

University of Groningen

ZDHHC11 and ZDHHC11B are critical novel components of the oncogenic MYC-miR-150-MYB network in Burkitt lymphoma

Dzikiewicz-Krawczyk, A; Kok, K; Slezak-Prochazka, I; Robertus, J-L; Bruining, J; Tayari, M M; Rutgers, B; de Jong, D; Koerts, J; Seitz, A

Published in:
Leukemia

DOI:
[10.1038/leu.2017.94](https://doi.org/10.1038/leu.2017.94)

IMPORTANT NOTE: You are advised to consult the publisher's version (publisher's PDF) if you wish to cite from it. Please check the document version below.

Document Version
Final author's version (accepted by publisher, after peer review)

Publication date:
2017

[Link to publication in University of Groningen/UMCG research database](#)

Citation for published version (APA):

Dzikiewicz-Krawczyk, A., Kok, K., Slezak-Prochazka, I., Robertus, J-L., Bruining, J., Tayari, M. M., Rutgers, B., de Jong, D., Koerts, J., Seitz, A., Li, J., Tillemma, B., Guikema, J., Nolte, I. M., Diepstra, A., Visser, L., Kluiver, J., & van den Berg, A. (2017). ZDHHC11 and ZDHHC11B are critical novel components of the oncogenic MYC-miR-150-MYB network in Burkitt lymphoma. *Leukemia*, 31, 1470-1473.
<https://doi.org/10.1038/leu.2017.94>

Copyright

Other than for strictly personal use, it is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), unless the work is under an open content license (like Creative Commons).

The publication may also be distributed here under the terms of Article 25fa of the Dutch Copyright Act, indicated by the "Taverne" license. More information can be found on the University of Groningen website: <https://www.rug.nl/library/open-access/self-archiving-pure/taverne-amendment>.

Take-down policy

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

Downloaded from the University of Groningen/UMCG research database (Pure): <http://www.rug.nl/research/portal>. For technical reasons the number of authors shown on this cover page is limited to 10 maximum.



ZDHHC11 and ZDHHC11B are critical novel components of the oncogenic MYC-miR-150-MYB network in Burkitt lymphoma

A Dzikiewicz-Krawczyk, K Kok, I Slezak-Prochazka, J-L Robertus, J Bruining, M M Tayari, B Rutgers, D de Jong, J Koerts, A Seitz, J Li, B Tillema, J E Guikema, I M Nolte, A Diepstra, L Visser, J Kluiver, A van den Berg

Cite this article as: A Dzikiewicz-Krawczyk, K Kok, I Slezak-Prochazka, J-L Robertus, J Bruining, M M Tayari, B Rutgers, D de Jong, J Koerts, A Seitz, J Li, B Tillema, J E Guikema, I M Nolte, A Diepstra, L Visser, J Kluiver, A van den Berg, ZDHHC11 and ZDHHC11B are critical novel components of the oncogenic MYC-miR-150-MYB network in Burkitt lymphoma, *Leukemia* accepted article preview 23 March 2017; doi: [10.1038/leu.2017.94](https://doi.org/10.1038/leu.2017.94).

This is a PDF file of an unedited peer-reviewed manuscript that has been accepted for publication. NPG are providing this early version of the manuscript as a service to our customers. The manuscript will undergo copyediting, typesetting and a proof review before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers apply.

ZDHHC11 and ZDHHC11B are critical novel components of the oncogenic MYC-miR-150-MYB network in Burkitt lymphoma

Running title Oncogenic role for *ZDHHC11* and *ZDHHC11B* in BL

Agnieszka Dzikiewicz-Krawczyk^{1,2}, Klaas Kok³, Izabella Slezak-Prochazka^{1,4}, Jan-Lukas Robertus¹, Jesper Bruining¹, Mina Masoumeh Tayari¹, Bea Rutgers¹, Debora de Jong¹, Jasper Koerts¹, Annika Seitz¹, Jun Li³, Berend Tillema¹, Jeroen E. Guikema⁵, Ilja M. Nolte⁶, Arjan Diepstra¹, Lydia Visser¹, Joost Kluiver^{1*}, Anke van den Berg^{1*}

¹Department of Pathology, ³Department of Genetics, ⁶Department of Epidemiology, University of Groningen, University Medical Center Groningen, The Netherlands

²Institute of Human Genetics, Polish Academy of Sciences, Poznan, Poland

⁴Biosystems Group, Institute of Automatic Control, Silesian University of Technology, Gliwice, Poland

⁵Department of Pathology, Academic Medical Center, University of Amsterdam, The Netherlands

*corresponding co-authors

Prof. Dr. Anke van den Berg and Dr. Joost Kluiver, Department of Pathology, University Medical Center Groningen, Hanzeplein 1, 9700 RB Groningen, The Netherlands. Tel: +31503611476; Fax: +31503619107; Email: a.van.den.berg01@umcg.nl and j.l.kluiver@umcg.nl

CONFLICT OF INTEREST

The authors declare no conflict of interest.

