MYC directly transactivates CR2/CD21, the receptor of the Epstein-Barr virus, enhancing the viral infection of Burkitt lymphoma cells

Ester Molina et al.

SUPPLEMENTARY TABLES AND FIGURES

Supplementary Table S1. Primers used in PCR**.** All correspond to human genes**.** The primers are written in the 5'→3' direction

Gene	**Forward**	**Reverse**	**Product Size (bp)**	**Use**
CR2	CCGACACGACTACCAACCTG	GACAATCCTGGAGCAATGGA	115	RT-qPCR
CR2 +325 bp	ATAAACCGCCTGGTCCTGAT	CCTCATGCAGTGGGTAGGTT	235	ChIP
CR2 +481 bp	AGGATTCTGCAGGTGCTCAT	TGCTTGGGAACCAGGTCTTA	196	ChIP
CR2 +141 bp	GGGTTTTCTTGGCTCTCGTC	ATTGCAGTGGTCCCTCAAAG	188	ChIP
CR2 -35 bp	GTGTGCGCTCAGAACTAGCA	CGACGAGAGCCAAGAAAACC	197	ChIP
CR2 -198 bp	AGTGTAGTGGGTTGCGTGGT	GCGGGCCCTTAAATAGTGTC	212	ChIP
CR2 -525 bp	CATGCAGAGAATCTGGGTGA	GTGCCATGCTGTGTTATGCT	246	ChIP
CR2 +2.1 Kb	GCATTCCTTGGAGAAACAGC	CAGTGGGAGCCCTCAAAATA	153	ChIP
CR2 +2.2 Kb	GGGTGCGGAAACAATGATAC	CAGTGGGAGCCCTCAAAATA	243	ChIP
CR2 Exon9	CAAGAAAGAGGCACCTGGAG	CAGCTTTGCAACGAATCTGA	199	ChIP
CR2 promoter	GCTTGTCCCACCCTCACC	GTTCCATCTTCCAGCGGATA	221	ChIP
EBNA1	GGTTCCAGCCAGAAATTTGA	CTCGCCTGAGGTTGTAAAGG	151	PCR
LDHA	TCCTGACTCAGGCTCATGGC	AGACAACCGACCGGCAGA	103	RT-qPCR
LMP1	CGCCTAGGTTTTGAGAGCAG	GATGAACACCACCACGATGA	154	PCR
MYC	TCGGATTCTCTGCTCTCCTC	CCTGCCTCTTTTCCACAGAA	157	RT-qPCR
CDKN1B/p27	CCGGCTAACTCTGAGGACAC	AGAAGAATCGTCGGTTGCAG	120	RT-qPCR
RPS-14	TATCACCGCCCTACACATCA	GGGGTGACATCCTCAATCC	135	RT-qPCR

Supplementary Table 2. Antibodies used in this work. IB, immunoblot; FC, flow cytometry.

Antigen	**Reference and specie**	**Concentration, Use**
MYC	N262, Sc-764, rabbit polyclonal Santa Cruz	1:1000 IB 1:40, FC
MYC	9402, , rabbit polyclonal Cell Signaling	1:3000, IB
MYC	D84C12, mouse monoclonal Cell Signaling	1:200, FC
CR2/CD21	C-20 Santa Cruz Biotech. sc-7025, goat polyclonal,	1:1000, IB
CR2/CD21	anti-CD21-APC ref 558658, Biolegend	1/100, FC
CD23	anti-CD23-PE ref 553139, Biolgened	1/100, FC
CD69	anti-CD69-bio-APC ref 553235) Biolegend	1/100, FC
B220	anti-B220-PE-Cy7, ref 103222 Biolegend	1:200 FC,
β-Actin	I-19, sc-1616, goat polyclonal, Santa Cruz Biotech.	1:1000, IB
β-Actin (C-4)	Sc-47778, mouse, Santa Cruz Biotech.	1:3000, IB
Cyclin A2 (H-432)	Sc-239, rabbit polyclonal, Santa Cruz Biotech.	1:1000, IB
PARP-1 (H-250)	Sc-7150, rabbit polyclonal, Santa Cruz Biotech.	1:1000, IB
CR2/CD21 FITC	Rat SD IgG2b,K, clon 7G6, BD Pharmingen	1:200, FC
CD23 biotin	Rat (LOU/M) IgG2a,K, clon B3B4, BD Pharmingen	1:200, FC
PE Streptavidin	BD Pharmingen	1:400, FC
CD19 APC eFluor780	Rat IgG2a,K, clon eBio1D3 (1D3), BD Pharmingen	1:100, FC

