

Suppression of BCL6 function by HDAC inhibitor mediated acetylation and chromatin modification enhances BET inhibitor effects in B-cell lymphoma cells

María G. Cortiguera^{1,2,4}, Lorena García-Gaipo^{1,4}, Simon D. Wagner³, Javier León¹, Ana Batlle-López^{2*} and M. Dolores Delgado^{1*}.

¹Instituto de Biomedicina y Biotecnología de Cantabria (IBBTEC) CSIC-Universidad de Cantabria, and Dpt. of. Biología Molecular, Universidad de Cantabria, Santander, Spain.

²Servicio de Hematología, Hospital Marqués de Valdecilla-IDIVAL, Santander, Spain.

³Leicester Cancer Research Centre and Ernest and Helen Scott Haematological Research Unit, University of Leicester, Leicester LE1 7HB, UK

⁴Both authors contributed equally to this work

*Corresponding authors

Prof. M. Dolores Delgado, maria.delgado@unican.es

Dr. Ana Batlle-López, mana.batlle@scsalud.es

Supplementary information

Supplementary Table S1. B-cell lymphoma cell lines used in this study.

Supplementary Table S2. Cell proliferation analysis (WST-1 method) in Ramos and Raji cells using different concentrations of Romidepsin and JQ1 to generate Combination Index (CI) plot.

Supplementary Table S3. Primers used for RT-PCR analysis.

Supplementary Figure S1. Romidepsin effects on BCL6 and PRDM1 expression in Ly03 cells.

Supplementary Figure S2. Synergistic effects of romidepsin and JQ1 in Toledo cells.

Supplementary Figure S3. Synergistic effects of romidepsin and JQ1 in DG75 cells.

Supplementary Figure S4. Full-length blots corresponding to Figure 1c.

Supplementary Figure S5. Full-length blots corresponding to Figure 2a.

Supplementary Figure S6. Full-length blots corresponding to Figure 2b.

Supplementary Figure S7. Full-length blots corresponding to Figure 2c.

Supplementary Figure S8. Full-length blots corresponding to Figure 2d.

Supplementary Figure S9. Full-length blots corresponding to Figure 3.

Supplementary Figure S10. Full-length blots corresponding to Figure 4.

Supplementary Figure S11. Full-length blots corresponding to Figure 5.

Supplementary Figure S12. Full-length blots corresponding to Figure 6d.

Supplementary Figure S13. Full-length blots corresponding to Figure 6e, f.

Supplementary Figure S14. Full-length blots corresponding to Figure 7.

Supplementary Figure S15. Full-length blots corresponding to Figure S1a.

Supplementary Figure S16. Full-length blots corresponding to Figure S2b.

Supplementary Figure S17. Full-length blots corresponding to Figure S2c.

Supplementary Figure S18. Full-length blots corresponding to Figure S3.

Supplementary Table S1. B-cell lymphoma cell lines used in this study

Cell line	Origin	FISH			BCL6 expression
		<i>BCL2</i>	<i>MYC</i>	<i>BCL6</i>	
Ramos	Burkitt lymphoma EBV negative	normal	rearrangement	gain	+++
DG75	Burkitt lymphoma EBV negative	gain	rearrangement	normal	++
Raji	Burkitt lymphoma EBV positive	normal	rearrangement	normal	++
Ly03	ABC-DLBCL	amplification	gain	gain	+
Toledo	GC-DLBCL	gain and rearrangement	gain	normal	-

Supplementary Table S2. Cell proliferation analysis (WST-1 method) in (a) Ramos and (b) Raji cells using different concentrations of Romidepsin and JQ1 to generate Combination Index (CI) plot

a

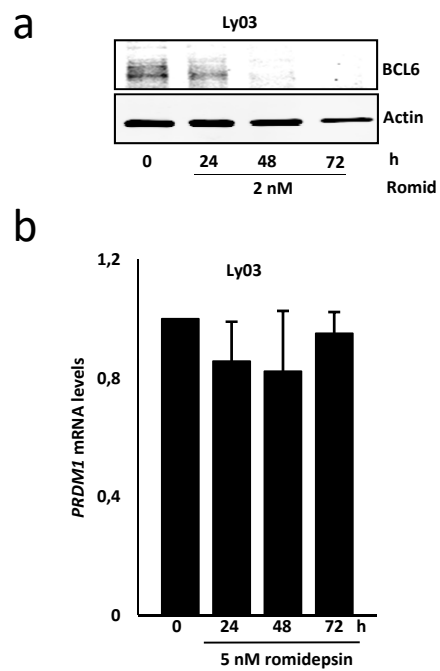
Romid (nM)	JQ1 (μM)	Relative Metabolic Activity	Combination Index (CI)
0	0	1.00	
1.0	0.1	0.94	1.32
5.0	1.0	0.26	0.73
10.0	1.0	0.27	1.16
2.0	0.5	0.55	0.76
5.0	0.5	0.27	0.59
10.0	0.5	0.28	1.03
15.0	1.5	0.27	1.77

b

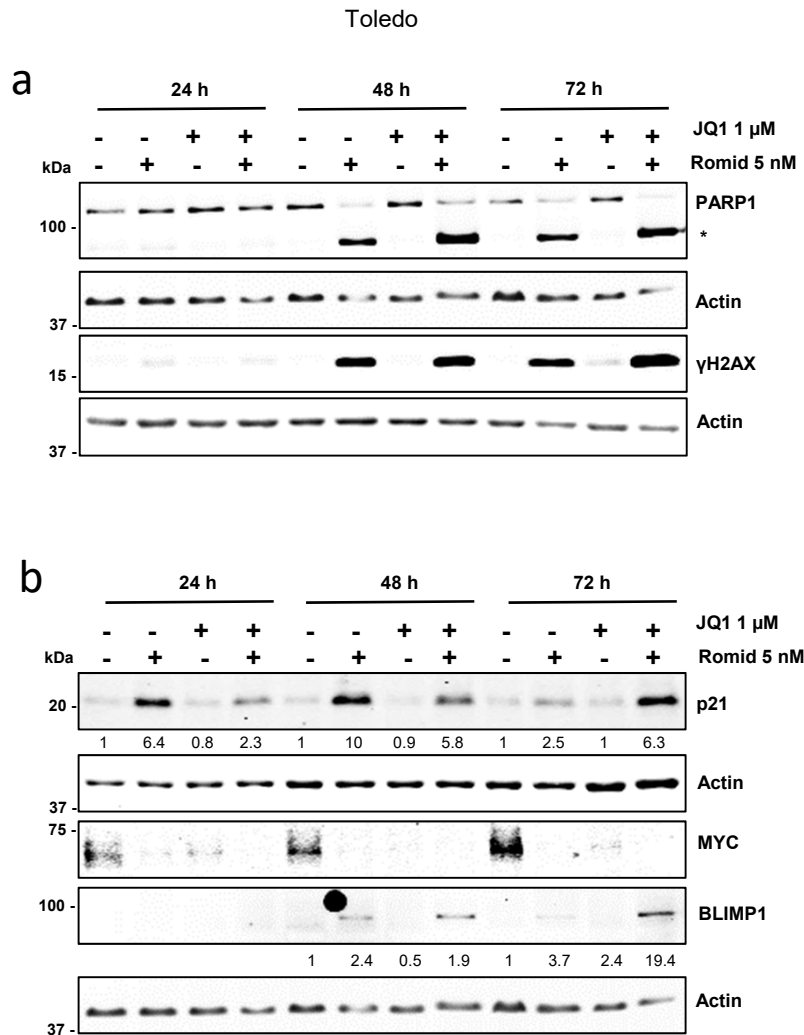
Romid (nM)	JQ1 (μM)	Relative Metabolic Activity	Combination Index (CI)
0	0	1.00	
1.0	0.1	0.57	0.80
5.0	1.0	0.29	0.50
10.0	1.0	0.29	0.99
2.0	0.5	0.29	0.20
5.0	0.5	0.29	0.52
10.0	0.5	0.28	0.98
15.0	1.5	0.28	1.40

