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Super-enhancer-driven LncRNA LINC02894 mediated by MORC2 promotes colorectal cancer progression

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Abstract

Super-enhancer associated LncRNAs (SE-lncRNAs) represent one of the most promising target classes in the emerging field of epigenetic and transcriptional therapy, which can integrate multiple signaling pathways onto a key super-enhancer (SE) driven oncogenic lncRNA to up-regulate expression of coding genes in tumor, yet the landscape of SE-driven lncRNA in colorectal cancer (CRC) remains incompletely mapped. Here, a previous uncharacterized *LINC02894*, as a novel SE-driven lncRNA was identified, which showed higher expression in CRC samples and indicated shorter survival of CRC patients. The oncogenic function of *LINC02894* dependent on SE-mechanism in CRC was validated both in vitro and in vivo. Mechanically, we found that the new function of MORC2 can upregulate LINC02894 transcription via recruiting BRD4 to occupy the SE of *LINC02894* in CRC. Moreover, *LINC02894* binds EZH2 to alleviate EZH2-mediated repression of downstream gene *ESRP1*, sustaining CRC malignant progression. Collectively, our study unveils a novel MORC2/SE-*LINC02894*/ESRP1 oncogenic axis as a pivotal epigenetic driver of colorectal carcinogenesis, presenting a potential therapeutic target.

Introduction

Colorectal cancer (CRC) is the third most common cancer globally, driven by a complex accumulation of genetic and epigenetic alterations(1). Despite advances in multimodal therapies, the 5-year survival rate for advanced-stage CRC remains below 15%(2), underscoring the urgent need for novel therapeutic targets. A large amount of evidence indicates that non-coding RNAs, particularly long non-coding RNAs (lncRNAs) are not merely bystanders but active drivers and suppressors of tumorigenesis(3). LncRNAs are dysregulated and emerge as central players in the molecular circuitry of colorectal cancer, contributing to all "hallmarks of cancer" such as proliferation, metastasis, drug resistance, and immune evasion(4–6), which offer significant potential as biomarkers(3) and therapeutic targets due to their tissue-and cancer-specific expression(7, 8). Super-enhancer-associated lncRNAs (SE-lncRNAs) that are long non-coding RNAs transcribed from the genomic loci of super-enhancers represent one of the most promising target classes in the emerging field of epigenetic and transcriptional therapy(9). Super-Enhancers (SEs) with large and dense clusters of enhancer elements (10, 11), occupied by high densities of master transcription factors, mediator complex, and histone marks like H3K27ac and BRD4(12). Studies indicated that SEs were as the sources of oncogenic lncRNAs. Therefore, targeting a SE-lncRNA offers a strategic path to indirectly inhibit "undruggable" oncogenes making them central to the next generation of cancer therapeutics(13), yet the landscape of SE-driven lncRNAs in CRC remains incompletely mapped.

Epithelial Splicing Regulatory Protein 1 (ESRP1) is a master molecular switch, sitting at the critical junction between epithelial integrity and mesenchymal plasticity, and its dysregulation is increasingly implicated in CRC pathogenesis, metastasis, and therapy response(14)， (15, 16).Unlike a straightforward tumor suppressor, recent studies suggested that ESRP1 exhibited a context-dependent, double-edged sword role in CRC(17). In the primary early-stage epithelial-like CRC, ESRP1 may paradoxically promote

tumorigenic traits such as cell proliferation and survival(18). Some lncRNAs or eRNA might regulate *ESRP1* expression forming integrated regulatory networks in CRC, but the mechanism remains unclear.

Microrchidia CW-type zinc finger 2 (MORC2), as a chromatin remodeler, has been established as a pivotal oncoprotein across diverse cancers(19), where it orchestrates a complex array of epigenetic mechanisms to drive tumorigenesis(20). Although the role of MORC2 in regulating protein-coding genes and histone modifications are increasingly recognized(21), however, whether MORC2 is involved in the regulation of long non-coding RNAs remains unexplored. Therefore, studying the role of MORC2 in the regulation of long non-coding RNAs is an important knowledge gap for understanding the new functions of MORC2.

Here, we characterize a novel SE-driven lncRNA with the oncogenic function, *LINC02894*, which is shown the promoting tumor capacity. Critically, we identify MORC2 as a master regulator that orchestrates *LINC02894* transcription by recruiting the canonical SE reader protein BRD4 to its cognate SE. Furthermore, we found that *LINC02894* functions as a molecular decoy to sequester EZH2, thereby alleviating its repressive effect on the promoter of ESRP1 and ultimately driving CRC cell proliferation. Collectively, our findings define a novel MORC2/SE-LINC02894/ESRP1 oncogenic axis essential for CRC proliferation, which reveals a new target vulnerability of therapeutic intervention.

Materials and methods

Cell culture and lentiviral productions

Human CRC cell lines SW480, SW620, and LoVo were acquired from the American Type Culture Collection (ATCC).Human normal colon epithelial cells NCM460 were obtained from Shanghai Fuheng Biotechnology of China (Cat. No. FH0541). All cell lines were cultured in Dulbecco's Modified Eagle Medium (DMEM; Gibco, USA) complete medium supplemented with 10% fetal bovine serum (FBS,Sangon Biotech, China) and 1% penicillin/streptomycin (Gibco, USA) in a constant-temperature incubator at 37°C with 5% CO_2. Recombinant lentivirus plasmids were infected into cells in the presence of polybrene. Selection with puromycin (2 μg/mL) was initiated 48 hours post-transduction to establish stable cell lines.

Plasmid construction and cell transfection

The pCMV-R8.74 and pMD2.G plasmids were commonly used for lentiviral packaging. pHAGE-EF1α-puro was used to generate overexpression LINC02894(LV-LINC02894) plasmid. The Lenti-CRISPR-V2 plasmid was employed to construct lentiviral vectors for stable MORC2 knockout (KO-MORC2), while the pLKO.1-puro plasmid was used to generate stable knockdown *LINC02894* expression(shRNA-LINC02894) constructs. These plasmids were all generously provided by Professor Fei Teng’s laboratory at Northeastern University. The BRD4 plasmid was purchased from Sino Biological Inc. (China). The pCMV6-Vector and pCMV6-MORC2 plasmids were obtained from OriGene Technologies. Plasmids were

transfected into cells using jetPRIME® transfection reagent (Polyplus-transfection®, France) according to the manufacturer's protocol. Cells were harvested at 48 hours and 72 hours post-transfection. All siRNA sequences in this study are listed in Supplementary Table 1. Quantitative real-time PCR (qPCR) was performed following the manufacturer's instructions, gene-specific PCR primers are listed in Supplementary Table 2–3. Detailed antibody information is provided in Supplementary Table 4. Some method introductions can be found in the Supplementary data.

Dual-Luciferase Reporter Assay

Firefly luciferase reporter plasmids were constructed by Synbio technologies (Suzhou, China). These plasmids include Super-enhancer elements E4/E5 (wild-type or BRD4-binding site mutants) cloned into the pGL3-Promoter vector and *LINC02894* promoter cloned into the pGL3-Basic vector. Cells were co-transfected with experimental firefly luciferase constructs and the pRL-TK Renilla luciferase control vector (Promega) using jetPRIME transfection reagent. After 48 h, lysates were analyzed using the Dual-Luciferase Reporter Assay System (Promega, Cat# E1910) according to the manufacturer's protocol. Luminescence was quantified on a GloMax Navigator microplate reader (Promega). Firefly luciferase activity was normalized to Renilla activity for each sample.

Xenograft Tumor Model

All animal procedures were approved by the Institutional Animal Care and Use Committee of China Medical University (Approval CMU20242028). Five-week-old female BALB/c nude mice (Beijing Hua fukang Bioscience) were maintained under SPF conditions. LoVo cells stably overexpressing *LINC02894* (LV-*LINC02894*) or empty vector control (LV-NC) were harvested, resuspended in PBS at 1×10^7 cells/mL, and mixed 1:1 with Matrigel (Corning). Mice (n = 5) received bilateral subcutaneous injections: 200 µL LV-NC mixture in the left flank and 200 µL LV-*LINC02894* mixture in the right flank. Tumor dimensions were measured every 3 days using calipers (volume = $\frac{1}{2} \times L \times W^2$), with simultaneous monitoring of body weight and clinical signs. After 4 weeks, mice were euthanized by cervical dislocation; tumors were excised, weighed, photographed, and flash-frozen in liquid nitrogen for downstream analysis.

