**Supplementary Materials and Methods**

***Cell lines and reagents.*** All cell lines were purchased from Shanghai Institute of Cell Biology, Chinese Academy of Sciences (Shanghai, China), and cultured in DMEM (Sigma) supplemented with 10% fetal bovine serum, 100 U/ml penicillin, and 0.1 mg/ml streptomycin at 37 °C with 5% $CO_2$. CGP53353, CGP57380 and doxorubicin hydrochloride were purchased from Sigma. Mouse anti myc monoclonal antibody was purchased from Invitrogen. Mouse anti RACK1 monoclonal antibodies were purchased from BD Bioscience and Santa Cruz. Mouse anti eIF6 monoclonal antibody was purchased from BD Bioscience. Rabbit anti eIF6, eIF2α, phospho-eIF2α (Ser51), eIF4E and phospho-eIF4E (Ser209) antibodies were purchased from Cell Signaling Technology. Mouse anti cyclin D1, c-myc, survivin and Bcl-2 monoclonal antibodies were purchased from Abcam. Mouse anti PKCβII antibodies were obtained from Santa Cruz and R&D.

***Real-time PCR analysis.*** Total RNA was prepared with Trizol reagent (Invitrogen) according to the manufacturer's protocol. Subsequent reverse transcription was performed using commercial kit (TaKaRa) according to the manufacturer's protocol. Quantitative real-time PCR was performed using the ABI Prism 7700 sequence detection system and a SYBR Green PCR core reagents kit. Primer pairs for target genes were RACK1, forward 5'-TGGGATGGAACCCTGCG-3', reverse 5'-GTATGGCCCACCAATCGCC-3'; cyclin D1, forward 5'-CCGTCCATGCGGAA GATC-3', reverse 5'-ATGGCCAGCGGGAAGAC-3'; c-myc, forward 5'-TCAAGA GGTGCCACGTCTCC-3', reverse 5'-T CTTGGCAGCAGGATAGTCCTT-3';

surviving, forward 5′-ATTTGAATCGCGGGACCC-3′, reverse 5′-GAGAAAGGGC TGCCAGGC-3′; Bcl-2, forward 5’-CTGCACCTGACGCCCTTCACC-3’, reverse 5’-CACATGACCCCACCGAACTCAAAGA-3’. β-actin, forward 5'-ACCGAGC GCGGCTACAG-3', reverse 5'-CTTAATGTCACGCACGATTTCC-3'.

***Plasmid construction, RNA interference and virus packaging.*** The human RACK1 cDNA was in frame subcloned into pcDNA3.1-Myc/His vector (Invitrogen). To support the knock-down effect of RACK1-shRNA, rescue-RACK1 expression vectors were constructed with 4 silent mismatches in the knockdown oligonucleotide sequence, 5'-CTCACGAGCGAAGAGACCA-3', corresponding to nucleotides 228–246 (mutation points are indicated by the underlines) by second step overlap extension PCR. Human eIF4E coding sequence was amplified using Hela cell cDNA as the template. The RACK1 mutants were generated using the mutanBEST kit (TaKaRa) according to the manufacturer’s protocol. To construct the shRNA vector for human RACK1, two synthetic oligonucleotides were annealed and introduced into the pSUPER.retro vector (Oligoengine) according to previous report (1). Virus packaging was carried out by transfecting pSUPER vectors into Phoenix packaging cells (Orbigen).

***Evaluation of cell apoptosis.*** HCC cells were treated with doxorubicin as indicated. In some experiments, Huh7 cells were pre-treated with CHX (50 nM), rapamycin (10 nM) or CGP53353 (1 μM) for translational suppression for 24 h. For annexin V-FITC and PI staining, cells were collected, washed with PBS and treated according to the manufacturer’s instructions (BD Biosciences). Stained cells were analyzed on a flow

cytometer, and assays were performed in triplicate.

***Co-immunoprecipitation and GST pull-down assay.*** For co-immunoprecipitation assay, Huh7 cells or purified ribosomes pretreated with RNase were solubilized with co-immunoprecipitation (Co-IP) buffer (50 mM Tris-HCl pH7.5, 150 mM NaCl, 0.1% NP-40, 15 mM $MgCl_2$, 1mM DTT), and Co-IP was performed as before (2). For GST-tagged recombinant proteins, the plasmids were constructed based on pcDNA3.0-GST vector and recombinant proteins were purified from HEK 293T cells using glutathione agarose beads (Amersham Bioscience). For His6-tagged recombinant proteins, corresponding coding sequences were constructed into pcDNA3.1-Myc/His (Invitrogen) and His6-tagged recombinant proteins were also purified from HEK 293T cells using Ni Sepharose (GE Healthcare Life Sciences). GST pull-down assay was performed according to our previous report (2).

***$M^7$GDP affinity chromatography.*** The protein extracts were prepared from growing Huh7 cells and incubated with $m^7$GDP sepharose 4B. The beads were pelleted and washed extensively with extraction buffer. The proteins retained were eluted with extraction buffer containing $m^7$GDP and applied to analysis.

***[$^{35}$S]-methionine incorporation assay.*** Huh7 cells were incubated with DMEM depleted of methionine for 30 min and then labeled with [$^{35}$S]-methionine (Perkin Elmer). For overall translation rate measurement, samples were subjected to TCA precipitation. The amount of radioactivity was determined by scintillation counting, and the counts were normalized to protein concentration. The assays were performed in quadruplicate (n=4). For rate of synthesis of individual proteins, cell extracts with

equal CPM were subjected to immunoprecipitation with relevant antibodies. The immunoprecipitates were eluted and applied to SDS-PAGE, and the gel were dried and visualized with phosphoimaging (Fujifilm). The data shown are representative of three independent experiments.

***In vitro* phosphorylation assay.** *In vitro* phosphorylation assay was performed according to previous report (3). Briefly, reactions were carried out in kinase buffer (50mM Tris-HCl pH 7.5, 15 mM $MgCl_2$, 1mM DTT, 50 mM ATP) containing recombinant eIF4E and active PKCβII kinase, with or without different doses of recombinant RACK1. After incubation for 30 min, samples were boiled and applied to analysis.

***Luciferase activity assay*.** The SV40-based monocistronic constructs was generated according to a previous report (4). For Luciferase activity assay, cell extracts were prepared and luciferase activities were detected according to the manufacture's intructions (Promega) using a Lumat LB 9507 luminometer. The relative luciferase activities of empty vector were normalized to 1. All assays were performed in triplicate.

