HiPSCs were cultured in an incubator with 5% CO₂ at 37 °C in ncTarget (Nuwacell, China) hPSC medium in matrigel (Corning) coated 6-well plates , and then cells were split using 0.5 mM EDTA-DPBS at 1:8 to 1:12 ratios every 4 to 5 days with ROCK inhibitor.