Communication between host organism and cancer cells is transduced by systemic sphingosine kinase 1/sphingosine 1-phosphate signaling to regulate tumor metastasis

Suriyan Ponnusamy, Shanmugam Panneer Selvam, Shikhar Mehrotra, Toshihiko Kawamori, Ashley J. Snider, Lina M. Obeid, Yuan Shao, Roger Sabbadini, and Besim Ogretmen

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Supporting information figure legends

Figure S1. Effects of genetic loss of SK1 on systemic Sph and dhSph in SK1-/- versus WT mice. (A-B) Sph (A) and dhSph (B) were measured in serum obtained from WT versus SK1-/- mice using LC/MS/MS. Data, obtained from duplicates at least in two independent trials, are represented as mean ± SEM. Error bars represent standard deviations. P <0.05 (*) was considered significant.

Figure S2. Loss of systemic SK1 inhibits lung colonization/metastasis of B16 melanoma cells in SK1-/- versus WT mice. Effects of systemic SK1 loss on lung colonization/metastasis of B16 melanoma cells were measured in WT and SK1-/- mice (n=3/group). Data are represented as mean ± SEM. Error bars represent standard deviations. P <0.05 (*) was considered significant.

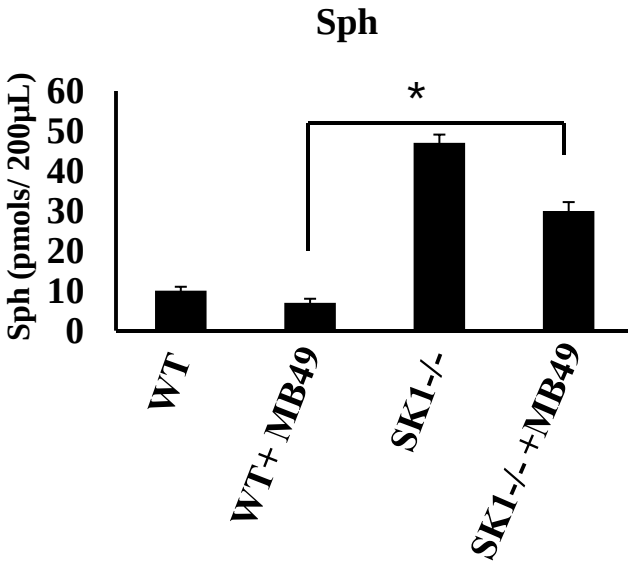
Figure S3. Effects of systemic loss of SK1 on expression of genes involved in the regulation of metastasis in MB49-derived lung tumors obtained from SK1-/- versus WT mice. Expression of 84 genes involved in the regulation of metastasis was measured using the Super Array Mouse Tumor Metastasis by Q-PCR in MB49-derived tumors obtained from SK1-/- compared to WT control mice. List of gene names and their relative expression in lung tumors of SK1-/- mice compared to WT controls are presented.

Figure S4. Effects of S1PR2 inhibition on Brms1 expression. (A) Effects of FTY720 versus JTE013 treatment on *BRMS1* mRNA at various concentrations, 10-100 nM for 24

h were measured by Q-PCR in A549 human lung adenocarcinoma cells (beta-actin was used as normalization control). (B) *Brms1* mRNA was measured using Q-PCR in response to S1PR2 inhibition in response to JTE013 treatment in MB49 cells (normalized to rRNA). (C) Efficiency of siRNA-mediated knock-down of *Brms1* mRNA compared to control Scr siRNAs at 100 nM was measured using Q-PCR. (D) Effects of neutralizing S1P by Sphingomab (0.2-1 μM) in WT and S1PR2-/- MEFs grown in control media were measured using Q-PCR compared to IgG-treated controls. Data, obtained from duplicates at least in two independent trials are represented as mean ± SEM. Error bars represent standard deviations. $P < 0.05$ (*) was considered significant.

