TRIB3 promotes MYC-associated lymphoma development through suppression of UBE3B-mediated MYC degradation

Ke Li1,2,5, Feng Wang3,5, Zhao-na Yang3, Ting-ting Zhang3, Yu-fen Yuan4, Chen-xi Zhao3, Zaiwuli Yeerjiang3, Bing Cui3, Fang Hua3, Xiao-xi Lv3, Xiao-wei Zhang3, Jiao-jiao Yu3, Shan-shan Liu3, Jin-mei Yu3, Shuang Shang3, Yang Xiao1 & Zhuo-wei Hu1,3

The transcription factor MYC is deregulated in almost all human cancers, especially in aggressive lymphomas, through chromosomal translocation, amplification, and transcription hyperactivation. Here, we report that high expression of tribbles homologue 3 (TRIB3) positively correlates with elevated MYC expression in lymphoma specimens; TRIB3 deletion attenuates the initiation and progression of MYC-driven lymphoma by reducing MYC expression. Mechanistically, TRIB3 interacts with MYC to suppress E3 ubiquitin ligase UBE3B-mediated MYC ubiquitination and degradation, which enhances MYC transcriptional activity, causing high proliferation and self-renewal of lymphoma cells. Use of a peptide to disturb the TRIB3-MYC interaction together with doxorubicin reduces the tumor burden in MycEmu mice and patient-derived xenografts. The pathophysiological relevance of UBE3B, TRIB3 and MYC is further demonstrated in human lymphoma. Our study highlights a key mechanism for controlling MYC expression and a potential therapeutic option for treating lymphomas with high TRIB3-MYC expression.

1 National Clinical Research Center for Metabolic Disease, Department of Metabolism and Endocrinology, the Second Xiangya Hospital, Central South University, 410011 Changsha, Hunan, China. 2 NHC Key Laboratory of Biotechnology of Antibiotics, Institute of Medicinal Biotechnology, Chinese Academy of Medical Sciences & Peking Union Medical College, 100050 Beijing, China. 3 Immunology and Cancer Pharmacology Group, State Key Laboratory of Bioactive Substance and Function of Natural Medicines, Institute of Materia Medica, Chinese Academy of Medical Sciences & Peking Union Medical College, 100050 Beijing, China. 4 Anyang Tumor Hospital, Henan University of Science and Technology, 300020 Anyang, China. 5 These authors contributed equally: Ke Li, Feng Wang. ✉email: huzhuowei@csu.edu.cn
Non-Hodgkin lymphoma represents a type of aggressive malignancy that originates in lymphocytes or lymph node (LN) tissues. Although advances in immunochemotherapy and supportive care have improved patient outcomes, a significant proportion of patients experience early treatment failure or relapse after initial therapy. The oncoprotein MYC is a transcription factor that drives cancer cell growth by controlling universal transcription programs, including the cell survival, cell cycle, and metabolism. MYC is deregulated in almost all human cancers, especially Burkitt lymphoma (BL), other aggressive B cell lymphomas (BCLs) and T cell lymphomas (TCLs). Although chromosomal translocation or amplification of MYC partially explains the altered MYC protein expression, a large proportion of lymphomas with high MYC protein expression rarely exhibit these MYC rearrangements, suggesting that mechanisms other than gene rearrangements are responsible for the elevated MYC expression in a considerable proportion of lymphoma cases. Moreover, high MYC expression is correlated with poor prognoses and drug resistance of lymphomas and other hematological malignancies. Targeting MYC, especially in combination with traditional therapies, is considered an attractive therapeutic strategy for lymphomas and other MYC-driven cancers.

Tribbles homologue 3 (TRIB3), a member of the pseudokinase family, acts as a stress sensor that responds to a diverse range of stresses, including inflammation, insulin, insulin-like growth factor 1, and ER stress. TRIB3 is also well known as a crucial “stress adjusting switch” that links homeostasis, metabolic disease, and cancer through its interactions with intracellular signaling and functional proteins. TRIB3 is emerging as a potential therapeutic target for cancer because abrogating its expression dramatically reduces tumorigenesis and cancer progression. Interestingly, the expression of TRIB3, another member of the pseudokinase family, is elevated in T-cell acute lymphoblastic leukemia (T-ALL), and TRIB2 has also emerged as a regulator of thymocyte cellular proliferation. TRIB3, the third member of this family, has a negative regulatory effect on immunoglobulin production in murine B cells. However, the role of TRIB3 in lymphomagenesis remains uncharacterized.

Despite its attractiveness as a cancer target, MYC has been considered “undruggable” and remains outside reach of pharmacological regulation, mainly due to its nuclear localization, lack of a defined ligand-binding site, and large protein-protein interaction (PPI) surface. Because targeting MYC itself is so challenging, efforts have focused on indirect targeting strategies. One evolving approach is the selective degradation of MYC by hijacking the degradation machinery or targeting specific E3 ligases of MYC. Utilizing peptides to overcome the limitations of small-molecule compounds, which can be inefficient in interfering with large PPI surfaces, is a promising strategy for MYC inhibition. We recently reported that TRIB3 enhances the stability of the oncoproteins PML-RARα and β-catenin/TCF4 to promote advanced precancerous lesions (APL) and colorectal cancer progression. In this work, we hypothesize that TRIB3 contributes to lymphoma pathogenesis by promoting MYC-deregulated lymphomagenesis. We examined the expression and roles of TRIB3 in primary lymphoma cells from patients and patient-derived xenograft (PDX) mice. We found that TRIB3 interacts with MYC to suppress E3 ubiquitin ligase UBEB3B-mediated MYC ubiquitination and degradation, which causes high proliferation and self-renewal of lymphoma cells. This study reveals several functional implications for MYC-associated lymphoma therapy.

Results
Deletion of TRIB3 suppresses lymphomagenesis. To examine the role of TRIB3 in lymphomagenesis, we searched the Oncomine database and found that TRIB3 expression was elevated in peripheral T-cell lymphoma (PTCL) and diffuse large B-cell lymphoma (DLBCL) compared to normal lymphocytes (Fig. 1a, b). Additionally, the copy number of the TRIB3 gene was increased in marginal zone BCL compared to lymphoid tissue (Fig. 1c). We sequenced the coding region of TRIB3 in 89 human lymphoma samples to identify nonsynonymous variants. The allele frequencies of nonsynonymous (Q48R) and synonymous (Y111Y and A323A) mutations in lymphoma patients were 38.2% and 47.2%, respectively (Fig. 1d and Supplementary Tables 1 and 2). Because of the unlikely biological significance of the two synonymous mutations (Y111Y and A323A), only the prevalent missense Q48R variant, in which a polar uncharged amino acid is substituted with a charged one, was further examined for association with lymphoma phenotypes. Interestingly, the TRIB3 Q48R mutant was shown to be more stable than wild-type (WT) TRIB3 (Supplementary Fig. 1a), which may result in high TRIB3 expression in human lymphomas. These data suggest the physiological significance of TRIB3 in human lymphoma. We crossed floxed Trib3 (Trib3fl/fl) mice with Cre+ lymphocyte-expressing Cre in thymocytes or CreCD19 mice expressing Cre in thymocytes or CreCD19 mice expressing Cre in B lymphocytes (Fig. 1e and Supplementary Fig. 1b). Reduction of TRIB3 expression was found in these cell lineages, and the heterozygous deletion of Trib3 reduced TRIB3 expression to a level similar to that resulting from the homozygous deletion of Trib3 (Supplementary Fig. 1c, d). The thymus and spleen sizes of 5-week-old CreLysmTrib3fl/fl, CreLckTrib3fl/fl, and CreCD19Trib3fl/fl mice did not differ substantially from those of age-matched WT mice (Supplementary Fig. 1e). Notably, Trib3 ablation in myeloid/thymocyte/B cell does not affect the total number of thymocytes, splenic B cell, T cell, and myeloid cell populations (Supplementary Fig. 1f–i), indicating that specific ablation of Trib3 in these cells does not affect lymphocyte and myeloid cell development.

We separately crossed CreLysmTrib3fl/fl, CreLckTrib3fl/fl, or CreCD19Trib3fl/fl mice with congenic MycF/F mice, the most widely studied model used to understand the genetic mechanisms of lymphomagenesis (Supplementary Fig. 1b). As expected, MycEμ mice developed BCL with an average latency of 140 days with enlarged spleens and LNs (Figs. 1e, f). However, the sizes and weights of spleens and LNs in MycEμCreCD19Trib3fl/fl and MycEμCreCD19Trib3fl/fl mice were significantly reduced compared with those in MycEμCreLysmTrib3fl/+ mice, MycEμCreLckTrib3fl/+ mice and MycEμ mice (Fig. 1f–h). Moreover, B lymphocyte-specific ablation of Trib3 resulted in the extended survival of mice with lymphoma (Fig. 1i). Thus, the loss of TRIB3 in B lymphocytes, but not in myeloid cells or thymocytes, reduces MYC-driven BCL development. To determine whether TRIB3 was required for not only lymphoma initiation but also the malignancy of established lymphomas, we crossed Trib3fl/fl mice with CreERT2 mouse to generate CreERT2Trib3fl/fl mice (Supplementary Fig. 1j), enabling temporally controlled Cre activation by tamoxifen treatment. These mice were further crossed with MycEμ mice to analyze BCL development. Upon the emergence of lymphoma, MycEμ CreERT2Trib3fl/+ mice were treated with vehicle or tamoxifen to monitor BCL progression (Fig. 1j). Although MycEμ CreERT2Trib3fl/+ mice became moribund as early as 53 days after vehicle dosing and 65% died or became moribund by day 120, none of the tamoxifen-treated MycEμ CreERT2Trib3fl/+ mice died within 180 days post treatment (Fig. 1k). Histological analysis revealed reduced tumor foci in the spleens and LNs of tamoxifen-treated MycEμ CreERT2Trib3fl/+ mice compared to lymphoma-matched vehicle recipients (Fig. 1l). This was caused by the tamoxifen-induced loss of TRIB3 in lymphoma tissues (Supplementary Fig. 1j). These data suggest that Trib3 deletion suppresses the initiation and progression of lymphoma.
of MYC-driven BCL and that Trib3 is essential for B cell lymphomagenesis.

