SUPPLEMENTAL INFORMATION FOR:

Crumbs3-mediated polarity directs airway epithelial cell fate through the Hippo pathway effector Yap

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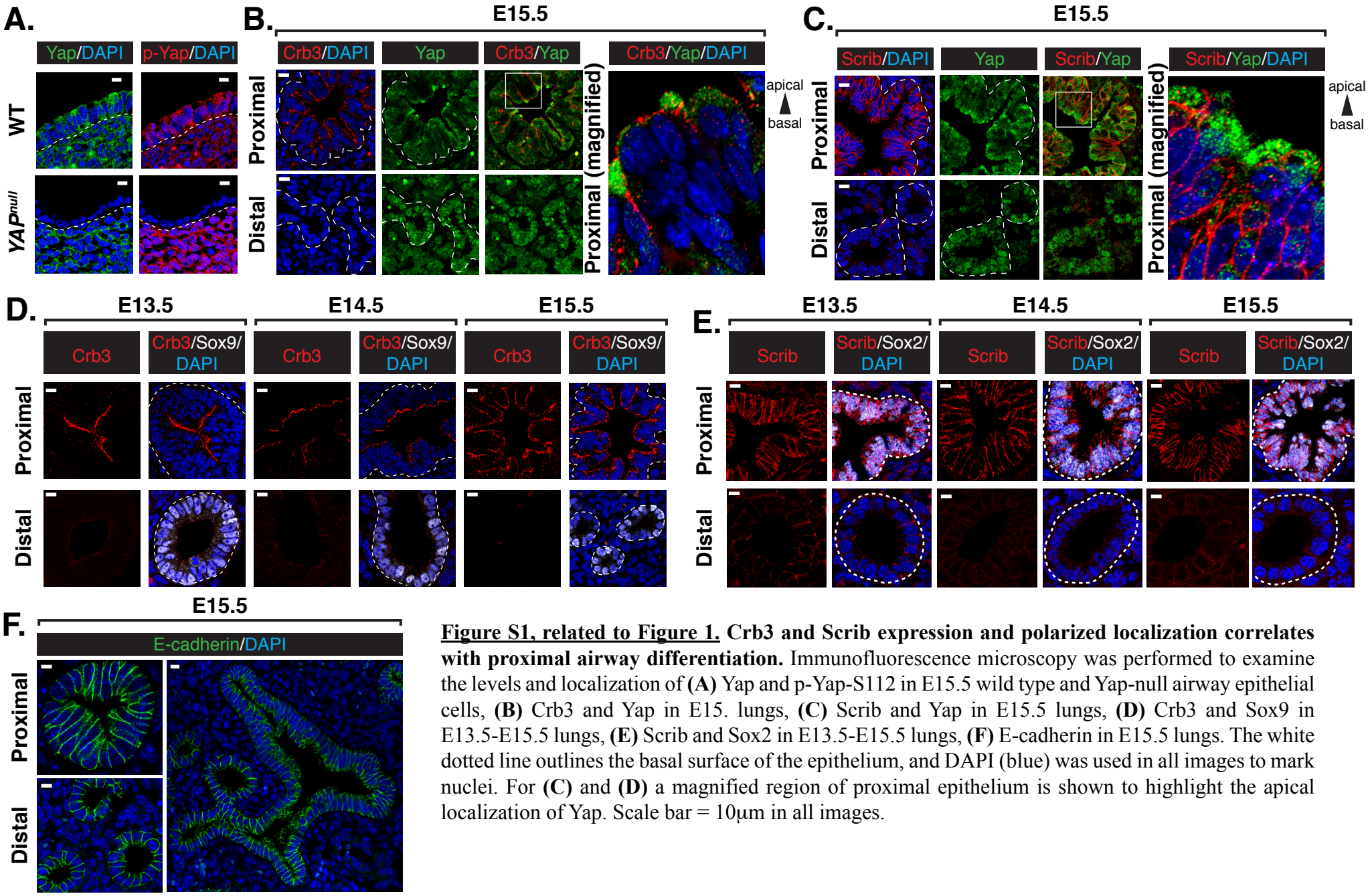


Figure S1, related to Figure 1. Crb3 and Scrib expression and polarized localization correlates with proximal airway differentiation. Immunofluorescence microscopy was performed to examine the levels and localization of **(A)** Yap and p-Yap-S112 in E15.5 wild type and Yap-null airway epithelial cells, **(B)** Crb3 and Yap in E15. lungs, **(C)** Scrib and Yap in E15.5 lungs, **(D)** Crb3 and Sox9 in E13.5-E15.5 lungs, **(E)** Scrib and Sox2 in E13.5-E15.5 lungs, **(F)** E-cadherin in E15.5 lungs. The white dotted line outlines the basal surface of the epithelium, and DAPI (blue) was used in all images to mark nuclei. For **(C)** and **(D)** a magnified region of proximal epithelium is shown to highlight the apical localization of Yap. Scale bar = 10μm in all images.

