Targeting TKI-Activated NFKB2-MIF/CXCLs-CXCR2 Signaling Pathways in FLT3 mutated Acute Myeloid Leukemia Reduced Blast Viability

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Supplementary Documents

List of Reagents				
Antibody/Reagents	Abbreviation/Name in the text	Cat. #	Company	Species Reactivity
Viability Dye eFluor™ 780	Viability Dye	65-0865-14	eBioscience	
CD13-PE-CY7	CD13	301712	Biolegend	Human
CD14-APC	CD14	325608	Biolegend	Human
CD33-PERCP	CD33	341640	BD	Human
CD33-APC	CD33	303408	Biolegend	Human
CD33-FITC	CD33	366620	Biolegend	Human
CD34-PE	CD34	348057	BD Biosciences	Human
CD44-FITC	CD44	338804	Biolegend	Human
CD44-PE-Cy7	CD44	338816	Biolegend	Human
CXCR2-PE	CXCR2	320705	Biolegend	Human
CXCR2-APC	CXCR2	320710	Biolegend	Human
CXCR2	CXCR2	20634-1-AP	Ptglab	Human
CD117-PERCP	CD117	313214	Biolegend	Human
Ki-67-PE	Ki-67	350504	Biolegend	Human
Ki-67-APC	Ki-67	350514	Biolegend	Human
Ki-67-PERCP	Ki-67	350519	Biolegend	Human
BrdU-PERCP	BrdU	364109	Biolegend	
BrdU (5-bromo-2'-deoxyuridine)	BrdU	142567	Abcam	
IgG1, κ Isotype Ctrl- FITC		400107	Biolegend	
IgG1, κ Isotype Ctrl- PE		400111	Biolegend	

IgG1, κ Isotype Ctrl- APC		400121	Biolegend	
IgG1, κ Isotype Ctrl- PERCP		400147	Biolegend	
Gilteritinib (ASP2215)	GILT	S7754	SELLECKCHEM	
Quizartinib	QUIZ	A10027	ADOOQ	
SB225002	CXCR2-I	HY-16711	MedChemExpress	
BAY11-7082 (BAY11-7821)	NFKB-I	S2913	SELLECKCHEM	
Recombinant Human MIF	MIF	300-69	Peprotech	Human
Human XL Cytokine Array Kit	Cytokine Array	ARY002B	R&D SYSTEMS	Human
siRNA Oligo NFKB2 p100/ p52	siRNA-NFKB2	SR303162	Origene	Human

Supplementary Table S1: List of Reagents used in this study.

#	Name (HUMAN)	Forward Sequence	Reverse Sequence
1	*CD44*	CCAGAAGGAACAGTGGTTTGGC	ACTGTCCTCTGGGCTTGGTGTT
2	*CD74*	AAGCCTGTGAGCAAGATGCGCA	AGCAGGTGCATCACATGGTCCT
3	*CXCR2*	TCCGTCACTGATGTCTACCTGC	TCCTTCAGGAGTGAGACCACCT
4	*CXCL1*	AGCTTGCCTCAATCCTGCATCC	TCCTTCAGGAACAGCCACCAGT
5	*CXCL5*	CAGACCACGCAAGGAGTTCATC	TTCCTTCCCGTTCTTCAGGGAG
6	*CXCL8 (IL8)*	GAGAGTGATTGAGAGTGGACCAC	CACAACCCTCTGCACCCAGTTT
7	*NFKB1*	GCAGCACTACTTCTTGACCACC	TCTGCTCCTGAGCATTGACGTC
8	*NFKB2*	GGCAGACCAGTGTCATTGAGCA	CAGCAGAAAGCTCACCACACTC
9	*MIF*	AGAACCGCTCCTACAGCAAGCT	GGAGTTGTTCCAGCCCACATTG
10	*CYCLIN E1*	TGTGTCCTGGATGTTGACTGCC	CTCTATGTCGCACCACTGATACC
11	*CDK4*	CCATCAGCACAGTTCGTGAGGT	TCAGTTCGGGATGTGGCACAGA

