Supplementary Material

Multiple Myeloma Cells Depend on the DDI2/NRF1-mediated Proteasome Stress Response for Survival

Tianzeng Chen[1], Matthew Ho[2], Jenna Briere[3], Maria Moscvin[1], Peter G. Czarnecki[4], Kenneth C. Anderson[3], T. Keith Blackwell[5] and Giada Bianchi[1*]

Gene editing via CRISPR/Cas9 ribonucleoprotein (RNP) delivery

CRISPR RNA (crRNA) were designed by using the MIT CRISPR tool (crDDI2-ASP: TGGCAATCCCTGCCCACCGA) or obtained as pre-designed gRNA from Integrated DNA Technologies (IDT) (crNFE2L1.1AA: GCACGGAACCTGCTAGTGGA and crDDI2.AA: GCTCGAAGTCGGCGTCGACC). A non-targeting crRNA was used as negative control (CGTTAATCGCGTATAATACG). ATTO550-labeled tracrRNA and recombinant Cas9 protein were obtained from Integrated DNA Technologies. Each crRNA or tracrRNA was resuspended in Nuclease-Free Duplex Buffer (30 mM HEPES, pH 7.5, 100 mM potassium acetate) as a 200 µM stock solution. Equimolar crRNA and tracrRNA were diluted in Nuclease-Free Duplex Buffer for a final concentration of 43.2µM and annealed by incubating at 95°C for 5 minutes to generate the crRNA:tracrRNA complex, followed by cooling to room temperature (15–25 °C). For each reaction, 0.2 µL of Resuspension Buffer R was added to 0.3 µL of a 62 µM Cas9 protein stock solution (18 pmol), and then the solution was mixed with 0.5 µL of 43.2 µM crRNA:tracrRNA complex (0.22pmol). The mixed solution was incubated at room temperature for 10-20 minutes to form the RNP complex. Meanwhile, 5 x 10^5 cells MM cells were collected in a 15 mL sterile falcon polypropylene tube and centrifugated at 1400xrpm for 5 minutes. The pellets were resuspended in 10 mL 1X Phosphate-Buffered Saline without $Ca^{2+}$and Mg^{2+}(PBS, Gibco) and pelleted again. Then cells were resuspended in 10 µL of Resuspension Buffer R and added to

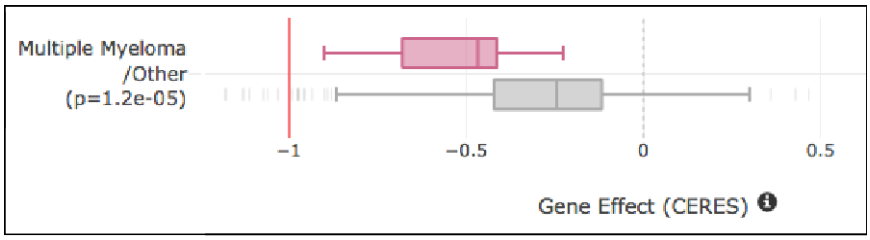
the RNP complex. The electroporation of the mixture was carried out by the Neon™ Transfection System (Thermo Fisher Scientific) with the following program (1 pulse, 1600 Voltz, 20ms pulse width) via a 10µL pipet tip (Neon 10µLkit Invitrogen) per reaction, according to manufacturer's instructions. Cells were seeded immediately after electroporation in a 6-well polystyrene tissue culture plate with 2mL of pre-warmed RPMI supplemented with 10% FBS and without antibiotics. Volumes and cell numbers were scaled up accordingly for multiple reactions. Cells were harvested 1-hour post electroporation and sorted based on DAPI negativity (viable cells) and ATTO550 positivity (transfected cells) either as a bulk population (growth competitive assays) or as single cells (AMO1-VR DDI2 KO clones).
Gene target knockout cells was validated by immunoblots and sequencing of genomic PCR amplicon across edited locus.

Immunoblotting

For whole cell lysates, cells were harvested, washed with PBS and lysed in RIPA buffer (Boston Bioproducts) containing protease inhibitors (cOmplete, Mini, Roche), 1mM EDTA, 1mM EGTA, protease inhibitors, and 10mM NEM (Thermofisher). Cytosolic and nuclear protein fractions were obtained by using NE-PER™ Nuclear and Cytoplasmic Extraction Reagents (Thermofisher), according to manufacturer's instructions. Protein concentration was determined by the Pierce™ BCA Protein Assay Kit (Thermofisher) and an equal amount of protein was loaded for each sample. SDS–PAGE was performed on NuPage Bis-Tris gels (Thermofisher) by using either MOPS or MES running buffers. Gels were wet-transferred on 0.45µm nitrocellulose membranes (0.2 µm for ubiquitin blots) (Amersham Hybond ECL, GE Healthcare) in Tris/Glycine, 0.1% SDS, 20%

