Supplementary Methods

qPCR and Microarray

All microarray and mRNA qPCR analysis was performed as described in Materials and Methods. For miRNA qPCR analysis, the indicated miRNAs were tested using Taqman miRNA qPCR Assays (Applied Biosystems, Foster City, CA) according to manufacturer's protocol.

Validation of iPSC pluripotency

Images of colony morphology and immunofluorescence of induced pluripotent stem cells (iPSCs) and fibroblast cell lines were performed as described in Materials and Methods. iPS cells were evaluated for the presence of tissue nonspecific alkaline phosphatase activity by fixing colonies in phosphate-buffered saline solution containing 0.5% glutaraldehyde, and washing 3 times in PBS. A staining buffer containing 100 mM Tris pH 8.5, 100 mM NaCl, 50 mM $MgCl_2$, 0.1 mg/mL 5-bromo-4-chloro-3-indolyl phosphate (xphos), and 1 mg/mL *p*-nitro-blue tetrazolium chloride (NBT; Sigma-Aldrich, St. Louis, MO) was used to detect tissue non-specific alkaline phosphatase activity.

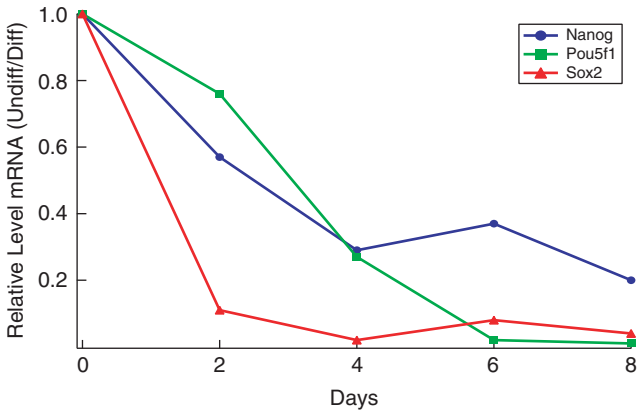
iPSC Teratoma formation

Induced pluripotent stem cells were detached from culture dishes with dispase (2 units/mL working concentration), 2×10^6 cells resuspended in F12:DMEM (1:1 mixture) medium without supplements, and injected into the femoral muscle of SCID-Beige mice (CB17.B6-*Prkdc*^{scid}*Lyst*^{bg}/Crl Charles River, Stock # 250). Mice were maintained under biosafety containment level 2 conditions and palpable tumor masses developed ~6 weeks later. When a tumor mass was palpable the mice were sacrificed, tumor tissue was fixed for several days in phosphate-buffered saline (PBS) solution containing 4% formaldehyde, and imbedded in paraffin. Sections of the tumor (5-µM thickness) were placed on slides and stained with hematoxylin and eosin using standard protocols. We are grateful to D. Miller for kindly providing Moloney leukemia retroviruses and human foreskin fibroblasts, and to D. Russell for supplying lentiviruses and human myocardial fibroblasts.

