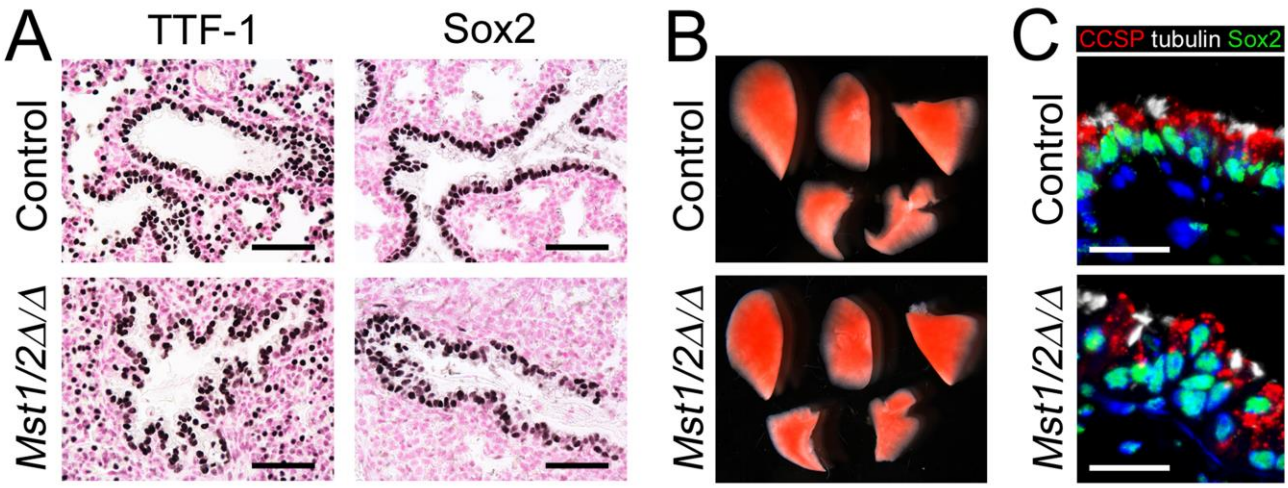
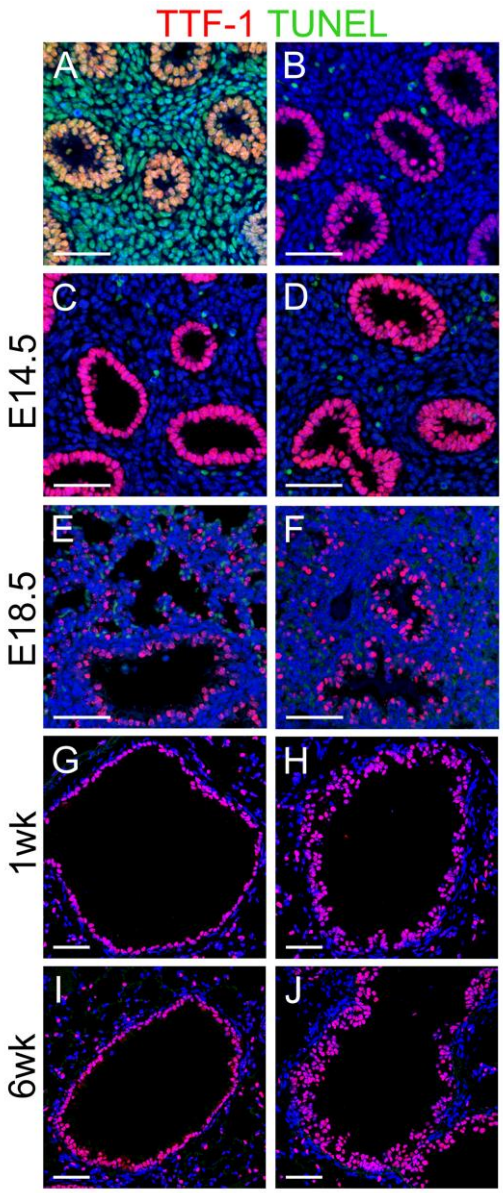
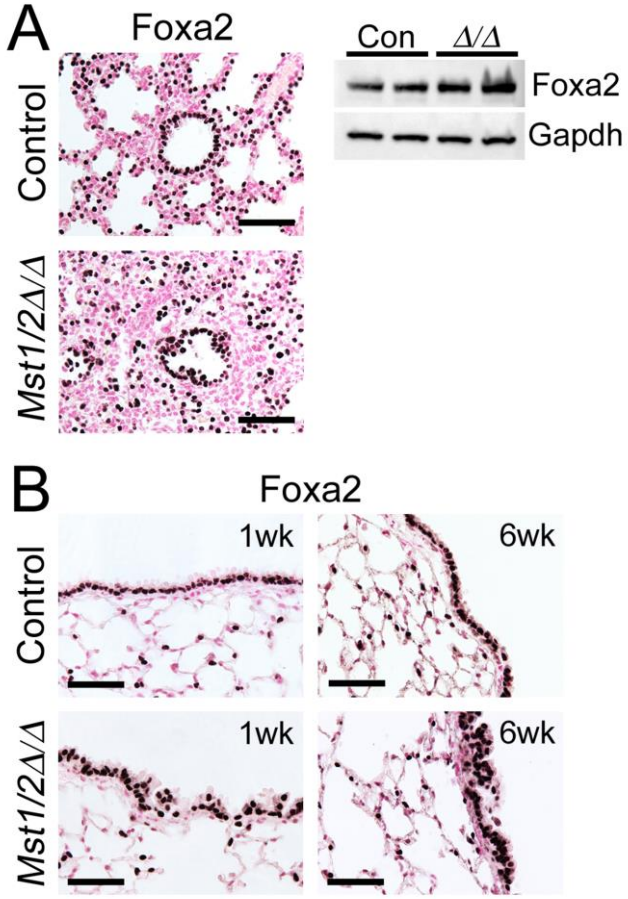


