Supplementary Methods S1

Cell culture

Human neuroblastoma SH-SY5Y and immortal human keratinocyte HaCaT cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) -F12, GlutaMAX (Gibco) or DMEM (Sigma-Aldrich), respectively, and all contained 10% FBS and 100 units/mL penicillin/ streptomycin. All cells were cultured at 37°C with 5% CO₂ and saturated humidity.

Transcript mapping of human HO-1 enhancer RNAs

To detect transcripts derived from HO-1 enhancer regions, we performed transcript mapping. Briefly, cDNA was synthesized using total RNA from DEM-treated HeLa cells as a template with random hexamers, and RT-PCR was performed with primer sets listed in Supplementary Table S1. In RT-PCR analysis, following samples were used as controls: HeLa genomic DNA was used as a positive control of PCR amplification; a reaction without reverse transcriptase was used as a negative control to rule out genome DNA contamination. RT-PCR products were electrophoresed on TAE-agarose gel, visualized by ethidium bromide staining, cloned using a Zero Blunt TOPO PCR Cloning kit (Life Technologies) and the cloned DNA was subjected to the DNA sequence analysis.

Determination of direction of hHO-1 eRNAs

To determine the direction of transcripts around the HO-1 enhancer regions, we performed a strand-specific RT reaction using internal primers followed by cDNA amplification using the primer sets. Briefly, cDNA was synthesized using total RNA from DEM-treated HeLa cells as a template with region specific forward or reverse primers, and subsequent PCR was performed with primer sets listed in Supplementary Table S1. Amplified RT-PCR products were electrophoresed on TAE-agarose gel, visualized by ethidium bromide staining, cloned using a Zero Blunt TOPO PCR Cloning kit (Life Technologies) and the cloned DNA was subjected to the DNA sequence analysis.

5' Rapid Amplification of cDNA Ends (5’ RACE)

5' RACE analysis was carried out using a Gene Racer Kit (Life Technologies) according to the manufacturer's protocol. Briefly, total RNA from DEM-treated HeLa cells was isolated, dephosphorylated and 5' Cap structure was removed. Then, the GeneRacer RNA oligo was ligated to de-capped 5' end of RNA and reverse transcription
was done with random hexamers. To amplify 5’ RACE product, RT-PCR was performed using the GeneRacer 5’ primer and reverse region specific primers listed in Supplementary Table S1. Subsequently, nested PCR was performed using the GeneRacer 5’ Nested primer and reverse region specific primers. Nested 5’ RACE PCR products were electrophoresed on TAE-agarose gel, visualized by ethidium bromide staining and sub-cloned using a Zero Blunt TOPO PCR Cloning kit. The DNA sequence of the inserts in isolated clones was validated by DNA sequencing. To determine 5’ ends, we analyzed DNA sequences of the junction where the GeneRacer RNA oligo was ligated.

**siRNA**

Stealth siRNAs against *eRNA E2-1* and *eRNA E2-2* were synthesized by Invitrogen with the following sequences: *eRNA E2-1* (1): 5’- AGU UGA UAC UCA CCG GGU CCC UUA A-3’, *eRNA E2-1* (2): 5’- CAC AGG CUC GGC AGC ACC GUG GGA A-3’. *eRNA E2-2* (1): 5’- CCU UUA GAG CUU AGA GAG UCG AAG A-3’, *eRNA E2-2* (2): 5’- UGA GUC ACG GUC CCG AGG UCU AUU U-3’. A stealth control siRNA was obtained from Invitrogen. Then, siRNA (final 20 nM) was transfected to cultured cells using the Lipofectamine RNAi MAX reagent according to the manufacturer’s protocol (Life Technologies). Twenty-four hours after transfection, the cells were sub-cultured and exposed to 100 µM DEM for the appropriate time.
### Supplementary Table S1. Primers used in this study

