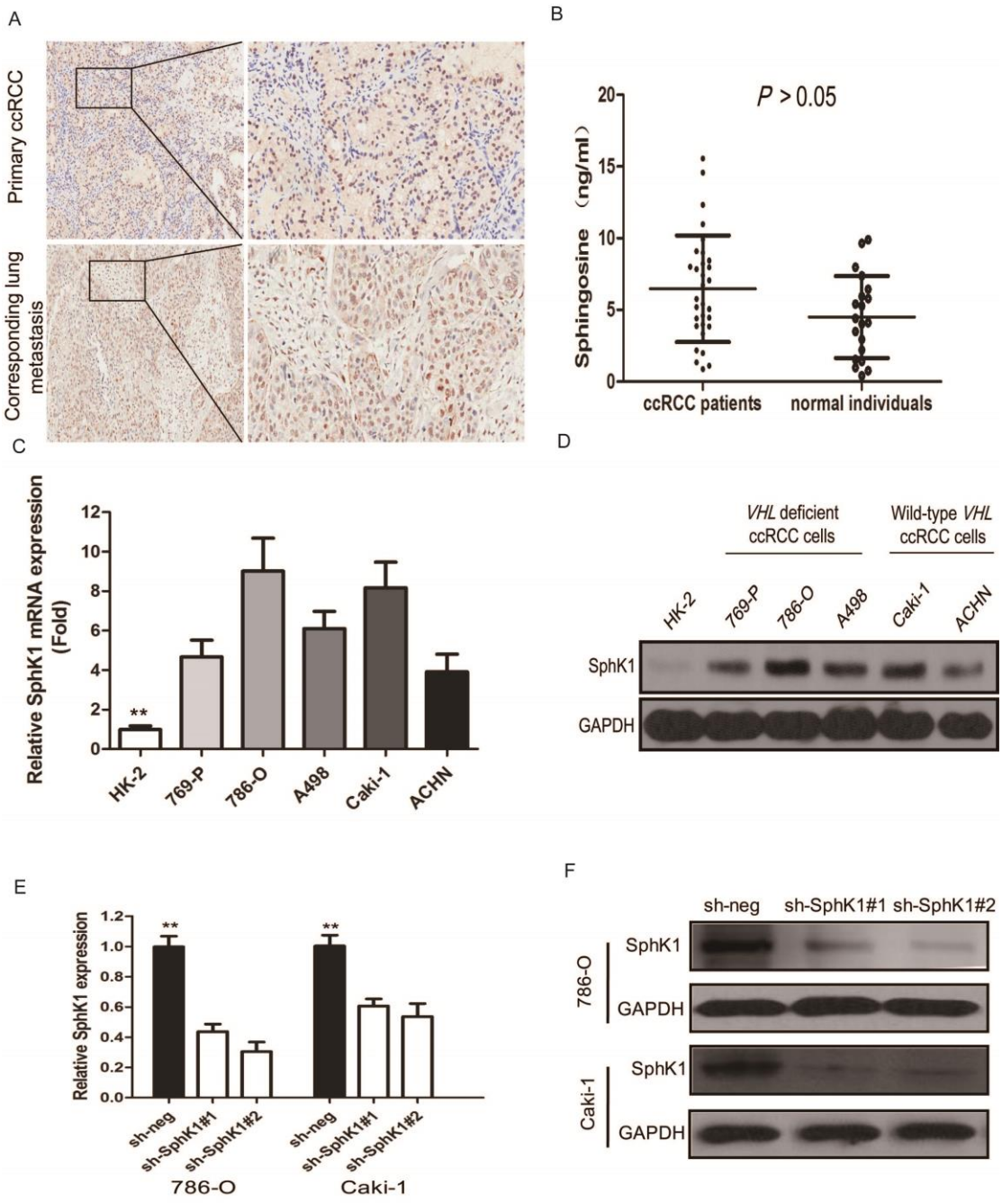


Figure S1 A. Representative immunohistochemistry micrographs of SphK1 expression from primary ccRCC tissue and the corresponding lung metastasis (left 200 × magnification, right 400 × magnification). B. LC-MSMS analysis for sphingosine levels in plasma from a small group of ccRCC patients (N=30) and normal individuals (N=20). C.

