

Supplementary Materials: Myc-Related Mitochondrial Activity as a Novel Target for Multiple Myeloma

Alejandra Ortiz-Ruiz^{1-2†}, Yanira Ruiz-Heredia^{1-2†}, María Luz Morales¹⁻², Pedro Aguilar-Garrido¹⁻², Almudena Garcia-Ortiz¹⁻², Antonio Valeri¹⁻², Carmen Bárcena³, Rosa María García-Martin³, Vanesa Garrido¹⁻², Laura Moreno¹⁻², Alicia Gimenez¹⁻², Miguel Ángel Navarro-Aguadero¹, María Velasco-Estevez¹, Eva Lospitao⁴, María Teresa Cedena², Santiago Barrio¹⁻², Joaquín Martínez-López^{1-2#}, María Linares^{1-2#} and Miguel Gallardo^{1-2*}

Patient therapies		MGUS (N=15)	SMM (N=19)	NDMM (N=27)	RRMM (N=22)
Therapy lines before sample collection (%)					
	RD	0	5,3	3,85	18,2
	VRD	0	0	0	22,7
	VMP	0	0	0	4,5
	VTD	0	0	0	4,5
	KRD	0	0	0	0
	KDD	0	0	0	0
	VBMCP	0	0	0	9,1
	Other therapies ^a	0	0	3,85	41
	No therapy	100	94,7	92,3	0
Therapy lines following sample collection (%)					
	RD	0	5,3	14,8	18,5
	VRD	0	5,3	0	18,5
	VMP	0	15,7	18,5	11,1
	VTD	0	5,3	3,7	3,7
	KRD	0	5,3	0	0
	KDD	0	0	0	0
	VBMCP	0	0	0	14,8
	Other therapies ^a	0	5,3	37,1	14,9
	No therapy	100	57,8	25,9	18,5

Table S1. Patient treatment regimens. Patient therapies and frequency of treatment according to each stage of the disease (N=123).

Abbreviations: KDD, carfilzomib daratumumab dexamethasone; KRD, carfilzomib lenalidomide dexamethasone; RD, lenalidomide dexamethasone; VBMCP, vincristine doxorubicin melphalan cyclophosphamide prednisone; VMP, bortezomib melphalan

prednisone; VRD, bortezomib lenalidomide dexamethasone; VTD, bortezomib thalidomide dexamethasone

^aOther therapies (22): lenalidomide dexamethasone and clarithromycin; melphalan + prednisone; isatuximab + velcade + clarithromycin + dexamethasone; velcade + dexamethasone; dexamethasone + cyclophosphamide; darzalex + velcade + melphalan + prednisone; velcade + cyclophosphamide + prednisone; clinical trial CC-92480; vincristine + carmustine + doxorubicine + dexamethasone; velcade + cyclophosphamide + dexamethasone; isatuximab; vincristine + carmustine + melphalan; cyclophosphamide + prednisone; clinical trial NKaes; pomalidomide + dexamethasone; bendamoustine + velcade + dexamethasone; darzalex + velcade + dexamethasone; clinical trial CASTOR; carfilzomib + dexamethasone; carfilzomib + cyclophosphamide + dexamethasone; elutuzumab + thalidomide + dexamethasone; clinical trial POMDEFIL; aplidin; clinical trial MK3475.

Antibodies	MW kDa	Used dilution	Source
OXPPOS	54, 48, 29, 22, 18	1:1000	Abcam
c-Myc	60	1:1000	Abcam
B-actin	45	1:1000	Cell Signaling
Goat polyclonal antibody anti-mouse	-	1:1000	Dako
Goat polyclonal antibody anti-rabbit	-	1:1000	Dako

Table S2. Antibodies used for western blotting.

