Supplemental figures

Targeting primary acute myeloid leukemia with a new CXCR4 antagonist IgG1 antibody (PF-06747143)

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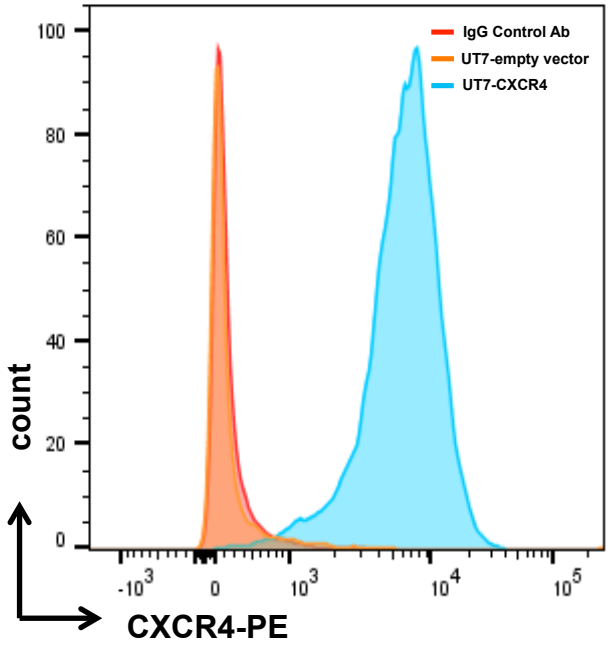
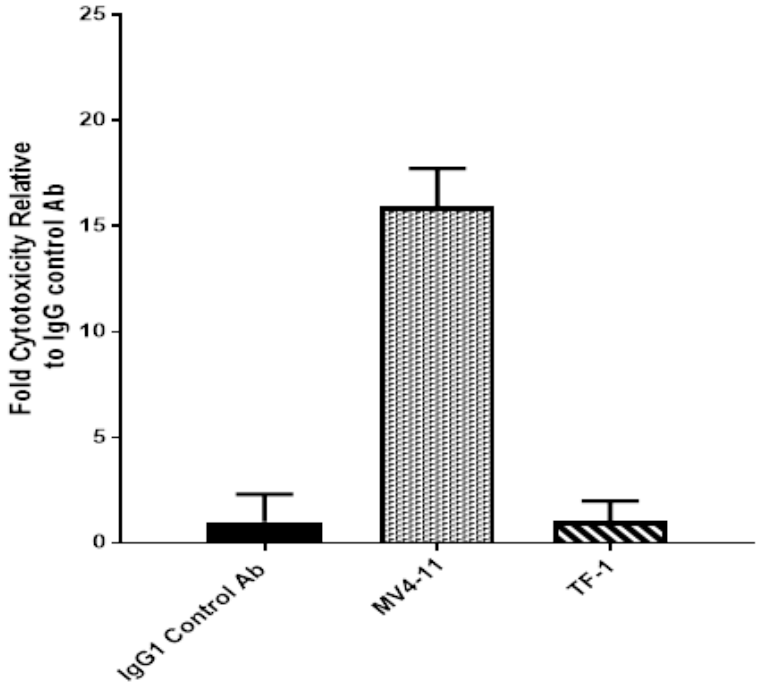