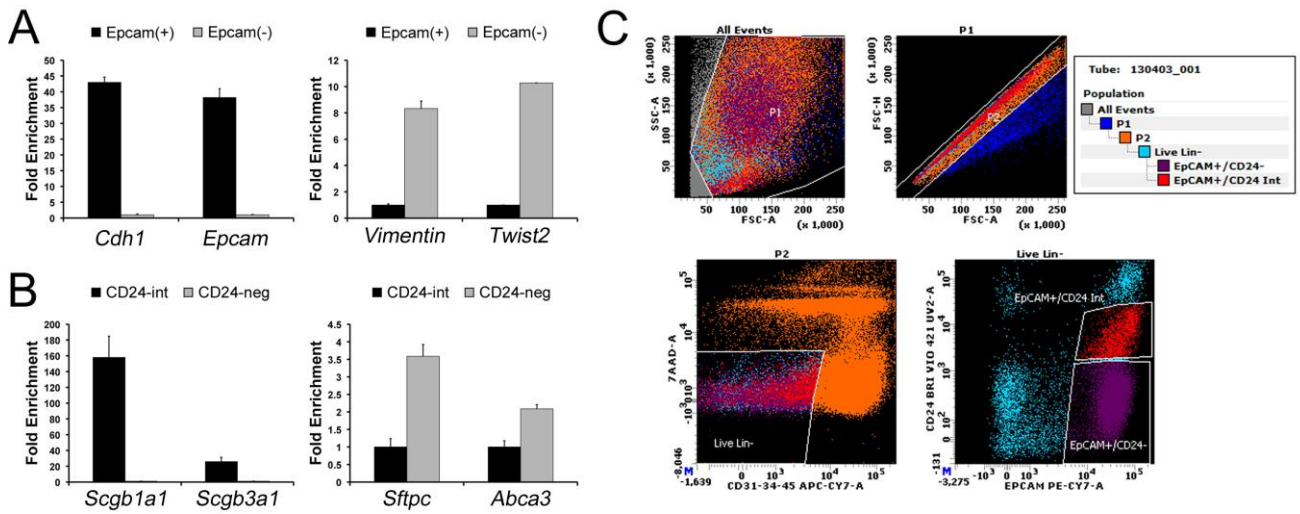
Supplementary Figure S1 Deletion of *Mst1/2* causes airway epithelial hyperplasia. Lungs from E18.5 (A-B) and adult (C) control and *Mst1/2* deleted mice are shown. (A) Immunostaining for TTF-1 and Sox2 showed that the bronchiolar epithelium in embryonic *Shh-Cre;Mst1/2Δ/Δ* lungs was pseudostratified and disorganized compared to the simple columnar epithelium in control airways. Sox2 was normally restricted to the proximal airway epithelium in *Mst1/2* deleted mice. (B) Lobe size was not affected by deletion of *Mst1/2*. (C) CCSP (red), acetylated tubulin (white) and Sox2 (green) immunofluorescence showed that the hyperplastic airway epithelium maintained bronchiolar identity following doxycycline-mediated deletion of *Mst1/2* in adult mice. Scale bars, 20μm (C); 50μm (A).