***Preparation of polyclonal antibody against phosphorylated eIF6 on serine 235*.** Briefly, two 11 amino acid–long peptides corresponding to amino acids 230–239 of eIF6 with a cysteine at the C terminal were synthesized, with or without the phosphorylation modification on serine 235. The amino acid sequence of the fragment were STIAT(pS)MRDS-C and STIATSMRDS-C. After four times immunization, the rabbits were bled and sera obtained, and antibodies were purified by affinity

chromatography using protein A and the phosphorylated peptide. Antibodies recognizing unphosphorylated form of eIF6 were further removed using the unphosphorylated peptide. The resultant antibody specificity was tested in an ELISA.

***Tumor xenograft experiments.*** 5-week old male BALB/C nude mice were obtained from Shanghai Laboratory Animal Center of Chinese Academy Sciences and housed in a specific pathogen-free room. $1\times10^7$ HCC stable cells (Huh7, BEL-7402 or PLC/PRF/5) in 100 μl of PBS were injected subcutaneously into the flank of mice (n=10 in each group). Vehicle control, doxorubicin (2 mg/kg) and retrovirus ($4\times10^7$ viral particles in 50 μl Optimum DMEM) were given by intratumoral injection. Tumor diameter in two dimensions was measured using calipers, and volume was estimated by the formula [length (mm) × width $(mm)^2$]/2.

***Immunohistochemical staining and scoring.*** Briefly, the slides were deparaffinized using a graded ethanol series, and endogenous peroxidase activity was blocked by soaking in 0.3 % hydrogen peroxide. Antigen retrieval was performed with 10 mM citrate buffer (121 °C, 20 minutes). Then slides were incubated overnight at 4 °C with diluted primary antibody, with anti-RACK1 mouse monoclonal antibody (diluted 1:200), anti-cyclin D1 mouse monoclonal antibody (diluted 1:100), anti-c-myc mouse monoclonal antibody (diluted 1:100), anti-survivin mouse monoclonal antibody (diluted 1:100) and anti-Bcl-2 mouse monoclonal antibody (diluted 1:100). Negative control slides were also processed in parallel using a nonspecific immunoglobulin IgG (Sigma Chemical Co., St. Louis, MO) at the same concentration as the primary antibody. All slides were processed using the peroxidase-antiperoxidase method

(Dako, Hamburg, Germany). Staining results were interpreted under microscopic fields of 200 or 400-fold magnification by two independent specialists. Immunohistochemical scoring was based on a semi-quantitative method according to the intensity and percentage of staining. The intensity of staining was scored on a scale of 0 to 3, in which 0 = negative staining, 1 = weakly positive staining, 2 = moderately positive staining, and 3 = strongly positive. The percentage of staining was estimated on a scale of 0 to 4, in which 0 = none, 1 = positive staining in 1–25% of cancer cells, 2 = positive staining in 26 –50%; 3 = positive staining in 51–75%; and 4 = positive staining in 76 –100%. The immunohistochemical score (IS) was calculated through multiplying the intensity score by the percentage score. Samples with IS between 0 and 1 were classified as Score 0, samples with IS between 2 and 4 were Score 1, samples with IS between 5 and 8 were Score 2, and samples with IS between 9 and 12 were Score 3. No sample from the 162 HCC cases was grouped as Score 0. All of the immunostained sections were evaluated in a blinded manner without knowledge of the clinical and pathological parameters of the patients.

***TUNEL assay.*** Terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) staining was performed by using in situ cell death detection kit (Roche Applied Science) according to manufacturer's instructions. Briefly, cryopreserved xenograft tumor tissue sections were fixed with 4% PFA/PBS for 1 h at room temperature and permeabilized with 0.1% Triton X-100 /PBS for 2 min on ice, then incubated with TUNEL reaction mixture that consisting of terminal deoxy- nucleotide at 37 °C for 60min followed by incubated with DAPI for 5 min. Coverslips were

mounted in Fluoromount (Sigma-Aldrich). Cells were visualized by fluorescence microscopy, and TUNEL positive cells were counted. Three areas per section were analyzed

**Supplementary Table 1. Summary of Clinicopathologic Variables of HCC patients**

| Characteristic | No. | Characteristic | No. |
|---|---|---|---|
| Sex | | Tumor size (cm) | |
| male | 142 | ≤5 | 53 |
| Female | 20 | >5 | 109 |
| Age(years) | | TNM stage | |
| ≤51 | 84 | I/II | 94 |
| >51 | 78 | III/IV | 68 |
| Hepatitics status | | Tumor number | |
| Yes | 131 | Single | 124 |
| No | 31 | Multiple | 38 |
| Liver cihhrosis | | Tumor capsulation | |
| Yes | 117 | Complete | 35 |
| No | 45 | Incomplete | 127 |
| p53 | | Vascular invasion | |
| + | 64 | Yes | 119 |
| - | 98 | No | 43 |
| AFP (ng/mL) | | Lymph node metastasis | |
| ≤100 | 58 | Yes | 5 |
| >100 | 104 | No | 157 |

TNM, tumor–node–metastasis.

**Supplementary Table 2. Relative RACK1 expression with TNM stage in clinical cases**

| Score (RACK1) | TNM stage | | |
|---|---|---|---|
| | I | II | III/IV |
| 1 | 22 | 15 | 4 |
| 2 | 15 | 34 | 16 |
| 3 | 1 | 7 | 48 |
| Total | 38 | 56 | 68 |

Note: One-way ANOVA analysis, $F=56.685$, $p<0.001$

**Supplementary Table 3. The correlation of RACK1 with Ki67 in clinical cases**

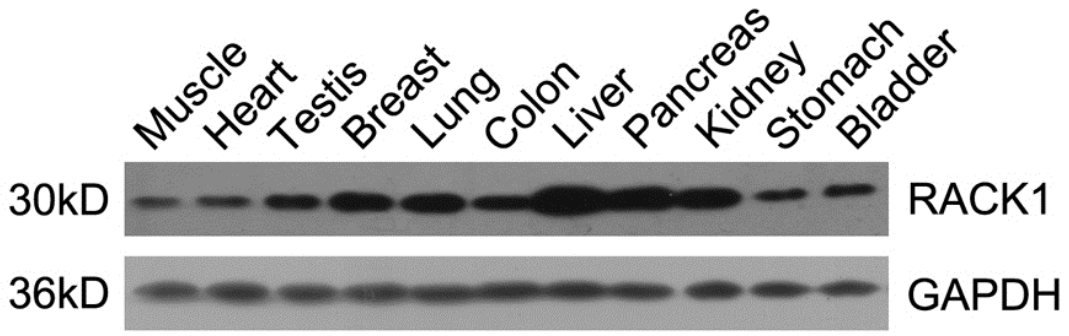
| Score | Ki67 | | |
|---|---|---|---|
| (RACK1) | + | ++ | +++ |
| 1 | 22 | 14 | 5 |
| 2 | 17 | 31 | 17 |
| 3 | 5 | 27 | 24 |
| total | 44 | 72 | 46 |

