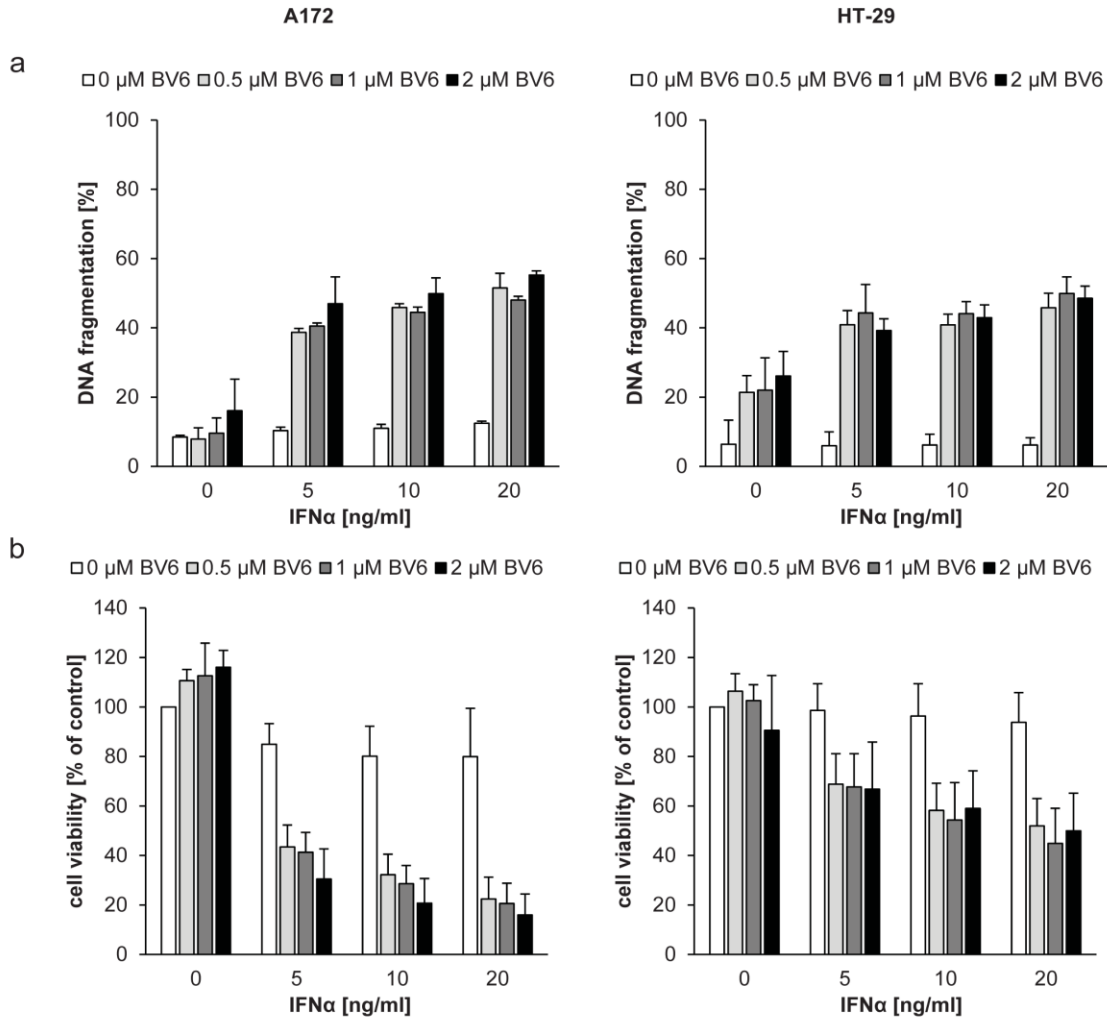


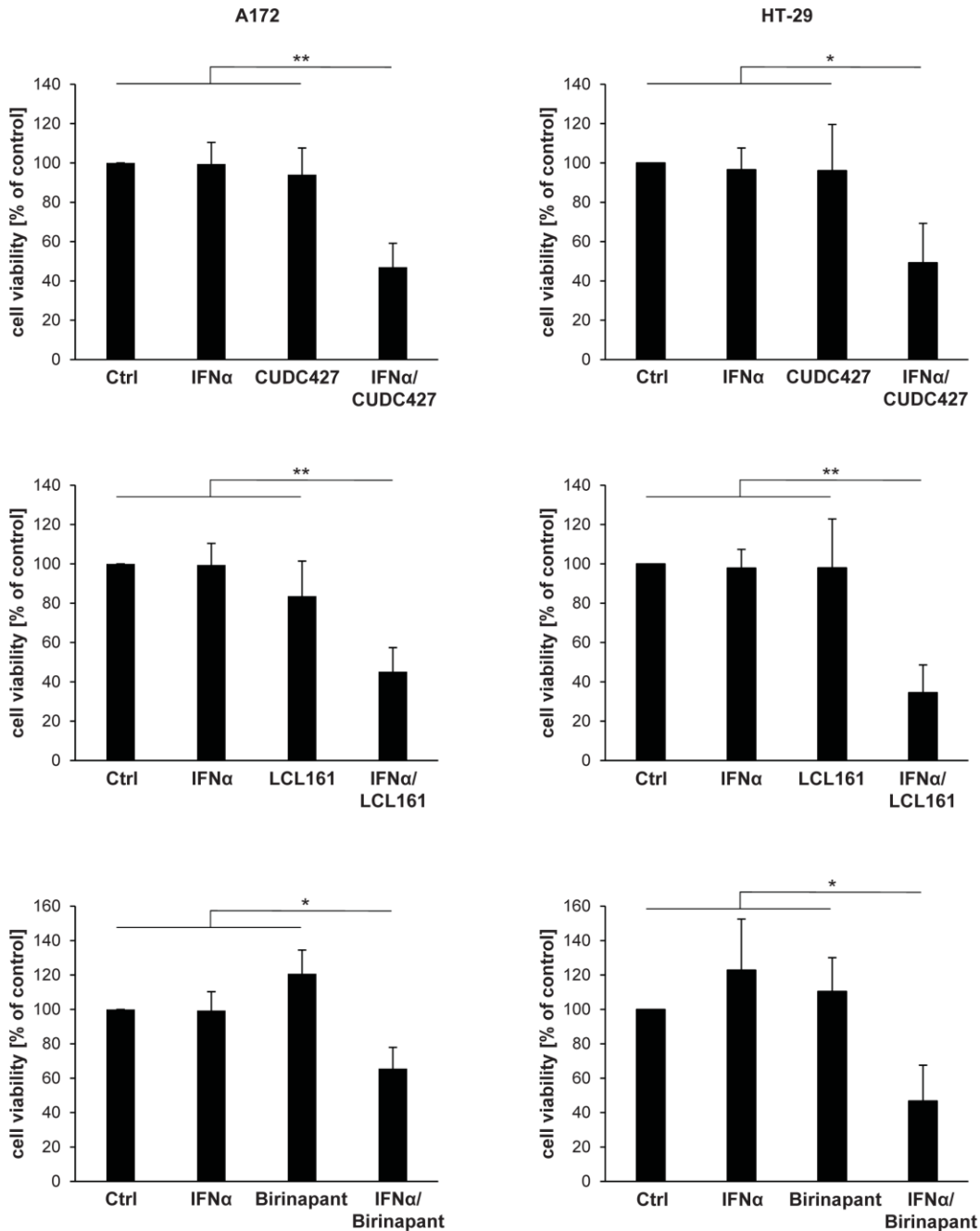
Cooperative TRAIL production mediates IFN α /Smac mimetic-induced cell death in TNF α -resistant solid cancer cells

Supplementary Material



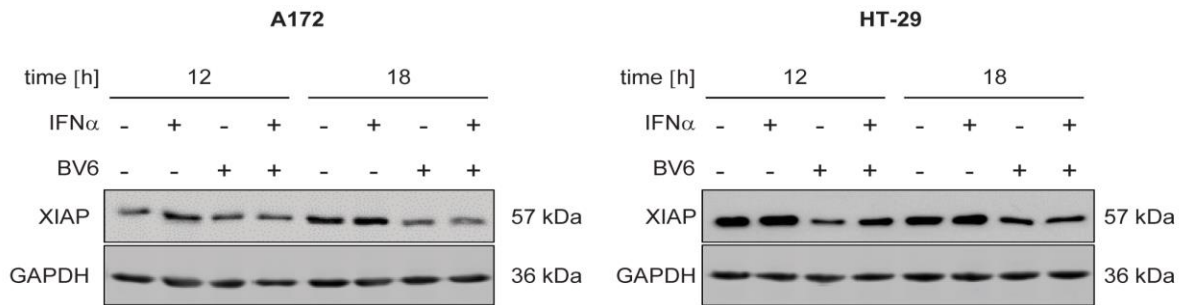
Suppl. Fig. 1. IFN α /BV6 cotreatment synergistically induces cell death in A172 and HT-29 cells.

A172 and HT-29 cells were treated for 72 hours with indicated concentrations of IFN α and/or BV6. Cell death was determined by analysis of DNA fragmentation using flow cytometry and mean + SD of three independent experiments performed in duplicate are shown (A). Cell viability was determined by MTT assay and data are shown as percentage of untreated control cells with mean + SD of three independent experiments performed in triplicate (B).



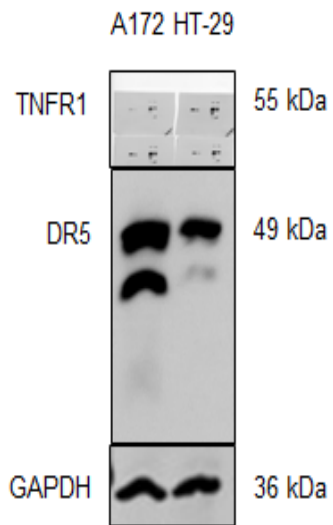
Suppl. Fig. 2. Different Smac mimetics cooperate with IFN α to reduce cell viability.

