Supporting Information

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SI Methods

Cell Culture. HuH-7 cells (a human hepatocellular carcinoma cell line, RCB1366) and HeLa cells (a human cervical carcinoma cell line, RCB0007) were from the RIKEN BioResource Center Cell Bank. Cells were maintained at 37 °C in 5% CO_2 in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% FBS (Sigma). Transfection efficiency of each cell line was about 70% and 30%, as judged by transient expression of an expression plasmid DNA encoding EGFP. FACS analysis was done on day 2 after transfection, and EGFP-positive cells were detected by the FL1 of FACSCalibur (Becton Dickinson).

Chemicals and Antibodies. FICZ (6-formylindolo[3,2-*b*]carbazole; Enzo Life Sciences), SP600125 (Sigma), SB202190 (Sigma), protease inhibitors (Roche), and phosphatase inhibitor mixture set (Calbiochem) were used. G418 (Sigma), hygromycin (Hygro; Sigma), puromycin (Puro; Sigma), and zeocin (Zeo; Invitrogen) were used after checking the sensitivity of each cell line to the respective antibiotic. Antibodies against aryl hydrocarbon receptor (AhR) (Santa Cruz Biotechnology), AhR nuclear translocator-1 (ARNT1) and ARNT2 (Santa Cruz Biotechnology), β-tubulin (NeoMarkers), γ-H2AX (Upstate Biotechnology), H2AX (Millipore), CREB and phosphorylated CREB (Cell Signaling Technology), C/EBP-β and phosphorylated C/EBP-β (Cell Signaling Technology), c-Jun and phosphorylated c-Jun (Cell Signaling Technology), FLAG (Sigma), EGFP (Medical & Biological Laboratories), and GAPDH (Trevigen) were used as first antibodies. Mouse IgG, horseradish peroxidase (GE Healthcare); rabbit IgG, horseradish peroxidase (GE Healthcare); and goat IgG, horseradish peroxidase (Santa Cruz Biotechnology) were used as second antibodies. An AhRR antibody and 3′-methoxy-4′-nitroflavone (MNF) were gifts from Mark Hahn (Woods Hole Oceanographic Institution, Woods Hole, MA) and Gabriele Vielhaber (Symrise, Holzminden, Germany), respectively.

Detection of L1-RTP. Colony assays were carried out according to the protocol described in Fig. 1*A* using pCEP4/L1*mneo*I/ColE1 (pL1-Neo^R), a kind gift from Nicolas Gilbert (University of Michigan Medical School, Ann Arbor, MI). pL1-Neo^R contains all components of human long interspersed nucleotide element-1 (L1), which are driven by the CMV promoter located in the 5′ region of the construct. It has an inversely inserted transcriptional unit that contains the CMV promoter, a neomycin-resistant (Neo^R) gene, and a poly(A) signal. The Neo^R gene is interrupted by an intron inserted in a sense orientation (arrow in Fig. 1*A*). The intron is spliced during the processing of retrotransposition of L1 (L1-RTP) (SD, splicing donor; SA, splicing acceptor; Fig. 1*A*), by which the Neo^R gene becomes functional. Cells were transfected with 8 μg of pL1-Neo^R and selected for 2 d with 25 μg/mL Hygro. On day 3 after transfection, the cells were replated and exposed for 2 d to various concentrations of FICZ or dimethyl sulfoxide (DMSO), which was used as a solvent of FICZ. Drug selection was started on day 5 using 800 μg/mL G418. After 14–21 d, Neo^R colonies were stained with methylene blue (Wako), counted, and subjected to statistical analysis. To explore the roles of cellular proteins in FICZ-induced L1-RTP, each siRNA was introduced on day 3 (indicated by the arrowhead in Fig. 1*A*) with the remainder of the protocol unchanged. Experiments were done more than twice, and in each experiment, test samples from six different plates were assayed. To examine the effects of MAPK inhibitors, SB202190 or SP600125 at concentrations of 1 μM or 100 μM, respectively, were added 30 min before the addition of 10 nM FICZ. TCDD (2,3,7,8-tetrachlorodibenzo-*p*-dioxin) was treated at 10, 1, 0.1, 0.01, 0.001, and 0.0001 μM.

The PCR-based assay was performed according to the protocol shown in Fig. 1*C* using pEF06R, which was kindly provided by Eline T. Luning Prak (University of Pennsylvania Medical Center, Philadelphia, PA). pEF06R contained essentially the same components as pL1-Neo^R, except that *EGFP* cDNA replaced the Neo^R gene (Fig. 1*C*). Cells were transfected with 8 μg of pEF06R and selected for 2 d with 0.5 μg/mL Puro. On day 3 after the transfection, the cells were replated, exposed for 1–6 d to 10 nM FICZ, and subjected to PCR analysis. Genomic DNA was isolated using the QuickGene DNA Tissue Kit S and QuickGene-800 (FujiFilm). To selectively amplify the product of L1-RTP, DNA was treated with PstI, which is present in the intron of the cDNA (Fig. 1*C*). L1 DNA was PCR-amplified using 5′-ACTGGGTGCTCAGGTAGTGGTT-3′ and 5′-GAAGAACGGCATCAAGGTGAA-3′ as the forward and reverse primers, respectively. Amplifications were performed using Ex Taq (TaKaRa) by an initial 15 min at 94 °C and 28–32 cycles of amplification for 30 s at 94 °C and 15 s at 68 °C. To detect *β-actin* as an internal control, 5′-TGAACCCCAAGGCCAACCGC-3′ and 5′-TTGTGCTGGGTGCCAGGGCA-3′ were used as forward and reverse primers, respectively. PCR amplification was done for an initial 5 min at 94 °C and 20 cycles of amplification for 30 s at 94 °C, 30 s at 62 °C, and 1 min at 72 °C. The PCR product was run on agarose gels and visualized with SYBR Green I Nucleic Acid Gel Stain (Lonza). The relative intensity (RI) was calculated based on the signal intensities of the amplified DNA derived from *EGFP* and *β-actin*.

