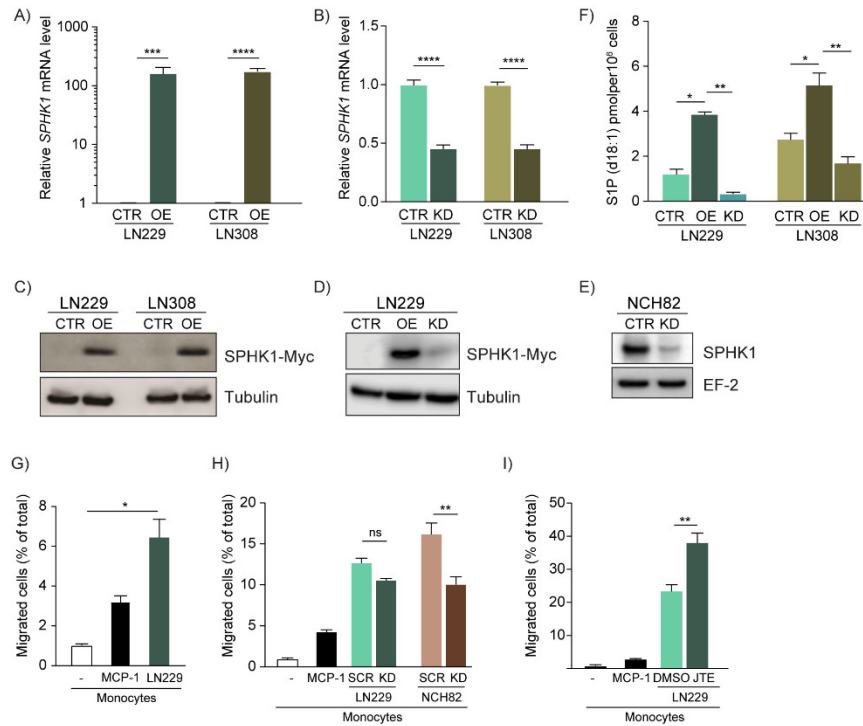
