SUPPLEMENTAL METHODS

Peptide Standard selection

Peptides for 10 proteins included in the assay were selected for immunization from tryptic digests of human MM1S, KG-1, K562, and Jurkat E6-1 cells previously analyzed by electrospray LC-MS/MS. Observed peptides were selected based on their response in electrospray LC-MS/MS, C18 reversed-phase chromatography retention time, possibility of missed cleavage (e.g dibasic residues, RR, KK, KR) and uniqueness to the target protein. In the case of IKZF1, IKZF3, CSNK1A1, RNF166, ZFP91, ZFP692, β-Actin, and GAPDH, selected peptides often span multiple protein isoforms for the given gene.

Generation of anti-peptide polyclonal antibodies and peptide standards

Twenty anti-peptide polyclonal antibodies were generated in New Zealand white rabbits following a standard 77-day protocol (New England Peptide) as previously described[1]. In brief, peptides were synthesized to 85% purity with an additional cysteine on the N-terminus and conjugated to KLH for immunization. Five peptides per protein were combined and two rabbits were immunized in descending doses over 70 days. Antisera titers of the final bleeds were measured by peptide ELISA. Peptides with the highest titer (typically 2 selected per protein), were serially purified from a pool of final bleeds by affinity chromatography using a Sulfolink column (Thermo) bound with the immunizing peptide. In short, the sera were bound to the column containing the peptide with the lowest titer; then the flow through, expected to contain antibodies specific to subsequent higher titer peptides, was bound to the column containing the peptide with the next highest titer in order to maximize yield for each antibody. After the antiserum was bound, an extended wash (> 100 CV) was used to reduce latent passenger peptide prior to elution with glycine buffer pH 2.5. Purified antibodies were dialyzed into 25% glycerol/1x PBS/0.1% NaN3 and stored at -20^{o} C until use.

Corresponding “heavy” peptides containing a stable isotope-labeled lysine or arginine at the C-terminus were synthesized, purified to greater than 95%, formulated in 30% acetonitrile/0.1% formic acid and quantified by amino acid analysis (New England Peptide). Light peptide sequences were also synthesized and used without further purification or AAA (Supplemental Table S1). An additional 2 peptides with similar sequence to 2 of the peptides for which antibodies were generated were also synthesized and included in the assay (see Supplemental results for details).

Preparation of chemically cross-linked antibody-Protein G-magnetic beads

To reduce the presence of residual light peptide used for purification of antibodies and increase capture reproducibility, antibodies were cross linked individually to 1um Protein G magnetic beads (Thermo) as described previously[2]. Briefly, each antibody was incubated with protein G beads using 1:1.5 (ug/uL) ratio and tumbled mixed in a final volume of 1.5 mL of 1x PBS/0.03% CHAPS for 16 hours at 4^{o}C. After mixing, the volume was reduced to 900 uL by isolating the beads to one side of the vial with an external magnet and removing 600uLs followed by transfer of the remaining solution to a 2mL deep 96 well plate. Ab-beads were crosslinked via primary amines by dimethyl pimelimidate (DMP) using a KingFisher^{TM} magnetic particle processor

(Thermo) equipped with a large magnetic head as follows: mix 5 min to pick up beads, 30 min in freshly made 20 mM DMP/200 mM triethanolamine pH 8.5 and quench 30 min in 150 mm monoethanolamine. Antibody-Protein G conjugated beads (Ab-beads) were washed twice for 5 min with 5% acetic acid/0.03% CHAPS followed by 5 min re-equilibration with 1x PBS/0.03% CHAPS. Ab-beads were finally resuspended to 0.5 mg/ml in 1x PBS/0.03% CHAPS/0.1% NaN3 and stored at 4°C until needed.

Cell Lysis and Digestion

Cell pellets were digested either manually following protocols described previously or in 96-well plate format on a Bravo automated liquid handling platform (Agilent Technologies)[3,4]. Cell pellets were first lysed on ice with 100uLs of lysis buffer containing 8M urea, 50mM Tris-HCl pH 8, 150mM NaCl, 200uM EDTA and protease inhibitors Aprotinin (2ug/mL), Leupeptin (10ug/mL) and PMSF (1mM). After 10 min on ice, cell lysates were clarified by centrifugation (20,000 x g) for 10 min at 4°C. Protein concentration for each sample was then determined in triplicate using Thermo Pierce's BCA assay following manufacturer's protocol. Then 100ug of each sample was delivered to a 2mL deep well plate with additional lysis buffer to a final volume of 100uLs and frozen in preparation for digestion the following day.

Time course and dose response curve samples for all the drugs tested were prepared, processed and analyzed in process triplicates. Each replicate of a given time course or dose curve series was processed on a separate plate while all the samples within a replicate were kept together on the plate but randomized to reduce systematic bias. This randomization was carried through the entire processing scheme including the run order during LC-MRM-MS analysis.

Once thawed, plates were centrifuged briefly and digestion was performed on Bravo platform configured with temperature controlled plate positions. Digestion program on Bravo has been modified for volumes, timed segments and some constituents but was otherwise described previously[5]. Briefly, samples were reduced by addition of 10uLs of DTT to a final concentration of 5 mM and incubated at 37°C for 45 min with shaking. After reduction, samples were cooled to room temperature and alkylated by addition of iodoacetamide (IAA) to a final concentration of 10 mM and incubated in the dark for 45 min. Urea concentration was reduced to less than 2M with 50mM Tris HCl pH 8.1 and, after checking that the pH remained at 8, LysC (Wako) was added at a 1:50 enzyme to substrate ratio and incubated for 2hr at 37°C at 850 rpm shaking. After 2hr, the sample was further diluted to 1M urea with additional 50mM Tris-HCl prior to addition of trypsin (Promega) at the same 1:50 enyzme to substrate ratio. Samples were left shaking overnight at 37°C. The following day, reactions were quenched with the addition of formic acid to a final concentration of 1%. Sample plates were then desalted using a positive pressure vacuum manifold (Waters) configured with an Oasis® HLB 10mg 96 well plate extraction cartridges using the following procedure: Plates were conditioned with 3x 500uLs of 80% acetonitrile/0.1% formic acid followed by equilibration with 4 x 750uLs 0.1% formic acid. After loading the plate with digested sample, wash each well with 4 x 750uLs of 0.1% formic

acid followed by elution with 3 x 500uLs 80% acetonitrile/0.1% FA. Plates were then frozen and dried by vacuum centrifugation and stored dry at -80°C.

Preparation of plates for immunoaffinity enrichment

Digested and dried cell lysates were then resuspended in 100uLs of 1x PBS/0.03% CHAPS for 10min with gentle shaking. After a brief spin, samples were transferred to a 250uL King Fisher 96 well plate (Thermo) by multichannel pipetman. An additional 100uLs of 1x PBS/0.03% CHAPS containing 0.3 fmol/uL heavy labeled peptides was then added to each well (30fm total per well) of the incubation plate. To ensure accuracy of the assay measurement and to compensate for higher levels of observed endogenous GSPT1, ACTB and GAPDH, additional heavy peptides for these proteins were spiked in at 0.15, 1.5 or 6 pmol total per well.
To evaluate the consistency of capture across all experiments, each plate also contained 1-3 wells of a common KG-1 digested cell lysate background added at the same 100ug input amount at the time of the immunoaffinity capture.

Automated Peptide Immunoaffinity Enrichment

Prior to addition to the capture plate, each of the 0.5ug/uL stock solutions of Ab-crosslinked magnetic beads were mixed gently and then combined equally as a "mastermix". Using an exterior magnet to isolate bead location and aid in buffer transfer and exchange, this Ab-bead mixture was washed twice briefly with 0.01N HCl as indicated previously and, following several rinses, then returned to the same original mixture volume and buffer, 1x PBS/0.03% CHAPS. This mastermix was used to distribute 1ug of each antibody to each sample well. The plates were sealed with aluminum adhesive seal mats and gently inverted on a Labquake® rotator (Thermo) overnight at 4 °C.

After overnight incubation, each plate was gently centrifuged briefly and transferred onto the King Fisher magnetic bead processor and processed as described previously (REF). Briefly, the beads were transferred from the incubation plate into a 250uL plate containing 1x PBS, 0.03% CHAPS and mixed for 1.5 min. The beads were subsequently transferred and mixed twice more, with the second wash plate containing one-tenth the salt concentration. In the final step, the beads were transferred into a 100uL PCR plate containing 50µL of 3% acetonitrile/5% acetic acid to elute any bound peptide.

Automated Desalting of Immunoaffinity Enriched Samples

Upon completion of post capture processing on the King Fisher system, the elution plate was immediately desalted using AssayMAP Bravo configured with Reverse Phase S (RPS) cartridges described previously[5]. Briefly, RPS cartridges were primed with 50 µL 90% acetonitrile/0.01% formic acid at 10 µL/min and equilibrated with 50 µL 0.1% formic acid at 10 µL/min. Samples were then loaded onto cartridges at 2 µL/min. Cartridges were washed 3 times with 50 µL of 0.1 % formic acid at 10µL/min and eluted with 50 µL 45% acetonitrile/0.01% formic acid at 5 µL/min. Eluates were transferred to individual autosampler vials, frozen, and dried down by vacuum centrifugation. Samples were resuspended in 6µL 3% acetonitrile/5% acetic acid prior to LC-MRM-MS analysis.

NanoLC-MRM-MS Analysis

All samples were analyzed on a Xevo TQ-S triple quadrupole mass spectrometer installed with a Nanospray source and coupled to a NanoAcquity LC (Waters, Milford MA). The NanoAcquity LC was primed with mobile phase A (0.1% formic acid) and mobile phase B (acetonitrile/0.1% formic acid). Samples were injected (1 µL) onto a 0.075 mm ID PicoFrit (New Objective) column pulled to a 10 um emitter and custom packed to ca. 25 cm with 1.9 µm 120 Å C18-AQ Reprosil beads (Dr. Maisch). The LC gradient was optimized for all peptides with an initial condition of 2.7% B followed by ramps of 2.7% B to 6.3% B in 3 min, 6.3% B to 22.5% B in 27 min, 22.5%B to 36%B in 7min and 36%B to 81%B in 3min. Inject-to-inject time was 75min in duration including a 30min re-equilibration period. Ion source was set to positive ion mode with source temperature of 90°C, spray voltage of 3660V, the cone voltage set to 35, and cone gas set to 50L/hr. A minimum of three fragment ions (transitions) were monitored per peptide by scheduled MRM (Table S1) using a 5 min RT scheduling window. Average dwell time per transition was 0.031 sec for 142 transitions with the average peak width set to 20sec and minimum number of data points per peak set to 12. Methionine sulfoxide versions of four of the peptides were also monitored using the same parameters as the non-oxidized peptide. Optimized collision energies were used for each transition. Extracted ion chromatograms (XIC) of all transition ions were integrated using a Skyline document (Skyline daily version 4.1. https://brendanx-uw1.gs.washington.edu/labkey/project/ home/software/Skyline/begin.view). Integrated peaks were manually inspected to confirm proper integration and detection of the transitions for the corresponding light and heavy peptides.

LC-MRM-MS data analysis

Light to heavy (L:H) peak area ratio (PAR) was calculated for each transition and the most abundant, interference-free transition was used for quantification. To account for any inconsistency during the sample processing all the data was normalized using PAR of control housekeeping proteins. A normalization factor for each sample was calculated using L:H peak area ratios of both β-actin peptides and the GAPDH.GAL peptide. The GAPDH.VPT peptide demonstrated inconsistent data quality and was therefore ignored for this analysis. For each sample and control peptide PAR was normalized to the median value of that experiment, i.e., dose response curve or time course. The median normalized L:H values for all three control housekeeping peptides were then averaged to generate a normalization factor for each sample. L:H PAR of CRBN and its substrate peptides were then divided by the normalization factor of that sample. Resulting ratio was used for all further analysis. Statistical analysis and visualization were performed in GraphPad Prism 7.

Quality Control of Anti-Peptide Antibodies

For performance evaluation of all anti-peptide antibodies after cross linking, mixtures of synthetic light and heavy peptides in buffer were used. A mixture of heavy peptides was spiked in at 100fm/peptide to a mixture of cross linked Ab-beads (1µg/each) prior to overnight capture followed by addition of 100fmol mixture of light peptides post-capture (see Methods section for details).

Captures were performed in triplicate using Abs chemically cross linked to beads and acid washed prior to capture. Samples were analyzed on Xevo-TQS triple quadrupole MS system (Waters) and peak integration was performed using Skyline. Performance of antibodies was assessed by the peak area ratio of pre-capture spiked heavy to post-capture spiked light peptides[5]. Median ratio of 0.8 was observed for all of the antibodies with the minimum ratio being 0.44 (data not shown).

Two of the antibodies were determined to successfully capture additional peptide sequences unique to other protein isoforms of interest. These peptides, DYICEFCAR (ZNF692) and MDDPTVNWSIER (GSPT2) varied by a single amino acid from the intended targets DYICEYCAR (ZFP91) and MDDPTVNWSNER (GSPT1), respectively. The former peptide was enriched with very similar efficiency while the efficiency of the latter was approximately about half.

