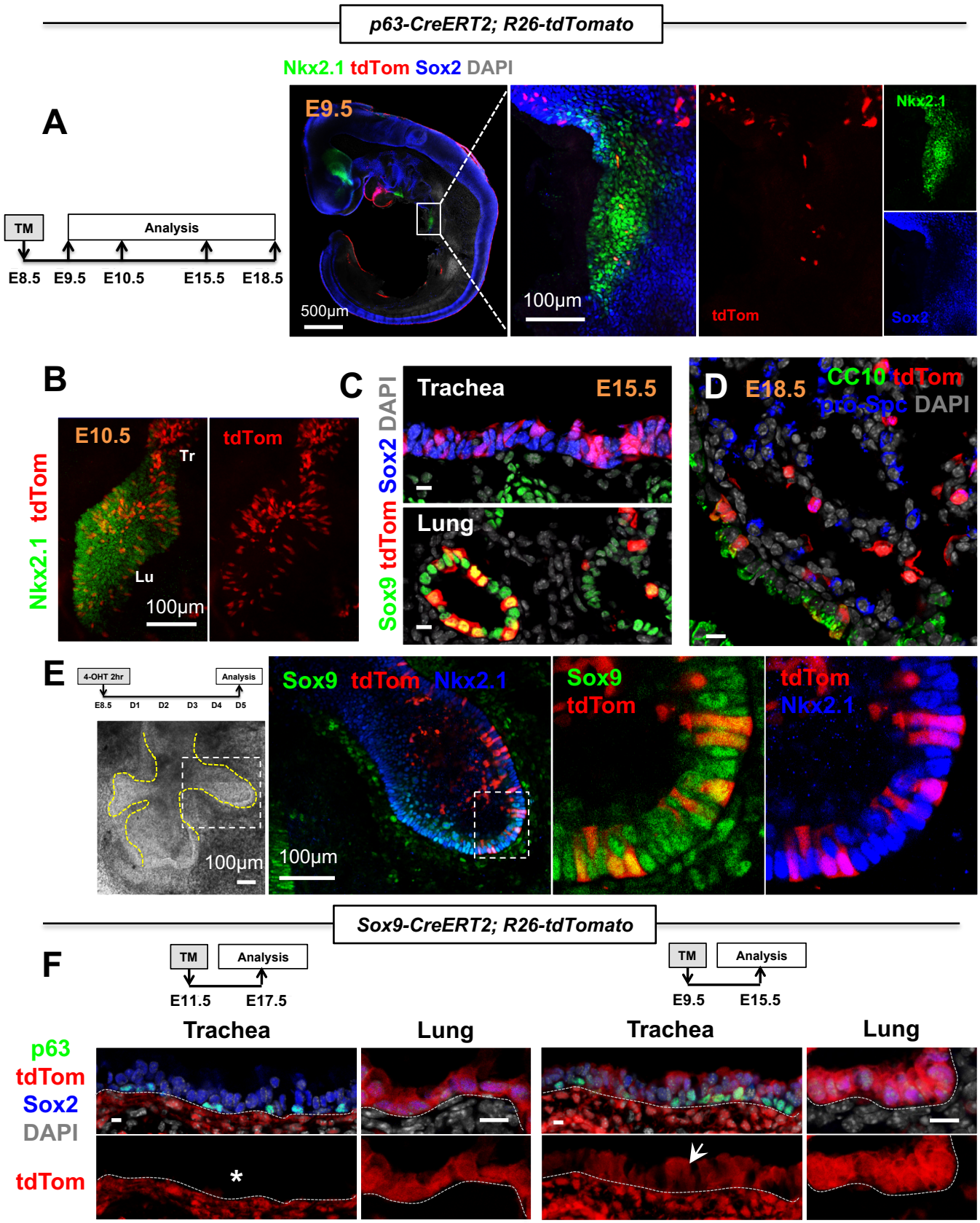

Fig. S1
Yang et al.

