

Supporting Information

Dong et al. 10.1073/pnas.1800505115

SI Materials and Methods

Cell Culture. HEK293F cells were purchased from Thermo Scientific (R79007). HEK293F, B2-1, and DTO cells were all cultured in DMEM (SH30081.01; HyClone) supplemented with 10% FBS (10099-141; Gibco), and were grown at 37 °C, 5% CO₂. Mycoplasma was removed before experiments by passaging the cells into the culture medium containing 1× MycoZap mycoplasma elimination reagent (VZA-2012; Lonza).

Generation of the Reporter Cell Line. The procedures used in construction the reporter cell line are shown in Fig. S14. Reporter vector pCMV-EGFP-IRES-Puro was methylated *in vitro* by SssI methyltransferase (M0226L; New England Biolabs) in the presence of 160 μM S-adenosylmethionine. Methylated pCMV-EGFP-IRES-Puro was mixed with pcDNA3.1-Hygro (+) (Invitrogen) at a ratio of 9:1, and then they were cotransfected into HEK293F cells. Stably transfected cells were selected using hygromycin B (J607; Amresco) in a final concentration of 700 μg/mL. Hygromycin-resistant clones that expressed EGFP upon 5-Aza treatment but not upon DMSO treatment were selected for further characterization. Finally, a clone with little EGFP background and strong response to 5-Aza treatment was chosen as the reporter cell line B2-1.

Compounds. 5-Aza (A3656), TSA (T1925), and SAHA (SML0061) were purchased from Sigma; the final working concentrations for these compounds were 5 μM, 100 nM, and 5 μM, respectively. Importazole and ivermectin were purchased from Selleck Chemicals (S8446 and S1351, respectively); the final working concentrations were 50 μM and 40 μM, respectively.

Antibodies. Mouse monoclonal anti-CSE1L (sc-271537) and mouse monoclonal anti-HDAC1 (sc-81598) were purchased from Santa Cruz. Rabbit polyclonal anti-CSEL1L (PA5-21468) was purchased from Pierce, Thermo Scientific. Mouse monoclonal anti-5-methylcytidine (BI-MECY-000) for MeDIP-seq was purchased from Eurogentec. Rabbit polyclonal anti-histone H3 (17168-1-AP) and rabbit polyclonal anti-Lamin B1 (12987-1-AP) were purchased from Proteintech. Mouse monoclonal anti-Flag (F3165) was purchased from Sigma. HRP-conjugated streptavidin (A0303) was purchased from Beyotime. Rabbit monoclonal anti-HDAC2 (ab32117) and rabbit polyclonal anti-β-tubulin (ab6046) were purchased from Abcam.

Bisulfite Sequencing. Bisulfite conversion was performed with the EpiTect Bisulfite Kit (59104; Qiagen). The converted DNA was amplified using ZymoTaq PreMix (E2003; ZYMO Research). Purified PCR products were then ligated into a cloning vector using the pCloneEZ-TA-Amp cloning kit (C5863; CloneSmart Technologies) for Sanger sequencing. Primers for bisulfite PCR were designed using Methyl Primer Express Software v1.0 (Thermo); primers for FOS were FOS-F, AAGGGGGAGATTTTTTATTTAGG and FOS-R, CCAACCCTAAAATAAACCCC. Primers

for RASD1 were RASD1-F, AAAAAGGTTTTTTTTAGGTTGTG and RASD1-R, AAAACAACAACCTAAACCAAA.

