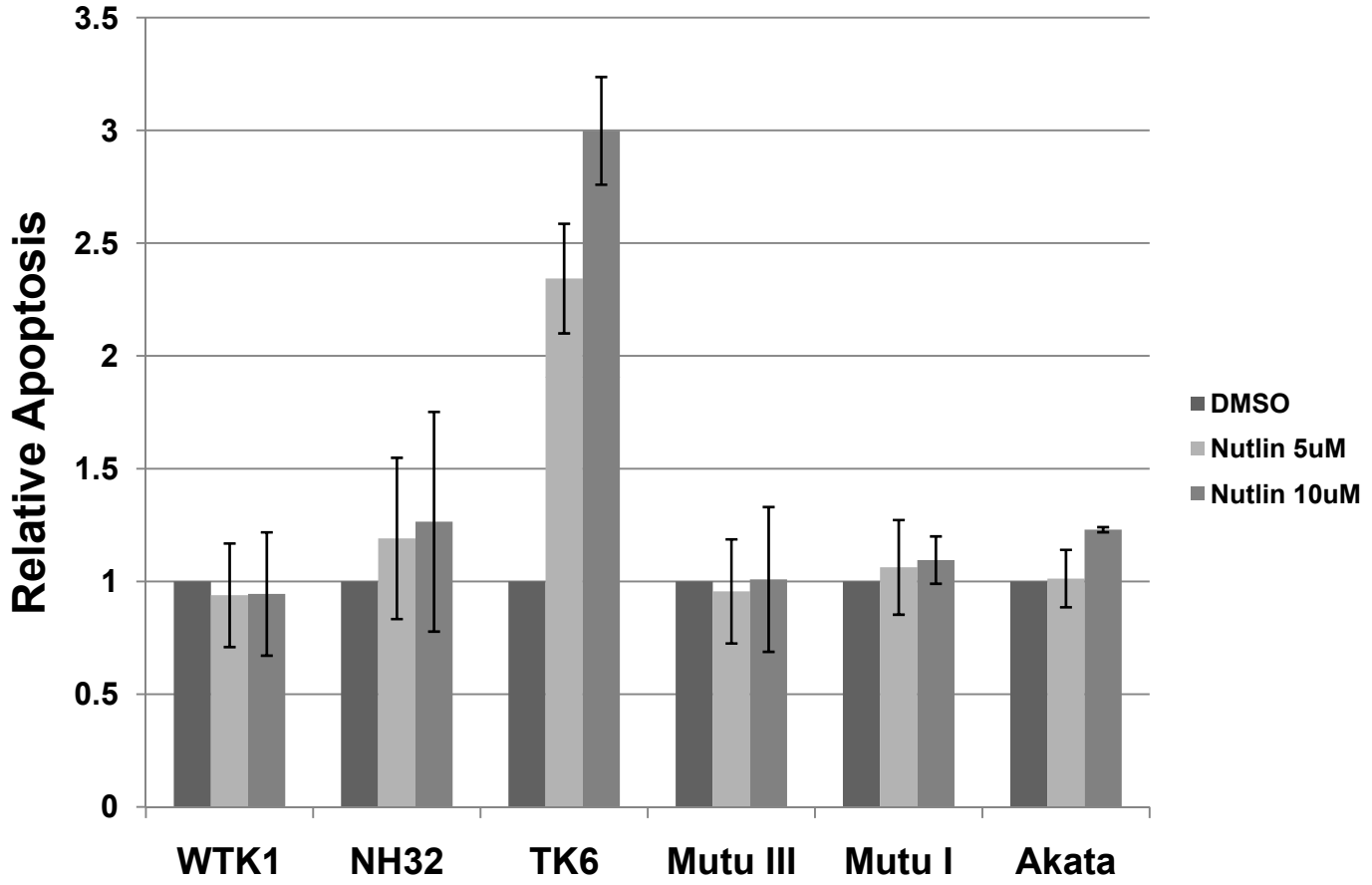
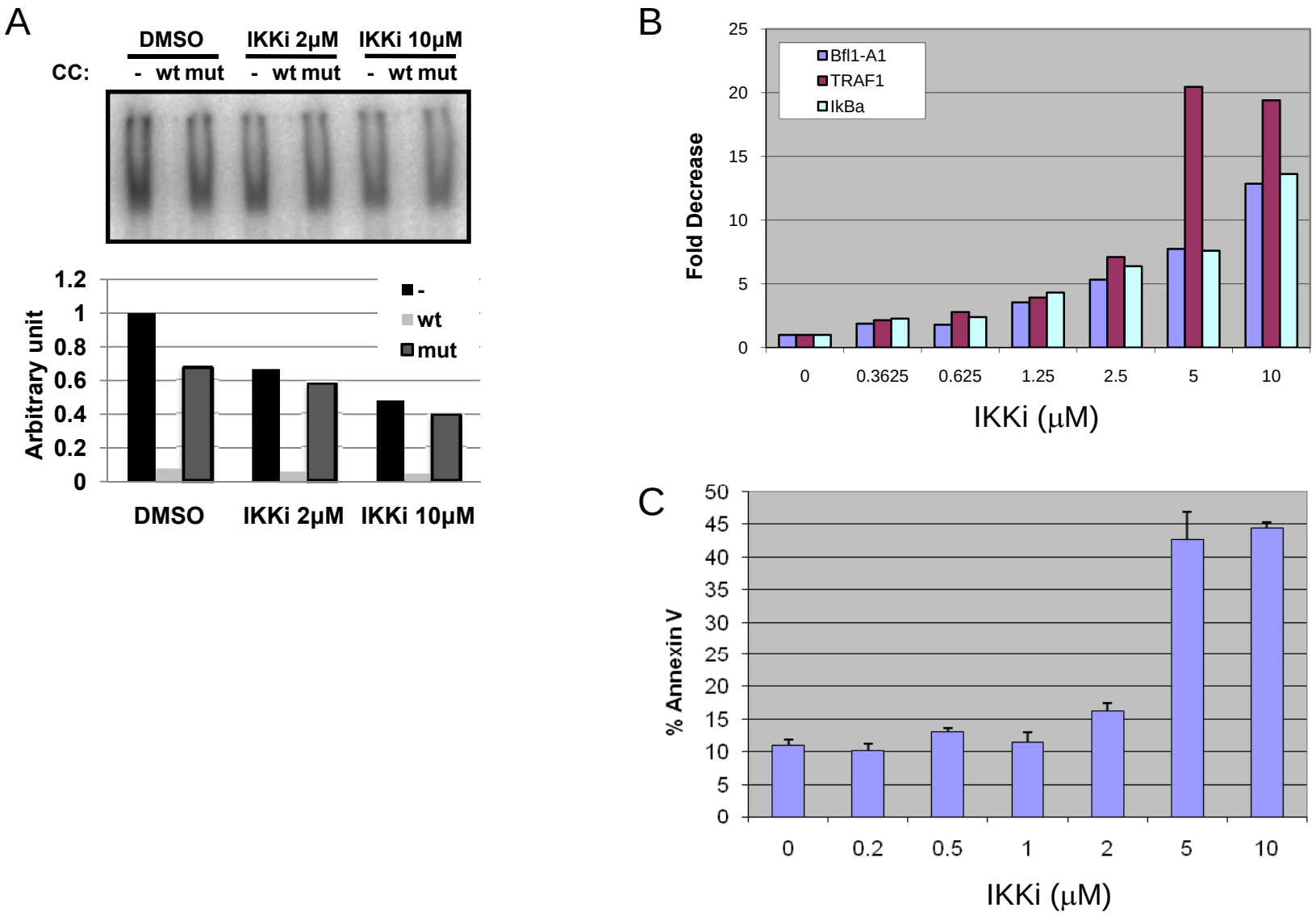
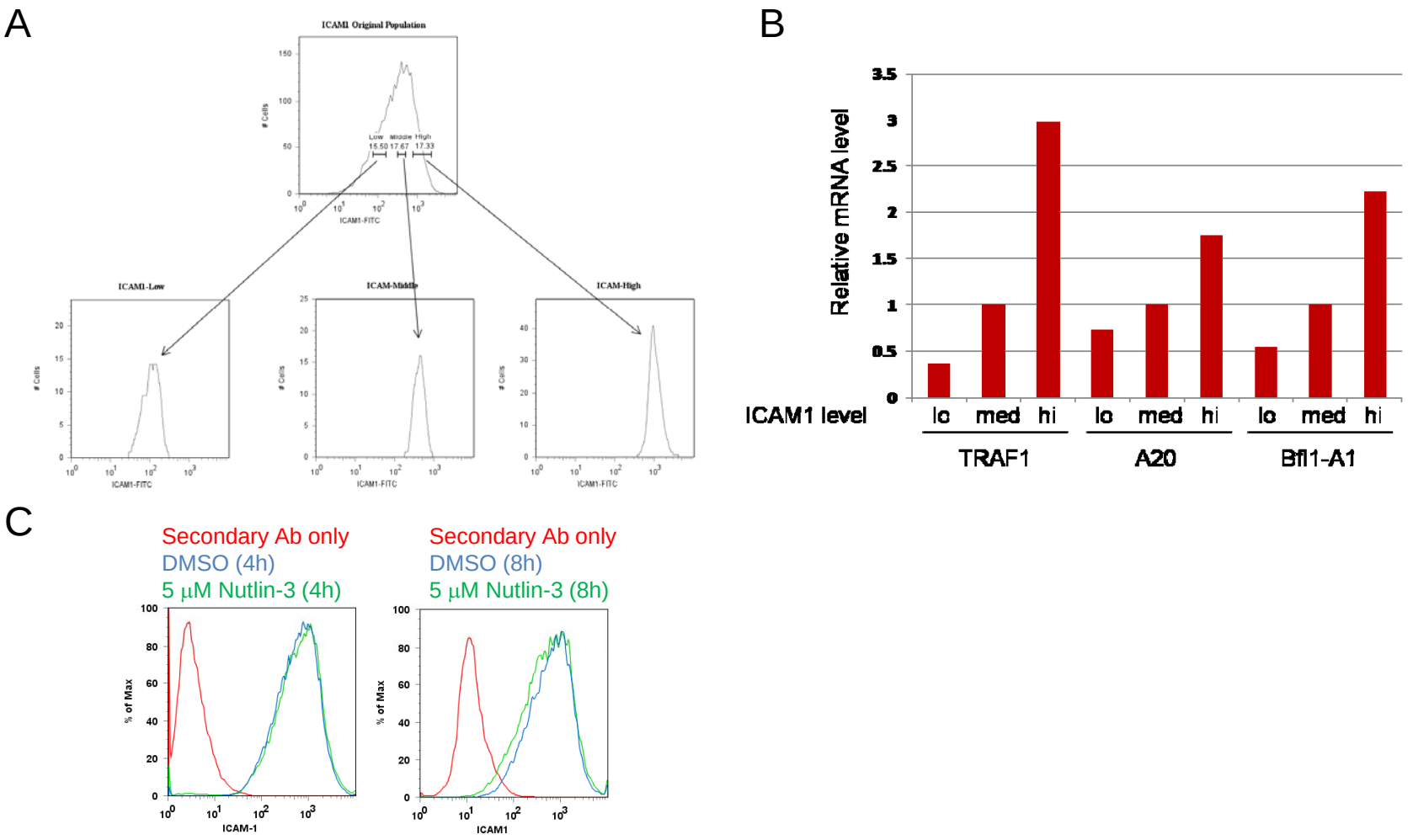


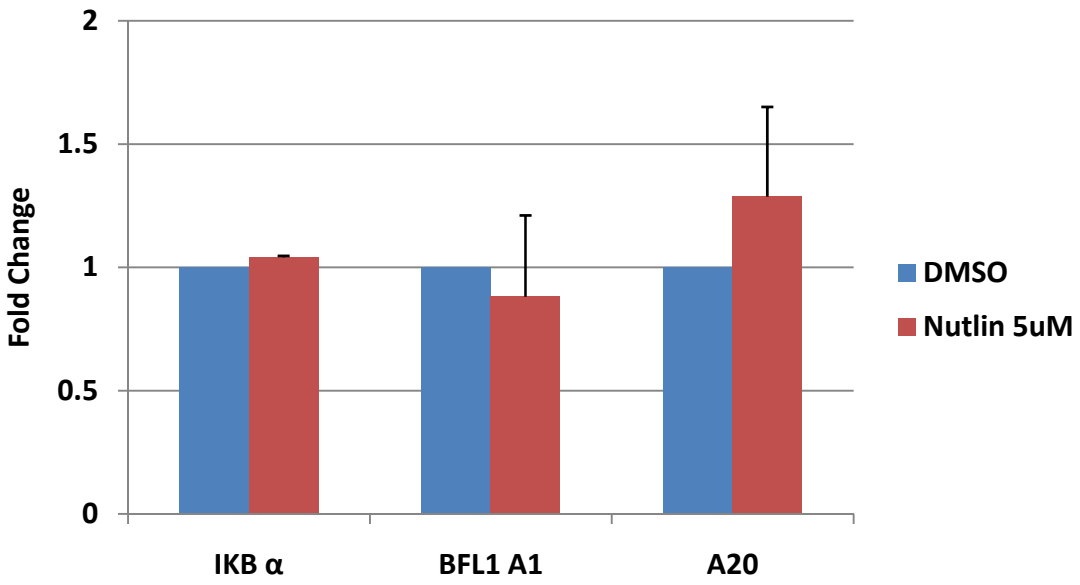
Supplemental Figure 1. Nutlin treatment of BL cells or LCLs with mutant p53 did not induce apoptosis. Each cell line was treated for 48h with DMSO, 5, or 10 μM Nutlin-3 followed by assaying for Annexin V staining (AnnexinV-APC, BD Biosciences). DMSO-treated values were set to 1 such that relative apoptosis (Annexin-positivity) is plotted on the y-axis. Values shown are averages +/- standard error of the mean from two independent experiments. WTK1 and NH32 are mutant p53 LCLs derived from the same donor as the WT LCL TK6. Mutu I and Akata are EBV latency I expressing BL cell lines and Mutu III is a latency III expressing BL line.



