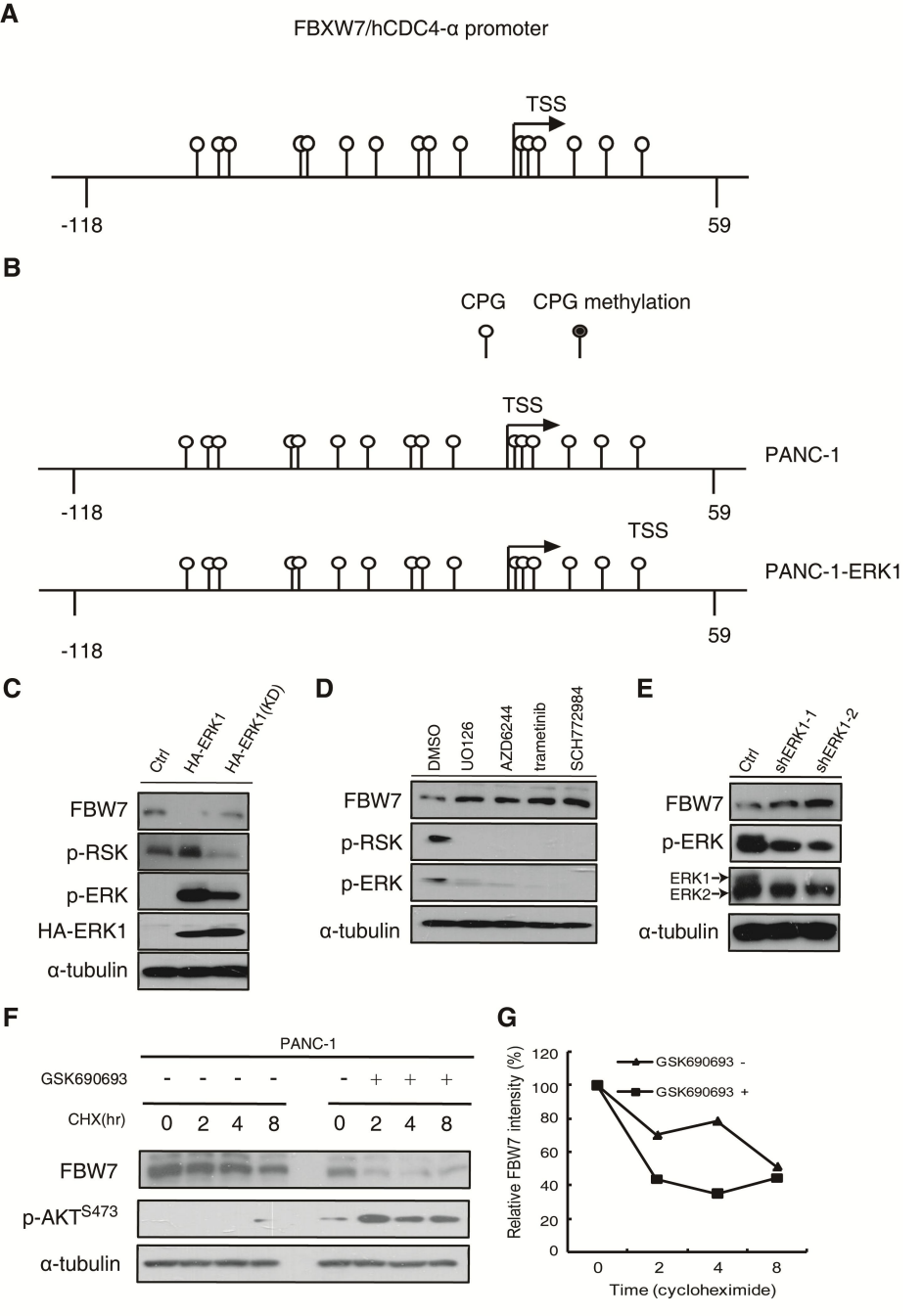
A
FBXW7/hCDC4-α promoter
TSS
-118
59
B
CPG
CPG methylation
TSS
PANC-1
-118
59
TSS
PANC-1-ERK1
-118
59
C
Ctrl
HA-ERK1
HA-ERK1(KD)
FBW7
p-RSK
p-ERK
HA-ERK1
α-tubulin
D
DMSO
UO126
AZD6244
trametinib
SCH772984
FBW7
p-RSK
p-ERK
α-tubulin
E
Ctrl
shERK1-1
shERK1-2
FBW7
p-ERK
ERK1
ERK2
α-tubulin
F
PANC-1
GSK690693
- - - - - + + +
CHX(hr)
0 2 4 8 0 2 4 8
FBW7
p-AKTS473
α-tubulin
G
GSK690693 -
GSK690693 +
Relative FBW7 intensity (%)
120
100
80
60
40
20
0
0 2 4 8
Time (cycloheximide)

Supplementary information, Figure S3 (related to Figure 3) ERK negatively regulates FBW7.

(A) Schematic map of the longest transcript of FBXW7/hCDC4-α promoter region around the transcription start sites (TSS). CpG dinucleotides are depicted. **(B)** FBXW7/hCDC4-α core promoter region containing 16 CpG sites in PANC-1 cells were seldom methylated after introduction of ERK1. **(C)** PANC-1 cells were infected with wild type ERK1 and kinase-dead mutant expressing virus, and then incubated with 1 ug/ml puromycin to eliminate the non-infected cells. FBW7 expression was analyzed by immunoblot. **(D)** PANC-1 cells were treated with indicated inhibitors for 12h and cell lysates were collected and immunoblots were performed with the indicated antibodies. **(E)** SW1990 cells were infected with indicated lenti-viral shRNA vectors, and then incubated with 1 ug/ml puromycin to eliminate the non-infected cells. Cell lysates were collected and immunoblots were performed with the indicated antibodies. **(F)** PANC-1 cells were treated with 20 μg/ml CHX in the presence or absence of 10 μM GSK690693, and whole cell lysates (WCL) were collected at the indicated time points for immunoblot analysis. **(G)** Semi-quantification with α-tubulin as a loading control and relative FBW7 levels at time 0 were set as 100%.