**SUPPLEMENTARY MATERIALS AND METHODS**

**Primers and Taqman probes**

Primers used in this study are listed below.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | **Gene ID** | **Reference** | **Application** | **Supplier** |
|  | BAIAP2L1 | Hs00989192\_m1 | qRT-PCR | Life technologies |
|  | CDH1 | Hs01023895\_m1 | qRT-PCR | Life technologies |
|  | DOCK9 | Hs00324508\_m1 | qRT-PCR | Life technologies |
|  | EMP1 | Hs00608055\_m1 | qRT-PCR | Life technologies |
|  | ENPP1 | Hs01054040\_m1 | qRT-PCR | Life technologies |
|  | EPHB2 | Hs00362096\_m1 | qRT-PCR | Life technologies |
|  | GAPDH | 4326317E | qRT-PCR | Life technologies |
|  | IGF2BP1 | Hs00198023\_m1 | qRT-PCR | Life technologies |
|  | MBOAT2 | Hs00294102\_m1 | qRT-PCR | Life technologies |
|  | MMSET | Hs00324599\_m1 | qRT-PCR | Life technologies |
|  | RCSD1 | Hs00364590\_m1 | qRT-PCR | Life technologies |
|  | SLFN5 | Hs00288058\_m1 | qRT-PCR | Life technologies |
|  | TNFRSF1B | Hs00961749\_m1 | qRT-PCR | Life technologies |
|  | VCAN | Hs00171642\_m1 | qRT-PCR | Life technologies |
|  | CDH1 | GPH1005252(+)01A | ChIP | Qiagen |
|  | CDH1 | GPH1005252(-)05A | ChIP | Qiagen |
|  | EMP1 | GPH1002985(-)01A | ChIP | Qiagen |
|  | ENPP1 | GPH1011593(-)01A | ChIP | Qiagen |
|  | ENPP1 | GPH1011593(-)05A | ChIP | Qiagen |
|  | EPHB2 | GPH1000222(-)01A | ChIP | Qiagen |
|  | GAPDH | GPH1002905(-)01A | ChIP | Qiagen |
|  | VCAN | GPH1010601(+)01A | ChIP | Qiagen |
|  | VCAN | GPH1010601(-)01A | ChIP | Qiagen |

**Chromatin Immunoprecipitation (ChIP)**

Cells were centrifuged and the pellet was resuspended in 1% formaldehyde in PBS for 8 minutes at room temperature. Cross-linking was quenched using 125mM glycine. Cells were washed twice in PBS and frozen. Following cell lysis and nuclei isolation, chromatin was sheared to 100-2000 bp fragments using a Covaris S220 ultrasonicator, and the total volume was split into 4 IPs. IPs were performed using automation (IPstar, Diagenode) with 20µL magnetic Protein A bead suspension and 2µg of antibodies as described in the figure legends. The IPs were processed using the wash and elution buffers listed below. Reverse cross-linking of IP elutions and inputs was done for 16h at 65°C, with additional NaCl to 160µM final concentration. Final samples after RNase treatment, Proteinase K digestion, and purification (Qiagen PCR purification kit) were analyzed using qRT-PCR, using the primers listed in the antibody and primer Tables.

Used Buffers:

Cells were lysed in cell lysis buffer (5 mM PIPES pH 8, 85 mM KCl, 0.5 % Nonidet P40) and nuclei were isolated using RIPA buffer. 2µg of antibodies anti-H3 (Abcam ab10799), anti-phosphoS5 RNA Pol II (Abcam ab5408) and rabbit IgG (Sigma), and 5µL anti-trimethyl-H3K27 (Cell Signaling #9733) were used per IP with the following buffers: bead wash (10 mM Tris-HCl pH 8, 1 mM EDTA, 0.02% Tween), wash 1 (20 mM Tris-HCl pH 8, 2 mM EDTA, 150 mM NaCl, 1 % Triton X-100, 0.1% SDS), wash 2 (20 mM Tris-HCl pH 8, 2 mM EDTA, 500 mM NaCl, 1 % Triton X-100, 0.1 % SDS), wash 3 (10 mM Tris-HCl pH 8, 1 mM EDTA, 250 mM LiCl, 1 % Nonidet P40, 1 % Sodium deoxycholate) and elute (0.1 M NaHCO3, 1 % SDS).