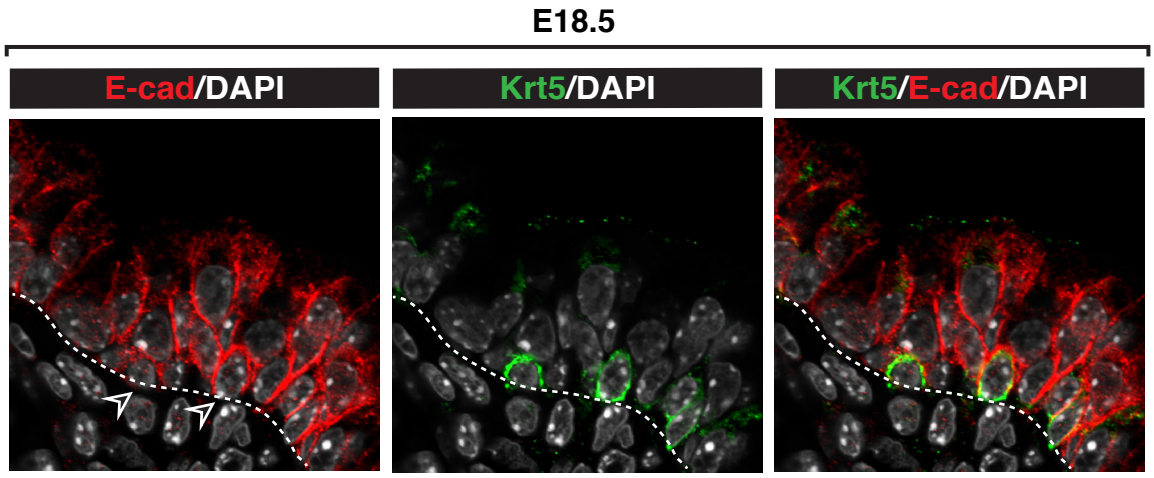
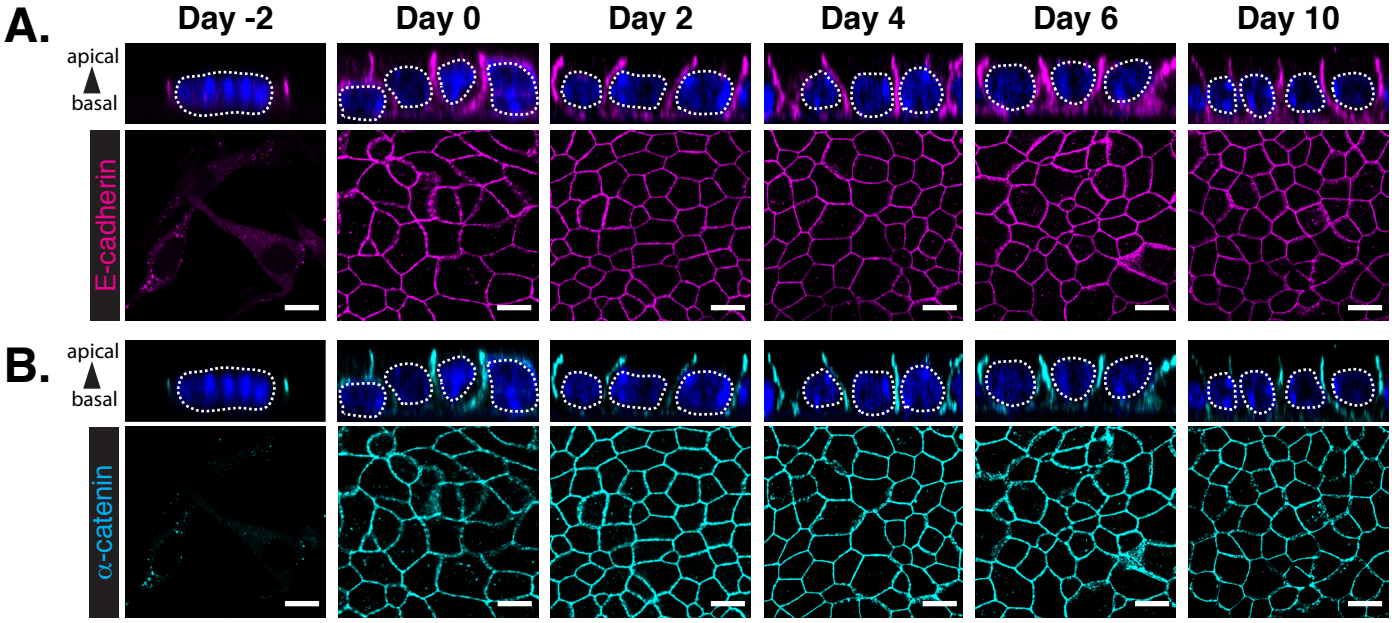


