

Pharmacological targeting of c-FLIP_L and Bcl-2 family members promotes apoptosis in CD95L-resistant cells

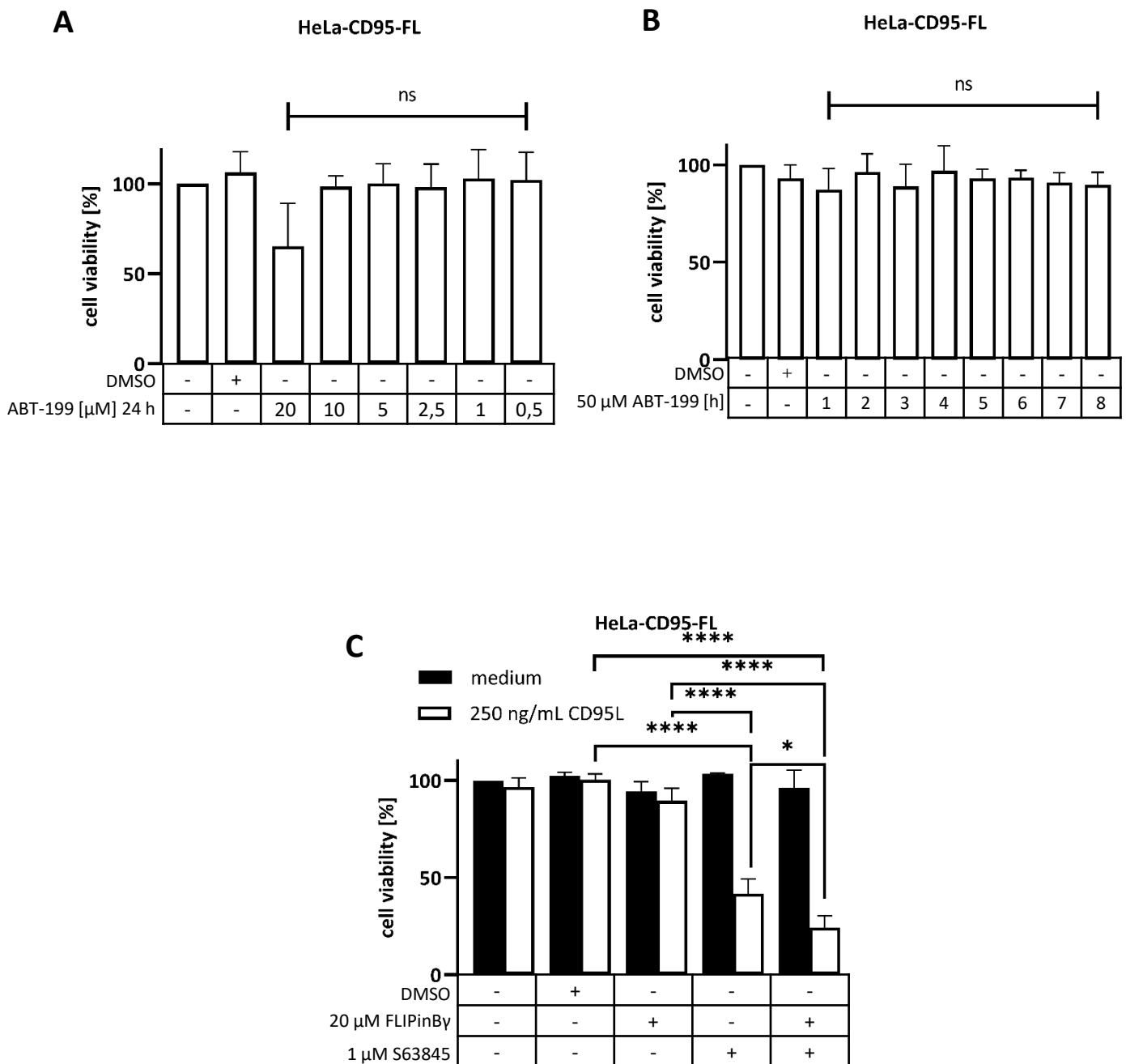
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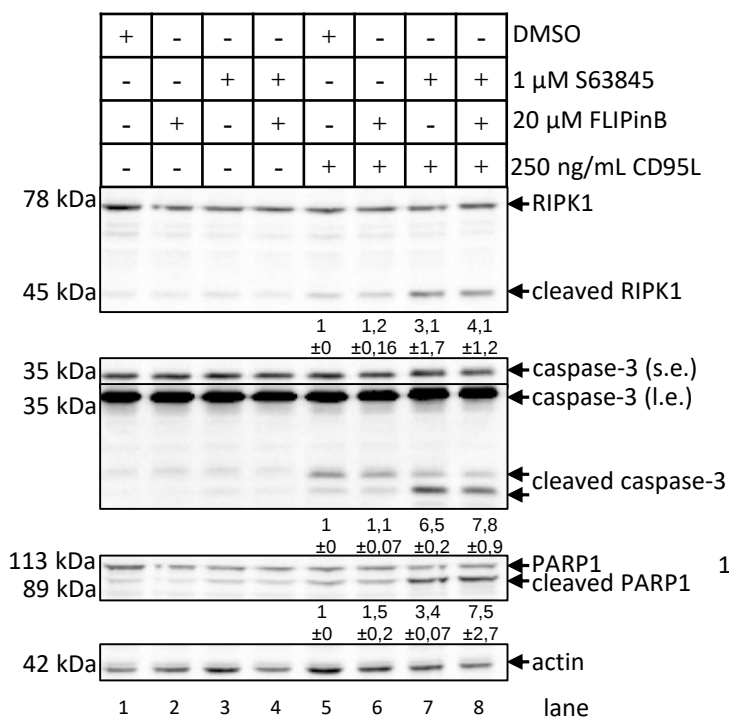


