Supplementary files

Carfilzomib Enhances the Suppressive Effect of Ruxolitinib in Myelofibrosis

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Supplementary Methods

Chemical validation of gene targets on HEL cell line

For MTS assay, 3-5 x 10^3 HEL cells were seeded in 100 μL of RF10 in each well of a 96-well plate and cultured for 72 h in presence of ruxolitinib 300 nM +/- with the proteasome inhibitor carfilzomib (CFZ) at 10 nM and 50 nM. For proteasome inhibitor IC_{50} determination on HEL cells, MTS assay was performed as described. Cells were exposed to increasing concentrations (0-80 nM) of bortezomib or CFZ for 72 h. Experiments contained 3-5 technical replicates.

Chemical validation of gene targets on primary cells

For the 72 h MTS assay, 2-5 x 10^4 $CD34^+$ cells isolated from healthy donor GCSF-stimulated leukapheresis product and $CD34^+$ cells isolated from peripheral blood from MF patients were suspended in 100 μL of StemSpan (STEMCELL Technologies, Canada) medium supplemented with CC100 in each well of a 96-well plate after 24 h expansion in absence of drug. Different concentrations of CFZ were tested with and without ruxolitinib 300 nM. Experiments were performed in triplicate.

Furthermore, the effect on viability of pulsed-proteasome inhibitor in the presence of ruxolitinib was assessed on both MNCs and $CD34^+$ cells derived from peripheral blood of MF patients.

For MF MNCs, cells were seeded in a 24-well plate at a density of 0.3 x 10^6/mL in StemSpan and incubated for 1h after the addition of (a) medium with no drug; (b) ruxolitinib 300 nM; (c) CFZ 300 nM; and (d) combination of the two drugs. After 1 h and wash with PBS, cells from the above conditions were resuspended in StemSpam supplemented with CC100 and treated with (a) no drug; (b) ruxolitinib; (c) no drug; and (d) ruxolitinib 300 nM. Trypan blue viability assay was performed after 48 h or 72 h incubation. Results of the trypan blue is presented as the mean of 2-4 counts along with the error bars.

For MF CD34+, cells were exposed for 1 h to CFZ 300 nM +/- ruxolitinib 300 nM (following the same procedure described above for MNCs) (Figure S3). Then, after washing with PBS, two experiments were carried out: 1) 2.5-3 × 10^4 cells were seeded in each well of a 96-well plate in 100 μL StemSpan + CC100 and treated with medium with (a) no drug; (b) ruxolitinib 300 nM; (c) no drug; and (d) ruxolitinib 300 nM; at 72h MTS was used to assess cell viability; 2) cells were resuspended in StemSpam + CC100 in a 24-well plate (0.3-0.5 x10^6 cells/mL) and treated with (a) no drug; (b) ruxolitinib 300 nM; (c) no drug and (d) ruxolitinib 300 nM; trypan blue viability assay was performed after 24 h. Results of the trypan blue is presented as the mean of 2-4 counts along with error bars.

Bioinformatics analysis of RNAseq experiment

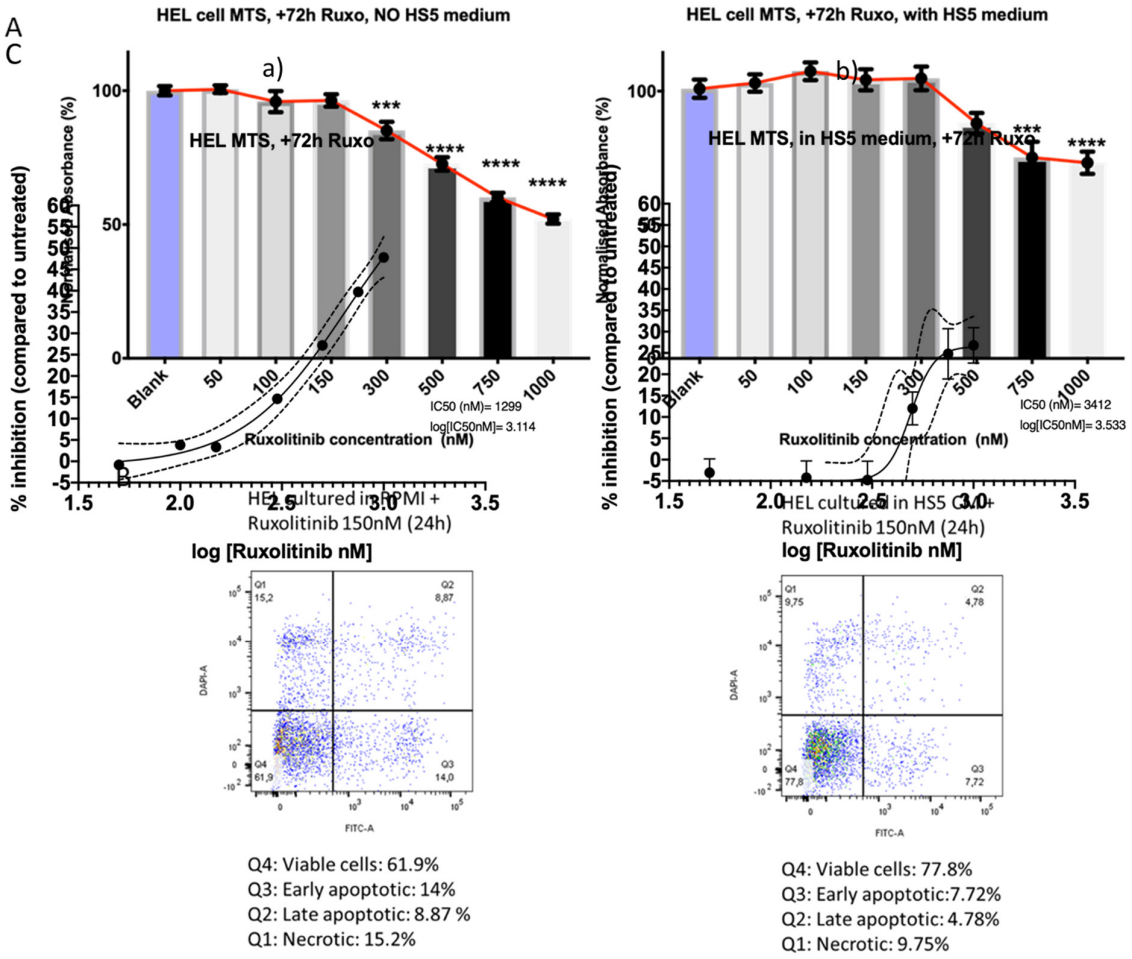
In order to identify gene regions matching the read sequences, sequencing alignment was performed by using the human genome reference GRCh38.p13 (https://www.ensembl.org/Homo_sapiens/Info/Index).

