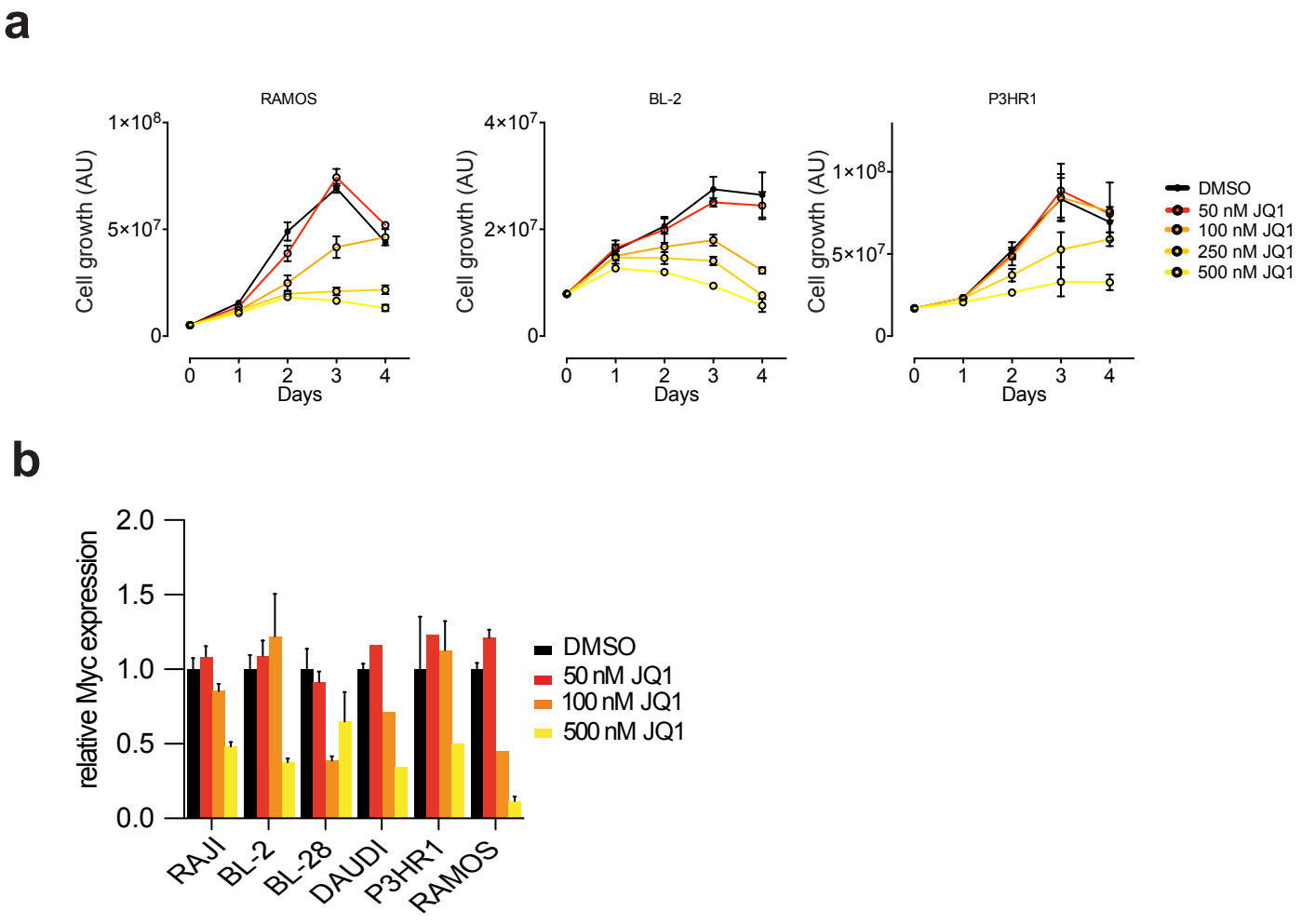
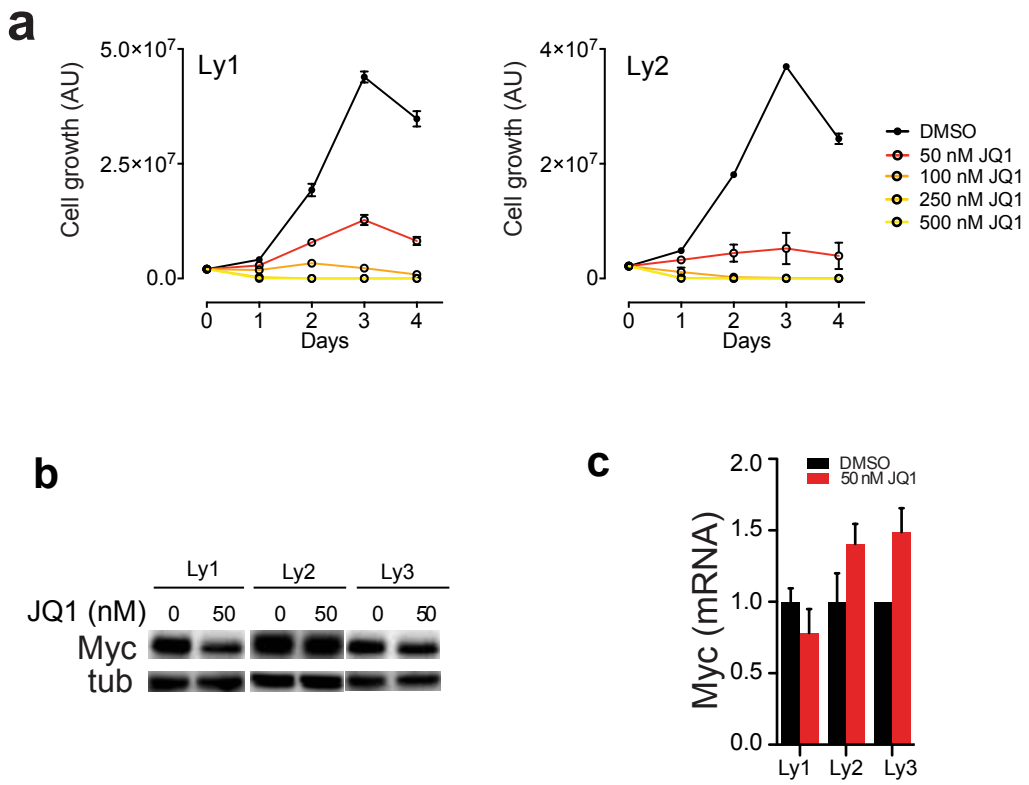
Supplementary Figure 1



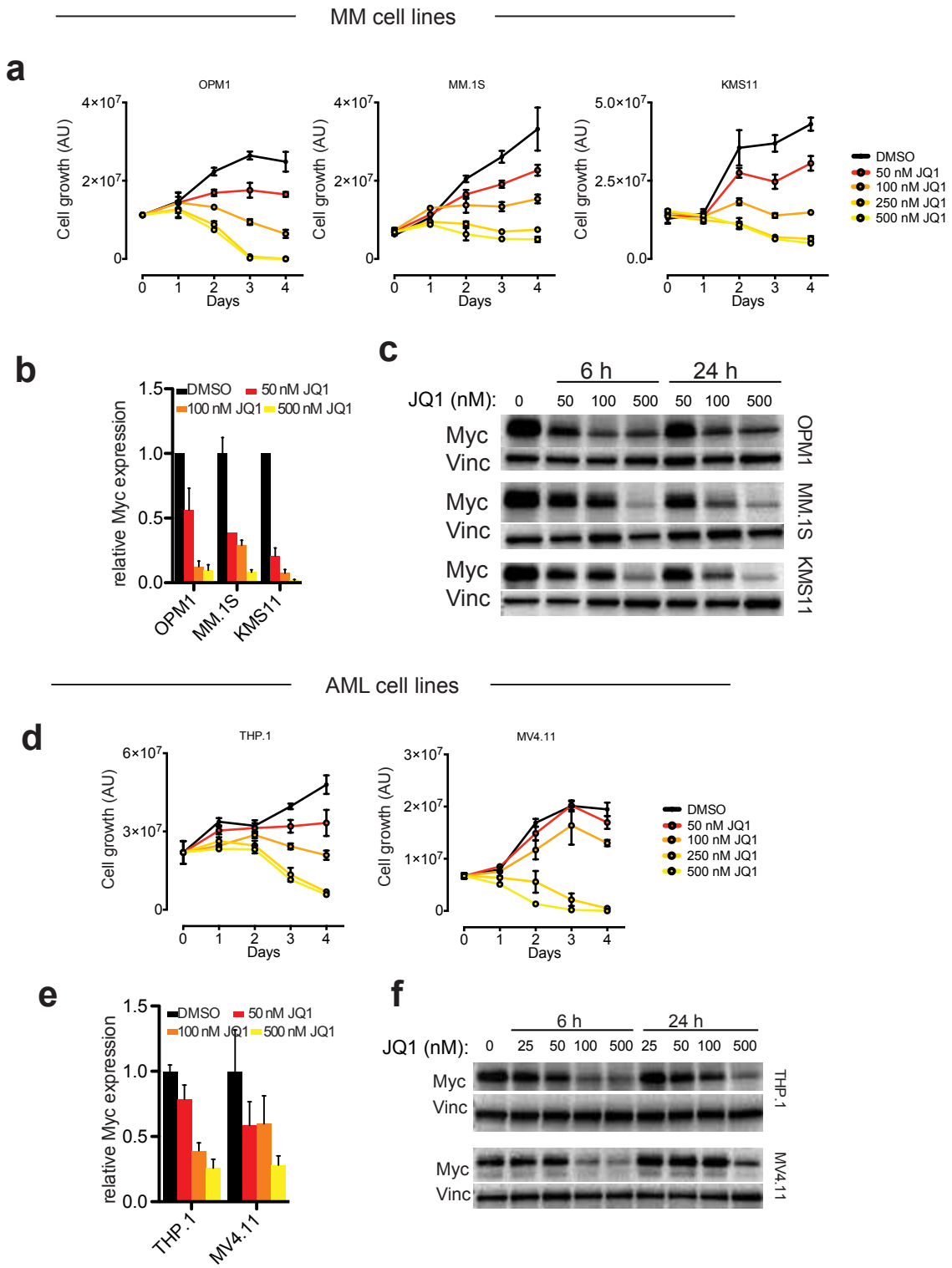
Supplementary Figure 1 | Antiproliferative effect of JQ1 in Burkitt's lymphoma cell lines
(**a**) Burkitt's lymphoma cell lines were grown in vitro in the presence of the indicated concentrations of JQ1 for up to 4 days. Growth was assessed by Cell titer glow. Each time point represents the average growth of three independent cultures. The error bar indicates the standard deviation.
(**b**) Myc mRNA levels were assessed by RTqPCR analysis. The expression values are reported as mean of technical triplicates, normalized to the RPLP0 gene and mock treated samples. The error bar is the standard deviation.

