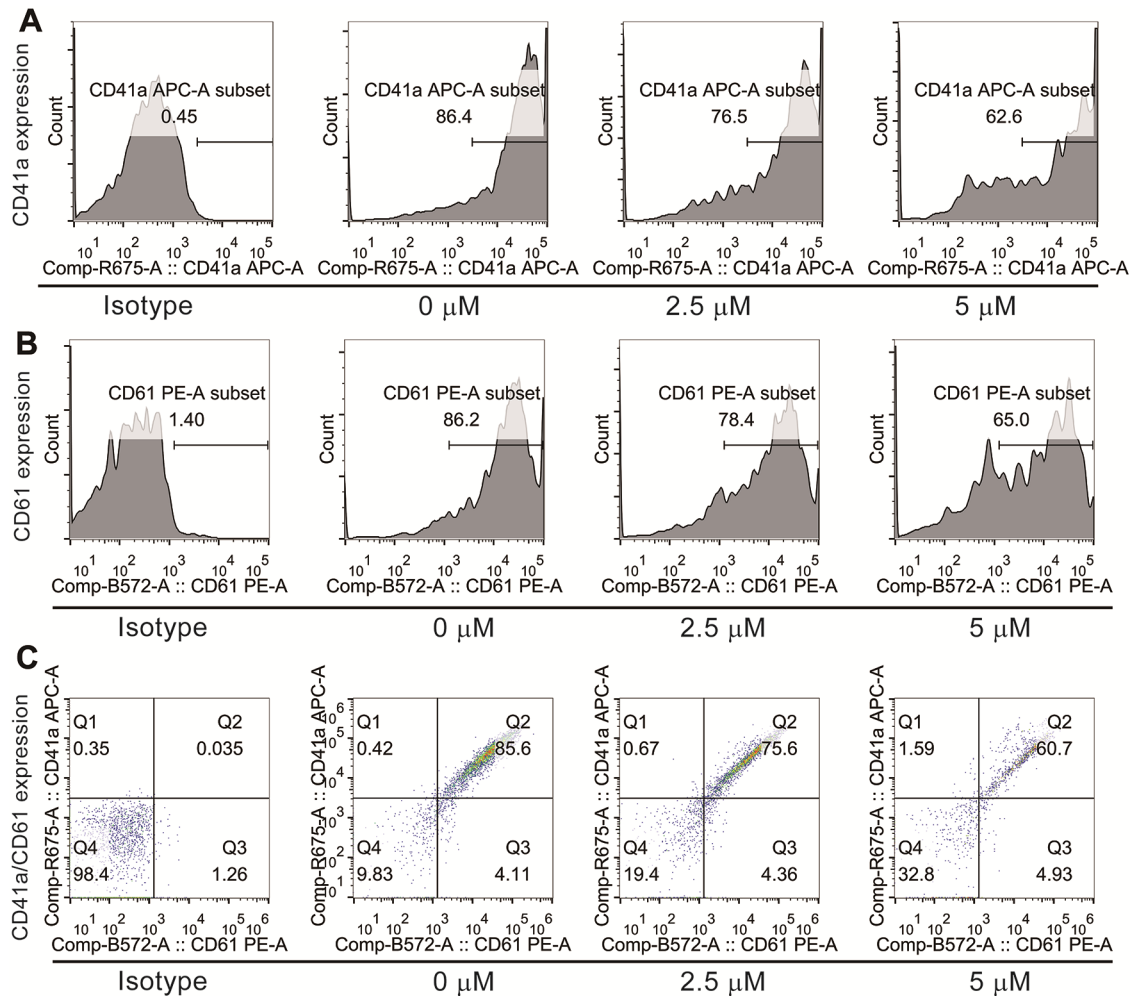