The alignment was performed with STAR (https://github.com/alexdobin/STAR version '2.7.5a') tool, with the '*--twopassMode Basic*' parameter enabled. The genome index was created from scratch with reference to the GRCh38 genome

(ftp://ftp.ensembl.org/pub/release-100/fasta/homo_sapiens/dna/, primary assembly version). In order to quantify gene expression, we counted the number of reads that map to each gene using 'featureCounts' (http://subread.sourceforge.net/ version '2.0.1'). The reads were normalised and the differential expression analysis was performed by using the 'DESeq2' package (version '1.26.0'). Heat maps were produced using the 'pheatmap' package on R3.6.0 and included only genes showing adjusted *p* values <0.05 and $\log_2$ fold change > 1 in absolute value.

The Gene Set Enrichment Analysis was performed on all differentially expressed genes (not filtered by thresholds on padj and fold change), by using the 'GSEA' software (https://www.gsea-msigdb.org/gsea/index.jsp version '4.0.3') with the '*Collapse/Remap to gene symbols*' parameter enabled in 'No_Collapse' mode. We used canonical pathways from KEGG and BIOCARTA as the reference database (version '7.1') in this GSEA analysis. Other parameters were used with default settings: 10 as min number of members, 500 as max number of members, and 1000 as number of permutations.

Supplementary Figures



Q4: Viable cells: 61.9%
Q3: Early apoptotic: 14%
Q2: Late apoptotic: 8.87 %
Q1: Necrotic: 15.2%

Q4: Viable cells: 77.8%
Q3: Early apoptotic:7.72%
Q2: Late apoptotic: 4.78%
Q1: Necrotic: 9.75%

Figure S1. The protective role of the microenvironment against ruxolitinib. Figure S1A shows the percent inhibition of proliferation (compared to the untreated control) by increasing concentration of ruxolitinib on HEL cell line cultured in

RF10, derived from 3 representative MTS assays as compared to the same experiment done in the presence of HS-5 medium; each point on the continuous black line represents the mean ± SEM (vertical bars). In the absence of CM there is a continuous reduction in the number of cells which correlated with the increased dose of ruxolitinib at 72h, but CM disrupted this gradual decrease and shifted the inhibitory dose of ruxolitinib to higher level for significant reduction. ***p < 0.001, **** p <0.0001, calculated by Tukey's multiple comparison test. Figure S1B shows the Annexin V-DAPI apoptosis assay on HEL cells treated with ruxolitinib 150nM in RF10 (left) or in presence of HS-5 medium (right); p = 0.02, Fisher's exact test. Figure S1C shows that the ruxolitinib IC_{50} of HEL cells cultured in HS-5 medium was nearly three-folds higher compared to culturing only in RF10 medium (3.4 μM versus 1.2 μM, respectively).

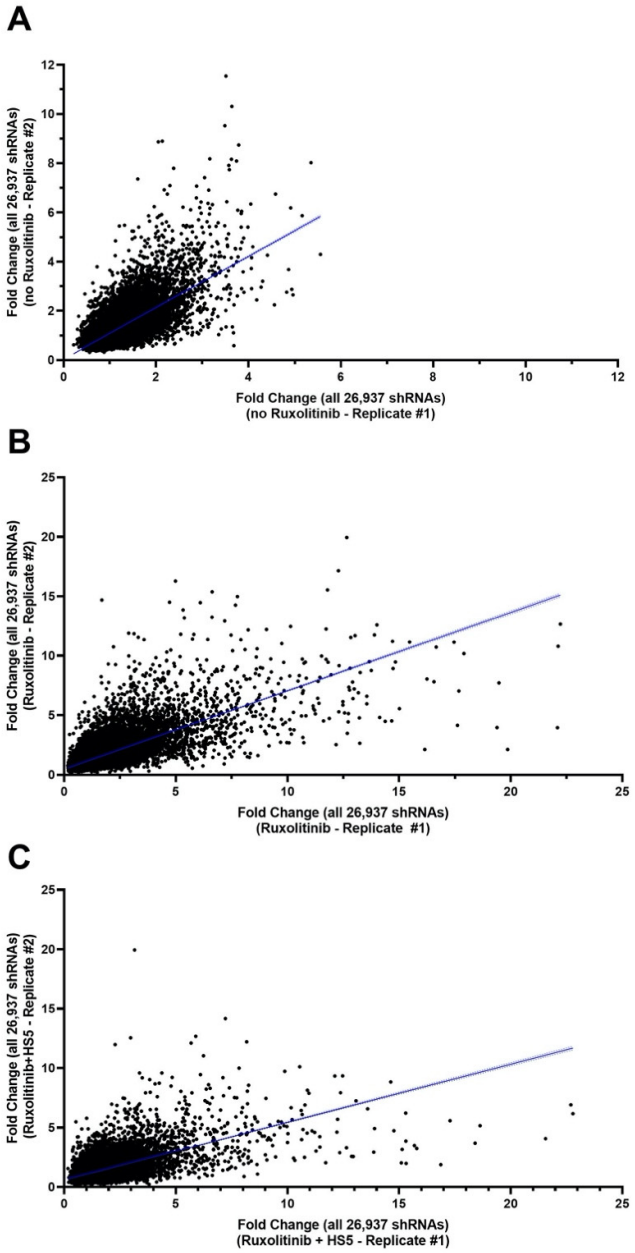


Figure S2. Correlation between experimental replicates. The correlation of fold change between experimental replicates is shown for (**A**) no ruxolitinib (setting 1), (**B**) ruxolitnib (setting 2), and (**C**) ruxolitinib plus HS-5 conditioned media. The fold change is calculated as described in the Methods, showing the change in reads present between baseline and at the end of the selection period for each of the 26,937 shRNAs targeting 4,974 genes. The X and Y axes represent the frequency of the normalised shRNA for replicate 1 and 2 for each setting. The Spearman correlations were for setting 1: r=0.635, p<0.0001; setting 2: r=0.720, p<0.0001; setting 3: r=0.574, p<0.0001.

