Bifurcated BACH2 control coordinates mantle cell lymphoma survival and dispersal during hypoxia

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Supplemental Methods

Cell lines and plasmids

Human MCL cell line Jeko was obtained from the ATCC (Manassas, VA). Human MCL SP53 cell line was a kind gift from MD Anderson Cancer Center (MDACC). HS5 bone marrow stromal cells (BMSCs) were a kind gift from Dr. B. Torok-Storb (Fred Huchinson Cancer Research Center, Seattle, WA). Cell lines were authenticated using short tandem repeats in Characterized Cell Line Core Facility at MDACC within 6 months. HA-HIF-1α-pcDNA3 expression plasmids were gifts from William Kaelin (Addgene plasmid # 18949)[1]. pEGFP-BACH2 plasmid was constructed by sub-cloning BACH2 coding sequence from human pLOC-BACH2 plasmid (GE Dharmacon; Clone ID: PLOHS_100066339) and inserting into pEGFP-N1 vector at XhoI and BamHI sites. The construction of pEGFP-BACH2 plasmid was further verified by sequencing.

Cell culture and MCL sample preparation

Cells were maintained under standard conditions (5% CO_2, 37°C) and cultured in complete RPMI1640 medium (Cellgro, Manassas, VA) supplemented with 10% fetal bovine serum (FBS), 2mM L-glutamine, and 100 U.I. /mL penicillin/streptomycin. Mononuclear cells from MCL patients were isolated from apheresis blood, bone marrow (BM) or spleen (SP) using standard Ficoll (GE Healthcare, Pittsburgh, PA) gradient separation methods. $CD19^+$ B cells were isolated as previously described[2].

Chemicals and Antibodies

Bortezomib was acquired from MDACC drug repository. Cycloheximide (CHX), $CoCl_2$ and MG132 were purchased from Sigma-Aldrich (St. Louis, MO). KC7F2 was purchased from Selleck Chemicals (Houston, TX). Fixable viability dye eFluor™ 780 was purchased from Thermo Fisher Scientific (Waltham, MA). Anti-human-CD45-APC, anti-human-CD19-FITC, anti-human-CD19-APC and anti-human-Ki-67-FITC were purchased from BD Biosciences (San Jose, CA) for FACS analysis. The following antibodies were used for immunoblots: anti-BACH2 (AbCam, Cambridge, MA), anti-IRF4 (eBioscience, San Diego, CA), anti-HIF-1α and anti-PHD3/EGLN3 (Novus Biologicals, Littleton, CO), anti-HO-1 and anti-GAPDH (Cell Signaling, Danvers, MA).

Generation of single guide RNA (sgRNA) expression vectors

To establish a lentiviral CRISPR-Cas9-mediated knockout system, the MIT CRISPR design software was used for the design of sgRNAs (http://crispr.mit.edu). sgRNA sequences with a minimal number of off-target sites in the human genome were selected. 25-bp oligonucleotides containing the sgRNA sequences were synthesized (Sigma-Aldrich, St. Louis, MO), and constructed into the LentiCRISPR vector. sgRNA sequences are as follows: *BACH2*, 5'-TCGCACAGCGCTAACGTGAG-3′; *HIF-1α*, 5'-GATCTCGGCGAAGTAAAGAA-3'; *PHD3*, 5'-CGATTCTGCGGGCGAGATGC-3'. The sgRNA expression vectors were generated and verified as previously described[3]

Generation of lentivirus constructs and infection

To generate BACH2 knockout ($BACH2^{KO}$), HIF-1α knockout ($HIF\text{-}1\alpha^{KO}$) or PHD3 knockout ($PHD3^{KO}$) lentiviruses, 293FT cells were transfected with LentiCRISPR plasmids specific for BACH2, HIF-1α or PHD3; a lentivirus empty vector LentiCRISPRv2 was used as a negative

control. To generate BACH2 overexpressed lentivirus, 293FT cells were transfected with human BACH2 (GE Dharmacon; Clone ID: PLOHS_100066339). Lentiviruses were produced as previously described[4]. Lentiviral-transduced cells were selected with puromycin (2 µg/mL) or blasticidin (5 µg/mL) for 10 days. Dead cells were removed by standard Ficoll gradient separation methods during antibiotics selection. Stable cells were generated and confirmed by immunoblot analysis.

Cell viability assay

To determine cell growth, viable cells were counted using Trypan Blue Solution (Corning, Manassas, VA). Cytotoxicity was assessed with Fluorimetric cell viability assay using CellTiter-Blue® (Promega, Madison, WI), as previously described[2].

Cell division and cell survival assays

BACH2^{ko} or control MCL cells were allowed to cycle for 6 days. During this time-course, cells were stained with PKH26 dye, and its intensity was tracked using a LSR-II flow cytometer (BD Biosciences, San Jose, CA). The FACS data were further analyzed using a FlowJo software. Meanwhile, cell survival was monitored by staining cells with Annexin V/7-AAD using a PE Annexin V Apoptosis Detection kit (BD Biosciences, San Jose, CA) according to the manufacturer's protocol.

Cell cycle analysis

MCL cells were stained with propidium iodide (PI). After staining, cells were promptly run through a LSR-II flow cytometer to determine the cell cycle distribution. The FACS data were further analyzed using a FlowJo software.

Quantitative real-time PCR (qRT-PCR) and primers

Procedures for mRNA analysis by qRT-PCR were performed as previously described[2]. The relative expression level of each gene was normalized to the *GAPDH* by the method of $2^{-\Delta\Delta Ct}$. The involved genes and primers are shown as follows: *PRDM1* 5'-GCCAAGTTCACCCAGTTTGT-3', 5'-GATTCGGGTCAGATCTTCCA-3'; *IRF4* 5'-GCCAGAGCAGGATCTACTGG-3', 5'-GGGTCTGGAAACTCCTCTCC-3'; *BACH2* 5'-ACACGAGGACTGCGAGAACT-3', 5'-GGGCTTCTTCCTTCTCTGCT-3'; *PHD3* 5'-TCAGATCGTAGGAACCCACA-3', 5'- CTTCAGTGAGGGCAGATTCA-3'; *S100A2* 5'-GGGAGAAAGTGGATGAGGAG-3', 5'- ACAGTGATGAGTGCCAGGAA-3'; *HMOX1* 5'-GAGACTCCCAGAGGGAAGC-3', 5'- TCACATGGCATAAAGCCCTA-3'; *PHD2* 5'-GCCCAGTTTGCTGACATTGAAC-3', 5'- CCCTCACACCTTTTTCACCTGTTAG-3'. Primers for cell cycle related genes, *IL-6*, *IL-6R*, *IL-6ST* and GAPDH were provided as described before[3].

MethoCult colony assay

MCL cells (5×10^3) were suspended in 1 mL of complete MethoCult medium and plated onto 35mm petri dishes. Colonies were cultured, counted and photographed as previously described[3].