Response curves to determine figures of merit of the assay

In order to establish lower levels of detection and quantitation (LLOD/LLOQ), linear range as well as reproducibility of the assay, we generated response curves as described previously[1]. Briefly, we prepared a bulk digest of MM1S and KG-1 cell lines for background as described in Methods. Ten-point calibration curves in both backgrounds were prepared: a serial dilution of 20 fmol of heavy peptides per µg of protein generated the following heavy peptide concentrations: 0, 0.003, 0.01, 0.03, 0.08, 0.25, 0.74, 2.22, 6.67, and 20fm/µg protein. Three 100µg aliquots of each concentration point were used for capture in 96-well plate format as described in Methods. The plate was then frozen at -80^{o}C overnight. The following day, the plate was thawed and 10µL of 10fm/µL light peptides were added along with the cross-linked Ab mixture containing 1 µg of each Ab-bead. The plate was then processed using our standard automated processing scheme (see Methods for details). Samples were subsequently desalted on the Bravo liquid handling robot configured with RPS-AssayMap cartridges (Agilent). Each calibration point was transferred to HPLC vials, frozen and dried via vacuum centrifugation. Each curve was then resuspended in 10µL 3% acetonitrile/0.1% formic acid and 1µL was injected onto the LC-MRM-MS system. Response curve samples were analyzed from lowest heavy spike-levels to highest, and each curve was run in entirety before started the next curve.

Response curve data was analyzed using Skyline and QuaSAR analysis package designed for the analysis of MRM-MS data to assess figures of merit of the assay such as lower levels of detection (LLOD), lower levels of quantitation (LLOQ), linear range, reproducibility, etc[6,7]. For calculations of measured concentrations, differences in light and heavy peptide stock concentrations as well as the presence of any observable endogenous peptide in both the MM1S and KG-1 cell lines were taken into consideration.

Supplemental figure 1A shows box-and-whisker plots of LLOQ values for all peptides in both MM1S and KG2 cell lysates. For MM1S, the LLOQ ranged from 5 to 195 amol/µg and the median was 12 amol/µg. For KG1, the LLOQ range was 4-254 amol/µg and the median was 10 amol/µg. The four peptides unique to the highly abundant control proteins ACTB and GAPDH were excluded from this analysis. Supplemental figure 2A-B shows response curves for all the 18 peptides (supplemental table 1) in MM1S cell line. The MS response for majority of peptides

was linear over five orders of magnitude (0.003 -20 fmol/μg) with a median R2 of 0.99 in both cell lines). For peptides with overall lower signal (GSPT2.MDD, RNF166.VQE), the linear range was reduced to four orders of magnitude, from 0.027 to 20fmol/μg range. Finally, the overall assay reproducibility was suitable. The coefficient of variation was less than 20% for concentrations above the LLOQ of the assay (~ 10fm/μg) (data not shown).

Development of a Capture Control sample as QC of immunoaffinity capture over time

To evaluate longitudinal performance variation of peptide capture, KG-1 cells treated with DMSO were prepared and digested in bulk to provide a common control background capture sample for each 96-well plate that was processed. The concentration of the digested background was determined by BCA and 100 μg aliquots were prepared and stored frozen at -80^{o}C until needed. One to three wells per 96-well sample plate were reserved for the analysis of capture control samples. Controls were processed identically with samples, utilizing the same mastermix of Ab-beads. GSPT2 and IKZF3 were not observed in the KG-1 cell line and were excluded from analyses. Supplemental Figure 1B shows excellent reproducibility of measurement for process replicates on the same plate (7.5%) while a two-fold higher %CV was observed when comparing all of the controls across the seven sample plates queried over almost 1 year.

Normalization of protein content by use of housekeeping proteins

In order to perform precise relative quantification across all the samples processed on different 96-well plates over time, the input protein amount as measure by BCA kept constant. To further account for possible variation during processing of samples, 2 peptides, each from the housekeeping proteins GAPDH and Actinβ were included in the assay for normalization. Individual, per sample normalization factors were generated using 3 of the 4 peptides by the following way: The observed L:H peak area ratio for each housekeeping peptide was normalized to the median value for that peptide within a replicate of the time course or dose curve. Then, these normalized peak area ratios (PARs) were averaged across the 3 peptides per experiment (well) and applied to the PARs observed for all of the measured targets. Supplemental Fig 1-C shows that the reproducibility of normalized-by-BCA improves two-fold when normalization is done using the peptides from the two housekeeping proteins. Statistical analysis and visualization was performed in R and GraphPad Prism version 8.0.2.

Dynamic BH3 profiling of thalidomide analogs

MM1S cells were exposed to lenalidomide, pomalidomide, avadomide or CC-885 for 20 hours and then resuspended in membrane extraction buffer (MEB) containing 0.001% digitonin (Sigma-Aldrich) to allow permeabilization of mitochondrial membrane. Cells were then transferred to a 384-well plate containing DMSO, Alamethacin or BIM-BH3 peptide and incubated for 60 minutes to allow peptide exposure. Cells were fixed, permeabilized, and stained for cytochrome c. For each drug treatment, the cytochrome c release of the non-drug-treated cells were subtracted from the cytochrome c release value of the drug-treated samples to derive the drug-induced change in priming or %Δ priming.

CRISPR-Cas9

The following gRNA sequences were used for MM1S knockdown experiments:
sgCRBN.g2: 5'-GGGGCGGTTACCAGGCAGGAG-3'
sgCRBN.g3: 5'-GTTACATACTGTATGTGATGT-3'
sgRNF166-1: 5'- GGGAGGTCTATCACCGGCCCG-3'
sgRNF166-3: 5'- GGCTGTGATGTGGGCACCAC-3'
sgRFN166-4: 5'- GACCTTCGCCTGCCCGTACTG-3'
sgZFP91-1: 5'- GCCCGACCTCCCCTCAGCACG-3'
sgZFP91-3: 5'- GGCTGCATCTAGACCTAGCCG-3'
sgZFP91-8: 5'- GTCACTGACCTGCAAATAGCG-3'
sgZNF692-5: 5'- GGCATGATGAGAGGACTCAAG-3'
sgZNF692-7: 5'- GTCACCTGCACACCTAAAGAG-3'
sgZNF692-8: 5'- GAGTCCTACCTAATTCTCTTG-3'
sgNTG: 5'-GACGGAGGCTAAGCGTCGCAA-3'

gRNAs targeting genes of interest were cloned using BsmBI restriction enzyme sites into the gRNA-vectors expressing either tagRFP or RFP657 driven by the SFFV promoter. Lentiviral production was performed using standard techniques. MM1S cells stably expressing Cas9 (pLentiCas9-Blast Addgene #52962) were infected with gRNA-containing vectors.

Co-immunoprecipitation

For each ZNF692 isoform a single 10cm plate of HEK293T was co-transfected with 9.5µg of each vector expressing FLAG-CRBN or HA-tagged substrate using TransIT-LT1 (MirusBio). After 48 hours of growth cells were harvested and lysed in IP lysis buffer (Pierce) containing DMSO or 1µM lenalidomide. Protein lysates were then incubated with 50µL anti-FLAG magnetic beads (Sigma-Aldrich M8823) in the presence of DMSO or 1µM lenalidomide overnight at 4°C. Beads were washed twice in IP wash buffer (150mM NaCl, 50mM Tris-HCl, pH7.4) in the presence of DMSO or 1µM lenalidomide and eluted in 80µL Laemmli sample buffer (Bio-Rad #1610737) diluted in IP lysis buffer, by boiling for 10 minutes. Eluates and whole cell lysate were run on a 4-15% polyacrylamide gel (Bio-Rad), transferred to a PVDF membrane (EMD Millipore), and immunoblotted for HA and FLAG.

SUPPLEMENTAL FIGURE LEGENDS:

Supplemental Figure S1: QC assessment of the iMRM assay

A. Box and whisker plots showing median and range of LLOQ values for all the peptides in MM1S and KG-1 cell lines. Only the best transition used for quantitation is included in the plot (supplemental Table 1). **B.** Box and whisker plot showing inter- and intra- plate median and range of coefficient of variation (CV) of capture control samples performed on sample plates. **C.** Histogram showing CV of data without normalization (No norm) and with normalization by housekeeping proteins (HK norm) across 1100 samples representing dose curve experiments. Reproducibility for 50 triplicate analyses from the five dose response curves improved two-fold with the employment of the normalization factors with median CV of 46.4 and 27.9 for data without and with normalization, respectively. Normalization factors ranged from 0.26 to 1.87 with a median value of 0.98 for all 150 dose curve experiments. Similar ranges and median values were observed for the time course data as well.

Supplemental Figure S2: Response curves for all peptides in MM1S cell lines

A. Response curves for CRBN, GSPT1, GSPT2, IKZF1 and IKZF3 peptides. **B.** Response curves for CK1α, RNF166, ZFP91 and ZNF692 peptides. Plots compare the measured concentration with the theoretical concentration in fmol/μg in $\log_{10}$ scale. Diagonal line on each plot represents the exact match in between the measured and theoretical concentrations. Different colored dots indicate response of all the transitions monitored for each peptide (supplemental Table 1).

Figure S3. An iMRM assay to measure thalidomide analog targets.

A. Plot of the ratio of substrate level in 10μM thalidomide over vehicle as measured in the iMRM assay and SILAC mass spectrometry. Line represents the linear regression ($R^2 = 0.85$). **B.** Levels of detected substrates in CD138 selected cell pellets from two patients with multiple myeloma. MM1 (100,000 cells) and MM2 (300,000 cells). L:H ratio is the ratio of the light to heavy (control) peptide as detected in the iMRM assay.

Figure S4. Dose response of degradation for all thalidomide analogs.

MM1S cells were treated with decreasing doses of drug or vehicle for 6 hours and then substrate degradation was measured using the iMRM assay. Protein levels were normalized to housekeeping proteins (β-actin and GAPDH) and the level in the vehicle treated sample was set to 1. Data are mean +/- S.E.M. (n = 3 replicates). Curves represent the non-linear regression.

Figure S5. Dose response of toxicity for thalidomide analogs.

A. Dose response curves for MM1S cells treated with lenalidomide, pomalidomide, avadomide or CC-885. MM1S cells were grown in the presence of the indicated drugs or vehicle and cells were split 1:1 into fresh media containing drug every 3-4 days. At the end of 10 days of treatment, the number of viable cells was determined using a FACSCanto fluorescence assisted cell sorter. The number of viable cells in the vehicle treated sample was set to 1 for each treatment. The IC_{50} for each drug was calculated (value in parentheses). Data are mean +/- S.E.M. (n = 3 replicates). Curves represent the non-linear regression. B. MM1S cells were treated with serial dilutions of lenalidomide, pomalidomide, avadomide or CC-885 for 20 hours. Cytochrome c release was then measured in response to DMSO or 0.3μM BIM peptide. The

cytochrome c release in the vehicle control was subtracted from the total to derive the drug-induced change in priming (% Δpriming). Data are mean +/- S.D. (n = 3 replicates).

Figure S6. BRET analysis.
A. 293T cells were transiently transfected with vectors carrying CRBN fused to NanoLuciferase and individual substrate proteins fused to HaloTag. They were then treated with decreasing doses of drug or vehicle for 6 hours and then the BRET signal was measured and the level in the vehicle treated sample was set to 1. Curves represent the non-linear regression. **B.** Full-length ZFP91 or ZNF692 were compared to their degron-deleted counterparts after treatment with vehicle, 1μM lenalidomide, 1μM pomalidomide, or 1μM CC-885 for 6 hours. ZFP91ΔZF, full length ZFP91 protein with the CRBN-interacting zinc finger (a.a. 400-422) deleted. ZNF692ΔZF, full length ZNF692 protein with the CRBN-interacting zinc finger (a.a. 417-439) deleted. Data are mean +/- S.E.M. ($n \geq 3$ replicates).

Figure S7. Kinetics of degradation for all thalidomide analogs and substrates.
Levels of substrate protein as measured by the iMRM assay following treatment with vehicle (gray), 10μM thalidomide (red), 1μM lenalidomide (black), 1μM pomalidomide (blue), 1μM avadomide (orange) or 0.1μM CC-885 (brown). Protein levels were normalized to housekeeping proteins (β-actin and GAPDH) and the level at the start of the experiment was set to 1. Data are mean +/- S.E.M. Curves represent the non-linear regression ($n \geq 3$ replicates).

Figure S8. Kinetics of CK1α degradation in KG-1 cells differs from MM1S cells.
A. KG-1 and MM1S cells were treated with 1μM lenalidomide and time points collected hourly for the first six hours and then at nine, 12 and 24 hours. Levels of substrate as measured by the iMRM assay. Curves represent the non-linear regression. $n \geq 3$ replicates. P values are from a sum of squares F-test. NS: non-significant (corrected for multiple hypothesis testing). **B.** The level of each protein in the vehicle treated samples was measured using iMRM and normalized by the level of housekeeping proteins (β-actin and GAPDH) to correct for differential total protein content. Data are mean +/- S.E.M. ($n \geq 3$ replicates).

Figure S9. Substrate degradation in response to lenalidomide requires CRBN.
A-C. MM1S cells that constitutively express CAS9 were transduced with lentivirus expressing sgRNAs and a fluor (tagRFP or RFP657). NTG, non-targeting guide. Western blotting **(A)** was performed to assess CRBN level and IKZF1 degradation after treatment with 1μM lenalidomide or vehicle for 24 hours. Competitive growth assays **(B)** were performed in the presence of 1μM lenalidomide (dashed lines) or vehicle (solid lines). The level of CRBN in the vehicle treated samples was measured using iMRM **(C)** and normalized by the level of housekeeping proteins (β-actin and GAPDH) to correct for differential total protein content. Data are mean +/- S.E.M. (n = 3 replicates).

Figure S10. Lenalidomide induced substrate degradation is dependant on a limiting pool of CRBN.

A-C. MM1S cells were transduced with retrovirus expressing GFP only or GFP and FLAG-tagged CRBN. The level of CRBN in the vehicle treated samples was measured using iMRM **(A)** and normalized by the level of housekeeping proteins (β-actin and GAPDH) to correct for differential total protein content. Data are mean +/- S.E.M. (n = 3 replicates). Levels of all substrate proteins as measured **(B)** by the iMRM assay. ($n \geq 3$ replicates). Protein levels were normalized to a housekeeping factor and the level at the start of the experiment was set to 1. EV = empty vector. Western blotting **(C)** was performed to assess CRBN level and IKZF1 degradation after treatment with 1μM lenalidomide for the specified time.

