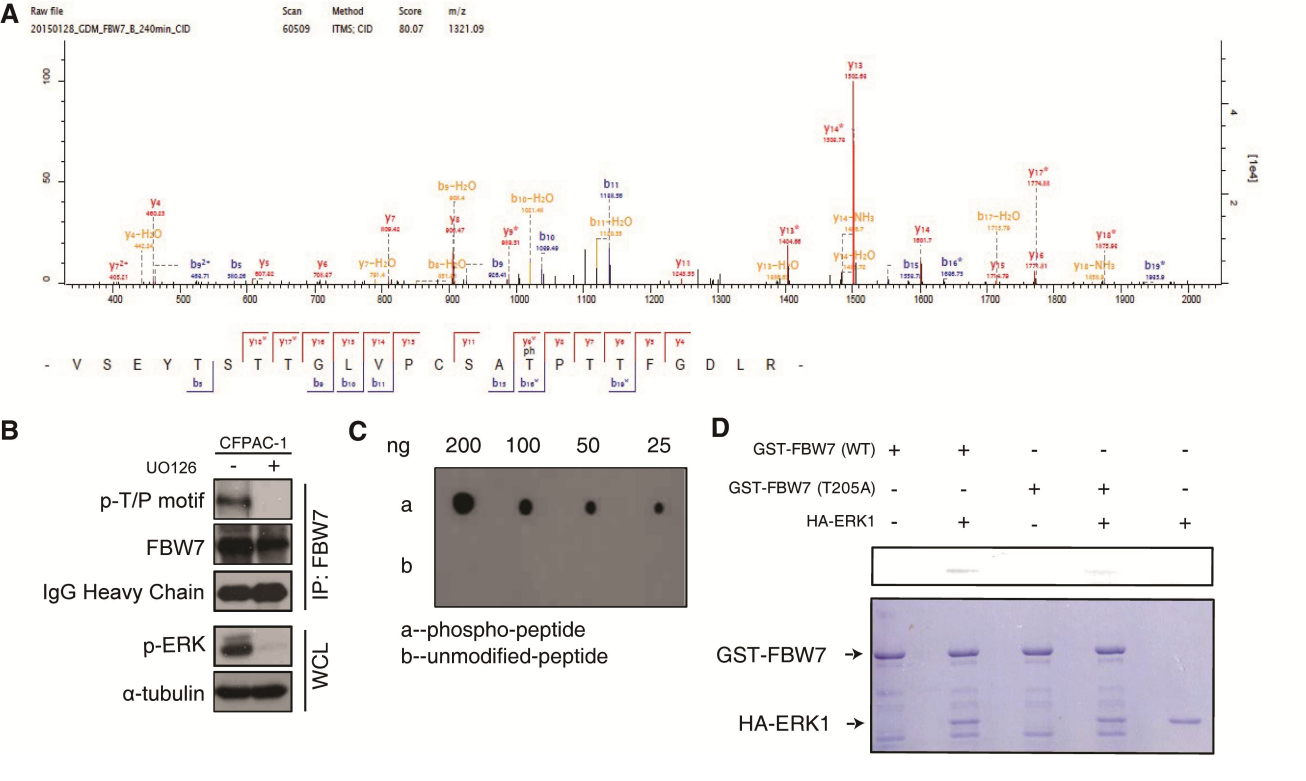
A
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B
CFPAC-1
UO126
- +
p-T/P motif
FBW7
IgG Heavy Chain
IP: FBW7
p-ERK
α-tubulin
WCL
C
ng 200 100 50 25
a
b
a--phospho-peptide
b--unmodified-peptide
D
GST-FBW7 (WT) + + - - -
GST-FBW7 (T205A) - - + + -
HA-ERK1 - + - + +
GST-FBW7
HA-ERK1

Supplementary information, Figure S4 (related to Figure 5) ERK phosphorylates FBW7 at T205.

(A) Detection of *in vivo* FBW7 T205 phosphorylation by mass spectrometry analysis. **(B)** CFPAC-1 cells were pretreated with the proteasome inhibitor MG132 and UO126, as indicated, overnight before harvest. Endogenous FBW7 phosphorylation status was examined by immunoblot analysis and immunoprecipitates (IP). The kinase reaction products were resolved by SDS-PAGE, and phosphorylation was detected by the phospho-Threonine-Proline monoclonal antibody (p-Thr-Pro-101). **(C)** Validation of phospho-T205-FBW7 antibody by dot blot assay. PVDF membrane was spotted with different amounts of phosphorylated or unmodified peptide and then blotted with p-FBW7 antibody. **(D)** ERK kinase phosphorylates FBW7 *in vitro* at T205. Purified ERK1 protein was incubated with 2 μg of indicated GST-FBW7 in the presence of γ-^{32}P-ATP. The kinase reaction products were resolved by SDS-PAGE, and phosphorylation was detected by autoradiography.