Expanded View Figures

Figure EV1. (related to Fig 1). Generation of naïve-like hiPSCs by 2a2iL induction.

A Schematic drawing of the strategy used to produce safe, transgene-free hiPSCs. After electroporation of the three episomal vectors that contained *OCT4*, *SOX2*, *KLf4*, *LIN28*, *L-MYC*, and *p53* shRNA into human dermal fibroblasts (HDF), we added two chemical agonists (2a), RJW101 and CD437, of nuclear receptors liver receptor homologue-1 (LRH-1) and retinoic acid receptor gamma (RARγ), respectively, to fibroblast medium for 5 days. The cells were re-plated on a mouse embryonic fibroblast (MEF)-coated plate on day 6 with serum-free medium that contained 2i and human LIF (2iL). Small colonies that had a distinct morphology from primed colonies were observed around day 20. After trypsinization and passage onto a feeder-coated plate, dome-shaped colonies were clearly visible. RT–PCR analysis showed the status of episomal expression in nRepi-iPSC1.

B Bright-field images of nRepi-iPSC1 colonies on day 20 after reprogramming and at passage 18 (scale bar: 200 μm).

C nRepi-iPSC1 showed alkaline phosphatase (ALP) activity (scale bar: 100 μm).

D nRepi-iPSC1 displayed a normal karyotype.

E Immunostaining showed the expressions of pluripotency markers OCT4, NANOG, TRA-1-81, and SSEA4 in addition to nuclear localization of STAT3 and TFE3 in nRepi-iPSC1 (scale bar: 50 μm).

F The teratoma formation assay showed the ability of nRepi-iPSC1 to differentiate into the derivatives of three embryonic germ cell layers as seen by: (i) rosette structures (ectoderm), (ii) cartilage (mesoderm), and (iii) primitive gut (endoderm; scale bar: 50 μm).

G Cloning efficiency assay of pRepi-iPSC4 and nRepi-iPSC1 in the absence of ROCK inhibitor (ROCKi) and Y27632. ***t*-test: $P < 0.01$. Error bar indicates SD ($n = 3$). nRepi-iPSC1, Naïve-like Royan episomal iPSC1; pRepi-iPSC4, Primed Royan episomal iPSC4.

