ARTICLE - Plasma Cell Disorders

Ribosomal protein S3 mediates drug resistance of proteasome inhibitor: potential therapeutic application in multiple myeloma

Gege Chen,[1*] Xuejie Gao,[1*] Xinyan Jia,[1] Yingcong Wang,[2] Li Xu,[1] Dandan Yu,[2] Shuaikang Chang,[1] Hui Deng,[1] Ke Hu,[1] Guanli Wang,[1] Bo Li,[3] Zhijian Xu,[3] Yumeng Lu,[2] Huaping Wang,[2] Ting Zhang,[2] Dongliang Song,[2] Guang Yang,[2] Xiaosong Wu,[2] Huabin Zhu,[2] Weiliang Zhu[3] and Jumei Shi[1]

[1]Department of Hematology, Shanghai East Hospital, Tongji University School of Medicine; [2]Department of Hematology, Shanghai Tenth People's Hospital, Tongji University School of Medicine and [3]CAS Key Laboratory of Receptor Research, State Key Laboratory of Drug Research, Drug Discovery and Design Center, Shanghai Institute of Materia Medica, Chinese Academy of Sciences, Shanghai, China

GC and XG contributed equally as first authors.

Correspondence: J. Shi
shijumei@tongji.edu.cn

W. Zhu
wlzhu@simm.ac.cn

Received: January 20, 2023.
Accepted: September 15, 2023.
Early view: September 28, 2023.

https://doi.org/10.3324/haematol.2023.282789

Published under a CC BY license

Supplementary Data

Ribosomal protein S3 mediates drug resistance of proteasome inhibitor: potential therapeutic application in multiple myeloma

Supplementary methods

Supplementary Table 1

Supplementary Figure 1-6

Supplementary References

Supplementary methods

Cell culture and patient samples

ARP-1, OCI-MY5, H929, RPMI-8226, and HEK293T cells were obtained from the American Type Culture Collection (Mananssas, VA, USA). The bortezomib-resistant cell lines H929R and RPMI-8226R5 have been described in our previous articles [1, 2]. MM cells were maintained in 1640 medium (Gibco, Carlsbad, CA, USA) supplemented with 10% fetal bovine serum (FBS; Gibco) and 1% penicillin-streptomycin (PS; Gibco). The HEK293T cell line was maintained in Dulbecco's modified Eagle's medium (Gibco) supplemented with 10% FBS and 1% PS. All cells were maintained in a humidified atmosphere containing 5% CO_2 at 37 ˚C.

Primary cells were obtained from bone marrow (BM) of patients with MM and the peripheral blood of normal healthy donors. Ficoll-Hypaque density gradient centrifugation was used to obtain bone marrow mononuclear cells and peripheral blood mononuclear cells. $CD138^+$ myeloma cells were selected from the BM of patients with MM using immunomagnetic microbeads coated with anti-CD138 monoclonal antibody (Miltenyi Biotech, Auburn, CA, USA). All samples were obtained from patients with MM and normal donors who gave informed consent according to procedures approved by the Shanghai Tenth People's Hospital Institutional Review Board.

Reagents and antibodies

DCZ0415 was synthesized at Shanghai Institute of Materia Medica, Chinese Academy of Sciences. Recombinant human TNF-α was obtained from R&D Systems (Minneapolis, MN, USA). Bortezomib was purchased from Sigma (St. Louis, MO, USA). The selective PKCδ inhibitor, BJE6-106, was obtained from MedChemExpress (Shanghai, China). Puromycin was purchased from Sigma.

Antibodies against PARP, phospho-Ikkβ, Ikkβ, CDC25C, DYKDDDDK Tag (Flag), and cyclinB1 were purchased from Cell Signaling Technology (Beverly, CA, USA). Antibodies against TRIP13, RPS3, phospho-NF-κB p65, NF-κB p65, phospho-IκBα, IκBα, cleaved caspase-3, cleaved caspase-8, cleaved caspase-9, CDK1, LaminB1, GAPDH, β-actin, HA tag, and PKCδ were purchased from Abcam (Cambridge, MA, UK). Antibodies against phosphorylated threonine (p-Thr), serine (p-Ser), and tyrosine (p-Tyr) were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). The peptide EPKDEILPTT(p)PI, which corresponds to the amino acid residues of the RPS3 protein, was synthesized by AtaGenix (Wuhan, China) and used to immunize four rabbits, resulting in the production of rabbit polyclonal antibody specific for RPS3 phosphorylated on T221.

Plasmids and viral transduction

The full-length cDNAs of human TRIP13 and RPS3 were synthesized and cloned into the pLVX-EGFP-IRESpuro (Invitrogen, Carlsbad, CA, USA). To

achieve TRIP13 and RPS3 stable overexpression cell lines, cells were infected with lentivirus and selected using 2 μg/mL puromycin for two weeks.

