SUPPLEMENTAL FIGURES AND LEGENDS

Figure S1. FP/obatoclax induces dose-dependent mitochondrial injury and cell death in MM cells, accompanied by down-regulation of Mcl-1 protein and mRNA. (A) Human MM RPMI8226 cells were exposed (3h) to 1 – 2.5 µM HA14-1 +/- 10 nM SCH727965, after which apoptosis was analyzed by flow cytometry after double staining with annexin V-FITC and PI. (B) U266 cells were incubated with 100 nM FP +/- 500 nM obatoclax for 16 hr, after which CTD phosphorylation (serine 2) of Pol II was monitored. (C) RPMI8226 cells were exposed to 500 – 750 nM obatoclax +/- 100 – 120 nM FP for 24h, and Immunoblot analysis was then performed to monitor Mcl-1 levels (left); or treated with 750nM obatoclax +/- 100 - 120nM FP for 16h, after which quantitative RT-PCR was performed to determine mRNA levels of Mcl-1, using GAPDH as control (right).

Figure S2. FP/obatoclax down-regulates Bcl-xL, but not Bcl-2, which plays a partial role in this regimen. U266 cells were treated as follows, (A) 300 – 750 nM obatoclax +/- 100 nM FP for 6h, 16h, and 24h; (B) 100 nM FP + 500 nM obatoclax, in the presence or absence of 1 µM CHX, for 16h; or (C) 75 – 100 nM FP +/- 500 – 750 nM obatoclax for 24h (left), or RPMI8226 cells were incubated with 100 – 120 nM FP +/- 500 - 750nM obatoclax for 24h (right). After drug treatment, Immunoblot analysis was performed to monitor protein levels of Bcl-xL and Bcl-2. (D) U266 cells stably transfected with human full-length Mcl-1 were incubated with 100 nM FP + 500 nM obatoclax for 24h, after which immunoblot analysis was performed to monitor expression of Mcl-1. Alternatively, IP was performed to assess Bax conformational change. (E) RPMI8226 cells were stably transfected with human full length Bcl-xL or empty vector (EV).
Cells were exposed to 120 nM FP +/- 500 nM obatoclax for 24h and 48h, and then subjected to flow cytometry for apoptosis.

**Figure S3. FP/obatoclax up-regulates the BH3-only proteins Bim, Noxa, and Bik/NBK, primary events for interaction between FP and obatoclax.** (A) After 24h-exposure to 75 – 100 nM FP +/- 500 – 750 nM obatoclax, BH3-only proteins were profiled in U266 cells using a BH3-only detection kit. (B) Cells were exposed (24h) to FP (U266, 75 -100 nM; 8226, 100 – 120 nM) +/- 500 – 750 nM obatoclax, or 4 nM bortezomib, respectively, after which Noxa expression was assessed by immunoblot analysis. (C) U266 cells were stably transfected with shRNA targeting human Noxa (shNoxa), Bim (shBim, clone #3), or a scrambled sequence as a negative control (shNC) (inset). Cells were then treated (24h) with 100 nM FP +/- 500 nM obatoclax, or 4 nM bortezomib for comparison, after which apoptosis was monitored by flow cytometry. *** P < 0.001. (D) Cells were exposed to the indicated concentrations of FP +/- 500 nM obatoclax for 3h (8226) and 6h (U266), respectively, after which quantitative RT-PCR was performed to determine mRNA levels of Bim, using β-actin as control. (E) U266 cells were transiently transfected with a construct encoding shRNA targeting human Bax or a scrambled sequence, and immunoblot analysis was performed to monitor expression of Bax at 30h after transfection. After 6h-recovery, cells were treated with 100 nM FP + 500 nM obatoclax for 24h and then subjected to flow cytometry to monitor cell death (7AAD-positive; * P = 0.0143). (F) U266 stably transfected with shRNA targeting Bim (shBim) or scrambled sequence as negative control (shNC) were treated with 100 nM FP +/- 500 nM obatoclax, After drug treatment, cytosolic (S-100) and mitochondria-enriched membrane (pellet) fractions were separated and subjected to immunoblot analysis to monitor Bax translocation. Alternatively, IP was performed to assess Bax conformational change.
SUPPLEMENTAL VIDEO

FP/obatoclax prevents hind-leg paralysis in an i.v. murine model, in which MM cells home to BM and cause bone disease. NOD scid gamma mice were intravenously injected via tail vein with 5x10^6 U266 cells carrying luciferase. Treatment was initiated after a luciferase signal was detected (14 days after injection of tumor cells). FP (5mg/kg, i.p.) ± obatoclax (3mg/kg, i.p.) were administered daily for the first five days, followed by FP (3mg/kg) ± obatoclax (3 mg/kg) twice every three days for additional seven cycles. As shown in the accompanying video, hind-leg paralysis was observed at 80 days (after injection of tumor cells) in control group, 90 days in FP group, and 120 days in obatoclax group, but was not seen at day 120 days in the FP+obatoclax group.