Supplemental Figures. S1-S7

Figure S1. Generation and characterization of the *Yap*^{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.

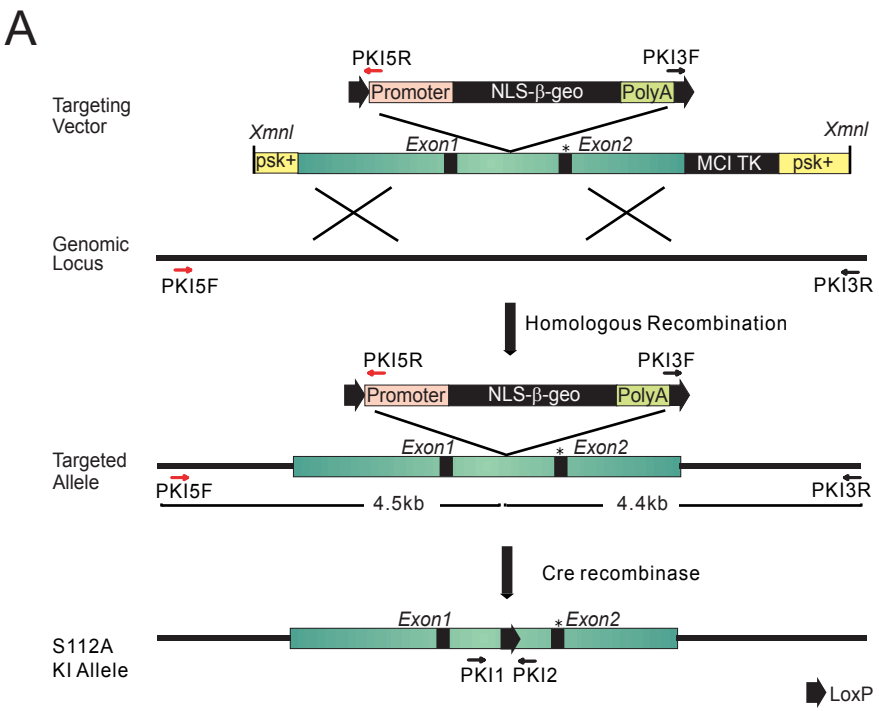
(C) PCR analysis of mouse tail DNA from the indicated genotypes. The positions of amplified fragments corresponding to the wild type and the *Yap*^{S112A} knockin alleles are indicated.

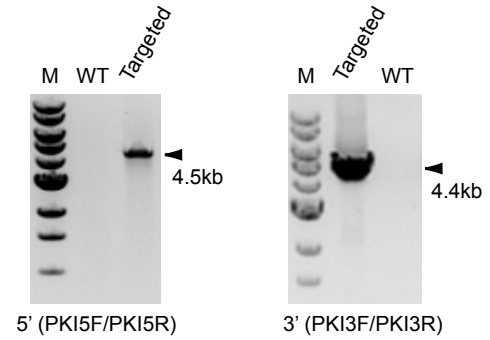
(D) Sequencing analysis of the indicated genotypes showing the expected Ser-to-Ala codon change at S112 of YAP.

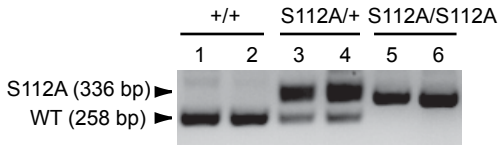
(E) Quantification of colonic crypt width (4-month-old) in wildtype and *Yap*^{S112A} distal colons reveals no difference between two groups. N=5. Data are mean ± SD.

