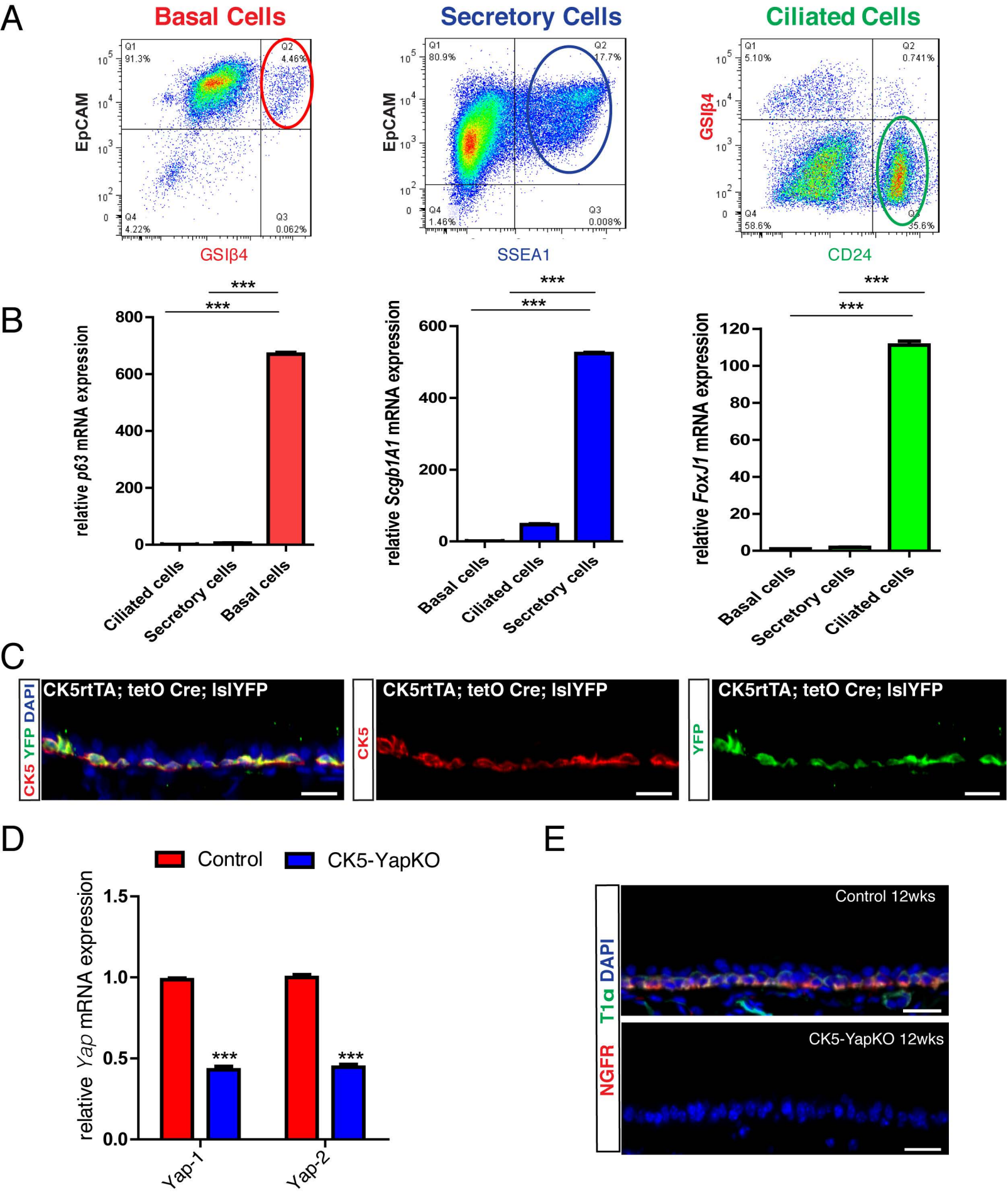
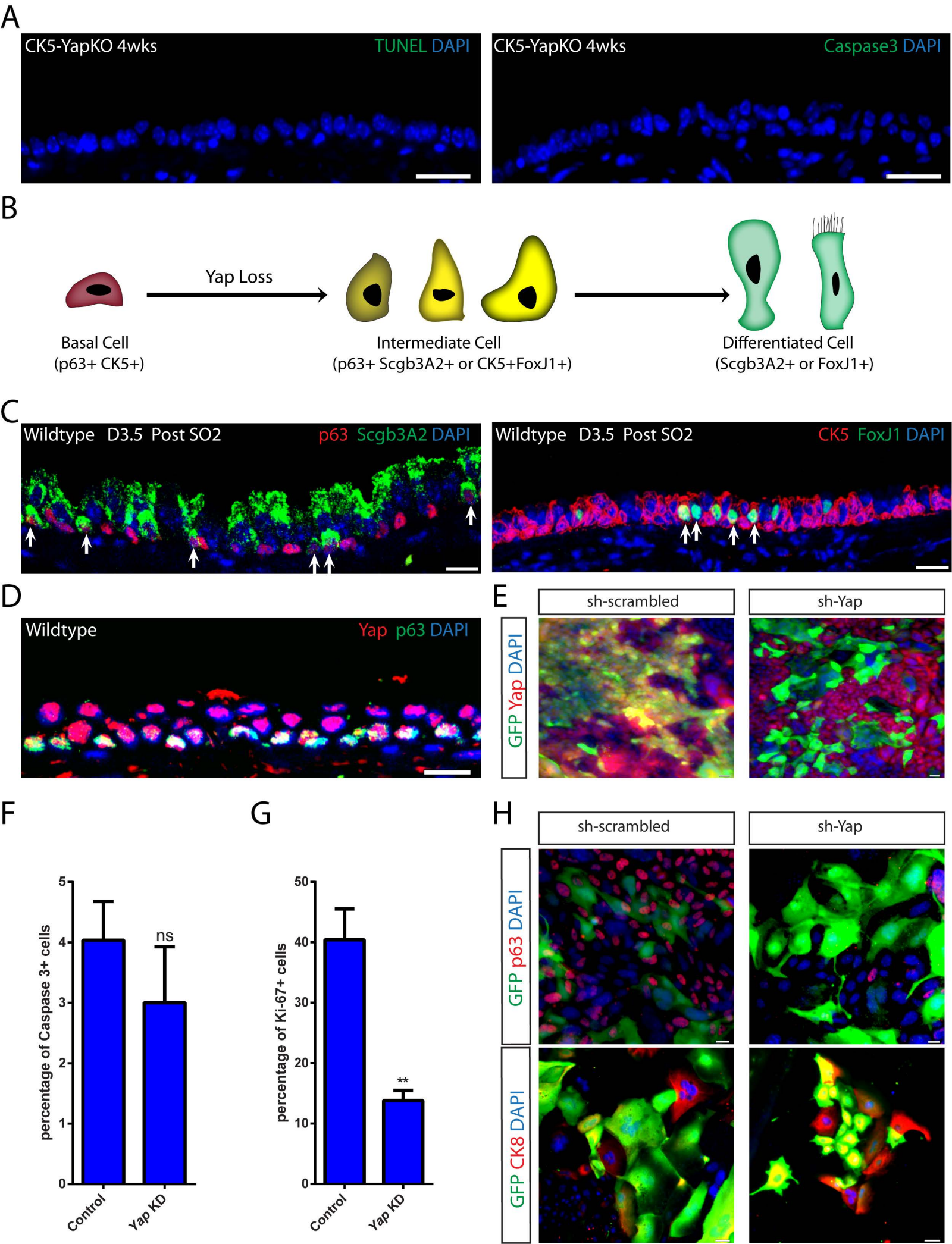
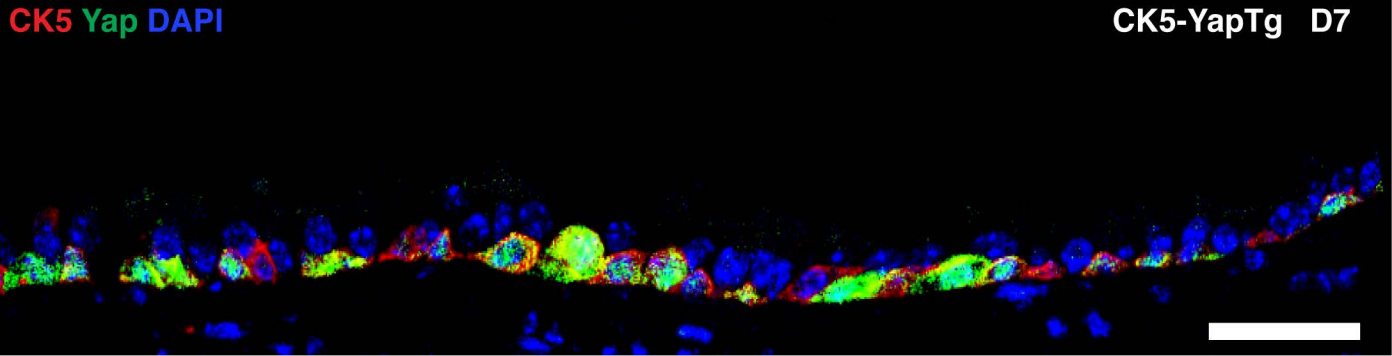