Clinical Tissue Sample Collection

Paired colorectal cancer (CRC) and adjacent non-tumor tissue samples were obtained from patients undergoing curative resection at the General Hospital of Northern Theater Command, China. All specimens were histologically evaluated by certified pathologists at the same institution. Written informed consent was obtained from all participants prior to tissue donation.

Datasets and Bioinformatics Analysis

LINC02894 expression patterns, clinical correlations, and gene-gene relationships were analyzed using the GEPIA web platform(22) (http://gepia.cancer-pku.cn). The CPAT(23) (http://rna-cpat.sourceforge.net/) and CPC(24) (https://cpc.gao-lab.org/) websites are used to predict coding potential and benchmarked against validated protein-coding transcripts and lncRNAs. The iLoc-LncRNA(25) (http://lin-group.cn/server/iLoc-LncRNA/predictor.php) and the lncLocator2.0(26) (http://www.csbio.sjtu.edu.cn/bioinf/lncLocator2/) websites are used to predict subcellular localization of lncRNAs. Publicly available ChIP-seq datasets (GSE106921, GSE126188, GSE73319, GSE136888) for H3K27ac and BRD4 in SW480/SW620/LoVo cells and H3K4me1 in colon adenocarcinoma (COAD) tissues were visualized with Integrative Genomics Viewer (IGV v2.12.3). SE elements were queried via SEdb 2.0(27) (https://bio.liclab.net/sedb/). The MORC2 protein interaction network was retrieved from BioGRID(28) (https://thebiogrid.org/). GeneCards(29) and JASPAR(30) were used to predicted the transcription factors (TFs) enriched at the ESRP1 promoter region. *LINC02894*-binding proteins were predicted using catRAPID omics v2(31) (http://service.tartaglialab.com), while EZH2 binding sites within the *ESRP1* promoter were identified via Human TFDB(32) (http://bioinfo.life.hust.edu.cn/HumanTFDB/). Kaplan-Meier survival analysis of ESRP1 in CRC was used the Kaplan-Meier Plotter database(33) (Dataset: GSE17538).

Results

1. LINC02894 is a novel nucleus-localized oncogenic lncRNA in CRC

To characterize the novel lncRNA, through GEPIA online analysis, we found that the *LINC02894* transcript (*RP11 − 22C11.2*) was highly expressed in various tumor tissues (Fig. 1A), and the CRC clinical patients with high *LINC02894* expression exhibited significantly shorter disease-free survival (DFS) and overall survival (OS) compared to the low-expression group (Fig. 1B). Therefore, we selected fresh 10 paired clinical CRC specimens to further confirm, the elevated expressions of *LINC02894* were observed in 10 CRC tissues compared to matched adjacent non-tumor tissues (Fig. 1C). Likewise, *LINC02894* showed high expression in CRC cell lines compared to normal colon epithelial cell NCM460 (Fig. 1D). These findings suggested that *LINC02894* expression is critically involved in CRC pathogenesis and progression. In order to further study its role and mechanism in CRC, we confirmed by comparative analysis that *LINC02894* exhibits weak protein-coding capacity, which is consistent with the established lncRNAs (Fig. 1E). Furthermore, *LINC02894* was found to be predominantly localized in the nucleus (Fig. 1F-1G). Collectively, these findings demonstrate that *LINC02894* is oncogenic lncRNA with nuclear localization in CRC.

2. LINC02894 is a SE-driven lncRNA hijacked by BRD4

Numerous studies in recent years have established that SE-driven lncRNAs play critical roles in tumorigenesis(34). To investigate whether *LINC02894* represents such a SE-driven lncRNA, we utilized the gene-based search function of the SEdb 2.0 database and identified an active SE proximal to the *LINC02894* locus across multiple human tissues, with high activity in colon tissue (sFigure 1A). To map functional SEs in CRC, we interrogated publicly available H3K27ac and H3K4me1 of ChIP-seq datasets

from the GEO database. Visualization using the IGV revealed pronounced H3K27ac and H3K4me1 signals within the 8q22.1 chromosomal region, specifically spanning approximately 0–12 kb upstream of the *LINC02894* transcription start site, in both CRC cell lines and primary tissues (Fig. 2A). Intriguingly, we also observed specific enrichment of BRD4 upstream of the *LINC02894* locus (Fig. 2A). BRD4 (bromodomain-containing protein 4), recognized as a canonical "reader" of SEs, is a key protein that binds and activates SEs to promote tumorigenesis(35), providing independent validation of this SE's presence. This genomic interval was confirmed as the LINC02894-proximal SE (SE ID: SE_01_009900695) within the SEdb 2.0 annotation. Subsequently, we constructed this *LINC02894*-proximal SE by partitioning it into five constituent enhancer modules (E1, E2, E3, E4 and E5) (Fig. 2A) and experimentally validated by ChIP-qPCR assays. The results showed that significant enrichment of H3K27ac (Fig. 2B) and H3K4me1(Fig. 2C) modifications was observed at regions E3, E4, and E5 in CRC cell lines (SW480 and LOVO), but there was not enriched in normal epithelial cell (NCM460) (Fig. 2D). These results suggested that *LINC02894* is extraordinary enriched with super-enhance elements in CRC cell rather than normal intestinal epithelial cell.

In addition, to determine whether *LINC02894* is indeed a SE-driven transcript. Dual-luciferase reporter assays were performed and showed that the enhancer containing E4 and E5 elements (exhibiting the highest H3K27ac enrichment) had significant activity to the promoter in CRC cells (sFigure 1B). To establish the functional dependence of SE activity on BRD4 binding, we generated luciferase reporter constructs harboring mutations within the predicted BRD4-binding sites of the E4 and E5 components (sFigure 1C), and the luciferase activity was significantly reduced upon mutation (Fig. 2E-2F and sFigure 1D). These results indicated that the functional activity of *LINC02894*-proximal E4 and E5 SE components in CRC cells depended on its binding to BRD4, which is required for maintaining SE-driven transcriptional activation.

Next, JQ1, a potent BET bromodomain inhibitor, specifically targets BRD4 by competitively inhibiting its acetyl-lysine recognition capability, which disrupts BRD4 binding to SEs, leading to defective transcriptional elongation and suppression of SE-driven gene expression(12). We found that CRC cells treated with JQ1 led to a significant reduction in expression of *LINC02894* (Fig. 2G and sFigure1E). GEPIA analysis showed a significant positive correlation between *BRD4* and *LINC02894* expression in CRC tissues (Fig. 2H). Furthermore, our experiment further validated that BRD4 overexpression significantly increased the transcriptional level of *LINC02894* in CRC cells (Fig. 2I-2J and sFigure 1F). Collectively, these findings demonstrated that BRD4 could functionally regulate *LINC02894* expression, suggesting that *LINC02894* is a SE-driven lncRNA.

3. **LINC02894 promotes CRC proliferation via SE-dependent mechanism**

To investigate the role of SE-driven *LINC02894* expression in CRC, we constructed the stable overexpressed *LINC02894* in SW480 and LoVo cells (sFigure 2A) and found that *LINC02894* overexpression significantly enhanced the proliferative capacity of CRC cells, which were partially reversed with JQ1 treatment by the results of the CCK8 (Fig. 3A), EdU (Fig. 3B and sFigure 2B) and

colony formation assays (Fig. 3C and sFigure 2C). Conversely, the stable *LINC02894*-knockdown (SW480 and LoVo) CRC cells (sFigure 2D) significantly suppressed the proliferative capacity of CRC cells compared to control group cells, which notably potentiated this proliferation-suppressive capacity with the SE inhibitor JQ1(Fig. 3D-3F and sFigure 2E-2F). Thus, these results demonstrate that *LINC02894* promotes CRC cell proliferation, and this proliferative capacity is dependent on SE activity.