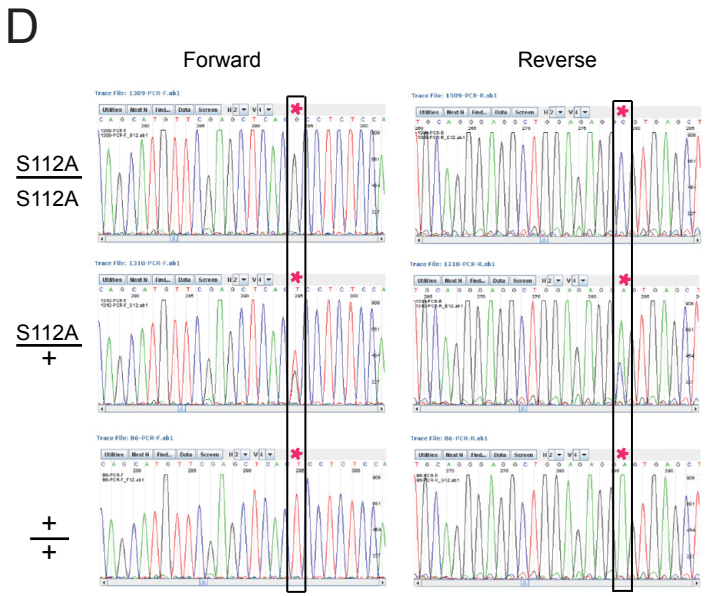
(F) Immunostaining of colon sections showing comparable Ki67-staining restricted to the crypt base cells (arrows) in wildtype and *Yap*^{S112A} mice. Scale bar = 50 μm.

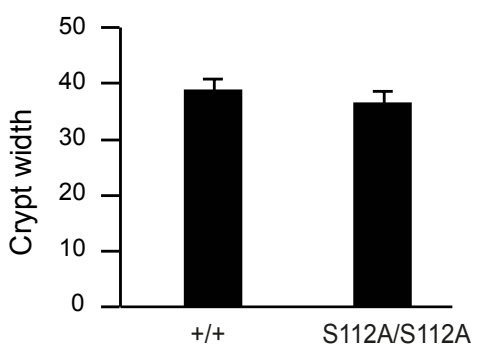
Figure S1


A
PKI5R
PKI3F
Promoter
NLS-β-geo
PolyA
Targeting Vector
XmnI
Exon1
Exon2
XmnI
psk+
MCI TK
psk+
Genomic Locus
PKI5F
PKI3R
Homologous Recombination
PKI5R
PKI3F
Promoter
NLS-β-geo
PolyA
Targeted Allele
Exon1
Exon2
PKI5F
4.5kb
4.4kb
PKI3R
Cre recombinase
Exon1
Exon2
S112A KI Allele
PKI1
PKI2
LoxP


B
M
WT
Targeted
4.5kb
5' (PKI5F/PKI5R)
M
Targeted
WT
4.4kb
3' (PKI3F/PKI3R)


C
+/+
S112A/+
S112A/S112A
1
2
3
4
5
6
S112A (336 bp)
WT (258 bp)