| Experiments | Primer names | S'-3' sequences | Purposes |
|-------------|--------------|-----------------|----------|
| **Transcription mapping, RT-PCR** | | | |
| map_E2_fwd_1 | CSG AGG TCG TGG TAT TAC AAC TGG | transcript mapping E2-1, strand specific cDNA synthesis and PCR |
| map_E2_rev_1 | CTA CTA GTC GAT ACT CAG CCG GGT | transcript mapping E2-1, strand specific cDNA synthesis and PCR |
| map_E2_fwd_2 | CAG TCC TCT GGG TGG CAC TCT | transcript mapping E2-2, strand specific cDNA synthesis |
| map_E2_rev_2 | GCT TAA ACC TGC AGG TGC ACG TCT | transcript mapping E2-2, strand specific cDNA synthesis |
| E2 Forward_1 | OTC TGG GGC TGC TGG TAT TAC AAC TCC | transcribe E2-3, strand specific cDNA synthesis, 5'RACE E2 transcript mapping, 3'RACE E2, 1stPCR, estimation of full-length eRNA E2; |
| 5'RACE E2 Reverse_1 | TGG AGG ATT GAT GAG AAG AGC GGG | transcript mapping E2-3, strand specific cDNA synthesis, 5'RACE E2, 1stPCR, estimation of full-length eRNA E2; |
| map_E3_rev_4 | CCC GTC ACG TGC CAT ACG AGG | transcript mapping E3-1, strand specific cDNA synthesis, human HO-1 E3 ChIP, transcript mapping, strand specific cDNA synthesis |
| map_E1_fwd_4 | ACA GAT ACC TCT AAG CCC AGG CCT | transcript mapping E1-4, strand specific cDNA synthesis |
| map_E1_rev_4 | CTA CAG TAC AAT CAG AAG CCG | transcript mapping E1-4, strand specific cDNA synthesis |
| **Transcription detection, nested PCR** | | | |
| map_E2_fwd_1 | CSG AGG TCG TGG TAT TAC AAC TGG | transcript mapping E2-1, strand specific cDNA synthesis and PCR |
| map_E2_rev_1 | CTA CTA GTC GAT ACT CAG CCG GGT | transcript mapping E2-1, strand specific cDNA synthesis and PCR |
| map_E2_fwd_2 | CAG TCC TCT GGG TGG CAC TCT | transcript mapping E2-2, strand specific cDNA synthesis |
| map_E2_rev_2 | GCT TAA ACC TGC AGG TGC ACG TCT | transcript mapping E2-2, strand specific cDNA synthesis |
| hHO-1 E1 Forward | CCC TGC TGA CCT AAG ACC TTT | transcript mapping E1-3, strand specific cDNA synthesis, human HO-1 E1 ChIP, transcript mapping, strand specific cDNA synthesis |
| map_E1_fwd_1 | AAT GGA ACT TCT AAG CCC AGG CCT | transcript mapping E1-4, strand specific cDNA synthesis |
| map_E1_rev_1 | CTA CAG TAC AAT CAG AAG CCG | transcript mapping E1-4, strand specific cDNA synthesis |
| E2-2, E2-3 overlap detection | - | | |
| map_E2_fwd_2(Nest) | TGC GCT TGA CCT AAG ACC CTG | strand specific RT-PCR E2-2 nested primer, E2-2, E2-3 overlap detection |
| map_E2_rev_2(Nest) | TCT GCT CAC TTC TGG GCT CAC TTA AGC CT | strand specific RT-PCR E2-3 nested primer |
| E1-3, E1-4 overlap detection | | | |
| E1-3, E1-4 overlap detection, human HO-1 E1 ChIP | CCC TGC TGA CCT AAG ACC TTT | E1-3, E1-4 overlap detection, human HO-1 E1 ChIP |
| 5'RACE E1 Reverse_1 | GAG GCT TCT GCC GTT TTC TA | transcript mapping E1-3, strand specific cDNA synthesis, 5'RACE E2, 1stPCR, estimation of full-length eRNA E2 |
| 5'RACE E1 Reverse_2 | GCT CTA GAT ACT TCT TGT AGC CCT | transcript mapping E1-3, strand specific cDNA synthesis, 5'RACE E2, 1stPCR, estimation of full-length eRNA E2 |
| E2 Reverse_1 | AGG AGA ATA TCC AGG CAA GGT CT | transcript mapping E2-1, strand specific cDNA synthesis, human HO-1 E1 ChIP, transcript mapping, strand specific cDNA synthesis |
| E2 Reverse_2 | AGT GAA ACT TCT AGA AAA CGG CAG AAG CCT | transcript mapping E2-1, strand specific cDNA synthesis, human HO-1 E1 ChIP, transcript mapping, strand specific cDNA synthesis |
| E2 Reverse_3 | CTA GCA ATG ACG CTC GAG GGA G | transcript mapping E2-1, strand specific cDNA synthesis, human HO-1 E1 ChIP, transcript mapping, strand specific cDNA synthesis |
| E2 Reverse_4 | AGA GCT AGG AAG AGC TGG CTT TA | transcript mapping E2-1, strand specific cDNA synthesis, human HO-1 E1 ChIP, transcript mapping, strand specific cDNA synthesis |