Subsequently, a xenograft mouse model was performed (Fig. 3G) showed that was consistent with *in vitro* results. LV-*LINC02894* + Control significantly enhanced tumor growth compared to LV-NC+Control. Notably, JQ1 treatment attenuated tumor growth in both LV-*LINC02894* + JQ1 and LV-NC+JQ1 groups (Fig. 3H-3J), Immunohistochemical staining for the proliferation marker Ki67 revealed the overexpression of *LINC02894* enhanced proliferative capacity. Importantly, JQ1 treatment reduced Ki67 expression in both LV-*LINC02894* + JQ1 and LV-NC+JQ1 groups (Fig. 3K). Meanwhile, *LINC02894* expression was confirmed and inhibited by JQ1 in excised tumors (Fig. 3L). These findings conclusively demonstrate that SE-driven *LINC02894* promotes CRC tumor growth *in vivo*, while inhibition of SE activity by JQ1 counteracts this oncogenic effect.

4. SE-driven LINC02894 is regulated by MORC2 via recruiting BRD4

Our previous lncRNA sequencing data in hepatocellular carcinoma cells revealed significant regulation of *LINC02894* by MORC2 (sFigure 3A). To validate whether this regulatory relationship exists in CRC, we first analyzed TCGA data by the GEPIA platform and demonstrated a significant high *MORC2* overexpression and positive correlation with *LINC02894* expression in CRC tissues (Fig. 4A). Studies had indicated that MORC2 was high expression and significantly correlated with poor prognosis in colon cancer patients (36, 37). Subsequently, we confirmed that overexpression of MORC2 (LV-MORC2) significantly increased the level of *LINC02894* (Fig. 4B and sFigure3B). Concurrently, knockout (KO)-MORC2 markedly suppressed *LINC02894* expression (Fig. 4C). Given that *LINC02894* is SE-driven, we hypothesized that MORC2 modulates *LINC02894* expression through the SE region. ChIP-qPCR results confirmed that MORC2 could bind to the E4 and E5 components of the SE (Fig. 4D). Furthermore, MORC2 overexpression significantly enhanced the activity of both E4 and E5 enhancer elements in CRC cells (Fig. 4E and sFigure 3C). These findings collectively demonstrated that MORC2 regulated LINC02894 expression through working on the SE region.

Reportedly, MORC2 typically interacts with transcription factors or recruits other transcriptional regulators to influence gene expression(19). BRD4, a key protein for binding and activating SEs(38), as shown in Fig. 2, significantly impacts the expression of SE-driven *LINC02894*. Therefore, we hypothesized that MORC2 might influence SE activity and *LINC02894* expression by recruiting BRD4. Interestingly, we found that BRD4 is indeed present within the MORC2 interaction network (sFigure 3D). Co-IP experiment confirmed that MORC2 interacts with BRD4 (Fig. 4F), and the two proteins exhibit co-localization (Fig. 4G). Dual-luciferase reporter gene assays revealed that both MORC2 and BRD4 enhance the luciferase activity of E4 and E5, and their co-expression further augmented this luciferase activity (Fig. 4H). Subsequently, we investigated the effect of MORC2 on BRD4 enrichment at SE regions. ChIP-qPCR

results demonstrated that MORC2 increases BRD4 enrichment at SE regions E4 and E5 (Fig. 4I). Conversely, MORC2 knockdown significantly reduced BRD4 enrichment at these SE regions (E4 and E5) (Fig. 4J). And, we found that MORC2 overexpression elevated *LINC02894* expression levels, whereas the addition of JQ1 partially suppressed this increase observed upon MORC2 overexpression (Fig. 4K). Furthermore, individual overexpression of either MORC2 or BRD4 increased *LINC02894* expression levels, and co-transfection of both further enhanced *LINC02894* expression (Fig. 4L). Collectively, these data demonstrated that MORC2 recruited BRD4 to the SE regions of *LINC02894*, thereby influencing SE activity and the expression of the SE-driven *LINC02894*.

5. LINC02894 upregulates ESRP1 expression to promote CRC cell proliferation.

Nuclear-localized lncRNAs often play crucial roles in regulating gene expression(39). Therefore, we investigated how *LINC02894* participates in regulating the expression of its downstream gene. LncRNA sequencing results suggested that *LINC02894* may regulate the expression of *ESRP1*. We subsequently found a positive correlation between *LINC02894* and *ESRP1* expression in CRC tissues within the TCGA database (Fig. 5A). Next, we experimentally validated these bioinformatic findings. Results demonstrated that overexpression of *LINC02894* increased both the mRNA (Fig. 5B and sFigure 4A) and protein (Fig. 5C and sFigure 4B) levels of ESRP1, while knockdown of LINC02894 decreased ESRP1 mRNA (Fig. 5D and sFigure 4C) and protein (Fig. 5E and sFigure 4D) expression. These data indicate that *LINC02894* participates in upregulating ESRP1 expression.

ESRP1 plays a pivotal role in maintaining epithelial cell identity and exhibits a pronounced dual nature in cancer, which primarily depends on cancer type, disease stage, cellular microenvironment, and regulated target gene networks(18). While its oncogenic role has been documented in CRC, this association remains contentious(40, 41). Therefore, to empirically validate the functional contribution of ESRP1 in CRC pathogenesis, we evaluated the impact of *ESRP1* knockdown in SW480 and LoVo cells (Fig. 5F and sFigure 4E) and demonstrated that *ESRP1* knockdown significantly inhibited cell proliferation (Fig. 5G), reduced colony formation capacity (Fig. 5H), and decreased DNA synthesis assessed by EdU assay (Fig. 5I and sFigure 4F). To further evaluate the clinical significance of ESRP1 in CRC, we analyzed TCGA and GTEx datasets and revealed that significant upregulation of *ESRP1* in COAD tissues compared to normal controls (sFigure 5G). Subsequent validation in 10 paired clinical specimens demonstrated consistently elevated ESRP1 expression in CRC tissues relative to matched adjacent non-tumor tissues (Fig. 5J). Kaplan-Meier survival analysis using the Kaplan-Meier Plotter database further indicated that high *ESRP1* expression correlates with poorer overall survival (OS) (Fig. 5K) and recurrence-free survival (RFS) (Fig. 5L) in CRC patients, suggesting that ESRP1 exhibits oncogenic role in CRC. Collectively, these results demonstrate that *LINC02894* upregulated *ESRP1* expression, which promoted CRC cell proliferation.

6. LINC02894 alleviates EZH2-mediated repression of the ESRP1 in CRC

Given that lncRNA-protein interactions are widely considered a primary mechanism for lncRNA function(42), we explored how *LINC02894* regulates ESRP1 expression. First, we predicted transcription factors (TFs) enriched at the *ESRP1* promoter region using GeneCards and JASPAR. Concurrently, the

catRAPID omics v2 database was used to predict proteins potentially interacting with *LINC02894* based on its binding domains and motifs (Fig. 6A). The top 500 predicted interacting proteins were ranked by score and intersected with the predicted TFs using a Venn diagram to identify overlapping candidates for further study (Fig. 6B). Considering the critical role of histone modifications in transcriptional regulation, and the established function of EZH2 (an H3K27me3-specific methyltransferase) and its catalytic product H3K27me3 in mediating transcriptional repression, we hypothesized that *LINC02894* might interact with EZH2 to regulate ESRP1 expression. To test this, we predicted the interaction regions between *LINC02894* and EZH2 using the catRAPID website, which revealed EZH2 binding sites enriched at the 5' end of LINC02894 (Fig. 6C). RNA immunoprecipitation (RIP) assays confirmed that EZH2 interacts with *LINC02894* (Fig. 6D). Then, we predicted EZH2 binding sites within the *ESRP1* promoter region using the Human TFDB website and designed specific primers targeting this promoter (Fig. 6E). Furthermore, ChIP-qPCR demonstrated significant enrichment of EZH2 at the *ESRP1* promoter (Fig. 6F). To assess the functional impact of EZH2 on ESRP1, we found that silencing EZH2 promoted ESRP1 expression (Fig. 6G), indicating that EZH2, acting as a histone methyltransferase, represses *ESRP1* transcription. We next performed ChIP assays to determine the effect of *LINC02894* on EZH2 occupancy at the *ESRP1* promoter. Results showed that overexpression of *LINC02894* significantly reduced the binding of EZH2 to the *ESRP1* promoter (Fig. 6H). Since EZH2 induces transcriptional repression by catalyzing H3K27me3 modification, we also examined H3K27me3 enrichment at the *ESRP1* promoter. ChIP-qPCR results indicated that *LINC02894* overexpression similarly reduced H3K27me3 enrichment in this region (Fig. 6I). Collectively, these findings indicate that *LINC02894* antagonizes the repressive effect of EZH2 on the *ESRP1* promoter. Thus, *LINC02894* functions as a molecular decoy to bind EZH2, which alleviates EZH2-mediated the inhibition of ESRP1, leading to CRC progression.

