

Table S1 Clinical cohorts analyzed in this study

	TCGA cohort	GSE17536	GSE19417	GSE84437	GSE103479
No. of patients	1021	177	64	433	154
Sex					
Female	407 (39.86%)	81 (45.76%)	18 (28.13%)	137 (31.64%)	67 (43.51%)
Male	614 (60.14%)	96 (54.24%)	46 (71.87%)	296 (68.36%)	87 (56.49%)
Age (years)					
<65	171 (16.75%)	78 (44.07%)		267 (61.66%)	49 (31.82%)
≥65	850 (83.25%)	99 (55.93%)		166 (38.34%)	105 (68.18%)
Stage					
I	73 (7.15%)	24 (13.56%)		11 (2.54%)	1 (0.65%)
II	190 (18.61%)	57 (32.20%)		38 (8.78%)	6 (3.90%)
III	597 (58.47%)	57 (32.20%)		92 (21.25%)	109 (70.78%)
IV	161 (15.77%)	39 (22.04%)		292 (67.43%)	38 (24.67%)
Cancer type					
COAD	430 (42.12%)				
ESCA	72 (7.05%)				
READ	153 (14.99%)				
STAD	366 (35.84%)				
Grade					
Moderate		134 (75.71%)	27 (42.19%)		117 (75.97%)
Well		16 (9.04%)	6 (9.38%)		8 (5.19%)
Poor		27 (15.25%)	31 (48.43%)		29 (18.84%)

TCGA, The Cancer Genome Atlas; ESCA, esophageal carcinoma; STAD, stomach adenocarcinoma; COAD, colon adenocarcinoma; READ, rectum adenocarcinoma.

