

ORIGINAL ARTICLE OPEN ACCESS

Novel TEAD1 Inhibitor VT103 Enhances Dabrafenib Efficacy in *BRAF* V600E Mutated Lung Adenocarcinoma via Survivin Downregulation

Kazutaka Hosoya¹  | Hiroaki Ozasa¹  | Hironori Yoshida¹ | Hitomi Ajimizu¹ | Takahiro Tsuji^{1,2}  | Masatoshi Yamazoe¹ | Tatsuya Ogimoto¹ | Kentaro Hashimoto¹ | Tomoko Funazo (Yamamoto)¹ | Keichiro Suminaga¹ | Yusuke Shima¹ | Hiroshi Yoshida¹ | Takashi Nomizo¹ | Hiroaki Ito³ | Kazuhiro Terada³ | Shigeto Nishikawa⁴ | Toshi Menju⁴ | Akihiko Yoshizawa⁵ | Hiroshi Date⁴ | Toyohiro Hirai¹

¹Department of Respiratory Medicine, Graduate School of Medicine, Kyoto University, Kyoto, Japan | ²Department of Anatomy and Molecular Cell Biology, Graduate School of Medicine, Nagoya University, Nagoya, Japan | ³Department of Diagnostic Pathology, Graduate School of Medicine, Kyoto University, Kyoto, Japan | ⁴Department of Thoracic Surgery, Graduate School of Medicine, Kyoto University, Kyoto, Japan | ⁵Department of Diagnostic Pathology, Graduate School of Medicine, Nara Medical University, Nara, Japan

Correspondence: Hiroaki Ozasa (ozahiro@kuhp.kyoto-u.ac.jp)

Received: 14 November 2024 | **Revised:** 21 March 2025 | **Accepted:** 24 March 2025

Funding: This study was supported by JST SPRING (grant number JPMJSP2110 [K.H.]), JSPS KAKENHI (grant numbers 23KJ1264 [K.H.], 19K08601 [H.O.], and 24K18484 [H.A.]) and Takeda Science Foundation (Hironori Y.).

Keywords: *BRAF* V600E | lung cancer | survivin | TEAD1 | YAP1

ABSTRACT

The *BRAF* V600E mutation is observed in 2% of the patients with lung adenocarcinoma (LUAD), and combination therapy targeting BRAF and mitogen-activated protein kinase (MEK) is the standard treatment for this population. However, acquired resistance inevitably develops, which highlights the need for novel therapeutic strategies. In this study, we established a patient-derived *BRAF* V600E-mutated LUAD cell line, KTOR81, and investigated the potential of targeting the Yes-associated protein 1 (YAP1)/transcriptional enhanced associate domain 1 (TEAD1) pathway in combination with BRAF inhibition. We observed that the novel TEAD1 inhibitor VT103 enhanced the efficacy of the BRAF inhibitor dabrafenib in KTOR81 cells and xenograft models. The combination of dabrafenib and VT103 downregulated the expression of the antiapoptotic protein survivin, which is transcriptionally regulated by the YAP1/TEAD1 complex, leading to increased apoptosis. Moreover, we used a LUAD tissue microarray to compare the staining patterns of YAP1, TEAD1, and survivin, and examined their association with prognosis. These analyses revealed a strong correlation between YAP1, TEAD1, and survivin expression in LUAD, suggesting the relevance of the YAP1/TEAD1-survivin axis beyond *BRAF* V600E-mutated cases. While no statistically significant association was observed between survivin expression and prognosis, when limited to driver oncogene-positive patients, high survivin expression was suggested to be associated with poor prognosis. These findings provide preclinical evidence for the efficacy of combining TEAD1 inhibition with BRAF-targeted therapy in *BRAF* V600E-mutated LUAD and highlight the YAP1/TEAD1-survivin axis as a potential therapeutic target especially in the driver oncogene-positive LUAD patients.

Abbreviations: ALK, anaplastic lymphoma kinase; BIRC5, baculoviral IAP repeat containing 5; BRAF, v-ras murine sarcoma viral oncogene homolog B; CTGF, connective tissue growth factor; DMSO, dimethyl sulfoxide; EGFR, epidermal growth factor receptor; ERK, extracellular signal-regulated kinase; HER2, human epidermal growth factor receptor 2; IC50, half-maximal inhibitory concentration; LUAD, lung adenocarcinoma; MEK, mitogen-activated protein kinase kinase; NSCLC, nonsmall cell lung cancer; OS, overall survival; PBS, phosphate-buffered saline; qRT-PCR, quantitative reverse transcription polymerase chain reaction; ROS1, c-ros oncogene 1; SD, standard deviation; siRNA, small interfering RNA; TAZ, transcriptional coactivator with PDZ-binding motif; TEAD, transcriptional enhanced associate domain; TMA, tissue microarray; TTF-1, thyroid transcription factor-1; YAP1, Yes-associated protein 1.

This is an open access article under the terms of the [Creative Commons Attribution-NonCommercial](https://creativecommons.org/licenses/by-nc/4.0/) License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited and is not used for commercial purposes.

© 2025 The Author(s). *Cancer Science* published by John Wiley & Sons Australia, Ltd on behalf of Japanese Cancer Association.

1 | Introduction

Nonsmall cell lung cancer (NSCLC) is the leading cause of cancer-related deaths worldwide [1]. The treatment paradigm has changed in recent decades with the identification of several driver oncogenes of NSCLC, especially lung adenocarcinoma (LUAD), and the development of targeted therapies such as epidermal growth factor receptor (*EGFR*) mutation, anaplastic lymphoma kinase (*ALK*) translocation, and c-ros oncogene 1 (*ROS1*) translocation [2], leading to the development of targeted therapies. For example, *EGFR*-tyrosine kinase inhibitors significantly prolong the prognosis of patients with *EGFR*-mutant LUAD at the metastatic stage [3].

The v-raf murine sarcoma viral oncogene homolog B (*BRAF*) V600E mutation has an incidence rate of 2% in patients with LUAD [4] and has been observed in other malignancies such as melanoma, colon adenocarcinoma, and thyroid adenocarcinoma [5, 6]. The combination of rapidly accelerated fibrosarcoma and mitogen-activated protein kinase kinase (MEK) inhibitors is the standard first-line treatment for *BRAF* V600E-mutated LUAD [7]. The response rate to therapy was reported to be 64% in a clinical trial, and most patients do not achieve a cure owing to the development of resistance. Therefore, novel therapeutic strategies, including combination therapy as first-line treatment, are needed. Moreover, no LUAD cell line harboring the *BRAF* V600E mutation is commercially available to date, which has hindered basic research focusing on novel combination therapies or resistance mechanisms.

The Hippo signaling pathway, which was initially identified as a controller of organ size during development, has been reported to contribute to tumor progression and drug resistance in cancer biology [8–10]. In the Hippo signaling pathway, transcriptional enhanced associate domain (TEAD) transcription factors are the core output components that form a complex with Yes-associated protein 1 (YAP1) or a transcriptional coactivator with PDZ-binding motif (TAZ). Therefore, the YAP1/TAZ-TEAD complex is a novel therapeutic target in cancer.

In this study, we established a LUAD patient-derived cell line harboring the *BRAF* V600E mutation, which is a rare and valuable resource for investigating this specific mutation. Using this cell line, we evaluated the efficacy of combination therapy of *BRAF* inhibitor dabrafenib with VT103, a newly developed TEAD inhibitor that selectively inhibits TEAD1 autopalmitylation [11] and we showed that downregulation of survivin, a transcription target of the YAP1/TEAD1 complex, could be a potential mechanism of action induced by the combination therapy leading to enhanced efficacy.

2 | Materials and Methods

2.1 | Procedures for Obtaining Informed Consent

The study protocol was prepared following the Declaration of Helsinki and approved by the Kyoto University Graduate School and Faculty of Medicine Ethics Committee (Kyoto, Japan; certification numbers: R0854, R0996, and R2163).

Written informed consent was obtained from the patient whose pleural effusion was used to generate a patient-derived cell line for this study. For the tissue microarray (TMA) analysis, we used previously collected specimens following Japanese guidelines.

2.2 | Establishment of a Patient-Derived Cell Line and Genetic Analysis

The KTOR81 cell line was established from a patient with *BRAF* V600E-mutated LUAD who was treated at the Kyoto University Hospital. Pleural effusion was obtained from the patient, tumor cells were isolated using centrifugation, and cells were then cultured and maintained. All in vitro experiments were performed using cells that were within 10 passages.

Genomic DNA was extracted from cells, *BRAF* exon 15 was PCR amplified using specific primers (Table S1), and the sequence was confirmed using Sanger sequencing.

2.3 | Reagents

Dabrafenib was purchased from Selleck Chemicals, alectinib from LC Laboratories, and osimertinib from ChemScene. VT103 was provided by Vivace Therapeutics Inc. All compounds were dissolved in dimethyl sulfoxide (DMSO).

2.4 | Cell Viability and Apoptosis Assays

Cells (2000 cells/well) were cultured in 96-well plates with stepwise concentrations of the candidate reagents for 168 h (in the experiment using VT103) or 72 h (in the experiment using small interfering RNA [siRNA]). Viable cells were measured using the CellTiter-Glo 2.0 Luminescent Cell Viability Assay (Promega).

For apoptosis analysis, Cells (1000 cells/well) were cultured in 384-well plates with the indicated drug concentration for 24 h. Caspase-3/7 activity was measured using the Caspase-Glo 3/7 Assay (Promega) with the subtraction of the negative control signal and normalization by the viable cell numbers.

