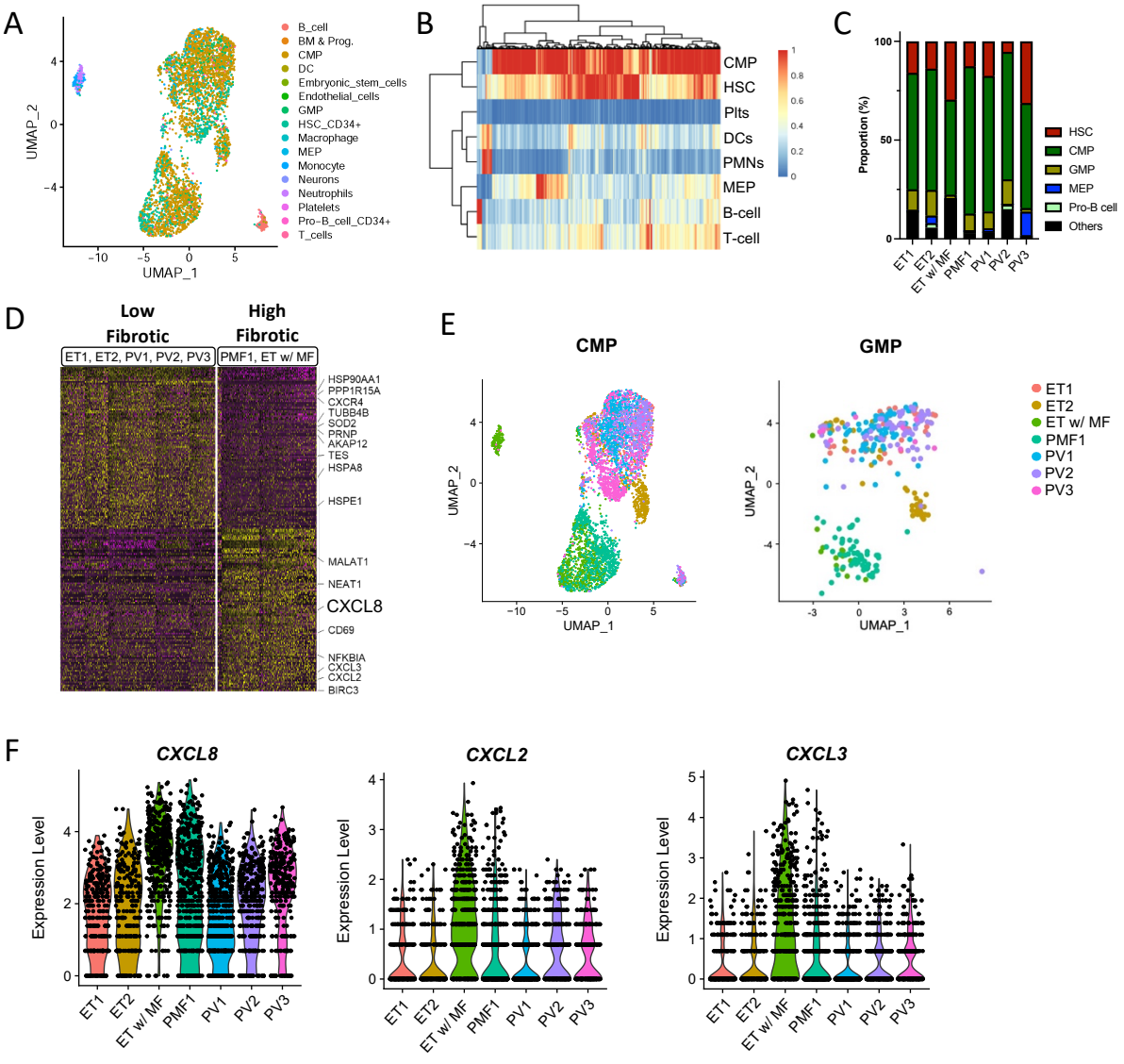
