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Supplemental Information

The FBXW7-SHOC2-Raptor Axis Controls the Cross-Talks between the RAS-ERK and mTORC1 Signaling Pathways

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SUPPLEMENTAL FIGURE LEGENDS

The FBXW7-SHOC2-Raptor axis controls the cross-talks between the RAS/ERK and mTORC1 signaling pathways

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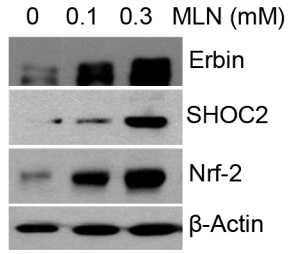
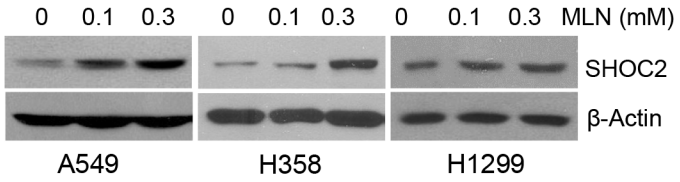
A



B



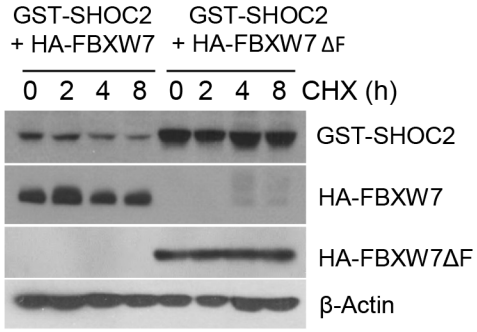
C

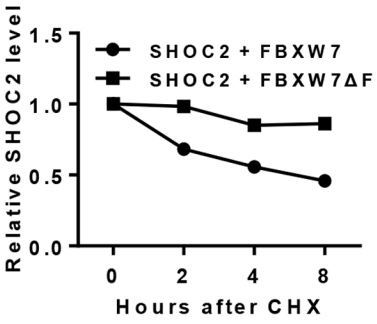
FBXW7 binding site

consensus	**L*TP**	**L*TP**
SHOC2 (Human)	238-CNLITLDV-245	503-ENLLTHLP-510
SHOC2 (Chimpanzee)	238-CNLITLDV-245	503-ENLLTHLP-510
SHOC2 (Cow)	238-CNLITLDV-245	503-ENLLTHLP-510
SHOC2 (Mouse)	238-CNLITLDV-245	503-ENLLTHLP-510
SHOC2 (Dog)	238-CNLITLDV-245	503-ENLLTHLP-510
SHOC2 (Chicken)	238-CNLITLDV-245	503-ENLLTHLP-510
SHOC2 (Xenopus)	233-CNLITLDV-240	498-ENLLTHLP-505
SHOC2 (Zebrafish)	217-CNLITLDV-224	482-ENLLQHLP-489

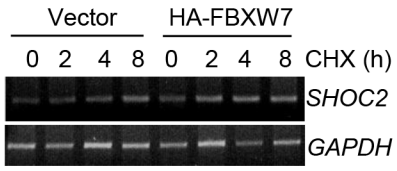
*=I or L or P

D





E



F

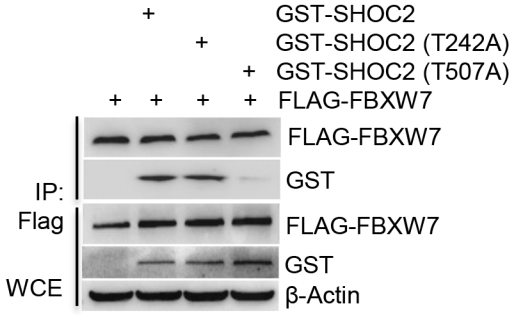


Figure S1. SHOC2 is a novel substrate of FBXW7 (Related to Fig 1)

(A&B) Mouse keratinocytes or lung cancer cells were treated with MLN4924 (MLN) for 24 hrs and then analyzed by IB.

(**C**) Evolutionary conservation of the FBXW7-binding motif on SHOC2 from eight different species.

(**D&E**) 293 (D) or H1299 (E) Cells were transfected with indicated plasmids for 48 hrs, followed by treatment with CHX for different time periods and harvested for IB (D) or RT-PCR (E). The band density was quantified by AlphaEaseFC software. The data shown are from a single representative experiment out of two repeats.

(**F**) 293 cells were transfected with indicated plasmids for 48 hrs, followed by IP and IB with indicated Abs. WCE, whole cell extract.

