In vitro differentiation

The induced pluripotent stem cells (iPSCs) were dissociated using an enzyme-free solution (ReLeSR) and then plated onto non-adherent surfaces in Embryoid Body (EB) media. This medium was composed of Knockout DMEM/F12, Knockout Serum Replacement, Glutamax, Antibiotic-Antimycotic, Minimum Essential Medium, and β-Mercaptoethanol, promoting EB formation. After 5–6 days, the EBs were harvested and transferred to matrigel-coated plates for spontaneous differentiation in DMEM-F12 media supplemented with 10% Fetal Bovine Serum. Following 5 days of culture, the cells were fixed with 4% PFA and subsequently stained with lineage-specific markers.

Immunofluorescence (IF) staining

The cells were cultured and fixed with 4% Paraformaldehyde (PFA) for 15 minutes at room temperature. Subsequently, the cells were permeabilized with 1% Triton X-100 for 10 minutes at room temperature, followed by a 30-minute incubation in blocking buffer (composed of 5% bovine serum albumin with 0.1% Triton X-100 in PBS) at room temperature. The appropriate primary antibody diluted in PBS with 1% BSA, was then applied to the cells and allowed to incubate overnight at 4°C. Cells were then treated with the respective secondary antibodies for 1 hour at room temperature. For nuclei staining, DAPI was added and incubated for 15 minutes at room temperature. Subsequently, the stained cells were visualized, and images were captured using fluorescence microscopy