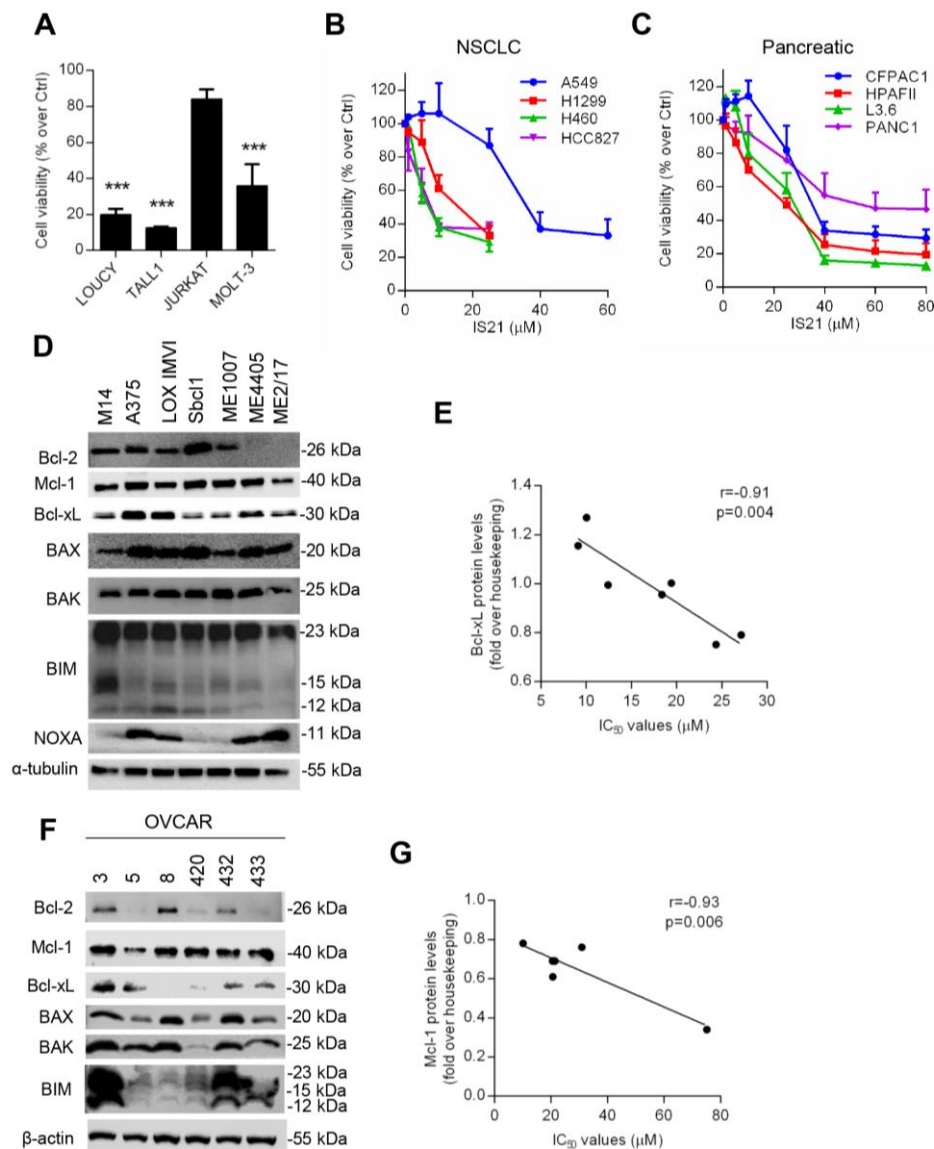
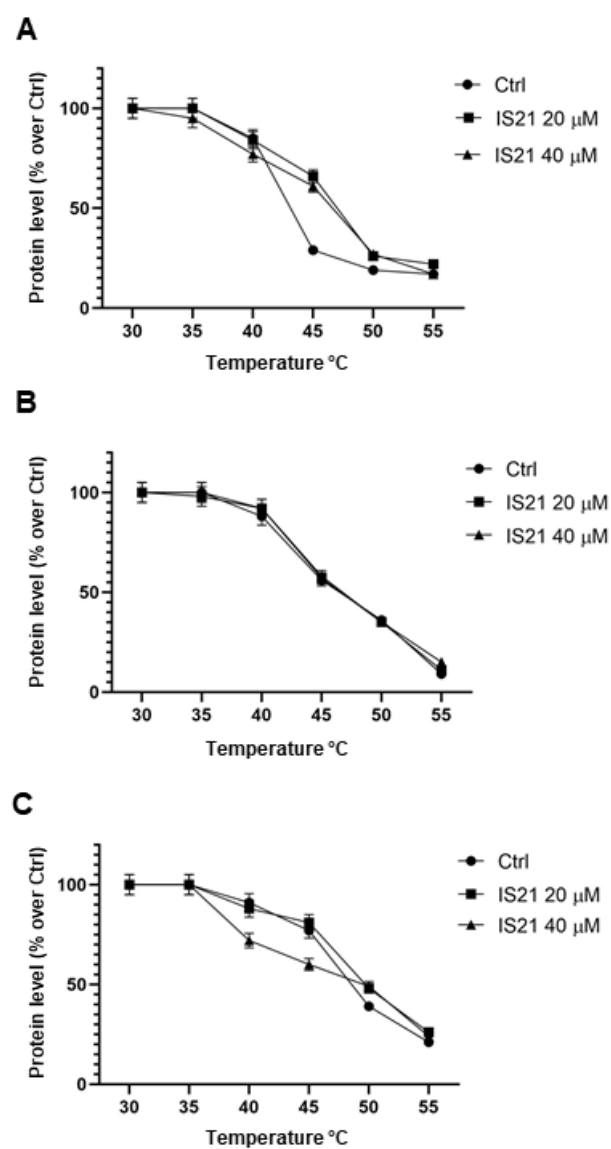


