Supplemental Data

Supplemental Methods

Western blot

Cells were washed twice with cold PBS and then resuspended in RIPA lysis buffer. The cell lysates were centrifuged at 12000 rpm for 15 min and the protein concentration was measured using BCA protein assay kit. Equal amounts of total protein were resolved by 10% SDS-PAGE and transferred to NC membrane. The membranes were incubated with primary antibodies followed by incubation with secondary Ig conjugated with HRP. Protein bands were visualized with the ECL kit (GE Healthcare). The following antibodies were used: RBMS1 (Abcam ab150353), xCT/SLC7A11 (CST 12691S), xCT/SLC7A11 (Abcam ab37185), FLAG (Sigma 1804), GST (ABclonal AE006), His (ABclonal AE003), eIF3d (Proteintech 10219-1-AP), eIF3m (Proteintech 11423-1-AP), eIF3i (Proteintech 11287-1-AP), VINCULIN (Proteintech 66305-1-Ig), GAPDH (Proteintech 60004-1-Ig), TUBULIN (ABclonal AC006), ACSL4 (SANTA SC-271800), GPX4 (Proteintech 67763-1-Ig), AIFM2 (Proteintech 20886-1-AP), DHODH (Proteintech 14877-1-AP).

Immunoprecipitation

A549 (H1299) cells were transfected with either pCDH-Flag-RBMS1 (pCDH-Flag-eIF3d, eIF3i, eIF3m) or empty vector for 24h using Sage LipoPlus reagent (Sage) according to the manufacturer's instructions. Cells were collected and lysised using IP lysise buffer then the supernatant was subjected to immunoprecipitation using anti-Flag M2 Affinity Gel (Sigma Aldrich) at 4˚C overnight, followed by five times washes with 1 ml of washing buffer. Where indicated, the affinity elute was subjected to SDS–PAGE and then either colloidal Coomassie blue staining or western blotting. Bands were excised and subjected to mass spectrometric sequencing.

Luciferase assay and translation efficiency

The RBMS1 stable knockdown or pLKO.1 control H1299 cells were transfected with SLC7A11 luciferase reporters and renilla control plasmid for 24h. The luciferase

activities were measured following dual luciferase reporter assay detection kit (Promega Corporation, USA). The parallel group cells were collected for RNA extraction and the levels of the transfected reporter RNA were quantified by RT-qPCR. FLuc activity was normalized to the renilla luciferase (RLuc) activity to evaluate reporter translation efficiency.

RNA immunoprecipitation

Flag-RBMS1 (Flag-eIF3d) or control H1299 cells were collected and cross-linked by 1% formaldehyde. Crosslinking reactions are blocked by the addition of glycine solution (pH 7.0) to a final concentration of 0.25 M for 5 min followed by two washes with ice-cold PBS. The cells are collected and resuspended in 1 mL of IP lysis buffer. After three rounds of sonication, solubilized cell lysate was precleared by mixing with Protein A-Sepharose beads along with nonspecific tRNA, and collected for immunoprecipitation with Anti-Flag M2 Affinity beads. After five or six times washes with 1 mL of RIPA buffer, the beads were resuspended in 100 μL RIP buffer and incubated at 70 °C for 45 min to reverse the crosslinks. The RNA was extracted using Trizol and reverse transcribed into cDNA for PCR detection.

GST pull down assay

BL21 *Escherihia coli* transformed with the plasmids of recombinant proteins were induced by IPTG at 20 °C overnight. The cell pellets were harvested by centrifugation at 5000 rpm at 4 °C for 15 min and resuspended in protease inhibitor containing lysis buffer and then lysised by sonication. His-tagged recombinant proteins were purified by Ni-NTA agarose (Qiagen 30210). The GST-tagged RBMS1 were purified by the Glutathione Magnetic Agarose Beads (Sigma G4510) according to the manufacturer's protocol. For GST pull down assay, equal amount of GST fusion RBMS1 or GST control bound to glutathione sepharose were incubated with purified recombinant His-tagged full-length eIF3d, eIF3i or eIF3m overnight at 4 °C, followed by five times washes with 1 mL of washing buffer. The target proteins were eluted with SDS loading buffer and detected by western blotting.

Proximity ligation assay (PLA)

The Proximity Ligation Assay (PLA) was performed according to manufacturer's instructions. FLAG-RBMS1-expressing A549 cells were deposited on glass slides for 24h and then fixed with 4% paraformaldehyde for 20 min. Next, cells were permeabilized with 0.2% Triton X-100 for 10min at RT and blocked with blocking solution for 60 min at 37 °C. Following primary antibodies incubation (anti-FLAG, Sigma, F3165, 1:100, and anti-eIF3d, Proteintech, 10219-1-AP, 1:50) overnight at 4°C, Cells were incubated with PLUS and MINUS PLA probes 1:5 (DUO92101 Sigma-Aldrich) for 1 hour at 37 °C after washing in 1x wash buffer A. Next, cells were incubated in Duolink ligation buffer for 30 min at 37 °C followed by washing in 1x wash buffer A, and incubated in Amplification buffer placed away from light at 37 °C which removed by 1× wash buffer B after 100 min. Finally, the slides were mounted with coverslips using a minimal volume of Duolink PLA Mounting Medium with DAPI for 15 min, and analyzed using Leica microscope (Leica Mi8, 40x).

Cell proliferation, EdU assays and colony formation assays

To measure cell proliferation, 5000 cells per well were plated in 24-well plates for incubation at 37 °C. Cell numbers were counted every one or two days to determine the cell growth curve. For EdU assays, A549 or H1299 cells were treated with 50 μM EdU reagent for 2h and detected by using Cell-Light EdU Apollo488 In Vitro Kit (RiboBio). For the colony formation assay, cells ($1x10^3$ cells/well) were seeded in the 10-cm dishes and incubate at 37 °C in humidified incubator. When cell colonies were clearly identifiable, the colonies were fixed with methanol and stained with crystal violet. After washing with water, cell colonies were photographed for statistics.

Xenograft Assays

The Institutional Animal Care and Use Committee of Dalian Medical University approved the experimental protocols performed on the animals. Doxorubicin (Dox)-inducible RBMS1 knockdown stable cells ($3×10^6$) were injected subcutaneously into the abdomen side of 6-week-old BALB/c nude mice (Vital River). Mice were fed either with sucrose water or sucrose water containing 0.1% doxycycline hyclate. H1299 vector，

H1299 pLKO.1 RBMS1 and H1299 pLKO.1 RBMS1/SLC7A11 cells (2.5×10^6) were injected subcutaneously into the abdomen side of 6-week-old BALB/c nude mice(Vital River). The xenograft tumour formation was monitored using callipers every 3 days. Tumor volume was calculated according to the formula: V = length × width^2/2. At the end of 32 days, mice were euthanized and excised tumors were weighed.

Irradiation and clonogenic survival assay

For Irradiation, cells were treated with ionizing radiation using an X-RAD 320ix Biological Irradiator (Precision X-ray Inc) and 50 cm from the source of radiation (SSD) at doses from 0 to 6 Gy. For all clonogenic survival assays, A549-IR cells knocking-down RBMS1 (750 cells per dish) were seeded in the 6-cm dishes. Next day, cells were pretreated with Ferr-1(2 μM) or DMSO for 24h, and then cells were irradiated. The culture media with inhibitor were changed every two days. After incubation for 9 days at 37 °C in humidified incubator, colonies were fixed with 4% paraformaldehyde and stained with crystal violet followed by washing with water. Finally, colonies were photographed and counted. The survival fraction was normalized to the cells without irradiation.

