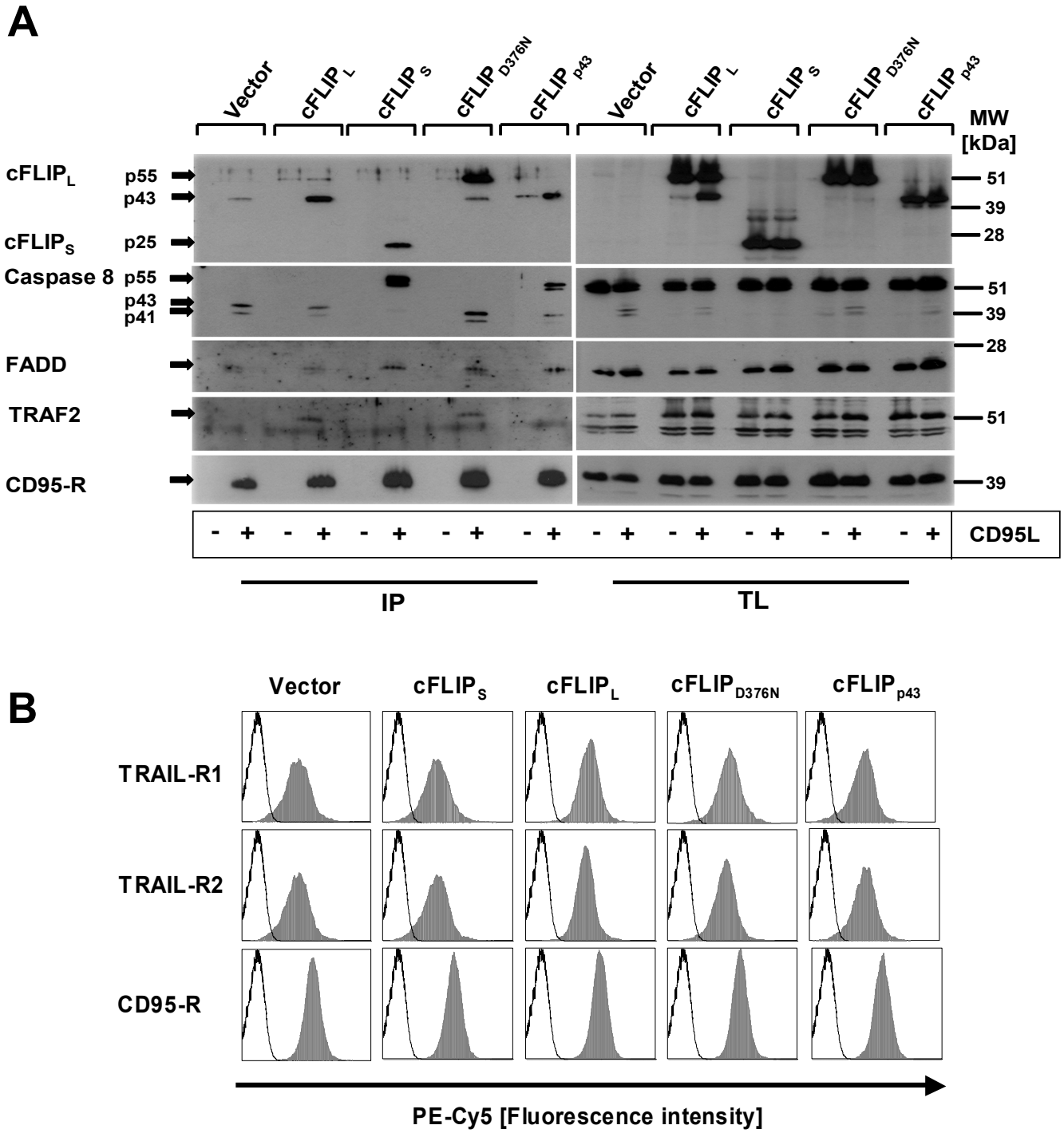
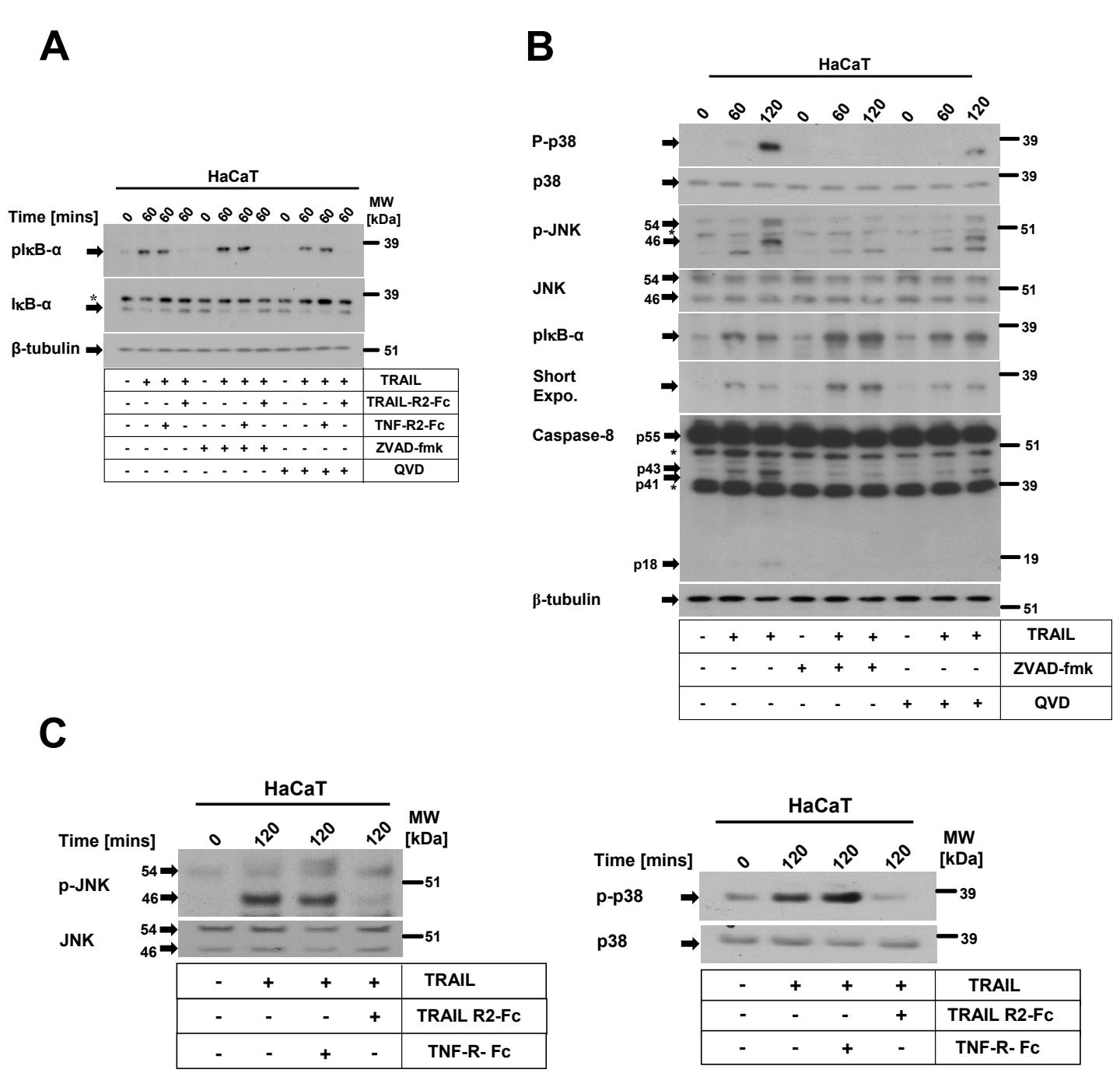
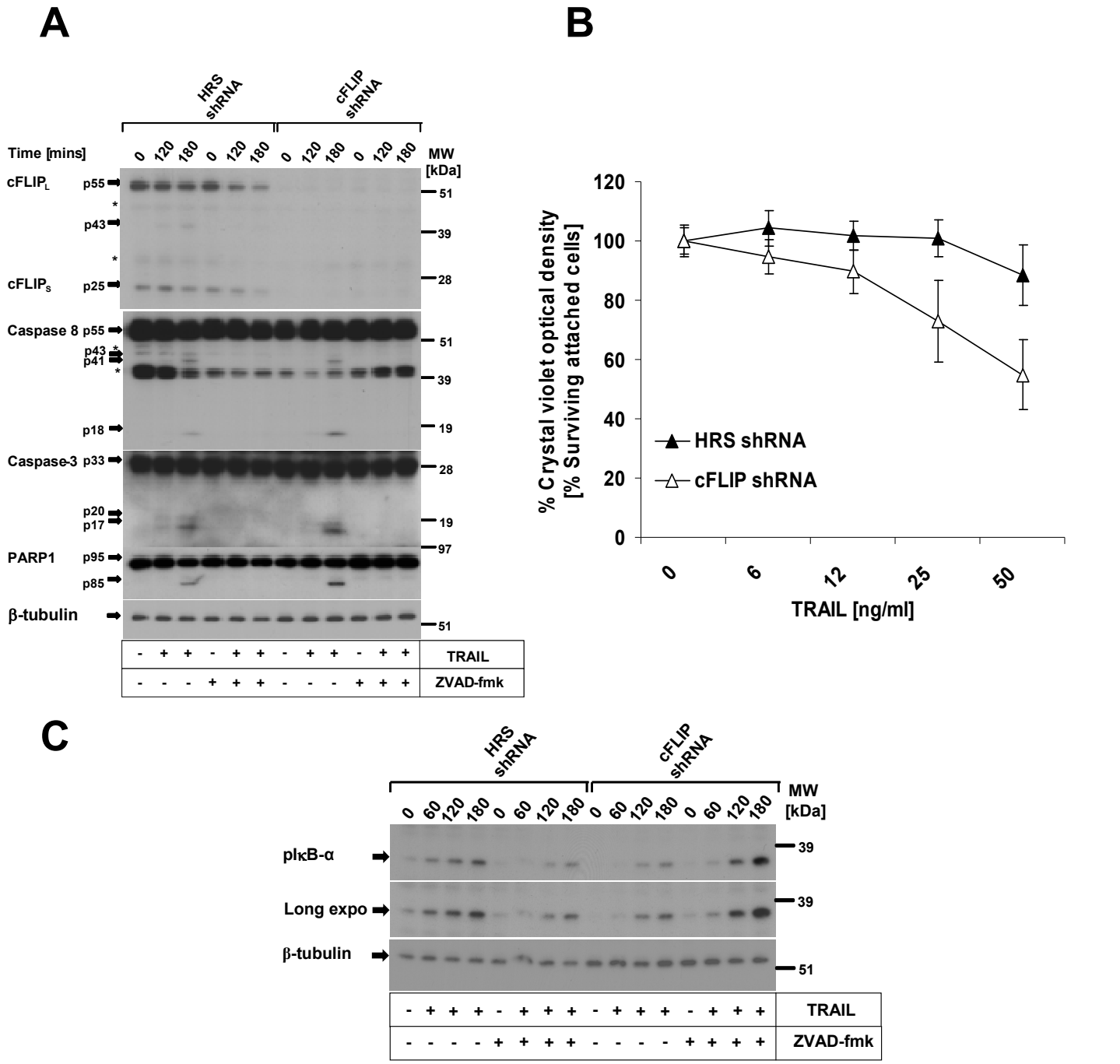


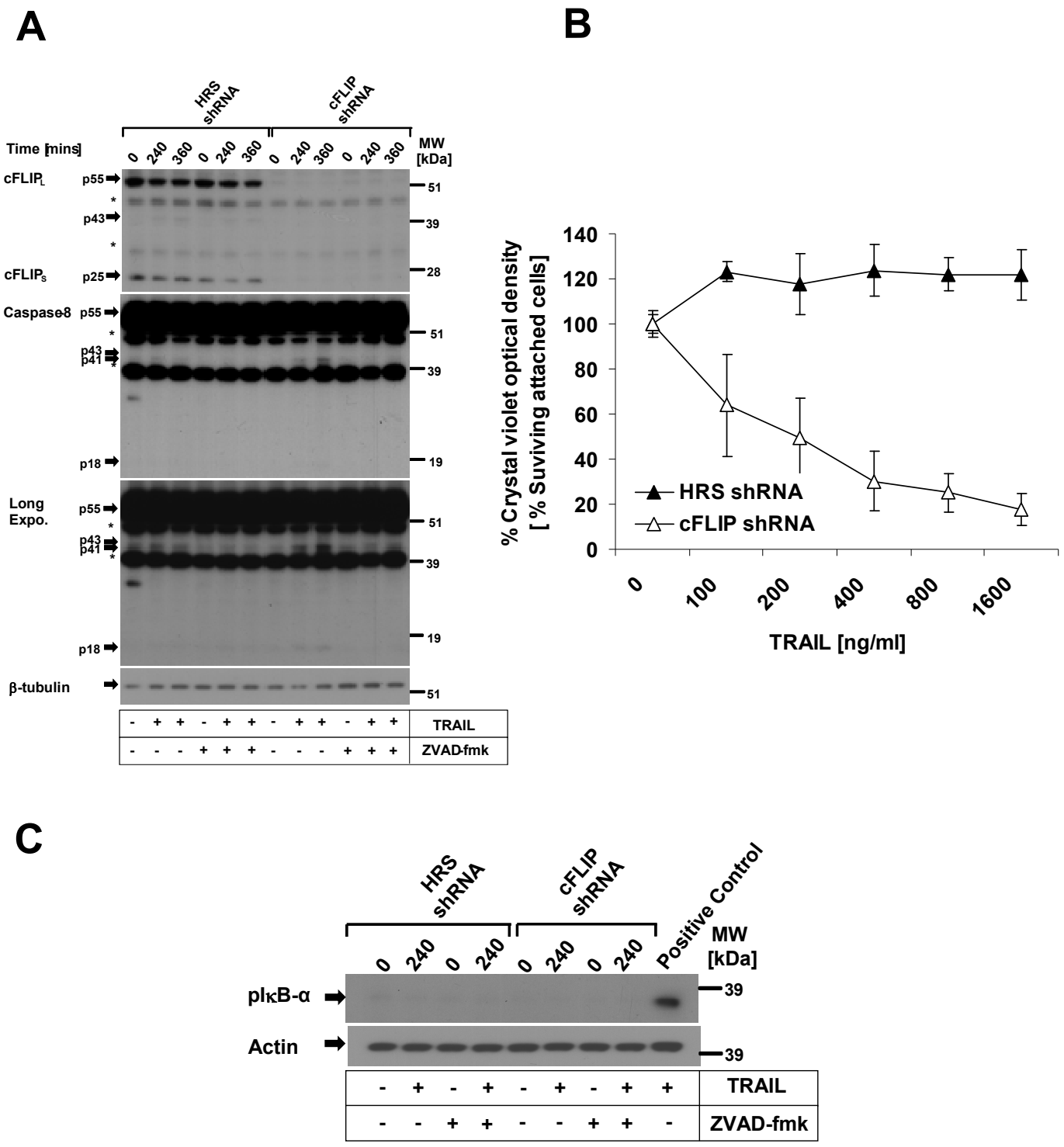
Supplemental Fig. 1 Different cFLIP isoforms modulate the composition of the CD95 DISC. Differential composition of the CD95 DISC **A)** in keratinocytes expressing different isoforms or mutants of cFLIPL-expression. DISC analysis (IP) was performed from a total of 5x106 cells. Precipitates of non-stimulated cells served as specificity controls for ligand affinity precipitates and were assayed for comparable immune precipitation of CD95 as a control. Cells were characterized for cFLIP, caspase-8, FADD, RIP-1, and TRAF2 recruitment by western blotting. Total cellular lysates (Lysates) were analyzed in parallel from all samples. **B)** TRAIL-R1 - TRAIL-R4 and CD95 cell surface expression of cFLIP isoforms/mutants overexpressing HaCaT keratinocytes were determined by FACS analysis. Cultured cells were stained with TRAIL-R1 (HS101), TRAIL-R2 (HS201), TRAIL-R3 (HS301), TRAIL-R4 (HS402), and CD95 (APO-1 IgG1) primary Abs as well as isotype-matched control Abs. Filled curves indicate receptor specific staining as compared to isotype-matched control staining (open curves).