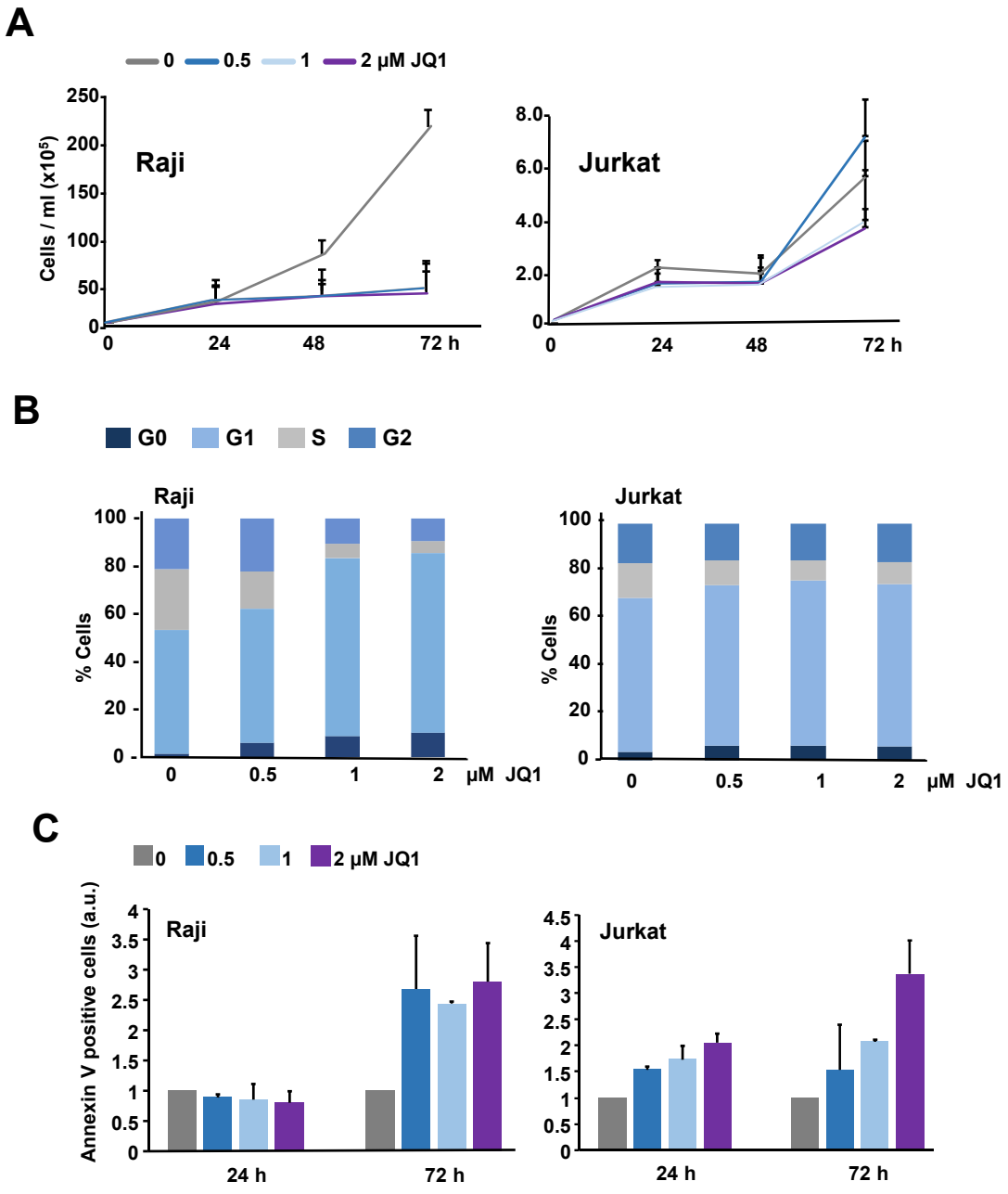
Supplementary Table 3. Plasmids used in this work

