PVT1 interacts with polycomb repressive complex 2 to suppress genomic regions with pro-apoptotic and tumour suppressor functions in multiple myeloma

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Abstract

Multiple myeloma is a heterogeneous hematological disease that originates from the bone marrow and is characterized by the monoclonal expansion of malignant plasma cells. Despite novel therapies, multiple myeloma remains clinically challenging. A common feature among patients with poor prognosis is the increased activity of the epigenetic silencer EZH2, which is the catalytic subunit of the PRC2. Interestingly, the recruitment of PRC2 lacks sequence specificity and, to date, the molecular mechanisms that define which genomic locations are destined for PRC2-mediated silencing remain unknown. The presence of a long non-coding RNA (lncRNA)-binding pocket on EZH2 suggests that lncRNA could potentially mediate PRC2 recruitment to specific genomic regions. Here, we coupled RNA immunoprecipitation sequencing, RNA-sequencing and chromatin immunoprecipitation-sequencing analysis of human multiple myeloma primary cells and cell lines to identify potential lncRNA partners to EZH2. We found that the lncRNA plasmacytoma variant translocation 1 (*PVT1*) directly interacts with EZH2 and is overexpressed in patients with a poor prognosis. Moreover, genes predicted to be targets of *PVT1* exhibited H3K27me3 enrichment and were associated with pro-apoptotic and tumor suppressor functions. In fact, *PVT1* inhibition independently promotes the expression of the PRC2 target genes *ZBTB7C*, *RNF144A* and *CCDC136*. Altogether, our work suggests that *PVT1* is an interacting partner in PRC2-mediated silencing of tumor suppressor and pro-apoptotic genes in multiple myeloma, making it a highly interesting potential therapeutic target.

Introduction

Multiple myeloma (MM) is a hematological malignancy characterized by aberrant monoclonal expansion of malignant plasma cells (PC) within the bone marrow.[1] Despite advances in treatment, disease management and therapeutic interventions remain challenging and non-curative. Global multi-omics analyses have revealed that MM cells exhibit complex intra-tumoral heterogeneity, have a diverse mutational landscape and undergo large scale epigenetic and metabolic reconfiguration during disease progression.[2,3] As a result, patients undergoing conventional treatment eventually develop drug resistance and relapse in disease.[4] In recent years, we and others have presented data suggesting that epigenetic regulatory mechanisms are key features in MM pathogenesis, and numerous drugs targeting epigenetic regulators have been developed and tested clinically or preclinically.[5] These drugs target epigenetic modifiers such as histone deacetylases (HDAC),[6] DNA methyltransferases (DNMT)[7] and histone metyltransferases (HMT).[8] For instance, transcriptional repression through epigenetic redistribution of histone H3 lysine 27 tri-methylation (H3K27me3), catalyzed by the polycomb repressive complex 2 (PRC2), has been reported to be a common feature of MM by us and others.[9-11] Accordingly, the catalytic component of PRC2, enhancer of zeste homolog 2 (EZH2), is overexpressed in

MM,[3] and increased methylation of H3K27 correlates with tumor progression according to the MM International Staging System (ISS).[1] We have previously demonstrated that EZH2 inhibition (EZH2i) reduces the viability of MM cells through the re-activation of microRNA (miRNA) which silence methionine cycling-associated genes and oncogenes involved in MM proliferation.[8] While the mechanisms behind enzyme-mediated epigenetic regulation of MM are in the process of being unravelled, potential regulatory mechanisms of a class of non-enzymatic epigenetic regulators, such as long non-coding RNA (lncRNA), remain largely unexplored.

Large-scale transcriptome efforts have identified an extensive number of lncRNA, that are involved in vital cellular processes for disease development such as malignant transformation, early tumor onset, chromatin reorganization, cell differentiation and gene expression modulation.[12] Recently, the first Cancer LncRNA Census was generated to effectively identify lncRNA with a putative causal role in cancers of various origin.[13] The lack of sequence specificity and the presence of a lncRNA binding pocket[14] on the enzymatic subunit EZH2 of the PRC2 complex suggests that lncRNA should be a highly interesting partner for PRC2 recruitment to specific genomic regions. One of the most interesting candidates among the list of potentially relevant lncRNA for MM is *PVT1*.[13]

In lung cancer and other diseases *PVT1* has been attributed the ability to promote transcriptional repression in a context-dependent manner by facilitating the deposition of H3K27me3 on various promoter regions through the recruitment of EZH2.[15] Moreover, *PVT1* has also been suggested to modulate gene expression patterns by stabilizing PRC2 in various cancers,[16-18] and its inhibition resulted in decreased EZH2 expression, promoted apoptosis and reduced tumor cell proliferation in a number of cancer types, including other hematological malignancies.[16,19] *PVT1* has previously been associated with relapse and drug resistance in MM[20] and its overexpression is connected to increased genomic stability in MM cells, providing enhanced protection against DNA damage.[21] In addition, *PVT1* expression can be transcriptionally activated by c-Myc binding to the PVT1 promoter.[22] Interestingly, a recent single-cell RNA sequencing (scRNA-seq)-based gene fusion analysis of immunoglobulin in MM reported immunoglobulin (Ig) loci fusion with either *MYC*, a known oncogene, or its downstream neighbor *PVT1*, resulting in gain of *MYC* expression.[23] Moreover, patients harboring PVT1-IGL translocation had worse prognosis than patients with MYC-IGL translocation.[23] However, a functional role of *PVT1* as a PRC2 collaborator has not yet been demonstrated in MM.

To date, the *PVT1*-EZH2 interaction has not been described in MM, and a comprehensive genome-wide understanding of the relationship between *PVT1* and PRC2-mediated silencing is lacking. In this study we determined that *PVT1* is overexpressed in MM patients and its expression is associated with a poor prognosis. Moreover, we determined that a physical interaction between *PVT1*-EZH2 exists in MM cells. This interaction occurs at specific gene locations and regulates genes associated with apoptosis as well as tumor suppressor genes (TSG) such as *CXCL14*, *RNF144A* and *ZBTB7C*, which are linked to oncogenic function and immune system evasion in MM. Taken together, our study identifies *PVT1*-mediated PRC2 targeting as a regulator of gene silencing in MM, thus highlighting *PVT1* as a potentially interesting therapeutic target for patients affected by this malignancy.

