Supplementary Figures and Legends

**Supplementary Fig. 1: Deferentially expressed proteins in drug-resistant MM cells**

**a** List of SILAC assay-identified 33 DEP proteins in proteomics and acetylome in BR and WT MM.1S cells. **b** GO enrichment analysis shows the biological processes and cellular functions of DEP proteins in the BR MM.1S cells. **c** Motif analysis of all identified acetylated sites in SILAC assay for the BR MM.1S cells. **d** QPCR detecting the genes of HP1 family genes in WT and BR MM.1S cells (mean ± s.d.; n=3 independent experiments). **e** Quantification of the positive staining in Figure 1K, DR vs CR. *P* values were determined by Student’s *t* test (**d, e**). Source data are provided as a Source data file.
**Supplementary Fig. 2: Effects of HP1γ on MM cell apoptosis.**

(a) Western blotting shows the efficacy of three shRNAs targeting HP1γ in HEK293T cells, and (b) validates the knockdown efficacy of shRNA#2 (HP1γ KD) and overexpression efficacy of HP1γ (HP1γ OE) in MM cells. 

(c) Alteration of IC50 to BTZ treatment in the Vector and HP1γ OE cells \((n = 3)\). 

(d) Representative flow cytometry assay to detect the percentage of apoptotic cells in the Vector, HP1γ OE, non-target control (NT Ctrl) and HP1γ KD MM cells induced by 5 nM BTZ for 48 hr. 

(e) Cleavage of PARP as the apoptotic marker in MM.1S and LP-1 cells with HP1γ expression manipulated by shRNA or ectopic expression vector, treated with increasing dosage of BTZ and carfilzomib (CFZ) for 24 hr. 

(f) Cell proliferation of LP-1 and MM.1S cells stably expressing vector (Vector) or HP1γ OE (mean ± s.d.; \(n=3\) independent experiments). Differences between groups were assessed by one-way ANOVA test. Source data are provided as a Source data file.

**Supplementary Fig. 3: Acetylation modification enhances ubiquitination of HP1γ.**

(a) Degradation of HP1γ-flag protein in HEK293T transfected with HDAC1 and treated with 20 μM cycloheximide (CHX) for 12hr. 

(b) Mass spectra of enriched acetylated peptides containing K5 sites in HP1γ-flag proteins. Upper: Degree of enrichment of acetylated lysine sequences of HP1γ in Vector and HDAC1 OE groups. PSM (peptide-spectrum matches) is positively correlated with abundance. 

(c) Coomassie blue staining
for flag-pulldown in HEK293T cells transfected with vector or HP1γ-flag for 48 hr. d Western blotting shows the HP1γ protein level in the HEK293T cells transfected with flag-tagged HP1γ WT, K5R and K5Q. e Levels of HP1γ protein in WT and BR MM.1S and LP-1 cells treated with gradient concentration of Romidepsin (Rom) for 24 hr. f Western blotting shows the levels of HP1γ protein in MM.1S and LP-1 cells treated with gradient concentration of LBH589 and Rom for 24 hr. g Western blotting shows HP1γ levels in LP-1 cell treated with DMSO, MG132 (20μM) and LLOME (L-leucyl-L-leucine methyl ester, 20μM) for 24hr. Ubiquitination status of HP1γ in HEK293T cells transfected with (h) HDAC1 for 48 hr, or (i) Rom (100nM) for 24 hr. Source data are provided as a Source data file.

Supplementary Fig. 4: MDC1 recruits HP1γ and HP1γ facilitates homologous recombination. a Comet assay images showing DNA damage in WT and BR MM.1S cells treated with DMSO or CPT, and (b) in Vector and HP1γ OE MM.1S cells treated with DMSO or CPT. c Western blotting shows p-NBS1 and p-ATM levels in WT and BR, and in (d) Vector and HP1γ OE MM.1S and LP-1 cells. e Cell cycle assay of the WT, BR and (f) stably expressing Vector or HP1γ OE MM.1S and LP-1 cells. g Cleaved PARP in NT Ctrl and HP1γ KD cell treated with HR inhibitor-YU238259 for 24 hr, and (h) in MM cell treated with increasing dosage of BTZ combined with YU238259 for
Co-IP assay shows bilateral interactions between exogenous HP1γ with TOPBP1. j Levels of MDC1 and TRIM28 in the WT and BR MM.1S and LP-1 cells. k Knockdown efficacy of MDC1 KD in CAG and HEK293A cells. l Left: immunofluorescence assay demonstrating the HP1γ recruited to DNA damage regions in HEK293A cells. The focused regions of DNA damage were produced by using a scanning laser system. Right: the graph represents the relative co-localization index of the HP1γ protein on the γH2AX track. Error bars represent S.E.M., n= 3 independent experiments and differences relative to NT Ctrl were calculated using student’s t test. Source data are provided as a Source data file.