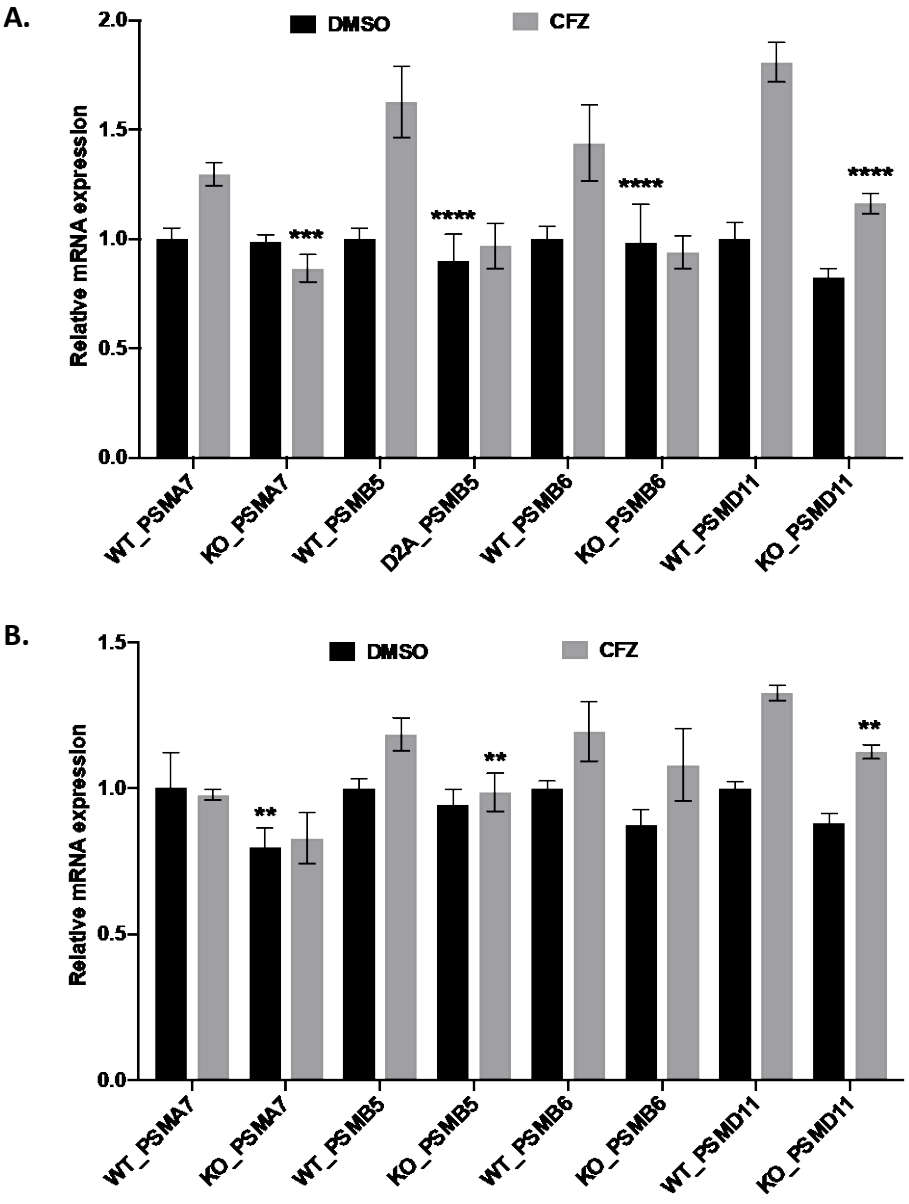
methanol transfer buffer. Following transfer, Ponceau S (Sigma Aldrich) staining was performed to assess for equal loading and adequate transfer and membranes were blocked in 5% BSA in T-BST, followed by incubation in primary antibody and secondary antibody in 5% BSA, T-BST. Detection of proteins was carried out with Amersham ECL (GE Healthcare) with antibodies listed in the main manuscript.

Supplementary Figure 1.



Supplementary Figure 1. Query of the dependency map database revealed that across 342 cancer cell lines assessed via genomic CRISPR screening, multiple myeloma cells are the most dependent on DDI2. Via this approach, a CERES score of -1 identifies an essential gene. MM cells are shown in the pink box plot, compared to all other cancer cell lines in grey.

Supplementary Figure 2.