**TRIB3 promotes lymphoma by supressing MYC activity.** To investigate the mechanism by which TRIB3 promotes B cell lymphomagenesis, BCL cells were isolated from the spleens of Myc<sup>EµLysm</sup>Trib3<sup>F/F</sup> mice and evaluated (Fig. 2a). Trib3 deletion decreased the number of Ki-67-positive cells (Fig. 2b). BCL cells from Myc<sup>Eµ CreCD19Trib3<sup>F/F</sup> mice yielded substantially fewer colonies than those from Myc<sup>Eµ</sup> mice (Fig. 2c).

Especially, there was significant difference between the survival rates of recipient mice transplanted from Myc<sup>Eµ CreCD19Trib3<sup>F/+</sup> and Myc<sup>Eµ</sup> mice for the transplantation cell dose of 5 x 10<sup>4</sup>, suggesting that BCL cells from Myc<sup>Eµ CreCD19Trib3<sup>F/+</sup> had a lower capacity for killing the recipient mice (Fig. 2d). Knocking out TRIB3 in primary human DLBCL (T69), BL (T4 and T5), and PTCL (T144) cells freshly isolated from patient tumors or lymphoma cell lines (Fig. 2e and Supplementary Table 1) reduced the colony-formation units (CFUs) and proliferation (Fig. 2f, g). We next detected the cell death and differentiation in Raji cells with
or without TRIB3 deletion. TRIB3 deletion did not induce a sub-G1 fraction of the cellular DNA content (Supplementary Fig. 2a), nor did it increase CD11b positive cells ratio in FACS analysis (Supplementary Fig. 2b), suggesting that TRIB3 deletion does not induce cell death or differentiation in lymphoma cells. Thus, TRIB3 promotes lymphoma by supporting lymphoma cell self-renewal ability and proliferation.

Through RNA sequencing (RNA-seq), we analyzed the gene expression profiles of lymphoma cells with or without Trib3 deletion. Gene set enrichment analysis (GSEA) showed highly enriched MYC target genes in BCL cells from MycCreCD19Trib3f/f mice (Supplementary Fig. 2c). qRT-PCR assays showed that TRIB3 deletion suppressed the transcription of MYC target genes (Supplementary Fig. 2d). Consistently, TRIB3 depletion decreased MYC transcriptional activity, while TRIB3 overexpression had the opposite effect (Fig. 2h). Cluster analysis of the heatmap revealed that the TRIB3-depleted and MYC-depleted groups had similar MYC target gene expression tendencies (Supplementary Fig. 2e). Because MYC activation has global effects on both promoter binding and elongation by RNA polymerase II (RNAPII) and, in some situations, globally (Supplementary Fig. 2f), we used representative images and colony numbers of LCs from the indicated groups with or without TRIB3 deletion. Data are presented as the means ± S.E.M from three independent experiments. Statistical significance was determined by two-tailed Student’s t-test.

Fig. 2 TRIB3 enhances the self-renewal and proliferation of LCs. a Approaches to defining self-renewal ability and proliferation in mouse BCL cells. b Cell proliferation was determined by Ki67 staining in BCL cells isolated from MycCre or MycCreCtrl mice (5 months old). Data are representative flow cytometry plots of Ki67 staining (left) and means ± S.E.M of three independent experiments. Statistical significance was determined by two-tailed Student’s t-test. P value: 0.0015. c Colony-forming ability of BCL cells from MycCre or MycCreCtrl mice (5 months old). Shown is means ± S.E.M of three independent experiments. Statistical significance was determined by two-tailed Student’s t-test. P value: 5.37 × 10^{-5}. d The tumorigenicity of BCL cells from the indicated mice was compared by limiting dilution transplantation into NSG mice; n = 8 per group. P = 0.0302 by Kaplan–Meier analysis (two-sided log-rank test) for comparison between the groups of the transplantation cell dose of 5 × 10^4. e Approaches to defining the effects of TRIB3 deletion on the self-renewal ability of primary human LCs and on the proliferation of lymphoma cell lines. f Representative images and colony numbers of LCs from the indicated groups with or without TRIB3 deletion. Data are presented as the means ± S.E.M from three independent experiments. Statistical significance was determined by two-tailed Student’s t-test. P value: 0.0005, 0.0032, 0.0097, and 0.0074. Scale bar, 50 μm. g Relative cell viabilities of Jurkat and Raji cells with or without TRIB3 deletion were detected by CCK-8 for the indicated times of three independent experiments. The colors represent different time points; the diameter indicates the relative cell viability. h Effects of TRIB3 deletion or overexpression on the transcriptional activity of MYC. CtrlCreCas9, Trib3Cas9, Ctrl, and Trib3OE Raji cells were transfected with E-box-dependent luciferase reporter genes of MYC transcription activity, and pTK-Renilla was used as an internal control. After 24 h of transfection, relative luciferase activities (R.L.A) were measured. Shown is means ± S.E.M of three independent experiments. Statistical significance was determined by two-tailed Student’s t-test. P value: 0.0014, 0.0034. i ChIP-sequencing tracks for CCNA2 from CtrlCas9 and Trib3Cas9 Raji cells normalized to spike-in controls. j Metagene plots of global RNAPII occupancy at gene bodies in CtrlCas9 and Trib3Cas9 Raji cells. k Heatmap showing occupancy of genome-wide RNAPII peaks in CtrlCas9 and Trib3Cas9 Raji cells in a window of ±10 kb surrounding the transcription start site (TSS). MycCreCD19Trib3f/f and MycCreCD19Trib3f/f mice are heterozygous for the Myc allele. Source data are provided as a Source Data file.

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TRIB3 disrupts UBE3B-mediated MYC ubiquitination and degradation. Although similar trends of MYC mRNA expression were observed in CtrlCas9 and TRIB3Cas9 Raji cells (Supplementary Fig. 2i), reduced MYC protein expression was detected in MYC1/fiCre-CreD19TRIB3f/+ mice compared to MYC1fi mice (Supplementary Fig. 2g). Notably, TRIB3 knockdown reduced MYC expression in most lymphoma lines and in some leukemia lines (Fig. 3a and Supplementary Fig. 2h). TRIB3 overexpression prolonged the degradation half-life of the MYC protein, while TRIB3 knockdown had the opposite effect, and the shortened MYC half-life induced by TRIB3 knockdown was reversed by the proteasome inhibitor MG132 (Fig. 3b, c and Supplementary Fig. 2i, j).

Serine 62 (S62) and threonine 58 (T58) phosphorylation of MYC is important for MYC-mediated gene regulation and its protein stability.36-40 We used phospho-specific antibodies to accurately quantify the level of Phospho-T58 (P-T58) and Phospho-S62 (P-S62) relative to total MYC in TRIB3 depleted cells. Upon TRIB3 knockdown in Jurkat and Raji cells, we observed a decrease in the P-S62 signal, but no change in the P-T58 signal (Supplementary Fig. 2k, l). Quantifying the ratio of P-T58 to total MYC revealed a substantial increase in the amount of T58 phosphorylated MYC with TRIB3 knockdown (Supplementary Fig. 2k, l). In addition, the signal for P-S62 relative to total MYC decreased in TRIB3 depleted cells compared with control group (Supplementary Fig. 2k, l). Similarly, TRIB3 overexpression increased S62 and decreased T58 phosphorylation relative to total MYC level in Raji cells (Supplementary Fig. 2m). Furthermore, the analysis of half-life of endogenous MYC protein revealed that TRIB3 deletion reduced the stability of MYC protein, especially accelerated P-T58 MYC protein degradation after cycloheximide treatment, as with an antibody against total RNAPII. TRIB3 deletion globally reduced the association of RNAPII with promoters (Fig. 2i–k). Thus, TRIB3 enhances BCL self-renewal and proliferation by enhancing MYC transcriptional activity and increasing the expression of its target genes.
cells were infected with MYC in human DLBCL cells (Fig. 3f, g). UBE3A (Fig. 3h, i). Knockdown of UBE3B inhibited UBE3B-enhanced MYC ubiquitination and proteolysis but increased MYC ubiquitination (Fig. 3i). Interestingly, TRIB3 decreased the stability and half-life of the MYC protein (Fig. 3h). In vitro ubiquitylation assay showed that UBE3B-catalyzed ubiquitination in vivo. HEK 293T cells were transfected with the indicated plasmids for 24 h. Extracts of cells were IP with an anti-HA Ab. Ubiquitinated MYC was detected by immunoblotting. The data are presented as representative from three independent experiments.

Effect of TRIB3 on MYC ubiquitination induced by UBE3B in vivo. HEK 293T cells were transfected with the indicated plasmids for 24 h. Extracts of cells were IP with an anti-Flag Ab. Ubiquitinated MYC was detected by immunoblotting. The data are presented as representative from three independent experiments.

In vitro ubiquitylation assays showed that purified UBE3B induces polyubiquitination of purified MYC in the presence of UBC3B (E2), ATP, Uba1 (E1), and biotinylated ubiquitin. The data are presented as representative from three independent experiments.

Mapping UBE3B regions binding to MYC. Left: deletion mutants of UBE3B. Right: HEK 293T cells were cotransfected with the indicated constructs of TRIB3–GFP and MYC (Flag tag). Cell extracts were IP with an anti-Flag Ab. The data are presented as representative from three independent experiments.

In vitro ubiquitylation assays showed that UBE3B mildly induces polyubiquitination of MYC in the presence of UBC3B (E2) but not in that of other E2s. The data are presented as representative from three independent experiments.