Figure S1, related to Figure 1. Lineage restriction of extrapulmonary (p63) and intrapulmonary (Sox9) multipotent progenitors in the lung. (A-F) *p63-CreERT2; R26-tdTomato* lineage labeling: TM administration at E8.5 *in vivo* **(A-D)** or *ex-vivo* **(E)**. IF of E9.5-E18.5 lungs: $Nkx2.1^+$ $tdTom^+$ cells in tracheal (Tr) and lung (Lu) primordia; at E15.5 $tdTom^+$ cells are abundant in tracheal epithelium but extending ectopically to distal ($Sox9^+$) lung buds (**C**, lower panel), by E18.5 $tdTom^+$ are present in intrapulmonary airways (double-labeled with CC10) and alveolar sacs (**D**, double-labeled with pro-Spc). N=6 embryos from 3 litters showing extensive alveolar labeling with TM exposure at E8.5. **(E)** Foregut explants from E8.5 reporter treated with 4-OHT and cultured for 5 days (left). IF: $tdTom^+$ distal lung buds double labeled with Sox9 or Nkx2.1. Bud with ectopic distal tdTom labeling (box) enlarged in right panels. N=8 explants from two independent experiments. **(F)** Lineage analysis of *Sox9-CreERT2; R26-tdTomato* mice *in vivo*. IF: E18.5 trachea and lung (intrapulmonary airways) from embryos exposed to TM at E11.5 (left) or E9.5 (right). Proper lineage restriction of distal Sox9 descendants to intrapulmonary airways confirmed by absence of $tdTom^+$ in tracheal epithelium (* $p63^+Sox2^+$) of TM-treated embryos at E11.5 but not at E9.5 (arrow: tdTom labeling extending to trachea).