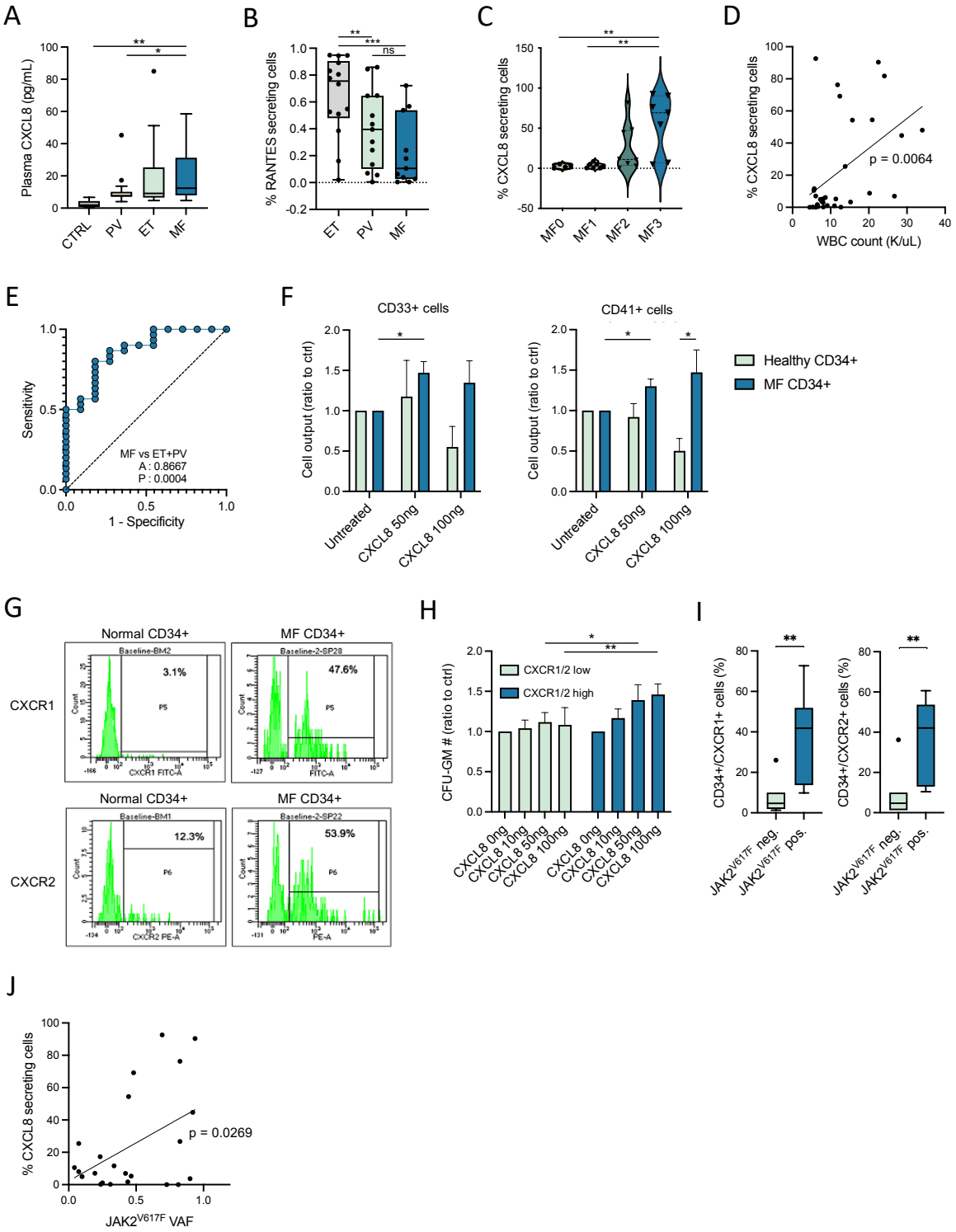
SUPPLEMENTAL FIGURES

