Supplementary Figure Legends

Figure S1. Related to Figures 1-4. **Severe injury to the tracheal SAE leads to expansion of αSMA$^+$ cell populations.**
Mice were injected with vehicle, 200 mg/kg naphthalene, or 300 mg/kg naphthalene and tracheas were harvest on day 1, 3, 5, and 7 following vehicle or naphthalene injection. **(A-C)** Immunofluorescent staining for αSMA expression at 3 days following **(A)** vehicle, **(B)** 200 mg/kg naphthalene, and **(C)** 300 mg/kg naphthalene injection. Arrowheads **(C)** mark a gland duct (white) and αSMA$^+$ cells in the SAE (red). Tracheal cartilage rings are marked as cricoid cartilage (C0) and cartilage ring 1 (C1). **(D)** The percentage of total SAE cells that are αSMA$^+$ at C0-C2 under the various injury conditions. **(E)** Fold change, relative to uninjured animals, in the percentage of αSMA$^+$ cells in the SMGs. Data are shown as mean ± SEM of N=3-6 mice from multiple sections >60 μm apart. Diamonds denote significance levels for Two-way ANOVA test: ◊ P<0.05 and ◊◊◊◊ P<0.0001. Asterisks denote significance levels for Holm-Sidak's multiple comparisons test: * P<0.05, ** P<0.01, *** P<0.001, and **** P<0.0001. **(F-H)** Lineage-tracing using **(F)** *ACTA2*-CreERT2:ROSA-TG and **(G,H)** *MYH11*-CreERT2:ROSA-TG. Mice were given 5 daily IP injections of tamoxifen, rested for 5 days, and then sacrificed for tracheal harvest and analysis by immunofluorescence. Tracheal sections were stained for nuclei, Tomato, GFP, and the indicated phenotypic markers: **(F and G)** αSMA and **(H)** SMMHC. **(I, J)** Multi-wavelength cell scoring was used to quantify the lineage-tracing efficiency for the two lines as the percentage of αSMA or SMMHC positive cells that also express the lineage marker GFP. Values represent the mean +/- SEM of N=7-10 mice per group.

Figure S2. Related to Figure 1. **MEC-derived cells emerge from SMGs and adopt a basal cell-like phenotype on the SAE of injured *MYH11*-Cre^{ERT2}:ROSA-TG mice.**
MYH11-Cre^{ERT2}:ROSA-TG mice were given 5 daily IP injections of tamoxifen, rested for 5 days, and then injured with naphthalene (300 mg/kg). **(A-E)** Tracheal sections at 21 days post-injury are oriented with the proximal region to the left and were stained for nuclei, Tomato, GFP, and the indicated phenotypic markers: **(A)** αSMA (**ai** and **aii:** enlarged images of the boxed regions shown in **A**); **(B)** Krt5 (**bi:** enlarged image of the boxed region shown in **B**); **(C)** Krt14; **(D)** DBA (**di** and **dii:** enlarged images of the boxed regions shown in **D**); and **(E)** αTubulin (arrows denote lineage-traced ciliated cells). **(F)** Quantification of the percentage of total SAE cells that are GFP+ (dotted line denotes background level of signal close to the basal lamina in uninjured controls). Values represent the mean +/- SEM of N=3 uninjured mice and N=6 injured mice. P-values indicate significance of **(F)** unpaired one-tailed Student's t-test, ***P*<0.01.

Figure S3. Related to Figures 1-3. **MEC-derived progenitors contribute to basal and luminal cells in the SAE following SO_2 injury.**
(A) Timeline of lineage-tracing of myoepithelial cells in *ACTA2*-Cre^{ERT2}:ROSA-TG mice induced with tamoxifen (Tmx) and injured with SO_2 (600 ppm). **(B-J)** Images of the GFP lineage trace with co-stained antigens as indicated for the **(B-G)** SAE and **(I-J)** SMGs. **(K,L)** Quantification of the percentage of total SAE cells that are GFP-positive in the **(K)** SAE and **(L)** SMGs. P-values indicate significance of one-way ANOVA followed by posttest for linear trend. **(M)** Quantification of the percentage of total GFP-positive cells that express each of the indicated markers. Values represent the mean +/- SEM of N=4-7 mice per group. Krt8$^+$ lineage-traced cells significantly increased over time (one-way ANOVA P=0.0064 with a posttest for linear trend P=0.0016). Micron bars: **(B-G)** 50 μm; **(H-J)** 25 μm.

Figure S4. Related to Figures 5 and 6. **Lef-1 expression in MECs using the *MYH11*-Cre^{ERT2} driver enhances lineage contribution to SAE and SMGs following airway injury.**

MYH11-Cre^{ERT2}:Lef-KI$^{+/-}$ mice were induced tamoxifen daily for 5 days, rested for 5 days, and then injured with naphthalene (300 mg/kg). Uninduced and induced/uninjured animals were used as controls. Animals were harvested at 21 days post-mock or naphthalene injury. **(A-H)** Tracheal images localizing the lineage trace (GFP) and αSMA for **(A-D)** uninjured and **(E-H)** injured animals. **(I)** Quantification of the percentage of total cells that are GFP-negative in the SAE and SMGs. Values represent the mean +/- SEM for the (N) mice. P-values indicate significance of Kruskal-Wallis and Dunn's post-test, **P*<0.05. Micron bars: 50 μm.

Figure S5. Related to Figure 7. **Basal cell transcriptional profile.**
(A) Surface airway epithelial cells were harvested and isolated by FACS into basal, club, and ciliated cell populations. Microarray analysis was performed on RNA collected from each cell population. **(B)** Principal component analysis of each sample indicates good separation of each cell type. **(C)** Unsupervised hierarchical clustering of genes showing distinct expression profiles for each cell type with at least 4 major groups of genes indicated by K-means++ gene clustering. **(D)** Examples of several canonical phenotypic markers indicated as being enriched (z-score>1.75) in each cell type.

