SUPPLEMENTAL DATA, FIGURES AND TABLES

SUPPLEMENTAL FIGURE LEGENDS

Supplemental Figure S1. Transactivation of the cyclin D2 promoter by c-maf in reporter NIH 3T3 cells. Wild type NIH 3T3 cells (black) infected with control vector, or MAF over-expressing NIH 3T3 cells (gray), were transfected with a luciferase reporter gene expressed from the cyclin D2 promoter (p*CCND2*-luc), the cyclin D2 promoter in reverse orientation (p*CCND2* reverse-luc), a β-Gal plasmid (b-GAL) or with no plasmid (buffer only control). After transfection, cells were lysed and luciferase activity determined. Endogenous transcription from the *CCND2* promoter was detected and was responsive to retroviral MAF; transcription from the reverse-orientation *CCND2* promoter was weak and unresponsive to MAF.

Supplemental Figure S2. Two color (CD138 | Annexin V) flow cytometric analysis of primary CD138+ myeloma cell response to Kinetin riboside
Examples of the apoptotic response of CD138-selected primary multiple myeloma bone marrow tumor cells to Kinetin riboside 10 μM or DMSO vehicle at 72h, analyzed by 2-color flow cytometry. While some primary tumor cells become Annexin V-positive simply from in vitro culture (and/or from treatment with DMSO vehicle) treatment with Kinetin riboside substantially enhances the apoptosis of primary myeloma cells, which become Annexin V-positive, CD138-negatve.

Supplemental Figure S3. Three-color (CD45 | CD38 | Annexin V) flow cytometric analysis of Kinetin riboside-induced apoptotic responses in primary MM patient bone marrow, by CD45 | CD38 compartments

(A) Example of a myeloma patient bone marrow specimen containing 20-30% primary tumor cells treated with Kinetin riboside 5-10 μM or DMSO vehicle for 72h and analyzed by 3-color flow cytometry. Forward scatter (FS) – side scatter (SS) (top, left column) and CD45 | CD38 subpopulations (top, right column) are shown. FS-SS gates for all cells (large gate) or cells with morphological characteristics of viable plasma cells (small gate) are illustrated; with Kinetin riboside treatment plasma cells become small and granular, suggesting cytotoxicity. All bone marrow cells in the large gate are separated by CD45 | CD38 characteristics (top, right column). Plasma cells represent >95% of CD38-positive cells. Gates on CD38-high, CD45-low/CD38-low and CD45-high/CD38-low subpopulations are shown; these subpopulations are further analyzed for Annexin V-positive apoptosis (bottom panels). Substantive apoptosis of the primary myeloma cell compartment occurs at low Kinetin riboside concentrations, alongside comparatively modest non-tumor cell apoptosis (most marked in CD45-high lymphocytes). (B) Compensation controls, showing cells stained with single antibodies, or no antibody, verifying absence of fluorochrome signal crossover between channels.

