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Supplementary Materials for

Inhibiting the oncogenic translation program is an effective therapeutic strategy in multiple myeloma

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The PDF file includes:

Other Supplementary Material for this manuscript includes the following:
(available at www.sciencetranslationalmedicine.org/cgi/content/full/9/389/eaal2668/DC1)

Supplemental Figure 1

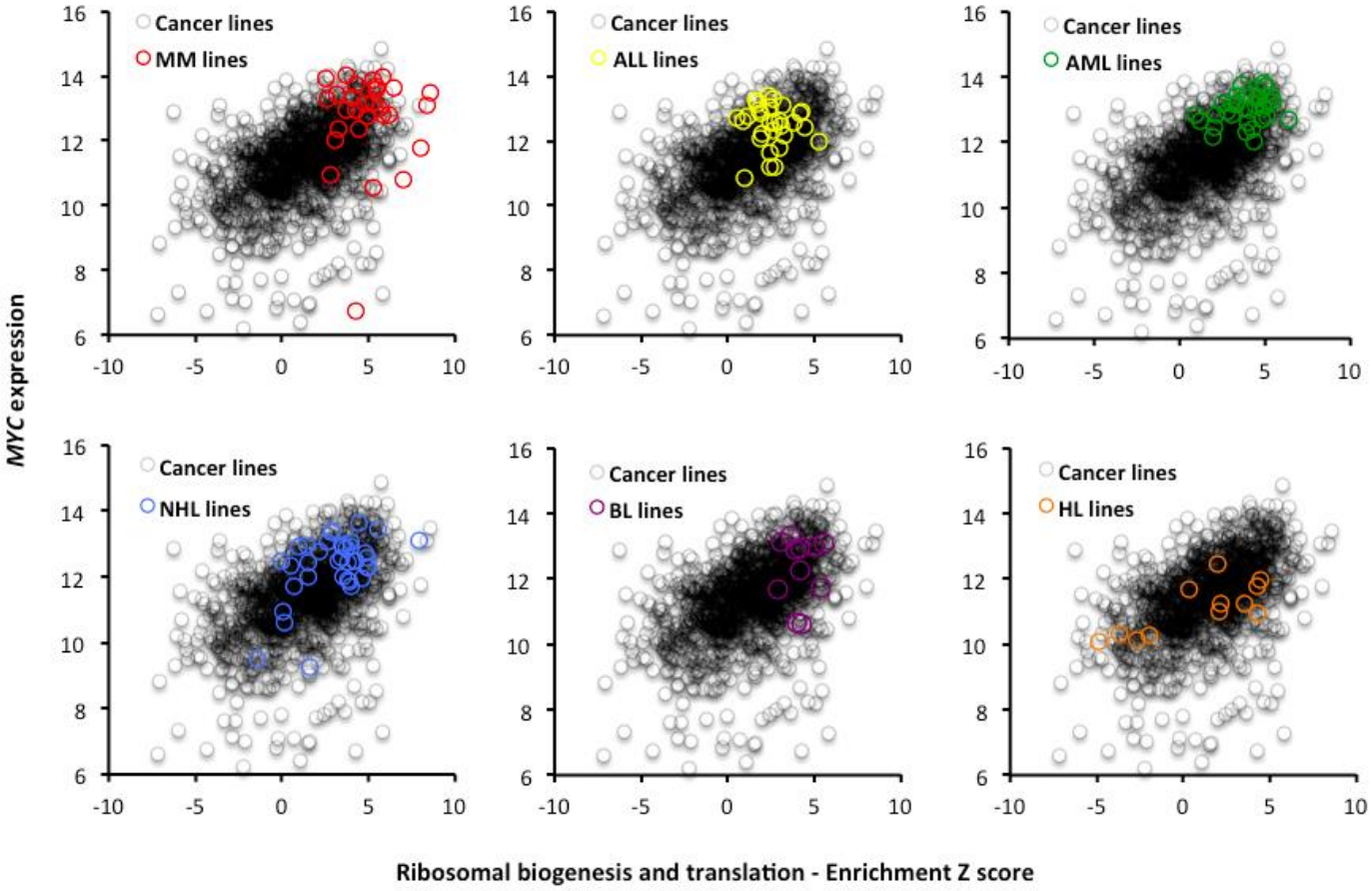
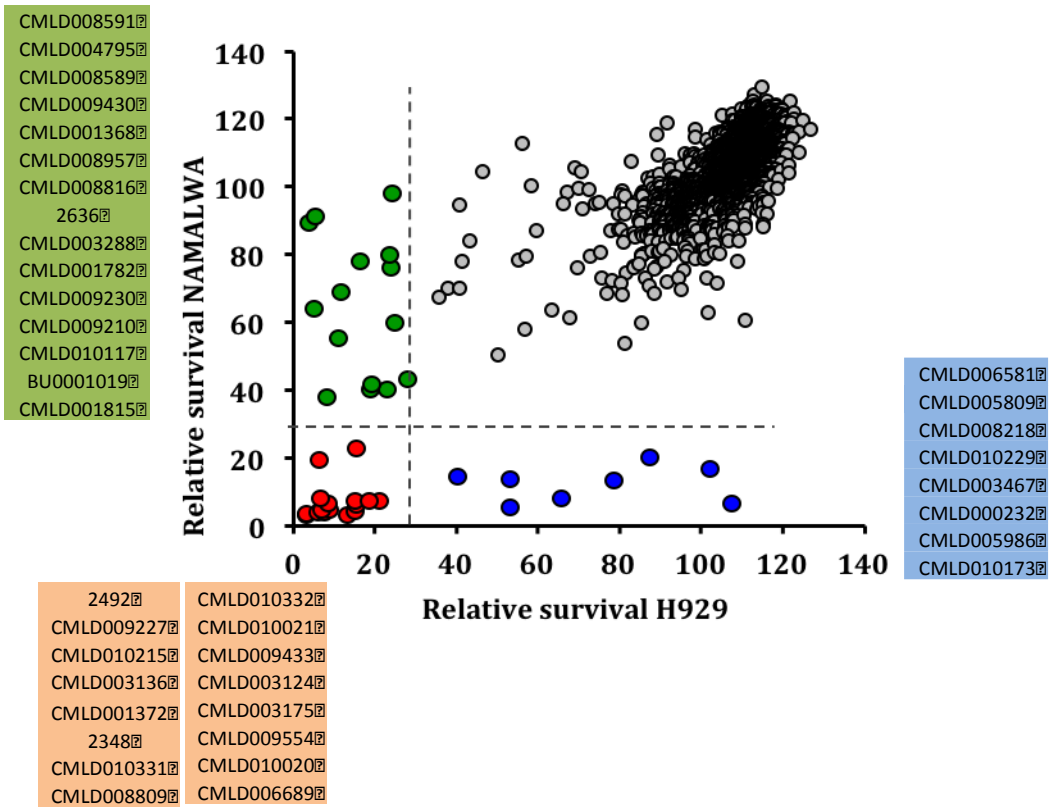


