Supplementary Figures.

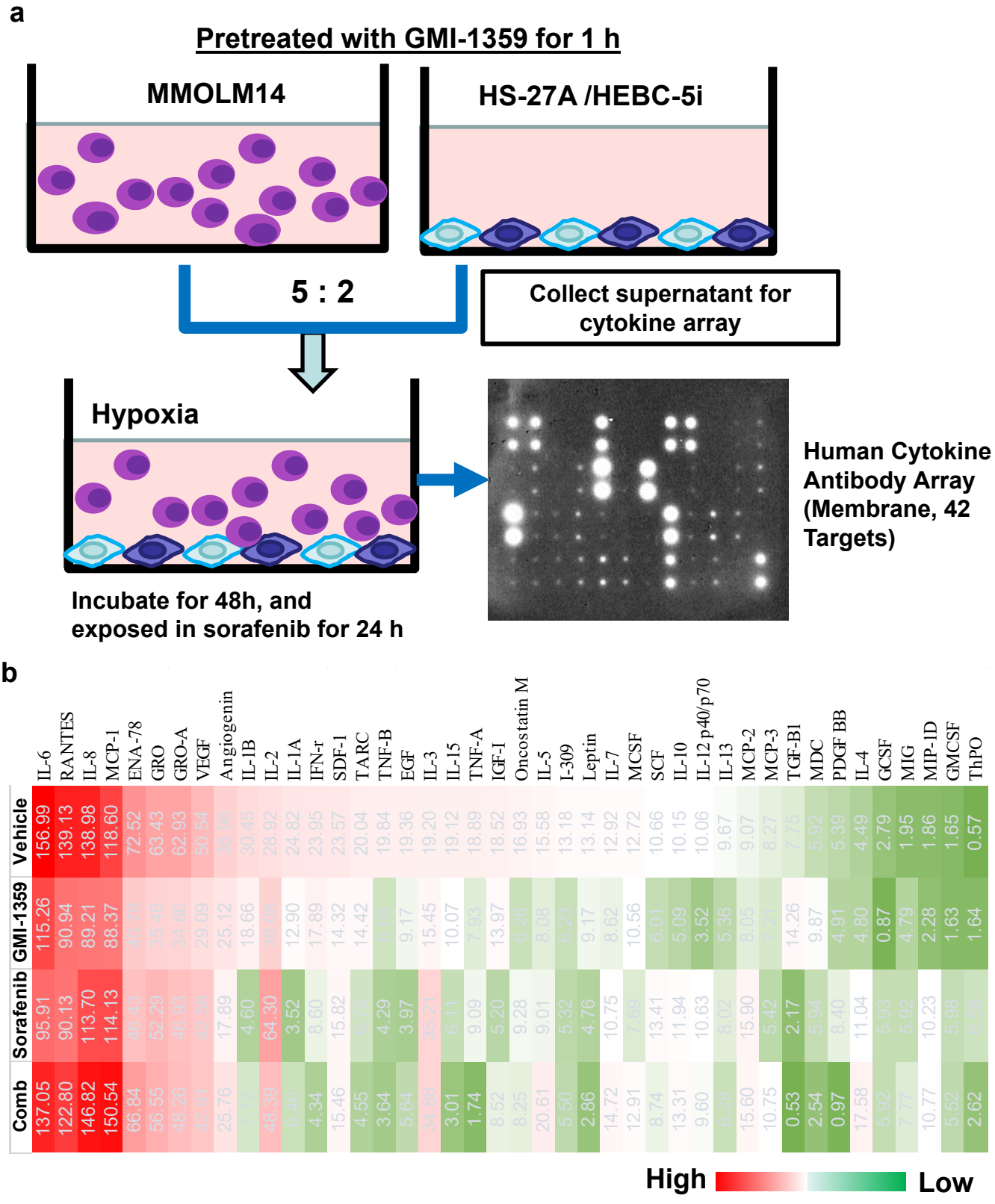


Fig. S1. (a) Schematic diagram of Cytokine antibody array and membrane result after development of antibody immunoblotting. (b) MOLM14 cells were exposed in sorafenib and/or GMI-1359 for 24 h in hypoxia condition. The levels of 42 targeted cytokines were determined by calculating dots intensity. The data were indicated as fold changes and normalized with vehicle control.

