Cell Reports, Volume *39*

Supplemental information

Progenitor potential of lung epithelial organoid cells in a transplantation model

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Figure S1

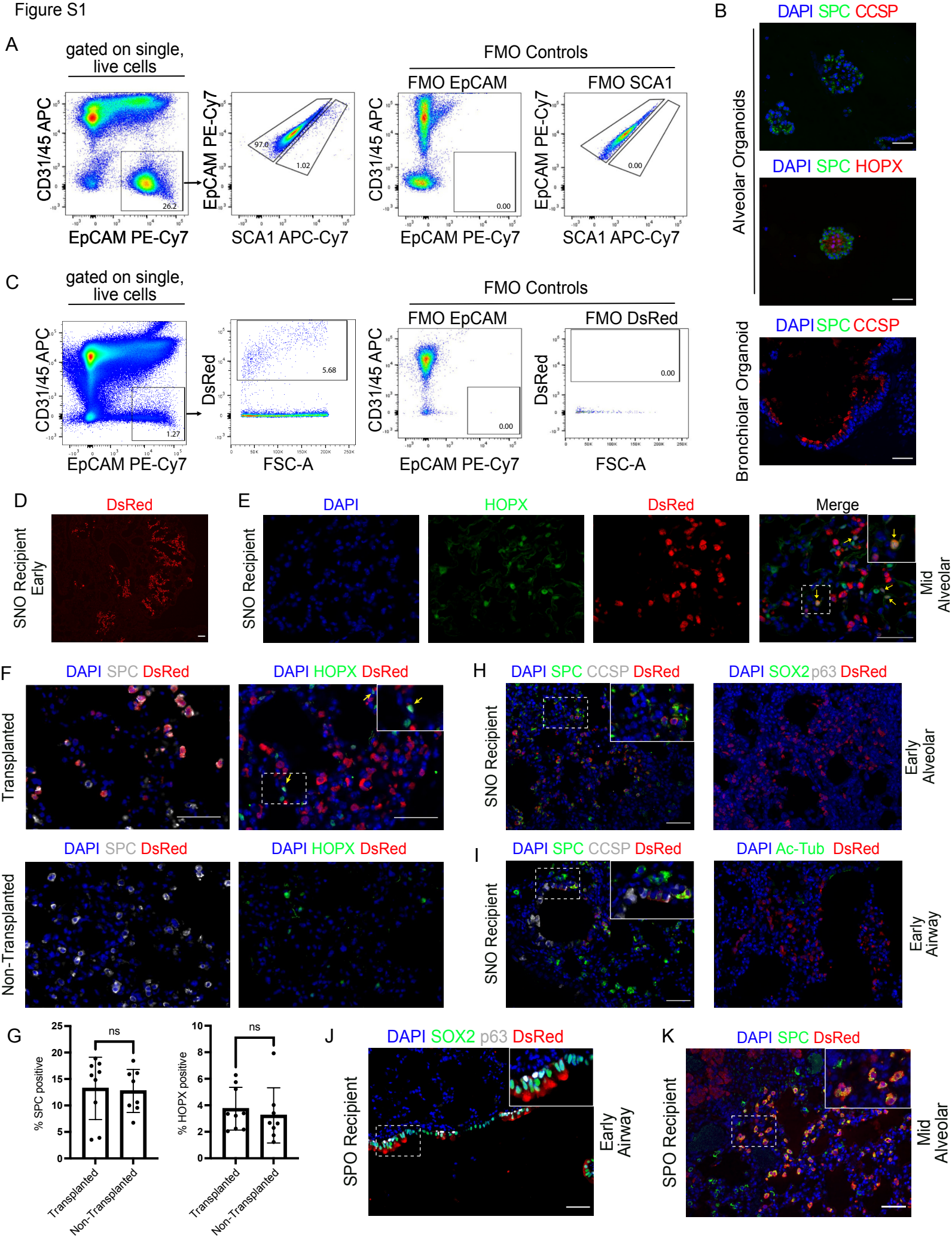
A
gated on single, live cells
FMO Controls
FMO EpCAM
FMO SCA1
CD31/45 APC
EpCAM PE-Cy7
SCA1 APC-Cy7
26.2
97.0
1.02
0.00
B
DAPI SPC CCSP
DAPI SPC HOPX
Alveolar Organoids
Bronchiolar Organoid
C
FMO DsRed
DsRed
FSC-A
1.27
5.68
D
DsRed
SNO Recipient Early
E
DAPI
HOPX
Merge
Mid Alveolar
F
DAPI SPC DsRed
DAPI HOPX DsRed
Transplanted
Non-Transplanted
H
DAPI SPC CCSP DsRed
DAPI SOX2 p63 DsRed
SNO Recipient
Early Alveolar
I
DAPI Ac-Tub DsRed
Early Airway
G
% SPC positive
% HOPX positive
ns
Transplanted
Non-Transplanted
J
DAPI SOX2 p63 DsRed
SPO Recipient
Early Airway
K
DAPI SPC DsRed
Mid Alveolar

