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Supplemental Information

Airway stem cell reconstitution by the transplantation of primary or pluripotent stem cell-derived basal cells

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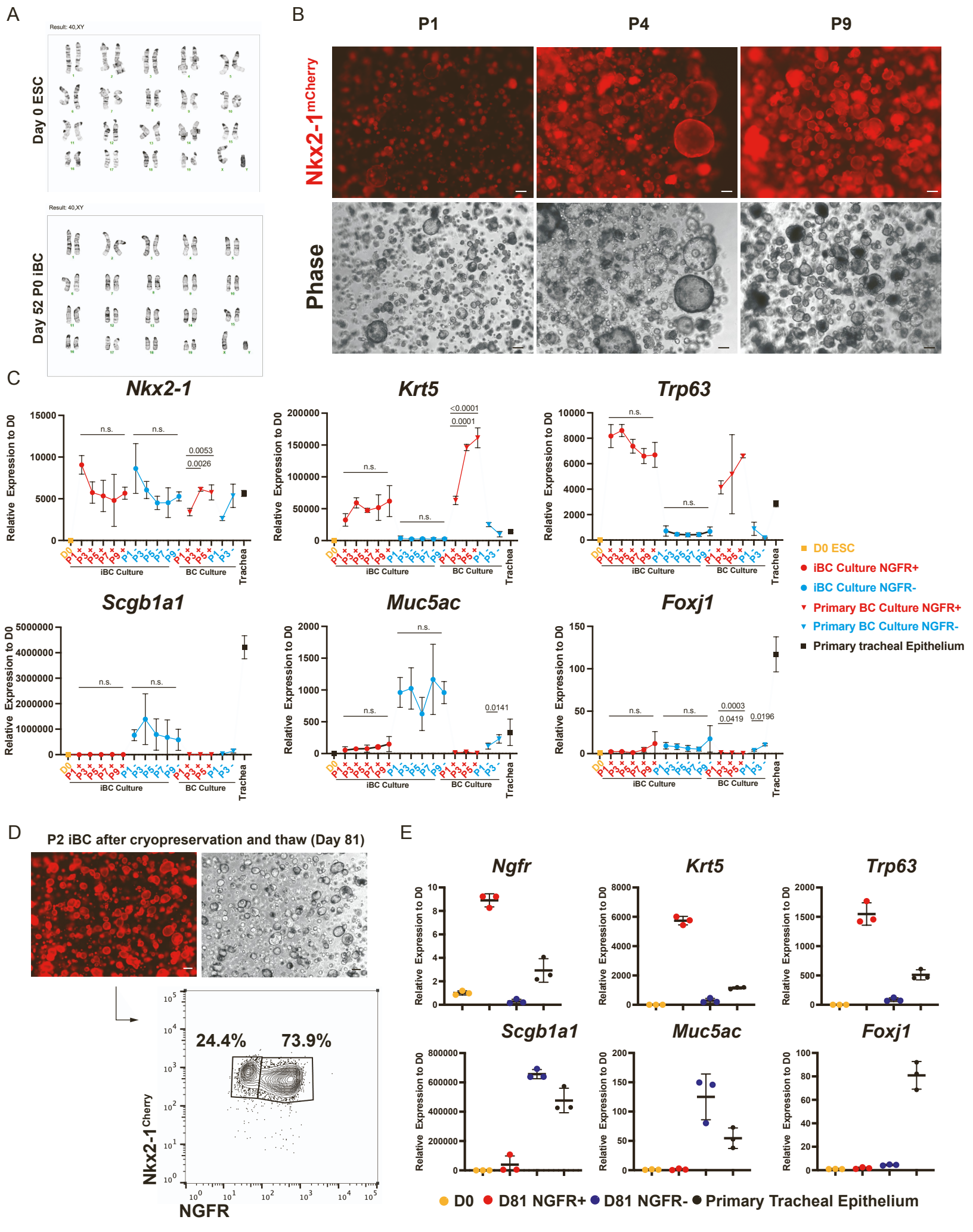
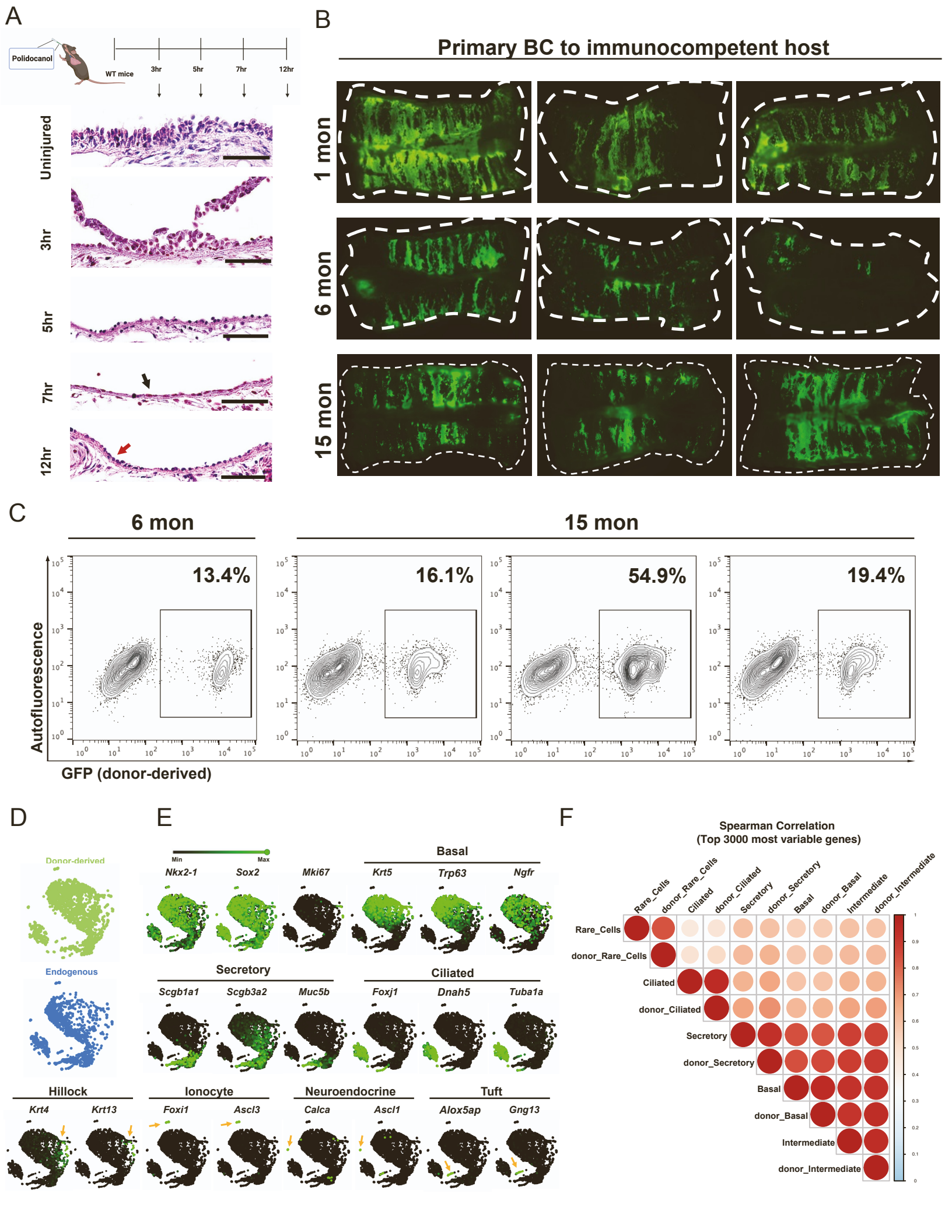

