Figure S1

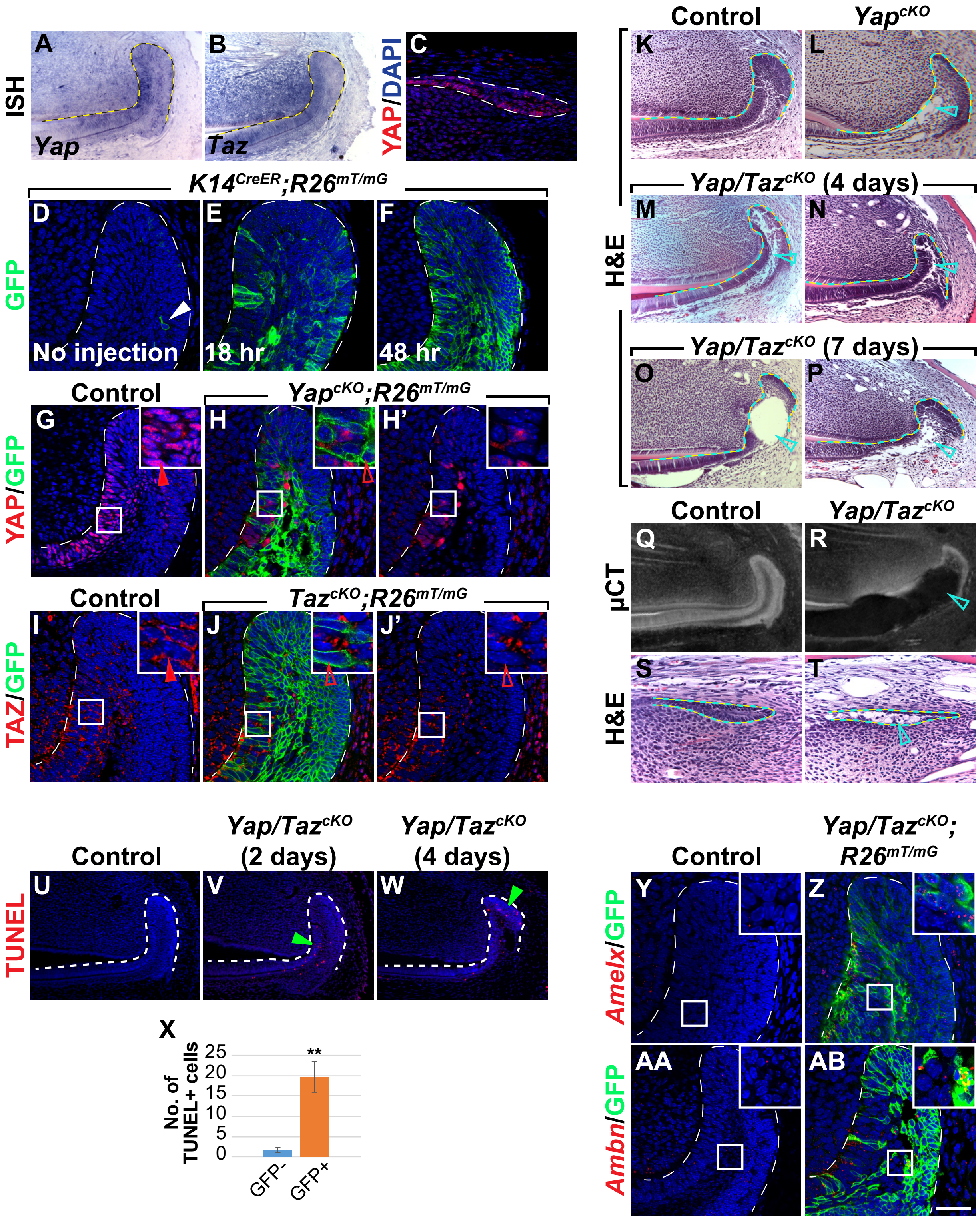
ISH
A
Yap
B
Taz
C
YAP/DAPI
K14CreER;R26mT/mG
GFP
D
No injection
E
18 hr
F
48 hr
Control
YapcKO;R26mT/mG
YAP/GFP
G
H
H’
Control
TazcKO;R26mT/mG
TAZ/GFP
I
J
J’
Control
YapcKO
K
L
H&E
Yap/TazcKO (4 days)
M
N
Yap/TazcKO (7 days)
O
P
Control
Yap/TazcKO
μCT
Q
R
H&E
S
T
Control
Yap/TazcKO (2 days)
Yap/TazcKO (4 days)
TUNEL
U
V
W
X
No. of TUNEL+ cells
25
20
15
10
5
0
**
GFP-
GFP+
Control
Yap/TazcKO; R26mT/mG
Amelx/GFP
Y
Z
Ambn/GFP
AA
AB

Figure S1. Expression of *Yap*/*Taz* and their functions. (related to Figures 1 and 2)

(A and B) *Yap* and *Taz in situ* hybridization.

(C) YAP immunostaining in the lingual CL (liCL).

(D-F) K14CreER activity in uninduced laCLs (D) and in tamoxifen-induced laCLs at 18 and 48 hours post injection (E and F), as indicated by the expression of the Cre-responsive reporter allele, $R26^{mT/mG}$. White arrowhead in (D) marks the occasional GFP-positive cells due to CreER leakage in uninduced laCLs.

(G-J') K14CreER ablates YAP and TAZ effectively. Closed and open red arrowheads, respectively, mark normal and reduced immunostaining. GFP labels Cre active cells.

(K and L) 3 out of 12 Yap^{cKO} samples exhibit tissue loss in the laCL (open cyan arrowhead) one week after Cre induction.