Figure S2, related to Figure 2. Expression and localization of the adherens junction protein E-cadherin in proximal airway epithelial cells. Similar E-cadherin (red) expression is observed between Krt5+ (green) basal progenitors and differentiated cells in developing proximal airways. Note, however, the cortical distribution of E-cadherin in the Krt5+ cells. White arrows point to the Krt5+ cells in the left image, the basal surface of the epithelium is outlined with a white dotted line, and DAPI (white) was used in all images to mark nuclei.



<u>Figure S3, Related to Figure 3.</u> Adherens junction formation precedes airway epithelial progenitor differentiation. Immunofluorescence confocal microscopy was performed to examine the levels and localization of **(A)** E-cadherin and **(B)** α-catenin in ALI cultures. The X-Y view (bottom panels) and the Z-plane apical-basal view (top panels) for each are shown. DAPI was used to mark the nuclei (blue), which are outlined with a thin white dotted line in the Z-plane images. Scale bar = 10μm.

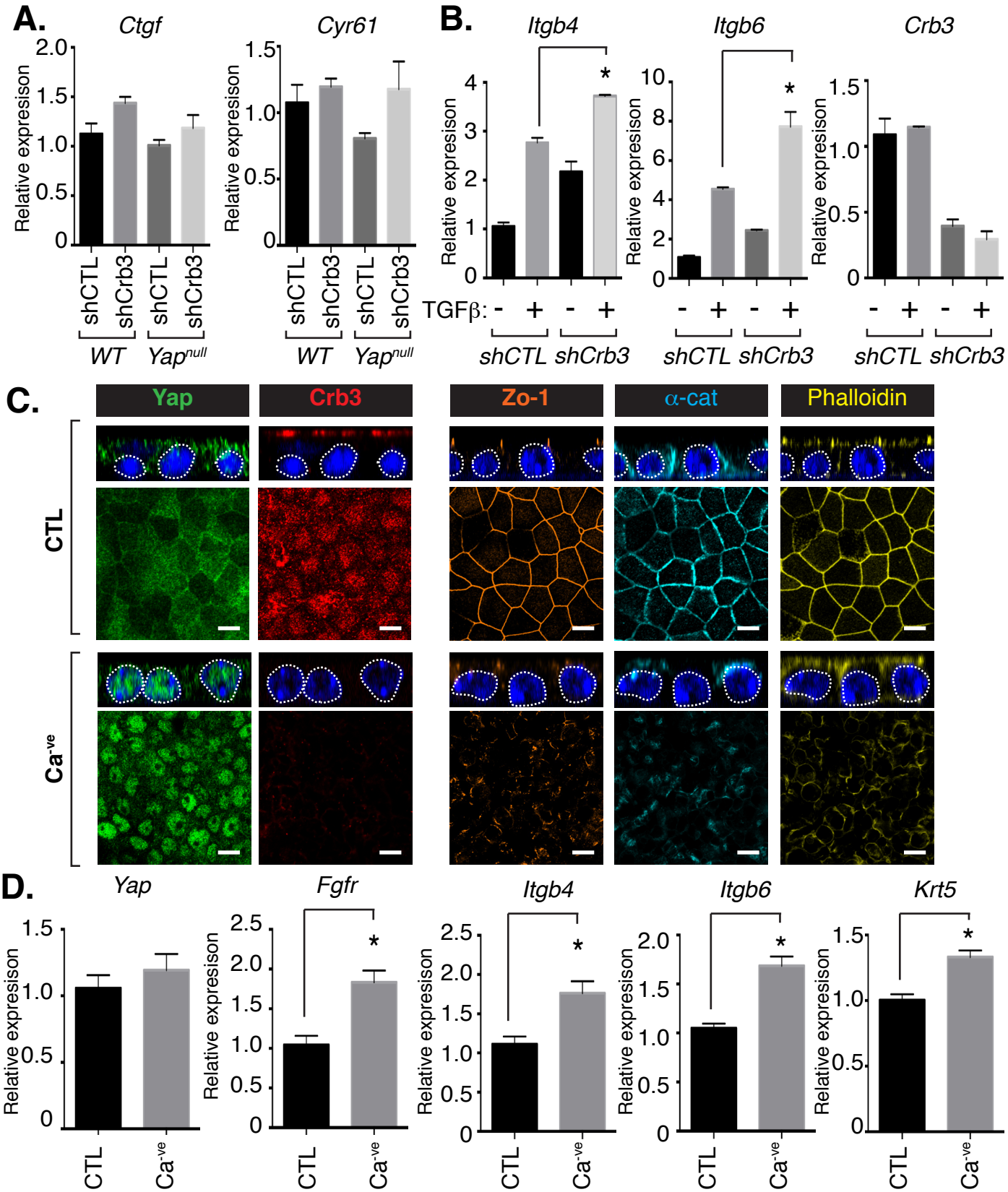


Figure S4, Related to Figure 4. **(A)** Lentiviruses were used to transduce control shRNA (shCTL) or shRNA targeting Crb3 in wild type (WT) or Yap-null airway progenitor cells, and following 6 days of ALI culture the expression of Ctgf and Cyr61 was determined from these cells (same samples as in Figure 4C). **(B)** Airway progenitors expresing shCTL or shCrb3 were treated with or without 500pMol TGFβ for 2 hours, and the expression of the indicated Yap-p63-regulated target genes was determined (n=3, average +SEM, * $p<0.01$). **(C)** Cadherin interactions were disrupted in differentated airway progenitors (10 day ALI cultures) by depleting calcium from the media (Ca-ve), and the levels and localization of the indicated proteins were determined by confocal immunofluorescence microscopy. DAPI was used to mark the nuclei (blue), which are outlined with a white dotted line in the Z-plane images. Scale bar = 10μm. **(D)** Cadherin interactions were disrupted in differentated airway progenitors (10 day ALI cultures) by depleting calcium from the media (Ca-ve) and the expression of Yap-p63-regulated target genes was determined (n=3, average +SEM, * $p<0.01$).

