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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Supplemental information
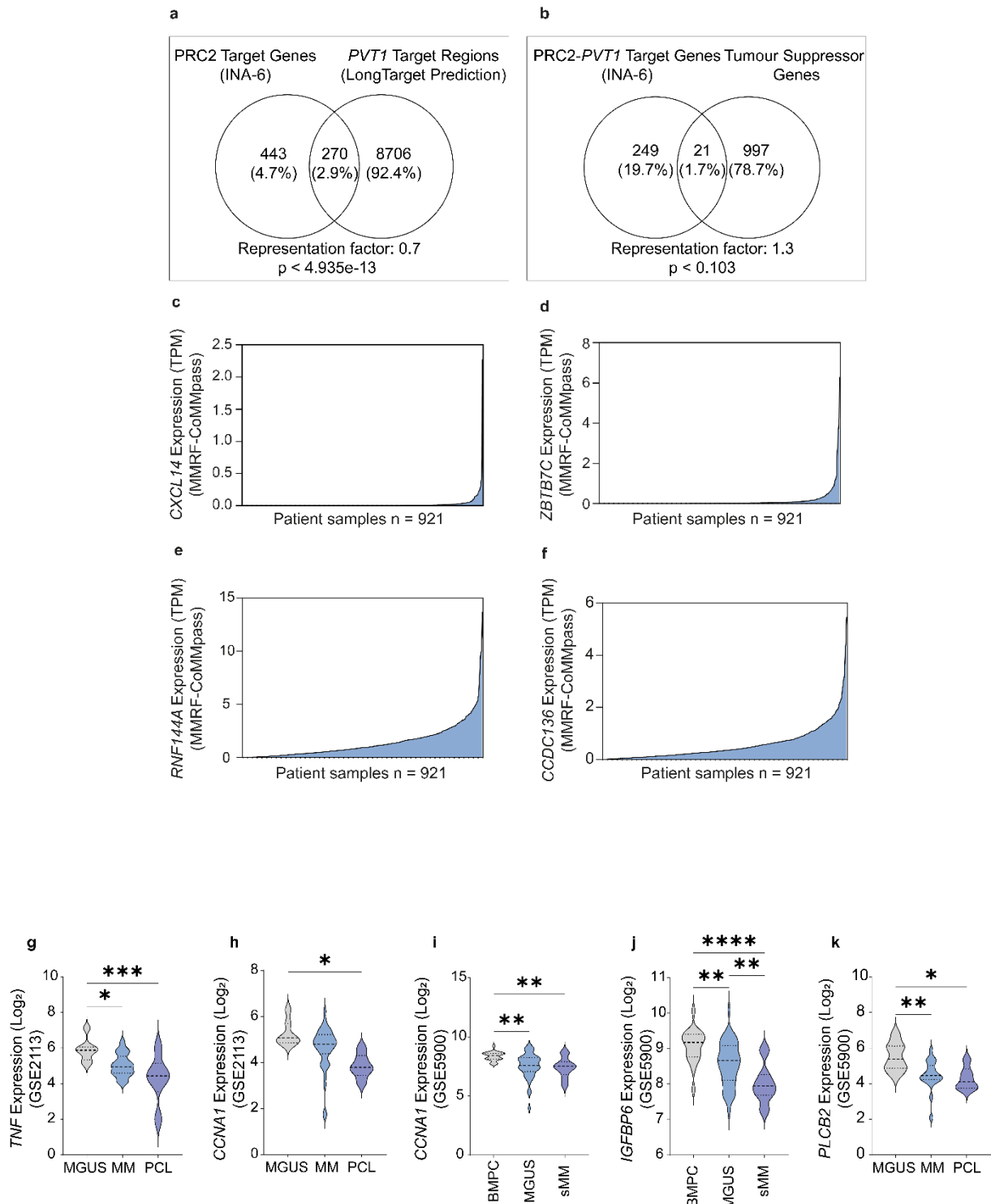
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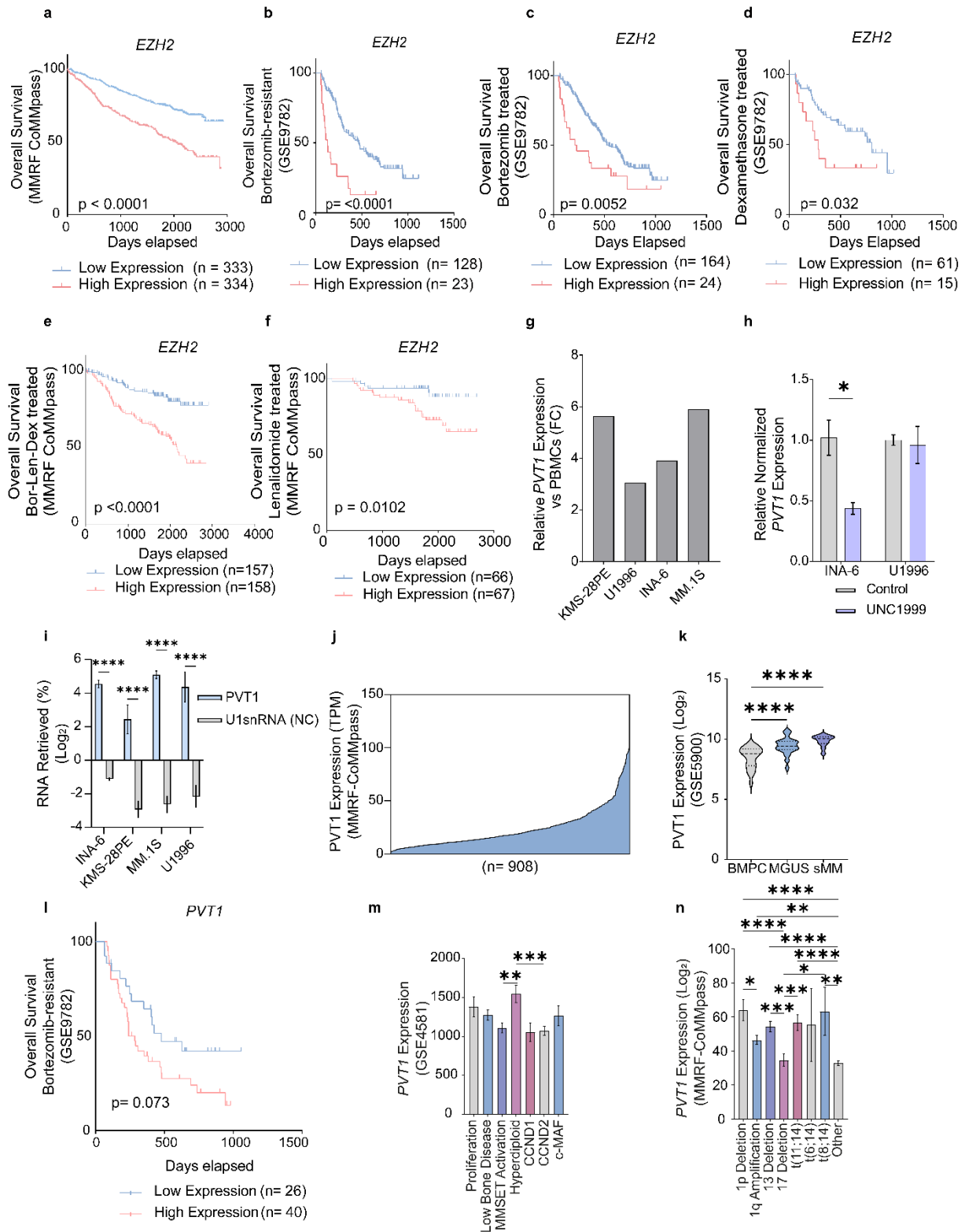
Supplemental figure 1. *EZH2* and *PVT1* expression is associated with poor prognosis in MM patients. **(a)** Overall survival data associated with *EZH2* expression in MM patients (MMRF-CoMMpass, n=667). Statistical test was performed with long-rank (Mantel-Cox test). **(b)** Overall survival of bortezomib-resistant MM patients stratified based on the normalized (MAS5) expression of *EZH2* (GSE9782, n=151). Statistical test was performed with long-rank (Mantel-Cox test). **(c)** Overall survival of bortezomib-treated MM patients stratified based on the normalized (MAS5) expression of *EZH2* (GSE9782, n=188).