Discussion

Our study establishes *LINC02894*, a previously uncharacterized lncRNA, as a SE-driven oncogene that promotes CRC proliferation through a defined epigenetic axis. We demonstrate that *LINC02894* is significantly upregulated in CRC tissues and cell lines, correlating with poor patient prognosis, implicating its clinical relevance.

Accumulating evidence reveals strong associations between SEs and lncRNAs. SEs represent large clusters of adjacent enhancers densely enriched with TFs, coactivators, and epigenetic modifiers(43). SE-lncRNAs defined as lncRNAs localized within 50 kb upstream or downstream of SEs, participate in diverse physiological and pathological processes(7). Super-enhancer-associated lncRNAs are the "voice" of the genome's most powerful regulatory elements(13). They translate the potent but spatially constrained activity of a super-enhancer into a *trans*-acting or *cis*-stabilizing regulatory signal(9, 44). In cancer, they are hijacked to lock cells into a malignant state. Two reported SE-lncRNAs in colorectal cancer, *AC005592.2* and *RP11-569A11.1*, exert opposing functions: the former promotes tumor progression by regulating OLFM4(45), while the latter inhibits progression and metastasis through modulating IFIT2(46).

To address this knowledge gap, we characterized a previously unstudied SE-lncRNA, *LINC02894*, which is driven by a tumor-specific SE proximal to its locus that orchestrates its oncogenic expression. Key evidence includes tissue-specific enrichment of H3K27ac/H3K4me1 histone marks at constituent enhancers (E3-E5), BRD4-dependent SE activity validated through binding site mutagenesis and pharmacological inhibition with JQ1, and strong correlation between *BRD4* and *LINC02894* expression in clinical cohorts. Crucially, we found that MORC2 physically interacts with BRD4 and recruits it to E4/E5 enhancer regions, amplifying *LINC02894* transcription. MORC2-dependent BRD4 enrichment at the SE *of LINC02894* synergistically enhance the expression of *LINC02894*. When cell treatment with JQ1, which completely abrogates of MORC2-mediated LINC02894 transcription. Thus, The MORC2-BRD4 partnership represents a novel regulatory paradigm for SE-driven oncogenes.

The subcellular localization of SE-driven lncRNAs critically determines their functional mechanisms(47). Nuclear-localized lncRNAs regulate splicing and cis/trans gene expression, while cytoplasmic SE-lncRNAs can interact with diverse protein partners, thereby influencing RNA stability, degradation, translation and mRNA splicing(48). Given the predominant nuclear localization of *LINC02894*, we focused on elucidating its molecular role in promoting CRC proliferation. LncRNA-seq results suggested *ESRP1* as a downstream target of LINC02894, which was experimentally validated. To investigate the underlying mechanism, we employed catRAPID omics v2 to predict LINC02894-interacting proteins and intersected these candidates with transcription factors regulating the *ESRP1* promoter. We prioritized EZH2, a histone methyltransferase that catalyzes repressive H3K27me3 modifications to silence gene expression(49). Subsequent experimental validation revealed that *LINC02894* binds EZH2, thereby alleviating its repressive effect on the *ESRP1* promoter. For example, SE-lncRNA DAW binds to EZH2 to counteract the inhibitory effect of Wnt2, thereby activating the Wnt/β-catenin signaling pathway(50). Our findings establish that *LINC02894* similarly functions as a molecular decoy to orchestrate *ESRP1*-dependent oncogenesis. ESRP1, a protein with a dual role in cancer whose suppression of epithelial-mesenchymal transition (EMT) is well-documented(51), has established oncogenicity in CRC(40). While we provide preliminary experimental validation here, a more comprehensive investigation of ESRP1 represents a limitation of this study. We demonstrated that SE-driven LINC02894 affects CRC proliferation, its impact on other tumor characteristics, such as invasion and metastasis, requires extensive future validation. In our experiment, we primarily utilized the top three LINC02894-high-expressing CRC cell lines (Lovo, SW480 and SW620). However, due to the dense growth and low single-cell survival rate of SW620 cells, assessing long-term proliferation via colony formation was challenging.

Conclusion

Our work first characterizes LINC02894 as a SE-driven oncogene promoting CRC cell proliferation. Mechanistically, LINC02894 is activated by MORC2 via recruiting BRD4 to bind its super-enhancer. Concurrently, *LINC02894* interacts with EZH2, as a decoy molecular to counteract EZH2-mediated repression of ESRP1 to promote the proliferative capacity of CRC cells. Therefore, our findings provide a novel theoretical foundation for targeting a SE-lncRNA like *LINC02894* offers a strategic path to indirectly inhibit "undruggable" oncogenes like *MORC2*, making them central to the next generation of cancer

therapeutics. While challenges remain, the SE-lncRNA emerging field is poised to significantly impact the future of cancer management, moving towards more personalized and effective strategies.

Abbreviations

ATCC: American Type Culture Collection; BRD4: Bromodomain-containing protein 4; CCK-8: Cell Counting Kit-8; ChIP: Chromatin Immunoprecipitation; COAD: Colon adenocarcinoma; Co-IP: Co-Immunoprecipitation; CRC: Colorectal cancer; DFS: Disease-free survival; E: Enhancer; EdU: 5-ethynyl-2'-deoxyuridine; EMT: Epithelial-mesenchymal transition; ESRP1: Epithelial splicing regulatory protein 1; EZH2: Enhancer of zeste homolog 2; H3K27ac: Histone 3 lysine 27 acetylation; H3K27me3: Histone H3 lysine 27 trimethylation; H3K4me1: Histone H3 lysine 4 mono-methylation; Kb: Kilobase; KO: Knock out; LncRNA: Long non-coding RNA; LV: Lentiviral vectors; MORC2: Microrchidia CW-type zinc finger protein 2; NC: Negative control; OD: Optical density; OE: Over-Expression; OS: Overall survival; RIP: RNA Immunoprecipitation; SE: Super enhancer; SE-lncRNAs: Super-enhancer-associated lncRNAs; Sh: Short hairpin; SiRNA: Short interfering RNA; TCGA: The Cancer Genome Atlas

Declarations

Ethics approval and consent to participate

The human CRC samples and adjacent normal tissues utilized in this study were approved by the Ethics Committee of General Hospital of Northern Theater Command of China (approval no.Y[2024]017).Informed consent was obtained from all participants and was conducted in compliance with the Helsinki Declaration. All animal procedures were approved by the Institutional Animal Care and Use Committee (IACUC) of China Medical University(approval no.CMU-2024-2028). We conducted animal experiments to evaluate the effect of *LINC02894* on tumor growth in nude mice with or without JQ1 treatment. Mice were injected with CRC cells and monitored for tumor development. Tumor volumes were calculated and analyzed statistically. Tumor size was measured in two perpendicular dimensions (D1 and D2), tumor volume was calculated using the formula $V = 4/3\pi[1/4(D1 + D2)]2$. Animal experiments were carried out in strict compliance with the Basel Declaration.