2.5 | Quantitative PCR

Total RNA was purified from the cultured cells, and gene expression was quantified using a qRT-PCR assay with primer pairs designed to amplify target mRNA (Table S1).

2.6 | Knockdown by siRNA

siRNA oligonucleotides for *YAP1*, *TEAD1-4*, and *BIRC5* were purchased from Thermo Fisher Scientific. Cells were transfected with siRNA oligonucleotides using reverse transfection methods.

2.7 | Immunoblotting

Proteins were separated on 10% sodium dodecyl sulfate–polyacrylamide gels and transferred onto polyvinylidene fluoride membranes. The membranes were washed and incubated with primary antibodies overnight at 4°C, and secondary antibodies at room temperature for 1 h (Table S2). Proteins were detected using chemiluminescence, and images were cropped using the Fiji/ImageJ software.

2.8 | Immunofluorescence Staining In Vitro

The cells were fixed, permeabilized, blocked, and incubated with the primary antibody solution at 4°C overnight, followed by incubation with the secondary antibody solution and Hoechst 33342 for 30 min at room temperature for nuclear counterstaining (Table S2).

2.9 | Chromatin Immunoprecipitation qPCR (ChIP-qPCR)

ChIP was performed using the SimpleChIP Plus Enzymatic Chromatin IP Kit following the manufacturer's instructions. Cells were cross-linked with 1% formaldehyde, permeabilized, and DNA was digested with Micrococcal Nuclease. Sonicated chromatin was immunoprecipitated with isotype control IgG, anti-TEAD1, or anti-YAP1 antibodies and collected with Protein G Magnetic Beads. DNA was purified from the immunoprecipitated chromatin using spin columns. Target regions containing YAP1/TEAD binding motifs (CATTCC or GGAATC) within 10 kbp upstream of the *BIRC5* transcription start site were quantified by qRT-PCR using SYBR Green (Figure S2A). Enrichment was calculated as percent Input using the formula: $2\% * 2^{(Ct\ 2\% \text{ Input Sample} - Ct\ \text{IP Sample})}$.

2.10 | Xenograft Models

Six-week-old female BALB/c-nu mice were purchased from Charles River Laboratories at 6 weeks of age. To generate the xenograft model, KTOR81 cells (5×10^6) were suspended in Matrigel (Corning) and injected subcutaneously into the backs of the mice. The width and length of the tumors were measured every 3 days. The tumor volume was calculated using the following formula: $(\text{length} \times \text{width}^2) \times 0.52$. Treatment was initiated when the tumor volume reached 200 mm³. All animal experiments were approved by the Animal Research Committee of Kyoto University (ID: MedKyo21272, MedKyo22519) and were conducted following the ARRIVE guidelines.

2.11 | Immunohistochemical Analysis

Patients diagnosed with LUAD who underwent surgical resection at the Kyoto University Hospital between January 2015 and April 2018 were included in the analysis. An expert pathologist reviewed and confirmed the pathological diagnoses of consecutive lung adenocarcinomas to prepare

TMA from formalin-fixed paraffin-embedded tissue blocks. Immunohistochemical staining was performed on 4-μm sections of the TMA using primary antibodies against YAP1, TEAD1, and survivin (Table S2). The stained TMA sections were analyzed using QuPath version 0.5.0. H-score, which considers both the intensity and percentage of positive tumor cells, ranges from 0 to 300, with higher scores indicating stronger target protein expression.

Xenograft tumor tissues were harvested on Day 7 from the start of treatment. Tumor tissues were fixed in 10% neutral-buffered formalin and embedded in paraffin. Immunohistochemical staining and quantification were conducted as described above.

2.12 | Prognostic Analysis of TMA-Stained Cases

To evaluate the prognostic significance of YAP1, TEAD1, and survivin expression, we performed survival analysis using the Kaplan–Meier method. Postrecurrence overall survival (OS) was defined as the time from recurrence of the resected tumor to death from any cause or the last follow-up. The H-scores for each marker were dichotomized into high- and low-expression groups using the median value as the cutoff point.

Initially, we analyzed the association between the expression of each protein and the postrecurrence OS in the entire cohort. Subsequently, we stratified the patients based on the presence or absence of driver oncogenic alterations (*EGFR*, *ALK*, *ROS1*, *BRAF*, or *HER2*) and examined the relationship between survivin expression and postrecurrence OS.

2.13 | TCGA Data Analysis

The Cancer Genome Atlas Lung Adenocarcinoma (TCGA-LUAD) dataset was analyzed to validate our findings in a broader population. RNA-sequencing data (TPM values) and clinical information were obtained using the TCGAbiolinks R package. Gene Set Variation Analysis (GSVA) was performed to evaluate Hippo-YAP pathway activity using the WikiPathways gene set. Correlation between pathway activity and *BIRC5* expression was assessed using Pearson correlation. For survival analysis, patients were stratified by *BIRC5* expression (high vs. low based on median expression) in both the entire cohort and the driver oncogene-positive subgroup (including *EGFR*, *KRAS*, *BRAF*, *MET* driver mutations, and *ALK* rearrangements).

2.14 | Statistical Analysis

Cell viability, cell proliferation, and qPCR assays were performed independently at least three times in at least triplicate wells. For clinical survival analysis, analyses were conducted using R (version 4.2.2) [12]. Other statistical analyses were performed using JMP Pro version 17.0.0 (SAS Institute Inc., Cary, NC, United States) and visualized using GraphPad Prism 8.

Detailed information can be found in Appendix S1.

3 | Results

3.1 | Establishment of a *BRAF* V600E-Mutated LUAD Patient-Derived Cell Line

We established a cell line, KTOR81, from malignant pleural effusion derived from a patient with *BRAF* V600E-mutated NSCLC. The patient was a 61-year-old male who underwent left upper lobe resection for a pathological diagnosis of LUAD. Hematoxylin and eosin staining of surgically resected specimens revealed the typical papillary morphology of LUAD, and thyroid transcription factor-1 (TTF-1) immunohistochemistry showed positive staining, indicating that the tumor originated from the lung (Figure 1A). The patient did not undergo adjuvant chemotherapy. 4 months later, the patient presented to our department with a cough and dyspnea, and chest computed tomography showed left pleural effusion (Figure 1B). The pleural effusion was pathologically confirmed to be malignant, as demonstrated by hematoxylin and eosin staining and TTF-1 immunohistochemistry of the cell block prepared from the pleural effusion (Figure 1C). At this time of disease recurrence, we collected the left pleural effusion and established the KTOR81 cell line before any targeted therapy was initiated (Figure 1D). Targeted next-generation sequencing of the surgically resected tumor, performed for clinical purposes, revealed the *BRAF* V600E mutation. Targeted therapy with oral dabrafenib (a *BRAF* inhibitor, 300 mg/day) and trametinib (a MEK inhibitor, 2 mg/day) was administered, resulting in a temporary decrease in the left pleural effusion after 4 weeks. However, 16 weeks after treatment initiation, right pleural effusion appeared, indicating disease progression.

Sanger sequencing of the PCR amplicon from *BRAF* exon 15 confirmed the substitution of valine (V) with glutamic acid (E) at position 600 in KTOR81 cells (Figure 1E). The cell viability assay revealed that dabrafenib inhibited the growth of KTOR81 cells with an IC₅₀ of 29.5 nM (Figure 1F). In contrast, the IC₅₀ values for other typical driver oncogene-targeted drugs in lung adenocarcinoma such as alectinib (against ALK) and osimertinib (against EGFR) were markedly higher at 9850 nM and 1070 nM, respectively, and osimertinib failed to induce sufficient cell death. (Figure S1A) Immunoblotting further demonstrated that dabrafenib successfully inhibited the phosphorylation of the downstream kinases *BRAF*, MEK, and extracellular signal-regulated kinase (ERK) in KTOR81 cells (Figure 1G).

3.2 | Novel TEAD1 Inhibitor VT103 Inhibits YAP1/TEAD1-Mediated Antiapoptotic Activity in KTOR81 Cells

We first evaluated the expression and localization of YAP1 in KTOR81 cells using immunofluorescence (Figure 2A). Immunofluorescence revealed that YAP1 was expressed and colocalized to the nuclear region, suggesting transcriptional activity of the YAP1/TEAD complex. To confirm the dependence of KTOR81 cells on YAP1 and TEAD1, we genetically knocked down *YAP1* and *TEAD1* using siRNAs. qRT-PCR analysis showed the effective knockdown of these genes (Figure S1B).

In cell proliferation assays, siYAP1 or siTEAD1 effectively inhibited the proliferation of KTOR81 cells with or without dabrafenib exposure (Figures 2B and S1C). Furthermore, the novel TEAD1 inhibitor VT103 successfully inhibited the transcriptional activity of the YAP1/TEAD complex, as measured by *CTGF* expression (Figure 2C), and inhibited the proliferation of KTOR81 when combined with dabrafenib (Figure 2D). Additionally, when trametinib was combined with dabrafenib, VT103 still demonstrated a significant additive inhibitory effect on cell proliferation (Figure S1D). VT103 monotherapy did not significantly inhibit cell proliferation (Figure S1E).

One possible mechanism by which YAP1-TEAD1 is involved in the drug resistance of tumor cells is the antiapoptotic pathway. In the evaluation of apoptotic activity measured by a caspase 3/7 assay, we found that the combination of dabrafenib and siYAP1, siTEAD1, or VT103 resulted in significant activation of apoptosis compared with the control (Figure 2E,F). However, treatment with siYAP1 or siTEAD1 without dabrafenib did not induce apoptosis (Figure S1F). These results suggested that YAP1/TEAD1 inhibition in combination with dabrafenib activated apoptosis and inhibited the proliferation of KTOR81 cells.