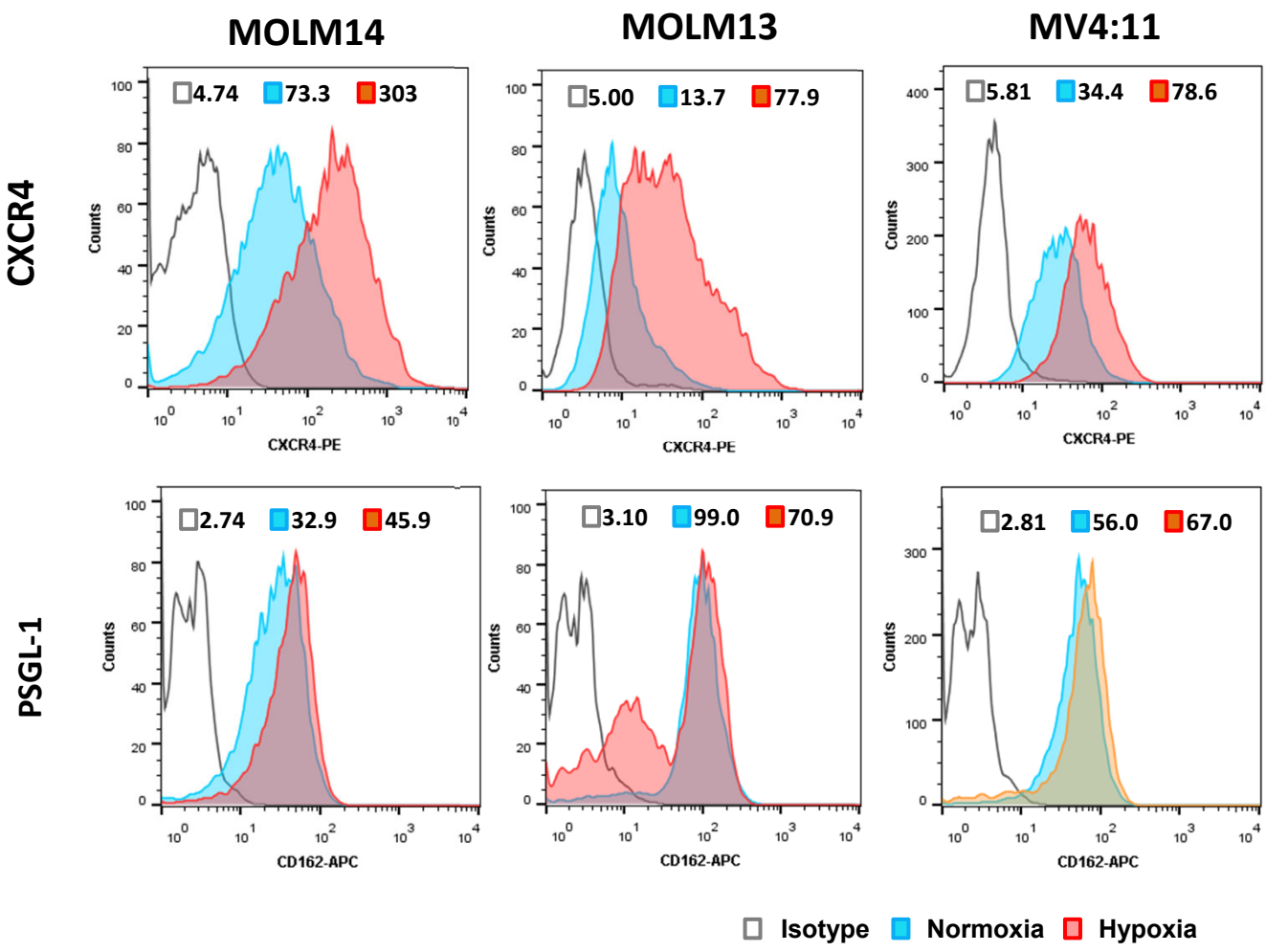


Fig. S2. FLT3 ITD mutated human leukemia cell lines MOLM13, MOLM14 and MV:11 were cultured in either normoxia or hyposia conditions for 48 h. Cell surface CXCR4 and E-selectin-L PSGL-1 were measured using flow cytometry after staining with their corresponding antibodies Isotype-PE was as background control. The numbers indicated mean fluorescence intensity (MFI).