Note: One-way ANOVA analysis, $F=13.998$, $p<0.001$

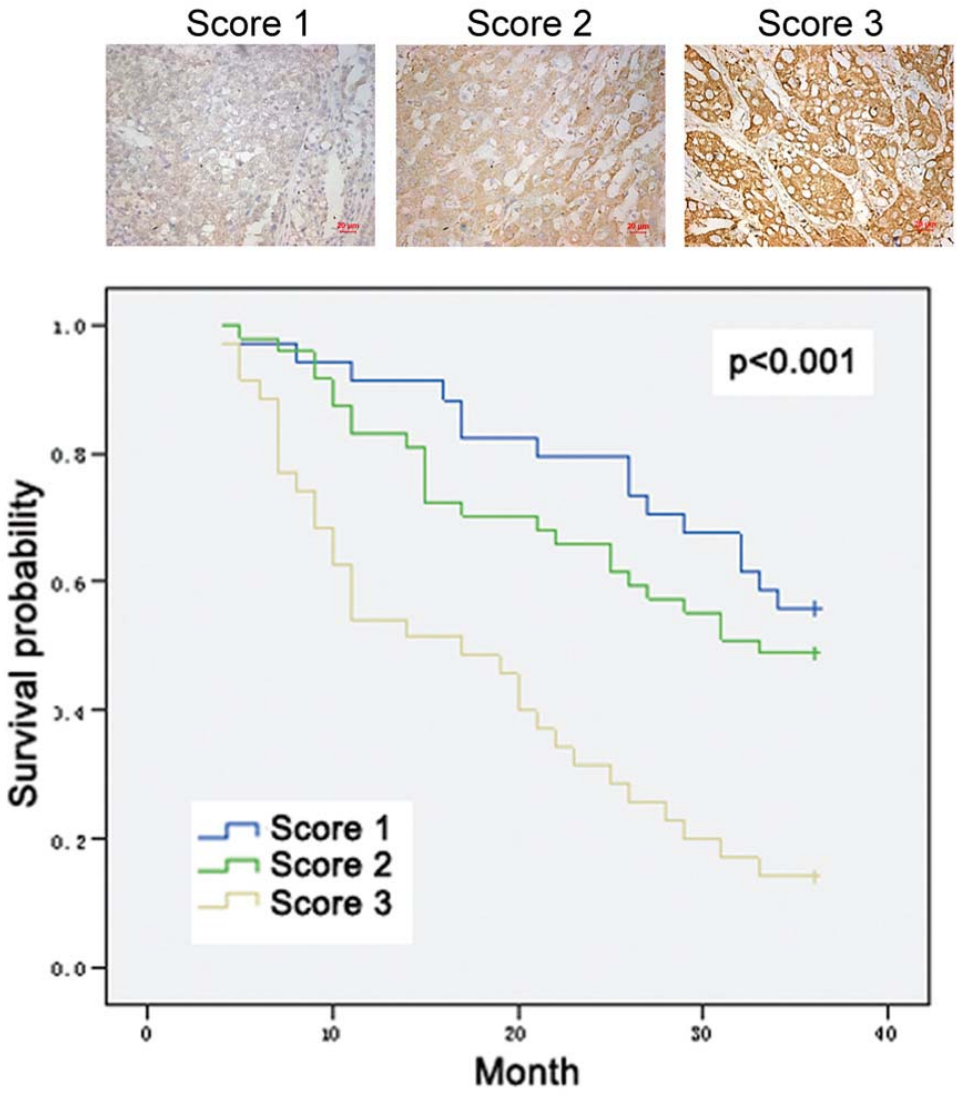
**Supplementary Table 4. The correlation of RACK1 with AFP in clinical cases**

| Score | AFP(ng/ml） | | |
|---|---|---|---|
| (RACK1) | － | 0～100 | ＞100 |
| 1 | 10 | 12 | 19 |
| 2 | 9 | 19 | 37 |
| 3 | 6 | 2 | 48 |
| total | 25 | 33 | 104 |

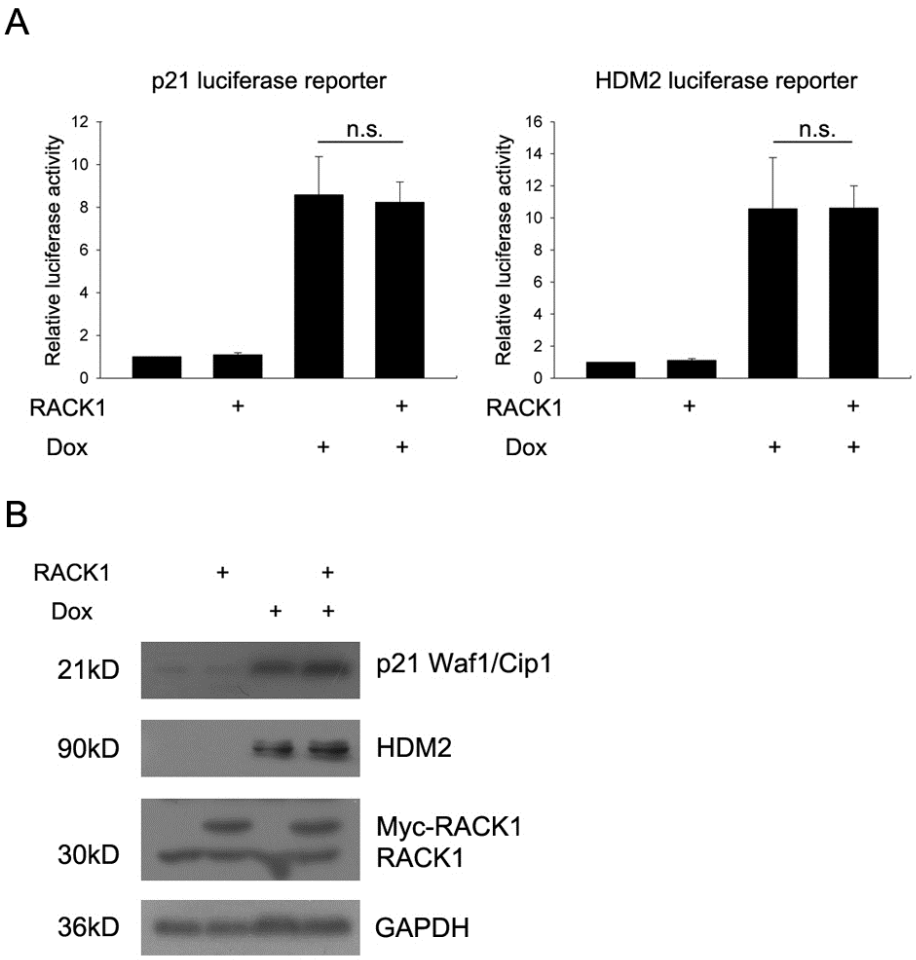
Note: One-way ANOVA analysis, F=6.680, p=0.002



