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**Extracellular vesicle-associated COMP from cancer-associated fibroblasts
promotes ferroptosis resistance in hepatocellular carcinoma**

Running title: Fibroblast Vesicles Promote Ferroptosis Resistance

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1 **Abstract**

2 Cancer-associated fibroblasts (CAFs) contribute to hepatocellular carcinoma (HCC)
3 progression through intercellular communication, but whether CAF-derived
4 extracellular vesicles (EVs) modulate ferroptotic susceptibility remains unclear. Here,
5 we investigated the role of CAF-EVs in ferroptosis resistance and HCC progression
6 and explored the EV-associated protein mechanism involved. Using EVs from primary
7 CAFs and matched normal fibroblasts, combined with molecular profiling, genetic and
8 pharmacological perturbations, mouse models, and spatial analysis of human HCC
9 tissues, we examined this stromal–tumor interaction across complementary
10 experimental systems. CAF-EVs reduced RSL3-induced oxidative and lipid-
11 peroxidative stress and decreased ferroptotic susceptibility while preserving aggressive
12 phenotypes in HCC cells, with SLC7A11 contributing to these effects. Proteomic
13 profiling and functional screening identified cartilage oligomeric matrix protein
14 (COMP) as a functionally relevant EV-associated protein that increased SLC7A11
15 expression. COMP increased HIF-1 α protein abundance without altering HIF1A mRNA
16 levels and was associated with RANBP2. COMP-enriched EVs enhanced HIF-1 α
17 SUMOylation, whereas RANBP2 knockdown or pharmacological inhibition of
18 SUMOylation attenuated COMP-induced HIF-1 α accumulation and SLC7A11
19 upregulation. Reciprocal COMP gain- and loss-of-function experiments, together with
20 RSL3 or ferrostatin-1 treatment, linked EV-associated COMP to tumor growth and
21 experimental lung colonization in vivo. In human HCC tissues, intratumoral α -
22 SMA⁺COMP⁺ CAFs and SLC7A11⁺PanCK⁺ tumor cells were independently

associated with poor clinical outcomes, and higher local densities of SLC7A11-positive tumor cells around α -SMA⁺COMP⁺ CAFs were associated with shorter overall survival. These findings support a model in which CAF-derived EV-associated COMP contributes to ferroptosis resistance through RANBP2/SUMO-dependent HIF-1 α accumulation and downstream SLC7A11 expression, linking stromal EV communication to tumor-cell redox adaptation and HCC progression.

Keywords:

Hepatocellular carcinoma (HCC); Cancer-associated fibroblasts (CAF); COMP; Extracellular vesicles (EV); Ferroptosis

1. Introduction

Hepatocellular carcinoma (HCC) is a leading cause of cancer-related death worldwide, and recurrence and metastasis remain major barriers to durable disease control (1, 2). Tumor progression requires malignant cells to adapt to fluctuating nutrient availability, oxidative stress, and other adverse conditions within the tumor microenvironment (TME). Understanding how HCC cells acquire such stress tolerance may therefore reveal mechanisms that support tumor growth and dissemination.

Ferroptosis is a form of regulated cell death driven by iron-dependent lipid peroxidation (3, 4). Susceptibility to ferroptosis depends on the balance between lipid oxidative damage and cellular antioxidant defenses. SLC7A11, the light chain subunit of system

xc⁻, supports cystine uptake and is a major determinant of ferroptosis resistance in cancer cells (4, 5). Ferroptosis vulnerability can also influence the survival of tumor cells during metastatic dissemination, although its biological consequences vary across tumor types and microenvironmental contexts (6, 7). HCC cells are exposed to substantial metabolic and oxidative stress, yet how signals from the surrounding stroma modify their susceptibility to ferroptosis remains poorly understood.

Cancer-associated fibroblasts (CAFs) constitute a major stromal population in HCC and influence tumor cell behavior through extracellular matrix remodeling, paracrine signaling, and metabolic exchange (8-10). CAFs can also communicate with cancer cells through extracellular vesicles (EVs), which carry proteins, lipids, and nucleic acids between cells (11). CAF-derived EVs have been implicated in tumor progression and therapy resistance in several cancer types (12). In particular, specific RNA cargo carried by CAF-derived EVs has been reported to suppress ferroptosis in gastric, lung, and colorectal cancers (13-15). Existing studies of CAF-EV-mediated ferroptosis resistance have therefore largely focused on RNA cargo. Whether CAF-EV-derived proteins modulate ferroptotic susceptibility in HCC remains unclear.

Here, we examined whether CAF-derived EVs alter ferroptosis susceptibility and aggressive phenotypes of HCC cells and sought to identify a functionally relevant EV-associated protein and its downstream signaling mechanism. Mouse models and spatial analyses of human HCC tissues were then used to assess their biological and clinical

relevance.

2. Materials and methods

2.1 Cell Culture and Experimental Treatments

Huh-7 cells were purchased from the American Type Culture Collection (ATCC, Manassas, VA, USA), whereas MHCC-97H hepatocellular carcinoma cells and HEK293T human embryonic kidney cells were obtained from the Cell Bank of the Chinese Academy of Sciences (Shanghai, China). All three cell lines were maintained in DMEM (GIBCO) containing 10% fetal bovine serum (FBS). Primary CAFs and NFs were cultured under the same conditions using DMEM supplemented with 10% FBS (GIBCO). Cells were grown at 37 °C in a humidified atmosphere containing 5% CO₂. Before conditioned medium (CM) treatment, MHCC-97H and Huh-7 cells were first maintained in fresh DMEM containing 10% FBS for 24 h. The medium was then replaced with a 1:1 mixture of fresh culture medium and either CAF-CM or NF-CM, and the cells were incubated for an additional 24 h. For the corresponding control, cells received an equal-volume mixture of fresh complete medium and serum-free DMEM. For extracellular vesicle (EV) treatment, CAF- or NF-derived EVs were applied to HCC cells at 20 µg/mL, based on total EV protein, for 24 h.

Ferroptosis was induced with RSL3 at 1 µM for a total of 24 h. In experiments evaluating the effects of CM or EVs on RSL3-induced ferroptosis, HCC cells were first exposed to RSL3 alone for 6 h. CM or EVs were subsequently introduced without

replacing the RSL3-containing medium, and incubation was continued for another 18 h. Thus, the overall duration of RSL3 exposure was 24 h. Cellular responses were assessed using colony formation, CCK-8 cell viability, wound-healing assay and Transwell assays, together with western blot analysis.

2.2 Establishment and Validation of Primary CAFs and Matched NFs

Primary CAFs and matched NFs were established from HCC specimens and the corresponding non-tumorous liver tissues, respectively, collected from three patients undergoing surgical resection. Immediately after collection, the tissues were thoroughly rinsed with D-Hanks' solution. Necrotic areas were removed, and the remaining tissue was cut into fragments of approximately 2 mm. The fragments were then transferred to culture vessels containing DMEM (GIBCO) supplemented with 10% fetal bovine serum (FBS) and allowed to adhere. Fibroblast-like cells began to grow out from the tissue explants after approximately 14 days. Following 2–3 passages, cultures with approximately 95% fibroblast purity were obtained. Cell identity was subsequently confirmed by immunofluorescence staining for α -smooth muscle actin (α -SMA) and fibroblast activation protein (FAP). Both CAFs and NFs tested negative for mycoplasma contamination using the Bimake mycoplasma detection kit.

2.3 Pharmacological Treatments

The pharmacological agents used in this study included the ferroptosis inducer RSL3 (1 μ M; HY-100218A, MedChemExpress), the HIF-1 α inhibitor LW6 (5 μ M; HY-13671,

MedChemExpress), the SUMOylation inhibitor TAK-981 (1 μ M; HY-111789, MedChemExpress), and the ferroptosis inhibitor ferrostatin-1 (1 μ M; HY-100579, MedChemExpress). Stock solutions were prepared in dimethyl sulfoxide (DMSO) or sterile water according to the manufacturer's instructions and were freshly diluted to the working concentration before treatment. Unless otherwise specified, cells were exposed to the indicated compounds for 24 h.

2.4 Conditioned Medium Preparation

After the cells had reached 80% confluence, they were washed three times with PBS and cultured in fresh medium containing 10% FBS for 24 h. Then, the supernatant was harvested and filtered through a 0.22- μ m membrane filter to remove cell debris. The resulting supernatant (termed conditioned medium, CM) was stored at -80 °C and used for experiments within 2 weeks.

2.5 EV Extraction, Characterization, and Protein Quantification

CAFs and NFs were cultured in DMEM containing 10% EV-depleted FBS. To remove bovine EVs, FBS was subjected to ultracentrifugation at $100\,000 \times g$ for 12 h at 4 °C (16). After 48 h of culture, conditioned media from CAFs (CAF-CM) and NFs (NF-CM) were harvested for EV isolation by sequential centrifugation. The conditioned media were centrifuged sequentially at $800 \times g$ for 10 min and $3000 \times g$ for 30 min at 4 °C to eliminate cells and cellular debris. The resulting supernatants were further centrifuged at $10\,000 \times g$ for 70 min, followed by ultracentrifugation at $100\,000 \times g$ for

70 min. The EV-containing pellets were washed twice with PBS and subjected to an additional centrifugation step at $100\,000 \times g$ for 70 min at 4 °C. The final pellets were resuspended in PBS and kept at -80°C until further use.

EV identity and physical characteristics were assessed as previously described (17). EV-associated markers were detected by western blotting, while vesicle morphology and particle-size distribution were examined by transmission electron microscopy (TEM) and nanoparticle tracking analysis (NTA), respectively. For EV protein quantification, the PBS-resuspended EV preparations were analyzed for total protein content using the Pierce BCA Protein Assay Kit (Thermo Fisher Scientific, Cat. #23225). Briefly, 1–5 μL of each EV preparation was combined with 200 μL of BCA working reagent and incubated at 37 °C for 30 min. Absorbance was subsequently measured at 562 nm for protein quantification.

2.6 EV Labeling and Tracing

EV uptake by HCC cells was examined using PKH67 fluorescence labeling as previously described (17). Purified EVs were mixed with PKH67 Green Fluorescent Cell Linker dye (Sigma-Aldrich, St. Louis, USA; Cat. #MINI67) for 5 min, and the labeling reaction was stopped by addition of serum. The labeled vesicles were washed twice with PBS, recovered by ultracentrifugation, and resuspended in PBS. EV protein concentration was measured by BCA assay and normalized to 1 mg/mL with PBS.

PKH67-labeled EVs were then diluted in culture medium containing 10% EV-free FBS to a final protein concentration of 50 µg/mL. The EV-containing medium was passed through a 0.22-µm syringe filter before being applied to MHCC-97H or Huh-7 cells cultured in 24-well plates. Cells were exposed to the labeled EVs for 24 h at 37 °C in a humidified atmosphere containing 5% CO₂. After incubation, cells were rinsed twice with PBS and fixed with 4% formaldehyde. Nuclei were counterstained with 4',6-diamidino-2-phenylindole (DAPI; Servicebio, Cat. #G1012), and F-actin was visualized using phalloidin (Beyotime, Cat. #12352207). Fluorescence images were acquired with a Zeiss LSM710 confocal microscope (Zeiss, Pleasanton, CA, USA).

2.7 Western Blotting

Total cellular proteins were extracted from HCC cells using RIPA lysis buffer supplemented with protease and phosphatase inhibitor cocktails. After centrifugation at 12 000 rpm for 10 min at 4 °C, the soluble protein fraction was recovered, and its concentration was measured by BCA assay. Equivalent amounts of protein were subjected to SDS-PAGE and subsequently transferred onto PVDF membranes (Merck Millipore, USA). The membranes were blocked in 5% nonfat dry milk for 1 h at room temperature and then probed with the indicated primary antibodies at 4 °C overnight. After removal of unbound antibodies by TBST washing, horseradish peroxidase-conjugated secondary antibodies were applied. The membranes were washed again with TBST before chemiluminescent signals were developed using SuperKine™ West Femto Maximum Sensitivity Substrate (Abbkine). Protein bands were recorded with a

Tanon imaging system (Shanghai, China). Information on all primary and secondary antibodies is provided in Table S1.

2.8 RNA Extraction and Quantitative Real-time PCR

Total RNA was extracted with TRIzol reagent (Invitrogen, Carlsbad, CA, USA). cDNA synthesis was performed using HiScript® III Reverse Transcriptase (Vazyme, Jiangsu, China; R302-01) according to the supplier's protocol. Quantitative PCR amplification was subsequently conducted with ChamQ Universal SYBR qPCR Master Mix (Vazyme; Q711-02). Relative transcript abundance was calculated by the $2^{-\Delta\Delta C_t}$ method. Relative transcript levels were normalized to *ACTB*. The primer sequences used for qRT-PCR are listed in Table S2.