Figure S6. Related to Figure 7. **MEC-derived progenitor cells are highly proliferative in primary cultures and Lef-1 expression enhances this phenotype.**
(A-F) *ACTA2*-Cre^{ERT2}:ROSA-TG mice were induced by five daily injections with tamoxifen and cells were isolated from the **(A-C)** SAE and **(D-F)** SMGs five days after the last tamoxifen injection. **(B,C)** SAE and **(E,F)** SMG cells were expanded from passage 0-10 (P0-P10) and the proportion of SAE cells expressing Tomato or GFP at each passage was quantified by FACS. **(G-L)** Mixing experiments of P3 **(G-I)** untraced (red/Tomato^{+}) or **(J-L)** lineage-traced (green/GFP^{+}) glandular progenitors isolated from induced *ACTA2*-Cre^{ERT2}:ROSA-TG mice and mixed with non-transgenic SAE progenitors at a ratio of 10% SMG:90% SAE. Mixed cultures were expanded from P3-P10 and the proportion of each phenotype was quantified by FACS at each passage. **(M-O)** *ACTA2*-Cre^{ERT2}:Lef-1KI$^{+/+}$ and *ACTA2*-Cre^{ERT2}:ROSA-TG mice were induced by five daily injections with tamoxifen and SMG cells were isolated five days later. FACS purified populations of MEC^{WT} (GFP^{+}) and MEC$^{Lef\text{-}1KI}$ (GFP^{-}) at P3 were mixed at a ratio of 15% MEC$^{Lef\text{-}1KI}$:85% MEC^{WT} and cultured to the 8th passage. The proportion of each phenotype was quantified by FACS at each passage. Data represents the mean +/-SEM for N=6 cultures.

Figure S7. Related to Figure 7. **Differentiation of WT and Lef-1KI$^{+/+}$ MECs in air-liquid interface cultures and tracheal xenografts.**
(A) Schematic of experimental procedure for isolation of GFP^{+} MEC^{WT} and GFP^{-} MEC$^{Lef\text{-}1KI}$ from glands of tamoxifen-induced *ACTA2*-Cre^{ERT2}:ROSA-TG and *ACTA2*-Cre^{ERT2}:Lef-KI$^{+/+}$ mice. Mice were induced by 5 sequential tamoxifen injections and rested for 5 days prior to harvest. **(B-I)** Phenotypes of cells in air-liquid interface (ALI) cultures established from a 50:50 mixture of MEC^{WT} and MEC$^{Lef\text{-}1KI}$ cells showing **(B-E)** orthogonal views and **(F-I)** maximum intensity projections of the ALI culture. Cultures were immunostained for the indicated markers of **(B,F)** club (Scgb1a1), **(C,G)** ciliated (αtubulin), and **(D,H)** Muc5AC and **(E,I)** Muc5B mucin secreting cells. **(J-L)** Denuded tracheal xenografts reconstituted with 90% non-transgenic SAE and 10% P2 *ACTA2*-Cre^{ERT2}:ROSA-TG labeled SMG cells (~4% GFP^{+} and ~6% Tomato^{+}). Phenotypic markers assessed by immunofluorescence were: **(J)** Krt14, **(K)** αtubulin, and **(L)** UEA-1. For panels **J,** boxed regions are enlarged and displayed to the right. **(M-Q)** Denuded tracheal xenografts reconstituted with FACS purified SMG cells isolated from tamoxifen-induced *ACTA2*-Cre^{ERT2}:ROSA-TG (GFP^{+}) and *ACTA2*-Cre^{ERT2}:Lef-KI$^{+/+}$ (GFP^{-}) mice and seeded at a ratio of 50:50. Sections are stained for the GFP and/or αtubulin.

