

Figure S1. Effect of ACF on cell survival. (A) U87, U251, U343, A549 and NCI-H69 cells were treated with various concentrations (0, 1, 2 and 10 μM) of ACF and cell viability was determined using the sulforhodamine B assay. (B) U87, U343, U251 and A549 cells were treated with ACF (2 μM) for 24 h and cell death was evaluated using Annexin V and 7-AAD staining followed by FACS. APC conjugated anti-human IgG was used as an isotype control. ACF, acriflavine; 7-AAD, 7-amino-actinomycin D; ISO, isotype control.

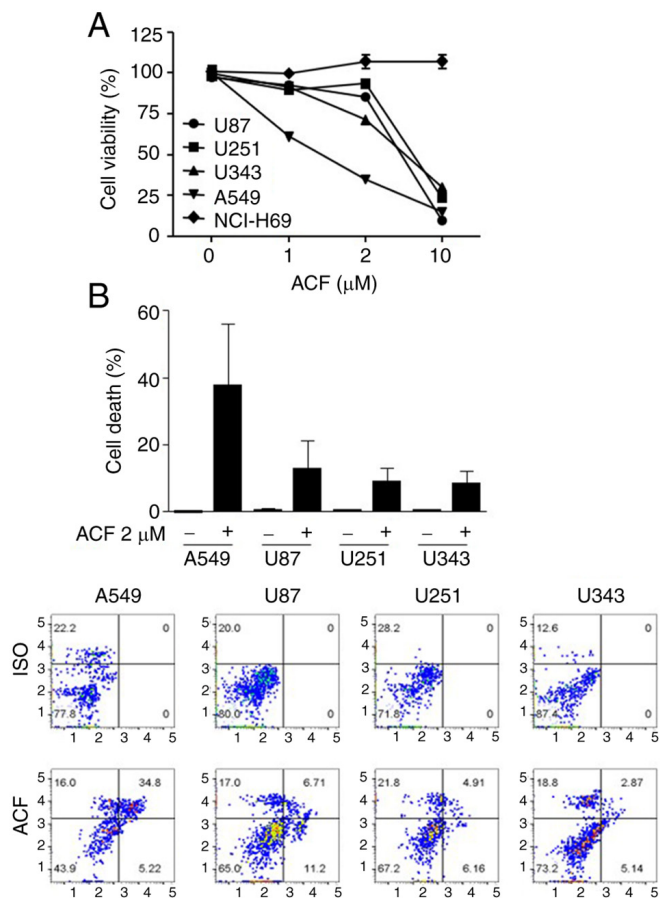


Figure S2. ACF-induced apoptosis is dependent on caspase-9 in triple-negative breast cancer and lung cancer cell lines. MDA-MB-231, HS578T and A549 cells were treated with 0 or 10 μ M ACF for 24 h, and the expression of caspase-8 and caspase-9 was detected using western blot analysis. ACF, acriflavine.