Cell Fractionation. Cell fractionation was performed as described (1, 2) with minor modifications. HEK293F cells were harvested with 0.05% trypsin-EDTA digestion. Dulbecco's PBS-washed cells were then lysed with cytoplasmic lysis buffer [10 mM Tris-HCl (pH 7.9), 0.34 M sucrose, 3 mM CaCl₂, 2 mM MgCl₂, 0.1 mM EDTA, 1 mM DTT, 0.2% Nonidet P-40, and Roche protease inhibitors]. Intact nuclei were pelleted at 3,000 × *g* for 20 min, washed with cytoplasmic wash buffer [10 mM Tris-HCl (pH 7.9), 0.34 M sucrose, 3 mM CaCl₂, 2 mM MgCl₂, 0.1 mM EDTA, 1 mM DTT, 0.1% Nonidet P-40, and Roche protease inhibitors], and then lysed in nuclei lysis buffer [20 mM Tris-HCl (pH 7.9), 3 mM EDTA, 10% glycerol, 150 mM KCl, 1.5 mM MgCl₂, Roche protease inhibitors]. The nuclear soluble fraction was cleared by centrifugation at 16,000 × *g* for 30 min at 4 °C. Next, the chromatin-containing pellet was washed with nuclei lysis buffer and then incubated for 3 h at 10 °C in a shaker (1,000 rpm) in nuclease buffer [150 mM Tris-HCl (pH 7.9), 5 mM MgCl₂, 150 mM NaAc, and Roche protease inhibitors] supplemented with Benzonase (Sigma) (125 U/mL) to release the chromatin-bound proteins. The suspension was cleared by centrifugation at 16,000 × *g* for 30 min at 4 °C. The pellet was then resuspended in BC500 [20 mM Tris-HCl (pH 7.9), 500 mM NaCl, 1 mM EDTA, 1 mM DTT, and Roche protease inhibitors] and incubated overnight in a rotator to extract the high-salt-soluble fraction, which was enriched with proteins bound to chromatin; the cleared suspension was collected by centrifugation at 16,000 × *g* for 30 min. The protein fractions were desalted and concentrated for Western blot analysis using the methanol-chloroform precipitation method described previously (3).

ChIP-Seq, Streptavidin-Mediated Biotinylated Protein Pull-Down Followed by Deep Sequencing, MeDIP-Seq, and Data Analysis.

ChIP, Streptavidin-mediated biotinylated protein pull-down followed by deep sequencing (BioChIP-seq), and MeDIP-seq were performed as previously described (4). MeDIP-seq reads (49 bp) were generated by Illumina HiSeq 4000 platforms with an average sequencing depth of 48 million single-ended reads (range: 44.5–52 million reads). Sequencing qualities were evaluated with FastQC software (Babraham Bioinformatics), and then the reads were aligned to hg19 genome sequences with a maximum of two mismatches allowed. Only the uniquely mapped reads were kept, and duplicate reads were removed. Mapped reads were extended to the average fragment sizes. Genome profile files were generated with igvtools (Integrative Genomics Viewer; Broad Institute) and linearly normalized to the same depth of 10 million reads. TSS annotation data were obtained from the refGene table of the University of California, Santa Cruz (UCSC) hg19 databases. The reads densities of MeDIP-seq for specific gene sets were averaged based on bigWig signals generated from reads-mapping files.

1. Lossaint G, et al. (2013) FANCD2 binds MCM proteins and controls replisome function upon activation of S phase checkpoint signaling. *Mol Cell* 51:678–690.
2. Aygün O, Svejstrup J, Liu Y (2008) A RECQ5-RNA polymerase II association identified by targeted proteomic analysis of human chromatin. *Proc Natl Acad Sci USA* 105: 8580–8584.

3. Wessel D, Flügge UI (1984) A method for the quantitative recovery of protein in dilute solution in the presence of detergents and lipids. *Anal Biochem* 138:141–143.
4. Xiong J, et al. (2016) Cooperative action between SALL4A and TET proteins in stepwise oxidation of 5-methylcytosine. *Mol Cell* 64:913–925.

