Supporting Information

Qian et al. 10.1073/pnas.1203185109

SI Materials and Methods

Plasmids. To generate a construct that inducibly expresses DEC1 shRNA, two pairs of oligos (sense-1, 5′-GATCCCC**GCAAGGAGACCTACAAATT**TTCAAGAGA**AATTTGTAGGTCTCCTTGC**TTTTTGGAAA-3′, and antisense-1, 5′-AGCTTTTCCAAAAA**GCAAGGAGACCTACAAATT**TCTCTTGAAA**ATTTGTAGGTCTCCTTGC**GGG-3′; sense-2, 5′-GATCCCC**CCTGAAGTCTTCGCAGCTT**TTCAAGAGA**AAGCTGCGAAGACTTCAGG**TTTTTGGAAA-3′, and antisense-2, 5′-AGCTTTTCCAAAAA**CCTGAAGTCTTCGCAGCT T**TCTCTTGAA**AAGCTGCGAAGACTTCAGG**GGG-3′), with siRNA targeting region shown in bold, were annealed and cloned into pBabe-H1 at BglII and HindIII sites. To generate a construct that stably expresses MIC-1 shRNA, one pair of oligos (sense, 5′-TCGAGGTCC**GACCCTCAGAGTTGCACTC**TTCAAGAGA**GAGTGCAACTCTGAGGGTC**TTTTTG-3′ and antisense, 5′-GATCCAAAAA**GACCCTCAGAGTTGCACTC**TCTCTTGAA**GAGTGCAACTCTGAGGGTC**GGACC-3′). with shRNA targeting region shown in bold (1), were annealed and cloned into pBabe-U6 at BamH1 and Xho1 sites. The p53 shRNA-expressing pBabe-U6 plasmid was generated previously (2).

HA-tagged DEC1, DEC1-R58P, and DEC1-M expression vectors in pcDNA3 were derived from pcDNA4-DEC1, -DEC1-R58P, and DEC1-M (3), respectively, with forward primer DEC1-1aa-F (5′-CGGAATTCGAGCGGATCCCCAGCGCGCAAC-3′) and reverse primer DEC1-412aa-R (5′-GCTCTAGAGGAAGGAAAGCAAAGCAGCAG-3′). DEC1(1–309) was amplified with forward primer DEC1-1aa-F and reverse primer DEC1-309aa-R (5′-GCTCTAGATTAGGAGCTGATCAGGTCACTGCTAG-3′). DEC1(110–412) was amplified with forward primer DEC1-110aa-F (5′-CGGAATTCCTAATTGATCAGCAGCAGCAG-3′) with reverse primer DEC1-412aa-R. DEC1(Δ66–109), which lacks the helix–loop–helix domain (amino acids 66–109), was amplified with forward primer DEC1-1aa-F and reverse primer DEC1-412aa-R from a mixture of two PCR fragments: fragment 1 was amplified from DEC1 cDNA with forward primer DEC1-1aa-F and reverse primer DEC1-65aa-R (5′-GCTGATCAATTAGACGTCTCTTTTTCTCGATGAGCCGGTGCG-3′); fragment 2 was amplified from DEC1 cDNA with forward primer DEC1-110aa-F2 (5′-GAGACGTCTAATTGATCAGCAGCAGCAG-3′) and reverse primer DEC1-412aa-R. These products were cloned into a pcDNA3-HA via EcoRI and XbaI sites.

To generate 2× FLAG-tagged p53, cDNA fragment was amplified from pcDNA3-p53 with forward primer p53-1aa-F (5′-CGGAATTCGAGGAGCCGCAGTCAGATCCTAG-3′) and reverse primer p53-393aa-R (5′-ATTACTCGAGTCAGTCTGAGTCAGGCCCTTCTG-3′). p53(1–324) was amplified with forward primer p53-1aa-F and reverse primer p53-324aa-R (5′-CCGCTCGAGTCAATCCAGTGGTTTCTTCTTTGGC-3′). p53(94–393) was amplified with forward primer p53-94aa-F (5′-CGGAATTCTCATCTTCTGTCCCTTCCCAG-3′) with reverse primer p53-393aa-R. p53(Δ325–356), which lacks the tetramerization domain (amino acids 325–356), was amplified with forward primer p53-1aa-F and reverse primer p53-393aa-R from a mixture of two PCR fragments: fragment 1 was amplified from p53 cDNA with forward primer p53-1aa-F and reverse primer p53-324aa-R2 (5′-CTGGCTCCTTATCCAGTGGTTTCTTCTTTGGCTG-3′); fragment 2 was amplified from p53 cDNA with forward primer p53-357aa-F (5′-GAAACCACTGGATAAGGAGCCAGGGGGGAGCAG-3′) and reverse primer p53-393aa-R. These products were cloned into a pcDNA3-2× FLAG vector via EcoRI and XhoI sites.

To generate luciferase reporters under the control of the *MIC-1* promoter (nt −976/+61, −585/+61, −232/+61, −97/+61, and −40/+61), genomic DNA from MCF7 cells was amplified with forward primer (MIC-1-P-976-F, 5′-ACAGGTACCGTCAGGCTAGTCTAGAACTCTTGAC-3′; MIC-1-P-585-F, 5′-AAGGTACCAGAGGAGGATGAGTAACTGC-3′; MIC-1-P-232, 5′-AAGGTACCTGGAATGGTGTCCTCATATCG-3′; MIC-1-P-97-F, 5′-AAGGTACCGTGTTTACTCTGCAGGCAGG-3′; and MIC-1-P-40-F, 5′-AAGGTACCAGTCCGGGGACTATAAAGG-3′) and reverse primer (MIC-1-P+61-R, 5′-ATCTCGAGTTCACCGTCCTGAGTTCTTGC-3′). To generate luciferase reporters under the control of the *MIC-1* promoter (nt −976/+41 and −585/+41), genomic DNA fragments from MCF7 cells were amplified with forward primer (MIC-1-P-976-F; MIC-1-P-585-F) and reverse primer (MIC-P+41-R, 5′-AATCTCGAGGGCATGGCTGTGCAGGTTG-3′).