Mouse model

The RBMS1 CKO mouse was generated by Shanghai Model Organisms Center, Inc. Strategy of RBMS1 CKO mouse model was illustrated in Supplemental Figure 6A. RBMS1 CKO mice were identified by PCR of tail-tip genomic DNA. For spontaneous lung tumor experiment, $KRAS^{G12D/WT}/RBMS1^{fl/fl}$ and $KRAS^{G12D/WT}/RBMS1^{WT}$ mice were used. Intratracheal delivery of Adeno-Associated Viral Vector expressing Cre-recombinase (AAV-Cre-GFP) (Hanbio Co.LTD) induced oncogenic Kras activation and led to the formation of aggressive adenocarcinomas. Briefly, 4-week-old mice were anesthetized by intraperitoneal injection of 4% Chloral hydrate. AAV-Cre-GFP at a dose of 9×10^{10} vg was delivered to the lung by intratracheal intubation. Mice were analyzed for tumor formation and progression at 8 weeks after infection.

Micro-CT imaging and processing

Micro-CT imaging was performed using a mCT machine (GE Healthcare) according to standard imaging protocol. In brief, mice were anesthetized using Chloral hydrate and a total of 720 views were obtained with a soft-tissue fast-scan setting. Raw image stacks were processed for lung reconstruction with the standard ROI tool (MicroView). Rendering and quantification were performed using render volume tool and measurement tool in MicroView.

Immunohistochemical staining

Tissue specimens were fixed in 10% formaldehyde solution overnight after being collected. Specimens were transferred into 70% ethanol after being washed with running water for 30 min followed by paraffin embedment including dehydration, clearing, wax immersion and embedding. And Paraffin-embedded tissue specimens were sectioned for HE and IHC staining. For IHC staining, tissue sections firstly were deparaffinized in xylene and rehydrated followed by antigen retrieval in sodium citrate. Next, sections were processed according to the manufacturer's instructions (SPlink Detection Kits, ZSGB-BIO), in which the antibody used was anti-4HNE (1:200, Abcam, ab46545), anti-RBMS1 (Abcam, ab150353, 1:50) and anti-SLC7A11 (Cell Signaling Technology, 12691S, 1:100).

Ribo-seq

Polysome profiling was carried out. Briefly, cells were grown to 70-80% confluence in 6×15-cm dishes. After treated with cycloheximide (CHX, 100 μg/mL) for 15min, cells were washed with CHX/PBS and lysed in polysome lysis buffer, followed by shearing 20 times with 26-gauge needles gently. 10% of cleared lysate was retained as input for total RNA extraction. The remained lysates from the polysome profiling procedure were treated with RNase I (Thermo Fisher Scientific, Waltham, USA) at room temperature for 5 min to obtain ribosome-protected mRNA fragments (RPF). The reaction was stopped by adding SUPERaseIn RNase Inhibitor (Invitrogen, Carlsbad, USA). Fractions containing 80S ribosome particles were combined and underwent RNA clean-up by TriZol reagent. The RNA sequencing library was prepared and were cleaned up and

subjected to next-generation sequencing on Illumina Hiseq X10 under the PE150 sequencing model.

Ribo-seq data were analyzed after removing adapters by Cutadapt (v2.4, key parameter: -m 20), reads aligning to rRNA sequences were removed using Bowtie (v1.1.2, key parameter: --seedlen=23). Gene expression was estimated from RSEM (version 1.3.1, default parameters) using transcriptome alignments and is reported in transcripts per kilobase million (TPM). Differential expression analysis was performed by DESeq2 and differential expressed genes (DEGs) were obtained with combined cut-off (p-value < 0.05, |Log2(FoldChange)| > 1.5) and used for gene ontology analysis with clusterProfiler package (version 3.16.1, default parameters). Clustering heatmap was assessed with the Euclidean distance measurement in column and the Z-normalization in row.

TMT-based quantitative proteome analysis

A549 cells with RBMS1 overexpression or knockdown were harvested and subjected to TMT-based quantitative proteome analysis. In brief, cell pellets were sonicated in lysis buffer, and proteins were reduced by incubation with tris (2-carboxyethyl) phosphine (TCEP, final concentration is 10 mM) (Thermo Scientific) at 55 °C for 1 h and then alkylated with iodoacetamide (final concentration is 18.75 mM) (Sigma) for 30 min at room temperature in the dark. After that, six volumes of pre-chilled acetone was added to precipitate the proteins overnight. The peptide mixture was fractionated into a total of 50 fractions by high pH reverse-phase HPLC, which were consolidated into 25, and then analyzed by Easy-nLC 1200 nano HPLC (Thermo Scientific, San Jose, CA) and Q Exactive Orbitrap mass spectrometer (Thermo Scientific, San Jose, CA). The acquired MS/MS data were analyzed against a UniProtKB *Homo Sapiens* (database released on Sep. 30, 2018) using Proteome Discoverer 2.1 (Thermo Scientific) with the Sequest HT search engine. Quantitative analyses were performed as described below. Reporter ion abundances of biological repeats were averaged for fold-change calculation. A two-tailed t-test was performed to calculate p-value. The cut-off for p-value and fold-change were set at 0.05 and 1.5 for considered statistically different.

Supplemental Figures

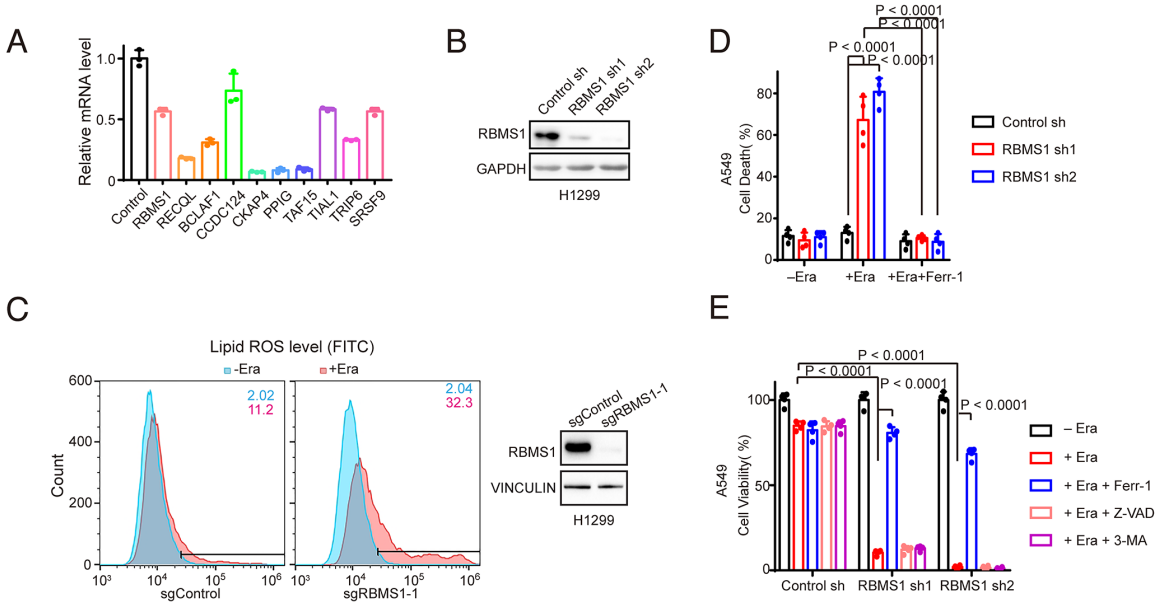

Figure S1. Loss of RBMS1 promotes ferroptosis. (**A**) The qPCR assay was utilized to determine the mRNA levels of different RBPs in A549 cells with depletion of ten distinct RBPs respectively. Error bars are mean ± SEM from three biologically independent samples. P values from a two-sided *t*-test. (**B**) A western blot assay was applied to examine the protein levels of RBMS1 in H1299 cells with stable depletion of RBMS1. **(C)** Lipid peroxidation was measured by flow cytometry after C11-BODIPY staining in H1299 cells with genetical deletion of RBMS1. The protein level of RBMS1 was determined with a western blot assay. **(D)** Cell death was measured after treatment with erastin (Era) (10 μM), Ferrostatin-1 (Ferr-1) (2 μM) and erastin (10 μM) in RBMS1 stably depleted A549 cells. **(E)** Bar graphs showing cell viability in RBMS1 stably depleted A549 cells treated with 5 μM erastin, or combined with 2 μM Ferr-1, 20 μM Z-VAD, or 2 mM 3-MA. Error bars are mean ± SEM, n = 3 (**A**), or n = 4 (**D**, **E**) independent repeats. P values were determined using One-way ANOVA with Tukey's multiple comparisons in **D**, **E**.