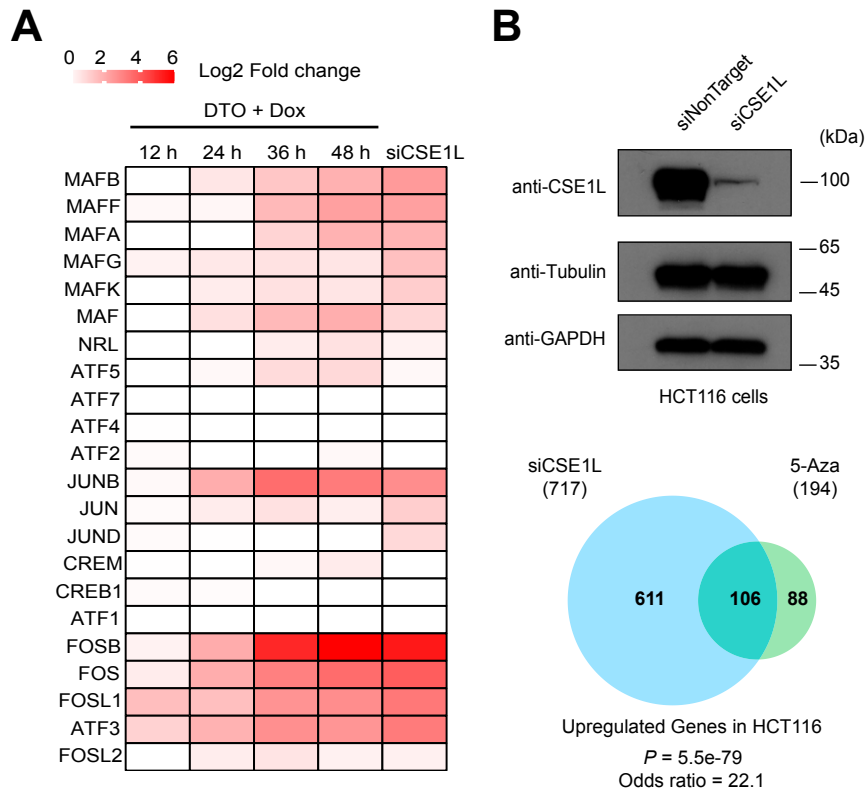


Fig. S2. CSE1L knockdown activated endogenous genes both in B2-1 cells and HCT116 cells. (A) Heat-map showing the change in the expression of AP1 superfamily transcription factors at different time points after the addition of Dox in DTO cells. (B, Upper) Western blot showing the CSE1L protein level in HCT116 cells transfected with siCSE1L. Tubulin and GAPDH were used as loading controls. (Lower) A Venn diagram showing the overlap of genes up-regulated by CSE1L knockdown and 5-Aza treatment ($P = 5.5e-79$).

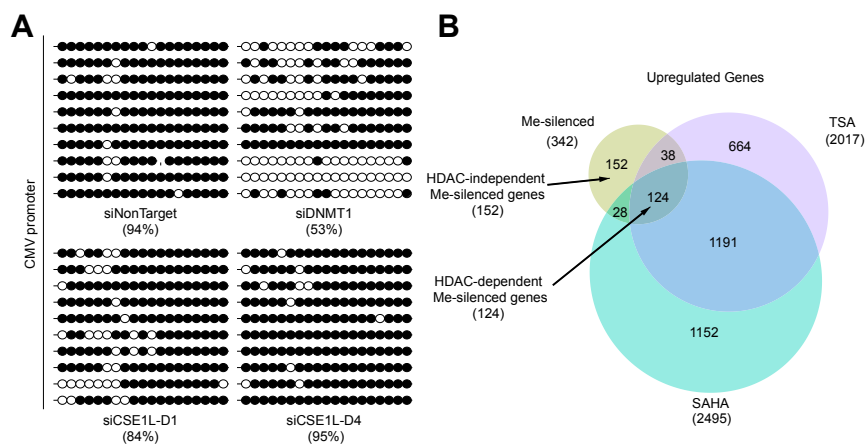


Fig. S3. Loss of CSE1L expression does not cause DNA demethylation. (A) There is no DNA demethylation at the CMV promoter of reporter genes when activated by siCSE1L. Shown are bisulfite sequencing results of the CMV promoter of the reporter gene in B2-1 cells treated with siNonTarget, siDNMT1, siCSE1L-D1, or siCSE1L-D4. Black and white circles represent methylated and unmethylated CpG sites, respectively. The percentages of methylated CpG sites are shown. (B) A Venn diagram showing the overlap of up-regulated genes in cells treated with 5-Aza and siDNMT1, TSA, or SAHA. Me-silenced genes are the genes commonly up-regulated in 5-Aza and siDNMT1 treatments. HDAC-dependent Me-silenced genes are the genes commonly activated by 5-Aza and siDNMT1, TSA, and SAHA treatments. HDAC-independent Me-silenced genes are the genes activated only by 5-Aza and siDNMT1 treatment but not by TSA or SAHA treatment.