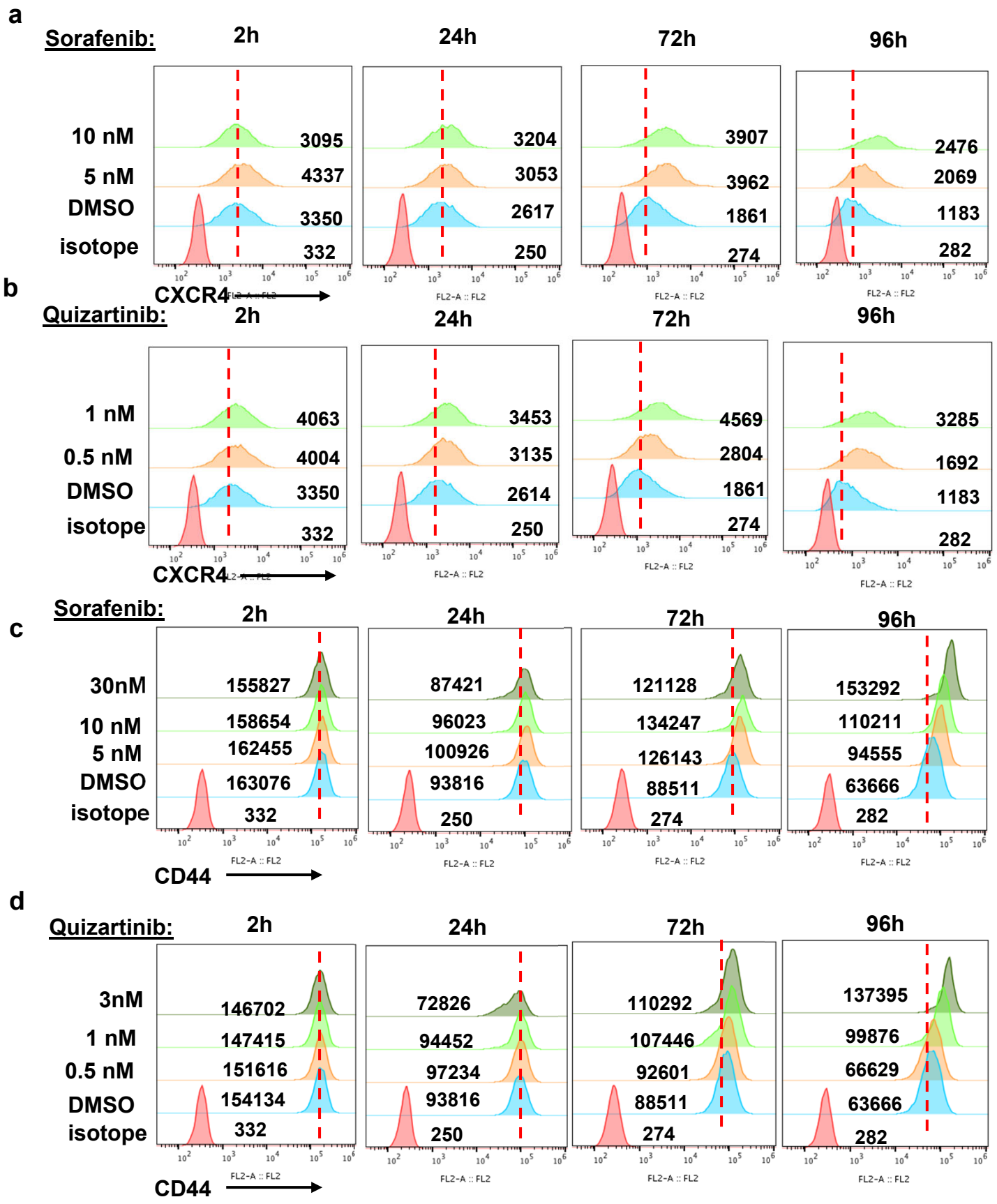


Fig. S3. MOLM14 cells were exposed in indicated concentrations of FLT3i sorafenib or quizartinib for 2, 24, 72 and 96 h. Cell surface CXCR4 (Sa, b) and CD44 (Sc, d) were measured using flow cytometry after staining with their corresponding antibodies Isotype-PE was as background control.

