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Figure S1 Validation of the specificity of antibodies against total or phosphorylated SPHK1 protein. A distinct band corresponding to the predicted molecular weight of SPHK1 protein (45 kDa) was evident in HEK293 cells transfected with a SPHK1 construct (pCMV6_XL4/SPHK1).

Figure S2 S1P promotes migration of C666-1 cells in wound healing assays. The effect of S1P on C666-1 cell migration was examined in wound healing assays using charcoal-stripped FBS (Biowest, France). Left panel: Representative images showing enhanced migration of C666-1 cells in the presence of S1P at 24 h. Right panel: Images were analysed using WimScratch (Wimasis Image Analysis) and the data are expressed relative to the wound area at 0 h (=100%). * $p < 0.05$.

Figure S3 S1P enhances invasion of SUNE1 cells in vitro. The invasive ability of SUNE1 was examined in pre-coated invasion chambers (8 μm pore size, BD BioCoat Matrigel) in the presence of S1P in the lower chamber.

Figure S4 Confirmation of the inhibition of AKT activation following treatment with a PI3K/AKT inhibitor, LY294002. Western blotting analyses of SUNE1 cells treated with 1 μM LY294002 or the vehicle control for 19 h.

Figure S5 Expression profiles of S1P receptors in HONE1 and SUNE1 cells. All five receptor mRNAs were detected in both cell lines.

Figure S6 Kaplan Meier survival analysis revealed higher expression of S1PR3 correlated with poor patient survival ($p < 0.05$).