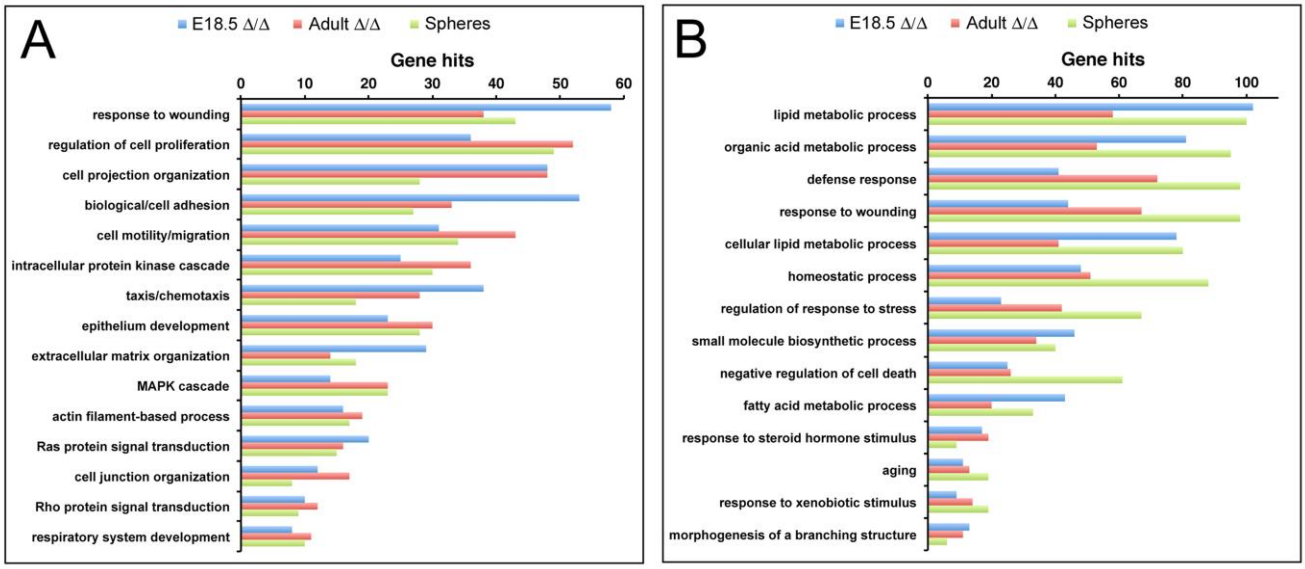
Supplementary Figure S2 Lack of apoptosis after deletion of *Mst1/2*. (A-J) Immunofluorescence staining for TTF-1 (red) and TUNEL (green) was performed on lungs from control (C,E,G,I) or *Mst1/2Δ/Δ* (D,F,H,J) mice. (A-B) TUNEL staining of a nuclease-treated positive control (A) and an unlabeled negative control (B) are shown. (C-F) TUNEL staining was not detected in epithelial or mesenchymal tissues from controls or *Mst1/2Δ/Δ* mice at E14.5 (C-D) or E18.5 (E-F). (G-J) Apoptotic cells were not observed in bronchiolar epithelial cells of adult mice at 1 week (G-H) or 6 weeks (I-J) after doxycycline. Scale bars, 50μm.



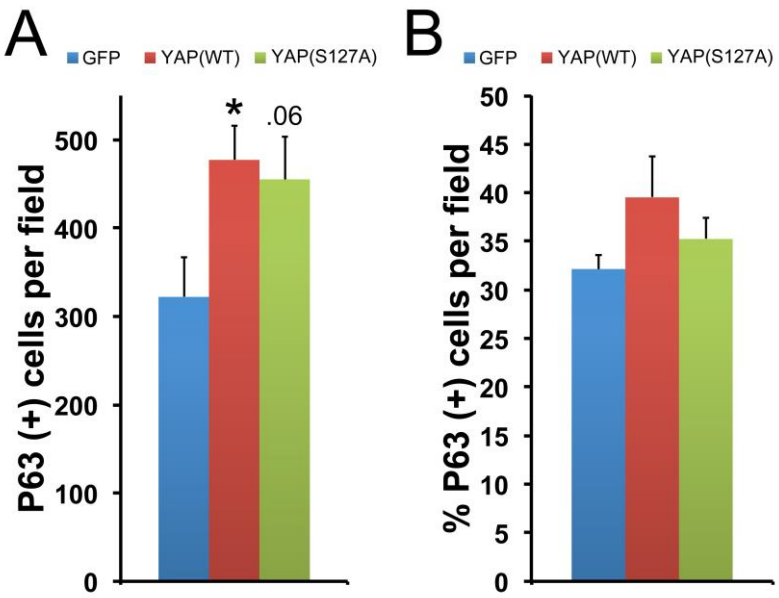
Supplementary Figure S3 Foxa2 is not affected after deletion of *Mst1/2*. (A) Foxa2 was assessed by immunostaining and Western blot. Foxa2 expression was unchanged in E18.5 *Mst1/2Δ/Δ* lungs. (B) Foxa2 staining was not affected in the hyperplastic bronchiolar epithelial cells 1 week or 6 weeks following *Mst1/2* deletion. Scale bars, 50μm.