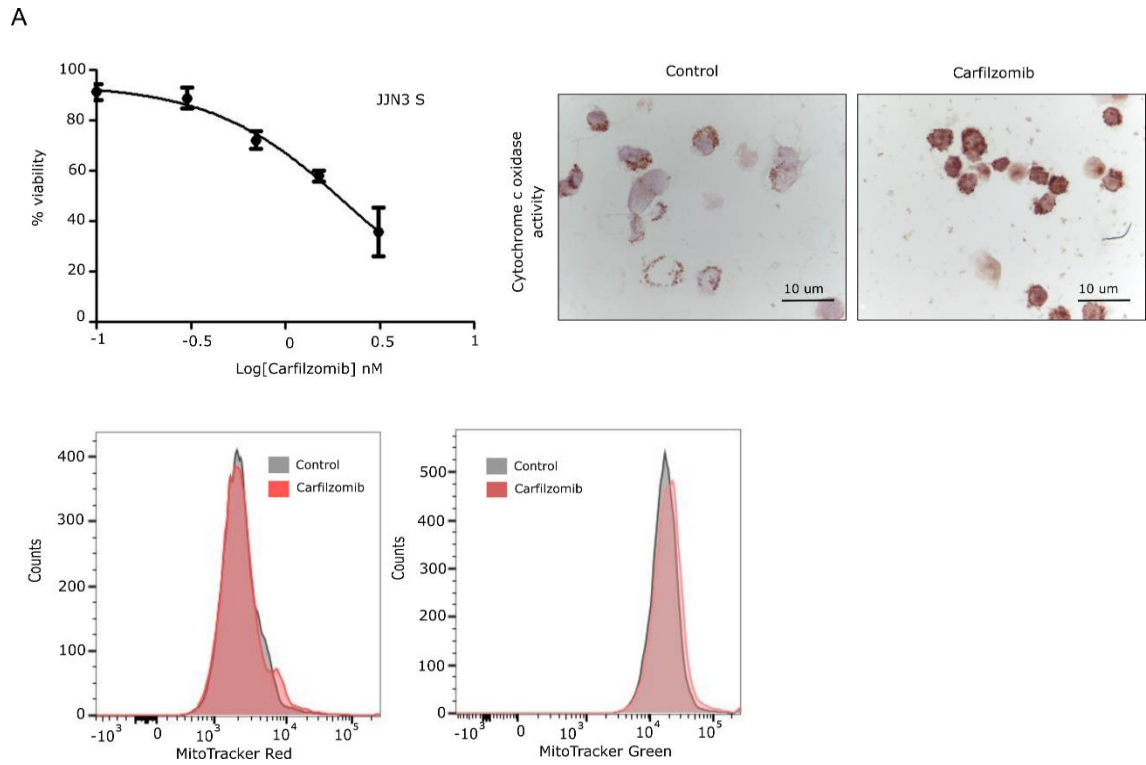


Figure S1. Contribution of carfilzomib treatment in mitochondrial activity features. **A** Study of carfilzomib cytotoxic effects in JJN3 S cells. Analysis of mitochondrial activity by histoenzymatic analysis of COX activity in JJN3 S cells treated with 0.1% DMSO (control) or 1.5 nM carfilzomib; and flow cytometry analysis of MitoTracker Red (left, mitochondrial membrane potential) and green (right, mitochondrial mass) in JJN3 S cells with 0.1% DMSO (control, grey) or 1.5 nM carfilzomib (red) (n=3).

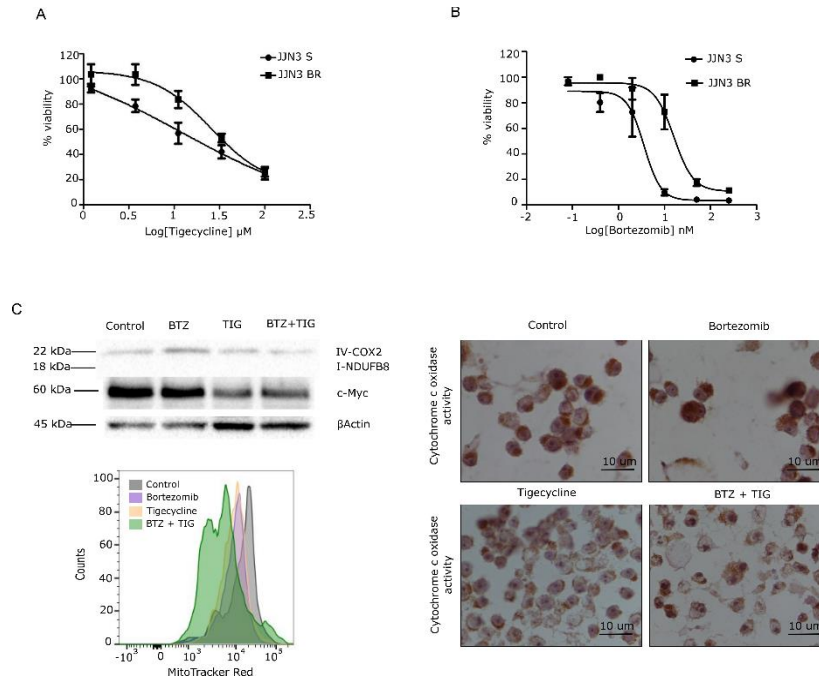


Figure S2. Effect of tigecycline and the combination with bortezomib on JLN3 BR cells. **A** Comparison of tigecycline activity in JLN3 S and JLN3 BR cells. **B** Comparison of bortezomib activity in JLN3 S and JLN3 BR cells. **C** Molecular validation of mitochondrial inhibition of JLN3 BR cells treated with 3.7 μ M tigecycline, 2 nM bortezomib and their combination (n=3): by WB of respiratory chain complexes I and IV and c-Myc; by HE-COX; and by Multiparametric Flow Cytometry (MFC) analysis of MitoTracker Red (mitochondrial membrane potential). All of the drug treatments were for 48 h; n= 3.

