The Oncoprotein BCL6 Enables Cancer Cells to Evade Genotoxic Stress

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1 Abstract

2 Genotoxic agents remain the mainstay of cancer treatment. Unfortunately, the clinical
3 benefits are often countered by a rapid tumor adaptive response. Here, we report that the
4 oncoprotein B cell lymphoma 6 (BCL6) is a core component that confers tumor adaptive
5 resistance to genotoxic stress. Multiple genotoxic agents promoted BCL6 transactivation,
6 which was positively correlated with a weakened therapeutic efficacy and a worse clinical
7 outcome. Mechanistically, we discovered that treatment with the genotoxic agent
8 etoposide led to the transcriptional reprogramming of multiple pro-inflammatory cytokines,
9 among which the interferon- α and interferon- γ responses were selectively and
10 substantially enriched in resistant cells. Our results further revealed that the activation of
11 interferon/signal transducer and activator of transcription 1 axis directly upregulated BCL6
12 expression. The increased expression of BCL6 further repressed the tumor suppressor
13 PTEN and consequently enabled resistant cancer cell survival. Accordingly, targeted
14 inhibition of BCL6 remarkably enhanced etoposide-triggered DNA damage and apoptosis
15 both *in vitro* and *in vivo*. Our findings highlight the importance of BCL6 signaling in
16 conquering tumor tolerance to genotoxic stress, further establishing a rationale for a
17 combined approach with genotoxic agents and BCL6-targeted therapy.

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1 Introduction

2 Genome instability is the major hallmark of chronic proliferating tumors (Hanahan &
3 Weinberg, 2011; Murai, Thomas, Miettinen, & Pommier, 2019). Conventional
4 genotoxic chemotherapy (e.g., topoisomerase II inhibitors, cisplatin, carboplatin) that
5 introduce DNA damage lesions, devastate genomic integrity and activate pro-apoptotic
6 pathways, are employed as the standard first-line treatment for a wide array of solid
7 malignancies (Cheung-Ong, Giaever, & Nislow, 2013). Despite initial therapeutic success,
8 intrinsic resistance or rapid adaptive resistance in cancer cells is a major hurdle,
9 hampering the clinical efficacy of these agents (O'Grady et al., 2014; Stebbing et al., 2018;
10 Trinh, Ko, Barengo, Lin, & Naora, 2013). Chemoresistance occurs due to complex
11 reasons, such as an increased DNA damage repair capacity, activation of pro-survival
12 pathways, and defects in caspase activity (Poth et al., 2010; Stebbing et al., 2018). While
13 several signaling effectors have been identified as predictive markers, such as *ABCA1*
14 (Koh et al., 2019) and *MAST1* (Jin et al., 2018), in tumor tolerance to genotoxic agents,
15 the majority of these studies lacked either an evaluation of the clinical correlation or an
16 explanation for how these effectors mediate pro-survival signals in the presence of
17 genotoxic stress.

18 The transcriptional repressor B cell lymphoma 6 (BCL6) has emerged as a critical
19 therapeutic target in diffuse large B-cell lymphomas (Parekh, Prive, & Melnick, 2008).
20 Increasing evidences indicate that BCL6 plays an oncogenic role in several human
21 hematopoietic malignancies and solid tumors (Beguelin et al., 2016; Cardenas et al., 2017;
22 Deb et al., 2017). BCL6 binds and represses different target genes to drive tumorigenesis

1 in a cell context-dependent manner (Ci et al., 2009). The constitutive expression of BCL6
2 sustains the lymphoma phenotype and promotes glioblastoma through transcriptional
3 repression of the DNA damage sensor ATR (Ranuncolo et al., 2007) and the p53 pathway
4 (Xu et al., 2017), respectively. According to data derived from The Cancer Genome Atlas
5 (TCGA), the BCL6 locus is also predominantly amplified in primary breast cancer and is
6 correlated with a worse prognosis (Walker et al., 2015). Recently, small molecular
7 inhibitors that target the interaction between BCL6 and its co-repressors or that trigger
8 BCL6 degradation effectively restored BCL6 target gene expression and impeded tumor
9 growth (Cardenas et al., 2016; Cheng et al., 2018; Slabicki et al., 2020).

10 The properties of BCL6 as a therapeutic target originate from its normal function in
11 sustaining the proliferative and the phenotype of stress-tolerant germinal center B cells
12 (Phan, Saito, Kitagawa, Means, & Dalla-Favera, 2007). BCL6 allows B cells to evade
13 ATR-mediated checkpoints and tolerate exogenous DNA damage by repressing the cell
14 cycle checkpoint genes *CDKN1A*, *CDKN1B*, and *CDKN2B*, and the DNA damage sensing
15 genes *TP53*, *CHEK1*, and *ATR* (Basso et al., 2010; Cardenas et al., 2017; Phan, Saito,
16 Basso, Niu, & Dalla-Favera, 2005). When genotoxic stress is accumulated to some extent,
17 BCL6 is phosphorylated by the DNA damage sensor ATM kinase and degraded through
18 the ubiquitin proteasome system, whereby the germinal center reaction is terminated
19 (Phan et al., 2007). The critical functions exerted by BCL6 during normal B cell
20 development could be hijacked by malignant transformation, thereby leading to lymphoma
21 (Basso & Dalla-Favera, 2012). Recent studies have suggested that BCL6 is involved in
22 stress tolerance and drug responses. In detail, BCL6 can be activated by heat shock

1 factor 1 to tolerate heat stress (Fernando et al., 2019). The aberrant expression of BCL6
2 can be provoked in leukemia cells in response to the tyrosine kinase inhibitor imatinib
3 (Duy et al., 2011). Our recent work additionally revealed that an increased expression of
4 BCL6 largely contributes to the resistance of *KRAS*-mutant lung cancer clinical BET
5 inhibitors (Guo et al., 2021). Given the fact that BCL6 plays an emerging role in DNA
6 damage tolerance and drug responses, we hypothesized that BCL6 might drive cancer
7 cell resistance to genotoxic agents.

8 Here, we report that the proto-oncogene BCL6 is a central component of the
9 resistance pathway in tumor response to genotoxic agents. We observed a striking
10 association between the activation of pro-inflammatory signals and BCL6 induction in
11 chemoresistant cancer cells. The tumor suppressor PTEN is further characterized as a
12 functional target gene of BCL6. Importantly, addition of BCL6-targeted therapy to the
13 genotoxic agent etoposide markedly restored the sensitivity of cancer cells to etoposide *in*
14 *vitro* and *in vivo*. Overall, our findings establish a rationale for targeting BCL6 to conquer
15 resistance to genotoxic stress in solid tumors.

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1 Results

2 Genotoxic agents promote BCL6 transcription.

3 While genotoxic agents have become the mainstay of clinical cancer treatments
 4 (Fillmore et al., 2015; Nitiss, 2009), many patients show a poor response to these drugs
 5 due to the emergence of a tumor rapid adaptive response (Wijdeven et al., 2015). To gain
 6 a comprehensive understanding of chemoresistance mechanisms, we initially measured
 7 the half inhibitory concentrations (IC₅₀s) of etoposide and doxorubicin, two well-validated
 8 topoisomerase II inhibitors for clinical use, in a panel of 22 cancer cell lines derived from
 9 four types of solid tumors, including lung, pancreatic, colorectal, and ovarian carcinomas.
 10 Some cell lines displayed apparent resistance to etoposide at doses up to 30 μ M (**Figure**
 11 **1A**) or to doxorubicin at doses up to 0.6 μ M (**Figure 1-figure supplement 1A**), while the
 12 remaining cell lines showed a gradient of sensitivity to them. The concentrations of 30 μ M
 13 and 0.6 μ M were chosen to define the resistance of multiple cancer cell lines to etoposide
 14 and doxorubicin, respectively, as these are the highest achievable concentration in the
 15 plasma of patients, which are likely to be clinically relevant (Kaul et al., 1995; Palle et al.,
 16 2006).

17 To decipher the mechanisms of tumor resistance to genotoxic therapy, we first
 18 performed RNA sequencing in etoposide-resistant cells (Capan-2 and H661) and
 19 etoposide-sensitive cells (PC9) in the presence or absence of etoposide. An in-depth
 20 comparison of the transcriptome was conducted to describe the transcriptional programs
 21 that were responsive to etoposide in sensitive cells but remained recalcitrant to treatment
 22 in a resistant population. By analyzing the significantly differentially expressed genes, we

1 strikingly found that etoposide treatment triggered a remarkable increase in BCL6
2 expression in etoposide-resistant Capan-2 and H661 cells, but not in etoposide-sensitive
3 PC9 cells (**Figure 1B**). Given that BCL6 signaling gene sets have not been fully defined in
4 solid tumors, several studies have focused on BCL6 transcriptional program (Ci et al.,
5 2009; Green et al., 2014). In addition to the well-known BCL6 target genes or
6 co-repressors in germinal centers and multiple tumors, such as *BMI1*, *EIF4E*, *NOTCH2*
7 and *BCL2* (Basso et al., 2010; Cerchietti et al., 2010; Ci et al., 2009; Dupont et al., 2016;
8 Valls et al., 2017), several other genes directly regulated by BCL6 have been recently
9 identified using chromatin immunoprecipitation followed by sequencing, including
10 BCL6-activated genes (e.g., *SYK*, *BANK1*, *BLK*, and *MERTK*) or BCL6-repressed genes
11 (e.g., *CDKN2C*, *CDKN1B*, *RB1*, and *PTPRO*) (Geng et al., 2015). We used comparative
12 BCL6 target gene selection to identify the genes that were differentially expressed
13 between resistant and sensitive cells in the presence or absence of etoposide. Our data
14 revealed that the BCL6 transcriptional program was dramatically affected by etoposide in
15 treated Capan-2 and H661 cells, but not in treated PC9 cells (**Figure 1B**). We further
16 verified the specificity of BCL6 increase in other etoposide-resistant cell lines (**Figure 1C**)
17 and the effects of etoposide on BCL6 target gene expression using qPCR analysis
18 (**Figure 1D**). Given that BCL6 transcription was induced in primary chemoresistant cells,
19 we next tested whether it could be provoked in acquired chemoresistant cells. Therefore,
20 we analyzed published microarray data (Januchowski, Zawierucha, Rucinski, Nowicki, &
21 Zabel, 2014; Moitra et al., 2012), and found that BCL6 upregulation was also observed in
22 acquired resistance process (**Figure 1E**)

1 To clarify whether the fact that transcriptional induction of BCL6 confers tolerance to
2 genotoxic stress was a general phenomenon, we treated five cell lines with a panel of
3 frontline genotoxic agents (Ettinger et al., 2017; Sandler et al., 2006; Tempero et al., 2017).
4 The results showed that BCL6 was upregulated in response to the majority of these
5 clinical agents (**Figure 1F** and **Figure 1-figure supplement 1B**). In addition, a high
6 expression of BCL6 was associated with a poor progression-free survival in patients who
7 received cisplatin, taxol, or both drugs (**Figure 1G**). These results collectively suggest that
8 an aberrant BCL6 expression might contribute to chemoresistance and is linked to a poor
9 prognosis.

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BCL6 transactivation is correlated with therapy resistance.

12 To further identify whether an increased BCL6 expression was associated with the
13 therapeutic efficacy of genotoxic agents, we first examined BCL6 protein expression in a
14 panel of solid tumor cell lines treated with etoposide or doxorubicin. In agreement with the
15 BCL6 transcription pattern observed in **Figure 1C**, BCL6 protein abundance was
16 dramatically and preferentially induced by etoposide in resistant cells, whereas it was
17 decreased or unchanged in sensitive cells (**Figure 2-figure supplement 1A**). Notably,
18 increased BCL6 protein levels were closely associated with increased etoposide IC₅₀
19 values (**Figure 2A**). Specifically, cells with higher BCL6 protein levels were prone to be
20 more tolerant to etoposide ($R^2 = 0.61$, $P < 0.0001$; **Figure 2B**). Similar results were also
21 obtained for doxorubicin (**Figure 2-figure supplement 1B**).

22 A more detailed observation demonstrated that BCL6 protein expression could also

1 be time-dependently provoked by a long-term exposure of resistant cells to etoposide
 2 (**Figure 2C**). This prompted us to examine the responsive role of BCL6 *in vivo*. Therefore,
 3 we set up a xenograft mouse model using human HCT116 cells and examined the BCL6
 4 expression shift in xenografts after etoposide treatment. Although etoposide impeded
 5 tumor growth at a dose of 10 mg/kg/day (**Figure 2-figure supplement 1C**), the protein
 6 level of phosphorylated H2AX, a DNA damage marker (Bonner et al., 2008), was overall
 7 decreased in etoposide-treated xenografts compared with that in the vehicle group
 8 (**Figure 2D**), implying the emergence of drug resistance. In contrast with the decreased
 9 level of phosphorylated H2AX, the BCL6 protein levels in the xenografts were dramatically
 10 increased by etoposide, which was consistent with our *in vitro* observations (**Figure 2C**),
 11 suggesting that a reciprocal alteration of BCL6 expression is associated with tumor
 12 responses to genotoxic agents.

