Supplementary Figure Legends

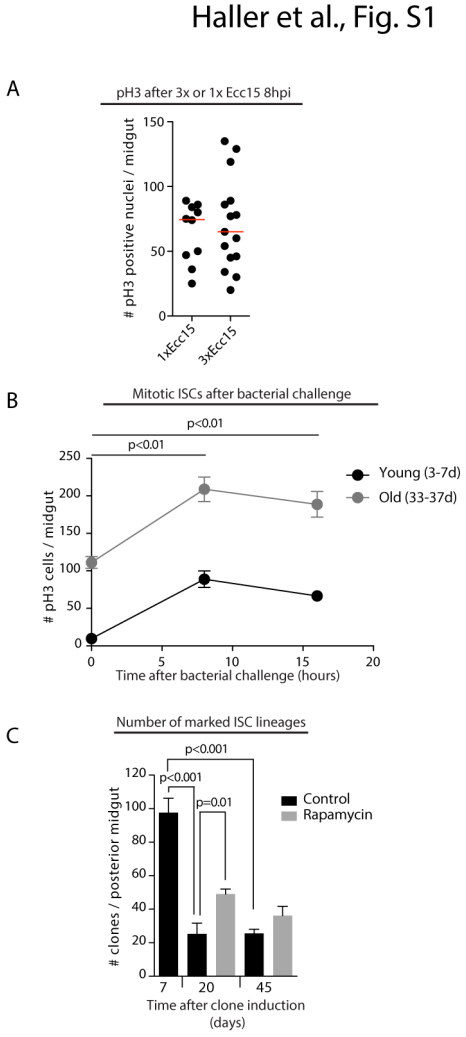

Figure S1 (relates to Figure 1). Influence of TOR signaling on epithelial regeneration in the fly intestine.

A. Quantification of mitotic figures (pH3 staining) at 8h post *Ecc15* infection in wild type flies during their first exposure to *Ecc15* (1xEcc15) or their third exposure to *Ecc15* (3xEcc15). Data points represent the total number of pH3 positive nuclei per midgut for one animal, red lines indicate the median (n>10). ANOVA U-test.

B. Time course of ISC proliferation (pH3+ cells) during *Ecc15* infection in young and old wild type flies. Data points represent the mean +/- SEM (n>10). ANOVA U-test.

C. Quantification of MARCM clones in wild type flies at 7, 20 and 45 days after clone induction and with or without Rapamycin treatment (started at 7 days after clone induction). Bars represent the mean +/- SEM (n>8). ANOVA U-test.

