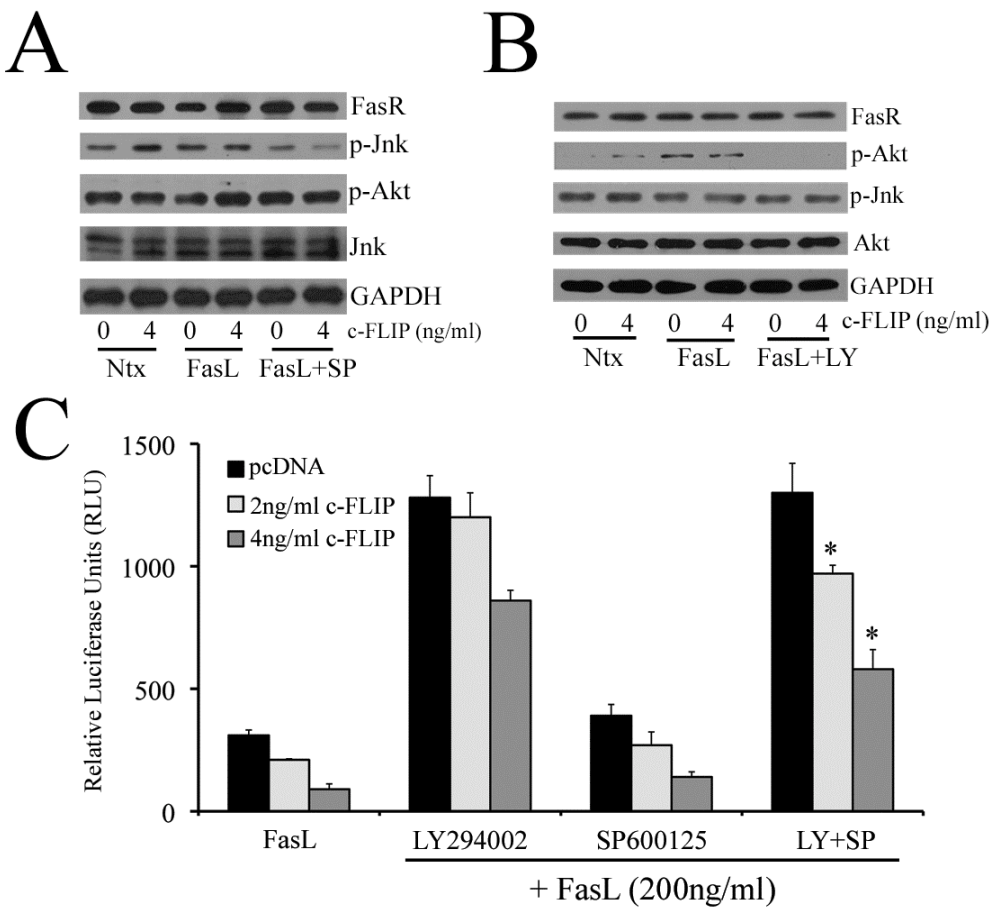
Supplemental Fig. 1. The effects of FasL on Akt- and c-FLIP-mediated inhibition of NF-κB are independent of JNK and Fas Receptor levels. *A, B.* 293T cells were transfected with either empty vector control pcDNA vector, or with c-FLIP plasmid using LipofectAMINE 2000 using standard protocols. Following transfection, cells were exposed to 200ng/ml FasL in the absence or presence of JNK inhibitor SP600125 (10μM) as indicated in (*A),* or with Akt inhibitor LY294002 (20μM) as shown in (*B)* for 12 h. Cells were lysed and assayed for levels of proteins indicated using immunoblot analysis. Blots were re-probed for GAPDH to ensure equal loading. Addition of FasL, and co-treatment with either JNK or Akt inhibitors did not have any significant effect on Fas receptor levels. Phospho-JNK *(A)* and phospho-Akt *(B)* levels were inhibited by SP600125 and LY294002 respectively, indicating that the inhibitors were active. Further, addition of SP600125 did not affect phospho-Akt levels in the absence or presence of FasL as shown in (*A)*. Similarly, Akt inhibitor LY294002 also did not affect levels of activated JNK either *(B)*, suggesting a lack of cross-talk between Akt and JNK in the context of Akt-mediated inhibition of NF-κB in the presence of FasL. *C.* 293T cells were co-transfected with 100ng/well NF-κB-Luc, 10ng/well pRL-tk normalization luciferase construct, and either empty pcDNA3 vector or increasing concentrations (2ng/ml, 4ng/ml) of c-FLIP. Total DNA in each well was kept equal by addition of empty control vector. Transfected cells were treated either with FasL alone (200ng/ml) or in the presence of Akt inhibitor LY294002 (20μM) or JNK inhibitor SP600125 (10μM) for 12 h. Cells were then harvested and assayed for NF-κB luciferase activity. Plots show relative NF-κB activity over non-treated control. Values are mean ± SD (n = 4). *P < 0.05 versus corresponding samples treated with LY294002 alone. Pre-

treatment with LY294002 released NF-κB from Akt-mediated repression in the presence of FasL. NF-κB levels remained comparable to SFM-treated controls (compare data sets for *Ntx* and *FasL+SP*) for cells treated with SP600125. However, treatment of cells with both LY294002 and SP600125 together did have a significant effect on NF-κB levels. SP294002 inhibited the increase in NF-κB levels observed using LY294002 alone (see data set for *FasL+LY*), suggesting a probable role for JNK in Akt-mediated effects on NF-κB. However, a lack of effect of SP294002 on Akt levels (see immunoblot in (*A*)) was also observed, suggesting that the effect of SP294002 on the levels of NF-κB, if any, may be independent of Akt levels.

Supplemental Fig. 2. Effects of FasL on Akt and c-FLIP and their cross-talk observed in 293T cells is recapitulated in Jurkat T-cell lymphocytes. *A.* 1 x 10^6 Jurkat T-cells were plated into a 6-well plate in complete medium, and transfected with 50ng/ml eGFP plasmid using Metafectene Pro reagent. Cells were imaged 24 h post transfection for green fluorescence. The image indicates low albeit measurable level of transfection of the cells. *B.* In addition to transfection with 200ng/well NF-κB-Luc and 20ng/well pRL-tk normalization luciferase construct, cells were transfected with either empty pcDNA3 vector or 50ng/ml c-FLIP. 24 h post transfection, cells were treated with 200ng/ml FasL in the absence or presence of 20 μM LY294002 for 12h, and assayed for NF-κB luciferase activity, and data plotted as shown. Values are mean ± SD (n = 3). *P < 0.05 versus non-treated control; *P < 0.05 and **P < 0.05 versus respective pcDNA controls as shown. The data shows that inhibition of Akt (*FasL+LY*) led to increased activation of NF-κB, but SP600125 did not have any significant effects. Transfection with c-FLIP could further inhibit FasL mediated activation of NF-κB in the presence and absence of

inhibitors, recapitulating the results obtained with 293T cells (see Fig. 5C). *C.* Jurkats treated with 50ng/ml c-FLIP in the absence or presence of FasL with or without LY294002 were assayed for several proteins using immunoblotting analysis. An increase in both phospho-I-κB and NF-κB are observed in the presence of Akt inhibitor LY294002, confirming the inhibitory role for Akt on NF-κB in the presence of FasL. *D.* Jurkats treated with FasL in the presence or absence of inhibitors showed no significant changes in FasR levels, suggesting that regulation of NF-κB by Akt may be independent of protein levels of death receptor.

Supplemental Figure 1:



Supplemental Figure 2: