Supplementary Materials

Genomic DNA extraction and copy number analysis

Genomic DNA extraction from tail tissue was performed using an UltraClean Tissue & Cells DNA Isolaion Kit (MOBIO) according to the manufacture's instruction. RT-PCR was done using the standard reagents and protocols (BIORAD). The following primers were used to analyze mRNA expressions in mouse tumor tissues: Apob, 5'-CACGTGGGCTCCAGCATT-3' (sense) and 5'-TCACCAGTCATTTCTGCCTTTG-3'; neu, 5'-CCCGAGTGTCAGCCTCAAA-3, (sense) and 5'-GCAGGCTGCACACTGATCA-3 (antisense). The relative copy number was analyzed by comparing relative target gene (neu) expression to Apolipoprotein B (Apob) reference gene using the 2-ΔΔCt method.

Primers

The following primers were used to analyze mRNA expressions in mouse tumor tissues: β-actin, 5'-ATGATGATATCGCCGC GCTC-3' (sense) and 5'-CACGATGGAGGGGAAGACG -3' (antisense); CLDN2, 5'-CAACTGGTGGGCTACATCCTA-3' (sense) and 5'-CCCTTGGAAAAGCCAACCG-3' (antisense). The following primers were used to analyze mRNA expressions in human cells: ßactin, 5'-ATGATGATAT CGCCGCGCTC-3' (sense) and 5'- CACGATGGAGGGGAAGACG-3' (antisense); SphK1, 5'-AGGCTGAAATCTCCTTCACGC-3' (sense) and 5'-GTCTCCAGACATGACCACCAG-3' (antisense); CLDN2, 5'- ATCGCTCCAACTACTACGATGC-3' (sense) and 5'-TGAACTCACTCTTGACTTTGGGA-3' (anti-sense).

Antibodies

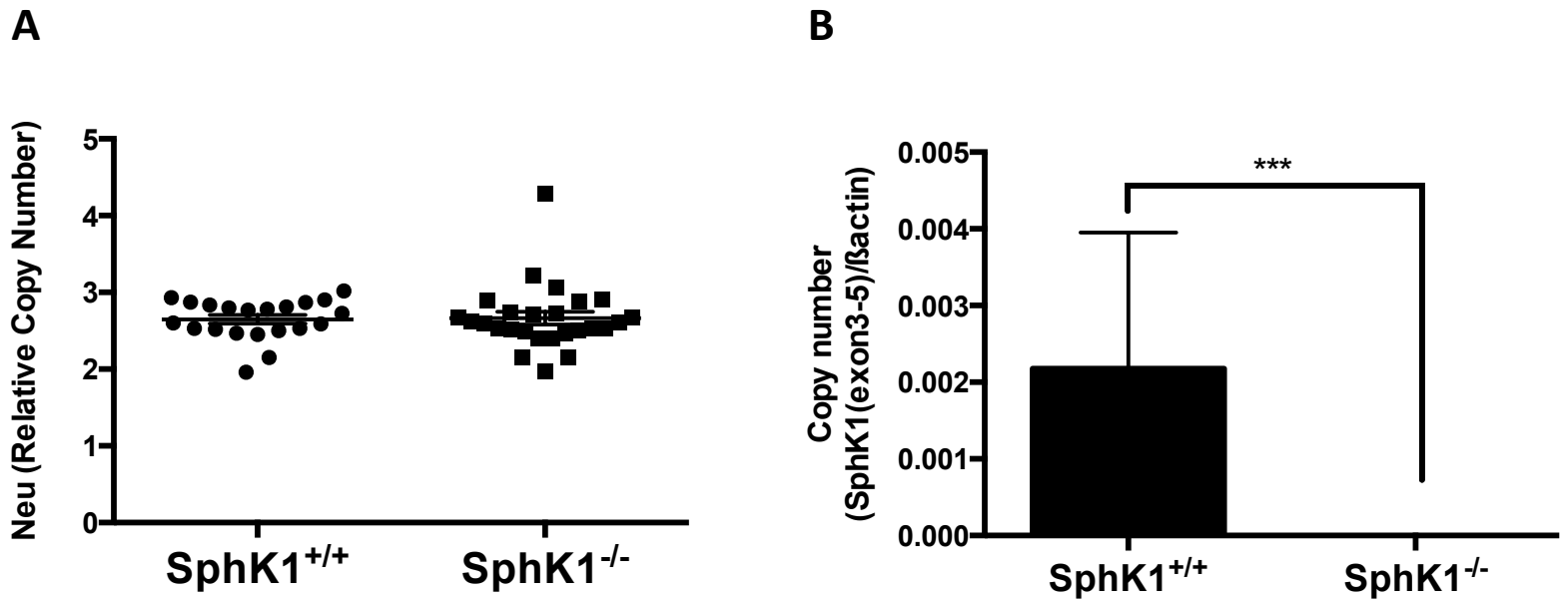
The following antibodies were used: Following antibodies were used. CD31 (1:400; BD Pharminogen, #550274, San Jose, CA), CD45 (1:200; BD Pharminogen, #550539), CLDN2 (1:100; Abcam, #ab53032, Cambridge, UK), ERBB2 (1:500; Santa Cruz Biotechnologies, #sc-284, Dallas, TX), ERα (1:500; Santa Cruz Biotechnologies, #sc-542), P53 (1:250; Novocastra, #NCL-p53-CM5p, Buffalo Grove, IL), PCNA (1:800, Santa Cruz Biotechnologies, #sc-56), PR (1:250; Abcam, #ab2764) and SphK1 (1:200; Abgent, #AP7237c, San Diego, CA).