Supplementary Figure 2



Supplementary Figure 2 | Antiproliferative effect of JQ1 in E-myc lymphomas
(**a**)Cell growth assay. Primary lymphomas derived from Eμ-myc mice were grown in vitro in the presence of the indicated concentrations of JQ1 for up to 4 days. Growth was assessed by Cell titer glow. Each time point represents the average growth of three independent cultures. The error bar indicates the standard deviation.(**b**) Western blotting analysis of c-Myc levels in different Eμ-myc lymphomas. Tubulin (Tub) was used as a loading control.
(**c**) RT-qPCR analysis of c-Myc mRNA levels in Eμ-myc lymphomas treated with JQ1. The expression values are reported as mean of technical triplicates, normalized to the RPLP0 gene and mock treated cells.

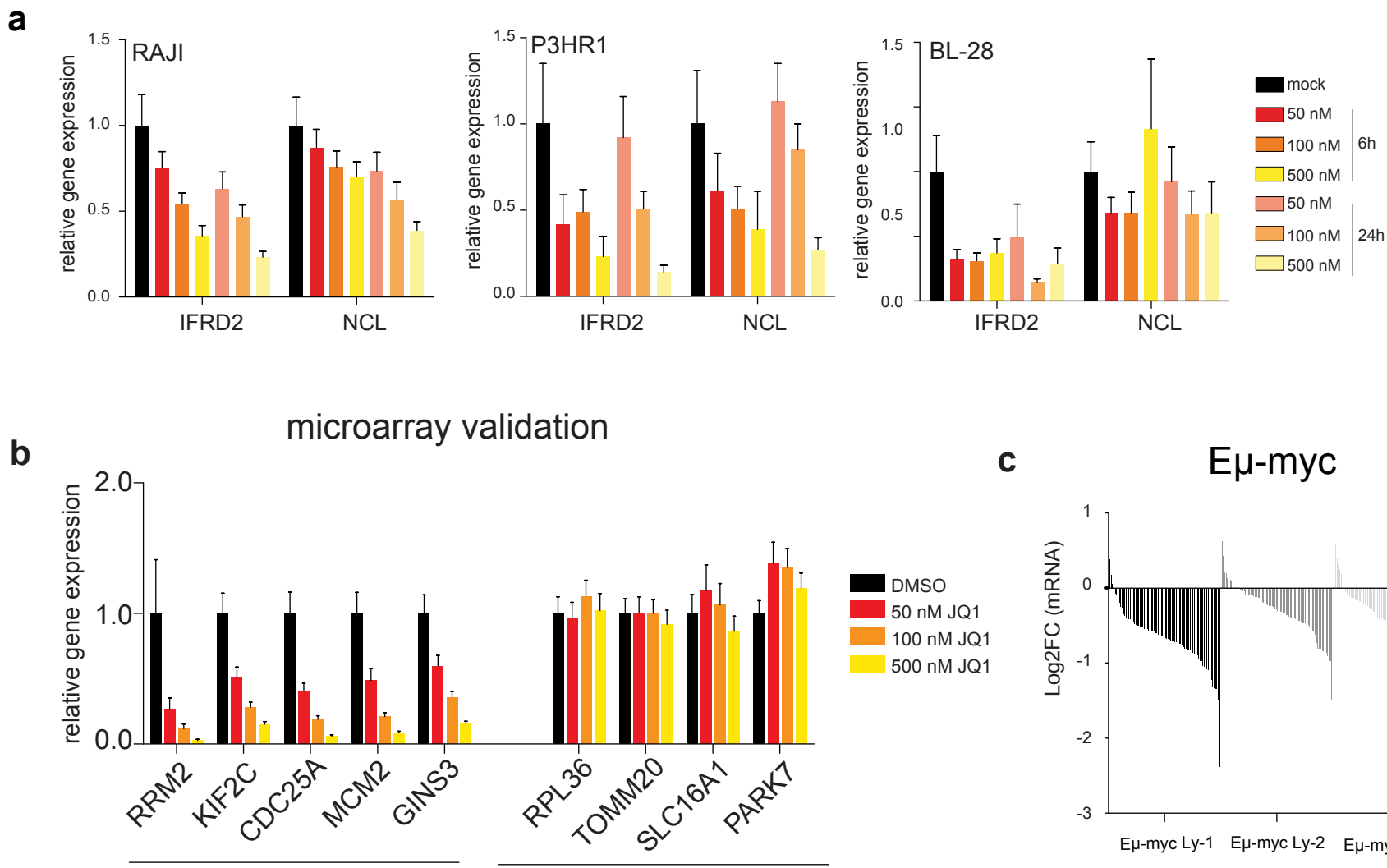
Supplementary Figure 3



Supplementary Figure 3 | Antiproliferative effect of JQ1 in Multiple Myeloma cell lines (MM) and Acute Myeloid Leukemia cell lines (AML)

(**a**) MM cell lines were grown in vitro in the presence of the indicated concentrations of JQ1 for up to 4 days. Growth was assessed by Cell titer glow. Each time point represents the average growth of three independent cultures. The error bar indicates the standard deviation. (**b**) Myc mRNA levels in MM cell lines were assessed by RTqPCR analysis. The expression values are reported as mean of technical triplicates, normalized to the RPLP0 gene and mock treated samples. The error bar is the standard deviation. (**c**) Western blotting analysis of c-Myc level assessed in different MM cell lines at 6 and 24 hours post JQ1 administration. Vinculin (vin) was used as a loading control. (**d**) AML cell lines were grown in vitro in the presence of the indicated concentrations of JQ1 for up to 4 days. Growth was assessed by Cell titer glow. Each time point represents the average growth of three independent cultures. The error bar indicates the standard deviation (**e**) Myc mRNA levels in AML cell lines were assessed by RTqPCR analysis. The expression values are reported as mean of technical triplicates, normalized to the RPLP0 gene and mock treated samples. The error bar is the standard deviation. (**f**) Western blotting analysis of c-Myc level assessed in different AML cell lines at 6 and 24 hours post JQ1 administration. Vinculin (vin) was used as a loading control.

