

Table S1. A list of guide sequences used for CRISPR-Cas9 KO

	Target gene	guide sequence
Control KO sequences	sgCTRL	ACTATCATGGCACCCAATTG
	sgLACZ	TGCGAATACGCCACGCGAT
Detoxifying enzyme screen	SEPHS2_g1	GGAGGGACGGCAGTGACCGG
	SEPHS2_g2	GGTGGTCTCCAGAGAAGAGG
	SEPHS2_g3	GGCAGGACTTCAATAACTCG
	DUT_g1	CCTGTACAGGTCGTAGCCCG
	DUT_g2	TTTGCAGCCAAGCCTGACCG
	DUT_g3	CCGGCTCTCCGAGCACGCCA
	IMPDH2_g1	GCACAGCAGCTCTTCAACTG
	IMPDH2_g2	TGGGATGGCCATAGCAATGG
	IMPDH2_g3	GAGAAAATCAATGTCCCTGG
	ALOX15B_g1	GCTGTTGCTGGAGCACAGGG
	ALOX15B_g2	GCGGTGTCAGCTGGAACCAG
	ALOX15B_g3	GGGGACCCTGGTGCTGCAGG
	GRHPR_g1	GCAGCTGTGAGGTGGAGCAG
	GRHPR_g2	GAGGAGGCAGAGCAGGCCGT
	GRHPR_g3	GGAGACTGCGAGTTCGGCGG
	ADO_g1	GACAAGCTAGACGCGGGCGG
	ADO_g2	GCTGCAGTGTGGCCTTGCGC
	ADO_g3	GCATGCCGTGCATGCCCGGG
	RDH8_g1	GGAGGTCACGAGTCCAGGGA
	RDH8_g2	GGGAAGAAGGAGACTG
	RDH8_g3	TCTGGGGCAGACCCTACCG
	ADSS_g1	GTTCGCCGAGACCTACCCGG
	ADSS_g2	GGGCGACGAAGGCAAAGGGA
	ADSS_g3	GGTGGTGGACCTGCTGGCGC
	NAMPT_g1	CCGGCCCGAGATGAATCCTG
	NAMPT_g2	TGTAGGAGTCGGTGGCCAGG
	NAMPT_g3	GGGATGGAACACTACATTCTTG
	COMT_g1	ACTGTGCCGCCATCACCCAG
	COMT_g2	GAACCACGTGCTGCAGCATG
	COMT_g3	CCTACTGCGAGCAGAAGGAG
	PGP_g1	GAACAGCAGCGTGTCCACGT
	PGP_g2	GAAGACCTCCAGGCTGGCGC
	PGP_g3	GAAGCCCAGGCGCTTGCCGC
GDPD5_g1	GAGGCCAGCGATGTATGCCA	
GDPD5_g2	GGTCATCCTGGCTTCCACGG	
GDPD5_g3	GGTGGTGGCCATGTCGGCCG	
Selenium metabolism related	SCLY_g1	GATGCCCGGCAGCAGTGGGG
	SCLY_g2	GAAGCAGCGCGTGGATGTGG
	SEPSECS_g1	GAACCGCGAGAGCTTCGCGG
	SEPSECS_g2	TGAGGTGCTCATGCGAGCGG
	SLC3A2_g1	TGAGTGGCAAAATATCACCA
	SLC3A2_g2	CATGAGCCAGGACACCGAGG
	PSTK_g1	AAACTGATCAGACACTCCGA
	PSTK_g2	GGAGGCCACAGAGGACGCGAG

