

BCL6 Inhibition Sensitizes Diffuse Large B-cell Lymphoma Cells to Ferroptosis

Lei Xu

0024x1@zidd.ac.cn

Shanghai Institute of Materia Medica

Yi Wen

Zhongshan City People's Hospital

Ran Zhong

Shanghai Institute of Materia Medica

Shun Zhang

Zhongshan City People's Hospital

Xuan Zhang

Shanghai Institute of Materia Medica

Jia Li

Shanghai Institute of Materia Medica, Chinese Academy of Sciences <https://orcid.org/0000-0003-3224-001X>

Article

Keywords: BCL6, Ferroptosis, DLBCL, IKE, ALOX5

Posted Date: May 11th, 2026

DOI: <https://doi.org/10.21203/rs.3.rs-9439017/v1>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License.
[Read Full License](#)

Additional Declarations: (Not answered)

BCL6 Inhibition Sensitizes Diffuse Large B-cell Lymphoma Cells to Ferroptosis

Yi Wen^{1#}, Yi-Ran Zhong^{2,3,4,5#}, Lai-Shun Zhang¹, Zhen-Xuan Zhang^{2,6}, Lei Xu^{2, *}, Jia Li^{2,3,4,5*}

¹Department of Pharmacy, Zhongshan City People's Hospital, Zhongshan 528403, China

²Zhongshan Institute for Drug Discovery, Shanghai Institute of Materia Medica, Chinese Academy of Sciences, Zhongshan 528400, China

³State Key Laboratory of Chemical Biology, Shanghai Institute of Materia Medica, Chinese Academy of Sciences, Shanghai 201203, China

⁴School of Pharmaceutical Science and Technology, Hangzhou Institute for Advanced Study, University of Chinese Academy of Sciences, Hangzhou 310024, China

⁵University of Chinese Academy of Sciences, Beijing 100049, China

⁶School of Life Sciences and Health Engineering, Jiangnan University, Wuxi 214122, China

*#Contributed equally, *These authors jointly supervised this work*

Correspondence: Lei Xu, Zhongshan Institute for Drug Discovery, Shanghai Institute of Materia Medica, Chinese Academy of Sciences, No. 8 Keji West Road, Zhongshan 528400, China. Email: 0024xl@zidd.ac.cn; Jia Li, Email: jli@simm.ac.cn

Abstract

Diffuse large B-cell lymphoma (DLBCL) is an aggressive non-Hodgkin lymphoma with a limited response to chemotherapy. Ferroptosis inducers have shown some efficacy in lymphoma treatment. However, there are some types of DLBCL cells that are resistant to ferroptosis through some unknown mechanism, which dramatically limits the application of this method. The transcriptional repressor BCL6 is a well-established oncogenic driver in a subset of DLBCL. We found that treatment with Imidazole Ketone Erastin (IKE), an inducer of ferroptosis, triggers a compensatory upregulation of BCL6. Pharmacological degradation or genetic knockdown of BCL6 synergistically sensitized DLBCL cells to IKE-induced ferroptosis in vitro and in vivo. Mechanism studies revealed that BCL6 knockdown aggravates mitochondrial damage, lipid peroxidation, and ROS accumulation in DLBCL. Proteomic analysis showed that the differentially expressed proteins after BCL6 knockdown were enriched in metabolic pathways, with SDHA and H6PD as core metabolic nodes, confirming that BCL6 mediates metabolic reprogramming in DLBCL cells. Public ChIP-seq data suggest that BCL6, together with SMRT and BCOR, may bind the ALOX5 promoter. In summary, the present study elucidates BCL6 as a critical adaptive resistor to ferroptosis and establishes the combined inhibition of SLC7A11 and BCL6 as a rational, synergistic strategy to overcome therapeutic resistance in DLBCL.

Keywords: BCL6; Ferroptosis; DLBCL; IKE; ALOX5

Introduction

Diffuse large B-cell lymphoma (DLBCL) is the most prevalent and aggressive subtype of non-Hodgkin lymphoma, accounting for approximately 30-40% of all newly diagnosed lymphoma cases worldwide^{1, 2, 3, 4}. The disease is defined by marked clinical and molecular heterogeneity, with at least four major genetic subtypes now recognized. Frontline immunochemotherapy with R-CHOP (rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisone) achieves durable remission in approximately 60% of patients; however, a substantial proportion develop refractory or relapsed disease with limited subsequent treatment options^{5, 6}. Although the therapeutic landscape has expanded with CAR-T cell therapies and antibody–drug conjugates such as polatuzumab vedotin, intrinsic and acquired resistance to these agents remains a major obstacle in DLBCL management^{7, 8, 9, 10}. This underscores the urgent need to identify novel therapeutic vulnerabilities.

Ferroptosis, an iron-dependent, non-apoptotic form of regulated cell death driven by uncontrolled peroxidation of polyunsaturated fatty acid (PUFA)-containing phospholipids, has emerged as a promising therapeutic vulnerability in cancer^{11, 12}. The central executioner of ferroptosis is GPX4, a glutathione (GSH)-dependent peroxidase that detoxifies lipid peroxides; its activity is critically sustained by cystine imported via the system Xc⁻ transporter, a heterodimer of SLC7A11 and SLC3A2^{13, 14, 15}. Notably, DLBCL cells harbor a defective transsulfuration pathway, rendering them uniquely dependent on system Xc⁻ for GSH biosynthesis and consequently hypersensitive to SLC7A11 inhibition-induced ferroptosis^{16, 17}. Imidazole ketone erastin (IKE), a metabolically stable SLC7A11 inhibitor, has demonstrated potent anti-tumor activity in preclinical lymphoma models^{18, 19}. Nevertheless, as observed with

other targeted agents, adaptive resistance mechanisms limiting sustained ferroptosis induction are anticipated but remain poorly characterized in DLBCL.

BCL6 is a POZ-domain zinc-finger transcriptional repressor and well-established oncogenic driver in DLBCL, particularly the germinal center B-cell-like (GCB) subtype, where it sustains lymphoma cell survival by silencing genes governing DNA damage responses, cell cycle arrest, and terminal differentiation^{20, 21, 22}. Beyond its canonical role in lymphomagenesis, emerging evidence implicates BCL6 as a broad mediator of stress adaptation and therapy resistance across multiple cancer types, capable of suppressing both apoptotic and senescence programs to enable tumor cell survival under cytotoxic pressure^{23, 24, 25, 26}. Whether BCL6 similarly operates as an adaptive transcriptional regulator against non-apoptotic cell death programs—particularly ferroptosis—has not been explored.

In this study, we investigated whether BCL6 functions as an adaptive mediator of ferroptosis resistance in DLBCL and whether its pharmacological or genetic inhibition could sensitize lymphoma cells to IKE-induced ferroptosis. We found that SLC7A11 inhibition triggers compensatory BCL6 upregulation, which in turn suppresses the ferroptosis machinery to limit cell death. Pharmacological degradation and genetic knockdown of BCL6 in DLBCL cell lines and xenograft models, combined with proteomic analyses, delineated the downstream mechanisms through which BCL6 confers this adaptive resistance.

Results

Targeting the SLC7A11–GPX4 axis induces ferroptosis and suppresses DLBCL growth

Analysis of public gene expression data (GSE56315) revealed that both SLC7A11 and GPX4, core components of the anti-ferroptosis defense, are significantly overexpressed in DLBCL patient samples compared to normal B cells (Fig. 1A, B). Furthermore, at the genetic perturbation level, CRISPR-based essentiality screening (DepMap) revealed that compared with 1,187 other tumor cell lines, DLBCL cell lines (n = 16) exhibited significantly stronger dependence on SLC7A11 and GPX4 (Fig. 1C, D). At the chemical perturbation level, we used IKE—a metabolically more stable SLC7A11 inhibitor and an analog of erastin—to evaluate its anti-proliferative effect on a panel of DLBCL cell lines (Fig. 1E). The results showed that among the ten cell lines tested, six were highly sensitive to IKE with IC₅₀ values below 1 μM. To further confirm the critical role of SLC7A11 in DLBCL, we performed proliferation inhibition assays using five patient-derived diffuse large B-cell lymphoma primary cell samples (provided by Hefei Pharmaceutical Co., Ltd., Approval No. SL-B2022-490-04). The results demonstrated that IKE exerted significant anti-proliferative activity against all five primary samples, with IC₅₀ values below 1 μM (Fig. 1F). Altogether, these results indicated that targeting the SLC7A11–GPX4 axis effectively suppresses DLBCL proliferation both in cell line models and in patient-derived primary cells.

IKE treatment triggers compensatory upregulation of the oncogenic transcriptional repressor BCL6

Acquired resistance remains a major challenge in targeted cancer therapies. Following confirmation of IKE's anti-tumor efficacy, we investigated potential adaptive resistance mechanisms. Given the established role of BCL6 in mediating stress adaptation and therapeutic resistance in lymphoid malignancies, we examined BCL6 expression in response to IKE

treatment. IKE induced significant, dose-dependent upregulation of BCL6 mRNA in multiple DLBCL cell lines, including SU-DHL-4, OCI-Ly10, and OCI-Ly1 (Fig. 2A–C). Time-course and dose-response Western blot analyses further demonstrated progressive and sustained BCL6 protein induction throughout IKE exposure (Fig. 2D–E). Consistently, BCL6 was upregulated at both the transcriptional and protein levels in IKE-sensitive DLBCL cell lines (Pfeiffer, SU-DHL-6) after 72 hours of treatment (Supplementary Fig. 1). Together, these findings indicate that DLBCL cells activate a BCL6-mediated transcriptional program as an adaptive survival response to IKE-induced ferroptotic stress.

BCL6 inhibition synergistically sensitizes DLBCL cells to IKE-induced cytotoxicity in vitro

Based on our finding that IKE upregulates BCL6 expression in DLBCL cells, we hypothesized that BCL6 induction attenuates IKE-induced ferroptosis and serves as an adaptive resistance mechanism. To test this, we evaluated combination treatments across multiple DLBCL cell lines. Co-treatment with IKE and the BCL6 inhibitor FX-1 yielded Bliss synergy scores of 14.63, 13.69, 13.71, 8.82, and 10.96 in SU-DHL-4, OCI-Ly1, OCI-Ly10, Toledo, and Pfeiffer cells, respectively (Fig. 3A). Similarly, IKE combined with the BCL6 degrader BI-3802 produced synergy scores of 12.16, 21.35, 11.43, and 10.12 in SU-DHL-4, OCI-Ly1, OCI-Ly10, and U-2932 cells (Fig. 3B). Notably, the BI-3802–IKE combination also showed synergistic activity in patient-derived cells (synergy score: 5.21) (Fig. 3C). To genetically validate BCL6's role, we established stable BCL6-knockdown SU-DHL-4 cells, with knockdown efficiency confirmed at both mRNA and protein levels (Fig. 3D–E). BCL6 knockdown dramatically enhanced IKE sensitivity, reducing the IC₅₀ from >20 μ M to 1.53 μ M (Fig. 3F). Collectively, these results demonstrate that pharmacological or genetic inhibition of BCL6 universally

potentiates IKE cytotoxicity, establishing BCL6 as a critical mediator of adaptive resistance to ferroptosis in DLBCL.