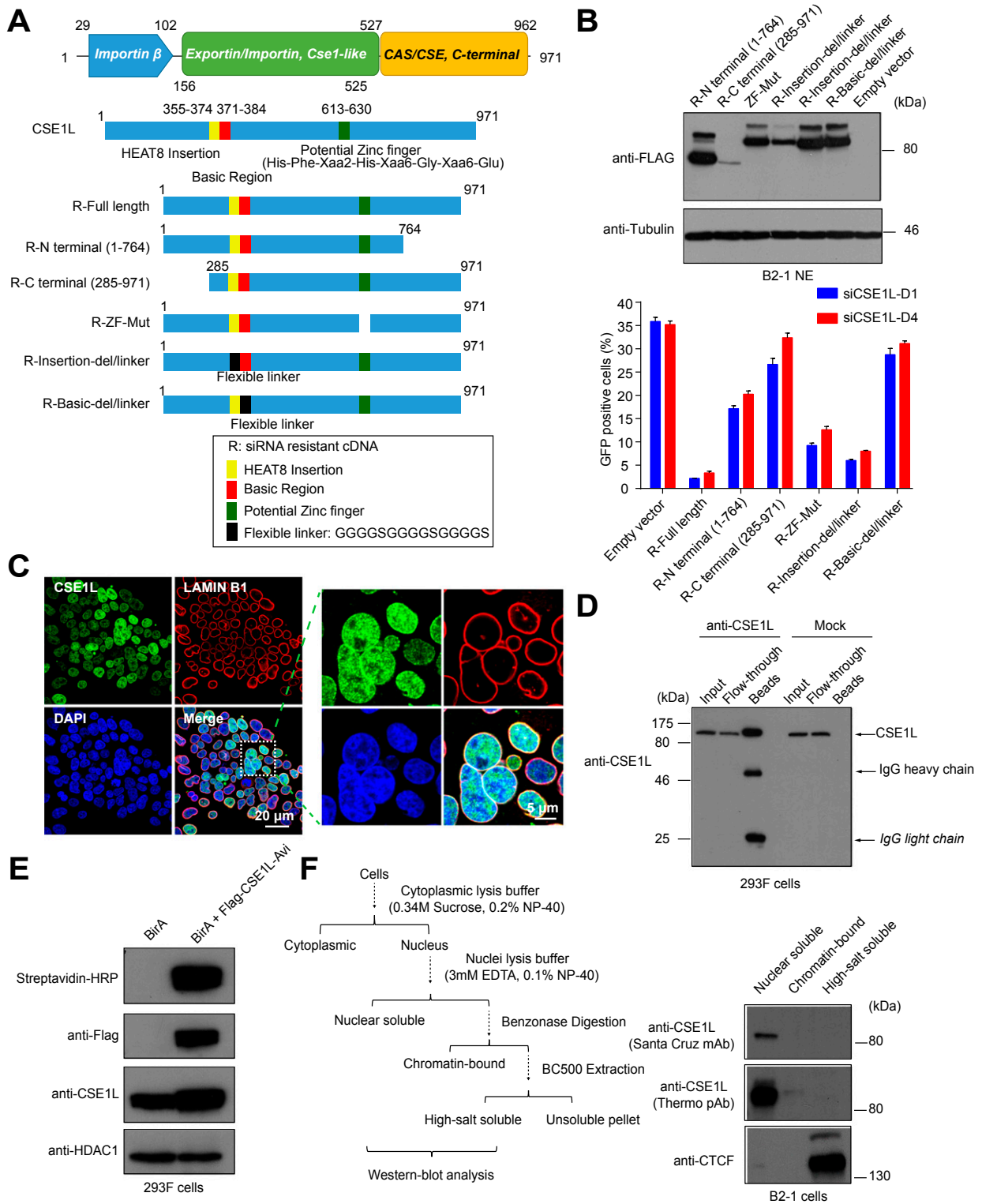


Fig. 54. CSE1L is not a chromatin-binding protein in HEK293F cells. (A) Schematic of CSE1L mutants used in the rescue assay. (B, Upper) Western blot of rescue assays performed in stable cell lines expressing truncation or deletion CSE1L mutants. (Lower) The percentage of EGFP-expressing cells was measured with FACS. Error bars represent the SD of three biological replicates. (C) Immunostaining image showing the cellular localization of CSE1L in HEK293F cells. The green signal indicates CSE1L, the red signal indicates LAMIN B1, and the blue signal indicates DAPI. (D) Western blot showing that endogenous CSE1L could be immunoprecipitated by CSE1L monoclonal antibody (Santa Cruz). (E) Western blot showing the protein level of endogenous and ectopically expressed biotinylated CSE1L in cells expressing only BirA and cells coexpressing BirA and Flag-CSE1L-Avi. (F) Cell fractionation experiment showing that nearly all CSE1L exists in the nuclear soluble fraction. (Left) Outline of the strategy for cell fractionation. (Right) Western blot of CSE1L in each nuclear fraction of B2-1 cells. CCCTC-binding factor (CTCF) was used as a positive control for chromatin-binding protein.