2.9 Immunofluorescence Staining

Cells were fixed with 4% paraformaldehyde for 10 min at room temperature. After fixation, the cells were washed three times with PBS and permeabilized with 0.1% Triton X-100 for 10 min. The cells were subsequently washed three times with PBS and blocked with 5% bovine serum albumin for 1 h. Primary antibodies were added for incubation overnight at 4 °C. After the samples were washed three times with PBS, they were incubated with secondary antibodies for 1 h at room temperature in the dark. The cells were subsequently washed three times with PBS and then stained with DAPI for 5 min at room temperature in the dark. After being washed five times with PBS, the cells were mounted and observed under a fluorescence microscope.

199

200 2.10 Wound Healing Assay

201 Cells were seeded in 12-well plates and allowed to form a confluent monolayer. The
202 cultures were then switched to medium containing 1% FBS, and a straight scratch was
203 introduced across the monolayer with a sterile pipette tip. Detached cells and debris
204 were gently removed by washing with PBS, and the cultures were subsequently
205 maintained under the indicated conditions. Cell migration into the scratched area was
206 documented at 0 and 24 h. The residual wound width was quantified using image
207 analysis software, and the change in wound healing over time was used to assess cell
208 migratory capacity.

209

210 2.11 Transwell Migration and Invasion Assays

211 Cell migration and invasion were evaluated using Transwell chambers (Millipore,
212 Billerica, MA, USA). For migration assays, a total of 5×10^4 cells were suspended in
213 serum-free medium and loaded into the upper compartment, while the lower chamber
214 was filled with 700 μ L of DMEM supplemented with 15% FBS as the chemoattractant.
215 For invasion assays, the inserts were coated with Matrigel diluted at 1:7 (BD
216 Biosciences, San Jose, CA, USA) before cell seeding. After 28 h (migration) or 32 h
217 (invasion), cells that had migrated or invaded through the membrane were fixed with
218 4% paraformaldehyde for 20 min and subsequently stained with crystal violet (catalog
219 #C0121; Beyotime, China). The membranes were then rinsed with PBS and allowed to
220 dry. Cells were counted in three randomly selected microscopic fields for quantification.

All experiments were independently performed in triplicate.

2.12 Colony Formation Assay

Cells were plated in 6-well plates at 1 000 cells per well and allowed to form colonies for approximately 10 days. At the end of the culture period, the colonies were fixed with 4% paraformaldehyde for 20 min and then stained with crystal violet (C0121; Beyotime, China). After staining, visible colonies were counted to evaluate clonogenic capacity.

2.13 CCK-8 Cell Viability Assay

The response of HCC cells to RSL3 was evaluated using a CCK-8 assay (catalog #40203ES92; Yeasen, Shanghai, China). Cells were seeded into 96-well plates at a density of 1 000 cells per well and allowed to adhere. The cultures were then treated with RSL3 at final concentrations of 0.01, 0.1, 1, 10, or 100 μ M. After 24 h of exposure at 37 °C, CCK-8 reagent was added to each well and the plates were incubated for 2 h. Absorbance at 450 nm was subsequently recorded using a Thermo Fisher microplate reader (Shanghai, China) to determine cell viability.

2.14 C11-BODIPY Assay

Cells were seeded in confocal imaging dishes and cultured until reaching 50–70% confluence. The cells were first exposed to 1 μ M RSL3 for 6 h, after which EVs were added directly to the RSL3-containing medium without medium replacement.

Incubation was continued for an additional 18 h, resulting in a total RSL3 exposure time of 24 h. After treatment, the medium was replaced with fresh medium containing 5 μ M C11-BODIPY 581/591 (Thermo Fisher, D3861), and the cells were stained for 30 min at 37 °C in the dark. After staining, the cells were gently washed and immediately imaged under light-protected conditions using a Zeiss super-resolution confocal microscope. C11-BODIPY fluorescence was used to evaluate cellular lipid peroxidation.

2.15 ROS Assay

A reactive oxygen species assay kit (Beyotime) was used to measure the intracellular ROS production following the manufacturer's guidelines. Cells were inoculated into six-well plates and incubated with a solution containing the fluorescent probe DCFH-DA for 30 min. After staining, cellular fluorescence was then measured using a flow cytometer (Beckman). Fluorescence signals were detected using the FITC channel (Ex/Em = 488/525 nm), and ROS levels were quantified based on the mean fluorescence intensity (MFI) of DCF fluorescence.

2.16 MDA Assay

MDA concentrations were measured using an MDA assay kit (Solarbio). Cells were seeded in 10-cm dishes at 1×10^5 cells per dish. After the addition of the MDA assay working solution at 100 °C, the cells were lysed and centrifuged, and the absorbance was measured at 532 nm.

265

266 2.17 Ferrous Ion Assay

267 Changes in the ferrous ion concentration were assessed using a ferrous ion content assay
268 kit (Solarbio, BC5415) according to the manufacturer's instructions.

269

270 2.18 DIA-based Quantitative Proteomic Analysis

271 EVs isolated from three patient-matched pairs of CAFs and NFs were subjected to DIA-
272 based quantitative proteomic analysis at SpecAlly Life Technology Co., Ltd. EV
273 proteins were extracted, quantified using a BCA assay, reduced with TCEP, alkylated
274 with CAA and digested overnight with trypsin. The resulting peptides were desalted,
275 separated using a Vanquish Neo UHPLC system and analyzed on an Orbitrap Astral
276 mass spectrometer in data-independent acquisition mode. Raw data were processed
277 using DIA-NN (version 1.9.2) in library-free mode against the UniProt human protein
278 database. Protein identification was controlled at a false discovery rate of 1%, and
279 protein abundance was normalized using the MaxLFQ algorithm. Differentially
280 abundant proteins were identified according to fold change and Student's t test
281 thresholds. The differentially abundant proteins between NF-EVs and CAF-EVs
282 identified by the proteomics analysis are listed in Table S3.

283

284 2.19 mRNA Sequencing and Analysis

285 To examine transcriptional changes induced by EV-associated COMP, Huh-7 cells were
286 cultured in medium supplemented with 1% FBS and treated for 24 h with 20 µg /mL of

EVs derived from COMP-overexpressing NFs (oeCOMP-EVs) or vector-control NFs (oeCtrl-EVs). Reduced-serum conditions were used to minimize background signaling from serum-derived factors. Total RNA was extracted using TRIzol reagent (9108; Takara), and the samples were subjected to mRNA purification, library preparation and paired-end sequencing at APExBIO Technology (Shanghai, China). Filtered reads were aligned to the human reference genome (GRCh38) using HISAT2, and gene expression was quantified using StringTie. Differentially expressed genes were identified using the DESeq2 package. Functional enrichment analysis was performed using Metascape (18). The gene expression matrix is presented in The NCBI SRA database at <https://www.ncbi.nlm.nih.gov>, reference number PRJNA1514655.

2.20 Analysis of Bulk RNA Sequencing Datasets

To investigate the relationship between gene expression levels and clinicopathological characteristics, publicly available expression profiles from the Gene Expression Omnibus (GEO) datasets GSE14520 (19) and GSE54236 (20) were retrieved for subsequent analysis. The TCGA-LIHC dataset was also obtained from the UCSC Xena database. The EPIC algorithm was utilized to measure CAF abundance using the R package IOBR (21).

2.21 Functional Enrichment Analysis

GSEA was conducted using clusterProfiler, and the results were visualized using the GseaVis package. The gene sets used for GSEA were obtained from the Molecular

Signatures Database (MSigDB) (22). For GSVA, RNA sequencing data from all TCGA-LIHC tumor samples were obtained from UCSC Xena. FPKM values were converted to TPM values and subjected to $\log_2(\text{TPM} + 1)$ transformation. GSVA scores were calculated using the GSVA R package (v2.6.2) for three HIF1A-related gene sets: GROSS_HYPOXIA_VIA_HIF1A_DN, PID_HIF1_TFPATHWAY and KEGG_MEDICUS_REFERENCE_HIF_1_SIGNALING_PATHWAY. Spearman correlations of these scores and the expression of 12 candidate genes were calculated. P values from all 36 comparisons were adjusted using the Benjamini–Hochberg method. None of the candidate genes were present in these gene sets; therefore, leave-one-gene-out recalculation was unnecessary.

2.22 Immunoprecipitation–Mass Spectrometry (IP–MS) and Co-IP

For immunoprecipitation, cells were lysed using NP40 lysis buffer supplemented with protease and phosphatase inhibitors. The cell lysates were then incubated with the indicated primary antibody overnight at 4 °C. The following day, protein A/G magnetic beads (HY-K0202, MCE) were washed three times with 0.5% PBST (PBS with 0.5% Tween-20) and then added to the antibody–lysate mixture. After a 4-hour incubation at 4 °C with gentle rotation, the beads were washed six times to remove nonspecifically bound proteins. For the IP–MS assay, lysates of HCC cells stably overexpressing COMP were incubated with an anti-COMP antibody (Abcam, ab260037), and the harvested beads were subjected to mass spectrometry analysis at Beijing Qinglian Biotech Co., Ltd. For the Co-IP assays, the harvested beads were eluted with loading

buffer and denatured at 100 °C for 10 min for western blot analysis. The primary and secondary antibodies used are listed in Table S1.

2.23 Lentivirus Production, Transduction and Plasmid Construction

For knockdown assays, short hairpin RNAs (shRNAs) were individually cloned and inserted into the pLKO.1-puro vector. *COMP*, *CD44*, *KYNU*, *PAIP1* and *RANBP2* plasmids were purchased from the MiaoLing Biology Sharing Platform (Wuhan, China). The *AK1* coding sequence was subjected to PCR amplification from cDNA and cloned and inserted into the pHAGE-CMV expression vector for overexpression. For Co-IP assays, domain deletion mutant plasmids were constructed by Nanjing Corues Biotechnology Co., Ltd. The primers employed for plasmid construction are listed in Table S2.

For transient transfection, plasmids were transfected into HEK293T cells using polyethylenimine linear (PEI) MW 40000 (40816ES02, YEASEN) or other cells using Lipofectamine 3000 (Invitrogen), following the manufacturer's instructions. For stable transfection, the packaging plasmid psPAX2, the envelope plasmid pMD2.G, and targeting plasmids were co-transfected into HEK293T cells. The viral supernatant was collected, filtered through a 0.45- μ m-diameter pore membrane (Millipore, USA), and added to cell culture medium supplemented with polybrene (Yeasten, Shanghai, China). Stably transfected cells were selected using 5 μ g/mL puromycin (ST551; Beyotime). NFs were stably transduced with a lentiviral vector encoding human COMP or the

corresponding control vector. CAFs were stably transduced with a lentiviral vector encoding a COMP-targeting short hairpin RNA (shRNA) or a matched nontargeting shRNA.

2.24 Animal Experiments

Four-week-old male BALB/c nude mice were purchased from GemPharmatech Co., Ltd. (Nanjing, China) and randomly assigned to groups using computer-generated numbers after acclimatization (n = 5 per group). All procedures were approved by the Laboratory Animal Welfare and Ethics Committee of Tongji Hospital, Huazhong University of Science and Technology (T3-2026-06-005). According to the requirements of the institutional animal ethics committee, the maximum permitted tumor burden was 10% of the animal's body weight, corresponding to a maximum subcutaneous tumor diameter of 17 mm in a 25 g mouse. Tumor ulceration or infection was also defined as a humane endpoint. No mouse in this study exceeded the permitted tumor burden or developed tumor ulceration or infection.

For the subcutaneous xenograft model, MHCC-97H cells were pretreated with the indicated EVs (20 $\mu\text{g ml}^{-1}$) for 24 h and injected subcutaneously into the mice (2×10^6 cells per mouse). The mice subsequently received the corresponding EVs intravenously (20 μg per mouse) twice weekly for 5 weeks. RSL3 (100 mg kg^{-1}) was administered intraperitoneally twice weekly, whereas ferrostatin-1 (Fer-1; 1 mg kg^{-1}) was administered intraperitoneally every 2 days. The vehicle groups received the

corresponding solvent, whereas the remaining groups did not receive any intraperitoneal injections. On day 35, the tumors were harvested and weighed, and the endpoint tumor volume was calculated as $V = (L \times W^2)/2$, where L and W denote the longest and shortest tumor diameters, respectively.

For the experimental lung colonization model, EV-pretreated MHCC-97H cells (1×10^6) were mixed with the corresponding EVs (20 μ g) and injected into the mice via the tail vein. The subsequent treatment schedule was identical to that used in the xenograft model. Lungs were collected on day 35, and pulmonary tumor foci in hematoxylin and eosin-stained sections were quantified. Ki-67 and 4-HNE staining intensity in xenograft tumors was evaluated in three randomly selected fields per mouse in a blinded manner, with field-level measurements averaged before statistical analysis.

2.25 Immunohistochemistry (IHC)

Immunohistochemical staining was performed as previously described (23) using the antibodies listed in Table S1. Briefly, formalin-fixed, paraffin-embedded tissue sections were deparaffinized, rehydrated and subjected to heat-induced antigen retrieval. After blocking endogenous peroxidase activity and nonspecific binding, the sections were incubated with the indicated primary antibodies overnight at 4 °C, followed by incubation with a horseradish peroxidase-conjugated polymer detection reagent. Immunoreactivity was visualized using 3,3'-diaminobenzidine, and the sections were counterstained with hematoxylin, dehydrated and mounted.