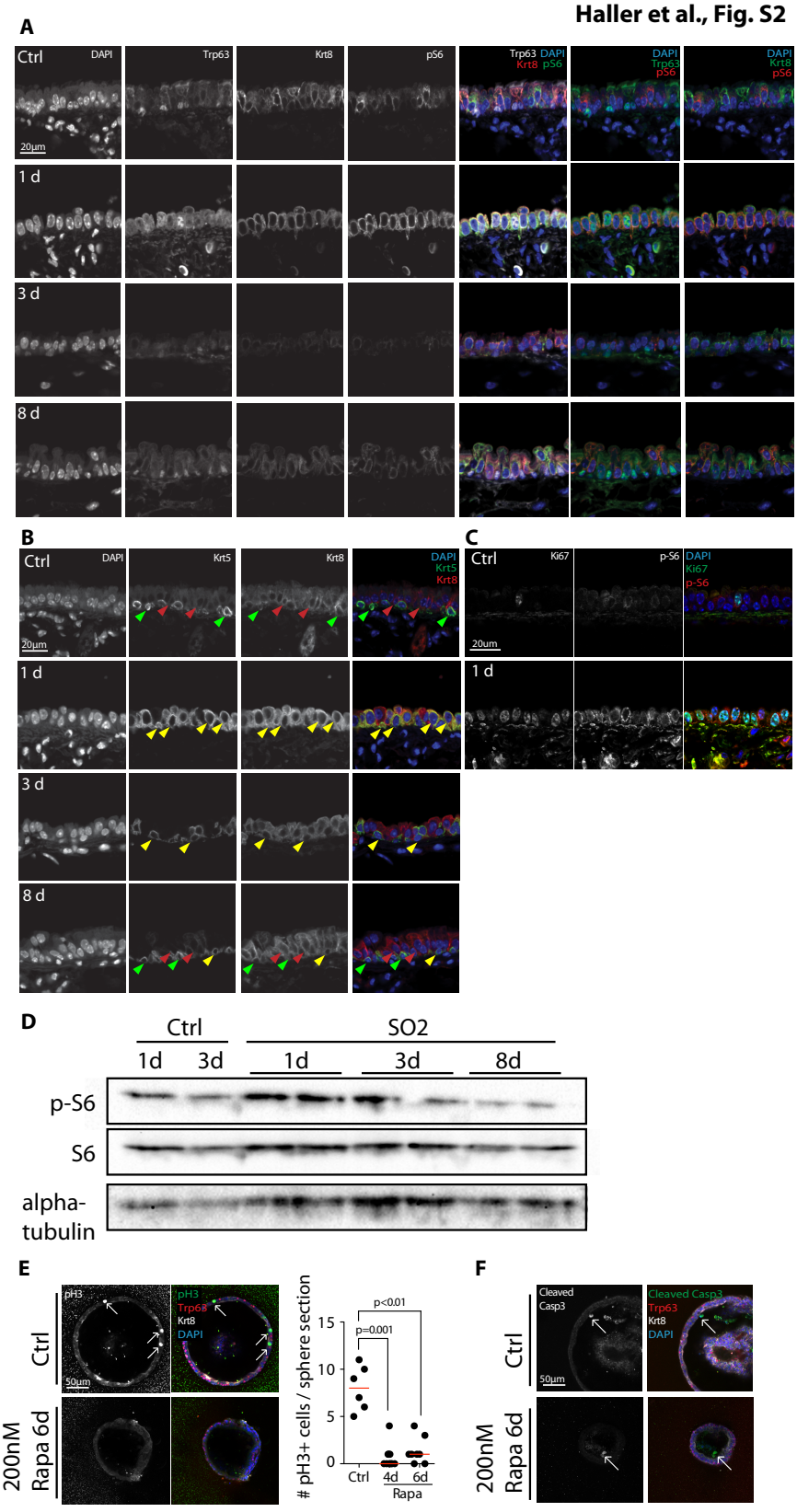
A
Ctrl
DAPI
Trp63
Krt8
pS6
Trp63 DAPI
Krt8 pS6
DAPI
Trp63
pS6
DAPI
Krt8
pS6
20μm
1 d
3 d
8 d
B
Ctrl
DAPI
Krt5
Krt8
DAPI
Krt5
Krt8
20μm
1 d
3 d
8 d
C
Ctrl
Ki67
p-S6
DAPI
Ki67
p-S6
20um
1 d
D
Ctrl
SO2
1d
3d
1d
3d
8d
p-S6
S6
alpha-
tubulin
E
Ctrl
200nM
Rapa 6d
pH3
pH3
Trp63
Krt8
DAPI
50μm
pH3+ cells / sphere section
p<0.01
p=0.001
15
10
5
0
Ctrl
4d
6d
Rapa
F
Ctrl
200nM
Rapa 6d
Cleaved
Casp3
Cleaved Casp3
Trp63
Krt8
DAPI
50μm

Figure S2 (relates to Figure 2). Tor signaling is activated in tracheal epithelial cells upon regeneration.

A. Complete set of panels for data shown in Fig.2B. Confocal images of a representative area of the tracheal epithelium of either control or SO_2 treated mice. IHC to detect Trp63 (BCs), Krt8 (differentiated cells) and p-S6 (Tor signaling activity). For each time-point pictures show one representative epithelium (from n=5 for SO_2 treated and n=3 for controls).

B. Complete set of panels for data shown in Fig.2D and Fig.2E. Confocal images of a representative area of the tracheal epithelium of either control or SO_2 treated mice. IHC to detect Krt5 (BCs) and Krt8 (differentiated cells). Arrowheads point to examples of Krt5+ cells (green), Krt8+ cells (red) and Krt5+/Krt8+ cells (yellow). For each time-point pictures show one representative epithelial section (from n=5 for SO_2 treated and n=3 for controls).