Tumor xenografts

Manipulated SP53 MCL cells were injected into NOD/SCID mice via subcutaneous injection (s.c., 3.5×10^6 /mouse) or intravenous injection (i.v., 3×10^6 /mouse). Mice were sacrificed 4 weeks (s.c.) or 8 weeks (i.v.) post-injection; the subcutaneous tumors, spleen and bone marrow were isolated for further analysis. The tumor volumes were measured as previously described[4]. The % population of human leukocyte cells from i.v. xenograft organs was determined by Anti-human-CD45-APC antibodies based on live cells stained with DAPI. The FACS data were analyzed using a FlowJo software. $CD45^+$ human cells from xenograft organs via s.c. injection were isolated by magnetic bead-activated cell sorting (MACS) using human CD45-MicroBeads

following the manufacturer's instructions (Miltenyi Biotec, Bergisch Gladbach, Germany). The characteristics of xenograft mice are described in detail in **supplemental Table 2**.

Cell adhesion assay

Cell adhesion assay was performed as previously described[2]. PKH26 dye intensity was measured using an Infinite®M1000 (TECAN, Morrisville NC) fluorescent plate reader. Representative pictures were taken using the Olympus DP71 digital camera on an Olympus IX70 microscope.

Ki-67 expression analysis

Primary cells from MCL patients were pre-stained with anti-human-CD19-APC antibodies and fixable viability dye eFluor™ 780 for 20 min on ice followed by fixation using a Fixation/Permeabilization Solution kit (BD Biosciences, San Jose, CA) according to the manufacturer's protocol. The fixed cells were then stained with anti-human-Ki-67-FITC antibodies for 20 min on ice before running through a LSR-II flow cytometer. $CD19^+$ B cells were gated based on live cells stained with fixable viability dye. Ki-67 levels were then determined within $CD19^+$ B cells. The FACS data were further analyzed using a FlowJo software.

Immunoblotting and semi-quantitative analysis

Total harvested cells were lysed to perform immunoblotting assay as previously described[2]. Immunoblotting was subjected to semi-quantitative analysis using an ImageJ software. The relative expression levels of proteins were normalized to GAPDH.

Generation of truncated promoter constructs

Four truncated human *BACH2* promoters containing different numbers of putative HREs sites (referred to as HRE1 [-510 to -215], HRE2 [-980 to -215], HRE3 [-1051 to -215] and HRE4 [-

1051 to +424], respectively) were amplified by PCR from genomic DNA. These PCR products were inserted into pGL2-basic vector (Promega, Madison, WI) at MluI and XhoI sites to make constructs of pGL2-HRE1, pGL2-HRE2, pGL2-HRE3 and pGL2-HRE4, respectively. These truncated promoter constructs were further verified by sequencing.

Luciferase activity assay

293T cells (3×10^4) were cultured in a 96-well plate overnight and transfected with firefly luciferase vectors using the calcium phosphate transfection method. The *Renilla* luciferase reporter pRL-SV40 was co-transfected as an internal control for normalization. The pGL2-basic empty vector was used as a negative control. During 48 h transfection, cells were either treated with $CoCl_2$ at 24 h prior to harvest or cultured at 1% O_2 for 4 h with or without KC7F2 treatment prior to harvest. Cells cultured under normal conditions or without chemical treatment were used as controls.

Chromatin immunoprecipitation (ChIP)

SP53 MCL cells were cultured at 1% O_2 for 4 hours or in normal conditions. Chromatin was immunoprecipitated with antibodies against HIF-1α or control IgG. Total chromatin before immunoprecipitation (input) was used as a positive control for PCR. The primers used for PCR amplification of immunoprecipitated chromatin fragments are listed in **supplemental Table 3**.

Statistical analysis

Data reported are described as experimental mean ± standard deviations. Statistical significance of differences between control and experimental groups was evaluated by the Student t-test, where $*p < 0.05$ and $**p < 0.01$ are considered statistically significant. All experiments and assays were repeated at least twice and performed in duplicate or triplicate.

References

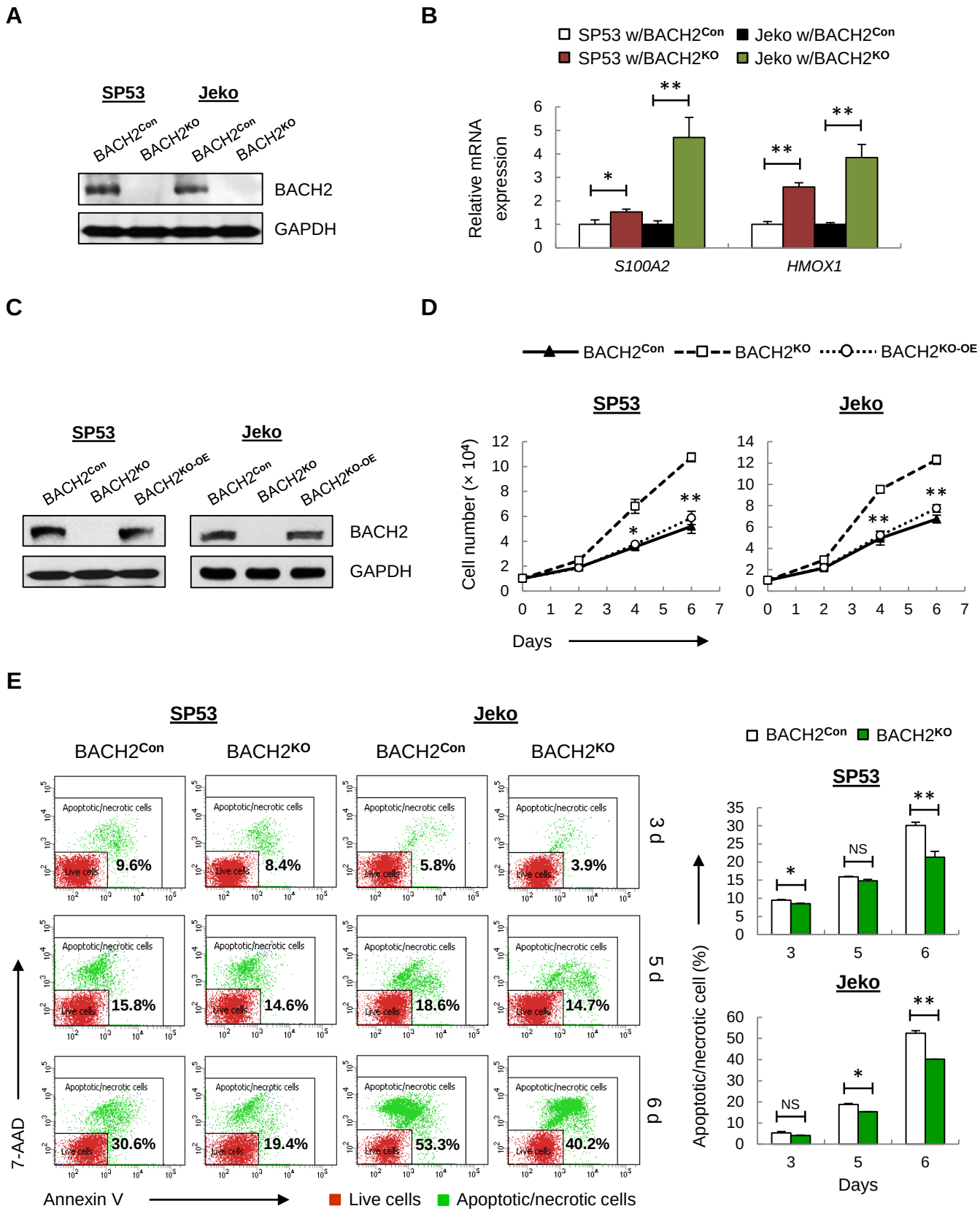
1. Kondo K, Klco J, Nakamura E, Lechpammer M, Kaelin WG, Jr. Inhibition of HIF is necessary for tumor suppression by the von Hippel-Lindau protein. *Cancer Cell*. 2002;1(3):237-246.