Supplementary Files

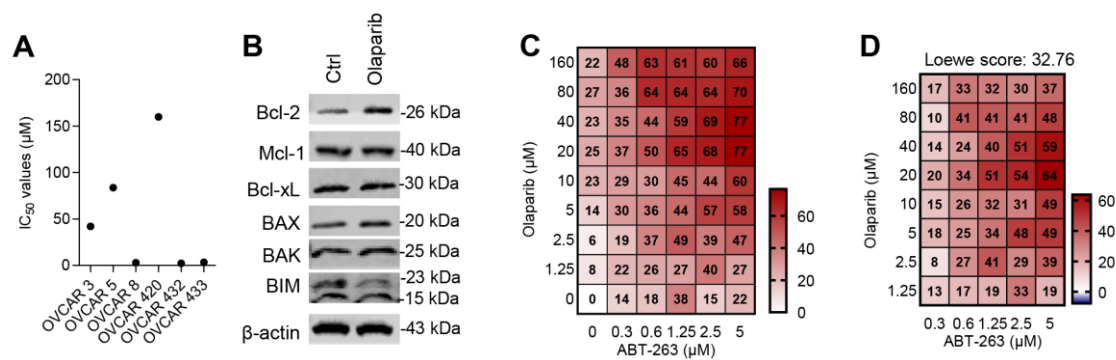


Supplementary Fig. 1. (A) Analysis of cell viability of the indicated T-ALL cell lines treated with ABT-199 (1 μM, 24h). *p*-values were calculated between control and treated cells, ****p* ≤ 0.001. (B,C) Analysis of cell viability of (B) NSCLC and (C) pancreatic cancer cell lines treated with increasing concentrations of IS21 for 72h. (A-C) The results are reported as “viability of treated cells/viability of control cells (Ctrl)” × 100, and as mean ± SD of three independent experiments. (D) Western blot analysis of basal Bcl-2, Mcl-1, Bcl-xL, BAX, BAK, BIM and NOXA protein expression levels in the indicated melanoma cell lines. (E) Correlation analyses between IC₅₀ IS21 values and the basal levels of Bcl-xL expression in melanoma cell lines. (F) Western blot analysis of basal Bcl-2, Mcl-1, Bcl-xL, BAX, BAK and BIM protein expression levels in the indicated ovarian cancer cell lines. (G) Correlation analyses between IC₅₀ values of IS21 and the basal levels of Mcl-1 expression in ovarian cancer cell lines. (D,F) Reported Western blot images are