A
Result: 40,XY
Day 0 ESC
Result: 40,XY
Day 52 P0 iBC
B
P1
P4
P9
Nkx2-1mCherry
Phase
C
Nkx2-1
Krt5
Trp63
Scgb1a1
Muc5ac
Foxj1
Relative Expression to D0
n.s.
0.0053
0.0026
<0.0001
0.0001
0.0141
0.0003
0.0419
0.0196
iBC Culture
BC Culture
Trachea
D0 ESC
iBC Culture NGFR+
iBC Culture NGFR-
Primary BC Culture NGFR+
Primary BC Culture NGFR-
Primary tracheal Epithelium
D
P2 iBC after cryopreservation and thaw (Day 81)
24.4%
73.9%
Nkx2-1Cherry
NGFR
E
Ngfr
Krt5
Trp63
Scgb1a1
Muc5ac
Foxj1
Relative Expression to D0
D0
D81 NGFR+
D81 NGFR-
Primary Tracheal Epithelium

Supplementary Figure 1: Characterization of murine iBC and primary BC cultures, Related to Main Figure 1

(A) Representative G-banding analysis indicating karyotypically normal undifferentiated murine ESCs and differentiated iBCs.
(B) Representative images of iBC cultures at P1, P4, and P9. Scale bars, 200 µm.
(C) RT-qPCR analysis of iBC and primary BC cultures. Bars indicate mean +/- SD. n = 3 biological replicates. For comparisons with three or more samples, one-way ANOVA was used. For comparisons with only two samples, paired two-tail Student's t-test was used.
(D) Representative image and corresponding FACS analysis of iBC culture two passages (P2; D81) after thawing from cryopreservation. Scale bars, 200 µm
(E) RT-qPCR analysis of iBC P2 after thawing from cryopreservation. Bars indicate mean +/- SD. n = 3 biological replicates.


A
Polidocanol
WT mice
3hr
5hr
7hr
12hr
Uninjured
3hr
5hr
7hr
12hr
B
Primary BC to immunocompetent host
1 mon
6 mon
15 mon
C
6 mon
15 mon
13.4%
16.1%
54.9%
19.4%
Autofluorescence
GFP (donor-derived)
D
Donor-derived
Endogenous
E
Min
Max
Nkx2-1
Sox2
Mki67
Basal
Krt5
Trp63
Ngfr
Secretory
Scgb1a1
Scgb3a2
Muc5b
Ciliated
Foxj1
Dnah5
Tuba1a
Hillock
Krt4
Krt13
Ionocyte
Foxi1
Ascl3
Neuroendocrine
Calca
Ascl1
Tuft
Alox5ap
Gng13
F
Spearman Correlation
(Top 3000 most variable genes)
Rare_Cells
donor_Rare_Cells
Ciliated
donor_Ciliated
Secretory
donor_Secretory
Basal
donor_Basal
Intermediate
donor_Intermediate

Supplementary Figure 2: Post-injury mouse tracheal histological time series assessment and syngeneic transplantation of murine primary BC into immunocompetent recipient mice, Related to Main Figure 2

(A) Representative H&E staining of mouse tracheas 3, 5, 7, and 12 hours post-polidocanol injury. At 7hrs post-injury, black arrow indicates flattened cells covering the basement membrane. At 12hrs post-injury, red arrow indicates clusters of epithelial cells. Scale bars, 50 μm.

(B) Tracheal whole mount fluorescence microscopy and FACS of recipients of GFP+ primary BCs, shown at various timepoints (1, 6 or 15 months, n = 9) post-transplantation.

