Supplementary Figure Legends

Figure S1. Vorinostat induces apoptosis and alters gene expression of Bcl-2 family members in different SCC cell lines. **A,** apoptosis is induced in a dose-dependent manner in HO1N1 cells following vorinostat treatment (24 hours). Percentages indicate the sum of Annexin V and/or PI-positive cells, assessed by flow cytometry. **B,** SCC cells exhibit various degree of sensitivity to vorinostat treatment. Cells were seeded at equal density, SRB assay was conducted after 24 hours treatment of vorinostat. Shown is the mean and SD of six replicates of a representative experiment. **C,** interaction patterns for major Bcl-2 family proteins in SCC cells. **D,** fold induction of BH3-only proteins and downregulation of Mcl-1 following vorinostat treatment do not correlate with vorinostat sensitivity. QRT-PCR of the indicated genes in the different SCC cell lines represented as fold change before and after vorinostat treatment (3 μM, 4 hours). The IC_{50} values for vorinostat are derived from Supplementary Fig. S1B. **E,** vorinostat alters protein levels of Bcl-2 family members. Immunoblots of Bcl-2 family members shown at basal level and after vorinostat treated (3 μM, 8 hours). Beta-tubulin (β-tub) serves as a loading control. Note: Noxa, Puma and Bim were induced at different levels for each cell line; the downregulation of Mcl-1 was most prominent for BICR-78, KYSE-150 and HO1N1 cells. EL: extra long; L: long; S: short.

Figure S2. Mcl-1 is essential for cell survival in SCC cells and vorinostat induces redistribution of Bim from Mcl-1 to Bcl-2/Bcl-xl. **A,** SCC cells are resistant to ABT-737. Cells were seeded at equal density; SRB assay was conducted 72 hours post ABT-737 treatment. Shown is the mean and SD of six replicates in a representative experiment. **B,** QRT-PCR of the indicated genes in three leukemia cell lines (HL60, SKNO1, THP1) and the seven SCC cell lines shown in Fig. 1D. Horizontal lines represent mean values. **C,** unique Mcl-1 dependence in SCC demonstrated in two additional SCC lines. SRB assay was conducted at 48 hours post siRNA transfection. Shown are mean and SD of four replicates in a representative experiment. **D,** densitometry quantitation of Fig. 2E, showing the change in the proportion of total Bim bound to each anti-apoptotic protein, expressed as IP/input for vorinostat treatment divided by IP/input for DMSO treatment. EL: extra long; L: long; S: short. **E,** replicate experiment as in Fig. 2E carried out in

JHU-O29 cells. EL: extra long; L: long; S: short. *Indicates Ig light chain. **F**, densitometry quantitation of Fig. S2E, calculated as for Fig. S2D. Note scale differs from Fig. S2D.

Figure S3. FBW7 mutant cancer cell lines lack Mcl-1 DNA copy number increase, express higher levels of Bim and are more sensitive to HDAC inhibitors. **A,** FBW7 ablation confers vorinostat sensitivity. JHU-O29 cells were transfected with FBW7-directed siRNA or control (Con), and 24 hours later were re-seeded for treatment with vorinostat (24 hours) at the indicated doses. Shown are mean and SD of triplicate samples in a representative experiment. *, $P < 0.05$; **, $P < 0.01$. **B,** FBW7 mutation and Mcl-1 DNA copy number increase are mutually exclusive. Mutation data of FBW7 mutant (n=41) and wild-type (WT; n=930) cancer cell lines and their corresponding DNA copy number data for Bcl-xl and Mcl-1 were obtained from the Broad Institute Cancer Cell Line Encyclopedia database (20). Group t test was used to calculate *P* values. Horizontal lines represent mean values. **C,** FBW7-mutant cell lines express a significant higher Bim level than FBW7 wild-type (WT) cell lines. Bim mRNA data was obtained from the Broad Institute Cancer Cell Line Encyclopedia database (20) only containing cell lines of non-hematological origin (mutant n=34; wild-type (WT) n=755). Group t test was used to calculate *P* value. Horizontal lines represent mean values. **D,** FBW7-mutant cells are significantly more sensitive to the pan-HDAC inhibitors AR-42 and belinostat than wild-type cells. IC_{50} values were obtained from internal database of MGH Center for Molecular Therapeutics for cell lines different origins and are shown as Log Normal (ln) (mutant n=10; wild-type (WT) n=174). Fisher's exact t test was used to calculate *P* values. Horizontal lines represent mean values. **E,** FBW7-mutant hematological cancers are more sensitive to pan-HDAC inhibitor AR-32 and belinostat as well as to class I-specific HDAC inhibitor MS-275. IC_{50} values were obtained from internal database of MGH Center for Molecular Therapeutics for cell lines different origins and are shown as Log Normal (ln). Fisher's exact t test was used to calculate *P* values. Horizontal lines represent mean values. **F,** FBW7-mutant cells are significantly more resistant to antitubulin drug epothilone. IC_{50} values were obtained from internal database of MGH Center for Molecular Therapeutics for cell lines different origins and are shown as Log Normal (ln). mutant n=32; wild-type (WT) n=1138). Fisher's exact t test was used to calculate *P* value. Horizontal lines represent mean values.