(M and N) 4 days after Cre induction, TA and SR regions begin to disintegrate in Yap/Taz^{cKO} laCLs (open cyan arrowheads).

(O and P) 7 days after Cre induction, a large hole develops in Yap/Taz^{cKO} laCLs (open cyan arrowheads).

(Q and R) μCT analysis confirms that one week after Cre induction, a large hole is formed in the Yap/Taz^{cKO} dental epithelium (open cyan arrowhead in R).

(S and T) liCL similarly disintegrates in the absence of *Yap*/*Taz* (open cyan arrowhead).

(U-W) Time course of TUNEL staining in Yap/Taz^{cKO} laCLs. Green arrowheads mark increased TUNEL signals.

(X) Quantification of number of TUNEL+ cells in GFP+ and GFP- population in Yap/Taz^{cKO}.

(Y-AB) GFP immunostaining and fluorescent *in situ* hybridization of *Amelx* and *Ambn* show increased *Amelx* and *Ambn* transcripts in Cre-recombined Yap/Taz^{cKO} cells.

Representative images are shown. Dashed lines outline the dental epithelium. Scale bar in AB represents 130 μm in (A, B, K-R, and U-W) and 50 μm in (C-J', S, T, and Y-AB).

Figure S2

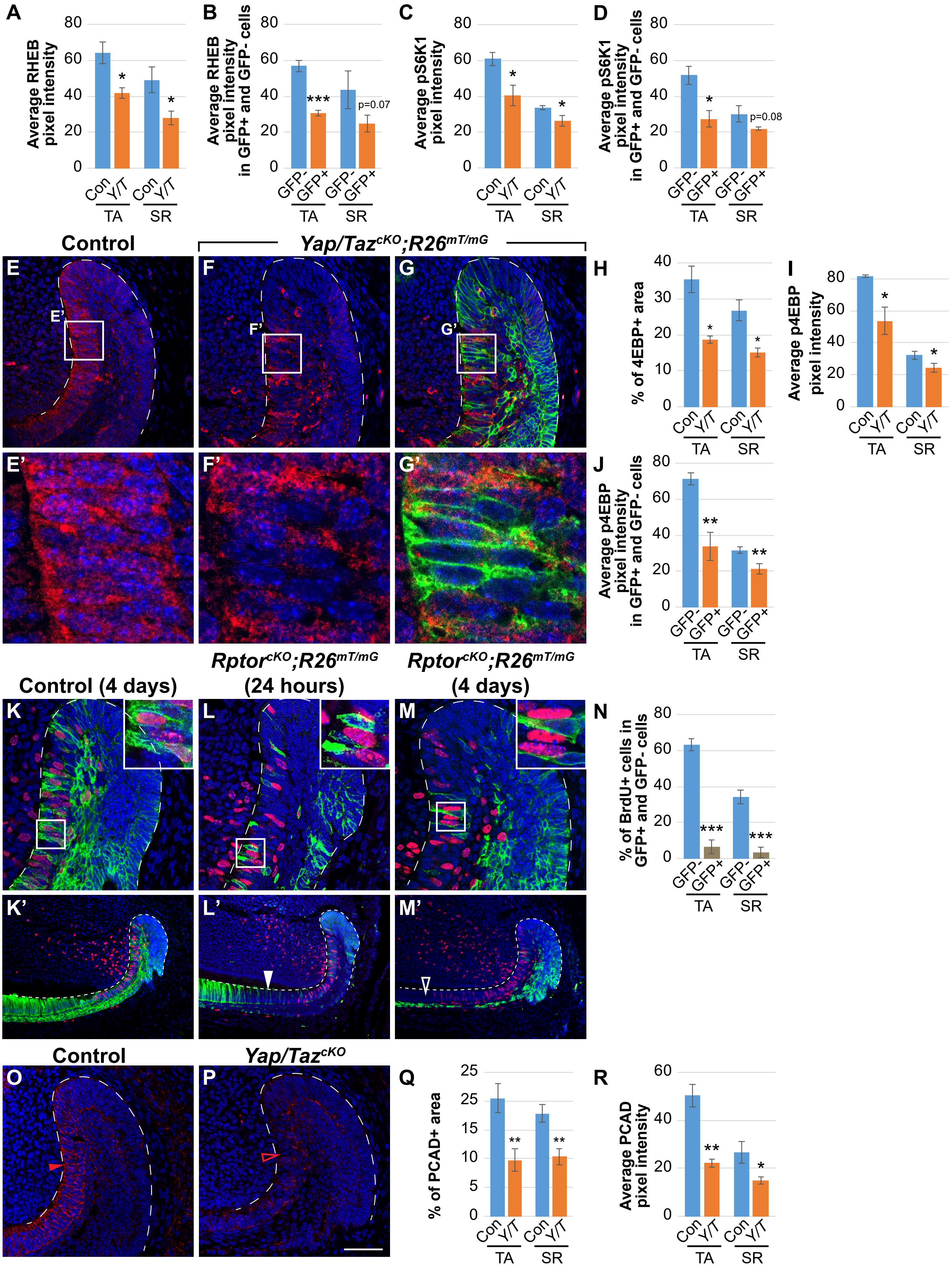
A
Average RHEB pixel intensity
80
60
40
20
0
*
*
Con
Y/T
Con
Y/T
TA
SR
B
Average RHEB pixel intensity in GFP+ and GFP- cells
***
p=0.07
GFP-
GFP+
GFP-
GFP+
TA
SR
C
Average pS6K1 pixel intensity
*
*
Con
Y/T
Con
Y/T
TA
SR
D
Average pS6K1 pixel intensity in GFP+ and GFP- cells
*
p=0.08
GFP-
GFP+
GFP-
GFP+
TA
SR
Control
Yap/Taz^cKO;R26^mT/mG
E
E'
F
F'
G
G'
p4EBP/GFP/DAPI
H
% of 4EBP+ area
40
30
20
10
0
*
*
Con
Y/T
Con
Y/T
TA
SR
I
Average p4EBP pixel intensity
80
60
40
20
0
*
*
Con
Y/T
Con
Y/T
TA
SR
J
Average p4EBP pixel intensity in GFP+ and GFP- cells
80
60
40
20
0
**
**
GFP-
GFP+
GFP-
GFP+
TA
SR
Control (4 days)
Rptor^cKO;R26^mT/mG (24 hours)
Rptor^cKO;R26^mT/mG (4 days)
K
L
M
K'
L'
M'
BrdU/GFP/DAPI
N
% of BrdU+ cells in GFP+ and GFP- cells
80
60
40
20
0
***
***
GFP-
GFP+
GFP-
GFP+
TA
SR
Control
Yap/Taz^cKO
O
P
PCAD/DAPI
Q
% of PCAD+ area
25
25
15
10
5
0
**
**
Con
Y/T
Con
Y/T
TA
SR
R
Average PCAD pixel intensity
60
40
20
0
**
*
Con
Y/T
Con
Y/T
TA
SR