| E2 Reverse_5 | CGG AGG AGA AGG AGG AGG | transcript mapping E2-1, strand specific cDNA synthesis, human HO-1 E1 ChIP, transcript mapping, strand specific cDNA synthesis |
| E2 Reverse_6 | CGG TCT CCA AGG AGG TGC CTA | transcript mapping E2-1, strand specific cDNA synthesis, human HO-1 E1 ChIP, transcript mapping, strand specific cDNA synthesis |
| E2 Reverse_7 | AGA GCT AGG AAG AGG CAA AGG AAG | transcript mapping E2-1, strand specific cDNA synthesis, human HO-1 E1 ChIP, transcript mapping, strand specific cDNA synthesis |
| E2 Reverse_8 | TTC AGG AAG AGG CAA AGG AAG | transcript mapping E2-1, strand specific cDNA synthesis, human HO-1 E1 ChIP, transcript mapping, strand specific cDNA synthesis |
| **Estimation of full-length eRNA E2** | | | |
| E2 Forward_1 | OTC TGG GGC TCG CAT ACG ACC TAT A | transcript mapping E2-3, strand specific cDNA synthesis, E2-2, E2-3 overlap detection, UPL_realtime PCR for eRNA E2-3 with universal probe library probe #17 |
| (i) 5'RACE E2 Reverse_1 | TGC TAT GCA ATG ACG CTC TTT CTG | transcript mapping E2-3, strand specific cDNA synthesis, E2-2, E2-3 overlap detection, UPL_realtime PCR for eRNA E2-3 with universal probe library probe #17 |
| (ii) E2 Reverse_1 | AGG GGT ACT TAT TTG AGA GGC | transcript mapping E2-3, strand specific cDNA synthesis, E2-2, E2-3 overlap detection, UPL_realtime PCR for eRNA E2-3 with universal probe library probe #17 |
| (iii) 5'RACE E2 Reverse_2 | TCC TGT CCT TCT TCT GCT | transcript mapping E2-3, strand specific cDNA synthesis, E2-2, E2-3 overlap detection, UPL_realtime PCR for eRNA E2-3 with universal probe library probe #17 |
| E2 Reverse_3 | AGG GGT ACT TAT TTG AGA GGC | transcript mapping E2-3, strand specific cDNA synthesis, E2-2, E2-3 overlap detection, UPL_realtime PCR for eRNA E2-3 with universal probe library probe #17 |
| E2 Reverse_4 | CGG TCT CCA AGG AGG TGC CTA | transcript mapping E2-3, strand specific cDNA synthesis, E2-2, E2-3 overlap detection, UPL_realtime PCR for eRNA E2-3 with universal probe library probe #17 |
| E2 Reverse_5 | AGA GCT AGG AAG AGG CAA AGG AAG | transcript mapping E2-3, strand specific cDNA synthesis, E2-2, E2-3 overlap detection, UPL_realtime PCR for eRNA E2-3 with universal probe library probe #17 |
| **UPL_realtime PCR** | | | |
| E2-1, UPL Forward | AAA AAG TCC CCA GGG TGC | UPL_realtime PCR for E2 E2-1 with universal probe library probe #33 |
| CAC GGG TGC CCT TAA A A | UPL_realtime PCR for E2-1 with universal probe library probe #33 |
| E2-2, UPL Forward | GCT GCC TGG GGC GAC TGA | UPL_realtime PCR for E2-2 with universal probe library probe #33 |
| GAC TCT TGC ACC AAG ACC TCA | UPL_realtime PCR for E2-2 with universal probe library probe #33 |
| eRNA_E2 UPL Reverse | GGC TAG AAG AAG GGG TGC | UPL_realtime PCR for eRNA E2-3 with universal probe library probe #48 |
| TAA AAG GGC | UPL_realtime PCR for eRNA E2-3 with universal probe library probe #48 |
| SYBR Green realtime PCR | | | |
| hSLC7A11 Forward | CCA TGA ACG GTG GTG TGT T | SYBR Green realtime PCR for SLC7A11 |
| hSLC7A11 Reverse | GAC CCT CTC GAG ACG CAA C | SYBR Green realtime PCR for SLC7A11 |
| hFTL Forward | GCC GAC TGG GAG AAG AGC | SYBR Green realtime PCR for FTL |
| hFTL Reverse | TGG AAG AAG TCA ACA AGG | SYBR Green realtime PCR for FTL |
| hSQSTM1 Forward | AGG GCC TCT ACA GCA ACC TCA | SYBR Green realtime PCR for SQSTM1 |
| hSQSTM1 Reverse | GCC AGA AAG TGG GCA TCA G | SYBR Green realtime PCR for SQSTM1 |
| hHO-1 Pro Forward | AGG GAG ACA TGG ACA CAG | SYBR Green realtime PCR for hHO-1 promoter ChIP |
| hHO-1 Pro Reverse | CAC CAC GAT CAA GCA GGA | SYBR Green realtime PCR for hHO-1 promoter ChIP |
| HGB2 Pro Forward | TGG GTA CTA GGA AGG AGG AAG | human gamma-globin gene promoter ChIP |
| HGB2 Pro Reverse | ATT GAT ACG TCT AAG CTT AGG AG | human gamma-globin gene promoter ChIP |
**Supplementary Figure S1**