Figure S11. CRBN level is associated with substrate degradation in MM cell lines.

A. MM cell lines were treated were treated with 1μM Lenalidomide and counted every 2-4 days and the fold proliferation plotted over time. **B-J.** MM cell lines were treated with 1μM lenalidomide or vehicle and the level of substrate measured using the iMRM assay. The ratio of substrate level in lenalidomide as compared to vehicle is plotted for IKZF1, IKZF3 and CK1α for all 10 cell lines **(B-C)**. The level of substrates in the vehicle treated samples was measured using iMRM and normalized by the level of housekeeping proteins (β-actin and GAPDH) to correct for differential total protein content. **D.** The ratio of new substrates (ZFP91, ZNF692 and RNF166) in the lenalidomide treated as compared to vehicle treated samples is plotted against the absolute CRBN level in vehicle treated samples. Points marked in red represent lenalidomide-sensitive cell lines. CRBN level positively correlates with degradation of all three substrates (R^2 for ZFP91 = 0.67; ZNF692 = 0.62; RNF166 = 0.8). Mean CRBN **(E)**, IKZF1 **(H)** and IKZF3 **(I)** levels in lenalidomide sensitive versus resistant cell lines is shown. The mean ratio of IKZF1 **(F)** and IKZF3 **(G)** level in lenalidomide as compared to vehicle in lenalidomide sensitive and resistant cell lines is plotted. P values from a Mann-Whitney test. NS = not significant. **J.** The level of CRBN in the vehicle lenalidomide treated samples was measured using iMRM and normalized by the level of housekeeping proteins (β-actin and GAPDH) to correct for differential total protein content. Mean level of CRBN in all 10 cell lines in vehicle and lenalidomide treated samples is plotted. P value from a Wilcoxon test. Data are mean +/- S.E.M.

Figure S12. Substrates compete for access to CRBN.

MM1S cells were transduced with lentivirus expressing GFP and HA-tagged full length, degron-deleted or a K^0 version of ZNF692. **A.** Western blotting of IKZF1 and HA after treating cells with 1μM lenalidomide for 1, 2, 3, 6, 11 or 24 hours or vehicle for 24 hours (-). **B.** 293T cells were transiently transfected with vectors expressing FLAG-tagged CRBN or HA-tagged full length, degron-deleted or a K^0 version of ZNF692. Lysates were made in the presence or absence of 1μM lenalidomide and immunoprecipitated with FLAG-beads. **C.** 293T cells were transiently transfected with vectors carrying CRBN fused to NanoLuciferase and full-length ZNF692, ZNF692ΔZF, or ZNF692 K^0 fused to HaloTag. They were then treated with vehicle, 1μM

lenalidomide, 1μM pomalidomide, or 1μM CC-885 for 6 hours. Data are mean +/- S.E.M. ($n \geq 3$ replicates). **D.** Relative protein level in MM1S cells as measured using the iMRM assay. Protein levels were normalized to a housekeeping factor and the level at the start of the experiment was set to 1. Curves represent the non-linear regression. P values are from a sum of squares F-test. Data are mean +/- S.E.M. ($n \geq 3$ replicates). ZNF692ΔZF, full length ZNF692 protein with the CRBN-interacting zinc finger (a.a. 417-439) deleted. ZNF692 K^0, full length ZNF692 with every lysine changed to arginine. **E.** MM1S cells were transduced with lentivirus expressing GFP and full length or degron-deleted versions of RNF166. Relative growth was assessed by means of flow cytometry after treatment with vehicle (solid lines) or 1μM lenalidomide (dashed lines). Data are mean +/- S.E.M. ($n \geq 3$ replicates). Data are representative of 2 independent experiments. F. Western blotting of IKZF1 and β-actin after treating MM1S cells overexpressing RNF166 or RNF166ΔZF with vehicle or 1μM lenalidomide for 18 hours. RNF166ΔZF, full length RNF166 protein with the CRBN-interacting zinc finger (a.a. 150-173) deleted.

Figure S13. Knockdown of non-essential substrates sensitizes cells to lenalidomide.
MM1S cells that constitutively express CAS9 were transduced with lentivirus expressing sgRNAs and a fluor (tagRFP or RFP657). Three separate sgRNAs were used to target each gene. Relative growth as compared to untransduced cells was assessed by means of flow cytometry after treatment with 0.2μM lenalidomide (red lines) or vehicle (black lines) for the indicated time. Data are representative of 2 independent experiments. NTG, non-targeting guide. Data are mean +/- S.E.M. (n = 3 replicates).

SUPPLEMENTARY TABLES:

Table S1. List of cell lines used.

Table S2. List of compounds used.

Table S3. List of antibodies used.

Table S4. List of vectors used.

Table S5. List of peptides included in immuno-MRM assay.

Peptide sequences used to generate antibodies for iMRM assay. Two peptides per target protein were chosen. GSPT2 (peptide #18) was detected using the antibody generated for GSPT1 (peptide #17), as these two sequences differ by only one amino acid. Differentiation between the two proteins was done at the mass spectrometry stage. ZNF692 peptide #13 was detected using the antibody for ZFP91 peptide #12 in a similar manner. [1]Bolded letters indicate heavy labeled (^{13}C, ^{15}N) amino acid. [2]All transitions are monitored by MRM-MS, but only italicized underlined transitions used for quantitation. [3]Collision energies are optimized on Xevo (Waters) triple quadrupole mass spectrometer.

Table S6. Limit of detection for iMRM substrates.

In order to determine the minimum number of input cells needed to reproducibly detect individual thalidomide analog substrate, the specified cell types (HEK293T, MM1S and KG-1) were counted and serial dilutions of decreasing cell numbers were made. Samples were processed and analyzed by iMRM and the minimal number of cells at which each substrate could be detected was determined by manual inspection of the data using the following criteria: 1) all 3 transitions are detected for the endogenous light version of the peptide; 2) height of the most abundant, interference free transition is more than 1000 counts (3x the noise); 3) light to heavy PAR > 0.01. nd = not detected.

SUPPLEMENTARY REFERENCES:

1. Kuhn E, Whiteaker JR, Mani DR, et al. Interlaboratory evaluation of automated, multiplexed peptide immunoaffinity enrichment coupled to multiple reaction monitoring mass spectrometry for quantifying proteins in plasma. *Mol Cell Proteomics*. 2012;11(6):M111 013854.
2. Whiteaker JR, Zhao L, Abbatiello SE, et al. Evaluation of large scale quantitative proteomic assay development using peptide affinity-based mass spectrometry. *Mol Cell Proteomics*. 2011;10(4):M110 005645.
3. Mertins P, Yang F, Liu T, et al. Ischemia in tumors induces early and sustained phosphorylation changes in stress kinase pathways but does not affect global protein levels. *Mol Cell Proteomics*. 2014;13(7):1690-1704.
4. Mertins P, Tang LC, Krug K, et al. Reproducible workflow for multiplexed deep-scale proteome and phosphoproteome analysis of tumor tissues by liquid chromatography-mass spectrometry. *Nat Protoc*. 2018;13(7):1632-1661.
5. Ippoliti PJ, Kuhn E, Mani DR, et al. Automated Microchromatography Enables Multiplexing of Immunoaffinity Enrichment of Peptides to Greater than 150 for Targeted MS-Based Assays. *Anal Chem*. 2016;88(15):7548-7555.
6. Keshishian H, Addona T, Burgess M, et al. Quantification of cardiovascular biomarkers in patient plasma by targeted mass spectrometry and stable isotope dilution. *Mol Cell Proteomics*. 2009;8(10):2339-2349.
7. Mani DR, Abbatiello SE, Carr SA. Statistical characterization of multiple-reaction monitoring mass spectrometry (MRM-MS) assays for quantitative proteomics. *BMC Bioinformatics*. 2012;13 Suppl 16:S9.

Supplemental Table 1

#	Cell Line	Tissue/Cell Type	Source	Identity Verified?
1	HEK293T	Human embryonic kidney cell	Genetic perturbations platform, Broad Institute	No
2	KG1	Acute myeloid leukemia (AML)	Broad Institute Cell Line Repository	Yes
3	MM1S	Multiple myeloma	Broad Institute Cell Line Repository	Yes
4	U266	Multiple myeloma	ATCC	Yes
5	KMS34	Multiple myeloma	JCRB Cell Bank	Yes
6	RPMI8226	Multiple myeloma	ATCC	Yes
7	LP1	Multiple myeloma	Kind gift of Dr. Ken Anderson	No
8	OPM2	Multiple myeloma	DSMZ	Yes
9	KMS11	Multiple myeloma	JCRB Cell Bank	Yes
10	JJN3	Multiple myeloma	Kind gift of Dr. Ken Anderson	No
11	KMS12BM	Multiple myeloma	JCRB Cell Bank	Yes
12	KMS26	Multiple myeloma	JCRB Cell Bank	Yes

Supplemental Table 2

#	Compound	Company	Cat. #
1	Thalidomide	SelleckChem	S1193
2	Lenalidomide	SelleckChem	No longer in stock
3	Pomalidomide	SelleckChem	S1567
4	Avadomide	MedChem Express	HY-100507
5	CC-885	Axon MedChem	2645

Supplemental Table 3

#	Target	Species	Mono/Poly	Company	Cat. #
1	human CRBN	rabbit	Polyclonal	Novus Biologicals	NBP1-91810
2	human β-Actin	mouse	Monoclonal	Abcam	ab20272
3	human IKZF1	rabbit	Polyclonal	Santa Cruz	No longer in stock
4	human IKZF1	rabbit	Polyclonal	Cell Signaling Technology	5443S
5	HA-HRP	mouse	Monoclonal	Miltenyi	130-091-972
6	FLAG-M2-HRP	mouse	Monoclonal	Sigma-Aldrich	A8592
7	FLAG-magnetic beads	mouse	Monoclonal	Sigma-Aldrich	M8823

Supplemental Table 4

#	Name	Contents	Resistance Marker	Source	Used for
1	Celery	SFFV.ClaI-BamHI-CloneSite.IRES.EGFP.cppt.EF1α.PuroR	AmpicillinR	Lentiviral (pLKO, taken from lentiGuide-Puro, Addgene #52963)	Full-length proteins, ZNF692, ZNF692ΔZF, ZNF692 K^0
2	pRSF91	ViralUTR.XbaI-MluI-CloneSite.IRES.CopGFP.T2A.PuroR	AmpicillinR	Retroviral vector pRSF91[2]	Full length proteins, CRBN, RNF166, RNF166ΔZF
3	pFN31K	CMV.NanoLuc.Flexi-CloneSite.SV40.NeoR	KanR	Promega	Full length CRBN fused to NanoLuciferase
4	pFC14K	CMV.Flexi-CloneSite.HaloTag.SV40.NeoR	KanR	Promega	Full length, ΔZF, and K^0 substrates fused to HaloTag
5	sgRNA-tagRFP	U6.gRNAclonesite.Scaffold.cppt.SFFV.tagRFP	AmpicillinR	Lentiviral (pLKO, taken from pLKO5.sgRNA Addgene #57823)	sgNTG guide
6	sgRNA-RFP657	U6.gRNAclonesite.Scaffold.cppt.SFFV.RFP657	AmpicillinR	Lentiviral (pLKO, taken from pLKO5.sgRNA Addgene #57824)	sgCRBN.g2 and sgCRBN.g3 guides

Supplemental table 5: List of peptides included in immunoMRM assay

[1]Bolded letters indicate heavy labeled (13C, 15N) amino acid.

[2]All transitions are monitored by MRM-MS, but only italicized underlined transitions used for quantitation

[3]Collision energies are optimized on Xevo (Waters) triple quadrupole mass spectrometer

Protein	Accession #	Sequence[1]	Precursor m/z	Charge State	Product m/z[2]	Fragment Ion	Collision Energy[3]
CRBN	Q96SW2	FHCANLTSWP**R**	463.555789	+3	646.33	y5	13
					545.28	y4	14
					458.25	y3	12
			466.891879		630.25	b5	14
					656.34	y5	13
					555.29	y4	14
					468.26	y3	12
					630.25	b5	14
		VAACLPIDDVL**R**	671.363387	+2	1100.58	y9	22
					940.55	y8	22
					827.46	y7	22
			676.367521		1110.59	y9	22
					950.55	y8	22
					837.47	y7	22
GSPT1	P15170	DIHFM(ox)PCSGLTGANI**K**	592.951768	+3	773.45	y8	17
					603.35	y6	15
					502.30	y5	15
			595.623168		660.28	b5	21
					781.47	y8	17
					611.36	y6	15
					510.31	y5	15
					660.28	b5	21
		DIHFMPCSGLTGANI**K**	587.62013	+3	773.45	y8	17
					603.35	y6	15
					502.30	y5	15
			590.291529		644.29	b5	21
					781.47	y8	17
					611.36	y6	15
					510.31	y5	15
					644.29	b5	21
		M(ox)DDPTVNWSNE**R**	740.31208	+2	1102.53	y9	30
					904.43	y7	29
					805.36	y6	29
			745.316214		1112.54	y9	30
					914.44	y7	29
					815.37	y6	29
		MDDPTVNWSNE**R**	732.314622	+2	1102.53	y9	30
					904.43	y7	29
					805.36	y6	29
			737.318757		1112.54	y9	30
					914.44	y7	29
					815.37	y6	29
GSPT2	Q8IYD1	M(ox)DDPTVNWSIE**R**	739.832648	+2	1101.57	y9	27
					903.47	y7	28
					804.40	y6	22
			744.836782		1111.58	y9	27
					913.48	y7	28
					814.41	y6	22
		MDDPTVNWSIE**R**	731.83519	+2	1101.57	y9	27
					903.47	y7	28
					804.40	y6	22
			736.839325		1111.58	y9	27
					913.48	y7	28
					814.41	y6	22
IKZF1	Q13422	SNHSAQDSAVENLLLLS**K**	642.669083	+3	*929.57*	y8	17
					800.52	y7	18
					686.48	y6	17
			645.340482		*937.58*	y8	17
					808.54	y7	18
					694.50	y6	17
		AASENSQDAL**R**	581.278366	+2	*803.40*	y7	20
					689.36	y6	21
					602.33	y5	15
			586.2825		*813.41*	y7	20
					699.37	y6	21
					612.33	y5	15
IKZF3	Q9UKT9	HVVSFDSSRPTSG**K**	501.923194	+3	1168.56	y11	19
					934.46	y9	20
					819.43	y8	20
			504.594594		1176.57	y11	19
					942.47	y9	20
					827.45	y8	20
		TFLQSTDPGDTASAEA**R**	883.913212	+2	1277.56	y13	30
					1089.48	y11	27
					974.45	y10	33
			888.917346		1287.57	y13	30
					1099.49	y11	27
					984.46	y10	33
			420.590939		905.53	y9	16
					692.42	y6	16