Alignment of partial UBE3B sequences (1024–1049 amino acids in human UBE3B) from the indicated species. The cysteine at position 1036 (C1036) of human UBE3B was mutated to alanine (C1036A). In vitro ubiquitylation assays showed that UBE3B mildly induces polyubiquitination of MYC. The data are presented as representative from three independent experiments.

In vitro assays show that the C terminus of UBE3B (UBE3B-C) mildly induces polyubiquitination of MYC. The data are presented as representative from three independent experiments.

Pie chart shows all the types and statistics of UBE3B mutations in 89 lymphoma patients. In vitro ubiquitylation assays showed that the UBE3B R346Q mutation decreased the polyubiquitination of MYC compared with the wild-type UBE3B. The data are presented as representative from three independent experiments. Source data are provided as a Source Data file.
ubiquitination, while UBE3B depletion enhanced the MYC protein stability regardless of the absence of FBXW7 (Supplementary Fig. 4i, j), indicating that two E3 ligases utilize an independent pathway for MYC degradation. In addition, the T58A mutation reduced binding of UBE3B to MYC (Supplementary Fig. 4k). Given that MYC T58 is phosphorylated by GSK3, a GSK3 inhibitor decreased UBE3B-mediated MYC ubiquitination by reducing T58 phosphorylation (Supplementary Fig. 4i). To test whether expression of T58A alleviates the dependence of MYC function and gene expression on TRIB3, we applied CRISPR/Cas9 technology to establish MYC$^{T58A}$ mutant Raji cells with or without TRIB3 depletion (Supplementary Fig. 5a), and further examined the function and gene expression profiling of these cells. The expression of phospho-T58 and total MYC were first examined in TRIB3 depleted and/or MYC$^{T58A}$ Raji cells. Similar with previous observation, TRIB3 depletion decreased total MYC expression and increased P-T58 relative to total MYC level. However, T58A mutation almost impeded TRIB3’s ability of reducing MYC expression (Supplementary Fig. 5b), suggesting that T58 phosphorylation is essential for the process of MYC expression regulated by TRIB3 depletion. Compared with Raji cells with wild-type MYC (MYC$^{WT}$), the MYC$^{T58A}$ mutant Raji cells exhibited enhanced proliferation (Supplementary Fig. 5c) as well as more and enlarged colonies (Supplementary Fig. 5d). Importantly, TRIB3 knockdown in MYC$^{T58A}$ mutant Raji cells did not significantly influence the abilities of proliferation and colony formation (Supplementary Fig. 5c, d), suggesting that the MYC$^{T58A}$ mutant Raji cells were much less sensitive to TRIB3 depletion compared with MYC$^{WT}$ Raji cells. In addition, we analyzed the gene expression profiles of MYC$^{T58A}$ cells with or without TRIB3 deletion through RNA-seq. GSEA data showed enriched MYC pathway-related genes in MYC$^{T58A}$ lymphoma cells compared with MYC$^{WT}$ lymphoma cells (Supplementary Fig. 5e). Similar with the observation of functional experiment, GSEA analysis shows no statistically significant difference between the MYC$^{T58A}$ mutant Raji cells with and without TRIB3 depletion (Supplementary Fig. 5f), suggesting that T58A mutation alleviated the dependence of MYC pathway-related gene expression on TRIB3. Taken together, these data indicated that K427 of MYC is critical for UBE3B-catalyzed MYC ubiquitination and the functions of TRIB3 in promoting lymphoma cell growth are dependent on T58 phosphorylation of MYC.

TRIB3 interacts with MYC to impair the E3 ligease function of UBE3B. To evaluate the biological consequences of UBE3B-mediated MYC ubiquitination and degradation, we examined the effect of the K427 mutation on MYC transactivation. The K427R mutation increased the transcriptional activity of MYC compared to WT MYC (Supplementary Fig. 6a). We next applied CRISPR/Cas9 technology to establish MYC$^{K427R}$ mutant Raji cell lines (Supplementary Fig. 6b), which were directly used to study MYC$^{K427R}$ function and activity without transfection of exogenous plasmids. The experiments of MYC ChIP-seq with spike-in normalization indicated that the K427 mutation increased the MYC occupancy at the promoters of its target genes (Fig. 4a, b and Supplementary Fig. 6c). Furthermore, UBE3B ablation increased MYC transcriptional activity, while UBE3B overexpression had the opposite effect (Supplementary Fig. 6d). qRT-PCR assays and GSEA showed that UBE3B overexpression suppressed the transcription of MYC target genes (Fig. 4c and Supplementary Fig. 6e). Inspection of multiple individual traces and global analyses showed that UBE3B overexpression globally reduced the association of RNAPII with promoters (Fig. 4d and Supplementary Fig. 6f). By examining the endogenous interactions with Co-IP, we found that MYC, UBE3B, and MAX (also known as MYC-associated factor X) formed the heterotrimer in lymphoma cells (Supplementary Fig. 6g). In the purified component system, MYC bound with UBE3B and MAX (Supplementary Fig. 6h), but no direct binding was detected between MAX and UBE3B (Supplementary Fig. 6i). UBE3B overexpression decreased the MYC/MAX interaction (Supplementary Fig. 6j), while the MYC K427R mutant bound more MAX than the WT MYC (Supplementary Fig. 6k). We examined MYC$^{K427R}$ biological function in the established MYC$^{K427R}$ mutant Raji cells with or without Ad-UBE3B infection. K427R mutation of MYC increased the proliferation of Raji cells, while UBE3B overexpression lost its ability of reduction in MYC$^{K427R}$ Raji cell number compared to that in MYC$^{WT}$ Raji cells (Supplementary Fig. 6l). These data indicate that UBE3B-mediated K427 ubiquitination of MYC interferes with the MYC/MAX interaction and plays an essential role in determining the biological output of UBE3B.

Compared with the Ctrl cells, UBE3B$^{OE}$ Raji cells developed fewer colonies (Fig. 4l) and exhibited reduced proliferation (Fig. 4f), TRIB3 overexpression reversed the antiproliferative effects of UBE3B overexpression in Raji cells (Fig. 4f). However, UBE3B ablation also impeded Raji cell growth (Supplementary Fig. 6m). We further detected the cell death ratio of Raji cells with UBE3B$^{OE}$ or UBE3B overexpression via Annexin V–APC/7-AAD staining. UBE3B overexpression did not induce apoptosis of Raji cells (Supplementary Fig. 6n). These data have indicated that the reduction in Raji cell number with UBE3B overexpression was due to reduced proliferation. We evaluated the effects of UBE3B and TRIB3 on lymphoma development in MYC$^{Eμ}$ mice (Fig. 4g). Ube3b overexpression reduced the enlarged spleens and LNs and reduced the number of Ki-67-positive cells in the spleens of lymphoma mice, which was reversed by TRIB3 overexpression (Fig. 4h–j). At the end of the experiment, BCLs were isolated from these mice and evaluated (Fig. 4k). During myelocellular culture, BCLs from Ad-UBE3B$^{OE}$ mice yielded fewer colonies and were less capable of killing the recipient mice than BCLs isolated from Ad-GFP mice; however, Trib3 overexpression diminished the antilymphoma effects of Ube3b overexpression (Fig. 4l, m). Overexpression of TRIB3 alone did not increase spleen weight, lymph node size or proliferation (Fig. 4h, i, j, l), which could be caused by endogenous levels of TRIB3 sufficient to suppress endogenous UBE3B activity. Additionally, knockout of Ube3b accelerated the death of Myc$^{Eμ}$ and Myc$^{EμCreCD19}$Trib3$^{−/+}$ mice (Fig. 4n, o and Supplementary Fig. 6o, p). Hence, the effects of Ube3b deletion on Myc$^{EμCreCD19}$Trib3$^{−/+}$ mice depended on reversing the reduced MYC expression and CFU number induced by Trib3 knockout (Fig. 4p, q).

In vitro ubiquitination assay showed that TRIB3 impeded MYC ubiquitination (Fig. 5a) and reduced the association of E3 with the MYC substrate but did not affect the association of UBE3B with UBC3 (Fig. 5b, c). Indeed, TRIB3 interacted with MYC in Raji cells (Fig. 5d), which was supported by the colocalization of TRIB3/MYC in human lymphoma cells (Fig. 5e). MYC mainly interacted with the kinase domain (KD) of the TRIB3 C terminus (Fig. 5f). Using three main truncations of MYC (N-terminal domain, central region, and C-terminal domain), we found that the C- and N-terminal domains, but not the central region (CT) of MYC mediated the interaction of MYC with either TRIB3 or UBE3B (Fig. 5g, h left). Among them, the C-terminal domain of MYC showed a major, but the N-terminal domain showed a minor contribution to its binding with TRIB3 or UBE3B (Fig. 5g, h, left). In addition, we performed the reverse Co-IP assay with anti-MYC truncation antibodies (anti-GFP) and examined the levels of TRIB3 of UBE3B in the IP samples. Similarly, the C-terminal MYC possessed the strongest
binding affinity to TRIB3 or UBE3B, while the N-terminal MYC weakly bind to TRIB3 or UBE3B, but the C-terminal domain of MYC did not exhibit any detectable level of binding (Fig. 5g, h, right). Thus, the TRIB3-MYC interaction interferes with the binding of MYC and UBE3B (Fig. 5i, j), likely due to TRIB3 occupying these MYC domains.

Given that the C-terminal domain of MYC also interacted with MAX, we examined the effect of TRIB3 on the MYC/MAX complex. Overexpression of TRIB3 increased the binding of MYC and MAX, as indicated by the Co-IP and surface plasmon resonance assays (Fig. 5k, l). Further, MYC, TRIB3, and MAX formed the heterotrimer in lymphoma cells (Fig. 5m).

Moreover, restored expression of full-length TRIB3 or the KDC domain of TRIB3 reversed the repressed proliferation of lymphoma cells induced by TRIB3 deletion (Fig. 5n), indicating that the KDC domain of TRIB3 interacts with MYC, which stabilizes MYC and enhances lymphoma cell proliferation.

