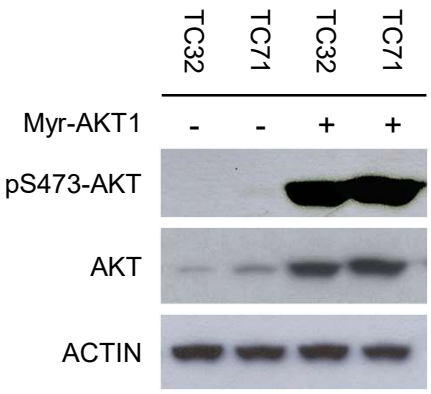

Figure S1. Expression of Myr-AKT1 in TC32 and TC71 cells. TC32 and TC71 cells were infected with control lentivirus or lentivirus directing expression of Myr-AKT1. Cells were selected with 1 μg/ml puromycin for 2 day prior to be analyzed for AKT expression.

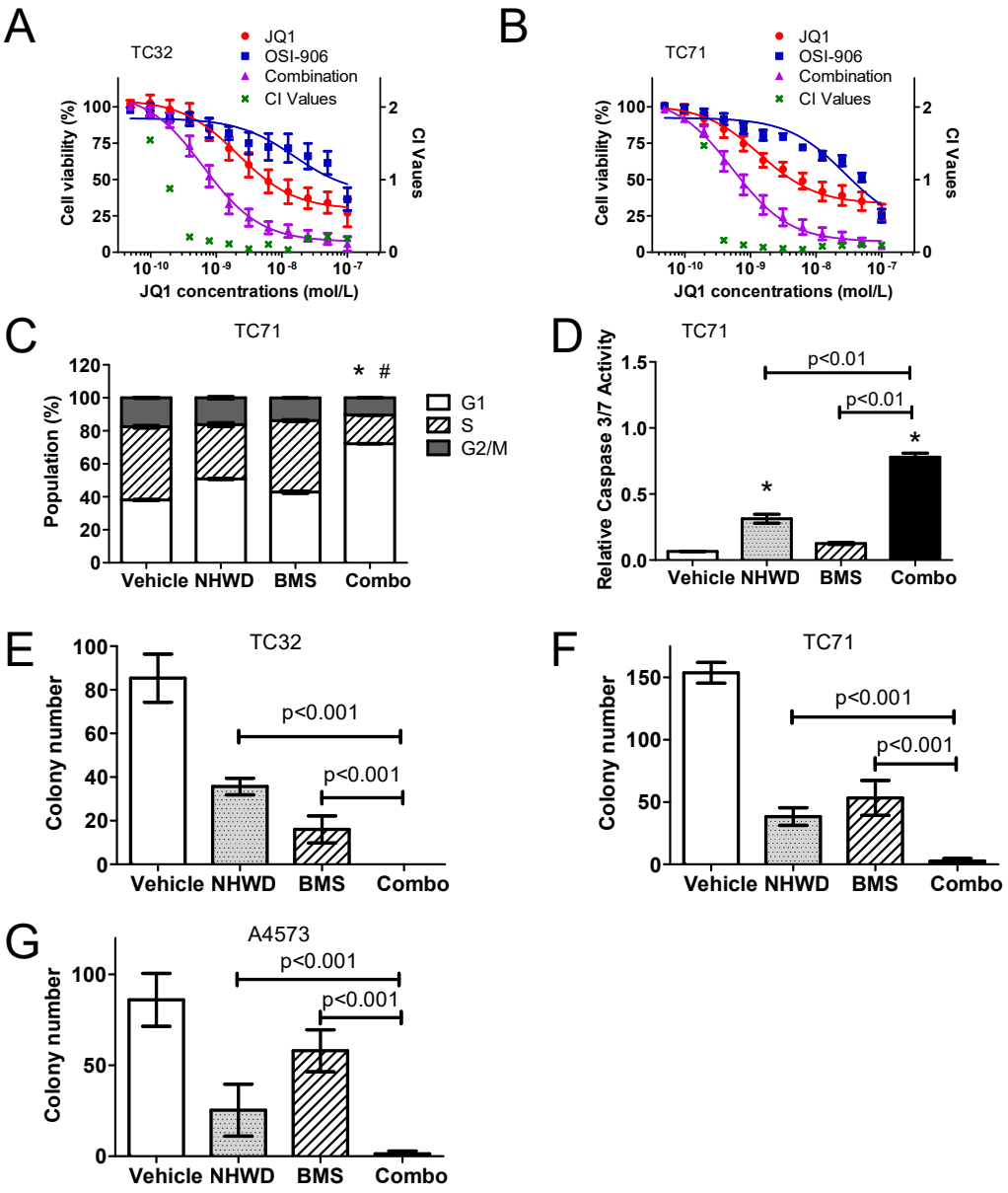

Figure S2. The blockade of IGF1R sensitizes Ewing sarcoma cells to BET inhibitors. (A) Dose response curves of JQ1 ± OSI-906 mixed at a fixed equal molar ratio in TC32 and (B) TC71 cells determined as described in Figure 2A. (C) The impact of NHWD870 and BMS754807 on cell cycle determined as described in Figure 2C. (D) Activation of caspase in TC71 cells determined as described in Figure 2D. (E-G) Colony numbers in TC32, TC71 and A4573 cells treated as described in Figure 2E. Data were generated from three replicates. *: $p < 0.05$ by Student's t-test, combination vs. vehicle. #: $p < 0.05$ by Student's t-test, combination vs. single agent.