13 Next, to examine whether BCL6 transactivation affects drug efficacy in resistant cells,
 14 we targeted BCL6 using two different small interfering RNAs and found that BCL6 genetic
 15 knockdown dramatically attenuated the clonogenic growth of HCT116 cells in the
 16 presence of etoposide (**Figure 2E**). In line with these results, inducible knockdown of
 17 BCL6 potentiated the killing effects of etoposide (**Figure 2F**). In addition, we
 18 overexpressed BCL6 using a lentiviral vector in etoposide-sensitive H522 cells and tested
 19 the cytotoxicity of etoposide. As expected, our results showed that BCL6 overexpression
 20 increased the etoposide IC₅₀ by up to 17-fold (**Figure 2G**). To further investigate the role
 21 of BCL6 in adaptive resistance, we introduced siBCL6 into acquired doxorubicin-resistant
 22 cells, and found that BCL6 depletion was sufficient to suppress cell proliferation of

1 MCF7/ADR cells (**Figure 2H**). Collectively, these data support the notion that BCL6
2 confers drug resistance and induces a targetable vulnerability in tumor cells.

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4 **Genotoxic stress activates interferon responses.**

5 To further elucidate the mechanisms of BCL6 feedback activation, we conducted
6 gene ontology enrichment analysis on the transcripts that were significantly activated by
7 the genotoxic agent etoposide. Intriguingly, the differentially genes related to inflammatory
8 and immune responses were enriched in resistant Capan-2 cells (**Figure 3-figure**
9 **supplement 1A**), raising the possibility that pro-inflammatory factors may play a causal
10 role in conferring chemoresistance. Gene set enrichment analysis further demonstrated a
11 significant upregulation of genes associated with interferon-alpha (IFN- α) response,
12 inflammatory response, and interferon-gamma (IFN- γ) response in etoposide-resistant
13 cells (**Figure 3, A-C**). Along with BCL6 upregulation, the expression of IFN
14 signaling-related genes was significantly increased accordingly (**Figure 3, D-E**).

15 Recent work has revealed that consistent DNA damage triggers an inflammatory
16 cytokine secretory phenotype in cultured cells (Rodier et al., 2009). To corroborate
17 whether IFN- α and IFN- γ were similarly induced because of genotoxic agents, we
18 assayed the gene expression of IFN- α and IFN- γ in treated cells. Our results showed that
19 etoposide exposure resulted in an evident upregulation of IFN- α (**Figure 3F**) and IFN- γ
20 transcription (**Figure 3G**) in etoposide-resistant H661, Capan-2, and PANC28 cells, but
21 not in etoposide-sensitive H522 cells. We further examined the cellular production of
22 IFN- α and IFN- γ in treated cells using a direct enzyme-linked immunosorbent assay, and

1 found that etoposide treatment evoked a significant increase in IFN- α and IFN- γ contents
2 in resistant cells (**Figure 3, H-I**).

3 Interferon regulatory factor 1 (IRF1), a key transcription factor that regulates cell
4 proliferation and immune responses, is an inducible gene of type I and type II interferon
5 (Castellaneta et al., 2014; Dery et al., 2014). To explore the effects of etoposide on IFN
6 signaling, we examined IRF1 expression in resistant cells. We found that etoposide not
7 only triggered a notable increase in IRF1 transcription itself, but also dramatically
8 enhanced IFN- α - and IFN- γ -induced IRF1 expression in resistant cells (**Figure 3, J-K**),
9 indicating the potent effect of etoposide on cellular interferon responses.

10 We next investigated the biological significance of IFN upregulation in the process of
11 tumor adaptive response to genotoxic agents. Our results showed that exogenous
12 addition of IFN- α and IFN- γ protected sensitive cells from etoposide-induced cell death
13 (**Figure 3, L-M**). In contrast, siRNA knockdown of the IFN- α receptor IFNAR1 led to an
14 enhanced sensitivity of resistant cells to etoposide, as indicated by impaired clonogenic
15 growth (**Figure 3N**) and decreased etoposide IC₅₀ values (**Figure 3-figure supplement**
16 **1B**). In line with these observations, antibodies against IFN- γ increased the killing ability of
17 etoposide towards resistant cells (**Figure 3O** and **Figure 3-figure supplement 1C**).
18 These results indicate that IFN activation provoked by genotoxic stress promotes tumor
19 cell survival, leading to a tumor resistance phenotype.

21 **The interferon/STAT1 axis directly regulates BCL6 expression.**

22 Accumulating evidences show that IFNs produce pro-survival effects and mediate

1 non-immune resistance to chemotherapy primarily through the transcriptional factor
2 STAT1 (Khodarev et al., 2007; Minn, 2015). Following this direction, we examined STAT1
3 expression in treated cells and found that etoposide treatment promoted STAT1 protein
4 abundance in etoposide-resistant PANC28 and HCT116 cells, but not in sensitive H522
5 cells (**Figure 4A**). Furthermore, genetic knockdown of STAT1 synergized with genotoxic
6 agents to inhibit the clonogenic growth of resistant cells (**Figure 4B**). These results
7 collectively suggest that the interferon/STAT1 axis is required for the therapeutic efficacy
8 of etoposide and plays an essential role in tumor response to genotoxic stress.

9 Activated STAT1 drives an interferon-related gene signature for DNA damage
10 tolerance (Minn, 2015), which prompted us to hypothesize that the interferon/STAT1 axis
11 might regulate BCL6 expression. Given that IFN- γ activated IFN-stimulated gene
12 expression specifically through the classical Janus kinase/STAT1 signaling, we first
13 incubated resistant cells with 5 or 10 ng/mL recombinant IFN- γ , and found that IFN- γ
14 significantly evoked a simultaneous increase in STAT1 and BCL6 protein expression
15 (**Figure 4C**), implying that these two factors might be functionally linked. When noted,
16 IFN- γ at the same concentrations evidently triggered BCL6 mRNA expression (**Figure 4D**).
17 To further clarify the role of interferon signaling in modulating BCL6 expression, we treated
18 resistant cells with etoposide in combination with IFN- α or IFN- γ , respectively. Our results
19 showed that etoposide-mediated BCL6 transactivation could be further enhanced in
20 etoposide-resistant H838 cells (**Figure 4, E-F**) and Capan-2 cells (**Figure 4-figure**
21 **supplement 1A-B**) by the addition of IFN- α or IFN- γ . Moreover, etoposide induced STAT1
22 and BCL6 protein expression in resistant cells, whereas these effects could be potentiated

1 by IFN- α (**Figure 4G**) or IFN- γ addition (**Figure 4H**), implying that etoposide-induced type
2 1 and type 2 interferon responses are required for STAT1 and BCL6 activation. Importantly,
3 an increased expression of BCL6 by etoposide was apparently suppressed by STAT1
4 genetic silencing (**Figure 4I**). These results collectively suggest that etoposide
5 transactivates BCL6 primarily through the interferon/STAT1 signaling pathway.

6 To elucidate the regulatory link of STAT1 on BCL6, we silenced STAT1 and found that
7 STAT1 knockdown led to a marked decrease in BCL6 protein expression (**Figure 4J**),
8 while STAT1 overexpression apparently increased BCL6 protein abundance (**Figure 4K**),
9 implying that STAT1 may be upstream of BCL6. To elucidate whether STAT1 is a direct
10 regulator of BCL6, we constructed a whole BCL6 promoter luciferase reporter and found
11 that STAT1 interference resulted in a decreased BCL6 reporter activity (**Figure 4L**). Our
12 chromatin immunoprecipitation coupled with qPCR analysis further revealed the
13 recruitment of STAT1 to three putative binding regions of the BCL6 locus (**Figure 4M**).
14 These results reinforced the direct regulation of the interferon/STAT1 signaling pathway
15 on BCL6 expression.

16

The tumor suppressor PTEN is a functional target of BCL6.

18 After characterizing STAT1 as an upstream regulator of BCL6, we next explored
19 BCL6 downstream signaling responsible for adaptive response to genotoxic stress.
20 Considering two lines of evidences showing that: (1) phosphatase and tensin homolog
21 (PTEN), a lipid phosphatase that antagonizes the phosphatidylinositol 3-kinase pathway
22 (Lee, Chen, & Pandolfi, 2018), was enriched in BCL6 promoter binding peaks in primary

germinal center B cells (Ci et al., 2009), and that (2) BCL6 directly binds to the promoter locus of PTEN in patient-derived acute lymphoblastic leukemia (Geng et al., 2015), we hypothesized that an increase in BCL6 expression by genotoxic stress might inhibit PTEN and subsequently promote cell survival. To this end, we performed transcriptome analysis and found an evident decrease in PTEN expression in Capan-2 and H661 cells exposed to etoposide (**Figure 5A**). The analysis of datasets from TCGA further revealed that PTEN deletion was mutually exclusive with BCL6 amplification (**Figure 5B**). Furthermore, our qPCR (**Figure 5C**) and immunoblotting analysis (**Figure 5D**) showed that the upregulation of BCL6 was accompanied by a decreased expression of PTEN at both the mRNA and protein levels. To further support the notion that BCL6 repressed the expression of PTEN, we overexpressed BCL6 and observed a significant decrease in PTEN (**Figure 5E**). In contrast, doxycycline-inducible knockdown of BCL6 increased PTEN expression (**Figure 5F**). Our ChIP-qPCR data further revealed that etoposide treatment significantly increased the occupancy of BCL6 at the promoter region of *PTEN* (**Figure 5G**). These results indicated that PTEN is a functional target of BCL6 and largely contributes to genotoxic stress tolerance in tumor cells.

It is well-known that PTEN acts as a tumor suppressor and hampers the activation of the proto-oncogenic mTOR pathway (Martelli et al., 2011). We further explored the effects of etoposide treatment on the mTOR signaling. Our immunoblotting results showed that phosphorylation of mTOR (S2448), S6K (T389) and S6 (S235/S236) was strikingly increased, along with an aberrant BCL6 expression in etoposide-treated resistant cells (**Figure 5H**). Similar results were also obtained in a long-term drug exposure assay

1 (Figure 5I). Notably, overexpression of PTEN enhanced the antitumor effects of etoposide
2 (Figure 5J). These results collectively suggest that the PTEN/mTOR pathway is a
3 downstream signaling of BCL6.

4

5 **BCL6 inhibition conquers resistance of cancer cells to genotoxic stress *in vitro*.**

6 Since tumor adaptive resistance to genotoxic stress was attributed to BCL6
7 transactivation, we tested whether pharmacological inhibition of BCL6 could restore the
8 sensitivity of resistant cancer cells to genotoxic agents. We suppressed BCL6's function
9 using two BCL6 pharmacological inhibitors, BI3802 and compound 7. BI3802 was
10 reported as a BCL6 degrader (Kerres et al., 2017; Slabicki et al., 2020), while compound 7
11 targeted the BCL6 BTB/POZ domain and prevented its partner binding (Kamada et al.,
12 2017). Our results showed that multiple resistant cell lines became vulnerable to
13 etoposide in the presence of BI3802 or compound 7 (Figure 6A). In addition, BI3802
14 addition could shift the IC₅₀ values of doxorubicin (Figure 6-figure supplement 1A).
15 Moreover, combination index values (CIs) were further employed to indicate drug synergy,
16 and our results showed that the majority of CIs at 50%, 75% and 90% of the effective dose
17 of each drug pair (etoposide plus BI3802, or etoposide plus compound 7) in five resistant
18 cell lines were all lower than 1 (Figure 6B), displaying a synergistic action of etoposide
19 and BCL6-targeted therapy. We further assessed the combined effects of etoposide and
20 the BCL6 inhibitor BI3802 in a long-term colony-formation assay. Our results showed that
21 the combination of etoposide and BI3802 led to a robust growth inhibition of cultured
22 colonies (Figure 6C). As expected, addition of BI3802 markedly enhanced the inhibitory

1 effects of etoposide on soft-agar colony formation (**Figure 6D**). A combinative synergy
2 was also obtained for doxorubicin and targeted BCL6 inhibition (**Figure 6-figure**
3 **supplement 1B-C**). All these data indicate that BCL6 blockage could restore the
4 sensitivity of cancer cells to genotoxic agents.

5 DNA damage potency triggered by genotoxic agents is a determinant of tumor
6 response to chemotherapy (Bouwman & Jonkers, 2012; Pearl, Schierz, Ward, Al-Lazikani,
7 & Pearl, 2015). The accumulation of DNA damage further causes genome instability and
8 consequently triggers cell apoptosis (Broustas & Lieberman, 2014). The fact that BCL6
9 upregulation was associated with reduced phosphorylated H2AX levels in HCT116
10 xenografts (**Figure 2E**) prompted us to explore whether the targeted inhibition of BCL6
11 could promote DNA damage in the presence of genotoxic agents. Our results showed that
12 the combined regimen of etoposide and BI3802 resulted in more poly (ADP-ribose)
13 polymerase cleavage and a higher phosphorylated H2AX expression (Ser139) than single
14 agent alone (**Figure 6E**). In addition, more DNA damage occurred as indicated by a
15 significantly higher tail moment observed in a comet assay in the combined treatment
16 group (**Figure 6F**). Consequently, an increase in the number of apoptotic cells was
17 observed in the drug pair group (**Figure 6G**). Taken together, these data suggest that
18 BCL6 blockade potentiates genotoxic agents by inducing DNA damage and growth
19 inhibition.

