In vitro three-germ-layer differentiation

iPSCs were dissociated using TrypLE™ Express (Gibco) and grown for 7 days as embryoid bodies (EBs) in low-attachment 6-well plates in DMEM/F-12, GlutaMAX™ supplement medium containing 20% KnockOut™ Serum Replacement (Gibco), 0.1 mM non-essential amino acids (Gibco), 0.1 mM 2-mercaptoetanol (Gibco) and 10 µM Y-27632 (Tocris). On day 8, EBs were dissociated and seeded on a Matrigel® (standard formulation, Corning) -coated 24-well plate and cultured in DMEM/F-12, GlutaMAX™ medium containing 10% fetal bovine serum (Biowest) and 1% Penicillin-Streptomycin (Gibco). After 7 days, the cells were fixed using 4% paraformaldehyde and stained for endoderm and mesoderm markers. We differentiated the PD patient-derived iPS cell lines into midbrain dopaminergic neurons as previously described in Kriks *et al.,* (2011). After 28 days of differentiation, the cells were fixed using 4% paraformaldehyde and stained for ectoderm markers.