Effects of siRNAs on the Expression of Endogenous Gene Products. siRNA was synthesized by Applied Biosystems. Three different siRNAs were prepared for each gene and their functions were evaluated. The nucleotide sequence of each siRNA is shown in Table S1. Each siRNA was introduced into cells in Opti-MEM (Invitrogen) by mixing 100 μL of the siRNA at a concentration ranging from 50 nM to 16 pM with 10 μL of Lipofectamine 2000 (Invitrogen) added to 6 mL of medium on a 10-cm plate. On days 2–6 after transfection, the cells were washed with phosphate-buffered saline (PBS) and resuspended in 50 mM Tris·HCl (pH 7.6), 150 mM NaCl, 0.1% SDS, 0.5% deoxycholic acid, 1% Nonidet P-40, and protease inhibitors (RIPA buffer). After sonication using a Bioruptor (UCD-250; Cosmo Bio) by ultrasonication for 12.5 min (10 s on, 20 s off) at a high level (250 W) at 4 °C, the soluble cellular extracts were recovered after centrifugation for 10 min at 16,000 × *g*. The protein concentration of each sample was determined using the BCA Protein Assay Reagent Kit (Thermo Scientific), and cell extracts were subjected to Western blot analysis. The blots were probed with the first antibody followed by the horseradish peroxidase-conjugated second antibody. The immune complex was visualized using the ECL Plus Western Blotting Detection System (GE Healthcare).

Construction of siRNA-Resistant Constructs of *ARNT1* and *CREB* cDNAs. *ARNT1* and *CREB* cDNAs were provided by Oliver Hankinson (Jonsson Comprehensive Cancer Center, University of California, Los Angeles, CA) and Kumiko Saeki (National Center for Global Health and Medicine), respectively. Both cDNAs were expressed by pcDNA3.1 Zeo (Invitrogen), which had been preinserted with an oligonucleotide cassette for generating the NheI-Kozak sequence ATG-3xFlag-XbaI (pFlagZeo).

To generate a construct that expresses siRNA-resistant *ARNT1* cDNA (pSi^R-*ARNT1*), a DNA fragment was first amplified from

a provided *ARNT1* cDNA (1) using an ARNT1-F/XbaI primer (5′-AAATCTAGAATGGCGGCGACTACTGC-3′) and an ARNT1-R/BamHI primer (5′-AAGGATCCCTATTCTGAAAAGGGGGGAAAC-3′) used as 5′ and 3′ primers, respectively. Next, an amplified DNA was treated with XbaI-BamHI and inserted into the same restriction enzyme sites of pFlagZeo (p*Flag-ARNT1*). On the other hand, a KpnI fragment of *ARNT1* cDNA, which contained target regions of *ARNT1* siRNA-1, was subcloned into pBluescriptII/KS+ vector (pKSII; Stratagene) and named pKSII/ARNT1/KpnI. Then, an oligonucleotide cassette of 5′-tcagcagtcttccatgagaatgtgtatgggctcaaggagatcgtttatttgccgaatgaggtgtggcagtagctctgtggacccCgtCtcCgtCaaCaggc-3′ (only the sense strand is depicted) was inserted into BbsI and BlpI sites of pKSII/ARNTI/KpnI. Capital letters in the oligonucleotide indicate mutated nucleotides, but the same amino acids were encoded as the wild-type ARNT1. Finally, a KpnI fragment of *ARNT1* cDNA with an siRNA-resistant sequence was inserted into the corresponding sites of p*Flag-ARNT1*.

To make a construct that expresses siRNA-resistant *CREB* cDNA (pSi^{R}-*CREB*), a XbaI-BamHI DNA fragment of *CREB* cDNA was excised from a gifted construct (2) and inserted into the same restriction enzyme sites of pFlagZeo to make p*Flag-CREB*. Then, an oligonucleotide of 5′-gcagtggacagtatattgccattacccagggaggagcaatacagctCgcCaaTaaCggtac-3′ (only the sense strand is depicted) was inserted into XcmI and KpnI of p*Flag-CREB*. Capital letters in the oligonucleotide indicate mutated nucleotides, but the same amino acids were encoded as the wild-type CREB.

The quality of each construct was checked by restriction mapping and nucleotide sequencing.

Construction of a Constitutively Active *CREB* (*CREB*^{Y134F}) cDNA. A plasmid DNA encoding the Y134F mutant (p*CREB*^{Y134F}), a constitutively active mutant with substituted phenylalanine at tyrosine 134 (3), was generated using a QuikChange Site-Directed Mutagenesis Kit (Stratagene) using p*Flag-CREB* as a template. After amplification of the plasmid using *PfuTurbo* DNA polymerase with primers of 5′-gaggccttcctTcaggaaaattttgaatgacttatcttctg-3′ and 5′-cagaagataagtcattcaaaatttttcctgAaggaaggcctc-3′ (the mutated nucleotide is indicated by a capital letter), the plasmid was treated with DpnI and then the PCR product was rescued in bacteria. A positive clone that contained tTc instead of tAc at codon 134 (a capital letter indicates the mutated nucleotide) was confirmed by nucleotide sequencing.

To evaluate the function of p*CREB*^{Y134F}, a *firefly* luciferase reporter construct was generated by inserting an oligonucleotide cassette of the cAMP-responsive element (CRE) of 5′-CTCGGGGGCGCCTCCTTGGCTGACGTCAGAGAGAGAGC-3′ into the pGL3 enhancer (pCRE-luc; Promega) (2). pCRE-luc was introduced into HuH-7 cells with phRL-SV40, which encoded *Renilla reniformis* luciferase (Promega). As a positive control of the promoter activity of CRE, the cells were treated with 40 ng/mL human TNF-α (R&D Systems) for 48 h (Fig. S5*B*, lane 2) (4). The luciferase activity was normalized to the activity of *R. reniformis* luciferase.