The TRIB3-MYC interaction confers a therapeutic target against lymphoma. Four α-helical peptides (CM1, CM2, CM3, and CM4) derived from C terminus of MYC were screened using the predictive I-TASSER server (Supplementary Fig. 7a), and only CM4 displayed a high binding affinity to TRIB3 (KD = 76.4 nM; Fig. 6a and Supplementary Fig. 7b, c). Homology modeling based on the TRIB1 crystal structure predicted a hydrogen bond between Glu344 (E344) of the TRIB3 protein and the CM4 peptide (Supplementary Fig. 7d), suggesting that the C terminus of TRIB3 is the binding domain of CM4 (Fig. 6b). We further performed sequence alignment within the C termini of TRIB family members and found that E344 of TRIB3 is not conserved at the same positions on TRIB1 and TRIB2 (Supplementary Fig. 7d), indicating that CM4 may be specific for TRIB3. Furthermore, CM4 itself, but not the scramble peptide of CM4, decreased the binding affinity of TRIB3 and MYC in vitro (Fig. 6c). Because the LZ and HLH domains of MYC bind to
MAX, we detected the abilities of these peptides to bind MAX, and CM1, but not CM2, CM3, or CM4, showed a high ability to bind MAX and decreased the binding of MYC and MAX (Supplementary Fig. 7f, g).

To determine whether the CM4 peptide disturbs the TRIB3-MYC interaction in lymphoma cells, a fused peptide, PCM4, was generated by linking CM4 to a cell-penetrating peptide43. High-resolution structured illumination microscopy (SIM) showed the TRIB3-MYC interaction in lymphoma cells treated with a penetrant control peptide (PCON), whereas PCM4 inhibited the colocalization and interaction of TRIB3 with MYC (Fig. 6d and Supplementary Fig. 7h) and rescued the interaction of MYC and UBE3B caused by TRIB3 expression (Fig. 6e, f and Supplementary Fig. 7i). Off-target effects of PCM4 were examined because TRIB3 has been shown to interact with AKT, p62, COP1, and PMI16-18. PCM4 did not disturb the interactions of TRIB3 with these proteins (Supplementary Fig. 7j), indicating that PCM4 specifically interrupts the TRIB3-MYC interaction. PCM4 rescued MYC ubiquitination and degradation (Fig. 6g-i and Supplementary Fig. 7k) and reduced the MYC abundance (Fig. 6d and Supplementary Fig. 7l) in lymphoma cells. Furthermore, we examined the effects of PCM4 on the colocalization and the interaction of MYC and MAX through confocal and CO-IP assays, respectively. The PLA assay showed the reduced foci of MAX-MYC colocalization in PCM4-treated Raji cells (Supplementary Fig. 7m). Interestingly, in the examination of PCM4’s effects on the interaction of MYC and MAX by CO-IP assay, if sufficient MAX antibody (5 μg) was added to the lysates to capture the MAX protein, the quantity of the precipitated MYC protein in control cells was much more than that in PCM4-treated cells (Supplementary Fig. 7n, left). However, if less anti-MYC antibody (1 μg) was added to capture the same quantity of MYC protein in the CO-IP assay, and the quantity of the precipitated MAX protein in control cells was identical with that in PCM4-treated cells (Supplementary Fig. 7n, right). Furthermore, we used the NanoBRET™ assay to identify whether CM4 interferes with the MYC-MAX protein interaction in a dose-dependent manner using the respective proteins tagged with NanoLuc® luciferase (NanoLuc®) and HaloTag® ligands. With 10058-F4 as a PPI inhibitor of MYC-MAX interaction, we found that CM4 showed no effects on the MYC/MAX heterodimer in this assay (Supplementary Fig. 7o). In addition, quantification of the MYC/MAX heterodimer using Proximity ligation assay (PLA) has been performed in the CM4-treated Raji cells with proteasome inhibitor MG132 reversing MYC degradation. CM4 treatment indeed did not affect the colocalization of MYC and MAX in MG132-treated Raji cells (Supplementary Fig. 7p). Overall, our results suggested that the low amount of MYC/MAX complex accounts for the reduced MYC transcriptional output, and CM4 shows specificity to MYC and TRIB3 but not to the MYC and MAX.

Consistent with these findings, PCM4-reduced MYC transcriptional activity (Fig. 6k). qRT-PCR and RNA-seq showed that PCM4 suppressed the transcription of MYC target genes (Fig. 6l and Supplementary Fig. 7q). Inspection of multiple individual traces and global analyses of RNAPII function showed that PCM4 globally reduced the association of RNAPII with promoters of its transcripted genes (Fig. 6m, n). Additionally, PCM4 reduced the intrinsic clonogenicity of human lymphoma cells (Fig. 6o and Supplementary Fig. 7r, s) and primary mouse BCL cells (Supplementary Fig. 7t) and inhibited the proliferation of most lymphoma and leukemia cells in a time-dependent manner (Fig. 6p). Interestingly, PCM4-sensitive cells expressed TRIB3 and MYC at higher levels than PCM4-resistant cells (Fig. 6q). The combination of PCM4 with doxorubicin (DOX), a chemotherapeutic agent for the treatment of lymphoma, synergistically inhibited Raji cell proliferation (Fig. 6r). These data indicate that disturbing the TRIB3-MYC interaction reduces MYC expression and blocks the lymphoma-promoting effects of MYC in vitro.

We examined the anti-BCL effect of PCM4 in MycEpi mice (Fig. 7a). On day 90 after treatment, dramatic reductions in the spleen/LN weights and numbers of Ki-67-positive cells in the spleens were observed in mice treated with PCM4 compared to those treated with PCON (Fig. 7a, d). Indeed, the secondary recipients from the PCM4 group had a better survival rate than those from the PCON group (Fig. 7e). The PCM4 group exhibited a decrease in the TRIB3-MYC interaction and MYC expression in the LNs and spleens of MycEpi mice (Fig. 7f, g). Additionally,
Fig. 5 TRIB3 interacts with MYC to inhibit the UBE3B:Myc axis. a In vitro ubiquitination assays show that the TRIB3 or TRIB3-KDC protein decreased polyubiquitination of MYC mediated by UBE3B and UBC3d. b The TRIB3 protein or the KDC domain of TRIB3 decreased the interaction of MYC and UBE3B in the purified system. c Neither the TRIB3 protein nor the KDC domain of TRIB3 affected the interaction of UHCH3 and UBE3B in the purified system. d The interaction of TRIB3 and MYC in Raji cells was evaluated by Co-IP assays. e Colocalization of TRIB3 and MYC in Raji cells was detected with immunostaining (PLA assay). Data are representative images from three independent experiments. Scale bar, 2 μm. f Mapping TRIB3 regions binding to MYC. Top: deletion mutants of TRIB3. Bottom: HEK 293T cells were cotransfected with the indicated constructs of MYC and TRIB3 or UBE3B. Cell extracts were IP with an anti-Flag Ab. g Mapping MYC regions binding to TRIB3 (HA tag) and MYC (Flag tag). Cell extracts were IP with an anti-Flag Ab. h MYC-Flag was captured by GFP pull-down. Cell extracts were IP with an anti-Myc Ab. i TRIB3 overexpression interfered with the interaction of UBE3B and MYC. HEK 293T cells were cotransfected with the indicated constructs. Cell extracts were IP with an anti-Myc Ab. j TRIB3 decreased the binding of UBE3B and MYC. Kinetic interactions of the UBE3B and MYC proteins were determined by SPR analyses with the BSA or TRIB3 proteins. k TRIB3 overexpression increased the interaction of MAX and MYC. HEK 293T cells were cotransfected with the indicated constructs. Cell extracts were IP with an anti-Myc Ab. l TRIB3 overexpression increased the binding of MAX and MYC. Kinetic interactions of the MAX and MYC proteins were determined by SPR analyses with the CTRL (GST) or TRIB3 (TRIB3-GST) protein. m The heterotrimers of MYC, TRIB3, and MAX in Raji cells were detected by Co-IP assays. Cellular extracts were IP with mouse IgG as a negative control or anti-Myc, anti-TRIB3, or anti-MAX antibodies. Western blots were performed with anti-Myc, anti-TRIB3, and anti-MAX antibodies. n Recovery of the TRIB3-HA wild-type or KDC-HA mutant enhanced the decreased cell viabilities of TRIB3Cas9 Jurkat cells for the indicated times. The colors represent different time points; the diameter indicates the relative cell viability. Source data are provided as a Source Data file.
PCM4 did not exert obvious toxic effects in these mice, as indicated by renal/liver function assays (Supplementary Fig. 8a, b). The combination of PCM4 and DOX enhanced the therapeutic efficacy of DOX, as indicated by the enhanced survival rate of Myc2/2 BCL mice treated with PCM4 and DOX (Fig. 7h). These data indicate that suppressing the TRIB3-MYC interaction and MYC expression attenuates the lymphoma-promoting effects of MYC in vivo.

We also examined the effects of PCM4 on established lymphoma PDX models derived from 10 patients with DLBCL (T64, T69, T169, T156, and T1), PTCL (T144), follicular lymphoma (FL; T151 and T174), T cell lymphoblastic lymphoma (T-LBL; T123) and TCL (T3; Fig. 8a, Supplementary Fig. 8c, and Table 1). PCM4 inhibited the tumor growth of PDXs (T64, T69, T144, T123, T174, T169, and T156) with high TRIB3 and MYC expression compared with PDXs (T64, T69, T144, T123, T174, T169, and T156) with low TRIB3 and MYC expression (Fig. 8b–i). In comparison with the benign LN tissues, most lymphoma specimens showed high expression of TRIB3 but not UBE3B (Fig. 8c) and Supplementary Fig. 8d). Notably, a positive correlation was observed between TRIB3 and MYC expression (Fig. 8k, l). These data indicate that TRIB3 plays a critical role in
MYC-driven lymphoma by interacting with MYC and that disturbing this interaction in combination with DOX treatment results in potent antilymphoma efficacy (Fig. 9).

