Small-molecule-induced polymerization triggers degradation of BCL6

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Effective and sustained inhibition of non-enzymatic oncogenic driver proteins is a major pharmacological challenge. The clinical success of thalidomide analogues demonstrates the therapeutic efficacy of drug-induced degradation of transcription factors and other cancer targets, but a substantial subset of proteins are resistant to targeted degradation using existing approaches. Here we report an alternative mechanism of targeted protein degradation, in which a small molecule induces the highly specific, reversible polymerization of a target protein, followed by its sequestration into cellular foci and subsequent degradation. BI-3802 is a small molecule that binds to the Broad-complex, Tramtrack and Bric-à-brac (BTB) domain of the oncogenic transcription factor B cell lymphoma 6 (BCL6) and leads to the proteasomal degradation of BCL6. We use cryo-electron microscopy to reveal how the solvent-exposed moiety of a BCL6-binding molecule contributes to a composite ligand–protein surface that engages BCL6 homodimers to form a supramolecular structure. Drug-induced formation of BCL6 filaments facilitates ubiquitination by the SIAH E3 ubiquitin ligase. Our findings demonstrate that a small molecule such as BI-3802 can induce polymerization coupled to highly specific protein degradation, which in the case of BCL6 leads to increased pharmacological activity compared to the effects induced by other BCL6 inhibitors. These findings open new avenues for the development of therapeutic agents and synthetic biology.

Small-molecule-induced protein degradation has emerged as a powerful therapeutic strategy, as demonstrated by the clinical efficacy of thalidomide analogues for the treatment of haematological malignancies. Thalidomide analogues such as lenalidomide and pomalidomide modulate the activity of the CUL4–RBX1–DDB1–CRBN (CRL4-DDB1) E3 ubiquitin ligase to recruit and ubiquitinate neo-substrates including IKZF1, IKZF3 and CK1α, which leads to their proteasomal degradation. Other small molecules, such as heterobifunctional degraders (also known as proteolysis-targeting chimeras; PROTACs), have been developed to degrade a wide range of clinically relevant targets including kinases, nuclear receptors and epigenetic enzymes. These small-molecule degraders engage both the E3 ligase and the target protein to promote the formation of a substrate–drug–ligase ternary complex. Although degraders can show notable efficacy and sustained depletion of targets, some proteins are resistant to this approach. One such example is BCL6, for which heterobifunctional degraders have shown insufficient modulation of the target protein to induce growth inhibition.

BCL6 is a promising drug target for non-Hodgkin lymphomas, including diffuse large B cell lymphoma (DLBCL) and follicular lymphoma. Pathologically increased expression of BCL6, as a result of somatic BCL6 translocation, exonic mutation, promoter mutation or mutations in regulatory pathways, is a common driver of B cell malignancies. In genetically engineered mice, overexpression of BCL6 is sufficient to drive lymphoma development. BCL6 acts as a master transcriptional repressor that enables the rapid expansion of germinal centre B cells and tolerance to the genomic instability that is caused by hypermutation of the immunoglobulin genes and class-switch recombination. BCL6 represses a broad range of genes involved in the DNA damage response, cell cycle checkpoints and differentiation. As expected, knockout of BCL6 in lymphoma cells results in tumour stasis. Several peptide and small-molecule inhibitors that target BCL6 have shown efficacy in vivo, but only at high concentrations, which has limited their translation into clinical therapeutic agents.

Screens for novel BCL6 inhibitors led to the identification of small molecules that, unexpectedly, induce the degradation of BCL6.
including BI-3802\(^2\). These molecules bind to the BCL6 BTB domain that mediates the homodimerization of BCL6 and its interactions with corepressor proteins\(^3\). Treatment with BI-3802 induces rapid ubiquitination and degradation of BCL6, resulting in marked derepression of BCL6 target genes and anti-proliferative effects in DLBCL cell lines. The effects of BI-3802 are comparable to those that result from a genetic knockout\(^4\), and are more pronounced than those induced by non-degrading BCL6 inhibitors (such as BI-3812) or by heterobifunctional BCL6 degraders\(^5,6\). To uncover the underlying basis of this superior pharmacology, we sought to determine the mechanism through which BCL6 is degraded by BI-3802.

**BI-3802 induces specific degradation of BCL6**

To determine the specificity of BI-3802 as a degrader of BCL6 (Fig. 1a), we performed quantitative-mass-spectrometry based proteomics in SuDHL4 cells (a DLBCL-derived cell line), after treatment with BI-3802 for 4 hours. BCL6 was the only protein with significantly decreased abundance after treatment with BI-3802 (Fig. 1b). BI-3802 efficiently depleted chromatin-bound BCL6 (Extended Data Fig. 1a) and did not alter the expression of BCL6 mRNA (Extended Data Fig. 1b). Treatment with the structurally similar BCL6 inhibitor BI-3812 (Fig. 1a) did not alter the abundance of any protein (Extended Data Fig. 1c).

To identify the critical region of BCL6 that mediates drug-induced degradation, we generated a fluorescent reporter system in HEK293T cells, in which the gene encoding the full-length BCL6 protein (BCL6(FL)) is fused in-frame with eGFP, followed by an internal ribosome entry site (IRES) and mCherry\(^23\) (Fig. 1c). Treatment with BI-3802 led to the degradation of eGFP–BCL6(FL), whereas treatment with BI-3812 did not alter the stability of the reporter. BI-3802-induced degradation of eGFP–BCL6(FL) was attenuated by chemical inhibition of the 26S proteasome with MG132 or by inhibition of the ubiquitin-activating enzyme UBA1 with MLN7243, but not by inhibition of the neddylation pathway—which is required for activity of the cullin-RING family of E3 ubiquitin ligases—with MLN4924 (Fig. 1d, Extended Data Fig. 1d, e).

An analysis of stepwise C-terminal truncations of the BCL6 protein in our reporter demonstrated that the first 275 amino acids, which include the drug-binding BTB domain, are sufficient for BI-3802-mediated degradation (Fig. 1e). These studies demonstrate that BI-3802 induces the selective degradation of BCL6; that this degradation is mediated by a non-cullin E3 ubiquitin ligase; and that a region of 275 amino acids within BCL6 is sufficient for drug-dependent degradation.

**BI-3802 induces cellular BCL6 foci**

We next used live-cell fluorescence microscopy to examine the cellular localization of the eGFP–BCL6 fusion construct after treatment with BI-3802. Notably, we observed the appearance of distinct eGFP-containing foci within minutes of BI-3802 treatment for both the full-length BCL6 construct and the minimal degradable construct (eGFP–BCL6(1–275)) (Fig. 1f, Supplementary Videos 1–7). The eGFP signal and foci subsequently disappeared, consistent with BCL6 degradation. Immunofluorescence studies in SuDHL4 cells confirmed that endogenous BCL6 also formed foci after treatment with BI-3802 (Extended Data Fig. 1f). Addition of an excess of the BCL6 inhibitor BI-3812, which competes for the same binding site on the BTB domain of BCL6, efficiently blocked BI-3802-induced degradation of BCL6 (Extended Data Fig. 1g). To investigate the dynamics of drug-induced foci formation, we generated a BTB-containing, non-degradable construct (eGFP–BCL6(1–250)), and found that this construct formed BI-3802-induced foci that persisted...
BI-3802 induces the formation of helical filaments of BCL6 in vitro.

The crystal structure of BCL6 BTB bound to BI-3802 (PDB 5MW2) was readily fitted into the cryo-EM density. At the interface between BCL6 dimers we observed density that represents BI-3802, overlapping with the location of the compound in the crystal structure (Extended Data Fig. 4a, b). BI-3802 binds at a groove between BCL6 dimers, directly in contact with Tyr58 of BTBα (Fig. 2e), and facilitates higher-order assembly through hydrophobic interactions of the compound with Cys84 on an adjacent BCL6 dimer (BCL6/6). In addition to the interaction that is mediated by BI-3802 between BCL6 dimers, the new intermolecular interface comprises a key interaction between Arg28 of BTBβ and Glu41 of BTBγ (Fig. 2e). The resulting interface from our cryo-EM model resembles the interface seen in the crystallographic lattice of the BCL6 BTB dimer in the BI-3802 co-crystal structure (PDB 5MW2; Extended Data Fig. 4c, d). When the structurally similar BCL6 inhibitor BI-3812 was modelled onto the cryo-EM structure, we found that there was a steric clash with the extended carboxamide group, which explains the lack of BCL6 polymerization (Fig. 2a, Extended Data Fig. 4e) and the consequent lack of degradation (Extended Data Fig. 1c).

To determine whether BCL6 polymerization is required for the formation of foci in cells, we introduced and assayed mutations designed to impair drug binding or the dimer–dimer interaction. Mutating the Tyr58 residue in BCL6 to alanine prevented the binding of BI-3802 in vitro (Extended Data Fig. 4f), and consequently blocked the formation of BCL6 foci in cells (Fig. 2f). Arg28 and Glu41 form a salt bridge that is critical for dimer–dimer interaction (Fig. 2e), and mutating either residue to alanine (R28A or E41A) prevented the formation of foci upon treatment with BI-3802 (Fig. 2f). Notably, the E41A substitution did not impair drug binding to BCL6 (Extended Data Fig. 4f). In addition, Cys84 forms a hydrophobic interaction with the methyl group of the neighbouring BCL6 molecule (Fig. 2e), and a C84A mutation notably reduced the formation of foci in cells (Fig. 2f). Together, these results demonstrate that mutation of the amino acids in BCL6 that are critical for dimer–dimer interactions disrupts drug-induced polymerization.