BCL6 inhibition potentiates IKE-induced ferroptosis

To determine whether the synergistic cytotoxicity was mediated through enhanced ferroptosis, we examined hallmarks of ferroptotic cell death. Transmission electron microscopy of SU-DHL-4^{BCL6KD} cells treated with IKE revealed pronounced mitochondrial pathology—including marked mitochondrial shrinkage, increased membrane electron density, and loss of cristae—classical ultrastructural features of ferroptosis—which were absent or minimal in IKE-treated SU-DHL-4^{NC} control cells (Fig. 4A). Consistent with these changes, BCL6 knockdown dramatically amplified IKE-induced lipid peroxidation (Fig. 4B) and intracellular ROS accumulation (Fig. 4C) in a dose-dependent manner. These data confirm that BCL6 functions as a key negative regulator of ferroptosis, and that its inhibition unlocks robust ferroptotic cell death in combination with SLC7A11 inhibition.

Proteomic profiling reveals BCL6-dependent reprogramming of metabolic and redox pathways

To elucidate the molecular mechanism underlying this synergistic effect, we performed unbiased proteomic profiling of BCL6-knockdown versus control SU-DHL-4 cells. Differentially expressed proteins (DEPs; FC > 1.5 or < 1/1.5, p < 0.05) were intersected with metabolic and redox databases, revealing 214 metabolism-associated and 334 redox-associated proteins (Fig. 5A). KEGG pathway enrichment analysis identified significant alterations in core metabolic pathways, including the TCA cycle, glycolysis/gluconeogenesis, pentose phosphate pathway, and lipid metabolism (Fig. 5B). Protein–protein interaction network

analysis further identified SDHA and H6PD as key regulatory nodes (Fig. 5C). SDHA, a rate-limiting enzyme in the mitochondrial TCA cycle, catalyzes succinate oxidation and regulates mitochondrial ROS generation^{27, 28}. H6PD participates in glucose metabolism and NADPH production, which is essential for maintaining GSH in its reduced state and resisting lipid peroxidation^{29, 30}. These findings suggest that BCL6 reprograms cellular metabolism by regulating SDHA and H6PD, thereby affecting TCA cycle flux, NADPH homeostasis, and oxidative stress responses. To validate the regulatory relationship between BCL6 and SDHA, we treated SU-DHL-4 cells with 100 nM BI-3802 for 18 days and assessed protein expression every 3 days. BI-3802 treatment progressively degraded BCL6 while concomitantly upregulating SDHA expression (Fig. 5D), confirming that BCL6 negatively regulates SDHA. Collectively, these findings indicate that BCL6 broadly reprograms cellular metabolism to maintain a pro-survival redox environment, and that its inhibition augments oxidative stress in a manner that cooperates with IKE-induced ferroptosis.

BCL6 directly represses transcription of the pro-ferroptotic enzyme ALOX5

To identify specific transcriptional mechanisms by which BCL6 suppresses ferroptosis, we interrogated publicly available BCL6 ChIP-seq data from the Cistrome DB database. This analysis revealed direct and cooperative binding of BCL6 together with its corepressors SMRT (NCOR2) and BCOR at the promoter region of ALOX5 (Fig. 6A). ALOX5 encodes arachidonate 5-lipoxygenase, a key enzyme catalyzing PUFA peroxidation and a critical initiating step of ferroptosis^{31, 32}. To functionally validate this regulatory relationship, SU-DHL-4 cells were treated with BI-3802 across a time course; BCL6 degradation resulted in a progressive, time-dependent increase in ALOX5 protein expression (Fig. 6B). Conversely,

transient overexpression of BCL6 in HEK293T cells led to significant reductions in both ALOX5 mRNA and protein levels (Fig. 6C, D). These results establish ALOX5 as a direct transcriptional target of BCL6, providing a specific mechanistic link between BCL6-driven repression and suppression of the lipid peroxidation cascade required for ferroptosis.

BCL6 inhibition synergizes with IKE to suppress DLBCL tumor growth in vivo

To evaluate the in vivo therapeutic potential of combined SLC7A11 and BCL6 inhibition, we established subcutaneous DLBCL xenograft models in NOD/SCID mice using OCI-Ly10 cells. When mean tumor volumes reached 100–300 mm³, mice were randomized into five treatment groups (n = 6 per group): vehicle control, IKE monotherapy (25 mg/kg, i.p., daily), FX-1 monotherapy (10 mg/kg, i.p., daily)³³, IKE plus FX-1 combination, and ibrutinib (20 mg/kg, p.o., daily) as an active comparator (Fig.7A) All regimens were well-tolerated, with no significant body weight loss observed throughout the study period (Fig. 7B). IKE monotherapy produced modest tumor growth inhibition at the dose tested, and FX-1 monotherapy demonstrated moderate anti-tumor activity. Strikingly, the IKE and FX-1 combination produced significantly greater tumor growth suppression than either agent alone, as demonstrated by tumor volume trajectories, endpoint tumor weights, and representative tumor images (Fig. 7C–E). These in vivo findings provide robust translational support for the synergistic therapeutic potential of dual SLC7A11 and BCL6 inhibition in DLBCL. To further validate our mechanistic findings, SU-DHL-4 cells were treated with IKE (20 μM), BI-3802 (1 μM), or their combination for 72 hours, and protein levels of BCL6, ALOX5, and SDHA were assessed by Western blotting. Consistent with previous results, IKE alone significantly upregulated BCL6. In contrast, both BI-3802 alone and the combination induced BCL6

192 degradation with concomitant upregulation of ALOX5 and SDHA. Notably, the combination
193 elicited greater upregulation of ALOX5 and SDHA than BI-3802 alone (Fig. 8A). These
194 findings provide robust validation for our proposed mechanistic model (Fig. 8B).

195

Discussion

A hallmark of DLBCL is its propensity to develop resistance to both standard immunochemotherapy and emerging targeted agents, underscoring the need to identify and preempt adaptive survival mechanism. In this study, we identify a previously unrecognized adaptive resistance circuit: treatment with the SLC7A11 inhibitor IKE triggers compensatory upregulation of the oncogenic transcriptional repressor BCL6, enabling DLBCL cells to withstand ferroptotic stress. Pharmacological or genetic inhibition of BCL6 profoundly sensitizes DLBCL cells to IKE both in vitro and in vivo, demonstrating that targeting this adaptive response can restore and amplify ferroptotic cell death.

Our data establish BCL6 as a mediator of resistance to SLC7A11 inhibition, extending a well-documented paradigm in oncology. BCL6 upregulation has previously been linked to resistance to HDAC inhibitors in DLBCL^{34, 35, 36}, to BCR-ABL1 kinase inhibitors in acute lymphoblastic leukemia^{37, 38}, to cytarabine in acute myeloid leukemia^{39, 40}, to paclitaxel in breast cancer⁴¹, and to BET bromodomain inhibitors in KRAS-mutant non-small cell lung cancer^{23, 42}. BCL6 induction has similarly been documented following treatment with imatinib in gastrointestinal stromal tumors^{43, 44}, EGFR inhibitors in NSCLC^{45, 46}, and chemo- or radiotherapy in glioblastoma, consistently correlating with reduced therapeutic efficacy^{47, 48, 49}. We extend this paradigm to ferroptosis—a non-apoptotic, iron-dependent cell death program—demonstrating that BCL6 constitutes a broad adaptive sensor capable of resisting mechanistically distinct cytotoxic insults.

Mechanistically, we identify ALOX5 as a direct transcriptional target of BCL6 in the context of ferroptosis. BCL6, together with its corepressors SMRT (NCOR2) and BCOR, binds the

218 ALOX5 promoter to suppress the expression of arachidonate 5-lipoxygenase, a critical enzyme
219 that catalyzes PUFA peroxidation—the initiating step of ferroptosis execution^{32, 50, 51}. By
220 repressing ALOX5, BCL6 effectively constrains the lipid peroxidation cascade, permitting cell
221 survival despite GSH depletion induced by IKE. In parallel, proteomic profiling reveals that
222 BCL6 broadly reprograms cellular metabolism, in part by regulating SDHA expression and
223 mitochondrial function, thereby reducing pro-oxidant tone. Consequently, BCL6 inhibitors
224 exert a dual pro-ferroptosis effect: de-repression of ALOX5 accelerates lipid peroxidation
225 while concurrent metabolic rewiring elevates ROS levels—a combination that synergistically
226 amplifies ferroptotic cell death when the primary antioxidant defense (GSH/GPX4) is
227 concurrently disabled by IKE.

228 The rapid and sustained induction of BCL6 following SLC7A11 inhibition suggests that
229 DLBCL cells have a pre-existing capacity to engage this oncogenic transcription factor as a
230 rapid adaptive response, targeting the precise molecular vulnerability exploited by ferroptosis
231 inducers. This is consistent with the emerging role of BCL6 as a central stress-sensing hub in
232 cancer, capable of integrating diverse cytotoxic signals and coordinating transcriptional
233 survival programs. These findings raise the possibility that BCL6 expression dynamics during
234 SLC7A11 inhibitor treatment could serve as a predictive biomarker for the emergence of
235 adaptive resistance, with potential utility for guiding combination therapy scheduling.

236 From a translational perspective, the mechanistic coherence of the IKE–BCL6 inhibitor
237 combination is compelling: IKE disables the primary antioxidant defense (GSH/GPX4), while
238 BCL6 inhibition concurrently removes the brake on lipid peroxidation and increases oxidative
239 burden. This dual-mechanism attack transforms a self-limiting monotherapy into a coordinated

240 oxidative assault that overwhelms cancer cell redox homeostasis. The observed in vivo synergy
241 in DLBCL xenograft models provides initial translational support for this strategy. Future
242 studies should evaluate combination efficacy across broader DLBCL molecular subtypes,
243 assess response biomarkers, and explore synergies with standard-of-care regimens.

244

Materials and Methods

Data collection and bioinformatics analysis

We downloaded the GSE56315 dataset from Gene Expression Omnibus (GEO) (<https://www.ncbi.nlm.nih.gov/geo/>), which contains clinical data and gene expression profiles of patients with DLBCL. This dataset was used to investigate whether there were differences in the expression levels of SLC7A11 and GPX4 genes in normal tissues and tumor tissues. The gene effect and expression of DLBCL cell lines were analyzed and downloaded from the Broad Institute DepMap website.