To generate a luciferase reporter under the control of the *MIC-1* promoter with deletion of the proximal E-boxes (nt +23/+61), two oligonucleotides (MIC-1-P+23-F, 5′-CAGAGCCCAACCTGCACAGCCATGCCCGGGCAAGAACTCAGGACGGTGAAC-3′; MIC-1-P+61-R′, 5′-TCGAGTTCACCGTCCTGAGTTCTTGCCCGGGCATGGCTGTGCAGGTTGCGGCTCTGGTAC-3′) were annealed and cloned into pGL2-basic vector. To generate luciferase reporters under the control of the *MIC-1* promoter with mutations in the proximal E-box elements, two pairs of primers were used (E1-M-F, 5′-AAGGTACCAGTCCGGGGACTATAAAGGCCGGTCCGGCA**GACTACAC**TCAGTCCCAGCTCAGAGCCGC-3′ and E1-M-R, 5′-ATCTCGAGTTCACCGTCCTGAGTTCTTGCCCGGGCATGGCTGTGCAGGTTGCGGCTCTGAGCTGGGACTGA-3′; E2-M-F, 5′-AAGGTACCAGTCCGGGGACTATAAAGGCCGGTCCGGCAG**CATCTG**GTCAGTCCTAGATCAGAGCCGC-3′ and E2-M-R, 5′-ATCTCGAGTTCACCGTCCTGAGTTCTTGCCCGGGCATGGCTGTGCAGGTTGCGGCTCTGATCTAGGACTGAC-3′. Mutated E-box elements are shown in bold.

To generate luciferase reporters under the control of the *Bax* promoter (nt −433/+388 and −433/+8), genomic DNA from MCF7 cells was amplified with forward primer (Bax-P-433-F, 5′-AAGGTACCGGGGTTATCTCTTGGGCTCAC-3′) and reverse primer (Bax-P+388-R, 5′-ACAAGCTTAGGAAGTGGTGCGGGCGACAAG-3′; Bax-P+8-R, 5′- ATCTCGAGTCACGTGAGAGCCCCGCTGAAC-3′).

Cell Line Generation. RKO-TR (clone 13), a derivative of RKO that expresses the tetracycline repressor, was generated previously in our laboratory (4). To generate RKO cell lines that inducibly express DEC1, RKO-TR (clone 13) cells were transfected with pcDNA4-DEC1. The resulting cell lines were designated RKO-DEC1 (clones 7 and 8). To generate RKO cell lines that inducibly knock down DEC1, RKO-TR (clone 13) cells were transfected with pBabe-HI-siDEC1-1 or -2, and the resulting cell lines were designated RKO-siDEC1 (clone 8 from shRNA-1 and clone 10 from shRNA-2). To generate RKO cell lines that stably knock down p53 or MIC-1 and inducibly express DEC1, RKO-DEC1 (clone 8) cells were transfected with pBabe-U6-sip53 or pBabe-U6-siMIC-1, and the resulting cell lines were designated RKO-sip53-DEC1 (clones 4 and 8) or RKO-siMIC-1-DEC1 (clones 2 and 10).

M7-TR (clone 7), M7-DEC1 (clones 6 and 16), M7-DEC1-R58P (clone 2), M7-siDEC1 (clones 1 and 34), M7-sip53-DEC1 (clones 7 and 12), and M7-HA-DEC1 (clone 2) were generated previously in our laboratory (3). To generate MCF7 cell lines that

stably knock down MIC-1 and inducibly express DEC1, M7-DEC1 (clone 6) cells were transfected with pBabe-U6-siMIC-1, and the resulting cell lines were designated M7-siMIC-1-DEC1 (clones 5 and 24).

Antibodies. Antibodies used in this study include anti-p53 monoclonal antibodies (DO-1, PAb1801, PAb240, and PAb421); anti-HA (HA11; Covance); anti-DEC1 (Bethyl Laboratories); anti-MIC-1, -HDAC1, and -γ-H2AX(S139) (Upstate); anti-Puma, -PolH, -GADD45α, -FDXR, -p21, -Bax, -Mdm2, -p53(FL393), and -GAPDH (Santa Cruz Biotechnology); anti-HIF1α (BD Biosciences); anti-actin, -FLAG, rabbit, and mouse IgG (Sigma); anti-mouse and -rabbit (BioRad).

Luciferase Reporter Assay. The dual luciferase assay was performed in triplicate according to manufacturer instructions (Promega) as previously described (3). To examine how DEC1 represses the ability of p53 to activate the *MIC-1* promoter, 0.2 μg of a luciferase reporter, 3 ng of an internal control *Renilla* luciferase assay vector pRL-CMV, and 0.2 μg of empty pcDNA3, or 0.2 μg of p53-expressing pcDNA3 along with an increasing amount of DEC1-expressing pcDNA3 were transfected into MCF7 cells. The total amount of DNA was normalized by empty pcDNA3. Luciferase activity was measured with the dual luciferase kit and Turner Designs luminometer. The fold change in relative luciferase activity is a product of the luciferase activity induced by DEC1 and/or p53 divided by that induced by an empty pcDNA3 vector.

ChIP Assay and Sequential ChIP-reChIP Assay. To test the binding of DEC1 or p53 to the target gene promoters, a ChIP assay was performed as previously described (3). To test DEC1 and p53 association on the promoters, a ChIP-reChIP assay was performed as described (5). Briefly, chromatin was collected from MCF7 cells with induction of HA-DEC1 along with treatment with camptothecin (CPT; 250 nM) for 16 h. The first ChIP performed with anti-p53 was re-ChIPed with anti-HA and control IgG. Conversely, the first ChIP performed with anti-HA was re-ChIPed with anti-p53 and control IgG. The first ChIP performed with IgG was only re-ChIPed with control IgG. Primers were described in Table S2.