The RPS3 mutation was generated by quick change point mutagenesis (Toyobo, Osaka, Japan) and confirmed by DNA sequencing. The primer sequences were: T221A: Forward: 5′-GTTCTGAGATGGGGGCGGTGGGCAGTATCTC -3′; Reverse: 5′-GAGATACTGCCCACCGCCCCCATCTCAGAAC -3′. The plasmids were transfected into MM cells using Lipofectamine 3000 (Invitrogen), following the manufacturer's instructions.

Cell viability assay and colony formation

Cell viability assay was performed as described previously [3]. Briefly, cells (2×10^5 cells/mL) were seeded in 96-well plates. Cell Counting Kit (CCK)-8 (Dojindo, Mashikimachi, Japan) was used to evaluate cell viability. CalcuSyn (Biosoft, Ferguson, MO, USA) was used to calculate the half-maximal inhibitory concentration (IC_{50}) for each drug and the combination index (CI) values. CI value <1 indicates synergism.

For the colony formation assay, cells pre-treated with the drugs or the medium with 0.33% agar were plated on top of a layer containing 0.5% agar in 12-well plates. After incubation for 2 weeks, the colonies were stained with 0.5% crystal violet (Sigma) for 1 h at room temperature and colony images were taken with a digital camera.

5-ethynyl-2′-deoxyuridine (EdU) cell proliferation assay

EdU cell proliferation assay were performed as described previously [3]. Briefly, MM cells (2 × 10^5 cells/mL) stably transfected with TRIP13-OE, EV, Sg-RPS3 or Sg-Control were collected, and EdU was incorporated using an EdU kit (RiboBio, Guangzhou, China) according to the manufacturer's protocol.

Apoptosis measurement

Apoptosis assay was performed using an FITC Annexin V Apoptosis Detection kit (BD Biosciences, Franklin Lakes, USA) as previously described [3]. Apoptotic cells were characterized as Annexin V^+ / PI^- (early apoptosis) and Annexin V^+ / PI^+ (late apoptosis).

Cell cycle analysis

MM cells stably transfected with Sg-RPS3 or Sg-Control were collected, washed with ice-cold phosphate-buffered saline (PBS, Sigma), and fixed overnight in pre-cooling 75% ethanol at -20 ˚C. Cells were then washed with PBS and stained with 300 μL PI/RNase staining buffer (BD Biosciences) for 30 min at 4 °C in the dark. The stained cells were detected using flow cytometry FACS Canto II flow cytometer (BD Biosciences).

Nuclear and cytoplasmic protein extraction

Cytoplasmic and nuclear proteins were extracted using the nuclear and cytoplasmic protein extraction kit (Beyotime Biotechnology, Shanghai, China) following the manufacturer's instructions. Briefly, cells were washed in ice-cold PBS and resuspended in 300 μL ice-cold cytoplasmic lysis buffer. The resuspended lysates were incubated on ice for 30 min and then collected for immunoblotting analysis. Nuclear pellets were washed in 500 μL ice-cold cell lysis buffer and resuspended in 50 μL nuclear extraction buffer. The nuclear extracts were then obtained after shaking at 4 °C for 30 min. Cytoplasmic lysates and nuclear extracts were quantified using a BCA Protein Assay kit (Beyotime). Antibodies against GAPDH and LaminB1 were used to normalize the expression of cytosolic and nuclear proteins, respectively.

Dual-luciferase reporter assay

EV and TRIP13-OE MM cells (2×10^5 cells/mL) were co-transfected with the p-NF-κB-TA-luc (Beyotime) and pRL-TK (Promega, WI, USA) plasmids at a 10:1 ratio. Lysates were subjected to luciferase activity assays using the dual-luciferase assay kit (Promega) following the manufacturer's protocol. The pRL-TK vector, which expresses Renilla luciferase, was used as the internal control to correct for the difference in transfection and harvest efficiencies.

RNA extraction and Quantitative RT-PCR (qRT-PCR)

Total RNA was extracted using an RNA-Quick purification kit (ES Science,

Shanghai, China). cDNA was synthesized from 1 μg total RNA using the PrimeScript RT reagent kit (TaKaRa, Shiga, Japan) following the manufacturer's instructions. mRNA was quantified using a SYBR-Green fluorescent-based qRT-PCR assay. Specific primers for TRIP13, RPS3, and GAPDH are shown in Supplementary Table 1. The qRT-PCR assays were carried out with the real-time PCR kit (TaKaRa) on a 7900HT fast RT-PCR detection system (Thermo Fisher Scientific, Waltham, MA, USA). The protocol comprised a polymerase activation step at 95 °C for 3 min, followed by 40 cycles of a denaturation step at 95 °C for 15 s and a primer annealing and elongation step at 60 °C for 1 min. Following amplification, the reaction specificity was evaluated via melting curve analysis. All reactions were performed in duplicates to obtain reproducible results. The $2^{-\Delta\Delta CT}$ relative quantification method was conducted for the analysis of mRNA expression levels, using GAPDH as an endogenous reference control for normalization purposes.