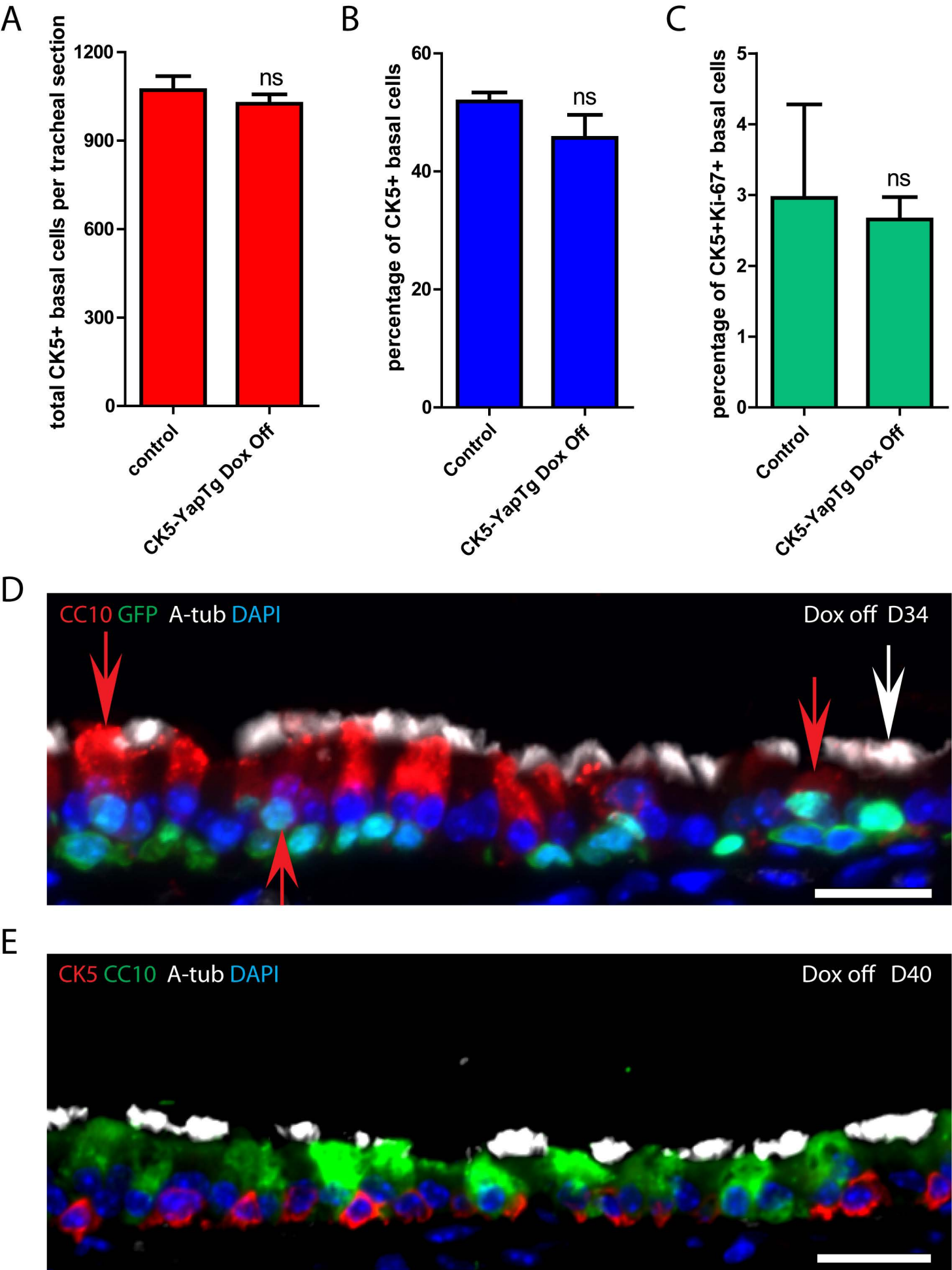
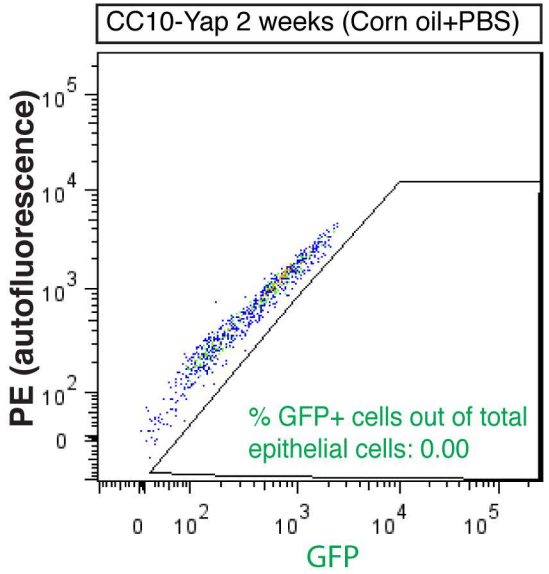