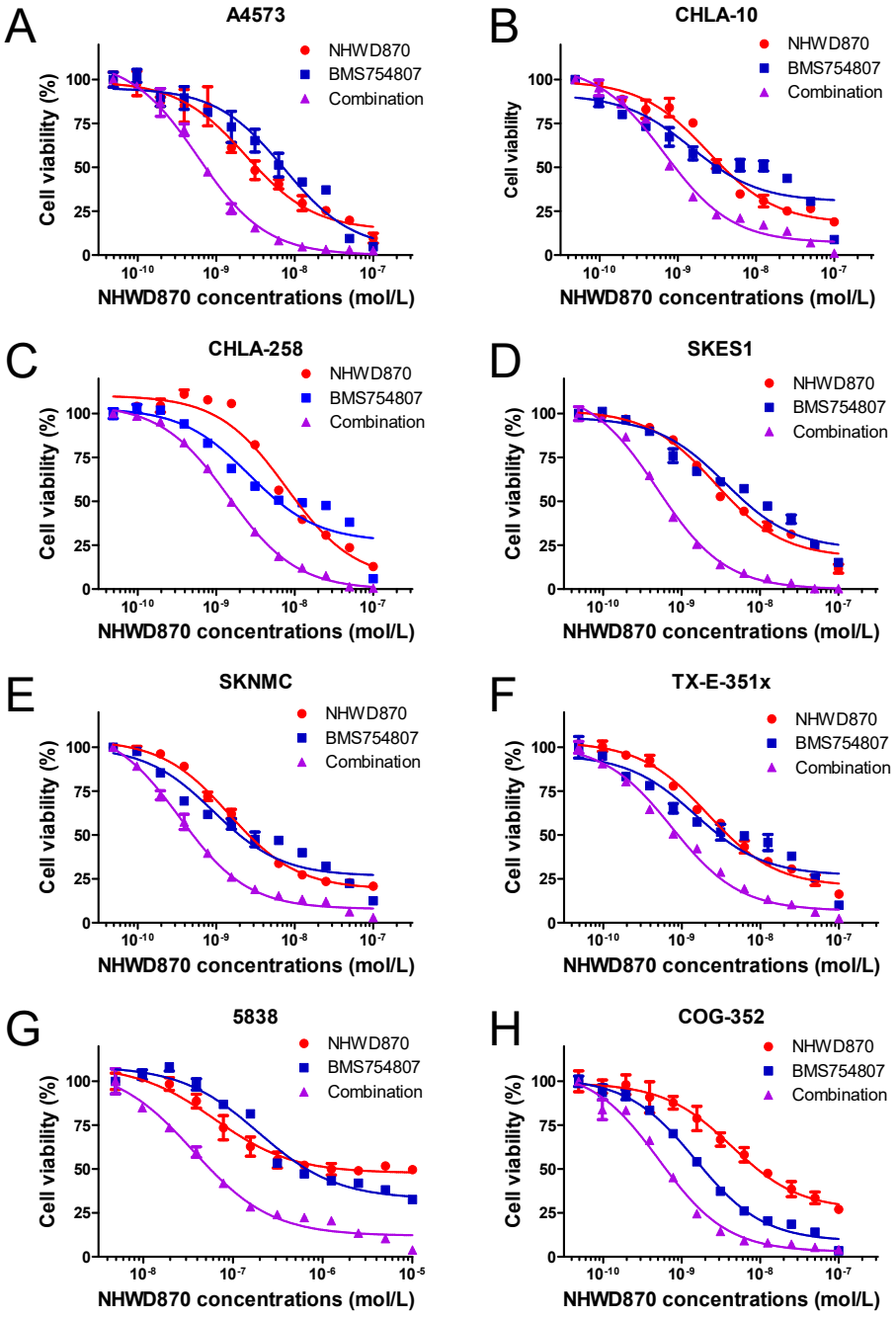

Figure S3. Dose-dependent response to the combination of BET inhibitors and IGF1R inhibitors in Ewing sarcoma cells. (A-H) Dose response curves determined in indicated Ewing sarcoma cell lines as described in Figure 2A.

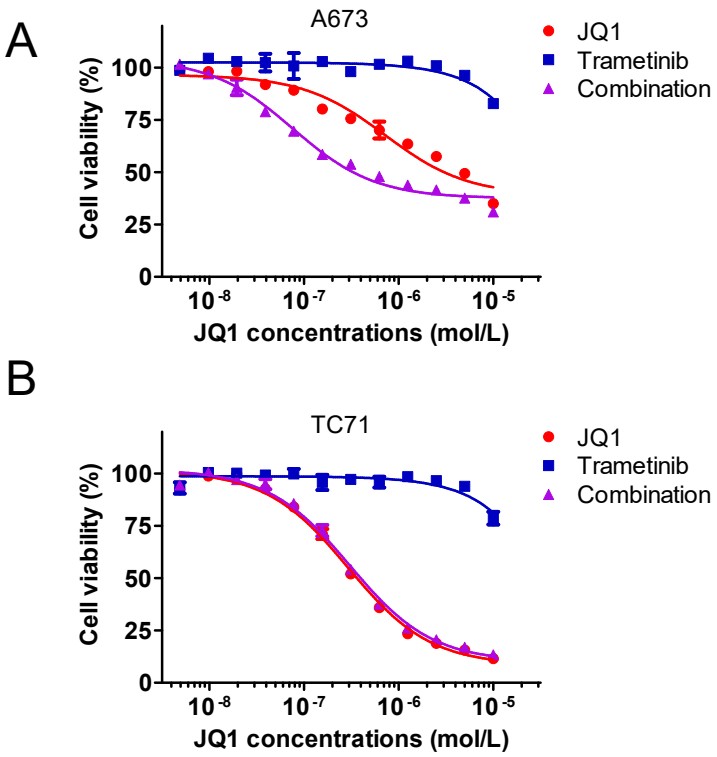

Figure S4. Dose-dependent response to the combination of BET inhibitors and MEK inhibitors in Ewing sarcoma cells. (A) Dose response curves of JQ1 and trametinib mixed at a fixed ratio of 100:1 were determined in A673 and (B) TC71 cells as described in Figure 2A.

