Figure S1. Related to Figure 1 and Figure 2

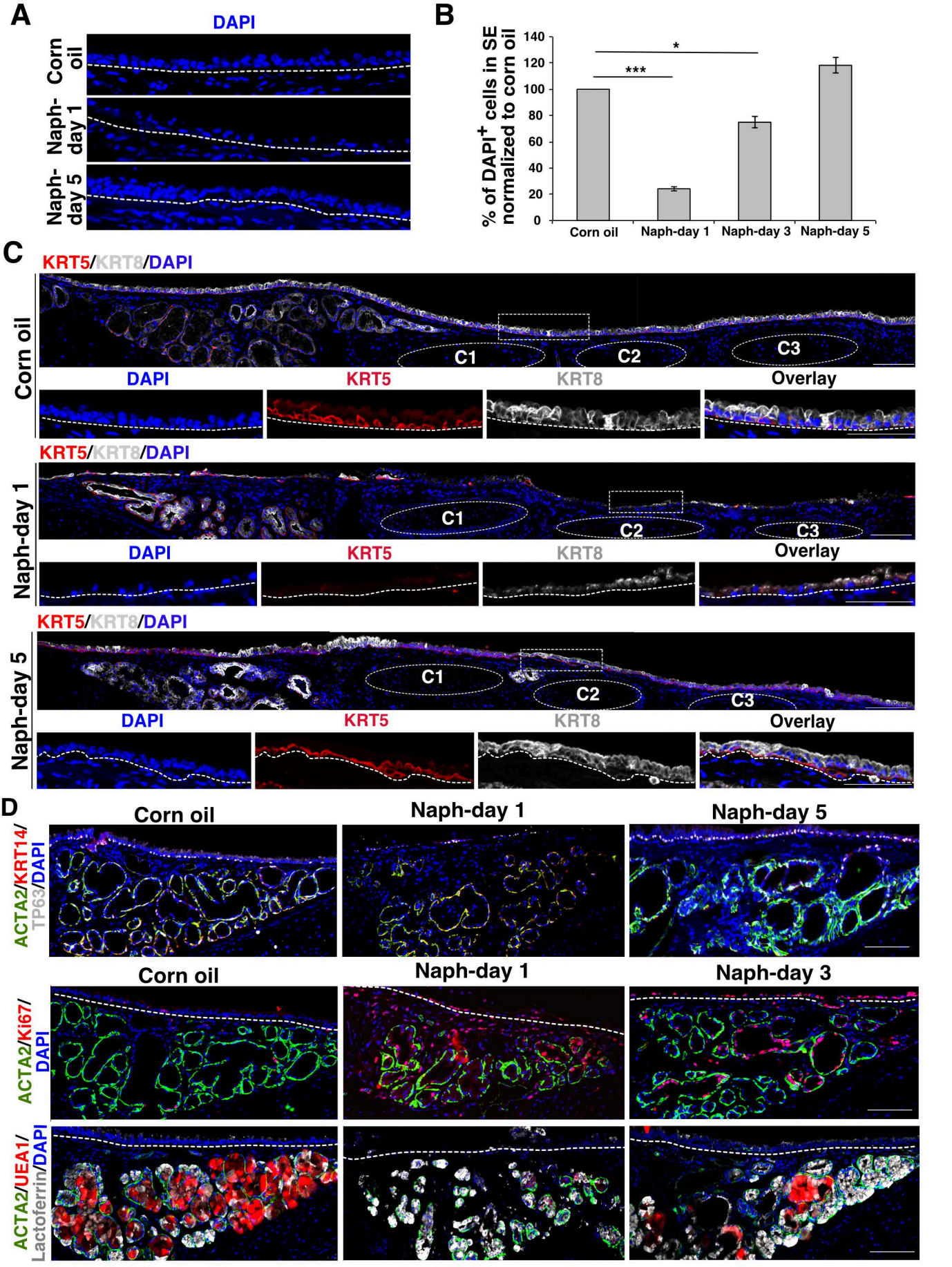