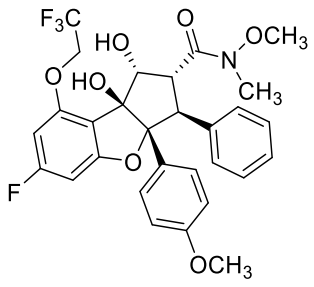
Fig. S1. Correlation between *MYC* expression and translation activation in different hematologic malignancies. Correlation analysis of *MYC* expression on the Y axis and the Z-score enrichment across over 1000 cell lines from the Cancer Cell Line Encyclopedia (CCLE) database. A Z-score was generated for each cell line by combining 2 KEGG canonical pathway gene sets: ribosomal biogenesis and translation.

Supplemental Figure 2

A



B



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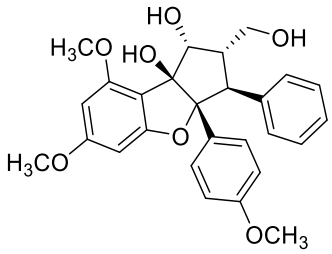

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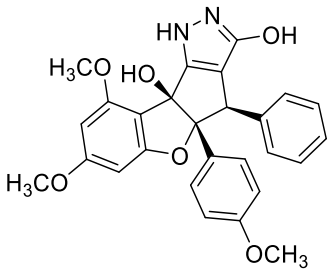