DNA Histogram Analysis. To measure the effect of ectopic expression of DEC1 on DNA damage-induced cell death, cells were seeded at 3×10^5 per well in 6-cm dishes without or with tetracycline to induce DEC1 expression for 12 h, and then untreated or treated with CPT or etoposide (ETP) for 48 h. To measure the effect of knockdown of DEC1 on DNA damage-induced cell death, cells were seeded at 5×10^4 per well in 6-cm dishes without or with tetracycline to knock down DEC1 for 72 h, and then untreated or treated with CPT or ETP for 30 h, ActD for 48 h, or incubated in an anaerobic chamber (model 1025; Forma Scientific) for 0 and 36 h. Both floating and attached cells were collected and fixed in precooled (−20 °C) ethanol (70%) overnight followed by propidium iodide staining. Samples were analyzed by fluorescence-activated cell sorting (BD Biosciences).

RT-PCR. The assay was performed as described (6). Primers for amplification of p53, MIC-1, DEC1, DEC2, and actin were shown in Table S2.

Cell Survival Assay and Colony Formation Assay. For cell survival assay, cells were seeded in six-well plates without or with tetracycline for 20 h, and then untreated or treated with various concentrations of CPT or ETP for 24 h. Attached cells were counted at the indicated times. For colony formation assay, cells were seeded at 4,000 per well in a six-well plate uninduced or induced to express DEC1 shRNA, and then untreated or treated with CPT or ETP for 9 h. For controls, cells were seeded at 1,000 per well and mock treated with DMSO. The assay was performed as described (7).

Northern Blot Analysis. The preparation of GAPDH probe and Northern blot analysis were described previously (8). MIC-1 probe was prepared from EST clone MHS1011-58735 purchased from Open Biosystems.

1. Appierto V, et al. (2007) Analysis of gene expression identifies PLAB as a mediator of the apoptotic activity of fenretinide in human ovarian cancer cells. *Oncogene* 26:3952–3962.
2. Yan W, Chen X (2006) GPX2, a direct target of p63, inhibits oxidative stress-induced apoptosis in a p53-dependent manner. *J Biol Chem* 281:7856–7862.
3. Qian Y, Zhang J, Yan B, Chen X (2008) DEC1, a basic helix-loop-helix transcription factor and a novel target gene of the p53 family, mediates p53-dependent premature senescence. *J Biol Chem* 283:2896–2905.
4. Xu Y, Yan W, Chen X (2010) SNF5, a core component of the SWI/SNF complex, is necessary for p53 expression and cell survival, in part through eIF4E. *Oncogene* 29: 4090–4100.
5. Furlan-Magaril M, Rincón-Arano H, Recillas-Targa F (2009) Sequential chromatin immunoprecipitation protocol: ChIP-reChIP. *Methods Mol Biol* 543:253–266.
6. Qian Y, Chen X (2008) ID1, inhibitor of differentiation/DNA binding, is an effector of the p53-dependent DNA damage response pathway. *J Biol Chem* 283: 22410–22416.
7. Qian Y, Jung YS, Chen X (2011) DeltaNp63, a target of DEC1 and histone deacetylase 2, modulates the efficacy of histone deacetylase inhibitors in growth suppression and keratinocyte differentiation. *J Biol Chem* 286:12033–12041.
8. Chen X, Bargonetti J, Prives C (1995) p53, through p21 (WAF1/CIP1), induces cyclin D1 synthesis. *Cancer Res* 55:4257–4263.

