SUPPLEMENTARY FIGURE 1

Silencing of c-FLIP induces spontaneous apoptosis in CaP cells

Bar graphs presenting the percentage of apoptotic cells detected in a 24h culture, following transfection with isoform-selective (FL:c-FLIP$_L$-targeted and FS:c-FLIP$_S$-targeted) or non-selective (FT:c-FLIP$_{L/S}$-targeted) siRNA oligonucleotides in (A) 22Rv1 and (B) LNCaP cells. All data points shown represent the mean ± S.E.M. value, calculated from three independent experiments. Statistically significant differences were determined using a Student's two-tailed t-test; *, $p<0.05$; **, $p<0.01$). Immunoblots confirming selective knockdown of c-FLIP isoforms using siRNA sequences at 10nM, together with a parallel assessment of PARP cleavage in (C) 22Rv1 cells or (D) LNCaP cells. Membranes were re-probed with anti-GAPDH to ensure equal loading.

SUPPLEMENTARY FIGURE 2

The HDAC inhibitor Droxinostat down-regulates c-FLIP expression in androgen-dependent CaP cells and potentiates bicalutamide-induced apoptosis

(A) Representative immunoblots comparing the sensitivity of droxinostat in decreasing c-FLIP$_L$ protein expression and promoting the induction of PARP cleavage in 22Rv1 (left panel) and LNCaP cells (right panel). Membranes were re-probed with anti-GAPDH to ensure equal loading. (B) Bar graphs illustrating the effect of droxinostat upon the levels of apoptosis detected in 22Rv1 (top) or LNCaP cells (bottom), in the absence or presence of bicalutamide. All data points represent mean ± S.E.M. value, calculated from three and four independent experiments, respectively. Statistically significant differences were determined using a Student's two-tailed t-test; *, $p<0.05$; **, $p<0.01$; ***, $p<0.001$. (C) Representative immunoblots comparing the effect of droxinostat, bicalutamide, or their combined administration upon the expression of c-FLIP and the induction of PARP cleavage in 22Rv1

(top panel) and LNCaP cells (bottom panel). Equal protein loading was confirmed by re-probing the membranes for GAPDH.

SUPPLEMENTARY FIGURE 3.

SAHA targets the AR pathway in androgen-dependent CaP cells

(A) Representative immunoblots illustrating the concentration-dependent effects of SAHA administration upon the expression of c-FLIP or the induction of PARP cleavage in 22Rv1 cells (left panel) or LNCaP cells (right panel). (B) Bar graphs illustrating the concentration-dependent effect of SAHA upon the transcript levels for c-FLIP isoforms detected within 22Rv1 (left panel) and LNCaP (right panel). (C) Bar graph illustrating the concentration-dependent effect of SAHA upon the induction of apoptosis in 22Rv1 and LNCaP cells, determined by detection of the sub-G0/G1 cell population by flow cytometry. (D) Bar graph depicting the concentration-dependent effect of SAHA in reducing the cell viability of 22Rv1 and LNCaP cells. Data points shown in (C) and (D) are representative of the mean ± S.E.M. value, calculated from three independent experiments. Statistically significant differences where indicated were determined by a two-tailed Student's t-test; *, $p<0.05$; **, $p<0.01$; ***, $p<0.001$.

SUPPLEMENTARY FIGURE 4

Effects of SAHA and bicalutamide in inducing apoptosis detected by Annexin-V labelling.

Bar graphs showing the measurement of apoptosis using detection of Annexin V expression in (A) 22Rv1 or (B) LNCaP cells following individual or combined treatment with SAHA or bicalutamide. Data points shown are the mean ± S.E.M. value, calculated from three

independent experiments. Statistically significant differences where indicated were determined by a two-tailed Student's t-test; *, $p<0.05$; **, $p<0.01$; ***, $p<0.001$.

SUPPLEMENTARY FIGURE 5.

Characterization of differential AR and NF-κB activity in prostate cancer cell lines and the response to SAHA and bicalutamide.

(A) Bar graph illustrating the increased AR-transcriptional activity in 22Rv1 cells relative to that in LNCaP cells. (B) Immunoblot depicting the relative expression of the AR in 22Rv1 and LNCaP cells. Equal protein loading was confirmed using GAPDH. The immunoblot is representative of at least three experiments. (C) Histogram comparing the basal activity of the NF-κB transcription factor determined by luciferase reporter assays conducted in 22Rv1 and LNCaP cells. (D) Bar graphs presenting the impact of SAHA, bicalutamide or their combined administration upon the transcript levels of the NF-κB target gene Bcl-2, (E) the AR-regulated gene PSA, and (F) the AP-1/NF-κB target gene CXCL8 in 22Rv1 cells and LNCaP cells. All data points shown in bar graphs represent the mean ± S.E.M. value, calculated from a minimum of 3-6 independent experiments. Statistically significant differences where indicated were determined by a two-tailed Student's t-test; *, $p<0.05$; **, $p<0.01$

SUPPLEMENTARY FIGURE 6

Castrate-resistant cells are insensitive to bicalutamide and SAHA.

(A) Histogram comparing the basal AR transcriptional activity in 22Rv1 and VCaP cells. Data points shown represent the mean ± S.E.M., calculated from three independent experiments. (B) Representative immunoblot comparing the intrinsic level of c-FLIP and Bcl-2 expression in 22Rv1, LNCaP and VCaP cells. (C) Bar graph showing the absence of an effect of SAHA administration upon the level of cell death detected in VCaP cells. (D)

Representative immunoblots illustrating the ineffectiveness of SAHA in decreasing c-FLIP protein levels or in promoting PARP cleavage upon treatment of VCaP cells. Membranes were re-probed with anti-GAPDH to ensure equal loading. (E) Bar graph showing the effect of adding increasing concentrations of SAHA upon the induction of caspase-8 and caspase-3/7 activity in treated VCaP cells. (F) Bar graph illustrating the effect of SAHA alone or in combination with bicalutamide upon the modulation of mitochondrial membrane depolarisation, determined by TMRE staining of treated VCaP cells. All data points represent the mean value ± S.E.M, determined from three independent experiments. Statistically significant differences were determined using a Student's two-tailed t-test; *, $p<0.05$; **, $p<0.01$

SUPPLEMENTARY FIGURE 7

Bar graphs illustrating the effect of SAHA, bicalutamide or their combined administration upon (A), the AR-target gene PSA or (B) the NF-κB target gene Bcl-2 in VCaP cells. Data points shown are the mean value ± S.E.M. of three independent experiments. (C) Bar graph characterizing the level of AR activity in LN-Abl cells relative to LNCaP and VCaP cells. Data points represent the mean value ± S.E.M, determined from three independent experiments. Statistically significant differences were determined using a Student's two-tailed t-test; **, $p<0.01$. (D) Bar graph characterizing the mRNA transcript expression level of C-FLIP$_L$ in LN-Abl cells relative to LNCaP and VCaP cells, calculated from three independent experiments. (E) Bar graph demonstrating the effect of repressing c-FLIP expression by FT-siRNA upon the viability of LN-Abl cells, in the absence and presence of 10μM bicalutamide. Data points represent the mean value ± S.E.M, determined from three independent experiments. Statistically significant differences were determined using a Student's two-tailed t-test; *, $p<0.05$.