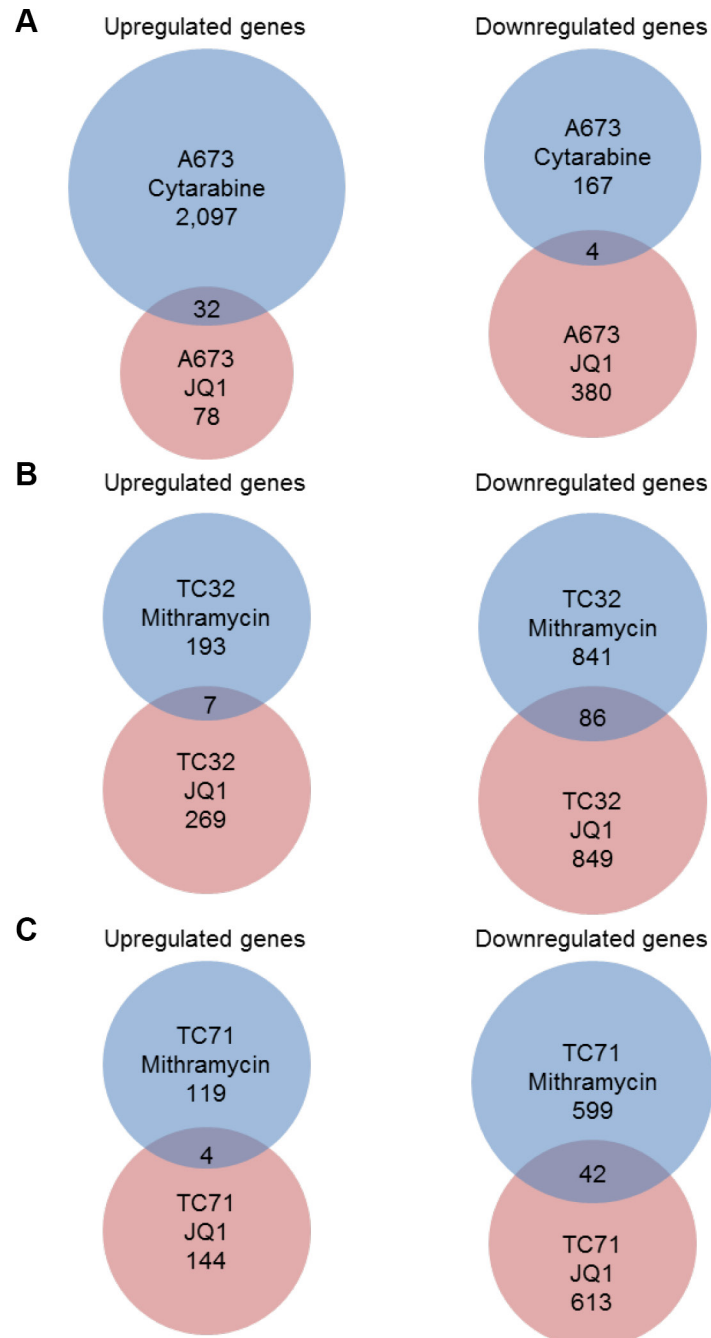
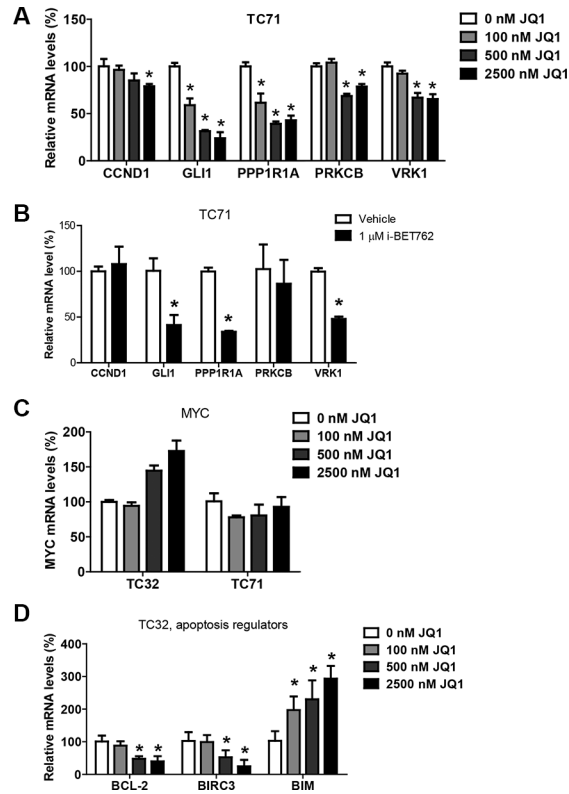