Supplementary Table S3. Primers used for RT-PCR analysis

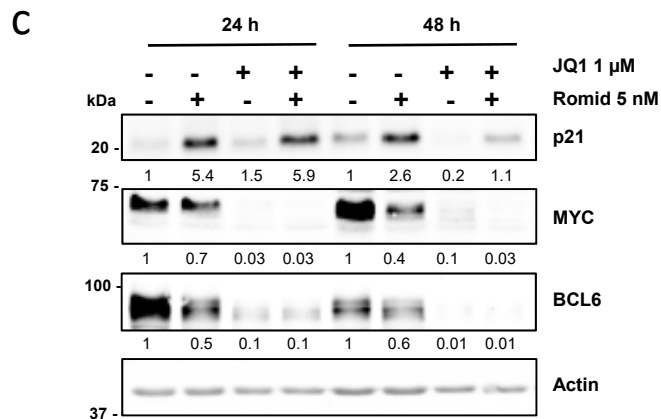
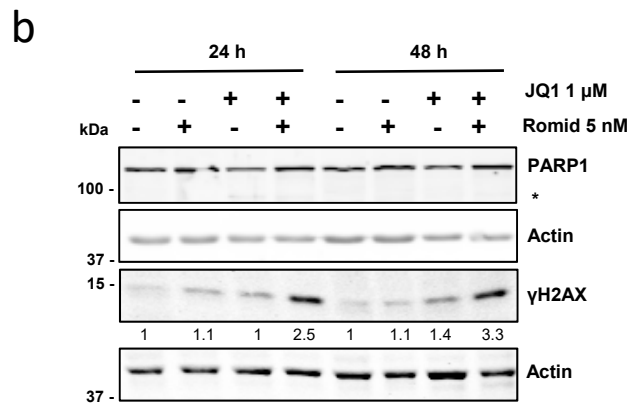
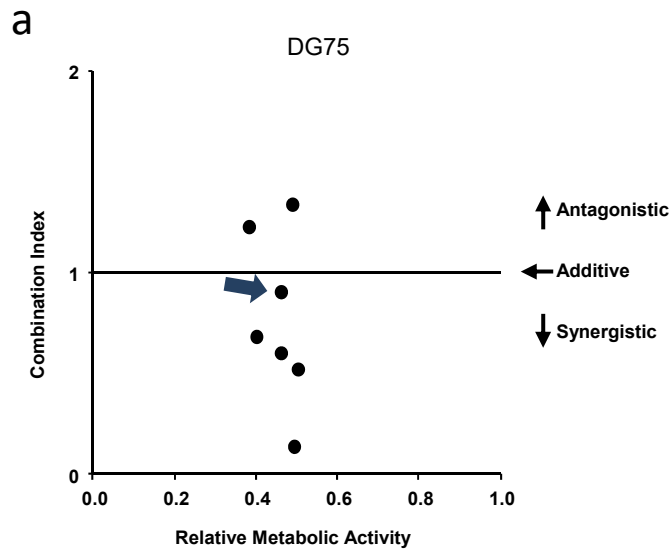
Name	Primers	Amplicon size
BCL6	5'-AGAGCCCATAAAACGGTCCT-3' 5'-AGTGTCCACAACATGCTCCA-3'	231 bp
CCND2	5'-ATATCCCGCACGTCTGTAGG-3' 5'-TGAGCTGCTGGCTAAGATCA-3'	176 bp
PRDM1	5'-CTGAGAGTGCACAGTGGAGA-3' 5'-TGGGTCTTGAGATTGCTGGT-3'	167 bp
PAX5	5'-AGACTTGTTACACAGCAGCA-3' 5'-AGATTGGCCTTCATGTCGTC-3'	165 bp
XBP1	5'-GGAGTTAAGACAGCGCTTGG-3' 5'-GAGATGTTCTGGAGGGGTGA-3'	168 bp
RPS14	5'-TCACCGCCCTACACATCAAAC-3' 5'-CTGCGAGTGCTGTCAGAGG-3'	157 bp



Supplementary Figure S1. Romidepsin effects on BCL6 and PRDM1 expression in Ly03 cells. (a) Western blot showing BCL6 downregulation in Ly03 cells treated with romidepsin for the indicated times. Actin was used as loading control. (b) RT-PCR showing PRDM1 mRNA expression in Ly03 cells treated with romidepsin as indicated.

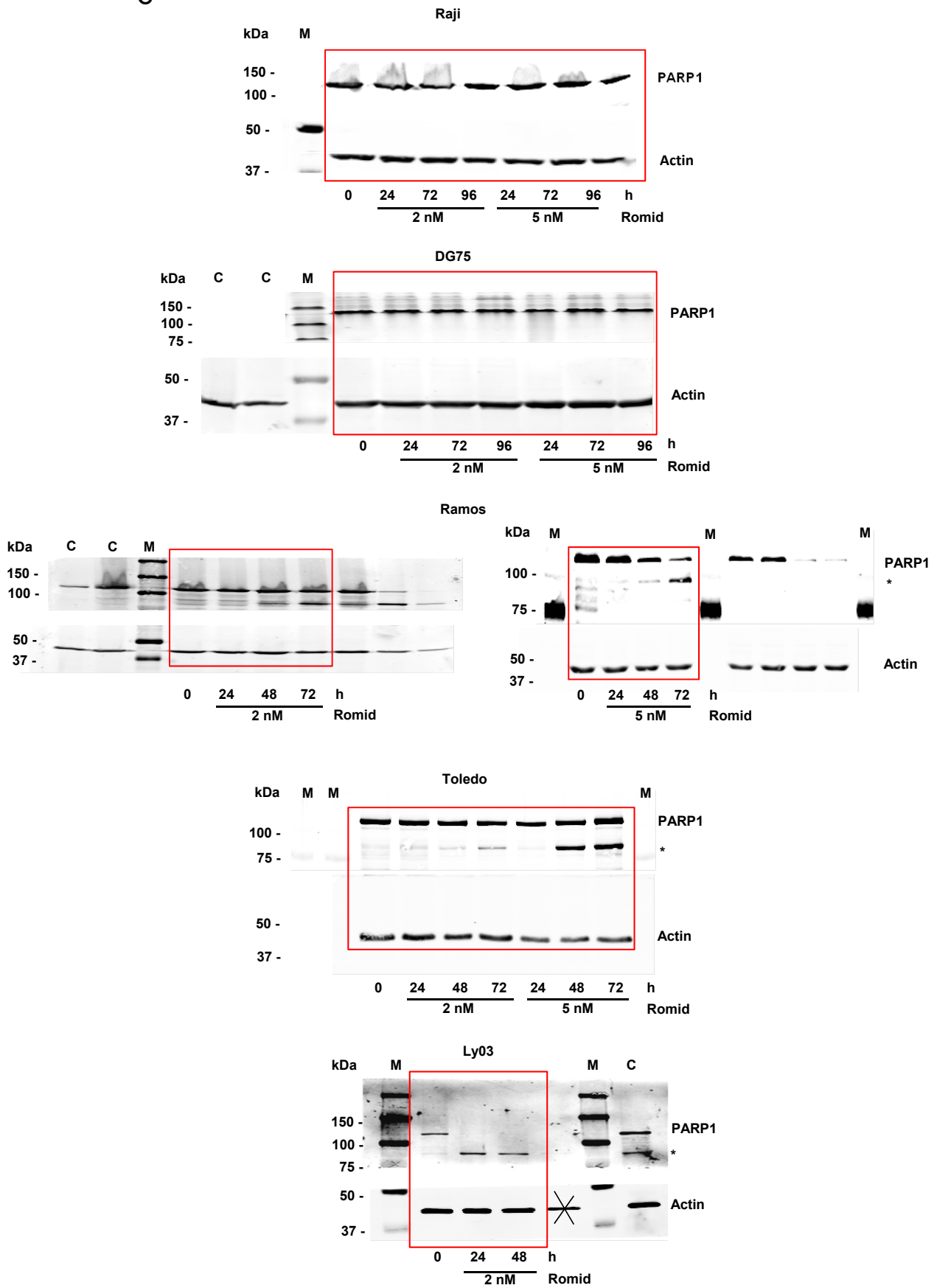


Supplementary Figure S2. Synergistic effects of romidepsin and JQ1 in Toledo cells. (a) Western blot showing cleaved PARP1 and γ H2AX protein levels in Toledo cells treated with romidepsin and/or JQ1 for the indicated times. Actin was used as loading control. (b) Western blot showing p21, MYC and BLIMP1 protein levels in Toledo cells treated as above.