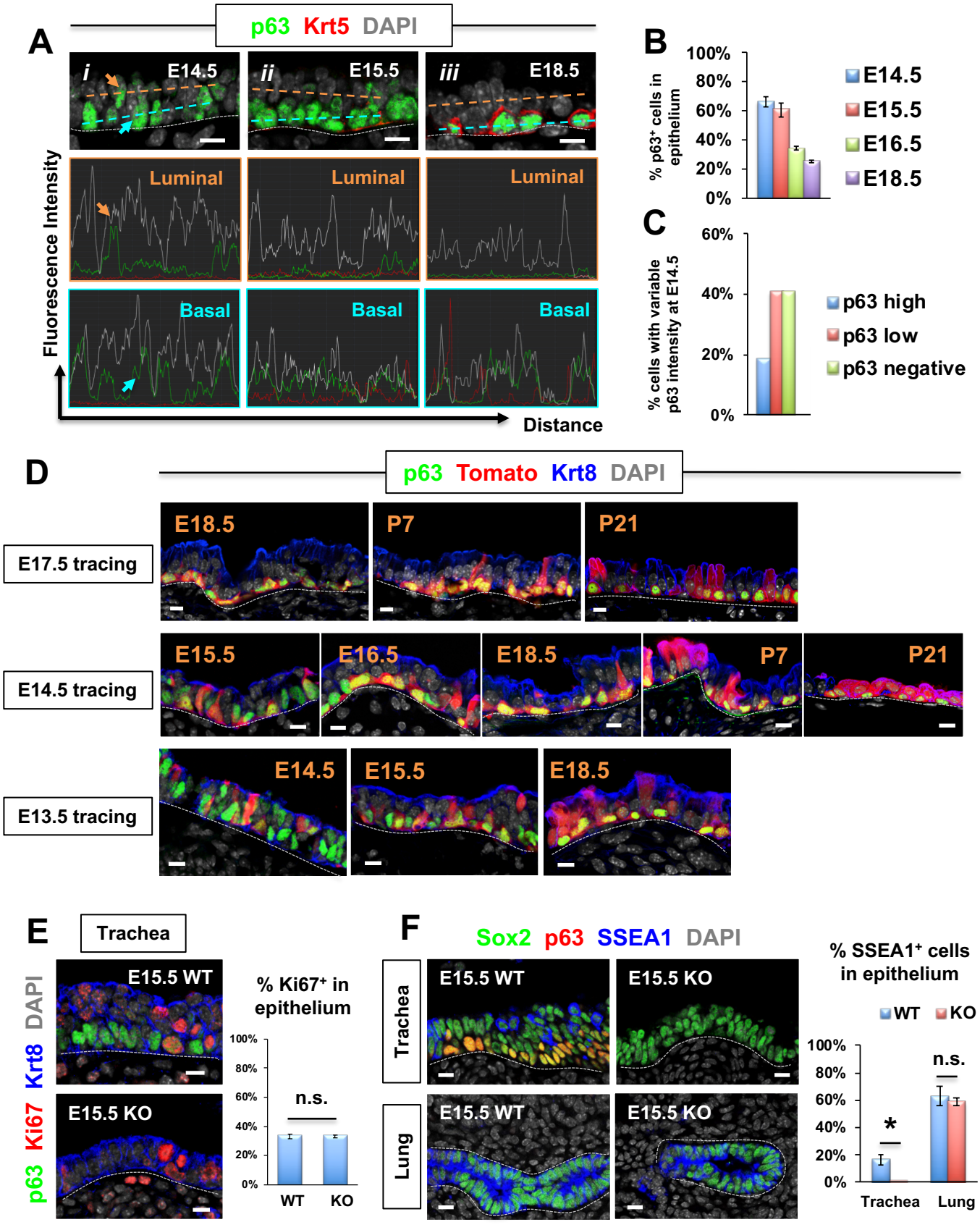

Fig. S2
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Figure S2, related to Figures 2 & 3. Dynamics of p63 expression pattern and lineage commitment in tracheal development. (A) Segregation of basal and luminal compartments in the pseudostratified trachea, profile analysis of p63 (green peaks) and Krt5 (red peaks) in both basal and luminal sides at E14.5, E15.5 and E18.5. Orange arrow showing luminal $p63^+$ cells with high intensity. Cyan arrow showing basal $p63^+$ cells with high intensity. **(B)** Graph showing % $p63^+$ cells in tracheal epithelium at indicated stages. **(C)** Graph showing % p63-expressing cells with variable fluorescence intensities at E14.5 from 150 randomly selected epithelial cells in trachea. **(D)** IF analysis of p63 lineage tracing in tracheal epithelium with TM exposure at E13.5, E14.5 and E17.5. **(E)** IF of E15.5 tracheas showing no difference in proliferation index (Ki67) in epithelium between WT and p63 KO mice. **(F)** IF at E15.5: marked reduction in SSEA1-expressing cells in KO selectively in trachea not in intrapulmonary airways (lung; note Sox2 epithelial labeling in all panels but p63 restricted in WT). Graphs in **(B)**, **(E)**, **(F)** are mean ± SEM from 6-12 fields per sample, N>=3, details in Table S1. Student's t-test: *P<0.05; n.s. non-significant. Scale bars: 10μm.

