

Title:

**Generation of sphingosine-1-phosphate is enhanced in biliary tract cancer patients and is associated with lymphatic metastasis**

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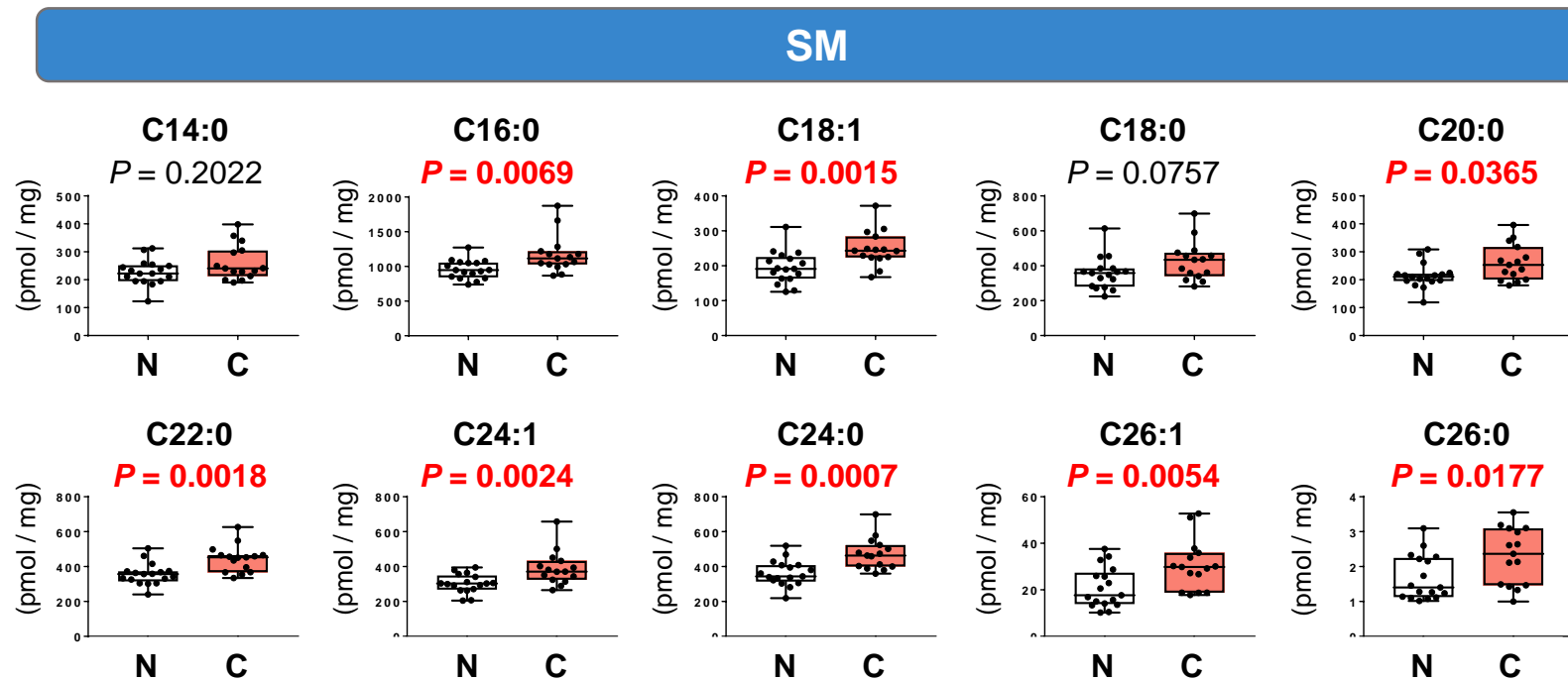