Statistical test was performed with long-rank (Mantel-Cox test). **(d)** Overall survival of dexamethasone-treated MM patients stratified based on the normalized (MAS5) expression of *EZH2* (GSE9782, n=76). Statistical test was performed with long-rank (Mantel-Cox test). **(e)** Overall survival of MM patients co-treated with bortezomib, dexamethasone and lenalidomide, and stratified based on the expression of *EZH2* (MMRF-CoMMpass, n=315). **(f)** Overall survival of lenalidomide-treated MM patients stratified based on the expression of *EZH2* (MMRF-CoMMpass, n=133). Statistical test was performed with long-rank (Mantel-Cox test). **(g)** Gene expression analysis of *PVT1* expression in a panel of MM cell lines compared to PBMCs by RT-qPCR. Normalization against actin. **(h)** *PVT1* expression by RT-qPCR in INA-6 and U1996 post UNC1999 treatment. Statistical test was performed with student t-test. Values are presented with SEM. **(i)** RIP-qPCR validation of EZH2-PVT1 interaction compared to U1snRNA (NC) in the MM cell lines INA-6, KMS-28PE, MM.1S and U1996. Statistical analysis was performed with 2way ANOVA. Values are presented with SEM. Samples were collected from 3 biological replicates. **(j)** *PVT1* expression data (TPM) of primary MM patients from MMRF-CoMMpass, excluding significant outliers (z-value ≤ 0.01). **(k)** Normalized (MAS5) *PVT1* expression data from BM collected CD138+ cells from MGUS and sMM patients (U133 Plus 2.0; Affymetrix, GSE5900) compared to BMPC. Statistical analysis was performed with one-way ANOVA with Tukey test for multiple comparisons. Values are presented with SEM. **(l)** Overall survival of bortezomib-resistant MM patients stratified based on the normalized (MAS5) expression of *PVT1* (GSE9782, n=66). **(m)** Normalized (MAS5) *PVT1* expression data from BM collected CD138^{+} MM patient cells categorized by molecular classification (GSE4581). Statistical analysis was performed with one-way ANOVA. Values are presented with SEM. **(n)** *PVT1* expression data (TPM) of primary MM patients from MMRF-CoMMpass categorized by sample cytogenetics. Statistical analysis was performed with one-way ANOVA. Values are presented with SEM. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$.*
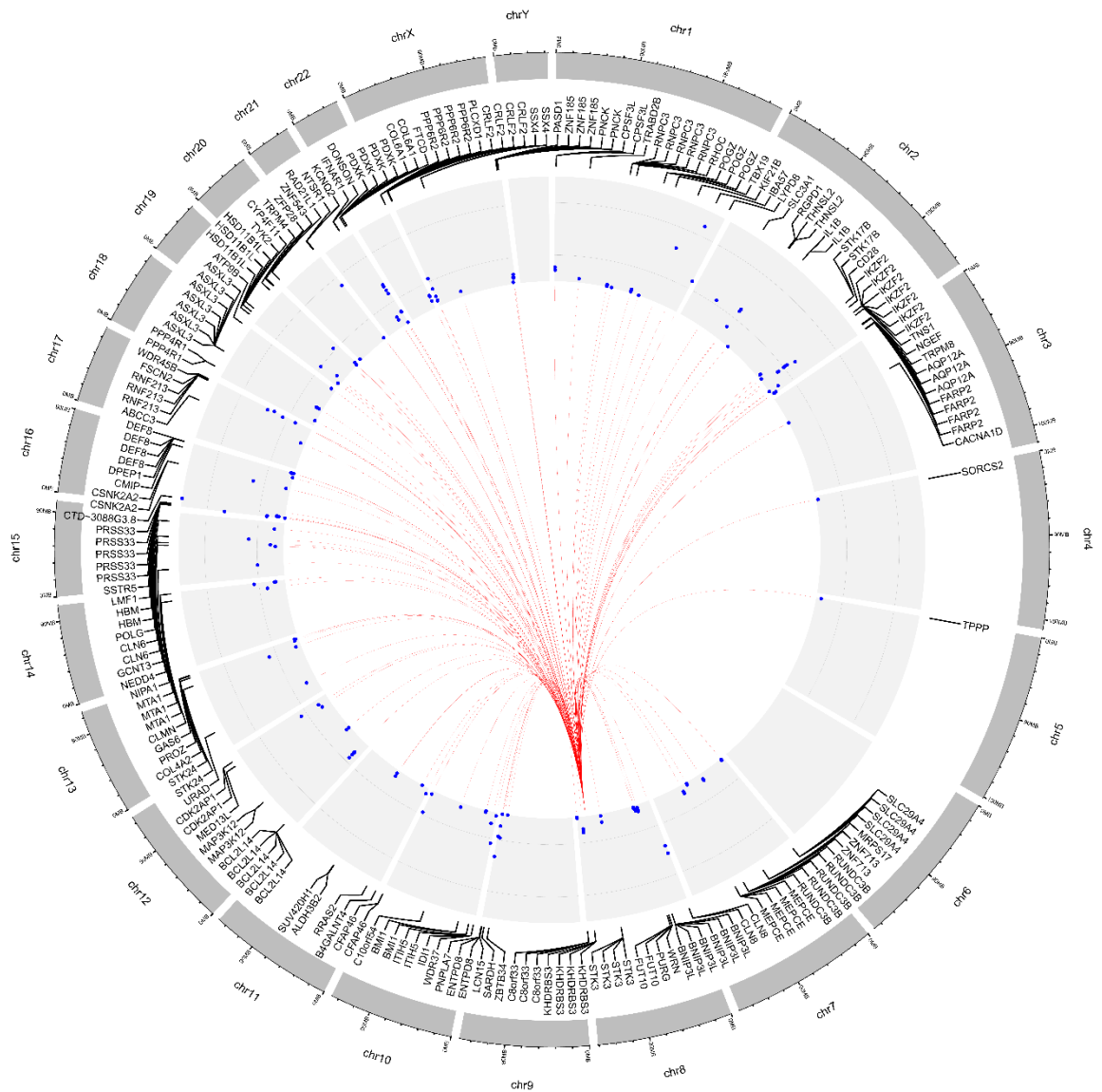
Supplemental figure 2. Predictive analysis of the EZH2-PVT1 axis in the human genome. Representative top 200 genome-wide interactions of PVT1-gDNA based on the prediction of triplex-forming oligonucleotides (LongTarget).

