Supplementary data

Supplementary Table S3: Different	ially expressed cell cycle associated ge	nes*
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Gene name	Description	Fold change (CD 138 ⁺ Vs CD138 ⁻)			
CDKN1C	Cyclin dependent kinase inhibitor 1C(p57)	+ 2.0666027			
CDKN1ACyclin dependent kinase inhibitor 1A (p21)		+ 2.1310976			
CDK2	Cyclin dependent Kinase 2	-2.0478663			
CDK4	Cyclin dependent Kinase 4	-2.7181473			
CDK8	Cyclin dependent Kinase 8	-2.292281			
CDK7	Cyclin dependent Kinase 7	-2.107182			
CDKN2A	Cyclin dependent kinase inhibitor 2A(p16)	-2.101037			
CDKN3	Cyclin dependent kinase inhibitor 3	-3.0566034			
CCNE2	Cyclin E2	-2.8048124			
CCNB2	Cyclin B2	-2.5280573			
CCNC	Cyclin C	-2.5377533			
CCNI	Cyclin I	-2.0644908			
CCNG1	Cyclin G1	-2.1613364			
CCNJL	Cyclin J like	-2.0111609			
CCNB11P	Cyclin B1 interacting protein	-2.5442545			
CCNDBP	CNDBPCyclin D type binding protein-2.0553572				
CDC2	Cell division cycle 2	-2.1113448			
CDC16	Cell division cycle 16 homologue (S.cerviciae)	-2.3152206			
CDC23	Cell division cycle 23 homologue (S.cerviciae)	-2.2285278			

Fold change 2.0 or more

Supplementary Table S4: list of primers used for q-PCR of selected PRC genes and their targets.

Genes	Primes
GMNN	Fp - GCCTTCTGCATCTGGATCTC
	RP –TGACTCCTGGGTGACTCCTC
SUZ12	FP-CTG CCT CCA TTC GAA ACA TT
	RP-AAC CAG GCT TGT TTT CCT GA
BMI	FP-ATG CAG CTC ATC CTT CTG CT
	RP- CCG ATC CAA TCT GTT CTG GT
SATB1	FP-GATGCCCCTGATGCTACAGT
	RP- CATTCCTCACTGTGGTGTGC
RYBP	FP- GACCAGCGAAACAAATCACA
	RP- TCGATGAGGAGCGAGTCTTT
CCND2	FP-TGAGCTGCTGGCTAAGATCA
	RP-ACGGTACTGCTGCAGGCTAT
EZH2	FP- TGATAGGGAAGCAGGGACTG
	RP- CCGAGAATTTGCTTCAGAGG
EED	Fp- GAGAGGGAAGTGTCGACTGC
	RP- GGTGTATCAGGGCGTTCAGT
BMP4	Fp- CTGGTCCACCACAATGTGAC
	RP- CGATCGGCTAATCCTGACAT
BMP3	FP- AGGCCGTAGATCATTGTTGG
	RP- CTGATACTGCACAGCCTCCA
BMP2	FP- GTCCTGAGCGAGTTCGAGTT
	RP-AGTGCCTGCGATACAGGTCT
CDKN2C	FP- GGACCCAGGACTATCCCTTC
(P18)	RP-TTTAGGGTCCCTTGTTCACG
NOTCH4	FP-CACGTGAACCCATGTGAGTC
	RP- TTGAGCAGTTCTGTCCATCG
PAX4	FP-GAGGGTCTGGTTTTCCAACA
	RP-TGCTGTGCAGAGATGATTCC
IGFBP1	Fp - CTGCGTGCAGGAGTCTGA
	RP- GTACTGATGGCGTCCCAAAG

Fig S1.