**Figure S1 Transcript mapping in HO-1 enhancer regions and their responsiveness to DEM.**

To detect transcripts around the HO-1 enhancer regions and their DEM response, we performed semi-quantitative RT-PCR. (A) and (C): Primer sets used in the mapping analysis are shown above the schematic presentation of the HO-1 enhancer regions. Forward primers are indicated in green and reverse primers are in red. The estimated sizes of PCR products are shown above the primer sets. (B), (D) and (E): Ethidium bromide staining of RT-PCR products.

We performed RT-PCR using the primer sets indicated in (A) and (C), and the following templates: g: genomic DNA of HeLa cells; 1: RT reaction with random hexamers and DEM-untreated HeLa total RNA; 2: RT reaction with random hexamers and DEM-treated HeLa total RNA; 3: RTase minus reaction with random hexamers and DEM-untreated HeLa total RNA; 4: RTase minus reaction with random hexamers and DEM-treated HeLa total RNA. Amplified RT-PCR products (asterisks) were subcloned using a ZeroBlunt TOPO PCR cloning Kit and the DNA sequence was analyzed. Note that the E2-4 PCR product was detected using genomic DNA as a template, but not using the RT reaction performed with random hexamer and DEM-treated HeLa total RNA (data not shown). On the other hand, E1-2 PCR product was not amplified even when using genomic DNA as a template (data not shown).
Figure S2 Determination of transcript direction in *HO-1* enhancer regions by strand-specific RT-PCR.

To determine the direction of transcripts that were detected around the *HO-1* enhancers, strand-specific RT-PCR was performed using strand-specific gene internal primers and total RNA from DEM-treated HeLa cells. (A) and (C): Primer sets used in RT-PCR analysis are shown above the schematic presentation of the *HO-1* enhancer regions. Forward primers are indicated in green and reverse primers in red. The direction of the transcripts was analyzed by strand-specific RT-PCR reactions. The direction of the transcript determined by the experiments in (B) and (D) is indicated by black arrow in (A) and (C), respectively. (B) and (D): Ethidium bromide staining of RT-PCR products. We performed RT-PCR using the following templates: g: genomic DNA of HeLa cells; 1: RTase minus reaction with random hexamers and DEM-treated HeLa total RNA; 2: RT reaction with random hexamers and DEM-treated HeLa total RNA; 3: RT reaction with a 3’end-directed cDNA synthesis primer and DEM-treated HeLa total RNA; 4: RT reaction with a 5’end-directed cDNA synthesis primer and DEM-treated HeLa total RNA. Amplified RT-PCR products (asterisks) were subcloned using a ZeroBlunt TOPO PCR cloning Kit and the DNA sequence was analyzed.
**Figure S3 Detection of overlapping RT-PCR signals in the HO-1 enhancer region.**