Immunoblotting analysis

Protein extracts were obtained from cells or human tissues using RIPA buffer (Sigma). Equivalent amounts of protein extracts were separated using 8–12.5% sodium dodecyl sulfate (SDS)–polyacrylamide gels and then transferred onto nitrocellulose membranes. Membranes were blocked with 5% fat-free milk in phosphate-buffered saline containing 0.05% Tween 20 at room

temperature for 1 h and incubated overnight at 4 °C with the specific primary antibodies. They were then incubated with the corresponding secondary antibody at room temperature for 1 h. Target proteins were detected with the Odyssey scanner (LI-COR Biosciences, Lincoln, NE, USA).

Immunofluorescence

Cells were harvested and fixed with 4% paraformaldehyde (Sigma) for 10 min. After being washed in PBS, cells were permeabilized with 0.5% TritonX-100 and then incubated with NF-κB p65 (1:250 dilution; Abcam), TRIP13 (1:500 dilution; Abcam), and RPS3 (1:250 dilution; Abcam) overnight at 4 °C. Subsequently, the cells were incubated with corresponding secondary antibodies against Cy™3 AffiniPure Donkey AntiMouse IgG or Alexa Fluor 647 AffiniPure Goat (1:400 dilution; Jackson Laboratory, Bar Harbor, ME) at room temperature for 1 h, followed by incubation with 4,6-diamidino-2-pheny-lindole (Sigma) for 5 min at room temperature. Images were captured using a confocal laser scanning microscope (Carl Zeiss, Jena, Germany).

Coimmunoprecipitation (Co-IP)

For endogenous Co-IP, cells were washed thrice with ice-cold PBS and then lysed with 300 μL IP lysis buffer (150 mM NaCl, 50 mM Tris pH 7.4, and 0.1% NP-40 supplemented with protease inhibitor cocktail). An equivalent number of proteins (1000 μg) were incubated with 30 μL Protein A/G agarose beads and specific antibodies on a rotator at 4 °C overnight. The samples were

then centrifuged to remove the supernatant, and after washing with IP lysis buffer three times, the pull-down complex was collected and boiled in 2*x* SDS loading buffer for immunoblotting analysis.

For exogenous co-immunoprecipitation, HEK293T cells were transfected with Flag-tagged TRIP13, HA-tagged RPS3, or both constructs; cultured for 48 h; and then processed for Co-IP analysis. The cell lysis procedure is the same as that of the endogenous Co-IP.

CRISPR/Cas9-mediated gene knockout

Knockout cells were generated using lentivirus-mediated CRISPR/Cas9 technology as described previously [4]. The single guided RNA (sgRNA) sequences targeting human TRIP13 was: #1, TGAGTAGCTTTCTAACACTC, #2, CCAAGCTGTCCCAAAGCCCA. The single guided RNA (sgRNA) sequences targeting human RPS3 were: #1, CTCACCTCTACACTGCCCTC, #2, CTTTCCAGAGGGCAGTGTAG. The sgRNA-encoding CRISPR lentivirus vectors were produced according to the protocol by the Trono lab (https://www.epfl.ch/labs/tronolab/laboratory-of-virology-and-genetics/lentivectors-toolbox/). To generate the knock-out cell lines, target cells were infected with sgRNA-encoding CRISPR lentivirus, cultured in 1640 medium with 10% FBS, and selected using puromycin (2 μg/mL) for two weeks. The expression of target proteins in infected cells was detected by immunoblotting to verify the knockdown.

RNA-sequencing

Total RNA was extracted using the RNA-Quick purification kit (ES Science) and preserved using TRIZOL (Ambion, Austin, TX, USA), which was sent to OE biotech Co., Ltd. (Shanghai, China) for RNA-Seq analysis. After determining the purity and quantification of total RNA, the cDNA libraries were constructed using VAHTS Universal V6 RNA-seq Library Prep Kit according to the manufacturer's instructions. The libraries were sequenced on an Illumina Novaseq 6000 platform. Differentially expressed genes were identified as those with FDR <0.05 and fold change >2 or fold change <0.5. Gene annotation and analysis was conducted using metascape (http://metascape.org/). Gene Set Enrichment Analysis (GSEA) was carried out according to the instructions from the OE biotech Institute. After gene expression was quantified by FPKM, log2 scaled fold change of all expressed genes was calculated between the negative control group and the overexpression group and imported into GSEA analysis. Graphic representations of results were generated using the packages in R (https://www.r-project.org/). The RNA-seq data generated in this study are deposited on the Gene Expression Omnibus (GEO accession no. GSE240978).

Animal study

For tumorigenesis, ARP-1 cells transfected with Sg1-RPS3, Sg2-RPS3, or

Sg-Control cells (3×10^6) in 100 μL serum-free culture medium were injected subcutaneously into athymic nude mice (5-week-old), and every three days the tumour volume was measured with a digital calliper. At the end of the study, mice were euthanized and tumours were resected and subjected to TUNEL and immunohistochemistry staining.