To identify amino acids that are critical for BI-3802 activity in an unbiased manner, we performed a systematic alanine scan of the BCL6 BTB domain (residues 32–99). We evaluated the effect of each mutation on BI-3802 cellular toxicity in SuDHL4 lymphoma cells (Extended Data Fig. 5a), and on BI-3802-induced degradation of the BCL6 reporter in HEK293T cells (Extended Data Fig. 5b, c), and on the basis of the results selected the top four variants for detailed validation (E41A, G55A, Y58A and C84A) (Fig. 2g). Overexpression of these variants in the BCL6-dependent SuDHL4 and Raji cell lines conferred resistance to BI-3802, but had no effect in the BCL6-independent DEL cell line (Extended Data Fig. 5d, e). In agreement with our structural analysis, the residues identified in this unbiased mutagenesis experiment were located either close to the BI-3802-binding site (G55A and Y58A),
Fig. 3 | BCL6 polymerization enhances SIAH1 interaction and ubiquitination. a, Correlation of P values for two genome-wide CRISPR–Cas9 knockout screens. The x-axis is a reporter screen for eGFP–BCL6 stability in HEK293T cells upon treatment with BI-3802, and the y-axis is a BI-3802 resistance screen in SuDHL4 cells. Guides were collapsed to gene level (n = 3; 4 guides per gene; two-sided empirical rank-sum statistics). b, Flow cytometry analysis of HEK293T cells expressing the indicated BCL6 BTB domain fusion construct and treated with DMSO or 1 μM BI-3802 for 7 h (bars represent mean, n = 3). BCL6 stability calculated as eGFP–BCL6/mCherry. c, Immunobots of eGFP immunoprecipitation (IP) in the presence of 2 μM BI-3802 or DMSO from HEK293T cells transduced with the indicated eGFP–BCL6 constructs and VS–SIAH1(C44S) (n = 2). d, Immunobots of the in vitro ubiquitination of strep-TagII–BCL6(5–360) by full-length SIAH1 in the presence of DMSO or 1 μM BI-3802 (n = 3). e, Bodipy-labelled BCL6(5–360) was titrated to 0.2 μM of the biotinylated SIAH1 substrate-binding domain (SIAH1(SBD)) in DMSO, 2 μM BI-3802 or 2 μM BI-3812, and the signal was measured by TR-FRET. Lines represent standard four-parameter log-logistic function fit (n = 3). f, HEK293T cells expressing eGFP–BCL6(1–275) and VS–SIAH1 were treated with 0.5 μM MLN7243 for 2 h, and 1 μM BI-3802 for 1 h. Cells were imaged by indirect immunofluorescence as indicated (n = 2). Scale bar, 5 μm.

BI-3802 or DMSO from HEK293T cells transduced with the indicated eGFP–BCL6 constructs and VS–SIAH1(C44S) (n = 2). d, Immunobots of the in vitro ubiquitination of strep-TagII–BCL6(5–360) by full-length SIAH1 in the presence of DMSO or 1 μM BI-3802 (n = 3). e, Bodipy-labelled BCL6(5–360) was titrated to 0.2 μM of the biotinylated SIAH1 substrate-binding domain (SIAH1(SBD)) in DMSO, 2 μM BI-3812 or 2 μM BI-3802, and the signal was measured by TR-FRET. Lines represent standard four-parameter log-logistic function fit (n = 3). f, HEK293T cells expressing eGFP–BCL6(1–275) and VS–SIAH1 were treated with 0.5 μM MLN7243 for 2 h, and 1 μM BI-3802 for 1 h. Cells were imaged by indirect immunofluorescence as indicated (n = 2). Scale bar, 5 μm.

SIAH1 degrades polymerized BCL6

We next sought to identify the cellular machinery that is necessary for the BI-3802-induced degradation of BCL6. We used two complementary, genome-scale CRISPR–Cas9 knockout screens to interrogate the mechanism of drug-induced BCL6 degradation. First, we performed a flow-cytometry-based BCL6 reporter screen in HEK293T cells, in which cells infected with the single-guide RNA (sgRNA) library were treated with BI-3802 or dimethyl sulfoxide (DMSO), and the cell populations were sorted with increased (highest 5% eGFP/mCherry ratio) or decreased (lowest 5% eGFP/mCherry ratio) levels of eGFP–BCL6(FL) were sorted from the bulk population (Extended Data Fig. 6a–d). Second, we performed a BI-3802 resistance screen in SuDHL4 cells (Extended Data Fig. 6e, f). The only gene that scored significantly in both screens was the non-cullin E3 ubiquitin ligase SIAH1 (Fig. 3a).

To validate the role of SIAH1 in drug-induced BCL6 degradation and resistance to BI-3802, we treated SIAH1 KO cells with multiple independent sgRNAs. Each sgRNA attenuated the degradation of eGFP–BCL6(FL) that resulted from BI-3802 treatment, and induced resistance to BI-3802 (Extended Data Fig. 6g, h). Overexpression of wild-type SIAH1 not only enhanced BI-3802-dependent degradation of BCL6, but also reduced the abundance of BCL6 in the absence of the drug (Extended Data Fig. 7a), suggesting that SIAH1 has a role in both drug-dependent and endogenous BCL6 degradation. The SIAH1 E3 ligase recognizes a VxP motif on substrate proteins25,26, and this motif is present in residues 249–251 of BCL6 (Extended Data Fig. 7b). Deletion of the VxP motif provides an explanation for our C-terminal truncation analysis, in which BCL6(1–275) was effectively degraded in the presence of BI-3802 but BCL6(1–250) was not, despite the ability of this shorter construct to form foci in the presence of drug (Fig. 1e, g). Direct C-terminal fusion of a VxP-containing peptide (BCL6(241–260)) to the BCL6 BTB domain (BCL6(1–129)) was sufficient for BI-3802-induced degradation mediated by SIAH1 (Extended Data Fig. 7c), and degradation was attenuated by mutation of the BCL6 VxP motif (VSP>GSA) (Fig. 3b, Extended Data Fig. 7d). In this BTB–SIAH1 degron construct, mutations in the BTB domain that affect critical residues for polymerization (R28A, E41A or C84A) or drug binding (G55A or Y58A) completely abolished BI-3802-induced degradation. Together, these data show that SIAH1 is involved in the BI-3802-induced degradation of BCL6.

To examine whether the BCL6 VxP motif mediates the interaction with SIAH1, we performed co-immunoprecipitation studies with catalytically inactive SIAH1(C44S). We found that BCL6 and SIAH1 co-immunoprecipitated in cells (Fig. 3c) and in vitro using recombinant proteins (Extended Data Fig. 8a), and that mutation or deletion of the VxP motif prevented this co-immunoprecipitation. The VxP-containing peptide alone (BCL6(241–260)) was sufficient for SIAH1 interaction (Extended Data Fig. 8b, c). In vitro ubiquitination assays with recombinant proteins showed that BCL6 is a substrate for SIAH1 (Extended Data Fig. 8d), and that the rate and magnitude of ubiquitination is accelerated by BI-3802 (Fig. 3d). Together, these data establish SIAH1 as a bona fide E3 ligase for BCL6.

To investigate how BI-3802-induced polymerization of BCL6 affects SIAH1-mediated degradation, we examined SIAH1 recruitment and BCL6 ubiquitination both in vitro and in cells. Using a time-resolved fluorescence energy transfer (TR-FRET) assay, we observed a moderate...
baseline affinity between BCL6 and SIAH1, which was strongly enhanced for BI-3802-polymerized BCL6 (apparent dissociation constant (K_{D}) = 0.2 μM) (Fig. 3e, Extended Data Fig. 8e). We found that BI-3802 increased the interaction between BCL6 and SIAH1 (half-maximum effective concentration (EC_{50}) = 64 nM) both in vitro (Extended Data Fig. 8f) and in cells (Extended Data Fig. 8g). By contrast, BI-3812 did not influence the interaction between BCL6 and SIAH1, despite BI-3802 and BI-3812 having a comparable affinity to BCL6 (Extended Data Fig. 8h).

Finally, in the presence of BI-3802, SIAH1 colocalized to BCL6 foci in a VxP-motif-dependent manner (Fig. 3f, Extended Data Fig. 8i). Together, our in vitro and cellular assays indicate that BI-3802-induced polymerization enhances the interaction between BCL6 and SIAH1, and leads to accelerated ubiquitination and degradation of BCL6.

### Discussion

Through a combination of functional screens, biochemical dissection and structural characterization, we have shown that binding of BI-3802 to the BTB domain of BCL6 triggers the higher-order assembly of BCL6 into filaments. Polymerization promotes the ubiquitination of BCL6 by SIAH1, an E3 ligase that recognizes a VxP motif distal to the drug-binding site, and proteasomal degradation. Treatment with BI-3802 results in the formation of intracellular foci that contain BCL6 and SIAH1. These findings represent a novel mechanism of targeted protein degradation, in which a small molecule inactivates a target protein through specific drug-induced polymerization and subsequent degradation.

Structurally, BI-3802 and BI-3812—a degrader and an inhibitor of BCL6, respectively—differ only in their solvent-exposed dimethylpiperidine moiety. BI-3802 induces the polymerization of the BCL6 BTB domain and the formation of foci in cells, whereas BI-3812 does not. The cryo-EM structure that we present here reveals that the dimethylpiperidine moiety of BI-3802 interacts directly with distal amino acids on an adjacent BTB-domain homodimer. Owing to the symmetry of the BTB domain, BI-3802 can iterate this dimer–dimer interaction to assemble supramolecular filaments. A previous study has shown that introducing amino acid mutations on the surface of symmetrical proteins can trigger supramolecular self-assembly.27 Because the solvent-exposed moiety in BI-3802 triggers polymerization of BCL6, it is possible that modification of the solvent-exposed part in a small molecule could induce new protein–protein interactions more generally, as we have recently shown for a kinase inhibitor.28 In the case of symmetrical proteins, small molecules have the potential to induce polymerization, which can then lead to degradation with exceptional specificity.

BI-3802—as well as structurally related bioavailable analogues29—exhibits a markedly increased activity against lymphoma cells compared to BI-3812, which suggests the combined effects of inhibiting co-activator binding, sequestering BCL6 into foci and degrading BCL6. It has previously been shown that inhibition of BCL6 or its degradation by PROTACs results in insufficient inhibition of downstream targets and consequently only minor anti-proliferative effects.3 The unique mechanism of action of BI-3802 overcomes these limitations and helps to explain its improved efficacy. Notably, the anti-proliferative and transcriptional effect of BI-3802 is comparable to knockout of BCL6 using an inducible CRISPR–Cas9 system.30 The molecular details provided here will enable the optimization of drugs towards this mechanism of action, which could lead to advances in the development of therapeutic agents that target malignancies driven by aberrant BCL6 activity.