CMLD009433

C

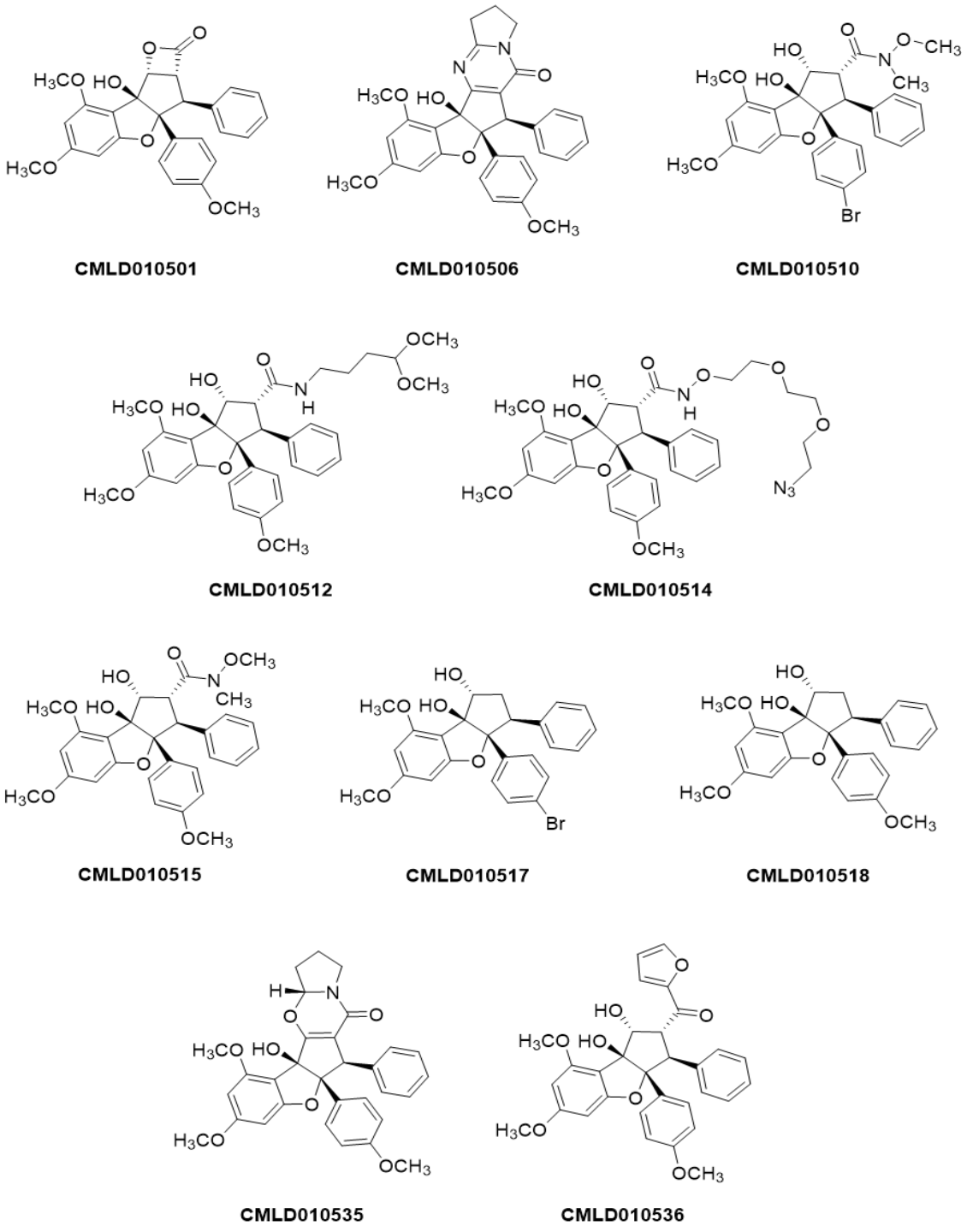


Fig. S2. Three potent rocaglate derivatives identified by an initial drug screen of a small-molecule compound library. (A) Comparison of the relative survival of lymphoid cell lines, NAMALWA *vs*. NCI-H929 after 72 h treatment with a library of 3000 small compounds. Compounds found effective in decreasing survival of NAMALWA and NCI-H929 cells are depicted respectively as green and blue dots along with the names of compounds shown in a box, while compounds effective in both cell lines are shown as red dots. In total, 45 compounds were found to potently inhibit proliferation in at least one of the cell lines. **(B)** Chemical structures of the 3 rocaglates identified by the validation screen. **(C)** Chemical structures of the 10 top rocaglates from the validation screen in addition to CMLD010509.

Supplemental Figure 3

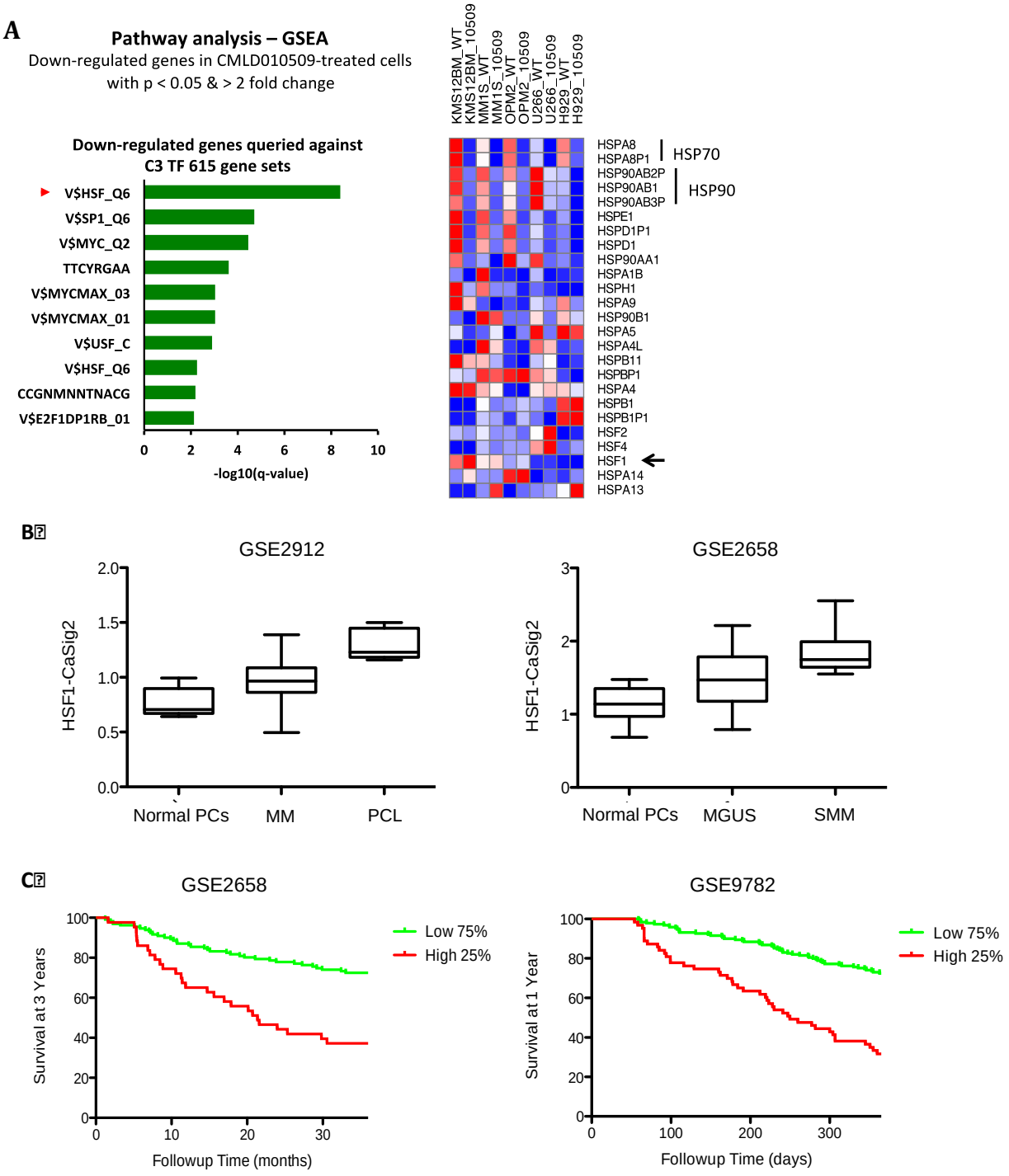
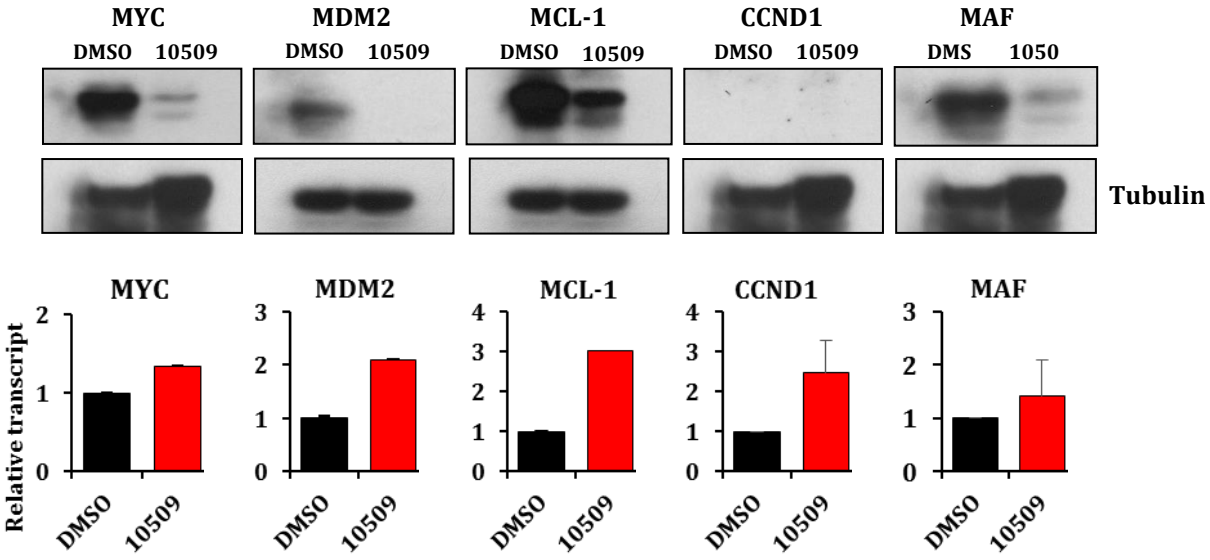