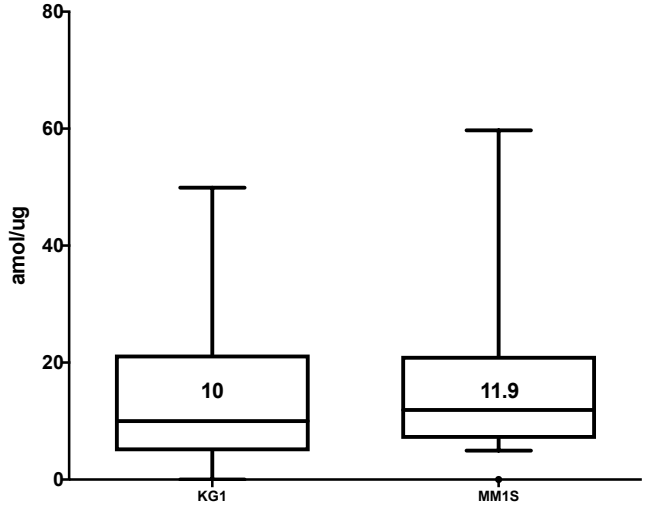
CSNK1α1	P48729	ILQGGVGIPHI**R**	423.927029	+3	*522.31*	y4	15
					915.54	y9	16
					702.43	y6	16
		TSLPWQGL**K**	515.290016	+2	*532.32*	y4	15
					728.41	y6	16
					631.36	y5	22
					445.28	y4	22
			519.297116		317.22	y3	23
					736.42	y6	16
					639.37	y5	22
					453.29	y4	22
					325.23	y3	23
RNF166	Q96A37	VQEQM(ox)ANCP**K**	610.773548	+2	*993.41*	y8	18
					864.37	y7	19
					736.31	y6	22
		VQEQMANCP**K**	614.780647	+2	*1001.43*	y8	18
					872.38	y7	19
					744.33	y6	22
			602.77609		*977.42*	y8	18
					848.38	y7	19
					720.32	y6	22
		STFACPYCGA**R**	606.78319	+2	*985.43*	y8	18
					856.39	y7	19
					728.33	y6	22
			645.273715		954.39	y8	21
					883.35	y7	21
					723.32	y6	21
			650.277849		964.40	y8	21
					893.36	y7	21
					733.33	y6	21
ZFP91	Q96JP5	TGSLQLIC**K**	510.281334	+2	774.45	y6	17
					661.37	y5	17
					533.31	y4	17
		DYICEYCA**R**	514.288434	+2	782.47	y6	17
					669.38	y5	17
					541.33	y4	17
			625.252448		971.41	y7	21
					858.32	y6	20
					698.29	y5	21
			630.256582		981.42	y7	21
					868.33	y6	20
					708.30	y5	21
ZNF692	Q9BU19	LLPSPVTCTP**K**	606.836474	+2	986.50	y9	17
					802.41	y7	22
					606.29	y5	26
		SFSCPEPACG**K**	610.843573	+2	401.71	y7	22
					994.51	y9	17
					810.43	y7	22
		DYICEFCA**R**	620.260273	+2	614.31	y5	26
					405.72	y7	22
					918.38	y8	20
					758.35	y7	20
			624.267372		661.30	y6	25
					532.25	y5	27
					926.39	y8	20
					766.36	y7	20
			617.254991		669.31	y6	25
					540.27	y5	27
					955.41	y7	21
					842.33	y6	19
			622.259125		682.30	y5	20
					965.42	y7	21
					852.34	y6	19
					692.31	y5	20
ACTB	P60709	AGFAGDDAP**R**	488.72778	+2	701.32	y7	15
					630.28	y6	15
					573.26	y5	15
		SYELPDGQVITIGNE**R**	493.731915	+2	711.33	y7	15
					640.29	y6	15
					583.27	y5	15
			895.949597		*1298.67*	y12	31
					1086.59	y10	37
					901.51	y8	36
			900.953732		*1308.68*	y12	31
					1096.60	y10	37
					911.52	y8	36
GAPDH	P04406	GALQNIIPASTGAA**K**	706.398815	+2	1042.59	y11	24
					928.55	y10	22
					815.46	y9	21
		VPTANVSVVDLTC**R**	710.405915	+2	1050.60	y11	24
					936.56	y10	22
					823.48	y9	21
			765.900865		*949.48*	y8	29
					862.45	y7	32
					763.38	y6	29
			770.904999		*959.49*	y8	29
					872.45	y7	32
					773.38	y6	29

Supplemental Table 6

Protein	293T	MM1.S	KG-1
CRBN	$5x10^4$	$5x10^5$	$5x10^4$
IKZF1	nd	$5x10^4$	$2.5x10^4$
IKZF3	$1x10^5$	$5x10^4$	nd
CSNK1α1	$2x10^4$	$1x10^5$	$2.5x10^4$
RNF166	$5x10^5$	nd	$5x10^5$
ZFP91	$2x10^4$	$5x10^4$	$2.5x10^4$
ZNF692	$2x10^4$	$1x10^5$	$2.5x10^4$
GSPT1	$5x10^4$	$1x10^5$	$2.5x10^4$
GSPT2	$5x10^5$	$5x10^5$	nd
ACTβ	$2x10^4$	$5x10^4$	$2.5x10^4$
GAPDH	$2x10^4$	$1x10^5$	$2.5x10^4$

Figures S1

A.



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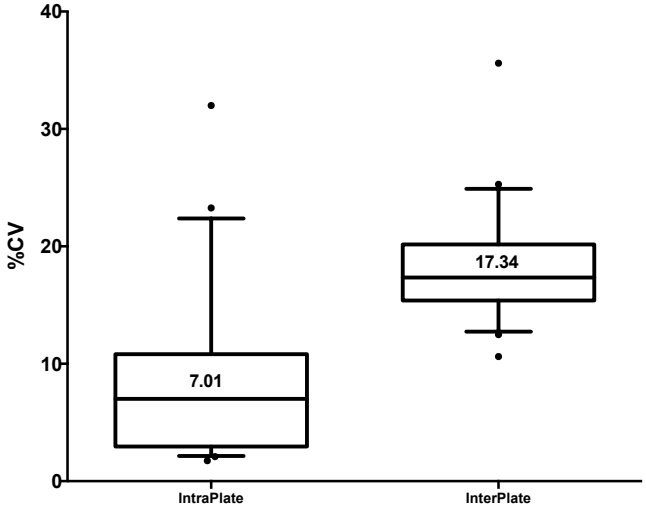

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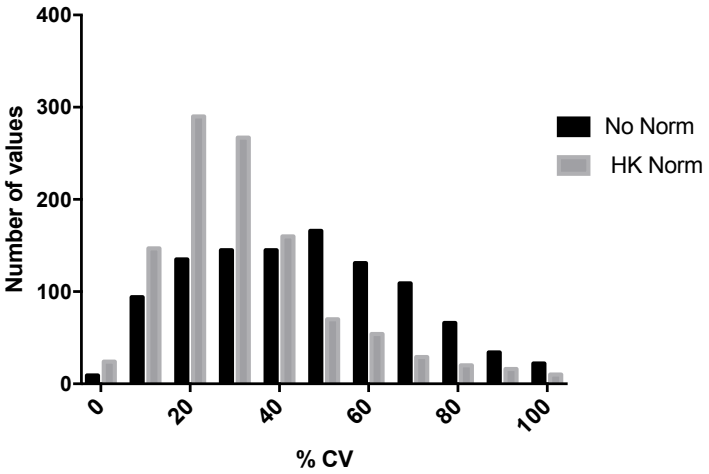
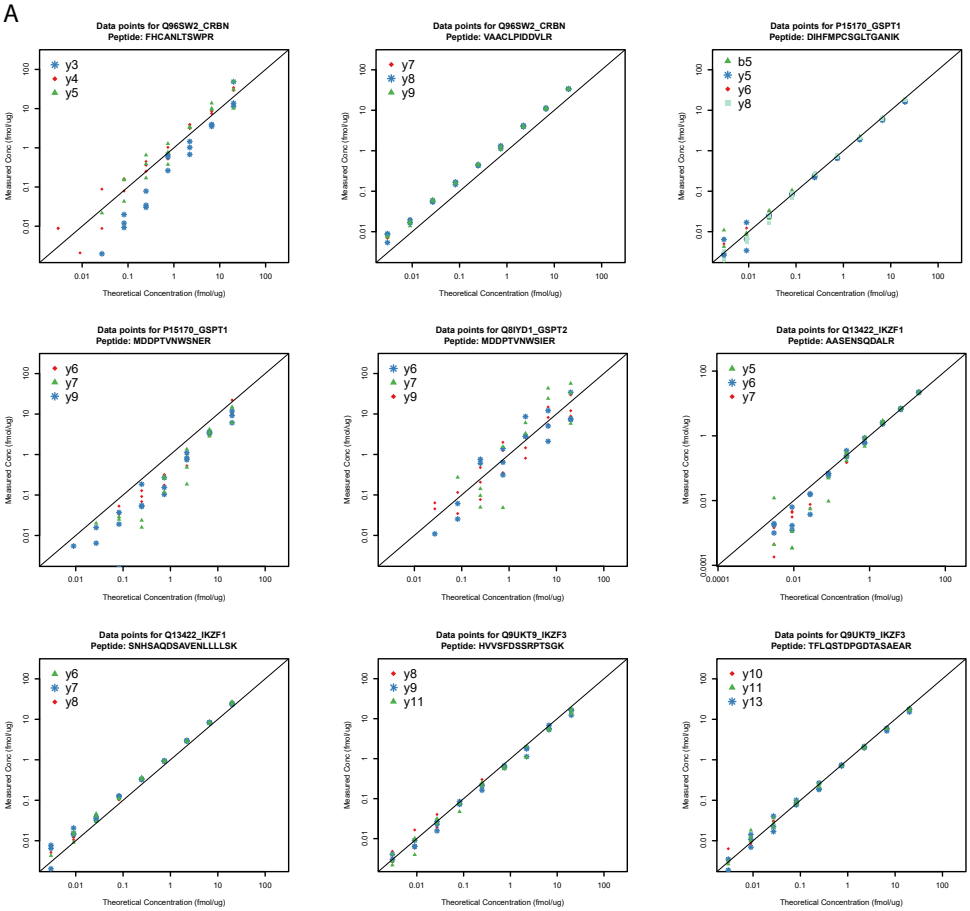


Figures S2

A



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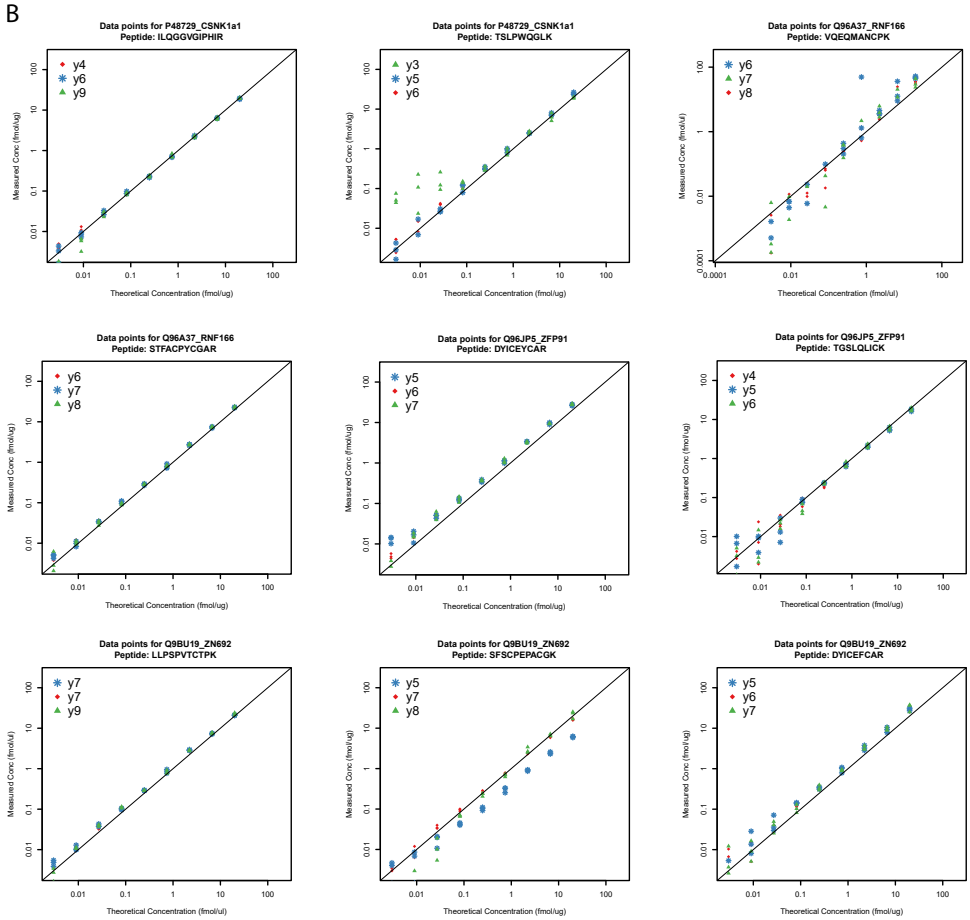
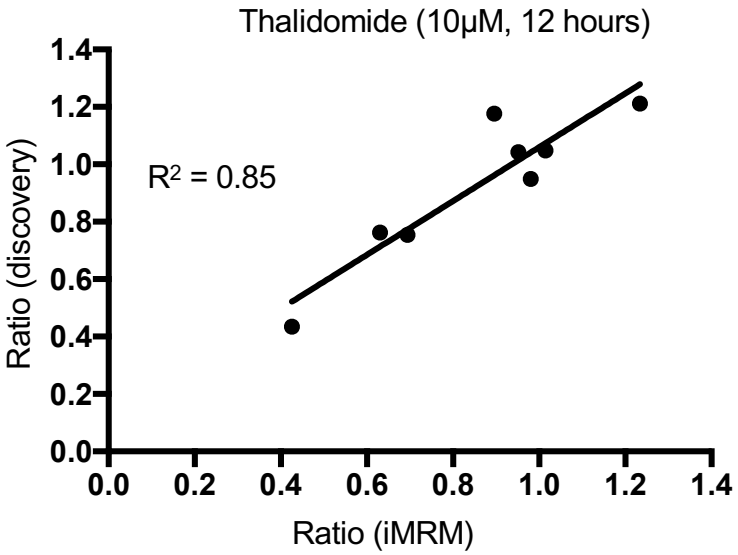


Figures S3

A.