Figure S2. Related to Figure 2

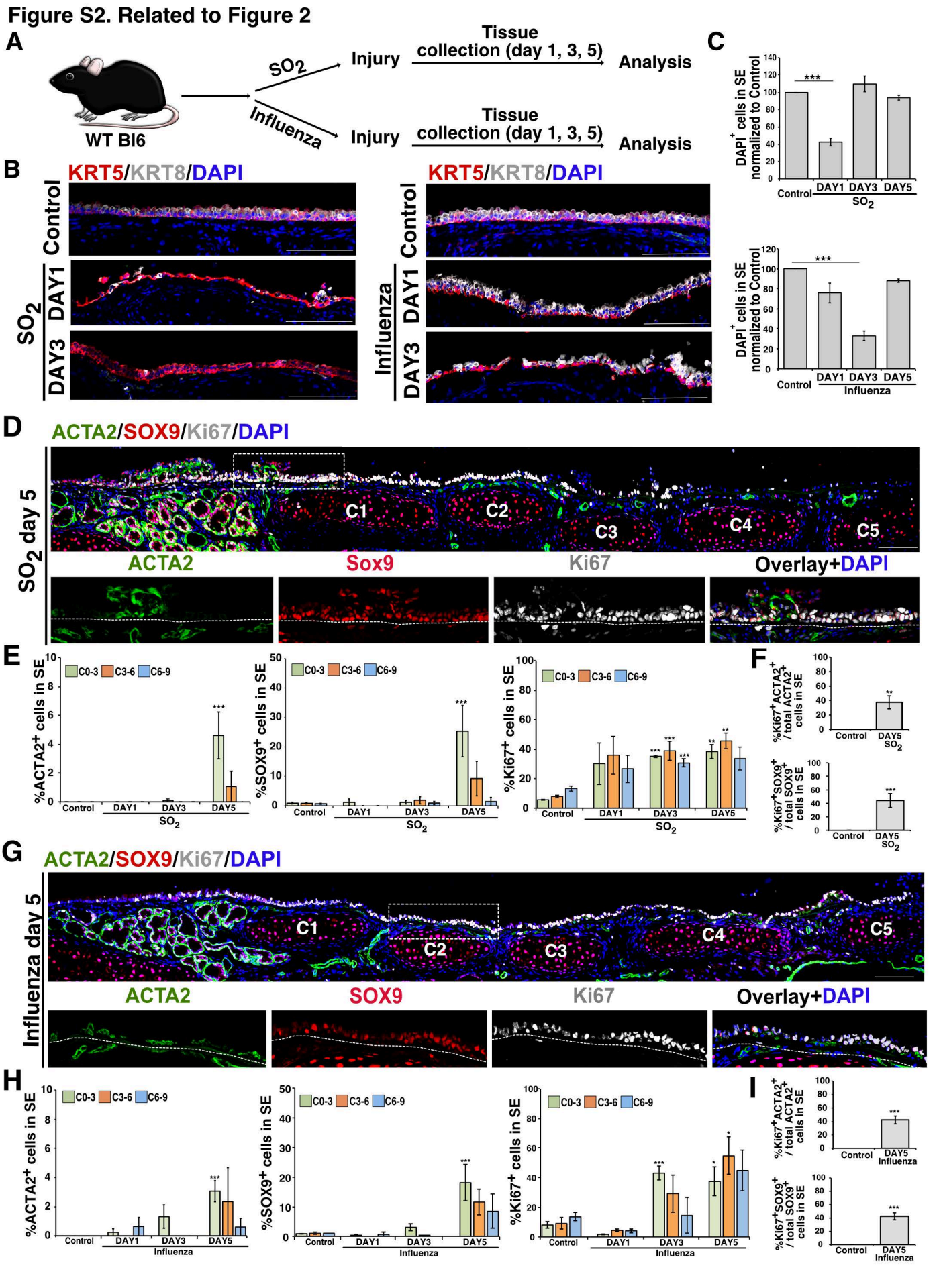


