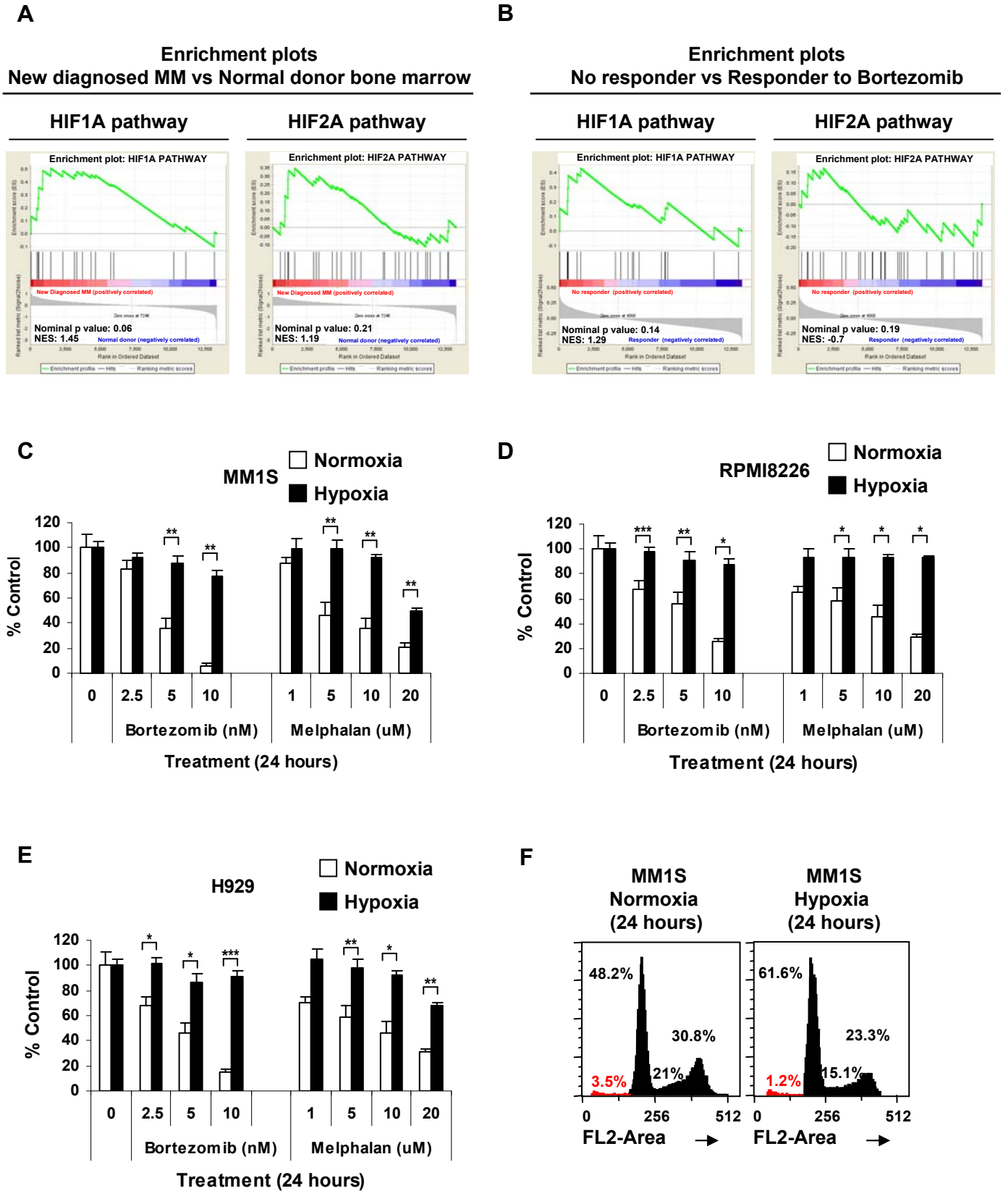