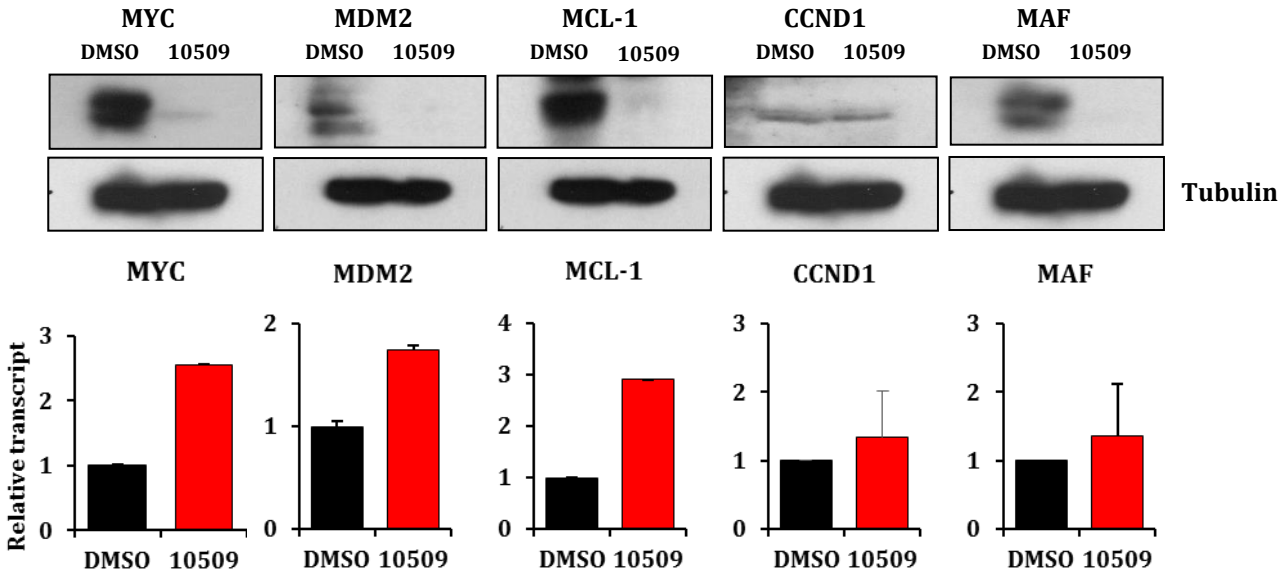
Fig. S3. Association of HSF1 activation with poor outcomes in MM. (A) Pathway analysis (RNA-seq) of genes downregulated in CMLD010509-treated cells identified by interrogating the MSigDB C3 Transcription Factor Dataset. Genes involved in heat shock response are represented in a heat map. Red arrow indicates HSF1 pathway and black arrow indicates *HSF1* gene. **(B)** HSF1 activation signature (HSF1-CaSig2) (*18*) was evaluated in 2 publicly available datasets of MM patients [GSE2912 (*19*) and GSE2658 (*20*)]. This signature represents the mean expression of 323 HSF1-bound genes identified through ChIP-seq of multiple human breast cancer cell lines. **(C)** High HSF1 activation signature is strongly correlated with poor clinical outcome in MM [GSE2658 (*20*) and GSE9782 (*21*)]. PCs: plasma cells; MM: multiple myeloma; PCL: plasma cell leukemia; SMM: smoldering multiple myeloma; MGUS: monoclonal gammopathy of undetermined significance.