Figure S3. Related to Figure 3

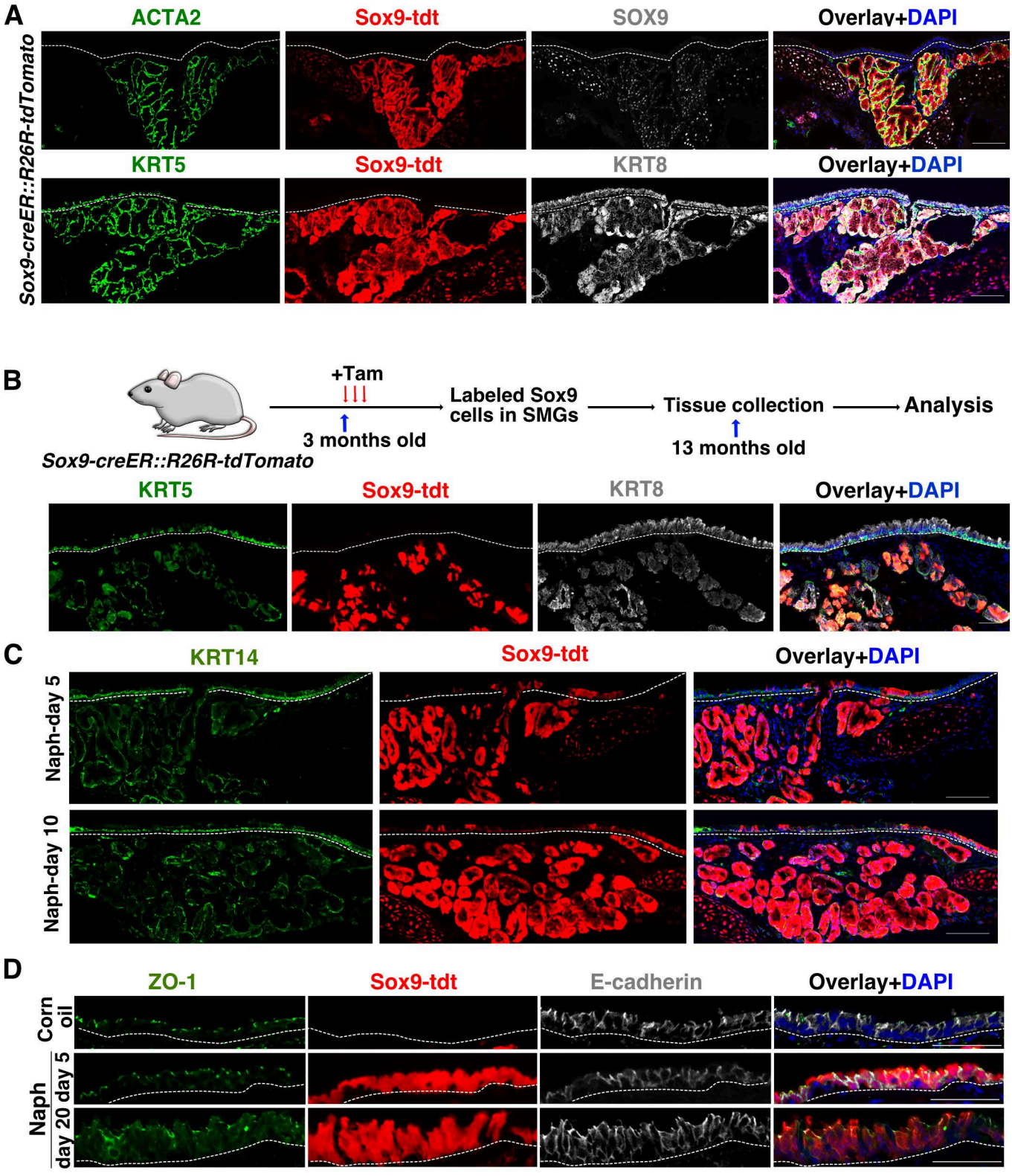


Figure S4. Related