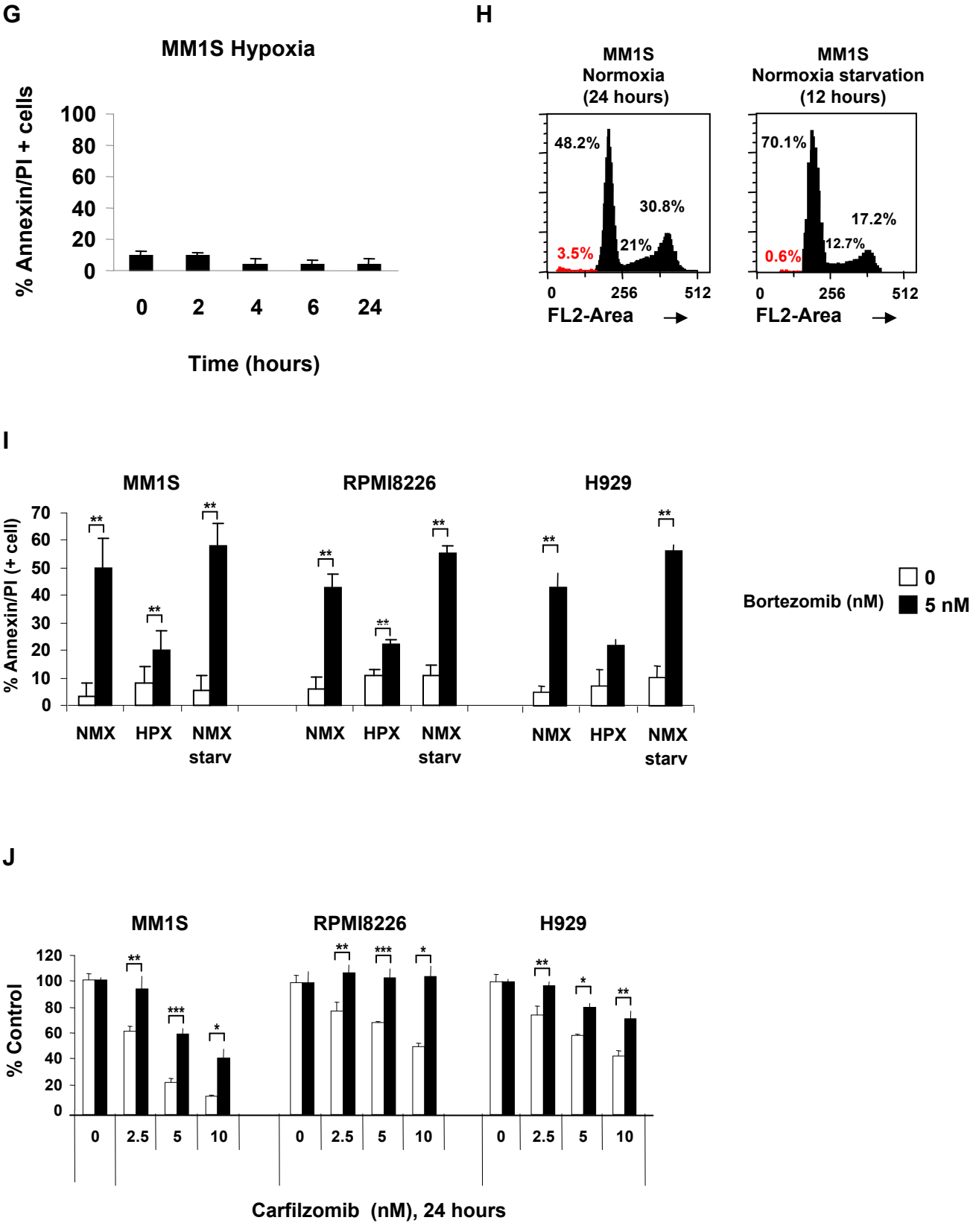
Supplemental Figure 1

