Supplemental experimental procedures

Immunocytochemical analyses. Cells performed monolayer culture were fixed in 4% paraformaldehyde at 4˚C for 30 min, then permeabilized with 0.2% Triton in PBS at 25˚C for 15 min and blocked with Blocking One Histo (Nacalai Tesque). Cells were then washed with PBS and incubated at 25˚C for 120 min with a primary antibody [NANOG (RCA0003P, Reprocell Inc.), Sox2 (ab97959, Abcam), or OCT4 (sc5279, SantaCruz Biotechnology, Dallas, TX, USA)] at a dilution of 1:100, 1:1,000, or 1:50. Next, the cells were again washed with PBS and incubated with anti-rabbit IgG (H + L) secondary antibody, Alexa Fluor 546 (A-11010, Thermo Fisher Scientific) at a 1:500 dilution, or with anti-mouse IgG (H + L) secondary antibody, CF488 (20014, BIOTUM, Vladimir, Russia) at a 1:500 dilution, for 30 min at room temperature, followed by another washing with PBS. We then stained the cells with the counterstain Fluoro-KEEPER Antifade Reagent containing 4',6-diamidino-2-phenylindole (DAPI; 12745-74, Nacalai Tesque).

Figure S1. hiPSC line, Toe, maintains its pluripotency. Pluripotency of hiPSC line, Toe, was confirmed using immunofluorescence staining with DAPI counterstaining to demonstrate the levels of NANOG, SOX2 and Oct4, pluripotency markers of hiPSCs, positivity and visualization of nuclear uptake (magnification, x400). SOX2, SRY-box 2; Oct4, Octamer-binding transcription factor 4; NANOG, homeobox protein NANOG; hiPSCs, human induced pluripotent stem cells.

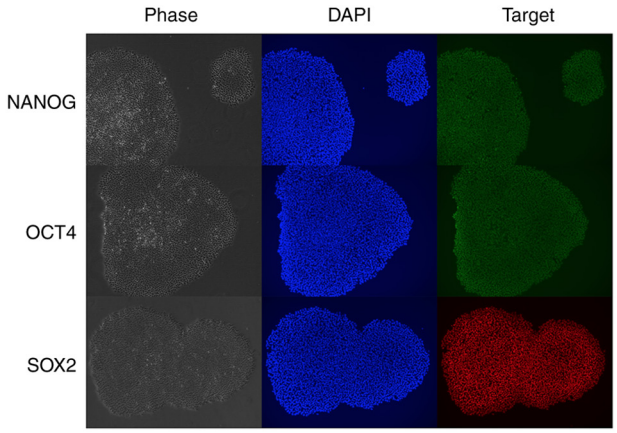

Figure S2. Analysis of marker gene expression and growth and death of hiPSC-derived cells during differentiation. (A) Timeline of hiPSCs cultured in monolayer under normal oxygen during cartilage differentiation for 10 days (n=3). (B) RT-qPCR analysis of the gene expression levels of *T* (immature mesodermal markers) and *sox9* (chondrogenic markers) on day 0, 3, 7 and 10. hiPSCs, human induced pluripotent stem cells; RT-qPCR, reverse transcription-quantitative PCR; T, Brachyury; sox9, Transcription factor SOX-9.