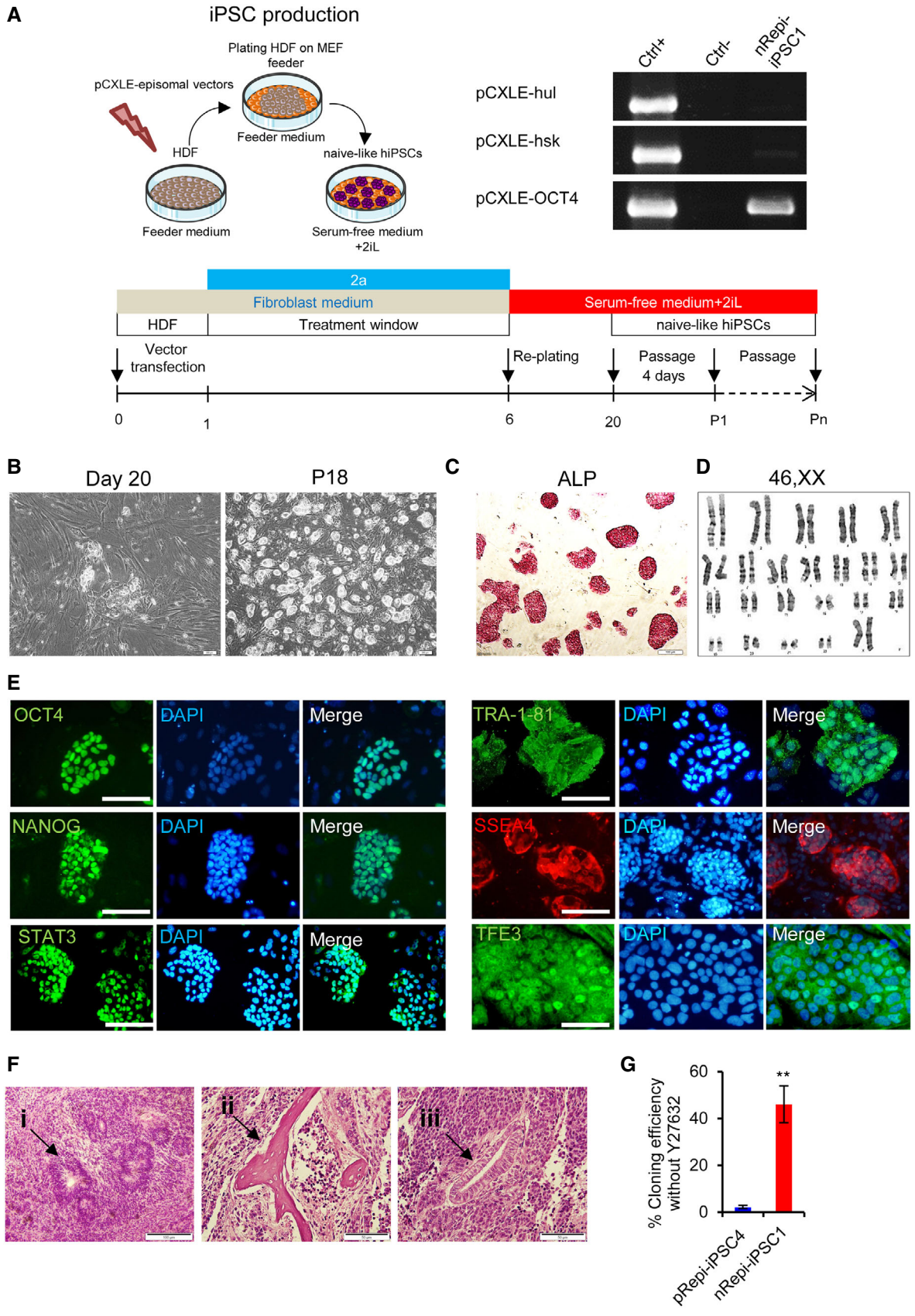

Figure EV1.

Figure EV2. (related to Fig 1). Conversion of primed hPSCs into the naïve-like state by 2a2iL induction.

A Morphological change of pRH6 during 14 days of induction with 2a2iL.

B Naïve-like colony morphology, alkaline phosphatase (ALP) staining, and immunostaining against pluripotency markers for 2a2iL-RiPSC1 and 2a2iL-RH5.

C A modified method for induction of naïve-like pluripotency by 2a2iL in some lines with impurity of mesenchymal-like cells (MLCs). (i) The hPSC line (pRH5) began to differentiate into mesenchymal-like cells (MLCs) after passage 4 or 5 (black and white arrows show naïve-like colonies and MLCs, respectively). (ii) Naïve-like cells could be separated from MLCs by differential adherence (iii) or by two rounds of single-cell passaging in the primed state before induction into the naïve-like state.

