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Supplemental Data

High-Throughput Screening Assay for the Identification of Compounds Regulating Self-Renewal and Differentiation in Human Embryonic Stem Cells

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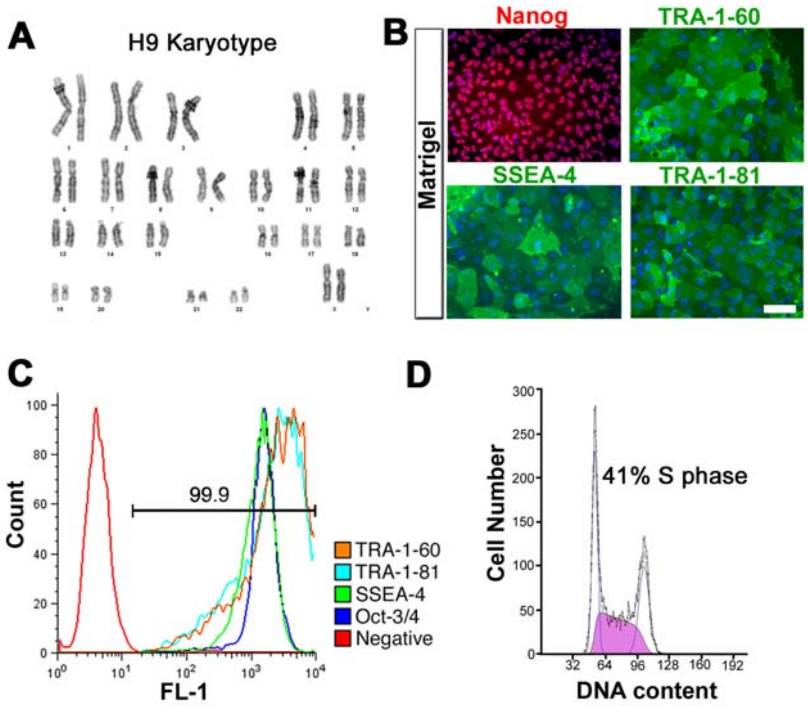

Figure S1 Characterization of H9 hESCs at the time of HTS plating. (**A**) Normal karyotype confirmed by G-banding of M-phase cells. (**B**) Representative images for expression of Nanog, Tra-1-60, SSEA-4, and Tra-1-81 in hESCs maintained on matrigel prior to HTS plating. (**C**) Quantification of the purity of H9 hESCs using flow cytometry. (**D**). Cell cycle analysis by flow cytometry using PI citrate. Scale bar in **B** corresponds to 100μm for all panels.