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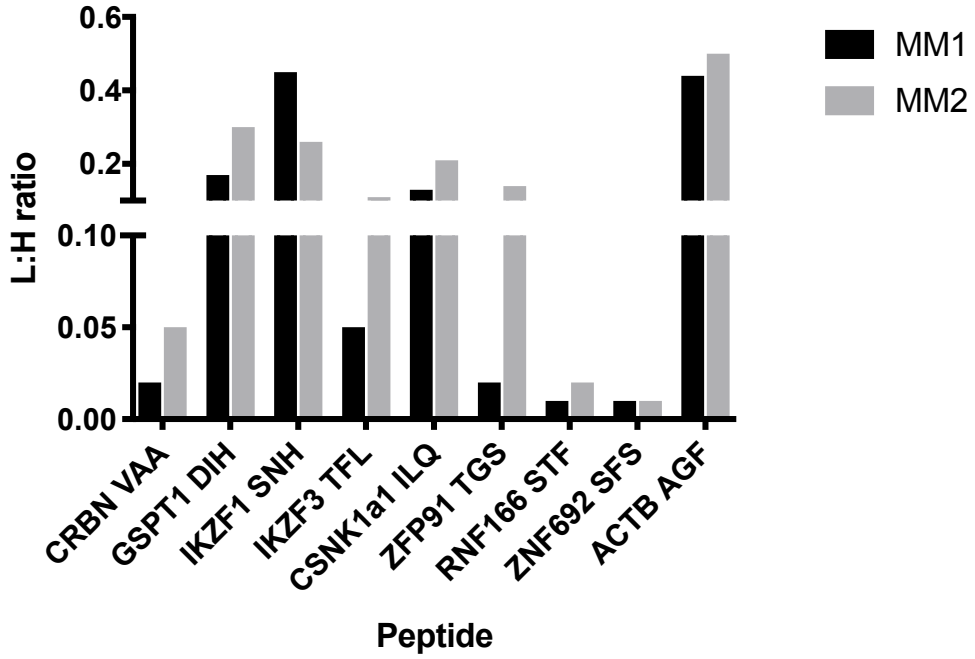


Figure S4

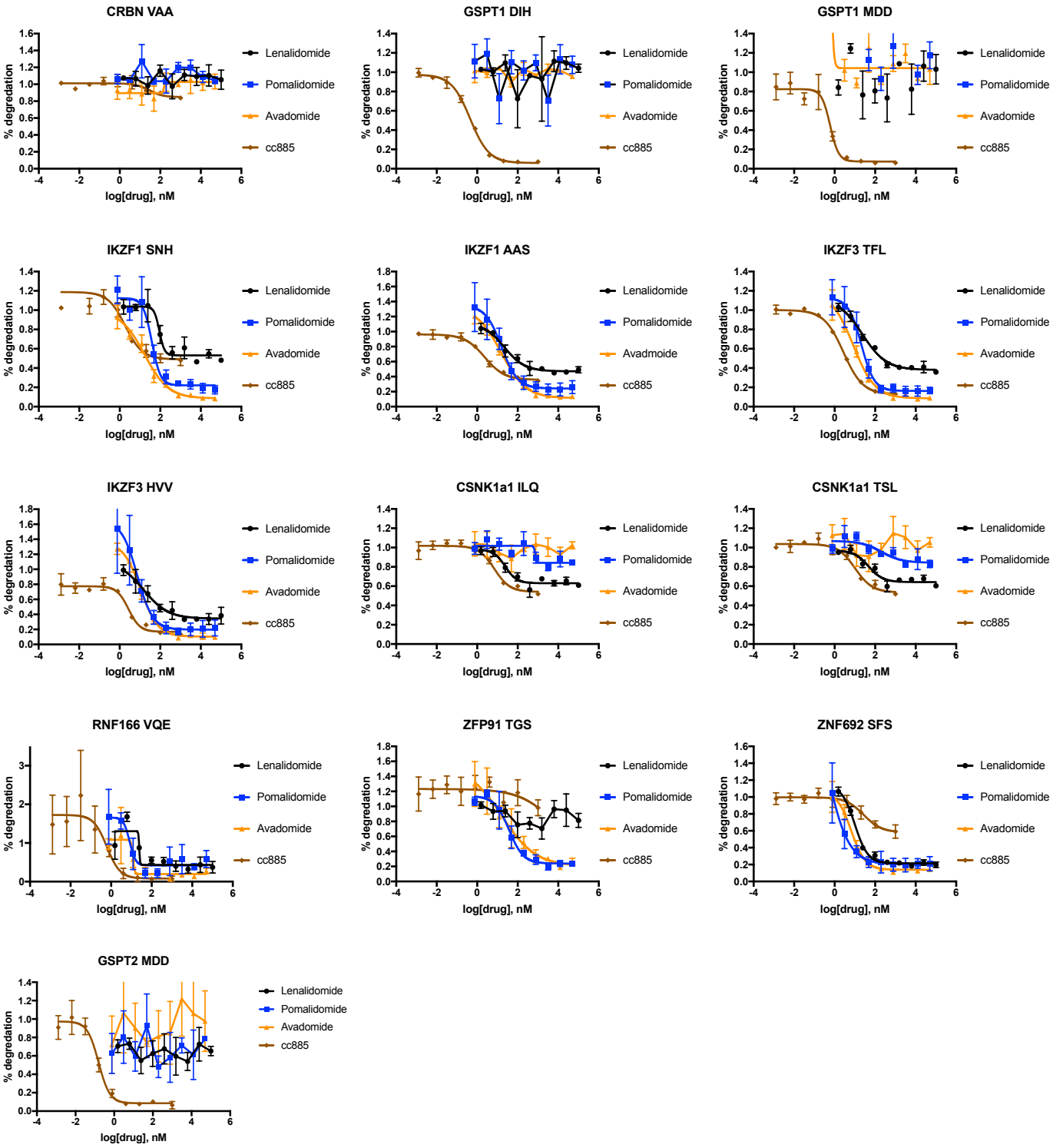
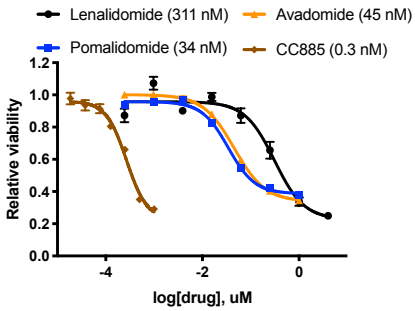

CRBN VAA
GSPT1 DIH
GSPT1 MDD
IKZF1 SNH
IKZF1 AAS
IKZF3 TFL
IKZF3 HVV
CSNK1a1 ILQ
CSNK1a1 TSL
RNF166 VQE
ZFP91 TGS
ZNF692 SFS
GSPT2 MDD
% degredation
log[drug], nM
Lenalidomide
Pomalidomide
Avadomide
Avadmoide
cc885

Figure S5

A.


Lenalidomide (311 nM)
Avadomide (45 nM)
Pomalidomide (34 nM)
CC885 (0.3 nM)
Relative viability
log[drug], uM

B.

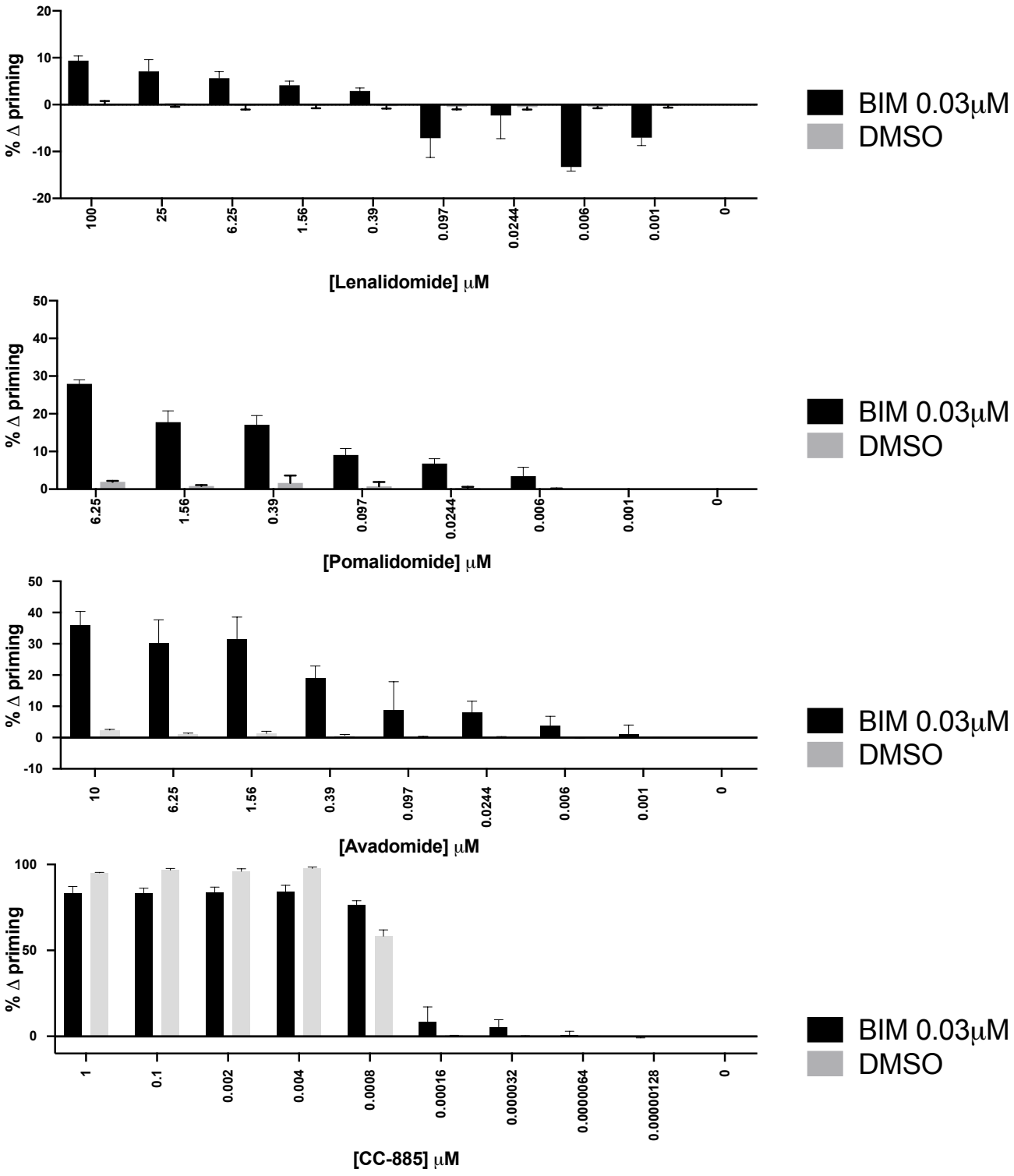
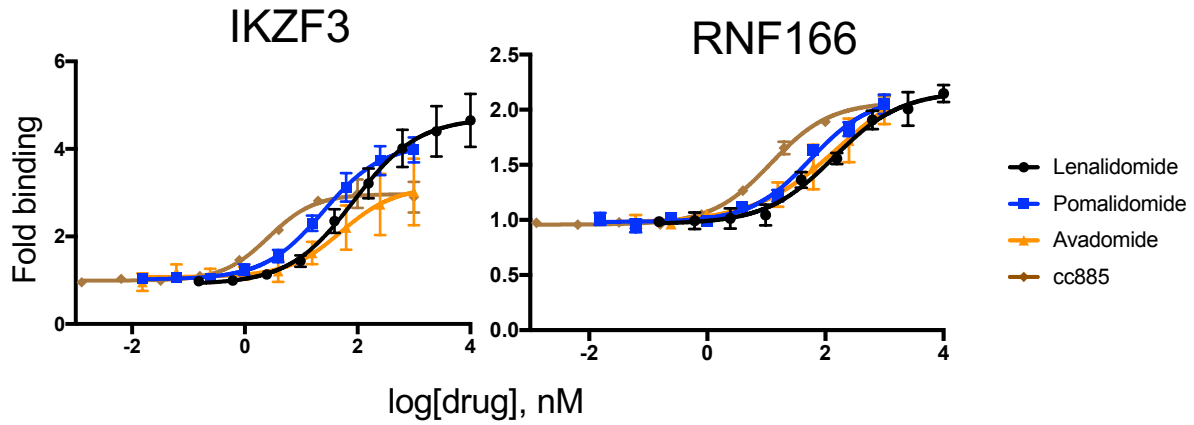

% Δ priming
[Lenalidomide] μM
[Pomalidomide] μM
[Avadomide] μM
[CC-885] μM
BIM 0.03μM
DMSO

Figure S6

A.