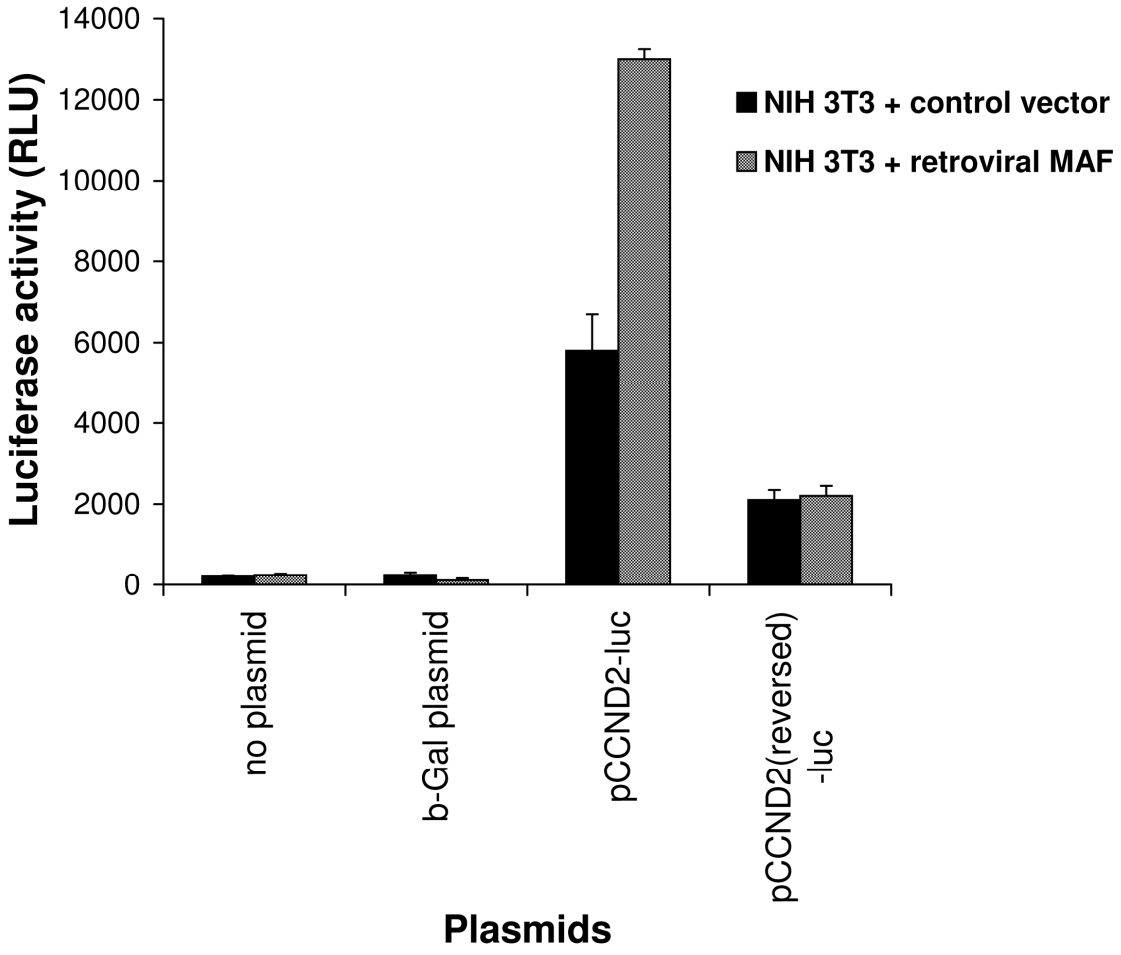
Supplemental Figure S4. Transcriptional repressor isoforms of the cyclic AMP response element modulator (CREM) are induced by Kinetin riboside. (A) Ensembl contig map of the human *CREM* gene, showing its modular structure, variant mRNA transcripts and *CREM*-specific Affymetrix U133_+2 probe-sets. (B) Relative expression of Affymetrix *CREM* 3'UTR- and exon-specific probe-sets in H929 or U266 cells 4h after treatment with Kinetin riboside, an unrelated cytotoxic agent, pristimerin, or DMSO vehicle. Three *CREM* probe-sets show specific increased expression of in both H929 and U266 cells after kinetin riboside treatment (indicated with *) indicating induction of specific CREM isoform(s). Probe-sets 228093_at and 210171_s_at, targeting exons found in CREM activator isoforms, are not up-regulated. (C) Correlation of *CREM* Affymetrix probe-set expression (normalized per tumor line to DMSO-treated samples) with *CREM* transcript variants curated by the Sanger Institute Vega Havana Group. (D) CREM variants (Vega) able to account for *CREM* probe-set expression profile changes induced by Kinetin riboside; all are repressors of the cAMP response element (CRE).

Supplemental Figure S5. Schematic of CRE and MARE sites in *CCND1* – *CCND3* promoters and model of repression of *CCND2* and *CCND1* by Kinetin riboside