D
Forward
Reverse
S112A
S112A
S112A
+
+
+


E
Crypt width
50
40
30
20
10
0
+/+
S112A/S112A

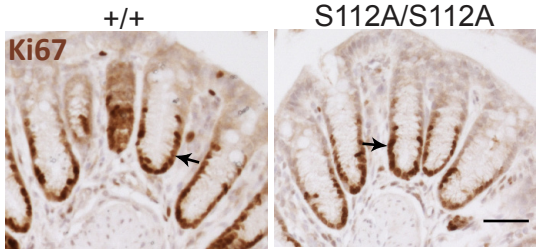

F
+/+
S112A/S112A
Ki67

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

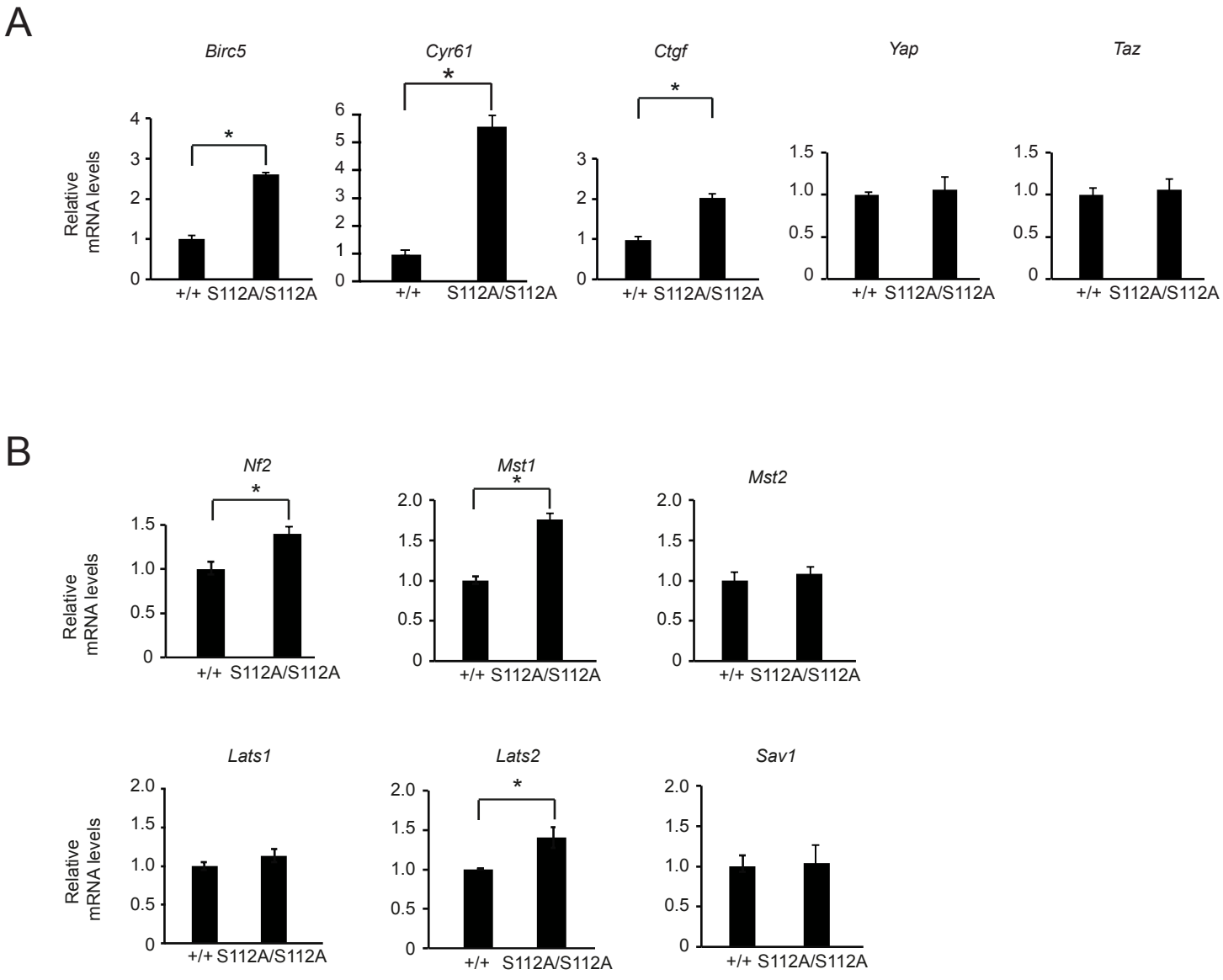

A
Birc5
Cyr61
Ctgf
Yap
Taz
Relative mRNA levels
+/+ S112A/S112A
B
Nf2
Mst1
Mst2
Lats1
Lats2
Sav1

Figure S3. Generation of *Lats2*^{flox} 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*$^{flox/flox}$ and *Lats1*$^{-/-}$*; Lats2*$^{flox/flox}$; *Yap*$^{flox/flox}$; *Taz*$^{flox/flox}$ mice 8 weeks after Adeno-Cre injection. Note that the small clusters of CK-positive cells remaining in the Adeno-Cre; *Lats1*$^{-/-}$*; Lats2*$^{flox/flox}$; *Yap*$^{flox/flox}$; *Taz*$^{flox/flox}$ 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