20 21 **Targeted inhibition of BCL6 sensitizes genotoxic agents *in vivo*.**

22 We next investigated whether our combined therapeutic approach is effective in

1 tumor preclinical mouse models. BI3802 was reported to possess a poor bioavailability
2 (Kerres et al., 2017). Therefore, we applied FX1, another BCL6 pharmacological inhibitor,
3 which disrupts the interaction between BCL6 and co-repressors with satisfactory
4 antitumor effects *in vivo* (Beguelin et al., 2016; Cardenas et al., 2016). We first set up a
5 xenograft mouse model using HCT116 cells. Once the average volume of xenografts
6 reached ~100 mm³, mice were treated with etoposide or the vehicle. We found that BCL6
7 was upregulated at both mRNA and protein levels in xenografts as early as 2 days after
8 drug administration, and intriguingly, this effect was sustained during the treatment period
9 (**Figure 7A**), implying the emergence of a resistance phenotype. Strikingly, the addition of
10 FX1 from day 2 significantly enhanced the therapeutic potency of etoposide, as indicated
11 by a decreased tumor volume and tumor weight (**Figure 7B**). Most importantly,
12 administration of 10 mg/kg etoposide and 5 mg/kg FX1 was well-tolerated in mice since
13 the levels of blood biochemical indicators were marginally affected (**Supplementary**
14 **Table 4**).

15 Immunoblot analysis of tumor lysates revealed a marked increase in p-mTOR
16 (S2448), p-P70S6K (T389), and p-S6 (S235/S236) expression levels in etoposide-treated
17 xenografts (**Figure 7C**), whereas addition of FX1 suppressed the activation of the mTOR
18 signaling pathway. Immunohistochemistry analysis additionally showed an increase in
19 p-H2AX (S139) expression and weaker Ki-67 signals in the xenografts from the drug pair
20 group (**Figure 7D**), suggesting a fundamental role of BCL6-targeted therapy in sensitizing
21 etoposide *in vivo*.

22 To evaluate the antitumor activity of FX1+ etoposide in a more clinically relevant

1 mouse model, we established a patient-derived xenograft model of lung adenocarcinoma
 2 harboring a G12V mutation in KRAS (LACPDx). Our results showed that the combination
 3 of etoposide and FX1 significantly suppressed tumor weight and tumor volume compared
 4 with single agent alone (**Figure 7E**), without causing systemic toxicity in mice
 5 (**Supplementary Table 5**). In agreement, addition of FX1 markedly decreased p-S6
 6 (S235/S236) expression and increased p-H2AX (S139) expression in LACPDx (**Figure**
 7 **7F**). These results collectively suggest that BCL6 is a crucial combinatorial target in the
 8 sensitization of resistant tumors to genotoxic agents *in vivo*.

9

10 Discussion

11 The exploration of underlying resistance mechanisms of genotoxic agents may allow
 12 the prediction of patient responses, the design of rational combination therapies and the
 13 implementation of re-sensitization strategies. Here, we show that BCL6 upregulation is a
 14 prominent mechanism to protect tumor cells from genotoxic killing. Our current findings
 15 support the notion that BCL6 functions as a central factor in mediating therapy resistance:
 16 (1) the interferon/STAT1 pathway serves as an upstream regulator of BCL6, (2) the tumor
 17 suppressor PTEN is identified as a functional target of BCL6, (3) the activation of BCL6
 18 signaling leads to a sustained pro-survival phenotype, whereas blocking it enhances the
 19 therapeutic efficacy of genotoxic agents. Our findings further establish a rationale for the
 20 concurrent targeting of BCL6 to conquer tumor tolerance to genotoxic stress, as
 21 evidenced by the striking synergy of genotoxic therapy and BCL6-targeted therapy *in vitro*
 22 and *in vivo* (**Figure 6** and **Figure 7**).

1 BCL6 acts as a gatekeeper to protect germinal center B cells from undergoing
2 somatic hypermutation and class-switch recombination against DNA damage (Duy et al.,
3 2010; Polo, Ci, Licht, & Melnick, 2008). In this study, we showed, for the first time, that
4 BCL6 was markedly upregulated by genotoxic agents in both *in vitro* and *in vivo* settings,
5 leading to a resistance phenotype (**Figure 1** and **Figure 2**). Furthermore, high BCL6
6 levels were positively associated with unfavorable clinical outcomes (**Figure 1G**). Our
7 results were conceptually in line with recent findings showing that BCL6 enabled heat
8 stress tolerance in vertebrates (Fernando et al., 2019) and conferred tyrosine kinase
9 inhibitor resistance in Ph⁺ acute lymphoblastic leukemia (Duy et al., 2011). As reported in
10 our recent work (Guo et al., 2021), BCL6 activation attenuated the antitumor efficacy of
11 clinical BET inhibitors in *KRAS*-mutant lung cancers. Combining these findings together,
12 we speculate that BCL6 may functionally program tumor pro-survival signals in drug
13 response and can be used as a predictive biomarker for therapy resistance. As an
14 essential transcription repressor, BCL6 suppresses rapid proliferation and survival of
15 germinal center B cells by recruiting co-repressors, such as BCOR, NCOR and SMRT, to
16 its BTB domain (Huang, Hatzi, & Melnick, 2013). Therapy targeting the BCL6 BTB domain
17 lateral groove displayed inhibitory effects in the treatment of lymphoma (Cheng et al.,
18 2018). Based on the substantial role of BCL6 in the tumor adaptive response to drug
19 treatments, we assessed the therapeutic efficacy of BCL6-targeted therapy in
20 combination with etoposide, which markedly strengthened DNA damage and tumor
21 growth inhibition (**Figure 6** and **Figure 7**), without causing obvious toxicity in mice,
22 providing a combinatorial strategy with translational significance.

1 BCL6 upregulation is required for maintaining B cells in germinal center
2 compartments (Basso & Dalla-Favera, 2012). Once expressed in B cells, BCL6 is tightly
3 controlled through an auto-regulatory circuit model, in which BCL6 negatively regulates its
4 own transcription by binding to its gene promoter (Kikuchi et al., 2000; Pasqualucci et al.,
5 2003). We recently reported that BRD3 maintained the auto-regulatory circuit of BCL6 by
6 directly interacting with BCL6. Aberrant genomic or expressional changes of BCL6 have
7 been detected in lymphomas and multiple solid tumors, including breast cancer,
8 glioblastoma or ovarian cancer (Walker et al., 2015; Y. Q. Wang et al., 2015; Xu et al.,
9 2017). Limited lines of evidence have revealed that the transcriptional factor STAT5
10 serves as a direct negative regulator of BCL6 in lymphomas (Walker, Nelson, & Frank,
11 2007), and FoxO3a promoted BCL6 expression in leukemia cells exposed to BCR-ABL
12 inhibitors (Duy et al., 2011; Fernandez de Mattos et al., 2004). However, the
13 transcriptional regulation pattern of BCL6 in solid tumors remains unexplored. Our
14 findings demonstrated that the genotoxic agent etoposide activated the interferon/STAT1
15 signaling axis, which directly upregulated BCL6 by recruiting STAT1 to the binding regions
16 of the BCL6 locus (**Figure 3** and **Figure 4**). The phenomenon that BCL6 could be
17 transactivated by STAT1 was partially observed in imatinib-treated chronic myeloid
18 leukemia cells (Madapura et al., 2017). These findings collectively suggest that BCL6 may
19 be concisely and dynamically regulated by a unique mechanism in the specific tumor
20 context.

21 While numerous cell-intrinsic processes are known to play critical roles in tumor
22 response to genotoxic agents, increasing attention has been paid to multiple cell-extrinsic

1 components of the tumor microenvironment that influence the malignant phenotype and
2 disease progression. During DNA damage, the production of cellular mitogenic growth
3 factors and proteases, such as HGF, EGF, and MMP, are programmed to facilitate tumor
4 growth (Bavik et al., 2006; Coppe et al., 2008). In addition to these pro-survival molecules,
5 the production of pro-inflammatory cytokines (e.g., IL6) provoked by chemotherapy, will
6 promote anti-apoptotic signaling and intrinsic chemo-resistance (Gilbert & Hemann, 2010;
7 Poth et al., 2010). In this study, we showed that, in response to genotoxic stress,
8 etoposide-resistant cells rapidly increased the production of IFN- α and IFN- γ , and more
9 importantly, the increase in IFNs was sufficient to protect cells from genotoxic killing
10 (**Figure 3**). These findings support the essential role of IFNs in the tumor
11 microenvironment of conferring drug resistance, along with the fact that the IFN-related
12 DNA damage resistance signature acts as a predictive marker for chemotherapy (Post et
13 al., 2018; Weichselbaum et al., 2008). Our results further delineate a mechanism by which
14 increased production of IFN- α or IFN- γ facilitated cancer cells to evade genotoxic stress
15 by activating the transcriptional factor STAT1 (**Figure 4**). Although genotoxic
16 therapy-induced damage to the tumor microenvironment promotes treatment resistance
17 through cell nonautonomous effects (Sun et al., 2012), whether targeting the biologically
18 notable upregulation of IFNs in conjunction with conventional therapy could enhance the
19 treatment response still requires additional experimentation.

20 The BCL6 transcriptional program for the direct silencing of multiple target genes has
21 been elaborated in primary B cells and lymphoma (Ci et al., 2009). However, few target
22 genes of BCL6 have been characterized in solid tumors. Our study identified PTEN, the

1 most frequently mutated tumor suppressor (Lee et al., 2018), as a functional target of
2 BCL6 in therapy resistance (**Figure 5**). We showed that the overexpression of BCL6
3 suppressed PTEN, while the knockdown of BCL6 increased the expression of PTEN
4 (**Figure 5, E-F**). Furthermore, the combination of BCL6 inhibitors and genotoxic agents
5 resulted in a marked suppression of the PTEN downstream component mTOR *in vivo*
6 (**Figure 7, C-D**), reinforcing that mTOR activation is an actionable mechanism that
7 confers drug resistance (Tanaka et al., 2011). When acting as a transcriptional repressor,
8 the BCL6 BTB domain recruits the co-repressors NCOR, SMRT, and BCOR (Ghetu et al.,
9 2008). The mechanism by which BCL6 mediated the repression of PTEN and whether this
10 action is dependent on the BCL6 BTB domain still requires further investigation.

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1 **Methods**

2 **Cell lines and culture.** H1975 (RRID:CVCL_1511), PC9 (RRID:CVCL_B260),
3 H661(RRID:CVCL_1577), H522 (RRID:CVCL_1567), HCC827 (RRID:CVCL_2063),
4 H838 (RRID:CVCL_1594), DLD-1 (RRID:CVCL_0248), HT-29 (RRID:CVCL_0320),
5 HCT-8 (RRID:CVCL_2478), HCT116 (RRID:CVCL_0291), LoVo (RRID:CVCL_0399),
6 AsPC-1 (RRID:CVCL_0152), BxPC-3 (RRID:CVCL_0186), Capan-2 (RRID:CVCL_0026),
7 PANC28 (RRID:CVCL_3917), ES-2 (RRID:CVCL_3509), OVCAR8 (RRID:CVCL_1629),
8 OVCA420 (RRID:CVCL_3935), HEY (RRID:CVCL_2Z96) and HEYA8 (RRID:CVCL_8878)
9 were purchased from the American Type Culture Collection (Manassas, VA, USA).
10 PANC-1 (RRID:CVCL_0480) and MIA PaCa-2 (RRID:CVCL_HA89) were purchased from
11 the Shanghai Cell Bank of the Chinese Academy of Sciences (Shanghai, China). All cell
12 lines were maintained in the appropriate culture medium supplemented with 10% fetal
13 bovine serum and 100 U/mL penicillin/streptomycin. Experiments were performed with
14 cells under 15 passages. All cell lines were authenticated by STR analysis and routinely
15 tested for *mycoplasma* by using the Mycoalert Detection Kit (Beyotime, Jiangsu, China).
16 The culture medium of cell lines is listed in **Supplementary Table 1**.