Plasmids	Construct	Origin
pmaxGFP	GFP gene	Amaxa
pGL3	*Firefly* sp. luciferase reporter gene with no promoter region	Promega
CR2-Luc	-315bp CR2 promoter in pGL3	[1]
Ebox1Mut-Luc	-315bp CR2 promoter with Ebox1 deletion in pGL3	[1]
Ebox2Mut-Luc	-315bp CR2 promoter with Ebox2 deletion in pGL3	[1]
4Ebox-Luc	*Firefly* sp. Luciferase reporter gene regulated by 4 E-box sequences	[2]
pRL-null	*Renilla* sp. luciferase reporter gene regulated by the T7 promoter	Promega
pCMV-VSV-G	VSV-G gene encoding enveloped lentiviral protein	Gift from Bob Weinberg, Addgene plasmid #8454 [3]
psPAX2	GAG and POL genes enconding packaging lentiviral proteins	Gift from Didier Trono, Addgene plasmid # 12260.
pLKO.1 control	Empty vector	Sigma- MISSION
pLKO.1 shMYC 1	Short hairpin RNA TRCN0000039640 against human MYC	Sigma- MISSION
pLKO.1 shMYC 2	Short hairpin RNA TRCN0000039642 against human MYC mRNA	Sigma- MISSION
pLKO.1 shCR2 1	Short hairpin RNA TRCN0000057113 against human CR2	Sigma- MISSION
pLKO.1 shCR2 2	Short hairpin RNA TRCN0000057114 against human CR2	Sigma- MISSION
pMX-cMyc-IRES-mOrange2	Retroviral vector carrying human MYC T58A ORF and mOrange2 ORF separated by an IRES sequence	This work
pMX-IRES-mOrange2	Retroviral vector carrying the mOrange2 gene and an IRES sequence	This work
pThy1-Brainbow3.2	Plasmid providing the mOrange2 ORF to generate pMX-cMyc-IRES-mOrange2	Gift from Joshua Sanes, Addgene plasmid # 45179 [4]
MSCV Myc T58A	Plasmid providing the MYC T58A ORF to generate pMX-cMyc-IRES-mOrange2	Gift from Scott Lowe, Addgene plasmid # 18773. [5],
Lv224-MYC	Lentiviral vector expressing MYC-IRES-Cherry-IRES-puromycin resistance gene	Genecopoiea
Lv224	Lentiviral vector expressing Cherry-IRES-puromycin resistance gene	Genecopoiea

References

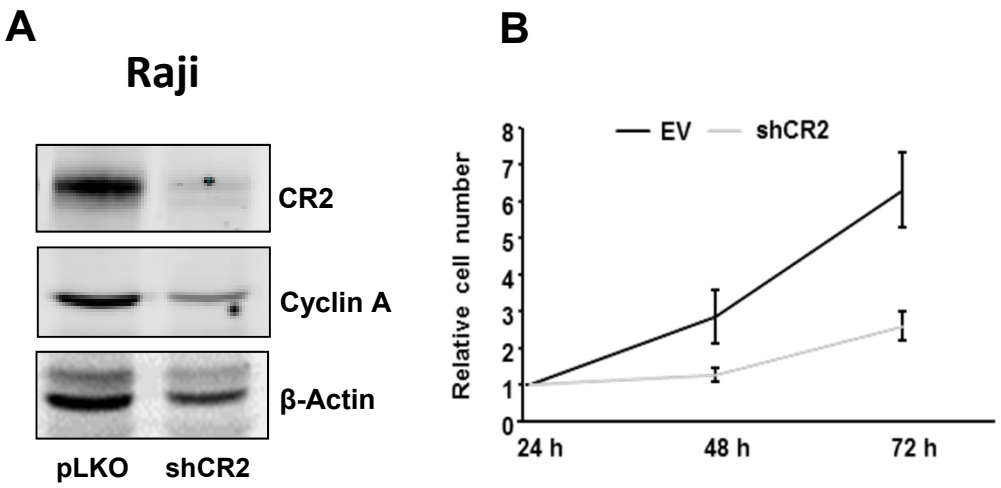
1. D. Ulgiati, C. Pham, V. M. Holers. Functional analysis of the human complement receptor 2 (CR2/CD21) promoter: characterization of basal transcriptional mechanisms. J Immunol. 2002; 168: 6279-6285
2. A. Kiessling, B. Sperl, A. Hollis, D. Eick, T. Berg. Selective inhibition of c-Myc/Max dimerization and DNA binding by small molecules. Chem Biol. 2006; 13: 745-751
3. S. A. Stewart *et al.* Lentivirus-delivered stable gene silencing by RNAi in primary cells. Rna. 2003; 9: 493-501
4. D. Cai, K. B. Cohen, T. Luo, J. W. Lichtman, J. R. Sanes. Improved tools for the Brainbow toolbox. Nat Methods. 2013; 10: 540-547
5. M. T. Hemann *et al.* Evasion of the p53 tumour surveillance network by tumour-derived MYC mutants. Nature. 2005; 436: 807-811