(A) Previously determined RT-PCR signals and primer sets used in the RT-PCR analysis are shown above the schematic representation of the HO-1 enhancer regions. Forward primers are indicated in green and reverse primers in red. The directions of the previously determined RT-PCR signals are indicated by black arrow. (B) Ethidium bromide staining of RT-PCR products. We performed RT-PCR using the following templates: g: genomic DNA of HeLa cells; 1: RT reaction with random hexamers and DEM-untreated HeLa total RNA; 2: RT reaction with random hexamers and DEM-treated HeLa total RNA; 3: RTase minus with random hexamers and DEM-untreated HeLa total RNA; 4: RTase minus reaction with random hexamers and DEM-treated HeLa total RNA. Amplified PT-PCR products (asterisks) were subcloned using a ZeroBlunt TOPO PCR cloning Kit and the DNA sequences were analyzed. Map E2 fwd_2(Nest) and eRNA E2_UPL_Reverse primers were used to amplify the overlapping region between E2-2 and E2-3, and hHO-1 E1_Forward and 5’ RACE E1 Reverse_1 primers were used to amplify the overlapping region between E1-3 and E1-4.
Supplementary Figure S4

DNA sequence and conservation of hHO-1 eRNA E2s.

5' RACE analysis was performed using total RNA from DEM-treated HeLa cells and the DNA sequences of obtained the 5' RACE clones were determined. (A) The 5' RACE reverse primer (pink arrow) is shown with the primer sets used in the RT-PCR analysis above the schematic representation of the HO-1 E2 enhancer region. The determined hHO-1 eRNA E2 5' end is indicated by a black arrow. (B and C): Conservation tracks of the region around the E2 enhancer (B) and the region adjacent to the putative hHO-1 eRNA E2 5' end (C) were obtained from the UCSC Genome Browser. In (B), the core HO-1 E2 enhancer region is underlined in pink and the determined hHO-1 eRNA E2 5' end is shown by a pink dot. (D) The DNA sequence adjacent to the human HO-1 E2 enhancer region and the primers used in this study are shown. TCIDs represent the transcription start sites in the CAGE data. Green letters represent the DNA portions that encode the possible ORF.
Figure S5  Sequences and conservation of hHO-1 eRNA E1s.
5' RACE was performed using total RNA from DEM-treated HeLa cells as a template and a 5' RACE reverse primer (cyan arrow). A schematic figure of the human HO-1 E1 enhancer region, the primers used in 5' RACE (A) and its conservation in vertebrates (B, C and D) are shown. Conservation tracks in the region of the E1 enhancer (B) and adjacent to the putative eRNA E1s 5' ends (C and D) were obtained from the UCSC Genome Browser. The determined DNA sequence and the 5' ends are indicated by black arrows. (E) The detailed DNA sequence adjacent to the human HO-1 E1 enhancer region and the primers used in this study are shown. TCID represents the transcription start site in the CAGE data.
**Figure S6 3’ primer walking analysis of *hHO-1* eRNA E2-3.**

To estimate the full-length of *eRNA E2-3*, cDNAs synthesized using random hexamers and total RNA of DEM-treated HeLa cells were examined by 3’ primer walking analysis. (A) The location of the primers used in 3’ primer walking analysis are shown. (A) A downward E2 Forward_1 primer (red arrow) and a series of upward primers ((i) to (viii)) were used. The primer sequences are listed in Supplementary Table S1. The sizes of the PCR products are indicated to the right of the primer name. (B) Ethidium bromide staining of PCR products in 3’ primer walking analysis. The PCR primers used for analysis are indicated in the figure. We performed RT-PCR using the following templates: 1: genomic DNA of HeLa cells; 2: RTase minus with random hexamer and DEM-treated HeLa total RNA; 3: RT reaction with random hexamers and DEM-treated HeLa total RNA. Amplified RT-PCR products (asterisks) were subcloned using a ZeroBlunt TOPO PCR cloning Kit and the DNA sequence was analyzed.

A PCR fragment of (v)-lane 3 contained *hHO-1* eRNA E2_L. We also confirmed by DNA sequencing that the PCR fragment of (i)-lane 3 contained DNA sequence corresponding to *eRNA E2-3*, whereas the PCR fragment of (ii)-lane 3 was a non-specific PCR amplification.
Figure S7 DNA sequence of hHO-1 eRNA E2_L.