Burkitt lymphoma (BL) is an aggressive subtype of B-cell non-Hodgkin lymphoma that most frequently affects young children¹. BL is characterized by a chromosomal translocation involving the *MYC* gene locus resulting in overexpression of *MYC*². *MYC* is a transcription factor involved in cell growth, proliferation and maintenance of stem cell-like properties^{3,4}. Besides protein-coding genes, *MYC* also regulates the expression of multiple microRNAs (miRNAs) and long non-coding (lnc)RNAs^{5,6}. In a previous study⁷, we identified a *MYC*-related miRNA expression profile in pediatric BL as compared to *MYC*-low chronic lymphocytic leukemia (CLL). In this study, we further examined the role of *MYC*-repressed miRNAs on growth of BL cells.

Eleven miRNAs were significantly differentially expressed between BL and CLL with >4-fold difference (Supplementary Figure S1a). The differences in miRNA levels were validated by qRT-PCR, and *MYC* regulation was confirmed using the P493-6 B-cell line, which harbors a tetracycline-repressible *myc* allele⁸ (Supplementary Figure S1b-d).

Next, we determined whether the *MYC*-repressed miRNAs influence BL cell growth. All six miRNAs that we could stably overexpress, affected growth of BL cells. Ectopic expression of miR-26a, miR-26b and miR-150 strongly reduced cell growth, similar to *MYC* inhibition (Supplementary Figure S2a-c). These results were confirmed in two independent biological replicates in the ST486 and DG75 BL cell lines (Supplementary Figure S2d-e). We have chosen to further investigate the role of miR-150 in BL, a miRNA with an important regulatory role in hematopoiesis⁹.

To identify miR-150 target genes, we performed AGO2-RNA immunoprecipitation (RIP) followed by quantitative RNA analysis in ST486 and DG75 BL cells upon stable overexpression of miR-150, and as a control in empty vector (EV)-transduced cells (Supplementary Materials and Methods). Efficiency of the AGO2-IP procedure was confirmed by Western blot and miRNA qRT-PCR (Supplementary Figure S3a-c). Eleven probes (ten genes) had a ≥ 2 -fold higher AGO2-IP enrichment in miR-150-overexpressing cells compared to EV-transduced cells in both cell lines (Supplementary Figure S3d, Supplementary Table S1). Among them we found *MYB*, a validated target of miR-150¹⁰. qRT-PCR for the five genes with the highest increase in IP enrichment upon miR-150 overexpression confirmed the microarray results (Supplementary Figure S3e-f).

The two genes with the highest IP enrichment in miR-150-cells compared to EV-cells were *ZDHHC11* and *ZDHHC11B*. These two highly homologous genes are located on chr5q15

separated by ~25 kb. Both genes encode protein-coding and non-coding transcripts (Figure 1a). The protein products of *ZDHHC11* and *ZDHHC11B* are poorly characterized members of a palmitoyltransferase family¹¹. Both protein-coding and non-coding *ZDHHC11* transcripts are expressed in BL cell lines. The *ZDHHC11B* non-coding transcript is expressed in BL cells, whereas the coding variant is below the detection limit of qRT-PCR. *ZDHHC11* levels are much higher in BL cell lines than *ZDHHC11B* (Supplementary Figure S4a). Expression of *ZDHHC11* and *ZDHHC11B* is significantly higher in primary BL cases compared to CLL, consistent with the low miR-150 levels in BL (Supplementary Figure S4b).