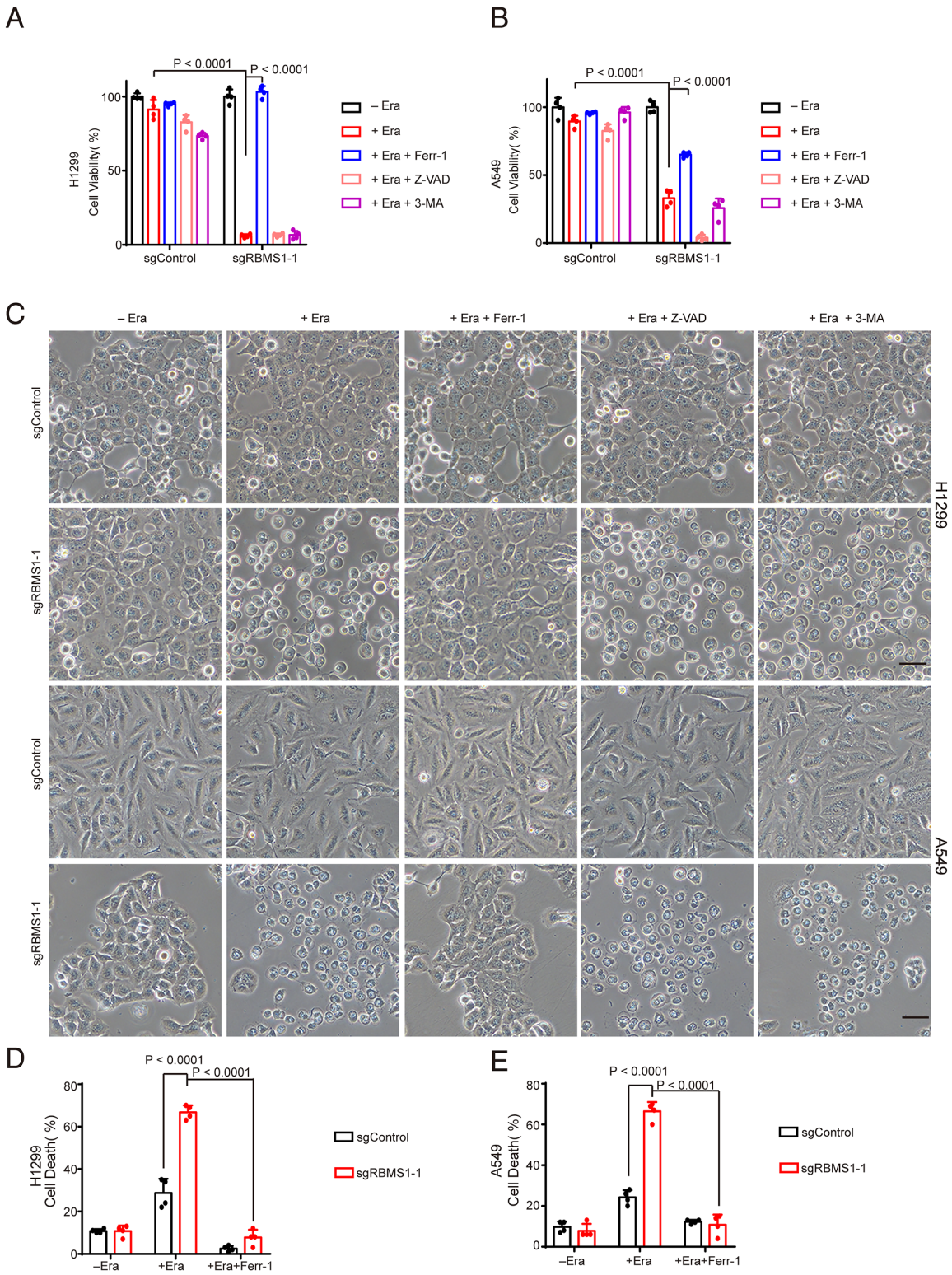

Figure S2. Deleted RBMS1 stimulates lung cancer cell ferroptosis. (A-B) Bar graphs showing cell viability in H1299 or A549 cells with genetical deletion of RBMS1 treated with 5 μM erastin, or combined with 2 μM Ferr-1, 20 μM Z-VAD, or 2 mM 3-MA

respectively. (**C**) Representative phase-contrast images of H1299 or A549 cells with genetical deletion of RBMS1 treated with erastin (3, or 5 μM), erastin (3 or 5 μM) and Ferr-1 (2 μM), erastin (3, or 5 μM) and Z-VAD-fmk (Z-VAD) (20 μM), erastin (3, or 5 μM) and 3-MA (2 mM) respectively. Scale bars, 100 μm. **(D-E)** Cell death was measured after treatment with earstin (Era) (3, or 5 μM), Ferrostatin-1 (Ferr-1) (2 μM) and erastin (3, or 5 μM) in H1299 (D) and A549 (E) cells with genetical deletion of RBMS1. Error bars are mean ± SEM, n = 4 (**A**, **B**, **D**, **E**) independent repeats. P values were determined using One-way ANOVA with Tukey's multiple comparisons in **A**, **B**, **D**, **E**.