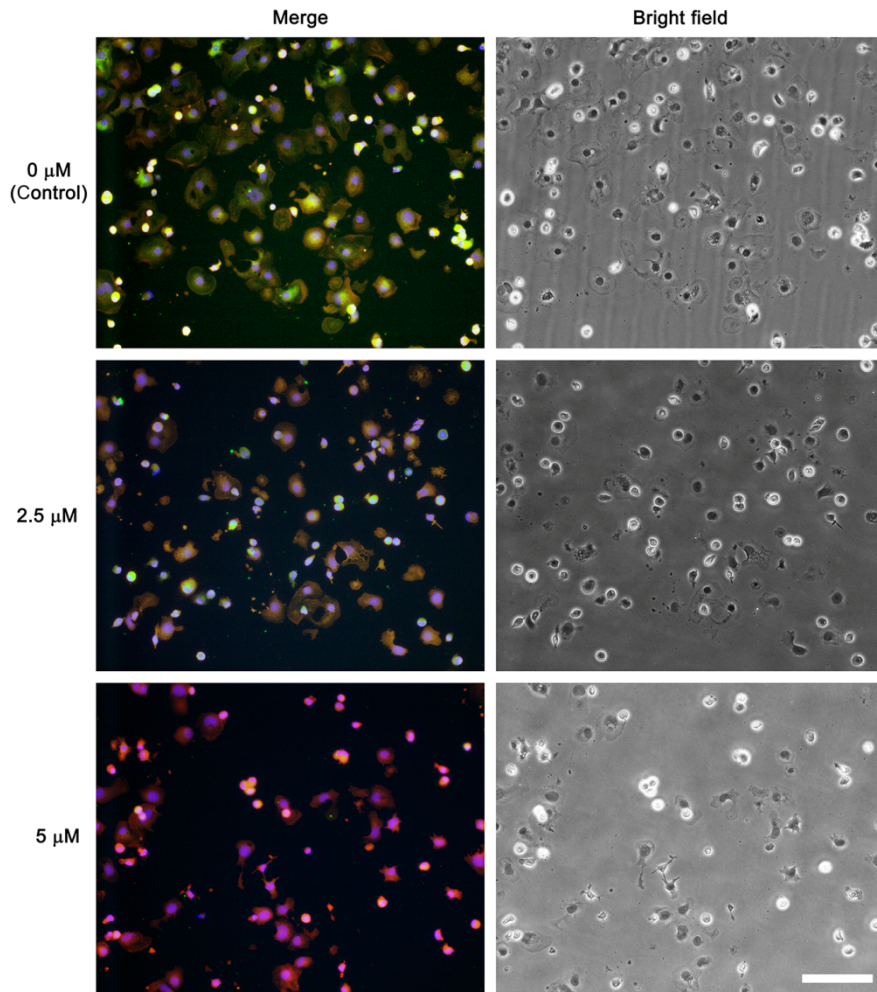