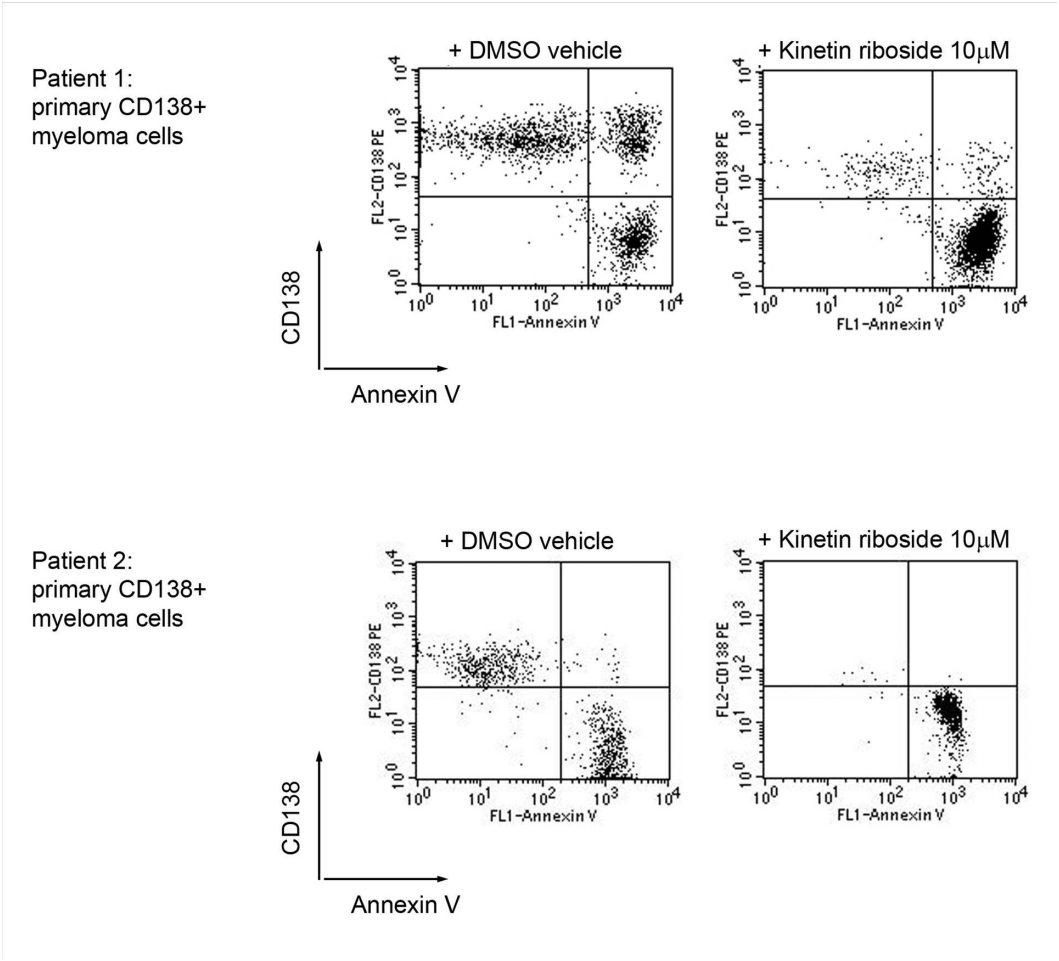
Evolutionarily-conserved cyclic AMP response elements (CRE) and MAF recognition elements (MARE) in *CCND1*, *CCND2* and *CCND3* 5' regulatory regions (1kb) are shown. The site have previously been identified and validated

by functional studies (1-7). The potent CRE repressor CREM / ICER is rapidly induced by Kinetin riboside (< 4 hrs) and is known to block the cAMP-response element (CRE) present in *CCND1* or *CCND2* (8, 9) located immediately 5' to the transcription initiation site. BACH2 is also induced and is a repressor of MARE and of the *IGH* enhancer (10-12) and thus is predicted to repress cyclin D gene expression when this is driven by MAF dysregulation or by translocation of a cyclin D gene to the *IGH* enhancer locus.