A
Basal Cells
Secretory Cells
Ciliated Cells
EpCAM
GSIβ4
SSEA1
CD24
Q1 91.3%
Q2 4.46%
Q4 4.22%
Q3 0.062%
Q1 80.9%
Q2 17.7%
Q4 1.46%
Q3 0.008%
Q1 5.10%
Q2 0.741%
Q4 58.6%
Q3 35.6%
B
relative p63 mRNA expression
relative Scgb1A1 mRNA expression
relative FoxJ1 mRNA expression
***
Ciliated cells
Secretory cells
Basal cells
C
CK5rtTA; tetO Cre; lslYFP
CK5 YFP DAPI
CK5
YFP
D
Control
CK5-YapKO
relative Yap mRNA expression
Yap-1
Yap-2
E
Control 12wks
CK5-YapKO 12wks
NGFR T1α DAPI

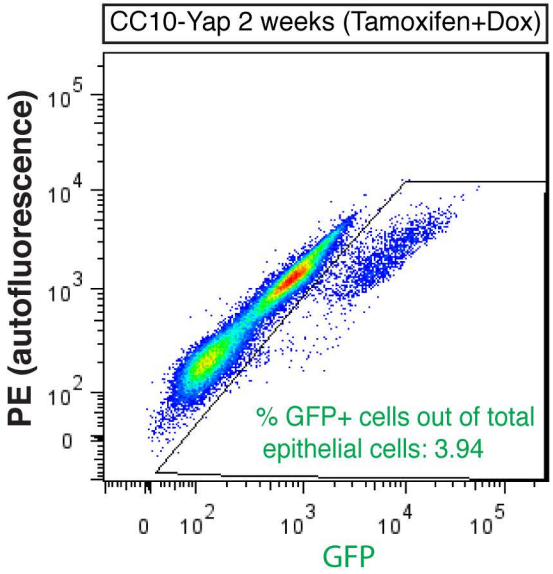

A
CK5-YapKO 4wks
TUNEL DAPI
CK5-YapKO 4wks
Caspase3 DAPI
B
Yap Loss
Basal Cell
(p63+ CK5+)
Intermediate Cell
(p63+ Scgb3A2+ or CK5+FoxJ1+)
Differentiated Cell
(Scgb3A2+ or FoxJ1+)
C
Wildtype D3.5 Post SO2
p63 Scgb3A2 DAPI
Wildtype D3.5 Post SO2
CK5 FoxJ1 DAPI
D
Wildtype
Yap p63 DAPI
E
sh-scrambled
sh-Yap
GFP Yap DAPI
F
percentage of Caspase 3+ cells
ns
Control
Yap KD
G
percentage of Ki-67+ cells
**
Control
Yap KD
H
sh-scrambled
sh-Yap
GFP p63 DAPI
GFP CK8 DAPI


CK5 Yap DAPI
CK5-YapTg D7

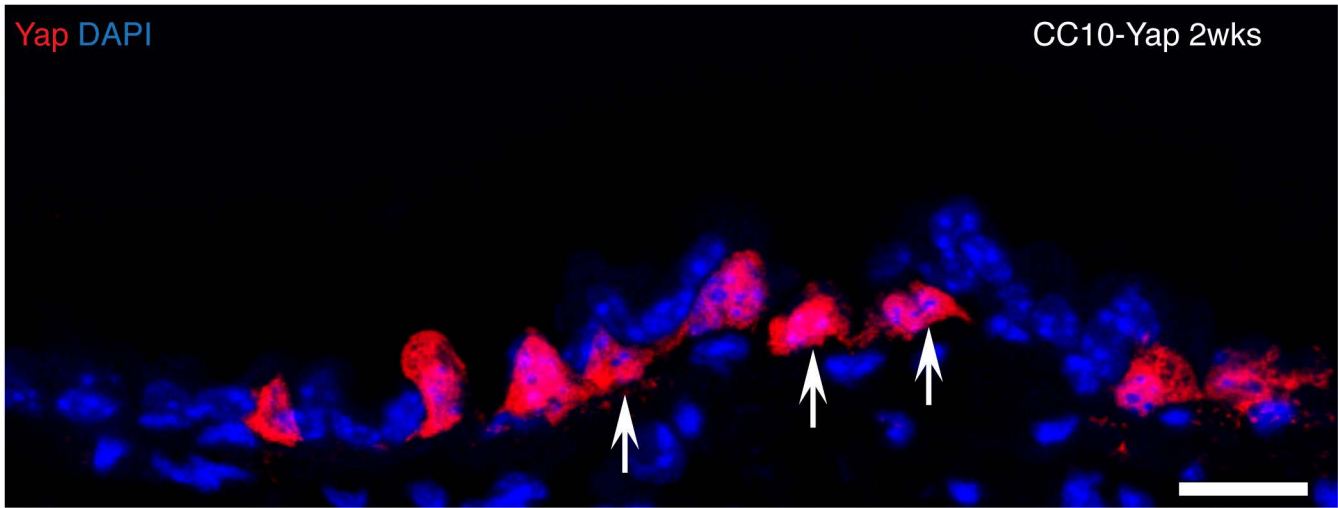

A
total CK5+ basal cells per tracheal section
1200
900
600
300
0
ns
control
CK5-YapTg Dox Off
B
percentage of CK5+ basal cells
60
40
20
0
ns
Control
CK5-YapTg Dox Off
C
percentage of CK5+Ki-67+ basal cells
5
4
3
2
1
0
ns
Control
CK5-YapTg Dox Off
D
CC10 GFP A-tub DAPI
Dox off D34
E
CK5 CC10 A-tub DAPI
Dox off D40