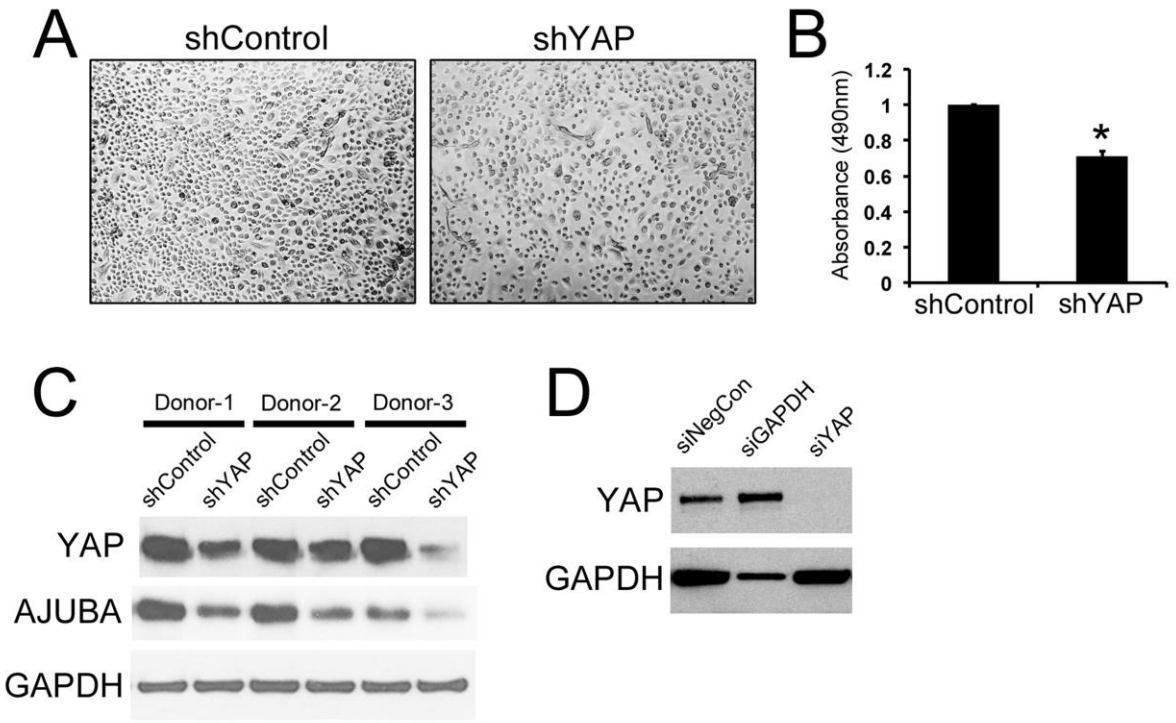
Supplementary Figure S4 Enrichment of respiratory epithelial cells from *Mst1/2* deleted mice by cell sorting. (A) qPCR of mRNA isolated from Epcam-positive and Epcam-negative cell populations sorted from E18.5 control lungs is shown. Epithelial-specific mRNAs *Cdh1* and *Epcam* were highly enriched in the Epcam-positive cells and *Vim* (*vimentin*) and *Twist2* were enriched in the Epcam-negative cells. (B-C) Epcam-positive/CD24-intermediate (bronchiolar) and Epcam-positive/CD24-negative (alveolar) epithelial cell populations were isolated from adult mouse lungs using FACS. (B) qPCR showed that mRNAs for bronchiolar epithelial markers *Scgb1a1* and *Scgb3a1* were enriched in the CD24-intermediate cells. Alveolar type II selective mRNAs *Sftpc* and *Abca3* were enriched in CD24-negative cells. (C) A FACS profile for isolation of live Lin-/Epcam+/CD24-intermediate and negative cells is shown.



