

Figure S1. *Yap* is important for epithelial expansion in the developing esophagus. (A) Deletion of *Yap* does not affect separation of the trachea and esophagus in *Shh-Cre; Yap^{loxP/loxP}* (*Yap KO*) mutants (n = 3). Note that deletion of *Yap* results in hypoplastic lungs with cystic features. (B-C) *Yap* deletion reduces the numbers of p63⁺ basal cells and Edu⁺ proliferating cells in the mutant esophagus at E12.5 (B) and E18.5 (C) (n = 3). Abbreviation: es, esophagus; tra, trachea. Scale bars: 50 μ m.

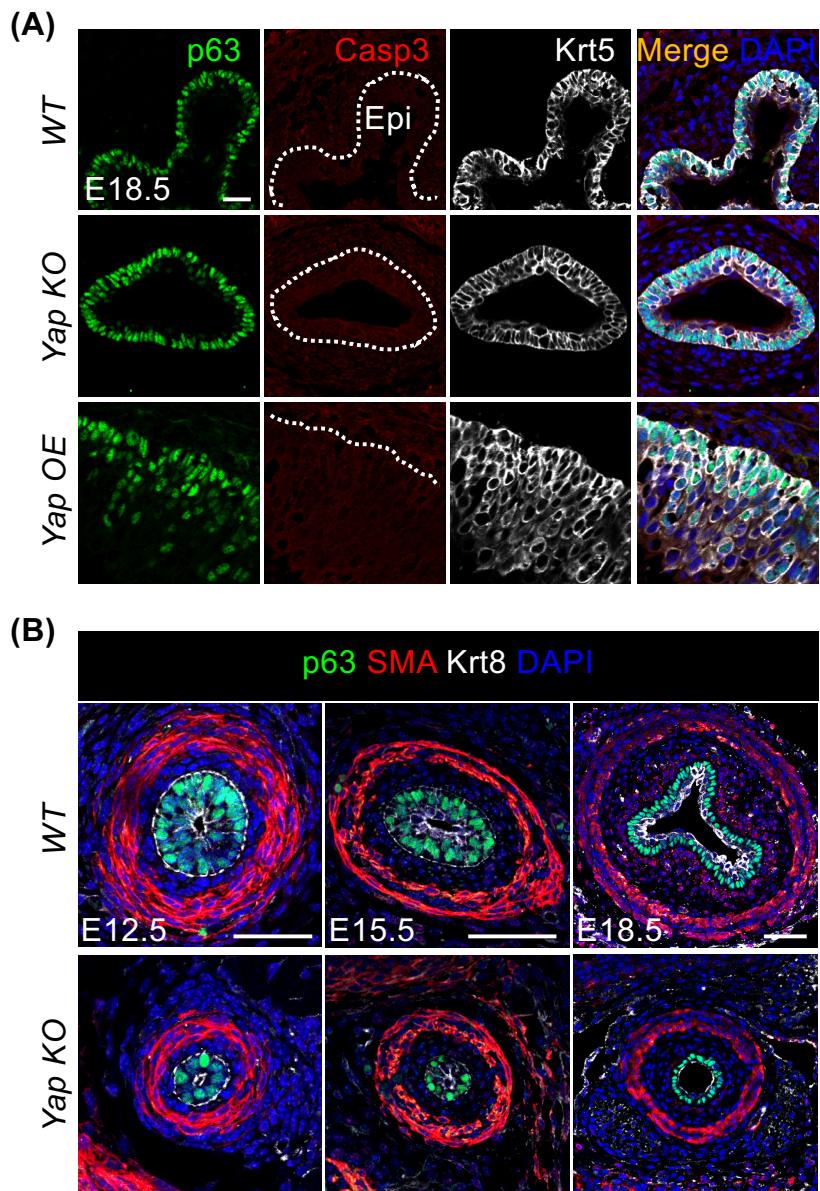


Figure S2. Loss of *Yap* does not affect apoptosis and mesenchymal differentiation in the developing esophagus. (A) Expression of cleaved Caspase-3 (Casp3), a marker for apoptosis, is not altered in the esophagus upon *Yap* deletion and overexpression at E18.5 (n = 3). (B) Expression of smooth muscle actin (SMA) is not altered in the esophagus upon *Yap* deletion at E12.5, E15.5 and E18.5 (n = 3 for each time point). Scale bars: 50 μ m.