C. Confocal images of an area of the tracheal epithelium of wild type mice, either mock treated or one day after SO2 exposure. IHC detecting Ki67 (green and white) and p-S6 (red and white). Images show representative epithelia (n>5).

D. Western blot to detect phosphorylated ribosomal protein S6 (p-S6) in whole trachea at indicated time points after SO_2 exposure. Total S6 and alpha-tubulin were detected as loading controls. Representative blot is shown; quantification is shown in Fig. 2C.

E & F Confocal images of tracheospheres treated with Rapamycin (200nM) for 6 days. In E, Spheres were stained for a mitotic marker (pH3, green and white), a BC marker (Trp63, red) and a differentiated cell marker (Krt8, white). Quantification of pH3+ cell numbers is shown in the chart (T test). In F, Spheres were stained for an apoptotic marker (Cleaved Caspase 3, green and white), a BC marker (Trp63, red) and a differentiated cell marker (Krt8, white). For both DAPI is shown in blue. Panels show one representative out of n≥20 spheres.

Haller et al., Fig. S3

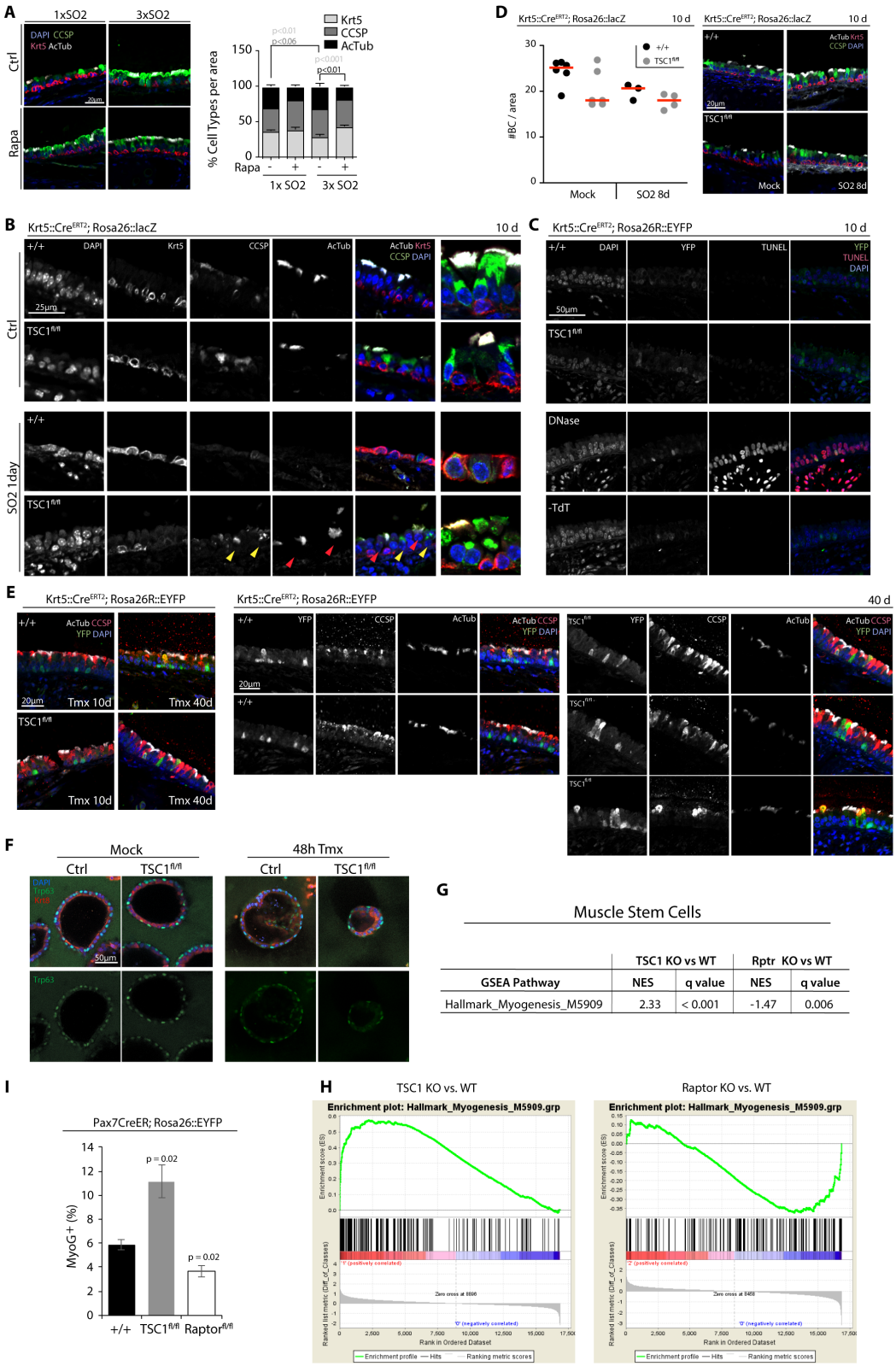

Figure S3 (Related to Figure 3). SC loss and differentiation in response to TOR activation in the mouse tracheal epithelium and muscle

A. Confocal images of an area of the tracheal epithelium and quantification of BCs (Krt5), secretory cells (CCSP) and ciliated cells (AcTub) in young animal two weeks after the first or third SO2 exposure (SO2 exposure was performed every other week). IHC detecting BCs (red), secretory cells (CCSP, green) and ciliated cells (AcTub, white). Images show representative epithelia (n=5). Bars representing the mean +/- SEM. ANOVA U-test (n>4).