Figure S2. Gene expression and mTOR signaling downstream of YAP/TAZ. (related to Figure 3)

(A-D) Average pixel intensity of RHEB and pS6K1 immunostaining between control and *Yap/Taz*^{cKO} laCLs (A and C) and average pixel intensity of RHEB and pS6K1 immunostaining between Cre-recombined (GFP+) and non-recombined (GFP-) cells in *Yap/Taz*^{cKO} laCLs (B and D) are compared.

(E-J) p4EBP is normally expressed in TA and inner SR cells (E and E') but downregulated when *Yap* and *Taz* are deleted (F-G'). The percentage of p4EBP positive area in TA and inner SR regions (H), average pixel intensity of p4EBP immunostaining between control and *Yap/Taz*^{cKO} laCLs (I), and average pixel intensity of p4EBP immunostaining between Cre-recombined (GFP+) and non-recombined (GFP-) cells in *Yap/Taz*^{cKO} laCLs (J) are quantified.

(K-N) BrdU labeling and GFP lineage tracing in *Rptor*^{cKO} laCLs reveals the absolute requirement for *Rptor* in expanding dental progenitor cells. GFP-positive Cre-recombined cells are mostly non-proliferative; this is quantified in (N). Closed white arrowhead in L' marks the proximal boundary of Cre-positive cells and open white arrowhead in M' marks the dramatic loss of GFP+ *Rptor*-deleted progeny in *Rptor*^{cKO} laCLs.

(O-R) P-Cadherin (PCAD) is reduced in *Yap/Taz*^{cKO} TA and inner SR cells (open red arrowhead in P) when compared to the control (red arrowhead in O). The corresponding quantification is shown in (Q and R).

Representative images and quantitative data are shown. Dashed lines outline laCLs. Scale bar in P represents 50 μm in (E-G, K-M, O and P), 9.76 μm in (E'-G'), and 130 μm in (K'-M'). All data are presented as mean ± SD. *p<0.05; **p<0.01; ***p<0.001.

Figure S3

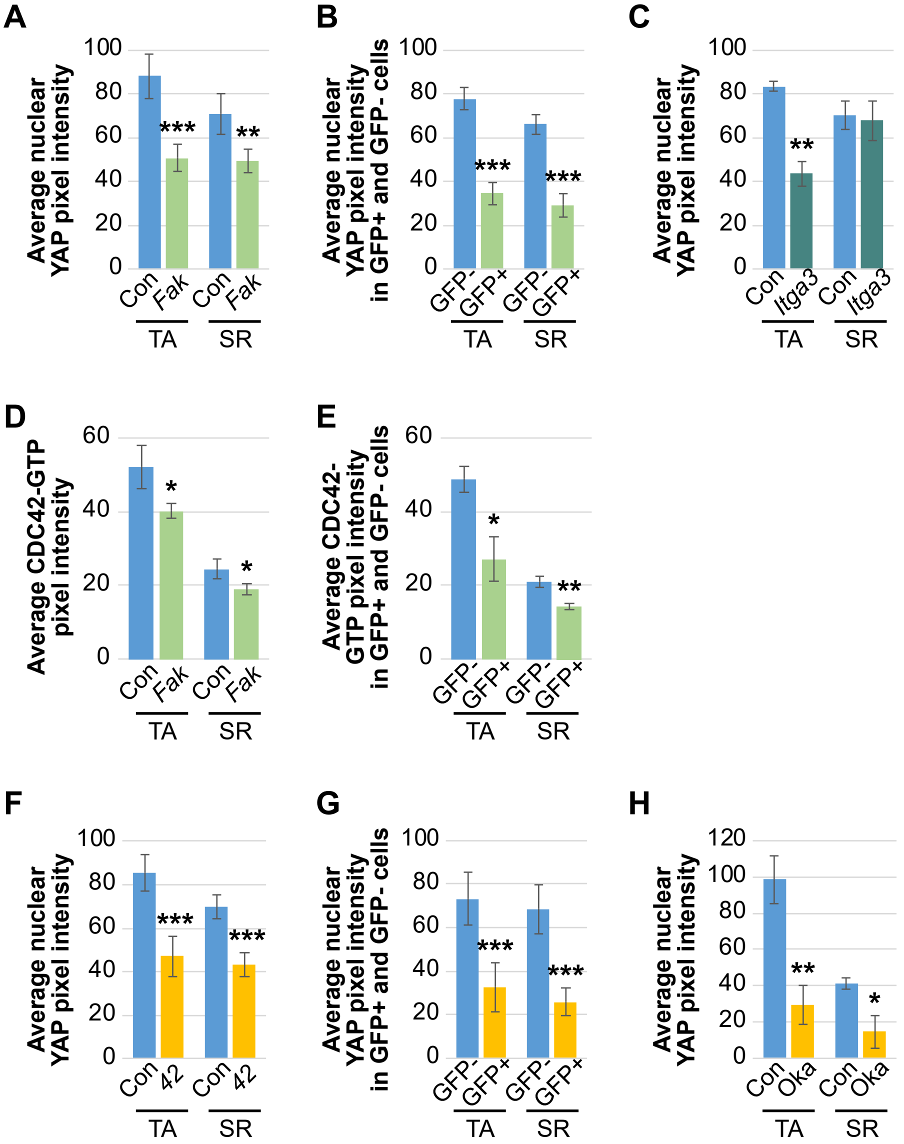
A
Average nuclear YAP pixel intensity
100
80
60
40
20
0
***
**
Con
Fak
Con
Fak
TA
SR
B
Average nuclear YAP pixel intensity in GFP+ and GFP- cells
100
80
60
40
20
0
***
***
GFP-
GFP+
GFP-
GFP+
TA
SR
C
Average nuclear YAP pixel intensity
100
80
60
40
20
0
**
Con
Itga3
Con
Itga3
TA
SR
D
Average CDC42-GTP pixel intensity
60
40
20
0
*
*
Con
Fak
Con
Fak
TA
SR
E
Average CDC42-GTP pixel intensity in GFP+ and GFP- cells
60
40
20
0
*
**
GFP-
GFP+
GFP-
GFP+
TA
SR
F
Average nuclear YAP pixel intensity
100
80
60
40
20
0
***
***
Con
42
Con
42
TA
SR
G
Average nuclear YAP pixel intensity in GFP+ and GFP- cells
100
80
60
40
20
0
***
***
GFP-
GFP+
GFP-
GFP+
TA
SR
H
Average nuclear YAP pixel intensity
120
100
80
60
40
20
0
**
*
Con
Oka
Con
Oka
TA
SR