Supplemental Figure 1 – Single-cell RNA-Seq (scRNA-Seq) identifies enrichment in CXCR2 signaling mediators in MF HSPCs



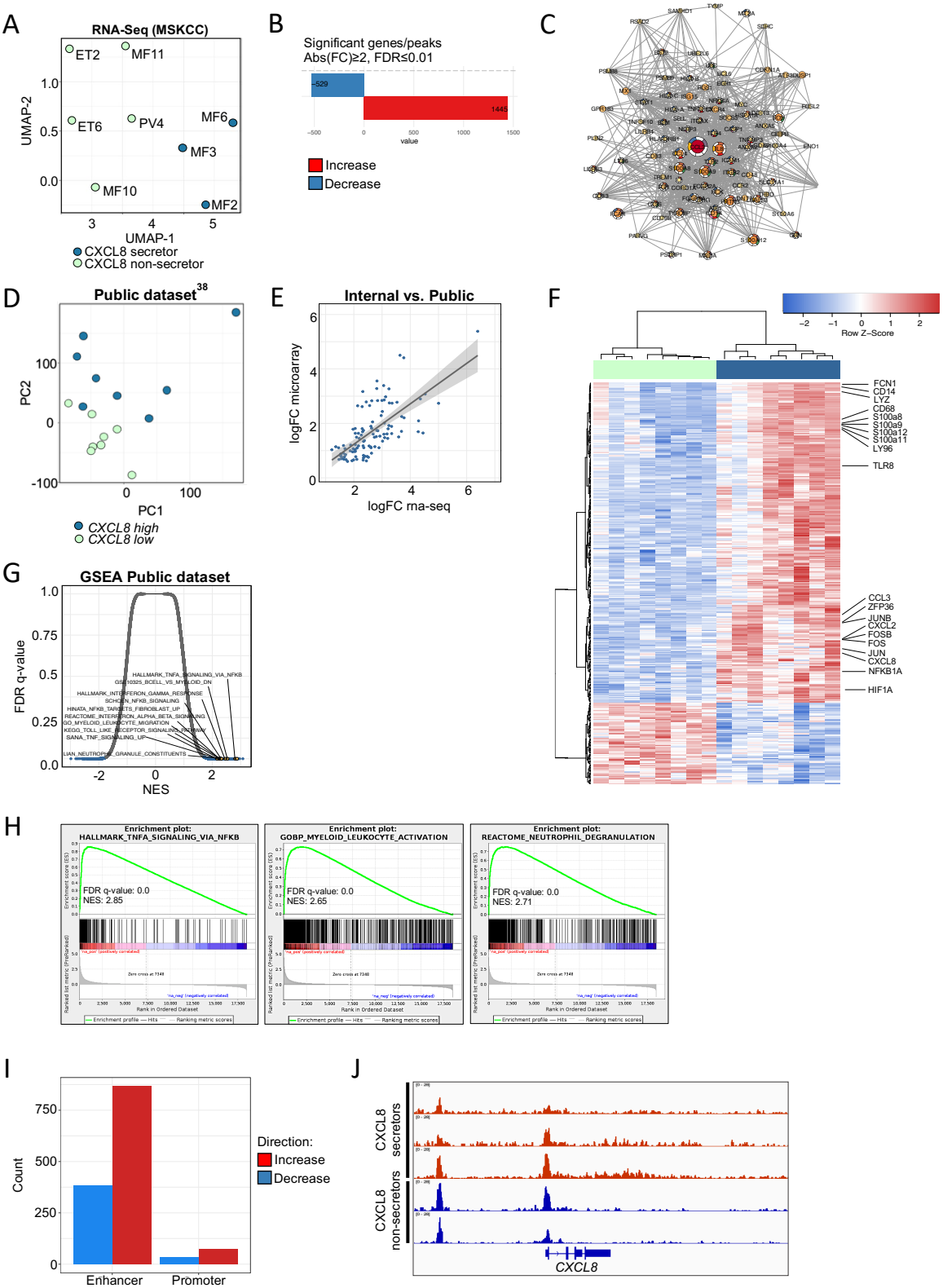
Supplemental Figure 1: Single-cell RNA-Seq (scRNA-Seq) identifies enrichment in CXCR2 signaling mediators in MF HSPCs. (A) Uniform manifold approximation and projection (UMAP) demonstrating clustering of detectable cell populations based on single-cell transcriptional profiling. Each dot represents a single cell with color corresponding to cell type (CMP, common myeloid progenitor; DC, dendritic cell; GMP, granulocyte monocyte progenitor; HSC, hematopoietic stem cell; MEP, megakaryocyte erythroid progenitor). **(B)** Hierarchical clustering of detectable cell populations based on transcriptional profiling (CMP, common myeloid progenitor; DC, dendritic cell; Plts: platelets; GMP, granulocyte monocyte progenitor; HSC, hematopoietic stem cell; MEP, megakaryocyte erythroid progenitor; PMN: polymorphonuclear neutrophils). Each hashmark represents a single cell. Colors represent normalized scores. **(C)** Percent frequency of detectable cell populations identified by scRNA-Seq per patient. **(D)** Hierarchical clustering of differentially expressed genes in “low fibrotic” and “high fibrotic” MPN patients. Adjusted p-value <0.01 (Wald test) and abs (log2-fold change) >1. Purple, negative values; yellow, positive values. N=2-5/group. **(E)** UMAPs of common myeloid progenitor (CMP; left panel) and granulocyte-monocyte progenitor (GMP; right panel) populations colored by patient (ET, essential thrombocythemia; PV, polycythemia vera; MF, myelofibrosis). Each dot represents a single cell. **(F)** Violin plots showing relative expression of CXCR2-mediated cytokines *CXCL8* (left panel), *CXCL2* (middle panel), and *CXCL3* (right panel) in CMPs of individual MPN patients by scRNA-Seq. Each dot represents a single cell.

