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Journal

iScience, 29(4)

ISSN

2589-0042

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Publication Date

2026-04-01

DOI

10.1016/j.isci.2026.115475

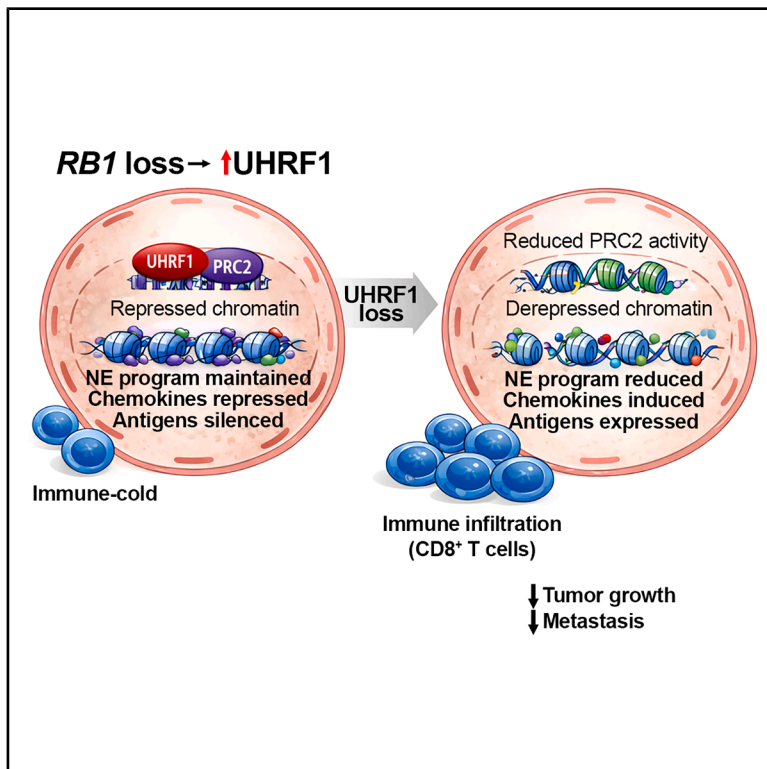
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UHRF1 drives subtype-independent aggressiveness and immune evasion in small cell lung cancer through PRC2 interactions

Graphical abstract



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In brief

Immunology; Biological sciences; Epigenetics; Cancer

Highlights

- UHRF1 promotes SCLC growth and metastasis across major molecular subtypes
- UHRF1 loss shifts tumors from neuroendocrine to inflamed transcriptional states
- UHRF1 loss induces chemokines and increases CD8⁺ T cell and myeloid infiltration
- UHRF1 interacts with PRC2 to repress inflammatory genes and tumor antigens



Article

UHRF1 drives subtype-independent aggressiveness and immune evasion in small cell lung cancer through PRC2 interactions

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<https://doi.org/10.1016/j.isci.2026.115475>

SUMMARY

Small cell lung cancer (SCLC) is an aggressive neuroendocrine (NE) carcinoma characterized by early metastasis and an immune-cold microenvironment. We show that UHRF1, a chromatin-associated effector of RB1/E2F signaling, promotes SCLC growth and metastasis across major molecular subtypes. Using human SCLC cell lines, orthotopic and intracardiac xenografts, and genetically engineered mouse models, we found that loss of UHRF1 reduces proliferation, invasion, tumor burden, and metastasis while reprogramming tumors away from NE states toward an inflamed transcriptional program. UHRF1-deficient tumors upregulate chemokines and exhibit increased infiltration of CD8⁺ T cells and myeloid populations. Mechanistically, UHRF1 interacts with PRC2 to reinforce NE lineage programs and suppress inflammatory gene expression. UHRF1 loss derepresses DNA-methylation-silenced tumor antigens, including MAGE-A4, highlighting a potential vulnerability that could be leveraged therapeutically. Together, these findings connect RB1 loss with chromatin repression, lineage control, and immune exclusion, highlighting UHRF1-dependent repression as a therapeutic vulnerability in SCLC.

INTRODUCTION

Small cell lung cancer (SCLC) is a highly aggressive neuroendocrine (NE) malignancy that accounts for approximately 15% of all lung cancer cases and is associated with a dismal 5-year survival rate below 6% for patients with extensive-stage disease.^{1,2} SCLC is characterized by rapid proliferation, early metastatic dissemination, and a profoundly immunosuppressive tumor microenvironment, often described as “immune-cold.”³ Despite a relatively stable and well-defined genomic landscape, therapeutic progress in SCLC has been limited, highlighting the need to better understand non-genetic mechanisms that sustain its aggressive behavior.

Recent integrative transcriptomic and epigenomic studies have revealed marked heterogeneity within SCLC, leading to a classification into distinct molecular subtypes based on lineage-defining transcription factors: ASCL1 (SCLC-A), NEUROD1 (SCLC-N), POU2F3 (SCLC-P), and in some contexts, YAP1 (SCLC-Y).^{4–7} These subtypes show unique patterns of NE marker expression, cellular plasticity, immune infiltration, and therapeutic vulnerabilities. Classical NE subtypes, such as SCLC-A and SCLC-N, express high levels of synaptophysin (SYP), chromogranin A (CHGA), neural cell adhesion molecule 1 (NCAM1),

and insulinoma-associated protein 1 (INSM1) and typically exhibit low immune cell infiltration. In contrast, non-NE variants, including SCLC-P and SCLC-Y, display reduced NE marker expression, increased inflammatory signaling, and enhanced immune engagement, features that have been associated with improved prognosis and therapeutic responsiveness.^{3,5–10} The ability of SCLC tumors to transition between these NE and non-NE states contributes to therapeutic resistance and disease progression.

Epigenetic mechanisms have emerged as key regulators of SCLC lineage identity and plasticity. Chromatin modifiers and transcriptional repressors, including MYC-Notch signaling components, SWI/SNF complex members, and Polycomb repressive proteins such as EZH2, play central roles in stabilizing NE programs while suppressing alternative differentiation states and immune-related gene expression.^{3,10–12} However, how these epigenetic programs are coordinated downstream of the defining genetic events in SCLC remains incompletely understood.

Genetically, SCLC is defined by near-universal loss of the tumor suppressors *RB1* and *TP53*.¹³ Beyond its canonical role in cell cycle control, *RB1* also regulates differentiation and epigenetic stability.^{14,15} Loss of *RB1* has been shown to promote



lineage plasticity and NE transdifferentiation in multiple cancer contexts, including lung adenocarcinoma and prostate cancer.^{16,17} Nevertheless, the downstream effectors that translate RB1 loss into stable epigenetic repression programs in SCLC have not been fully elucidated.

Ubiquitin-like with PHD and Ring Finger domains 1 (UHRF1) is a direct transcriptional target of the RB/E2F pathway and a central mediator of epigenetic silencing in proliferative cells.¹⁸ UHRF1 maintains DNA methylation by recruiting DNMT1 to hemi-methylated DNA via histone H3 ubiquitination and interacts with multiple chromatin-modifying complexes, including histone deacetylases and histone methyltransferases, to reinforce transcriptional repression.^{19–22} While UHRF1 expression is normally restricted to stem and progenitor cells, it is aberrantly upregulated in many RB1-deficient cancers, where it has been implicated in tumor growth and metastasis.^{23,24} Despite these observations, the role of UHRF1 in SCLC biology, lineage maintenance, and tumor-immune interaction has not been thoroughly investigated.

Here, we identify UHRF1 as a subtype-independent oncogenic driver in SCLC that integrates RB1 loss with chromatin-based repression to promote tumor aggressiveness and immune evasion. Using human SCLC cell lines, patient datasets, and genetically engineered mouse models (GEMMs), we show that UHRF1 is broadly overexpressed across SCLC subtypes and that its loss impairs tumor growth and metastatic progression. Mechanistically, UHRF1 interacts with the Polycomb Repressive Complex 2 (PRC2) to enforce NE identity while suppressing inflammatory and immune-related gene expression. Loss of UHRF1 reprograms tumors toward a less NE, more inflamed state characterized by increased chemokine production, immune cell infiltration, and derepression of epigenetically silenced tumor antigens, including MAGE-A4. These findings establish UHRF1 as a central epigenetic regulator that links RB1 inactivation to NE identity, metastatic behavior, and immune suppression in SCLC, highlighting UHRF1-dependent chromatin repression as a potential therapeutic vulnerability in this otherwise refractory disease.

RESULTS

UHRF1 is broadly overexpressed in SCLC and correlates with adverse clinical outcomes

To establish the clinical relevance of UHRF1 in SCLC, we first evaluated its expression in patient tumors and its association with clinical outcome. Analysis of publicly available SCLC datasets (Database: GSE60052, HRA003419) revealed that UHRF1 mRNA and protein levels are significantly elevated in SCLC tumors compared with adjacent normal lung tissue (Figure 1A).^{7,25} Consistent with UHRF1 being a downstream target of RB/E2F signaling, RB1 protein levels were inversely correlated with UHRF1 protein expression across SCLC samples (Figure 1B). These findings confirm that UHRF1 upregulation is a common feature of human SCLC.

Kaplan-Meier survival analysis demonstrated that patients with higher UHRF1 expression exhibited significantly reduced overall survival compared with those with lower expression, identifying UHRF1 as a negative prognostic marker in SCLC (Figure 1C). This association suggests that UHRF1 expression

is linked not only to tumor presence but also to aggressive clinical behavior.

To determine whether UHRF1 expression is restricted to specific molecular subtypes, we examined transcriptomic data from 212 human lung cell lines in the DepMap Public 25Q3 database, including 60 SCLC lines. UHRF1 is robustly expressed across the majority of SCLC cell lines, irrespective of subtype classification (Figure 1D). Based on these findings, we selected three RB1-mutant human SCLC cell lines representing the major transcriptional NE subtypes, DMS79 (SCLC-A; ASCL1), NCI-H446 (SCLC-N; NEUROD1), and NCI-H526 (SCLC-P; POU2F3), for functional. All three lines exhibited high expression of canonical NE markers (SYP, CHGA, NCAM1, and INSM1) and elevated UHRF1 protein levels compared with the proliferative but non-malignant lung fibroblast line MRC5 (Figures 1E and 1F).

UHRF1 is required for SCLC proliferation, clonogenicity, and invasive behavior across molecular subtypes

Having established that UHRF1 is broadly overexpressed in SCLC, we next investigated whether UHRF1 is functionally required for malignant phenotypes across distinct SCLC molecular subtypes *in vitro*. For this, we generated CRISPR/Cas9-mediated UHRF1 knockout (KO) clones in all three SCLC cell lines. Inducible KO led to reduced cell viability at 72 h, suggesting UHRF1 is essential for cell survival (Figure S1A).²⁶ Despite efficient editing at the DNA level, we were unable to recover any full UHRF1 KO clones after screening nearly a hundred single-cell-derived colonies. All viable clones retained low but detectable UHRF1 protein, consistent with partial editing and confirmed by inference of CRISPR edits analysis (Figures 2A and S1B–S1D). This indicates that full loss of UHRF1 is incompatible with SCLC cell viability. Even these partially edited KO cells exhibited a significant decrease in proliferation rates, as measured by growth curves and EdU incorporation (Figures 2B–2D). Clonogenic potential was assessed using soft agar assays, where UHRF1 KO cells formed fewer and smaller colonies compared to vector control (VC) cells (Figures 2E and 2F).

