Genetic alterations of SENP6 in multiple myeloma disrupt genome and proteome stability, sensitizing to proteasome inhibition

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SUPPLEMENTAL FIGURES

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Frederik Herzberg[1,2*], Michael Korenkov[1,2*], Konstandina Isaakidis[1,2], Chuanbing Zang[1,2], Matthias Wirth[1,2,3,4], Stefan Müller[4,5], Ulrich Keller[1,2,4,6,7], Uta M. Demel[1,2,8]

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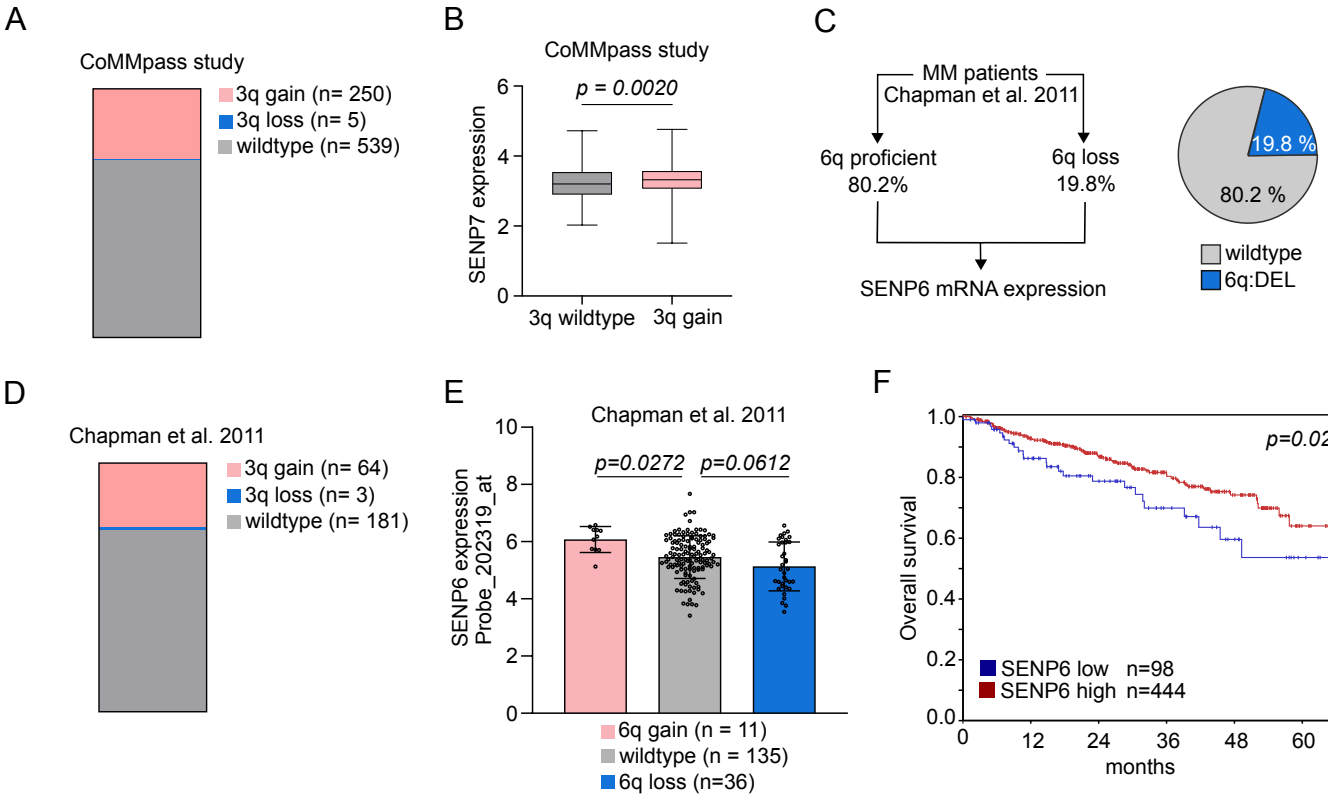