Discussion
Although MYC rearrangements partially explain the high MYC expression in all BLs and non-Burkitt high-grade BCLs, there is no consensus regarding the causes of high MYC levels in lymphomas without MYC rearrangement. In this study, we observed that TRIB3 expression was elevated in more than 50% of human lymphoma cases. Using transgenic mice with Trib3 deletion in different cell lineages, we found that TRIB3 participated in MYC-driven lymphoma tumorigenesis and pathogenesis. TRIB3 knockout suppresses the cell proliferation and self-renewal ability of MYC-associated lymphoma by reducing MYC protein stability and abundance in these cells. Our study thus provides insights into the pathogenesis of MYC-associated lymphomagenesis.

TRIB3 deletion in B lymphocytes, but not in myeloid cells or T lymphocytes, inhibited lymphoma development. Several mechanisms may account for TRIB3 ablation-induced lymphoma inhibition. First, TRIB3 depletion restores UBE3B-mediated MYC ubiquitination and degradation, which suppresses MYC expression and MYC-related oncogenic activities. Second, TRIB3 depletion increases the activity of the autophagy and ubiquitin proteasome system (UPS), p53-dependent senescence, PML nuclear body formation and regulation of cancer metabolism, which may collaborate to participate in the inhibition of lymphomagenesis. Third, TRIB3 deletion decreases TRIB2 expression in lymphocytes, while TRIB2 functions as an oncogene in acute myeloid lymphoma (AML) by regulating C/EBPα expression and degradation. Thus, TRIB3 depletion-reduced TRIB2 expression in lymphoma cells may contribute to lymphoma pathogenesis in MycFipCreCD19Trib3F1/− mice. TRIB2 was reported to act downstream of Wnt/TCF, and Wnt/β-catenin activation was shown to induce TRIB2 expression in cancer cells. Our recent study indicated that TRIB3 depletion decreased β-catenin/TCF transcriptional activity by reducing the recruitment of TCF and β-catenin to the promoter region of genes regulated by Wnt in colon cancer cells. Hence, we presumed that the decreased protein expression of TRIB2 is caused by TRIB3 knockout-reduced TRIB2 transcription through suppressing the activity of Wnt/β-catenin in lymphoma cells. Obviously, it is worth further dissecting the molecular mechanism accounting for the TRIB3 regulation of TRIB2 expression.

The MYC level is tightly controlled by E3 ligase-regulated protein degradation. FBXW7 is the most important E3 ligase for MYC ubiquitination and proteasomal degradation in hematologic malignancies, which is dependent on T58 phosphorylation of MYC. However, mutation of T58 to a nonphosphorylatable residue stabilizes MYC more than the ablation of FBXW7, suggesting that additional E3 ligases also stimulate the proteolysis of MYC T58 phosphorylation. Indeed, we found that UBE3B, a newly identified E3 ligase of MYC, drives the proteolytic turnover of MYC dependent on T58 phosphorylation. TRIB3 sustained MYC stability specifically via UBE3B but not via other known E3 ligases. Indeed, UBE3B overexpression reduces MYC abundance and suppresses its transcriptional activity, resulting in lymphoma inhibition. Interestingly, our work indicates that UBE3B may not represent a viable drug target because UBE3B deletion in lymphoma cells decreases tumor cell proliferation.

Loss of UBE3B function causes Kaufman oculocerebrofacial syndrome, a rare developmental disorder. The relationship between UBE3B deregulation and cancer has not yet been reported or validated. Indeed, we did not find altered UBE3B expression in human lymphoma samples compared to benign LNs. Thus, high MYC expression may be mainly caused by a decreased interaction of UBE3B and MYC, which is induced by elevated TRIB3 expression in lymphoma cells. Moreover, a loss-of-function mutation of UBE3B (R346Q) is found in MYC-associated lymphoma patients and impedes MYC degradation. Notably, TRIB3 knockdown decreases MYC expression in most blood cancer cells but not in U937 AML cells or bjab BL cells. One possible reason is that the E3 ligase FBXW7 or SKP2, but not UBE3B, mediates MYC ubiquitination and degradation in these cells. Another reason may be that these cells express very low levels of UBE3B. Thus, it is worth examining the expression of MYC-related E3 ligases when MYC expression is evaluated in lymphoma cells. The synergetic effect of PCM4 and DOX on the cell viability of Raji cells. Source data are provided as a Source Data file.
Fig. 7 Disturbing the TRIB3/MYC interaction attenuates lymphoma in mice. a The strategy for investigating the anti-BCL effects of PCM4 on MycEi mice in vivo. b, c The data represent the statistical analyses of spleen and LN weights in MycEi mice (5 months old; n = 7 per group) treated with the indicated agents. Data are represented as means ± SEM. Statistical significance was determined by two-tailed Student’s t test. P value: 0.016; 0.0108, and 0.0038 (b); 0.003, 0.0003, 7.34 × 10⁻⁵ (c). d Representative images of Ki67 staining (left) and statistical analyses of Ki67-positive cells (right) in the spleens of MycEi mice (5 months old; n = 12 per group) treated with the indicated agents. Scale bar, 50 μm. Data are represented as means ± SEM. Statistical significance was determined by two-tailed Student’s t test. P value: 1.28 × 10⁻⁹, 7.86 × 10⁻¹⁰, and 1.13 × 10⁻⁹. e The tumorigenicity of BCL cells from the indicated mice was compared by limiting dilution transplantation into NSG mice; n = 8 mice per group. f PCM4 disturbed the TRIB3/MYC interaction in vivo. Spleen lysates from the indicated mice (5 months old) were IP with an anti-TRIB3 Ab and blotted with an anti-MYC Ab. The data are representative from three independent experiments. g Kaplan-Meier survival curves for MycEi BCL mice treated with the indicated agents (n = 10 per group). Statistical difference was determined by two-sided log-rank test. P value: 0.0214 (DOX + PCM4 vs. DOX + PCON). Source data are provided as a Source Data file.

difficult, several MYC-directed therapies, including bromodomain, extraterminal (BET) inhibitors, and the Omomyc peptide, have been assessed in preclinical settings and in clinical trials (NCT01713582) involving hematological malignancies with durable responses⁵¹,⁵². However, acquired resistance will limit responsiveness to BET inhibitor treatment through the rewiring of transcriptional programs or activation of the WNT pathway⁵³,⁵⁴. In this study, we found that lymphoma cells with high TRIB3 and MYC expression were more sensitive to PCM4 than those with low TRIB3 or MYC expression and that PCM4 increased the sensitivity of lymphoma cells to DOX due to decreased MYC expression. Notably, the CM4 peptide corresponds to aa 436–450 of the LZ region of MYC, which is the MAX-binding region domain. However, CM4 specifically bound TRIB3 and subsequently disturbed the MYC-TRIB3 and MYC-MAX interactions. The PCM4-reduced interaction and colocalization of MYC-MAX in lymphoma cells may largely be caused by a reduction in MYC abundance induced by PCM4. Although the short-term toxicity of this peptide is not obvious in lymphoma mice, the long-term toxicity and target specificity of PCM4 need to be further investigated in normal proliferating tissues. Moreover, PCM4 is not a viable option for human trials due to its intrinsic weaknesses, including poor pharmacochemical stability and a short circulating plasma half-life. Hence,
stabilization of the α-helix peptide, substitution of amino acids and other modifications to improve the physicochemical properties of PCM4 are further needed to obtain the desired druggable properties.

In summary, our study indicates that TRIB3 is a reasonable molecular target for the treatment of high MYC-expressing lymphoma and that interruption of the MYC-TRIB3 and MYC-MAX interactions has therapeutic potential for this lymphoma.
Fig. 9 Schematic diagram illustrates the TRIB3-UBE3B-MYC axis driving lymphoma development and progression. TRIB3 expression is enhanced in human lymphoma cells due to the increased copy number in response to various stresses and nonsynonymous (Q84R) mutation of TRIB3. Whereas, UBE3B, an E3 ubiquitin ligase, and UBCH3, an E2 ubiquitin-conjugated enzyme, have been identified to target MYC for proapoptotic degradation. The enhanced TRIB3 interacts with MYC to decrease the UBE3B-mediated ubiquitination of MYC K427 and increase MYC stability; this interaction also supports the formation of MYC and MAX heterodimer. These actions enhance MYC transcriptional activity, causing high proliferation and self-renewal of lymphoma cells. Interfering with the TRIB3/MYC interaction enhances MYC degradation and suppresses the development and progression of high TRIB3/MYC-associated lymphoma.

Furthermore, our results raise the intriguing possibility of targeting this interaction to treat other MYC-associated malignancies by reducing MYC abundance.