Supplementary Figure 1: Co-treatment with ABT-199 and CD95L/S63845/FLIPinBy of HeLa-CD95-FL cells

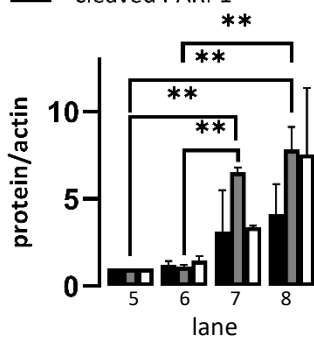
A, B) HeLa-CD95-FL cells were stimulated with the indicated concentrations of ABT-199 over 24 h (A) or for 8 h with 50 μM ABT-199 (B). **C)** HeLa-CD95-FL cells were treated with 20 μM of FLIPinBy and 1 μM S63845 for 2 h. Afterwards the cells were treated with CD95L for 6 h. The cell viability was measured using the Cell Titer-Glo-Luminescent Cell Viability Assay. Unstimulated cells were taken as 100 %. Mean and standard deviation are shown (n=3). For statistical analysis ANOVA Post Hoc Tukey tests were used. ****p<0,0001; ***p<0,0005; **p<0,005; *p<0,05; ns: not significant

A

HeLa-CD95-FL

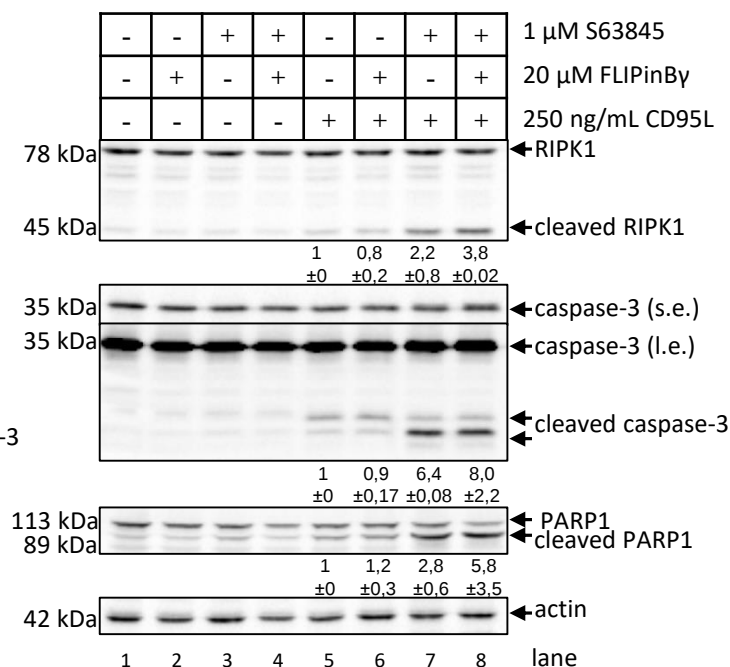


■ cleaved RIPK1 ■ p17
□ cleaved PARP1

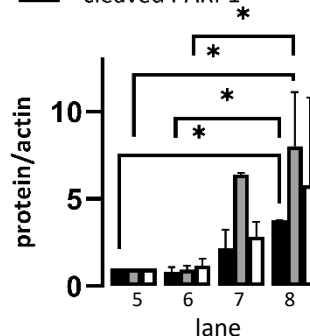


B

HeLa-CD95-FL

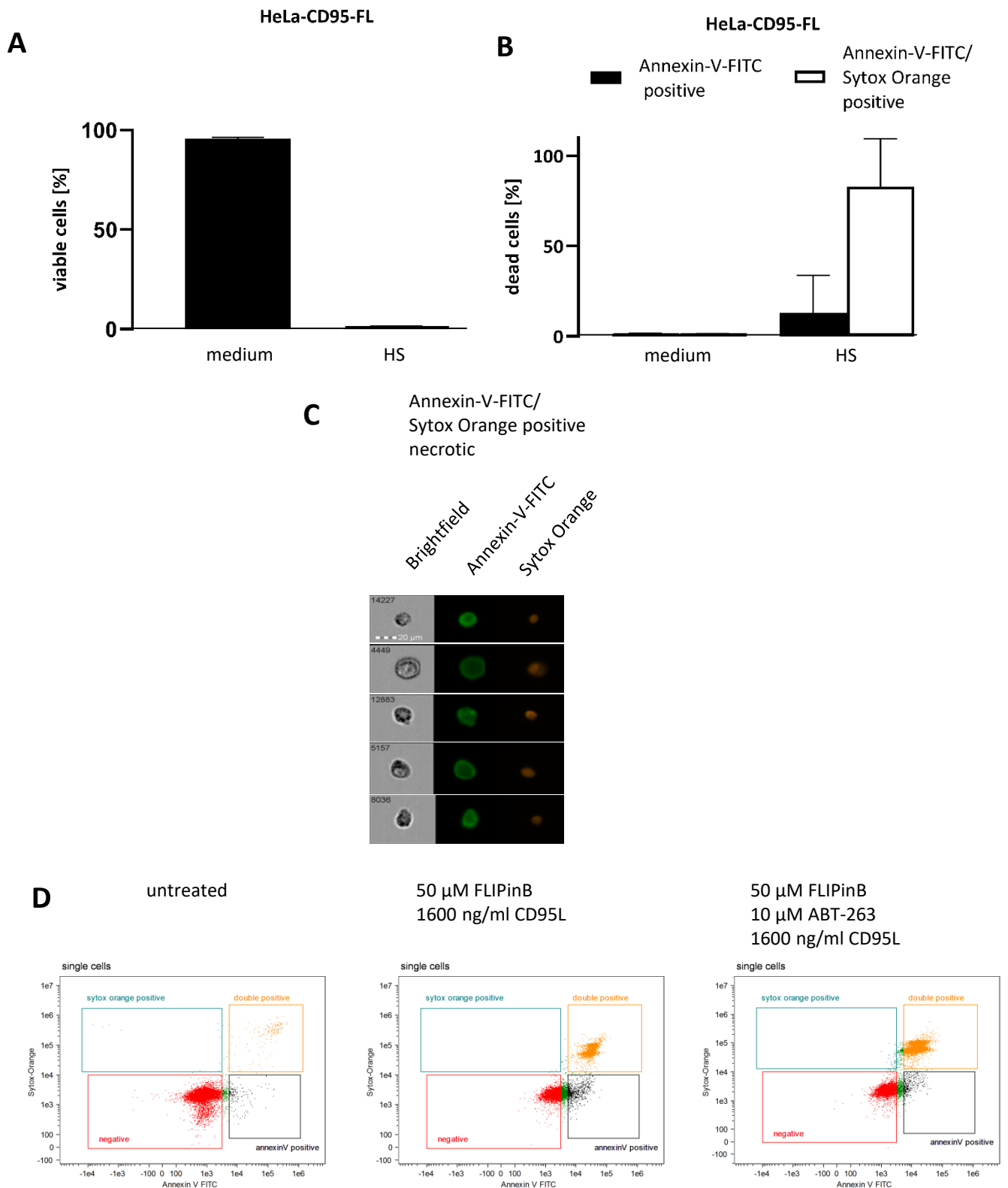


■ cleaved RIPK1 ■ p17
□ cleaved PARP1



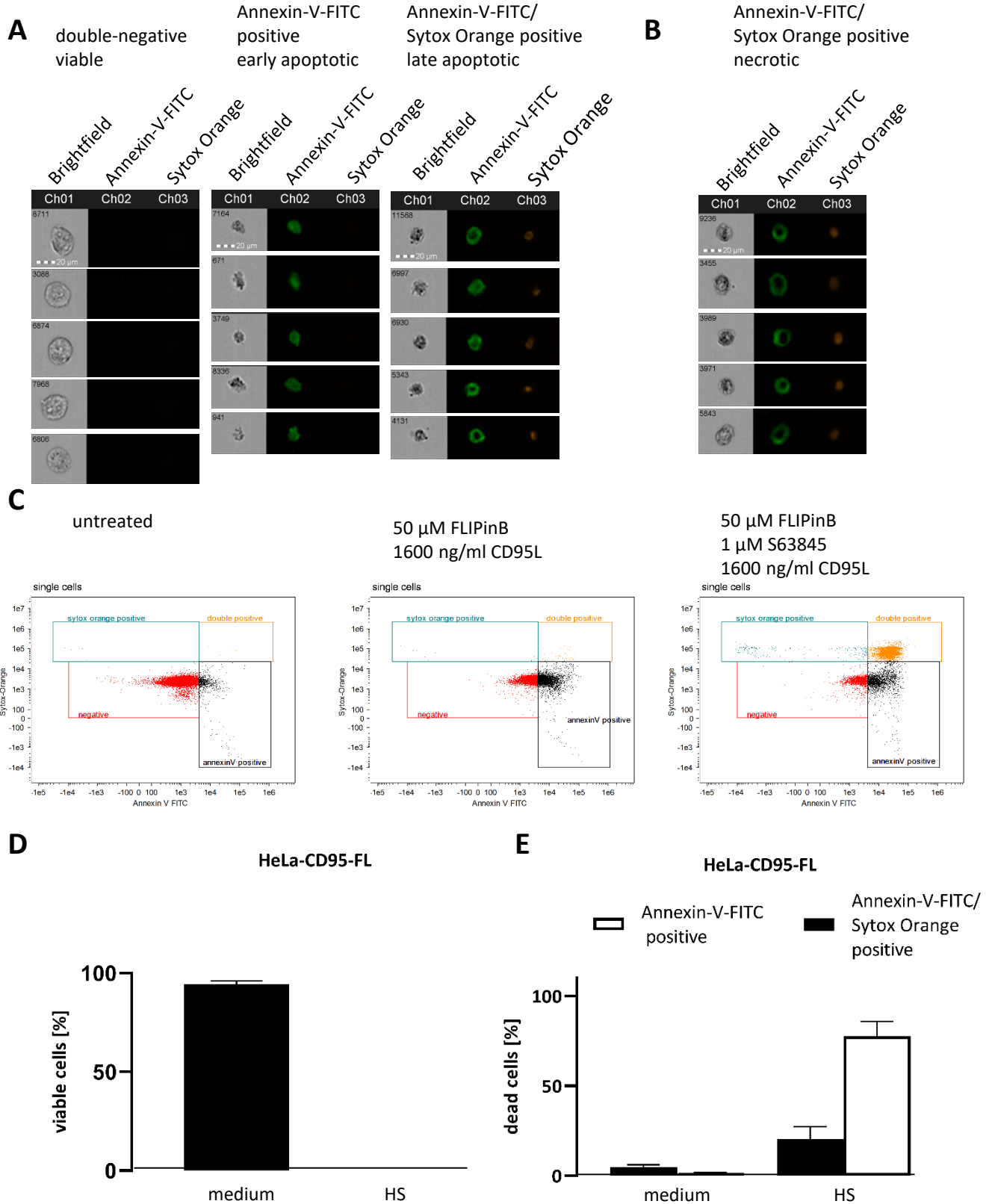
Supplementary Figure 2: Co-treatment leads to an increase of cleavage products of apoptotic key proteins.