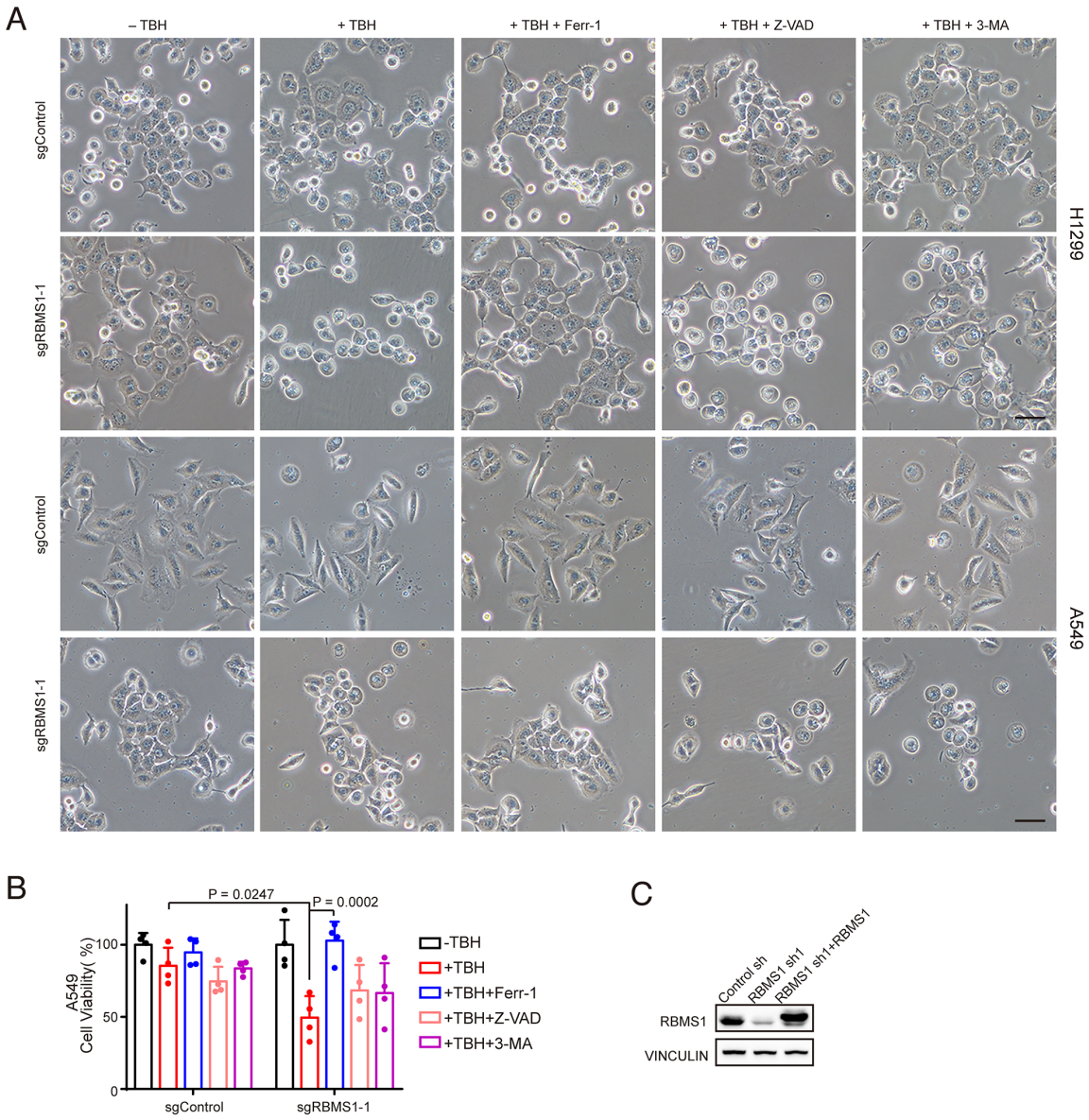

Figure S3. Deletion of RBMS1 promotes TBH-induced ferroptosis. (A) Representative phase-contrast images of H1299 or A549 cells with genetical deletion of RBMS1 treated with TBH (20, or 150 μM), TBH (20, or 150 μM) and Ferr-1 (2 μM), TBH (20, or 150 μM) and Z-VAD-fmk (Z-VAD) (20 μM), TBH (20, or 150 μM) and 3-MA (2 mM) respectively. Scale bars, 100 μm. **(B)** Bar graphs demonstrating cell viability in RBMS1 stably deleted A549 cells treated with 90 μM tert-butyl hydroperoxide (TBH) combined with 2 μM Ferr-1, 20 μM Z-VAD, or 2 mM 3-MA respectively. **(C)** A western blot assay was applied to examine the protein levels of RBMS1 in H1299 cells with stable depletion of RBMS1 in the absence or presence of re-expression of RBMS1. Error

bars are mean ± SEM, n = 4 (**B**) independent repeats. P values were determined using One-way ANOVA with Tukey's multiple comparisons in **B**.

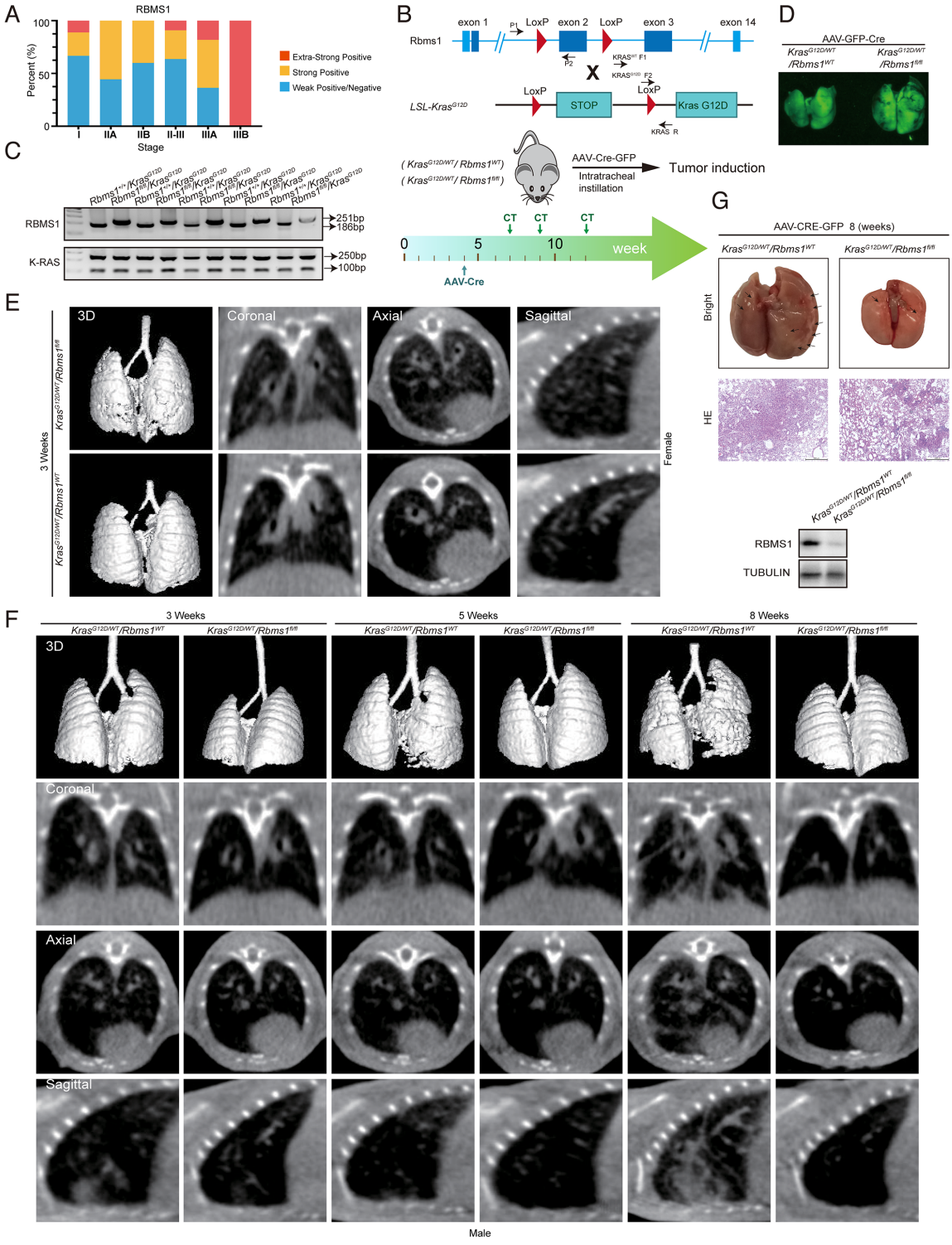

Figure S4. Loss of RBMS1 inhibits lung cancer progression. (A) The quantification of RBMS1 and protein level in lung cancer patient tissues with different clinical stages. **(B)** The schematic of generation of *Kras*$^{G12D/WT}$/*Rbms1*$^{fl/fl}$ mice. The lungs of four-week-old mice were infected with 9 × 10^{10} vg AAV-GFP-Cre virus. Micro-CT images were taken

at 3, 5, or 8 weeks respectively post infection of virus to monitor the lung tumor growth. (**C**) Genotyping of the *Kras*$^{G12D/WT}$/*Rbms1*^{WT} and *Kras*$^{G12D/WT}$/*Rbms1*$^{fl/fl}$ CKO mice. (**D**) The equal introduction of AAV-GFP-Cre signals were monitored to exhibit in the lung. **(E-F)** Micro-CT images in indicated planes from mice with or without *Rbms1* deletion (*Kras*$^{G12D/WT}$/*Rbms1*^{WT} or *Kras*$^{G12D/WT}$/*Rbms1*$^{fl/fl}$ CKO) in lung at 3-week (female) (E); or 3-, 5-, and 8- weeks (male) (F) post infection of 9×10^{10} vg pfu AAV-GFP-Cre. Three-dimensional rendering of micro-CT data with lungs in gray, lost part represented tumor. **(G)** Representative images of lung lesions, and H&E staining from the indicated groups. The protein levels of RBMS1 in tumors from RBMS1 wild type or deletion mice were measured with a western blot assay.