Supplemental figure 3. Genes targeted by the EZH2-PVT1 axis were downregulated in MM patients. (a) Overlap between identified PRC2 and PVT1 target genes. **(b)** Overlap of genomic regions characterized as PRC2-PVT1 target regions and previously identified tumour suppressor genes. **(c-f)** *CXCL14*, *ZBTB7C*, *RNF144A* and *CCDC136* expression data (TPM) of primary MM patients from MMRF-CoMMpass. **(g-k)** Normalized (MAS5) gene expression data from BM collected CD138+ cells from BMPC, MGUS, sMM, MM and plasma cell leukaemia (PCL) patients (U133 Plus 2.0; Affymetrix, GSE5900 and GSE2113) compared to BMPC and/or MGUS for genes (g) TNF, (h-i) CCNA1, (j) IGFBP6 and (k) PLCB2. Statistical analysis

was performed with one-way ANOVA with Tukey test for multiple comparisons. Values are presented with SEM. $*p < 0.05$, $**p < 0.01$, $***p < 0.001$, $****p < 0.0001$.

Supplemental tables

Supplemental table 1. Upregulated lncRNAs in MM patients.

lncRNA			
LINC01239	LINC01850	ACVR2B-AS1	CDC42-IT1
MIR99AHG	LINC01361	LINC01735	ST7-OT4
MIR325HG	CCDC39	AC009506.1	H1-10-AS1
LINC01518	LINC02718	FLJ46284	AC008982.2
AC012494.1	PRNCR1	LINC00996	LINC00494
AP000962.2	KLF3-AS1	LINC01359	CDKN2B-AS1
SPART-AS1	LNCTAM34A	LINC00899	IQCH-AS1
LINC02389	PRKG1-AS1	RASSF8-AS1	SNHG22
HOMER3-AS1	LINC02609	ST3GAL6-AS1	PSMA3-AS1
NECTIN3-AS1	SAMD12-AS1	MIR34AHG	PVT1
DNAJB8-AS1	LINC00222	RNU11	
LINC00997	LINC01565	PKIA-AS1	
SLIT2-IT1	LINC02328	LINC01725	
KCNQ1DN	LMNTD2-AS1	PCAT1	
LINC01752	SP2-AS1	KDM7A-DT	
ZNF341-AS1	LINC01055	LINC00304	
LINC01119	CXXC4-AS1	AC135050.5	
AC007036.5	LINC01781	VPS9D1-AS1	
LINC02882	TTLL7-IT1	CRNDE	

Supplemental table 2. EZH2-lncRNA interactions in MM cells.

lncRNA						
MALAT1	LINC02506	AL354993.2	AC115837.2	AC079089.1	AC011632.1	AC087854.1
AC010761.1	AC096536.2	AL133342.1	PLCG1-AS1	AC026979.4	SAMD12-AS1	
AC006064.4	TTTY20	AL391095.1	AL121832.2	C8orf37-AS1	AC100861.2	
AC125611.3	AC009899.1	RBM38-AS1	LINC01603	AC084083.1	AC018953.1	
AC011603.3	AC100781.1	AL034548.2	AC090579.1	LINC00534	AC027117.2	
LINC00399	AC090771.2	CSE1L-AS1	AL121832.3	AC087855.1	AF121898.1	
NEAT1	AC104985.2	CEP250-AS1	AC090578.1	AC133634.1	AC100803.4	
AC009133.5	AC119868.2	AL109984.1	NOL4L-DT	AZIN1-AS1	AC115485.1	
AC009133.2	BCL2L1-AS1	AL035458.2	LAMP5-AS1	OTUD6B-AS1	RDH10-AS1	
AL359762.3	CEBPB-AS1	NXT1-AS1	PVT1	AL356299.2	AL662797.1	
PABPC4-AS1	AL109824.1	SNHG17	ADNP-AS1	AC083964.1	AP006545.1	
AL160408.2	AL354813.1	AL109840.2	AL096828.1	AC023590.1	MIR3150BHG	
GAS5	AL079338.1	LINC01606	AC104561.4	AF230666.1	AC104370.1	
AL590133.2	NRSN2-AS1	LINC02055	AC027031.2	AL162458.1	AL450423.1	
LINC-PINT	AL110114.1	AC011773.1	MIR2052HG	HMBOX1-IT1	AC018953.2	

AL365181.3	AL035106.1	PCAT1	MCPH1-AS1	AC078906.1	AC064807.1
AL645728.1	DLGAP4-AS1	AL157838.1	AC068587.4	AC246817.1	AC022784.1
LINC01036	AL121906.2	AF279873.3	AC090993.1	AC015687.1	AC084125.2
AL390038.1	RALY-AS1	AC087855.2	GASAL1	AC011124.2	ZNF236-DT

Supplemental table 3. Top 200 *PVT1* genomic binding sites.

