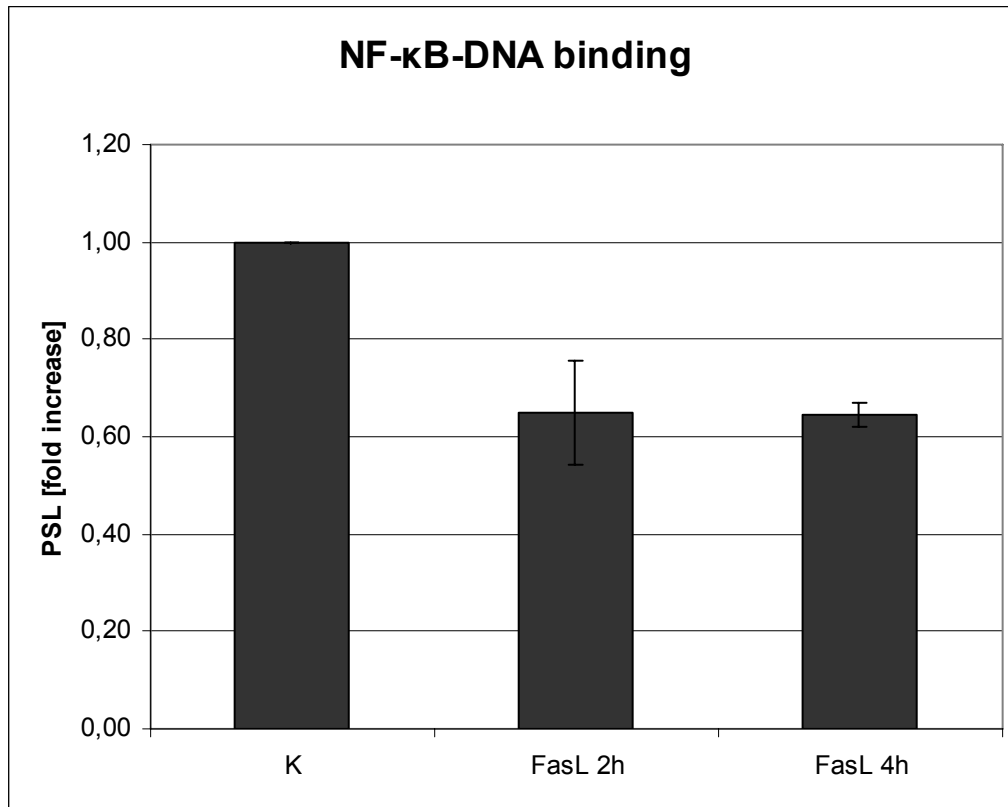


## Supplementary Experiments to ‘ON/OFF and Beyond - a Boolean Model of Apoptosis’

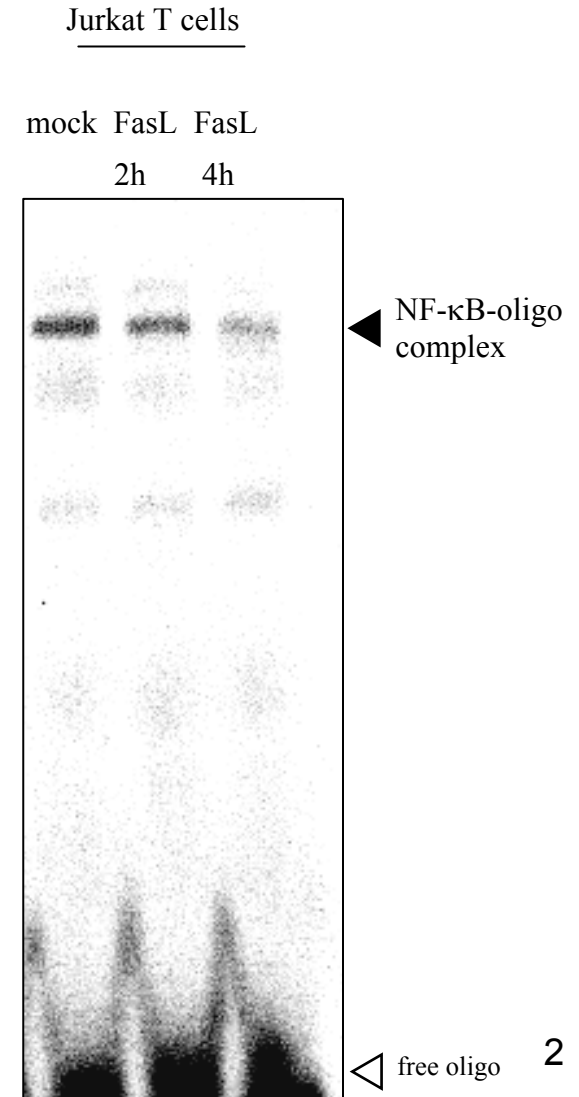
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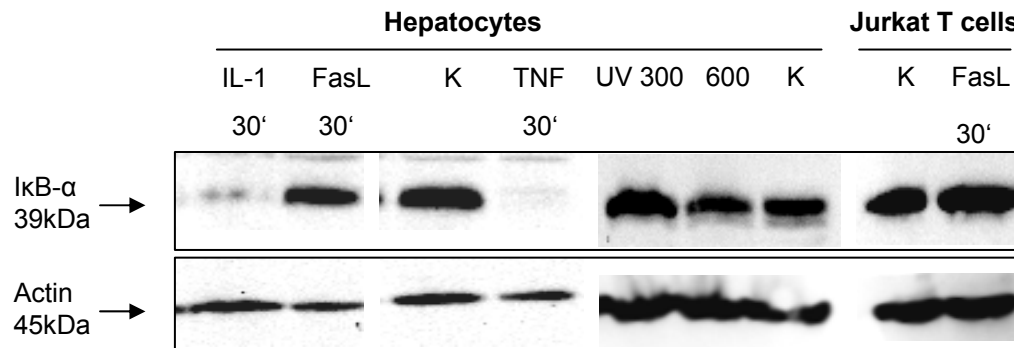
# NF- $\kappa$ B-DNA binding in response to FasL treatment in Jurkat T cells



Jurkat T cells were treated with 25ng/ml FasL for the indicated times and equal amounts of nuclear protein were subjected to EMSA. NF- $\kappa$ B-DNA binding is measured as PSL and expressed as fold increase of untreated cells. Means of two independent experiments with sd are shown.