A172 and HT-29 cells were treated with 10 ng/ml IFN α and/or CUDC427, LCL161 (A172: 30 μ M; HT-29: 10 μ M) or 5 μ M Birinapant for 72 hours. Cell viability was determined by MTT assay and data are shown as percentage of untreated cells with mean + SD of three independent experiment performed in triplicate; * P < 0.05; ** P < 0.01.



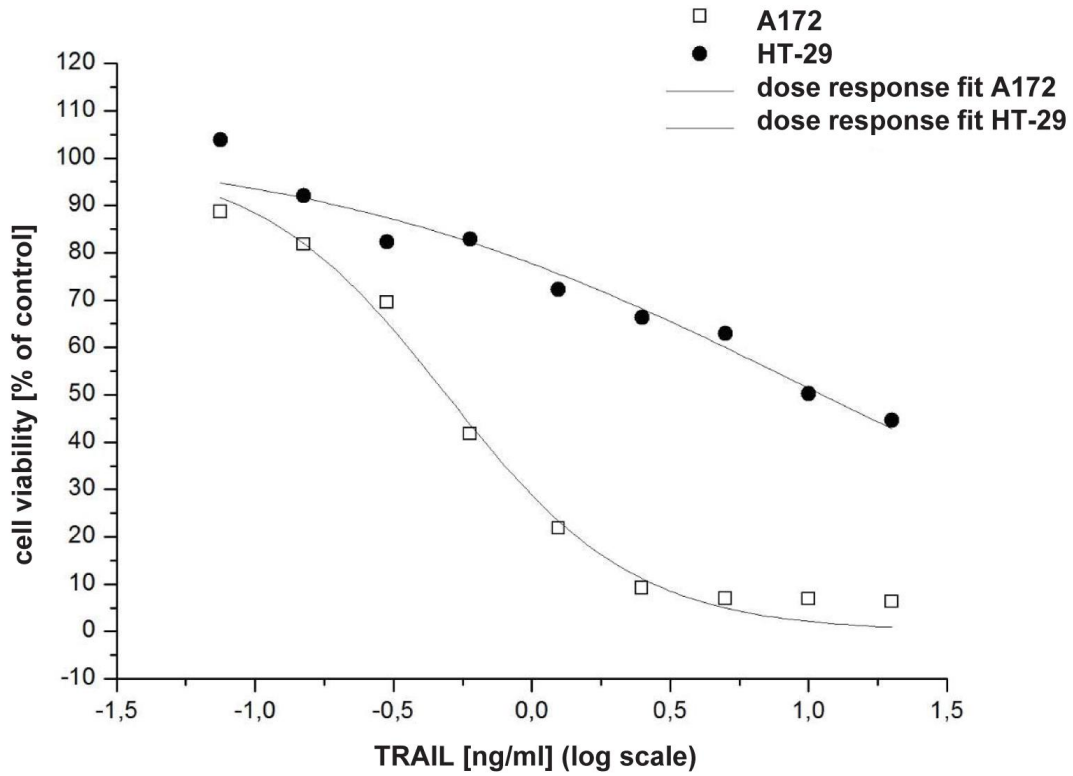
Suppl. Fig. 3. Effect of IFN α and/or BV6 on XIAP protein expression.

Cells were treated for 12 and 18 hours with IFN α (A172: 5 ng/ml, HT-29: 10 ng/ml) and/or 1 μ M BV6. Protein levels of XIAP were assessed by Western blotting, GAPDH was used as loading control.



Suppl. Fig. 4. TNFR1 and DR5 expression.

Constitutive protein levels of TNFR1 and DR5 were assessed in untreated cells by Western blotting, GAPDH was used as loading control.



Suppl. Fig. 5. Calculation of IC₅₀ values for TRAIL/BV6 cotreatment.
 IC₅₀ values for TRAIL/BV6 cotreatment were calculated by Origin data analysis software (OriginLab, Northampton, MA, USA).

Suppl. Tab. 1. Synergistic induction of cell death by IFN α and BV6.

Combination indices (CI) were calculated as described in Materials and Methods for DNA fragmentation (A) and cell viability (B) shown in suppl. Fig. 1 after treatment with indicated concentrations of IFN α and BV6. CI <0.9 indicates synergism, 0.9-1.1 additivity and >1.1 antagonism.

A172

HT-29

a

		BV6 [μ M]		
		0.5	1	2
IFN α [ng/ml]	5	0.04	0.007	0.011
	10	0.002	0.005	0.007
	20	0.001	0.003	0.003

		BV6 [μ M]		
		0.5	1	2
IFN α [ng/ml]	5	0.006	0.006	0.034
	10	0.006	0.006	0.015
	20	0.002	0.002	0.005

b

		BV6 [μ M]		
		0.5	1	2
IFN α [ng/ml]	5	0.064	0.04	0.056
	10	0.042	0.075	0.072
	20	0.027	0.051	0.076

		BV6 [μ M]		
		0.5	1	2
IFN α [ng/ml]	5	0.067	0.026	0.044
	10	0.068	0.022	0.039
	20	0.021	0.023	0.038