Supplementary information, Data S1 Materials and Methods

Antibodies.

Primary antibodies against Flag (Monoclonal ANTI-FLAG M2，Sigma，F1804)， *RGS4*（a gift from Dr. Mumby of University of Texas Southwest Medical Center） and *RAIDD* (Santa cruz Biotech., sc-7880), *cyclin G1* (Santa cruz Biotech., sc-7865), *cyclin D2* (Santa cruz Biotech., sc-593), *cyclin D3* (Santa cruz Biotech., sc-182), *ATF3* (Santa cruz Biotech., sc-188), *MLH1* (Santa cruz Biotech., sc-582), *BCL10* (Santa cruz Biotech., sc-5611), *stat3* (Santa cruz Biotech., sc-482), *CDK7* (Santa cruz Biotech., sc-856), *AP2A1* (Santa cruz Biotech., sc-8975), *p35* (Santa cruz Biotech., sc-821) , *PSMC1* (Proteintech, 11196-1-AP), *PSMD10* (Proteintech, 12342-2-AP), *SOST* (Santa cruz Biotech., sc-130258), *FASN* (Bioworld, BS6050) were obtained from indicated sources. All chemicals were from Sigma, if not otherwise indicated.

Cell culture.

Human embryonic kidney cells 293FT (Invitrogen) and murine *UBR1/UBR2* double knockout Cells, (*UBR1*$^{-/-}$*UBR2*$^{-/-}$), were maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS), L-glutamine (2 mM), penicillin (100 UI/ml), and streptomycin (10 mg/ml) at 37 ºC in 5% CO_2 atmosphere. Human multiple myeloma CZ-1 cells and their Bortezomib (BTZ)-resistant derivatives, CZ-1/R cells, were maintained in PRMI 1640 supplemented with 10% fetal bovine serum (FBS), L-glutamine (2 mM), penicillin (100 UI/ml), and streptomycin (10 mg/ml) at 37ºC in a 5% CO_2 atmosphere. The BTZ-resistant CZ-1/R cells were derived from CZ-1 cells by culturing them in the media that contained 0.5 μM Bortezomib for approximately three months, followed by maintaining the cells in medium containing 1 μM Bortezomib for three additional months before freezing storage. Afterwards, the BTZ-resistant CZ-1/R cells were switched to regular growth media, if not otherwise indicated.

Construction of the parental vector, pCDH-ccdB-mEGFP$_{fu}$-mRFP$_{f}$.

The plasmid, pAG426GAL-attR1-ccdB-attR2-EGFP, originally constructed in Dr. Lindquist's lab at Massachusetts Institute of Technology[1], was obtained from Addgene *Inc*. EGFP was replaced by DNA fragments coding monomeric Enhanced Green Fluorescent protein (mEGFP), double FLAG epitopes, human ubiquitin with all lysines substituted with arginines, monomeric Red Fluorescent Protein (mRFP) and double FLAG epitopes, mEGFP-$(FLAG)_2$-Ub_{k0}-mRFP-$(FLAG)_2$. Then, the fragment containing attR1-ccdB-attR2-mEGFP-$(FLAG)_2$-Ub_{k0}-mRFP-$(FLAG)_2$ was subcloned into the lentivirus vector,

pCDH-CMV-MCS-EF1a-Puro (System Biosciences), using XbaI and Nhe1 restriction sites, to obtain the parental vector, pCDH-ccdB-mEGFP$_{fu}$-mRFP$_{f}$, for mammalian expression.

Construction of the virus-based human ORF expression library.

For ProTA (Protein Turnover Assay), ORFs of 15,000 cDNAs from human ORFeome V5.1 in pDONR223 (OpenBiosystems) were cloned into the parental plasmid, pCDH-ccdB -mEGFP$_{fu}$-mRFP$_{f}$, using Gateway cloning technique (Invitrogen). To ensure the complexity of the library, each Gateway reaction should give >20000 colonies and total $2*10^6$ colonies were obtained.

The ProTA reporter cell library construction.

To generate the reporter cell library, 293FT cells were infected with lentiviruses carrying ORFs-mEGFP$_{fu}$-mRFP$_{f}$ supplemented with 8 μg/ml polybrene (Sigma).Viruses were removed 18 hrs later, and cells expressing mRFP were isolated by FACS 72 hrs after infection. In order to prevent multiple insertion events, multiplicity of infection was controlled so that < 0.5% of cells were mRFP positive (MOI ~ 0.05). To ensure the complexity of the library, 3×10^6 mRFP-positive cells were collected after infection and a minimal representation of 3×10^5 cells were maintained during each cell passage. Diversity of the ProTA reporter cell library was further confirmed through microassay analysis and also PCR reactions performed with the primer sets and genomic DNAs from randomly picked colonies of the cultured ProTA library (see details on "**Genomic DNA preparation and microarray hybridization" section**).