Figure S4. Synergy between vorinostat and ABT-737 *in vitro* and *in vivo*. **A,** synergistic induction of apoptosis by vorinostat and ABT-737. Percentages indicate the sum of Annexin V and/or PI-positive cells at 24 hours post vehicle treatment, 1.5 μM vorinostat, 3 μM ABT-737 or the combination, assessed by flow cytometry. **B,** strong synergy between vorinostat and ABT-737 in SCC cell lines. Calculation of combination indices by the Chou-Talalay method (36) using Fig. 4A data at the indicated concentrations. Values < 0.7 and < 0.3 indicate synergy and strong synergy, respectively**.** CalcuSyn program was employed for calculation. **C,** combination vorinostat and ABT-737 treatment leads to regression in the majority of xenograft tumors. Quantitation of Fig. 4C, showing mean increase (yellow) or decrease (red) in tumor size and the proportion of tumors in each category. **D,** QRT-PCR of control JHU-O29 tumors of Fig. 4B 6 hours after the last indicated treatments at day 12 for the indicated genes is shown. Horizontal lines representing mean of 7-8 samples per group.

Figure S5. Organization and function of Bcl-2 family members is recapitulated in primary HNSCC. **A,** stable overexpression of Mcl-1 protein by retroviral infection in the indicated SCC cell lines. Beta-tubulin (β-tub) serves as a loading control. **B,** successful knockdown of Noxa by siRNA transfection (48 hours) in the indicated SCC cell lines. **C,** Noxa is a key effector in the response to vorinostat and ABT-737 in SCC. Dose-response analysis as in (**5A**) was performed following transfection of control (siCon) or Noxa-directed (siNoxa). **D,** successful knockdown of Bim protein by siRNA transfection (48 hours) in the indicated SCC cell lines. **E,** overview of the IC_{50} values of vorinostat in the presence of 3 μM ABT-737 in different SCC cell lines measured by SRB assay at 24 hours of treatment. **F,** homeostasis between pro-apoptotic Bim and the sum of the anti-apoptotic genes Bcl-2, Bcl-xl and Bcl-w (left), between Bim and anti-apoptotic Mcl-1 mRNA (middle) as well as between the sum of Bim and Noxa mRNA and Mcl-1 (right) in primary HNSCC tumors (n=28), determined by QRT-PCR. **G,** disruptive *FBW7* mutation in one primary HNSCC tumor. Sanger sequencing chromatogram shows wild-type (WT) sequence and mutation within the substrate binding domain. **H,** correlation between Bcl-2 mRNA levels and clinical outcome of HNSCC tumors treated with taxane/platinum chemotherapy and radiation. CR: complete response (n=6); PR: partial response; SD: stable disease; PD: progressive disease; PR+SD+PD: n=12. Horizontal lines represent mean values and error bars show SEM.

Table S1. QRT-PCR primer and Taqman probes.