TIF1β is phosphorylated at serine 473 in colorectal tumor cells through p38 mitogen-activated protein kinase as an oxidative defense mechanism

TIF1β is phosphorylated at Serine 473 in colorectal tumor cells through p38 mitogen-activated protein kinase as an oxidative defense mechanism

Larina Tzu-Wei Shen[1,4], Han-Yi E. Chou[2,3], and Mitsuyasu Kato[4,5*]

[1]Ph.D. Program in Human Biology, School of Integrative and Global Majors, University of Tsukuba, Tsukuba, Japan. [2]Graduate Institute of Oral Biology, School of Dentistry, National Taiwan University, Taipei, Taiwan. [3]Center for Biotechnology, National Taiwan University, Taipei, Taiwan. [4]Department of Experimental Pathology and [5]Transborder Medical Research Center, Faculty of Medicine, University of Tsukuba, 1-1-1 Tennodai, Tsukuba, Ibaraki 305-8575, Japan.

Word Count: 4104

Figure number: 4

* To whom all correspondence should be addressed:

Mitsuyasu Kato, M.D., Ph.D.

Department of Experimental Pathology

Faculty of Medicine

University of Tsukuba

Tel: 81-29-853-3159

Fax: 81-29-853-3944

E-Mail: mit-kato@md.tsukuba.ac.jp

Abbreviations:

TIF1β; Transcription Intermediary Factor 1 beta, ROS; reactive oxygen species, CRC; colorectal cancer, MAPK; mitogen-activated protein kinase, HP1; heterochromatin protein 1

ABSTRACT

TIF1β is a pleiotropic regulator of a diverse range of cellular processes such as DNA repair or gene repression in stem cells. This functional switch depends on phosphorylation at serine residue 473 and multiple pathways exist to accomplish this. However, the effects of exogenous reactive oxygen species (ROS) generated by bacterial flora and dietary metabolites in the colonic lumen or chemotherapy on TIF1β have not been determined. We report here that exposure of colorectal cancer (CRC) cell lines DLD-1 and HCT116 to hydrogen peroxide specifically induces TIF1β Ser473 phosphorylation. Hydrogen peroxide also induces primarily p38 MAPK and some p42/44 MAPK phosphorylation. Chemical inhibition of p38 MAPK and p42/44 MAPK reduced phosphorylation of TIF1β serine 473 and increased CRC cell death upon peroxide exposure. Taken together, this suggests that it is primarily peroxide-induced p38 MAPK that mediates Ser473 phosphorylation and activation of TIF1β to enable more efficient DNA repair to assist in tumor cell survival against exogenous ROS.

Keywords: TIF1β phosphorylation, chromatin, oxidative stress, MAPKs, colorectal cancer

1. Introduction

The protein Transcription Intermediary Factor 1 beta (TIF1β) is an epigenetic modulator that represses errant gene expression via complexing with heterochromatin protein 1 (HP1), allowing it to interact with the Krüppel-associated box (KRAB) repressor domain found in zinc-finger transcription factors [1]. It is highly expressed in hematopoietic stem cells (as a repressive maintenance factor) [2] as well as several types of cancers [3,4,5] and high expression of TIF1β is associated with poor prognosis in colorectal cancer patients [6]. A mediator of multiple homeostatic processes, TIF1β has another key function where it influences DNA repair through HP1 proteins [7] and mediates cellular survival after DNA damage in MCF-7 and HCT116 cells [8]. Whether TIF1β serves as a repressor or a repair modulator seems to be site-dependent, as the phosphorylation site of TIF1β at serine 473 (TIF1β-S473) has been found to regulate TIF1β-HP1 interaction [9] and activate its role in efficient DNA repair and cell survival [10].

Bacterial flora and dietary metabolites produce ROS in the colon and rectum [11] and inadequate antioxidant defense could result in loss of tissue homeostasis and subsequent tumor formation. To this end, several reports have been published that point to ROS involvement in the pathogenesis of colorectal cancer (CRC) [12,13,14]. The ROS hydrogen peroxide (H_2O_2) can modify proteins and nucleic acids and it is thought to work at low levels as a signaling molecule regulating cell survival and growth through modulation of transcription factors [15]. However, even if compensatory mechanisms are activated, microsatellite instability from excessive H_2O_2 would induce DNA damage [16] associated with colorectal cancer development. Therefore, peroxides are implicated in both the

regulation of and progression of diseases and the role they play is dependent on their concentration [17,18]. Although the cellular damage caused by ROS in colorectal cancer has been well studied, less well known is the role of these potential second messengers in regulating gene transcription via phosphorylation of repressor proteins.

In the case of TIF1β, several factors have been reported to induce phosphorylation on the primary activation site Ser473. For example, mitogen-activated protein kinase-activated protein (MAPKAP) kinase 2 (MK2) regulates phosphorylation of TIF1β-S473 in Kaposi's sarcoma-associated herpes virus (KSHV) infection [19]. In early S-phase under normal culture conditions, protein kinase Cδ (PKCδ) regulates TIF1β-S473 phosphorylation for cell cycle progression and cellular proliferation [9]. Temporally dynamic TIF1β-S473 phosphorylation has been found upon growth factor stimulation [20]. On the other hand, TIF1β-S473 phosphorylation may also be induced by nutrient depletion in breast cancer [21]. This diverse pool of regulatory elements establishes Serine 473 as the common activation hub for TIF1β to fulfill its roles of chromatin regulation (DNA damage response) and cell survival and growth. However, little is known about the effect of oxidative stress as an additional modulator of phosphorylation on this crucial site and if activation of the DNA repair mechanism by basal or exogenous ROS could upregulate TIF1β to protect tumor cells by increasing efficiency of DNA repair.

In this study, we hypothesized that, at physiologically relevant concentrations, TIF1β phosphorylation will increases, blunting peroxide-induced cell damage. We found that, in fact, H_2O_2-induced CRC cell death was intensified in TIF1β knockdown conditions. We then discovered that H_2O_2 dose induce phosphorylation of TIF1β-S473. In addition, activation of

p38 MAPK and secondary activation of p42/44 MAPK by ROS may regulate TIF1β-S473 phosphorylation in response to oxidative stress to protect CRC cells from ROS-mediated death.

2. Material and methods

2.1 Cell Lines and Cell Culture

Human colorectal adenocarcinoma HCT116 cells and human embryonic kidney (HEK) 293T cells were maintained in Dulbecco's modified Eagle's medium (DMEM, Invitrogen) supplemented with 10% fetal bovine serum and 1% of a penicillin-streptomycin solution (Gibco) at 37°C in a 5% CO_2-humidified atmosphere. Human colorectal adenocarcinoma cells (DLD-1) were cultured in RPMI 1640 medium (Invitrogen) supplemented with 10% fetal bovine serum and 1% of a penicillin-streptomycin solution at 37℃ in a 5% CO_2-humidified atmosphere. Multiple frozen aliquots were established upon the acquisition and all experimental cells were passaged for fewer than 30 passages after reviving from liquid N2. Cells were washed once after cell growth to a subconfluent state and then made quiescent by incubation in serum- and supplement-free medium for 18 hours (hr) before further experiments.