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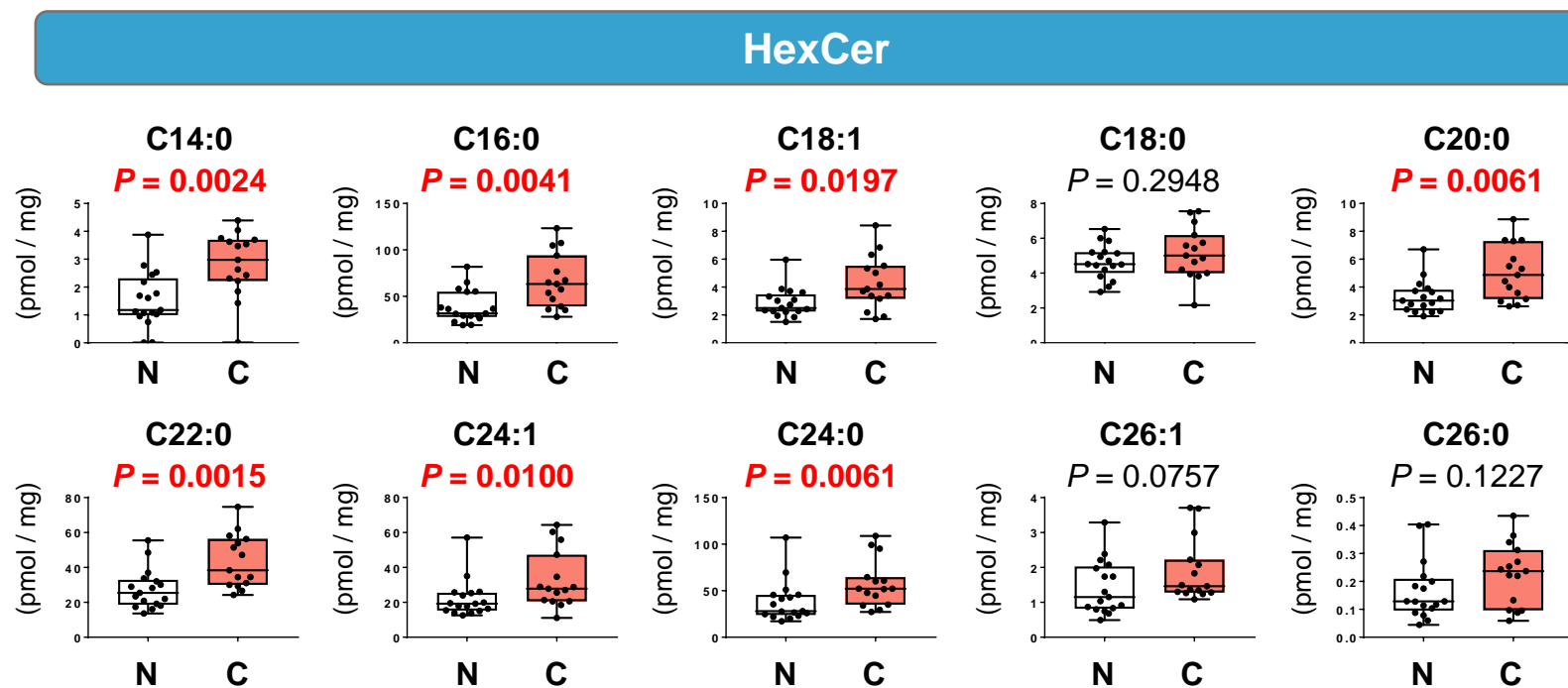
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# Supplementary Figure 1