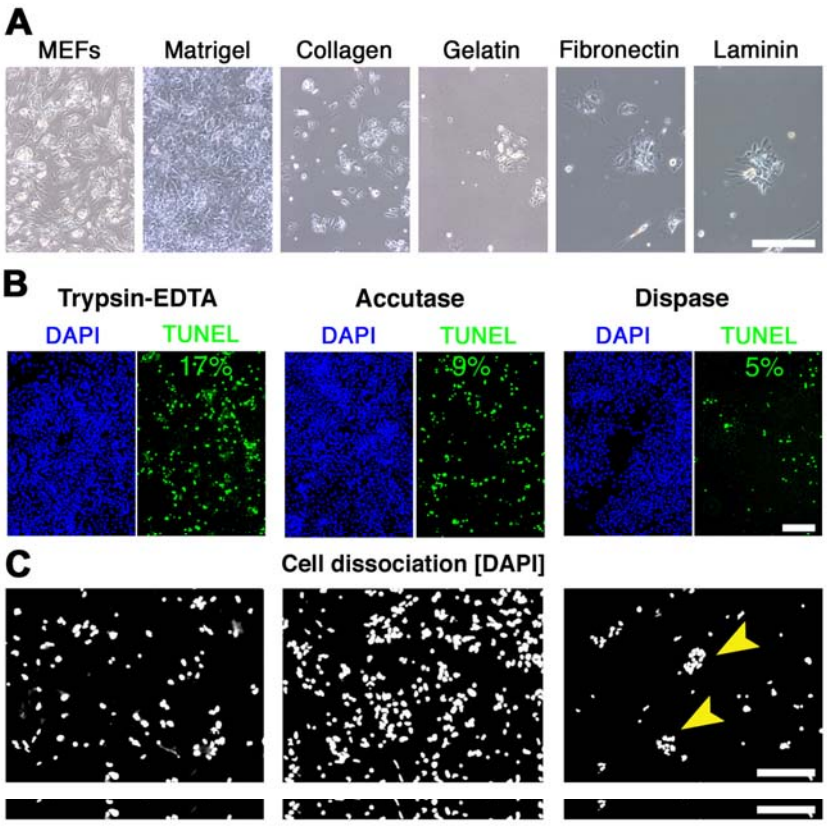

Figure S2 Optimization of hESC dissociation and attachment. (**A**) A number of substrates were tested on 96 and 384 well plates followed by analysis of cell morphology 24hrs after plating. (**B**) Comparison of various dissociation regimens followed by plating and analysis of cell death. (**C**) Analysis of cell density and cell dispersion following dissociation and plating. Arrowheads indicate cell clusters not dissociated by dispase. Scale bars correspond to 50μm.

A

	FGF2			
	Plate 1	Plate 2	Plate 3	Avg
Avg	1745	1772	1458	1658
SD	359	237	265	254
CV	15	13	18	15

	BMP4			
	Plate 1	Plate 2	Plate 3	Avg
Avg	251	246	247	248
SD	47	42	45	45
CV	19	17	18	18

Z'	0.37

B

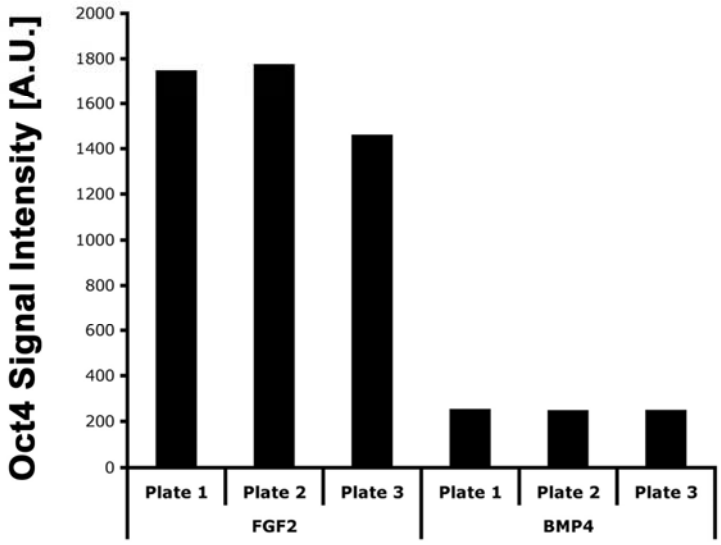

Figure S3 Validation of high and low controls – calculation of Z' score. (**A**). Quantification of Signal Intensity values for Oct4 followed by statistical analysis. (**B**) Graph of mean value for each FGF2 and BMP4 plates.