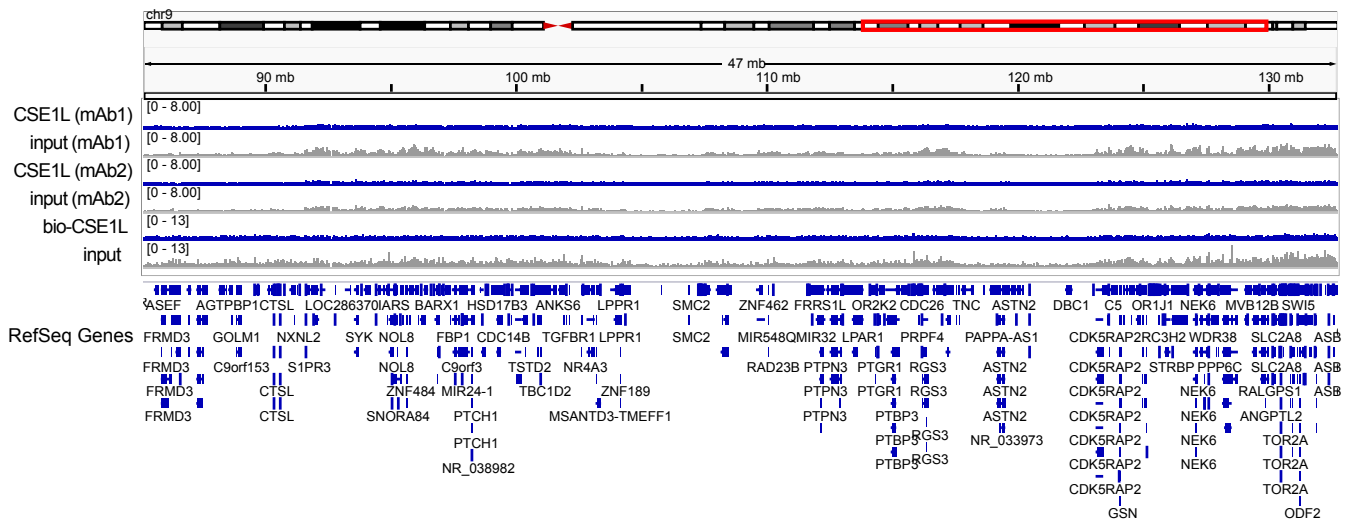


Fig. S5. CSE1L shows no localization enrichment in the genome. A 47-Mb representative region (chr9: 86,000,000–133,000,000) shows no CSE1L peak. Data include two replicates of ChIP-seq with antibody against endogenous CSE1L and a Biotin ChIP-seq using cells overexpressing biotinylated CSE1L.