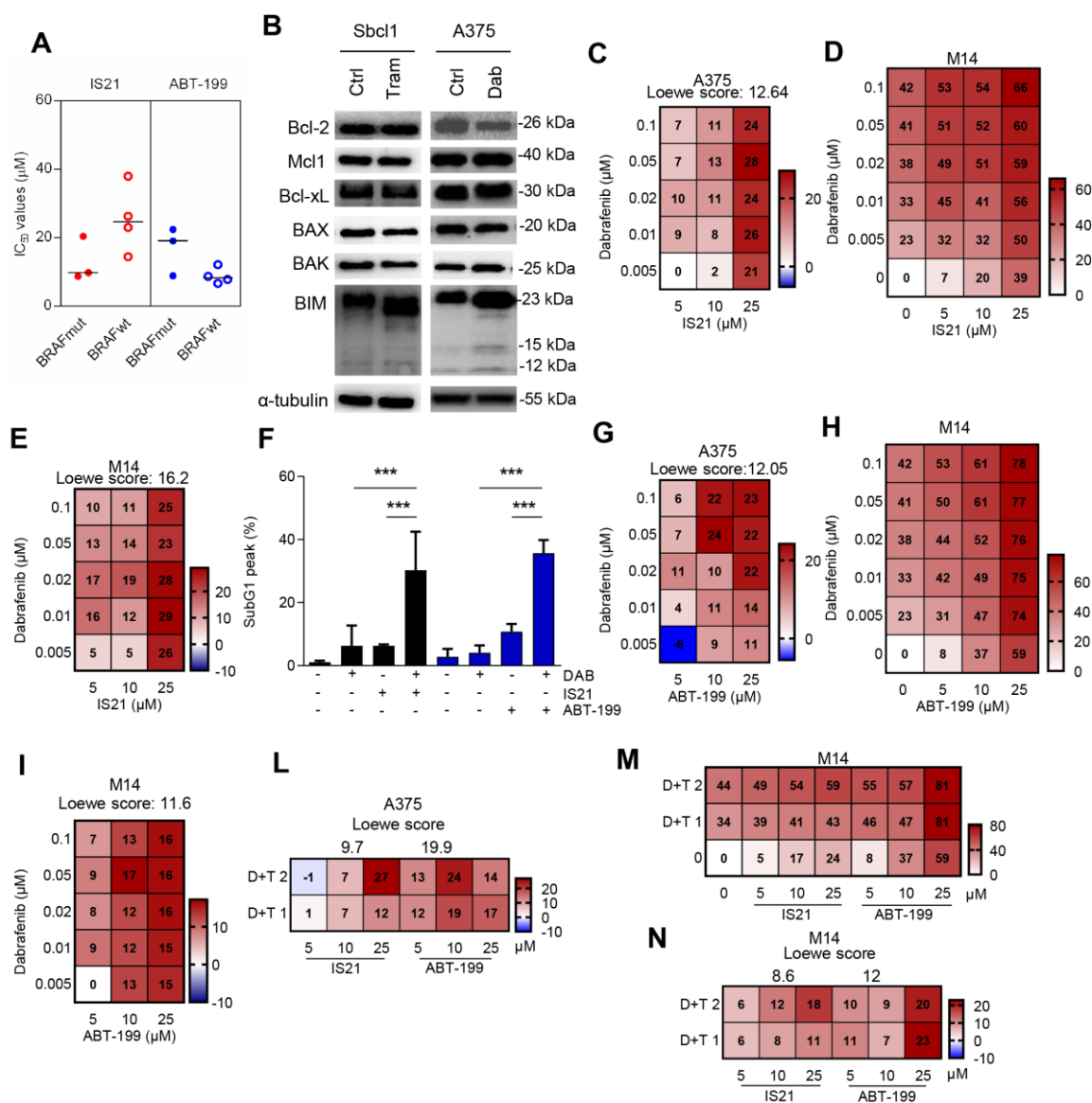
representative of two independent experiments with similar results. β -actin and α -tubulin are shown as loading and transferring control, molecular weights are expressed in kilodalton (kDa).



Supplementary Fig. 2. Intracellular thermal denaturation curves obtained for (A) Bcl-2, (B) Bcl-xL and (C) Mcl-1 in A375 cells underwent a 4 h treatment with IS21 or untreated (Ctrl).