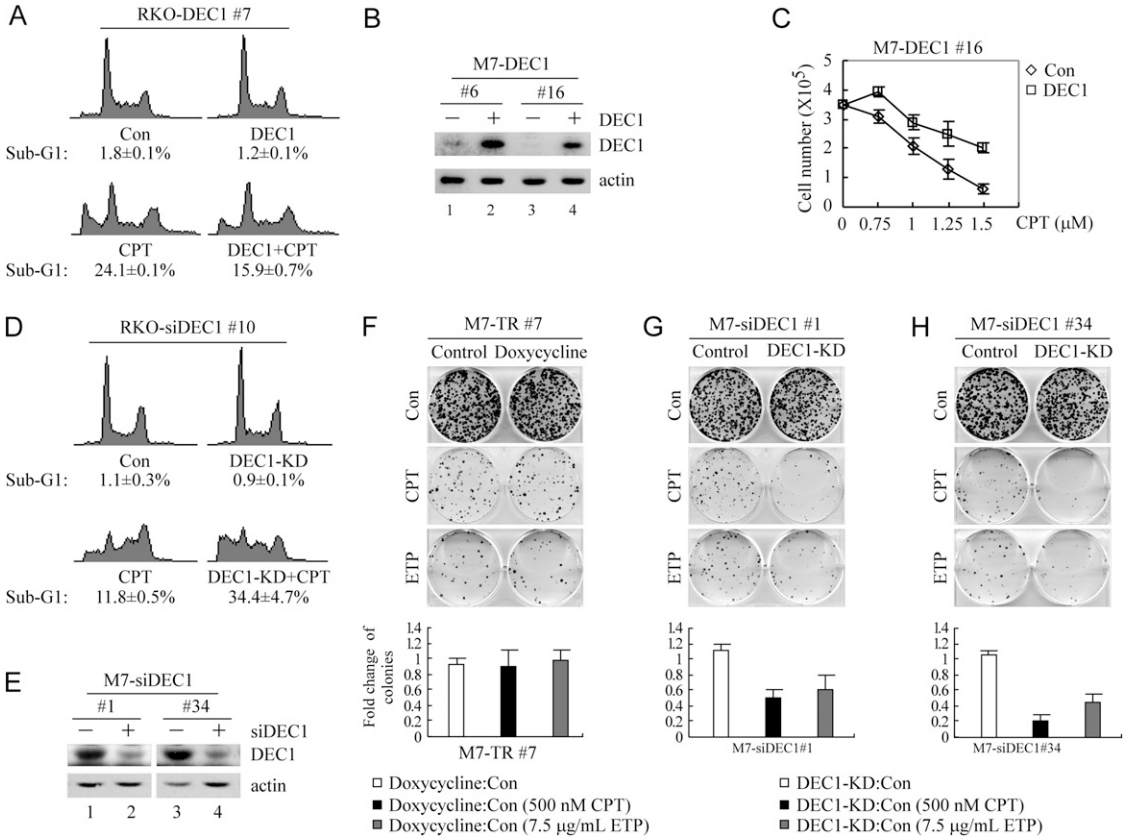

Fig. S1. DEC1 inhibits DNA damage-induced cell death. (*A*) DNA histogram analysis was performed with RKO cells uninduced (–) or induced (+) to express DEC1 for 12 h along with mock treatment or treatment with CPT (250 nM) for 48 h. (*B*) Western blots were prepared with extracts from MCF7 cells uninduced or induced to express DEC1 for 24 h. (*C*) MCF7 cells were uninduced or induced to express DEC1 for 12 h along with mock treatment or treatment with CPT (0.75, 1, 1.25, and 1.5 μM) for 24 h, and then survival cells were counted (mean ± SD; $n = 3$). (*D*) DNA histogram analysis was performed with RKO cells uninduced or induced to express DEC1 shRNA for 72 h along with mock treatment or treatment with CPT (500 nM) for 30 h. (*E*) Western blots were prepared with extracts from MCF7 cells uninduced or induced to express DEC1 shRNA for 72 h. (*F*) (*Upper*) Colony formation assay was performed with MCF7 cells untreated (–) or treated (+) with doxycycline for 72 h along with mock treatment or treatment with CPT (500 nM) or ETP (7.5 μg/mL) for 9 h, and then maintained for 12 d. Colonies were stained with 0.02% crystal violet. (*Lower*) Quantification of colonies in *Upper* presented as the ratio of colonies formed in cells treated with doxycycline vs. that in control cells (mean ± SD; $n = 3$). (*G* and *H*) The assay was performed as in *F* except that these cells were uninduced or induced to express DEC1 shRNA for 72 h.

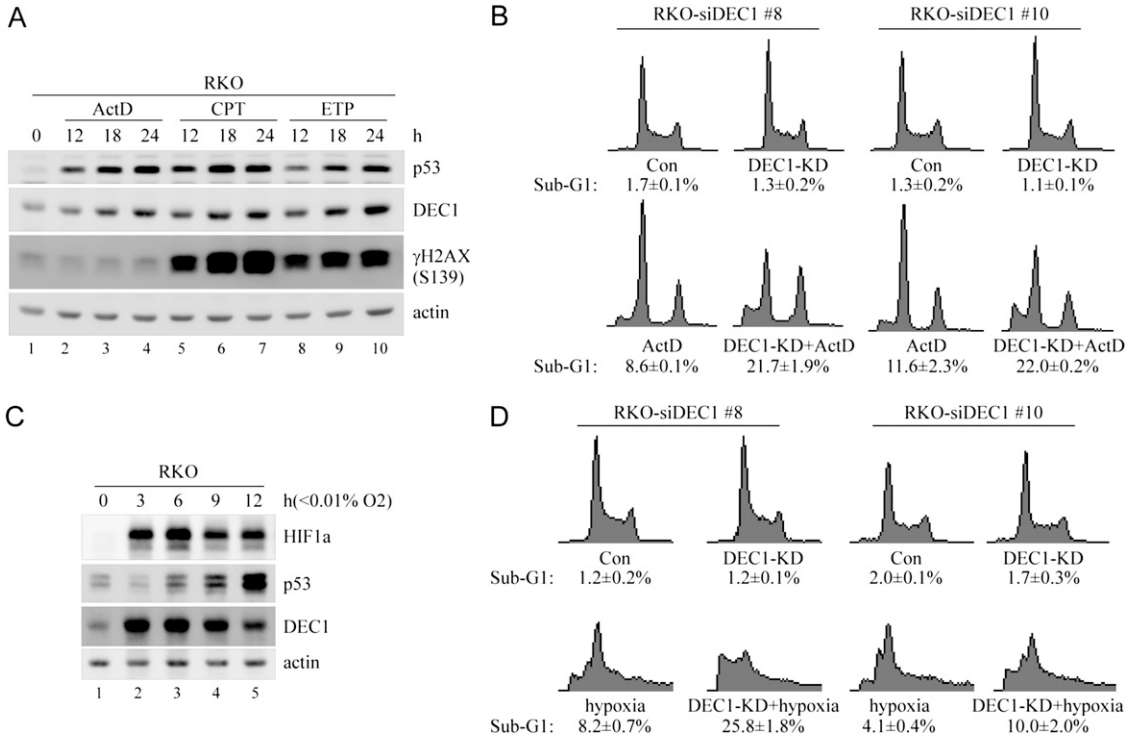

Fig. S2. DEC1 inhibits ribosomal stress- and hypoxia-induced cell death. (*A*) DEC1 is induced by ribosomal stress. Western blots were prepared with extracts from RKO cells mock treatment or treatment with ActD (5 nM), CPT (250 nM), or ETP (5 μg/mL) for 12, 18, and 24 h. p53, DEC1, phosphorylated γH2AX (S139), and actin were detected by their respective antibodies. (*B*) DNA histogram analysis was performed with RKO cells uninduced (–) or induced (+) to express DEC1 shRNA for 72 h along with mock treatment or treatment with ActD (5 nM) for 48 h. (*C*) DEC1 is induced by hypoxia. Western blots were prepared with extracts from RKO cells incubated in a hypoxia chamber (<0.01% O_2) for 0, 3, 6, 9, and 12 h. p53, DEC1, HIF1α, and actin were detected by their respective antibodies. (*D*) DNA histogram analysis was performed with RKO cells uninduced or induced to express DEC1 shRNA for 72 h along with incubation in a hypoxia chamber (<0.01% O_2) for 0 and 36 h.