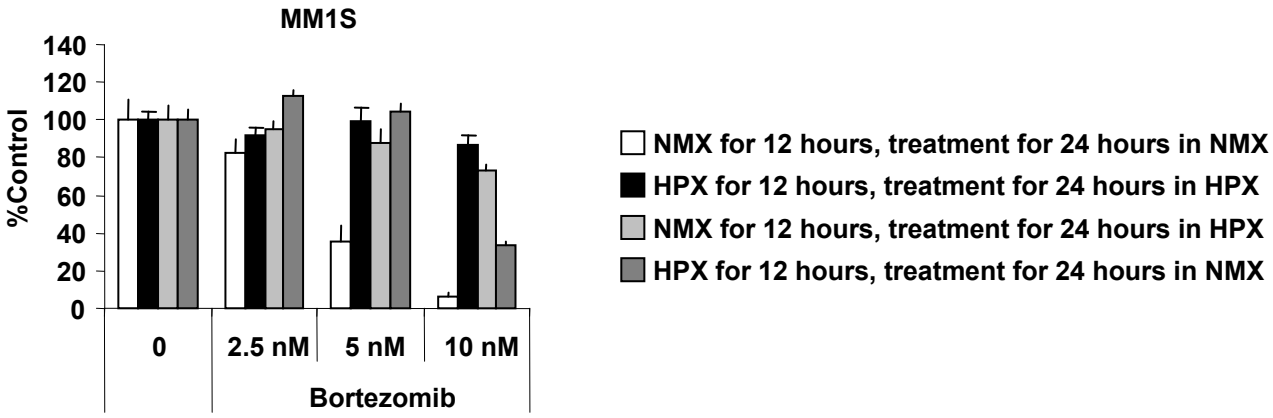


Supplemental Figure 1



Supplemental Figure 1

K



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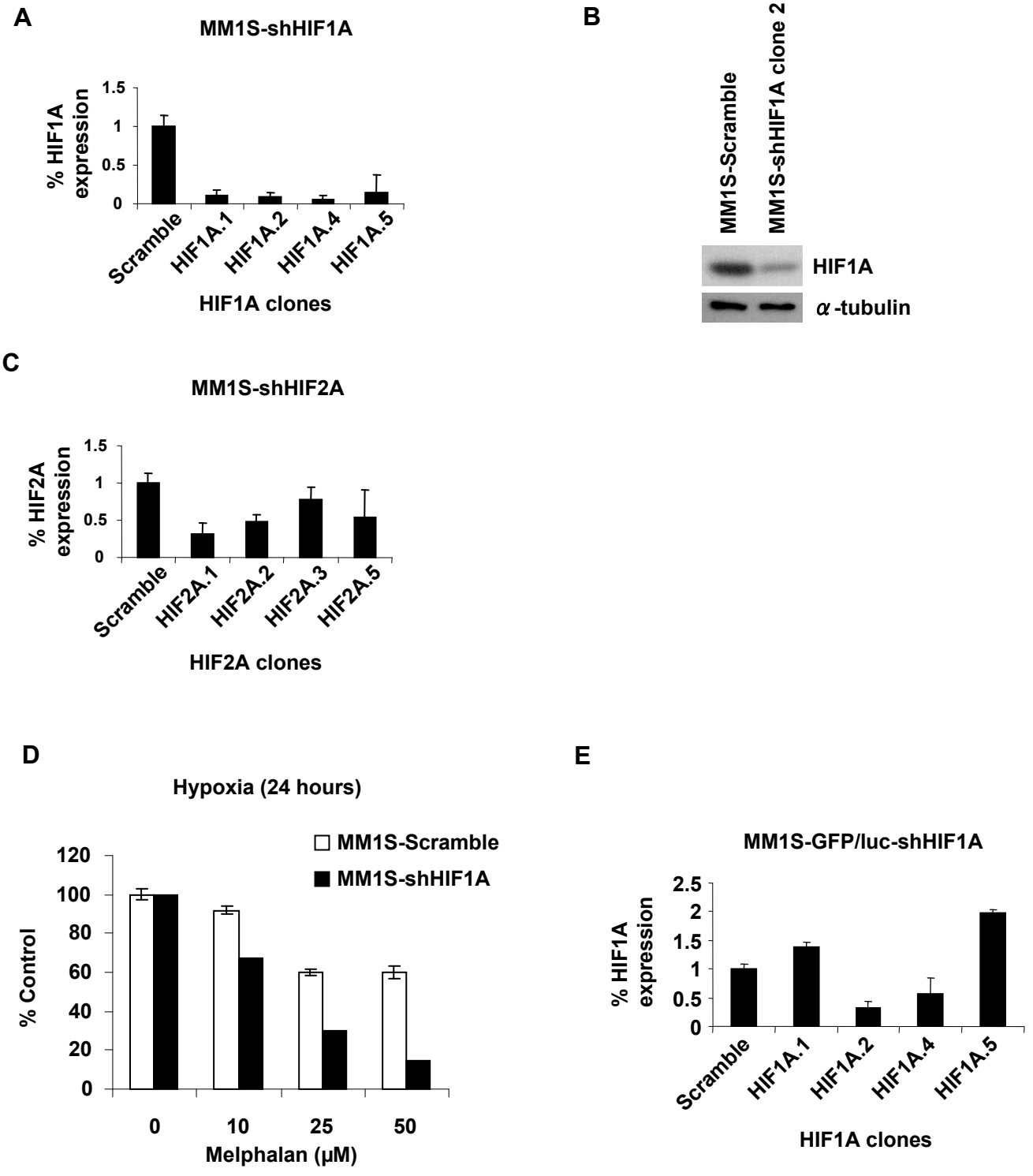