2.2 H_2O_2 Treatment

A 1M stock solution of H_2O_2 was initially made by diluting a 30% H_2O_2 solution (WAKO) with ultrapure water. For H_2O_2 treatment experiments, cells were serum starved for 18 hr then incubated with 1 mM of H_2O_2 for the indicated periods or with differing concentrations of H_2O_2 for 15 min, and sampled for Western blot analysis. For kinase inhibitor experiments, after serum starvation for 18 hr, cells were incubated with p42/44 MAPK Inhibitor U0126 (10 μM, SIGMA) and/or p38 MAPK inhibitor SB203580 (10μM, Cayman Chemical) for 1 hr, respectively, before H_2O_2 challenge.

2.3 Antibodies

A FLAG epitope tag (M2, Sigma-Aldrich) and β-actin (Sigma-Aldrich) were used. Mouse monoclonal antibodies against TIF1β (20A1) and rabbit polyclonal antibodies against TIF1β-S473 phosphorylation (Poly 6446) were used (BioLegend). Rabbit polyclonal antibodies against p44/42 MAPK (Erk1/2, #9102), phospho-p44/42 MAPK (Thr202/Tyr204) (#9101S), phospho-p38 MAPK (Thr180/Tyr182) (#9211S), and p38 MAPK (#9212) were purchased from Cell Signaling Technologies.

2.4 Transfection and Plasmids

For plasmid transfection, HEK293T cells were grown to 85% confluence and transfected with Lipofectamine 2000 reagent (Invitrogen) in serum-free DMEM for 6 hr, after which the medium was replaced with fresh culture medium. After a further 24-48 hr of incubation, the transfected cells were used in experiments. Expression constructs for FLAG-TIF1β-wild-type, FLAG-TIF1β S473A mutants, and FLAG-TIF1β S473E mutants were previously reported [9]. All plasmids were sequenced before use.

2.5 Western Blotting

Cells were solubilized in NP-40 lysis buffer (20 mM Tris-HCl, ph7.5, 150 mM NaCl, 1% Nonidet P-40, 2000 KIU/ml aprotinin, 1 μg/ml leupeptin). After clearing by centrifugation, total cell lysates or immunoprecipitates obtained using the indicated antibodies were subjected to 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE).

Proteins were electrotransferred to a mixed ester nitrocellulose membrane (Hybond-C Extra, GE Healthcare) and incubated with indicated primary antibodies. Bound antibodies were detected with horseradish peroxidase (HRP)-conjugated antibodies specific for mouse IgG and rabbit IgG (GE Healthcare) and with SuperSignal West Dura Extended Duration Substrate (Thermo Fisher Scientific). An LAS-3000 Image Analyzer (Fuji Photo Film) was used to image the membranes.

2.6 Transient Knockdown of TIF1β Expression

DLD-1 cells were transfected with 40 nM of small interference RNA (siRNA) directed against TIF1β or control siRNA using Lipofectamine 3000 (Invitrogen) according to the manufacturer's protocol. A mixture of three TIF1β siRNAs (Stealth RNA, Invitrogen) was used: siTIF1β #1 (HSS115468), siTIF1β #2 (HSS115469), and siTIF1β #3 (HSS115470). Control siRNA was purchased from Invitrogen (Stealth RNAi Negative Universal Control Medium).

2.7 Cell Viability Assay

We used the MTT assay to measure cell viability. Cells were plated in regular growth medium at $5x10^3$ cells per well in 96-well plates. The following day, cells were incubated with indicated concentrations of H_2O_2 for 15 min, then changed to the culture medium and further incubated for 3 days. At each time point, 10 µl MTT [3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (5 µg/ml; Wako)] was added to each well and incubated for 4 hr. Precipitated formazan crystals were dissolved by the addition of 100 µl isopropanol and the absorbance was measured at 570 nm with a

microplate reader (MTP-300, Corona Electric). Each experiment was conducted at least in triplicate.

3. Results

3.1 Knockdown of TIF1β reduces cell survival of DLD-1 cells after H_2O_2 treatment

To evaluate the effect of hydrogen peroxide on cell survival, we measured cell survival rates using the MTT assay at 72 hr after H_2O_2 treatment. Exposure of human DLD-1 colorectal cancer cells to differing concentrations of H_2O_2 resulted in a dose-dependent decrease of cell survival (Fig. 1A). We then transfected DLD-1 cells with siRNA (siControl and siTIF1β) for 48hr and then exposed them to H_2O_2. These siTIF1β-transfected DLD-1 cells showed a partial knockdown of TIF1β as well as increased sensitivity to H_2O_2 and had lower survival rates than siControl-transfected DLD-1 cells in the range of 0.25 - 1 mM H_2O_2 (Fig. 1B). These results indicate that TIF1β contributes to the resistance to H_2O_2-induced cell death in colorectal cancer cells.

3.2 Hydrogen peroxide induces the specific phosphorylation of TIF1β-S473

To explore the effects of hydrogen peroxide on TIF1β-S473 phosphorylation in human colorectal cancer cell lines DLD-1 and HCT116, cells were treated with differing concentrations of H_2O_2 for 15 minutes (Fig. 2A and 2B) or treated with 1mM of H_2O_2 over a time course (Fig. 2C and 2D). H_2O_2 induced TIF1β-S473 phosphorylation in a dose dependent manner at least up to 2 mM (Fig. 2A and 2B). Increased TIF1β phosphorylation was also observed over the time course, with increased phosphorylation maintained through the 2hr timepoint in both DLD-1 and HCT116 cells (Fig. 2C and 2D).

To verify the specificity of the phospho-TIF1β antibody, ectopically expressed FLAG-TIF1β-wild-type or TIF1β mutants in HEK293T cells were immunoprecipitated with

anti-FLAG M2 antibody-conjugated beads and subjected to Western blotting with anti-phospho-TIF1β-S473 antibody. Unlike wild type TIF1β, phosphorylation of TIF1β-S473 mutants was not detected by the anti-phospho-TIF1β-S473 antibody (Fig. S1A). To further verify the phosphorylation status of the band detected by the phospho-TIF1β specific antibody, cell lysates from HCT116 cells were incubated with lambda phosphatase to remove phosphorous. As shown in Fig. S1B, the band corresponding to TIF1β phosphorylation is reduced with lambda phosphatase treatment, confirming that phospho-TIF1β antibody was specifically detecting the phosphorylated TIF1β-S473.

