Title: Dimethyl fumarate dampens IL-17-ACT1-TBK1 axis-mediated phosphorylation of Regnase-1 and suppresses IL-17–induced IκB-ζ expression

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Footnotes

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[1] **Abbreviations used in this article:**

3′-UTR, 3′-untranslated region

DMF, dimethyl fumarate

DMSO, dimethyl sulfoxide

IκB-ζ, inhibitor of nuclear factor kappa B zeta

IKKε, IκB kinase ε

IL-17, interleukin 17A

NAC, N-acetylcysteine

NRF2, nuclear factor erythroid-derived 2-like 2

qPCR, quantitative real-time PCR

siRNA, small interfering RNA

TBK1, TANK-binding kinase 1

Abstract

The signaling elicited by the cytokine interleukin-17A (IL-17) is important for antimicrobial defense responses, whereas excessive IL-17 production leads to autoimmune diseases such as psoriasis and multiple sclerosis. IL-17–induced stabilization of mRNAs has been recognized as a unique and important feature of IL-17 signaling. Previously, we demonstrated that IL-17 signaling protein ACT1 is required to counteract constitutive inhibitor of nuclear factor kappa B zeta (IκB-ζ) mRNA degradation by the ribonuclease Regnase-1. However, information about the mechanism of mRNA stabilization in IL-17–stimulated cells remains insufficient. In the present study, we aimed to clarify the mechanism in more detail and identify an agent that can inhibit IL-17–induced mRNA stabilization. Experiments using small interfering RNA and an inhibitor of TANK-binding kinase 1 (TBK1) revealed that TBK1 was required for IκB-ζ mRNA stabilization through Regnase-1 phosphorylation. Intriguingly, this TBK1-mediated phosphorylation of Regnase-1 was suppressed by the addition of dimethyl fumarate (DMF), an electrophilic small molecule that has been used to treat IL-17–related autoimmune diseases. Confocal microscopic observation of the cellular localization of ACT1 revealed that DMF treatment resulted in the disappearance of ACT1 nuclear dots and perinuclear accumulation of ACT1. These results suggested that DMF is a small molecule that compromises IL-17–induced activation of the ACT1-TBK1 pathway, thereby inhibiting IL-17–induced mRNA stabilization.

Keywords: IL-17, mRNA stability, dimethyl fumarate, IκB-ζ, Regnase-1

Introduction

Interleukin 17A (IL-17) is an inflammatory cytokine. IL-17–mediated inflammation is crucial for microbial clearance, whereas excessive activation of IL-17 signaling can promote autoimmune diseases and cancer progression [1,2]. ACT1 (also known as CIKS, encoded by *TRAF3IP2*) is an essential adaptor protein in IL-17–dependent signaling in autoimmune and inflammatory diseases [3]. ACT1 is critical for IL-17–induced signaling events, including activation of transcription factors such as NF-κB and C/EBPβ [1] and post-transcriptional stabilization of certain mRNAs [4-7].

IL-17/ACT1-induced stabilization of mRNAs has been recognized as a unique and important feature of IL-17 signaling. Several mechanisms of IL-17/ACT1-induced mRNA stabilization have been suggested. TANK-binding kinase 1 (TBK1) and IκB kinase ε (IKKε; also known as IKKi, encoded by *IKBKE*) have been revealed to be activated upon IL-17 stimulation, interacting directly with ACT1 and thereby modulating IL-17–induced events [4,7-9]. Importantly, Tanaka et al. have reported that IL-17 induces the phosphorylation of the endoribonuclease Regnase-1 (also known as MCPIP1, encoded by *ZC3H12A*) in an ACT1-TBK1/IKKε-dependent manner and that phosphorylated Regnase-1 loses its mRNA degradation function, leading to the expression of IL-17 target genes [7]. In our previous study, we reported that IL-17/ACT1 signaling counteracts the constitutively occurring Regnase-1–mediated degradation of inhibitor of nuclear factor kappa B zeta (IκB-ζ, encoded

by *NFKBIZ*) mRNA [10]. The RNA binding protein Arid5a has been demonstrated to be induced upon IL-17 stimulation and to stabilize IL-17–induced cytokine transcripts by binding to their 3′-untranslated regions (3′-UTRs) and also counteract mRNA degradation mediated by Regnase-1 [11]. These findings suggested that the inactivation of Regnase-1–mediated mRNA degradation is an important mechanism of IL-17–induced mRNA stabilization and that the inhibition of Regnase-1 phosphorylation may keep Regnase-1 active and suppress IL-17–induced inflammatory responses. Thus, it would be useful to identify the mechanism and inhibitory agents for IL-17–induced Regnase-1 phosphorylation.