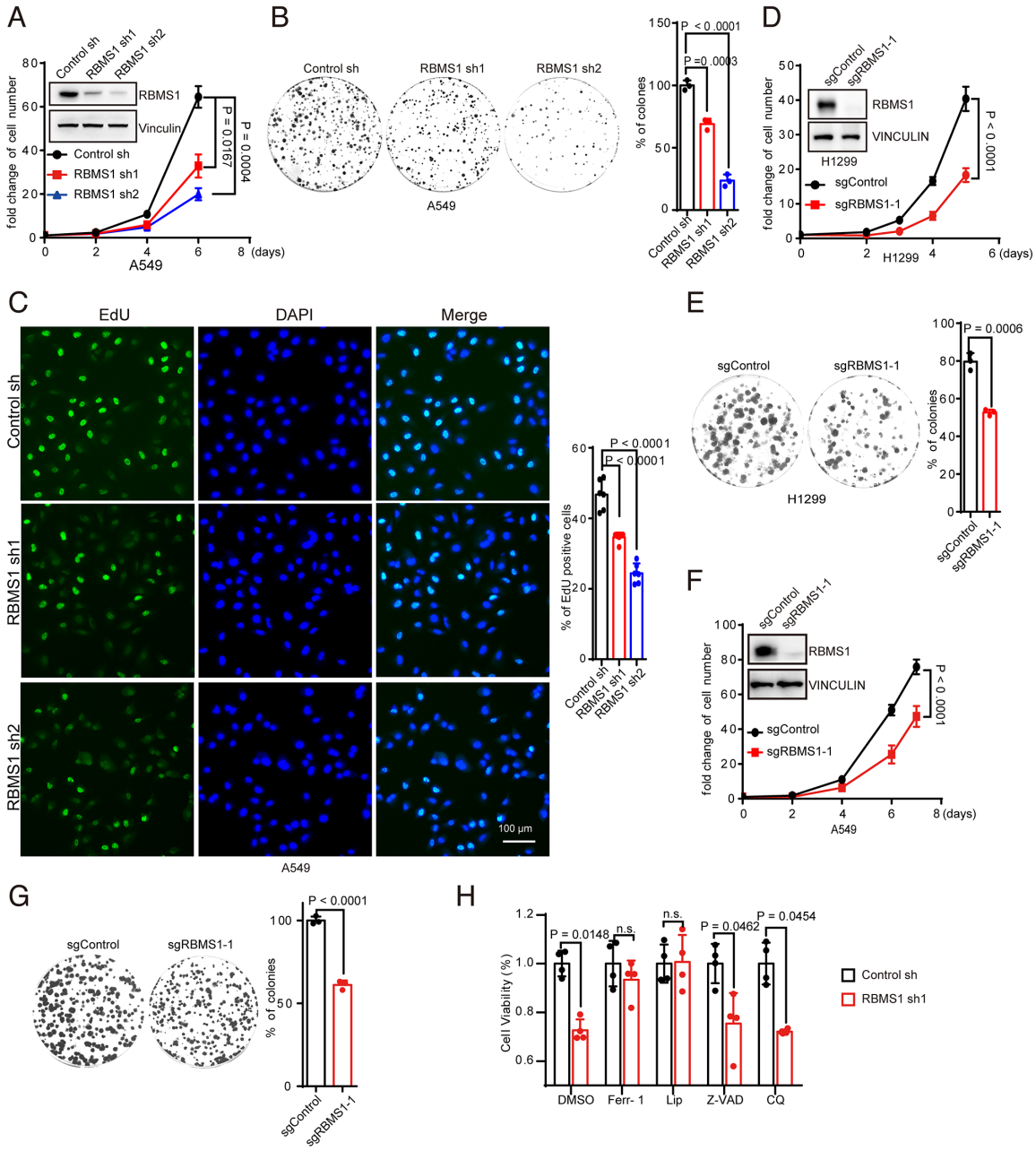

Figure S5. Depleted RBMS1 suppressed A549 lung cancer cell progression. (A) A549 cells with stable depletion of RBMS1 or control were grown for six days, with cell numbers counted every two days. The changes of cell numbers were compared to day 0. The mean ± SEM from three experiments was plotted. (**B**) Colony formation assays using A549 cells with stable depletion of RBMS1. Images of the whole plate are shown. Three experiments were carried out with mean ± SEM of relative colony numbers plotted. (**C**) The proliferative abilities of RBMS1 stably depleted A549 cells were measured with EdU staining assay. Six experiments were conducted with mean ± SEM of percentage of EdU

positive cells plotted. (**D**) H1299 cells with deletion of RBMS1 or control were grown for five days, with cell numbers counted. The changes of cell numbers were compared to day 0. The mean ± SEM from three experiments was plotted. The level of RBMS1 was examined with a western blot assay. (**E**) Colony formation assays using H1299 cells with deletion of RBMS1. Images of the whole plate are shown. Three experiments were carried out with mean ± SEM of relative colony numbers plotted. (**F**) A549 cells with deletion of RBMS1 or control were grown for five days, with cell numbers counted. The changes of cell numbers were compared to day 0. The mean ± SEM from three experiments was plotted. The level of RBMS1 was examined with a western blot assay. (**G**) Colony formation assays using H1299 cells with deletion of RBMS1. Images of the whole plate are shown. Three experiments were carried out with mean ± SEM of relative colony numbers plotted. (**H**) Bar graphs showing cell viability in RBMS1-depleted H1299 cells treated with Ferr-1, liproxstatin-1 (Lip), Z-VAD, or CQ respectively. P values were determined using One-way repeated measures ANOVA in **A**, **D, F**; One-way ANOVA with Dunnett multiple comparisons in **B**, **C**; unpaired t-test in **E, G, H**.

