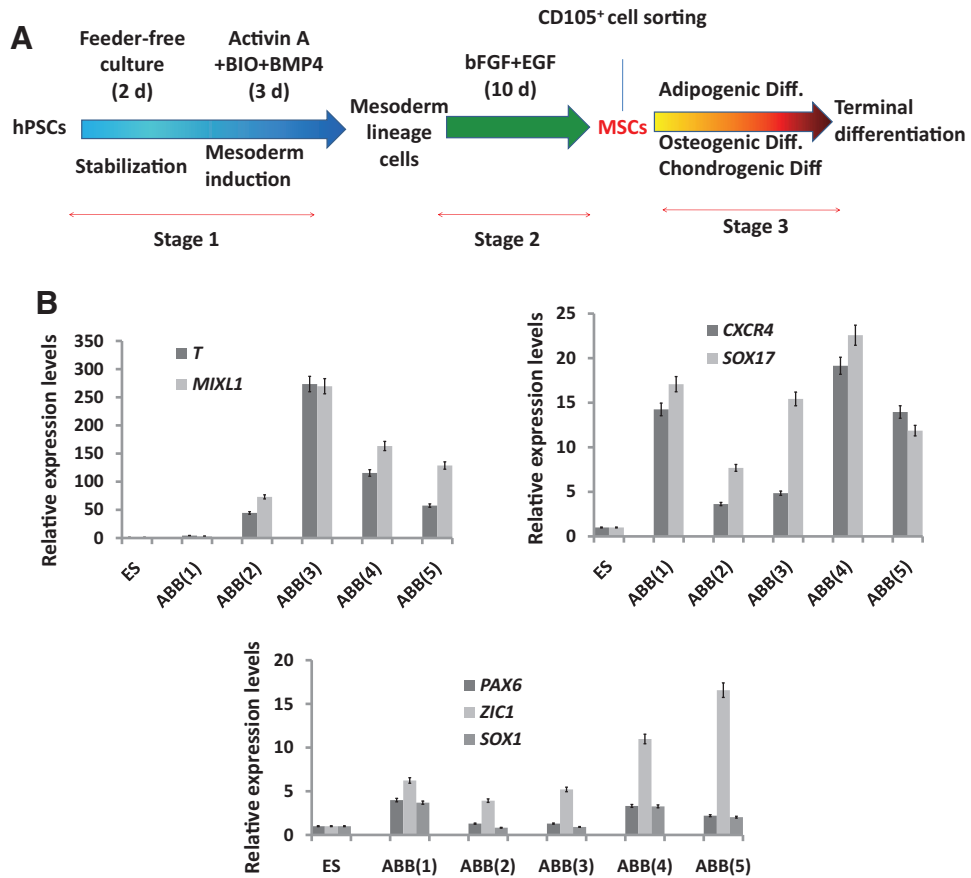
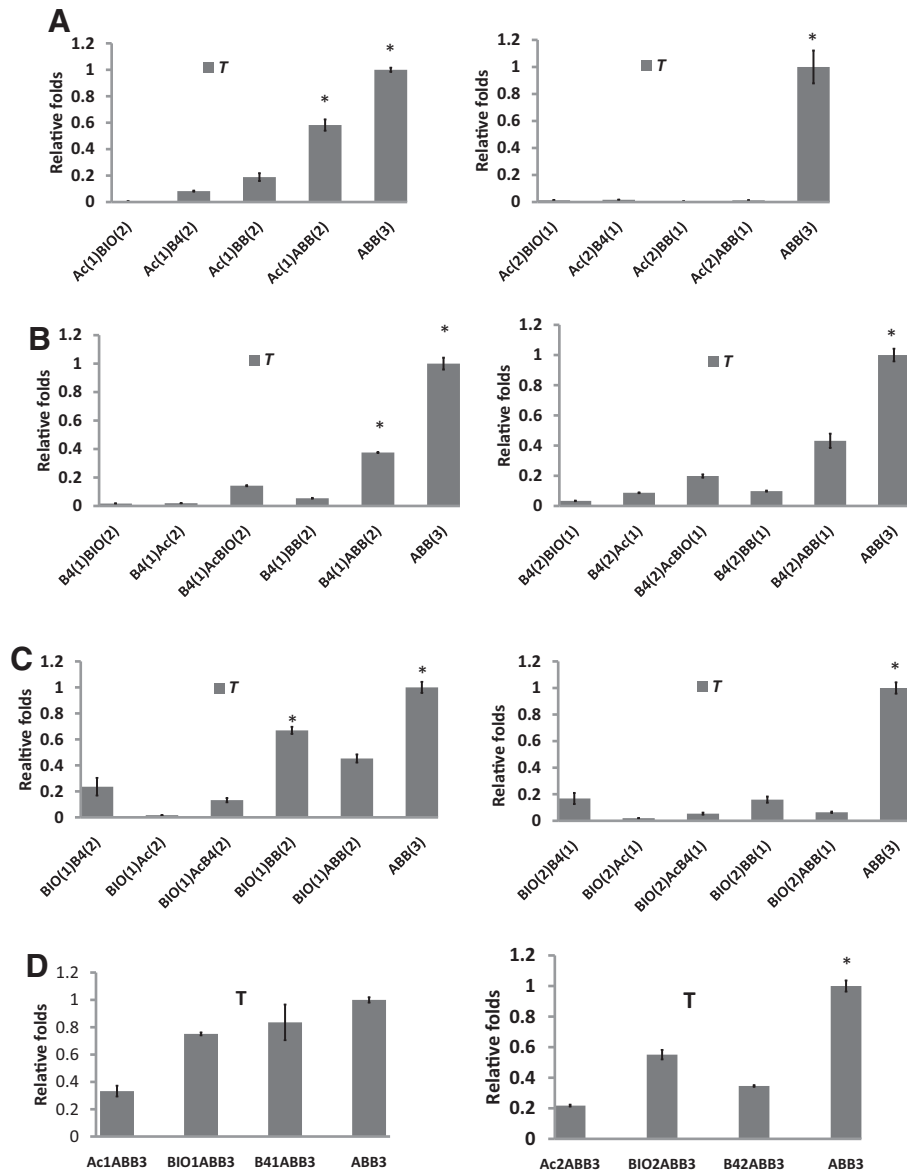