Methods

Cell culture

Human MM authenticated cell lines were cultured and supplemented as previously described.[8] Potential mycoplasma infections were investigated before the start of each experimental procedure utilizing MycoAlert™ Mycoplasma Detection Kit (Lonza; Basel, Switzerland; cat. no. LT07118).

RNA immunoprecipitation sequencing

RNA immunoprecipitation was conducted utilizing Magna Nuclear RIP kit (Millipore; Billerica, MA, USA; cat. no. 17-10520 and 17-700) as described by the manufacturer. In brief, $1.0x10^7$ INA-6 cells were collected and cross-linked with formaldehyde with a final concentration of 0.3% for 10 minutes at room temperature. Excess formaldehyde was quenched with glycine. Post outer membrane and nuclear membrane lysis, the cells were sonicated for ten cycles on Pico Bioruptor™ (Diagenode) (30 seconds on/30 seconds off). Immunoprecipitation of EZH2 targets was conducted by incubating the samples with 5 μg of anti-EZH2 (cat. no. 17-662, Millipore) and anti-IgG Mouse (cat. no. CS200621, Millipore) antibodies over night at 4°C. RNA was purified and cleaned by using RNeasy Micro Kit (Netherlands, Qiagen; cat. no. 74004). Complementary DNA conversion and quantitative polymerase chain reaction analysis were performed as previously described[8] with primers found in the *Online Supplementary Table S6*.

RNA immunoprecipitation sequencing library preparation and analysis

RNA concentration was measured using Qubit™ (Thermo Scientific). One hundred ng of RNA was used for sample library preparation using TruSeq Stranded Total RNA Gold (Illumina) with non-poly-A selection. Samples were then sequenced 50 cycles pair-end on one lane of a SP flow cell on NovaSeq 6000 system and v1 sequencing chemistry (Illumina). The fastq files from three biological replicates of RNA immunoprecipitation sequencing (RIP-seq) were concatenated per read pair to generate one pooled fastq file. The read mapping was then carried out using the nf-core[24] RNA sequencing (RNA-seq) pipeline (*https://doi.*

org/10.5281/zenodo.3503887) in version 1.4.2 using default parameters for paired-end sequencing, but with additional flags -reverseStranded - removeRiboRNA.[25] The BAMS were used by RIPSeeker[26] to statistically infer RIP regions for each strand given the background of input RNA, with parameter setting minBinSize=200 and maxBinSize=10,000. The RIP regions were selected at an estimated false discovery rate (eFDR) of 5%.

Transfection

One hundred and sixty thousand cells/mL MM.1S cells were seeded in Opti-MEM™ Reduced Serum Media (Gibco; Thermo Fisher Scientific, Inc., Waltham, MA, USA; cat. no. 31985070) and were allowed to attach over 24 hours (h) before transfection. HiPerFect transfection reagent (Netherlands, Qiagen; cat. no. 301704) and PVT1 GapmeR (200 nM) (Qiagen, Netherlands, cat. no. 339517) were added (1:3,000) and the cells were incubated for 72 h at 37°C in a humidified 5% CO_2 in-air atmosphere. Transfection efficiency was evaluated 72 h post transfection using 5-FAM-labeled positive/negative control GapmeR (Qiagen, Netherlands, cat. no. 339515) on Cytoflex LX (Beckman Coulter, Brea, CA, USA). Data was analyzed utilizing CytExpert v.2.4.0.28 (Beckman Coulter). Validation of *PVT1* inhibition was evaluated by real-time quantitative polymerase chain reaction.

Other methods

Additional methods are described in the *Online Supplemental Appendix*.

Results

Long non-coding RNA *PVT1* directly binds EZH2 in multiple myeloma cells

The expression of the catalytic subunit of PRC2 (EZH2) has been associated with disease progression from premalignant to malignant MM,[27] and patients exhibiting high expression of EZH2 have a significantly poorer prognosis (*Online Supplementary Figure S1A*). In addition, high expression of EZH2 correlates with poor survival in patients treated with bortezomib, dexamethasone, lenalidomide as monotherapies, or in combination therapies (*Online Supplementary Figure S1B-F*). To date, the mechanisms which mediate the recruitment of PRC2 to specific genomic regions have not been elucidated, however, prior studies have suggested that lncRNA may be putative partners contributing to PRC2 genomic binding and consequently to silencing of genes in selected cancer types.[14,28,29]

In order to evaluate whether dysregulation of lncRNA is a contributing mechanism to PRC2 targeting in MM, we first analyzed lncRNA expression in MM patients using transcriptomic data from the Blueprint Consortium Cohort (BCC) and identified 67 dysregulated lncRNA (5% false discovery rate [FDR]) with a putative role in the tumor (Figure 1A; *Online Supplementary Table S1*). In order to determine whether a functional and direct interaction may exist between PRC2 and the identified lncRNA in MM, we performed RNA immunoprecipitation coupled with sequencing (RIP-seq) against EZH2 in the INA-6 MM cell line and found 101 lncRNA (5% FDR) that physically interacted with EZH2 (Figure 1B; *Online Supplementary Table S2*). By overlapping the list of EZH2-bound lncRNA with the list of lncRNA overexpressed in MM patients, we identified the lncRNA *PVT1*, *PCAT1* and *SAMD12-AS1* as potential mediators of PRC2 targeting to chromatin in MM (Figure 1C).

One interesting candidate among the list of potentially relevant lncRNA for MM is *PVT1*, which has been previously reported to regulate the expression of EZH2 and has been associated with relapse and drug resistance in various cancers.[29,30] However, its interaction to EZH2 has not yet been resolved in MM cells. In order to elucidate whether EZH2 and *PVT1* interact in MM, we first validated *PVT1* overexpression in MM cell lines compared to peripheral blood mononuclear cells (PBMC) (*Online Supplementary Figure S1G*). Interestingly, EZH2i resulted in decreased *PVT1* expression in the EZH2i-sensitive MM cell line INA-6, but not in the EZH2i-resistant U1996 MM cells (*Online Supplementary Figure S1H*). Accordingly, pull-down of EZH2 by RIP-quantitative polymerase chain reaction (RIP-qPCR) confirmed a direct *PVT1*-EZH2 interaction in the MM cell lines INA-6, KMS-28PE, MM.1S and U1996 (*Online Supplementary Figure S1I*).

