**Supplementary methods and materials**

**Quantitative RT-PCR**

Total RNA was extracted using RNeasy mini kit (Qiagen), and 1 μg aliquots were used for cDNA synthesis using the qScript™ cDNA Synthesis Kit (Quanta Biosciences). The cDNA templates were subjected to PCR amplification on CFX96 qPCR System (Biorad). Expression of each gene was normalized to GAPDH, and quantified using 2−delta(Ct) method. Primers used for quantitative RT-PCR are as follows:

|  |  |  |
| --- | --- | --- |
|  | Fwd | Rev |
| GAPDH | GTCTCCTCTGACTTCAACAGCG | ACCACCCTGTTGCTGTAGCCAA |
| HJURP | CAGACCAGGAAGAGTCAGTTGC | CTTCCAGCTCTGTTACCTGCAC |
| NSD2 | GAGAAGGACAGTTTGAAAAATTATGC | CCCACATAGAGAAAGGTGAACTTG |

## **Immunoblotting assay**

Cells were lysed in RIPA lysis and extraction buffer (Thermo Fisher Scientific), supplemented with proteinase inhibitor cocktail and phosphatase inhibitor cocktail (Roche) for 30 min on ice. Protein quantification was determined by the Bradford assay. Immunoblotting was performed using SDS-PAGE followed by protein transfer to PVDF membrane (Bio-Rad). Primary antibodies were incubated overnight in cold room. Secondary antibodies were incubated for 1 h at room temperature. The following antibodies were used: GAPDH (1: 500, SANTA CRUZ BIOTECHNOLOGY, sc-47724); β-Actin (1:1000, Cell Signaling Technology, #4970); HJURP: (1:1000, Sigma Aldrich, HPA008436); PARP (1:1000, Cell Signaling Technology, #9542); Cleaved caspase-3 (1:500, Cell Signaling Technology, #9664); Cleaved caspase-7 (1:500, Cell Signaling Technology, #8438); Cleaved caspase-9 (1:1000, Cell Signaling Technology, #20750); NSD2 (1:1000, Abcam, ab75359); BRD4 (1:1000, Abcam, ab75359); CAS9 (1:1000, Cell Signaling Technology, #14697). The signals were detected with SuperSignal reagents (Thermo Fisher Scientific) on an ImageQuant LAS500 (GEHealthcare).

**shRNA knockdown and transfection.**

The two-independent NSD2 shRNA vectors and pLkO.1 scrambled shRNA were purchased from Sigma-Aldrich - Mission TRC shRNA library. Cells were transfected with shRNA targeting HJURP or a scramble shRNA control using the Neon Transfection System (Life Technology) according to manufacturer’s protocol (KMS11: 1050V, 30 ms, 1 pulse; H929: 1150V, 10 ms, 5 pulse). Cells were harvested at 72 hours post-transfection for protein extraction.

**Luciferase reporter assay**

Luciferase reporter assays were performed as previously described. Briefly, the pGL3-enhancer Firefly luciferase reporter constructs were co-transfected using lipofectamine 2000 reagent (Life technologies, USA) with the vector phRG-TK (Promega, USA) which expresses synthetic Renilla luciferase to normalize the transfection efficiency. Luciferase activities were measured using the Dual-Luciferase Reporter Assay reagent (Promega) on a LB 960 Centro XS3 luminometer (Berthold Technologies, Germany). Relative luciferase activities were expressed as ratios between Firefly and Renilla luciferase activities.

## **Flow cytometry for cell-cycle and cell apoptosis analysis**

Cell death was evaluated by the loss of membrane integrity (high PI fluorescence) after treatment with PI solution. Phosphatidylserine exposure was determined using Annexin V-FITC/PI double staining kit (BD Biosciences, USA) and analyzed by flow cytometry. Analysis of stained cells can distinguish cells into four groups, namely viable (annexin V− PI−), early apoptotic (annexin V+ PI−), late apoptotic (annexin V+PI+) and necrotic (annexin V− PI+) cells. Data were analyzed using FlowJo 7.6 software (Tree Star). Cells were harvested and washed twice with PBS for cell-cycle analysis, and the Cell Cycle Staining Kit (MULTISCIENCES, CCS012) was used. Cell apoptosis and cell-cycle analyses were performed by flow cytometry (Accuri model C6).