3.3 | *BIRC5*/Survivin Could Be a Functional Antiapoptotic Gene/Molecule in KTOR81 Cells

Next, we sought to identify the mediator of apoptosis transcriptionally regulated by the YAP1/TEAD1 complex. By qRT-PCR analysis of major apoptosis-related genes previously reported to be regulated by the YAP1/TEAD complex, *BIRC5* was found significantly downregulated by the combination of dabrafenib and VT103 (Figure 3A). To verify direct regulation of *BIRC5* by the YAP1/TEAD1 complex, we performed ChIP-qPCR analysis. Both YAP1 and TEAD1 showed significant enrichment at putative binding motifs within an enhancer region upstream of the *BIRC5* gene, confirming direct interaction of YAP1 and TEAD1 with the *BIRC5* regulatory elements (Figure S2A–D). Immunoblotting analysis showed downregulation of survivin, which is encoded by the *BIRC5* gene, caused by the combination therapy of dabrafenib and gene knockdown of *YAP1/TEAD1* or dabrafenib and VT103 (Figure 3B,C). Furthermore, dabrafenib and VT103 led to higher levels of downregulation than gene knockdown of *YAP1/TEAD1* alone (Figure S3A). To further validate the importance of the YAP1/TEAD1 pathway in regulating *BIRC5*/survivin transcription, we performed knockdown experiments of TEAD1-4. siTEAD1, with two different sequences, consistently suppressed *BIRC5* transcription, and this transcriptional inhibition was maintained when combined with dabrafenib. In contrast, knockdown of TEAD2-4 did not consistently affect *BIRC5* expression (Figure S3B–D). To confirm the function of survivin, we evaluated the phenotypic changes caused by the gene knockdown of *BIRC5* (S3E). In cell proliferation and caspase 3/7 assays, siBIRC5, in combination with dabrafenib, significantly inhibited proliferation and activation of apoptosis (Figures 3D,E and S3F). These results suggested that the downregulation of *BIRC5*/survivin could be a potential mechanism of apoptosis induced by the combination of dabrafenib and VT103 in KTOR81 cells.

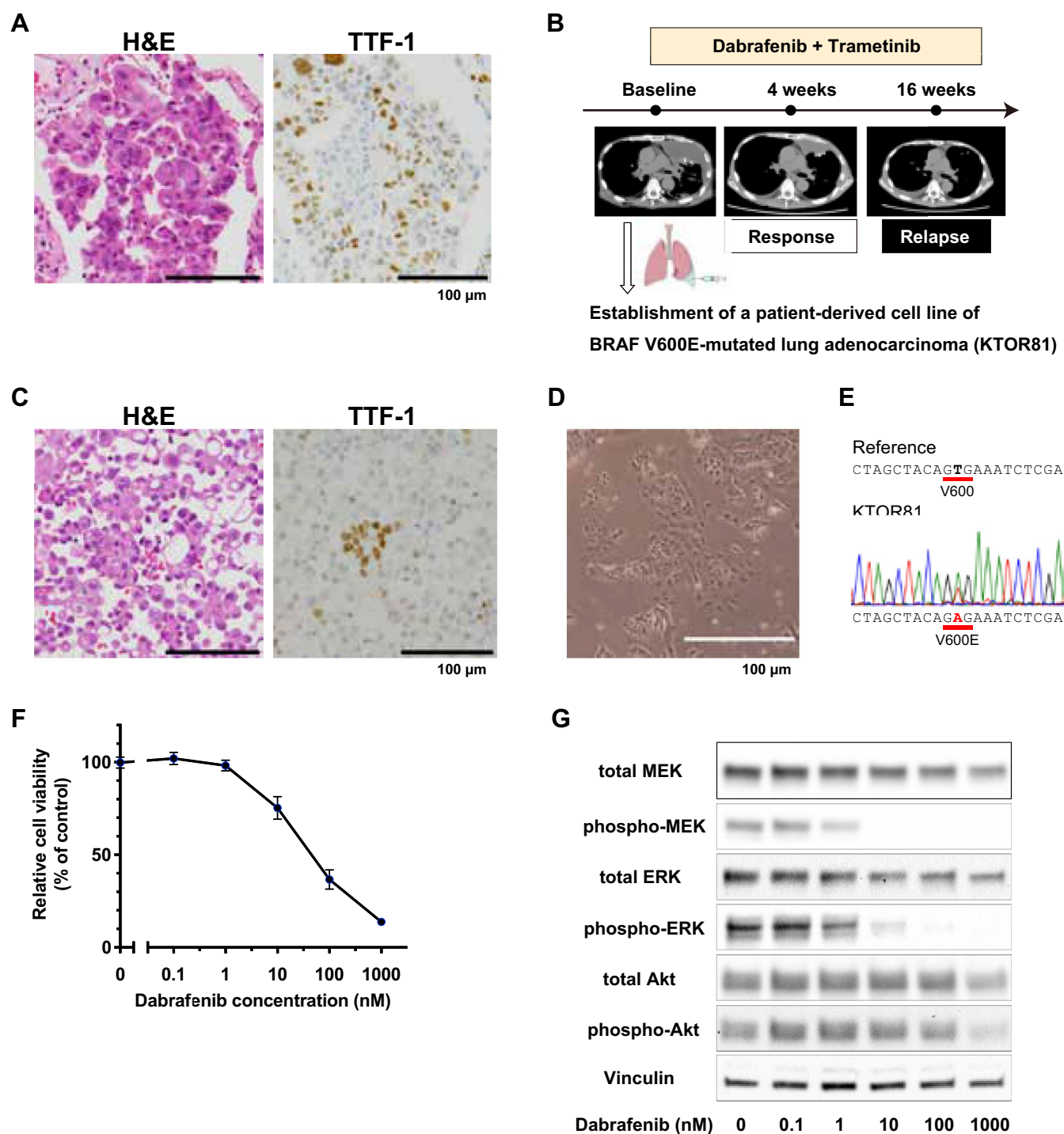


FIGURE 1 | Establishment of a BRAF V600E-mutated patient-derived LUAD cell line. (A) Representative hematoxylin and eosin (H&E) staining and TTF-1 immunohistochemistry of the surgically resected specimen from a patient with BRAF V600E-mutated LUAD. Scale bar, 100 μ m. (B) Chest computed tomography showing left pleural effusion in the patient 4 months after surgery (baseline, left image), at 4 weeks during dabrafenib + trametinib treatment (middle image), and at 16 weeks with disease progression (right image). (C) H&E staining and TTF-1 immunohistochemistry of the cell block prepared from the patient's malignant pleural effusion. Scale bar, 100 μ m. (D) Phase-contrast micrograph of the KTOR81 cell line established from the patient's pleural effusion. Scale bar, 100 μ m. (E) Sanger sequencing of BRAF exon 15 confirming the V600E mutation in KTOR81 cells. (F) Cell viability assay of KTOR81 cells treated with dabrafenib for 72 h. Data are expressed as mean $\pm$ SD. (G) Immunoblotting analysis of KTOR81 cells treated with DMSO or dabrafenib at the indicated concentrations for 24 h.