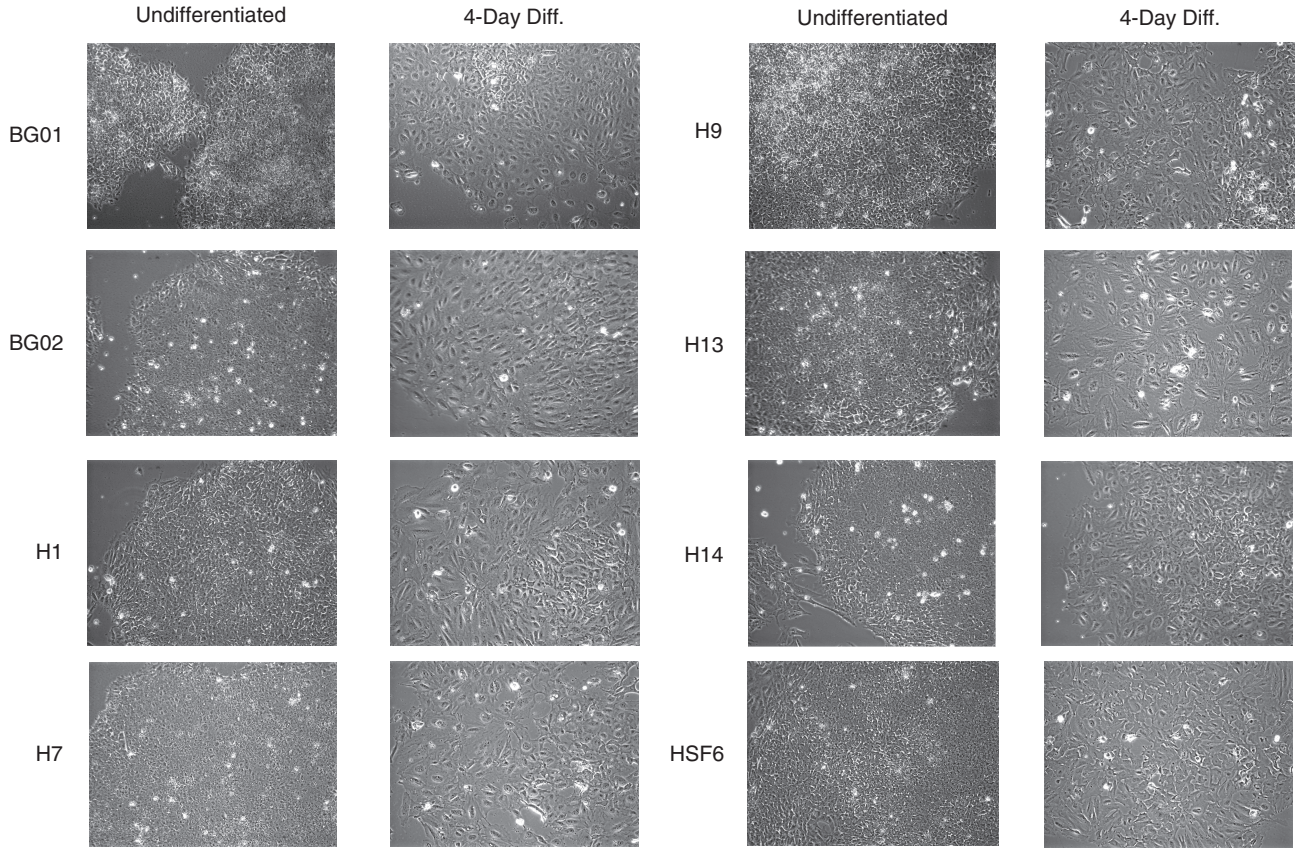
Supplementary Table 1. Top 20 Up-Regulated and Down-Regulated miRNAs Following Early Commitment of hESCs

Top 20 miRNAs Down-regulated		*Top 20 miRNAs Up-regulated*	
hESCs	NTera-2	hESCs	NTera-2
miR-124a	miR-367	miR-30a-5p	miR-99a
miR-367	miR-302b	miR-24	miR-675
miR-498	miR-373	miR-145	miR-21
miR-19b	miR-372	miR-181d	miR-10a
miR-19a	miR-302a	miR-22	miR-100
miR-18a	miR-630	miR-125b	miR-181b
miR-18b	miR-302a*	miR-622	miR-30a-5p
miR-17*	miR-302c	miR-29a	miR-149
miR-141	miR-302d	miR-125a	miR-99b
miR-302b*	miR-371	miR-23a	miR-766
miR-148a	miR-302b*	miR-27a	miR-22
miR-302c	miR-302c*	miR-28	miR-125a
miR-20a	miR-9	miR-181b	miR-29b
miR-302a	miR-205	miR-21	miR-181a
miR-302d	miR-199a	miR-149	miR-133b
miR-101	miR-422b	miR-500	miR-29a
miR-363	miR-381	miR-193b	miR-331
miR-20b	miR-663	miR-486	miR-133a
miR-302a*	miR-18b	miR-503	miR-30c
miR-302b	miR-17*	miR-222	miR-342
C19MC (512-3p, 512-5p, 515-3p, 516-5p, 517b, 517c, 518b, 518c, 518f, 518f*, 519b, 519c, 519d, 519e*, 520a*, 520b, 520c, 520f, 521, 523, 525, 526a, 527)*			

