SUPPLEMENTAL FIGURE LEGENDS

Fig S1 (A) KMS18 and **(B)** INA6 cells were treated with PBS-1086 (0.5 and 5 μM) for the indicated times (1 and 2 hours). NF-κB DNA binding activity in KMS18 nuclear extracts was measured by ELISA. NF-κB canonical activity included p65 (■), p50 (□), and c-Rel (■); NF-κB non-canonical activity included p52 (■) and RelB (□). Treatment with TNF-α (10 ng/ml) for 1 hour served as a positive control of NF-κB activity for both time points, 1 and 2 hours. The results of ELISA are expressed as relative absorbance. Data represent mean +/- SD of three independent experiments.

Fig S2 MM.1S cells were cultured with PBS-1086 (1 μM) for the indicated times (2 to 8 hours). Cytoplasmic extracts were subjected to Western blotting using IκBα, P-IκBα, and GAPDH antibodies. GAPDH was used as a loading control for cytoplasmic extracts. Blots are representative of three independent experiments.

Table S3 PBS-1086 with bortezomib triggers synergistic or additive cytotoxicity in bortezomib-resistant MM cells. Dox40 and ANBL-VR5 cells were treated with bortezomib and/or PBS-1086. Cytotoxicity was assessed by MTT assay. Combination indices (CI) were calculated using CompuSyn software. CI < 0.9 indicates synergistic effects. 0.9 < CI < 1.1 indicates additive effects.

Fig S4 (A) ANBL6-VR5 cells were cultured with PBS-1086 (0.15-40 μM) and bortezomib (0.62-160 nM) for 24 (—◆—), 48 (- □ -) and 72 hours (··▲··). Cell viability was assessed by MTT assay of triplicate cultures, expressed as percentage of untreated control. Data represent mean +/- SD viability. **(B)** Bortezomib-sensitive ANBL6-wt and bortezomib-resistant ANBL6-VR5 MM cell lines were treated for 48 hours with increasing concentrations of bortezomib (0.62-160 nM) and PBS-1086 (0.15-40 μM). Cell viability was assessed by MTT assay of triplicate cultures. The IC_{50} values of PBS-1086 and bortezomib for both cell lines were measured. The bar graph shows the IC_{50} ratio (ANBL6-VR5/ANBL6-wt). Data represent mean +/- SD viability of three independent experiments.

(C) ANBL6-VR5 cells were cultured for 4 and 8 hours with PBS-1086 (1 μM) in the presence or absence of bortezomib (Bort) (40 nM). Whole cell lysates were subjected to Western blotting using IκBα, P-IκBα (Ser 32/36), p38, and α-Tubulin antibodies. α-Tubulin was used as a loading control. Blots are representative of three independent experiments.

(D) The densitometric analysis of scanned immunoblotting images for IκBα (■) and P-IκBα (□) was performed with the NIH imageJ Software and expressed as fold change relative to non-treated cells.

Fig S5 Cytoplasmic extracts from mature osteoclasts were cultured for 2 hours with PBS-1086 at 1 and 5 μM. Control non-treated osteoclasts were stimulated with RANKL and M-CSF. Cytoplasmic extracts were subjected to Western blotting using JNK, ERK, and α-Tubulin antibodies. α-Tubulin was used as loading control, and blots are representative of three independent experiments.