Supplemental Figure 4

A. NAMALWA

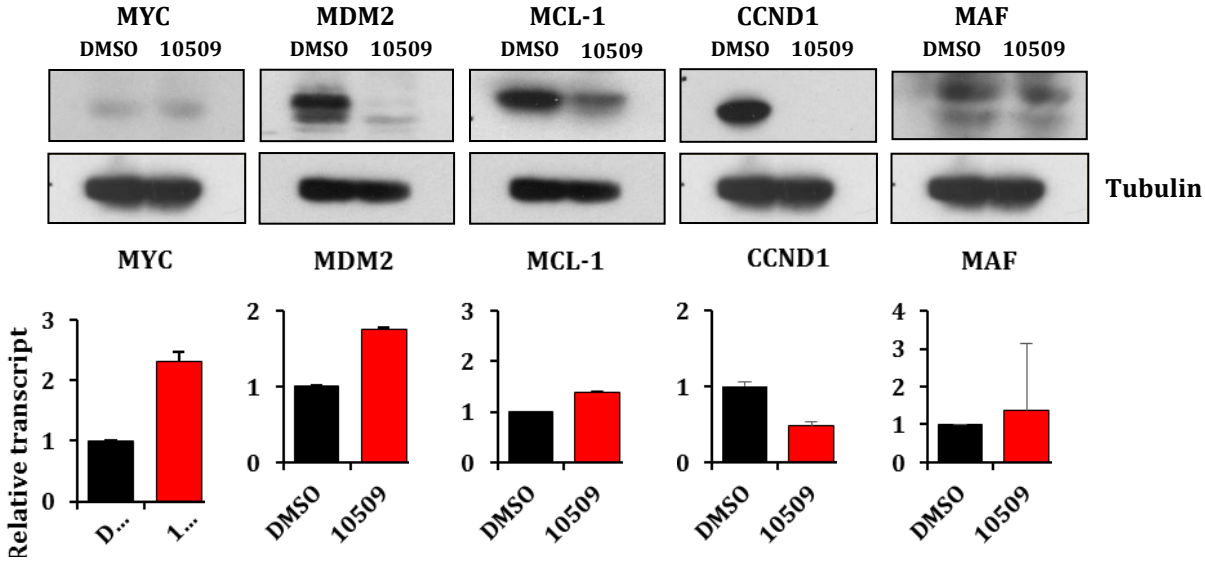


B. KMS18

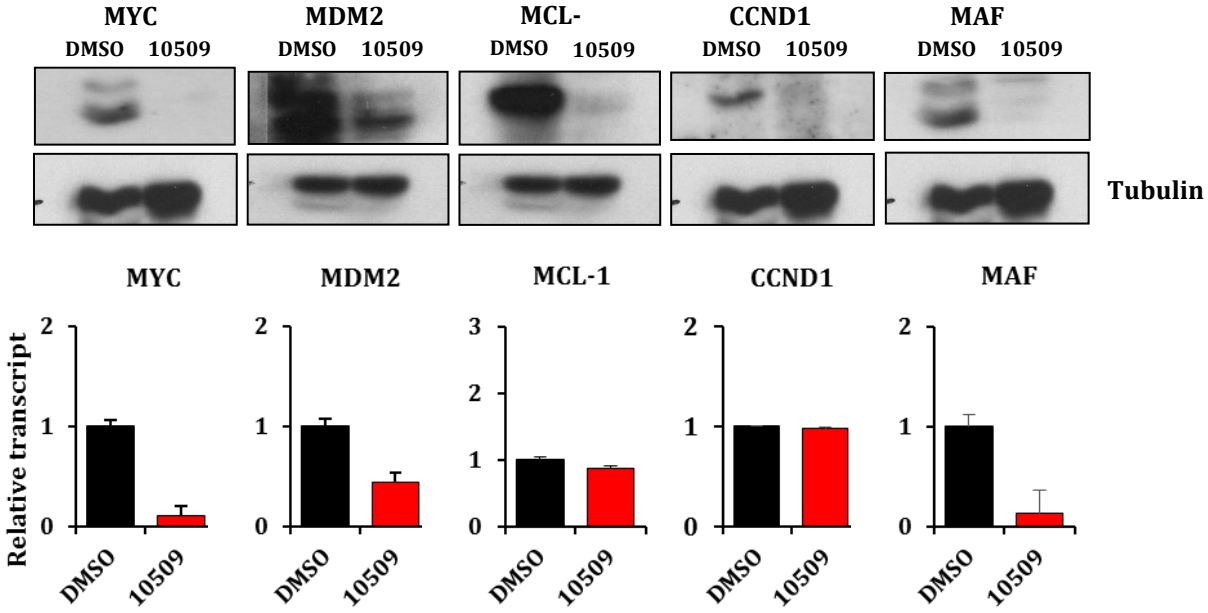


Supplemental Figure 4 – continuation

C. U266

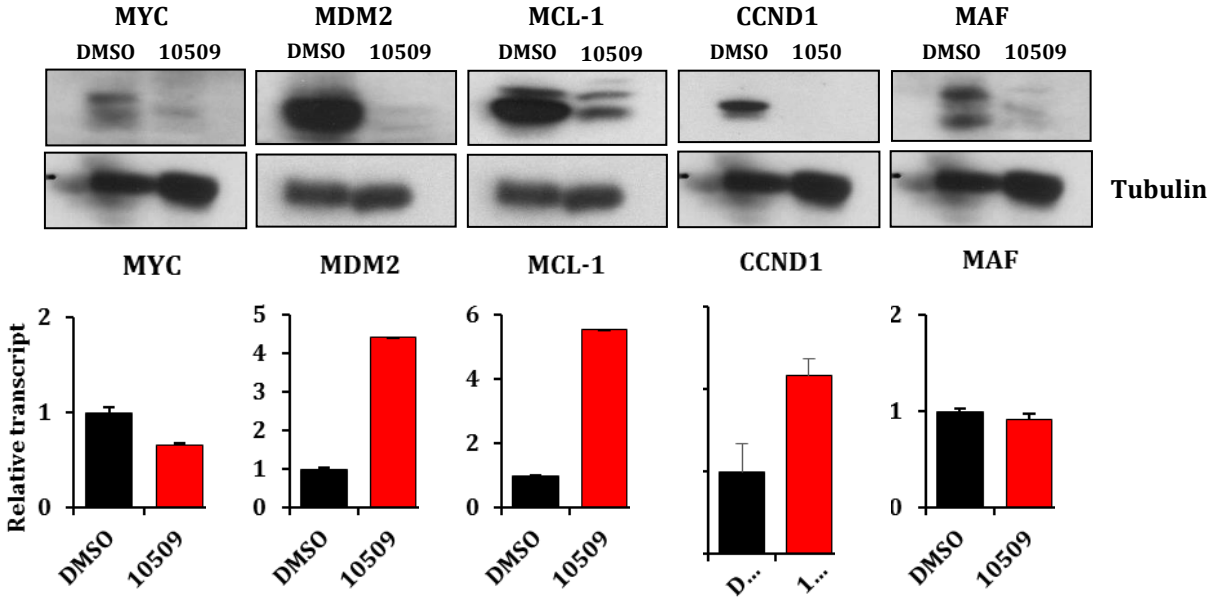