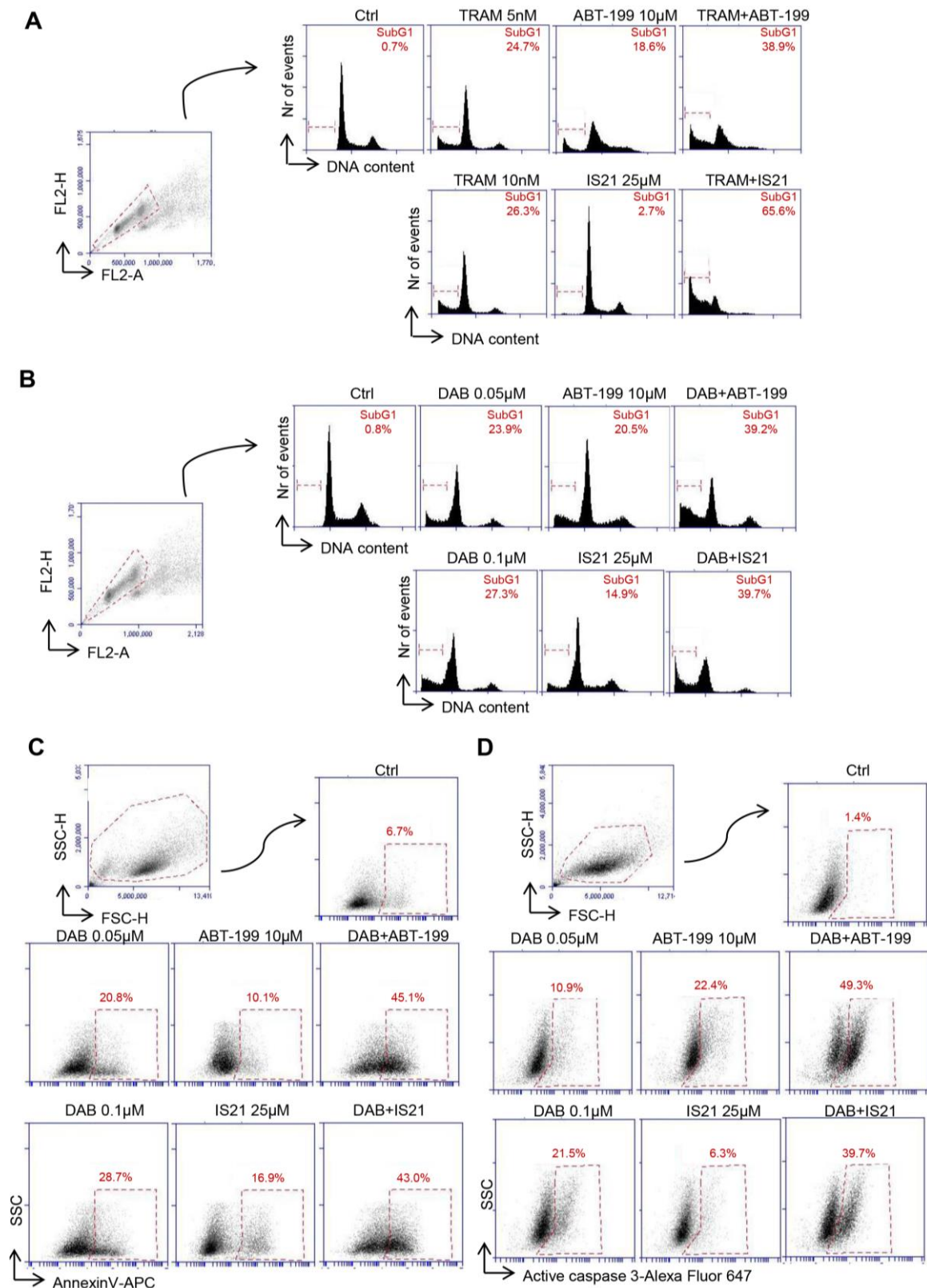
Supplementary Fig. 3. (A) IC_{50} values of the indicated ovarian cancer cell lines treated with increasing concentration of olaparib (0.3-5 μ M, 6 days). (B) Western blot analysis of Mcl-1, Bcl-xL, Bcl-2, BAX, BIM and BAK protein expression levels in OVCAR 5 ovarian cancer cell line treated with olaparib (5 μ M, 72h). Reported Western blot images are representative of two independent experiments with similar results. β -actin is shown as loading and transferring control, molecular weights are expressed in kilodalton (kDa). (C,D) Heat map graphs showing (C) the cell growth inhibitory effect, analyzed by MTS assay, and (D) Loewe scores of OVCAR 5 cells treated with the indicated concentrations of ABT-263 and olaparib alone or in combination for 6 days. (C) The results are reported as inhibition of treated cells/inhibition of control cells $\times$ 100 and represented the mean of three independent experiments. (D) The numbers inside the squares indicate Loewe scores in every experimental point.