Supplementary Table S2: List of Primers (Origene, etc.) used in this study.
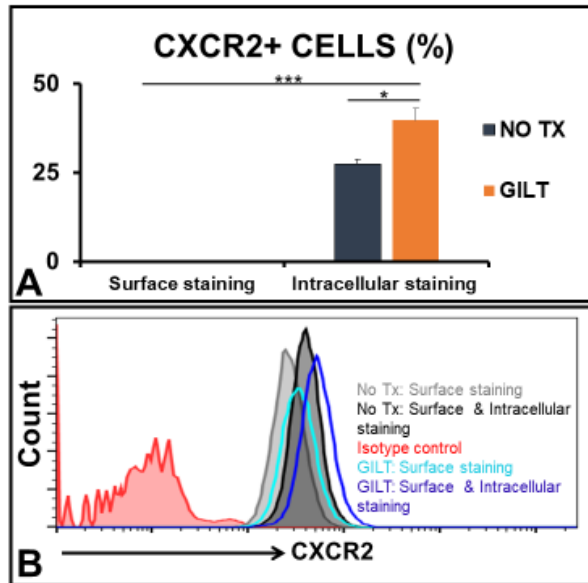
Supplementary Figure S1. Increased CXCR2 expression in survived GILT-treated MV4-11 cells *in vitro*.

A) Cumulative FC percentage data of viable CXCR2+ MV4-11 cells after 3 days' GILT-treatment, which were analyzed by either surface staining or intracellular staining;

B) Representative FC histogram plot of CXCR2 expressions in viable GILT-treated MV4-11 cells *in vitro*;

Briefly, MV4-11 cells were treated with 80nM GILT or without GILT (No Tx) for three days, and then underwent medium change to remove GILT and continuous culture for another week to let survived GILT-treated cells recover. Then GILT-treated cells were collected for FC staining including surface staining and/or intracellular staining to detect CXCR2 expressions. Color curves indicate different staining methods and show increased expressions of CXCR2 in viable GILT-treated MV4-11 cells (light or dark blue curves) when compared to the non-treatment controls (gray or black curves);

$*P<0.05$, $***P<0.005$.
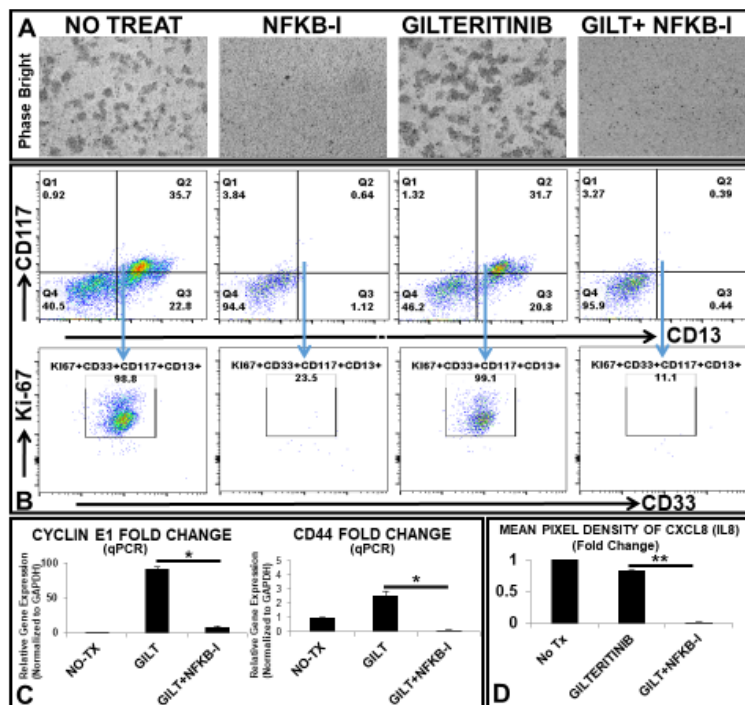
Supplementary Figure S2. Therapeutic effect of the combination of 80nM GILT and 50uM NFKB-I on a refractory AML-FLT3 BMMNC (Patient #6) *ex vivo*.

A) Representative phase-bright images showing tumorigenic cluster formation in different experimental groups 3 days after the treatment of different TKI drugs *ex vivo*;

B) Representative FC plots of viable CD117+CD13+ primary blasts in different treatment groups; Blue arrows (Gating) indicate further analyses of Ki-67 expression of these viable CD117+CD13+ primary blasts;

C) 3 days after the treatment *ex vivo*, the cells were collected for RNA isolation and gene expressions were analyzed by qPCR. Data show mRNA expressions of the genes of *CYCLIN E1* and *CD44*;

D) Cumulative Mean Pixel Densities (Fold Change) of CXCL8, which were acquired from proteome analyses of cell-free supernatants from treated groups of primary AML BMMNC cells. *P<0.05, **P<0.01.