Figure EV1. Comp A inhibits B16 tumor growth in vivo.

A The murine melanoma cell line B16 was mixed with matrigel and vehicle (control) or Comp A (2 μM) and then subcutaneously injected into the flank region of recipient mice. Histological analysis was performed on day 12 by H&E staining.

B Histological analysis of implanted tumors (Fig 1A) stained using a Ki-67 or cleaved (cl.) caspase-3 antibody (red); nuclei were counterstained with DAPI (blue). The mean number of Ki-67-positive cells and total number of nuclei in tumors were calculated on tumor sections using ImageJ software. Error bars show SEM for n ≥ 5.

C The relative cell growth of B16 cells treated with Comp A (2 μM) alone or combined with TNF (20 ng/ml) was analyzed by using JuLi Br Live Cell Analyzer (Peqlab) for 48 h.

D The canonical NF-κB activation in B16 cells was measured by Western blotting using specific anti-phospho IkB and IkB antibodies or by NF-κB p65 ELISA after treatment with Comp A (2 μM) at indicated time points. Error bars show SEM for n = 3.

Data information: *P < 0.05, two-sided unpaired Student’s t-test.

Source data are available online for this figure.
Comp A inhibits B16 tumor vasculature in vivo without altering immune cell infiltration.

The murine melanoma cell line B16 was mixed with matrigel and vehicle (control) or Comp A (2 μM) and then subcutaneously injected into the flank region of recipient mice (Fig 1A). Histological analysis of implanted tumors was performed.

A The number of hyaluronan receptor LYVE (lymphatic vessels) and CD68 (monocytes)-, CD45 (leukocytes)-, GR1 (neutrophils)-, CD11b (NK cells)-, CD16 (neutrophils, macrophages and NK cells)-, CD8 (T cells)-, and CD3 (T cells)-expressing cells was estimated according to the intensity of specific staining and was arbitrarily set as the following:  , not expressed; +, low expression; ++, moderate expression; +++, strong expression.

B Sections of intra-tumoral (i) and peri-tumoral (p) regions of A on day 12 are illustrated. Nuclei were counterstained with DAPI (blue).

Figure EV2.
Figure EV3. Comp A promotes TNF-induced endothelial cell death.

A Measurement of clonogenicity of HUVEC cells treated as indicated. After 48 h, medium was changed to normal growth media. Clonogenicity was assessed by crystal violet staining of adherent colonies 12 days after treatment.

B Flow cytometric analysis for cleaved caspase-3 staining (secondary Alexa 594-conjugated polyclonal goat anti-rabbit antibody) was performed on HUVECs at indicated time points and treated as indicated. Error bars show SEM for n = 3.

C qRT-PCR of angiogenesis-related genes after stimulation of HUVECs with TNF (20 ng/ml) and TNF combined with Comp A (2 μM) at indicated time points using specific primers for VEGF-A, VEGF-C, VCAM-1, and GAPDH. Error bars show SEM for n ≥ 4.

D HUVEC cells were seeded on matrigel and treated with Comp B (2 μM) or with Comp B and TNF (20 ng/ml) for 48 h. Pictures were taken with a bright-field microscope.
Figure EV4. IAP antagonization potentiates TNF-induced vascular disruption in vivo.
Matrigel was mixed with vehicle (control) or Comp A (2 μM) and subcutaneously injected into the flank region of recipient TNFR-1/2−/− mice. Intra-tumoral vasculature of tumors on day 12 was analyzed by multiphoton microscopy using FITC–dextran staining of vessels. FITC–dextran was injected into the tail vein of mice 30 min before sacrificing the mice. A 3D rendered model is illustrated.