PVT1 expression is associated with disease progression and poor prognosis in multiple myeloma patients

In order to assess the clinical relevance of *PVT1* in MM, we analyzed expression data collected from three independent data sets of MM patients. Using the BCC, we found that MM cells expressed higher levels of *PVT1* than normal plasma cells (Figure 1D). Interestingly, PVT1 expression levels were heterogeneous across MM patients (*Online Supplementary Figure S1J*) and positively correlated with increased ISS stage of MM (CoMMpass cohort) (Figure 1E). In accordance with previously published data,[20] we found that premalignant stages of MM such as monoclonal gammopathy of undetermined significance (MGUS) and smouldering MM (sMM) harbor increased levels of *PVT1* as compared to normal bone marrow plasma cells (BMPC) (*Online Supplementary Figure S1K*), suggesting a potential role of *PVT1* already in the early stages of tumor development.

Finally, high expression of *PVT1* was associated with poor overall survival in newly diagnosed MM patients (CoMMpass cohort; Figure 1F), and patients that were resistant to conventional bortezomib treatment (GSE97582 cohort; *Online Supplementary Figure S1L*). Stratification of patients based on molecular classification revealed an increased expression of *PVT1* in patients with a hyperdiploid karyotype (GSE4581 cohort) (*Online Supplementary Figure S1M*), while patient grouping based on cytogenetics revealed that patients with

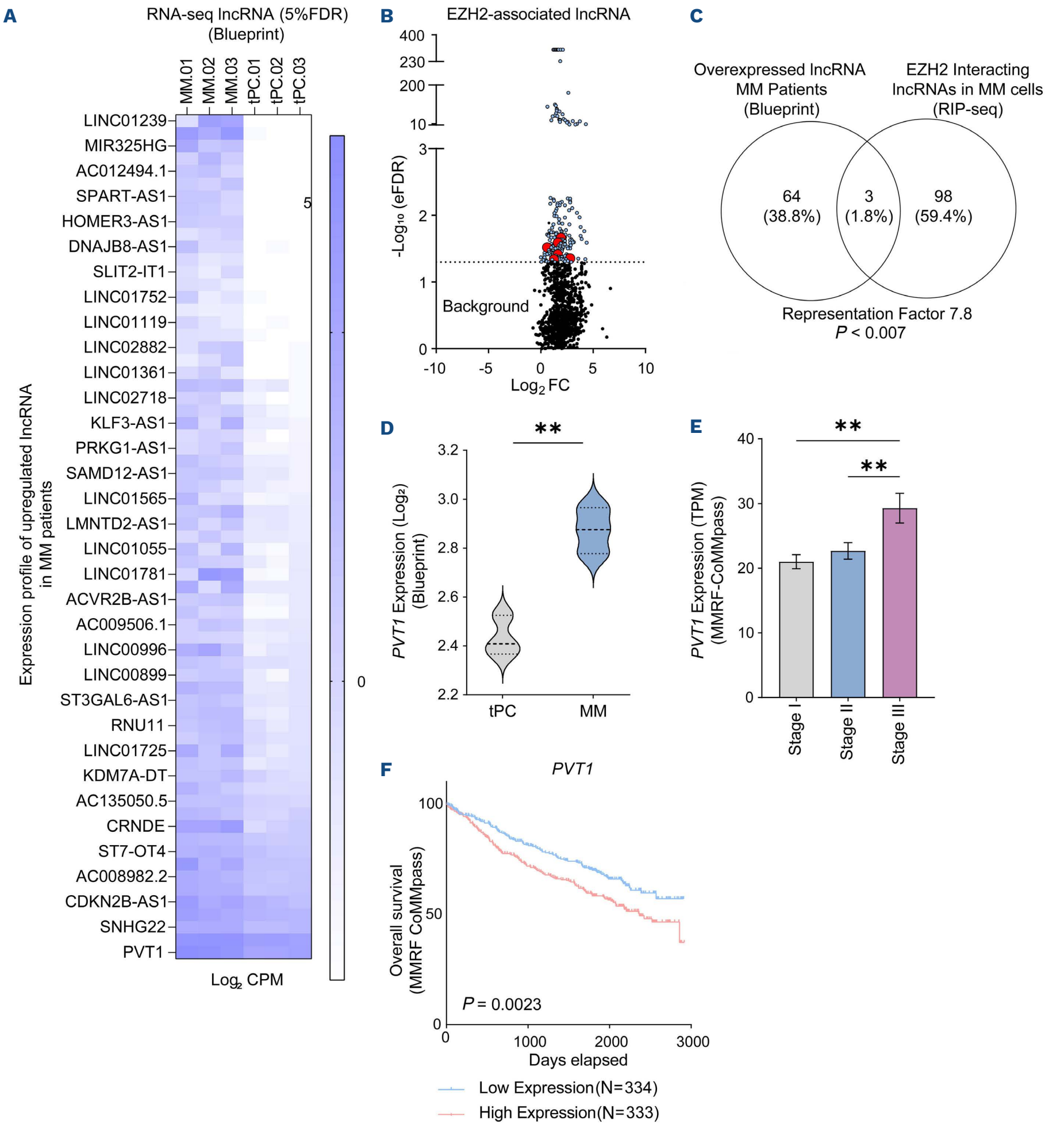

Figure 1. RNA immunoprecipitation sequencing analysis determined a physical interaction between EZH2 and the long non-coding RNA *PVT1*. (A) Expression profile of upregulated long non-coding RNA (lncRNA) in multiple myeloma (MM) patients from RNA-sequencing (RNA-seq) data with $P<0.05$ and 5% false discovery rate (FDR) cutoff, collected from the Blueprint Consortium ($N_{mm}=3$, $N_{tPC}=3$). (B) EZH2-RNA interactome collected from RNA immunoprecipitation sequencing (RIP-seq) in the MM cell line INA-6 with 5% estimated FDR (eFDR) cutoff. Samples collected from 3 biological replicates. Red circles indicate EZH2-*PVT1* interaction. (C) Overlap between lncRNA overexpressed in MM patients and lncRNA interacting with EZH2. (D) Log_2 normalized expression data of *PVT1* in MM patients compared to tonsil plasma cells (tPC) from the Blueprint Consortium dataset. Statistical analysis was performed with student *t* test. (E) *PVT1* expression categorized by Internation Staging System (ISS) stage from the MMRF-CoMMpass dataset. Statistical analysis was performed with one-way ANOVA. Values are presented with standard error of the mean. (F) Overall survival data associated with *PVT1* expression (MMRF-CoMMpass, N=667). Statistical test was performed with log rank (Mantel-Cox test). *$P<0.05$; **$P<0.01$; ***$P<0.001$; ****$P<0.0001$.

