Supplementary Figure 1. WTX reduces proliferation of HCC cells.

HepG2 and Huh7 cells were transduced with WTX expression plasmid (pWTX) or pcDNA3.1 as indicated. (A) Cell proliferation was determined by MTS assay. Data represent the means ± SEM. **P < 0.01.

Supplementary Figure 2. Silencing WTX promotes proliferation, migration, invasion and autophagy of HCC cells.

HepG2 and Huh7 cells were transfected with siWTX or siCon. (A) Levels of WTX and LC3B were detected by western blot. (B) Cell proliferation was determined by MTS assay. (C) The expression levels of the cell proliferation marker Ki67 were detected by immunofluorescence. (D) Effect of WTX on cell proliferative abilitys was examined by EdU incorporation assay. (E, F) Cell metastasis was determined by Scratch wound assays (E) or Transwell migration and Matrigel invasion assays (F). (G) Cell autophagy was detected using an autophagosome detection kit. Data represent the means ± SEM. **P < 0.01.

Supplementary Figure 3. miR-454-3p inhibitor reduces proliferation of HCC cells.

HepG2 and Huh7 cells were transduced with miR-454-3p inhibitor NC or miR-454-3p inhibitor. (A) Cell proliferation was determined by MTS assay. Data represent the means ± SEM. **P < 0.01.

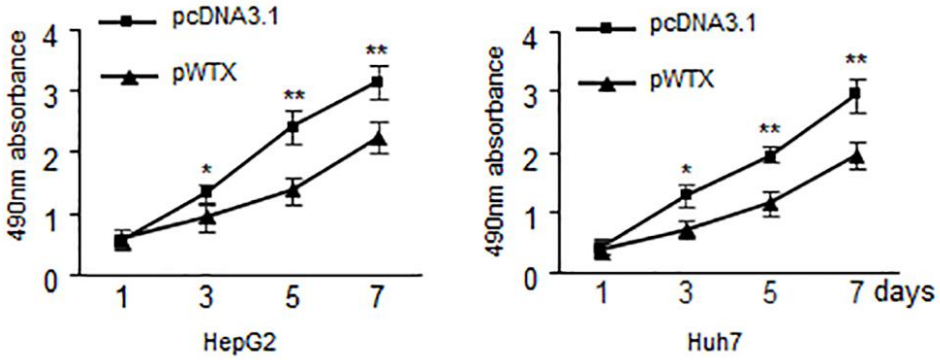
Supplementary Figure 4. miR-454-3p inhibitor reduces proliferation, migration, invasion and autophagy of HCC cells.

HepG2 and Huh7 cells were transduced with miR-454-3p inhibitor NC or miR-454-3p inhibitor. (A) Levels of WTX were detected by western blot. (B) Levels of LC3B were detected by western blot. (C) The expression levels of the cell proliferation marker Ki67 were detected by immunofluorescence. (D) Effect of miR-454-3p inhibitor on cell proliferative abilitys was examined by EdU incorporation assay. (E, F) Cell metastasis was determined by Scratch wound assays (E) or Transwell migration and Matrigel invasion assays (F). (G) Cell autophagy was detected using an autophagosome detection kit. Data represent the means ± SEM. **P < 0.01.

Supplementary Figure 5. WTX inhibits the expression of TGFβ2.

(A) RT-qPCR of selected genes related to the ranked pathways andtumorigenesis in WTX-knockdown Huh7 cells. (B) The decay of TGFβ2 mRNA was monitored by RT-qPCR in WTX overexpression or miR-454-3p suppression Huh7 cells by blocking mRNA synthesis using actinomycin D (ActD, 5 μg/mL) for the indicated time points upon normalization to RNA input levels. (C) The mRNA levels of TGFβ2 were determined in Huh7 cells transfected with miR-454-3p mimics. (D) The mRNA levels of TGFβ2 were determined in Huh7 cells cotransfected with pWTX and miR-454-3p mimics. Data represent the means ± SEM. **P < 0.01.