There is an important role for IL-17 in the pathogenesis of autoimmune diseases such as psoriasis and multiple sclerosis [2]. Fumaric acid esters, such as dimethyl fumarate (DMF) have been used to treat these diseases [12-15]. The mechanisms of the anti-inflammatory effects of DMF involve activation of the anti-oxidative transcription factor nuclear factor erythroid-derived 2-like 2 (NRF2, encoded by *NFE2L2*); however, it is also known that NRF2-independent effects may exist [12,15]. Interestingly, dimethyl itaconate, which has similarities in chemical structure and electrophilicity to DMF, has been demonstrated to suppress IκB-ζ expression at the post-transcriptional stage through an NRF2-independent mechanism [16]. Collectively, these findings imply that the anti-inflammatory mechanism of

DMF may involve the suppression of IL-17–induced mRNA stabilization at the post-transcriptional stage.

In the present study, we demonstrated that IL-17–induced IκB-ζ expression requires ACT1 and TBK1. The ACT1-TBK1 pathway participates in Regnase-1 phosphorylation. DMF suppressed ACT1-TBK1–mediated Regnase-1 phosphorylation through its electrophilic properties. These data suggested that TBK1 inhibitors and fumarate esters such as DMF can be regarded as inhibitors of IL-17–induced mRNA stabilization.

Materials and methods

Plasmid construction and reagents

The firefly luciferase reporter pGL3-mIκB-ζ 3′-UTR and pFLAG-CMV2-mZc3h12a (mRegnase-1) were kindly provided by Dr. Osamu Takeuchi (Kyoto University, Kyoto, Japan) [17]. The pFN21A clone expressing an N-terminal HaloTag fusion of human full-length ACT1 (HaloTag-ACT1) was obtained from the Kazusa DNA Research Institute (Kisarazu, Japan). Recombinant human IL-17 was purchased from R&D Systems (Minneapolis, MN, USA).

Cell culture and transfection of small interfering RNA (siRNA)

The human keratinocyte cell line HaCaT and the human cervix carcinoma cell line HeLa were maintained in DMEM containing 10% FCS. For siRNA transfection, Lipofectamine 2000 reagent (Life Technologies, Carlsbad, CA, USA) was used. The following siRNA duplexes (Shanghai GenePharma, Shanghai, China) were used: negative control, 5′-UUCUCCGAACGUGUGACGUTT-3′ (sense) and 5′-ACGUCACACGUUCGGAGAATT-3′ (antisense); human TBK1, 5′-GGGAACCUCUGAAUACCAUTT-3′ (sense) and 5′-AUGGUAUUCAGAGGUUCCCTT-3′ (antisense); human IKBKE (IKKε), 5′-GGAGAACGGCAUUGUGCAUTT-3′ (sense) and

5′-AUGCACAAUGCCGUUCUCCTT-3′ (antisense); human TRAF3IP2 (ACT1), 5′-GGAUGAGCAUGGCUUACAUTT-3′ (sense) and 5′-AUGUAAGCCAUGCUCAUCCTT (antisense); and human NFE2L2 (NRF2), 5′-GCCCAUUGAUGUUUCUGAUTT-3′ (sense) and 5′-AUCAGAAACAUCAAUGGGCTT-3′ (antisense).

RNA isolation and quantitative real-time PCR (qPCR)

Cells were harvested, and total RNA was prepared using TRI Reagent (Molecular Research Center, Cincinnati, OH, USA). First-strand cDNA was synthesized from 1 μg of total RNA with ReverTra Ace (TOYOBO, Osaka, Japan). qPCR analysis of transcripts was conducted using a KAPA SYBR FAST qPCR master mix (KAPA Biosystems, Woburn, MA, USA) with a Mx3005P real-time PCR system (Stratagene, Santa Clara, CA, USA). The following primer pairs were used: *ACTB* (beta-actin), 5′-TGTTACCAACTGGGACGACA-3′ (sense) and 5′-GGGGTGTTGAAGGTCTCAAA-3′ (antisense); *NFKBIZ* (IκB-ζ), 5′-TGGAGTCCCGGTCGAGAG-3′ (sense) and 5′-ACCTTGTGTCTTAAAATCATCCACA-3′ (antisense); *TBK1*, 5′-TCGTCCAGTGGATGTTCAAA-3′ (sense) and 5′-ATTCATTCCACCCACCACAT-3′ (antisense); *IKBKE* (IKKε), 5′-GAGCTAATGTTTCGGGGGCT (sense) and 5′-AGAGGACCTCCGCTAGAGTC (antisense); and *NFE2L2* (NRF2), 5′-GCGACGGAAAGAGTATGAGC-3′ (sense) and 5′-GTTGGCAGATCCACTGGTTT-3′

(antisense). Data were normalized to the amount of *ACTB* mRNA.

