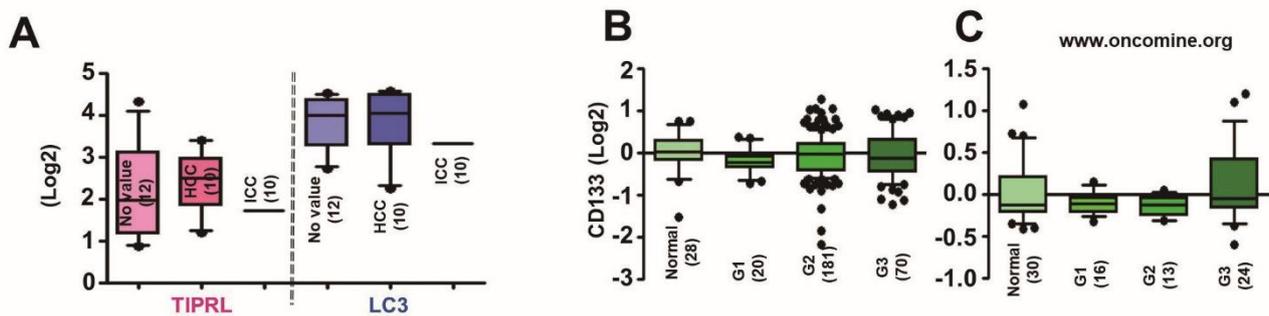
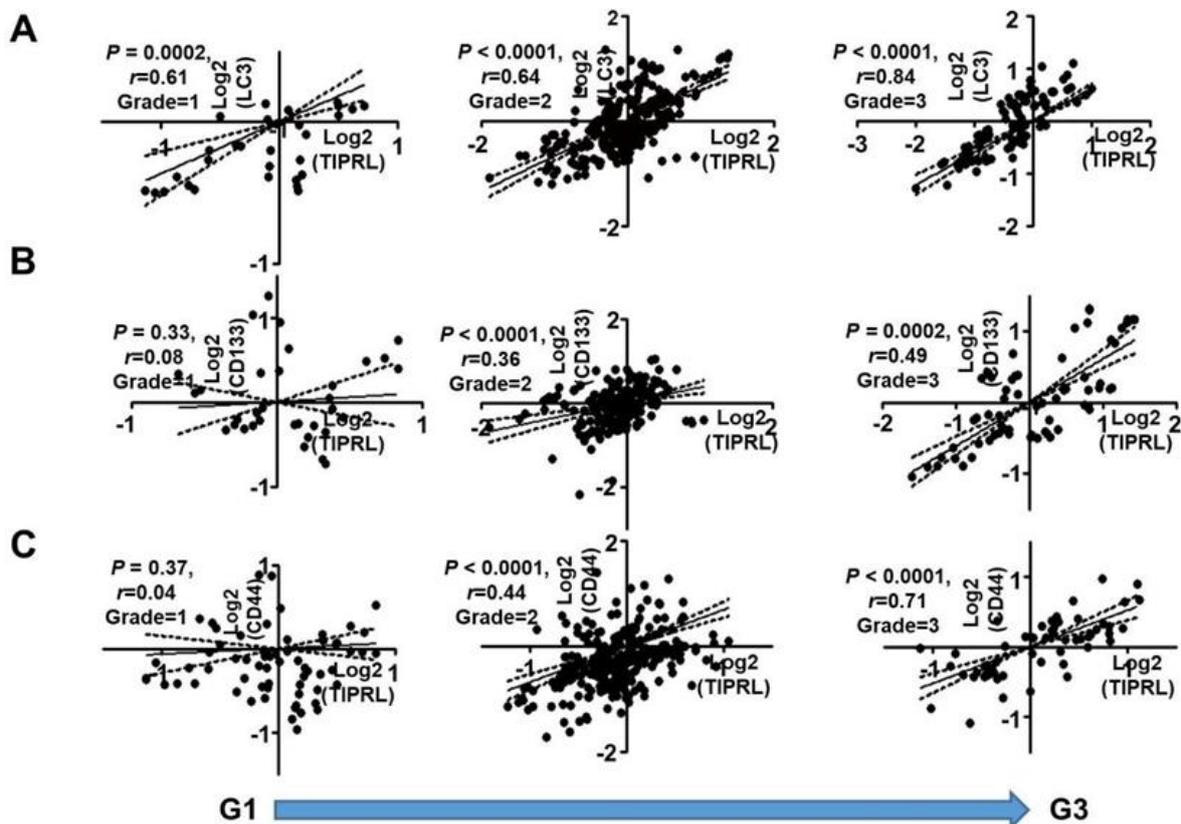


# The Human TOR Signaling Regulator is the Key Indicator of Liver Cancer Patients' Overall Survival: TIPRL/LC3/CD133/CD44 as Potential Biomarkers for Early Liver Cancers

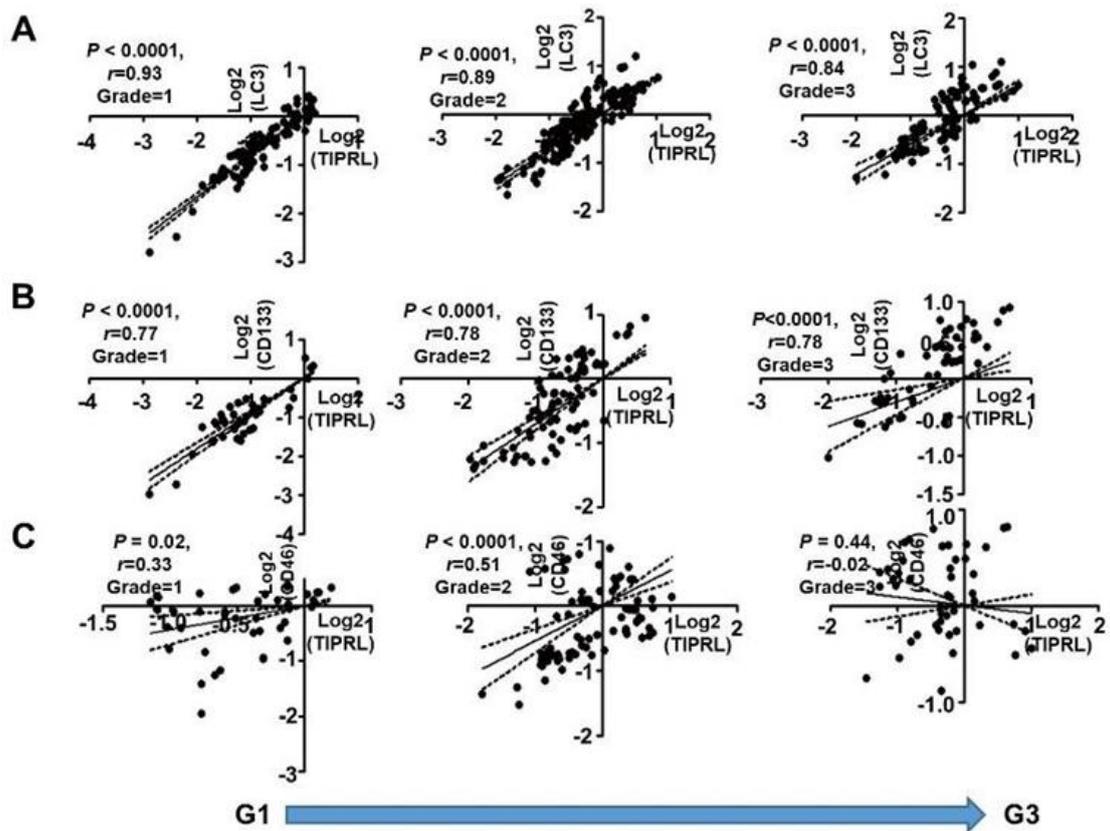
Soo Young Jun, Hyang Ran Yoon, Ji-Yong Yoon, Su-Jin Jeon, Jeong-Ju Lee, Debasish Halder, Jin-Man Kim and Nam-Soon Kim