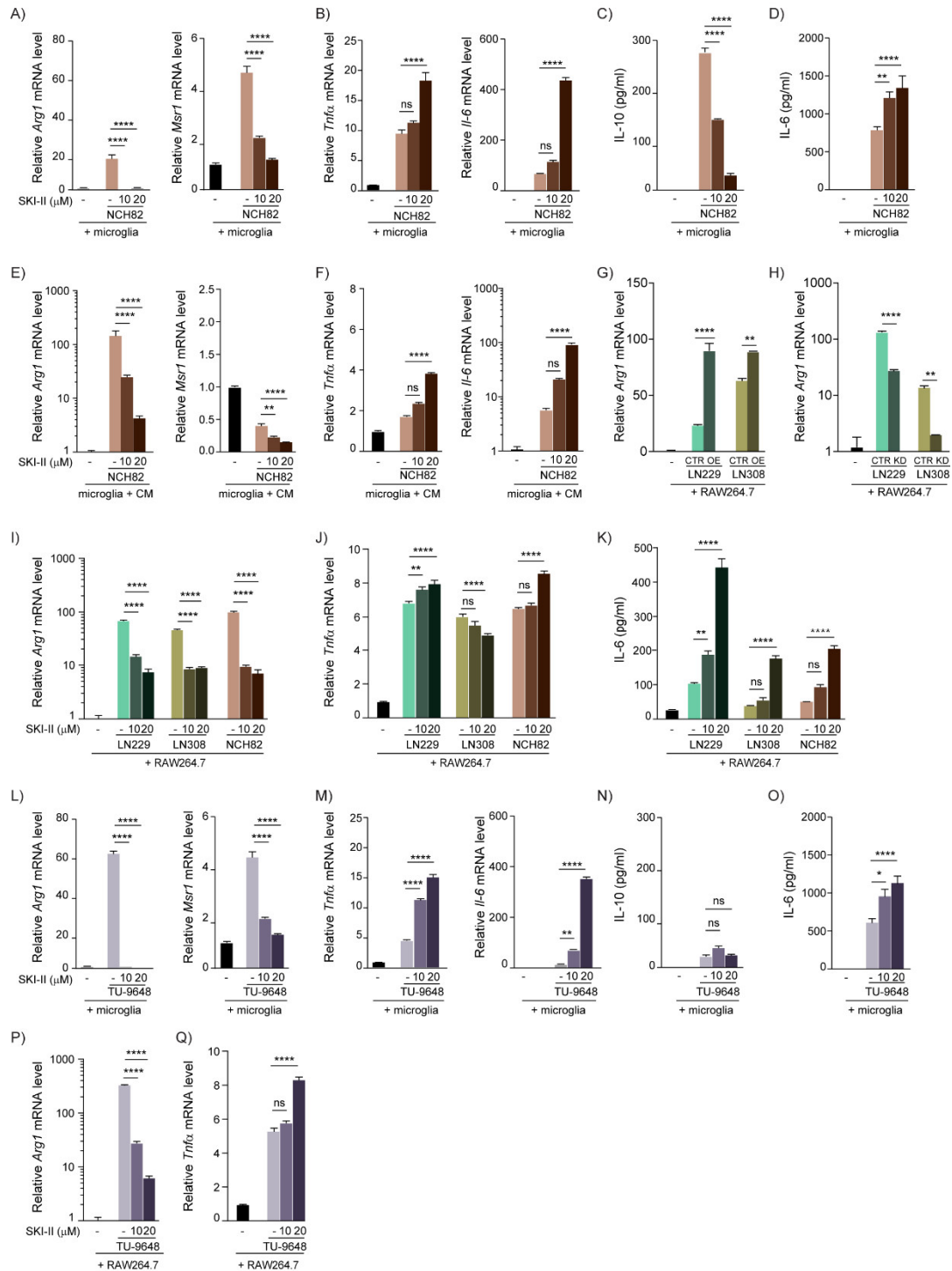