17
18 **Plasmids and reagents.** The inducible BCL6 shRNA vectors were generated based on a
19 pLVX-TetOne-Puro vector (RRID: Addgene 124797) according to standard protocols. All
20 constructs were verified by sequencing. shRNAs sequence targeting BCL6 are available
21 in **Supplementary Table 2**. Recombinant human IFN- α 1 (z02866) was purchased from
22 Genscript (Nanjing, China). Recombinant IFN- γ (300-02) and anti-human IFN- γ antibody

(506532) were purchased from PeproTech (Rocky Hill, USA). Etoposide (HY-13629, a topoisomerase II inhibitor), doxorubicin (HY-15142, a topoisomerase II inhibitor), cisplatin (HY-17394, a DNA synthesis inhibitor), carboplatin (HY-17393, a DNA synthesis inhibitor), taxol (HY-B0015, a microtubule association inhibitor) and gemcitabine (HY-17026, a DNA synthesis inhibitor) were purchased from MedChemExpress (Monmouth Junction, USA).

6

Cell viability assay. Cell viability was assessed using the sulforhodamine B (SRB) assay.

Cells (2,000 - 5,000 cells per well) were seeded onto 96-well plates in appropriate cell culture medium, allowed to attach overnight, and treated with the indicated drug concentrations. Approximately 48 h later, the cells were fixed in 50% trichloroacetic acid at 4°C for 1 h, stained with 0.4% SRB, and dissolved in a 10 mM Tris solution. The absorbance (optical density, OD) was read at a wavelength of 515 nm. The IC₅₀ values were calculated using GraphPad Prism 8.0 (RRID:SCR_002798), and the CI values were evaluated using CalcuSyn software (Version 2; Biosoft).

15

Two-dimensional clonogenic assay. Cells (1,000-2,000 cells per well) were seeded onto 12-well plates. After 24 h, cells were treated with the indicated drug for about 7 - 10 days. When grown into visible clones, the cells were washed with phosphate-buffered saline (PBS), fixed with 4% paraformaldehyde, stained with 0.2% crystal violet and photographed. Stained cells were then dissolved in 10% acetic acid. The absorbance of the stained solution was read at a wavelength of 595 nm in a 96-well plate. The relative viability was calculated by setting that of untreated cells as 100%.

1

2 **Soft-agar colony formation assay.** The soft-agar colony formation assay was conducted
3 to evaluate the inhibitory effects of different treatments on the anchorage-independent
4 growth of tumor cells. The bottom layer of soft agar was prepared by mixing 2 × growth
5 medium and 1.5% noble agar (BD Biosciences, San Jose, CA) at a 1:1 ratio and the
6 mixture was poured into 6-well plates. Cells (1,000 - 2,000 cells per well) were suspended
7 in the second soft agar layer that contained 0.5% low melting point agar mixed with growth
8 media and spread over the bottom layer. After solidification, the growth medium was
9 added into each well. After incubation for 5-7 days, cells were treated with various drugs
10 for 10-15 days. When grew into visible clones, cells were imaged using a fluorescence
11 microscope and counted to evaluate cell viability.

12

13 **Cell apoptosis assays.** Cell apoptosis was quantified using flow cytometry (FACSCalibur,
14 BD) as described previously (Elkabets et al., 2013). For cell apoptosis, the cells exposed
15 to drugs for the indicated times were washed twice with PBS, re-suspended in 400-500 µL
16 of 1× binding buffer (BD), and stained with 5 µL of Annexin V–FITC and 5 µL of propidium
17 iodide (PI, Sigma; 5 µg/mL) for 15 min at room temperature in the dark. Cells were
18 detected using flow cytometry (FACS Calibur, BD) and quantitative analysis was carried
19 out using FlowJo software (RRID:SCR_008520).

20

21 **RNA interference.** For siRNA transfection, the cells were plated at a confluence of
22 approximately 40%-60% in basal culture medium and transfected with siRNA duplex

1 using Lipofectamine TM 2000 reagent (ThermoFisher Scientific) according to the
2 manufacturer's instructions for 6 h. After that, the medium of the transfected cells were
3 replaced by complete medium, and the cells were plated into wells and exposed to the
4 drugs. Western blotting was applied to detect the interference efficiency of target genes.

5

6 **RNA isolation and RT-qPCR analysis.** Total RNA from cell lines was isolated using
7 TRIzol extraction (Invitrogen). cDNA was then prepared using the PrimeScript RT reagent
8 kit (TaKaRa). QPCR reactions were performed according to the manufacturer's
9 instructions using SYBR® Premix Ex Taq kit (TaKaRa). All reactions were performed in
10 triplicates. The CT difference values between the target gene and housekeeping gene
11 (*GAPDH*) were calculated using the standard curve method. The relative gene expression
12 was calculated. The sequences of primers used for qPCR are listed in **Supplementary**
13 **Table 3.**

14

15 **ChIP analysis.** ChIPs were performed using cross-linked chromatin from Capan-2 cells
16 and either anti-BCL6 antibodies (1;1000, Cell Signaling Technology Cat# 14895,
17 RRID:AB_2798638), anti-STAT1 antibodies (1;1000, Abclonal Cat# A12075, RRID:
18 AB_2758978), or normal rabbit IgG (CST, 2729), using SimpleChIP Plus Enzymatic
19 Chromatin Immunoprecipitation kit (agarose beads) (Cell Signaling Technology, 9004).
20 The enriched DNA was quantified by qPCR analysis using the primers listed in
21 **Supplementary Table 3.**

22

Western blotting assay. The preparation of cell lysis was performed according to standard methods. Cells were treated with the respective concentrations of drug for indicated times. Afterward, the cells were washed slightly with ice-cold PBS, and then lysed with radio-immunoprecipitation assay (RIPA) buffer containing protease and phosphatase inhibitor cocktail (Calbiochem). The protein concentrations of cell lysate supernatants were assayed using a BCA protein assay kit (Thermo Scientific). Protein samples were resolved on 8–12% SDS–polyacrylamide gels and transferred to nitrocellulose membranes (Millipore). Subsequently, the membranes were blocked using 5% BSA (bovine serum albumin) for 1 h at room temperature and then hybridized sequentially using the primary antibodies and fluorescently labeled secondary antibodies. Signals were detected using the Odyssey infrared imaging system (Odyssey, LI-COR). The antibodies used are listed as follows: anti-BCL6 (1;1000, Cell Signaling Technology Cat# 14895, RRID:AB_2798638), anti-phospho-mTOR^{S2448} (1;1000, Cell Signaling Technology Cat# 2971, RRID:AB_330970), anti-mTOR (1;1000, Cell Signaling Technology Cat# 2972, RRID:AB_330978), anti-phospho-p70S6K^{T389} (1;1000, Cell Signaling Technology Cat# 9206, RRID:AB_2285392), anti-p70S6K (1;1000, Cell Signaling Technology Cat# 9202, RRID:AB_331676), anti-phospho-S6^{S235/S236} (1;1000, Cell Signaling Technology Cat# 2211, RRID:AB_331679), anti-S6 (1;1000, Cell Signaling Technology Cat# 2217, RRID:AB_331355), anti-phospho-γ-H2AX^{S139} (1;1000, Cell Signaling Technology Cat# 9718, RRID:AB_2118009), anti-PTEN (1;1000, Cell Signaling Technology Cat# 9559, RRID:AB_390810), anti-GAPDH (1;10000, Abcam Cat# ab181602, RRID:AB_2630358), anti-STAT1 (1;1000, Abclonal Cat# A19563, RRID:AB_2862669), and anti-IFNAR1

1 (1;1000, Proteintech Cat# 13083-1-AP, RRID:AB_2122626). The immunoblots are
2 representative of three independent experiments.

3

4 **Enzyme-linked immunosorbent assay.** To detect the cellular IFN- α and IFN- γ contents,
5 cell lysates were extracted using RIPA buffer. The total protein concentrations were
6 determined using BCA protein assay kit (Thermo Scientific), and IFN- α and IFN- γ protein
7 concentrations were measured using a human IFN- α ELISA kit (1110012) and a human
8 IFN- γ ELISA kit (1110002) from Dakewe Biotech, according to the manufacturer's
9 protocol.

10

11 **RNA sequencing.** RNA-seq data were produced by Novogene (Beijing, China). Capan-2,
12 H661, and PC9 cells were treated with dimethyl sulfoxide (DMSO) or etoposide at their
13 respective IC₅₀s for 24 h. Cells were harvested, and the total RNA was extracted using
14 TRIzol reagent (Invitrogen) following the manufacturer's protocol. A total of 1 μ g RNA per
15 sample was used as the input material for the RNA sample preparations. Libraries were
16 prepared using the NEBNext Ultra™ RNA Library Prep kit for Illumina (NEB, USA) and
17 library quality was assessed using the Agilent Bioanalyzer 2100 system. The clustering of
18 the index-coded samples was performed using a cBot cluster generation system and a
19 TruSeq PE cluster kit (Illumina) according to the manufacturer's instructions. After cluster
20 generation, the library preparations were sequenced on an Illumina platform and 150 bp
21 paired-end reads were generated. Differential expression was analyzed using DESeq2
22 (RRID:SCR_000154). Pathway analysis was performed using gene set enrichment

1 analysis (GSEA; <http://software.broadinstitute.org/gsea/index.jsp>).

2

3 **Single cell gel electrophoresis (comet) assay.** Single cell electrophoresis (Neutral) was
 4 performed according to the manufacturer's instructions (Trevigen). HCT116 and Capan-2
 5 cells were treated with 10 μ M etoposide, 10 μ M BI3802, or both for 48 h. Afterward, cells
 6 were re-suspended in PBS at 2×10^5 cells/mL and mixed with molten LMAgarose (at 37°C)
 7 at a ratio of 1:10. A 50 μ L mixture was pipetted onto comet slides. The slides were
 8 solidified, and successively immersed in lysis solution and neutral electrophoresis buffer.
 9 The slides were then performed to electrophoresis, placed in a DNA precipitation solution,
 10 and stained using diluted SYBR® Gold. Signals were captured using a fluorescence
 11 microscope. DNA damage was quantified for 50 cells using the mean for each
 12 experimental condition, which was obtained by using Comet Score (TriTek) software.

13

14 **Animal experiments.** For the human cancer cell xenograft mouse model, 6-week-old
 15 male BALB/cA nude mice were purchased from the National Rodent Laboratory Animal
 16 Resources (Shanghai, China). HCT116 cells (3 million per mouse) were injected
 17 subcutaneously into the flanks of nude mice. The primary *KRAS*-mutant lung cancer
 18 xenograft mouse model (LACPDx) was established as previously described (J. Wang et
 19 al., 2016). The patient-derived tumor tissues were cut into ~15 mm³ fragments and
 20 implanted subcutaneously into BALB/cA nude mice using a trocar needle. For these two
 21 different xenograft mouse models, the tumors were measured using electronic calipers
 22 every other day, and the body was measured in parallel. When the tumor volume reached

1 approximately 100 - 200 mm³, mice were randomized and treated with vehicle (dissolved
2 in sterile water supplied with 0.5% CMC-Na), etoposide (10 mg/kg, orally, dissolved in
3 corn oil), FX1 (5 mg/kg, intraperitoneally, dissolved in sterile water supplied with 0.5%
4 CMC-Na) or etoposide + FX1. The tumor volumes were calculated using the formula,
5 volume=length×width²×0.52. On day 16 or 24, the mice were sacrificed, and tumor tissues
6 were excised, weighed and snap-frozen in liquid nitrogen for qPCR analysis, Western
7 blotting analysis, and biochemistry testing. All animal experiments were conducted
8 following a protocol approved by the East China Normal University Animal Care
9 Committee.

10

11 **Statistical analysis.** The data are presented as the mean ± S.E.M. unless otherwise
12 stated. Statistical tests were performed using Microsoft Excel and GraphPad Prism
13 Software version 8.0. For comparisons of two groups, a two-tailed unpaired *t*-test was
14 used. For comparisons of multiple groups, one-way analysis of variance was used.
15 Significance levels were set at * *P* < 0.05, ** *P* < 0.01, *** *P* < 0.001. Other specific tests
16 applied are included in figure legends.

17

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22

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Availability of supporting data

RNA-seq data sets and the processed data that support the findings of this study have been deposited to the Gene Expression Omnibus (GEO) under accession ID: GSE161803.

Authors' Contributions

Y. L. designed and performed experiments, analyzed data and wrote the manuscript. J.F., performed experiments and revised the manuscript. K.Y., Y.L., K.L. performed and assisted with experiments. J.G., J.C. C.M. provided experimental supports and revised the manuscript. X.P. led the project, analyzed data and wrote the manuscript.

Ethics Approval and Consent to participate

This study was approved by the Ethics Committee of the East China Normal University.

Competing interests

1 The authors declare that they have no competing interests

2

3 **References**

4 Basso, K., & Dalla-Favera, R. (2012). Roles of BCL6 in normal and transformed germinal center B cells.

5 *Immunol Rev*, 247(1), 172-183. doi:10.1111/j.1600-065X.2012.01112.x

6 Basso, K., Saito, M., Sumazin, P., Margolin, A. A., Wang, K., Lim, W. K., . . . Dalla-Favera, R. (2010).

7 Integrated biochemical and computational approach identifies BCL6 direct target genes

8 controlling multiple pathways in normal germinal center B cells. *Blood*, 115(5), 975-984.

