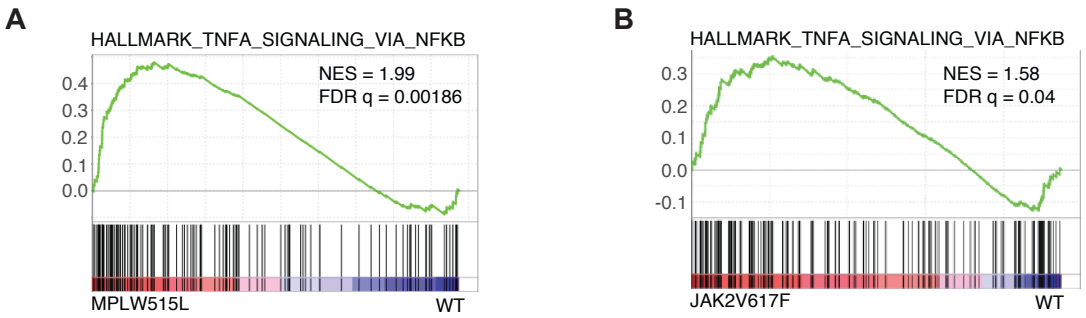


Figure S1, related to Figure 1. TNF/NF-κB signaling axis in MF progenitors. A, B) GSEA pathways analysis of H3K27ac marks at enhancers from MPLW515L-positive **(A)** and JAK2V617F-positive **(B)** MEPs. NES, normalized enrichment score; FDR, false-discovery rate.