**Figure S1. High SPHK1 expression strongly correlates with higher microglia recruitment.** A) Kaplan-Meier estimates overall survival in patients with glioblastoma divided into high and low SPHK1 expression. Data analyzed using the Affymetrix 540 MASS 5.0-u133 array available in the online R2: Genomics Analysis and Visualization Platform (<http://r2.amc.nl>). B) Heatmap representation of microglia gene signature as reported in Butovsky et al., 2014 in TCGA RNAseq data of GB dataset and sorted according to SPHK1 expression level. C) Scatter plot showing the correlation between SPHK1 and SLC2A5 gene expression. Data analyzed using the RNAseq-IlluminaHiSeq GB data available in the online tool XENA from UCSC (<https://xena.ucsc.edu/>). p value in Kaplan Meier results from scan modulus test.

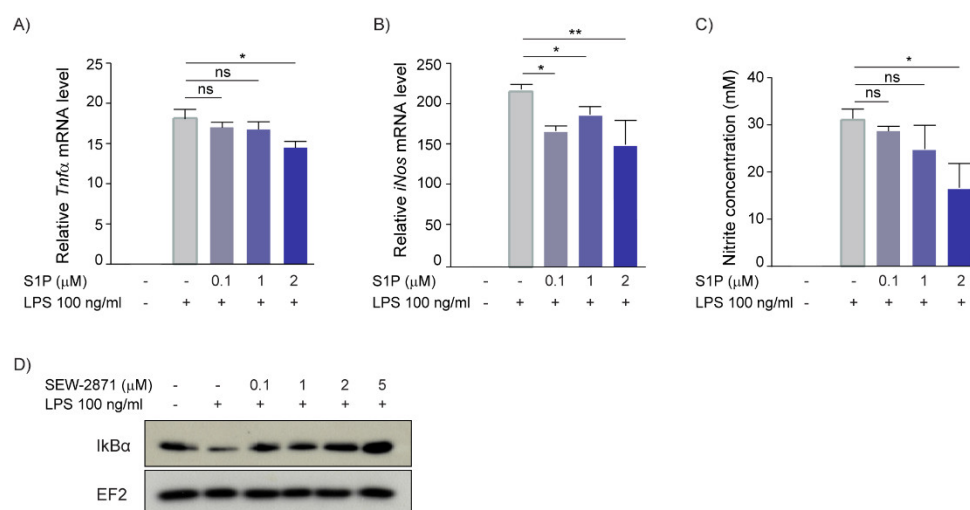


**Figure S2.** SPHK1 modulation is effective both at the expression and protein level and results in altered S1P secretion and higher monocytes chemoattraction. A, B) Quantitative RT-PCR analysis of SPHK1 in human glioma cells, LN229 and LN308, stably transfected with SPHK1-myc to induce SPHK1 overexpression (OE, A) or with shSPHK1 to promote the knockdown (KD, B). EGFP and scramble sh were used as control (CTR), respectively. The target gene was normalized to the mean of two housekeeping genes (PPIA, GAPDH). Data are expressed as fold-change over corresponding controls. C-E) Western blot analysis of SPHK1 expression in LN229, LN308 and NCH82 stably transfected with either SPHK1-myc (OE) or with shSPHK1 (KD) compared with respective controls (CTR). Tubulin or EF-2 served as loading controls. F) Quantification of S1P level by LC-MS/MS in LN229 and LN308 stably transfected with control, SPHK1-myc (OE) or with shSHK1 (KD) vectors. Data are expressed as S1P [d18:1] pmol per 10<sup>6</sup> cells. G, H) Chemoattraction assay of human monocytes towards LN229 and NCH82 either wild type (G) or knockdown for SPHK1 (H) versus corresponding control. I) Chemoattraction assay of human monocytes towards LN229 treated with JTE-013 or solvent control. MCP-1 was used as a positive control for chemoattraction. Data are expressed as percent increased or decreased migration in a time frame of 3 hrs. Mean with SEM are shown, n=3, \*p<0.05, \*\*p<0.01, \*\*\*p<0.001, \*\*\*\*p<0.0001 by Mann-Whitney test in A and B, Kruskal-Wallis test in C, G-I.

