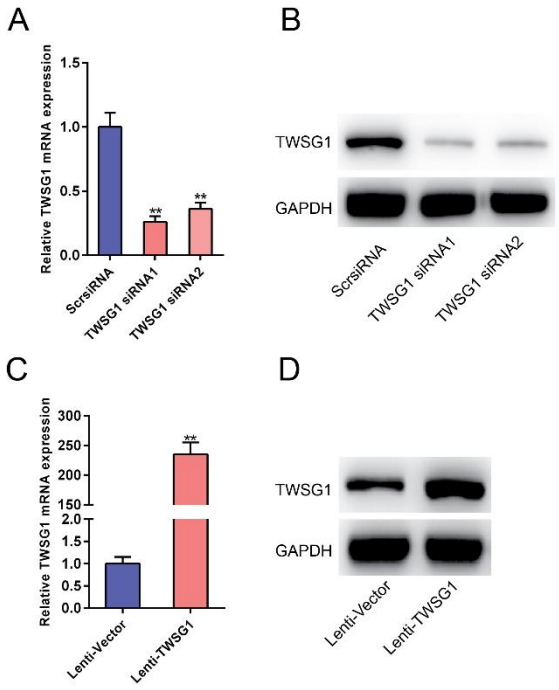

Fig. S1 TWSG1 knockdown and overexpression in K1 and TPC1 cells respectively.

(A) qRT-PCR analysis of mRNA expression levels of TWSG1 in K1 cells transfected with TWSG1 specific siRNA by lipofectamine. Data was expressed as means±S.D of three independent experiments. "**" indicates P<0.01. (B) Western blot analysis of TWSG1 in K1 cells transfected with TWSG1 specific shRNA. GAPDH was used as loading control. Representative images of three repeated experiments were shown. (C) qRT-PCR analysis of mRNA expression levels of TWSG1 in TPC1 cells infected with Lenti-Vector or Lenti-TWSG1. Data was expressed as means±S.D of three independent experiments. "**" indicates P<0.01. (D) Western blot analysis of TWSG1 in TPC1 cells Lenti-Vector or Lenti-TWSG1. GAPDH was used as loading control. Representative images of three repeated experiments were shown.

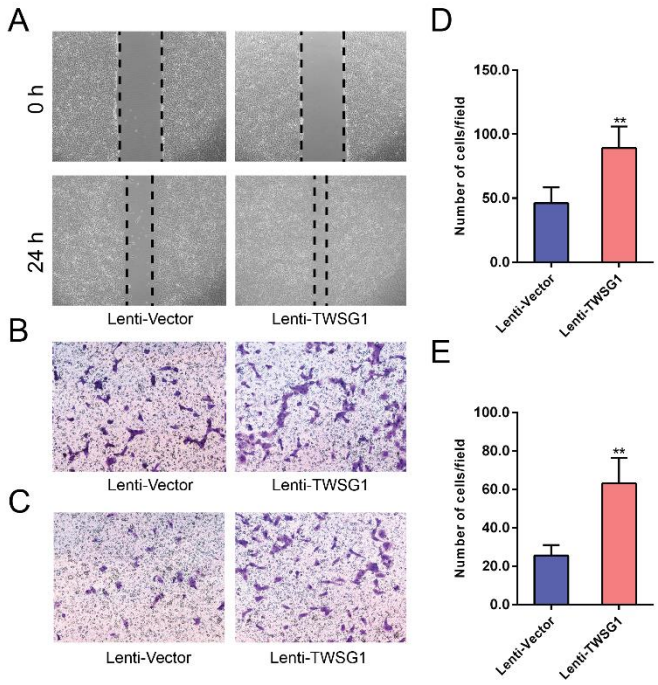

Fig. S2 TWSG1 overexpression promotes the migration and invasion of TPC1 cells.

(A) Wound healing assays were used to determine the migratory ability of TPC1 cells overexpressing TWSG1. Representative images at 0 h and 24 h of three repeated experiments are shown. (B) Transwell assays were performed to determine the migratory ability of TPC1 cells overexpressing TWSG1. Representative images of migrated cells in the lower chamber stained with crystal violet. (C) Transwell assays were performed to determine the invasive ability of TPC1 cells overexpressing TWSG1. Representative images of invasive cells in the lower chamber stained with crystal violet. (D) The quantification of cell migration is presented as migrated cell numbers. All data are expressed as the mean ± S.D. of three independent experiments. ** indicates $P<0.01$. (E) The quantification of cell invasion is presented as invasive cell numbers. All data are expressed as the mean ± S.D. of three independent experiments. ** indicates $P<0.01$.