We next examined whether UHRF1 influences migratory and invasive behaviors. Using scratch-wound assays, UHRF1 KO cells demonstrated reduced closure of the wound area over 24 h compared to VC cells, a phenotype reversed by UHRF1 re-expression (Figures 2G and 2H). Transwell invasion assays using Matrigel-coated inserts showed that UHRF1-deficient cells had impaired capacity to invade through the extracellular matrix, further confirming a role for UHRF1 in promoting motility and invasive potential (Figures 2I and 2J).

Loss of UHRF1 suppresses tumor growth and metastatic dissemination *in vivo*

To evaluate whether UHRF1 influences tumorigenicity *in vivo*, we performed orthotopic lung xenografts and intracardiac metastasis assays using luciferase-labeled SCLC cells. In the orthotopic model, UHRF1 KO tumors grew significantly slower and reached smaller endpoint volumes compared to VC tumors (Figures 3A–3C, S2A, S3A, S3B, S4A, and S4B). *Ex vivo* bioluminescent imaging of major organs showed a marked reduction in metastatic burden in the UHRF1 KO group, with fewer

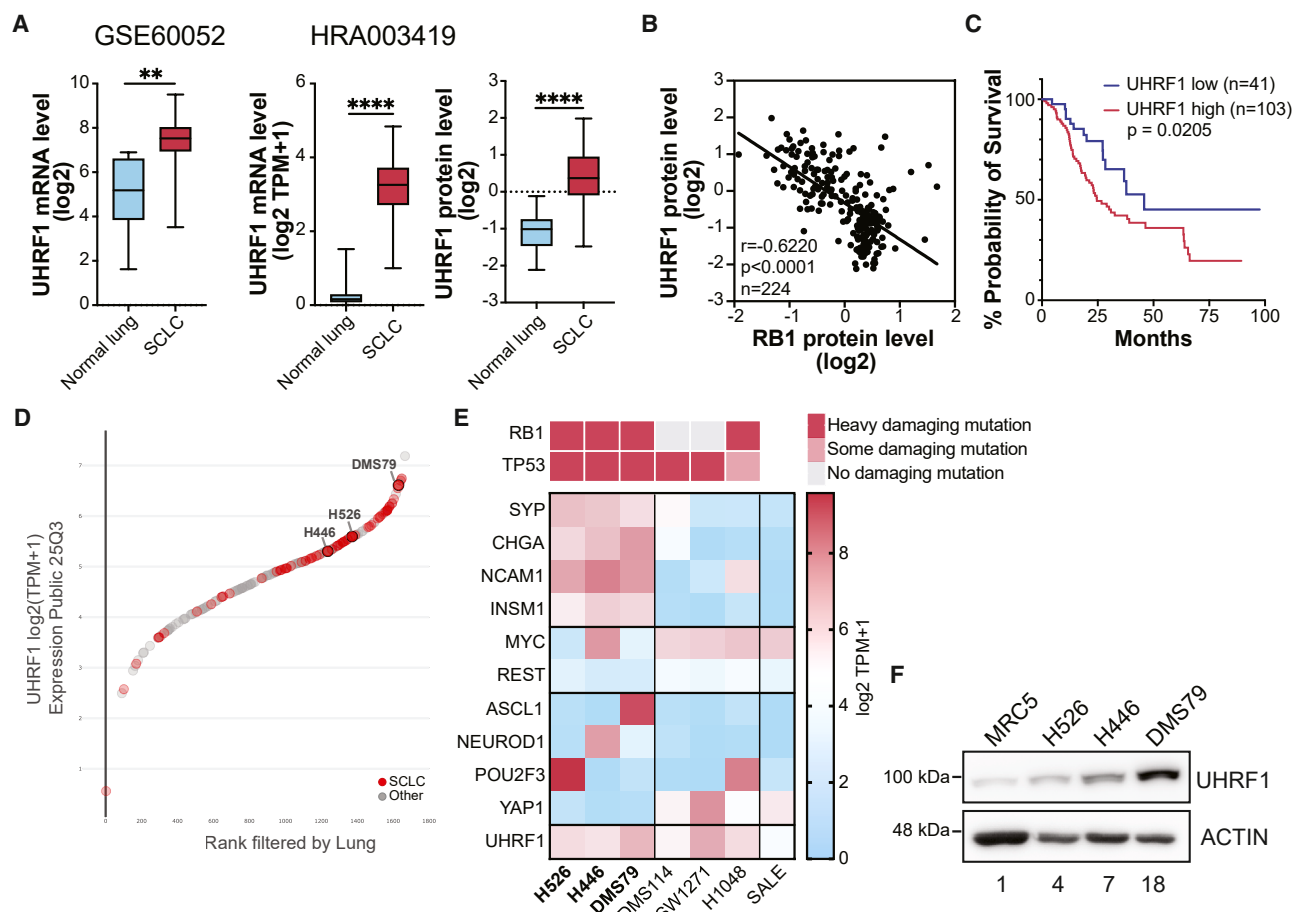


Figure 1. UHRF1 is broadly overexpressed in SCLC and inversely correlates with RB1 protein expression

(A) UHRF1 mRNA and protein expression, derived from datasets GSE60052 and HRA003419, were compared between human SCLC tumors and normal lung tissues. For GSE60052 normal lung $n = 7$, SCLC $n = 79$. For HRA003419 normal lung $n = 106$, SCLC $n = 107$. $^{**}p < 0.01$ and $^{****}p < 0.0001$ by unpaired t test with Welch's correction.

(B) Correlation analysis of RB1 and UHRF1 protein expression demonstrating a significant inverse relationship, consistent with UHRF1 acting downstream of RB1 loss. (C) Kaplan-Meier overall survival analysis of patients with SCLC stratified by high (red) vs. low (blue) UHRF1 mRNA expression. Survival curves were analyzed using log rank (Mantel-Cox) test. $p = 0.0205$.

(D) UHRF1 mRNA expression across human lung cancer cell lines, including 60 SCLC lines (red), from the DepMap Public 25Q3 database. $^{****}p < 0.0001$ by Spearman correlation.

(E) Overview of RB1 and TP53 mutation status (top), and expression profiles of NE markers (SYP, CHGA, NCAM1, and INSM1) and non-NE markers (MYC and REST), lineage-specific transcription factors (ASCL1, NEUROD1, POU2F3, and YAP1), and UHRF1 across three SCLC cell lines used in this study (H526, H446, and DMS79), three non-NE SCLC cell lines (DMS114, SW1271, and H1048), and one non-malignant lung epithelial cell line (SALE).

(F) Western blot analysis of UHRF1 protein levels in normal lung fibroblast (MRC5) and three NE SCLC cell lines. Actin was used as a loading control. Semi-quantitative values of UHRF1 protein intensity relative to MRC5 are shown in numbers below.

metastases detected in the liver, bone, adrenal glands, and reproductive organs (Figures 3D–3F, S3C–S3E, and S4C–S4E).

To control for primary tumor growth effects, we also employed an intracardiac injection model for distant metastasis using NCI-H526 cells. UHRF1 KO cells exhibited reduced colonization of distant organs, with significantly lower bioluminescent signals and fewer metastatic foci (Figures 3G–3J). Immunohistochemical analysis of CD31 revealed reduced vascular density in UHRF1 KO tumors (Figures S2C and S2D), suggesting a possible role for UHRF1 in promoting angiogenesis, as previously reported.²⁴ Furthermore, long-term survival studies revealed a significant increase in overall survival and decreased metastatic burden in

UHRF1 KO mice (Figures 3K and S2B), reinforcing UHRF1's contribution to SCLC tumor progression and dissemination.

Uhrf1 deletion delays tumor initiation and prolongs survival in SCLC GEMMs

To directly evaluate the role of UHRF1 in SCLC initiation and progression within an intact tissue and immune context, we employed GEMMs with the conditional deletion of *Uhrf1*. We generated GEMMs with the deletion of *Tp53* and *Rb1* ($Tp53^{lox/lox}$, $Rb1^{lox/lox}$; TR DKO) or *Tp53*, *Rb1*, and *Uhrf1* ($Tp53^{lox/lox}$, $Rb1^{lox/lox}$, $Uhrf1^{lox/lox}$; TRU TKO) using intranasal delivery of adenoviral-Cre.²⁷ TRU TKO mice developed fewer

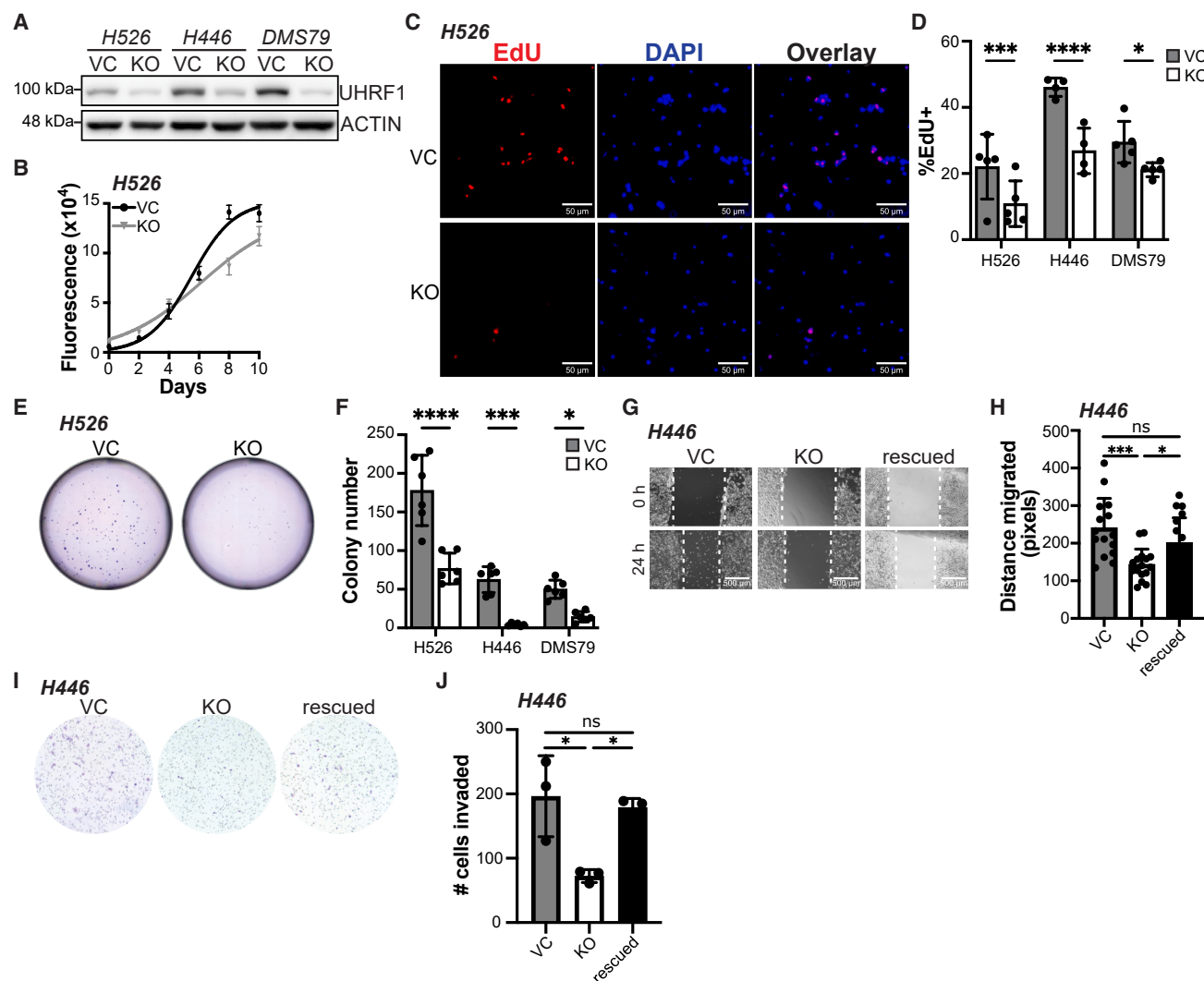


Figure 2. UHRF1 promotes proliferation, anchorage-independent growth, migration, and invasion in SCLC cells