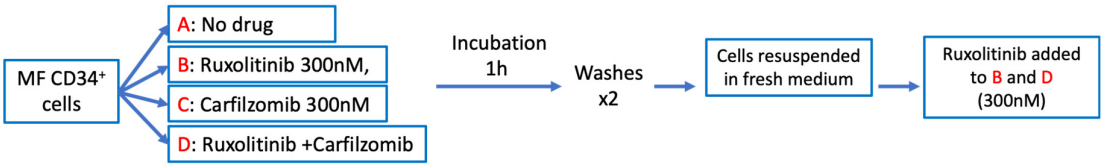


Figure S3. The 1h-pulsed CFZ in vitro experiment.The principle of the experiment for mimicking pulsed therapy is shown.

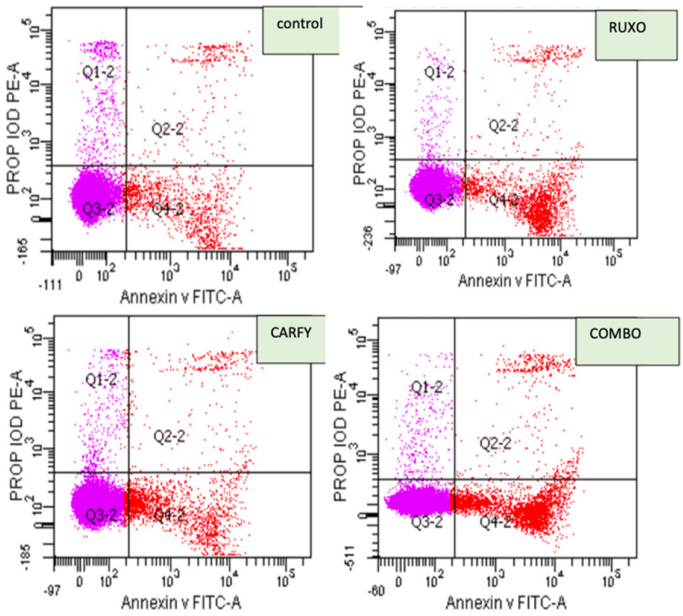


Figure S4. A. The flowcytometry analysis of the Annexin V/Iodide assay. The apoptotic cells are highly positive for Annexin which are detected strongly by FITC channel and weakly for the propidium iodide PE-A channel (lower right windows).

Supplementary Tables

Table S1. Characteristics of MF patients investigated in this study.

ID	Diagnosis	Sex, Age at sampling	Mutation status (VAF)	Ongoing therapies	WCC (x 10^9)	Hb (g/L) & Plts (x10^9)	Spleen size (cm)/response (c/pr/n)	Symptoms response	Therapy at sample collection?
MF#3	Primary MF	F, 76	***JAK2*^{V617F}** (94%) ***TET2*** (44%)	momelotinib	23.1	90 & 375	16.1/n	no	Yes
MF#5	Primary MF	M, 75	***JAK2*^{V617F}** (36%)	ruxolitinib (stopped 3 weeks before)	13.6	94 & 34	NA*	no	No
MF#8	Primary MF	M, 53	***MPL*** ^{W515L} (74%) ***ASXL1*** (34.4%)	ruxolitinib	14.6	121 & 191	28/n	no	Yes
MF#9	Post-ET MF	M, 66	**Triple-NEG** ***ASXL1*** (36%)	ruxolitinib	14.6	99 & 262	20.1/n	no	Yes
MF#10	Primary MF	M, 80	***JAK2*^{V617F}**(48 %)	ruxolitinib	23	133 & 138	17/pr at 5y	yes	Yes
MF#14	Post-PV MF	F, 75	***JAK2*^{V617F}**(n. d.)	ruxolitinib	11.7	137 & 250	19/pr at 6y	no	Yes
MF#15	Primary MF	M, 83	***JAK2*^{V617F}**(80 %)	ruxolitinib	15.9	109 & 221	normal/c	no	Yes
MF#19	Primary MF	F, 75	***JAK2*^{V617F}**(83 %) ***ASXL1*** (10%)	ruxolitinib	23.2	75 & 130	23/pr	no	Yes
MF#20	Post-ET MF	F, 60	***JAK2*^{V617F}** (94%)	ruxolitinib	22.3	130 & 91	30/pr	yes	Yes
MF#24	Primary MF	F, 62	***JAK2*^{V617F}** (n.d.)	ruxolitinib	20.7	98 & 179	17/pr	no	Yes
MF#28	Post-PV MF	M, 77	***JAK2*^{V617F}** (94%)	None	59.8	117 & 278	17/NA	NA	No

VAF = variant allele frequency; WCC = white cell count; Hb = haemoglobin; Plts = platelets; spleen response: c = complete, n = no, pr = progressive splenomegaly. Reported spleen size derives from abdomen ultrasound study. NA = not applicable; nd = not determined. * = patient previously splenectomised. Both spleen response and symptom burden response to treatment are according to the revised IWG-MRT and ELN response criteria (Tefferi A, et al. Blood. 2013;122(8):1395-8).

Table S2. Top essential genes for viability of HEL cells under various conditions. Listed are the top 25 essential genes for the viability or proliferation of HEL cells in the presence or absence of ruxolitinib and conditioned medium (CM) as identified by a pooled-shRNA library targeting 4,974 genes for two experimental replicates of each condition.