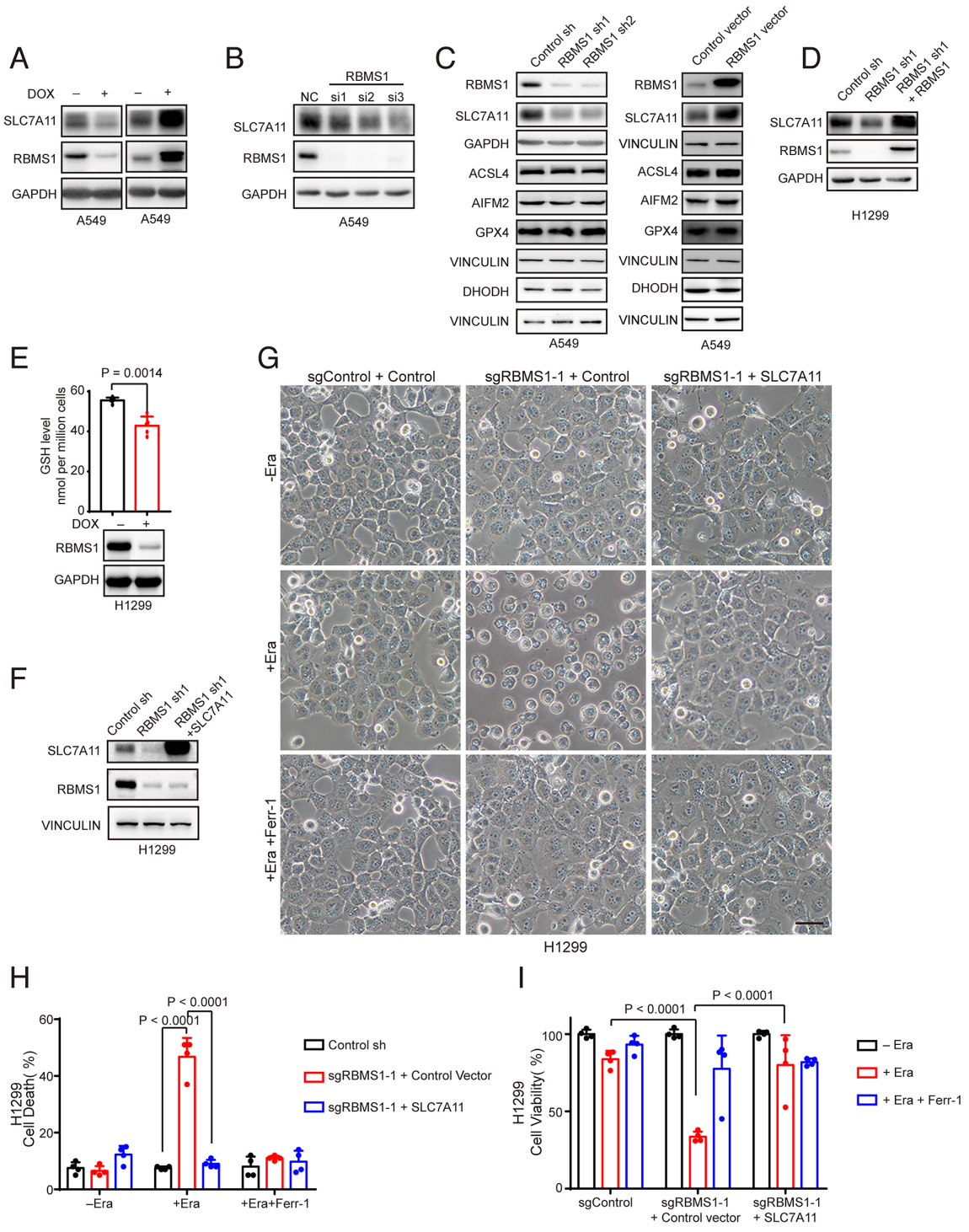

Figure S6. Reduced RBMS1 stimulates ferroptosis through repressed SLC7A11. (**A**) The protein levels of RBMS1 and SLC7A11 in A549 cells with doxycycline-induced depletion or overexpression of RBMS1 were measured with a western blot assay. **(B)** The protein levels of SLC7A11 and RBMS1 were examined in RBMS1 transiently depleted

A549 cells. (**C**) The protein levels of SLC7A11, ACSL4, AIFM2, GPX4, DHODH, and RBMS1 were examined in RBMS1 stably depleted or overexpressed A549 cells. (**D**) The protein levels of SLC7A11, and RBMS1 were examined in RBMS1 stably depleted H1299 cells with or without re-expression of RBMS1. **(E)** Bar graph demonstrating intracellular GSH levels in tetracycline induced RBMS1 depletion H1299 cells. **(F)** The protein levels of SLC7A11 and RBMS1 were examined with a western blot assay in RBMS1 stably depleted H1299 cells with or without re-expression of SLC7A11. **(G)** Representative phase-contrast images of the H1299 cells with genetic deletion of RBMS1 with or without re-expression of SLC7A11, treated with erastin (3 μM), erastin (3 μM) and Ferr-1 (2 μM) respectively. Scale bars, 100 μm. (**H**) Cell death was measured after treatment with erastin (2 μM), Ferr-1 (2 μM) and erastin (2 μM) in H1299 cells with genetical deletion of RBMS1, RBMS1 deletion and re-expression of SLC7A11. (**I**) Bar graphs showing cell viability in H1299 cells with genetical deletion of RBMS1, RBMS1 deletion and re-expression of SLC7A11 treated with 3 μM erastin, and erastin combined with 2 μM Ferr-1. P values were determined using unpaired t-test in **E**; One-way ANOVA with Tukey's multiple comparisons in **H**, **I**.

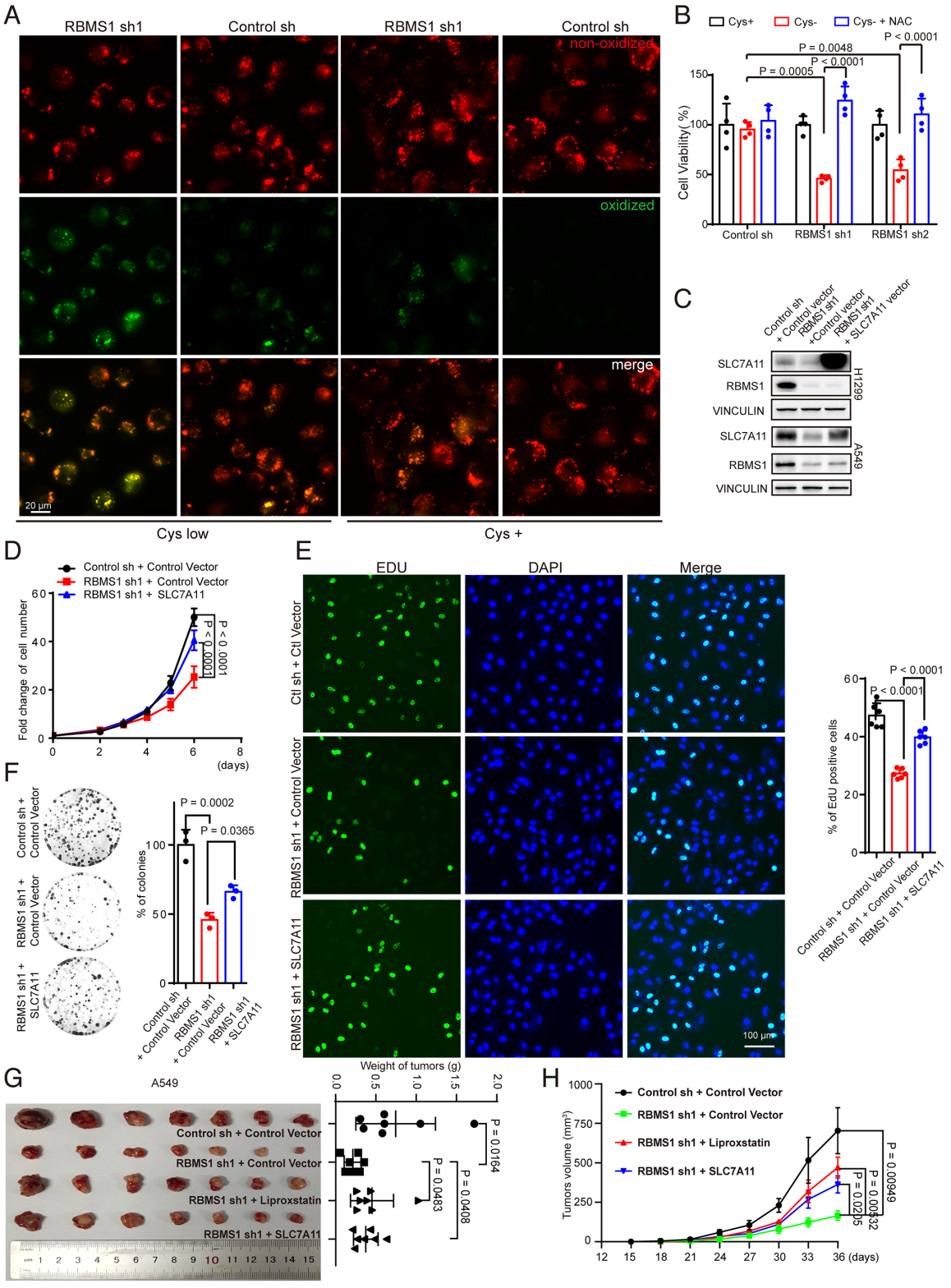

Figure S7. Decreased RBMS1 suppresses lung cancer through repressed SLC7A11 induced ferroptosis. (**A**) Immunofluorescence microscopy showed the non-oxidized

