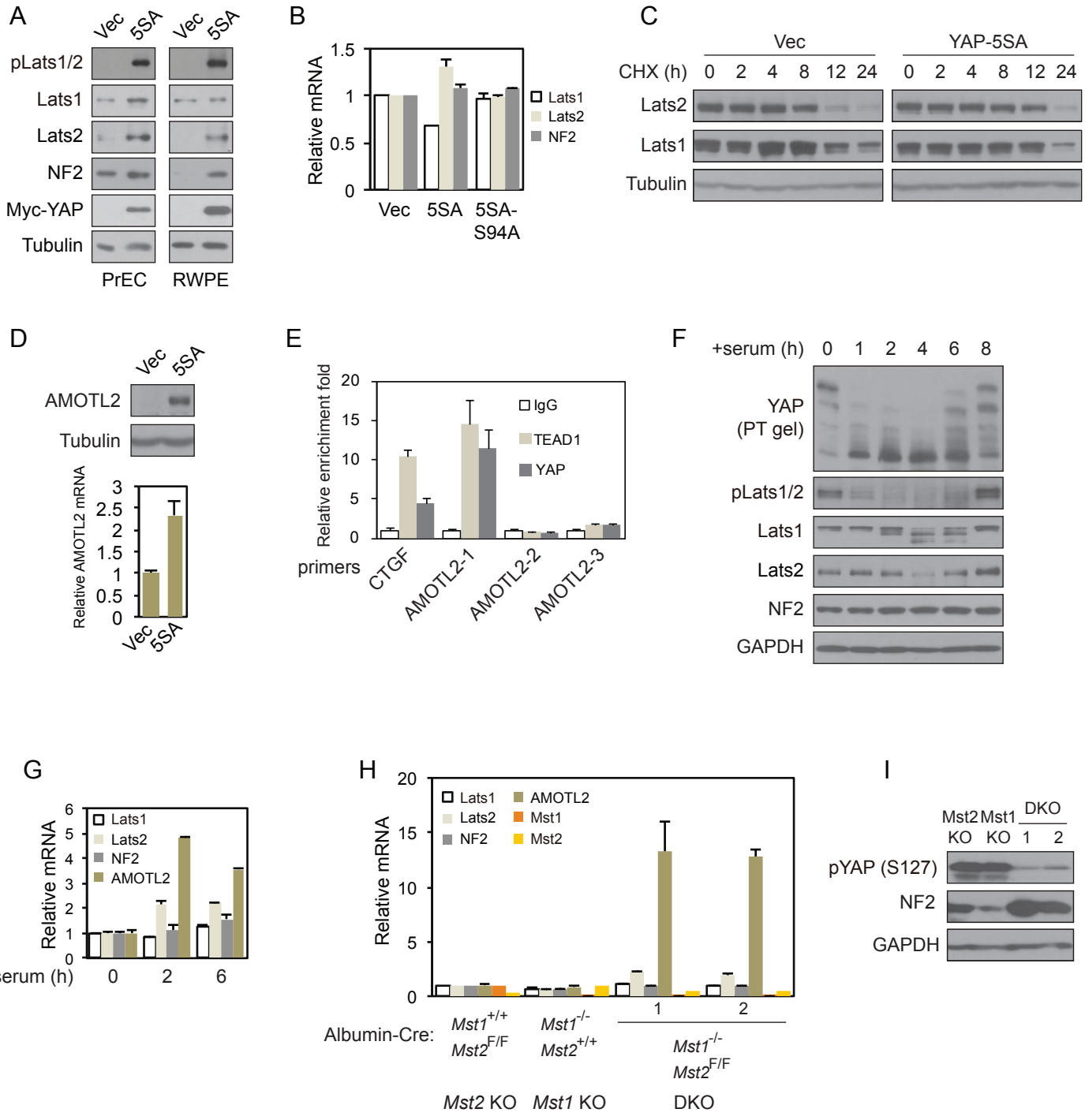
Figure S1


A
Vec
5SA
Vec
5SA
pLats1/2
Lats1
Lats2
NF2
Myc-YAP
Tubulin
PrEC
RWPE
B
Relative mRNA
1.5
1
0.5
0
Lats1
Lats2
NF2
Vec
5SA
5SA-S94A
C
Vec
YAP-5SA
CHX (h)
0 2 4 8 12 24
0 2 4 8 12 24
Lats2
Lats1
Tubulin
D
Vec
5SA
AMOTL2
Tubulin
Relative AMOTL2 mRNA
3
2.5
2
1.5
1
0.5
0
Vec
5SA
E
Relative enrichiment fold
20
15
10
5
0
IgG
TEAD1
YAP
primers
CTGF
AMOTL2-1
AMOTL2-2
AMOTL2-3
F
+serum (h)
0 1 2 4 6 8
YAP (PT gel)
pLats1/2
Lats1
Lats2
NF2
GAPDH
G
Relative mRNA
6
5
4
3
2
1
0
Lats1
Lats2
NF2
AMOTL2
+serum (h)
0
2
6
H
Relative mRNA
20
15
10
5
0
Lats1
Lats2
NF2
AMOTL2
Mst1
Mst2
1
2
Albumin-Cre:
Mst1+/+ Mst2F/F
Mst1-/- Mst2+/+
Mst1-/- Mst2F/F
Mst2 KO
Mst1 KO
DKO
I
Mst2 KO
Mst1 KO
DKO 1
DKO 2
pYAP (S127)
NF2
GAPDH

Supplementary information, Figure S1, Related to Figure 1.