Cell lines and cell culture

HT, SU-DHL-1, Farage, Toledo, SU-DHL-4, OCI-Ly10, Pfeiffer, U-2932, and HEK293T cells were obtained from the American Type Culture Collection (ATCC, Manassas, VA, USA). The cell lines OCI-Ly1 and WSU DLCL2 were obtained from Cobioer Biosciences Company (Nanjing, China). The HEK293T-BCL6 cells were generated as described previously: a BCL6-EGFP fusion construct was introduced into HEK293T cells via a lipofectamine 3000 delivery system, and stable expression cells were selected with 5 µg/mL puromycin. Human DLBCL primary cell experiments were approved (Approval No. SL-B2022-490-04; Hefei PreceDo Pharmaceuticals Co., Ltd., Hefei, China). All DLBCL cell lines were maintained in a humidified incubator at 37°C with 5% CO₂. OCI-Ly1 and Pfeiffer cells were cultured in RPMI-1640 supplemented with 20% heat-inactivated fetal bovine serum (FBS; Cellcook, Guangzhou, China) and 0.1% Penicillin-Streptomycin (Pen-Strep; Ameresco Inc., Solon, OH, USA). HT, SU-DHL-1, and Farage cells were grown in RPMI-1640 (HyClone, Logan, UT, USA) containing 10% FBS (Avantor, #76294-180) and 1% Pen-Strep (Gibco, #15140-122, Waltham,

MA, USA). SU-DHL-4 cells were cultured in RPMI-1640 (ATCC modification) with 10% FBS and 0.1% Pen-Strep. OCI-Ly10 cells were maintained in IMDM (Gibco) supplemented with 20% FBS and 0.1% Pen-Strep. U-2932 cells were grown in RPMI-1640 (Gibco) with 10% FBS and 0.1% Pen-Strep. WSU DLCL2 cells were cultured in Leibovitz's L-15 medium (ATCC, #30-2008) containing 10% FBS and 1% Pen-Strep, and incubated in a CO₂-free environment. All cell lines were routinely tested for mycoplasma contamination and maintained in exponential growth phase for experiments.

Chemicals

IKE (HY-114481), BI-3802 (HY-108705), and FX-1 (HY-102027) were purchased from Selleck China (Shanghai, China).

Cell proliferation assay

DLBCL cells were resuspended and seeded at the appropriate density in 384-well plates. Semi-adherent and semi-floating cells were incubated overnight at 37°C and 5% CO₂; suspended cells were not incubated overnight. IKE was diluted in a gradient and added to the plates, which were then incubated for 3 days. SU-DHL-4, OCI-Ly10, and OCI-Ly1 cells were seeded in 96-well plates and treated with IKE and BI-3802 at specified concentrations for 7 days. Cell survival rate (%) = (experimental group viability – blank group viability) / (control group viability – blank group viability) × 100%. IC₅₀ values were calculated using GraphPad Prism 10.1.2 (GraphPad Software, La Jolla, CA, USA). Cell survival rates from combined treatment groups were analyzed using the SynergyFinder website (<https://synergyfinder.aittokallio.group/>), and synergy scores were calculated using the Bliss model.

Patient-derived cell processing and viability assays

Primary DLBCL cells were isolated from consented patient tumor samples via enzymatic dissociation and mechanical mincing. Patient-derived DLBCL cells in logarithmic growth phase were seeded in 384-well plates at 4,000 cells per well in 50 μ L of complete medium and allowed to adhere overnight. Compounds were serially diluted in culture medium and added in a final volume of 50 μ L per well. Cells were treated for 7 days. Cell viability was assessed using the CellCounting-Lite 2.0 Luminescent Cell Viability Assay (Vazyme, DD1101-02, Nanjing, China) according to the manufacturer's instructions. Luminescence was measured using an EnVision 2015 Multilabel Plate Reader (PerkinElmer, Waltham, MA, USA).

RNA extraction and quantitative RT-PCR

RNA was extracted using the FastPure Cell/Tissue Total RNA Isolation Kit V2 (Vazyme, RC112-01). Reverse transcription was performed using the HiScript IV All-in-One Ultra RT SuperMix for qPCR kit (Vazyme, R433-01) to obtain cDNA. Real-time PCR was conducted using the ChamQ Universal SYBR qPCR Master Mix kit (Vazyme, Q711) on a real-time fluorescence quantitative PCR instrument (Thermo Scientific, Waltham, MA, USA). Results were analyzed using the $\Delta\Delta$ CT method. Primer sequences are provided in Table S1.

Western blot analysis

Cells were lysed in 100 μ L of 4 $\times$ Laemmli Sample Buffer (Bio-Rad, #1610747, Hercules, CA, USA) and heated at 100°C for 10 min. Proteins were separated by SDS-PAGE and transferred to a nitrocellulose membrane. After 2 h of blocking with 5% skimmed milk or 5% bovine serum albumin, membranes were incubated with primary antibodies overnight at 4°C, followed by a

2-h incubation at room temperature with secondary antibodies. Bands were detected with Enhanced Chemiluminescence (ECL) developing solution and imaged using an e-Blot Touch imager. Antibodies used: BCL6 (Santa Cruz, sc-7388, Dallas, TX, USA); ALOX5 (ABclonal, A2877, Wuhan, China); SDHA (Cell Signaling Technology, #11998, Danvers, MA, USA); Peroxidase AffiniPure Goat Anti-Rabbit IgG (Jackson ImmunoResearch, 111-035-003, West Grove, PA, USA); Peroxidase AffiniPure Goat Anti-Mouse IgG (Jackson ImmunoResearch, 115-035-003).

Generation of stable BCL6-knockdown SU-DHL-4 cells

SU-DHL-4 cells were transduced with lentivirus expressing either a non-targeting control shRNA (GL427NC3) or a BCL6-targeting shRNA (Y21565; sequences in Table S1) in the presence of 5 µg/mL polybrene. At 72 h post-transduction, stable cell lines were selected using puromycin (2 µg/mL); selective medium was refreshed every 2–3 days.

Animals and tumor xenograft model

Female NOD SCID mice (age 6–8 weeks, weight 20 ± 2 g) were purchased from ZhuHai Bestest Biotechnology Co., Ltd. (Zhuhai, China) and housed under specific pathogen-free (SPF) conditions with a 12-h light/dark cycle, controlled temperature ($22 \pm 2^\circ\text{C}$) and humidity ($50 \pm 10\%$), and free access to food and water. All animal procedures were approved by the Institutional Animal Care and Use Committee (IACUC) of the Zhongshan Institute for Drug Discovery (IACUC approval no. 2023-01-LJ-01) and conducted in accordance with relevant guidelines. OCI-Ly10 cells in logarithmic growth phase were harvested, washed, and resuspended in PBS. A volume containing 1×10^7 cells was injected subcutaneously into the right flank of each mouse. Tumor growth was monitored until the average volume reached

100–300 mm³, at which point mice were randomized into experimental groups. Tumor volume was calculated as $V = (\text{length} \times \text{width}^2) / 2$.

Transmission electron microscopy

Cell samples were fixed in 2.5% glutaraldehyde at 4°C for 2 h. Following phosphate buffer (PB) washes, samples were post-fixed in 1% osmium tetroxide, dehydrated through a graded ethanol series, and embedded in SPI 812 epoxy resin. Ultrathin sections (60–80 nm) were stained with uranyl acetate and lead citrate. Images were acquired using a Hitachi HT7800 transmission electron microscope (Hitachi, Tokyo, Japan) operated at 80 kV. Mitochondrial morphology was assessed in multiple cells per condition.

Lipid peroxidation measurement

Lipid peroxidation levels were measured using the Image-iT® Lipid Peroxidation Kit (Thermo Fisher Scientific, C10445, Waltham, MA, USA). After treating SU-DHL-4NC and SU-DHL-4BCL6KD cells with IKE for 24 h, cells were washed twice with 1× PBS, centrifuged at 600 × g for 5 min at 4°C, and resuspended in serum-free medium containing the Image-iT® Lipid Peroxidation Sensor (1:1,000 dilution). After 30 min incubation at 37°C, cells were washed once with 1× PBS and analyzed by flow cytometry (NovoCyte Quanteon VBYR, Agilent Technologies, Santa Clara, CA, USA). Results were analyzed with FlowJo v10.8.1 (BD Biosciences, Ashland, OR, USA).

Reactive oxygen species measurement

Intracellular ROS levels were measured using the Reactive Oxygen Species Detection Reagent kit (Thermo Fisher Scientific, MP 36103). After treating SU-DHL-4BCL6NC and SU-DHL-

4BCL6KD cells with IKE for 24 h, cells were washed twice with 1× PBS, centrifuged at 600 × g for 5 min at 4°C, and resuspended in serum-free medium containing CM-H₂DCFDA (1:1,000 dilution). After 30 min incubation at 37°C, cells were washed once with 1× PBS and analyzed by flow cytometry as described above.

Proteomics analysis

Proteomic sequencing was performed by Hangzhou Jingjie Biotechnology Co., Ltd. (Hangzhou, China). After SU-DHL-4NC and SU-DHL-4BCL6KD cells were treated with IKE for 24 h, they were washed twice with 1× PBS and centrifuged to collect cell pellets. Differential protein expression was analyzed by liquid chromatography–tandem mass spectrometry (LC-MS/MS). Significantly differentially expressed proteins were defined by fold change (FC): up-regulation threshold FC > 1.5; down-regulation threshold FC < 1/1.5 (with appropriate P value cutoff). Downstream characterization of differential proteins employed KEGG pathway enrichment, Venn diagram, and protein-protein interaction (PPI) analyses.

Transcription factor binding analysis

BigWig and BED files for BCL6, BCOR, and SMRT were downloaded from the public ChIP-seq database Cistrome DB (<http://cistrome.org/db/>). The Integrative Genomics Viewer (IGV) software was used to assess BCL6, BCOR, and SMRT binding peaks in the promoter region of ALOX5. BED files were used to annotate significant peaks.

Statistical analysis

Data are presented as mean $\pm$ standard deviation (SD) and were analyzed using GraphPad Prism 10.1.2 (GraphPad Software, La Jolla, CA, USA). Comparisons between two groups were performed using an unpaired two-tailed Student's t-test; comparisons among three or more groups were performed using one-way analysis of variance (ANOVA) with appropriate post-hoc tests. P values < 0.05 were considered statistically significant.

Acknowledgements

The authors thank the patients and their families for their participation. We gratefully acknowledge Hefei PreceDo Pharmaceuticals Co., Ltd. for providing patient-derived primary DLBCL cell samples.

Conflict of Interest

The authors declare no conflict of interest.

Author Contributions

Y.W.: methodology, data curation, formal analysis, figure collation, writing—original draft and editing, funding acquisition. Y.R.Z.: data curation, formal analysis, figure collation. L.S.Z.: methodology. Z.X.Z.: bioinformatic analysis, figure collation. L.X.: formal analysis, supervision, project administration. J.L.: supervision, resources, project administration. All authors read and approved the final manuscript.

Ethics Statement

All animal procedures were approved by the Institutional Animal Care and Use Committee (IACUC) of the Zhongshan Institute for Drug Discovery, China (IACUC approval no. 2023-

01-LJ-01) and conducted in accordance with relevant guidelines and regulations. Human DLBCL primary cell experiments were approved by the relevant ethics committee (Approval No. SL-B2022-490-04) and performed in accordance with the principles of the Declaration of Helsinki. Informed consent was obtained from all participants.