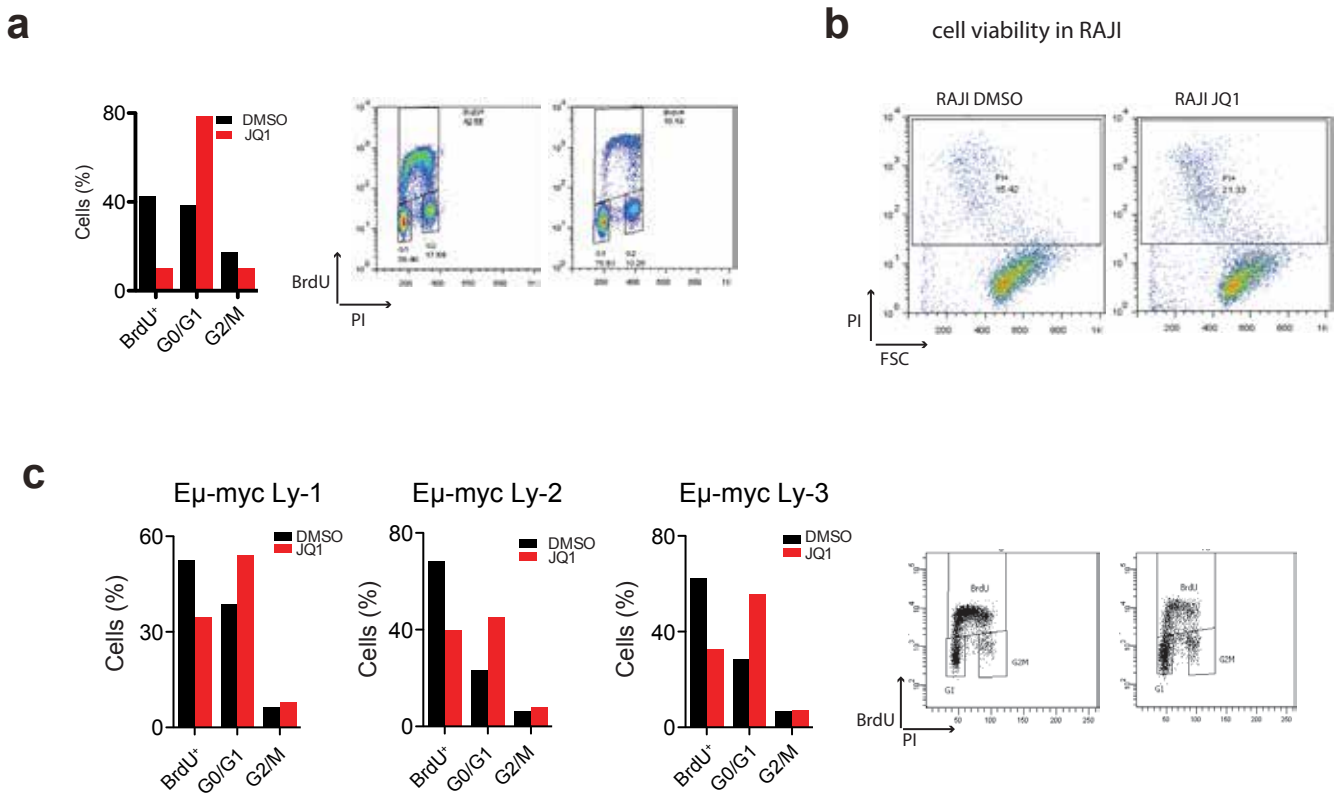
Supplementary Figure 4



Supplementary Figure 4 | Gene expression analyses of RAJI and Eμ-myc lymphomas

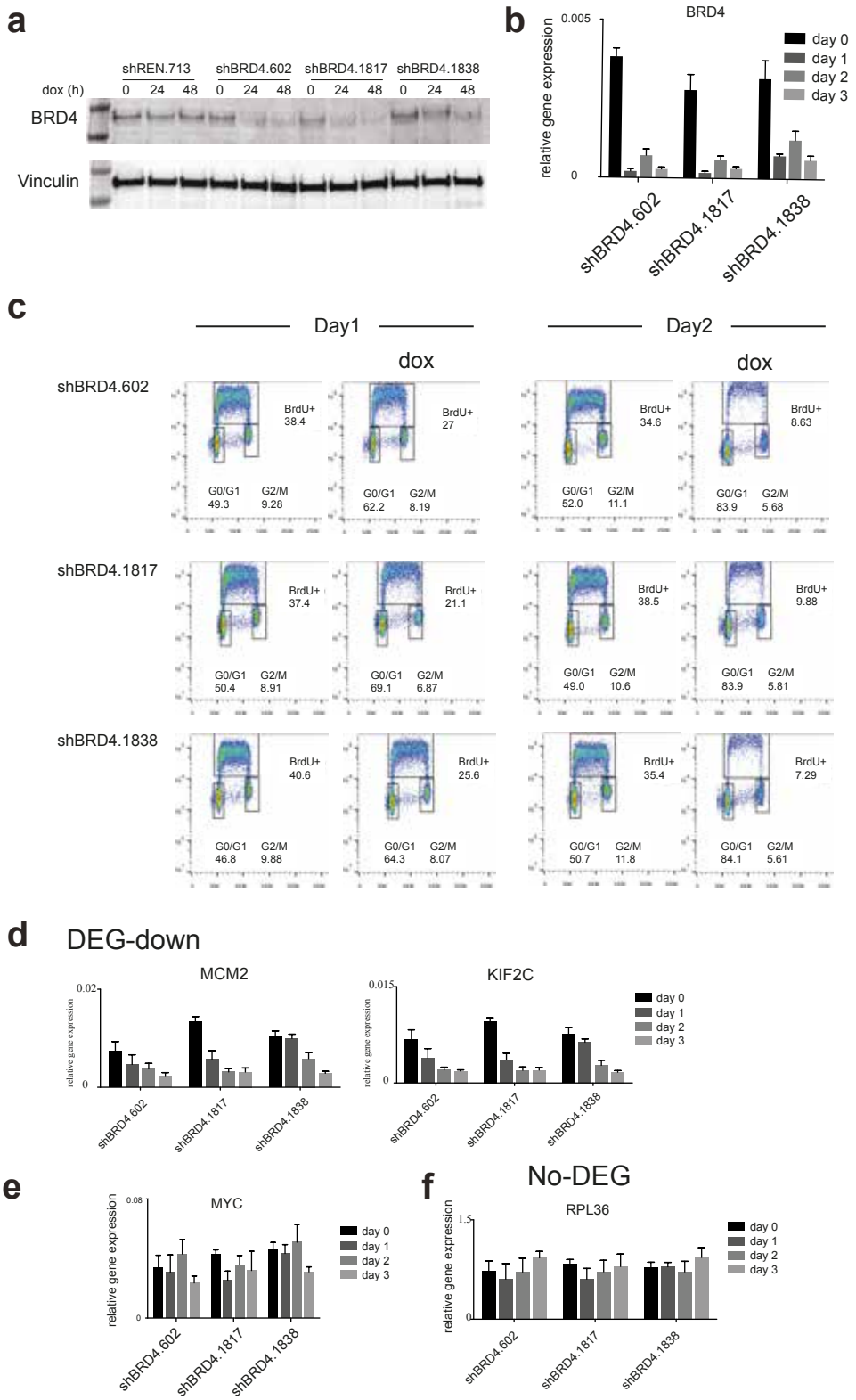
(**a**) Expression analysis by RT-qPCR of selected Myc target genes performed on representative BL cell lines at either 6 or 24 hours post JQ1 treatment. The expression values are reported as mean of technical triplicates, normalized to the RPLP0 gene and mock treated samples (**b**) Expression analysis by RT-qPCR of selected DEG-down and No-DEG genes performed in RAJI treated with JQ1 at the indicated concentration fro 24 hours. The expression values are reported as mean of technical triplicates, normalized to the RPLP0 gene and mock treated samples. (**c**) Expression of a subset of Myc target genes assessed by NanoString in three independent Eμ-Myc lymphomas treated with 50 nM JQ1 for 24 hours. The graph reports the Log2 fold change relative to mock treated cells (Log2FC) measured in three independent lymphomas. Genes were ranked descendingly, based on their Log2FC.

Supplementary Figure 5



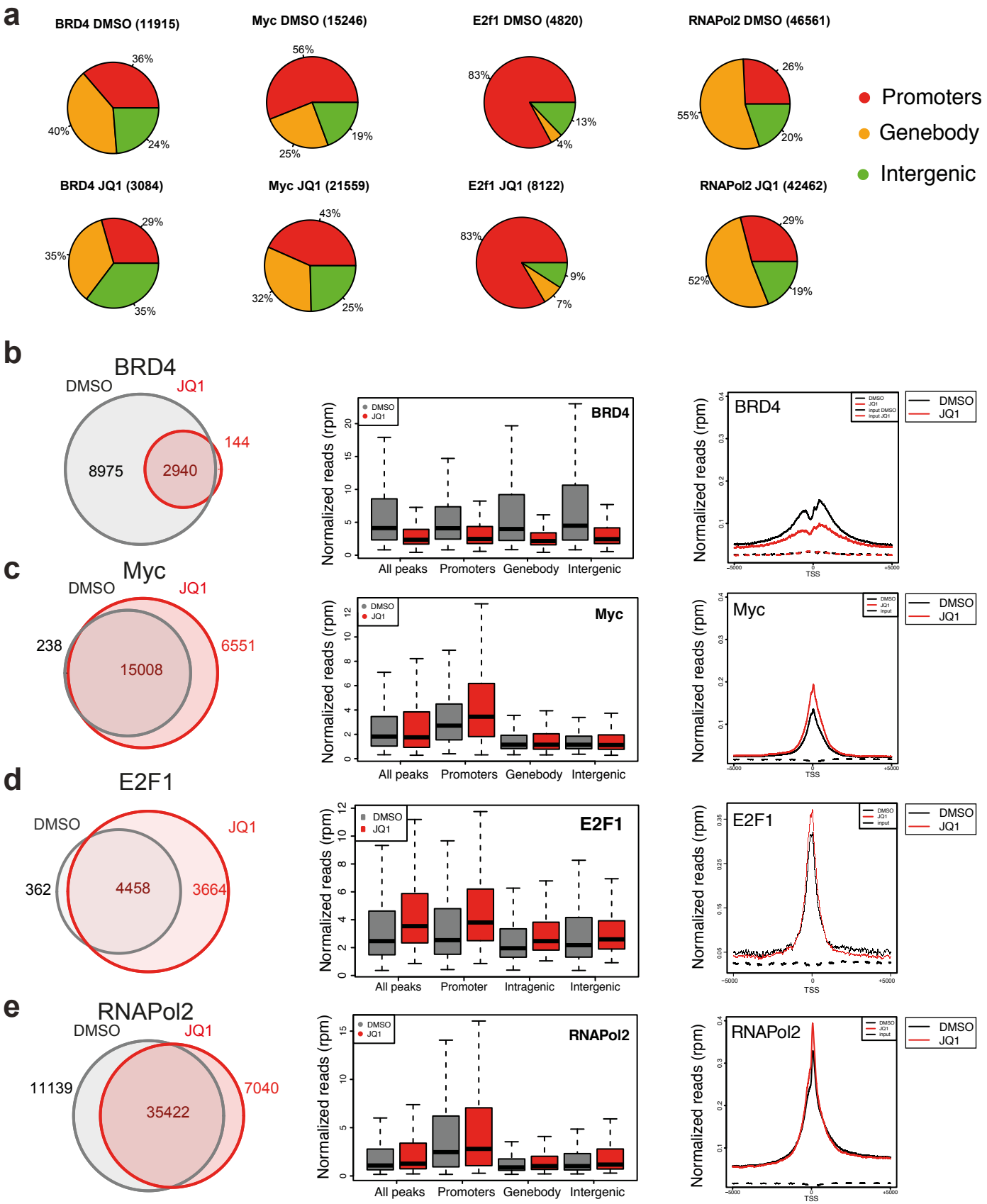
Supplementary Figure 5 | JQ1 is cytostatic (short-term)
Treatment of RAJI or E-myc lymphomas cells in vitro for short term is mainly cytostatic rather than cytotoxic. (**a**) Cell cycle distribution of RAJI cells grown in vitro for 24 hours in the presence of 100 nM JQ1, measured by FACS. Cultures were pulsed with BrdU 15 minutes before collection. The bar graph reports the percentage of cells in each phase of the cell cycle for either mock (DMSO) or JQ1 treated cells. Representative FACS profiles are shown on the right. (**b**) Viability of RAJI cells treated with 100 nM JQ1 for 24 hours as measured by propidium iodide incorporation (PI). Mock treated cells are indicated as DMSO. (**c**) Cell cycle distribution measured by FACS of three independent E-myc lymphomas grown in vitro for 24 hrs in the presence of 50 nM JQ1. Cultures were pulsed with BrdU 15 min before collection. The bar graphs report the percentage of cells in each phase of the cell cycle for either mock (DMSO) or JQ1 treated cells. A representative FACS profile is shown on the right.

Supplementary Figure 6



Supplementary Figure 6 | shRNA mediated Knock-down of BRD4 in RAJI mimics BET's inhibition
RAJI cells were infected with three independent conditional shRNAs targeting BRD4 or luciferase (shREN, control). ShRNA production was triggered by the addition of doxycycline (dox) to the cell cultures. (**a**) Western blotting analysis of BRD4 levels in cells transduced with the indicated ShRNAs and treated with doxycycline (dox) for the indicated times. Vinculin was used as a loading control. (**b,e**) RTqPCR analysis of BRD4 (**b**) and c-Myc (**e**) mRNA levels in cells transduced with the indicated shRNAs and treated with doxycycline (dox) for the indicated times. The expression values are reported as mean of technical triplicates, normalized to the RPLP0 gene and mock treated samples. The error bar is the standard deviation. (**c**) Cell cycle analysis of cells transduced with conditional shRNAs targeting BRD4. Cells were analysed by FACS 24 or 48 hours post-induction of the shRNAs by the addition of dox. Cells were pulsed with BrdU 15 min before collection. (**d,f**) Expression of selected gene identified in RAJI as either downregulated after JQ1 treatment (DEG-down, (**d**)) or not affected by the treatment (No-DEG, (**f**)) was assessed by RTqPCR at different time points post BRD4 silencing. The expression values reported are the mean value of technical triplicates, normalized to the RPLP0 gene and mock treated samples. The error bar is the standard deviation.