The 3' regions of both genes contain a strikingly high number of tandemly repeated miR-150 binding sites (BS): 18 in *ZDHHC11* and 62 in *ZDHHC11B*. The consensus sequence of the miR-150 BS is CACAGUACUGGGAAUUGGGAG, with differences in 1 to 4 nucleotides between individual repeats. Complementarity of miR-150-5p to *ZDHHC11* and *ZDHHC11B* extends beyond the 6-mer seed region enhancing the miRNA-RNA interaction (Figure 1a). The dbSNP database reports several indel polymorphisms of one or more of the miR-150 BS repeats in both *ZDHHC11* and *ZDHHC11B*. Screening of the miR-150 BS region by PCR revealed a variation in the length of the repeat region both in lymphoma cell lines and control samples (Supplementary Figure S4c).

To confirm that *ZDHHC11* and *ZDHHC11B* are targeted by miR-150, we cloned the 18 miR-150 BS of *ZDHHC11* and the 62 miR-150 BS of *ZDHHC11B* into the psiCheck2 luciferase vector. Co-transfection of the constructs along with miR-150-5p precursor into ST486 and DG75 cells resulted in significantly lower relative luciferase activity (35-60% decrease) compared to the negative control. Moreover, *ZDHHC11* mRNA and protein levels were downregulated upon miR-150 overexpression (Figure 1b).

The high number of miR-150 BS suggested that *ZDHHC11* and *ZDHHC11B* could function as endogenous sponges. Since circular RNA transcripts containing high number of miRNA binding sites have been described^{12,13}, we set out to identify potential circular transcripts arising from the *ZDHHC11* and *ZDHHC11B* loci. We designed divergent primers located at the ends of the last exon of *ZDHHC11* and *ZDHHC11B*, which contains the miR-150 BS region. The resulting PCR product from ST486 and DG75 cells contained the two last *ZDHHC11* exons joined by back-splicing, with 129 additional nucleotides downstream of the last exon. Presence of *circ-ZDHHC11*

was confirmed with two additional primer sets (Figure 1a). The *circ-ZDHHC11* was also present in primary BL cases, albeit at variable levels (Supplementary Figure S4d). Despite much lower *circ-ZDHHC11* levels compared to the linear *ZDHHC11* transcripts, its enrichment in the AGO2-IP fraction of DG75-miR-150 cells was more pronounced (13.6-fold compared to EV) than the linear protein-coding (1.6-fold) and non-coding (3.3-fold) transcripts (Supplementary Figure S4e). Altogether, this suggests that the non-coding *ZDHHC11* and *ZDHHC11B* transcripts, especially the circular RNA, are efficiently targeted by miR-150 and could function as an endogenous sponge for this miRNA.

We next explored whether *ZDHHC11*, *ZDHHC11B* and MYB are involved in the strongly impaired growth of BL cells observed upon miR-150 overexpression. shRNA knockdown of MYB severely impaired growth of ST486 cells, even stronger than miR-150 overexpression, demonstrating high dependency of BL cell growth on MYB (Figure 1c). Downregulation of *ZDHHC11* and *ZDHHC11B* with three shRNAs targeting all transcripts of *ZDHHC11/B* also resulted in a significantly reduced cell growth, with a 33-91% decrease in GFP+ cells after 22 days (Figure 1c). Remarkably, MYB protein levels were also decreased by 19-46% in ST486 cells upon *ZDHHC11/B* knockdown and the strength of the phenotype induced by individual *ZDHHC11/B* shRNAs correlated with the degree of MYB downregulation (Figure 1c). In DG75 cells, downregulation of MYB and *ZDHHC11/B* also copied the phenotype of miR-150 overexpression (Supplementary Figure S6). The observed effect of *ZDHHC11/B* downregulation on BL cell growth and MYB levels can be reconciled by a model in which *ZDHHC11* and *ZDHHC11B* function as miR-150 sponges or competing endogenous RNAs¹⁴. Alternatively, the *ZDHHC11* palmitoyltransferase protein can be involved. Palmitoylation affects protein localization and protein-protein interactions¹¹ and there is some evidence for a role of palmitoylation in hematological malignancies¹⁵.

Finally, we explored the relevance of the miR-150-*ZDHHC11/B*-MYB network for other types of lymphoma. Expression analysis of the individual network components in a panel of diffuse large B-cell, Hodgkin and Burkitt lymphoma cell lines in comparison to germinal center B-cells (GCB) revealed that *MYC*, *ZDHHC11/B* and *MYB* are upregulated and miR-150 is downregulated in all lymphoma types as compared to GCB (Figure 2a). This pattern is similar to that observed in

P493-6 cells upon induction of MYC (Figure 2a), which suggests that MYC regulates all three components. Overall, these data show that the relevance of our findings likely extends beyond BL.