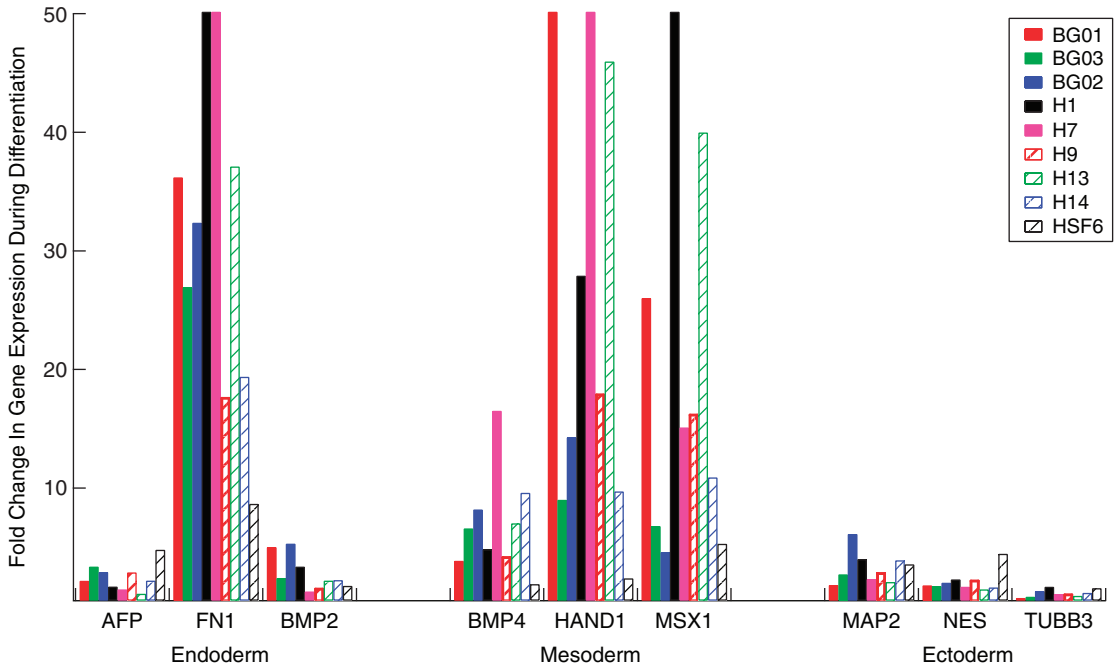
Microarray expression data was used to determine fold changes for each microRNA (miRNA) gene in each human embryonic stem cells (hESCs) line. The miRNAs most highly down-regulated or up-regulated upon 4-day differentiation (7 of 9 hESC lines with at least a 1.5-fold change in expression) are shown in comparison with NTera-2. The down-regulated miRNAs from C19MC are denoted in italics because expression levels for these miRNAs in differentiated cell lines were generally at or below the threshold of detection, preventing accurate fold-change levels to be determined.