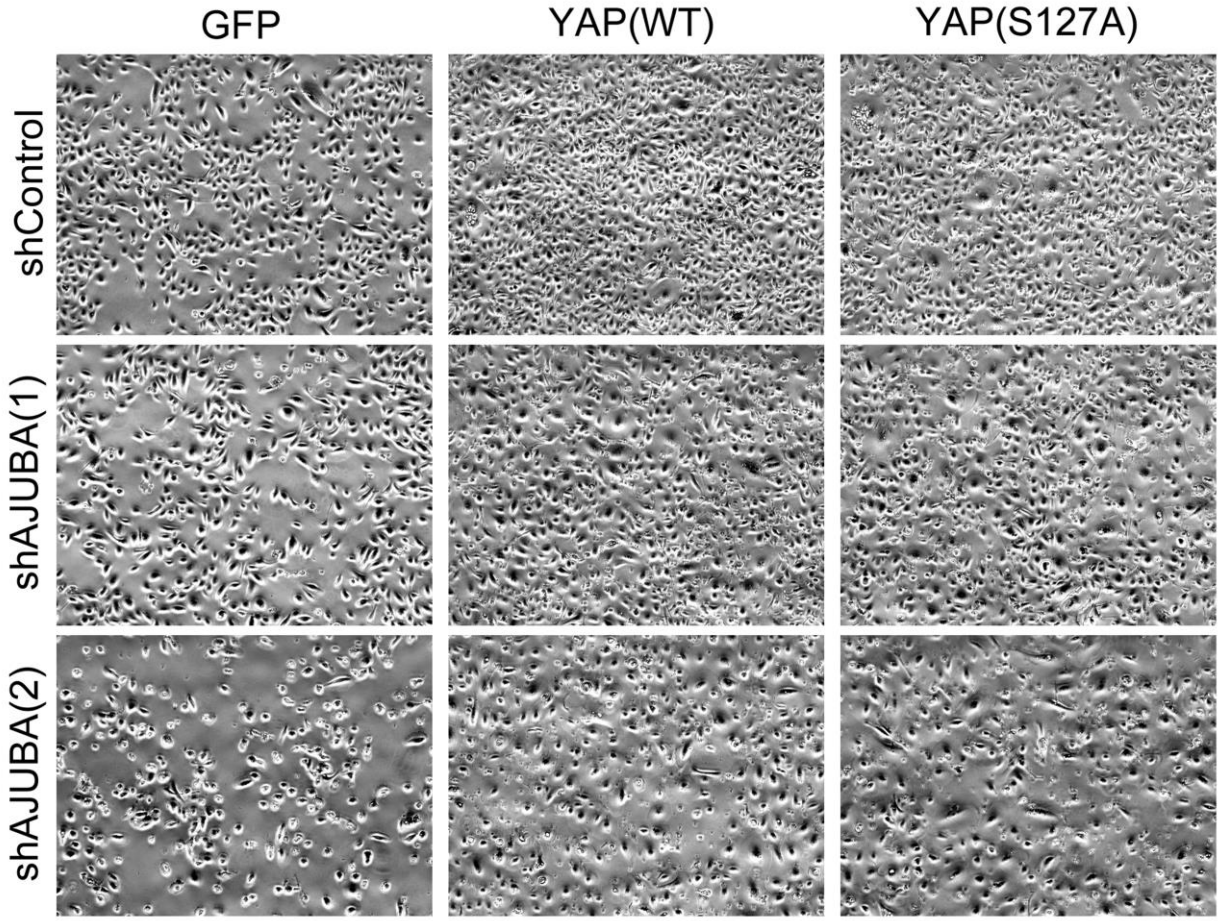
Supplementary Figure S5 Common biological processes are regulated following *Mst1/2* deletion *in vivo* and YAP expression *in vitro*. (A-B) Enrichment of biological processes associated with mRNAs that were increased (A) and decreased (B) in RNA microarray and RNA-seq analyses of *Mst1/2* deficient epithelial cells and YAP(S127A) bronchospheres were identified using ToppFun.



Supplementary Figure S6 Quantification of P63-positive cell numbers in air-liquid interface cultures. Total P63-positive cells (A) and percent P63-positive cells per DAPI-positive nuclei (B) per field (40X) were quantified from 3-4 week ALI cultures. P-value is shown (A) and asterisk indicates statistical significance ($p < 0.05$).



Supplementary Figure S7 YAP inhibition decreases proliferation and AJUBA expression. (A) Images of primary HBECs at 72h following transduction with control or shYAP lentiviruses showed fewer cells following YAP inhibition. (B) MTS assays indicated that inhibition of YAP reduced the number of viable primary HBECs after 72h. (C) Western blots of primary HBEC lysates demonstrated that shRNA-mediated inhibiton of YAP caused decreased AJUBA expression. (D) Western blot analysis of HBEC-3KT cell lysates showing decreased YAP expression at 72h following targeted siRNA transfection.



Supplementary Figure S8 Ajuba mediates the proliferative effects of YAP *in vitro*. Images of HBEC-3KT cells infected with control, YAP, or shAJUBA lentiviruses are shown. Inhibition of AJUBA blocked control and YAP-induced cell proliferation.

Supplementary Table S1 Antibody information.

Antibody	Company; Catalog number	Raised in	Antigen Retrieval	Dilution (application)
Yap	Cell Signaling; 4912	Rabbit polyclonal	Citrate	1:75 (IHC;+TSA for IF)
Yap	Santa Cruz; sc-101199	Mouse monoclonal	Citrate	1:100 (IHC)
phospho Yap (S127)	Cell Signaling; 4911	Rabbit polyclonal	Citrate	1:100
phospho Yap (S127)	Cell Signaling; 13008	Rabbit monoclonal (D9W2I)	Citrate	1:1500 (IHC)
CCSP	Seven Hills Bioreagents	Guinea pig polyclonal	Tris-EDTA	1:800 (IF)
CCSP	Santa Cruz; sc-9772	Goat polyclonal	Citrate	1:3000 (IHC)
proSP-C	Seven Hills Bioreagents	Rabbit polyclonal		1:400 (IHC)
acetylated tubulin	Sigma-Aldrich; T7451	Mouse monoclonal (clone 6-11B-1)		1:3K (IHC; IF)
BrdU	DSHB; G3G4	Mouse monoclonal	Citrate	1:100 (IF)
Krt8/Endo-A	DSHB; TROMA-1	Rat monoclonal	Tris-EDTA	1:250 (IF)
E-cadherin	Cell Signaling; 4065	Rabbit polyclonal	Tris-EDTA	1:200 (IF)
T1-alpha /Podoplanin	DSHB; MA5-16113	Hamster monoclonal		1:400 (IHC)
TTF-1	Seven Hills Bioreagents	Guinea pig polyclonal	Citrate	1:200 (IF)
TTF-1	Seven Hills Bioreagents	Mouse monoclonal (8G7G3-1)	Citrate	1:2000 (IHC)
PAR3	EMD Millipore; 07-330	Rabbit polyclonal		1:50 (IF)
P63	Santa Cruz; sc-8431	Mouse monoclonal (clone 4A4)		1:50 (IF)
β-catenin	Santa Cruz; sc-7199	Rabbit polyclonal		1:100 (IF)
Ajuba	Novus; NBP 1-89570	Rabbit polyclonal	Tris-EDTA	1:200 (IHC); 1:40 +TSA (IF)
Ajuba	Cell Signaling; 4897	Rabbit polyclonal	Citrate	1:80 (IHC)
Foxa2	Seven Hills Bioreagents	Rabbit polyclonal	Citrate	1:1500 (IHC)
Sox2	Seven Hills Bioreagents	Guinea pig polyclonal	Citrate or Tris-EDTA	1:100-500 (IF)
Hopx	Santa Cruz; sc-30216	Rabbit polyclonal	Tris-EDTA	1:200 (IF)
Phospho-Histone H3	(Santa Cruz)	Rabbit polyclonal	Citrate	1:300 (IF)
GFP	Abcam; 13970	Chicken polyclonal		1:300 (IF)