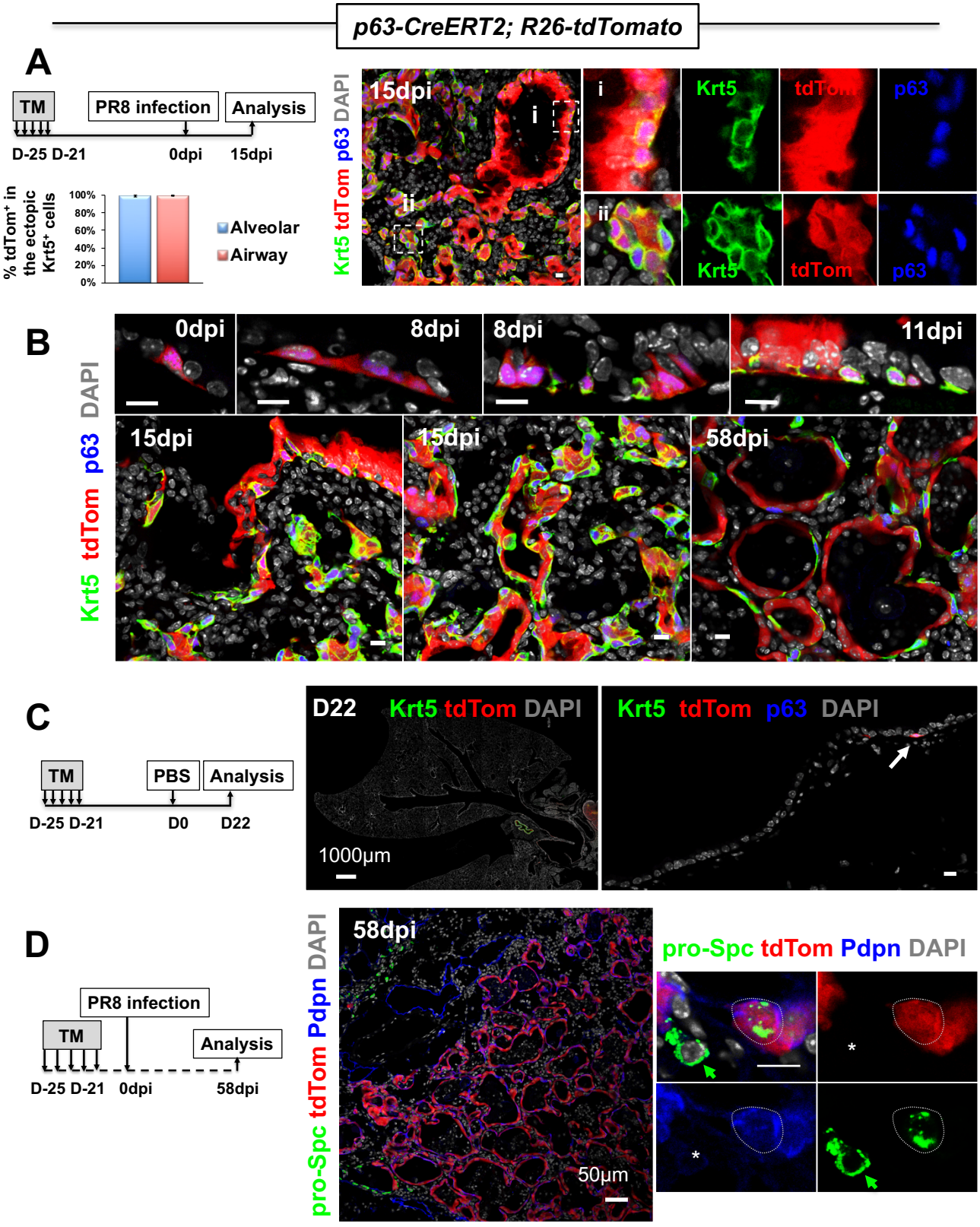

Fig. S3
Yang et al.

Figure S3, related to Figure 4. p63 lineage-labeled cells generating the Krt5^{+} pods with long-term persistence after H1N1 challenge (A, B, D) Lineage labeling of *p63-CreERT2; R26-tdTomato* mice infected with H1N1-PR8 21 days after TM administration in adulthood. **(A)** IF analysis of 15dpi lungs showing continuous trail of tdTom^{+} cells from large airway to alveolar space with nearly 99% tdTom^{+} labeling of the ectopic Krt5^{+} cells in the airway (i) and alveolar (ii) compartments. Graphs: mean ± SEM from 10-12 fields per sample. n=5 from 3 independent experiments. **(B)** representative images showing time course response of intrapulmonary p63^{+}Krt5^{-}tdTom^{+} cells to H1N1: expansion of endogenous p63^{+}tdTom^{+} cells (0dpi-8dpi), acquisition of Krt5 (8dpi-11dpi), extensive tdTom labeling of the airway epithelium with migrating trail towards adjacent alveolar space to form the p63^{+}Krt5^{+}tdTom^{+} clusters and honeycomb-like structures (15 dpi) which persisted by 58dpi. **(C)** PBS mock-infection showing the rare lineage-labeled p63^{+}Krt5^{-} intrapulmonary progenitors (arrow) unaltered after 22 days. **(D)** IF analysis of 58 dpi lungs showing persisted Pdpn^{+}tdTom^{+} cysts with minimal contribution to alveolar regeneration. *no fluorescent signal. Scale bars: 10μm unless noted.