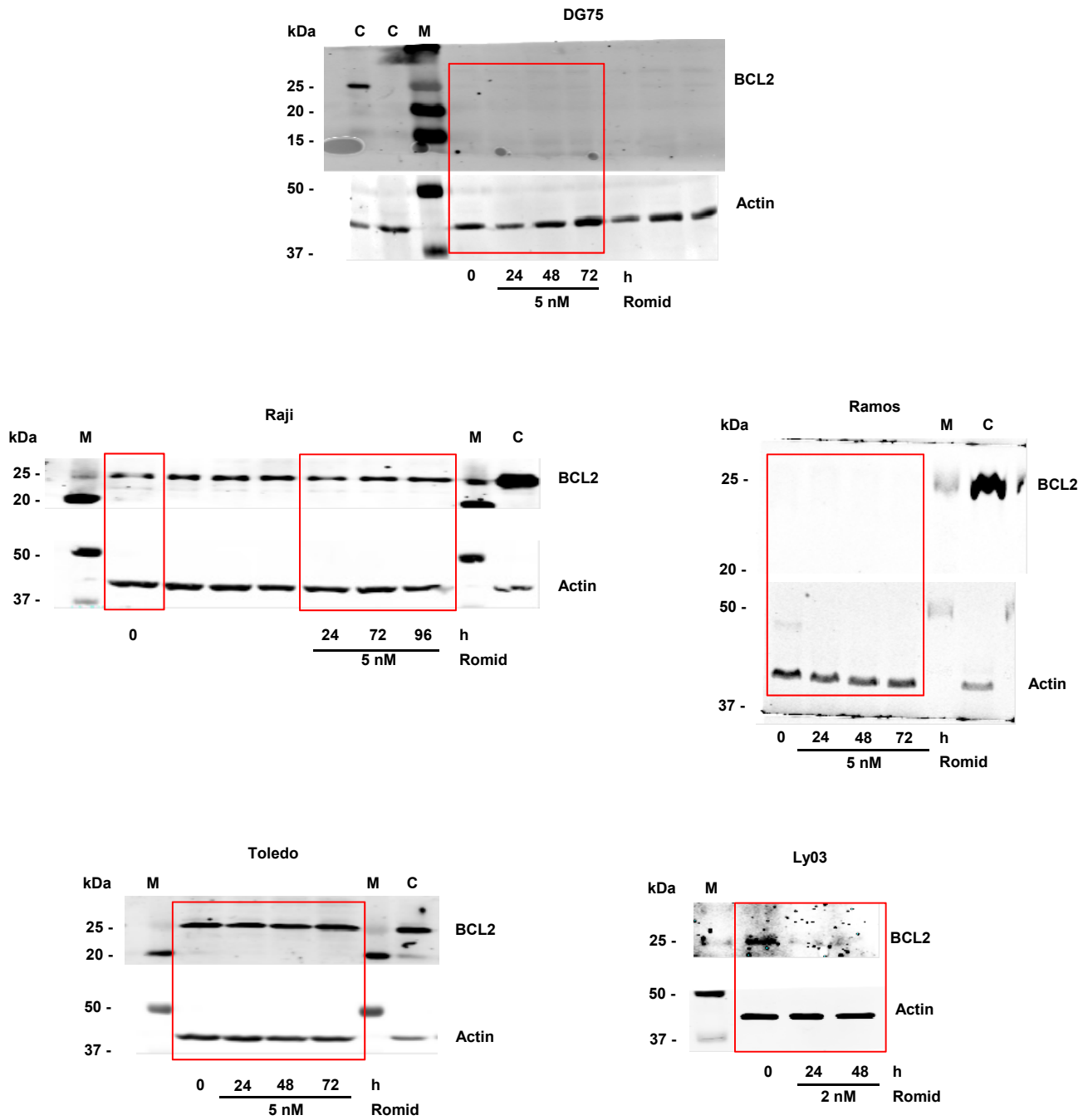
Supplementary Figure S3. Synergistic effects of romidepsin and JQ1 in DG75 cells. (a) Combination index plot showing synergistic effect of romidepsin plus JQ1 on the proliferation of DG75 cells. (b) Western blot showing PARP1 and γ H2AX protein levels in DG75 cells treated with romidepsin and/or JQ1 for the indicated times. Actin was used as loading control. (c) Western blot showing p21, MYC and BCL6 protein levels in DG75 cells treated as above.

C



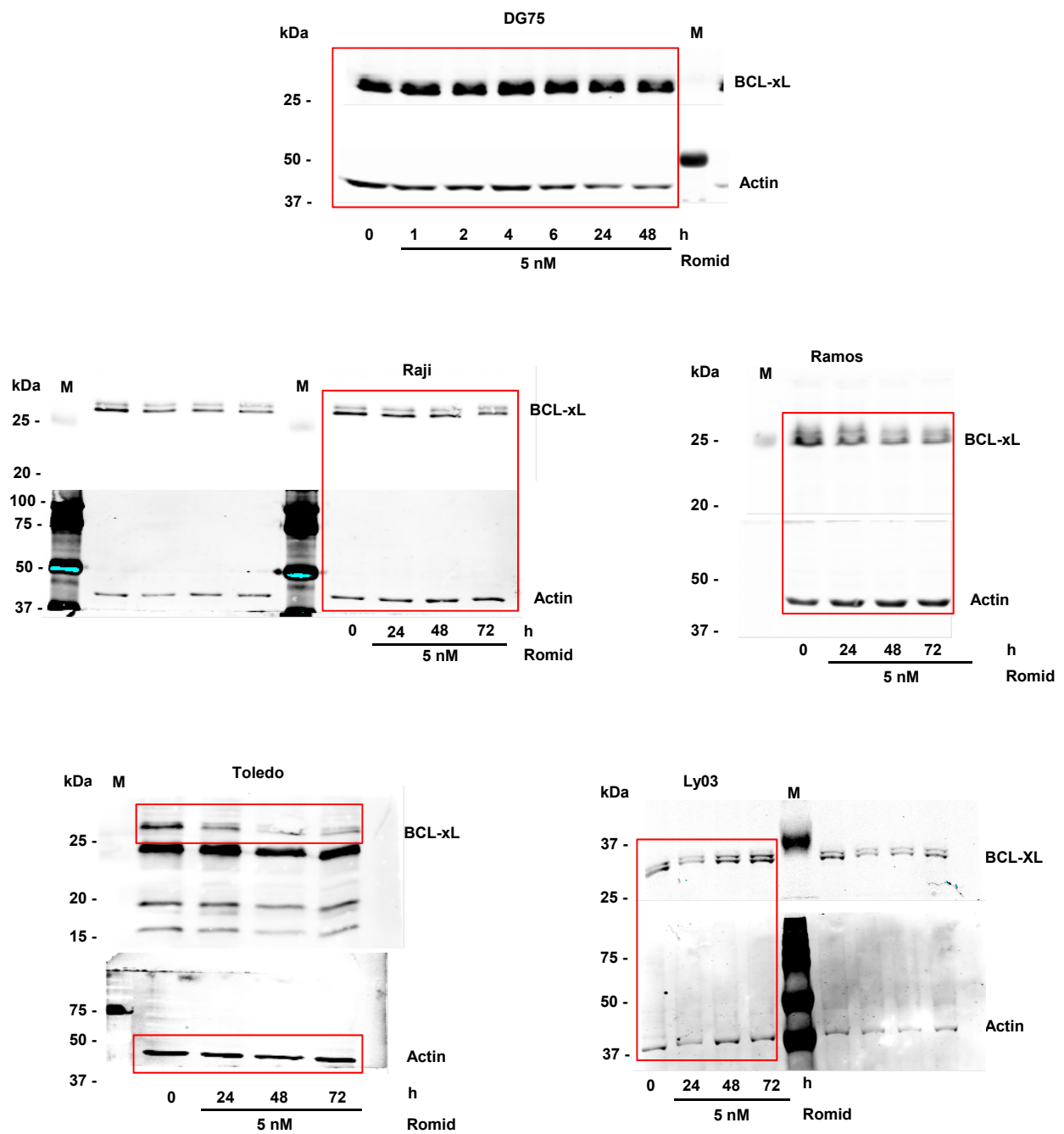
Supplementary Figure S4. Full-length blots corresponding to Figure 1c. (M, markers. C, positive control)