Supplementary Table S2 Taqman primer list.

Sftpc	Mm00488144_m1
Sftpb	Mm00455681_m1
Abca3	Mm00550501_m1
Sox9	Mm00448840_m1
Id2	Mm00711781_m1
Hopx	Mm00558630_m1
Pdpn	Mm00494716_m1
Aqp5	Mm00437578_m1
Ccnd1	Mm00432359_m1
Ccna2	Mm00438063_m1
Ccnb1	Mm00838401_g1
Cdk1	Mm00772472_m1
Mycn	Mm00476449_m1
Mst1	Mm00451755_m1
Mst2	Mm00490480_m1
Scgb1a1	Mm00442046_m1
Scgb3a1	Mm00446493_m1
Foxj1	Mm00807215_m1
Trp63	Mm00495788_m1
Epcam	Mm00493214_m1
Cdh1	Mm00486906_m1
Twist2	Mm00492147_m1
Muc5ac	Mm01276725_g1
Vimentin	Mm01333430_m1
YAP1	Hs00902712_g1
AJUBA	Hs01036974_m1
GPR126	Hs01089210_m1
MUC20	Hs00416321_m1
CTGF	Hs01026927_g1
SCGB1A1	Hs00171092_m1
SCGB3A1	Hs00369360_g1
MUC5AC	Hs01365616_m1
MUC5B	Hs00861595_m1
KRT14	Hs00265033_m1
TRP63	Hs00978340_m1
AURKB	Hs00945858_g1
CCNB1	Hs00259126_m1
CCNE1	Hs01026536_m1
CDC25A	Hs00947994_m1
CDC25B	Hs01550934_m1
FOXM1	Hs00153543_m1
PLK1	Hs00153444_m1
CMYC	Hs00153408_m1
CCND1	Hs00277039_m1

Supplementary Methods

Immunohistochemistry and immunofluorescence

Tissue sections were deparaffinized and rehydrated through a graded ethanol series Endogenous peroxidase activity was inactivated in 1.5% H_2O_2 in methanol. Microwave antigen retrieval was performed when required using 10mM citrate buffer, pH 6.0 or 10mM Tris/0.5mM EDTA/0.5% Tween-20, pH 9.0. Sections were blocked for 1-2h in 4% normal goat or donkey serum in PBS-0.1% Triton X-100 (PBST) followed by primary antibody incubation overnight at 4°C. Sections were then washed and incubated with biotinylated secondary antibodies (1:200; Vector Labs) followed by incubation in ABC reagent (Vector Labs). Antigen localization was detected with nickel-diaminobenzidine and enhanced with Tris-Cobalt. Sections were counterstained with 0.1% of Nuclear Fast Red and coverslipped using Permount (Fisher Scientific). Immunofluorescence was performed as described above with the omission of peroxidase treatment and the use of fluorophore-conjugated secondary antibodies (Alexa Fluor-488, Alexa Fluor-568, and Alexa Fluor-647; Molecular Probes). TUNEL staining for apoptosis was performed using a TdT in situ apoptosis kit (R&D) per the manufacturer protocol. Nuclei were stained with DAPI and sections were mounted with ProLong Gold anti-fade reagent (Life Technologies).