The Conflict of Interest Statement

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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CRediT authorship contribution statement

Xiuyan Gong: Writing–original draft, Visualization, Data curation, Conceptualization. Hao Xu: Writing – review & editing, Validation, Resources, Methodology. Chenshuang Dong: Resources, Methodology, Data curation. Jia Liu:Resources, Data curation. Zhan Wang: Data curation, Formal analysis. Miao He: Resources. **Guiling Wang** : Conceptualization, Writing – review & editing, Investigation, Funding acquisition, Supervision, Project administration, Funding acquisition.

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We have used Biorender.com to prepare the figure illustrations.

Data Availability

All data supporting the findings of this study are available within the paper and its Supplementary Information.

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Figures

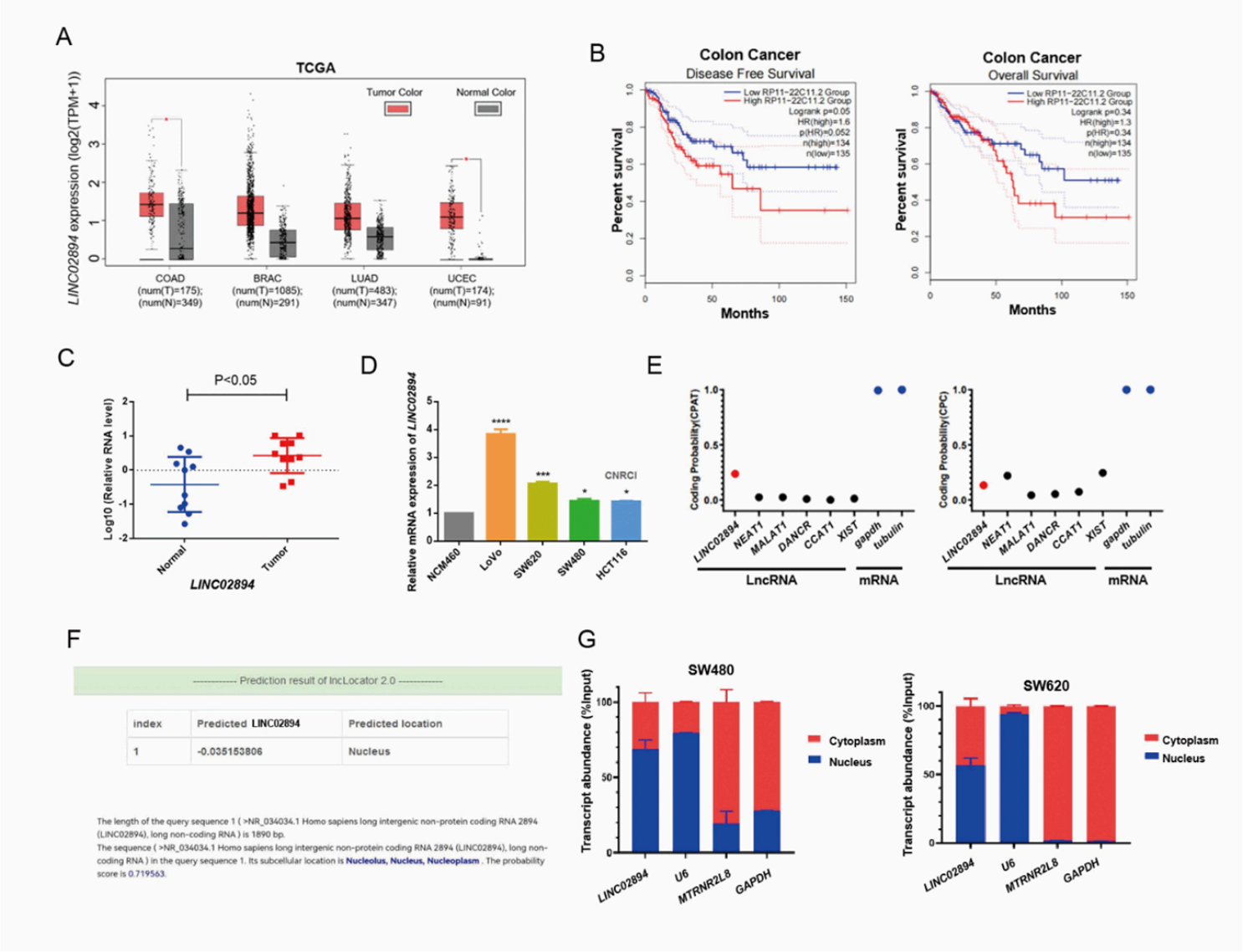

Figure 1

LINC02894 is a novel oncogenic lncRNA in CRC

(A) The expression of *LINC02894* in multiple human tumor types according to TCGA data. **(B)** The LINC02894 transcript (*RP11−22C11.2*) expression in 269 CRC clinical patients (TCGA-COAD cohort) exhibited significantly shorter disease-free survival (DFS) and overall survival (OS) compared to the low-expression group by Kaplan-Meier survival analysis, *P*-value for DFS and OS is indicated (Log-rank test). **(C)** RT-qPCR detection of *LINC02894* expression in CRC (n = 10) paired tissues. **(D)** RT-qPCR detection of *LINC02894* expression in multiple CRC cells and in normal colon epithelial cell. **(E)** Assessment of *LINC02894* coding potential using CPAT and CPC tools, compared to known protein-coding transcripts (Pos) and lncRNAs (Neg). **(F)** In silico prediction of *LINC02894* subcellular localization using iLoc-LncRNA and lncLocator2.0. **(G)** RNA nuclear-cytoplasmic separation（PARIS™ Kit, Life Tech.,USA） experiment validated subcellular localization of *LINC02894*. GAPDH and U6 serve as cytoplasmic and nuclear controls, respectively. The data are shown as the mean ± S.D. of at least three replicates, **P* < 0.05, ***P* < 0.01, ****P* < 0.001, ns: not significant.