B. Confocal images of an area of the tracheal epithelium of controls (Krt5::Cre^{ERT2}, Rosa26::lacZ / +) or TSC1 deletor mice (Krt5::Cre^{ERT2}, Rosa26::lacZ / TSC1$^{fl/fl}$) of SO2 or Mock treated mice at one day after exposure. The SO2 exposure was performed 10 days after the last Tamoxifen injection (4 IP injections every other day). IHC detecting BCs (Krt5, red and white), Secretory Cells (CCSP, green and white) and Ciliated Cells (AcTub, white). Images show representative epithelia (n≥4).

C. Confocal images of an area of the tracheal epithelium of controls (Krt5::Cre^{ERT2}, Rosa26::eYFP / +) or TSC1 deletor mice (Krt5::Cre^{ERT2}, Rosa26::eYFP / TSC1$^{fl/fl}$) at 10 days after the last Tamoxifen injection (3 injections, every other day). TUNEL staining detecting apoptotic cells (red and white) was performed in addition to a positive control (DNase treated section) and a negative control (without the TdT enzyme). Images show representative epithelia (n≥4).

D. On the left, quantification of BCs (Trp63 positive cells) in the tracheal epithelium of controls (Krt5::Cre^{ERT2}, Rosa26::lacZ / +) or TSC1 deletor mice (Krt5::Cre^{ERT2}, Rosa26::lacZ / TSC1$^{fl/fl}$), mock or SO2 treated. The SO2 exposure was performed 10 days after the last Tamoxifen injection (4 IP injections every other day). Each data point represents the mean for one trachea. Red lines represent the median. ANOVA U-test. On the right, confocal images of representative tracheal epithelia at 8 days after SO2 stained with different cell type markers (Krt5 in red for BCs, CCSP in green for secretory cells and AcTub in white for ciliated cells). n≥3.

E. Confocal images of an area of the tracheal epithelium of controls (Krt5::Cre^{ERT2}, Rosa26::eYFP / +) or TSC1 deletor mice (Krt5::Cre^{ERT2}, Rosa26::eYFP / TSC1$^{fl/fl}$) at 10 or 40 days after the last Tamoxifen injection (3 injections, every other day). IHC detecting eYFP clones (green), Secretory Cells (CCSP, red) and Ciliated Cells (AcTub, white). Images show representative epithelia (n≥4).

