Supplemental Data

Figure S1. Related to Figure 1: Silencing USP7 Decreases NEK2-induced Bortezomib Resistance

OCI-MY5 cells transduced with EV, NEK2-OE or NEK2-OE + USP7-shRNA were treated with DOX to suppress USP7 expression in NEK2-OE cells. After 72 hours, cells were treated with bortezomib (5nM) for further 24 hours and cell viability was measured using trypan blue stain.

Figure S2. Related to Figure 2: USP7 Deubiquitinizes NEK2 Protein

(A) H1299 cells were transfected with USP7-shRNA, after 72-hour induction with DOX, followed by protein extraction. Western blot were performed using anti-USP7, NEK2 and β-actin antibodies. (B) ARP1 cells were treated with P5091 overnight at 16μM. Cells were lysed, and protein extract was analyzed by western blot using USP7, USP47, NEK2, and GAPDH antibodies. (C) ARP1 cells were treated with P5091 overnight at 16μM. Cells were lysed, total RNA was extracted and analyzed for NEK2 mRNA expression by qRT-PCR (NS: No Significance). (D & E) OCI-MY5 (D) and H1299 (E) cells were treated with P5091 at different doses or a combination with the proteasome inhibitor MG132 for 5 hours. NEK2 protein levels were analyzed by western blot using NEK2 and β-actin antibodies. (F) Input controls for immunoprecipitation of Figure 2H. H1299 cells were co-transfected with HA-UB and NEK2-OE vectors with or without a USP7-OE plasmids. Cells were lysed in the presence of NEM and protein extracts were analyzed by western blot using NEK2, USP7, and β-actin antibodies.

Figure S3. Related to Figure 3: Overexpression of NEK2 increases phosphorylatated p-65 in myeloma cell lines. ARP1 and OCI-MY5 myeloma cells overexpressing NEK2 were lysed. NEK2 and p65-S536 phosphorylation levels were analyzed between NEK2-OE and EV cells by western blot.

Figure S4. Related to Figure 4: NEK2 Activates NF-κB signaling via AKT

(A) H1299 cells transfected with EV or NEK2-OE were lysed and analyzed by western blot using NEK2, P-IkBα, and GAPDH antibodies. (B) H1299 cells transfected with EV or NEK2-OE were treated with vehicle or MK-2206 2HCl for 30 minutes. Protein was extracted and analyzed by western blot using P-p65-S536, and β-actin antibodies. (C) H1299 cells transfected with NEK2-shRNA or Scramble were induced with DOX for 48 hours and then treated with tautomycin for another 24 hours. Cells were lysed and analyzed by western blot using NEK2, P-p65-S536, P-PP1α, and P-AKT-S473 antibodies.

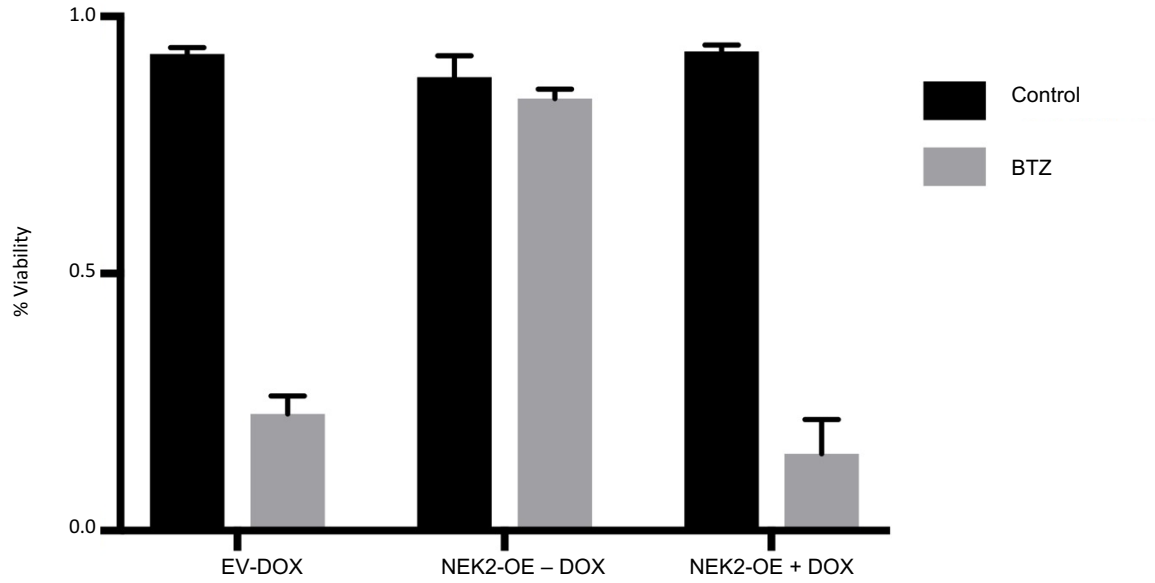
Figure S5. Related to Figure 7: INH1 and P5091 Overcome NEK2-induced Bortezomib Resistance

