

Supplemental Data for

Prolonged early G1 arrest by selective CDK4/CDK6 inhibition sensitizes myeloma cells
to cytotoxic killing through cell cycle-coupled loss of IRF4

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SUPPLEMENTAL FIGURE LEGENDS AND TABLE

Figure S1. Induction of early G1 arrest by CDK4/CDK6 inhibition is reversible

FACS analysis of BrdU uptake and DNA content (% of S phase cells) in HMCL CAG (A) and KMS12-PE (B) cells treated with PD (0.5 μ M) for 24 h (pG1) and released into fresh media (pG1-S) for time indicated. (C) BrdU-uptake and DNA content in MM1.S cells at 15 h after release from incubation with PD (0.3 μ M) for time indicated. (D) Percentages of Annexin V⁺ cells and live cells cultured with or without PD continuously for days indicated. PD (0.3 μ M) was added on days 0, 2, 3, 4 and 5.

Figure S2. Global gene expression in pG1 and pG1-S

(A) Hierarchical clustering analysis of gene expression in MM1.S cells treated with PD for time indicated. (B) Fold change plots of *TK1*, *PLK1*, *NEK2*, *BUB1*, *AURKA*, *AURKB* and *MCM2* shown in Figure 1E. (C) q-RT-PCR analysis of *TK1*, *ASS1* and *SCD* mRNA

Figure S3. pG1 enhances bortezomib killing

The dexamethasone-resistant MM1.R cells were cultured as diagrammed and analyzed for viable cells and percentage of apoptotic cells by JC-1 staining.

Figure S4. Lack of response to PD 0332991 in Rb-deficient cells

(A) Percentage of S phase Rb-negative REC-1 mantle cell lymphoma cells treated with PD (0.5 μ M) for 24 h was determined by PI staining. (B) BTZ killing of REC1 cells in pG1 (pretreated with PD for 24 h) was determined at 24 after incubation with BTZ (10 nM) and expressed as percentages of live cells and MT⁻ cells.

Figure S5. pG1 enhances interaction of Bim with Mcl-1 and Bcl-2

MM1.S cells in pG1 or left untreated were incubated with BTZ (4 nM) for 12 h. For immunoprecipitation, cells were lysed in a buffer containing 10 mM Tris-HCl, pH 7.5, 150 mM NaCl, 5 mM EDTA, 1% Triton X-100, and a protease inhibitor cocktail (Roche).

Lysates (50 μ g of protein) were precleared with 20 μ l of Protein G PLUS-agarose beads

(Santa Cruz Biotechnology, Santa Cruz, CA) and incubated with antibody overnight at 4°C. The immune complexes were washed 5 times with the lysis buffer and analyzed by immunoblotting. The antibodies used are summarized in Supplementary Table S1.

Figure S6. Sensitization of human primary myeloma cells to bortezomib by PD 0332991

(A-B) Primary MM cells cultured with PD (0.5 µM) for 24 h, or left untreated, were incubated with BTZ at indicated concentrations for 24 h and analyzed for viable cells. Data represent mean ± s.d. in triplicate. p-value was determined by two-tailed or one-tailed* t-test. ns, not-significantly different.

Figure S7. Cooperative tumor suppression by PD 0332991 and bortezomib

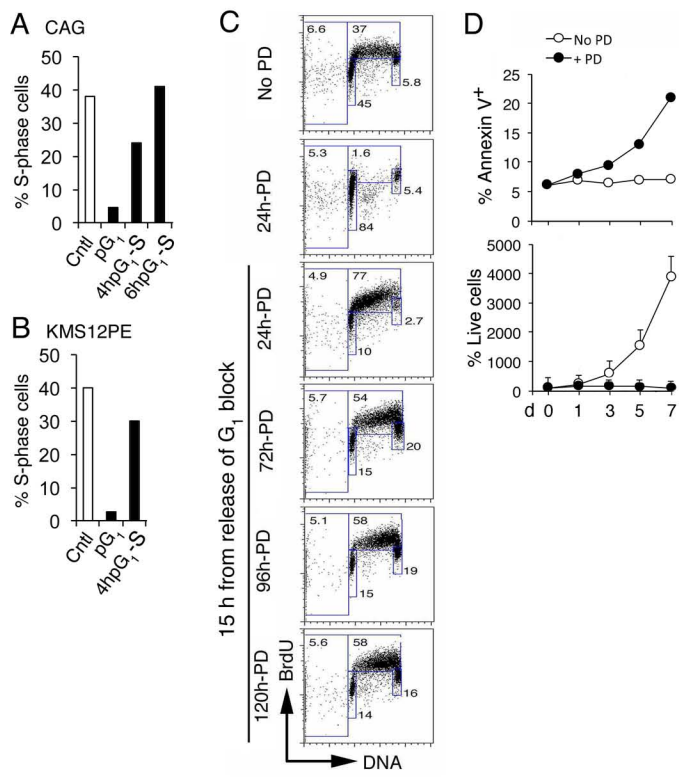
BLI analysis of tumor mass on day 9 of NOD/SCID mice treated with PD and BTZ as diagrammed in Figure 7A. V, ventral; D, dorsal.

