Embryoid body (EB) differentiation

The confluent culture of hiPSC was dissociated using a ReLeSR dissociation reagent. EBs were generated by aggregating the cells, using ultra-low attachment plates, in an E8 medium containing 10 µm ROCK inhibitor Y-27632 (Santa Cruz Biotechnologies) for two days on an orbital shaker. Cell aggregates were then further cultured in DMEM containing 20 % fetal bovine serum, 2 mM L-glutamine, 55 µM ß-mercaptoethanol, and 1x non-essential amino acids. The differentiation medium was changed every other day for 3 months. Spontaneous differentiated EBs were fixed at 4 % PFA and processed according to standard procedures for paraffin embedding and hematoxylin/eosin staining.