**Supplementary Materials: TM4SF1 Promotes Proliferation, Invasion, and Metastasis in Human Liver Cancer Cells**

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**Figure S1.** Expression of TM4SF1 in HepG2 cells after transfection with TM4SF1-expressing plasmids. HepG2 cells were transfected with TM4SF1-expressing plasmids, maintained in 5% CO₂ at 37 °C for 72 h and 96 h, and then observed by fluorescence microscopy (400×) and light microscopy (400×). At 72 h (A,B) and 96 h (C,D), the total cell number (A,C) and number of fluorescent cells (B,D) were determined. The results indicate that transfection
efficiency (fluorescent cells/total cells) was greater than 80% at both time points. HepG2 cells were transfected with TM4SF1-expressing plasmids or blank vectors, and real-time PCR and Western blotting were used to measure expression of mRNA (E) and protein (F); Non-transfected HepG2 cells were controls. Densitometric quantification of levels of TM4SF1 (G) was performed for three independent experiments, and protein expression of the indicated proteins normalized to levels of GAPDH was presented as arbitrary units (mean ± SD). Transfection with TM4SF1-expressing plasmids led to significantly higher expression than transfection with blank vectors or controls (★ p < 0.01).

Figure S2. Expression of TM4SF1 in HepG2 cells after transfection with TM4SF1-siRNAs. HepG2 cells were transfected with TM4SF1-siRNA, maintained in 5% CO₂ at 37°C for 24 h and 48 h, and observed by fluorescence microscopy (400×) and light microscopy (400×). At 24 h (A,B) and 48 h (C,D), the total cell number (A,C) and the number of fluorescent cells (B,D) were determined. The results indicate that transfection efficiency (fluorescent cells/total cells) was greater than 80% at both time points. HepG2 cells were transfected with different TM4SF1-siRNAs (TM4SF1-siRNA-497, -733, and -813) or control siRNA. Non-transfected HepG2 cells were controls. Real-time PCR and Western blotting were used to measure expression of mRNA (E) and protein (F); Densitometric quantification of levels of
TM4SF1 (G) was performed for three independent experiments, and protein expression of the indicated proteins normalized to levels of GAPDH was presented as arbitrary units (mean ± SD). Protein and mRNA expression were lower after transfection with TM4SF1-siRNA-733 (a TM4SF1-siRNA) relative to non-transfected cells and cells transfected with TM4SF1-siRNA-497, TM4SF1-siRNA-813, and control siRNA (★ p < 0.001).

**Figure S3.** Effect of TM4SF1 expression on proliferation of HepG2 cells. Cells transfected with siRNA-TM4SF1, TM4SF1-expressing plasmids, or blank vectors, and those without transfection were harvested and cultured for 5 days, followed by MTT assays to evaluate cell proliferation. The number of HepG2 cells after transfection with siRNA-TM4SF1 (Δ) was significantly reduced compared with other groups (p < 0.01). The number of HepG2 cells after transfection with TM4SF1-expressing plasmids (★) was dramatically increased compared with other groups (p < 0.01). The experiment was performed 4 times and similar findings were observed.

**Figure S4.** TM4SF1 plays an important role in tumor growth and progression of HCC. HepG2 cells over-expressing TM4SF1 showed reduced the expression of caspase-3 and caspase-9, but led to increased expression of PCNA, cyclin D1, MMP-2, MMP-9, VEGF, and uPA, whereas siRNA-mediated silencing of TM4SF1 had the opposite effect.