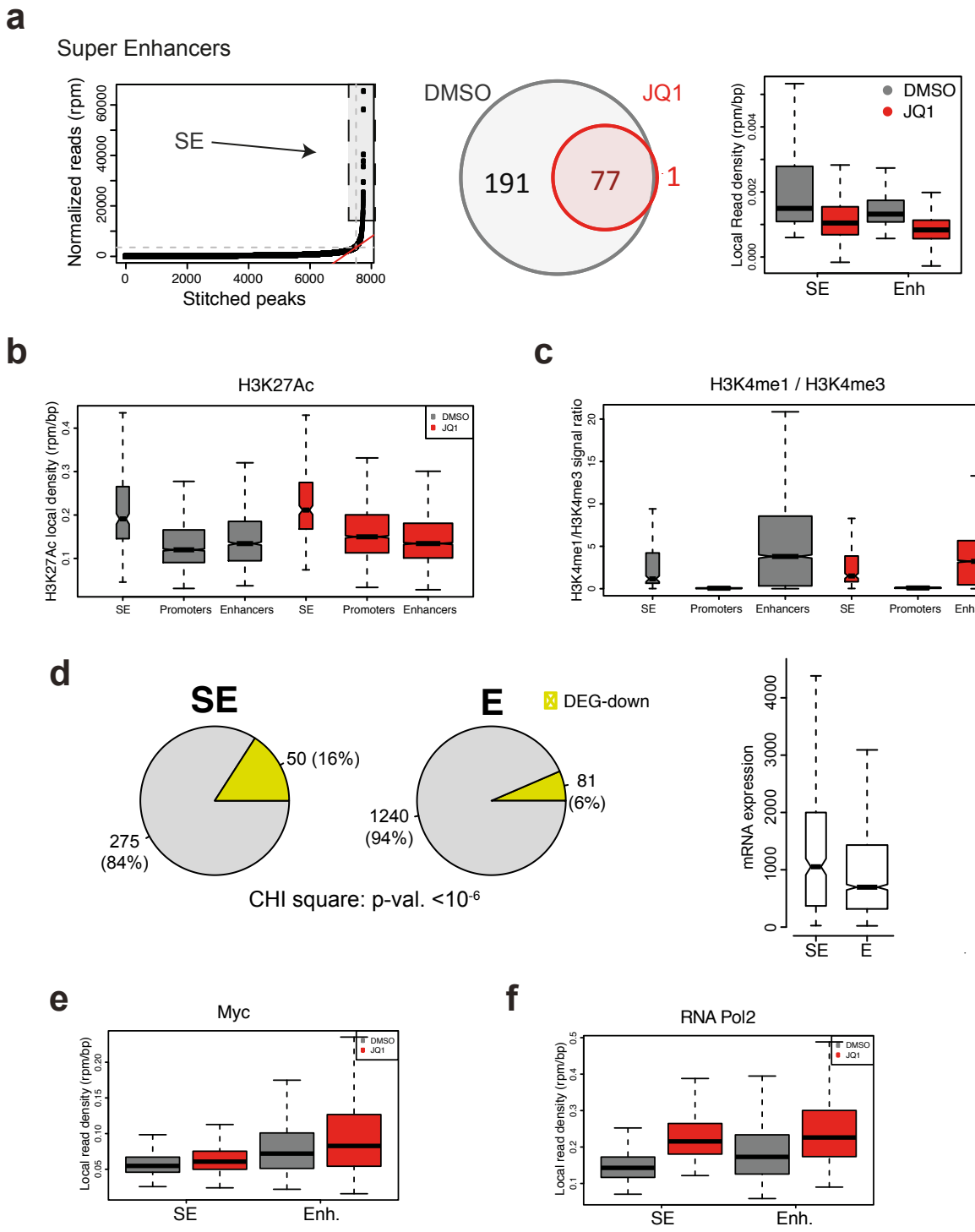
Supplementary Figure 7



Supplementary Figure 7 | ChIPseq analyses in RAJI

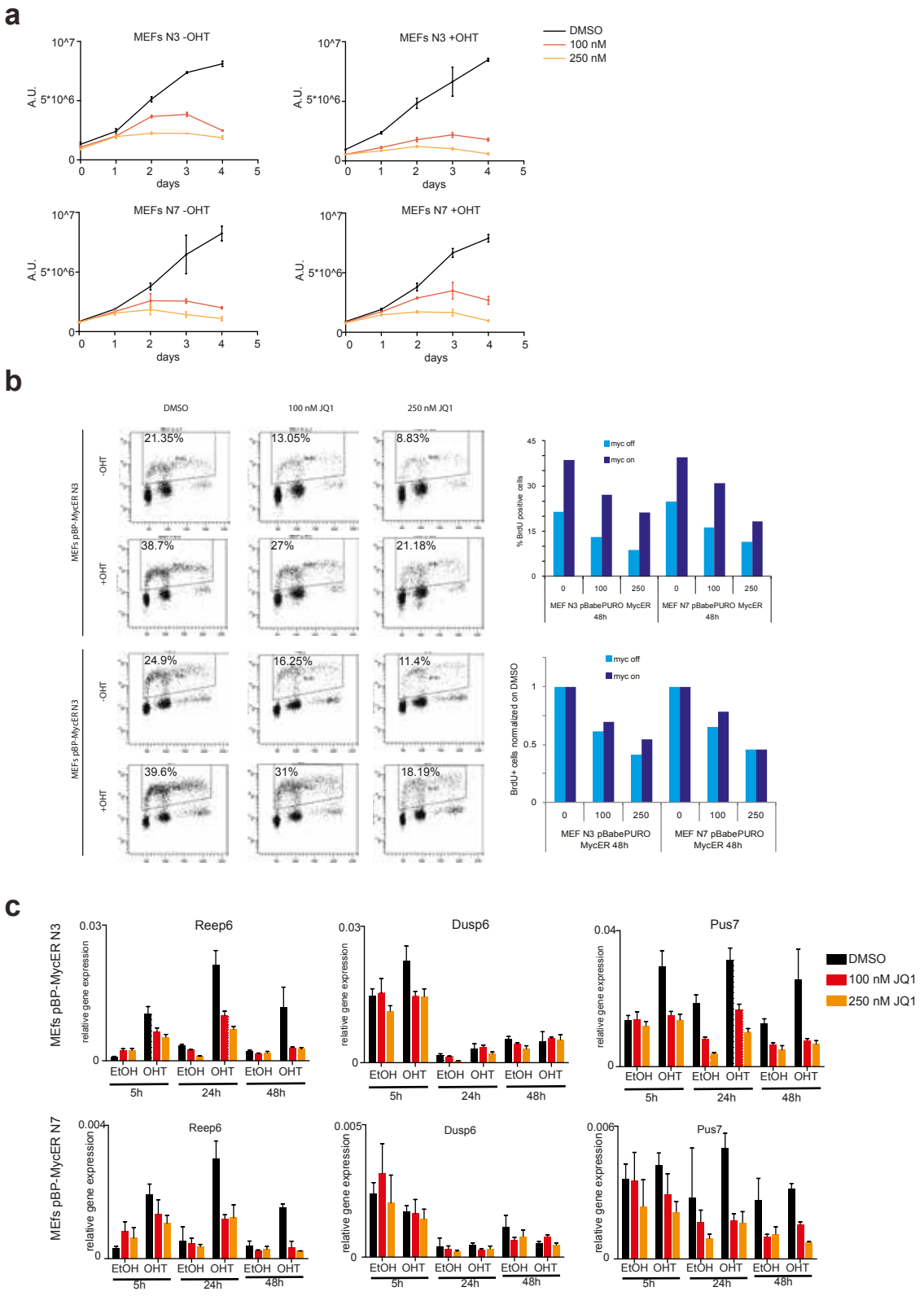
(**a**) Pie chart analysis of ChIPseq peak distribution in cells treated with either JQ1 or DMSO (mock treated). Peak numbers are reported within brackets. (**b**) ChIPseq of BRD4. Left, venn diagram reports the overlap of BRD4 peaks determined in either mock (black) or JQ1 (red) treated cells. Center, the box plot reports the enrichments as determined by reads count, for all the statistically significant peaks and their relative subsets based on their genomic annotation. Right, cumulative distribution of ChIPseq reads relative to the total peaks identified by ChIPseq. (**c**) ChIPseq of c-Myc, as in (**b**) (**d**) ChIPseq of E2F1, as in (**b**) (**e**) ChIPseq of RNAPol2, as in (**b**)

Supplementary Figure 8



Supplementary Figure 8 | Computational analyses of super-enhancers (SE) identified in RAJI cells
(**a**) Identification of Super-enhancers (SEs) based on the enrichment of BRD4 peaks as described in material and methods. The Venn diagram reports the number of BRD4 SEs identified in mock treated or JQ1 treated cells. The boxplot represent the relative enrichments of BRD4 peaks mapped on SEs (SE) compared to BRD4 peaks found in regular enhancers (Enh). (**b**) Box plot of the reads density of the H3K27Ac signals identified within SE, promoters and regular enhancers in either untreated RAJI a in cell grown with 100 nM JQ1 for 24 hours. (**c**) Box plot of the ratio of the H3K4 me1/H3K4me3 signals identified within SE, promoters and regular enhancers in either untreated RAJI or in cells grown with 100 nM JQ1 for 24 hours. (**d**) Pie chart showing all the genes found in the proximity (i.e. within 50Kb) of super-enhancers (SE) or regular enhancer (E). The fraction of genes that are down-regulated by JQ1 is indicated in red. Numbers indicate the number of genes associated to either super-enhancers or enhancers and their relative percentage (within brackets). The bar graph on the left shows how the expression of SE associated genes is on average higher than the expression of genes associated to regular enhancers. (**e,f**) Box plot of the enrichments of Myc (**e**) or RNAPol2 (**f**) peaks within SE or regular enhances (Enh.), determined in either untreated RAJI or in cells grown with 100 nM JQ1 for 24 hours. As observed for promoter associated peaks, the enrichment of RNAPol2 and Myc peaks contained in SEs was increased following BET's inhibition based on microarray analysis performed in RAJI cells.