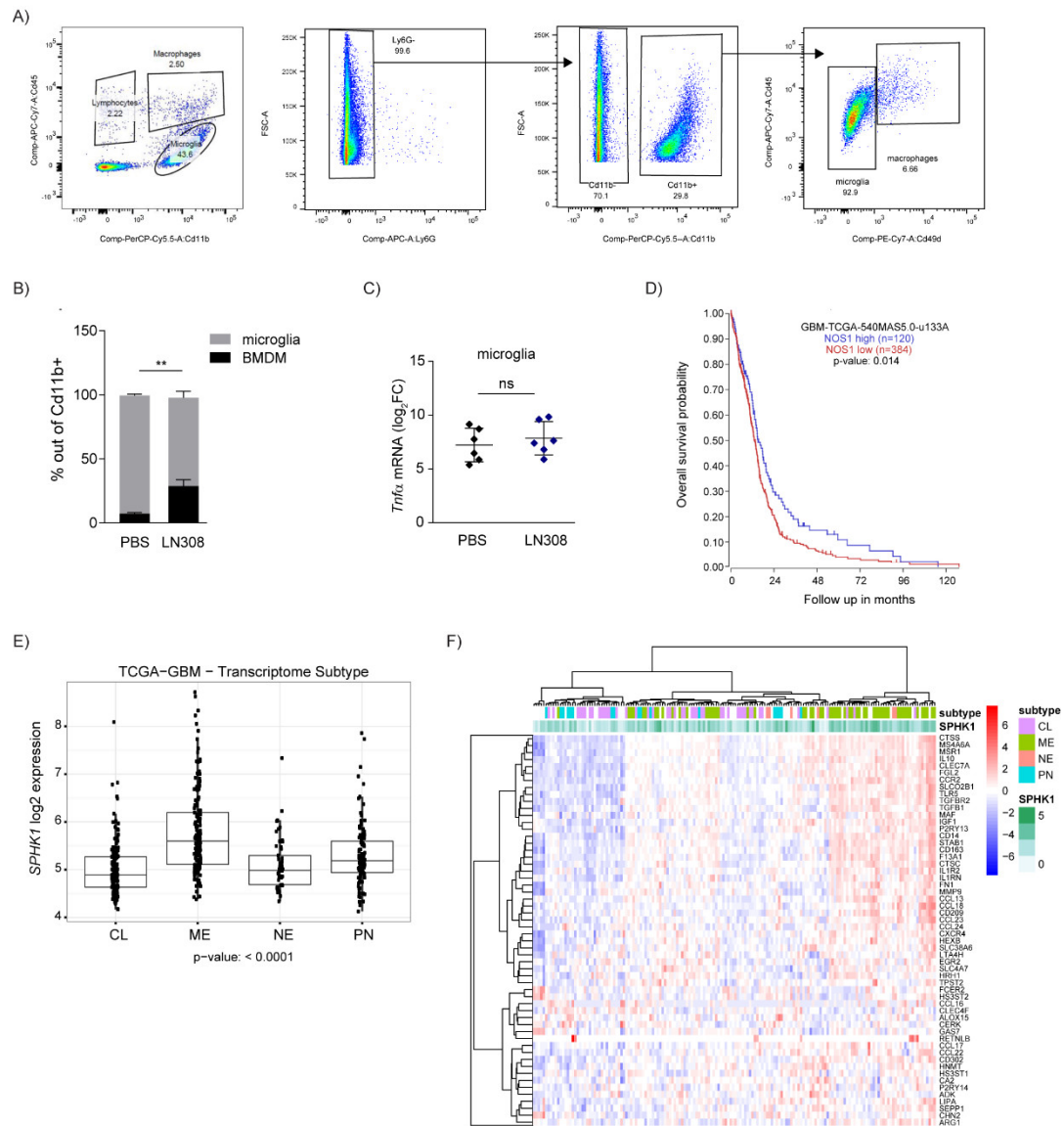


**Figure S3.** S1P shifts TAMs to an anti-inflammatory phenotype, whereas inhibition of SPHK1 activity reverts this phenotype into a pro-inflammatory one. A, B) Quantitative RT-PCR analysis of M2 markers *Arg1* and *Msr1* (A) and M1 markers *Tnf $\alpha$*  and IL-6 (B) in murine primary microglia co-cultured with human NCH82 glioma cells and treated for 24h with 10 or 20  $\mu$ M of SKI-II or corresponding solvent control. C, D) ELISA of M2 cytokine IL-10 (C) and M1 cytokine IL-6 (D) in cell-free supernatant of murine primary microglia co-cultured with NCH82, upon treatment with SKI-II for 24 hrs. E, F) Quantitative RT-PCR analysis of *Arg1* and *Msr1* (E) and *Tnf $\alpha$*  and IL-6 (F) in murine microglia cultured in media conditioned by NCH82 (CM), upon treatment for 24 hrs with 10 or 20  $\mu$ M of SKI-II or corresponding control. G, H) Quantitative RT-PCR analysis of *Arg1* in murine RAW264.7 macrophages co-cultured with LN229 or LN308, overexpressing (OE, G) or knockdown (KD, H) for SPHK1. I, J) Quantitative RT-PCR analysis of *Arg1* (I) and *Tnf $\alpha$*  (J) in RAW264.7 macrophages co-cultured with LN229, LN308 and NCH82, and treated for 24 hrs with 10 and 20  $\mu$ M of SKI-II or corresponding control. K) ELISA of M1 cytokine IL-6 in cell-free supernatant of RAW264.7 macrophages co-cultured with LN229, LN308 and NCH82, upon treatment with SKI-II for 24 hrs. L, M) Quantitative RT-PCR analysis of *Arg1* and *Msr1* (L) and