Funding

This study was supported by the National Natural Science Foundation of China (Grant No. 82204451), the China Postdoctoral Science Foundation (Grant No. 2022M713652), the Zhongshan City Social Welfare and Basic Research Project (Project No. 2022B3003), and the Youth S&T Talent Support Programme of Guangdong Provincial Association for Science and Technology (Project No. SKXRC2025146).

Data Availability Statement

The datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Figure 1. Targeting the SLC7A11/GPX4 axis inhibits DLBCL cell viability. (A, B)

Expression of *SLC7A11* (A) and *GPX4* (B) in DLBCL patient tumor samples compared to normal B cells from the GSE56315 dataset. Data are presented as log₂-transformed expression values. *** $P < 0.001$, two-tailed Student's t -test. (C, D) DepMap CRISPR essentiality scores for *SLC7A11* (C) and *GPX4* (D) across 1 187 tumor cell lines (black) and 16 DLBCL cell lines (red). A more negative score indicates greater gene dependency. (E) Dose-response viability curves for a panel of DLBCL cell lines treated with IKE for 72 h. IC₅₀ values are indicated. Data are presented as mean $\pm$ SD ($n = 3$). (F) Dose-response viability curves of patient-derived DLBCL primary cells (PDCs) treated with IKE for 7 days. IC₅₀ values are indicated.

Figure 2. IKE treatment upregulates BCL6 expression in a dose- and time-dependent

manner. (A, B) *BCL6* mRNA levels measured by RT-qPCR in SU-DHL-4 (A) and OCI-Ly10 (B) cells treated with the indicated concentrations of IKE or DMSO for 24 h. Data are presented as $-\Delta\Delta C_t$ values relative to the DMSO control; $n = 3$. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, one-way ANOVA. (C) *BCL6* mRNA levels in OCI-Ly1 cells treated with the indicated concentrations of IKE for 24 h; $n = 3$. * $P < 0.05$, ** $P < 0.01$, one-way ANOVA. (D) Western blot analysis of *BCL6* protein expression in SU-DHL-4 cells treated with increasing concentrations of IKE for 72 h. α -Tubulin serves as the loading control. (E) Time-course Western blot analysis of *BCL6* protein in SU-DHL-4 cells treated with 20 μ M IKE for the indicated durations. Tubulin serves as the loading control.

Figure 3. BCL6 inhibition synergizes with IKE in DLBCL. (A) Synergy matrices and

corresponding Bliss synergy landscapes for DLBCL cells (SU-DHL-4, OCI-Ly1, OCI-Ly10, Toledo, Pfeiffer) treated with the indicated concentrations of IKE and FX-1 for 7 days. (B)

Synergy matrices and corresponding Bliss synergy landscapes for DLBCL cells (SU-DHL-4, OCI-Ly1, OCI-Ly10, U-2932) treated with the indicated concentrations of IKE and BI-3802 for 7 days. (C) Synergy matrix and Bliss synergy landscape for a patient-derived DLBCL primary cell culture (PDC, ZML-250072) treated with IKE and BI-3802 for 7 days. (D) *BCL6* mRNA levels in SU-DHL-4^{NC} and SU-DHL-4^{BCL6KD} cells measured by RT-qPCR; $n = 3$. *** $P < 0.001$, two-tailed Student's t -test. (E) *BCL6* protein expression confirmed by Western blot. α -Tubulin serves as the loading control. (F) Dose-response viability curves for SU-DHL-4^{NC} and SU-DHL-4^{BCL6KD} cells treated with IKE for 72 h: $n = 3$. Calculated IC₅₀ values are indicated.

Figure 4. BCL6 deficiency potentiates IKE-induced ferroptosis. (A) Representative TEM images of SU-DHL-4^{NC} and SU-DHL-4^{BCL6KD} cells treated with DMSO or 20 μ M IKE for 72 h. Arrowheads indicate shrunken mitochondria with condensed membranes and loss of cristae, characteristic ultrastructural features of ferroptosis. Scale bar: 2.0 μ m. (B) Lipid peroxidation levels measured by flow cytometry using the Image-iT® Lipid Peroxidation Sensor in SU-DHL-4^{NC} and SU-DHL-4^{BCL6KD} cells treated with the indicated concentrations of IKE for 72 h. MFI, mean fluorescence intensity. (C) Intracellular ROS levels measured by flow cytometry using CM-H₂DCFDA in SU-DHL-4^{NC} and SU-DHL-4^{BCL6KD} cells treated with the indicated concentrations of IKE for 72 h. Data in (B) and (C) are presented as mean $\pm$ SD ($n = 3$); * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$, two-way ANOVA.

Figure 5. Proteomic profiling identifies BCL6-regulated metabolic and redox pathways.

(A) Venn diagrams showing overlap between DEPs in SU-DHL-4^{BCL6KD} versus SU-DHL-4^{NC} cells and curated databases of redox-related (left) and metabolism-related (right) proteins. (B)

KEGG pathway enrichment analysis of the 214 redox-associated and 334 metabolism-associated overlapping DEPs from (A). Top 20 enriched pathways are shown. (C) Protein-protein interaction network of overlapping DEPs from (A), constructed using the STRING database. Node color (red intensity) and size correspond to betweenness centrality; hub proteins SDHA and H6PD are labeled. (D) Time-course Western blot analysis of SDHA protein expression in SU-DHL-4 cells treated with BI-3802 (5 μ M) for the indicated number of days. α -Tubulin serves as the loading control.

Figure 6. BCL6 transcriptionally represses the pro-ferroptotic gene *ALOX5*. (A) IGV snapshot of publicly available ChIP-seq tracks from Cistrome DB showing binding of BCL6 and its corepressors SMRT (NCOR2) and BCOR at the *ALOX5* promoter region. (B) Western blot analysis of ALOX5 protein in SU-DHL-4 cells treated with BI-3802 (5 μ M) for the indicated number of days. α -Tubulin serves as the loading control. (C) RT-qPCR analysis of *BCL6* and *ALOX5* mRNA levels in HEK293T cells transfected with a BCL6 overexpression plasmid or empty vector control; $n = 3$. Data are presented as mean $\pm$ SD; *** $P < 0.001$, two-tailed Student's t -test. (D) Western blot confirming BCL6 overexpression and its suppressive effect on ALOX5 protein in HEK293T cells transfected as in (C).

Figure 7. Combined SLC7A11 and BCL6 inhibition suppresses DLBCL tumor growth in vivo. NOD/SCID mice bearing subcutaneous OCI-Ly10 xenograft tumors were randomized into five treatment groups ($n = 6$ per group): vehicle, IKE (25 mg/kg, i.p., daily), FX-1 (10 mg/kg, i.p., daily), IKE + FX-1, or ibrutinib (20 mg/kg, p.o., daily).

(A) Schematic illustration of OCI-Ly10 subcutaneous xenograft model establishment and experimental design. **(B)** Body weight changes of OCI-Ly10 xenograft mice during treatment.

(C) Relative tumor volume changes of OCI-Ly10 xenograft mice during treatment. (D) Excised OCI-Ly10 xenograft tumor weights at endpoint. Statistical comparisons were performed between the IKE, FX-1, IKE+FX-1, and ibrutinib groups versus the vehicle control group. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ by one-way ANOVA. (E) Representative images of excised OCI-Ly10 xenograft tumors at endpoint.

Figure 8. Mechanism model of BCL6 inhibition synergistically enhancing IKE. (A) Western blot analysis of BCL6, ALOX5, and SDHA protein levels in SU-DHL-4 cells treated with 20 μ M IKE, 1 μ M BI-3802, or their combination for 72 hours. Quantification of band intensities using ImageJ. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$ by one-way ANOVA. (B) Schematic model illustrating the proposed mechanism.

487 **References**

488

- 489 1. International Non-Hodgkin's Lymphoma Prognostic Factors P. A predictive model for
490 aggressive non-Hodgkin's lymphoma. *N Engl J Med* 1993, **329**(14): 987–994.
491
- 492 2. Alizadeh AA, Eisen MB, Davis RE, Ma C, Lossos IS, Rosenwald A, *et al.* Distinct
493 types of diffuse large B-cell lymphoma identified by gene expression profiling. *Nature*
494 2000, **403**(6769): 503–511.
495
- 496 3. Schmitz R, Wright GW, Huang DW, Johnson CA, Phelan JD, Wang JQ, *et al.* Genetics
497 and Pathogenesis of Diffuse Large B-Cell Lymphoma. *N Engl J Med* 2018, **378**(15):
498 1396–1407.
499
- 500 4. Chapuy B, Stewart C, Dunford AJ, Kim J, Kamburov A, Redd RA, *et al.* Molecular
501 subtypes of diffuse large B cell lymphoma are associated with distinct pathogenic
502 mechanisms and outcomes. *Nat Med*, vol. 24: United States, 2018, pp 679–690.
503
- 504 5. Dickinson MJ, Carlo-Stella C, Morschhauser F, Bachy E, Corradini P, Iacoboni G, *et*
505 *al.* Glofitamab for Relapsed or Refractory Diffuse Large B-Cell Lymphoma. *N Engl J*
506 *Med* 2022, **387**(24): 2220–2231.
507
- 508 6. Shi Y, Xu Y, Shen H, Jin J, Tong H, Xie W. Advances in biology, diagnosis and
509 treatment of DLBCL. *Ann Hematol* 2024, **103**(9): 3315–3334.
510
- 511 7. Tilly H, Morschhauser F, Sehn LH, Friedberg JW, Trněný M, Sharman JP, *et al.*
512 Polatuzumab Vedotin in Previously Untreated Diffuse Large B-Cell Lymphoma. *N Engl*
513 *J Med* 2022, **386**(4): 351–363.
514

8. Kim J, Cho J, Lee MH, Yoon SE, Kim WS, Kim SJ. CAR T cells vs bispecific antibody as third- or later-line large B-cell lymphoma therapy: a meta-analysis. *Blood* 2024, **144**(6): 629–638.
9. Laurent C, Syrykh C, Hamon M, Adelaide J, Guille A, Escudie F, *et al.* Resistance of B-Cell Lymphomas to CAR T-Cell Therapy Is Associated With Genomic Tumor Changes Which Can Result in Transdifferentiation. *Am J Surg Pathol*, vol. 46: United States, 2022, pp 742–753.
10. Sworder BJ, Kurtz DM, Alig SK, Frank MJ, Shukla N, Garofalo A, *et al.* Determinants of resistance to engineered T cell therapies targeting CD19 in large B cell lymphomas. *Cancer Cell* 2023, **41**(1): 210–225 e215.
11. Dixon SJ, Lemberg KM, Lamprecht MR, Skouta R, Zaitsev EM, Gleason CE, *et al.* Ferroptosis: an iron-dependent form of nonapoptotic cell death. *Cell* 2012, **149**(5): 1060–1072.
12. Yang WS, Kim KJ, Gaschler MM, Patel M, Shchepinov MS, Stockwell BR. Peroxidation of polyunsaturated fatty acids by lipoxygenases drives ferroptosis. *Proc Natl Acad Sci U S A*, vol. 113: United States, 2016, pp E4966–4975.
13. Yang WS, SriRamaratnam R, Welsch ME, Shimada K, Skouta R, Viswanathan VS, *et al.* Regulation of ferroptotic cancer cell death by GPX4. *Cell* 2014, **156**(1-2): 317–331.
14. Bannai S. Exchange of cystine and glutamate across plasma membrane of human fibroblasts. *J Biol Chem*, vol. 261: United States, 1986, pp 2256–2263.
15. Ursini F, Maiorino M, Valente M, Ferri L, Gregolin C. Purification from pig liver of a protein which protects liposomes and biomembranes from peroxidative degradation

and exhibits glutathione peroxidase activity on phosphatidylcholine hydroperoxides.