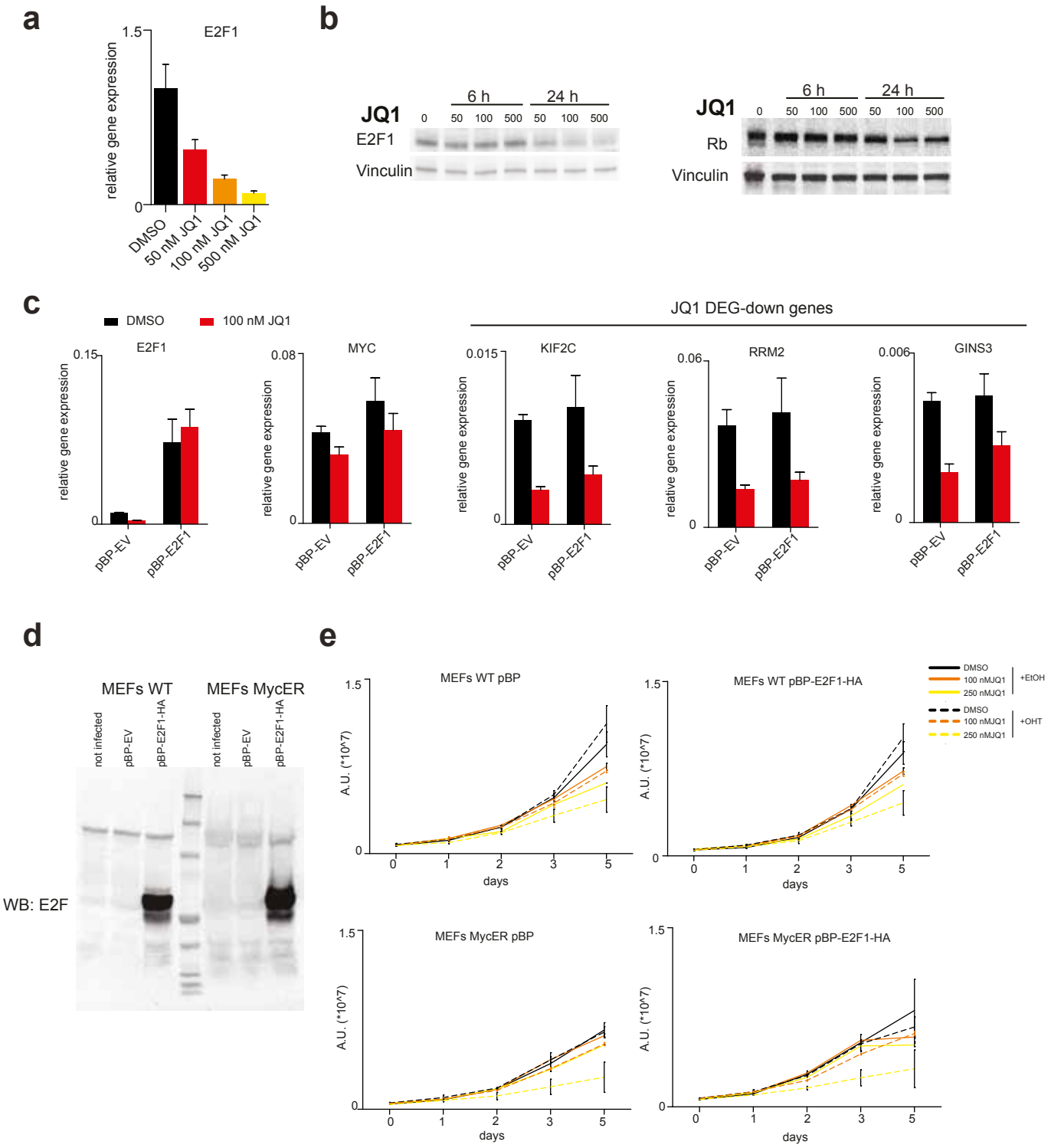
Supplementary Figure 9



Supplementary Figure 9 | Ectopic activation of Myc in MEF does not rescue transcription, cell cycle or the cell growth defect due to JQ1 treatment

(**a**) Primary MEFs transduced with the Myc-ER chimera (MycER-MEF) were grown in vitro in the presence of the indicated concentrations of JQ1 for up to 4 days. Ectopic Myc was activated by addition of OHT at day 0. Growth was assessed by Cell titer glow. Two independent MEFs culture were used. Each time point represents the average growth of three independent cultures. The error bar indicates the standard deviation. (**b**) Cell cycle analysis of MycER-MEF. Cells were analysed by FACS 24 hours post-Myc activation by OHT and grown in the presence of the indicated concentration of JQ1. Cells were pulsed with BrdU 15 min before collection. (**c**) The expression level of Myc target genes was assessed by RTqPCR in the indicated MycER-MEFs treated with JQ1. JQ1 and OHT were added to the culture at time 0. The expression values are reported as mean of technical triplicates, normalized to the RPLP0 gene and mock treated samples. The error bar is the standard deviation.

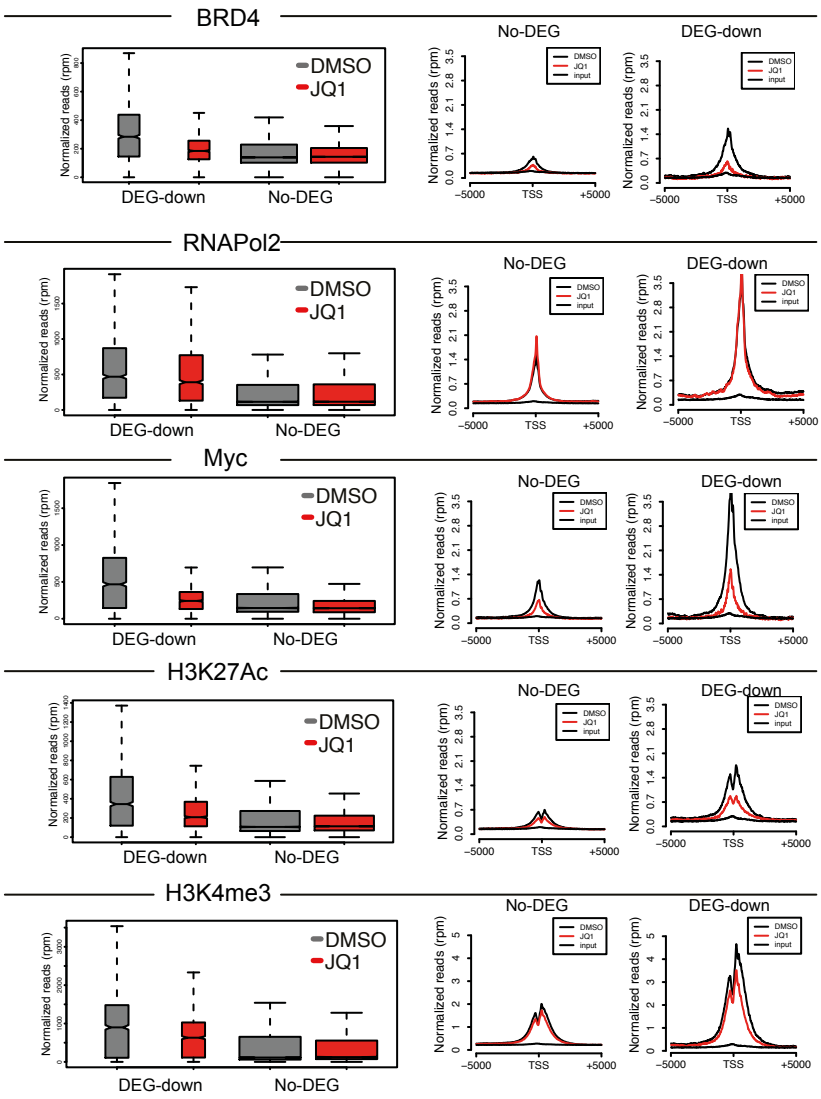
Supplementary Figure 10



Supplementary Figure 10 | Ectopic over-expression of E2F1 and Myc in RAJI or MEFs fails to rescue transcription and cell growth defect due to JQ1 treatments

(**a**) E2F1 mRNA levels in RAJI were assessed by RTqPCR analysis. The expression values are reported as mean values of technical triplicates, normalized to the RPLP0 (**b**) Western blotting analysis of E2F1 and the retinoblastoma protein (pRb) levels in RAJI cells treated with the indicated concentration of JQ1 for either 6 or 24 hours. Vinculin was used as a loading control. (**c**) RAJI cells transduced with either HA-tagged E2F1 (pBP-E2F1) or empty vector (pBP-EV) were trated with 100 nM JQ1 for 24 hours. Expression level of the indicated genes was assessed by RTqPCR as in (**a**). (**d**) Western blotting analysis of E2F1 levels in either MEFs or MycER-MEFs transduced with either HA-tagged E2F1 (pBP-E2F1) or empty vector (pBP-EV). (**e**) wild-type and MycER-MEFs were transduced with either HA-tagged E2F1 (pBP-E2F1) or empty vector (pBP-EV). Cells were grown in vitro in the presence of the indicated concentrations of JQ1 for up to 5 days. Ectopic Myc was activated by addition of OHT at day 0. Growth was assessed by Cell titer glow. Two independent MEFs culture were used. Each time point represents the average growth of three independent cultures. The error bar indicates the standard deviation.