a



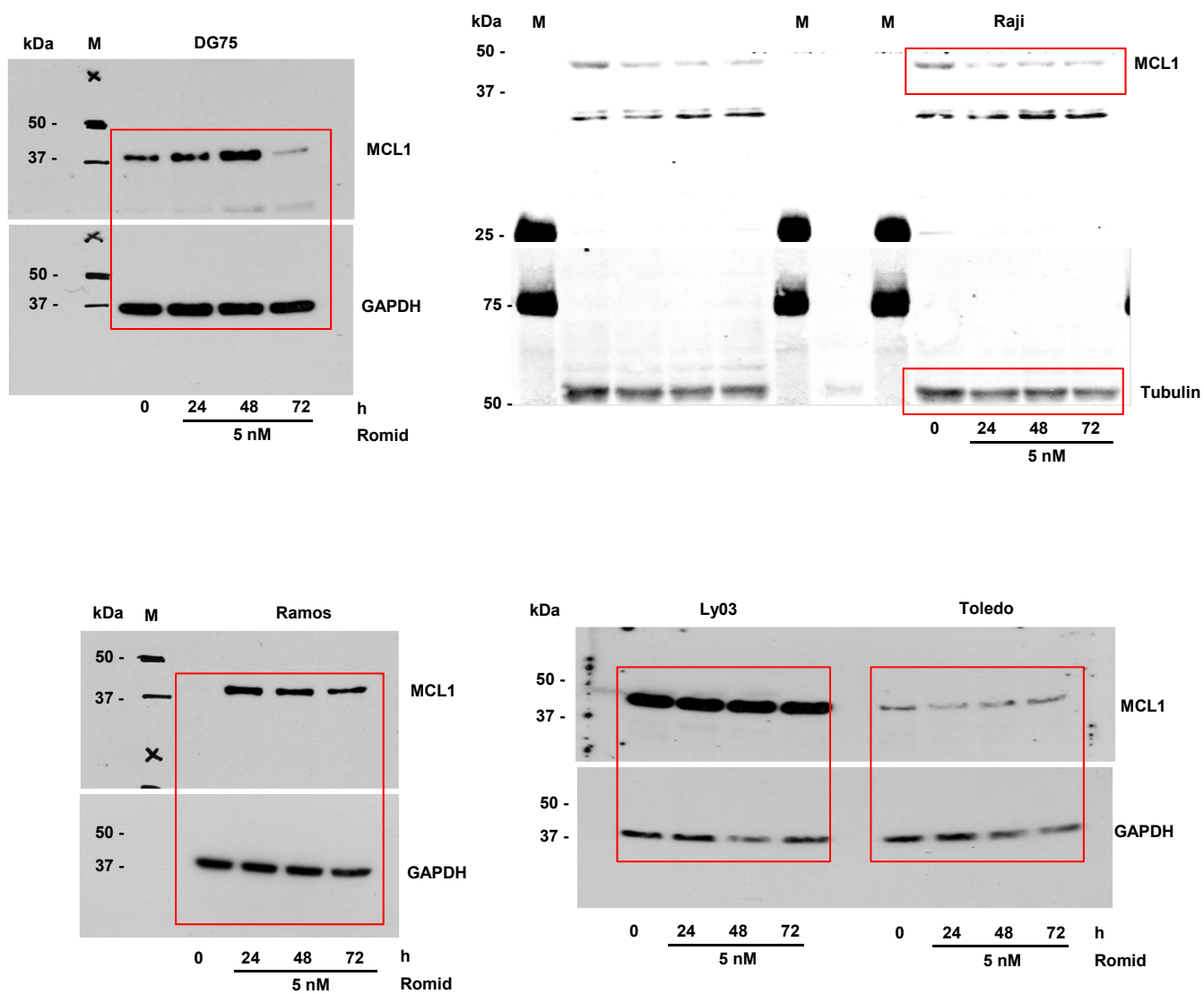
Supplementary Figure S5. Full-length blots corresponding to Figure 2a. (M, markers. C, positive control).

b



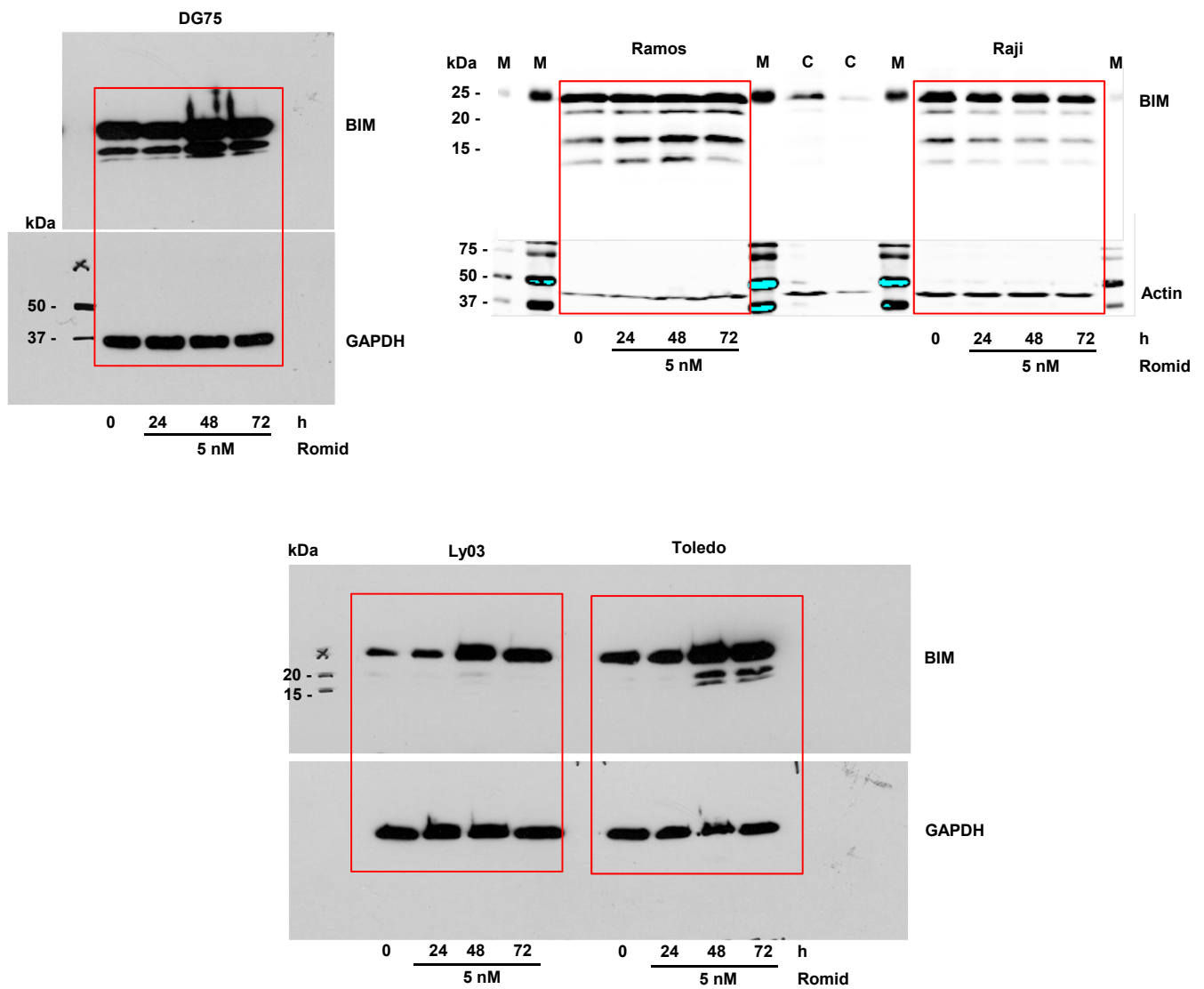
Supplementary Figure S6. Full-length blots corresponding to Figure 2b. (M, markers. C, positive control).

