

Reporting Summary

Nature Portfolio wishes to improve the reproducibility of the work that we publish. This form provides structure for consistency and transparency in reporting. For further information on Nature Portfolio policies, see our [Editorial Policies](#) and the [Editorial Policy Checklist](#).

Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

n/a	Confirmed
☐	☒ The exact sample size (<i>n</i>) for each experimental group/condition, given as a discrete number and unit of measurement
☐	☒ A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
☐	☒ The statistical test(s) used AND whether they are one- or two-sided <i>Only common tests should be described solely by name; describe more complex techniques in the Methods section.</i>
☒	☐ A description of all covariates tested
☐	☒ A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
☐	☒ A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
☐	☒ For null hypothesis testing, the test statistic (e.g. <i>F</i> , <i>t</i> , <i>r</i>) with confidence intervals, effect sizes, degrees of freedom and <i>P</i> value noted <i>Give P values as exact values whenever suitable.</i>
☒	☐ For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
☒	☐ For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
☐	☒ Estimates of effect sizes (e.g. Cohen's <i>d</i> , Pearson's <i>r</i>), indicating how they were calculated

Our web collection on [statistics for biologists](#) contains articles on many of the points above.

Software and code

Policy information about [availability of computer code](#)

Data collection	Tanon (4600), Image pro plus (version 6.0), MaxQuant (version 2.0.3.0), Alphafold (version3.0), Analyst (version 1.6.2)
Data analysis	MultiQuant 2.1.1 software, Graph Pad Prism (Version 9.5.0), Image J (version 1.53k), SPSS Statistics (version 21.0)

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors and reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Portfolio [guidelines for submitting code & software](#) for further information.

Data

Policy information about [availability of data](#)

- All manuscripts must include a [data availability statement](#). This statement should provide the following information, where applicable:
- Accession codes, unique identifiers, or web links for publicly available datasets
 - A description of any restrictions on data availability
 - For clinical datasets or third party data, please ensure that the statement adheres to our [policy](#)

TCGA database for expression of ACSL4 database: <http://gepia.cancer-pku.cn/>
GSE23400 dataset: <https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE23400>
GSE44021 dataset: <https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE44021>
The pictures of Figure 3b and Supplemental Figure 4a were download from GEPIA database (<http://gepia.cancer-pku.cn/>). And the parameters were set as: gene

symbol, ACSL4/ACSL1/ACSL3/ACSL5; |log2FC|, 1; P value, 0.01; Jitter size, 0.4. The quantification information is not available in the GPEIA database.

Research involving human participants, their data, or biological material

Policy information about studies with [human participants or human data](#). See also policy information about [sex, gender \(identity/presentation\), and sexual orientation](#) and [race, ethnicity and racism](#).

Reporting on sex and gender	These reports have shown Acs14 gene is located X- chromosome. we did not observe a surplus of tumor formation in females over males due to no a marked divergence of ACSL4 protein expression between genders
Reporting on race, ethnicity, or other socially relevant groupings	No
Population characteristics	In this investigation, samples were collected from patients diagnosed with precancerous lesions (n=17), ESCC (n=22) and corresponding adjacent tissues (n=16) based on pathological diagnosis results and tissue H&E staining.
Recruitment	The formalin-fixed paraffin-embedded tissue samples from patients diagnosed as esophageal squamous cell carcinoma or precancerous lesions were collected. The selection of participants was based solely on the pathological diagnosis results and tissue H&E staining. There was no bias in the participant selection process.
Ethics oversight	This study were approved by Ethics Committee of Affiliated Cancer Hospital of Zhengzhou University (Zhengzhou, China) Written informed consent was provided by all patients for the use of the tissue samples.