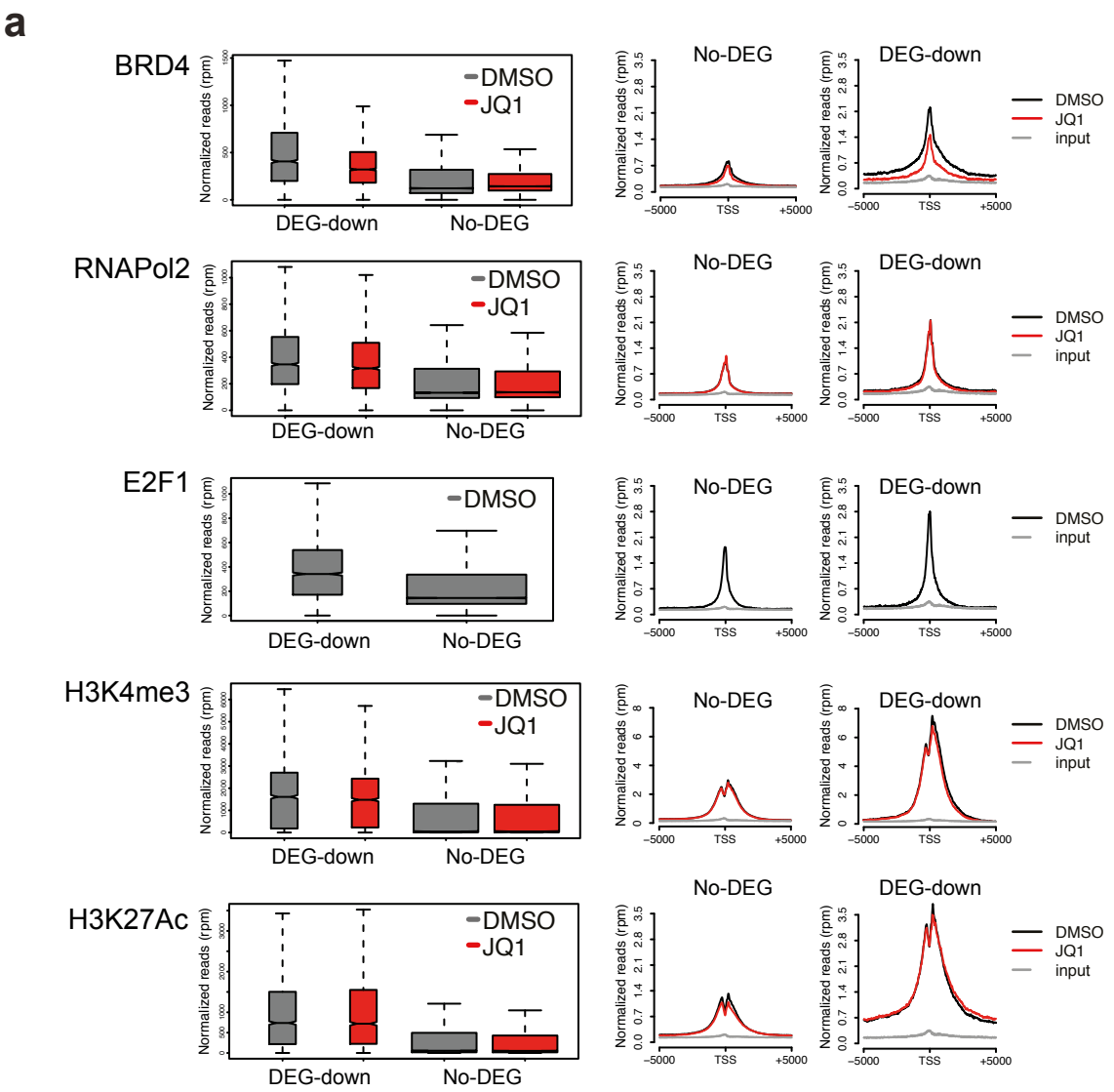
Supplementary Figure 11



Supplementary Figure 11 | Computational analysis of ChIPseq and genome wide expression data sets relative to MM.1S treated with JQ1

Datasets relative to MM.1S treated with JQ1 were retrieved from GEO (GSE31365 (expression data), GSE42355 (ChIPseq data relative to BRD4, RNAPol2, Cdk9 and MED1), GSE42161 (Myc ChIPseq), GSE43743 (RNAPol2 ChIPseq in CDK9i treated cells)) and analysed as described in the Methods section. (**a**) Left, box-plot of the peak signal intensity. Right panel, cumulative distribution plot of ChIPseq peaks. ChIPseq peaks were subsetted based on their association with promoters of either genes down-regulated by JQ1 (DEG-down) or not affected by JQ1 (No-DEG). (**b**) Digital heat-maps showing the signals of the indicated transcription factors, RNAPol2 and chromatin marks. ChIPseq peaks were subsetted in DEG-down or No-DEG based on their overlap with expressed genes.

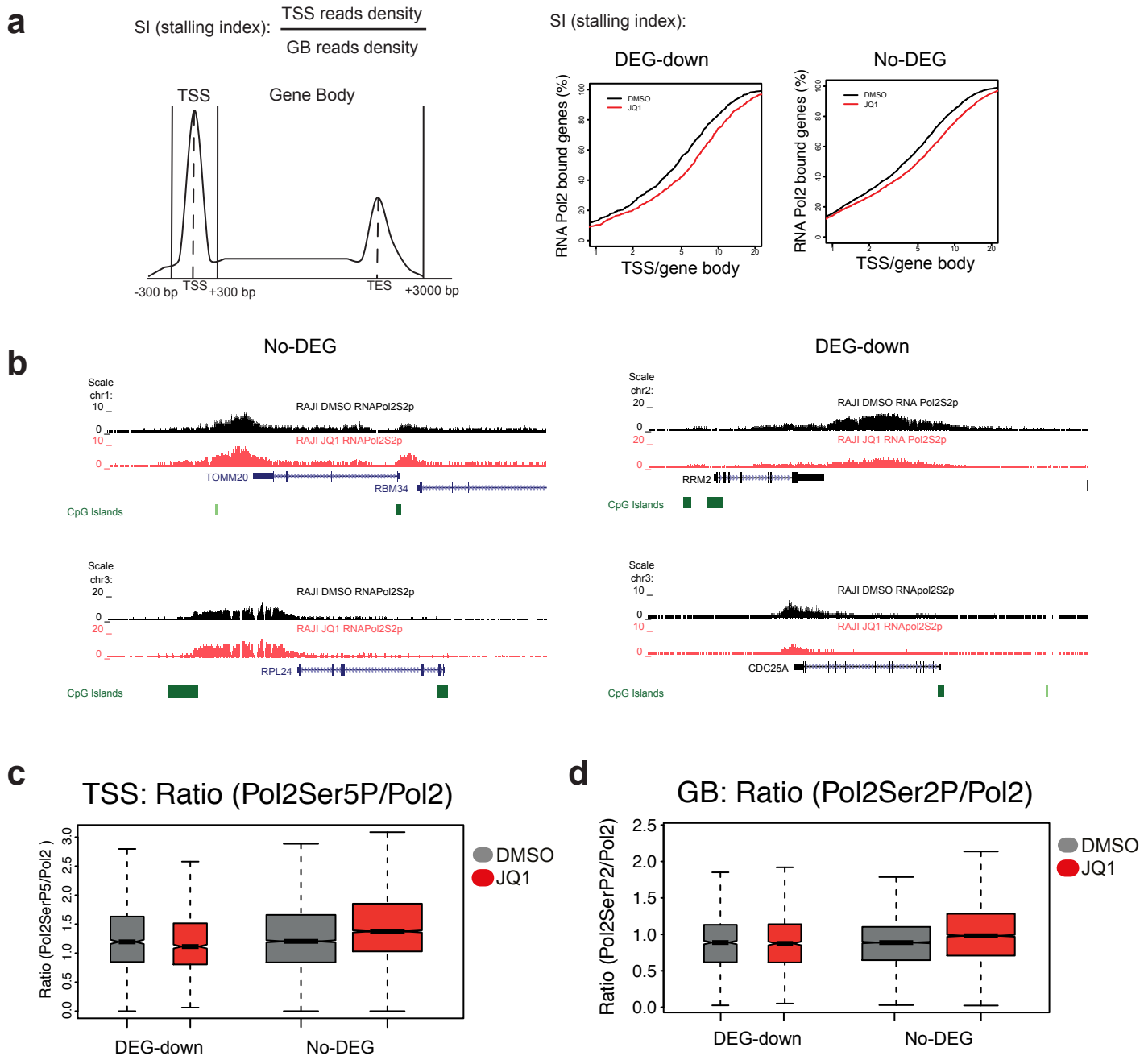
Supplementary Figure 12



Supplementary Figure 12 | Computational analysis of ChIPseq and genome wide expression data sets relative to OCLY cells treated with JQ1

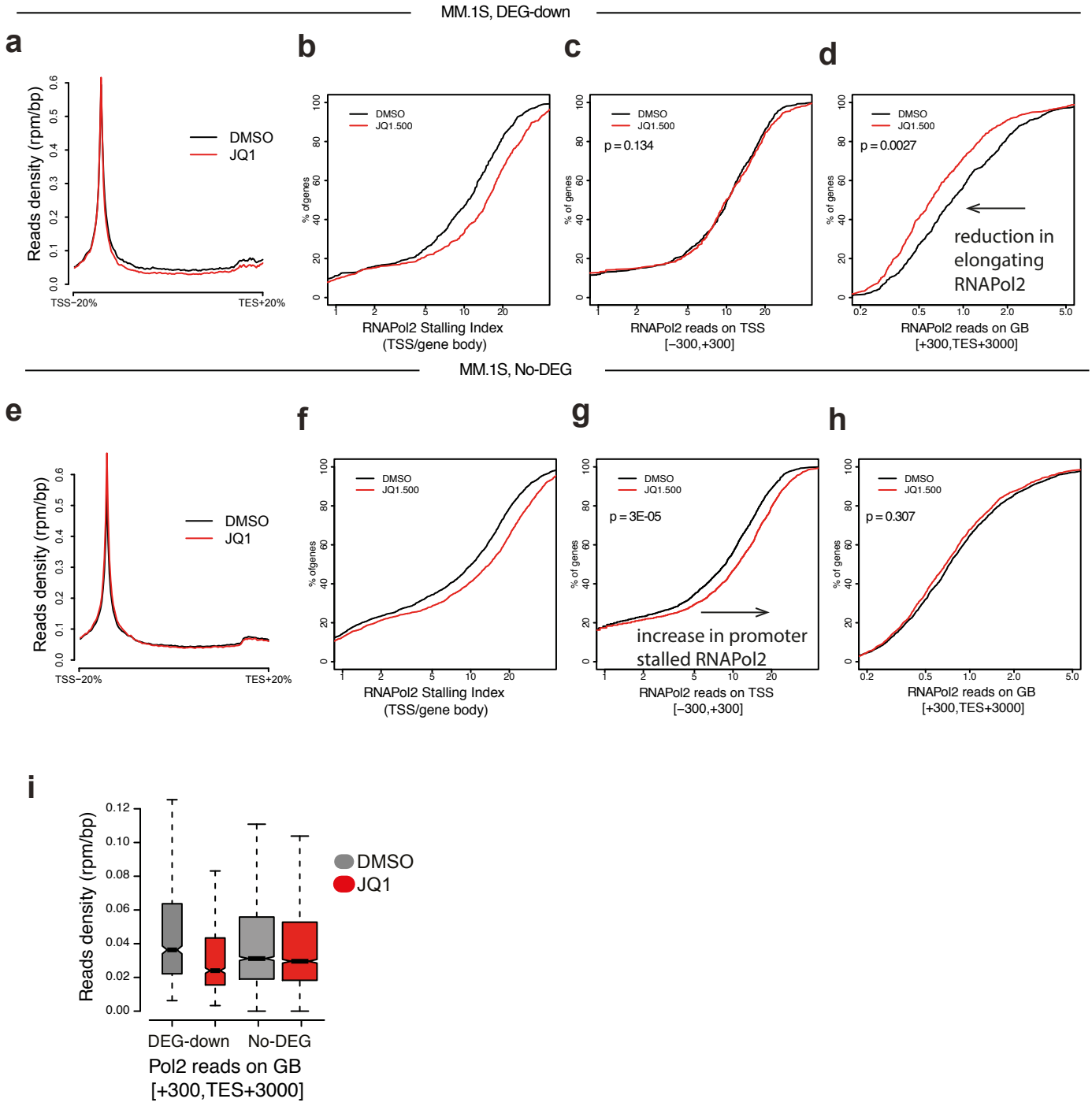
Datasets relative to OCLY treated with JQ1 were retrieved from GEO (GSE45630 (expression data), GSE46663 (ChIPeq)) and analysed as decribed in the Methods section. Left, box-plot of the peak intensity. Right panel, cumulative distribution plot of ChIPseq peaks. ChIPseq peaks were subsetted based on their association with promoters of either genes down-regulated by JQ1 (DEG-down) or not affected by JQ1 (No-DEG).

