Supplemental Figure Legends

Figure S1. Plasma exposure levels of MKC-3946 in mice.

Plasma exposure levels from individual mice 4 hours post IP administration at the indicated dose mg/kg. Data represent mean concentration from 3 independent mice. Blood was drawn by cardiac puncture at the time of sacrifice.

Figure S2. Normal mononuclear cells from healthy donors are not affected by MKC-3946.

Mononuclear cells isolated from four healthy donors were cultured with MKC-3946 (0-12.5 μM) for 48 hours. Cell viability was assessed by MTT assay of triplicate cultures, expressed as percentage of untreated control. Data represent mean ± SD.

Figure S3. MKC-3946 inhibits XBP1 splicing induced by bortezomib even when MM cells are co-cultured with BMSCs.

INA6 cells were treated with bortezomib (10 nM) in combination with MKC-3946 (10 μM) in the presence or absence of BMSCs for 8 hours. Total RNA was extracted, and XBP1 and β-actin mRNA was evaluated by RT-PCR.

Figure S4. Apoptotic signaling induced by 17-AAG is enhanced with MKC-3946, in association with increased CHOP.

RPMI8226 cells were treated with 17-AAG (AAG) (1 μM), in the presence or absence of MKC-3946 (10 μM) for the indicated times. Whole cell lysates were

subjected to western blotting using anti-CHOP, PARP, caspase3, and GAPDH Abs.

Figure S5. MKC-3946 treatment maintains IRE1α kinase function and enhances IRE1α/TRAF2 binding and downstream JNK pathway.

RPMI8226 cells were treated with bortezomib (10 nM) in the presence or absence of MKC-3946 (10 μM) for 8 hours.

(A) Cell lysates were immunoprecipitated (IP) with anti-TRAF2 Abs, followed by immunoblotting (IB) with anti-IRE1α and TRAF2 Abs. Whole cell lysates served as protein loading controls.

(B) Whole cell lysates were subjected to western blotting using anti-IRE1α, phospho-IRE1α (p-IRE1α), SAPK/JNK, phospho-SAPK/JNK (p-SAPK/JNK), and α-tubulin Abs.

Figure S6. MKC-3946 treatment does not affect body weight of mice.

Body weight of mice treated with MKC-3946 100 mg/kg, bortezomib 0.15 mg/kg IV, combination of MKC3946 with bortezomib, and control vehicle was expressed as percentage of baseline. Data represent mean ± SD.

Figure S7. MKC-3946 inhibits XBP1 splicing in plasmacytoma in vivo.

Total RNA was prepared from plasmacytoma of RPMI8226 harvested from MKC-3946- (100 mg/kg) and vehicle control- treated mice at day 3. XBP1 and β-actin mRNA was examined using RT-PCR. Each lane represents a single mouse.

Figure S8. MM cells have greater ER stress in vivo than in vitro.

Total RNA was prepared from RPMI8226 or INA6 cells either from in vitro cultures or harvested from plasmacytoma or human bone chips in untreated SCID mice. CHOP mRNA was determined by real time quantitative PCR. Data represent mean ± SD changes relative to β-actin mRNA in triplicate RPMI8226 or INA6 samples in vivo versus in vitro.

Figure S9. MKC-3946 inhibits XBP1 splicing induced by hypoxia, and triggers growth inhibition even under hypoxic conditions in vitro.

(A) RPMI8226 cells were treated with or without MKC-3946 (10 μM) under hypoxic (0.1% O_2) conditions for 24 hours. Cells cultured in normoxia served as controls. Total RNA was extracted, and XBP1 mRNA was evaluated by RT-PCR of triplicate cultures in each condition.

(B) RPMI8226 and INA6 cells were treated with MKC-3946 0 μM (□), 5 μM (■) or 10 μM (■) under normoxic or hypoxic (0.1% O_2) conditions for 48 hours. Cell proliferation was assessed by [^{3}H]-thymidine uptake of quadruplicate cultures, expressed as percentage of untreated normoxic controls. Data represent mean ± SD.