Figure S1

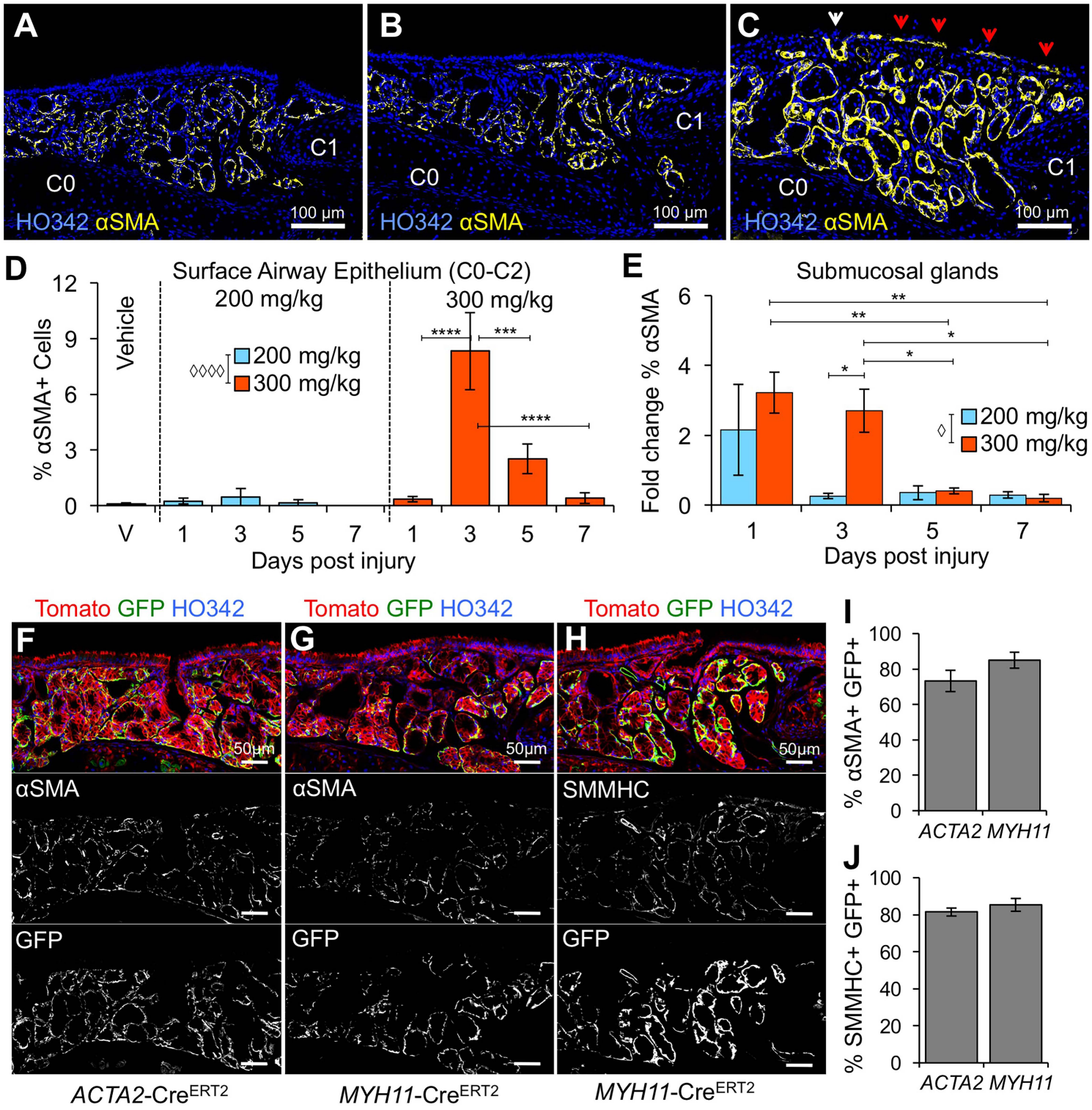
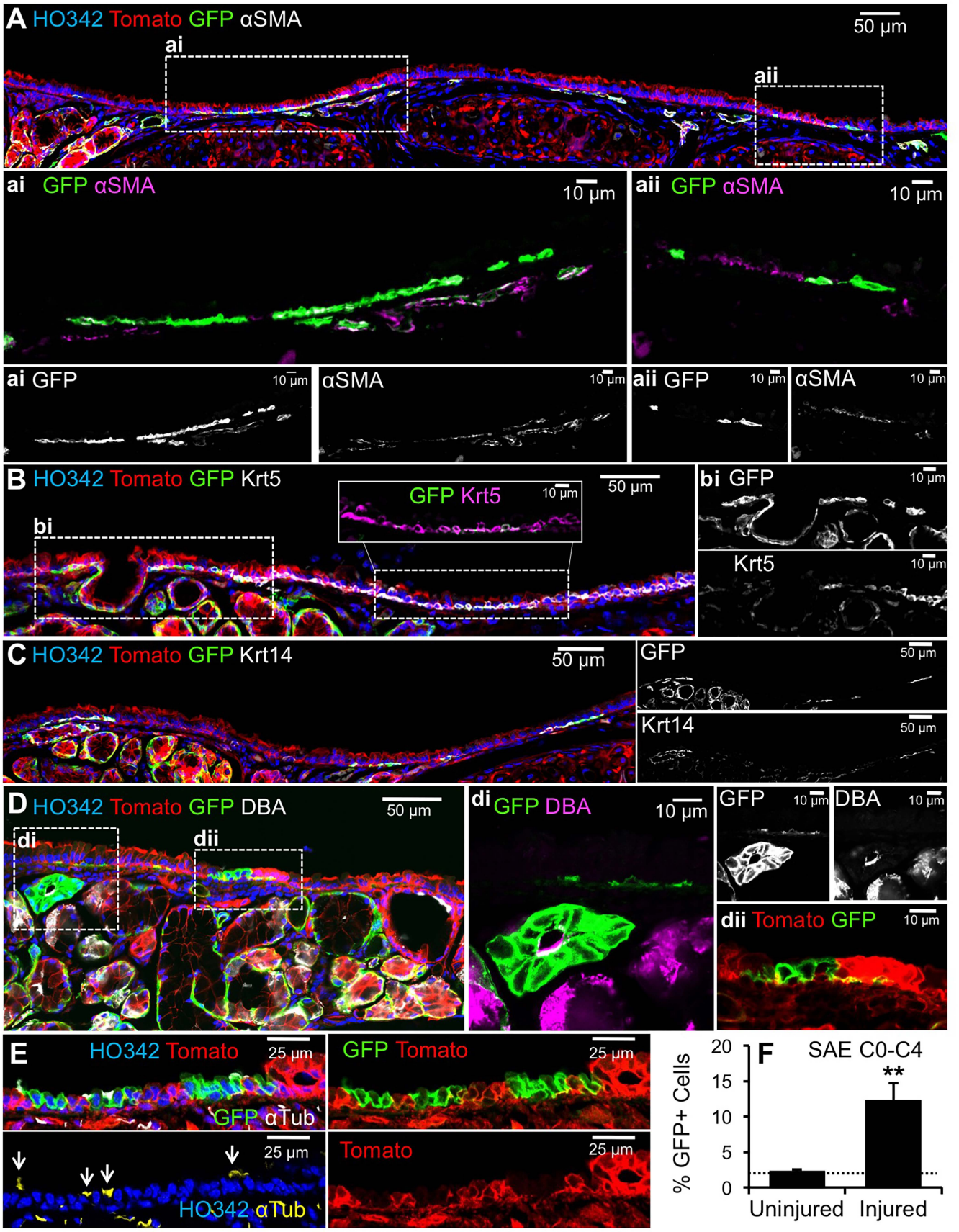
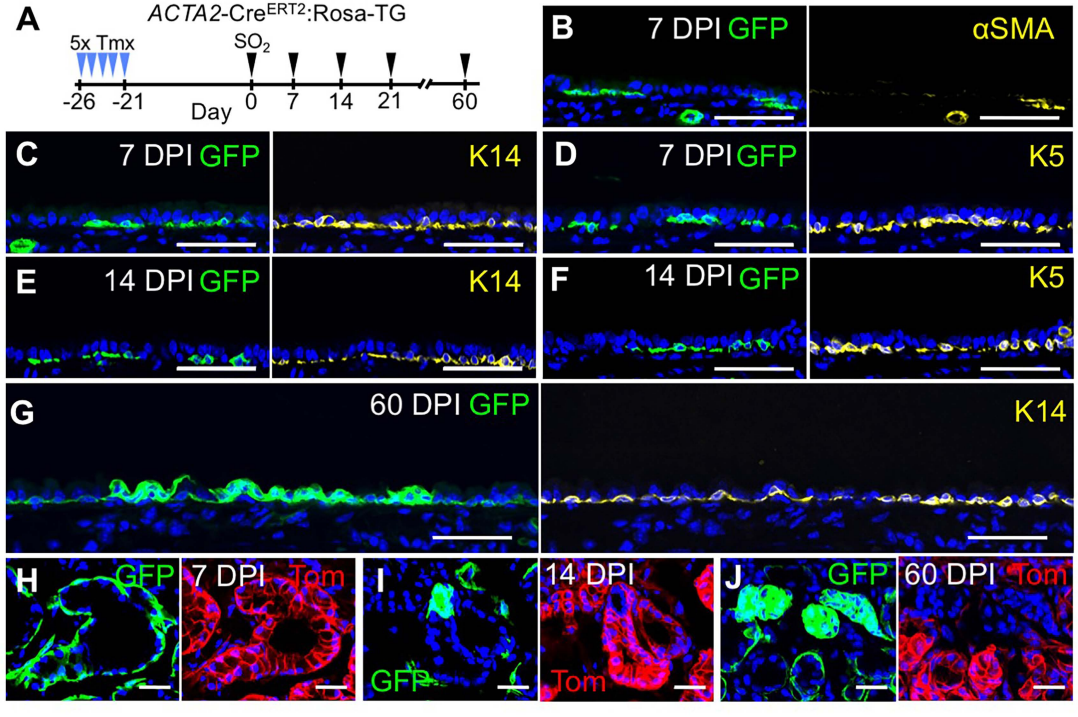
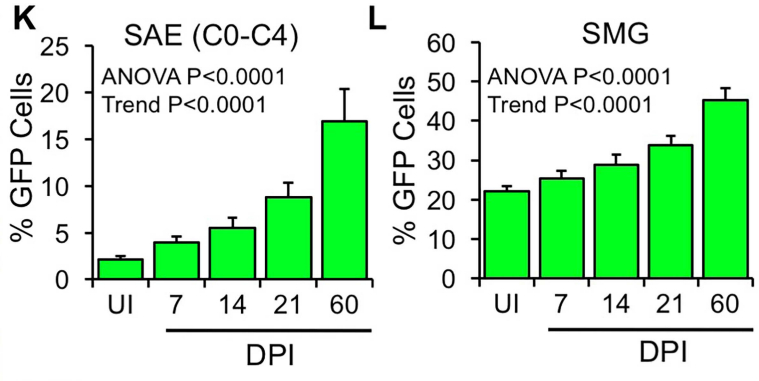