Quantitative RT-PCR assay for SphK1 mRNA level in a panel of ccRCC-derived cell lines. SphK1 mRNA was higher in the ccRCC cell lines, including Caki-1, ACHN, A498, 786-O, and 769-P than in the immortalized human renal tubular epithelial cells line HK-2 which is not tumorigenic. ** $P < 0.01$. D. Western blot analysis of SphK1 protein in a panel of ccRCC-derived cell lines and HK-2 cell line. E. Quantitative RT-PCR assay for SphK1 mRNA level in 786-O and Caki-1 cells stably transfected with shRNAs. ** $P < 0.01$. F. Western blot analysis of SphK1 protein in 786-O and Caki-1 cells stably transfected with shRNAs.

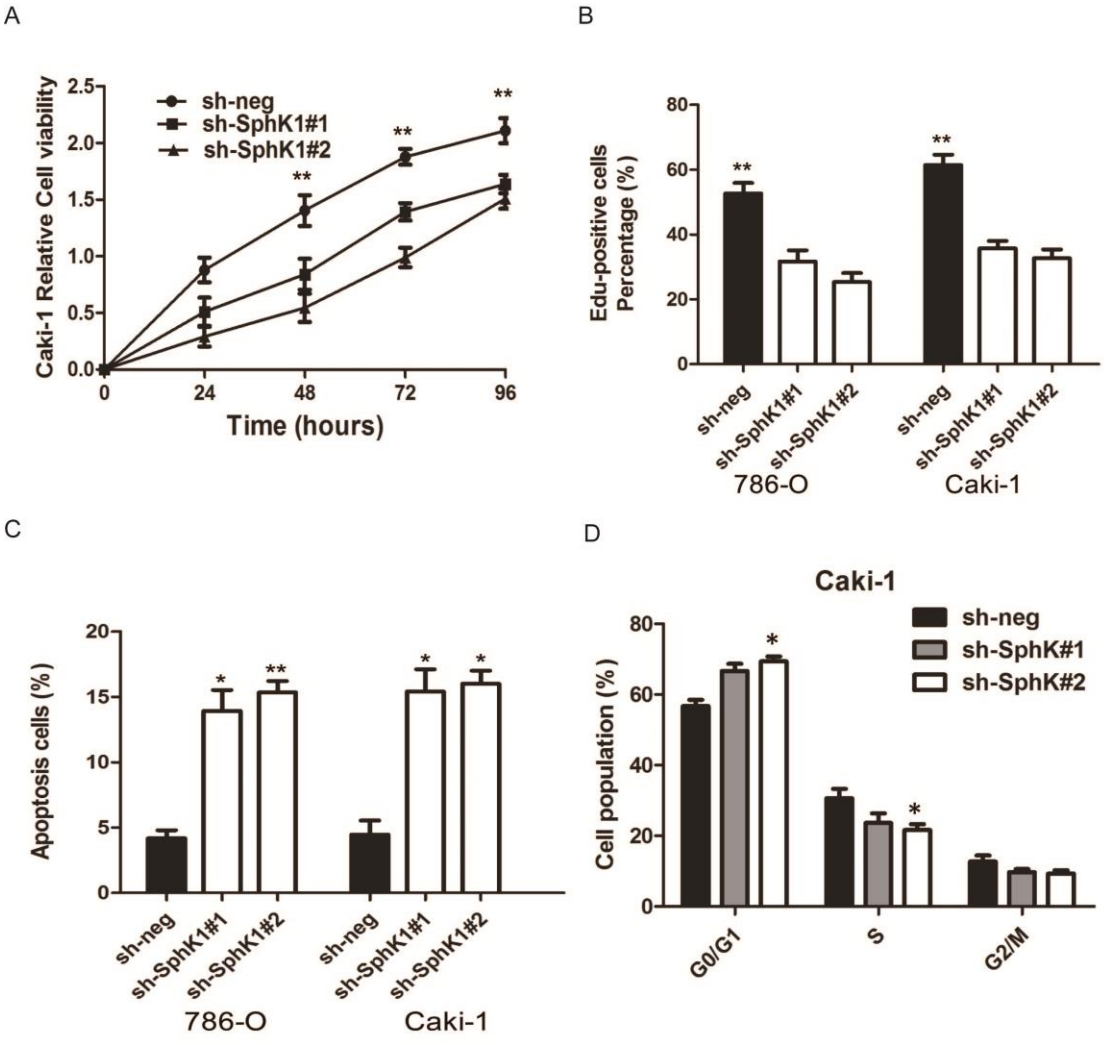

Figure S2 A. Cell viability was measured by MTT assay at 0, 24, 48, 72 and 96 hours in Caki-1 cells stably transfected with SphK1 short hairpin RNAs (sh#1 and #2) or control shRNA (sh-neg). The data are presented as the means ± S.D. from three independent experiments. B. The bar chart represents the percentage of EdU incorporation assay of 786-O and Caki-1 cells stably expressing SphK1 shRNAs (sh#1 and #2) or control shRNA. ** $P < 0.01$. C. The percentage of apoptotic 786-O and Caki-1 cells stably expressing SphK1 shRNAs (sh#1 and #2) or control shRNA was determined by flow cytometric analysis. The data represent the means ± SD from three independent experiments. *$P < 0.05$, ** $P < 0.01$. D. The bar chart represents the percentage of Caki-1 cells stably expressing SphK1 shRNAs (sh#1 and #2) or control shRNA in the G0/G1, S, or G2/M phase, as indicated. The data are presented as the means ± SD from three independent experiments. *$P < 0.05$.