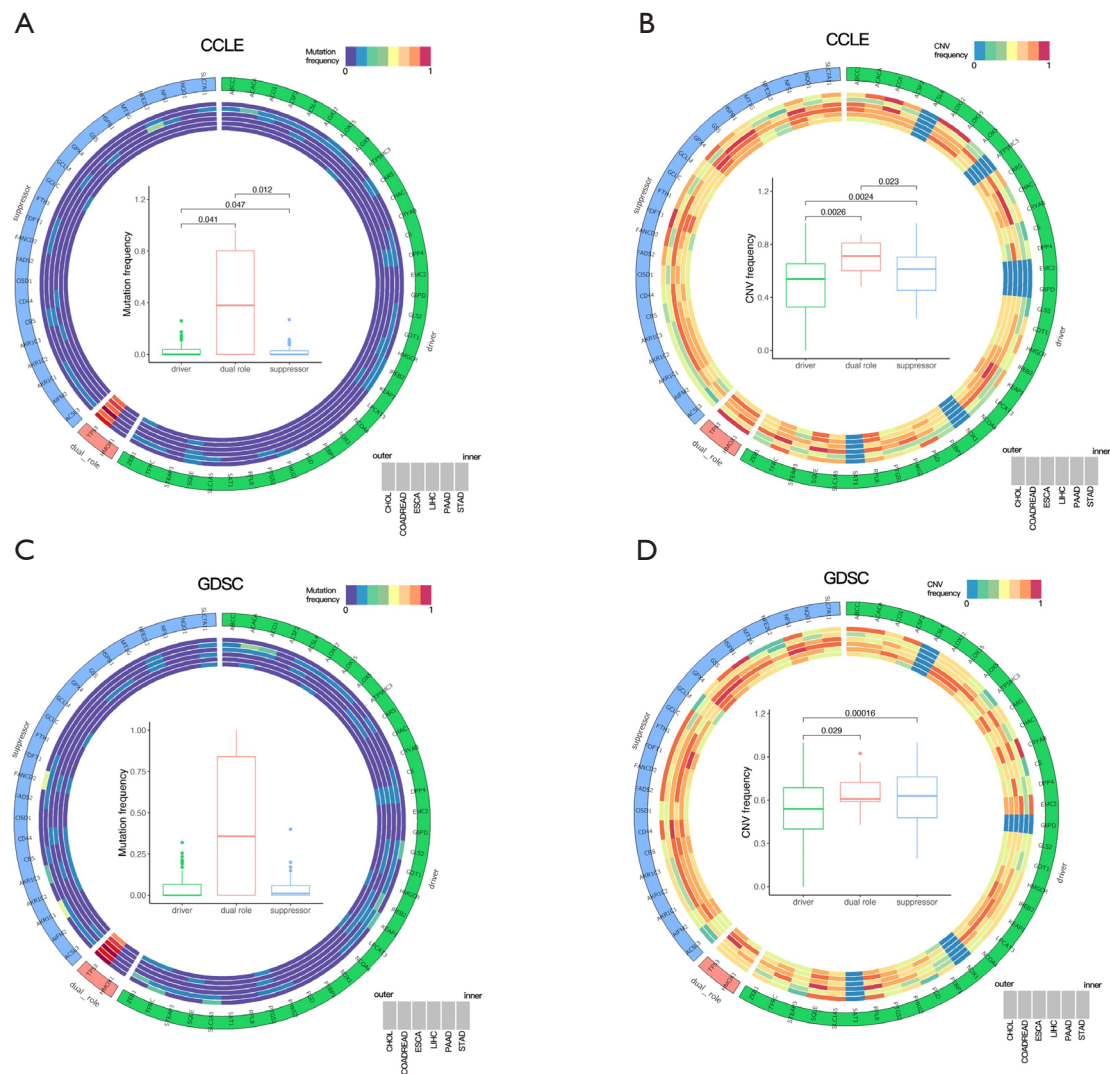
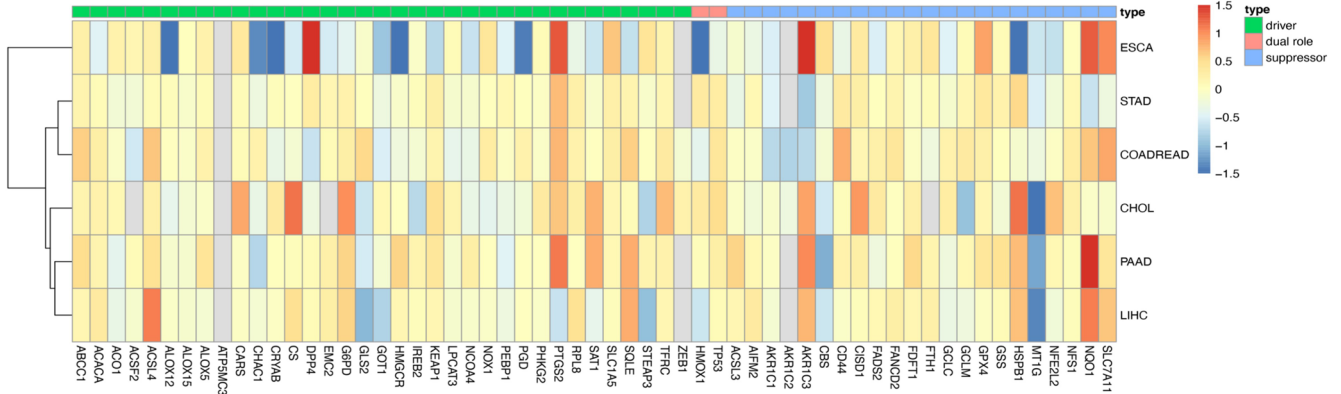


Figure S1 Genomic alterations of ferroptosis-associated signature in different cancer cell lines. (A) Mutation frequency of the ferroptosis-related genes in the cell lines of CCLC. (B) Copy number variation (CNV) of the ferroptosis-related genes in the cell lines of The Cancer Cell Line Encyclopedia (CCLE) database. (C) Mutation frequency of the ferroptosis-related genes in the cell lines of The Genomics of Drug Sensitivity in Cancer (GDSC) database. (D) CNV of the ferroptosis-related genes in the cell lines of GDSC. CCLC, The Cancer Cell Line Encyclopedia; GDSC, The Genomics of Drug Sensitivity in Cancer; ESCA, esophageal carcinoma; STAD, stomach adenocarcinoma; COAD, colonic adenocarcinoma; READ, rectum adenocarcinoma; PAAD, pancreatic adenocarcinoma; CHOL, intrahepatic cholangiocarcinoma; LIHC, liver hepatocellular carcinoma.