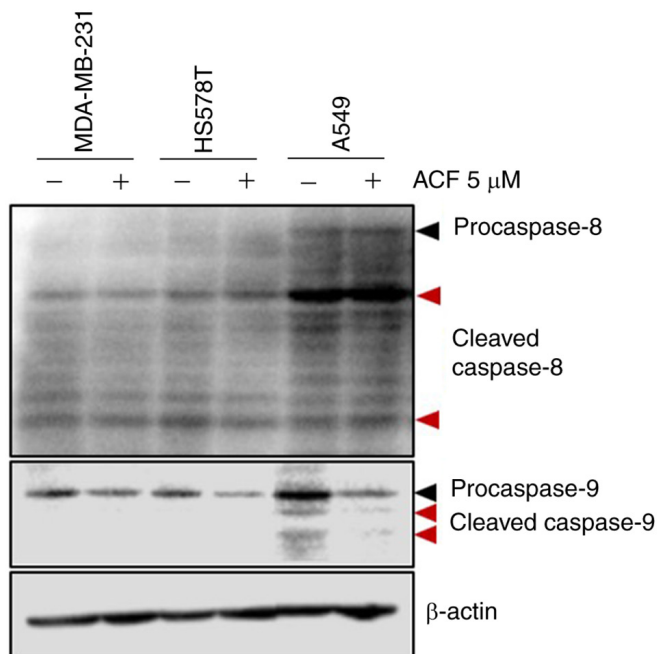


Figure S3. ACF downregulates MCL-1 in normoxia. MDA-MB-231, HS578T and HCC-70 cells were cultured in CoCl_2 ($600 \mu\text{M}$)-induced normoxic or in hypoxic conditions. The hypoxic condition was induced with CoCl_2 ($600 \mu\text{M}$). Cells cultured in normal normoxic conditions were treated with 0 or $10 \mu\text{M}$ of ACF for 24 h and determination of the expression of MCL-1 was performed using western blot analysis. ACF, acriflavine; MCL-1, myeloid cell leukemia sequence 1; p-, phosphorylated.

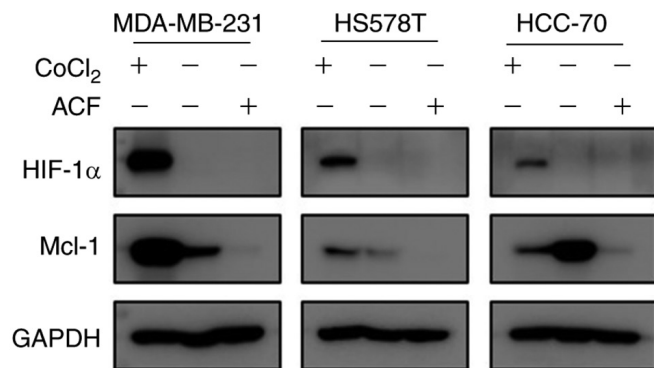


Figure S4. MCL-1 downregulation by ACF is independent of GSK3 β . MDA-MB-231, HS578T and HCC-70 cells were treated with 0 or 10 μ M ACF for 24 h, and determination of the expression of p-GSK-3 β , GSK-3 β , p-MCL-1 and MCL-1 was performed using western blot analysis. MCL-1, myeloid cell leukemia sequence 1; ACF, acriflavine; p-, phosphorylated.

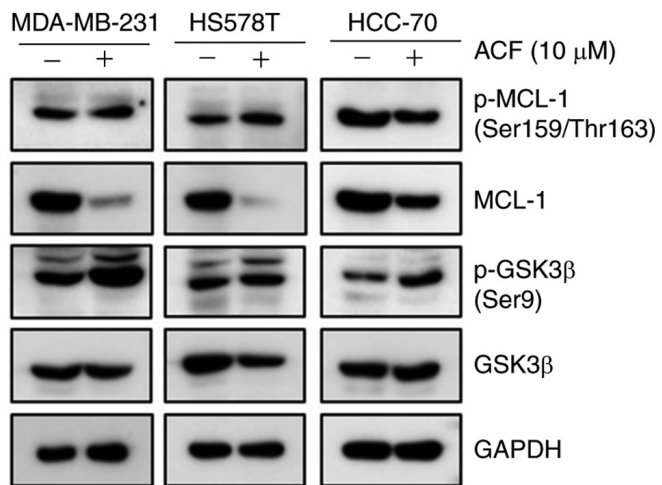


Figure S5. CI reveals a synergistic effect in triple-negative breast cancer, glioblastoma multiforme and lung cancer cell lines. CI of acriflavine and ABT-263 treatment on MDA-MB-231, HS578T, HCC-70, U87, U251, U343 and A549 cells was plotted using CompuSyn software. $CI < 1$ indicates a synergistic effect, $CI = 1$ indicates an additive effect and $CI > 1$ indicates an antagonistic effect. CI, Combination index; ACF, acriflavine.