9 doi:10.1182/blood-2009-06-227017

10 Bavik, C., Coleman, I., Dean, J. P., Knudsen, B., Plymate, S., & Nelson, P. S. (2006). The gene

11 expression program of prostate fibroblast senescence modulates neoplastic epithelial cell

12 proliferation through paracrine mechanisms. *Cancer Res*, 66(2), 794-802.

13 doi:10.1158/0008-5472.CAN-05-1716

14 Beguelin, W., Teater, M., Gearhart, M. D., Calvo Fernandez, M. T., Goldstein, R. L., Cardenas, M. G., . . .

15 Melnick, A. M. (2016). EZH2 and BCL6 Cooperate to Assemble CBX8-BCOR Complex to

16 Repress Bivalent Promoters, Mediate Germinal Center Formation and Lymphomagenesis.

17 *Cancer Cell*, 30(2), 197-213. doi:10.1016/j.ccell.2016.07.006

18 Bonner, W. M., Redon, C. E., Dickey, J. S., Nakamura, A. J., Sedelnikova, O. A., Solier, S., & Pommier, Y.

19 (2008). GammaH2AX and cancer. *Nat Rev Cancer*, 8(12), 957-967. doi:10.1038/nrc2523

20 Bouwman, P., & Jonkers, J. (2012). The effects of deregulated DNA damage signalling on cancer

21 chemotherapy response and resistance. *Nat Rev Cancer*, 12(9), 587-598. doi:10.1038/nrc3342

22 Broustas, C. G., & Lieberman, H. B. (2014). DNA damage response genes and the development of

1 cancer metastasis. *Radiat Res*, 181(2), 111-130. doi:10.1667/RR13515.1

2 Cardenas, M. G., Oswald, E., Yu, W., Xue, F., MacKerell, A. D., Jr., & Melnick, A. M. (2017). The

3 Expanding Role of the BCL6 Oncoprotein as a Cancer Therapeutic Target. *Clin Cancer Res*,

4 23(4), 885-893. doi:10.1158/1078-0432.CCR-16-2071

5 Cardenas, M. G., Yu, W., Beguelin, W., Teater, M. R., Geng, H., Goldstein, R. L., . . . Melnick, A. M.

6 (2016). Rationally designed BCL6 inhibitors target activated B cell diffuse large B cell lymphoma.

7 *J Clin Invest*, 126(9), 3351-3362. doi:10.1172/JCI85795

8 Castellaneta, A., Yoshida, O., Kimura, S., Yokota, S., Geller, D. A., Murase, N., & Thomson, A. W. (2014).

9 Plasmacytoid dendritic cell-derived IFN-alpha promotes murine liver ischemia/reperfusion injury

10 by induction of hepatocyte IRF-1. *Hepatology*, 60(1), 267-277. doi:10.1002/hep.27037

11 Cerchietti, L. C., Hatzi, K., Caldas-Lopes, E., Yang, S. N., Figueroa, M. E., Morin, R. D., . . . Melnick, A.

12 (2010). BCL6 repression of EP300 in human diffuse large B cell lymphoma cells provides a

13 basis for rational combinatorial therapy. *J Clin Invest*, 120(12), 4569-4582.

14 doi:10.1172/JCI42869

15 Cheng, H., Linhares, B. M., Yu, W., Cardenas, M. G., Ai, Y., Jiang, W., . . . Xue, F. (2018). Identification of

16 Thiourea-Based Inhibitors of the B-Cell Lymphoma 6 BTB Domain via NMR-Based Fragment

17 Screening and Computer-Aided Drug Design. *J Med Chem*, 61(17), 7573-7588.

18 doi:10.1021/acs.jmedchem.8b00040

19 Cheung-Ong, K., Giaever, G., & Nislow, C. (2013). DNA-damaging agents in cancer chemotherapy:

20 serendipity and chemical biology. *Chem Biol*, 20(5), 648-659.

21 doi:10.1016/j.chembiol.2013.04.007

22 Ci, W., Polo, J. M., Cerchietti, L., Shaknovich, R., Wang, L., Yang, S. N., . . . Melnick, A. (2009). The

1 BCL6 transcriptional program features repression of multiple oncogenes in primary B cells and
2 is deregulated in DLBCL. *Blood*, 113(22), 5536-5548. doi:10.1182/blood-2008-12-193037

3 Coppe, J. P., Patil, C. K., Rodier, F., Sun, Y., Munoz, D. P., Goldstein, J., . . . Campisi, J. (2008).
4 Senescence-associated secretory phenotypes reveal cell-nonautonomous functions of
5 oncogenic RAS and the p53 tumor suppressor. *PLoS Biol*, 6(12), 2853-2868.
6 doi:10.1371/journal.pbio.0060301

7 Deb, D., Rajaram, S., Larsen, J. E., Dospoy, P. D., Marullo, R., Li, L. S., . . . Wu, L. F. (2017). Combination
8 Therapy Targeting BCL6 and Phospho-STAT3 Defeats Intratumor Heterogeneity in a Subset of
9 Non-Small Cell Lung Cancers. *Cancer Res*, 77(11), 3070-3081.
10 doi:10.1158/0008-5472.CAN-15-3052

11 Dery, K. J., Kujawski, M., Grunert, D., Wu, X., Ngyuen, T., Cheung, C., . . . Shively, J. E. (2014). IRF-1
12 regulates alternative mRNA splicing of carcinoembryonic antigen-related cell adhesion molecule
13 1 (CEACAM1) in breast epithelial cells generating an immunoreceptor tyrosine-based inhibition
14 motif (ITIM) containing isoform. *Mol Cancer*, 13, 64. doi:10.1186/1476-4598-13-64

15 Dupont, T., Yang, S. N., Patel, J., Hatzi, K., Malik, A., Tam, W., . . . Cerchiatti, L. (2016). Selective
16 targeting of BCL6 induces oncogene addiction switching to BCL2 in B-cell lymphoma.
17 *Oncotarget*, 7(3), 3520-3532. doi:10.18632/oncotarget.6513

18 Duy, C., Hurtz, C., Shojaei, S., Cerchiatti, L., Geng, H., Swaminathan, S., . . . Muschen, M. (2011). BCL6
19 enables Ph+ acute lymphoblastic leukaemia cells to survive BCR-ABL1 kinase inhibition. *Nature*,
20 473(7347), 384-388. doi:10.1038/nature09883

21 Duy, C., Yu, J. J., Nahar, R., Swaminathan, S., Kweon, S. M., Polo, J. M., . . . Muschen, M. (2010). BCL6
22 is critical for the development of a diverse primary B cell repertoire. *J Exp Med*, 207(6),

1 1209-1221. doi:10.1084/jem.20091299

2 Elkabets, M., Vora, S., Juric, D., Morse, N., Mino-Kenudson, M., Muranen, T., . . . Baselga, J. (2013).

3 mTORC1 inhibition is required for sensitivity to PI3K p110alpha inhibitors in PIK3CA-mutant

4 breast cancer. *Sci Transl Med*, 5(196), 196ra199. doi:10.1126/scitranslmed.3005747

5 Ettinger, D. S., Wood, D. E., Aisner, D. L., Akerley, W., Bauman, J., Chirieac, L. R., . . . Hughes, M. (2017).

6 Non-Small Cell Lung Cancer, Version 5.2017, NCCN Clinical Practice Guidelines in Oncology. *J*

7 *Natl Compr Canc Netw*, 15(4), 504-535. doi:10.6004/jnccn.2017.0050

8 Fernandez de Mattos, S., Essafi, A., Soeiro, I., Pietersen, A. M., Birkenkamp, K. U., Edwards, C. S., . . .

9 Lam, E. W. (2004). FoxO3a and BCR-ABL regulate cyclin D2 transcription through a

10 STAT5/BCL6-dependent mechanism. *Mol Cell Biol*, 24(22), 10058-10071.

11 doi:10.1128/MCB.24.22.10058-10071.2004

12 Fernando, T. M., Marullo, R., Pera Gresely, B., Phillip, J. M., Yang, S. N., Lundell-Smith, G., . . . Cerchietti,

13 L. (2019). BCL6 Evolved to Enable Stress Tolerance in Vertebrates and Is Broadly Required by

14 Cancer Cells to Adapt to Stress. *Cancer Discov*, 9(5), 662-679.

15 doi:10.1158/2159-8290.CD-17-1444

16 Fillmore, C. M., Xu, C., Desai, P. T., Berry, J. M., Rowbotham, S. P., Lin, Y. J., . . . Kim, C. F. (2015). EZH2

17 inhibition sensitizes BRG1 and EGFR mutant lung tumours to Topolli inhibitors. *Nature*,

18 520(7546), 239-242. doi:10.1038/nature14122

19 Geng, H., Hurtz, C., Lenz, K. B., Chen, Z., Baumjohann, D., Thompson, S., . . . Muschen, M. (2015).

20 Self-enforcing feedback activation between BCL6 and pre-B cell receptor signaling defines a

21 distinct subtype of acute lymphoblastic leukemia. *Cancer Cell*, 27(3), 409-425.

22 doi:10.1016/j.ccell.2015.02.003

- 1 Ghetu, A. F., Corcoran, C. M., Cerchietti, L., Bardwell, V. J., Melnick, A., & Prive, G. G. (2008). Structure
2 of a BCOR corepressor peptide in complex with the BCL6 BTB domain dimer. *Mol Cell*, 29(3),
3 384-391. doi:10.1016/j.molcel.2007.12.026
- 4 Gilbert, L. A., & Hemann, M. T. (2010). DNA damage-mediated induction of a chemoresistant niche. *Cell*,
5 143(3), 355-366. doi:10.1016/j.cell.2010.09.043
- 6 Green, M. R., Vicente-Duenas, C., Romero-Camarero, I., Long Liu, C., Dai, B., Gonzalez-Herrero, I., . . .
7 Sanchez-Garcia, I. (2014). Transient expression of Bcl6 is sufficient for oncogenic function and
8 induction of mature B-cell lymphoma. *Nat Commun*, 5, 3904. doi:10.1038/ncomms4904
- 9 Guo, J., Liu, Y., Lv, J., Zou, B., Chen, Z., Li, K., . . . Pang, X. (2021). BCL6 confers KRAS-mutant
10 non-small-cell lung cancer resistance to BET inhibitors. *J Clin Invest*, 131(1).
11 doi:10.1172/JCI133090
- 12 Hanahan, D., & Weinberg, R. A. (2011). Hallmarks of cancer: the next generation. *Cell*, 144(5), 646-674.
13 doi:10.1016/j.cell.2011.02.013
- 14 Huang, C., Hatzi, K., & Melnick, A. (2013). Lineage-specific functions of Bcl-6 in immunity and
15 inflammation are mediated by distinct biochemical mechanisms. *Nat Immunol*, 14(4), 380-388.
16 doi:10.1038/ni.2543
- 17 Januchowski, R., Zawierucha, P., Rucinski, M., Nowicki, M., & Zabel, M. (2014). Extracellular matrix
18 proteins expression profiling in chemoresistant variants of the A2780 ovarian cancer cell line.
19 *Biomed Res Int*, 2014, 365867. doi:10.1155/2014/365867
- 20 Jin, L., Chun, J., Pan, C., Li, D., Lin, R., Alesi, G. N., . . . Kang, S. (2018). MAST1 Drives Cisplatin
21 Resistance in Human Cancers by Rewiring cRaf-Independent MEK Activation. *Cancer Cell*,
22 34(2), 315-330 e317. doi:10.1016/j.ccell.2018.06.012

- 1 Kamada, Y., Sakai, N., Sogabe, S., Ida, K., Oki, H., Sakamoto, K., . . . Matsui, J. (2017). Discovery of a
2 B-Cell Lymphoma 6 Protein-Protein Interaction Inhibitor by a Biophysics-Driven
3 Fragment-Based Approach. *J Med Chem*, 60(10), 4358-4368.
4 doi:10.1021/acs.jmedchem.7b00313
- 5 Kaul, S., Igwezie, L. N., Stewart, D. J., Fields, S. Z., Kosty, M., Levithan, N., . . . et al. (1995).
6 Pharmacokinetics and bioequivalence of etoposide following intravenous administration of
7 etoposide phosphate and etoposide in patients with solid tumors. *J Clin Oncol*, 13(11),
8 2835-2841. doi:10.1200/JCO.1995.13.11.2835
- 9 Kerres, N., Steurer, S., Schlager, S., Bader, G., Berger, H., Caligiuri, M., . . . Koegl, M. (2017). Chemically
10 Induced Degradation of the Oncogenic Transcription Factor BCL6. *Cell Rep*, 20(12), 2860-2875.
11 doi:10.1016/j.celrep.2017.08.081
- 12 Khodarev, N. N., Minn, A. J., Efimova, E. V., Darga, T. E., Labay, E., Beckett, M., . . . Weichselbaum, R. R.
13 (2007). Signal transducer and activator of transcription 1 regulates both cytotoxic and
14 prosurvival functions in tumor cells. *Cancer Res*, 67(19), 9214-9220.
15 doi:10.1158/0008-5472.CAN-07-1019
- 16 Kikuchi, M., Miki, T., Kumagai, T., Fukuda, T., Kamiyama, R., Miyasaka, N., & Hirose, S. (2000).
17 Identification of negative regulatory regions within the first exon and intron of the BCL6 gene.
18 *Oncogene*, 19(42), 4941-4945. doi:10.1038/sj.onc.1203864
- 19 Koh, J., Itahana, Y., Mendenhall, I. H., Low, D., Soh, E. X. Y., Guo, A. K., . . . Itahana, K. (2019). ABCB1
20 protects bat cells from DNA damage induced by genotoxic compounds. *Nat Commun*, 10(1),
21 2820. doi:10.1038/s41467-019-10495-4
- 22 Lee, Y. R., Chen, M., & Pandolfi, P. P. (2018). The functions and regulation of the PTEN tumour

1 suppressor: new modes and prospects. *Nat Rev Mol Cell Biol*, 19(9), 547-562.