(A) Western blot analysis of UHRF1 expression in vector control (VC) and UHRF1 knockout (KO) cells across three SCLC cell lines. (B) Growth proliferation curves measured by AlamarBlue assay comparing NCI-H526 VC (black line; doubling time = 1.42 days) and UHRF1 KO (gray line; doubling time = 2.68 days). (C) Representative images of EdU incorporation assay (proliferating cells; red) in NCI-H526 VC and UHRF1 KO cells. Nuclei were counterstained with DAPI (blue). Scale bars, 50 μ m. (D) Quantification of the proportion of EdU-positive cells across all three SCLC cell lines. ($n = 3$). (E) Representative soft agar colony formation images from NCI-H526 VC and UHRF1 KO. (F) Quantification of colony numbers for each of the SCLC cell lines in the soft agar assay. (G) Representative images from scratch wound healing assays at 0 and 24 h in NCI-H446 VC, UHRF1 KO, and UHRF1-rescued cells. Scale bars, 500 μ m. (H) Quantification of wound closure in the migration assay ($n = 15$). (I) Representative images from Transwell invasion assays with crystal violet-stained cells (purple) in NCI-H446 VC, UHRF1 KO, and UHRF1-rescued groups. (J) Quantification of cells invaded in the transwell assay ($n = 6$). For all histograms, each bar represents the mean $\pm$ SD; ns = not significant, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, and **** $p < 0.0001$ by unpaired two-tailed t test.

and smaller lung tumors 30 weeks post-induction (Figures 4A–4C). Histological examination confirmed SCLC morphology (Figure 4D). RNA *in situ* hybridization and western blotting confirmed reduced Uhrf1 expression in TRU TKO tumors (Figures 4E–4G). Notably, overall survival was significantly improved in the TRU TKO group, supporting the oncogenic function of UHRF1 in SCLC (Figure 4H).

UHRF1 maintains NE lineage programs while repressing non-NE and inflammatory states

Given the established link between NE identity, tumor aggressiveness, and immune exclusion in SCLC, we next examined whether UHRF1 regulates transcriptional programs associated with lineage state and inflammatory signaling. Transcriptomic correlation analysis using the GSE60052 dataset revealed

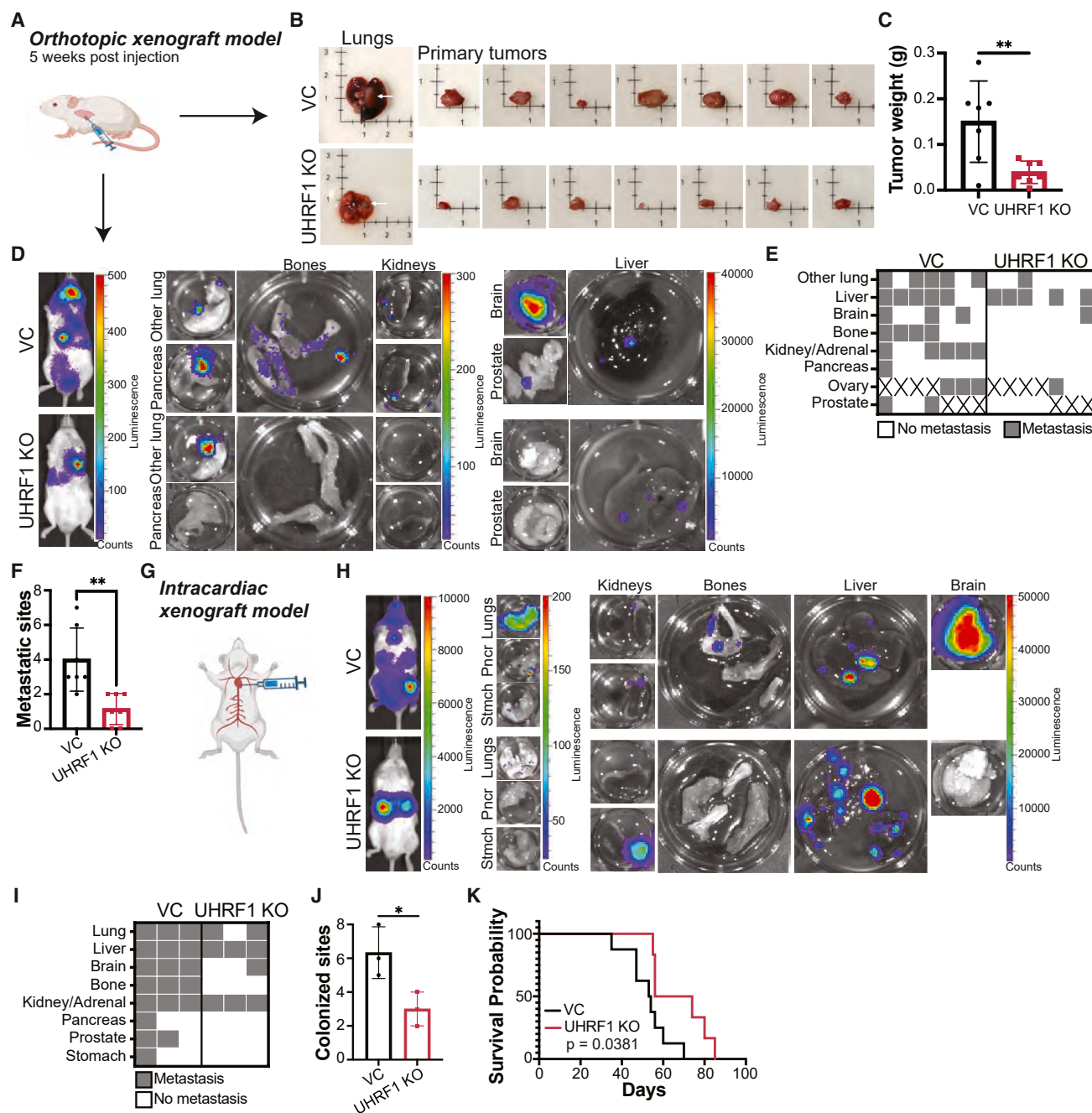


Figure 3. UHRF1 promotes tumor growth and metastatic spread in SCLC xenograft models

All results represent the NCI-H526 cell line.

(A) Scheme of the orthotopic xenograft mouse model.

(B) Representative images of lungs and primary lung tumors harvested at the study endpoint.

(C) Comparison of primary tumor weights between vector control (VC; black) and UHRF1 KO (red) groups. Bars represent mean $\pm$ SD ($n = 7$). ** $p < 0.01$ by unpaired two-tailed t test.

(D) Representative bioluminescent imaging of mice and dissected major organs from the orthotopic model.

(E) Heatmap-style binary presence (gray) or absence (white) chart summarizes the frequency and distribution of metastasis across orthotopic xenografts.

(F) Quantification of the number of metastatic sites observed across orthotopic xenografts. VC in black; UHRF1 KO in red. Bars represent mean $\pm$ SD ($n = 7$). ** $p < 0.01$ by unpaired two-tailed t test.

(G) Schematic of the NCI-H526 intracardiac injection xenograft model.

(H) Representative bioluminescent images of mice and major organs from the intracardiac model.

(I) Heatmap illustrates the distribution of metastases across organs in the intracardiac xenograft model.

(legend continued on next page)

positive associations between UHRF1 and NE markers (*SYP*, *CHGA*, *NCAM1*, and *INSM1*) and negative associations with non-NE genes (*YAP1*, *MYC*, *REST*) (Figures 5A and 5B). To validate these findings functionally, we performed bulk RNA-seq on tumors derived from GEMMs and NCI-H526 orthotopic xenografts. Gene Set Enrichment Analysis (GSEA) demonstrated that UHRF1 KO tumors exhibited significant downregulation of NE signatures and upregulation of non-NE and inflammatory response gene sets (Figures 5C, 5D, 6A, and S5A–S5C).²⁸ UHRF1-deficient tumors had a reduced NE score and displayed reduced expression of NE markers (*SYP* and *NCAM1*) and increased expression of non-NE markers (*REST* and *YAP1*), as confirmed by IHC and western blotting (Figures 5E–5H, S3F–S3J, and S4F–S4I).²⁸ No significant enrichment of non-NE gene programs was observed in UHRF1-deficient SCLC cells maintained *in vitro* (Figure S3K).

UHRF1 cooperates with PRC2 to reinforce repressive chromatin states in SCLC

To further understand how UHRF1 preserves NE identities, we explored its interaction with chromatin-modifying complexes. We focused particularly on the polycomb repressive complex 2 (PRC2) and its catalytic subunit EZH2, given previous reports implicating EZH2 in the maintenance of NE phenotypes and immune suppression in SCLC.^{29,30} EZH2 is a known driver of H3K27 trimethylation (H3K27me3), a histone mark associated with the transcriptional repression of lineage-inappropriate genes, and is frequently overexpressed in SCLC.^{31,32} We observed a strong positive correlation between UHRF1 and EZH2 mRNA expression in clinical datasets and our GEMM models (Figures 5I and 5J). Co-immunoprecipitation assays confirmed a physical interaction between recombinant UHRF1 and PRC2 components EZH2 and SUZ12 in 293T cells (Figure S6A), as previously reported.³³ Western blot analysis showed reduced EZH2 protein levels in UHRF1 KO tumors (Figures 5K and 5L). Interestingly, global H3K27me3 levels were consistently decreased in NCI-H446 but not NCI-H526 or DMS79 UHRF1 KO cells, suggesting context-dependent modulation of PRC2 activity by UHRF1 (Figure S6B).

