

SUPPLEMENTAL FIGURES

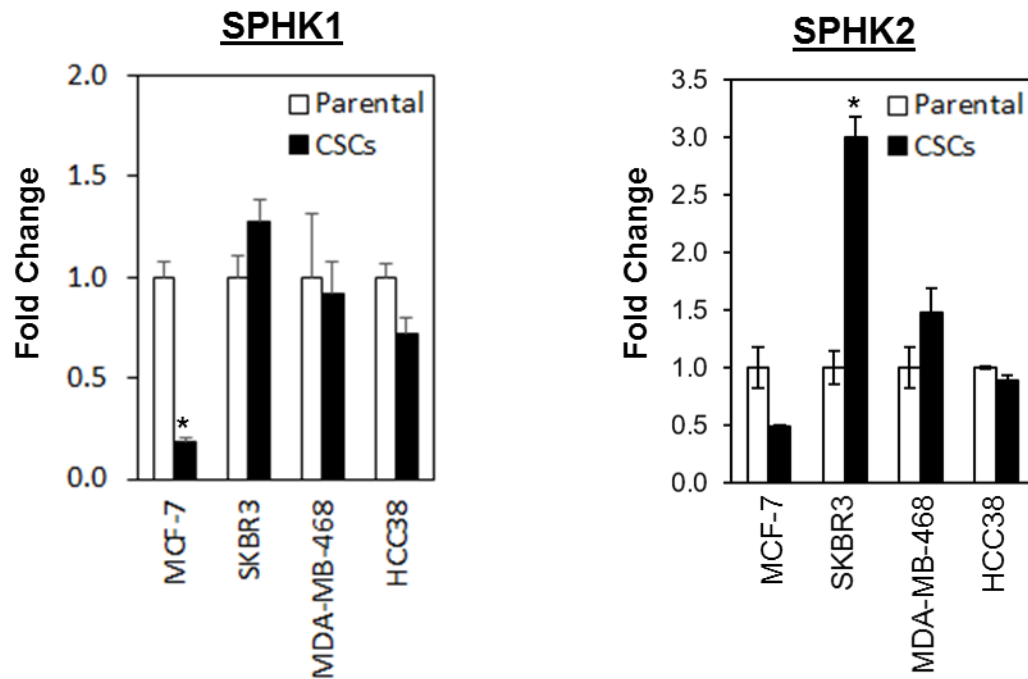


Figure S1: mRNA Expression of SPHK1 and SPHK2 in breast CSCs and non-CSCs. mRNA from both parental and CSCs were harvested using RNeasy Mini Kit (Qiagen, USA). High Capacity RNA-to-cDNA Master Mix (Applied Biosystems, USA) was used to prepare cDNA. Gene expression was measured using FastStart Universal SYBP Green Master reagent (Roche, USA) in Bio-Rad iQ5 real-time quantitative real-time polymerase chain reaction (qPCR) detector system (BioRad Laboratories, USA). The forward and reverse primers used in qPCR reactions are shown in Table S5. The following qPCR reactions were applied: 94°C (3 minutes), then a total of 40 cycles of 94°C (40 seconds), 60°C (40 seconds), and 72°C (25 seconds). All qPCR results were normalized using GAPDH, the house keeping gene. Bars represent the means \pm s.d. of 3 independent experiments. Asterisks (*) indicate statistical significance compared with non-silencing (NS) control cells ($P < 0.01$, Student's *t*-test).