2. Zhang H, Chen Z, Neelapu SS, Romaguera J, McCarty N. Hedgehog inhibitors selectively target cell migration and adhesion of mantle cell lymphoma in bone marrow microenvironment. *Oncotarget*. 2016;7(12):14350-14365.

3. Zhang H, Chen Z, Miranda RN, Medeiros LJ, McCarty N. TG2 and NF-kappaB Signaling Coordinates the Survival of Mantle Cell Lymphoma Cells via IL6-Mediated Autophagy. *Cancer Res*. 2016;76(21):6410-6423.

4. Teo AE, Chen Z, Miranda RN, McDonnell T, Medeiros LJ, McCarty N. Differential PAX5 levels promote malignant B-cell infiltration, progression and drug resistance, and predict a poor prognosis in MCL patients independent of CCND1. *Leukemia*. 2016;30(3):580-593.

Supplemental Figure 1



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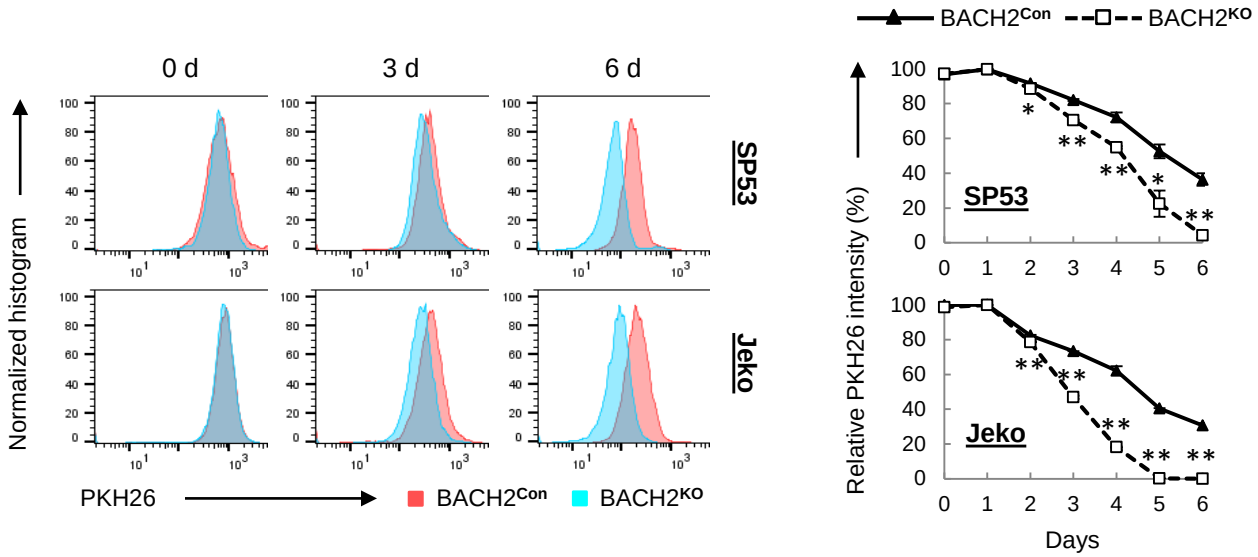
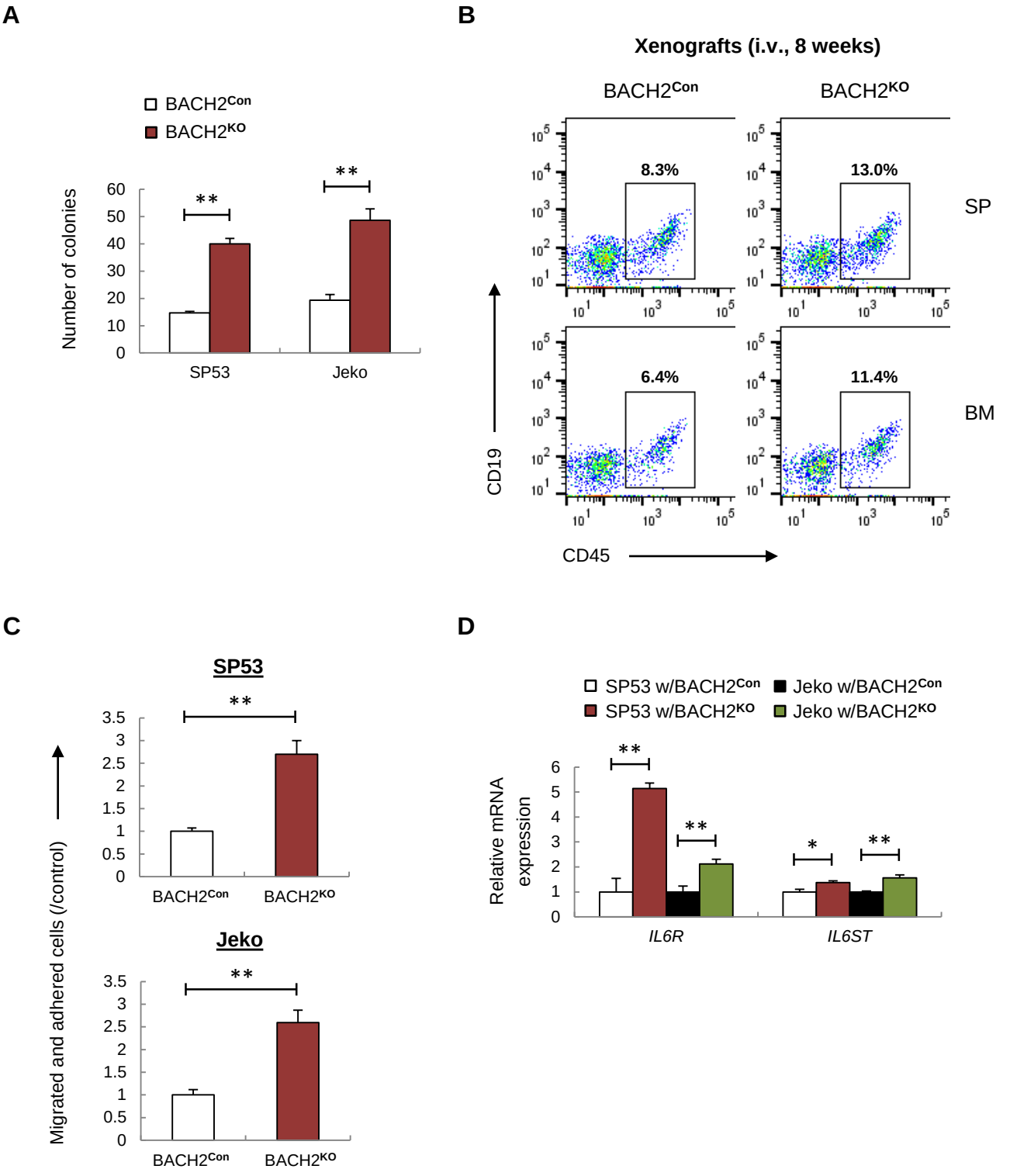