to Figure 4

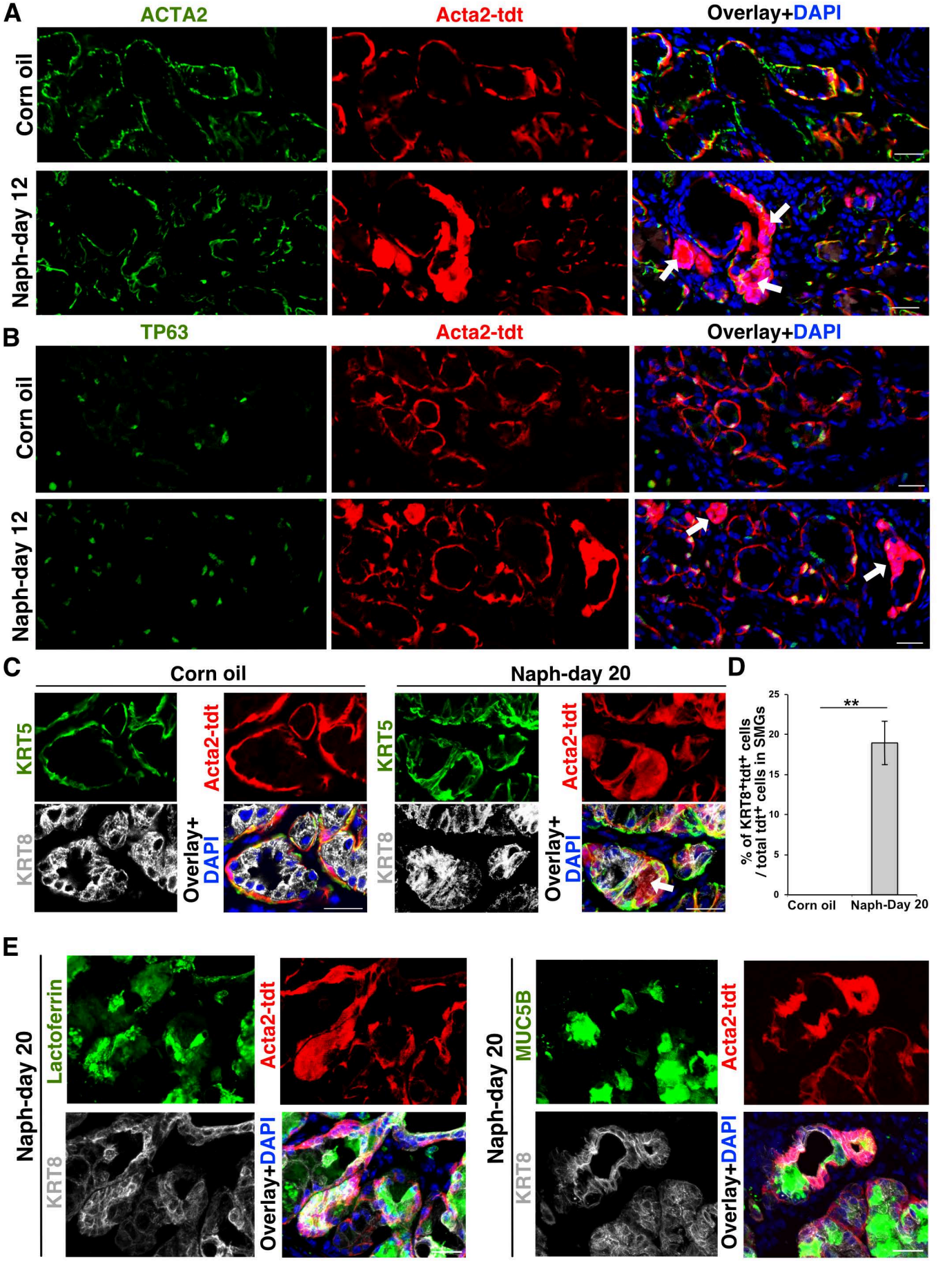


Figure S5. Related to Figure 5