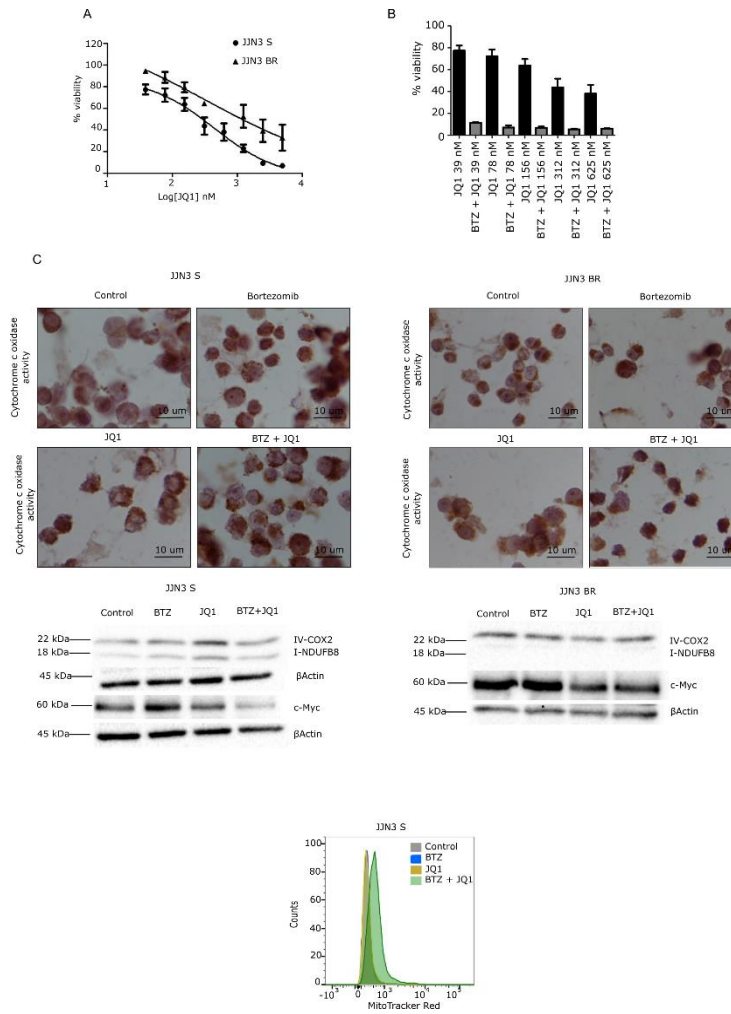


Figure 3C

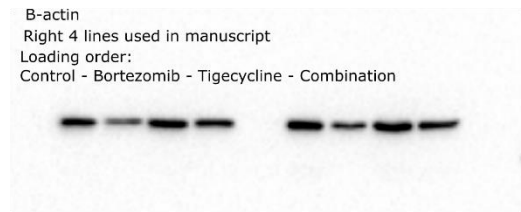
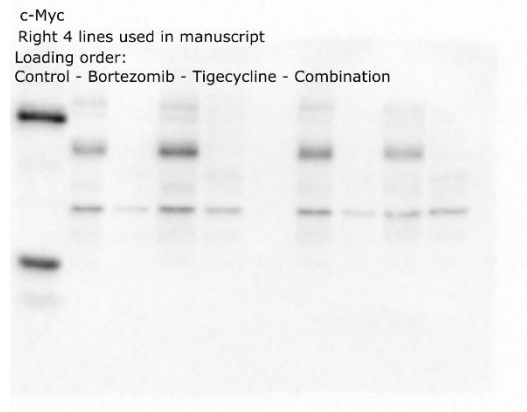
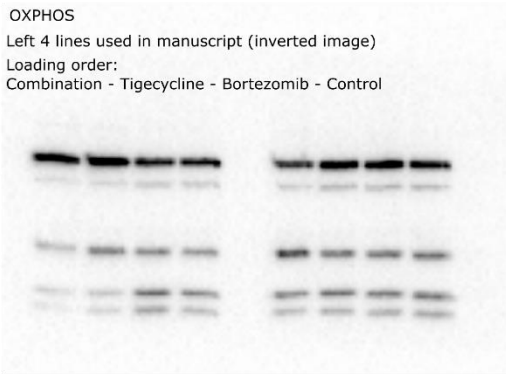


Figure S2B

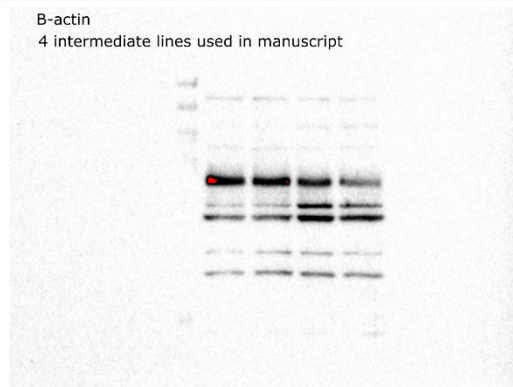
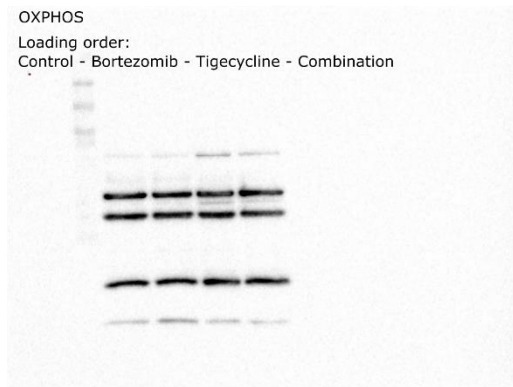