Supplemental Figure 1

(A) The knockout efficiency of BACH2 in MCL cells using immunoblots with GAPDH as a loading control. The control cells were mock-transfected cells (BACH2^{Con}). **(B)** *S100A2* and *HMOX1* mRNA levels were measured using qRT-PCR in MCL cells with BACH2^{KO} or BACH2^{Con}. Each value in qRT-PCR was normalized to *GAPDH* and represents the mean ± S.D. from three independent experiments. *p < 0.05; **p < 0.01 (vs. control cells; Student's *t*-test). **(C)** Validation of re-introduction of BACH2 in BACH2^{KO} MCL cells using immunoblotting. **(D)** Cell growth in each cell line was detected using Trypan Blue staining. *p < 0.05; **p < 0.01 (vs. BACH2^{KO} cells; Student's *t*-test). **(E)** BACH2^{KO} or control MCL cells were allowed to cycle for 6 days. During this time-course, cell survival was monitored by staining cells with Annexin V/7-AAD at days 3, 5 and 6. Representative FACS analyses are shown (*Left*). The % population of apoptotic/necrotic cells in each group is shown as the mean ± S.D. (*Right*). **(F)** BACH2^{KO} or control MCL cells were stained with PKH26 fluorescent dye, which was analyzed every day using FACS. Representative FACS analyses at days 0, 3 and 6 are shown (*Left*). The relative PKH26 intensity in each group is shown (*Right*). NS, not significant; *p < 0.05; **p < 0.01 (vs. control cells; Student's *t*-test).

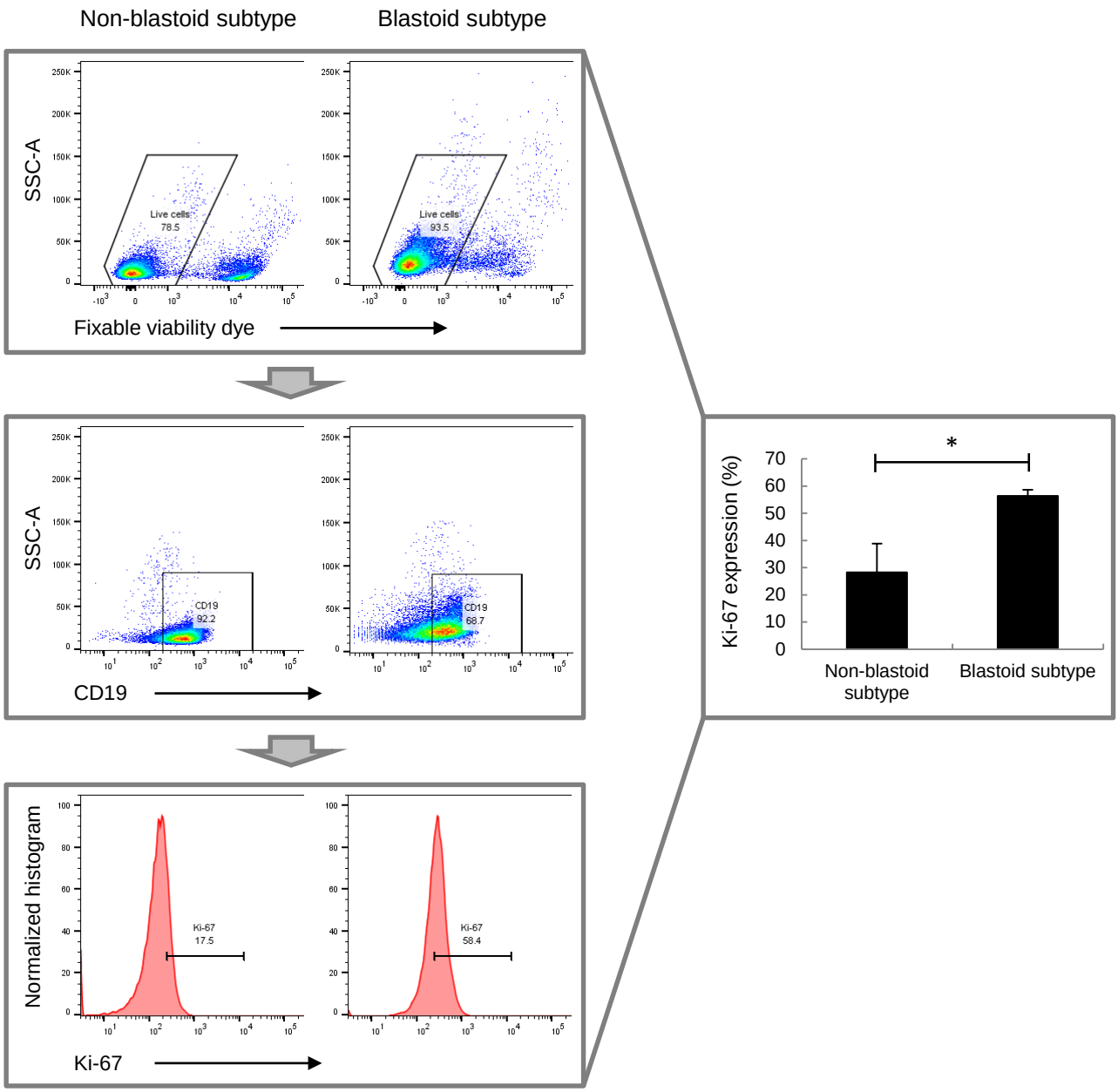
Supplemental Figure 2



Supplemental Figure 2

(A) The average number of colonies from three parallel assays in each group is shown in the diagram. **(B)** Representative FACS analyses of SP and BM cells from xenograft mice injected with BACH2^{Con} or BACH2^{KO} SP53 MCL cells (i.v., 3 x 10^6/mouse) after 8 weeks. Black boxes represent CD45$^+$ human leukocyte cells. **(C)** PKH26 dye intensity in BACH2^{KO} cells were normalized to the control. The results are shown as the mean $\pm$ S.D. **(D)** The mRNA levels of *IL6R* and *IL6ST* in BACH2^{KO} or BACH2^{Con} MCL cells upon serum starvation for 48 h. Each value in qRT-PCR was normalized to *GAPDH* and represents the mean ± S.D. *p < 0.05; **p < 0.01 (vs. control cells; Student's *t*-test).