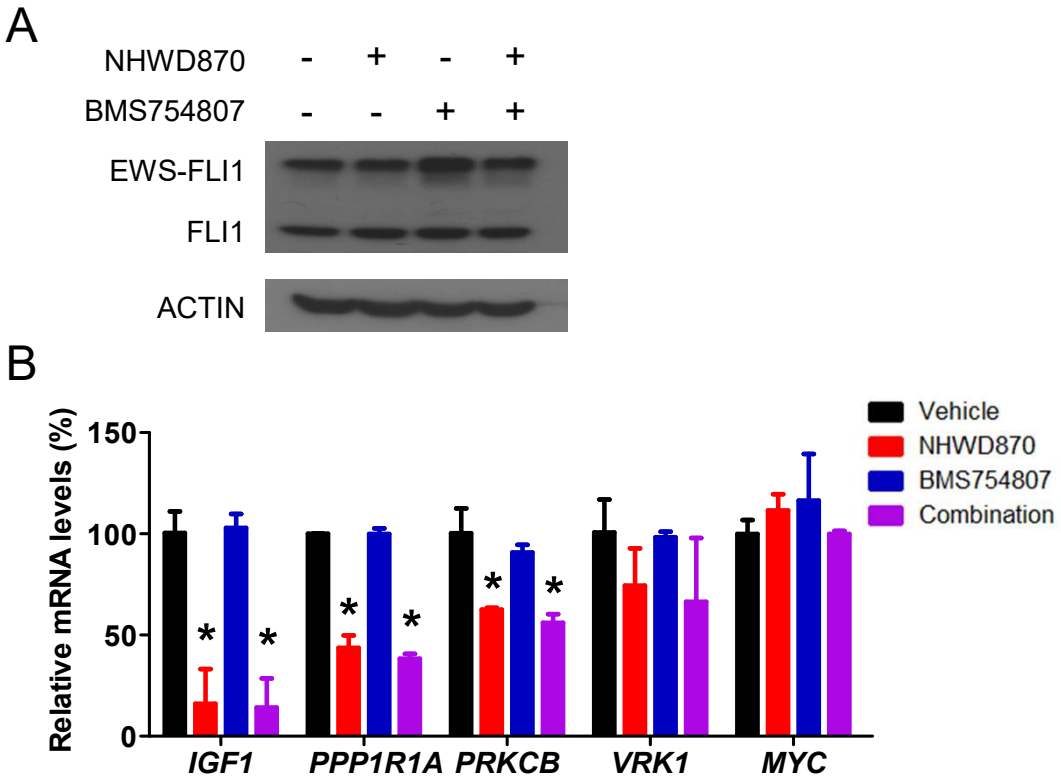

Figure S5. IGF1R inhibition does not change expression of EWS-FLI1 or its target genes. (A) Immunoblotting of EWS-FLI1 and endogenous FLI1 in TC71 cells were treated with 10 nmol/L NHWD870 ± 100 nmol/L BMS754807 for 24 hours. (B) TC32 cells were treated with 10 nmol/L NHWD870 ± 100 nmol/L BMS754807 for 24 hours prior to RNA preparation and RT-PCR analysis. *: $p < 0.05$ by Student's t-test, treated vs. vehicle.