In addition, we constructed another tumour transplantation model. RPS3-OE or EV ARP-1 cells (3×10^6) in 100 μL serum-free culture medium were subcutaneously injected into the upper flank region of the athymic nude mice. The size and volume of the tumours were measured with a digital calliper every three days. When the tumours were measurable, mice were randomly assigned into two groups and treated with bortezomib (1 mg/kg dissolved in 200 μL of the vehicle, thrice/week) or the vehicle (200 μL of saline containing 1% dimethyl sulfoxid). The tumour volume (mm^3) was calculated using the following formula: length×width^2/2. The number of surviving nude mice was recorded, and survival analysis was performed using a Kaplan-Meier survival curve.

For drug sensitivity assay, another animal model was constructed via injecting serum-free culture medium containing H929R cells (3×10^7) into the upper flank region of athymic nudemice. Tumour growth was monitored by measurement of the tumour diameters using a digital calliper every other day and the tumour volume was calculated as described above. Mice were randomly assigned into four groups and treated with bortezomib (1 mg/kg

dissolved in 200 μL of the vehicle, thrice/week), DCZ0415 (50 mg/kg dissolved in 200 μL of the vehicle, twice/week), or a combination of the two. Experimental mice were euthanized, and the tumours were resected, weighed and photographed, at the end of the treatment.

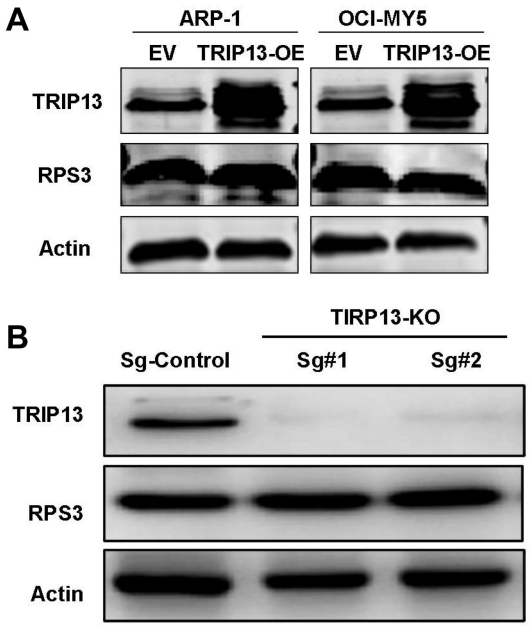
All animal procedures were approved by the Institutional Review Board of the Shanghai Tenth People's Hospital (Approval No. SHDSYY-2018-991).

Supplementary Table 1

Supplemental Table 1. The sequences of primers used in qRT-PCR

Gene	Sequences (5′-3′)
TRIP13	Forward:ATCCCATCTCCTCGATTAGTGA
	Reverse:GGGCTAACGCTTTACACAGGG
RPS3	Forward:GCCAAAGGCTGCGAGGTTGTGGTGTCTG
	Reverse:GCGGCTCTGGCTTCCCACCCTTCTGTTC
GAPDH	Forward:GGAGCGAGATCCCTCCAAAAT
	Reverse:GGCTGTTGTCATACTTCTCATGG

Supplementary Figures



Supplemental Figure 1. TRIP13 has no obvious effects on NF-κB non-Rel subunit RPS3 protein levels. (A) Immunoblotting detection of protein levels of RPS3 in EV and TRIP13-OE ARP-1/OCI-MY5 cells. Actin served as a loading control. **(B)** Immunoblotting detection of the protein levels of RPS3 in TRIP13-knockout (KO) and the corresponding control ARP-1 cells. Actin served as a loading control.

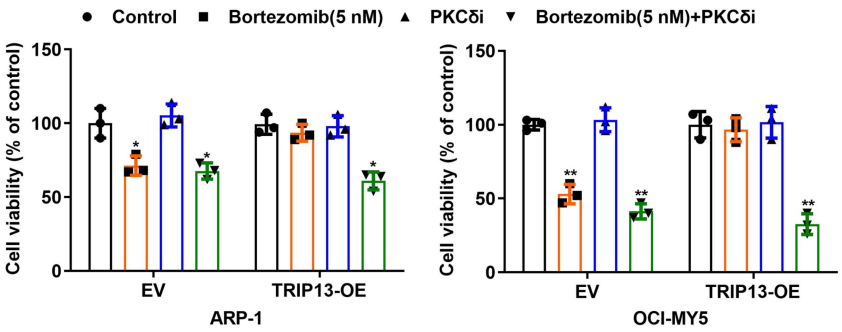
A

Protein IDs	Gene names	Amino acid	Sequence window	Phospho (STY) Probabilities	Score	Positions
P23396;P23396-2	RPS3	T	DHVSIVEPKDEILPTTPISEQKGGKPEPPAM	DEILPTT(0.979)PIS(0.002)EQK	132.6	221