Chromosome	Binding Site Start	Binding Site End	Gene Name
chr11	11246475	11247047	CTD-3224I3.3
chr16	11493802	11494200	CTD-3088G3.8
chr7	151873969	151874563	PRKAG2-AS1
chr1	228174092	228174534	IBA57
chr7	151873948	151874563	PRKAG2-AS1
chr12	132835799	132836600	RNU6-327P
chr20	62755049	62756013	NTSR1
chr16	164672	165036	HBM
chr1	201006820	201007438	KIF21B
chr8	1763272	1763731	CLN8
chr8	1763293	1763731	CTD-2336O2.1
chr8	1763293	1763731	CTD-2336O2.1
chr8	1763324	1763731	CTD-2336O2.1
chr8	1763272	1763731	CLN8
chr17	80260497	80261023	RNF213
chr17	80260497	80261023	RNF213
chr13	44142120	44142313	SMIM2-AS1
chr21	43741121	43741494	PDXK
chr21	43741121	43741494	PDXK
chr9	137440379	137441266	ENTPD8
chr17	82639629	82640004	WDR45B
chr4	188796597	188797301	RP11-756P10.2
chr21	43741121	43741494	PDXK
chr21	44484791	44485021	AP001065.15
chr2	217809068	217809715	TNS1
chr16	58197688	58198049	CSNK2A2
chr2	88181097	88181571	THNSL2
chr21	43741121	43741494	PDXK
chr2	88181097	88181571	THNSL2
chr15	68257750	68258611	CLN6
chr15	68257750	68258611	CLN6
chr1	248746558	248747931	LYPD8
chr13	98576719	98577446	STK24
chr12	123274956	123275299	CDK2AP1
chr17	80260497	80261023	RNF213
chr14	105458985	105460188	MTA1
chr10	1046495	1047629	WDR37
chr2	213151634	213151940	IKZF2
chr2	44286477	44286876	SLC3A1
chrX	1212858	1213724	CRLF2

chr2	31805725	31806045	AL133249.1
chr19	49181936	49182533	TRPM4
chr2	213151634	213151930	IKZF2
chr2	213151634	213151930	IKZF2
chr19	10377314	10378042	TYK2
chr2	213151634	213151930	IKZF2
chr15	22770158	22770744	NIPA1
chr13	44142128	44142313	SMIM2-AS1
chr13	44142128	44142313	SMIM2-AS1
chr2	213151634	213151930	RP11-105N14.1
chr9	133668371	133669342	SARDH
chr10	132912100	132912651	CFAP46
chr9	137440379	137441261	ENTPD8
chr11	76718669	76719197	RP11-672A2.1
chr12	12047942	12048180	BCL2L14
chr2	213151634	213151930	IKZF2
chr2	213151634	213151930	IKZF2
chr2	213151634	213151930	IKZF2
chr14	95319798	95320133	CLMN
chr16	164672	165036	HBM
chrX	1212846	1213724	CRLF2
chr16	1075824	1076353	SSTR5
chr17	81535212	81535959	FSCN2
chr2	318873	319491	AC079779.6
chr12	12047942	12048178	BCL2L14
chr12	12047942	12048180	BCL2L14
chr8	135457308	135457717	KHDRBS3
chr8	135457308	135457717	RP11-343P9.1
chr12	12047942	12048178	BCL2L14
chr2	86914281	86914961	RGPD1
chr8	135457308	135457717	KHDRBS3
chr1	1323907	1324289	CPSF3L
chr12	12047942	12048178	BCL2L14
chr21	45980517	45981110	COL6A1
chr22	50340055	50340614	PPP6R2
chr15	59610372	59611332	GCNT3
chr21	45980517	45981110	COL6A1
chr22	50340055	50340614	PPP6R2
chr22	50340055	50340614	PPP6R2
chr9	136769170	136769693	LCN15
chr8	135457308	135457717	KHDRBS3
chr8	135457308	135457611	KHDRBS3
chr10	7666842	7667340	ITIH5
chr10	7666842	7667340	ITIH5
chr12	123274980	123275288	CDK2AP1
chr1	1323920	1324289	CPSF3L
chr21	33323115	33324051	IFNAR1
chr21	38742962	38743361	LINC00114

chr2	233927707	233927987	TRPM8
chr14	105458985	105459837	MTA1
chr13	27990754	27991399	URAD
chrX	1212846	1213724	CRLF2
chr12	116277737	116278174	MED13L
chr15	89325034	89325312	POLG
chrX	153677508	153677905	PNCK
chr2	868278	869374	LINC01115
chr14	105458981	105460069	MTA1
chr13	27990705	27991398	RN7SL272P
chr21	38742962	38743361	LINC00114
chr13	29491789	29492249	MTUS2-AS1
chr16	2786838	2787486	PRSS33
chr7	100426621	100427262	MEPCE
chr8	98825679	98825912	STK3
chr19	15932919	15934077	CYP4F11
chr15	55993665	55993890	NEDD4
chr14	101555703	101555940	DIO3OS
chr22	50340055	50340611	PPP6R2
chr21	33577599	33577705	DONSON
chr16	2786838	2787486	PRSS33
chr16	2786838	2787486	PRSS33
chr16	2786838	2787486	PRSS33
chr7	100426621	100427262	MEPCE
chr7	100426621	100427262	MEPCE
chr7	55887726	55887934	MRPS17
chr7	55887726	55887934	ZNF713
chr17	80313669	80314911	CTD-2047H16.2
chr13	112195636	112196395	LINC01070
chrX	152911610	152912055	ZNF185
chr8	26383494	26383753	BNIP3L
chr7	152435921	152436249	AC005631.1
chr18	33578214	33578531	ASXL3
chr3	140489998	140490175	AC048346.1
chr11	67669831	67670384	ALDH3B2
chr16	2786838	2787486	PRSS33
chr7	100426621	100427262	MEPCE
chrX	153677508	153677905	PNCK
chrX	1212879	1213724	CRLF2
chr11	374683	375241	B4GALNT4
chrX	152911610	152912055	ZNF185
chr8	26383494	26383753	BNIP3L
chrX	152911610	152912055	ZNF185
chr7	55887726	55887934	ZNF713
chr18	33578214	33578531	ASXL3
chrX	280273	281179	PLCXD1
chr2	241355597	241355970	AC005104.3
chrX	48381096	48381553	SSX4