Supplemental Table S1.

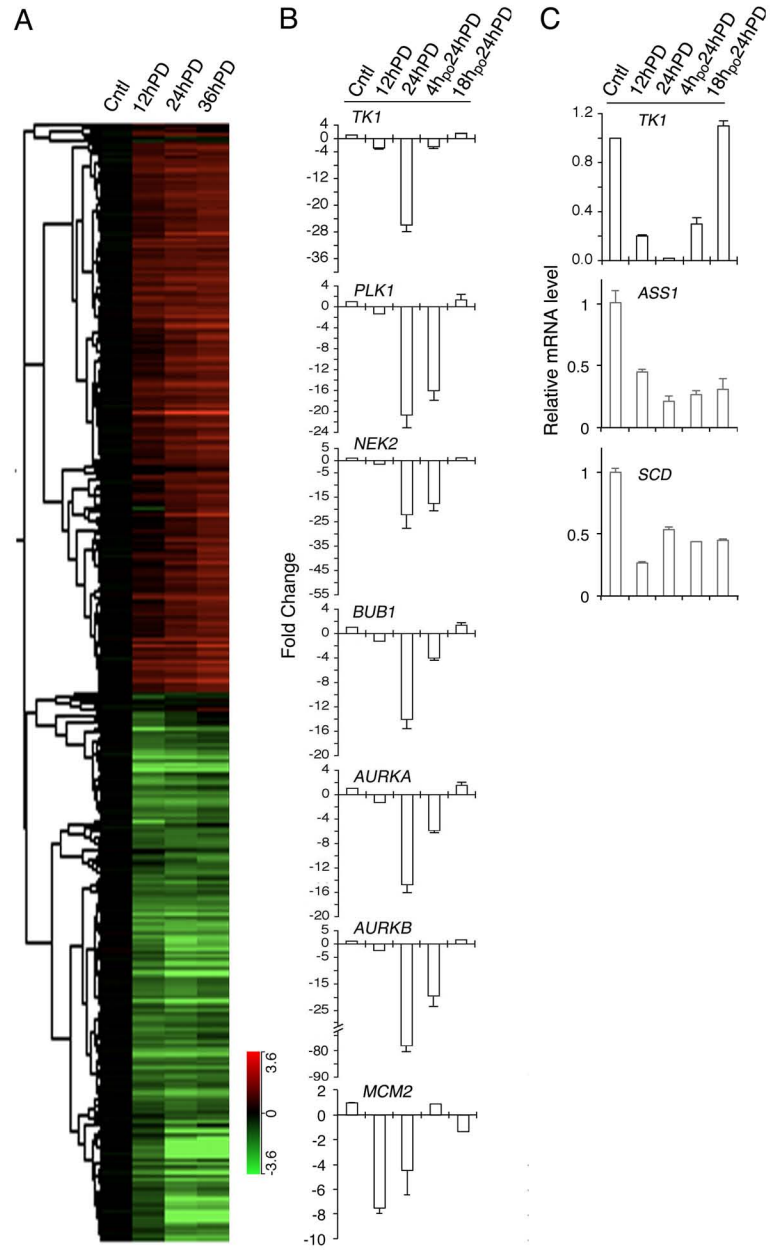
Antibodies Used in Immunoblotting and Immunoprecipitation