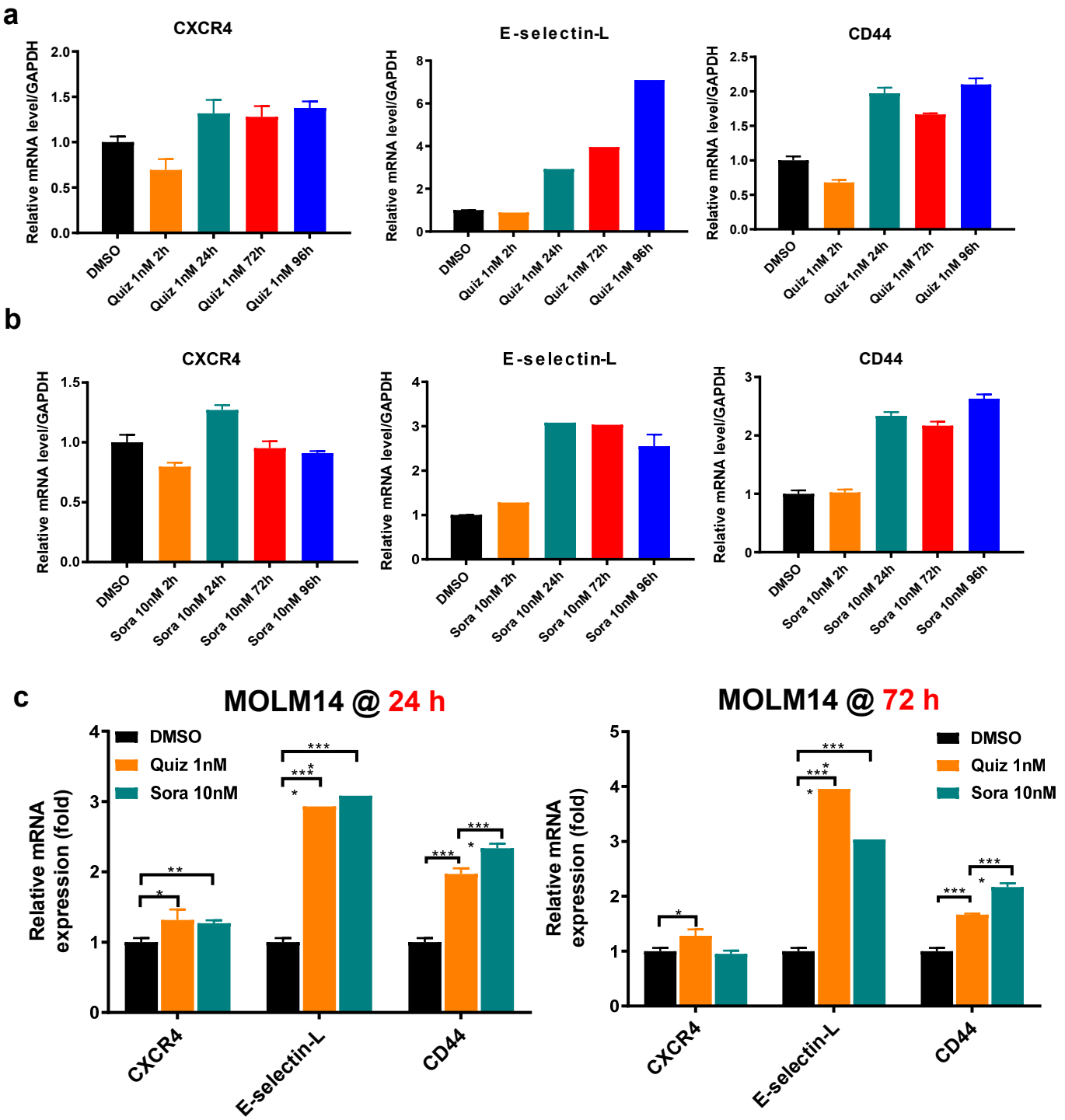


Fig. S4. MOLM14 cells were exposed in indicated concentrations of sorafenib (S3a) or quizartinib (S3b) for 2, 24, 72 and 96 h. The mRNA levels of CXCR4, E-selectin-L and CD44 were measured by using qPCR. (S3c) The mRNA levels of CXCR4, E-selectin-L and CD44 were compared at 24 h and 72 h treatment. The numbers of x-axes indicated relative mRNA levels (fold) of normalizing with housekeeping gene GAPDH at DMSO groups Error bars presented as the means ± standard deviation from three independent experiments.

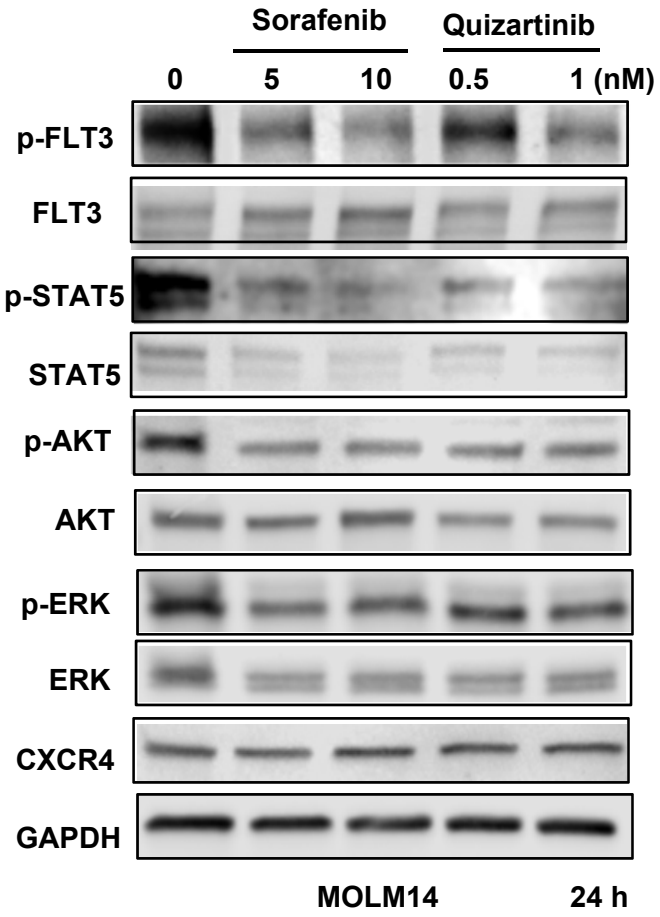


Fig. S5. MOLM14 cells were exposed in indicated concentrations of sorafenib or quizartinib for 24 h. The protein levels of FLT3 and its downstream signaling pathway were determined by using immunoblotting.