Figure S3. Quantification of YAP and CDC42-GTP immunostaining. (related to Figures 4,5, and 6)

(A and B) Average pixel intensity of nuclear YAP between control and *Fak*^{cKO} IaCLs (A) and average pixel intensity of nuclear YAP between Cre-recombined (GFP+) and non-recombined (GFP-) cells in *Fak*^{cKO} IaCLs (B) are compared.

(C) Average pixel intensity of nuclear YAP in control and *Itga3*^{cKO} IaCLs is quantified.

(D and E) Average pixel intensity of CDC42-GFP between control and *Fak*^{cKO} IaCLs (D) and average pixel intensity of CDC42-GFP between Cre-recombined (GFP+) and non-recombined (GFP-) cells in *Fak*^{cKO} IaCLs (E) are compared.

(F and G) Average pixel intensity of nuclear YAP between control and *Cdc42*^{cKO} IaCLs (F) and average pixel intensity of nuclear YAP between Cre-recombined (GFP+) and non-recombined (GFP-) cells in *Cdc42*^{cKO} IaCLs (G) are compared.

(H) Average pixel intensity of nuclear YAP in control and okadaic acid-treated IaCLs is quantified.

All data are presented as mean ± SD. $^{*}p<0.05$; $^{**}p<0.01$; $^{***}p<0.001$.