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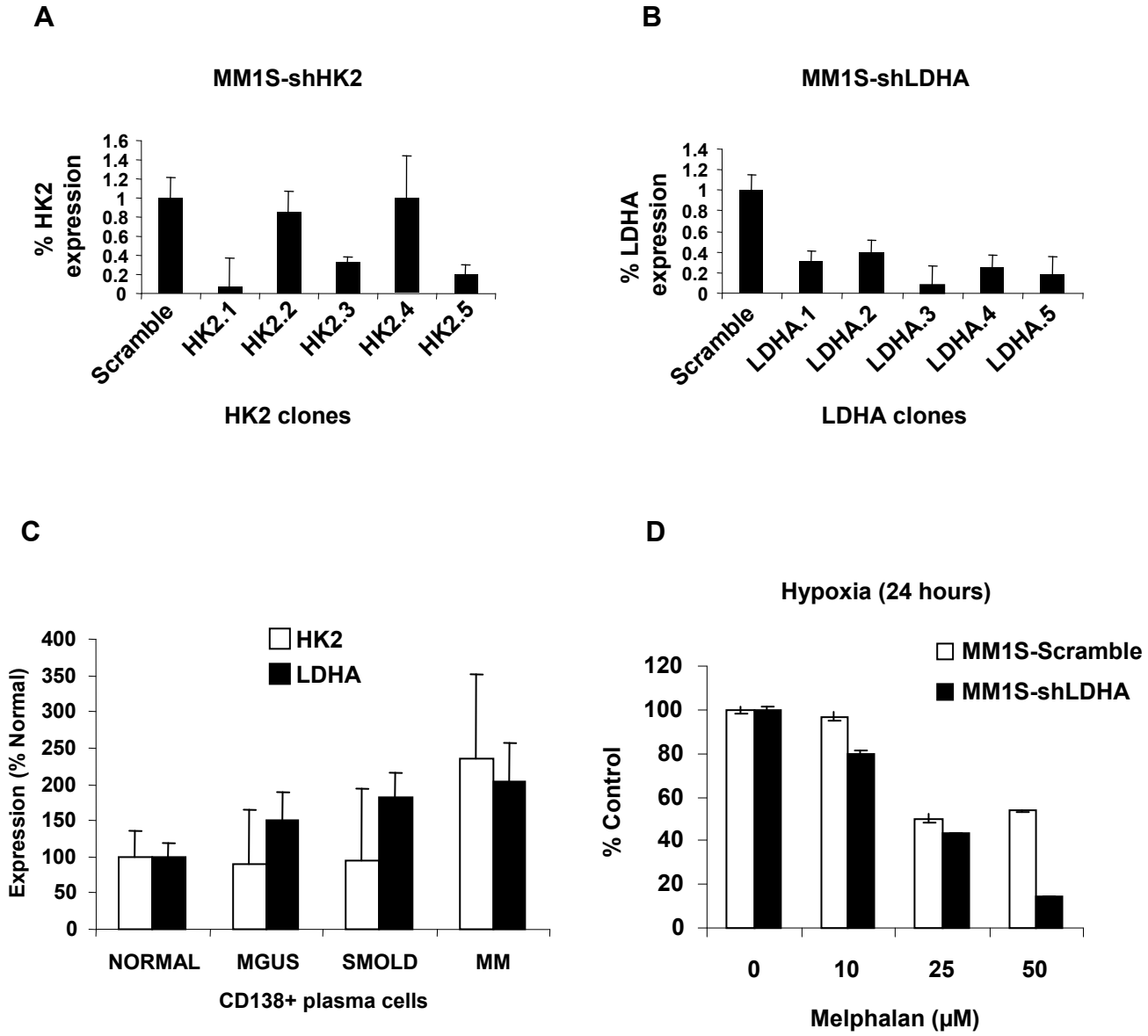
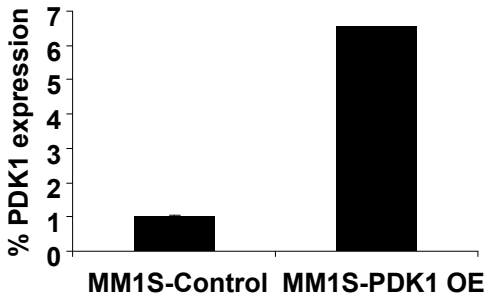


