Supplementary Information

Targeting PP2A-dependent autophagy enhances sensitivity to ruxolitinib in JAK2^{V617F} myeloproliferative neoplasms

Courdy et al.

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	ID study	From	$JAK2^{V617F}$ allelic burden at diagnosis	Follow-up treatment
$JAK2^{V617F}$-positive MPN patients	12	Toulouse University Hospital	"Intermediate"	Phlebotomy
	13		45%	Phlebotomy
	16		60%	Phlebotomy
	17		36%	Phlebotomy
	18		23%	Phlebotomy
	19		45%	Phlebotomy
	20		13%	Phlebotomy
	22		45%	Phlebotomy
	23		87%	Phlebotomy
	29		85%	Phlebotomy + pegylated interferon
	31		43%	Phlebotomy
	32		<10%	Phlebotomy
	33		27%	Phlebotomy
	34		10%	Phlebotomy
	35		39%	Phlebotomy
	36		>50%	Phlebotomy
	37		21%	Phlebotomy
	38		23%	Phlebotomy
	39		9%	Phlebotomy
	40		<10%	Phlebotomy
	41		Not referred	Phlebotomy
	42		7%	Phlebotomy
$JAK2^{WT}$ healthy donors	A	EFS, Toulouse	None	None
	B		None	None
	C		None	None
	D		None	None
	E		None	None
	F		None	None

Supplementary Table 1. Primary MPN patient and healthy donor characteristics.

Figure S1:

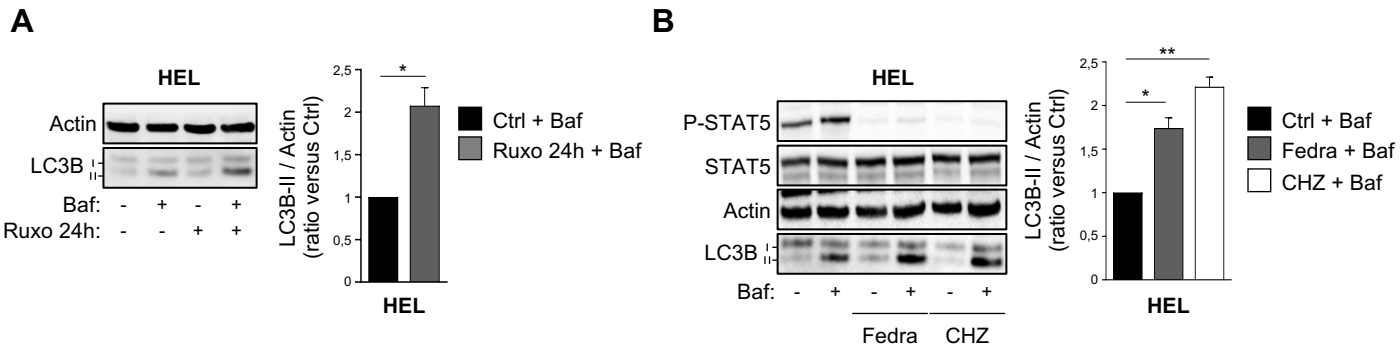
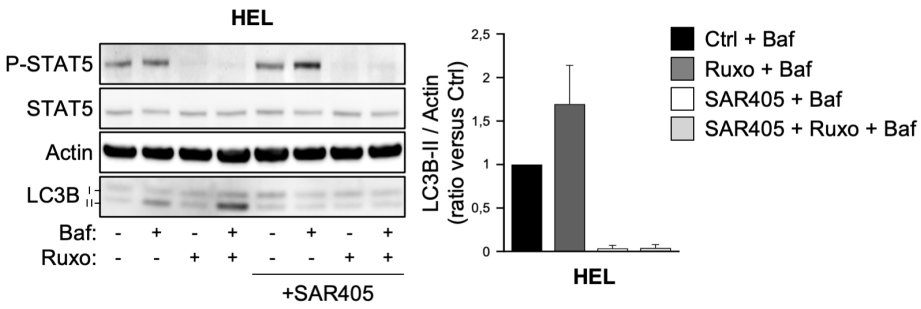