Supplemental Figure 3

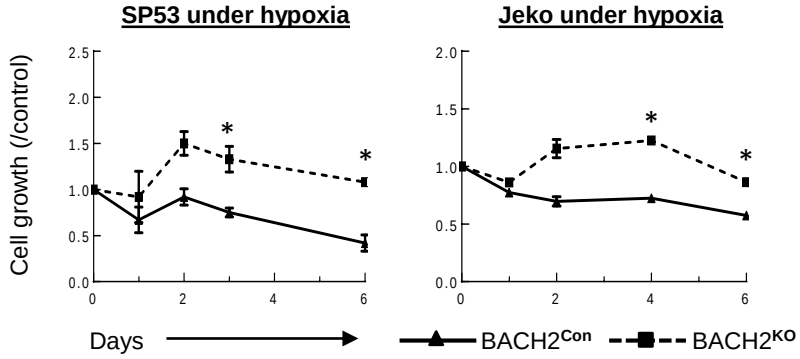


Supplemental Figure 3

Ki-67 expression levels were measured using FACS in MCL patients (n=6, 3 for blastoid and 3 for non-blastoid subtypes). Levels were analyzed in live CD19⁺ B cells.. Representative FACS analyses of Ki-67 levels from 2 MCL patients are shown (*Left*). The % population of Ki-67⁺ cells in 6 MCL patients are shown as the mean ± S.D. (*Right*). *p < 0.05 (vs. non-blastoid subtypes; Student's t-test).

Supplemental Figure 4

A



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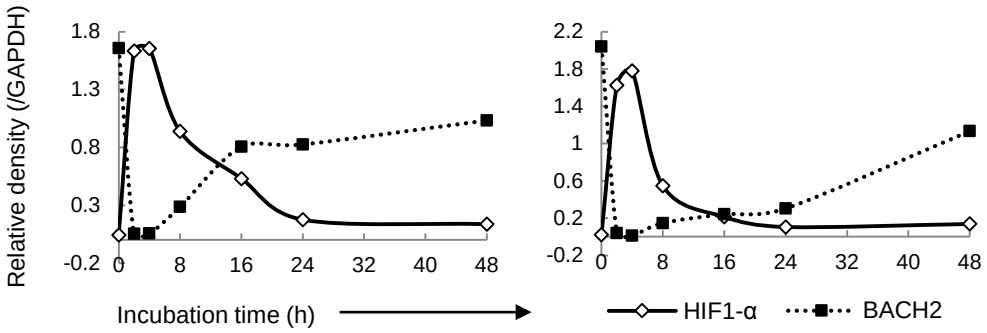


Supplemental Figure 4

(A) Cell growth of BACH2^{KO} or BACH2^{Con} MCL cells under hypoxia (at 1% O_2) was determined by cell counting using Trypan Blue staining. The data represent the mean ± S.D. from three independent experiments. *p < 0.05 (vs. control cells; Student's t-test). **(B)** Trend charts generated with relative HIF-1α and BACH2 expression levels based on densitometry analyses of immunoblots in Figure 4C are as indicated against incubation time.

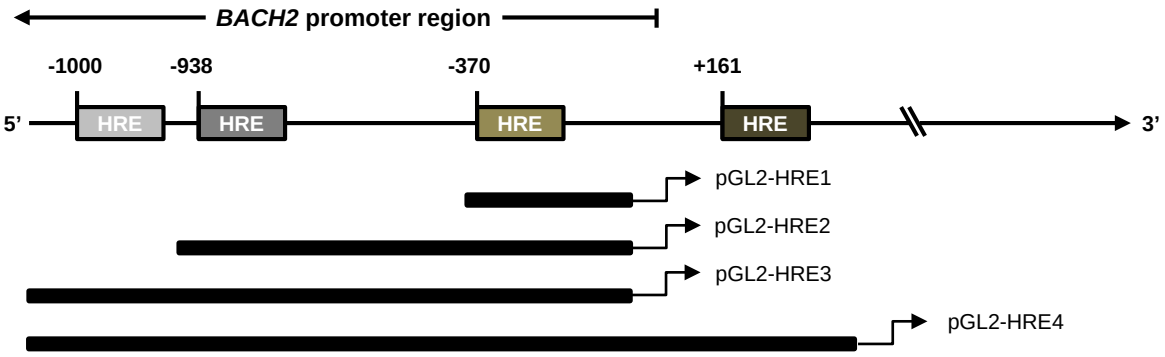
Supplemental Figure 5

A

ACGTGTAATAATATGGAATACTGCCTGTACACAAGAGGGCCAAGTTATAG	-950
GTGTTTAGGGAAACGTGGCAGAATTTTCTGGCCTTGGCAAGAGGTAAAGT	-900
TGCAGGCACCAGCACAACACTAACACAACCTTATTTTATTGCAAATGTAC	-850
ATTCAGCCTAAATCCTAATTACCTTCTGTAAAATCCTAACCACCACCAAG	-800
CTTTCTTCTGACAGGGCACTCACACGACGGCCTTTAAGGGCAGCAACAAC	-750
ATCCTAAGAGCCGCAGAAAGGAAAGGCAGGGGAGAGTGGCTGGAAGGCAG	-700
TAGGAGCTGGGGCTGAAGCCCGGGAGGTTGGCTTTTAATGTCCTCCTGAA	-650
ATTGACCACTCTCTTTCCAAAAACACTGGCTTGGGGGCTTAAAGGTTCGC	-600
TCAGTAGGGCAGGCGGAGGGCCTCCTGAAAAGGATTGAGAAGTCAACATT	-550
CAAAAGGCTGCTTTTACTCGCCCGGTCCCCACCCAAAAGGGAGTCCGGTC	-500
AGCTTTGTGGGGGCCAGTGGAGCATCTTTTCTTGCCCTTCCGGGAAAACG	-450
CTAGCTAACCCTGGGATCCCCGGTCCCGACGTCTGCTCTCCTTCCCCAGG	-400
TGCAGCACCTAGGGCTCTGGCCCCCTCCCCACGTGCCACACCAGCCCTCA	-350
CCACTACTCTCACCCTGCTATGCCCCCAACCCTCGTCTTTCGTGGCCGGG	-300
AAGGCCAGGCCGAGCGGGGCGGTCAGCCGGGCCGGGAGGAGGGGGCTCTC	-250
CGGGCCGCGCGGCCGCACAAAGCGGGGAGATCTGCTGCTGCGGCGGCATG	-200
AGGCGCGCCCCCTCCCGCGAGCCAGCGGAGCGCGCGCGCCCGCCCCCGTC	-150
CCCCGCCCCCCGCCCCAGCCCCCGGAGCGCCATTCGCGGAGCGGCTTACG	-100
CTAGTCGCCGGGCGTACCGCGCCCCAGCGGCCGGGGAGGTGCGCTGCCCG	-50
GCTCCCGTAAAGTTATTGTGAATGGGGAGCGGGTGACGTCAGCGCCGAAT	0
GTCAACAATGTAGCGATTGAGAGTGTGGGCGTTCCGGGGAGAGCGCAAGC	+50
CGCGCGGCGCGGAGCAAACAGCGCCGAGCCGCCGCCGCCTCAGCAGCAGC	+100
AGCAGCAGCAGCAGCGGCAGCAGCGGCCGTGCACGCCCGGGCTGCGGTCG	+150
CACAGCGCTAACGTGAGCGGCCGCCGCCCTCGCCACCCCGCCTGCCCACT	+200