Loss of UHRF1 induces chemokine expression and promotes immune cell infiltration in SCLC tumors

Given the upregulation of inflammatory gene signatures following UHRF1 loss (Figure 6A), we next evaluated whether these transcriptional changes were accompanied by alterations in the tumor immune microenvironment. IHC analysis of GEMM-derived tumors revealed increased infiltration of F4/80+ macrophages and CD3+ T cells in TRU TKO tumors relative to TR DKO controls (Figures 6B–6E). To comprehensively characterize immune cell subsets, we performed flow cytometry analysis on allografted primary SCLC tumors derived from TR and TRU mice, using tumors generated from the corresponding

UCI-ScTR-1 and UCI-ScTRU-1 cell lines (Figure 6F). To control for tumor burden, UCI-ScTRU-1-derived tumors (0.14 ± 0.08 g) were allowed to grow longer prior to analysis to achieve tumor weights comparable to UCI-ScTR-1-derived tumors (0.18 ± 0.10 g), enabling assessment of immune infiltration independent of tumor size ($p = 0.377$). Loss of *Uhrf1* resulted in the elevated infiltration of both lymphoid (B cells, CD8+ T cells) and myeloid (monocytes, neutrophils, cDC1s) populations (Figures 6G–6N, S5D, and S5E). Notably, the ratio of cytotoxic to regulatory T cells was significantly increased in the UCI-ScTRU-1 group, suggesting a shift toward a more immunostimulatory tumor microenvironment (Figure 6J).

To investigate the mechanism underlying this immune cell influx, we profiled secreted chemokines in conditioned media from UCI-ScTR-1 and UCI-ScTRU-1 cells using a cytokine array. *Uhrf1*-deficient TRU cells exhibited increased secretion of several chemokines known to modulate immune trafficking, including CCL6, CCL27, IL-16 (T cell recruitment), CXCL1, GCP-2 (neutrophil chemotaxis), CCL12 (inflammatory monocytes), and CXCL10 and CXCL12 (broadly involved in T cell and dendritic cell migration) (Figures 6O and S7). Conversely, the expression of CXCL13, an oncogenic chemokine associated with immune exhaustion and metastatic competence, was reduced. These findings suggest that *Uhrf1* acts as a suppressor of chemokine-mediated immune infiltration in SCLC, and that its loss creates a more permissive immune microenvironment that may support improved responsiveness to immunotherapy.³⁴

UHRF1 loss leads to DNA hypomethylation and derepression of tumor-associated antigens

Given the central role of UHRF1 in DNA methylation maintenance,^{35,36} we finally assessed whether UHRF1 loss alters global methylation patterns and derepresses epigenetically silenced tumor-associated antigens (TAAs). We performed 5-methylcytosine (5 mC) dot blot assays, which revealed a global reduction of DNA methylation levels in UHRF1 KO cells compared to controls (Figure S8B). Consistently, the reduced 5 mC signal suggests a genome-wide erosion of methylation patterns.

RNA-seq-based differential expression analysis comparing UHRF1-intact and UHRF1-deficient SCLC tumors (Figure S8A) uncovered significant upregulation of multiple TAAs, including members of the cancer/testis antigen family, including MAGE-A4, as well as immunogenic proteins such as TGFBI (Figure 6P), while other popular SCLC TAAs showed no change or downregulation in UHRF1-deficient tumors (Figures S8H–S8M). To determine whether the derepression of these upregulated genes was directly linked to demethylation, we performed methylation-specific PCR. The promoters of MAGE-A4 and TGFBI exhibited marked demethylation in UHRF1 KO conditions (Figures 6Q, 6R, S8C, and S8D), confirming epigenetic reactivation. Furthermore, the treatment of wild-type SCLC cells with the

(J) Quantification of the number of metastatic organ sites in each group. VC in black; UHRF1 KO in red. Bars represent mean $\pm$ SD ($n = 3$). ** $p < 0.01$ by unpaired two-tailed t test.

(K) Kaplan-Meier survival analysis show overall survival probabilities for the orthotopic xenograft model for VC (black) and UHRF1 KO (red). Survival curves were analyzed using the log rank (Mantel-Cox) test. $p = 0.0381$.

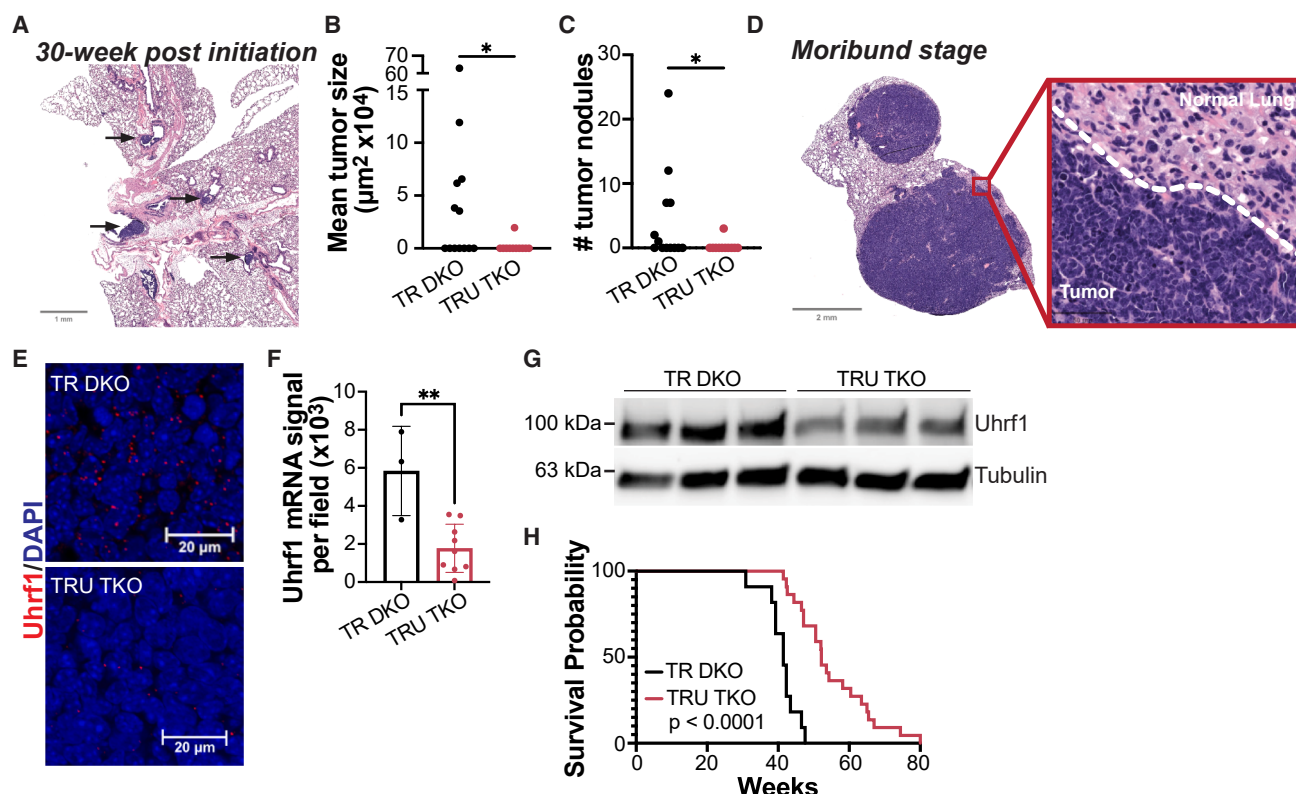


Figure 4. Genetic deletion of *Uhrf1* suppresses early tumor development and extends survival in an SCLC GEMM model

(A) Representative H&E-stained lung section from mice at 30 weeks post-initiation. Scale bars, 1 mm.
 (B and C) Quantification of tumor burden in TR DKO (black; $n = 13$) and TRU TKO (red; $n = 11$) GEMM lungs at 30 weeks: (B) average tumor size and (C) number of tumor nodules per lung. $*p < 0.05$ by unpaired t test.
 (D) H&E-stained lung section from a moribund GEMM animal study endpoint. Scale bars, 2 mm.
 (E) Representative fluorescent *in situ* hybridization (RNAscope) images showing *Uhrf1* mRNA (red) and counterstained nuclei (blue, DAPI) in GEMM tumors. Scale bars, 20 μ m.
 (F) Quantification of *Uhrf1* mRNA signal intensity across TR DKO ($n = 3$) and TRU TKO ($n = 9$) GEMM tumor samples. $**p < 0.01$ by unpaired t test.
 (G) Western blot analysis of *Uhrf1* protein levels in GEMM tumors. Tubulin used as loading control.
 (H) Kaplan-Meier survival analysis compares overall survival between TR DKO and TRU TKO GEMM cohorts. Survival curves were analyzed using the log rank (Mantel-Cox) test. $p < 0.0001$.

DNA methyltransferase inhibitor 5-azacytidine (5-aza) induced MAGE-A4 and TGBI expression, whereas little to no further increase was observed in UHRF1 KO cells, indicating that their expression is dependent on DNA methylation status maintained by UHRF1 (Figures S8S and S8E).

To explore the functional consequences of TGBI derepression, we overexpressed TGBI in NCI-H446 cells and observed a significant reduction in cell migration (Figures S8F and S8G). This suggests that TGBI may act as a tumor suppressor whose silencing by UHRF1 contributes to SCLC invasiveness. Collectively, these data identify UHRF1 as a critical regulator of methylation-dependent antigen silencing and underscore its potential as a target to boost tumor immunogenicity.

DISCUSSION

In this study, we identify UHRF1 as a subtype-independent epigenetic driver of SCLC progression that integrates RB1 loss with chromatin-based repression to sustain NE identity, promote

aggressiveness, and limit immune engagement. Using complementary *in vitro* models, xenografts, and GEMMs, we demonstrate that UHRF1 is broadly overexpressed across SCLC subtypes and that its loss consistently impairs proliferation, invasion, metastatic dissemination, and tumor development. While previous studies have linked UHRF1 to aggressive phenotypes in select non-NE SCLC models,³⁷ our data expand this role across the major molecular subtypes. These findings establish UHRF1 as a central regulator of SCLC malignancy and highlight epigenetic repression as a key determinant of disease behavior.