A
B
C
C0
C1
HO342 αSMA
100 µm
D
Surface Airway Epithelium (C0-C2)
200 mg/kg
300 mg/kg
Vehicle
% αSMA+ Cells
200 mg/kg
300 mg/kg
Days post injury
E
Submucosal glands
Fold change % αSMA
Tomato GFP HO342
F
G
H
50µm
αSMA
SMMHC
GFP
ACTA2-CreERT2
MYH11-CreERT2
I
% αSMA+ GFP+
ACTA2 MYH11
J
% SMMHC+ GFP+

Figure S2


A HO342 Tomato GFP αSMA
50 μm
ai
aii
ai GFP αSMA
10 μm
aii GFP αSMA
10 μm
ai GFP
10 μm
αSMA
10 μm
aii GFP
10 μm
αSMA
10 μm
B HO342 Tomato GFP Krt5
50 μm
GFP Krt5
10 μm
bi
bi GFP
10 μm
Krt5
10 μm
C HO342 Tomato GFP Krt14
50 μm
GFP
50 μm
Krt14
50 μm
D HO342 Tomato GFP DBA
50 μm
di
dii
di GFP DBA
10 μm
GFP
10 μm
DBA
10 μm
dii Tomato GFP
10 μm
E
HO342 Tomato
25 μm
GFP αTub
GFP Tomato
25 μm
25 μm
HO342 αTub
Tomato
25 μm
F
SAE C0-C4
**
% GFP+ Cells
20
15
10
5
0
Uninjured
Injured


A
ACTA2-CreERT2:Rosa-TG
5x Tmx
SO2
-26 -21 Day 0 7 14 21 60
B 7 DPI GFP αSMA
C 7 DPI GFP K14
D 7 DPI GFP K5
E 14 DPI GFP K14
F 14 DPI GFP K5
G 60 DPI GFP K14
H GFP 7 DPI Tom
I GFP 14 DPI Tom
J GFP 60 DPI Tom


K
SAE (C0-C4)
ANOVA P<0.0001
Trend P<0.0001
% GFP Cells
UI 7 14 21 60
DPI
L
SMG
ANOVA P<0.0001
Trend P<0.0001
% GFP Cells
UI 7 14 21 60
DPI