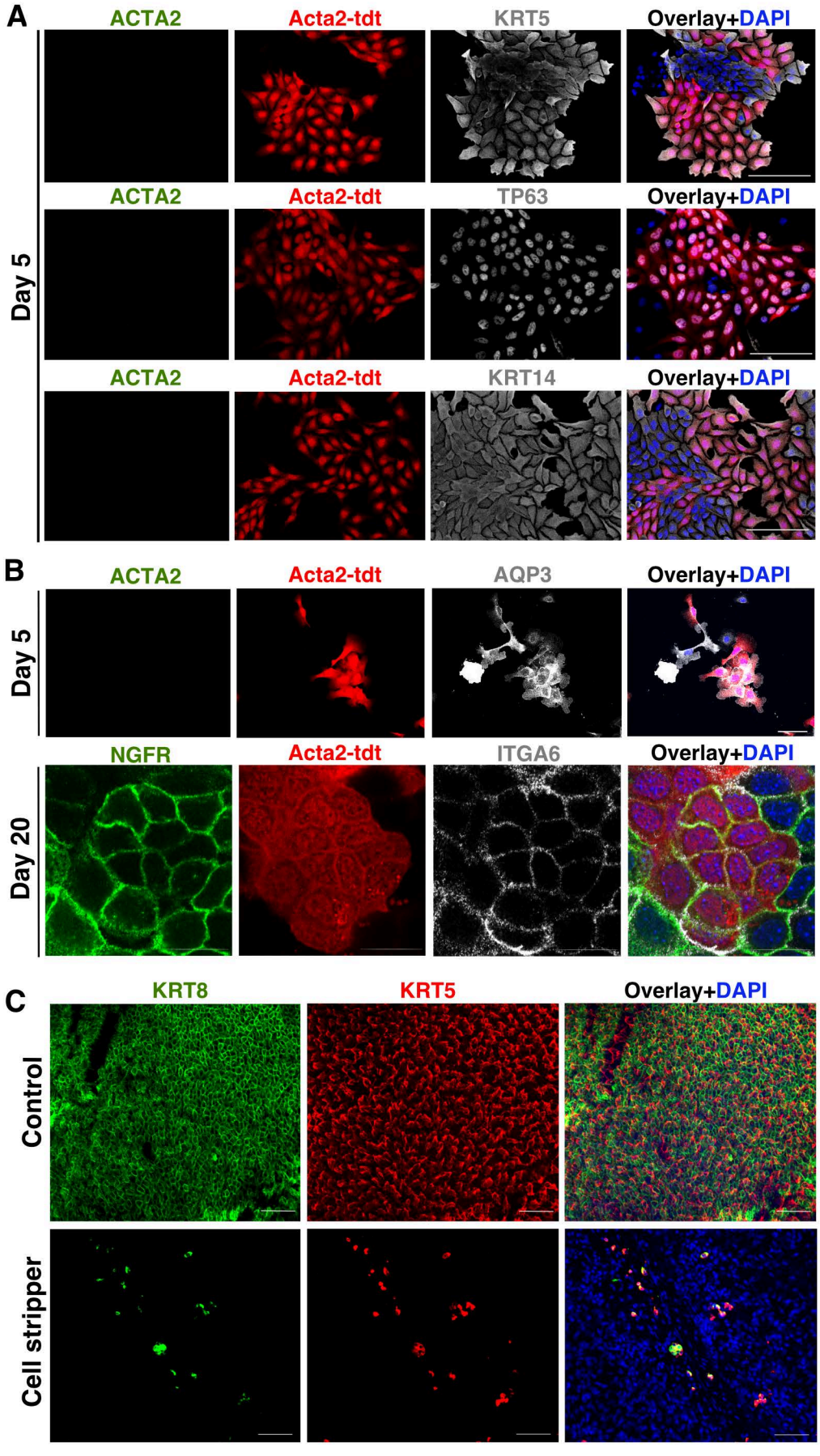


Figure S6. Related to Figure 6