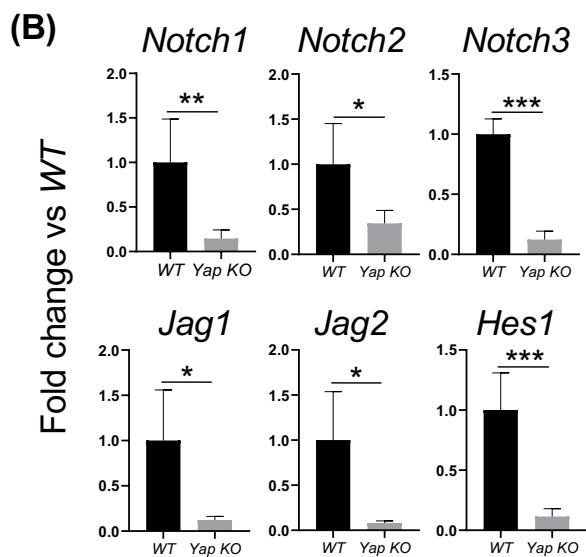
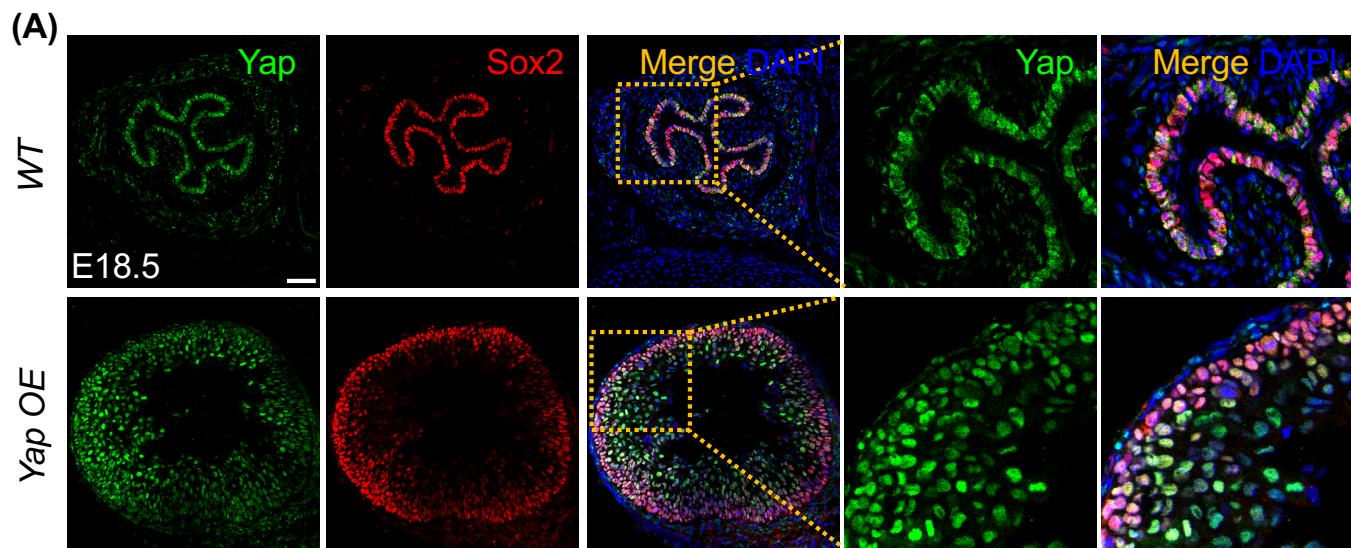
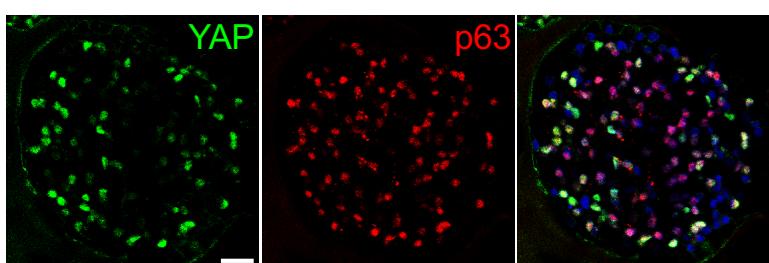
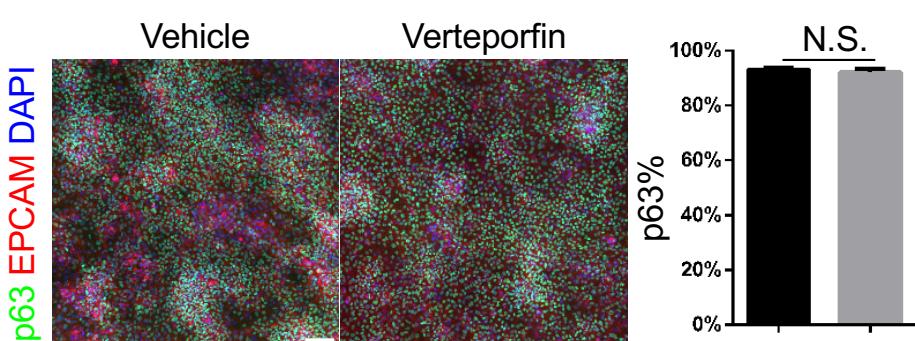