HRE binding site: NCGTG

B



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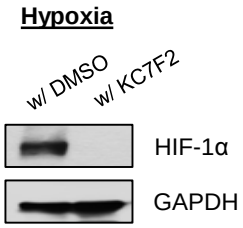

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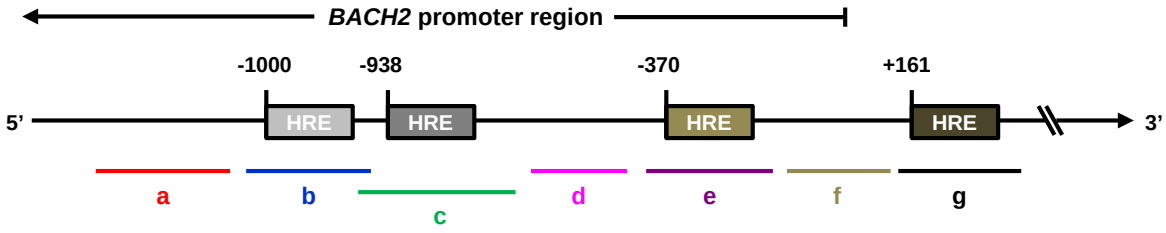

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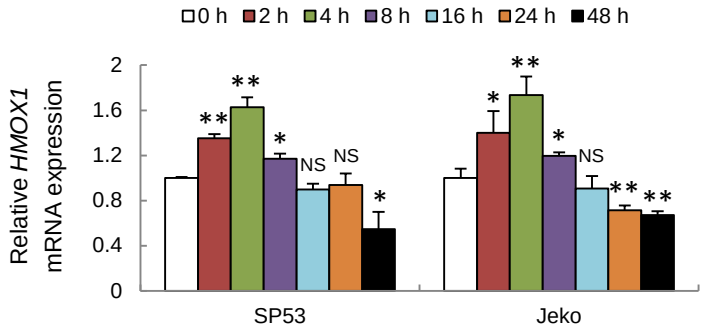
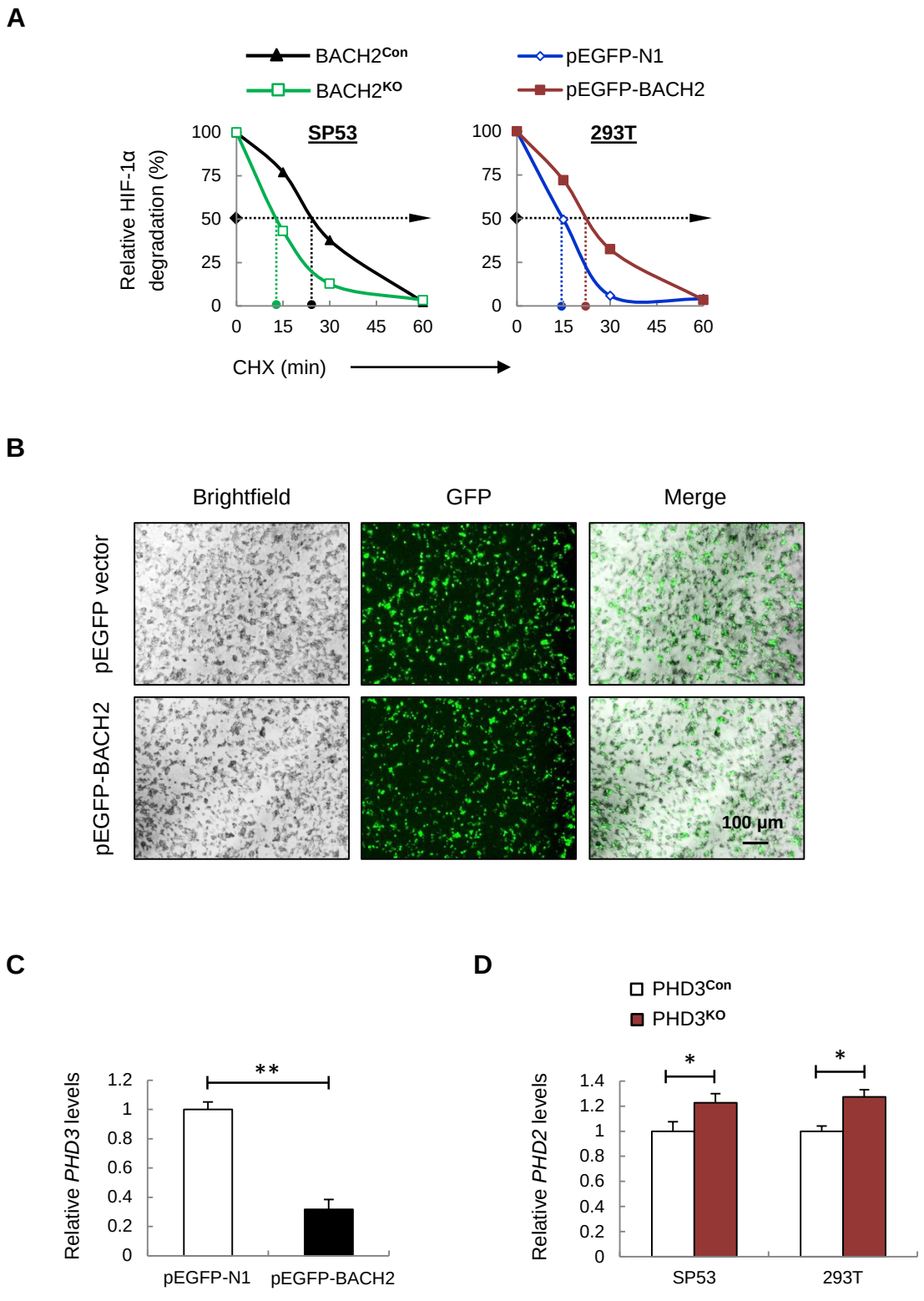


