Supplementary Information

Materials and Methods

qRT-PCR analysis

Total RNA was extracted from 2×10^5 cells using 0.2 ml TRIzol (Life Technologies) and purified according to the manufacturer's manual. Complementary DNA was synthesized using the iScript Reverse Transcription kit (Bio-Rad). Each 20 μl reaction contained 4 μl of 5× Mixture, 1 μl reverse transcriptase, 1 μg total RNA, and nucleasefree water to 20 μl. The reaction conditions for reverse transcription were as follows: 25℃ for 5 min, 42℃ for 30 min, 85℃ for 5 min, and hold at 4℃. qPCR was performed using Fast Start Universal SYBR Green Master (Roche) on a CFX96 real-time system (Bio-Rad). Each 15 μl reaction contained 7.5 μl of 2× Mixture, 0.4 μl of each primer (10 μM), 4.7 μl H_2O, and 2 μl complementary DNA. qPCR conditions were as follows: denaturation at 95℃ for 10 min, followed by 45 cycles of 95℃ for 15 sec, 58℃ for 30 sec, and 72℃ for 30 sec. The mRNA encoding glyceraldehyde 3-phosphate dehydrogenase (*GAPDH*) served as an internal control. All the used primers were listed in Supplementary Table S1.

Western blot analysis

Cells were lysed in RIPA buffer (Sigma) with shaking on ice for 30 min. After centrifugation (12,000 ×g, 4℃, 5 min), the protein concentration was determined by the classical BCA method. Proteins were denatured at 100℃ for 5 min and then subjected to SDS-PAGE (5% separating gel, 10% stacking gel) on a Bio-Rad MiniPROTEAN Tetra Cell electrophoresis system. Proteins were transferred to a PVDF membrane using a Bio-Rad Trans-Blot cell in 1× TBST (10 mM Tris-HCl, pH 8.0, 150 mM NaCl, and 0.05% Tween 20). Thereafter, membranes were incubated overnight at 4℃ with anti-SOX2 (diluted 1 : 1000; Cat. # sc-365823, Santa Cruz Biotechnology), anti-OCT4 (diluted 1 : 1000; Cat. # sc-5279, Santa Cruz Biotechnology), anti-NANOG (diluted 1 : 1000; Cat. # sc-293121, Santa Cruz Biotechnology), anti-P21 (diluted 1 : 100; Cat. # sc- 6246, Santa Cruz Biotechnology), and anti-GAPDH (diluted 1 : 200; Cat. # KC-5G4, Kang Chen Inc) mouse monoclonal antibodies in blocking solution (1× TBST containing 5% nonfat milk powder). Membranes were then washed with 1× TBST, incubated with horseradish peroxidase–onjugated goat anti-mouse IgG H&L (diluted 1 : 1000; Cat. # ZB-2305, ZSBIO) in blocking solution, washed again with 1× TBST and finally developed using an Amersham ECL Prime western blotting detection kit (Cat. # RPN2232, GE Healthcare). Signals were quantified using an ImageQuant LAS4000 mini system. Molecular weights were calculated by reference to the pre-stained protein ladder (Cat. # 26626, PageRuler). Grayscale contrast analysis was performed by Gel- Pro analyzer. All the used antibodies were listed in Supplementary Table S2.

Table S1. Primers used for qRT-PCR analysis

Gene	Forward primer (5′→3′)	Reverse primer (5′→3′)
GAPDH	GTCTCCTCTGACTTCAACAGCG	ACCACCCTGTTGCTGTAGCCAA
vWF	CCTTGAATCCCAGTGACCCTGA	GGTTCCGAGATGTCCTCCACAT
GATA1	CACGACACTGTGGCGGAGAAAT	TTCCAGATGCCTTGCGGTTTCG
GATA2	CAGCAAGGCTCGTTCCTGTTCA	ATGAGTGGTCGGTTCTGCCCAT
RUNX1b/c	TCTGCAGAACTTTCCAGTCG	GTCGGGGAGTAGGTGAAGG
CD34	AACATCTCCCACTAAACCCTA	TCTTAAACTCCGCACAGCTG
LMO2	CTCATAGGCACGAATCCGCTTG	GACCTCTCTGTGAAGCAACTGC
CDKN1A	CTGTCTTGTACCCTTGTGCCTC	TGGAGTGGTAGAAATCTGTCATG