F. Confocal images of tracheospheres derived from control (Krt5::Cre^{ERT2}, Rosa26::lacZ / +) or TSC1 deletor mice (Krt5::Cre^{ERT2}, Rosa26::lacZ / TSC1$^{fl/fl}$). Spheres were grown for 9 days, then exposed to Tamoxifen for 2 days. Trp63+ cells (BCs, green) and differentiated cells (Krt8+, red) are detected by IHC (DAPI blue).

G., H. mTORC1 signaling promotes expression of genes involved in skeletal muscle myogenesis in MuSCs. Data are displayed as normalized enrichment scores (NES) and FDR-adjusted p-values (q value) from gene-set enrichment analysis (GSEA) of gene expression profiles of total RNA purified from YFP+ MuSCs immediately after isolation

from adult WT, TSC1 KO, or Raptor KO mice. Gene expression profiling experiments were described in Rodgers et al., Nature 2014 and the data are deposited as GSE55490.

I. mTORC1 signaling promotes expression of MyoG in MuSCs. Data are presented as percent MyoG MuSCs, 12 hours after FACS isolation of YFP+ cells from adult WT, TSC1 KO, Raptor KO mice, as measured by IF staining. Mean and sem, P values were calculated using two tailed students tTest (WT; n =3, TSC1 KO; n = 3, Raptor KO; n = 4).

WT: *Pax7CreER /+;rosa26EYFP /+*; TSC1 KO: *$TSC1^{fl/fl}$; Pax7CreER /+;rosa26EYFP /+*; Raptor KO: *$RPTR^{fl/fl}$; Pax7CreER /+;rosa26EYFP /+*.

