

## **SUPPLEMENTARY INFORMATION**

### **Targeting FTL regulates ferroptosis and remodels lymph node metastasis microenvironment in esophageal squamous cell carcinoma**

#### **Supplementary materials and methods**

##### **Preparation for single-cell data**

The gene expression matrix metadata was converted to a Seurat object using Seurat R package (v 4.0). To remove low quality cells, the various metrics distribution of the data was visualized and the following thresholds were applied:  $nUMI \geq 300$ ,  $nGene \geq 200$ ,  $\log_{10}GenesPerUMI > 0.8$  and  $mitoRatio < 0.2$ . Doublets were removed by DoubletFinder (v 2.0). From the remaining cells, the gene expression dataset was normalized, scaled, and subsequently dimensionally reduced. Major cell lineages, including epithelial cells, T cells, B cells, myeloid cells, fibroblasts, and endothelial cells, were projected on the two dimensional UMAP representation and annotated using well recognized signatures. FindMarkers were used to compare differentially expressed genes between different groups. Venn plot showed the intersection of differentially expressed genes from different comparisons.

##### **Preparation for public RNA-seq datasets**

ESCC cohort in TCGA database were collected for detailed RNA-seq analysis. Eighty-one patients were classified into two groups according to the expression of FTL, top 25 high or low FTL expression samples were used for differential gene analyses. And the high expressed genes related to FTL was defined as the FTL related genes for the GO and GSEA analyses. In Gene Set Enrichment Analysis (GSEA), the error finding rate (FDR) and P-value are used to determine statistical significance. Genotype-Tissue Expression (GTEx) database was an online analysis database for

different genes expression in normal, tumor and metastasis group.

## **Cell culture**

All cell lines were maintained in a humidified incubator at 37°C containing 5% CO<sup>2</sup>. In everyday cultivation, RPMI-1640 Medium (Gibco BRL, Grand Island, NY, USA) supplemented with 1% 200mM L-glutamine (Gibco BRL, Grand Island, NY, USA) and 10% fetal bovine serum (FBS) (Gibco BRL, Grand Island, NY, USA) was used for the six ESCC cell lines (KYSE30, KYSE140, KYSE180, KYSE410, KYSE510 and KYSE520). Sometimes we would withdraw or reduce FBS. High glucose Dulbecco's Modified Eagle Medium (Gibco BRL, Grand Island, NY, USA) supplemented with 1% 200mM L-glutamine (Gibco BRL, Grand Island, NY, USA) and 10% FBS (Gibco BRL, Grand Island, NY, USA) was used to support the other cell lines (EC18, HKESC1, HEK-293T).

## **Lentivirus production**

For lentivirus production, HEK-293T packaging cells were transfected with lentiviral vectors using Lipofectamine 2000 Reagent (Invitrogen). After 72 h of incubation, the viral supernatant was collected and filtered. ESCC cells were incubated overnight with the viral supernatant and supplemented with 10 µg/ml polybrene. Stable clones were selected using puromycin and blasticidin.

## **Western blotting and qPCR**

Denatured protein samples with molecular weight markers were packed into 8-12% SDS-PAGE gel Wells. After electrophoresis, the protein was transferred to PVDF membrane at 200A constant current for 2h. The PVDF membrane was sealed in TNS-T with 5% skim milk for more than 1 h and incubated overnight with primary antibody at 4°C. After TBS-T washing, PVDF membrane was incubated with the corresponding secondary antibody for 1 hour. Immunoassays were performed using ECL Western Blotting Detection Kit and Hyperfilm (Amersham Biosciences, Piscataway, NJ). Total RNA was extracted via TRIZOL Reagent (Invitrogen), and the

1 cDNA was synthesized from 4ul of total RNA by PrimeScript RT Master Mix (Takara  
2 Bio). Quantitative real-time PCR (qPCR) was performed using the TB Green Premix  
3 Ex Taq II (TAKARA) and a LightCycler® 480 System (ROCHE). The mRNA  
4 expression of FTL, GPX2, GPX4, TXNRD1, NQO1, PRDX1, SLC40A1, GSTM3,  
5 GSTM4, HMOX1, FTH1 and  $\beta$ -Actin was assessed by qPCR with the primers  
6 (Supplementary Table 2).

#### 7 **XTT cell proliferation assay**

8 The cells were planted in 96-well plates at the confluence point of  $1 \times 10^3$  cells per  
9 well. Cell proliferation XTT kit (Roche Applied Science, Penzberg, Germany) assays  
10 cell proliferation rate according to manufacturer's instructions. Each cell line was  
11 triploid sown and measured daily. Curves are represented by three averages. The two  
12 sets of data at each time point were statistically analyzed by t-test, and the results  
13 were obtained by comparing multiple sets of data.

#### 14 **Cell proliferation and drug resistance assay**

15 In vitro cell proliferation rate was assessed by monitoring the cell number for 5–7  
16 days in cell culture. The cells in each condition were seeded at 1,000 cells/well for  
17 proliferation assay or 8,000 cells/well for drug resistance assay in 96-well in triplicate  
18 and incubated in a 37 °C humidified CO<sub>2</sub> incubator with the drug or vehicle  
19 containing medium refreshed every other day. The number of cells was determined by  
20 XTT kit (Roche Diagnostics, Indianapolis, IN).