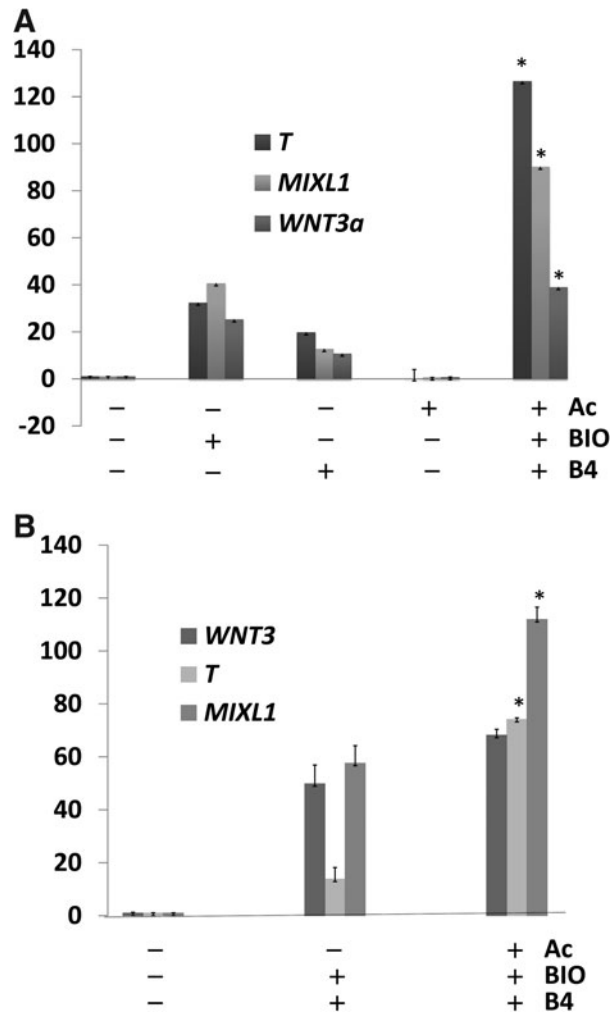
## Supplementary Data



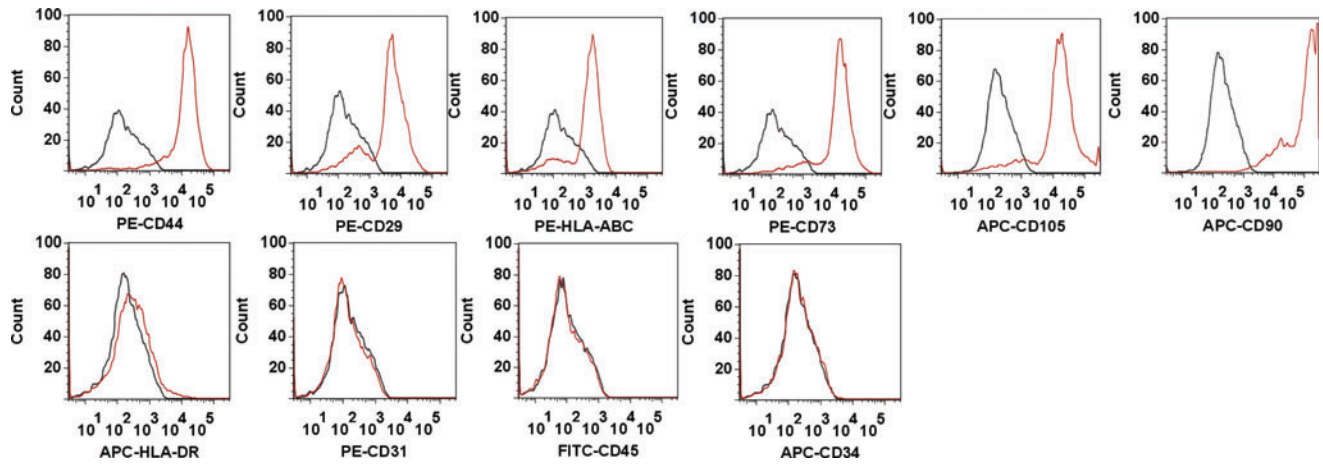
**SUPPLEMENTARY FIG. S1.** (A) Experimental scheme. (B) Expression of early development genes in ABB-treated hESCs; transcripts of mesoderm-lineage genes (*T* and *MILX1*), endoderm-lineage genes (*CXCR4* and *SOX17*), and ectoderm-lineage genes (*PAX6*, *ZIC1*, and *SOX1*) were analyzed by real-time PCR in ABB-treated hESCs (from 1 to 5 days) compared with untreated hESCs. ABB, combination of Activin A, BIO, and BMP4; hESCs, human embryonic stem cells; BIO, 6-bromoindirubin-3'-oxime; BMP4, bone morphogenetic protein 4; PCR, polymerase chain reaction.



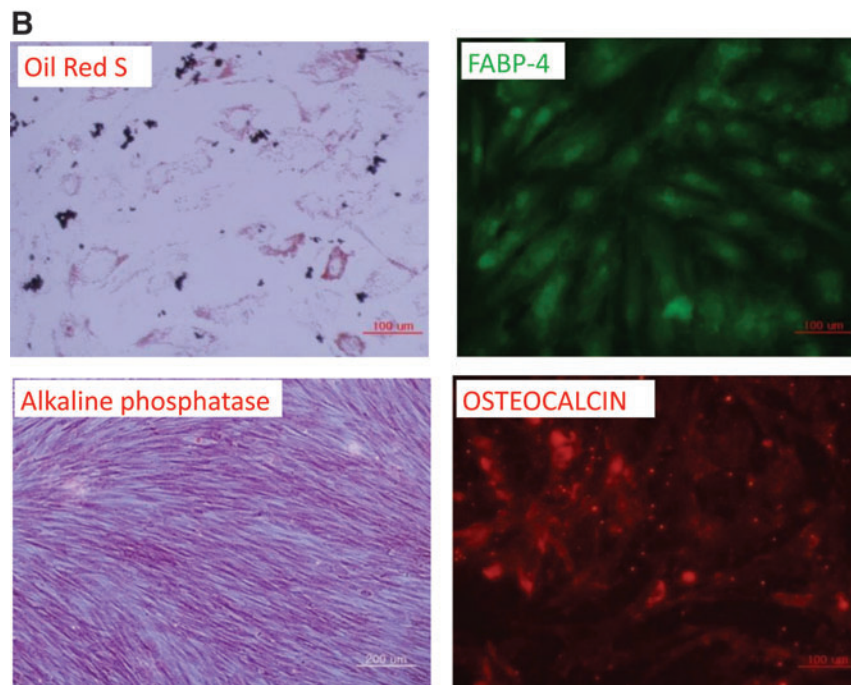
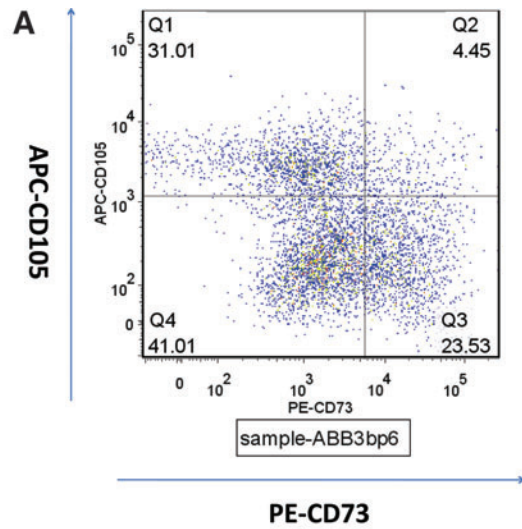
**SUPPLEMENTARY FIG. S2.** Over a time course of 3 days (A) hESCs were treated with Activin A (Ac) for 1 or 2 days, and then with other combinations: BIO, BMP4 (B4), BIO+BMP4 (BB), and Activin A+BIO+BMP4 (ABB) for 2 or 1 day, respectively. (B) hESCs were treated with BMP4 for 1 or 2 days and then with other combinations: BIO, Activin A (Ac), BIO+Activin A (AcBIO), BIO+BMP4 (BB), and Activin A+BIO+BMP4 (ABB) for 2 or 1 day, respectively. (C) hESCs were treated with BIO for 1 or 2 days and then with other combinations: Activin A (Ac), BMP4 (B4), ActivinA+BMP4 (AcB4), BIO+BMP4 (BB), and Activin A+BIO+BMP4 (ABB) for 2 or 1 day, respectively. (D) In this experiment, hESCs were treated with Activin A, BIO, or BMP4 for the first day or first 2 days and then with ABB for 3 days. These experimental groups were analyzed by real-time PCR to estimate the expression of gene *T* and were compared with the positive control groups (ABB-treated cells). Statistical significance among samples was evaluated by ANOVA (\* $P < 0.05$ ). ANOVA, analysis of variance.