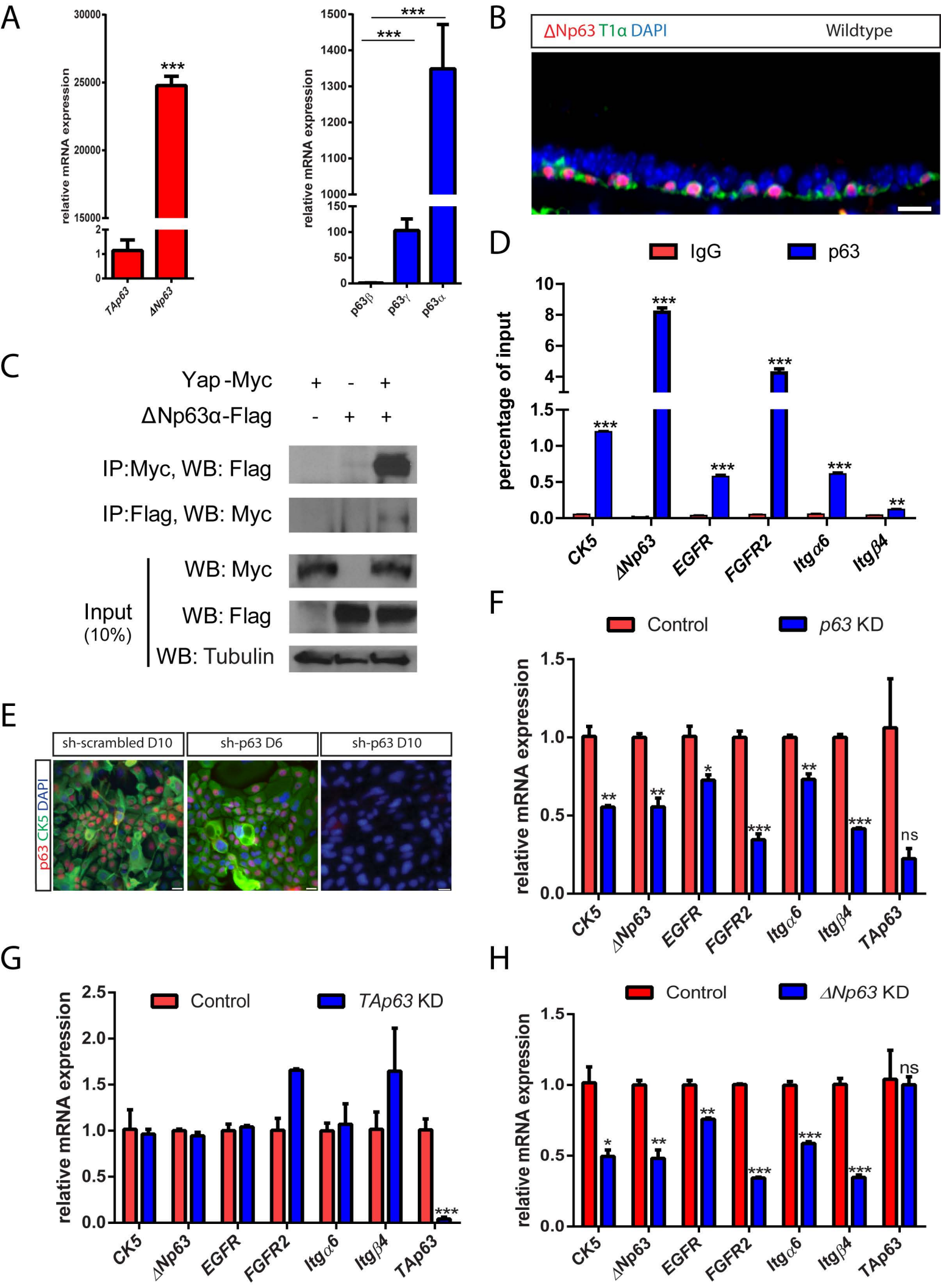
A

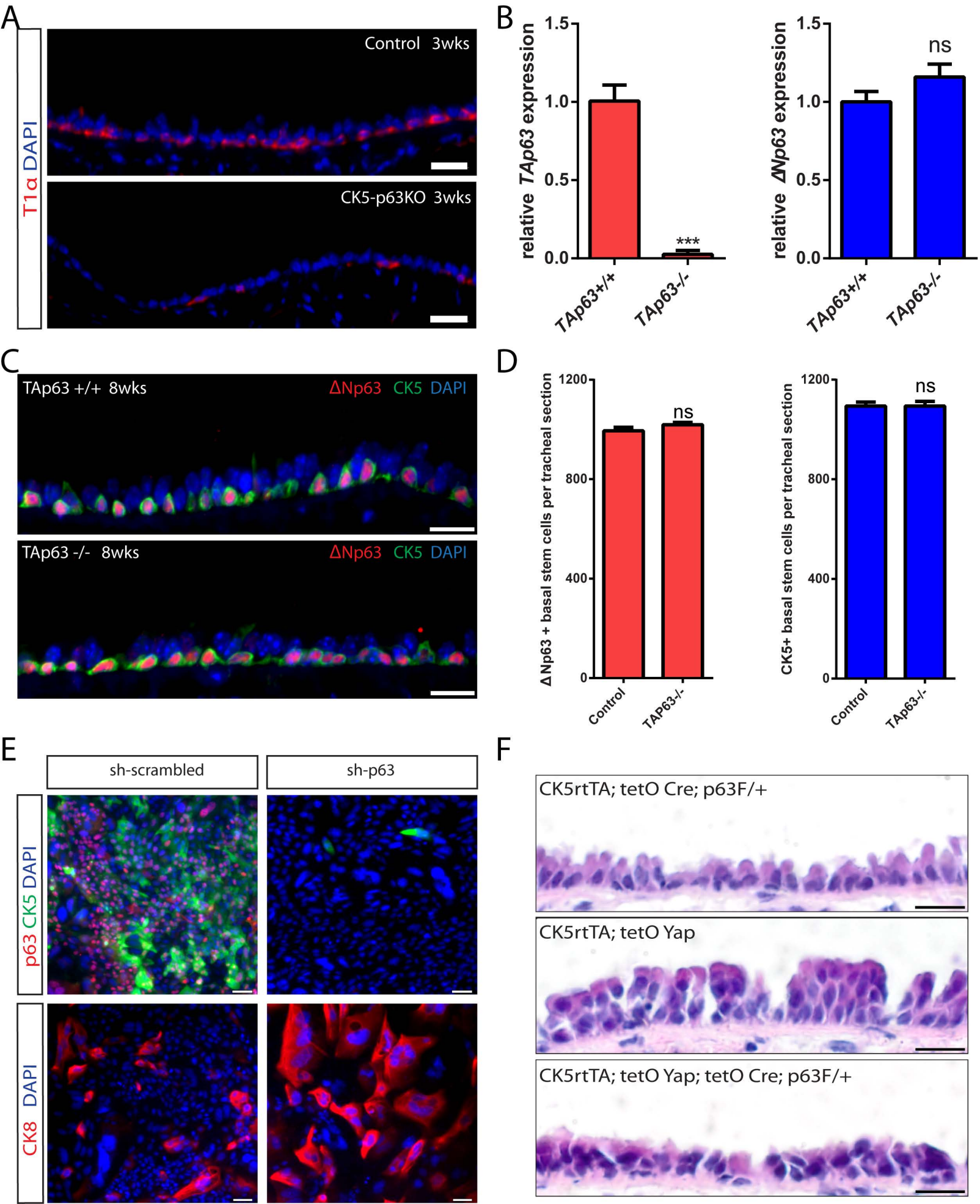

CC10-Yap 2 weeks (Corn oil+PBS)
PE (autofluorescence)
GFP
% GFP+ cells out of total epithelial cells: 0.00