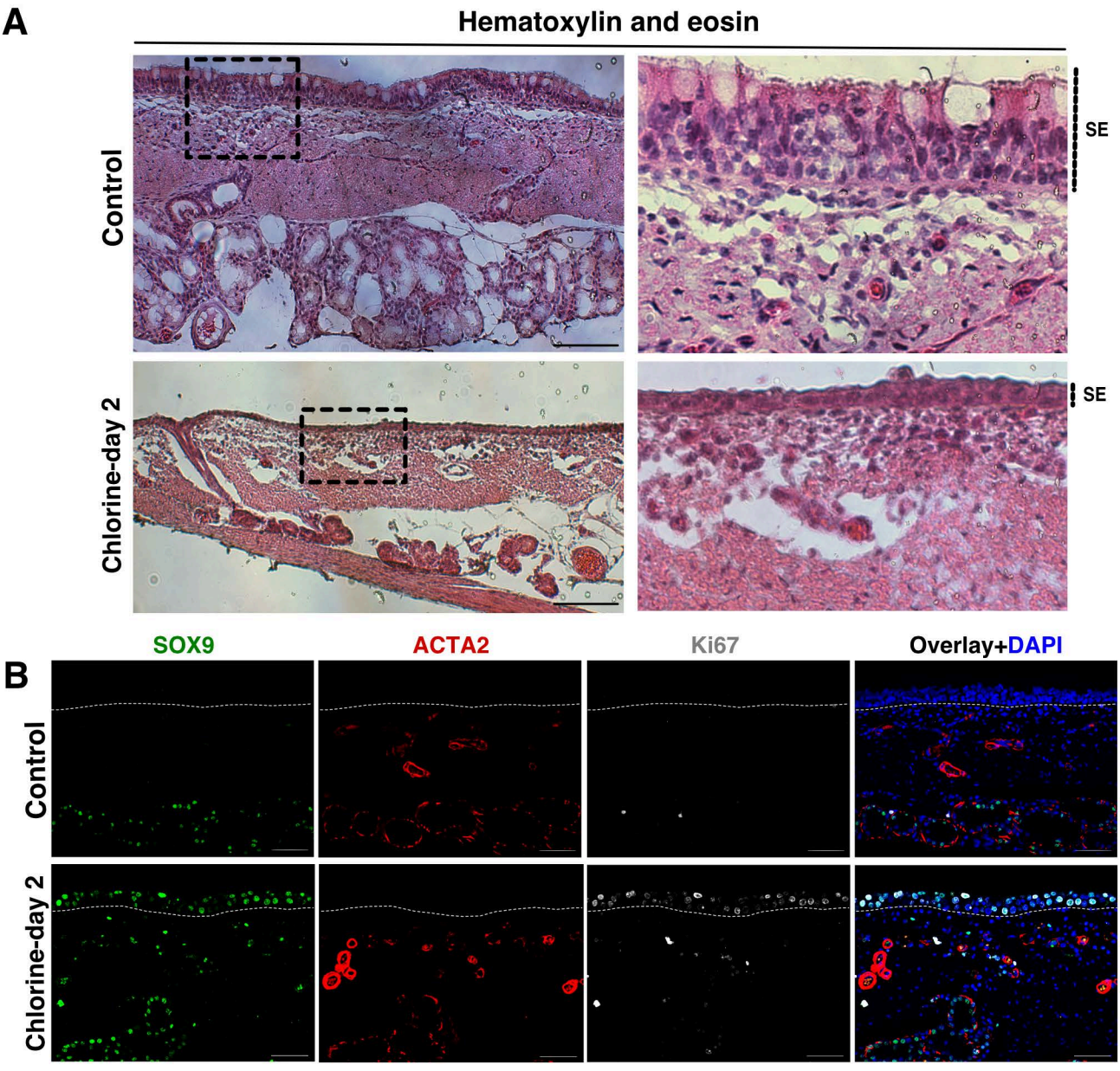
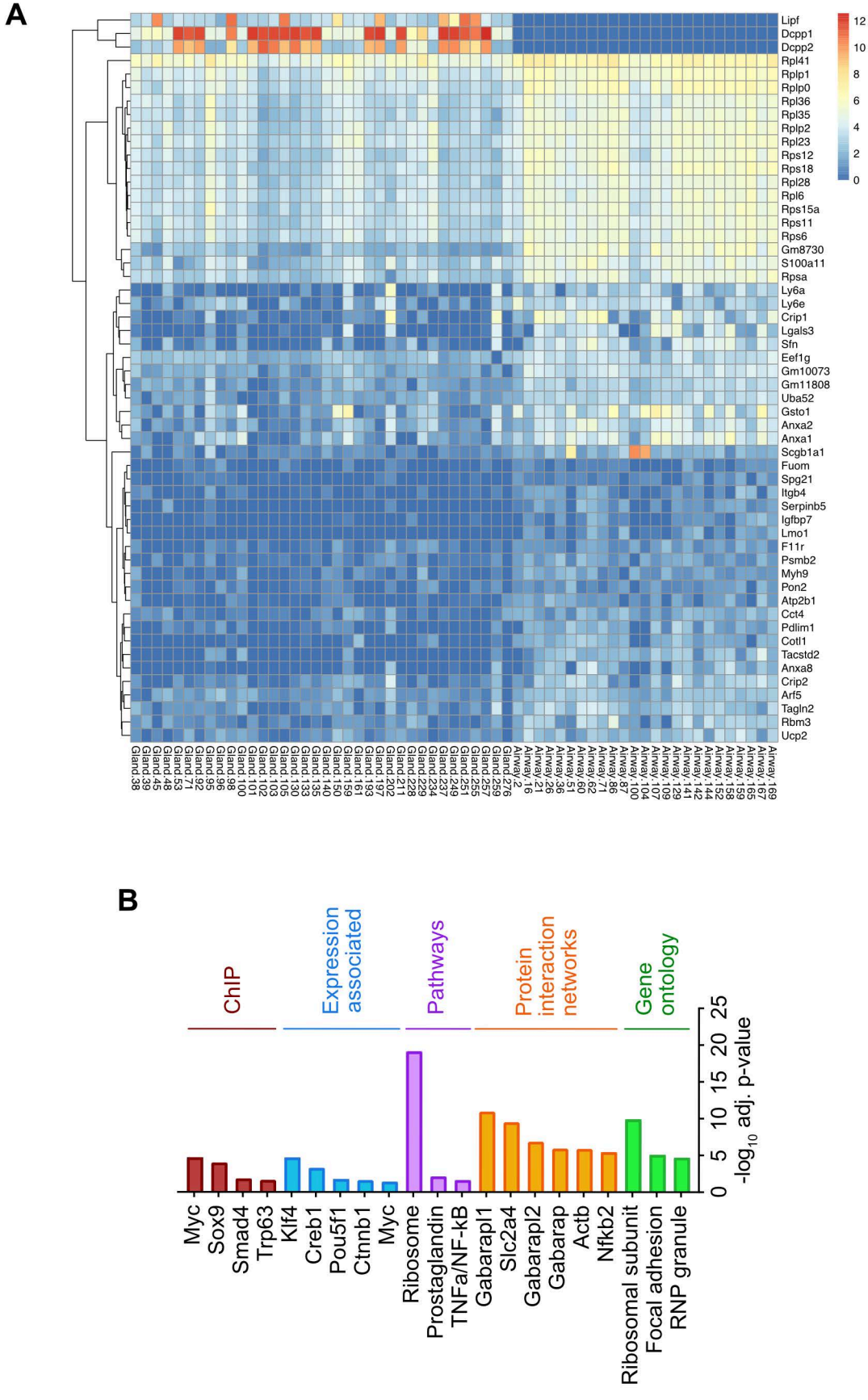