Haller et al., Fig. S4

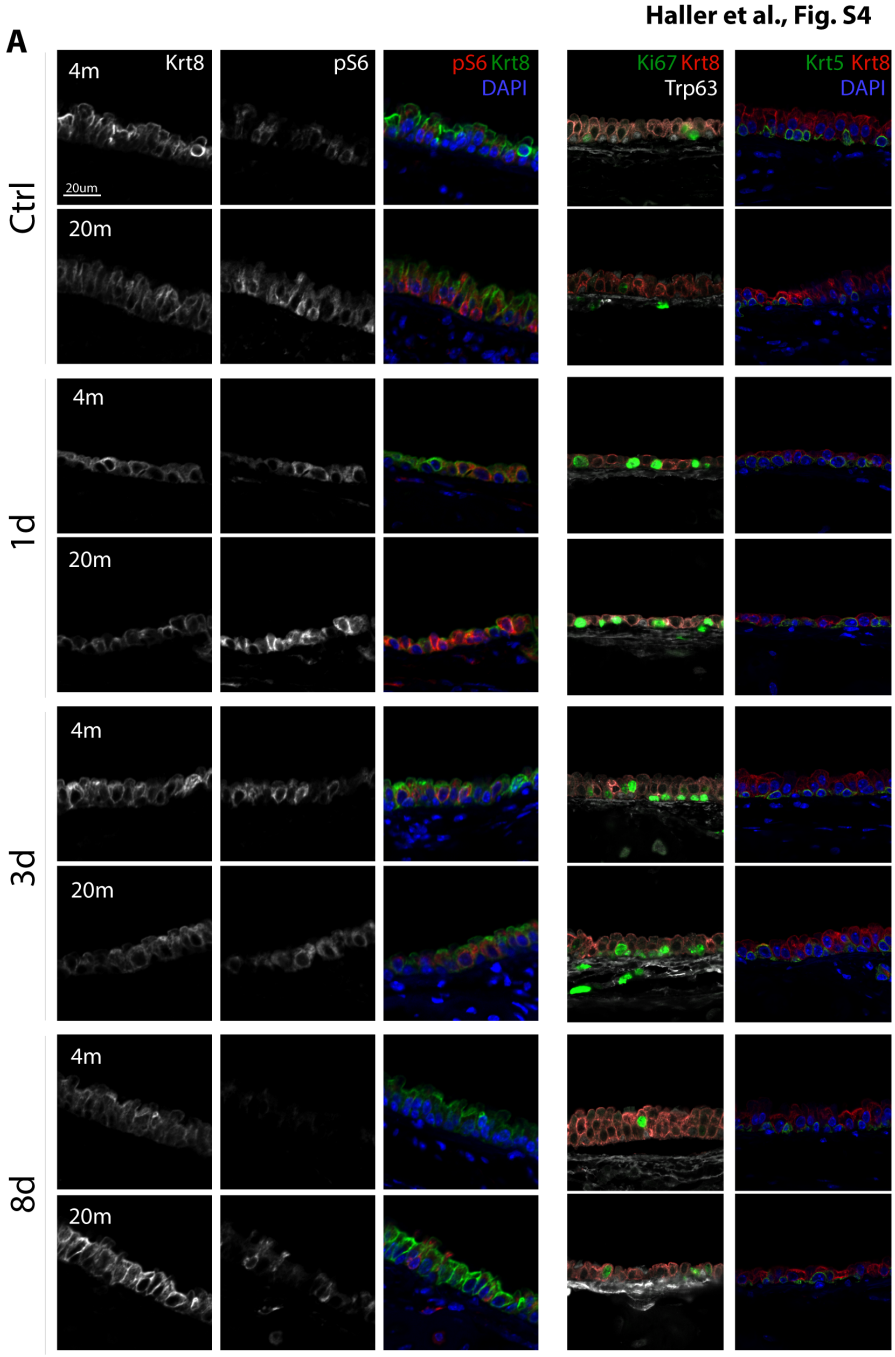

Figure S4 (related to Figure 4). Delayed regeneration of the airway epithelium of old mice.

A: Images corresponding to the quantifications in Fig.4A. Confocal images of an area of the tracheal epithelium of either 4 month (4m) or 20 month (20m) old wild-type (C57BL/6J) mice at the indicated timepoints after SO2 exposure. IHC detecting Trp63 (white), pS6 (red), Krt5 (green), Krt8 (red or green), and Ki67 (green). Images show representative epithelia (n≥3 mice).

B: Quantification of p-S6 levels in the BCs (fluorescence intensity normalized to background) in the conditions shown in A. Mean and SEM are shown, ANOVA U-Test.