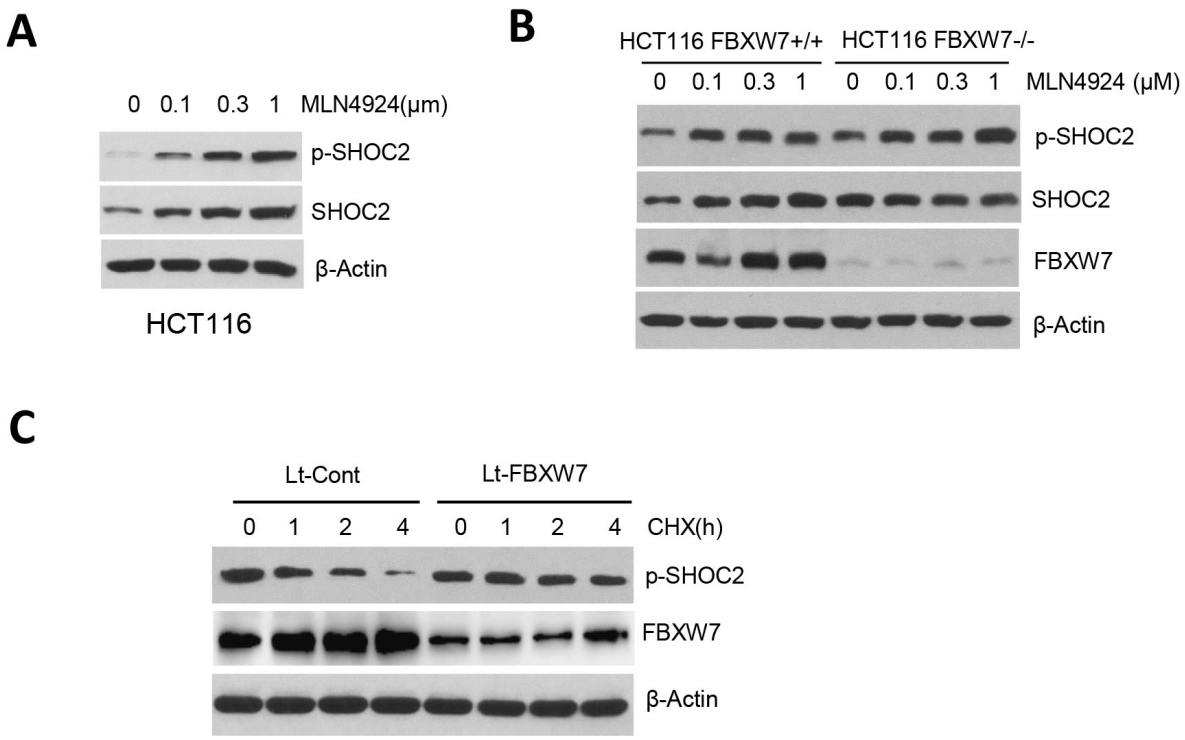


Figure S2. SHOC2 is phosphorylated on Thr507 by ATR upon DNA damage (Related to Fig 2)

(**A&B**) Cells were treated with MLN4924 (MLN) for 24 hrs and then analyzed by IB.

(**C**) H1299 cells were transfected with Control shRNA or FBXW7 shRNA for 48 hrs, followed by treatment with CHX for different time periods and harvested for IB.