(A) YAP activates Lats1/2 in multiple cell lines. PrEC and RWPE noncancerous prostate epithelial cells expressing YAP-5SA mutant were lysed and analyzed by western blots.

(B) YAP induces Lats2 transcription in a TEAD-dependent manner. mRNA was extracted from indicated HMLE stable cells. The mRNA levels of Lats1, Lats2, and NF2 were analyzed by quantitative RT-PCR.

(C) YAP does not significantly stabilize Lats1/2. Vector and YAP-5SA-expressing HMLE cells were treated with 100μg/ml cycloheximide (CHX) for the indicated time. Cell lysates were analyzed by western blots. Exposures of similar intensity were chosen for blots of the same protein for comparison of protein stability.

(D) Active YAP induces AMOTL2 mRNA and protein. Vector and YAP-5SA-expressing HMLE cells were analyzed for AMOTL2 expression by western blots and quantitative RT-PCR.

(E) YAP and TEAD1 directly bind to AMOTL2 promoter. HepG2 cells were processed for ChIP with control IgG or antibodies against TEAD1 or YAP followed by quantitative PCR. CTGF was used as a positive control.

(F) YAP activation upon serum stimulation activates Lats1/2. HMLE cells were serum starved overnight and then serum stimulated for the indicated time. Cell lysates were examined by western blots.

(G) YAP activation upon serum stimulation induces Lats2 and AMOTL2 transcription. HMLE cells were serum starved overnight and then serum stimulated for the indicated time. mRNA was extracted and analyzed by quantitative RT-PCR.

(H) Liver-specific *Mst1/2* DKO induces Lats2 and AMOTL2 mRNA transcription. Liver-specific *Mst1* and *Mst2* single or double knockout livers of the indicated genotypes were harvested at two months of age for analysis by quantitative RT-PCR. 1 and 2 are two different mice. Incomplete knockout of *Mst2* is likely due to the presence of non-hepatocytes in livers.

(I) NF2 protein level was elevated in *Mst1/2* DKO livers. Tissues the same as these in (H) were analyzed by western blots.

Supplementary Materials and Methods

Antibodies, plasmids, mice and other materials

Anti-YAP and Sav antibodies were obtained from Santa Cruz Biotechnology. Anti- pYAP (S127), pLats1/2, Mst1, Mst2, GST, Lats1 and Lats2 were obtained from Cell Signaling Technologies. Anti-Lats1, Lats2 and AMOT were obtained from Bethyl laboratories. Anti-TEAD1 antibody was from BD Biosciences. Anti-NF2 was from Abcam. Anti-α-Tubulin, GAPDH and Flag antibodies were obtained from Sigma. Anti-Myc and anti-HA antibodies were obtained from Covance. Anti-pAMOT antibody was previously described[1]. Horseradish peroxidase-conjugated secondary antibodies were obtained from GE Healthcare.

YAP wt and mutants were subcloned into the pLVX-HA lentiviral vector or pQCXIH-Myc retroviral vector. The Act-PB Transposase plasmid was previously described[2]. The PB[CMV-myc-YAP-5SA]DS plasmid was constructed by excising Act-RFP from the PB[Act-RFP]DS plasmid and ligating the corresponding fragments excised from pQCXIH vectors.

Mst1$^{-/-}$*Mst2*$^{F/F}$ mice were from Drs. Yingzi Yang and Zengqiang Yuan. Albumin-Cre mouse line is a gift from Dr. Yong Cang.

Common chemicals were from Sigma or Sangon Biotech.

Cell culture, transfection, and viral infection

HepG2 and MDCK cells were cultured in DMEM (Life Technologies) containing 10% FBS (Life Technologies) and 50μg/mL penicillin/streptomycin (P/S). MCF10A and immortalized human breast epithelial (HMLE) cells were cultured in DMEM/F12 (Life Technologies) supplemented with 5% horse serum (Life Technologies), 20ng/mL EGF, 0.5μg/mL hydrocortisone, 10μg/mL insulin, 100ng/mL cholera toxin, and 50μg/mL P/S. PrEC and RWPE cells were cultured in Clonetics PrEGM prostate epithelial cell growth medium (Lonza).

Transfection of plasmids was performed using Lipofectamine (Life Technologies) according to the manufacturer's instructions.

To generate all sorts of stable cells, lentiviral infection was used. Briefly, HEK293T cells were co-transfected with viral vectors and packaging plasmids. 48

hours after transfection, culture medium was supplemented with 5μg/ml polybrene, filtered through a 0.45μm filter, and used to infect cells of interest. 36 hours after infection, cells were selected with 2μg/ml puromycin in culture medium.