A
HEL
Actin
LC3B I
II
Baf: - + - +
Ruxo 24h: - - + +
LC3B-II / Actin (ratio versus Ctrl)
2,5
2
1,5
1
0,5
0
*
HEL
Ctrl + Baf
Ruxo 24h + Baf
B
HEL
P-STAT5
STAT5
Actin
LC3B I
II
Baf: - + - + - +
Fedra
CHZ
LC3B-II / Actin (ratio versus Ctrl)
2,5
2
1,5
1
0,5
0
*
**
HEL
Ctrl + Baf
Fedra + Baf
CHZ + Baf

Figure S2:

A


HEL
P-STAT5
STAT5
Actin
LC3B I
II
Baf: - + - + - + - +
Ruxo: - - + + - - + +
+SAR405
LC3B-II / Actin (ratio versus Ctrl)
2,5
2
1,5
1
0,5
0
HEL
Ctrl + Baf
Ruxo + Baf
SAR405 + Baf
SAR405 + Ruxo + Baf

B

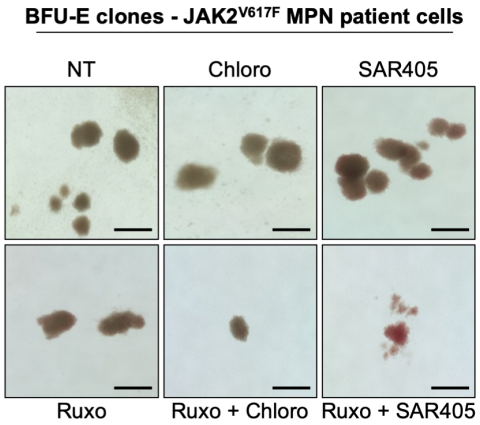

BFU-E clones - JAK2V617F MPN patient cells
NT
Chloro
SAR405
Ruxo
Ruxo + Chloro
Ruxo + SAR405

Figure S3:

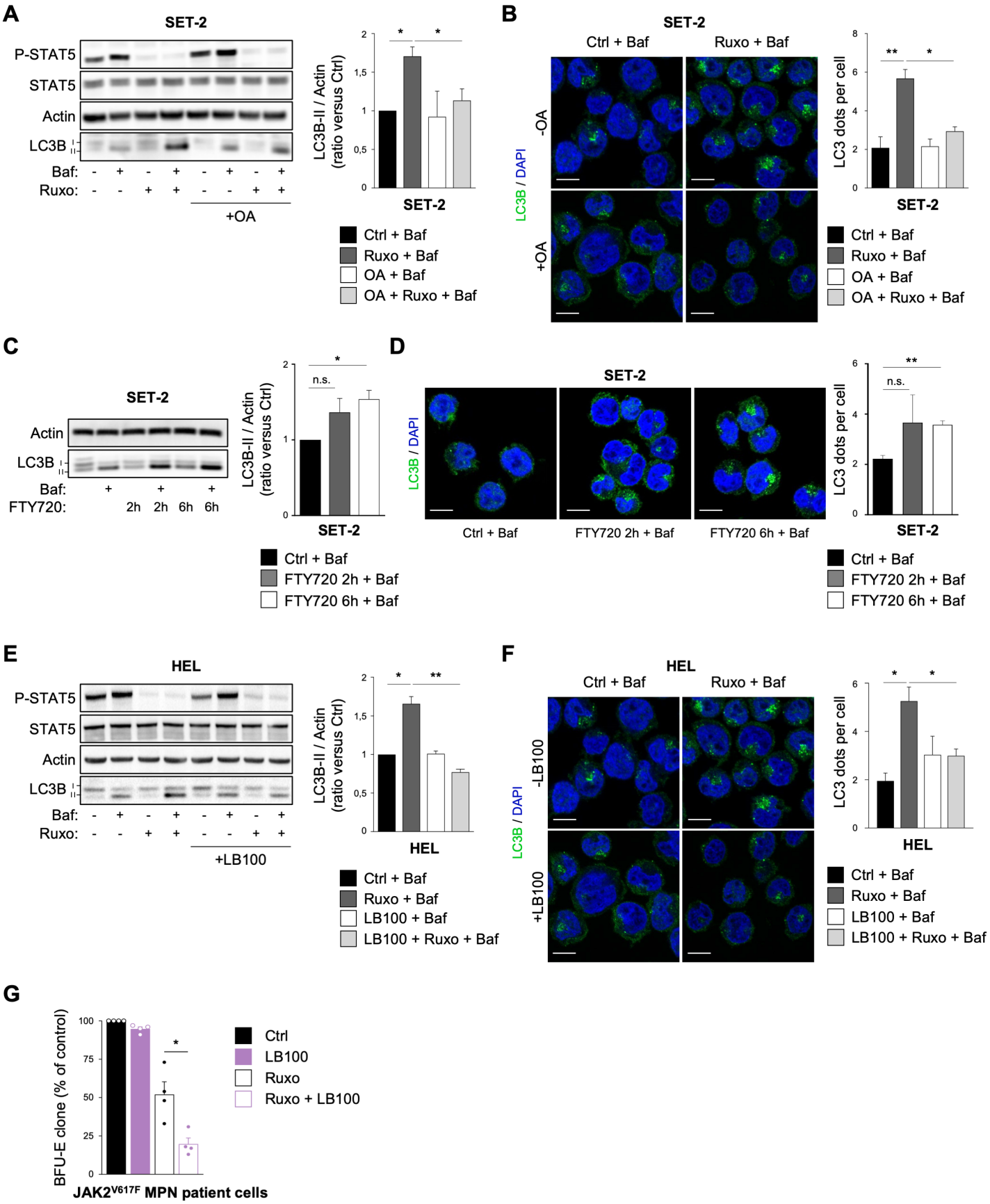
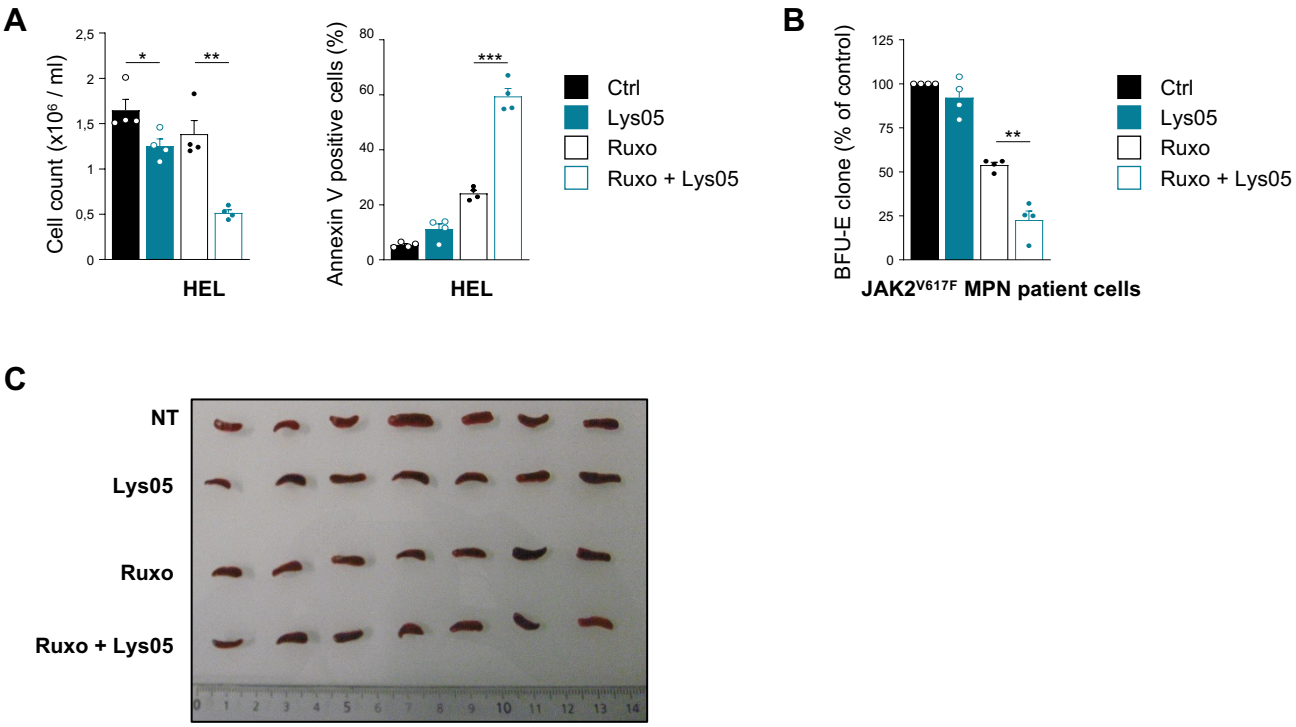