3.3 Hydrogen peroxide induces the phosphorylation of p42/44 MAPK and p38 MAPK

To determine which kinases are responsible for TIF1β phosphorylation under oxidative stress condition, we examined the phosphorylation of p42/44 MAPK and p38 MAPK after incubation with various concentrations of H_2O_2. Both p42/44 MAPK and p38 MAPK showed dose-dependent phosphorylation in response to H_2O_2 in both DLD-1 cells (Fig. 3A) and HCT116 cells (Fig. 3B). However, the p42/44 MAPK phosphorylation was observed even in the absence of H_2O_2 treatment and dosage-dependent increase of the phosphorylation levels was weaker than that of p38 MAPK in both DLD-1 and HCT116 cells. This indicates that ROS insult does affect phosphorylation status of the MAPK family with p38 MAPK being most susceptible to peroxide-induced phosphorylation and p42/44 MAPK being only tangentially affected.

3.4 Hydrogen peroxide induced phosphorylation of TIF1β-S473 is mediated by both p38

MAPK and p42/44 MAPK

Since we found peroxide-induced-p42/44 MAPK and p38 MAPK phosphorylation in both DLD-1 cells and HCT116 cells, the involvement of the MAPK family in TIF1β phosphorylation upon peroxide treatment was subsequently explored. We used U0126 (a specific inhibitor of p42/44 MAPK) and SB203580 (a specific inhibitor of p38 MAPK) to block activity. U0126 blocked p42/44 MAPK phosphorylation and marginally inhibited TIF1β phosphorylation in DLD-1 cells (Fig. 4A) and in HCT116 cells (Fig. 4B). Inhibition of p38 MAPK by SB203580 suppressed TIF1β phosphorylation more clearly than p42/44 MAPK inhibition and combined use of both inhibitors further suppressed TIF1β phosphorylation in both DLD-1 and HCT116 cells (Fig. 4A and 4B, respectively). These kinase inhibitors (U0126 and SB203580) were found to act in a dose dependent manner in DLD-1 and HCT116 cells (Fig. S2). These results indicate that p38 MAPK serves as the primary modulator of peroxide-induced TIF1β phosphorylation and p42/44 MAPK may play a secondary or backup role.

Discussion

ROS are naturally and ubiquitously generated in all aerobic organisms and primarily serve to trigger the inflammatory response [22] and oxidant-induced stress conditions [23]. However, reactive oxygen also acts as a secondary messenger and is involved in many cellular functions such as apoptosis [24], cell survival [25] and proliferation [26]. Within the scope of colorectal cancer, ROS damage leading to carcinogenesis has been well studied but the effect of reactive oxygen in a signaling or secondary messenger setting has not yet been fully explored.

Recently, several reports have suggested that high expression of TIF1β is related to poor prognosis in colorectal cancer patients [6] as TIF1β induction in tumor cells could engage DNA repair mechanisms in response to chemotherapy [10]. In this case, suppression of TIF1β may make chemotherapy much more effective as we discovered that H_2O_2-induced cell death in CRC cells was enhanced by TIF1β knockdown. In addition, H_2O_2 induced TIF1β phosphorylation at the crucial serine 473 primarily through p38 MAPK with p42/44 MAPK showing secondary involvements. Two critical subgroups of mitogen-activated protein kinases (MAPK) involved in the potential control of tumor cell growth are the extracellular signal-regulated kinases (ERKs or p42/44 MAPK) and the p38 MAPK [27]. ROS, such as hydrogen peroxide, have been reported to activate p42/44 MAPK and p38 MAPK [28] which is well in line with our results. This leads to the conclusion that activation of TIF1β via p38 MAPK may contribute the resistance of cancer cells against ROS at both physiological levels (e.g., ROS generated by oxidized lipids within the feces or from intestinal flora) and chemotherapeutic levels.

The mechanism of this resistance may not be due to TIF1β's ability to suppress gene transcription but rather its ability to enhance DNA repair after complexing with HP1 [29]. TIF1β-mediated recruitment of HP1 to histones, the Ser473 phosphorylation-dependent binding of HP-1 to TIF1β, and phosphorylation-dependent release of HP-1 from TIF1β are required for efficient DNA repair [7]. This cascade relaxes the winding of genomic DNA around the histones, facilitating effective repair. Therefore, any exogenous factor that decreases the affinity of TIF1β for HP1 by increasing phosphorylation at Ser473 would be expected to active this DNA repair function over the repressor function. In line with this expectation, we found evident TIF1β-S473 phosphorylation after hydrogen peroxide treatment. Peroxide also strongly induced p38 MAPK phosphorylation with a smaller effect on p42/44 MAPK, and as these affect Ser473 phosphorylation, it stands to reason that ROS can and do force a switching of TIF1β's function from repressor to oxidative defense.

However, whether TIF1β is a direct substrate for p42/44 MAPK and p38 MAPK under peroxide stress or not is unclear at this moment. Future studies will need to consider how phosphorylation of TIF1β-S473 is regulated by the multiple pathways it connects with and if feedback regulatory mechanics take a central or secondary role to ROS-mediated, MAPK-driven activation.

In summary, our data demonstrated that hydrogen peroxide activates MAPK, particularly p38 MAPK and stimulates phosphorylation of TIF1β-S473 that will force HP1 dissociation and subsequent protection of CRC cells from oxidative stress by an increase in DNA repair efficiency.

Figure Legends

Fig. 1. H_2O_2-induced cell death is enhanced by TIF1β knockdown in DLD-1 cells. (A) DLD-1 cells were treated for 15 min with differing concentrations of H_2O_2 as indicated. Cell viability was determined at 72 hr after H_2O_2 treatment by MTT assay. Data are presented as mean ± SD, n=3. (B) DLD-1 cells were transfected with siRNA (control and TIF1β). After 48 hr, cells were challenged with H_2O_2 for 15 min. Cell viability was determined at 72 hr after H_2O_2 treatment by MTT assay. Data are presented as mean ± SD, n=3. **P<0.01, ***P<0.001. Right panel shows Western blot data indicating total TIF1β after siRNA transfection.

Fig. 2. H_2O_2 induces TIF1β serine 473 phosphorylation in DLD-1 and HCT116 cells. DLD-1 and HCT116 cells were incubated with the indicated concentrations of H_2O_2 for 15 min (A, B) or with 1mM of H_2O_2 for the indicated periods (C, D). Whole cell lysates were subjected to Western blot analyses using antibodies against phosphorylated TIF1β (pTIF1β (S473)) and total TIF1β (TIF1β). β-actin antibody was used as a loading control.

Fig. 3. H_2O_2 induces p42/44 MAPK and p38 MAPK phosphorylation in DLD-1 and HCT116 cells. DLD-1 cells (A) and HCT116 cells (B) were incubated with the indicated concentrations of H_2O_2 for 15 min. Whole cell lysates were subjected to Western blot analyses using first antibodies against phosphorylated p38 (p-p38), total p38 (p38), phosphorylated p42/44 (p-p42/44), and total p42/44 (p42/44). β-actin antibody was used as a loading control.