Figure S1

MKC-3946 IP dose (mg/kg)	10	30	60
mean plasma concentration (μM)	2.9	3.8	13.1

Figure S2

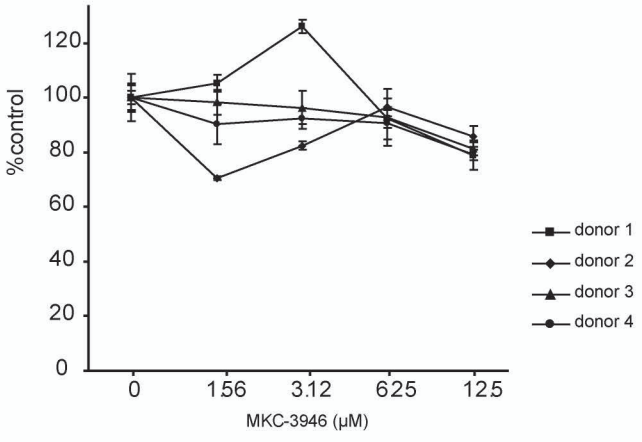


Figure S3

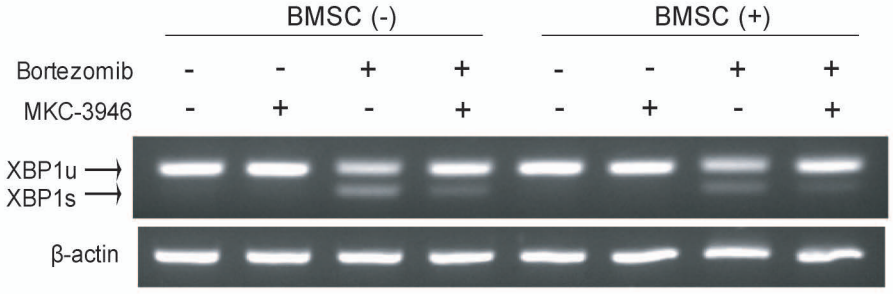


Figure S4

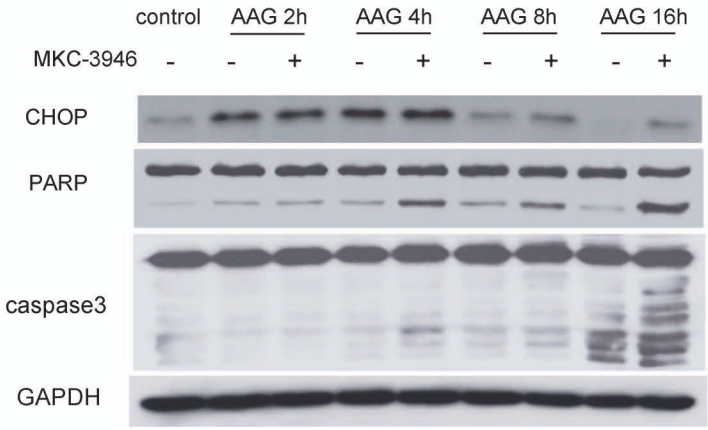
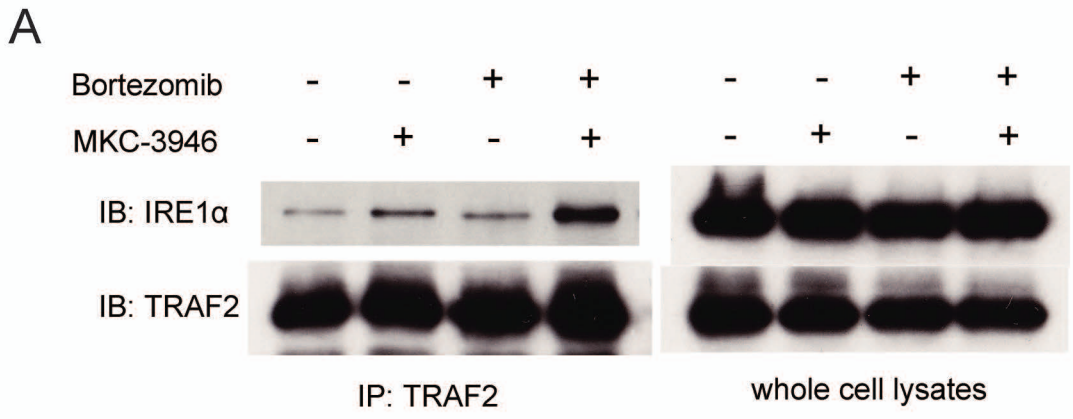
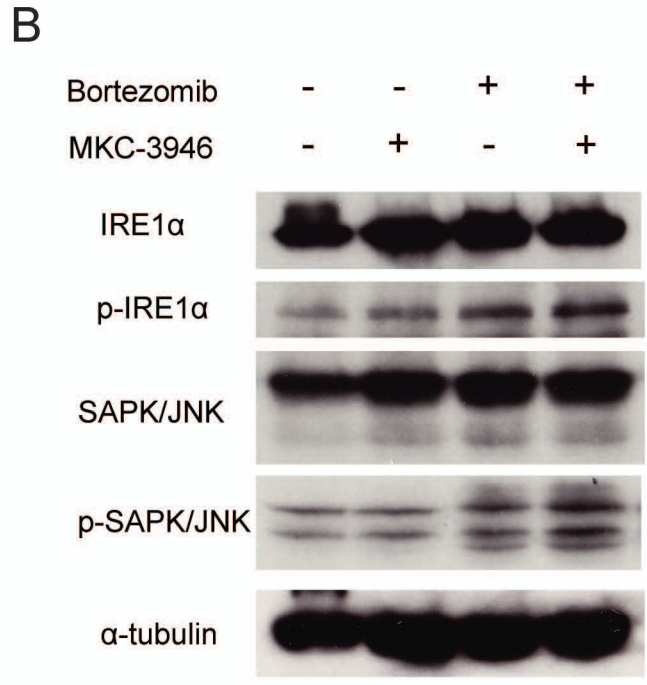