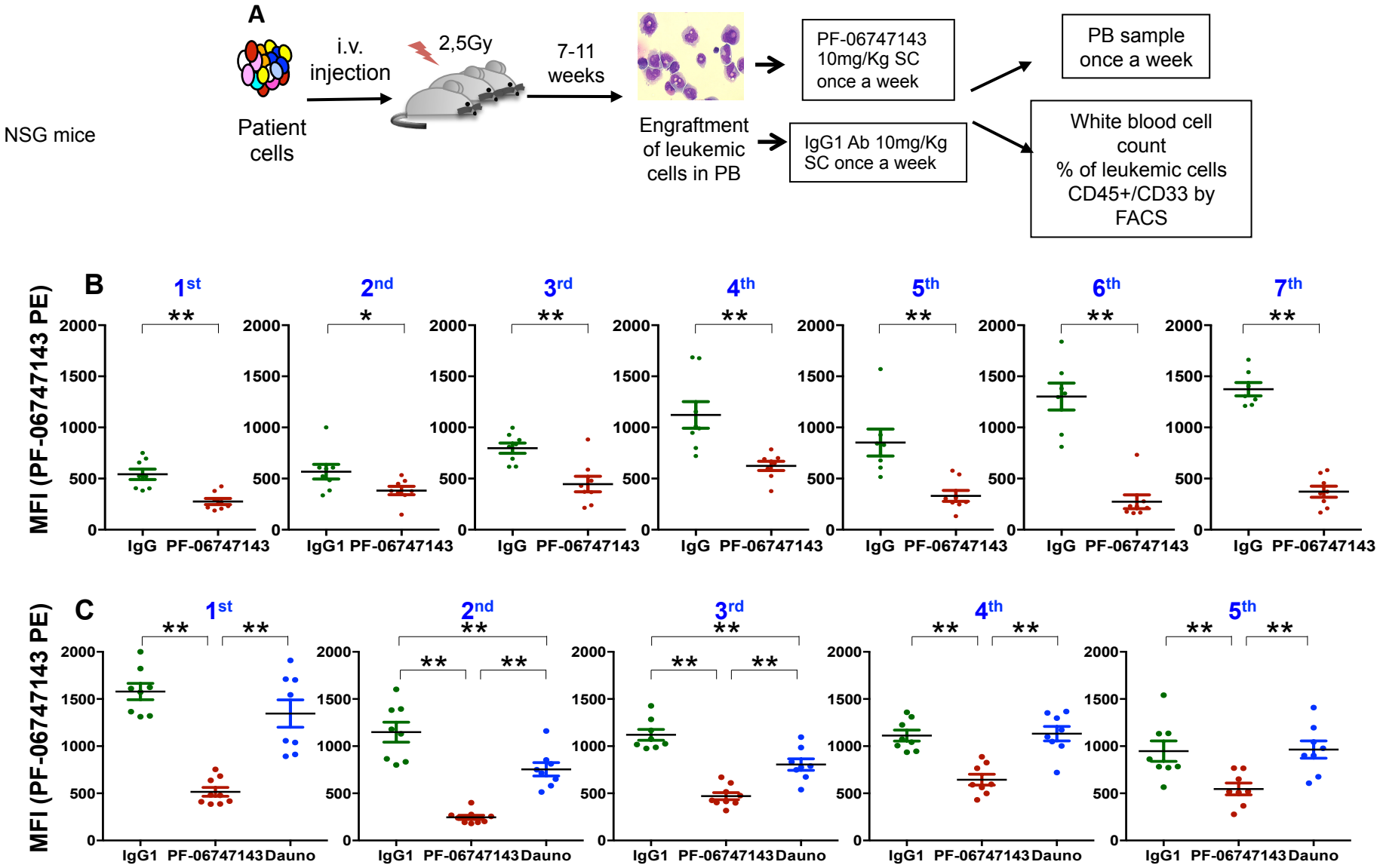
Supplemental Figure 1

Zhang Y et al



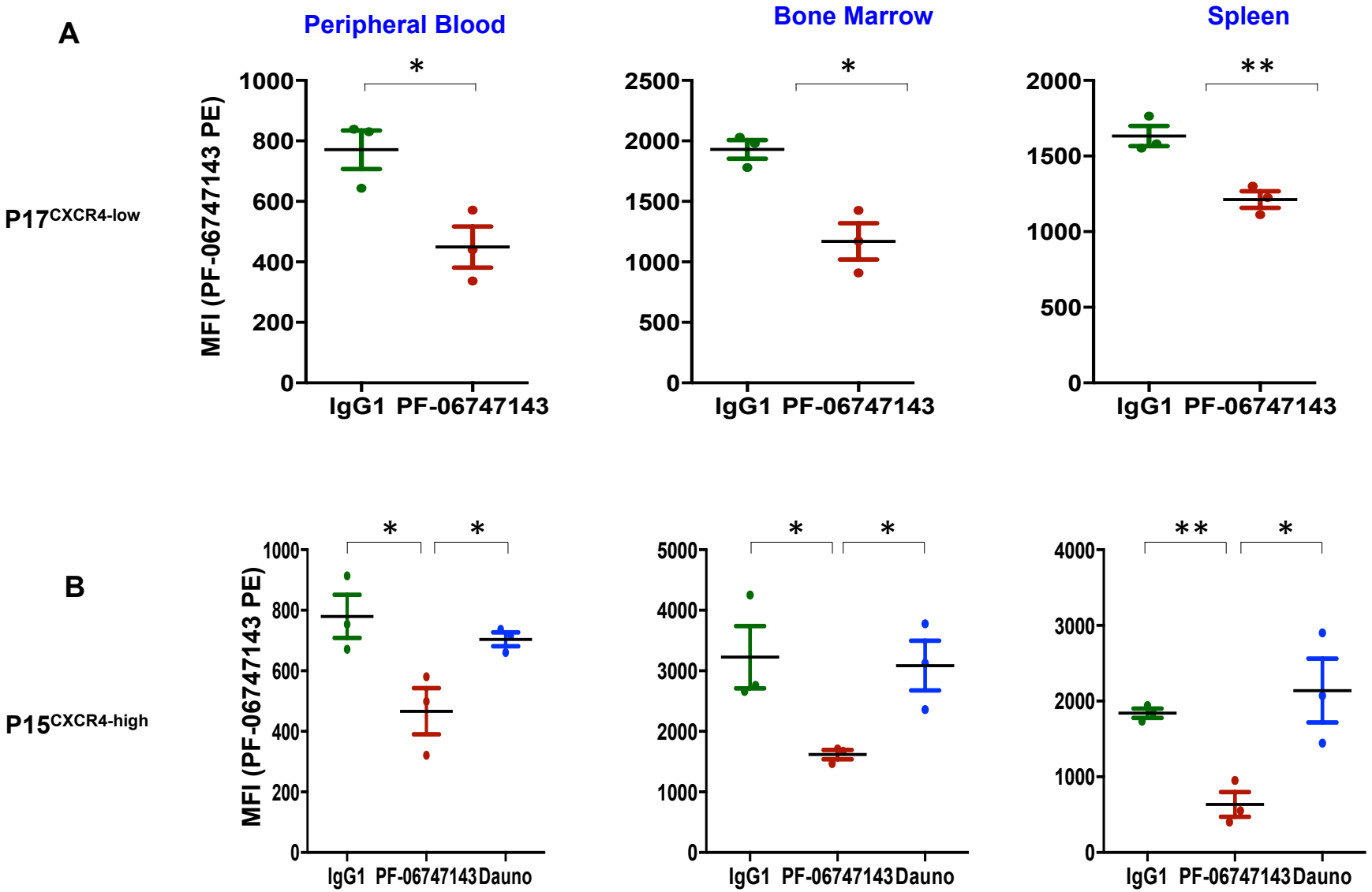
Supplemental Figure 2

Zhang Y et al



Supplemental Figure 3

Zhang Y et al



Supplemental Figure 4

Zhang Y et al

Supplemental Table I: Characteristics of the two AML patient samples studied in PDX model

patients (No.)	Gender	age	P/S AML*	FAB	Karyotype	WBC(x109/L)
$P15^{CXCR4\text{-}high}$	M	81	S	5	Normal	68
$P17^{CXCR4\text{-}low}$	F	32	S	5	-7, Inv3, -11p13	25,5

***: primary leukemia, S: secondary leukemia**

Supplemental figure legends

Supplemental Figure 1: Specificity of PF-06747143 antibody binding to CXCR4. Representative histograms of flow cytometry comparing PF-06747143 antibody binding to the UT7 CXCR4-negative human AML cell line transduced with empty vector (negative control) or, transduced with CXCR4 vector (UT7-CXCR4).

Supplemental Figure 2: PF-06747143 did not induce ADCC on a CXCR4-negative AML TF-1 cell line. ADCC activity was evaluated by incubating PF-06747143 (100nM) with CXCR4-negative AML TF-1 cell line or CXCR4-positive MV4-11 AML cells in the presence of NK92 158V effector cells.

Supplemental Figure 3: (A) Experimental design for evaluation of PF-06747143 efficacy on leukemic development. Mice were engrafted with CXCR4 low ($P17^{CXCR4\text{-}low}$) and CXCR4 high ($P15^{CXCR4\text{-}high}$) primary patient