BET bromodomain inhibitors suppress EWS-FLI1-dependent transcription and the IGF1 autocrine mechanism in Ewing sarcoma

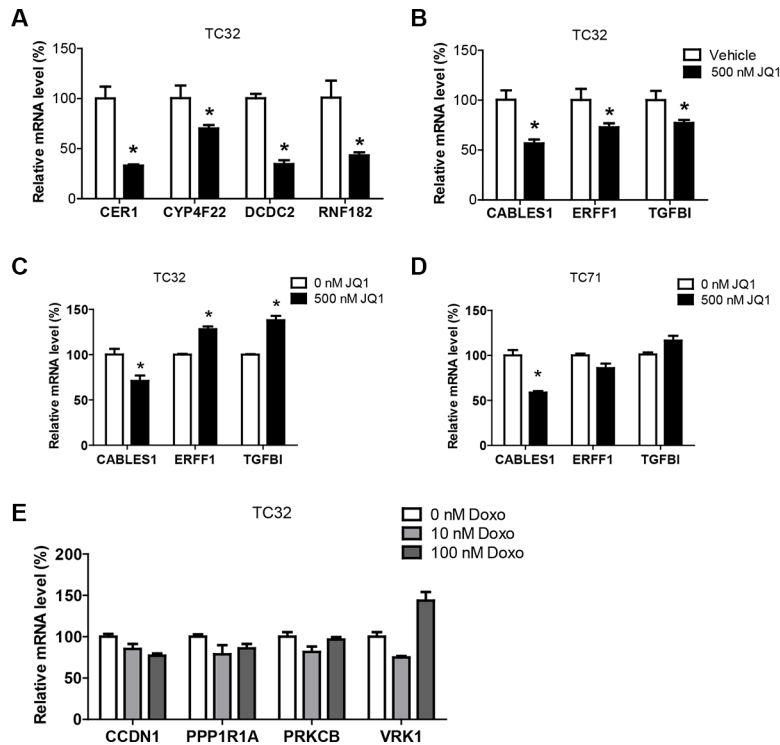
Supplementary Materials



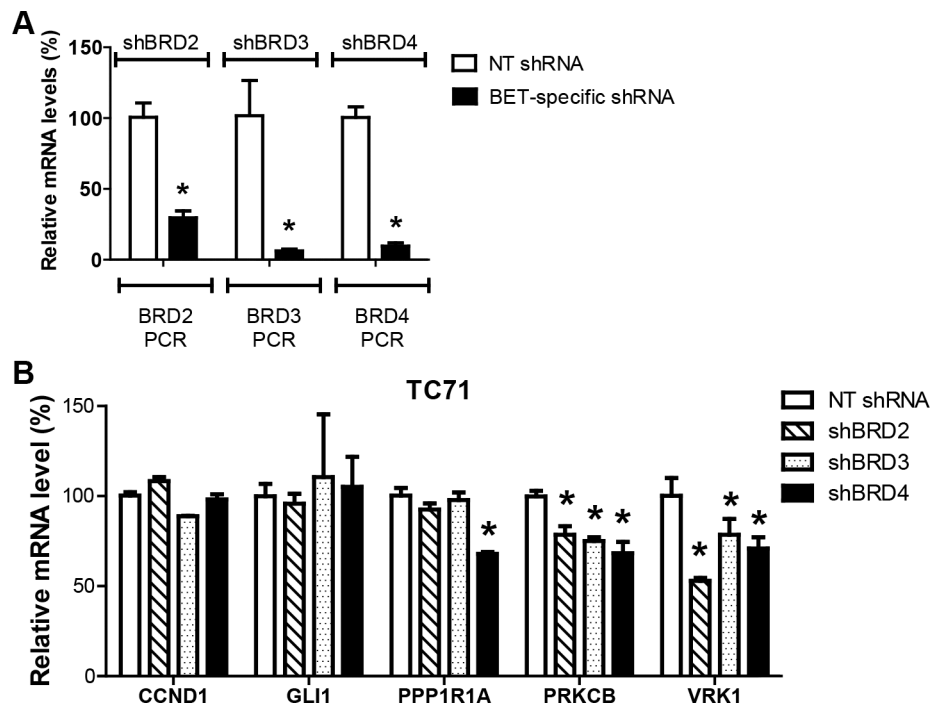
Supplementary Figure S1: Comparison of global gene expression following JQ1 treatment or chemotherapy drugs. (A–C) Venn diagrams summarize the genes that were differentially or commonly affected by JQ1, cytarabine (GEO: GSE6930) or mithramycin (GEO: GSE25127). Genes changed by more than 2 fold were selected for analyses.



