SUPPLEMENTAL MATERIALS AND METHODS

Small-molecule compounds

DPI (#81050), PER (#16982) and MET (#13118) were from Cayman Chemical.

Antibodies

HK1 (#2024), HK2 (#2867), cleaved PARP (#D64E10), and GAPDH (#5174) antibodies were from Cell Signaling Technology.

Colony Formation Assay

Colony formation assays were carried out in semisolid methyl cellulose medium as described (1). 2000 cells were seeded in triplicates in 12-well culture plates, in 1 ml methylcellulose medium (MethoCult 4230, StemCell Technologies) with 10% FBS. Colonies were detected after 15 days of culture by adding 3 mg/mL MTT reagent.

Cell Cycle Profiling

Following 6-day treatment with ASOs, MM cells were harvested and spun down. The supernatant was removed as completely as possible by aspiration. 0.5 mL of cold hypotonic DNA staining solution (1 mg/mL sodium citrate, 0.3% Triton-X 100, 0.1 mg/mL PI, 20 ug/mL RNaseA) was added to each sample and mixed well. Sample were run on a BD FACSVERSE flow cytometer after 15 min, but no later than 1 hr. Analysis was performed using FlowJo software. All reagents were purchased from Sigma-Aldrich.

Intracellular Metabolite Extraction and Analysis

The experiments were performed as described previously (2). Briefly, cells were seeded in 6-well plates, and metabolites were extracted at 70-80% confluence. After 8 hr exposure to the indicated treatments, cells were washed with ice-cold 150 mM ammonium acetate, and scraped off the plate in 800 µl ice-cold 80% methanol. 10 nmol norvaline was added as an internal standard. After vigorous vortexing, the samples were centrifuged at 12,000 rpm. Supernatants were transferred into glass vials and the metabolites were dried under vacuum. Metabolites were resuspended in 50 µl 70% acetonitrile (ACN); 5 µl samples of these solutions were used for mass spectrometer-based analyses. The analyses were performed on a Q Exactive (Thermo Scientific) instrument, with polarity-switching (+3.50 kV/-3.50 kV) in full scan mode. Separation was achieved using A) 5 mM NH_4AcO (pH 9.9) and B) ACN. The gradient started with 15% A) going to 90% A) over 18 min, followed by an isocratic step for 9 min and reversal to the initial 15% A) for 7 min. Metabolites were quantified with TraceFinder 3.3 using accurate mass measurements (≤ 3 ppm), as well as retention times and fragmentation patterns of purchased compounds.

Western blotting

Cells with indicated treatments were washed with ice-cold PBS, and lysed in cold lysis buffer (20 mM Tris-HCl, 150 mM NaCl, 1 mM EDTA, 1% Triton X-100, pH 7.5) with 1x protease and phosphatase inhibitors. Protein extracts (15 µg) were resolved on 10% SDS-PAGE and then electrotransferred to Immun-Blot PVDF membrane (Bio-Rad, Hercules, CA, USA). After blocking with 5% milk in TBS-T, membranes were probed

with the indicated primary antibodies at 4℃ overnight, and then with horseradish peroxidase-conjugated secondary antibody at room temperature for 1 h. Blots were developed using Pierce ECL Substrate (Thermo Scientific, Rockford, IL) and exposed to X-ray films.

Differential Gene Expression Analysis

Gene expression data for the CCLE multiple myeloma cell lines was obtained through the Translational Genomics Research Institute. The results published here are based upon data generated by the Cancer Target Discovery and Development (CTD) Network (https://ctd2.nci.nih.gov/dataPortal/CTD2_DataPortal.html) established by the National Cancer Institute's Office of Cancer Genomics. Briefly, transcript- and gene-level abundance was quantified using Salmon [3] 0.4.2 with reference genome Homo Sapiens GRCh37.74. Differential expression analysis was carried out using the DESeq2 package for R (4). Cell lines were stratified based on HK1 status, which was determined by a HK1 gene expression threshold of 1500 raw counts. Lowly expressed genes (average raw count < 10) and genes with zero variance were filtered from analysis. Heatmaps were generated using the pheatmap package in R and clustering of genes was done with default settings (Euclidean distance measure with complete linkage clustering).