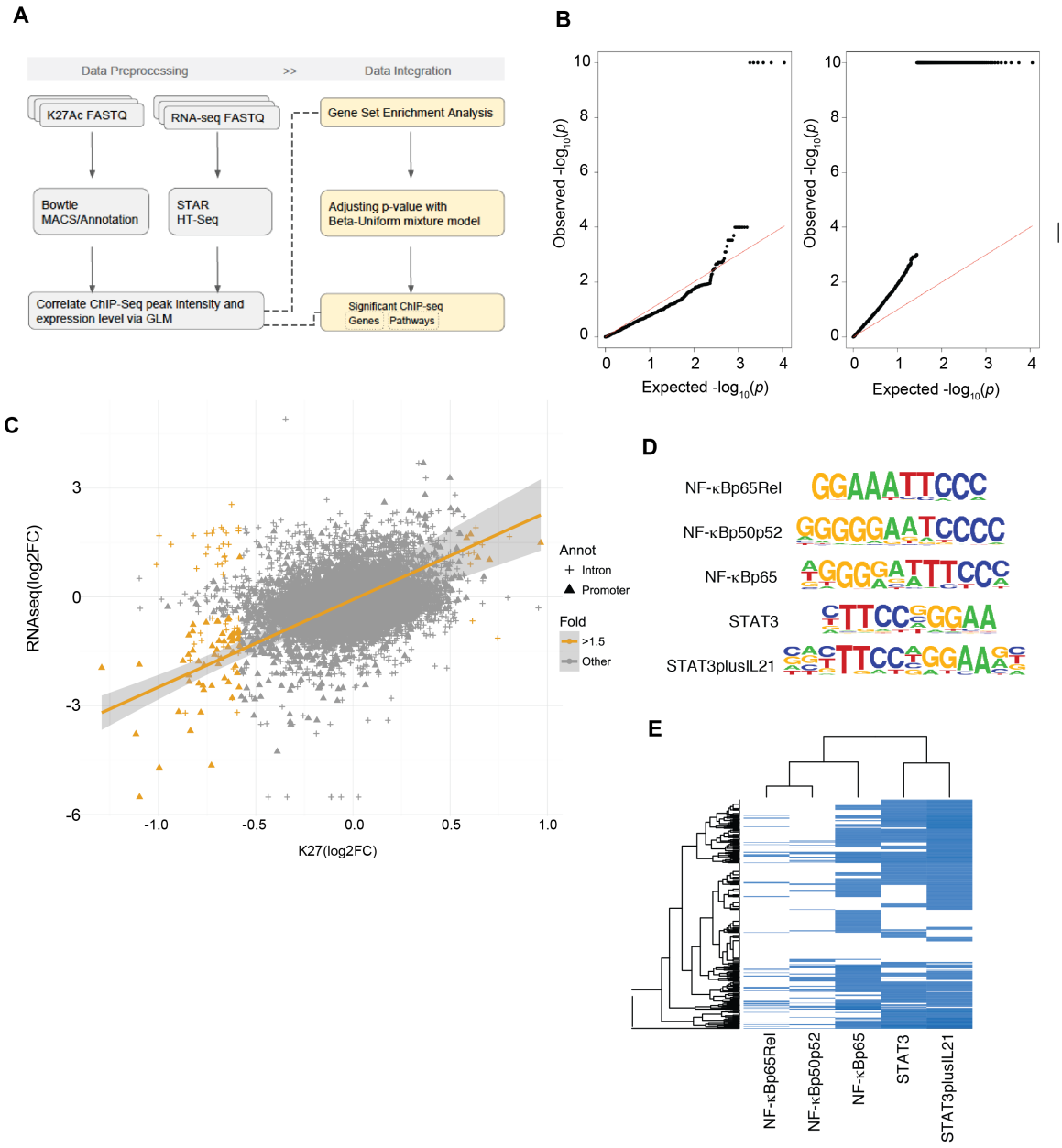


Figure S2, related to Figure 2. Integrative analysis of ChIP-seq and gene expression data. A) RNA-seq and ChIP-seq data analysis workflow. GLM, generalized linear model. **B)** QQ plot of GSEA p values. **C)** Differentially expressed genes (log2-fold change) are plotted versus differentially enriched H3K27ac peaks (log2-fold change). K27, H3K27ac; log2FC, log2-fold change. **D)** DNA motifs used for transcription factor binding analysis. **E)** Analysis of the STAT3 (STAT3, STAT3plusIL21) and NF-κB (NF-κBp65Rela, NF-κBp50p52, and NF-κBp65) transcription factor bindings sites in the regulatory regions of the DEGs in JAK2V617F-positive MEPs.

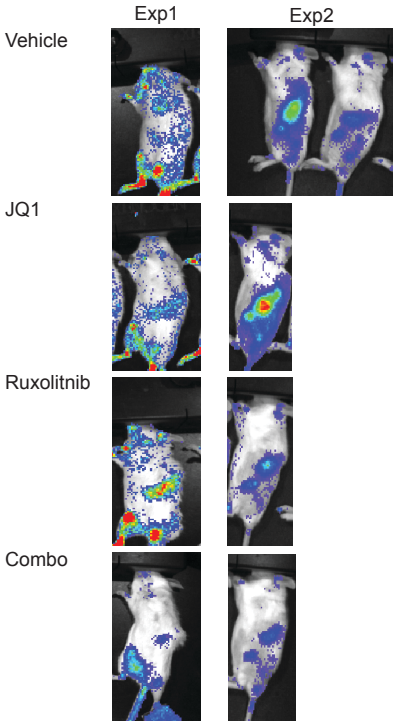


Figure S3, related to Figure 3. BET and JAK inhibition alone and in combination reduces NF-κB pathway activation *in vivo*. *In vivo* BLI of MPLW515L-diseased mice treated with ruxolitinib (60 mg/kg, BID), JQ1 (50 mg/kg, QD), ruxolitinib plus JQ1, or vehicle for three days. Mice were imaged 2-3 hours after last drug administration. Images shown are from two independent experiments.