Table S2. Antibodies used for FACS analyses

Antibodies	Brand	Antibody channels	Cat #
CD309 (KDR)	BD	PE	560494
CD34	BD	APC	555824
CD43	BD	PE	560199
CD43	BD	PE-CY7	563522
CD45	BD	PE-CY7	557748
CD235a (GPA)	BD	APC	551336
CD71	BD	PE	561938
7-AAD	BD	7-AAD	559925

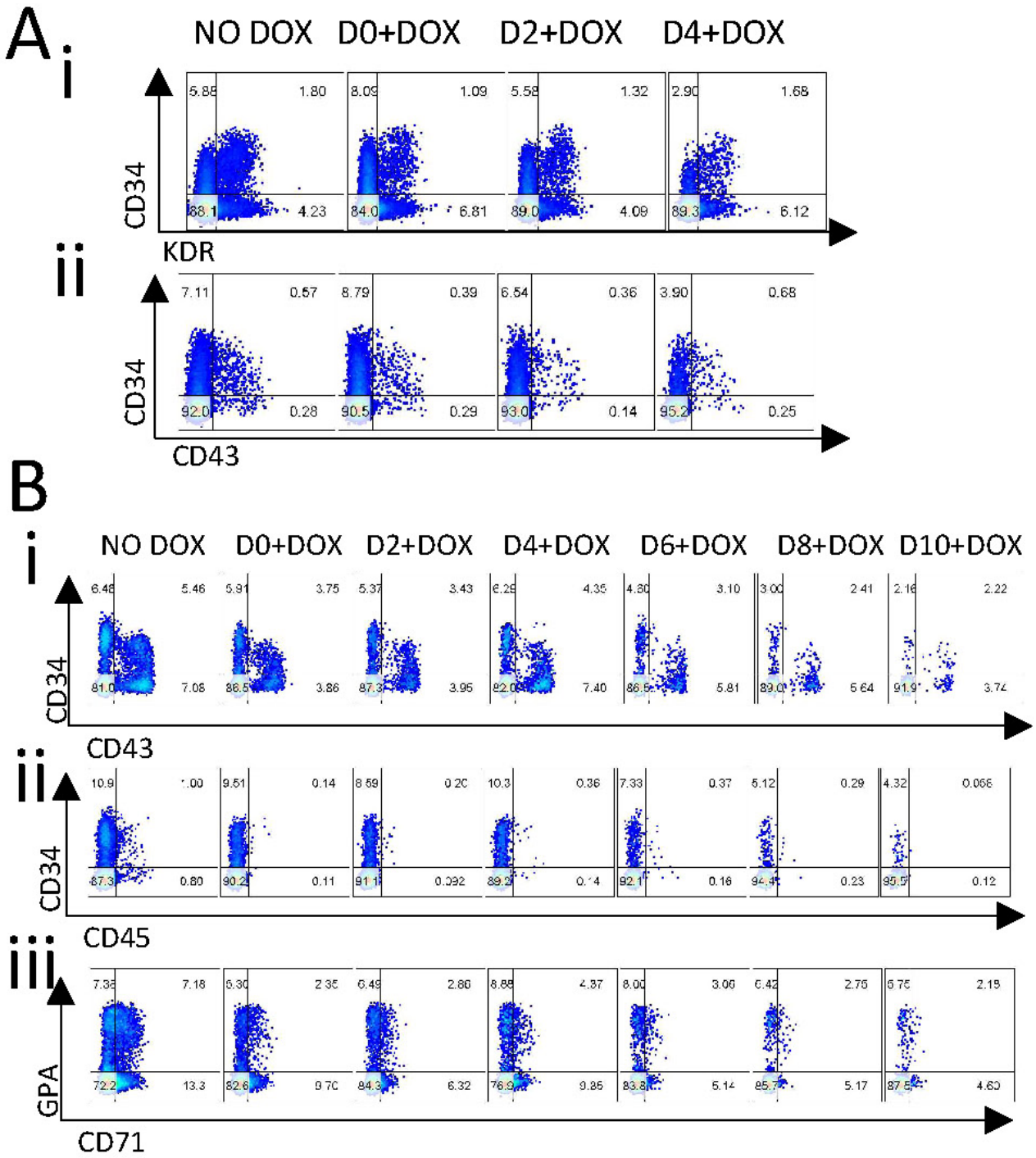

Fig. S1. Overexpression of p21 from D0 blocks hematopoiesis. Co-cultured p21/hESCs were treated with DOX from D0, D2, D4, D6, D8, or D10, and analyzed by FACS using antibodies against (A) CD34/KDR/CD43 at D6 or (B) CD34/CD43, CD34/CD45, and GPA/CD71 at D14. Non-induced co-cultures and the GFP+ fraction of co-cultures treated with DOX were compared by FACS.