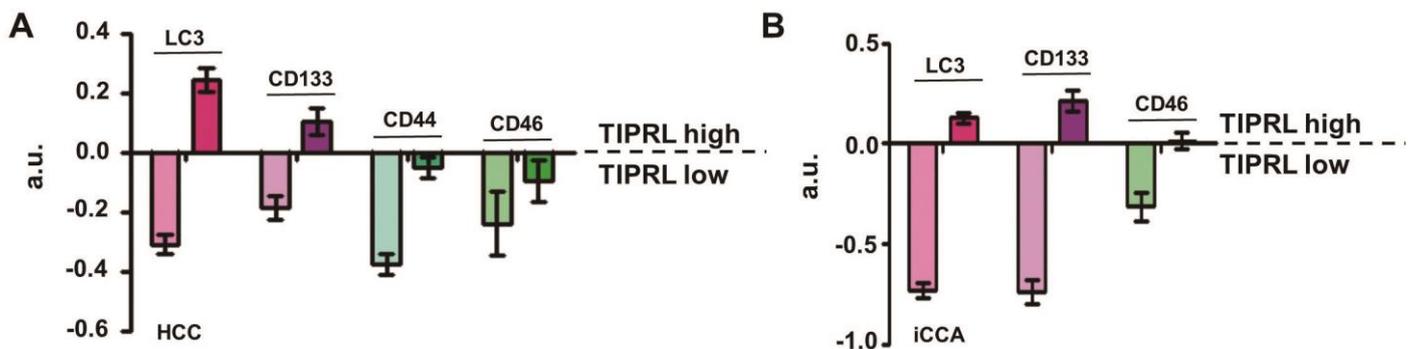
**Figure S1.** The expression level of the variables in HCCs and iCCA. (A) The levels of the variables in HCCs and iCCA were obtained from the public database (www.oncomine.org). The level of CD133 in each grade from our cohort (B) and the public database (C). (n), the number of the sample. No value, tissues cannot be categorized to any grade.