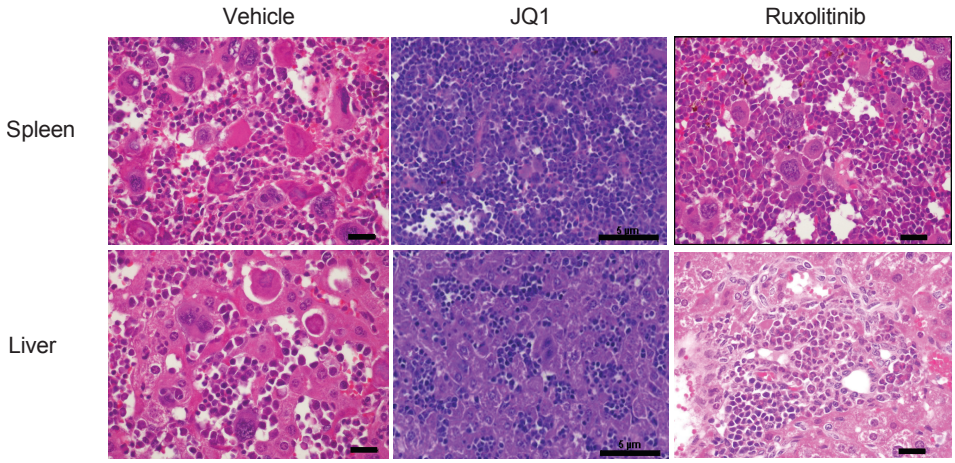
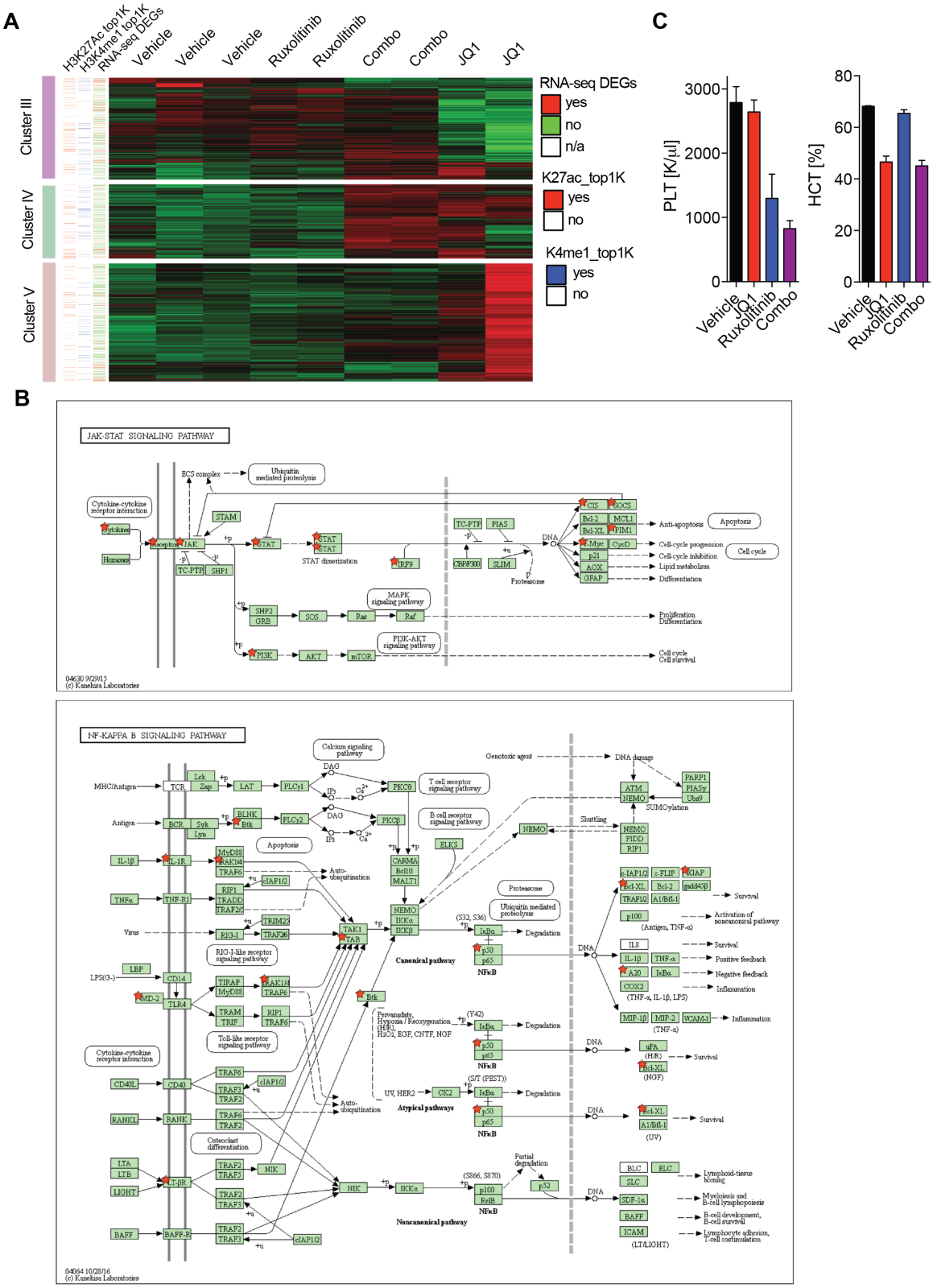


Figure S4, related to Figure 4. BET and JAK inhibition alone and in combination reduces EMH in liver and spleen of MPLW515L-diseased mice. Representative images showing H&E stain of spleen and liver from MF mice treated for 21 (vehicle and JQ1) or 28 (ruxolitinib) days. Data shown are representative of 4-5 mice per group. Scale bar, 10 μM (vehicle and ruxolitinib), 5 μM (JQ1).