Supplemental Figure 5

(A) The proximal promoter and 5' UTR region (-1000bp/+200bp) of human *BACH2* gene. Blue, putative hypoxia-responsive elements (HRE, NCGTG). **(B)** Four putative HREs binding sites within *BACH2* promoter and 5'UTR regions along with serial deletion of promoter constructs are indicated. **(C)** Immunoblots of HIF-1α in 293T cells upon KC7F2 treatment (40 μmol/L) at 1% O2 for 4 h. GAPDH was used as a loading control. **(D)** The schematic of the DNA fragments (a-g) on the *BACH2* promoter. Among them, fragments b, c, e and g contain HIF-1α binding sites (HRE). **(E)** *HMOX1* mRNA levels were measured by qRT-PCR using MCL cells cultured under hypoxia at the indicated time. The values compared to the controls (at 0 h) are indicated as the mean ± S.D.. NS, not significant; *p < 0.05; **p < 0.01 (vs. control cells; Student's *t*-test).

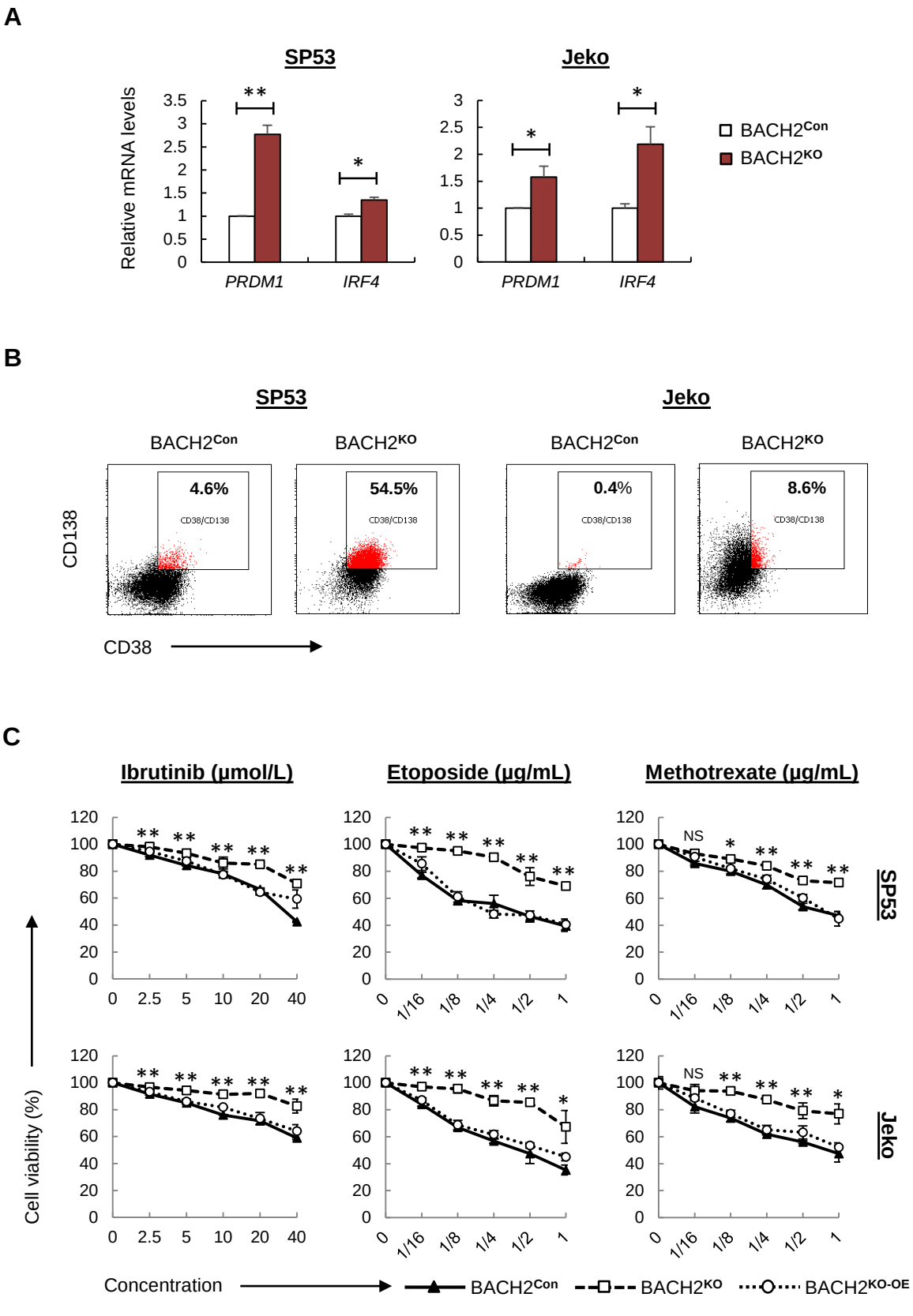
Supplemental Figure 6



Supplemental Figure 6

(A) The relative half-life ($t_{1/2}$) of HIF-1α (50% of degradation, black arrow) as shown in Figure 6B and 6D is indicated in the diagram. Green dotted line and black dotted line represent $t_{1/2}$ of HIF-1α in BACH2^{KO} and control SP53 MCL cells, respectively. Red dotted line and blue dotted line represent $t_{1/2}$ of HIF-1α in 293T cells transfected with pEGFP-BACH2 and pEGFP-N1, respectively. **(B)** 293T cells were transiently transfected with HIF-1α and BACH2 expression plasmids for 48 h. The pEGFP-N1 empty vector was used as a control. Transfection efficiency was confirmed after 48 h, and GFP^{+} cells were imaged under microscope. Scale bar, 100 μm. **(C)** The *PHD3* mRNA levels were measured in 293T cells transiently transfected with the pEGFP-BACH2 plasmids for 48 h, with pEGFP-N1 empty vector as a control. Each value in qRT-PCR was normalized to *GAPDH* and represents the mean ± S.D. **(D)** *PHD2* mRNA levels were measured using qRT-PCR in PHD3^{KO} or PHD3^{Con} cells. Each qRT-PCR value was normalized to *GAPDH* and is presented as the mean ± S.D. *$p < 0.05$; **$p < 0.01$ (vs. control cells; Student's *t*-test).