Figure S4

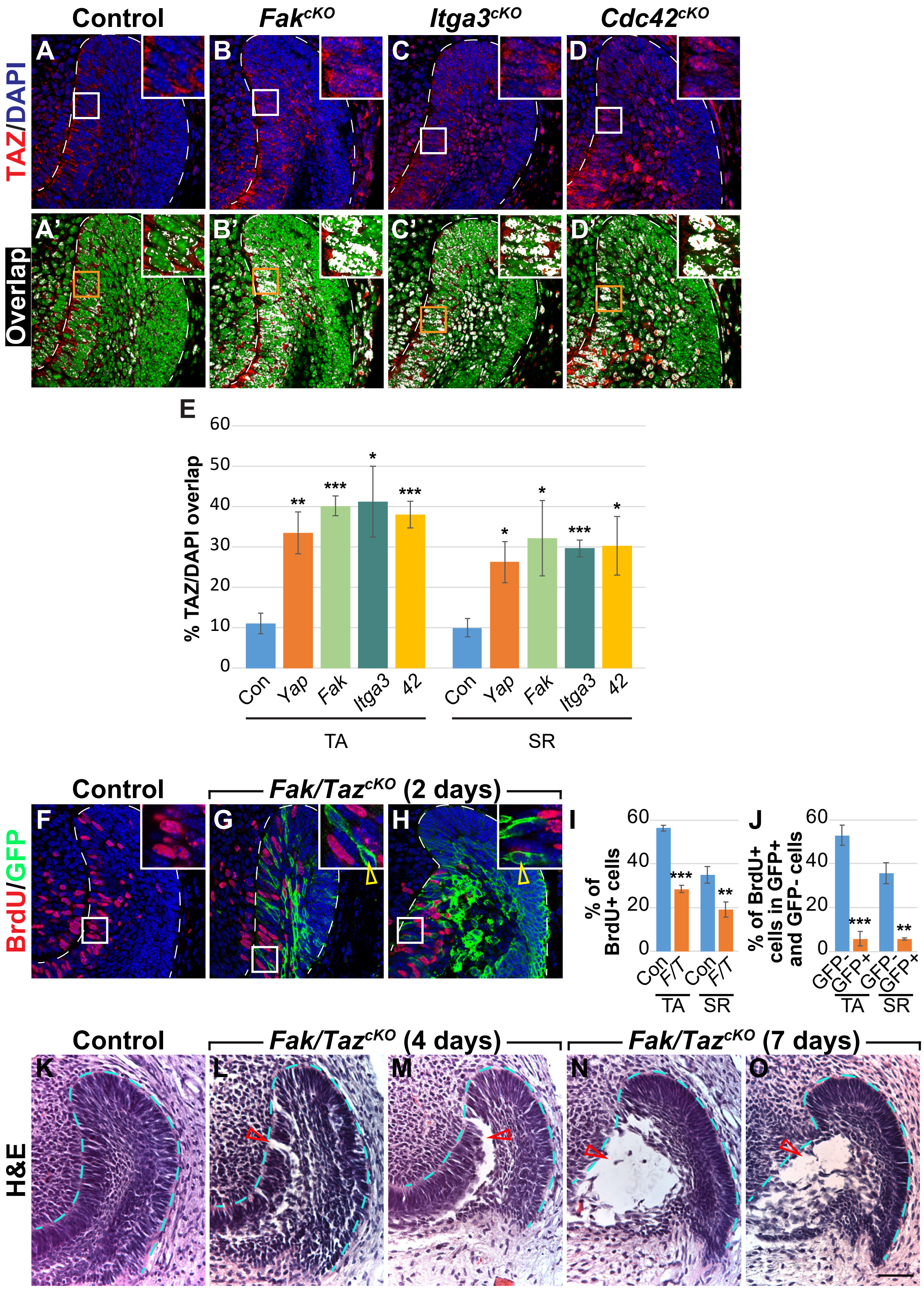
Control
Fak^{cKO}
Itga3^{cKO}
Cdc42^{cKO}
TAZ/DAPI
A
B
C
D
TAZ/DAPI Overlap
A'
B'
C'
D'
E
% TAZ/DAPI overlap
60
50
40
30
20
10
0
**
***
*
***
*
*
***
*
Con
Yap
Fak
Itga3
42
Con
Yap
Fak
Itga3
42
TA
SR
Control
Fak/Taz^{cKO} (2 days)
BrdU/GFP
F
G
H
I
% of BrdU+ cells
60
40
20
0
***
**
Con
F/T
Con
F/T
TA
SR
J
% of BrdU+ cells in GFP+ and GFP- cells
60
40
20
0
***
**
GFP-
GFP+
GFP-
GFP+
TA
SR
Control
Fak/Taz^{cKO} (4 days)
Fak/Taz^{cKO} (7 days)
H&E
K
L
M
N
O

Figure S4. Expression of TAZ in different mutants. (related to Figures 4 and 5)

(A-D') TAZ is normally restricted in the cytoplasm in the laCL (A and A') but translocates to the nucleus in *Fak*^{cKO} (B and B'), *Itga3*^{cKO} (C and C'), and *Cdc42*^{cKO} (D and D') mutant laCLs.

(E) Quantification of TAZ/DAPI overlap in mutant laCLs.

(F-J) BrdU labeling in control and *Fak/Taz*^{cKO} laCLs (F-H). Open yellow arrowheads in (G and H) mark non-proliferative Cre-recombined GFP+ cells. Quantification is made by calculating the percentage of BrdU-positive (+) cells per section in control and *Fak/Taz*^{cKO} laCLs (I) and by comparing the percentage of BrdU+ cells between Cre-recombined (GFP+) and non-recombined (GFP-) cells in mutant laCLs (J).

(K-O) H&E staining of control and *Fak/Taz*^{cKO} laCLs at different timepoints. Open red arrowheads mark tissue loss in the mutants.

Representative images and quantitative data are shown. Dashed lines outline laCLs. Scale bar in O represents 50 µm in (A-D', F-H, and K-O). Quantitative data are presented as mean ± SD. $^{*}p<0.05$; $^{**}p<0.01$; $^{***}p<0.001$.