Anti-ferroptosis factors	GPX4_g1	AGAGATCAAAGAGTTCGCCG
	GPX4_g2	GTGGATGAAGATCCAACCCA
	FSP1_g1	GGAGAGCAGCCACATTGTGG
	FSP1_g2	GCAGCTGCACGCCCTTCCGG
Transporter screen	IMPDH1_g1	GCCCTGCAGGTACCTCACTG
	DBH_g1	GAATGTCAGCTACACCCAGG
	SLC26A11_g1	CAGGAGGGACATAATGGCGG
	SLC26A11_g2	GTAGAGCCTCACCCAAACGC
	SLC4A1_g1	GGAGCTGAGATGGATGGAGG
	SLC4A1_g2	GCTTCCTCAAACACCAGCAG
	SLC4A3_g1	GAAGTAGATGAAGAGCACAG
	SLC4A3_g2	GAGGACGCCGAGCACAGCGG
	SLC7A11_g1	AATGTAGCGTCCAATGCCA
	SLC7A11_g2	CATGGAGCCAAAGCAGGAGA
	SLC39A10_g1	CAAGATGATGATGCAAACGA
	SLC39A10_g2	AACAGAGACCAATAAAACCC
	SLC39A14_g1	GGAGAATGAGCAGACGGAGG
	SLC39A14_g2	GGCCCACTCCCACATCCAGG
	SLC39A6_g1	GGCCTGCACAATTTAGCGA
	SLC39A6_g2	GAAGACAGATGACTGAAAAG
	SLC39A8_g1	AGAGCTCCTTACCTAACTCG
	SLC39A8_g2	GCCAGGGCTAGCCTCAGCG
	SLC26A10_g1	GAAGTGCATTTAAGTCCTGG
	SLC26A10_g2	AGTGTAGAGTCCAACACCG
	SLC26A4_g1	CAGGTCATGGCAGCGCCAGG
	SLC26A4_g2	GTTGTGAAGCCACCAACCAA
	SLC26A9_g1	GAAGGACCGGACATACCCAG
	SLC26A9_g2	AATCAGGATCTGCAGGCCGG
	SLC26A2_g1	GGAGCCACTGCAAAACAGGA
	SLC26A2_g2	GCAGGGAATAAGCAATGGAC
	SLC26A5_g1	GACATGCACAGCTGCTGCGG
	SLC26A5_g2	AAATCCTTGCAGCAACCCAG
	SLC1A4_g1	GAGTTGCGCAATGAACACCG
	SLC1A4_g2	GCAGCAGGCGTGCCAGCTGG
	STARD8_g1	GAGCAGGAGGCACATTCAGG
	STARD8_g2	GTAGATCTGGAGGAAAGTGG
	SLC13A3_g1	GGAGGGCAAGATGCCCATGA
	SLC13A3_g2	CCACATGGACAAGAACGAGG
	GABRA3_g1	ATCAAGACGACAAGAACCCG
	GABRA3_g2	GCATTTGGAAGATTTTCCCA
	KCNE4_g1	GGTGAGGTGCACGTCCGGGG
	KCNE4_g2	GCTACATGAAATCCAAGAGG
	ABCB4_g1	AGAGGGGCCAGCTGAGTGG
	ABCB4_g2	TGGCTGTGGGAAGAGCACGG
SLC26A1_g1	GAGCCTCTGCAGCAGGGCAG	
SLC26A1_g2	AGCATCTCACGCAGACCCCG	
SLC26A7_g1	GCAGTTCAACAGGTGACCCA	
SLC26A7_g2	CAAGTGCTGCTGCCATGGGA	
Etc.	SEPHS1_g1	GCAGGAACATCTGTAACAGG
	SEPHS1_g2	CTGGATTGCTCCTGGGAGGAG

Table S2. List of cell lines used in the study, their sensitivity to the toxicity of SEPHS KO, and relevant information (source, validation, culture conditions)