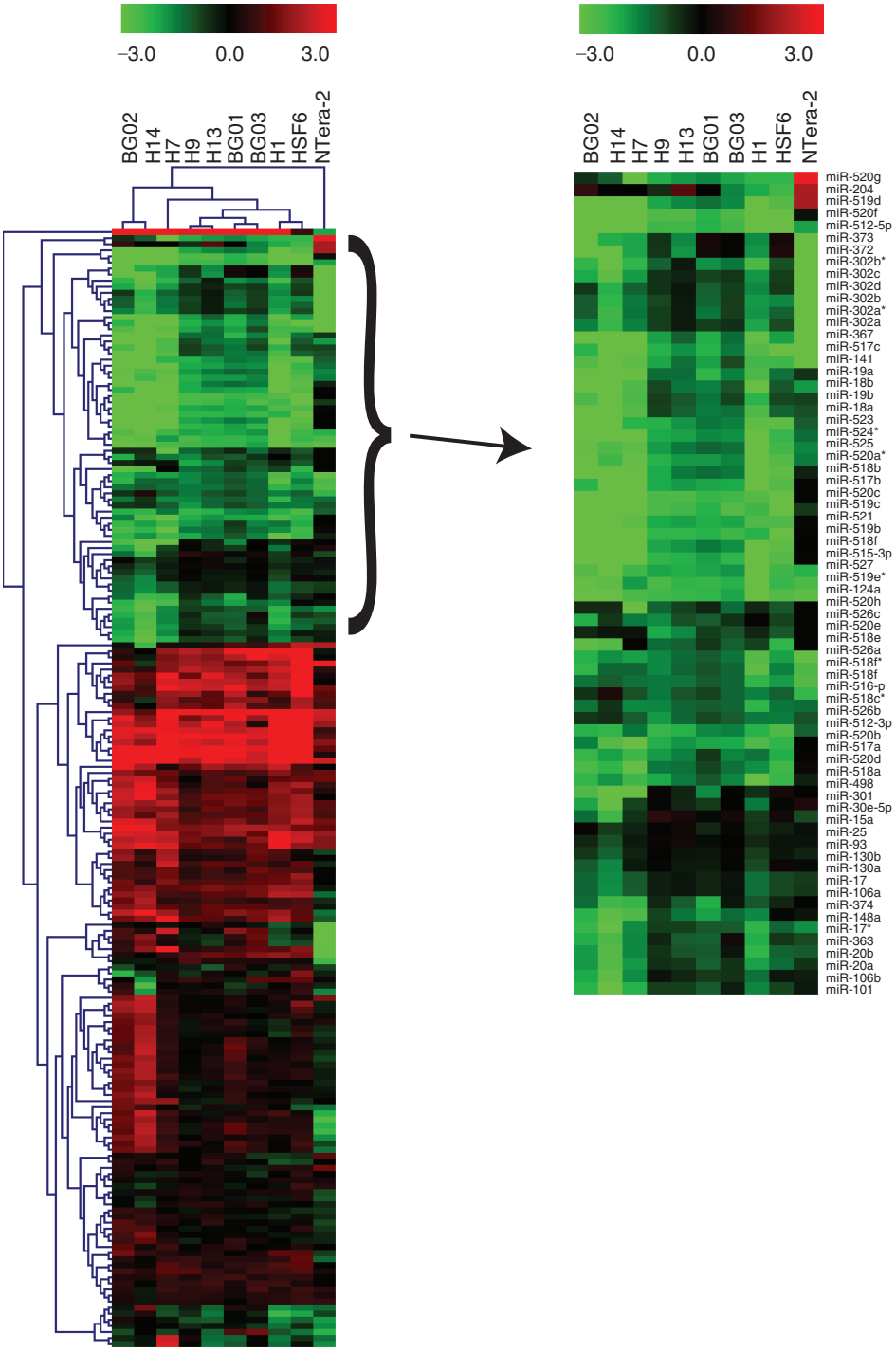
SUPPLEMENTARY FIG. 1. Assessing stem cell marker expression during serum-induced differentiation. Expression of pluripotent stem cell markers, Nanog, Sox2, and Oct4 (Pou5f1) were monitored in undifferentiated and differentiated H1 cells over 8 days by qRT-PCR. All samples are internally normalized using β-actin levels and expression levels are reported as the fold change in expression (Undiff/Diff).