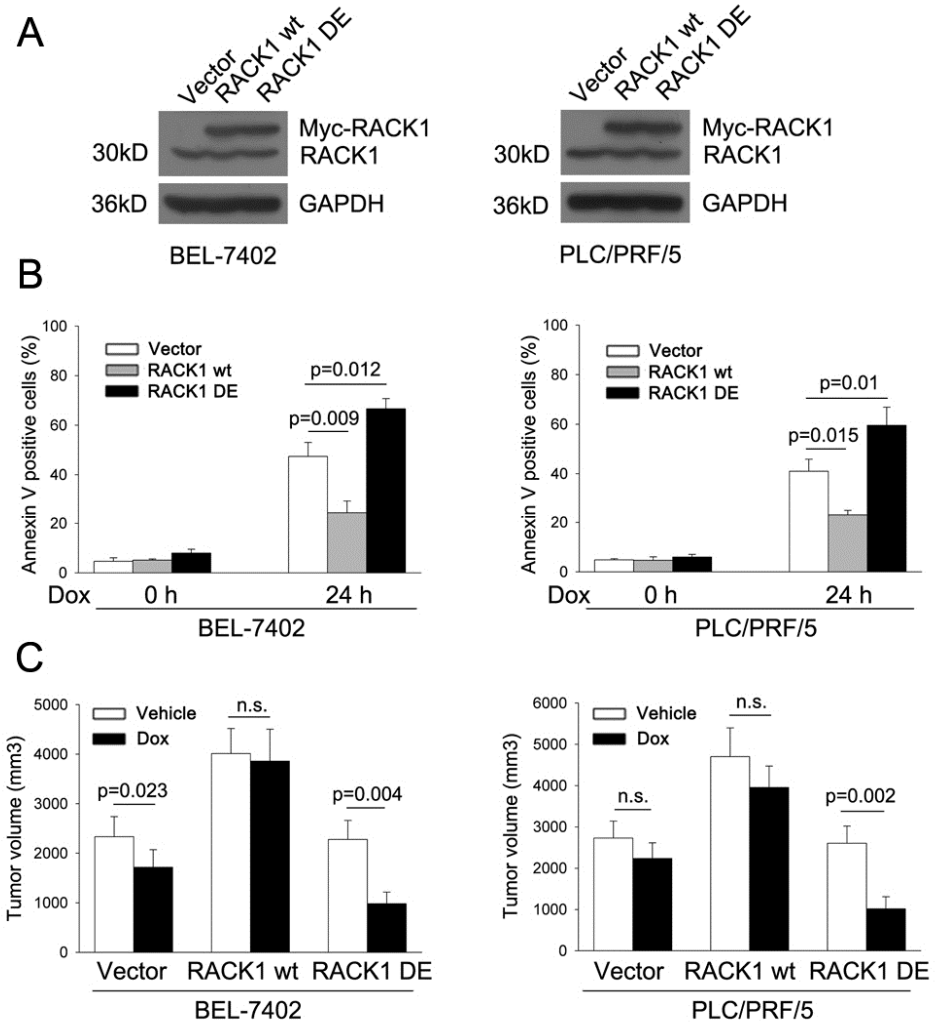

**Supplementary Figure 1** RACK1 is highly expressed in normal liver. Total protein was extracted from fresh tissues of adult BALB/C mouse. Western blot was performed to assess RACK1 expression with GAPDH as the internal control.



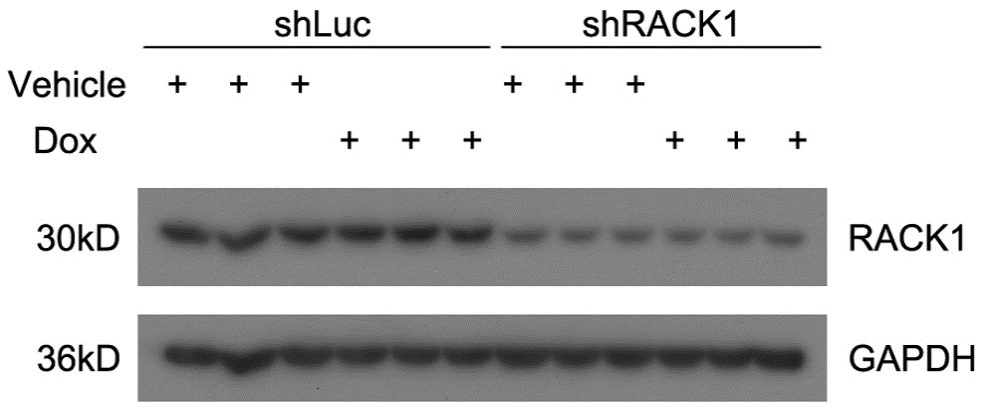

**Supplementary Figure 2** RACK1 expression in HCC is associated with poor prognosis. 116 out of 162 primary HCC patients, who were diagnosed and received treatment before the year of 2007, were selected. Kaplan–Meier analysis of survival was applied in the 116 HCC cases stratified by RACK1 expression level ($p<0.001$; log-rank test for significance).