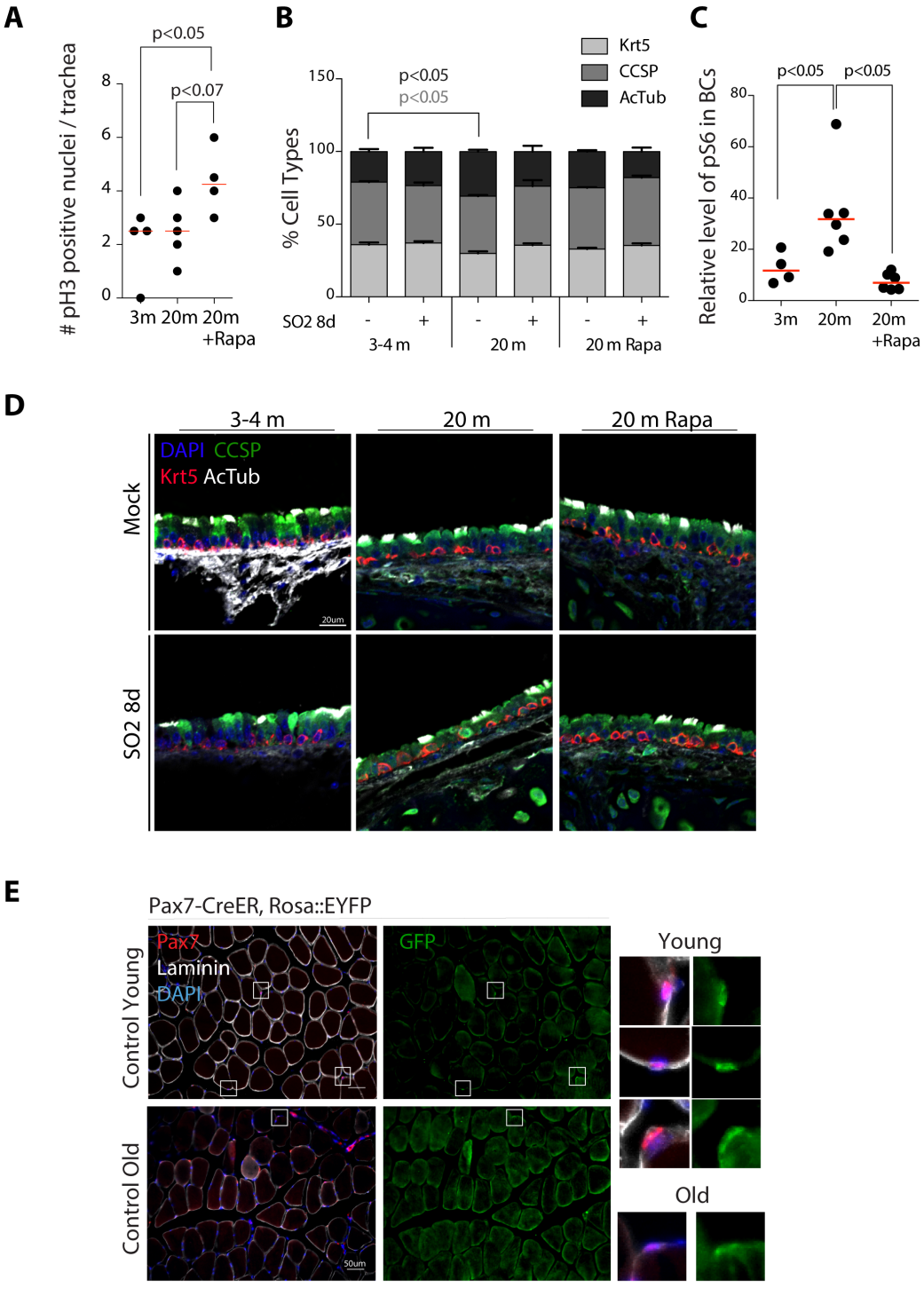
A
pH3 positive nuclei / trachea
p<0.05
p<0.07
3m 20m 20m +Rapa
B
% Cell Types
Krt5
CCSP
AcTub
p<0.05
p<0.05
SO2 8d
3-4 m
20 m
20 m Rapa
C
Relative level of pS6 in BCs
p<0.05
p<0.05
3m 20m 20m +Rapa
D
3-4 m
20 m
20 m Rapa
DAPI CCSP
Krt5 AcTub
Mock
SO2 8d
20um
E
Pax7-CreER, Rosa::EYFP
Pax7
Laminin
DAPI
GFP
Control Young
Control Old
Young
Old
50um

Figure S5 (related to Figure 5). Age-related stem cell phenotypes in trachea and muscle

A. Quantification of mitotic figures (pH3 staining) in young (3 months old), old (20 months old) and old Rapamycin treated (3 month of Rapamycin 42ppm) epithelia (no injury). Each data point represents the mean for one trachea. Red lines represent the median. ANOVA U-test.

B. Quantification of BCs (Krt5), Secretory cells (CCSP) and Ciliated Cells (AcTub) in an area of the tracheal epithelium of young, old and old Rapamycin treated mice after SO2 exposure. Bars representing the mean +/- SEM. ANOVA U-test (n>4).

C. Quantification of p-S6 levels (fluorescence, normalized to the background) in BCs of young (3m old), old (20m old) and old Rapamycin treated mice (3 month of Rapamycin starting at 17 months of age). Each data point represents the mean for one trachea. Red lines represent the median.

D. Confocal images of an area of the tracheal epithelial of young (3 month), old (20 month) and old Rapamycin treated for 3 month (20 month) at 8 days after SO2 exposure or mock exposed. IHC detecting BCs (red), secretory cells (CCSP, green) and ciliated cells (AcTub, white). Images show representative epithelia (n≥4).

E. Representative images of IHC staining of TA (tibial anterior) muscles from young and aged control mice. White squares surround GFP^{+}-$Pax7^{+}$ MuSCs. Higher resolution images of these cells are displayed on the right. The scale bar represents 50μm.