For whole mount immunofluorescence staining of primary HBEC grown at ALI, cultures were transwell cultures washed with 0.1M PBS and fixed using 4% PFA in PBS, overnight at 4°C. The samples were permeabilized with 1% Triton X-100 in PBS for 15 min at room temperature and blocked in PBS containing 4% normal donkey serum for 3 hours at room temperature. The samples were incubated with primary antibodies for 72 hours at 4°C, washed with PBS, followed by incubation with the respective fluorophore-conjugated

secondary antibodies for 4 hours at room temperature. The samples were counterstained with DAPI (1μg/ml) and mounted with PBS onto a slide with a No. 1.5 coverslip. Z-stack immunofluorescence images were acquired sequentially using channel series with a Nikon A1Rsi inverted confocal microscope. Z-intensity correction was used to compensate for reduced signal intensity at increasing Z-depth. Z-stacks were projected in slice view using the Nikon NIS-Elements software. P63 positive cell counts were determined using images of ALI cultures from three independent donors and Imaris software.

Lentiviruses

Mammalian expression vectors p2xFLAGhYAP2 and p2xFLAGhYAP2-S127A encoding for YAP(WT) and YAP(S127A) were obtained from Addgene (plasmids 17793 and 17794). EcoRI fragments containing the YAP coding region were isolated from the p2xFLAGhYAP plasmids and inserted by blunt end ligation into the ClaI site of SSIN-SFFVmcs-ires-eGFPn lentivirus vector (kindly provided by Dr. Punam Malik, Cincinnati Children's Hospital) to generate SFFV-FLAG-hYAP(WT) and SFFV-FLAG-hYAP(S127A) lentiviral plasmids. SFFVmcs-ires-eGFPn empty vector was used as a control in YAP lentivirus experiments. Lentiviral shRNA plasmids obtained from Sigma included shNon-target control (SHC016), shYAP (TRCN0000107265), shAJUBA(1) (TRCN0000074208), and shAJUBA(2) (TRCN0000074209). Lentiviral production was performed using the Cincinnati Children's Hospital Viral Vector Core.

Cell culture

Primary human bronchial epithelial cells (HBECs) were grown in Bronchial Epithelial Growth Media (BEGM) on Purecol (Advanced Biomatrix) coated tissue-culture dishes. Primary

HBECs at 50-70% confluence were transduced with control or YAP expressing lentivirus (MOI=2). For shRNA-mediated inhibition of AJUBA, HBECs were transduced with control or YAP lentivirus (MOI=2) for 24h prior to lentiviral shRNA infection (MOI=5). HBECs were passaged at 80-90% confluency and plated for ALI or bronchosphere cultures (Rock et al., 2009). For ALI cultures, passage 2 cells were plated at 1.6 x 10^5 cells/cm^2 onto type IV collagen-coated (Sigma-Aldrich) Transwell-Clear inserts (Corning) in ALI medium. When confluent (5-7 days), the culture medium was removed from the apical chamber. The cultures were harvested for analyses after being maintained at an ALI for 3-4 weeks. For bronchosphere cultures, passage 2 HBECs were resuspended in a 50-50 mixture of ALI medium-Cultrex BME reduced growth factor (RGF) (Trevigen Inc.) and plated at a density of 1 x 10^4 cells per type IV collagen-coated Transwell-Clear 12mm inserts (Corning) in a total volume of 200l/cm^2. ALI medium was added to the basal compartment of the plates and the cultures were maintained for 18-20 days. Lentiviral expression of YAP in bronchosphere and ALI cultures was repeated three times, and YAP/shAJUBA experiments were repeated twice using primary HBECs from independent donors. For sphere number and size quantification, images of bronchospheres in culture were obtained on day 17-18 using an Olympus IX70 or Axiovert 100M inverted microscope (Ziess). The perimeter of spheres was measured using the Nikon Elements general analysis module and spheres with perimeters of 150μm or greater were counted.

For siRNA-mediated inhibition of YAP, HBEC-3KT cells were seeded at 3 x 10^4 cells per well in 12-well culture plates. After 24h, cells were transfected with siRNA (10nM final concentration) using Lipofectamine 2000 (Life Technologies) per the manufacturer protocol. Silencer Select siRNAs (Ambion) included a negative control (4390843), siGAPDH (4390849), and siYAP (s20368). For lentiviral expression of YAP(WT) and YAP(S127A), HBEC-3KT cells

were seeded at 1 x 10^5 cells per well in 6-well culture plates and infected with control or YAP-expressing lentivirus (MOI=4) the following day. For YAP/shAJUBA experiments, HBEC-3KT cells were transduced with control or YAP-expressing lentiviruses (MOI=3) and passaged at 72h post-infection. At 24h after passage, cells were infected with shRNA lentiviruses (MOI=3). Lentiviral and siRNA experiments in HBEC-3KT cells were repeated three times and cells were harvested after 72h for analyses. Cell culture images were obtained using an Olympus IX70 inverted microscope equipped with an Evolution VF camera and Image-Pro Plus 7.0 software (Media Cybernetics).