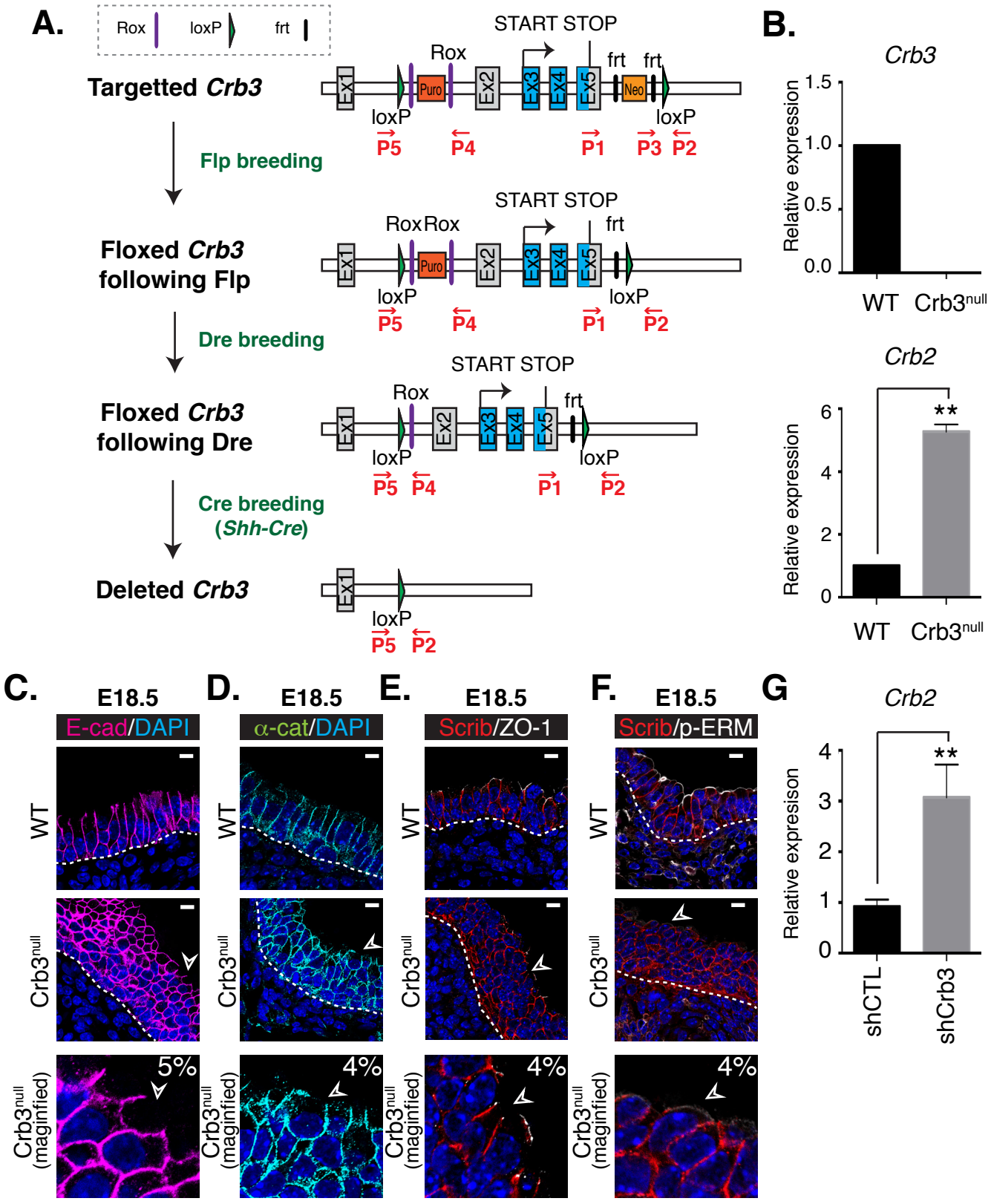


Figure S5, Related to Figure 5. Conditional knockout of Crb3 in the mouse lung epithelium. (A) Schematic of the targeting strategy used to delete the Crb3 gene. Genotyping primer combinations are depicted as red arrows (see Supplementary Experimental Procedures for further details). **(B)** Wild type or Crb3null tissues from E15.5 mice were examined by quantitative real-time PCR for Crb3 and Crb2 expression levels (n=3, average +SEM, **p<0.0001). **(C, D, E, F)** E18.5 wild type (WT) or Crb3-null proximal airways were analyzed by immunofluorescence microscopy for the localization of **(C)** E-cadherin, **(D)** α-catenin, **(E)** Scrib and ZO-1, and **(F)** Scrib and p-ERM. An arrow marks cells that appeared to have an apical domain, and the quantitated percentage of these cells is shown on the top right of the panels (n=400 cells over three experiments). The basal surface of the epithelium is outlined with a thick white dotted line, and DAPI (blue) was used to mark nuclei. Scale bar = 10μm. **(G)** Crb2 levels were determined by qPCR in Crb3 knockdown ALI cultures. (n=3, average +SEM, **p<0.0001)