Protocol of Back-Transfection Experiments Using siRNA-Resistant cDNA Clones. The basic protocol for these experiments is illustrated in Fig. 1*A*. To evaluate the roles of the target gene products, cells were transfected with siRNA for each gene on day 3 after the first transfection of pL1-Neo^{R} or pEF06R (Fig. 1*A*, arrowhead). On day 4, pSi^{R}-*ARNT1* or pSi^{R}-*CREB* was further introduced with Lipofectamine 2000, and the cells were again replated on day 5. The cells were then treated with FICZ for 2 d, and G418 selection was started on day 7 after the first transfection with pL1-Neo^{R}. For the PCR-based assay, cells were harvested on day 7.

Construction of Plasmid DNAs Expressing ORF1-TAP or EGFP-TAP and Detection of Chromatin Recruitment of ORF1. To prepare a construct to express ORF1-TAP (tandem affinity purification) (pORF1-TAP), a BamHI-EcoRI fragment of *TAP-tag* cDNA was excised from pZome-1-C (5) and inserted into the same sites of pcDNA3.1 Zeo (pcDNA/TAP). Then, a KpnI-BamHI codon-optimized ORF1 cDNA fragment was amplified using pBudORF1$_{syn}$ (6) as a template and inserted into the same restriction enzyme sites of pcDNA/TAP. To prepare a plasmid DNA encoding EGFP-TAP (pEGFP-TAP), a KpnI-BglII fragment of EGFP cDNA was amplified from pBOSH2BGFP-N1 (7) and inserted into KpnI-BamHI sites of pcDNA/TAP. The quality of the constructs was verified by restriction mapping and nucleotide sequencing. On day 2 of transfection with these plasmid DNAs, the chromatin fraction was isolated using a Subcellular Protein Fractionation Kit (Thermo Scientific). First, a prepared nuclear insoluble fraction was washed three times in the supplied buffer supplemented with NaCl giving 300 mM as the final concentration. Then, samples were treated for 30 min with 300 U micrococcal nuclease (Thermo Scientific) at 37 °C and centrifuged for 10 min at 16,000 × *g*. The recovered supernatant was used as samples as the chromatin fraction. TAP-tagged proteins were detected by horseradish peroxidase-conjugated goat anti-human IgG (Jackson ImmunoResearch). As a positive control of a protein present in the chromatin fraction, H2AX was detected.

Molecular Interaction of ARNT1 and ORF1. To prepare a construct to express a chimeric protein of ORF1-EGFP (p*ORF1-EGFP*), a BamHI-NotI DNA fragment of *EGFP* cDNA excised from pBOSH2BGFP-N1 (7) was first inserted into the same restriction enzyme sites of pcDNA3.1 Zeo (pcDNA/*EGFP*). Then, a codon-optimized *ORF1* cDNA amplified from pBudORF1$_{syn}$ (6) was inserted in-frame into KpnI-BamHI sites of pcDNA/*EGFP*. A plasmid DNA expressing *Flag-streptag-EGFP* (p*FS-EGFP*) was generated by inserting an oligonucleotide cassette encoding -WSHPQFEK-WSHPQFEK-M- (amino acids are depicted by single letters for streptag, with spacers shown by "-") into pFlag-CMV2 (Sigma), which had been inserted with an *EGFP* cDNA. Restriction mapping and nucleotide sequencing verified the quality of the constructs. HuH-7 cells transfected with p*FS-EGFP* or p*ORF1-EGFP* were further treated with 10 nM FICZ or 0.001% DMSO. Cells were recovered the following day and suspended in 20 mM Tris·HCl (pH 7.6), 5 mM EDTA, 150 mM NaCl, 0.5% Nonidet P-40, and 10% glycerol. After incubation on ice for 30 min, each sample was sonicated, as described. After centrifugation at 16,000 × *g*, the supernatants were used as whole-cell extracts. For immunoprecipitation (IP), each 500 μg of cell extract was reacted with 4 μg of αARNT1 (αA) or αEGFP (αE), and the immune complex was recovered using protein G beads (GE Healthcare). As an "input" sample, about one-tenth of each extract subjected to IP was loaded and simultaneously analyzed.

Detection of *CYP1A1* mRNA by RT-PCR. Total RNA from HuH-7 cells treated with 10 nM FICZ for 6 h was isolated using an RNeasy Mini Kit (Qiagen). The RNA was treated for 30 min with RNase-free DNase (Qiagen) at 25 °C, and then 350 ng of the total RNA was reverse-transcribed for 60 min at 37 °C in a 20-μL volume using a High Capacity RNA-to-cDNA Kit (Applied Biosystems). The synthesized cDNA was used for PCR reactions. Amplifications were performed using Ex Taq for an initial 15 min at 94 °C and 20 cycles of amplification consisting of 15 s at 94 °C, 25 s at 60 °C, and 30 s at 72 °C. Human *CYP1A1* mRNA was detected by 5′-TAGACACTGATCTGGCTGCAG-3′ and 5′-GGGAAGGCTCCATCAGCATC-3′ used as forward and reverse primers, respectively.