A Somatic mutation



B

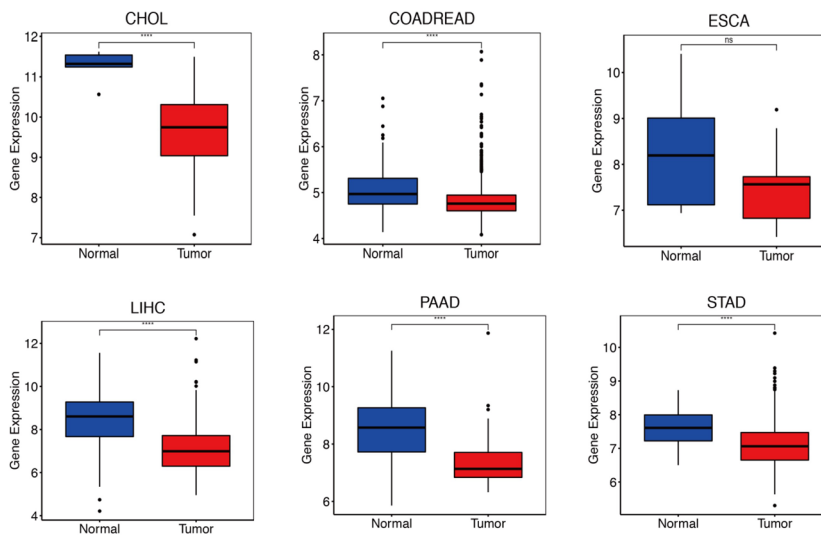


Figure S2 Genomic alterations in ferroptosis-related signature in the Gene Expression Omnibus (GEO) cohort. (A) RNA expressions of the ferroptosis-related signature in six cancers. (B) Box plots of MTIG expression across six cancers. **** $P < 0.0001$. ESCA, esophageal carcinoma; STAD, stomach adenocarcinoma; COADREAD, colon adenocarcinoma/rectum adenocarcinoma; LIHC, liver hepatocellular carcinoma; PAAD, pancreatic adenocarcinoma; CHOL, cholangiocarcinoma; ns, not significant.

