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**Effect of perifosine on tumor vasculature by perifosine restores the vascular
permeability of TRAIL-expressing CD34⁺ cells**

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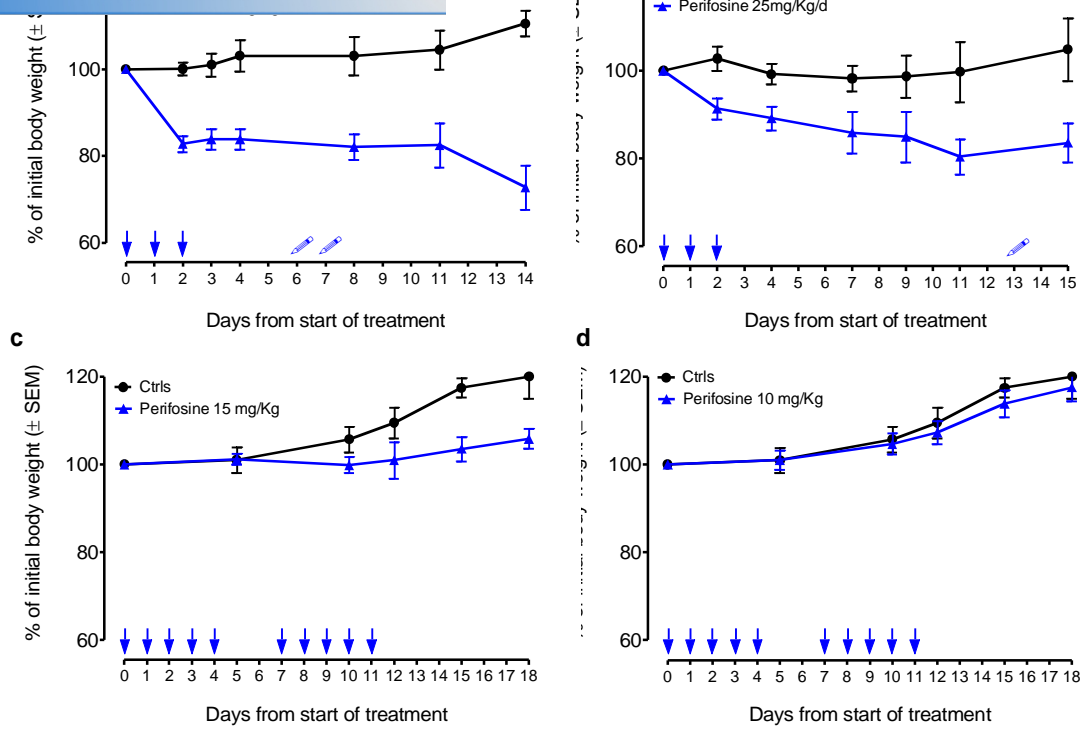


Fig. S1 In vivo dose finding of perifosine

As an index of systemic toxicity, changes in body weight after oral perifosine administration were assessed. (a) One 3-days course of oral (PO) perifosine at 35 mg/Kg/day caused a significant loss of body weight and was lethal for 2/5 of treated animals. (b) One 3-days course of oral (PO) perifosine at 25 mg/Kg/day caused a significant loss of body weight and was lethal for 1/6 of treated animals. (c) Two 5-days courses of oral (PO) perifosine at 15 mg/Kg/day and (d) two 5-days courses of oral (PO) perifosine at 10 mg/Kg/day were well tolerated and did not cause any significant loss of body weight. Skulls indicate dead animals. Autopsy of dead animals revealed swollen stomach and intestines and yellowish-white brittle liver, suggesting gastrointestinal toxicity as cause of death

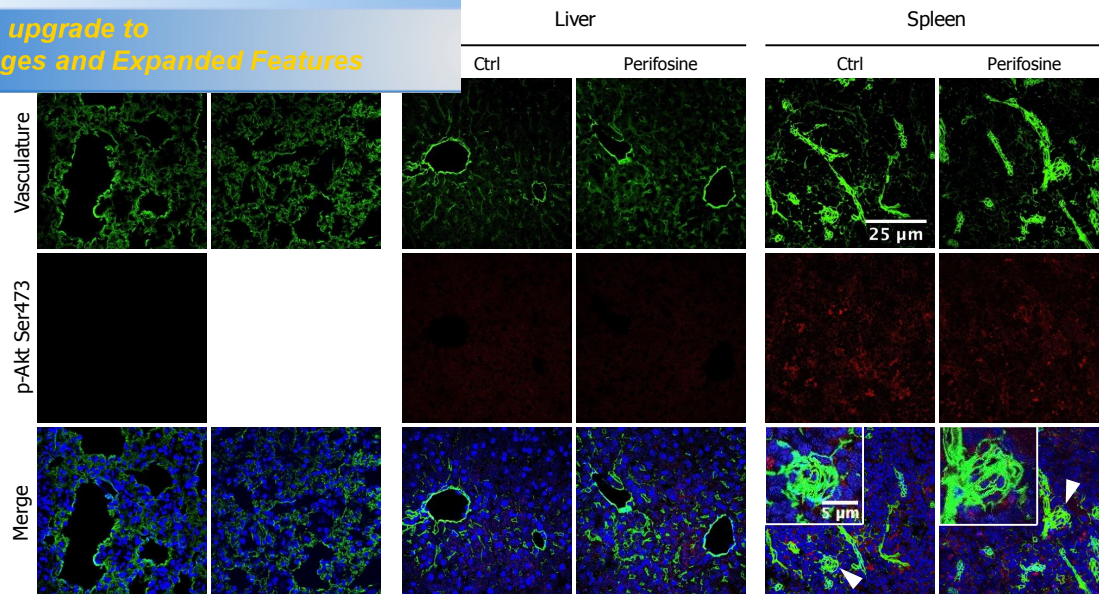


Fig. S2 Perifosine does not affect phospho-Akt expression in healthy organs

NOD/SCID mice bearing subcutaneous tumor nodules were treated with perifosine at 15 mg/kg (once daily for 5 days, PO), or the vehicle control. Immediately prior to sacrifice, mice were IV injected with 0.2 ml of sulfo-NHS-LC-biotin (5 mg/ml) to biotinylate functional vasculature. Healthy organs (including lungs, livers and spleens) were then harvested, freshly snap-frozen in isopentane and analyzed for phospho-Akt expression. Representative confocal images of lungs, livers and spleens from perifosine-untreated and treated mice processed by triple immunofluorescence staining are shown. Biotinylated endothelial cells were detected by green staining with Alexa 488-conjugated streptavidin; phosphorylated Akt was detected by red coloration incubating sections with an anti-mouse phospho-Akt antibody followed by the appropriate Alexa Fluor 568-conjugated secondary antibody; cell nuclei were detected in blue with TO-PRO-3 nuclear dye. Arrowheads indicate the field magnified in inserted images. Objective lens, original magnification: 1.0 NA oil objective, 40x; scale bar 25 μm. Inserted images objective lens, original magnification: 1.4 NA oil objective, 60x; scale bar 5 μm

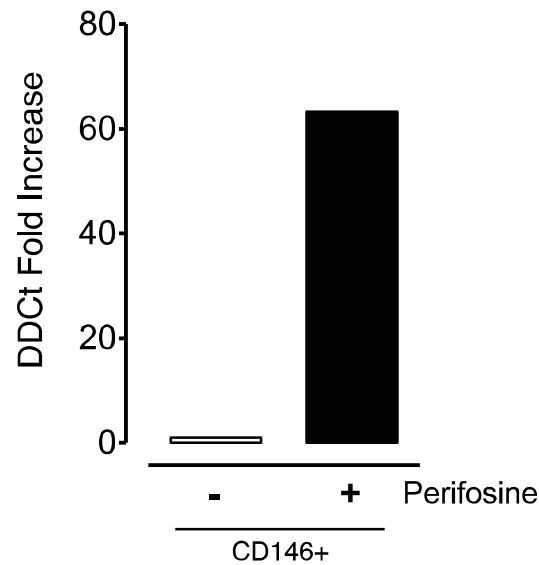


Fig. S3 DR5 expression in TECs is modulated by perifosine at mRNA level

CD146+ subpopulations obtained from SU-DHL-4V tumor nodules from mice receiving the vehicle control or perifosine at 15 mg/kg (once daily for 5 days, PO) were processed for RT-PCR analysis to evaluate relative fold increase in DR5 expression. Data in the graph are the results obtained by the analysis of TECs derived from a pool of three tumors per group

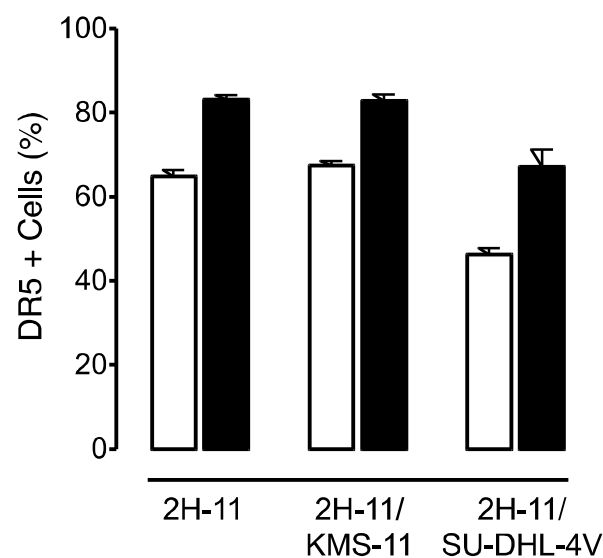
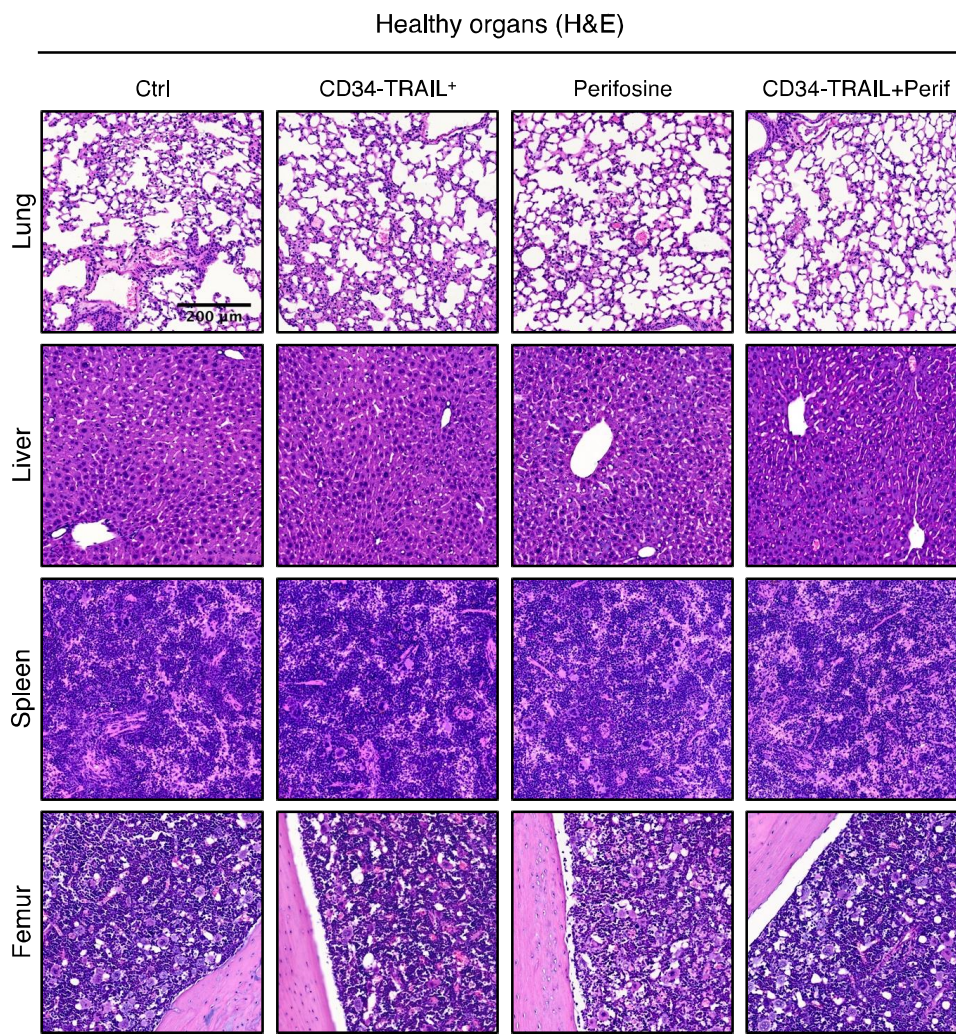


Fig. S4 Tumor cells differentially affect TECs phenotype

2H-11 cells were co-cultured for 72 hours with either SU-DHL-4V or KMS-11 cells (2H-11: tumor cells ratio 1:4) in presence (black bars) or absence (white bars) of perifosine 5 μ M and analyzed for DR5 expression by flow cytometric analysis. Represented are the mean \pm SD percentage of positive cells. The percentages of positive cells were measured according to gates set on the basis of isotype control



NOD/SCID mice bearing subcutaneous tumor nodules were randomly assigned to the different treatment groups consisting of the administration of one injection of CD34-TRAIL⁺ cells (3 x 10⁶ cells/mouse, IV); perifosine at 15 mg/kg (once daily for 5 days, PO); CD34-TRAIL⁺ cells in combination with perifosine; or the vehicle control. Seventy-two hours after CD34-TRAIL⁺ cell treatment and three hours after the last perifosine administration, healthy organs (including lungs, livers, spleens and femurs) were harvested, fixed in formalin, embedded in paraffin and analyzed for the presence of hemorrhages and necrosis. Representative histological images of healthy organs from untreated and treated animals are shown. Hematoxylin and eosin staining demonstrates the absence of tissue or vascular damage. Objective lens, original magnification: 0.40 NA dry objective, 10x; scale bar: 200µm

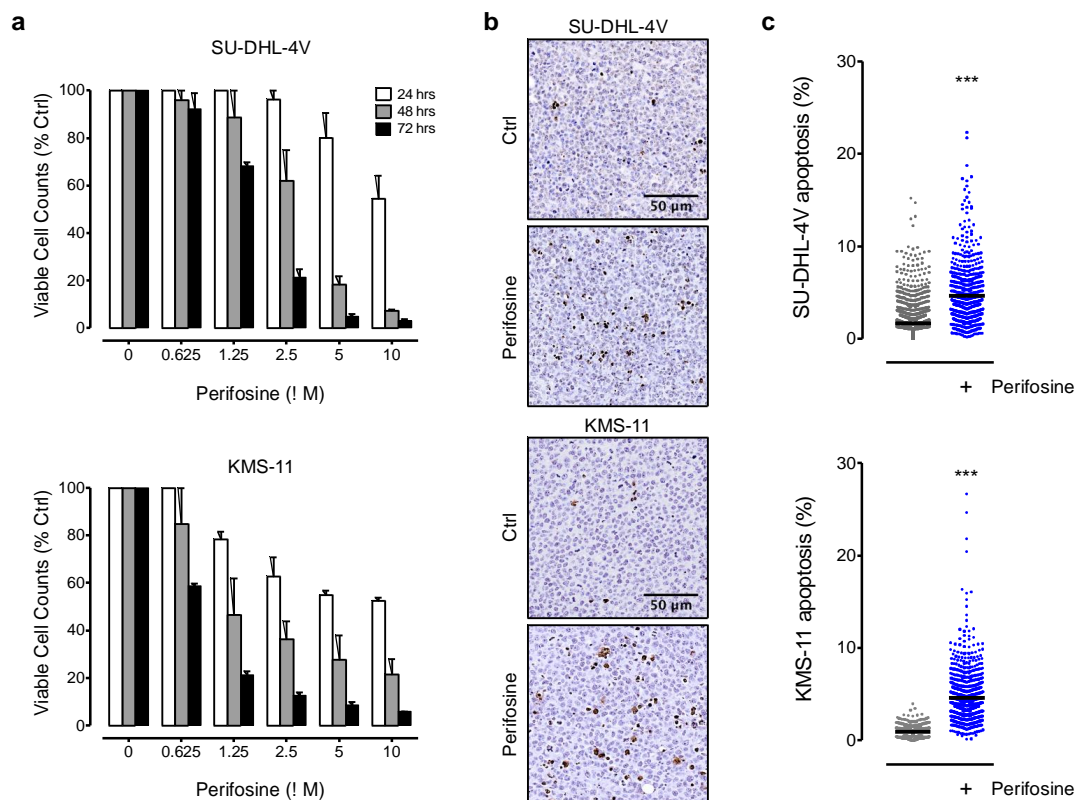


Fig. S6 In vitro and in vivo effects of perifosine on tumor cells

(a) SU-DHL-4V and KMS-11 cells were exposed for 24, 48 and 72 hours to increasing doses of perifosine and analyzed by Annexin-V-fluorescein isothiocyanate/propidium iodide (PI) double

cells by flow cytometry. The mean \pm SD percentages are

cutaneous tumor nodules were randomly assigned to the

different treatment groups consisting of the administration of perifosine at 15 mg/kg (once daily for 5 days, PO) or the vehicle control. Three hours after the last perifosine administration, tumor nodules were harvested, fixed in formalin, embedded in paraffin and analyzed for the presence of apoptotic cells by TUNEL staining. Representative histological images of TUNEL-stained tumors sections from untreated and perifosine-treated animals are shown. Objective lens, original magnification: 0.40 NA dry objective, 20x; scale bar: 50 μ m. (c) TUNEL stained SU-DHL-4V and KMS-11 whole tumor sections were digitally acquired and images analyzed using ImageJ for quantification of the percentage of apoptotic cells. At least three tumor sections from different animals were analyzed per treatment group. Each dot represents the value obtained from the analysis of a single tissue field, and the lines indicate the mean values. *** $P < 0.001$ compared with controls