Drug-induced polymerization expands the repertoire of pharmacological modalities that mediate the targeted degradation of proteins, as shown here for BCL6, and this approach is likely to be applicable to other transcription factors and proteins with internal symmetry that have traditionally been difficult to drug. A subtle derivatization in the solvent-exposed moiety distinguishes BI-3802 from BCL6 inhibitors that do not induce degradation, providing a potential path towards the rational design of molecules that induce polymerization.

### Online content

Any methods, additional references, Nature Research reporting summaries, source data, extended data, supplementary information, acknowledgements, peer review information; details of author contributions and competing interests; and statements of data and code availability are available at https://doi.org/10.1038/s41586-020-2925-1.

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Methods

Mammalian cell culture
The human HEK293T, SuDHL4Cas9, RajiCas9 and DELCas9 cell lines were provided by the Genetic Perturbation Platform, Broad Institute. HEK293T cells were previously published. HEK293T cells were cultured in DMEM (Gibco) and SuDHL4Cas9, RajiCas9 and DELCas9 cells in RPMI (Gibco), with 10% fetal bovine serum (FBS) (Invitrogen), glutamine (Invitrogen) and penicillin–streptomycin (Invitrogen) at 37 °C and 5% CO2.

Compounds
BI-3802 and BI-3812 were obtained from opnMe, Boehringer Ingelheim; MLN7243 (CT-M7243) from ChemieTek; MLN4924 (HY-70062) from MedChem Express; MG132 (S2619) from Selleck Chemicals; and chloroquine (C6628) from Sigma-Aldrich.

Primers
All primers used in this study are listed in Supplementary Table 1.

Antibodies
The following antibodies were used in this study: anti-BCL6 (Santa Cruz Biotechnology, sc-7388), anti-β-tubulin (Cell Signaling, 2146S), anti-HSP90 (Cell Signaling, 4874S), anti-HDAC1 (Cell Signaling, 2062S), anti-histone H3 (Cell Signaling, 12648S), anti-eGFP (Cell Signaling, 2956), anti-V5-tag (Thermo Fisher Scientific, MAS-15253), anti-strep-Tag II HRP conjugate (Sigma, S7199-3), anti-mouse 800CW (LI-COR Biosciences, 926-32211), anti-rabbit 680LT (LI-COR Biosciences, 925-68021), anti-mouse Alexa Fluor 633 (Thermo Fisher Scientific, A21052) and Alexa anti-mouse 488 (Biolegend, 405319).

Whole-proteome quantification using tandem mass tag mass spectrometry
A total of 10^6 SuDHL4Cas9 cells were treated with DMSO, 1 μM BI-3802 or 1 μM BI-3812 for 4 h and cells were collected by centrifugation. Samples were processed, measured and analysed as previously described. Data are available in the PRIDE repository (PXD016185) and in Supplementary Data 1.

Cellular fractionation
A total of 1 × 10^6 SuDHL4Cas9 cell were treated with DMSO or 1 μM BI-3802 for 24 h and fractionated using the Celllytic NuCLEAR Extraction Kit (Sigma-Aldrich) according to the manufacturer’s protocol, resolved on a polyacrylamide gel and immunoblotted for the indicated targets.

Quantitative PCR
A total of 1 × 10^6 SuDHL4Cas9 cells were treated with DMSO or 1 μM BI-3802 for 1 h, collected by centrifugation, washed with phosphate-buffered saline (PBS) and flash-frozen in dry ice. mRNA was isolated using the QiAGEN RNA kit (Qiagen, 74106). For cDNA synthesis, total RNA was reverse-transcribed with SuperScript VILO Master Mix (Invitrogen, 4444557) for BCL6 (Sigma, Hs02758991_g1, Life Technologies) and GAPDH (TaqMan, Hs99999905_m1, Life Technologies) and GAPDH (TaqMan, Hs02758991_g1, Life Technologies) and GAPDH (TaqMan, Hs02758991_g1, Life Technologies). Reactions were run and analysed on the QStudio 6 FLX Real-Time PCR System (Thermo Fisher Scientific).

Immunoblots
SuDHL4Cas9 cell were treated as indicated in figure legends. A total of 2 × 10^6 cells were collected (1,000 rpm, 5 min) and flash-frozen in dry ice. Cells were lysed in 150 μl of lysis buffer (PBS + 0.25% NP-40 +125 U/ml benzonase (Invitrogen), 1:100 Halt Protease and Phosphatase Inhibitor Cocktail (Thermo Fisher Scientific)) for 2 min at room temperature. The soluble fraction was separated by centrifugation (5,000 rpm, 5 min). Protein lysates were mixed with Laemmli (SDS-sample) buffer (reducing, 6X) (Boston BioProducts), resolved on a polyacrylamide gel and immunoblotted for the indicated targets (Supplementary Fig. 1).

Lentivirus production
In a 6-well plate format, 500,000 HEK293T cells were seeded per well in 2 ml medium. The next day, 3 μl of TransIT-LT1 (Mirus, MIR2305) was added to 15 μl of OPTI-MEM (Invitrogen), incubated for 10 min and combined with a mixture consisting of 500 ng of the desired plasmid, 500 ng psPAX2 and 50 ng pVSV-G in 32.5 μl OPTI-MEM. The solution was incubated for 30 min at room temperature and 50 μl was added to HEK293T cells in a dropwise manner. The lentivirus-containing medium was collected two days after transfection and stored at −80 °C.

Lentiviral transduction
Cells were infected by spin infection. Two million cells per well in 2 ml of culture medium were transferred to a well of a 6-well plate. For constructs for which puromycin selection was possible, 20% (volume/volume) of virus was added. For constructs for which puromycin selection was not possible, 50% (volume/volume) of virus was added. The plates were centrifuged for 2 h (2,000 rpm, 37 °C).

Degradation of BCL6 reporter constructs in HEK293T cells
The eGFP–BCL6(FL) BCL6 stability vector was constructed by shuffling BCL6 from pDONR223-FL-BCL6 (Broad Institute human ORFeome library) into a gateway-compatible version of an ‘Articheck’ vector by a LR gateway reaction. eGFP–BCL6(1–250), eGFP–BCL6(1–275), eGFP–BCL6(1–360), eGFP–BCL6(1–500), eGFP–BCL6(1–129 + linker +241–260) and eGFP–BCL6(1–129 + linker+241–260 VSP–GSA) inserts were synthesized or PCR-amplified with BsmBI sites and ligated into a ‘Cilantro2’ vector (Addgene 74450) by golden-gate assembly. eGFP–BCL6 E41A, G55A, Y58A, C84A and R28A mutations were designed on the minimal construct containing the BTB domain fused to a linker and the SIAHI-binding site (BCL6(1–129 + linker +241–260 VSP–GSA)), synthesized through Integrated DNA Technologies and ligated into Cilantro2 by golden-gate assembly. Lentivirus was packaged in HEK293T cells using TransIT (Mirus) and subsequently used for spin infection. HEK293TCas9 cells expressing the indicated constructs in Articheck or Cilantro2 stability reporter vectors (PGK or SFFV target–eGFP–IRES–mCherry, puromycin resistance) were plated in 96-well plates and treated for the indicated times. Expression of eGFP–BCL6 and mCherry was quantified by flow cytometry (Supplementary Fig. 2) (CytoFLEX, Beckman or LSR Fortessa flow cytometer; BD Biosciences). All degradation assays were done in at least triplicate. Geometric means of eGFP and mCherry fluorescence signals for live and mCherry-positive cells were exported using flow cytometry analysis software (FlowJo, BD Biosciences). Ratios of eGFP to mCherry were normalized to the average of DMSO-treated controls.

Live-cell imaging
A total of 1 × 10^4 HEK293T cells per cm² were seeded in a μ-Slide 8 Well chamber (ibidi) and cultured for 18–24 h under standard growth conditions. Cell culture medium was exchanged to CO2-independent medium (Gibco) and imaged with the DeltaVision Ultra High-Resolution Microscope (GE Healthcare, 100× lens, oil refraction index = 1.520). The following acquisition parameters were used: image size 896 × 896 pixels, binning 1 × 1, GFP exposure time 0.08 s, and the neutral density filter (ND) 32%. To capture foci within the whole-cell volume, around 26 μm per cell was imaged every 0.4–0.5 μm. Images were deconvolved (10 cycles, conservative conditions) and projected using maximal intensity by softWoRx v.7.0.0. Images for videos were taken every 10 min and combined to a video by QuickTime.

CytoSpin
SuDHL4Cas9 cells (0.8 × 10^6 cells/ml) were treated with DMSO or 0.5 μM E1 Inhibitor (3 h) + 1 μM BI-3802 (1 h) and 200 μl of the cell suspension was plated in a 6-well plate and centrifuged for 2 h (2,000 rpm, 37 °C).
was immobilized on a slide using the Cytospin 4 Cytocentrifuge (Cytospin 4, A7830003; 6,000 rpm, 6 min). Medium was aspirated and cells were fixed with 4% formaldehyde diluted in warm PBS for 15 min at room temperature. Slides were washed three times for 5 min with PBS, blocked and permeabilized with blocking solution (5% Normal Goat Serum (Cell Signaling), 0.3% Triton X-100 in PBS) for 60 min and stained with anti-BCL6 antibody in blocking solution overnight at 4 °C. Cells were washed three times with PBS for 5 min each, incubated with Alexa Fluor 488–conjugated anti-mouse antibodies, washed with three times with PBS for 5 min each and covered with coverslip slides using ProlongGold Antifade Reagent (Thermo Fisher Scientific, P36934). Cells were imaged using the DeltaVision microscope as described above.