397

398 Multiple nonoverlapping representative fields from each tumor section were analyzed
399 under identical imaging conditions and evaluated by an investigator who was blinded
400 to the treatment groups. The proportion of the positively stained area was graded as 1
401 ($\leq 25\%$), 2 (26–50%), 3 (51–75%) or 4 ($>75\%$), whereas the staining intensity was
402 graded as 1 (weak), 2 (moderate) or 3 (strong). An IHC score was calculated for each
403 field by multiplying the positive area grade by the staining intensity grade, yielding a
404 score ranging from 1 to 12. A score of 0 was assigned when no specific staining was
405 detected. Scores from all evaluated fields of the same tumor were averaged to obtain
406 one value for each mouse, and individual mice were treated as biological replicates.

407

408 2.26 Patient Samples

409 HCC tissue microarrays (HLivH165Su01) were acquired from Shanghai Outdo Biotech
410 Company (Shanghai, China) with approval from the China Human Genetic Resources
411 Management Office. Surgical samples for primary NF and CAF isolation and related
412 experiments were collected from 3 HCC patients who received curative surgical
413 treatment at Tongji Hospital, Huazhong University of Science and Technology (Wuhan,
414 China). The Ethics Committee of Tongji Hospital granted approval for all the
415 experimental protocols (TJ-IRB202511042), and written informed consent was
416 obtained from all the patients.

417

418 2.27 mIHC Staining

Paraffin-embedded tissue sections were deparaffinized in xylene and rehydrated through a graded ethanol series. Multiplex immunohistochemistry (mIHC) was performed using a five-color multiplex immunofluorescence staining kit (AFIHC035; AiFang Biological, China). Antigen retrieval was carried out by microwave heating according to the manufacturer's protocol. Endogenous peroxidase activity was blocked with 3% hydrogen peroxide for 15 min at room temperature, followed by incubation with 10% goat serum for 15 min.

The first primary antibody was applied to the sections and allowed to react overnight at 4 °C. After three washes with PBST, the sections were incubated with polymer-HRP anti-mouse/rabbit universal secondary antibody IgG (AFIHC001; AiFang Biological) for 30 min at room temperature. Following PBST washing, TYR fluorescent dye was applied for 8 min. The sections were then washed three times with PBST, and the bound antibody complex was removed by microwave treatment before the next staining cycle. After another blocking step with goat serum, the subsequent primary antibody was applied. This sequential staining procedure was repeated until all target proteins, including COMP (Abcam, ab260037), α -SMA (CST, #19245), SLC7A11 (Proteintech, 26864-1-AP), and PanCK (Proteintech, 26411-1-AP), had been visualized.

After completion of the multiplex staining cycles, nuclei were counterstained with DAPI for 10 min at room temperature in the dark. The sections were washed three times with PBST and mounted using an antifade mounting medium. Whole-slide fluorescence

images were acquired with an eight-channel fluorescence digital slide scanner (AF-KL-20-8; AiFang Biological, China).

2.28 mIHC Staining Image Acquisition and Quantification

For image acquisition, an eight-channel fluorescence digital slide scanner (AF-KL-20-8; AiFang Biological, China) was used to collect images of the mIHC slides, and the acquired images were saved in .kfbf format. For image data analysis, custom algorithms were developed in HALO Image Analysis Software (Indica Labs, HALO v4.2.26399.152). To ensure the consistency of the analysis results, fixed and uniform threshold parameters were set through the HALO application to identify and quantitatively analyze the fluorescence intensity and distance. After the analysis was completed, the results were exported.

2.29 Spatial Quantification of CAF-associated Tumor Cell Density

For spatial analysis, α -SMA⁺COMP⁺ cells were defined as CAF reference regions, and SLC7A11⁺PanCK⁺ cells were identified as tumor cells. The spatial distribution of SLC7A11⁺PanCK⁺ tumor cells surrounding α -SMA⁺COMP⁺ CAFs was quantified by generating CAF-associated neighborhoods extending 20, 40 and 60 μ m from the boundary of each α -SMA⁺COMP⁺ CAF region. The number of SLC7A11⁺PanCK⁺ tumor cells located within these CAF-associated regions was counted. When multiple CAF-associated regions overlapped, overlapping areas were merged and duplicate regions were excluded to avoid repeated counting. The CAF-proximal

SLC7A11⁺PanCK⁺ tumor cell density was calculated as the total number of SLC7A11⁺PanCK⁺ tumor cells within CAF-associated regions divided by the total effective analyzed area and expressed as cells/mm².

2.30 Statistical Analysis

Statistical analyses were performed using R software (version 4.4.3) and GraphPad Prism (version 11.0.2). Unless otherwise indicated, experiments were independently repeated at least three times. Quantitative data are presented as mean $\pm$ SD. Two-group comparisons were performed using two-sided paired or unpaired Student's t-tests for approximately normally distributed data and Wilcoxon signed-rank or rank-sum tests for paired or independent nonparametric data, respectively. Comparisons among more than two groups were performed using one-way ANOVA followed by Tukey's multiple-comparisons test when appropriate. Spearman's rank correlation was used to assess associations between continuous variables. Kaplan–Meier curves were compared using the log-rank test, and hazard ratios with 95% confidence intervals were estimated using Cox proportional hazards models. For continuous variables used in survival analyses, the optimal cutoff value was determined with the R package “survminer”. Harrell's C-index was used to assess prognostic discrimination. Pairwise survival comparisons were adjusted using the Holm method where indicated. A two-sided $P < 0.05$ was considered statistically significant.

3. Results

3.1 CAF-derived extracellular vesicles promote malignant phenotypes in HCC cells

Primary CAFs and paired normal fibroblasts (NFs) were isolated from human HCC specimens and corresponding non-tumorous liver tissues, respectively, and characterized by immunofluorescence staining. CAFs showed stronger staining of the fibroblast activation markers α -SMA and FAP than NFs, supporting their activated fibroblast phenotype (Figure S1A).

To determine whether factors released by CAFs alter the behavior of HCC cells, MHCC-97H and Huh-7 cells were treated with CAF-conditioned medium (CAF-CM) or NF-conditioned medium (NF-CM). CAF-CM reduced E-cadherin expression while increasing N-cadherin and vimentin levels in both HCC cell lines, consistent with a shift toward a more mesenchymal phenotype (Figure S1B). CAF-CM also increased migration and invasion in Transwell assays and accelerated wound closure in wound-healing assays (Figure S2A, B). Colony formation and CCK-8 assays further showed that CAF-CM increased clonogenic growth and cell viability relative to NF-CM (Figure S2C, D). These results show that factors released by CAFs promote aggressive phenotypes in HCC cells.

We next asked whether EVs released by CAFs could reproduce these effects. EVs were isolated from CAF-CM and NF-CM and characterized by complementary approaches. Nanoparticle tracking analysis (NTA) showed particle-size distributions within the

expected range (Figure S1C), while transmission electron microscopy (TEM) revealed typical membrane-bound vesicular structures (Figure S1D). Western blotting detected CD9, CD81, and TSG101 in both NF-derived EVs (NF-EVs) and CAF-derived EVs (CAF-EVs) (Figure S1E). Following incubation with fluorescently labeled EVs, punctate green fluorescence was detected in MHCC-97H and Huh-7 cells, consistent with cellular uptake of both NF-EVs and CAF-EVs (Figure S1F).

Compared with NF-EVs, CAF-EVs reduced E-cadherin and increased N-cadherin and vimentin in both HCC cell lines (Figure S1G). CAF-EVs also increased migration and invasion, accelerated wound closure, and enhanced colony formation and cell viability (Figure S3A–D). These findings show that CAF-derived EVs reproduce multiple aggressive phenotypes induced by CAF-CM.

3.2 SLC7A11 contributes to CAF-EV-mediated ferroptosis resistance in HCC cells

Studies have shown that cargo delivered by CAF-derived EVs can reduce the susceptibility of cancer cells to ferroptosis through distinct molecular mechanisms (13, 14, 24). To determine whether CAF abundance is associated with ferroptosis-related transcriptional states in HCC tumors, TCGA-LIHC tumors were stratified according to median EPIC-inferred CAF abundance and analyzed by gene set enrichment analysis (GSEA). Epithelial–mesenchymal transition (EMT) and angiogenesis signatures were enriched in CAF-high tumors, whereas the ferroptosis-associated gene set was enriched in CAF-low tumors (Figure 1A). These findings prompted us to examine whether CAF-

derived EVs alter HCC-cell susceptibility to ferroptotic stress.

Following RSL3 exposure, MHCC-97H and Huh-7 cells were treated with NF-EVs or CAF-EVs. RSL3 increased DCFH-DA fluorescence in both cell lines, whereas CAF-EVs largely reduced this increase relative to RSL3 alone or RSL3 plus NF-EVs (Figure 1B). RSL3 also increased malondialdehyde (MDA) levels and intracellular ferrous ion (Fe^{2+}) content in both cell lines. Although NF-EVs partially reversed these changes, CAF-EVs produced a more pronounced decrease in both readouts (Figure 1C, D). Similarly, C11-BODIPY 581/591 imaging showed increased probe oxidation after RSL3 treatment, which was substantially reduced by CAF-EVs (Figure 1E). Thus, CAF-derived EVs attenuate multiple oxidative and lipid peroxidation features associated with RSL3-induced ferroptotic stress.

This biochemical protection was accompanied by reduced sensitivity to RSL3. Compared with NF-EVs, CAF-EVs markedly preserved cell viability during RSL3 exposure (Figure S4A). RSL3 dose-response analyses further showed a rightward shift in the dose-response curve for cells treated with CAF-EVs: the estimated IC_{50} increased from 6.55 μM with NF-EVs to 11.64 μM with CAF-EVs in MHCC-97H cells and from 3.18 μM with NF-EVs to 9.93 μM with CAF-EVs in Huh-7 cells (Figure S4B). RSL3 treatment also reduced migration, invasion, and wound closure, whereas CAF-EVs partially restored these phenotypes in both cell lines (Figure S4C, D).

To identify candidate regulators underlying this effect, we profiled a panel of ferroptosis-related genes in HCC cells treated with NF-EVs or CAF-EVs (25). Among the genes examined, *SLC7A11* was the most prominently and consistently induced in both MHCC-97H and Huh-7 cells following treatment with CAF-EVs (Figure 1F, G). Immunoblotting further showed higher SLC7A11 protein levels in CAF-EV-treated cells than in PBS control or NF-EV-treated cells (Figure 1H). Under RSL3 exposure, CAF-EVs maintained higher SLC7A11 levels and partially reversed RSL3-induced changes in E-cadherin, N-cadherin, and vimentin (Figure 1I).

We therefore examined whether SLC7A11 contributed to the effects of CAF-EVs. SLC7A11 knockdown blunted the CAF-EV-induced EMT-associated protein changes, restoring E-cadherin while reducing N-cadherin and vimentin (Figure 1J). SLC7A11 knockdown reduced migration and invasion and weakened the effects of CAF-EVs on both phenotypes (Figure S5A). A similar effect was observed in wound-healing assays (Figure S5B). In the presence of RSL3, SLC7A11 knockdown also reduced cell viability and weakened the protective effect of CAF-EVs (Figure S5C). These results support a functional contribution of SLC7A11 to CAF-EV-mediated resistance to RSL3 and to the associated aggressive phenotypes.

3.3 EV-associated COMP contributes to ferroptosis resistance in HCC cells

Having established that CAF-derived EVs reduce RSL3 sensitivity and increase SLC7A11 expression, we next sought to identify the EV-associated proteins that might

contribute to these effects. Comparative proteomic profiling identified several proteins enriched in CAF-EVs relative to NF-EVs (Figure 2A, B). Five top-ranked proteins—COMP, CD44, KYNU, KAD1, and PAIP1—were selected for functional screening. Overexpression of each candidate in HEK293T cells was confirmed by qRT-PCR (Figure S6A). EVs derived from HEK293T cells overexpressing each candidate were then applied to MHCC-97H and Huh-7 cells. Among the candidates tested, COMP-enriched EVs produced the most consistent increase in SLC7A11 protein levels in both cell lines (Figure 2C), leading us to focus on COMP in subsequent experiments.

We then investigated the relationship between COMP expression and ferroptosis-related transcriptional programs in HCC tumors. GSEA of TCGA-LIHC tumors stratified by COMP expression showed enrichment of ferroptosis-associated gene set in COMP-low tumors (Figure 2D). By contrast, angiogenesis and EMT signatures were enriched in COMP-high tumors (Figure S6B, C). COMP expression was also positively correlated with EPIC-inferred CAF abundance (Spearman $\rho = 0.75$), ACTA2 expression ($\rho = 0.61$), and FAP expression ($\rho = 0.80$) in TCGA-LIHC (Figure S6D–F). Thus, high COMP expression is associated with greater inferred CAF abundance and with EMT- and angiogenesis-related transcriptional programs in HCC.