chrX	48381096	48381553	SSX4
chr8	31032843	31033320	WRN
chr2	240693786	240694099	AQP12A
chr13	98576719	98577444	STK24
chr5	693114	693540	TPPP
chr16	87492247	87492573	RP11-482M8.1
chr18	33578237	33578531	ASXL3
chr18	33578237	33578531	ASXL3
chr8	26383494	26383753	BNIP3L
chr18	33578237	33578531	ASXL3
chr18	33578237	33578531	ASXL3
chr18	79356829	79357987	ATP9B
chr17	50673895	50674086	ABCC3
chr10	71759823	71760342	C10orf54
chr12	53498902	53499020	MAP3K12
chr16	58197688	58198049	CSNK2A2
chr8	31032843	31033320	PURG
chr13	110456780	110457328	COL4A2
chr10	6580103	6580492	PRKCQ-AS1
chr10	6580103	6580492	PRKCQ-AS1
chr2	196171631	196171889	STK17B
chr1	47997206	47997622	TRABD2B
chr13	113836192	113836959	GAS6
chr1	151458547	151459053	POGZ
chr18	33578237	33578531	RP11-258B16.1
chr6	169724301	169724782	RP1-266L20.4
chr18	33578237	33578531	ASXL3
chr10	132837288	132837902	CFAP46
chr10	1046897	1047629	IDI1
chr16	89948732	89949178	DEF8
chr16	58197688	58198049	RP11-459F6.1
chr2	241355597	241355970	FARP2
chr2	241355597	241355970	FARP2
chr2	241355597	241355970	FARP2
chr2	241355597	241355970	FARP2
chr2	196171631	196171887	STK17B
chr11	68212842	68213223	SUV420H1
chr19	57319165	57319523	ZNF543
chr17	60042355	60043083	AC005702.1
chr2	240693786	240694099	AQP12A
chr2	240693786	240694099	AQP12A
chr8	26383494	26383753	BNIP3L
chr8	26383494	26383753	BNIP3L
chr8	26383494	26383753	BNIP3L
chr18	76611714	76612090	LINC00683
chr13	113161705	113162834	PROZ
chr19	5681424	5681899	HSD11B1L
chr19	5681424	5681899	HSD11B1L

chr16	89948732	89949178	DEF8				
chr8	33379904	33380264	FUT10				
chr19	5681424	5681899	HSD11B1L				
chr7	87626175	87626843	RUNDC3B				
chr7	87626175	87626843	RUNDC3B				
chr7	87626175	87626843	RUNDC3B				
chr16	89618911	89619832	DPEP1				
chr1	151458547	151459053	POGZ				
chr1	103525466	103525710	RNPC3				
chr1	103525466	103525710	RP11-153F1.1				
chr17	60042355	60043083	AC005702.3				
chr20	63472721	63473080	KCNQ2				

Supplemental table 4. Genes regulated by the EZH2-*PVT1* axis in MM patients.

Gene Name

CYS1	CACNA2D3	ARHGEF4	FAM81A	PPP4R4	CPM	BAIAP2L1	CTSH
KLHL29	GPR137B	ETV7	SOX9-AS1	SWAP70	MYO1E	ANKRD18B	NPTX1
EPCAM	RFTN1	CR1	MYT1	ATP12A	ANKRD34B	CORO2B	C19orf81
CPXM1	ITGB2	EBI3	PRSS21	PXDN	TDRD9	EBF1	IGF2BP3
SLC1A1	RASGRF1	WDFY4	LMO2	TVP23A	SORL1	TSPAN18	PPP1R9A
ATP8B1	CR2	IRX6	AFF3	NDRG4	SERP2	APCDD1	APLP1
TNF	PROK2	FBXW10	ALOX12B	SLC45A1	COL18A1	AMER2	CIITA
DPY19L2	SHANK2	ESPN	SMIM10	CRYBB1	PFKP	HECW2	CSMD2
RANBP17	ABCC8	SLC16A10	LRRC32	MBP	KCNQ3	DNAJA4	ILDR2
AEBP1	ZAP70	ARC	ZNF677	CR1L	ANGPTL6	STAT5B	RET
TJP3	PTK2	MYO7A	DLGAP1	PDGFD	CDH1	CENPV	FHOD3
LHFPL2	CLIP2	MUC4	AC226118.1	MUC16	PALLD	ZNF667	B4GALT6
ZKSCAN7	CD83	IRAK3	ADAMTS7	TRPV3	EGLN3	TTC39C	PTPRN2
AKR1E2	TRPM2	RGL3	HOXA1	AUTS2	ATP6V1C2	SGCB	PTPRU
CDKN2C	PIK3CD	AFAP1L2	STX1B	SCARF1	STXBP1	IFI27L2	PLCH2
EGR3	SCPEP1	VDR	FBP1	RGS3	MYO10	MYLK3	RASSF4
ZEB2	NINJ2	EVL	ITGAE	CDH23	CTPS2	PIM1	GSN
CPNE5	HK1	SCYL3	FKBP1A	TERF2			

Supplemental table 5. EZH2i-mediated upregulated genes targeted by *PVT1* in MM cells.