Supplemental Fig. 1 Effects of abivertinib on apoptosis of MKs.

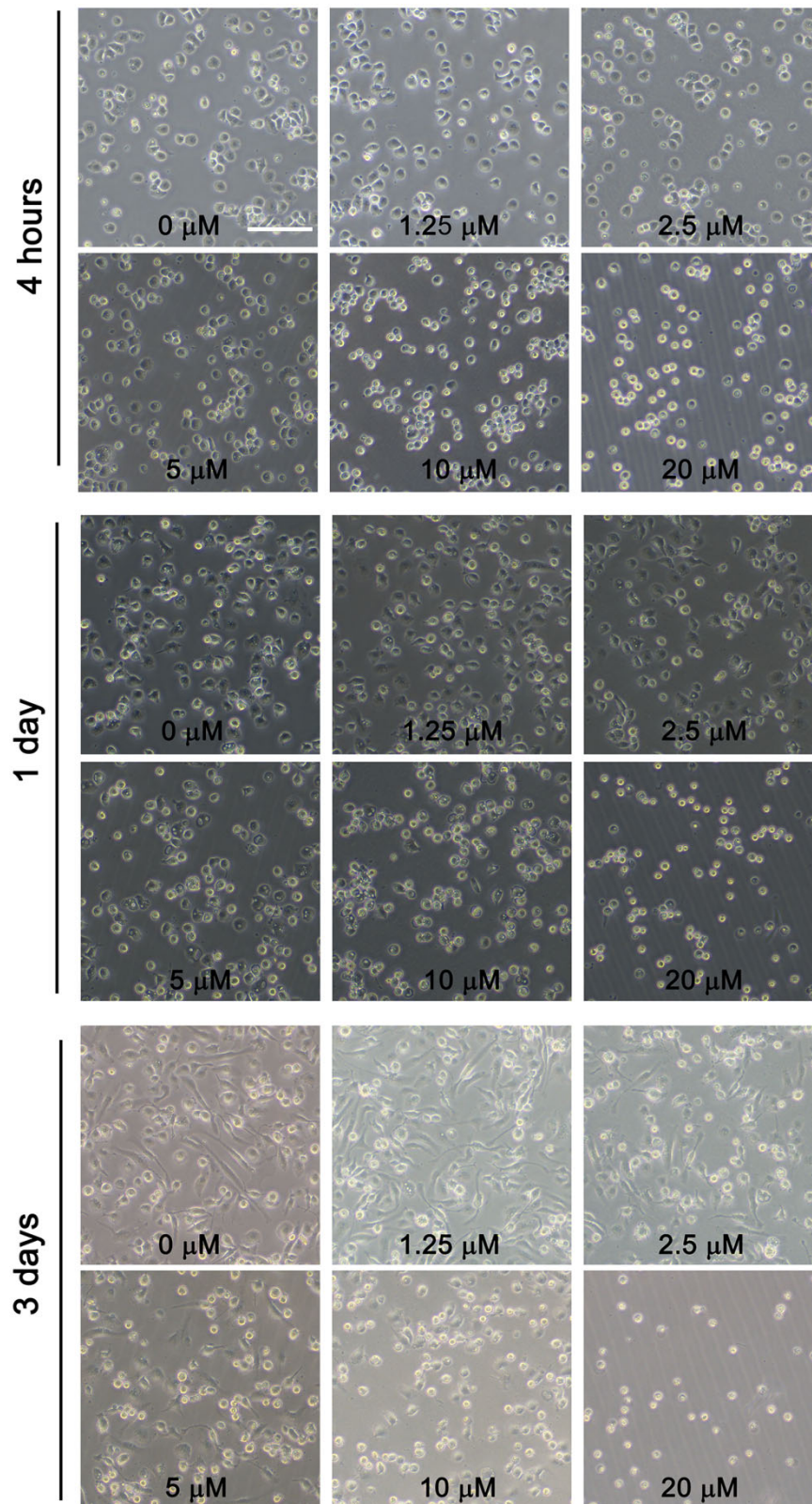
(A) CD34⁺ HSCs were cultured with SCF/IL-3/IL-6/IL-9/TPO. Different doses (0 μ M, 2.5 μ M, 5 μ M) of abivertinib or DMSO were added to the medium on day 0. Then, 50 μ M Z-VAD-FMK or 1 μ M staurosporine was added to the medium on day 4. Apoptosis of the total cell population at day 6 was analyzed by flow cytometry. Statistical histograms show the percentage of Annexin V-positive apoptotic cells. Abivertinib-induced apoptosis can be slightly reduced by Z-VAD-FMK but significantly enhanced by staurosporine. (B) The mitochondrial membrane potential (MMP) at day 6 was analyzed by flow cytometry. The data for at least three independent experiments are presented as bar graphs. The abivertinib-induced decline in the MMP can be slightly reversed by Z-VAD-FMK but increased by staurosporine. (C) Meg-01 cells treated with different doses (0 μ M, 2.5 μ M, 5 μ M) of abivertinib followed by 50 μ M Z-VAD-FMK or 1 μ M staurosporine for 48 hours. The percentage of Annexin V-positive apoptotic cells is shown. Similar to panel A, the results were obtained in Meg-01 cells. (D) The MMP is shown. Similar to panel B, the results were obtained in Meg-01 cells. * $P < 0.05$, ** $P < 0.01$ compared with relevant abivertinib.



Supplemental Fig. 2 Effects of abivertinib on CD41a and CD61 expression in CD34⁺ HSC-derived MKs. (A) Cord blood CD34⁺ HSCs were induced to undergo MK differentiation in StemSpan SFEM II medium supplemented with SCF, IL-3, IL-6, IL-9, and TPO and were cultured in the presence or absence of abivertinib. On day 12, the expression of CD41a was analyzed by flow cytometry. CD41a expression was inhibited by abivertinib in a dose-dependent manner. (B) On day 12, CD61 expression was analyzed by flow cytometry. CD61 expression was inhibited by abivertinib in a dose-dependent manner. (C) The percentage of MKs (CD41a positive/CD61 positive) in the total cell population was analyzed by flow cytometry. The percentage of MKs in the total cell population was decreased by abivertinib in a dose-dependent manner.