To determine whether COMP contributes to the ability of CAF-derived EVs to protect HCC cells from ferroptotic stress, EVs from control shRNA-transduced CAFs (shNC-EVs) were compared with EVs from COMP-knockdown CAFs (shCOMP-EVs). RSL3

increased intracellular Fe²⁺ content and MDA levels in both HCC cell lines, and both increases were reduced by shNC-EVs (Figure 2E, F). This protection was attenuated when COMP was knockdown in CAF-EVs, supporting a functional contribution of COMP to the ferroptosis-resistant phenotype.

Consistent with these findings, shNC-EVs reduced RSL3-induced C11-BODIPY oxidation, whereas this effect was largely lost following COMP knockdown (Figure 2G). DCFH-DA fluorescence was similarly reduced by shNC-EVs, while COMP knockdown weakened this effect in both MHCC-97H and Huh-7 cells (Figure 2H). These loss-of-function data support a functional contribution of EV-associated COMP to the protection conferred by CAF-derived EVs against RSL3-induced ferroptotic stress.

3.4 EV-associated COMP promotes ferroptosis resistance through the HIF-1 α -SLC7A11 axis in HCC cells

Having identified EV-associated COMP as a contributor to ferroptosis resistance, we next examined the downstream signaling pathway involved. Transcriptomic profiling of Huh-7 cells exposed to EVs from COMP-overexpressing or control NFs (oeCOMP-EVs and oeCtrl-EVs, respectively) identified HIF-1 signaling and ferroptosis among the significantly enriched KEGG pathways (Figure 3A). Previous studies have linked HIF-1 α signaling to the regulation of SLC7A11 and GPX4 in other cellular contexts (26, 27), suggesting that HIF-1 α might contribute to the ferroptosis-protective effect of

COMP. Increased doses of oeCOMP-EVs produced progressive increases in HIF-1 α , SLC7A11, and GPX4 protein levels in both MHCC-97H and Huh-7 cells (Figure 3B). HIF-1 α knockdown attenuated the induction of SLC7A11 and GPX4 by oeCOMP-EVs (Figure 3C), supporting a functional role of HIF-1 α in this response.

We next tested whether HIF-1 α contributed to the protection conferred by oeCOMP-EVs during ferroptotic challenge. RSL3 increased DCFH-DA fluorescence in both cell lines, whereas oeCOMP-EVs reduced this increase relative to oeCtrl-EVs. HIF-1 α knockdown markedly weakened this effect (Figure 3D). Similar patterns were observed for intracellular Fe²⁺ content and MDA levels: both increased after RSL3 treatment, were reduced by oeCOMP-EVs, and rose again following HIF-1 α knockdown (Figure 3E, F). C11-BODIPY 581/591 imaging likewise showed that oeCOMP-EVs reduced RSL3-induced lipid oxidation, whereas this protection was largely lost after HIF-1 α knockdown (Figure 3G). These results support HIF-1 α as a downstream mediator of the ferroptosis-protective effect of EV-associated COMP.

We further examined whether HIF-1 α contributed to the aggressive phenotypes associated with oeCOMP-EVs. oeCOMP-EVs increased migration and invasion and accelerated wound closure in both MHCC-97H and Huh-7 cells, whereas HIF-1 α knockdown attenuated these effects (Figure S7A, B). oeCOMP-EVs treatment was also accompanied by reduced E-cadherin and increased N-cadherin and vimentin, changes that were attenuated by HIF-1 α knockdown (Figure S7C). Pharmacological inhibition

of HIF-1 α with LW6 suppressed COMP-induced increases in migration, invasion, and wound healing and reduced SLC7A11 expression while reversing the associated changes in EMT marker expression (28) (Figure S8A-C). RSL3 treatment also reduced the oeCOMP-EV-induced increases in migration, invasion, and wound closure and partially reversed the associated changes in SLC7A11 and EMT-related proteins (Figure S8A, B, D).

These results support HIF-1 α as a downstream mediator of EV-associated COMP that sustains SLC7A11 expression and limits ferroptotic stress. HIF-1 α also contributes to the aggressive phenotypes associated with oeCOMP-EV treatment.

3.5 EV-associated COMP promotes HIF-1 α accumulation through RANBP2-dependent SUMOylation signaling

We next examined how EV-associated COMP increases HIF-1 α protein abundance. Although oeCOMP-EVs increased HIF-1 α protein expression, no corresponding increase in *HIF1A* mRNA levels was detected at 6, 12, or 24 h in either MHCC-97H or Huh-7 cells (Figure 4A), suggesting that the increase in HIF-1 α occurs through a post-transcriptional mechanism.

To identify proteins that might link COMP to HIF-1 α regulation, COMP immunoprecipitation followed by mass spectrometry (IP-MS) was performed in MHCC-97H and Huh-7 cells stably overexpressing COMP, with matched IgG

immunoprecipitates as controls. Fourteen proteins were shared between the two datasets, and 12 with quantifiable signals in both COMP immunoprecipitates were retained for further analysis (Figure 4B). We next examined the relationships between these candidates and three HIF-1 α -related transcriptional signatures in the TCGA-LIHC cohort. Several candidates showed positive correlations with these HIF-1 α -related transcriptional scores, with RANBP2 showing concordant positive associations across all three signatures (Spearman ρ = 0.59, 0.49, and 0.55, respectively; Figure 4C). Given its established SUMO E3 ligase activity and previous evidence linking RANBP2 and SUMO1 to HIF-1 α regulation (29-31), RANBP2 was prioritized for experimental validation. Following oeCOMP-EV treatment, immunoprecipitation with an anti-COMP antibody co-recovered RANBP2 in both MHCC-97H and Huh-7 cells (Figure 4D). These data supported an association between COMP and RANBP2.

Given the SUMO E3 ligase activity of RANBP2, we hypothesized that SUMOylation contributes to COMP-induced HIF-1 α accumulation. Co-immunoprecipitation showed increased association of HIF-1 α with SUMO1 in both MHCC-97H and Huh-7 cells following oeCOMP-EV treatment, whereas total SUMO1 levels remained unchanged (Figure 4E). To determine whether SUMOylation contributes to this response, we inhibited SUMO conjugation using TAK-981, a specific inhibitor (32). This treatment attenuated oeCOMP-EV-induced increases in HIF-1 α and SLC7A11 protein levels in both cell lines (Figure 4F). We next examined whether RANBP2 was required for these effects. RANBP2 knockdown similarly reduced HIF-1 α accumulation and SLC7A11

expression following oeCOMP-EV treatment (Figure 4G). Together, these findings suggest that RANBP2-dependent SUMOylation contributes to COMP-induced HIF-1 α accumulation and downstream SLC7A11 upregulation.

To further characterize the COMP–RANBP2 association, FLAG-tagged full-length COMP and a series of domain-deletion constructs were co-expressed with HA-tagged RANBP2 in HEK293T cells. Deletion of the C-terminal TSP_C domain markedly reduced COMP co-precipitation with RANBP2, whereas constructs lacking the other examined domains retained detectable association with RANBP2 (Figure 4H). These results indicate that the TSP_C region contributes to efficient COMP–RANBP2 association under these conditions.

Collectively, these results support a model in which EV-associated COMP interacts with RANBP2 and engages SUMOylation signaling to promote HIF-1 α accumulation and downstream SLC7A11 expression.

3.6 EV-associated COMP promotes HCC growth and experimental lung colonization by suppressing ferroptotic stress *in vivo*

We next examined whether the ferroptosis-protective effect of EV-associated COMP contributes to HCC progression *in vivo*. For gain-of-function experiments, MHCC-97H cells were pretreated with EVs derived from vector control or COMP-overexpressing NFs before subcutaneous implantation, followed by continued administration of the

corresponding EV preparations (Figure 5A). Mice receiving oeCOMP-EVs developed larger tumors, with greater endpoint tumor volume and weight than mice receiving oeCtrl-EVs (Figure 5B–D). In the oeCOMP-EV groups, RSL3 reduced both endpoints relative to vehicle treatment, supporting a contribution of ferroptotic susceptibility to the tumor-promoting effect of EV-associated COMP (Figure 5B–D).

We next performed reciprocal loss-of-function experiments using shNC-EVs and shCOMP-EVs. shCOMP-EVs reduced endpoint tumor volume and weight relative to shNC-EVs (Figure 5E–G). Fer-1 restored both endpoints in mice receiving shCOMP-EVs relative to the matched vehicle-treated group (Figure 5E–G). These reciprocal pharmacological responses support the functional contribution of ferroptosis suppression to the growth-promoting activity of EV-associated COMP *in vivo*.

Histological analysis showed corresponding changes in proliferation and lipid peroxidative stress. Tumors exposed to oeCOMP-EVs had higher Ki-67 and lower 4-HNE staining scores than control tumors, consistent with increased proliferative activity and reduced lipid peroxidative damage (Figure 5H, I). RSL3 reversed both patterns. Conversely, COMP knockdown in CAF-EVs reduced Ki-67 and increased 4-HNE scores, whereas Fer-1 counteracted these changes (Figure 5H, I).

We next examined the effect of EV-associated COMP on experimental lung colonization. EV-pretreated MHCC-97H cells were mixed with the corresponding EV

preparation and injected through the tail vein, followed by continued EV administration and the indicated ferroptosis-modulating treatments (Figure 5J). oeCOMP-EVs increased the number of pulmonary tumor foci relative to control EVs, while RSL3 reduced this effect compared with the matched vehicle-treated group (Figure 5K). In the reciprocal experiment, shCOMP-EVs reduced the number of pulmonary tumor foci, whereas Fer-1 partially restored foci formation (Figure 5L). These findings show that EV-associated COMP promotes HCC growth and experimental lung colonization, at least in part, by limiting ferroptotic stress *in vivo*.

3.7 Multiplex tissue profiling identifies intratumoral α -SMA⁺COMP⁺ CAFs and SLC7A11⁺PanCK⁺ tumor cells as adverse prognostic features of HCC

To assess the clinical relevance of COMP and SLC7A11 in human HCC, we first analyzed independent transcriptomic cohorts. In GSE54236, both genes showed higher expression in tumor samples than in their paired non-tumor counterparts (Figure S9A, B). Higher COMP expression was associated with shorter OS in the TCGA-LIHC and GSE14520 cohorts and shorter RFS in GSE14520 (Figure S9C). Similarly, higher SLC7A11 expression was associated with shorter OS in both cohorts and shorter RFS in GSE14520 (Figure S9D). These findings linked elevated COMP and SLC7A11 expression to adverse clinical outcomes in patients with HCC. However, bulk transcriptomic data do not resolve the cellular sources or spatial distribution of these molecules within the TME.

We therefore performed multiplex immunohistochemistry (mIHC) staining for COMP, α -SMA, SLC7A11, and PanCK in an HCC tissue microarray to determine their cellular and compartment-specific patterns. Digital image analysis enabled tumor–stroma segmentation, single-cell segmentation, and phenotypic identification of α -SMA⁺COMP⁺ CAFs and SLC7A11⁺PanCK⁺ tumor cells (Figure 6A). No significant difference in α -SMA⁺ cell density was observed between tumor and stromal regions. By contrast, the densities of COMP⁺, α -SMA⁺COMP⁺, SLC7A11⁺, and SLC7A11⁺PanCK⁺ cells were higher in tumor than in stromal regions (Figure 6B–F).

We next examined the clinical associations of these markers. High α -SMA expression was correlated with vascular invasion, T stage, and AJCC stage (Table S4). Survival analyses showed that high tumor α -SMA⁺ density was associated with shorter OS but not DFS, whereas stromal α -SMA⁺ density was not associated with either endpoint (Figure S10A, B). The prognostic associations of COMP and SLC7A11 were more pronounced in the tumor compartment. High tumor COMP⁺ density was associated with shorter DFS and OS, whereas stromal COMP⁺ density was not associated with either outcome. Similarly, high tumor SLC7A11⁺ density was associated with shorter DFS and OS, while stromal SLC7A11⁺ density showed no significant association with either endpoint (Figure S10A, B). High COMP expression was also associated with older age (Table S5).

We next focused on the cellular phenotypes most closely aligned with the proposed

signaling axis. Tumor α -SMA⁺COMP⁺ CAF density and tumor SLC7A11⁺PanCK⁺ tumor-cell density showed higher Harrell C-indices than their corresponding stromal measurements, with values of 0.642 and 0.649 for DFS and 0.672 and 0.699 for OS, respectively (Figure 6G, H). Kaplan–Meier analysis showed that high tumor α -SMA⁺COMP⁺ density was associated with shorter DFS and OS; similar associations were observed for high tumor SLC7A11⁺PanCK⁺ density (Figure S10C). Associations with the corresponding stromal phenotypes were less consistent across the two survival endpoints. High tumor α -SMA⁺COMP⁺ density was also associated with older age and an incomplete tumor capsule (Table S6), whereas tumor SLC7A11⁺PanCK⁺ density was not significantly associated with the available clinicopathological variables (Table S7).