A, B) HeLa-CD95-FL cells were pretreated for 2 h with the indicated concentrations of S63845 and FLIPinB (A) or FLIPinBy (B). Afterwards the cells were stimulated with 250 ng/ml CD95L for 4 h. Total cell lysates were analyzed by Western Blot with the indicated antibodies. Actin served as a loading control. The Western Blot Quantification is shown with mean and standard deviation under the corresponding Western Blot and as diagrams below the Western Blot. (n=2). For statistical analysis ANOVA Post Hoc Tukey tests were used. ****p<0,0001; ***p<0,0005; **p<0,005; *p<0,05; ns: not significant



Supplementary Figure 3: Heat shock treatment was used as a positive control for CD95L/ABT-263/FLIPinB treatment of HeLa-FL cells, which have shown double positive staining

A) HeLa-CD95-FL cells were incubated for 10 min at 56°C for heat shock (HS). The unstimulated and heat shock-treated samples were measured using AMNIS FlowSight and gated for double-negative cells (viable cells) **B)** The HS samples were measured using AMNIS FlowSight and gated for Annexin-V-FITC/Sytox Orange positive and Annexin-V FITC cells. **C)** Representative pictures of the treated cells. Shown are Annexin-V-FITC/Sytox Orange positive cells. Mean, standard deviation and representative pictures are shown (n=3). **D)** The gating strategy is shown for double-negative, Annexin-V-FITC positive and Annexin-V-FITC/Sytox Orange positive populations after the indicated stimulation. The samples were measured using AMNIS FlowSight.



Supplementary Figure 4: Heat shock treatment used as a positive control for CD95L/S63845/FLIPinB treatment results in double positive staining of HeLa-CD95-FL cells.

A, B Representative pictures of double-negative (viable), Annexin-V-FITC-positive and Annexin-V-FITC/Sytox Orange positive cells after treatment (A) or Annexin-V-FITC/Sytox Orange positive cells after HS (B) are shown. **C** Shown is the gating strategy for double-negative, Annexin-V-FITC positive and Annexin-V-FITC/Sytox Orange positive populations after the indicated stimulation. Samples were measured with AMNIS FlowSight. **D** HeLa-CD95-FL cells were incubated for 10 min at 56°C for heat shock (HS). Samples were measured using AMNIS FlowSight and gated for double-negative cells (viable cells). **E** Unstimulated (medium) and HS samples were measured using AMNIS FlowSight and gated for Annexin-V-FITC/Sytox Orange positive and Annexin-V FITC cells.