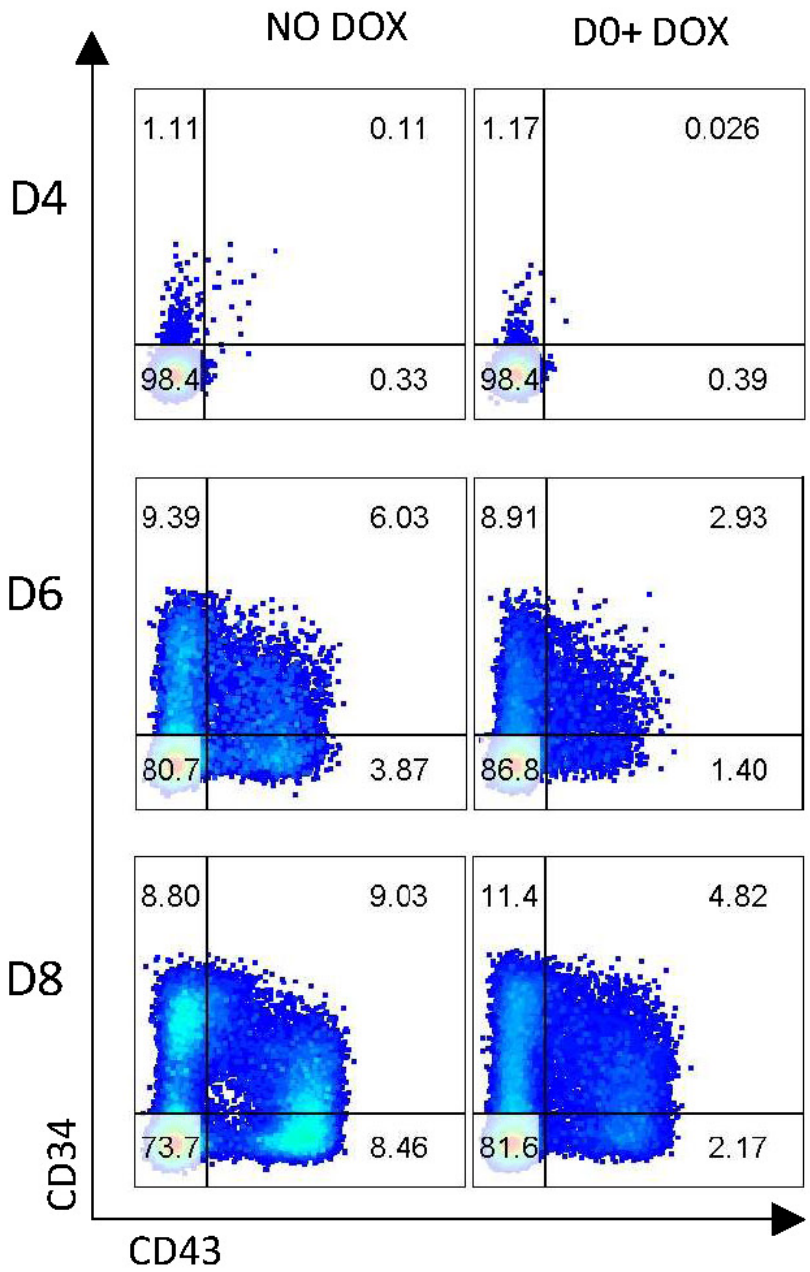

Fig. S2. Overexpression of p21 from D0 blocks the emergence of CD43+ cells. Co-cultured p21/hESCs treated with DOX from D0 were analyzed by FACS using antibodies against CD34/CD43 at D4, D6, or D8. Non-induced co-cultures and the GFP+ fraction of co-cultures treated with DOX were compared by FACS.