17p deletion exhibit the lowest PVT1 expression (*Online Supplementary Figure S1N*).

In summary, *PVT1* expression increases gradually across premalignant and progressive stages of MM, suggesting a potential role of *PVT1* in MM disease progression.

PVT1-PRC2-mediated silencing of genes involved in tumor suppression and apoptosis signaling can be reversed in multiple myeloma

We then sought to study the relationship between *PVT1* and PRC2. First, we investigated potential genomic binding sites for *PVT1* using LongTarget,[31] a lncRNA-genomic DNA interaction tool which predicts potential triplex-forming oligos and triplex target sites. We identified 8,976 potential *PVT1*-binding sites and their closest corresponding genes (*Online Supplementary Figure S2*; *Online Supplementary Table S3*).[32] Next, we overlapped the obtained list of predicted *PVT1* gene targets with genes that fulfilled the requirements of being downregulated in MM patients (Blueprint cohort, 5% FDR) and genes that are enriched for H3K27me3 in MM patients (Blueprint cohort, 5% FDR). The analysis resulted in a list of 141 genes which are predicted to be both PRC2 targets (H3K27me3-enriched) and *PVT1* targets, suggesting that this subset of genes may be subjected to *PVT1*-PRC2 regulation in MM (Figure 2A, B; *Online Supplementary Table S4*).

In order to validate which of these genes could be regulated by the *PVT1*-PRC2 complex, we treated INA-6 MM cells with the EZH2 inhibitor UNC1999 and performed RNA-seq. We identified 713 genes that gained expression post-EZH2 inhibition (Figure 3A), 270 of which were also predicted *PVT1* genomic binding sites (*Online Supplementary Figure S3A*; *Online Supplementary Table S5*). Interestingly, 21 of the identified genes had a known TSG function based on

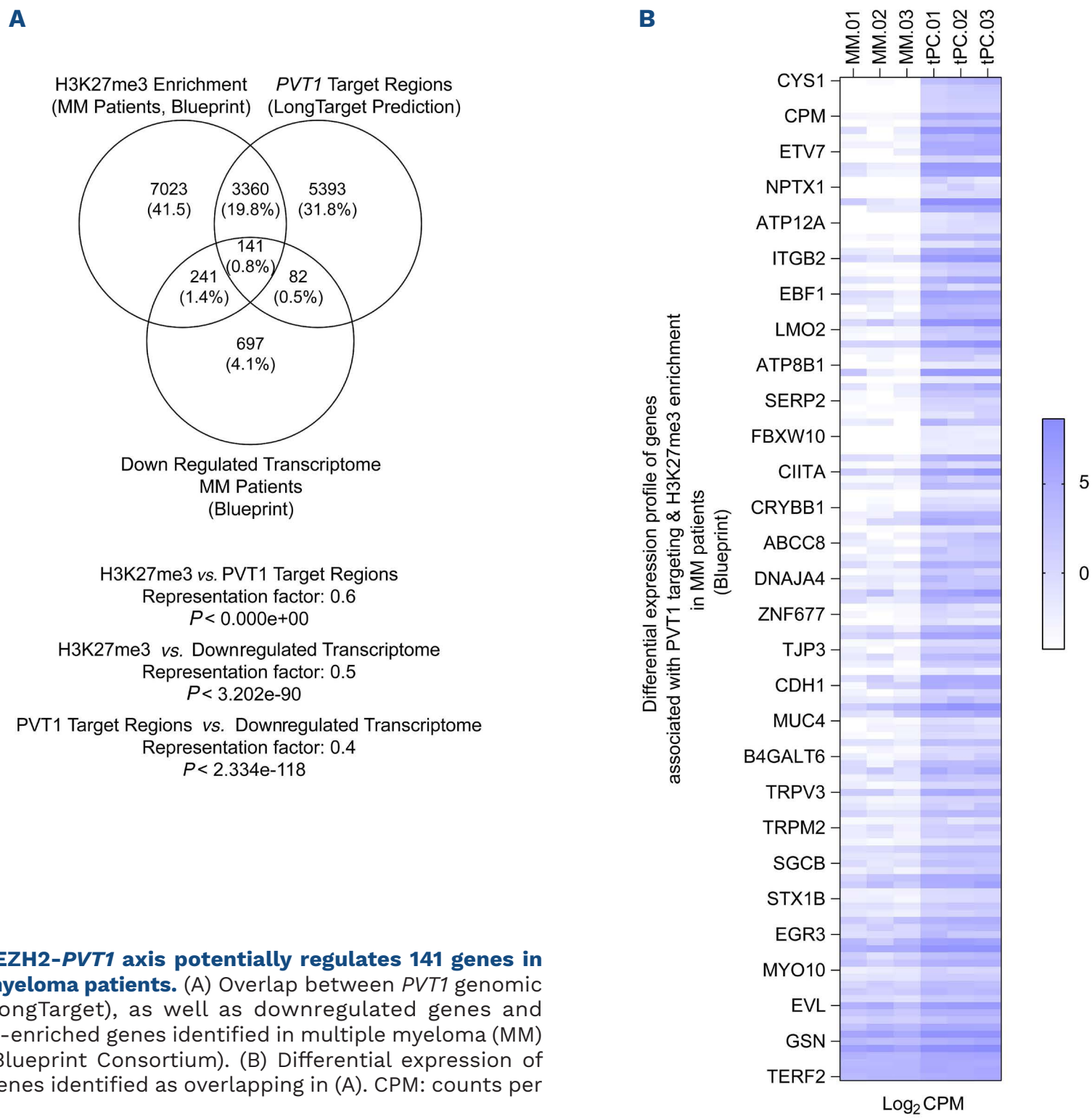

Figure 2. EZH2-*PVT1* axis potentially regulates 141 genes in multiple myeloma patients. (A) Overlap between *PVT1* genomic targets (LongTarget), as well as downregulated genes and H3K27me3-enriched genes identified in multiple myeloma (MM) patients (Blueprint Consortium). (B) Differential expression of selected genes identified as overlapping in (A). CPM: counts per million.