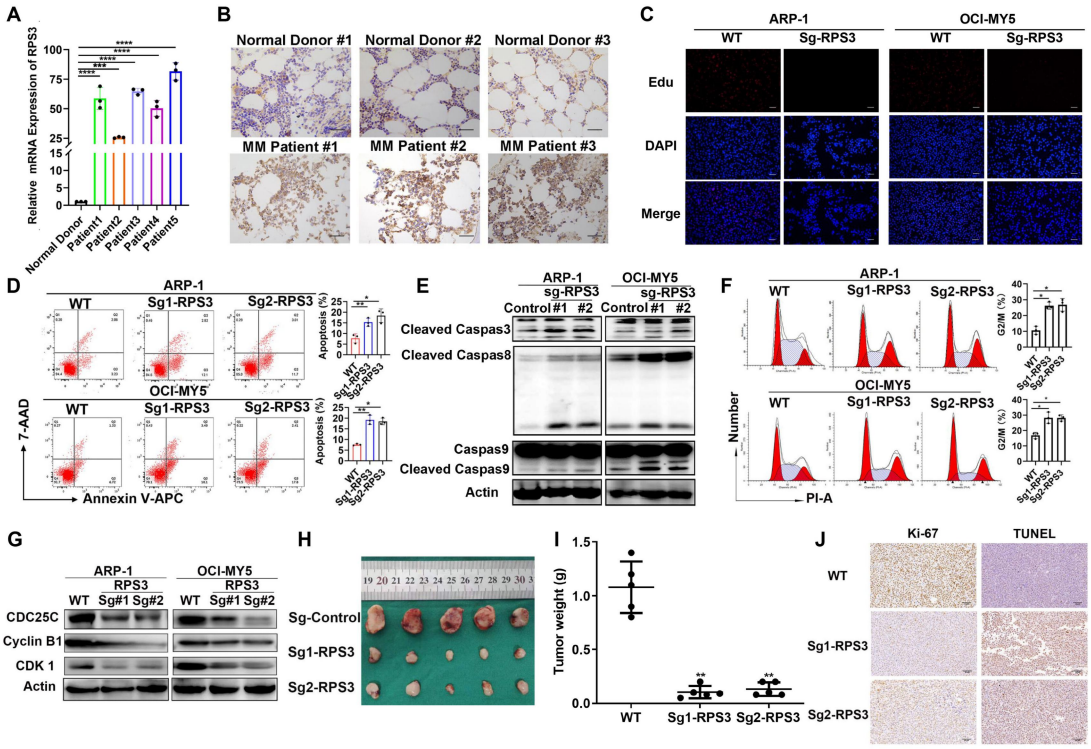
>sp|P23396|RS3_HUMAN 40S ribosomal protein S3 OS=Homo sapiens OX=9606 GN=RPS3 PE=1 SV=2
MAVQISKKRKFVADGIFKAELNEFLTRELAEDGYSGVEVRVTPTRTEIIILATRTQNVLG
EKGRRIRELTAVVQKRFGFPEGSVELYAEKVATRGLCAIAQAESLRYKLLGGLAVRRACY
GVLRFIMESGAKGCEVVVSGKLRGQRAKSMKFVDGLMIHSGDPVNYYVDTAVRHVLLRQG
VLGIKVKIMLPWDPTGKIGPKKPLPDHVSIVEPKDEILPTTPISEQKGGKPEPPAMPQPV
PTA

>sp|P23396-2|RS3_HUMAN Isoform 2 of 40S ribosomal protein S3 OS=Homo sapiens OX=9606 GN=RPS3
MAVQISKKRKFVADGIFKAELNEFLTRELAEDGYSGVEVRVTPTRTEIIILATRTQNVLG
EKGRRIRELTAVVQKRFGFPEGSVELKIMVMVTGYPLLPLKLYAEKVATRGLCAIAQAES
LRYKLLGGLAVRRACYGVLRFIMESGAKGCEVVVSGKLRGQRAKSMKFVDGLMIHSGDPV
NYYVDTAVRHVLLRQGVLGIKVKIMLPWDPTGKIGPKKPLPDHVSIVEPKDEILPTTPIS
EQKGGKPEPPAMPQPVPTA