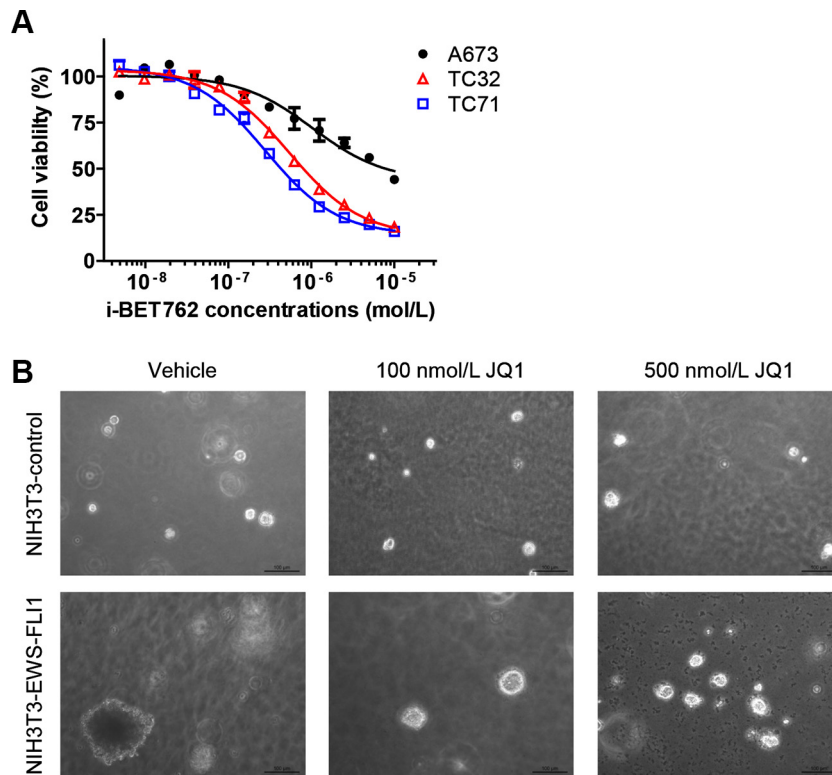
Supplementary Figure S2: BET inhibition represses expression of EWS-FLI1 target genes. (A) Expression of EWS-FLI1 target genes in TC71 cells treated with JQ1 at indicated concentrations or (B) 1 μ M i-BET762 for 24 hours, as assessed by qRT-PCR. (C) Expression of MYC in TC32 and TC71 cells treated as described above. (D) Expression of apoptotic regulators in TC32 cells treated as described above. * $p < 0.05$ by Student's *t*-test compared with control samples.



Supplementary Figure S3: JQ1 but not doxorubicin selectively suppresses EWS-FLI1-activated genes. (A) TC32 cells were treated with 500 nmol/L JQ1 for 4 hours. Expression of EWS-FLI1-activated and (B) repressed genes were determined by qRT-PCR. Actin was used as loading control. * $p < 0.05$ by Student's *t*-test compared with control samples. (C) TC32 or (D) TC71 cells were treated with 500 nmol/L JQ1 for 24 hours. Expression of selected genes repressed by EWS-FLI1 were determined by qRT-PCR. (E) TC32 cells were treated with doxorubicin at 10 or 100 nmol/L for 24 hours. Expression of selected EWS-FLI1 target genes were shown by qRT-PCR.



