Supplemental Figures. S1-S7

Figure S1. Generation and characterization of the Yap\textsuperscript{S112A} mice.
(A) Gene targeting strategy. Exons and LoxP sites are marked by black blocks and solid arrows, respectively. The S112A mutation is marked by an asterisk in Exon 2. Also shown are PCR primers used for genotyping.
(B) PCR genotyping of mice with the targeted allele. Primers PKI5F and PKI5R amplify a PCR product of ~ 4.5 kb from the 5’ region; primers PKI3F and PKI3R amplify a PCR product of ~ 4.4 kb from the 3’ region. See (A) for schematic position of the PCR primers.
(C) PCR analysis of mouse tail DNA from the indicated genotypes. The positions of amplified fragments corresponding to the wild type and the Yap\textsuperscript{S112A} knockin alleles are indicated.
(D) Sequencing analysis of the indicated genotypes showing the expected Ser-to-Ala codon change at S112 of YAP.
(E) Quantification of colonic crypt width (4-month-old) in wildtype and Yap\textsuperscript{S112A} distal colons reveals no difference between two groups. N=5. Data are mean ± SD.
(F) Immunostaining of colon sections showing comparable Ki67-staining restricted to the crypt base cells (arrows) in wildtype and Yap\textsuperscript{S112A} mice. Scale bar = 50 μm.
Figure S1

A

Targeting Vector

Genomic Locus

Targeted Allele

S112A  KI Allele

B

MWT

5' (PKI5F/PKI5R) 3' (PKI3F/PKI3R)

4.5kb 4.4kb

M Targeted

Forward Reverse

S112A

C

KI Allele

Targeting Vector

Genomic Locus

Targeted Allele

S112A  KI Allele

CRE recombinase

D

Forward Reverse

S112A  S112A

S112A  +

+/+

S112A/S112A

E

Crypt width

+/+  S112A/S112A

F

Ki67

+/+  S112A/S112A
Figure S2. Quantification of mRNA in wildtype and $Yap^{S112A}$ MEFs and livers.

(A) Real-time PCR analysis of $Birc5$, $Cyr61$, $Ctgf$, $Yap$ and $Taz$ mRNA levels in wildtype and $Yap^{S112A}$ MEFs. Data are mean ±SD. N=3 for each genotype. (*) P<0.05, $t$-test.

(B) Real-time PCR analysis of $NF2$, $Mst1$, $Mst2$, $Lats1$, $Lats2$ and $Sav1$ mRNA levels in wildtype and $Yap^{S112A}$ liver tissues. Data are mean ±SD. N=3 for each genotype. (*) P<0.05, $t$-test.
Figure S2

A

**Relative mRNA levels**

- **Birc5**: +/+ S112A/S112A  vs. +/+ S112A/S112A
- **Cyr61**: +/+ S112A/S112A  vs. +/+ S112A/S112A
- **Ctgf**: +/+ S112A/S112A  vs. +/+ S112A/S112A
- **Yap**: +/+ S112A/S112A  vs. +/+ S112A/S112A
- **Taz**: +/+ S112A/S112A  vs. +/+ S112A/S112A

B

**Relative mRNA levels**

- **Nf2**: +/+ S112A/S112A  vs. +/+ S112A/S112A
- **Mst1**: +/+ S112A/S112A  vs. +/+ S112A/S112A
- **Mst2**: +/+ S112A/S112A  vs. +/+ S112A/S112A
- **Lats1**: +/+ S112A/S112A  vs. +/+ S112A/S112A
- **Lats2**: +/+ S112A/S112A  vs. +/+ S112A/S112A
- **Sav1**: +/+ S112A/S112A  vs. +/+ S112A/S112A
Figure S3. Generation of *Lats2*^floxc^ mice and characterization of *Lats1/2;Yap;Taz* mutant livers.

(A) Gene targeting strategy. Exon 5 and 6 of the *Lats2* gene are indicated by black blocks. LoxP and FRT sites are indicated by black and blue arrows, respectively. Also shown are the neomycin resistance gene (Neo) and PCR primers used for genotyping. (B) Long-template PCR genotyping of mice with the targeted allele. Primers PL5F and PL5R amplify a PCR product of ~4.4 kb from the 5’ region; primers PL3F and PL3R amplify a PCR product of ~4.5 kb from the 3’ region. See (A) for schematic position of the PCR primers.