Antibody	Source	Species	Cat.# (clone)	Isotype
Phospho-Rb (Ser807/811)	Cell Signaling	Rabbit	9803	
Rb	BD Pharmingen	Mouse	554136 (G3-245)	IgG1
Cyclin D2 (M20)	Santa Cruz	Rabbit	sc-593	IgG
CDK4	Alexis Biochemicals	Rat	ALX804527 (3C5)	IgG2 α κ
CDK6	Cell Signaling	Rabbit	4582	
Cyclin E1	Cell Signaling	Rabbit	2002	
Cyclin A	Cell Signaling	Rabbit	4976	
CDK2	Santa Cruz	Rabbit	sc-593	IgG
p27(C-19)	Santa Cruz	Rabbit	sc-528	IgG
SKP2	Santa Cruz	Rabbit	sc-7164	
IRF4(M-17)	Santa Cruz	Goat	sc-6059	
Noxa	Alexis Biochemicals	Mouse	ALX804408 (114C307.1)	IgG1
Bim*	Alexis Biochemicals	Rat	ALX804527 (3C5)	IgG2 α κ
Bim	Cell Signaling	Rabbit	4582	
Bid	Cell Signaling	Rabbit	2002	
Puma	Cell Signaling	Rabbit	4976	
Bcl-2*	DakoCytomation	Mouse	M0887 (124)	IgG1
Bcl-2	Cell Signaling	Rabbit	2876	
Bcl-XL	Cell Signaling	Rabbit	2762	
Mcl-1**	Santa Cruz	Rabbit	sc-819	IgG
Caspase-9	Cell Signaling	Rabbit	9502	
Caspase-8	Cell Signaling	Mouse	9746	IgG1
Cleaved caspase-3	Cell Signaling	Rabbit	9661	IgG1
PARP	Cell Signaling	Rabbit	9542	
Tubulin	Cell Signaling	Rabbit	2144	
Actin	Santa Cruz	Rabbit	sc-1615-R	IgG

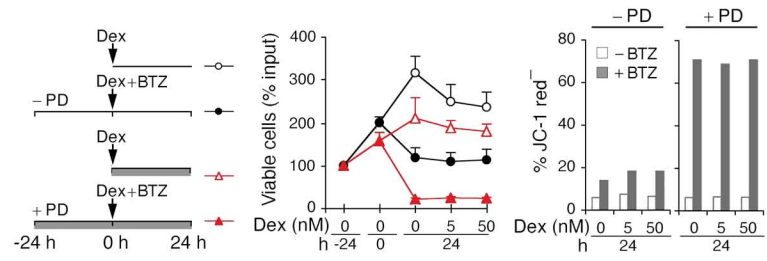
Antibodies were used for immunoblotting, immunoprecipitation (*), or both (**).



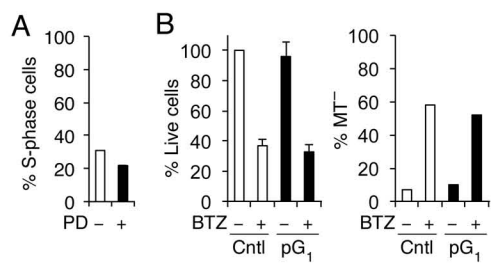
Huang et al_Figure S1



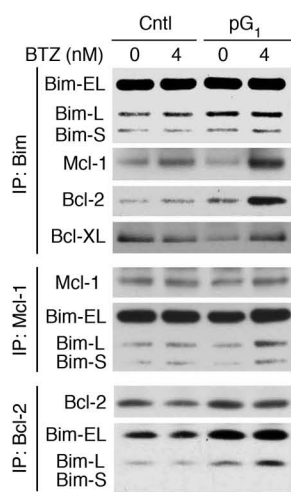
Huang et al_Figure S2



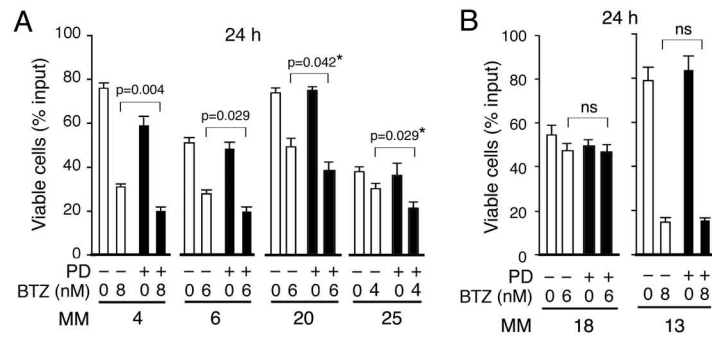
Huang et al_Figure S3



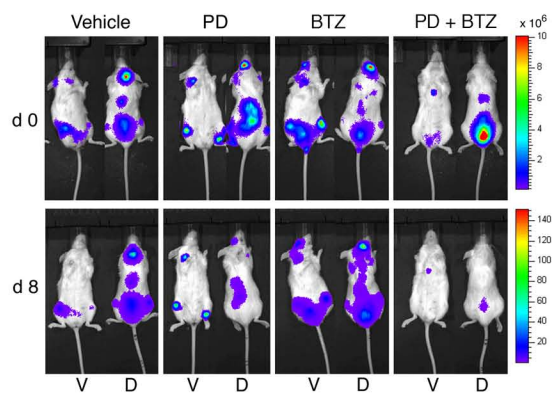
Huang et al_Figure S4



Huang et al_Figure S5



Huang et al_Figure S6



Huang et al_Figure S7