Figure S1. Lung organoid cells are retained in distinct lung compartments after transplantation, Related to Figure 1.

(A) Representative FACS plot showing cell sorting strategy to isolate progenitor cells for organoid 3D co-culture. FMO = fluorescence minus one.

(B) Representative picture of IF staining on organoids. Top and bottom: Blue, DAPI; Green, SPC; Red, CCSP. Middle: Blue, DAPI; Green, SPC; Red, HOPX. Scale bar = 50 μm.

(C) Representative flow cytometry plot showing gating strategy to quantify donor cell retention in transplanted lungs. FMO = fluorescence minus one.

(D) Representative picture of IF staining on mouse lung that was transplanted with SNO cells and harvested at early time point after transplantation. Red, DsRed. Scale bar = 100 μm.

(E) Representative picture of IF staining on mouse lung that was transplanted with SNO cells and harvested at a middle (mid) time point after transplantation. Arrowheads point to cells that dually express HOPX and DsRed. Blue, DAPI; Green, HOPX; Red, DsRed. Scale bar = 50 μm.

(F) Representative pictures of IF staining on engrafted and non-engrafted areas of mouse lung that was transplanted with SNO cells. Images on left: Blue, DAPI; White, SPC; Red, DsRed. Images on right: Blue, DAPI; Green, HOPX; Red, DsRed. Scale bar = 50 μm.

(G) Quantification of SPC and HOPX positive cells normalized to total cells (DAPI positive). Quantification was performed on 3 fields of engrafted or non-engrafted regions of SNO recipient mouse lungs. n=9. P-value was determined by student's T-test. ns = $p>0.05$,

(H) (I) Representative picture of IF staining on mouse lung that was transplanted with SNO cells and harvested at an early time point after transplantation. Images show cells retained in the **(H)** distal alveolar region or **(I)** airway region. **(H** and **I)** Left: Blue, DAPI; Green, SPC; Grey, CCSP; Red, DsRed; **(H)** Right: Blue, DAPI; Green, SOX2; Grey, p63; Red, DsRed; **(I)** Right: Blue, DAPI; Green, Ac-Tub; Red, DsRed. Scale bar = 50 μm.

(J) Representative picture of IF staining on mouse lungs that were transplanted with SPO cells and analyzed at an early time point after transplantation. Blue, DAPI; Green, SOX2; Grey, p63; Red, DsRed. Scale bar = 50 μm.

(K) Representative picture of IF staining on mouse lungs that were transplanted with SPO cells and analyzed at middle time point after transplantation. Blue, DAPI; Green, SPC; Red, DsRed. Scale bar = 50 μm.