(C) H&E, Pan-CK, and YAP/TAZ staining of serial liver sections from *Lats1*^-/-^; *Lats2*^floxcflax^ and *Lats1*^-/-^; *Lats2*^floxcflax^, *Yap*^floxcflax^, *Taz*^floxcflax^ mice 8 weeks after Adeno-Cre injection. Note that the small clusters of CK-positive cells remaining in the Adeno-Cre; *Lats1*^-/-^; *Lats2*^floxcflax^, *Yap*^floxcflax^, *Taz*^floxcflax^ liver (arrows) were positive for YAP/TAZ staining (arrows) indicating that they were escaper cells that failed to delete *Yap/Taz*. Scale bar = 100 µm.
Figure S3

A

Conditional Targeting Vector

Genomic Locus

Targeted Allele

Flox Allele

KO Allele

Homologous Recombination

Flp recombinase

Cre recombinase

B

M WT WT WT WT WT WT WT WT WT WT Targeted WT WT WT 3' (PL3F/PL3R)

4.4kb → 4.5kb

C

Lats1/2 Lats1/2; Yap; Taz

Pan-CK Pan-CK

YAP/TAZ YAP/TAZ
Figure S4. S112 is required for LatB-induced cytoplasmic translocation of YAP in MEFs.

Wildtype and $Yap^{S112A/S112A}$ MEFs treated with vehicle (ethanol, control) or LatB (10μm for 1h) were immunostained for endogenous YAP (green), actin (red) and nuclear dye DAPI (blue). Endogenous YAP shows nuclear-to-cytoplasmic translocation after LatB treatment in the wildtype MEFs, but not in the $Yap^{S112A/S112A}$ MEFs. Scale bar = 20 μm.
Figure S4

|          | YAP | Actin | DAPI | Merge |
|----------|-----|-------|------|-------|
| **WT MEF** | ![Image](image1.png) | ![Image](image2.png) | ![Image](image3.png) | ![Image](image4.png) |
| **Control** | ![Image](image5.png) | ![Image](image6.png) | ![Image](image7.png) | ![Image](image8.png) |
| **WT MEF** | ![Image](image9.png) | ![Image](image10.png) | ![Image](image11.png) | ![Image](image12.png) |
| **LatB (1µg/ml, 1h)** | ![Image](image13.png) | ![Image](image14.png) | ![Image](image15.png) | ![Image](image16.png) |
| **S112A MEF** | ![Image](image17.png) | ![Image](image18.png) | ![Image](image19.png) | ![Image](image20.png) |
| **Control** | ![Image](image21.png) | ![Image](image22.png) | ![Image](image23.png) | ![Image](image24.png) |
| **S112A MEF** | ![Image](image25.png) | ![Image](image26.png) | ![Image](image27.png) | ![Image](image28.png) |
| **LatB (1µg/ml, 1h)** | ![Image](image29.png) | ![Image](image30.png) | ![Image](image31.png) | ![Image](image32.png) |
Figure S5. S112 is required for epinephrine-induced cytoplasmic translocation of YAP in MEFs.

Wildtype and Yap$^{S112A/S112A}$ MEFs treated with vehicle (DMSO, control) or epinephrine (10μm for 1h) were immunostained for endogenous YAP (green), actin (red) and nuclear dye DAPI (blue). Endogenous YAP shows nuclear-to-cytoplasmic translocation after Epinephrine treatment in the wildtype MEFs, but not in the Yap$^{S112A/S112A}$ MEFs. Scale bar = 20 μm.
Figure S5

YAP  Actin  DAPI  Merge

WT MEF Control

WT MEF epinephrine (10µM, 1h)

S112A MEF Control

S112A MEF epinephrine (10µM, 1h)
Figure S6. S112 is required for forskolin-induced cytoplasmic translocation of YAP in MEFs.

Wildtype and $Yap^{S112A/S112A}$ MEFs treated with vehicle (DMSO, control) or forskolin (10μm for 1h) were immunostained for endogenous YAP (green), actin (red) and nuclear dye DAPI (blue). Endogenous YAP shows nuclear-to-cytoplasmic translocation after forskolin treatment in the wildtype MEFs, but not in the $Yap^{S112A/S112A}$ MEFs. Scale bar = 20 μm.
Figure S6

WT MEF
Control

WT MEF
Forskolin
(10μM, 1h)

S112A MEF
Control

S112A MEF
Forskolin
(10μM, 1h)
Figure S7. S112 is required for Y-27632 induced cytoplasmic translocation of YAP in MEFs.
Wildtype and Yap<sup>S112A/S112A</sup> MEFs treated with vehicle (DMSO, control) or Y-27632 (10μm for 2h) were immunostained for endogenous YAP (green), actin (red) and nuclear dye DAPI (blue). Endogenous YAP shows nuclear-to-cytoplasmic translocation after Y-27632 treatment in the wildtype MEFs, but not in the Yap<sup>S112A/S112A</sup> MEFs. Scale bar = 20 μm.