#### 21 **Migration, and invasion assay**

22 Migration and invasion assays were determined to evaluate cell motility (BD  
23 Biosciences, San Jose, CA). Matrigel was used in invasion assays. Approximately  $8 \times$   
24  $10^4$  cells were seeded in the medium without FBS on transwell upper chambers, and  
25 the lower chamber was supplied with a medium of complete 10% FBS. After 24-48 h,  
26 the lower membrane surface was stained using crystal violet to count the cell number  
27 under a microscope.

## **Immunohistochemistry (IHC)**

Standard streptavidin-biotin-peroxidase complex method was used for immunohistochemistry. Soon, the tissue sections were dewaxed with xylene and then rehydrated with a reduced concentration of ethanol. Endogenous peroxidase was blocked with H<sub>2</sub>O<sub>2</sub> (0.3%) for 10 min. Tissue slides were cultured in a 10 mM citric acid buffer at 98°C for 10-20 min and cooled for several hours or antigen recovered. After the slices were taken, the tissue slides were sealed with 10% normal goat serum for more than 1 h and incubated overnight with primary antibody at 4°C. On the second day, the tissue sections were rinsed with PBS 4 times for 15 min each, and incubated with secondary antibody at 37°C for 30 min. Incubate with hematoxylin and prepared DAB (DAKO, Carpinteria, CA) for 3-10 seconds and stain the nucleus.

## **Immunofluorescence (IF)**

Slides containing adherent cells or retrieved tissues were fixed with 4% paraformaldehyde for 10 min. In some cases, such as nucleoprotein staining, 1-5 minutes (optional) of infiltration is performed using 0.05% Triton X-100. The slides were blocked with protein blocks (DAKO, Carpinteria, CA) for 10 min. After washing by TBS, the slides were loaded with primary antibody and incubated overnight at 4°C. On the second day, the primary antibody was rinsed with TBS, and the slides were incubated with the fluorophore-coupled secondary antibody in a dark and humid environment for 30 min. Finally, DAPI stain the nucleus (Invitrogen, Carlsbad, CA).

## **Iron assay**

Intracellular ferrous iron (Fe<sup>2+</sup>) level was determined using the iron assay kit (E-BC-k733-M) purchased from Elabscience according to the manufacturer's instructions. Cells were seeded onto 10 cm plate ( $5 \times 10^6$  cells per plate) and treated with corresponding drugs for 24 h. Cells were collected and washed in ice-cold PBS and

homogenized in iron assay buffer 1#, Ultrasonic crushing 4 °C, then centrifuged (10,000 × g, 10 min) at 4 °C. Collect the supernatant and add iron assay buffer 2# to each sample, mix, and incubate for 10 min at 37°C, then centrifuged (10,000 × g, 10 min). Add 300µl of each sample to 96-well plates. Measure the absorbance at 593 nm using a microplate reader.

#### **Malondialdehyde (MDA) assay**

The relative MDA concentration in cell lysates was assessed using a lipid peroxidation assay kit (S0131S) purchased from Beyotime according to the manufacturer's instructions. Cells were seeded in 10 cm plate ( $5 \times 10^6$  cells per plate) and treated with corresponding drugs for 24 h. Cells were washed with ice-cold PBS and homogenized on ice in Western and IP cell lysates (P0013, Beyotime), then centrifuged (12,000 × g, 20 min) to obtain its supernatant from each homogenized sample into a microcentrifuge tube. Add 0.2ml of the assay working solution of MDA into each sample. After mixing, the mixture was heated at 100°C or in a boiling water bath for 15 minutes. The water bath was cooled to room temperature and centrifuged at 1000g for 10 min at room temperature. 0.2ml of the supernatant was added to a 96-well plate, and absorbance was subsequently measured at 532nm using a microplate reader.

#### **Lipid ROS assay using flow cytometer**

Lipid ROS level was analyzed by flow cytometry using a reactive oxygen species assay kit (S0033S) purchased from Beyotime according to the manufacturer's instructions. DCFH-DA was diluted in serum-free medium at 1:1000 to give a final concentration of 10 micromoles/liter. The cell culture medium was removed and the appropriate volume of diluted DCFH-DA was added. The volume added was appropriate to cover the cells adequately, and usually no less than 1 ml of diluted DCFH-DA was added for one well of a six-well plate. The cells were incubated in a cell incubator at 37°C for 20 min. Cells were washed three times with serum-free cell culture medium to adequately remove DCFH-DA that did not enter the cells. Use

ROS positive controls and corresponding drugs to stimulate cells. The intensity of fluorescence before and after stimulation was detected in real time or by time point using the 488nm excitation wavelength and 525nm emission wavelength of a flow cytometer (ACEA NovoCyte Quanteon).