Figure S3. *Yap* gain- and loss-of-function leads to changes in esophageal epithelium and Notch signaling activities. (A) *Yap* overexpression promotes the expansion of esophageal epithelium. Note ectopic *Yap* is sequestered to the nuclei of esophageal basal cells in *Shh-Cre; Yap^{loxP/loxP}; R26-Yap5SA* (*Yap OE*) mutant (n = 3). (B) *Yap* deletion leads to reduced Notch signaling as evidenced by the reduced transcript levels of Notch receptors, ligands and the downstream target *Hes1*. Data represent mean ± SEM (n = 3). *p < 0.05, **p < 0.01, ***p < 0.001 by unpaired, two-tailed Student's t test. Scale bar: 50 μm.

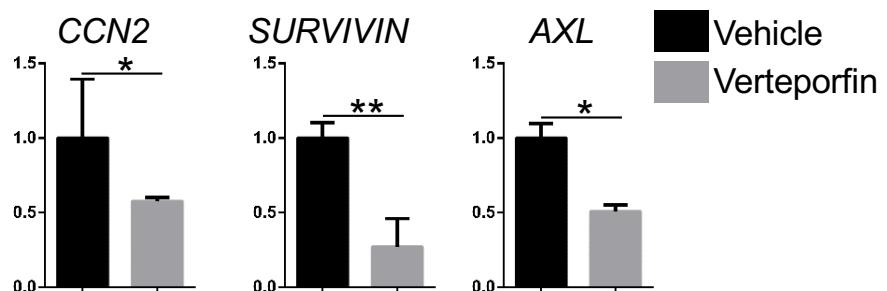
(A)



(B)



(C)



(D)

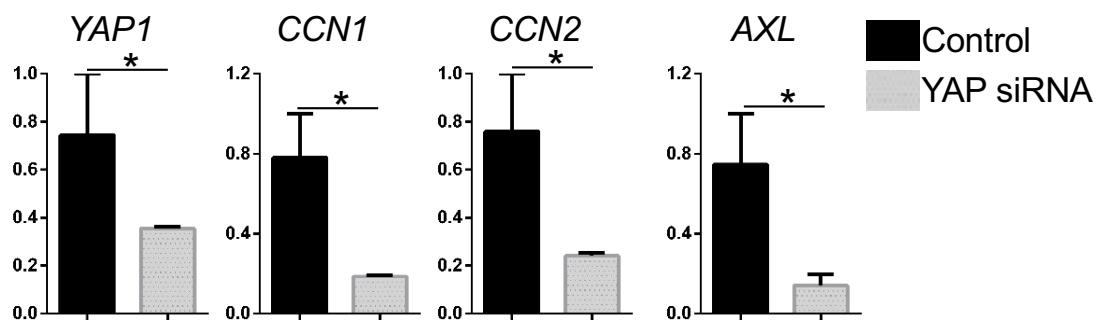


Figure S4. YAP is essential for the development of hPSC-derived esophageal organoids.

(A) Yield of p63⁺ EPCs is not affected by Verteporfin treatment during EPC differentiation from the hPSC-derived anterior foregut endoderm. The number of p63⁺ cells was normalized to EPCAM⁺ epithelial cells. Data represent mean \pm SEM (n = 3). N.S., not significant. Scale bar: 100 μ m. (B) Expression of YAP is mainly localized in the nuclei and higher in the peripheral cells of esophageal organoids. Scale bar: 20 μ m. (C-D) YAP inhibition by Verteporfin (C) or knockdown by siRNA (D) significantly reduces the transcript levels of the YAP downstream targets *CCN1*, *CCN2*, *SURVIVIN* and *AXL*. The transcript levels were represented by fold change as compared to vehicle or control siRNA. Data represent mean \pm SEM (n = 3). *p < 0.05, **p < 0.01 by unpaired, two-tailed Student's t test.