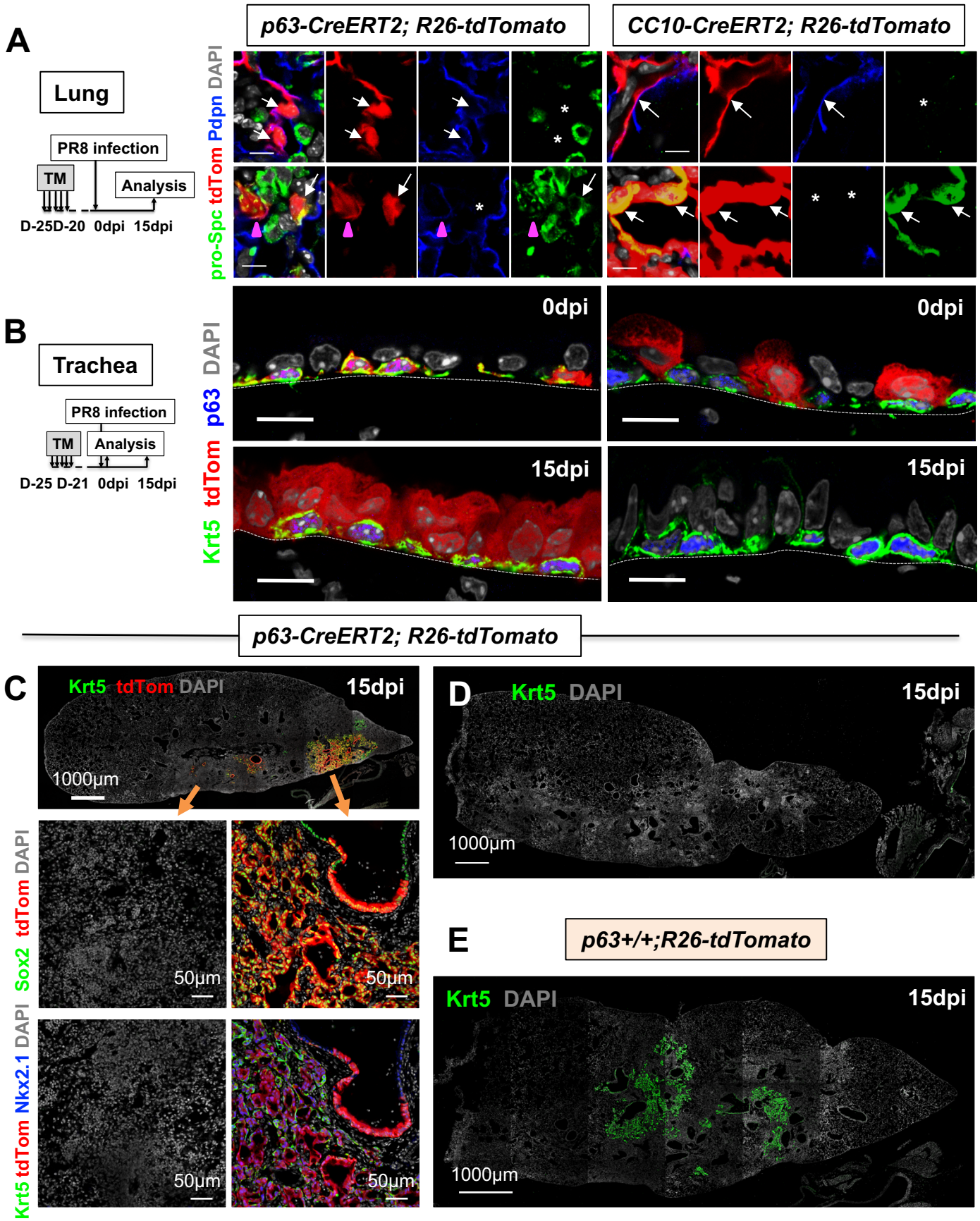

Fig. S4
Yang et al.