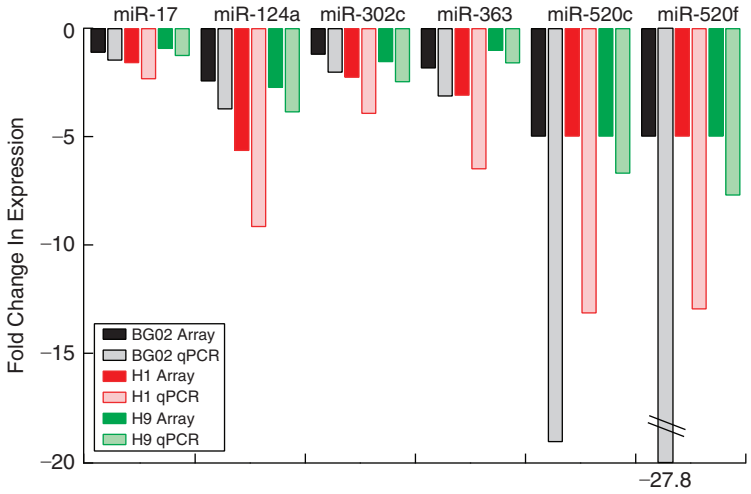
SUPPLEMENTARY FIG. 2. Differentiation of human embryonic stem cell (hESC) colonies. Phase-contrast images of undifferentiated and differentiated hESC during the differentiation protocol. All images shown were captured using a 10× magnification.



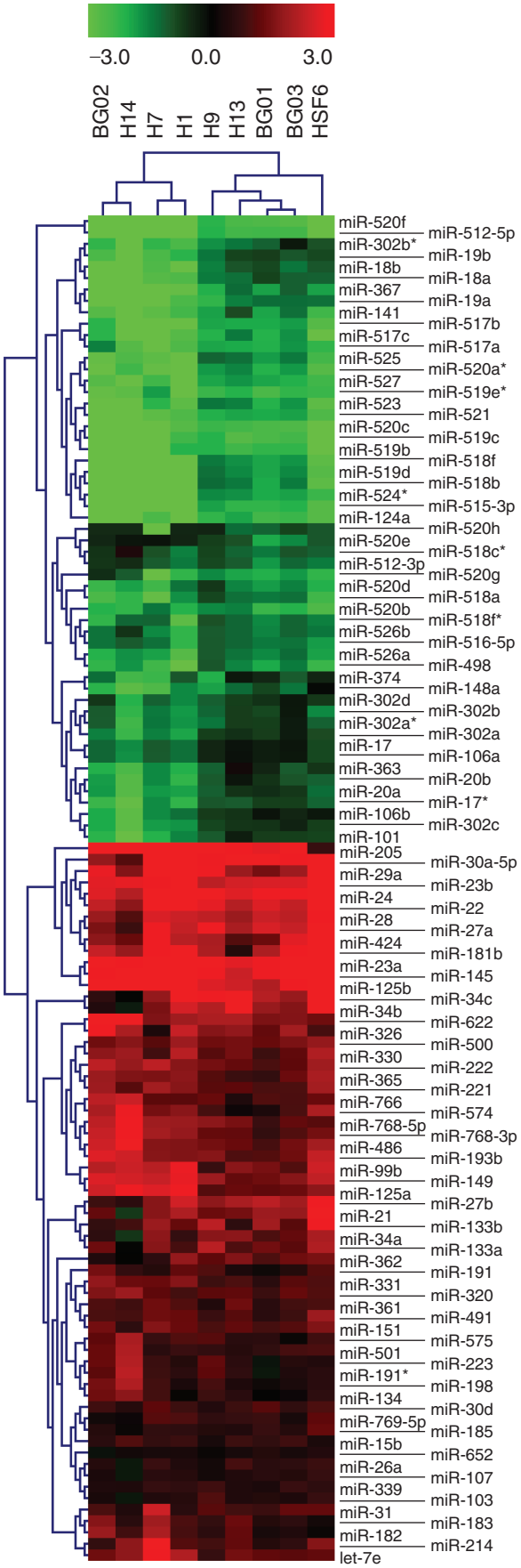
SUPPLEMENTARY FIG. 3. Germ layer marker expression during differentiation. Expression of several markers for each germ layer was monitored in all undifferentiated and differentiated human embryonic stem cell (hESC) lines by microarray analysis. Data are expressed as the change in the level of gene expression during 4-day differentiation (Diff/Undiff).