SEPHS2 Sensitivity (1.0 equals viability of CTRL control cells)										
type	origin	name	SEPHS2 g1	SEPHS2 g2	AVERAGE 2 GUIDES	CTRL Growth Rate	RRID	source	STR profiling	culture medium
non-cancer	immortalized liver	THLE-2	0.93	0.96	0.95	1.13	CVCL_3803	Kind gift of the Kwan Yong Choi lab (POSTECH, KOREA)	Yes, Confirmed	BEBM, 10% FBS, 5ng/ml EGF, 70ng/ml phosphoethanolamine, additives (include BPE, insulin, hydrocortisone, retinoic acid, transferrin, triiodothyronine and hEGF/ discard GA-1000 and Epinephrine) from BEGM Bullet Kit (Lonza)
	normal colon fibroblast	CCD-18Co	1.18	0.97	1.08	2.35	CVCL_2379		Yes, Confirmed	EMEM, 10% FBS, 100units/ml penicillin and streptomycin
	immortalized breast	MCF10A	0.94	0.87	0.90	5.12	CVCL_0598		Yes, Confirmed	DMEM/F12, 5 %horse serum, 20ng/ml EGF, 0.5µg/ml hydrocortisone, 100ng/ml cholera toxin, 10µg/ml insulin, 100units/ml penicillin and streptomycin
	immortalized breast	MCF12A	0.88	0.83	0.86	1.79	CVCL_3744	Kind gift of the Michael Green lab (UMMS, USA)	Yes, Confirmed	DMEM/F12, 5 %horse serum, 20ng/ml EGF, 0.5µg/ml hydrocortisone, 100ng/ml cholera toxin, 10µg/ml insulin, 100units/ml penicillin and streptomycin
	prostate	PNT1a	0.93	0.81	0.87	2.36		Kind gift of the Arthur Mercurio lab (UMMS, USA)	Yes, Confirmed *(PubMed=11416159)	RPMI, 10% FBS, 100 units/ml penicillin and streptomycin
	Primary lung	Lung Fibroblast (LF), human	0.99	0.84	0.92	2.24		ATCC	purchased in 2018 December	Fibroblast Basal Medium, 2% FBS, 5 ng/ml rhFGFb, 7. mM L-Glutamine, 50µg/mL, Hydrocortisone Hemisuccinate1 µg/mL, rh Insulin5 µg/mL
	Primary lung	Lung Smooth Muscle (LSM), human	1.05	0.92	0.98	2.76			purchased in 2018 December	Vascular Cell Basal Medium, 5% FBS, 5ng/mL rh FGF-basic, 5µg/mL rh Insulin, 50µg/mL Ascorbic acid, 10mM L-Glutamine, 5ng/mL rh EGF
cancer	bone	U2OS	0.54	0.42	0.48	6.46	CVCL_0042	Kind gift of the David Sabatini lab (MIT, USA)	Yes, Confirmed	DMEM, 10% FBS, 20mM L-Glutamin, 100 units/ml penicillin and streptomycin
	brain	U251	0.36	0.37	0.37	9.59	CVCL_0021		Yes, Confirmed	
	brain	LN229	1.11	0.86	0.99	5.35	CVCL_0393		Yes, Confirmed	
	breast	CAL120	0.63	0.55	0.59	4.85	CVCL_1104		Yes, Confirmed	
	breast	MDA-MB415	0.92	1.04	0.98	2.60	CVCL_0621		Yes, Confirmed	
	breast	MDA-MB468	0.66	0.56	0.61	2.47	CVCL_0419		Yes, Confirmed	
	breast	MDA-MB231	0.46	0.33	0.39	4.19	CVCL_0062		Yes, Confirmed	
	breast	Hs578T	1.27	0.81	1.04	2.69	CVCL_0332		Yes, Confirmed	
	colon	SW620	0.75	0.66	0.70	10.35	CVCL_0547		Yes, Confirmed	
	connective tissue	HT1080	0.60	0.33	0.47	5.71	CVCL_0317		Yes, Confirmed	
	kidney	A498	0.40	0.42	0.41	3.47	CVCL_1056		Yes, Confirmed	
	kidney	Caki-1	0.76	0.69	0.73	3.52	CVCL_0234		Yes, Confirmed	
	skin	A2058	0.39	0.37	0.38	2.87	CVCL_1059		Yes, Confirmed	
	prostate	DU145	0.56	0.35	0.46	4.37	CVCL_0105		Yes, Confirmed	
	brain	U118**	0.75	0.71	0.73	6.55	CVCL_0633		Yes, Confirmed	
	kidney	A549	0.94	0.68	0.81	6.95	CVCL_0023		Yes, Confirmed	
	skin	WM115	1.06	1.01	1.04	1.67	CVCL_0040		Yes, Confirmed	
	breast	Sum159	0.90	0.71	0.81	4.79	CVCL_5423	Kind gift of the Leslie Shaw lab (UMMS, USA)	Untested	
	breast	MDA-MB157	0.80	0.53	0.67	2.61	CVCL_0618	Kind gift of the Michael Lee lab (UMMS, USA)	Yes, Confirmed	
	breast	HCC1395	0.42	0.38	0.40	6.20	CVCL_1249		Untested	
breast	MDA-MB436	0.69	0.49	0.59	2.00	CVCL_0623	Untested			
liver	Huh7	0.52	0.52	0.52	1.19	CVCL_0336	Kind gift of the Kwan Yong Choi lab (POSTECH, KOREA)	Yes, Confirmed*		
breast	MCF10CA1h	0.34	0.32	0.33	37	CVCL_6683	Kind gift of the Michael Green lab (UMMS, USA)	Untested	DMEM/F12, 5 %horse serum, 100units/ml penicillin and streptomycin	

* 4/4 loci matched but that reference data was not available for other loci that we tested.