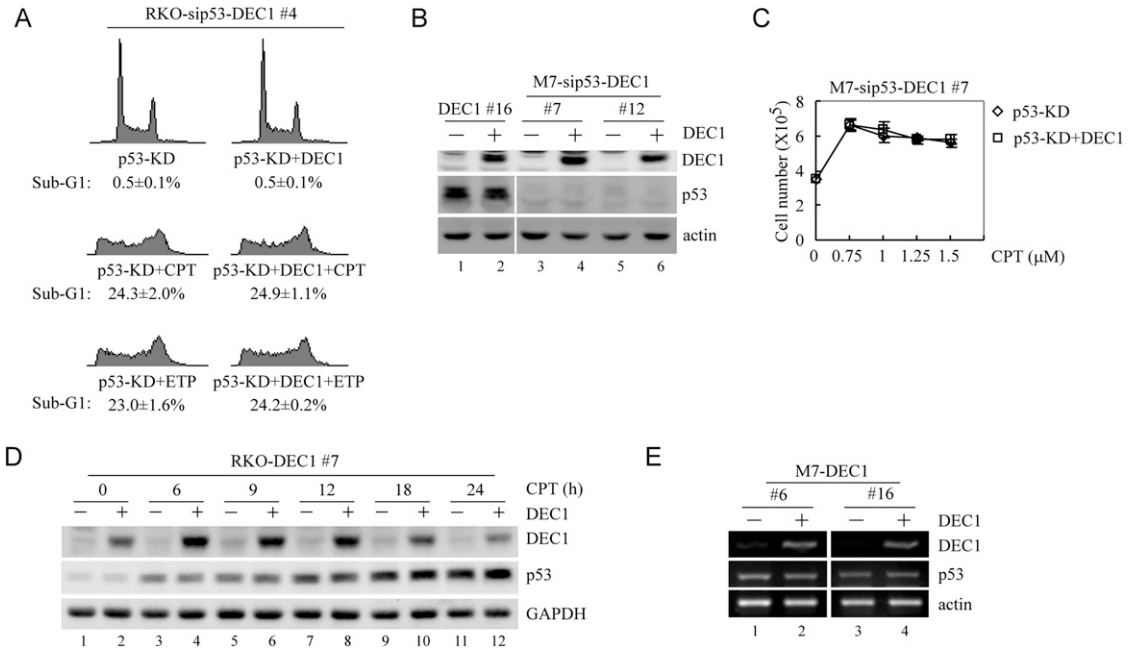

Fig. S3. The anti–cell-death activity of DEC1 is p53 dependent. (*A*) Knockdown of p53 abrogates the anti–cell-death activity of DEC1 upon DNA damage. DNA histogram analysis was performed with p53-KD RKO cells uninduced or induced to express DEC1 for 12 h along with mock treatment or treatment with CPT (250 nM) and ETP (20 μg/mL) for 48 h. (*B*) Western blots were prepared with extracts from p53-WT and p53-KD MCF7 cells uninduced (–) or induced (+) to express DEC1 for 24 h. (*C*) Knockdown of p53 diminishes the prosurvival activity of DEC1. p53-KD MCF7 cells were uninduced or induced to express DEC1 for 12 h along with mock treatment or treatment with CPT (0.75, 1, 1.25, and 1.5 μM) for 24 h, and then survival cells were counted (mean ± SD; $n = 3$). (*D*) Western blots were prepared with extracts from RKO cells uninduced or induced to express DEC1 for 12 h along with mock treatment or treatment with CPT (250 nM) for 6, 9, 12, 18, and 24 h. (*E*) The level of transcripts for DEC1, p53, or actin was measured by RT-PCR with RNAs purified from MCF7 cells uninduced or induced to express DEC1 for 24 h.