Biochim Biophys Acta, vol. 710: Netherlands, 1982, pp 197–211.

16. Iglehart JK, York RM, Modest AP, Lazarus H, Livingston DM. Cystine requirement of continuous human lymphoid cell lines of normal and leukemic origin. *J Biol Chem*, vol. 252: United States, 1977, pp 7184–7191.

17. Qiu H, Liu J, Shao N, Zhao J, Chen C, Jiang Y, *et al.* SLC7A11 as a bridge between ferroptosis and disulfidptosis: a promising target for tumor treatment. *Cell Commun Signal*, vol. 23: England, 2025, p 460.

18. Zhang Y, Tan H, Daniels JD, Zandkarimi F, Liu H, Brown LM, *et al.* Imidazole Ketone Erastin Induces Ferroptosis and Slows Tumor Growth in a Mouse Lymphoma Model. *Cell Chem Biol* 2019, **26**(5): 623–633 e629.

19. Larraufie MH, Yang WS, Jiang E, Thomas AG, Slusher BS, Stockwell BR. Incorporation of metabolically stable ketones into a small molecule probe to increase potency and water solubility. *Bioorg Med Chem Lett* 2015, **25**(21): 4787–4792.

20. Ye BH, Lista F, Lo Coco F, Knowles DM, Offit K, Chaganti RS, *et al.* Alterations of a zinc finger-encoding gene, BCL-6, in diffuse large-cell lymphoma. *Science* 1993, **262**(5134): 747–750.

21. Phan RT, Dalla-Favera R. The BCL6 proto-oncogene suppresses p53 expression in germinal-centre B cells. *Nature*, vol. 432: England, 2004, pp 635–639.

22. Nurieva RI, Chung Y, Martinez GJ, Yang XO, Tanaka S, Matskevitch TD, *et al.* Bcl6 mediates the development of T follicular helper cells. *Science*, vol. 325: United States, 2009, pp 1001–1005.

23. Guo J, Liu Y, Lv J, Zou B, Chen Z, Li K, *et al.* BCL6 confers KRAS-mutant non-small-cell lung cancer resistance to BET inhibitors. *J Clin Invest* 2021, **131**(1).
24. Fernando TM, Marullo R, Pera Gresely B, Phillip JM, Yang SN, Lundell-Smith G, *et al.* BCL6 Evolved to Enable Stress Tolerance in Vertebrates and Is Broadly Required by Cancer Cells to Adapt to Stress. *Cancer Discov* 2019, **9**(5): 662–679.
25. Parekh S, Polo JM, Shaknovich R, Juszczynski P, Lev P, Ranuncolo SM, *et al.* BCL6 programs lymphoma cells for survival and differentiation through distinct biochemical mechanisms. *Blood* 2007, **110**(6): 2067–2074.
26. Ranuncolo SM, Polo JM, Melnick A. BCL6 represses CHEK1 and suppresses DNA damage pathways in normal and malignant B-cells. *Blood Cells Mol Dis* 2008, **41**(1): 95–99.
27. Panizza E, Ercolino T, Mori L, Rapizzi E, Castellano M, Opocher G, *et al.* Yeast model for evaluating the pathogenic significance of SDHB, SDHC and SDHD mutations in PHEO-PGL syndrome. *Hum Mol Genet*, vol. 22: England, 2013, pp 804–815.
28. Van Vranken JG, Bricker DK, Dephoure N, Gygi SP, Cox JE, Thummel CS, *et al.* SDHAF4 promotes mitochondrial succinate dehydrogenase activity and prevents neurodegeneration. *Cell Metab* 2014, **20**(2): 241–252.
29. Salvemini F, Franze A, Iervolino A, Filosa S, Salzano S, Ursini MV. Enhanced glutathione levels and oxidoresistance mediated by increased glucose-6-phosphate dehydrogenase expression. *J Biol Chem*, vol. 274: United States, 1999, pp 2750–2757.
30. Rogoff D, Black K, McMillan DR, White PC. Contribution of hexose-6-phosphate dehydrogenase to NADPH content and redox environment in the endoplasmic

reticulum. *Redox Rep*, vol. 15: England, 2010, pp 64–70.

31. Rouzer CA, Matsumoto T, Samuelsson B. Single protein from human leukocytes possesses 5-lipoxygenase and leukotriene A4 synthase activities. *Proc Natl Acad Sci U S A* 1986, **83**(4): 857–861.

32. Liu T, Xu X, Li J, Bai M, Zhu W, Liu Y, *et al.* ALOX5 deficiency contributes to bladder cancer progression by mediating ferroptosis escape. *Cell Death Dis*, vol. 14: England, 2023, p 800.

33. Cardenas MG, Yu W, Beguelin W, Teater MR, Geng H, Goldstein RL, *et al.* Rationally designed BCL6 inhibitors target activated B cell diffuse large B cell lymphoma. *J Clin Invest* 2016, **126**(9): 3351–3362.

34. Fan G, Zhang Y, Li Q, Rong R, Chen S, He L, *et al.* BCL6 confers resistance to HDAC inhibitors in DLBCL. *Biochem Pharmacol* 2024, **227**: 116466.

35. Winkler R, Magdefrau AS, Piskor EM, Kleemann M, Beyer M, Linke K, *et al.* Targeting the MYC interaction network in B-cell lymphoma via histone deacetylase 6 inhibition. *Oncogene*, vol. 41: England, 2022, pp 4560–4572.

36. Yang H, Green MR. Epigenetic Programing of B-Cell Lymphoma by BCL6 and Its Genetic Deregulation. *Front Cell Dev Biol* 2019, **7**: 272.

37. Duy C, Hurtz C, Shojaee S, Cerchietti L, Geng H, Swaminathan S, *et al.* BCL6 enables Ph⁺ acute lymphoblastic leukaemia cells to survive BCR-ABL1 kinase inhibition. *Nature* 2011, **473**(7347): 384–388.

38. Druker BJ, Sawyers CL, Kantarjian H, Resta DJ, Reese SF, Ford JM, *et al.* Activity of a specific inhibitor of the BCR-ABL tyrosine kinase in the blast crisis of chronic

myeloid leukemia and acute lymphoblastic leukemia with the Philadelphia chromosome. *N Engl J Med*, vol. 344: United States, 2001, pp 1038–1042.

39. Zhang L, Wu M, Guo W, Zhu S, Li S, Lv S, *et al.* A small molecule BCL6 inhibitor as chemosensitizers in acute myeloid leukemia. *Biomed Pharmacother* 2023, **166**: 115358.

40. Kawabata KC, Zong H, Meydan C, Wyman S, Wouters BJ, Sugita M, *et al.* BCL6 maintains survival and self-renewal of primary human acute myeloid leukemia cells. *Blood*, vol. 137: United States, 2021, pp 812–825.

41. Sultan M, Nearing JT, Brown JM, Huynh TT, Cruickshank BM, Lamoureaux E, *et al.* An in vivo genome-wide shRNA screen identifies BCL6 as a targetable biomarker of paclitaxel resistance in breast cancer. *Mol Oncol* 2021, **15**(8): 2046–2064.

42. Liu Y, Feng J, Yuan K, Wu Z, Hu L, Lu Y, *et al.* The oncoprotein BCL6 enables solid tumor cells to evade genotoxic stress. *Elife* 2022, **11**.

43. Zeng X, Zhao F, Jia J, Ma X, Jiang Q, Zhang R, *et al.* Targeting BCL6 in Gastrointestinal Stromal Tumor Promotes p53-Mediated Apoptosis to Enhance the Antitumor Activity of Imatinib. *Cancer Res*, vol. 83: United States, 2023, pp 3624–3635.

44. Schlemmer M, Bauer S, Schutte R, Hartmann JT, Bokemeyer C, Hosius C, *et al.* Activity and side effects of imatinib in patients with gastrointestinal stromal tumors: data from a German multicenter trial. *Eur J Med Res*, vol. 16: England, 2011, pp 206–212.

45. Zhou Tran Y, Minozada R, Cao X, Johansson HJ, Branca RM, Seashore-Ludlow B, *et al.* Immediate Adaptation Analysis Implicates BCL6 as an EGFR-TKI Combination

Therapy Target in NSCLC. *Mol Cell Proteomics* 2020, **19**(6): 928–943.

46. Chi A, Remick S, Tse W. EGFR inhibition in non-small cell lung cancer: current

evidence and future directions. *Biomark Res*, vol. 1: England, 2013, p 2.

47. Tribe AKW, Peng L, Teesdale-Spittle PH, McConnell MJ. BCL6 is a context-dependent

mediator of the glioblastoma response to irradiation therapy. *Int J Biol Macromol* 2024,

270(Pt 1): 131782.

48. Song W, Wang Z, Kan P, Ma Z, Wang Y, Wu Q, *et al.* Knockdown of BCL6 Inhibited

Malignant Phenotype and Enhanced Sensitivity of Glioblastoma Cells to TMZ through

AKT Pathway. *Biomed Res Int* 2018, **2018**: 6953506.

49. Fabre MS, Stanton NM, Slatter TL, Lee S, Senanayake D, Gordon RMA, *et al.* The

oncogene BCL6 is up-regulated in glioblastoma in response to DNA damage, and

drives survival after therapy. *PLoS One*, vol. 15: United States, 2020, p e0231470.

50. Ghetu AF, Corcoran CM, Cerchietti L, Bardwell VJ, Melnick A, Prive GG. Structure

of a BCOR corepressor peptide in complex with the BCL6 BTB domain dimer. *Mol*

Cell, vol. 29: United States, 2008, pp 384–391.

51. Liu T, Xu X, Li J, Bai M, Zhu W, Liu Y, *et al.* ALOX5 deficiency contributes to bladder

cancer progression by mediating ferroptosis escape. *Cell Death & Disease* 2023, **14**(12):

800.