Fig. 4. Effects of p42/44 MAPK and p38 MAPK kinase inhibitors on H_2O_2-induced TIF1β –S473 phosphorylation in DLD-1 and HCT116 cells. DLD-1 cells (A) and HCT116 cells (B) were incubated with DMSO (vehicle), U0126, and SB203580 as indicated for 1 hr before 1 mM H_2O_2 treatment for 15 min. UNT; untreated. Whole cell lysates were subjected to Western blot analyses using first antibodies against phosphorylated TIF1β (pTIF1β (Ser473)), total TIF1β (TIF1β), phosphorylated p42/44 (p-p42/44), phosphorylated p38 (p-p38) and total p38 (p38). β-actin antibody is used as a loading control.

Acknowledgements

This research was funded by Grants-in-Aid for Scientific Research (JP25293092, JP26116707, and JP15K29070) and Basic Science and Platform Technology Program for Innovative Biological Medicine from the Japanese Ministry of Education, Culture, Sports, Science, and Technology. We also thank Dr. Bryan J. Mathis (Medical English Communication Center, University of Tsukuba) for critical proofreading of the manuscript.

Conflict of Interest

The authors declare no conflicts of interest.

References

[1] S. Iyengar, P.J. Farnham, KAP1 protein: an enigmatic master regulator of the genome, J Biol Chem 286 (2011) 26267-26276.

[2] S. Miyagi, S. Koide, A. Saraya, G.R. Wendt, M. Oshima, T. Konuma, S. Yamazaki, M. Mochizuki-Kashio, Y. Nakajima-Takagi, C. Wang, T. Chiba, I. Kitabayashi, H. Nakauchi, A. Iwama, The TIF1beta-HP1 system maintains transcriptional integrity of hematopoietic stem cells, Stem Cell Reports 2 (2014) 145-152.

[3] L. Liu, E. Zhao, C. Li, L. Huang, L. Xiao, L. Cheng, X. Huang, Y. Song, D. Xu, TRIM28, a new molecular marker predicting metastasis and survival in early-stage non-small cell lung cancer, Cancer Epidemiol 37 (2013) 71-78.

[4] L.F. Lin, C.F. Li, W.J. Wang, W.M. Yang, D.D. Wang, W.C. Chang, W.H. Lee, J.M. Wang, Loss of ZBRK1 contributes to the increase of KAP1 and promotes KAP1-mediated metastasis and invasion in cervical cancer, PLoS One 8 (2013) e73033.

[5] C. Yu, L. Zhan, J. Jiang, Y. Pan, H. Zhang, X. Li, F. Pen, M. Wang, R. Qin, C. Sun, KAP-1 is overexpressed and correlates with increased metastatic ability and tumorigenicity in pancreatic cancer, Med Oncol 31 (2014) 25.

[6] S. Fitzgerald, K.M. Sheehan, A. O'Grady, D. Kenny, R. O'Kennedy, E.W. Kay, G.S. Kijanka, Relationship between epithelial and stromal TRIM28 expression predicts survival in colorectal cancer patients, J Gastroenterol Hepatol 28 (2013) 967-974.

[7] D. White, I.U. Rafalska-Metcalf, A.V. Ivanov, A. Corsinotti, H. Peng, S.C. Lee, D. Trono, S.M. Janicki, F.J. Rauscher, 3rd, The ATM substrate KAP1 controls DNA repair in heterochromatin: regulation by HP1 proteins and serine 473/824 phosphorylation,

Mol Cancer Res 10 (2012) 401-414.

[8] K. Okamoto, I. Kitabayashi, Y. Taya, KAP1 dictates p53 response induced by chemotherapeutic agents via Mdm2 interaction, Biochem Biophys Res Commun 351 (2006) 216-222.

[9] C.W. Chang, H.Y. Chou, Y.S. Lin, K.H. Huang, C.J. Chang, T.C. Hsu, S.C. Lee, Phosphorylation at Ser473 regulates heterochromatin protein 1 binding and corepressor function of TIF1beta/KAP1, BMC Mol Biol 9 (2008) 61.

[10] C. Hu, S. Zhang, X. Gao, X. Xu, Y. Lv, Y. Zhang, Z. Zhu, C. Zhang, Q. Li, J. Wong, Y. Cui, W. Zhang, L. Ma, C. Wang, Roles of Kruppel-associated Box (KRAB)-associated Co-repressor KAP1 Ser-473 Phosphorylation in DNA Damage Response, J Biol Chem 287 (2012) 18937-18952.

[11] P.J. Turnbaugh, M. Hamady, T. Yatsunenko, B.L. Cantarel, A. Duncan, R.E. Ley, M.L. Sogin, W.J. Jones, B.A. Roe, J.P. Affourtit, M. Egholm, B. Henrissat, A.C. Heath, R. Knight, J.I. Gordon, A core gut microbiome in obese and lean twins, Nature 457 (2009) 480-484.

[12] T. Obtulowicz, M. Swoboda, E. Speina, D. Gackowski, R. Rozalski, A. Siomek, J. Janik, B. Janowska, J.M. Ciesla, A. Jawien, Z. Banaszkiewicz, J. Guz, T. Dziaman, A. Szpila, R. Olinski, B. Tudek, Oxidative stress and 8-oxoguanine repair are enhanced in colon adenoma and carcinoma patients, Mutagenesis 25 (2010) 463-471.

[13] K. Oka, T. Tanaka, T. Enoki, K. Yoshimura, M. Ohshima, M. Kubo, T. Murakami, T. Gondou, Y. Minami, Y. Takemoto, E. Harada, T. Tsushimi, T.S. Li, F. Traganos, Z. Darzynkiewicz, K. Hamano, DNA damage signaling is activated during cancer progression in human colorectal carcinoma, Cancer Biol Ther 9 (2010) 246-252.

[14] T. Sato, H. Takeda, S. Otake, J. Yokozawa, S. Nishise, S. Fujishima, T. Orii, T. Fukui, J. Takano, Y. Sasaki, K. Nagino, D. Iwano, T. Yaoita, S. Kawata, Increased plasma levels of 8-hydroxydeoxyguanosine are associated with development of colorectal tumors, J Clin Biochem Nutr 47 (2010) 59-63.

[15] K.M. Holmstrom, T. Finkel, Cellular mechanisms and physiological consequences of redox-dependent signalling, Nat Rev Mol Cell Biol 15 (2014) 411-421.

[16] A.L. Jackson, R. Chen, L.A. Loeb, Induction of microsatellite instability by oxidative DNA damage, Proc Natl Acad Sci U S A 95 (1998) 12468-12473.

[17] G. Slupphaug, B. Kavli, H.E. Krokan, The interacting pathways for prevention and repair of oxidative DNA damage, Mutat Res 531 (2003) 231-251.

[18] M.S. Cooke, M.D. Evans, M. Dizdaroglu, J. Lunec, Oxidative DNA damage: mechanisms, mutation, and disease, FASEB J 17 (2003) 1195-1214.