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☒ Life sciences ☐ Behavioural & social sciences ☐ Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see [nature.com/documents/nr-reporting-summary-flat.pdf](https://www.nature.com/documents/nr-reporting-summary-flat.pdf)

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	Statistical methods were not employed for pre-determining the sample size of experiments. The specific sample sizes for experiments are detailed either in the Methods section or in the Figure legends. Maximum available samples were utilized for all experiments.
Data exclusions	No data were excluded.
Replication	The number (n) of biological replicates or number of mice was presented in Results or Figure legends. For example, in CDX models of KYSE150, each group had eight rats. For cell experiments, all results were repeated three times.
Randomization	Each mouse can be assigned a number, and then allocated to different treat group according to the number in a random number table. In mice experiments, mice were randomly divided into different group with treatment. Each mouse can be assigned a number, and then allocated to different treat group according to the number in a random number table. Mice were randomly divided into mock, sh-1, and sh-5 group in CDX models, and mice also were randomly divided into water and 4NQO treatment group according to the principle as above.
Blinding	Blinding was not relevant to our study as the experimental design did not involve any subjective assessments or interventions. Therefore, collection and analysis of in vivo and vitro experiments involving different experimental groups, we were not blinded to the group allocations. For image analysis, we need to find the relevant time points of 4NQO treatment or distinguish adjacent and cancer when taking picture.

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

n/a	Involved in the study
☐	☒ Antibodies
☐	☒ Eukaryotic cell lines
☒	☐ Palaeontology and archaeology
☐	☒ Animals and other organisms
☒	☐ Clinical data
☒	☐ Dual use research of concern
☒	☐ Plants

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☒	☐ Flow cytometry
☒	☐ MRI-based neuroimaging

Antibodies

Antibodies used

GAPDH (ZSGB-BIO, TA-08, 1:3000 for WB)
 β -Tubulin (ZSGB-BIO, TA-10 1:3000 for WB)
 β -Tubulin (HUABIO, EM0103 1:5000 for WB)
p-AKT S473 (CST, 4060S, 1:1000 for WB)
p-p38 (T180/Y182) (CST, 9211S, 1:1000 for WB, 1:100 for IHC)
p-ERK1/2 (Thr202/Tyr204) (CST,4370S, 1:2000 for WB, 1:200 for IHC)
p-STAT3 S727 (CST, 9134S, 1:1000 for WB)
p-JNK2 (T183/Y185) (CST, 4668S, 1:1000 for WB)
T-AKT (CST, 9272S, 1:1000 for WB)
T-p38 (CST, 9212S, 1:1000 for WB)
T-p38 (abcam, ab170099, 1:50 for IP)
T-ERK1/2 (CST, 4695S, 1:1000 for WB)
T-JNK (CST, 9252S, 1:1000 for WB)
Ki67 (Servicebio, GB151499-100, 1:400 for IHC)
Flag-tag (MBL,M185-3MS, 1:50 for IP, 1:10000 for WB)
V5-tag (CST, 13202S, 1:1000 for WB, 1:50 for P)
HA-tag (Santa cruz, sc-7392, 1:100 for IP, 1:1000 for WB)
GST-tag (Santa cruz, sc-138, 1:1000 for WB)
normal mouse IgG (Santa cruz, sc-2025, 1:100 for IP)
normal rabbit IgG (CST, 2729S, 1:100 for IP)
ACSL4 (proteintech, 1:2000 for WB)
ACSL4 (Santa cruz, sc-365230, 1:250 for IP)
p-ACSL4 (Biolyx, AB007346-1, 1:500 for WB, 1:200 for IHC) Thr679-ACSL4 phosphorylation-specific antibody p-ACSL4 (Thr679) was ordered and produced by BioLyx Technology (<https://www.biolyxntec.com/advantage>) (Product ID: AB007346), and the sequence is CRLSPEPWTPETGLVT.
T-SRC (CST, 2108S, 1:1000 for WB)
p-SRC S416 (CST, 6943S, 1:1000 for WB)
p-SRC S416 (Gene Tex, GTX24816, 1:100 for IHC)
HSPA1A (Santa cruz, sc-59569, 1:500 for WB)
HSP9 β (Abcam, ab203085, 1:1000 for WB)
HSPA8 (CUSABIO, CSB-PA03249A0Rb, 1:1000 for WB)
ACSL1 (Huabio, HA601112, 1:1000 for WB)
ACSL3 (Proteintech, 20710-1-AP, 1:1000 for WB)
ACSL5 (Proteintech, 15708-1-AP, 1:1000 for WB)
ACSL6 (CUSABIO, CSB-PA892139LA01HU, 1:500 for WB)
Goat anti-rabbit IgG H & L (HRP) (ZSGB-Bio, ZB2306, 1:5000 for WB), Goat anti-mouse IgG H & L (HRP) (ZSGB-Bio, ZB2305, 1:5000 for WB)