TSGene 2.0 (*https://bioinfo.uth.edu/TSGene/*) (*Online Supplementary Figure S3B*). Among these, *CXCL14* and *ZBTB7C* showed *de novo* activation and *RNF144A* together with *CCDC136* demonstrated an increase in expression post EZH2 inhibition (Figure 3B). Low expression profiles were observed for *CXCL14*, *ZBTB7C*, *RNF144A* and *CCDC136* in primary MM samples (*Online Supplementary Figure S3C-F*) and were associated with poor prognosis in MM patients (Figure 3C-F). Similarly, decreased expression of these genes was identified in MGUS and sMM, the asymptomatic prestages of MM (Figure 4A-D). In order to evaluate the functional relationship between EZH2 and *PVT1,* we knocked down *PVT1* expression by transfecting MM cells with GapmeR directly targeting the *PVT1* transcripts (*Online Supplementary Table S7*). Inhibition of *PVT1* expression was successful after 72 h of transfection (Figure 4E). Interestingly, *PVT1* inhibition (*PVT1*i) promoted a gain of expression of *ZBTB7C*, *RNF144A* and *CCDC136*, consistent with EZH2i in MM cells (Figure 4F). In addition, gene set enrichment analysis (GSEA) of the overall list of PRC2 target genes showed significant enrichment of

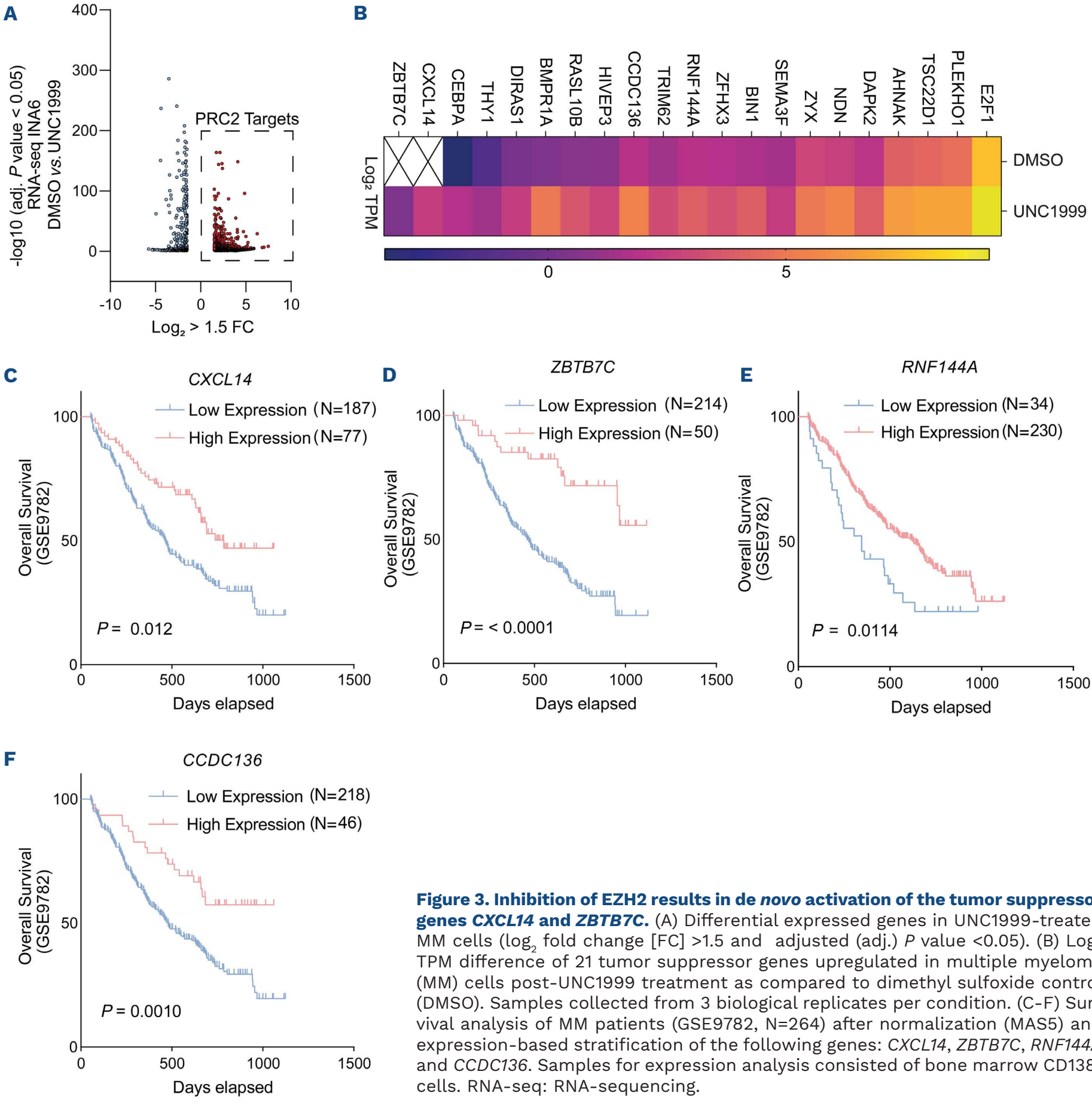

Figure 3. Inhibition of EZH2 results in de *novo* activation of the tumor suppressor genes *CXCL14* and *ZBTB7C*. (A) Differential expressed genes in UNC1999-treated MM cells (log_2 fold change [FC] >1.5 and adjusted (adj.) *P* value <0.05). (B) Log_2 TPM difference of 21 tumor suppressor genes upregulated in multiple myeloma (MM) cells post-UNC1999 treatment as compared to dimethyl sulfoxide control (DMSO). Samples collected from 3 biological replicates per condition. (C-F) Survival analysis of MM patients (GSE9782, N=264) after normalization (MAS5) and expression-based stratification of the following genes: *CXCL14*, *ZBTB7C*, *RNF144A* and *CCDC136*. Samples for expression analysis consisted of bone marrow $CD138^+$ cells. RNA-seq: RNA-sequencing.

genes regulating apoptosis in INA-6 cells that underwent EZH2 inhibition (Figure 5A). Among these, we identified a subset of PRC2-*PVT1* targets, such as *TNF, CCNA1, IGFBP6, SATB1* and *PLCB2* (Figure 5B). Importantly, we found that decreased expression of these genes was associated with a poor prognosis (Figure 5C-F) and advanced stages of the disease (*Online Supplementary Figure S3G-K*), while *SATB1* showed no correlation to poor prognosis in MM patients. Taken together, our data suggests that *PVT1*-mediated PRC2 targeting regulates apoptosis and mediates the silencing of a selected number of TSG in MM.