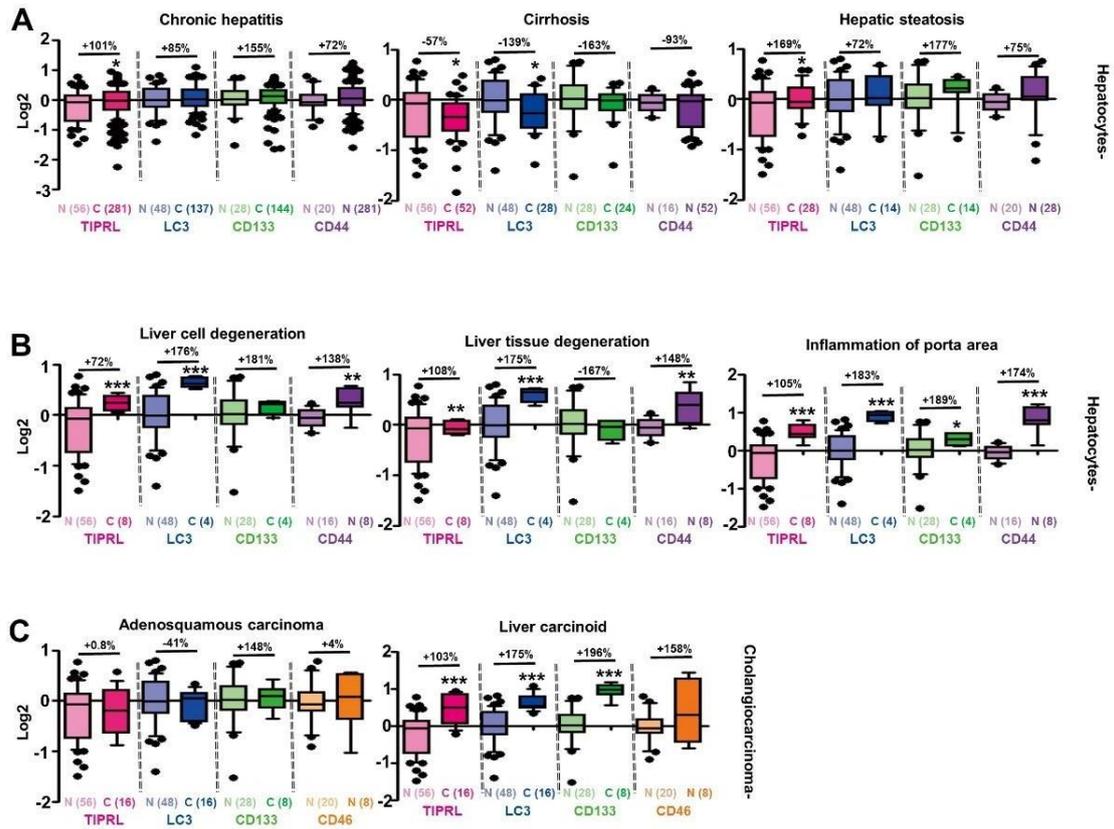
**Figure S2.** The significant association between TIPRL and LC3/CD133/CD44 in HCCs. HCCs were categorized into their cancer grades and then evaluated for the correlation with the variables according to cancer grades. x, TIPRL(Log2); y, LC3 (Log2, A), CD133 (Log2, B), CD44 (Log2, C), P-values, Spearman r, and Grade were represented. A single dot indicates one sample.