Validation

ZSGB-BIO TA-08 validated for WB by manufacturer. <http://www.zsbio.com/product/TA-08>
ZSGB-BIO, TA-10 validated for WB by manufacturer. <http://www.zsbio.com/product/TA-10>
CST, 4060 validated for WB by manufacturer. <https://www.cellsignal.cn/products/primary-antibodies/phospho-akt-ser473-d9e-xp-rabbit-mab/4060>
CST, 9211S validated for WB and IHC by manufacturer. <https://www.cellsignal.com/products/primary-antibodies/phospho-p38-mapk-thr180-tyr182-antibody/9211>
CST,4370S validated for WB and IHC by manufacturer. <https://www.cellsignal.cn/products/primary-antibodies/phospho-p44-42-mapk-erk1-2-thr202-tyr204-d13-14-4e-xp-rabbit-mab/4370>
CST, 9134S validated for WB by manufacturer. <https://www.cellsignal.cn/products/primary-antibodies/phospho-stat3-ser727-antibody/9134>
CST, 4668S validated for WB by manufacturer. <https://www.cellsignal.cn/products/primary-antibodies/phospho-stat3-ser727-antibody/9134>
CST, 9272S validated for WB by manufacturer. <https://www.cellsignal.com/products/primary-antibodies/akt-antibody/9272>
CST, 9212S validated for WB by manufacturer. <https://www.cellsignal.cn/products/primary-antibodies/p38-mapk-antibody/9212>

abcam, ab170099 validated for IP by manufacturer. <https://www.abcam.cn/products/primary-antibodies/p38-alphamapk14-antibody-e229-ab170099.html>

CST, 4695S validated for WB by manufacturer. <https://www.cellsignal.com/products/primary-antibodies/p44-42-mapk-erk1-2-137f5-rabbit-mab/4695>

CST, 9252S <https://www.cellsignal.cn/products/primary-antibodies/sapk-jnk-antibody/9252>

Servicebio, GB151499-100 validated for IHC by manufacturer. <https://www.servicebio.cn/goodsdetail?id=21813>

MBL, M185-3MS validated for WB and IP by manufacturer. <https://ruo.mbl.co.jp/bio/dtl/A?pcd=M185-3MS>

CST, 13202S validated for WB and IP by manufacturer. <https://www.cellsignal.cn/products/primary-antibodies/v5-tag-d3h8q-rabbit-mab/13202>

Santa cruz, sc-7392 validated for WB and IP by manufacturer. <https://www.scbt.com/zh/p/ha-probe-antibody-f-7>

Santa cruz, sc-138 validated for WB by manufacturer. <https://www.scbt.com/p/gst-antibody-b-14>

Santa cruz, sc-2025 validated for IP by manufacturer. <https://www.scbt.com/p/normal-mouse-igg>

CST, 2729S validated for IP by manufacturer. <https://www.cellsignal.cn/products/primary-antibodies/normal-rabbit-igg/2729>

Proteintech, 22401-1-AP validated for WB by manufacturer. <https://www.ptgcn.com/Products/ACSL4-Antibody-22401-1-AP.htm>

Santa cruz, sc-365230 validated for IP by manufacturer. <https://www.scbt.com/zh/p/acsl4-antibody-f-4>