The control plasmid pCDH-mEGFP$_{fu}$-mRFP$_{f}$ was obtained by removing attR1-ccdB-attR2 cassette from the ProTA parental vector to allow in-frame expression of the mEGFP$_{fu}$-mRFP$_{f}$ fusion protein. And ORFs of human *RGS4*, *TP53*, *CDC25A*, *ARC*, *NF-KbIB* or *ANXA1* gene were cloned into the parental vector, resulting in the ProTA mammalian expression constructs, pCDH-*RGS4*-mEGFP$_{fu}$-mRFP$_{f}$, pCDH-*TP53*-mEGFP$_{fu}$-mRFP$_{f}$, pCDH-*CDC25A* -mEGFP$_{fu}$-mRFP$_{f}$, pCDH-*ARC*-mEGFP$_{fu}$-mRFP$_{f}$, pCDH-*NF-KbIB*-mEGFP$_{fu}$-mRFP$_{f}$, pCDH-*ANXA1*-mEGFP$_{fu}$-mRFP$_{f}$ were obtained through Gateway reactions.

Construction the the individual ProTA reporter cell lines.

To generate the stable ProTA reporter cell lines, 293FT cells were infected with the invidual lentiviruses to express $mEGFP_{fu}$-$mRFP_f$, *RGS4*-$mEGFP_{fu}$-$mRFP_f$, *TP53*-$mEGFP_{fu}$-$mRFP_f$, *CDC25A*-$mEGFP_{fu}$-$mRFP_f$, *ARC*-$mEGFP_{fu}$-$mRFP_f$, *NF-KbIB*-$mEGFP_{fu}$-$mRFP_f$, or *ANXA1*-$mEGFP_{fu}$-$mRFP_f$, respectively, in the presence of 8 μg/ml polybrene. Viruses were removed 18 hrs later, and cells expressing mRFP were sorted out using FACS 72 hrs after infection. All cell lines were then maintained in puromycin (1 ug/ml). In all the experiments to profile Bortezomib (BTZ, *LC Laboratories*) induced change in protein stability, cells were treated with Bortezomib (1 μM, final concentration) for 6 h.

For transient expression of the human ORFs tagged to $mEGFP_{fu}$-$mRFP_f$, 293FT cells were transfected with the ProTA parental vectors containing the respective ORFs in typical ProTA configuration.

Fluorescence-activated cell sorting (FACS).

To prepare cells for FACS, live cells were harvested, re-suspended in DMEM with 10% FBS and filtered using a 40 μm cell strainer (BD Falcon). Cell sorting was performed on a BD FACS Aria II Cell Sorting System. Flow cytometry analyses were carried out on BD LSR II System, using FlowJo (version 6.3) for data analysis.

Genomic DNA preparation and microarray hybridization.

To prepare genomic DNAs, cell pellets from each FACS subpopulation were lysed in DNA extraction solution (10 mM Tris-HCl pH 8.0,10 mM EDTA, 0.5% SDS, 0.2 mg/ml proteinase K and 25 μg/ml RNAse A) at 55°C for 16 hrs, followed by phenol-chloroform /chloroform extractions. Genomic DNAs were precipitated in ethanol, air-dried and dissolved in TE buffer (10 mM Tris-HCl, pH 8.0, 0.1 mM EDTA), following the standard procedure[2]. To amplify the chromosomally integrated human ORFs in ProTA, PCR amplifications were performed with aforetime extracted genomic DNAs as the template. For the PCR, the following primer sets were used: the forward primer: 5'-CCTGGAGACGCC ATCCACGCTG-3' and the reverse primer: 5'-<u>TAATACGACTCACTATAG</u>GGAG CTCCTCGCCCTTGCTCACCATTAAGCT-3'. The reverse primer contains the promoter sequence for T7 RNA polymerase (underlined). EX-Taq (Takara，DRR001A) was used for PCR amplification. PCR was performed with the following program: 98°C for 3 min, 22 cycles of "98°C for 10 sec, 62°C for 40sec, 72°C for 7min" and a final step of 72°C for 10 min. The resulted PCR products were then used as template for *in vitro* transcription to prepare complementary RNA (cRNAs) for hybridization, using T7 MEGAscript (Ambion，AM1334). The transcribed RNAs were extracted and labeled with Cy3 or Cy5（GE

healthcare Bioscience，PA13105）and purified using RNeasy mini kit（QIAGEN，74106）. All procedures were performed following the manufacturers' instructions.