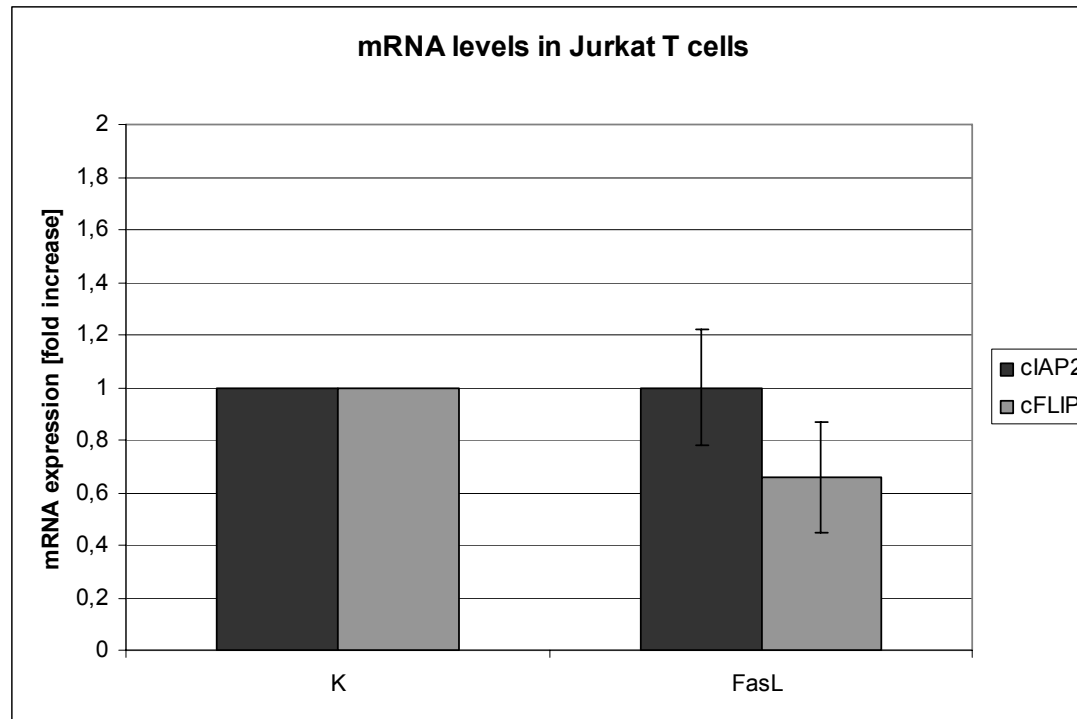


# I $\kappa$ B- $\alpha$ Western Blot in hepatocytes and Jurkat T cells



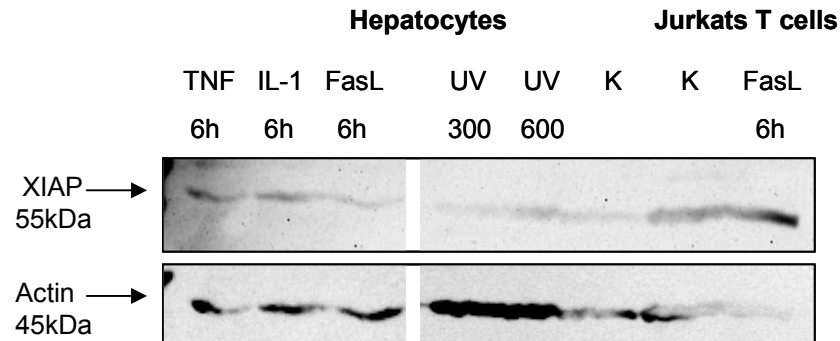
Hepatocytes were stimulated with TNF- $\alpha$  25ng/ml, IL-1 $\beta$  20ng/ml, FasL 50ng/ml, UV irradiation 300J/m<sup>2</sup> or 600 J/m<sup>2</sup> and Jurkat Tcells with FasL 50ng/ml, respectively and total extracts were subjected to western blotting targeting I $\kappa$ B- $\alpha$  and after stripping actin as a loading control. Note that cells treated with UV radiation 600 J/m<sup>2</sup> reveal higher I $\kappa$ B- $\alpha$  levels then the model predicted. After UV radiation cells had to be cultivated further before preparing total extracts so that initial NF- $\kappa$ B activity already induced protein newsynthesis of its negative feedback regulator I $\kappa$ B- $\alpha$ . NF- $\kappa$ B is still active in this state which is shown by EMSA in Fig.2.