Discussion

EZH2 is the catalytic subunit of PRC2 and is responsible for the deposition of methyl groups to histone H3 lysine 27. We and others have previously demonstrated the clinical relevance of targeting PRC2 in MM and that PRC2-mediated gene silencing is a key feature of MM pathogenesis.[2,3] One challenging aspect of defining target genes of PRC2 is that this complex lacks sequence specificity; thus, the molecular mechanisms of its genomic localization are largely unknown. Prior studies have suggested that EZH2-lncRNA interactions could promote PRC2's functional capacity to bind chromatin. Indeed, while EZH2 does not contain a conventional RNA-binding motif, it includes a RNA-binding domain in residues 342–368 of the protein,[28] as well as a major RNA-binding site within its N-terminal helix.[14] Increasing evidence highlights the physiological and pathological impact that lncRNA have on cancer cell proliferation, metastasis, invasion, relapse, resistance, and genomic stability.[21] Dysregulation of lncRNA has been observed in various cancers, including MM, and numerous studies have provided insight into the diversity of the biological functions that lncRNA can have an impact on during cancer patho-

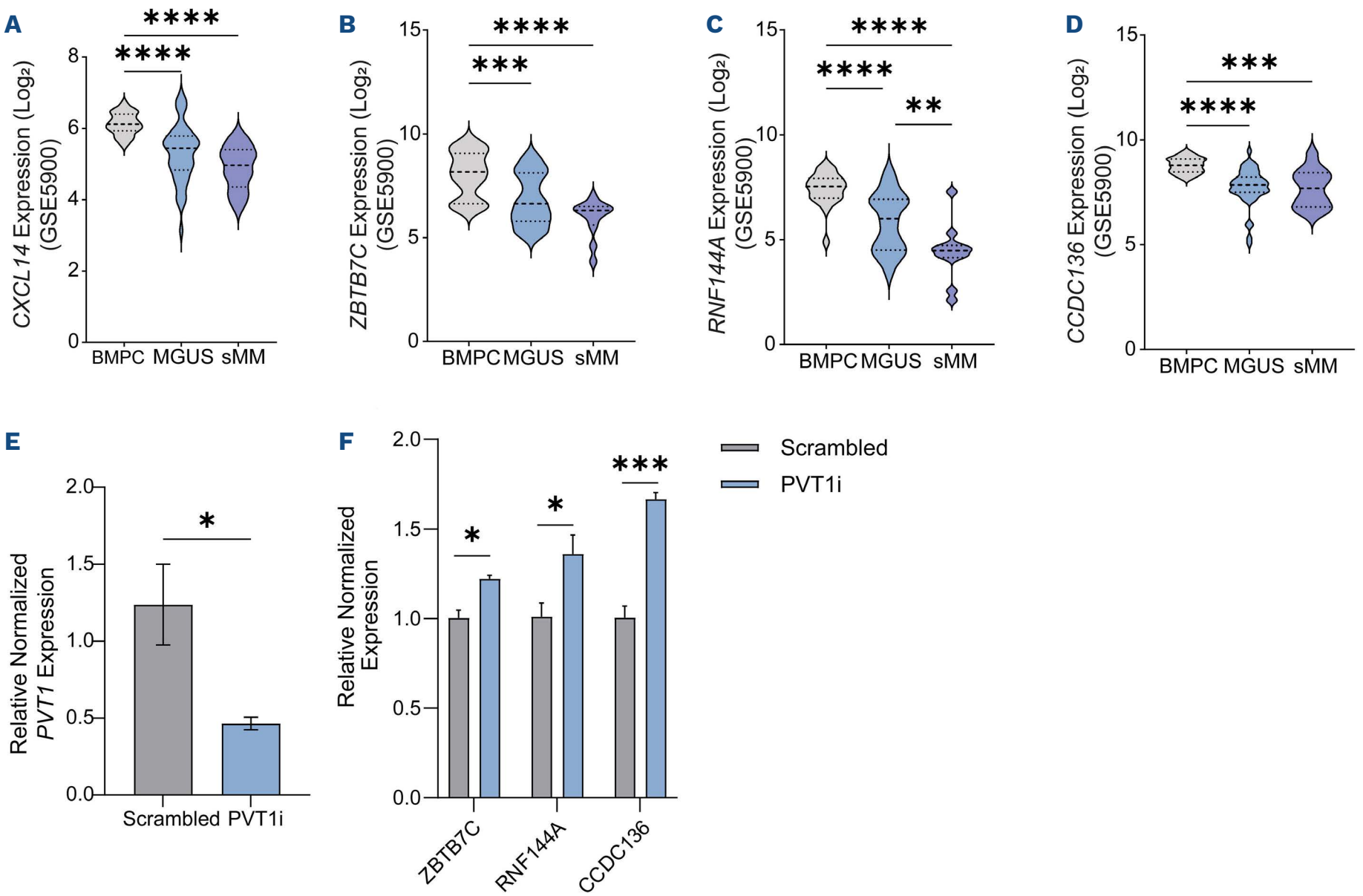

Figure 4. Loss of the EZH2-*PVT1* regulatory axis promotes expression of the tumor suppressor genes *ZBTB7C*, *RNF144A* and *CCDC136*. (A-D) Normalized (MAS5) *CXCL14*, *ZBTB7C*, *RNF144A* and *CCDC136* expression data from bone marrow-collected CD138$^+$ cells from monoclonal gammopathy of undetermined significance (MGUS) and smoldering multiple myeloma (sMM) (GSE5900) compared to bone marrow plasma cells (BMPC). (E) Normalized relative expression of *PVT1* post 72 hours transfection of GapmeR targeting *PVT1* in MM.1S MM cells. (F) Normalized relative expression of *ZBTB7C*, *RNF144A* and *CCDC136* post 72 hours PVT1i in MM.1S MM cells. Statistical analysis was performed with one-way ANOVA with Tukey test for multiple comparisons or multiple *t* test. Values are presented with standard error of the mean.*P<0.05; **P<0.01; ***P<0.001; ****P<0.0001. GSEA: gene set enrichment analysis.