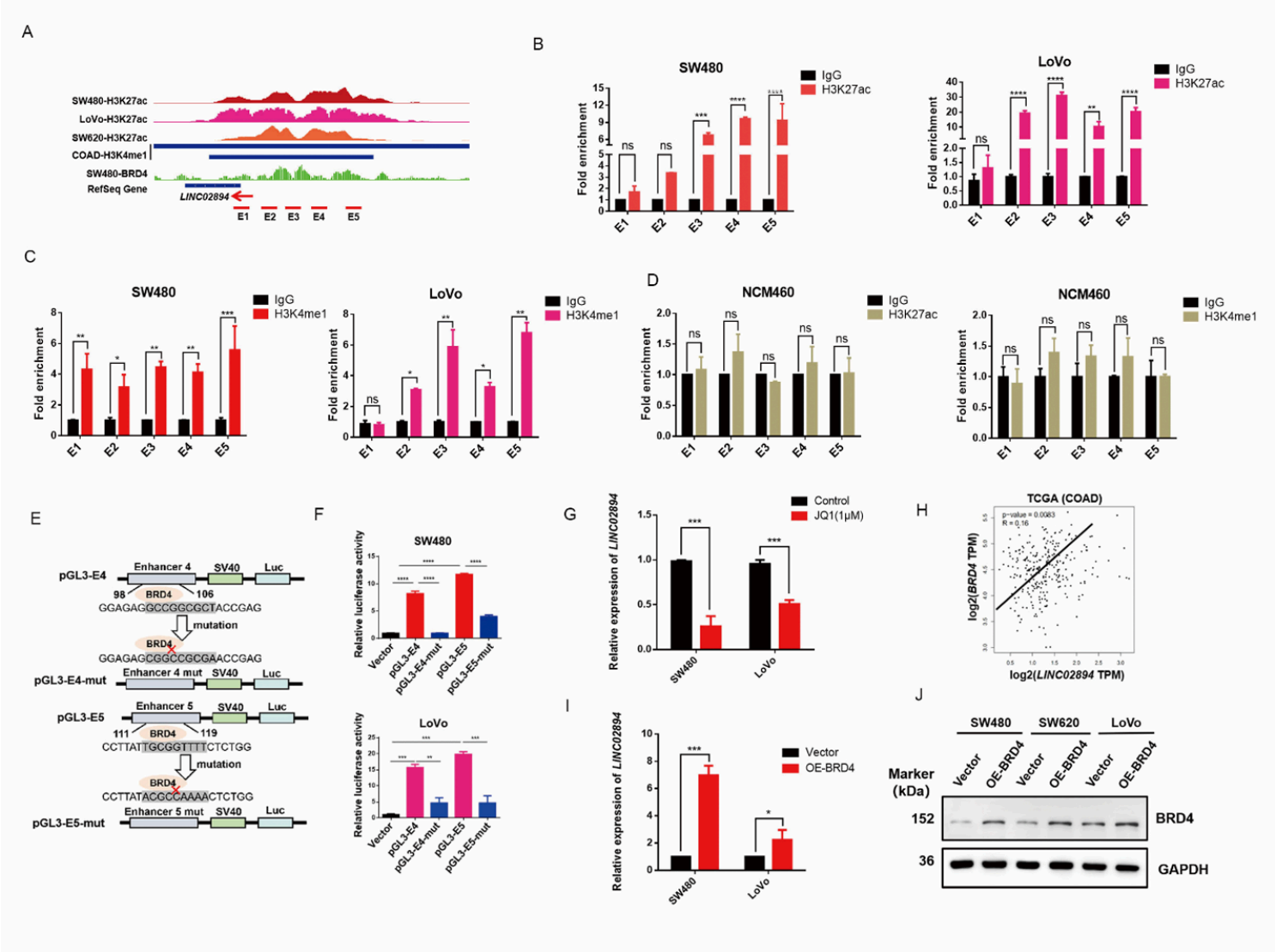

Figure 2

***LINC02894* is a SE-driven lncRNA dependent on BRD4 in CRC**

(A) Schematic of the *LINC02894*genomic locus (8q22.1). IGV visualization of H3K27ac, H3K4me1 and BRD4 (GEO dataset) ChIP-seq enrichment profiles within the *LINC02894* SE region in CRC cell lines/tissues. **(B-D)** ChIP-qPCR validation of H3K27ac **(B)** and H3K4me1 **(C)** enrichment at the *LINC02894* SE modules (E1-E5) in SW480 and Lovo CRC cell lines, and NCM460 normal colon epithelial cell (**D**). **(E)** Schematic of wild-type (WT) and BRD4-binding site mutant (Mut) E4 and E5 reporter constructs. **(F)**Dual-luciferase assays showing significantly reduced activity of E4-Mut and E5-Mut reporters compared to WT controls in SW480 and Lovo CRC cells. **(G)**RT-qPCR analysis showed that *LINC02894* expression was suppressed when CRC cells (SW480 and Lovo) treated with the BRD4 inhibitor JQ1 (1 μM, 24h). **(H)** Correlation analysis between *BRD4* and *LINC02894* expression in colon cancer patients from the TCGA database. (I-J) BRD4 overexpression increased *LINC02894* level in SW480 and Lovo cells by RT-qPCR assays. The data are shown as the mean ± S.D. of at least three replicates, **P* < 0.05, ***P* < 0.01, ****P* < 0.001, ns: not significant.

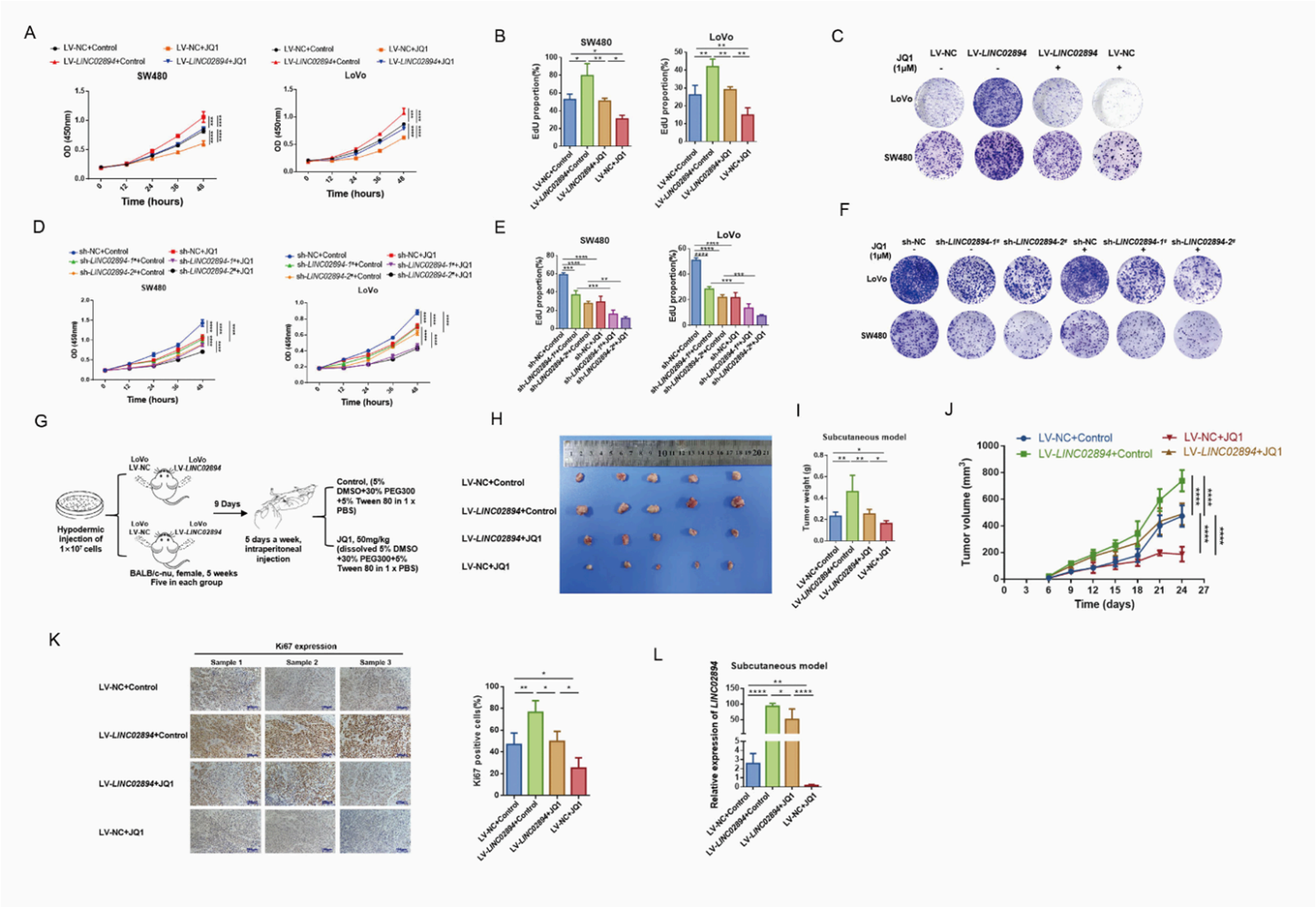

Figure 3

LINC02894 promotes CRC proliferation in vitro and in vivo

(A-C) CCK-8 **(A)** and EdU **(B)** and Colony formation **(C)**assays were performed to evaluate the effects of LINC02894 overexpression (LV-LINC02894) on CRC cell proliferation with or without JQ1 treatment. **(D-F)** CCK-8 (D) and EdU **(E)** and Colony formation **(F)** assays were performed to evaluate the effects of Knockdown LINC02894 (sh-*LINC02894*) on CRC cell proliferation with or without JQ1 treatment. **(G)** Schematic diagram of the tumor-bearing mouse experimental procedure. (H) Representative tumor images from nude mice bearing xenografts. **(I)** Tumor weights at endpoint. **(J)** Tumor growth curves (volume = 1/2 × length × width²). **(K)** Representative IHC images (left) and quantification (right) of the proliferation marker Ki67 in xenograft tumors. Scale bar: 100 μm. **(L)** RT-qPCR confirming sustained *LINC02894* overexpression in LV-*LINC02894* tumors. The data are shown as the mean ± S.D. of at least three replicates, **P* < 0.05, ***P* < 0.01, ****P* < 0.001, ns: not significant.