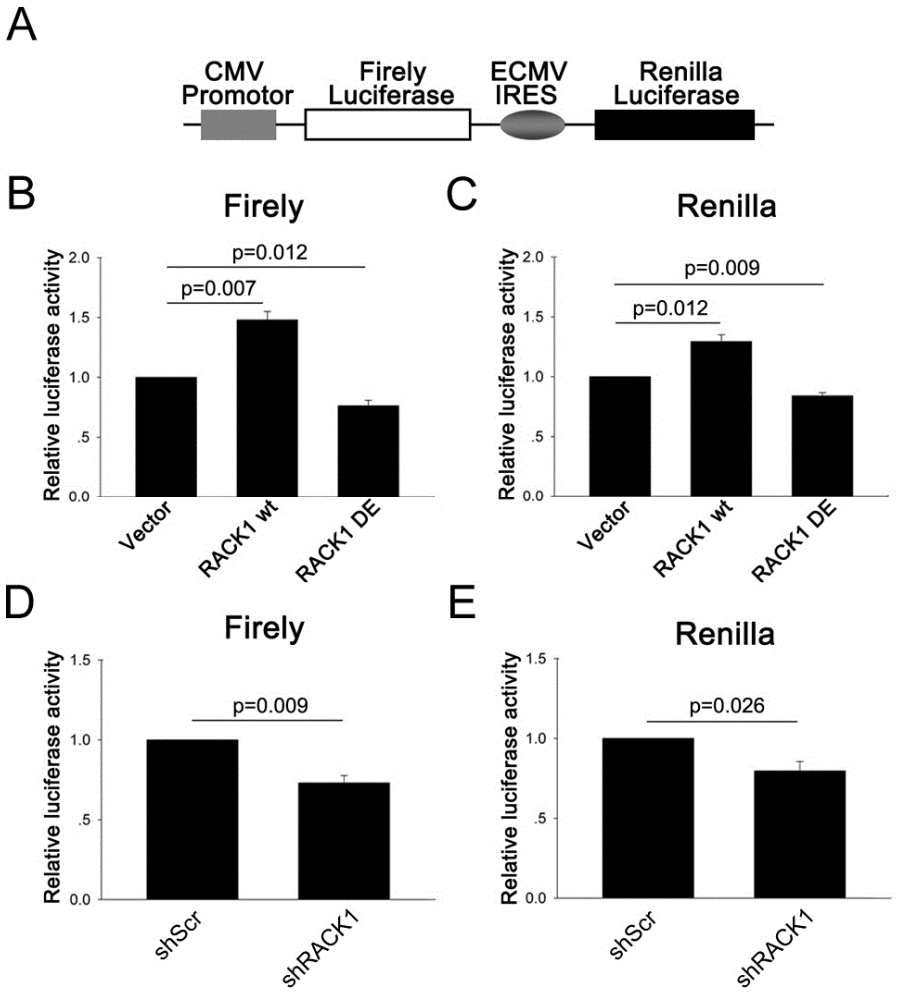

**Supplementary Figure 3** Effect of RACK1 on p53 activity in HepG2 cell line. (A) Effect of RACK1 on luciferase activity driven by the p53-responsive promoter of p21 or HDM2. The p21 or HDM2 luciferase reporter construct was transfected into HepG2 cells along with empty vector or wild-type RACK1. 48 h after transfection, cells were treated with or without doxorubicin for 12 h, washed, lysed and applied to the detection of luciferase activities. (B) Effect of RACK1 on p21 or HDM2 expression. HepG2 cells were transfected with empty vector or wild-type RACK1. 48 h later, cells were treated with or without doxorubicin for 12 h, washed, lysed and applied to the detection of p21 or HDM2 expression. n.s., no statistical significance.