Table S2 The 59 ferroptosis-related genes used in the ferroptosis-score model

Signature	Description	Entrez gene summary	Included in the ferroptosis score
<i>ACSL4</i>	acyl-CoA synthetase long-chain family member 4	The protein encoded by <i>ACSL4</i> is an isozyme of the long-chain fatty-acid-coenzyme A ligase family.	bad
<i>AKR1C1</i>	aldo-keto reductase family 1 member C1	This enzyme catalyzes the reaction of progesterone to the inactive form 20-alpha-hydroxy-progesterone. <i>AKR1C1</i> shares high sequence identity with three other gene members and is clustered with those three genes at chromosome 10p15-p14.	not included
<i>AKR1C2</i>	aldo-keto reductase family 1 member C2	<i>AKR1C2</i> shares high sequence identity with three other gene members and is clustered with those three genes at chromosome 10p15-p14.	not included
<i>AKR1C3</i>	aldo-keto reductase family 1 member C3	<i>AKR1C3</i> is clustered with those three genes at chromosome 10p15-p14. Three transcript variants encoding different isoforms have been found for this gene.	good
<i>ALOX15</i>	arachidonate 15-lipoxygenase	The encoded enzyme and its reaction products have been shown to regulate inflammation and immunity.	not included
<i>ALOX5</i>	arachidonate 5-lipoxygenase	Mutations in the promoter region of <i>ALOX5</i> lead to a diminished response to antileukotriene drugs used in the treatment of asthma and may also be associated with atherosclerosis and several cancers.	bad
<i>ALOX12</i>	arachidonate 12-lipoxygenase	The encoded enzyme acts on different polyunsaturated fatty acid substrates to generate bioactive lipid mediators including eicosanoids and lipoxins.	not included
<i>ATP5MC3</i>	ATP synthase membrane subunit c locus 3	<i>ATP5MC3</i> encodes a subunit of mitochondrial ATP synthase. Mitochondrial ATP synthase catalyzes ATP synthesis, utilizing an electrochemical gradient of protons across the inner membrane during oxidative phosphorylation.	good
<i>CARS</i>	Cysteinyl-tRNA synthetase	Predicted to enable several functions, including ATP binding activity; cysteine-tRNA ligase activity; and protein homodimerization activity. Predicted to be involved in cysteinyl-tRNA aminoacylation.	good
<i>CBS</i>	cystathionine beta-synthase	The protein encoded by <i>CBS</i> acts as a homotetramer to catalyze the conversion of homocysteine to cystathionine, the first step in the transsulfuration pathway.	not included
<i>CD44</i>	CD44 molecule	The protein encoded by this gene is a cell-surface glycoprotein involved in cell-cell interactions, cell adhesion and migration.	bad
<i>CHAC1</i>	ChaC glutathione-specific gamma-glutamyl acyltransferase 1	<i>CHAC1</i> encodes a member of the gamma-glutamylcyclotransferase family of proteins. The encoded protein has been shown to promote neuronal differentiation by deglaciation of the Notch receptor, which prevents receptor maturation and inhibits Notch signaling.	good
<i>CISD1</i>	CDGSH iron sulfur domain 1	<i>CISD1</i> encodes a protein with a CDGSH iron-sulfur domain and has been shown to bind a redox-active [2Fe-2S] cluster. The encoded protein has been localized to the outer membrane of mitochondria and is thought to play a role in regulation of oxidation.	not included
<i>CS</i>	citrate synthase	The protein encoded by <i>CS</i> is a Krebs tricarboxylic acid cycle enzyme that catalyzes the synthesis of citrate from oxaloacetate and acetyl coenzyme A.	good
<i>DPP4</i>	dipeptidyl-dipeptidase-4	The <i>DPP4</i> gene encodes dipeptidyl peptidase 4, which is identical to adenosine deaminase complexing protein-2, and to the T-cell activation antigen CD26.	good

Table S2 (continued)