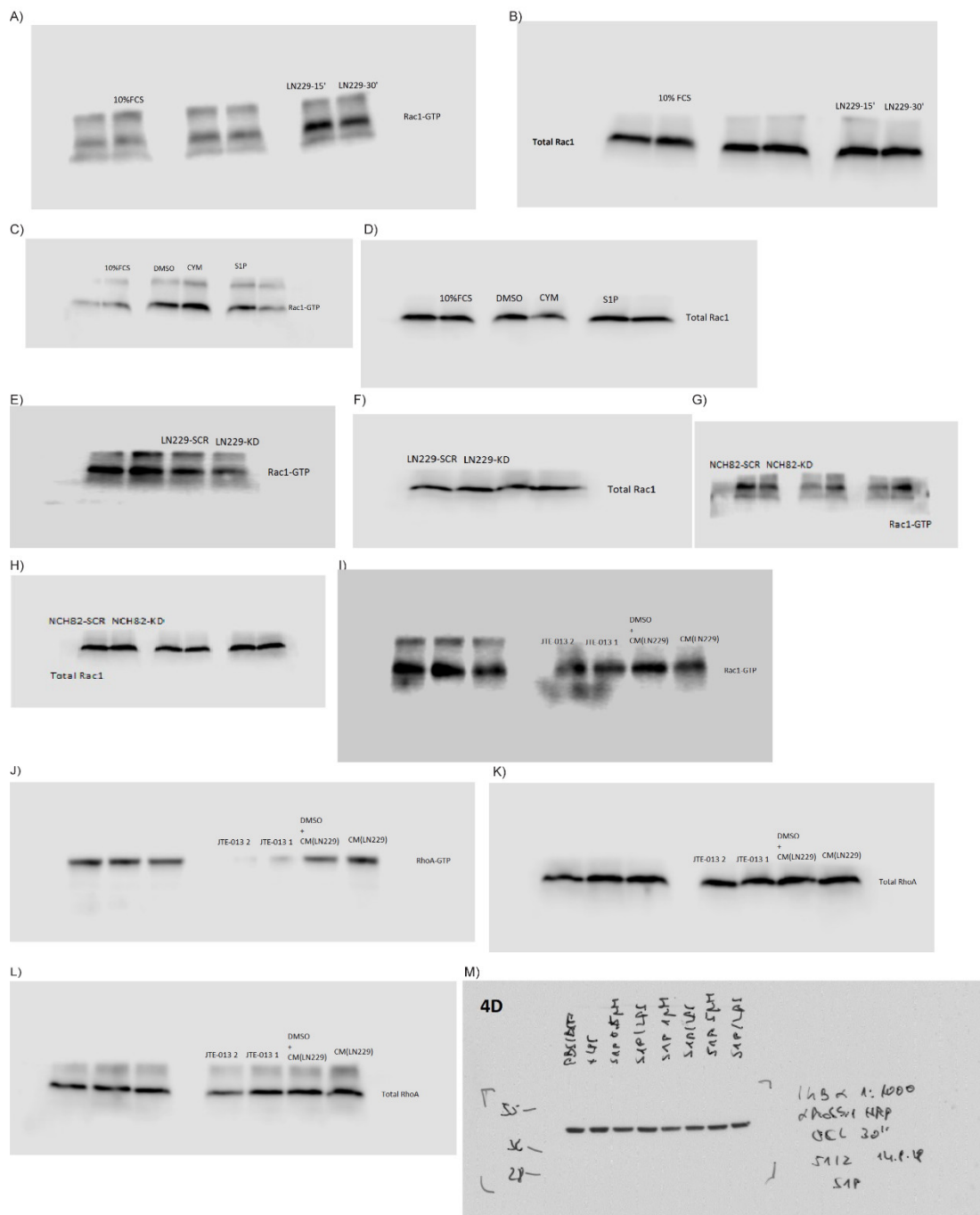
Tnf $\alpha$  and Il-6 (M) in murine primary microglia co-cultured with murine TU-9648 glioma cells and treated for 24 hrs with 10 or 20  $\mu$ M of SKI-II or corresponding solvent control. N, O) ELISA of IL-10 (N) and IL-6 (O) in cell-free supernatant of murine primary microglia co-cultured with TU-9648 cells, upon treatment with SKI-II for 24 hrs. P, Q) Quantitative RT-PCR analysis of Arg1 (P) and Tnf $\alpha$  (Q) in RAW264.7 macrophages co-cultured with TU-9648 cells, and treated for 24 hrs with 10 and 20  $\mu$ M of SKI-II or corresponding control. In all the qPCR analysis, target genes were normalized to the mean of two housekeeping genes (B2m, Hprt). Data are expressed as fold-change over microglia or RAW264.7 cells cultured without glioma cells or conditioned medium. In all the ELISA, data are expressed as pg/ml as plotted against a standard curve for each cytokine. Mean with SEM are shown, n=3, ns: not significant, \*p<0.05, \*\*p<0.01, \*\*\*p<0.001, \*\*\*\*p<0.0001 by One-way ANOVA. .