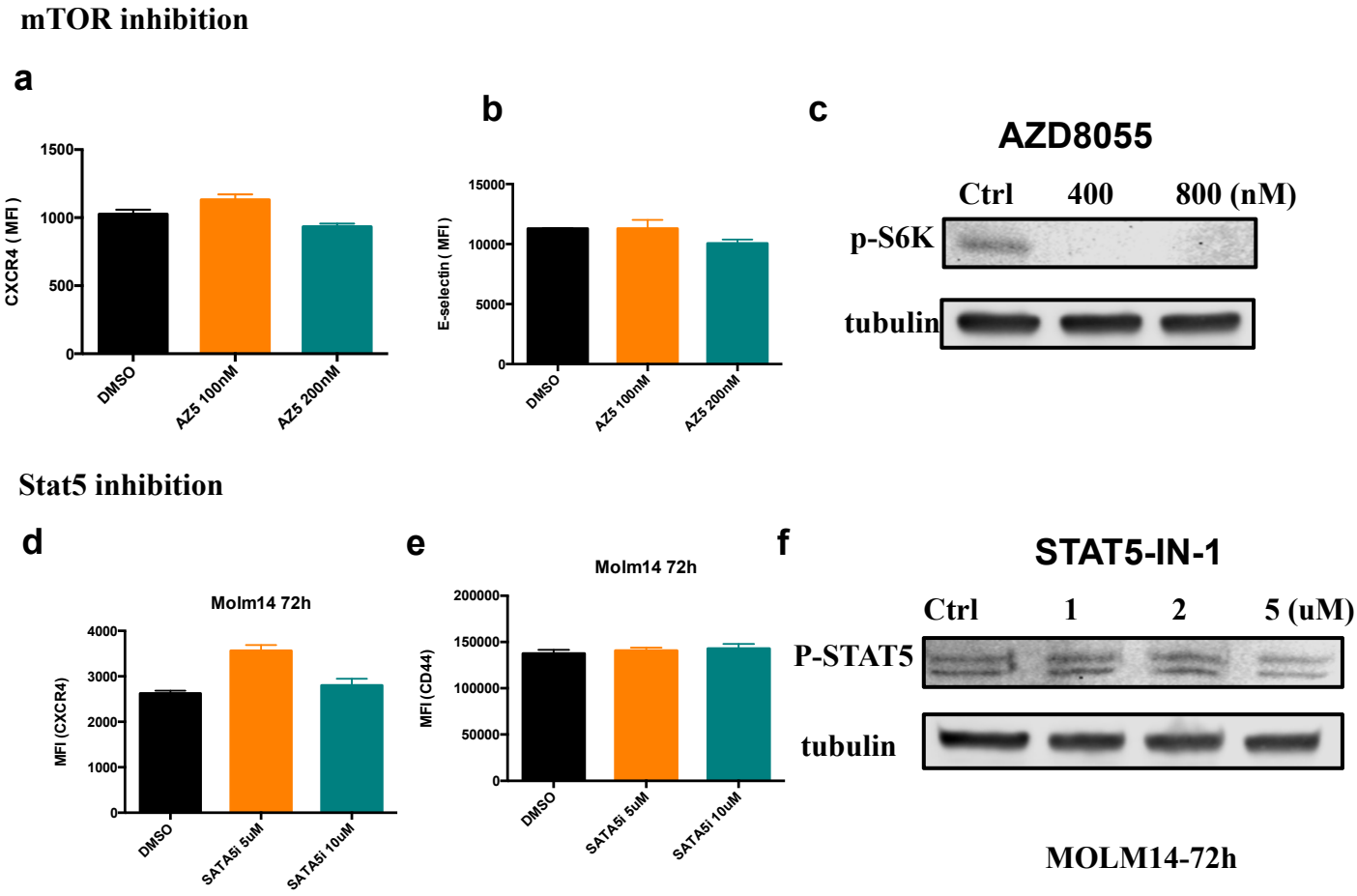


Fig. S6. MOLM14 cells were exposed in indicated concentrations of either (S5a-c) mTORi AZD8055 or (S5d-f) STAT5i STAT5-IN-1 for 72 h. The surface CXCR4 and E-selectin-L (CD44) were measured by using flow cytometry. Immunobloting analysis indicated phospho-S6k and –STAT5 were suppressed by their specific inhibitors at indicated concentrations.