Immunoblotting

Immunoblotting was performed using standard protocol. Briefly, cells were lysed with 1% SDS lysis buffer and protein concentration was determined using the BCA Assay Kit (Pierce). Protein samples were resolved on SDS-PAGE and then transferred to PVDF membranes. The membranes were blocked with 5% BSA and then incubated sequentially with primary and secondary antibodies, then washed. Protein expression was detected by ECL Detection Reagent (Pierce).

Immunoprecipitation and kinase assay

For Lats1/2 kinase assays, cells were lysed with lysis buffer [50mM HEPES (pH 7.5), 150mM NaCl, 1mM EDTA, 1% NP-40, 10mM pyrophosphoate, 10mM glycerophosphate, 50mM NaF, 1.5mM Na_3VO_4, protease inhibitor cocktail (Roche), 1mM DTT, 1mM PMSF], and immunoprecipitated with anti-Lats1 or anti-Lats2 antibodies. The immunoprecipitates were washed 3 times with lysis buffer, followed by once with wash buffer [40mM HEPES, 200mM NaCl] and once with kinase assay buffer [30mM HEPES, 50mM potassium acetate, 5mM $MgCl_2$]. The immunoprecipitates were subjected to a kinase assay in the presence of 500μM ATP and 1μg GST-YAP expressed and purified from *E.coli* as substrate. The reaction mixtures were incubated at 30°C for 30 min, terminated with SDS sample buffer, and subjected to SDS-PAGE and western blots.

Hydrodynamic injection

Male ICR mice of 4-week-old from Shanghai SLAC Laboratory Animal Company were used for injection. For each mouse, 50μg of total transposon plasmids together with 10μg of transposase-expressing plasmids were diluted in sterile Ringer's solution in a volume equal to 10% of body weight. The mixture was injected within

5 to 7 seconds through the tail vein.

Chromatin immunoprecipitation

ChIP assay was performed using the EZ-ChIP kit from Millipore according to the manufacturer's instructions. Briefly, cells were cross-linked, lysed, and sonicated to generate DNA fragments with an average size of 0.5 kb. ChIP was performed using control IgG or 5μg antibodies against YAP or TEAD1. IPs were then washed and eluted. The eluents were then de-crosslinked and DNA was purified for PCR analysis. PCR primers are as follow:

	primer name	sequence	start	stop
hLats2	hLats2-chip-1F	GCAGAATATTGGCTGGAGAC	-1470	
	hLats2-chip-1R	GAAACCATCATCGTGCAGCT		-1356
	hLats2-chip-2F	ACTGTGAATCTTTGCACCAT	-961	
	hLats2-chip-2R	GAAGTATTTTATGGTGTAGGG		-819
	hLats2-chip-3F	CACCAAGACTGGTATGAGATAC	-311	
	hLats2-chip-3R	TTTGACTGGCTGCTATCTGC		-192
hLats1	hLats1-chip-1F	AGGTATCACCATTCCCACAC	-1575	
	hLats1-chip-1R	GGTGGAATGATCAGGAAGAG		-1454
	hLats1-chip-2F	CCGTCACCGGTATTCCAATT	-425	
	hLats1-chip-2R	GTGAGCTTCCAGCATCTGTAT		-276
NF2	hNF2-chip-1F	CCGTTAGAACCCTTGATGAC	-1090	
	hNF2-chip-1R	GGTTGTTGAATTTCAGCGCAC		-914
	hNF2-chip-2F	GGGTCGGTATATGTGATTAAC	-476	
	hNF2-chip-2R	GGGAATTGGGTCCTTCGTT		-343
GAPDH	GAPDH Promoter F	ATCGGGCCAATCTCAGTCCCTTC	-146	
	GAPDH Promoter R	AGGTCTTGAGGCCTGAGCTACG		12
AMOTL2	AMOTL2-chip-1F	ATCACAGCACAGTATATGATTCT	-4715	
	AMOTL2-chip-1R	AGGTGTTATCTACCTCATTCC		-4539
	AMOTL2-chip-2F	GAGGATCTTTCCAGGCAGCA	-3037	
	AMOTL2-chip-2R	CTGGGAAAGAATGGGGTGGG		-2865

	AMOTL2-chip-3F	ATATCCCGTAGCTTGGCACAAA	-641	
	AMOTL2-chip-3R	CAAGCTTAATTGCCAGGGACAT		-474

RNA isolation and real-time PCR

For determining the mRNA expression levels of genes, total RNA was isolated from cultured cells using Trizol reagent (Life Technologies). cDNA was synthesized by reverse transcription using random hexamers and subjected to real-time PCR with gene-specific primers in the presence of SYBR Green (Applied Biosystems). Relative abundance of mRNA was calculated by normalization to hypoxanthine phosphoribosyltransferase 1 (HPRT) mRNA.

Supplementary References

1 Dai X, She P, Chi F *et al.* Phosphorylation of angiomotin by Lats1/2 kinases inhibits F-actin binding, cell migration, and angiogenesis. *J Biol Chem* 2013; **288**:34041-34051.

2 Ding S, Wu X, Li G, Han M, Zhuang Y, Xu T. Efficient transposition of the piggyBac (PB) transposon in mammalian cells and mice. *Cell* 2005; **122**:473-483.