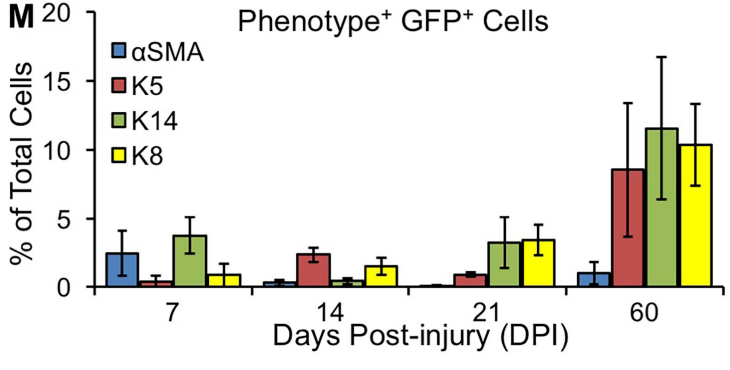

M
Phenotype+ GFP+ Cells
αSMA
K5
K14
K8
% of Total Cells
7 14 21 60
Days Post-injury (DPI)

Figure S4

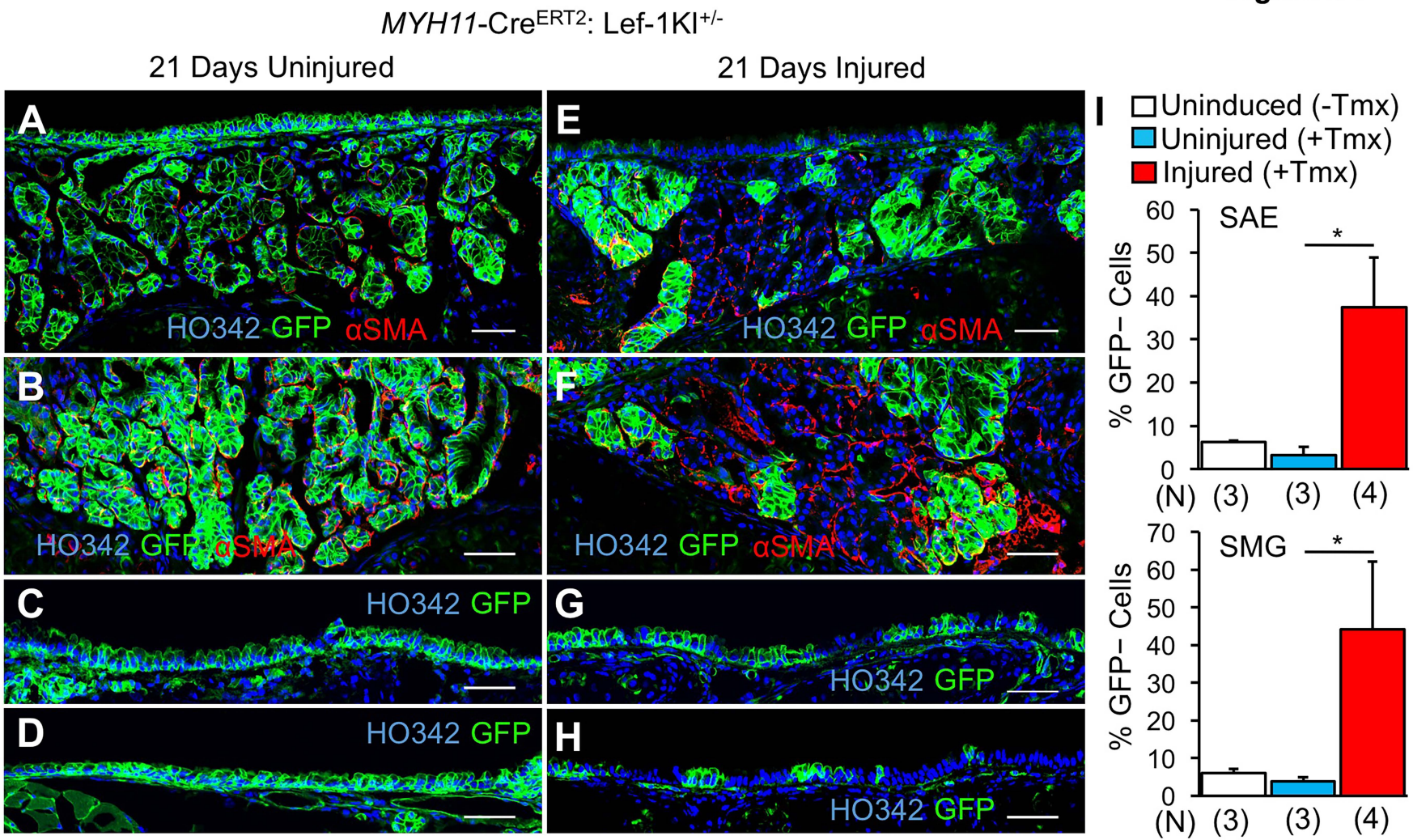

MYH11-CreERT2: Lef-1KI+/-
21 Days Uninjured
21 Days Injured
A
HO342 GFP αSMA
B
HO342 GFP αSMA
C
HO342 GFP
D
HO342 GFP
E
HO342 GFP αSMA
F
HO342 GFP αSMA
G
HO342 GFP
H
HO342 GFP
I
Uninduced (-Tmx)
Uninjured (+Tmx)
Injured (+Tmx)
SAE
% GFP– Cells
60
50
40
30
20
10
0
*
(N) (3) (3) (4)
SMG
% GFP– Cells
70
60
50
40
30
20
10
0
*
(N) (3) (3) (4)