B

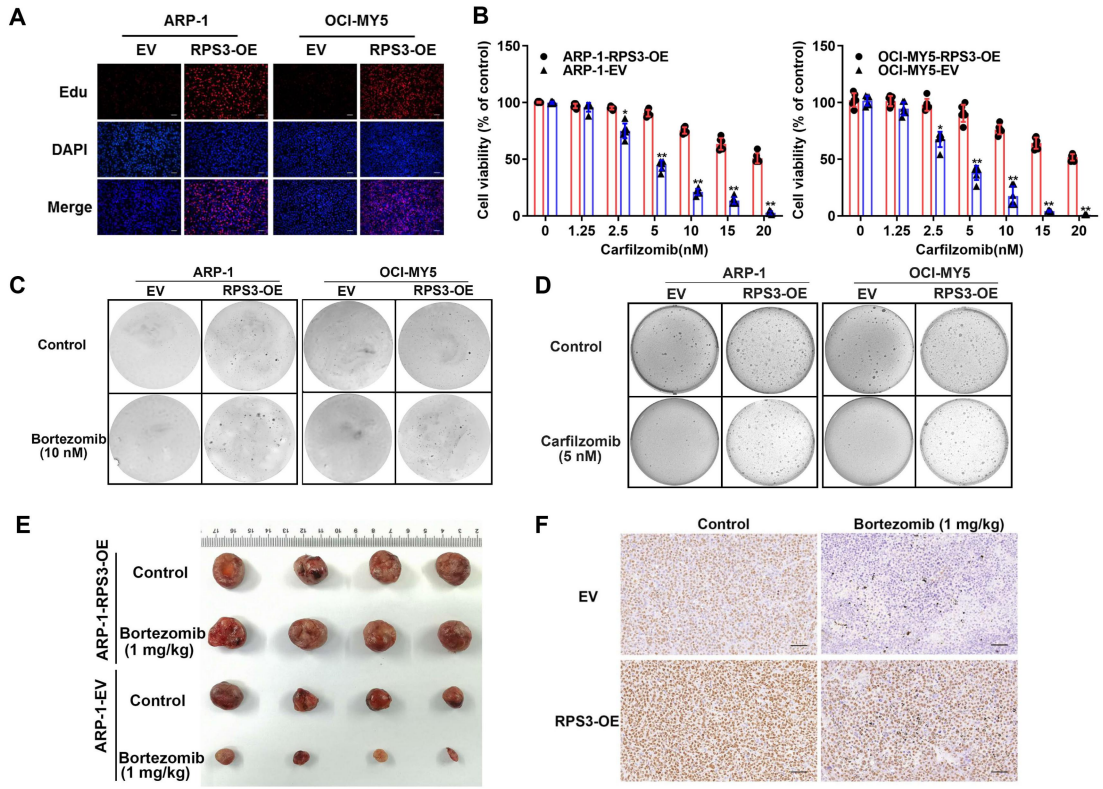


Supplemental Figure 2. TRIP13 regulates the phosphorylation of RPS3, and PKCδ involves in TRIP13-medicated drug resistance. (A) Mass spectrometry phosphoproteomic analysis identified phosphorylated threonine 221 (T221, upper panel, marked in red) on RPS3 with a score of 0.979 as a specific site that was affected by TRIP13. Mapping the site of phosphorylation on RPS3 using mass spectrometry (lower panel, marked in red). **(B)** EV and TRIP13-OE ARP-1 (left panel) and OCI-MY5 (right panel) MM cells were treated with vehicle, BJE6-106 (0.1 μM), and bortezomib (5 nM) alone or in combination for 48 h, followed by CCK-8 assay analysis of cell viability. Data are shown as mean ± SD with P value based on unpaired t-test. (n = 3, *P < 0.05, **P < 0.01). The experiments were repeated three times.



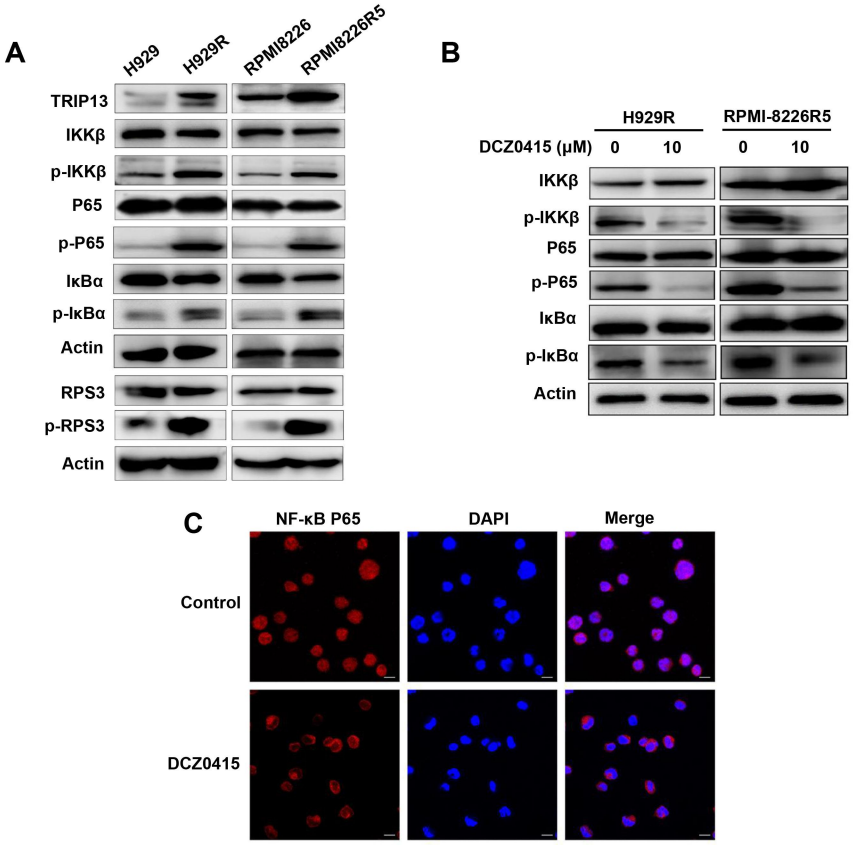
Supplementary Figure 3: RPS3 is highly expressed in MM and is required for MM survival *in vitro* and *in vivo*. (A) qRT-PCR confirmed high levels of RPS3 mRNA in patients with MM compared with that of the normal group. Data are shown as mean ± SD with P value based on unpaired t-test. (n = 3, *** P < 0.001, **** P < 0.0001). The experiments were repeated three times. **(B)** Immunohistochemistry analysis of RPS3 expression in bone marrow biopsies from patients with MM (lower panel) and normal donors (upper panel). Scale bars = 50 μM, Original magnification ×40. **(C)** EdU assay detection of cell proliferation of ARP-1/OCI-MY5 stably transfected with Sg-RPS3 or Sg-Control. Scale bars = 200 μM, Original magnification: ×20. **(D and F)** Flow cytometry detection of cell apoptosis and cell cycle of ARP-1/OCI-MY5 stably transfected with Sg-RPS3 or Sg-Control. Data are shown as mean ± SD with P value