Luciferase assay for post-transcriptional stabilization of NFKBIZ mRNA

HeLa cells were transfected with the luciferase reporter plasmid pGL3 containing the mouse IκB-ζ 3′-UTR (full length: 1353 nucleotides after the stop codon), together with the expression plasmid for *Renilla* luciferase as an internal control. After 24 h of cultivation, treatment with 10 ng/mL IL-17, and subsequent incubation for 1.5 h, cells were lysed, and luciferase activity in lysates was determined using the Dual-Luciferase Reporter Assay System (Promega, Madison, WI, USA).

Immunoblotting

Immunoblotting was performed as described previously [18]. Briefly, cell lysates were resolved via SDS-PAGE and transferred to PVDF transfer membranes (PerkinElmer; Boston, MA, USA). The filters were then immunoblotted with each antibody. Immunoreactive proteins were visualized using an enhanced chemiluminescence detection system (Millipore; Bedford, MA, USA). Anti-IκB-ζ (#9244) antibody was obtained from Cell Signaling Technologies (Beverly, MA, USA). Anti-ACT1 (H-300) antibody was purchased from Santa Cruz Biotechnology (Santa Cruz Biotechnology, CA, USA). Anti-TBK1 antibody (EP611Y) was acquired from Abcam (Cambridge, UK). Anti-ZC3H12A (Regnase-1) antibody was

procured from GeneTex (Irvine, CA, USA). Anti-FLAG-tag (M2) and anti-actin (AC15) antibodies were obtained from Sigma-Aldrich (St. Louis, MO, USA).

Indirect immunofluorescence microscopy

HeLa cells seeded on a glass plate were fixed with 4% paraformaldehyde, permeabilized with PBS containing 1% Triton X-100 and reacted with rabbit anti-ACT1 antibody (1:100, H-300, Santa Cruz Biotechnology) overnight at 4°C. The cells were then reacted with an Alexa Fluor 488-conjugated F(ab′)2 fragment of goat anti-rabbit IgG (Invitrogen). Nuclei were stained with 4′,6-diamidino-2-phenylindole (Wako, Osaka, Japan). Images were obtained using a confocal laser-scanning microscope (FluoView FV10i; Olympus, Osaka, Japan). Images were collected with a ×60 water objective lens (numerical aperture = 1.3) (Olympus) and acquired using FV10-ASW software (Olympus).

Statistical evaluation

Data are expressed as the mean ± SEM. For statistical comparison of two groups, Student's *t*-test was performed. $p < 0.05$ denoted statistical significance.

Results and discussion

Involvement of TBK1 in IL-17–induced IκB-ζ accumulation

The IκB-ζ protein is highly expressed in the epidermal keratinocytes of psoriatic lesions and is considered to be involved in the pathogenesis of psoriasis [19]. We previously reported that IκB-ζ is an IL-17–inducible protein that mediates IL-17–induced gene expression [20]. We assessed IκB-ζ induction as an indicator of IL-17–induced responses and investigated whether TBK1 and/or IKKε is involved in IκB-ζ induction. HaCaT cells were transfected with non-targeting negative control (siControl), TBK1 (siTBK1), or IKKε siRNA (siIKKε) and stimulated with IL-17 for 3 h. IL-17–induced accumulation of IκB-ζ mRNA was observed in siControl-transfected cells. Such accumulation of IκB-ζ mRNA was significantly reduced in cells transfected with siTBK1 and was enhanced in those with siIKKε, respectively (Fig. 1A). These results implied that TBK1 and IKKε have distinct roles in the IL-17–induced accumulation of IκB-ζ mRNA. IκB-ζ protein accumulation was detected over time in HaCaT cells upon IL-17 stimulation (Fig. 1B). The effect of TBK1 depletion on IL-17–induced IκB-ζ protein accumulation was investigated (Fig. 1C). IL-17–induced IκB-ζ accumulation was attenuated in siTBK1-transfected HaCaT cells, confirming that TBK1 may mediate IκB-ζ induction.