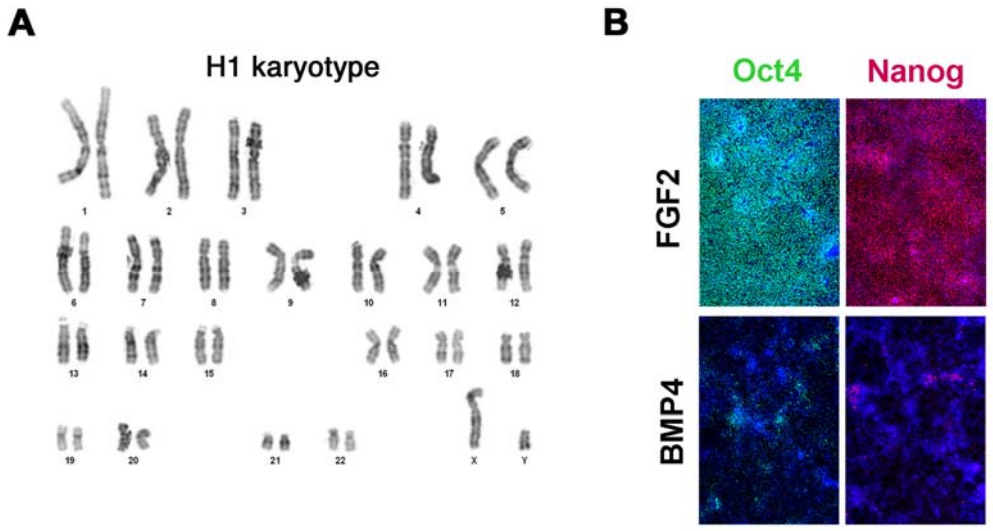

Figure S4 Validation of H1 cells in HTS assay. (**A**) Normal karyotype of H1 cell line prior to 384 well plating was confirmed by G-banding. (**B**). Effect of high and low controls on the expression of self-renewal factors Oct4 and Nanog. Scale bar in **B** corresponds to 50μm for all panels.

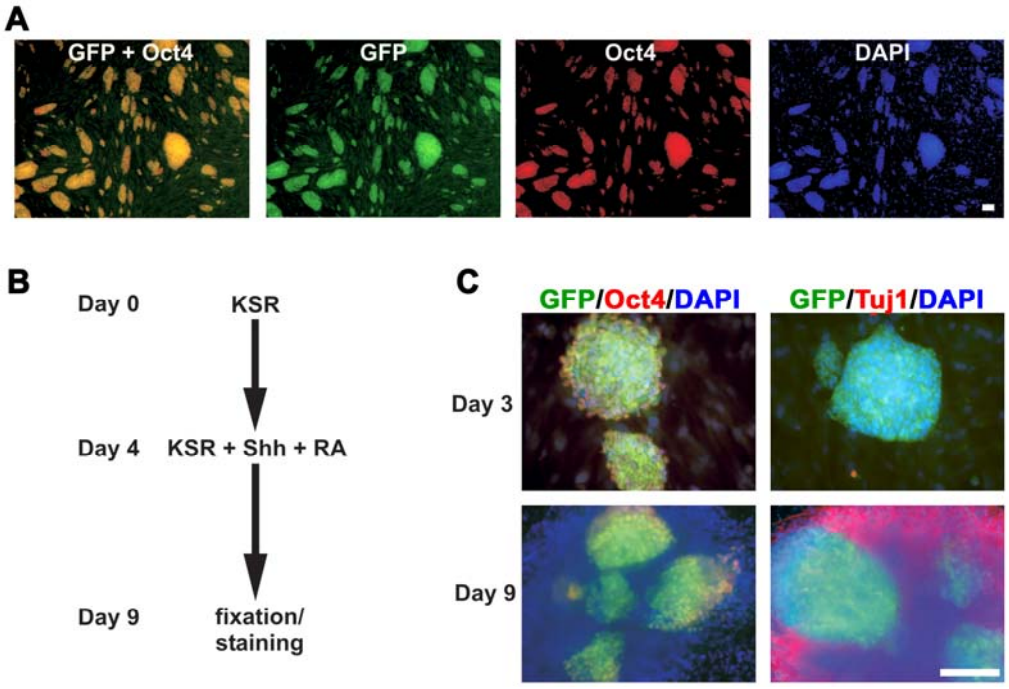

Figure S5 Generation of BAC transgenic *Oct4::eGFP* cell lines for validating activator compounds in mouse ESCs. (**A**) BAC transgenic *Oct4::eGFP* mouse ESCs showing robust colocalization of GFP (green) with the endogenous Oct4 (red) immunoreactivity. When subjected to FACS analysis, 85% of the mouse ES cells were positive for GFP. (**B**) Protocol for mouse ESC differentiation on MS5 feeders toward spinal motor neuron identity (Barberi et al., 2003). Briefly, single mouse ES cells were plated on MS5 feeders in KSR (knockout serum replacement) medium. On day 4, sonic hedgehog (SHH) and retinoic acid (RA) were added to the medium. (**C)** Progressive loss of GFP+ (green) undifferentiated ESCs during differentiation on MS5. On day 3, the small cell clusters were still positive for Oct4 (red) and GFP, while there was no Tuj1 immunoreactivity. On day 9, however, the much larger colonies contained only limited foci of Oct4 and GFP+ undifferentiated cells, with abundant Tuj1 immunoreactivity on the periphery. Scale bars, 100 μm.