Figure S3C

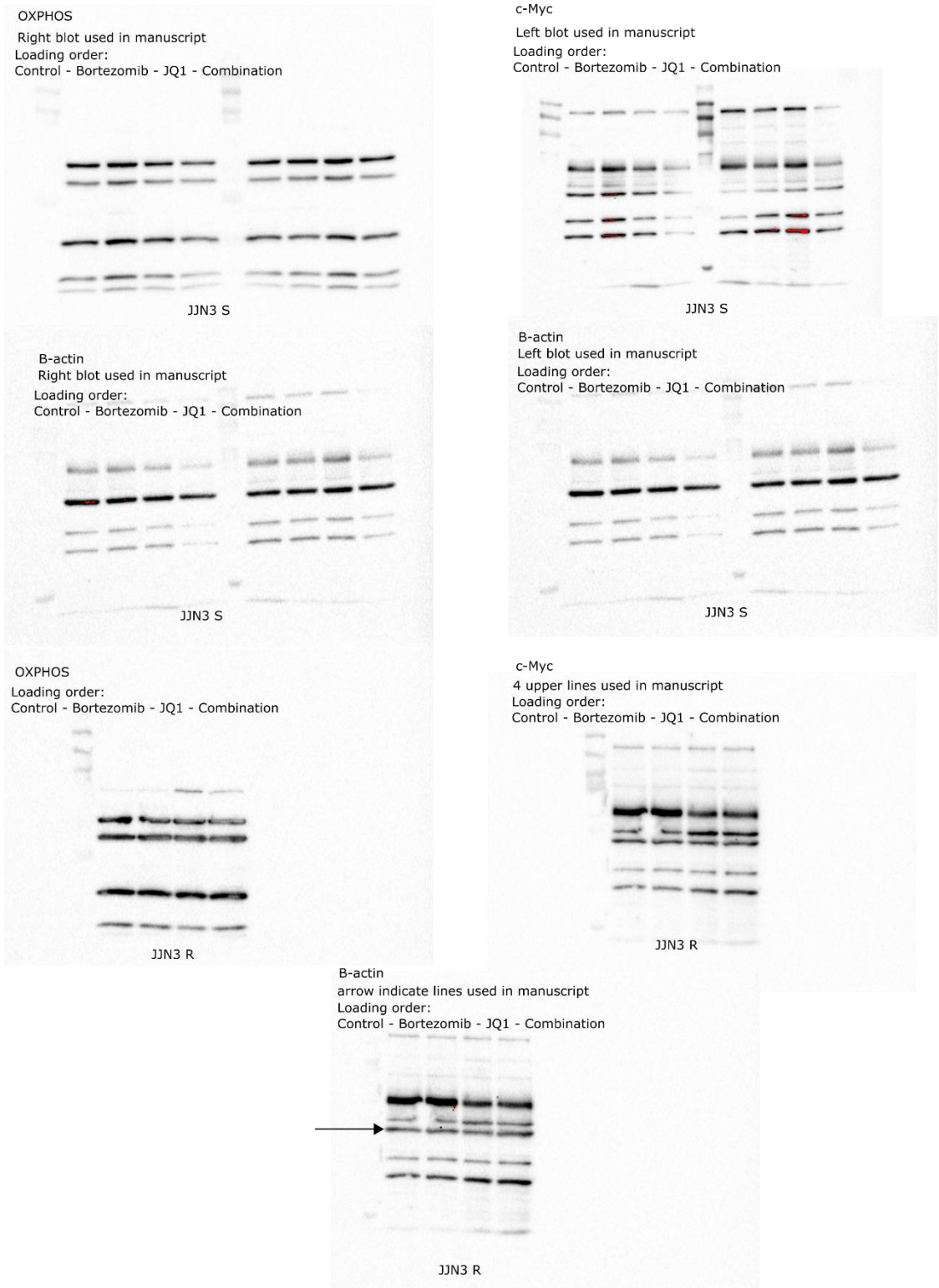
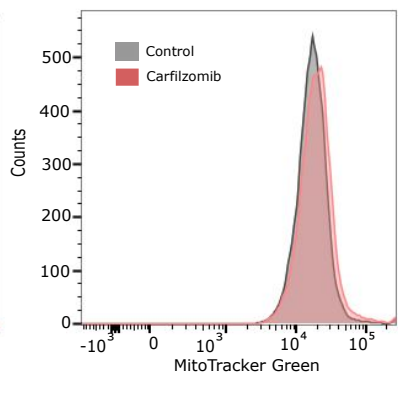
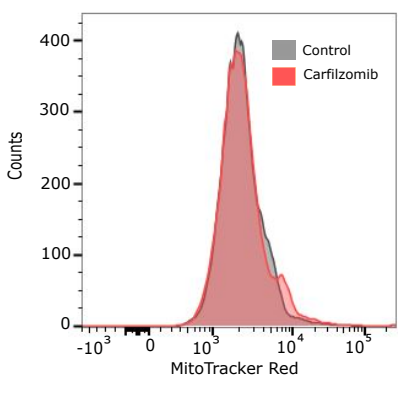
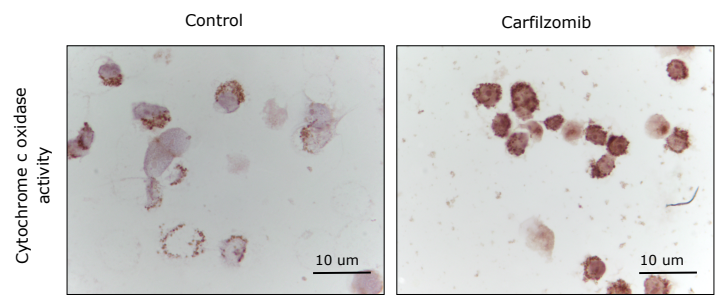
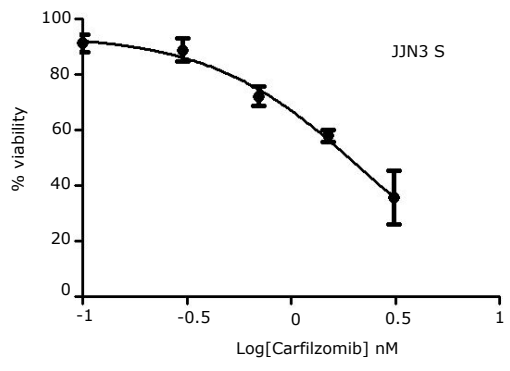
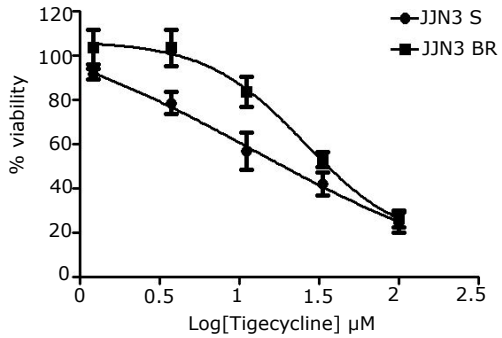


Figure S4: uncropped western blot.