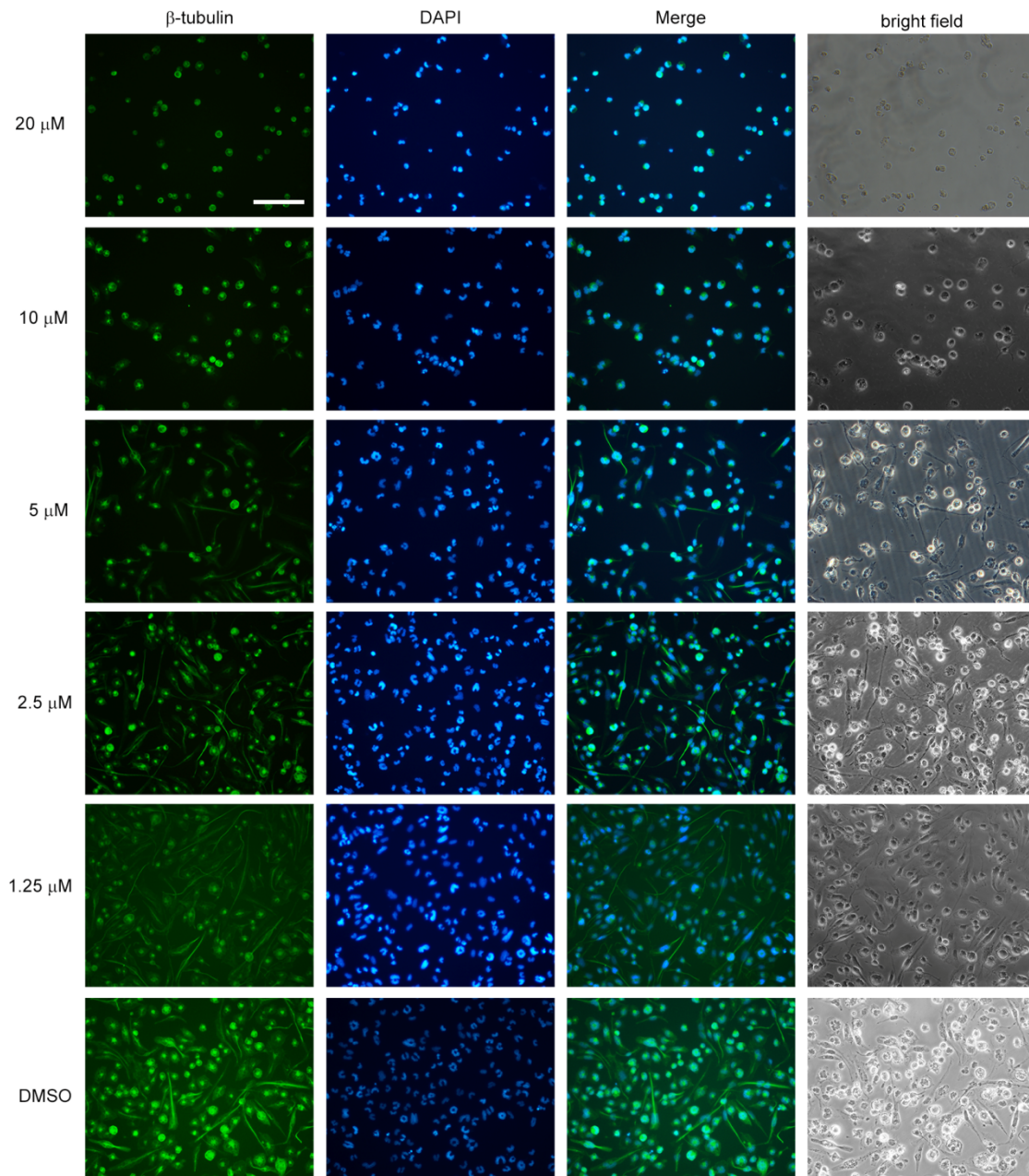


Supplemental Fig. 3 Effects of abivertinib on the morphologic features of MKs. Cord blood CD34⁺ HSC-derived MKs cultured in the presence or absence of abivertinib were allowed to adhere to a fibrinogen-coated coverslip for 2 days. Representative images of merged images (actin/red, CD41a/green, and nuclei/blue) and bright field images are shown. The scale bar is 80 μm.



Supplemental Fig. 4 Effects of abivertinib on Meg-01-derived MK differentiation. Meg-01 cells were treated with 10 nM PMA to induced differentiation and were cultured in the presence or absence of abivertinib (1.25 μM, 2.5 μM, 5 μM, 10 μM and 20 μM) for four days. All figures show representative images of the morphologic features of Meg-01 cells at the indicated times (4 hours, 1

day, and 3 days). The scale bar is 60 μm .



Supplemental Fig. 5 Effects of abivertinib on the morphologic features of Meg-01-derived MKs. Meg-01 cells were cultured with 10 nM PMA for 4 days in the presence or absence of abivertinib (1.25 μ M, 2.5 μ M, 5 μ M, 10 μ M and 20 μ M). The cells were then stained with FITC-conjugated β -tubulin antibody. The cytoplasmic extensions of Meg-01 cells were inhibited with increasing abivertinib concentrations compared to the control (DMSO). Nuclei were stained with DAPI. In the control groups, Meg-01 cells had lobulated nuclei, the number of which decreased sharply with increasing abivertinib concentrations. The scale bar is 60 μ m.