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Signature	Description	Entrez gene summary	Included in the ferroptosis score
<i>FANCD2</i>	Fanconi anemia complementation group D2	The Fanconi anemia complementation group (FANC) currently includes <i>FANCA</i> , <i>FANCB</i> , <i>FANCC</i> , <i>FANCD1</i> (also called <i>BRCA2</i>), <i>FANCD2</i> , <i>FANCE</i> , <i>FANCF</i> , <i>FANCG</i> , <i>FANCI</i> , <i>FANCIJ</i> (also called <i>BRIP1</i>), <i>FANCL</i> , <i>FANCM</i> and <i>FANCN</i> (also called <i>PALB2</i>).	good
<i>GCLC</i>	glutamate-cysteine ligase catalytic subunit	Glutamate-cysteine ligase, also known as gamma-glutamylcysteine synthetase is the first rate-limiting enzyme of glutathione synthesis.	not included
<i>GCLM</i>	glutamate-cysteine ligase modifier subunit	Glutamate-cysteine ligase, also known as gamma-glutamylcysteine synthetase, is the first rate limiting enzyme of glutathione synthesis. The enzyme consists of two subunits, a heavy catalytic subunit and a light regulatory subunit.	good
<i>GLS2</i>	glutaminase 2	The protein encoded by <i>GLS2</i> is a mitochondrial phosphate-activated glutaminase that catalyzes the hydrolysis of glutamine to stoichiometric amounts of glutamate and ammonia.	good
<i>GPX4</i>	glutathione peroxidase 4	The protein encoded by <i>GPX4</i> belongs to the glutathione peroxidase family, members of which catalyze the reduction of hydrogen peroxide, organic hydroperoxides and lipid hydroperoxides, and thereby protect cells against oxidative damage.	not included
<i>GSS</i>	glutathione synthetase	The protein encoded by <i>GSS</i> functions as a homodimer to catalyze the second step of glutathione biosynthesis, which is the ATP-dependent conversion of gamma-L-glutamyl-L-cysteine to glutathione.	good
<i>HMGCR</i>	3-hydroxy-3- methylglutaryl-CoA reductase	HMG-CoA reductase is the rate-limiting enzyme for cholesterol synthesis and is regulated via a negative feedback mechanism mediated by sterols and non-sterol metabolites derived from mevalonate, the product of the reaction catalyzed by reductase.	good
<i>HSPB1</i>	heat shock protein beta 1	This gene encodes a member of the small heat shock protein (HSP20) family of proteins.	bad
<i>CRYAB</i>	crystallin alpha B	Mammalian lens crystallins are divided into alpha, beta, and gamma families. Alpha crystallins are composed of two gene products: alpha-A and alpha-B, for acidic and basic, respectively.	bad
<i>LPCAT3</i>	lysophosphatidylcholine acyltransferase 3	Enables 1-acylglycerophosphocholine O-acyltransferase activity; 1-acylglycerophosphoethanolamine O-acyltransferase activity; and 1-acylglycerophosphoserine O-acyltransferase activity.	good
<i>MT1G</i>	metallothionein-1G	Enables zinc ion binding activity. Involved in cellular response to metal ion; cellular response to vascular endothelial growth factor stimulus; and negative regulation of growth.	good
<i>NCOA4</i>	nuclear receptor coactivator 4	<i>NCOA4</i> encodes an androgen receptor coactivator. The encoded protein interacts with the androgen receptor in a ligand-dependent manner to enhance its transcriptional activity.	not included
<i>PTGS2</i>	prostaglandin-endoperoxide synthase 2	<i>PTGS2</i> encodes the inducible isozyme. It is regulated by specific stimulatory events, suggesting that it is responsible for the prostanoid biosynthesis involved in inflammation and mitogenesis.	bad
<i>RPL8</i>	ribosomal protein L8	The protein belongs to the L2P family of ribosomal proteins. It is located in the cytoplasm.	good

Table S2 (continued)