Supplementary Fig. 5: Deacetylation enhances nuclear condensation of HP1γ. a Fluorescence intensity of full length GFP-HP1γ (FL) in HEK293T cells before and after treatment with 3% 1,6-hexanediol (1,6-Hex) at different time. b Fluorescence recovery after photobleaching (FRAP) assay of HP1γ-GFP in HEK293T cells (mean ± s.d.; n= 3 independent experiments). c Western blotting shows the overexpression efficacy of HDAC1 in HEK293T cells. d Representative images for HP1γ foci in HEK293T cells with or without HDAC1 OE (mean ± s.d.; n= 3 independent experiments). Quantification of puncta count is shown in images. Two-sided P values were determined by Student’s t test. e Analysis of the HP1γ protein sequence for intrinsically disordered regions using the program VL3-BA. Red dots, potential acetylated lysine sites. f Sequence alignment of CBX3 (HP1γ) -WT, K5Q, K5R and KallQ. The site of lysine
acetylation mutants indicated by red arrows. g Coomassie blue staining shows in vitro expression of GFP, (GFP-HP1γ) -WT, -K5Q, -K5R, -KallQ fusion protein, and (h) GST-GFP, (GST-GFP-HP1γ-IDR1) -WT, -K5Q, -K5R, -KallQ fusion protein. Source data are provided as a Source data file.

Supplementary Fig. 6: Enhancing nuclear condensation of HP1γ promote drug resistance to BTZ. a Visualization of turbidity associated with droplet formation in vitro for the GFP-vector control (Ctrl), (HP1γ-IDR1) -WT, K5Q, K5R or KallQ. b Microscopy images of GFP fusion proteins of (HP1γ-IDR1) -WT, -K5Q and -K5R before and after partial droplet photobleaching in vitro (mean ± s.d.; n= 3 independent experiments), and two-sided $P$-values of the comparisons between the final extent of recovery after photobleaching (i.e. 60 s) were performed using one-way ANOVA. c Turbidity (OD600) of WT, K5Q, K5R, and KallQ mutants of HP1γ-IDR1 in 200 mM NaCl and 10% PEG was measured (mean ± s.d.; n= 3 independent experiments). $P$ values were determined by Pearson’s coefficient and Student’s t test. d Quantitative assessment of HR activity in the HEK293T cells expressing vector control or K5Q-HP1γ via flow cytometry assay for the percentage of GFP+ cells among RFP+ cells (mean ± s.d.; n= 3 independent experiments). e Co-IP assay for HP1γ-WT, -K5Q or -K5R with MDC1, HDAC1 and H3K9me3 in HEK293T cells. Input, 2% whole lysate; IP, M2-flag antibody. f Alteration of IC50 to BTZ treatment in the HP1γ-WT, -K5R and -K5Q in MM.1S cells (mean ± s.d.; n= 3 independent experiments). g Cell proliferation of MM.1S cells stably expressing HP1γ-WT, -K5Q or -K5R for 72 hr. $P$ values were
determined by two-way ANOVA test (c, g) and Student’s t test (d). Source data are provided as a Source data file (mean ± s.d.; n= 3 independent experiments).

**Supplementary Fig. 7: Interaction with MDC1-IDR1 enhances HP1γ droplet formation in vitro.**  

**a** Analysis of the MDC1 protein sequence for intrinsically disordered regions using the program VSL2.  

**b** Coomassie blue staining to show in vitro expression of GFP and HP1γ-GFP-FL fusion protein.  

**c** Schematic illustration of strategy to construct HP1γ truncations.  

**d** Immunoprecipitation assay to show interactions of full length HP1γ (FL) or truncations with HDAC1 or MDC1 in HEK293T cells.  