[19] C.A. King, Kaposi's sarcoma-associated herpesvirus kaposin B induces unique monophosphorylation of STAT3 at serine 727 and MK2-mediated inactivation of the STAT3 transcriptional repressor TRIM28, J Virol 87 (2013) 8779-8791.

[20] J.V. Olsen, B. Blagoev, F. Gnad, B. Macek, C. Kumar, P. Mortensen, M. Mann, Global, in vivo, and site-specific phosphorylation dynamics in signaling networks, Cell 127 (2006) 635-648.

[21] C.T. Cheng, C.Y. Kuo, C. Ouyang, C.F. Li, Y. Chung, D.C. Chan, H.J. Kung, D.K. Ann, Metabolic Stress-Induced Phosphorylation of KAP1 Ser473 Blocks Mitochondrial Fusion in Breast Cancer Cells, Cancer Res 76 (2016) 5006-5018.

[22] C. Wittmann, P. Chockley, S.K. Singh, L. Pase, G.J. Lieschke, C. Grabher, Hydrogen

peroxide in inflammation: messenger, guide, and assassin, Adv Hematol 2012 (2012) 541471.

[23] S.S. Wijeratne, S.L. Cuppett, V. Schlegel, Hydrogen peroxide induced oxidative stress damage and antioxidant enzyme response in Caco-2 human colon cells, J Agric Food Chem 53 (2005) 8768-8774.

[24] M. Singh, H. Sharma, N. Singh, Hydrogen peroxide induces apoptosis in HeLa cells through mitochondrial pathway, Mitochondrion 7 (2007) 367-373.

[25] M. Tochigi, T. Inoue, M. Suzuki-Karasaki, T. Ochiai, C. Ra, Y. Suzuki-Karasaki, Hydrogen peroxide induces cell death in human TRAIL-resistant melanoma through intracellular superoxide generation, Int J Oncol 42 (2013) 863-872.

[26] C. Polytarchou, M. Hatziapostolou, E. Papadimitriou, Hydrogen peroxide stimulates proliferation and migration of human prostate cancer cells through activation of activator protein-1 and up-regulation of the heparin affin regulatory peptide gene, J Biol Chem 280 (2005) 40428-40435.

[27] T. Ravingerova, M. Barancik, M. Strniskova, Mitogen-activated protein kinases: a new therapeutic target in cardiac pathology, Mol Cell Biochem 247 (2003) 127-138.

[28] J.A. McCubrey, M.M. Lahair, R.A. Franklin, Reactive oxygen species-induced activation of the MAP kinase signaling pathways, Antioxid Redox Signal 8 (2006) 1775-1789.

[29] R.F. Ryan, D.C. Schultz, K. Ayyanathan, P.B. Singh, J.R. Friedman, W.J. Fredericks, F.J. Rauscher, 3rd, KAP-1 corepressor protein interacts and colocalizes with heterochromatic and euchromatic HP1 proteins: a potential role for Kruppel-associated box-zinc finger proteins in heterochromatin-mediated gene

silencing, Mol Cell Biol 19 (1999) 4366-4378.

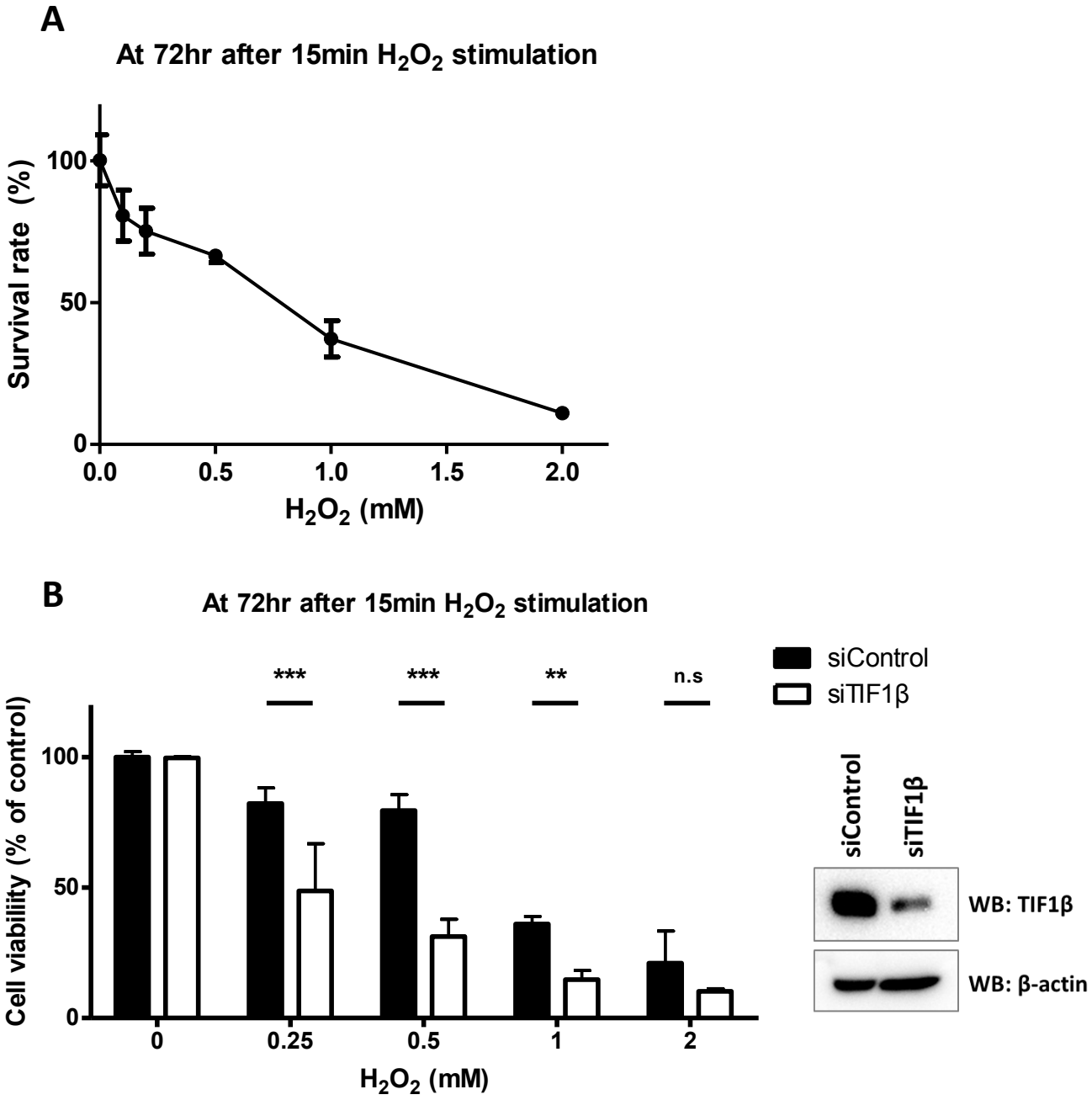

Fig. 1. H_2O_2-induced cell death is enhanced by TIF1β knockdown in DLD-1 cells. (A) DLD-1 cells were treated for 15 min with differing concentrations of H_2O_2 as indicated. Cell viability was determined at 72 hr after H_2O_2 treatment by MTT assay. Data are presented as mean ± SD, n=3. (B) DLD-1 cells were transfected with siRNA (control and TIF1β). After 48 hr, cells were challenged with H_2O_2 for 15 min. Cell viability was determined at 72 hr after H_2O_2 treatment by MTT assay. Data are presented as mean ± SD, n=3. **P<0.01, ***P<0.001. Right panel shows Western blot data indicating total TIF1β after siRNA transfection.