Figure S4, related to Figure 5. Comparison of *p63-CreERT2* and *CC10-CreERT2* mice showing distinct response to H1N1 challenge due to p63 haploinsufficiency. (A) IF analysis of 15 dpi lungs showing rare tdTom-labeled alveolar Type I ($Pdpn^+pro\text{-}Spc^-$ with line structure, upper panels) and Type II cells ($Pdpn^-pro\text{-}Spc^+$, lower panels) in both *p63-CreERT2* and *CC10-CreERT2* mice. *no fluorescent signal. Magenta arrowhead showing the $tdTom^+$ intermediate cell co-expressing Pdpn and pro-Spc. (**B)** Tracheal sections of adult *p63-CreERT2* (left panel) or *CC10-CreERT2* (right panel) mice showing no $p63^+CC10$ lineage-labeled cells in the uninjured tracheal epithelium (present in the uninjured intrapulmonary epithelium; compare upper panels with Fig.5E). Bottom panels: H1N1 injury results in extensive epithelial repair at 15dpi by p63 lineage-labeled but not CC10 lineage-labeled $tdTom^+$ cells in trachea. **(C)** IF analysis of 15dpi lung sections of *p63-CreERT2* mice: cells in the pods are $Sox2^+$ and $Nkx2.1^+$, while the surrounding parenchyma devoid of pods is absent of lung epithelial cells. **(D-E)** IF analysis of 15dpi lung sections of *p63-CreERT2* mice or the *p63+/+* WT littermates. N=10 for *p63+/+* animals showing ectopic $Krt5^+$ cells in lung after H1N1 challenge in two independent experiments. Note some *p63-CreERT2* mice failed to generate the ectopic $Krt5^+$ cells in response to H1N1 challenge, while this phenomenon was never observed in *p63+/+* WT animals. Scale bars: 10μm unless noted.