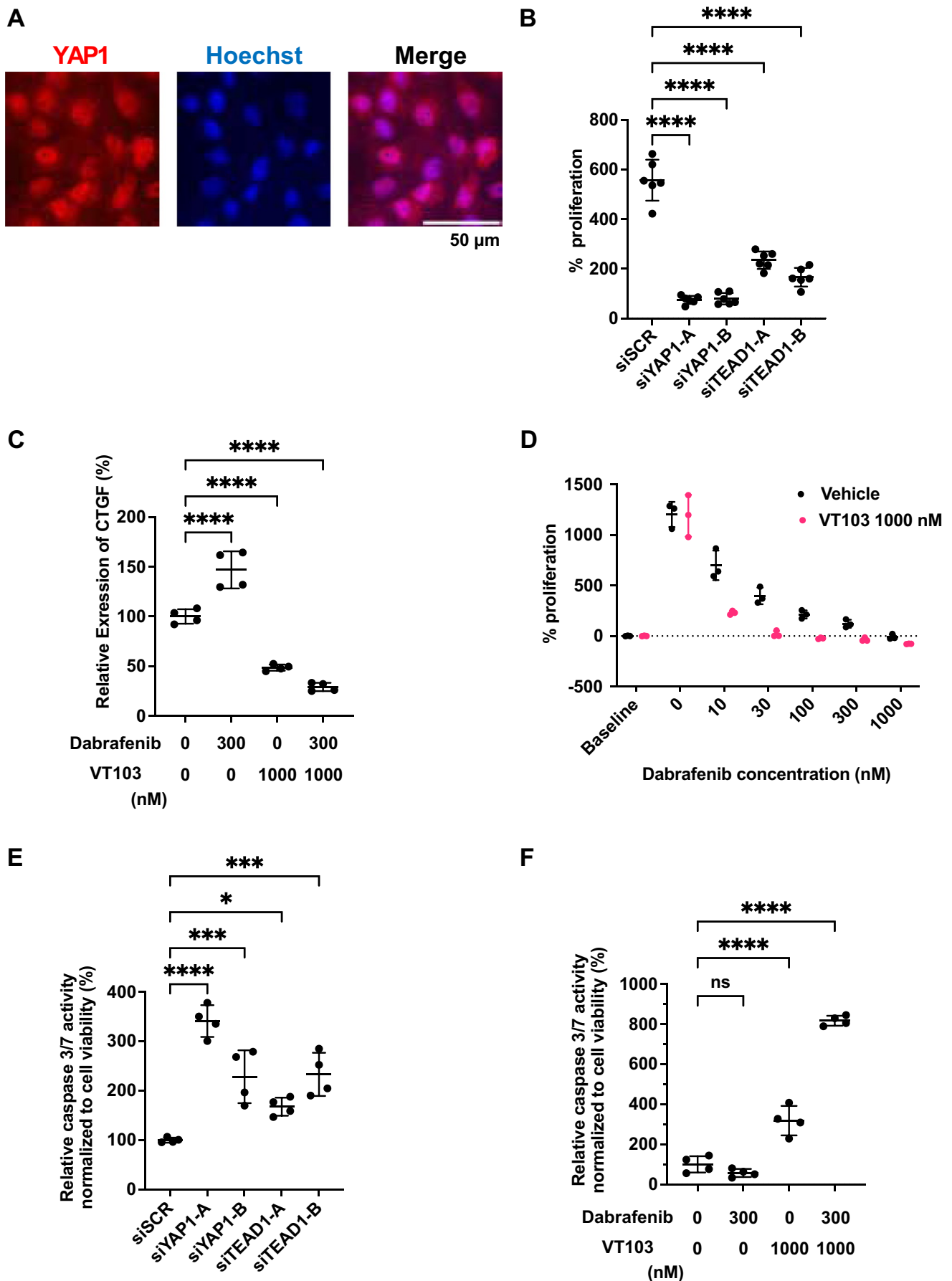


FIGURE 2 | Legend on next page.

FIGURE 2 | Novel TEAD1 inhibitor VT103 inhibits YAP1/TEAD1-mediated antiapoptotic activity in KTOR81 cells. (A) Immunofluorescence staining of YAP1 (red) and nuclei (blue) in KTOR81 cells. Scale bar, 50 μ m. (B) Cell proliferation assay of KTOR81 cells transfected with siRNA targeting scrambled control, YAP1, or TEAD1 and treated with 300 nM dabrafenib for 72 h. Data are expressed as mean $\pm$ SD. (C) qRT-PCR analysis of CTGF expression in KTOR81 cells treated with DMSO, dabrafenib, VT103, or dabrafenib + VT103 for 72 h. Data are expressed as mean $\pm$ SD. (D) Cell proliferation assay of KTOR81 cells treated with dabrafenib compared between with and without VT103 supplementation for 168 h. Data are expressed as mean $\pm$ SD. (E, F) Caspase-3/7 activity in KTOR81 cells transfected with siRNA targeting YAP1 or TEAD1 (E) or treated with VT103 (F) in combination with 300 nM dabrafenib for 24 h. Data are expressed as mean $\pm$ SD. Asterisks indicate statistical significance: * p < 0.05, ** p < 0.01, *** p < 0.001, **** p < 0.0001.

3.4 | Xenograft Models Showed That Dabrafenib + VT103 Combination Therapy Could Be Effective and Tolerable

Furthermore, we confirmed the effectiveness of the dabrafenib and VT103 combination therapy in a xenograft model. We inoculated KTOR81xeno cells, which were derived from successfully engrafted xenograft tumors, subcutaneously into female BALB/c-nu mice. During 14 days of therapy, both dabrafenib monotherapy and dabrafenib and VT103 combination therapy induced significant tumor shrinkage (Figure 4A). The dabrafenib monotherapy group showed significant tumor regrowth 42 days after treatment initiation. In contrast, the dabrafenib + VT103 combination therapy showed a sustained response during the observation period (Figure 4B). No significant adverse effects were observed on the body weight of the mice, suggesting the tolerability of the combination therapy (Figure 4C).

Additionally, we performed immunohistochemical staining for YAP1, TEAD1, and survivin in xenograft tumors harvested on day 7 (Figure S4A,B). The expression levels of survivin were significantly lower in the dabrafenib + VT103 group than in the vehicle group, which was consistent with the proposed mechanism of action.

In summary of the preclinical experiments on KTOR81 cells, the combination of dabrafenib and VT103 successfully enhanced therapeutic efficacy over dabrafenib monotherapy and that this effect was induced by the apoptotic activity as a result of BIRC5/survivin (Figure 4D).

3.5 | TMA and TCGA-LUAD Analyses Reveal Correlation Between YAP1/TEAD1 Activity and Survivin Expression in LUAD and Suggest BIRC5/Survivin as a Prognostic Marker in Driver Oncogene-Positive Patients

Because of the rarity of the *BRAF* V600E mutation in LUAD, it is challenging to conduct large-scale studies focusing on this specific subset. To investigate whether the YAP1/TEAD1-survivin mechanism observed in KTOR81 cells was generally observed in LUAD, we performed immunohistochemical staining for YAP1, TEAD1, and survivin in a TMA consisting of 329 LUAD cases. The patient characteristics were summarized in Table S3. The expression levels of YAP1, TEAD1, and survivin were quantified using H-scores. Figure 5A shows two representative cases of immunohistochemical staining for YAP1, TEAD1, and survivin in LUAD tissues. Pearson's correlation analysis confirmed that the H-scores of YAP1 and TEAD1 (Figure 5B), as well as

those of TEAD1 and survivin (Figure 5C) or YAP1 and survivin (Figure S5A), were highly correlated (YAP1 vs. TEAD1: $R^2=0.37$, $p<0.001$; TEAD1 vs. survivin: $R^2=0.70$, $p<0.001$, YAP1 vs. survivin: $R^2=0.34$, $p<0.001$), suggesting that the expression levels of YAP1, TEAD1, and survivin are strongly associated in LUAD tissues, supporting the existence of the YAP1/TEAD1-survivin axis. Among the 329 patients, 80 patients who experienced recurrence were included in the survival analysis (Table S4). Kaplan–Meier survival analysis using TMA data showed that the expression levels of YAP1 (Figure S5B), TEAD1 (Figure S5C), and survivin (Figure 5D) did not significantly correlate with postrecurrence OS. However, when we stratified patients based on the presence or absence of driver oncogenes (*EGFR*, *ALK*, *ROS1*, *BRAF*, or *HER2*), high survivin expression was associated with substantially shorter postrecurrence OS in patients with LUAD with driver oncogenes (Figure 5E, HR 1.68 [95% CI 0.17–2–12], $p=0.42$), while this trend was not observed in patients with LUAD without driver oncogenes (Figure S5D). To further validate these findings in a more broad population, we analyzed public TCGA-LUAD dataset. Gene Set Variation Analysis (GSVA) of the Hippo-YAP signaling pathway from WikiPathways revealed a significant positive correlation with *BIRC5* expression across all cases (Figure S6A). Survival analysis demonstrated that high *BIRC5* expression was significantly associated with shorter overall survival in the entire cohort (Figure S6B). In the subset of patients with driver oncogene-positive LUAD, these trends persisted but did not reach statistical significance (Figure S6C,D). Our TMA analysis suggests that the YAP1/TEAD1-survivin axis is widely activated in LUAD tissues and that high survivin expression could serve as a negative prognostic biomarker for postrecurrence OS, especially in patients with driver oncogene-positive LUAD. The TCGA analysis provides additional support for the broader clinical relevance of *BIRC5*/survivin expression in LUAD prognosis.

4 | Discussion

In this study, we established a patient-derived LUAD cell line harboring the *BRAF* V600E mutation. Using this unique resource, we demonstrated the potential additive effects of VT103, a novel TEAD1 inhibitor, in combination with standard *BRAF*-targeted therapy. Moreover, immunohistochemical analysis suggested the existence of a YAP1/TEAD1-survivin axis in a broader population of patients with LUAD, highlighting its potential as a therapeutic target.

The *BRAF* V600E mutation is a rare oncogenic driver of LUAD, accounting for only 2% of all LUAD cases [4]. To our knowledge, only one cell line, HCC364, has been previously reported but