based on unpaired t-test. (n = 3, * P < 0.05, ** P < 0.01). The experiments were repeated three times. **(E and G)** Immunoblotting analysis of apoptosis (cleaved caspase 3/8/9) and G2/M-phase-related proteins (CDC25C, Cyclin B1 and CDK1) expression in ARP-1/OCI-MY5 transfected with Sg-RPS3 or Sg-Control. Actin served as a loading control. **(H - J)** ARP-1 cells transfected with Sg-RPS3 or Sg-Control were subcutaneously injected into nude mice. **(H)** Photographs of tumours in nude mice. **(I)** Weight of tumour. Data are shown as mean ± SD with P value based on unpaired t-test. (n = 5, ** P < 0.01). **(J)** Tumour tissues were stained with Ki-67 (left panel) or TUNEL (right panel). Scale bars = 50 μM, Original magnification: ×40.



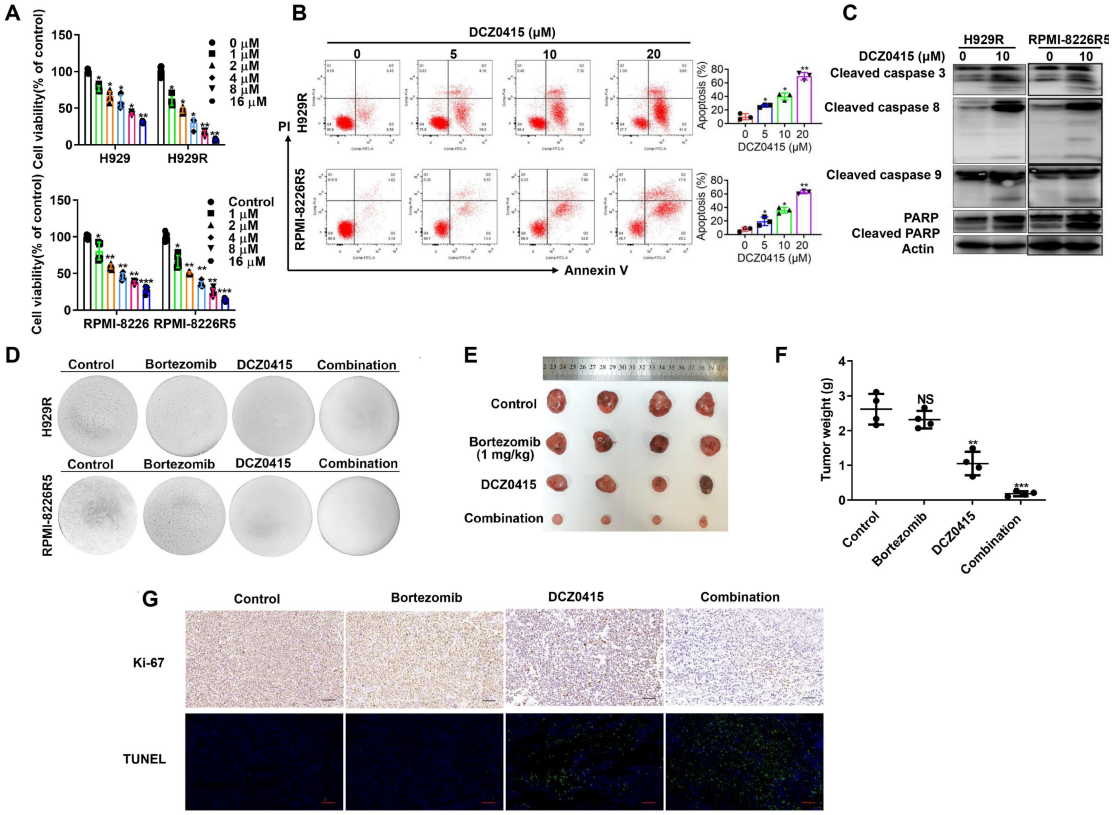
Supplementary Figure 4: RPS3 overexpression induces proteasome