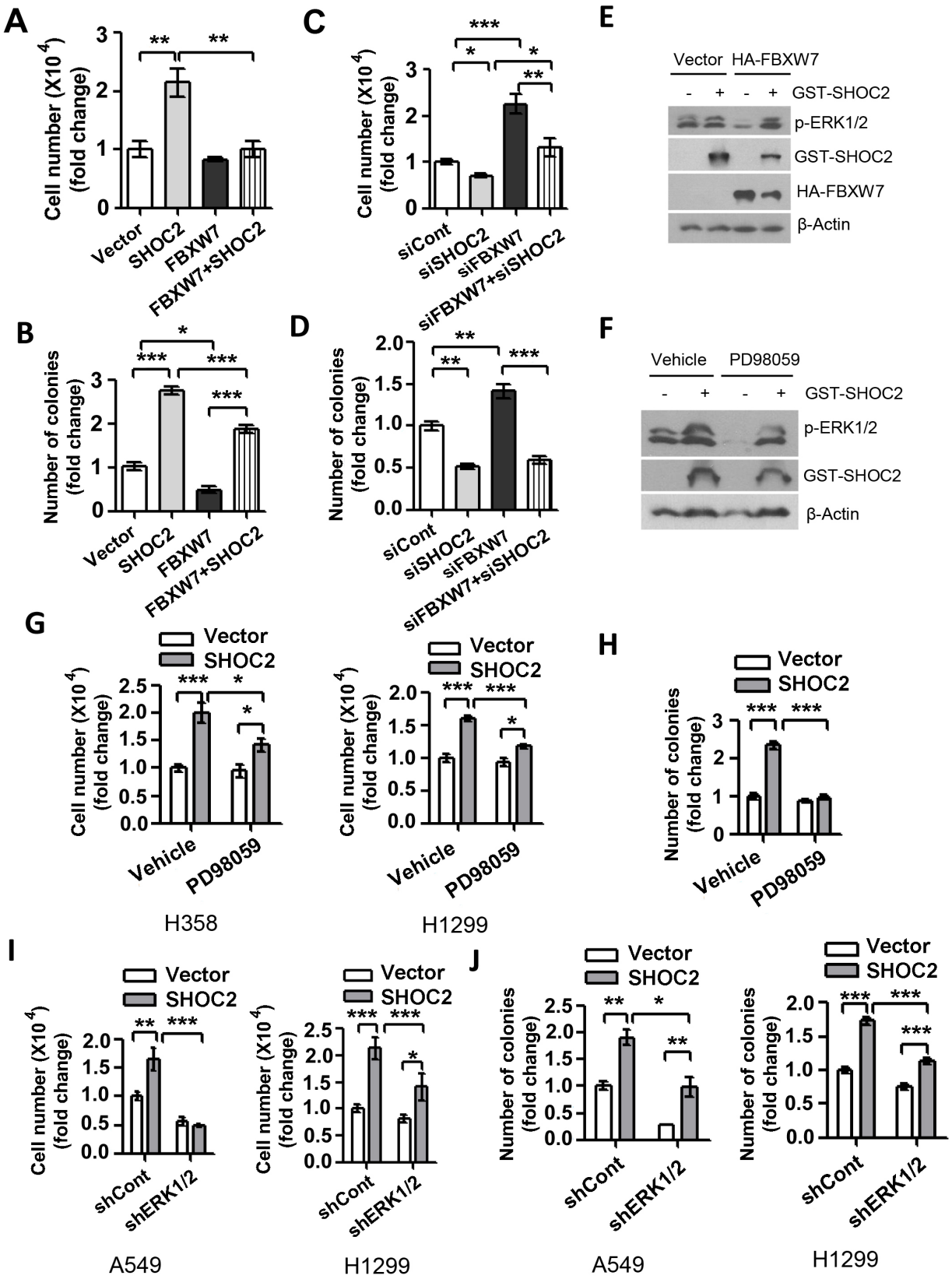

A
Cell number (X10 4) (fold change)
Vector
SHOC2
FBXW7
FBXW7+SHOC2
B
Number of colonies (fold change)
C
siCont
siSHOC2
siFBXW7
siFBXW7+siSHOC2
D
E
Vector
HA-FBXW7
GST-SHOC2
p-ERK1/2
HA-FBXW7
β-Actin
F
Vehicle
PD98059
G
H358
H1299
H
I
shCont
shERK1/2
A549
J

Figure S3. SHOC2 promotes proliferation via ERK activation, blocked by FBXW7 (Related to Fig 3)

(**A-D**) A549 cells were transfected with indicated plasmids or siRNA oligoes for 48 hrs. Cells were seeded and cell numbers counted 48 hrs later (A&C), or colonies counted 9-13 later (B&D).

(**E-H**) H358 (E&F, G left panel) or H1299 (H, G right panel) cells were transfected with indicated plasmids for 48 hrs, followed by IB with indicated Abs (E), or transfected with indicated plasmids for 24 hrs, then treated with PD98059 for 24 hrs, followed by IB (F), cell count (G) or colonies counted 9-13 later (H).

(**I&J**) Cells were transfected with shRNA targeting ERK1/2 (shERK1/2) in the presence or absence of SHOC2 overexpression for 48 hrs. Cells were plated, and cell number counted 48 hrs later (I) or colonies counted 9-13 days later (J). Error bar represents SEM.*$p< 0.05$, **$p< 0.01$,***$p< 0.001$.