**Cryo-EM sample preparation and data collection**

Strep-Tag II–Avi–BCL6(5–360) (0.6 mg/ml, 13.4 μM) in buffer (25 mM HEPES pH 7.4, 200 mM NaCl, 1 mM TCEP) was incubated with 20 μM BI-3802 (1.5 molar excess, 1% DMSO) for 1 h at room temperature. The sample was diluted (tenfold) and concentrated again to decrease total DMSO concentration (0.1%). This process yielded polymerized BCL6 protein (0.48 mg/ml) as confirmed by negative-stain electron microscopy. The sample was further mixed with CHAPS (0.8 mM final concentration) to yield a final sample for vitrification. Four microlitres of the sample was applied twice to glow-discharged 1.2/1.3 Quantifoil grids and the grids were blotted for 1.3 s after each application and vitrified using a Leica EM GP (10 °C, 95% relative humidity). A total of 67,552 movies (60 frames each) were collected on a FEI Titan Krios operated at 300 kV with a Gatan Quantum Image filter (20 eV slit width) and a post-GIF Gatan K3 camera, at a nominal magnification of 105,000× in counting mode with a pixel size of 0.825 Å/pixel. Per stage position, two movies were acquired in four holes, resulting in eight image acquisition groups. Movies were recorded in a defocus range from −1.0 to −2.5 μm over an exposure time of 3 s and with a total dose of 63.4 e/Å².

**Image processing and model building**

The movie frames were aligned and initially Fourier-cropped by a factor of 2, yielding a pixel size of 1.65 Å with MotionCor2, and CTF parameters were estimated with CTFIND4. Particle picking was carried out using cryoYLO for 1,610,413 initial particles. All subsequent processing steps were performed with RELION v.3.0. Multiple rounds of 2D classification were used to clean the data, after which 274,999 particles were pooled for initial 3D classification. After re-extraction of the particles from the uncropped micrograph (final pixel size of 0.825 Å), three rounds of 3D classification at 7.5° sampling and an additional round of 3D classification at 3.7° sampling left 128,526 particles that were used for 3D refinement, followed by CTF refinement and Bayesian polishing. Polished particles led to a reconstruction at 4.1-Å nominal resolution (using the Fourier shell correlation (FSC) = 0.143 threshold criterion). The polished particles were subjected to one additional round of 3D classification with application of a soft mask (angular sampling = 7.5°, regularization = 7), which led to one major class with 95% of the particles (112,048 particles). Three-dimensional refinement and subsequent beam-tilt correction resulted in the final reconstruction at 3.7-Å nominal resolution (using the FSC = 0.143 threshold criterion). Local resolution was estimated using RELION v.3.0. Because of a highly preferred orientation of the ribbon-like filament on the electron microscopy grid, the reconstruction suffers from an anisotropic resolution distribution. However, eight instances of the BCL6 BTB domain bound to BI-3802 from PDB 5MW2 could unambiguously be fitted into the cryo-EM density using Coot. The resulting model was refined using global minimization, rigid body and ADP refinement implemented in phenix.real_space_refine, with reference constraints to the high-resolution crystal structure (SMW2). Owing to the limited resolution of the map, the placement of side chains is approximated from the crystal structure (SMW2). The model resolution at FSC = 0.5 appears low (8.1 Å), which is due to a dip in the model FSC versus the map FSC resulting from the preferred orientation.

**Construction of the BCL6 BTB domain alanine-scan library**

An alanine-scan library, in which each amino acid of full-length BCL6 between positions 32 and 99 was individually mutated to alanine and each alanine to arginine, was introduced into the eGF-B–BCL6(FL) stability reporter (Fig. 1c). Two 176-bp-long oligo libraries were synthesized (Twist Bioscience) in one oligo pool, encoding BCL6 BTB variants. The first library covered BCL6 amino acids 32–65 (5′-TCCGGAGTCGAGACATCCTGG...AAATGCAACCTTAGTGTGATCAATC-3′) and the second library covered BCL6 amino acids 66–99 (5′-CTATAGCATTTACAGACCAGTTG...
BI-3802 resistance alanine-scan screen

The pDONR-BCL6 plasmid backbone was amplified with NEBNext polymerase (NEB M0541, 98 °C for 30 s, 26 cycles of [98 °C for 10 s, 64 °C for 10 s, 72 °C for 6 min], 72 °C for 2 min).

The pDONR-BCL6 plasmid backbone was amplified with NEBNext polymerase (98 °C for 30 s, 6 cycles of [98 °C for 10 s, 59 °C for 10 s, 72 °C for 150 s], 24 cycles of [98 °C for 10 s, 64 °C for 10 s, 72 °C for 150 s], 72 °C for 2 min), depurinated with Dpn1 (NEB) and purified by gel purification using the QiAquick Gel Extraction Kit (Qiagen). Libraries were transferred into Stbl3 chemically competent bacteria (Invitrogen) and plated on LB plates with carbenicillin for chemical selection. The resulting colonies were scraped, pooled and purified using the QIAprep Spin Miniprep Kit (Qiagen). To shuffle the alanine-scan library into the Artichoke expression backbone, 150 μg of the pDONR BCL6 BTB alanine-scan library and 150 μg of the gateway-pArtichoke vector were incubated overnight with LR clonase (Thermo Fisher Scientific) at room temperature. After Proteinase K treatment, salts were removed by dialysis (membrane filter, 0.025 μm pore size, Millipore). Libraries were transformed into Stbl3 chemically competent bacteria (Invitrogen) and plated on LB plates with carbenicillin for chemical selection. The resulting colonies were scraped, pooled and purified using the QIAprep Spin Miniprep Kit (Qiagen). Lentivirus for the BCL6 BTB alanine-scan library was packaged using HEK293T cells.

Targeted BCL6 reporter screen in HEK293T cells

BI-3802 resistance alanine-scan screen

A total of 6 × 10^6 SuDHL4 cells were transduced with 5% (v/v) alanine-scan 1 or alanine-scan 2 libraries, and selected with 2 μg/ml of puromycin 24 h later. Forty-eight hours after infection, cells were treated with either DMSO or 1 μM BI-3802. Cells were split every 3–4 days for 21 days; 1 × 10^6 cells were collected for each time point, flash-frozen in dry ice and subsequently subjected to direct lysis buffer adding 1 × 10^6 cells/100 μl (1 mM CaCl₂, 3 mM MgCl₂, 1 mM EDTA, 1% Triton X-100, Tris pH 7.5) with freshly supplemented 0.2 mg/ml proteinase. A 20-μl volume of this mix was used for library amplifications in each sorted sample, resulting in 48 first PCR amplifications with 8 staggered primers in a 50 μl reaction volume (0.04U Titanium Taq (Takara Bio 639210), 0.5× Titanium Taq buffer, 0.5 μl dNTP mix, 200 mM P5-SBS3 forward primer, 200 nM SBS12-xPXR003 reverse primer), 94 °C for 5 min, 15 cycles of [94 °C for 30 s, 58 °C for 15 s, 72 °C for 30 s], 72 °C for 2 min. Two microliters of the first PCR reaction was used as the template for 15 cycles of the second PCR, in which Illumina adapters and barcodes were added (0.04U Titanium Taq (Takara Bio 639210), 1× Titanium Taq buffer, 0.8 μl dNTP mix, 200 nM SBS2-Stagger-xPXR003 forward primer, 200 nM P7-barcode-xSBS2 reverse primer). An equal amount of all samples was pooled and subjected to preparative agarose electrophoresis followed by gel purification (Qiagen). Eluted DNA was further purified by NaOAc and isopropanol precipitation. Amplified alanine-scan libraries were quantified by Illumina novaseq sp_100 platform with 123 cycles from SBS3 and 6 barcodes from SBS12 (Supplementary Data 2). The numbers of forward and reverse reads were combined and analysed as described below in ‘Data analysis of CRISPR–Cas9 knockout screens and alanine scans’.

BCL6 stability alanine-scan screen

A total of 6 × 10^6 HEK293T cells were transduced with 5% (v/v) alanine-scan 1 or alanine-scan 2 libraries and 24 h later were selected with 2 μg/ml of puromycin. Six days after infection cells were treated either with DMSO or 1 μM BI-3802 for 18 h and sorted using by fluorescence-activated cell sorting (FACS). Four populations were collected (top 5%, top 5–15%, bottom 5–15% and bottom 5%) on the basis of the eGFP–BCL6/mCherry ratio (Supplementary Fig. 2). For each condition, at least 100 × 10^6 cells were subjected to sorting.

GGCAACATCGGCTGTGAT-3’. The two libraries were amplified from the oligo pool by PCR with the NEBNext polymerase (NEB M0541, 98 °C for 30 s, 26 cycles of [98 °C for 10 s, 64 °C for 10 s, 72 °C for 6 min], 72 °C for 2 min).

The puromycin resistance cassette of the eGFP–BCL6(FL) construct was swapped to a neomycin resistance cassette (eGFP–BCL6(FL)–Neo). Five per cent (v/v) of the human genome-scale CRISPR-knockout Brunello library with 0.4 μl polybrene/ml was added to 440 × 10^6 HEK293Tbcl6 cells expressing eGFP–BCL6(FL)–Neo in 220 ml of RPMI medium. The culture was divided into three, replicated and transduced (2,400 rpm, 2 h, 37 °C). Twenty-four hours after infection sgRNA cells were selected with 2 μg/ml of puromycin for two days. On the seventh day, cells were treated with either DMSO or 1 μM BI-3802 and then sorted on day eight (Supplementary Fig. 2). Sorted cells were collected by centrifugation and subjected to direct lysis, library preparation and sequencing as specified above.

Genome-scale BI-3802 resistance screen in SuDHL4 cells

The pDONR-BCL6 plasmid backbone was amplified with NEBNext polymerase (NEB M0541, 98 °C for 30 s, 6 cycles of [98 °C for 10 s, 59 °C for 10 s, 72 °C for 150 s], 24 cycles of [98 °C for 10 s, 64 °C for 10 s, 72 °C for 150 s], 72 °C for 2 min), depurinated with Dpn1 (NEB) and purified by gel purification using the QiAquick Gel Extraction Kit (Qiagen). Libraries were transferred into Stbl3 chemically competent bacteria (Invitrogen) and plated on LB plates with carbenicillin for chemical selection. The resulting colonies were scraped, pooled and purified using the QIAprep Spin Miniprep Kit (Qiagen). To shuffle the alanine-scan library into the Artichoke expression backbone, 150 μg of the pDONR BCL6 BTB alanine-scan library and 150 μg of the gateway-pArtichoke vector were incubated overnight with LR clonase (Thermo Fisher Scientific) at room temperature. After Proteinase K treatment, salts were removed by dialysis (membrane filter, 0.025 μm pore size, Millipore). Libraries were transformed into Stbl3 chemically competent bacteria (Invitrogen) and plated on LB plates with carbenicillin for chemical selection. The resulting colonies were scraped, pooled and purified using the QIAprep Spin Miniprep Kit (Qiagen). Lentivirus for the BCL6 BTB alanine-scan library was packaged using HEK293T cells.