## **Mice model**

For the subcutaneous injection model,  $5 \times 10^6$  FTL transfected cells, shFTL-transfected cells, and their corresponding control cells were injected subcutaneously into different sides of 4–6 week-old C57/6N or NOD/SCID mice. The tumor size was measured every 5–7 days with a caliper and calculated with the formula  $0.5 \times \text{length} \times \text{width} \times \text{width}$ . Mice were sacrificed 4 weeks. For the metastasis mice model,  $1 \times 10^6$  FTL-transfected cells, shNRF2-transfected cells, and their corresponding control cells were injected through the hock injection of 4–6-week-old NOD/SCID mice.

Mice were sacrificed around 3 months. As the NOD/SCID mice do not have any B and T cells, normal inguinal lymph nodes were very small and transparent, of which were very different from the large and solid metastatic lymph nodes. Thus, we placed only the mice with lymph node metastasis. For the subcutaneous injection model for immune function exploration,  $5 \times 10^6$  FTL transfected cells and their corresponding control cells were injected subcutaneously into different sides of 4–6 week-old C57/6N mice. Mice were sacrificed on the 10th day for its tumors. The tumors were put into a 2ml EP tube containing 300-700ul of tissue digestion solution respectively, and the tissues were cut into pieces as much as possible. The tissue was bathed in water at 37°C for 25min. During the period, the tissue block was removed and shaken every 5min for 30s. The cell suspension was transferred to a flow tube at 100w cells/tube, and 1.5-3ml PBS was added according to the amount of red liquid, followed by centrifugation at 300g for 5min. Live/dead dye and corresponding macrophage immunophenotyping marker flow antibodies were added, and then left at room temperature for 15min and 30min. Add 1ml FACS, centrifuge at 300g for 5min, centrifuge, pour supernatant and shake off the wall hanging droplets, and finally add

1 200ul FACS to resuspension for flow cytometry analyses respectively.

## 2 **Brusatol**

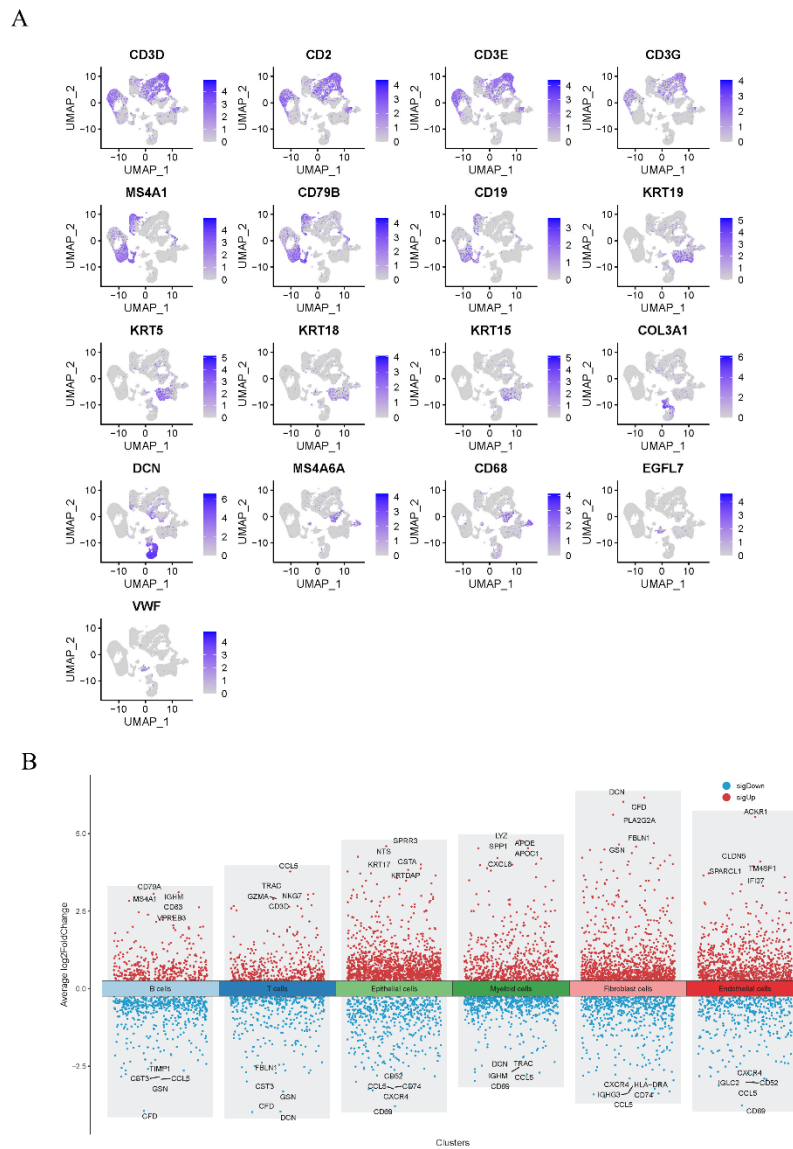
3 The treatment of tumors via traditional Chinese medicine (TCM) is an important way  
4 to develop new drugs, and there are many studies showing the effects of Brucea  
5 javanica on tumor development, metastasis and ferroptosis<sup>1,2</sup>. Brusatol is a  
6 diterpenoid, a critical component extracted from the Brucea javanica plant<sup>3</sup>, which has  
7 been shown to inhibit NRF2 activity in previous literatures<sup>4,5</sup>. As to the in vitro  
8 experiments, it has been reported that the viability of cancer cells was reduced in a  
9 dose-dependent manner<sup>6</sup>. Also, we do the dose-dependent of Brusatol in mEC2 and  
10 mEC3 cell lines (Figure 7F) for double check. As to the in vivo experiments, it has  
11 been reported Nude mice are injected with cancer cells to induce tumor growth,  
12 followed by a single injection of 2 mg/kg Brusatol. Nrf2 protein levels are  
13 significantly decreased at 24 h or 48 h postinjection<sup>7</sup>.

