

Supplementary information, Figure S2 Characterization of iPS1.HEMd and fluorescence staining of WA09 cells with other lectins.

(a) Colonies of iPS1.HEMd cells were stained using anti-POU5F1, anti-SOX2, SSEA-4 and Tra-1-81 antibodies. The iPS1.HEMd cells are positive for all four well-established pluripotency biomarkers.

(I, II, III) Cell colony positive for POU5F1. (IV, V, VI) Cell colony positive for SOX2. (VII, VIII, IX) Cell colony positive for SSEA-4. (X, XI, XII) Cell colony positive for Tra-1-81. DAPI (I, IV, VII, X), pluripotent biomarker (II, V, VIII, XI) and merge (III, VI, IX, XII) images are shown. (b) Embryoid bodies (EBs) and teratomas were generated using iPS1.HEMd cells. Antibodies against biomarkers for all three germ layers (human NG2: ectoderm, SMA: mesoderm, AFP: endoderm) were used to stain EBs. (I, II, III) Cells positive for human NG2. (IV, V, VI) Cells positive for SMA. (VII, VIII, IX) Cells positive for AFP. DAPI (I, IV, VII), germ layer markers (II, V, VIII), and merged images (III, VI, IX) are shown. (X, XI, XII) Hematoxylin/eosin-stained sections from iPS1.HEMd-derived teratomas showing ectoderm, mesoderm, and endoderm tissue. (c) WA09 cells were spiked into a cell suspension of HDFs to create a mixed cell population. The cells were fixed on adhesion slides and then subjected to staining with anti-POU5F and SSEA-4 antibodies as well as biotinylated AOL lectin. WA09 cells expressing the pluripotency biomarkers POU5F1 or SSEA-4 are indicated by arrowheads and positive AOL staining. It is noteworthy that a sub-population of the cells positive for POU5F1 were negative for AOL staining, suggesting that glycan marks recognized by AOL may only exist on a subset of the total WA09 cell population. (I, II, III, IV) Cells subjected to DAPI/AOL/POU5F1 staining. (V, VI, VII, VIII) Cells subjected to DAPI/AOL/SSEA-4 staining. DAPI (I, V), AOL (II, VI), POU5F1 (III), SSEA-4 (VII) and merge (IV, VIII) images are shown. Insets, magnified images of the double-positive cells. (d) A mixed population of HDF and WA09 were fixed on adhesion slides and then subjected to staining by anti-POU5F1 and SSEA-4 antibodies as well as biotinylated TJA-II lectin. WA09 cells expressing POU5F1 or SSEA-4 are indicated by arrowheads and positive TJA-II staining. (I, II, III, IV) Cells subjected to DAPI/TJA-II/POU5F1 staining. (V, VI, VII, VIII) Cells subjected to DAPI/TJA-II/SSEA-4 staining. DAPI (I, V), TJA-II (II, VI), POU5F1 (III), SSEA-4 (VII) and merge (IV, VIII) images are shown. Insets, magnified images of the double-positive cells.