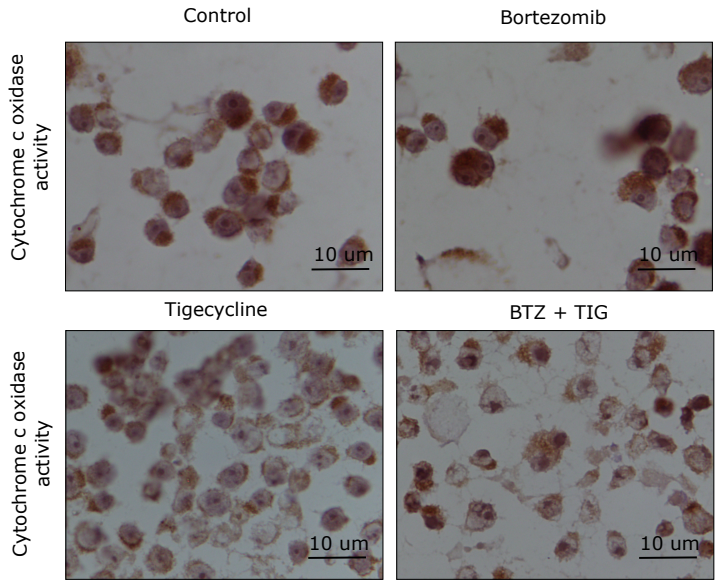
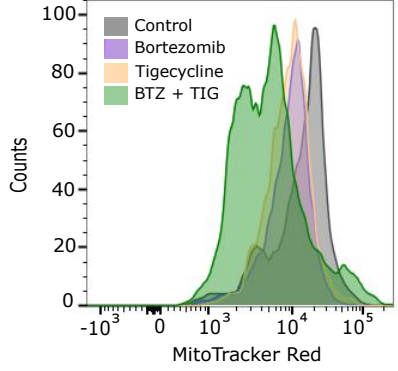
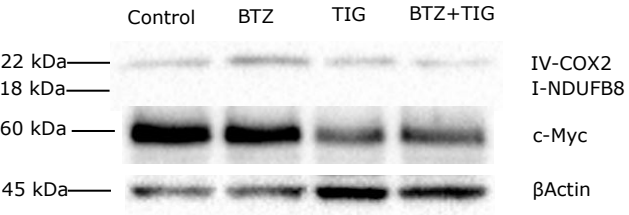
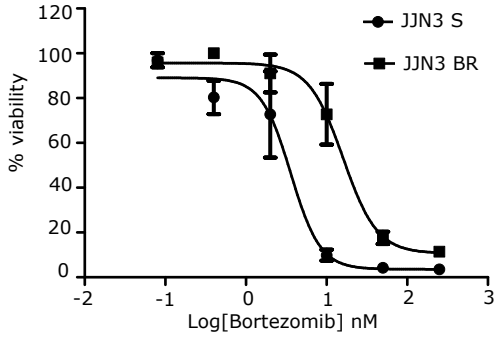
A