(C) FACS analysis of primary BC recipient at 6- or 15-months post-transplantation, demonstrating persistence of donor-derived cells in recipient tracheas.

(D) SPRING visualization of scRNA-Seq of primary BC recipient, separated by sample origin (also shown in main Figure 2E).

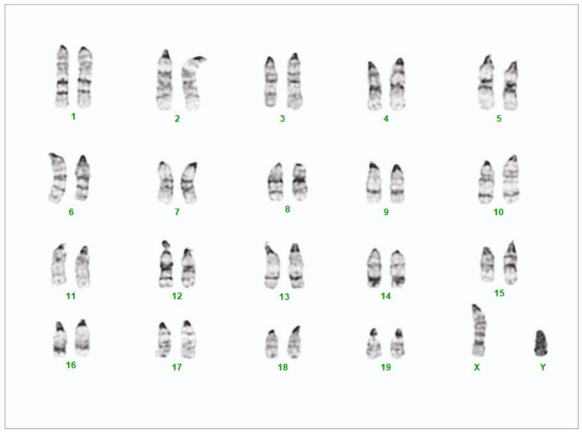
(E) Selected canonical marker gene expression levels (in green) overlayed on combined SPRING plots from (C).

(F) Spearman correlation coefficients of single cell transcriptomic profiles of donor-derived and endogenous cells in recipients of primary mouse BCs (69 days post transplantation) based on the Top 3000 most variable genes.

A

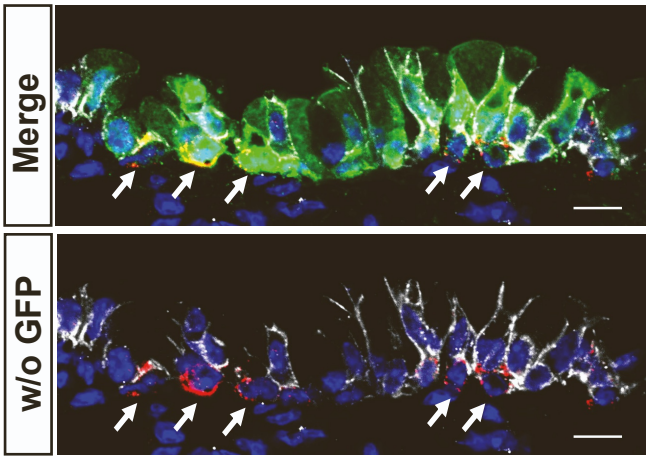
Day 106 iBC post-lentiviral transduction and freeze-thaw

