Chetty et al., http://www.jcb.org/cgi/content/full/jcb.201502035/DC1

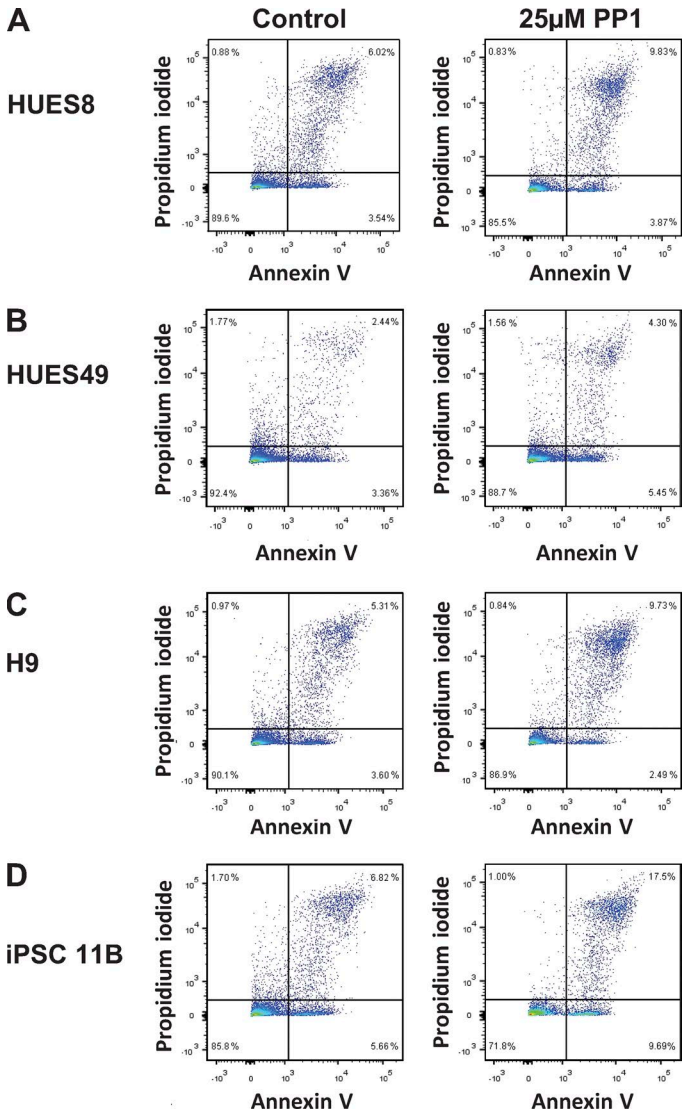

Figure S1. **The viability of hPSCS remains high after the PP1 treatment.** Percentages of hPSCs that are viable (Annexin V/Propidium iodide–), apoptotic (Annexin V+), or necrotic (Annexin V/Propidium iodide+) after no treatment (Control) or a 24-h 25 μM PP1 treatment in the HUES8 (A), HUES49 (B), H9 (C), and iPSC 11b (D) hPSC lines.