Customized microarrays with 60-mer probes against ORFs in human ORFeome v5.1 were manufactured by Agilent at a density of 4 x 44,000. Microarray hybridizations were performed following the standard Agilent hybridization protocol. Eight hybridizations were performed with ORFs from untreated cells labeled with Cy5 against from treated cells labeled with Cy3. Other details were just performed following our ProTA pipeline.

Data acquisition and normalization.

The hybridized arrays were scanned using Agilent Microarray Scanner (Agilent technologies, G2565BA) and Feature Extraction software 10.7 (Agilent technologies) with default settings. Background-subtracted fluorescence log-ratios were normalized within each array using composite loess normalization that was available in the Bioconductor package limma. Composite loess normalization corrects the expression log-ratios for intensity-based trends by subtracting from each expression log-ratio the corresponding value of the loess curve. The loess curve is constructed by performing a series of local regressions. One local regression was carried out for each spike-in control spot on the corresponding MA-plot, using the statistical environment R.

Quality assessment of array data set.

To assess the overall quality of the array hybridization, we first examined the sum of the Cy5 signals from all eight hybridizations. In principle, when the cells expressing a specific ORF-mEGFP$_{fu}$ fusion is distributed across the eight subpopulations after FACS, its total Cy5 signal should be equal to the sum of the Cy5 signals from the eight individual chips. In all the Cy5 signals checked for every ORF in ProTA library, 94.3% of probes displaying 3-fold above-background signal, suggesting that library complexity did not decrease during our experimental manipulations. Therefore, the cutoff was set to 3-fold above background to ensure the quality of data for subsequent analyses.

Microarray data processing in ProTA.

For each ORF, the sum of the eight ratios was first normalized to 1. PSI values were then generated using the formulas $\mathrm{PSI}=\sum_{i=1}^{8} \mathrm{Ri} * \mathrm{i}$ and $\mathrm{SD}=\sqrt{\sum_{i=1}^{8} \mathrm{Ri} * (\mathrm{i}-\mathrm{PSI})^2}$, where *i* denotes the fraction number of the subpopulation in FACS sorting, and Ri is the fraction of

the signal present for a gene in that given subpopulation i. (also see the Result section). Data were further filtered to remove those bearing one of the following characteristics: (1) a STDEV greater than 1.5; (2) a maximal value of the Ri less than 0.3; (3) a difference between PSI and the fraction number i with the maximal Ri greater than 1. This finally left us with 5,894 ORFs that have a single-peak pattern in PSI analysis.

When ProTA was applied to profile bortezomib (BTZ)-induced changes in protein stability, PSI values were obtained with all the human ORF-encoded proteins in cells treated with or without BTZ (PSI_{BTZ} and PSI_0, respectively), the increases in PSIs (ΔPSI) after BTZ treatment were calculated using the formula, $\Delta PSI = PSI_{BTZ} - PSI_0$. Proteins were then ranked according to their respective $\Delta PSI/PSI_0$ values in descending order, resulting in the ProTA-BTZ dataset indexing BTZ-induced changes in protein stability (Supplementary information, **Table S2**).

Microarray analysis to identify bortezomib-induced changes at transcriptome level.

To identify bortezomib-induced changes at transcriptome level, 293FT cells were treated with or without Bortezomib (1 μM, 6h). Total RNAs were extracted using Trizol (Invitrogen). cRNAs were prepared and hybridized to the Whole Human Genome Oligo Microarray **(**Agilent) following the manufacturer's instruction. Data were normalized with Gene-Spring (Agilent). Microarray hybridization and analysis were carried out with assistance from ShanghaiBio Inc. These genes whose expression levels changed at least threefold upon BTZ treatment were selected for subsequent analyses.

Gene Ontology (GO) analysis.