cells, 5 $x10^6$ /per mouse. After leukemia establishment, at 7 weeks for $P17^{CXCR4\text{-}low}$ and 10 weeks for $P15^{CXCR4\text{-}high}$ the mean percentage of AML cells in the peripheral blood was 7% and 2%, respectively. Mice were then divided into two or three groups, 8 mice/group, which received weekly subcutaneous treatment with IgG1 control, PF-06747143 Ab or daunorubicin (only for $P15^{CXCR4\text{-}high}$ PDX model). Twenty-four hours after each weekly treatment, quantitative analyses of PF-06747143-PE binding (mean fluorescence on human PB $CD45^+$ cells were performed. Each group comprises 8 mice. **B)** $P17^{CXCR4\text{-}low}$ PDX and **C)** $P15^{CXCR4\text{-}high}$ PDX. Data for each animal is shown and the horizontal bar represents the mean. * *P*<0,05 ; ** *P*<0,01 assessed by t-test**.**

Supplemental Figure 4: CXCR4 receptor occupancy by injected PF-06747143 antibody on blood, BM, and spleen AML cells at the end of in PDX efficacy studies. Quantitative analyses of PF-06747143-PE were performed on human $CD45^+$ cells derived from BM, spleen, and blood at 24 hours post last antibody treatment, for each PDX model: **A)** $P17^{CXCR4\text{-}low}$ PDX and **B)** $P15^{CXCR4\text{-}high}$ PDX. Each group comprises 8 mice. Data for each animal is represented and the horizontal bar is the average. * *P*<0.05 and ** *P*<0,01 assessed by t-test.