CC10-Yap 2 weeks (Tamoxifen+Dox)
PE (autofluorescence)
GFP
% GFP+ cells out of total epithelial cells: 3.94

B


Yap DAPI
CC10-Yap 2wks


A
relative mRNA expression
30000
25000
20000
15000
2
1
0
***
TAp63
ΔNp63
1500
1400
1300
1200
1100
1000
150
100
50
0
***
***
p63β
p63γ
p63α
B
ΔNp63 T1α DAPI
Wildtype
C
Yap -Myc + - +
ΔNp63α-Flag - + +
IP:Myc, WB: Flag
IP:Flag, WB: Myc
Input (10%)
WB: Myc
WB: Flag
WB: Tubulin
D
IgG
p63
percentage of input
10
8
6
4
1.5
1.0
0.5
0.0
***
***
***
***
***
**
CK5
ΔNp63
EGFR
FGFR2
Itgα6
Itgβ4
E
sh-scrambled D10
sh-p63 D6
sh-p63 D10
p63 CK5 DAPI
F
Control
p63 KD
relative mRNA expression
1.5
1.0
0.5
0.0
**
**
*
***
**
***
ns
CK5
ΔNp63
EGFR
FGFR2
Itgα6
Itgβ4
TAp63
G
Control
TAp63 KD
relative mRNA expression
2.5
2.0
1.5
1.0
0.5
0.0
***
CK5
ΔNp63
EGFR
FGFR2
Itgα6
Itgβ4
TAp63
H
Control
ΔNp63 KD
relative mRNA expression
1.5
1.0
0.5
0.0
*
**
**
***
***
ns
CK5
ΔNp63
EGFR
FGFR2
Itgα6
Itgβ4
TAp63


A
T1α DAPI
Control 3wks
CK5-p63KO 3wks
B
relative TAp63 expression
1.5
1.0
0.5
0.0
***
TAp63+/+
TAp63-/-
relative ΔNp63 expression
ns
C
TAp63 +/+ 8wks
ΔNp63 CK5 DAPI
TAp63 -/- 8wks
D
ΔNp63 + basal stem cells per tracheal section
1200
800
400
0
Control
TAP63-/-
CK5+ basal stem cells per tracheal section
TAp63-/-
E
sh-scrambled
sh-p63
p63 CK5 DAPI
CK8 DAPI
F
CK5rtTA; tetO Cre; p63F/+
CK5rtTA; tetO Yap
CK5rtTA; tetO Yap; tetO Cre; p63F/+

SUPPLEMENTAL INFORMATION

SUPPLEMENTAL FIGURE LEGENDS

Figure S1. Pure Sorts of Airway Epithelial Cell Populations and Stem Cell Loss Following Basal Stem Cell-Specific *Yap* Deletion. Related to Figure 1.

(A) Sorting of basal, secretory, and ciliated cells based upon surface expression of GSIβ4, SSEA1 and CD24 respectively. EpCAM is a marker of all airway epithelial cells. (B) Quantitative PCR of *p63*, *Scgb1a1* and *FoxJ1* transcripts verifies that sorted basal, secretory, and ciliated cells are pure. Data are represented as mean +/- SEM. (C) Immunostaining for the basal stem cell-specific marker CK5 (red) and a YFP reporter (green) shows that YFP expression is induced solely in basal stem cells following doxycycline induction in mice carrying *CK5rtTA*, *tetO Cre* and *LSL-YFP* alleles. (D) Quantitative PCR using two pairs of primers (Yap-1 and Yap-2) in control and post-recombination CK5-YapKO sorted basal stem cells one month after doxycycline treatment. Control mice possess only the *CK5 rtTA* and *tetO Cre* alleles. Data are represented as mean +/- SEM. (E) Immunostaining for T1α (green) and NGFR (red) reveals a loss of basal stem cells in CK5-YapKO trachea following three months of doxycycline administration. Scale bars represent 15 μm in C and E.

Figure S2. Basal Stem Cells Undergo Differentiation Upon Yap Loss. Related to Figure 2.

(A) There is no increase in TUNEL (green) or activated Caspase 3 (green) staining in CK5-YapKO tracheal epithelium 4 weeks after doxycycline administration. (B) Hypothetical schema showing that, following *Yap* loss, basal stem cells undergo differentiation transiting through intermediate cells positive for both basal and differentiated cell markers. (C) Transient transitional cells double-positive for p63 (red) and Scgb3A2 (green) as well as for CK5 (red) and

FoxJ1 (green) are frequently detected in the tracheal epithelium 3.5 days after sulfur dioxide (SO2) injury. White arrows point to double-positive cells. (D) Immunostaining for Yap (red) following tyramide signal amplification (TSA). Yap staining reveals clear signals in both basal stem cells marked by p63 (green) and suprabasal differentiated cells. Here the Yap antibody dilution has intentionally been increased to 1:100 to detect even minimal or residual Yap expression. (E) Immunostaining of GFP (green) and Yap (red) in basal stem cell cultures after *Yap* lentiviral transfection demonstrates efficient *Yap* knockdown (GFP+ cells that are infected with knockdown *Yap* virus do not express Yap). (F) Quantification of the percentage of Caspase 3+ cells within the GFP+ virally infected cell population reveals no difference in the degree of apoptosis following *Yap* knockdown. (G) Quantification of the percentage of GFP+ virally infected cells that are also positive for Ki-67 reveals that *Yap* knockdown decreases cell proliferation. Data are represented as mean +/- SEM. ns represents not significant. (H) Immunostaining of GFP (green), basal stem cell marker p63 (red) and differentiation marker CK8 (red) in basal stem cell cultures after *Yap* lentiviral knockdown demonstrates that stem cells differentiate following *Yap* loss. Scale bar represents 20 μm in A, E and H, and 10 μm in C and D.

Figure S3. Yap Overexpression in Basal Stem Cells. Related to Figure 3.

Unamplified Yap immunostaining (green) identifies Yap-overexpressing basal cells marked by CK5 (red) 7 days after doxycycline administration to CK5-YapTg mice. Scale bar represents 30 μm.

Figure S4. Normalizing Yap Expression Restores Basal Stem Cell Proliferation and Differentiation. Related to Figure 4.