ACT1 and TBK1 were required for Regnase-1 phosphorylation and IκB-ζ mRNA stabilization

The 3′-UTR downstream of the protein-coding region of IκB-ζ mRNA affects IκB-ζ mRNA stability [17]. We demonstrated that IL-17 signaling counteracts Regnase-1–mediated post-transcriptional mRNA degradation and that IL-17 signaling poorly stimulates the promoter activity of the IκB-ζ gene [10]. A reporter plasmid (IκB-ζ 3 ′-UTR Luc), in which a 3 ′-UTR sequence derived from IκB-ζ mRNA was inserted downstream of a luciferase gene expressed under the constitutively active CMV promoter (Fig. 2A and [17]), was used to determine the effects of TBK1 depletion by siRNA (Fig. 2B). TBK1 depletion, the efficacy of which (data not shown) was similar as that shown in Fig. 1A and 1C, resulted in considerable suppression of IL-17–induced reporter activity (Fig. 2B). BX-795 is a small molecule that inhibits the kinase activity of TBK1 [21]. IL-17–induced activation of the IκB-ζ 3′-UTR reporter was significantly suppressed by BX-795 treatment (Fig. 2C, left panel). Luciferase expression from original pGL3 reporter vector, which does not carry the IκB-ζ 3′-UTR, was unresponsive to both IL-17 and BX-795 (Fig. 2C, right panel).

It has been reported that IL-17 induces Regnase-1 phosphorylation in an ACT1-TBK1/IKKε-dependent manner and that phosphorylated Regnase-1 loses its mRNA-degrading activity, leading to the expression of IL-17 target genes [7]. Phosphorylation of Regnase-1 in cells stimulated with cytokines including IL-17 can be

detected as a band with slower mobility via Western blotting [7,22]. Similarly, we detected a slower mobility band of overexpressed Regnase-1 in cells stimulated with IL-17, whereas we could not clearly detect the mobility shift of endogenous Regnase-1 in our methods used (Fig. 2D). In addition, this IL-17–induced retardation of Regnase-1 band mobility was cancelled by ACT1 siRNA introduction (Fig. 2D) and BX-795 pretreatment (Fig. 2E). These results confirmed that the findings of the previous study [7] can be applied to IL-17–induced IκB-ζ accumulation and demonstrated that the ACT1-TBK1 axis mediates IL-17–induced Regnase-1 inactivation.

DMF suppressed Regnase-1 phosphorylation

DMF has been revealed to inhibit IκB-ζ induction in LPS-stimulated macrophages via an electrophilic stress-related mechanism, although the detailed mechanism has not been completely elucidated [16]. We investigated the effects of DMF pretreatment on IL-17–induced IκB-ζ induction and found that DMF inhibited this induction (Fig. 3A). IL-17–induced activation of the IκB-ζ 3′-UTR reporter was suppressed by DMF treatment, and this effect was cancelled by the small molecule thiol N-acetylcysteine (NAC), which is known to react directly with and trap DMF via Michael-type addition [23] (Fig. 3B), suggesting that DMF acts through its electrophilic property and modulates the function of thiol-containing molecules or the levels of glutathione in cells. DMF-induced effects are

mediated by oxidative stress-induced activation of the NRF2-dependent antioxidant response pathway [12]. We next tested whether NRF2 is involved in the DMF-induced inhibition of IL-17–induced IκB-ζ mRNA stabilization. NRF2 depletion in HeLa cells did not alleviate DMF-mediated IκB-ζ suppression (Fig. 3C). Of note, we found that DMF inhibited IL-17–induced Regnase-1 phosphorylation (Fig. 3D) and that co-treatment with NAC cancelled this effect (Fig. 3D). These data suggested that DMF treatment compromises IL-17–induced activation of the ACT1-TBK1 pathway independently of NRF2 function, thereby inhibiting IκB-ζ accumulation.

DMF altered the subcellular localization of ACT1

The subcellular localization of ACT1 is reportedly shifts to nucleus and cytoplasmic granules upon IL-17 stimulation [4,24]. To explore the effect of DMF on the subcellular distribution of endogenous ACT1, the cellular localization of ACT1 was observed via confocal microscopy. Regardless of IL-17 stimulation, ACT1 was found to localize to the cytosol and nuclear punctate structures (Fig. 4). Pretreatment with 100 μM DMF for 12 h resulted in the disappearance of ACT1 nuclear dots and perinuclear accumulation of ACT1 (Fig. 4), suggesting that DMF alters the localization of ACT1 and thereby compromises IL-17 responses.