A
H3K27Ac top1K
H3K4me1 top1K
RNA-seq DEGs
Vehicle
Vehicle
Vehicle
Ruxolitinib
Ruxolitinib
Combo
Combo
JQ1
JQ1
Cluster III
Cluster IV
Cluster V
RNA-seq DEGs
yes
no
n/a
K27ac_top1K
yes
no
K4me1_top1K
yes
no
C
PLT [K/µl]
3000
2000
1000
0
HCT [%]
80
60
40
20
0
Vehicle
JQ1
Ruxolitinib
Combo
B
JAK-STAT SIGNALING PATHWAY
Cytokine-cytokine receptor interaction
Cytokine
Hormone
Receptor
JAK
STAM
STAT
STAT dimerization
TC-PTP
SHP1
SHP2
GRB
SOS
Ras
Raf
MAPK signaling pathway
PI3K
AKT
mTOR
PI3K-AKT signaling pathway
IRF9
ISGF3
CBP/P300
SLIM
Proteasome
DNA
CIS
SOCS
Bcl-2
MCL1
Bcl-XL
PIM1
c-Myc
CyeD
p21
AOX
GFAP
Anti-apoptosis
Apoptosis
Cell-cycle progression
Cell cycle
Cell-cycle inhibition
Lipid metabolism
Differentiation
Proliferation
Differentiation
Cell cycle
Cell survival
04630 9/29/15
(c) Kanehisa Laboratories
NF-KAPPA B SIGNALING PATHWAY
Calcium signaling pathway
MHC/Antigen
TCR
Lck
Zap
LAT
PLCγ1
PKCθ
T cell receptor signaling pathway
Antigen
BCR
Syk
Lyn
BLNK
Btk
PLCγ2
PKCβ
B cell receptor signaling pathway
Apoptosis
ELKS
IL-1β
IL-1R
MyD88
IRAK1/4
TRAF6
TNFα
TNF-R1
RIP1
TRADD
TRAF2/5
cIAP1/2
Auto-ubiquitination
Virus
RIG-I
TRIM25
TRAF26
RIG-I-like receptor signaling pathway
LBP
LPS(G-)
CD14
MD-2
TLR4
TRAP
TRAM
TRIF
Toll-like receptor signaling pathway
CARMA
Bcl10
MALT1
NEMO
IKKα
IKKβ
TAK1
TAB
Canonical pathway
IκBα
Degradation
p50
p65
NFκB
Pervanadate, Hypoxia / Reoxygenation (H/R), H2O2, EGF, CNTF, NGF
(Y-42)
UV, HER2
CK2
(S/T (PEST))
Atypical pathways
Cytokine-cytokine receptor interaction
CD40L
CD40
RANKL
RANK
Osteoclast differentiation
LTA
LTB
LIGHT
T-βR
BAFF
BAFF-R
NIK
IKKα
(S866, S870)
p100
RelB
p52
Partial degradation
Noncanonical pathway
Genotoxic agent
DNA damage
PARP1
PIASy
Ubc9
ATM
NEMO
SUMOylation
Shuttling
NEMO
PIDD
RIP1
c-IAP1/2
c-FLIP
XIAP
Bcl-XL
Bcl-2
gadd45β
TRAF1/2
A1/Bfl-1
Survival
p100
Activation of noncanonical pathway
(Antigen, TNF-α)
IL8
IL-1β
TNF-α
Positive feedback
A20
IκBα
Negative feedback
COX2
Inflammation
(TNF-α, IL-1β, LPS)
MIP-1β
MIP-2
VCAM-1
Inflammation
(TNF-α)
uPA
(H/R)
Survival
(NGF)
Bcl-XL
A1/Bfl-1
Survival
(UV)
BLC
ELC
Lymphoid-tissue homing
SLC
SDF-1α
Myelopoiesis and B-cell lymphopoiesis
BAFF
B-cell development, B-cell survival
ICAM
Lymphocyte adhesion, T-cell costimulation
(LT/LIGHT)
04064 10/28/16
(c) Kanehisa Laboratories