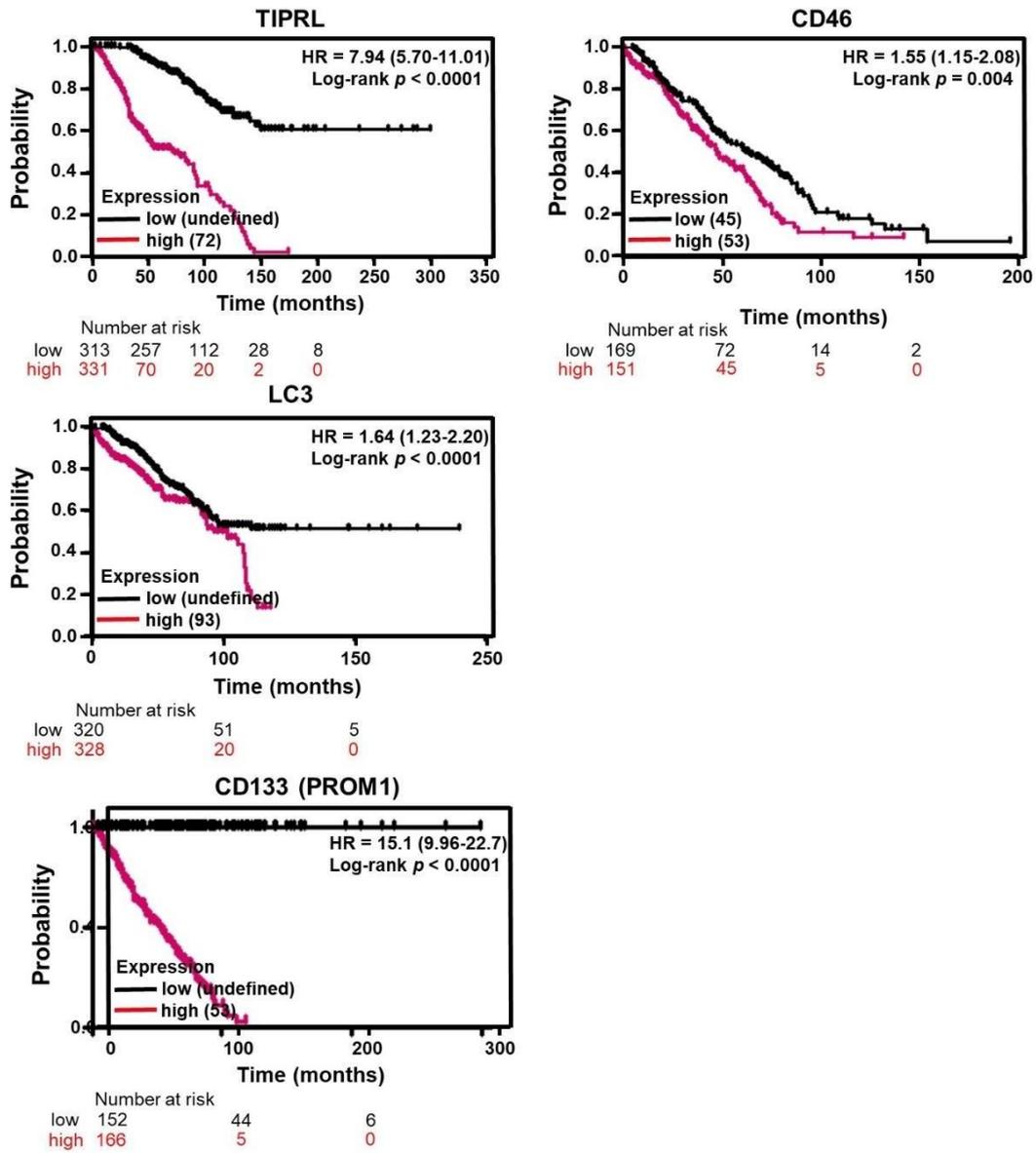
**Figure S3.** The significant grade-dependent increase in the association between TIPRL and LC3/CD133 in iCCA. iCCA were grouped into their cancer grades and then evaluated for the correlation with the variables according to cancer grades. x, TIPRL(Log2); y, LC3 (Log2, A), CD133 (Log2, B), CD46 (Log2, C),  $P$ -values, Spearman  $r$ , and Grade were represented. A single dot indicates one sample.



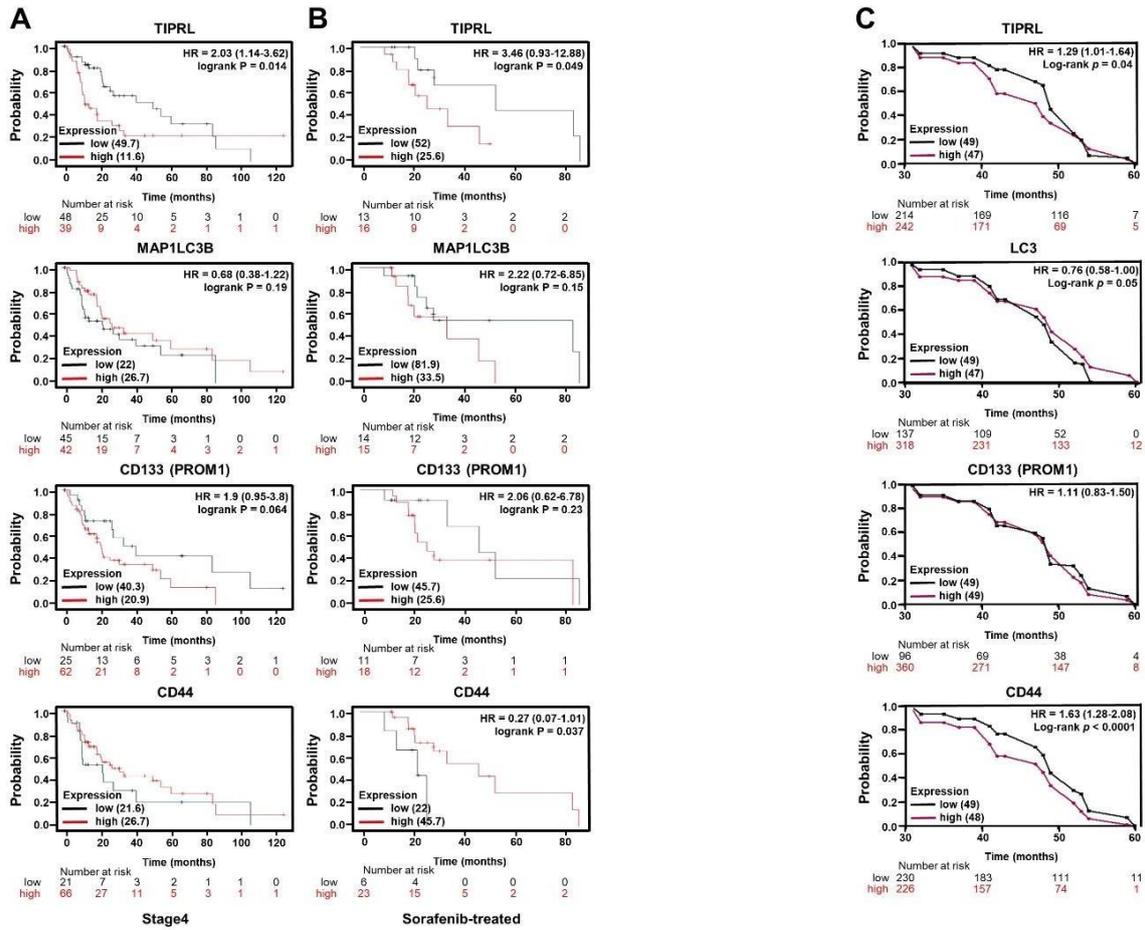
**Figure S4.** The significant relationship between TIPRL and LC3/CD133 in HCCs and iCCA. TIPRL level was subdivided into high and low expressed group, and then the level of the variables, LC3/CD133/CD44/CD46 (A), and LC3/CD133/CD46 (B) was evaluated in the TIPRL low and high expressed group. a.u. artificial unit and standard error.



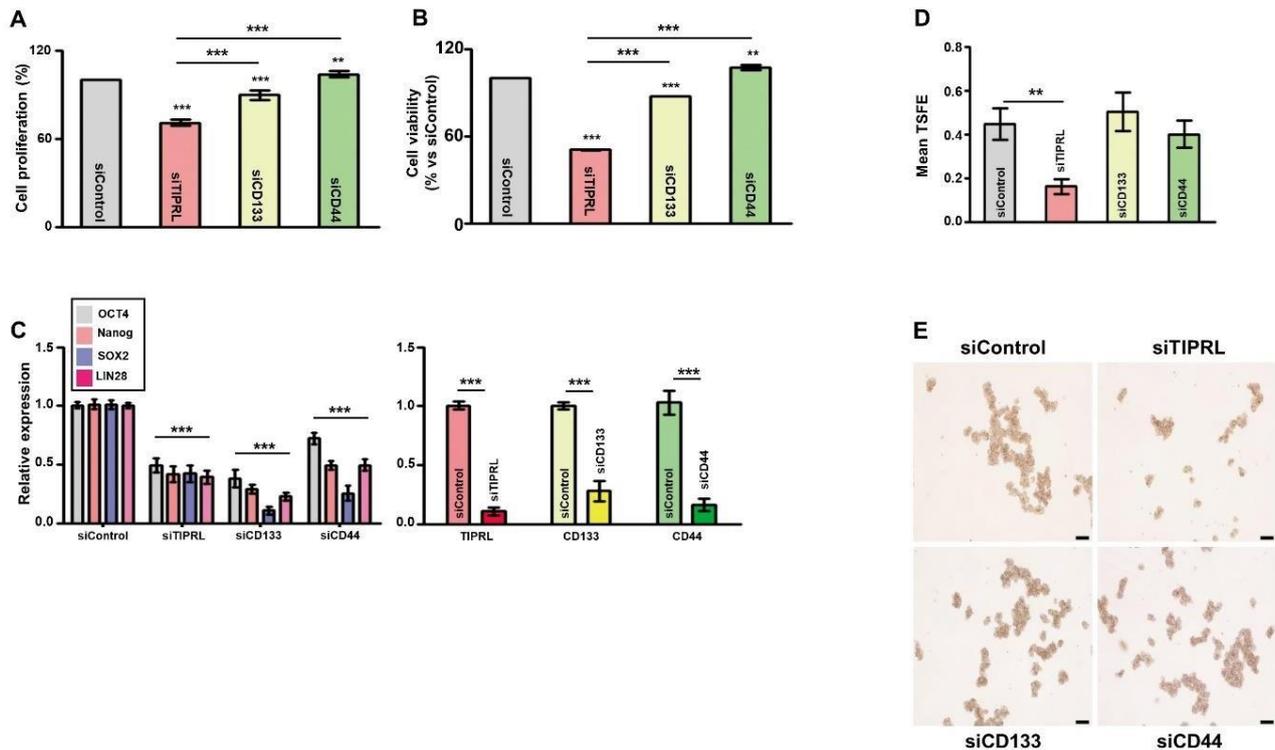
**Figure S5.** Differential expression of the variables in different liver disease tissues. The levels of the variables in different liver disease tissues (Table S2-3) were categorized into each group according to their disease name. Each set was grouped, given its origins, such as hepatocytes-originated (**A**, **B**) and cholangiocarcinoma-originated (**C**). \*  $p < 0.05$ , \*\*  $p < 0.01$ , \*\*\*  $p < 0.001$  were determined by a paired t-test, and % differences are noted. ( $n$ ), the number of the sample.