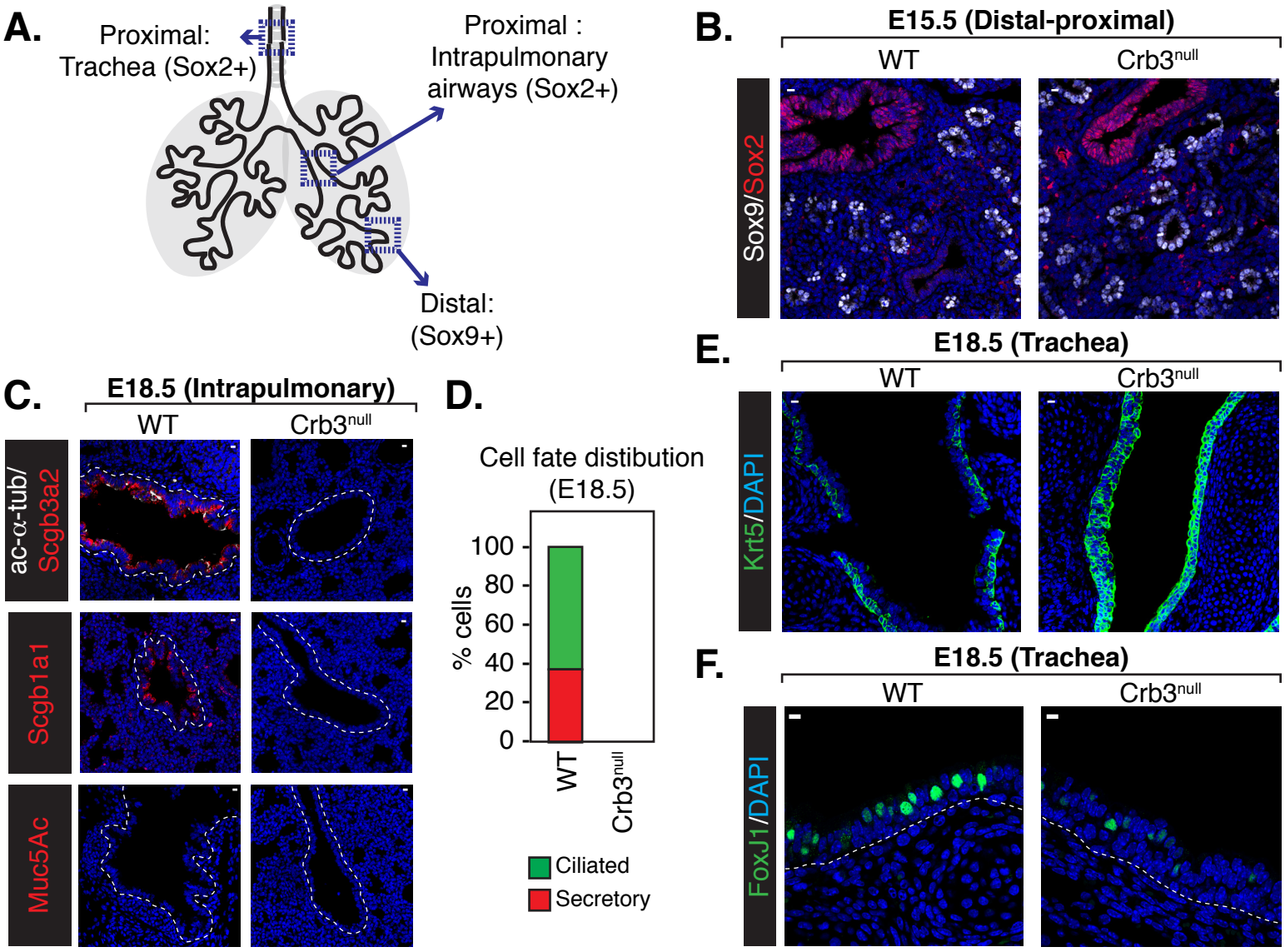