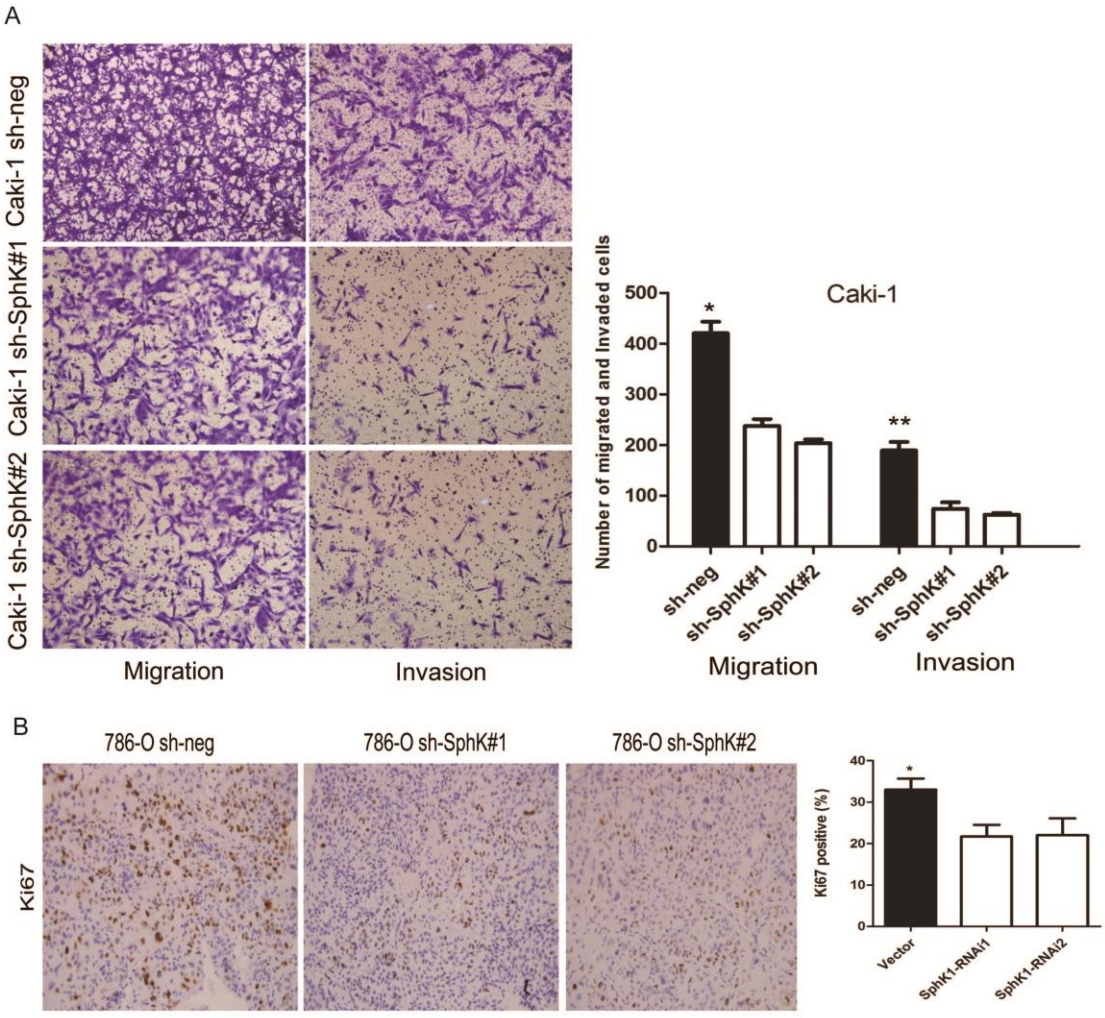


Figure S3 A. Representative images of migration and invasion assays in Caki-1 cells stably expressing SphK1 shRNAs (sh#1 and #2) or control shRNA (left) and quantification of the relative migration and invasion cell number (right). Scale bar = 100 μm. ***** $P < 0.05$, ****** $P < 0.01$. B. Representative immunohistochemistry micrographs of Ki67 expression from SphK1-shRNA xenograft tumors (left) and quantification of the relative Ki67 positive cell number (right).

