

Quantitative analysis of BMF mRNA levels

Total RNA was extracted using TRIZOL (Invitrogen). cDNA synthesis was performed using Omniscript™ from Qiagen and quantitative real-time PCR for *BMF* expression was performed using a Taqman kit from Applied Biosystems (hs.00372938_m1 human Bmf).

Bisulfite modification and BMF DNA methylation analysis

Bisulfite modification was performed using the EZ DNA Methylation-Gold Kit (Zymo Research). MethyLight PCR analysis was done as described previously with COL2A1 as reference gene.²⁸ Primers and probes for BMF were determined with the assistance of the computer program Primer Express version 2.0.0 (Applied Biosystems) to produce a 90-base-pair PCR amplicon in the promoter region of BMF transcription variant 1 with a mean distance of 146 base pairs to the transcription start site (Table S1). The primer specificity was verified on SssI treated (100% hypermethylated) and on whole genome amplified DNA (100% hypomethylated) bisulfite-modified DNA and on unmodified genomic DNA. The preparation of the hyper and hypomethylated control DNA was described recently.²⁹ Primers for bisulfite sequencing were designed using the Methyl Primer Express software version 1.0 (Applied Biosystems). PCR was performed in a 25 µl final volume under standard conditions with an annealing temperature of 54°C. Each reaction included 100 ng of bisulfite modified DNA, 250 nM of each primer, 200 µM of each dNTP, 1× PCR-Buffer and 1 U Hotstart Taq polymerase (Qiagen). PCR products were subcloned into pGEM-T easy (Promega) and inserts were sequenced using standard techniques at Microsynth (Switzerland).

Table S1. Primers and probe for MethyLight PCR and bisulfite sequencing

Reaction	Primer	Sequence	Nucleotide positions as defined by NCBI Reference Sequence NT_010194.16/Hs15_10351
MethyLight PCR	Forward	5'- CGAACTAAACCGCTACCAAAAAC -3'	11.191.823 – 11.191.733
	Reverse	5'- GGCGACGGTCGGAATTT-3'	
	Probe	5'FAM- CGAACCTTCCCATAAA AACATTACAAAAACCGA-3'BHQ1	
Bisulfate sequencing reaction 1	Forward	5'- GAAGAGGATTTAAGGGTTTTTTT-3'	11.192.198 – 11.191.774
	Reverse	5'- CCTTCCCATAAAAACATTACAA-3'	
Bisulfate sequencing reaction 2	Forward	5'- TGTAATGTTTTTATGGGAAGGT-3'	11.191.795 – 11.191.347
	Reverse	5'-ACCCA ACTATAAATTCTACACCC-3'	

Figure S1. Growth of Bmf- or Bad-deficient tumors in wt recipients

Primary lymphoma cells (10^5 /recipient mouse) from ill *E μ -myc/bad^{-/-}* (A) or *E μ -myc/bmf^{-/-}* (B) mice were injected i.v. into three each of *wt* and *bad^{-/-}* or *wt* and *bmf^{-/-}* recipient mice respectively. Recipient mice were monitored for disease onset and the results from two individual lymphomas are presented in Kaplan-Meyer plots.

Figure S2. Induction of Bmf mRNA by 5-aza-2-deoxycytidine in Daudi and Raji cells

Raji and Daudi cells were incubated for the indicated times with 5 μ M of 5'-Aza-deoxycytidine. RNA was isolated and reverse transcribed prior qPCR-based quantification of BMF and the TATA-binding protein (TBP) mRNA. Bars depict the ratio of BMF/TBP transcript levels and represent means of triplicates. One experiment out of two yielding similar results is shown.

Figure S3. Induction of Bmf and of apoptosis by SAHA and serum deprivation in Raji and Dadi cells cells

(A) Western blot analysis of Bmf levels in Raji and Daudi cell lines treatment with 2 μ M SAHA or after serum deprivation for the indicated times. (B) Cell death determined by AnnexinV/7-AAD staining in Daudi and Raji cells treatment with 2 or 4 μ M SAHA for the indicated times. Values represent mean \pm SE of 2 independent experiments. (C) Cell death determined by AnnexinV/7-AAD staining in Daudi and Raji cells after serum deprivation for the indicated times. Values represent mean \pm SE of 2 independent experiments.