The prognostic relevance of these two tumor compartment phenotypes persisted after adjustment for clinicopathological variables. High tumor α -SMA⁺COMP⁺ density was independently associated with shorter DFS (HR, 5.882; 95% CI, 2.068–16.732; $P < 0.001$) and OS (HR, 9.506; 95% CI, 2.451–36.861; $P = 0.001$). High tumor SLC7A11⁺PanCK⁺ density was likewise independently associated with shorter DFS (HR, 2.284; 95% CI, 1.064–4.900; $P = 0.034$) and OS (HR, 4.403; 95% CI, 1.382–14.028; $P = 0.012$; Figure S11A, B).

Tumor α -SMA⁺COMP⁺ CAF density was positively correlated with tumor SLC7A11⁺PanCK⁺ cell density (Spearman $r = 0.312$, $P = 0.008$), whereas no significant correlation was detected between the corresponding stromal densities ($r = 0.098$, $P =$

0.416; Figure 6I, J). The patients were therefore classified into High/High, High/Low, Low/High, and Low/Low subgroups according to tumor α -SMA⁺COMP⁺ CAF and SLC7A11⁺PanCK⁺ tumor-cell densities, respectively. Survival differed significantly among the four subgroups for both DFS and OS, with the High/High subgroup showing the poorest outcomes (Figure 6K). Apart from age, the available clinicopathological characteristics were comparable across the four subgroups (Table S8). In multivariable analyses using the High/High subgroup as the reference, the Low/High (HR, 0.13; 95% CI, 0.03–0.55; P = 0.006) and Low/Low (HR, 0.14; 95% CI, 0.05–0.44; P < 0.001) subgroups had lower hazards in the DFS analysis. For OS, only the Low/Low subgroup remained significantly different from the High/High reference group (HR, 0.05; 95% CI, 0.0078–0.28; P < 0.001; Figure S11C, D). These results show that concomitant enrichment of intratumoral α -SMA⁺COMP⁺ CAFs and SLC7A11⁺PanCK⁺ tumor cells is associated with particularly adverse clinical outcomes in HCC.

3.8 CAF-proximal SLC7A11⁺PanCK⁺ tumor-cell density is associated with shorter OS

We next examined the local distribution of SLC7A11⁺PanCK⁺ tumor cells around α -SMA⁺COMP⁺ CAFs. Using α -SMA⁺COMP⁺ CAFs as spatial reference cells, CAF-proximal SLC7A11⁺PanCK⁺ tumor-cell density was quantified within 0–20, 0–40, and 0–60 μ m neighborhoods. Representative mIHC images and spatial maps illustrate the distribution of these cell populations in tumors with high and low α -SMA⁺COMP⁺ CAF densities (Figure 7A). Tumors with high α -SMA⁺COMP⁺ CAF density showed higher

CAF-proximal SLC7A11⁺PanCK⁺ tumor-cell density at all three radii than tumors with low CAF density (Figure 7B).

We next assessed the prognostic relevance of this spatial measure. Although the HR increased with neighborhood radius, none of the DFS comparisons reached statistical significance (Figure 7C). By contrast, high CAF-proximal SLC7A11⁺PanCK⁺ tumor-cell density was associated with shorter OS at 0–20 μ m (HR, 2.57; 95% CI, 1.07–6.13; $P = 0.027$), 0–40 μ m (HR, 2.90; 95% CI, 1.18–7.13; $P = 0.015$), and 0–60 μ m (HR, 2.90; 95% CI, 1.18–7.13; $P = 0.015$; Figure 7D). These results show that higher CAF-proximal SLC7A11⁺PanCK⁺ tumor-cell density is associated with shorter OS in HCC.

These findings support a CAF-to-HCC pathway in which EV-associated COMP promotes HIF-1 α SUMOylation and SLC7A11 expression through RANBP2/SUMO signaling, thereby supporting ferroptosis resistance, HCC growth, and experimental lung colonization (Figure 8).

4. Discussion

This study identifies EV-associated COMP as a stromal regulator of ferroptosis resistance in HCC. CAF-derived EVs reduced sensitivity to RSL3, with SLC7A11 contributing to this protective phenotype. Functional screening identified COMP as a relevant EV-associated protein, and subsequent experiments linked COMP-induced HIF-1 α accumulation to RANBP2/SUMO signaling. Reciprocal COMP gain- and loss-

of-function experiments further connected this pathway to tumor growth and experimental lung colonization in vivo. Human tissue analyses showed that intratumoral α -SMA⁺COMP⁺ CAFs and SLC7A11⁺PanCK⁺ tumor cells were associated with adverse outcomes and clinically relevant spatial patterns.

CAFs influence HCC cells through extracellular matrix remodeling, paracrine signaling, metabolic interactions, and EV-mediated communication (33, 34). Our conditioned-medium experiments showed that CAF-derived factors promote several aggressive phenotypes, while isolated CAF-EVs reproduced many of these effects. This does not imply that EVs account for the entire activity of the CAF secretome, because soluble proteins, metabolites, and matrix-associated signals may act in parallel. Instead, the data identify EVs as one route through which CAFs modify HCC cell behavior. Comparative proteomic profiling followed by functional candidate testing identified COMP as a relevant EV-associated protein. Although COMP is best known as an extracellular matrix-associated protein and has been linked to cancer progression (35, 36), our findings suggest an additional role in EV-mediated stromal–tumor communication. COMP knockdown weakened the ferroptosis-protective activity of CAF-EVs, whereas COMP upregulation enhanced this phenotype.

EV-associated COMP increased HIF-1 α protein abundance without a corresponding increase in *HIF1A* mRNA, indicating post-transcriptional regulation. IP–MS and Co-IP identified RANBP2 as a COMP-associated protein, and deletion mapping implicated

the C-terminal TSP_C region in this association. RANBP2 is a multifunctional nucleoporin with SUMO E3 ligase activity (29, 37). COMP-enriched EVs enhanced HIF-1 α SUMOylation, increasing its stability and sustaining HIF-1 α -dependent transcriptional activity, including SLC7A11 upregulation. RANBP2 knockdown or inhibition of SUMO conjugation attenuated the increases in HIF-1 α protein and SLC7A11 expression. These results implicate RANBP2 in the ability of EV-associated COMP to regulate HIF-1 α through SUMOylation, connecting stromal signaling to the antioxidant defenses of HCC cells.

Functionally, HIF-1 α knockdown reduced SLC7A11 induction and weakened the ferroptosis-protective effect of COMP-enriched EVs. This is consistent with previous evidence linking HIF-1 α -dependent programs to redox adaptation and SLC7A11 regulation (38, 39). However, the present experiments do not independently demonstrate direct HIF-1 α binding to the *SLC7A11* promoter. SLC7A11 knockdown also reduced migration and invasion, attenuated wound closure, and weakened protection during RSL3 exposure. The residual effects observed after SLC7A11 knockdown suggest that additional ferroptosis-defense pathways may contribute, consistent with the multiple layers of lipid metabolism, iron handling, and antioxidant defense that determine ferroptotic susceptibility (25, 40). Importantly, the *in vivo* experiments provided reciprocal pharmacological support for the ferroptosis component of this phenotype. The corresponding changes in 4-HNE staining intensity were consistent with altered lipid peroxidative stress *in vivo*.

881

882 The human tissue analyses provide an independent clinical context for this mechanism.
883 Public transcriptomic cohorts showed higher COMP and SLC7A11 expression in HCC
884 tumors and linked higher expression to shorter survival, but bulk data cannot resolve
885 cellular sources. Multiplex tissue profiling addressed this limitation by identifying α -
886 SMA⁺COMP⁺ CAFs and SLC7A11⁺PanCK⁺ tumor cells within tumor and stromal
887 compartments. High intratumoral densities of both phenotypes were associated with
888 adverse outcomes, and their combined assessment identified a High/High subgroup
889 with particularly poor survival. Their densities were also positively correlated within
890 the tumor compartment. Spatial analysis further showed that CAF-rich tumors
891 contained greater SLC7A11⁺PanCK⁺ tumor-cell density within CAF-associated
892 neighborhoods, and higher CAF-proximal tumor-cell density was associated with
893 shorter OS across the radii examined. These observations are consistent with the
894 stromal–tumor relationship identified experimentally, but they do not demonstrate that
895 individual CAFs directly induce SLC7A11 expression in neighboring tumor cells or
896 that EV transfer accounts for the spatial association. Shared hypoxia, matrix
897 organization, or other paracrine signals may contribute to the same pattern.

898

899 Several limitations should be considered. The mechanistic experiments were performed
900 in two HCC cell lines, and the in vivo studies used immunodeficient mice, limiting
901 assessment of the immune microenvironment. In addition, the tail-vein model evaluates
902 experimental lung colonization rather than spontaneous metastasis. The clinical and

spatial analyses were performed in a single tissue cohort and require validation in independent cohorts. Despite these limitations, our findings support a model in which CAF-derived EV-associated COMP promotes RANBP2-dependent HIF-1 α SUMOylation, thereby increasing SLC7A11 expression and ferroptosis resistance in HCC. This pathway links stromal EV communication to tumor-cell redox adaptation and HCC progression.

5. List of abbreviations

α -SMA, α -smooth muscle actin; 4-HNE, 4-hydroxynonenal; AJCC, American Joint Committee on Cancer; ANOVA, analysis of variance; BCA, bicinchoninic acid; CAF, cancer-associated fibroblast; CCK-8, Cell Counting Kit-8; CI, confidence interval; CM, conditioned medium; COMP, cartilage oligomeric matrix protein; Co-IP, co-immunoprecipitation; DAPI, 4',6-diamidino-2-phenylindole; DCFH-DA, 2',7'-dichlorodihydrofluorescein diacetate; DFS, disease-free survival; DIA, data-independent acquisition; DMEM, Dulbecco's modified Eagle medium; EMT, epithelial–mesenchymal transition; EV, extracellular vesicle; FAP, fibroblast activation protein; FBS, fetal bovine serum; FDR, false discovery rate; Fer-1, ferrostatin-1; FPKM, fragments per kilobase of transcript per million mapped reads; GEO, Gene Expression Omnibus; GPX4, glutathione peroxidase 4; GSEA, gene set enrichment analysis; GSVA, gene set variation analysis; H&E, hematoxylin and eosin; HCC, hepatocellular carcinoma; HIF-1 α , hypoxia-inducible factor 1 α ; HR, hazard ratio; IHC, immunohistochemistry; IP–MS, immunoprecipitation–mass spectrometry; KEGG,

Kyoto Encyclopedia of Genes and Genomes; MDA, malondialdehyde; MFI, mean fluorescence intensity; mIHC, multiplex immunohistochemistry; MSigDB, Molecular Signatures Database; NF, normal fibroblast; NTA, nanoparticle tracking analysis; OS, overall survival; PanCK, pan-cytokeratin; PBS, phosphate-buffered saline; qRT-PCR, quantitative reverse transcription polymerase chain reaction; RANBP2, RAN binding protein 2; RFS, recurrence-free survival; ROS, reactive oxygen species; SD, standard deviation; SEM, standard error of the mean; shRNA, short hairpin RNA; SLC7A11, solute carrier family 7 member 11; SUMO, small ubiquitin-like modifier; TCGA-LIHC, The Cancer Genome Atlas Liver Hepatocellular Carcinoma; TEM, transmission electron microscopy; TME, tumor microenvironment; TPM, transcripts per million.

Ethics approval and consent to participate

The collection and use of clinical HCC specimens were authorized by the Ethics Committee of Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology (TJ-IRB202511042). Written informed consent was obtained from all participants. All animal studies were conducted with approval from the Laboratory Animal Welfare and Ethics Committee of Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology (protocol No. T3-2026-06-005).

Availability of data and materials

All publicly available datasets were downloaded directly from the indicated websites.

The proteomics expression data was provided in Table S3. The expression matrix of RNA-sequencing data is openly available in The NCBI SRA database at <https://www.ncbi.nlm.nih.gov>, reference number PRJNA1514655. The data generated and analyzed during the present study are available from the corresponding author on reasonable request. No original code was generated in this study.

Conflict of Interest

The authors declare no conflicts of interest.

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Authors' contributions

Conceptualization, B.C, S.X. Formal analysis, QD.Z. Methodology, QD.Z, QX.M, W.P. Investigation, QD.Z, TT.X, LY.L, XZ.Q, W.P, H.H, JM.J, S.X, W.C, R.W and YC.Z. Resources, YC.Z, Z.L, B.C and S.X. Writing -- Original Draft, QD.Z, B.C and S.X. Writing -- Review & Editing, QD.Z, YC.Z, and SY.B. Supervision, B.C. and S.X. All authors read and approved the final manuscript.

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Supplementary Information

Additional File 1 (.xlsx): Supplementary Tables S1–S3, including antibody and primer information and differentially abundant proteins identified by quantitative proteomic analysis of extracellular vesicles derived from matched normal fibroblasts and cancer-associated fibroblasts.

Additional File 2 (.docx): Supplementary Figures S1–S11 and Tables S4–S8, providing additional experimental validation, ferroptosis-related functional assays, COMP-associated mechanistic analyses, and clinical and survival analyses of human hepatocellular carcinoma samples.