IKZF3
RNF166
Fold binding
log[drug], nM
Lenalidomide
Pomalidomide
Avadomide
cc885

B.

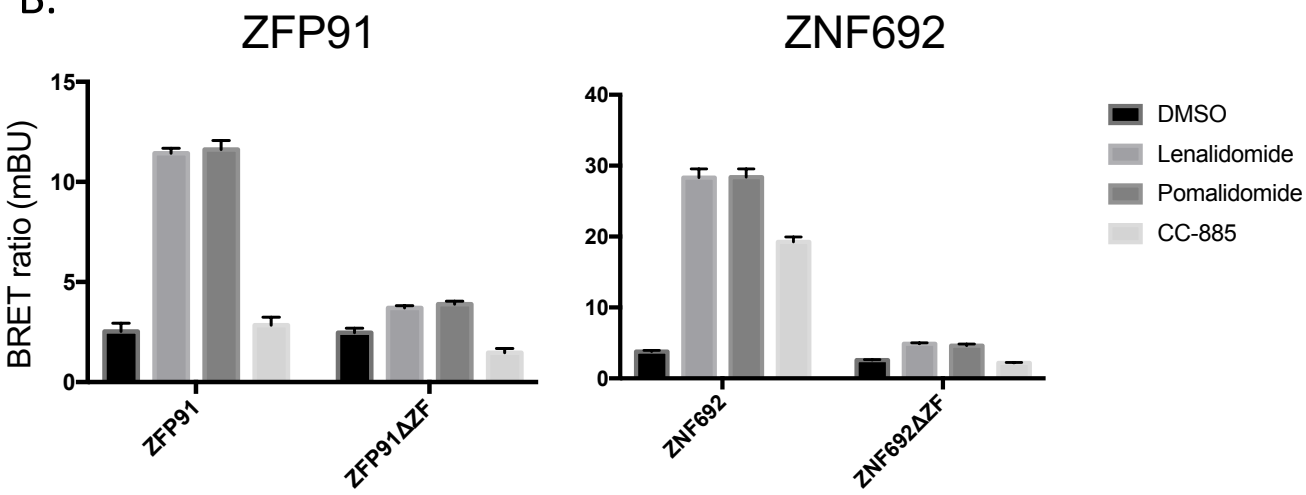

ZFP91
ZNF692
BRET ratio (mBU)
ZFP91
ZFP91ΔZF
ZNF692
ZNF692ΔZF
DMSO
Lenalidomide
Pomalidomide
CC-885

Figure S7

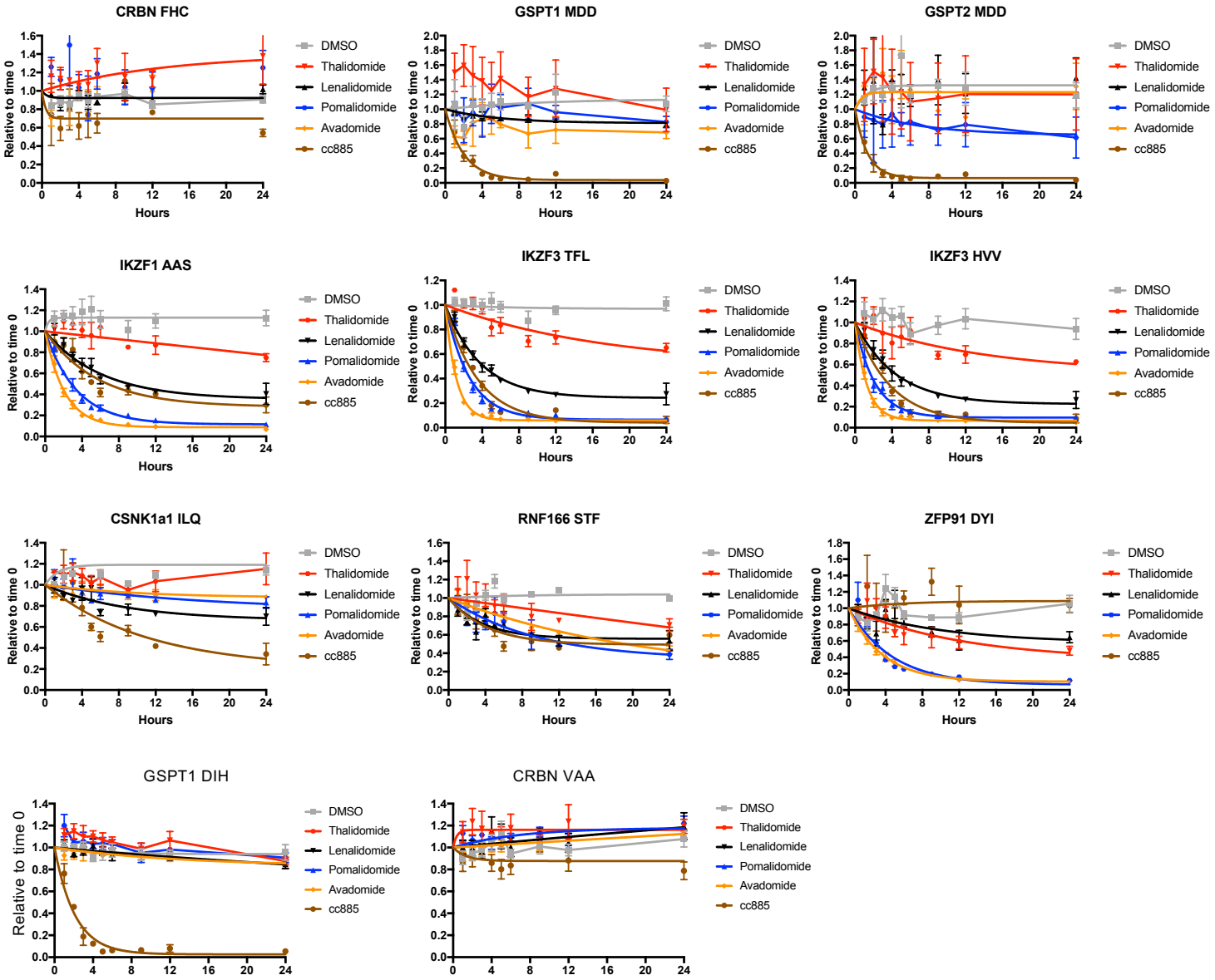
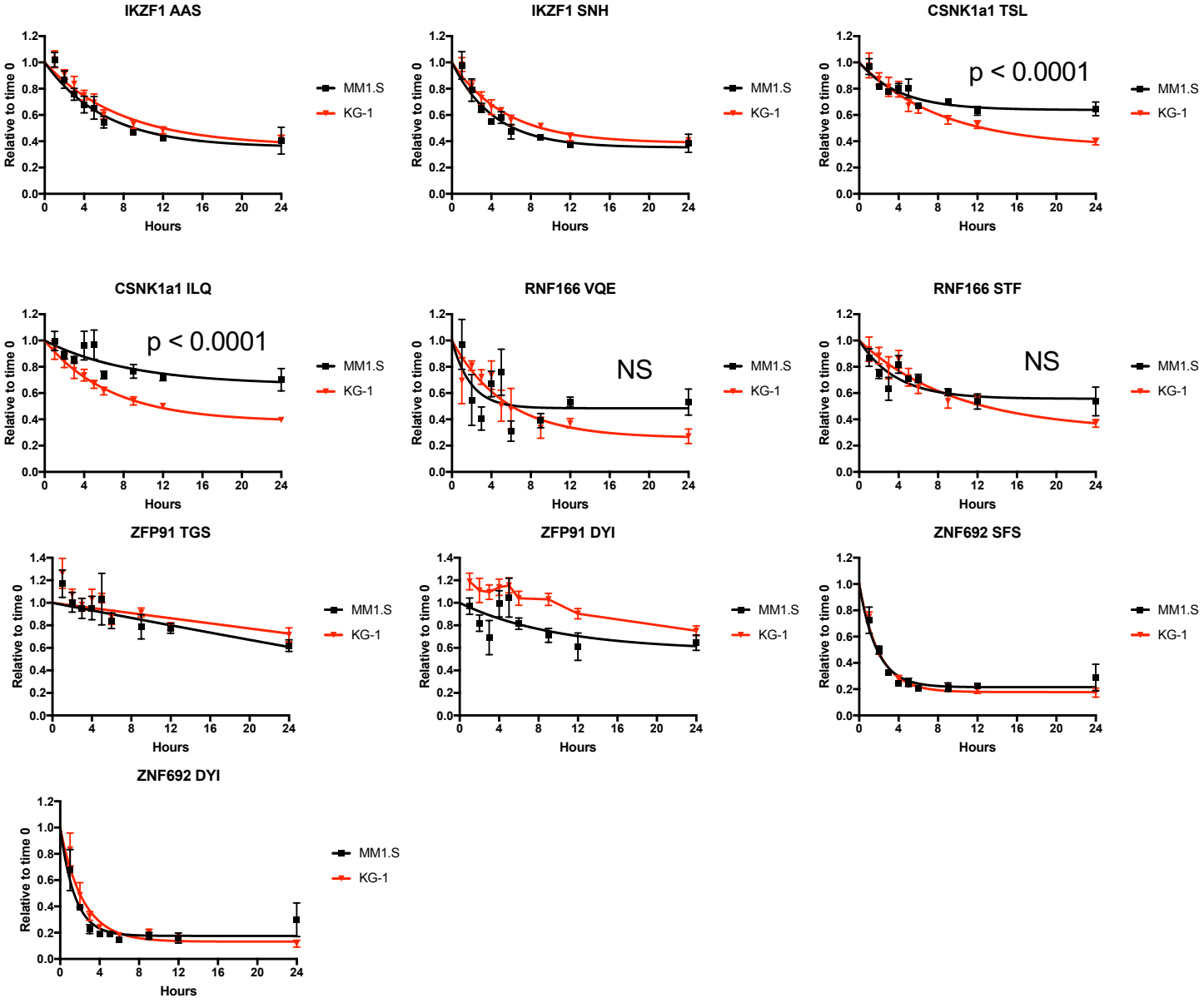

CRBN FHC
GSPT1 MDD
GSPT2 MDD
IKZF1 AAS
IKZF3 TFL
IKZF3 HVV
CSNK1a1 ILQ
RNF166 STF
ZFP91 DYI
GSPT1 DIH
CRBN VAA
Relative to time 0
Hours
DMSO
Thalidomide
Lenalidomide
Pomalidomide
Avadomide
cc885

Figure S8

A.


IKZF1 AAS
IKZF1 SNH
CSNK1a1 TSL
p < 0.0001
CSNK1a1 ILQ
p < 0.0001
RNF166 VQE
NS
RNF166 STF
NS
ZFP91 TGS
ZFP91 DYI
ZNF692 SFS
ZNF692 DYI
Relative to time 0
Hours
MM1.S
KG-1

B.

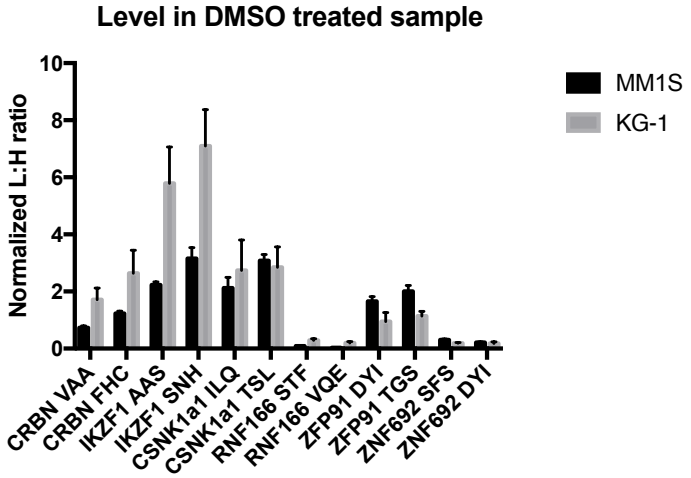
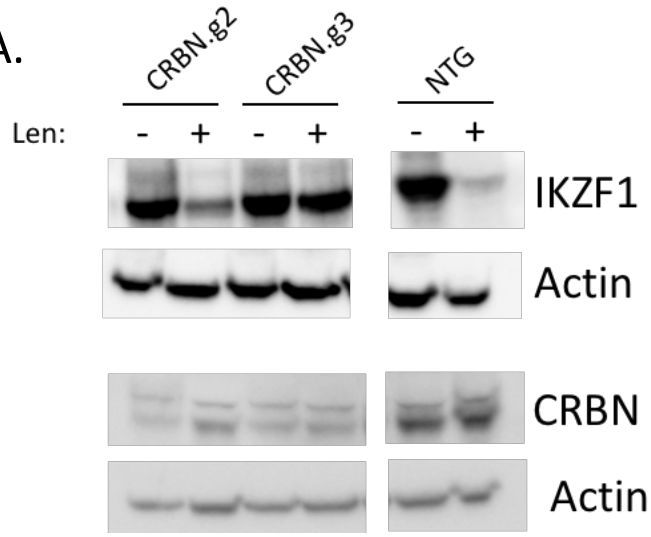
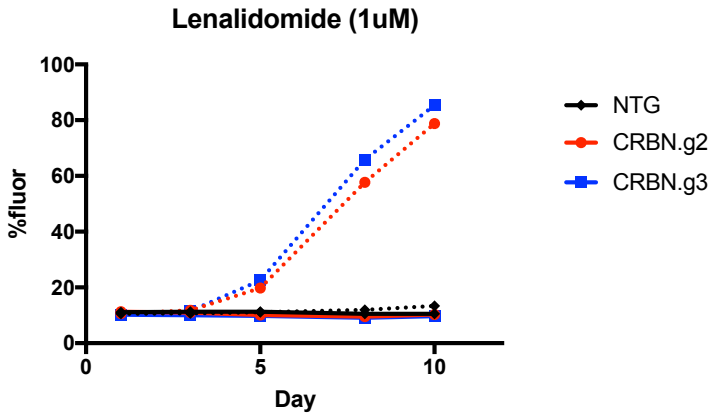

Level in DMSO treated sample
Normalized L:H ratio
MM1S
KG-1
CRBN VAA
CRBN FHC
IKZF1 AAS
IKZF1 SNH
CSNK1a1 ILQ
CSNK1a1 TSL
RNF166 STF
RNF166 VQE
ZFP91 DYI
ZFP91 TGS
ZNF692 SFS
ZNF692 DYI

Figure S9

A.