Quantitative RT-PCR to Detect L1 mRNA. Total RNA was purified using an RNeasy Mini Kit (Qiagen) and treated for 30 min with

RNase-free DNaseI (Qiagen) at 25 °C. First-strand cDNA was prepared using random hexamers or oligo-dT. An Omniscript RT Kit (Qiagen) was used for reverse transcription, and amplification of DNAs was monitored and quantified by SYBR Premix Ex Taq (TaKaRa) and a 700 Sequence Detection System (Applied Biosystems) according to the manufacturers' instructions. All data were normalized with respect to *β-actin* (*ACTB*). For the quantification of both endogenous and exogenous *ORF2* mRNA, a primer set of L1-EGFP+5653F (5′-CCAAATGTCCAACAATGATAGACTG-3′)/L1-EGFP+5762R(5′-CCATGTCCCTACAAAGGATATGAAC-3′) was used. For the quantification of mature or precursor mRNA of *EGFP*, primer sets of L1-EGFP+6342F (5′-TAGTGGTTGTCGGGCAGCAG-3′)/L1-EGFP+7351R(TTCAAGATCCGCCACAACATC-3′) or L1-EGFP+7222F(5′-TGGAAGCTGGGTGTGTAGTTATCTG-3′)/L1-EGFP+7365R(5′-GGCATCAAGGTGAACTTCAAGATC-3′) were used, respectively. Transcripts derived from the 3′ CMV promoter region of pEF06R were quantified using the primer set L1-EGFP+8245F(5′-TGGCATATGATACACTTGATGTACTGC-3′)/L1-EGFP+8349R(5′-TATGTTCCCATAGTAACGCCAATAGG-3′). For amplification of *ACTB*-derived DNA, a primer set of h-ACTB-F(5′-GAGTACTTGCGCTCAGGAGGA-3′)/h-ACTB-R(5′-CATTGCCGACAGGATGCA-3′) was used. To evaluate the levels of mRNA, each sample was prepared in triplicate, and statistical analyses were performed based on the relative intensities of the mRNA levels normalized with respect to the *ACTB* levels.

To correctly assess the mature form of L1 mRNA from pEF06R, it was required to exclude the effects of antisense mRNA transcribed from the inverted 3′ CMV promoter (Fig. S7*A*, lower arrow). For this purpose, we prepared a construct that lacked the corresponding region (pEF06RΔ3′CMV; Fig. S7*C*). A BamHI fragment of pEF06R, which had the 3′ CMV promoter, was subcloned into the BamHI site of pBSII, digested with HindIII, and recovered after self-ligation (pBSII/Δ3′ CMV/BamHI fragment). The Δ3′ CMV/BamHI fragment was then prepared and ligated into BamHI sites of pBSII-L1, which had most of pEF06R except for the 5′ CMV promoter (pL1-*EGFP*Δ3′ CMV). Finally, a NotI-ApaI fragment of pL1-*EGFP*Δ3′ CMV was excised and inserted into the same restriction enzyme sites of pcDNA3.1 Hygro. The quality of the obtained product was confirmed by restriction mapping and nucleotide sequence analysis.

Statistics. Statistical significance was evaluated using the Mann–Whitney *U* test. Numbers of test samples were more than four. Values of <0.05 were considered to be statistically significant.

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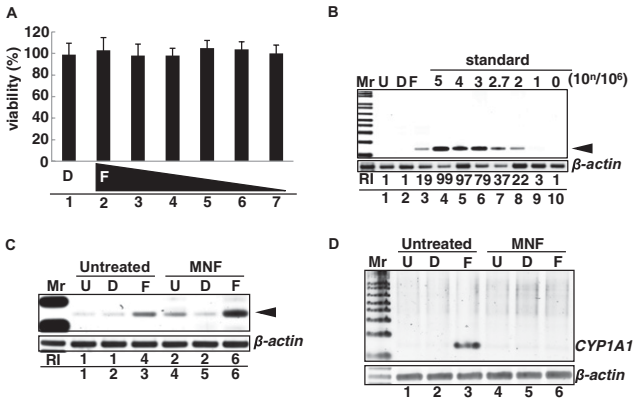

Fig. S1. FICZ induces L1-RTP without cytotoxic effects. (*A*) Plating efficiency after treatment with FICZ. HuH-7 cells were treated for 2 d with FICZ at doses ranging from 100 nM to 1 pM, and then 500 cells of each sample were plated onto 6-cm plates. Cells were cultured for an additional 10 d in the presence of the same concentrations of FICZ, fixed, and stained with methylene blue. Cells were treated with 0.01% DMSO (lane 1) or FICZ at 100 nM, 10 nM, 1 nM, 100 pM, 10 pM, and 1 pM (lanes 2–7). Numbers of colonies were counted and the means ± SD were calculated. Relative viability was compared with respect to the sample treated with DMSO and shown by %. (*B*) Frequency of FICZ-induced L1-RTP. HuH-7 cells transfected with pEF06R were treated with 10 nM FICZ and subjected to the PCR-based assay. Standard samples were prepared by making a mixture of 10^5 to 10^0 EGFP-positive and 10^6 EGFP-negative cells (lanes 4–10, $10^n/10^6$, n = 5–0), and subjected to the analysis. An arrowhead indicates the 140-bp band corresponding to L1-RTP. RI indicates relative intensity, calculated based on signal intensities of the 140-bp bands normalized to the PCR bands derived from *β-actin*. Untreated control (U, lane 1), 0.001% DMSO (D, lane 2), 10 nM FICZ (F, lane 3). (*C*) Effects of AhR antagonist on FICZ-induced L1-RTP. Cells were treated with 10 μM MNF for 30 min before the addition of FICZ (lanes 4–6), and then the PCR-based assay was performed. As a control, 0.001% DMSO was included (lanes 1–3). Untreated control (U, lanes 1 and 4), DMSO (D, lanes 2 and 5), FICZ (F, lanes 3 and 6). (*D*) Effects of MNF on FICZ-induced expression of *CYP1A1* mRNA. HuH-7 cells were treated for 30 min with 10 μM MNF and then exposed to 10 nM FICZ supplemented in fresh culture medium. After 6 h (lanes 4–6), RNAs were extracted and analyzed by RT-PCR. As a control, 0.001% DMSO was included (lanes 1–3). Untreated control (U, lanes 1 and 4), DMSO (D, lanes 2 and 5), FICZ (F, lanes 3 and 6).