We performed a 3’ primer walking analysis as shown in Figure S6 and the DNA sequences of the plasmid clones were determined. (A) A schematic representation of the putative transcript (hHO-1 eRNA E2_L) is shown by bold black arrows. (B) The sequence obtained from the subclone of PCR using E2 Forward_1 and (v) E2 Reverse_5 is underlined against the HO-1 E2 enhancer region sequences. Putative intron is indicated by hatching. We identified CAGC sequences at the both sides of the intron/exon junction of genomic DNA (shown in red). The hHO-1 eRNA E2_L contains only one of these. The DNA sequence that encode the putative ORF is indicated by green letters. The 5’ end of hHO-1 eRNA E2-3 is indicated by a magenta letter. The primers used in this experiment are shown by arrows below the sequence.
**Figure S8**  *hHO-1* eRNAs expression in human cultured cells.

We analyzed the expression of *hHO-1* eRNAs in HaCaT and SH-SY5Y cells. HaCaT (A) and SH-SY5Y (B) cells were either untreated (-) or treated with 100 µM DEM for 6 hours (+) and total RNA samples were isolated. The cDNAs were synthesized using random hexamers with total RNA as a template. The arbitrary RNA levels of eRNA E2-1, eRNA E2-3 and eRNA E1-4 were measured by real-time RT-PCR using specific primers and Universal Probe Library Probes. The value was normalized to the expression of the cyclophilin A gene, and the arbitrary RNA level was expressed as the mean ± SEM of three independent assays. *: P<0.05; **: P<0.01 (two-tailed unpaired Student’s t-test).
Figure S9 Effect of eRNA E2-2 knockdown in HeLa cells.

(A) The effect of eRNA E2-3 knockdown (KD) on eRNA E2-2 expression. (B and C) The effect of eRNA E2-2-KD on eRNA E2-2 (B) or eRNA E2-3 (C) expression. HeLa cells were transfected with control siRNA (Ctrl) or two siRNAs against eRNA E2-2 (E2-2(1) or E2-2(2)). Then the cells were either untreated (-) or treated with 100 μM DEM for 6 hours (+). The RNA level was analyzed by real-time RT-PCR using specific primers and Universal Probe Library Probes. The effect of eRNA E2-2-KD on the expression of HO-1 (D) and TXNRD1 (E). The expression of HO-1 and TXNRD1 was analyzed by real-time RT-PCR. Each value was normalized to cyclophilin A gene expression and the arbitrary RNA level was expressed as the mean ± SEM of three independent assays.

*: P<0.05; **: P<0.01 (two-tailed unpaired Student's t-test) compared to control siRNA without DEM (Ctrl (-)). #: P<0.05, ##: P<0.01; NS: no significance compared to the value of control siRNA with 100 μM DEM for 6 hours (Ctrl (+)) (one-way ANOVA followed by a Dunnett's post-hoc test for multiple parameter comparisons).
**Figure S10** Effect of *eRNA E2-3* knockdown on *HO-1* expression in HaCaT cells.

(A) The effect of *eRNA E2-3* knockdown (KD) on *eRNA E2-3* expression in HaCaT cells. HaCaT cells were transfected with control siRNA (Ctrl) or two siRNAs against *eRNA E2-3* (E2-3(1) or E2-3(2)). The cells were untreated (-) or treated with 100 μM DEM for 6 hours (+). The *eRNA E2-3* level was analyzed by real-time RT-PCR using specific primers and Universal Probe Library Probe #17. (B) The effect of *eRNA E2-3-KD* on the expression of *HO-1*. The expression of *HO-1* was analyzed by real-time RT-PCR. Each value was normalized to cyclophilin A gene expression, and the arbitrary RNA level was expressed as the mean ± SEM of four independent assays. *: \( P < 0.05 \); **: \( P < 0.01 \) (two-tailed unpaired Student’s *t*-test) compared to control siRNA without DEM (Ctrl (-)). #: \( P < 0.05 \); ##: \( P < 0.01 \); NS: not significant compared to the value of control siRNA with 100 μM DEM for 6 hours (Ctrl (+)) (one-way ANOVA followed by a Dunnett’s *post-hoc* test for multiple parameter comparisons). (C) The effect of *eRNA E2-3-KD* on *HO-1* protein expression in HaCaT cells. Whole-cell lysates were separated by SDS-PAGE, and *HO-1* protein expression was analyzed by immunoblotting using a *HO-1*-specific antibody (Abcam, ab68477). Actin was used as a loading control (Sigma-Aldrich, A1978).