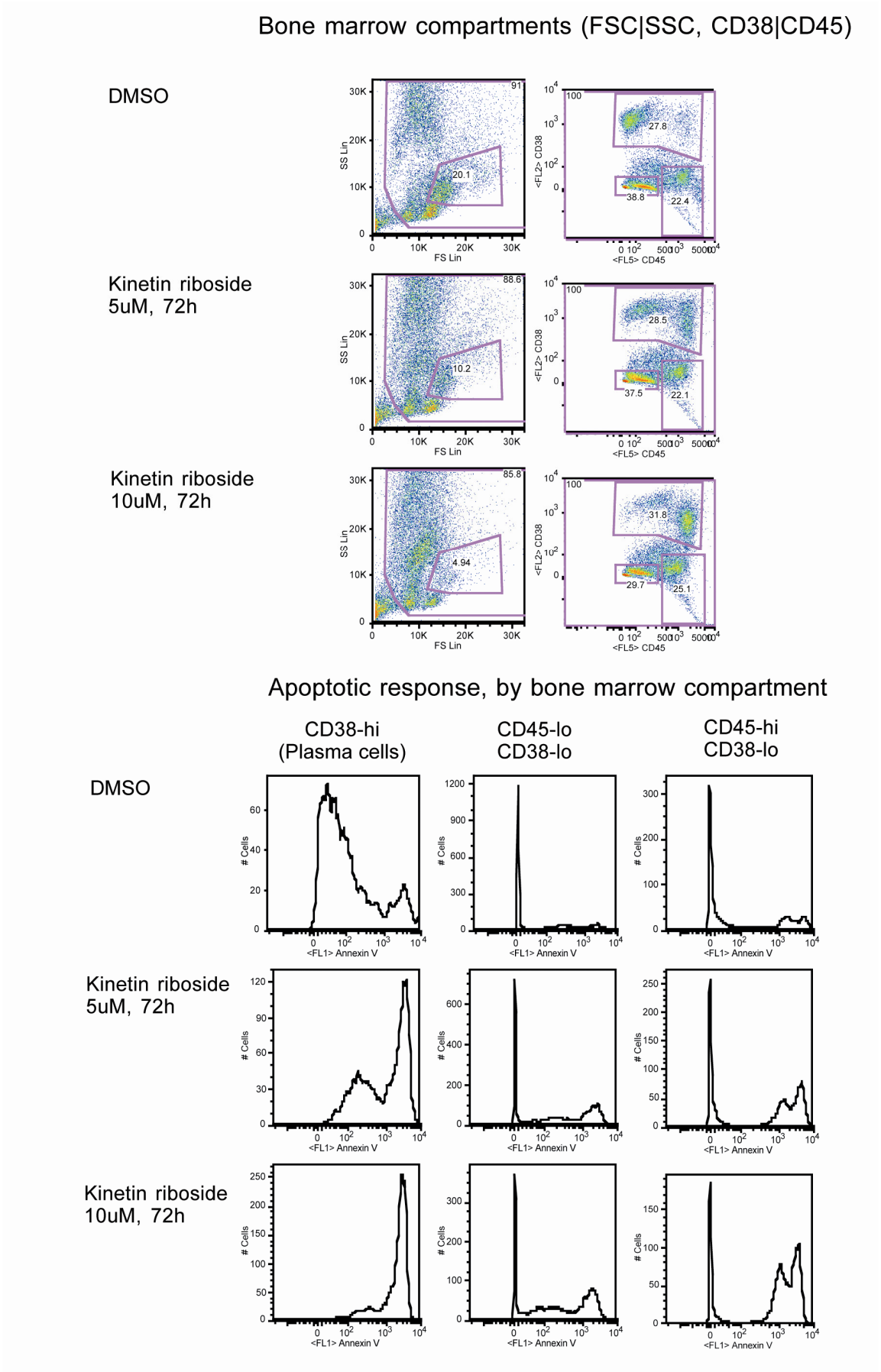
Supplemental Figure S1



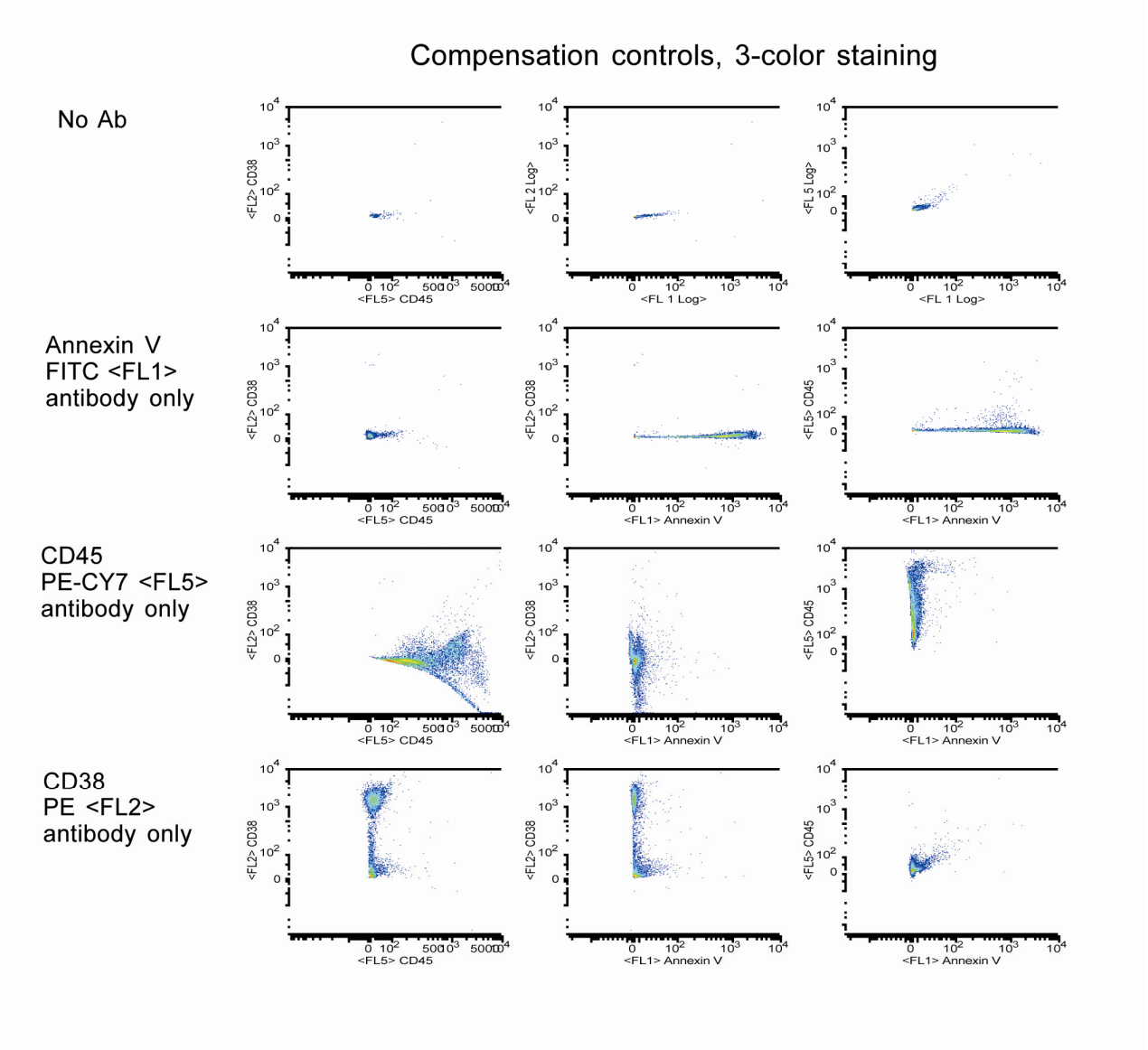
Supplemental Figure S2