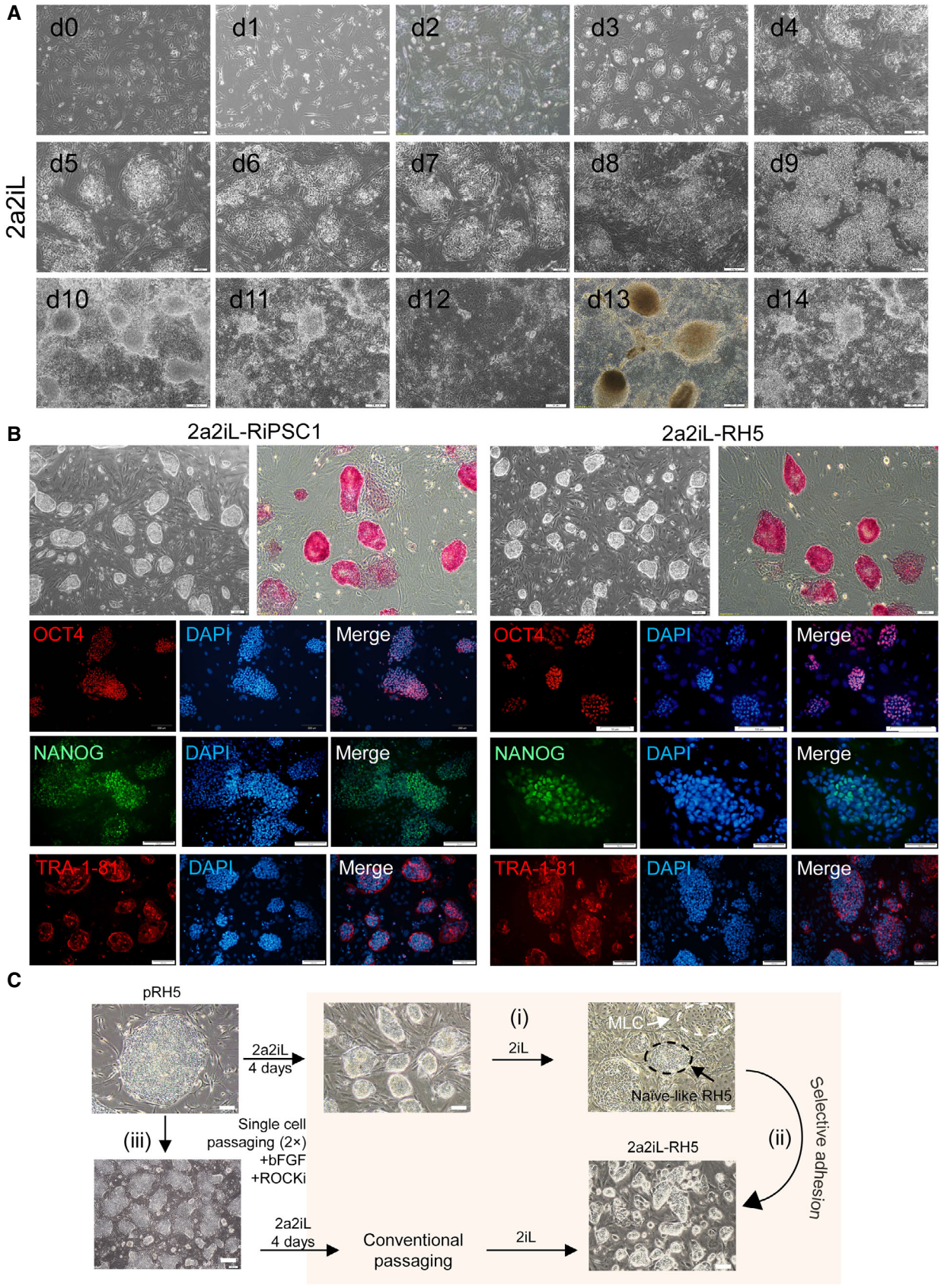

Figure EV2.

Figure EV3. (related to Figs 1 and 3). Conversion of pRH6 into the naïve-like state by 2a2iL under feeder-free conditions.

A The same protocol as Fig 1B was used for conversion of primed hESCs into naïve-like cells without a feeder layer. Phase contrast morphology of 2a2iL-RH6 and immunostaining for OCT4 and NANOG in nRH6 (left panel) and TFE3 and STAT3 indicated nuclear localization (right panel) scale bar: 50 μM.

B Reversion of 2a2iL-RH6 into the primed state (re-primed RH6) with cytoplasmic localization of the TFE3 and STAT3 transcription factors.

C Fluorescence images of tissue sections of non-injected mouse in E10.5 (negative control group) showed no green or red signals representing GFP and human nuclear antigen (HNA) in different parts of the embryo.