Figure S5

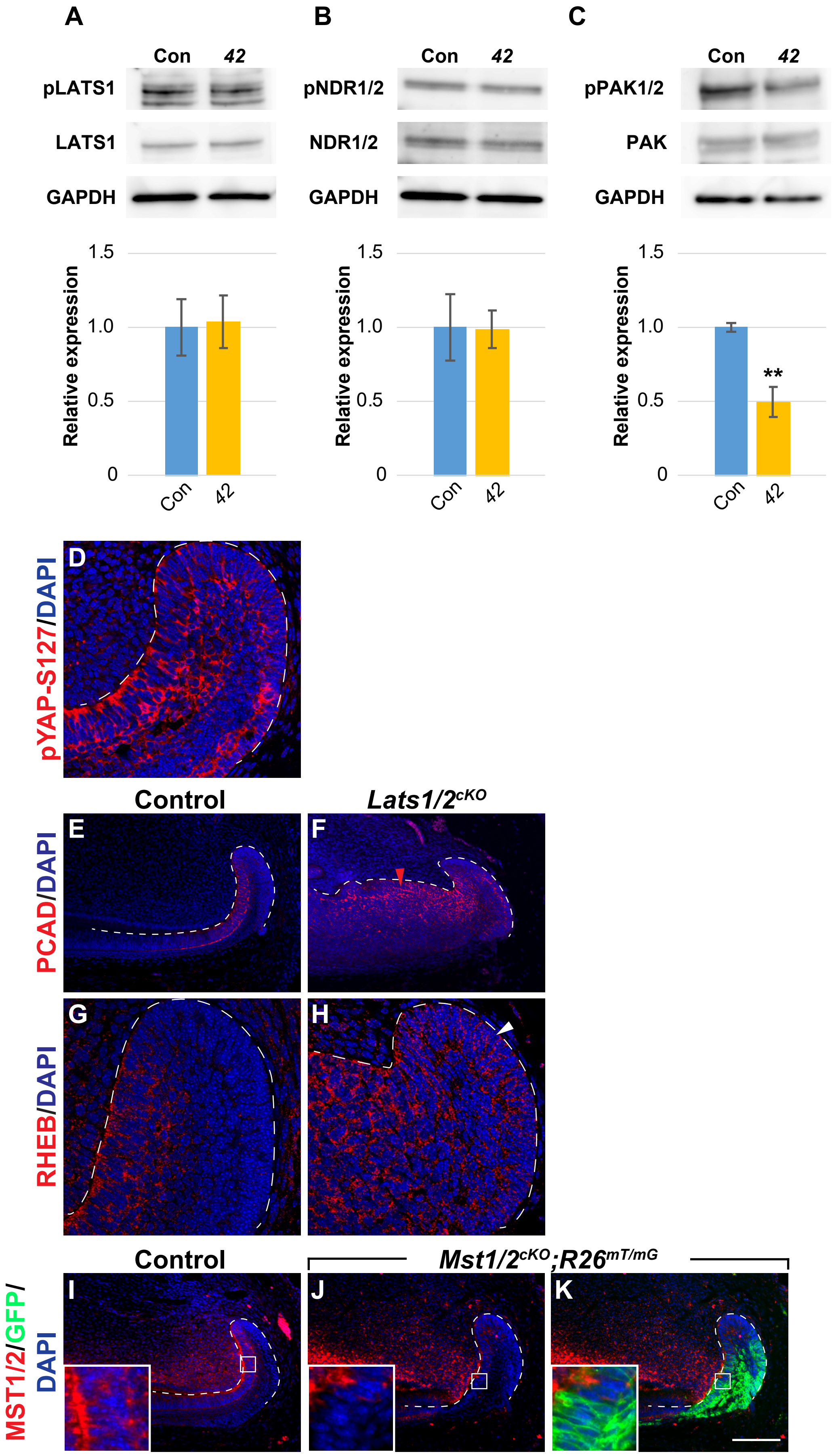
A
B
C
Con
42
pLATS1
LATS1
GAPDH
pNDR1/2
NDR1/2
pPAK1/2
PAK
Relative expression
1.5
1.0
0.5
0
**
D
pYAP-S127/DAPI
Control
Lats1/2cKO
E
F
PCAD/DAPI
G
H
RHEB/DAPI
Control
Mst1/2cKO;R26mT/mG
I
J
K
MST1/2/GFP/DAPI

Figure S5. LATS1/2, but not MST1/2, function in parallel to CDC42. (related to Figure 6 and 7)

(A and B) Western blot analysis and corresponding quantification show that levels of pLATS1 and pNDR1/2 remain unchanged in *Cdc42*^{cKO} IaCLs.

(C) pPAK1/2 level is decreased in *Cdc42*^{cKO} IaCLs.

(D) Immunostaining of pYAP-S127 in IaCLs.

(E and F) Deletion of *Lats1/2* results in expansion of the TA region, which can be marked by PCAD (red arrowhead in F).

(G and H) RHEB expression is upregulated in the OEE region of *Lats1/2*^{cKO} IaCLs (white arrowhead).

(I-K) MST1/2 expression in control and *Mst1/2*^{cKO} IaCLs. GFP marks cells that have undergone Cre recombination.

Representative images, cropped blots and quantitative data are shown. Dashed lines outline the dental epithelium. Scale bar in K represents 50 μm in (D, G, and H) and 130 μm in (E, F, and I-K). Quantitative data are presented as mean ± SD. $^{**}p<0.01$

Figure S6

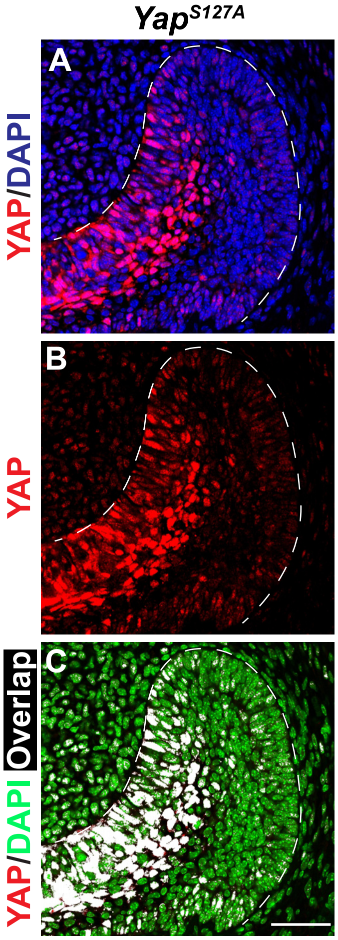

Figure S6. Overexpression of *Yap*^{S127A}. (related to Figures 6)

(A-C) Overexpression of *Yap*^{S127A} does not effectively drive nuclear YAP in the OEE. Representative images are shown. Dashed lines outline laCLs. Scale bar in (C) represents 50 μm in (A-C).

Table S1. Gene Set Enrichment Analysis (GSEA) results for downregulated genes. Related to Figure 2.