**Figure S4. S1P reduces LPS-induced inflammation.** A, B) Quantitative RT-PCR analysis of M1 markers Tnf $\alpha$  (A) and iNos (B) in RAW264.7 macrophages co-treated with LPS and increasing concentrations of S1P (0 – 2  $\mu$ M) for 5 hrs. Target genes were normalized to the mean of two housekeeping genes (B2m, Hprt). Data are expressed as fold-change over cells treated with solvent control. C) Nitrite levels (NO $_2^-$ ) assessed by Griess reagent assay in RAW264.7 macrophages co-treated with LPS (100 ng/ $\mu$ L) and increasing concentration of S1P (0 – 2  $\mu$ M) for 24 hrs. Mean with SEM are shown, n=3, ns: not significant, \*p<0.05, \*\*p<0.01, \*\*\*p<0.001 by Kruskal-Wallis test. D) Western blot analysis of I $\kappa$ B $\alpha$  amounts in murine primary microglia treated with LPS and increasing concentrations of S1P analogue SEW-2871 (0 – 5  $\mu$ M, C) for 1 hr. EF2 served as loading control.



**Figure S5. Increased expression of pro-inflammatory genes correlates with a better survival in GB.** A) Gating strategy used to define microglia (Cd11b+/Cd45low/Cd49dlow) and BMDM (Cd11b+/Cd45high/Cd49dhigh) populations from dissociated mouse brains. B) The amount of microglia (Cd11b+/Cd45low/Cd49dlow) and BMDM (Cd11b+/Cd45high/Cd49dhigh) are quantified by FACS based on the abundance of the corresponding expression markers and expressed as percentage out of Cd11b+ cells. C) Quantitative RT-PCR analysis of M1 marker *Tnfa* in FACS-sorted microglia population. The target gene was normalized to the mean of two housekeeping genes (*B2m*, *Hprt*). Data are expressed as log<sub>2</sub>FC normalized to PBS mice. Mean with SD are shown, n=6, ns: not significant, \*\*p<0.01 by unpaired t-test. D) Kaplan-Meier estimates overall survival in patients with GB divided into high and low NOS1 expression. Data analyzed using the Affymetrix 540 MASS 5.0-u133 array available in the online R2: Genomics Analysis and Visualization Platform (<http://r2.amc.nl>). E) SPHK1 expression measured in classical (CL), mesenchymal (ME), neural (NE) and proneural (PN) GB subtypes as in the TCGA Transcriptome GB dataset. F) Heatmap of M2 genes in TCGA Affimetrix GB dataset. Samples are clustered according to subtypes classification and SPHK1 expression level. p value in Kaplan Meier results from scan modulus test.