C

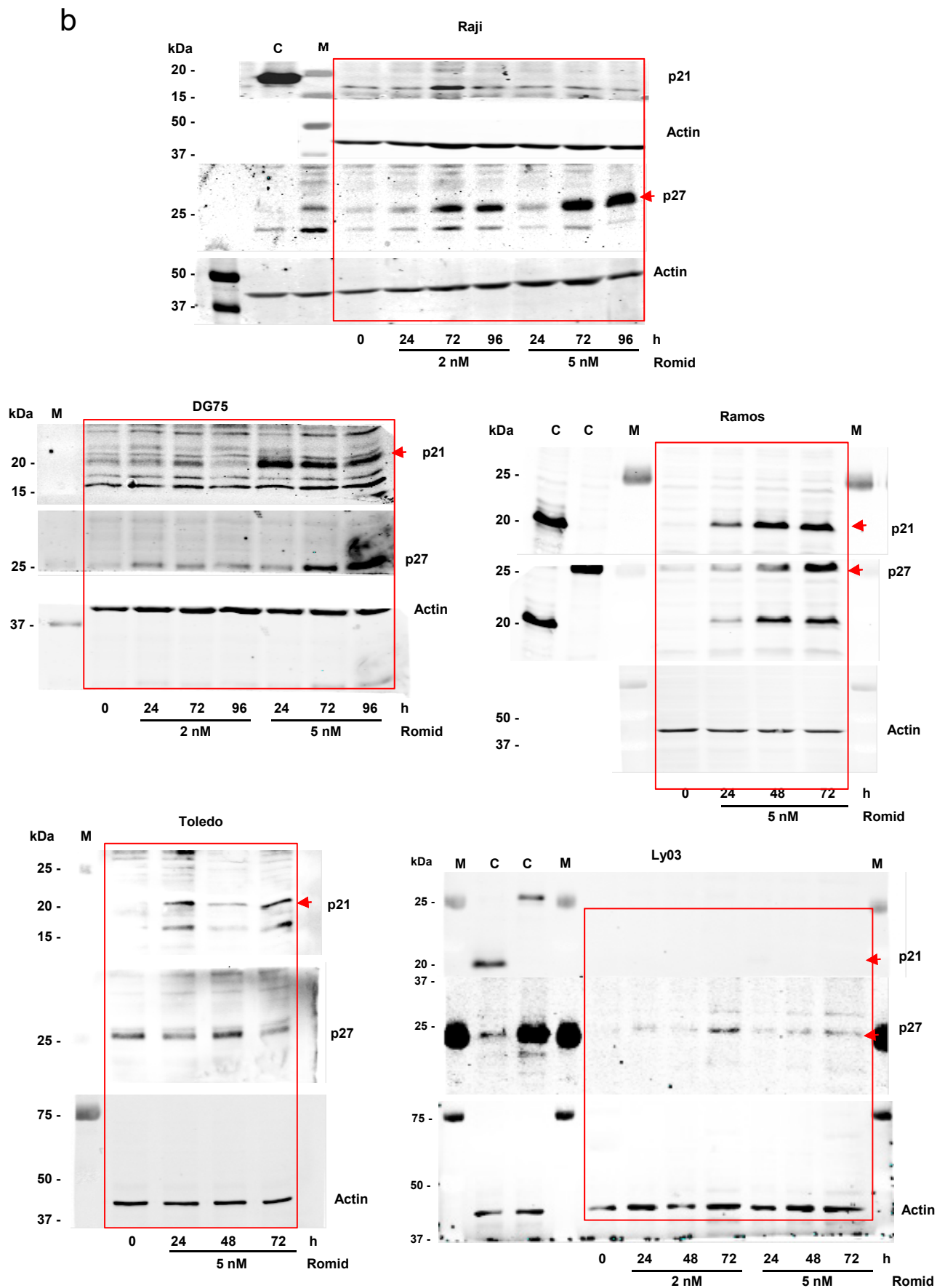


Supplementary Figure S7. Full-length blots corresponding to Figure 2c. (M, markers. C, positive control).

d

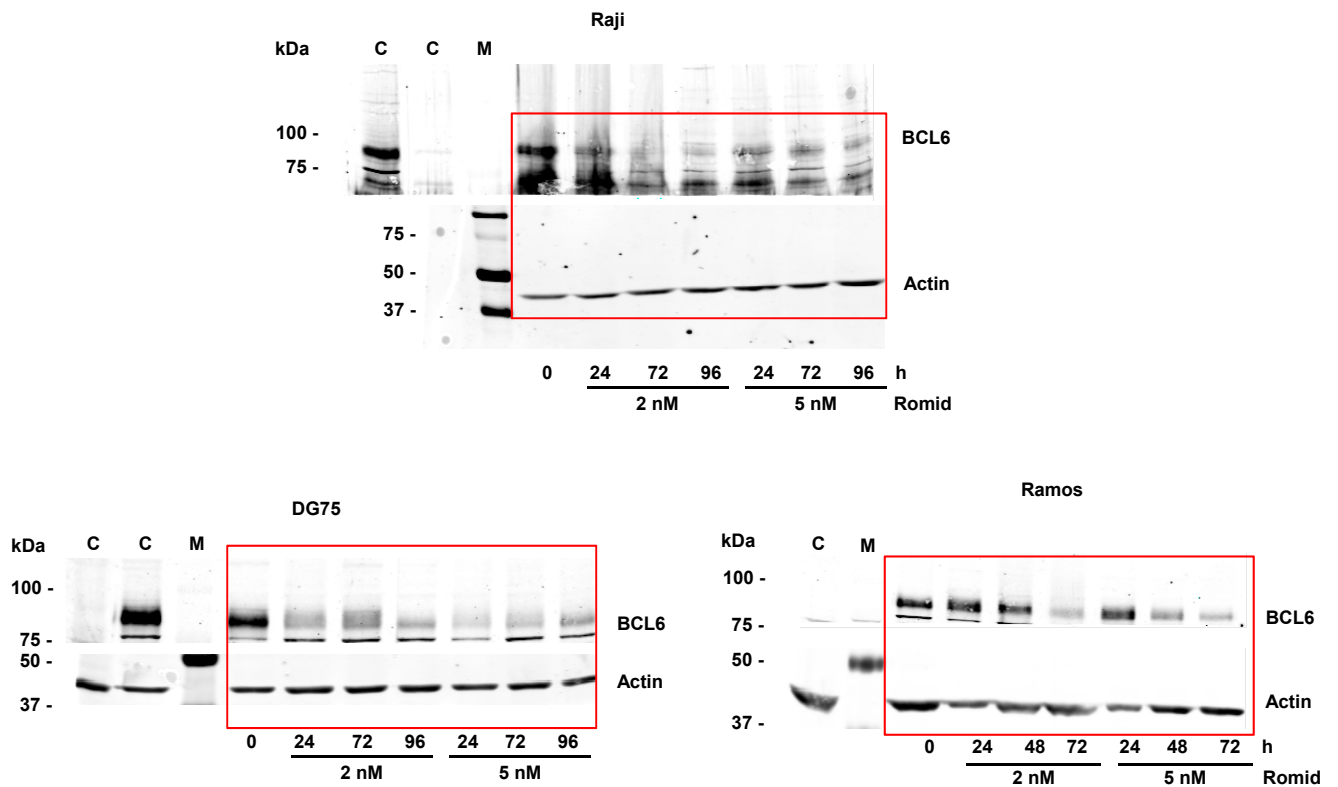


Supplementary Figure S8. Full-length blots corresponding to Figure 2d. (M, markers. C, positive control).