	No ruxolitinib (setting 1)				Ruxolitinib (setting 2)				Ruxolitinib + CM (setting 3)			
	Experiment 1		Experiment 2		Experiment 1		Experiment 2		Experiment 1		Experiment 2	
Rank	Gene	Fold Change	Gene	Fold Change	Gene	Fold Change	Gene	Fold Change	Gene	Fold Change	Gene	Fold Change
1	*CSE1L*	4.1	***PSMD1***	8.7	***PSMA2***	12.7	*RAN*	11.7	*ABCB5*	11.4	*RTF1*	9.2
2	*RRM1*	4	***PSMB2***	7.7	*RPS6*	12	***PSMC1***	9.7	***PSMD3***	9	***PSMB7***	8.6
3	*SNRPN*	3.9	***PSMA2***	7.4	*RAN*	11.3	***PSMB2***	9.7	*PRDX2*	8.4	***PSMA2***	8.1
4	***PSMA2***	3.9	*SNRPN*	6.6	*EIF2S2*	10.6	***PSMA2***	9.7	***PSMD1***	8.3	*RAN*	7.6
5	*RAN*	3.7	*RRM1*	6.3	***PSMC1***	9.2	*RPS13*	9.6	*SNRNP200*	8.2	*SNRPN*	7.5
6	***PSMD1***	3.6	*SNRPD2*	6.2	*TUBA1C*	9.1	***PSMD11***	9.1	*PHB2*	8.2	*RPS13*	7
7	*RTF1*	3.6	*HSPE1*	5.5	*RUVBL2*	9	***PSMA3***	9	***PSMA2***	8.1	***PSMA3***	6.9
8	***PSMB2***	3.5	*TUBA1C*	5.4	***PSMB4***	9	*SNRPD2*	8.9	*GATA1*	8	***PSMD1***	6.5
9	***PSMA3***	3.2	*RPS13*	5.4	*SNRPD2*	8.9	*RRM1*	8.6	*RRM1*	7.9	*RRM1*	6.3
10	*EEF2*	3	***PSMA3***	5.1	*RPL14*	8.8	*HSPE1*	8.4	*RPL11*	7.9	*RPL12*	6.1
11	*MPL*	3	*SNRNP200*	4.9	*RPS11*	8.6	*INPP4A*	8.3	*TUBA1C*	7.7	*RPS3A*	5.9
12	*RPS13*	3	***PSMB7***	4.9	*RTF1*	8.6	*RTF1*	7.9	*SIN3A*	7.6	*SNRPD2*	5.9
13	*SNRPD2*	2.9	*RAN*	4.7	***PSMA3***	8.6	*RPL12*	7.7	*RAN*	7.4	***PSMB2***	5.8
14	*EIF2S2*	2.9	*EEF2*	4.6	***PSMD1***	8.4	*SNRNP200*	7.6	***PSMB2***	7.3	*RPL10*	5.7
15	*JAK2*	2.8	***PSMB4***	4.6	***PSMD6***	7.9	*RPL4*	7.5	*MPL*	7.2	***PSMC6***	5.6
16	*RPL12*	2.8	*BCL2L1*	4.4	*HSPA9*	7.9	*EIF2S2*	7.4	*EIF2S2*	7.2	*SNRNP200*	5.5
17	*SEM1*	2.7	*RPL14*	4.3	*RPL12*	7.7	*HSPA9*	7.4	*RUVBL2*	7	*KPNB1*	5.3
18	*NEDD8*	2.7	*EIF2S2*	4.3	*RPS13*	7.7	*MPL*	7.3	*BCL2L1*	6.8	*TUBA1C*	5.2
19	*PHKB*	2.7	*RPL13*	4.3	*ACTA2*	7.6	*FLI1*	7.2	***PSMA4***	6.7	*RPS11*	5.2
20	*CCNG1*	2.7	*RPSA*	4.3	*PRPF40A*	7.6	*RPS6*	7.2	***PSMB7***	6.6	*RPL8*	5.1
21	*SNRNP200*	2.7	*RTF1*	4.2	*RPSA*	7.5	*PRPF40A*	7	*STX1A*	6.4	*RPL11*	5
22	*CDC40*	2.7	*KPNB1*	4.1	*PHB2*	7.3	*OGDH*	7	*INPP4A*	6.4	*RUVBL2*	5
23	*FLI1*	2.7	***PSMD6***	4.1	*SNRNP200*	7.3	*JAK2*	6.9	*DDX23*	6.2	*PRDX2*	4.7
24	*PAFAH1B1*	2.7	*PHB2*	4.1	*EIF1AX*	7.3	***PSMB7***	6.9	*RPS13*	6.2	***PSMA7***	4.7
25	*RPS11*	2.7	*CSE1L*	4	*AQR*	7.2	*PDHX*	6.8	*CD247*	6	***PSMC3***	4.7

Fold change represents the change in reads observed between baseline and follow-up, with a fold change >1 indicating a depletion from baseline in observed cells having the incorporated shRNA for each gene, as explained in the Methods. A total of 33 proteasomal genes were among the 4,974 targeted in the shRNA library. Additional observations of proteasomal gene family members beyond the top 25 but within the top 1% for each setting and experimental replicate were: experiment 1, setting 1: *PSMD3*, *PSMD7*, and *PSMB3*; experiment 2, setting 1: *PSMC6*, *PSMD2*; experiment 1, setting 2: *PSMB7*, *PSMA4*, *PSMB2*, *PSMD11*; experiment 2, setting 2: *PSMD6*, *PSMD2*, *PSMD1*, *PSMC6*; experiment 1, setting 3: *PSMA3*, *PSMB1*; experiment 2, setting 3: *PSMD2*, *PSMB4*, *PSMD11*, *PSMD6*, *PSMB1*. *JAK2* and *MPL*, which are crucial drivers of MPN pathogenesis, ranked within the top 1.25% of fold change depletions for at least one of the experiments for all settings. Two genes which were in the top 1% of both experiments for setting 3 were not in the top 1% of any experiments for settings 1 and 2: *PSMB1*, *SIN3A*; similarly, unique to setting 2 was only one gene: *AQR*; and similarly unique to setting 1 were two genes: *GTF2F1*, *RACK1*.