Overproduction of IL-17 is one of major pathogenesis of autoimmune diseases. IL-17

mediates signals to induce Regnase-1 phosphorylation and IκB-ζ mRNA accumulation in ACT1-TBK1-dependent manner. In the present study, we demonstrated that treatment with small molecules such as a TBK1 inhibitor or DMF can suppress IL-17–induced responses as demonstrated by IκB-ζ expression. This finding is important because small molecule-mediated suppression of Regnase-1 phosphorylation has been described as a novel strategy for treating IL-17-associated diseases [7]. That study, using TBK1-deficient, IKKε-deficient, and TBK1/IKKε double-deficient mouse embryonic fibroblasts, clearly demonstrated that both TBK1 and IKKε are responsible for Regnase-1 phosphorylation, in response to IL-17 [7]. However, our results of qPCR experiment implied that TBK1 and IKKε have distinct roles in the IL-17–induced accumulation of IκB-ζ mRNA (Fig. 1A). The reason for this discrepancy remains at present unexplained; it might be related to differences in cell lines used.

DMF is clinically used to treat patients with autoimmune diseases. From a pharmacological viewpoint, our findings suggested that the prevention of Regnase-1 inactivation exists as a previously unrecognized mechanism of the anti-inflammatory effects of DMF. This effect of DMF may rely on the disturbance of the cellular localization of ACT1. However, it is unclear whether DMF directly reacts with the cysteine residues in the ACT1 protein and alters the localization of ACT1. Further research will be needed to clarify the mechanism by which DMF affects the subcellular localization of ACT1. Our findings could

help us to understand how DMF acts in the inflammatory lesions in body and to identify additional molecular targets for therapeutic intervention in IL-17/ACT1-related diseases.

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Disclosures

The authors have no financial conflicts of interest.

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Figure captions

Figure 1. Involvement of TANK-binding kinase 1 (TBK1) in interleukin-17 (IL-17)–induced inhibitor of nuclear factor kappa B zeta (IκB-ζ) induction

(A) HaCaT cells transfected with 20 pmol of TBK1 (siTBK1), IKKε (siIKKε), or control small interfering RNA (siControl) were stimulated with IL-17 (10 ng/mL) for 1.5 h. The mRNA levels of IκB-ζ, TBK1, and IKKε in cells were determined by quantitative real-time PCR. Transcript expression is shown as relative values. Data are presented as the mean ± SEM (n = 3). * $p < 0.05$ compared with siControl. (B) HaCaT cells stimulated with IL-17 (10 ng/mL) for the indicated times were harvested and subjected to Western blotting using anti-IκB-ζ antibody. β-actin served as the loading control. IB: immunoblot. (C) HaCaT cells transfected with siControl or siTBK1 were stimulated with IL-17 (10 ng/mL) for 3 h. Total cell lysates were prepared and analyzed via Western blotting using the indicated antibodies.

Figure 2. TANK-binding kinase 1 (TBK1) mediates ACT1-mediated Regnase-1 inactivation.

(A) Schematic representation of IκB-ζ 3′-UTR Luc. The full-length IκB-ζ 3′-untranslated region (3′-UTR) was inserted immediately after the stop codon of the firefly luciferase coding sequence. (B) HeLa cells were transfected with 20 pmol of control (siControl) or TBK1 (siTBK1) small interfering RNA (siRNA). After 6 h, the IκB-ζ 3′-UTR Luc reporter construct was transfected into the cells. After 39 h of incubation, the cells were treated with

interleukin-17 (IL-17) (10 ng/mL) for an additional 3 h. The cells were lysed and subjected to the reporter luciferase assay. Data are presented as the mean ± SEM of three independent experiments relative to the levels in non-stimulated siControl-transfected cells. * $p < 0.05$. (C) HeLa cells were transfected with the IκB-ζ 3′-UTR Luc reporter construct (left) or original pGL3 reporter (pGL3 Luc) control (right). After 44 h of incubation, cells were pretreated with the TBK1 inhibitor BX-795 (10 μM) or solvent (dimethyl sulfoxide; DMSO) for 1 h and then stimulated with IL-17 (10 ng/mL) for 3 h. The cells were lysed and subjected to the reporter luciferase assay. Data are presented as the mean ± SEM ($n = 3$). * $p < 0.05$. (D) HeLa cells were transfected with 20 pmol of TRAF3IP2 (ACT1) siRNA (siACT1) or siControl. After 6 h, the expression plasmid for FLAG-tagged Regnase-1 (Reg1) or empty plasmid was transfected into the cells. After 39 h of incubation, the cells were treated with IL-17 (10 ng/mL) for the indicated times. Total cell lysates were prepared and analyzed via Western blotting using the indicated antibodies. Arrowheads indicate the positions of phosphorylated or unphosphorylated Regnase-1. (E) The expression plasmid for FLAG-tagged Regnase-1 was transfected into HeLa cells. After 44 h of incubation, cells were pretreated with BX-795 (10 μM) or DMSO for 1 h and then stimulated with IL-17 (10 ng/mL) for 3 h. Total cell lysates were prepared and analyzed using Western blotting with the indicated antibodies.