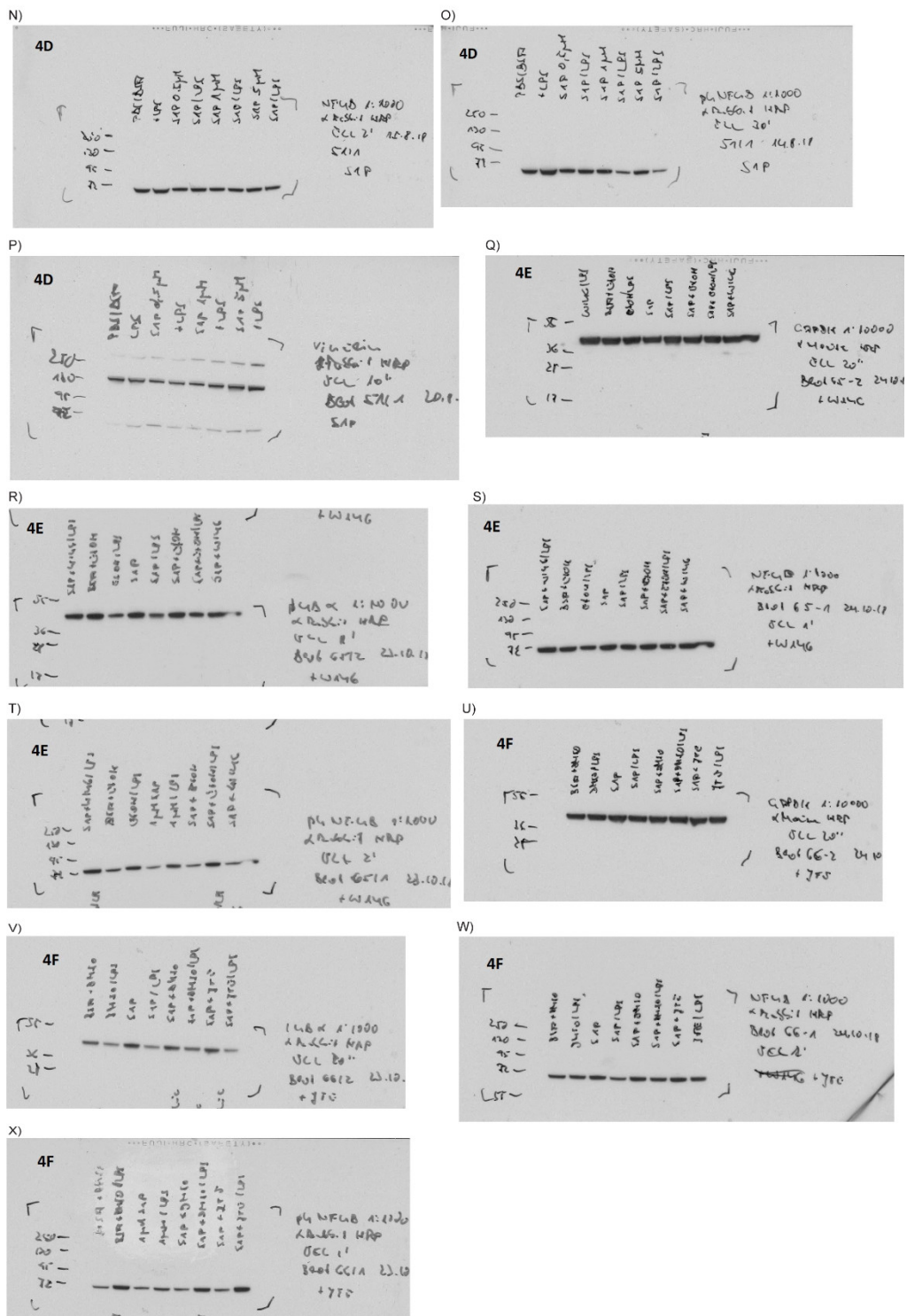


Figure S6. Original WB files.

## Supplementary Tables

Supplementary Table S1: Cell lines

CELL LINE	SOURCE
HEK293 (RRID:CVCL_0045)	ATCC, USA
RAW264.7 (RRID:CVCL_0493)	ATCC, USA
LN229 (RRID:CVCL_0393)	ATCC, USA
LN308 (RRID:CVCL_0394)	ME Hegi (Lausanne University, Switzerland)

Supplementary Table S2: Plasmids

NAME	DESCRIPTION
pLKO.1-SPHK1	shRNA against SPHK1
pLKO.1-shCTR	Control shRNA
pLVX- SPHK1-myc	SPHK1 with N-terminal myc-tag
pLVX-tRFP-2A-Luc	Control; CDS fusion of tRFP and FLuc with porcine teschovirus-1 2A

Supplementary Table S3: Short hairpin RNA

	NAME	TARGET SEQUENCE 5'-3'
hSPHK1 overexpression	Sphk1shRNAHum-F	CCG GAC CTA GAG AGT GAG AAG TAT CCT CGA GGA TAC TTC TCA CTC TCT AGG TTT TTT TG
	Sphk1shRNAHum-R	CTA GCA AAA AAA CCT AGA GAG TGA GAA GTA TCC TCG AGG ATA CTT CTC ACT CTC TAG GT
hSPHK1 knockdown	hSPHK1-A-shRNA-F	CCG GCC TGA CCA ACT GCA CGC TAT TCT CGA GAA TAG CGT GCA GTT GGT CAG GTT TTT G
	hSPHK1-A-shRNA-R	AAT TCA AAA ACC TGA CCA ACT GCA CGC TAT TCT CGA GAA TAG CGT GCA GTT GGT CAG G
shCTR	control	CAACAAGATGAAGAGCACCAA