Supplemental Figure S3A

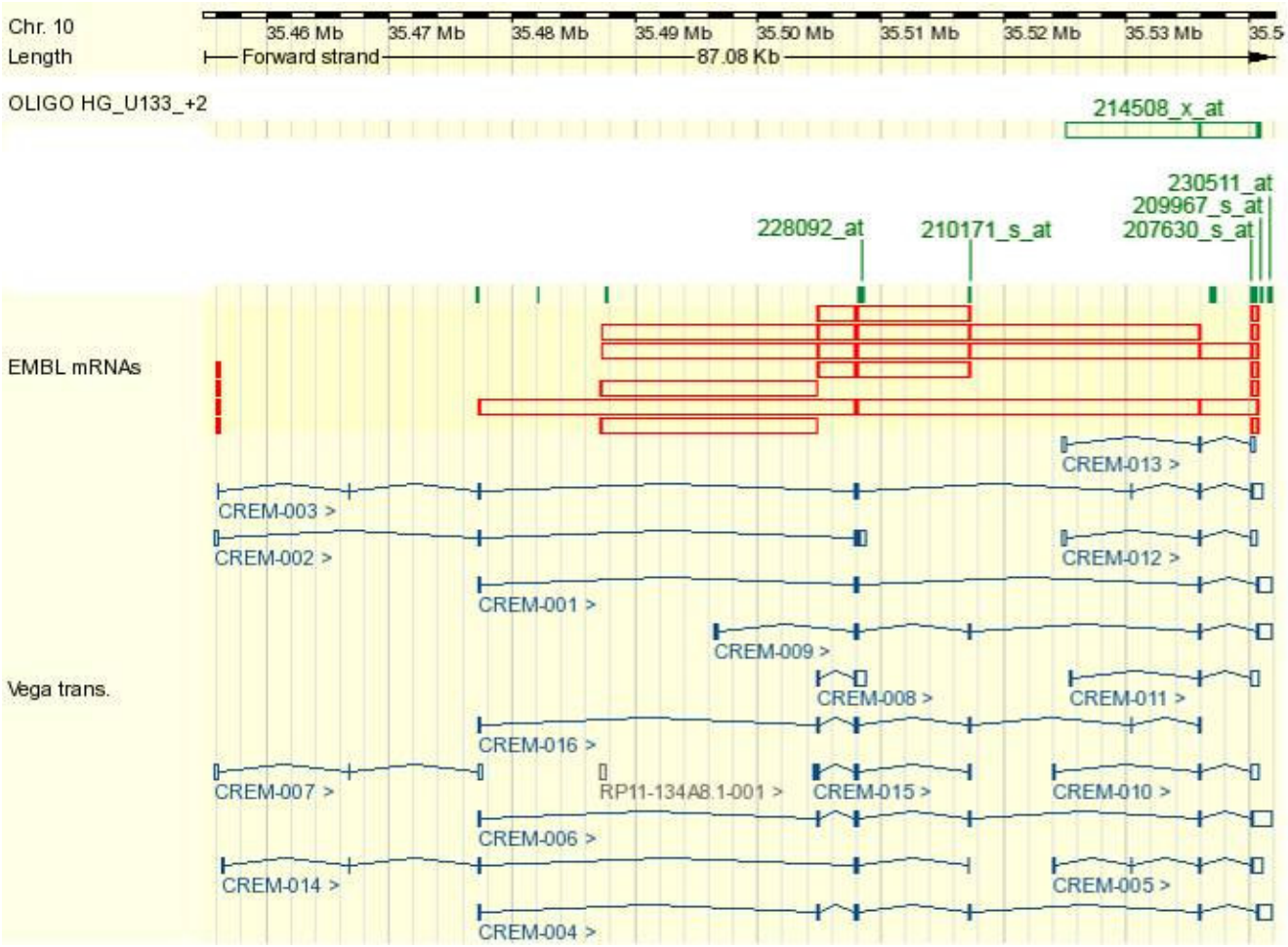


Supplemental Figure S3B