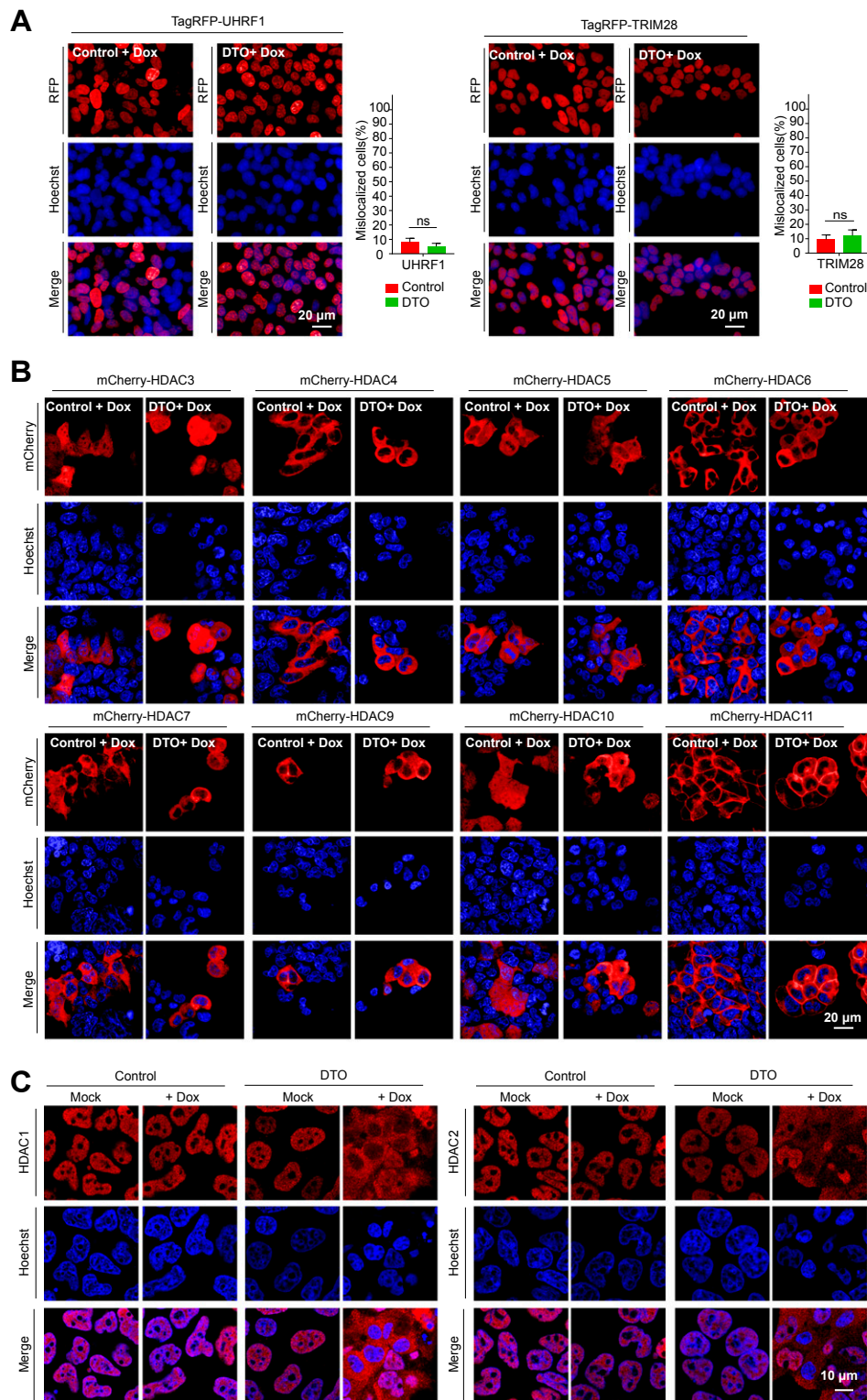


Fig. S6. The localization of certain proteins was not affected by CSE1L knockdown. (A, *Left*) Nuclear localization of TagRFP-UHRF1 was not disrupted when expression of CSE1L was turned off. (*Right*) Nuclear localization of TagRFP-TRIM28 was not disrupted when expression of CSE1L was turned off. Fluorescence images and the statistics results for mislocalized cells are shown; error bars represent the SD. An unpaired two-tailed t test was used for statistical calculation. ns, not significant. (B) The localization of HDAC3, 4, 5, 6, 7, 9, 10, and 11 was not affected when the expression of CSE1L was turned off. (C) Nuclear localization of endogenous HDAC1 (*Left*) and HDAC2 (*Right*) was disrupted in DTO cells after CSE1L was turned off for 36 h.