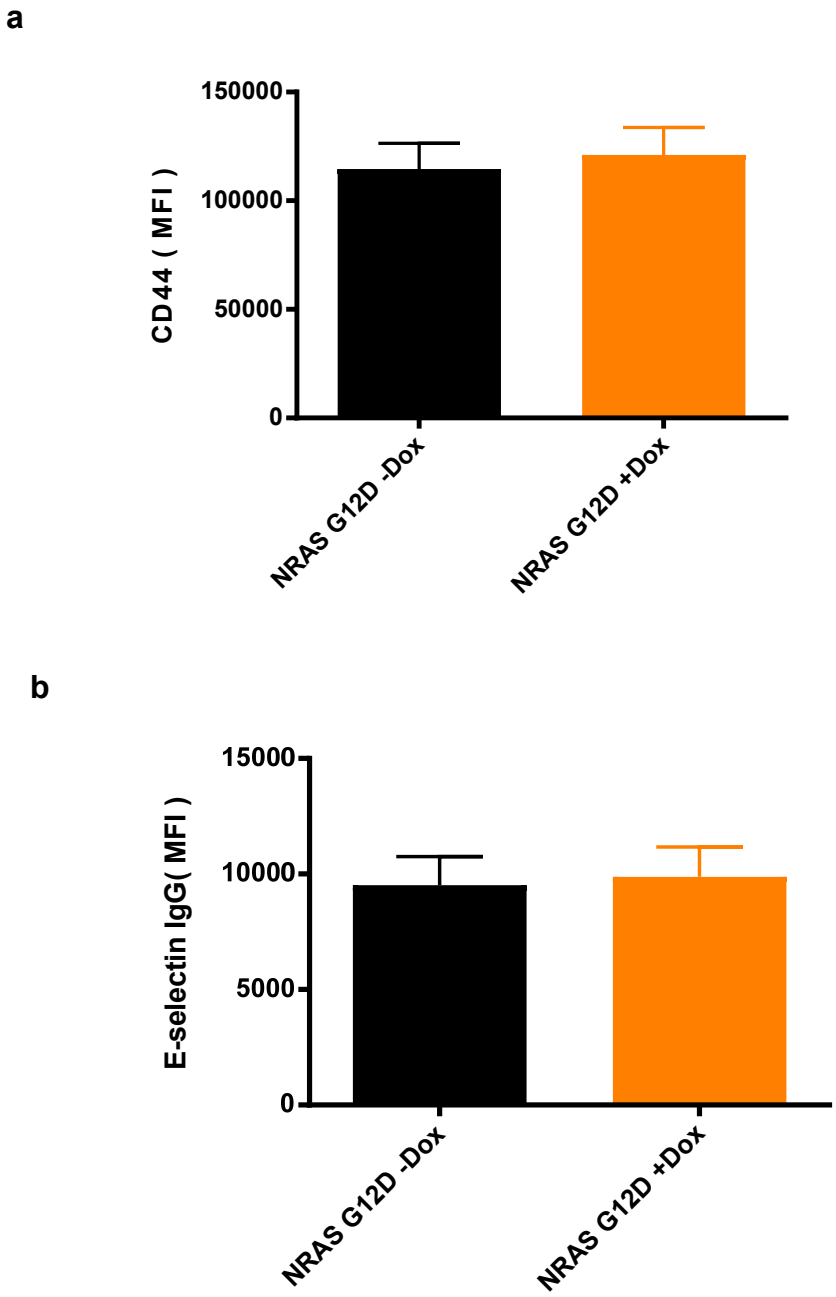
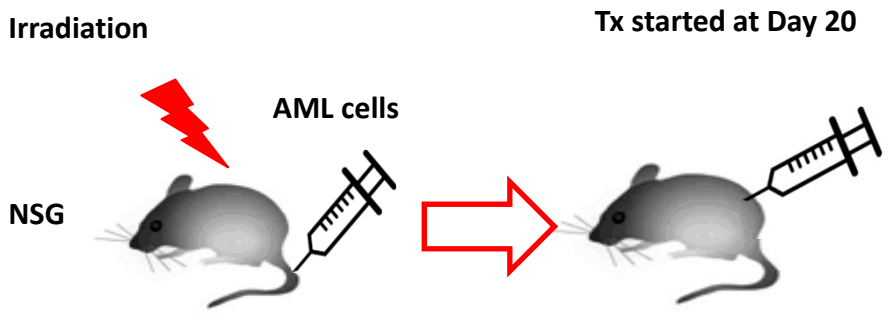


Fig. S7. Dox-inducible MOLM13-NRAS(G12D) mutant cells were cultured with/without Dox for 72 h. The MFI of the cell surface (a) CD44 or (b) E-selectin-L were measured using flow cytometry..