Table S3. The calculated *p* value of the comparison between various condition in primary samples treated with ruxolitinib or CFZ. The *p* value of comparing any two conditions in these experiments was calculated by unpaired t-test analysis using GraphPad v.9.

Sample ID	*p* value					
	Control v Ruxolitinib	**Control v CFZ 10**	**Control v CFZ 50**	**Ruxolitinib v CFZ 10**	**Ruxolitinib v CFZ 50**	**CFZ 10 v CFZ 50**
MF#3	< 0.0001	< 0.0001	< 0.0001	< 0.0003	< 0.0001	< 0.0001
MF#10	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
MF#15	< 0.0001	0.004	< 0.0001	< 0.0001	0.07	< 0.0001

Table S4. The calculated *p* value of the comparison between various condition in primary samples treated with ruxolitinib, pulsed CFZ and the combination. The *p* value of comparing any two conditions in these experiments was calculated by unpaired t-test analysis using GraphPad v.9.

Sample ID	*p* value				
	Control v Ruxolitinib	**Control v CFZ 300**	**Ruxolitinib v CFZ 300**	**Ruxolitinib v Ruxolitinib + CFZ 300**	**CFZ 300 v Ruxolitinib + CFZ 300**
MF#3	0.8	0.2	0.2	0.04	0.1
MF#20	0.7	0.0009	0.02	<0.0001	0.001
MF#19	0.02	0.049	0.03	0.01	0.001
MF#10	0.004	0.004	0.1	0.002	0.009

Table S5. Differentially expressed genes in $CD34^+$ MF cells treated with ruxolitinib. This table lists the genes from the most downregulated to the most upregulated ones having a $\log_2$ fold change >1 in absolute value and an adjusted *p* value <0.05.

Gene	$\log_2$ Fold Change	Standard Error ($\log_2$ fold change)	*p* value	Adjusted *p* value
H19	-6.745	1.296	1.93E-07	2.07E-05
PAK3	-5.975	1.449	3.71E-05	1.41E-03
IL7R	-4.402	0.82	7.82E-08	9.53E-06
STC2	-3.812	0.907	2.63E-05	1.08E-03
LRRC77P	-3.316	0.575	7.99E-09	1.42E-06
ADM2	-3.261	0.561	6.06E-09	1.14E-06
GLI2	-3.123	0.631	7.32E-07	6.35E-05
IL17RB	-3.071	0.433	1.25E-12	6.40E-10
KCNJ15	-3.07	0.87	4.15E-04	9.21E-03
CHAC1	-2.971	0.688	1.57E-05	7.21E-04
CBS	-2.891	0.413	2.52E-12	1.16E-09
FHAD1	-2.801	0.835	7.96E-04	1.47E-02
IGFBP5	-2.794	0.534	1.68E-07	1.86E-05
CCL24	-2.577	0.639	5.52E-05	1.96E-03
TNFRSF18	-2.569	0.241	1.81E-26	7.90E-23
SLAMF1	-2.568	0.424	1.42E-09	3.31E-07
HSPA7	-2.49	0.812	2.18E-03	3.14E-02
ERFE	-2.49	0.348	8.31E-13	5.37E-10
SERPINA1	-2.477	0.562	1.05E-05	5.25E-04
IL19	-2.415	0.573	2.47E-05	1.03E-03
CCL2	-2.4	0.362	3.36E-11	1.22E-08
ZNF804B	-2.389	0.707	7.29E-04	1.39E-02
LINC01948	-2.317	0.477	1.21E-06	9.39E-05
AL138916.1	-2.304	0.8	3.99E-03	4.76E-02
EGR1	-2.292	0.543	2.46E-05	1.03E-03
AC109466.1	-2.231	0.522	1.93E-05	8.50E-04

RUBCNL	-2.186	0.603	2.86E-04	6.96E-03
CCDC3	-2.155	0.559	1.17E-04	3.50E-03
ELFN1	-2.144	0.488	1.10E-05	5.45E-04
FPR1	-2.118	0.432	9.55E-07	7.85E-05
IL2RA	-2.086	0.458	5.17E-06	3.00E-04
PSAT1	-2.051	0.246	7.23E-17	1.05E-13
DHH	-1.981	0.686	3.88E-03	4.66E-02
GGT5	-1.964	0.424	3.57E-06	2.22E-04
FAM47E-STBD1	-1.931	0.288	1.87E-11	7.10E-09
HP	-1.93	0.655	3.23E-03	4.12E-02
LINC00032	-1.926	0.552	4.82E-04	1.02E-02
CHDH	-1.883	0.475	7.32E-05	2.45E-03
ENPP2	-1.863	0.495	1.69E-04	4.66E-03
PPFIA4	-1.851	0.376	8.47E-07	7.16E-05
CCDC170	-1.842	0.356	2.34E-07	2.37E-05
GLP1R	-1.836	0.604	2.37E-03	3.33E-02
IGFBP4	-1.818	0.626	3.68E-03	4.48E-02
IER3	-1.809	0.333	5.79E-08	7.48E-06
SIGLEC12	-1.808	0.422	1.82E-05	8.18E-04
CD80	-1.796	0.418	1.77E-05	8.00E-04
FPR2	-1.786	0.37	1.40E-06	1.05E-04
CD163	-1.783	0.591	2.54E-03	3.49E-02
IL2RB	-1.774	0.567	1.77E-03	2.68E-02
SOCS3	-1.738	0.423	3.93E-05	1.48E-03
TGFBI	-1.725	0.413	2.98E-05	1.18E-03
SLC9A2	-1.711	0.479	3.58E-04	8.24E-03
HORMAD2	-1.695	0.44	1.17E-04	3.51E-03
OSM	-1.692	0.457	2.11E-04	5.54E-03
AF127936.1	-1.69	0.512	9.69E-04	1.71E-02
MT-RNR1	-1.688	0.216	5.48E-15	4.78E-12
LOXL1	-1.665	0.187	4.91E-19	1.43E-15
SERTM2	-1.663	0.526	1.57E-03	2.46E-02
LINC02204	-1.646	0.393	2.82E-05	1.14E-03
CFH	-1.645	0.3	4.30E-08	5.73E-06
AL109837.3	-1.641	0.297	3.17E-08	4.50E-06
AL031293.1	-1.625	0.364	8.31E-06	4.41E-04
ULBP1	-1.623	0.487	8.61E-04	1.56E-02
PIM1	-1.616	0.381	2.25E-05	9.67E-04
AC015911.11	-1.612	0.497	1.18E-03	1.99E-02
RNVU1-31	-1.609	0.393	4.18E-05	1.55E-03
BATF3	-1.604	0.32	5.20E-07	4.72E-05
CACNA2D1	-1.596	0.394	5.21E-05	1.86E-03
MMP9	-1.583	0.273	6.64E-09	1.22E-06
ADGRG3	-1.582	0.427	2.08E-04	5.47E-03
IL18RAP	-1.578	0.377	2.78E-05	1.13E-03
ST6GALNAC3	-1.569	0.394	6.95E-05	2.35E-03
MT-ATP8	-1.553	0.217	8.88E-13	5.53E-10
BCYRN1	-1.534	0.529	3.70E-03	4.50E-02
TNFRSF8	-1.532	0.378	5.01E-05	1.81E-03
GSG1L	-1.529	0.396	1.14E-04	3.44E-03
CLEC7A	-1.527	0.482	1.53E-03	2.42E-02