**U118 is on the list of commonly misidentified lines as it is thought to be identical to U138, another glioma cell line

Table S3. Materials used in the study

Type of material	Source	Catalogue #
Chemicals and kits		
Sodium selenite	Sigma-Aldrich	Cat#S5261
Sodium selenide	Santa Cruz	Cat#sc-272495
L-Glutathione reduced	Sigma-Aldrich	Cat#G4251
tert-Butyl hydroperoxide	Sigma-Aldrich	Cat#458139
Hydrogen peroxide	Sigma-Aldrich	Cat#H1009
Ferostatin-1	Sigma-Aldrich	Cat#SML0584
Liproxatin	Sigma-Aldrich	Cat#SML1414
Z-VAD-FMK	Santa Cruz	Cat#sc3067
Necrostatin-1	Cayman	Cat#11658
L-Cysteine	Sigma-Aldrich	Cat#C7352
Polyvinylpyrrolidone	Sigma-Aldrich	Cat#81440
Silver nitrate	Sigma-Aldrich	Cat#209139
Lead acetate	Sigma-Aldrich	Cat#467863
MOPS-SDS Running Buffer	Boston Bio Products	Cat#BP-178
TBS-T	Boston Bio Products	Cat#BB-181
Borg Decloaker	Biocare medical	Cat#BD1000
Crystal violet	Sigma-Aldrich	Cat#C0775
Erastin	Sigma-Aldrich	Cat#E7781
5,5'-Dithiobis(2-nitrobenzoic acid)	Sigma-Aldrich	Cat#D8130
Basement Membrane Matrix	Trevigen	Cat#3432-005-01
X-tremeGENE 9 Transfection reagent	Roche	Cat#XTG9-RO
Polybrene	EMD Millipore	Cat#TR-1003-G
Puromycin	Corning	Cat#61-385-RA
Blasticidin	Thermo Fisher Scientific	Cat#R21001
Nitric acid (HNO ₃)	Sigma-Aldrich	Cat# 438073
Perchloric acid (HClO ₄)	Sigma-Aldrich	Cat# 311413
Hydrochloric acid (HCl)	Sigma-Aldrich	Cat# 320331
Ethylenediaminetetraacetic acid (EDTA)	EMD Millipore	Cat# 324503
2,3-diaminonaphthalene-HCl (DAN)	ChemSavers	23DANH1G
Cyclohexane	Sigma-Aldrich	Cat# 650455
CellTiter-Glo solution	Promega	Cat#G7570
ABC HRP Kit	Vector Laboratories	Cat#PK-6200
DAB substrate kit	Abcam	Cat#ab64238
RNAscope Pretreatment reagents (hydrogen peroxide, protease plus, and target retrieval)	Advanced Cell Diagnostics	Cat#322381
RNAscope Multiplex fluorescent detection reagents v2 (AMP1, AMP2, AMP3, HRP-C1 and HRP-blocker)	Advanced Cell Diagnostics	Cat#323110
RNAscope Multiplex TSA buffer	Advanced Cell Diagnostics	Cat#322809
TSA plus fluorescein	Perkin Elmer	Cat#NEL741001KT
SEPHS2 (human) RNAscope probe	Advanced Cell Diagnostics	Cat#843901
RNAscope 3-plex positive control probe	Advanced Cell Diagnostics	Cat#320881
RNAscope 3-plex negative control probe	Advanced Cell Diagnostics	Cat#320871
Vectashield antifade mounting media with DAPI	Vector Laboratories	Cat#H-1200
FITC Annexin V Apoptosis Detection Kit I	BD Pharmingen	Cat#556547
DCFDA / H2DCFDA - Cellular ROS Assay Kit	Abcam	Cat#ab113851
Peptide		
Acetyl-[Sec]K[Sec]NLNGRKKRRQRRRPQ	ThermoFisher	Cat#PEP95MODINTO T
Recombinant DNA		
lentiCRISPR v2	(Sanjana et al., 2014)	Addgene plasmid # 52961
pLV-EF1a-IRES-Blast	(Hayer et al., 2016)	Addgene plasmid # 85133
pMD154	Scot Wolfe Lab	N/A
Mice		
Athymic NCI-nu, Female	Charles River Laboratories	N/A
Oligonucleotides		
Primer for cloning SEPHS2 cDNA,		
Forward:		
GGCGGATCCGCCACCATGGCGGAAGCCTCGGCGACGGGCGCCTGC	This paper	N/A
Reverse:		
GGCGGCGCCGCCACCTCACGAGCTAGGCTCAGAGGAGGCATTTGA		
Primer for cloning CAT cDNA,		
Forward: GGCACGCGTGCACCATGGCTGACAGCCGGGATCCCGCCAGCGAC	This paper	N/A
Reverse: GGCGTAAACGCCACCTCACAGATTTGCCCTTCCCTTGCCGCCAA		
Primer for SDM SEPHS2 U60C		
Forward: CCGGCATGAAGGGCTGTGGCTGC	This paper	N/A
AAGGTCCCGCAGGAGGCGC (Genewiz)		
Reverse: GCGGACCTTGACGACACAGCCC		
TTCATGCCGGAAGCCC (Genewiz)		
Primer for SDM CRISPR resistant SEPHS2 clone 1		
Forward:		
GATGCGGCTGAGGAAGGAGGGACGGCAGTTACTGGTGGGCAAACGGTGGTCAACCCCTGG	This paper	N/A
Reverse:		
CCAAGGGTTGACCACCGTTTGCCACACAGTAACTGCCCTCCCTCCTCAGCCGCATC		
Primer for SDM CRISPR resistant SEPHS2 clone 1		
Forward:		
GATGCGGCTGAGGAAGGAGGGACCGCGTTACTGGTGGGCAAACGGTGGTCAACCCCTGG	This paper	N/A
Reverse:		
CCAAGGGTTGACCACCGTTTGCCACACAGTAAACGGCGGTCCTCCTCCTCAGCCGCATC		
Guide sequences for CRISPR	(Wang et al., 2014)	N/A
See Supplementary Table S1		
Software		
Prism version 8	GraphPad	N/A
Image J version 1.52o	N/A	N/A
FlowJo version 10	BD	N/A
ChemDraw Professional	Version 18.0.0.231	N/A