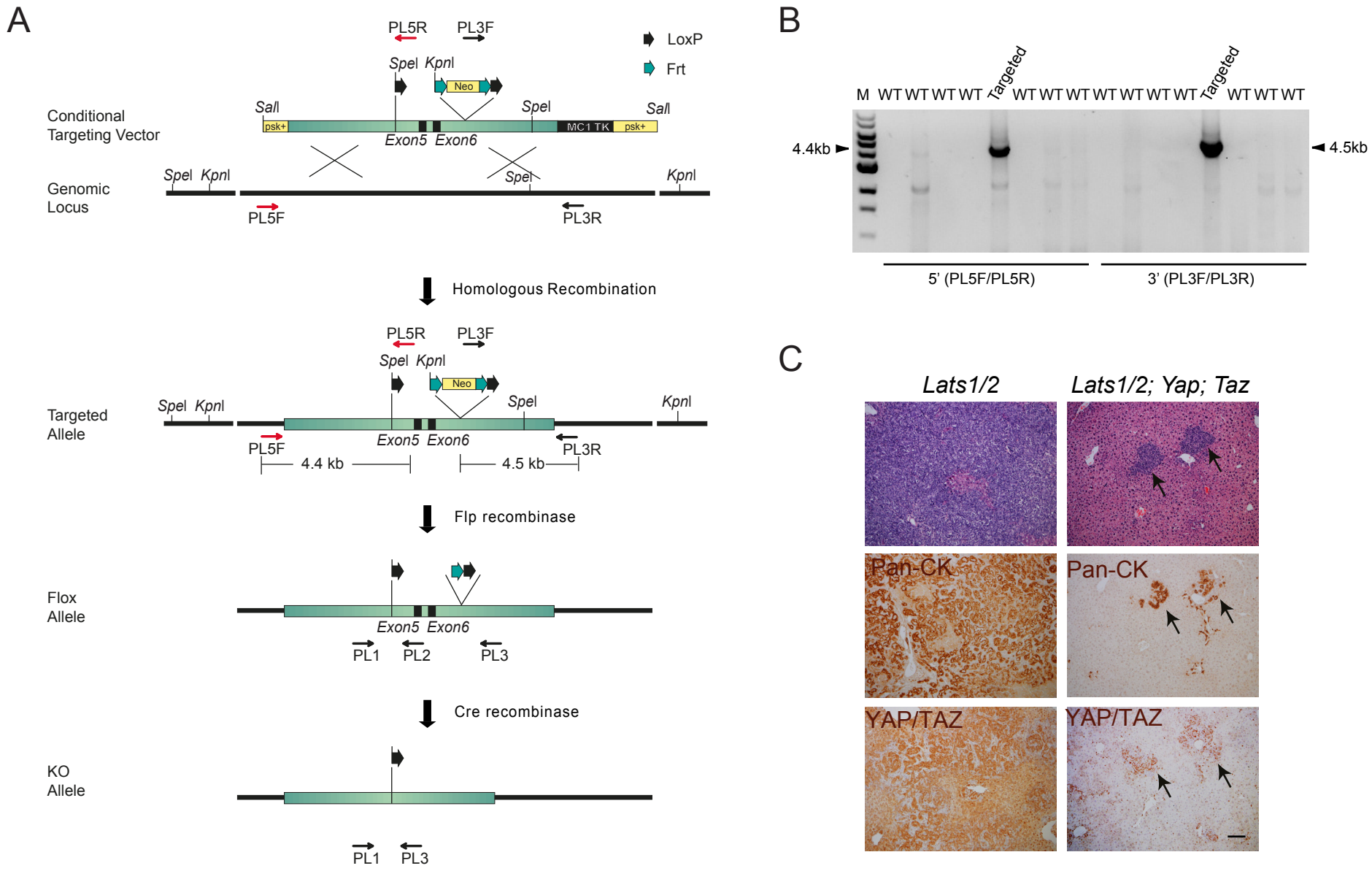

A
PL5R
PL3F
LoxP
Frt
SpeI KpnI
Neo
SalI
SpeI
SalI
Conditional
Targeting Vector
psk+
MC1 TK
psk+
Exon5 Exon6
SpeI KpnI
SpeI
KpnI
Genomic
Locus
PL5F
PL3R
Homologous Recombination
PL5R
PL3F
SpeI KpnI
Neo
Targeted
Allele
SpeI KpnI
SpeI
KpnI
PL5F
Exon5 Exon6
PL3R
4.4 kb
4.5 kb
Flp recombinase
Flox
Allele
Exon5 Exon6
PL1
PL2
PL3
Cre recombinase
KO
Allele
PL1
PL3
B
M WT WT WT WT Targeted WT WT WT WT WT WT WT Targeted WT WT WT
4.4kb
4.5kb
5' (PL5F/PL5R)
3' (PL3F/PL3R)
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