Immunohistochemistry

Tissue sections were mounted on glass slides, deparaffinized with xylene, and hydration with graded alcohol (100%, 95% and 70% ethanol). Antigen retrieval was accomplished with 10 mM citrate buffer (pH 6). Endogenous peroxidase was blocked with 3% hydrogen peroxidase. Subsequently, slides were blocked with 1% BSA or casein buffer (BIOCARE MEDICAL). The conditions for primary antibodies were 0.5-2 hours at room temperature (ERα, ERBB2, P53, PCNA, and SphK1) and

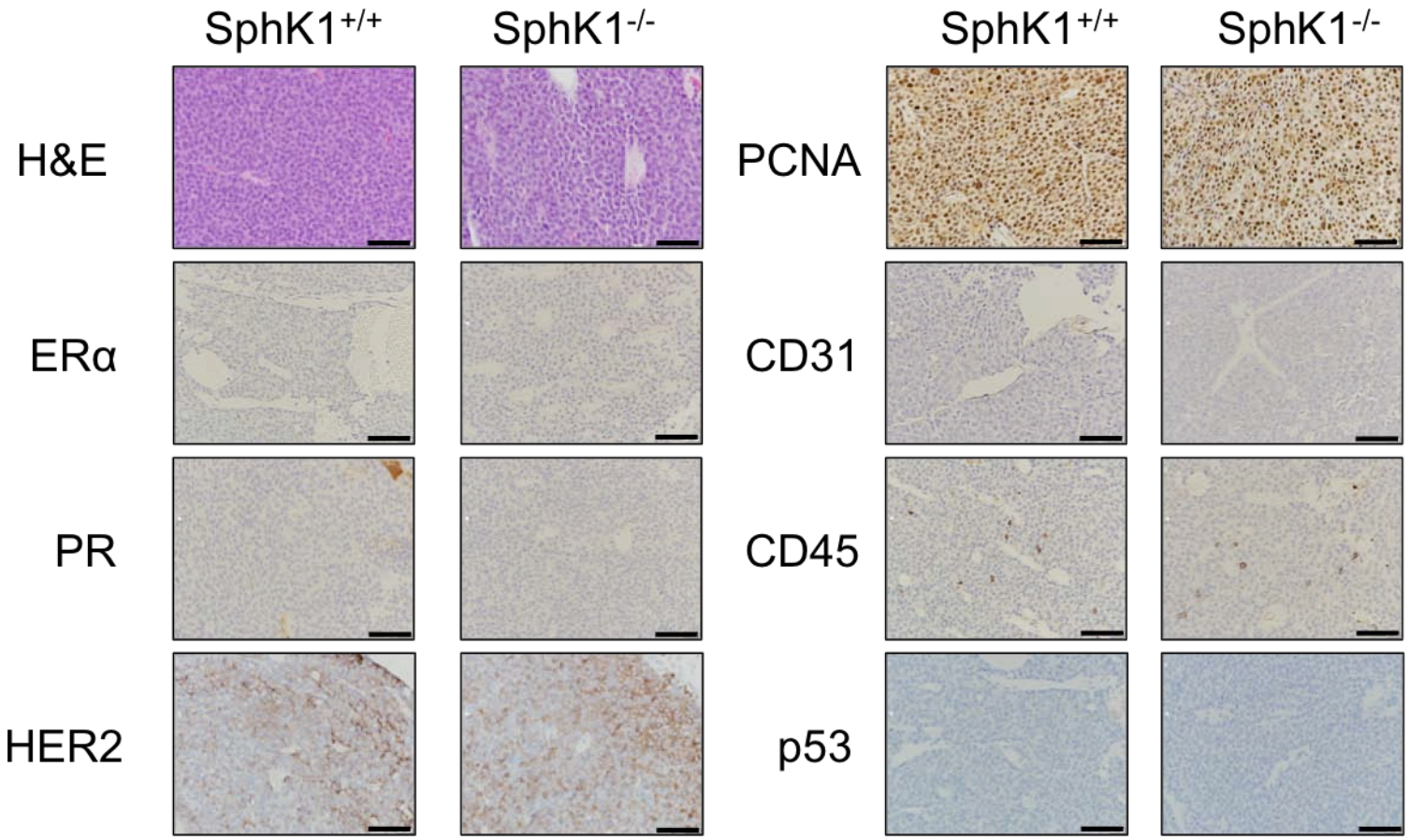
overnight at 4˚C (CD31, CD45, CLDN2, and PR). Slides were then incubated with biotinylated secondary antibody, followed by Vectastain ABC reagents (Vector Laboratories), 1,1’-diaminobenzidine, and hydrogen peroxide and countertained with hematoxylin.