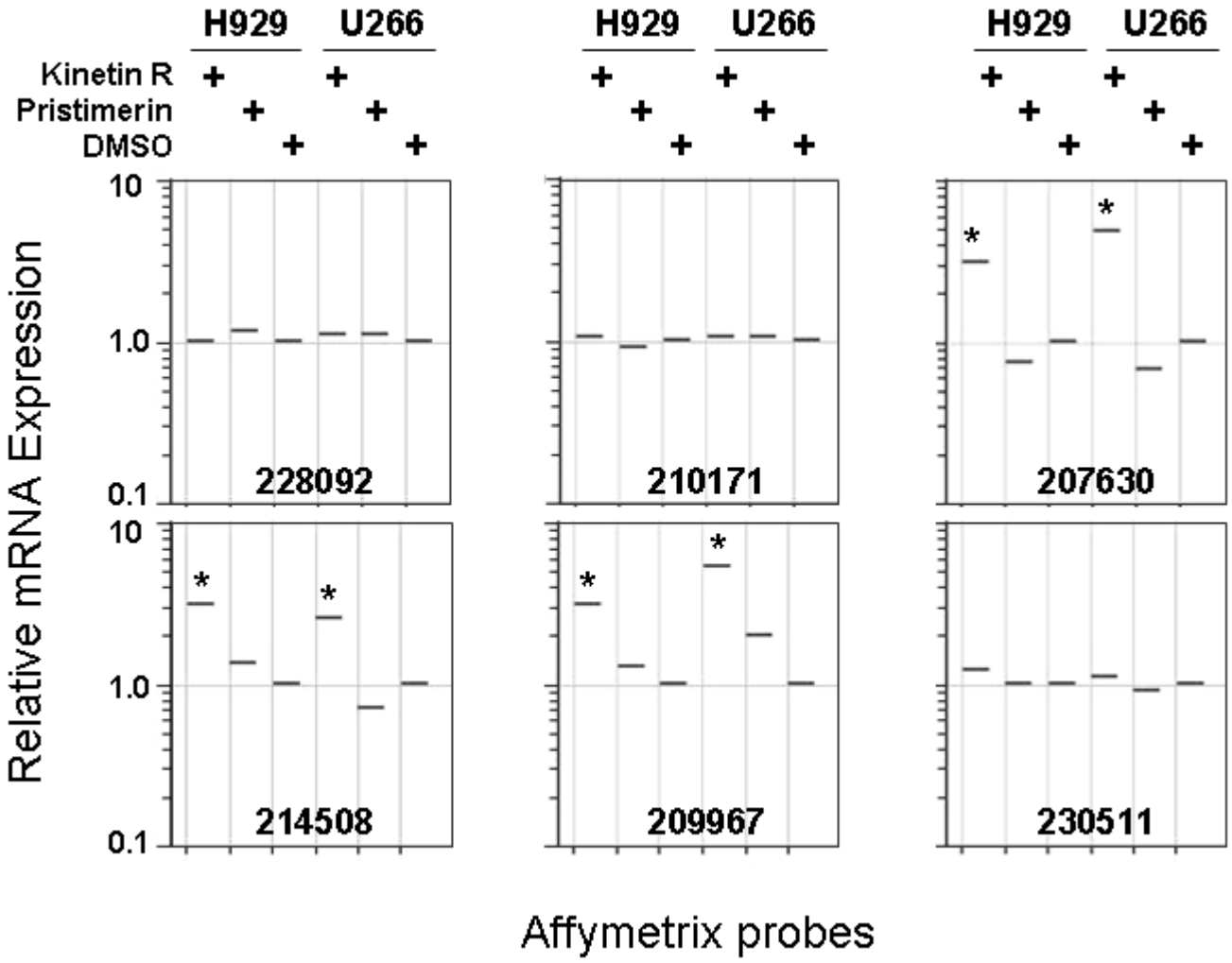
Supplemental Figure S4

A



Supplemental Figure S4 cont.

B



Supplemental Figure S4 cont.