Methods

**Animal studies.** NOD-scid IL2Rgnull (NSG) mice (4–6 weeks old, male) were purchased from the Nanjing Biomedical Research Institute of Nanjing University (Nanjing, China). Trib3−/− knockout (B6;129P2-Tg(CAG-CreERT2/CreloxP)) mice (5–6 weeks old, male) were generated as described in our previous study (Li et al.15). CreERT2 (B6; Cg-Tg(CAG-cre/Esx15Amp4)) mice (5–6 weeks old, 1 male and 2 females) (The Jackson Laboratory, 004682) were obtained from The Jackson Laboratory (CA, USA). When CreERT2 transgenic mice bred with mice containingloxP-flanked sequences, tamoxifen-inducible Cre-mediated recombination results in deletion of the floxed sequences in widespread tissues/organisms of the offspring. CreERT2 (B6; Cg-Tg(Lck-cre)548Jxm/J) mice (5–6 weeks old, 1 male and 2 females; The Jackson Laboratory, 003802), and CreERT2D19 (B6;129P2-CdCd19tm1(Cre)/Cgrla) mice (5–6 weeks old, 1 male and 2 females; The Jackson Laboratory, 006785) were obtained from the Shanghai Research Center for Model Organisms (Shanghai, China). During breeding, Trib3−/− knockout mice were generated by crossing Trib3ERT2 mice with CreERT2 mice, CreERT2 mice, and CreERT2 mice, respectively. The generation of inducible Trib3−/− knockout mice was performed by breeding Trib3ERT2 mice with CreERT2 mice and treating them with tamoxifen to induce Trib3 gene deletion. Lymphomagenesis was induced by transgenic E9-driven Myc (MYC−E9). MYC−E9 mice were obtained from the Nanjing Biomedical Research Institute of Nanjing University (Nanjing, China, N000116). To determine the role of Trib3 in lymphomagenesis, MYC−E9 mice were crossed with CreERT2Trib3ERT2, CreERT2Trib3ERT2, CreERT2Trib3ERT2, and CreERT2Trib3ERT2 mice. For the induced deletion of Trib3 in MYC−E9CreERT2Trib3ERT2 mice, 3-month-old MYC−E9CreERT2Trib3ERT2 mice were treated with tamoxifen (Sigma Aldrich, 06734) at a dose of 250 mg/kg i.p. for 6 days, followed by tamoxifen injection once per week until killing at 6 months. Deletion of Trib3 was corroborated by quantitative PCR and immunoblot analysis. Animals were housed in groups of 4–6 mice per individually ventilated cage in a 12 h light/dark cycle (07:30–19:30 light, 19:30–7:30 dark), with controlled room temperature (23 ± 2°C) and relative humidity (40–50%).

All mice were maintained in the animal facility at the Institute of Materia Medica under specific-pathogen free (SPF) conditions. For animal studies, the mice were divided equally before grouping and then randomly separated into groups by an independent person; however, no particular method of randomization was used. Sample size was predetermined empirically according to previous experience using the same strains and treatments. Generally, we used n ≥ 6 mice per genotype and condition. We ensured that the experimental groups were balanced in terms of animal age, sex, and weight. All animal procedures were conducted in accordance with the guidelines of the Institutional Committee for the Ethics of Animal Care and Treatment in Biomedical Research of Chinese Academy of Medical Sciences and Peking Union Medical College (PUMC). The animal study was also performed according to the ARRIVE guidelines22.

**Human lymphoma tissues/samples and cell culture.** Human lymphoma patient specimens were obtained from Anyang Tumor Hospital, Henan University of Science and Technology and the Institute of Hematology and Blood Diseases Hospital of PUMC. Informed consent was obtained from all participants in accordance with the Declaration of Helsinki. The procedure was approved by the institutional review board at Anyang Tumor Hospital and the Ethics Committee of the Institute of Hematology and Blood Diseases Hospital of PUMC (K2019055-EC-1). All participants provided written informed consent to publish information that identifies individuals. Our study is compliant with the ‘Guidance of the Ministry of Science and Technology (MOST) for the Review and Approval of Human Genetic Resources’, which requires formal approval for the export of human genetic material or data from China. Patient-related information is provided in Supplementary Table 1.

9 (T lymphoma cells), NALMALWA (BL cells), K562 (chronic myelogenous leukemia) cells, MOLT-4 (acute lymphoblastic leukemia) cells, bjab (BL cells), Jurkat (acute T cell leukemia) cells, Sup-B15 (acute B cell leukemia) cells, U937 (myeloid leukemia) cells, NALMALWA (BL cells), K562 (chronic myelogenous leukemia) cells, Rai (BL), and HEK 293T cells were purchased from Shanghai Biolae Biotech Co., Ltd., where they were recently authenticated by short tandem repeat (STR) profiling and characterized by mycoplasma and cell vitality detection. These cells were cultured and maintained in RPMI 1640 medium supplemented with 10% fetal bovine serum (FBS, Invitrogen, CA, USA) under 5% carbon dioxide. All cell lines were verified negative for mycoplasma contamination by MycoAlertTM Mycoplasma Detection Kit (Lonza, LT07419). No cell lines used here appear in the database of commonly misidentified cell lines (the International Cell Line Authentication Committee). For generation of cells stably expressing MycΔ5 or shUBE3B lentivirus particles were purchased from TransOMIC Technologies Inc. The gRNA sequence targeting TRIB3 was 5’-CATGGTTATCGGACAGAGC-3’. And the shRNA sequence targeting UBE3B was 5’-GAGAGAGATTGCTAGCTT-3’. After 24 h of infection, stable cells were selected in medium containing 1 μg/ml puromycin (Gibco) for 14 days. After 2–3 passages in the presence of puromycin, the collected cells were used for experiments without cloning. The Amaxa™ 4D-Nucleofector system was used to transiently transfect plasmids into primary lymphoma cells or suspension cells following the standard instructions (Lonza).

**Plasmid construction.** The Human MYC-Flag-tagged (HG11346-CF), SKP2-Flag-tagged (HG15079-CF), TRIM6-untagged (HG18626-UT), TRIM52-untagged (HG18626-UT), TRIM21-Flag-tagged (HG18010-CF), FXW7-His-tagged (HG10648-HM), UBE3B-N (HG12885-CH), COPI-FLAG-tagged (HG19467-CH), TRIB2-FLAG-tagged (HG10725-CF), UBC13 (UBE2K)-Myc-tagged (HG16296-CM), UBC13 (CDC4)-Myc-tagged (HG11443-CM), UBC13 (UBE2D1)-Myc-tagged (HG11432-CM), UBC13 (UBE2D2)-Flag-tagged (HG17832-CF), UBC13C (UBE2D3)-Myc-tagged (HG11628-CM), UBC16-Myc-tagged (HG20023-CM), UBC7 (UBE2C)-Myc-tagged (HG12005-CM), UBC8 (UBE2L6)-Myc-tagged (HG16791-CM), UBC10 (UBE2D2)-Myc-tagged (HG16911-CM), UBC13-His-tagged (HG16310-CM), and UBC-HA-tagged (HG16831-NY) plasmids were purchased from Sino Biological Inc. (Beijing, China). The human UBE3B-Flag-tagged (OHu09547) and HACE1-Flag-tagged (HG23533-CF) plasmids were purchased from GenScript Inc. (Nanjing, China). The Human HACE1-UBE2H-Myc-DYK-DKD (RC205216) was purchased from Origene. The truncations of MYC-MYCN (amino acids 1–360), ΔLZ (amino acids 1–426), ΔTAD (amino acids 144–445), ΔTAD–ΔLZ (amino acids 144–426), and central region (amino acids 144–360) were inserted into the pCDNA3.1-his vector (Invitrogen, Carlsbad, CA, USA) by standard ligation. The truncations of MYC-MYCN (amino acids 1–144), MYC-C (amino acids 360–426), and central region (amino acids 144–360) were inserted into the pGEP-C1 vector by standard subcloning. TRIB3-FL (amino acids 1–359) and the truncations of TRIB3, T3-N (amino acids 1–179), T3-C (amino acids 180–359), and KD (amino acids 72–315) plasmids were inserted into the pCDNA3.1-N-HA vector (GenScript, Nantong, China) by standard subcloning. TRIB3-FL was cloned into the pCDNA3.1-N-HA vector by standard subcloning. TRIB3-Flag-tagged (HG10731-CF) was purchased from Sino Biological Inc. (Beijing, China). MYC-TS8A based on the isofrom of MYC with 439 aa was inserted into the pCDNA3.1-Myc vector by subcloning. The truncations of UBE3B, UBE3B-N (amino acids 1–144), and UBE3B-C (amino acids 245–1068), UBE3B-L (amino acids 1–862), and HECT (amino acids 682–1068) were inserted into the pCDNA3.1-Myc vector by standard...
The control-3XFlag (Empty-Flag) vector (E4151) was purchased from Sigma Aldrich. p–TRIB3-luc was generated as described in the previous study. His-tagged MYC and His-tagged MAX were inserted into the pCDNA3.1-Myc-His vector by standard subcloning. HA- and Flag-tagged ubiquitin were purchased from Sino Biological Inc. (Beijing, China).

E3 ligase library screening. The E3 ligase library including 71 E3 ligase expression plasmids (Flag-tagged) was constructed by standard subcloning into the control-3XFlag vector. Most clone plasmids of these E3 ligases were purchased from Sino Biological Inc. and Origene Inc. Detailed information on the E3 ligases is provided in Supplementary Table 3. For E3 ligase screening, HEK 293T cells were seeded into six-well plates. After 12 h, HEK 293T cells were co-transfected with MYC (Myc-tagged) and different E3 ligases separately. After 24 h of transfection, Co-IP experiments were performed to detect the interaction of MYC and different E3 ligases.

Luciferase reporter assays of MYC transcriptional activity. Cells were seeded in 12-well plates and transfected with E-box-dependent luciferase reporters (Yeasen Inc.) using VivoFect (Vigorose Biotechnology Beijing, China). pT7-Remila was used as an internal control. Luciferase activity was measured 20–24 h after transfection using the Dual-luciferase Reporter Assay System (Promega, USA).

Proximity ligation assay. A total of 1 × 10⁶ cells were seeded overnight in a six-well plate. The next day, cells were collected, fixed in 4% paraformaldehyde, permeabilized with 0.5% Triton-X100, blocked with Duolink® Blocking Solution, and probed with antibodies directed against MYC (R&D, NB600-302, 1:100), TRIB3 (Novus, H0057761-M03, 1:100), UBE3B (Invitrogen, PAS-59930, 1:100), and MAX (Cell Signaling Technology, #4739, 1:100). The cells were then treated with the Duolink In Situ Red Starter Mouse/Rabbit kit (Sigma Aldrich, DU9201-1KT) according to the manufacturer’s instructions. Images were captured with an OLYMPUS confocal microscope.