A
SET-2
P-STAT5
STAT5
Actin
LC3B
Baf:
Ruxo:
+OA
LC3B-II / Actin (ratio versus Ctrl)
SET-2
Ctrl + Baf
Ruxo + Baf
OA + Baf
OA + Ruxo + Baf
B
SET-2
Ctrl + Baf
Ruxo + Baf
-OA
+OA
LC3B / DAPI
LC3 dots per cell
SET-2
C
SET-2
Actin
LC3B
Baf:
FTY720:
2h 2h 6h 6h
LC3B-II / Actin (ratio versus Ctrl)
n.s.
SET-2
Ctrl + Baf
FTY720 2h + Baf
FTY720 6h + Baf
D
SET-2
LC3B / DAPI
Ctrl + Baf
FTY720 2h + Baf
FTY720 6h + Baf
LC3 dots per cell
n.s.
SET-2
E
HEL
P-STAT5
STAT5
Actin
LC3B
Baf:
Ruxo:
+LB100
LC3B-II / Actin (ratio versus Ctrl)
HEL
Ctrl + Baf
Ruxo + Baf
LB100 + Baf
LB100 + Ruxo + Baf
F
HEL
Ctrl + Baf
Ruxo + Baf
-LB100
+LB100
LC3B / DAPI
LC3 dots per cell
HEL
G
BFU-E clone (% of control)
JAK2V617F MPN patient cells
Ctrl
LB100
Ruxo
Ruxo + LB100

Figure S4:


A
Cell count (x10⁶ / ml)
2,5
2
1,5
1
0,5
0
*
**
HEL
Annexin V positive cells (%)
80
60
40
20
0
***
HEL
Ctrl
Lys05
Ruxo
Ruxo + Lys05
B
BFU-E clone (% of control)
125
100
75
50
25
0
**
JAK2V617F MPN patient cells
Ctrl
Lys05
Ruxo
Ruxo + Lys05

C
NT
Lys05
Ruxo
Ruxo + Lys05

Figure S1. Ruxolitinib increases autophagy in JAK2^{V617F} cells. **A.** HEL cells were treated with ruxolitinib (1μM) for 24 hours in the presence or absence of bafilomycin (25nM) during 2 hours to monitor autophagy flux and processed for western blot analysis of LC3B. Actin was used as a loading control. Graphs represent the LC3B-II/actin ratios obtained by densitometric analysis (n=3 ± s.e.m.). **B.** HEL cells were treated with fedratinib (1μM) or CHZ868 (500nM) for 2 hours in the presence or absence of bafilomycin (25nM) to monitor autophagy flux and processed for western blot analysis of P-STAT5, STAT5 and LC3B. Actin was used as a loading control. Graphs represent the LC3B-II/actin ratios obtained by densitometric analysis (n=3 ± s.e.m.).