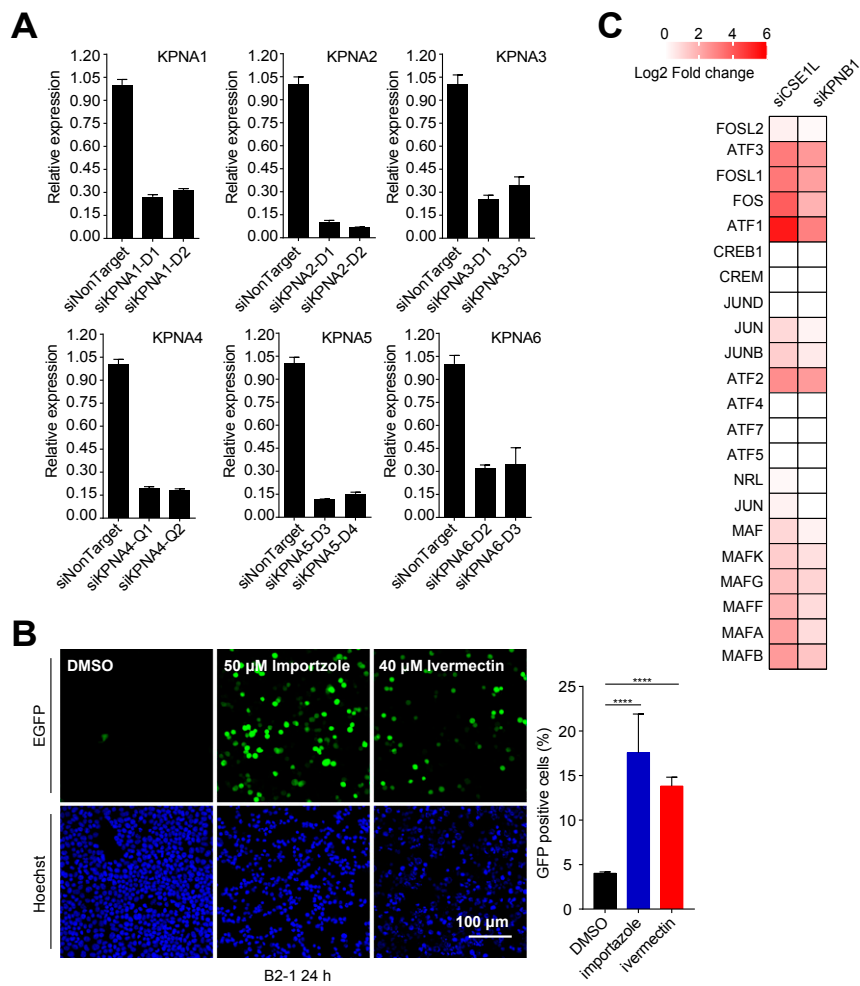


Fig. S7. The knockdown efficiency of siKPNA and the expression of AP-1 superfamily transcription factors in KPNA1-knockdown cells. (A) The knockdown efficiency of siKPNA was measured by qRT-PCR in B2-1 cells. Error bars represent the SD. (B, Left) Treatment with importazole or ivermectin for 20 h in B2-1 cells reactivates the silenced EGFP. (Right) The percentage of EGFP-positive cells was also calculated by FACS. Error bars represent the SD. An unpaired two-tailed *t* test was used for statistical calculation. *****P* < 0.0001. (C) Heat-map showing the expression change of AP1 superfamily transcription factors in siKPNA1-treated cells.

Table S2. siRNA used in this study

Target gene	Sequence of sense strand (5' to 3')	Source
<i>siNonTarget</i>	UUCUCCGAACGUGUCACGU	Qiagen
<i>siDNMT1-09</i>	CAAUGAGACUGACAUCAAA	This study
<i>siDNMT1-16</i>	GGAAGUGAAUGGACGUCUA	This study
<i>siUHRF1-297</i>	GGAUCAUCUUCGUGGACGA	This study
<i>siUHRF1-290</i>	GGACGGCGCGGGAACUCUA	This study
<i>siCSE1L-D1</i>	GCAGUUAAGUGAUGCAAUU	Dharmacon
<i>siCSE1L-D2</i>	GGACUGAAAUAAGCUUGU	Dharmacon
<i>siCSE1L-D3</i>	GCAAAUCUGCAAACACUAA	Dharmacon
<i>siCSE1L-D4</i>	AUAGUGCACUUGAUGCUUA	Dharmacon
<i>siCSE1L-Q1</i>	GGAUAAUGUUAUCAAGUA	Qiagen
<i>siCSE1L-Q2</i>	AAUGAACUUGUAAACCUAA	Qiagen
<i>siCSE1L-Q3</i>	CGGUUUGGAUUAUCUAAA	Qiagen
<i>siCSE1L-Q4</i>	CGAUCGAGUGGCCAUUAAA	Qiagen
<i>siKPNB1-1</i>	GGACUUAUGUACAGCAUUU	Dharmacon
<i>siKPNB1-2</i>	GGAAGGAUGUACUGAAAUU	Dharmacon
<i>siKPNB1-3</i>	UCACACAGAUGGAGUAGUA	Dharmacon
<i>siKPNB1-4</i>	GACGAGAAGUCAAGAACUA	Dharmacon
<i>siNOVA1-1</i>	GCGAAGACGGCCAGUAUUU	Qiagen
<i>siHDAC1-1</i>	CUAAUGAGCUUCCAUACAA	Dharmacon
<i>siHDAC2-3</i>	GCAAAGAAAAGCUAGAAUUG	Dharmacon
<i>siNOVA1-2</i>	GCUGCUGGAUCUUAUUUUG	Qiagen
<i>siKPN1-D1</i>	GCACUGGCAUUAACCCUUA	Dharmacon
<i>siKPN1-D2</i>	CAACAUUGCUGGAGAUAGU	Dharmacon
<i>siKPN2-D1</i>	GAAAUGAGGGCUGCGAGAA	Dharmacon
<i>siKPN2-D2</i>	GAAGCUACGUGGACAAUGU	Dharmacon
<i>siKPN3-D1</i>	GCAACAAGUUAAGCUGUA	Dharmacon
<i>siKPN3-D3</i>	GAACGUCACAUGGGUCAUU	Dharmacon
<i>siKPN4-Q1</i>	GGGAUCUGCUGCUGCAUUA	Qiagen
<i>siKPN4-Q2</i>	AGAUCUUUGCAUGAGGUAA	Qiagen
<i>siKPN5-D3</i>	GCAUGGGCAUUAACAAAUA	Dharmacon
<i>siKPN5-D4</i>	GGAUGGAUUUCAACUUUAA	Dharmacon
<i>siKPN6-D2</i>	GAAGCUGCCAUGUUCGAUA	Dharmacon
<i>siKPN6-D3</i>	GAUUACGUCUUGAACUGUU	Dharmacon