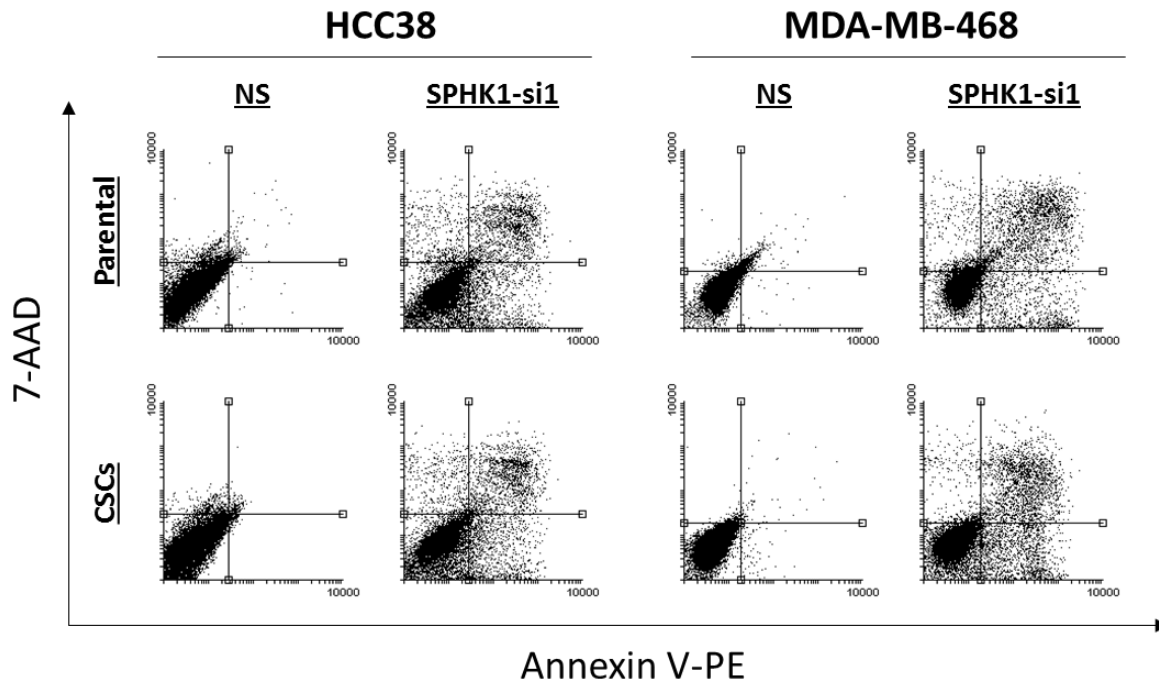


Figure S2: Induction of apoptosis in breast CSC and non-CSC cultures following depletion of SPHK1. Representative flow cytometry analysis of cells transduced with either SPHK1-directed shRNA (SPHK1-si1) or a non-specific shRNA control (NS). Unfixed cells were stained with annexin V-PE and 7-aminoactinomycin D (7-AAD) at 72 hours following SPHK1 knock-down, then analyzed by flow cytometry.

SUPPLEMENTAL TABLES

Table S1: List of proteins that were up-regulated in HEK-293 cells following ectopic expression of SPHK1 (log₂ ratio of SPHK1/vector > 2).

Gene Symbol	Full Name
ABL1	Tyrosine-protein kinase ABL1
AFP	Alpha-fetoprotein
AHSG	Alpha-2-HS-glycoprotein;Alpha-2-HS-glycoprotein chain A;Alpha-2-HS-glycoprotein chain B
ALB	Serum albumin
AMBP	Protein AMBP;Alpha-1-microglobulin;Inter-alpha-trypsin inhibitor light chain;Trypstatin
ARHGAP45	Minor histocompatibility protein HA-1;Minor histocompatibility antigen HA-1
ASNS	Asparagine synthetase [glutamine-hydrolyzing]
C11orf96	Chromosome 11 open reading frame 96
C4B	Complement C4-B;Complement C4 beta chain;Complement C4-B alpha chain;C4a anaphylatoxin;C4b-B;C4d-B;Complement C4 gamma chain
CBSL	Cystathionine beta-synthase like
CD40	Tumor necrosis factor receptor superfamily member 5
CFAP100	Coiled-coil domain-containing protein 37
COMP	Cartilage oligomeric matrix protein
CTH	Cystathionine gamma-lyase
DCAF13	DDB1- and CUL4-associated factor 13
DKK3	Dickkopf-related protein 3
EFEMP1	EGF-containing fibulin-like extracellular matrix protein 1
EIF4EBP1	Eukaryotic translation initiation factor 4E-binding protein 1
GC	Vitamin D-binding protein
GPX1	Glutathione peroxidase 1
GPX4	Phospholipid hydroperoxide glutathione peroxidase, mitochondrial
ITIH2	Inter-alpha-trypsin inhibitor heavy chain H2
KNG1	Kininogen-1;Kininogen-1 heavy chain;T-kinin;Bradykinin;Lysyl-bradykinin;Kininogen-1 light chain;Low molecular weight growth-promoting factor
LGALS13	Galactoside-binding soluble lectin 13

PPP4R4	Serine/threonine-protein phosphatase 4 regulatory subunit 4
PSAT1	Phosphoserine aminotransferase
QPRT	Nicotinate-nucleotide pyrophosphorylase [carboxylating]
SELENOF	15 kDa selenoprotein
SELENOH	Selenoprotein H
SERPINA1	Alpha-1-antitrypsin;Short peptide from AAT
SERPINF2	Alpha-2-antiplasmin
SLC7A1	High affinity cationic amino acid transporter 1
SLFNL1	Schlafen-like protein 1
TXNIP	Thioredoxin-interacting protein
ZNF717	Zinc finger protein 717

Table S2: List of proteins that were down-regulated in HEK-293 cells following ectopic expression of SPHK1 (log₂ ratio of SPHK1/vector < -2).