Figure S7. Related to Figure 7



Supplementary Figure legends:

Figure S1: Naphthalene-induced injury causes loss of airway SE.

(A) DAPI (blue) staining on control and naphthalene day-1 and 5 injured tracheal sections. The dashed line separates the SE cell layer from the mesenchyme. Scale bars: 50μm.

(B) Quantification of percent $DAPI^+$ cells in SE (up to cartilage 9) in control and naphthalene (day-1 and 5) treated airways. Data shown are mean ± SEM (n= 3; *$p \leq 0.05$; **$p \leq 0.01$).

(C) IHC for KRT5 (green), KRT8 (red), and DAPI (blue) on longitudinal sections of airways collected from control or naphthalene injured mice. The dashed line separates the SE cell layer from the mesenchyme. Single markers shown for regions delineated by dotted box Dotted circles indicate cartilage rings marked as C1-C3. Scale bars: 100μm

(D) Co-staining of ACTA2 (green), KRT14 (red), TP63 (grey) and DAPI (blue): upper panel - ACTA2 (green) and Ki67 (red); middle panel - ACTA2 (green), UEA-1 (red-mucous cell marker) and lactoferrin (grey-serous cell marker); lower panel - tracheal sections collected from control and naphthalene injured mice. Scale bar: 100μm

Figure S2: *SMG-like cells appear in SE in multiple injury models*

(A) Schematic of experimental design for sulphur dioxide (SO_2) or H1N1 PR8 infection injury models.

(B) IHC for KRT5 (red), KRT8 (grey) and DAPI (blue) on trachea sections from control, SO_2 and influenza day-1 and 3 injured mice. The dashed line separates the SE cell layer from the mesenchyme. Scale bars: 50μm.

(C) Quantification of percent $DAPI^+$ cells in SE (up to cartilage 9) in control, SO_2 (day-1, 3 and 5) and influenza (day-1, 3 and 5) injured airways. Data shown as mean ± SEM (n = 3; $^{**}p \leq 0.01$).

(D) Co-staining of ACTA2 (green), SOX9 (red), Ki67 (grey), DAPI (blue) in SO_2 injured trachea. The dotted line separates the SE and mesenchyme. Scale bar: 100μm.

(E) Quantification of $ACTA2^+$, $SOX9^+$ and $Ki67^+$ cells among total $DAPI^+$ cells in SE of control and SO_2 injured mice. Data shown as mean ± SEM (n=3; $^{*}p \leq 0.05$; $^{**}p \leq 0.01$; $^{***}p \leq 0.001$). C0-3, C3-6, and C6-9 refer to SE spanning cartilage rings between 0-3, 3-6 or 6-9 respectively. *p*- values were determined compared to controls.

(F) Quantification of $ACTA2^+$, $SOX9^+$ co-expressing $Ki67^+$ cells in SE of control and day-5 post SO_2 injury. Data shown as mean ± SEM (n=3; $^{**}p \leq 0.01$; $^{***}p \leq 0.001$). *p*- values were determined compared to controls.

(G) Co-staining of ACTA2 (green), SOX9 (red), Ki67 (grey), DAPI (blue) in influenza injured trachea. The dotted line separates the SE and mesenchyme. Scale bar: 100μm.

(H) Quantification of $ACTA2^+$, $SOX9^+$ and $Ki67^+$ cells among total $DAPI^+$ cells in SE of control and influenza injured mice. Data shown as mean ± SEM (n=3; $^{*}p \leq 0.05$; $^{**}p \leq 0.01$). C0-3, C3-6, and C6-9 refer to SE spanning cartilage rings between 0-3, 3-6 or 6-9 respectively. *p*- values were determined compared to controls.