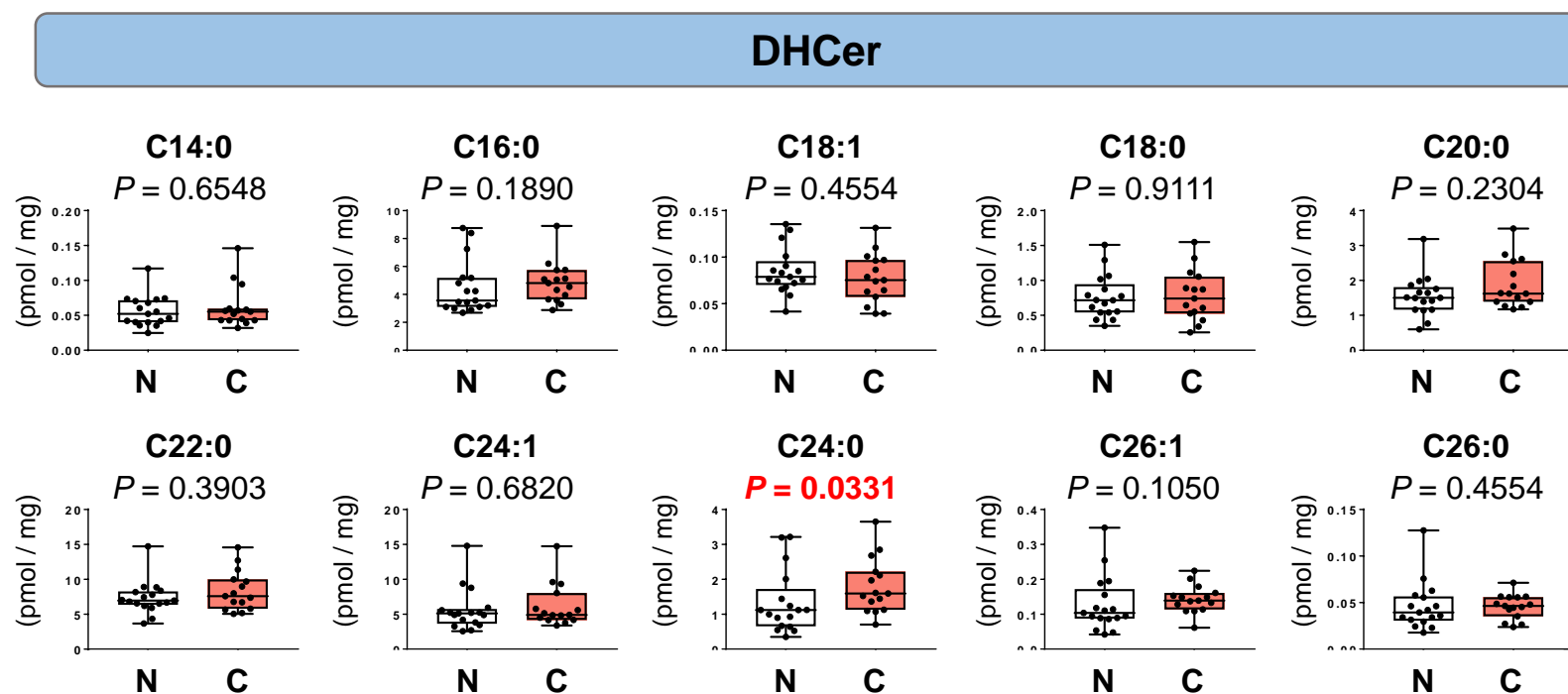
**Supplementary Figure 1.** The level of SM species (C14:0, C16:0, C18:1, C18:0, C20:0, C22:0, C24:1, C24:0, C26:1, and C26:0) in biliary tract cancer (n = 15) and normal biliary tract tissue (n = 17) were determined by LC-ESI-MS/MS. Mean values are shown by the horizontal lines. Two-sided  $P$  values < 0.05 were considered statistically significant (Mann-Whitney U test). N: normal biliary tract tissue; C: biliary tract cancer tissue.