## cFLIP and cIAP2 mRNA levels in Jurkat T cells



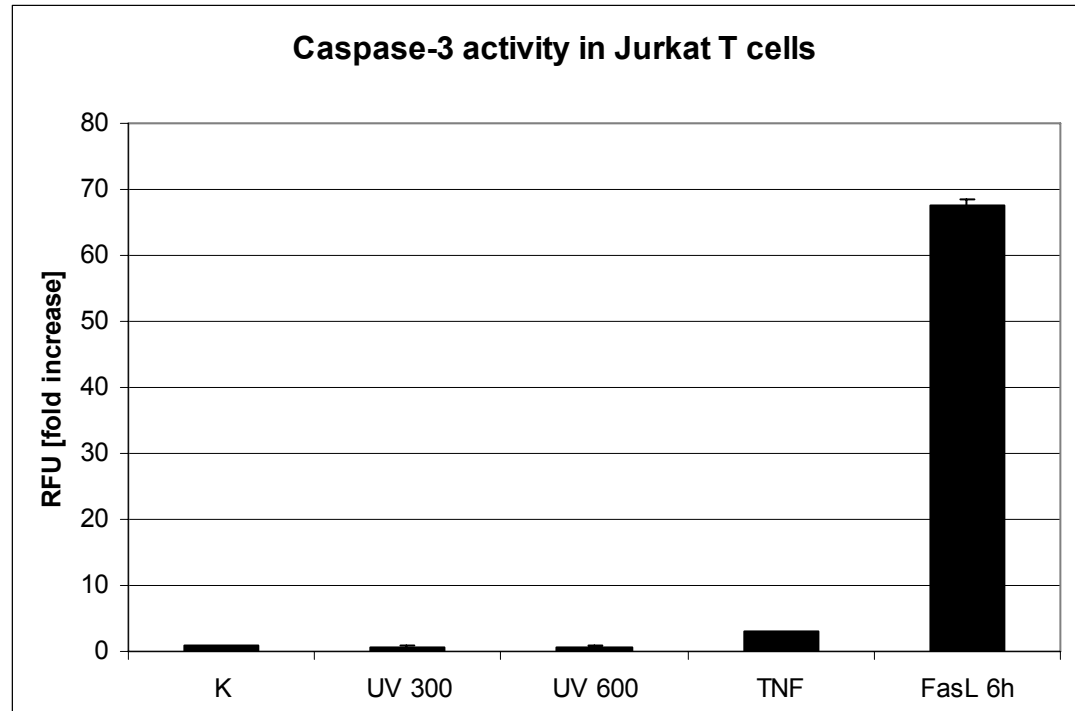
Jurkat T cells were treated with FasL 50ng/ml for 2h, total RNA was isolated and cIAP2 and cFLIP mRNA levels were determined by qRT-PCR. Means of two independent experiments with sd are shown.

# XIAP Western Blot in hepatocytes and Jurkat T cells



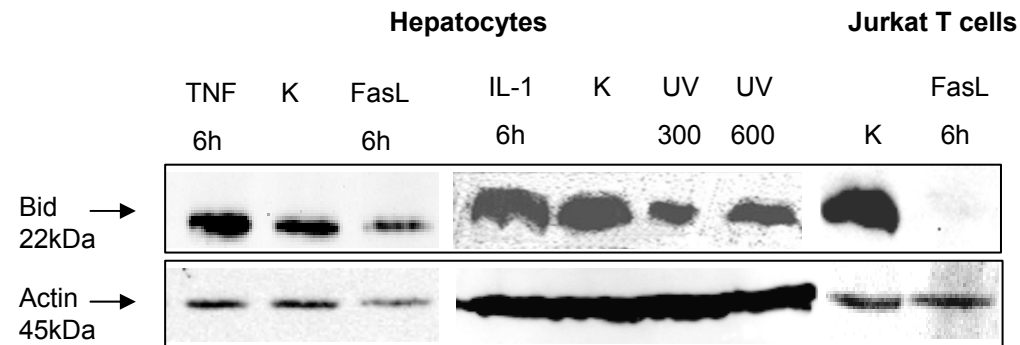
Hepatocytes were stimulated with TNF- $\alpha$  25ng/ml, IL-1 $\beta$  20ng/ml, FasL 50ng/ml, UV irradiation 300J/m<sup>2</sup> or 600 J/m<sup>2</sup> and Jurkat T cells with FasL 50ng/ml, respectively, for the indicated times. Total extracts were subjected to western blotting targeting XIAP and after stripping actin as a loading control.

