Supplementary Figure 1

(A) Identification of nuclear (i and iii) and cytoplasmic (ii and iv) compartments in stroma (i and ii) and adjacent tumour tissue (iii and iv) in a representative core from the tissue microarrays used in the study. In this example, the expression levels of FLIP in the nuclear and cytoplasmic compartments in the stroma and tumour tissues are indicated. This technique allows us to assess expression in both the cytoplasm and nucleus on a cell by cell basis.

Artefacts are excluded by means of a strict feature filter in which size, shape, compactness, haematoxylin and biomarker values are used to distinguish between true cellular features and artefacts.
Supplementary Figure 1 (continued)

(B) Absolute expression of FLIP and procaspase-8 in nuclei and cytoplasm in all patients, sub-divided into adenocarcinoma and squamous. Mann-Whitney non-parametric $t$ test, two-tailed. (C) Correlations between stromal expression of FLIP and procaspase-8 in nuclei (left) and cytoplasm (right), as determined using Pearson’s correlation. Scatterplots with correlations boxes are presented.
Supplementary Figure 2

Correlations of overall survival with the level of cytoplasmic FLIP and procaspase-8 expression in tumor tissue, subdivided into (A) squamous and (B) adenocarcinoma histology. Kaplan-Meier curves were and analyzed using log-rank (Mantel-Cox) test.
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(A) Protein lysates from H460 and A549 cells treated with indicated doses of panobinostat for either 6h or 24h were immunoblotted for FLIP, PARP and procaspase-8. (B) The expression of a panel of apoptosis-regulating proteins was analysed by Western blotting in H460 and A549 cells treated with indicated doses of vorinostat for either 6h or 24h. (C) DEVDase (caspase-3-like) activity assay performed on lysates from cells treated with 5μM vorinostat for time periods indicated. (D) Immunoblotting of cell lysates from 34LU cells treated with indicated doses of panobinostat for 24h. (E) Procaspase-8 silencing was confirmed by Western blot for the experiments presented in Figures 4E and 5E.
Supplementary Figure 4

(A) Real-time PCR quantification of FLIP mRNA levels normalized to GAPDH for H460 and A549 cells treated with 5μM vorinostat for the indicated time points

(B) Western blot analysis of FLIP expression in H460 and A549 cells pre-treated with z-VAD for 1h before treatment with 5μM vorinostat for 6h.

(C) Western blot analysis of FLIP expression in H460 and A549 cells pre-treated with the MG-132 for 1h before treatment with 5μM vorinostat for 6h.

(D) Western blot analysis of XIAP and cIAP1 expression and PARP cleavage in H460, A549 and 34LU cells pre-treated with vorinostat or entinostat for 6h followed by the SMAC mimetic birinapant for 24h.
Supplementary Figure 5

(A) DEVDase (caspase-3-like) activity assay of H460 cells overexpressing FLIPs or empty vector control treated with vorinostat alone, cisplatin alone or co-treated with vorinostat and cisplatin (combo) for 48h. Statistical significance was determined by Student’s t test.

(B) Sub-G1 flow cytometric analysis of H460 cells overexpressing FLIPs or empty vector control pre-treated with vorinostat for 6h followed by treatment with TRAIL for a further 16h, or as single agents. Statistical significance determined by Student’s t test.

(C) Colony counts for clonogenic assays depicted in Figures 4B and 5B. Ø denotes zero colonies.
Supplementary Figure 6

(A) H460 Parental or cisplatin resistant (CisR) cells were transfected with siRNA targeting FLIP (FT) or scrambled control (SC) and immunoblot analysis performed after 48h. (B) 48h after FLIP silencing, apoptosis was detected by flow cytometry and caspase-3/7 activity assays. Statistical significance determined by student’s t test. (C) Sub-G1 analysis of apoptosis in parental and cisplatin-resistant H460 cells treated for 48h with increasing doses of vorinostat. (D) Sub-G1 analyses of apoptosis after treatment with either vorinostat for 6h followed by TRAIL for 18h or co-treatment with vorinostat and cisplatin. Statistical significance was determined by Student’s t test.