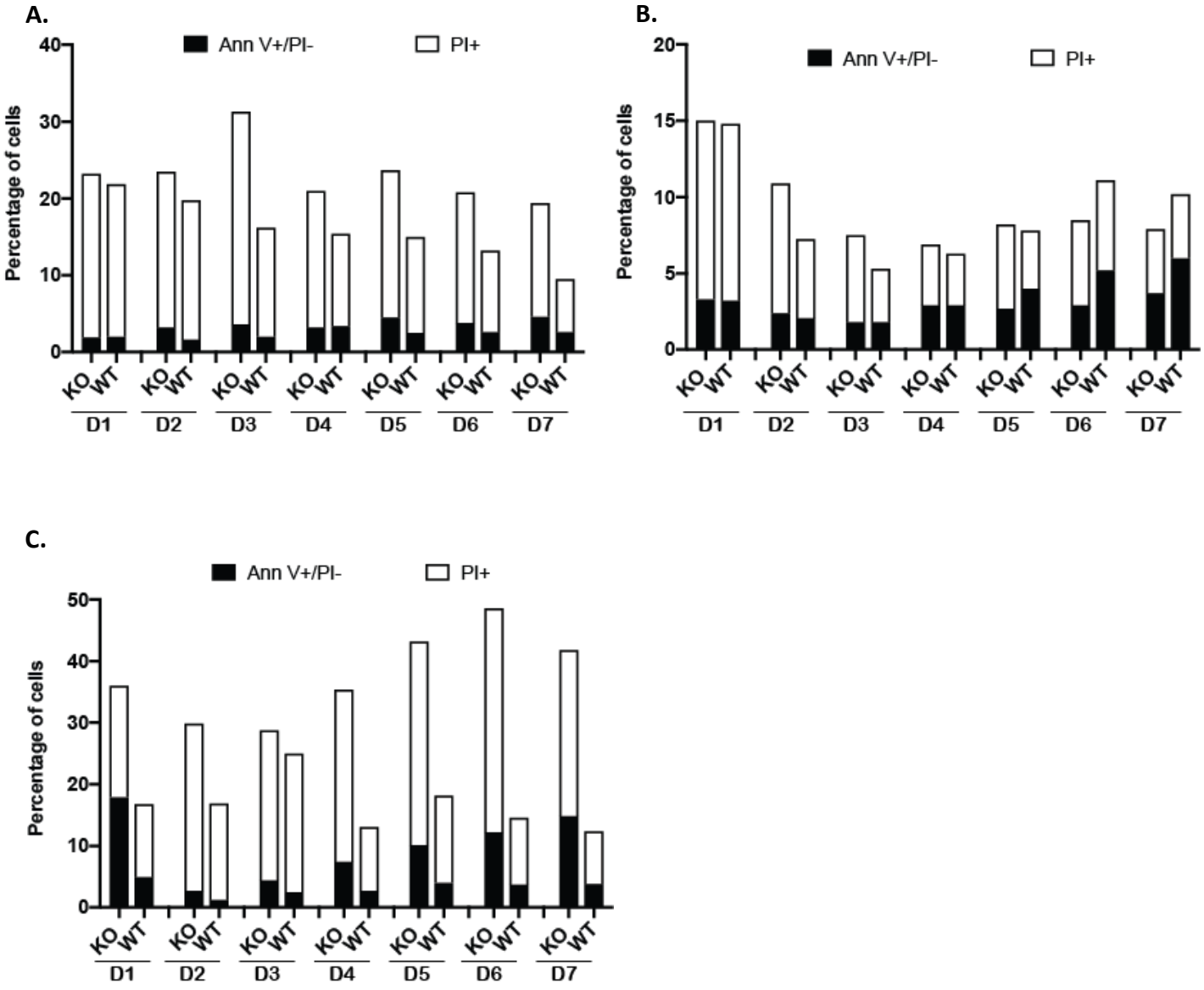


Supplementary Figure 2. Real time PCR showing expression of mRNA coding for proteasome subunits PSMA7, PSMB5, PSMB6 and PSMD11 in DDI2 WT (WT) versus DDI2 KO (KO) H929 **(A)** and AMO1 **(B)** cells. Black columns reflect RNA level in cells treated with DMSO, light grey columns represent RNA level in cells treated with carfilzomib. For each gene, RNA level in WT DDI2 cells treated with DMSO was used as control for normalization. DDI2 KO causes reduced

mRNA transcription across all proteasome subunits in baseline conditions and upon treatment with carfilzomib. P value was calculated for each paired condition (same proteasome subunit and same treatment) for DDI2 WT versus KO. P values: **<0.01; ***<0.001; ****<0.0001. One representative

experiment out of 3 biological replicates with triplicate conditions each.

Supplementary Figure 3.



Supplementary Figure 3. H929 **(A)**, AMO1 **(B)** and KMS20 **(C)** cells were subjected to RNP-based gene editing with a non-targeting gRNA (WT) or a gRNA targeting DDI2 (KO). Cells were assessed for apoptosis via annexin V (Ann V) and propidium iodide (PI) staining every 24 hours starting one day (D1) and until 7 days (D7) post transfection. Bars represent cumulative percentage of cells

undergoing apoptosis with black segments representing early (Ann V+/PI-) and white segments representing late (PI+) apoptosis. One of three representative experiments shown.