Supplemental Figure 2. Chemical inhibition of IκB kinase β decreased NFκB DNA binding, target gene expression and induced apoptosis in LCLs. (A) LCLs were treated overnight with DMSO or the indicated concentration of IKKi IV. Nuclear proteins were extracted and subjected to EMSA using a ^{32}P-labeled consensus NFκB oligo from the HIV-1 LTR. Each sample was incubated with hot probe and either no competing oligo, wild-type , or mutant cold competitor. Quantification of DNA binding activity was performed using a Molecular Dynamics Typhoon Phosphor-imager and plotted below the EMSA. (B) IKKβ inhibitor IV (Calbiochem) was added to LCLs at the noted concentrations. 48h following treatment, cells were assayed for NFκB target gene expression by quantitative RT-PCR. (C) LCLs were treated with noted IKK inhibitor concentrations and assayed at 48h for apoptosis by Annexin V-positivity.



Supplemental Figure 3. ICAM1 is an endogenous reporter of NFκB activity in LCLs. (A) LCLs were sorted based on ICAM1 surface expression levels. Representative FACS histograms are shown for one sorting experiment. Below the initial LCL population, separated (hi, middle, and low) populations were analyzed for ICAM1 expression following sorting. (B) Sorted LCL populations (ICAM lo, med, hi) were assayed by quantitative RT-PCR for NFκB target gene expression. The levels of the detected mRNAs correlated positively with the ICAM1 surface expression. (C) LCLs were treated with DMSO or 5 μM Nutlin-3 for 4h (left) or 8h (right) and assayed for ICAM1 expression level. Red histogram traces indicate secondary antibody alone, while blue and green represent ICAM1 primary and Alexa 488 anti-mouse secondary staining for DMSO or Nutlin-treated cells. Mean fluorescent intensity for Nutlin-treated cells did not vary from DMSO-treated cells by more than 10%.



Supplemental Figure 4. NFκB target genes are not affected by Nutlin treatment in LCLs. LCLs were treated with DMSO or 5 μM Nutlin for 8h. RNA was harvested and subjected to quantitative RT-PCR for three NFκB target genes (IκBα, Bfl1-A1, and A20). GUS-normalized mRNA levels are shown on the y-axis relative to the DMSO treated cells.