Human iPSC derivation, expansion and maintenance procedures

Full thickness punch biopsy was taken aseptically from the retro-auricular skin of a healthy patient volunteers under local anesthesia, with their informed consent. The biopsies were used to establish explant cultures of human dermal fibroblasts (HDFs) in DMEM containing 10% FBS and antibiotics. Recombinant retroviruses were prepared in HEK 293T cells by transfecting individual pMXs retroviral vectors carrying *OCT3/4, SOX2, KLF4* and *MYC* (OSKM) transgenes (Addgene No. 17217, 17218, 17219, 17220) and the helper plasmids (Addgene No. 8455, 12259) using Fugene 6 (Roche Ltd., Basel, Switzerland) as per manufacturer's instructions. The HDFs at passage 3 were seeded 12h before transduction at a density of $1X10^5$ cells per 10 cm^2. The retroviral cocktail was used to transduce HDFs at an approximate MOI of 2. The cells were then split and transferred to fresh dishes containing mouse embryonic fibroblast feeders (MEFs) and cultured with standard human ESC medium. The cultures were treated with 1mM valproic acid on the first week of reprogramming (day 3-10) and the treatment was discontinued subsequently. The clones that emerged after 3 weeks were manually picked based on colony morphology and five clones were passaged for further expansion under standard hESC conditions on Matrigel™ (Corning, USA) coated plates using mTeSR™1 kit, as per manufacturer's instructions (STEMCELL Technologies, USA). The reprogramming efficiency was 0.005%.

Only undifferentiated iPSC colonies with clear margins were selectively picked and manually passaged as few cell clusters till passage 5, using a sterile flame-pulled glass Pasteur pipette with hooked tips, to enable gentle cutting and splitting of large colonies. The stably expanding clones are subsequently passaged as cell suspensions prepared using 1X cell dissociation solution (1X DPBS containing 0.5 mM EDTA and 30 mM NaCl) treatment for 6-7

minutes. The cultures are regularly passaged when they reach 70-80% confluence. A split ratio of 1:6 to 1:10 was routinely followed to maintain a split interval of 4 days. The iPSC clone hiPSC-F2-3F1 was expanded beyond 25 passages and characterized for the stemness, pluripotency and genomic integrity.