# Caspase-3 activity in response to FasL and UV irradiation in Jurkat T cells



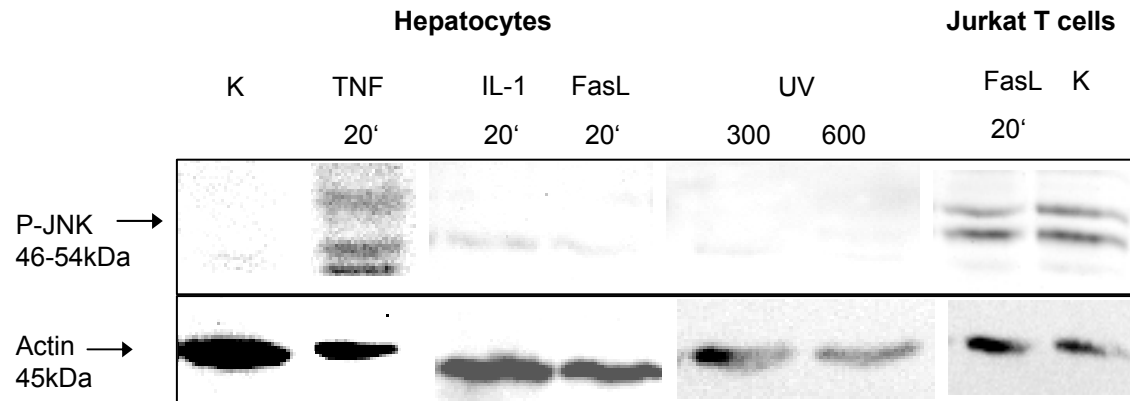
Jurkat T cells were treated with 25ng/ml TNF- $\alpha$ , 50ng/ml FasL, UV irradiation 300 J/m<sup>2</sup> or 600 J/m<sup>2</sup> or 100nM insulin for the indicated times and caspase-3 activity was measured.

# Bid Western Blot in hepatocytes and Jurkat T cells



Hepatocytes were stimulated with TNF- $\alpha$  25ng/ml, IL-1 $\beta$  20ng/ml, FasL 50ng/ml, UV irradiation 300J/m<sup>2</sup> or 600 J/m<sup>2</sup> and Jurkat T cells with FasL 50ng/ml, respectively, for the indicated times. Total extracts were subjected to western blotting targeting Bid and after stripping actin as a loading control.

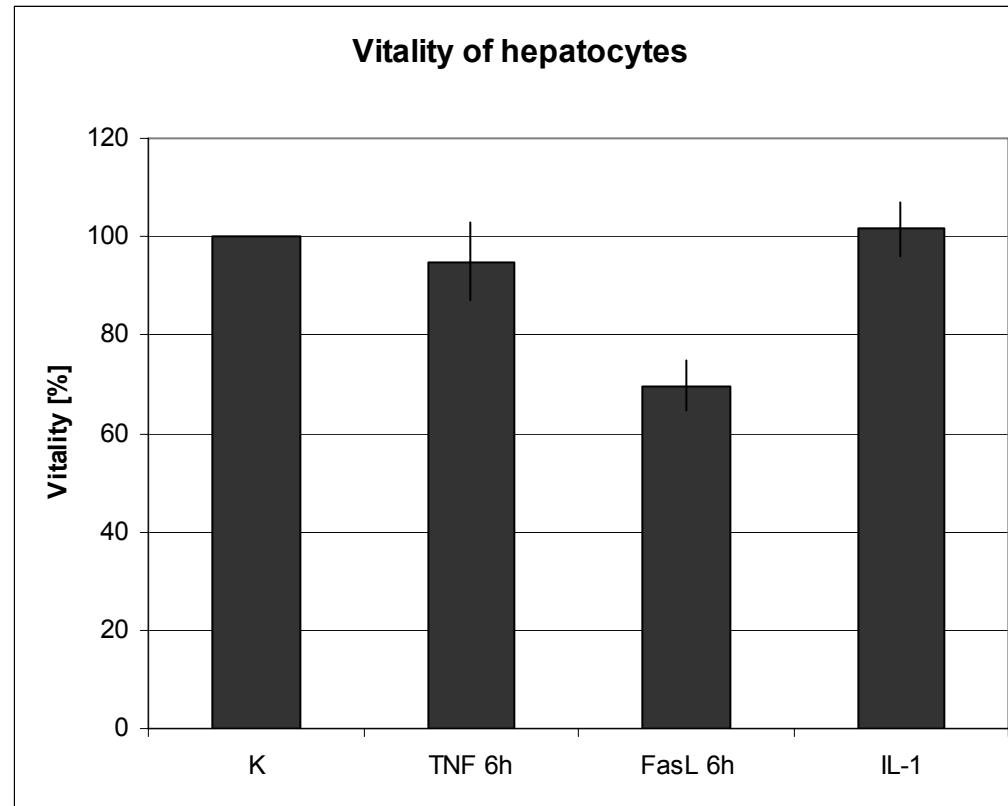
# P-JNK Western Blot in hepatocytes and Jurkat T cells



Hepatocytes were stimulated with TNF- $\alpha$  25ng/ml, IL-1 $\beta$  20ng/ml, FasL 50ng/ml, UV irradiation 300J/m<sup>2</sup> or 600 J/m<sup>2</sup> and Jurkat T cells with FasL 50ng/ml respectively for the indicated times. Total extracts were subjected to western blotting targeting P-JNK and after stripping actin as a loading control.