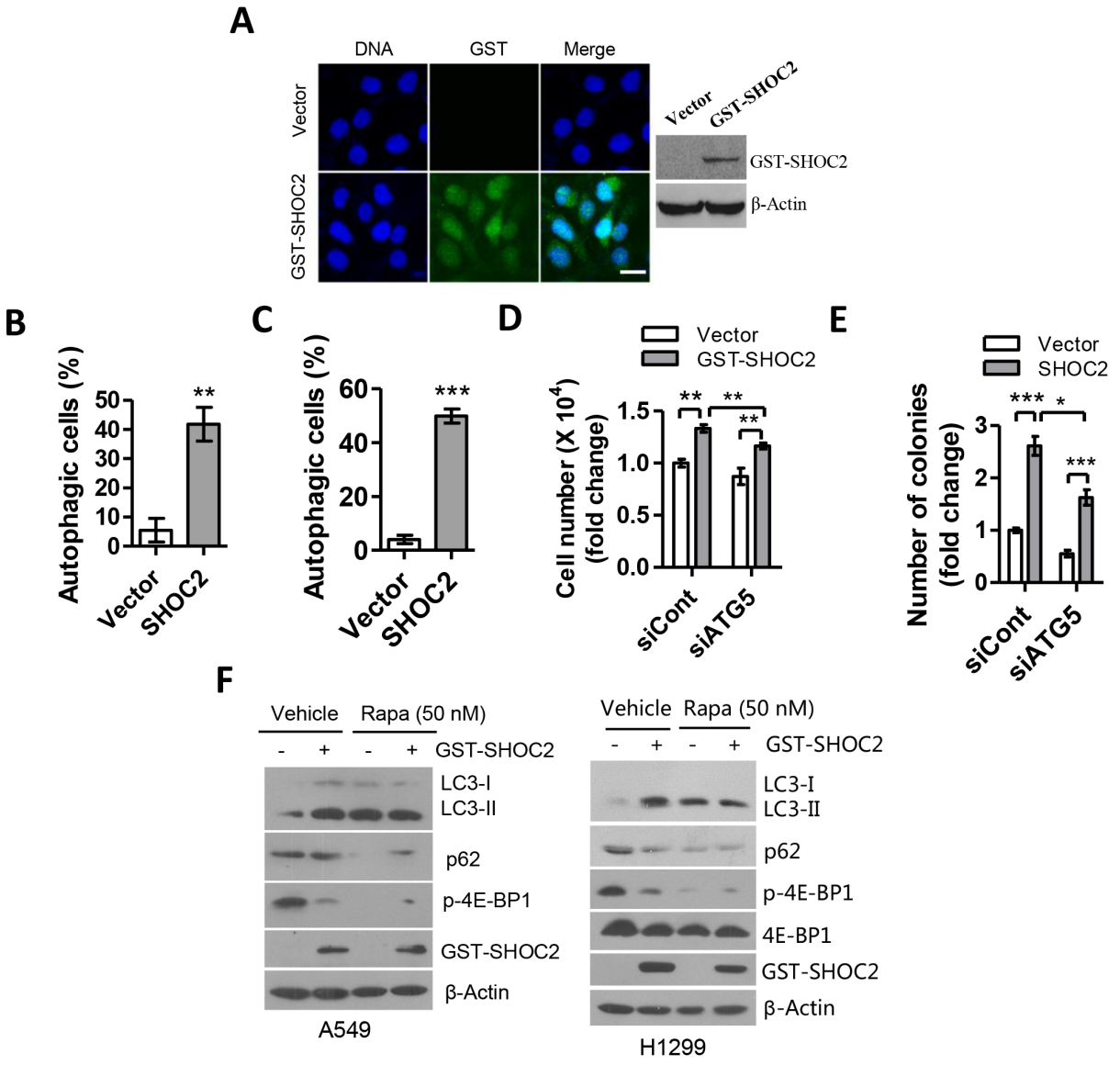


Figure S4. SHOC2 induces autophagy via inactivation of mTORC1(Related to Fig 4)

(**A-C**) H1299 (A) or A549 (B&C) cells were transfected with GST-SHOC2 for 48 hrs and analyzed by IB (A, left panel) or stained with GST antibody (A, right panel), Cyto-ID Autophagy detection kit (B) or with LC3 antibody (C). Cells containing more than 10 autophagic vacuoles (B) or five LC3 dots (C) were counted as autophagic cells. The data shown are from a single representative experiment out of three repeats. For the experiment shown, at least 100 cells were counted in each group. Error bar represents SEM. ***P*< 0.01, ****P*< 0.001. Scale bar, 20 μm.

(**D&E**) A549 cells were transfected with indicated plasmids or siATG5 for 48 hrs, followed by assays for cell growth (D) or clonal survival (E). The error bars represent SEM from three independent experiments, **p*<0.05, ***p*<0.01, ****p*<0.001.

(**F**) Cells were transfected with GST-SHOC2 for 24 hrs, followed by exposure to 50 nM Rapamycin (Rapa) for 24 hrs, followed by IB.