**e** Coomassie blue staining to show *in vitro* expression of GST-GFP-HP1γ-FL, GST-MDC1-mC (mCherry) -IDR1 and GST-MDC1-mC-IDR1 protein, and  

**f** mC and mC-MDC1-IDR3 protein.  

**g** Visualization of turbidity associated with droplet formation *in vitro* for mC and mC-MDC1-IDR3 protein.  

**h** Representative images for HP1γ and MDC1 foci in WT and BR MM cells, and (i) MM cells treated with or without Rom, and (j) CPT. Quantification of puncta count or relative puncta FI (fluorescence intensities) in 30 cells are shown in images. Two-sided *P* calculated using Student’s t test; mean ± s.d.; n= 3 independent experiments. Source data are provided as a Source data file.
Supplementary Fig. 8: HP1 promotes transcription of resistance genes. a Venn diagram shows the number of overlapped genes between WT and BR cells in HPIγ-ChIP seq. b KEGG analysis for differentially expressed genes with a $P < 0.05$ using DAVID methods. c QPCR shows expression of selected genes which overlapped between ChIP seq and RNA seq in WT and BR MM cells, and (d) FOS, JUN and CD40 genes expression in NT Ctrl and HP1γ KD cells treated with DMSO or Rom, mean ± s.d.; n= 3 independent experiments, and $P$ values were determined by Student’s t test. Source data are provided as a Source data file.
**Supplementary Fig. 9: HP1γ facilitates chromatin accessibility of genes governing drug sensitivity.** a Gene tracks showing representative ChIP-Seq profiles for the indicated proteins and histone marks at the CD40, and (b) JUN gene loci. ChIP-qPCR assay to show enrichment of HP1γ, H3K9me3 and H3K36me3 at gene loci of (c) JUN, and (e) CD40 in the Vector and HP1γ overexpressing LP-1 cells, (d) JUN, and (f) CD40 in WT and BR LP-1 cells (mean ± s.d.; n= 3 independent experiments). g Alteration of IC50 to BTZ treatment in the NT Ctrl, CD40 KD, JUN KD and FOS KD LP-1 cells (n = 3), and (h) in the Vector, CD40 OE, JUN OE and FOS OE LP-1 cells with HP1γ KD stably (mean ± s.d.; n= 3 independent experiments). i Correlation of FOS, JUN and CD40 expression with progression free survival (PFS), (j) overall survival (OS) in patients after receiving BTZ-based treatment regimens from the coMMpass cohort. P values were determined by Pearson’s coefficient and log-rank test. Source data are provided as a Source data file.

**Supplementary Fig. 10: Flow cytometry gating schemes.** a Gating scheme used for analysis of apoptosis. First debris were excluded (FSC-A vs SSC-A), and apoptosis cells were assessed for Annexin V and PI expression, then cells were separated into viability (Annexin V- PI-), early apoptosis (Annexin V+ PI-) and late apoptosis (Annexin V+ PI+). Gating is related to Fig. 1m, Fig. 8a and Supplementary Fig. 2d.
### Supplementary Tables