Figure 3. Dimethyl fumarate (DMF) suppresses interleukin-17 (IL-17)–induced Regnase-1

phosphorylation.

(A) HaCaT cells were pretreated with increasing amounts of DMF (25, 50, or 100 μM) or dimethyl sulfoxide (DMSO) for 12 h and then stimulated with IL-17 (10 ng/mL) for 3 h. Total cell lysates were prepared and analyzed via Western blotting using anti-IκB-ζ antibody. (B) HeLa cells were transfected with the IκB-ζ 3′-UTR Luc reporter construct. After 33 h of incubation, the cells were pretreated with either DMF (100 μM) alone, N-acetylcysteine (NAC, 1 mM) alone, DMF plus NAC, or DMSO for 12 h and then stimulated with IL-17 (10 ng/mL) for 3 h. The cells were lysed and subjected to the reporter luciferase assay. Data are presented as the mean ± SEM (n = 3). * $p < 0.05$. (C) (left) HeLa cells were transfected with 20 pmol of nuclear factor erythroid-derived 2-like 2 (NRF2) (siNRF2) or control (siControl) small interfering RNA. The cells were then transfected with the IκB-ζ 3′-UTR Luc reporter construct. After 27 h of incubation, the cells were pretreated with either DMF (100 μM) or DMSO for 12 h and then stimulated with IL-17 (10 ng/mL) for 3 h. The cells were lysed and subjected to the reporter luciferase assay. Data are presented as the mean ± SEM (n = 3). * $p < 0.05$. (right) The amounts of NRF2 mRNA in the cells at the time of the luciferase assay were determined using quantitative real-time PCR. Data are presented as the mean ± SEM (n = 3). # $p < 0.05$ compared with siControl. (D) The expression plasmid for FLAG-tagged Regnase-1 was transfected into HeLa cells. After 33 h of incubation, the cells were pretreated with either DMF (100 μM), DMF plus NAC (1 mM), or DMSO for 12 h and then stimulated

with IL-17 (10 ng/mL) as indicated. Total cell lysates were prepared and analyzed via Western blotting using anti-Regnase-1 and anti-FLAG antibodies.

Figure 4. Dimethyl fumarate (DMF) alters the subcellular localization of ACT1.

Confocal imaging of HeLa cells incubated without or with interleukin-17 (IL-17, 10 ng/mL) for 3 h in the presence of DMF (100 μM) or dimethyl sulfoxide (DMSO). The cells were fixed and stained with anti-ACT1 antibody followed by Alexa Fluor 488-conjugated anti-rabbit Ig antibody (green). Blue, 4′,6-diamidino-2-phenylindole (DAPI) nuclear staining. Original magnification: ×600.