A



B



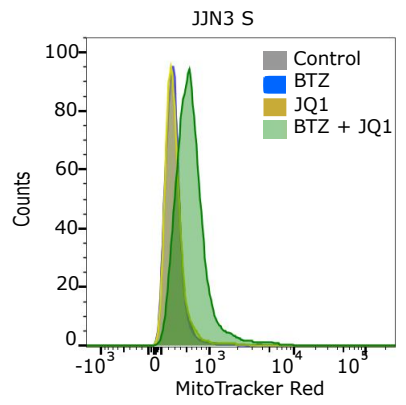
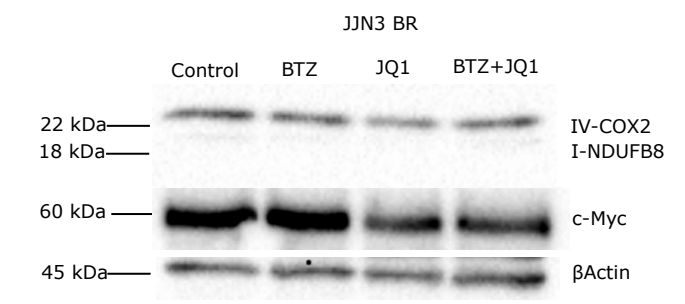
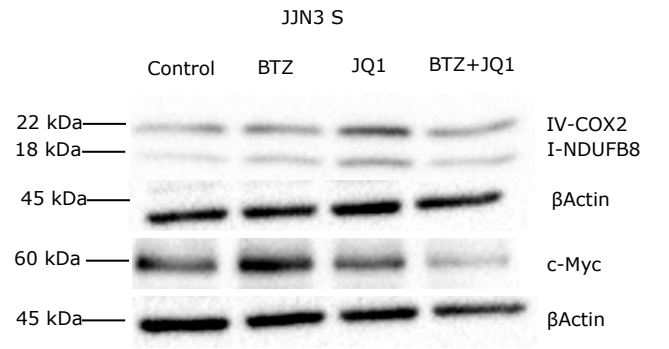
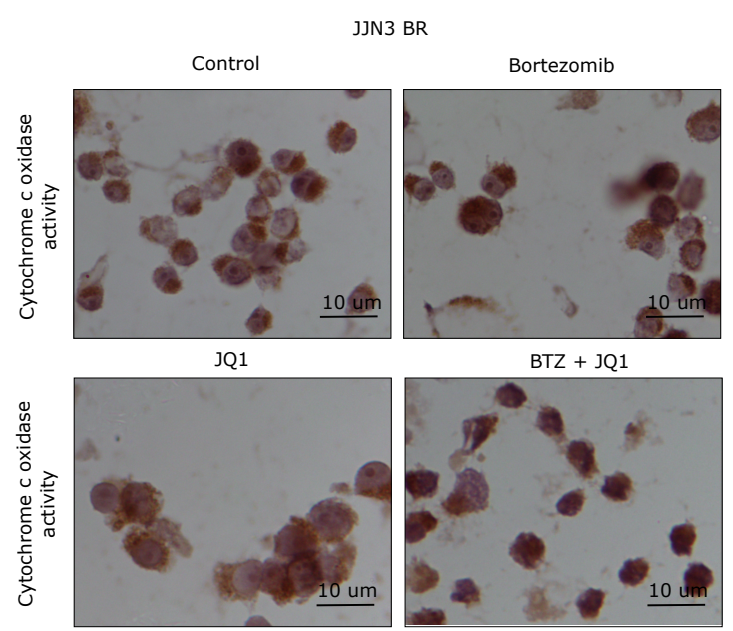
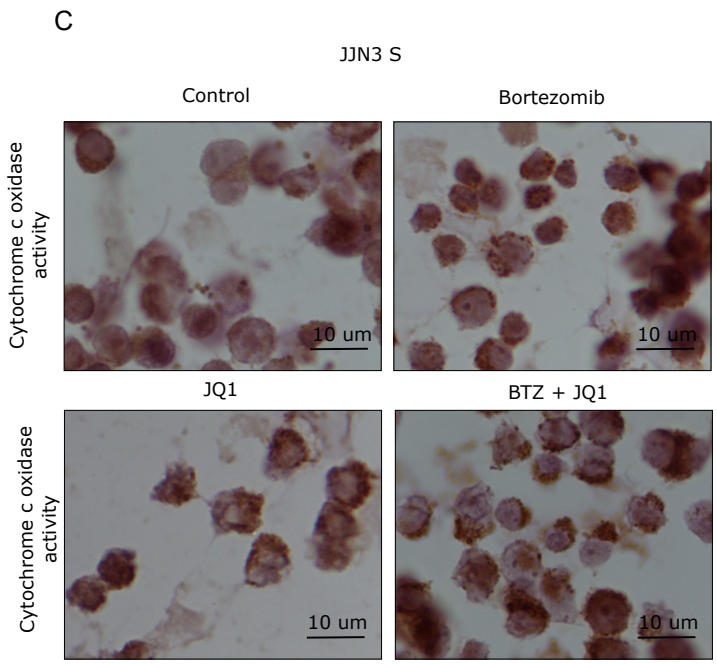
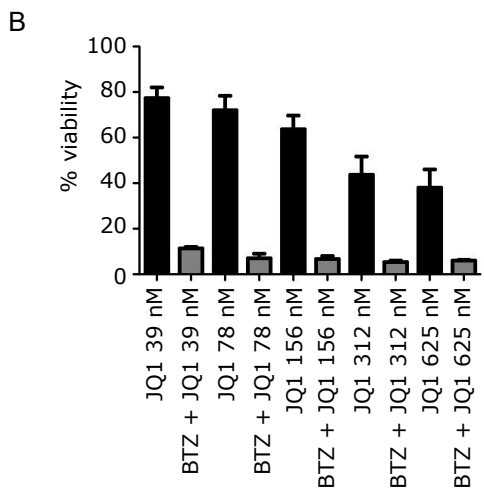
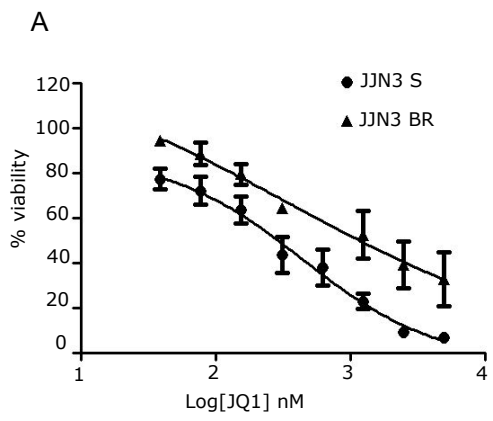


Figure 3C

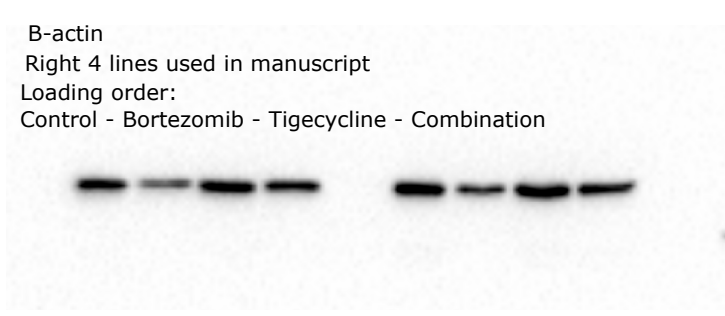
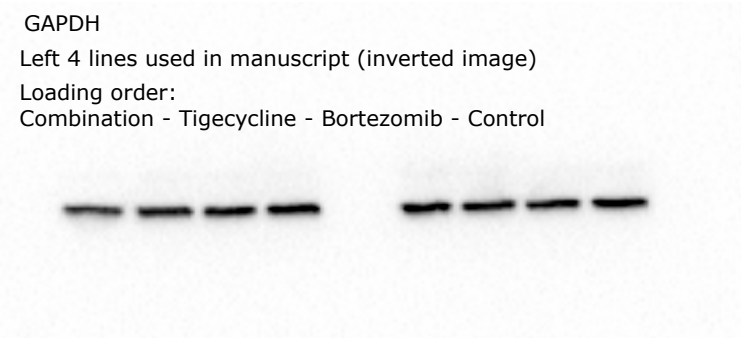
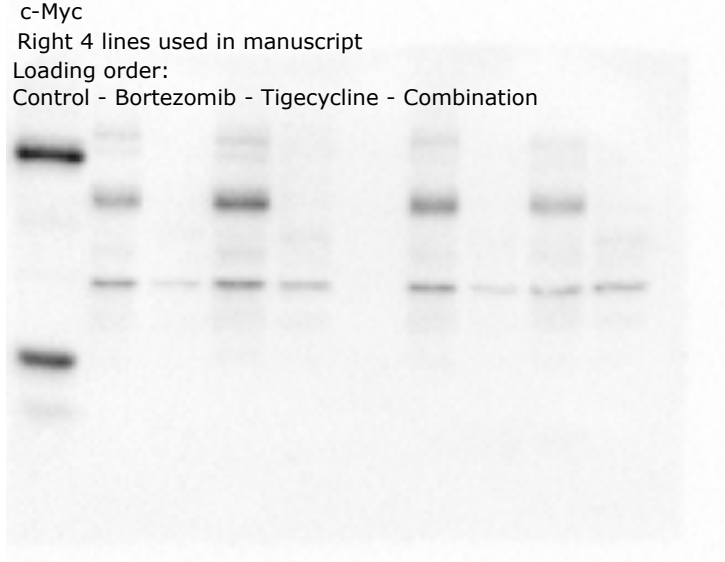
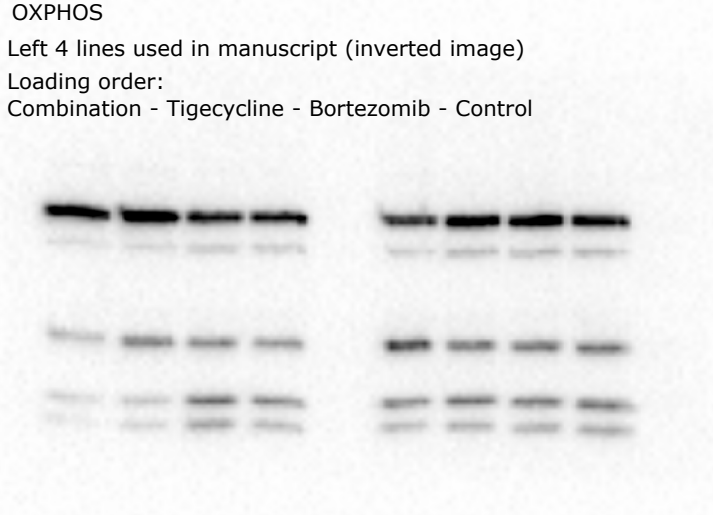