Result: 40,XY



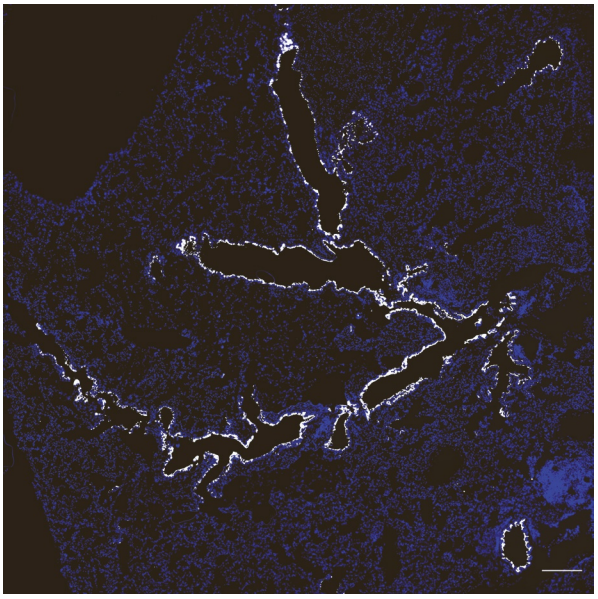
B

GFP E-Cadherin KRT5 Nuclei



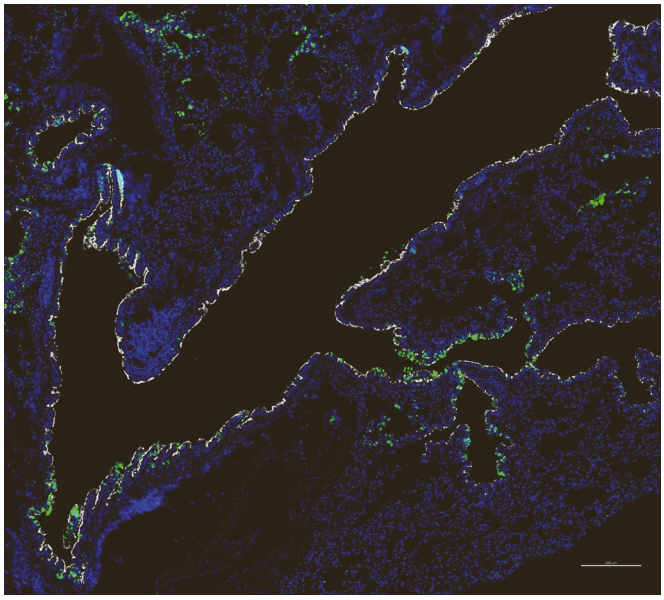
C

GFP ACTUB Nuclei



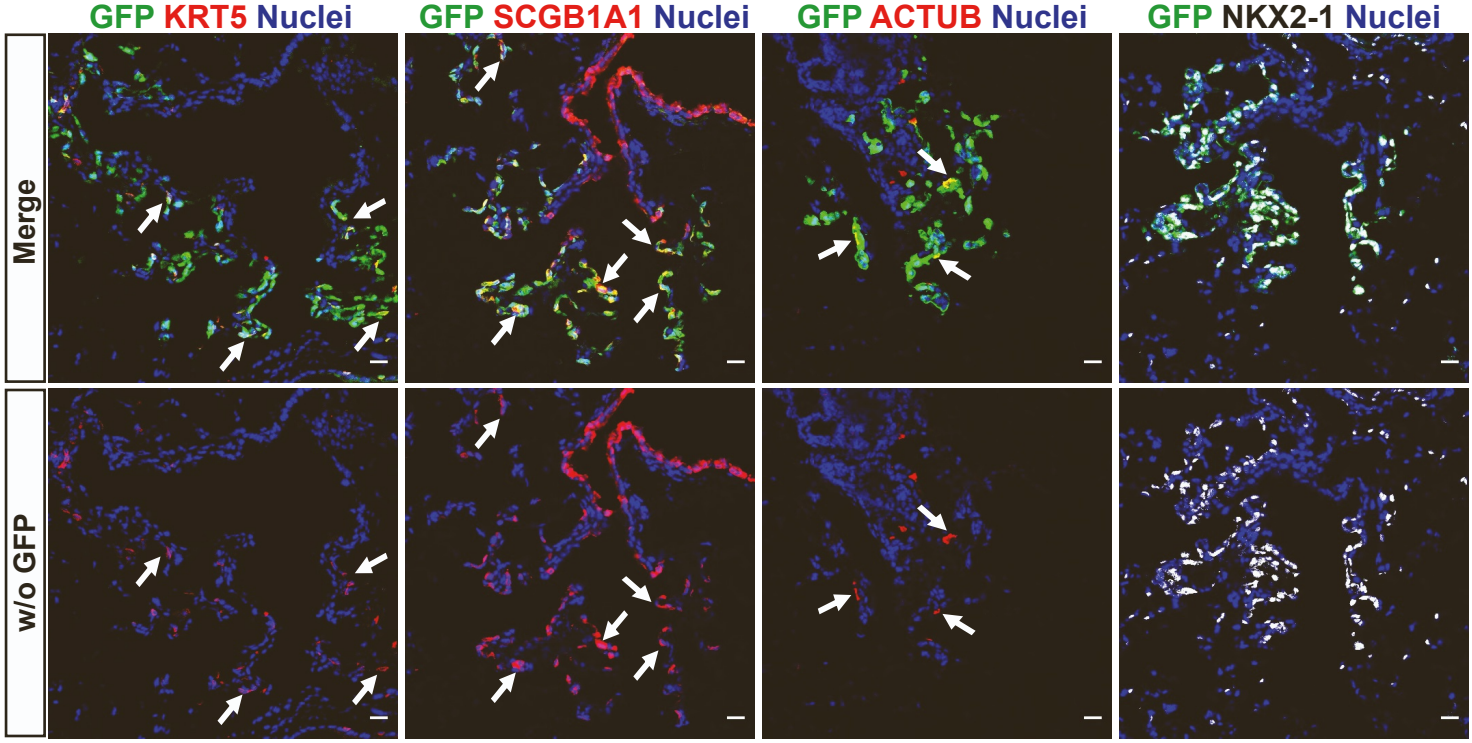
Majority cases (14/19)
Specific sample from 192 dpt (Fig.3C)

GFP ACTUB Nuclei



Minority cases (5/19)
Specific sample from 310 dpt

D



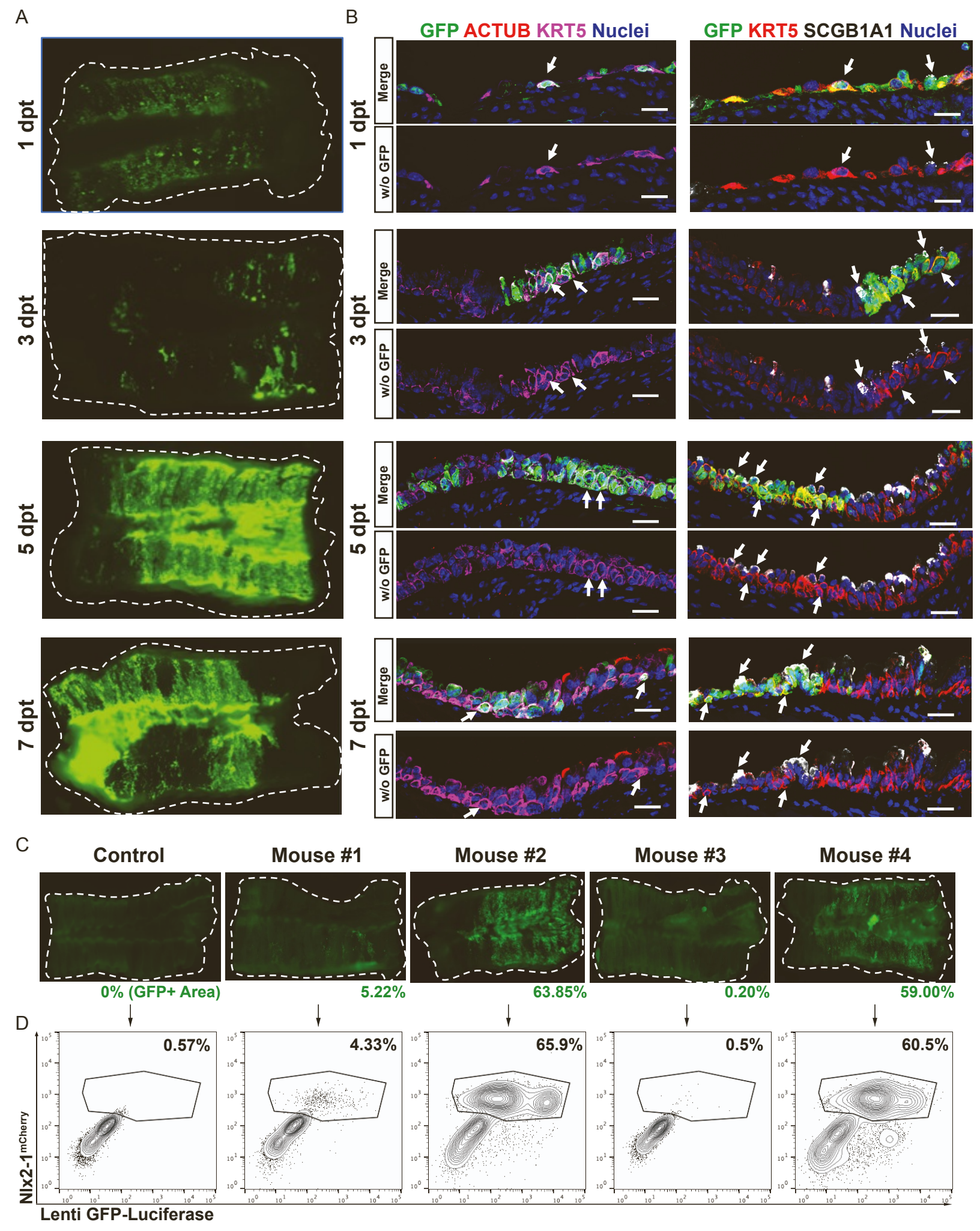
Supplementary Figure 3: Syngeneic transplantation of murine iBC into immunocompetent recipient mice, Related to Main Figure 3