In summary, our results establish a novel network involving MYC, miR-150, *ZDHHC11*, *ZDHHC11B* and MYB that promotes proliferation of BL cells (Figure 2b). We propose that MYC ensures elevated levels of MYB required for the high proliferation rate of BL cells via two mechanisms. First, MYC downregulates miR-150 which unleashes MYB from the miR-150 mediated repression. Second, MYC induces *ZDHHC11* and *ZDHHC11B* which further ensures the maintenance of high MYB levels. Together, our results demonstrate a key role for *ZDHHC11* and *ZDHHC11B* in maintaining the oncogenic MYC-miR-150-MYB axis in BL.

ACKNOWLEDGEMENTS

This work was supported by SKOG grants 11-001 (to J.K. and A.v.d.B.) and 14-001 (to J.K., K.K. and A.v.d.B.).

AUTHOR CONTRIBUTIONS

A.D.K., J.K., K.K. and A.v.d.B. planned and supervised the project, A.D.K., J.K., I.S.P., J.L.R., J.B., M.M.T., B.R. D.d.J., J.K., A.S., B.T. collected data, analyzed by A.D.K., J.K., I.M.N. and J.L., A.D. revised pathological tissues, J.E.G. contributed tissue specimens and A.D.K., J.K. and A.v.d.B. with help of A.D., L.V. and K.K. prepared the manuscript.

Supplementary information is available on *Leukemia's* website.

REFERENCES

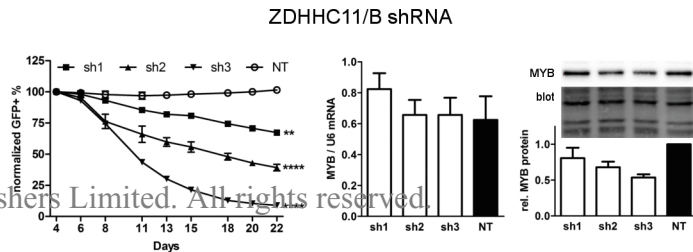
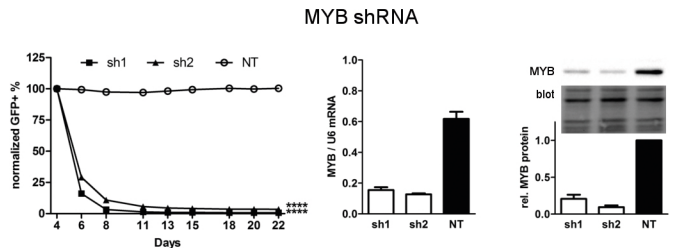
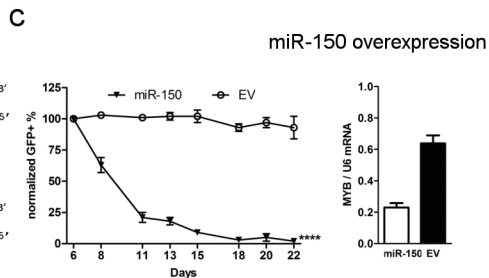
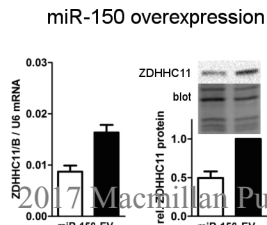
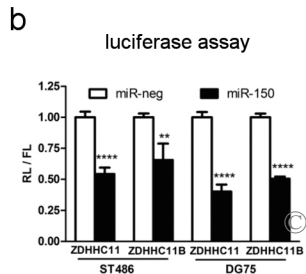
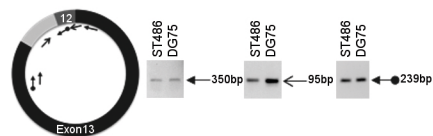
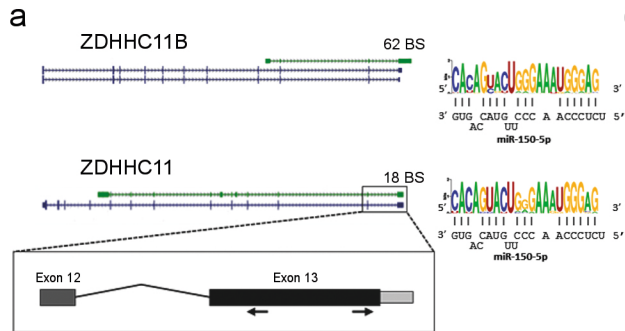
1. Harris NL, Jaffe ES, Stein H, Banks PM, Chan JK, Cleary ML, *et al.* A revised European-American classification of lymphoid neoplasms: a proposal from the International Lymphoma Study Group. *Blood* 1994; **84**: 1361-92.
2. Molyneux EM, Rochford R, Griffin B, Newton R, Jackson G, Menon G, *et al.* Burkitt's lymphoma. *Lancet* 2012; **379**: 1234-44.