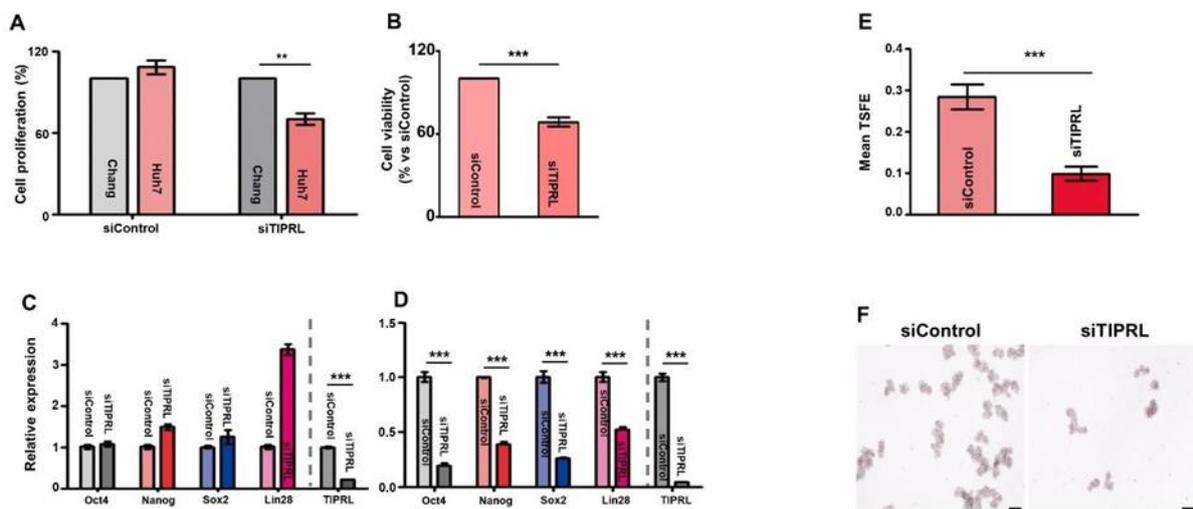
**Figure S6.** The survivability of liver disease patients in the validation set. The survival time of liver disease patients in the validation set was determined using the Kaplan Meier estimator. HR, (95%CI), Log-rank, and  $p$ -values are noted.



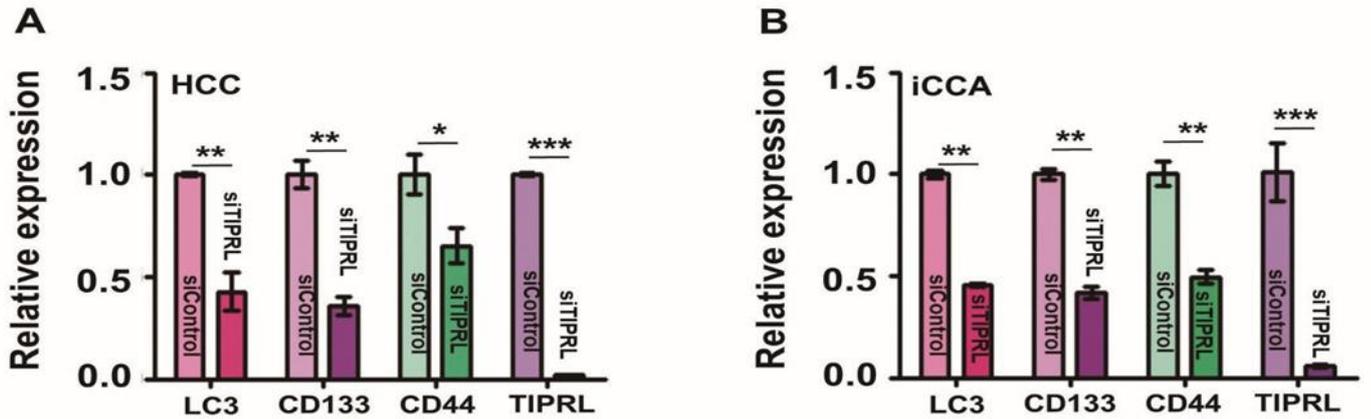
**Figure S7.** TIPRL has a prominent effect on liver cancer patients' survival. A public database ([www.kmplot.com](http://www.kmplot.com) accessed on 12–28 January 2021) was used to investigate the relationship between the level of the variables and patients with stage 4 liver cancers (A) or sorafenib-treated liver cancer patients (B). (C) The DSS of liver cancer patients in the training set was determined using the Kaplan Meier estimator. HR (95%CI), Log-rank, and *p*-values are noted.