CD22	-1.516	0.422	3.27E-04	7.75E-03
MUC1	-1.485	0.266	2.25E-08	3.42E-06
SOCS1	-1.477	0.353	2.85E-05	1.15E-03
SCN2A	-1.47	0.373	8.18E-05	2.67E-03
TREM1	-1.469	0.472	1.87E-03	2.80E-02
LIF	-1.461	0.419	4.96E-04	1.04E-02
AZIN2	-1.456	0.27	6.85E-08	8.66E-06
MT-TF	-1.454	0.326	8.43E-06	4.44E-04
ENPP6	-1.453	0.437	8.75E-04	1.58E-02
NDUFA4L2	-1.448	0.421	5.74E-04	1.16E-02
LTB	-1.447	0.356	4.83E-05	1.75E-03
ADAMTS14	-1.412	0.383	2.30E-04	5.89E-03
AL034397.2	-1.411	0.333	2.26E-05	9.67E-04
ALDH1L2	-1.394	0.264	1.30E-07	1.51E-05
RPL41P1	-1.392	0.412	7.29E-04	1.39E-02
MT-ND3	-1.385	0.226	9.37E-10	2.24E-07
SYN1	-1.375	0.476	3.85E-03	4.63E-02
AC005324.3	-1.365	0.351	1.01E-04	3.16E-03
SLAMF8	-1.359	0.286	2.04E-06	1.41E-04
MT-TL1	-1.288	0.269	1.73E-06	1.24E-04
TNFRSF4	-1.277	0.327	9.56E-05	3.04E-03
TMEM273	-1.276	0.346	2.26E-04	5.84E-03
AC011246.1	-1.26	0.177	1.05E-12	5.73E-10
STAT4	-1.259	0.157	8.87E-16	1.03E-12
TM4SF1	-1.257	0.336	1.82E-04	4.96E-03
GDF15	-1.249	0.3	3.15E-05	1.24E-03
AL591506.1	-1.245	0.419	2.97E-03	3.89E-02
CHRM3-AS2	-1.244	0.321	1.04E-04	3.22E-03
SPP1	-1.243	0.218	1.22E-08	2.02E-06
MTATP6P1	-1.226	0.181	1.42E-11	5.62E-09
MT-ND4L	-1.225	0.186	5.13E-11	1.69E-08
LINC01091	-1.213	0.267	5.44E-06	3.09E-04
ID1	-1.203	0.364	9.52E-04	1.69E-02
FCRLB	-1.201	0.252	1.85E-06	1.31E-04
AL109615.3	-1.187	0.332	3.53E-04	8.16E-03
MT-ND2	-1.175	0.178	3.91E-11	1.39E-08
MT-ATP6	-1.171	0.17	5.34E-12	2.22E-09
CIB3	-1.17	0.397	3.17E-03	4.08E-02
CD69	-1.154	0.383	2.55E-03	3.49E-02
RN7SL471P	-1.153	0.204	1.50E-08	2.40E-06
ICAM1	-1.151	0.377	2.24E-03	3.20E-02
MT-ND4	-1.151	0.166	4.12E-12	1.75E-09
MT-ND5	-1.123	0.162	3.80E-12	1.66E-09
ENOX1	-1.118	0.33	7.09E-04	1.36E-02
CISH	-1.117	0.148	3.64E-14	2.76E-11
F5	-1.108	0.38	3.54E-03	4.37E-02
DHRS9	-1.108	0.173	1.48E-10	4.08E-08
MT-ND6	-1.108	0.213	1.86E-07	2.02E-05
TRDC	-1.086	0.353	2.11E-03	3.06E-02
PPM1E	-1.066	0.349	2.27E-03	3.23E-02
MB21D2	-1.065	0.254	2.81E-05	1.14E-03