Name	Size	ES	NES	Nom P-Val	FDR Q-Val	FWER P-Val
Mitotic Spindle	45	0.3849198	1.9285586	0.002074689	0.08329048	0.079
Mtorc1 Signaling	41	0.37824142	1.877489	0.00533049	0.0646663	0.119
Xenobiotic Metabolism	27	0.40863594	1.7669948	0.007650273	0.12305588	0.297
Estrogen Response_Late	21	0.40807262	1.6800778	0.02027027	0.1707811	0.484
Epithelial Mesenchymal Transition	32	0.35450116	1.6387851	0.027262814	0.17580047	0.566
Oxidative Phosphorylation	32	0.3431483	1.5768838	0.04849138	0.22019507	0.724
Myc Targets V1	51	0.30922395	1.5741473	0.03305785	0.19229464	0.729
E2f Targets	48	0.30326316	1.54611	0.045454547	0.19782263	0.778
G2m Checkpoint	44	0.31142673	1.5308156	0.061458334	0.19116285	0.802

ES, enrichment score; NES, normalized enrichment score; Nom P-Val, nominal p-value; FDR Q-Val, false discovery rate q-value; FWER, familywise-error rate.

Table S2. Chemical concentrations and culture time. Related to Figures 3, 4, and 6, and STAR Methods.

Experiments	**Chemicals**	**Targets**	**Working concentration**	**Culture time**
Functional test of mTOR signaling in laCLs (Figures 3N-3P)	Rapamycin	mTORC1	1 µM	24 hours
Screening upstream regulators of YAP (Figures 4A-4F)	(-)-Blebbistatin	Non-muscle myosin II	50 µM	24 hours
	Erlotinib HCl	EGFR	10 µM	24 hours
	Ki16425	LPA receptor	10 µM	24 hours
	Latrunculin A, Latrunculia magnifica	Actin polymerization	0.5 µM	24 hours
	PF-573228	Focal adhesion kinase (FAK)	5 µM	24 hours
	PP2	SRC kinase	20 µM	24 hours
	Y-27632	Rho-associated protein kinase (ROCK)	50 µM	24 hours
Functional test of PP1A in YAP localization (Figures 6K-6M)	Okadaic acid, Prorocentrum sp.	Protein phosphatase 1 and 2A (PP1 and PP2A)	0.05 µM	12 hours

Table S3. Primer sequences. Related to STAR Methods.

Target	**Forward**	**Reverse**
Genotyping		
Cdc42	ATGTAGTGTCTGTCCATTGG	TCTGCCATCTACACATACAC
Cre	GCAAAACAGGCTCTAGCGTTCG	CTGTTTCACTATCCAGGTTACGG
dnRock2	ACTCATCTCAGAAGAGGATCTG	TTAGCTTGGCTTGTTTGGAGC
Fak	GAGAATCCAGCTTTGGCTGTT	GAATGCTACAGGAACCAAATAAC
Itga3	TGATGACTATACCAACCGGAC	ACTCCAAGCCACATATCCTC
Lats1	TTGTTGCTGGTGTTGTTTCC	ATGAATGAACCTGAGGCTGC
Lats2	ATCCTAGCACTCAGGAGGCA	ACACATTCCCCTCCACTGAC
Mst1	CCTGCTTCAGTGTTGGCTCTTGATTTTCCT	TAGACCAGCCAGGGCTAGAGTGAAACCTTG
Mst2	GTTCAGGGTCCCACCAAGAGTCGCTTCATT	TGTCTAGCTGCTGATGACACTGAACTTCTGGC
R26$^{mT/mG}$	CTCTGCTGCCTCCTGGCTTCT	CGAGGCGGATCACAAGCAATA
R26-rtTA	AAAGTCGCTCTGAGTTGTTAT	GCGAAGAGTTTGTCCTCAACC
		GGAGCGGGAGAAATGGATATG
Rac1	TCCAATCTGTGCTGCCCATC	GATGCTTCTAGGGGTGAGCC
RhoA	AGCCAGCCTCTTGACCGATTTA	TGTGGGATACCGTTTGAGCAT
Rptor	CTCAGTAGTGGTATGTGCTCAG	GGGTACAGTATGTCAGCACAG
Taz	CCCACAGTTAAATGCTTCTCCCAAGACTGGG	GGCTTGTGACAAAGAACCTGGGGCTATCTGAG
Yap	ACATGTAGGTCTGCATGCCAGAGGAGG	AGGCTGAGACAGGAGGATCTCTGTGAG
Yap^{S127A}	CCCTCCATGTGTGACCAAGG	GCACAGCATTGCGGACATGC
	GCAGAAGCGCGGCCGTCTGG	
qPCR		
Ambn	GAGCTGATAGCACCAGATGAG	CGGTTGGAAATTGTGGATCAG
Amelx	GCATACACTCAAAGAACCATCAAG	CACCTCATAGCTTAAGTTGATATAACC
Cdh3	CTTGATGCCAACGATAACGC	ACTGTCAGCCTCTGTACCTC
Ppia	CAAACACAAACGGTTCCCAG	TTCACCTTCCCAAAGACCAC
Rheb	GCAGATACCTATTATGTTGGTTGG	AGCAGTTTGATTTTCTTTAGCAGA
Yap	CAGACGCTGATGAATTCTGC	GGATGTGGTCTTGTTCTTATGGT.

Table S4. Antibody sources and concentrations. Related to STAR Methods.