Genome-scale BI-3802 resistance screen in SuDHL4 cells

The resistance screen was performed similarly to the genome-scale BI-3802 reporter screen in HEK293T cells with the following modifications. For three replicates, 500 × 10^6 SuDHL4 cells in 200 ml of RPMI medium were transduced with 3.5 ml of the human genome-scale CRISPR KO Brunello library with 0.4 μl/ml polybrene. Twenty four hours after infection, cells were selected with 1 μg puromycin/ml for four days. Eight days after infection, cells were treated with either 1 μM BI-3802 or DMSO. The cells were then cultured for 20 more days until collection, with one split every 3–4 days, at which point fresh drug was added. Genomic DNA was purified with the QIAamp DNA Maxi kit (Qiagen) and up to 3 μg of DNA was submitted for multiple reactions: 94 °C for 2 min, 18 cycles of [94 °C for 30 s, 58 °C for 15 s, 72 °C for 30 s], 72 °C for 2 min.

Targeted BCL6 reporter screen in HEK293T cells

The BISON CRISPR library targets 713 E1, E2 and E3 ubiquitin ligases, deubiquitinases and control genes and contains 2,852 gRNAs. It was cloned into the pXPR003 vector as previously described. The virus for the library was produced in a F-175 flask format, as described above in ‘Lentivirus production’. Amplified alanine-scan libraries were quantified using the Illumina Novaseq platform (Supplementary Data 2).

Data analysis of CRISPR–Cas9 knockout screens and alanine scans

The CRISPR–Cas9 knockout screen and alanine-scan data were analysed using R (v.3.5.1) and Rstudio (v.1.1.453) with the following packages: tidyverse (v.1.2.1), ggrepel (v.0.8.1), GGally (v.1.4.0), and ShortReads (Bioconductor v.3.10). The data analysis pipeline comprised the following steps: (1) Reads per guide or alanine variant codon for each sample were normalized to the total number of reads across all samples for comparison. (2) For each guide or alanine variant codon, the ratio of reads in the stable versus the unstable sorted gate was calculated, which was then used to rank gRNAs or alanine variant codons. (3) The replicates were combined by summing up the ranks across replicates for each individual guide or alanine variant codon. (4) P-values were calculated by simulating a distribution with gRNAs or alanine variant codons that had randomly assigned ranks over 100 iterations (Supplementary Data 2). R scripts are available at

P values were calculated by simulating a distribution with gRNAs or alanine variant codons that had randomly assigned ranks over 100 iterations (Supplementary Data 2).
Individual validation of alanine-scan variants

The E41A, G55A, Y58A and C84A mutations in eGFP–BCL6(FL) were introduced by Q5 site-directed mutagenesis (NEB) in pDONR223-BCL6 and then shuffled into the Articchoke stability reporter. After the lentivirus production, SuDHL4<sub>carr</sub>, Raji<sub>carr</sub> and DEL<sub>carr</sub> cells were infected with the indicated BCL6 variants and treated with 1 μM BI-3802 or DMSO over 21 days. The percentage of mCherry-positive cells was monitored over time by flow cytometry.

Overexpression of SIAH1 in HEK293T cells

HEK293T<sub>carr</sub> cells expressing eGFP–BCL6(FL) were transfected with V5–SIAH1 or V5–SIAH1(C44S). Cells were trypsinized 72 h after infection and flow cytometry and mCherry expression quantified by flow cytometry (Supplementary Fig. 2). For construction of V5–SIAH1 expression vectors, inserts were PCR-amplified with attP sites and cloned into pDONR221 by a BP clonase reaction and then transferred into the pLEX_307 (Addgene 41392) expression vector by an LR clonase reaction. To construct V5–SIAH1(C44S), mutations were introduced by site-directed mutagenesis in pDONR221-SIAH1 and then transferred into pLEX_307 (Addgene 41392).

Co-immunoprecipitation

HEK293T<sub>carr</sub> cells expressing eGFP–BCL6(FL), eGFP–BCL6(FL:249–251/VSP>SA), eGFP–BCL6(1–250) and eGFP–BCL6(1–275) constructs were transfected with V5–SIAH1(C44S). A total of 1 x 10<sup>7</sup> cells were plated into 10-cm dishes, cultured for one day, treated with 0.5 μM MLN7249 for 2 h, and then treated with either 2 μM BI-3802 or DMSO for 1 h. The cells were collected and lysed in RIPa lysis buffer (Thermo Fischer Scientific, 89900) infused with protease inhibitor (Thermo Fischer Scientific, Halt Protease Inhibitor Cocktail 78438) for 30 min at 4 °C. BI-3802 (5 μM) was infused to all buffers used for the BI-3802-treated arm. Lysates were cleared by centrifugation (17,000g, 20 min, 4 °C). Twenty microlitres of pre-cleaned GFP-Trap magnetic agarose beads (Chromotek, gmta-20) were added to the lysates. The beads–lysate mixture was incubated at 4 °C for 30 min. Proteins were eluted in 2× sample mix (Promega, gmta-20) and ligated into linearized vector backbone. Constructs were transformed into XL10-Gold ultra-competent E. coli (Stratagene or Agilent Technologies) and plasmids were purified using the MiniPrep Kit (Qiagen) and validated by Sanger sequencing. Lentivirus was produced as described above. HEK293T<sub>carr</sub> or SuDHL4<sub>carr</sub> cells were transfected with sgRNAs. For BCL6 reporter assays, the effect of the knockdown was determined by quantifying the GFP/mCherry ratios in BFP or RFP657 positive and negative populations by flow cytometry seven days after infection. For competition assays, the percentage of BFP-positive cells was monitored over time by flow cytometry (Supplementary Fig. 2).

Isothermal titration calorimetry

All calorimetric experiments were carried out using an Affinity isothermal calorimeter from TA Instruments equipped with auto sampler in a buffer containing 20 mM HEPES, pH 7.5, 150 mM NaCl and 0.5 mM TCEP at 25 °C. For the BCL6–SIAH1 interaction, 25 μM BCL6(5–360) protein solution in the calorimetric cell was titrated by injecting 2 μl of 250 μM SIAH1(SBD) protein solution in 200-s intervals with stirring speed of 125 rpm. For the isolated BCL6 peptide (residues 241–260) and SIAH1 interaction, 25 μM SIAH1(SBD) protein solution in the calori- metric cell was titrated by injecting 2 μl of 250 μM BCL6(241–260) peptide solution in the same set-up. The resulting isotherm was fitted with a single-site model to yield a K<sub>d</sub> using NanoAnalyze software (TA instruments).

BCL6–SIAH1 TR-FRET

Titrations of compounds to induce the BCL6(5–360)–SIAH1 complex were carried out by mixing 200 nM biotinylated SIAH1(SBD), 200 nM Bodipy FL-labelled BCL6(5–360) variants and 2 nM terbium-coupled streptavidin (Invitrogen) in an assay buffer containing 50 mM Tris pH 8.0, 200 mM NaCl, 0.1% Pluronic F-68 solution (Sigma), 0.5% bovine serum albumin (BSA) (w/v) and 1 mM TCEP. After dispensing the assay mixture (15 μl volume), increasing concentrations of compounds were dispensed in a 384-well microplate (Corning, 4514) using a D300e Digital Dispenser (HP) normalized to 1% DMSO. After excitation of terbium fluorescence at 337 nm, emission at 490 nm (terbium) and 520 nm (Bodipy FL) were recorded with a 70-μs delay over 600 μs to reduce background fluorescence, and the reaction was followed over 60 cycles of each data point using a PHERAsart FS microplate reader (BMG Labtech). The TR-FRET signal of each data point was extracted by calculating the 520/490 nm ratio. The EC<sub>50</sub> values were estimated using dose–response analysis standard four-parameter log-logistic curves, fitted to the experimental data using the dr4pl R package.

Titrations of Bodipy FL-labelled BCL6(5–360) were performed by mixing 400 nM biotinylated SIAH1(SBD), 2 μM compounds or equivalent volume of DMSO, and 4 nM terbium-coupled streptavidin in the same assay buffer. After dispensing the assay mixture, an increasing concentration of Bodipy FL-labelled BCL6(5–360) was added to the SIAH1 mixture in a 1:1 volume ratio (7.5 μl each, total assay volume 15 μl). The 520/490 nm ratios were measured as described above and plotted to calculate the K<sub>d</sub> values using dose–response analysis standard four-parameter log-logistic curves using the dr4pl R package.

BCL6–BCoR compound-binding assay

Competitive titration of BI-3802 or BI-3812 was carried out by mixing 100 nM biotinylated BCL6(5–129), 100 nM N-terminal fluorescent isothiocyanate (FITC)-labelled BCoR peptide (sequence: RSEIISTAPSS-WVPPG) and 2 nM terbium-coupled streptavidin in the same assay buffer. After dispensing the assay mixture (15 μl volume), increasing concentrations of compounds were dispensed in a 384-well microplate (Corning, 4514) using a D300e Digital Dispenser (HP) normalized to 1% DMSO. The 520/490 nm ratios were measured as described above in the ‘BCL6–SIAH1 TR-FRET’ section and plotted to calculate the K<sub>d</sub> values using dose–response analysis standard four-parameter log-logistic curves using the dr4pl R package.

Bioluminescence resonance energy transfer analysis

Bioluminescence resonance energy transfer (BRET) experiments were performed using a NanoBRET PPI starter kit (Promega NIS21) according to the manufacturer’s instructions and as previously described<sup>43</sup>.
In vitro ubiquitination

In vitro ubiquitination for identification of compatible E2 conjugating enzymes was performed using a K-982 E2 Ubiquitin Conjugation Kit (Boston Biochem), following the manufacturer’s instructions, using Strep II-Avi-BCL6(5–360) and Flag–SIAH1(F1L). Time-course in vitro ubiquitination was performed by mixing the substrate (BCL6, 2 μM), E3 (SIAH, 0.3 μM), E1 (UBA1, Boston Biochem, 0.2 μM), E2 (UBE2D1, Boston Biochem, 0.5 μM), and ubiquitin (Boston Biochem, 50 μM) with a reaction buffer (B-71, Boston Biochem) containing BI-3802 or DMSO (normalized to 1% DMSO) in a 15 μl volume for each. Reactions were initiated by adding 5 μl of Mg-ATP solution (B-20, Boston Biochem), incubated for up to 60 min at 37 °C and analysed by western blot using a strep-tag II antibody–HRP conjugate (71591-3 Sigma) at 1:4,000.