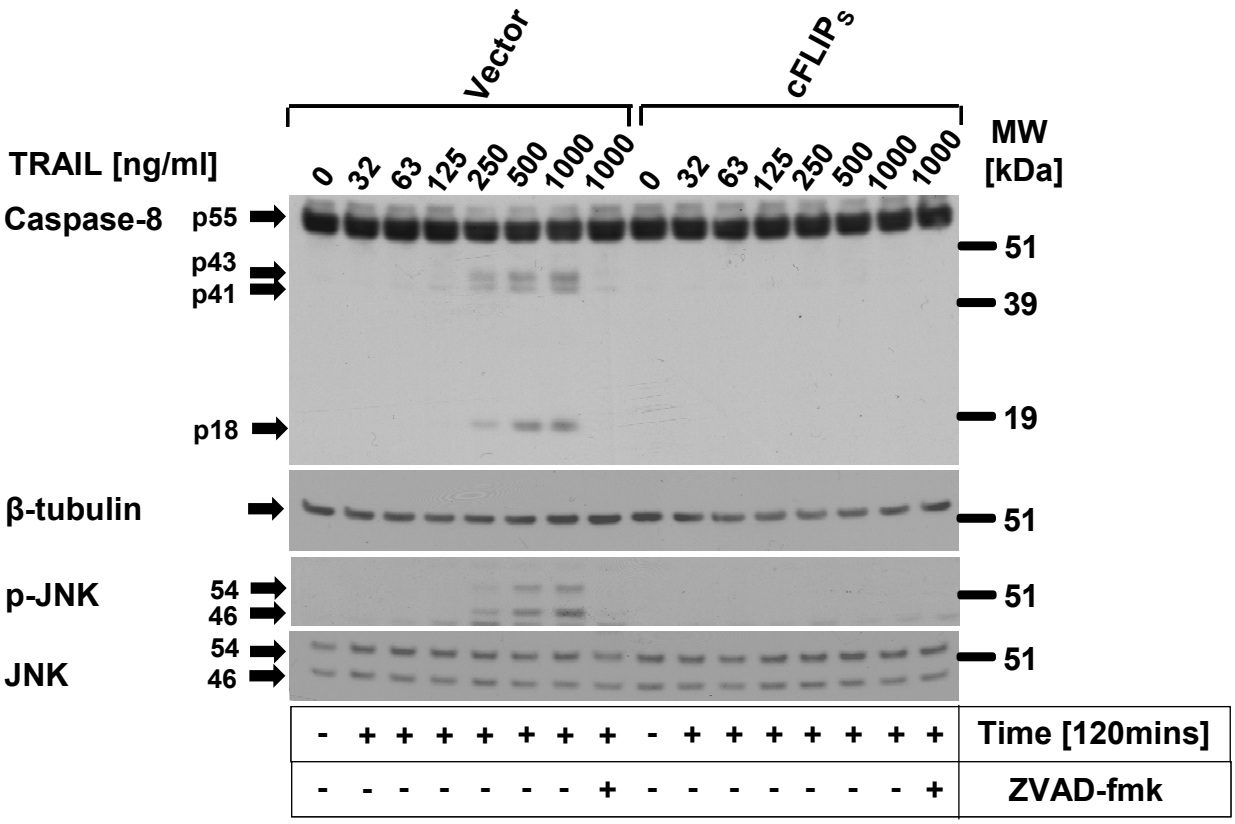
Supplemental Fig. 2 TRAIL-induced non-apoptotic signalling pathways are independent of an autocrine loop of TNF activated by ZVAD-fmk. A) HaCaT keratinocytes were pre-incubated with either ZVAD-fmk or TRAIL-R2-Fc or TNF-R2-Fc, or the combination of caspase inhibitor and receptor fusion proteins as indicated for 1hr. Cells were subsequently stimulated with TRAIL (0.1μg/ml) for the indicated time points. Cellular lysates were analysed for pIκB-α and IκB-α degradation. β-tubulin served as loading control. Asterisk indicates non-specific band. **B)** HaCaT keratinocytes were pre-incubated with either ZVAD-fmk (10 μM) or QVD (10 μM) for 1hr and stimulated with TRAIL (0.1μg/ml) for the indicated time points. Cellular lysates were analysed for pIκB-α, p-p38, p-JNK, respectively. β-tubulin, p38, or JNK served as loading controls. **C)** HaCaT keratinocytes were pre-incubated with either TRAIL-R2-Fc (20μg/ml) or TNF-R2-Fc (20μg/ml) for 1hr and stimulated with TRAIL (0.5μg/ml) for the indicated time points. Cellular lysates were analysed for p-JNK (left), or p-p38 (right) activation. p38, and JNK served as loading controls.



Supplemental Fig. 3 Knockdown of cFLIP proteins leads to increased sensitivity to TRAIL-mediated apoptosis and NF-κB activation in A5RT3 keratinocytes. A5RT3 were infected with cFLIP-specific siRNA using recombinant retroviruses as explained earlier in the methods section. Cells were subsequently stimulated with 50ng of recombinant TRAIL for indicated time points and analysed for **A)** cFLIP, caspase-8, and caspase-3 expression and cleavage. Arrows indicate molecular weights of protein cleavage fragments. Western blotting of the caspase substrate PARP-1 and detection of the 85kDa fragment served as marker for caspase activity under those conditions. Analysis of β-tubulin protein expression served as loading control. **B)** Knockdown of cFLIP sensitizes to TRAIL-induced cell death. A5RT3 generated as described in A) were treated