DDN	-1.057	0.319	9.30E-04	1.66E-02
AC084082.1	-1.054	0.311	7.02E-04	1.35E-02
ASIC1	-1.048	0.265	7.62E-05	2.52E-03
RNY1	-1.046	0.181	7.24E-09	1.30E-06
BATF2	-1.039	0.296	4.59E-04	9.86E-03
RAB20	-1.038	0.176	3.94E-09	8.08E-07
GPR84	-1.034	0.353	3.35E-03	4.22E-02
CTSL	-1.03	0.214	1.53E-06	1.11E-04
MT-TM	-1.023	0.229	8.23E-06	4.39E-04
AC234582.1	-1.019	0.225	5.87E-06	3.30E-04
HRH2	-1.011	0.345	3.41E-03	4.27E-02
ARG2	-1	0.293	6.48E-04	1.27E-02
AP002992.1	1.012	0.305	9.20E-04	1.65E-02
FLT3	1.015	0.316	1.33E-03	2.17E-02
KANK4	1.018	0.302	7.48E-04	1.41E-02
AC092979.2	1.041	0.33	1.60E-03	2.48E-02
MIR3142HG	1.059	0.16	4.06E-11	1.42E-08
AL138963.4	1.059	0.245	1.49E-05	6.93E-04
MMP28	1.064	0.179	2.64E-09	5.89E-07
TRIM73	1.075	0.243	1.00E-05	5.07E-04
COL9A2	1.082	0.168	1.21E-10	3.45E-08
TOB1-AS1	1.082	0.344	1.65E-03	2.53E-02
LGI4	1.088	0.299	2.72E-04	6.70E-03
AC083870.1	1.094	0.373	3.37E-03	4.24E-02
TMPRSS9	1.095	0.243	6.37E-06	3.53E-04
AP000695.2	1.104	0.275	5.97E-05	2.07E-03
LINC00920	1.106	0.384	4.01E-03	4.77E-02
CRHBP	1.16	0.237	9.87E-07	7.94E-05
AP002907.1	1.165	0.388	2.67E-03	3.61E-02
USP30-AS1	1.177	0.353	8.66E-04	1.57E-02
AC234782.4	1.243	0.266	2.89E-06	1.87E-04
PDZD7	1.249	0.394	1.50E-03	2.37E-02
NTN5	1.254	0.372	7.47E-04	1.41E-02
FSIP2-AS1	1.261	0.368	6.03E-04	1.21E-02
LTK	1.293	0.265	1.06E-06	8.40E-05
TNK2-AS1	1.297	0.405	1.37E-03	2.23E-02
CASC15	1.303	0.219	2.53E-09	5.73E-07
AF165147.1	1.324	0.366	2.99E-04	7.22E-03
LINC02082	1.342	0.443	2.47E-03	3.42E-02
RHBDF1	1.353	0.465	3.59E-03	4.40E-02
GAL3ST4	1.36	0.209	7.79E-11	2.41E-08
AC020656.2	1.373	0.457	2.64E-03	3.58E-02
LINC02718	1.383	0.322	1.73E-05	7.86E-04
AC009560.4	1.432	0.487	3.27E-03	4.16E-02
MEGF10	1.457	0.492	3.03E-03	3.96E-02
LINC02147	1.461	0.495	3.13E-03	4.05E-02
TAS1R3	1.462	0.37	7.72E-05	2.54E-03
ECEL1P1	1.485	0.46	1.24E-03	2.06E-02
LINC02801	1.539	0.276	2.35E-08	3.50E-06
CCDC187	1.573	0.362	1.38E-05	6.54E-04
WSCD2	1.612	0.546	3.16E-03	4.07E-02

SDK2	1.665	0.56	2.95E-03	3.87E-02
AL136181.1	1.82	0.534	6.63E-04	1.30E-02
AC010745.5	1.924	0.608	1.55E-03	2.43E-02
CNTNAP2	2.1	0.262	9.90E-16	1.08E-12
ADCY2	3.22	0.833	1.10E-04	3.38E-03
MIR3648-2	3.665	1.255	3.48E-03	4.33E-02
PRLR	3.71	1.087	6.39E-04	1.26E-02

Table S6. Downregulated signalling pathways by ruxolitinib in CD34⁺ MF cells. Listed are all gene sets having a nominal *p* value <0.05. Further descriptions of each column variable are provided at: https://www.gsea-msigdb.org/gsea/doc/GSEAUserGuideTEXT.htm#_Detailed_Enrichment_Results.

Gene set name (GS)	SIZE	ES	NES	NOM p-val	FDR q-val	FWER p-val	RANK AT MAX	LEADING EDGE
KEGG_OXIDATIVE_PHOSPHORYLATION	101	-0.773	-2.386	0	0	0	2506	tags=51%, list=14%, signal=60%
KEGG_SYSTEMIC_LUPUS_ERYTHEMATOSUS	96	-0.744	-2.298	0	0	0	2538	tags=53%, list=15%, signal=62%
KEGG_PARKINSONS_DISEASE	105	-0.711	-2.215	0	0	0	1926	tags=43%, list=11%, signal=48%
KEGG_HUNTINGTONS_DISEASE	147	-0.666	-2.16	0	0	0	2501	tags=38%, list=14%, signal=44%
KEGG_CYTOKINE_CYTOKINE_RECEPTOR_INTERACTION	140	-0.656	-2.129	0	0	0	1169	tags=18%, list=7%, signal=19%
KEGG_SPLICEOSOME	119	-0.666	-2.091	0	0	0	2935	tags=51%, list=17%, signal=61%
KEGG_PROTEASOME	40	-0.766	-2.013	0	0.00015	0.001	2886	tags=73%, list=17%, signal=87%
KEGG_AMINOACYL_TRNA_BIOSYNTHESIS	39	-0.756	-2.005	0	0.00013	0.001	2225	tags=49%, list=13%, signal=56%
KEGG_ALZHEIMERS_DISEASE	131	-0.619	-1.979	0	0.00061	0.005	2501	tags=34%, list=14%, signal=39%
KEGG_DNA_REPLICATION	31	-0.775	-1.979	0	0.00055	0.005	2688	tags=71%, list=15%, signal=84%
KEGG_CARDIAC_MUSCLE_CONTRACTION	48	-0.714	-1.953	0	0.00109	0.01	2475	tags=44%, list=14%, signal=51%
KEGG_GLYCINE_SERINE_AND_THREONINE_METABOLISM	25	-0.793	-1.926	0	0.00184	0.019	799	tags=28%, list=5%, signal=29%
BIOCARTA_TH1TH2_PATHWAY	17	-0.845	-1.919	0	0.00203	0.023	193	tags=12%, list=1%, signal=12%
KEGG_MISMATCH_REPAIR	22	-0.783	-1.896	0	0.00278	0.033	2688	tags=73%, list=15%, signal=86%
KEGG_JAK_STAT_SIGNALING_PATHWAY	94	-0.608	-1.858	0	0.00559	0.072	1277	tags=17%, list=7%, signal=18%
KEGG_CITRATE_CYCLE_TCA_CYCLE	27	-0.719	-1.831	0	0.00793	0.104	1360	tags=26%, list=8%, signal=28%
KEGG_SELENOAMINO_ACID_METABOLISM	22	-0.763	-1.82	0.00358	0.00859	0.119	2152	tags=36%, list=12%, signal=41%
KEGG_FRUCTOSE_AND_MANNOSE_METABOLISM	28	-0.717	-1.811	0	0.00973	0.142	3254	tags=57%, list=19%, signal=70%
KEGG_AMINO_SUGAR_AND_NUCLEOTIDE_SUGAR_METABOLISM	39	-0.693	-1.809	0.00175	0.00951	0.147	3525	tags=46%, list=20%, signal=58%
KEGG_PYRIMIDINE_METABOLISM	86	-0.597	-1.806	0	0.00965	0.156	3415	tags=47%, list=20%, signal=58%
KEGG_PPAR_SIGNALING_PATHWAY	40	-0.671	-1.806	0	0.00925	0.157	2224	tags=35%, list=13%, signal=40%
KEGG_CYSTEINE_AND_METHIONINE_METABOLISM	24	-0.74	-1.768	0.00173	0.01519	0.256	1904	tags=33%, list=11%, signal=37%
KEGG_PYRUVATE_METABOLISM	33	-0.67	-1.757	0	0.01719	0.3	1068	tags=27%, list=6%, signal=29%
KEGG_RNA_POLYMERASE	28	-0.694	-1.746	0.00349	0.01904	0.339	1443	tags=36%, list=8%, signal=39%
KEGG_GLYCOLYSIS_GLUCONEOGENESIS	38	-0.652	-1.742	0.00333	0.01889	0.35	980	tags=29%, list=6%, signal=31%
KEGG_GLUTATHIONE_METABOLISM	39	-0.65	-1.713	0.00348	0.02724	0.489	1708	tags=28%, list=10%, signal=31%