Supplementary Fig. 4. (A) IC_{50} values of different melanoma cell lines (BRAFwt and BRAFmut) treated with IS21 or ABT-199 (from 5 μ M to 25 μ M for 72 h). The bar indicates the median of IC_{50} for each condition. (B) Western blot analysis of Mcl-1, Bcl-xL, Bcl-2, BIM, BAX and BAK protein expression levels in Sbc11 and

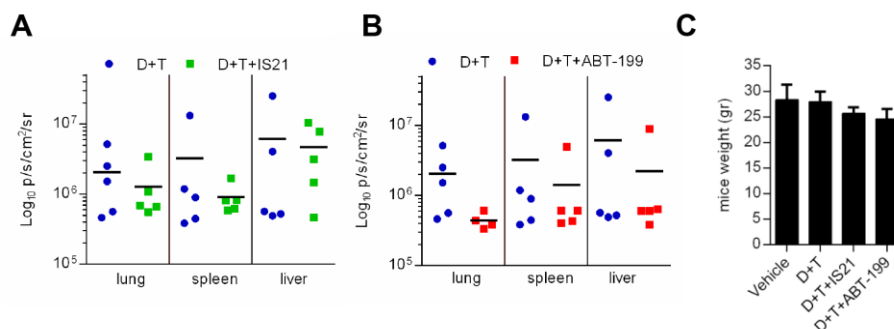
A375 cancer cell lines treated with trametinib (10nM) or with dabrafenib (0.1 μ M) for 48h, respectively. Reported Western blot images are representative of two independent experiments with similar results. α -tubulin is shown as loading and transferring control, molecular weights are expressed in kilodalton (kDa).

(C) Heat map graph showing Loewe scores of A375 cells treated for 48h with the indicated concentrations of dabrafenib (DAB) and IS21 alone or in combination. Heat map graphs showing (D) cell growth inhibitory effect and (E) Loewe scores of M14 cells treated for 48h with the indicated concentrations of dabrafenib and IS21 alone or in combination. (F) Cytofluorimetric quantification of subG1 peak of cell cycle distribution in M14 cells treated with IS21 (25 μ M) or ABT-199 (10 μ M) alone or in combination with Dabrafenib (0.1 μ M for IS21 combination and 0.05 μ M for ABT-199). Data represented the mean $\pm$ SD of three independent experiments. p-values were calculated between single treatments and combination ones, ***p<0.001. (G) Heat map graph showing Loewe scores of A375 cells treated for 48h with the indicated concentrations of dabrafenib and ABT-199 alone or in combination. Heat map graphs showing (H) cell growth inhibitory effect and (I) Loewe scores of M14 cells treated with the indicated concentrations of dabrafenib and ABT-199 alone or in combination. (L) Heat map graph showing Loewe scores of A375 cells treated with IS21 or ABT-199 alone or in combination with dabrafenib+trametinib (D+T 1, dabrafenib 0.01 μ M+trametinib 0.001 μ M; D+T 2, dabrafenib 0.05 μ M+trametinib 0.005 μ M) for 48h. Heat map graphs showing (M) cell growth inhibitory effect and (N) Loewe scores of M14 cells treated with IS21 or ABT-199 alone or in combination with dabrafenib+trametinib (D+T 1, dabrafenib 0.01 μ M+trametinib 0.001 μ M; D+T 2, dabrafenib 0.05 μ M+trametinib 0.005 μ M) for 48h. (D,H,M) The results are reported as inhibition of treated cells/inhibition of control cells $\times$ 100 and represented the mean of three independent experiments. (C,E,G, I,L,M) The numbers inside the squares indicate Loewe and inhibition values in every experimental point.



Supplementary Fig. 5. Representative images of flow cytometric analysis of subG1 peak in (A) *Sbc11* cells after treatment with the indicated concentrations of trametinib (TRAM), IS21, ABT-199 alone or in combination (TRAM+IS21, TRAM+ABT-199) for 48h and (B) A375 cells treated with the indicated concentrations of dabrafenib (DAB), IS21, ABT-199 alone or in combination (DAB+IS21, DAB+ABT-199)

for 48h. Representative images of (C) AnnexinV and (D) active caspase 3 staining of A375 treated as reported in (B).



Supplementary Fig. 6. Analysis of the metastatization measured by ex vivo imaging on lung, spleen and liver of mice treated for three weeks with dabrafenib+trametinib (D+T) and (A) D+T+IS21 or (B) D+T+ABT-199. (C) Analysis of mice weight after treatment reported in (A,B).

Bcl Protein Type	pdb entry code	Docking Energy (kcal/mol)*		KD ₁ (μM)
		Raw	Minimized	
Bcl-2	2VM6	-7.5	-7.72	0.32
Bcl-xL	3FDL	-8	-8.1	0.42
Mcl-1	2PQK	-6.7	-7.39	3.9

Supplementary Table 1. Predicted docked energy by the VINA scoring function for IS21 as docked into Bcl-2, Bcl-xL and Mcl-1 proteins extracted from complexes BH3 alpha-helix. *Data are the average values from two independent docking runs from two different randomized starting conformations.