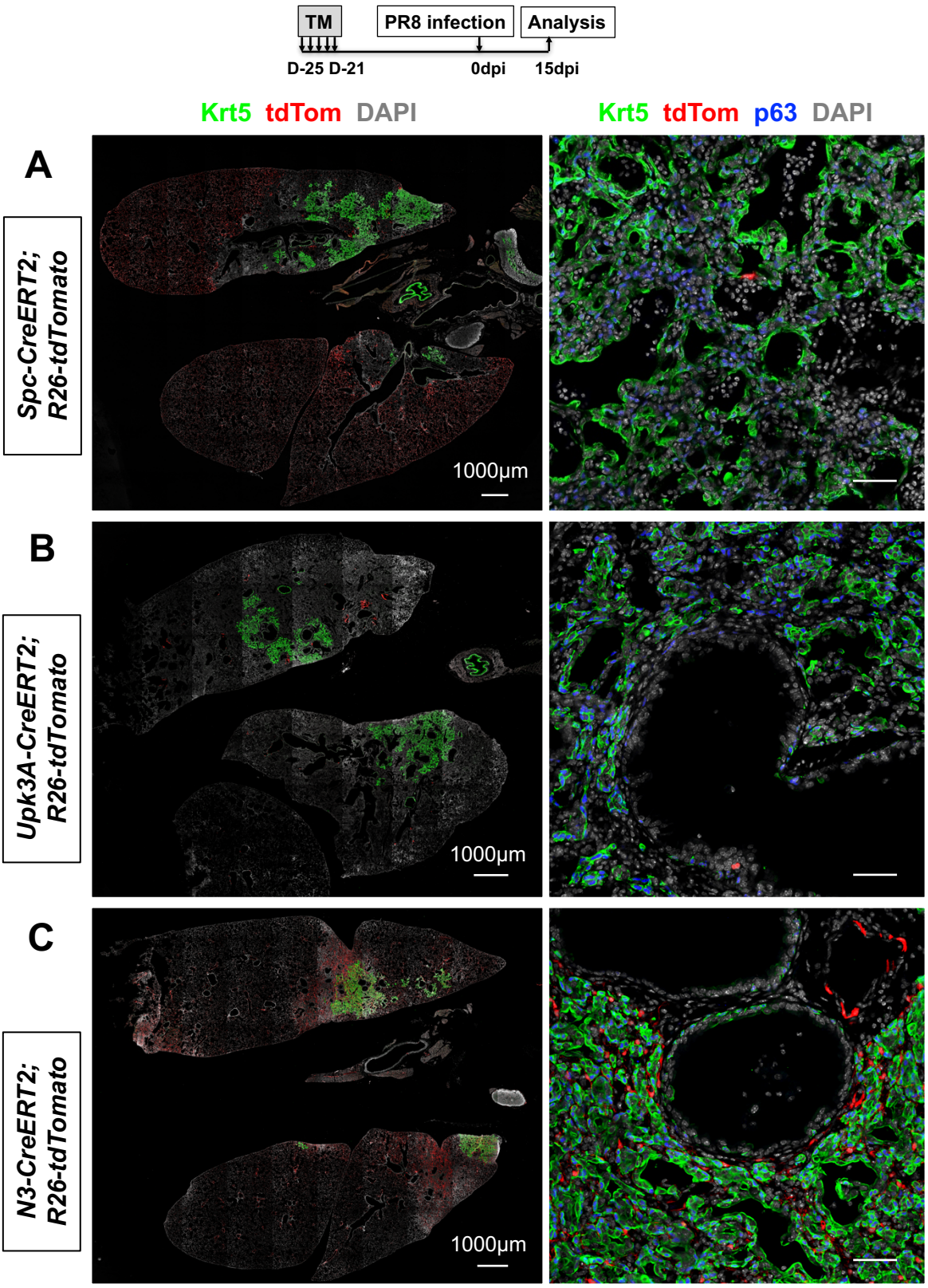

Fig. S5
Yang et al.

Figure S5, related to Figure 5. Variable Cre lines of lung lineages show minimal labeling in the H1N1-induced ectopic Krt5^{+} cells. (A-C) IF analysis of 15dpi lungs showing non-overlapped pattern between tdTom lineage label and Krt5 cells. Left panel showing tile scanning images of whole lung sections; right panels showing areas of Krt5^{+} pods. **(A)** *Spc-CreERT2* line labeling the descendants of alveolar Type II cells. N=2 from two independent experiments. **(B)** *Upk3A-CreERT2* line labeling the descendants of secretory cells associated with neuroendocrine bodies and terminal bronchioles. N=5 from 3 independent experiments. **(C)** *N3-CreERT2* line labeling the descendants of mesenchymal and scattered epithelial cells in airways. N=3 from one experiment. Scale bars: 50μm unless noted.

Table S1, related to STAR Methods, and Figures 2-5 and S2-4. Morphometric analysis.

Table S2, related to Figures 5 and S4. *p63-CreERT2* haploinsufficiency shows an attenuated response to H1N1 challenge in generating Krt5$^+$ pods compared to the *p63+/+* littermates and *other Cre lines*. Number of mice from each reporter line showing ectopic Krt5$^+$ cells or not in injured lungs at least 15 days after infection.

	K5	no K5	K5 frequency
p63-CreERT2; R26-tdTomato	28	38	42.4%
p63+/+; R26-tdTomato	10	0	100.0%
CC10-CreERT2; R26-tdTomato	11	0	100.0%
Spc-CreERT2; R26-tdTomato	2	0	100.0%
Upk3A-CreERT2; R26-tdTomato	5	0	100.0%
N3-CreERT2; R26-tdTomato	3	0	100.0%

Movies S1-4, related to Figures 1, 4 and S1. Confocal z-stack images of whole mount IF staining showing p63 expression in early development. p63 is weakly expressed in the earliest Nkx2.1-GFP$^+$ lung field at E9.0 (Movie S1) and E9.5 (Movie S2), and then proximally restricted to tracheal domain at E10.5 (Movie S3). At E12.5 p63 is broadly expressed in extrapulmonary airways and gradually reduced towards distal region along main bronchi in intrapulmonary airways (Movie S4). *Nkx2.1-GFP* embryos were used in Movie S1, S2 and S3: green showing GFP; red showing p63; blue showing Sox2. WT embryo was used in Movie S4: green showing GFP; red showing Ecad; blue showing Sox2.