(A) Heat map depicting enriched gens (H929 cell line)

H929_CD138- H929_CD138+	HSC sig	nature	H929_CD138- H626_CD138-	PC signa	ature			
	SFRS18	121		HAUSE				
	EIES SCAMOL	SC4MOL		TM9SF3	TM9SF3			
	OGT	OGT		MRE11A	MREIIA			
	MLLT3	MLLT3		TPB	TPR			
	KETEDS	KBTBDS		MINPPI HBS1L	MINPPI HBS1L			
	KTAA0125	KTAA0125		CHEK1 SUPTION	CHEK1 SUPTLEH			
	MLL	MLL		BFC3	BFC3			
	DDX5	DDX5		HSPSOAAI	HSPSOAAI			
	ZDHHC21 SLC25A36	SLC25A36		HMGB1	HMGB1			
	MYST3	MYST3		CALU	CALU			
	GUCY1A3	GUCY1A3		IDE	IDE			
	PCNX DNA IRG	PCNX DNA IRG		CKAP5	CKAP5			
	ITSN2	ITSN2		CDK6	CDK6			
	ALCAM YESI	YES1		ARMCS KIFAP3	ARMC8 KIFAP3			
	BNF125	BNF125		BRM1 MTMB2	BBM1 MTMB2			
	LONP2	EBM 2		PHES	PHES			
	TMEM38B	TMEM38B		TXNED1	TXNED1 MTHED2			
	CRIMI	CRIMI		TPOS	LDUP			
	EPC1	EPC1		SMC1A	SMC1A			
	SPTBN1 DAM	SPTBN1 DAM		HNENPE	FIFOCI	gi gi		
	DAPK1	DAPK1		METAPS	METAP2	귀귀		
	FAM169A	RUNX2		POLAT	POLAT	88		
	SOCS2	SOCS2		SSX2TP	SSX2IP	cr lor	I	
	MST2	MST2		XK	XK	22		
	TMEM200A	MEISI		DTL.	DTL	비원		
	WBP5 MDFG	WBP5		TMEM48	TMEM48		LOC SIGR	lature
	HOXA5	HOXA5		AHCYL1 NDC80	AHCYLI			
	ERG	ERG		FAM82B	FAM82B		UBR5	
	ZEB1 ABCB1	ABCB1		MRPL35	MBPL35		PTCD2	PTCD2
	BCLIIA	BCLIIA		EIF2AK1	EIF2AK1		EIF2S3	EIF2S3
	JUN	JUN		ANKED32	ANKED32		TOGAP2	TOGAP2
	FRMD4B PDE10A	FRMD4B PDE10A		GPB125	GPB125		ARFGEF1	ARFGEF1
	CD109	CD109		FECH	FECH		PPIG	PPIG
	PTK2	PTK2		DUT	DUT		MAP3K7	MAP3K7
	LPP FLT3	FLT3		BFX7	CDE7		NF1	NF1
	KLF4	KLF4		KCNQ5	KCNQ5		RABGAP1	RABGAPI
	TFPI	TFPI		UMPS	UMPS		NAB1	NAB1
	ASXL1	ASXL1		ANKED27	ANKED27		CLN5	CLN5
	DACH1 RAALC	DACH1 RAALC		LEEFIP2	LEEFIP2		ZNF304	ZNF304
	FGD.5	FGD.5		TMEM97	TMEM97		SLC9A7	SLC9A7
	CALNI	CALNI		TPM1	TPMI		<u>ZFP30</u>	<u>ZFP30</u>
	ZNF165 COL541	ZNF165 COLSA1		ODC1	ODC1		FRMD46	FRMD48
	BIMKLB			ZNF225	ZNF225		APT 198	PARTISE NDIO
	F0X01	28184		CCNJ	CCNJ		ARL3 CETER	ARL3
	CACNB2 BBBMS	CACNB2 PRPMS		HIBA	HIBA		DICUI	DICUI
	PRKCH	PRKCH		WASE1	WASF1		VCLLA	VCLLA
	WDB91			BTK	BTK		DAODE	DAODE
	SMARCA1 BTBD11	SMARCA1 BTBD11		MIPEP	MIPEP		TRAFSIP2	TPAESTP2
	ATPSB4 HTB1F	ATPSB4 HTB1F		TNIK	TNIK		FLJ13197	FLJ13197
	KSB1	KSR1		GINS2	GINS2		C2CD2	
	SPINK2	SPINK2		STEAP3	STEAP3		ZBTB39	ZBTB39
	INPP4B MCTP1	MCTP1		TAL1	TALI		RBPMS	RBPMS
	TMEM107	TMEM107		LANCL2	LANCL2		ABCG1	ABCG1
	PLSCR4	PLSCR4		BAMBI	BAMBI		ZNE500	ZNE500
	DLK1 HIST1H2BC	DLK1 HIST1H2BC		SLC27A2	SLC27A2		TGIF2	TGIF2
	HES1 REVI	HES1		BYB3	BYB3		LERCSE	LERCSE
	HOXB3	HOXB3		EBEG	EREG		GPR56	GPR56
	PROM1 HIST1H2BD	PROM1 HIST1H2BD		BHAG	BHAG		PPP1R10	PPP1R10
	GCNT2 HOYB2	GCNT2 HOYB2		FAMITIAL	PR.X.5		PNPLA4	PNPLA4
	PPP1B16B	PPP1B16B		PDLIMI	PDLIM1		LEBC61	LBBC61