## 14 **Statistical analysis and reproducibility**

15 Statistical analysis was performed, and plots were generated using GraphPad  
16 Prism 8 and IBM SPSS Statistics 25. For data with a sample size  $\leq 6$ , bar plots were  
17 used and all data points were shown. For data with a sample size  $> 6$ , box & whiskers  
18 plots were used to visualize the data distribution. Bar plot data were presented as the  
19 mean values  $\pm$  SD. Box & whiskers plots were presented as the median values  $\pm$  IQR  
20 (25th and 75th percentiles) and whiskers encompass 1.5 times the IQR, as determined  
21 by the Tukey method. Violin plots were presented as kernel density estimations  
22 (KDE) annotated with median values  $\pm$  IQR. For parametric data, P values were  
23 evaluated by the two-sided Student's t-test. For non-parametric data from scRNA-seq  
24 data, P values were evaluated by the two-sided Wilcoxon signed-rank test or Kruskal-  
25 Wallis one-way analysis of variance. Two-sided Pearson and Spearman correlation  
26 analysis was used to compute the correlation and statistical significance between two  
27 variables. In GSEA, both false discovery rates (FDR) and P values were used to

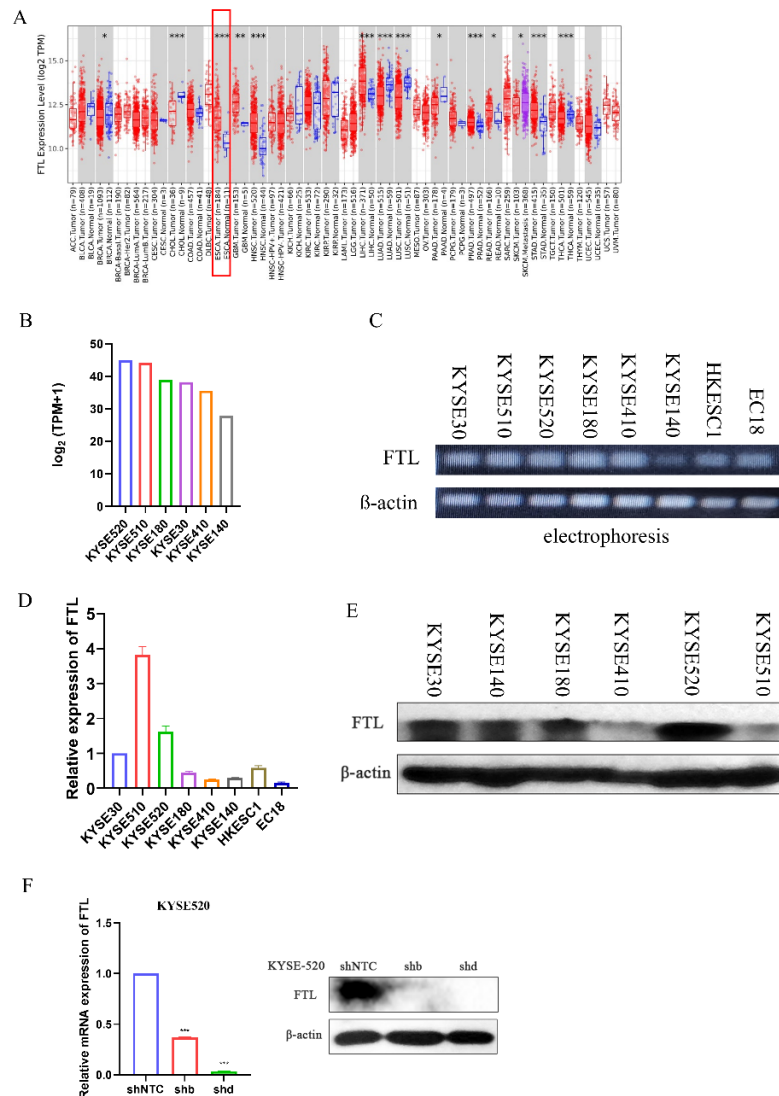
1 determine statistical significance. In survival analysis, P values were evaluated by the  
2 two-sided log-rank test.