Supplementary Figure S1

A



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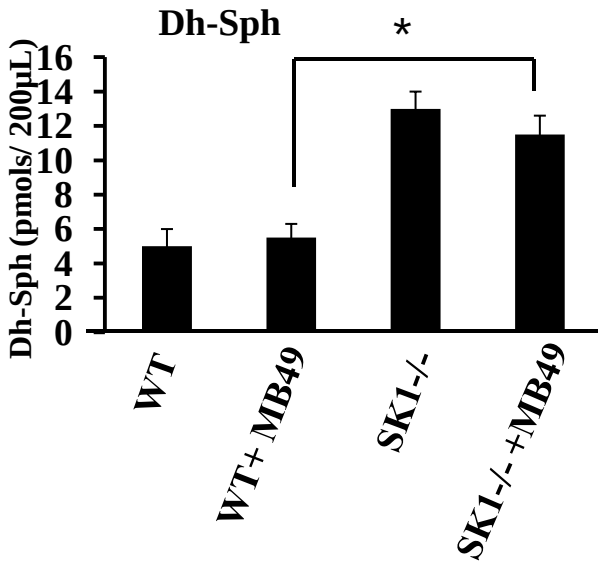
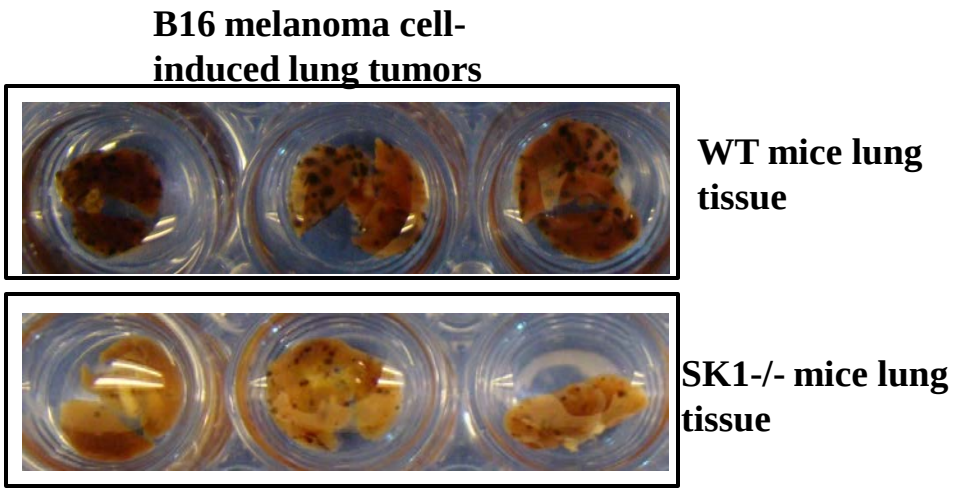
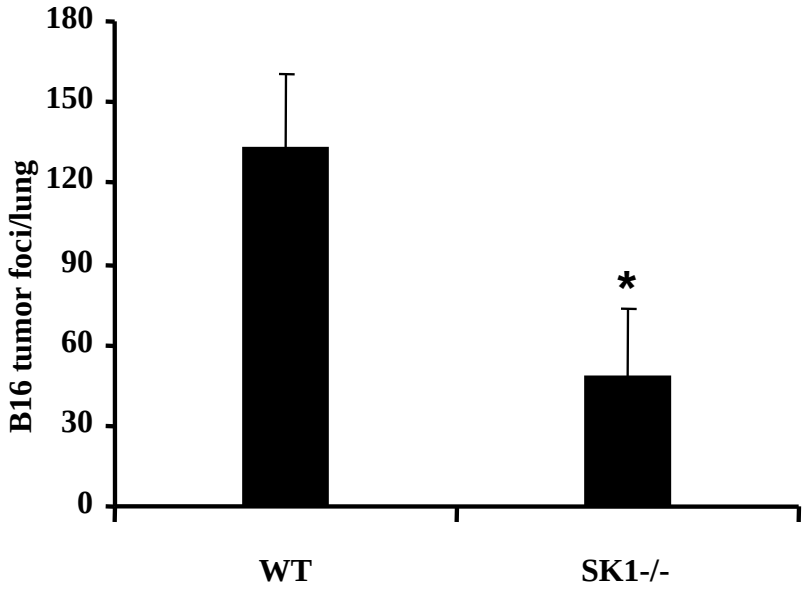


Supplementary Figure S2





Supplementary Figure S3

Pathway specific Super array for Mouse Tumor Metastasis

Apc	Brms1	Ccl7	Cd44	Cdh1	Cdh11	Cdh6	Cdh8	Cdkn2a	Chd4	Col4a2	Csf1
0.1	8.99	1.01	1.0	1.11	0.87	0.79	0.10	0	-0.23	0.59	1.27
Ctbp1	Ctnna1	Ctsk	Ctsl	Cxcl12	Cxcr4	Denr	Ela2	Ephb2	Etv4	Ewsr1	Fat1
0.09	-1.6	0.45	-.98	0.79	0.77	1.17	2.1	1.9	1.09	-0.92	0.24
Fgfr4	Flt4	Fn1	Fxyd5	Gpnmb	Kiss1r	Hgf	Hpse	Hras1	Htatip2	Igf1	Il18
1.19	1.67	0.0	1.0	0.5	1.56	2.7	0.19	1.21	0.19	-2.0	0.12
Il1b	Il8rb	Itga7	Itgb3	Cd82	Kiss1	Kras	Rpsa	Mycl1	Mcam	Mdm2	Met
0.0	1.27	0.06	0.23	1.24	1.1	1.45	0.18	-0.92	0.75	4.8	2.1
Mmp10	Mmp11	Mmp13	Mmp2	Mmp3	Mmp7	Mmp9	Mta1	Mtss1	Myc	Nf2	Nme1
3.4	-0.19	1.18	1.0	-2.9	1.87	1.09	0.98	-1.56	0.87	0.46	1.29
Nme2	Nme4	Nr4a3	P2ry5	Plaur	Pnn	Pten	Rb1	Rorb	Set	Smad2	Smad4
-.58	-1.2	0.87	-0.94	0.69	-2.18	0.38	0.90	0.37	2.49	1.11	2.27
Src	Sstr2	Syk	Tcf20	Tgfb1	Timp2	Timp3	Timp4	Tnfsf10	Trp53	Tshr	Vegfa
1.01	2.41	-093	0.57	1.07	0.08	0.39	2.34	-0.91	2.0	0.88	1.09

Supplementary Figure S4