a



b

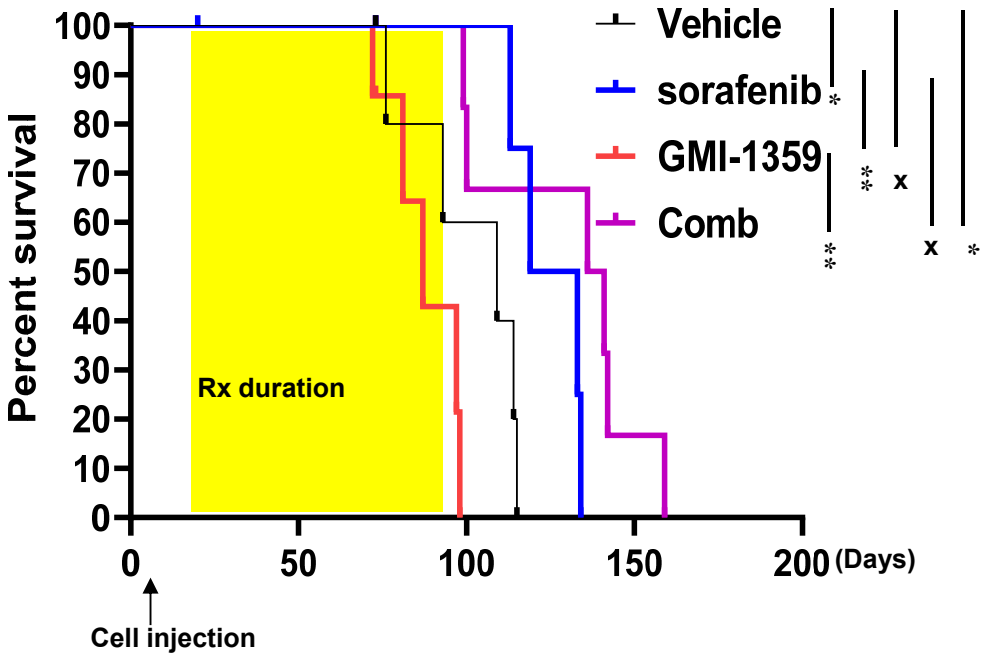


Fig. S8. (a) A PDX AML mouse model was established by injecting FLT3 ITD-mutated AML patient tissue samples into NSG mice after irradiation. The mice received sorafenib (8 mg/kg, orally) and/or GMI-1359 (40 mg/kg, i.p.) following a qd × 5 days/week schedule. (b) Mouse survival duration was estimated using the Kaplan-Meier method. The yellow area indicates the treatment duration.