D. OPM2

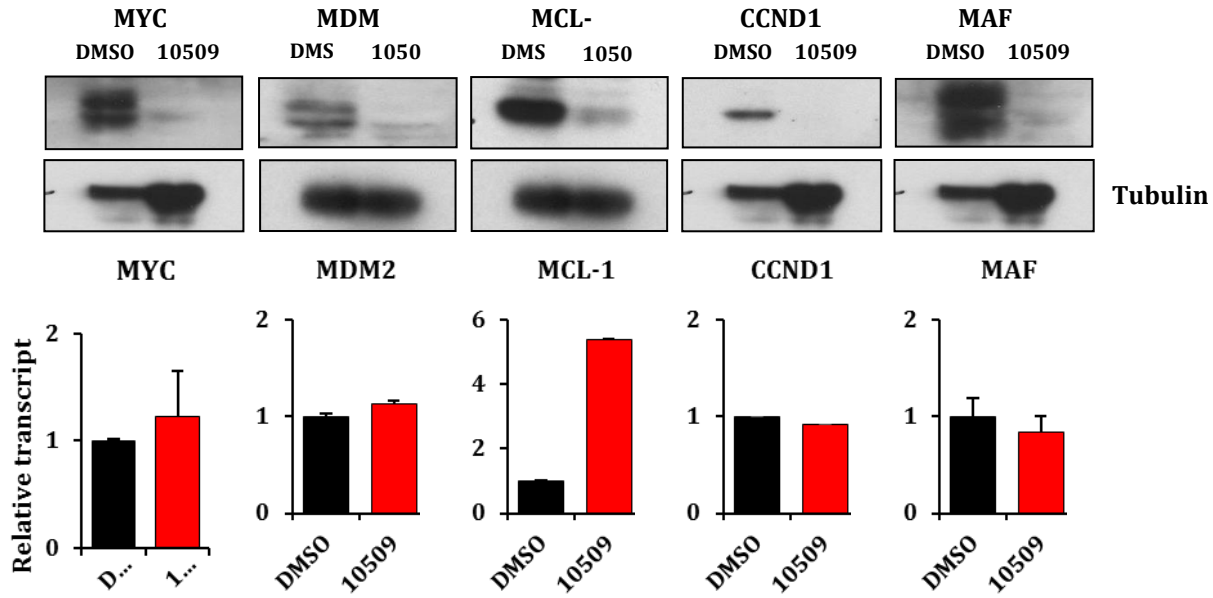


Supplemental Figure 4 – continuation

E. MM1R

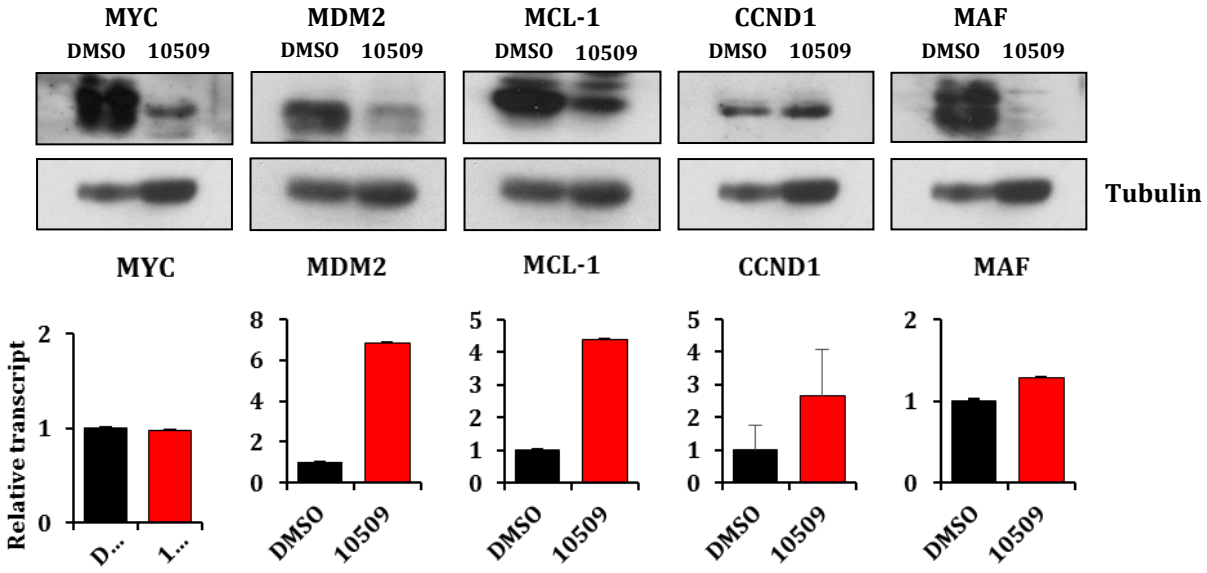


F. KMM-1



Supplemental Figure 4 – continuation

G. MM1S



H.