Protein purification. Purified His-tagged MYC (TP760019) was purchased from Origene Inc. GST-tagged TRIB3 (10731-H90B) and KDC domains, GST-tagged MAX (12885-H20B) and His-tagged MAX (12269-H08H) were purchased from Sinobiological Inc. The recombinant proteins UBE3B (His-tagged), UBE3B-C (His-tagged), UBE3B R346Q, and UBE3B C1036A were produced in FreestyleTM 293-F MAX (12885-H20B) and His-tagged MAX (12269-H08H) were purchased from Biolog Inc. (Beijing, China).

Flow cytometry. Primary cell suspensions from the thymus, spleen, or LNs or Raji cells with or without TRIB3 deletion were directly labeled. Fluorescently labeled antibodies against the following surface proteins were used for human/mouse cell staining: FITC anti-human CD19 antibody (Biologend, 392508, 1:100), PE anti-human CD3 antibody (Biologend, 981004, 1:100), APC anti-mouse CD3 antibody (Biologend, 100236, 1:100), FITC anti-mouse-human CD45R/B220 antibody (Biologend, 103205, 1:100), FITC anti-mouse Ki-67 antibody (Biologend, 652410, 1:100), PE anti-mouse-human CD11b antibody (Biologend, 101207, 1:100), FITC anti-human CD11b antibody (Biologend, 301350, 1:100). Data were acquired using a FACSCalibur or FACSQuant II flow cytometer (BD); FCS EXPRESS software was used for data analysis.

Primary cell purification/isolation. For protein analysis or other assays, B cells from mouse spleens or LNs were isolated by Dynabeads® Mouse Pan B (B220; Dynabeads; Invitrogen, 11441D). Primary human BCL cells were purified using MACS CD19 MicroBeads (Miltenyi Biotec, 130-050-301) at 4 °C, and the percentage of BCL cells (CD19+ and CD3−) was determined by flow cytometry. Primary human TCL cells were isolated with the EasySep® Humian T Cell Isolation Kit with immunomagnetic negative selection according to standard instructions (StemCell Inc. #17951). Thymocytes were isolated from mice as described below: the thymus was removed, trimmed clean of connective tissue and brown fat (if present) and transferred into Hanks’ Balanced Salt Solution (Sigma) containing 10% (v/v) FBS. A single cell suspension was prepared by passage through a 70-μm nylon sieve (Falcon).

Immunoprecipitation, immunoblotting, immunostaining, and lymphoma tissue microarrays. Co-IP experiments were performed as described previously. Briefly, cells were collected and lysed for 30 min on ice. Soluble lysates were incubated with the indicated antibodies at 4 °C overnight, followed by incubation with Protein A/G Plus-Agarose (Santa Cruz Biotechnology, TX, USA) at 4 °C for 3 h. Soluble lysates were incubated with the indicated Anti-Myc magnetic beads (Bimake.com, B23102) or Anti-HA Afﬂiction Gel (Bimake.com, B23102) or Anti-HA Afflition Gel (Bimake.com, B23302) at 4 °C overnight. Immunocomplexes were separated from the beads and then boiled for 10 min. The precipitated proteins were subjected to SDS-PAGE and blotted with specific antibodies. For immunoblotting assays, proteins were extracted from cells, spleens, or lymphoma tissues using RIPA buffer (Cell Signaling Technology, MA, USA). A BCA Protein Assay Kit was used to determine protein concentrations. Protein extracts were separated by SDS-PAGE, transferred onto PVDF membranes, and subjected to immunoblot analysis. Western blots were performed on 5000 units of the Hanks’ Balanced Salt Solution (Sigma) containing 10% (v/v) FBS. A single cell suspension was prepared by passage through a 70-μm nylon sieve (Falcon).

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For immunofluorescence staining, cells seeded on coverslips were briefly washed with PBS and fixed with 4% buffered paraformaldehyde for 15 min, permeabilized with 0.5% Triton X-100 for 15 min, blocked with 3% BSA for 30 min at 37 °C, and stained with specific primary antibodies followed by corresponding secondary antibodies. Nuclei were counterstained with DAPI. Images were captured using a confocal fluorescent microscope (Olympus Microsystems, CA, USA). Quantitative image analysis was performed with the Imaris 9.3.1 software. Pearson's coefficient was used to analyze colocalization between two target proteins.

For immunohistochemistry analysis, the paraffin-embedded tissue sections were deparaffinized with xylene and hydrated through graded alcohols into water. Antigen retrieval was obtained with 10 mM citrate buffer, pH 6.0, at sub-boiling temperature for 15 min. The sections were permeabilized with 0.5% Triton-100/PBS for 20 min. Endogenous peroxidase activity was blocked with 3% H2O2 solution for 10 min, followed by washing three times with PBS. Blocking buffer (3% BSA/PBS) was added to the sections and incubated for 30 min. Slides were then incubated with indicated primary antibodies at 4 °C overnight. After washing three times, sections were incubated for 30 min with corresponding secondary antibodies at room temperature. Signals were detected with freshly made DAB substrate solution (ZSGB-Bio, Company, Beijing, China). Sections were then counterstained with hematoxylin, dehydrated, and mounted with coverglasses. Images were analyzed by Olympus DP72 microscope (Olympus Microsystems, CA, USA) and analyzed by Image-Pro Plus 5.1.

For immunoblotting, the following antibodies were used: anti-TRIB3 (Abcam, ab75846, 1:100, anti-TRIB3 (ThermoFisher, PA-517408, 1:1000), anti-TRIB3 (Abcam, ab137526, 1:200), TRB-3 Antibody (D-4) (Santa cruz Biotechnology, sc-635842, 1:500), anti-GAPDH (ZSGB-BIO TA-08, 1:200), anti-Myc (Cell Signaling Technology, 1:1000), anti-HA Ubiquitin (CST, #3933 S, 1:1000), anti-Fbxw7 (Abcam, ab109617, 1:500), anti-Max (Abcam, ab83834, 1:1000), anti-UBE3B (365842, 1:500), anti-GAPDH (ZSGB-BIO TA-08, 1:2000) anti-c-Myc Antibody (CST, #9402 S, 1:1000), anti-UBE3B (365842, 1:500), anti-GAPDH (ZSGB-BIO TA-08, 1:2000) anti-c-Myc (D3N8F) (Olympus Microsystems, CA, USA) and analyzed by Image-Pro Plus 5.1. Background noise, an anti-MYC antibody (Cell Signaling Technology, 1:1000) was used to confirm protein bands with some electrophoresis. To obtain accurate results, the PAGE gel was run for at least 3 h to ensure that protein bands run off the bottom line of the gel as judged by the protein molecular weight marker. Then, the protein bands were cut out with a cold knife and elution buffer. ChIP-seq libraries were generated by DNA fragmentation, end repair, dA-tailing, adapter ligation and PCR amplification. The Qubit® 3.0 fluorometer was used for quantitation of the ChIP library. The Agilent 2100 Bioanalyzer was used to test the insert sizes of the library. The StepOnePlus™ Real-Time PCR system was used to assess the mobility of the library, which must exceed 100 bp for sequencing.

ChIP-sequence analysis. The ChIP libraries were sequenced on Illumina HiSeq platform in 150 bp paired-end reads. After quality control, the clean reads were aligned to the human reference genome (Homo sapiens. GRCh38.p2) with Bowtie v2.1.0. Unambiguously mapped reads were retained for subsequent generation of binding profiles, heatmaps and calling of peaks. MACS2 (Model-based Analysis of ChIP-seq, version 2) was used to identify regions in ChIPed samples of the signal enriched over the background signal from the corresponding input sample, and P value of 10^-9 was used as a cutoff to identify statistically significant peaks. Mapped reads were visualized using the Integrative Genomics Viewer (IGV). We used the mouse spike-in data to normalize the peak calling in each sample. The total number of reads aligned to the mouse mm9 genome assembly was used as a normalization factor to scale ChIP-seq data sets produced from equal cell numbers. Bambi-liquidator (https://github.com/BrudnerLab/pipeline/wiki/bamliquidator, version 1.0) was used to calculate the ChIP-seq read coverage over a defined genomic coordinate. Heatmaps and genome-wide correlation analyses were generated using DeepTools2. To create density distributions around TSS or ChIP peaks and heatmaps indicating c-Myc or RNA polymerase II occupancies, plotHeatmap was used. Peak annotations were achieved using the ‘closestBed’ feature from the Bedtools suite v2.20.1.
Proliferation and methylcellulose colony assay. For analysis of the effects of PC4 on cell viability, cells were seeded in 6-well plates at a concentration of 3000 cells per well in triplicate, and CCK-8 (Dojindo Laboratories, Beijing, China, CK04) was used to assess cell proliferation according to the manufacturer's instructions. Mouse and human lymphoma cells were dispersed in methylcellulose-based medium MethoCult™ M3630 (StemCell, 03630) or MethoCult™ SF H4336 (StemCell, 04536). A representative plate was then washed and cells were resuspended and replated. After an additional 14 days, colonies were counted (P2).

