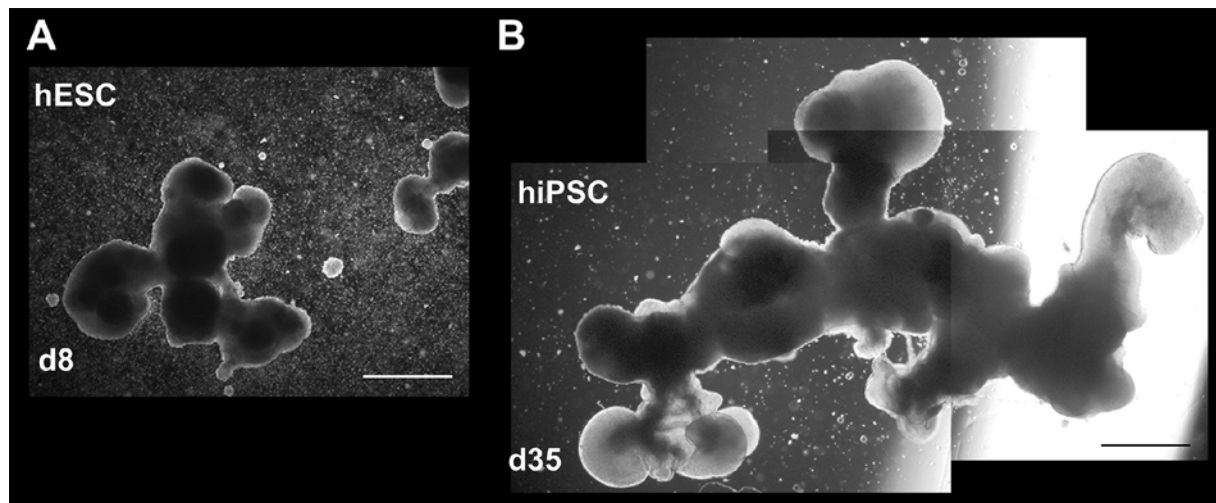


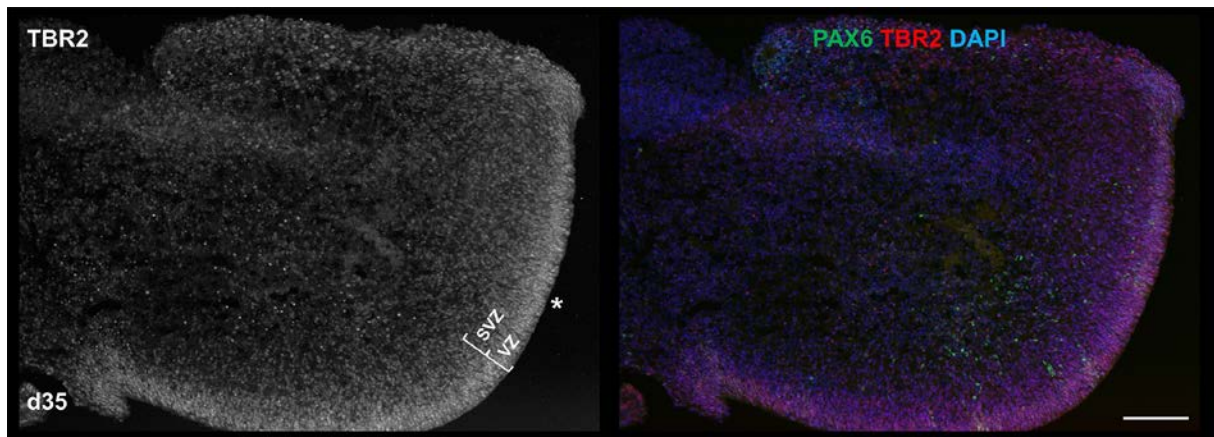
SUPPLEMENTARY FIGURES

Figure S1



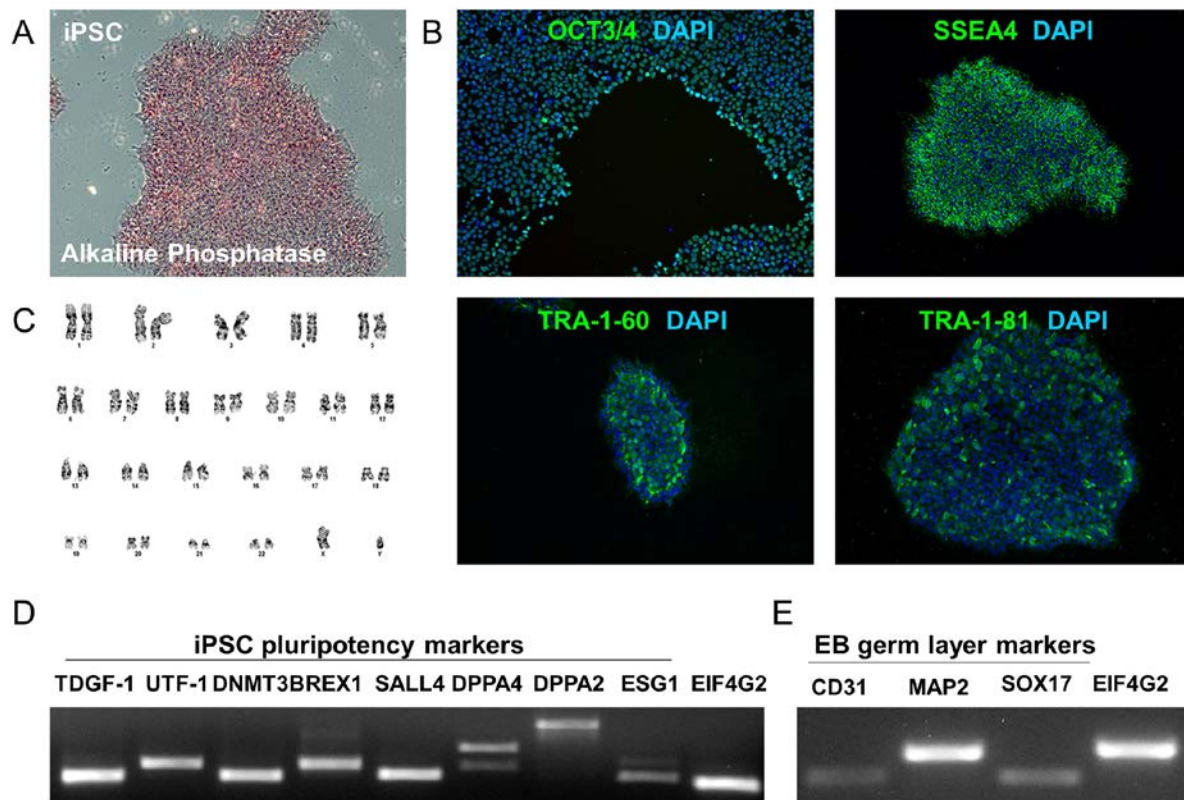
3D culture show variation in aggregate size and complexity. Brightfield images showing (A) hESC-derived aggregates at d8 in culture, with one enlarged likely by early merger of smaller aggregates, and (B) iPSC-derived aggregate demonstrating general extent of growth and complexity for 3D products, though portions may have resulted from early mergers between aggregates and other portions potentially missing, due to breaking off during cell feeding or transfer. Due to size of aggregate, the complete image is constructed by combining three separate brightfield images. Scale bar = 200 μ m.

Figure S2



3D products demonstrate large scale ventral zone-like structure. An hESC-derived aggregate at d35 in culture, positive for early neural markers PAX6 (green) and TBR2 (red) which are associated *in vivo* with ventral zones (VZs) and subventral zones (SVZs), respectively. A dense band of PAX6+/TBR2+ cells lines the outer edge of the aggregate running from the left bottom to upper right corner, with curled lip at one end and a slight outward bump at the other. (A) TBR2 staining signal with asterisk (*) marking the apical side of a VZ-like region lining the edge of the aggregate and brackets indicating depth/division of VZ/SVZs. (B) Merged signals indicate VZ-like structure. Small scattered sections of PAX+/TBR2- cells increase in size toward the bump at the upper right end of the VZ. Scale bar = 100 μ m.

Figure S3



iPSC characterization confirms pluripotency. (A) Alkaline Phosphatase staining (red) is an indicator of pluripotency; (B) ICC staining images for markers of pluripotency (in green) OCT3/4 (upper left), SSEA4 (upper right), TRA-1-60 (lower left), and TRA-1-81 (lower right); (C) Karyotype analysis image showing normal chromosome integrity; (D) PCR results for markers of pluripotency: TDGF1, UTF1, DNMT3B, REX1, SALL4, DPPA4, DPPA2, ESG1 and reference gene EIF4G2; (E) PCR results for markers of different germ layers on EBs: CD31 (mesoderm), MAP2 (ectoderm), SOX17 (endoderm), and reference gene EIF4G2. Characterization images are from iPSC line hvs-88.