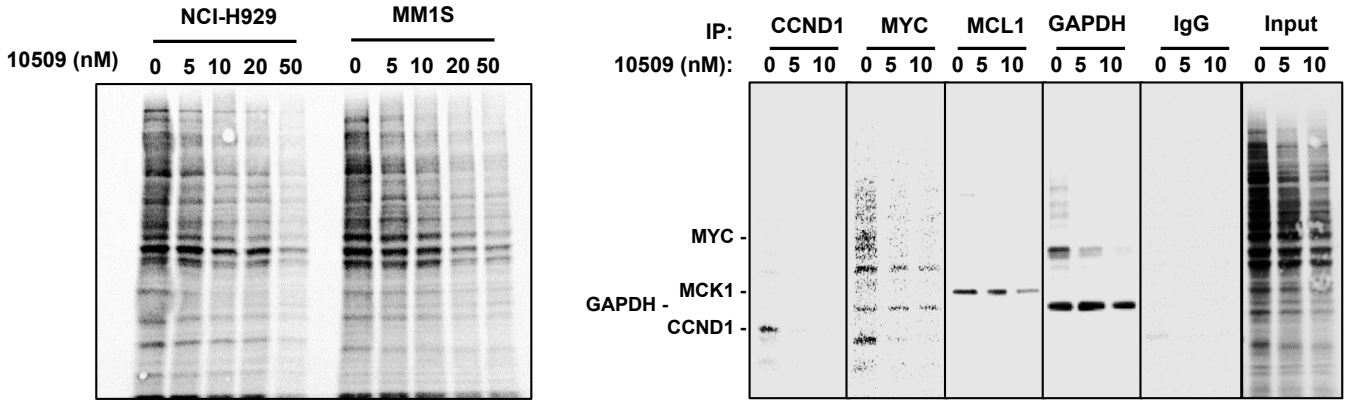


Fig. S4. RNA-seq and TMT proteomic data validation in several MM cell lines. Immunoblots (upper panels) and qRT-PCR analysis (lower panels) for MYC, MDM2, MCL-1, CCDN1, and MAF in 6 MM cell lines and NAMALWA after 3 hours of treatment with CMLD010509 or control vehicle. (**A**) NAMALWA, (**B**) KMS18, (**C**) U266, (**D**) OPM2, (**E**) MM1R, (**F**) KMM-1, and (**G**) MM1S. Error bars indicate mean +/- SD of triplicate experiments. **(H)** S35 pulse-chase labeling of MM1S and NCI-H929 cells treated with different concentrations of CMLD010509. Left panel: bulk of S35 labeled MM1S and NCI-H929 cells; right panel: immunoprecipitated CCND1, MYC, MCL1, GAPDH, and IgG as a control.

Supplemental Figure 5

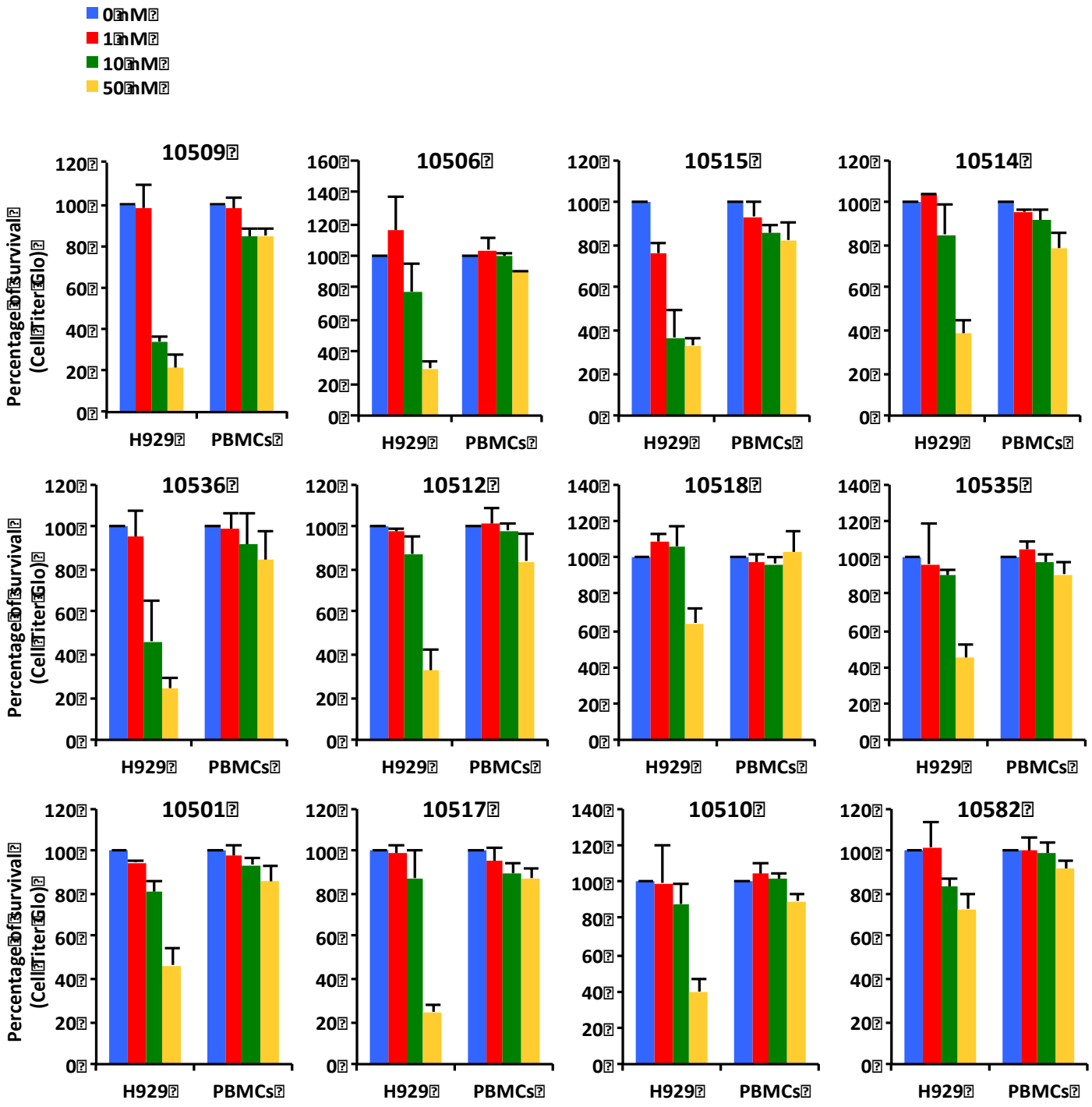


Fig. S5. Assessment of CMLD010509 toxicity on PBMCs. MM cell line, NCI-H929, and normal donor PBMCs were treated with 0, 1, 10, or 50 nM of 12 rocaglate derivatives for 72 hours. Percentage of survival was determined by Cell Titer Glo and normalized to untreated group for each different cell type. Error bars indicate mean+/-SD of triplicates.