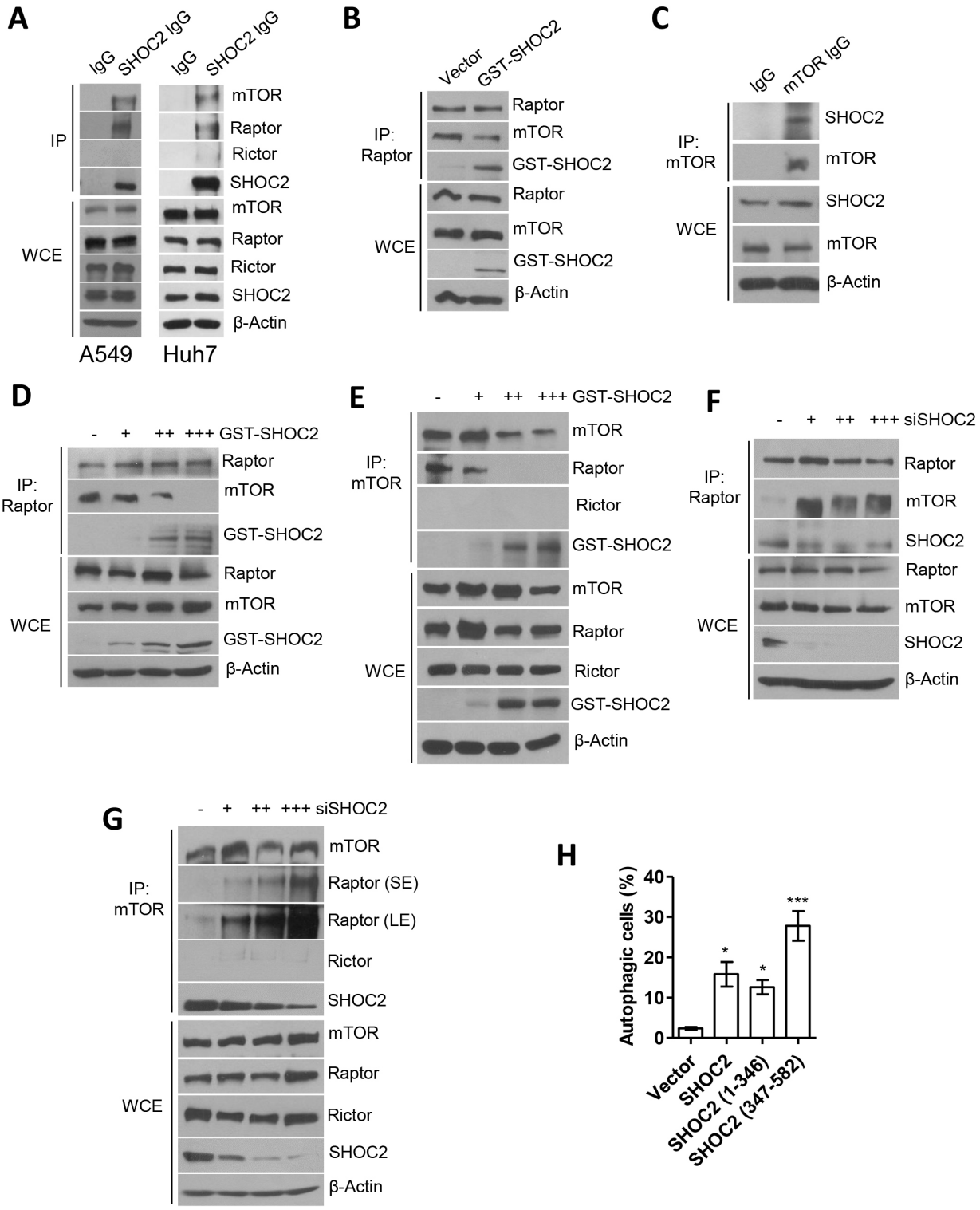

A
IgG
SHOC2 IgG
IgG
SHOC2 IgG
IP
WCE
mTOR
Raptor
Rictor
SHOC2
mTOR
Raptor
Rictor
SHOC2
β-Actin
A549
Huh7
B
Vector
GST-SHOC2
IP: Raptor
WCE
Raptor
mTOR
GST-SHOC2
Raptor
mTOR
GST-SHOC2
β-Actin
C
IgG
mTOR IgG
IP: mTOR
WCE
SHOC2
mTOR
SHOC2
mTOR
β-Actin
D
- + ++ +++ GST-SHOC2
IP: Raptor
WCE
Raptor
mTOR
GST-SHOC2
Raptor
mTOR
GST-SHOC2
β-Actin
E
- + ++ +++ GST-SHOC2
IP: mTOR
WCE
mTOR
Raptor
Rictor
GST-SHOC2
mTOR
Raptor
Rictor
GST-SHOC2
β-Actin
F
- + ++ +++ siSHOC2
IP: Raptor
WCE
Raptor
mTOR
SHOC2
Raptor
mTOR
SHOC2
β-Actin
G
- + ++ +++ siSHOC2
IP: mTOR
WCE
mTOR
Raptor (SE)
Raptor (LE)
Rictor
SHOC2
mTOR
Raptor
Rictor
SHOC2
β-Actin
H
Autophagic cells (%)
0
10
20
30
40
*
*
***
Vector
SHOC2
SHOC2 (1-346)
SHOC2 (347-582)

Figure S5. SHOC2 binds to Raptor and competitively inhibits Raptor-mTOR binding (Related to Fig 5)

(**A**) Cells were left untransfected plasmids. Cell lysates were prepared for IP and IB with indicated Abs, or for direct IB. WCE, whole cell lysates.

(**B&C**) A549 (B) or H1299 (C) cells were left untransfected (C) or transfected (B) with indicated plasmids. Cell lysates were prepared for IP and IB with indicated Abs, or for direct IB. WCE, whole cell lysates.

(**D-G**) A549 (D&F) or H1299 (E&G) cells were transfected with increasing doses of GST-SHOC2 or siSHOC2 oligoes, followed by IP and IB with indicated Abs, or direct IB.

(**H**) A549 cells were transfected with indicated plasmids for 48 hrs, stained with Cyto-ID Autophagy detection kit, and autophagic cells counted. Error bars represent SEM. *$p< 0.05$, ***$p< 0.001$.