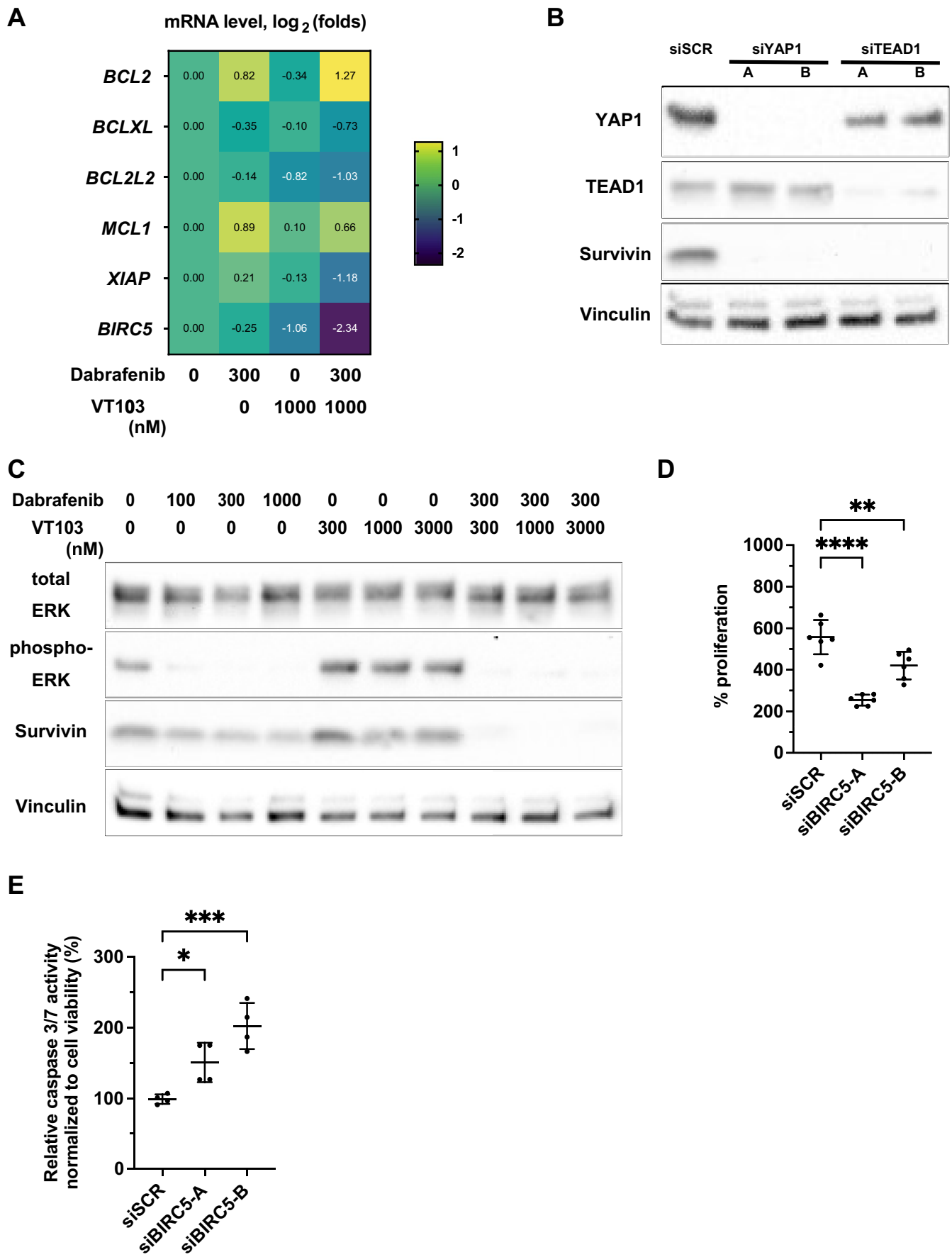


FIGURE 3 | Legend on next page.

FIGURE 3 | BIRC5/Survivin is a functional antiapoptotic gene/molecule in KTOR81 cells. (A) qRT-PCR analysis of apoptosis-related genes in KTOR81 cells treated with DMSO, dabrafenib, VT103, or dabrafenib + VT103 for 72 h. (B) Immunoblotting analysis of YAP1, TEAD1, and survivin in KTOR81 cells transfected with siRNA targeting scrambled control, YAP1, or TEAD1 and treated with dabrafenib for 72 h. (C) Immunoblotting analysis of YAP1, TEAD1, and survivin in KTOR81 cells treated with indicated doses of dabrafenib and/or VT103 for 72 h. (D) Cell proliferation assay of KTOR81 cells transfected with siRNA targeting scrambled control or BIRC5 and treated with DMSO or dabrafenib for 72 h. (E) Caspase-3/7 activity in KTOR81 cells transfected with siRNA targeting scrambled control or BIRC5 and treated with dabrafenib for 24 h. Asterisks indicate statistical significance: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ and **** $p < 0.0001$.

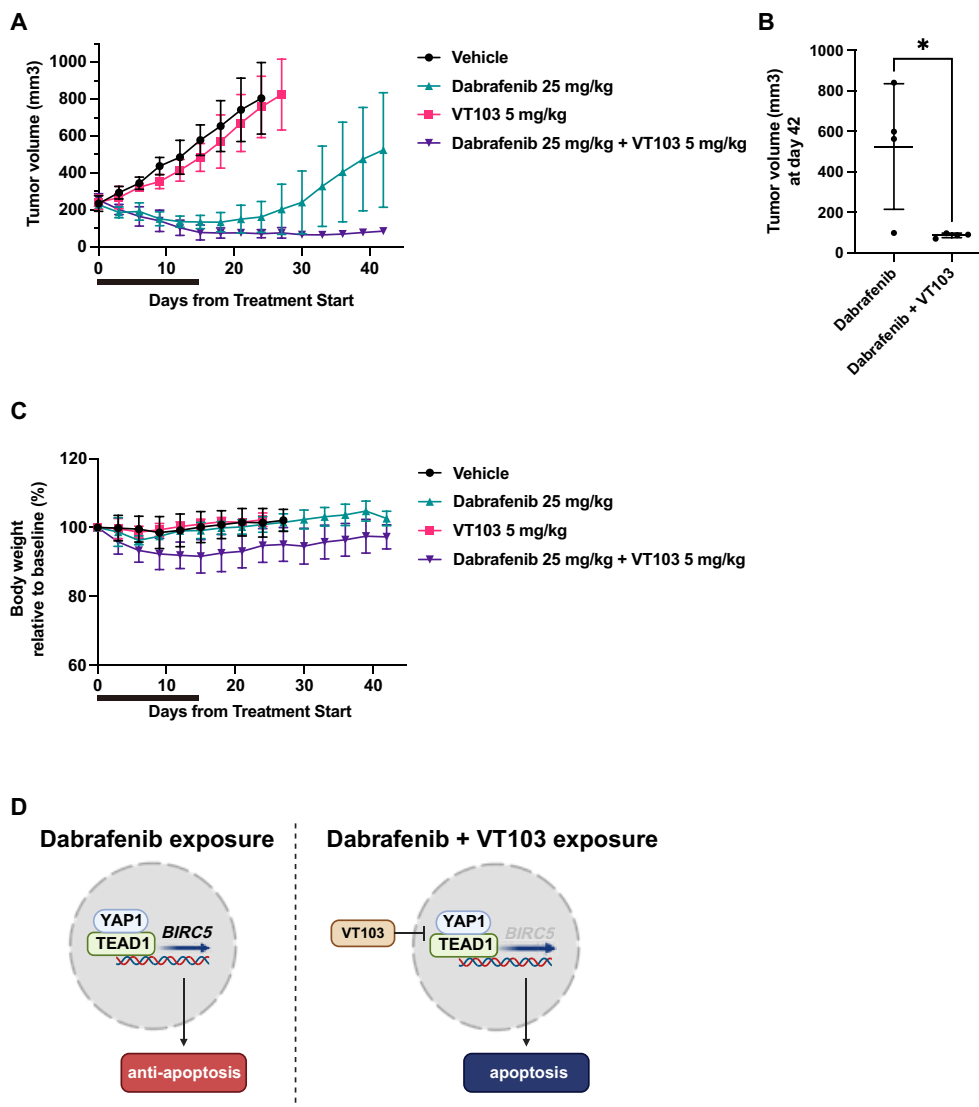


FIGURE 4 | Dabrafenib + VT103 combination therapy is effective and tolerable in xenograft models. (A) Tumor volume of KTOR81xeno xenografts treated with vehicle, dabrafenib, VT103, or dabrafenib + VT103 for 14 days ($n = 6$ per group). (B) Tumor volumes of KTOR81xeno xenografts at Day 42 treated with dabrafenib with or without VT103. (C) Body weight of mice bearing KTOR81xeno xenografts treated as in (A) for 14 days ($n = 6$ per group). (D) Schematic summary of the proposed mechanism by which the combination of dabrafenib and VT103 enhances therapeutic efficacy in BRAF V600E-mutated LUAD cells. Asterisks indicate statistical significance: * $p < 0.05$.

appears to have been used exclusively in the United States and is not commercially available [13]. Therefore, our KTOR81 cell line provides a valuable resource for investigating BRAF V600E-mutated LUAD.

The Hippo pathway, with YAP1-TEAD complexes as key downstream effectors, has attracted considerable interest in cancer biology since its discovery in developmental studies [8, 9].

Numerous reports have implicated YAP1 and TEADs in resistance to targeted therapies [14–19]. However, verteporfin, a classical YAP1 inhibitor, has shown limitations in clinical applications due to photosensitivity and short half-life [20–22]. Recently, several novel YAP1-TEAD inhibitors have been developed to address these limitations. For example, IAG933, which disrupts YAP1-TEAD interaction, demonstrated efficacy against RAS-MAPK altered cancer cells when combined with MAPK