Supplementary Figure S4: Knockdown of BET genes downregulates EWS-FLI1 target genes. (A) Expression of BRD2, BRD3 and BRD4 in TC71 cells infected with lentivirus directing expression of shRNA sequences specific to BET genes, as measured by qRT-PCR. Knockdown efficiency in TC32 cells was similar (data not shown). (B) Expression of EWS-FLI1 in TC71 cells following knockdown of individual BET genes. * $p < 0.05$ by Student's t -test compared with control samples.



Supplementary Figure S5: BET inhibition impairs Ewing sarcoma cell viability and blocks NIH3T3 transformation. (A) Dose response curves of i-BET762 in Ewing sarcoma cell lines generated as described in Figure 5A. (B) NIH3T3 cells were infected with control lentivirus or lentivirus directing expression of EWS-FLI1. Following puromycin selection, cells were plated at 10,000 cells per well in 0.35% agar in 6-well plates. Cells were treated with 100 or 500 nmol/L JQ1 for 5 days and further incubated for 2 weeks in the absence of JQ1. Representative images are presented. Bars shown in the images represent 100 μ m.

Supplementary Table S1: Sequences of qRT-PCR primers used in this study

Primer sequences		
ACTIN	forward	5'-TCGTGCGTGACATTAAGGA
	reverse	5'-AGGAAGGAAGGCTGGAAGAG
CCND1	forward	5'-CCGCTGGCCATGAACTACCT
	reverse	5'-ACGAAGGTCTGCGCGTGT
GLI1	forward	5'-CTGGAGAACCTTAGGCTGGA
	reverse	5'-CGGCTGACTGTGTAAGCAGA
PPP1R1A	forward	5'-GCAACGGAAGAAGATGACAAG
	reverse	5'-AGGTTCTCTCCTTGCTGCT
PRKCB	forward	5'-CTTCAAGCAGCCACCTTCT
	reverse	5'-TCCCCGAAGCCCCAGATG
VRK1	forward	5'-CCAACGAGCTGCAAAACC
	reverse	5'-TGTCATGTAGACCAGACCCC
BCL2	forward	5'-ATGTGTGTGGAGAGCGTCAACC
	reverse	5'-TGAGCAGAGTCTTCAGAGACAGCC
BIRC3	forward	5'-TTGAACAGCTGCTATCCACATC
	reverse	5'-TCCAGGTTCAAAATGGATAATTG
BIM	forward	5'-CACAAACCCCAAGTCTCCTT
	reverse	5'-TTCAGCCTGCCTCATGGAA
CER1	forward	5'-GAAATGCGGGTCTGTTCATT
	reverse	5'-CAGGCAAACAGTGAGAGCAG
CYP4F221	forward	5'-TTGGATGCTGTTCAATTTGG
	reverse	5'-GGCAGCTGAGTCAGATCGT
DCDC2	forward	5'-GAATTCACAAGAAACCATTCCAA
	reverse	5'-CCCCGTGTTTCAGACCTC
RNF182	forward	5'-CCGTTCTTAGAGGCAGGACTT
	reverse	5'-GGCCAGCTCTAGCAAACCTA
ERRF1	forward	5'-ACCCACTGAAGTAGCTCATCG
	reverse	5'-TTCCATCTGAGTCTAACGTACCC
TGFBI	forward	5'-CGAGTGCTGTCCTGGATATG
	reverse	5'-CCCAGGGTCTCGTAAAGGTT
CABLES1	forward	5'-CACCGCAGCCTCTCCATA
	reverse	5'-GGGGTCATCCAAGAGATTTG
EWS-FLI1 primer set 1 [35]	forward	5'-TAGTTACCCACCCAAACTGGAT
	reverse	5'-GGGCCGTTGCTCTGTATTCTTAC
EWS-FLI1 primer set 2 [36]	forward	5'-TCCTACAGCCAAGCTCCAAGTC
	reverse	5'-GAATTGCCACAGCTGGATCTGC
EWS-FLI1 primer set 3	forward	5'-CGACTAGTTATGATCAGAGCAGT
	reverse	5'-CCGTTGCTCTGTATTCTTACTGA