(B) Heat map depicting enriched gens (RPMI8226 cell line)

8226_CD138-	HSC signature	8226 (D138- 8226 (D138-	PC sign	ature			
	TMEM38B TMEM38B		MINPPI	MINPPI			
	SC4MOL SC4MOL		SLC27A2	SLC27A2			
	ZDHHC21 ZDHHC21		PAICS	PAICS			
	MREG		DLAT	DLAT			
	BNF125 BNF125		FECH FAM82B	FECH FAM82B			
	FAM3C FAM3C		MTMR2	MTMB2 TMCSF2			
	CYLD CYLD DNAJB9 DNAJB9		RRM1	REMI			
	ALCAM ALCAM		HS2ST1	HS2ST1			
	KETEDS KETEDS		BAMBI	BAMBI			
	MLLT3 MLLT3		PSMD1	PSMD1			
	PAM PAM		SSX2IP	SSX2IP			
	EIFS EIFS		AHCYL1	AHCYL1			
	TMEMIO7 TMEMIO7		TMEM48	TMEM48			
	INSIGI INSIGI		FBX07	FBX07			
	SOCS2 SOCS2		TMEM97	TMEM97			
	METSI MEISI		TRITI	TRITI			
	RPL31 RPL31		XK	XK			
	GPR126 GPR126		CHEK2	CHEK2			
	PCNX PCNX		LEPPEC	LEPPEC			
	LONP2		CDK7	CDK7			
	EPC1 EPC1		CALU	CALU			
	MCTPI MCTPI FLV3 FLV3		POLE2 DUT	DUT			
	ITSN2 ITSN2		MTHFD2	MTHFD2			
	CRIMI CRIMI		SUPTION	SUPTLEH			
	WDR91		HBS1L	TFRC HBS1L			
	OGT OGT		ANKED32 TYMED1	ANKED32 TYMED1			
	FNBP1 FNBP1		TPOS				
	BCL11A BCL11A		CHEK1	CHEK1			
	CALNI CALNI		PHE6 MIPEP	PHE6 MIPEP			
	FRMD4B FRMD4B		EIFSB	EIFSB	러 하		
	SFRS18 SPTENI SPTENI		SORD	SORD	10 C		
	KTAAD125 KTAAD125		MLFITP	MLFITP	e e		
	ASXL1 ASXL1 PROM1 PROM1		SCD	SCD METARS		I	
	WBP5 WBP5		TMOD3	TMOD3	292		
	HISTIH2BD HISTIH2BD		PEX5	PEX5	88		
	DST DST		SAPT3	SART3		LSC sign	nature
	ZBTB4 ZBTB4		MBEIIA	MBEILA			
	BUNX2 BUNX2		GPB125	GPR125		CLN5	CLN5
	GCNT2 GCNT2		CKAP5 HMGXB4	CKAP5		PPIG	PPIG
	TFPI TFPI		PDLIM1 HMGB1	PDLIM1 HMGB1		ARFGEF1	AR FGE F1
	DDX5 DDX5		DDAH1	DDAH1		EIF2S3	EIF2S3
	ZNE165 ZNE165 TNPP4B TNPP4B		RACGAPI	BACGAP1		ZNF304	ZNF304
	SPINK2 SPINK2		RFC3 HNRNPR	RFC3		NAB1	NABI
	KSB1 KSB1		METTL14	MET 1		PAMI198	CETER 1