Supplemental Figure 7



Supplemental Figure 7

(A) The mRNA levels of *PRDM1* and *IRF4* were determined using qRT-PCR. Each value was normalized to *GAPDH* and represents the mean ± S.D. **(B)** CD38 and CD138 levels in BACH2^{KO} or BACH2^{Con} MCL cells were measured using FACS analysis, and the % population of double-positive cells (CD38^{+}/CD138^{+}) are indicated. **(C)** BACH2^{KO}, BACH2$^{KO\text{-}OE}$ or mock-transfected control MCL cells (SP53 and Jeko) were treated with ibrutinib, etoposide, and methotrexate for 24 h. Cell viability was determined using MTT assays. The data represent the mean ± S.D. NS, not significant; *p < 0.05; **p < 0.01 (BACH2^{KO} vs. control cells; Student's *t*-test).

Supplemental Table 1

	Patient #	Sex	Age (yr)	Extranodal involvement	Morphologic variant [a]	Treatment status [b]	Tissue collection [c]
mRNA levels	MCL1	M	64	Bone marrow, other	Blastoid	Untreated	PB
	MCL2	F	64	Bone marrow	Unk	Treated	PB
	MCL3	M	70	Bone marrow	Blastoid	Untreated	PB
	MCL4	F	66	Bone marrow	Blastoid	Untreated	PB
	MCL5	M	61	Bone marrow, other	Blastoid	Treated	PB
	MCL6	F	83	Bone marrow, other	Blastoid	Treated	PB
	MCL7	M	68	Bone marrow, other	Blastoid	Treated	PB
	MCL8	M	63	Bone marrow	Blastoid	Treated	PB
	MCL9	M	62	Bone marrow	Blastoid	Untreated	PB
	MCL10	M	64	Bone marrow	Unk	Treated	PB
	MCL11	M	53	Bone marrow, other	Blastoid	Treated	PB
	MCL12	Unk	Unk	Bone marrow, other	Blastoid	Treated	PB
	MCL13	M	51	Bone marrow	Classical	Treated	PB
	MCL14	M	80	Bone marrow	Classical	Treated	PB
	MCL15	M	62	Bone marrow	Classical	Untreated	PB
	MCL16	F	70	Bone marrow	Classical	Untreated	PB
	MCL17	M	58	Bone marrow	Classical	Untreated	PB
	MCL18	M	78	Bone marrow	Classical	Treated	PB
	MCL19	M	65	Bone marrow	Classical	Treated	PB
	MCL20	M	53	Bone marrow, other	Classical	Treated	PB
	MCL21	M	35	Bone marrow, other	Classical	Treated	PB
	MCL22	M	53	Unk	Classical	Treated	PB
	MCL23	Unk	Unk	Unk	Unk	Unk.	PB
	MCL24	Unk	Unk	Unk	Unk	Unk.	PB
	MCL25	M	75	Unk	Unk	Treated	PB
	MCL26	Unk	Unk	Unk	Unk	Unk.	PB
	MCL27	Unk	Unk	Unk	Unk	Unk.	PB
	MCL28	M	78	Bone marrow	Classical	Treated	BM
	MCL29	M	66	Bone marrow, other	Classical	Treated	BM
	MCL30	F	58	Bone marrow	Classical	Treated	BM
	MCL31	M	56	Bone marrow	Classical	Untreated	BM
	MCL32	M	64	Unk	Classical	Treated	BM
	MCL33	M	63	Unk	Classical	Treated	BM
	MCL34	M	64	Bone marrow, other	Unk	Treated	BM
	MCL35	M	66	Bone marrow, other	Classical	Treated	BM
Heme content	1	M	53	Unk	Classical	Treated	PB
	2	Unk	Unk	Bone marrow, other	Blastoid	Treated	PB
	3	Unk	Unk	Unk	Unk	Unk	SP
	4	Unk	Unk	Unk	Unk	Unk	SP
	5	M	56	Bone marrow	Classical	Untreated	BM
	6	M	66	Bone marrow, other	Classical	Treated	BM

[a] Lymphoma staging verified by MD Anderson pathologist.

[b] Treatment status of patients upon sample collection.

[c] Cells were isolated from either fresh or previously frozen peripheral blood ,bone marrow, or spleen.

Unk, unknown status; PB, peripheral blood; BM, bone marrow; SP, spleen; M, male; F, female; yr, year.

Supplemental Table 1

MCL patient samples (n=35) were evaluated for *BACH2* mRNA levels. Total of 27 PB and 8 BM samples were used. Additional 6 MCL patient samples (n=6, 2 from PB, 2 from BM and 2 from SP) were evaluated for cellular heme content. All data were recorded and collected at MD Anderson Cancer Center.

Supplemental Table 2

	Mice	Sex	Age (w)	# of cells injected	Injected region	Time period (w)	Tissue examined
s.c. injection	#1	F	7	3.5×10^6	Neck	4	SP / BM
	#2	M	6	3.5×10^6	Neck	4	SP / BM
	#3	F	7	3.5×10^6	Neck	4	SP / BM
	#4	M	6	3.5×10^6	Neck	4	SP / BM
	#5	F	7	3.5×10^6	Neck	4	SP / BM
	#6	M	6	3.5×10^6	Neck	4	SP / BM
	#7	F	7	3.5×10^6	Neck	4	SP / BM
	#8	M	6	3.5×10^6	Neck	4	SP / BM
	#9	F	6	3.5×10^6	Neck	4	SP / BM
	#10	M	6	3.5×10^6	Neck	4	SP / BM
i.v. injection	#11	M	7	3×10^6	Tail vein	8	SP / BM
	#12	M	7	3×10^6	Tail vein	8	SP / BM
	#13	F	7	3×10^6	Tail vein	8	SP / BM
	#14	F	6	3×10^6	Tail vein	8	SP / BM
	#15	M	7	3×10^6	Tail vein	8	SP / BM
	#16	M	7	3×10^6	Tail vein	8	SP / BM
	#17	F	7	3×10^6	Tail vein	8	SP / BM
	#18	F	6	3×10^6	Tail vein	8	SP / BM

BM, bone marrow; SP, spleen; s.c., subcutaneous; i.v., intravenous; M, male; F, female; w, week.

Supplemental Table 3

Fragments	ChIP-qRT-PCR primers	
	Forward primer (5'-3')	Reverse primer (5'-3')
BACH2-ChIP-a	GGACCTCAGAAGCAGGGTGT	AACAGCACTGCAGAGTCCCTG
BACH2-ChIP-b	GGACGCTTCACTGCTTCCTCA	GGCCCTCTTGTGTACAGGCAG
BACH2-ChIP-c	CTGCCTGTACACAAGAGGGCC	GCCCTGTCAGAAGAAAGCTTGG
BACH2-ChIP-d	GGGGCTTAAAGGTTCGCTCAGT	AAGCTGACCGGACTCCCTTT
BACH2-ChIP-e	AACGCTAGCTAACCCTGGGATC	CTTCCCGGCCACGAAAGA
BACH2-ChIP-f	CCGGCTCCCGTAAAGTTATTGTG	GCTCGGCGCTGTTTGCT
BACH2-ChIP-g	GCACAGCGCTAACGTGAGC	CATCACATGGCAGCTCGTTCC