### 3 **Supplementary figure legends**



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5 **Supplementary Figure 1. Single-cell transcription atlas analyses of primary and**  
6 **metastatic ecosystems. (A)** Umaps of specific marker genes, including B cells  
7 (MS41, CD79B, and CD19), T cells (CD3D, CD2, CD3E and CD3G), epithelial cells  
8 (KRT5, KRT15, KRT18 and KRT19), fibroblast cells (DCN and COL3A1), myeloid  
9 cells (MS4A6A, and CD68) and endothelial cells (EGFL7 and VWF). (B) Volcano

1 plot showing high expression genes in different cell types. The upper genes were the  
2 higher expressed group and the lower genes were the lower expressed group.



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4 **Supplementary Figure 2. FTL expression in different cell lines.** (A) FTL

5 expression levels in different tumor types via TIMER tools. (B) qPCR of FTL in

6 different ESCC cell lines according to Cancer Cell Line Encyclopedia. (C)

7 Electrophoresis showing FTL expression in different ESCC cell lines. (D) qPCR of

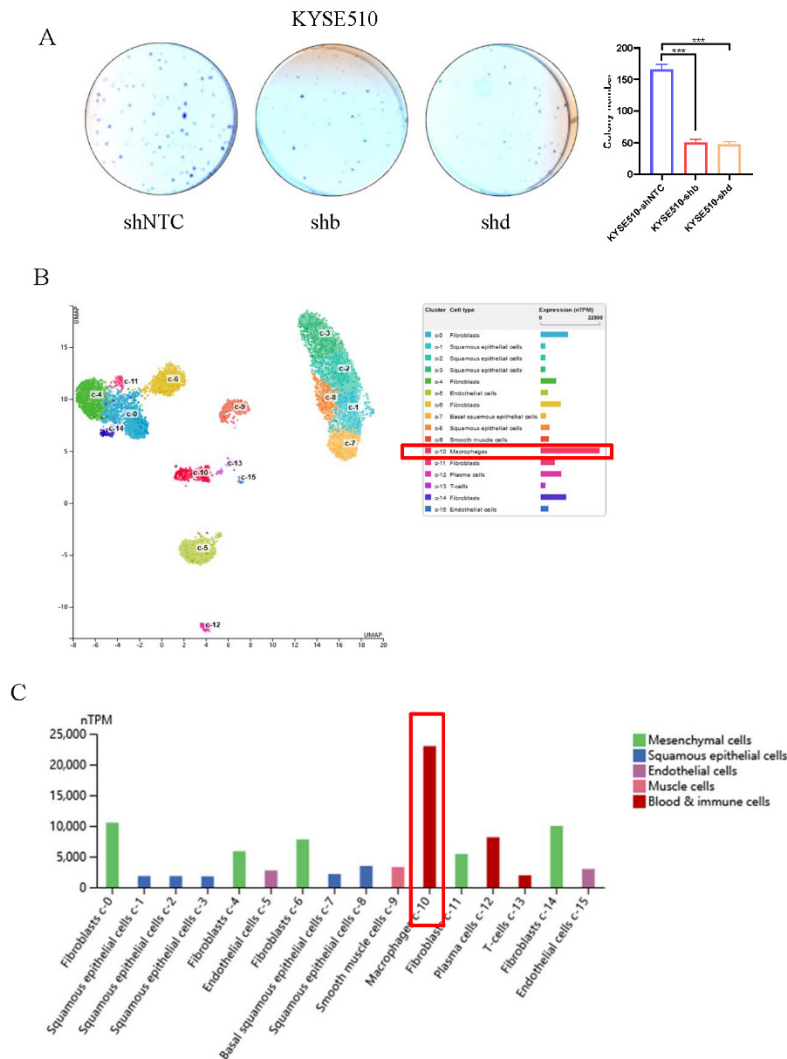
8 FTL in different ESCC cell lines. (E) Western blotting of FTL in different ESCC cell

9 lines. β-Actin was used as a loading control. (F) qPCR and Western blotting of FTL

10 in KYSE520 ESCC cells transfected with lentiviruses expressing control shRNA

11 (shNTC) and two independent shRNAs targeting FTL (shb and shd). β-Actin was

- 1 used as a loading control.
- 2 Statistical significances: \*,  $P < 0.05$ ; \*\*,  $P < 0.01$ ; \*\*\*,  $P < 0.001$ .



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- 4 **Supplementary Figure 3. FTL expression in its infiltrating different cell types.**
- 5 (A) Foci formation assay showing different colony numbers in KYSE 510 cells
- 6 transfected with shNTC, shb and shd. (B, C) Single cell analysis of FTL
- 7 expression in different cell types in online public database.
- 8 Statistical significances: \*,  $P < 0.05$ ; \*\*,  $P < 0.01$ ; \*\*\*,  $P < 0.001$ .