(A) Representative G-banding analysis indicating karyotypically normal donor iBCs after lentiviral transduction, cryopreservation, and thawing prior to transplantation.

(B) Immunofluorescence confocal microscopy for GFP, KRT5, and E-Cadherin in iBC recipient trachea 1-year post-transplantation. Nuclei are counterstained with Hoechst33342. White arrows indicate GFP+ cells co-expressing KRT5. Lower panels have the GFP channel removed. Scale bars,10 μm.

(C) Left: representative distal lung immunofluorescence microscopy of iBC recipient. Right: in rare cases, GFP+ signal was observed in the intrapulmonary large and small (distal) airways. Nuclei are counterstained with Hoechst33342. Scale bars, 200 μm.

(D) Immunofluorescence confocal microscopy for GFP, ACTUB, SCGB1A1, KRT5, and NKX2-1 in iBC recipient intrapulmonary airway. Nuclei are counterstained with Hoechst33342. White arrows indicate GFP+ cells co-expressing canonical airway epithelial markers. Lower panels have the GFP channel removed. Scale bars, 20 μm.


A
B
GFP ACTUB KRT5 Nuclei
GFP KRT5 SCGB1A1 Nuclei
1 dpt
3 dpt
5 dpt
7 dpt
Merge
w/o GFP
C
Control
Mouse #1
Mouse #2
Mouse #3
Mouse #4
0% (GFP+ Area)
5.22%
63.85%
0.20%
59.00%
D
0.57%
4.33%
65.9%
0.5%
60.5%
Nlx2-1mCherry
Lenti GFP-Luciferase

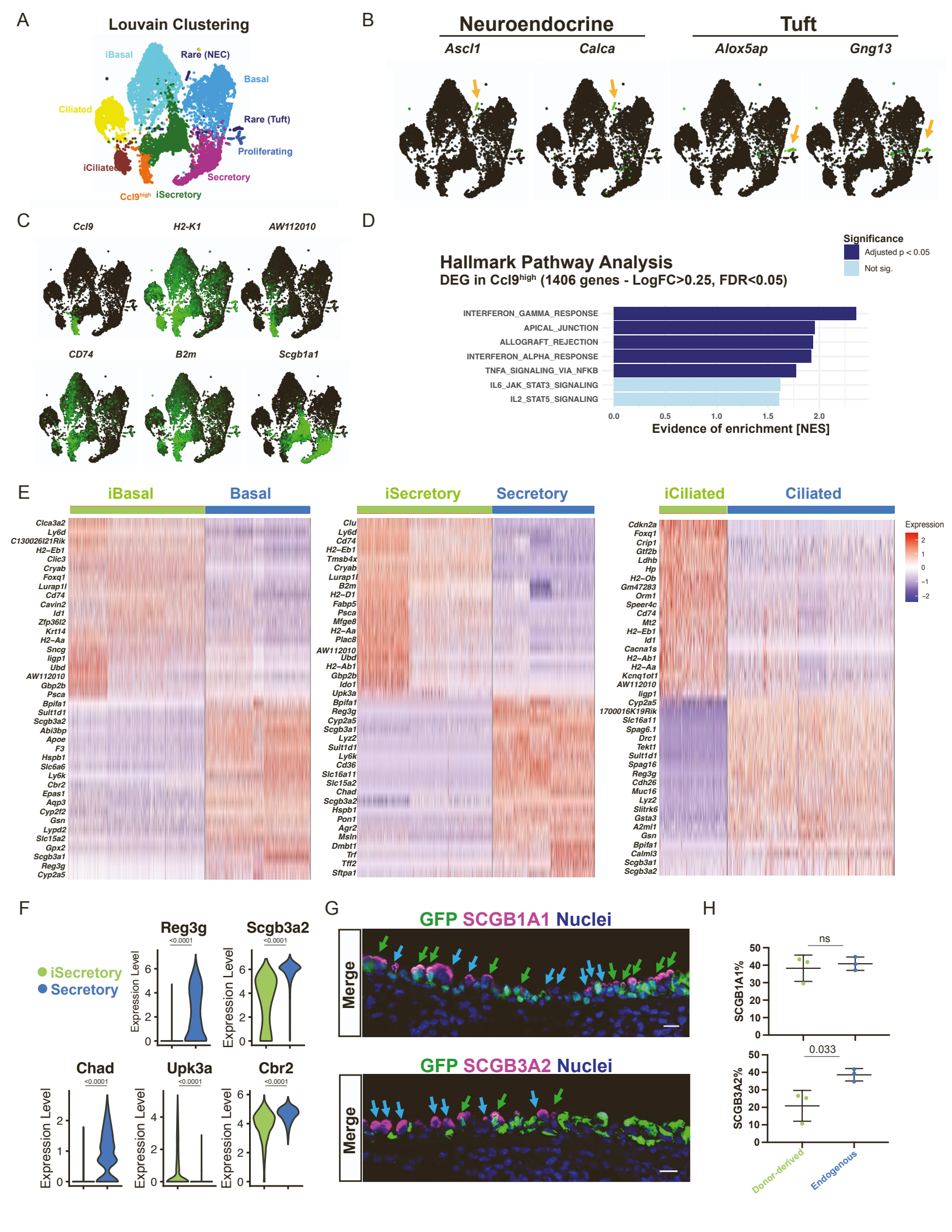
Supplementary Figure 4: Dynamic changes post-murine iBC transplantation, Related to Main Figure 3