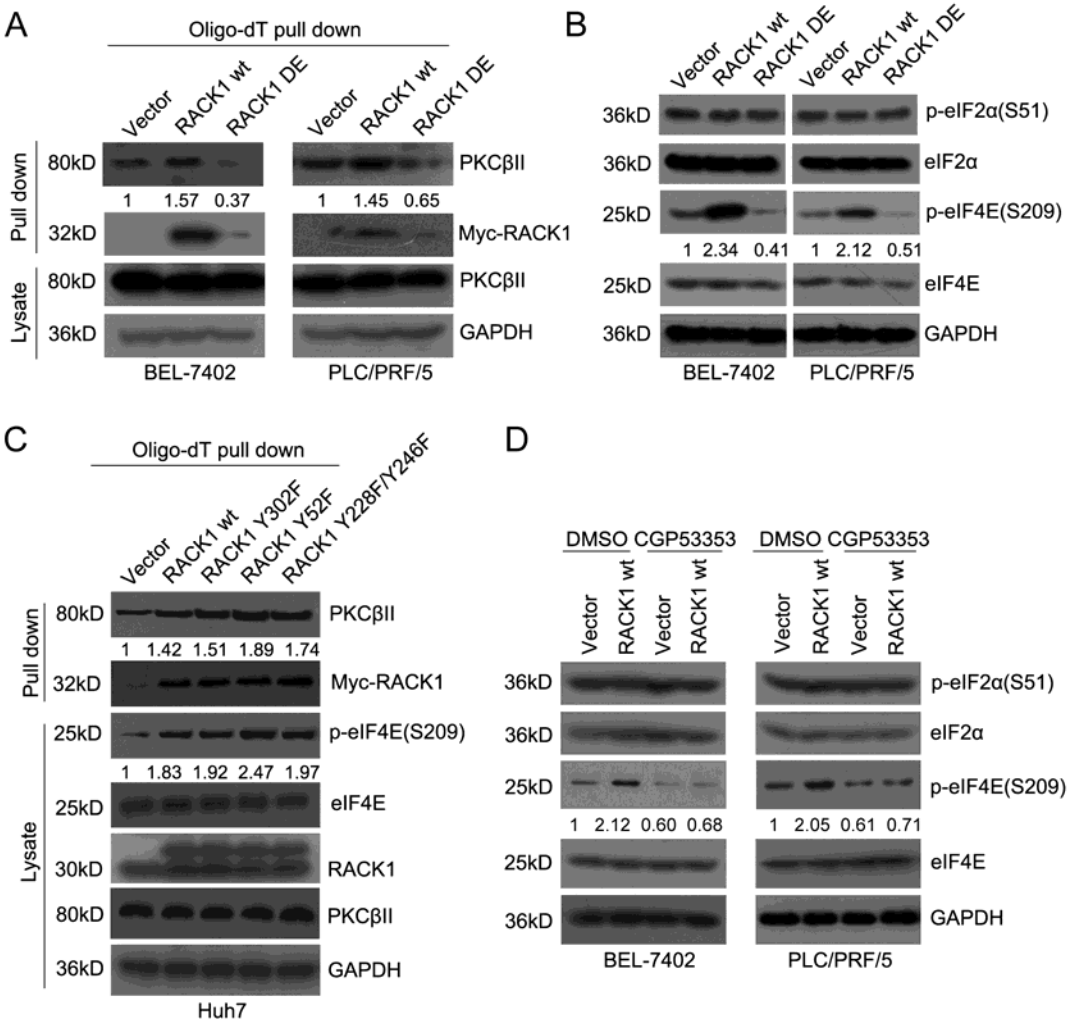

**Supplementary Figure 4** Role of ribosomal RACK1 in *in vitro* chemo-sensitivity and *in vivo* tumor behavior of HCC. (A) Identification of BEL-7402 and PLC/PRF/5 stable cell lines. (B) Role of ribosomal RACK1 on chemo-sensitivity of BEL-7402 and PLC/PRF/5 cells *in vitro*. Cells were treated with or without doxorubicin for 24 h, and apoptosis was determined by annexin V staining. (C) Role of ribosomal RACK1 on tumor behavior *in vivo*. Xenografts were generated using stable BEL-7402 or PLC/PRF/5 cells. After 2 weeks, doxorubicin was given by intratumoral injection, and tumor volume was assessed 3 weeks later. n.s., no statistical significance.



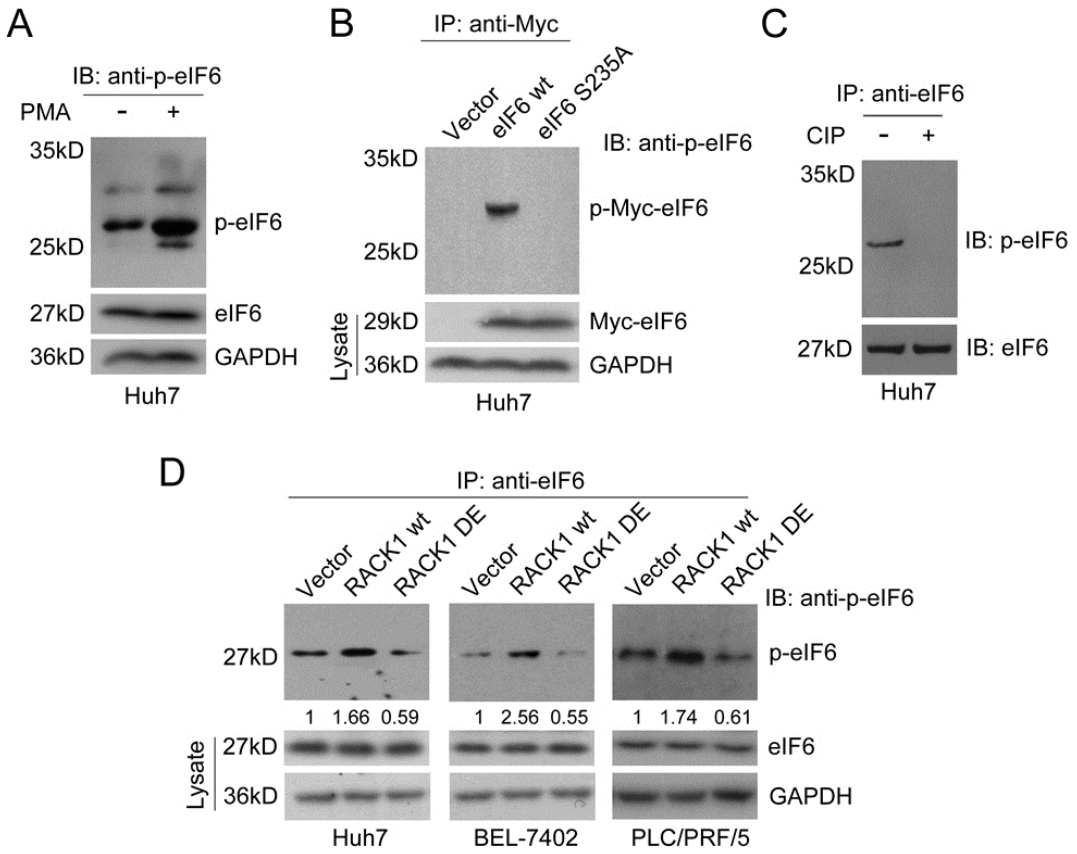

**Supplementary Figure 5** Expression pattern of RACK1 in tumor xenografts. On the day of harvest, tumor xenografts were collected, lysed and applied to western-blot analysis to examine the expression of RACK1.



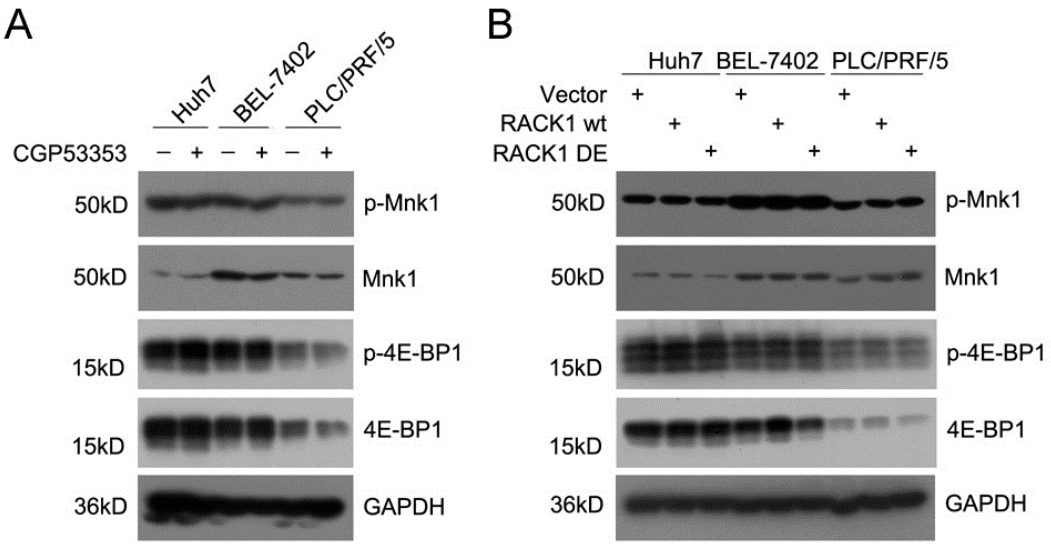

**Supplementary Figure 6** Effect of RACK1 on the cap- and IRES-mediated translation. (A) Schematic diagram of the pcDNA/Fluc/IRES/Rluc construct. (B, C) The bicistronic reporter pcDNA/Fluc/IRES/Rluc construct was transfected into Huh7 cells along with empty vector, wild-type RACK1 or the DE mutant. 48 h after transfection, cells were collected, lysed and applied to the detection of luciferase activities. (D, E) The bicistronic reporter pcDNA/Fluc/IRES/Rluc construct was transfected into Huh7 cells along with shLuc or shRACK1 construct. 72 h later, cells were collected, lysed and applied to the detection of luciferase activities.