Mouse weigh was calculated at the starting and ending of the injection of NEK2-OE ARP1 cells expressing luciferase into NOD-Rag1^{null} mice. After one-week injection of NEK2-OE cells, mice were treated with (1) vehicle, (2) BTZ (3mg/kg, IP, 2 times/week), (3) INH1 (100mg/kg, IP, 3 times/week), (4) P5091 (10mg/kg, IV, 2 times/week), (5) P5091 + BTZ, and (6) INH1 + BTZ Mice for 28 days.

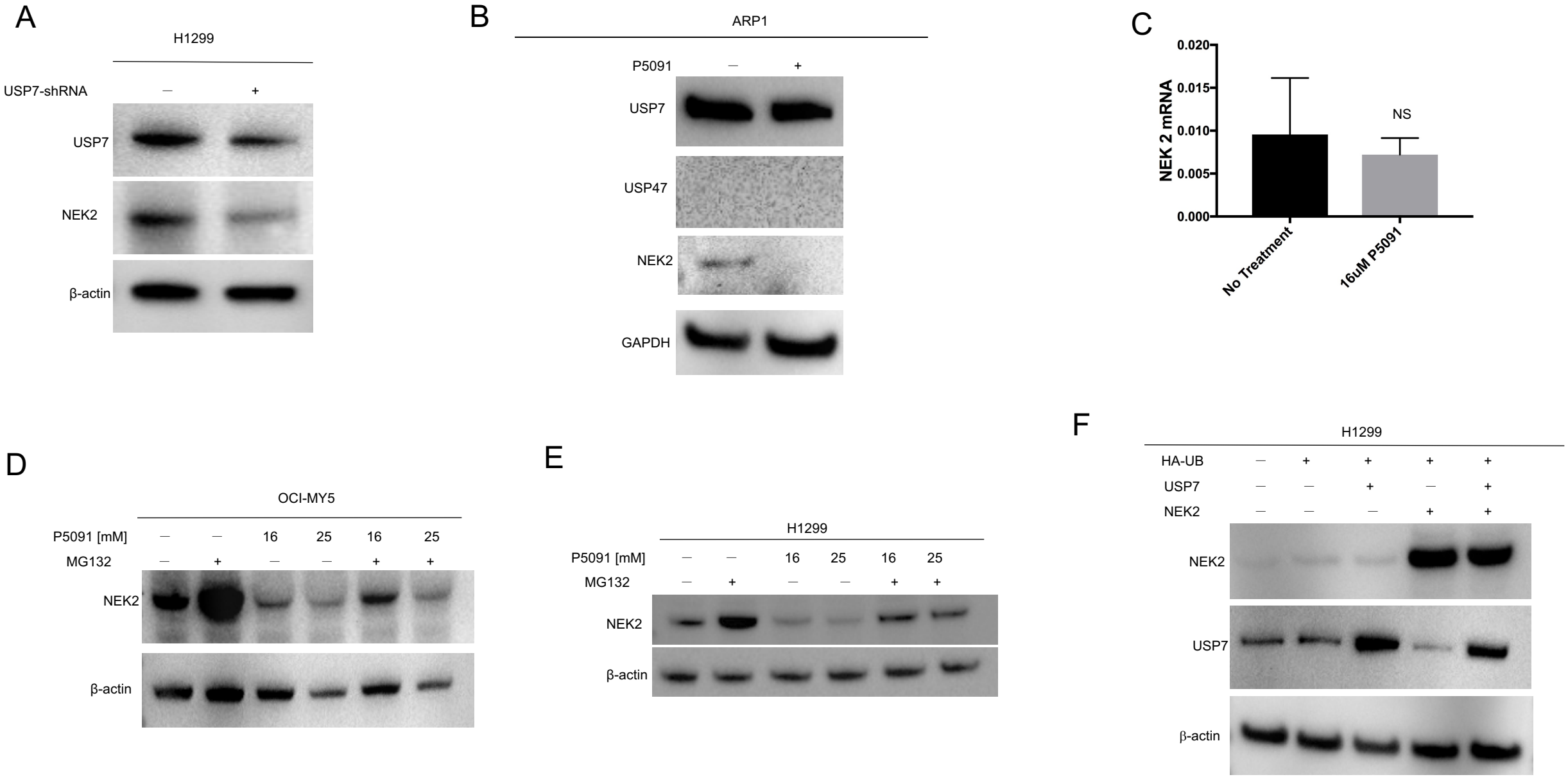
Table S1. Related to Figure 1: Protein List Identified by TAP-MS Bound to NEK2.