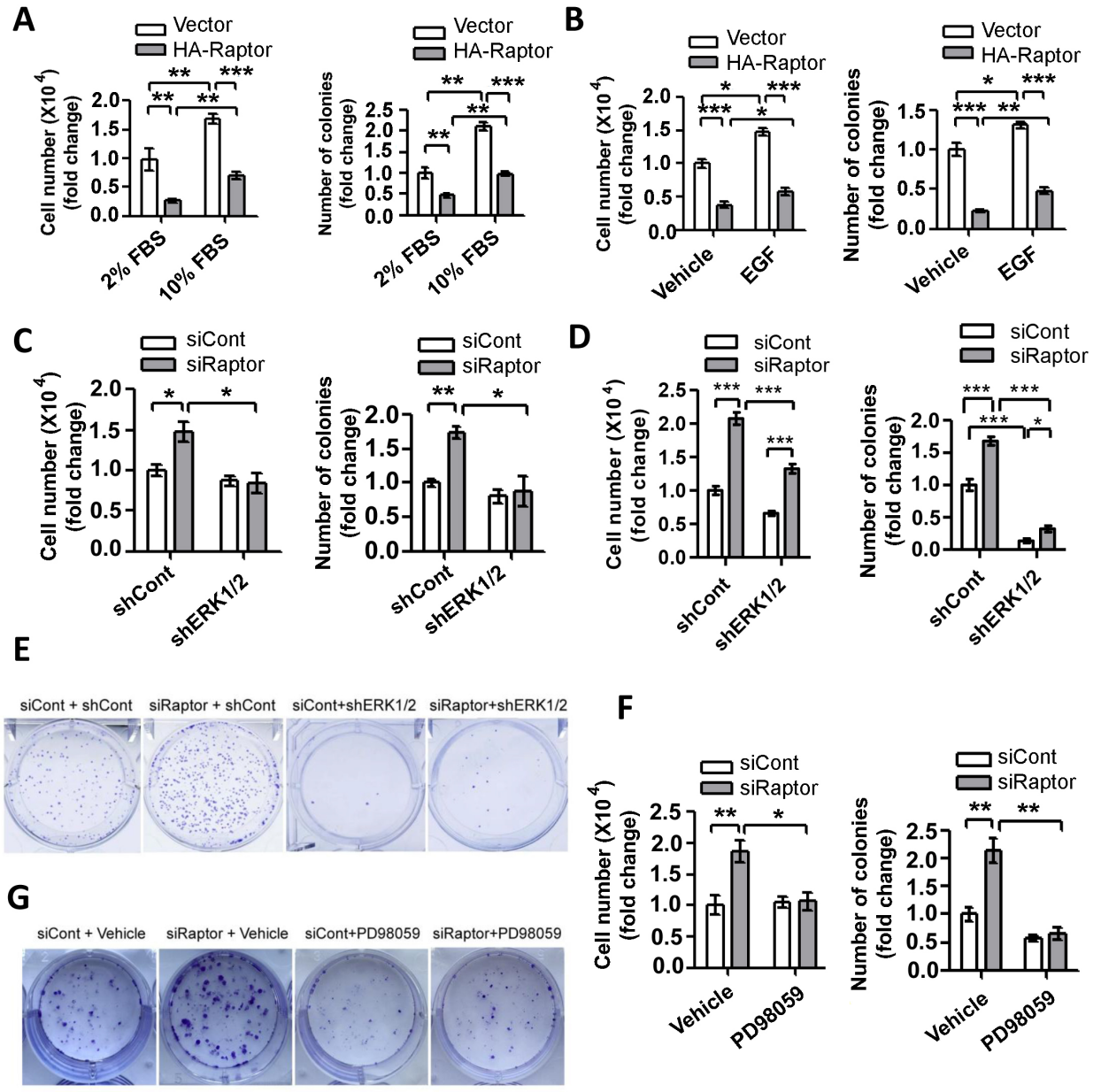


Figure S6. Raptor negatively regulates the RAS/ERK signal pathway via SHOC2 binding (Related to Fig 6)

(**A&B**) H1299 cells were transfected with HA-Raptor in the presence of 2% FBS or EGF (100 ng/ml) for 48 hrs. Cells were plated into 6-well plate for cell counting after 48 hrs, or colonies counting after 9 days.

(**C-E**) H1299 (C) or A549 (D&E) cells were transfected with indicated siRNA oligoes or shRNAs for 48 hrs. Cells were plated into 6-well plate for cell counting after 48 hrs, or colonies counting after 9 days.

(**F&G**) H1299 cells were transfected with siRNA oligoes targeting Raptor (siRaptor) for 24 hrs, followed by treatment with PD98059 (10 μM) for 24 hrs. Cells were plated and cell number counted 48 hrs later or colonies counted 9-13 days later. Error bars represent SEM. *$p< 0.05$; **$p< 0.01$.