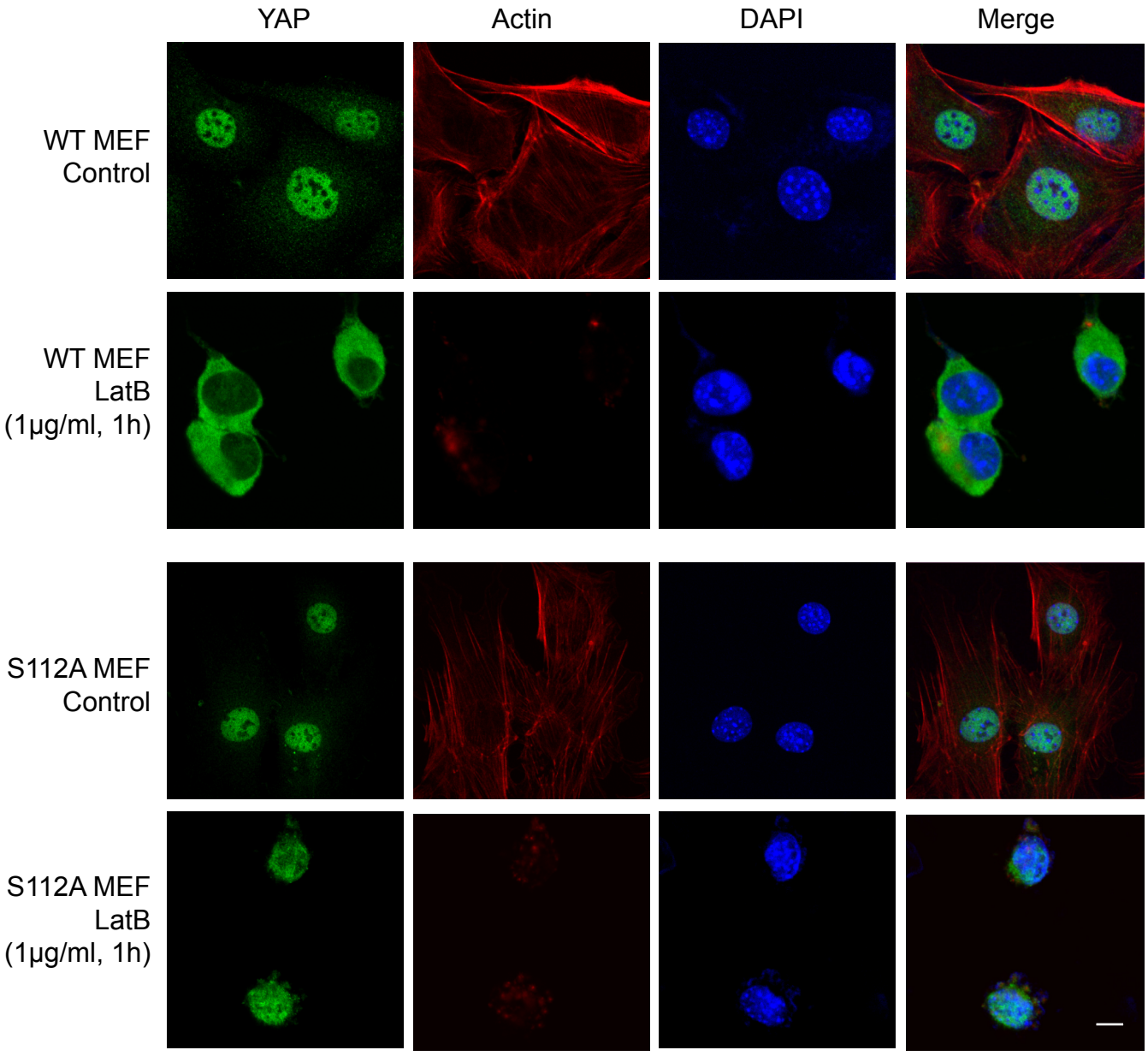

YAP
Actin
DAPI
Merge
WT MEF
Control
WT MEF
LatB
(1µg/ml, 1h)
S112A MEF
Control
S112A MEF
LatB
(1µg/ml, 1h)