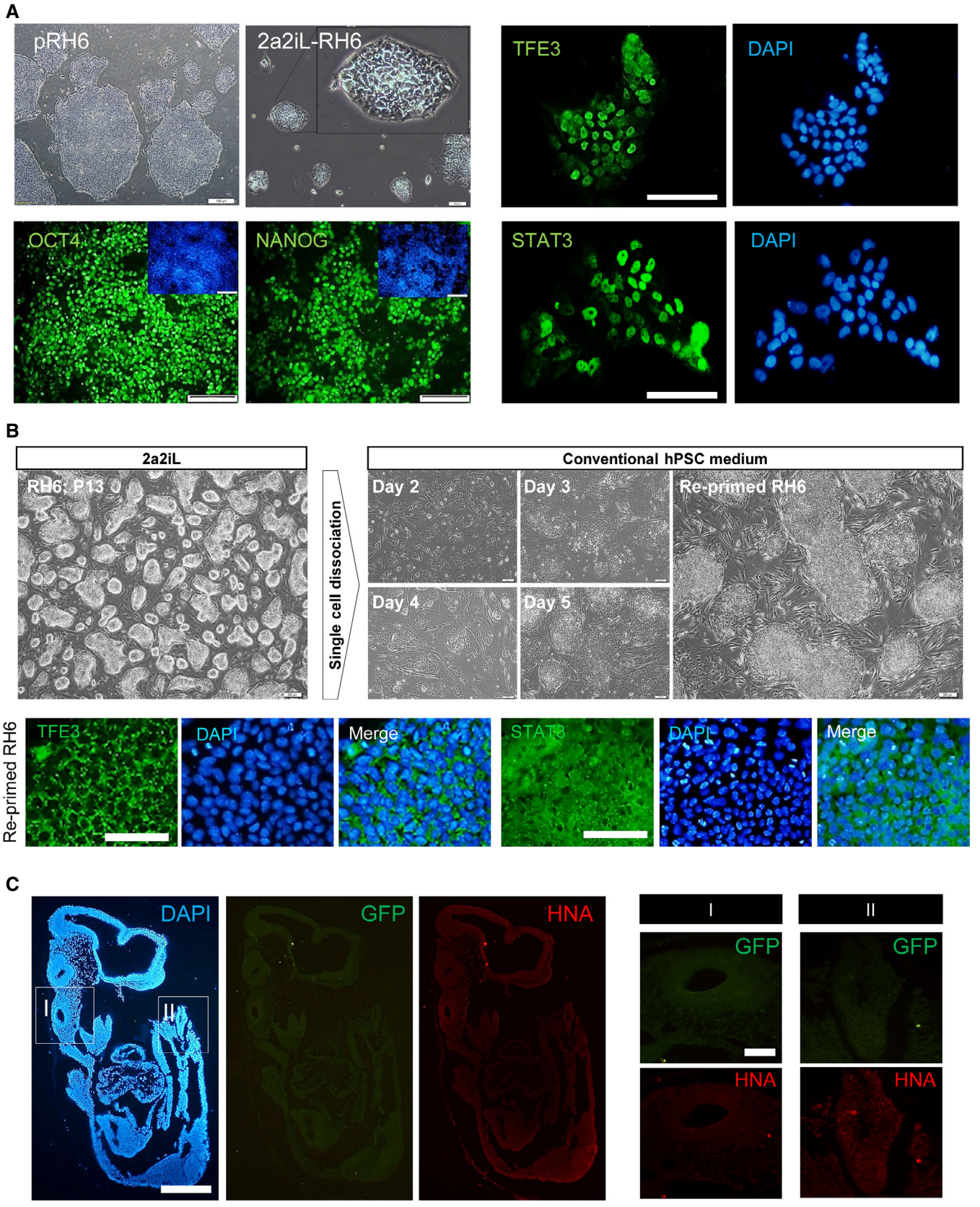

Figure EV3.