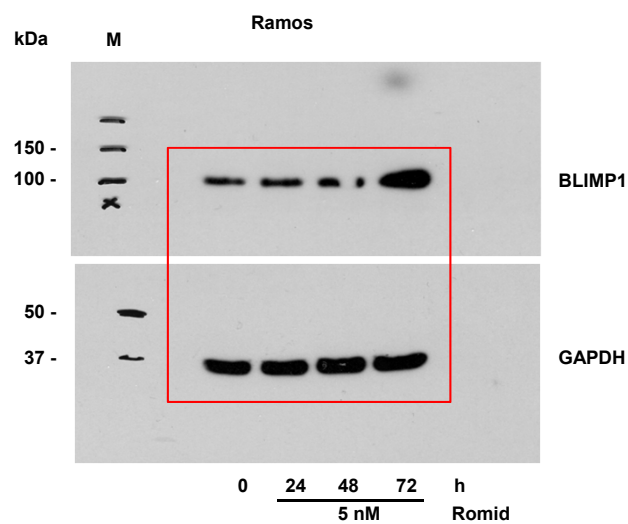


Supplementary Figure S9. Full-length blots corresponding to Figure 3. (M, markers. C, positive control)

a

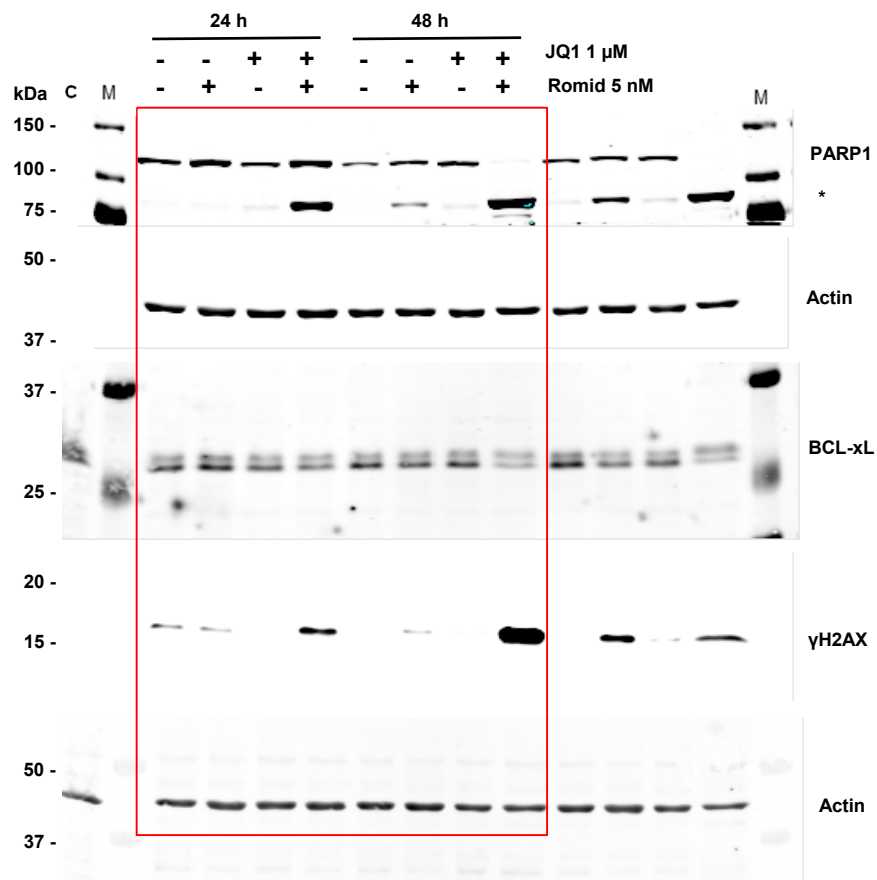


d



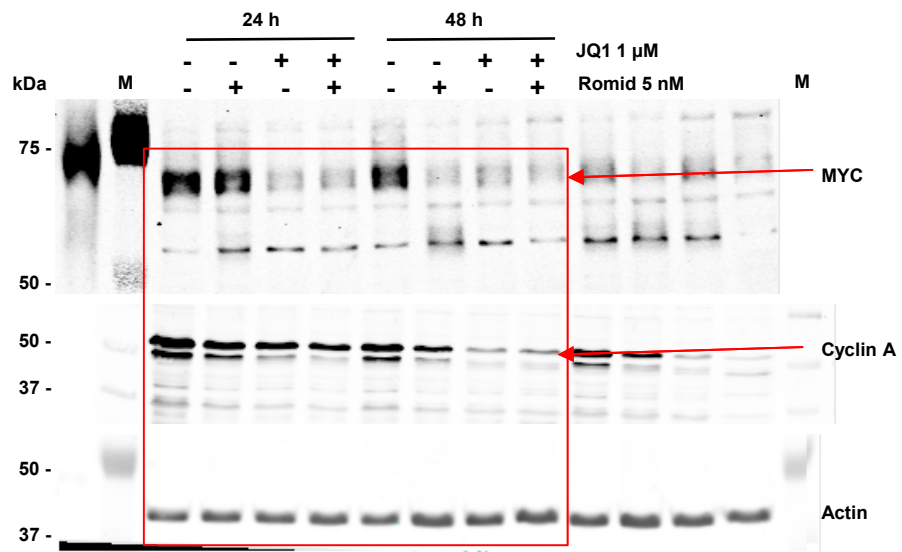
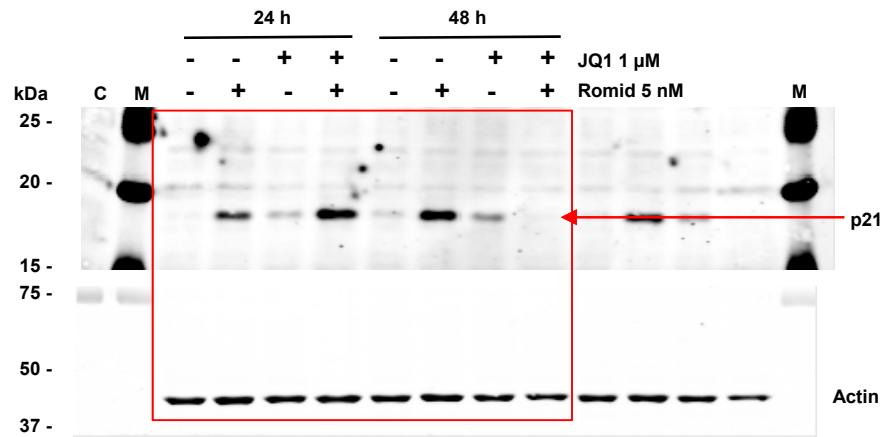
Supplementary Figure S10. Full-length blots corresponding to Figure 4. (M, markers. C, positive control).

d

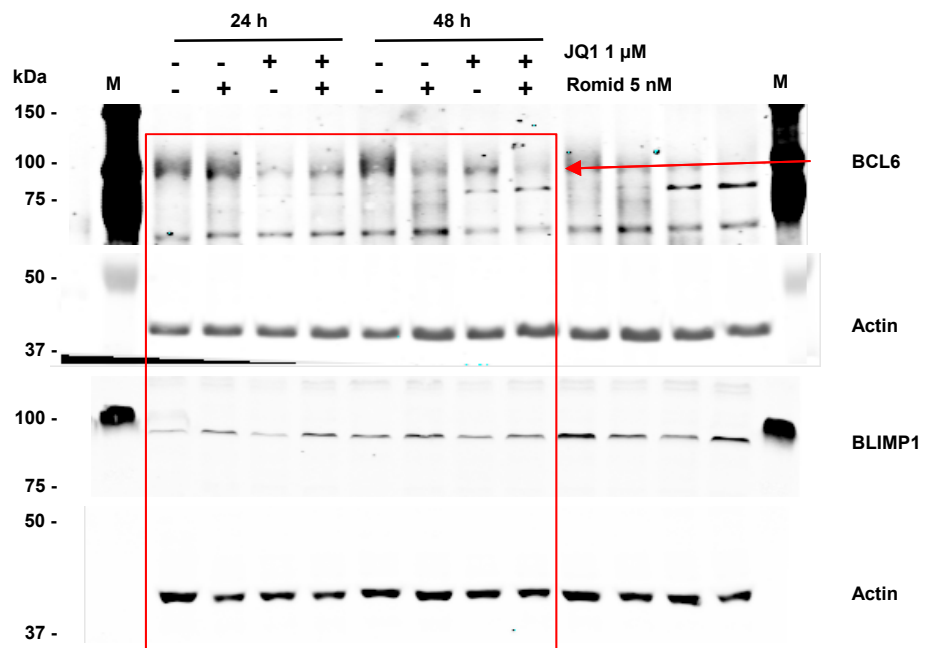


Supplementary Figure S12. Full-length blots corresponding to Figure 6d. (M, markers. C, positive control).