Supplementary Figure 13



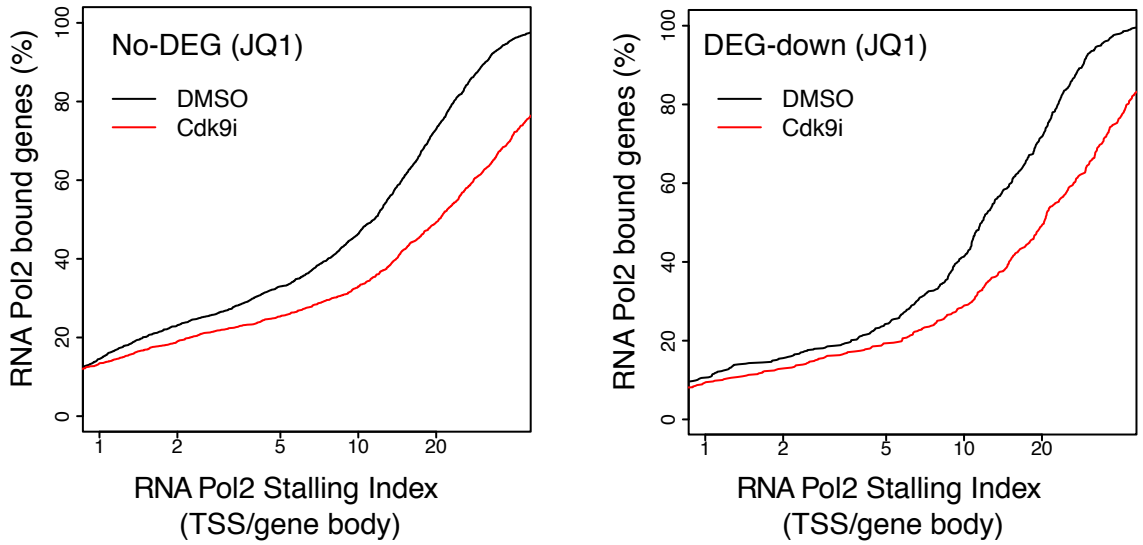
Supplementary Figure 13 | RNAPol2 stalling index in RAJI cells
ChIPseq analysis of RAJI cells treated with 100 nM JQ1 for 24 hours. (**a**) Schematic representation of the calculation of the stalling index (SI) (left panel) and cumulative calculation of the stalling index for either DEG-down genes or No-DEG genes, in either mock treated (DMSO) or JQ1 treated cells. (**b**) Genome browser tracks of Pol2Ser2P of representative DEG-down (right) and No-DEG genes (left). (**c**) Box plot showing the amount of Pol2Ser5P normalized on total RNAPol2 detected on TSS of either DEG-down or No-DEG genes. (**d**) Box plot showing the amount of Pol2S2P normalized on total RNAPol2 detected on the gene body (GB) of either DEG-down or No-DEG genes.

Supplementary Figure 14



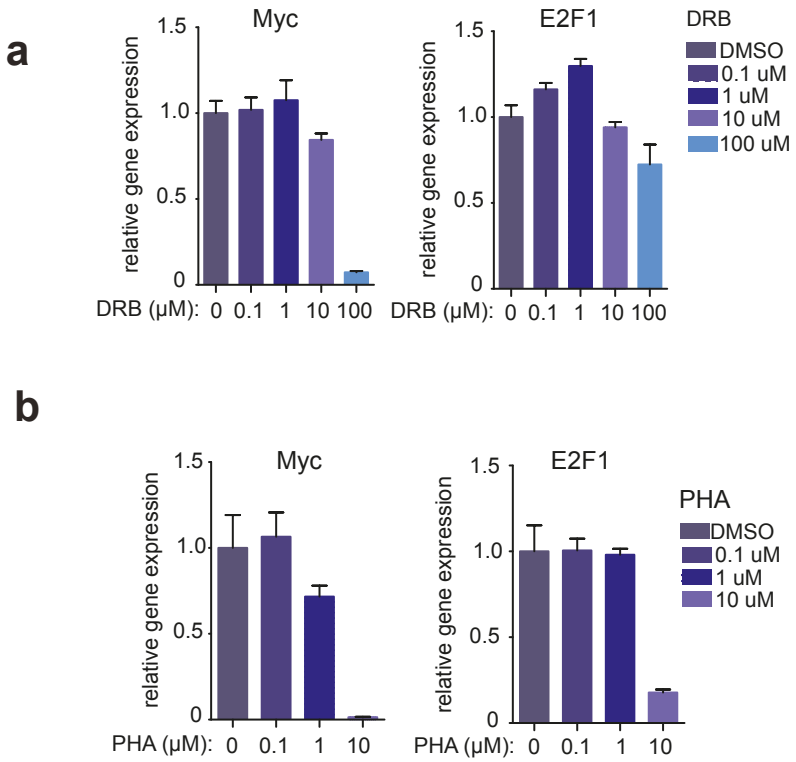
Supplementary Figure 14 | RNAPol2 stalling index in MM.1S cells
In silico analysis of RNAPol2 distribution in genes subsetted for their differential expression upon JQ1 treatment, base on published datasets (GSE42355 (ChIPseq), GSE31365 (expression)). (**a,e**) distribution of RNA Pol2 read counts along either DEG-down (**a**) or No-DEG genes (**e**). (**b,f**) Cumulative plot of the stalling index determined for RNAPol2 associated to DEG-down and No-DEG genes, in either mock treated (DMSO) or JQ1 treated MM.1S. (**c,g**) Cumulative plot of the promoter associated RNAPol2 determined in DEG-down and No-DEG genes, in either mock treated (DMSO) or JQ1 treated MM.1S. (**d,h**) Cumulative plot of the gene body associated RNAPol2 determined in DEG-down and No-DEG genes, in either mock treated (DMSO) or JQ1 treated MM.1S. (**i**) Box plot of the RNAPol2 enrichment calculated at gene bodies (GB) of the indicated class of genes.