Immunofluorescence

HEK293T cells expressing eGFP–BCL6(1–250) and eGFP–BCL6(1–275) constructs were transduced with V5–SIAH(C44S) (infection rate > 70%). A total of 0.1 × 10⁶ cells were plated per chamber of a four-well chamber slide, cultured overnight and pre-treated with 0.5 μM MLN7243 for 2 h, followed by treatment with either DMSO or 2 μM BI-3802 for 1 h. The cells were fixed with 4% formaldehyde for 15 min and permeabilized with 0.1% Triton X100 for 30 min. Epitopes were blocked with 10% BSA for 10 min. Anti-V5 antibodies were added and incubated on slides overnight at 4 °C. After removal of the primary antibodies and washes, Alexa-Fluor 633-conjugated anti-mouse antibodies were added and incubated at room temperature for 45 min. Finally, the slides were stained with DAPI (BD Biosciences, 564907, 1:5,000 in H2O) and mounted with SlowFade Diamond Antifade Mountant (Thermo Fisher Scientific, S36963). Cells were imaged with the Leica TCS SPE confocal microscope.

Statistics and reproducibility

The n numbers denoted in the figure legends refer to independent experiments after genetic perturbation or drug treatment, which were also performed as independent replicates for each experiment. For micrographs, immunoblots and microscopy images, a representative image from n replicates is shown. For pooled CRISPR-knockout screens and alanine scans, n denotes independent experiments with four unique sgRNAs per gene or four codons per substitution for the alanine scan that were infected in a pool but then treated separately throughout the screen. No statistical methods were used to predetermine sample size. The experiments were not randomized and the investigators were not blinded to allocation during experiments and outcome assessment.

Reporting summary

Further information on research design is available in the Nature Research Reporting Summary linked to this paper.

Data availability

Structural data have been deposited to the Electron Microscopy Data Bank (EMDB; EMDB-22265) and the RCSB PDB (6XM4). Proteome quantification data are available in the PRIDE repository (https://www.ebi.ac.uk/pride/archive; PXD016185). Uncropped gel and western blot data are shown in Supplementary Fig. 1, and the flow cytometry gating strategy is shown in Supplementary Fig. 2.

Code availability

The scripts used for modelling and analysis in this study are available on Github (https://github.com/fischerlab/scripts-publications/tree/master/2020_BCL6_polymerization).

Additional information

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Extended Data Fig. 1 | Characterization of BI-3802-induced BCL6 degradation. a, Immunoblots of BCL6 levels in cytoplasmic, nuclear or chromatin-bound fractions of SuDHL4Cas9 cells after treatment with DMSO or 1 μM BI-3802 for 24 h (n = 2). b, mRNA levels quantified by qPCR in SuDHL4Cas9 cells after treatment with 1 μM BI-3802 or DMSO for 1 h (bars represent mean and s.d., n = 3). c, Whole-proteome quantification of SuDHL4Cas9 cells treated with 1 μM BI-3812 (n = 1) or DMSO (n = 3) for 4 h (two-sided moderated t-test, n = 3). d, Immunoblots of BCL6 levels in SuDHL4Cas9 cells treated with 10 μM MG132 (26S proteasome inhibitor) for 1 h, 1 μM BI-3802 for 45 min or 10 μM BI-3812 for 10 min. A subset of the polymerized BCL6 was insoluble and lost during the western blot sample preparation, however, treatment with an excess of BI-3812 shortly before protein collection reverted polymerization, solubilized BCL6 and allowed for reliable quantification (n = 2). e, Immunoblots of BCL6 levels in SuDHL4Cas9 cells treated with DMSO, 10 μM MLN7243 (ubiquitin activating enzyme inhibitor), 10 μM MG132 (26S proteasome inhibitor), 10 μM chloroquine (lysosomal inhibitor) or 5 μM MLN4924 (neddylation inhibitor) for 15 min; then, for indicated samples, 1 μM BI-3802 was added and 35 min later, 10 μM BI-3812 was added for the final 10 min, resulting in a total of 1 h treatment with MLN7243, MG132, chloroquine or MLN4924, 45 min with BI-3802 and 10 min with BI-3812 (n = 2). f, Cytospin immunofluorescence images of SuDHL4Cas9 cells treated with DMSO (left) or 0.5 μM MLN7243 for 2 h and 1 μM BI-3802 for 1 h. Scale bar, 5 μm (n = 2). g, Flow cytometry analysis of HEK293TCas9 cells expressing eGFP-BCL6(1–275) that were exposed simultaneously to the indicated concentrations of BI-3802 and BI-3812 for 24 h. Lines represent standard four-parameter log-logistic curve fit (n = 3).
Extended Data Fig. 2 | Computational docking of BCL6 helical filament models with distinct binding modes. Visualization of top-scoring BCL6 BTB domain filament model from three different binding modes: end-to-end (E2E), face-to-end (F2E) and face-to-face (F2F). Each BTB monomer used for building the tetramer model is labelled in a distinct colour. BI-3802 is visualized as a sphere. The interface score is an estimate of the binding energy between the dimers. The helical pitch was calculated by extending the tetramer. Sub-angstrom variations in the F2F binding mode have a profound effect on helical pitch (more than 10 nm).
Extended Data Fig. 3 | Structure determination of BCL6 filaments by cryo-EM.  

a, Representative cryo-EM micrograph at −2 μm defocus. Micrograph was low-pass-filtered. Scale bar, 100 nm. 
b, Local-resolution map of the final reconstruction with a threshold of 0.0154 (Chimera) calculated using RELION v.3.0. 
c, Data-processing scheme for the BCL6 filaments. Iterative 2D classifications resulted in 274,999 particles. Multiple subsequent rounds of 3D classification, refinement, and polishing improved map resolution to a final overall resolution of 3.7 Å. Percentages refer to the particles in each class. 
d, FSC plots for unmasked and masked maps. Overall resolution is indicated at FSC = 0.143. 
e, Histogram and directional FSC plot for BCL6 cryo-EM map. 
f, g, Regions of the cryo-EM model for the BCL6 filament fit into the density map, demonstrating side-chain density for multiple residues. Each density is shown at a threshold of 0.0178 (from Chimera). 

Red density maps indicate the classes that were used for the next round of processing, and blue density maps are from 3D refinements.
Extended Data Fig. 4 | Structural details of BI-3802-induced BCL6 filaments.

**a**, Density for BI-3802 in the 3.7Å cryo-EM reconstruction. The crystal structure of BCL6 bound to BI-3802 (PDB 5MW2) was docked into the cryo-EM map and refined using phenix.real_space_refine. The cryo-EM density is shown in grey at a threshold of 0.0178 (from Chimera).

**b**, Density of BI-3802 and key interacting residues (Arg28, Glu41, Tyr58, Cys84) for BCL6 polymerization. Each density in mesh is shown at a threshold of 0.0178 (from Chimera).

**c, d**, Comparison of the cryo-EM model of polymerized BCL6 (white) with the BCL6 crystallographic lattice (yellow, PDB 5MW2) for dimer–dimer (c), and filament (d).

**e**, Superimposed structures of BI-3802 (yellow) and BI-3812 (orange) bound to the BCL6 filament. BI-3812 was docked to the crystal structure of BCL6 BTB (PDB 5MW2), which was then aligned to the BI-3802-mediated BCL6 filament model. The solvent-exposed moiety of the inhibitor is clashing with the adjacent BCL6 dimer (grey).

**f**, Preassembled 0.1 μM FITC-labelled BCoR peptide and 0.1 μM biotinylated BCL6(5–129) variants were treated with an increasing concentration of BI-3802, and the signal was measured by TR-FRET. The interaction of BCL6 with the BCoR co-repressor peptide was used to quantitively determine drug binding. Lines represent standard four-parameter log-logistic curve fit (n = 3).
**Extended Data Fig. 5** | Analysis of BCL6 BTB variants in vivo. 

**a**, Schematic of alanine mutagenesis resistance screen of the BCL6 BTB domain in SuDHL4 Cas9 cells. 

**b**, Schematic of alanine mutagenesis reporter screen of the BCL6 BTB domain in HEK293T Cas9 cells. 

**c**, Alanine mutagenesis screen of the BCL6 BTB domain for impaired BI-3802 induced degradation at 1 μM BI-3802 in HEK293T Cas9 cells. Mutations that confer resistance are labelled. Four different codons were collapsed to each unique amino acid position (greater than threefold enrichment, \( P < 10^{-4}; n = 2 \); four codons per position; two-sided empirical rank-sum test-statistics). 

**d**, Correlation of BCL6 mRNA expression (transcripts per million (TPM)) and BCL6 dependency (CERES score) in a set of 559 cancer cell lines from the Dependency Map Project. Cell lines chosen for experiments are labelled. 

**e**, SuDH4Cas9, RajiCas9 (both BCL6-dependent) and DELCas9 (BCL6-independent) cells were infected with the indicated BCL6 variants and treated with 1 μM BI-3802 or DMSO over 21 days. Lines represent measurement from each replicate \( (n = 2) \). 

**f**, BI-3802 in the polymerization interface. Residues identified in the alanine scan are highlighted, with the following colour code: orange, Gly55, Tyr58 (residues involved in drug binding); magenta, Glu41, Cys84 (residues involved in polymerization). Hydrogen atoms in Gly55 are depicted as spheres.
Extended Data Fig. 6 | Genome-wide CRISPR–Cas9 screens to identify the molecular machinery involved in BI-3802-induced degradation of BCL6.