e

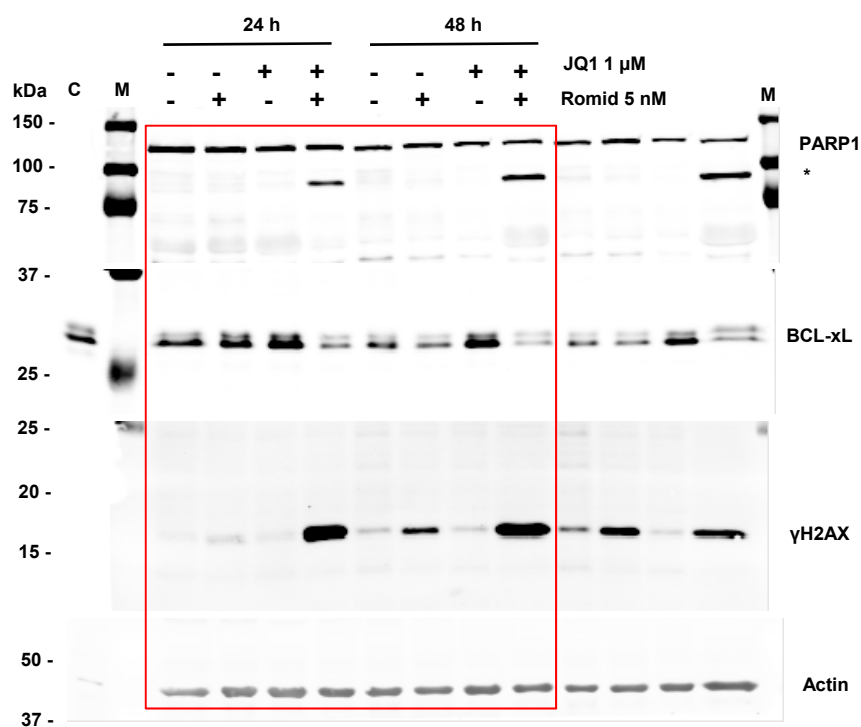


f

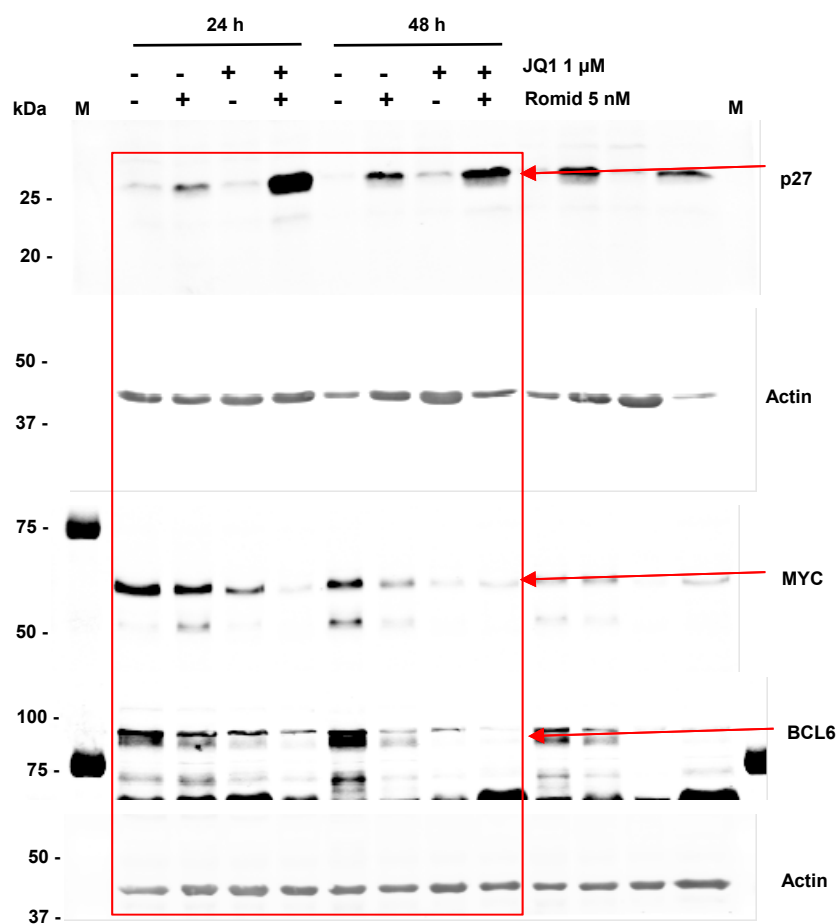


Supplementary Figure S13. Full-length blots corresponding to Figure 6e and f. (M, markers. C, positive control).

b

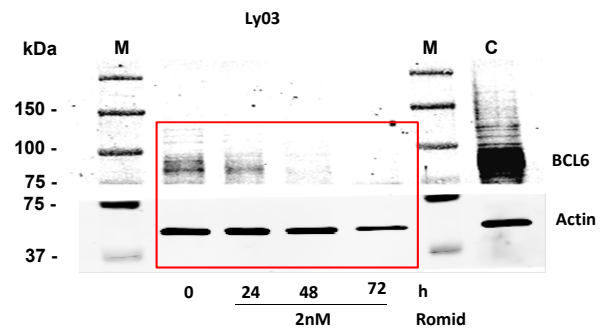


C



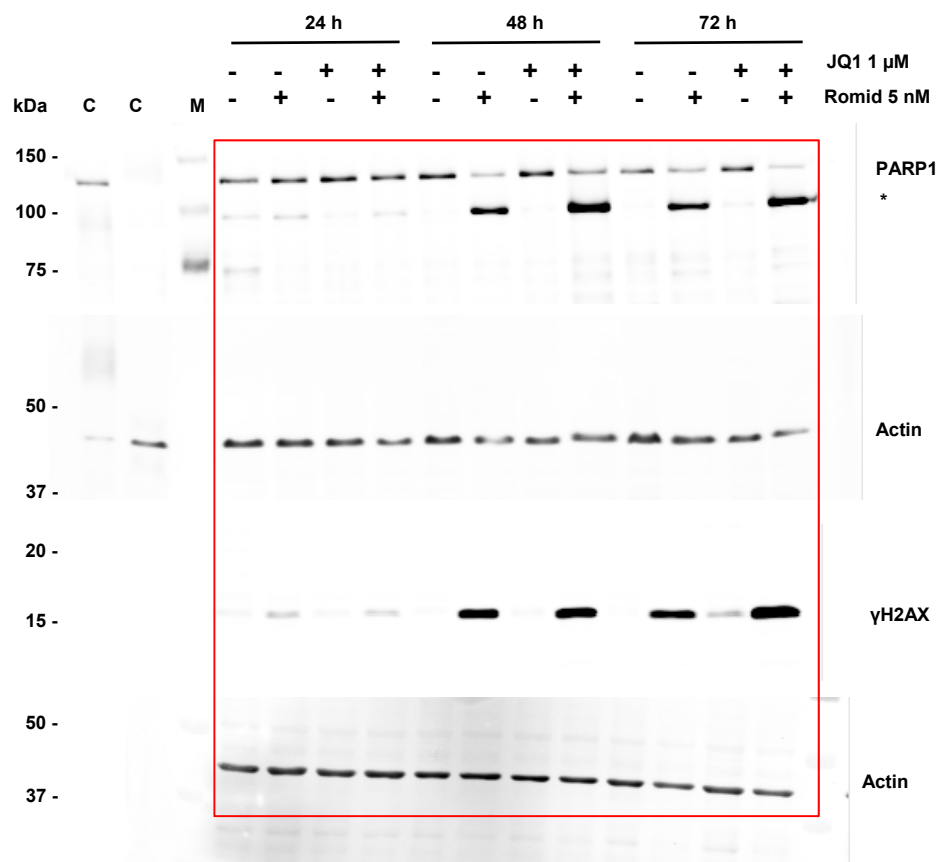
Supplementary Figure S14. Full-length blots corresponding to Figure 7 (M, markers. C, positive control).