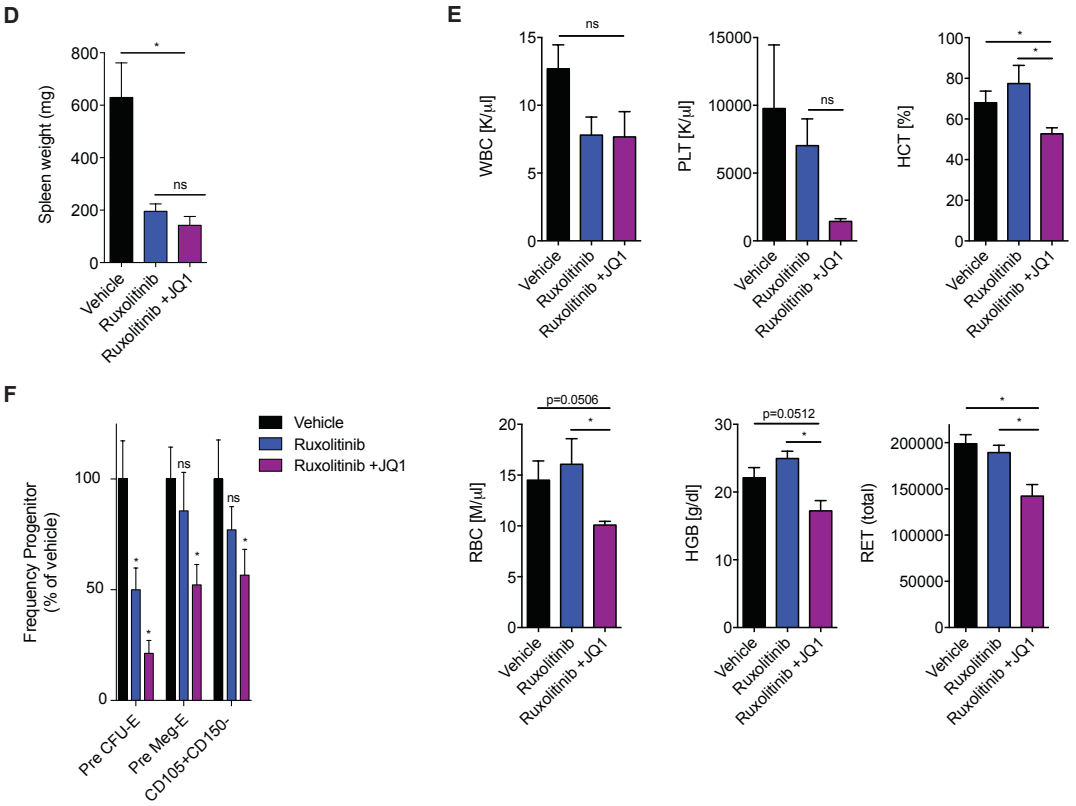


Figure S5, **related to Figure 5. Combined BET and JAK inhibition abrogates murine MPN. A)** Heatmap depicting RNA-sequencing data for indicated samples. Data from biological duplicates or triplicates are shown. Color code indicates whether a gene is differentially expressed (DEGs) or contains an H3K27ac or H3K4me1 peak belonging to the top 1000 significant differential peaks (K27ac_top1K or K4me1_top1K, respectively). **B)** Representation of the KEGG JAK/STAT and NF-κB Signaling Pathway, showing differentially regulated genes between MF and control cells (red stars). **C)** PLT counts (K/μl) and HCT levels (%) of MF mice treated as indicated for 21 days. **D)** Bar graph showing spleen weights of primary JAK2V617F mice (2-4 months) treated with vehicle, ruxolitinib, or combination for 28 days. **E)** White blood cell counts (WBC, K/μl), platelet counts (PLT, K/μl), hematocrit levels (HCT, %), red blood cell counts (RBC, M/K/μl), hemoglobin level (HGB, g/dl) and reticulocyte count (RET, total count) of primary JAK2V617F mice treated with vehicle, ruxolitinib, or ruxolitinib plus JQ1 for 28 days. **F)** Flow analysis of megakaryocytic-erythroid progenitors ($Lin^-Sca1^-cKit^+CD41^-FcgR^-$) in the bone marrow from JAK2V617F mice treated as indicated for 28 days. Frequency of progenitors in control mice represents 100%. Data shown in this figure are from two different experiments with a totel of n=4-5 mice/group. ns= not significant, *, p value <0.05. Bar graphs represent mean ± S.E.M.

Table S4, related to Figure 5. GO term enrichment analysis using DAVID

Cluster	Term	Benjamini
I	cell division	4.80E-17
	DNA repair	4.20E-14
	RNA splicing	5.10E-13
	NK/NK-kappaB signaling	3.90E-10
	tumor necrosis factor-mediated signaling pathway	2.70E-04
	MAPK cascade	7.80E-03
II	mitotic cell cycle	8.90E-01
	cell division	7.30E-02
	RNA splicing	5.20E-01
III	cytokine-mediated signaling pathway	7.20E-02
	type I interferon signaling pathway	1.80E-01
	positive regulation of NF-kappaB import into nucleu	2.70E-01
	regulation of small GTPase mediated signal transdu	1.70E-01
	regulation of dendritic cell cytokine production	7.20E-01
	positive regulation of chemokine production	7.30E-01
	I-kappaB phosphorylation	9.40E-01
IV	innate immune response	1.80E-01
	MAPK cascade	9.00E-01
	NIK/NF-kappaB signaling	1.50E-01
	tumor necrosis factor-mediated signaling pathway	3.10E-01