Loss of RB1 is a defining genetic event in SCLC and is known to drive uncontrolled proliferation through deregulated E2F activity. Beyond cell-cycle control, RB1 also plays an essential role in maintaining epigenetic stability and cellular identity.^{14,15} Our data position UHRF1 as a critical downstream effector of RB1/E2F signaling in SCLC. The inability to recover complete UHRF1 KO clones, despite efficient genome editing, underscores the essential nature of UHRF1 in SCLC cell survival and suggests that even partial attenuation of UHRF1 function is

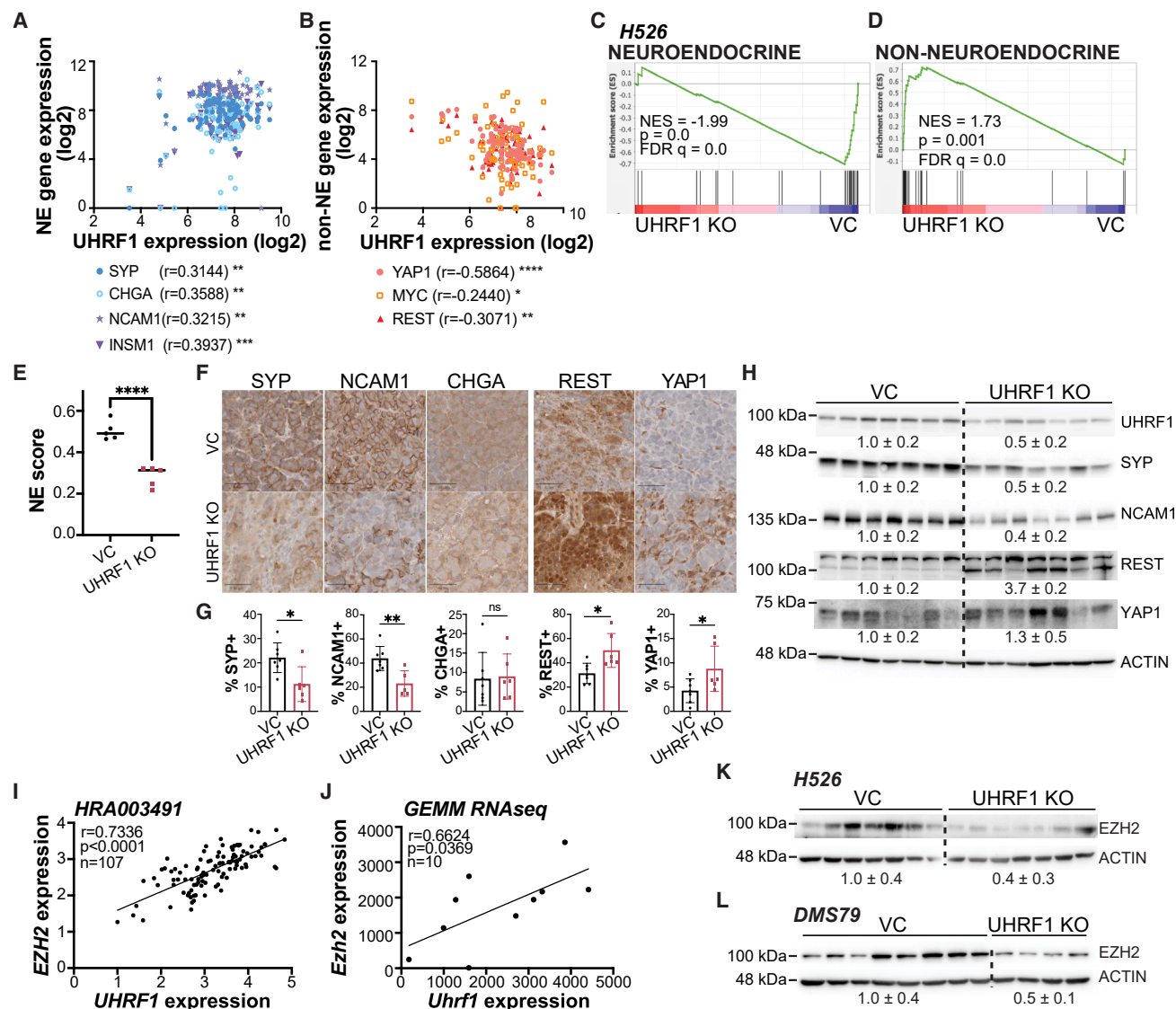


Figure 5. UHRF1 expression is associated with the NE phenotype in SCLC

(A and B) Pearson's correlation (r) analyses between UHRF1 expression and canonical markers of (A) NE and (B) non-NE phenotypes using human SCLC tumor data (GSE60052). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, and **** $p < 0.0001$ by two-tailed Pearson's correlation.

(C and D) Gene Set Enrichment Analysis (GSEA) compares (C) NE signature enrichment and (D) non-NE signature enrichment between NCI-H526 vector control (VC) and UHRF1 KO orthotopic tumors.

(E) NE score comparison between NCI-H526 VC and UHRF1 KO tumors. **** $p < 0.0001$ by two-tailed unpaired t test.

(F) Representative immunohistochemistry images of NE markers (SYP, NCAM1, CHGA) and non-NE markers (REST, YAP1) in VC and UHRF1 KO tumors. Scale bars, 50 μ m.

(G) Quantifications of immunohistochemistry signal intensities. Bars represent mean $\pm$ SD; VC ($n = 7$), UHRF1 KO ($n = 6$). ns = not significant, * $p < 0.05$, ** $p < 0.01$ by unpaired two-tailed t test.

(H) Western blot analysis shows the expression of UHRF1, NE markers (synaptophysin, NCAM1), non-NE markers (REST, YAP1), and actin as a loading control in NCI-H526 VC and UHRF1 KO tumor samples. Relative average protein abundance shown below each blot.

(I and J) Pearson's correlation analyses between EZH2 and UHRF1 expression in (I) clinical SCLC dataset HRA003491 and (J) GEMM tumors.

(K and L) Western blot analysis of EZH2 levels in UHRF1 KO versus VC tumors derived from (K) NCI-H526 and (L) DMS79 xenografts. Actin was used as a loading control. Relative average protein abundance is shown below each blot.

sufficient to compromise tumorigenic capacity. Importantly, UHRF1 loss reduced aggressive phenotypes, supporting a broader role in maintaining malignant cell states rather than serving merely as a proliferation-associated factor.

A major challenge in SCLC biology is its pronounced transcriptional and phenotypic heterogeneity, which has led to classification into distinct molecular subtypes. Despite this diversity, our findings reveal a shared dependency on UHRF1 across

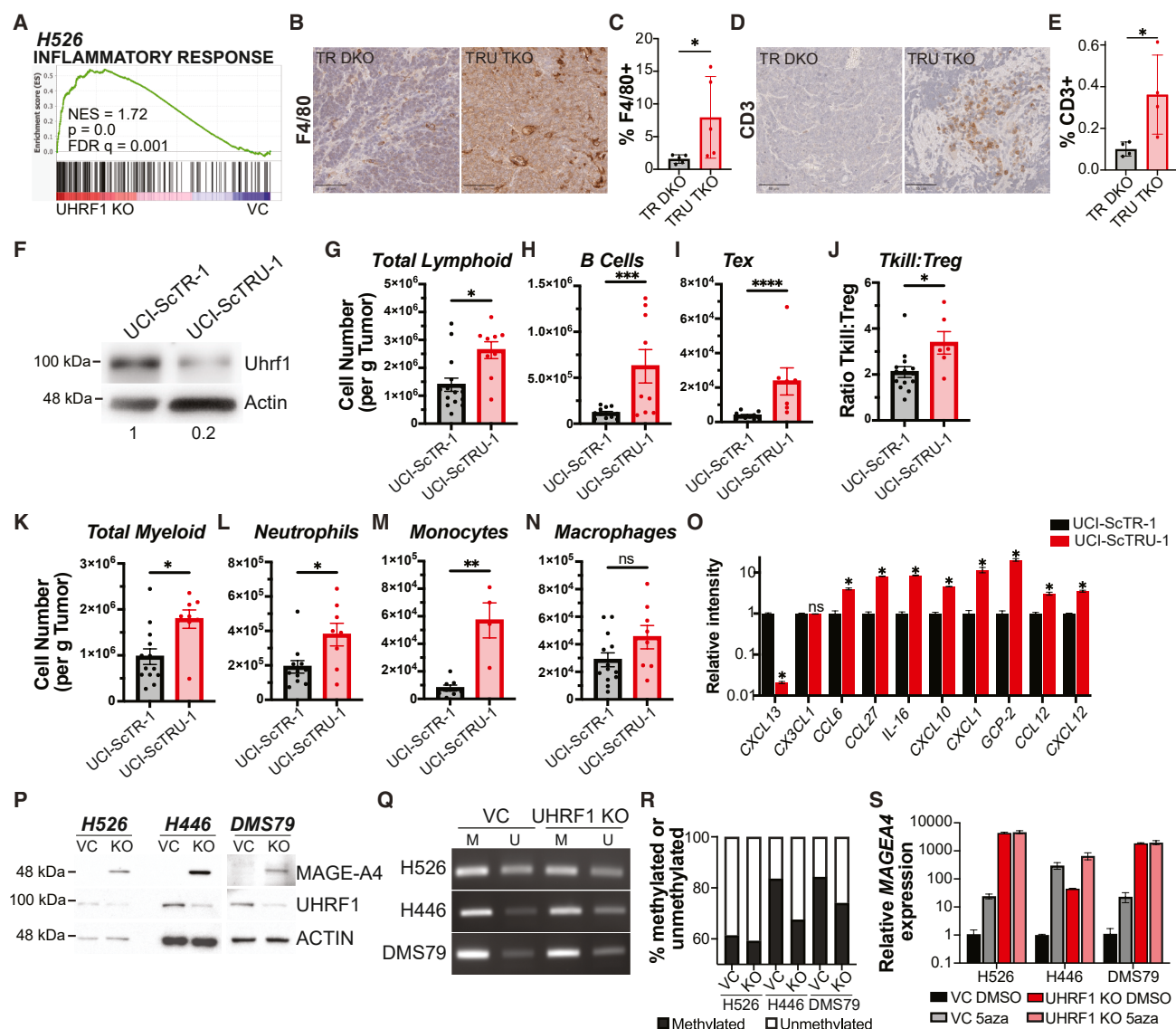


Figure 6. Loss of UHRF1 leads to more immune cell infiltration

(A) Gene Set Enrichment Analysis (GSEA) comparing inflammatory response signature enrichment between NCI-H526 vector control (VC) and UHRF1 KO or-thotopic tumors.

(B) Representative IHC images for F4/80 macrophage staining. Scale bars, 50 μ m; and, (C) signal quantification on Tp53/Rb1 (TR) DKO and Tp53/Rb1/Uhrf1 (TRU) TKO GEMM tumors. Bars represent mean $\pm$ SD (n = 5). *p < 0.05 by unpaired two-tailed t test.

(D) Representative IHC images for CD3 T cell staining. Scale bars, 50 μ m; and (E) signal quantification on GEMM tumors. Bars represent mean $\pm$ SD (n = 5). *p < 0.05 by unpaired two-tailed t test.

(F) Western blot shows UHRF1 protein levels in primary mouse SCLC cells generated from GEMM tumors.

(G–N) Quantification of various immune cell populations in TR DKO (n = 14) and TRU TKO (n = 9) tumor allografts. Bars represent mean $\pm$ SD. ns = not significant, *p < 0.05, **p < 0.01, ***p < 0.001, and ****p < 0.0001 by unpaired two-tailed t test.

(O) Quantification of the chemokines immunoassay analysis (n = 2 independent batches). Bars represent mean $\pm$ SD *p < 0.05 by unpaired two-tailed t test.

(P) Western blot analysis of MAGE-A4 in VC and UHRF1 KO cells of all three SCLC cell lines.

(Q) Methylation-specific PCR (MSP) analysis of specific CpG loci on MAGE-A4 in VC and UHRF1 KO cells of all three SCLC cell lines. M: methylated. U: unmethylated.

(R) Quantification of MAGE-A4 MSP.

(S) RT-qPCR analysis of MAGE-A4 expression in 5-azacytidine (5aza) treated and untreated (DMSO) VC and UHRF1 KO cells of all three SCLC cell lines. Bars represent mean $\pm$ SD.