B.



C.

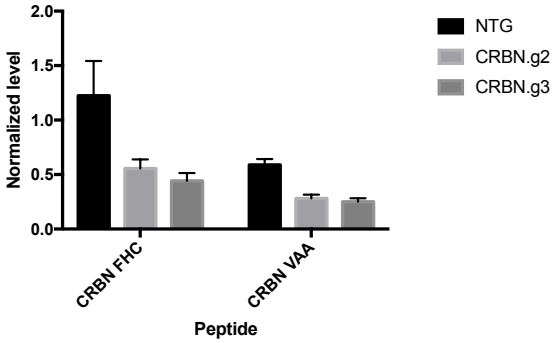
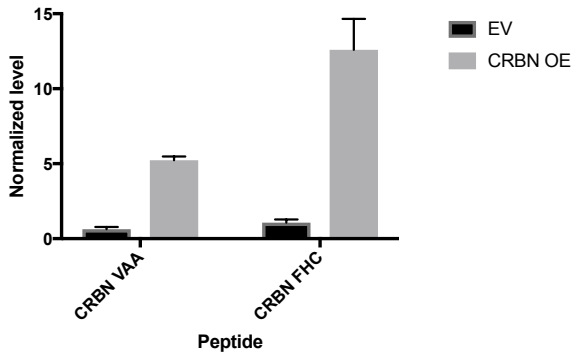
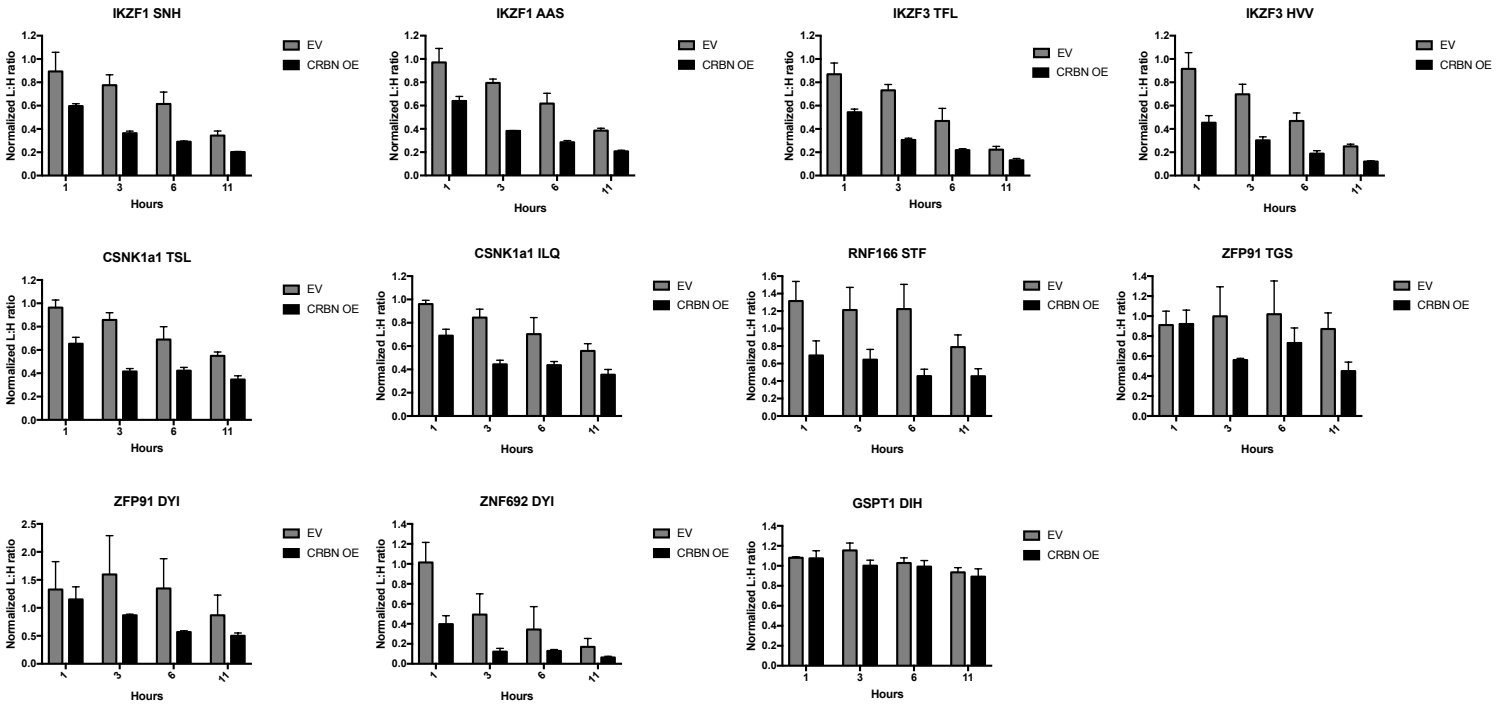


Figure S10

A.



B.



C.