Fig. 1

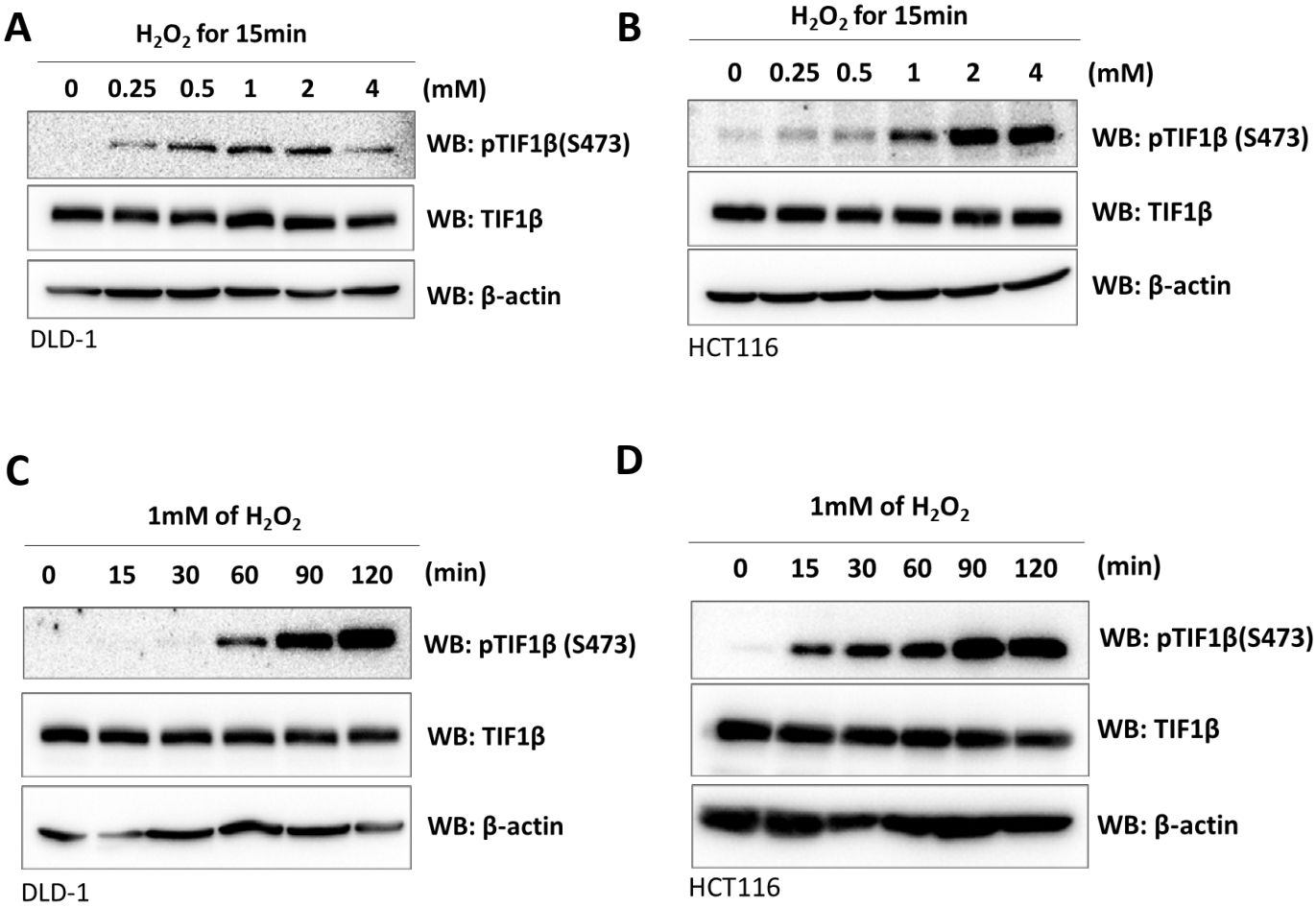

Fig. 2. H_2O_2 induces TIF1β serine 473 phosphorylation in DLD-1 and HCT116 cells. DLD-1 and HCT116 cells were incubated with the indicated concentrations of H_2O_2 for 15 min (A, B) or with 1mM of H_2O_2 for the indicated periods (C, D). Whole cell lysates were subjected to Western blot analyses using antibodies against phosphorylated TIF1β (pTIF1β (S473)) and total TIF1β (TIF1β). β-actin antibody was used as a loading control.