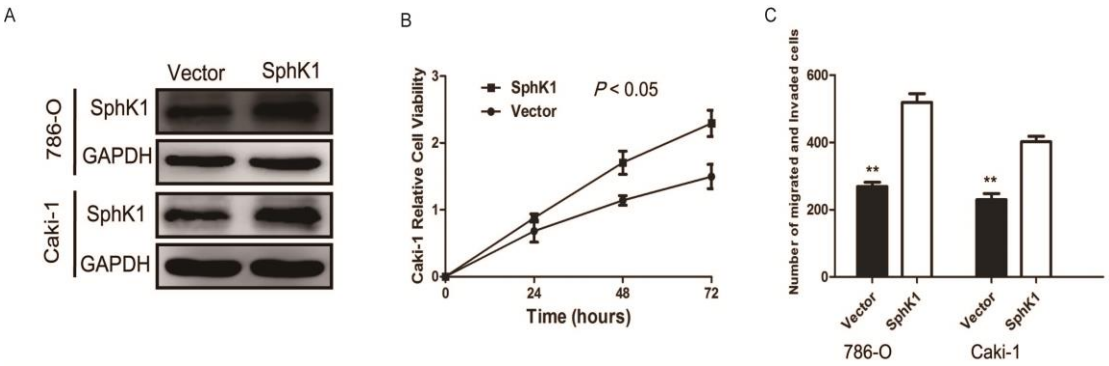


Figure S4 A. Western blot analysis of SphK1 protein in 786-O and Caki-1 cells with overexpression of SphK1 or control vector. B. Cell viability was measured by MTT assay at 0, 24, 48 and 72 hours in Caki-1 cells with overexpression of SphK1 or control vector. The data are presented as the means ± S.D. from three independent experiments. C. Quantification of the relative migration and invasion of 786-O and Caki-1 cells with overexpression of SphK1 or control vector. ** $P < 0.01$.