# Supplementary Figure 2



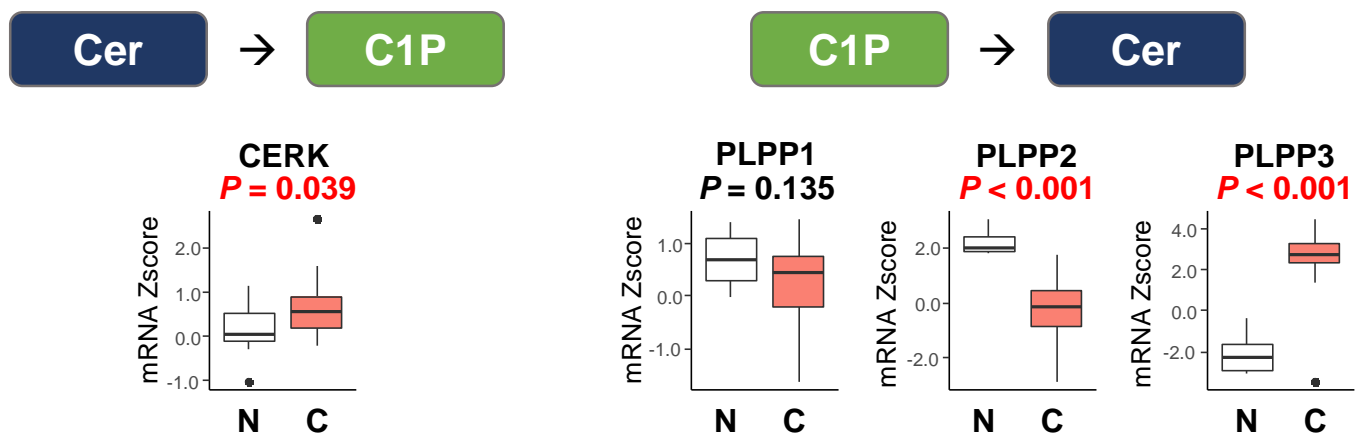
**Supplementary Figure 2.** The level of HexCer species (C14:0, C16:0, C18:1, C18:0, C20:0, C22:0, C24:1, C24:0, C26:1, and C26:0) in biliary tract cancer (n = 15) and normal biliary tract tissue (n = 17) were determined by LC-ESI-MS/MS. Mean values are shown by the horizontal lines. Two-sided *P* values < 0.05 were considered statistically significant (Mann-Whitney U test). N: normal biliary tract tissue; C: biliary tract cancer tissue.

# Supplementary Figure 3



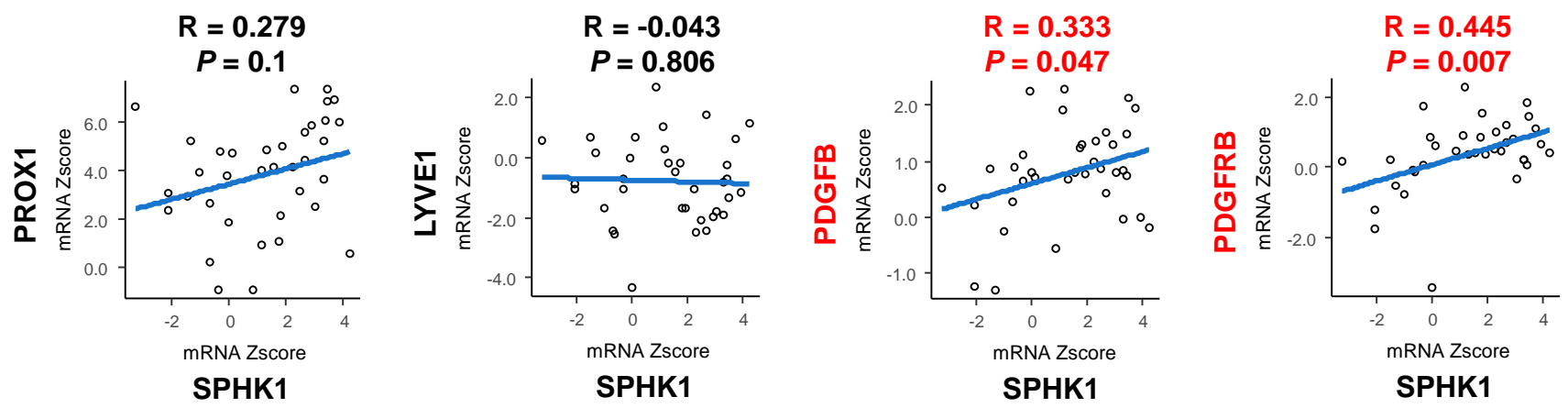
**Supplementary Figure 3.** The level of DHCer species (C14:0, C16:0, C18:1, C18:0, C20:0, C22:0, C24:1, C24:0, C26:1, and C26:0) in biliary tract cancer (n = 15) and normal biliary tract tissue (n = 17) were determined by LC-ESI-MS/MS. Mean values are shown by the horizontal lines. Two-sided  $P$  values < 0.05 were considered statistically significant (Mann-Whitney U test). N: normal biliary tract tissue; C: biliary tract cancer tissue.