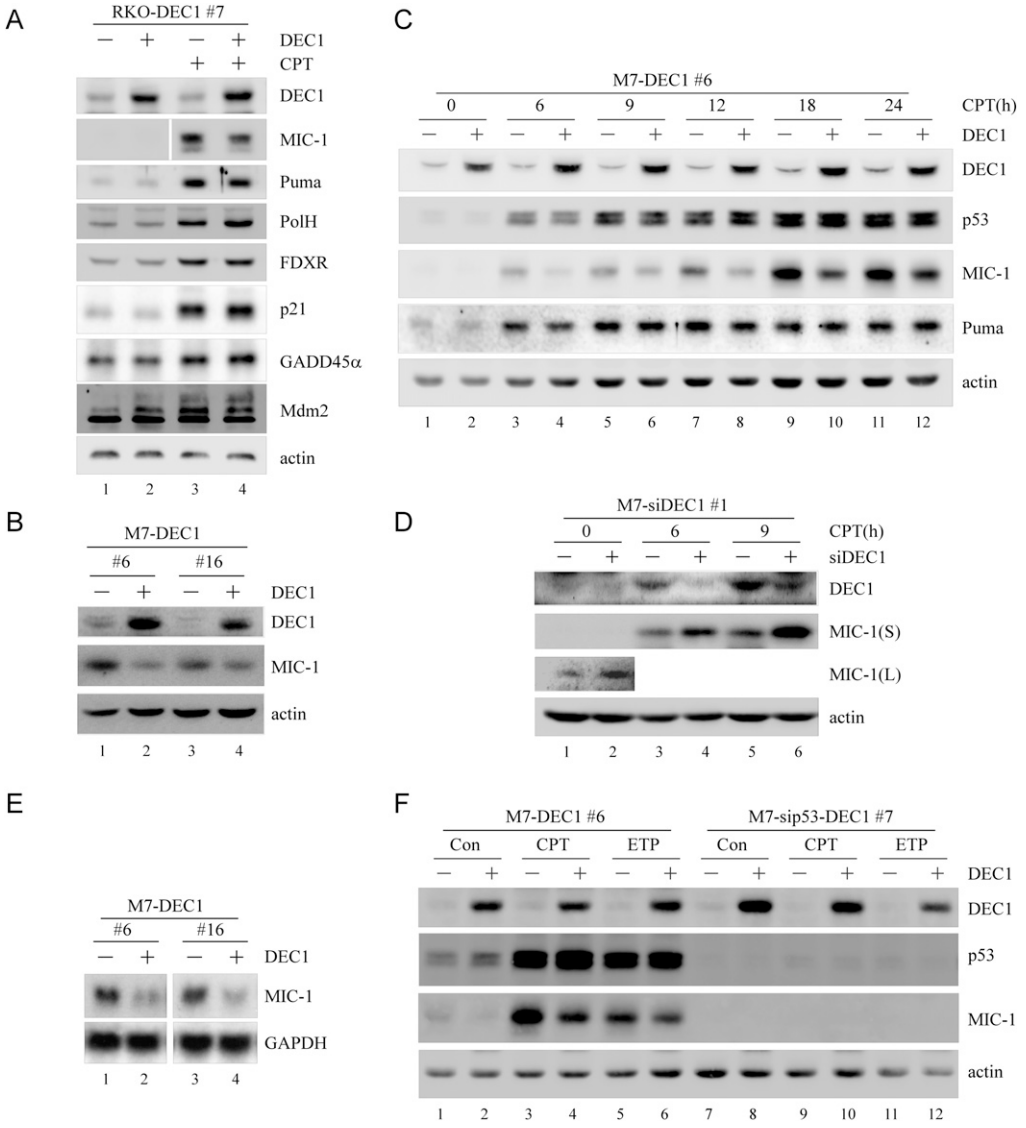

Fig. S4. MIC-1 expression is repressed by DEC1. (*A*) Overexpression of DEC1 inhibits MIC-1 expression. Western blots were prepared with extracts from RKO cells uninduced (–) or induced (+) to express DEC1 for 12 h along with mock treatment or treatment with CPT (250 nM) for 18 h. DEC1, p53, MIC-1, Puma, PolH, FDXR, p21, GADD45α, Mdm2, and actin were detected by their respective antibodies. (*B*) Western blots were prepared with extracts from MCF7 cells uninduced or induced to express DEC1 for 24 h. (*C*) Western blots were prepared with extracts from MCF7 cells uninduced or induced to express DEC1 for 12 h along with mock treatment or treatment with CPT (250 nM) for 6, 9, 12, 18, and 24 h. (*D*) Western blots were prepared with extracts from MCF7 cells uninduced or induced to express DEC1 shRNA for 72 h along with mock treatment or treatment with CPT (250 nM) for 6 and 9 h. (*E*) Northern blots were prepared with RNAs from MCF7 cells uninduced or induced to express DEC1 for 24 h. The blots were probed with cDNAs derived from MIC-1 and GAPDH genes, respectively. (*F*) Western blots were prepared as in *C* except that both MCF7 and p53-KD MCF7 cells were uninduced or induced to express DEC1 for 12 h along with mock treatment or treatment with CPT (250 nM) or ETP (5 μg/mL) for 12 h.