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Signature	Description	Entrez gene summary	Included in the ferroptosis score
<i>SAT1</i>	spermidine/spermine N1-acetyltransferase 1	The protein encoded by this gene belongs to the acetyltransferase family, and is a rate-limiting enzyme in the catabolic pathway of polyamine metabolism.	not included
<i>SLC7A11</i>	solute carrier family 7 member 11	This gene encodes a member of a heteromeric, sodium-independent, anionic amino acid transport system that is highly specific for cysteine and glutamate.	good
<i>FDFT1</i>	farnesyl-diphosphate farnesyltransferase 1	<i>FDFT1</i> encodes a membrane-associated enzyme located at a branch point in the mevalonate pathway.	good
<i>TFRC</i>	transferrin receptor	<i>TFRC</i> encodes a cell surface receptor necessary for cellular iron uptake by the process of receptor-mediated endocytosis	good
<i>TP53</i>	tumor protein 53	<i>TP53</i> encodes a tumor suppressor protein containing transcriptional activation, DNA binding, and oligomerization domains.	not included
<i>EMC2</i>	ER membrane protein complex subunit 2	Contributes to membrane insertase activity. Involved in protein insertion into ER membrane by stop-transfer membrane-anchor sequence and tail-anchored membrane protein insertion into ER membrane.	not included
<i>AIFM2</i>	apoptosis inducing factor mitochondria associated 2	<i>AIFM2</i> encodes a flavoprotein oxidoreductase that binds single stranded DNA and is thought to contribute to apoptosis in the presence of bacterial and viral DNA.	good
<i>PHKG2</i>	phosphorylase kinase catalytic subunit gamma 2	Phosphorylase kinase is a polymer of 16 subunits, four each of alpha, beta, gamma and delta. The alpha subunit includes the skeletal muscle and hepatic isoforms, encoded by two different genes.	good
<i>ACO1</i>	aconitase 1	The protein encoded by <i>ACO1</i> is a bifunctional, cytosolic protein that functions as an essential enzyme in the TCA cycle and interacts with mRNA to control the levels of iron inside cells.	not included
<i>FTH1</i>	ferritin heavy chain 1	<i>FTH1</i> encodes the heavy subunit of ferritin, the major intracellular iron storage protein in prokaryotes and eukaryotes.	good
<i>STEAP3</i>	six-transmembrane epithelial antigen of prostate 3	<i>STEAP3</i> encodes a multipass membrane protein that functions as an iron transporter. The encoded protein can reduce both iron (Fe ³⁺) and copper (Cu ²⁺) cations.	not included
<i>NFS1</i>	cysteine desulfurase	<i>NFS1</i> uses alternate in-frame translation initiation sites to generate mitochondrial forms and cytoplasmic/nuclear forms.	good
<i>ACSL3</i>	acyl-CoA synthetase long-chain family member 3	The protein encoded by <i>ACSL3</i> is an isozyme of the long-chain fatty-acid-coenzyme A ligase family.	good
<i>ACACA</i>	Acetyl-CoA carboxylase alpha	The enzyme is under long term control at the transcriptional and translational levels and under short term regulation by the phosphorylation/ dephosphorylation of targeted serine residues and by allosteric transformation by citrate or palmitoyl-CoA.	good
<i>PEBP1</i>	phosphatidylethanolamine-binding protein 1	<i>PEBP1</i> encodes a member of the phosphatidylethanolamine-binding family of proteins and has been shown to modulate multiple signaling pathways, including the MAP kinase (MAPK), NF-kappa B, and glycogen synthase kinase-3 (GSK-3) signaling pathways.	good
<i>ZEB1</i>	zinc finger E-box-binding homeobox 1	<i>ZEB1</i> encodes a zinc finger transcription factor. The encoded protein likely plays a role in transcriptional repression of interleukin 2.	good

Table S2 (continued)