A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pWTX
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3
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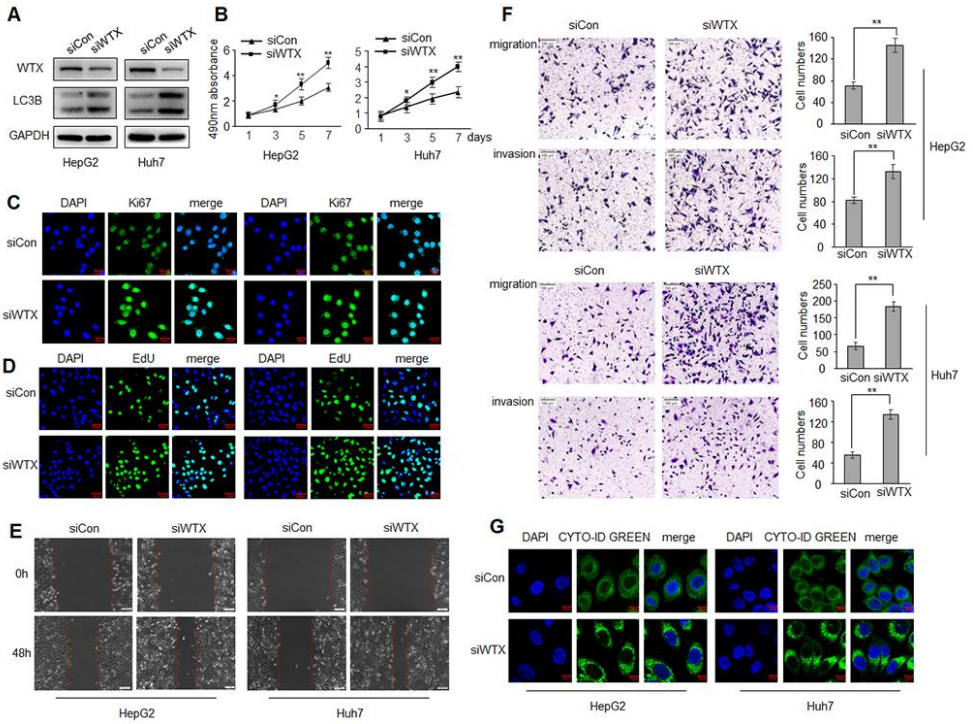
490nm absorbance
0
1
2
3
4
*
**
**
1
3
5
7 days
Huh7


A
siCon
siWTX
siCon
siWTX
WTX
LC3B
GAPDH
HepG2
Huh7
B
siCon
siWTX
490nm absorbance
HepG2
Huh7
days
C
DAPI
Ki67
merge
DAPI
Ki67
merge
siCon
siWTX
D
DAPI
EdU
merge
DAPI
EdU
merge
siCon
siWTX
E
siCon
siWTX
siCon
siWTX
0h
48h
HepG2
Huh7
F
siCon
siWTX
migration
invasion
Cell numbers
siCon
siWTX
HepG2
Huh7
G
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CYTO-ID GREEN
merge
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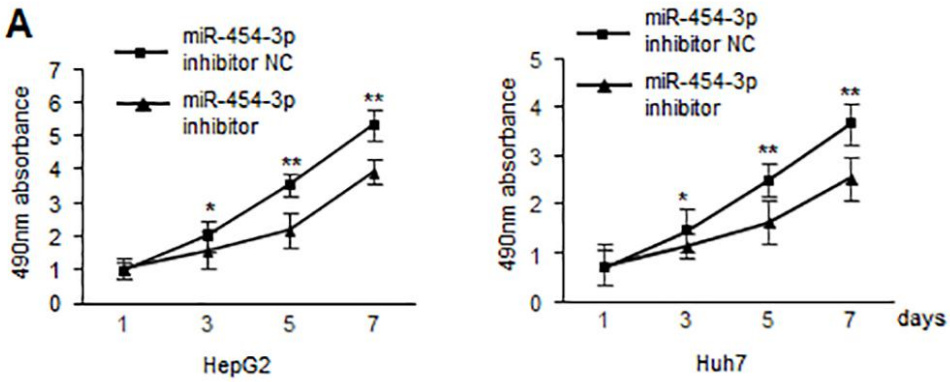
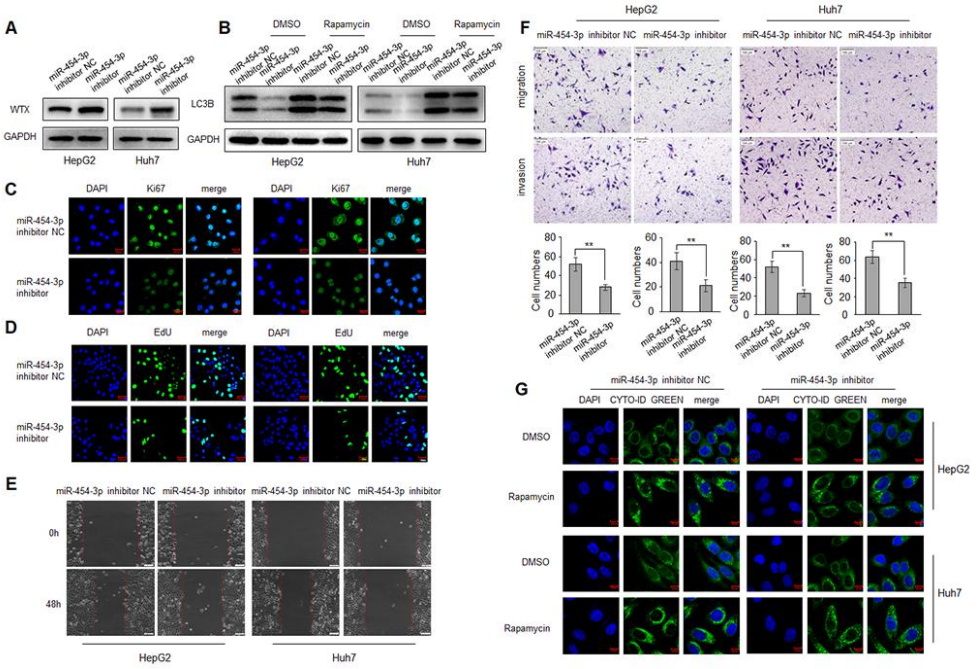