Supplemental figure 1



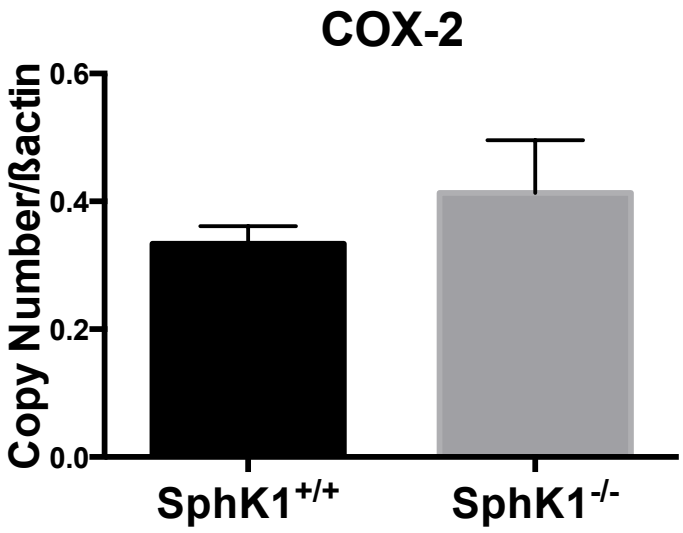
Supplemental figure 1. No copy number variance and confirmation of SphK1 knockdown. A, Real-time qPCR analysis of neu gene in tumors from SphK1$^{+/+}$ and SphK1$^{-/-}$ mice tail tissue was analyzed by one-way ANOVA. **B,** qRT-PCR analysis of SphK1 gene in tumors from SphK1$^{+/+}$ and SphK1$^{-/-}$ mice tail tissue was analyzed by unpaired t-test. ; ***, $p < 0.001$.