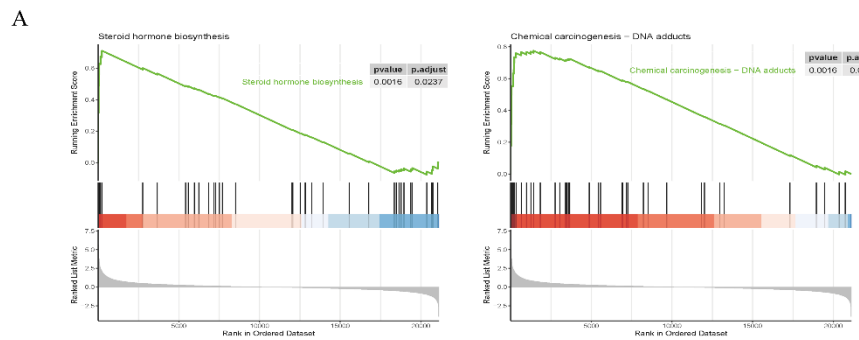
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6 **Supplementary Figure 4. Exploration of upstream and downstream genes of**  
7 **FTL. (A) GSEA analysis of ESCC FTLhigh and FTLlow groups in TCGA database.**

8 Statistical significances: \*,  $P < 0.05$ ; \*\*,  $P < 0.01$ ; \*\*\*,  $P < 0.001$ .

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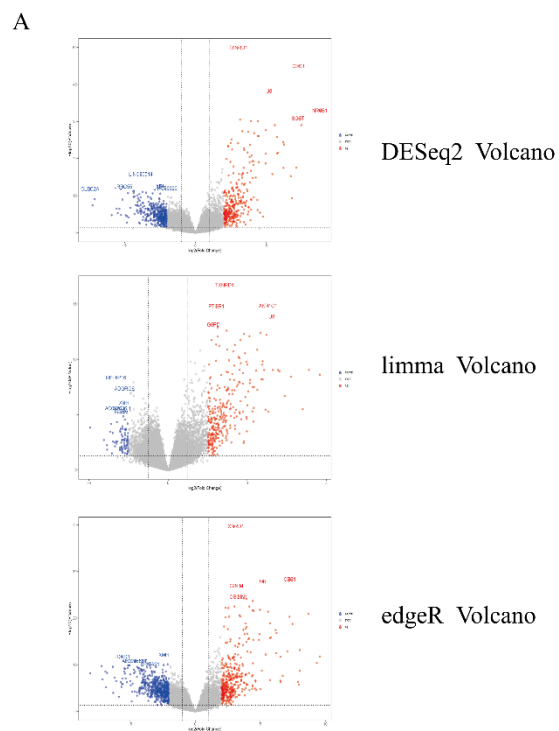
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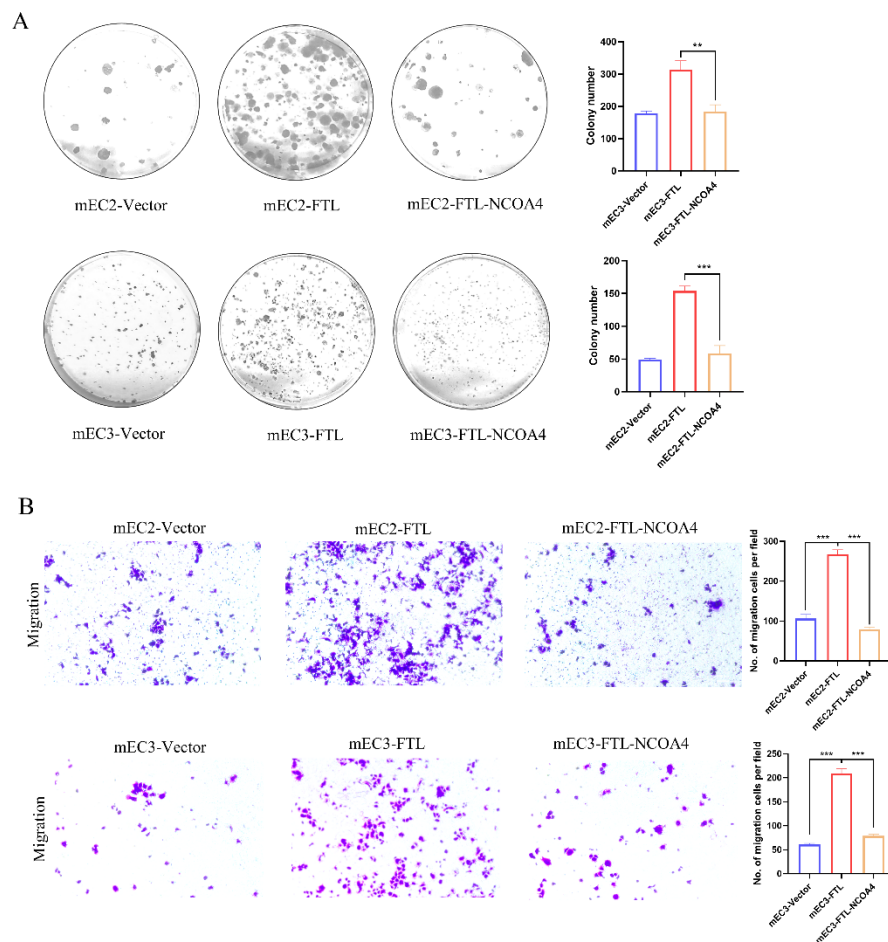
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6 **Supplementary Figure 5. Exploration of downregulated and upregulated genes**  
7 **of FTL in ESCC.** (A) Volcano plot showing upstream and downstream genes of FTL  
8 via different algorithm.

