Supporting Information

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SI Materials and Methods

Retrovirus. Full-length cDNA of HDAC4 and BCL6 was cloned into Murine Stem Cell Virus (MSCV) containing IRES and GFP (pMIG). Retrovirus was produced in Phoenix cells maintained in DMEM with 10% (vol/vol) FBS. DLBCL cell lines were transduced with 2 mL of virus per 1×10^6 by spin-infection with 10 μg/mL polybrene (SCBT). Briefly, the cells containing virus were centrifuged at 37 °C at 930 × *g* for 90 min, and incubated with virus for additional 4–5 h, after which it was replaced with fresh media for recovery overnight. After 3–4 d, GFP positive cells were sorted using BD FACS Aria and cultured for colony formation, apoptosis and proliferation assays.

Luciferase Reporter Assay. miR-155 target prediction was according to the Targetscan [Release 3.0 (1)] and RNA22 algorithms (http://cbcsrv.watson.ibm.com/rna22.html) (2). The 3′UTR of the human and mouse HDAC4 and BCL6 containing the two miR-155 binding sites was PCR amplified with *XbaI* flanked primers. The PCR products were purified, digested, and cloned downstream of the luciferase coding region in the pGL3 control vector (Promega). Human HEK293T, HeLa, Jurkat, and mouse NIH 3T3 cells were cotransfected with respective luciferase constructs and miR-155 or scrambled (Scr) oligos along with Renilla luciferase expression plasmid (pRL-TK) as a transfection control using Lipofectamine (Invitrogen). Deletion and mutant HDAC4 luciferase constructs were prepared by deleting the miR-155 binding sites in the original 3′UTR construct, respectively. After 48 h, cells were lysed and analyzed for relative luciferase activity using the Dual Luciferase Assay Kit (Promega). Results are representative of three independent experiments.

The primer sequences for luciferase contructs are as follows:

hsHdac4_WT3utr_L: ACCTCTAgAAgATTCTATTTTCAgAAAgTgAgAg; hsHdac4_WT3utr_R: ACCTCTAgAAgggTTTCCCTCAAAgggATATAA; mmHdac4_3utr_L: ACCTCTAGAACATCATGAGAGGGGAAAGAAA; mmHdac4_WT3utr_R: ACCTCTAGAGAGCTAAGACAGAGGGTGGAGA; mmBcl6_WT3utr_L: AACtctagaGACTTTTCAATGTTTTATTCTTATGTTTTGTACA; mmBcl6_WT3utr_R: AACtctagaATGAAGCATGGAGTGTTCCT; hsBcl6_WT3utr_L: AACtctagaCCAGTTTGACTTTTCAACATTTTATTCTTATATT; hsBcl6_WT3utr_R: AACtctagaAGCATGGAGTGTTGATGCTTT); hsHdac4_m155Del7-mer_L: AGCTTCACAAATGTGCTGAGCTGTGTAGCCTTTTCTTTGA; hsHdac4_m155Del7-mer_R: TCAAAGAAAAGGCTACACAGCTCAGCACATTTGTGAAGCT); hsHdac4_m155Mut7-mer_L: CACAAATgTgCTgAggAACATACTgTgTAgCCTTTTC; and hsHdac4_m155Mut7mer_R: gAAAAggCTACACAgTATgTTCCTCAgCACATTTgTg).

qRT-PCR. For qRT-PCR total mRNA from sorted cells was isolated using Phenol-free total RNA isolation kit (Amresco) and reverse transcribed using iScript first strand cDNA synthesis kit. About 1/20th of diluted cDNA was used for gene taqman analysis using respective taqman assays for mouse or human *Hdac4*, *Bcl6, cMyc*, and *Mxd1* (ActinB was used as normalizer). For miR expression analysis, total RNA was reverse transcribed using TaqMan miRNA reverse transcription kit using the manufacturer's protocol, followed by miRNA RT-PCR for miR-155 and sno-135 (normalizer). BCL6 target genes (*IL6*, *Id2*, *Ccnd1*, and *Ccl3*/*Mip1α*) were detected using sybr-green-based RT-PCR using primers picked from the RTprimer database (http://medgen.ugent.be/rtprimerdb/). The list of primers are as follows: mActin_ForSybr: ATG CTC CCC GGG CTG TAT; mActin_RevSybr: CAT AGG AGT CCT TCT GAC CCA TTC; mId2_ForSybr: AAG ACT TTT GTT ATC AGC CAT TT; mId2_RevSybr: GAC GAT CAT CCT TAG TTT TCC CGC TTT CTT; mIL6_ForSybr: TCC AGT TGC CTT CTT GGG AC; mIL6_RevSybr: GTG TAA TTA AGC CTC CGA CTT G; mCCL3_ForSybr: CGT TCC TCA ACC CCC ATC; and mCCL3_RevSybr: TGT CAG TTC ATG ACT TTG TCA TCA T. All RT-PCR assays were performed on the Biorad CFX96. Data were analyzed using $2^{-\Delta Ct}$ method to calculate the relative amounts or $2^{-\Delta\Delta Ct}$ for fold change. The overlap between up-regulated genes in mice and known BCL6 targets (3) in humans was calculated using Gene Cluster 3.0. The clustered genes were mean centered, and average linkage was performed using Euclidean distance, after filtering the genes with SD > 1.