Barberi, T., Klivenyi, P., Calingasan, N.Y., Lee, H., Kawamata, H., Loonam, K., Perrier, A.L., Bruses, J., Rubio, M.E., Topf, N., et al. (2003). Neural subtype specification of fertilization and nuclear transfer embryonic stem cells and application in parkinsonian mice. Nat. Biotechnol. 21, 1200–1207.

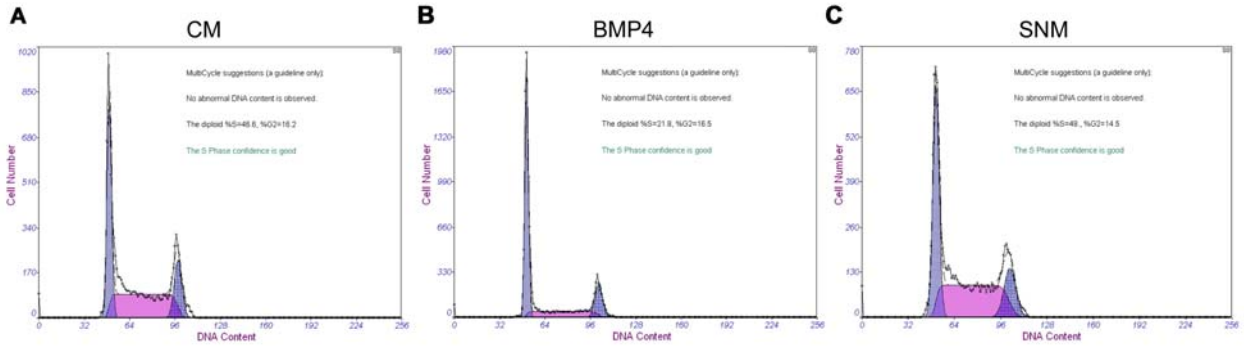

Figure S6 Representative Cell Cycle Profiles of hESCs after self-renewal compound treatments. (**A-C**) Cell cycle analysis using FACS after PI-citrate solution treatment of hESCs in CM (**A**) as positive control, BMP4 (**B**) as negative control and SNM (**C**).