Tables S2. Related to Figure 5: p65 Binding Sites to Direct Target Genes Regulated by NEK2 Identified by ENCODE at UCSC.

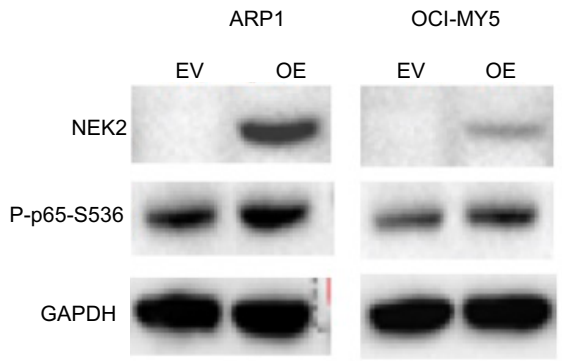
Supplemental Figure 1



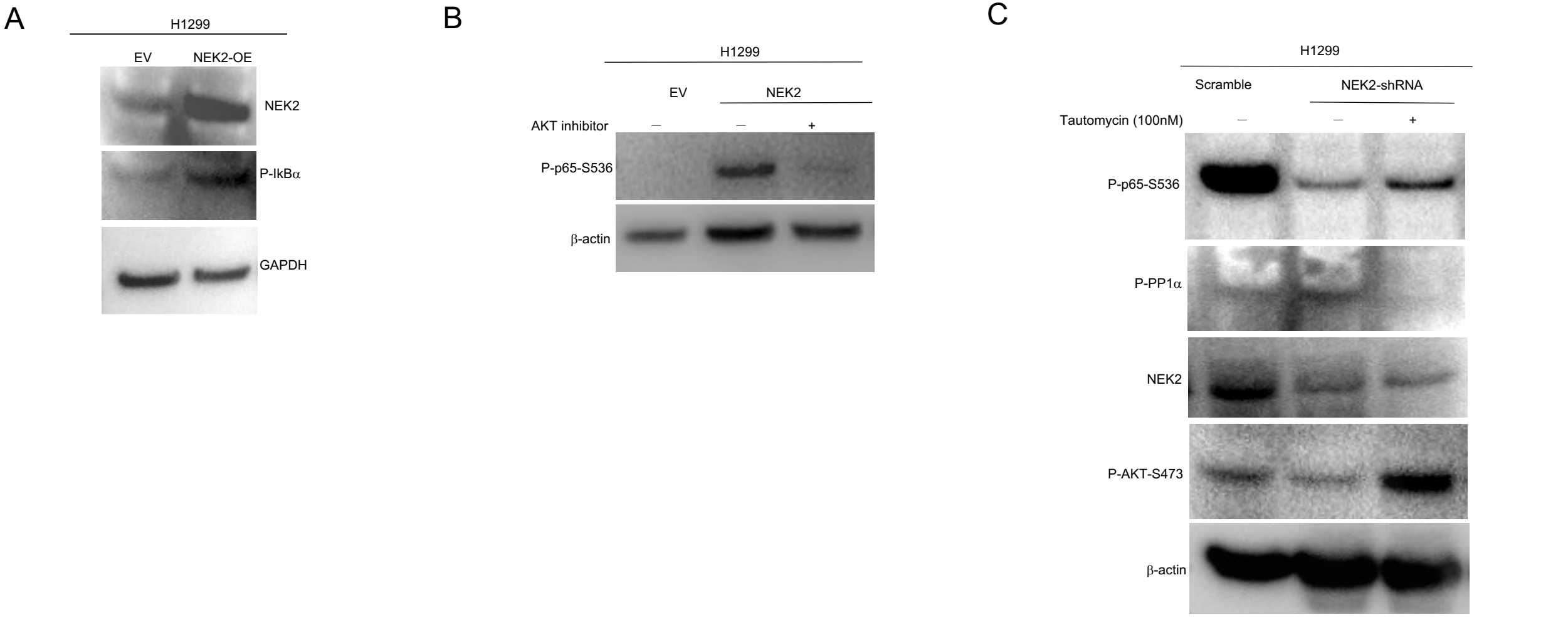
Supplemental Figure 2



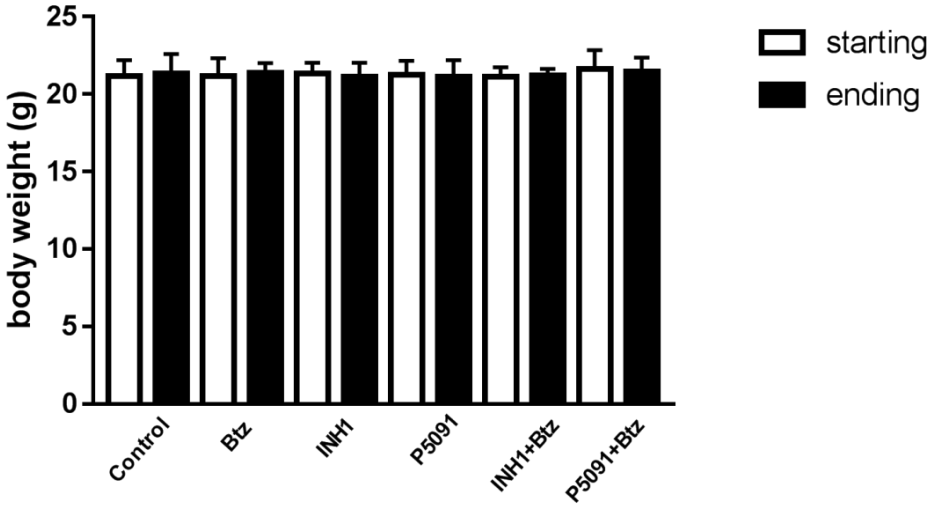
Supplemental Figure 3



Supplemental Figure 4



Supplemental Figure 5



Supplemental Table 2

Gene Symbol	p65 binding site*
HPSE	chr4:84,262,139-84,262,508
HMGB1	chr13:31,038,167-31,038,595
LCP1	chr13:46,739,634-46,740,590
DARS2	chr1:173,793,487-173,794,076
NEDD1	chr12:97,285,732-97,286,132
PRKDC	chr8:48,790,935-48,791,348
CDCA2	chr8:25,316,433-25,316,836
CDC2	chr14:50,810,854-50,811,264
TPX2	chr20:30,311,226-30,312,035
SMC4	chr3:160,117,270-160,117,688
PPP1R12A	chr12:80,328,739-80,329,571
MKI67	chr10:129,924,754-129,925,158
PLAC8	chr4:84,031,300-84,031,974
DYRK2	chr12:68,042,402-68,042,777
PTEN	chr10:89,623,165-89,623,590
NLK	chr17:26,382,567-26,382,950
MLL	chr6:168,106,886-168,107,255
ALCAM	chr3:105,085,406-105,086,113
NFE2L1	chr17:46,126,327-46,126,777
GLG1	chr16:74,486,191-74,486,614
TGOLN2	chr2:85,545,632-85,546,001
CYLD	chr16:50,775,635-50,776,546
ALDH2	chr12:112,211,700-112,212,283
DCP2	chr5:112,312,074-112,312,787
TMED4	chr7:44,621,599-44,622,341
RC3H2	chr9:125,667,614-125,668,078
EXT2	chr11:44,117,125-44,117,528
IL15	chr4:142,556,807-142,557,225
TMEM179B	chr11:62,554,693-62,555,096
TTC17	chr11:43,380,226-43,380,814
C14ORF100	chr14:59,910,898-59,911,267

* Human Feb. 2009 (GRCh37/hg19 from UCSC Genome Browser) Assembly, Track Name: GM12878+TNFa RELA Cell line: GM12878 (B cell)