Supplemental Figure 2 – CXCL8 is aberrantly secreted by MF CD34+ cells and correlates with clinical parameters.



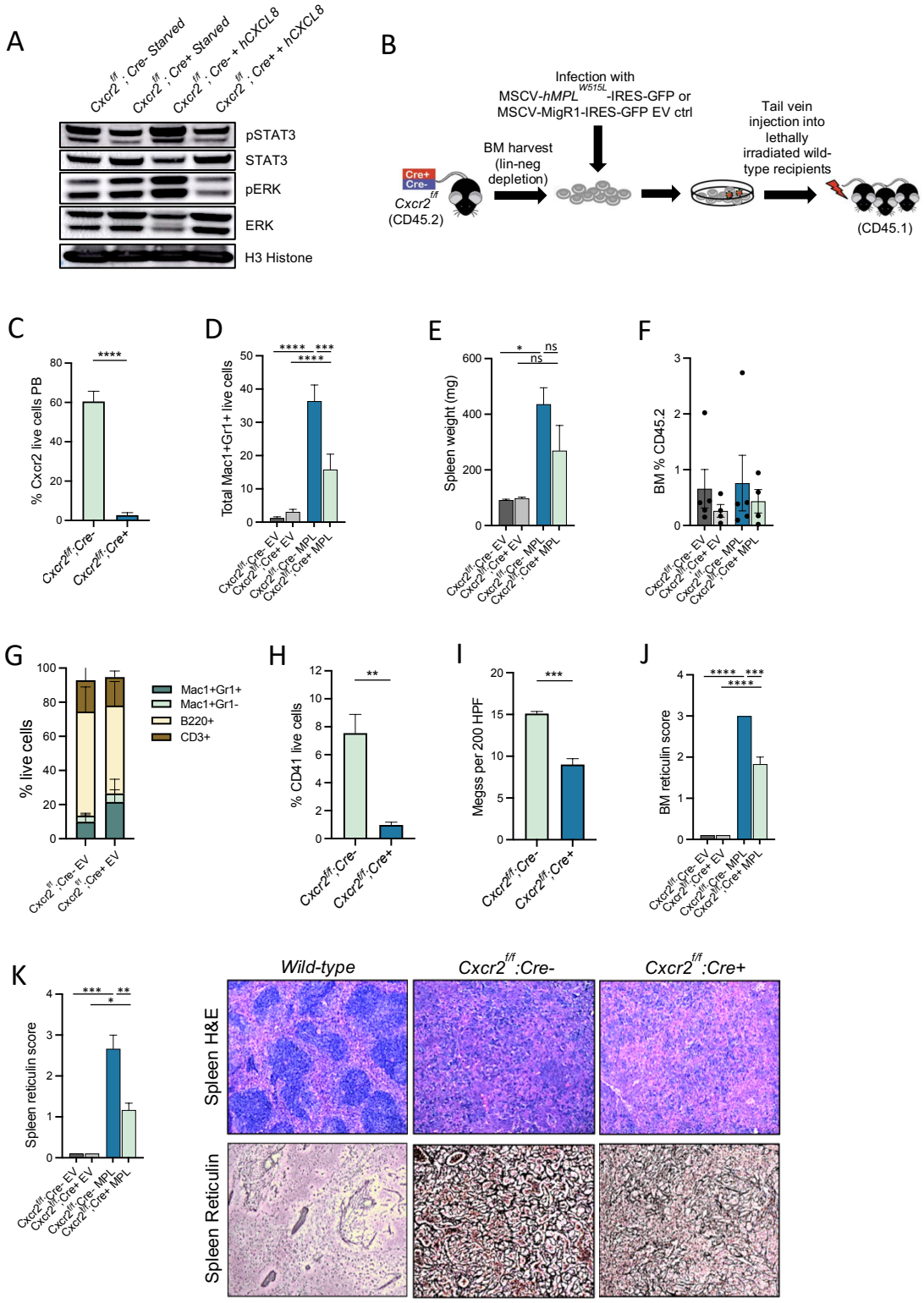
Supplemental Figure 2: CXCL8 is aberrantly secreted by MF CD34+ cells and correlates with clinical parameters. (A) CXCL8 levels as detected by ELISA in isolated plasma from PV, ET, and MF MPN patients in comparison to healthy controls (CTRL). (CTRL N=8, PV N=16, ET N=16, MF N=35). *p<0.05, **p<0.01. Data shown represent mean ± SD. **(B)** Box plots depicting correlation between MPN sub-type and percent fraction of RANTES-only secreting cells as detected by single-cell cytokine analysis. **p<0.01, ***p<0.001, ns=non-significant. **(C)** Violin plot depicting correlation between bone marrow reticulin score (MF0-3) of MF patients and percent fraction of detected CXCL8-only secreting cells by single-cell cytokine analysis (MF0 N=5, MF1 N=7, MF2 N=8, MF3 N=7). **p<0.01. **(D)** Linear correlation analysis of percent fraction of CXCL8-only secreting CD34+ cells detected by single-cell cytokine analysis and total white blood cell (WBC) count at time of sample collection. **(E)** Receiver operator curve demonstrating association between percent CXCL8-only secreting cells and MF sub-type in comparison to PV/ET. A=area under the curve; P=p-value. **(F)** Ratio of total CD33+ cell output (left panel) and CD41+ cell output (right panel) relative to untreated of cultured healthy donor (light blue) vs. MF (dark blue) CD34+ cells in response to exogenous CXCL8 as detected by flow cytometry. Representative of duplicate experiments from 3 healthy donor (HD) and 6 individual MF cases. *p<0.05. Data shown represent mean ± SD. **(G)** Representative histograms demonstrating fraction and intensity of MF CD34+ cells expressing CXCR1/2 receptors. **(H)** Ratio of granulocyte-macrophage progenitor (CFU-GM) colony output to control of cultured MF CD34+ cells in methylcellulose in response to increasing doses of exogenous CXCL8 stratified by degree of CXCR1/2 surface expression ("low:" <10% of cells, light blue; "high:" $\geq$10% of cells, dark blue). Representative of duplicate experiments from 4 of individual MF cases with low-CXCR1/2 and 6 of individual MF cases with high-CXCR1/2. *p<0.05, **p<0.01. **(I)** Percentage of total MF CD34+ cells expressing CXCR1 (left panel) or CXCR2 (right panel) as detected by flow cytometry based on $JAK2^{V617F}$ mutational status of JAK2WT MF samples (light blue; N=5) vs. JAKV617F+ MF samples (dark blue; N=10). **p<0.01. **(J)** Linear correlation analysis of percent fraction of CXCL8-only secreting CD34+ cells detected by single-cell cytokine analysis and associated $JAK2^{V617F}$ variant allele fraction (VAF) of bone marrow mononuclear cells at time of bone marrow biopsy.

Supplemental Figure 3 – Extended RNA-Seq/ATAC-Seq patient data and publicly available MF gene expression dataset