2 doi:10.1038/s41580-018-0015-0

3 Madapura, H. S., Nagy, N., Ujvari, D., Kallas, T., Krohnke, M. C. L., Amu, S., . . . Salamon, D. (2017).

4 Interferon gamma is a STAT1-dependent direct inducer of BCL6 expression in imatinib-treated

5 chronic myeloid leukemia cells. *Oncogene*, 36(32), 4619-4628. doi:10.1038/onc.2017.85

6 Martelli, A. M., Evangelisti, C., Chappell, W., Abrams, S. L., Basecke, J., Stivala, F., . . . McCubrey, J. A.

7 (2011). Targeting the translational apparatus to improve leukemia therapy: roles of the

8 PI3K/PTEN/Akt/mTOR pathway. *Leukemia*, 25(7), 1064-1079. doi:10.1038/leu.2011.46

9 Minn, A. J. (2015). Interferons and the Immunogenic Effects of Cancer Therapy. *Trends Immunol*, 36(11),

10 725-737. doi:10.1016/j.it.2015.09.007

11 Moitra, K., Im, K., Limpert, K., Borsa, A., Sawitzke, J., Robey, R., . . . Dean, M. (2012). Differential gene

12 and microRNA expression between etoposide resistant and etoposide sensitive MCF7 breast

13 cancer cell lines. *PLoS One*, 7(9), e45268. doi:10.1371/journal.pone.0045268

14 Murai, J., Thomas, A., Miettinen, M., & Pommier, Y. (2019). Schlafen 11 (SLFN11), a restriction factor for

15 replicative stress induced by DNA-targeting anti-cancer therapies. *Pharmacol Ther*, 201, 94-102.

16 doi:10.1016/j.pharmthera.2019.05.009

17 Nitiss, J. L. (2009). Targeting DNA topoisomerase II in cancer chemotherapy. *Nat Rev Cancer*, 9(5),

18 338-350. doi:10.1038/nrc2607

19 O'Grady, S., Finn, S. P., Cuffe, S., Richard, D. J., O'Byrne, K. J., & Barr, M. P. (2014). The role of DNA

20 repair pathways in cisplatin resistant lung cancer. *Cancer Treat Rev*, 40(10), 1161-1170.

21 doi:10.1016/j.ctrv.2014.10.003

22 Palle, J., Frost, B. M., Peterson, C., Gustafsson, G., Hellebostad, M., Kanerva, J., . . . Oncology. (2006).

1 Doxorubicin pharmacokinetics is correlated to the effect of induction therapy in children with
2 acute myeloid leukemia. *Anticancer Drugs*, 17(4), 385-392.
3 doi:10.1097/01.cad.0000198911.98442.16

4 Parekh, S., Prive, G., & Melnick, A. (2008). Therapeutic targeting of the BCL6 oncogene for diffuse large
5 B-cell lymphomas. *Leuk Lymphoma*, 49(5), 874-882. doi:10.1080/10428190801895345

6 Pasqualucci, L., Migliazza, A., Basso, K., Houldsworth, J., Chaganti, R. S., & Dalla-Favera, R. (2003).
7 Mutations of the BCL6 proto-oncogene disrupt its negative autoregulation in diffuse large B-cell
8 lymphoma. *Blood*, 101(8), 2914-2923. doi:10.1182/blood-2002-11-3387

9 Pearl, L. H., Schierz, A. C., Ward, S. E., Al-Lazikani, B., & Pearl, F. M. (2015). Therapeutic opportunities
10 within the DNA damage response. *Nat Rev Cancer*, 15(3), 166-180. doi:10.1038/nrc3891

11 Phan, R. T., Saito, M., Basso, K., Niu, H., & Dalla-Favera, R. (2005). BCL6 interacts with the transcription
12 factor Miz-1 to suppress the cyclin-dependent kinase inhibitor p21 and cell cycle arrest in
13 germinal center B cells. *Nat Immunol*, 6(10), 1054-1060. doi:10.1038/ni1245

14 Phan, R. T., Saito, M., Kitagawa, Y., Means, A. R., & Dalla-Favera, R. (2007). Genotoxic stress regulates
15 expression of the proto-oncogene Bcl6 in germinal center B cells. *Nat Immunol*, 8(10),
16 1132-1139. doi:10.1038/ni1508

17 Polo, J. M., Ci, W., Licht, J. D., & Melnick, A. (2008). Reversible disruption of BCL6 repression complexes
18 by CD40 signaling in normal and malignant B cells. *Blood*, 112(3), 644-651.
19 doi:10.1182/blood-2008-01-131813

20 Post, A. E. M., Smid, M., Nagelkerke, A., Martens, J. W. M., Bussink, J., Sweep, F., & Span, P. N. (2018).
21 Interferon-Stimulated Genes Are Involved in Cross-resistance to Radiotherapy in
22 Tamoxifen-Resistant Breast Cancer. *Clin Cancer Res*, 24(14), 3397-3408.

doi:10.1158/1078-0432.CCR-17-2551

Poth, K. J., Guminski, A. D., Thomas, G. P., Leo, P. J., Jabbar, I. A., & Saunders, N. A. (2010). Cisplatin treatment induces a transient increase in tumorigenic potential associated with high interleukin-6 expression in head and neck squamous cell carcinoma. *Mol Cancer Ther*, 9(8), 2430-2439. doi:10.1158/1535-7163.MCT-10-0258

Ranuncolo, S. M., Polo, J. M., Dierov, J., Singer, M., Kuo, T., Greally, J., . . . Melnick, A. (2007). Bcl-6 mediates the germinal center B cell phenotype and lymphomagenesis through transcriptional repression of the DNA-damage sensor ATR. *Nat Immunol*, 8(7), 705-714. doi:10.1038/ni1478

Rodier, F., Coppe, J. P., Patil, C. K., Hoeijmakers, W. A., Munoz, D. P., Raza, S. R., . . . Campisi, J. (2009). Persistent DNA damage signalling triggers senescence-associated inflammatory cytokine secretion. *Nat Cell Biol*, 11(8), 973-979. doi:10.1038/ncb1909

Sandler, A., Gray, R., Perry, M. C., Brahmer, J., Schiller, J. H., Dowlati, A., . . . Johnson, D. H. (2006). Paclitaxel-carboplatin alone or with bevacizumab for non-small-cell lung cancer. *N Engl J Med*, 355(24), 2542-2550. doi:10.1056/NEJMoa061884

Slabicki, M., Yoon, H., Koeppel, J., Nitsch, L., Roy Burman, S. S., Di Genua, C., . . . Ebert, B. L. (2020). Small-molecule-induced polymerization triggers degradation of BCL6. *Nature*, 588(7836), 164-168. doi:10.1038/s41586-020-2925-1

Stebbing, J., Shah, K., Lit, L. C., Gagliano, T., Ditsiou, A., Wang, T., . . . Giamas, G. (2018). LMTK3 confers chemo-resistance in breast cancer. *Oncogene*, 37(23), 3113-3130. doi:10.1038/s41388-018-0197-0

Sun, Y., Campisi, J., Higano, C., Beer, T. M., Porter, P., Coleman, I., . . . Nelson, P. S. (2012). Treatment-induced damage to the tumor microenvironment promotes prostate cancer therapy

1 resistance through WNT16B. *Nat Med*, 18(9), 1359-1368. doi:10.1038/nm.2890

2 Tanaka, K., Babic, I., Nathanson, D., Akhavan, D., Guo, D., Gini, B., . . . Mischel, P. S. (2011). Oncogenic

3 EGFR signaling activates an mTORC2-NF-kappaB pathway that promotes chemotherapy

4 resistance. *Cancer Discov*, 1(6), 524-538. doi:10.1158/2159-8290.CD-11-0124

5 Tempero, M. A., Malafa, M. P., Al-Hawary, M., Asbun, H., Bain, A., Behrman, S. W., . . . Darlow, S. (2017).

6 Pancreatic Adenocarcinoma, Version 2.2017, NCCN Clinical Practice Guidelines in Oncology. *J*

7 *Natl Compr Canc Netw*, 15(8), 1028-1061. doi:10.6004/jnccn.2017.0131

8 Trinh, B. Q., Ko, S. Y., Barengo, N., Lin, S. Y., & Naora, H. (2013). Dual functions of the homeoprotein

9 DLX4 in modulating responsiveness of tumor cells to topoisomerase II-targeting drugs. *Cancer*

10 *Res*, 73(2), 1000-1010. doi:10.1158/0008-5472.CAN-12-3538

11 Valls, E., Lobry, C., Geng, H., Wang, L., Cardenas, M., Rivas, M., . . . Melnick, A. (2017). BCL6

12 Antagonizes NOTCH2 to Maintain Survival of Human Follicular Lymphoma Cells. *Cancer*

13 *Discov*, 7(5), 506-521. doi:10.1158/2159-8290.CD-16-1189

14 Walker, S. R., Liu, S., Xiang, M., Nicolais, M., Hatzi, K., Giannopoulou, E., . . . Frank, D. A. (2015). The

15 transcriptional modulator BCL6 as a molecular target for breast cancer therapy. *Oncogene*,

16 34(9), 1073-1082. doi:10.1038/onc.2014.61

17 Walker, S. R., Nelson, E. A., & Frank, D. A. (2007). STAT5 represses BCL6 expression by binding to a

18 regulatory region frequently mutated in lymphomas. *Oncogene*, 26(2), 224-233.

19 doi:10.1038/sj.onc.1209775

20 Wang, J., Hu, K., Guo, J., Cheng, F., Lv, J., Jiang, W., . . . Liu, M. (2016). Suppression of KRas-mutant

21 cancer through the combined inhibition of KRAS with PLK1 and ROCK. *Nat Commun*, 7, 11363.

22 doi:10.1038/ncomms11363

1 Wang, Y. Q., Xu, M. D., Weng, W. W., Wei, P., Yang, Y. S., & Du, X. (2015). BCL6 is a negative prognostic
2 factor and exhibits pro-oncogenic activity in ovarian cancer. *Am J Cancer Res*, 5(1), 255-266.

3 Weichselbaum, R. R., Ishwaran, H., Yoon, T., Nuyten, D. S., Baker, S. W., Khodarev, N., . . . Minn, A. J.
4 (2008). An interferon-related gene signature for DNA damage resistance is a predictive marker
5 for chemotherapy and radiation for breast cancer. *Proc Natl Acad Sci U S A*, 105(47),
6 18490-18495. doi:10.1073/pnas.0809242105

7 Wijdeven, R. H., Pang, B., van der Zanden, S. Y., Qiao, X., Blomen, V., Hoogstraat, M., . . . Neefjes, J.
8 (2015). Genome-Wide Identification and Characterization of Novel Factors Conferring
9 Resistance to Topoisomerase II Poisons in Cancer. *Cancer Res*, 75(19), 4176-4187.
10 doi:10.1158/0008-5472.CAN-15-0380

11 Xu, L., Chen, Y., Dutra-Clarke, M., Mayakonda, A., Hazawa, M., Savinoff, S. E., . . . Koeffler, H. P. (2017).
12 BCL6 promotes glioma and serves as a therapeutic target. *Proc Natl Acad Sci U S A*, 114(15),
13 3981-3986. doi:10.1073/pnas.1609758114

Figure 1. Genotoxic agents promote BCL6 expression. (A) Cell sensitivity to etoposide (ETO). Cancer cells were treated with etoposide at gradient concentrations for 48 h. IC₅₀s were measured using Sulforhodamine B (SRB) assays. Values are expressed as mean ± SEM of three independent experiments. ETO-resistant cell lines are marked in red. Cell sensitivity to doxorubicin (ADR) was also examined (see **Figure 1-figure supplement**

1 **1A). (B)** Heat map illustrating expression of BCL6 target genes in Capan-2, H661 and
2 PC9 cell lines. Cells were treated with etoposide at their respective 1/2 IC₅₀s for 24 h.
3 mRNA was isolated from treated cells and sequenced. Z-scores were calculated based on
4 counts of exon model per million mapped reads. BCL6 target genes were identified by a
5 cutoff of $P < 0.05$, $n = 3$. **(C)** BCL6 mRNA expression in ETO-resistant and -sensitive cells.
6 Cells were treated with etoposide at their respective 1/2 IC₅₀s for 24 h. QPCR assays were
7 subsequently performed. Values are expressed as mean $\pm$ SEM of three independent
8 experiments. $*P < 0.05$, $**P < 0.01$ and $***P < 0.001$, unpaired, two tailed t -test.
9 ETO-resistant cell lines are marked in red. **(D)** Validation of differentially expressed target
10 genes of BCL6 in Capan-2 and H661 cells using qPCR analysis. Values are expressed as
11 mean $\pm$ SEM of three independent experiments. $*P < 0.05$, $**P < 0.01$ and $***P < 0.001$,
12 unpaired, two tailed t -test. **(E)** Normalized BCL6 mRNA expression levels in MCF7 and
13 MCF7/ETO (required ETO-resistant MCF7), or A2780 and A2780/ADR (required
14 ADR-resistant A2780). Values are expressed as mean $\pm$ SEM of three independent
15 experiments. $*P < 0.05$, $***P < 0.001$, unpaired, two tailed t -test. **(F)** BCL6 protein
16 expression levels in different cancer cell lines in response to various genotoxic agents.
17 Cells were treated with indicated genotoxic agents for 24 h. BCL6 protein expression
18 levels were detected and normalized to GAPDH expression using immunoblotting
19 analysis. Representative images related to **Figure 1-figure supplement 1B**. The ratio of
20 genotoxic agent-treated groups to the control group was calculated. CDDP, cisplatin;
21 Carbo, carboplatin; GEM, gemcitabine. **(G)** Kaplan-Meier curves of ovarian cancer

1 patients treated with cisplatin, taxol or both drugs. The curves were stratified by BCL6
2 (215990_s_at) expression.