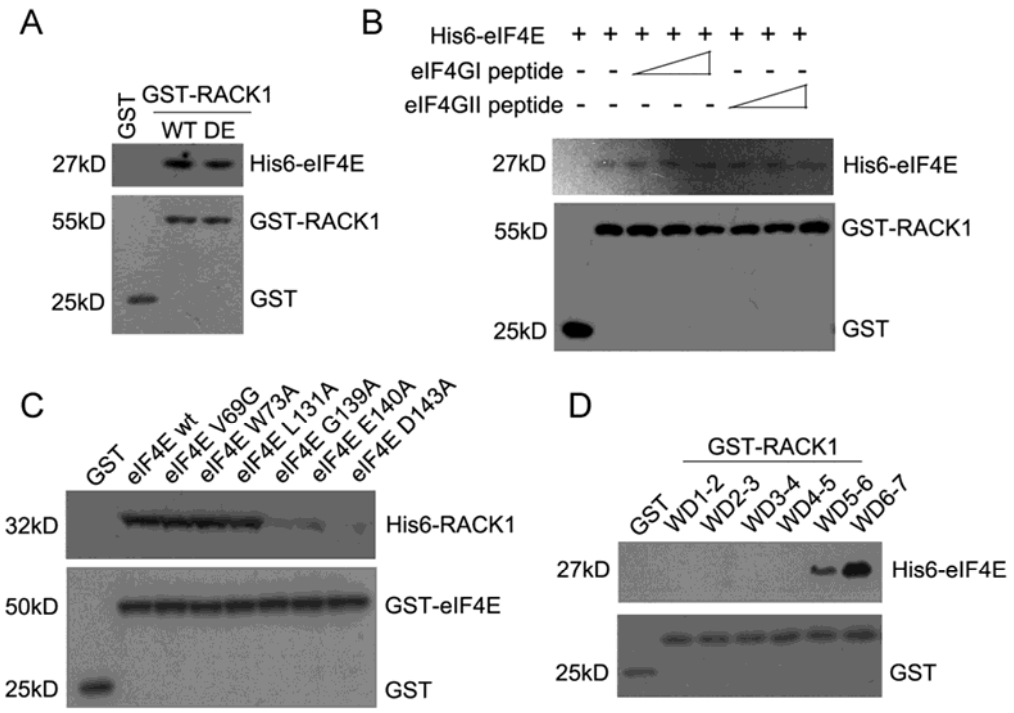

**Supplementary Figure 7** Ribosomal RACK1 contributes to PKCβII recruitment and eIF4E phosphorylation. (A) Ribosomal RACK1 enriches PKCβII with poly(A) mRNA. Stable BEL-7402 or PLC/PRF/5 cells were applied to oligo-dT pull-down assay. (B) The effect of ribosomal RACK1 on the phosphorylation of eIF2α and eIF4E. Stable BEL-7402 or PLC/PRF/5 cells were harvested, and lysates were applied to western blot analysis. (C) Effect of RACK1 mutants on PKCβII recruitment and eIF4E phosphorylation. Huh7 were transiently transfected as indicated. 72 h later, cells were collected, lysed, and applied to oligo-dT pull-down assay and western blot analysis. (D) PKCβII activity is required for RACK1-mediated phosphorylation of eIF4E. Stable BEL-7402 or PLC/PRF/5 cells were incubated with or without CGP53353 (5 μM) for 1 h, and cell lysates were applied to western blot analysis.



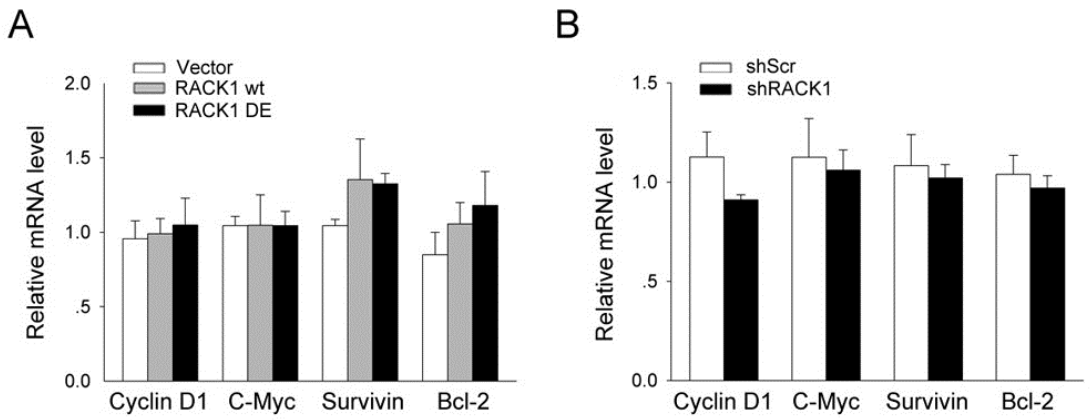

**Supplementary Figure 8** Ribosomal RACK1 contributes to eIF6 phosphorylation on Serine 235. (A) Characterization of the antibody in western-blot analysis. Huh7 cells were treated with PMA (100 nM) for 1 h, and cell lysates were applied to western-blot using the eIF6 phospho-S235 pAb as primary antibody. A major band migrating at about 27kD and responsive to PMA treatment was detected. (B) Characterization of the antibody specificity on S235 phosphorylation of eIF6. Huh7 cells were transfected with empty vector, Myc-tagged wild-type eIF6 or Myc-tagged eIF6 S235A mutant. 48 h later, cell lysates were harvested and subjected to immunoprecipitation using the anti-Myc antibody. Immunoprecipitates were analyzed by western-blot using the eIF6 phospho-S235 pAb. (C) Characterization of the antibody specificity on phosphorylation of eIF6. Lysates of Huh7 cells were subjected to immunoprecipitation using the anti-eIF6 antibody, and immunoprecipitates were treated with or without CIP, followed by western-blot using the eIF6 phospho-S235 pAb. (D) Effect of ribosomal RACK1 on eIF6 phosphorylation. Huh7, BEL-7402 or PLC/PRF/5 cells transfected with empty vector, wild-type RACK1 or the DE mutant were harvested, and cell lysates were applied to immunoprecipitation for eIF6, followed by western-blot analysis using the eIF6 phospho-S235 pAb.



