Supplementary information, Data S1 Materials and Methods

Plasmids

FLAG or HA tagged wild type FBW7 and its relative T205A, T205E and R465H mutants were cloned into pCDH-CMV-MCS-EF1-Puro vector to generate expression constructs. Coding sequence of ERK1 with FLAG or HA tag and the kinase dead mutant of ERK1 with mutation of K71 to A (ERK1-KD) were cloned into pCDH-CMV-MS-EF1-Puro to generate the lentiviral expressing constructs.

DNA methylation analysis

Methylation pattern of the FBW7 promoter region（-118 - 59）was examined in control or ERK1 overexpression PANC-1 cell lines were examinedas previously described [1]. Bisulfite-modified genomic DNA was prepared and CpG methylation was analysed by bisulfite-sequence analysis. Sequencing primers were Forward: 5'-TTG AAA AGA TTT AGG AAG AGG AAA AG-3' or Reverse: 5'-AAA CCA CTC ACA CTT TTA AAA AAA AA-3'.

Cell migration assay

Cell migration assay was performed as previously described [2].

Hot kinase assay

2μg purified GST-FBW7 protein (wild type and T205A mutant) were incubated with ERK1 kinase in the presence of 5 μCi of [γ-^{32}P] ATP and 20 μM unlabelled ATP in the kinase reaction buffer for 30 min. The reaction was stopped by the addition of SDS-containing lysis buffer, resolved by SDS–PAGE and detected by autoradiography.

Next-generation sequencing

60 formalin-fixed paraffin-embedded (FFPE) tissues were enriched for tumor by macrodissection using scalpels under visualization guided by a contiguous hematoxylin and eosin (H&E) section. Genomic DNA was extracted using the QIAamp DNA FFPE Tissue Kit (Qiagen) following the manufacturer's instructions. DNA quality and quantity were assessed by the Qubit® 2.0 Fluorometer (Life Technologies) and gel electrophoresis. Hotspot regions, including approximately 2,800 COSMIC mutations of 50 oncogenes and tumor suppressor genes, were amplified from 10 ng of genomic DNA with the Ion AmpliSeq™ Library Kit and the Ion AmpliSeq™ Cancer Hotspot Panel v2 (Life Technologies). The resulting amplicons were treated with FuPa Reagent to partially digest the primers and phosphorylate the amplicons. The amplicons were then ligated to Ion Adapters with barcodes, and purified with AMpure XP beads (Sigma). After amplification and purification, libraries were quantified with the Qubit® 2.0 Fluorometer (Life Technologies) and library DNA quality are assessed by Agilent 2100 Bioanalyzer (Agilent). The completed libraries were combined and diluted appropriately, and then subject to Ion template preparation using the Ion One Touch 2 System (Life Technologies). Sequencing was performed on Ion Torrent PGM/Proton platforms (Life Technologies). After sequencing completion, the data was de-multiplexed by Torrent Suite v4.0.1 into fastq files and bam files. SNP and mutations detection and annotation were performed by the in-house developed software pipeline.

Reference

1. Akhoondi S, Lindström L, Widschwendter M, *et al*. Inactivation of FBXW7/hCDC4-β expression by promoter hypermethylation is associated with favorable prognosis in primary breast cancer. *Breast Cancer Res* 2010; **12**:R105.

2. Shair KH, Schnegg CI, Raab-Traub N. EBV latent membrane protein 1 effects on plakoglobin, cell growth, and migration. *Cancer Res* 2008; **68**:6997-7005.