Figure S5





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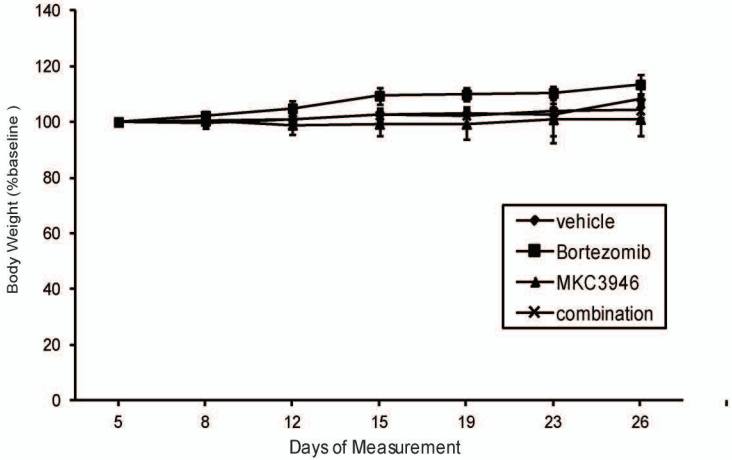

Figure S7

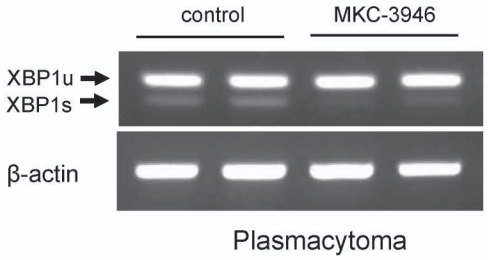

Figure S8

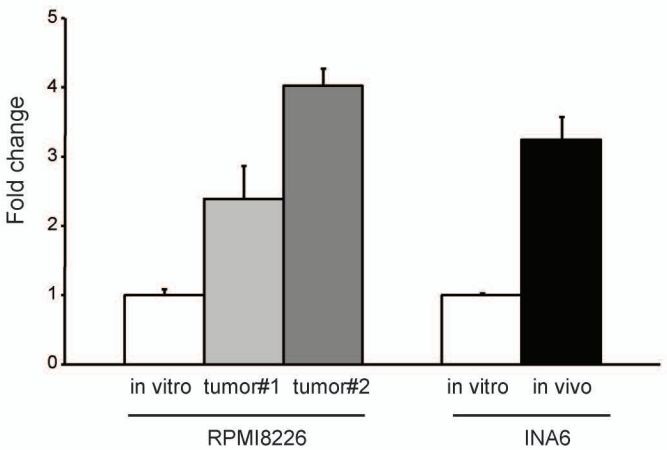

Figure S9