**a.** Schematic of the BCL6 stability reporter-based sorting screen.

**b, c.** Genome-wide CRISPR–Cas9 knockout screen for eGFP–BCL6 stability in HEK293T Cas9 cells after 16 h of treatment with 1 μM BI-3802 or DMSO. Results for SIAH1 and FBXO11 (a previously reported E3 ligase involved in BCL6 endogenous degradation) are labelled. Guides were collapsed to gene level ($n = 3$; four guides per gene; two-sided empirical rank-sum test-statistics).

**d.** Normalized read counts in each sorted gate for 4 sgRNAs targeting SIAH1 and 4,000 non-targeting controls (NTC). Symbols indicate the mean normalized read numbers for each sgRNA ($n = 3$).

**e.** Flow cytometry analysis of HEK293TCas9 cells expressing the full-length eGFP–BCL6 reporter and individual sgRNAs after 4 h treatment with DMSO or 1 μM BI-3802. Bars represent mean ($n = 3$).

**f.** Schematic of the genome-wide CRISPR–Cas9 resistance screen.

**g.** Genome-wide CRISPR–Cas9 knockout screen for resistance to BI-3802. Guides were collapsed to gene level ($n = 3$; four guides per gene; two-sided empirical rank-sum test-statistics).

**h.** Flow cytometry analysis of SuDHL4Cas9 cells expressing sgRNAs and blue fluorescent protein (marker) treated with DMSO or 1 μM BI-3802. Lines represent measurement from each replicate ($n = 3$).
Extended Data Fig. 7 | SIAH1 induces degradation of BCL6 through the VxP motif. 

**a**, Flow cytometry analysis of HEK293T<sub>Cas9</sub> cells expressing full-length eGFP–BCL6 stability reporter and vectors expressing no-insert control, SIAH1 or SIAH1(C44S), treated with DMSO or BI-3802 for 2 h. Bars represent the mean (n = 3). 

**b**, Alignment of the BCL6 SIAH1-recognition site with previously published peptide sequences recognized by SIAH1 with inferred consensus SIAH1-binding site. 

**c**, CRISPR–Cas9 knockout screen with the Bison library for eGFP–BCL6(1–129 + 241–260) stability in HEK293T<sub>Cas9</sub> cells after 16 h of treatment with 1 μM BI-3802 or DMSO. Guides were collapsed to gene level (n = 1; four guides per gene; two-sided empirical rank-sum test-statistics). 

**d**, Flow cytometry analysis of HEK293T<sub>Cas9</sub> cells expressing eGFP–BCL6(FL) or eGFP–BCL6(FL;VSP>GSA) treated with DMSO or 1 μM BI-3802 for 7 h (bars represent mean, n = 3).
Extended Data Fig. 8 | Characterization of SIAH1-mediated degradation of polymerized BCL6. 

a, SDS–PAGE gel analysis of the in vitro pull-down between recombinant SIAH1(SBD) and recombinant Strept–BCL6 in the presence of BI-3802 or DMSO. Strept, strep•Tag II (n = 2). b, Titration of BCL6(241–260) peptide binding to SIAH1(SBD) using isothermal calorimetry (n = 1). c, Titration of SIAH1(SBD) binding to BCL6(5–360) using isothermal calorimetry (n = 1). d, Recombinant strep•Tag II–BCL6(5–360) was combined with full length SIAH1 and a panel of E2 enzymes (Boston Biochem) and screened for ubiquitination activity in vitro. Samples were analysed by western blot and visualized by strep•Tag II antibody–HRP conjugate (n = 1). e, Bodipy-labelled BCL6(5–360) variants (WT, E41A, Y58A) were titrated to 0.2 μM biotinylated SIAH1(SBD) in the presence of 2 μM BI-3802, and the signal was measured by TR-FRET. Dots represent mean. Lines represent standard four-parameter log-logistic curve fit (n = 3). f, Preassembled 0.2 μM Bodipy-labelled BCL6(5–360) and 0.2 μM biotinylated SIAH1(SBD) were treated with an increasing concentration of BI-3802 or BI-3812, and the signal was measured by TR-FRET. Dots represent mean. Lines represent standard four-parameter log-logistic curve fit (n = 3). g, HEK293T cells transiently transfected with nano-luciferase-tagged SIAH1(C44S) and HaloTag-labelled BCL6 constructs were treated with DMSO, 1 μM BI-3802 or 1 μM BI-3812 for 2 h and the mBRET signal was measured. Bars represent mean (n = 3). One-sided t test. h, Preassembled 0.1 μM FITC-labelled BcoR peptide and 0.1 μM biotinylated BCL6(5–129) were treated with an increasing concentration of BI-3802 or BI-3812, and the signal was measured by TR-FRET. Lines represent standard four-parameter log-logistic curve fit (n = 3). i, HEK293TCas9 cells expressing the eGFP–BCL6(1–250) stability reporter and V5–SIAH1 were treated with 0.5 μM MLN7243 for 2 h and 1 μM BI-3802 for 1 h. Cells were imaged by indirect immunofluorescence as indicated. Scale bar, 5 μm (n = 2).
Extended Data Table 1 | Cryo-EM data collection, refinement and validation statistics

| Data collection and processing | Polymerized BCL6 bound to BI-3802 (EMD-22265) |
|--------------------------------|-----------------------------------------------|
| Magnification                 | 105,000                                       |
| Voltage (kV)                  | 300                                           |
| Electron exposure (e-/Å²)      | 63.4                                          |
| Defocus range (µm)            | -1 to -2.5                                    |
| Pixel size (Å)                | 0.825                                         |
| Symmetry imposed              | C1                                            |
| Initial particle images (no.) | 1,610,413                                     |
| Final particle images (no.)   | 112,048                                       |
| Map resolution (Å)            | 3.7                                           |
| FSC threshold                 | 0.143                                         |
| Map resolution range (Å)      | 3.65 to 5.25                                  |

**Refinement**

| Initial model used (PDB code) | 8 x 5MW2                                      |
| Model resolution (Å)          | 8.1<sup>a</sup>                               |
| FSC threshold                 | 0.5                                           |
| Model resolution range (Å)    | 3.65 to 5.25                                  |
| Map sharpening B-factor (Å²)  | -98.3                                         |
| Model composition             |                                               |
| Non-hydrogen atoms            | 8072                                          |
| Protein residues              | 976                                           |
| Ligands                       | 8                                             |
| B factors (Å²)                |                                               |
| Protein                       | 23.42                                         |
| Ligand                        | 22.21                                         |
| R.m.s. deviations             |                                               |
| Bond lengths (Å)              | 0.012                                         |
| Bond angles (°)               | 1.112                                         |
| Validation                    |                                               |
| MolProbity score              | 1.36                                          |
| Clashscore                    | 6.48                                          |
| Poor rotamers (%)             | 0.89                                          |
| Ramachandran plot             |                                               |
| Favored (%)                   | 98.33                                         |
| Allowed (%)                   | 1.67                                          |
| Disallowed (%)                | 0                                             |

<sup>a</sup>Strongly preferred orientation causes a dip in the model versus the map FSC, leading to low model resolution (8.1 Å) estimation at FSC = 0.5.
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Last updated by author(s): Aug 12, 2020

Reporting Summary

Nature Research wishes to improve the reproducibility of the work that we publish. This form provides structure for consistency and transparency in reporting. For further information on Nature Research policies, see our Editorial Policies and the Editorial Policy Checklist.

Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

- The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement
- A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
- The statistical test(s) used AND whether they are one- or two-sided
- A description of all covariates tested
- A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
- A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
- For null hypothesis testing, the test statistic (e.g. F, t, r) with confidence intervals, effect sizes, degrees of freedom and P value noted
- Give P values as exact values whenever suitable.
- For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
- For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
- Estimates of effect sizes (e.g. Cohen's d, Pearson's r), indicating how they were calculated

Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection
- Proteins were identified and quantified using Proteome Discoverer 2.2 (Thermo Fisher Scientific): RRID:SCR_014477
- QPCR signal was quantified with QStudio 6 FLX real-Time PCR System (Thermo Fisher Scientific).
- Western blot data were imaged on the Odyssey Imaging System, Image Studio (Li-Cor).
- Microscopy images were acquired on a DeltaVision Ultra High-Resolution Microscope (GE Healthcare) or a TCS SP5 confocal microscope (Leica).
- The luminescent BRET signals were acquired on a EnVision Multilabel Plate Reader (Perkin Elmer).
- TR-FRET signals were acquired on a PHERAstar FS microplate reader (BMG Labtech).
- Negative stain images were collected on a JEM-1400 (Plus) Transmission Electron Microscope (Jeol).
- Cryo movies were collected on a Titan Krios Transmission Electron Microscope (Thermo Fisher Scientific).
- Flow data were collected on BD FACSDiva 8.0 (BD Biosciences) or CytExpert Software 2.3 (Beckman Coulter Life Sciences).

Data analysis
- Bioinformatic, CRISPR screen data analyses and data visualization were done using the R programming language (3.5.1) and RStudio (1.1.453) with the following packages: tidyverse (1.2.1), ggrepel (0.8.1), GGally (1.4.0), do4pl (1.1.11), ShortReads (Bioconductor 3.10) and Statistical Analysis Limma Package 3.42.2 (Bioconductor 3.0). Custom R scripts were used to analyze the data, which are attached as Supplementary Code.
- Flow data were analyzed with FlowJo v10 (BD).
- Microscopy images were processed with Fiji/ImageJ (2.0.0-rc-69/1.52p) or softWoRx® 7.0.0 and combined to make movies using QuickTime.
- Structural data from cryo-EM were processed with Relion 3.0, crYOLO 1.6.1, Phenix 1.17.1-3660, Chimera 1.14, PyMOL 2.3, COOT 0.8.9.2-pre-revision-7884. Computational modeling data were processed with RosettaDock 4.0, Rosetta 3.12, HELFIT (only one version exists).
- All scripts used in this study have been uploaded in the github: https://github.com/fischerlab/scripts-publications/tree/master/2020_BCL6_polymerization

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors and reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research guidelines for submitting code & software for further information.
Data

Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A list of figures that have associated raw data
- A description of any restrictions on data availability

Structural data have been deposited to the EMDB and RCSB (EMD-22265, PDB-6XMX). SMW2 was downloaded from the Protein Data Bank (PDB). Proteome quantification data are available in the PRIDE repository (PXD016185). Functional genomics data, uncropped gels, western blot source data, and the flow cytometry gating strategy can be found in the Supplementary Information.