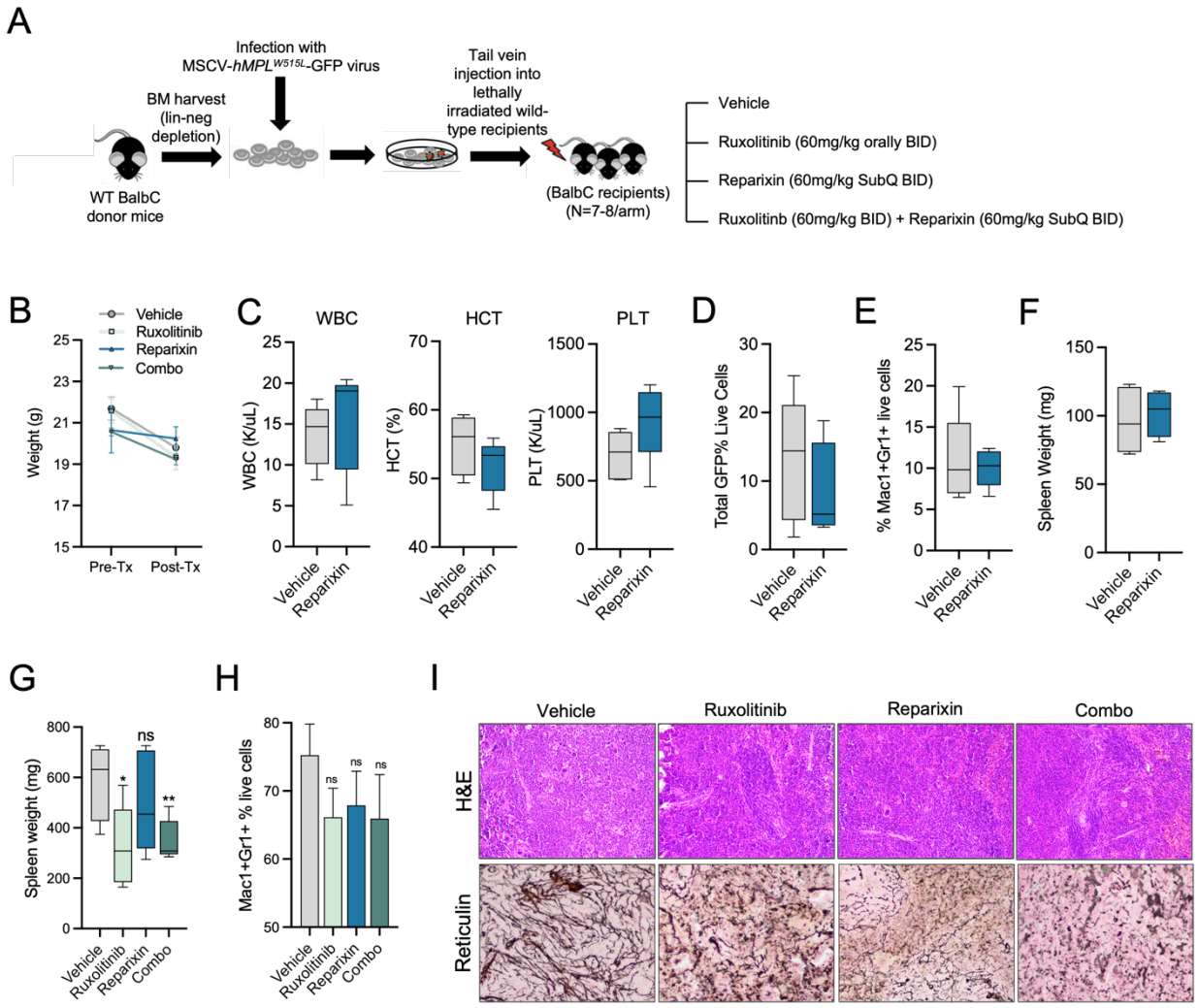
Supplemental Figure 3: Extended RNA-Seq/ATAC-Seq patient data and publicly available MF gene expression dataset. (A) Uniform manifold approximation and projection (UMAP) visualization of N=8 MPN patient samples (see Supp. Table S3) available for gene expression (RNA-Seq) analysis. Each dot represents an individual patient. Light blue represents CXCL8 "non-secretors;" dark blue represents CXCL8 "secretors" as identified by their single-cell cytokine secretion analysis. **(B)** Total number of differentially regulated genes (blue represents significantly down-regulated genes; red significantly up-regulated genes) of CXCL8 secretor vs. non-secretor MPN patients by a fold change cutoff of ±2 and a FDR of 1%. **(C)** Graphical representation of optimized gene expression sub-network analysis from gene expression profiles showing key nodes enriched by RNA-Seq in CXCL8 secretor vs. non-secretor MPN patients. **(D)** PCA plot demonstrating clustering of myelofibrosis patients segregated by CXCL8 expression levels and stratified by <20% and >80% from a publicly available microarray gene expression data set.[77] Each dot represents an individual patient. Dark blue represents "*CXCL8* high" (>80%) MF patients (N=8); light blue represents "*CXCL8* low" (<20%) MF patients (N=8) as based on their relative *CXCL8* expression level. **(E)** Correlation analysis comparing the internal (MSKCC) RNA-Seq dataset to the publicly-available MF gene expression data set. **(F)** Hierarchal clustering demonstrating differentially expressed genes in select MF patients stratified by *CXCL8* expression level (light blue = *CXCL8* low; dark blue = *CXCL8* high). Blue, negative values; red, positive values. **(G)** Gene Set Enrichment Analysis (GSEA) demonstrating enriched pathways in "*CXCL8* high" vs. "*CXCL8* low" MF patients from the publicly-available dataset presented as normalized enrichment score (NES) by FDR q-value. N=8/arm. **(H)** Representative GSEA plots demonstrating positive enrichment in the pro-inflammatory Hallmark TNFα via NF-κB and mature leukocyte activation gene sets in CXCL8-high MF from the publicly-available dataset. **(I)** Total number of differential ATAC peaks segregating enhancers from promoter regions (defined as within 2kb of the transcription start site [TSS]) of CXCL8-high (N=3) vs. CXCL8-low (N=2) MPN patients. **(J)** Representative accessibility peaks at the *CXCL8* locus extending from 6kb prior to the TSS to the 8kb beyond the 3'-UTR of CXCL8-secretor vs. non-secretor MPN patients.