CST, 2108S validated for WB by manufacturer. <https://www.cellsignal.cn/products/primary-antibodies/src-antibody/2108>

CST, 6943S validated for WB by manufacturer. <https://www.cellsignal.com/products/primary-antibodies/phospho-src-family-tyr416-d49g4-rabbit-mab/6943>

Gene Tex, GTX24816 validated for IHC by manufacturer. <https://www.genetex.cn/Product/Detail/Src-phospho-Tyr416-antibody/GTX24816>

Santa cruz, sc-59569 validated for WB by manufacturer. <https://www.scbt.com/p/hsp-70-antibody-4g4>

Abcam, ab203085 validated for WB by manufacturer. <https://www.abcam.cn/products/primary-antibodies/hsp90-beta-antibody-epr16621-ab203085.html#:~:text=Immunohi>

CUSABIO, CSB-PA03249A0Rb, <https://www.cusabio.com/Recombinant-Protein/Recombinant-Human-Heat-shock-cognate-71-kDa-protein-HSPA8--12927175.html#:~:text=The%20human%20heat%20shock%20cognate%2071%20kDa%20protein>

Huabio, HA601112, <http://www.huabio.cn/products/ACSL1-antibody-HA601112>

Proteintech, 20710-1-AP <https://www.ptgcn.com/products/ACSL3-Antibody-20710-1-AP.htm>

CUSABIO, CSB-PA892139LA01HU, <https://www.cusabio.cn/Polyclonal-Antibodies/ACSL6-Antibody-3133935.html>

ZSGB-Bio, ZB2306, <http://www.zsbio.com/product/ZB-2306>

ZSGB-Bio, ZB2305, <http://www.zsbio.com/product/ZB-2305>

Eukaryotic cell lines

Policy information about [cell lines and Sex and Gender in Research](#)

Cell line source(s)	Human ESCC cell line including KYSE150, KYSE70, and KYSE450, human normal esophageal epithelium cell line HET-1A, HEK293T cell line and HEK293F cells were preserved and donated by Professor Ziming Dong (the Department of Pathophysiology, school of basic medical sciences of Zhengzhou University).
Authentication	Human normal esophageal epithelium cell line HET-1A, and human ESCC cell line including KYSE150, KYSE70, and KYSE450 were cytogenetically tested by STR authenticated.
Mycoplasma contamination	All cell lines were confirmed to be mycoplasma-free.
Commonly misidentified lines (See ICLAC register)	No such misidentified lines were used in the study.

Animals and other research organisms

Policy information about [studies involving animals](#); [ARRIVE guidelines](#) recommended for reporting animal research, and [Sex and Gender in Research](#)

Laboratory animals	SCID/cb17 female mice (6-7 weeks) were obtained from Vital River Laboratory Animal Technology (Beijing). C57BL/6 mice (6-7 weeks) were acquired from The Animal Experimental Center of Zhengzhou University. Mice were kept in a specific pathogen-free (SPF) environment, subjected to a 12-hour light/12-hour dark cycle. The room temperature was controlled at 20 °C ± 2 with 60% humidity. Mice had free access to water and rodent diet (Beijing keao xieli feed co., LTD, 1016706476803973120). All animal procedures in this study were approved by the Zhengzhou University Institutional Animal Care and Use Committee.
Wild animals	C57BL/6 mice (7 weeks) were acquired from The Animal Experimental Center of Zhengzhou University, and Genotyping of Acsl4 wild-type (WT) was acquired from mice in the process of Acsl4 knock out.
Reporting on sex	Based on gender, we categorized the experimental groups: WT-male, WT-female, CKO-Male, and CKO-Female for Supplemental Figure 6.
Field-collected samples	No field-collected samples were used in the study.
Ethics oversight	This study was approved by the Research Ethics Committee of Zhengzhou University. All animal procedures in this study were approved by the Zhengzhou University Institutional Animal Care and Use Committee (ZZUIRB2022-70).

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Plants

Seed stocks

n

Novel plant genotypes

n

Authentication

n