Gene Name

FGR	RASGRP2	PRTFDC1	TJP3	KISS1R	GNAZ	MYO1F

SEMA3F	CLTCL1	GGT1	PBX4	TRIM62	CCDC136	AZIN2
TMEM176A	AP3M2	CARD10	PON3	KLF7	CHN1	PTPRF
PRKAR2B	PTPN21	PLEK2	TFR2	PIGZ	GAD1	DMRTB1
BAIAP2L1	ARHGAP10	DHRS2	TMEM176B	C1orf198	TMOD2	IGSF3
TBXA2R	LLGL2	E2F1	PRKAG2	PGF	SHBG	CELSR2
CYTH3	ADD2	GABRE	DNM1	KLHL29	PLD2	ILDR2
ETV7	PAG1	TSC22D1	SHB	MOB3B	DOCK6	MBOAT2
OSBPL5	NEBL	CBLN1	DNMBP	TMEM54	LAMA5	SFXN5
PLEKHO1	CHRNA3	CRISPLD2	BMPR1A	LPGAT1	HABP4	GALNT13
DAPK2	CDC14B	NME4	NMU	FAM210B	KHDRBS3	DLX1
MYOM2	ULK2	SYT17	KLF3	DEK	IQCA1	ACKR3
CYP46A1	MECOM	CA2	DTX4	AHNAK	HSPA12B	HES6
USP2	SNX10	TRPA1	EXPH5	BFSP1	PCNA	MYO10
HOXC8	LPCAT2	NIPAL2	BCL7A	FAM110A	ARHGEF11	ARHGAP26
CTPS2	GNA11	JPH1	CD83	ID1	WASF3	CXCL14
PRDM6	TMEM40	GDAP1	SEMA5A	KIRREL2	CCNA1	CPLX2
ROGDI	CERS4	KLC3	ZAP70	HIVEP3	TSPAN2	ENDOD1
INPP5A	TYRO3	FSD1	PASK	FGFRL1	ETS1	GLB1L2
CACHD1	ZNF704	CYP4F22	IFNL1	SCN8A	KCNH3	ST14
CHAF1B	ADCY1	ACOT12	SATB1	PARVA	SEMA4F	FAM124A
ATP13A2	STOX1	OVOL1	NDN	ZNF71	KLHL36	LYPD6B
ALDH4A1	PACSIN3	GXYLT2	RGS6	SULT1C2	ABHD17C	PRSS23
CHCHD6	TTC7B	RHOD	CRIP2	NRARP	CIB2	ADAMTS12
ZYX	SMCO4	VANGL1	SPATA13	ZNF521	LIMD2	NR3C2
LYPD5	AMOTL1	SNX33	NUDT14	ZNF358	BIN1	RNF144A
LRRC3	GLB1L3	SH3PXD2B	SCN5A	NOS1AP	HS6ST1	CCDC122
ITGB2	CCDC68	MSRA	ZBTB7C	SP5	RALGPS1	ASTN1
PTH1R	LOXHD1	ARL4D	JAG2	DENND1C	SLC2A8	PTPN14
PLXDC1	IGFBP6	HSD11B2	ANO9	GPC2	ARRB1	TMEM163
FAM171A2	TTC39C	DIRAS1	PDE6G	ZNF579	PLCB2	THY1
ADCY9	TSPAN5	MAGEF1	RXRA	TNF	PAK6	PRKCA
SLC1A7	ATOH8	ZNF114	QRFPR	LYRM9	ARHGAP24	FAM81A
DAPL1	NLGN2	CA8	GCNT1	PNMA6A	FAM222A	SKI
HDAC11	FABP6	LDLRAD3	BCAM	CEBPA	TPM1	GALNT14
APBB2	SP7	FJX1	ARL4C	EPPK1	ZFHX3	RHPN1
GRIK3	PAQR8	CUEDC1	ASMT	TAPT1-AS1	NFIC	CLSTN2
KIF6	TSNARE1	ZNF467	ZNF777	MIR4505	ADAMTS10	DUSP23
RUNDC3A-AS1	RASL10B	CD24	TSC22D1-AS1			

Supplemental table 6. Primers for gene and RIP-qPCR relative expression assays.