(A) Quantification reveals no significant changes in total p63+ basal stem cell numbers between control and CK5-YapTg animals 20 days after doxycycline withdrawal (CK5-YapTg Dox Off). Control animals bearing only the CK5 rtTA driver underwent the same treatment as experimental animals, namely 20 days of doxycycline followed by a 20-day washout period. (B) The proportion of basal stem cells (marked by CK5) as a fraction of the total epithelial cells in the CK5-YapTg animals three weeks after doxycycline withdrawal is not statistically different than that in control animals. (C) The percentage of proliferating CK5+ basal stem cells in CK5-YapTg animals after doxycycline withdrawal is not statistically different from that in control animals. Data are represented as mean +/- SEM in A, B and C. ns indicates a lack of significance. (D) Co-immunostaining of H2BGFP (green) with the secretory cell marker CC10 (red) and the ciliated cell maker acetylated tubulin (A-tub, white) shows that H2BGFP lineage labeled stem and progenitor cells that previously overexpressed Yap terminally differentiate after Yap expression is normalized. Red arrows point to the cells double-positive for H2BGFP and CC10. White arrows point to the cells double-positive for H2BGFP and A-tub. (E) CK5+ basal stem cells (red), CC10+ secretory cells (green) and A-tub+ ciliated cells (white) occur in a normal distribution in a pseudostratified epithelium following Yap normalization. Scale bar = 20 μm in D and E.

Figure S5. Yap Overexpression in Secretory Cells Causes Them to Adopt a Basal-like Identity. Related to Figure 5.

(A) FACS analysis of control CC10-Yap animals treated with corn oil and PBS (left panel) and experimental CC10-Yap animals treated with tamoxifen followed by doxycycline (right panel). No GFP+ cells are detected in the corn oil and PBS treated CC10-Yap animals (left panel), whereas 3.94% of total epithelial cells are GFP+ in experimental animals. X axis represents GFP

expression and Y axis represents PE, which is used to gate out cells exhibiting autofluoresence. (B) Staining for ectopic Yap using unamplified Yap staining (red) in CC10-YapTg animals demonstrates that some Yap-overexpressing secretory cells altered their morphology to resemble pyramidal basal stem cells (arrows). Scale bar = 10 μm in B.

Figure S6. Yap and p63 Interact and Share Common Target Genes. Related to Figure 6.

(A) Quantitative-PCR analysis shows that *ΔNp63α* is the major p63 transcript in cultured basal stem cells. (B) Expression of ΔNp63 protein (red) occurs exclusively in T1α+ (green) basal stem cells. (C) Yap interacts with ΔNp63α in HEK cells. (D) p63 binds to the promoters of its target genes in basal stem cells. (E) Immunohistochemical analysis reveals that p63 expression (red) is decreased at day 6 and is totally abolished at day 10 after *p63* knockdown in basal cell cultures. Immunostaining reveals no significant difference in CK5 (green) between control and *p63* knockdown at day 6. However, CK5 protein expression is no longer detectable 10 days after *p63* knockdown. (F) Decrease in the mRNA expression of p63 target genes following *p63* knockdown in basal stem cells cultured for 6 days. (G) No significant decrease in the mRNA expression of p63 target genes is detected following *TAp63* knockdown. (H) Decreases in mRNA expression of p63 target genes are observed following *ΔNp63* knockdown. Data are represented as mean +/- SEM. ns represents not significant. Scale bar represents 10 μm in B and E.

Figure S7. p63 and Yap Genetically Interact. Related to Figure 7.

(A) There is a loss of basal stem cells marked by T1α (red) in CK5-p63KO tracheal epithelium after 3 weeks of doxycycline administration. Transgenic animals bearing only *CK5 rtTA* and *tetO Cre* alleles are used as controls. (B) Quantitative-PCR analysis confirms the lack of *TAp63* in *TAp63* knockout mice. No discernible change in the expression of *ΔNp63* was observed with

TAp63 deletion. (C) Immunostaining for ΔNp63 and CK5 reveals that basal stem cell maintenance is not affected by the absence of *TAp63*. (D) Quantification of cells positive for ΔNp63 and CK5 reveals that the total number of basal stem cells remains unchanged in the absence of *TAp63*, suggesting that TAp63 is dispensable for homeostatic basal stem cell maintenance in the airway. (E) Immunostaining for p63 (red) confirms that *p63* is efficiently knocked down by lentiviral short hairpin RNAs 10 days after viral infection. A representative picture showing the lack of CK5+ (green) cells (upper right panel) confirms the loss of basal stem cells after *p63* knockdown. The increase in CK8-expressing cells (red) at day 10 is evidence for basal stem cell differentiation into suprabasal progenitors following *p63* knockdown (bottom panel). (F) H & E staining of tracheal sections reveals that the epithelial stratification caused by Yap overexpression is suppressed by removing one copy of *p63*. Data is presented as mean+/- SEM in B and D. DAPI is in blue. Scale bar represents 20 μm in A and E, and 10 μm in C and F.

EXTENDED EXPERIMENTAL PROCEDURES

Mouse Tracheal Epithelial Cell Dissociation and Sorting of Different Fractions of Epithelial Cells

Minced mouse tracheal fragments were incubated in a dissociation solution containing Papain (20U/ml), EDTA (1.1 mM), 2-Mercaptoethanol (0.067 mM), Cysteine-HCl (5.5 Mm) and DNAse I (100 U/mL) for 1 hour and 30 minutes. The reaction was stopped with Ovomucoid protease inhibitor (Worthington biochemical Corporation, Cat LK003182) on a rocker at 4ºC for 20 minutes. Cells were centrifuged and resuspended in FACS buffer (2.0% FBS in PBS). Cells were then stained with EpCAM-PECy7 (1:100; 25-5791-80, eBiosciences), GSIβ4-FITC (1:100; L2895, Sigma), SSEA1-Alexa Fluor® 647 (1:50; 125608, BioLegend), and CD24-PE (1:75; 553262, BD Pharmingen) for 30 minutes in on ice. Cell specific surface markers for individual

cell populations were identified by screening 40 cell surface markers. Cell specificity was confirmed by immunostaining of sorted cell populations after cytospin. After selecting the most specific and reliable markers, basal cells were sorted as EpCAM+ GSIβ4+ cells. Ciliated cells were sorted as EpCAM+ CD24+ cells. Secretory cells were sorted as EpCAM+ SSEA1+ cells. The purity of the sorted basal (96.71%), secretory (95.74%), and ciliated cell (96.87%) populations was established using p63, CC10 (Scgb1A1)/Scgb3A2, and FoxJ1 immunostaining respectively.

Immunofluorescence and Microscopy

Tracheas were dissected and fixed in 4% PFA for 2 hours at 4°C followed by three washes in PBS. For frozen section preparations, tracheal samples were then placed in 30% sucrose overnight at 4˚C and embedded in OCT. For paraffin sections, fixed tracheal samples were dehydrated and embedded in paraffin. Frozen sections or deparaffinized sections (6 μm) were permeabilized with 0.1% Triton X-100 in PBS, blocked in 1% BSA for 30 minutes at room temperature, incubated with primary antibodies for 2 hours at room temperature or 4ºC overnight. The sections were then incubated with secondary antibodies diluted in blocking buffer for 45 min at room temperature and counterstained with 4', 6-diamidino-2-phenylindole (DAPI, 1μg/ml). Cells stained as whole mounts on a tissue culture dish were fixed with 4% PFA, washed with PBS, and stained as described above.