Supplemental Figure 4 – *Cxcr2*$^{-/-}$ knock-out validation, experimental set-up, and extended analysis of *VavCxcr2*$^{-/-}$ hMPL^{W515L} mice



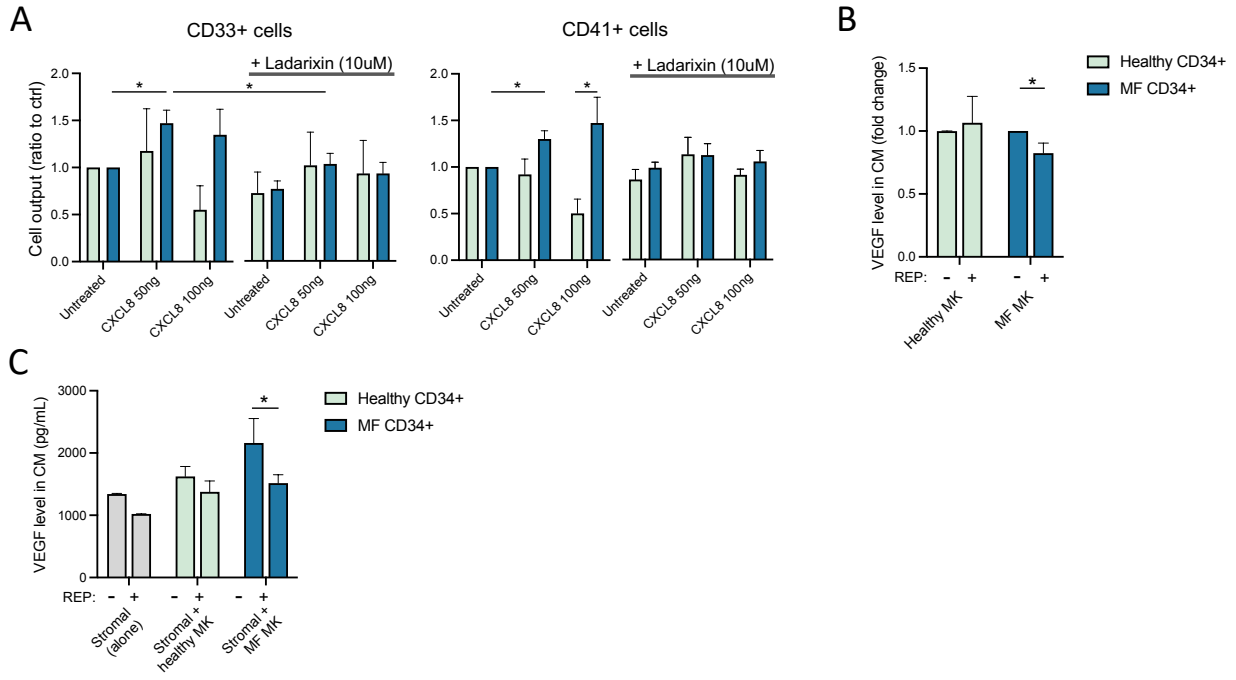
Supplemental Figure 4: *Cxcr2*$^{-/-}$ knock-out validation, experimental set-up, and extended analysis of *Cxcr2*$^{-/-}$ hMPL^{W515L} mice. (A) Western blot analysis of canonical downstream Cxcr2 mediators in harvested murine bone marrow after 2 hours cultured with or without exogenous human CXCL8 (hCXCL8) for both *Cxcr2*$^{f/f}$*;Cre*$^{-}$ wild-type (WT) and *Cxcr2*$^{f/f}$*;Cre*$^{+}$ knock-out (KO) cells. Representative blots of N=3 mice/arm. **(B)** Schematic of experimental set up of the *Cxcr2*$^{-/-}$ KO hMPL^{W515L} transplant studies. BM=bone marrow, lin-neg=lineage-negative, EV ctrl=empty vector control. **(C)** Confirmation of Cxcr2 loss on the surface of live peripheral blood (PB) mononuclear cells from *Cxcr2*$^{f/f}$*;Cre*$^{+}$ primary KO mice. N=6/arm. ****p<0.0001. Unpaired t-test. Data shown represent mean ± SEM. **(D)** Absolute Mac1+Gr1+ mature neutrophil populations in peripheral blood of *Cxcr2*$^{f/f}$*;Cre*$^{+}$ vs. *Cxcr2*$^{f/f}$*;Cre*$^{-}$ hMPL^{W515L} vs. empty vector (EV) transplanted mice at time of sacrifice. N=4-5 mice/arm. ***p<0.001, ****p<0.0001. Data shown represent mean ± SEM. Two-way ANOVA. **(E)** Spleen weights (mg) of *Cxcr2*$^{f/f}$*;Cre*$^{+}$ KO vs. *Cxcr2*$^{f/f}$*;Cre*$^{-}$ WT hMPL^{W515L} or EV control mice. N=4-5 mice/arm. *<0.05, ns-non-significant. Data shown represent mean ± SEM. **(F)** Homing assay assessing frequency of donor CD45.2 mutant cells in bone marrow of lethally irradiated recipient mice 48 hours following transplant of *Cxcr2*$^{f/f}$*;Cre*$^{+}$ KO vs. *Cxcr2*$^{f/f}$*;Cre*$^{-}$ WT hMPL^{W515L} or EV control cells. N=4-5 mice/arm. **(G)** Peripheral blood mature cell fractions of mice transplanted with *Cxcr2*$^{f/f}$*;Cre*$^{+}$ KO vs. *Cxcr2*$^{f/f}$*;Cre*$^{-}$ WT EV cells 9 weeks post-transplant. N=4-5 mice/arm. **(H)** Percent CD41+ live cells as detected by flow cytometry in bone marrow of *Cxcr2*$^{f/f}$*;Cre*$^{+}$ vs. *Cxcr2*$^{f/f}$*;Cre*$^{-}$ hMPL^{W515L} transplanted mice at time of sacrifice 9 weeks post-transplant. N=4-6/arm. **p<0.01. Unpaired t-test. Data shown represent mean ± SEM. **(I)** Recorded bone marrow megakaryocyte number (MKs) per high powered field (HPF) from *Cxcr2*$^{f/f}$*;Cre*$^{+}$ KO vs. *Cxcr2*$^{f/f}$*;Cre*$^{-}$ WT hMPL^{W515L} transplanted mice. N=4-6/arm. ***p<0.001. **(J)** Bone marrow grade reticulin scores of *Cxcr2*$^{f/f}$*;Cre*$^{+}$ vs. *Cxcr2*$^{f/f}$*;Cre*$^{-}$ hMPL^{W515L} or EV transplanted mice. N=4-6/arm. **p<0.01. Two-way ANOVA. Data shown represent mean ± SEM. **(K)** Spleen grade reticulin scores (left) and representative hematoxylin and eosin (H&E) and reticulin images of spleen sections (right) from *Cxcr2*$^{f/f}$*;Cre*$^{+}$ KO vs. *Cxcr2*$^{f/f}$*;Cre*$^{-}$ WT hMPL^{W515L} mice in comparison to wild-type. 20X magnification.