Additional File 3 (.pdf): Full-length uncropped original western blot images corresponding to the immunoblotting experiments presented in the main and supplementary figures.

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Figure Legends

Figure 1 | SLC7A11 contributes to CAF-EV-mediated resistance to RSL3-induced ferroptotic stress

(A) GSEA of TCGA-LIHC tumors stratified by median EPIC-inferred CAF abundance, showing enrichment of the ferroptosis-associated gene set in CAF-low tumors and enrichment of EMT and angiogenesis signatures in CAF-high tumors. (B) DCFH-DA fluorescence in MHCC-97H and Huh-7 cells under the indicated RSL3 and EV treatment conditions. (C, D) MDA levels (C) and intracellular Fe^{2+} content (D) under the indicated conditions. (E) C11-BODIPY 581/591 imaging and quantification of lipid oxidation. Scale bar, 100 μm . (F, G) qRT-PCR profiling of ferroptosis-associated genes in MHCC-97H (F) and Huh-7 (G) cells after treatment with NF-EVs or CAF-EVs. (H) Western blot analysis of SLC7A11 protein levels in PBS control, NF-EV-treated, and CAF-EV-treated cells. (I) Western blot analysis of E-cadherin, N-cadherin, vimentin, and SLC7A11 after RSL3 and/or CAF-EV treatment. (J) Western blot analysis of indicated proteins after SLC7A11 knockdown with or without CAF-EV treatment. For (A), GSEA was performed using the TCGA-LIHC cohort ($n = 374$ tumors). For (B–G), data are presented as mean $\pm$ SD from $n = 3$ independent experiments and were analyzed using Student's t-test for two-group comparisons or one-way ANOVA followed by Tukey's post hoc test for multiple comparisons, as appropriate. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Figure 2 | EV-associated COMP contributes to CAF-EV-mediated ferroptosis resistance in HCC cells

(A) Heatmap of the 15 proteins most strongly enriched in CAF-EVs relative to NF-EVs isolated

from paired primary fibroblasts. (B) Volcano plot showing differentially abundant proteins between CAF-EVs and NF-EVs. (C) Western blot analysis of SLC7A11 in MHCC-97H and Huh-7 cells following treatment with indicated EVs. (D) GSEA showing enrichment of the ferroptosis-associated gene set in COMP-low HCC tumors. (E, F) Intracellular Fe^{2+} content (E) and MDA levels (F) in MHCC-97H and Huh-7 cells under the indicated RSL3 and EV treatment conditions. (G) C11-BODIPY 581/591 imaging and quantification of lipid oxidation. (H) DCFH-DA fluorescence under the indicated conditions. Scale bar, 100 μm . EV proteomic analyses in (A, B) were performed using EVs from $n = 3$ patient-matched pairs of CAFs and NFs. GSEA in (D) was performed using the TCGA-LIHC cohort ($n = 374$ tumors). For the experimental data in (E–H), data are presented as mean $\pm$ SD from $n = 3$ independent experiments. Statistical analysis was performed using Student's t-test for two-group comparisons or one-way ANOVA followed by Tukey's post hoc test for multiple comparisons, as appropriate. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Figure 3 | EV-associated COMP promotes ferroptosis resistance through HIF-1 α -SLC7A11 signaling

(A) KEGG pathway enrichment analysis of differentially expressed genes in Huh-7 cells treated with oeCOMP-EVs versus oeCtrl-EVs. Functional enrichment analysis was performed using Metascape. (B) Western blot analysis of HIF-1 α , SLC7A11, and GPX4 in MHCC-97H and Huh-7 cells treated with increasing doses of oeCOMP-EVs. (C) Western blot analysis of HIF-1 α , SLC7A11, and GPX4 after HIF-1 α knockdown with or without oeCOMP-EV treatment. (D) DCFH-DA fluorescence in MHCC-97H and Huh-7 cells under the indicated conditions. (E)

Intracellular Fe²⁺ content in MHCC-97H and Huh-7 cells. (F) MDA levels in MHCC-97H and Huh-7 cells. (G) C11-BODIPY 581/591 imaging and quantification of lipid oxidation in MHCC-97H and Huh-7 cells. Scale bar, 100 μ m. RNA-sequencing analysis in (A) was performed using n = 3 biological replicates per group. For (D–G), data are presented as mean $\pm$ SD from n = 3 independent experiments. Statistical analysis was performed using Student's t-test for two-group comparisons or one-way ANOVA followed by Tukey's post hoc test for multiple comparisons, as appropriate. *P < 0.05, **P < 0.01, ***P < 0.001.

Figure 4 | EV-associated COMP promotes HIF-1 α accumulation through RANBP2/SUMO signaling.

(A) *HIF1A* mRNA levels in MHCC-97H and Huh-7 cells after treatment with the indicated EV preparations for 6, 12, or 24 h. (B) Venn diagram of proteins identified by COMP IP–MS in COMP-overexpressing MHCC-97H and Huh-7 cells after exclusion of proteins detected in matched IgG controls. Fourteen proteins were shared between the two cell lines, 12 of which were quantifiable in both COMP immunoprecipitates. (C) Spearman correlations between the expression of the 12 candidate genes and three HIF-1 α -related GSVA scores in TCGA-LIHC tumors (n = 374). Values indicate Spearman's ρ . (D) Co-immunoprecipitation of COMP and RANBP2 in MHCC-97H and Huh-7 cells following oeCOMP-EV treatment. (E) HIF-1 α immunoprecipitation followed by western blot for SUMO1 in cells treated with oeCtrl-EVs or oeCOMP-EVs. (F) Western blot analysis of HIF-1 α and SLC7A11 under the indicated combinations of oeCOMP-EV and TAK-981 treatment. (G) Western blot analysis of RANBP2, HIF-1 α , and SLC7A11 after RANBP2 knockdown with or without oeCOMP-EV treatment. (H)

Schematic of FLAG-tagged full-length COMP and the indicated deletion mutants. HA-RANBP2 was immunoprecipitated, and co-precipitated FLAG-tagged COMP constructs were detected by western blotting in HEK293T cells. Blank, empty-vector control. For (C), correlation analyses were performed using TCGA-LIHC tumors (n = 374). Data are presented as mean ± SD. *P < 0.05, **P < 0.01, ***P < 0.001.

Figure 5 | EV-associated COMP promotes HCC growth and experimental lung colonization *in vivo*.

(A) Schematic diagram of EV treatment and the subcutaneous xenograft model. (B–D) Excised tumors (B), endpoint tumor volume (C), and tumor weight (D) in the oeCOMP-EV/RSL3 series. (E–G) Excised tumors (E), endpoint tumor volume (F), and tumor weight (G) in the shCOMP-EV/Fer-1 series. (H) Representative H&E, Ki-67, and 4-HNE staining of xenograft tumors. (I) Quantification of Ki-67 and 4-HNE staining scores. Scale bars, 50 µm. (J) Schematic of EV treatment and the tail-vein experimental lung colonization model. (K, L) Representative H&E-stained lung sections and quantification of pulmonary tumor foci in the oeCOMP-EV/RSL3 (K) and shCOMP-EV/Fer-1 (L) series. Black, red, and blue scale bars indicate 2 mm, 200 µm, and 100 µm, respectively. Data are presented as mean ± SD; n = 5 mice per group, with each point representing one mouse. Field-level measurements for Ki-67 and 4-HNE were averaged within each mouse before statistical analysis. *P < 0.05, **P < 0.01, ***P < 0.001.

Figure 6 | Intratumoral α -SMA⁺COMP⁺ CAF and SLC7A11⁺PanCK⁺ tumor-cell densities are associated with poor outcomes in HCC.

(A) Representative low-power and high-power field images stained with five-color mIHC panel (DAPI in blue, COMP in red, α -SMA in green, SLC7A11 in yellow, and PanCK in purple) (left two panels), tissue segmentation map of tumor (red) and stromal (green) regions (upper right panel), and cell segmentation map (nucleus in blue, membrane outlined in corresponding color, and cytoplasm located between nucleus and membrane) (low-right panel). Scale bar: 50 μ m. (B-F) Paired comparisons of α -SMA⁺ (B), COMP⁺ (C), α -SMA⁺COMP⁺ (D), SLC7A11⁺ (E), and SLC7A11⁺PanCK⁺ (F) cell densities between tumor and stromal compartments. P values were calculated using the Wilcoxon signed-rank test. (G, H) Harrell C-indices with 95% CIs for DFS (G) and OS (H), estimated from univariable Cox proportional hazards models using the indicated stromal and tumor cell-density measures (n=71). (I) Spearman correlation matrix of compartment-specific cell densities; values indicate Spearman ρ . (J) Correlations between tumor SLC7A11⁺PanCK⁺ tumor-cell density and tumor or stromal α -SMA⁺COMP⁺ CAF density. (K) Kaplan–Meier curves for DFS and OS according to the four tumor α -SMA⁺COMP⁺ CAF/tumor SLC7A11⁺PanCK⁺ tumor-cell subgroups. Log-rank test and Holm’s test. All analyses were performed using n = 71 patients.

Figure 7 | CAF-proximal SLC7A11⁺PanCK⁺ tumor-cell density is associated with poor OS in HCC.

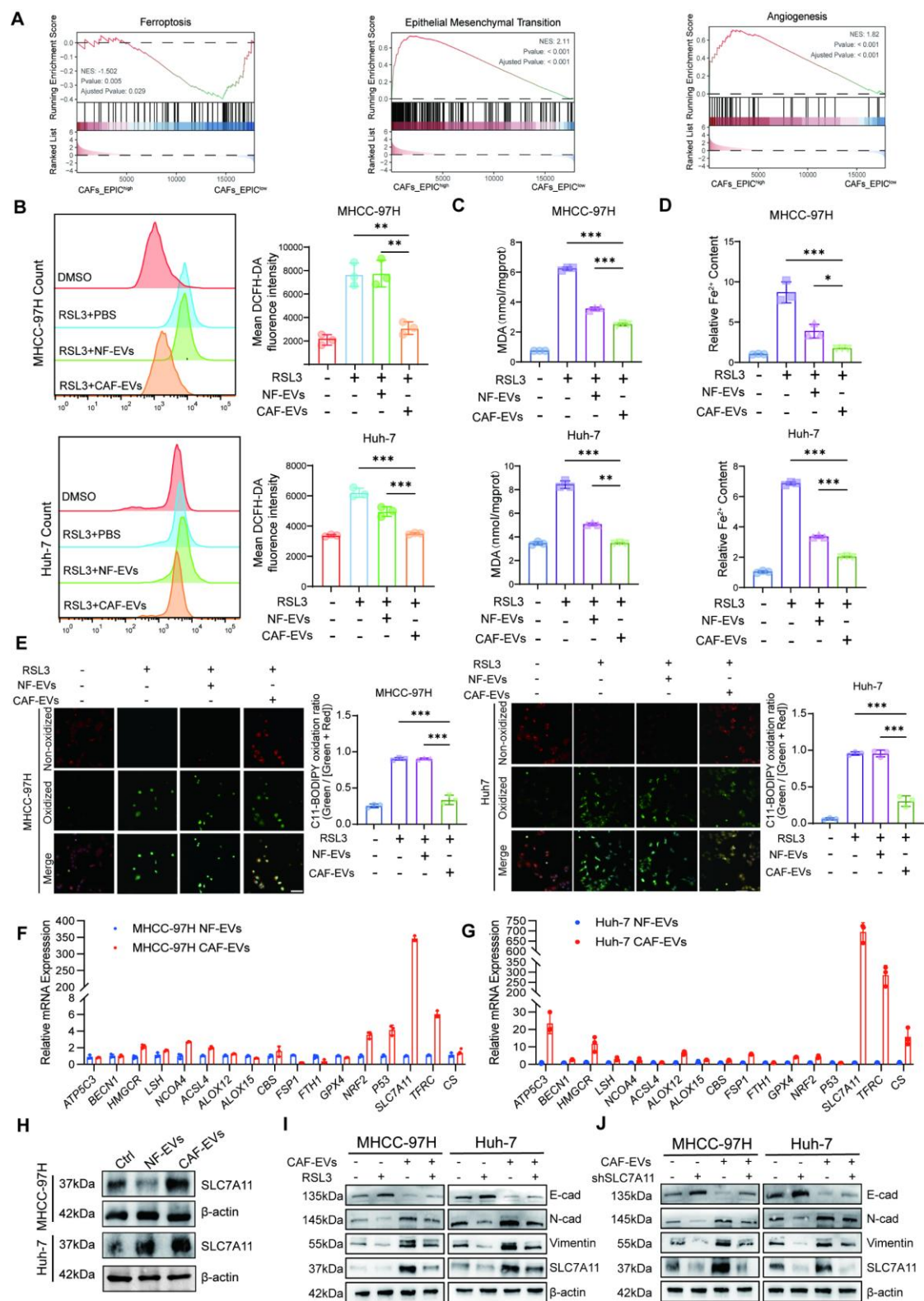
(A) Schematic of the spatial analysis and representative mIHC images with corresponding spatial maps. Concentric neighborhoods of 20, 40, and 60 μ m were defined around each α -SMA⁺COMP⁺ CAF, and SLC7A11⁺PanCK⁺ TCs located within this region were considered CAF-proximal TCs. Red dots indicate α -SMA⁺COMP⁺ CAFs and yellow dots indicate CAF-

1205 proximal SLC7A11⁺PanCK⁺ tumor cells. Scale bars, 50 μ m. (B) CAF-proximal
1206 SLC7A11⁺PanCK⁺ tumor-cell density at the indicated radii in tumors with low or high α -
1207 SMA⁺COMP⁺ CAF density. Data are presented as mean $\pm$ SEM. Two-sided Wilcoxon rank-
1208 sum test. (C, D) Kaplan–Meier analyses of DFS (C) and OS (D) according to CAF-proximal
1209 SLC7A11⁺PanCK⁺ tumor-cell density at the indicated radii. Survival differences were assessed
1210 using the log-rank test; HRs and 95% CIs were estimated using univariable Cox proportional
1211 hazards models. Spatial and survival analyses were performed using n = 71 patients.