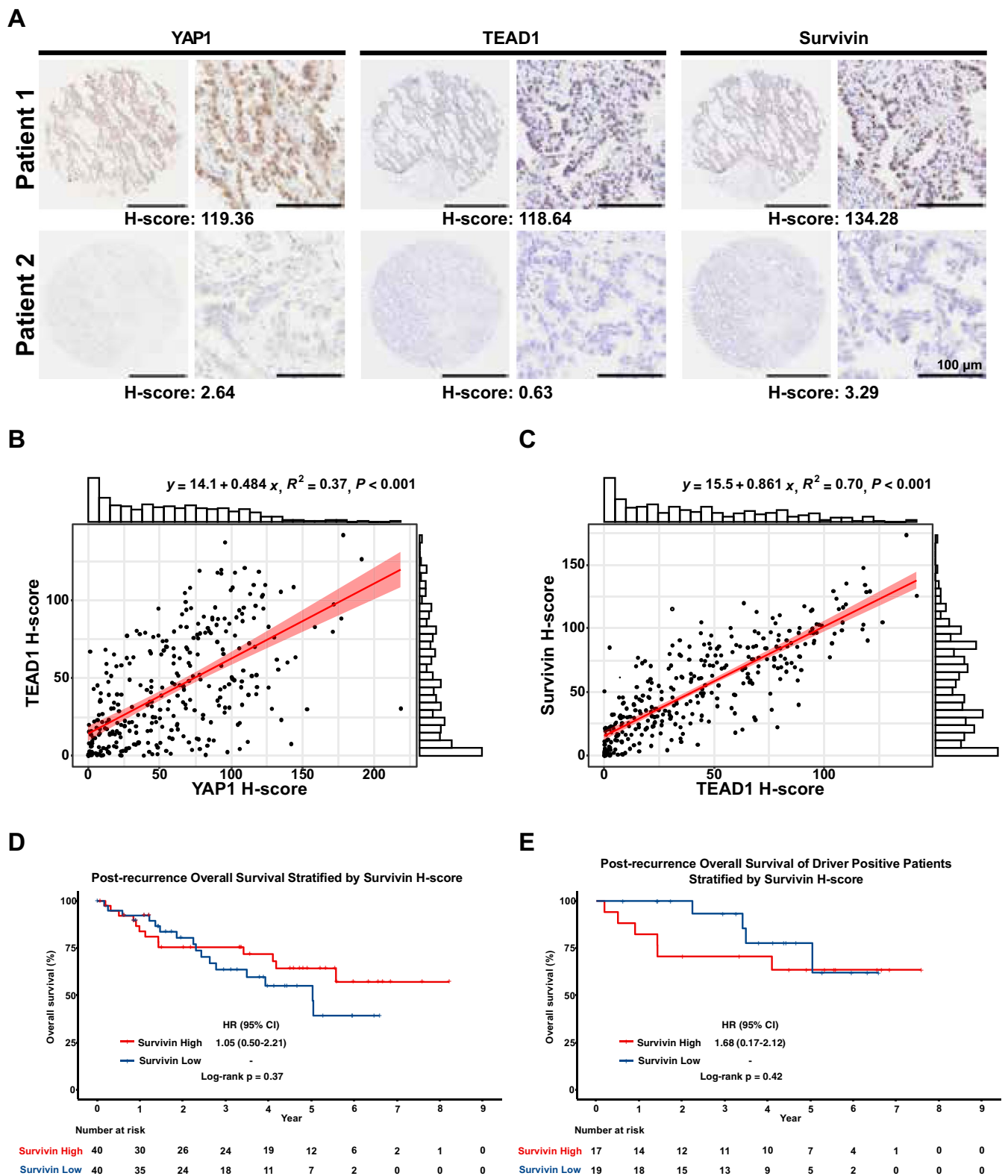


FIGURE 5 | YAP1, TEAD1, and survivin expression levels are highly correlated in LUAD tissue microarrays. (A) Representative immunohistochemical staining of YAP1, TEAD1, and survivin in two LUAD cases from the tissue microarray. Scale bar, 100 μ m. (B,C) Scatter plots and Pearson's correlation analysis of the H-scores for YAP1 and TEAD1 (B) or TEAD1 and survivin (C) in the LUAD tissue microarray. (D) Kaplan-Meier survival analysis of postrecurrence overall survival of patients with LUAD stratified by survivin expression level in the tissue microarray. (E) Kaplan-Meier survival analysis of postrecurrence overall survival of patients with driver oncogene-positive LUAD stratified by survivin expression level in the tissue microarray.

pathway inhibitors [23]. Similarly, GNE-7883 proved effective against *KRAS* G12C mutated cells when used with *KRAS* G12C inhibitors [24]. Adding to these developments, VT103, a TEAD1-selective autopalmitoylation inhibitor, has shown promising efficacy against NF2-deficient mesothelioma in preclinical models [11]. In the current study, VT103 was effective in combination with dabrafenib against *BRAF* V600E-mutated LUAD cells. No adverse effects of VT103, as measured by body weight, were observed in the preclinical studies using xenograft models. These findings highlight the potential of TEAD inhibitors as a promising strategy for targeting the YAP1-TEAD pathway in cancer treatment, particularly in *BRAF* V600E-mutated LUAD. Although our study is limited to a single cell line model, multiple independent studies have consistently shown that combining MAPK pathway inhibitors with YAP1/TEAD inhibitors yields promising results in various driver oncogene-positive cancers [13, 15, 18, 19, 23, 24]. This collective evidence across different research contexts supports the validity of our approach, despite the constraints of our experimental model.

Survivin, encoded by the *BIRC5* gene, is a well-known antiapoptotic molecule transcriptionally regulated by the YAP1/TEAD1 complex, and it functions through direct or indirect binding to caspases [25, 26]. Survivin is overexpressed in a wide range of human cancers [27], and its overexpression has been associated with poor prognosis in several malignancies [28–30] and chemotherapy resistance [31, 32]. These findings suggest that survivin is a promising target for cancer therapy. Several clinical trials have investigated the inhibition of survivin as a therapeutic strategy; however, these attempts have yielded negative results. For example, YM155, a small-molecule inhibitor of survivin that disrupts the interaction between the upstream transcription factor specificity protein 1 and the promoter region of *BIRC5* [33, 34], has been evaluated in several clinical trials. Despite its promising mechanism of action, YM155 has failed to demonstrate significant clinical benefits [35–37], possibly because of the uncertainty of its molecular target and its short half-life [33]. In the current study, we demonstrated that inhibiting the upstream YAP1/TEAD1 complex with VT103 suppressed survivin transcription, thereby enhancing the antitumor effects of dabrafenib in a *BRAF* V600E-mutated LUAD cell line. Our findings suggest that indirect inhibition of survivin by targeting YAP1/TEAD1 could be a more effective approach.

While previous studies using HCC364 cells identified Bcl-xL as a key mediator of antiapoptotic effects through the YAP/TEAD pathway [13], our study highlighted survivin as the most significant mediator. This discrepancy could be attributed to differences in cell lines or specific inhibitors used and underscores the importance of further validation in additional *BRAF* V600E-mutated LUAD models.

Immunohistochemical analysis of TMA revealed a strong correlation between the expression levels of YAP1, TEAD1, and survivin, suggesting that the YAP1/TEAD1-survivin axis may be active in LUAD beyond *BRAF* V600E-mutated cases. This was further supported by transcriptome analysis of the TCGA-LUAD dataset, which demonstrated a significant positive correlation between Hippo-YAP pathway activity and *BIRC5* expression. These findings suggest that YAP1/TEAD1 inhibition strategies, such as the combination of VT103 with targeted

therapies, may be effective in a broader subset of patients with LUAD. Although the expression levels of these proteins were not prognostic indicators in our entire LUAD cohort, we suggest that survivin expression could be a prognostic biomarker, especially in patients with driver oncogene-positive LUAD. Consistently, analysis of the TCGA-LUAD cohort showed that high *BIRC5* expression was associated with poor survival in the entire cohort, with a similar trend observed in driver oncogene-positive cases, although not reaching statistical significance. Previous studies on the prognostic significance of YAP1 and survivin have shown variable results [38–41]. The lack of correlation in our entire cohort might reflect our unique study design using early-stage surgical specimens to evaluate postrecurrence survival, unlike previous studies that focused on advanced-stage tumors and palliative treatment outcomes. Given that driver oncogene-positive tumors represent a biologically distinct population [42, 43], our observation that high survivin expression may be associated with poor prognosis specifically in the driver oncogene-positive population is reasonable. This finding suggests that targeting the YAP1/TEAD1-survivin axis could be particularly promising for patients with driver oncogene-positive LUAD. While multiple factors are known to regulate survivin expression, our comprehensive analyses using both TMA and TCGA-LUAD dataset demonstrate that Hippo-YAP pathway activation substantially contributes to survivin expression regulation in LUAD. This mechanistic insight provides a rational basis for exploring TEAD1 inhibition as a therapeutic strategy.

Our study had several limitations. First, we used only one cell line, which is a constraint given the rarity of *BRAF* V600E mutations and the lack of commercially available cell lines. Second, we used dabrafenib monotherapy in preclinical models, which differs from the clinical combination with MEK inhibitors that was developed after early melanoma trials revealed paradoxical secondary malignancies with *BRAF* inhibitor monotherapy [44–46]. Although this combination therapy has become standard for *BRAF* V600E-mutated cancers, preclinical studies, including ours, often use *BRAF* inhibitor monotherapy as a baseline to avoid off-target effects and experimental complexity [13, 47–49]. Third, we focused solely on apoptosis, although other pathways such as epithelial-mesenchymal transition and metabolic regulation could be involved in the antitumor effects of YAP1/TEAD1 inhibition. Despite these limitations, our findings provide a foundation for future studies to validate the efficacy of YAP1/TEAD1 inhibition in combination with *BRAF*-targeted therapy in a broader context and to investigate additional mechanisms of action.

In conclusion, we established a new clinically derived *BRAF* V600E-mutated LUAD cell line and validated the additive effects of the novel YAP1/TEAD1 inhibitor VT103 on standard *BRAF* inhibition therapy with possible involvement of survivin. While our study's primary limitation lies in the use of a single cell line model, our TMA analyses revealed the fundamental role of the YAP1/TEAD1-survivin axis across a broader range of LUAD cases. These findings, demonstrated through our experimental model, TMA analyses, and supported by previous reports, suggest that targeting this pathway may have therapeutic implications beyond *BRAF*-mutated cases and provide a foundation for future therapeutic developments.