Figure

Figure 1

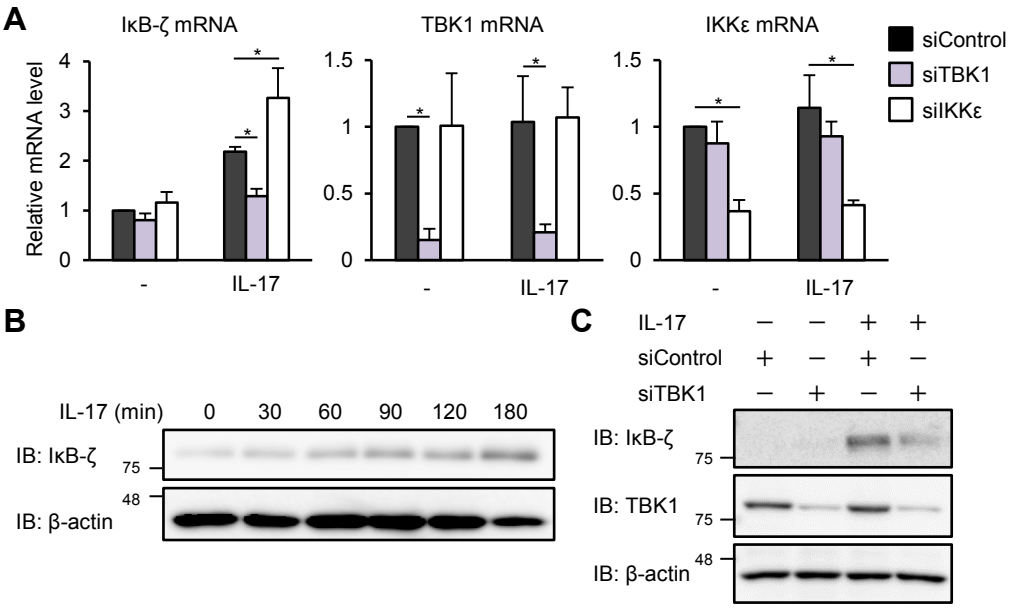
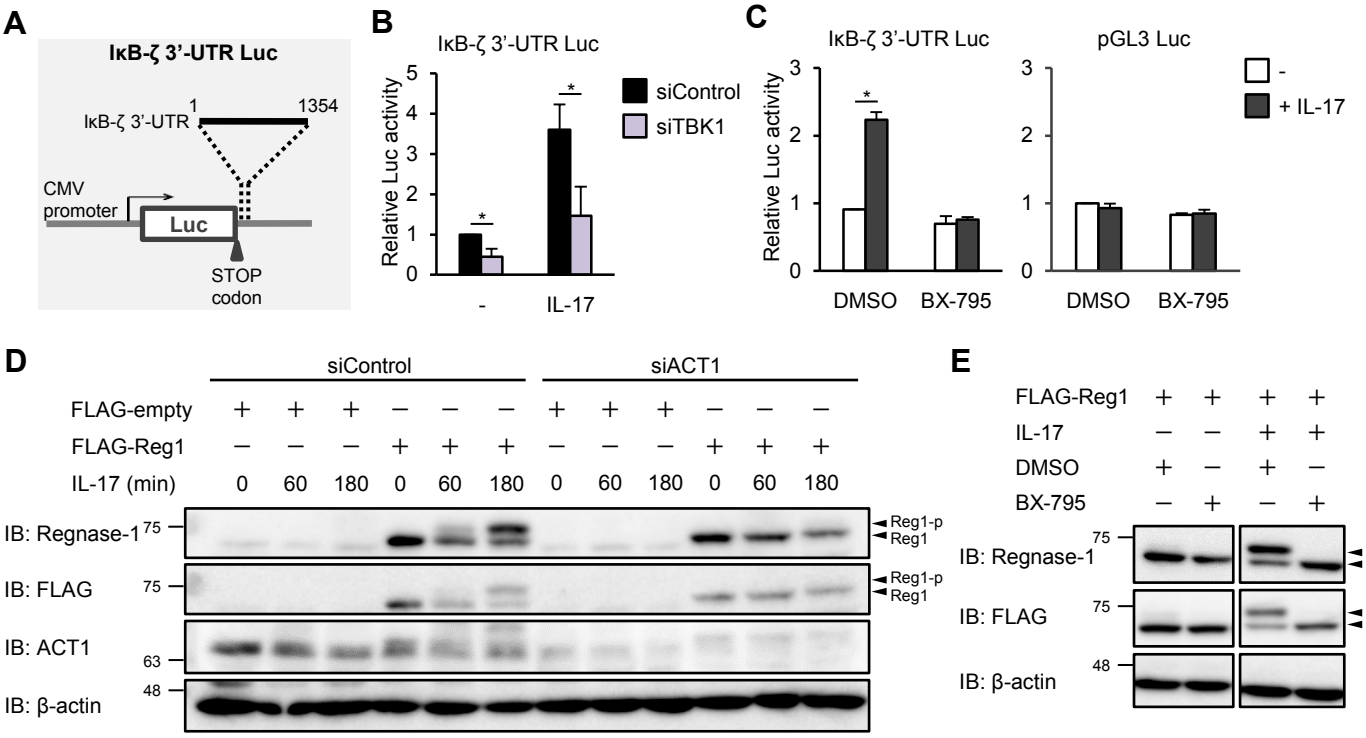