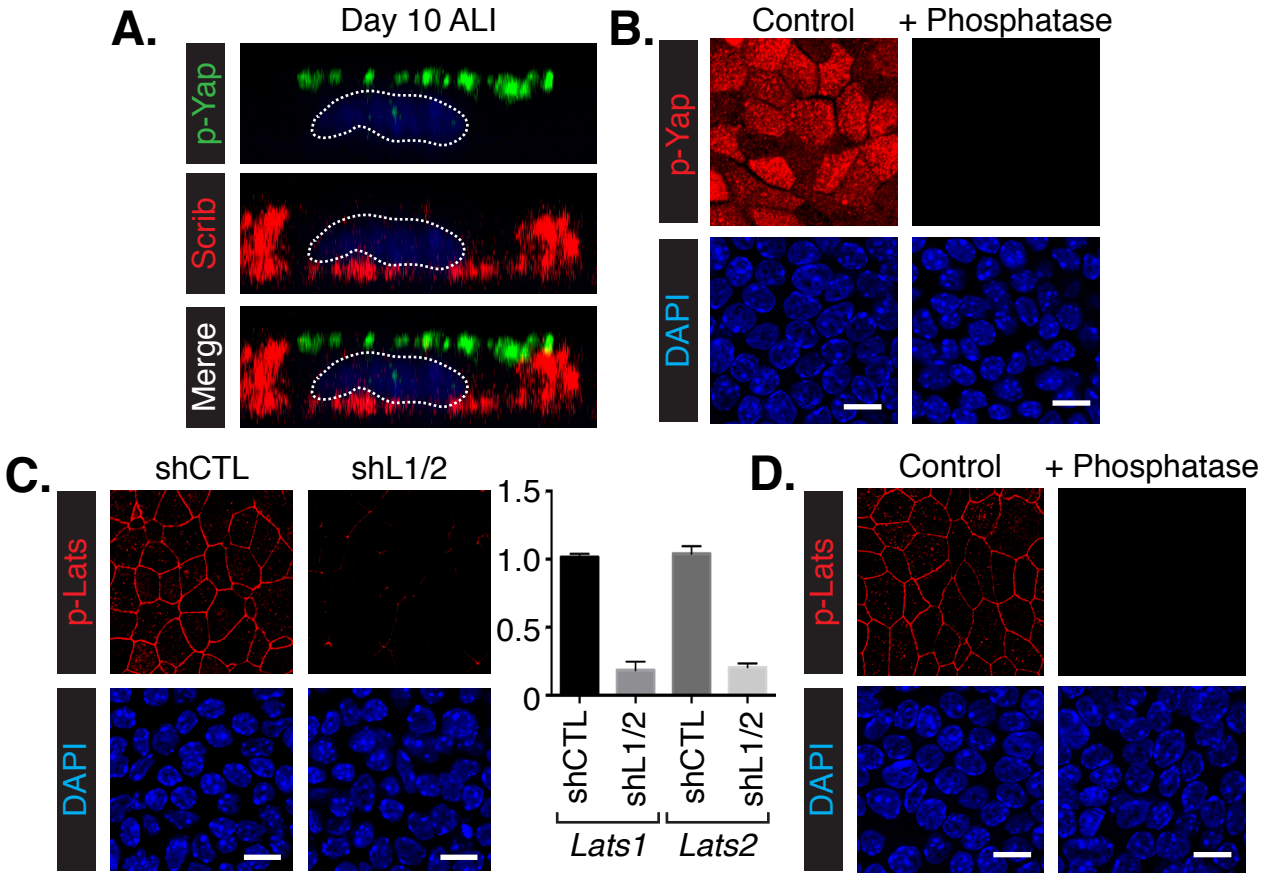