3. Singh AM, Dalton S. The cell cycle and Myc intersect with mechanisms that regulate pluripotency and reprogramming. *Cell Stem Cell* 2009; **5**: 141-9.
4. Yap CS, Peterson AL, Castellani G, Sedivy JM, Neretti N. Kinetic profiling of the c-Myc transcriptome and bioinformatic analysis of repressed gene promoters. *Cell Cycle* 2011; **10**: 2184-96.
5. Winkle M, van den Berg A, Tayari M, Sietzema J, Terpstra M, Kortman G, *et al.* Long noncoding RNAs as a novel component of the Myc transcriptional network. *FASEB J* 2015; **29**: 2338-46.
6. Chang TC, Yu D, Lee YS, Wentzel EA, Arking DE, West KM, *et al.* Widespread microRNA repression by Myc contributes to tumorigenesis. *Nat Genet* 2008; **40**: 43-50.
7. Robertus JL, Kluiver J, Weggemans C, Harms G, Reijmers RM, Swart Y, *et al.* MiRNA profiling in B non-Hodgkin lymphoma: a MYC-related miRNA profile characterizes Burkitt lymphoma. *Br J Haematol* 2010; **149**: 896-9.
8. Pajic A, Spitkovsky D, Christoph B, Kempkes B, Schuhmacher M, Staeger MS, *et al.* Cell cycle activation by c-myc in a burkitt lymphoma model cell line. *Int J Cancer* 2000; **87**: 787-93.
9. He Y, Jiang X, Chen J. The role of miR-150 in normal and malignant hematopoiesis. *Oncogene* 2014; **33**: 3887-93.
10. Xiao C, Calado DP, Galler G, Thai TH, Patterson HC, Wang J, *et al.* MiR-150 controls B cell differentiation by targeting the transcription factor c-Myb. *Cell* 2007; **131**: 146-59.
11. Korycka J, Lach A, Heger E, Boguslawska DM, Wolny M, Toporkiewicz M, *et al.* Human DHHC proteins: a spotlight on the hidden player of palmitoylation. *Eur J Cell Biol* 2012; **91**: 107-17.
12. Hansen TB, Jensen TI, Clausen BH, Bramsen JB, Finsen B, Damgaard CK, *et al.* Natural RNA circles function as efficient microRNA sponges. *Nature* 2013; **495**: 384-8.
13. Memczak S, Jens M, Elefsinioti A, Torti F, Krueger J, Rybak A, *et al.* Circular RNAs are a large class of animal RNAs with regulatory potency. *Nature* 2013; **495**: 333-8.
14. Salmena L, Poliseno L, Tay Y, Kats L, Pandolfi PP. A ceRNA hypothesis: the Rosetta Stone of a hidden RNA language? *Cell* 2011; **146**: 353-8.

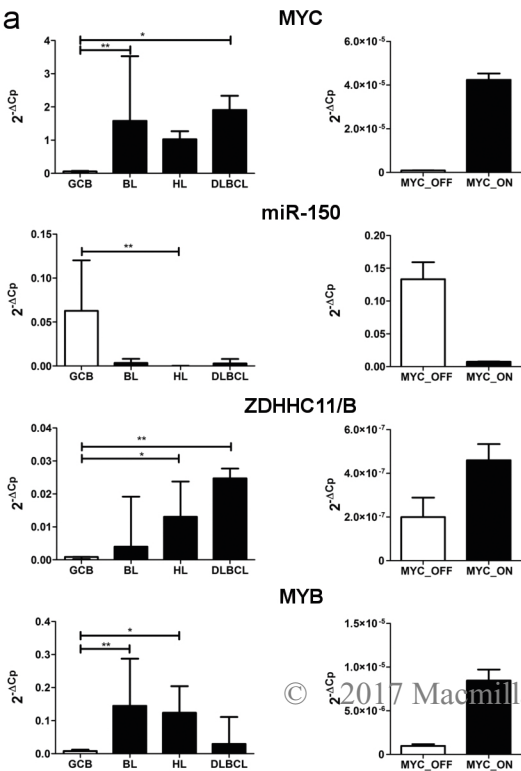
15. Yu L, Reader JC, Chen C, Zhao XF, Ha JS, Lee C, *et al.* Activation of a novel palmitoyltransferase ZDHHC14 in acute biphenotypic leukemia and subsets of acute myeloid leukemia. *Leukemia* 2011; **25**: 367-71.