Supplementary Figure 1



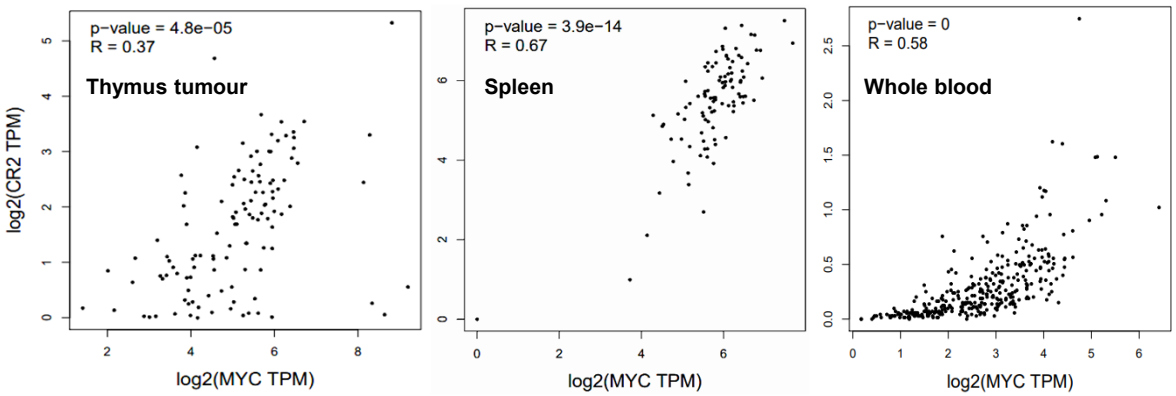
Supplementary Figure 1. Effect of JQ1 on proliferation and apoptosis of Raji and Jurkat cells. A. Proliferation of Raji and Jurkat cells treated with JQ1 at the indicated concentrations for up to 72 h, estimated by cell counting. Data represent mean values ±S.D. (n = 3). **B.** Cell cycle distribution of Ramos and Jurkat cells treated for 48 h with JQ1 at the indicated concentrations. Cells were stained wit iodide propidium and analyzed by flow cytometry. Data are mean values from two independent experiments ±SD. **C.** Apoptosis of Raji and Jurkat cells assayed by annexin V binding and measured by flow cytometry. Data represent means values ±S.D. (n=2). Graphs represent the fraction of positive cells normalized to the value of untreated cells.

Supplementary Figure 2



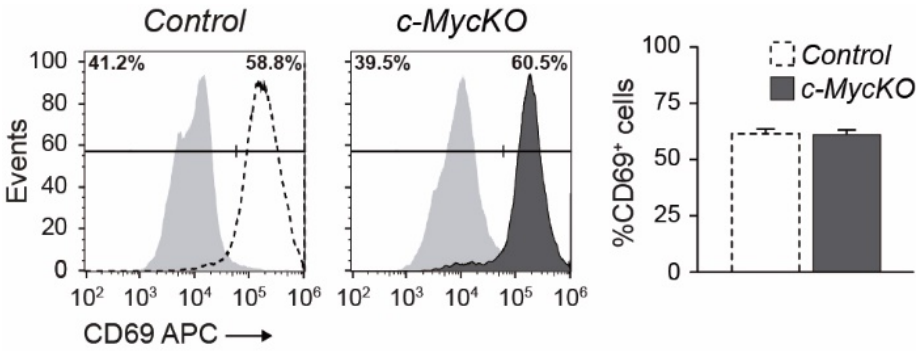
Supplementary Figure 2. Silencing of CR2 impairs Raji cell proliferation. **A.** Cells were infected with a mixture of two lentiviral vectors encoding for CR2 short-hairpin constructs as well as the pLKO empty vector (EV). After 36 h puromycin was added (1 μM final concentration) and the cells were further incubated for 72 h. Cells were then harvested and the indicated proteins analyzed by immunoblot. Expression of the indicated proteins 48 h after the addition of puromycin. **B**. Viable cell densities, as determined by trypan blue at the indicated time points