## Supplementary Figure 4



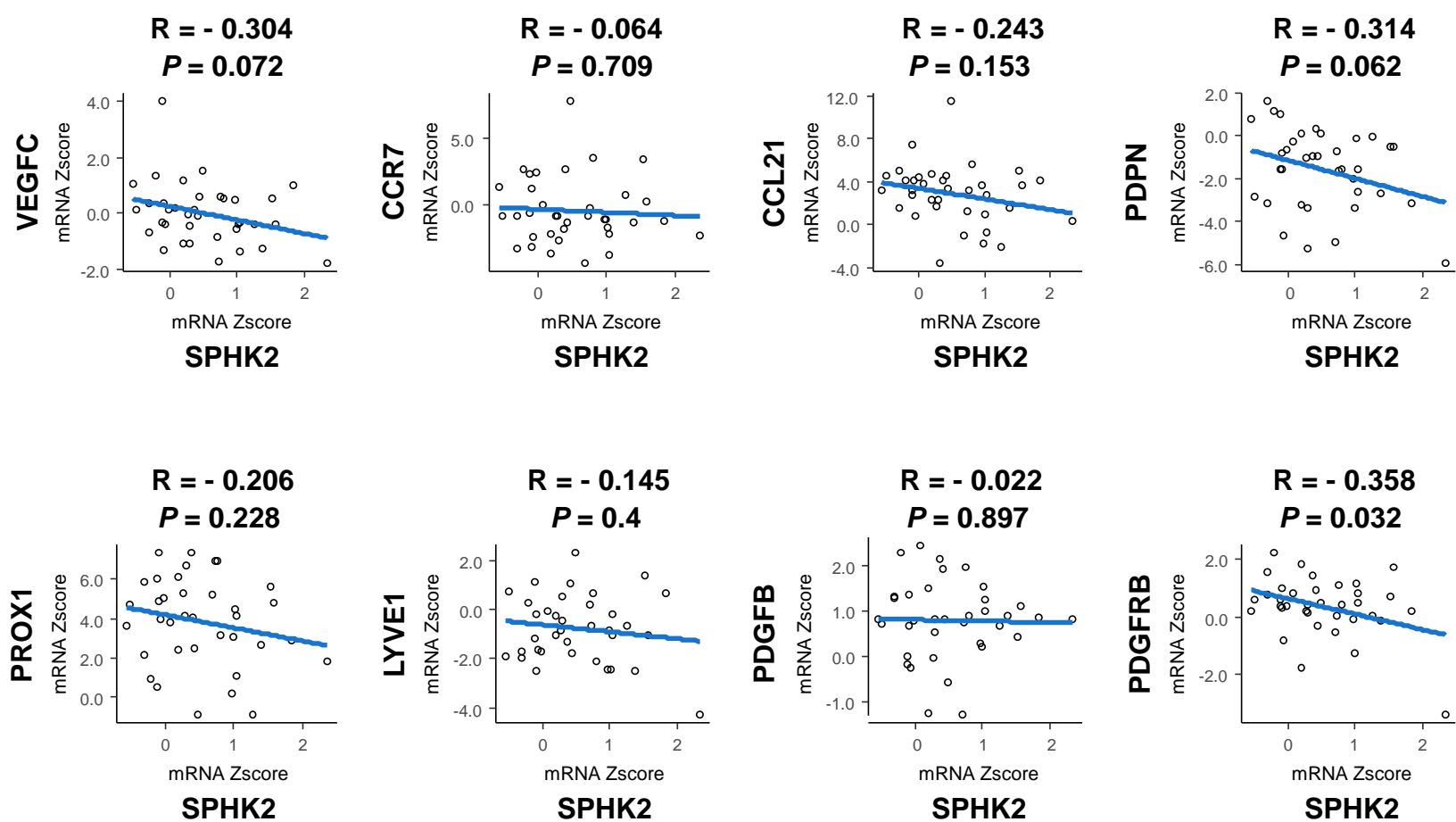
**Supplementary Figure 4.** The gene expression level of CERK and PLPPs in TCGA cohort (36 and 9 samples of cancer and non-cancer tissue in cholangiocarcinoma cohort) were analyzed. Mean values are shown by the horizontal lines. Two-sided  $P$  values  $< 0.05$  were considered statistically significant (Mann-Whitney U test). N: normal biliary tract tissue; C: biliary tract cancer tissue.