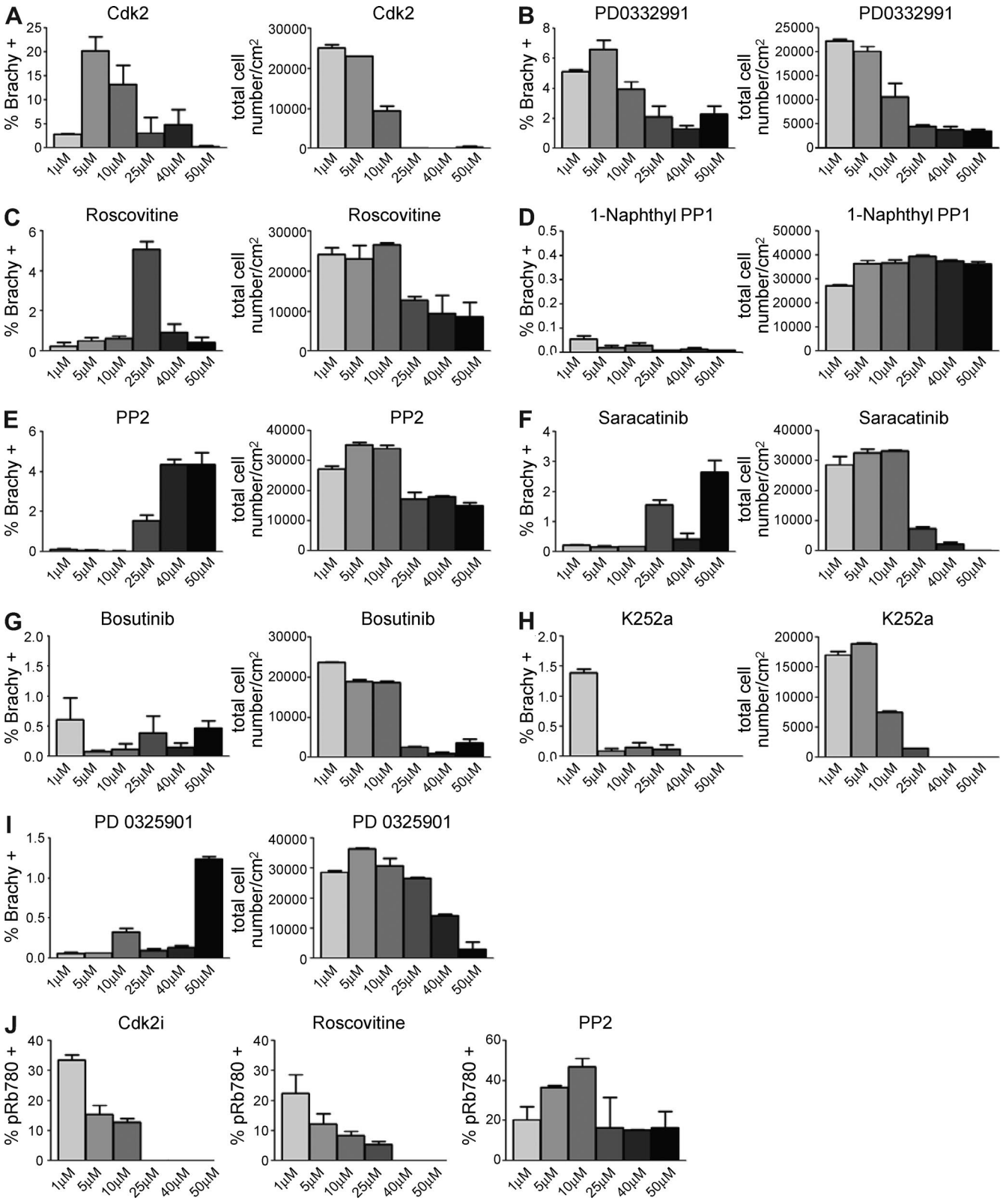

Figure S2. **The differentiation capacity of hPSCs is differentially regulated by various cell cycle–related kinase inhibitors.** (A–I) Percentages of HUES6 hPSCs differentiating into Brachy+ cells after a 24-h treatment with various inhibitors at increasing doses in relation to the total cell numbers. (J) Percentage of HUES6 hPSCs expressing the hyperphosphorylated Rb (pRb780) after a 24-h treatment with various inhibitors at increasing doses. Error bars indicate SEM of 2–3 replicates.