Supplemental Figure 5 – Pharmacologic inhibition of CXCR1/2 improves hematologic parameters and reticulin fibrosis in the hMPL^{W515L} adoptive transfer model of myelofibrosis



Supplemental Figure 5: Pharmacologic inhibition of CXCR1/2 improves hematologic parameters and reticulin fibrosis in the hMPL^{W515L} adoptive transfer model of myelofibrosis. (A) Schematic of experimental outline for the reparixin/ruxolitinib *in vivo* drug trials. WT=wild-type, BM=bone marrow, lin-neg=lineage-negative. **(B)** Weights observed across treatment arms comparing the beginning and end of the 3-week trial period. N=6/arm. **(C)** White blood cell counts (WBC, K/uL), hematocrit levels (Hct, %), and platelet counts (PLT, K/uL), **(D)** % GFP fraction in peripheral blood, **(E)** % Mac1+Gr1+ fraction in peripheral blood, and **(F)** Spleen weights of lethally irradiated mice transplanted with wild-type MSCV-MigR1-IRES-GFP empty vector transfected bone marrow cells and treated with an identical dosing schedule and duration of reparixin (60mg/kg BID x21d) as hMPL^{W515L} mice. N=5/arm. **(G)** Spleen weights (mg) of hMPL^{W515L}-diseased mice treated with either vehicle, ruxolitinib, reparixin, or combination therapy. N=6/arm. *p<0.05, **p<0.01, ns=not significant. Data shown represent mean ± SEM. **(H)** Percentage of Mac1+Gr1+ neutrophil fractions in peripheral blood by flow cytometry of treated mice in comparison to vehicle at trial end point. ns=non-significant. Data shown represent mean ± SEM. **(I)** Representative H&E and reticulin images of hMPL^{W515L}-diseased spleen treated with ruxolitinib, reparixin, or combination therapy compared with vehicle-treated hMPL^{W515L} mice. N=6/arm. 20X magnification.

Supplemental Figure 6 – CXCR1/2 inhibition demonstrates therapeutic efficacy in primary MF patient cells *in vitro*.



Supplemental Figure 6: CXCR1/2 inhibition demonstrates therapeutic efficacy in primary MF patient cells *in vitro*. (A) Ratio of total CD33+ cell (left) and CD41+ cell (right) output relative to untreated of cultured healthy donor (HD; light blue) vs. myelofibrosis (MF; dark blue) CD34+ cells in response to exogenous CXCL8 doses (50ng vs. 100ng) with or without the CXCR1/2 inhibitor Ladarixin (10uM). Representative of duplicate experiments from 3 healthy donor (HD) and 6 individual MF cases. *p<0.05. Data shown represent mean ± SD. **(B)** Fold-change in detectable VEGF levels in conditioned media (CM) elicited by either healthy donor vs. MF megakaryocytes (MKs) with or without the addition of reparixin (REP; 10uM). Representative of duplicate experiments from 3 healthy donor (HD) and 4 individual MF cases. *p<0.05. Data shown represent mean ± SD. **(C)** Total levels of VEGF in conditioned media (CM) of cultured stromal cells, either alone or together with healthy vs. MF MKs with or without the addition of reparixin (REP; 10uM). Representative of duplicate experiments from 3 healthy donor (HD) and 3 individual MF cases. *p<0.05. Data shown represent mean ± SD.