SCLC-A, SCLC-N, and SCLC-P models. UHRF1 loss consistently reduced NE marker expression and promoted a shift toward non-NE transcriptional programs, including the upregulation of REST and YAP1. These changes did not reflect nonspecific dedifferentiation but rather a structured reprogramming away from NE lineage identity. This observation supports the idea that epigenetic constraints, enforced by UHRF1, help stabilize NE states across otherwise heterogeneous SCLC subtypes and may underlie the capacity of tumors to maintain aggressive behavior despite transcriptional diversity. Notably, non-NE transcriptional programs were not induced by UHRF1 loss *in vitro*, indicating that the shift toward non-NE states is reinforced within the *in vivo* tumor microenvironment rather than being an intrinsic consequence of UHRF1 depletion alone. Prior studies have shown that NE-to-non-NE lineage plasticity in SCLC is often revealed *in vivo*, consistent with a role for tumor microenvironmental cues that are absent in standard cell culture systems.^{38,39}

Mechanistically, our data indicate that UHRF1 cooperates with PRC2 to reinforce repressive chromatin states in SCLC. We observed strong correlations between UHRF1 and EZH2 expression, physical interaction between UHRF1 and PRC2 components, and reduced EZH2 protein levels following UHRF1 loss. Although global H3K27me3 levels were not uniformly altered across all cell lines, this context-dependent effect is consistent with a model in which UHRF1 modulates PRC2 function at specific genomic loci rather than acting as a universal regulator of Polycomb activity. Such locus-specific cooperation likely enables the repression of lineage-inappropriate and immune-related genes while preserving essential transcriptional programs required for tumor maintenance.

One notable consequence of UHRF1 loss was the induction of inflammatory and chemokine expression programs accompanied by increased immune cell infiltration in tumors. UHRF1-deficient tumors displayed elevated recruitment of lymphoid and myeloid populations, including CD8⁺ T cells, monocytes, neutrophils, and dendritic cells. While some of these cell types can exhibit tumor-promoting functions in other cancer contexts, particularly within immunosuppressive microenvironments, their coordinated increase here likely reflects broader immune activation driven by epigenetic derepression. Importantly, our data do not imply that immune infiltration alone is sufficient for tumor eradication; rather, they suggest that UHRF1 contributes to immune exclusion by repressing chemokine expression and inflammatory signaling pathways that would otherwise permit immune engagement.

SCLC is increasingly recognized as a tumor type that actively enforces immune evasion despite a high mutational burden. Prior studies have identified multiple, non-mutually exclusive mechanisms contributing to the immune-cold phenotype of SCLC, including reduced antigen presentation, suppressed interferon signaling, impaired chemokine expression, and enrichment of immunosuppressive myeloid populations. Comprehensive analyses of human SCLC tumors and mouse models have demonstrated that classical NE SCLC subtypes exhibit particularly low expression of major histocompatibility complex (MHC) class I molecules and antigen processing machinery, accompanied by limited T cell infiltration and diminished

responsiveness to immune checkpoint blockade.^{40,41} In parallel, epigenetic repression has emerged as a key contributor to immune exclusion in SCLC.^{3,19,31,42,43} Polycomb-mediated chromatin silencing has been shown to suppress antigen presentation and inflammatory signaling programs, thereby limiting immune recognition.⁴⁴ Restoration of inflammatory signaling and antigen expression can enhance immune engagement; however, these changes are frequently insufficient to produce durable tumor control, underscoring the complexity of immune modulation in this disease.⁴⁵

Our findings place UHRF1 within this established framework of SCLC immune evasion by identifying it as an upstream epigenetic regulator that coordinates multiple immune-suppressive features. Rather than acting through a single immune pathway, UHRF1 loss resulted in the derepression of chemokine expression, increased immune cell recruitment, and re-expression of epigenetically silenced tumor antigens. Importantly, these changes are consistent with prior reports linking NE identity to immune exclusion and support a model in which UHRF1-dependent chromatin repression reinforces the immune-cold state characteristic of aggressive SCLC subtypes. While immune infiltration alone was not sufficient to eradicate tumors in our models, the convergence of chemokine induction, altered lineage state, and antigen derepression suggests that UHRF1 operates at a regulatory nexus connecting epigenetic control to immune accessibility.

Consistent with its established role in maintaining DNA methylation, UHRF1 loss also resulted in global hypomethylation and derepression of epigenetically silenced genes, including the cancer-testis antigen MAGE-A4.^{22,26,36} While MAGE-A4 re-expression alone does not predict therapeutic response, it serves as a proof-of-principle example of how UHRF1-dependent chromatin repression limits antigen visibility in SCLC. This finding aligns with prior observations that SCLC tumors are characterized by low antigenicity and poor immunogenicity despite high mutational burden. Our data suggest that epigenetic silencing, rather than antigen absence, may contribute to this immune-cold phenotype.

Together, these findings have potential therapeutic implications. Rather than targeting individual downstream pathways or subtype-specific transcription factors, disrupting UHRF1-dependent epigenetic repression may represent a strategy to broadly modulate lineage state, tumor aggressiveness, and immune accessibility in SCLC. Given the essential role of UHRF1 in tumor maintenance, direct inhibition may pose challenges; however, indirect approaches that perturb UHRF1-associated chromatin complexes or DNA methylation dynamics could prove effective. Importantly, any such strategies would need to be carefully evaluated in combination with existing therapies, including chemotherapy and immunotherapy, and guided by appropriate biomarkers.

In summary, our study defines UHRF1 as a central epigenetic regulator that links RB1 inactivation to NE lineage maintenance, tumor progression, and immune exclusion in SCLC. By integrating chromatin repression, lineage identity, and tumor-immune interactions into a unified framework, these findings provide new insight into the epigenetic vulnerabilities of SCLC and establish a foundation for future therapeutic exploration.

Limitations of the study

Complete UHRF1 loss was not tolerated in SCLC cells, restricting mechanistic analysis to partial depletion models. Additionally, while increased immune infiltration was observed, we did not directly assess immune cell functionality or response to immune checkpoint blockade. Finally, PRC2 modulation by UHRF1 appeared context dependent, underscoring the complexity of epigenetic regulation in SCLC and the need for locus-specific analyses in future work.

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact, Claudia A. Benavente (claudia.benavente@uci.edu).

Materials availability

All unique reagents and materials generated in this study, including cell lines and GEMMs, are available from the [lead contact](#) upon reasonable request and completion of appropriate material transfer agreements (MTAs).

Data and code availability

Transcriptomics data have been deposited in the Gene Expression Omnibus (GEO) Database: GSE303043. All other datasets are available from the [lead contact](#) on reasonable request. This paper does not report original code.

ACKNOWLEDGMENTS

This work was supported by grants to C.A.B. from NIH (CA229696) and the American Lung Association (LCD-1034555). J.D.L.T. was supported by the NIAMS/NIH Interdisciplinary Skin Biology T32 Training Program (AR080622). F.G. and J.M.V. were supported by the NIH-MARC USTAR training grant (T34GM136498). This work utilized resources of the UCI Genomics Research and Technology Hub (GRT Hub), parts of which are supported by NIH grants to the Comprehensive Cancer Center (P30CA-062203) and the UCI Skin Biology Resource Based Center (P30AR075047) at the University of California, Irvine, as well as to the GRT Hub for instrumentation (1S10OD010794-01 and 1S10OD021718-01).

AUTHOR CONTRIBUTIONS

C.A.B. conceived the project. Y.G., Y.F., J.M.V., F.G., and C.A.B. performed the experiments. J.D.L.T. and R.T. performed and analyzed the immune panel data. Y.G. and C.A.B. wrote the manuscript. All authors reviewed the manuscript.

DECLARATION OF INTERESTS

C.A.B. serves on the iScience advisory board.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

- [KEY RESOURCES TABLE](#)
- [EXPERIMENTAL MODEL AND STUDY PARTICIPANT DETAILS](#)
 - Patient datasets and survival analysis
 - Cell lines and culture conditions
 - Animals
- [METHOD DETAILS](#)
 - Transfection, lentivirus production and transduction
 - CRISPR KO single cloning
 - Growth curve and EdU immunocytochemistry

- Soft-agar colony formation assay
- Scratch-wound healing assay
- Transwell invasion assay
- Western blotting
- Real-time RT-qPCR
- Mouse SCLC primary cell lines generation
- Orthotopic SCLC xenografts and intracardiac metastasis model
- RNA sequencing
- Immunohistochemistry
- *In situ* hybridization
- Immune panel
- Chemokine array
- Pull down assay
- 5-mC dot blot
- Methylation-specific PCR
- 5-aza experiment

● QUANTIFICATION AND STATISTICAL ANALYSIS

SUPPLEMENTAL INFORMATION

Supplemental information can be found online at <https://doi.org/10.1016/j.isci.2026.115475>.

Received: July 29, 2025

Revised: January 30, 2026

Accepted: February 23, 2026

Published: March 24, 2026

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Antibodies		
UHRF1	Santa Cruz Biotechnology	sc-373750
β-Actin	Sigma-Aldrich	A1978
Synaptophysin (SYP)	Cell Signaling Technology	36406
NCAM1	Cell Signaling Technology	99746
Chromogranin A (CHGA)	Invitrogen	PA5-77917
REST	Bioss	BS-2590R
YAP1	Cell Signaling Technology	14074
F4/80	Cell Signaling Technology	70076
CD3	Cell Signaling Technology	99940
CD31	Cederlane DIA310	NC1630455
5-Methylcytosine (5-mC)	Cell Signaling Technology	28692
MAGE-A4	Cell Signaling Technology	82491
Anti-Flag M2	Sigma-Aldrich	F1804
GATA2	Cell Signaling Technology	79802
EZH2	Cell Signaling Technology	5246
SUZ12	Cell Signaling Technology	3737
goat anti-rabbit, HRP	Vector Laboratories	PI-1000
horse anti-mouse, HRP	Vector Laboratories	PI-2000
goat anti-rabbit, biotinylated	Vector Laboratories	BA-1000
goat anti-rat, biotinylated	Invitrogen	31830
Streptavidin-HRP	PerkinElmer	FP1047
Fixable Viability Stain 510	Fisher	BDB564406
anti-mouse CD16/32 Antibody	Biolegend	101302
PE anti-mouse/human CD44	Biolegend	103008
PerCP anti-mouse CD45.2	Biolegend	109826
PE/Cyanine7 anti-mouse CD279	Biolegend	109110
BV 421™ anti-mouse CD4	Biolegend	100443
APC/Cyanine7 anti-mouse CD19	Biolegend	115530
BV 605™ anti-mouse CD366	Biolegend	119721
BV 785™ anti-mouse CD8a	Biolegend	100750
Alexa Fluor® 488 anti-mouse Ly-6G	Biolegend	127626
PE anti-mouse Siglec H	Biolegend	129606
PE/Cyanine7 anti-mouse F4/80	Biolegend	123114
APC anti-mouse Ly-6C	Biolegend	128016
APC/Cyanine7 anti-mouse CD3	Biolegend	100222
APC/Cyanine7 anti-mouse NK-1.1	Biolegend	156510
BV 421™ anti-mouse I-A/I-E	Biolegend	107631
Brilliant Violet 605™ anti-mouse/human CD11b	Biolegend	101257
BV 785™ anti-mouse CD11c	Biolegend	117336
FOXP3 Monoclonal (FJK-16s), APC	Fisher	5015209
TCF1/TCF7 (C63D9) mAb, Alexa Fluor® 488	Cell Signaling Technology	6444S
Bacterial and virus strains		
Ad5-CMV-Cre	University of Iowa Viral Vector Core	