3 The following source data and figure supplements are available for figure 1:

4 **Figure 1-Source data 1.** Genotoxic agents promote BCL6 expression.

5 **Figure 1-figure supplement 1.** Genotoxic agents promote BCL6 expression.

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Figure 1-figure supplement 1

Figure 1-figure supplement 1. Genotoxic agents promote BCL6 expression. (A) Cell sensitivity to doxorubicin (ADR). Various cancer cell lines were treated with ADR at gradient concentrations for 48 h. IC₅₀s were measured using SRB assays. Values are expressed as mean ± SEM of three independent experiments with triplicates. ADR-resistant cell lines are marked in red. **(B)** BCL6 protein expression levels in different cancer cell lines in response to genotoxic agents. Cells were treated with indicated genotoxic agents at their respective IC₅₀s for 24 h. Proteins lysates from each cell line were blotted individually. CDDP, cisplatin; Carbo, carboplatin; ETO, etoposide; GEM, gemcitabine.

Figure 2

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2 **Figure 2.** BCL6 transactivation is correlated with therapy resistance. (A) Association
3 between BCL6 upregulation with ETO sensitivity in various cancer cell lines.
4 Representative images related to **Figure 2-figure supplement 1A**. Left vertical axis,
5 IC_{50} s of etoposide in different cancer cell lines; right vertical axis, relative BCL6 protein
6 levels compared with that of the control group; horizontal axis, cancer cell lines. (B)

1 Correlation analysis. Correlation between BCL6 upregulation levels and ETO IC₅₀s or
2 ADR IC₅₀s (see **Figure 2-figure supplement 1B**). (C) Etoposide induced BCL6 protein
3 expression in a time-dependent manner. ETO-resistant or -sensitive cells were treated
4 with etoposide at their respective 1/4 IC₅₀s for 2, 4 or 6 days. Cell lysates were collected
5 and probed with specific antibodies using Western blotting assays. ETO-resistant cell
6 lines are marked in red. (D) Etoposide increased BCL6 expression and decreased the
7 phosphorylated levels of γ-H2AX (S139) in HCT116 xenografts treated with 10 mg/kg
8 etoposide for 14 days. At the end of the experiment, tumor tissues were isolated and
9 subjected to immunoblotting analysis. Six biologically independent samples of each group
10 are shown. Tumor volume curves and tumor weight are shown in **Figure 2-figure**
11 **supplement 1C**. (E) Relative clonogenic growth of ETO-resistant cells. HCT116 cells
12 were transfected with BCL6 siRNAs or the control siRNA, followed by the treatment of 0.2
13 μM etoposide for 7 days. The expression of BCL6 was detected by immunoblotting
14 analysis (*right*). Values are expressed as mean ± SEM of three independent experiments
15 by setting the control group as 100%. ****P* < 0.001, unpaired, two tailed *t*-test. (F) Relative
16 clonogenic growth of ETO-resistant cells. HCT116 cells stably transfected with shRNA
17 targeting BCL6 were exposed to etoposide (0.2 or 0.4 μM) with or without doxycycline
18 (Dox) for 7 days. The clonogenic growth were examined. The BCL6 expression levels
19 were detected by an immunoblotting assay (*right*). Values are expressed as mean ± SEM.
20 ****P* < 0.001, unpaired, two tailed *t*-test. (G) BCL6 overexpression decreased the
21 sensitivity of H522 cells to etoposide (*left*). ETO-sensitive H522 cells were transfected
22 with pcDNA3.1-BCL6 or pcDNA3.1 control plasmid, and then treated with etoposide at

1 gradient concentrations for 48 h. The etoposide IC₅₀s were detected by SRB assays.
 2 BCL6 overexpression efficiency was examined by an immunoblotting assay (*right*). (H)
 3 Cell viability curves of required doxorubicin-resistant cells in response to BCL6
 4 knockdown. MCF7/ADR cells were transfected with siRNAs targeting BCL6 or the control
 5 siRNA. Data are presented as mean ± SEM of six independent experiments by setting the
 6 control group as 1. *** $P < 0.001$, unpaired, two tailed *t*-test (*right*).

7 The following source data and figure supplements are available for figure 2:

8 **Figure 2-Source data 1.** BCL6 transactivation is correlated with therapy resistance.

9 **Figure 2-figure supplement 1.** BCL6 upregulation is associated with therapy resistance.

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Figure 2-figure supplement 1

Figure 2-figure supplement 1. BCL6 upregulation is associated with therapy resistance.

(A) ETO induced BCL6 protein expression. ETO-resistant or -sensitive cancer cells were treated with etoposide at their 1/2 IC₅₀s or IC₅₀s for 24 h, respectively. Proteins lysates from each cell line were blotted individually. (B) Correlation between BCL6 upregulation levels and ADR IC₅₀s in various cancer cell lines. (C) Tumor volume curves and mean tumor weight on day 14. Data are expressed as mean ± SEM. **P* < 0.05, ***P* < 0.01, unpaired, two tailed *t*-test, *n* = 6.

Figure 3. Genotoxic stress activates interferon responses. **(A)** Gene set enrichment analysis of pathways significantly upregulated in Capan-2 cells treated with 50 μ M etoposide for 24 h ($n = 3$). GO analysis are shown in **Figure 3-figure supplement 1A**. **(B)** and **(C)** Enrichment plots for genes associated with interferon α (IFN- α , **B**) and interferon γ

(IFN- γ , **C**) responses in etoposide-treated Capan-2 cells. (**D**) Heat map illustrating of representative gene expression of IFN- α and IFN- γ responses in treated Capan-2 cells. Z-scores were calculated based on counts of exon model per million mapped reads. Upregulated and downregulated genes were identified by a cutoff of $P < 0.05$. (**E**) Validation of expression of inflammation-related genes in (**D**). Capan-2 cells were treated with 50 μ M etoposide for 24 h. QPCR assays were subsequently performed. Values are expressed as mean $\pm$ SEM of three independent experiments. $*P < 0.05$, $**P < 0.01$ and $***P < 0.001$, unpaired, two tailed t -test. (**F** and **G**) IFN- α (**F**) and IFN- γ (**G**) mRNA expression levels in treated cells. ETO-sensitive and -resistant cells were treated with etoposide at their respective 1/2 IC_{50} s for 24 h, and qPCR analysis was further performed. Values are expressed as mean $\pm$ SEM of three independent experiments. $*P < 0.05$, $**P < 0.01$, unpaired, two tailed t -test. ETO-resistant cell lines are marked in red. (**H** and **I**) IFN- α (**H**) and IFN- γ (**I**) production in ETO-resistant cells. Cells were treated with etoposide at their respective 1/2 IC_{50} s for 48 h. The concentrations of IFN- α and IFN- γ in cell lysates were measured using an ELISA assay. Values are expressed as mean $\pm$ SEM of three independent experiments. $*P < 0.05$, $***P < 0.001$, unpaired, two tailed t -test. (**J** and **K**) Relative IRF1 mRNA levels in Capan-2 cells. Capan-2 cells were treated with 50 ng/mL IFN- α (**J**) or 10 ng/mL IFN- γ (**K**) in the presence or absence of 50 μ M etoposide. IRF1 mRNA levels were detected by qPCR assays. Values are expressed as mean $\pm$ SEM of three independent experiments. $*P < 0.05$, $**P < 0.01$, unpaired, two tailed t -test. (**L** and **M**) Relative cell viability. ETO-sensitive H522 cells were treated with etoposide alone, 50 ng/mL IFN- α (**L**), 10 ng/mL IFN- γ (**M**) or their combinations. Cell viability were examined

1 by SRB assays. Values are expressed as mean $\pm$ SEM of three independent experiments
2 by setting the control group as 100%. $**P < 0.01$, $***P < 0.001$, unpaired, two tailed *t*-test.
3 (N) Clonogenic growth of Capan-2 cells treated with siIFNAR1, 0.4 μ M etoposide, or their
4 combinations (*left*). IFNAR1 silencing efficiency was examined using immunoblotting
5 analysis (*right*). Data are expressed as mean $\pm$ SEM of three independent experiments.
6 $***P < 0.001$, unpaired, two tailed *t*-test. Cell viability curves are shown in **Figure 3-figure**
7 **supplement 1B**. (O) Clonogenic growth showing the relative survival of PANC28 cells
8 treated with 0.2 μ M etoposide, 10 μ g/mL anti-IFN- γ or both. Values are expressed as
9 mean $\pm$ SEM of three independent experiments. $***P < 0.001$, unpaired, two tailed *t*-test.
10 Cell viability curves are shown in **Figure 3-figure supplement 1C**.

11 The following source data and figure supplements are available for figure 3:

12 **Figure 3-Source data 1**. Genotoxic stress activates interferon responses.

13 **Figure 3-figure supplement 1**. Genotoxic stress activates interferon responses.

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Figure 3-figure supplement 1

Figure 3-figure supplement 1. Genotoxic stress activates interferon responses. (A) GO analysis of RNA-seq data (ETO treatment group versus the control group). The top ten upregulated pathways in Capan-2, as indicated ($n = 3$). Graph displays category scores as $\log_{10}$ (P value) from Fisher's exact test. **(B)** Silencing of IFNAR1 enhanced Capan-2 cells sensitivity to etoposide. Capan-2 cells were transfected with IFNAR1 siRNAs or the control siRNA for 48 h. Transfected cells were then exposed to etoposide at gradient concentrations for 48 h. Cell viability was detected using SRB assays. Values are expressed as mean $\pm$ SEM of two independent experiments. **(C)** Anti-IFN- γ antibody increased etoposide cytotoxicity. PANC28 cells were treated with etoposide at gradient concentrations for 48 h in the presence or absence of 10 μ g/mL anti-IFN- γ antibody. Cell viability was detected using SRB assays. Values are expressed as mean $\pm$ SEM of three independent experiments.