A
miR-454-3p inhibitor NC
miR-454-3p inhibitor
490nm absorbance
HepG2
Huh7
days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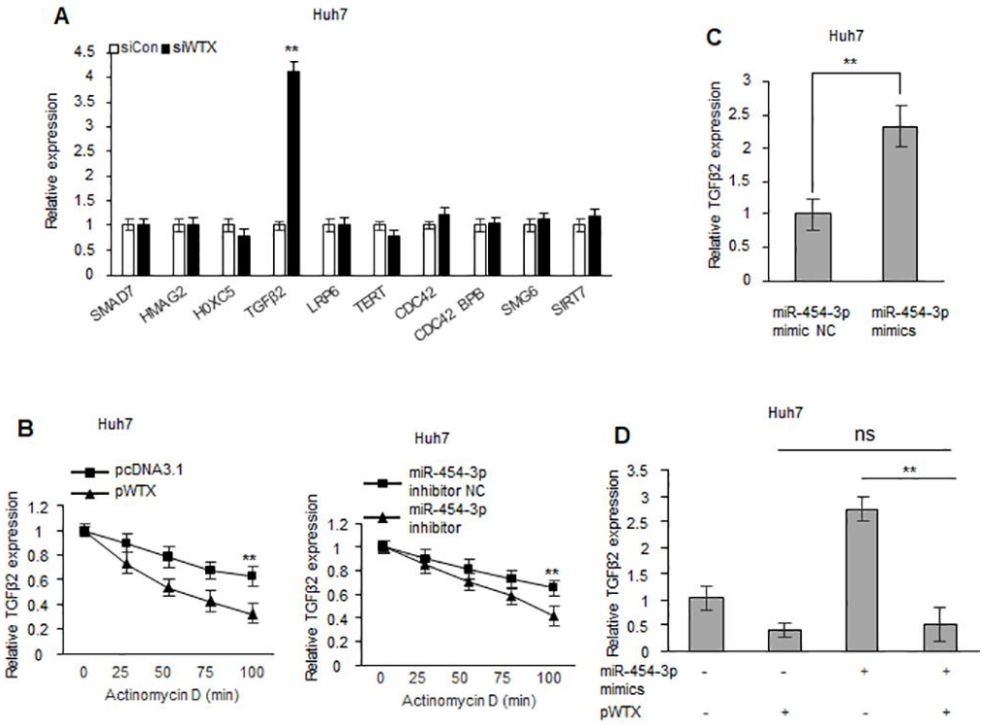
miR-454-3p inhibitor NC
miR-454-3p inhibitor
WTX
GAPDH
HepG2
Huh7
B
DMSO
Rapamycin
LC3B
GAPDH
C
DAPI
Ki67
merge
D
EdU
E
0h
48h
F
migration
invasion
Cell numbers
G
CYTO-ID GREEN


A
Huh7
siCon
siWTX
Relative expression
SMAD7
HMAG2
HOXC5
TGFβ2
LRP6
TERT
CDC42
CDC42 BPB
SMG6
SIRT7
B
Huh7
pcDNA3.1
pWTX
Relative TGFβ2 expression
Actinomycin D (min)
Huh7
miR-454-3p inhibitor NC
miR-454-3p inhibitor
Relative TGFβ2 expression
Actinomycin D (min)
C
Huh7
Relative TGFβ2 expression
miR-454-3p mimic NC
miR-454-3p mimics
D
Huh7
ns
Relative TGFβ2 expression
miR-454-3p mimics
pWTX