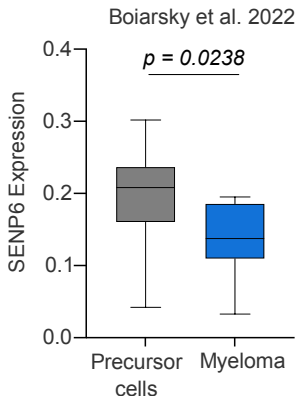

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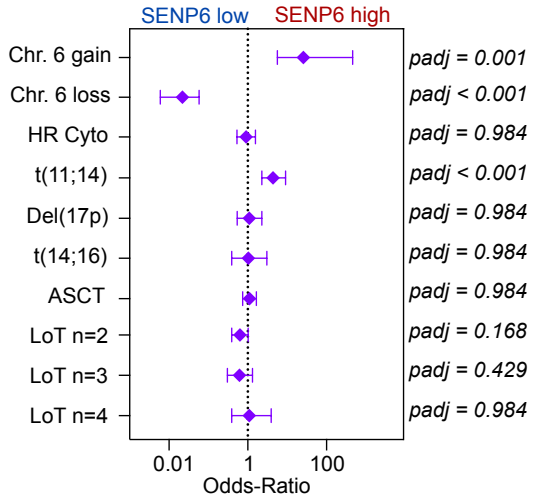
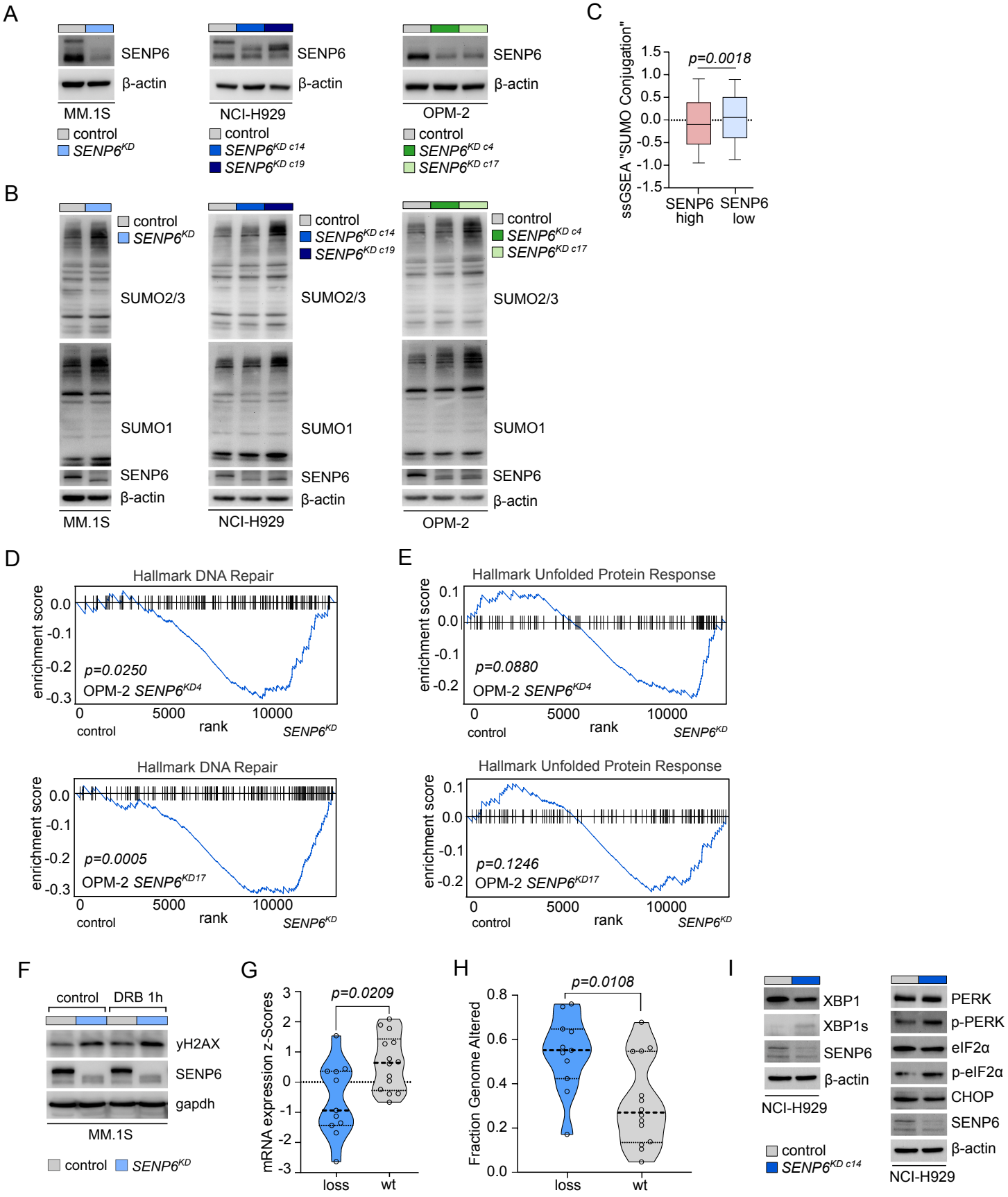
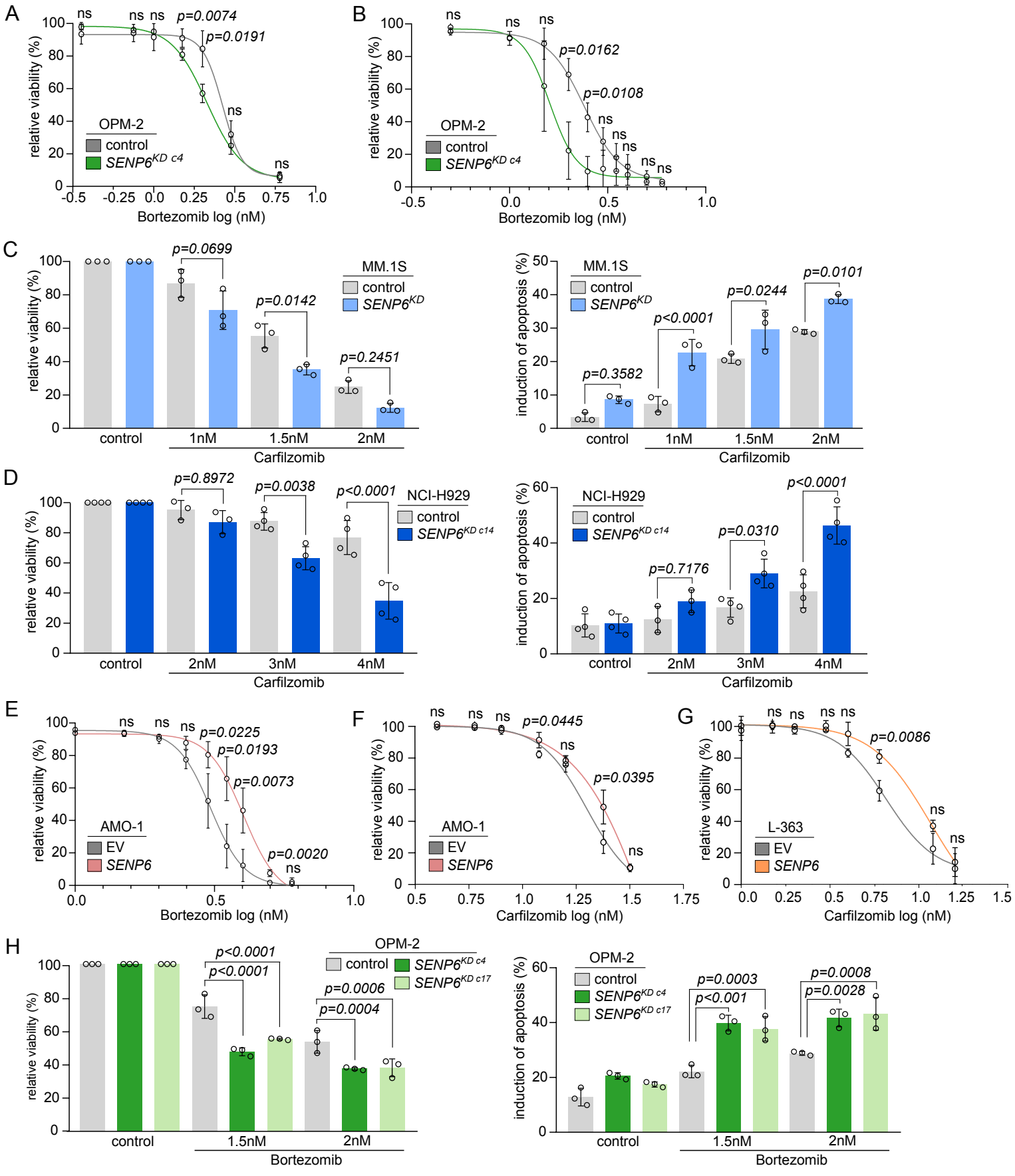
Characteristic	Overall	*SENP6* low	*SENP6* high
Chr. 6 gain	80 (10.7%)	1 (0.6%)	79 (13.9%)
Chr. 6 loss	51 (6.8%)	47 (26.7%)	4 (0.7%)
Genetic Characteristics			
High-risk cytogenetics	214 (30.2%)	65 (38.5%)	149 (27.6%)
t(11;14)	147 (20.2%)	14 (8.0%)	133 (24.1%)
Del(17p)	94 (12.6%)	30 (17.0%)	64 (11.2%)
t(14;16)	27 (3.7%)	9 (5.1%)	18 (3.3%)
Treatment Characteristics			
ASCT	441 (51.3%)	113 (52.8%)	328 (50.9%)
Lines of therapy			
n=1	574 (66.8%)	127 (59.3%)	447 (69.3%)
n=2	161 (18.7%)	59 (27.6%)	102 (15.8%)
n=3	70 (8.1%)	21 (9.8%)	49 (7.6%)
n=4	54 (6.3%)	7 (3.3%)	47 (7.3%)