Gene Symbol	Full Name
ADM	ADM;Adrenomedullin;Proadrenomedullin N-20 terminal peptide
APOL2	Apolipoprotein L2
BRD2	Bromodomain-containing protein 2
BRD3	Bromodomain-containing protein 3
SHFL	Shiftless antiviral inhibitor of ribosomal frameshifting
CCDC175	Coiled-coil domain-containing protein 175
CD14	Monocyte differentiation antigen CD14;Monocyte differentiation antigen CD14, urinary form;Monocyte differentiation antigen CD14, membrane-bound form
CD274	Programmed cell death 1 ligand 1
CLIC3	Chloride intracellular channel protein 3
CMPK2	UMP-CMP kinase 2, mitochondrial
CTSF	Cathepsin F
DDX58	Probable ATP-dependent RNA helicase DDX58
DHRS2	Dehydrogenase/reductase SDR family member 2, mitochondrial
DTX3L	E3 ubiquitin-protein ligase DTX3L
ECHDC3	Enoyl-CoA hydratase domain-containing protein 3, mitochondrial

EPS8L1	Epidermal growth factor receptor kinase substrate 8-like protein 1
FADS3	Fatty acid desaturase 3
GBP1	Interferon-induced guanylate-binding protein 1
GJB3	Gap junction beta-3 protein
HERC3	Probable E3 ubiquitin-protein ligase HERC3
HLA-B	HLA class I histocompatibility antigen, B-8 alpha chain
HLA-B	HLA class I histocompatibility antigen, B-7 alpha chain
HLA-C	HLA class I histocompatibility antigen, Cw-7 alpha chain
HLA-C	HLA class I histocompatibility antigen, Cw-1 alpha chain
HLA-F	HLA class I histocompatibility antigen, alpha chain F
HS3ST1	Heparan sulfate glucosamine 3-O-sulfotransferase 1
IFI35	Interferon-induced 35 kDa protein
IFI6	Interferon alpha-inducible protein 6
IFIT1	Interferon-induced protein with tetratricopeptide repeats 1
IFIT2	Interferon-induced protein with tetratricopeptide repeats 2
IFIT3	Interferon-induced protein with tetratricopeptide repeats 3
IFIT5	Interferon-induced protein with tetratricopeptide repeats 5
IFITM3	Interferon-induced transmembrane protein 3
IL1RN	Interleukin-1 receptor antagonist protein
IRF9	Interferon regulatory factor 9
ISG15	Ubiquitin-like protein ISG15
KRT14	Keratin, type I cytoskeletal 14
KRT17	Keratin, type I cytoskeletal 17
KRT3	Keratin, type II cytoskeletal 3
LGALS3BP	Galectin-3-binding protein
LY75	Lymphocyte antigen 75
MGARP	Protein MGARP
MGST3	Microsomal glutathione S-transferase 3
MNDA	Myeloid cell nuclear differentiation antigen
MX1	Interferon-induced GTP-binding protein Mx1;Interferon-induced GTP-binding protein Mx1, N-terminally processed
NIP7	60S ribosome subunit biogenesis protein NIP7 homolog
NMI	N-myc-interactor

NPEPL1	Probable aminopeptidase NPEPL1
NWD2	NACHT and WD repeat domain-containing protein 2
OAS1	2'-5'-oligoadenylate synthase 1
OAS2	2'-5'-oligoadenylate synthase 2
OAS3	2'-5'-oligoadenylate synthase 3
PARP12	Poly [ADP-ribose] polymerase 12
PARP14	Poly [ADP-ribose] polymerase 14
PARP9	Poly [ADP-ribose] polymerase 9
PIN4	Peptidyl-prolyl cis-trans isomerase NIMA-interacting 4
PLEKHA6	Pleckstrin homology domain-containing family A member 6
PIIG	Peptidyl-prolyl cis-trans isomerase G
PSMB9	Proteasome subunit beta type-9
PYCARD	Apoptosis-associated speck-like protein containing a CARD
RUBCN	Run domain Beclin-1 interacting and cysteine-rich containing protein
SAA1	Serum amyloid A-1 protein;Amyloid protein A;Serum amyloid protein A(2-104);Serum amyloid protein A(3-104);Serum amyloid protein A(2-103);Serum amyloid protein A(2-102);Serum amyloid protein A(4-101)
SAA2	Serum amyloid A-2 protein
SAMD9	Sterile alpha motif domain-containing protein 9
SIX4	Homeobox protein SIX4
SP110	Sp110 nuclear body protein
SRSF4	Serine/arginine-rich splicing factor 4
SRSF6	Serine/arginine-rich splicing factor 6
ST14	Suppressor of tumorigenicity 14 protein
STAP2	Signal-transducing adaptor protein 2
STAT1	Signal transducer and activator of transcription 1-alpha/beta
TLR3	Toll-like receptor 3
TMEM40	Transmembrane protein 40
TRIM22	E3 ubiquitin-protein ligase TRIM22
UBE2L6	Ubiquitin/ISG15-conjugating enzyme E2 L6
XAF1	XIAP-associated factor 1
ZRANB2	Zinc finger Ran-binding domain-containing protein 2

Table S3: List of pathways that were significantly enriched in HEK-293 cells following ectopic expression of SPHK1 (P < 0.05).