C **Correlation of *CREM* gene transcripts and Affymetrix *CREM* probe-sets induced by Kinetin riboside**

	Fold change in expression after Kinetin riboside treatment*			
Hg_U133_plus2 probe-set	H929	U266	*CREM* transcript variants targeted by probe-set†	Interpretation of transcript expression
228092	1.04	1.14	002, 008	Not induced
210171	1.09	1.04	004, 006, 009, 015, 016	Not induced
207630	3.18	4.88	003, 005, *006*, 010, 011, 012	At least one: 003, 005, 010, 011,012 Induced
214508	3.18	2.65	*001*, 003, *004*, 005, *006*, *009,* 010, 011	At least one: 003, 005, 010, 011 Induced
209967	3.12	5.41	*001*, 003, *004*, *009*	003 Induced
230511	1.23	1.14	001, 004, 006, 009	Not induced

*Expression of probe-set target mRNA sequences was quantified by gene expression profiling 4hrs after treatment and is shown as the ratio of expression in Kinetin riboside-treated cells to DMSO vehicle-treated cells

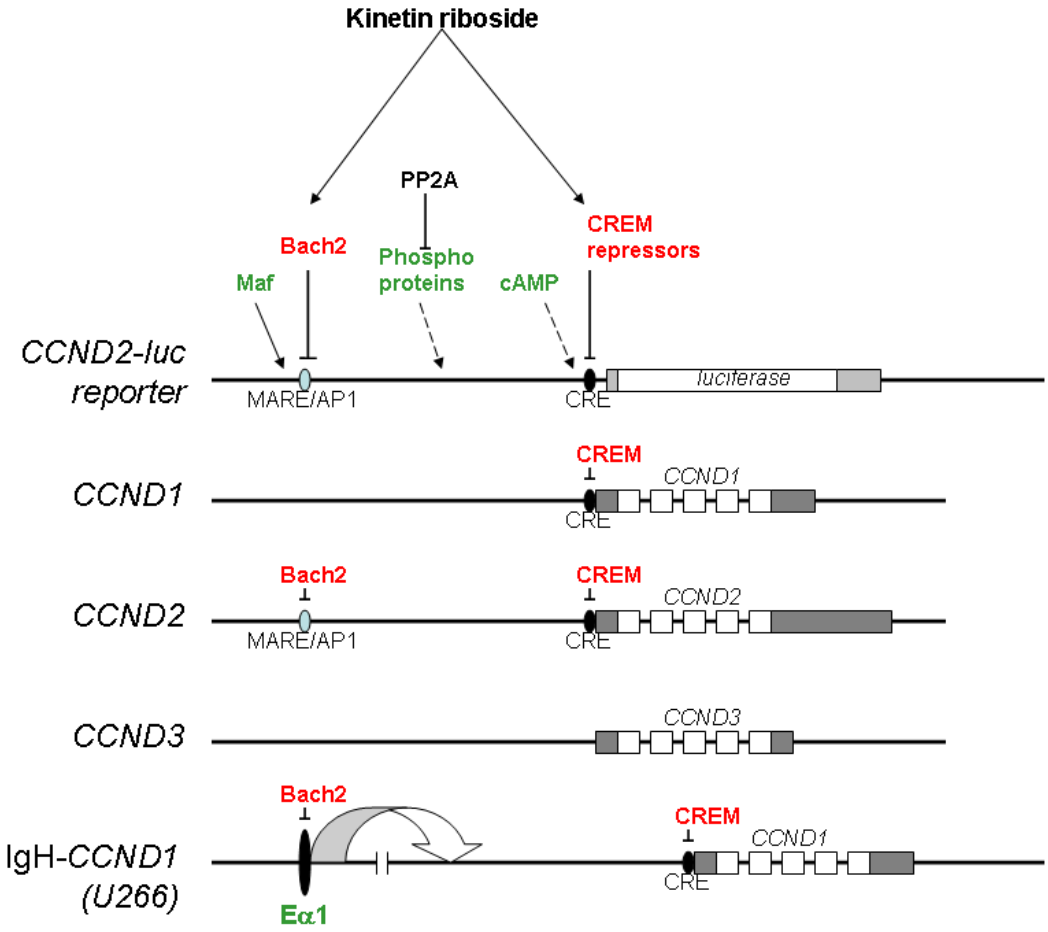
†Vega transcript nomenclature from Sanger Institute Havana Group (curated). Transcript variants in italics are potential targets of the indicated probe-set but are also targets of probe-sets that show no induced expression, excluding them as the induced transcript

D **CREM variants induced by Kinetin riboside are repressors of the cAMP response element (CRE)**

CREM transcript Variant*	Protein Isoform / Name	Function
003	hCREM type1 alpha (isoform s)	type alpha repressor isoform
005	ICER1 hCREM 2alpha-a (isoform d)	early repressor ICER isoform
010, 011, 012	Non-coding / no isoform	

*Transcript nomenclature from Sanger Institute's Havana Group, Vega database.