with the indicated concentrations of TRAIL for 24 hours. Viability was subsequently examined by crystal violet assay. Shown are mean ± SD of two independent experiments. **C)** Down regulation of cFLIP leads to enhanced pIκB-α activation, Transduced cells were incubated with TRAIL for 50ng for indicated time points and subsequently analysed for the activation of pIκB-α. β-tubulin served as a loading control.



Supplemental Fig. 4 Knockdown of cFLIP proteins leads to increased sensitivity to TRAIL-mediated apoptosis and but NF-κB activation is unchanged in IGR melanoma cells. IGR were infected with cFLIP-specific siRNA using recombinant retroviruses as explained earlier in the methods section. Cells were subsequently stimulated with TRAIL (400 ng/ml) for indicated time points and analysed for **A)** cFLIP, caspase-8 expression and cleavage. Arrows indicate molecular weights of protein cleavage fragments. Analysis of β-tubulin protein expression served as loading control. **B)** Knockdown of cFLIP sensitizes to TRAIL-induced cell death. IGR were generated as described in (A) and were treated with the indicated concentrations of TRAIL for 24 hours. Viability was subsequently examined by crystal violet assay. Shown are mean ± SD of two independent experiments **C)** Downregulation of cFLIP does not allow for activation of NF-κB activation as determined by phosphorylation of IκB-α. Transduced cells were incubated with TRAIL (400ng/ml) for the indicated time points.



Supplemental Fig. 5 cFLIP confers resistance to TRAIL- induced apoptosis and completely blocks TRAIL-induced caspase-8 and JNK activation in HaCaT keratinocytes. A) Control cells or $cFLIP_S$-expressing HaCaT cells were preincubated with caspase inhibitor (ZVAD-fmk) or left untreated. Cells were then treated with TRAIL (0,5 µg/ml; left panel) Subsequently, 3µg of total cellular lysates were analyzed by western blotting for caspase-8, JNK activation. β-tubulin served as a control for comparable loading of cellular protein.