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REAGENT or RESOURCE	SOURCE	IDENTIFIER
Critical commercial assays		
RNAscope Multiplex Fluorescent Reagent Kit	Advanced Cell Diagnostics	323100
RNAscope Target Probe C1 (MmUhrf1 and Hs-UHRF1)	Advanced Cell Diagnostics	300031
Lung Dissociation Kit	Miltenyi Biotec	
Proteome Profiler Mouse Chemokine Array Kit	R&D Systems	ARY020
Deposited data		
RNAseq human SCLC and normal control	GEO	GSE60052
GSA for human	CNCB	HRA003419
RNAseq	This paper	GSE303043
Experimental models: Cell lines		
NCI-H526	ATCC	CRL-5811
NCI-H446	ATCC	HTB-171
DMS79	ATCC	CRL-2049
MRC5	ATCC	CCL-171
HEK293T	Dharmacon	HCL4517
UCI-ScTR-1	This paper	
UCI-ScTRU-1	This paper	
Experimental models: Organisms/strains		
Mouse:		
Mouse: <i>Uhrf1</i> ^{flox/flox}	CA Benavente (UC Irvine) Wu et al. ²⁴	N/A
Mouse: NOD scid gamma	Jackson Laboratories	005557
Oligonucleotides		
hUHRF1 gRNA1 TGCTCGGGACACGAACATGG	IDT DNA	
hUHRF1 gRNA3 GGCTACCCCGACACACGT	IDT DNA	
hUHRF1 gRNA4 CTACAACCCCGACAACCCCA	IDT DNA	
MAGEA4-methylated	IDT DNA	
FWD: 5'-TTGATTAGTAATTCGAGATTACGG-3'; REV: 5'-CTCTTAAATCCTAACTTTAACGCC-3'		
MAGEA4-unmethylated	IDT DNA	
FWD: 5'-TTTGATTAGTAATTTGAGATTTATGG-3'; REV: 5'-CTCTTAAATCCTAACTTTAACACC-3'		
TGFBI-methylated	IDT DNA	
FWD: 5'-GAAAATTGAGTACGGGTATAGTGC-3'; REV: 5'-CCAAATTAATAAACTACGAACGAA-3'		
TGFBI-unmethylated	IDT DNA	
FWD: 5'-AAAATTGAGTATGGGTATAGTGTGG-3'; REV: 5'-CCAAATTAATAAACTACAAACAAA-3'		
hMAGEA4	IDT DNA	
FWD: 5'-GAGCTTCTGCGTCTGACTCG-3'; REV: 5'-AGGCAAGAGTGCAGGCAAAA-3'		
hTGFBI	IDT DNA	
FWD: 5'-GTCTACACAGTCTTTGCTCCC REV: 5'-TCCGCTAACGAGATTTCATC-3'		
hGPI	IDT DNA	
FWD: 5'-CTGTGACTCCCTTTCTCTTC-3'; REV: 5'-CCAAGCCCAACAACAGAG-3'		
Recombinant DNA		
TLCV2 plasmid	Addgene	87360
pLenti-PGK V5-LUC Neo	Addgene	21471
pCMV-VSV-G	Addgene	8454

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REAGENT or RESOURCE	SOURCE	IDENTIFIER
pCMV-dr8.2-dvpr	Addgene	8455
pCMV6-UHRF1-tGFP	Origene	RG217766
pCMV6-AC-tGFP	Origene	PS100010
Software and algorithms		
Prism 10	Graphpad	
ImageJ 1.52k	NIH	
QuPath 5.0	University of Edinburgh	
FlowJo 10	BD Life Sciences	
R	R Foundation	

EXPERIMENTAL MODEL AND STUDY PARTICIPANT DETAILS

Patient datasets and survival analysis

Transcriptomic and clinical data from SCLC patients were obtained from public repositories (GSE60052 and HRA003419).^{7,25} Patients were stratified into high and low expression groups based on median UHRF1 level for Kaplan-Meier survival analyses using Prism 10. Statistical significance was determined by the log-rank test.

Cell lines and culture conditions

Human SCLC cells (NCI-H526, NCI-H446 and DMS79) and MRC5 fibroblasts were obtained from ATCC. HEK293T cells were obtained from Dharmacon and cultured in DMEM (Gibco) supplemented with 10% bovine calf serum (BCS) and 1% penicillin-streptomycin. Human SCLC cell lines and mouse SCLC primary cells were cultured in RPMI1640 with 10% fetal bovine serum (FBS) and 1% penicillin and streptomycin at 37°C with 5% CO₂. Cell line authentication was verified by STR profiling; all cells were routinely screened for mycoplasma (SouthernBiotech).

Animals

The *Tp53*^{lox/lox} and *Rb1*^{lox/lox} mice (B6/129 background) were obtained from the Mouse Models of Human Cancer Consortium at the National Cancer Institute; *Uhrf1*^{lox/lox} mice were generated as previously described.²⁴ Conditional mouse SCLC initiation was achieved using intranasal adenoviral delivery of Cre recombinase (Ad5-CMV-Cre, University of Iowa Viral Vector Core) into the lungs, following an established protocol.²⁷ Briefly, adenovirus was diluted to 45 μ L per mouse with a final titer of 2.5×10^7 PFU in serum-free MEM. The diluted virus was then incubated with 5 μ L of 2.5 M CaCl₂ at room temperature for 20 min to form calcium phosphate precipitates. Prepared viruses were dispensed dropwise to the opening of one nostril using a micropipette until all the volume has been inhaled. Lungs were collected 30 weeks post initiation to detect early tumor lesions and collected at moribund status (15% or above weight loss or showing signs of difficult breathing) for survival study.

NOD scid gamma (NSG) mice were obtained from The Jackson Laboratories. 10-to-12-week-old NSG mice were used for orthotopic or intracardiac implantation.

The University of California Irvine Institutional Animal Care and Use Committee approved all animal procedures (Protocol # AUP-23-058). Male and female mice were utilized and analyzed in all *in vivo* studies, with no sex differences identified.

METHOD DETAILS

Transfection, lentivirus production and transduction

HEK293T were transfected using the calcium phosphate method. Lipofectamine 3000 (Invitrogen) was utilized for transfection in NCI-H446.

Human *UHRF1* gRNA sequences (Table S1) were cloned into doxycycline-inducible CRISPR/Cas9 plasmid TLCV2 (#87360, Addgene). pLenti-PGK V5-LUC Neo (#21471, Addgene) was used for bioluminescence imaging. All lentiviruses were produced by calcium phosphate co-transfection with lentiviral envelop plasmid pCMV-VSV-G (#8454, Addgene), packaging plasmid pCMV-dr8.2-dvpr (#8455, Addgene) and lentiviral transfer plasmid into HEK293T cells. Supernatant containing virus were collected 24 and 48 hr post-transfection and filtered through 0.45 μ m PES filter (Nalgene) for ultracentrifugation at 23,000 RPM for 1.5 hours at 4°C. Lentiviral pellets were resuspended in cold PBS and stored in aliquots at -80°C. Following titering, lentiviral particles were added to cells in serum-free condition for 6 hours at MOI 0.5 or lower. 72 hours post transduction, antibiotic selection was performed.

CRISPR KO single cloning

Following antibiotic selection on the transduced pools, KO cells were single cell cloned by seeding at a density of 0.5 cells per well in a 96 well plate. Single clones were screened for target protein expression using Western blotting. Clones with the lowest protein expression levels were further screened for CRISPR editing efficiency using Tracking of Indels by Decomposition (TIDE) or Inference of CRISPR Edits (ICE).

Growth curve and EdU immunocytochemistry

Cells were seeded 3000-8000 cells/well in 96-well plates. Alamarblue™ Cell Viability Reagent (Invitrogen) was incubated with cells for 3 hours and the plates were then read by BioTek Cytation5 cell imaging reader every 24 hours.

Human SCLC cells were attached to poly-L-lysine (Sigma-Aldrich) pretreated coverslips and were allowed to recover overnight. Click-iT™ EdU Alexa Fluor™ 488 Imaging Kit was used for EdU staining. Slides were counterstained with Hoechst 33342 or DAPI (Roche) and mounted with Gelvatol with DABCO. At least four randomly picked areas per sample were imaged with BZ-X700 Keyence microscope and EdU positive cells were counted in Image J.

Soft-agar colony formation assay

A bottom layer of 0.75% agarose in full serum RPMI media was added to 6-well plate and a top layer of 3000 cells single cell suspension (per well) mixed in 0.36% agarose with full RPMI was then added. Colonies were given three to five weeks to grow. Colonies embedded in agarose were fixed and stained with 0.1% crystal violet in 10% ethanol at room temperature for 15 min, followed by multiple rounds of Milli-Q water destaining. The plates were imaged with a BZ-X700 Keyence microscope and the colonies were counted manually under a dissection microscope.

Scratch-wound healing assay

NCI-H446 cells were seeded at 50% confluency and allowed to grow to 70-80% confluency in a monolayer for scratch-wound. 2 hours prior scratch, cells were treated with 5 µg/mL mitomycin C (Sigma) to block cell division. A scratch was made by a 100 µL pipette tip. Old media containing mitomycin C and floating cells were replaced with fresh media. The wounds were imaged immediately (0 h) and 24 hours post scratch at the same location. The distance between the boundaries of the wound were measured using ImageJ or QuPath.

Transwell invasion assay

Inserts with 8 µm pore size (Thermo Fisher) were coated with 25 µg of Matrigel (Corning) per insert and solidified in the incubator for at least 1 hour. Mitomycin C pretreated NCI-H446 cells were trypsinized, neutralized, washed and resuspended into serum-free RPMI. 120,000 cells in 100 µL serum-free RPMI per insert were added on top of the solidified Matrigel. RPMI with 10% FBS was added to the outside of the insert. 24 hours post initiation of chemotaxis, cells invaded to the other side of the inserts were fixed with 70% ethanol and stained with 0.2% crystal violet. The inserts were destained with Milli-Q water and imaged using BZ-X700 Keyence microscope. The invaded cells were counted manually in ImageJ.

Western blotting

Cell pellets or homogenized tumors or tissues were lysed for 30 min on ice for protein extraction using RIPA buffer supplemented with Protease Inhibitor Cocktail (Thermo Fisher). Lysates were cleared after centrifugation at 14,000 RPM, 30 min at 4°C. The concentration of lysates were quantified by Pierce BCA Protein Assay kit (Thermo Scientific) with a bovine serum albumin (Thermo Scientific) standard curve. 10-20 µg total lysates were loaded and resolved on SDS-PAGE gel (Bio-Rad). Proteins were transferred onto methanol-activated PVDF membrane (EMD Millipore) by semi-dry transfer. The membrane was blocked for 1 hour at room temperature in 5% non-fat dry milk in TBS-T, followed by primary antibody incubation (Table S3) in 0.5% milk/TBS-T overnight at 4°C on a shaker. Next day, membrane was washed three time with TBS-T and incubated with 1:1000 to 1:5000 diluted HRP-conjugated secondary antibodies (goat anti-rabbit; horse anti-mouse; Vector Laboratories) for 1 hour with shaking at room temperature. After another three TBS-T washes, the membranes were developed with SuperSignal West Pico or Femto Chemiluminescent Substrate (Thermo Scientific) and imaged with Syngene G:BOX. The signal intensity was quantified using ImageJ. If needed, the membranes were stripped, washed and blocked for another primary antibody incubation.