Figures

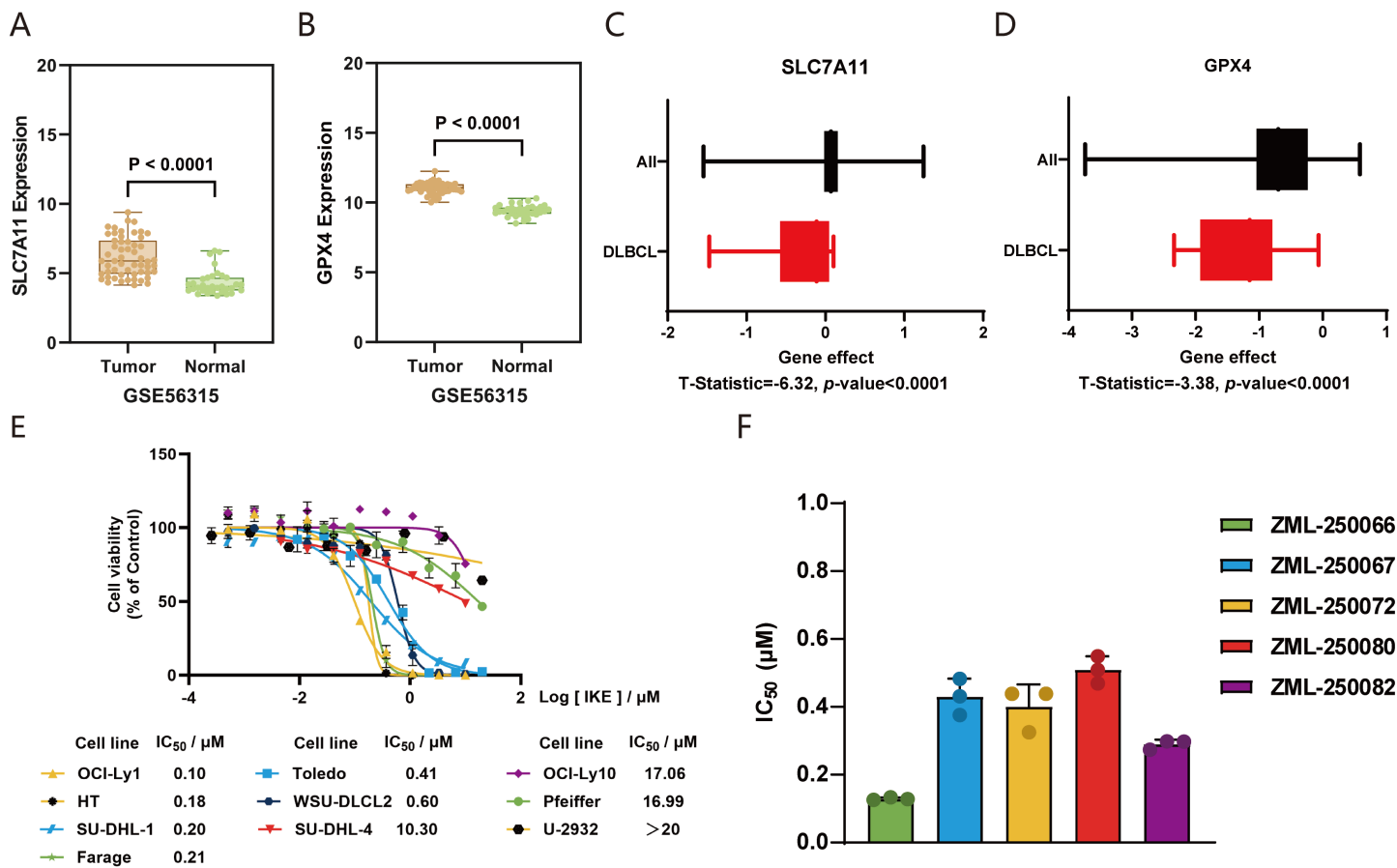


Figure 1

Figure 1. Targeting the SLC7A11/GPX4 axis inhibits DLBCL cell viability. (A, B) Expression of SLC7A11 (A) and GPX4 (B) in DLBCL patient tumor samples compared to normal B cells from the GSE56315 dataset. Data are presented as log₂-transformed expression values. *** $P < 0.001$, two-tailed Student's t-test. (C, D) DepMap CRISPR essentiality scores for SLC7A11 (C) and GPX4 (D) across 1 187 tumor cell lines (black) and 16 DLBCL cell lines (red). A more negative score indicates greater gene dependency. (E) Dose-response viability curves for a panel of DLBCL cell lines treated with IKE for 72 h. IC_{50} values are indicated. Data are presented as mean $\pm$ SD (n = 3). (F) Dose-response viability curves of patient-derived DLBCL primary cells (PDCs) treated with IKE for 7 days. IC_{50} values are indicated.

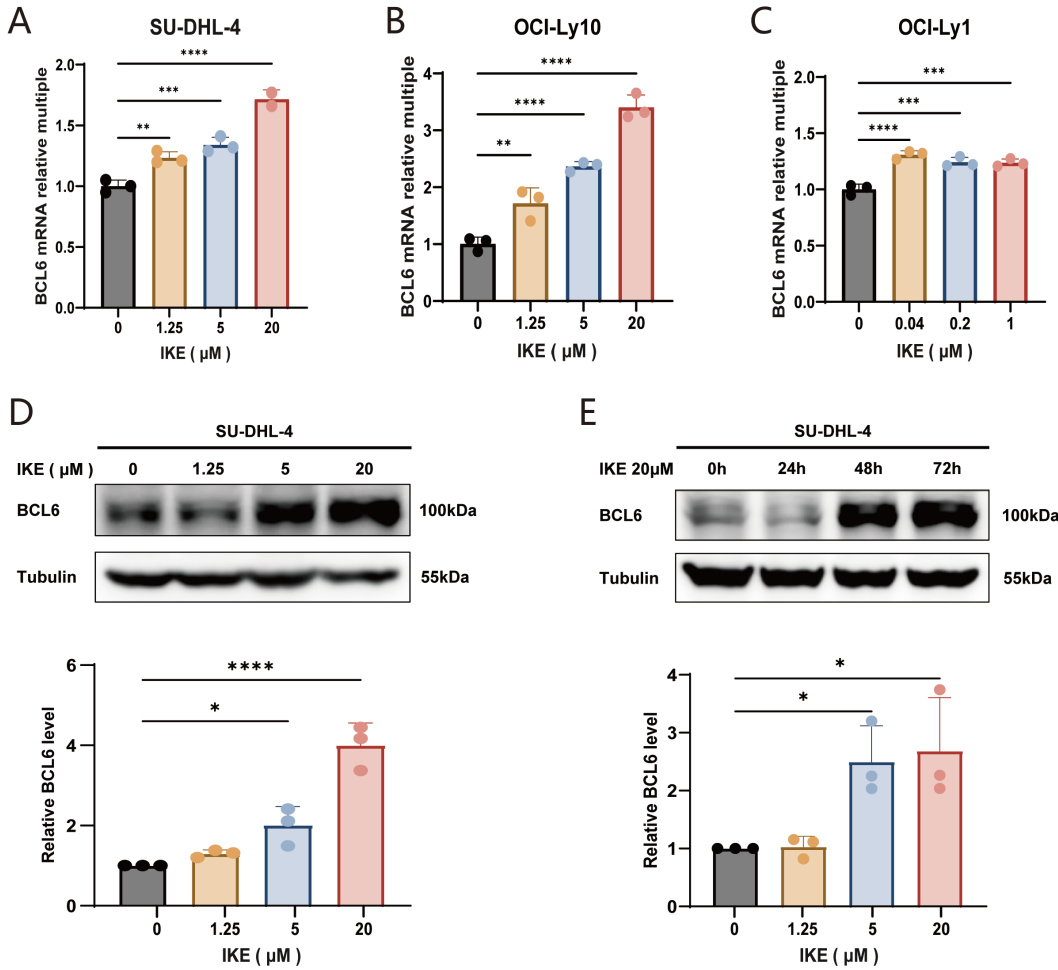


Figure 2

Figure 2. IKE treatment upregulates BCL6 expression in a dose- and time-dependent manner. (A, B) BCL6 mRNA levels measured by RT-qPCR in SU-DHL-4 (A) and OCI-Ly10 (B) cells treated with the indicated concentrations of IKE or DMSO for 24 h. Data are presented as $-\Delta\Delta C_t$ values relative to the DMSO control; $n = 3$. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, one-way ANOVA. (C) BCL6 mRNA levels in OCI-Ly1 cells treated with the indicated concentrations of IKE for 24 h; $n = 3$. * $P < 0.05$, ** $P < 0.01$, one-way ANOVA. (D)

Western blot analysis of BCL6 protein expression in SU-DHL-4 cells treated with increasing concentrations of IKE for 72 h. α -Tubulin serves as the loading control. (E) Time-course Western blot analysis of BCL6 protein in SU-DHL-4 cells treated with 20 μ M IKE for the indicated durations. Tubulin serves as the loading control.