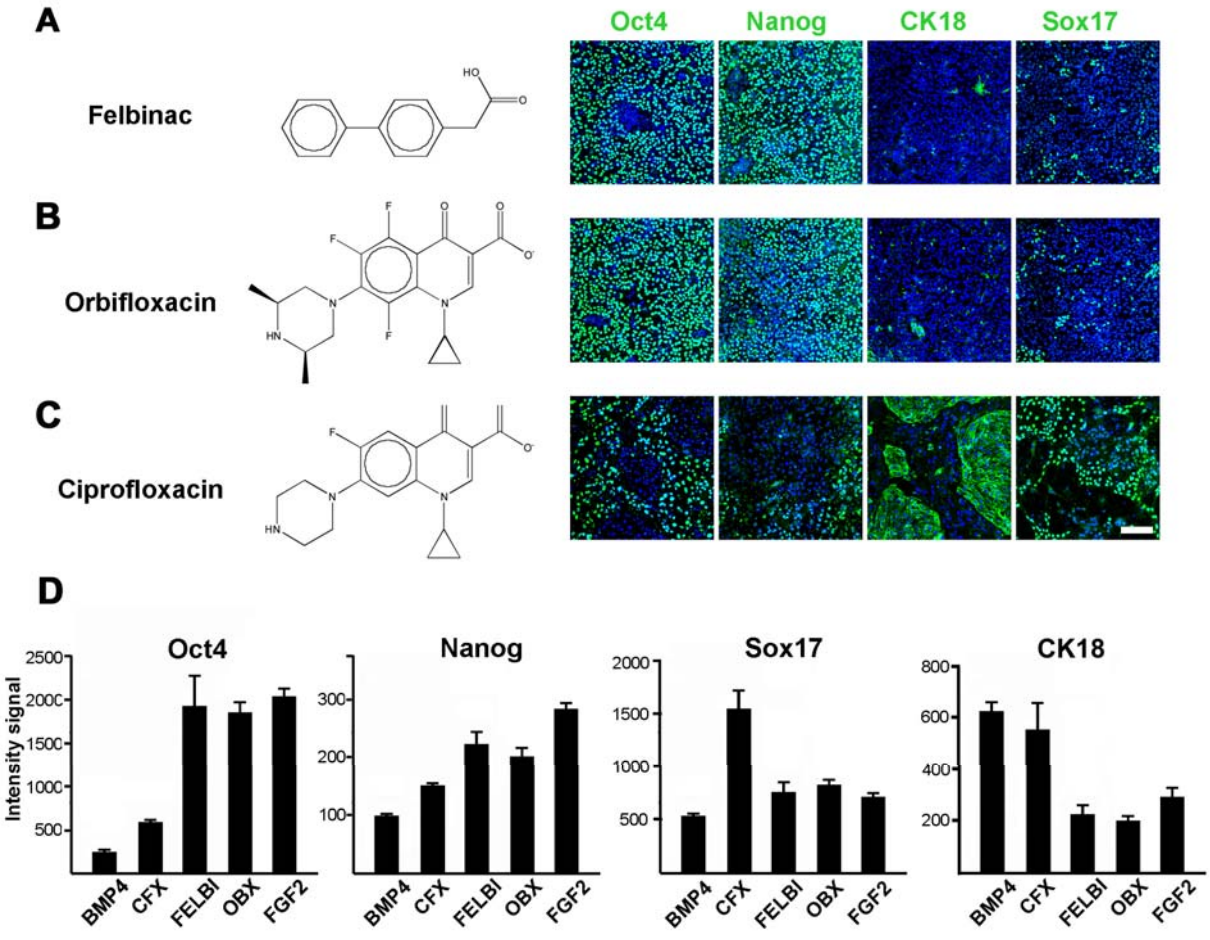

Figure S7 Agonist and antagonist actions of derivatives of hit compounds. (**A-C**) Felbinac (derivative of Flurbiprofen) and Orbifloxacin and Ciprofloxacin (derivatives of Gatifloxacin) were tested for the induction of self-renewal and differentiation markers. (**D**) Quantification of the immunocytochemical data acquired on INCell 3000 analyzer. Data for each marker are represented from independent duplicate cultures. Scale bar corresponds to 50μm for all panels.

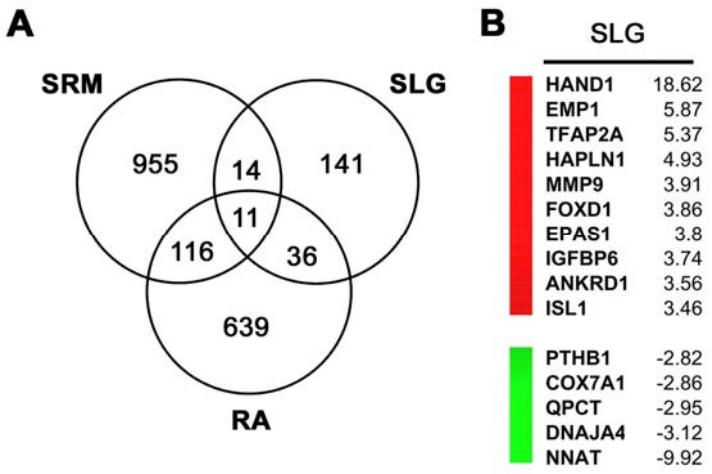

Figure S8 Venn diagram (**A**) and list of most differentially upregulated and downregulated transcripts (**B**) in hESCs exposed to Selegiline (SLG) compared to cells exposed to Tretinoin (RA) or Sarmentogenin (SRM) treatment. Differentially expressed genes were identified as those significantly regulated compared with hESCs grown in KSR medium alone.