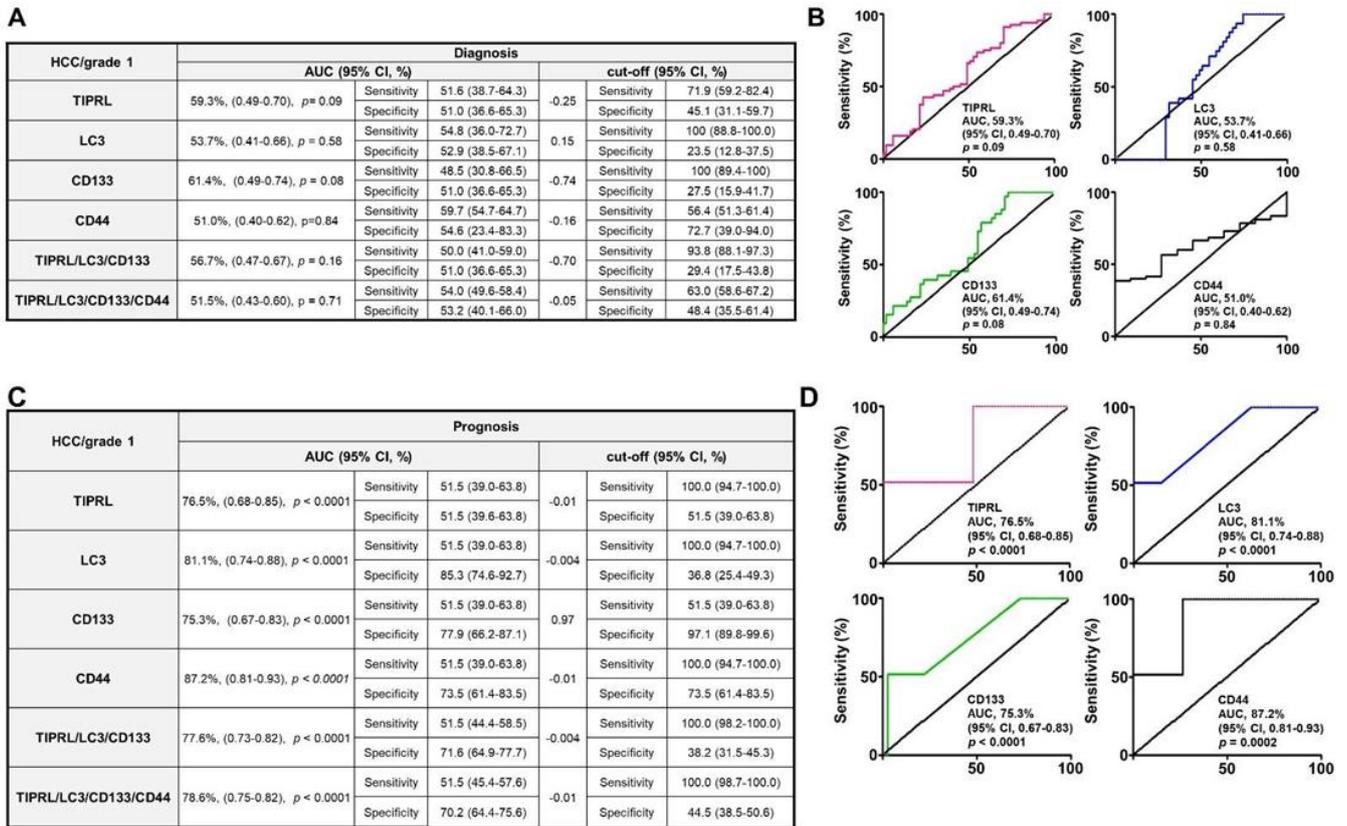
**Figure S8.** TIPRL is a crucial player in SNU1097, iCCA cell viability and stemness. SNU1097 cells were seeded in a 96-well plate (A) or anoikis plates (B–E) followed by transfected with the indicated siRNAs. For cell proliferation (A) and viability (B) assays, 48 hours after siRNA transfection, an MTT assay was performed. For quantification analysis of expression in stemness-related genes, we performed RT-qPCR using primers (C; Table S12). (D–E) We counted the numbers of spheroids after 72 hours siRNAs transfection. TSFE, tumorsphere formation efficiency: [(the number of tumorspheres formed/the initial number of cells seeded) × 100]. All experiments were independently repeated three times. \*\*  $p < 0.01$ , \*\*\*  $p < 0.001$  by unpaired *t*-test.