| Antibodies                                      | Source                          | Cat. No. |
|------------------------------------------------|---------------------------------|----------|
| Anti-rabbit HP1γ                                | Cell signaling technology       | 2619     |
| Anti-rabbit Acetylated-Lysine                   | Cell signaling technology       | 9441     |
| Anti-rabbit CBX3                                | Abclonal                        | A2248    |
| Anti-mouse HP1γ,clone 42s2                      | millipore                       | 05-690   |
| Anti-rabbit GFP (D5.1)                          | Cell signaling technology       | 2956     |
| Anti-rabbit HDAC1                               | Abclonal                        | A0238    |
| Anti-rabbit HDAC2                               | Abclonal                        | A2084    |
| Anti-mouse phospho-Histone H2A.X                | millipore                       | 05-636   |
| Anti-mouse MDC1                                 | millipore                       | 05-1572  |
| Anti-PARP                                       | Cell signaling technology       | 9532     |
| Anti-rabbit β-actin                             | Abclonal                        | AC006    |
| Anti-mouse Ub                                  | Cell signaling technology       | 3936     |
| Anti-Histone H3 (tri methyl K9) antibody-ChIP Grade | Cell signaling technology     | 13969    |
| Anti-H3K36me3 antibody [D5A7] - ChIP Grade     | Cell signaling technology       | 4909     |
| Anti-rabbit HA-Tag(C29F4)                       | Cell signaling technology       | 3724     |
| Anti-rabbit GFP-Tag pAb                        | Abclonal                        | AE011    |
| Anti-mouse GST-Tag mAb                          | Abclonal                        | AE001    |
| Anti-rabbit KAP1/TRIM28 pAb                     | Abclonal                        | A2245    |
| Anti-rabbit Phospho-p95/NBS1 (Ser343)           | Cell signaling technology       | 3001     |
| Anti-rabbit Phospho-ATM (Ser1981) (D6H9) mAb    | Cell signaling technology       | 5883     |
| Anti-rabbit GAPDH pAb                           | Abclonal                        | AC001    |
| Anti-rabbit Histone H3 (D1H2) XP mAb            | Cell signaling technology       | 4499     |
| Goat Anti-Rabbit IgG- HRP                      | Sigma-Aldrich                   | A0545    |
| ANTI-FLAG® M2-Peroxidase                        | Sigma-Aldrich                   | A8592    |
| Rabbit Anti Mouse IgG- HRP                      | Sigma-Aldrich                   | A9044-2ML|
| Alexa Fluor® 555 donkey anti-rabbit IgG (H+L)   | Invitrogen                      | A31572   |
| Alexa Fluor® 488 donkey anti-rabbit IgG (H+L)   | Invitrogen                      | A11034   |
| Anti-rabbit IgG                                 | Proteintech                     | 30000-0-AP|
| Anti-mouse IgG                                  | Proteintech                     | B900620  |

### Chemicals, Peptides and Recombinant Proteins

| Product                          | Source          | Cat. No. |
|----------------------------------|-----------------|----------|
| 3FLAG peptide                   | Sigma-Aldrich   | F4799    |
| FLAG Peptide                    | Sigma-Aldrich   | F3290    |
### Drugs

| Name                  | Supplier                | Code   |
|-----------------------|-------------------------|--------|
| Bortezomib (PS-341)   | SelleckChem             | S1013  |
| Romidepsin            | MedChemExpress          | HY-15149 |
| Puromycin 2HCL        | SelleckChem             | S7417  |
| Cycloheximide         | Sigma-Aldrich           | C7698  |
| Camptothecin          | Gift from Dr. Lei Shi, Tianjin Medical University |

### Enzymes

| Name                        | Supplier                | Code   |
|-----------------------------|-------------------------|--------|
| RNase A, DNase and protease-free | Thermo Fisher          | EN0531 |
| Proteinase K Solution, ChIP grade | Thermo Fisher         | 26160  |
| Benzonase Nuclease          | Sigma-Aldrich           | E1014-25KU |
| NotI-HF                     | NewEngland Biolabs      | R3189S |
| BamHI                       | NewEngland Biolabs      | R0136S |
| EcoRI                       | NewEngland Biolabs      | R0101S |
| Kpnl-HF                     | NewEngland Biolabs      | R3142S |
| XhoI                        | NewEngland Biolabs      | R0146S |
| AgeI                        | NewEngland Biolabs      | R3552S |
| CutSmart Buffer             | NewEngland Biolabs      | 137204S |
| T4 DNA Ligase               | NewEngland Biolabs      | M0202S |
| 10×Buffer for T4 DNA ligase | NewEngland Biolabs      | B0202S |
| Multiscribe Reverse Transcriptase | ABI                  | 4308228 |
| dNTP mix                    | ABI                     | 362275 |