Cell count and viability assays

HBEC bronchospheres were harvested after 18-19 days and spheres were released from culture gels with dispase II in saline (50U/ml; Life Technologies). The intact spheres were centrifuged (300rpm, 1 min), washed with 1X PBS, and dissociated in an enzyme cocktail [0.3% trypsin with 1mM EDTA in PBS (Sigma), 0.1% pronase (Roche), 0.05% protease XIV (Sigma), and 0.1% collagenase type IV (Life Technologies)] in PBS for 1 hour at 37°C. Spheres were then mechanically dissociated with a sterile syringe and 19G1½" needle. The dissociated single cell suspensions were pelleted, washed with PBS and filtered through a 40μm nylon cell strainer (MidSci). HBEC-3KT cells were harvested using trypsin-EDTA digestion (Lonza). HBEC and HBEC-3KT cells were pelleted by centrifugation and resuspended in PBS. Cell counts and viability were determined using Trypan blue staining and a hemocytometer, CellTiter 96 AQueous One Solution MTS assay (Promega), or a Muse Count and Viability Assay Kit and a Muse Cell Analyzer (Millipore) per the manufacturer recommendations.

Cell sorting

Non-cilated bronchiolar epithelial cells were isolated by fluorescence cell sorting for Lin^-/$CD326^+$/CD24-intermediate cells from control (n=4) and *Mst1/2* deleted (n=4) mice after 16 days of doxycycline treatment as previously described (H. Chen et al., 2012). Cell sorting was performed using a BD FACSVantage SE flow cytometer and Cell Quest software (BD Biosciences). Live Lin^-/$CD326^+$ epithelial cells were separated into CD24-intermediate and CD24-negative populations to enrich for CCSP-expressing airway cells and alveolar type 2 cells, respectively. Isolation of epithelial cells from whole lungs of E18.5 control (n=7) and *Mst1/2* deleted (n=4) mice was performed using a CD326 (Epcam)-biotin antibody (clone caa7-9G8) and anti-biotin magnetic microbeads (Miltenyi Biotec) and an AutoMACS Pro Separator as previously described (Lange et al., 2014).

RNA analyses

Microarray analysis was performed on RNA isolated from Epcam-positive cells from control (n=3) and *Mst1/2* deleted (n=3) embryo lungs using Mouse Gene 1.0 ST Arrays (Affymetrix, Santa Clara, CA). Comparison of lung mRNAs regulated during normal perinatal lung maturation (E15.5-birth) (Xu et al., 2012) and those changed E18.5 *Mst1/2* deficient epithelial cells was used to generate a heat map depicting 50 inversely regulated mRNAs. RNA-seq analysis was performed using mRNA from primary HBEC bronchospheres harvested at day 18 (n=2 independent donors), and pooled mRNAs isolated from Lin^-/$CD326^+$/CD24-intermediate cells of control (n=4) and *Mst1/2* deleted (n=4) adult mice following 16 days of doxycycline treatment. RNA-sequencing was performed by the DNA sequencing and genotyping core at Cincinnati Children's Hospital using an established NGS pipeline. Total RNA was used to generate amplified cDNA with the Ovation RNA-Seq System V2 kit (NuGEN). A sequencing library was prepared using the Nextera XT DNA Sample Preparation

Kit (Illumina) and next-generation sequencing of equimolar pools of cDNA libraries was performed using a single read 50 rapid flow cell on a HiSeq 2500 sequencing platform (Illumina) with greater than 30 million raw reads per sample. RNA quantification was performed using the DEseq algorithm. RNA-seq data was normalized to RPKM (reads per kilobase per million mapped reads) with the median of all samples as baseline. Raw counts less that 20 were omitted and differentially expressed mRNAs were identified by unpaired t-test ($p \leq 0.05$; fold change ≥ 1.5) using GeneSpring 12.6 (Agilent Technologies). Differential expression analysis by FPKM (fragments per kilobase of exon per million fragments mapped) was determined using Cufflinks software to generate a transcriptome assembly of the aligned RNA-seq reads. mRNA changes identified in RNA-seq analyses were confirmed by qPCR. Biological process enrichment of differentially expressed RNAs was determined using ToppFun software available from the ToppGene Suite (http://toppgene.cchmc.org/) (J. Chen et al., 2009).

Western blots

Total cellular protein was extracted from primary HBECs and HBEC-3KT cells using RIPA buffer (40mM Tris-HCl, pH8.0; 280mM NaCl; 20% glycerol; 1% NP-40; 4mM EDTA; 5μl/ml protease and phosphatase inhibitor cocktails, Sigma). SDS-PAGE and immunoblotting were performed according to standard protocols using 10-20% Tris-Glycine gels (Novex) and nitrocellulose membranes (BioRad). Primary antibodies included rabbit anti-YAP (Cell Signaling; 1:1000), rabbit anti-AJUBA (Novus; 1:1000), and rabbit anti-GAPDH (1:10,000; Bethyl Laboratories). Following incubation with peroxidase-conjugated secondary antibodies (Calbiochem; 1:5000), chemiluminescent bands were detected using Luminata Forte Western HRP Substrate (Millipore) and HyBlot CL autoradiography film (Denville).

Supplementary References

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