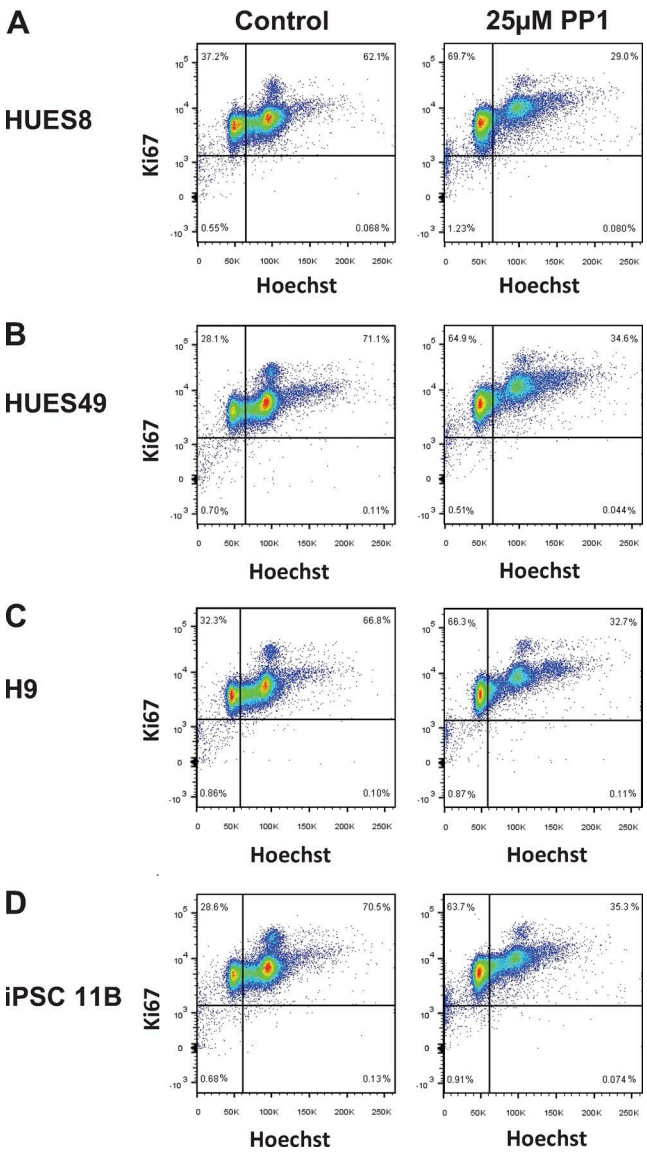

Figure S3. **Enrichment of hPSC lines in the G1 phase of the cell cycle after the PP1 treatment.** Distribution of hPSCs in the G1 phase relative to the S/G2/M phases of the cell cycle after no treatment (Control) or a 24-h 25 μM PP1 treatment in the HUES8 (A), HUES49 (B), H9 (C), and iPSC 11b (D) hPSC lines as assessed by the Hoechst cell cycle dye and the Ki67 proliferation marker.