Pathway	P-value
Type I interferon signaling pathway	3.05E-05
Negative regulation of DNA damage response by p53	2.37E-03
Response to virus	3.51E-03
Release of cytochrome c from mitochondria	7.42E-03
Interferon-gamma-mediated signaling pathway	7.59E-03
Intracellular protein transport	1.09E-02
Positive regulation of cell growth	1.20E-02
Positive regulation of atpase activity	1.32E-02
Defense response to virus	1.43E-02
Negative regulation of apoptotic process	1.47E-02
Single organismal cell-cell adhesion	1.96E-02
Response to interferon-beta	4.98E-02

Table S4: shRNA target sequences for SPHK1 and STAT1.

	Target sequence
SPHK1-si1	5'-CCGGGCAGCTTCCTTGAACCATTATCTCGAGATAATGGTTCAAGGAAGCTGCTTTTTG-3'
SPHK1-si2	5'-CCGGGCAGGCATATGGAGTATGAATCTCGAGATTCATACTCCATATGCCTGCTTTTTG-3'
STAT1-si1	5'-CCGGCCCAAAGTATCAGGACGAGAACTCGAGTTCTCGTCCTGATACTTTGGGTTTTT-3'

Table S5: Forward and reverse primer sequences for quantitative RT-PCR.

Target Genes	Sequence (5'-3')	
	Primer (Forward)	Primer (Reverse)
SPHK1	CGGCTGCGAAGTTGAGCGAA	GGTGACCTGCTCATAGCCAGC
SPHK2	CCCAGTGTTGGAGAGCTGAAGG	CGAGGTAGCAGGTCAGGGGT
GAPDH	GTCTCCTCTGACTTCAACAGCG	ACCACCCTGTTGCTGTAGCCAA

SUPPLEMENTAL METHODS

Protein extraction

Total protein was harvested by trichloroacetic acid (TCA) method followed by acetone precipitation. Briefly, cells were washed twice with PBS and proteins precipitated in 6% TCA solution in 100 mM ammonium bicarbonate. The lysates were centrifuged at maximum speed for 10 minutes at 4°C, and the cell pellet washed twice in 100% ice-cold acetone.

Enzymatic digestion

Pellets were resuspended in 8M Urea/100mM ammonium bicarbonate, reduced by incubation with 5mM dithiothreitol (DTT) at room temperature for 30 min and then alkylated with 15 mM iodoacetamide for 30 min at room temperature in the dark. Iodoacetamide was quenched with an additional 5mM DTT. Samples were diluted to 2M Urea using 100 mM ammonium bicarbonate prior to digestion with Lysyl endopeptidase (LysC, Promega) for 6 hours at room temperature. Samples were further diluted to 1M Urea and digested overnight with trypsin at 37 °C. Digests were acidified by the addition of 10% trifluoroacetic acid (TFA) to 0.5% final concentration and the peptides were desalted on 100mg C18 Sep-Pak cartridges (Waters, USA) and dried in a centrifugal evaporator.

TMT Labeling and peptide fractionation by high pH reverse phase chromatography

Peptides were TMT-labelled and pre-fractionated in the manner described in Huang et.al (2017, <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5336479/>). Briefly, peptides were resuspended in 0.2 M HEPES buffer, pH 8.5. TMT reagents (only 3 channels) were added to samples and incubated at room temperature for 1 hr, quenched by the addition of 5% hydroxylamine for 15 min and immediately acidified with 100% FA. Reaction mixtures were combined in a 1:1:1 ratio and desalted on a StageTip. Following pre-fractionation using high pH reverse phase chromatography, peptides were analyzed by previously described LC-MS3 methods on a Thermo Orbitrap Fusion Tribrid mass spectrometer (Thermo Fisher Scientific) equipped with an Easy nLC-1000 UHPLC (Thermo Fisher Scientific) (Huang et.al (2017, <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5336479/>)).

Data Analysis

Mass spectra were processed, filtered, and quantified using a SEQUEST-based software pipeline (<https://www.ncbi.nlm.nih.gov/pubmed/21183079/>). Briefly, peptide-spectrum matches were adjusted to a 1% false discovery rate (FDR). PSM filtering was carried out using a linear discriminant analysis. TMT quantitation was conducted by extracting a signal-to-noise ratio for each TMT channel; PSMs were identified, quantified, and collapsed to a 1% peptide FDR and further collapsed to the protein level using a 1% protein FDR. Signal-to-noise ratios of < 200 were eliminated from the dataset. A filter of at least 2 or more peptides for each protein identification was set before pathway analysis.