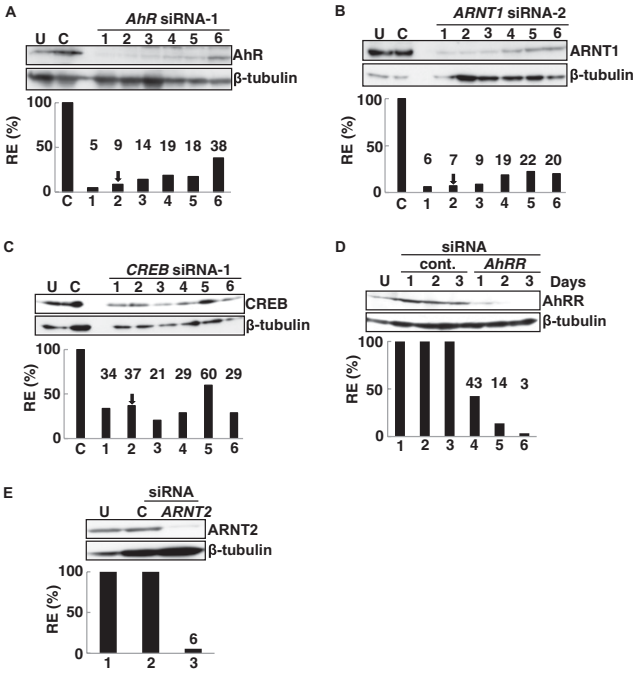

Fig. S2. Functional evaluation of siRNAs. (*A*–*C*) Effects of *AhR*, *ARNT1*, and *CREB* siRNAs on endogenous protein expression. Western blot analysis was done on day 2 after the introduction of each siRNA at concentrations of 50 nM, 10 nM, 2 nM, 400 pM, 80 pM, and 16 pM (lanes 1–6). C, control siRNA; U, untreated. Relative expression (RE) was calculated based on signal intensities of samples treated with control siRNA (100%) and the siRNA targeting a specific gene. Numbers shown within each panel indicate RE depicted by % obtained by each concentration of siRNA. By using 50 nM siRNAs, the RE was about 5%, 6%, and 34% by *AhR*, *ARNT1*, and *CREB* siRNAs, respectively. When 10 nM siRNA was used, it was about 9%, 7%, and 37% for *AhR*, *ARNT1*, and *CREB* siRNAs, respectively. Arrows indicate the concentration (10 nM) used in most of the subsequent experiments. (*D* and *E*) Down-regulation of endogenous proteins by siRNAs of *AhRR* and *ARNT2*. After transfection of 50 nM *AhRR* siRNAs, the RE reduced to about 43%, 14%, and 3% on days 1, 2, and 3 (lanes 4–6). By 50 nM *ARNT2* siRNA, the RE was reduced to 6% on day 2 (lane 3). C, control siRNA; U, untreated.

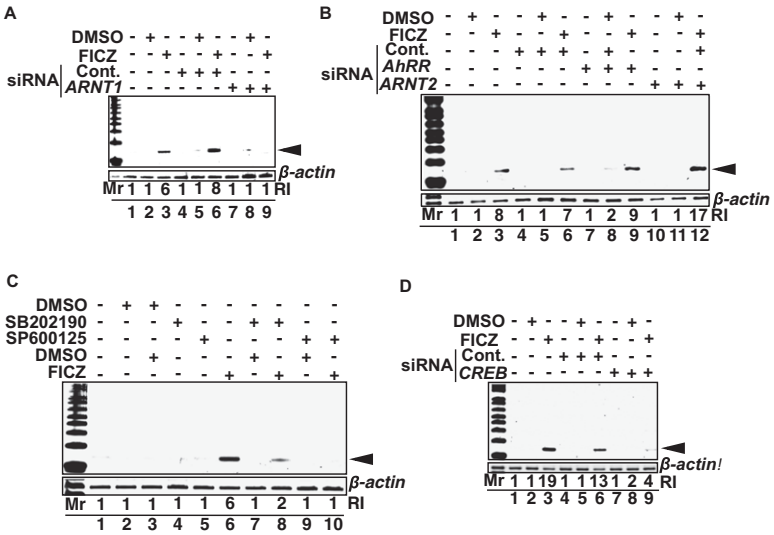

Fig. S3. PCR-based assays to detect the effects of siRNAs or MAPK inhibitors on FICZ-induced L1-RTP. Before FICZ treatment, HuH-7 cells were transfected with 10 nM siRNAs or treated for 30 min with 1 μM SB202190 or 100 μM SP600125, treated with FICZ, and analyzed using the PCR-based assay. (*A*) Effects of *ARNT1* siRNA. A mixture of *ARNT1* siRNA-1 and -2 was used. (*B*) Effects of *AhRR* and *ARNT2* siRNAs. Each 10 nM siRNA was transfected. (*C*) Effects of MAPK inhibitors. (*D*) Effects of *CREB* siRNA. A mixture of *CREB* siRNA-1 and -2 was used. Arrowheads indicate the 140-bp band of the L1-RTP. *β-Actin* was amplified as an internal control. The RI was calculated based on the signal intensities of the 140-bp band and *β-actin*.