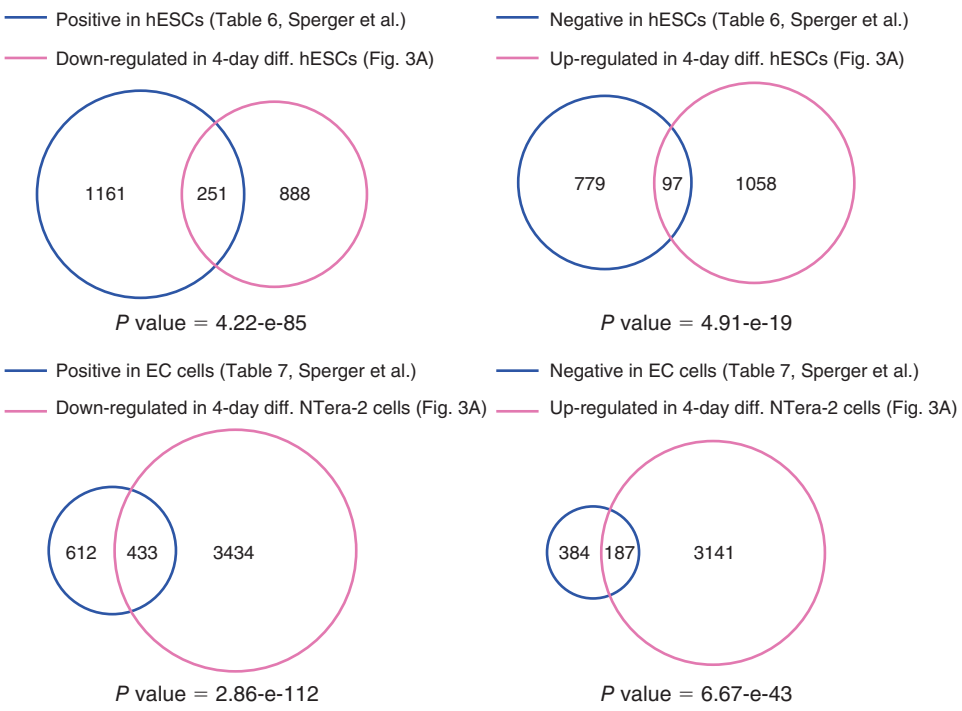
SUPPLEMENTARY FIG. 4. MicroRNA (miRNA) expression levels in human embryonic stem cells (hESCs) and NTera-2. Expression profiling analysis for the 9 hESC lines and the NTera-2 embryonal carcinoma cell line is displayed. The $\log_2$ ratio of differentiated/undifferentiated signal for each cell line was subjected to hierarchical clustering by Euclidean distance metric using average linkage.