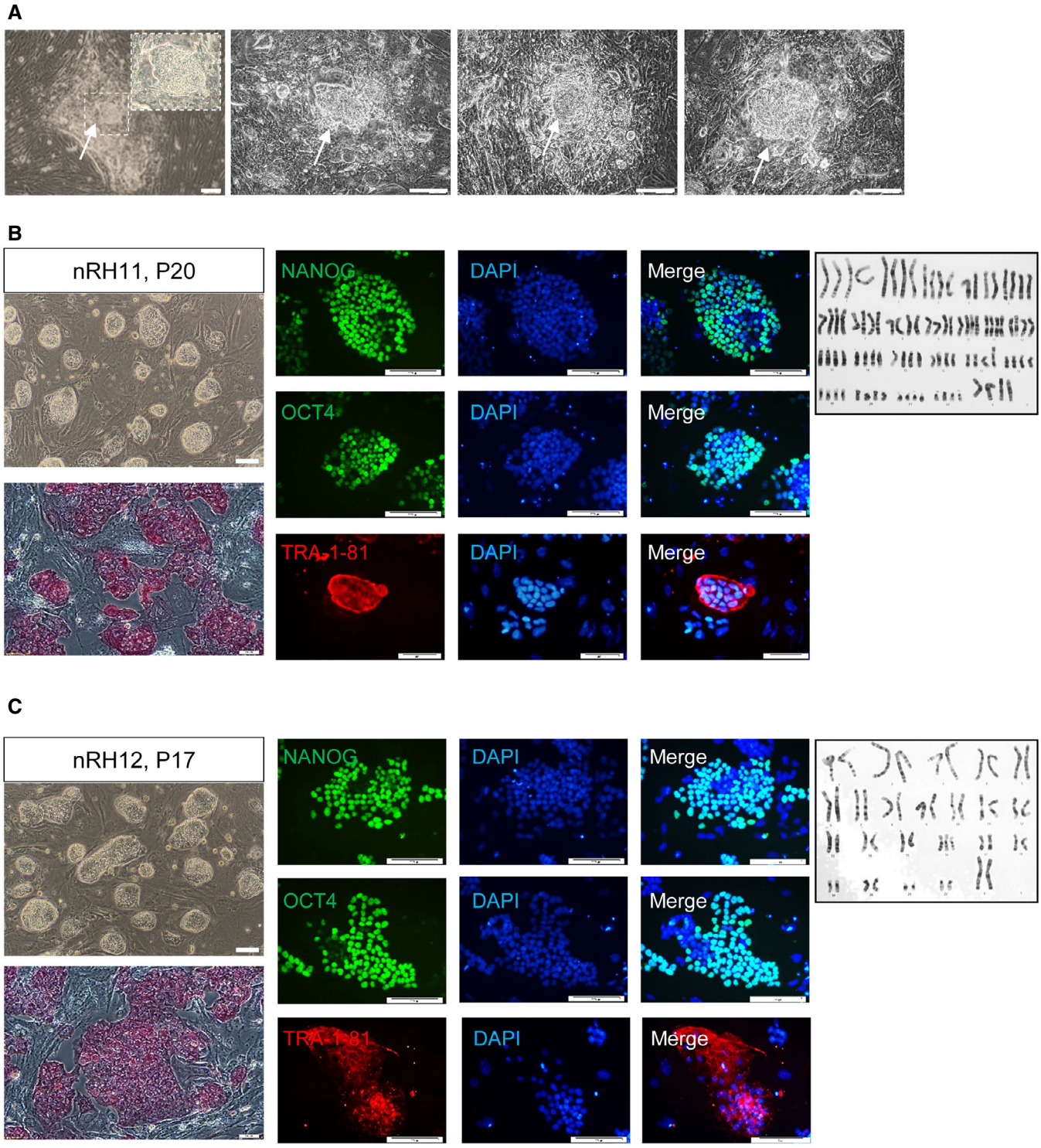

Figure EV4. (related to Fig 4). Characterization of 2a2iL-hESCs that were derived directly from human blastocysts.

A Whole blastocyst outgrowths after 5 days of culture in the 2a2iL condition. Despite the formation of naïve-like ICM outgrowths, there were no cell lines established by this protocol, which was most likely due to the presence of trophectoderm (TE) cells. More than 100 early, poor quality human embryos were used in this study.

B, C Colony morphology, ALP staining, immunostaining against pluripotency markers, and karyotype in the 2a2iL-derived nRH11 and nRH12 cell lines.