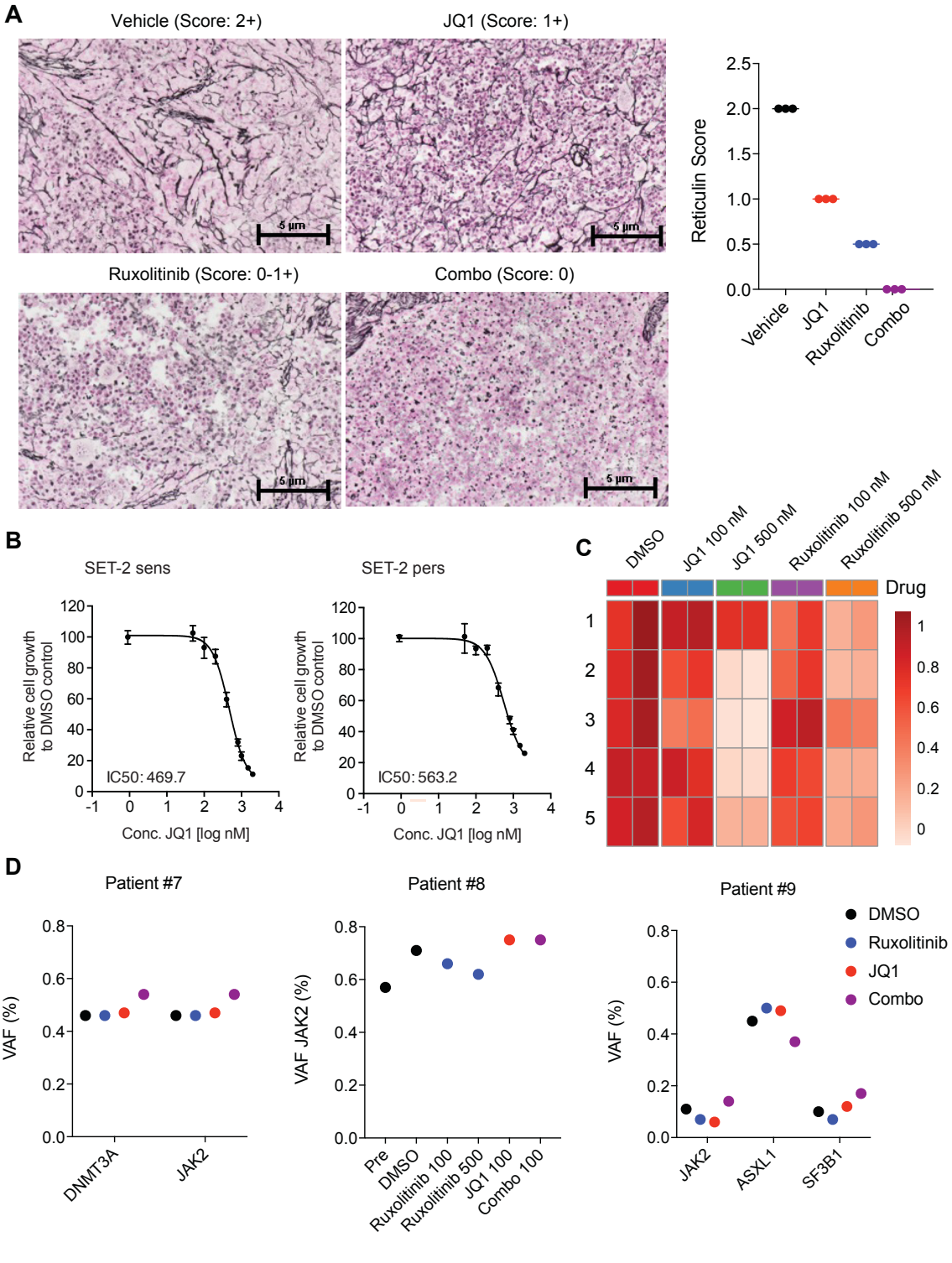

A
Vehicle (Score: 2+)
JQ1 (Score: 1+)
Ruxolitinib (Score: 0-1+)
Combo (Score: 0)
5 µm
Reticulin Score
2.5
2.0
1.5
1.0
0.5
0.0
Vehicle
JQ1
Ruxolitinib
Combo
B
SET-2 sens
SET-2 pers
Relative cell growth to DMSO control
IC50: 469.7
IC50: 563.2
Conc. JQ1 [log nM]
C
DMSO
JQ1 100 nM
JQ1 500 nM
Ruxolitinib 100 nM
Ruxolitinib 500 nM
Drug
1
2
3
4
5
D
Patient #7
Patient #8
Patient #9
VAF (%)
VAF JAK2 (%)
DNMT3A
JAK2
Pre
DMSO
Ruxolitinib 100
Ruxolitinib 500
JQ1 100
Combo 100
ASXL1
SF3B1
DMSO
Ruxolitinib
JQ1
Combo