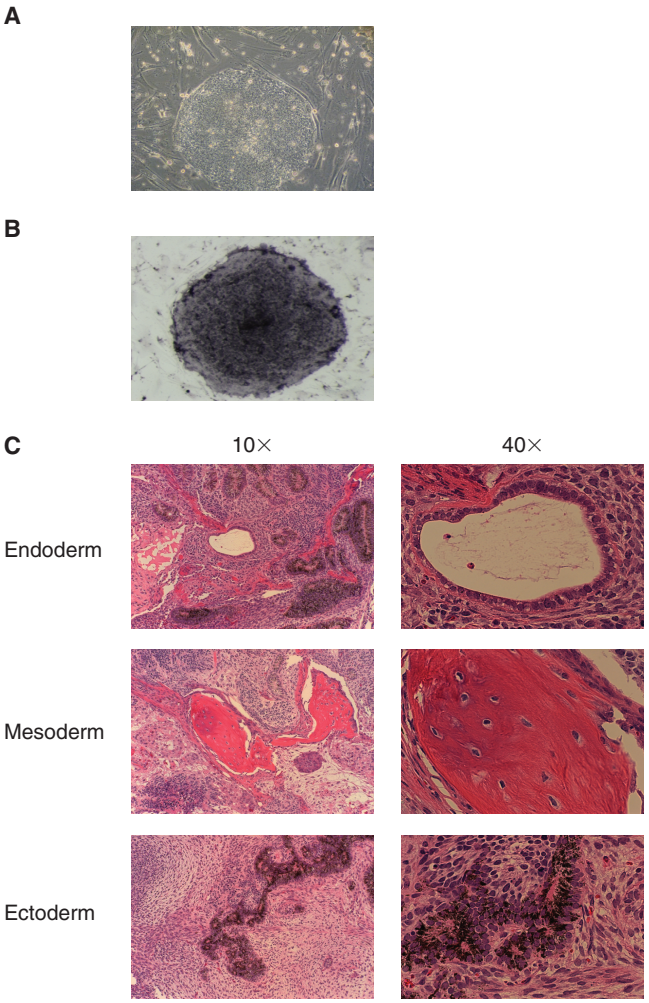
SUPPLEMENTARY FIG. 5. qRT-PCR and microarray validation of human embryonic stem cell (hESC)-enriched microRNA (miRNA) expression data. Additional samples for hESC lines BG02, H1, and H9 subjected to the same differentiation protocol as described for the 9 hESC lines were prepared. Select low-expression miRNAs demonstrating differential expressions in the initial samples were analyzed by both microarray and miRNA-specific qRT-PCR in the new samples to confirm the initial findings. The high expression, differentially expressed miRNAs, miR-302c and miR-363, are included as positive controls, while miR-17, which did not significantly change expression during the 4-day differentiation scheme, was included as a negative control. All qPCR samples were internally normalized to a small RNA control (RNU66; Applied Biosystems, Foster City, CA) and the data are expressed as fold change in expression during differentiation.



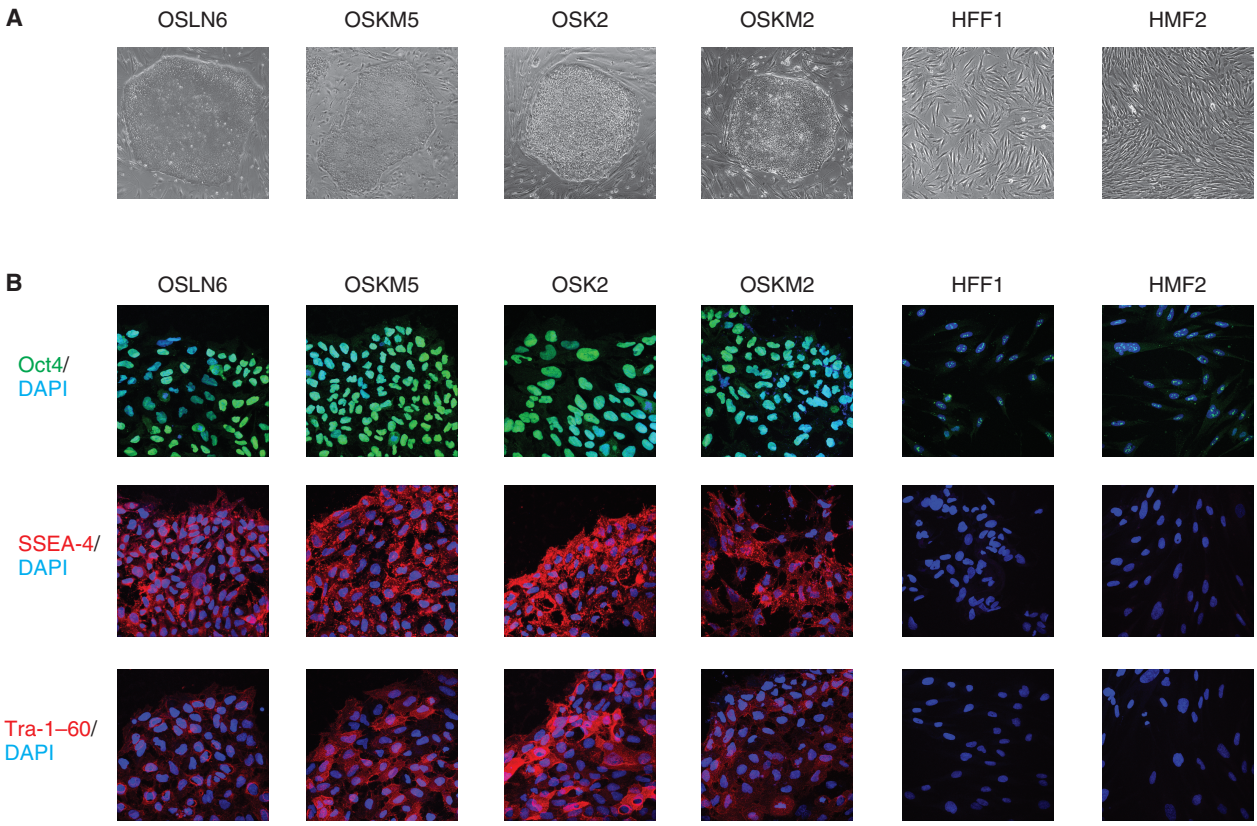
SUPPLEMENTARY FIG. 6. MicroRNAs (miRNAs) most significantly regulated in human embryonic stem cells (hESCs). A one-class *T*-test analysis was performed to determine the miRNAs whose expression was most significantly regulated during the 4-day differentiation protocol for the 9 hESC lines examined. The significantly regulated genes were further subjected to hierarchical clustering by Euclidean distance metric using average linkage.