Supplemental Figure 3

E



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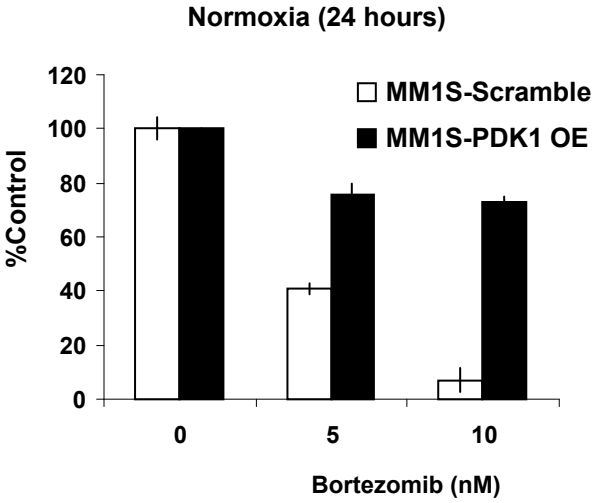

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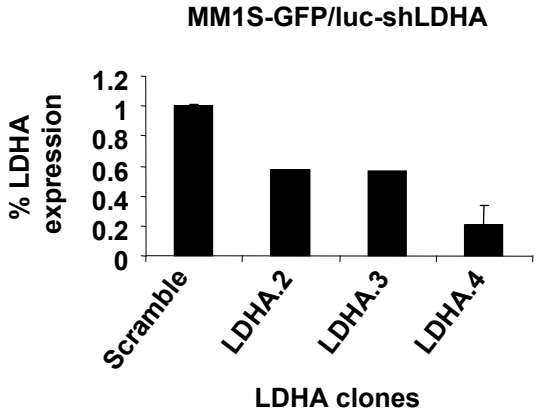
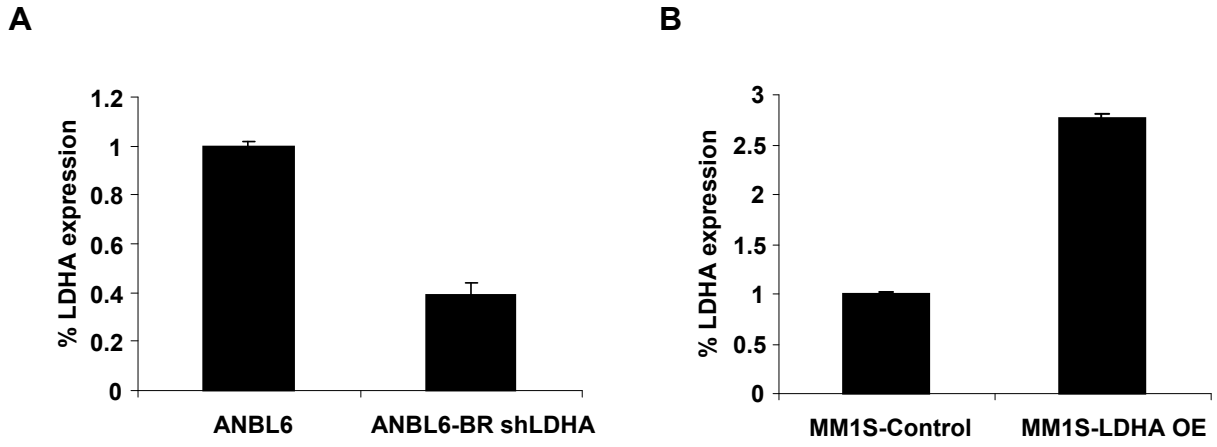


Supplemental Figure 4



SUPPLEMENTAL FIGURES

Supplemental Figure 1. Hypoxia-induced drug resistance on MM cells.