Figure 4

Figure 4. The interferon/STAT1 axis directly regulates BCL6 expression. **(A)** STAT1 protein levels by immunoblotting analysis. ETO-resistant and -sensitive cells were treated with etoposide at their respective 1/2 IC₅₀s for indicated time points. Cell lysates were collected and subjected to immunoblotting analysis. ETO-resistant cell lines are marked in

1 red. **(B)** Clonogenic growth of H838 cells treated with siRNAs targeting STAT1, 0.2 μ M
2 etoposide, or their combinations. STAT1 silencing efficiency was examined using
3 immunoblotting analysis (*right*). Values are expressed as mean $\pm$ SEM of three
4 independent experiments. *** $P < 0.001$, unpaired, two tailed t -test. **(C)** IFN- γ increased
5 BCL6 and STAT1 protein levels. H838 cells were treated with 5 or 10 ng/mL IFN- γ for 24 h.
6 Cell lysates were subjected to immunoblot analysis with indicated antibodies. **(D)** Relative
7 BCL6 mRNA expression. H838 cells were treated with 5 or 10 ng/mL IFN- γ for 24 h. BCL6
8 mRNA levels were detected by qPCR assays. Values are expressed as mean $\pm$ SEM of
9 three independent experiments. ** $P < 0.01$, *** $P < 0.001$, unpaired, two tailed t -test. **(E)**
10 and **(F)** Relative BCL6 mRNA expression. H838 cells were treated with 50 ng/mL IFN- α **(E)**
11 or 10 ng/mL IFN- γ **(F)** in the presence or absence of 50 μ M etoposide. BCL6 mRNA levels
12 were detected. Values are expressed as mean $\pm$ SEM of three independent experiments.
13 * $P < 0.05$, *** $P < 0.001$, unpaired, two tailed t -test. The same experiments were also
14 repeated in Capan-2 cells (see **Figure 4-figure supplement 1A-B**). **(G and H)**
15 Immunoblotting analysis for BCL6 and STAT1 protein expression. PANC28 or H838 cells
16 were treated with 50 ng/mL IFN- α **(G)** or 10 ng/mL IFN- γ **(H)** in the presence or absence of
17 etoposide for 48 h. Cell lysates were subjected to immunoblotting analysis with specific
18 antibodies against BCL6, STAT1 and GAPDH. **(I)** STAT1 knockdown impaired
19 etoposide-induced BCL6 activation. STAT1 silencing was performed by RNA interference
20 in H838 cells. Transfected cells were treated with 50 μ M etoposide for 24 h, and cell
21 lysates were subjected to immunoblotting analysis. **(J)** Silencing of STAT1 decreased
22 BCL6 expression in ETO-resistant PANC28 and Capan-2 cells. **(K)** Overexpression of

1 STAT1 increased BCL6 expression in Capan-2 cells. (L) Relative luciferase activity.
 2 siRNAs targeting STAT1 and BCL6n-luc vector were transiently co-transfected into
 3 ETO-resistant H838 and PANC28 cells. Luciferase activity was measured 48 h
 4 post-transfection. Bar graphs represent the mean $\pm$ SEM of three independent
 5 experiments. *** $P < 0.001$, unpaired, two tailed t -test. (M) ChIP-qPCR data showing the
 6 enrichment of STAT1 binding to the BCL6 promoter region in Capan-2 cells. Values are
 7 expressed as mean $\pm$ SEM of three independent experiments. The experiments were
 8 performed twice.

9 The following source data and figure supplements are available for figure 4:

10 **Figure 4-Source data 1.** The interferon/STAT1 axis directly regulates BCL6 expression.

11 **Figure 4-figure supplement 1.** The interferon/STAT1 axis directly regulates BCL6
 12 expression.

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Figure 4-figure supplement 1

Figure 4-figure supplement 1. The interferon/STAT1 axis directly regulates BCL6 expression. **(A and B)** Relative BCL6 expression. Capan-2 cells were treated with 50 ng/mL IFN-α **(A)** or 10 ng/mL IFN-γ **(B)** in the presence or absence of 50 μM etoposide. BCL6 mRNA levels were detected by qPCR assays. Values are expressed as mean ± SEM of three independent experiments. * $P < 0.05$, *** $P < 0.001$, unpaired, two tailed t -test.

Figure 5

Figure 5. The tumor suppressor PTEN is a functional target of BCL6. **(A)** Normalized PTEN expression levels in etoposide-resistant Capan-2 and H661 cells treated with etoposide at their respective IC₅₀s for 24 h. RNA-seq tag count at exons was plotted as counts of exon model per million mapped reads. Values are expressed as mean ± SEM of three independent experiments. ***P* < 0.01, ****P* < 0.001, unpaired, two tailed *t*-test. **(B)**

1 Genomic alteration of BCL6 and PTEN according to TCGA database ($n = 1144$). The
2 percentage of gene alteration is shown. **(C)** Relative mRNA expression of BCL6 and
3 PTEN. Capan-2 and H661 cells were exposed to etoposide at their respective $1/2$ IC_{50} s for
4 24 h. QPCR analysis was further carried out. Values are expressed as mean $\pm$ SEM of
5 three independent experiments. $*P < 0.05$, $**P < 0.01$, $***P < 0.001$, unpaired, two tailed
6 t -test. **(D)** BCL6 and PTEN protein levels in Capan-2, PANC-28 and H838 cells. Cells
7 were treated with etoposide at their respective $1/4$ IC_{50} s for 2 or 4 days. Cell lysates are
8 subjected to immunoblotting analysis. **(E)** BCL6 overexpression decreased PTEN mRNA
9 and protein levels in HCT116 cells. Cells were transfected with pcDNA3.1-BCL6 or
10 pcDNA3.1 control plasmid. Total mRNA and protein were extracted and subjected to
11 qPCR analysis (*left*) and immunoblotting analysis (*right*). Values are expressed as mean $\pm$
12 SEM of three independent experiments. $*P < 0.05$, $**P < 0.01$, unpaired, two tailed t -test.
13 **(F)** BCL6 inducible knockdown increased PTEN expression. Immunoblotting analysis of
14 PTEN in HCT116 cells treated with or without doxycycline. **(G)** BCL6 binding levels at the
15 promoter region of *PTEN* examined by ChIP-qPCR assays. Values are expressed as
16 mean $\pm$ SEM of three independent experiments. $***P < 0.001$, unpaired, two tailed t -test.
17 **(H)** Etoposide activated mTOR signaling components in etoposide-resistant Capan-2,
18 PANC-28 and H838 cells. Cells were treated with etoposide at their respective $1/2$ IC_{50} s
19 for 6 or 12 h. Whole-cell lysates were prepared and subjected to immunoblotting analysis.
20 **(I)** A long-term treatment with etoposide activated mTOR signaling components in
21 ETO-resistant cells. Capan-2 and PANC-28 cells were treated with 10 μ M etoposide for 2
22 or 4 days. Cell lysates were subjected to immunoblotting analysis. **(J)** PTEN

1 overexpression increased the sensitivity of etoposide-resistant cells to etoposide.
 2 PANC28 cells were transfected with pCDH-PTEN or pCDH control plasmid. PTEN
 3 overexpression efficiency was measured immunoblotting analysis (*up*). Quantification of
 4 clonogenic growth after 7 days treatment with 0.2 μ M etoposide (*down*). Values are
 5 expressed as mean $\pm$ SEM of three independent experiments. *** $P < 0.001$, unpaired, two
 6 tailed t -test.

7 The following source data are available for figure 5:

8 **Figure 5-Source data 1.** The tumor suppressor PTEN is a functional target of BCL6.

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Figure 6

Figure 6. Therapeutic suppression of BCL6 sensitizes genotoxic agents. (A) Pharmacological inhibition of BCL6 increased ETO sensitivity. Various types of cancer cells were treated with etoposide at gradient concentrations for 48 h in the presence of 10 μM BI3802 or 20 μM Compound 7 ($n = 2$ biological replicates). IC_{50} s were measured using SRB assays. For graphs, $\log(IC_{50})$ of control cells was subtracted from $\log(IC_{50})$ of BI3802

1 or Compound 7-treated cells and multiplied by ten to be depicted as log fold change $\times 10$.

2 Targeted inhibition of BCL6 also increased ADR sensitivity (see **Figure 6-figure**

3 **supplement 1A**). **(B)** Synergistic interaction between BCL6 inhibitors (BI3802 or

4 Compound 7) and ETO. Growth inhibition was averaged and input into CalcuSyn software

5 to extrapolate combinational index values (CI) at 50% effective dose (ED50), 75%

6 effective dose (ED75) and 90% effective dose (ED90). CI values < 1 represent synergism.

7 The synergy between BI3802 and ADR was also detected in H838, Capan-2 and AsPC-1

8 cells (see **Figure 6-figure supplement 1B**). **(C)** Inhibition of clonogenic growth by the

9 combined regimen. Representative long-term clonogenic images (*up*) and quantified

10 clonogenic growth inhibition results (*down*) for cells treated with ETO, BI3802, or their

11 combinations. Data are presented as mean of three independent experiments. The same

12 experiments were also conducted for ADR (see **Figure 6-figure supplement 1C**). **(D)**

13 Inhibition of soft-agar colony growth by the combined regimen. HCT116 cells were

14 exposed to 0.2 μM etoposide, 2 μM BI3802, or their combinations. Representative images

15 of soft-agar colonies (*left*) and the relative clonogenic growth (*right*) are shown. Scale bar,

16 100 μm . Values are expressed as the mean $\pm$ SEM of three independent experiments.

17 *** $P < 0.001$, unpaired, two tailed t -test. **(E)** Immunoblotting analysis showing the protein

18 expression of BCL6, p- γ -H2AX^{S139} and cleaved-PARP in Capan-2 and H661 cells treated

19 with 15 μM etoposide, 10 μM BI3802 or their combinations for 48 h. Cell lysates were

20 subjected to immunoblotting analysis. **(F)** Comet assays. HCT116 and Capan-2 cells were

21 treated with etoposide, BI3802, or their combinations for 48 h. The tail moment was

22 quantified for 50 cells for each experimental condition (*right*). Scale bar, 100 μm . Values

1 are expressed as mean $\pm$ SEM. *** $P < 0.001$, unpaired, two tailed t -test. (G) Quantification
 2 of apoptotic cells in Capan-2 cells analyzed by flow cytometry. Cells were exposed to 15
 3 μ M etoposide, 10 μ M BI3802 or their combinations for 48 h. Percentage of positive cells
 4 was counted. Values are expressed as mean $\pm$ SEM of three independent experiments.
 5 *** $P < 0.001$, unpaired, two tailed t -test.

6 The following source data and figure supplements are available for figure 6:

7 **Figure 6-Source data 1.** Therapeutic suppression of BCL6 sensitizes genotoxic agents.

8 **Figure 6-figure supplement 1.** BCL6 inhibition sensitizes cancer cells to doxorubicin.

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Figure 6-figure supplement 1

Figure 6-figure supplement 1. BCL6 inhibition sensitizes cancer cells to doxorubicin. (A)

Increased sensitivity of cancer cells to doxorubicin. ADR-resistant cancer cells were

treated with doxorubicin at gradient concentrations for 48 h in the presence of 10 μ M

BI3802. IC_{50} s were measured using SRB assays. Values are expressed as mean $\pm$ SEM

of two independent experiments. ADR-resistant cell lines are marked in red. **(B)** Cell

viability of ADR-resistant cancer cells treated with different concentrations of doxorubicin

in the combination with BI3802. Growth inhibition for three independent biological

replicate experiments was averaged and input into CalcuSyn software to extrapolate CI

values. CI values < 1 represent synergism. Values are expressed as mean $\pm$ SEM of three

independent experiments. ADR-resistant cell lines are marked in red. **(C)** Representative

1 long-term clonogenic assays (*left*) and quantified clonogenic growth inhibition data (*right*)
 2 for H838 and OVCAR8 cells treated with ADR, BI3802, or their combinations. Data are
 3 presented as mean of three independent experiments.

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Figure 7

Figure 7. Pharmacological inhibition of BCL6 synergizes etoposide *in vivo*. **(A)** Etoposide increased BCL6 mRNA (*up*) and protein (*down*) expression in HCT116 xenografts. Tumor tissues were isolated on day 2, 4 or 8 after etoposide treatment. QPCR and immunoblotting analysis for BCL6 expression were conducted. BCL6 mRNA expression values represent mean of three independent replicates $\pm$ SEM. $*P < 0.05$, unpaired, two tailed *t*-test. **(B)** Tumor growth curves. Mice bearing HCT116 xenografts were treated with

1 vehicle, etoposide (10 mg/kg body weight), and etoposide plus FX1 (5 mg/kg body weight)
2 for indicated times. Average tumor weight on day 16 is shown in the inset. Values are
3 expressed as mean $\pm$ SEM, $n = 8$. $*P < 0.05$, $***P < 0.001$, one-way ANOVA with
4 Tukey's multiple-comparisons test. **(C)** Protein expression of BCL6 and mTOR signaling
5 components in HCT116 xenografts. Tumors were harvested at the end of treatment and
6 subjected to immunoblotting analysis. Four biologically independent samples per group
7 are shown. **(D)** Representative immunohistochemical staining of tumors in HCT116
8 xenografts. Tumor tissues from HCT116 xenografts on day 16 were examined for the
9 expression of Ki-67, p- γ -H2AX^{S139}, and p-S6^{S235/S236}. Scale bar, 50 μ m. **(E)** Tumor growth
10 curves. Mice bearing primary *KRAS*-mutant lung cancer xenografts (LACPDx) were
11 treated with vehicle, etoposide (10 mg/kg body weight), FX1 (5 mg/kg body weight) or
12 both drugs for 24 days. Average tumor weight on day 24 is shown in the inset ($n = 5$).
13 Values are expressed as mean $\pm$ SEM. $*P < 0.05$, $**P < 0.01$, $***P < 0.001$, one-way
14 ANOVA with Tukey's multiple-comparisons test. **(F)** Representative immunohistochemical
15 staining of LACPDx tumors. Tumor tissues from LACPDx on day 24 were evaluated for
16 the expression of Ki-67, p-S6^{S235/S236} and p- γ -H2AX^{S139}. Scale bar, 50 μ m.

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