Gene	Forward Primer	Revere Primer
PVT1	GGGTGACCTTGGCACATACA	CAAGCAGCTCAAAGGGGAGA
EZH2	GACCTCTGTCTTACTTGTGGAGC	CGTCAGATGGTGCCAGCAATAG
ZBTB7C	GGAGAAGCCATACATGTGCACC	ACGAACTTGGCGTTGCAGTGGA

RNF144A	*GGAGCAGATGACAACCATAGCC*	CTGTTTAGGGCAGGCAGCATCT
CCDC136	*TGAGGTGCTTCGGTTTCAGACC*	CTCGTTCTGCTCATCCTGGCTA
GAPDH	*GTCTCCTCTGACTTCAACAGCG*	*ACCACCCTGTTGCTGTAGCCAA*
ACTIN	CCAACCGCGAGAAGATGA	TCCATCACGTGCCAGTG
RIP-qPCR	**Forward Primer**	**Reverse Primer**
NEAT1	CTTCCTCCCTTTAACTTATCCATTCAC	CTCTTCCTCCACCATTACCA CAATAC
PVT1	GGGTGACCTTGGCACATACA	CAAGCAGCTCAAAGGGGAGA
U1_snRNA	GGGAGATACCATGATCACGAAGGT	CCACAAATTATGCAGTCGAGTTTCCC

Supplemental table 7. Sequence of *PVT1* GapmeR

GapmeR Type	**Sequence**
PVT1 GapmeR	5-FAM/*T*C*G*C*G*G*T*G*A*C*T*A*A*A*A*T

Online supplemental methods

UNC1999 treatment of MM cells

Two MM authenticated cell lines (INA-6 and U1996) were selected for UNC1999 treatment. 100,000 cells/mL were seeded into flasks. 24h post seeding, 1 µM of UNC1999 or DMSO (Sigma-Aldrich; cat. No 317275) was added to the cells. Media, supplements, and drug were renewed every 3rd day until end point at day 5. All experiments were conducted in 3 biological replicates.

RNA extraction, cDNA synthesis and quantitative-PCR

RNA extraction was done by utilizing RNeasy Micro and/or Mini Kit (Netherlands, Qiagen; cat. no 74004/74104) as previously described[1]. cDNA conversion was performed as previously described[1] and using 0.25 µM of forward and reverse primers (supplemental table 6). Gene expression quantification was done with the $2^{-\Delta\Delta CT}$ method using actin or GAPDH as the reference gene.

RNA sequencing library preparation and analysis

1 µg of total RNA was used for sequencing library preparation using TruSeq stranded total RNA library preparation kit with ribosomal depletion using RiboZero Gold (Illumina Inc.). Samples were then sequenced 50 cycles pair-end on a SP flow cell on NovaSeq 6000 SP lane (Illumina). The RNA-Seq analysis was performed using the nf-core[2] RNA-seq pipeline (https://doi.org/10.5281/zenodo.3503887) in version 1.4.2 using default parameters for paired-end sequencing. Differential expression analysis was performed using the DESeq2 workflow. The differentially expressed genes were selected based on a FDR of 5%.

In silico **analysis**

LongTarget Analysis

Binding site interactions between *PVT1* and genomic DNA were obtained by utilizing LongTarget[3]. The analysis was performed in the region of -3500~+1500 bp upstream/downstream TSS of all the transcripts in hg38. The transcripts whose promoter had a predicted binding site for *PVT1* (max peak area value>= 500) were analysed.

Patient gene expression and survival data

Patient gene expression and survival data were collected from genomicscape.com, spanning the Arkansas, myeloma cohort (GSE2113), the Mattioli myeloma cohort (GSE5900) and the Mulligan myeloma cohort (GSE9782)[4–6]. Patient expression data of relapsed MM was analysed from the GSE6477 dataset (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE6477). The GSE4581 dataset was used for *PVT1* expression in correlation with patient molecular classification http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE4581. All data was analysed in Graphpad Prism V.9.1.0.

MMRF gene expression and clinical data

The expression, clinical value, and overall survival data of *EZH2* and *PVT1* were assessed by using publicly available datasets from newly diagnosed MM patients from the Multiple Myeloma Research Foundation CoMMpass study. (https://research.themmrf.org/; release IA17).

BLUEPRINT data analysis

MM samples and human genome build 38 (hg38) were downloaded from the BLUEPRINT Consortium of hematopoietic epigenomes. RNA-seq differential analysis was performed by utilizing the nf-core[2] pipelines (https://doi.org/10.5281/zenodo.3503887) in version 1.4.2 using default parameters for paired-end sequencing. Differential expression analysis was performed using the DESeq2 workflow. The differentially expressed genes were selected based on a FDR of 5%. H3K27me3 ChIP-seq processing of the samples was done by utilizing the nf-core[2] pipelines (https://doi.org/10.5281/zenodo.3966161). Peak calling analysis for ChIP-seq data was performed utilizing ChIPSeeker[7]. Data was visualized and processed in Graphpad Prism V.9.1.0.

Gene set enrichment analysis

Gene set enrichment analysis (GSEA) was conducted utilizing GSEA V.4.0.3, using the hallmarks for cancer dataset.

Statistical analysis

One-way ANOVA was utilized for data generated from genomicscape.com. Student t-test was used on extrapolated RIP-seq data and UNC1999-treated cell viability data. Two-way ANOVA was used for RIP-qPCR, patient expression data from MMRF. Outliers were identified utilizing https://www.graphpad.com/quickcalcs/grubbs1/. Gene set enrichment analysis was used to identify pathways based of genes that were differentially expressed. Differences in

overall survival were identified using the log-rank test and survival curves were plotted utilizing

the Kaplan-Meier method.

References supplemental methods

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