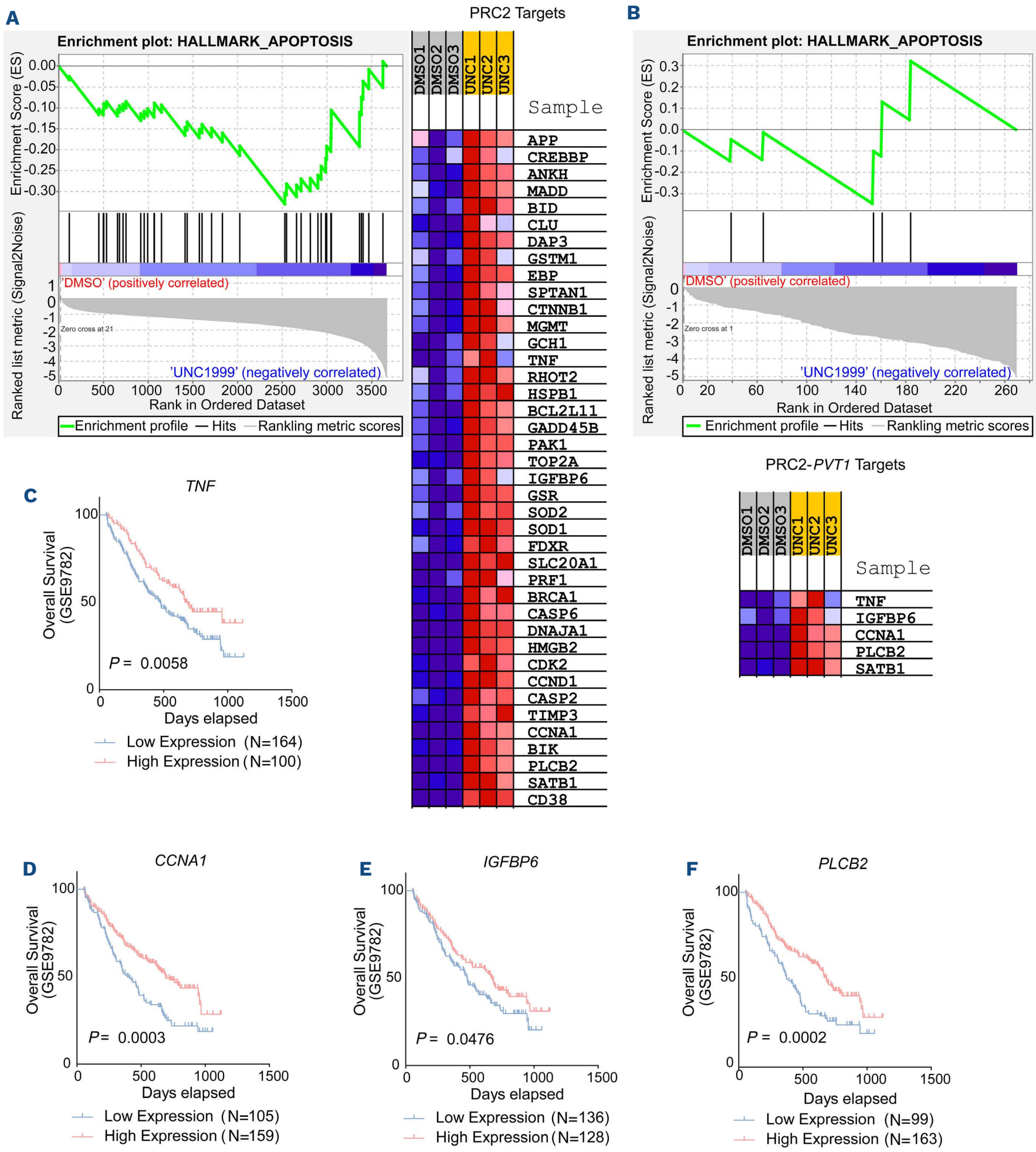

Figure 5. PRC2-*PVT1* axis regulates genes associated with apoptosis signaling. (A) Gene set enrichment analysis (GSEA) of PRC2 target genes in INA-6 multiple myeloma (MM) cells. (B) GSEA of PRC2-*PVT1* target genes in INA-6 MM cells. (C-F) Survival analysis of MM patients (GSE9782, N=264; GSE9782, N=262) after normalization (MAS5) and expression-based stratification of the following genes: *TNF*, *CCNA1*, *IGFBP6* and *PLCB2*. Samples for expression analysis consisted of bone marrow CD138$^+$ cells.

genesis.[20,33-35] Therefore, we sought to evaluate a potential lncRNA-mediated targeting mechanism of PRC2 in MM. Herein, three of the 67 lncRNA upregulated in MM primary cells *PVT1*, *PCAT1* and *SAMD12-AS1* were found to interact with the EZH2 protein. *PVT1* was overexpressed in MGUS, sMM and MM compared to normal PC, and its expression