(A) Whole mount fluorescence of iBC recipients 1, 3, 5, or 7 days post-transplantation.

(B) Immunofluorescence confocal microscopy for GFP, ACTUB, SCGB1A1, KRT5, NKX2-1 in iBC recipient tracheas at 1, 3, 5, or 7days post-transplantation. Nuclei are counterstained with Hoechst33342. White arrows indicate GFP+ cells co-expressing canonical airway epithelial markers. Lower panels have the GFP channel removed. Scale bars, 10 μm.

(C) Whole mount tracheal fluorescence and corresponding FACS analysis (gated from all live cells harvested from the digest) of recipients of iBCs labeled with GFP-Luciferase expressing lentivirus as shown in Figure 3H. Transplantation efficiencies measured by GFP+% area over total trachea area (marked by dotted line) are shown below each recipient trachea image.

(D) Corresponding FACS plots for tracheas shown in (C).


A
Louvain Clustering
iBasal
Rare (NEC)
Basal
Ciliated
Rare (Tuft)
Proliferating
iCiliated
Secretory
Ccl9high iSecretory
B
Neuroendocrine
Tuft
Ascl1
Calca
Alox5ap
Gng13
C
Ccl9
H2-K1
AW112010
CD74
B2m
Scgb1a1
D
Significance
Adjusted p < 0.05
Not sig.
Hallmark Pathway Analysis
DEG in Ccl9high (1406 genes - LogFC>0.25, FDR<0.05)
INTERFERON_GAMMA_RESPONSE
APICAL_JUNCTION
ALLOGRAFT_REJECTION
INTERFERON_ALPHA_RESPONSE
TNFA_SIGNALING_VIA_NFKB
IL6_JAK_STAT3_SIGNALING
IL2_STAT5_SIGNALING
0.0
0.5
1.0
1.5
2.0
Evidence of enrichment [NES]
E
iBasal
Basal
iSecretory
Secretory
iCiliated
Ciliated
Expression
F
iSecretory
Secretory
Reg3g
Scgb3a2
Chad
Upk3a
Cbr2
<0.0001
Expression Level
G
GFP SCGB1A1 Nuclei
Merge
GFP SCGB3A2 Nuclei
H
SCGB1A1%
SCGB3A2%
ns
0.033
Donor-derived
Endogenous

Supplementary Figure 5: Single cell transcriptomic profiling of murine iBC recipient tracheas, Related to Main Figure 4

(A) SPRING Visualization of iBC recipient tracheas profiled by scRNA-Seq. Cells are colored by Louvain clustering (resolution = 0.2) and annotated as in Figure 4C.

(B) Expression of rare cell canonical gene markers (green) overlayed on SPRING plots from (A).

(C) Expression of selected genes associated with H2-K1+ Scgb1a1^{low} airway progenitor cells[24] by scRNA-Seq.