## Supplementary Figure 5



**Supplementary Figure 5.** Pearson correlations were calculated between gene expression level of SPHK1 and those of PROX1, LYVE1, PDGFB, and PDGFR $\beta$  in biliary tract cancer in TCGA cohort (36 samples of cancer tissue in cholangiocarcinoma cohort). All tests were two-sided, and  $P$  values  $< 0.05$  were considered statistically significant.

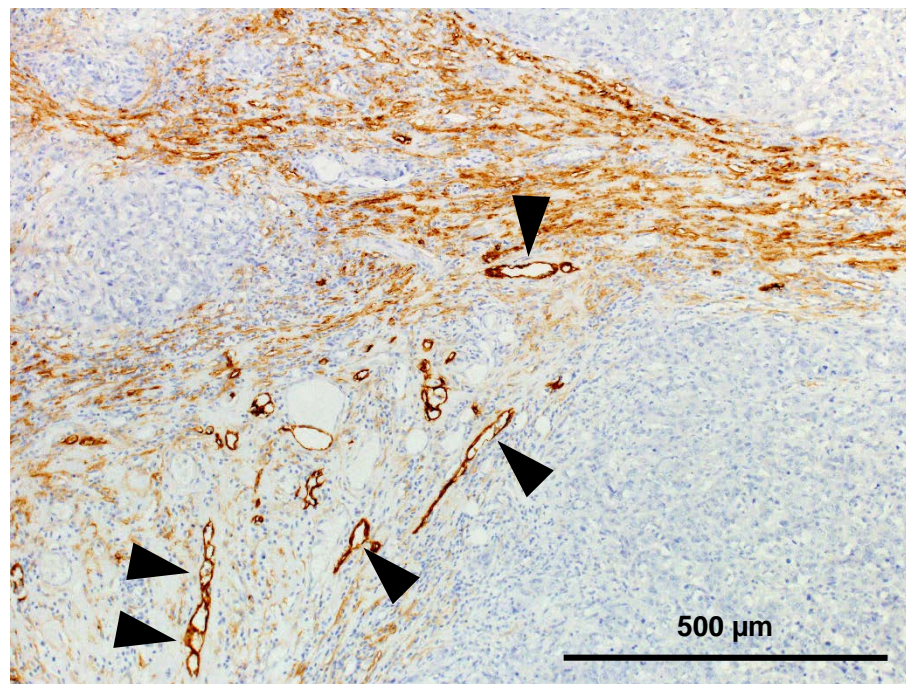
## Supplementary Figure 6



**Supplementary Figure 6.** Pearson correlations were calculated between gene expression level of SPHK2 and those of VEGFC, CCR7, CCL21, PDPN, PROX1, LYVE1, PDGFB, and PDGFR $\beta$  in biliary tract cancer in TCGA cohort (36 samples of cancer tissue in cholangiocarcinoma cohort). All tests were two-sided, and  $P$  values  $< 0.05$  were considered statistically significant.

# Supplementary Figure 7

a



b

	No. of patients with		<i>P</i> value
	pSPHK1 high expression	pSPHK1 low expression	
Intratumoral lymphatic vessel density			> 0.999
High	6	2	
Low	9	3	

**Supplementary Figure 7.** (a) Immunohistochemical staining of D2-40 in biliary tract cancer is shown. Arrow heads shows lymphatic vessels in gallbladder cancer. Scale bar = 500  $\mu\text{m}$ . (b) The association between the pSPHK1 expression and intratumoral lymphatic vessel density in gallbladder cancer tissues with ( $n = 10$ ) or without lymph node metastasis ( $n = 10$ ) was evaluated by the Fisher's exact test. All tests were two-sided, and  $P$  values  $< 0.05$  were considered statistically significant.