**SUPPLEMENTARY FIG. S3.** (A) In this figure, transcripts of mesoderm-lineage genes (*T*, *MIXL1*, and *WNT3a*) in hESCs treated with a single chemical: BIO, BMP4 (B4), or Activin A (Ac) for 3 days were analyzed by real-time PCR and compared with ABB-treated hESCs. Statistical significance among samples was evaluated by ANOVA ( $*P < 0.05$ ). (B) Transcripts of mesoderm-lineage genes (*T*, *MIXL1*, and *WNT3a*) in hESCs treated with BB (BIO+BMP4) and ABB for 3 days were analyzed by real-time PCR. Statistical significance between BB and ABB samples was evaluated by *t*-test ( $*P < 0.05$ ).



**SUPPLEMENTARY FIG. S4.** Surface-antigen profiles of bone marrow-derived MSCs. FACS was employed with the following antibodies: PE-CD29, PE-CD44, APC-CD90, PE-CD73, PE-HLA-ABC, PE-CD31, FITC-CD45, APC-HLA-DR, and APC-CD34. MSCs, mesenchymal stem cells.



**SUPPLEMENTARY FIG. S5.** (A) Proportion of CD105<sup>+</sup> and CD73<sup>+</sup> cells in differentiated hESCs. PE-CD73 and APC-CD105 were used to double-stain differentiated hESCs. (B) Adipogenic and osteogenic potentials of the CD105<sup>+</sup>CD73<sup>-</sup> cell population. Oil-Red S staining and FABP-4 immunostaining were used to analyze adipocytes (*top figures*). Alkaline phosphatase staining and osteocalcin immunostaining were used to analyze osteoblasts (*bottom figures*). Scale bar is 100 μm.

SUPPLEMENTAL TABLE S1. PRIMER LIST USED IN THIS STUDY

<i>Genes</i>		<i>Primer sequence (5'-3')</i>	<i>Product size (bp)</i>	<i>Accession number</i>
<i>PAX6</i>	Sense	GTGTCCAACGGATGTGTGAG	254	NM_001127612
	Antisense	CTAGCCAGGTTGCGAAGAAC		
<i>ZIC1</i>	Sense	GCGCTCCGAGAATTTAAAGA	212	NM_003412
	Antisense	CGTGGACCTTCATGTGTTG		
<i>T (BRACHYURY)</i>	Sense	ATCACAAAGAGATGATGGAGGAA	250	NM_003181
	Antisense	GGTGAGTTGTCAGAATAGGTTGG		
<i>WNT3</i>	Sense	CTGCCAGGAGTGATTCGCATC	223	NM_030753
	Antisense	GAGAGCCTCCCCGTCCACAG		
<i>MIXL1</i>	Sense	GGTACCCCGACATCCACTT	120	NM_031944
	Antisense	TGGAAGGATTTCCCACTCTG		
<i>SOX1</i>	Sense	TACAGCCCCATCTCCAATC	199	NM_005986
	Antisense	GCTCCGACTTCACCAGAGAG		
<i>CXCR4</i>	Sense	GGTGGTCTATGTTGGCGTCT	200	NM_001008540.1
	Antisense	TGCAATAGCAGGACAGGATG		
<i>SOX17</i>	Sense	CGCACGGAATTTGAACAGTA	181	NM_022454.3
	Antisense	GGATCAGGGACCTGTCACAC		
<i>GAPDH</i>	Sense	TGGTATCGTGGAAGGACTCA	250	NM_002046
	Antisense	CCTGCTTCACCACCTTCTTG		