Supplementary Methods

Cell culture

Early passage fibroblasts (less than 10 passages) in growth phase were used for derivation of iPS cells. Established iPS cell lines were maintained in one of the following combinations: EB (embryoid body) medium (20% knock out serum replacement in DMEM/F12 with 1 mM GLUTAMAX, 1 mM NEAA, penicillin-streptomycin and 0.1 mM β-mercaptoethanol (ThermoFischer - Life technologies) or; mTESR1 (Stemcell Technologies) or; recombinant vitronectin and Essential 8 (E8) medium (ThermoFischer - Life technologies).

Immunoblotting

The cells were lysed in RIPA buffer (SIGMA) 24～48 hours of transfection supplemented with proteinase inhibitors (Roche). Total protein was quantified using BCA protein assay kit (Thermo Scientific) and the same amount of protein was loaded into gels (NuPAGE 10% Bis-Tris, Novex Life Technologies) for all the samples. Secondary HRP-conjugated antibodies used: polyclonal goat anti-mouse HRP and polyclonal goat anti-rabbit HRP at a concentration of 1 : 10000. Blocking was carried out in 5% milk/0.1% tween in PBS (blocking solution) for 1 h prior to incubation with secondary antibody overnight at 4°C. The following day, secondary antibodies were diluted in blocking solution and incubated at 1 h at room temperature. The signal was developed with the ECL chemiluminescence kit (GE HealthCare, Amersham UK).

Immunofluorescence

Briefly, the cells fixed with 4% paraformaldehyde for 20 min and were blocked for 30 mins with 1% goat serum, 0.1% bovine serum albumin (Sigma-Aldrich) in PBS. If performing nuclear staining, cells were permeabilised with ice-cold 100% methanol for 5 mins prior to blocking. After secondary antibody incubation, DAPI was added (100 ng/μl) (Sigma-Aldrich) was added for 2～5 mins washed with PBS before visualising using Axiovision microscope equipped with 10×objective. For alkaline phosphatase staining, cells were stained with the alkaline phosphatase staining kit (Millipore) as per manufacturer's instructions.