Supplementary Table S4: Primers

GENE NAME	PRIMER NAME	TARGET SEQUENCE 5'-3'
<b>Human primers</b>		
SPHK1	SPHK1_F	GAG CAC CGG TGT CAT TCC
	SPHK1_R	CAG ACG TGG GCT GAG CTT
GAPDH	GAPDH_F	AGA AGG CTG GGG CTC ATT TG
	GAPDH_R	AGG GGC CAT CCA CAG TCT TC
PPIA	PPIA_F	TTC TGC TGT CTT TGG GAC CT
	PPIA_R	CAC CGT GTT CTT CGA CAT TG
<b>Mouse primers</b>		
B2m	B2m_F	TTC AGT ATG TTC GGC TTC CC
	B2m_R	TGG TGC TTG TCT CAC TGA CC
Gapdh	Gapdh_F	TTG ATG GCA ACA ATC TCC AC
	Gapdh_R	CGT CCC GTA GAC AAA ATG GT
ActB	ActB_F	ATG GAG GGG AAT ACA GCC C



	ActB_R	TTC TTT GCA GCT CCT TCG TT
Hprt	Hprt1_F	CAT AAC CTG GTT CAT CAT CGC
	Hprt1_R	TCC TCC TCA GAC CGC TTT T
Arg1	Arg1_F	GTG AAG AAC CCA CGG TCT GT
	Arg1_R	GCC AGA GAT GCT TCC AAC TG
Msr1	Msr1_F	TTT CCC AAT TCA AAA GCT GA
	Msr1_R	CCT CCG TTC AGG AGA AGT TG
Il-6	Il-6_F	TGG TAC TCC AGA AGA CCA GAG G
	Il-6_R	AAC GAT GAT GCA CTT GCA GA
iNos	iNos_F	TTC TGT GCT GTC CCA GTG AG
	iNos_R	TGA AGA AAA CCC CTT GTG CT
Il1b	Il1β_F	GGT CAA AGG TTT GGA AGC AG
	Il1β_R	TGT GAA ATG CCA CCT TTT GA
Tnfα	Tnfα_F	AGG GTC TGG GCC ATA GAA CT
	Tnfα_R	CCA CCA CGC TCT TCT GTC TAC
Cd206	Cd206_F	GCAAATGGAGCCGTCTGTGC
	Cd206_R	CTCGTGGATCTCCGTGACAC

Supplementary Table S5: Antibodies

NAME	CLONE	CATALOGUE NO	MANUFACTURER
Cd11b	M1/70	45-0112-82	eBioscience
Cd45	30-F11	103116	Biolegend
Cd49d	R-2	103617	Biolegend
CD14	M5E2	301804	Biolegend
EF2	C-14	sc-13004	Santa Cruz Biotechnologies
GAPDH	6C5	CB1001-500UG	Merck Millipore
IκBα		9242	Cell Signaling Technology
Ly6G	1A8	17-9668-82	eBioscience
NFκB-p65	D14E12	8242S	Cell Signaling Technology
Phosphor-NFκB-p65	93H1	3033S	Cell Signaling Technology
Rac1		ARC03	Cytoskeleton
RhoA		ARH04	Cytoskeleton
Sphk1		71700	Abcam
Tubulin		T9026	Sigma-Aldrich
Vinculin	N1N3	gtx113294-100	GeneTex

Supplementary Table S6: Measurements of S1P derivatives analyzed by UPLC-ESI-(QqQ)-MS

Time [min]	solvent A [%]	solvent B [%]
0	70	30
1	70	30
2.5	30	70
4	20	80
5	20	80
6.5	10	90
6.6	0	100
7.5	0	100
7.6	70	30
9	70	30

Solvent A: Acetonitrile/water (1:4) + 0.1% formic acid

Solvent B: Acetonitrile/isopropanol (1:4) + 0.1% formic acid

Supplementary Table 7: Multiple reaction monitoring (MRM) for S1P measurements

	Precursor Ion	Product Ion	
S1P(d17:1) (internal standard) (Rt = 2.70 min)	m/z 422	m/z 60	
	m/z 422	m/z 113	Quantifier
	m/z 422	m/z 127	
S1P(d18:1) (Rt = 2.89 min)	m/z 436	m/z 60	
	m/z 436	m/z 113	Quantifier
	m/z 436	m/z 127	