Supplemental Figure S5



SUPPLEMENTAL TABLE S1. Lentivirus clones and shRNA used in functional studies

Gene	Lentivirus clone	shRNA
CCND1	NM_053056.1-2322	CCGGGCCAGGATGATAAGTTCCTTTCTCG AGAAAGGAACTTATCATCCTGGCTTTTTG
CCND2	NM_001759.2-380	CCGGCCTTCCGCAGTGCTCCTACTTCTCGA GAAGTAGGAGCACTGCGGAAGGTTTTTG

SUPPLEMENTARY TABLE S2. Genes up- or down-regulated >3 fold in H929 and U266 myeloma tumor lines in response to Kinetin riboside (KR) 10μM vs control DMSO treatment at 4h, by Affymetrix microarray

Probeset	Gene	Fold change in expression		MAS5 Present(P)/Absent(A) flag			
		H929 KR:DMSO	U266 KR:DMSO	H929 DMSO	H929 KR	U266 DMSO	U266 KR
221641_s_at	ACOT9	3.4	3.9	P	P	P	P
212543_at	AIM1	7.2	6.8	A	P	P	P
208836_at	ATP1B3	3.2	3.2	P	P	P	P
218631_at	AVPI1	5.7	3.4	P	P	P	P
221234_s_at	BACH2	15.1	7.5	P	P	P	P
207571_x_at	C1orf38	6.9	4.0	P	P	P	P
210785_s_at	C1orf38	7.0	3.3	P	P	P	P
1555950_a_at	CD55	7.9	3.4	P	P	A	P
204637_at	CGA	34.4	13.0	A	P	A	P
207630_s_at	CREM	3.2	4.9	P	P	P	P
209967_s_at	CREM	3.1	5.4	P	P	A	P
206085_s_at	CTH	5.3	3.4	P	P	P	P
217127_at	CTH	5.0	3.8	P	P	P	P
212503_s_at	DIP2C	3.9	3.2	A	P	P	P
204014_at	DUSP4	7.9	22.9	P	P	A	P
204015_s_at	DUSP4	13.8	17.1	P	P	A	P
205409_at	FOSL2	3.9	4.6	A	P	A	P
218880_at	FOSL2	7.8	10.8	A	P	A	P
218881_s_at	FOSL2	4.0	5.6	A	P	A	P
225262_at	FOSL2	4.9	19.1	A	P	A	P
203397_s_at	GALNT3	9.5	5.4	P	P	P	P
214430_at	GLA	3.1	12.2	P	P	P	P
202814_s_at	HEXIM1	4.2	5.4	P	P	P	P
213793_s_at	HOMER1	3.9	7.4	P	P	P	P
226651_at	HOMER1	3.4	5.2	P	P	P	P
226352_at	JMY	4.2	3.5	P	P	P	P
241985_at	JMY	15.3	10.3	P	P	P	P
201751_at	JOSD1	4.6	3.0	P	P	P	P
212733_at	KIAA0226	3.5	3.2	P	P	P	P
219371_s_at	KLF2	0.2	0.3	P	P	P	M
222068_s_at	LRRC50	17.3	6.5	P	P	P	P
202340_x_at	NR4A1	7.3	4.1	A	P	A	P

207978_s_at	NR4A3	12.7	12.2	A	P	A	P
209959_at	NR4A3	11.4	10.5	A	P	A	P
1555167_s_at	PBEF1	6.5	4.6	P	P	P	P
217739_s_at	PBEF1	6.5	5.4	P	P	P	P
209621_s_at	PDLIM3	5.8	4.0	P	P	P	P
210170_at	PDLIM3	5.5	9.1	A	P	P	P
238592_at	PDLIM3	6.0	7.7	P	P	P	P
218319_at	PELI1	7.1	3.1	P	P	P	P
202388_at	RGS2	10.2	8.6	A	P	A	P
1553962_s_at	RHOB	5.2	3.7	A	P	P	P
212099_at	RHOB	5.2	21.4	P	P	P	P
230333_at	SAT	9.5	6.3	P	P	A	P
203455_s_at	SAT1	20.9	39.1	P	P	P	P
210592_s_at	SAT1	23.8	34.1	P	P	P	P
213988_s_at	SAT1	16.6	19.7	A	P	A	P
218872_at	TESC	6.5	3.6	P	P	P	P
224836_at	TP53INP2	4.3	3.7	A	P	P	P
204141_at	TUBB2A	6.2	3.5	P	P	A	P
208622_s_at	VIL2	4.7	3.2	P	P	P	P
210561_s_at	WSB1	3.2	3.1	P	P	P	P
217783_s_at	YPEL5	5.0	5.4	P	P	P	P
222408_s_at	YPEL5	4.6	6.6	P	P	P	P
226034_at	---	9.1	25.4	P	P	A	P
227200_at	---	4.3	3.3	P	P	P	P
227539_at	---	7.3	4.6	A	P	P	P
228188_at	---	13.9	12.7	A	P	A	P
228498_at	---	5.2	4.5	P	P	P	P
239277_at	---	3.3	3.7	M	P	P	P

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