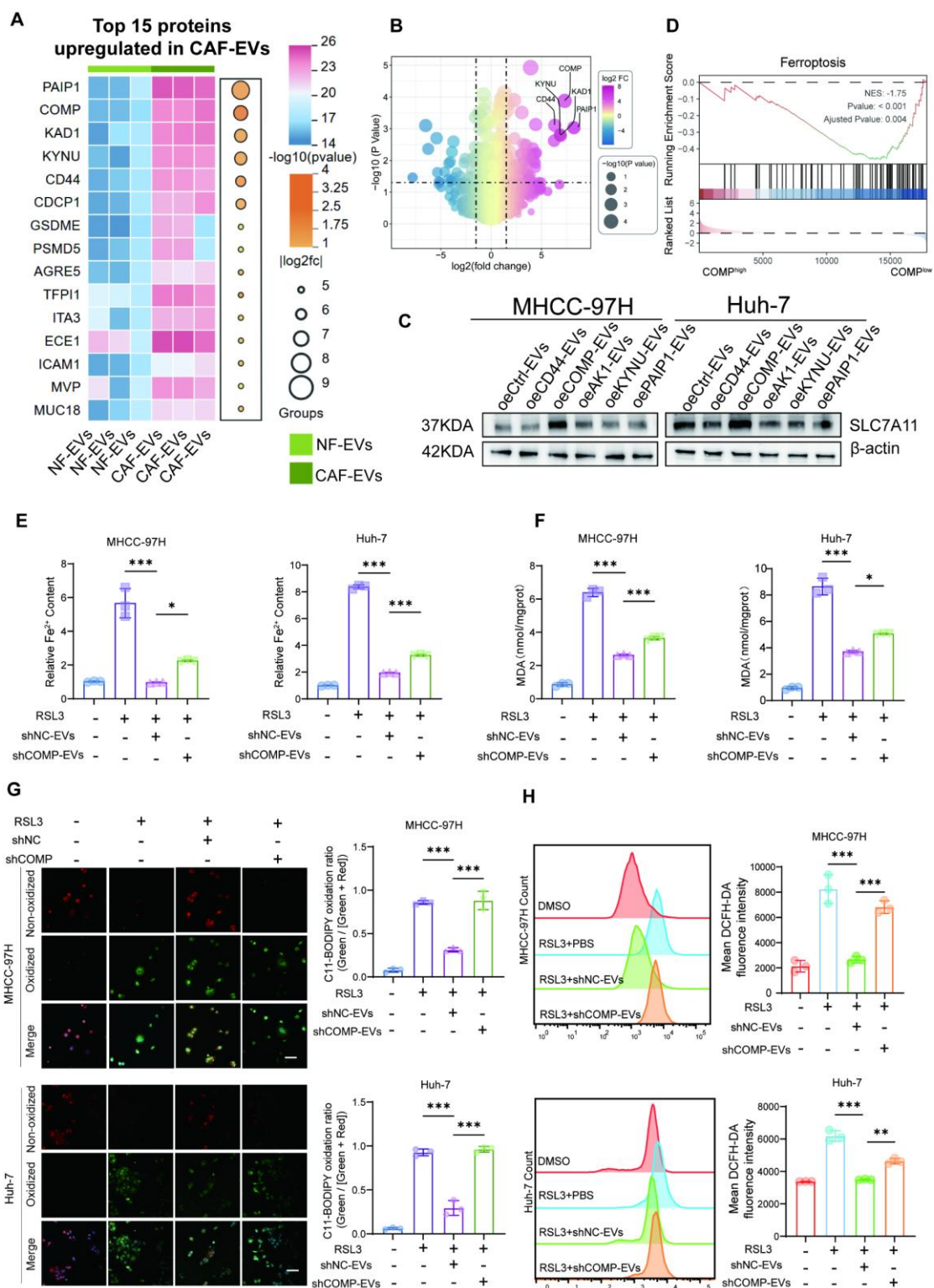
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1213 **Figure 8 | Proposed model of the CAF-EV-associated COMP–RANBP2/SUMO–HIF-1 α –**
1214 **SLC7A11 pathway in HCC.**

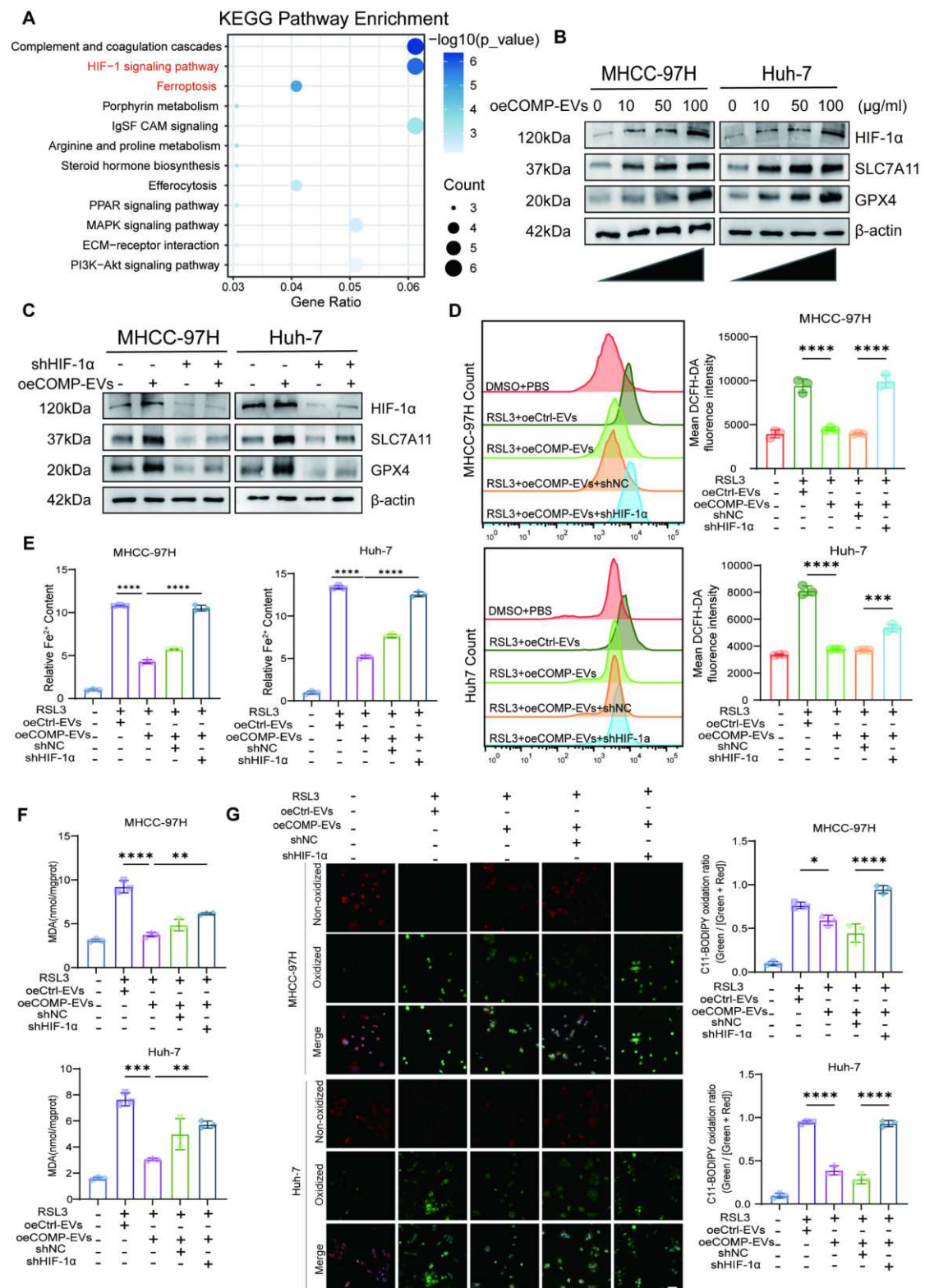
1215 CAF-derived EV-associated COMP contributes to reduced ferroptosis susceptibility in HCC
1216 cells. Functional experiments implicate HIF-1 α and SLC7A11 in this response and support the
1217 involvement of RANBP2 and SUMOylation in HIF-1 α accumulation.