Primary antibodies	**Sources**	**Dilutions**	**Secondary antibodies used**
Immunostaining			
Ameloblastin	Santa Cruz (sc-50534)	1:100	Goat anti-rabbit 555 (1:500)
Amelogenin	Santa Cruz (sc-32892)	1:100	Goat anti-rabbit 555 (1:500)
BrdU	Abcam (ab6326)	1:200	Goat anti-rat 555 (1:500)
CDC42-GTP	NewEast (26905)	1:50	Biotinylated anti-mouse (1:1000)
GFP	Abcam (ab13970)	1:500	Goat anti-chick 488 (1:500)
ITGA3	Aggarwal et al., 2014	1:100	Biotinylated anti-rabbit (1:1000)
Ki67	Thermo (RM-9106)	1:100	Goat anti-rabbit 555 (1:500)
p4EBP	Cell Signaling (2855)	1:100	Biotinylated anti-rabbit (1:1000)
P-cadherin	Thermo (135800)	1:200	Biotinylated anti-mouse (1:1000)
pFAK	Assay Biotech (A0925)	1:100	Biotinylated anti-rabbit (1:1000)
pMerlin	Rockland (600-401-414)	1:100	Biotinylated anti-rabbit (1:1000)
pS6K1	Assay Biotech (A0533)	1:100	Biotinylated anti-rabbit (1:1000)
pSRC	Signalway (11091)	1:100	Biotinylated anti-rabbit (1:1000)
pYAP-S127	Cell Signaling (4911)	1:100	Biotinylated anti-rabbit (1:1000)
RHEB	ProSci (3501)	1:50	Biotinylated anti-rabbit (1:1000)
SerpinH1	ABclonal (A-2517)	1:100	Biotinylated anti-rabbit (1:1000)
TAZ	Sigma (HPA007415)	1:100	Biotinylated anti-rabbit (1:1000)
YAP	Cell Signaling (4912)	1:100	Biotinylated anti-rabbit (1:1000)
YAP (human)	Abcam (ab52771)	1:100	Goat anti-rabbit 555 (1:500)
Western Blot			
4EBP	Cell Signaling (4923)	1:500	Anti-rabbit HRP (1000)
Ameloblastin	Santa Cruz (sc-50534)	1:300	Anti-rabbit HRP (1000)
Amelogenin	Santa Cruz (sc-32892)	1:300	Anti-rabbit HRP (1000)
CDC42	Santa Cruz (sc-87)	1:300	TrueBlot anti-rabbit, HRP (1:1000)
GAPDH	Acris (ACR001P)	1:3000	Anti-mouse HRP (1000)
Merlin	Cell Signaling (12888)	1:500	Anti-rabbit HRP (1000)
NDR1	Santa Cruz (sc-46184)	1:300	Anti-goat HRP (1000)
p4EBP	Cell Signaling (2855)	1:500	Anti-rabbit HRP (1000)
PAK1/2/3	Cell Signaling (2604)	1:500	Anti-rabbit HRP (1000)
pLATS-T1079	Bioss (bs-7913R)	1:500	Anti-rabbit HRP (1000)
pMerlin	Cell Signaling (13281)	1:500	Anti-rabbit HRP (1000)
pNDR1/2	Biorbyt (orb335842)	1:500	Anti-rabbit HRP (1000)
PP1	Santa Cruz (sc-7482)	1:300	TrueBlot anti-mouse, HRP (1:1000)
PP2A	Santa Cruz (sc-6110)	1:300	SmartBlot anti-goat, HRP (1:1000)
pPAK1-S423	Cell Signaling (2601)	1:500	Anti-rabbit HRP (1000)
pS6K1	Cell Signaling (9205)	1:1000	Anti-rabbit HRP (1000)
pYAP-S127	Cell Signaling (13008)	1:500	Anti-rabbit HRP (1000)
pYAP-S397	Cell Signaling (13619)	1:500	Anti-rabbit HRP (1000)
S6K1	Cell Signaling (2708)	1:500	Anti-rabbit HRP (1000)
Immunoprecipitation			
CDC42-GTP	NewEast (26905)	1.5 µg/IP	n/a
YAP (63.7)	Santa Cruz (sc-101199)	2 µg/IP	n/a

Table S5. Numbers of samples used for quantification. Related to STAR Methods.

Experiments	**Sample origins**	**N numbers**
Colony formation assay	Control and *Yap/Taz*^{cKO}	3 experiments performed with 4 laCLs from 2 animals used per experiment
BrdU labeling	Control and *Yap/Taz*^{cKO}	3 animals per genotype, with the middle 3 sections used for each laCL
	Control and Rptor^{cKO}	3 animals per genotype
	Control and *Fak/Taz*^{cKO}	3 animals per genotype
Ki67 staining	Control and Rapamycin treated	3 laCLs per condition, with the middle 3 sections used for each laCL
TUNEL	Control and *Yap/Taz*^{cKO}	3 animals per genotype
Microarray	Control and *Yap/Taz*^{cKO}	3 experiments with 8 laCLs from 2 males and 2 females per genotype in each experiment
qPCR	Control and *Yap/Taz*^{cKO}	6 experiments with 1 animal used per genotype in each experiment
YAP immunostaining	Control and *Fak*^{cKO}	6 animals per genotype
	Control and *Itga3*^{cKO}	3 animals per genotype
	Control and *Cdc42*^{cKO}	6 animals per genotype
	Control and Okadaic acid treated	3 laCLs per condition
	Electroporated samples	135 cells from 9 laCLs for *Yap*; 166 cells from 13 laCLs for *Yap*^{S127A}; 161cellsl from 11 laCLs for *Yap*^{S397A}; and 128 cells from 9 laCLs for *hYap*^{S127A,S397A}
TAZ immunostaining	Control and *Fak*^{cKO}	3 animals per genotype
	Control and *Itga3*^{cKO}	3 animals per genotype
	Control and *Cdc42*^{cKO}	3 animals per genotype
Immunostaining for RHEB, pS6K1, p4EBP, CDC42-GFP, and PCAD	Control and mutants	3 animals per genotype
Immunoblotting	Control and corresponding mutants	3 experiments with 7 animals used per genotype in each experiment
Co-immunoprecipitation	Control and corresponding mutants	3 experiments with 14 animals used per genotype in each experiment