The primary antibodies used were: anti-Acetylated tubulin (1:500; T6793, Sigma); rabbit anti-CK5 (1:1000; ab53121, Abcam); mouse anti-CK5 (1:250; M7237, DAKO); anti-CK8 (1:50; #TROMA 1, Developmental Studies Hybridoma Bank); anti-caspase3 (1:100; 9661, Cell Signaling); anti-ΔNp63 (1:50; 619001, BioLegend); rabbit anti- ΔNp63 (1:100, kindly provided by Satrajit Sinha); anti-FoxJ1 (1:100; 14-9965, eBioscience); chicken anti-GFP (1:500; GFP-

1020, Aves Labs); rabbit anti-GFP (1:100; A11122, Invitrogen); mouse anti-Ki-67 (1:500; 550609, BD Pharmingen); rabbit anti-Ki-67 (1:200; ab15580, Abcam); anti-NGFR (1:100; ab8875, Abcam); goat anti-CC10 (also named SCGB1A1) (1:5000; kindly provided by Barry Stripp); rabbit anti-Scgb3A2 (1:3000; kindly provided by Shioko Kimura); anti-SSEA1 (1:100; 125602, BioLegend); anti-T1α (1:50; 8.1.1, Developmental Studies Hybridoma Bank); mouse anti-p63 (1:100; sc-56188, Santa Cruz); rabbit anti-p63 (1:100; SC8343, Santa Cruz), mouse anti-Yap (1:100; 101199, Santa Cruz); mouse anti-Yap (1:100; H00010413-M01, Abnova), and rabbit anti-Yap (1:100; kindly provided by Joseph Avruch). All secondary antibodies were Alexa Fluor® conjugates (488, 594 and 647) and were used at 1:500 dilutions (Life Technologies). DeadEnd™ Fluorometric TUNEL System (Promega, G3250) was used to detect apoptosis.

Images were obtained using an Olympus IX81 Inverted microscope (Olympus, Center Valley, PA). Confocal images were obtained using a Nikon A1R-A1 Confocal Microscope System (Nikon Instruments Inc., NY).

Yap TSA Amplification

Immunohistochemical staining for Yap protein was performed using a TSA Biotin Tyramide Amplification system (SAT700001EA, Perkin Elmer). Samples were PFA fixed and prepared as above. Antigen retrieval was performed in citrate buffer (10mM Citric Acid, 0.1% Tween 20, pH 6.0) using a high pressure controlled retrieval machine. Samples were then treated for 15 minutes with peroxidase blocking solution (S2003, Dako), and then incubated with primary Yap antibodies diluted in a blocking buffer containing PBS with 0.1% Triton X-100 (PBST) and 1% BSA at 4ºC overnight. Following primary antibody staining, samples were washed thoroughly with PBST and then incubated with anti-primary HRP conjugated secondary antibodies diluted at 1:100 in blocking buffer for 3 hours at room temperature. Following secondary antibody

staining, samples were washed with PBST. Active biotinylated tyramide solution (biotinylated tyramide reagent dissolved in DMSO) was added. The tyramide deposition reaction was halted using three PBS washes. Samples were then treated with a 1:250 dilution of streptavidin conjugated to Alexa Fluor® 594 (Invitrogen) in PBST for 30 minutes at room temperature. Immunoflourescent stains of additional proteins could then be achieved by sequential application of the standard immunofluorescence protocol. The following Yap antibodies were used for TSA amplification: mouse anti-Yap (101199, Santa Cruz); mouse anti-Yap (H00010413-M01, Abnova), and rabbit anti-Yap (kindly provided by Joseph Avruch).

Mouse Tracheal Epithelial Cell Culture

Sorted basal and secretory cells were plated on transwells with polyester membranes (3470, Corning) in MTEC medium (You et al., 2002). Rho kinase inhibitor was added for the initial two days following plating.

Plasmid and Lentiviral Infection

The pLKO.1 backbone vector, with either a GFP reporter or a Puromycin selection cassette, was used for lentivirally mediated gene knockdown. The sequences of the short hairpins for the scrambled control and *Yap* were described previously (Zhang et al., 2011). For *p63* knockdown, the following short hairpins were used: shp63-1 tgatcgatgccgtgcgcttta and shp63-2 gaatgaacagacgtccaattt. For *TAp63* knockdown, the following short hairpins were used: shTAp63-1 tatccgcatgcaagactcaga and shTAp63-2 tgtatccgcatgcaagactca. For *ΔNp63* knockdown, the following short hairpins were used: shΔNp63-1 aacaatgcccagactcaattt and shΔNp63-2 aatgcccagactcaatttagt. Cells infected with shRNA virus were selected with 1 ug/mL puromycin (Sigma) for 5 days before they were subjected to further analysis.

Quantitative PCR analysis

Total RNA was isolated using Trizol (Invitrogen) and cDNA was produced using the Superscript III kit (Invitrogen). Quantitative Real Time PCR (qPCR) was done following the instructions in the Brilliant II SYBR® Green QPCR Master Mix kit (600828, Agilent Technologies), and the samples were analyzed on a StepOnePlus™ Real-Time PCR System (Applied Biosystems). Sequence information for qPCR primers is provided below:

Gene name	Primer sequence
β-actin	Forward: TGGAATCCTGTGGCATCCATGAAAC
	Reverse: TAAAACGCAGCTCAGTAACAGTCCG
Scgb1A1	Forward: GATCGCCATCACAATCACTG
	Reverse: CTCTTGTGGGAGGGTATCCA
ΔNp63	Forward: GGAAAACAATGCCCAGACTC
	Reverse: GATGGAGAGAGGGCATCAAA
EGFR	Forward: GAACATCACC TGTACAGGCA
	Reverse: GGCATCTGCATACTTCCAGA
FGFR	Forward: CCTCGATGTCGTTGAACGGTC
	Reverse: CAGCATCCATCTCCGTCACA
FoxJ1	Forward: GAGCTGGAACCACTCAAAGG
	Reverse: GGTAGCAGGGCAGTTGATGT
GAPDH	Forward: AACTTTGGCATTGTGGAAGG
	Reverse: ACACATTGGGGGTAGGAACA
Itgα6	Forward: GCTGTTCTTG CCGGGATTCT
	Reverse: AGTATGGATCTCAGCCTTGT

Itgβ4	Forward: ACGATTGCCC CTTTAAAGTC
	Reverse: GCAACAGGAGGAAGATGAGC
p63α	Forward: GCTGCTCATCATGCCTGGA
	Reverse: CAGACTTGCCAAATCATCCA
p63β	Forward: GCATGGGAGCCAACATTCCTA
	Reverse: TGCCAAATCCTGACAATGCTG
p63γ	Forward: CTGCTGTACCTACCAGTGAGA
	Reverse: TGAAGCAGGCTGAAAGGAGA
TAp63	Forward: TTACAGATCTGCCATGTCGC
	Reverse: CCCAGATATGCTGGAAGACC
Yap-1	Forward: TACATAAACCATAAGAACAAGACCACA
	Reverse: GCTTCACTGGAGCACTCTGA
Yap-2	Forward: CATCT TCTGGTCAAA GATACTTC
	Reverse: CAGAATTCATCAGCGTCTG

Sulfur Dioxide Injury

The standardization of the sulfur dioxide injury model has been previously described (Kim et al., 2012; Rock et al., 2011). The exposure time was modified to 3 hours and 40 minutes of 500ppm SO2 in the strains we used so that the suprabasal epithelium was completely eliminated without damaging the basal cell layer.