(A and B) GSEA analysis was performed in gene expression data from two independent sets of CD138+ cells, figures show the GSEA profiles of the HIF1A and HIF2A pathways in **(A)** newly diagnosed myeloma patients and normal donors (GSE6477) and in **(B)** no responder and responder patients to bortezomib (GSE9782). **(C and D)** Cytotoxicity was assessed by MTT assay in MM cell lines. **(C)** MM1S **(D)** RPMI8226 and **(E)** H929 were cultured in 20% O_2 (normoxia, NMX) or 1% O_2 (hypoxia, HPX) for 12 hours before treatment with bortezomib (2.5-10 nM) or melphalan (1-20 nM) and then continued for 24 hours more. The average proliferation values of control untreated samples were taken as 100%. Points, mean of quadruplicates of an experiment that was repeated at least three times. **(F)** Effect of hypoxia on cell cycle after 24 hours in MM1S cells. **(G)** Effect of hypoxia on apoptosis was performed using Annexin/PI staining and flow cytometric analysis after 2, 4, 6 and 24 hours in MM1S cells. **(H)** Effect of hypoxia on cell cycle after 12 hours of serum starvation in MM1S cells. **(I)** MM1S, RPMI8226 and H929 cells were treated with bortezomib (5 and 10 nM) under normoxic and hypoxic conditions for 24 or starved for 12 hours and apoptosis was performed using Annexin/PI staining and flow cytometric analysis. *$p<0.05$, ** $p<0.005$, and *** $p<0.0005$. **(J)** Cytotoxicity was assessed by MTT assay in MM cell lines. MM1S, RPMI8226 and H929 cells were cultured with carfilzomib (2.5-10 nM) hours. The average proliferation values of control untreated samples were taken as 100%. Points, mean of quadruplicates of an experiment that was repeated at least three times. **(K)** Cytotoxicity was assessed by MTT

assay in MM cell lines. MM1S were cultured in 20% O_2 (normoxia, NMX) or 1% O_2 (hypoxia, HPX) for 12 hours. After this time, we incubate the cells with bortezomib (2.5-10 nM) or melphalan (1-20 nM) for 24 hours more under normoxic and hypoxic conditions. The average proliferation values of control untreated samples were taken as 100%. Points, mean of quadruplicates of an experiment that was repeated at least three times. *p<0.05, ** p<0.005, and *** p<0.0005.

Supplemental Figure 2. HK2, PFKFB3, PFKFB4 and LDHA expression in MM cell lines. Relative levels of glycolytic enzymes (HK2, PFKFB3, PFKFB4, and LDHA) were measured by qRT-PCR in **(A)** MM1S, **(B)** H929 and **(C)** RPMI8226 cell lines under normoxic and hypoxic conditions.

Supplemental Figure 3. (A-B) HIF1A expression in MM1S-shHIF1A. Relative levels of HIF1A measured by (A) qRT-PCR and (B) western blot in MM1S. **(C) HIF2A expression in MM1S-shHIF2A.** Relative mRNA levels of HIF2A in MM1S cells measured by qRT-PCR. **(D) Stable knockdown of HIF1A restores the effect of melphalan under hypoxic conditions.** MM1S scramble and MM1S-shHIF1A cells were incubated with Melphalan (10, 25 and 50 μM) for 24 hours under hypoxic conditions and cell viability was evaluated by MTT. Graphs and bars represent the mean ± s.d. for cell viability in three independent experiments performed in four replicates. **(E) HIF1A expression in MM1S-shHIF1A.** Relative mRNA levels of HIF1A in MM1S cells measured by qRT-PCR.

Supplemental Figure 4. (A-B) HK2 and LDHA expression in MM1S-shHK2, MM1S-shLDHA. Relative mRNA levels of HK2 **(A)** and LDHA **(B)** in MM1S measured by qRT-PCR. **(C)** Relative mRNA levels of HK2 and LDHA in CD138 + cells

from normal donors bone marrow, MGUS, Smoldering myeloma and newly diagnosed myeloma (GSE6477). **(D) Stable knockdown of LDHA partially restore the effect of melphalan under hypoxic conditions.** MM1S scramble, MM1S-shHIF1A and MM1S-shLDHA cells were incubated with Melphalan (10, 25 and 50 μM) for 24 hours under hypoxic conditions and cell viability was evaluated by MTT. Graphs and bars represent the mean ± s.d. for cell viability in three independent experiments performed in four replicates. **(E) PDK1 expression in MM1S-PDK1 expressing cells.** Relative mRNA levels of PDK1 in MM1S cells measured by qRT-PCR. **(F) Stable overexpression of PDK1 induces resistance to bortezomib in MM1S cells.** MM1S scramble and MM1S-PDK1 expressing cells were incubated with Bortezomib (5 and 10 nM) for 24 hours and cell viability was evaluated by MTT analysis. Graphs and bars represent the mean ± s.d. for cell viability in three independent experiments performed in four replicates. **(G)** Relative mRNA levels of LDHA in MM1S-GFP/luc cells measured by qRT-PCR.