Author Contributions

Kazutaka Hosoya: conceptualization, formal analysis, funding acquisition, investigation, methodology, visualization, writing – original draft, writing – review and editing. **Hiroaki Ozasa:** conceptualization, funding acquisition, methodology, project administration, supervision, writing – review and editing. **Hironori Yoshida:** funding acquisition, writing – review and editing. **Hitomi Ajimizu:** funding acquisition, writing – review and editing. **Takahiro Tsuji:** methodology, writing – review and editing. **Masatoshi Yamazoe:** writing – review and editing. **Tatsuya Ogimoto:** writing – review and editing. **Kentaro Hashimoto:** writing – review and editing. **Tomoko Funazo (Yamamoto):** writing – review and editing. **Keiichiro Suminaga:** writing – review and editing. **Yusuke Shima:** writing – review and editing. **Hiroshi Yoshida:** writing – review and editing. **Takashi Nomizo:** writing – review and editing. **Hiroaki Ito:** methodology, resources, writing – review and editing. **Kazuhiro Terada:** methodology, resources, writing – review and editing. **Shigeto Nishikawa:** resources, writing – review and editing. **Toshi Menju:** resources, writing – review and editing. **Akihiko Yoshizawa:** methodology, resources, writing – review and editing. **Hiroshi Date:** resources, writing – review and editing. **Toyohiro Hirai:** supervision, writing – review and editing.

Acknowledgments

We thank Tracy T. Tang from Vivace Therapeutics Inc. for providing us with VT103, developed by their company, and for proofreading and valuable comments for this paper. We thank Kentaro Tsuji from the Department of Diagnostic Pathology, Graduate School of Medicine, Kyoto University, for the valuable assistance with providing the clinical histopathology images. We thank Yuko Maeda from the Department of Respiratory Medicine, Graduate School of Medicine, Kyoto University, for her assistance with the experiments. We thank the Division of Histological Study, Center for Anatomical Studies, Kyoto University, for preparing and staining the microscope slides. We thank the Medical Research Support Center, Graduate School of Medicine, Kyoto University, for research support (use of microscope, Sanger sequencing). The conceptual diagrams in Figures 1B and 4D were created with BioRender.com.

Ethics Statement

Approval of the research protocol by an institutional review board: 0854, R0996, and R2163. Animal Studies: MedKyo21272 and MedKyo22519.

Consent

All informed consent was obtained from the subjects.

Conflicts of Interest

The authors declare no conflicts of interest.

References

1. R. L. Siegel, K. D. Miller, H. E. Fuchs, and A. Jemal, “Cancer Statistics, 2022,” *CA: A Cancer Journal for Clinicians* 72, no. 1 (2022): 7–33, <https://doi.org/10.3322/caac.21708>.
2. A. C. Tan and D. S. W. Tan, “Targeted Therapies for Lung Cancer Patients With Oncogenic Driver Molecular Alterations,” *JCO* 40, no. 6 (2022): 611–625, <https://doi.org/10.1200/JCO.21.01626>.
3. S. S. Ramalingam, J. Vansteenkiste, D. Planchard, et al., “Overall Survival With Osimertinib in Untreated, EGFR-Mutated Advanced NSCLC,” *New England Journal of Medicine* 382, no. 1 (2020): 41–50, <https://doi.org/10.1056/NEJMoa1913662>.
4. P. K. Paik, M. E. Arcila, M. Fara, et al., “Clinical Characteristics of Patients With Lung Adenocarcinomas Harboring BRAF Mutations,”

Journal of Clinical Oncology 29, no. 15 (2011): 2046–2051, <https://doi.org/10.1200/JCO.2010.33.1280>.

5. H. Davies, G. R. Bignell, C. Cox, et al., “Mutations of the BRAF Gene in Human Cancer,” *Nature* 417, no. 6892 (2002): 949–954, <https://doi.org/10.1038/nature00766>.
6. J. Owsley, M. K. Stein, J. Porter, et al., “Prevalence of Class I–III BRAF Mutations Among 114,662 Cancer Patients in a Large Genomic Database,” *Experimental Biology and Medicine* (Maywood, N.J.) 246, no. 1 (2021): 31–39, <https://doi.org/10.1177/1535370220959657>.
7. D. Planchard, E. F. Smit, H. J. M. Groen, et al., “Dabrafenib Plus Trametinib in Patients With Previously Untreated BRAFV600E-Mutant Metastatic Non-Small-Cell Lung Cancer: An Open-Label, Phase 2 Trial,” *Lancet Oncology* 18, no. 10 (2017): 1307–1316, [https://doi.org/10.1016/S1470-2045\(17\)30679-4](https://doi.org/10.1016/S1470-2045(17)30679-4).
8. S. Wu, J. Huang, J. Dong, and D. Pan, “Hippo Encodes a Ste-20 Family Protein Kinase That Restricts Cell Proliferation and Promotes Apoptosis in Conjunction With Salvador and Warts,” *Cell* 114, no. 4 (2003): 445–456, [https://doi.org/10.1016/S0092-8674\(03\)00549-X](https://doi.org/10.1016/S0092-8674(03)00549-X).
9. K. F. Harvey, C. M. Pflieger, and I. K. Hariharan, “The Drosophila Mst Ortholog, Hippo, Restricts Growth and Cell Proliferation and Promotes Apoptosis,” *Cell* 114, no. 4 (2003): 457–467, [https://doi.org/10.1016/S0092-8674\(03\)00557-9](https://doi.org/10.1016/S0092-8674(03)00557-9).
10. P. C. Calses, J. J. Crawford, J. R. Lill, and A. Dey, “Hippo Pathway in Cancer: Aberrant Regulation and Therapeutic Opportunities,” *Trends Cancer* 5, no. 5 (2019): 297–307, <https://doi.org/10.1016/j.trecan.2019.04.001>.
11. T. T. Tang, A. W. Konradi, Y. Feng, et al., “Small Molecule Inhibitors of TEAD Auto-Palmitoylation Selectively Inhibit Proliferation and Tumor Growth of NF2-Deficient Mesothelioma,” *Molecular Cancer Therapeutics* 20, no. 6 (2021): 986–998, <https://doi.org/10.1158/1535-7163.MCT-20-0717>.
12. R. Ihaka and R. Gentleman, “R: A Language for Data Analysis and Graphics,” *Journal of Computational and Graphical Statistics* 5, no. 3 (1996): 299–314.
13. L. Lin, A. J. Sabnis, E. Chan, et al., “The Hippo Effector YAP Promotes Resistance to RAF- and MEK-Targeted Cancer Therapies,” *Nature Genetics* 47, no. 3 (2015): 250–256, <https://doi.org/10.1038/ng.3218>.
14. M. R. Yun, H. M. Choi, Y. W. Lee, et al., “Targeting YAP to Overcome Acquired Resistance to ALK Inhibitors in ALK-Rearranged Lung Cancer,” *EMBO Molecular Medicine* 11, no. 12 (2019): e10581, <https://doi.org/10.15252/emmm.201910581>.
15. T. Tsuji, H. Ozasa, W. Aoki, et al., “YAP1 Mediates Survival of ALK-Rearranged Lung Cancer Cells Treated With Alectinib via Pro-Apoptotic Protein Regulation,” *Nature Communications* 11, no. 1 (2020): 74, <https://doi.org/10.1038/s41467-019-13771-5>.
16. K. J. Kurppa, Y. Liu, C. To, et al., “Treatment-Induced Tumor Dormancy Through YAP-Mediated Transcriptional Reprogramming of the Apoptotic Pathway,” *Cancer Cell* 37, no. 1 (2020): 104–122.e12, <https://doi.org/10.1016/j.ccell.2019.12.006>.
17. M. B. Nilsson, H. Sun, J. Robichaux, et al., “A YAP/FOXO1 Axis Mediates EMT-Associated EGFR Inhibitor Resistance and Increased Expression of Spindle Assembly Checkpoint Components,” *Science Translational Medicine* 12, no. 559 (2020): eaaz4589, <https://doi.org/10.1126/scitranslmed.aaz4589>.
18. M. Yamazoe, H. Ozasa, T. Tsuji, et al., “Yes-Associated Protein 1 Mediates Initial Cell Survival During Lorlatinib Treatment Through AKT Signaling in ROS1-Rearranged Lung Cancer,” *Cancer Science* 114, no. 2 (2023): 546–560, <https://doi.org/10.1111/cas.15622>.
19. T. Ogimoto, H. Ozasa, T. Tsuji, et al., “Combination Therapy With EGFR Tyrosine Kinase Inhibitors and TEAD Inhibitor Increases Tumor Suppression Effects in EGFR Mutation-Positive Lung Cancer,”