Figure S6, **related to Figure 6. SET-2 cells are sensitive to BET inhibitor JQ1. A)** *Left:* Representative images of the degree of fibrosis in the spleen of MF mice treated as indicated. *Right:* Scoring of the degree of fibrosis in the spleen of MF mice treated as indicated. n=3 mice per group. Scale bar, 5 μM. **B)** SET-2 cells were treated with increasing concentrations of JQ1 for 48 hours. Data are representative of three independent experiments. IC50, drug concentration to reduce cell growth by 50 %. sens, SET-2 parental cells not previously exposed to ruxolitinib; pers, ruxolitinib-persistent SET-2 cells. Graphs represent mean values ± S.E.M. **C)** CFU assays of CD34-positive cells from MPN patients. Each row represents the data from one patient with colony counts normalized to DMSO control wells. n=2 wells/condition are shown. Genetic information for each patient is listed in **Table S5. D)** Variant allele frequencies (VAF, %) from propagated colonies of 3 MPN patients post exposure to different drug conditions are shown.

Table S5, related to Figure 6. Information for patient samples used for CFU assays

No	Disease	Gender	Age	Mutations	VAF
1	MF	M	42	JAK2 (NM_004972) exon 14 p.V617F (c.1849G>T)	N/A
				DNMT3A N802S	N/A
				SF3B1 K666N	N/A
				TP53 V172A	N/A
2	MF	F	70	JAK2 (NM_004972) exon 14 p.V617F (c.1849G>T)	0.49
3	MF	F	55	JAK2 (NM_004972) exon 14 p.V617F (c.1849G>T)	N/A
				CBL splicing exon 16	N/A
				del20q	N/A
4	MF	M	77	JAK2 (NM_004972) exon 14 p.V617F (c.1849G>T)	N/A
5	post ET-MF	F	76	CALR exon 9	N/A
6	PV (high-grade)	F	57	EZH2 (NM_004456) exon12 p.R475T (c.1424G>C)	N/A
				JAK2 (NM_004972) exon14 p.V617F (c.1849G>T)	0.25
7*	PV	M	82	DNMT3A (NM_022552) exon23 p.R882H (c.2645T>A)	N/A
				JAK2 (NM_004972) exon14 p.V617F (c.1849G>T)	0.737
8*	MF	F	69	JAK2 (NM_004972) exon 14 p.V617F (c.1849G>T)	0.83
				RUNX1 (NM_001754) exon 5 p.F163L (c.487T>C)	0.15
9*	MF	F	64	JAK2 (NM_004972) exon 14 p.V617F (c.1849G>T)	0.23
				DNMT3A (NM_022552) exon 20 p.K783E (c.2347A>G)	0.06
				SF3B1 (NM_012433) exon 2 p.D34N (c.100G>A)	0.24
10	MF	M	40	PHF6 (NM_032458) exon 6 p.H149L (c.446A>T)	N/A

* Patients used for detailed mutational analysis

Table S6, related to Figure 6. Mutational profiling results using a targeted myeloid-disease s