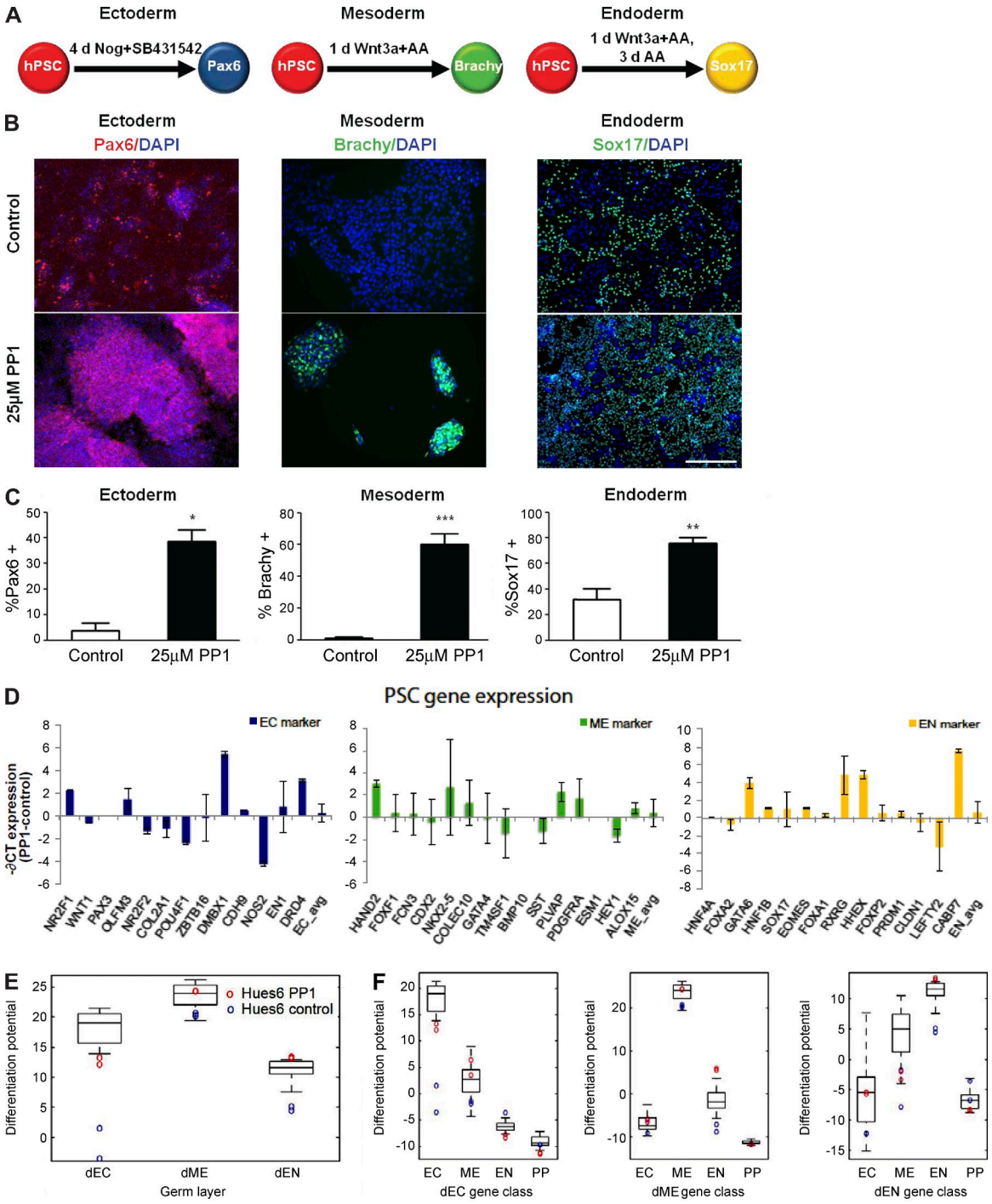

Figure S4. **PP1 treatment improves the differentiation capacity of hPSCs across all germ layers.** (A) Schematic of directed differentiation of HUES6 hPSCs into Pax6+ ectodermal, Brachy+ mesodermal, or Sox17+ endodermal cells after no treatment (Control) or a 24-h 25 μM PP1 treatment. (B) Immunostaining for the indicated differentiation markers after directed differentiation in control and PP1-treated cultures. Bar, 200 μM. (C) Percentages of cells expressing the indicated differentiation markers in control and PP1-treated cultures. Error bars indicate SEM of 2–3 replicates. *, $P \leq 0.05$; **, $P \leq 0.01$; ***, $P \leq 0.001$. (D) Difference in mean expression between PP1-treated and control samples, $-\partial CT = -(CT_{PP1} - CT_{CONTROL})$, for selected ectoderm (left), mesoderm (center), and endoderm (right) marker genes in HUES6 hPSCs before directed differentiation into the three germ layers. All markers with $-\partial CT \geq 1.9$ or ≤ -1.9 are displayed, where $-\partial CT = 2$ represents a fourfold increase in mean expression for PP1-treated versus control samples. The mean difference in expression for all ectoderm, mesoderm, and endoderm markers on the Scorecard panel (Life Technologies) is displayed as the rightmost bar. Error bars indicate standard deviation in difference in expression of two replicates. (E) Box plots show the distribution of differentiation potential for a reference set of 14 hPSC lines (Bock et al., 2011) after directed differentiation into ectodermal (dEC), mesodermal (dME), and endodermal (dEN). Circles show the differentiation potential of PP1 treated (red) and control (blue) samples in replicate after differentiation of the hPSC line HUES6 into dEC, dME, and dEN. (F) More detailed box plots of the distribution of differentiation potential for a reference set of 14 hPSC lines after directed differentiation into dEC (left), dME (middle), and dEN (right). Differentiation potential for each germ layer was calculated (Bock et al., 2011) using gene expression signatures from four gene classes (EC, ectoderm; ME, mesoderm; EN, endoderm; PP, pluripotent). Circles show the differentiation potential of PP1 treated (red) and control (blue) samples in replicate.