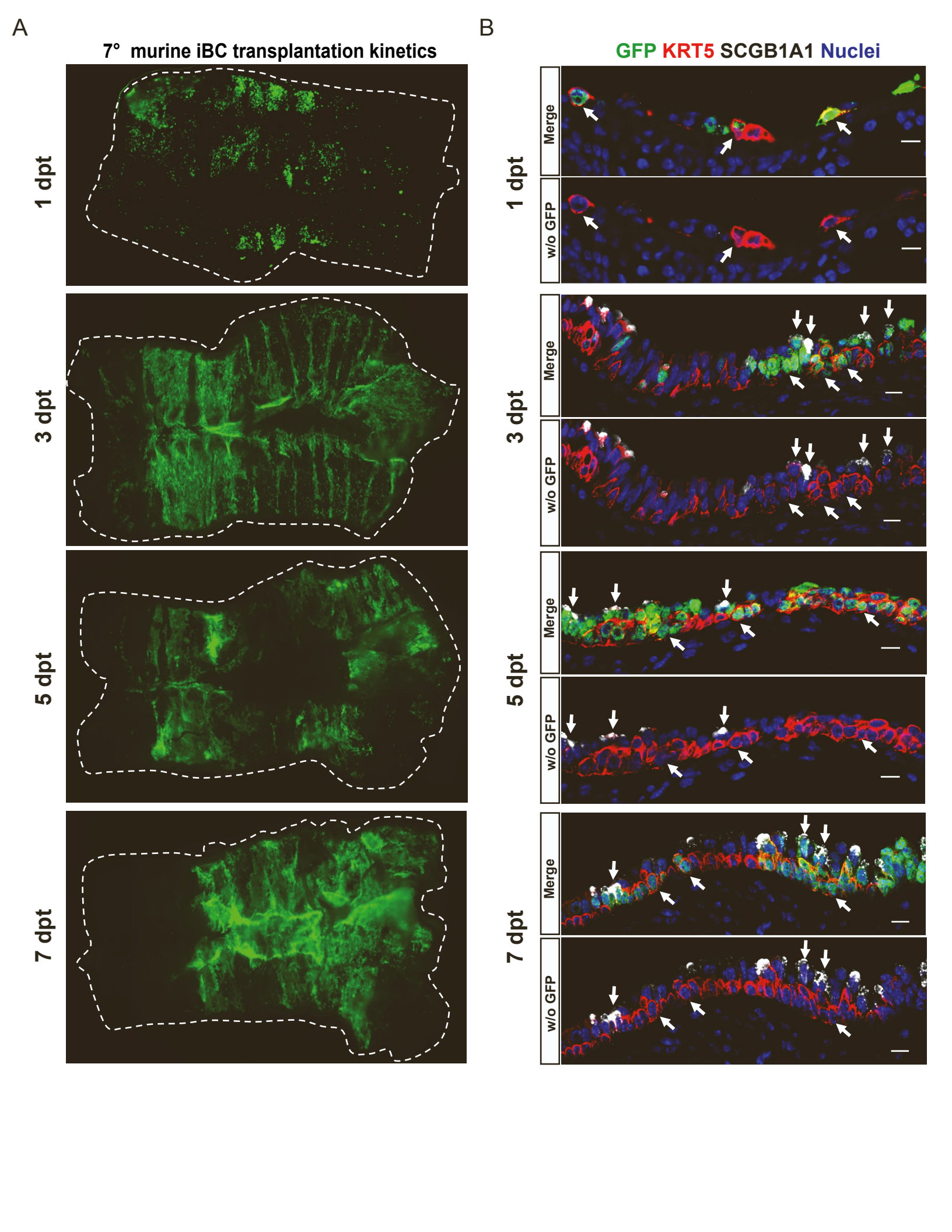
(D) Gene set enrichment analysis of top differentially expressed gene (1406 total) in the Ccl9^{high} cluster. NES = normalized enrichment score.

(E) Heatmap of Top 20 differentially expressed genes (DEG) between each of the indicated iBC-derived lineages and their endogenous counterparts.

(F) Violin plots comparing club cell proximal-distal patterning markers[45] between endogenous and donor-derived secretory cells. Statistical analysis done with Wilcoxon signed-rank test, with adjusted p values shown only for significantly different expression levels.

(G) Immunofluorescence confocal microscopy for GFP and SCGB1A1 or SCGB3A2 in iBC recipient tracheas. Nuclei are counterstained with Hoechst33342. Green arrows indicate GFP+ cells co-expressing SCGB1A1 or SCGB3A2. Blue arrows indicate GFP- (endogenous) cells expressing SCGB1A1 or SCGB3A2. Scale bar, 10 μm.

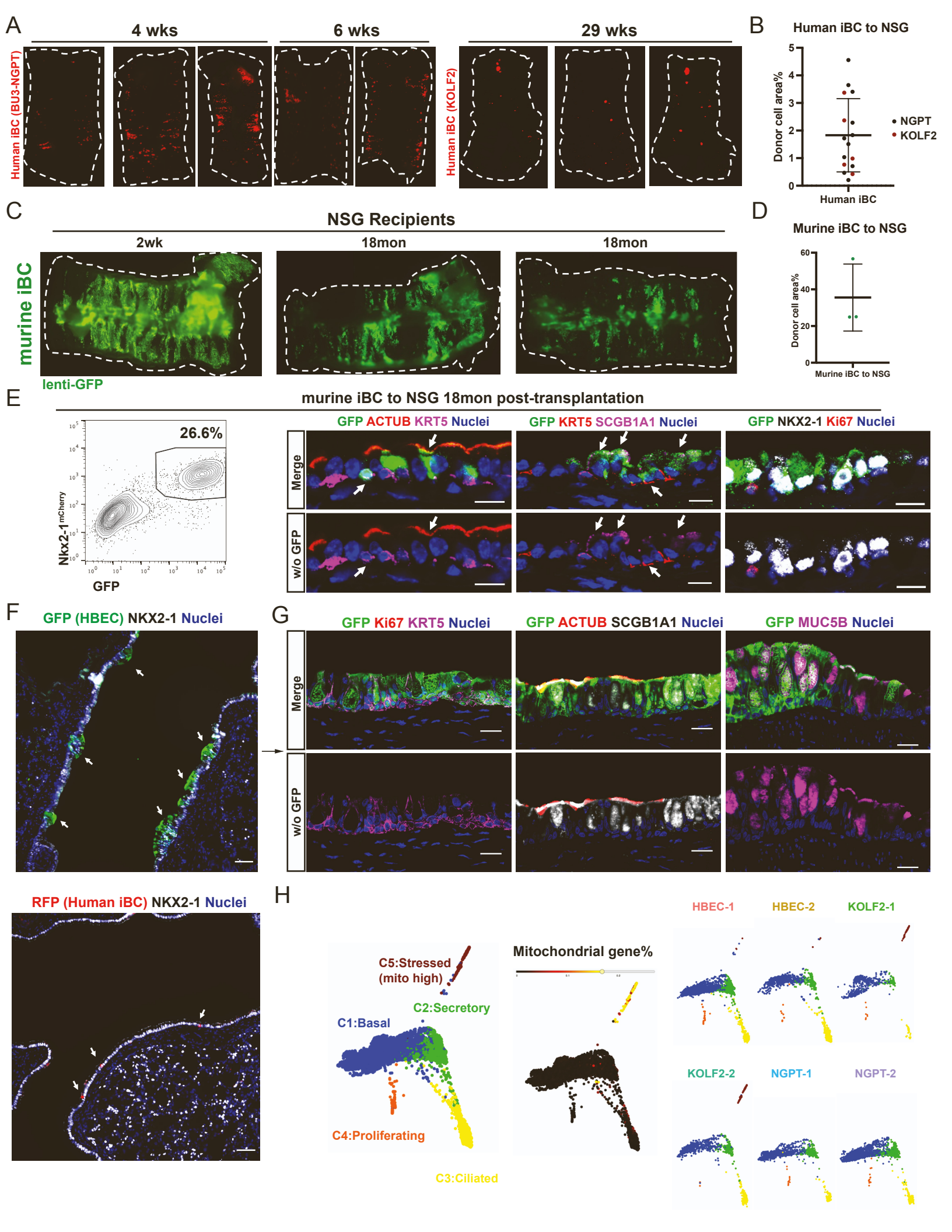
(H) Quantification of SCGB1A1+ or SCGB3A2+ cells among donor-derived (GFP+) or endogenous cells. Bars indicate mean +/- SD. Statistical analysis performed by unpaired, two-tailed Student's t-test (n = 3 animals, 1165 donor-derived cells, 934 endogenous cells).