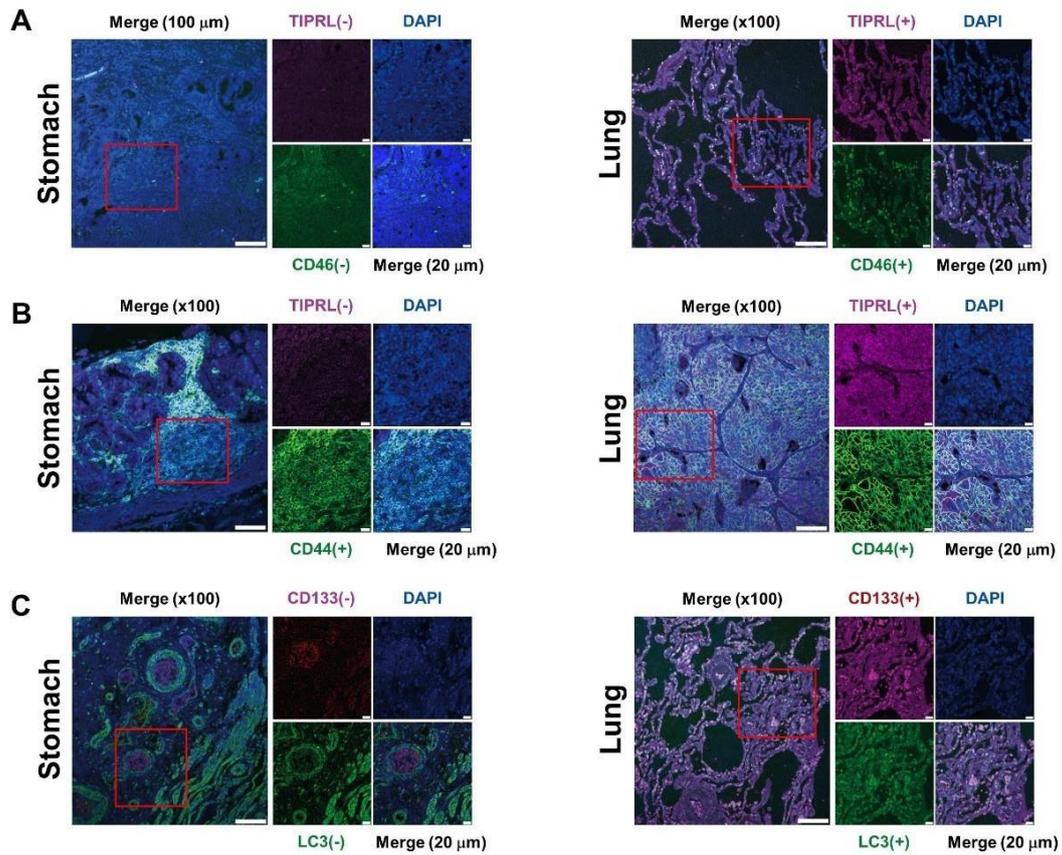
**Figure S9.** The critical role of TIPRL in huh7, HCC cell viability and stemness. Cells were transfected with second siTIPRL (Table S12) were seeded in a 96-well plate (A) or anoikis plates (B–F). For cell proliferation (A) and viability (B) assays, 48 hours after transfection, an MTT assay was performed. For quantification analysis of expression in stemness-related genes, we performed RT-qPCR using primers (C–D; Table S12). (E–F) We counted the numbers of spheroids after 72 hours siRNAs transfection. TSFE, tumorsphere formation efficiency: [(the number of tumorspheres formed/the initial number of cells seeded) × 100]. All experiments were independently repeated three times. \*\*  $p < 0.01$ , \*\*\*  $p < 0.001$  by unpaired *t*-test.



**Figure S10.** The depletion of TIPRL decreased significantly expression of LC3, CD133, and CD44. Huh7, HCC (A) and SNU-1097, iCCA (B) cells were transfected with siTIPRL (100 nM). 72 hrs after that, rt-qPCR analyses for the indicated primers (Table S12) were performed.  $n = 3$ . \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$  by two-way anova with bonferroni post hoc test.  $n = 3$ .



**Figure S11.** The prominent efficiency of TIPRL, LC3, CD133, and CD44 as the prognostic marker. ROC analysis was used to determine diagnostic (A–B) and prognostic (C–D) abilities of the variables in grade 1 HCCs. AUC, the area under the curve, (95% CI), and  $p$ -values are noted.



**Figure S12.** Demonstration of the antibody specificity using human stomach and lung tissues. Human stomach and lung tissues were stained with the indicated antibodies and then observed under confocal microscope. DAPI was used for staining nucleus, and scale bars, 20 and 100  $\mu\text{m}$ . (-) and (+) indicate negative and positive staining, and the information were obtained from "The human protein atlas (<https://v15.proteinatlas.org/cancer> accessed on 1 June 2021).