### Plasmids

| Name                        | Supplier                | Code   |
|-----------------------------|-------------------------|--------|
| hU6-MSC-Ubiquitin-eGFP      | Shanghai genechem       | GV298  |
| CBX3-shRNA1                 | Shanghai genechem       | PSC57295-1 |
| CBX3-shRNA2                 | Shanghai genechem       | PSC57297-1 |
| CBX3-shRNA3                 | Shanghai genechem       | PSC57296-1 |
| pLKO.1                      |                         |        |
| HDAC1-flag                  | Gift from Dr. Dr. Robert Orlowski, UT MD Anderson Cancer Center |
| HR-GFP receptor             | Gift from Dr. Jun Li, Peking Union Medical College |
| NHEJ-GFP receptor           | Gift from Dr. Jun Li, Peking Union Medical College |
| pcDNA-3×FALG                | Gift from Dr. Michael Naksi lab, UT Health Science Center at San Antonio |
| pITA                        | Gift from Dr. Yupeng Chen, Tianjin Medical University |
| psPAX2                      | Gift from Dr. Xudong Wu, Tianjin Medical University, Dept. Cell Biology |
| pMD2.2.G                    | Gift from Dr. Xudong Wu, Tianjin Medical University, Dept. Cell Biology |
| pEGFP-C1                    | Gift from Dr. Yupeng Chen, Tianjin Medical University |
| pGEX-6p-1-GFP-A206K         | Gift from Dr. Yupeng Chen, Tianjin Medical University |
| pITA insert-CBX3-N-FLAG     | Self-construction       |        |
| pITA insert-CBX3-C-FLAG     | Self-construction       |        |
| pEGFP-C1-CBX3-WT            | Self-construction       |        |
| Vector | Self-construction |
|--------|------------------|
| pEGFP-C1-CBX3-K5Q |  |
| pEGFP-C1-CBX3-K5R |  |
| pGEX-5X-3-CBX3 |  |
| pGEX-6p-1-GFP-A206K-CBX3-IDR1-WT |  |
| pGEX-6p-1-GFP-A206K-CBX3-IDR1-K5Q |  |
| pGEX-6p-1-GFP-A206K-CBX3-IDR1-K5R |  |
| pGEX-6p-1-GFP-A206K-CBX3-IDR1-KAllQ |  |
| MDC1 shRNA1 |  |
| MDC1 shRNA2 |  |

**Critical Commercial Assays**

- EvaGreen 2X qPCR MasterMix: ABI, MasterMix-R
- 5×All-In-One RT MasterMix: abm, G490
- Pierce BCA Protein Assay Kit: Thermo SCIENTIFIC, 23225
- AxyPrep DNA Extraction Kit: AXYGEN, 295 AP-GX-250G
- AxyPrep Plasmid Miniprep Kit: AXYGEN, 183 AP-MN-P-250G
- Plasmid Maxi Kit (25): QIAGEN, 12163
- EnVision G12 Doublestain System, Rabbit/Mouse (DAB+/Permanet Red): Dako, K5361
- SuperSignal West Dura Extended Duration Substrate: ThermoFisher, 34580
- 9002 SimpleCHIP® Kit: Cell Signaling, 22188S
- Simple CHIP® Kits-20C-Reagents: Cell Signaling, 45061S
- ChIP-grade Protein A/G Magnetic Beads: Thermo SCIENTIFIC, 26162
- ANTI-FLAG M2 Affinity Gel: Sigma-Aldrich, A2220
- CellTiter 96 Aqueous One Solution: Promega, G358B
- NuPAGE 4-12% Bis-Tris Gel: Invitrogen, NP0335BOX
- Phosphatase Inhibitor Cocktail (100×): Cell Signaling, 5870S
- Annexin V-FITC Apoptosis Kit: Sigma-Aldrich, APOAF-50TST
- Pierce® Protein G Plus Agarose: Thermo Scientific, 22852
- Human CD20 MicroBeads: Miltenyi Biotec, MB17-R0829
- Human CD138 MicroBeads: Miltenyi Biotec, 130-105-961
- DeadEnd™ Fluorometric TUNEL System: Promaga, G3250
- Ficoll-Paque PLUS endotoxin tested: GE Healthcare, 17-1440-02
- LS Columns (25 columns): Miltenyi Biotec, 130-042-401
- TRIzol Reagent: Ambion, Life Science, 15596018
- Opti-MEM®I (1×) Reduced Serum: Gibco, Life Technologies, 31985-070
| Protein/Ladder Kit                                      | Brand                | Catalogue |
|--------------------------------------------------------|----------------------|-----------|
| Cell Cycle and Apoptosis Analysis Kit                   | Beyotime             | C1052     |
| Opti-protein XL Marker                                  | ABM                  | G266      |
| PageRuler Prestained protein Ladder                     | ThermoFisher Scientific | 26616     |
| 1Kb Ladder DNA Marker                                   | Biomed               | MD114     |
| 1Kb DNA Ladder                                         | TIANCEN              | MD111     |
| 100bp DNA Ladder                                       | TRANS                | BM301     |
| BM15000 DNA Marker                                     | Biomed               | MD106     |
| 1Kb Plus DNA Ladder                                     | Solarbio             | M1500     |
| PEI-Transferrinfection Kit                             | ThermoFisher Scientific | BMS1003   |