Glucose Consumption and Lactate Production Assays

Media were collected from culture plates and analyzed for glucose and lactate concentrations using a Biomedical Bioprofile Analyzer (Nova Biomedical). Cells seeded

in 6-well plates received treatments described in Results and Figure Legends. 24 hr before the analysis the media were refreshed. Medium added to wells with no cells was used as a blank control. After 24 hr incubation, 1 ml of medium was collected from each sample and the blank control, and media samples were analyzed in the Bioprofile Analyzer. Values were normalized to cell number and time intervals.

CRISPR Cas9 mediated HK1 Knockout

Mouse HK1 gRNA-1 (GATCACGTCGCTGAATGCCT) and gRNA-2 (CTCAGGCCGTACTGATCACG) were purchased from GenScript. Lentiviruses carrying the lentiCRISPRv2-gRNA were packaged in 293FT cells using the ViraPower Lentiviral Packaging Mix (ThermoFisher), and harvested after 72 hr. Two days after lentivirus infection, target cancer cells were selected in 2 μg/mL puromycin for 5 days. After puromycin selection, cells were seeded in 96-well plates at 1 cell/well density, and allowed to grow into colonies. Single colonies were isolated and validated for HK1 knockout by Western blotting analysis of HK1 protein expression.

Bone marrow cell extraction

Mouse femurs and tibias were harvested as described (5), with minor modifications. Briefly, muscle and surrounding tissue was removed with sterile forceps and scissors, wiping clean with sterile gauze. Intact bones were soaked in 70% ethanol for 1-2 min and then washed twice in 1x PBS before being placed back in RPMI complete medium (RPMI-1640 medium supplemented with 10% heat-inactivated fetal calf serum (FCS), 2 mM L-glutamine, 100 U/mL penicillin, 100 μg/mL streptomycin, and 50 μM 2-

mercaptoethanol). Bones were cut at the knee joint and placed cut side down in 0.5 mL Eppendorf tubes with an 18 G hole in the bottom of the tube and then placed in a 1.5 mL Eppendorf tube. Nested tubes were spun at >10,000 x g for 15 sec and visually inspected for marrow removal. Red blood cells were lysed in 1.5 mL ACK lysis buffer and transferred to a 15 mL conical tube and incubated at room temperature (RT) for 5 min before 5 mL of RPMI complete medium was added and cells centrifuged at 400 x g for 5 min. Supernatant was removed and cells were washed once in RPMI complete medium and centrifuged as above. Cells were then resuspended in RPMI complete medium and counted. 10^6 cells were washed in 1x PBS and then stained with a fixable viability dye, Zombie aqua (BioLegend; 1:800 in 1x PBS), for 15 min at RT and washed in 10 mL of FACS buffer (1% BSA-0.1% Sodium Azide-PBS). Cells were fixed in 400 uL of 1% PFA and flow cytometric data were collected on the FacsVerse (BD) and analyzed using FCS Express (De Novo Software).

References

1. Dhyani A, Machado-Neto JA, Favaro P, Saad ST. ANKHD1 represses p21 (WAF1/CIP1) promoter and promotes multiple myeloma cell growth. Eur J Cancer **2015**;51:252-9
2. Krall AS, Xu S, Graeber TG, Braas D, Christofk HR. Asparagine promotes cancer cell proliferation through use as an amino acid exchange factor. Nat Commun **2016**;7:11457
3. Patro R, Duggal G, Love MI, Irizarry RA, Kingsford C. Salmon provides fast and bias-aware quantification of transcript expression. Nat Methods **2017**;14:417-9
4. Love MI, Huber W, Anders S. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. Genome Biol **2014**;15:550
5. Amend SR, Valkenburg KC, Pienta KJ. Murine Hind Limb Long Bone Dissection and Bone Marrow Isolation. J Vis Exp **2016**