Supplemental figure 2



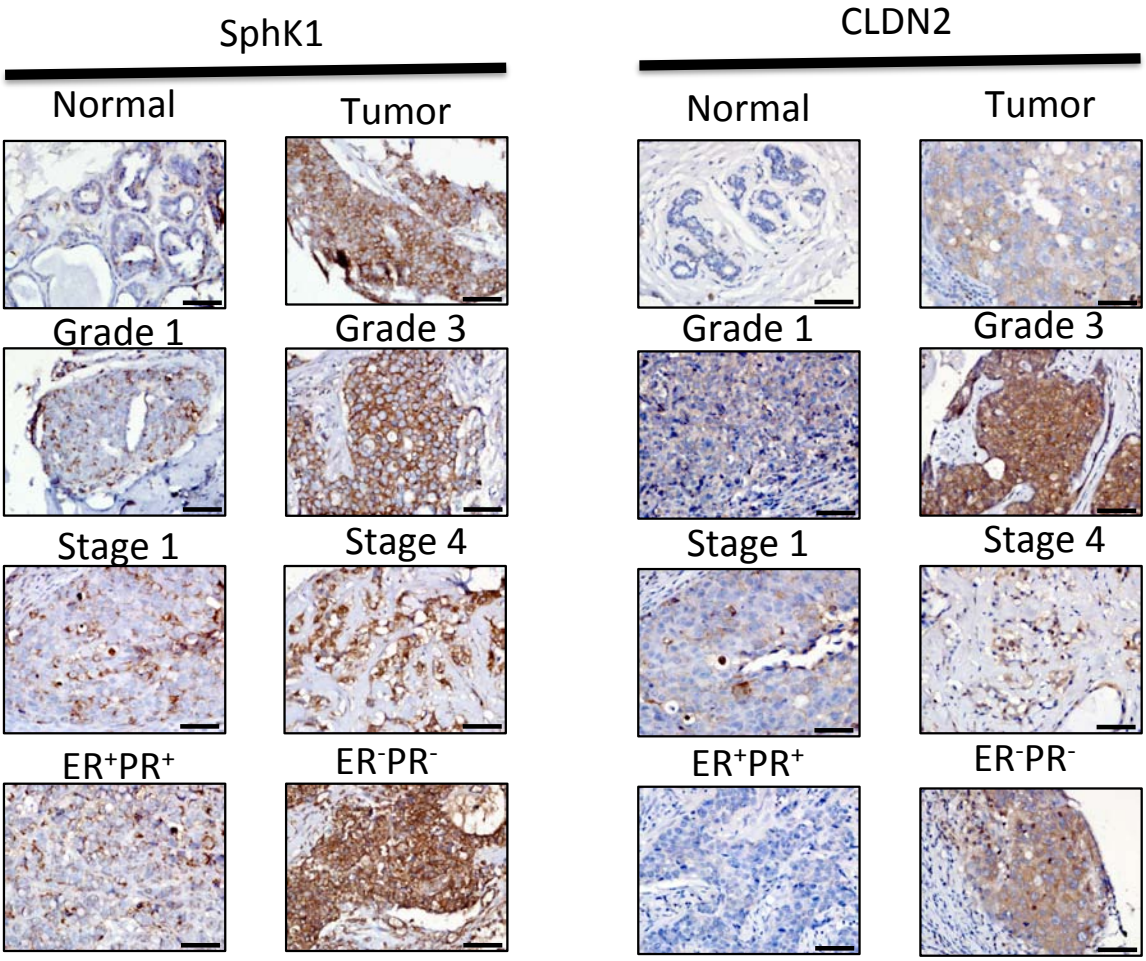
Supplemental figure 2. Genetic deletion of SphK1 had no effect on proliferation, angiogenesis and inflammation in tumors. Representative paraffin-tumor sections immunostained for PCNA, CD31, CD45 and p53, and were counterstained with hematoxylin (magnification 400X). Bar = 60 μm.

Supplemental figure 3



Supplemental figure 3. Genetic deletion of SphK1 had no effect COX-2 expression in the tumors.
qRT-PCR analysis of COX-2 gene in tumors from SphK1$^{+/+}$ and SphK1$^{-/-}$mice tumor tissue was analyzed by unpaired t-test.; No significant difference.

Supplemental figure 4



Supplemental figure 4. SphK1 and CLDN2 levels are increased in HER2-positive breast cancers in humans. Tissue microarray containing breast cancer samples from 92 HER2-positive breast cancer patients and matched adjacent normal breast tissues (n = 34) are stained for SphK1 and CLDN2. Staining was graded based on the intensity (1-3) and proportion (1-3) and the total scores were used for the analysis. Representative image of SphK1 and CLDN2 IHC staining in tissues with different pathological features (grade, stage and hormone receptor status) are shown.

Supplemental Table 1. Top 5 cellular function (A) and canonical pathway (B).
A.

Cellular functions	p-value	# of molecules	Molecules
Cellular Movement	3.32E-07-4.67E-03	31	CRYAB, ANGPT2, PALLD, CTGF, HLA-A, HSPA1A/HSPA1B, Cxcl9, TUBB2B, HLA-G, VEGFA, FLNA, TIMP1, EFNA5, ST6GALNAC2, MGP, Ceacam10, STAT1, TACSTD2, TIMP2, ELN, BGN, VWF, IRF7, CYTIP, ACTA2, ANXA3, THBS2, IGFBP3, FBN1, EDNRA, IFIT2
Protein Synthesis	6.68E-07-3.04E-03	12	DHX58, ELN, Igtp, RSAD2, LGALS3BP, IGFBP3, PSMB8, HBA1/HBA2, STAT1, IFIT2, TAP1, Gpihbp1
Antigen Presentation	1.95E-06-1.95E-06	3	PSMB8, STAT1, TAP1
Cell-To-Cell Signaling and Interaction	1.64E-05-4.67E-03	27	ANGPT2, CRYAB, CTGF, PALLD, HLA-A, HSPA1A/HSPA1B, PSMB8, TAP1, Cxcl9, Gpihbp1, VEGFA, FLNA, TIMP1, EFNA5, SLC6A2, TIMP2, ELN, BGN, VWF, UBE2L6, IRF7, LGALS3BP, THBS2, ESM1, IGFBP3, FBN1, CLDN2
Cell Death and Survival	2.34E-05-4.67E-03	38	HIST1H1C, Igtp, ANGPT2, USP18, CRYAB, CTGF, PALLD, HLA-A, HSPA1A/HSPA1B, RNASE1, PSMB8, HBA1/HBA2, Cxcl9, TAP1, VEGFA, HLA-G, TIMP1, FLNA, EFNA5, MGP, STAT1, PLAC8, TIMP2, KRT14, DHX58, OAS1, BGN, VWF, Hbb-b1, IRF7, LGALS3BP, THBS2, Hbb-b2, IGFBP3, EDNRA, FBN1, AATK, HLA-E