**Primer sequences**

| Human GAPDH-F                                           | TTGCCCTCAACGACCACACTTT |
| Human GAPDH-R                                          | TGGTCCAGGGGCTCTTTCTCC |
| Human CBX3-F                                            | TGCTGCTGACAAACCAAGAG  |
| Human CBX3-R                                           | CACAAAGTCTGCATCTCTG   |
| Human CBX5-F                                            | GCACACAGATTCTGTGCTG   |
| Human CBX5-R                                           | GATGCATATGCTACCTTC    |
| Human CBX1-F                                            | AAGGGAGAGGAGGACGACC   |
| Human CBX1-R                                           | TTGACATTGGCTCTTTGCG   |
| Mouse Fos-F                                            | TGTGTTCCTGGCAATAGGT   |
| Mouse Fos-R                                            | ACCACCTGACAATGCATGA   |
| Mouse Jun-F                                            | GGGAGCTTGGAGAGCTTC    |
| Mouse Jun-R                                            | TTTGCAAAAAGTTCCTCAG   |
| Mouse Cd40-F                                           | TGTGTTGAGCCTTCTTCCT   |
| Mouse Cd40-R                                           | CACATGCTTCGCAATCTTTG  |
| Human FOS-F                                            | GGGGCAAGGTTGGAACGTTAT |
| Human FOS-R                                            | AGGTGGCAATCTGGTCTG    |
| Human JUN-F                                            | GCCAGGTGCGCATAGTC     |
| Human JUN-R                                            | GAACTGCTGCACTTCCTCC   |
| Human CD40-F                                           | ACTGATGTGGCTGCTGGC    |
| Human CD40-R                                           | GGGGCCACCTTTTTTGATAAGGACG |
| FOS-A-F                                                | GCGGGGGCGGGCGGGGAGGCC |
| FOS-A-R                                                | CACCTTCCACTCTGCGGCG  |
| FOS-B-F                                                | ACAACCACTGCCACTCCAGGCT |
| FOS-B-R                                                | GCCTACGTAATGCTGCGC   |
| JUN-A-F                                                | GCAACTGCGCAGCGGATGGAG |
| JUN-A-R                                                | GGGGGCAATTTATAGGGG    |
| JUN-B-F                                                | GCAACTGCGCAGCGGATGGAG |
| JUN-B-R                                                | GGGGCCAGTTTTTATAGGGG  |
| CD40-A-F                                               | TTCCATCTCCCTGATCTGAGGTT |
| CD40-A-R                                               | CTCCACGTTGGCGGAGCTGCCTCA |
| CD40-B-F                                               | GGGGAGAAATCTCTCATCAT |
| CD40-B-R                                               | CTTTCTACCCCTTCAAGGTC  |
| pITA-CBX3-N-flag-KpnI-F                                 | CGGGGTACCATGGATTACAAGGATGACGAGCA |
| DNA Sequence | Description |
|-------------|-------------|
| TAAGGCCTCCAACAAAACTAC | pITA-CBX3-N-flag-BamHI-R |
| CGCGGATCTTTATTTGAGCTTCATCTTCTTG | pITA-CBX3-C-flag-KpnI-F |
| CGCGGATCTTTATTTGAGCTTCATCTTCTTG | pGEX-5X-3-CBX3-BamHI-F |
| CGGAGGATCTTTATTTGAGCTTCATCTTCTTG | pGEX-5X-3-CBX3-K5Q-R |
| CGGAGGATCTTTATTTGAGCTTCATCTTCTTG | pGEX-6p-1-GFP-A206K-CBX3-IDR1-KAllQ-M |
| TTTTTTGCAATGTAGTCCTGG | Human MDC1-shRNA1-F |
| TTTTTTGCAATGTAGTCCTGG | Human MDC1-shRNA3-F |
| AATCTACACTACAGGT | Human MDC1-shRNA1-R |
| AATCTACACTACAGGT | Human MDC1-shRNA3-R |