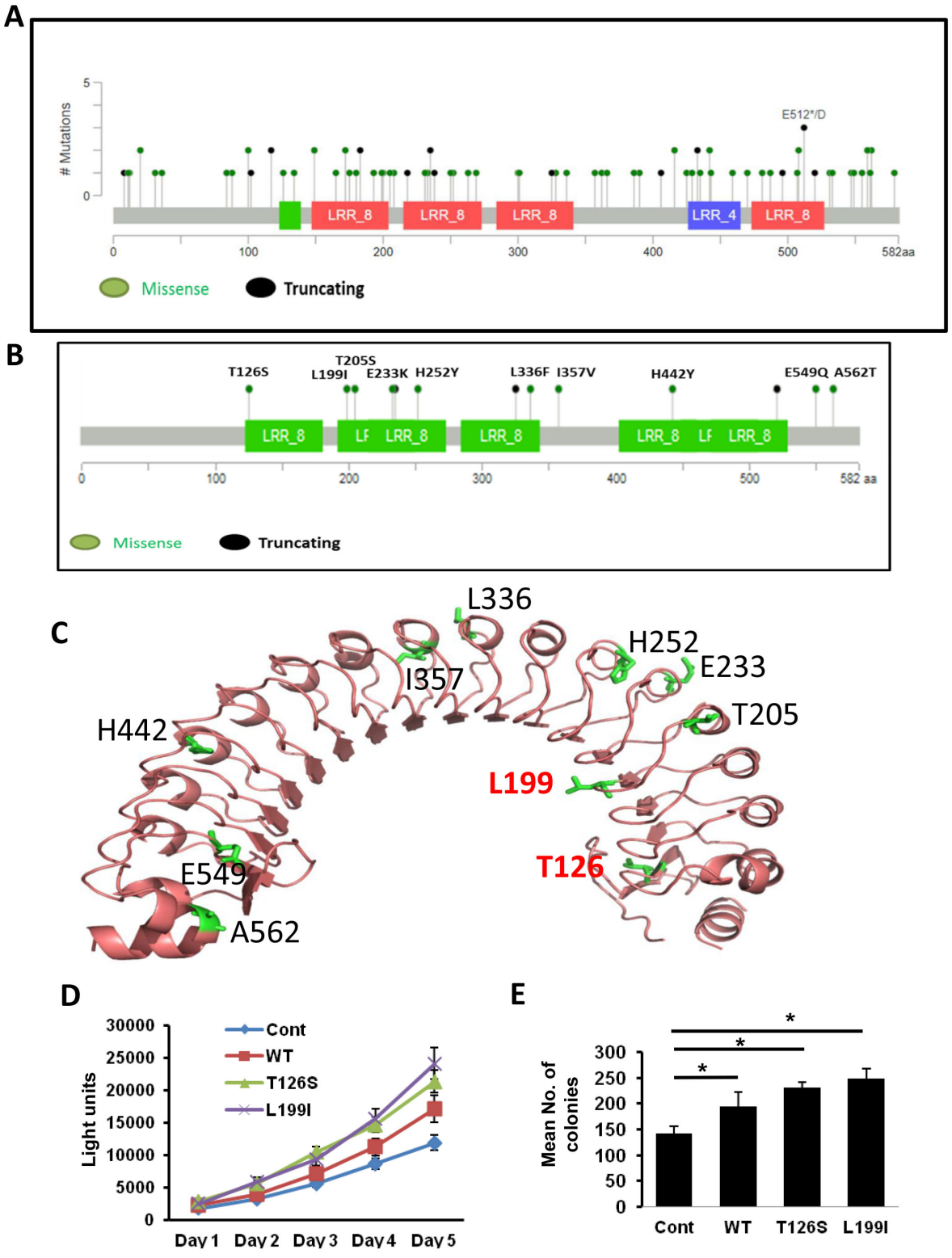

A
Mutations
5
0
E512*/D
LRR_8
LRR_8
LRR_8
LRR_4
LRR_8
0
100
200
300
400
500
582aa
Missense
Truncating
B
T126S
T205S
L199I
E233K
H252Y
L336F
I357V
H442Y
E549Q
A562T
LRR_8
LF
LRR_8
LRR_8
LRR_8
LF
LRR_8
0
100
200
300
400
500
582 aa
Missense
Truncating
C
L336
H252
E233
I357
T205
H442
L199
T126
E549
A562
D
Light units
30000
25000
20000
15000
10000
5000
0
Cont
WT
T126S
L199I
Day 1
Day 2
Day 3
Day 4
Day 5
E
Mean No. of colonies
300
250
200
150
100
50
0
*
*
*
Cont
WT
T126S
L199I

Figure S7. Gain-of-function SHOC2 mutations found in human lung adenocarcinoma tissues (Related to Fig 7)

(A) Distribution of SHOC2 mutations observed in all cancer types. Data obtained from the cbioportal.org.

(B) Distribution of SHOC2 mutations found in human lung cancer from TCGA and COSMIC databases.

(C) Computer modeling of SHOC2 structure and locations of two mutations. SHOC2 3D model was simulated by Phyre2, a structure prediction software. Residues shown as sticks are SHOC2 mutations found in human lung cancer.