Figures that have associated raw data:

Fig. 1b and Extended Data Fig. 1c are associated with the proteome quantification data.
Fig. 2b, Fig. 3a and Extended Data Fig. 5d, 6b, 6c, 6g are associated with the functional genomics data.
Extended Data Fig. 5e is associated with data from the Dependency Map Project (https://depmap.org/).

Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

- Life sciences
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Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size

No sample size calculation was performed. The sample size (n) of each experiment is provided in the figure captions. Each quantitative experiment was performed in at least 3 replicates, each qualitative experiment was performed in at least two replicates. The expected effect sizes were large therefore 3 replicates were fully sufficient (e.g. degraded versus non-degraded).

Data exclusions

No data was excluded from the analysis.

Replication

The "n" denoted in the figure legends refer to independent experiments following genetic perturbation or drug treatment, which were also performed as independent replicates for each experiment. For micrographs, immunoblots, and microscopy images, a representative image from n replicates is shown. For pooled CRISPR-knockout screens and alanine scans, n denotes independent experiments with 4 unique sgRNAs/gene or 4 codons/substitution for the alanine scan that were infected in a pool but then treated separately throughout the screen. All replicates experiments were consistent across multiple replicates on different days.

Randomization

No randomization was performed. Internal controls were employed for quantitative comparisons and the proteomics and functional genomics experiments were performed in a pooled setup – which is intrinsically randomized (i.e. all sgRNAs are expressed in random cells depending on which cell was infected with which sgRNA containing lentivirus).

Blinding

Investigators were not blinded during data collection or analysis. However, controls and samples were analyzed in exactly the same way using the same computational pipeline. Due to the nature of pooled experiments (proteomics and functional genomics), the outcome of the experiment was blinded.

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

| n/a | Involved in the study |
|-----|----------------------|
| ☒ Antibodies |
| ☒ Eukaryotic cell lines |
| ☒ Palaeontology and archaeology |
| ☒ Animals and other organisms |
| ☒ Human research participants |
| ☒ Clinical data |
| ☒ Dual use research of concern |

Methods

| n/a | Involved in the study |
|-----|----------------------|
| ☒ ChIP-seq |
| ☒ Flow cytometry |
| ☒ MRI-based neuroimaging |
Antibodies

The following antibodies were used in this study: anti-BCL6 (Santa Cruz Biotechnology, sc-7388, dilution 1:200), anti-beta-tubulin (Cell Signaling, 21465, dilution 1:1000), anti-Hsp90 (Cell Signaling, 4875S, dilution 1:1000), anti-HDAC1 (Cell Signaling, 2062S, dilution 1:1000), anti-Histone H3 (Cell Signaling, 12648S, dilution 1:2000), anti-eGFP (Cell Signaling, 2956, dilution 1:1000), anti-V5-tag (ThermoFisher Scientific, MA5-15253, dilution 1:1000), anti-Strep tag II-HRP conjugate (Sigma, 71591-3, dilution 1:4000), anti-mouse Alexa Fluor 633 (ThermoFisher Scientific, A-21052, dilution 1:500), Alexa anti-mouse 488 (Biolegend, 405319, dilution 1:500).

Validation

All antibodies are commercially available and were tested by their manufacturer.

- anti-BCL6 (Santa Cruz Biotechnology, sc-7388) was tested by the manufacturer by near-infrared western blot analysis of Bcl-6 expression in Ramos, U-698-M, Raji, BJAB and NAMALWA whole cell lysates. [https://datasheets.scbt.com/sc-7388.pdf](https://datasheets.scbt.com/sc-7388.pdf)
- anti-beta-tubulin (Cell Signaling, 21465) was tested by the manufacturer by western blot analysis of extracts from various cell lines using β-tubulin Antibody. [https://www.cellsignal.com/products/primary-antibodies/b-tubulin-antibody/2146](https://www.cellsignal.com/products/primary-antibodies/b-tubulin-antibody/2146)
- anti-Hsp90 (Cell Signaling, 4875S) was tested by the manufacturer by western blot analysis of extracts from various cell lines. [https://www.cellsignal.com/products/primary-antibodies/hsp90-antibody/4874](https://www.cellsignal.com/products/primary-antibodies/hsp90-antibody/4874)
- anti-HDAC1 (Cell Signaling, 2062S) was tested by the manufacturer by western blot analysis of COS or HeLa cell lysates. [https://www.cellsignal.com/products/primary-antibodies/histone-deacetylase-1-hdac1-antibody/2062](https://www.cellsignal.com/products/primary-antibodies/histone-deacetylase-1-hdac1-antibody/2062)
- anti-Histone H3 (Cell Signaling, 12648S) was tested by the manufacturer by western blot analysis of extracts from various cell lines. [https://www.cellsignal.com/products/primary-antibodies/histone-h3-d1h2-xp-rabbit-mab-hrp-conjugate/12648](https://www.cellsignal.com/products/primary-antibodies/histone-h3-d1h2-xp-rabbit-mab-hrp-conjugate/12648)
- anti-eGFP (Cell Signaling, 2956) was tested by the manufacturer by western blot analysis of extracts from HCC827 cells, untransfected or GFP-transfected. [https://www.cellsignal.com/products/primary-antibodies/gfp-d5-1-rabbit-mab/2956](https://www.cellsignal.com/products/primary-antibodies/gfp-d5-1-rabbit-mab/2956)
- anti-V5-tag (ThermoFisher Scientific, MA5-15253) was tested by the manufacturer by western blot analysis of V5 Epitope Tag was performed from E.coli lysate. [https://www.thermofisher.com/antibody/product/V5-Tag-Antibody-clone-E10-V4RR-Monoclonal/MA5-15253-HP](https://www.thermofisher.com/antibody/product/V5-Tag-Antibody-clone-E10-V4RR-Monoclonal/MA5-15253-HP)
- anti-Strep tag II-HRP conjugate (Sigma, 71591-3) was tested by the manufacturer by a dot blot detecting < 5 ng of GFP-Strep. [https://ca.vwr.com/assetsvc/asset/en_CA/id/10003428/contents](https://ca.vwr.com/assetsvc/asset/en_CA/id/10003428/contents)
- anti-Mouse 800CW (LI-COR Biosciences, 926-32211) was tested by the manufacturer by western blot of cellular fractions from Stauroporine (STS) treated and non-treated HeLa cells. [https://www.licor.com/bio/reagents/irdye-800cw-goat-anti-mouse-igg-secondary-antibody](https://www.licor.com/bio/reagents/irdye-800cw-goat-anti-mouse-igg-secondary-antibody)
- anti-Rabbit 680LT (LI-COR Biosciences, 925-68021) was tested by the manufacturer by western blot detection of p38 in Jurkat lysate. [https://www.licor.com/bio/reagents/irdye-680lt-goat-anti-rabbit-igg-secondary-antibody](https://www.licor.com/bio/reagents/irdye-680lt-goat-anti-rabbit-igg-secondary-antibody)
- anti-mouse Alexa Fluor 633 (ThermoFisher Scientific, A-21052) was tested by the manufacturer by immunofluorescence analysis of MCF-7 cells stained with Cytokeratin 19 (RCK108) Mouse Monoclonal Primary Antibody. [https://www.thermofisher.com/antibody/product/Goat-anti-Mouse-IgG-H-L-Highly-Cross-Adsorbed-Secondary-Antibody-Polyclonal-A-21052](https://www.thermofisher.com/antibody/product/Goat-anti-Mouse-IgG-H-L-Highly-Cross-Adsorbed-Secondary-Antibody-Polyclonal-A-21052)
- Alexa anti-mouse 488 (Biolegend, 405319) was tested by the manufacturer by immunofluorescence of frozen mouse intestine stained with purified anti-Ankyrin-B. [https://www.biolegend.com/de-de/products/alexa-fluor-488-goat-anti-mouse-igg-minimal-x-reactivity-9282](https://www.biolegend.com/de-de/products/alexa-fluor-488-goat-anti-mouse-igg-minimal-x-reactivity-9282)

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)

All cell lines mentioned in the manuscript, except for HEK293T cells used for viral packaging, are versions of the cell line that stably express Cas9. The human HEK293T, SuDHHL4-Cas9, Raji-Cas9, and DEL-Cas9 cell lines were provided by the Genetic Perturbation Platform, Broad Institute (please refer to reference 30 in the methods section on how Cas9 was initially introduced in the cell lines by the Genetic Perturbation Platform). The HEK293T-Cas9 cell line was previously published (see methods section and reference 12).

Authentication

The HEK293T, HEK293T-Cas9 and SuDHHL4-Cas9 cell lines were authenticated by short tandem repeat (STR) profiling. Raji-Cas9, DEL-Cas9 were not authenticated.

Mycoplasma contamination

Mycoplasma negative.
Commonly misidentified lines
(See ICLAC register)
None of commonly misidentified lines were used in this study.

Flow Cytometry

Plots

Confirm that:

☑ The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).

☐ The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a 'group' is an analysis of identical markers).

☑ All plots are contour plots with outliers or pseudocolor plots.

☐ A numerical value for number of cells or percentage (with statistics) is provided.

Methodology

Sample preparation
Adherent cells were trypsinized, collected, and the cell pellets resuspended in PBS. Suspension cells were washed with PBS or directly subjected to analysis without fixation.

Instrument
Cytoflex LX (Beckman), MA900 Cell Sorter (Sony), Fortessa FACS (BD Biosciences), FACSCanto (BD Biosciences).

Software
BD FACSDiva 8.0 (BD Biosciences), CytExpert Software (Beckman), FlowJo 10 (BD).

Cell population abundance
Round cells (population with forward and side scatter properties consistent with the alive, non-treated cell line) were usually > 50% in most measurements (rarely lower due to drug toxicity), singlets were > 90%. For reporter assays, mCherry positive cells > 50%.

Gating strategy
Cells were first gated for live cells based on forward and side scatter. Single cells were discriminated based on the area vs. height of the side scatter. Finally, reporter positive cells were gated based on the mCherry expression.

☑ Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information.