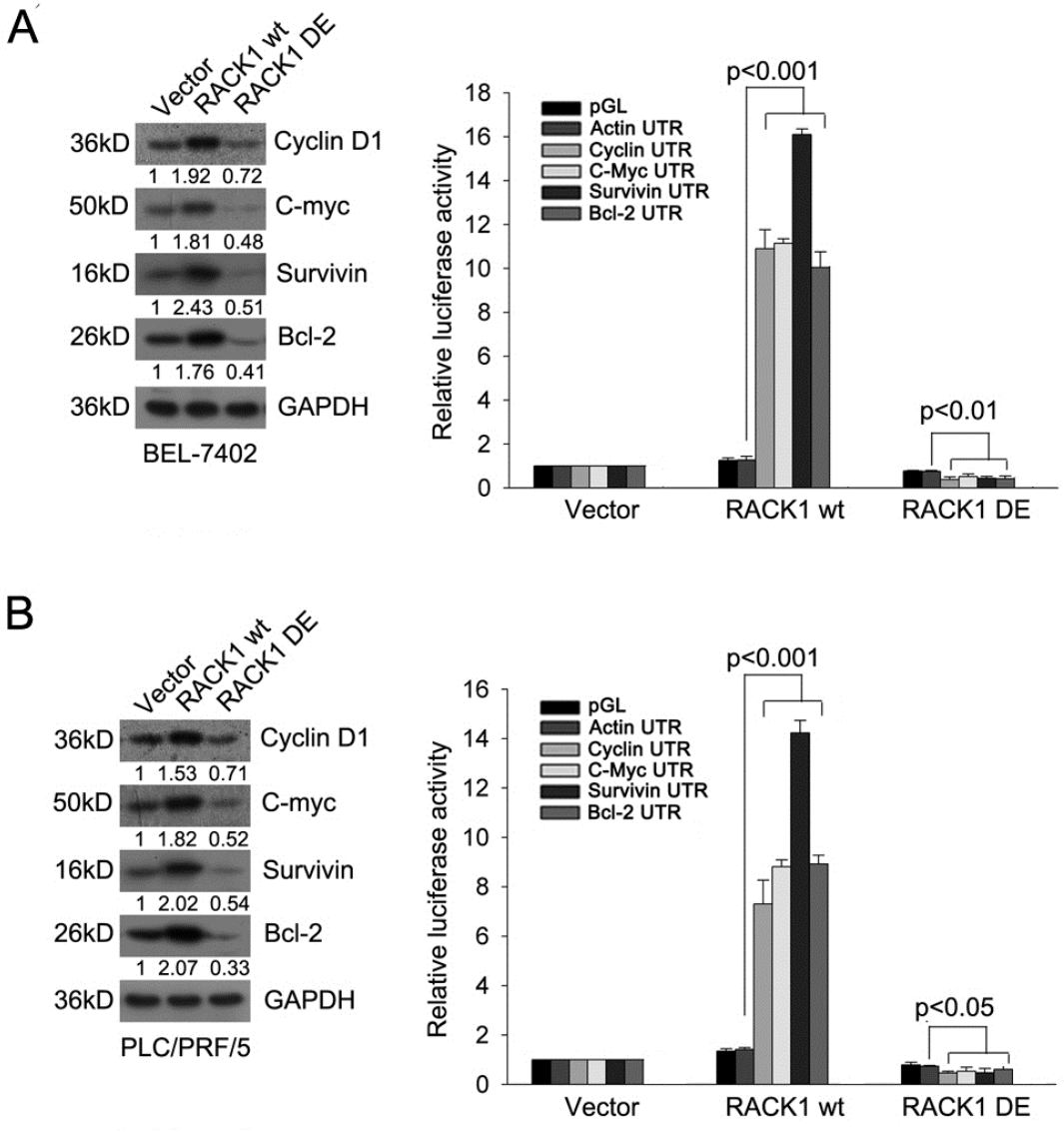

**Supplementary Figure 9** Effect of PKCβII and ribosomal RACK1 on the phosphorylation of Mnk1 and 4E-BP1 in HCC. Cells treated with CGP53353 (A) or transfected with indicated plasmids (B) were collected, lysed and applied to western-blot analysis to detect Mnk1 and 4E-BP1 phosphorylation.




**Supplementary Figure 10** Characterization of RACK1 and eIF4E association *in vitro*. (A) Wild-type RACK1 or the DE mutant associates with eIF4E *in vitro*. (B) RACK1 and eIF4G do not compete for binding to eIF4E. Different doses of eIF4GI peptide (LEEKKR**Y**DREF**LL**GFQFIF) or eIF4GII peptide (KKQ**Y**DREF**LL**DFQFMPA) that mimics the binding site for eIF4E was added to the mixture for competition binding assay. (C) Mutation analysis of eIF4E dorsal surface for RACK1 binding. Accessible residues that are conserved on the eIF4E dorsal surface were mutated for analysis. (D) Characterization of the eIF4E binding site of RACK1. GST pull-down assay was carried out by using the truncated form of RACK1.




**Supplementary Figure 11** Effect of RACK1 on the mRNA levels of cyclin D1, c-myc, survivin and Bcl-2. (A) Huh7 cells were transiently transfected with empty vector, wild-type RACK1 or the DE mutant. Total RNA was prepared and the mRNA levels of cyclin D1, c-myc, survivin and Bcl-2 were examined as described in the "Supplemental Methods". (B) Huh7 cells were transiently transfected with shScr or shRACK1, and total RNA was prepared and examined as in (A).




**Supplementary Figure 12** Ribosomal RACK1 preferentially promotes the translation of potent growth and survival factors. The effect of ribosomal RACK1 on the expression and translational activity of cyclin D1, c-myc, survivin and Bcl-2 in BEL-7402 (A) and PLC/PRF/5 (B) cells was checked. 24 h after transient transfection with indicated monocistronic constructs, cell extracts were prepared and luciferase activities were detected. The relative luciferase activities of empty vector were normalized to 1. Western-blot analysis was carried out in the lysates of stable cells.

## Supplementary References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