lipid (red) and oxidized lipid (green) in RBMS1-depleted H1299 cells that in the presence or absence of Cystine. **(B)** Cell viability of H1299 cells with RBMS1-depletion were measured after culturing in cystine-deficiency-medium combined with or without addition of N-acetylcysteine (NAC). **(C)** The protein levels of RBMS1 and SLC7A11 were examined in RBMS1 stably depleted H1299 or A549 cells with or without re-expression of SLC7A11 using a western blot assay. (**D**) A549 cells with stable depletion of RBMS1, or stably depleted RBMS1 with re-expression of SLC7A11 were grown for five days, with cell numbers counted every day. The changes of cell numbers were compared to day 0. The mean ± SEM from three experiments was plotted. (**E**) The proliferative abilities A549 cells with stable depletion of RBMS1, or stably depleted RBMS1 with re-expression of SLC7A11 were measured with EdU staining assay. Six experiments were conducted with mean ± SEM of percentage of EdU positive cells plotted. **(F)** Colony formation assays using A549 cells with stable depletion of RBMS1, or stably depleted RBMS1 with re-expression of SLC7A11. Images of the whole plate are shown. Three experiments were carried out with mean ± SEM of relative colony numbers plotted. **(G)** H1299 cells with RBMS1-knockdown, combined with SLC7A11 re-expression, were subcutaneously injected into the flank of nude mice. When the volume of tumors reached 50 ~ 100 mm^3, Liproxstatin-1 was diluted in PBS and injected intraperitoneally into mice daily at a dose of 10 mg/kg. Pictures of the tumors removed were shown and tumors were weighed and plotted. (**H**) The average sizes of xenograft-tumors were measured (n=7). P values were determined using One-way ANOVA with Tukey's multiple comparisons in **B**; One-way repeated measures ANOVA in **D**, **H**; One-way ANOVA with Dunnett multiple comparisons in **E**, **F**, **G**.

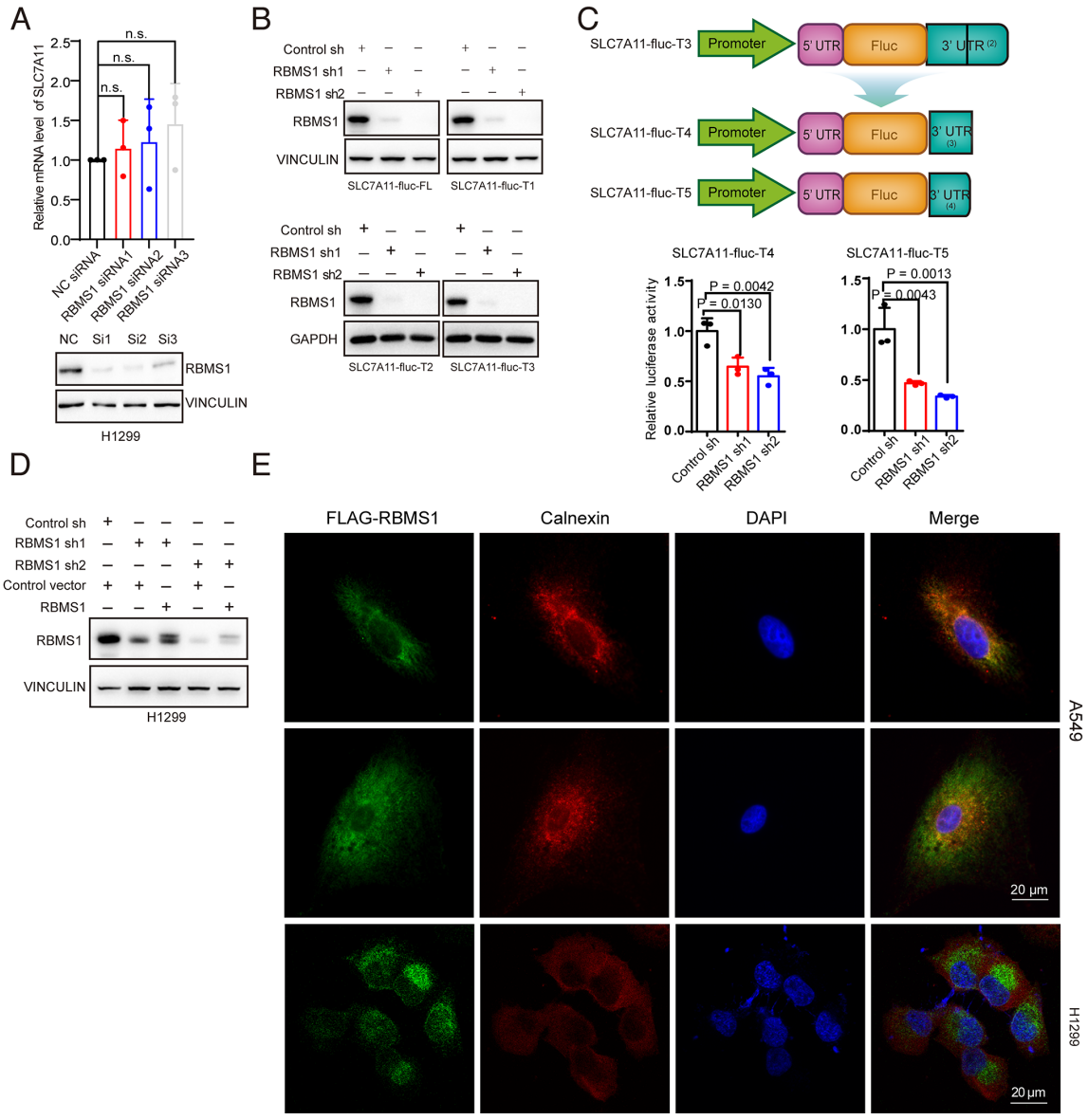

Figure S8. RBMS1 affects translation of SLC7A11. (**A**) The mRNA expression levels of SLC7A11 in RBMS1 transiently knocked down H1299 cells were investigated using qRT-PCR. The knockdown efficiency of RBMS1 was determined with a western blot assay. Error bars are mean ± SEM, n = 3 independent repeats. **(B)** The protein level of RBMS1 was examined in RBMS1 stably depleted H1299 cells with transient expression of different luciferase reporters of *SLC7A11* using a western blot assay. **(C)** The schematic of the *SLC7A11* reporters (SLC7A11-fluc-T4 and SLC7A11-fluc-T5) with truncated 3'-UTRs. SLC7A11-fluc-T4 and SLC7A11-fluc-T5 were transiently transfected into RBMS1-depleted H1299 cells respectively. The relative luciferase activities were determined by calculating the ratio of firefly-luciferase over renilla-

luciferase activities. (**D)** The protein level of RBMS1 in RBMS1 stably depleted H1299 cells with re-expression of RBMS1 was measured using a western blot assay. (**E)** The localization of RBMS1 and Calnexin were examined with anti-Flag and anti-Calnexin antibodies using confocal microscopy in A549 and H1299 cells with stable expression of RBMS1. P values were determined using One-way ANOVA with Dunnett multiple comparisons in **A, C**.