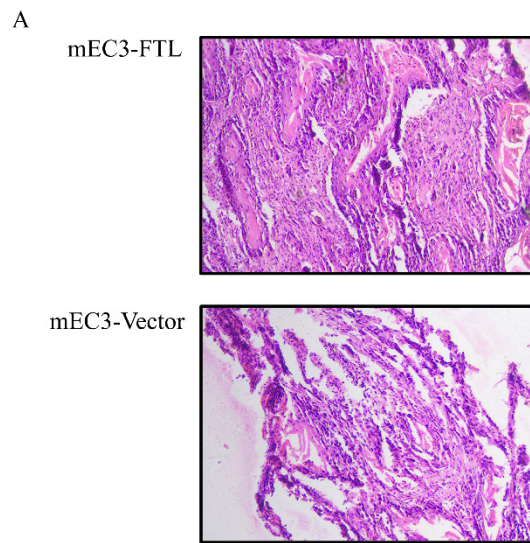
9 Statistical significances: \*,  $P < 0.05$ ; \*\*,  $P < 0.01$ ; \*\*\*,  $P < 0.001$ .



**Supplementary Figure 6. Upregulation of NCOA4 impairs FTL oncogenic and metastasis effects in ESCC.** (A) Foci formation assay showing different colony numbers in mEC2 and mEC3 cells transfected with vector, FTL isoform, FTL-NCOA4 isoform. (B) Representative images of transwell migration assays (left) and their summaries (right) in mEC2 and mEC3 cells transfected with vector, FTL isoform, FTL-NCOA4 isoform.

Statistical significances: \*,  $P < 0.05$ ; \*\*,  $P < 0.01$ ; \*\*\*,  $P < 0.001$ .

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3 **Supplementary Figure 7. H&E staining of lymph node metastases lesions in**

4 **different groups. (A) Representative H&E images of the lymph node metastasis**

5 **(LNM) for hock injection mice model in vector, FTL isoform groups.**

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**Supplementary Table 1. List of primers used in this study.**

| Gene                 | Primer  | Sequence                                                        |
|----------------------|---------|-----------------------------------------------------------------|
| For Real-time PCR    |         |                                                                 |
| FTL                  | Forward | ATGATGTGGCTCTGGAAGGC                                            |
|                      | Reverse | TTCATGGCGTCTGGGGTTTT                                            |
| SLC40A1              | Forward | TGACTGTCCTGGGCTTTGAC                                            |
|                      | Reverse | AGACCTGTCCGAACCAAACC                                            |
| GPX4                 | Forward | GAGGCAAGACCGAAGTAAACTAC                                         |
|                      | Reverse | CCGAAGTGGTTACACGGGAA                                            |
| IREB2                | Forward | TCGATGTATCTAAACTTGGCACC                                         |
|                      | Reverse | GCCATCACAATTTTCGTACAGCAG                                        |
| TXNRD1               | Forward | ATATGGCAAGAAGGTGATGGTCC                                         |
|                      | Reverse | GGGCTTGTCTTAACAAAGCTG                                           |
| NQO1                 | Forward | GAAGAGCACTGATCGTACTGGC                                          |
|                      | Reverse | GGATACTGAAAGTTCGCAGGG                                           |
| GPX2                 | Forward | GGTAGATTTCAATACGTTCCGGG                                         |
|                      | Reverse | TGACAGTTCTCCTGATGTCCAAA                                         |
| GSTM4                | Forward | CAATGATGCAGCACTTCTCACA                                          |
|                      | Reverse | GATCTTCTCCAAGCCCTCAAAGC                                         |
| GSTM3                | Forward | CGTGCGAGTCGTCTATGGTT                                            |
|                      | Reverse | CATAGTCAGGAGCTTCCCCG                                            |
| FTH1                 | Forward | CGCCTCCTACGTTTACCTGT                                            |
|                      | Reverse | GAAGATTCGGCCACCTCGTT                                            |
| HMOX1                | Forward | AAGACTGCGTTCCTGCTCAAC                                           |
|                      | Reverse | AAAGCCCTACAGCAACTGTCG                                           |
| PRDX1                | Forward | CCACGGAGATCATTGCTTTCA                                           |
|                      | Reverse | AGGTGTATTGACCCATGCTAGAT                                         |
| For clone gene       |         |                                                                 |
| FTL-isoform          | Forward | GACACCGACTCTAGAGGATCCGCCACCATG<br>AGCTCCCAGATTCGTCAGA           |
|                      | Reverse | CGCGGGCCCTCTAGACTCGAGTTAGTCGTG<br>CTTGAGAGTGAGCC                |
| Knockdown-<br>NRF2-1 | Forward | CCGGCTTGAAGTCTTCAGCATGTTACTCGA<br>GTAACATGCTGAAGACTTCAAGTTTTTG  |
|                      | Reverse | AATTCAAAAAGCTTGAAGTCTTCAGCATGTTA<br>CTCGAGTAACATGCTGAAGACTTCAAG |
| Knockdown-<br>NRF2-2 | Forward | CCGGGCCTTACTCTCCCAGTGAATACTCGA<br>GTATTCAGTGGGAGAGTAAGGCTTTTTG  |
|                      | Reverse | AATTCAAAAAGCCTTACTCTCCCAGTGAATA<br>CTCGAGTATTCAGTGGGAGAGTAAGGC  |