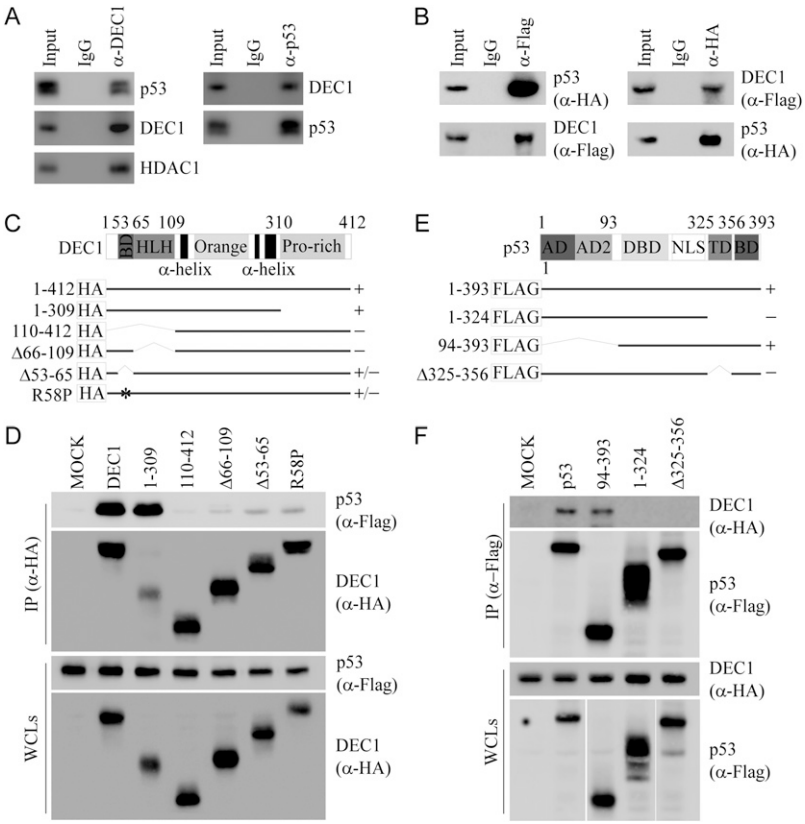

Fig. S5. DEC1 physically interacts with p53. (*A*) (*Left*) Extracts from RKO cells were immunoprecipitated with anti-DEC1 or a control IgG, which were then used to detect p53 and DEC1 along with whole-cell lysates as input control. (*Right*) Extracts from RKO cells were immunoprecipitated with anti-p53 or a control IgG, which were then used to detect p53 and DEC1 along with whole-cell lysates as input control. (*B*) The experiment was performed as in *A* except that anti-Flag was used to immunoprecipitate Flag-tagged DEC1 (*Left*) and anti-HA was used to immunoprecipitate HA-tagged p53 (*Right*) in RKO cells transfected with HA-p53 and Flag-DEC1. (*C*) Schematic presentation of DEC1 domains and deletion constructs. BD, the basic and DNA binding domain; HLH, helix–loop–helix; orange, orange domain; pro-rich, proline-rich domain; α-helix, α-helix motif. (*D*) MCF7 cells were transfected with Flag-p53 along with WT or mutant HA-DEC1 shown in *C*. At 36 h posttransfection, cells lysates were immunoprecipitated with anti-HA and then immunoblotted with the indicated antibodies. WCLs, whole-cell lysates. (*E*) Schematic presentation of p53 domains and deletion constructs. AD1 and AD2, activation domain 1 and 2; BD, basic domain; DBD, DNA-binding domain; NLS, nuclear localization signal; TD, tetramerization domain. (*F*) MCF7 cells were transfected with HA-DEC1 along with WT or mutant Flag-p53 shown in *E*. At 36 h posttransfection, cell lysates were immunoprecipitated with anti-Flag and immunoblotted with the indicated antibodies.

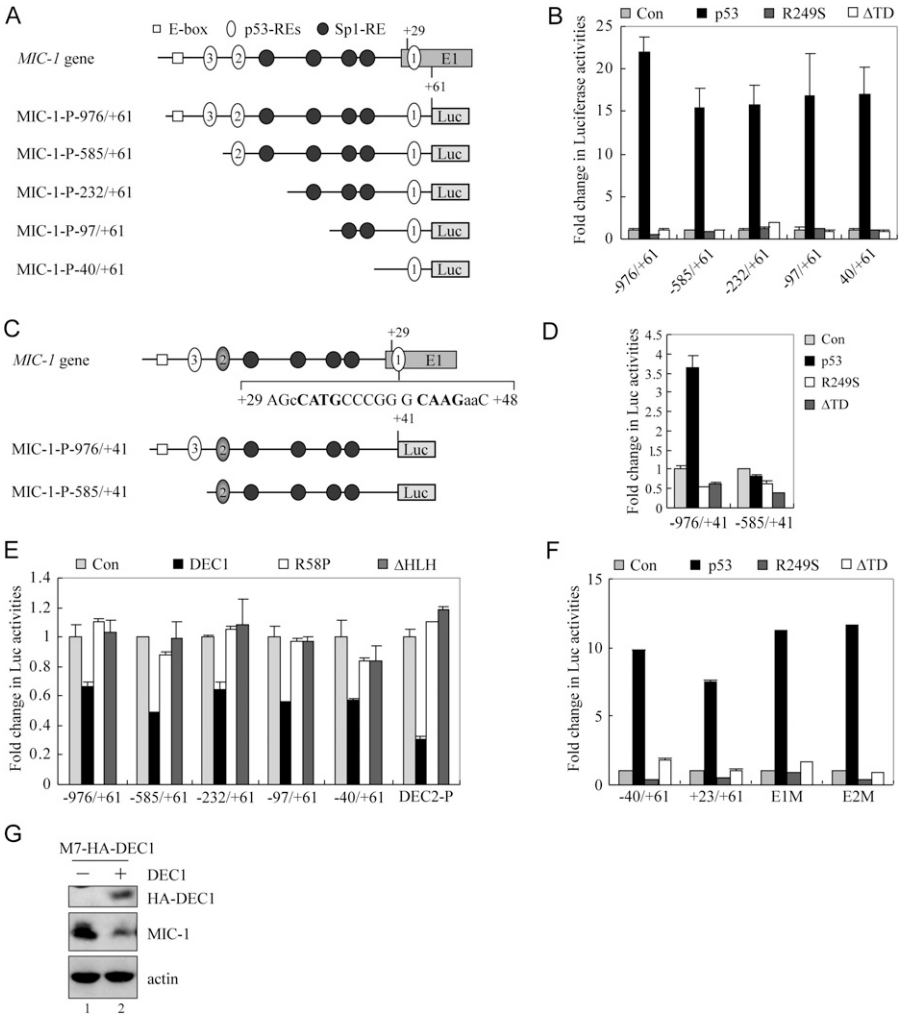

Fig. S6. MIC-1 is a direct target of DEC1. (*A*) Schematic presentation of the *MIC-1* promoter along with locations for an E-Box, p53-REs, and Sp-1 sites and luciferase reporter constructs under the control of the *MIC-1* promoter. (*B*) The *MIC-1* promoter is responsive to p53 in the presence of p53-RE1. The luciferase activity was measured in the presence of WT and mutant p53. (*C*) Schematic presentation of the *MIC-1* promoter along with the sequence of p53-RE1 and luciferase reporter constructs under the control of the *MIC-1* promoter with deletion of p53-RE1. (*D*) The *MIC-1* promoter without p53-RE1 is weakly responsive to p53. The luciferase activity was measured in the presence of WT and mutant p53. (*E*) The *MIC-1* promoter is responsive to DEC1. The luciferase activity was measured in the presence of WT or mutant DEC1. The effect of DEC1 on the *DEC2* promoter was measured as a control. (*F*) The luciferase activity was measured in the presence of WT or mutant p53. (*G*) Western blots were prepared with extracts from MCF7 cells uninduced (–) or induced (+) to express HA-DEC1 for 24 h.