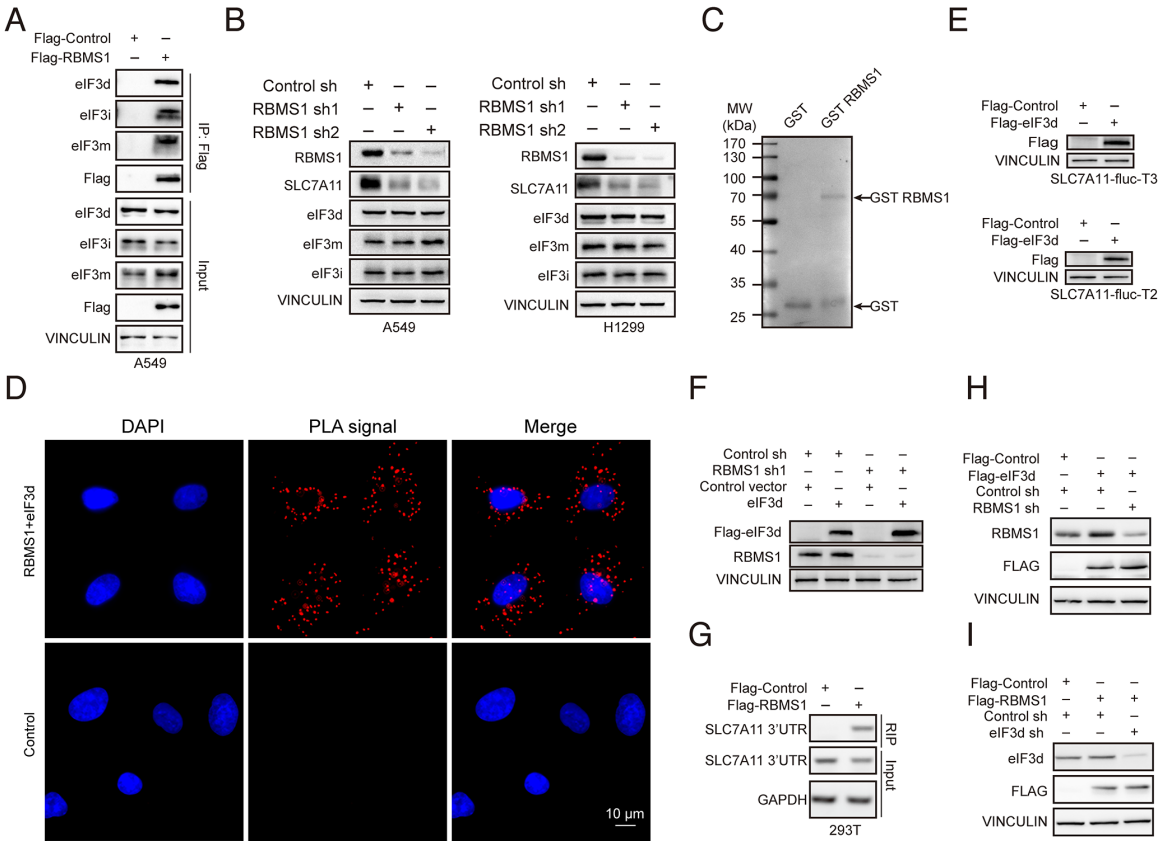

Figure S9. RBMS1 regulates SLC7A11 translation through interacting with eIF3d. (**A**) Immunoprecipitation assay was conducted with RBMS1 stably expressed A549 cells using anti-Flag M2 beads, and the precipitated complexes were analyzed by Western blot with anti-eIF3d, anti-eIF3m, and anti-eIF3i antibodies. **(B)** The protein levels of RBMS1, SLC7A11, eIF3d, eIF3m, and eIF3i were determined in RBMS1 stably depleted A549 or H1299 cells using a western blot assay. **(C)** The protein expression levels of purified GST and GST-RBMS1 were examined with a PAGE gel and stained with Coomassie blue staining. **(D)** PLA was performed to examine the endogenous interaction between RBMS1 and eIF3d. PLA signals were shown in red and the nuclei in blue. **(E)** The expression level of exogenously expressed eIF3d was examined in H1299 cells with stable overexpression of eIF3d and transient transfection of different luciferase reporters. **(F)** The protein expression levels of RBMS1 and eIF3d in RBMS1 stably depleted H1299 cells with or without re-expression of eIF3d were examined with a western blot assay. **(G)** Binding of *SLC7A11* 3′-UTR with RBMS1 is examined by RNA-immunoprecipitation assay in 293T cells exogenously expression FLAG-RBMS1 or

vector control. **(H)** The protein level of RBMS1 was examined in A549 cells exogenously expressed FLAG-eIF3d with or without RBMS1 depletion. **(I)** The protein level of eIF3d was examined in A549 cells exogenously expressed FLAG-RBMS1 with or without eIF3d depletion.

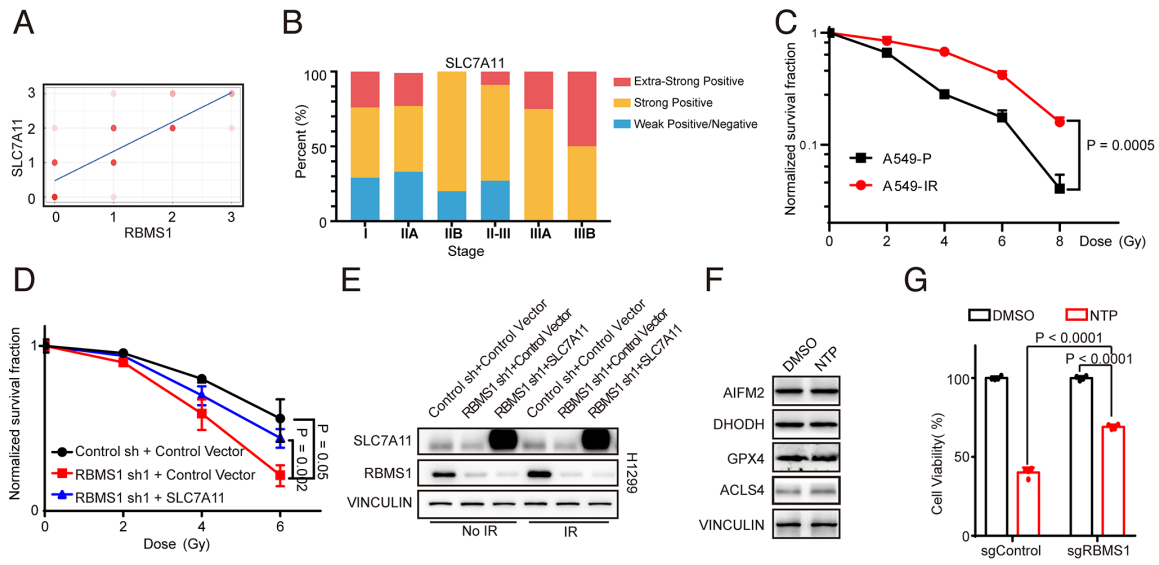

Figure S10. The correlation of RBMS1 and SLC7A11 in clinical samples and radioresistance. **(A)** The correlation of the protein levels of RBMS1 and SLC7A11 from immunohistochemical staining in lung cancers (n = 60) and matched adjacent normal tissues (n = 60) were analyzed and plotted. **(B)** The quantification of SLC7A11 protein level in lung cancer patient tissues with different clinical stages. **(C)** Clonogenic survival was measured with a colony formation assay in parental or IR-resistant A549 cells in the presence of distinct dosages of irradiation. (**D**) Clonogenic survival was measured with a colony formation assay in IR-resistant A549 cells with stable knock down of RBMS1, or stably depleted RBMS1 with re-expression of SLC7A11, treated with different dosages of irradiation. (**E**) A western blot assay was applied to examine the protein levels of RBMS1 and SLC7A11 in RBMS1 stably depleted H1299 cells with or without re-expression of SLC7A11 in the absence or presence of irradiation. (**F**) The levels of ferroptosis-related genes were examined in H1299 cells in the presence or absence of NTP treatment. (**G**) Cell viability of RBMS1-deleted H1299 cells were measured after treatment with or without NTP. P values were determined using one-way repeated measures ANOVA in C, D; One-way ANOVA with Tukey's multiple comparisons in G.