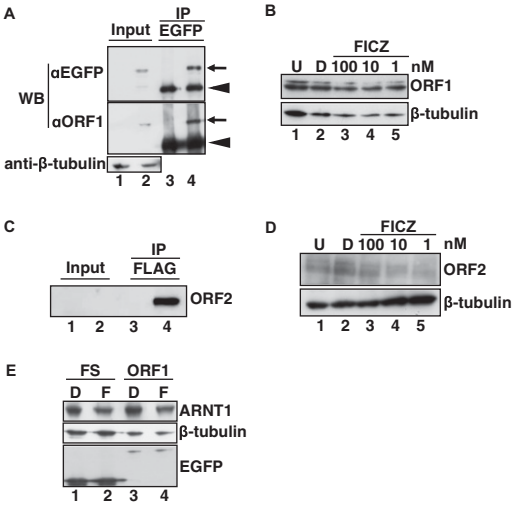

Fig. S4. FICZ did not increase the expression levels of ORF1 or ORF2. (*A*) Detection of ORF1 by a newly generated antibody. A rabbit antibody against ORF1 (αORF1) was produced by a peptide MGKKQNRKTGNSKTQSAC (amino acids depicted by single letters) used as an immunogen by conjugating with keyhole limpet hemocyanin. HuH-7 cells were transfected with an expression vector encoding a fusion protein of codon-optimized ORF1 (6) and EGFP. Cell extracts were first immunoprecipitated with αEGFP (lanes 3 and 4), and then the recovered proteins were probed with αEGFP (*Upper*) or αORF1 (*Middle*). αORF1 detected the band recovered by αEGFP (arrow in middle panel). Arrowheads indicate IgG heavy chain. Input samples and β-tubulin were analyzed as loading controls. (*B*) FICZ did not increase the level of ORF1. HuH-7 cells were transfected with pEF06R, and FICZ was added on day 2. The total cell extracts were prepared 24 h after the addition of 100, 10, and 1 nM FICZ and subjected to Western blot analysis. αORF1 was used as the first antibody. β-Tubulin was included as a loading control. (*C*) Functional evaluation of αORF2. HEK293T cells (a human cell line derived from fetal kidney with exogenous expression of SV40 T antigen) were transfected with a plasmid DNA expressing a *Flag-tagged ORF2*. This construct was generated from pBudORF2$_{syn}$ (6), which encodes a codon-optimized ORF2 of human L1 (a kind gift from Astrid M. Roy-Engel, Tulane University Health Sciences Center, New Orleans, LA). An oligonucleotide encoding 3xFlag tags was inserted in the 3′ region of pBudORF2$_{syn}$. The cellular extracts were prepared 2 d after transfection, immunoprecipitated with αFlag (M2) (Sigma), and subjected to Western blot analysis. αORF2, a kind gift of John Goodier (University of Pennsylvania School of Medicine, Philadelphia, PA), was used as the first antibody. (*D*) FICZ did not increase the expression of ORF2. HuH-7 cells were transfected with pEF06R, treated for 24 h with FICZ, and subjected to Western blot analysis. Untreated control (U, lane 1), 0.01% DMSO (D, lane 2), 100, 10, and 1 nM FICZ (F, lanes 3–5). (*E*) FICZ did not increase ARNT1 expression. HuH-7 cells were transfected with p*FS-EGFP* (FS, lanes 1 and 2) or p*ORF1-EGFP* (ORF1, lanes 3 and 4) and treated with 0.01% DMSO (D, lanes 1 and 3) or 10 nM FICZ (F, lanes 2 and 4). After 24 h, cell extracts were prepared and subjected to Western blot analysis. In addition to ARNT1, EGFP and β-tubulin were analyzed as loading controls.

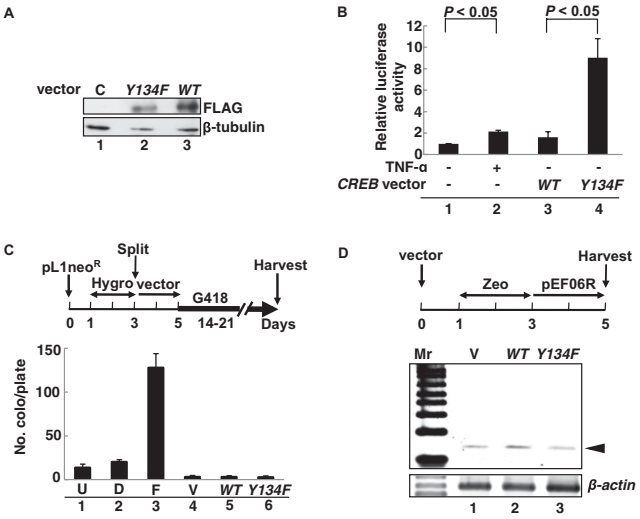

Fig. S5. Constitutively active CREB (Y134F) does not induce L1-RTP. (*A*) Detection of protein expression of a constitutively active CREB. p*CREB*^{Y134F} was constructed as described in *SI Methods*. HuH-7 cells were transfected with a vector control (C, lane 1), p*CREB*^{Y134F} (Y134F, lane 2), or p*CREB*^{WT} (WT, lane 3), and subjected to Western blot analysis. αFlag was used as the first antibody. β-Tubulin was included as a loading control. (*B*) Increased promoter activity by CREB^{Y134F}. pGL-Luc (Promega) was inserted with a reported nucleotide sequence that had cAMP-responsive element (CRE) (pCRE-Luc). pCRE-Luc was introduced into HuH-7 cells with p*CREB*^{WT} or p*CREB*^{Y134F} (lanes 3 or 4). Then, *firefly* luciferase activity was measured. As a positive control for CRE promoter activity, cells were treated with 40 ng/mL human TNF-α (R&D Systems) for 48 h (lane 2) (4). The luciferase activity was normalized to the activity of *R. reniformis* luciferase expressed by cotransfecting phRL-SV40 (Promega). The fold increase was calculated with respect to the control sample (lane 1). The relative activity of CREB^{Y134F} was statistically significant ($P < 0.05$). (*C*) A colony assay detected no induction of L1-RTP by the forced expression of p*CREB*^{Y134F}. HuH-7 cells were first transfected with 8 μg of pL1-Neo^R and selected for 2 d with Hygro. On day 3, cells were further transfected with 8 μg of vector control, p*CREB*^{WT} (*WT*), or p*CREB*^{Y134F} (*Y134F*), and G418 selection was started on day 5. Values shown are the means ± SD of the numbers of Neo^R colonies counted; no significant increase of Neo^R colonies was detected. Untreated control (U, lane 1), 0.001% DMSO (D, lane 2), 10 nM FICZ (F, lane 3), vector control (V, lane 4), p*CREB*^{WT} (*WT*, lane 5), p*CREB*^{Y134F} (*Y134F*, lane 6). (*D*) The PCR-based assay detected no induction of L1-RTP by CREB^{Y134F}. HuH-7 cells were transfected with 8 μg of vector, p*CREB*^{WT}, or p*CREB*^{Y134F}, and selected for 2 d with 25 μg/mL Zeo. Then, cells were further transfected with 8 μg of pEF06R on day 3, further cultured for an additional 2 d without Puro, and subjected to the PCR-based assay on day 5. The arrowhead indicates the position of the 140-bp fragment amplified as a result of L1-RTP. *β-Actin* was amplified as an internal control.