Real-time RT-qPCR

RNA was isolated from cell pellets or homogenized tumors or tissues using Trizol (Invitrogen)-chloroform method. 1 µg RNA was used with SuperScript II or III Reverse Transcriptase (Invitrogen) for a 20 µL reaction, following manufacturer's protocol. Quantitative PCR amplification was performed using 1 µL cDNA in Power SYBR Green PCR Master Mix (Life Technologies) for a 10 µL reaction. Three replicates per gene per sample were performed. Reaction was carried out using QuantStudio 6 or 7 Real-Time PCR system (Applied Biosystems). Ct values were normalized to endogenous 18S or GPI controls and further analyzed following $\Delta\Delta C_t$ method. Primers used are listed in Table S2.

Mouse SCLC primary cell lines generation

Mouse SCLC primary cell lines UCI-ScTR-1 and UCI-ScTRU-1 were derived from a Tp53/Rb1 DKO and a Tp53/Rb1/Uhrf1 TKO SCLC tumor, respectively. Briefly, SCLC tumors were dissected and minced, followed by collagenase D and DNase I incubation for 15 min at 37°C, 300 RPM. Enzyme-digested tumors were then neutralized in RPMI with 10% FBS, filtered through 70 µm cell strainer, pelleted and cultured in RPMI with 10% FBS, P/S.

Orthotopic SCLC xenografts and intracardiac metastasis model

NOD scid gamma (NSG) mice were obtained from The Jackson Laboratories. 10-to-12-week-old NSG mice were injected orthotopically through intercostals into the left lung lobe with 50 µL luciferase-labeled tumor cells (0.6 million cells per injection for NCI-H526; 1 million for NCI-H446; 2 million for DMS79; 1.2 million for mSCLC primary cells) in 10% Matrigel/PBS, following established surgery procedures.⁴⁶ The wound was treated with P/S and the incision was closed using VetBond Tissue Adhesive (3M). Mice were given buprenorphine (PAR pharmaceutical) i.m. for pain-relieving and were monitored for recovery post operation. Primary tumor growth and spontaneous metastasis were monitored weekly through bioluminescent imaging with D-luciferin substrate (Thermo Scientific) using IVIS Lumina or AML-HT system. Primary tumors were collected and, in some studies, ex-vivo imaging was performed to confirm metastasis.

For intracardiac metastasis models, 1×10^5 luciferase-labeled tumor cells in 100 µL Mg^{2+} and Ca^{2+} -free PBS were injected into left ventricle of the mouse, following established procedures.⁴⁷ A small thoracic skin midline incision with scissors after preparing skin was made for better localization of injection point. When the tip of the needle was correctly inserted into the left ventricle, pulsation of bright-red blood was observed. The cell suspension was very slowly injected. The injection point was pressed with cotton swab for hemostasis and the incision was closed with Vetbond Tissue Adhesive. Mice were monitored for recovery post operation. Metastasis were monitored biweekly through bioluminescent imaging and ex-vivo imaging was performed. The University of California Irvine Institutional Animal Care and Use Committee approved all animal procedures.

RNA sequencing

Total RNA from homogenized tumors was isolated using RNeasy Mini Kit (Qiagen). After quality check with Agilent BioAnalyzer and Qubit, 1 µg RNA per sample was used for poly(A) mRNA isolation, followed by cDNA library construction (New England Biolabs). Bar-code-indexed libraries were purified with AMPure XP beads (Beckman Coulter) and checked for library quality using Agilent BioAnalyzer DNA Chip and quantified using KAPA qPCR. A paired-end 100-bp sequencing run was conducted on Illumina NovaSeq X Plus with 50 million PE reads. The differential expression gene analysis was conducted using limma-voom.

Immunohistochemistry

Fixed tissues were paraffin embedded and sectioned at Experimental Tissue Resource, UC Irvine. Slides were baked for 1 hour at 60°C, followed up deparaffinize and hydrating steps. Slides were sub-boiled in sodium citrate buffer for 10 min and cooled for 30 min for antigen retrieval. Endogenous HRP was quenched by 3% H_2O_2 incubation and the slides were blocked in 5% goat serum/TBS-T for 1 hour at room temperature, followed by 1:100 primary antibody incubation (Table S3) in 3% goat serum/TBS-T overnight in humidified chamber at 4°C. Next day, the slides were washed and then incubated with 1:200 biotinylated secondary antibody for 30 min at room temperature. After three times of wash, the slides were incubated for 30 min with avidin/biotin-based peroxidase VECTASTAIN ABC kit (Vector Laboratories). After wash, 3,3-diaminobenzidine DAB Quanto (Epredia) was added to the slides, followed by a hematoxylin counterstain. Slides were mounted with Gelvatol and scanned using Ventana DP200. All histological analysis was conducted using QuPath.

Alternatively, fluorescence-based IHC was performed. After biotinylated secondary antibody incubation, slides were washed and then incubated with 1:100 SA-HRP (Perkin) for 30 min. The signal was then amplified and developed with Tyramid Signal Amplification - Cyanine 3 system (Akoya Biosciences) per manufacturer's protocol. Slides were counterstained with DAPI, mounted with Gelvatol with DABCO and scanned using BZ-X700 Keyence microscope.

In situ hybridization

mUhrf1 RNA *in situ* hybridization was performed with RNAscope Multiplex Fluorescent Reagent Kit (Advanced Cell Diagnostics) per manufacturer's instructions. Briefly, FFPE slides were baked, deparaffined and underwent H_2O_2 incubation and target retrieval steps as described in immunohistochemistry section. Samples were then treated with protease for 30 min at 40°C. Mouse Uhrf1-specific probes (customized by Bio-Techne) and RNAscope Positive Control Probe Mm-PP1B and Negative Control Probe DapB were used. 1:1000 TSA Plus Cyanine 3 (Akoya Biosciences) were used to develop Mm-Uhrf1 signal and DAPI was used for counterstain. Slides were mounted with Gelvatol with DABCO and imaged with Olympus FV3000 Confocal Laser Scanning Microscope. The signals were quantified in ImageJ.

Immune panel

Mouse SCLC primary cells were orthotopically injected into the left lung lobes of syngeneic mice. Primary lung tumors were collected when mice showed signs of difficulty in breathing or more than 10% weight loss. Tumor were kept in RPMI with 10% FBS on ice before processing. Tumors were minced by scissor and further dissociated using Lung Dissociation Kit, mouse (Miltenyi Biotec)

in gentleMACS machine (Miltenyi Biotec) for 40 min. Digested tumors were passed through 70 μ m cell strainers and 10 μ L cells were mixed with Propidium iodide (PI) and loaded on NovoCyte (Agilent) for live singlet cell counts. Approximately 2×10^6 cells in 100 μ L wash media were added into separate wells in a 96 well plate. Cells were stained with fixable viability dye followed by Fc blocking, surface antibody staining, FOXP3 fixation/permeabilization, and intracellular antibody staining. Antibodies used for panels were listed in [Table S3](#). Samples were run on NovoCyte 3000 Flow Cytometer (Agilent) and analyzed with FlowJo™ v10.10 Software (BD Life Sciences).

Chemokine array

Conditioned media from UCI-ScTR-1 and UCI-ScTRU-1 cells was collected for chemokine profiling using the Proteome Profiler Mouse Chemokine Array Kit (ARY020, R&D Systems), following manufacturer instructions.

Pull down assay

pCMV6-UHRF1-tGFP (RG217766; Origene) and control plasmid pCMV6-AC-tGFP (PS100010; Origene) were transfected into HEK293T cells. 48 hours post transfection, lysates were collected for pull-down using ChromoTek TurboGFP-trap Magnetic Agarose (Proteintech) per manufacturer's protocol. The inputs and the eluted pull-down fractions were analyzed through Western blotting.

5-mC dot blot

Genomic DNA was purified using Wizard SV96 Genomic DNA purification system (Promega) and was sonicated using Bioruptor Pico device (Diagenode) into 200-500 bp fragments. The fragmented DNA was denatured and loaded to the assembled dot blot apparatus. Gentle vacuum pressure was applied to draw solution through the Amersham Hybond-N⁺ membrane (Cytiva). After UV-cross-linking, the membrane was stained with methylene blue, blocked with 5% milk/TBS-T, incubated with 1:1000 5-mC antibody (#28692; CST), and followed the same steps as Western blotting.

Methylation-specific PCR

Purified genomic DNA was bisulfite-converted and extracted using EZ DNA Methylation-Direct Kit (Zymo). Methylation-specific PCR primers were designed by MethPrimer and primer sequences were listed in [Table S2](#). For PCR reaction, 1 μ L bisulfite-converted DNA was used with ZymoTaq Premix (Zymo) with an annealing temperature of 45°C. The PCR products were analyzed by gel electrophoresis and imaged with InGenius 3 Imager (Syngene). The signal intensity was analyzed in ImageJ.

5-aza experiment

SCLC cells were treated with 12 μ M 5-Aza-2'-deoxycytidine (Sigma Aldrich) or same volume of DMSO every day for a total of 5 days. RNA was extracted using the Trizol-chloroform method. RT-qPCR was performed as described above. Primer sequences were listed in [Table S2](#).

QUANTIFICATION AND STATISTICAL ANALYSIS

All statistical analyses were performed using GraphPad Prism (v10.0, GraphPad Software). RNA-seq differential expression analyses were conducted using the limma-voom pipeline in R. Correlation analyses were performed using Pearson or Spearman correlation coefficients as indicated. Data are presented as mean $\pm$ standard deviation (SD) unless otherwise specified. For survival analyses, Kaplan-Meier curves were generated and compared using the log-rank (Mantel-Cox) test. For comparisons between two groups, unpaired two-tailed Student's t tests were used. When variance between groups was unequal, Welch's correction was applied. For comparisons involving more than two groups, one-way ANOVA followed by appropriate post hoc multiple comparisons testing was performed. Correlation significance was determined using two-tailed Pearson or Spearman tests as appropriate to data distribution. The exact value of *n* is indicated in the corresponding figure legends and represents independent biological replicates. For *in vitro* experiments, *n* refers to independent experiments or independently cultured cell samples. For *in vivo* studies, *n* represents the number of individual mice. For flow cytometry and immune profiling experiments, *n* represents individual tumors derived from independent animals. Technical replicates (e.g., qPCR triplicates) were averaged prior to statistical analysis and are not considered independent *n* values. Statistical significance is denoted as: ns (not significant), **p* < 0.05; ***p* < 0.01; ****p* < 0.001; *****p* < 0.0001. Exact statistical tests and sample sizes are reported in the corresponding figure legends and, where applicable, within the [results](#) section and Figure Legends.