Supplementary Figure 3



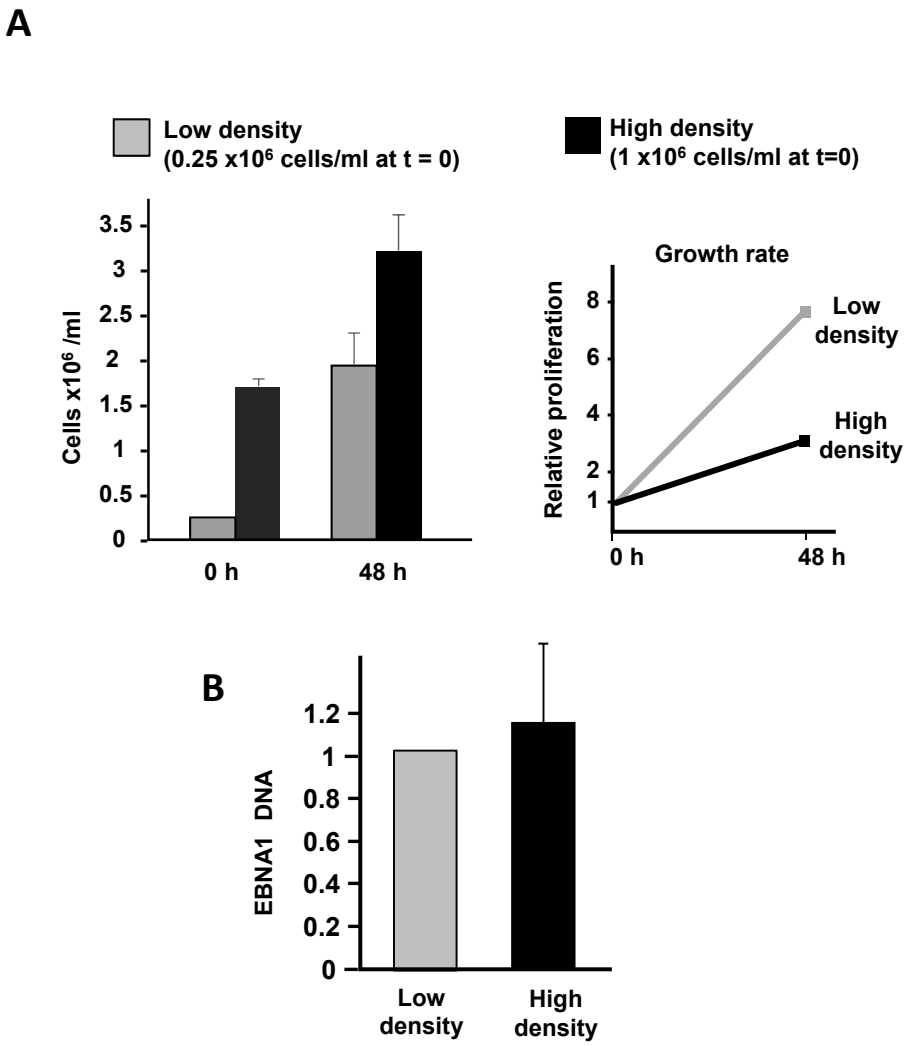
Supplementary Figure 3. Expression correlation between mRNA levels of MYC and CR2 in human thymus tumours (TCGA database), normal spleen (TCGA database) and spleen (GTEx database) according to the Gene Expression Profiling Interactive Analysis (http://gepia.cancer-pku.cn/) using the GTEx tissues database. R, Pearson correlation coefficient