Figure S2B

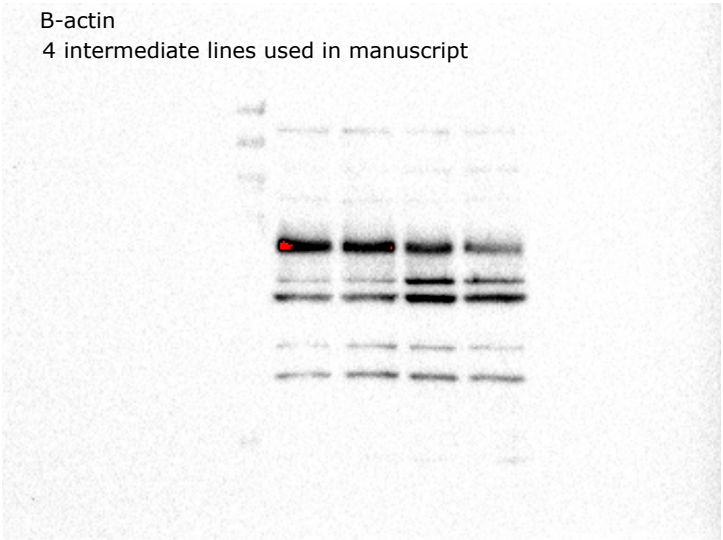
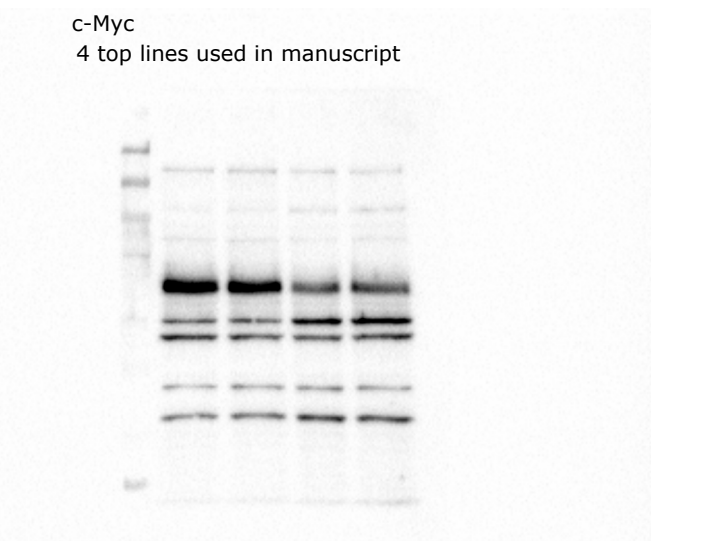


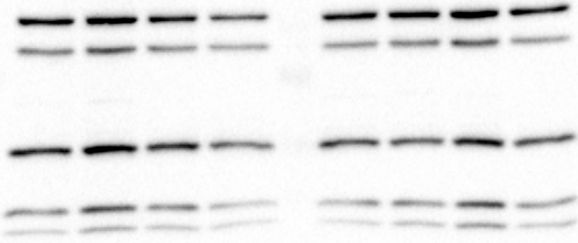
Figure S3C

OXPHOS

Right blot used in manuscript

Loading order:

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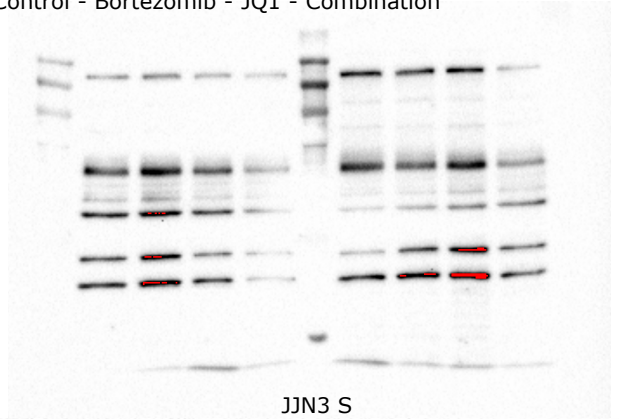
JJN3 S

c-Myc

Left blot used in manuscript

Loading order:

