

In vitro spontaneous differentiation

iPSCs were detached with 0.15% Type IV Collagenase (Thermo Fisher Scientific), seeded onto 100 mm dishes coated with 1% agarose in KnockOut DMEM supplemented with 15% KoSR, 0.1 mM NEAA, 2 mM GlutaMax, 100 U/ml penicillin-streptomycin and cultivated for 9-10 days. Then embryoid bodies were plated onto Matrigel-treated 8-well Chambered Coverglass plates (Thermo Fisher Scientific) for another 7-9 days.

Immunofluorescence staining

Adherent cells growing on 8-well Chambered Coverglass plates were fixed in 4% paraformaldehyde for 10 min at room temperature (RT), permeabilized in 0.5% Triton-X100 for 30 min at RT, then incubated with Blocking Buffer (1% BSA in PBS) for 30 min at RT. Fixed cells were incubated with primary antibodies overnight at 4 °C, washed twice with PBS and incubated with secondary antibody for 1.5–2 h at RT. All antibodies were diluted in PBS with 1% BSA. Nuclei were counterstained with DAPI (Sigma-Aldrich). Micrographs were taken using Nikon eclipse Ti-E microscope and NIS Elements software.