Figure S6, Related to Figure 6. Knockout of Crb3 results in airway epithelium cell fate defects. (A) Illustration depicting the different regions examined for cell fate defects in the Crb3-null lung epithelium. **(B)** E15.5 wild type (WT) or Crb3-null lungs were analyzed by immunofluorescence microscopy for Sox9 and Sox2 exprssion. **(C)** The intrapulmonary regions of E18.5 WT or Crb3-null proximal airways were analyzed by immunofluorescence microscopy for the presence of cells expressing Scgb1a1, Scgb3a2, Muc5A, or apical acetylated α-tubulin, and **(D)** based on these markers the fate of the epithelial cells was quantitated (n=800 cells from 3 experiments) **(E, F)** The tracheas of E18.5 wild type (WT) or Crb3-null proximal airways were analyzed by immunofluorescence microscopy for the presence of **(E)** cells expressing the progenitor marker Krt5, and **(F)** the multi-ciliated cell marker FoxJ1. DAPI (blue) was used to mark nuclei in all images, and the basal epithelial surface in **(C and F)** is outlined with a white dotted line. Scale bar = 10μm.



<u>Figure S7, Related to Figure 7</u>. p-YAP and p-Lats1/2 antibody specificity. (A) p-Yap and Scrib localization was assessed by confocal immunofluorescence microsopy in a single differentiated airway epithelial cell (10 days of ALI culture). The Z-plane view is shown to highlight the apical accumulation of p-Yap and the basal-lateral localization of Scrib. DAPI marked nuclei are outlined with a white dotted line. **(B)** Differentiated airway epithelial cells (10 days of ALI culture) were treated with or without Antarctic phosphatase for 2 h and p-Yap levels were determined by confocal immunofluorescence microscopy. **(C)** Lentiviruses were used to transduce control shRNA (shCTL) or shRNA targeting Lats1 and Lats2 (shL1/2) in airway progenitor cells isolated from mouse tracheas, and following 6 days of differentiation, the levels and localization of p-Lats1/2 was determined (the X-Y view is shown), and the level of Lats1 and Lats2 knockdown was determined in parallel cultures by qPCR (n=3, average+SEM). **(D)** Differentiated airway epithelial cells (10 days of ALI culture) were treated with or without Antarctic phosphatase for 2 h, and p-Lats1/2 levels were assessed using immunofluorescence microscopy. The X-Y view is shown. DAPI was used to mark nuclei. Scale bar = 10μm in all images.