- Molecular Cancer Therapeutics* 23, no. 4 (2024): 564–576, <https://doi.org/10.1158/1535-7163.MCT-23-0371>.
20. J. M. Houle and H. A. Strong, “Duration of Skin Photosensitivity and Incidence of Photosensitivity Reactions After Administration of Verteporfin,” *Retina* 22, no. 6 (2002): 691–697, <https://doi.org/10.1097/00006982-200212000-00002>.
 21. J. M. Houle and A. Strong, “Clinical Pharmacokinetics of Verteporfin,” *Journal of Clinical Pharmacology* 42, no. 5 (2002): 547–557, <https://doi.org/10.1177/0091270022011607>.
 22. Treatment of Age-Related Macular Degeneration With Photodynamic Therapy (TAP) Study Group, “Photodynamic Therapy of Subfoveal Choroidal Neovascularization in Age-Related Macular Degeneration With Verteporfin: One-Year Results of 2 Randomized Clinical Trials—TAP Report 1,” *Archives of Ophthalmology* 117, no. 10 (1999): 1329–1345, <https://doi.org/10.1001/archophth.117.10.1329>.
 23. E. A. Chapeau, L. Sansregret, G. G. Galli, et al., “Direct and Selective Pharmacological Disruption of the YAP-TEAD Interface by IAG933 Inhibits Hippo-Dependent and RAS-MAPK-Altered Cancers,” *Nature Cancer* 5, no. 7 (2024): 1102–1120, <https://doi.org/10.1038/s43018-024-00754-9>.
 24. T. J. Hagenbeek, J. R. Zbieg, M. Hafner, et al., “An Allosteric Pan-TEAD Inhibitor Blocks Oncogenic YAP/TAZ Signaling and Overcomes KRAS G12C Inhibitor Resistance,” *Nature Cancer* 4, no. 6 (2023): 812–828, <https://doi.org/10.1038/s43018-023-00577-0>.
 25. S. Shin, B. J. Sung, Y. S. Cho, et al., “An Anti-Apoptotic Protein Human Survivin Is a Direct Inhibitor of Caspase-3 and -7,” *Biochemistry* 40, no. 4 (2001): 1117–1123, <https://doi.org/10.1021/bi001603q>.
 26. Z. Song, X. Yao, and M. Wu, “Direct Interaction Between Survivin and Smac/DIABLO Is Essential for the Anti-Apoptotic Activity of Survivin During Taxol-Induced Apoptosis,” *Journal of Biological Chemistry* 278, no. 25 (2003): 23130–23140, <https://doi.org/10.1074/jbc.M300957200>.
 27. V. E. Velculescu, S. L. Madden, L. Zhang, et al., “Analysis of Human Transcriptomes,” *Nature Genetics* 23, no. 4 (1999): 387–388, <https://doi.org/10.1038/70487>.
 28. H. Kawasaki, D. C. Altieri, C. D. Lu, M. Toyoda, T. Tenjo, and N. Tanigawa, “Inhibition of Apoptosis by Survivin Predicts Shorter Survival Rates in Colorectal Cancer,” *Cancer Research* 58, no. 22 (1998): 5071–5074.
 29. H. S. Swana, D. Grossman, J. N. Anthony, R. M. Weiss, and D. C. Altieri, “Tumor Content of the Antiapoptosis Molecule Survivin and Recurrence of Bladder Cancer,” *New England Journal of Medicine* 341, no. 6 (1999): 452–453, <https://doi.org/10.1056/NEJM199908053410614>.
 30. D. Grossman, J. M. McNiff, F. Li, and D. C. Altieri, “Expression and Targeting of the Apoptosis Inhibitor, Survivin, in Human Melanoma,” *Journal of Investigative Dermatology* 113, no. 6 (1999): 1076–1081, <https://doi.org/10.1046/j.1523-1747.1999.00776.x>.
 31. J. J. Virrey, S. Guan, W. Li, A. H. Schönthal, T. C. Chen, and F. M. Hofman, “Increased Survivin Expression Confers Chemoresistance to Tumor-Associated Endothelial Cells,” *American Journal of Pathology* 173, no. 2 (2008): 575–585, <https://doi.org/10.2353/ajpath.2008.071079>.
 32. L. Chen, L. Liang, X. Yan, et al., “Survivin Status Affects Prognosis and Chemosensitivity in Epithelial Ovarian Cancer,” *International Journal of Gynecological Cancer* 23, no. 2 (2013): 256–263, <https://doi.org/10.1097/IGC.0b013e31827ad2b8>.
 33. T. Nakahara, A. Kita, K. Yamanaka, et al., “YM155, a Novel Small-Molecule Survivin Suppressant, Induces Regression of Established Human Hormone-Refractory Prostate Tumor Xenografts,” *Cancer Research* 67, no. 17 (2007): 8014–8021, <https://doi.org/10.1158/0008-5472.CAN-07-1343>.
 34. Q. Cheng, X. Ling, A. Haller, et al., “Suppression of Survivin Promoter Activity by YM155 Involves Disruption of Sp1-DNA Interaction in the Survivin Core Promoter,” *International Journal of Biochemistry and Molecular Biology* 3, no. 2 (2012): 179–197.
 35. G. Giaccone, P. Zatloukal, J. Roubec, et al., “Multicenter Phase II Trial of YM155, a Small-Molecule Suppressor of Survivin, in Patients With Advanced, Refractory, Non-Small-Cell Lung Cancer,” *Journal of Clinical Oncology* 27, no. 27 (2009): 4481–4486, <https://doi.org/10.1200/JCO.2008.21.1862>.
 36. A. W. Tolcher, D. I. Quinn, A. Ferrari, et al., “A Phase II Study of YM155, a Novel Small-Molecule Suppressor of Survivin, in Castration-Resistant Taxane-Pretreated Prostate Cancer,” *Annals of Oncology* 23, no. 4 (2012): 968–973, <https://doi.org/10.1093/annonc/mdr353>.
 37. R. J. Kelly, A. Thomas, A. Rajan, et al., “A Phase I/II Study of Sepantronium Bromide (YM155, Survivin Suppressor) With Paclitaxel and Carboplatin in Patients With Advanced Non-Small-Cell Lung Cancer,” *Annals of Oncology* 24, no. 10 (2013): 2601–2606, <https://doi.org/10.1093/annonc/mdt249>.
 38. B. Vischioni, P. van der Valk, S. W. Span, F. a E. Kruij, J. A. Rodriguez, and G. Giaccone, “Nuclear Localization of Survivin Is a Positive Prognostic Factor for Survival in Advanced Non-Small-Cell Lung Cancer,” *Annals of Oncology* 15, no. 11 (2004): 1654–1660, <https://doi.org/10.1093/annonc/mdh436>.
 39. B. Lu, A. Gonzalez, P. P. Massion, et al., “Nuclear Survivin as a Biomarker for Non-Small-Cell Lung Cancer,” *British Journal of Cancer* 91, no. 3 (2004): 537–540, <https://doi.org/10.1038/sj.bjc.6602027>.
 40. Y. Wang, Q. Dong, Q. Zhang, Z. Li, E. Wang, and X. Qiu, “Overexpression of Yes-Associated Protein Contributes to Progression and Poor Prognosis of Non-Small-Cell Lung Cancer,” *Cancer Science* 101, no. 5 (2010): 1279–1285, <https://doi.org/10.1111/j.1349-7006.2010.01511.x>.
 41. K. Sun, X. de Zhang, X. Y. Liu, and P. Lu, “YAP1 Is a Prognostic Biomarker and Correlated With Immune Cell Infiltration in Pancreatic Cancer,” *Frontiers in Molecular Biosciences* 8 (2021): 625731, <https://doi.org/10.3389/fmolb.2021.625731>.
 42. W. Roh, Y. Geffen, H. Cha, et al., “High-Resolution Profiling of Lung Adenocarcinoma Identifies Expression Subtypes With Specific Biomarkers and Clinically Relevant Vulnerabilities,” *Cancer Research* 82, no. 21 (2022): 3917–3931, <https://doi.org/10.1158/0008-5472.CAN-22-0432>.
 43. J. Z. Xu, C. Gong, Z. F. Xie, and H. Zhao, “Development of an Oncogenic Driver Alteration Associated Immune-Related Prognostic Model for Stage I-II Lung Adenocarcinoma,” *Frontiers in Oncology* 10 (2021): 593022, <https://doi.org/10.3389/fonc.2020.593022>.
 44. L. Boussemaert, E. Routier, C. Mateus, et al., “Prospective Study of Cutaneous Side-Effects Associated With the BRAF Inhibitor Vemurafenib: A Study of 42 Patients,” *Annals of Oncology* 24, no. 6 (2013): 1691–1697, <https://doi.org/10.1093/annonc/mdt015>.
 45. C. Robert, B. Karaszewska, J. Schachter, et al., “Improved Overall Survival in Melanoma With Combined Dabrafenib and Trametinib,” *New England Journal of Medicine* 372, no. 1 (2015): 30–39, <https://doi.org/10.1056/NEJMoa1412690>.
 46. R. Dummer, P. A. Ascierto, H. J. Gogas, et al., “Encorafenib Plus Binimetinib Versus Vemurafenib or Encorafenib in Patients With BRAF-Mutant Melanoma (COLUMBUS): A Multicentre, Open-Label, Randomised Phase 3 Trial,” *Lancet Oncology* 19, no. 5 (2018): 603–615, [https://doi.org/10.1016/S1470-2045\(18\)30142-6](https://doi.org/10.1016/S1470-2045(18)30142-6).
 47. K. L. Jameson, P. K. Mazur, A. M. Zehnder, et al., “IQGAP1 Scaffold-Kinase Interaction Blockade Selectively Targets RAS-MAP Kinase-Driven Tumors,” *Nature Medicine* 19, no. 5 (2013): 626–630, <https://doi.org/10.1038/nm.3165>.
 48. R. Yaeger, Z. Yao, D. M. Hyman, et al., “Mechanisms of Acquired Resistance to BRAF V600E Inhibition in Colon Cancers Converge on

RAF Dimerization and Are Sensitive to Its Inhibition,” *Cancer Research* 77, no. 23 (2017): 6513–6523, <https://doi.org/10.1158/0008-5472.CAN-17-0768>.

49. M. Salton, W. K. Kasprzak, T. Voss, B. A. Shapiro, P. I. Poulikakos, and T. Misteli, “Inhibition of Vemurafenib-Resistant Melanoma by Interference With Pre-mRNA Splicing,” *Nature Communications* 6 (2015): 7103, <https://doi.org/10.1038/ncomms8103>.

Supporting Information

Additional supporting information can be found online in the Supporting Information section.