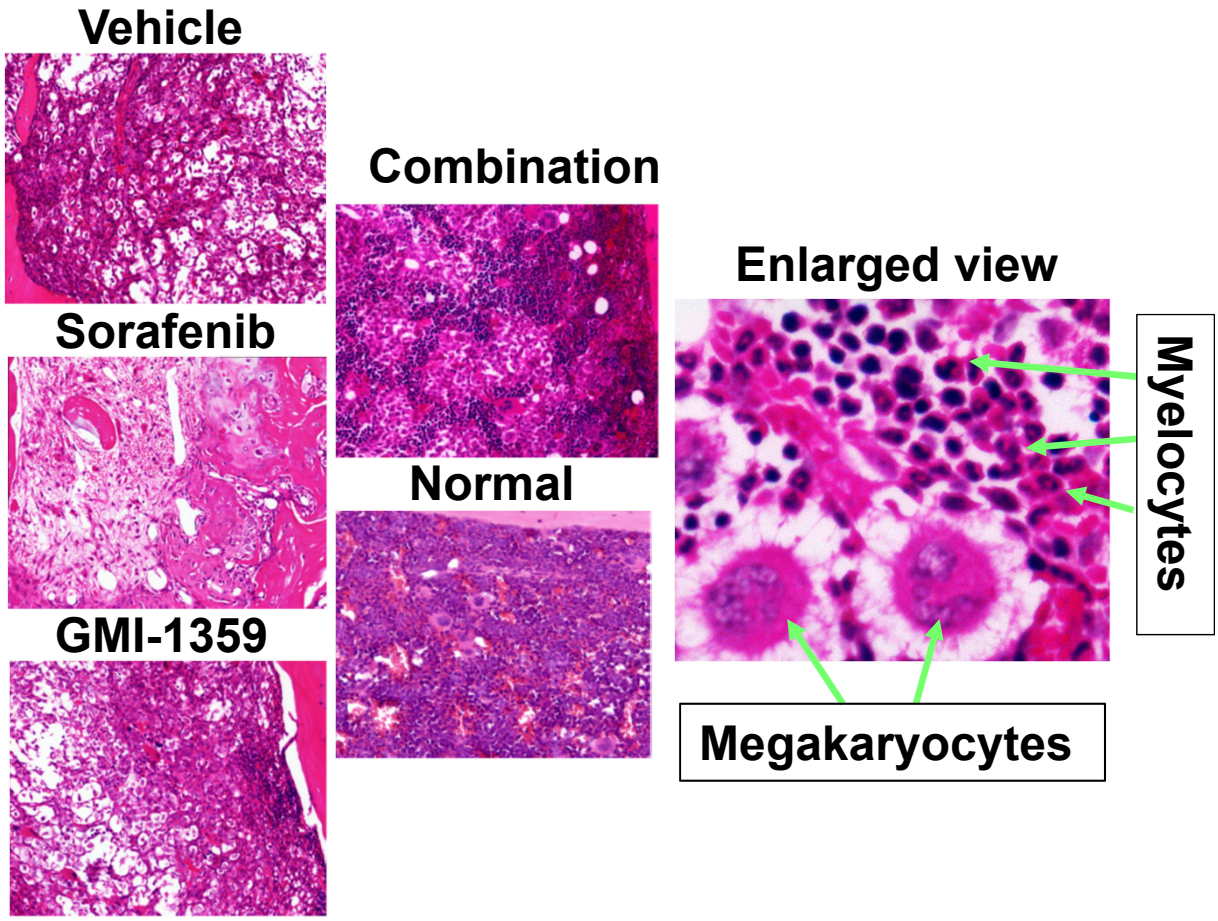


Fig. S9. BM samples from a PDX murine model, which received sorafenib and/or GMI-1359 administration for 53 days, were stained with H&E. Morphological analysis of megakaryocytes and myelocytes were performed under microscopy. Enlarged view showed the morphological features of the megakaryocytes and myelocytes (green arrows).

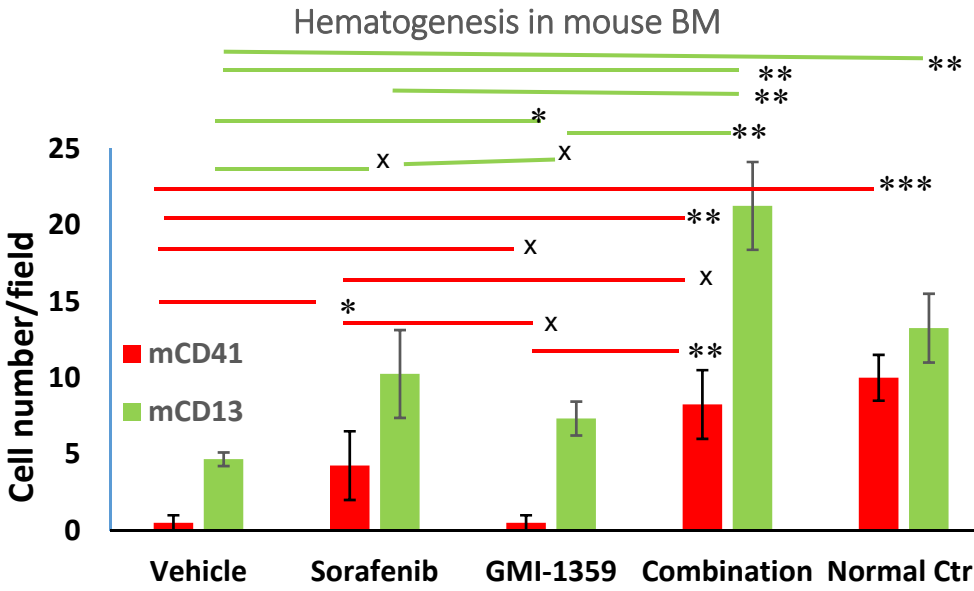


Fig. S10. A semiquantitative analysis of immunofluorescence images from BM samples by counting mCD41- and mCD13-positive cell numbers. Error bars are presented as the means ± standard deviation.

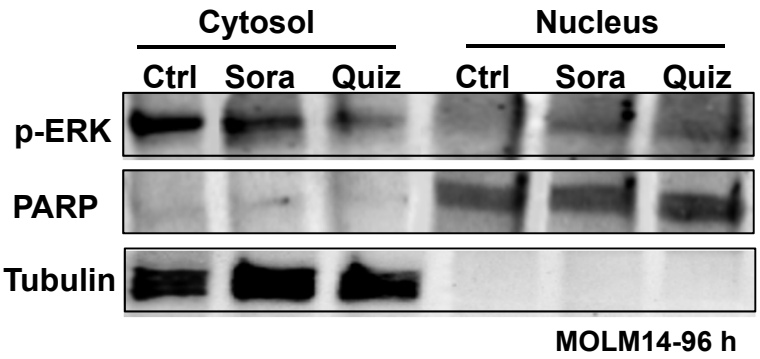


Fig. S11. MOLM14 cells were exposed to sorafenib (10 nM) or quizartinib (1 nM) for 96 h. The protein levels in cytosol and nuclear fractions were determined by using immunoblotting.