SUPPLEMENTARY FIG. 7. Comparative analysis of mRNA regulation during differentiation protocols. A Fisher's exact test was used to compare the overlap between genes regulated in this study (Fig. 4A) and those generated by Sperger and colleagues. Venn diagrams of the data analysis were generated in MatLab and the *P* values are displayed below each comparison demonstrating the high similarity in the overlapping gene sets in these analyses. Abbreviation: hESCs, human embryonic stem cells.



SUPPLEMENTARY FIG. 8. Validation of the M83.9 induced pluripotent stem cell (iPSC) line. The M83.9 iPSC line was induced by the retroviral expression of Oct4, Sox2, and Klf4 in HFF1 fibroblasts. Stem cell characterization of this line includes the formation of human embryonic stem cell (hESC)-like colonies (**A**) and the presence of alkaline phosphatase staining in the cells (**B**). (**C**) Pluripotency of the M83.9 line is further demonstrated by the formation of teratomas upon intramuscular injection of M83.9 cells into NOD/SCID mice. Endoderm-derived tissue is identified by a gut-like structure outlined by smooth muscle, parenchymal tissue, and lined with columnar endothelium. Mesoderm-derived tissue is identified by bone, and ectoderm-derived tissue is identified by the presence of pigmented neural epithelium. Photos show at 10× and 40× magnifications.



SUPPLEMENTARY FIG. 9. Validation of the OSLN6, OSKM5, OSK2, and OSKM2 induced pluripotent stem cell (iPSC) lines. The names of the indicated iPSC lines were lines are derived from the cocktail of pluripotency factors used to induce the IPSCs (O = Oct4, S = Sox2, L = Lin28, N = Nanog, M = c-Myc). (**A**) Light microscopic images demonstrate that the iPSC lines adopt human embryonic stem cell (hESC)-like colony morphology, and that the parental fibroblast lines, HFF1 and HMF2, do not. (**B**) Immunofluorescent microscopy of iPSC lines and parental fibroblast lines indicates the presence of the stem cell markers, Oct4, SSEA-4, and Tra-1–60 in the iPSC lines.