inhibitor resistance of MM *in vitro* and *in vivo*. (A) EdU assay detection of cell proliferation of ARP-1/OCI-MY5 stably transfected with RPS3-OE or EV. Scale bars = 200 μM, Original magnification: ×20. **(B)** CCK-8 assay examined cell viability of RPS3-OE or EV MM cells treated with increasing doses of carfizomib (0–20 nM) for 48 h. Data are shown as mean ± SD with P value based on unpaired t-test. (n = 6, **P < 0.01). The experiments were repeated three times. **(C and D)** Colony formation of RPS3-OE and EV ARP-1 (left panel) and OCI-MY5 (right panel) MM cells treated with or without bortezomib (10 nM) or carfizomib (5 nM). **(E and F)** RPS3-OE and EV ARP-1 cells implanted subcutaneously in nude mice were treated with bortezomib (1 mg/kg, twice/week) or vehicle. **(E)** Photographs of tumours in nude mice. **(F)** Tumour tissues were stained with Ki-67. Scale bars = 50 μM, Original magnification: ×40.



Supplementary Figure 5: DCZ0415 inactivates NF-κB signaling. (A) Immunoblotting detection of proteins involved in canonical NF-κB signalling (IKKβ, p-IKKβ, P65, p-P65, IκBα, and p-IκBα) in H929/H929R (left panel) and RPMI-8226/RPMI-8226R5 (right panel) cells. Actin served as a loading control. **(B)** H929R (left panel) and RPMI-8226R5 (right panel) cells were treated with vehicle or DCZ0415 (10 μM) for 72 h. Immunoblotting detection of proteins involved in canonical NF-κB signalling (IKKβ, IKKβ, P65, p-P65, IκBα, and p-IκBα). Actin served as a loading control. **(C)** Confocal micrographs of H929R cells treated with vehicle (upper panel) or DCZ0415 (10 μM, lower panel) for 72 h. The fixed cells were stained with anti-NF-κB p65 (red) and the nuclear

dye DAPI (blue). Scale bars = 10 μM.



Supplementary Figure 6: DCZ0415 treatment re-sensitizes Bortezomib-resistant MM cells *in vitro* and *in vivo*. (A) H929/H929R and RPMI-8226/RPMI-8226R5 cells were treated with increasing doses of DCZ0415 for 72 h, followed by CCK-8 assay analysis of cell viability. Data are shown as mean ± SD with P value based on one-way ANOVA (n = 6, *P < 0.05, **P < 0.01, ***P < 0.001). The experiments were repeated three times. **(B)** H929R and RPMI-8226R5 cells were treated with increasing doses of bortezomib for 72 h, followed by flow cytometry detection of cell apoptosis. Data are shown as mean ± SD with P value based on one-way ANOVA (n = 3, *P < 0.05, **P < 0.01). The experiments were repeated three times. **(C)** H929R

and RPMI-8226R5 cells were treated with vehicle or DCZ0415 (10 μM), followed by immunoblotting analysis of relative apoptosis proteins (cleaved caspase 3/8/9 and PARP). Actin served as a loading control. **(D)** Colony formation of H929R and RPMI-8226R5 cells that were treated with vehicle, bortezomib (20 nM), and DCZ0415 (10 μM) alone or in combination. **(E - G)** Nude mice bearing H929R MM tumours were treated with vehicle, bortezomib (1 mg/kg, twice/week), and DCZ0415 (50 mg/kg, twice/week) alone or in combination. **(E)** Photographs of tumours in nude mice. **(F)** Weight of tumour. Data are shown as mean ± SD with P value based on unpaired t-test. (n = 4, ** $P < 0.01$, *** $P < 0.001$, NS, nonsignificant). **(G)** Tumour tissues were stained with Ki-67 or TUNEL. Scale bars = 50 μM, Original magnification × 40.

Supplementary References

1. Hu L, Li B, Chen G, et al. A novel M phase blocker, DCZ3301 enhances the sensitivity of bortezomib in resistant multiple myeloma through DNA damage and mitotic catastrophe. J Exp Clin Cancer Res. 2020;39(1):105.

2. Xie Y, Wang Y, Xu Z, et al. Preclinical validation and phase I trial of 4-hydroxysalicylanilide, targeting ribonucleotide reductase mediated dNTP synthesis in multiple myeloma. J Biomed Sci. 2022;29(1):32.

3. Chen G, Hu K, Sun H, et al. A novel phosphoramide compound, DCZ0847, displays in vitro and in vivo anti-myeloma activity, alone or in combination with bortezomib. Cancer Lett. 2020;478(45-55.

4. Wang Y, Huang J, Li B, et al. A Small-Molecule Inhibitor Targeting TRIP13 Suppresses Multiple Myeloma Progression. Cancer Res. 2020;80(3):536-548.