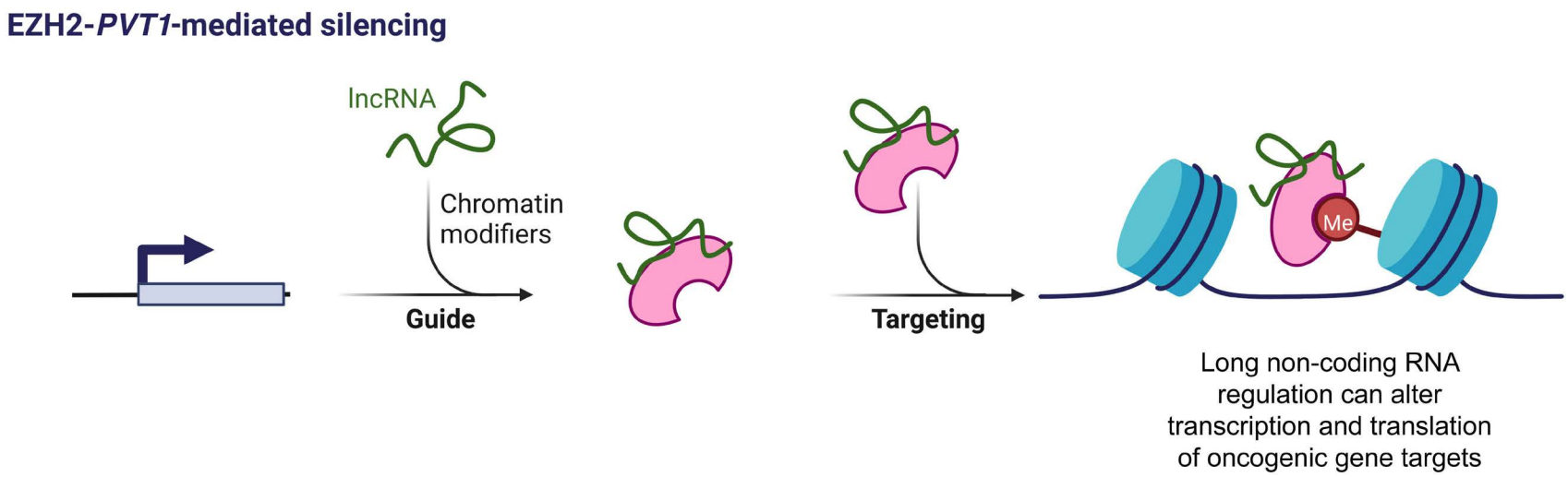

Figure 6. Suggested mechanism of action of EZH2-*PVT1*-mediated silencing. Adapted from "Epigenetic Deregulation in Cancer" by BioRender.com (2023). Retrieved from https://app.biorender.com/biorender-templates. lncRNA: long non-coding RNA.

gradually increased with ISS staging. In this paper, we show in two independent large MM cohorts that *PVT1* expression is also associated with poor prognosis. In our previous work we found no correlation between Polycomb-mediated expression signatures and specific genetic alterations,[3,11] which suggests that the epigenetically regulated signature mediated by *PVT1* is likely independent from these genetic alterations.

The functional implication of EZH2-*PVT1* interaction has not been fully investigated in the context of hematological malignancies, including MM. Thus, we sought to unravel the relationship between PRC2 and *PVT1* target genes in this malignancy. EZH2 inhibition in MM cells resulted in downregulation of *PVT1* expression, further solidifying the functional relationship between EZH2 and *PVT1*. Moreover, we found that 270 PRC2 target regions overlapped with genomic targets for *PVT1*. This suggests that *PVT1* plays a pivotal role in mediating EZH2 targeting in MM, which is in line with what has been reported for non-small cell lung cancer.[15]

Previous studies reported that treatment of MM cells with a *PVT1* inhibitor resulted in decreased cell proliferation and induction of apoptosis.[36] In line with this finding, we now suggest an important role for *PVT1* as an interacting partner to PRC2 by showing that EZH2 inhibition resulted in increased expression of PRC2-*PVT1* target genes associated with apoptosis regulation, such as *TNF*, *IGFBP6*, *CCNA1*, *PLCB2* and *SATB1*. Induced TNF expression has been reported to promote cell death in MM cell lines through the NFκB pathway,[37] and PLCB2 expression has been associated with a favorable prognosis in other hematological malignancies such as AML.[38] *CCNA1* has previously been identified as a PRC2 target in AML, and decreased expression of SATB1 resulted in increased cell proliferation in AML.[39,40] Importantly, we also show that these genes are downregulated in more advanced stages of MM, highlighting the potential relevance of their silencing as the disease progresses.

Loss of the EZH2-*PVT1* axis was also associated with the upregulation of 21 tumor suppressor genes. Interestingly, *CXCL14* and *ZBTB7C* showed *de novo* activation. Downregulation of CXCL14 is an important step in malignancy transformation within the bone marrow.[41,42] Indeed, previous studies have suggested that CXCL14 is needed for trafficking natural killer cells to sites of inflammation or oncogenesis as well as for the inhibition of the CXCL12-CXCR4 axis, which is critical for the migration of malignant cells.[41,42] Strikingly, similar to the effects observed with EZH2 inhibition, *PVT1* inhibition in MM cells resulted in the gain of expression of *ZBTB7C*, *RNF144A* and *CCDC136*, suggesting a co-regulatory relationship of these genes by the proposed PRC2-*PVT1* functional axis. ZBTB7C binds to p53 in solid tumors to prevent p53-mediated activation of CDKN1A, a known oncogene in both Burkitt lymphoma and MM, suggesting that ZBTB7C repression is of importance for MM oncogenesis.[43,45] Interestingly, suppression of the E3 ligase RNF144A has previously been described to increase survival of glioblastoma cells in stressful microenvironments and disruption of EZH2-mediated silencing in these cells assisted in overcoming drug resistance.[45] CCDC136 has been identified as a putative TSG and is frequently deleted in various malignancies such as gastric cancer,[46] however, its exact function in the cancer setting remains unclear. Studies in zebrafish have shown that CCDC136 promotes enhanced Wnt/β-catenin activity during zebrafish development.[47]

In summary, our study demonstrates that the *PVT1*-mediated EZH2 recruitment to genomic loci is responsible for the targeted silencing of genes associated with apoptosis (Figure 6) and regulates the expression of important oncogenes in MM. This makes *PVT1* an attractive candidate for targeted therapy in MM.

Disclosures

No conflicts of interest to disclose.

Contributions

PN, AAP, AK and HJW conceptualized the project. PN, BGZ, GMH and AAP acquired data. PN, PTP and LV performed formal analysis of the data. JJ and AM provided reagents.

PN, BGZ, AAP, AK and HJW assisted in project investigation. HJW provided acquisition of funding. FÖ, AK and HJW supervised the project. PN and HJW administrated the project. PN visualized all the data. PN organized and integrated the data. PN wrote the original manuscript draft. All authors read and approved the final manuscript.

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Data-sharing statement

RIP-sequencing and RNA-sequencing data have been deposited at the ArrayExpress platform with the accession numbers E-MTAB-13135 and E-MTAB-13136, respectively.

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