Supplementary Figure S1. (A) Frequency of SENP7 (3q) copy-number alterations in patients with multiple myeloma (MM) from the CoMMpass study. **(B)** SENP7 mRNA expression stratified by copy-number status in the CoMMpass cohort. **(C)** Frequency of SENP6 (6q) copy-number alterations in MM patients from the Chapman dataset. **(D)** Frequency of SENP7 (3q) copy-number alterations in MM patients from the Chapman dataset. **(E)** SENP6 mRNA expression stratified by copy-number status in the Chapman cohort. **(F)** Kaplan-Meier overall survival of patients grouped by SENP6 expression in the Hanamura cohort (GSE2658). **(G)** SENP6 mRNA expression in pseudobulked single plasma cells from precursor (MGUS, SMM) and MM from Bojarsky et al. 2022. **(H)** Patient characteristics table of patients from CoMMpass study. MM patients were grouped by SENP6 mRNA expression levels. **(I)** Forest plot of adjusted odds ratios (95% CI) from a multivariable logistic regression of SENP6 high vs low groups including covariates from **(H)**; LoT: Line of Therapy, HR Cyto: High risk cytogenetics. BH FDR has been applied across coefficients. P-values are reported as adjusted (padj). Bar plot represent mean ± standard deviation in **(B, E)**. P values calculated by Mann Whitney U test **(B, G)**, ANOVA with Tukey's post hoc test **(E)** and log-rank Mantel-Cox test **(F)**.