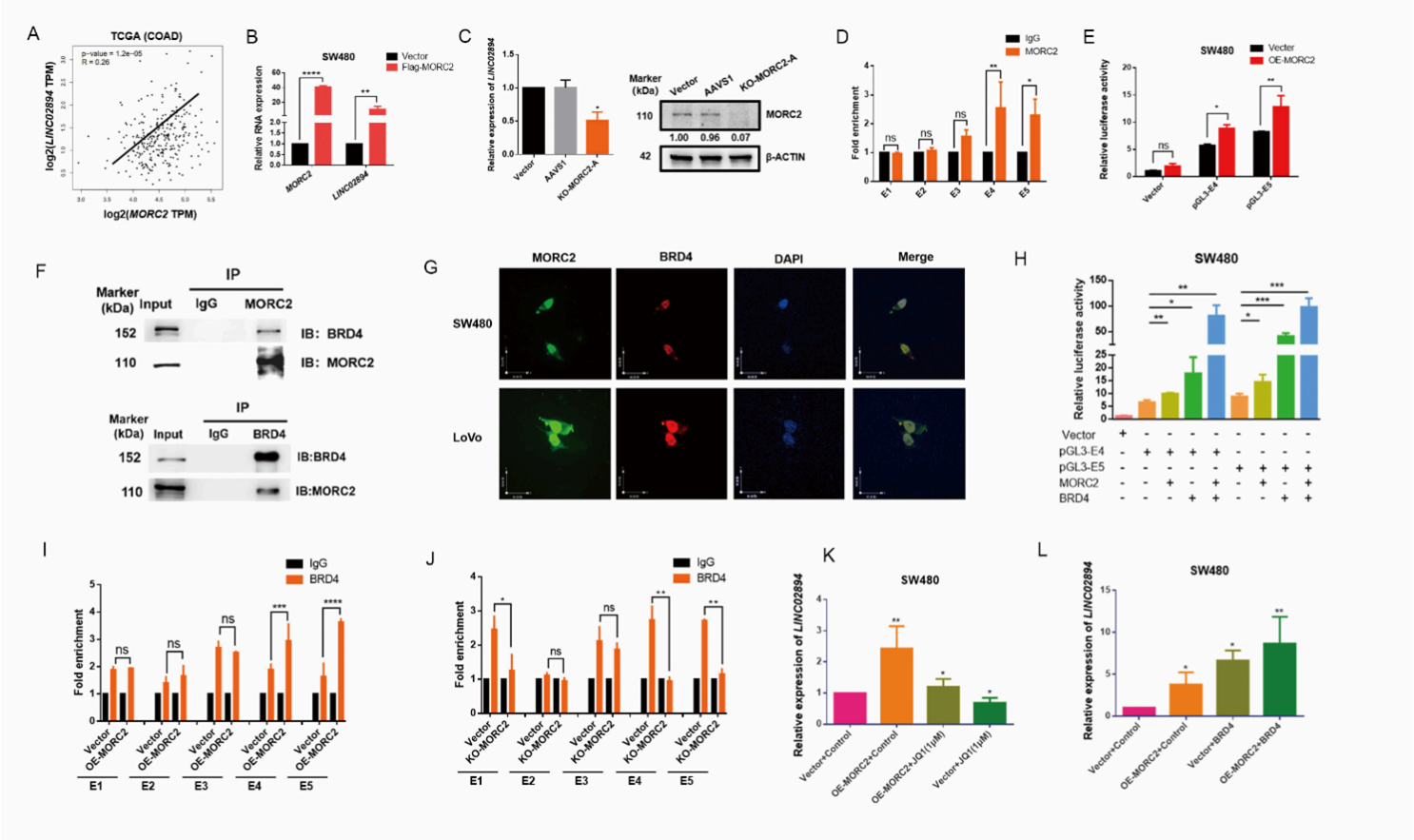

Figure 4

MORC2 recruits BRD4 to the *LINC02894* SE region to drive its expression

(A) GEPIA showing a significant positive correlation between MORC2 and *LINC02894* expression in CRC via analyzing TCGA data. **(B)** RT-qPCR analysis indicated that MORC2 overexpression increased *LINC02894* level in SW480 cells. **(C)** RT-qPCR showed that MORC2-KO significantly inhibited *LINC02894* expression and western blot validation of MORC2 knockout (KO). **(D)** ChIP-qPCR results indicated MORC2 bind to the E4 and E5 components of the *LINC02894* SE in CRC cells. **(E)** Dual-luciferase reporter assays detected the impact of MORC2 overexpression on the activity of E4 and E5 enhancer elements in SW480 cells. **(F)** Co-IP assays confirming the interaction between endogenous MORC2 and BRD4 in CRC cells. **(G)**Immunofluorescence staining demonstrating nuclear co-localization of MORC2 (green) and BRD4 (red) in CRC cells. Nuclei counterstained with DAPI (blue). Scale bar: 10 μm. **(H)** Dual-luciferase reporter assays demonstrating enhanced activity of E4 and E5 elements by individual or combined overexpression of MORC2 and BRD4. **(I, J)** ChIP-qPCR analysis showing BRD4 enrichment at the *LINC02894* SE regions (E4, E5) upon MORC2 overexpression **(I)** and KO **(J)**. **(K)** RT-qPCR analysis of *LINC02894*expression in SW480 cells overexpressing MORC2 with or without BRD4 inhibition (JQ1, 1 μM). **(L)** RT-qPCR showing synergistic upregulation of *LINC02894*by co-overexpression of MORC2 and BRD4 in SW480 cells. The data are shown as the mean ± S.D. of at least three replicates, **P* < 0.05, ***P* < 0.01, ****P* < 0.001, ns: not significant.