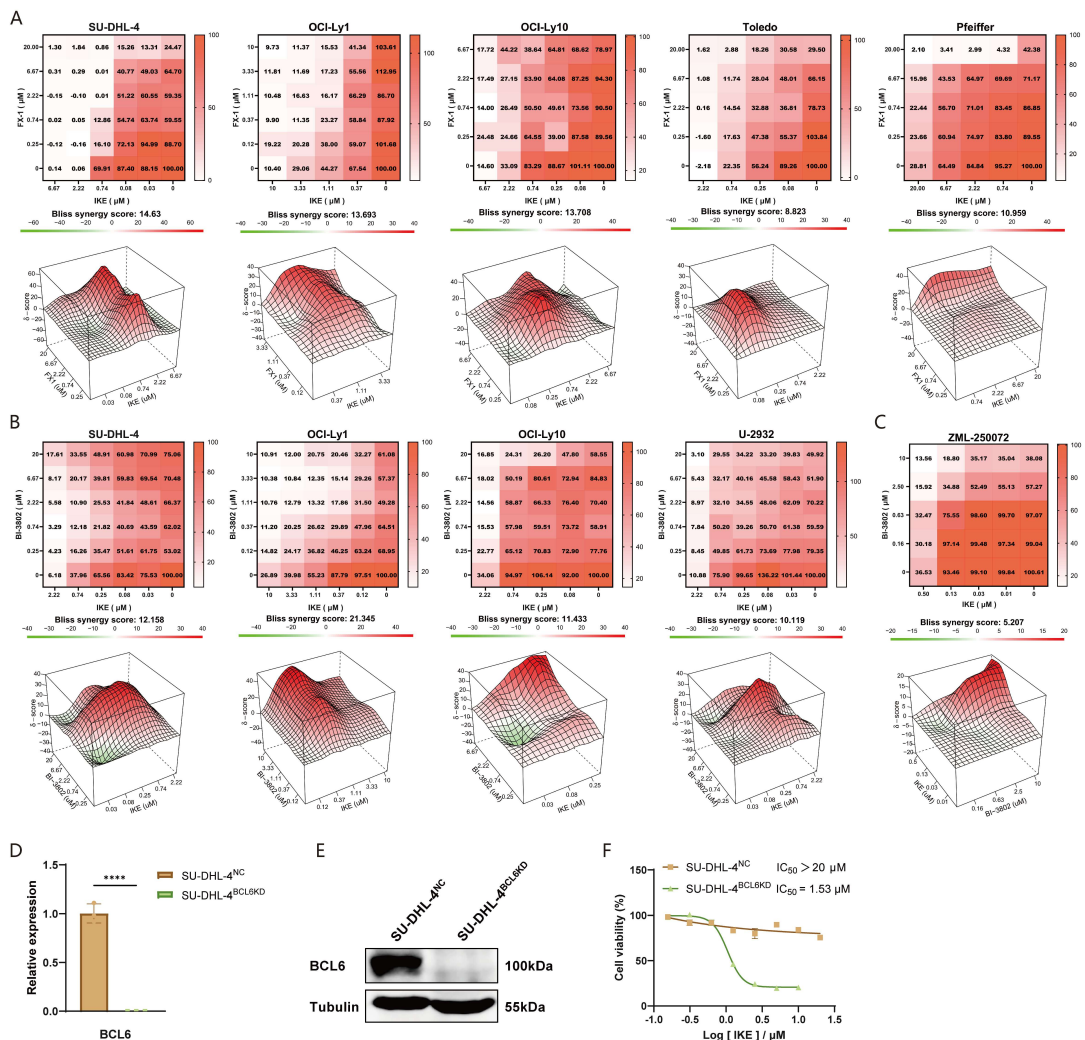


Figure 3

Figure 3. BCL6 inhibition synergizes with IKE in DLBCL. (A) Synergy matrices and corresponding Bliss synergy landscapes for DLBCL cells (SU-DHL-4, OCI-Ly1, OCI-Ly10, Toledo, Pfeiffer) treated with the indicated concentrations of IKE and FX-1 for 7 days. (B) Synergy matrices and corresponding Bliss synergy landscapes for DLBCL cells (SU-DHL-4, OCI-Ly1, OCI-Ly10, U-2932) treated with the indicated concentrations of IKE and BI-3802 for 7 days. (C) Synergy matrix and Bliss synergy landscape for a patient-derived DLBCL primary cell culture (PDC, ZML-250072) treated with IKE and BI-3802 for 7 days. (D) BCL6 mRNA levels in SU-DHL-4^{NC} and SU-DHL-4^{BCL6KD} cells measured by RT-qPCR; n = 3. ***P < 0.001, two-tailed Student's t-test. (E) BCL6 protein expression confirmed by Western blot. α -Tubulin serves as the loading control. (F) Dose–response viability curves for SU-DHL-4^{NC} and SU-DHL-4^{BCL6KD} cells treated with IKE for 72 h: n = 3. Calculated IC₅₀ values are indicated.

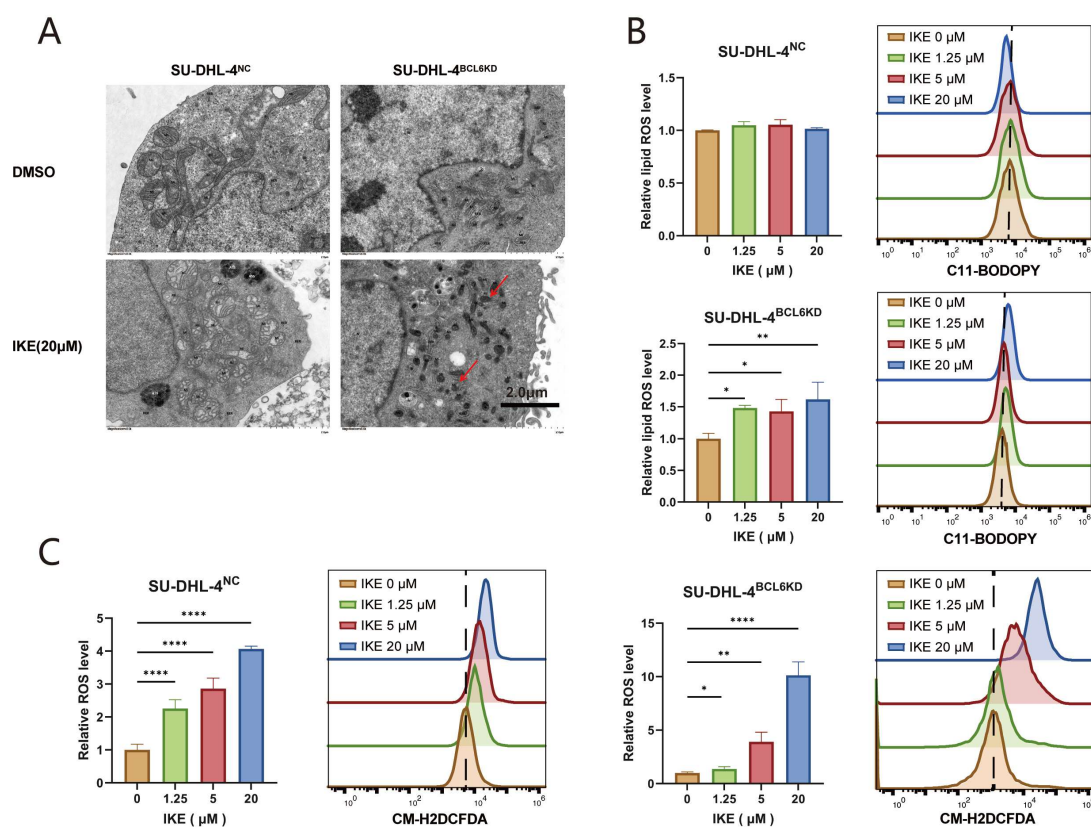


Figure 4

Figure 4. BCL6 deficiency potentiates IKE-induced ferroptosis. (A) Representative TEM images of SU-DHL-4^{NC} and SU-DHL-4^{BCL6KD} cells treated with DMSO or 20 μ M IKE for 72 h. Arrowheads indicate shrunken mitochondria with condensed membranes and loss of cristae, characteristic ultrastructural features of ferroptosis. Scale bar: 2.0 μ m. (B) Lipid peroxidation levels measured by flow cytometry using the Image-iT[®] Lipid Peroxidation Sensor in SUDHL- 4^{NC} and SU-DHL-4^{BCL6KD} cells treated with the

indicated concentrations of IKE for 72 h. MFI, mean fluorescence intensity. (C) Intracellular ROS levels measured by flow cytometry using CM-H₂DCFDA in SU-DHL-4NC and SU-DHL-4BCL6KD cells treated with the indicated concentrations of IKE for 72 h. Data in (B) and (C) are presented as mean ± SD (n = 3); *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001, two-way ANOVA.

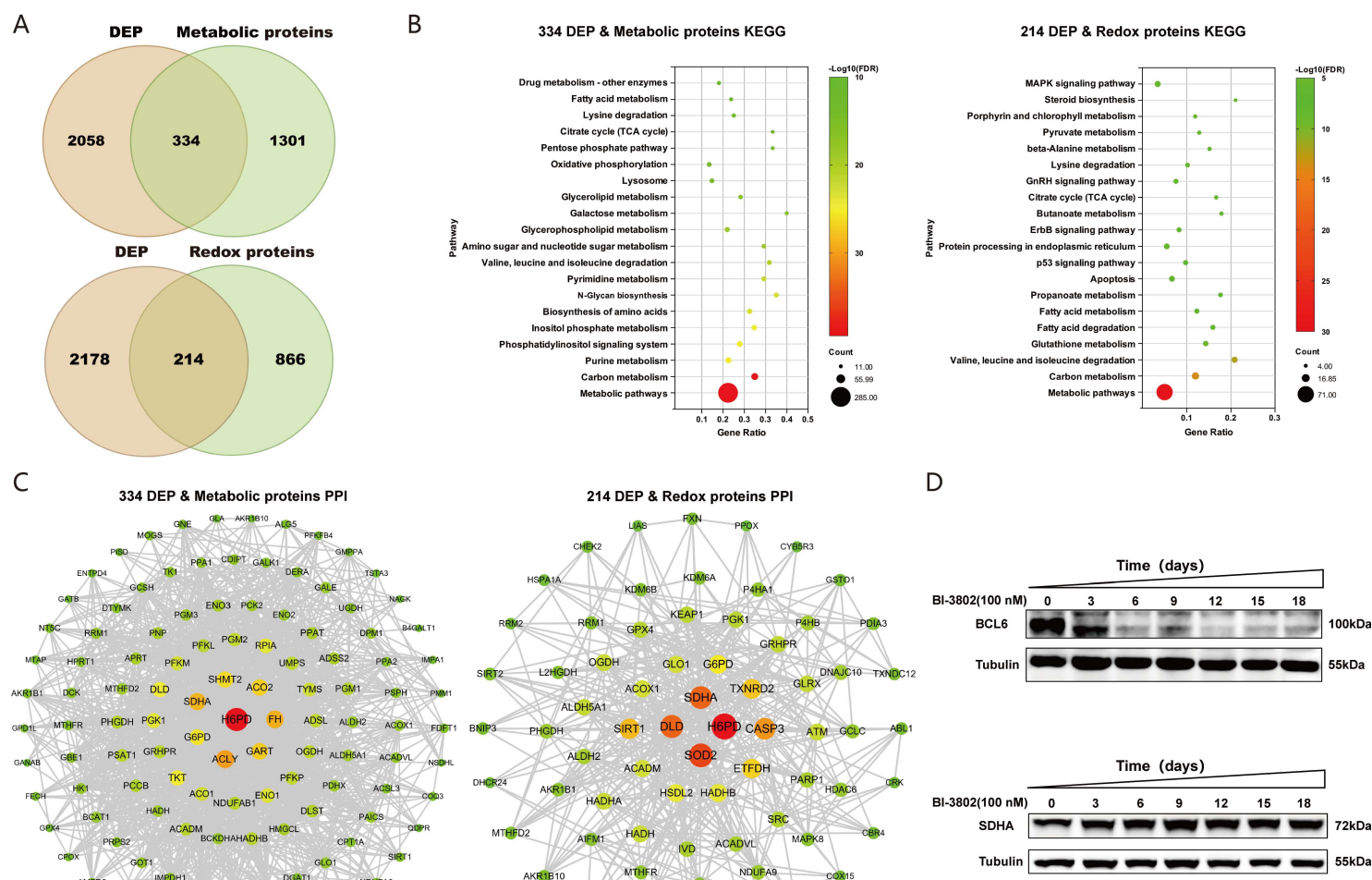


Figure 5

Figure 5. Proteomic profiling identifies BCL6-regulated metabolic and redox pathways. (A) Venn diagrams showing overlap between DEPs in SU-DHL-4BCL6KD versus SU-DHL-4NC cells and curated databases of redox-related (left) and metabolism-related (right) proteins. (B) KEGG pathway enrichment analysis of the 214 redox-associated and 334 metabolism-associated overlapping DEPs from (A). Top 20 enriched pathways are shown. (C) Protein-protein interaction network of overlapping DEPs from (A), constructed using the STRING database. Node color (red intensity) and size correspond to betweenness centrality; hub proteins SDHA and H6PD are labeled. (D) Time-course Western blot analysis of SDHA protein expression in SU-DHL-4 cells treated with BI-3802 (5 μM) for the indicated number of days. α-Tubulin serves as the loading control.