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

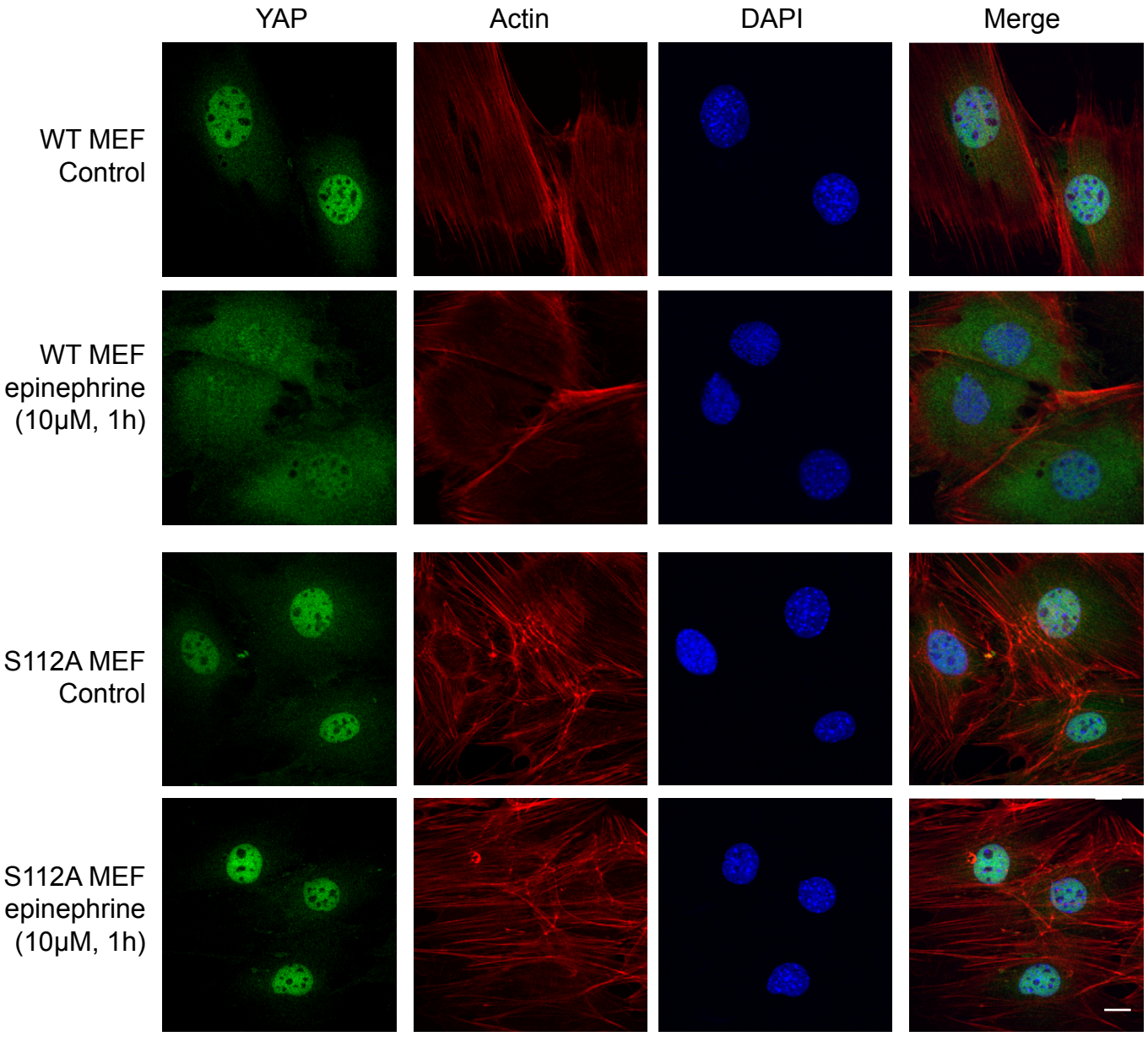

YAP
Actin
DAPI
Merge
WT MEF
Control
WT MEF
epinephrine
(10μM, 1h)
S112A MEF
Control
S112A MEF
epinephrine
(10μM, 1h)