**Supplementary Table 2. List of antibodies used in this study.**

| Antibody              | Size<br>(kDa) | Vendor         | Cat No.       | Application             |
|-----------------------|---------------|----------------|---------------|-------------------------|
| Rabbit anti-FTL       | 10-20         | ABclonal       | A1768         | WB, 1: 1000; IHC, 1:100 |
| Mouse anti-FTL        | 10-20         | Novus          | H00002512-M16 | IF, 1: 100; IP, 1 µg/mg |
| Rabbit anti-NRF2      | 95-140        | Novus          | NBP1-32822    | IF, 1:500; WB, 1:1000   |
| Rabbit anti-KEAP1     | 60-75         | ABclonal       | A1820         | WB, 1:1000              |
| Rabbit anti-HO-1      | 25-35         | ABclonal       | A11102        | WB, 1:1000              |
| Rabbit anti-SLC7A11   | 50-70         | ABclonal       | A2413         | WB, 1:1000              |
| Rabbit anti-IREB2     | 100-150       | ABclonal       | A6382         | WB, 1:1000              |
| Rabbit anti-FTH1      | 20            | ABclonal       | A19544        | WB, 1:1000              |
| Mouse anti-NCOA4      | 50-75         | Sigma-Aldrich  | SAB1409837    | IF, 1:100; WB, 1:1000   |
| Rabbit anti-NCOA4     | 71-117        | Abcam          | ab86707       | IP, 1 µg/mg; WB, 1:1000 |
| Mouse anti-E-cadherin | 100-140       | Abcam          | ab76055       | WB, 1:1000              |
| Rabbit anti-β-catenin | 80-100        | Cell signaling | CST8480       | WB, 1:1000              |
| Rabbit anti-Vimentin  | 50-60         | Cell signaling | CST5741s      | WB, 1:1000              |
| Rabbit anti-Snail     | 30            | Cell signaling | CST3879s      | WB, 1:1000              |
| Rabbit anti-Slug      | 30            | Cell signaling | C19G7         | WB, 1:1000              |
| Mouse anti-β-actin    | 43            | Abcam          | ab6276        | WB, 1:5000              |

- <sup>1</sup> He T, Zhou F, Su A, Zhang Y, Xing Z, Mi L, Li Z, Wu W. Brusatol: A potential sensitizing agent for cancer therapy from *Brucea javanica*. *Biomed Pharmacother*. 2023 Feb;158: 114134. doi: 10.1016/j.biopha.2022.114134.
- <sup>2</sup> Xing S, Nong F, Wang Y, Huang D, Qin J, Chen YF, He DH, Wu PE, Huang H, Zhan R, Xu H, Liu YQ. Brusatol has therapeutic efficacy in non-small cell lung cancer by targeting Skp1 to inhibit cancer growth and metastasis. *Pharmacol Res*. 2022 Feb;176: 106059. doi: 10.1016/j.phrs.2022.106059.
- <sup>3</sup> Lee KH, Okano M, Hall IH, Brent DA, Soltmann B. Antitumor agents XLV: Bisbrusatolyl and brusatolyl esters and related compounds as novel potent antileukemic agents. *J Pharm Sci*. 1982 Mar;71(3):338-45. doi: 10.1002/jps.2600710320.
- <sup>4</sup> Park JE, Leem YH, Park JS, Kim SE, Kim HS. Astrocytic Nrf2 Mediates the Neuroprotective and Anti-Inflammatory Effects of Nootkatone in an MPTP-Induced Parkinson's Disease Mouse Model. *Antioxidants (Basel)*. 2023 Nov 13;12(11):1999. doi: 10.3390/antiox12111999.
- <sup>5</sup> Yan J, Li Z, Liang Y, Yang C, Ou W, Mo H, Tang M, Chen D, Zhong C, Que D, Feng L, Xiao H, Song X, Yang P. Fucoxanthin alleviated myocardial ischemia and reperfusion injury through inhibition of ferroptosis via the NRF2 signaling pathway. *Food Funct*. 2023 Nov 13;14(22):10052-10068. doi: 10.1039/d3fo02633g.
- <sup>6</sup> Chen HM, Lai ZQ, Liao HJ, Xie JH, Xian YF, Chen YL, Ip SP, Lin ZX, Su ZR. Synergistic antitumor effect of brusatol combined with cisplatin on colorectal cancer cells. *Int J Mol Med*. 2018 Mar;41(3):1447-1454. doi: 10.3892/ijmm.2018.3372.
- <sup>7</sup> Ren D, Villeneuve NF, Jiang T, Wu T, Lau A, Toppin HA, Zhang DD. Brusatol enhances the efficacy of chemotherapy by inhibiting the Nrf2-mediated defense mechanism. *Proc Natl Acad Sci U S A*. 2011 Jan 25;108(4):1433-8. doi: 10.1073/pnas.1014275108.