Fig. 2

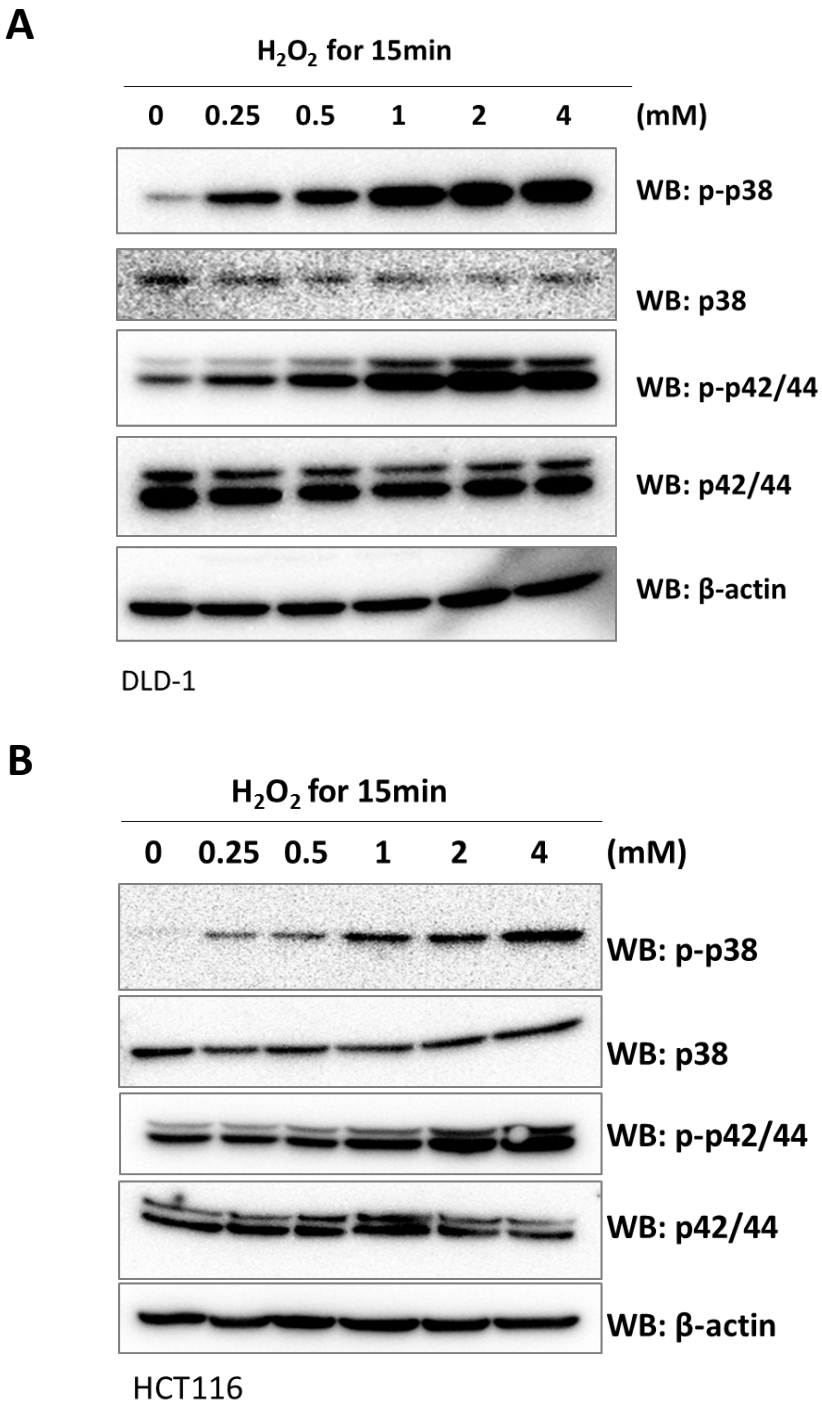

Fig. 3. H_2O_2 induces p42/44 MAPK and p38 MAPK phosphorylation in DLD-1 and HCT116 cells. DLD-1 cells (A) and HCT116 cells (B) were incubated with the indicated concentrations of H_2O_2 for 15 min. Whole cell lysates were subjected to Western blot analyses using first antibodies against phosphorylated p38 (p-p38), total p38 (p38), phosphorylated p42/44 (p-p42/44), and total p42/44 (p42/44). β-actin antibody was used as a loading control.

Fig. 3

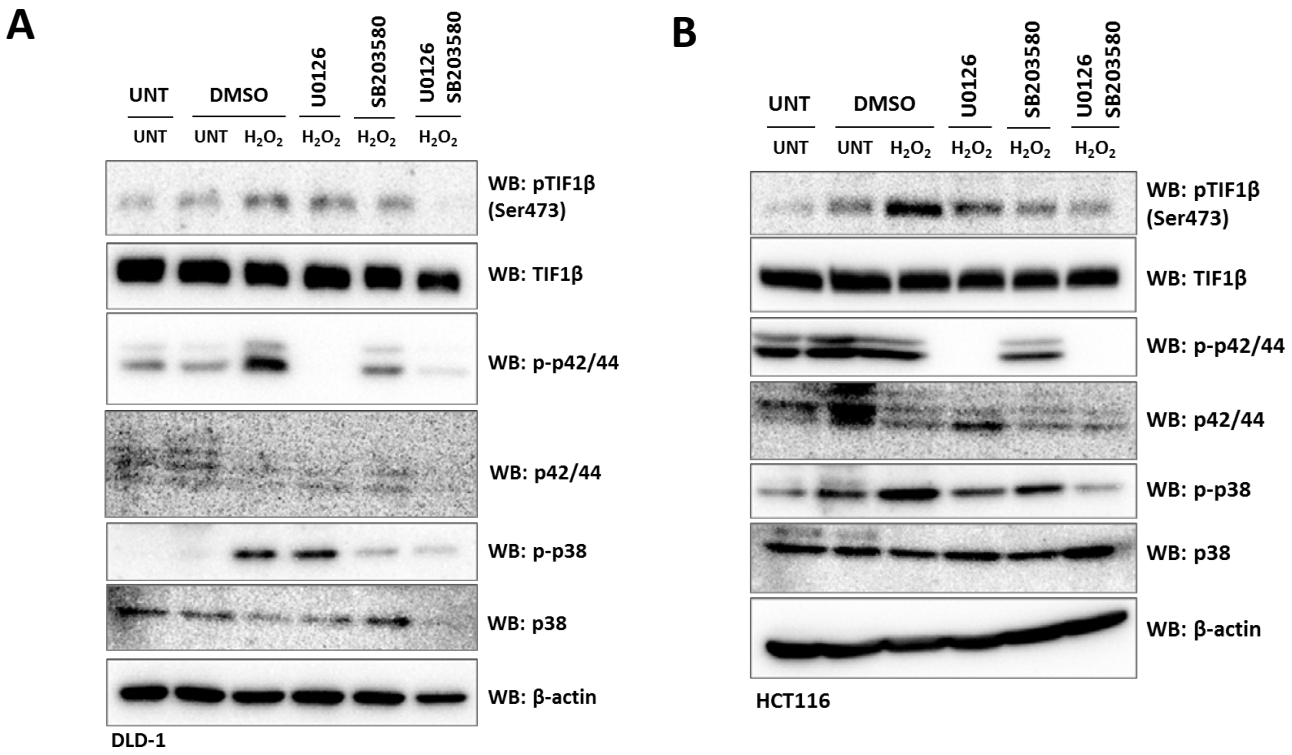

Fig. 4. Effects of p42/44 MAPK and p38 MAPK kinase inhibitors on H_2O_2-induced TIF1β –S473 phosphorylation in DLD-1 and HCT116 cells. DLD-1 cells (A) and HCT116 cells (B) were incubated with DMSO (vehicle), U0126, and SB203580 as indicated for 1 hr before 1 mM H_2O_2 treatment for 15 min. UNT; untreated. Whole cell lysates were subjected to Western blot analyses using first antibodies against phosphorylated TIF1β (pTIF1β (Ser473)), total TIF1β (TIF1β), phosphorylated p42/44 (p-p42/44), phosphorylated p38 (p-p38) and total p38 (p38). β-actin antibody is used as a loading control.