Gen5 software	BioTek	N/A
Antibodies for Western blots		
Rabbit polyclonal anti-SEPHS2	Sigma-Aldrich	Cat#SAB2700271
Rabbit polyclonal anti-PSTK	Invitrogen	Cat#PA5-58101
Rabbit monoclonal anti-GPX4	Abcam	Cat#ab125066
Rabbit polyclonal anti-GPX1	Abcam	Cat#ab22604
Rabbit monoclonal anti-xCT/SLC7A11	Cell signaling Technology	Cat#12691
Rabbit polyclonal anti-SLC3A2	Sigma-Aldrich	Cat#HPA017980
Rabbit polyclonal anti-SELS (C11ORF31)	Sigma-Aldrich	Cat#HPA048362
Rabbit polyclonal anti-SELH (VIMP)	Sigma-Aldrich	Cat#HPA010025
Rabbit monoclonal anti-Catalase (D4P7B)	Cell signaling Technology	Cat#12980T
Mouse monoclonal anti-SEPHS1 (F-6)	Santa Cruz	Cat#sc-365945
Mouse monoclonal anti-AMID (B-6)	Santa Cruz	Cat#sc-377120
Mouse monoclonal anti- β -Actin	Sigma-Aldrich	Cat#A2228
Rabbit monoclonal anti- β -Actin (13E5)	Cell signaling Technology	Cat#4970
Antibodies for ICC and IHC		
Rabbit polyclonal anti-Cleaved Caspase-3 (Asp175)	Cell signaling Technology	Cat#9661
Goat anti-Rabbit IgG H&L (Alexa Fluor® 488)	Abcam	Cat#ab150077
Rabbit monoclonal anti-GPX4	Abcam	Cat#ab125066
Rabbit polyclonal Anti-CA9	Sigma-Aldrich	Cat#HPA055207
Mouse monoclonal PECAM-1 Antibody (10G9)	Santa Cruz	Cat#sc-13537
Mouse monoclonal anti-MKI67	Sigma-Aldrich	Cat#AMAB90870