Figure S2. Autophagy inhibition enhances ruxolitinib efficacy in JAK2^{V617F} cells. **A.** HEL cells were treated with ruxolitinib (1μM) and SAR405 (3μM) for 2 hours in the presence or absence of bafilomycin (25nM) to monitor autophagy flux and processed for western blot analysis of P-STAT5, STAT5 and LC3B. Actin was used as a loading control. Graphs represent the LC3B-II/actin ratios obtained by densitometric analysis (n=2 ± s.e.m.). **B.** Representative picture of BFU-E clones after 14 days of CD34+ MPN patients'cells cultured in semi-solid medium supplemented or not with ruxolitinib (250nM) and chloroquine (3μM) or SAR405 (3μM). Scale bar: 300μm.

Figure S3. PP2A inhibition enhances ruxolitinib efficacy in JAK2^{V617F} cells. **A-B.** SET-2 cells were treated with ruxolitinib (1μM) and OA (5nM) for 2 hours in the presence or absence of bafilomycin (25nM) to monitor autophagy flux. Cells were processed for western blot analysis of P-STAT5, STAT5 and LC3B. Actin was used as a loading control. Graphs represent the LC3B-II/actin ratios obtained by densitometric analysis (n=4 ± s.e.m.), (**A**). Cells were stained for LC3B and analyzed by confocal microscopy. Graphs represent the number of LC3B dots per cell (n=4 ± s.e.m.), (**B**). Scale bar: 10μm. **C-D.** SET-2 cells were treated with FTY720 (2.5μM) for 2 or 6 hours in the presence or absence of bafilomycin (25nM) to monitor autophagy flux. Cells were processed for western blot analysis of LC3B. Actin was used as a

loading control. Graphs represent the LC3B-II/actin ratios obtained by densitometric analysis (n=3 ± s.e.m.), (**C**). Cells were stained for LC3B and analyzed by confocal microscopy. Graphs represent the number of LC3B dots per cell (n=3 ± s.e.m.), (**D**). Scale bar: 10µm. **E-F.** HEL cells were treated with ruxolitinib (1µM) and LB100 (2.5µM) for 2 hours in the presence or absence of bafilomycin (25nM) to monitor autophagy flux. Cells were processed for western blot analysis of P-STAT5, STAT5 and LC3B. Actin was used as a loading control. Graphs represent the LC3B-II/actin ratios obtained by densitometric analysis (n=3 ± s.e.m.), (**E**). Cells were stained for LC3B and analyzed by confocal microscopy. Graphs represent the number of LC3B dots per cell (n=3 ± s.e.m.), (**F**). **G.** CD34+ cells from $JAK2^{V617F}$ MPN patient samples were plated for colony forming assay in semi-solid medium supplemented or not with ruxolitinib (250nM) and LB100 (1µM). After 14 days, clonogenic potential was assessed by counting the number of BFU-E clones per dish. Data are represented as percent of control (n=4 ± s.e.m.).

Figure S4. Autophagy inhibitor Lys05 enhances ruxolitinib efficacy in $JAK2^{V617F}$ cells *in vitro* and *in vivo*. **A.** HEL cells were treated or not with ruxolitinib (1µM) in the presence or absence of Lys05 (5µM). After 3 days, the number of cells was assessed by trypan blue exclusion counting (left panel; n=4 ± s.e.m.) and the percentage of cell death was determined by Annexin-V labelling and flow cytometry analysis (right panel; n=4 ± s.e.m.). **B.** CD34+ cells from $JAK2^{V617F}$ MPN patient samples were plated for colony forming assay in semi-solid medium supplemented or not with ruxolitinib (250nM) and Lys05 (1µM). After 14 days, clonogenic potential was assessed by counting the number of BFU-E clones per dish. Data are represented as percent of control (n=4 ± s.e.m.). **C.** HEL cells were injected in NSG mice and, after 14 days of the indicated treatments their spleens were collected.