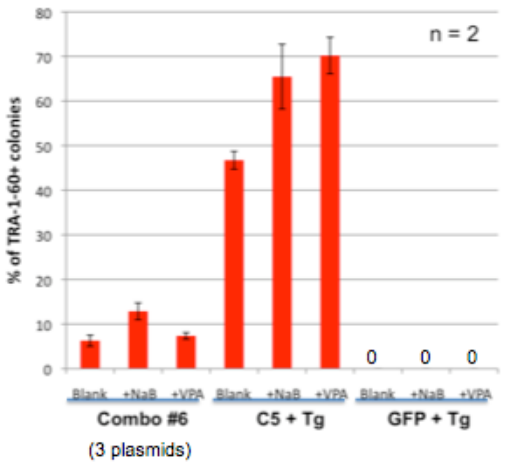

A
Plasmid nucleofection
Transfer to MEF plates
Use ESC medium, +/- NaB
Use MEF CM
Live stain for TRA-1-60
Pick up colonies
Cell culture and prime
Day 0
2
3
9
14
0.2x10^5 CD34+ cells
10^5 CD34+ cells with 10 µg plasmids
MEF medium
ESC medium
MEF CM


B
Images of culture at day 10 (left) and day 12 (right) by the Combo #6
Live TRA-1-60 staining at day 14, post nucleofection by the Combo #6
TRA-1-60
C
Live TRA-1-60 staining at day 14, post nucleofection by our pEB-C5 + pEB-Tg plasmids
TRA-1-60


D
# of TRA-1-60+ colonies / 10^5 nucleofected cells
n = 2
0 0 0
Blank +NaB +VPA Blank +NaB +VPA Blank +NaB +VPA
Combo #6 (3 plasmids)
C5 + Tg
GFP + Tg


E
% of TRA-1-60+ colonies
n = 2
0 0 0
Blank +NaB +VPA Blank +NaB +VPA Blank +NaB +VPA
Combo #6 (3 plasmids)
C5 + Tg
GFP + Tg

Supplementary information, Figure S3 Reprogramming of cord blood (CB) CD34+ cells by various combinations of episomal vectors.

(A). A procedure for reprogramming and identifying iPSC-like cells. Purified CD34+ cells were expanded and primed for 4 days, and then nucleofected once by 2 or 3 plasmids (a total of 10 μg DNA) at day 0. Transfected cells were cultured for 2 more days before transferring onto 6 wells coated with mouse embryonic fibroblast (MEF) feeder cells. At day 3, the ESC culture medium was used in the presence or absence of sodium butyrate (NaB, 0.25 mM) or Valproic acid (VPA, 0.5 mM). After day 9 when the original MEF feeder cells deteriorated, fresh MEF conditioned medium (CM) was used to substitute plain ESC medium. Starting at day 10, live staining for TRA-1-60 cell surface marker, which is expressed in human ESCs and bona fide iPSCs, could be performed every other day. Typically, we performed TRA-1-60 live staining at day 14, numerated colonies of both TRA-60 positive and negative, and picked up colony afterward. (**B**). Morphology of colonies emerged at day 10, 12 and 14 after nucleofection by the combination #6 (3 episomal vectors) described by Thomson/Yu (Yu et al., 2009). Scale bar: 100 μm (throughout). Yellow arrows and lines delineate candidate colonies. (**C**). Phase and fluorescence images of a colony after TRA-1-60+ staining 14 day after one-time nuecleofection of the two plasmids: pEB-C5 (C5) and pEB-Tg (Tg) episomal vectors. (**D).** Reprogramming efficiency (measured as numbers of TRA-1-60+, ESC-like colonies at day 14 per 10^6 nucleofected CD34+ cells) by either Thomson/Yu combo #6 (3 plasmids expressing 7 genes) or the C5+Tg vector combination. A pEB-GFP (GFP) was used as a negative control for C5. The percentages of total colonies that are TRA-1-60+ are also plotted (**E**). Data are plotted as mean +/-SEM (n=2).