Figure S1

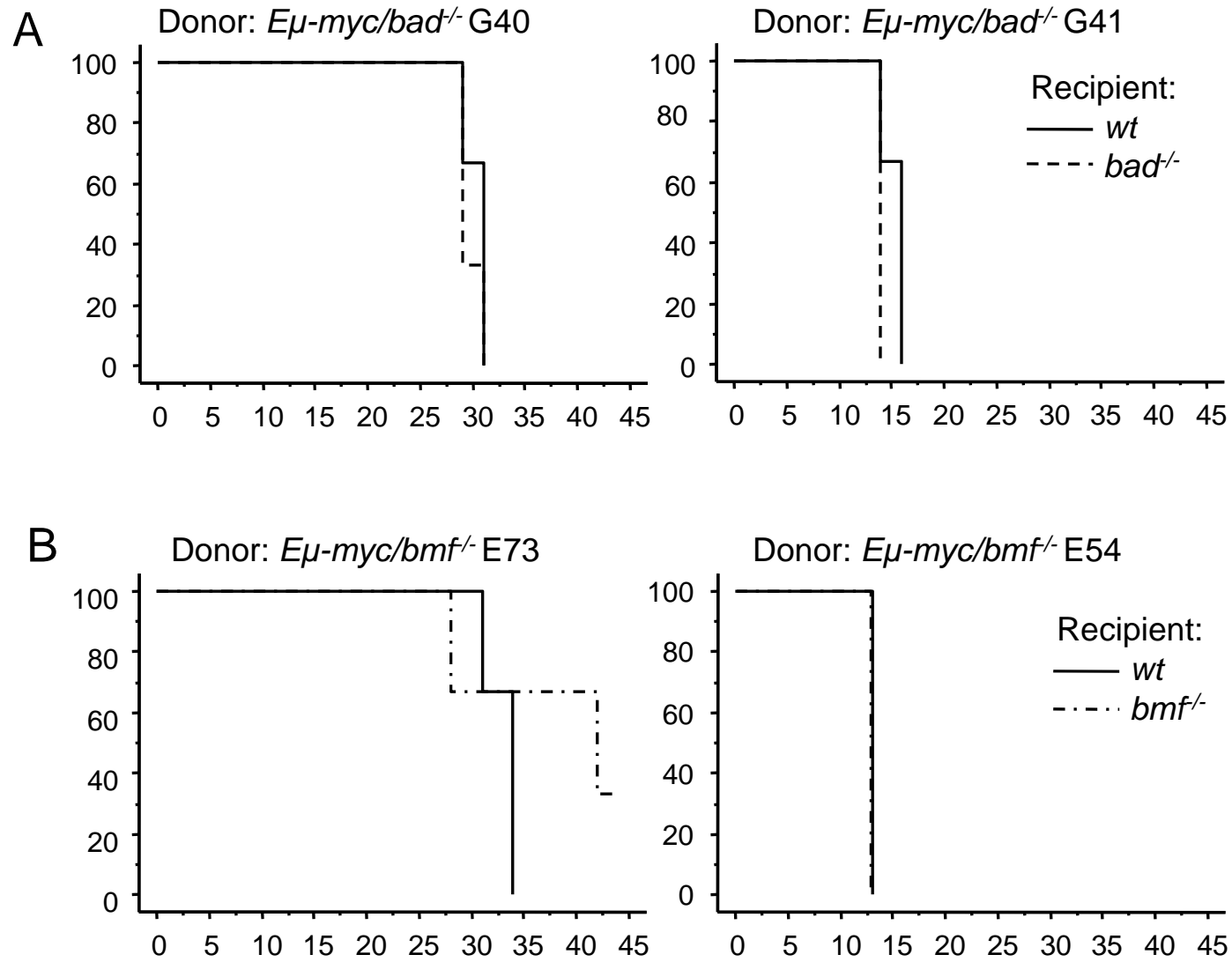


Figure S2

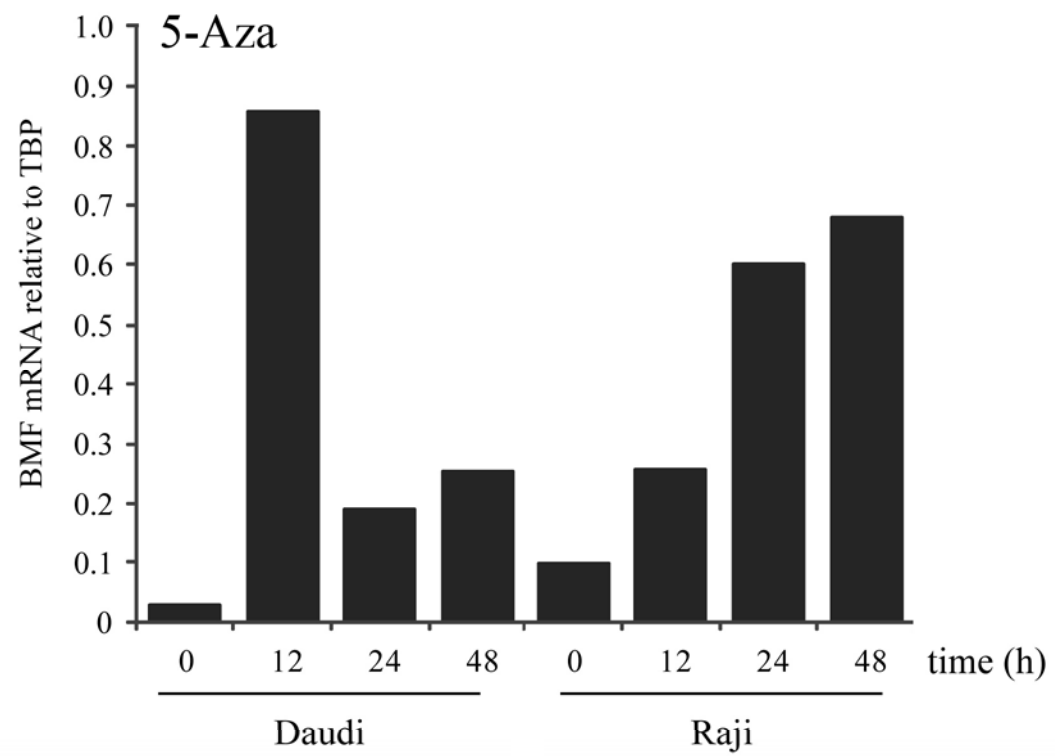


Figure S3