Supplementary Figure 15

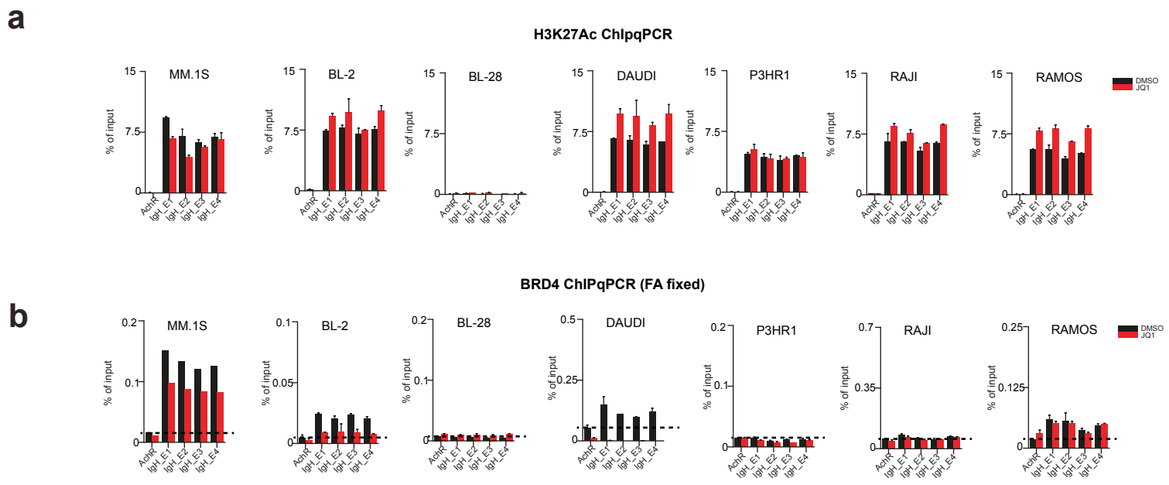


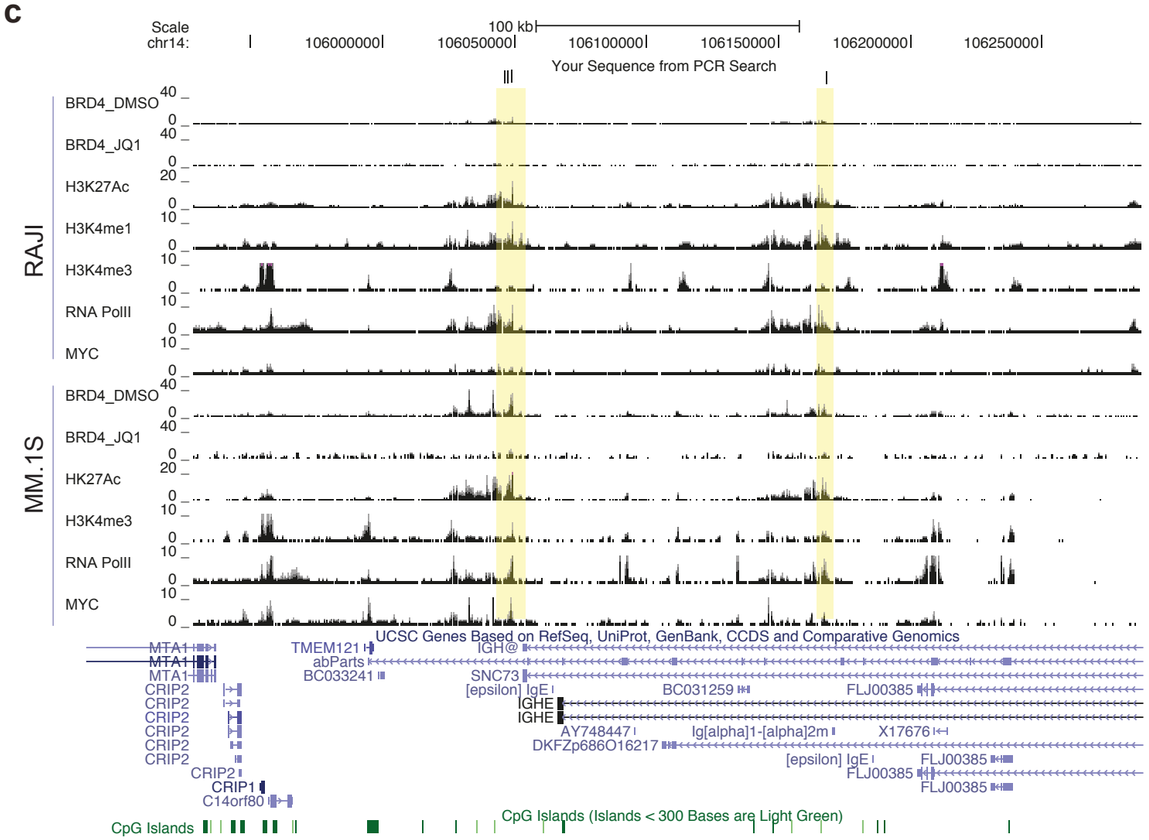
Supplementary Figure 15 | Stalling index of RNAPol2 MM.1S cells treated with CDK9i.
Stalling index was calculated based on RNAPol2 ChIPseq performed in MM.1S cells treated with CDK9i based on published data (GSE43743). Genes were subset in DEG-down and No-DEG based on their differential gene expression upon JQ1 treatment (GSE31365).

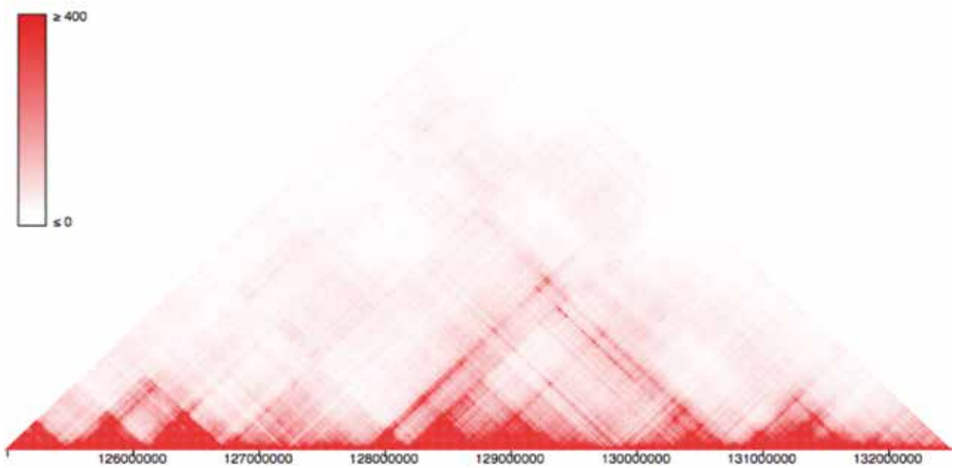
Supplementary Figure 16

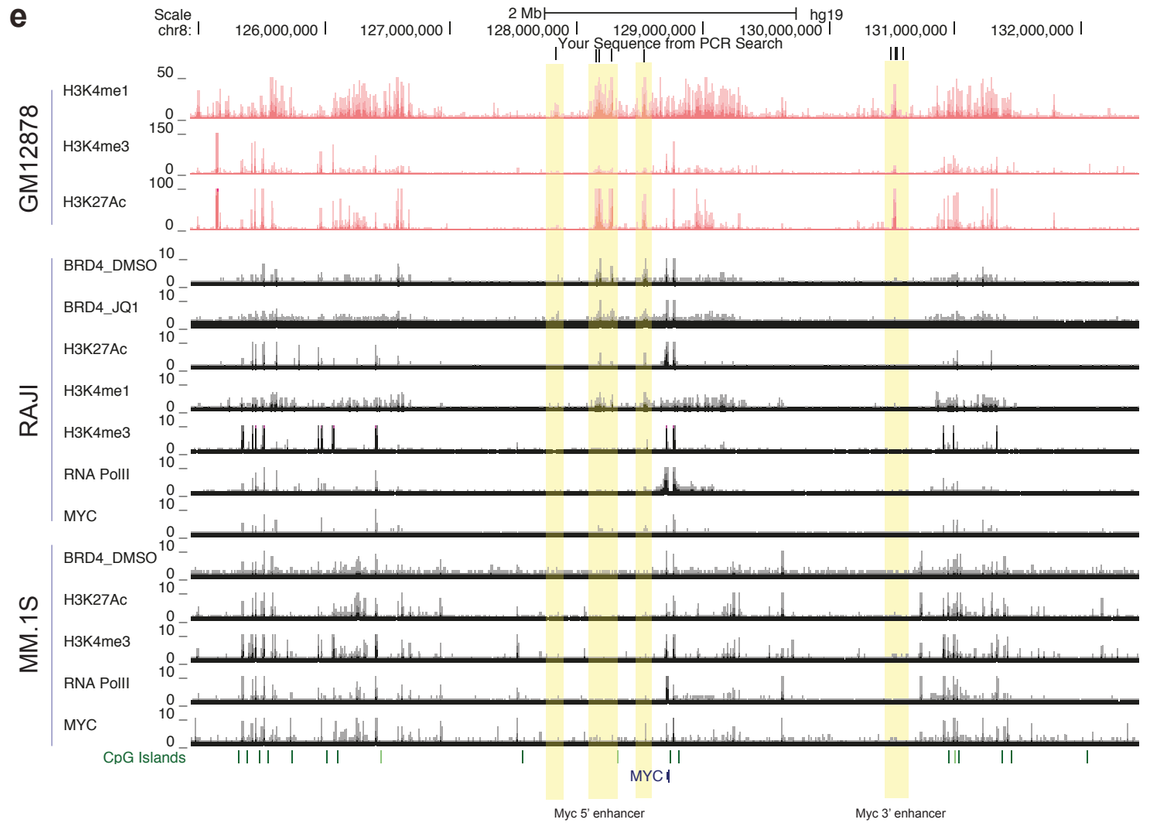


Supplementary Figure 16 | Myc and E2F mRNA levels upon inhibition of elongation
(a,b) Expression analysis by RT-qPCR of c-Myc and E2F1 in RAJI treated with DRB (**a**) or PHA-767491 (**b**). The expression values are reported as mean of technical triplicates, normalized to the RPLP0 gene and mock treated samples.









Supplementary Figure 17 | Comparative analyses of potential distal elements regulating Myc expression in BL cell lines.
(**a,b**) The activity of IgH enhancer regions was assessed by ChIPqPCR for H3K27Ac (**a**) and BRD4 (**b**) on BL lines (BL-2, BL-28, DAUDI, P3HR1, RAJI, RAMOS) and MM.1S, here used as reference, since the activity of IgH enhancers were already reported[1]. Cells were treated for 24h with vehicle (DMSO) or BRD4 (100 nM). The mean and the standard deviations of 3 technical replicates are reported. Acetylcholine Receptor (AchR) is used as negative control. (**c**) Genome Browser view of IgH regulatory regions. Tracks for BRD4, H3K27Ac, H3K4me1, H3K4me3, RNA PolII and Myc are shown. In order to compare the levels of BRD4 biding at the IgH enhancer in RAJI and MM.1S cells, BRD4 ChIPqPCR was performed on RAJI cells fixed for 10' with formaldheyde. MM.1S ChIPseq data used in this analysis was from Loven et al.[2]. The regulatory regions identified in Delmore et al.[1] are highlighted in yellow. (**d**) Hi-C data from GM12878 (B lymphoblastoid cells)[3] showing TADs of chromosome 8 in the proximity of the c-myc gene. The Hi-C graph was generated using the online tool http://www.3dgenome.org. (**e**) Genome Browser view of regulatory regions in the proximity of the c-Myc gene. Tracks for GM128783; RAJI; and MM.1S2 are shown. Regulatory regions identified as Myc enhancer[1, 4] are highlighted in yellow.

Supplementary figure references

1. Delmore JE, Issa GC, Lemieux ME, Rahl PB, Shi J, Jacobs HM, et al. BET bromodomain inhibition as a therapeutic strategy to target c-Myc. Cell 2011 Sep 16; 146(6): 904-917.
2. Loven J, Hoke HA, Lin CY, Lau A, Orlando DA, Vakoc CR, et al. Selective inhibition of tumor oncogenes by disruption of super-enhancers. Cell 2013 Apr 11; 153(2): 320-334.
3. Dixon JR, Selvaraj S, Yue F, Kim A, Li Y, Shen Y, et al. Topological domains in mammalian genomes identified by analysis of chromatin interactions. Nature 2012 May 17; 485(7398): 376-380.
4. Shi J, Whyte WA, Zepeda-Mendoza CJ, Milazzo JP, Shen C, Roe JS, et al. Role of SWI/SNF in acute leukemia maintenance and enhancer-mediated Myc regulation. Genes Dev 2013 Dec 15; 27(24): 2648-2662