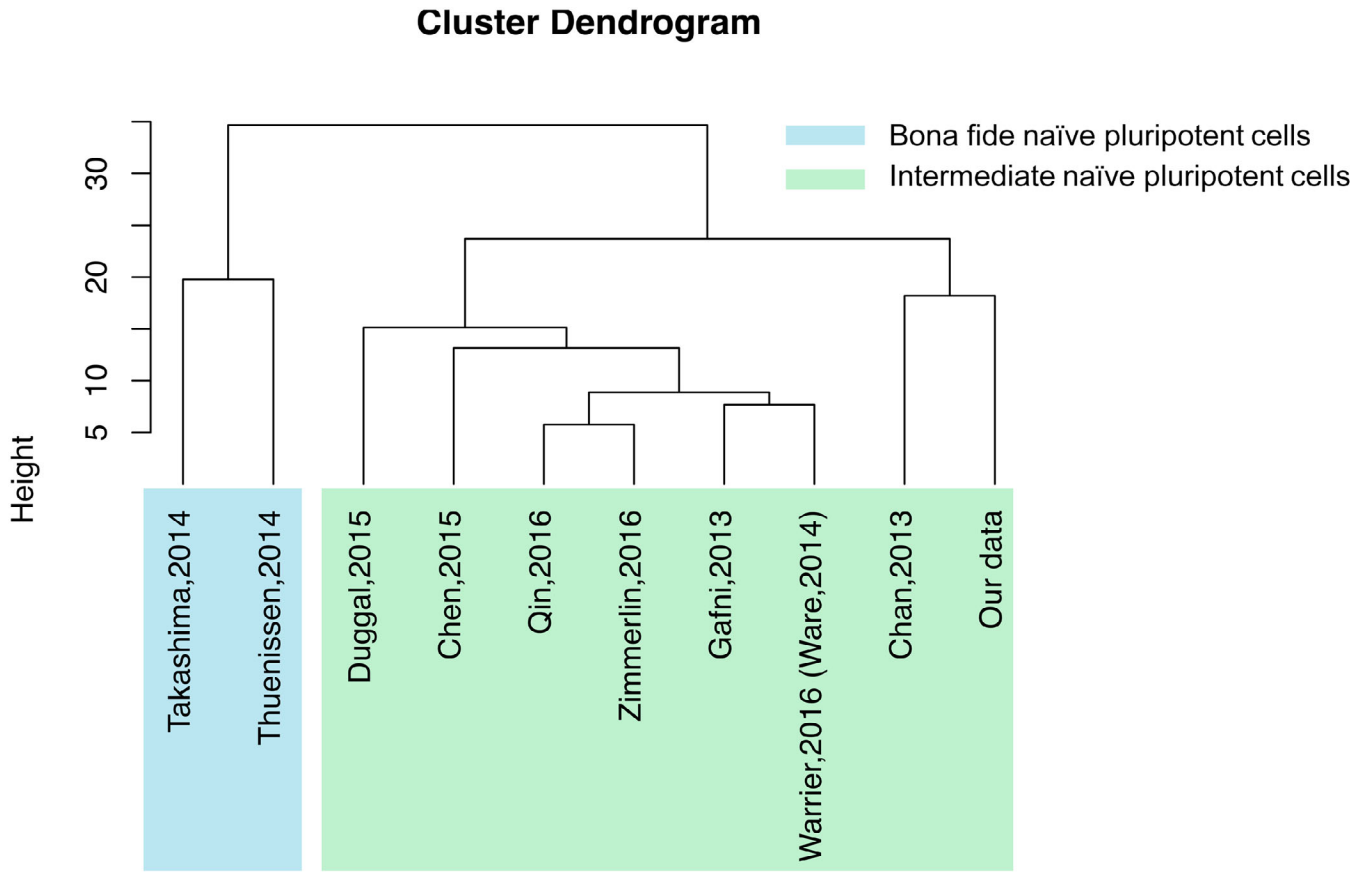

Figure EV5. (related to discussion). Dendrogram clustering.

Hierarchical clustering of naÿve, primed, and lineage specific-associated marker genes in 10 studies. The color blue indicates bona fide and green indicates intermediate states of human naÿve pluripotency. This clustering shows our study in the intermediate state of human naivety.