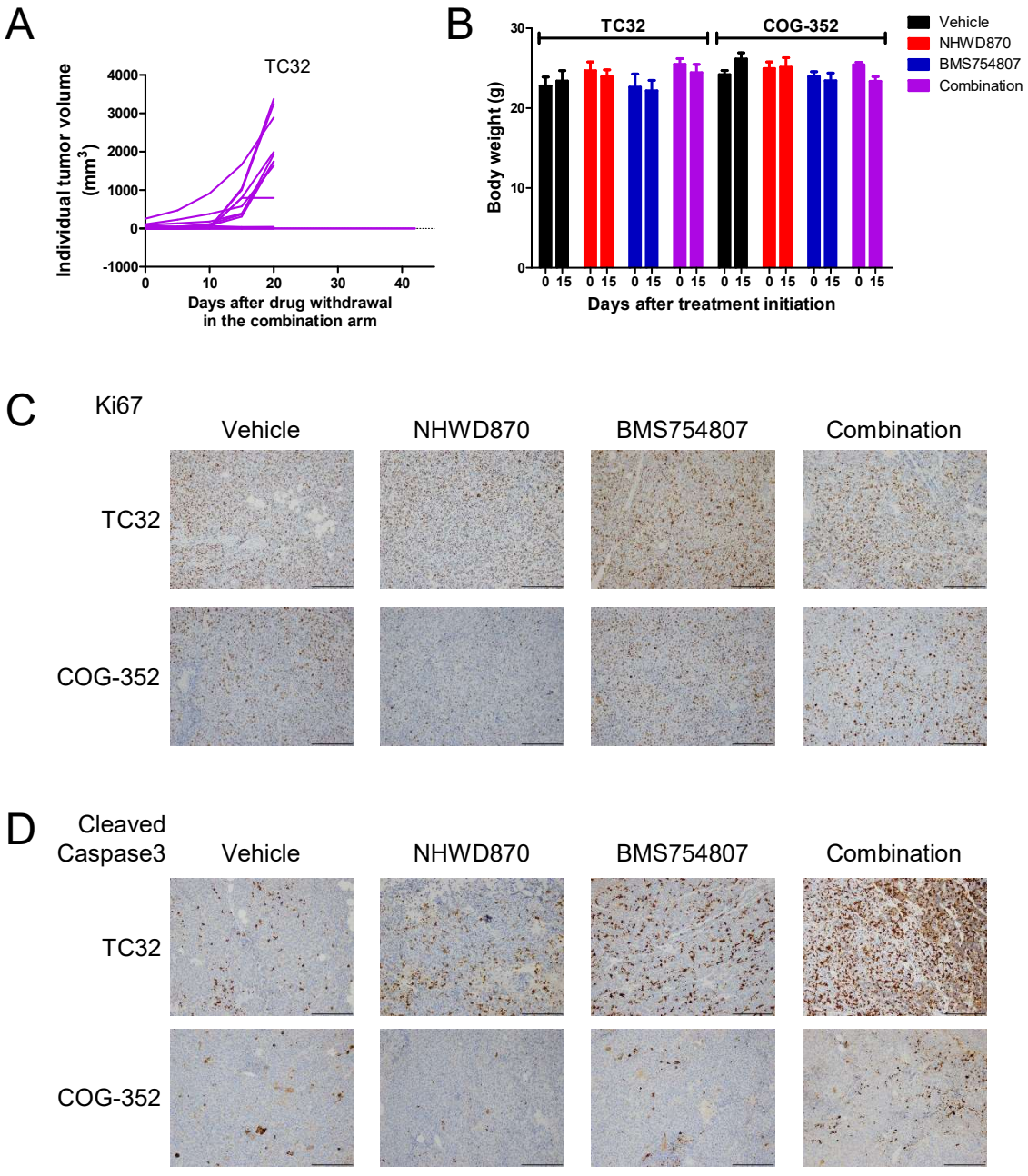

Figure S6. (A) TC32 xenograft tumor growth after discontinuation of drug administration in the arm treated with NHWD870 and BMS754807. (B) Representative image of Ki67 and (C) cleaved caspase 3 staining in TC32 and COG-352 xenograft tumors following 3-day treatment. The bars represent 100 μm in the images.

Primer sequences		
ACTIN	forward	5’-TCGTGCGTGACATTAAGGA
	reverse	5’-AGGAAGGAAGGCTGGAAGAG
IGF1	forward	5’-GCAATGGGAAAAATCAGCAG
	reverse	5’-GAGGAGGACATGGTGTGCA
PPP1R1A	forward	5’-GCAACGGAAGAAGATGACAAG
	reverse	5’-AGGTTCCTCTCCTTGCTGCT
PRKCB	forward	5’-CTTCAAGCAGCCCACCTTCT
	reverse	5’-TCCCCGAAGCCCCAGATG
VRK1	forward	5’-CCAACGAGCTGCAAAACC
	reverse	5’-TGTCATGTAGACCAGACCCC
MYC	forward	5’-TCAAGAGGCGAACACACAAC
	reverse	5’-GGCCTTTTCATTGTTTTCCA
BCL2	forward	5’-ATGTGTGTGGAGAGCGTCAACC
	reverse	5’-TGAGCAGAGTCTTCAGAGACAGCC
BIRC3	forward	5’-TTGAACAGCTGCTATCCACATC
	reverse	5’-TCCAGGTTCAAAATGGATAATTG
BIM	forward	5’-CACAAACCCCAAGTCCTCCTT
	reverse	5’-TTCAGCCTGCCTCATGGAA
EWS-FLI1 primer set 1	forward	5'-TAGTTACCCACCCCAAACTGGAT
	reverse	5'-GGGCCGTTGCTCTGTATTCTTAC
EWS-FLI1 primer set 2	forward	5'-TCCTACAGCCAAGCTCCAAGTC
	reverse	5'-GAATTGCCACAGCTGGATCTGC
EWS-FLI1 primer set 3	forward	5'-CGACTAGTTATGATCAGAGCAGT
	reverse	5'-CCGTTGCTCTGTATTCTTACTGA

Supplementary Table S1. Sequences of qRT-PCR primers.