Table S3. Sample size for image analysis of EGFP-positive cells calculation (10× air)

Figure	Samples	Sample size
Fig. 1C	<i>siNonTarget</i>	Six wells (or replicates), 108 fields, total 192,880 cells
	<i>siCSE1L-Q1</i>	Six wells (or replicates), 108 fields, total 62,791 cells
	<i>siCSE1L-Q2</i>	Six wells (or replicates), 108 fields, total 196,447 cells
	<i>siCSE1L-Q3</i>	Six wells (or replicates), 108 fields, total 304,521 cells
	<i>siCSE1L-Q4</i>	Five wells (or replicates), 90 fields, total 54,316 cells
	<i>siCSE1L-D1</i>	Six wells (or replicates), 108 fields, total 69,816 cells
	<i>siCSE1L-D2</i>	Six wells (or replicates), 108 fields, total 45,368 cells
	<i>siCSE1L-D3</i>	Six wells (or replicates), 108 fields, total 88,454 cells
	<i>siCSE1L-D4</i>	Six wells (or replicates), 108 fields, total 83,806 cells
Fig. 6A	<i>siNonTarget</i>	Six wells (or replicates), 108 fields, total 192,880 cells
	<i>siKPNB1-D1</i>	Six wells (or replicates), 108 fields, total 46,152 cells
	<i>siKPNB1-D2</i>	Six wells (or replicates), 108 fields, total 35,369 cells
	<i>siKPNB1-D3</i>	Six wells (or replicates), 108 fields, total 57,119 cells
Fig. S1G	Control mock	Four wells (or replicates), 56 fields, total 66,154 cells
	Control + Dox	Four wells (or replicates), 56 fields, total 35,369 cells
	DTO mock	Four wells (or replicates), 56 fields, total 73,111 cells
	DTO + Dox	Four wells (or replicates), 56 fields, total 52,864 cells

Table S4. Sample size for image analysis of mislocalized cargo calculation (60× water)

Figure	Samples	Sample size
Fig. 5C	TagRFP-NOVA1 control Control + Dox	Six wells (or replicates), 540 fields, total 12,247 cells
	TagRFP-NOVA1 DTO #C1 DTO + Dox #C1	Six wells (or replicates), 540 fields, total 23,790 cells
	TagRFP-NOVA1 DTO #C2 DTO + Dox #C2	Six wells (or replicates), 540 fields, total 21,298 cells
Fig. 5D	mCherry-HDAC1 control + Dox	Two wells (or replicates), 12 fields, total 103 cells
	mCherry-HDAC1 DTO + Dox	Two wells (or replicates), 13 fields, total 120 cells
	mCherry-HDAC2 control + Dox	Two wells (or replicates), nine fields, total 79 cells
	mCherry-HDAC2 DTO + Dox	Two wells (or replicates), 10 fields, total 90 cells
	mCherry-HDAC8 control + Dox mCherry-HDAC8 DTO + Dox	Two wells (or replicates), seven fields, total 67 cells Two wells (or replicates), eight fields, total 100 cells
Fig. S6A	TagRFP-UHRF1 Control + Dox	Six wells (or replicates), 540 fields, total 14,992 cells
	TagRFP-UHRF1 DTO + Dox #C1	Six wells (or replicates), 540 fields, total 20,034 cells
	TagRFP-TRIM28 Control + Dox	Six wells (or replicates), 540 fields, total 10,953 cells
	TagRFP-TRIM28 DTO + Dox #C1	Six wells (or replicates), 540 fields, total 23,567 cells