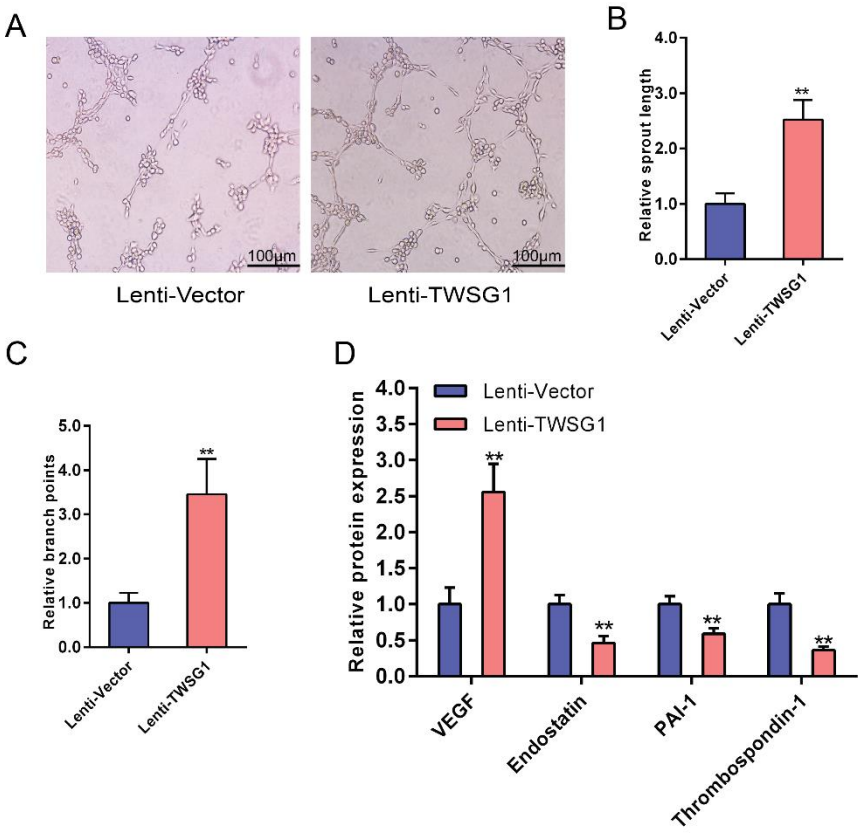

Fig. S3 TWSG1 overexpression promotes the impact of TPC1 cells on endothelial cell function.

(A) TWSG1 overexpression in TPC1 cells affects tube formation of endothelial cells. HUVECs were incubated with the supernatant of TPC1 cells with or without TWSG1 overexpression. Thereafter, HUVECs were subjected to Matrigel assays. Representative micrographs of HUVECs stimulated with supernatants of TWSG1-overexpressing TPC1 cells are shown. Scale bar= 100 μm. (B) Cumulative sprout length and (C) branch points of capillary-like structures were measured after 4 h and quantified. All data are expressed as the mean ± S.D. of three independent experiments. ** indicates $P<0.01$. (D) The expression levels of VEGF, endostatin, PAI-1 and thrombospondin-1 in TPC1 cells with TWSG1-overexpressing supernatant measured by ELISA. All data are expressed as the mean ± S.D. of three independent experiments. ** indicates $P<0.01$.