Supplemental Figure 6

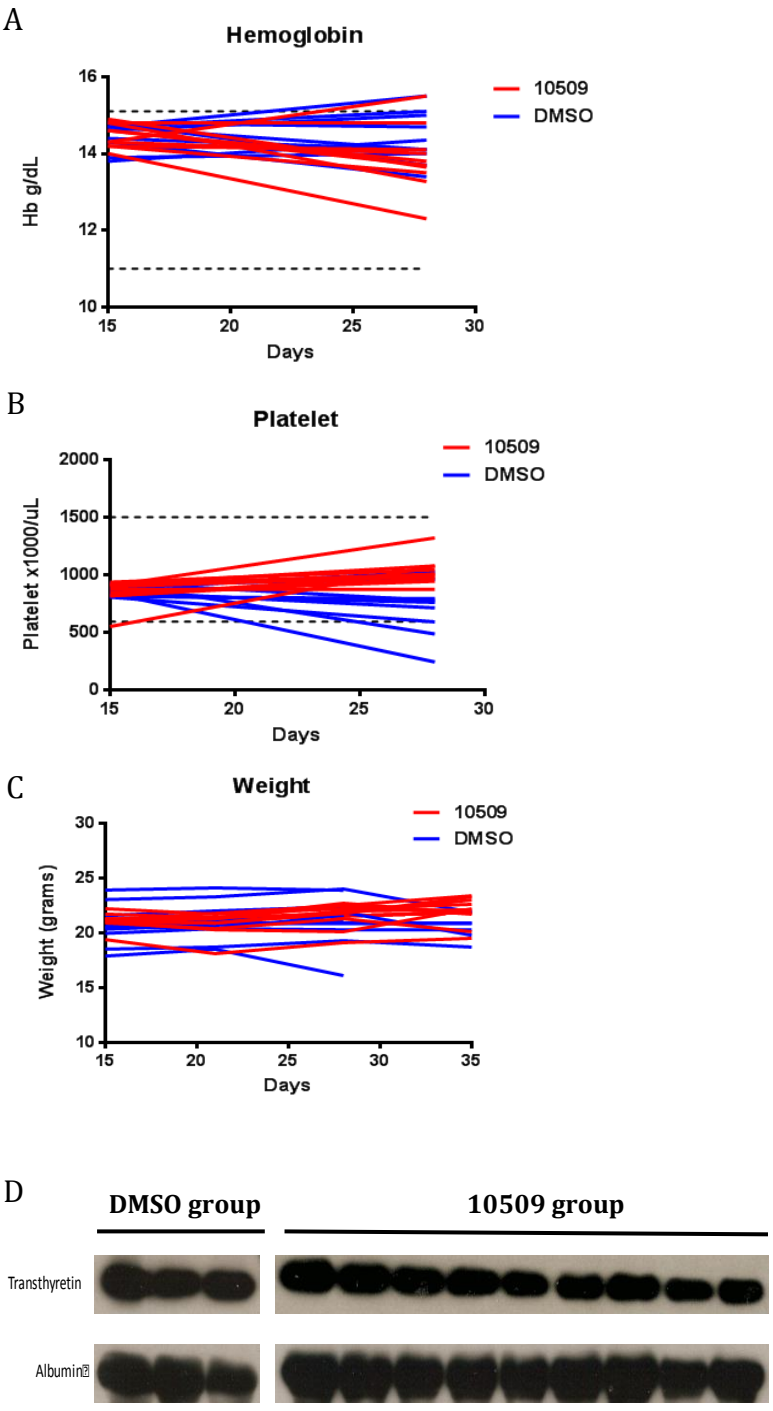


Fig. S6. Evaluation of CMLD010509 toxicity in vivo. Peripheral blood was collected from SCID mice receiving DMSO (n=10) or 10509 (n=10) every week and processed for blood (**A**) hemoglobin concentration and (**B**) platelet count. (**C**) Mice in both groups were weighed every week. (**D**) Immunoblotting for transthyretin and albumin. Serum samples were collected from the peripheral blood of BL-6 mice receiving DMSO (n=3) or 10509 (n=9) at 6 weeks after tumor cell injection. Transthyretin and albumin expression was similar in the two groups, as evaluated by western blot analysis for transthyretin and albumin.

Supplementary Table Legends

Table S1. RNA-seq of MM cells treated with CMLD010509 (provided as an Excel file). NCI-H929, NAMALWA, U266, MM1S, and OPM2 cells were treated with 50 nM CMLD010509 or vehicle control for 6 hours and further processed for RNA-seq.
(A) 845 genes that were significantly up-regulated in CMLD010509-treated cells compared to vehicle control cells ($p<0.05$).
(B) 475 genes that were significantly up-regulated in CMLD010509-treated cells compared to vehicle control cells ($p<0.05$).
(C) The whole RNA-seq dataset was queried against MSigDB C5 (GO Biological Process, GO Cellular Component, GO Molecular Function) from GSEA (http://software.broadinstitute.org/gsea/index.jsp) and represented by a network enrichment map using cytoscape (http://www.cytoscape.org).
(D) Top 20 enriched pathways in the 845 up-regulated genes (table S1A) in CMLD010509-treated cells among MSigDB C5 gene sets.
(E) Top 20 enriched pathways in the 475 down-regulated genes (table S1B) in CMLD010509-treated cells among MSigDB C5.
(F) The LINCS cloud database (http://apps.lincscloud.org/signin) was interrogated for our CMLD010509 signature (corresponding to all up- and down-regulated genes with $p<0.05$ and a fold change higher than 2). Top 100 connections are listed here.

Table S2. TMT proteomic analysis of NCI-H929 treated with CMLD010509 (provided as an Excel file). NCI-H929 cells were treated in triplicate with CMLD010509 (100 nM) or vehicle control for 2 hours. Proteins were isolated and further processed for quantitative proteomic analysis by Tandem Mass Tag (TMT) with mass spectrometry (MS).
(A) Top 54 down-regulated proteins in 10509-treated NCI-H929 cells with p value <0.05 and $FC<0.5$, among 7013 identified proteins.
(B) Significantly enriched pathways in the top 54 depleted proteins (table S2A) when queried against canonical KEGG pathways.
(C) Top 10 pathways enriched in the top 54 depleted proteins (table S2A) when queried against MSigDB C2 genesets.