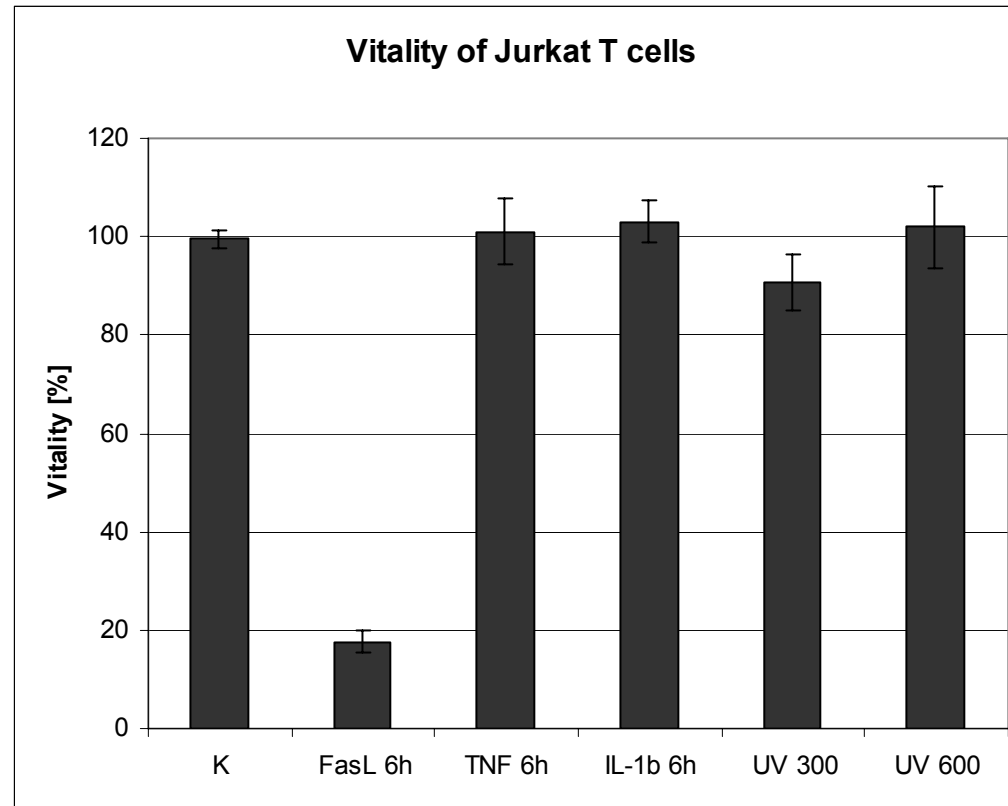


# Cytotoxicity of TNF- $\alpha$ , FasL and IL-1 $\beta$ in hepatocytes



Primary mouse hepatocytes were treated with 25ng/ml TNF- $\alpha$ , 50ng/ml FasL or 20ng/ml IL-1 $\beta$  for the indicated times and vitality was measured by MTT assay and referred to untreated cells. Means of at least 3 independent experiments are shown.

# Cytotoxicity of TNF- $\alpha$ , FasL, UV irradiation and IL-1 $\beta$ in Jurkat T cells



Jurkat T cells were treated with 25ng/ml TNF- $\alpha$ , 50ng/ml FasL, UV irradiation 300 J/m<sup>2</sup> or 600 J/m<sup>2</sup> or 20ng/ml IL-1 $\beta$  for the indicated times and vitality was measured by MTT assay and referred to untreated cells. Means of at least 3 independent experiments are shown.