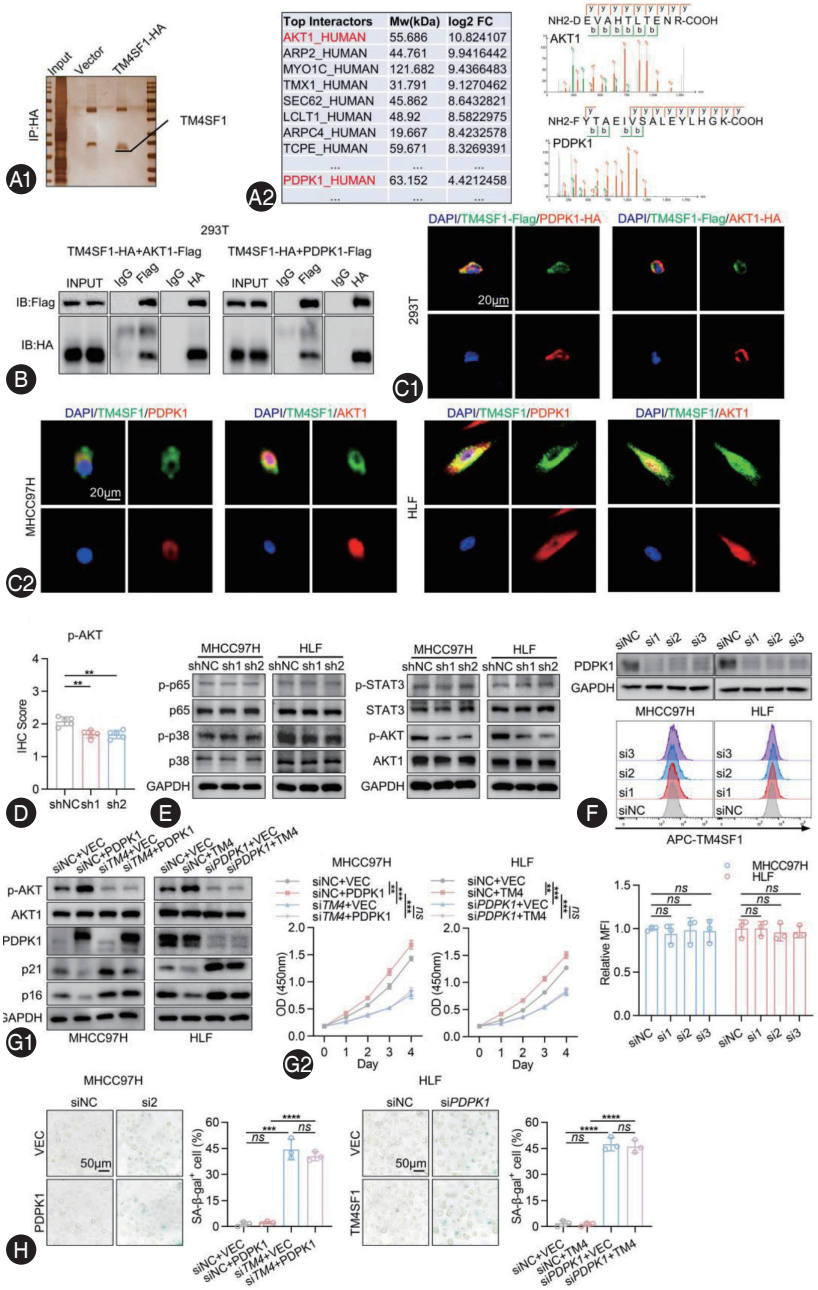

Supplementary Figure 3. TM4SF1 interacts with AKT1 and PDPK1, and promotes AKT phosphorylation. (A) 1) Verification of co-immunoprecipitation products by silver staining; 2) Top interactors of TM4SF1 identified by mass spectrometry and the peptide fragments of AKT1 and PDPK1. (B) The interaction of TM4SF1-AKT1 and TM4SF1-PDPK1, detected by co-immunoprecipitation in HEK293T cells following transfection with the corresponding plasmids. (C) 1) The co-localization of TM4SF1-AKT1 and TM4SF1- PDPK1 in HEK293T cells following transfecting plasmids, using confocal immunofluorescence demonstrating; 2) The co-localization of endogenous TM4SF1-AKT and TM4SF1-PDPK1 in MHCC97H and HLF cells by confocal immunofluorescence imaging. (D) IHC intensity of p-AKT-S473 in the orthotopic tumor model, related to Figure 3B2. (E) The changes in the phosphorylation levels of p38, p65 and STAT3 following TM4SF1 knockdown. (F) MFI of TM4SF1 following PDPK1 siRNA transfection. (G) 1) Expression level of p-AKT-S473, PDPK1, p21 and p16 in MHCC97H and HLF transfected with TM4SF1 or PDPK1 siRNAs, followed by overexpression of PDPK1 or TM4SF1, respectively; 2) CCK-8 assay of MHCC97H and HLF transfected with TM4SF1 or PDPK1 siRNAs, followed by overexpression of PDPK1 or TM4SF1, respectively. (H) SA-β-gal staining of MHCC97H and HLF transfected with TM4SF1 or PDPK1 siRNAs, followed by overexpression of PDPK1 or TM4SF1, respectively. Data are represented as means±standard deviation. *P*-values were calculated using the Student's *t*-test (unpaired). ns>0.05, **P*<0.05, ***P*<0.01, ****P*<0.001, *****P*<0.0001. TM4SF1, transmembrane 4 L six family member 1; PDPK1, 3-phosphoinositide dependent protein kinase 1; MFI, Mean fluorescence intensity; IHC, immunohistochemistry; ns, not significant.

 https://doi.org/10.3350/cmh.2024.0934