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

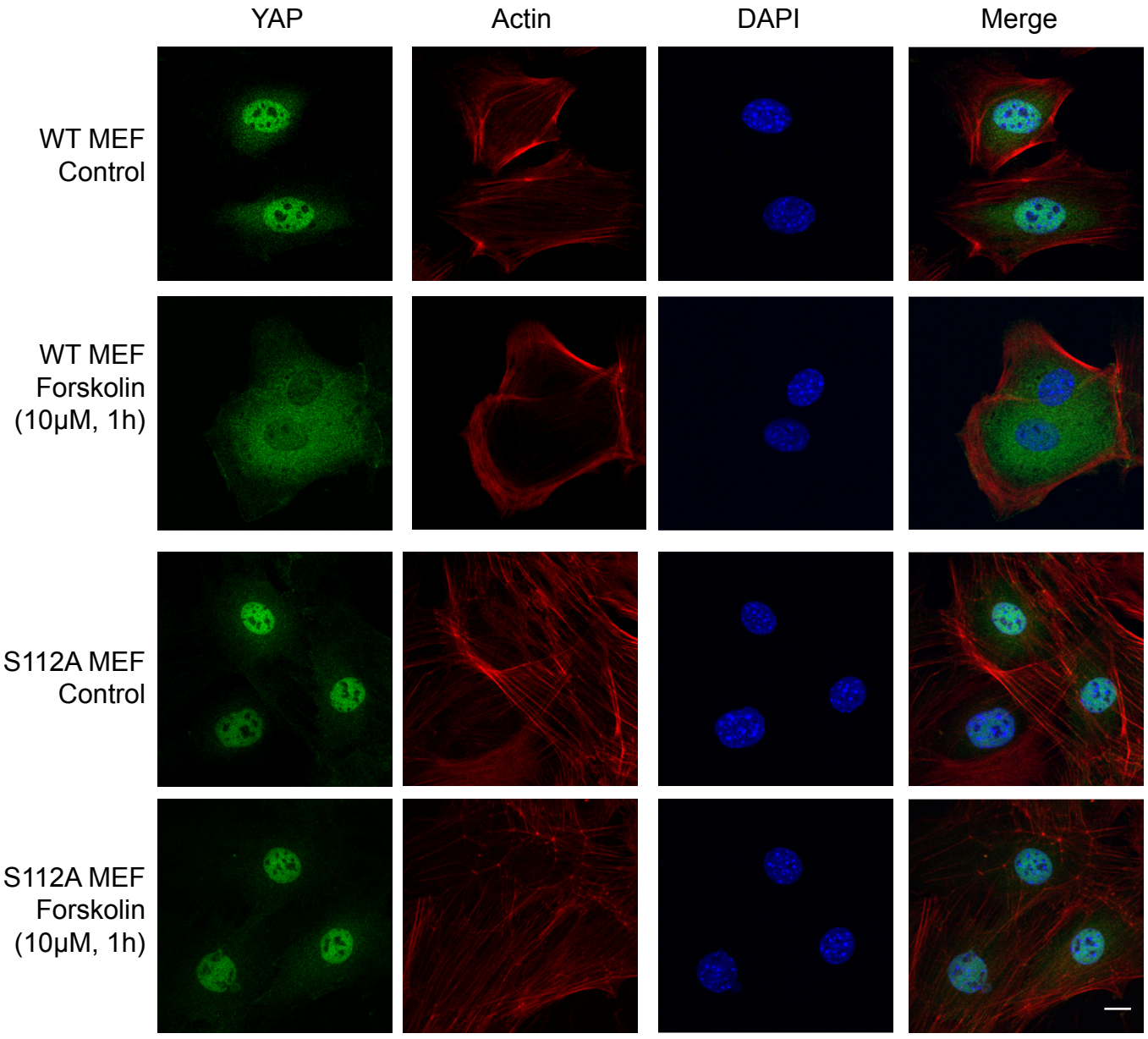
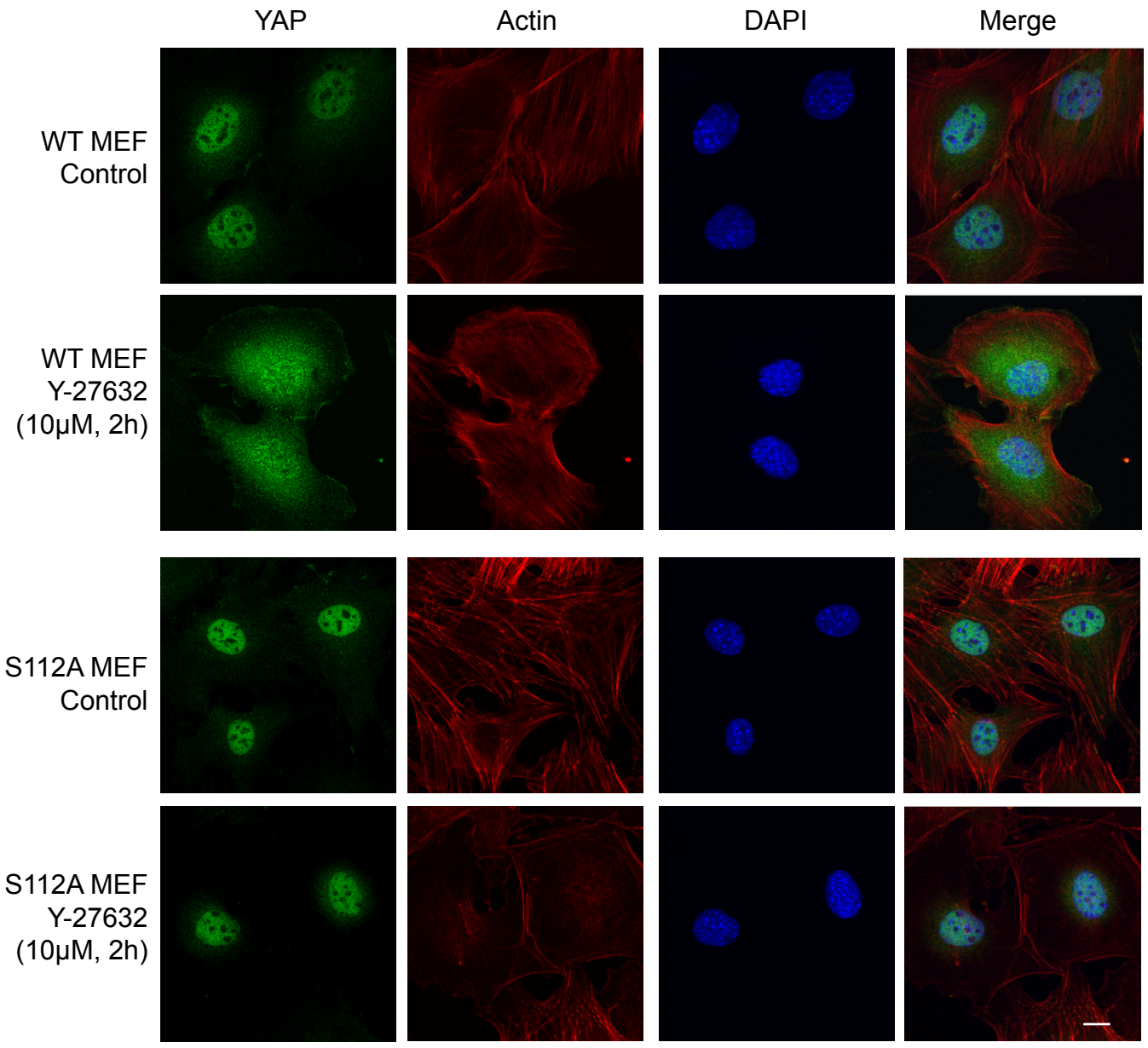

YAP
Actin
DAPI
Merge
WT MEF
Control
WT MEF
Forskolin
(10μM, 1h)
S112A MEF
Control
S112A MEF
Forskolin
(10μM, 1h)

Figure S7. S112 is required for Y-27632 induced cytoplasmic translocation of YAP in MEFs.

Wildtype and *Yap*$^{S112A/S112A}$ 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*$^{S112A/S112A}$ MEFs. Scale bar = 20 μm.

Figure S7


YAP
Actin
DAPI
Merge
WT MEF
Control
WT MEF
Y-27632
(10μM, 2h)
S112A MEF
Control
S112A MEF
Y-27632
(10μM, 2h)