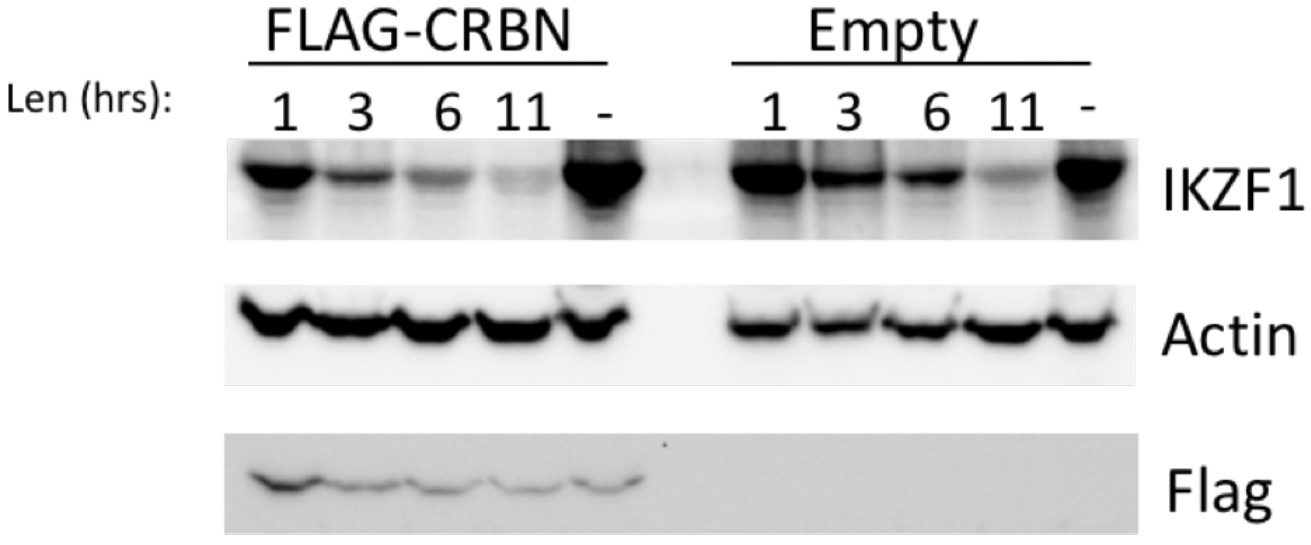


Figure S11

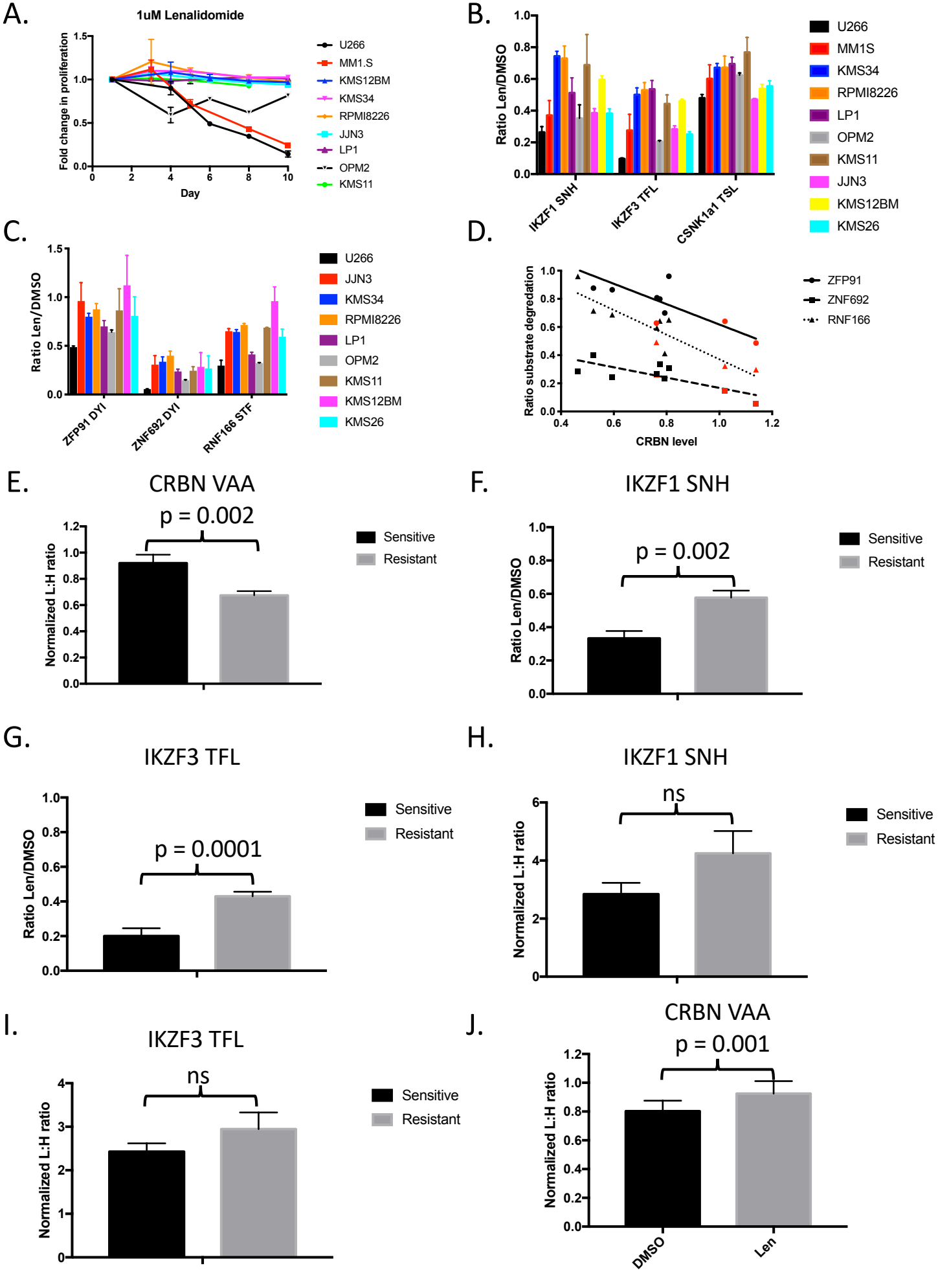
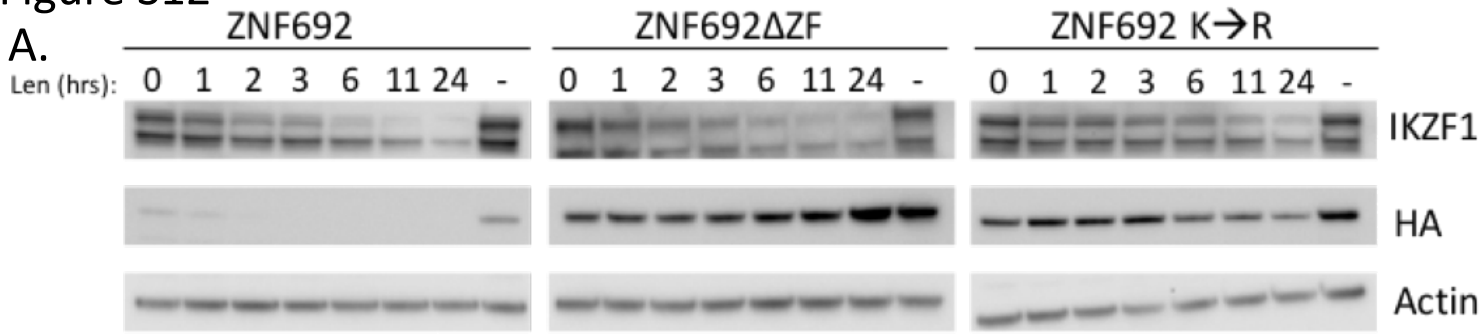
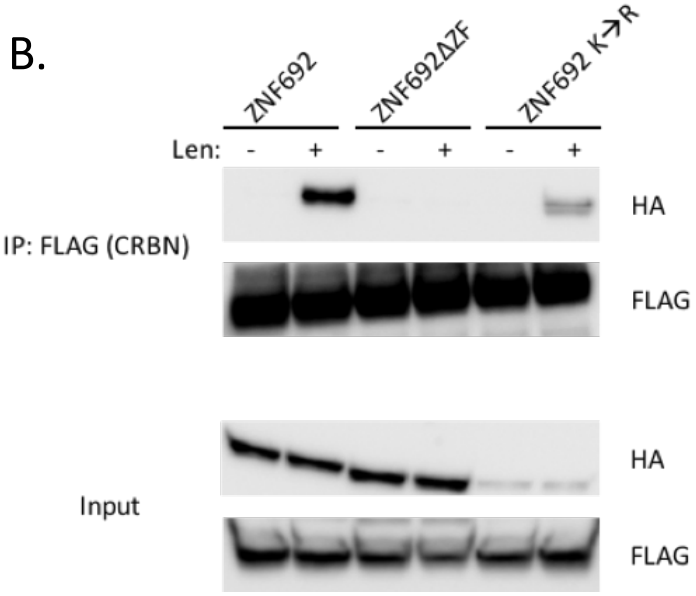
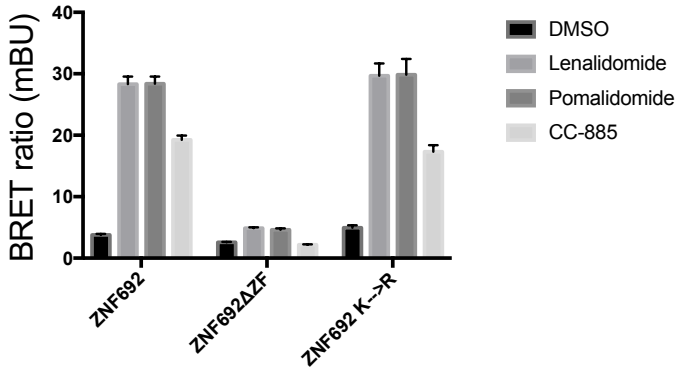
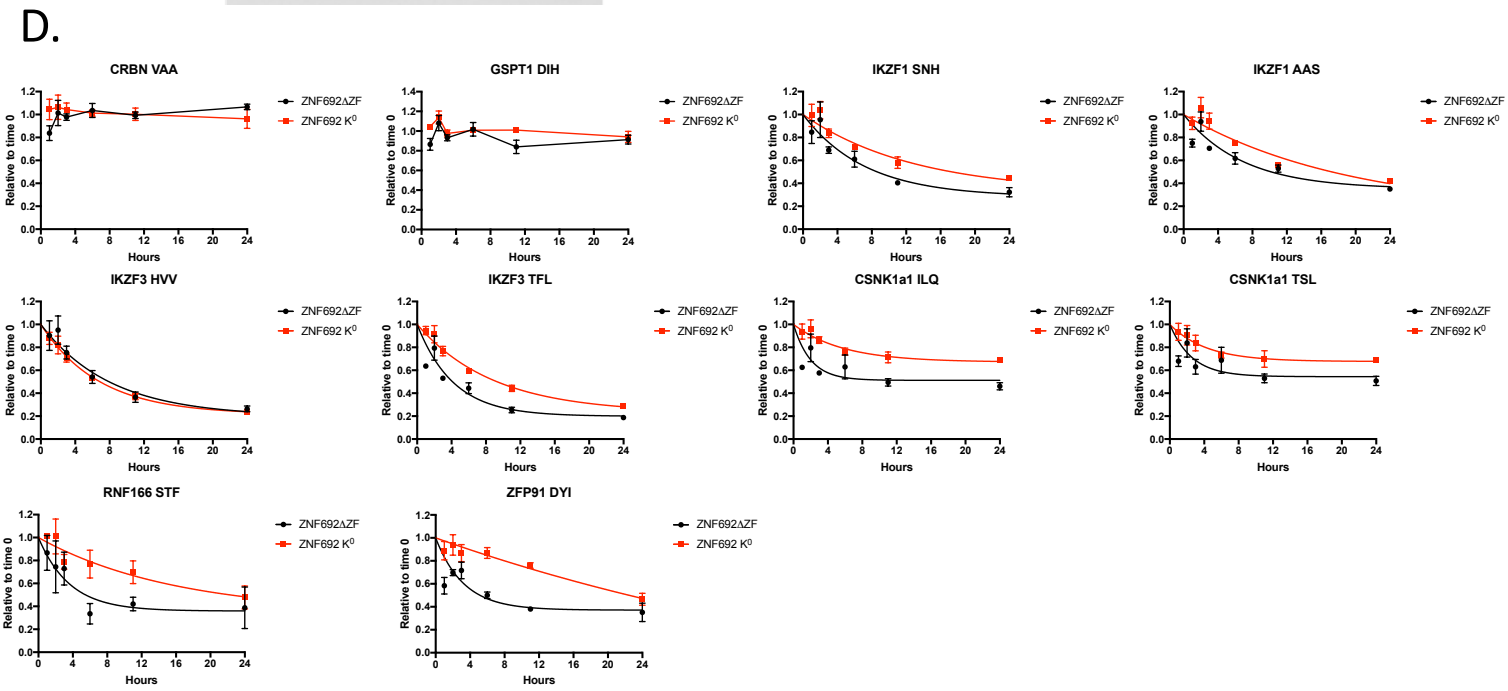

A.
1uM Lenalidomide
Fold change in proliferation
Day
U266
MM1.S
KMS12BM
KMS34
RPMI8226
JJN3
LP1
OPM2
KMS11
B.
Ratio Len/DMSO
IKZF1 SNH
IKZF3 TFL
CSNK1a1 TSL
U266
MM1S
KMS34
RPMI8226
LP1
OPM2
KMS11
JJN3
KMS12BM
KMS26
C.
Ratio Len/DMSO
ZFP91 DYI
ZNF692 DYI
RNF166 STF
U266
JJN3
KMS34
RPMI8226
LP1
OPM2
KMS11
KMS12BM
KMS26
D.
Ratio substrate degredation
CRBN level
ZFP91
ZNF692
RNF166
E.
CRBN VAA
p = 0.002
Normalized L:H ratio
Sensitive
Resistant
F.
IKZF1 SNH
p = 0.002
Ratio Len/DMSO
Sensitive
Resistant
G.
IKZF3 TFL
p = 0.0001
Ratio Len/DMSO
Sensitive
Resistant
H.
IKZF1 SNH
ns
Normalized L:H ratio
Sensitive
Resistant
I.
IKZF3 TFL
ns
Normalized L:H ratio
Sensitive
Resistant
J.
CRBN VAA
p = 0.001
Normalized L:H ratio
DMSO
Len

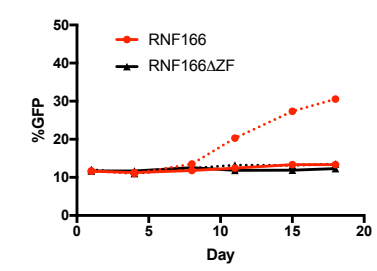
Figure S12


A.
ZNF692
ZNF692ΔZF
ZNF692 K→R
Len (hrs): 0 1 2 3 6 11 24 -
IKZF1
HA
Actin


B.
ZNF692
ZNF692ΔZF
ZNF692 K→R
Len: - + - + - +
IP: FLAG (CRBN)
HA
FLAG
Input
HA
FLAG


C.
BRET ratio (mBU)
DMSO
Lenalidomide
Pomalidomide
CC-885
ZNF692
ZNF692ΔZF
ZNF692 K-->R


D.
CRBN VAA
GSPT1 DIH
IKZF1 SNH
IKZF1 AAS
IKZF3 HVV
IKZF3 TFL
CSNK1a1 ILQ
CSNK1a1 TSL
RNF166 STF
ZFP91 DYI
Relative to time 0
Hours
ZNF692ΔZF
ZNF692 K0


E.
RNF166
RNF166ΔZF
%GFP
Day

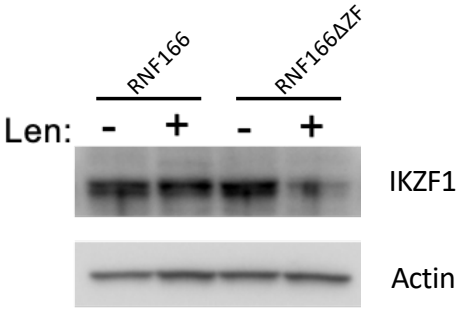
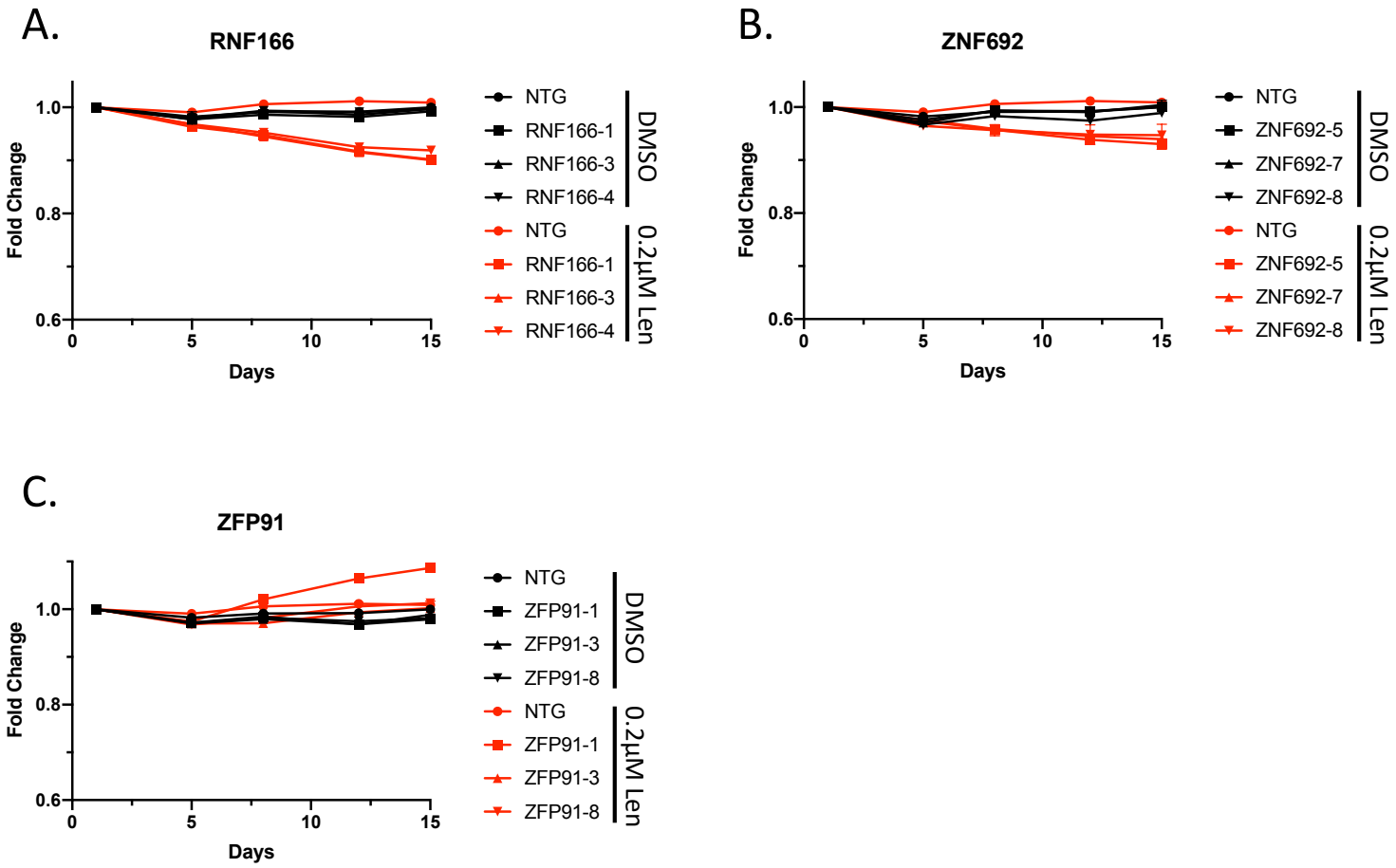

F.
RNF166
RNF166ΔZF
Len: - + - +
IKZF1
Actin

Figure S13


A.
RNF166
Fold Change
1.0
0.8
0.6
0
5
10
15
Days
NTG
RNF166-1
RNF166-3
RNF166-4
DMSO
NTG
RNF166-1
RNF166-3
RNF166-4
0.2μM Len
B.
ZNF692
Fold Change
1.0
0.8
0.6
0
5
10
15
Days
NTG
ZNF692-5
ZNF692-7
ZNF692-8
DMSO
NTG
ZNF692-5
ZNF692-7
ZNF692-8
0.2μM Len
C.
ZFP91
Fold Change
1.0
0.8
0.6
0
5
10
15
Days
NTG
ZFP91-1
ZFP91-3
ZFP91-8
DMSO
NTG
ZFP91-1
ZFP91-3
ZFP91-8
0.2μM Len