Figure S5

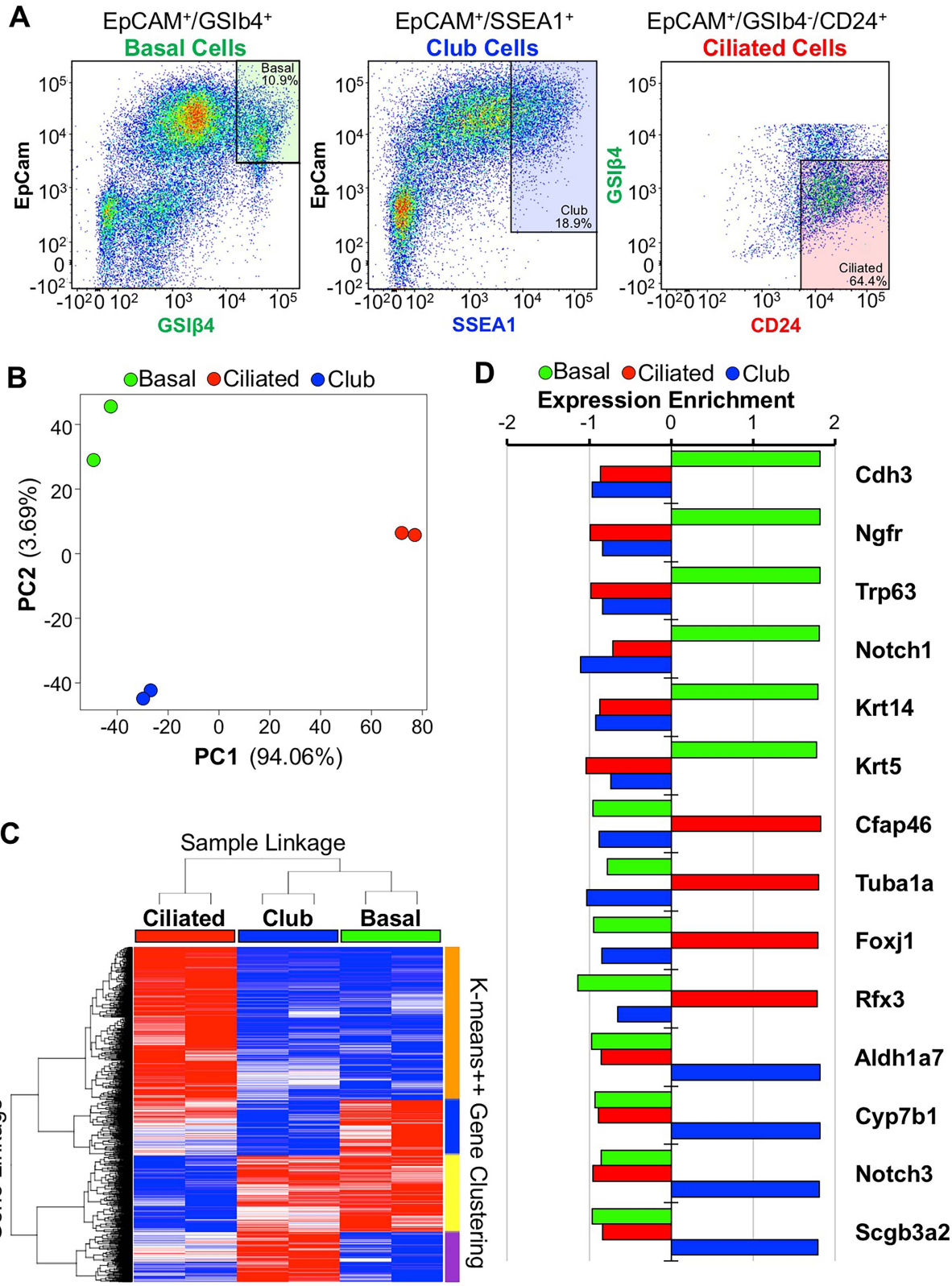

A
EpCAM+/GSIb4+
Basal Cells
Basal
10.9%
EpCam
GSIβ4
EpCAM+/SSEA1+
Club Cells
Club
18.9%
EpCam
SSEA1
EpCAM+/GSIb4-/CD24+
Ciliated Cells
GSIβ4
Ciliated
64.4%
CD24
B
Basal
Ciliated
Club
PC2 (3.69%)
PC1 (94.06%)
C
Sample Linkage
Ciliated
Club
Basal
Gene Linkage
K-means++ Gene Clustering
D
Basal
Ciliated
Club
Expression Enrichment
Cdh3
Ngfr
Trp63
Notch1
Krt14
Krt5
Cfap46
Tuba1a
Foxj1
Rfx3
Aldh1a7
Cyp7b1
Notch3
Scgb3a2