Figure 2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-
IL-17
C
IκB-ζ 3'-UTR Luc
pGL3 Luc
Relative Luc activity
-
+ IL-17
DMSO
BX-795
D
siControl
siACT1
FLAG-empty
FLAG-Reg1
IL-17 (min)
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IB: FLAG
IB: ACT1
IB: β-actin
75
63
48
Reg1-p
Reg1
E
FLAG-Reg1
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IB: Regnase-1
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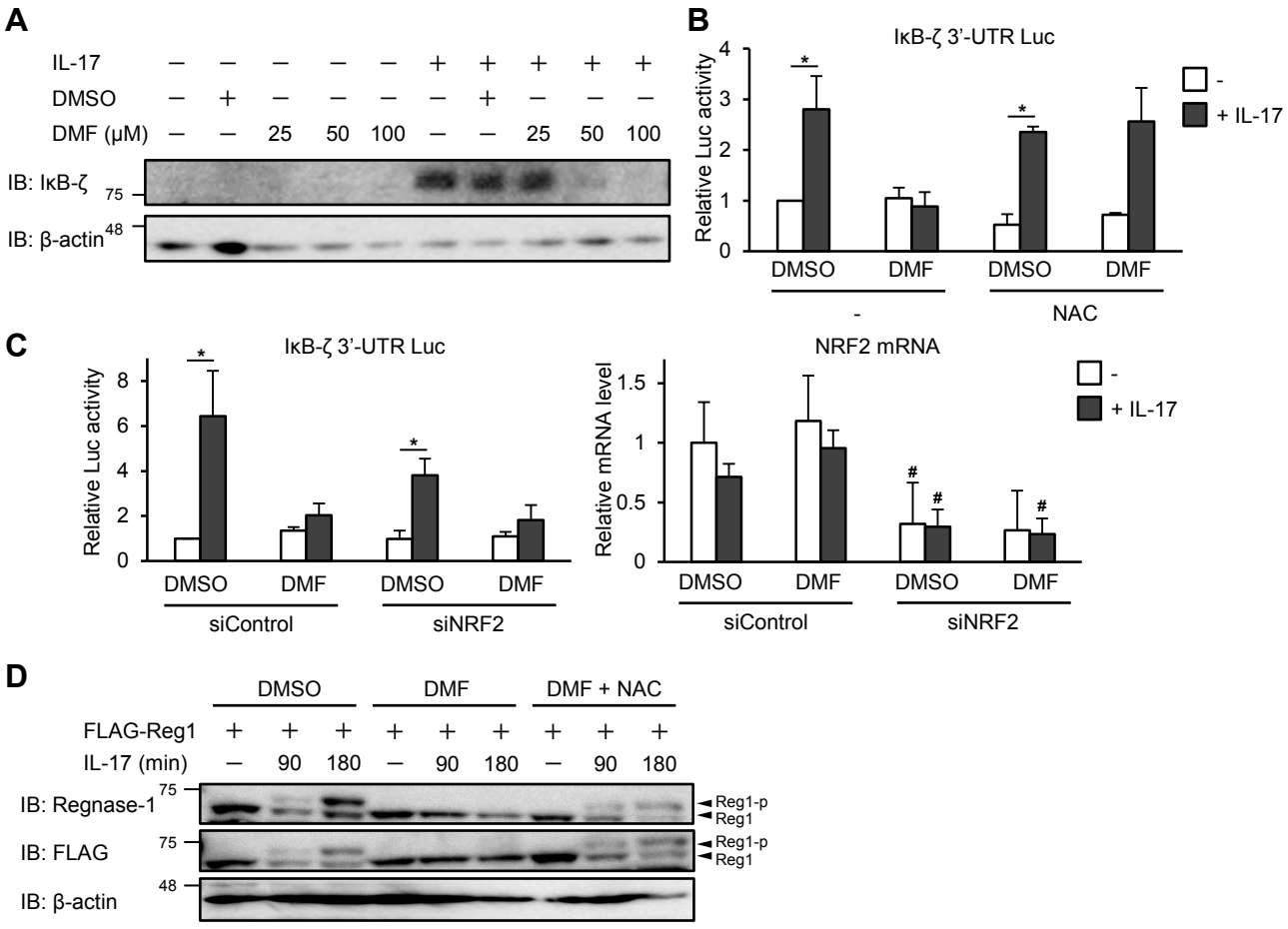
75
48
Reg1-p
Reg1

Figure 3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IL-17
DMSO
DMF (μM)
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IB: β-actin
B
IκB-ζ 3'-UTR Luc
Relative Luc activity
DMSO
DMF
NAC
+ IL-17
C
IκB-ζ 3'-UTR Luc
NRF2 mRNA
Relative mRNA level
siControl
siNRF2
D
DMSO
DMF
DMF + NAC
FLAG-Reg1
IL-17 (min)
IB: Regnase-1
IB: FLAG
Reg1-p
Reg1

Figure 4