SUPPLEMENTAL EXPERIMENTAL PROCEDURES

Genotyping details for the *Crb3*-null mice

Primers

Primer Name	Nucleotide sequence (5'– 3')
1. Crb3-wtflpF	**GAAGGGAAGAAAGTTCTTCAAAGACC**
2. Crb3-R	**CCTCATCCACCACTCCAAGTGACC**
3. Crb3-vF	**GCTGGGGCTCGACTAGAGCTTG**
4. Crb3-dreR	**GGAAGTGTCTGTGAGGAAGCATGG**
5. Crb3-F	**CAAGCTGTGCCCATACATCTGTGG**

Genotyping PCRs

Primer Combinations	PCR product size (bp)	Genotype
1 & 2	236	Wild Type
2 & 3	340	Targeted Floxed *Crb3* mutant
1 & 2	601	Floxed *Crb3* mutant following Flp
4 & 5	228	Floxed *Crb3* mutant following Dre
2 & 5	351	Floxed *Crb3* mutant following Cre ($Crb3^{null}$)

Antibodies used in the study

Antigen	Species	Company	Cat #	Dilution	Lot #	Use
Acetylated alpha-tubulin	Mouse	Sigma	T7415	1/1000		IF
Crb3	Rabbit	Custom	Targeted sequence: VGARAPPPPNLK LPPEERLI	1/5000	N/A	IF
CTNNA1 (α-catenin)	Mouse	BD Bioscience	610194	1/100	20174	IF
Phospho-Ezrin/Radixin Moesin (41A3)	Rabbit	Cell Signaling Technologies	3149S	1/100	8	IF
FoxJ1	Mouse	eBioscience	14-9965-80	1/300	E10108-1630	IF
GAPDH	Rabbit	Sigma	G8795	1/5000		IB
Ki67	Mouse	BD Bioscience	550609	1/100	38	IF
Krt5	Rabbit	Covance	PRB-160P-100	1/300	D12KF03035	IF
Krt8	Rat	Developmental Studies Hybridoma Bank	TROMA-1c	1/500	42004	IF
p63	Mouse	Santa Cruz	8431	1/50	I1112 & D0412	IF
Phospho-LATS1 T1079	Rabbit	Cell Signaling Technologies	9159S	1/100	1	IB
Phospho-LATS1/2 (T1079/T1041)	Rabbit	Assay BioTech	A8125	1/100	118125	IF, PLA
Scgb1a1	Goat	Santa Cruz	9772	1/300	C2913	IF
Scgb3a2	Rabbit	Custom antibody	N/A	1/5000	N/A	IF
Scrib	Goat	Santa Cruz	11049	1/100	L0513	IF
Sox2	Rabbit	Seven Hills	WRAB-1236	1/500	1013A	IF
Sox9	Goat	R&D Systems	AF3075	1/100	WIL0413111	IF
Yap	Mouse	Santa Cruz	101199	1/100	B2713 & A0512	IF, PLA
Yap-p-S127 (recognizes mouse p-S112)	Rabbit	Cell Signaling Technologies	13008S	1/100	1 & 2	IF
Zo-1	Rabbit	Zymed	61-7300	1/100		IF

Primers used for SYBR Green RT-qPCR

Target	Direction	Sequence
Gapdh	Forward	TGTTCCTACCCCCAATGTGT
Gapdh	Reverse	GGTCCTCAGTGTAGCCCAAG
Yap	Forward	AATGTGGACCTTGGCACACT
Yap	Reverse	ACTCCACGTCCAAGATTTCG
Crb2	Forward	GATCCTAACAGCTTCCGTTGC
Crb2	Reverse	GCACTCGTAGTGATCTGCCA
Crb3	Forward	CCGGACCCTTTCACAAATAG
Crb3	Reverse	TCGCATGAGCAGAAACAGTC
Lats1	Forward	GCGATGTCTAGCCCATTCTC
Lats1	Reverse	GGTTGTCCCACCAACATTTC
Lats2	Forward	ACAGAGACGCAGCTGAAGGT
Lats2	Reverse	CACAGCTTCGTGATGAGGTC
Ctgf	Forward	GGGCCTCTTCTGCGATTTC
Ctgf	Reverse	ATCCAGGCAAGTGCATTGGTA
Cyr61	Forward	TAAGGTCTGCGCTAAACAACTC
Cyr61	Reverse	CAGATCCCTTTCAGAGCGGT
Fgfr	Forward	CCTCGATGTCGTTGAACGGTC
Fgfr	Reverse	CAGCATCCATCTCCGTCACA
Itgα6	Forward	GCTGTTCTTGCCGGGATTCT
Itgα6	Reverse	AGTATGGATCTCAGCCTTGT
Itgβ4	Forward	ACGATTGCCCCTTTAAAGTC
Itgβ4	Reverse	GCAACAGGAGGAAGATGAGC