Figure S6

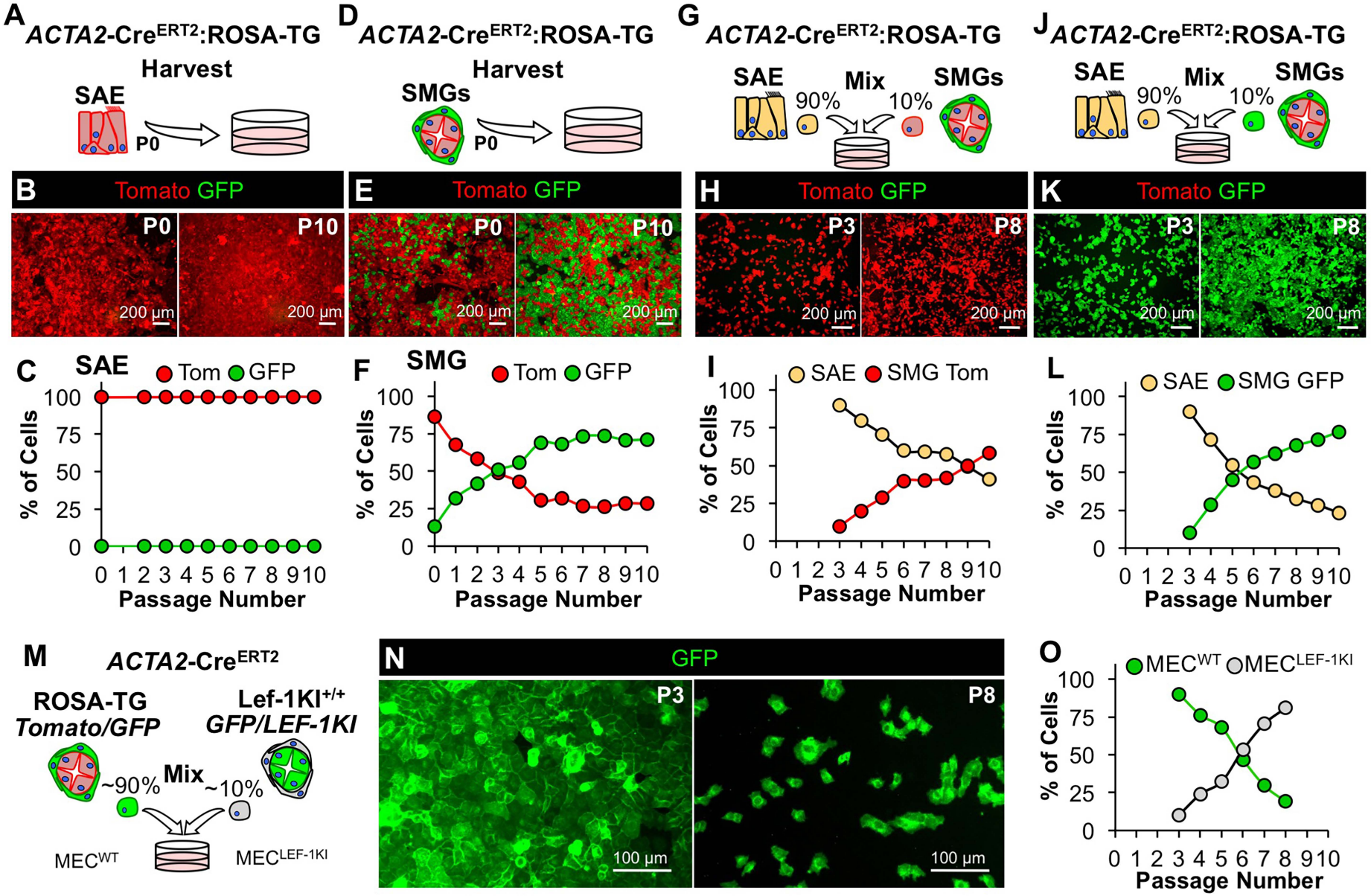

A
ACTA2-CreERT2:ROSA-TG
Harvest
SAE
P0
D
ACTA2-CreERT2:ROSA-TG
Harvest
SMGs
P0
G
ACTA2-CreERT2:ROSA-TG
SAE
Mix
SMGs
90%
10%
J
ACTA2-CreERT2:ROSA-TG
SAE
Mix
SMGs
90%
10%
B
Tomato GFP
P0
P10
200 μm
E
Tomato GFP
P0
P10
200 μm
H
Tomato GFP
P3
P8
200 μm
K
Tomato GFP
P3
P8
200 μm
C
SAE
Tom GFP
% of Cells
Passage Number
F
SMG
Tom GFP
% of Cells
Passage Number
I
SAE SMG Tom
% of Cells
Passage Number
L
SAE SMG GFP
% of Cells
Passage Number
M
ACTA2-CreERT2
ROSA-TG
Tomato/GFP
Lef-1KI+/+
GFP/LEF-1KI
~90%
Mix
~10%
MECWT
MECLEF-1KI
N
GFP
P3
P8
100 μm
O
MECWT MECLEF-1KI
% of Cells
Passage Number