Patient ID	Treatment cc	GENE	CHR	START	END	REF	ALT	TRANSCRIPT	RNA_CHANGE	cDNA_CHANGE	PROTEIN_CHANGE	EFFECT	TARGET_VAF	TARGET_DEPTH	NORMAL_VAF	NORMAL_DEPTH
7	DMSO	*DNMT3A*	2	25457242	25457242	C	T	CCDS33157.1	r.2983g>a	c.2645G>A	p.R882H	non_synonymous_codon	0.46	1223	0	765
	Rux 100nM	*DNMT3A*	2	25457242	25457242	C	T	CCDS33157.1	r.2983g>a	c.2645G>A	p.R882H	non_synonymous_codon	0.46	885	0	765
	JQ1 100nM	*DNMT3A*	2	25457242	25457242	C	T	CCDS33157.1	r.2983g>a	c.2645G>A	p.R882H	non_synonymous_codon	0.47	891	0	765
	Combo 100n	*DNMT3A*	2	25457242	25457242	C	T	CCDS33157.1	r.2983g>a	c.2645G>A	p.R882H	non_synonymous_codon	0.54	692	0	765
	DMSO	*JAK2*	9	5073770	5073770	G	T	CCDS6457.1	r.2343g>u	c.1849G>T	p.V617F	non_synonymous_codon	0.55	945	0	841
	Rux 100nM	*JAK2*	9	5073770	5073770	G	T	CCDS6457.1	r.2343g>u	c.1849G>T	p.V617F	non_synonymous_codon	0.49	720	0	841
	JQ1 100nM	*JAK2*	9	5073770	5073770	G	T	CCDS6457.1	r.2343g>u	c.1849G>T	p.V617F	non_synonymous_codon	0.56	727	0	841
	Combo 100n	*JAK2*	9	5073770	5073770	G	T	CCDS6457.1	r.2343g>u	c.1849G>T	p.V617F	non_synonymous_codon	0.54	612	0	841
8#	Before platin	*JAK2*	9	5073770	5073770	G	T	CCDS6457.1	r.2343g>u	c.1849G>T	p.V617F	non_synonymous_codon	0.57	569	0	841
	DMSO	*JAK2*	9	5073770	5073770	G	T	CCDS6457.1	r.2343g>u	c.1849G>T	p.V617F	non_synonymous_codon	0.71	879	0	841
	Rux 100nM	*JAK2*	9	5073770	5073770	G	T	CCDS6457.1	r.2343g>u	c.1849G>T	p.V617F	non_synonymous_codon	0.66	740	0	841
	Rux 500nM	*JAK2*	9	5073770	5073770	G	T	CCDS6457.1	r.2343g>u	c.1849G>T	p.V617F	non_synonymous_codon	0.62	690	0	841
	JQ1 100nM	*JAK2*	9	5073770	5073770	G	T	CCDS6457.1	r.2343g>u	c.1849G>T	p.V617F	non_synonymous_codon	0.75	828	0	841
	Combo 100n	*JAK2*	9	5073770	5073770	G	T	CCDS6457.1	r.2343g>u	c.1849G>T	p.V617F	non_synonymous_codon	0.75	726	0	841
9	DMSO	*JAK2*	9	5073770	5073770	G	T	CCDS6457.1	r.2343g>u	c.1849G>T	p.V617F	non_synonymous_codon	0.11	961	0	841
	Rux 100nM	*JAK2*	9	5073770	5073770	G	T	CCDS6457.1	r.2343g>u	c.1849G>T	p.V617F	non_synonymous_codon	0.07	955	0	841
	JQ1 100nM	*JAK2*	9	5073770	5073770	G	T	CCDS6457.1	r.2343g>u	c.1849G>T	p.V617F	non_synonymous_codon	0.06	840	0	841
	Combo 100n	*JAK2*	9	5073770	5073770	G	T	CCDS6457.1	r.2343g>u	c.1849G>T	p.V617F	non_synonymous_codon	0.14	790	0	841
	DMSO	*ASXL1*	20	31023598	31023598	C	T	CCDS13201.1	r.3507c>u	c.3083C>T	p.S1028L	non_synonymous_codon	0.45	1227	0	900
	Rux 100nM	*ASXL1*	20	31023598	31023598	C	T	CCDS13201.1	r.3507c>u	c.3083C>T	p.S1028L	non_synonymous_codon	0.5	1134	0	900
	JQ1 100nM	*ASXL1*	20	31023598	31023598	C	T	CCDS13201.1	r.3507c>u	c.3083C>T	p.S1028L	non_synonymous_codon	0.49	1125	0	900
	Combo 100n	*ASXL1*	20	31023598	31023598	C	T	CCDS13201.1	r.3507c>u	c.3083C>T	p.S1028L	non_synonymous_codon	0.37	870	0	900
	DMSO	*SF3B1*	2	198288627	198288627	C	T	CCDS33356.1	r.192g>a	c.100G>A	p.D34N	non_synonymous_codon	0.1	1509	0	1341
	Rux 100nM	*SF3B1*	2	198288627	198288627	C	T	CCDS33356.1	r.192g>a	c.100G>A	p.D34N	non_synonymous_codon	0.07	1523	0	1341
	JQ1 100nM	*SF3B1*	2	198288627	198288627	C	T	CCDS33356.1	r.192g>a	c.100G>A	p.D34N	non_synonymous_codon	0.12	1458	0	1341
	Combo 100n	*SF3B1*	2	198288627	198288627	C	T	CCDS33356.1	r.192g>a	c.100G>A	p.D34N	non_synonymous_codon	0.17	1147	0	1341

Runx1 mutation not detected during mutational profiling of propagated colonies

*ASXL1 mutation newly identified in this patient, DNMT3A mutation not detected during mutational profiling