	MI.I. MI.I.		AC01	AC01		DDD1D1C	DDD1D1C
	ANK3 ANK3		HIRA	HIRA		TDDCOD	TDDCOD
	CRHBP CRHBP		ANKED27 LANCL2	ANKED27 LANCL2		UBR 5	
	HOXB3 HOXB3		LHFPL2	LHFPL2 CTNNPL1		MAP3K7	MAP3K7
	FGFE1 FGFE1		HSPSOAAI	HSPSDAAI		TOGAP2	TOGAP2
	BEX1 BEX1		PERDC	PEKDC		ABL3	ABL3
	HOXB2 HOXB2		TIPIN NCBP1	TIPIN NCBP1		NF1	NF1
	GUCY1A3 GUCY1A3 DACH1 DACH1		SMCIA	SMCIA		RABGAP1	RABGAP1
	PLSCR4 PLSCR4		KCNQS	KCNQ5		ABCG1	ABCG1
	FOXO1 DAPKI DAPKI		ZNF225 POLAI	ZNF225 POLA1		VGLL4	VGLL4
	FLT3 FLT3		BMS1 KNTC1	KNTCI		FRMD4B	FRMD4B
	ATPSB4 ATPSB4		WASF1	WASE1		BTCD2	BTCD2
	FGD5 FGD5		TPR	TPR		PICD2	PICD2
	RIMKLB ARCRI		MICAL2 TNIK	TNIK		TGIE2	TGIE2
	PPPIRIAB PPPIRIAB		CTPS STEADS	CTPS STEAD3		7BTB39	7BTB39
	MY05C MY05C		CCNJ	CCNJ		SLC9A7	SLC9A7
	KLF4 KLF4		DLC1	DLC1		C2CD2	
	BTBD11 BTBD11		RHAG	RHAG MYCN		PLCH1	PLCH1
	PDEIGA PDEIGA		FAM171A1	DVD2		ZFP30	ZFP30
	TMEM200A		EREG	EREG		LEBC61	LBBC61
	LPP LPP		ANKI	ANK1		FLJ13197	FLJ13197
	TAMA COA		TALL	TAL1		ZNF500	ZNF500
	PAPILONA		CMPTPI				
	PTK2 PTK2 CACNB2 CACNB2		CNRIP1 PRKAR2B	PEKAE2B		RBPMS	RBPMS
	PARTINGA PTK2 PTK2 CACNB2 CACNB2 ERG ERG		CNRIPI PRKAR2B SPTA1 ASEGL1	PRKAR2R SPTA1 ASEGL1		RBPMS TRAF3IP2	TRAF31P2

Figure S1. Heat map of lead genes of GSEA of CD138⁺ and CD138⁻ populations in H929 cells (A) and RMPI 8226 cells (B).



Figure S2. CD 138⁻ population is heterogeneous and represents different stages of B cell differentiation. Human myeloma cell lines H929 (A) or RPMI 8226 (B) were treated with antihuman CD138 FITC, CD27 PE and CD19 APC antibodies and subjected to flow cytometry. Percentage of each subset is depicted on the corresponding quarter for H929 and RPMI cell lines.