(I) Quantification of $ACTA2^+$, $SOX9^+$ co-expressing $Ki67^+$ cells in SE of control and day-5 post PR8 injury. Data shown as mean ± SEM (n=3; $^{***}p \leq 0.001$). *p*- values were determined compared to controls.

Figure S3: Sox9-expressing SMG cells contribute to the SE repair following naphthalene-induced injury.

(A) Representative images of Sox9-tdt SMG after tamoxifen treatment co-stained with ACTA2 and SOX9 (green) upper panel (Scale bar: 100μm) and KRT5 (green) and KRT8 (grey) lower panel. Scale bar: 50μm.

(B) Three months old Sox9-tdt mice received three doses of tamoxifen and were sacrificed at 13 months. Sox9-tdt (red) Tracheal sections were co-stained for KRT5 (green) and KRT8 (grey) and nuclear DAPI (blue). Scale bar: 50μm

(C) IHC for KRT14 (green) and Sox9-tdt (red) on airway sections from day 5 and 10 post naphthalene injury. Scale bar: 100μm.

(D) Co-staining of ZO-1 (green), CDH1 (grey) and Sox9-tdt (red) from control and day-5 and day-20 naphthalene injured Sox9-tdt trachea mice. Scale bar: 50μm.

Figure S4: SMG-derived ACTA2-lineage labeled MECs repair SMGs and contribute to both basal and luminal cells *in vivo* following injury

(A) Acta2-lineage labeling and IHC for ACTA2 (green) on control and naphthalene day 12 injured tracheal SMGs. The arrows indicate luminal Acta2-tdt lineage labeled cells in injured trachea. Scale bar: 20μm.

(B) Acta2-lineage labeling and IHC for basal cell marker TP63 (green) on control and naphthalene day 12 injured tracheal SMGs. The arrows indicate TP63^{-}Acta2-tdt lineage labeled cells in injured tracheal SMGs. Scale bar: 20μm.

(C) Co-staining of KRT5 (green) and KRT8 (grey) in control or naphthalene-day 20 Acta2-tdt mice. The arrows indicate luminal cells derived from Acta2-tdt lineage labeled cells in injured trachea. Scale bar: 20μm.

(D) Quantification of $KRT8^{+}Acta2\text{-}tdt^{+}$ cells among total $Acta2\text{-}tdt^{+}$ cells in SMGs in control and naphthalene day- 20 injured mice. Data shown as mean ± SEM (n=3; ** $p \leq 0.01$).

(E) Lactoferrin (green; left panel) and Muc5B (green; right panel) co-stained with KRT8 (grey), DAPI (blue) in naphthalene injured day-20 Acta2-tdt (red) mice. Scale bar: 20μm

Figure S5: SMG cells culture and engraftment models.

(A) Isolated Acta2-tdt myoepithelial cells were cultured for 5 days, then fixed and stained for ACTA2 (green), KRT5, TP63, and KRT14 (grey), nuclear DAPI (blue). Scale bar: 50μm.

(B) Acta2-tdt cells cultured for 5 or 20 days followed by co-IHC for ACTA2 (green) and AQP3 (grey) –upper panel or NGFR (green) and ITGA6 (grey) – lower panel.

(C) KRT8 (green) and KRT5 (grey) whole mount IF on trachea treated with PBS (control) or cell stripper. Scale bar: 50μm

Figure S6: Chlorine gas-inhalation causes severe damage to porcine airway tissues.

(A) Hematoxylin and eosin staining on filtered air or chlorine gas administered pig airways on day-2 post exposure. Scale bar: 100μm. Black box indicates magnified area shown in right panels.

(B) Co-staining of SOX9 (green), ACTA2 (red), Ki67 (grey), and DAPI (blue) in chlorine injured porcine trachea. The dotted line separates the SE and mesenchyme. Scale bar: 50μm

Figure S7: ***Single cell RNA-seq analysis identifies differentially expressed genes and pathways.***

(A) Heatmap shows differentially expressed genes between Acta2^{+} cells from homeostatic SMGs and Acta2^{+} cells on SE following naphthalene-induced injury, as identified through scRNA-seq analysis.

(B) Enricher analysis identifies Gene Ontology terms, pathways, genomic and proteomic regulatory networks associated with differentially expressed genes between Acta2^{+} cells from homeostatic SMGs and Acta2^{+} cells on SE following naphthalene-induced injury.