With the whole genome background (DAVID default) as a reference, gene ontology (GO) enrichment analysis was performed using the Database for Annotation, Visualization and Integrated Discovery (DAVID, National Institutes of Health)[3-5]. For Supplementary information, Figure S3, the top 1500 hits from the ProTA-BTZ dataset were used in the GO enrichment analysis. To detect significantly over-represented GO biological processes, the DAVID functional annotation clustering tool was used by choosing the 'PANTHER_BP_ALL', 'GOTERM_CC_FAT', 'PANTHER_MF_ALL' options. The combined list of official gene symbols corresponding to the hits from the ProTA-BTZ dataset

was used for input. Similarly, for Supplementary information, Figure S7, all the transcriptional hits of FC (fold change) > 3 were used as input.

Enriched pathway analysis.

For enriched pathway analysis, the Database for Annotation, Visualization and Integrated Discovery (DAVID) 2007 Functional Annotation Clustering was used to search Kyoto Encyclopedia of Genes and Genomes (KEGG) and Biocarta pathway, in order to identify significantly changed pathways in the subset of the more stable genes. The thresholds used in this study included: Count (2); EASE (0.1); (P-values from modified Fisher's exact test; more information can be found in http://david.abcc.ncifcrf.gov/); Gene Symbol, Fold Enrichment, and FDR were also given in the Table 1.

Protein-protein interaction (PPI) analysis.

The initial PPI network was retrieved from STRING[6] (v. 9.0) (http://string.embl.de/), one of the largest database of known and predicted protein interactions. For Figure.4A, the top 250 hit genes from ProTA-BTZ dataset were used. The following active prediction methods were employed: neighborhood, co-expression, gene fusion, experiments, co-occurrence, database, and text mining, with a medium confidence score (0.400). The PPI networks were then visualized with Cytoscape[7], and the MCODE (v1.32) clustering algorithm[8] was employed to identify highly connected nodes that constituted many functional sub-networks. To further annotate these sub-networks, modules were defined using the BiNGO 2.44 Cytoscape plug-in[9]. The top 500 hit genes in ProTA-BTZ dataset were used to generate Supplementary information, Figure S4; The hit genes from transcriptional analysis (fold changes, FC>3） were used to generate Supplementary information, Figure S8**,** with text mining skipped.

Chemical-protein interaction (CPI) analysis.

The initial CPI network was retrieved from STITCH [4] (version 2.0) (http://stitch2.embl.de/), an established resource to explore known and predicted interactions between chemicals and proteins. The following active prediction methods were employed: neighborhood, co-expression, gene fusion, experiments, co-occurrence, database and text mining, with a medium confidence score (0.400). The CPI network was then visualized using Cytoscape.

Immunoblotting analysis.

293FT Cells and the derivative lines that stably expressed dual fluorescence-tagged genes were seeded in 6-well cell culture plates (Corning). After 48h, cells were either treated with or without Bortezomib (1uM) for 6 h. Then, cells were washed with PBS (Gibco, 14040-133) and lysed in ice-cold lysis buffer (20 mM Tris-Cl, 150 mM NaCl, 1 mM EDTA, 1 mM EGTA, 1% (v/v) Triton X-100, 2.5 mM sodium pyrophosphate, 1 mM β-glycerolphosphate, 1 mM Na_3VO_4 , pH 7.5) with protease inhibitor cocktail (Roche), followed by sonication on Bioruptor UCD-200 (Diagenode). Samples were centrifuged at 4°C, 15, 000 *g* for 10 minutes to collect supernatants. Cell extracts were subjected to 12% sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) and blotted onto PVDF membrane (Millipore). Membranes were blotted with mouse monoclonal M2 anti-FLAG antibody. To determine whether ProTA system was applicable for monitoring the stability of N-end rule substrates, 293FT or *UBR1*$^{-/-}$*UBR2*$^{-/-}$cells were transiently transfected with the vectors pCDH-*RGS4*-mEGFP$_{fu}$-mRFP$_f$ or pcDNA3.1-EGFP-*RGS4*, separately. 48h after transfection, cells with or without Bortezomib treatment were collected and subject to immunoblotting analysis with M2 anti-FLAG or anti-*RGS4*. To validate some hits from PPI analysis, 293FT cells were transiently transfected with the ProTA vectors containing indicated ORFs. To determine the level of endogenous proteins, CZ-1 or CZ-1/R cells with or without Bortezomib treatment were collected, lyzed and subjected to immunoblotting analysis with respective antibodies.