Supplementary Figure S2. (A–B) Immunoblot analysis of global SUMO2/3 and SUMO1 modification in MM.1S, NCI-H929, and OPM-2 cells following CRISPR/Cas9-mediated SENP6 knockdown (KD) **(B)**; **(A)** shows SENP6 protein expression in the indicated cell lines upon SENP6KD. **(C)** ssGSEA analysis of gene set "SUMO Conjugation" of expression data derived from the GDC MMRF CoMMpass dataset. SENP6 was grouped according to mRNA expression levels as follows: SENP6 low = 1st quartile (n= 211), SENP6 high = 2nd to 4th quartile (n= 633). **(D, E)** Gene Set Enrichment Analysis (GSEA) of expression data from transcriptome profiling of OPM-2 control and SENP6KD cells for the gene sets **(D)** "DNA Repair" and **(E)** "Unfolded Protein Response". **(F)** Immunoblot analysis of DDR-related protein γH2AX in MM.1S control and SENP6KD following doxorubicin (DRB, 0.5 μM) treatment at indicated time points. **(G)** Expression analysis of SENP6 mRNA in MM cell lines listed in the cancer cell line encyclopedia (CCLE) (n=28). Groups were classified according to their SENP6 copy number status. **(H)** Analysis of somatic copy number alterations (SCNAs) in MM cell lines (n=28). Groups were classified according to their SENP6 copy number status. **(I)** Immunoblot analysis of indicated UPR markers in NCI-H929 control and SENP6KD cells. Box and whisker plots indicate minimum to maximum distribution and the mean in **(C)**. P values were calculated by Mann-Whitney U test **(C, G, H)** and by Kolmogorov-Smirnov test **(D, E)**.



Supplementary Figure S3. (A) Bortezomib dose-response curves of control and SENP6KD OPM-2 cells. Cells were treated for 72h and viability was determined by DAPI flow cytometry measurement. **(B)** Bortezomib dose-response curves of control and SENP6KD OPM-2 cells. Cells were treated for 72h and viability was determined by MTT cell viability assay. **(C, D)** Quantification of viable and apoptotic fractions in control vs. SENP6KD cells after 72 h carfilzomib treatment in MM.1S **(C)** and NCI-H929 **(D). (E)** Bortezomib dose-response curves of upon SENP6 reconstitution versus EV transduction in AMO-1 cells. Cells were treated for 72h and viability was determined by MTT cell viability assay. **(F-G)** Carfilzomib dose-response curves of upon SENP6 reconstitution versus EV transduction in AMO-1 **(F)** and L-363 **(G)** cells. Cells were treated for 72h and viability was determined by DAPI flow cytometry measurement. **(H)** Relative viability and apoptosis in control and SENP6KD OPM-2 cells after bortezomib treatment for 72h. Bar plots and dose–response curves represent mean ± standard deviation **(A-H)**. P values determined by unpaired t-test **(A, B, E-G)** or one-way ANOVA with Tukey's post hoc test **(C, D, H)**.