**Immunofluorescence assay**

HMCLs (H929 and U266) were cultured in 1640 medium and plated in 6-well plates at a density of 1 × 105 cells per well; then, 2–4 glass coverslips were placed in each of the wells. The plates were incubated for 24 to 48 hours until reaching 30–40% confluence before fixation; they were then fixed with 4% paraformaldehyde for 10 min and permeabilized with 0.1% Triton X-100 in PBS. The slides were washed three times with PBS and blocked with 5% BSA in PBS for 30 min at room temperature. The cells were incubated with the HJURP (1:200, Sigma Aldrich, HPA008436) or CD138 antibody (1:200; Abcam; ab128936) overnight at 4°C, which was followed by incubation with a fluorescein isothiocyanate (FITC)-conjugated anti-rabbit secondary antibody (1:100; Thermo Scientific) for 30 min in the dark. Nuclei were counterstained with DAPI for 2-3min and then analyzed using a fluorescent microscope.

**Chromatin immunoprecipitation (ChIP) assay coupled with quantitative PCR**

ChIP assays were performed using reagents obtained from Cell Signaling Technology (SimpleChIP® Enzymatic Chromatin IP Kit (Magnetic Beads) #9003). Chromatin was used for immunoprecipitation with anti-NSD2(ab75359, Abcam); anti-BRD4 (ab243862, Abcam), anti-H3K27ac (ab4729, Abcam), anti-H3K27me3 (07-449 Millipore), anti-H3K36me2 (07-369, Millipore) and normal rabbit-IgG antibody as negative control. ChIP-enriched DNA was measured using real-time PCR, and the primer sets were designed as follows:

|  |  |  |
| --- | --- | --- |
|  | Fwd | Rev |
| HJURP Promoter-1 (P1) | GCAACCCAGAACTGCTTGTG | ATGACAGAACACCGGCCTTT |
| HJURP Promoter-2 (P2) | ATTTTTCATTCCCGCCTGCC | TCCCAATGTAAGACGCAGGG |
| HJURP SE-1 (E1) |

|  |
| --- |
| GGACTCACGCACAGGCTATT |

 | GTGGTCTGGTGGCTGAGTAT |
| HJURP SE-2 (E2) | CAGACCAGCATTTCATCGCC | ACCAAACCACTCCTTCTATGGC |
| HJURP SE-3 (E3) |

|  |
| --- |
| AACTCCTTCTGCTTGACGGG |

 | TTGACGTTGGGACAGAAGGG |

**Detail information of public myeloma datasets used in this study**

HMCL (aCGH & GEP): Supplementary Information of Keats JJ, Fonseca R, Chesi M, Schop R, Baker A, et al. (2007) Promiscuous Mutations Activate the Noncanonical NF-kB Pathway in Multiple Myeloma. Cancer Cell 12: 131–144.

Mayo (GEP, GSE6477): Chng WJ, Kumar S, Vanwier S, Ahmann G et al. Molecular dissection of hyperdiploid multiple myeloma by gene expression profiling. Cancer Res 2007 Apr 1;67(7):2982-9.

UAMS (GEP, GSE2658): Zhan F, Huang Y, Colla S, Stewart JP, Hanamura I, et al. (2006) The molecular classification of multiple myeloma. Blood 108: 2020–2028.

APEX (GEP, GSE9782): Mulligan G, Mitsiades C, Bryant B, Zhan F, Chng W-J, et al. (2007) Gene expression profiling and correlation with outcome in clinical trials of the proteasome inhibitor bortezomib. Blood 109: 3177–3188.

HOVON (GEP, GSE19784): Broyl A, Hose D, Lokhorst H, de Knegt Y, Peeters J, et al. (2010) Gene expression profiling for molecular classification of multiple myeloma in newly diagnosed patients. Blood 116: 2543–2553.

MMRC (aCGH: GSE26849; GEP: GSE26760) :Chapman MA, Lawrence MS, Keats JJ, Cibulskis K et al. Initial genome sequencing and analysis of multiple myeloma. Nature 2011 Mar 24;471(7339):467-72.

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