Fig. 4

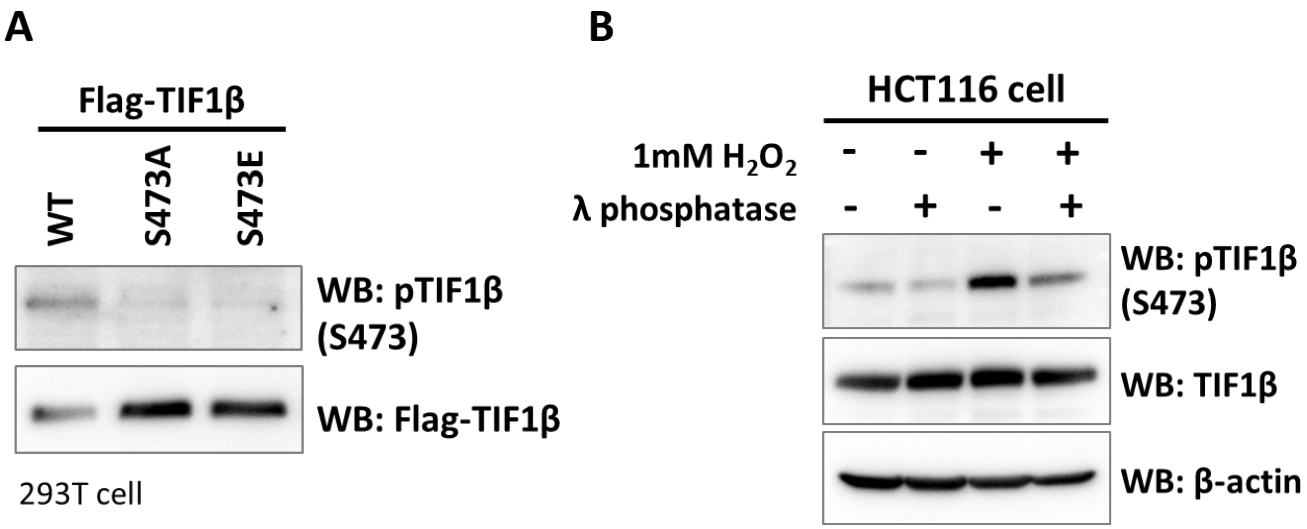

Figure S1. Characterization of anti-TIF1β-Ser473 phosphorylation antibody under oxidative stress. (A) HEK293T cells were transfected with TIF1β-wild type, S473A and S473E mutant, respectively. (B) HCT116 cells were incubated with H_2O_2 for 15 min and treated with or without λ phosphatase for 30 min. Whole cell lysates were immunoblotted to detect endogenous expression of phosphorylated TIF1β, total TIF1β and β-actin, respectively.

Fig. S1

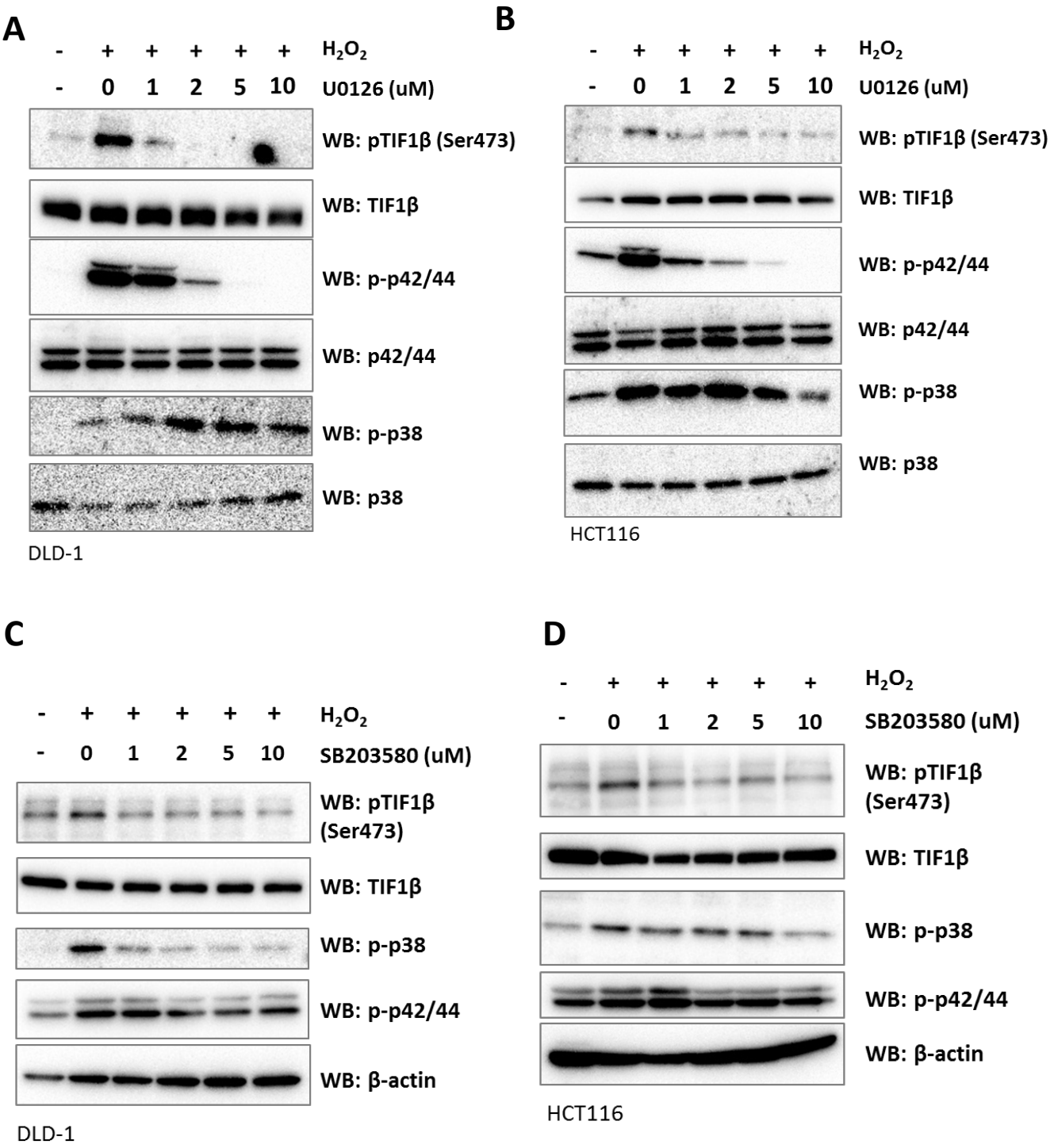

Figure S2. Effect of doses of kinase inhibitors on H_2O_2-induced phosphorylation in colorectal cancer DLD-1 and HCT116 cells. .

DLD-1 cells and HCT116 cells were incubated with U0126 (A-B) or SB203580 (C-D) for the indicated concentration for 1hour before H_2O_2 stimulation. Whole cell lysates were immunoblotted to detect endogenous expression of phosphorylated TIF1β (pTIF1β (Ser473)), total TIF1β (TIF1β), phosphorylated p42/44 (p-p42/44), phosphorylated p38 (p-p38) and total p38 (p38). β-actin antibody is used as a loading control.

Fig. S2