Transfection

For transient transfections, 293T cells were transfected with pLPC-ΔNp63α-Flag and pQCXIH-Yap-Myc (Zhao et al., 2007) (#33091, Addgene) using Fugene 6 (E2691, Promega) according to manufacturer's instructions. Forty-eight hours post transfection, cells were harvested and nuclear extracts were prepared for co-immunoprecipitations.

Human Basal Cell Culture

This study was approved by the Partners Human Research Committee (IRB Protocol No. 2010P001354/MGH). Primary human airway epithelial cells were isolated from the discarded trachea and bronchi of donor lungs using the papain dissociation method described above and cultured in LHC-9 medium (12680-013, Life Technologies). These cultured cells were stained at each passage. All of the cells expressed the pan epithelial marker EpCAM. Over 96% of the cells were positive for the basal stem cell markers p63 and CK5.

Co-Immunoprecipitation

Nuclear extracts were prepared by suspending human basal cells in buffer A (10mmol/L Tris-HCl pH 7.5, 1.5 mmol/L MgCl2, 10 mmol/L KCl) for 20 minutes, followed by douncing. Pelleted nuclei were first suspended in 1 volume of 20 mM KCl nuclear buffer (20 mM Tris-HCl pH 7.5, 1.5 mM MgCl2, 0.2 mM EDTA, 25% glycerol). One volume of 1.2 mol/L KCl nuclear buffer was then added dropwise followed by incubation for 30 minutes at 4°C with rotation. Nuclear extracts were pre-cleared with equilibrated protein G beads. Cleared nuclear lysates were incubated with antibody and protein G beads for 4 hours at 4°C, and were then washed with wash buffer (50 mM Tris-HCl pH 7.5, 5 mM MgCl2, 0.2 mM EDTA, 0.1% NP-40, 10% glycerol) containing different concentrations of KCl (100 mM, 250 mM, 250 mM, and 100 mM). The following antibodies were used: anti-c-Myc (9E10, DSHB), anti-Flag (M2 clone, Sigma),

mouse anti-YAP (H00010413-M01, Abnova), rabbit anti-Yap (NB110-58358, Novus), mouse anti-p63 (4A4, Santa Cruz Biotechnology), rabbit anti-p63 (H129, Santa Cruz Biotechnology), rabbit anti-TAp63 (618902, Biolegend) and beta-tubulin (D-10, Santa Cruz).

Chromatin Immunoprecipitation

Human basal cells were subjected to additional cross-linking with 2 mM EGS (Ethylene Gycol-bis (Succinimidylsuccinate)) in PBS for 30 minutes at room temperature followed by washing in PBS and cross-linking with 1% (v/v) formaldehyde at room temperature for 15 minutes. Cross-linking was stopped by adding 125 mM glycine. Cells were then lysed in RIPA buffer (10 mM Tris-HCl pH 7.5, 150 mM NaCl, 1 mM EDTA, 1% (w/v) sodium deoxycholate, 0.1% (w/v) SDS, 1% (v/v) NP40, proteinase inhibitor cocktail and phosphatase inhibitor cocktail) for 2hr at 4^0C. Nuclear lysates were sonicated for 5 min 8-10 times in a bioruptor (Diagenode). Sonicated chromatin was precleared with Protein G sepharose beads (GE healthcare), pre-blocked with BSA and sonicated salmon sperm DNA (15632-011, Invitrogen). Samples were incubated with 2 μg antibody overnight at 4^0C and with beads for an additional 2 hrs. Rabbit anti-Yap (NB110-58358, Novus) and rabbit anti-p63 (H129, Santa Cruz Biotechnology) were used. Beads were washed in wash buffer 1 (150 mM NaCl, 20 mM Tris pH 8.1, 2 mM EDTA, 0.1% (w/v) SDS, 1% (v/v) Triton-X-100), wash buffer 2 (500 mM NaCl, 20 mM Tris pH 8.1, 2 mM EDTA, 0.02% (w/v) SDS, 1% (v/v) Triton-X-100), wash buffer 3 (250 mM LiCl ,10 mM Tris pH 8.1, 1 mM EDTA, 1%(w/v) sodium deoxycholate, 1% (v/v) NP40), and 10 mM Tris pH 7.9, 1 mM EDTA. After the wash, beads were incubated for 3 hours at 55^0C, then overnight at 65^0C in 10 mM Tris pH 7.9, 1 mM EDTA), 0.5% (w/v) SDS, 10μg RNase A, and 10 μg Proteinase K. DNA was purified via QIAquick PCR purification kit (28106, Qiagen) as per manufacturer's instructions. PCR was performed using IQ SYBR Green Supermix reagent (Bio-Rad) in a

MX300P machine (Stratagene). Percentage of input from the IPs was calculated by using 10% as a standard. ChIP primers for *FGFR2, EGFR* were previously described (McDade et al., 2012). ChIP-PCR primers are listed below:

ACTB	Forward: CCAGGAATTCCGACTTTCAA
	Reverse: CAGGACTGGTGAAGTGCTCA
CK5	Forward: AGCCTTGGCCCCCATCCCTTAGATT
	Reverse: GGTGTGCCATGAGCAAAACACCTTTCA
ΔNp63	Forward: GCTTGACTACCGTGGAGAGC
	Reverse: TTTTCCCAAACTCCAACCTG
EGFR	Forward: GGTTAGGGCAGCTCCTCTTT
	Reverse: TGGAATTAAGCCTCTCTGCAA
FGFR2	Forward: AAATGAGCGCGCAAGTTAGA
	Reverse: CGAACTGGACCGACTTTTTC
Itgα6	Forward: TTATAGGTCCACAAGCGGTGAATGC
	Reverse: TCCCCATGACTAAAGCAAGTCCACACC
Itgβ4	Forward: GAGCCGCAGCCCTTTCCG
	Reverse: ACGAGGCGGGCAGCGCTTTAT

SUPPLEMENTAL REFERENCES

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