(D&E) H1299 cells were transfected with wild-type and different SHOC2 mutants. Cell proliferation was assayed by ATPlite (D) and cell survival measured by clonogenic assay (E). Error bars represent SEM. *$p < 0.05$.

Table S1. Primers for RT-qPCR. Related to Figure 3 and Figures S1.

Gene	Sequence
SHOC2 forward	5'-GGAAAGAAGGACTCCAGTGCTG-3'
SHOC2 reverse	5'- ACTAAACATCCCACCTCTGCTGG-3'
FBXW7 forward	5' -CCGAGTCTGGGATGTGGA-3'
FBXW7 reverse	5'- TCTGATGC TTGCTGGGACC-3'
GAPDH forward	5'-GTTGCCATCAATGACCCCTTC-3
GAPDH reverse	5'-GCAGGGATGATGTTCTGG-3'

Table S2. Sequences of siRNAs and shRNAs. Related to Figures 1-6 and Figures S3-6.

Gene	Sequence	Cat No.
SHOC2 siRNA-1	Targeting 5'-AAGCTGCGGATGCTTGATT-3'	N/A
SHOC2 siRNA-2	Targeting 5'-TACCTTCGCTTTAATCGTATA-3';	N/A
FBXW7 siRNA	Targeting 5'-ACAGGACAGTGTTTACAAA-3'	N/A
H1 promoter-driven *ERK1* shRNA	Targeting 5'-CATGAAGGCCCGAAACTAC-3'	N/A
H1 promoter-driven *ERK2* shRNA	Targeting 5'-GCGCTTCAGACATGAGAAC-3'	N/A
ATG5 siRNA	Targeting 5'-GGATGAGATAACTGAAAG-3′	N/A
MEK1 siRNA	Targeting 5'-GCAGGCAAGTCAAAGACAA-3'	N/A
MEK2 siRNA	Targeting 5'-GCCATCGAATATACTTGTT-3	N/A
Raptor siRNA	including 4 siRNA duplexes	SC-44069

Key Resources