SUPPLEMENTAL TABLES

Supplemental Table 1 – Selected patients for single cell transcriptional (scRNA-Seq) analysis

ID	Diagnosis	Age	Sex	Driver Mutation	$JAK2^{V617F}$ VAF	Grade reticulin fibrosis	Sequenced cell number (after filtration)	Sample Origin
MF1	Primary myelofibrosis	76	F	$JAK2^{V617F}$	82.5%	3+/3	801	Patient Blood
PV1	Polycythemia vera	59	F	$JAK2^{V617F}$	69.4%	0-1+/3	892	Patient Blood
PV2	Polycythemia vera	41	M	$JAK2^{V617F}$	14.2%	1-2+/3	772	Patient Blood
PV3	Polycythemia vera	46	M	$JAK2^{V617F}$	25.1%	0/3	700	Patient Blood
ET1	Essential thrombocythemia	74	F	Triple neg.	N/A	1+/3	532	Patient Blood
ET2	Essential thrombocythemia	40	M	CALR	N/A	0-1+/3	505	Patient Blood
ET3 (ET w/ MF)	Essential thrombocythemia with fibrosis	73	M	$JAK2^{V617F}$	7.6%	2+/3	997	Patient Blood

MF: myelofibrosis; PV: polycythemia vera; ET: essential thrombocythemia; VAF: variant allele fraction

Supplemental Table 2 – Selected patients for single-cell cytokine analysis

ID	Diagnosis	Age	Sex	Driver mutation	Grade reticulin fibrosis	% CXCL8 secreting CD34+ cells
MF1	PMF	76	F	JAK2^{V617F}	3+/3	44.74
MF2	PMF	74	F	JAK2^{V617F}	2-3/3	81.79
MF3	PV to MF	67	F	JAK2^{V617F}	3+/3	92.63
MF4	ET to MF	53	F	JAK2^{V617F}	3+/3	69.22
MF5	PMF	68	M	CALR	2-3/3	76.32
MF6	PMF	72	M	MPL	3+/3	90.38
MF7	ET to MF	72	F	CALR	3+/3	4.67
MF8	PMF	81	M	JAK2^{V617F}	1+/3	8.79
MF9	PMF	64	F	JAK2^{V617F}	2+/3	17.31
MF10	ET to MF	67	F	MPL	1-2+/3	18.0
MF11	PV to MF	75	M	JAK2^{V617F}	2-3+/3	6.98
PV1	PV	59	F	JAK2^{V617F}	0-1+/3	25.49
PV2	PV	41	M	JAK2^{V617F}	1-2+/3	1.38
PV3	PV	46	M	JAK2^{V617F}	0/3	2.66
PV4	PV	70	M	JAK2^{V617F}	1-2+/3	3.30
PV5	PV	63	F	JAK2^{V617F}	2-3+/3	54.46
PV6	PV	57	M	JAK2^{V617F}	2+/3	48.05
PV7	PV	71	M	JAK2^{V617F}	1-2+/3	11.62
PV8	PV	66	M	JAK2^{V617F}	NA	54.27
PV9	PV	50	M	JAK2^{V617F}	1-2+/3	6.15
PV10	PV	84	F	JAK2^{V617F}	NA	1.09
PV11	PV	70	F	JAK2^{V617F}	NA	8.06
PV12	PV	69	M	JAK2^{V617F}	1+/3	5.25
PV13	PV	62	F	JAK2^{V617F}	NA	7.07
ET1	ET	74	F	Tripe neg.	1+/3	1.68
ET2	ET	40	M	CALR	0-1+/3	3.71
ET3	ET	73	M	JAK2^{V617F}	2+/3	14.6
ET4	ET	51	F	JAK2^{V617F}	1+/3	0.18
ET5	ET	75	M	JAK2^{V617F}	0+/3	0.44
ET6	ET	73	M	MPL	1+/3	0.85
ET7	ET	49	M	JAK2^{V617F}	0-1/3	0.16
ET8	ET	71	F	JAK2^{V617F}	1-2/3	10.55
ET9	ET	73	F	CALR	1+/3	4.92
ET10	ET	72	F	JAK2^{V617F}	N/A	0
ET11	ET	72	F	JAK2^{V617F}	0/3	5.29
ET12	ET	72	F	Triple neg.	1+/3	0.18
ET13	ET	61	F	JAK2^{V617F}	1-2+/3	0
ET14	ET	66	M	JAK2^{V617F}	1+/3	0.93

PMF: primary myelofibrosis; PV: polycythemia vera; ET: essential thrombocythemia

Supplemental Table 3 – Selected patients for bulk RNA-Seq (+ ATAC-Seq) analysis

ID	Diagnosis	Age	Sex	Driver mutation	JAK2^{V617F} VAF	Grade reticulin fibrosis	%CXCL8 secreting CD34+ cells	Sample Origin
CXCL8 secretors								
MF2*	PMF	74	F	JAK2^{V617F}	N/A	2-3+	81.79%	Patient Blood
MF3*	PV to MF	67	F	JAK2^{V617F}	0.69	3+/3	92.63%	Patient Blood
MF6*	PMF	72	M	MPL	0.94	3+/3	90.38%	Patient Blood
CXCL8 non-secretors								
MF10	ET to MF	67	F	MPL	0.24	1-2/+3 to 3+/3	18.0%	Patient Blood
MF11	PV to MF	75	M	JAK2^{V617F}	0.42	2-3+/3	6.98%	Patient Blood
PV4*	PV	70	M	JAK2^{V617F}	N/A	1+, focal 2+/3	3.30%	Patient Blood
ET2*	ET	40	M	CALR	0.9	0-1+/3	3.71%	Patient Blood
ET6	ET	73	M	MPL	0.24	1+/3	0.85%	Patient Blood

MF: primary myelofibrosis; PV: polycythemia vera; ET: essential thrombocythemia. VAF: variant allele
*Denotes patients on whom integrated RNA-Seq/ATAC-Seq analysis was performed