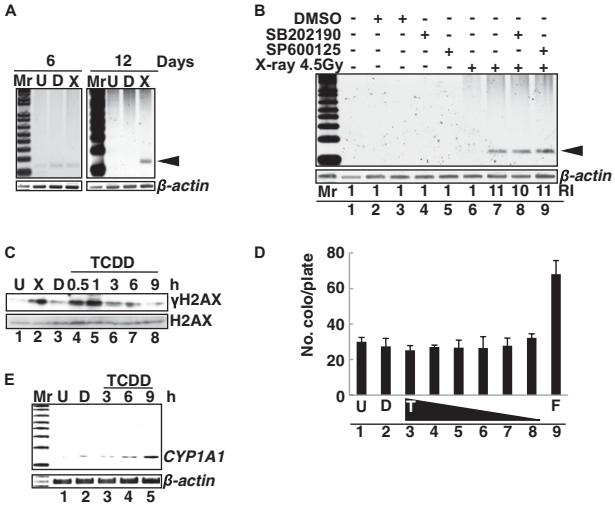

Fig. S6. FICZ-induced L1-RTP depends on cellular cascades different from those involved in double-strand-break-induced L1-RTP. (*A*) L1-RTP triggered by DNA damage required longer incubation periods than that triggered by FICZ. The PCR-based assay was carried out using HuH-7 cells after X-ray irradiation at 4.5 Gy. The arrowhead indicates the 140-bp fragment, and *β-actin* was amplified as an internal control. Note that L1-RTP was not detected after 6 d, but was detected after 12 d of cell culture. In contrast, FICZ-induced L1-RTP was detected within 1–2 d (Fig. 1 *E* and *F*). D, 0.01% DMSO; U, untreated; X, 4.5 Gy. (*B*) L1-RTP triggered by DNA damage was not sensitive to MAPK inhibitors. HuH-7 cells were irradiated without (lanes 6 and 7) or with (lanes 8 and 9) treatment of MAPK inhibitors. As a control, HuH-7 cells cultured for 12 d with no reagents (lane 1), 0.001% and 0.1% DMSO (lanes 2 and 3), or MAPK inhibitors (lanes 4 and 5) were included. Cells were harvested on day 6 (lane 6) or day 12 (lanes 1–5 and 7–9), and subjected to the PCR-based assay. Notably, MAPK inhibitors did not attenuate the signals of the 140-bp band (lanes 8 and 9). *β-Actin* was amplified as an internal control and the RI was calculated based on signal intensities of the 140-bp band and *β-actin*. (*C*) TCDD induced expression of γ-H2AX. HuH-7 cells were treated with 10 μM TCDD for 0.5, 1, 3, 6, and 9 h (lanes 4–8) and lysed in RIPA buffer supplemented with phosphatase inhibitors. After 15 min on ice, cell extracts were recovered by centrifugation at 16,000 × *g*. Western blot analyses were performed using anti-γH2AX and anti-H2AX. Untreated control (U, lane 1), X-ray irradiation at 4.5 Gy (X, lane 2), 0.1% DMSO (D, lane 3). H2AX was included as a loading control. (*D*) TCDD did not induce L1-RTP. A colony assay was carried out on HuH-7 cells after treatment for 2 d with concentrations of TCDD (100, 10, 1, 0.1, 0.01, and 0.001 μM; lanes 3–8). Cells were treated with no reagents (U, lane 1), 0.001% DMSO (D, lane 2), or 10 nM FICZ as a positive control (F, lane 9). (*E*) TCDD induced *CYP1A1* mRNA expression. HuH-7 cells were treated with 10 μM TCDD, and the induction of *CYP1A1* mRNA was detected by RT-PCR. RNA was extracted at time t = 3, 6, or 9 h (lanes 3–5) after the addition of 10 μM TCDD. Untreated control (U, lane 1), 0.1% DMSO (D, lane 2).