Supplementary Figure 4



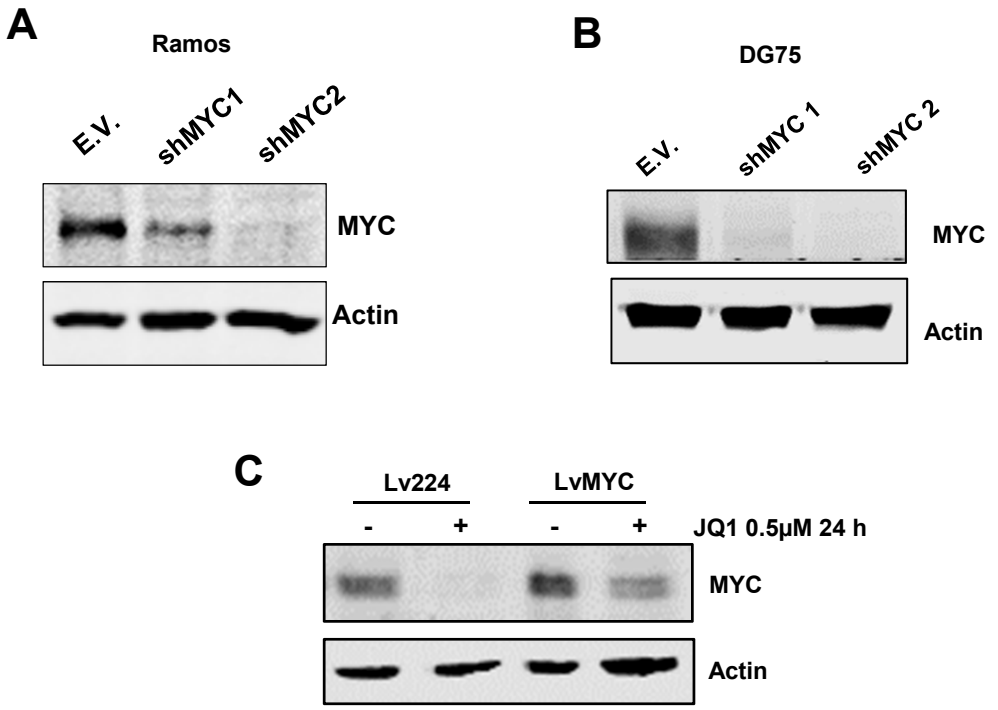
Supplementary Figure 4. CD69 staining of mouse splenic B cells. Mature B lymphocytes from the spleens of *Myc*$^{flox/flox}$;*Max*$^{flox/+}$;*cd19*$^{cre/+}$;*Rosa26*$^{gfp/gfp}$ (MycKO, *n*=3) homozygous and *Myc*$^{flox/+}$;*Max*$^{flox/+}$;*Cd19*$^{cre/+}$;*Rosa26*$^{gfp/gfp}$ heterozygous control mice (*n*=3) were activated with LPS and IL-4 for 48h. GFP^{+} cells (*Myc*-depleted B lymphocytes) were gated and surface expression of CD69 was analysed by flow cytometry. Absolute numbers of CD69^{hi} are shown, ±S.D. (n=3). ***p<0.001.

Supplementary Figure 5



Supplementary Figure 5. Ramos cells proliferation rate does not affect the infection by EBV. **A** Cell proliferation rate of the cells infected at high and low density cultures. Ramos cells were grown until a density of 0.5 x 10^5 cells/ml /low density) or 2 x 10^6 cells/ml (high density), infected with EBV (1:1 vol de B95-8 supernatant) and further incubated for 48 h. The cells were then harvested, washed and the total DNA extracted, The cell densities are shown at the left graph and the relative growth rate at the right graph (n = 5). **B**.The levels of viral genomic DNA were determined by PCR with primers of the EBNA1 gene. Data was normalized to the mean DNA levels of LDH and CR2 genomic DNA (n=5)

Supplementary Figure 6



Supplementary Figure 6. Silencing of MYC in BL cells. (**A**) Ramos cells where infected with a mixture of shMYC1 and shMYC2 lentivirus as described in Methods. The cells were harvested, lysed and the expression of MYC protein was analyzed by immunoblot (**B**) DG75 cells were infected by shMYC1 and shMYC2 lentivirus as described in Methods and the expression of MYC protein was analyzed by immunoblot. The expression of β-actin was analyzed as loading control. E.V:, empty vecpr (pLKO.1) (**C**) DG75 cells were infected with lentivirus expressing MYC (Lv224-MYC) and the empty vector, selected for 10 days with puromycin 0.75 μg/ml and treated with JQ1 (0.5 μM) for 24 h to suppress endogenous MYC expression. The expression of MYC and β-actin (as loading control) was analyzed by immunoblot.