Co-Immunoprecipitation

293FT cells tranfected with PSMB5-Flag and the HA-PSMC1 were grown to 85% confluence in DMEM containing 10% FBS and 1% Pen/strep, then trypsinized, and washed three times with PBS buffer. The cell pellets were collected and lysed in buffer A [100 mM sodium chloride, 50 mM sodium phosphate, 10% glycerol, 5 mM ATP, 1 mM DTT, 5 mM $MgCl_2$, protease inhibitor cocktail (Roche), phosphatase inhibitor, and 0.5% NP-40 (pH 7.5)]. The lysates were centrifuged at 13 000 rpm for 15 min to remove cell debris, and the supernatant was incubated with Anti-FLAG M2 Magnetic Beads overnight at 4°C.
The Anti-FLAG M2 Magnetic Beads were then washed with 20 bed volumes of the lysis buffer. Beads were incubated in 2 bed volumes of Flag Peptide 20ug/ul at 30°C for 30min. The 26S proteasome complex was eluted from the beads.

Cell viability assay.

A total of approximately 5,000 cells were plated in each well of the 96-well plates and cultured until exposed to Bortezomib, 17-AAG (Selleck Chemicals) or C75 (Cayman Chemical) or Merck-5 (SYNKINASE) at indicated concentrations. 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT, 10 µl, 5 mg/ml) solution in PBS was added to each well at the specified time points. After 4 h, 100 µl of diffusion solution was added to each well to dissolve the formazan crystals. The absorbances at 570 nm were recorded for each well on a Multiskan MK3 microplate reader (Thermo Scientific). Data were presented as mean ±S.E.M (n = 6; $P < 0.0001$).

Cycloheximide chase

293FT cells transfected with *PSMC1*-mEGFP$_{fu}$-mRFP$_{f}$ or *PSMD10*-mEGFP$_{fu}$-mRFP$_{f}$ were pretreated with vehicle or the proteasome inhibitor BTZ (1.0 µM) for 4 h before addition of cycloheximide (100mg/ml) for the time indicated. Cellular extracts were subjected to Western blotting with an anti-FLAG antibody, Protein samples were normalized based on cell number. The protein levels were quantified using NIH ImageJ software and the relative protein remaining (%) was plotted against the indicated time course.

Enzyme-linked immunosorbent assay (*ELISA*).

The ELISAs were performed with Human *IL-6* ELISA Ready-SET-Go kit (eBioscience), following the manufacturer's instruction. Samples were collected from the indicated groups of cells sonicated in lysis buffer (20 mM Tris-Cl, 150 mM NaCl, 1 mM EDTA, 1 mM EGTA, 1% TritonX-100 (v/v), 2.5 mM sodium pyrophosphate, 1 mM β-glycerolphosphate, 1 mM Na_3VO_4, and protease inhibitor cocktail (Roche), pH 7.5), followed by ELISA analysis immediately. Data were presented as mean ±S.E.M (n=4; $P < 0.02$).

Combination index analysis

The dose-effect relationship between bortezomib and 17-AAG or C75 was analyzed using CompuSyn software (http://www.combosyn.com/). The combination index equation was based

on the following multiple drug effect equation of Chou-Talalay (11). Combination index = 1, >1, or <1 is considered additive, antagonistic, or synergistic, respectively. Drug combination studies were based on the fraction of cells affected relative to untreated control. To evaluate the relative contribution of each agent, CZ-1 cells were seeded at $5*10^3$per well and exposed to five concentrations of BTZ, C75 or 17-AAG, or the indicated combinations. After 72 hours at 37℃, cytotoxicity was measured by 3-(4,5-dimethylthiazol-2-yl) -2,5-diphenyltetrazolium bromide assay as previously described.

Statistical Methods

Data were analyzed by using Prism 5 (GraphPad Software, Inc). Unpaired T-test was performed using GraphPad Prism version 5.0 for Windows (GraphPad Software). Results were presented as means ± S.E.M.

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Supplementary legends

Supplementary information, Figure S1 Immunoblotting analysis confirmed bortezomib induced changes in stability of individual fusion proteins.

The steady state levels of ORF-mEGFP$_{fu}$-mRFP$_{f}$ proteins from cells treated with or without bortezomib were assessed with immunoblotting analysis using anti-FLAG

Supplementary information, Figure S2 Immunoblotting analysis confirmed bortezomib induced changes in stability of endogenous proteins.

The steady state levels of endogenous proteins in HEK293 FT cells, treated with or without bortezomib, were assessed with immunoblotting analysis using indicated antibodies.