KEGG_TRYPTOPHAN_METABOLISM	31	-0.677	-1.688	0.00175	0.03671	0.601	3560	tags=32%, list=20%, signal=40%
KEGG_GALACTOSE_METABOLISM	22	-0.701	-1.672	0.00517	0.04256	0.657	3435	tags=50%, list=20%, signal=62%
BIOCARTA_G2_PATHWAY	24	-0.678	-1.665	0.01541	0.04512	0.698	3528	tags=50%, list=20%, signal=63%
KEGG_HOMOLOGOUS_RECOMBINATION	26	-0.669	-1.66	0.00694	0.04609	0.715	3149	tags=62%, list=18%, signal=75%
KEGG_NUCLEOTIDE_EXCISION_REPAIR	41	-0.617	-1.645	0.00664	0.05254	0.775	2724	tags=46%, list=16%, signal=55%
KEGG_BIOSYNTHESIS_OF_UNSATURATED_FATTY_ACIDS	21	-0.678	-1.642	0.0123	0.05283	0.791	3473	tags=48%, list=20%, signal=59%
BIOCARTA_NKT_PATHWAY	18	-0.708	-1.59	0.0239	0.08493	0.915	2	tags=6%, list=0%, signal=6%
KEGG_ONE_CARBON_POOL_BY_FOLATE	15	-0.725	-1.573	0.0239	0.09671	0.947	857	tags=33%, list=5%, signal=35%
KEGG_ARGININE_AND_PROLINE_METABOLISM	40	-0.586	-1.555	0.01868	0.11145	0.974	2056	tags=35%, list=12%, signal=40%
KEGG_DRUG_METABOLISM_OTHER_ENZYMES	21	-0.657	-1.538	0.04203	0.12427	0.981	3657	tags=43%, list=21%, signal=54%
KEGG_BLADDER_CANCER	39	-0.577	-1.531	0.01498	0.12943	0.988	3528	tags=38%, list=20%, signal=48%
KEGG_HEMATOPOIETIC_CELL_LINEAGE	55	-0.538	-1.522	0.01557	0.13438	0.991	456	tags=9%, list=3%, signal=9%
BIOCARTA_VEGF_PATHWAY	25	-0.633	-1.51	0.02574	0.14453	0.993	3085	tags=40%, list=18%, signal=49%
KEGG_COMPLEMENT_AND_COAGULATION_CASCADES	35	-0.568	-1.494	0.04085	0.15907	0.996	1104	tags=14%, list=6%, signal=15%
KEGG_BASAL_TRANSCRIPTION_FACTORS	28	-0.592	-1.489	0.03846	0.16166	0.996	1915	tags=21%, list=11%, signal=24%
KEGG_FC_GAMMA_R_MEDIATED_PHAGOCYTOSIS	84	-0.497	-1.488	0.01463	0.15863	0.996	2927	tags=23%, list=17%, signal=27%
KEGG_RNA_DEGRADATION	55	-0.527	-1.484	0.02254	0.1588	0.996	3572	tags=45%, list=20%, signal=57%
KEGG_CELL_CYCLE	117	-0.464	-1.479	0.0121	0.16189	0.997	3589	tags=43%, list=21%, signal=53%
KEGG_PURINE_METABOLISM	129	-0.466	-1.468	0.0104	0.17087	0.997	2747	tags=34%, list=16%, signal=40%
KEGG_N_GLYCAN_BIOSYNTHESIS	46	-0.529	-1.467	0.03959	0.16828	0.997	2589	tags=35%, list=15%, signal=41%

SIZE = Number of genes in the gene set; ES = Enrichment score; NES = Normalized enrichment score; NOM p-val = Nominal *p* value; FDR q-val = False discovery rate; FWER p-val = Familywise-error rate; RANK AT MAX = The position in the ranked list at which the maximum enrichment score occurred; LEADING EDGE = Three statistics used to define the leading-edge subset.