a



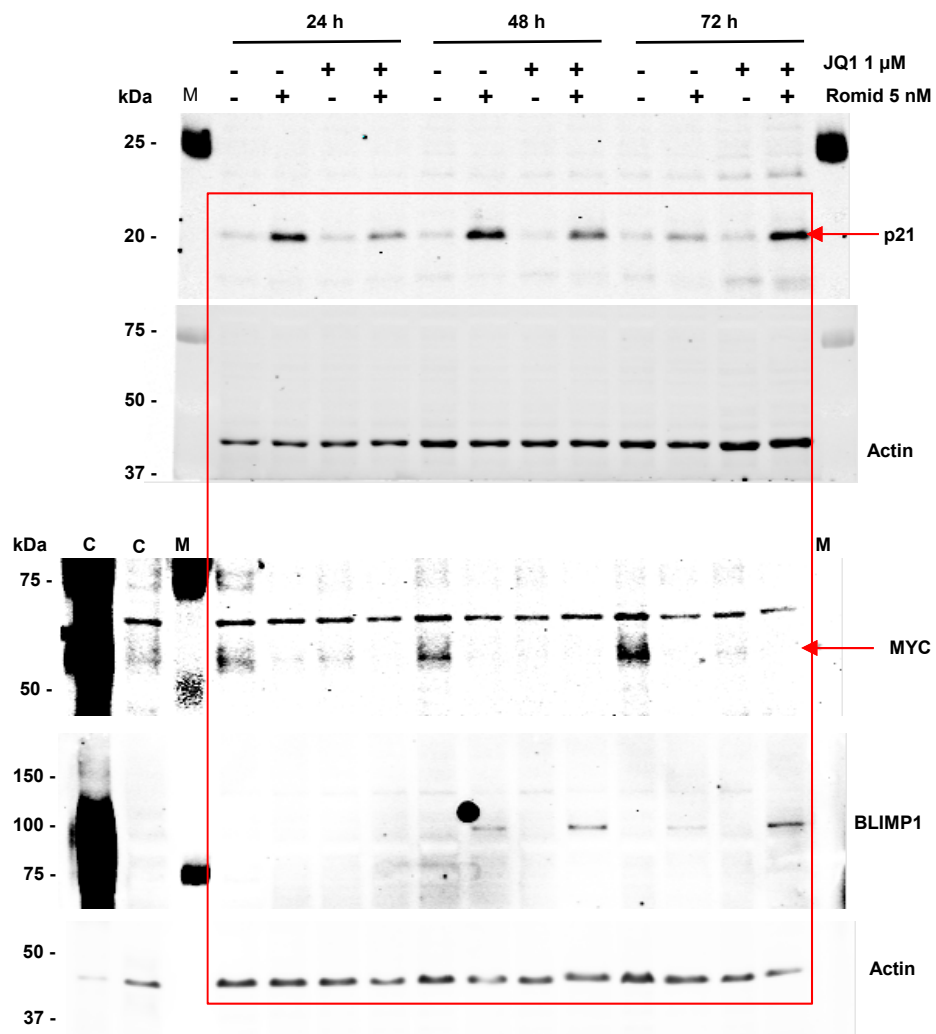
Supplementary Figure S15. Full-length blots corresponding to Figure S1a. (M, markers; C, positive control).

b



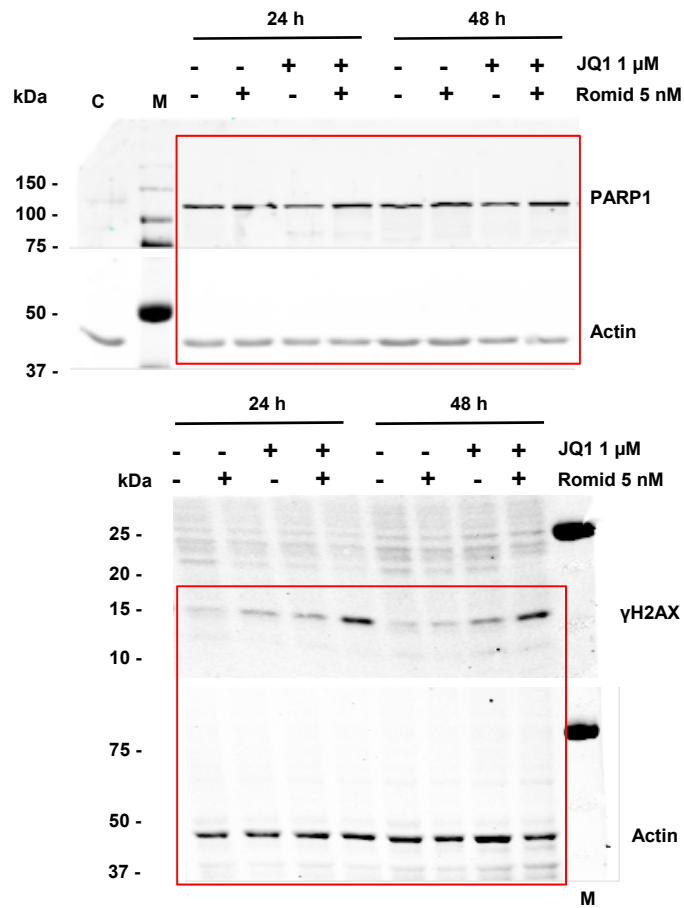
Supplementary Figure S16. Full-length blots corresponding to Figure S2b. (M, markers; C, positive control).

C

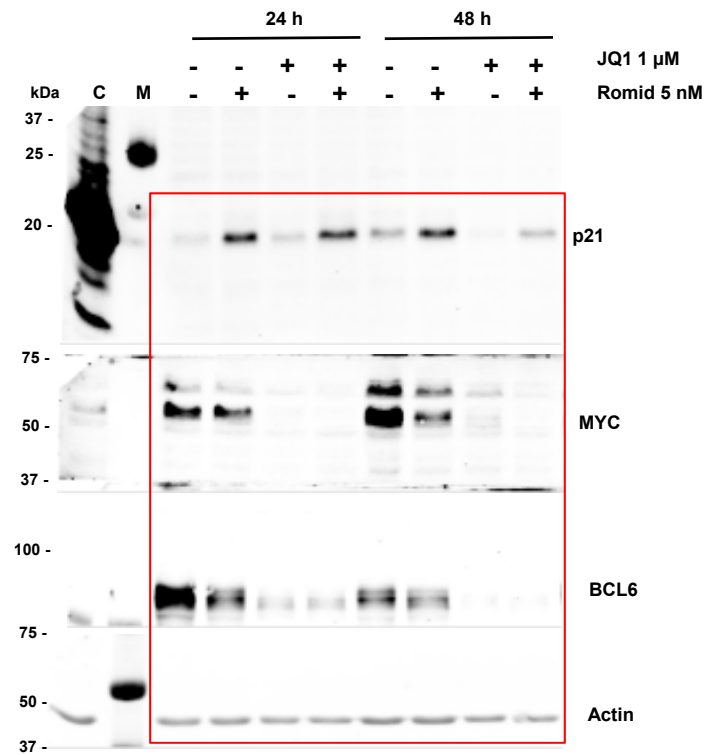


Supplementary Figure S17. Full-length blots corresponding to Figure S2c. (M, markers; C, positive control).

b



c



Supplementary Figure S18. Full-length blots corresponding to Figure S3. (M, markers; C, positive control).