A
7° murine iBC transplantation kinetics
1 dpt
3 dpt
5 dpt
7 dpt
B
GFP KRT5 SCGB1A1 Nuclei
Merge
w/o GFP

Supplementary Figure 6: Dynamic changes post-murine iBC serial transplantation into 7th generation (7°) recipients, Related to Main Figure 5

(A) Whole mount fluorescence of iBC recipients 1, 3, 5 or 7days post-transplantation。

(B) Immunofluorescence confocal microscopy for GFP, SCGB1A1, and KRT5 in 7° iBC recipient tracheas at 1, 3, 5, or 7 days post-transplantation. Nuclei are counterstained with Hoechst33342. White arrows indicate GFP+ cells co-expressing canonical airway epithelial markers. Lower panels have the GFP channel removed. Scale bars, 10 μm.


A
4 wks
6 wks
29 wks
Human iBC (BU3-NGPT)
Human iBC (KOLF2)
B
Human iBC to NSG
Donor cell area%
NGPT
KOLF2
Human iBC
C
NSG Recipients
2wk
18mon
18mon
murine iBC
lenti-GFP
D
Murine iBC to NSG
Donor cell area%
Murine iBC to NSG
E
murine iBC to NSG 18mon post-transplantation
26.6%
Nkx2-1mCherry
GFP
GFP ACTUB KRT5 Nuclei
GFP KRT5 SCGB1A1 Nuclei
GFP NKX2-1 Ki67 Nuclei
Merge
w/o GFP
F
GFP (HBEC) NKX2-1 Nuclei
RFP (Human iBC) NKX2-1 Nuclei
G
GFP Ki67 KRT5 Nuclei
GFP ACTUB SCGB1A1 Nuclei
GFP MUC5B Nuclei
Merge
w/o GFP
H
C5:Stressed (mito high)
C2:Secretory
C1:Basal
C4:Proliferating
C3:Ciliated
Mitochondrial gene%
HBEC-1
HBEC-2
KOLF2-1
KOLF2-2
NGPT-1
NGPT-2

Supplementary Figure 7: Characterization of human or mouse cells post-transplantation into injured NSG recipients, Related to Main Figure 7

(A) Whole mount fluorescence of iBC (BU3-NGPT and KOLF2) recipient tracheas (BU3 NGPT n =11; KOLF2 n = 5) at various harvest timepoints (4, 6 , or 29 weeks).

(B) Quantification of human iBC (BU3-NGPT indicated by black dots and KOLF2 indicated by red dots) transplantation efficiency, shown as RFP+% area over total recipient trachea area. Bars indicate mean +/- SD.

(C) Whole mount fluorescence of NSG recipient tracheas that received murine iBC transplantation at various harvest timepoints (2 weeks or 18 months).

(D) Quantification of murine iBC into NSG recipient transplantation efficiency, shown as GFP+% area over total recipient trachea area. Bars indicate mean +/- SD.

(E) FACS and Immunofluorescence confocal microscopy for GFP, ACTUB, SCGB1A1, Ki67, and KRT5 in NSG recipient that received murine iBC 18 months post-transplantation. Nuclei are counterstained with Hoechst33342. White arrows indicate GFP+ cells co-expressing canonical airway epithelial markers. Lower panels have GFP channel removed. Scale bar, 10 μm.

(F) Immunofluorescence confocal microscopy for RFP (iBC) or GFP (HBEC) and NKX2-1 in the intrapulmonary airway of NSG recipients. White arrows indicate RFP+ or GFP+ donor-derived cells. Scale bar, 50 μm.

(G) Immunofluorescence confocal microscopy for GFP, KI67, KRT5, SCGB1A1, ACTUB, and MUC5B in the intrapulmonary airway of NSG recipient. Nuclei are counterstained with Hoechst33342. Lower panels have GFP channel removed. Scale bar, 10 μm.

(H) SPRING visualization of scRNA-Seq of human cell transplantation recipients, colored by Louvain clustering (resolution = 0.1) or sample of origin as in Figure 7. Mitochondrial gene percentage of human cells profiled by scRNA-seq six weeks after transplantation into mouse recipient tracheas is also shown.