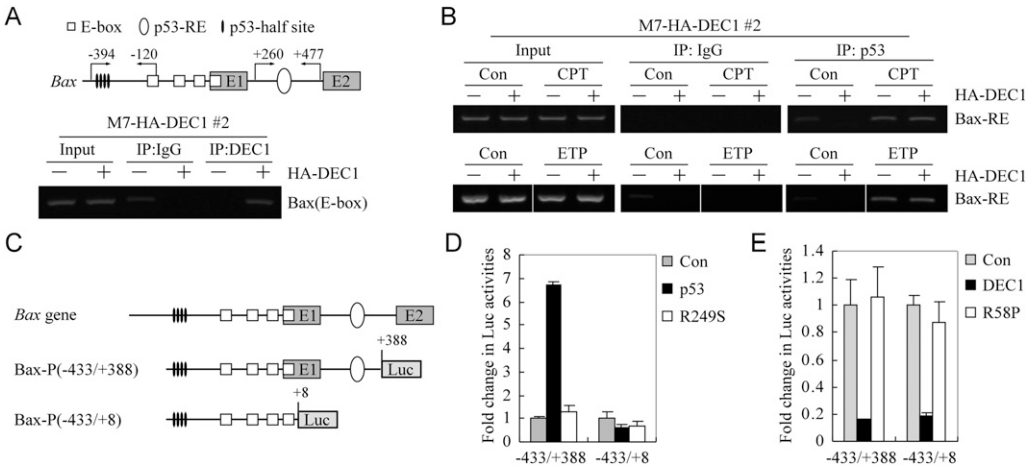

Fig. S7. DEC1 inhibits the *Bax* promoter activity. (*A*) DEC1 binds to the *Bax* promoter. MCF7 cells uninduced or induced to express HA-DEC1 for 18 h were cross-linked with formaldehyde followed by sonication. Chromatin was immunoprecipitated (IP) with anti-HA to precipitate HA-DEC1, or a control IgG. The binding of DEC1 to the *Bax* promoter was quantified by PCR. (*B*) DEC1 does not affect p53 binding to the *Bax* promoter upon DNA damage. The experiment was performed as in *A* except that anti-p53 was used to precipitate p53–DNA complexes, and MCF7 cells were mock treated or treated with CPT (250 nM) or ETP (5 μg/mL) for 12 h. (*C*) Schematic presentation of the *Bax* promoter along with locations for an E-Box and p53-RE and luciferase reporter constructs under the control of the *Bax* promoter. (*D*) The intronic p53-RE is responsive to p53. The luciferase activity was measured in the presence of WT and mutant p53. (*E*) The *Bax* promoter is responsive to DEC1. The luciferase activity was measured in the presence of WT and mutant DEC1.

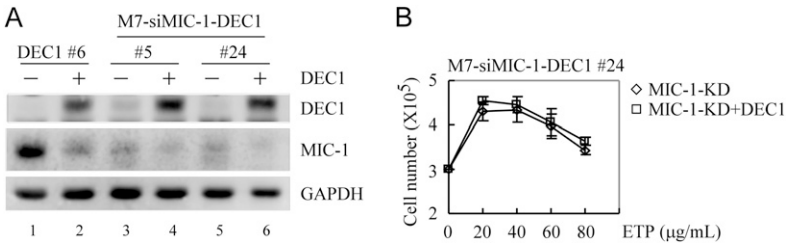

Fig. S8. MIC-1 is required for DEC1 to inhibit DNA damage-induced cell death. (*A*) Western blots were prepared with extracts from MIC-1-WT or MIC-1-KD MCF7 cells uninduced (–) or induced (+) to express DEC1 for 24 h. (*B*) MIC-1-KD MCF7 cells were uninduced or induced to express DEC1 for 12 h along with mock treatment or treatment with ETP (20, 40, 60, and 80 μg/mL) for 24 h, and then survival cells were counted (mean ± SD; $n = 3$).

Table S1. E-box elements on the promoters of p53 targets

Gene symbol	E-box sequence	Location	No.	Distance from p53-RE, nt
MIC1	CAGCTC	Exon 1	1	17
p21	CAGCTG	Promoter	5	>800
Mdm2	CACGTG	Intron 1	1	282
Bax	CACGTG	Promoter and exon 1	4	>346

Table S2. Primers for RT-PCR and ChIP assay

Primer name	Sequence
p53-RT-F	5′-GACCGGCGCACAGAGGAAGAGAATC-3′
p53-RT-R	5′-GAGTTTTTTATGGCGGGAGGTAGAC-3′
MIC1-RT-F	5′-CCAGAGCTGGGAAGATTCGAACACC-3′
MIC1-RT-R	5′-AGATTCTGCCAGCAGTTGGTCCGAC-3′
DEC1-RT-F	5′-ATCTGGCCAAGCACGAGAACAC-3′
DEC1-RT-R	5′-GATTCTCCTCCATAGCCACTGTC-3′
actin-RT-F	5′-CTGAAGTACCCCATCGAGCACGGCA-3′
actin-RT-R	5′-GGATAGCACAGCCTGGATAGCAACG-3′
MIC1-ChIP-F	5′-CCAGCTGTGGTCATTGGAGTG-3′
MIC1-ChIP-R	5′-CCATTCACCGTCCTGAGTTC-3′
p21-RE1-ChIP-F	5′-GGTCTGCTACTGTGTCCTCC-3′
p21-RE1-ChIP-R	5′-CATCTGAACAGAAATCCCAC-3′
p21-Ebox-ChIP-F	5′-TATTGTGGGGCTGTTCTGGA-3′
p21-Ebox-ChIP-R	5′-CTGTTAGAATGAGCCCCCTTT-3′
Mdm2-ChIP-F	5′-GGGAGTTCAGGGTAAAGGTCA-3′
Mdm2-ChIP-R	5′-CCTTTTACTGCAGTTTCG-3′
DEC2-ChIP-F	5′-ATGGTACGTTCCGCACGTGAGC-3′
DEC2-ChIP-R	5′-TCTCTCGCTCTCCCTCTTCAGTGC-3′
GAPDH-ChIP-F	5′-AAAAGCGGGGAGAAAGTAGG-3′
GAPDH-ChIP-R	5′-AAGAAGATGCGGCTGACTGT-3′
Bax-Ebox-ChIP-F	5′-GGCGTGGGCTATATTGCTAGATCC-3′
Bax-Ebox-ChIP-R	5′-CGTGACTGTCCAATGAGCATCTCC-3′
Bax-RE-ChIP-F	5′-AGGTTCCTGGCTCTCTGATCC-3′
Bax-RE-ChIP-R	5′-GAAAGAGGCTAGGGGAACGC-3′