M1. Immuno-histochemistry Liver tissues harvested from the mice were fixed in 4% paraformaldehyde for overnight and then placed in Tissue-Tek biopsy uni-cassettes and embedded in wax using the Tissue-Tek tissue processor. After which, the tissues were sectioned into 4 μ m longitudinal sections (Leica RM 2165). The sections were dipped in Histo-Clear (National Diagnistics, USA) for 5minutes twice and 30 seconds four times to remove the wax. The sections were then dipped twice in 100%, 95% and 75 % ethanol (1 min each) and subsequently rehydrated by immersing in water. The sections were then immersed in Dako Target Retrieval solution (citrate buffer pH6) and samples were heated to 110^{0} C for 10min in a T/T mega Multifunctional Histoprocessor. After the antigen retrieval step, 3% of H₂O₂ solution was added to the sections for 10min to quench the peroxidase activity of the tissue. The tissue sections were then washed twice in water and once in Tris borate saline (TBS) and incubated with mouse

monoclonal antibody to human syndican-1 (Abcam, ab 82200) at 4 ^oC for overnight. After incubation the sections were washed for 5 min thrice in TBS and incubated with secondary anti mouse antibody, Dako Envision system with HRPO (Dako K4000) for 1hr at room temperature. After incubation, sections were washed in TBS thrice and immunodetection was done using liquid DAB substrate chromagen system (Dako K3468), visualized and imaged under Olympus CK 40 microscope.

Bone marrow: Bone marrow samples were resuspended in PBS and cell were then pelletted at 1000rpm for 3 min. The pellets were resuspended in RBC lysis buffer (8.36 g of NH₄Cl, 1g of KHCO₃, 0.04g of EDTA in 500 ml ddH₂O) and incubated at room temperature for about 30min. The cells were spun down and washed once with PBS and made into cell spots on glass slides using cytospin (Cytospin 4, Thermo Scientific). The cells were fixed using absolute methanol for 15 min and allowed to dry. Dried slides were stored at 4^{0} C until immunodetected and imaged

M2. Survival analysis of MM CD138⁻ signature using UAMS and Bortezomib datasets

Method

- 1. Associating Affymetrix HuGene v1 probes to HG-U133 Plus 2 probesets.
- a. Duplicates of CD138- and CD138+ clones from MM cell lines 8226 and H929 (totally 8 chips of Affymetrix HuGene v1 platform) were processed with RMA. Fold changes in 8226 and H929 cell lines were estimated independently and those whose fold change values in 8226 and H929 were commonly over $2^{1.3} = 2.46$ were selected for further analysis. At this step, 5323 and 358 probes were selected from cell lines 8226 and H929, respectively. As a whole, 169 probes were in common.

- b. Associated genes for those probes were searched from NetAffx annotation using EntrezGene id as the key. At this step, 93 of them were not associated with any gene but 74 of them were associated with unique gene and 2 with 2 genes.
- c. Probesets of Affymetrix HG-U133 Plus 2 platform were looked for those uniquely matched genes. At this step, 72 of the genes had associated probesets in HG-U133 Plus 2 platform; 14 of them were associated with unique probesets; 18 with 2 probesets; 40 with more than 2 probesets.
- d. For multiply associated genes, unique association was established by choosing those with maximum median expression in MMRC reference sample gene expression data.
- 2. Survival association.
- a. MAS5 preprocessed gene expression profiling data for UAMS dataset (GSE2658) and Bortezomib dataset (GSE9782) were normalized with probeset-wise median normalization; for each probeset, we first determined its median MAS5 intensity level over all samples and expression value is estimated by the logarithm (base 2) of MAS5 intensity divided by median level (log2ratio).
- b. Expression measure of a sample was calculated as the median of log2ratio for signature probesets.
- c. Survival association was assessed by the Cox proportional hazard regression analysis using signature groups obtained by dividing signature index values into 4 equally spaced segments across the whole range. The number of samples in each segment was as follows: UAMS dataset: 51 (top range), 445 (2nd range), 56 (3rd range), and 7 (bottom range); Bortezomib dataset: 18 (top range), 91 (2nd range), 70 (3rd range), 9 (bottom range).