Supplementary Materials and Methods

Plasmids. pSIREN-RetroQ-CDK5RAP3 expressing CDK5RAP3 shRNA was kindly provided by Dr. Honglin Li (3). Human cDNA encoded the full length form of CDK5RAP3 (clone IMAGE: 5760597) was cloned into pCMV-Tag 3B (Stratagene, La Jolla, CA) and pGEX4T2 (Amersham Pharmacia, Piscataway, NJ). Human cDNA encoded the full length form of PAK4 (clone IMAGE: 3867403) was cloned into pEGFPC1 (Clontech, Palo Alto, CA) and pET28c (EMD Biosciences, San Diego, CA).

Antibodies. Mouse anti-c-Myc (9E10), Rabbit anti-c-Myc (A-14), rabbit anti-GFP and mouse anti-His were purchased from Santa Cruz Biotechnology. Goat anti-GST was obtained from Pharmacia Biotech. Rabbit anti-PAK4 and anti-phospho-PAK4/5/6 (S474) were obtained from Cell Signaling Technology. Mouse anti-β-actin was purchased from Sigma-Aldrich.

Clinical HCC Samples. Human primary HCCs and their corresponding nontumorous liver tissues from Chinese patients were collected at the time of surgical resection at Queen Mary Hospital, the University of Hong Kong (Pokfulam, Hong Kong), from 1991 to 2000. The patient tissue samples

were collected according to approved research guidelines. All samples, after collection from surgical resection, were snap-frozen in liquid nitrogen before storage at -80^{o}C.

Clinicopathological Correlation. The clinicopathologic features of HCC patients included sex, age, tumor size, cellular differentiation according to the Edmondson grading, venous invasion, direct liver invasion, tumor microsatellite formation, liver cirrhosis, resection margin, tumor encapsulation, tumor stage, number of tumor nodules, serum hepatitis B surface antigen and hepatitis B core antigen status were analyzed by SPSS for Windows 17.0 (SPSS Inc., Chicago, IL). Categorical data were analyzed by Chi square test or Fisher's exact test, whereas independent *t* or Mann-Whitney test was used for continuous data wherever appropriate. After resection, all patients were followed up monthly in the first year and quarterly thereafter. Disease-free survival was measured from the date of hepatic resection to the date when recurrent disease was diagnosed or, in the absence of detectable tumor, to the date of death or the last follow up. Actuarial survival was measured from the date of hepatic resection to the date of death or the last follow up. The survival curves were assessed by Kaplan-Meier method and the statistical difference between two groups was evaluated by log-rank test. Tests were considered significant when the *P* value was less than 0.05.

SiRNA Oligonucleotide Transfection. Small interfering RNA (siRNA) was transfected into cells using 200 pmole per well of 6-well plate by LipofectAMINE 2000. CDK5RAP3-1 siRNA duplex 5'-AGUCUAUCCCAUCACCUCCAGGAUC-3' (sense strand) and the CDK5RAP3-3 siRNA duplex 5'-AACAGGUGUUGCAGCUGAAGACUGG-3' (sense strand) and siRNA negative control, Hi GC duplex, were purchased from Invitrogen. CDK5RAP3-1 siRNA and CDK5RAP3-3 siRNA were transfected together as a mixture for each experiment. SiRNA targeting *PAK4* 5'-TTCTGCTCGTGCTGGTCGAAG-3' (sense strand) and siRNA negative control 5'-UAAGGCUAUGAAGAGAUAC-3' (sense strand) were purchased from Qiagen.

Supplementary Data Figure Legend

Supplementary Fig. S1. Characterization of CDK5RAP3 antibody. (*A*) The pre-immune rabbit serum, anti-CDK5RAP3 rabbit serum (1:2000), and rabbit anti-myc antibody were used for Western blotting. No CDK5RAP3 specific bands were detected with the use of pre-immune serum. By blocking the anti-CDK5RAP3 antibody in serum with 100 μg GST-CDK5RAP3, no CDK5RAP3 bands were detected, indicating the specificity of CDK5RAP3 antibody. Lane (1) 20 μg protein lysate from SMMC-7721 cells and (2) 20 μg protein lysate from SMMC-7721 cells transfected with Myc-CDK5RAP3. (*B*) Endogenous CDK5RAP3 protein level in HCC cell lines was examined by

Western blotting using affinity purified rabbit polyclonal anti-CDK5RAP3 antibody (1:1000). Twenty microgram of total cell lysates from HCC cell lines was loaded in each lane.

Supplementary Fig. S2. Knockdown of CDK5RAP3 inhibited the tumorigenicity of SMMC-7721 cells. (*A*) The shRNA specific for CDK5RAP3 was transfected into SMMC-7721 cells and selected for stable CDK5RAP3 knockdown SMMC-7721 cells for cell proliferation assay. *Top,* Western blotting using anti-CDK5RAP3 antibody showing knockdown of CDK5RAP3 in SMMC-7721 cells; *bottom,* the curve showed the proliferation rate of two stable CDK5RAP3 knockdown SMMC-7721 cells. (*B*) Two stable CDK5RAP3 knockdown clones were used for soft agar growth assay, *, $P<0.005$ compared with SMMC-7721-vector, *t*-test. Error bars: mean±SD.

Supplementary Fig. S3. Representative immunohistochemical staining for p-PAK4 (Ser474) in HCC cells of the nude mice xenograft. All photos were of 20X magnification.