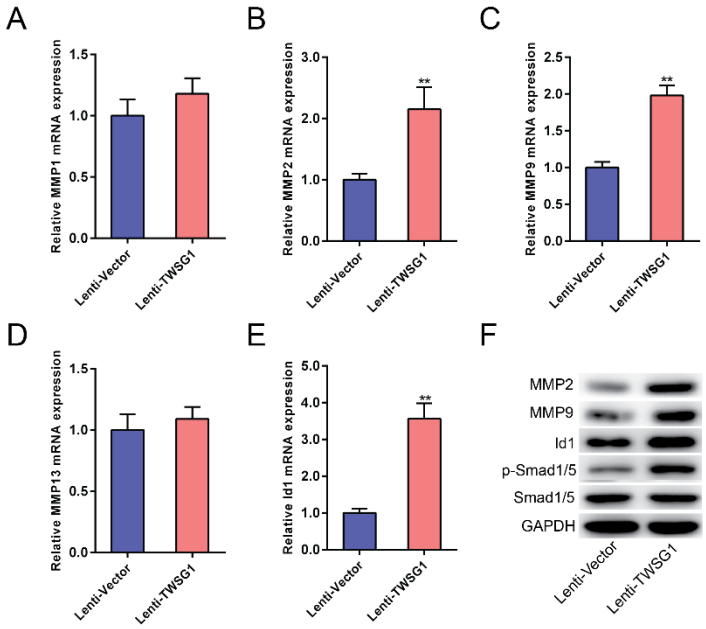

Fig. S4 TWSG1 overexpression promotes the expression of MMPs and the BMP target gene Id1.

qRT-PCR analysis of the mRNA expression levels of MMP1 (A), MMP2 (B), MMP9 (C), MMP13 (D) and Id1 (E) in TPC1 cells with TWSG1 overexpression. The values were normalized to GAPDH mRNA expression. Data are expressed as the mean ± S.D. of three independent experiments. ** indicates P<0.01. (F) Western blot analysis of MMP2, MMP9, Id1 and p-Smad1/5/8 in TPC1 cells overexpressing TWSG1. Representative images of three repeated experiments are shown.

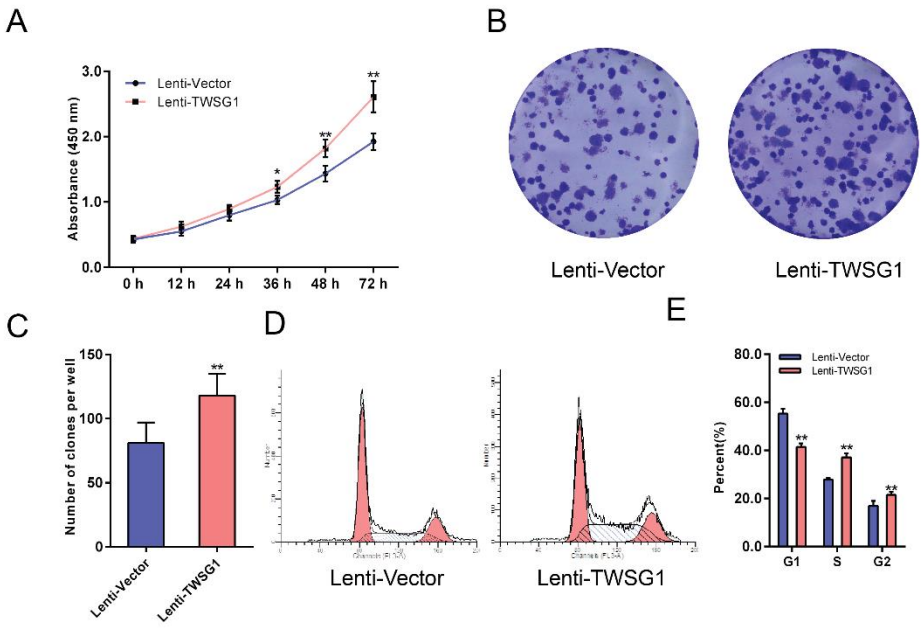

Fig. S5 TWSG1 overexpression promote the proliferation of TPC1 cells.

(A) CCK-8 assays were performed to assess TPC1 cell proliferation after 12 h, 24 h, 36 h, 48 h and 72 h of TWSG1 overexpression. (B) Colony formation assays were performed to measure the proliferation of TPC1 cells overexpressing TWSG1. The colonies were identified and counted. (C) The colony formation assay results are presented as histograms. Data were derived from independent experiments performed in triplicate and are presented as the mean ± S.D. (D) Flow cytometry images of the cell cycle in TPC1 cells. (E) Cell cycle quantification is shown as a percentage of total cells. Data are expressed as the mean ± S.D. of three independent experiments. All data are expressed as the mean ± S.D. of three independent experiments. * indicates $P<0.05$, ** indicates $P<0.01$.