B.

Pathway	***P*-value**	**Ratio**
Antigen Presentation Pathway	3.52E-10	7/37
Interferon Signaling	1.53E-08	6/36
Hepatic Fibrosis / Hepatic Stellate Cell Activation	3.53E-07	9/197
Protein Ubiquitination Pathway	2.57E-05	8/255
Allograft Rejection Signaling	5.14E+00	5/86

Supplemental table 2. Influence of SphK1 and CLDN2 expression on HER2-positive breast cancer clinicopathological features. (Corresponding to Figure 4A and B)

Variable	SphK1 IHC Score			Cumulative Logit Model			
	Low	Moderate	High	OR	95% LCL	95% UCL	p
Normal	49%	41%	10%	1.00			
Tumor	10%	41%	48%	8.21	3.88	17.35	**.0000**
Grade 1	33%	52%	16%	1.00			
Grade 2	21%	54%	25%	1.82	0.57	5.85	.32
Grade 3	6%	37%	57%	7.00	2.41	20.38	**.0004**
Stage 1	10%	43%	48%	1.79	0.83	3.88	.14
Stage 2A	7%	37%	56%	2.47	1.29	4.72	**.006**
Stage 2B-4	16%	50%	34%	1.00			
ER-PR-	3%	23%	74%	1.00			
ER-PR+	9%	45%	46%	0.30	0.11	0.79	**.02**
ER+PR-	23%	55%	23%	0.10	0.03	0.32	**.0001**
ER+PR+	17%	54%	29%	0.14	0.08	0.28	**.0000**
CLDN2 IHC Score							
Low	43%	43%	13%	1.0			
Moderate	3%	19%	78%	22.6	10.6	48.2	**.0000**
High	0%	1%	99%	509.0			

The model estimated percent of cases for the SphK1 IHC scores are shown
OR is the odds ratio from a cumulative logit model with IHC score as the outcome.
Only tumor tissue data were used for Grade, Stage, ER/PR, and CLDN2 analyses.

Supplemental table 2 continued. Influence of CLDN2 expression on HER2-positive breast cancer clinicopathological features.

Variable	Percent of Cases CLDN2 IHC Score			Cumulative Logit Model			
	Low	Moderate	High	OR	95% LCL	95% UCL	p
Normal	91%	8%	1%	1.00			
Tumor	45%	43%	12%	11.65	4.03	33.68	**.0000**
Grade 1	82%	16%	2%	1.00			
Grade 2	57%	37%	6%	3.50	0.70	17.58	.13
Grade 3	39%	48%	12%	7.14	1.50	33.90	**.01**
Stage 1	46%	44%	10%	1.60	0.84	3.03	.15
Stage 2A	38%	48%	14%	2.21	1.32	3.72	**.003**
Stage 2B-4	57%	36%	7%	1.00			
ER-PR-	32%	53%	15%	1.00			
ER-PR+	49%	42%	8%	0.48	0.21	1.08	.08
ER+PR-	53%	40%	7%	0.41	0.11	1.48	.17
ER+PR+	54%	39%	7%	0.40	0.26	0.63	**.0001**
SphK1 IHC Score							
Low	93%	7%	0%	1.0			
Moderate	57%	43%	0%	10.4	5.4	20.0	**.0000**
High	11%	85%	4%	109.0			

The model estimated percent of cases for the CLDN2 IHC scores are shown.
OR is the odds ratio from a cumulative logit model with IHC score as the outcome.
Only tumor tissue data were used for Grade, Stage, ER/PR, and SphK1 analyses.
The CLDN2-SphK1 correlation (square root of the generalized R-square) is 0.50