Table S1. Antibody list

YAP	Cell Signaling Technology	Cat#14074S; RRID: AB_2650491
Ki67	BD Biosciences	Cat#550609; RRID: AB_393778
Ki67	Thermo Fisher Scientific	Cat#14-5698-82; RRID: AB_10854564
Rabbit monoclonal anti-KRT13	abcam	Cat#ab92551; RRID: AB_2134681
Mouse monoclonal APC anti-human CD326 (EPCAM)	BioLegend	Cat#324208; RRID: AB_756082
Mouse monoclonal PE anti-human CD104 (ITGβ4)	BioLegend	Cat#327807; RRID: AB_2129147
Rabbit monoclonal anti-p63	Santa Cruz	Cat#sc-8343; RRID: AB_653763
Mouse monoclonal anti-p63	BioLegend	Cat#687202; RRID: AB_2616941
Rabbit monoclonal anti-p63-α	Cell Signaling	Cat#13109; RRID: AB_2637091
Chicken polyclonal anti-Keratin 5	BioLegend	Cat#905901; RRID: AB_2565054
Rabbit monoclonal anti-Keratin 13	abcam	Cat#ab92551; RRID: AB_2134681
Keratin 8	abcam	Cat#ab107115; RRID: AB_10976462
Keratin 8	abcam	Cat#53280; RRID: AB_869901
α-Smooth muscle actin (α-SMA)	Sigma-Aldrich	Cat#A2547; RRID: AB_476701
Notch3	Cell Signaling Technology	Cat#5276S; RRID: AB_10560515
Cleaved Notch1 (NICD1)	Cell Signaling Technology	Cat#4147S; RRID: AB_2612342
Cleaved Caspase-3	Cell Signaling Technology	Cat#9664S; RRID: AB_2070042
SOX2	Thermo Fisher Scientific	Cat#14-9811-82; RRID: AB_11219471
phospho-Histone H3	Sigma-Aldrich	Cat#H9908; RRID: AB_260096
Donkey anti-rabbit, Alexa Fluor 488	Thermo Fisher Scientific	Cat#A-21206; RRID: AB_2535792
Goat anti-chicken, Alexa Fluor 647	Thermo Fisher Scientific	Cat#A-21449; RRID: AB_2535866
Donkey anti-mouse, Alexa Fluor 568	Thermo Fisher Scientific	Cat#A10037; RRID: AB_2534013
Donkey anti-rat, Alexa Fluor Cy3	Jackson ImmunoResearch	Cat#712-165-150; RRID: AB_2340666
Donkey anti-rat, Alexa Fluor 647	Jackson ImmunoResearch	Cat#712-606-153; RRID: AB_2340696

Table S2. qRT-PCR primer sequences

Genes	Forward primers	Reverse primers
YAP	CCAGATGACTCCTGAACAG	CCATCTCCTCCAGTGTCC
CCN1	CAAGGAGCTGGATTGATG	AAAGGGTTGTATAGGATGCGAG
CCN2	ACCAATGACAACGCCTCC	TTGGAGATTTGGGAGTACGG
SURVIVIN	CCACCGCATCTCTACATTCAAG	CAAGTCTGGCTCGTTCTCAG
AXL	TTTATGACTATCTGCCAGG	TGTGTTCTCAAATCTCCCG
Notch1	TGCCTGGACAAGATCAATGAG	CAGGTGTAAGTGTGGGTCC
Notch2	ACCCTCACCTTGTCAATG	ACAAACCCCTCCATTCTGAC
Notch3	CTGCGAAGTGAACATTGACG	TGATCTCCACGTTACAAAGGG
Jag1	CTGTCCCCTGGTTCTCTG	GTTCTGCCCTCATAGTCCTC
Jag2	TCGTCATTCCCTTCAGTCG	GTCATTGTCCCAGTCCCAG
Hes1	TGGTACCCAGTGCTTTGAG	CTCCGATAGCCATAGCAAGG