Supplemental Figure 5. LDHA and HIF1A expression in ANBL6-BR-shLDHA, MM1S-HIF1A OE and in MM1S-LDHA OE cells. Relative mRNA levels of LDHA in (A) ANBL6-BR and in (B) MM1S-LDHA OE cells measured by qRT-PCR. **(C) HIF1A expression in MM1S-HIF1A OE cells measured by western blot. (D) LDHA expression in MM1S-LDHA OE cells measured by western blot.**

SUPPLEMENTAL MATERIALS AND METHODS

Metabolite profiling

Data were acquired using a 5500 QTRAP triple quadrupole mass spectrometer (AB/Sciex) coupled to a Prominence UFLC system (Shimadzu) via selected reaction monitoring (SRM) of a total of 289 endogenous water soluble metabolites for steady-state analyses of samples.

Metaboanalyst software was used for analysis. Metabolite levels were normalized to the total of all metabolites detected on a triplicate set of cells treated identically to the experimental cells.

RNA purification, reverse transcription and quantitative RT-PCR (qRT-PCR)

The primers sequences for the qRT-PCR are:

Gene	**Primers**
human *HIF1A*	F: TTGGACACTGGTGGCTCATTAC
	R: TGAGCTGTCTGTGATCCAGCAT
human *HIF2A*	F: GTGTTGTGGACACTGCAGACTTGT
	R: ATGACTCCACTGCTCGGATTGTCA
human *HK2*	F: AGCCCTTTCTCCATCTCCTT
	R: AACCATGACCAAGTGCAGAA
human *LDHA*	F: GGAGATCCATCATCTCTCC
	R: GGCCTGTGCCATCAGTATCT

human *18S*	F: TCAACTTTCGATGGTAGTCGCCGT
	R: TCCTTGGATGTGGTAGCCGTTTCT

***In vivo* studies**

Mice were treated with bortezomib, 0.75 mg/Kg in PBS once weekly by intraperitoneal (IP) injection beginning 10 days after tumor implantation until moribund. Mice with different stages of tumor development based on tumor size detected by bioluminescence (BLI) were treated with the hypoxia marker pimonidazole hydrochloride (PIMO; 100 mg/kg by IP injection; Hypoxyprobe Store). After 4 hours, BM was isolated from one femur by flushing with cold PBS and prepared for RNA isolation as described; the other femur was used for immunohistochemistry (IHC).

In vivo tumor growth has been assessed by using *in vivo* bioluminescence imaging. Mice were injected with 75 mg/kg of Luciferin (Xenogen, Hopkington, MA), and tumor growth was detected by bioluminescence 3 min after the injection, using Xenogen In Vivo Imaging System (Caliper Life Sciences, Hopkinton, MA). Mice were monitored and sacrificed when they developed side effects of tumor burden in accordance with approved protocol of the Dana-Farber Cancer Institute (DFCI) Animal Care and Use Committee.

Knockdown constructs

A scrambled shRNA sequence was used as control (TRCN0000072212).

Lentiviral shRNAs were obtained from The RNAi Consortium (TCR) collection of the Broad Institute. The TCR numbers for the shRNA used are:

Gene	Clones
human *HIF1A*	TRCN0000003809
	TRCN0000003810
	TRCN0000010819
	TRCN0000318675
	TRCN0000349634
human *HIF2A*	TRCN0000003803
	TRCN0000003804
	TRCN0000003805
	TRCN0000352630
	TRCN0000342501
human *HK2*	TRCN0000037670
	TRCN0000195171
	TRCN0000196260
	TRCN0000232927
	TRCN0000232928
human *LDHA*	TRCN0000026538
	TRCN0000026536
	TRCN0000158762
	TRCN0000164922
	TRCN0000166246

Statistics

P values described in the *in vitro* assays are based on T-tests (two-tailed; α 0.05). *P* values are provided for each figure.