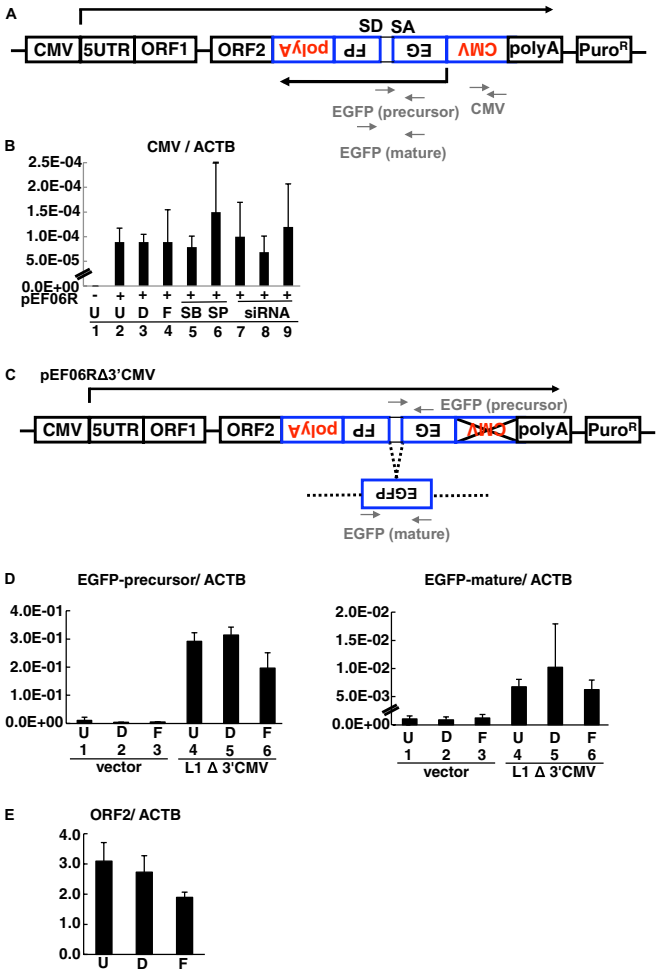

Fig. S7. Effects of FICZ, siRNA, and MAPK inhibitors on L1 mRNA. (*A*) PCR primers for quantitative RT-PCR (RT-PCR) analysis. It was difficult to detect L1 mRNA using two sets of PCR primers in *EGFP* cDNA, probably due to the presence of an antisense L1 mRNA transcribed from the 3′ CMV promoter of the construct (lower arrow). To exclude the effects of antisense mRNA, we designed PCR primers in the region that is out of the transcription initiation site of the 3′ CMV promoter. By using "CMV" PCR primers, we could successfully detect L1 mRNA. (*B*) No change in the transcript after the addition of FICZ, MAPK inhibitors, or siRNAs. SB, SB202190; SP, SP600125. qRT-PCR using CMV PCR primers detected no increase in the level of the transcript after treatment of FICZ (lane 4), MAPK inhibitors (lanes 5 and 6), or transfection of siRNAs of *AhR* (lane 7), *ARNT1* (lane 8), or *CREB* (lane 9). The background signal was subtracted in each case. RNA samples were prepared in triplicate for statistical analyses. (*C*) Schematic diagram of a construct without the 3′ CMV promoter region. To exclude the effects of antisense *EGFP* mRNA, we constructed pEF06RΔ3′ CMV (*SI Methods*). (*D*) No effects of FICZ on the expression of precursor L1 mRNA and on splicing. HuH-7 cells were transfected with pEF06RΔ3′ CMV or pcDNA3.1 Hygro as a control, and qRT-PCR was done. No change in either precursor or mature *EGFP* mRNAs was detected. Untreated control (U, lanes 1 and 4), 0.001% DMSO (D, lanes 2 and 5), 10 nM FICZ (F, lanes 3 and 6). (*E*) No induction of endogenous *ORF2* mRNA by FICZ. After treatment of HuH-7 cells for 6 h with FICZ, RNAs were extracted and qRT-PCR was performed. Nucleotide sequences of PCR primers are described in Table S1.

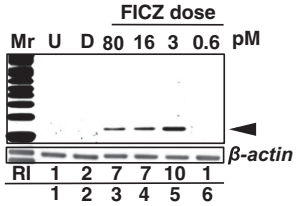

Fig. S8. Picomolar levels of FICZ induce L1-RTP. HuH-7 cells were transfected with pEF06R, treated for 2 d with various doses of FICZ, and analyzed by the PCR-based assay. Note that 3 pM FICZ induced L1-RTP (lane 5). The arrowhead indicates the 140-bp fragment. *β-Actin* was included as a control. Untreated (U, lane 1), 0.001% DMSO (D, lane 2), FICZ at 80, 16, 3, and 0.6 pM (F, lanes 3–6). RI, relative intensity.

Table S1. Summary of siRNAs used in the current study

Genes		Nucleotide sequences (5′→3′)
AhR-1	Se	GCAUGAUAGUUUUCCGGCUTT
	AS	AGCCGGAAAACUAUCAUGCCA
-2	Se	GGCUCUUUCAAGAUAGUAATT
	AS	UUACUAUCUUGAAAGAGCCCT
-3	Se	CGGAUGAAAUCCUGACGUATT
	AS	UACGUCAGGAUUUCAUCCGTT
ARNT1-1	Se	GGCGUAUCCUGGAUCUAAATT
	AS	UUUAGAUCCAGGAUACGCCCT
-2	Se	CAGUUUCUGUGAAUAGGCUTT
	AS	AGCCUAUUCACAGAAACUGGG
CREB-1	Se	GCUGGCUAACAAUGGUACCTT
	AS	GGUACCAUUGUUAGCCAGCTG
-2	Se	CCAAUCCCUUGAGUUAUAUTT
	AS	AUAUAACUCAAGGGAUUGGTT
-3	Se	GGUGGAAAAUGGACUGGCUTT
	AS	AGCCAGUCCAUUUUCCACCTT
AhRR-1	Se	GGGACGAUAUUUUAUGCAUTT
	AS	AUGCAUAAAAUAUCGUCCCTT
-2	Se	CCCAAUUACUCAGCAGGAATT
	AS	UUCCUGCUGAGUAAUUGGGTT
-3	Se	GGAUGCACCUGAAAACAGATT
	AS	UCUGUUUUCAGGUGCAUCCTG
ARNT2-1	Se	CCAGAGAGAUGGAUUGUCATT
	AS	UGACAAUCCAUCUCUCUGGTG
-2	Se	CUAUAACAUCGAAGACUUUTT
	AS	AAAGUCUUCGAUGUUAUAGTT
-3	Se	CACUGUACAGGAUACAUCATT
	AS	AAAGUCUUCGAUGUUAUAGTT

Nucleotide sequences of both sense (Se) and antisense (AS) strands are shown.