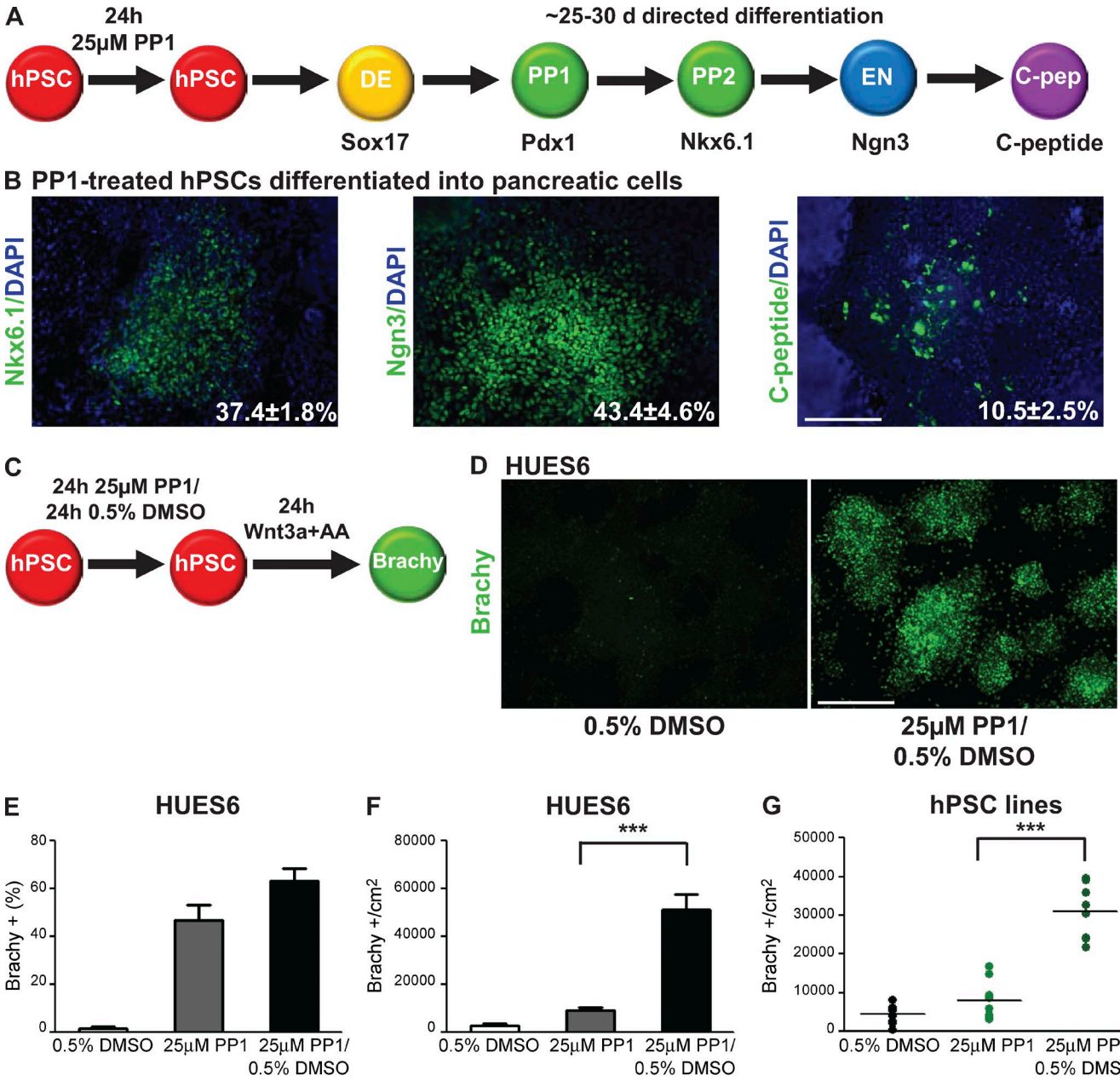

Figure S5. **Beneficial impacts of the PP1 treatment on the differentiation capacity of hPSCs.** (A) Schematic of stepwise differentiation along the pancreatic lineage into terminally differentiated hormone-expressing C-peptide (C-pep+) cells following a 25–30-d directed differentiation protocol after a 24-h 25 μM PP1 treatment. (B) Immunostaining and quantification for Nkx6.1+, Ngn3+, and C-peptide+ pancreatic cells in PP1-treated cultures after directed differentiation. Results are SEM of three replicates. (C) Schematic of directed differentiation of hPSCs into Brachy+ cells after no treatment or a 25-μM PP1 treatment for 24 h and a subsequent 0.5% DMSO treatment for 24 h. (D) Immunostaining for Brachy in DMSO-treated control (0.5% DMSO) and DMSO-treated PP1 cultures (25 μM PP1/0.5% DMSO) after directed differentiation. (E and F) Percentages (E) and absolute numbers (F) of DMSO-treated control, PP1-treated, and DMSO-treated PP1 HUES6 hPSCs differentiating into Brachy+ cells. (G) Absolute numbers of DMSO-treated control, PP1-treated, and DMSO-treated