Real-time PCR and RNA interference. Total RNA was extracted using TRIzol (Invitrogen, CA, USA) according to the manufacturer's instructions. Reverse transcription of total cellular RNA was carried out using oligo (dT) primers and M-MLV reverse transcriptase (Transgen Biotech, Beijing, China). PCR was performed with a Mycycler thermal cycler and analyzed using LineGene960. The PCR primer sequences were as follows: MYC forward, 5′-TTCTGGTGGAAAGGACGG-3′; MYC reverse, 5′-TGGGTGATTGCTGCGATG-3′; GAPDH forward, 5′-GACGATCCGCAAGAGGCGAAGAG-3′; GAPDH reverse, 5′-GGGAGAAGCCATTTGGTG-3′; TPAD forward, 5′-GCTGATGCTCGGGGATAGTG-3′; TPAD reverse, 5′-GTCGCCCTTTTGGACAAATTC-3′; PD6 forward, 5′-GCTTGAGGCTCTGGCTTCTGGC-3′; PD6 reverse, 5′-TACCTGTACCCCTGCTTGGC-3′; CCNA1 forward, 5′-GGGTGTGACTGAAATGACCG-3′; CCNA1 reverse, 5′-GAAACGTCGGAAAGGATGCACCC-3′; CCND1 forward, 5′-CCGCTTATGCCGAGG-3′; CCND1 reverse, 5′-ATGGCCGGCGCCGAG-3′; CCNE2 forward, 5′-TTCCTGAGGACACCCTCTCTGGAGCC-3′; and CCNE2 reverse, 5′-TCTGCACTATCGGAAGAACCC-3′. The human FBXW7 siRNA sequence was 5′-GGGACATACAGGTGGAGTA-3′. The human TRIB3 siRNA sequence was 5′-GAGAATACGCAAGAAGGAG-3′, and the human GAPDH siRNA sequence was 5′-GAGGAGCATTACGGAAGAATAGC-3′. The human TRIB3 RNA interference MAX Transfection Reagent (Life Technologies, CA, USA) was used according to the manufacturer's instructions.

Structured illumination microscopy. Cells (~20,000) were grown on coverslips and fixed with formalin after the indicated treatment. Subsequently, cells were washed in Tris-buffered saline (TBS) and permeabilized for 5 min in 2% Triton X-100 in TBS. The cells were quenched for 10 min with 50 μM glycine in TBS and blocked for 30 min with 5% normal mouse serum or normal goat serum in a 0.2% TBS-EP buffer (GE Healthcare) at pH 7.4. TRIB3/MYC antibody (Abcam, ab75846, 1:100) dilutions were placed on Paraflin-coated slides. The cells were quenched for 10 min with 50 nM glycine in TBS and incubated overnight at 4 °C. The next day, the coverslips were lifted by adding a small volume (200 μl) of TBS under the coverslip. After washing with 5x for 20 min with 1.02 g/mL TBS, Alexa Fluor 488 (Thermo Fisher, B37114, 1:200) and Alexa Fluor 555 (Thermo Fisher, A-31572, 1:200) secondary antibody incubation was performed as described for the primary antibody, and the coverslips were incubated for 1 h at room temperature. Following this, 10 min washes with 1 ml of 0.2% TBS-gelatin and one wash with regular TBS, the slides were mounted using soft set mounting medium with DAPI (Vectashield) and sealed with nail polish. Images were acquired using a confocal microscope (Olympus Microsystems, CA, USA) or a GE Healthcare 3D structured illumination microscope. Intensity plots of individual pixels taken from a straight line in the indicated immunofluorescence images were generated by twin slicer analysis using Imaris 3D & 4D image software (Bitplane AG, Switzerland). Images were cropped and processed in Adobe Photoshop. When comparisons were made between images of the same experiment, all levels were adjusted equally, and the ratio between the levels was not altered.

Analysis of surface plasmon resonance. Binding kinetics between MYC and UBE3B, TRIB3 and the indicated a helical peptides, MAX and the indicated a helical peptides, or MYC and MAX were analyzed using a BiACore T200 instrument (GE Healthcare, CA, USA) as described previously. Briefly, binding reactions were performed in HBS-EP buffer (GE Healthcare) at pH 7.4. TRIB3/MYC/MAX protein was coated on the flow cell of a CM5 sensor chip by direct immobilization. Another flow cell, not coated with an immobilized protein, was used to evaluate nonspecific binding. The binding analyses were performed at a flow rate of 25 μl/min at 25 °C. A total of 0–200 nM peptide or 0–500 nM TRIB3 protein was passed over the surface of the sensor chip. The association rate constant (Ka) and the dissociation rate constant (Kd) were calculated according to the BIA evaluation software. The dissociation constant (Kd) was determined by Ka/Kd.

RNA-Seq library preparation and sequencing. RNA purity was evaluated using the Kaino K5500 spectrophotometer (Kaino, Japan). For analysis, the RNA integrity and concentration were assessed using the RNA Nano 6000 Assay Kit and the Bioanalyzer 2100 system (Agilent Technologies, CA, USA). An RNA integrity number (RIN) > 7.5 was required for all samples. Sequencing libraries were generated using the NEBNext UltraTM RNA Library Prep Kit for Illumina* (E7530L, NEB, USA) according to the manufacturer's recommendations, and index codes were added to attribute sequences to each sample. In brief, mRNA was purified from 2 μg of each total RNA sample using poly-T oligo-attached magnetic beads. Fragmentation was performed using divalent cations at an elevated temperature in a NEB Next First Strand Synthesis Reaction Buffer (SX). First-strand cDNA synthesis was performed using random hexamer primers and RNase H. Second-strand cDNA synthesis was subsequently performed using buffer, dNTPs, DNA polymerase I, and RNase H. The library fragments were purified with QiaQuick PCR kits and eluted with EB buffer; then, terminal repair, A-tailing and adapter addition were implemented. The intended products were retrieved, and PCR was performed, at which point the library construction was complete.

Gene set enrichment analysis. We ranked the genes by their association with the Si-CTRL (n = 3), Si-TRIB3 (n = 3), and Si-MYC groups; BCLs from Myc +/− mice were compared with Myc +/− mice (n = 3) and Ad-UBE3B (n = 3) groups; the PCON (n = 3) and PC4 (n = 3) groups; the MYCWT (n = 3) and MYCSiRNA (n = 3) groups; and the MYCWT + shTRIB3 (n = 3), MYCSiRNA + shTRIB3 (n = 3), MYCSiRNA + shCTRL (n = 3) groups, using the signal-to-noise measurement determined by GSEA according to log2-fold changes. MYC target gene sets were collected from the database (http://software.broadinstitute.org/gsea/msigdb/index.jsp).

NanoBRET assay. HEK 293T cells (8 × 10^6 per well) were seeded into six-well culture plate and allowed to attach for 6 h. These cells were then cotransfected with 2 μg Max-HaloTag Fusion Vector (Promega, N1870) and 0.2 μg MYC-Nano Luciferase Fusion Vector (Promega, N1870). Six hours after transfection, cells were then harvested and resuspended in OptiMEM (Life Technologies) supplemented with 4% FCS at a density of 3 × 10^6 cells/ml in the absence or presence of 100 nM HaloTag NanoBRET 618 fluorescent ligand (Promega, G9801). Cells were then seeded into white, 96-well plates, with 100 μl of cell suspension per well. 10058-F4 or PC4 (n = 3) was added to these cells at various concentrations. Plates were incubated for 3 h at 37 °C, 5% CO2. NanoBRET Nano-Glo Substrate (Promega, N1572) was added to both control and experimental samples at a final concentration of 10 μM. Plates were read within 10 min using a Synergy H1 plate reader (BioTek) equipped with a 450/60-nm bandpass and 610-nm long-pass filter module. A corrected BRET ratio was calculated, defined as the ratio of the emission at 610 nm/450 nm for experimental samples (i.e., those treated with HaloTag NanoBRET 618 fluorescent ligand) minus the emission at 610 nm/450 nm for control samples (i.e., those not treated with HaloTag NanoBRET 618 fluorescent ligand). BRET ratios were then calculated as milliBRET units (mBU), where 1 mBU corresponds to the corrected BRET ratio multiplied by 1000.

Apoptosis analysis. Apoptotic cells were detected by Apoptosis Detection kit (BioGenes,62700-80). Cells were collected and washed twice with stain buffer, resuspended in the Annexin V Binding Buffer and stained with 5 μl Annexin V and 5 μl 7-AAD (BD,559925) for 15 min at RT (25°C) in the dark, followed by washing with 500 μl Annexin V Binding buffer. Data were acquired using a FACSCan™ II flow cytometer (BD).

Cell cycle analysis. Cells were collected and washed twice with PBS, pelleted, and fixed with cold 70% ethanol alcohol for at least 30 min. After being washed twice with cold PBS, cells were incubated with 200 μg/ml RNase A for 30 min at 37 °C. The cells were stained with 100 μg/ml propidium iodide (PI) for 30 min at room temperature. The samples were immediately analyzed by flow cytometry. Cell cycle phase distribution was determined using FCS EXPRESS software.

Statistical analysis. Data are expressed as the mean ± standard error of the mean (SEM). Comparisons between two groups were performed by unpaired Student’s t test or one-way ANOVA. Correlations between groups were determined by Pearson’s correlation test. Both were performed with the GraphPad Prism software. The sample number (n) indicates the number of independent biological samples in each experiment. Sample numbers and experimental repeats are indicated in the figures and figure legends. Generally, all experiments were carried out with n ≥ 3 biological replicates. p < 0.05 was considered statistically significant. Analyses were performed using GraphPad Prism 6.0 software.
**Data availability**

The RNA-seq and ChIP-seq data have been uploaded to the NCBI Gene Expression Omnibus (GEO) database under accession numbers GSE117128, GSE126528, GSE143862, GSE143863, GSE143864, and GSE143866, respectively. The TRIB3 mRNA expression was analyzed on the following accession code GSE6338, GSE4881 and the web site http://lmpp.nih.gov/DBLC/. All other supporting the findings of this study are available from the corresponding author upon reasonable request.

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Author contributions

Z.W.H. conceptualized the study and participated in the overall design, supervision and coordination of the study. K.L. and F.W. designed and performed most of the experiments. X.X.L., F.H., B.C., J.I. Y., S.S.L., T.T.Z., Z.Y., Z.N.Y., and C.X.Z., participated in the molecular and cellular biological experiments. S.S., J.M.Y., Y.X., and X.W.Z. performed the animal studies. Y.F.Y. followed up the patients and collected clinical information and materials. Z.W.H. and K.L. wrote the manuscript. All authors read and approved the manuscript.

Competing interests

The authors declare no competing interests.

Additional information

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Correspondence and requests for materials should be addressed to Z.-w.H.

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