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Signature	Description	Entrez gene summary	Included in the ferroptosis score
<i>SQLE</i>	squalene monooxygenase	Squalene epoxidase catalyzes the first oxygenation step in sterol biosynthesis and is thought to be one of the rate-limiting enzymes in this pathway.	bad
<i>FADS2</i>	fatty acid desaturase 2/acyl-CoA 6-desaturase	The protein encoded by <i>FADS2</i> is a member of the fatty acid desaturase (FADS) gene family. Desaturase enzymes regulate unsaturation of fatty acids through the introduction of double bonds between defined carbons of the fatty acyl chain.	good
<i>NFE2L2</i>	nuclear factor, erythroid 2 like 2	<i>NFE2L2</i> encodes a transcription factor which is a member of a small family of basic leucine zipper (bZIP) proteins.	not included
<i>KEAP1</i>	kelch-like ECH- associated protein 1	<i>KEAP1</i> encodes a protein containing KELCH-1 like domains, as well as a BTB/POZ domain.	not included
<i>NQO1</i>	quinone oxidoreductas e-1	<i>NQO1</i> is a member of the NAD(P)H dehydrogenase (quinone) family and encodes a cytoplasmic 2-electron reductase.	good
<i>NOX1</i>	NADPH oxidase 1	<i>NOX1</i> encodes a member of the NADPH oxidase family of enzymes responsible for the catalytic one-electron transfer of oxygen to generate superoxide or hydrogen peroxide.	good
<i>ABCC1</i>	ATP binding cassette subfamily C member 1	The protein encoded by <i>ABCC1</i> is a member of the superfamily of ATP-binding cassette (ABC) transporters. ABC proteins transport various molecules across extra-and intra-cellular membranes.	good
<i>SLC1A5</i>	solute carrier family 1 member 5	<i>SLC1A5</i> encodes a sodium-dependent neutral amino acid transporter that can act as a receptor for RD114/type D retrovirus	bad
<i>GOT1</i>	glutamic-oxaloacetic transaminase 1	<i>GOT1</i> plays a role in amino acid metabolism and the urea and tricarboxylic acid cycles.	good
<i>G6PD</i>	glucose-6-phosphate dehydrogenase	<i>G6PD</i> encodes glucose-6-phosphate dehydrogenase. This protein is a cytosolic enzyme encoded by a housekeeping X-linked gene whose main function is to produce NADPH, a key electron donor in the defense against oxidizing agents and in reductive biosynthetic reactions.	good
<i>PGD</i>	phosphoglycerate dehydrogenase	<i>PGD</i> is the second dehydrogenase in the pentose phosphate shunt. Deficiency of this enzyme is generally asymptomatic, and the inheritance of this disorder is autosomal dominant.	good
<i>IREB2</i>	iron response element-binding protein 2	The protein encoded by <i>IREB2</i> is an RNA-binding protein that acts to regulate iron levels in the cells by regulating the translation and stability of mRNAs that affect iron homeostasis under conditions when iron is depleted.	good
<i>HMOX1</i>	heme oxygenase 1	Heme oxygenase, an essential enzyme in heme catabolism, cleaves heme to form biliverdin, which is subsequently converted to bilirubin by biliverdin reductase, and carbon monoxide, a putative neurotransmitter.	not included
<i>ACSF2</i>	acyl-CoA synthetase family member 2	Enables medium-chain fatty acid-CoA ligase activity. Predicted to be involved in fatty acid metabolic process. Predicted to be located in mitochondrial matrix.	not included

Table S3 Prognostic effect of the ferroptosis-score in multivariable models

Parameter	Univariable analysis		Multivariable analysis	
	HR (95% CI)	P value	HR (95% CI)	P value
TCGA cohort				
Age (years, ≥65 vs. <65)	1.40 (1.10-1.78)	0.007	1.57 (1.22-2.01)	<0.001
Sex (male vs. female)	1.34 (1.05-1.70)	0.02	1.10 (0.86-1.41)	0.46
Tumor stage (III&IV vs. I&II)	1.45 (1.10-2.01)	0.009	1.89 (1.38-2.57)	<0.001
Cancer types (dummy variable)		<0.001		<0.001
ESCA vs. COAD	2.94 (1.98-4.38)	<0.001	3.01 (1.94-4.68)	<0.001
READ vs. COAD	0.84 (0.54-1.29)	0.42	1.03 (0.70-1.51)	0.89
STAD vs. COAD	2.61 (2.01-3.40)	<0.001	2.80 (2.14-3.66)	<0.001
Ferroptosis-score (high vs. low)	0.67 (0.52-0.87)	0.002	0.75 (0.57-0.98)	0.04
GSE17536				
Age (years, ≥65 vs. <65)	0.96 (0.60-1.52)	0.85		
Sex (male vs. female)	0.91 (0.57-1.44)	0.68		
Tumor stage (III&IV vs. I&II)	4.22 (2.39-7.46)	<0.001	3.84 (2.16-6.82)	<0.001
Grade (moderate & well vs. poor)	0.46 (0.26-0.80)	0.006	0.53 (0.30-0.94)	0.03
Ferroptosis score (high vs. low)	0.57 (0.34-0.96)	0.04	0.56 (0.33-0.94)	0.03
GSE103479				
Age (years, ≥65 vs. <65)	2.98 (1.51-5.90)	0.02	3.27 (1.64-6.51)	0.001
Sex (male vs. female)	0.97 (0.59-1.62)	0.92		
Tumor stage (III&IV vs. I&II)	0.73 (0.26-2.01)	0.54		
Grade (Moderate & Well vs. Poor)	0.78 (0.40-1.50)	0.45		
Ferroptosis score (high vs. low)	0.55(0.32-0.96)	0.04	0.47 (0.27-0.83)	0.009
GSE84437				
Age (years, ≥65 vs. <65)	1.40 (1.07-1.84)	0.02	1.44 (1.09-1.89)	0.009
Sex (male vs. female)	1.25 (0.93-1.70)	0.14		
Tumor stage (III&IV vs. I&II)	3.74 (1.92-7.29)	<0.001	3.55 (1.82-6.93)	<0.001
Ferroptosis score (high vs. low)	0.68 (0.51-0.90)	0.006	0.67 (0.52-0.91)	0.009
GSE19417				
Sex (male vs. female)	1.26 (0.68-2.32)	0.46		
Grade (moderate & well vs. poor)	0.68 (0.39-1.17)	0.16		
Ferroptosis score (high vs. low)	0.53 (0.28-1.01)	0.05	0.53 (0.28-1.01)	0.05