PP1 hPSCs differentiating into Brachy+ cells across the HUES8, HUES49, H9, and iPSC 11b hPSC lines. Error bars indicate SEM of 2–3 replicates. Bars, 200 μM. ***, $P \leq 0.001$.

Table S1. **Complementary DNA PCR primer sequences**

Human gene	Forward sequence	Reverse sequence
Cyclin A1	GAGGTCCCGATGCTTGTCAG	GTTAGCAGCCCTAGCACTGTC
Cyclin A2	CGCTGGCGGTACTGAAGTC	GAGGAACGGTGACATGCTCAT
Cyclin D1	GCTGCGAAGTGGAAACCATC	CCTCCTTCTGCACACATTTGAA
Cyclin E1	AAGGAGCGGGACACCATGA	ACGGTCACGTTTGCCTTCC
Cdk2	CCAGGAGTTACTTCTATGCCTGA	TTCATCCAGGGGAGGTACAAC
Cdc6	CCAGGCACAGGCTACAATCAG	AACAGGTTACGGTTTGGACATT
E2f1	ACGCTATGAGACCTCACTGAA	TCCTGGGTCAACCCCTCAAG
p21	TGTCCGTCAGAACCCATGC	AAAGTCGAAGTTCCATCGCTC
p27	AACGTGCGAGTGTCTAACGG	CCCTCTAGGGGTTTGTGATTCT
GAPDH	TGTTGCCATCAATGACCCCTT	CTCCACGACGTACTCAGCG

Reference

Bock, C., E. Kiskinis, G. Verstappen, H. Gu, G. Boulting, Z.D. Smith, M. Ziller, G.F. Croft, M.W. Amoroso, D.H. Oakley, et al. 2011. Reference Maps of human ES and iPS cell variation enable high-throughput characterization of pluripotent cell lines. *Cell.* 144:439–452. http://dx.doi.org/10.1016/j.cell.2010.12.032