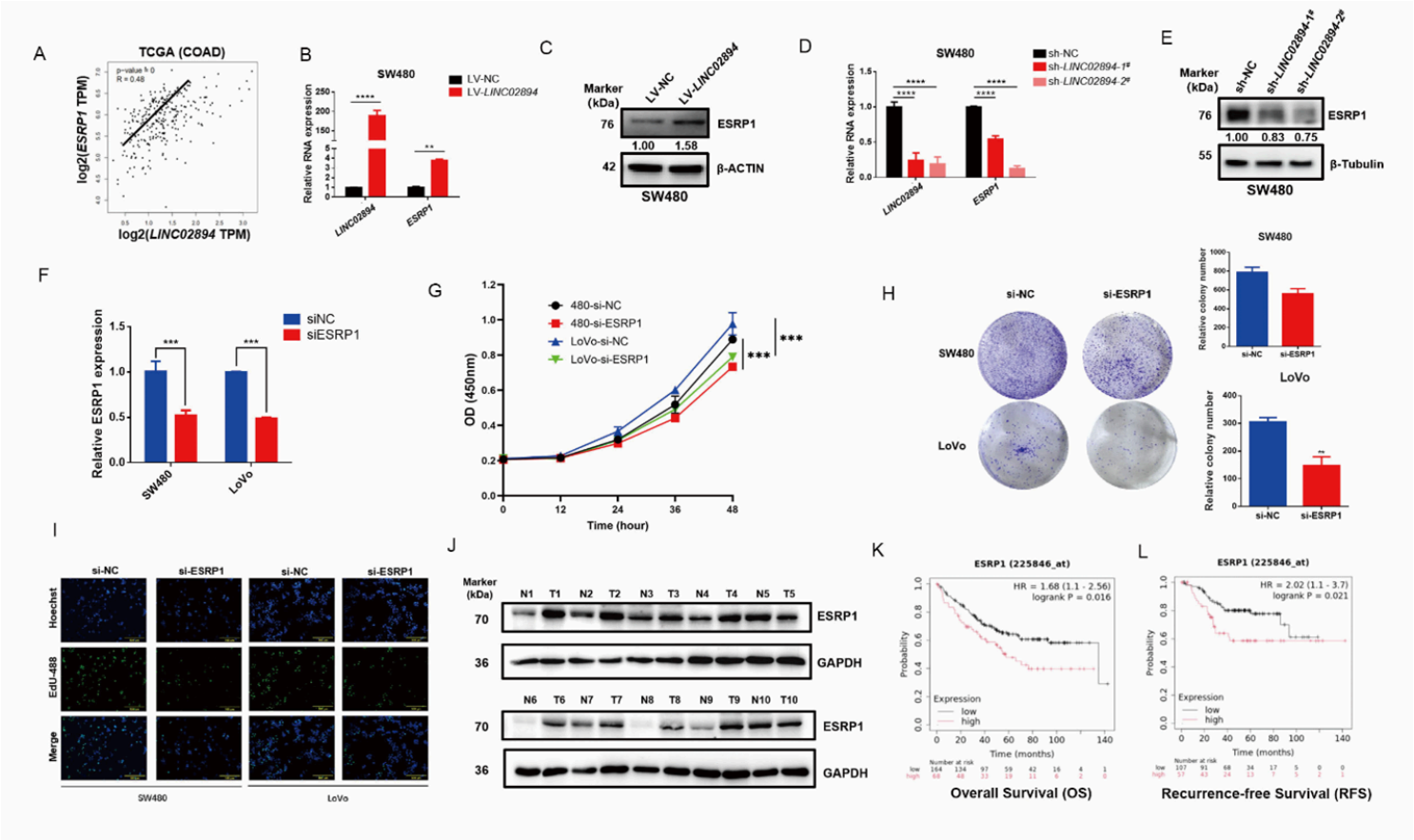

Figure 5

LINC02894 upregulates *ESRP1* expression to promote CRC cell proliferation

(A) GEPIA analysis of TCGA data showing positive correlation between *LINC02894* and *ESRP1* expression in CRC tissues. **(B, C)** RT-qPCR and Western blot analysis the effect of *overexpression* LINC02894 (LV- LINC02894) on ESRP1 expression. **(D, E)** RT-qPCR and Western blot analysis the effect of knockdownLINC02894 (sh-LINC02894) on ESRP1 expression in CRC cells. **(F-I)** siESRP1 **(F)** inhibited cell proliferation in SW480 and Lovo cells by performing CCK-8 proliferation assays **(G)**, Colony formation assays(H) and EdU incorporation assays **(I).** Scale bar: 100 μm. **(J)** Western blot validation of elevated *ESRP*1 expression in 10 paired clinical CRC tumor tissues (T) versus adjacent non-tumor tissues (N). **(K, L)** Kaplan-Meier survival curves from the Kaplan-Meier Plotter database indicating that high *ESRP1*expression correlates with poorer overall survival (OS) **(K)** and recurrence-free survival (RFS) **(L)** in CRC patients (Data source: GSE17538). Log-rank P-values are shown. The data are shown as the mean ± S.D. of at least three replicates, **P* < 0.05, ***P* < 0.01, ****P* < 0.001, ns: not significant.

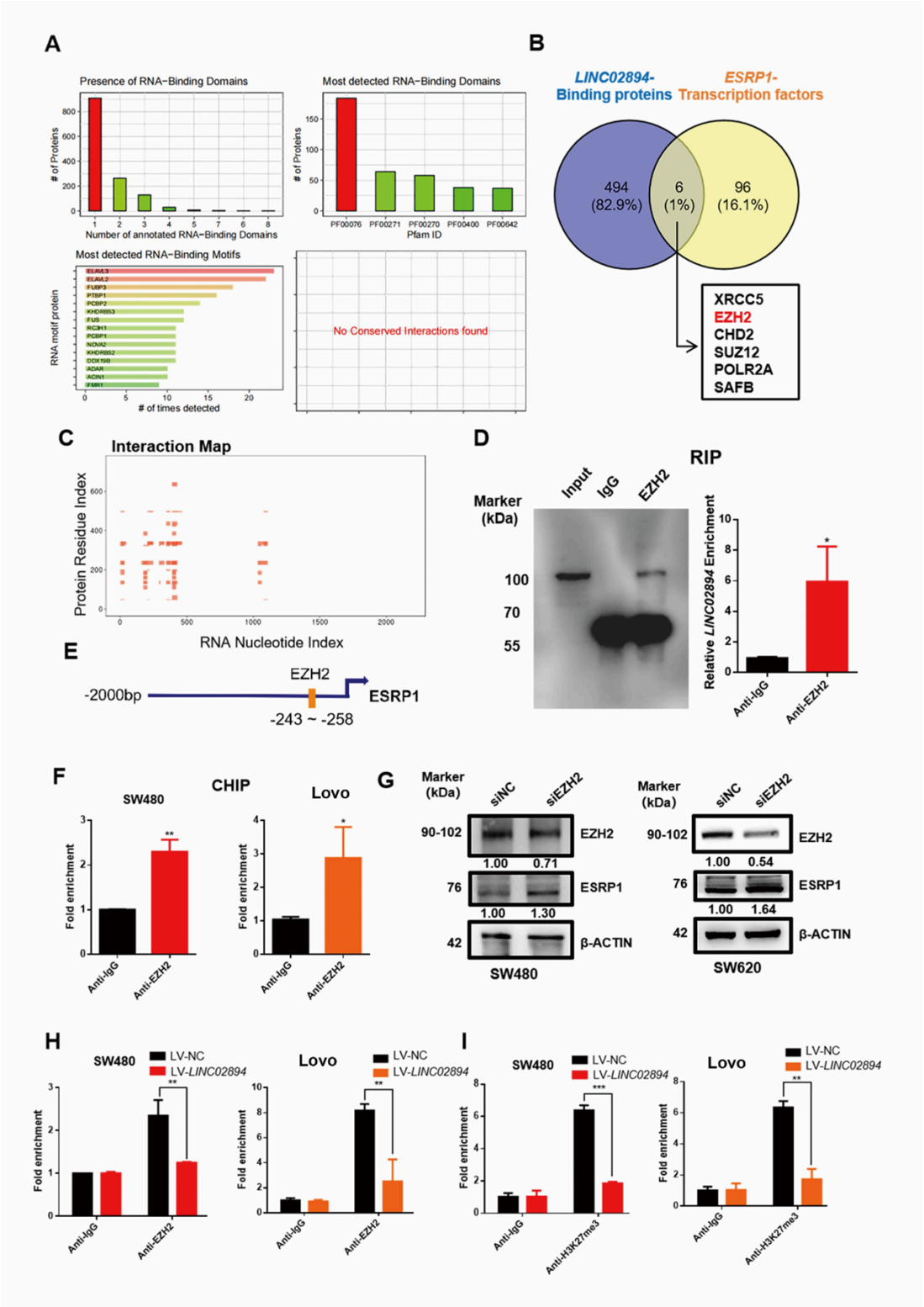

Figure 6

LINC02894 functions to alleviate EZH2-mediated repression of ESRP1

(A) CatRAPID omics v2 is used for predicting *LINC02894*-binding proteins **(B)**Venn diagram intersecting *LINC02894*-binding protein predictions and transcription factors binding the *ESRP1* promoter, identifying EZH2 as a candidate regulator. **(C)** In silico prediction of EZH2 binding sites within the *LINC02894*

transcript (5' end) via catRAPID. **(D)** RNA immunoprecipitation (RIP) assays confirming direct binding between EZH2 and*LINC02894* in CRC cells. Left panel: Antibody binding efficacy validation diagram. **(E)** Schematic of EZH2 binding sites predicted within the*ESRP1* promoter region. **(F)** ChIP-qPCR validation of EZH2 enrichment at the *ESRP1* promoter. **(G)** RT-qPCR showing *ESRP1*upregulation upon *EZH2* knockdown. **(H)** ChIP-qPCR demonstrating reduced EZH2 binding to the *ESRP1* promoter upon *LINC02894*overexpression. **(I)** ChIP-qPCR showing decreased H3K27me3 enrichment at the *ESRP1* promoter in *LINC02894*-overexpressing cells. The data are shown as the mean ± S.D. of at least three replicates, *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, ns: not significant.

Supplementary Files

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