Figure 1. ZDHHC11 and ZDHHC11B are novel targets of miR-150 essential for growth of BL cells. (a) *ZDHHC11* and *ZDHHC11B* genes encode for protein-coding (blue) and non-coding (green) transcripts. The 3' regions of *ZDHHC11* and *ZDHHC11B* contain tandemly repeated miR-150 binding sites (18 and 62, respectively). Consensus sequence of the miR-150 binding sites in *ZDHHC11* and *ZDHHC11B* is shown. A circular RNA is generated from the *ZDHHC11* locus. RT-PCR in BL cell lines with divergent primers located in the last exon of *ZDHHC11* revealed a circular transcript consisting of exons 12 and 13, exon 13 being 129 nt longer than annotated for the linear transcript (light grey box). Presence of the circular transcript was confirmed with two additional indicated primer sets. See also Supplementary Figure S4. (b) ST486 and DG75 cell lines were transfected with psiCheck2-*ZDHHC11* or psiCheck2-*ZDHHC11B* and miRNA negative control precursor or pre-miR-150-5p (left graph). Average Renilla/firefly luciferase ratio from three independent experiments is shown \pm SD, values for the negative control were set to 1 (two-sided t-test, ** $p < 0.01$; **** $p < 0.0001$). ST486 cells were transduced with miR-150 overexpression vector or control EV (middle and right graph). *ZDHHC11/B* mRNA was quantified by qRT-PCR relative to *U6*. *ZDHHC11* protein levels were quantified relative to the total protein. A representative Western blot is shown with quantification (mean \pm SD) from three experiments. (c) ST486 cells were transduced with lentiviral miR-150 overexpression vector or control EV, shRNA vectors targeting *MYB* or *ZDHHC11/B* and control NT vectors. Effect on cell growth was assessed by following the percentage of GFP+ cells over three weeks post-transduction (left panel; mixed model analysis, ** $p < 0.01$, **** $p < 0.0001$). Mean \pm SD from two independent experiments is shown. *MYB* mRNA levels were quantified by qRT-PCR relative to *U6*. *MYB* protein levels were quantified relative to the total protein. A representative Western blot is shown with quantification (mean \pm SD) from at least two experiments (right panel). See also Supplementary Figures S5 and S6.

Figure 2. The MYC-miR-150-ZDHHC11/B-MYB network in B-cell lymphoma. (a) Expression of *MYC*, *miR-150*, *MYB* and *ZDHHC11/B* was analysed in germinal center B-cells (GCB) sorted from tonsils (n=3) compared to Burkitt lymphoma (BL, n=8), Hodgkin lymphoma (HL, n=7) and diffuse large B-cell lymphoma (DLBCL, n=7) cell lines (left panel), and in P493-6 cells (right panel). RNA levels of *MYC*, *ZDHHC11/B* and *MYB* are upregulated in lymphoma cells compared to GCB, while *miR-150* is downregulated. In P493-6 cells *MYB* and *ZDHHC11/B* are upregulated upon MYC induction, while *miR-150* is downregulated, suggesting direct regulation of all three components by MYC. Data are presented as median with interquartile range (left panel, Kruskal-Wallis test with Dunn's post test, *p<0.05, **p<0.01, ***p<0.001) or mean ±SD (right panel). (b) Model of the MYC-miR-150-ZDHHC11/B-MYB network. In normal mature B-cells high levels of miR-150 repress MYB, *ZDHHC11* and *ZDHHC11B*. In BL cells high levels of MYC repress miR-150, which leads to de-repression of MYB, *ZDHHC11* and *ZDHHC11B* and promotes proliferation. *ZDHHC11* and *ZDHHC11B* further ensure high MYB levels, possibly by acting as a competing endogenous RNA for miR-150.



a



b