HR, hazard ratio; 95% CI: 95% confidence interval; TCGA, The Cancer Genome Atlas; ESCA, esophageal carcinoma; COAD, colonic adenocarcinoma; READ, rectum adenocarcinoma; STAD, stomach adenocarcinoma.

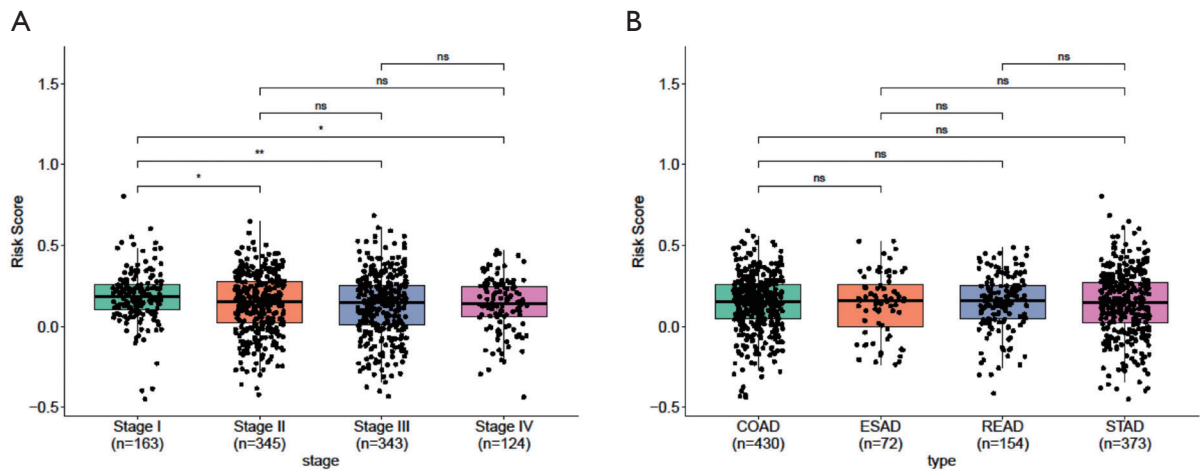


Figure S3 The relationship of the ferroptosis-score with tumor stage and cancer types. (A) The box plots indicate that there was no difference in tumor stage between clusters with high and low ferroptosis scores. (B) The box plots indicate that there was no difference in 4 cancer types between clusters with high and low ferroptosis scores. * $P < 0.05$; ** $P < 0.01$. COAD, colon adenocarcinoma; ESAD, esophageal adenocarcinoma; READ, rectum adenocarcinoma; STAD, stomach adenocarcinoma; ns, not significant.