Supplementary information, Figure S3 Categorization of bortezomib-induced changes in global protein stability.

Gene ontology (GO) analysis revealed the "functional gene categories" of the genes whose products were stabilized upon BTZ treatment (**A**). These genes were further categorized into the categories of "cell proliferation and differentiation" (**B**), "apoptosis" (**C**), and "an overview of recorded molecular function" (D), "subcellular localizations" (**E**).

Supplementary information, Figure S4 Protein-protein interaction (PPI) network analysis of bortezomib-induced changes in protein stability.

PPI network analysis with STRING categorized (500) genes whose products were most significantly stabilized upon BTZ treatment into several functional sub-network or modules (see "Methods" for definition). The sub-networks were visualized with Cytoscape.

Supplementary information, Figure S5 Characterization of BTZ-sensitive cells (CZ-1) or BTZ-resistant cells (CZ-1/R) from a multiple myeloma (MM) patient.

CZ-1 or CZ-1/R cells were treated with BTZ for 24 h at the indicated concentrations. Cell viability was determined using MTT assay, with the percentages of viable cells

in the untreated control set as 100%.

Supplementary information, Figure S6 More functional proteasomes were assembled upon BTZ treatment.

(A) The stability of PSMC1 and PSMD10 were assayed by cycloheximide (CHX) chase in CZ-1 cells. (**B**) Level of endogenous *PSMB5* proteins in CZ-1 was unaltered upon bortezomib treatment. (**C**) The basal levels of endogenous *PSMB5* proteins were higher in drug-resistant CZ-1/R cells than in CZ-1 cells. (**D**)The stability of PSMB5 were assayed by cycloheximide (CHX) chase in CZ-1 cells. (**E**) The steady state levels of PSMB5-flag and HA-PSMC1 proteins from cells treated with or without bortezomib were assessed with immunoblotting analysis. (**F**) Cell lysates were subjected to a Co-IP assay with anti-Flag beads and were subjected to immunoblotting analysis with anti-HA or anti-Flag antibodies.

Supplementary information, Figure S7 Categorization of bortezomib-induced changes in gene transcriptions.

Gene ontology (GO) analysis categorized the (FC>3) genes whose transcriptions changed most significantly upon BTZ treatment into the categories of “biological process” (**A**), “subcellular localization” (**B**) and “molecular function” (**C**).

Supplementary information, Figure S8 Protein-protein interaction (PPI) network analysis of bortezomib-induced changes in gene transcriptions.

PPI network analysis with STRING categorized the (FC>3) genes whose transcriptions changed most significantly upon BTZ treatment (see “Methods” for more details). The network was visualized with Cytoscape.

Supplementary information, Figure S9 Chemical-protein interaction (CPI) network analysis of bortezomib-induced changes in protein stability.

CPI network analysis with STITCH categorized (250) genes whose products were most significantly stabilized upon BTZ treatment (see “Methods” for more details). The network was visualized with Cytoscape.

Supplementary information, Figure S10 Combined chemotherapy using BTZ and drugs that targeted the ProTA identified key genes

BTZ-resistant CZ-1/R cells (A) were treated with 5 nM BTZ alone or in combination with 10 μg/ml C75, 48h. BTZ-resistant CZ-1/R cells (B) were treated with 10 nM BTZ or in combination with 50 μM 17-AAG, 48h. Viability of the myeloma cells in each group was assessed using MTT assay. Data were represented as mean ±S.E.M (n = 6; *, $P < 0.1$; n=6; ***, $P < 0.0001$).

Supplementary information, Figure S11 C75, 17-AAG and BTZ exhibited synergistic, cytotoxic effects on the MM cell line CZ-1/R

(A) CZ-1/R cells were exposed to BTZ alone or in combination with 10 μg/ml of C75 for 24 h, and survival was assessed by an MTT assay (B) CZ-1/R cells were exposed to BTZ alone or in combination with 50 μM of 17-AAG for 24 h, and survival was assessed by an MTT assay. The combination index plots (CI) were generated by CompuSyn software (http://www.combosyn.com/) based upon the Chou-Talalay method. The dashed line indicates additive affect (CI = 1).Antagonism (above dashed line) and synergism (below dashed line). Tables were included to provide the drug concentrations tested.

Supplementary Tables

Supplementary information, Table S1 Annotations for the genes whose symbols were frequently used in this work.

Supplementary information, Table S2 A complete list of the ProTA-identified protein stability profiles in 293FT cells treated with or without bortezomib.