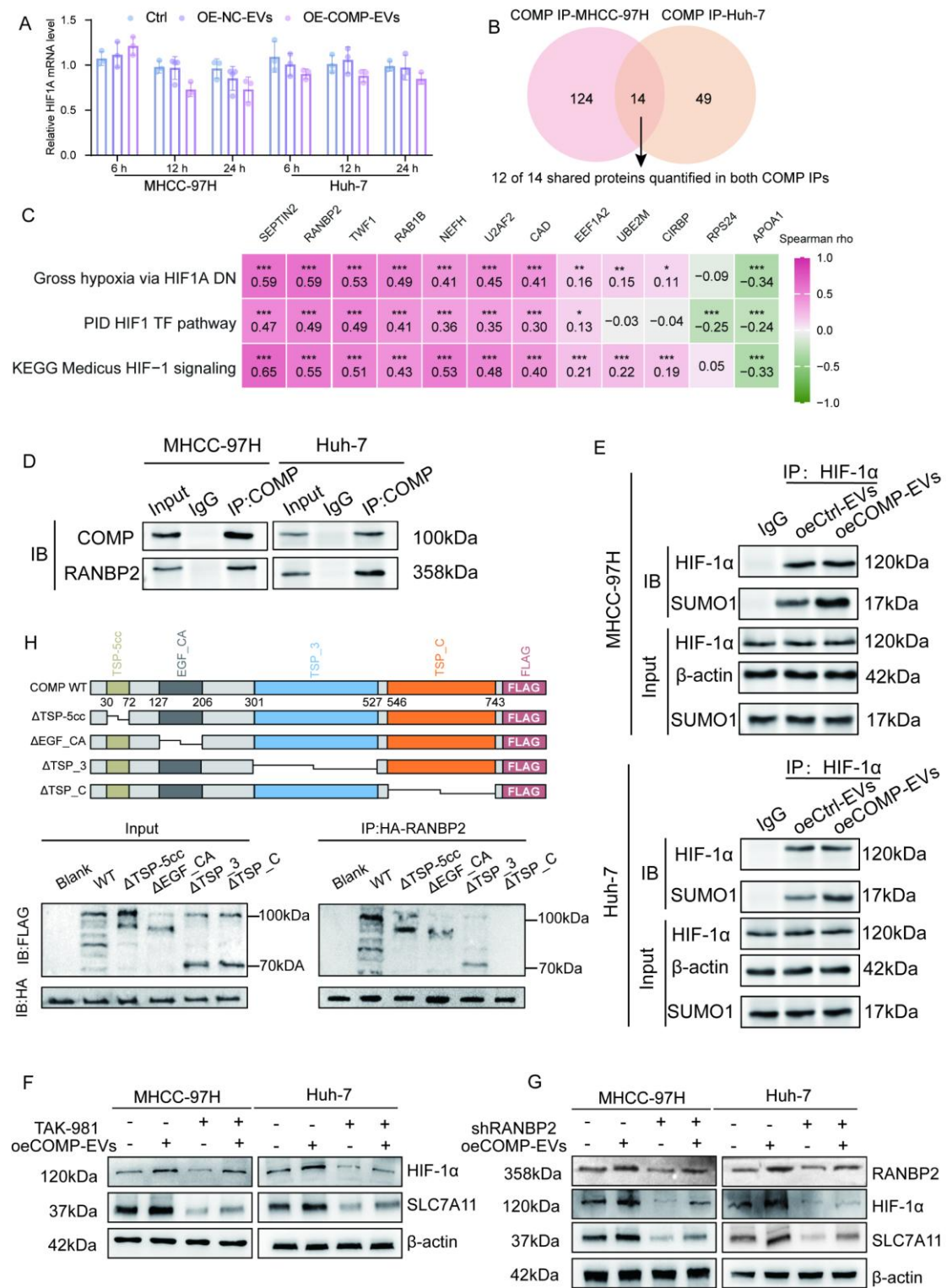
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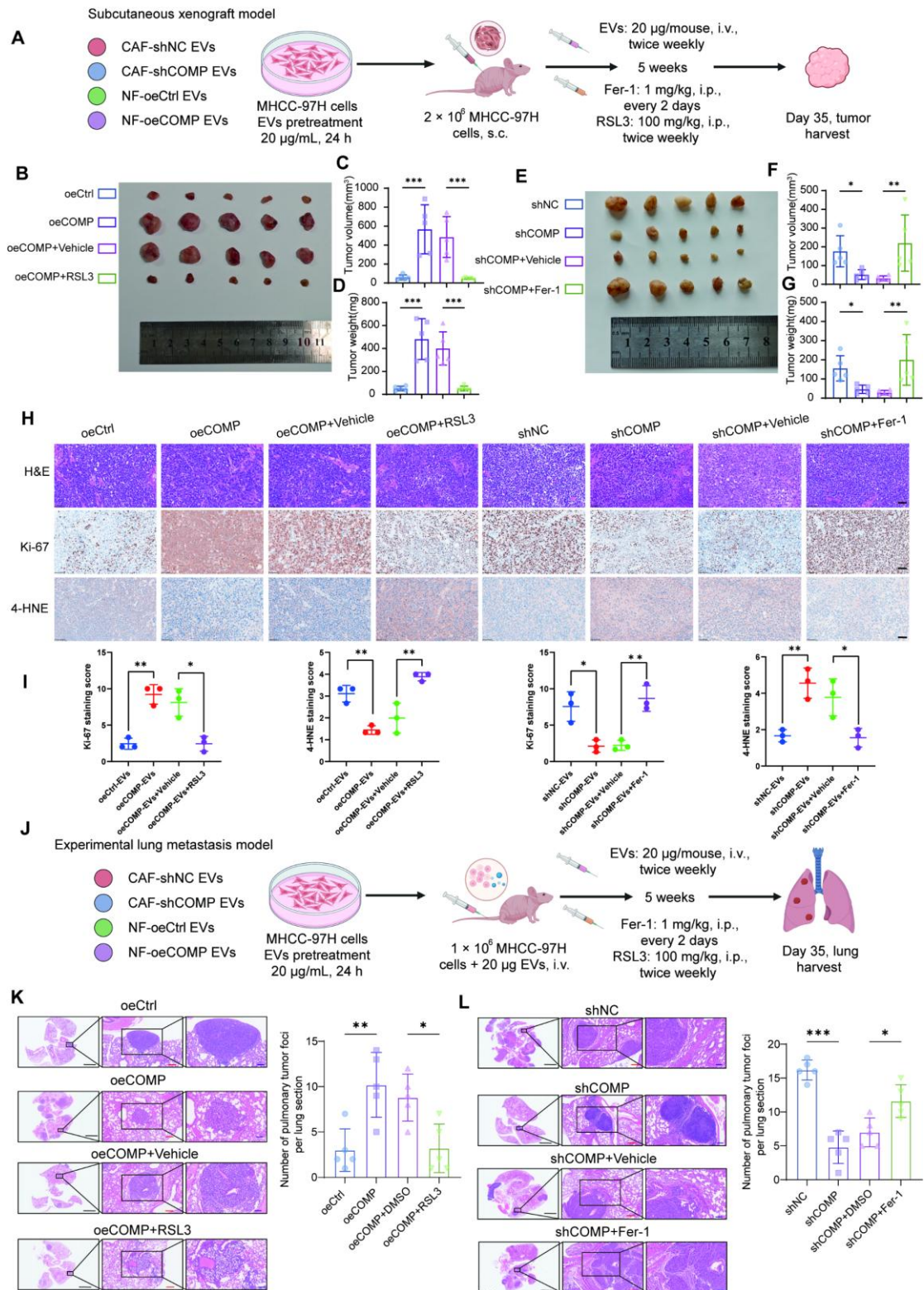
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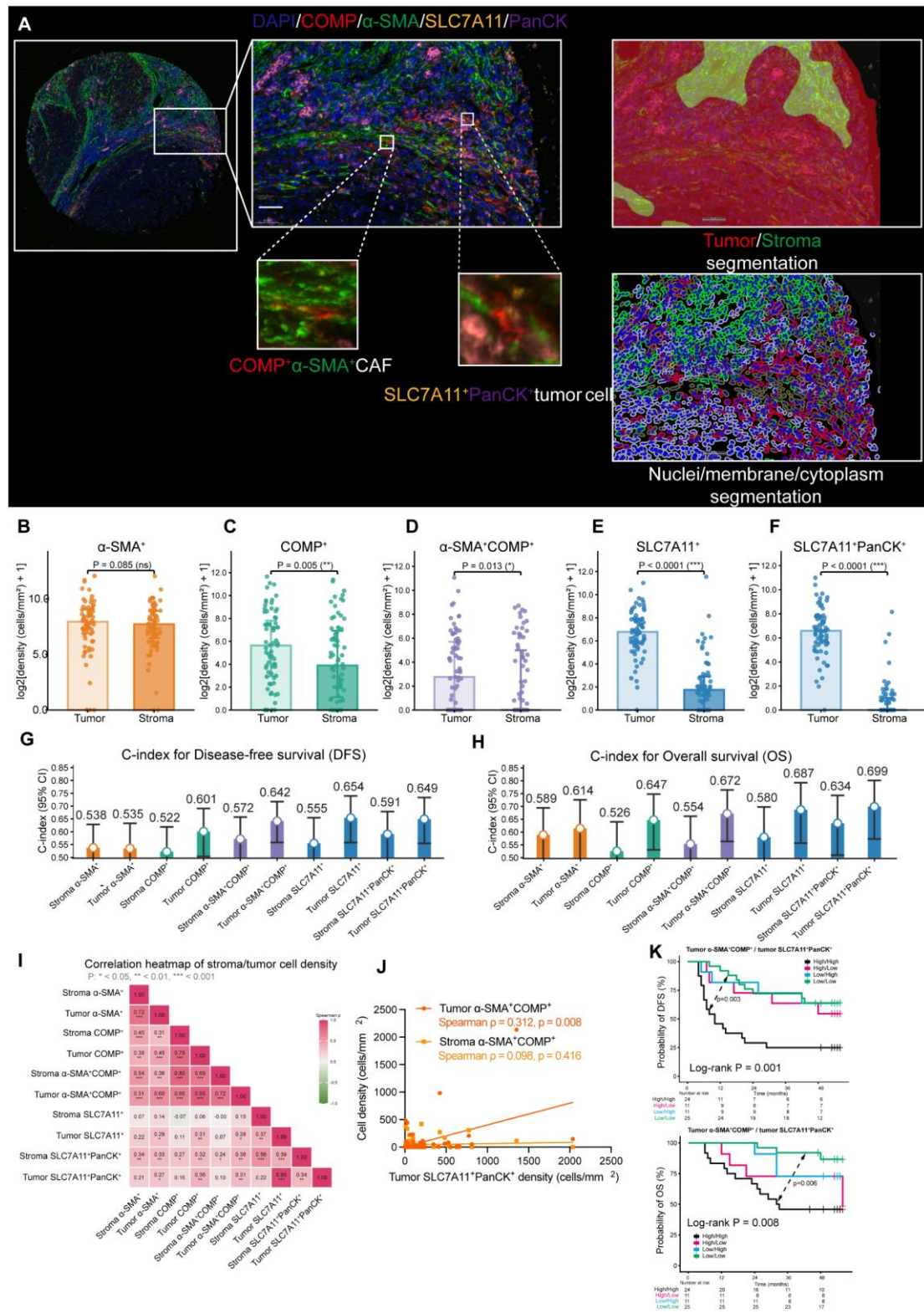


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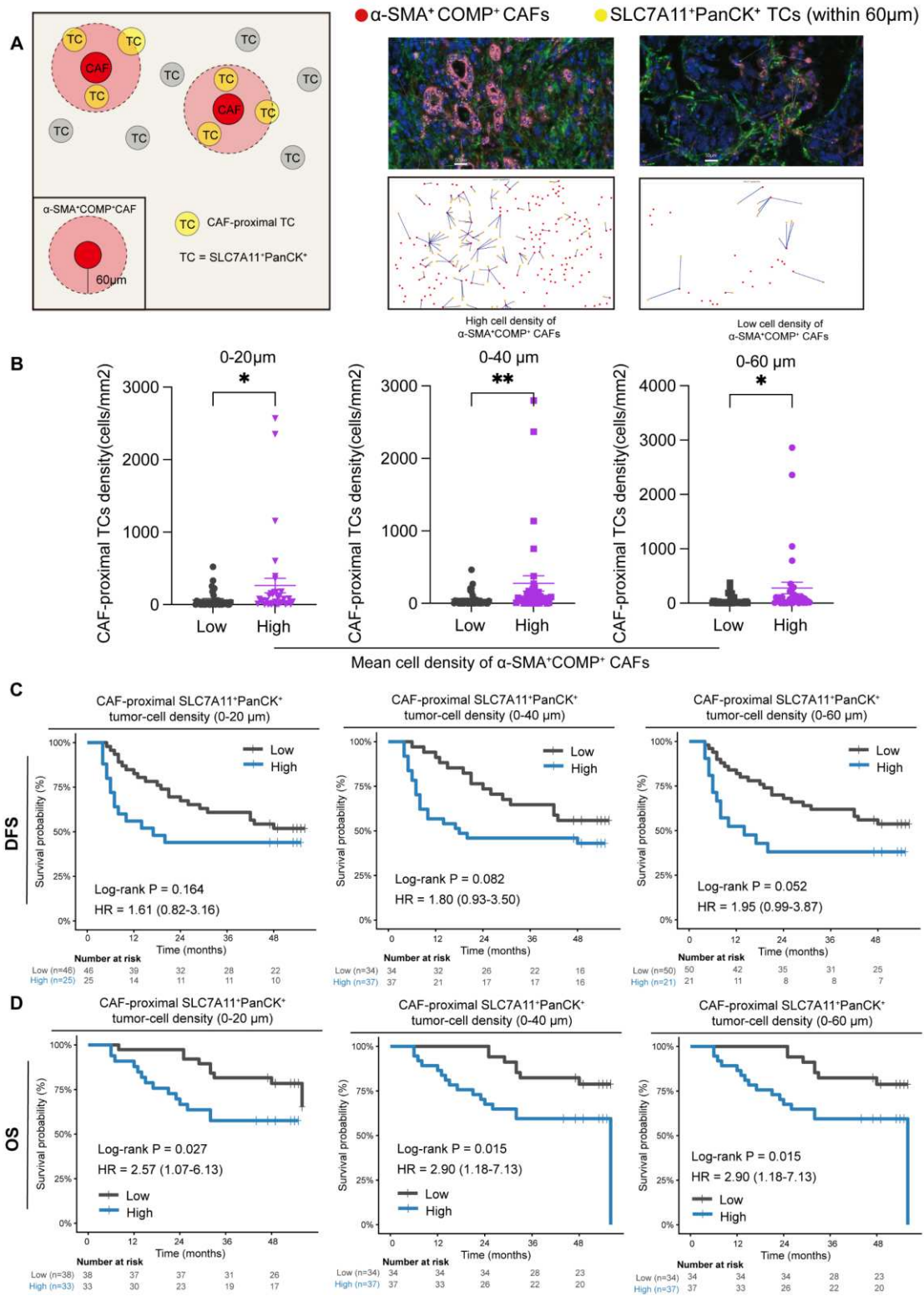


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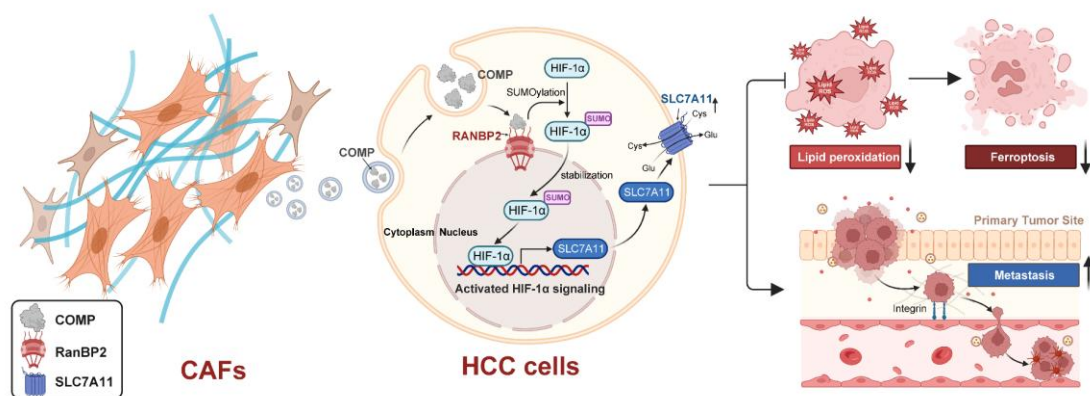




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