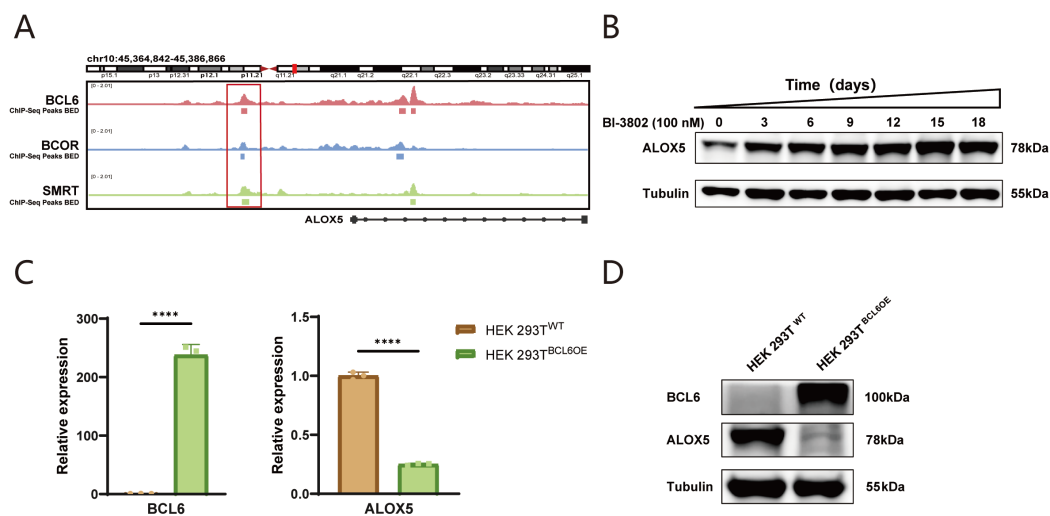


Figure 6

Figure 6. BCL6 transcriptionally represses the pro-ferroptotic gene ALOX5. (A) IGV snapshot of publicly available ChIP-seq tracks from Cistrome DB showing binding of BCL6 and its corepressors SMRT (NCOR2) and BCOR at the ALOX5 promoter region. (B) Western blot analysis of ALOX5 protein in SU-DHL-4 cells treated with BI-3802 (5 μ M) for the indicated number of days. α -Tubulin serves as the loading control. (C) RT-qPCR analysis of BCL6 and ALOX5 mRNA levels in HEK293T cells transfected with a

BCL6 overexpression plasmid or empty vector control; n = 3. Data are presented as mean ± SD; ***P < 0.001, twotailed Student's t-test. (D) Western blot confirming BCL6 overexpression and its suppressive effect on ALOX5 protein in HEK293T cells transfected as in (C).

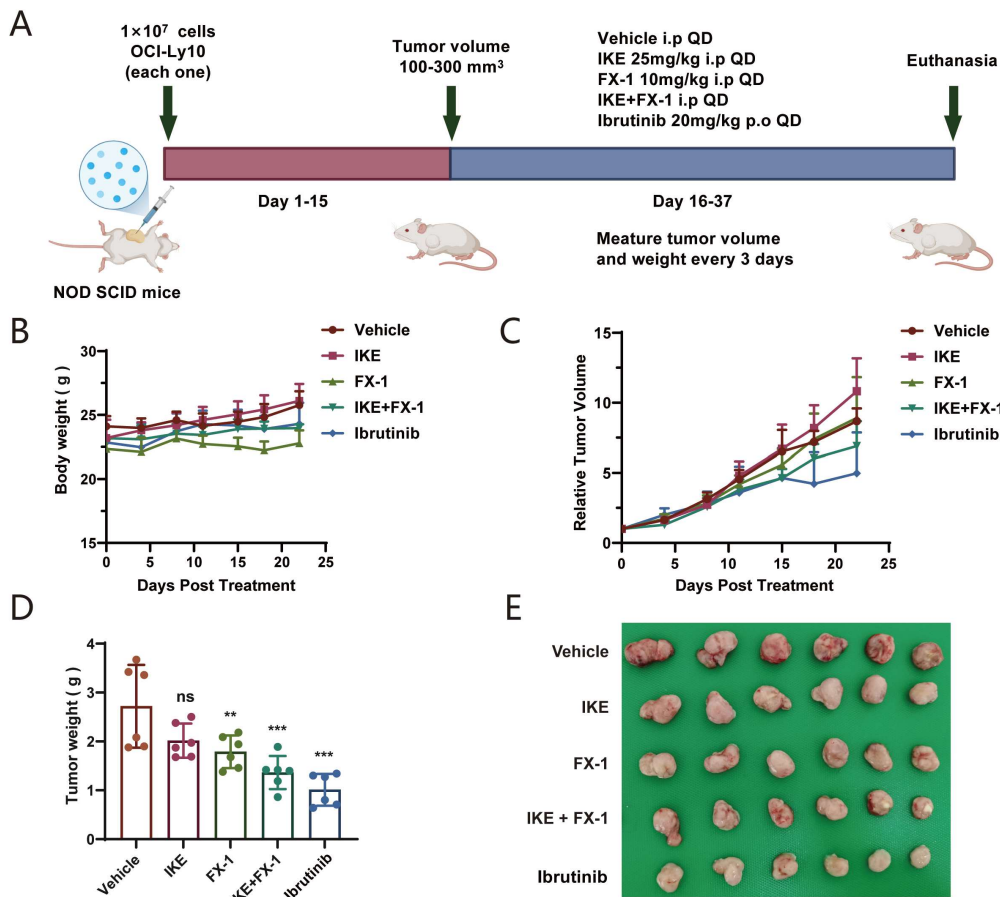


Figure 7

Figure 7. Combined SLC7A11 and BCL6 inhibition suppresses DLBCL tumor growth in vivo. NOD/SCID mice bearing subcutaneous OCI-Ly10 xenograft tumors were randomized into five treatment groups (n = 6 per group): vehicle, IKE (25 mg/kg, i.p., daily), FX-1 (10 mg/kg, i.p., daily), IKE + FX-1, or ibrutinib (20 mg/kg, p.o., daily). (A) Schematic illustration of OCI-Ly10 subcutaneous xenograft model establishment and experimental design. (B) Body weight changes of OCI-Ly10 xenograft mice during treatment. (C) Relative tumor volume changes of OCI-Ly10 xenograft mice during treatment. (D) Excised OCI-Ly10 xenograft tumor weights at endpoint. Statistical comparisons were performed between the IKE, FX-1, IKE+FX-1, and ibrutinib groups versus the vehicle control group. *p < 0.05, **p < 0.01, ***p < 0.001 by one-way ANOVA. (E) Representative images of excised OCI-Ly10 xenograft tumors at endpoint.

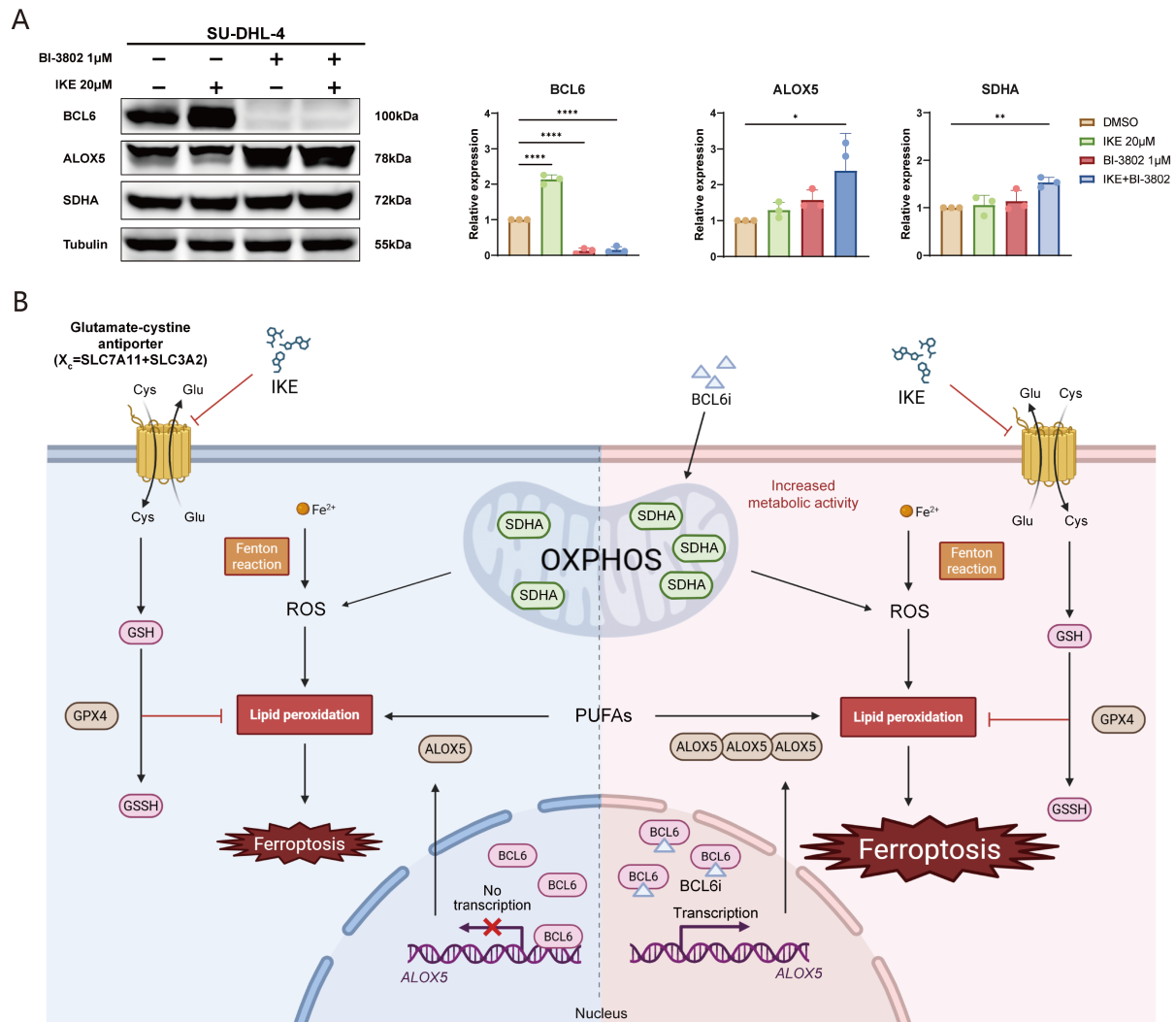


Figure 8

Figure 8. Mechanism model of BCL6 inhibition synergistically enhancing IKE. (A) Western blot analysis of BCL6, ALOX5, and SDHA protein levels in SU-DHL-4 cells treated with 20 μ M IKE, 1 μ M BI-3802, or their combination for 72 hours. Quantification of band intensities using ImageJ. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$ by one-way ANOVA. (B) Schematic model illustrating the proposed mechanism.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [SupplementaryTableS1.docx](#)
- [Originalfulllengthwesternblots.pdf](#)