Primers locations are indicated in the scheme below:

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**SUPPLEMENTARY FIGURE LEGEND**

**Supplementary Figure S1: MMSET expression analysis in MM cell lines. A.** Western blot of MMSET (Abcam ab75359) showing the two MMSET isoforms I (75 kDa) and II (150kDa). Molecular weight markers are shown in lines 1 and 15, and molecular weights (kDa) were indicated in the left part of the figure. **B.** MMSET qRT-PCR expression levels relative to GAPDH tested in MM t(4;14) positive (black) and t(4;14) negative (grey) cell lines.

**Supplementary Figure S2: E7438 inhibits the proliferation of several multiple myeloma cell lines and reduces H3K27me3 levels.** **A.** Structure of E7438. **B.** Proliferation assays at days 3 (black) and 7 (grey) of seven multiple myeloma cell lines (KMS-28BM, U-266, KMS-12PE, LP-1, L-363, MM.1S and OPM-2). Fluorescence values at days 3 and 7 were expressed as a percentage of the DMSO control value and plotted against compound concentrations. The absolute IC50 was calculated by fitting a dose-response curve using GraphPad software. **C.** Cell cycle distribution detected by flow cytometry of the five cell lines that showed anti-proliferative effect treated for 7 days using different E7438 concentrations: KMS20 3 µM, KMS-28BM 15 µM, MOLP-8 5 µM, RPMI8226 2 µM and U266B1 20 µM. **D.** Apoptosis and cell death induced by E7438 (using same conditions as in C) detected by flow cytometry quantification of Annexin V-FITC and Propidium Iodide (PI) staining.**E.** Western blot of HK27me3 (Cell Signaling #9733) in seven multiple myeloma cell lines (KMS-28BM, U-266, KMS-12PE, LP-1, L-363, MM.1S and OPM-2) tested after 3 days treatment with DMSO, or 0.5 µM or 2 µM of E7438. Histone H3 (Abcam ab10799) is included as a loading control.

**Supplementary Figure S3: Treatment of KMS-11 cell line with different EZH2 inhibitors. A.** Proliferation assays at days 3 (black) and 7 (grey) of KMS-11 cell line showing the percentage of proliferation relative to DMSO control at different E7438, CPI169, GSK126 and GSK343 concentrations. **B.** Western blot of H3K27me3 (Cell Signaling #9733) in KMS-11 cell line tested after 3 days of treatment with DMSO, or 0.5 µM and 2µM E7438. Histone H3 (Abcam ab10799) is included as a loading control.

**Supplementary Figure S4: Effects of GSK126 treatment in MOLP-8 cell line. A.** qRT-PCR expression levels relative to GAPDH of 12 significantly upregulated genes (from MOLP-8 with false discovery rate (FDR) < 0.1 and fold change > 1.5) tested in the MOLP-8 cell line after 3 days of treatment with DMSO (white), 2µM E7438 (black) and 2µM GSK126 (grey). *P* values were calculated using *t*-test (\**P* ≤ 0.05 and \*\**P* ≤ 0.01) compared to DMSO. **B.** H3K27me3 (Cell Signaling #9733) and **C.** Histone H3 (Abcam ab10799) ChIP signal reported as percent of input at *CDH1, EMP1, ENPP1, EPHB2, VCAN* and *GAPDH* gene promoter regions in MOLP-8 cells after 3 days of treatment with DMSO (white) or 2µM GSK126 (black). *P* values were calculated using *t*-test (\**P* ≤ 0.05 and \*\**P* ≤ 0.01) compared to DMSO. **D.** xCelligence adherence measurement showing the cell index of adherent cells treated with DMSO (grey) or 2 µM GSK126 (black). Measurements were taken over 4 days using different numbers of starting cells (7500 and 10000).

**Supplementary Figure S5: E7438 induces local reduction of H3K27me3 in promoter regions of upregulated genes.** **A.** Enrichment of total histone H3 and IgG (background binding control) as percent of input in *CDH1, EMP1, ENPP1, EPHB2, VCAN* and *GAPDH* promoter regions.

**Supplementary Figure S6: *in vivo* inhibition of tumor growth with E7438. A**. Effect of E7438 on body weight of MOLP-8 xenograft mice treated with vehicle, 250 mg/kg or 500 mg/kg of E7438 p.o. BID for 16 days after tumor inoculation.

**Supplementary Tables S1:** Overlap of the significantly (false discovery rate (FDR) < 0.1 and fold change > 1.5) upregulated (A) and downregulated (B) probes in RPMI-8226, KMS-20, MOLP-8, KMS-28BM, U-266, KMS-11, KMS-12-PE, KMS-34, LP-1 and NCI-H929 cell lines.