1. Lewis BP, Burge CB, Bartel DP (2005) Conserved seed pairing, often flanked by adenosines, indicates that thousands of human genes are microRNA targets. *Cell* 120(1): 15–20.
2. Lewis BP, Shih IH, Jones-Rhoades MW, Bartel DP, Burge CB (2003) Prediction of mammalian microRNA targets. *Cell* 115(7):787–798.
3. Basso K, et al. (2010) Integrated biochemical and computational approach identifies BCL6 direct target genes controlling multiple pathways in normal germinal center B cells. *Blood* 115(5):975–984.

3' ugGGGAUAGUGC-UAAUCGU-AAUu 5' hsa-miR-155 |::|||:: | | |||| ||| 595:5' auCUUUAUUGAGAAAGAGCACUUAa 3' BCL6	mirSVR score: -1.2878 PhastCons score: 0.8451
3' ugGGGAUAG-UGUUAAUCGU-AAUu 5' mmu-miR-155 |::|||: | || |||| ||| 591:5' auCUUUAUUGAGAAAGAGCACUUAa 3' Bcl6	mirSVR score: -1.1787 PhastCons score: 0.7444

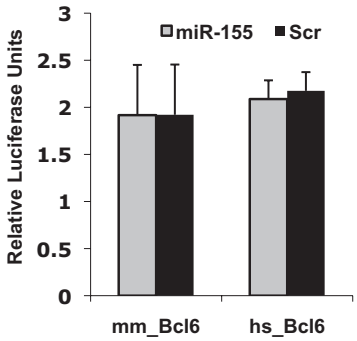

Fig. S1. Sequence alignment of 3′UTR of human and mouse Bcl6 with respect to miR-155 mature sequence (1), showing possible interaction through a less conserved binding site (*Upper*). Luciferase reporter assay showing no difference in pGL3-Bcl6-3′UTR (mouse or human) reporter activity by miR-155 or empty vector (*Lower*).

1. Miranda KC, et al. (2006) A pattern-based method for the identification of microRNA binding sites and their corresponding heteroduplexes. *Cell* 126(6):1203–1217.

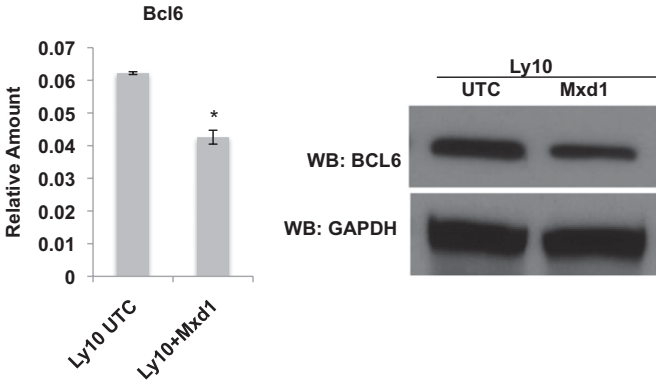

Fig. S2. qRT-PCR (*Left*) and immunoblot (*Right*) analysis of full-length Mxd1 cDNA-transfected OCI-Ly10 cells.

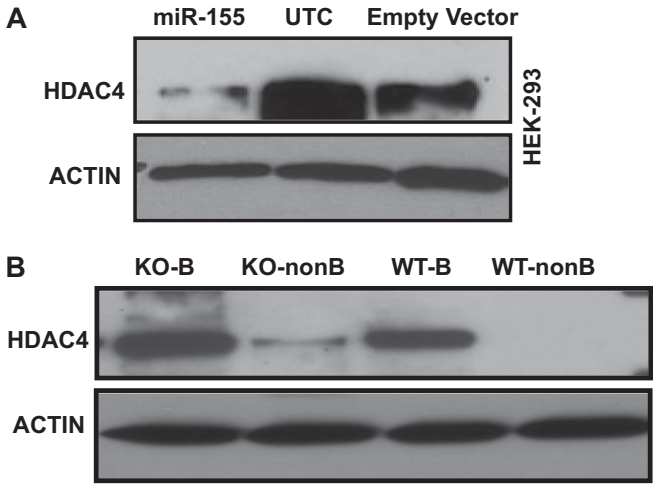

Fig. S3. (*A*) Ectopic miR-155 expression into HEK293T cells significantly down-regulates endogenous HDAC4. (UTC, Untransfected Control). (*B*) Immunoblot analysis of HDAC4 levels in miR-155–deficient CD19 cells from *bic*/miR-155 knock-out mice showing increased expression. βactin was used as a loading control in all immunoblots unless indicated otherwise. (KO-B, miR-155 knock out/bic$^{-/-}$ mice CD19+ B cells; KO-nonB, miR-155 knock out/bic$^{-/-}$ mice non-B-cells, CD19-; WT, wild type).

Other Supporting Information Files

Dataset S1 (XLSX)