Supplemental Figure 1. Structure of biotiniylated birinapant.

Supplemental Figure 2. Concentration-dependent degradation of GFP-cIAP1/2 by birinapant. (A) Quantitative measurement of GFP-cIAP1 and GFP-cIAP2 degradation by FACS analysis. (B) Synchronized degradation of GFP-cIAP1 and GFP-cIAP2 with endogenous cIAP1 and cIAP2, respectively, by birinapant in A375 cells. In order to increase the level of endogenous cIAP2 for evaluation, cells were stimulated with TNF for 16 hours.

Supplemental figure 3. Representative examples of data analysis from 111 cell line panel of birinapant in combination with TRAIL analyzed using the MacSynergy II program. Data generated by the treatment of (A) MDA-MB-231 cells and (B) HT-1376 cells with birinapant and TRAIL. Areas above 0% interaction represent synergy, areas below represent antagonism, and the area at 0% is additive. Synergy was measured in (A) and no synergy was measured in (B).

Supplemental figure 4. Cells which are sensitive to birinapant as a single agent produce TNF in response to birinapant treatment. Indicated cell lines were seeded into 96-well plates at an approximate density of 50,000 cells /well and allowed to adhere overnight. Next day, cells were treated with birinapant or left untreated for 16 hours. TNF in culture supernatants was measured by ELISA (BD OptEIA) according to manufacturer's instructions.

Supplemental Figure 5. Birinapant induces cell death as a single agent in a TNF-dependent manner.

SK-OV-3 and EVSA-T cell lines were pretreated for 1 hour with 10 μg/mL anti-TNF antibody followed by birinapant treatment. Viability was measured by MTT assay following 72 hours incubation.

Supplemental Figure 6. Preferential degradation of TRAF2-associated cIAP1 and cIAP2 by birinapant. (A) HeLa cells were treated with 10 ng/mL TNF for 20 hours. Cells were next treated with indicated concentrations of birinapant for 2 hours. Immunoprecipitation was carried out using anti-TRAF2 antibody and cIAP2 levels were analyzed by western blot. (B) HeLa cells were treated with indicated concentrations of birinapant for 2 hours. Immunoprecipitation was carried out using anti-TRAF2 antibody and cIAP1 levels were analyzed by western blot.

Supplemental Figure 7. Birinapant inhibits the degradation of IkBα in cells stimulated with TNF.

A375 cells stably overexpressing GFP-cIAP1 or GFP-cIAP2 were utilized to test birinapant to inhibit the degradation of IκBα induced by TNF. Cells were treated with 100 nM of birinapant 2 hours prior to the TNF (20 ng/mL) stimulation for the time

indicated. The total cell lysate was subjected to the western blot analysis using the antibodies indicated.

Supplemental table 1.

Kd values of birinapant and Smac-AVPI peptide for IAP BIR domains. A fluorescently labeled peptide (a-Abu-RPFK(5-FAM)-NH2) which binds to the Smac binding groove of BIR domains was incubated with indicated BIR domains. Birinapant or AVPI peptide was added at various concentrations and displacement of the peptide from the BIR domain by birinapant was measured by FP and an IC_{50} value was generated. K_d values were calculated from the IC_{50}.

Supplemental table 2

Sensitivity of a 111 cell line panel to birinapant as a single agent and in combination with TNF and TRAIL. Single agent activity is indicated as IC_{50} value. Combination activity with TNF or TRAIL was evaluated by treating cells with a range of concentrations of both birinapant and TNF or TRAIL in a matrix format. Data was analyzed using the MacSynergyII program, which performs a 3 dimensional analysis of drug interactions based on a Bliss-independence calculation. Combinations resulting in synergy volumes

>100 were considered significant. All data was generated by MTT assay following 72 hours compound exposure. + indicates IC_{50} < 100 ng/mL. – indicates IC_{50} > 100 ng/mL.