Figure S7

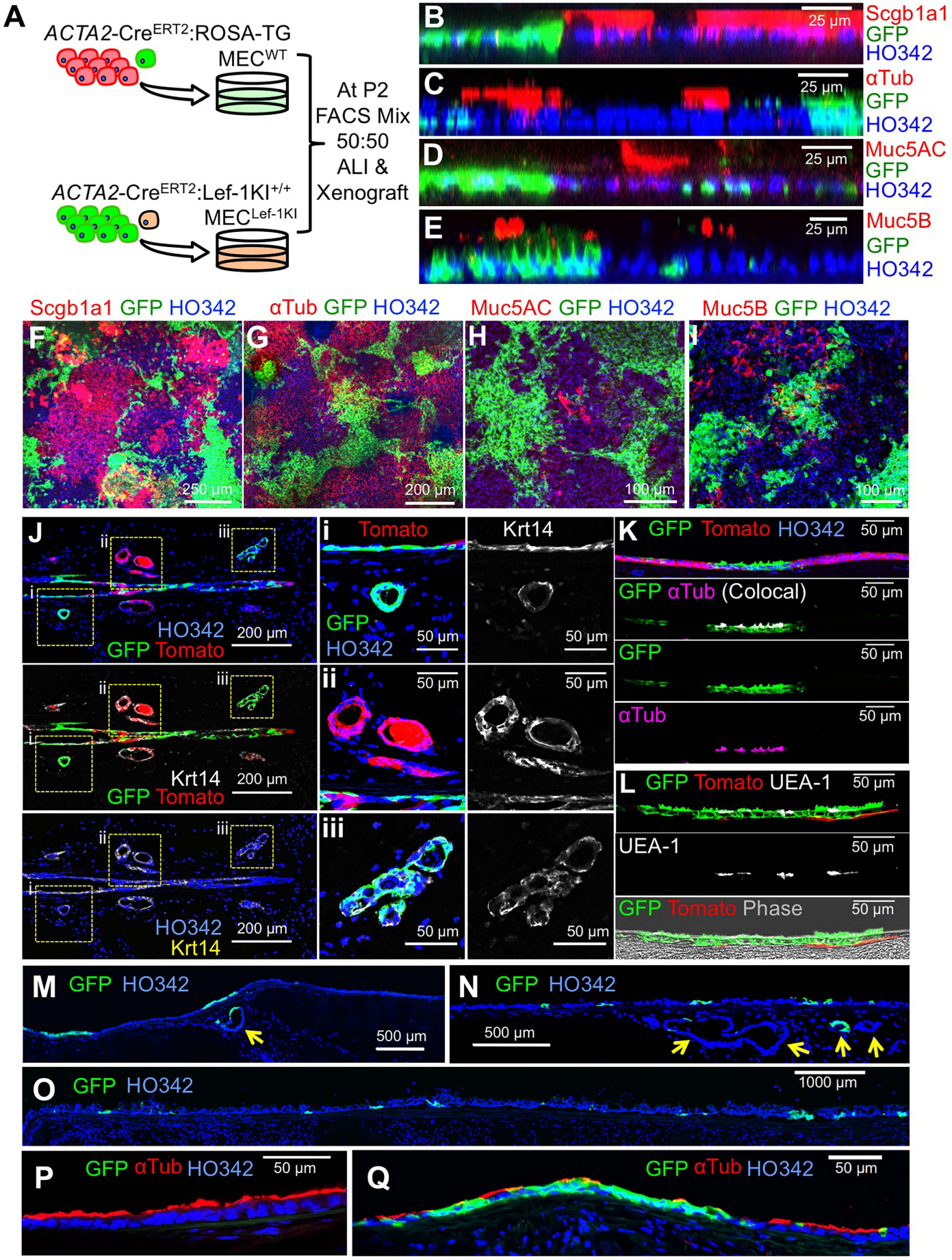

A
ACTA2-CreERT2:ROSA-TG
MECWT
ACTA2-CreERT2:Lef-1KI+/+
MECLef-1KI
At P2
FACS Mix
50:50
ALI &
Xenograft
B
25 μm
Scgb1a1
GFP
HO342
C
25 μm
αTub
GFP
HO342
D
25 μm
Muc5AC
GFP
HO342
E
25 μm
Muc5B
GFP
HO342
Scgb1a1 GFP HO342
αTub GFP HO342
Muc5AC GFP HO342
Muc5B GFP HO342
F
250 μm
G
200 μm
H
100 μm
I
100 μm
J
ii
iii
i
HO342
GFP Tomato
200 μm
Krt14
200 μm
HO342
Krt14
200 μm
i
Tomato
GFP
HO342
50 μm
Krt14
50 μm
ii
50 μm
50 μm
iii
50 μm
50 μm
K
GFP Tomato HO342
50 μm
GFP αTub (Colocal)
50 μm
GFP
50 μm
αTub
50 μm
L
GFP Tomato UEA-1
50 μm
UEA-1
50 μm
GFP Tomato Phase
50 μm
M
GFP HO342
500 μm
N
GFP HO342
500 μm
O
GFP HO342
1000 μm
P
GFP αTub HO342
50 μm
Q
GFP αTub HO342
50 μm

Table S4: Related to STAR Methods. **Summary of mouse experiments.**

Figure	Mouse Line	Induction Time	Chase Time	Injury Type	Injury Quantity	Time Post-injury to Harvest
Figure 1	*ACTA2*-Cre^{ERT2}:ROSA-TG	5 days	5 days	Naphthalene	Single	21 days
Figure 2	*ACTA2*-Cre^{ERT2}:ROSA-TG	5 days	5 days	Naphthalene	Single	7, 14, 21, 60 days
Figure 3	*ACTA2*-Cre^{ERT2}:ROSA-TG	5 days	5 days	Naphthalene	Single and Double	60 days
Figure 4	C57BL/6	N/A	N/A	Naphthalene	Single	12 and 24 hours
Figure 5 (Panels A-F)	*ACTA2*-Cre^{ERT2}:ROSA-TG and *ACTA2*-Cre^{ERT2}:Lef-1KI$^{+/+}$	5 days	0 days	N/A	N/A	N/A
Figure 5 (Panels G-U)	*ACTA2*-Cre^{ERT2}:Lef-1KI$^{+/-}$ and *ACTA2*-Cre^{ERT2}:Lef-1KI$^{+/+}$	5 days	5 days	Naphthalene	Single	21 days
Figure 6	*ACTA2*-Cre^{ERT2}:Lef-1KI$^{+/-}$ and *ACTA2*-Cre^{ERT2}:Lef-1KI$^{+/+}$	5 days	21 days	SO_2	Single and Double	21 and 42 days
Figure S1 (Panels A-E)	C57BL/6	N/A	N/A	Naphthalene	Single	1, 3, 5, 7 days
Figure S1 (Panels F-J)	*ACTA2*-Cre^{ERT2}:ROSA-TG and *MYH11*-Cre^{ERT2}:ROSA-TG	5 days	5 days	N/A	N/A	N/A
Figure S2	*MYH11*-Cre^{ERT2}:ROSA-TG	5 days	5 days	Naphthalene	Single	21 days
Figure S3	*ACTA2*-Cre^{ERT2}:ROSA-TG	5 days	21 days	SO_2	Single	7, 14, 21, 60 days
Figure S4	*MYH11*-Cre^{ERT2}:Lef-1KI$^{+/-}$	5 days	5 days	Naphthalene	Single	21 days