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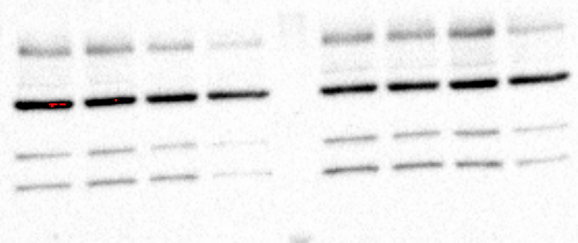
JJN3 S

B-actin

Right blot used in manuscript

Loading order:

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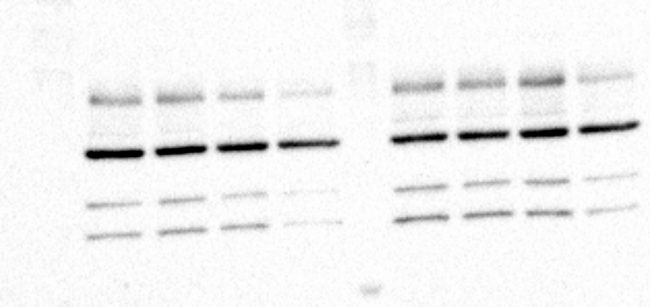
JJN3 S

B-actin

Left blot used in manuscript

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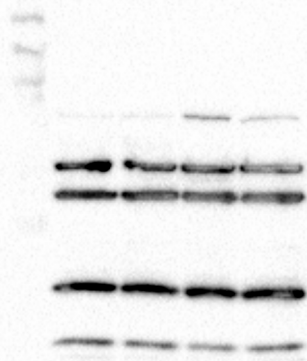


JJN3 S

OXPHOS

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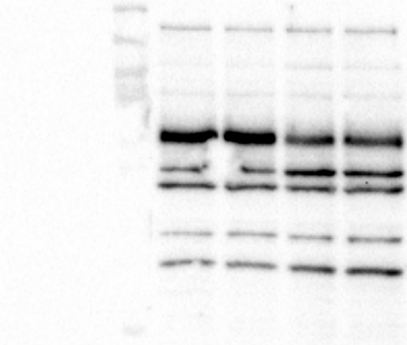
JJN3 R

c-Myc

4 upper lines used in manuscript

Loading order:

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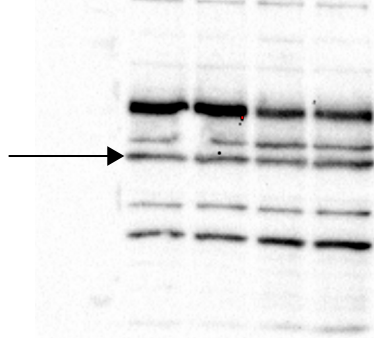
JJN3 R

B-actin

arrow indicate lines used in manuscript

Loading order:

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JJN3 R

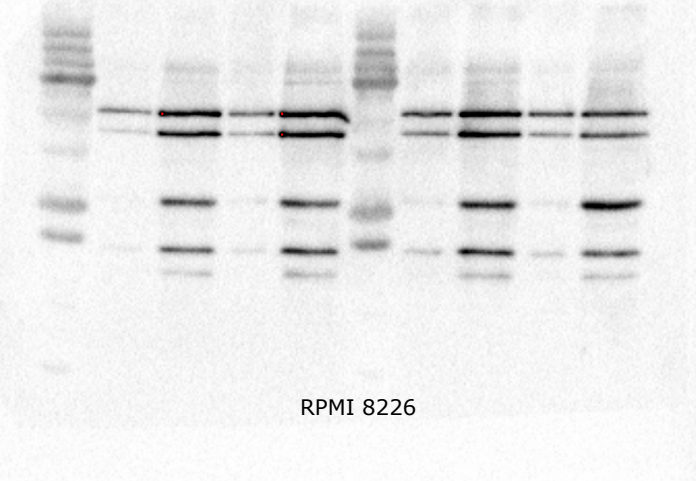
Figure S4A

OXPHOS

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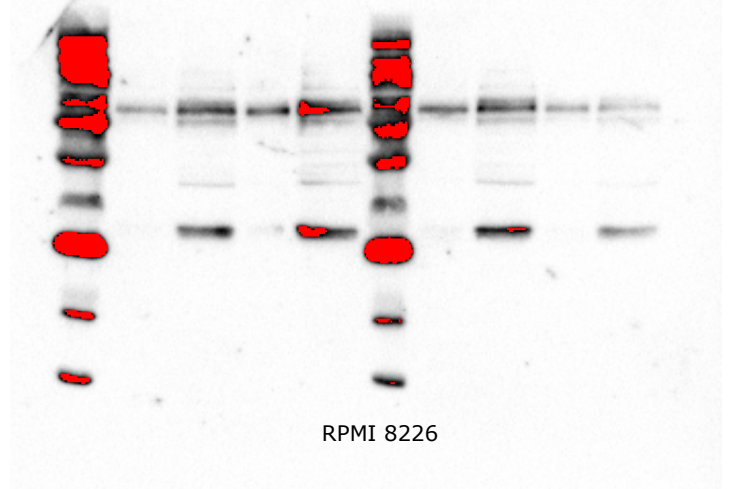


c-Myc

Right blot used in manuscript, lanes 7 and 8

Loading order:

Control - Daratumumab



B-actin

Right blot used in manuscript, lanes 7 and 8

Loading order:

Control - Daratumumab