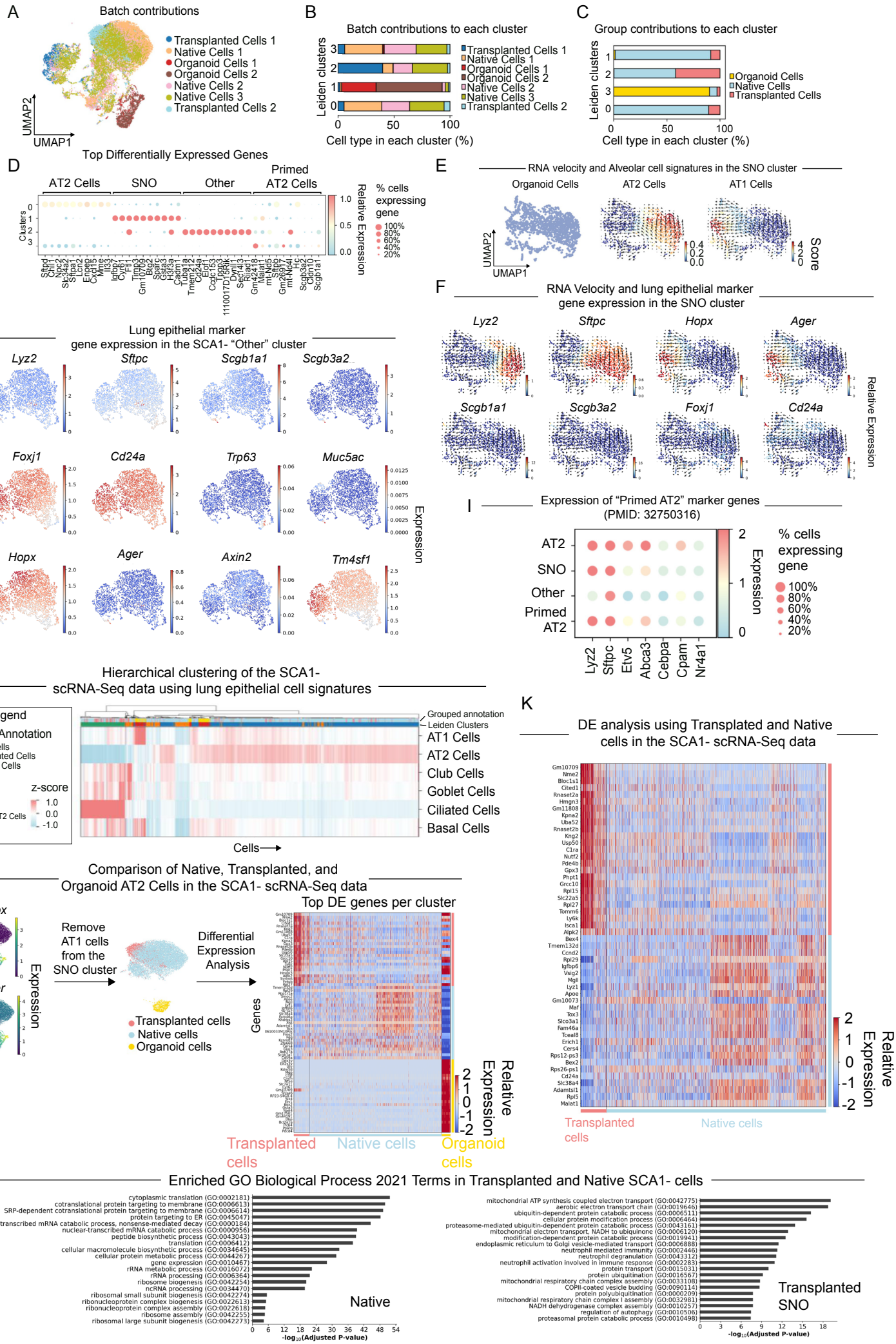
A
Batch contributions
Transplanted Cells 1
Native Cells 1
Organoid Cells 1
Organoid Cells 2
Native Cells 2
Native Cells 3
Transplanted Cells 2
UMAP2
UMAP1
B
Batch contributions to each cluster
Leiden clusters
Cell type in each cluster (%)
C
Group contributions to each cluster
Organoid Cells
Native Cells
Transplanted Cells
Cell type in each cluster (%)
D
Top Differentially Expressed Genes
AT2 Cells
SNO
Other
Primed AT2 Cells
Clusters
Relative Expression
% cells expressing gene
E
RNA velocity and Alveolar cell signatures in the SNO cluster
Organoid Cells
AT2 Cells
AT1 Cells
Score
F
RNA Velocity and lung epithelial marker gene expression in the SNO cluster
Lyz2
Sftpc
Hopx
Ager
Scgb1a1
Scgb3a2
Foxj1
Cd24a
Relative Expression
G
Lung epithelial marker gene expression in the SCA1- "Other" cluster
Lyz2
Sftpc
Scgb1a1
Scgb3a2
Foxj1
Cd24a
Trp63
Muc5ac
Hopx
Ager
Axin2
Tm4sf1
"Other" cluster
Expression
UMAP2
UMAP1
H
Hierarchical clustering of the SCA1- scRNA-Seq data using lung epithelial cell signatures
Legend
Grouped Annotation
Native Cells
Transplanted Cells
Organoid Cells
Leiden clusters
0 AT2 Cells
1 Primed AT2 Cells
2 Other
3 SNO
z-score
Grouped annotation
Leiden Clusters
AT1 Cells
AT2 Cells
Club Cells
Goblet Cells
Ciliated Cells
Basal Cells
Cells
I
Expression of "Primed AT2" marker genes (PMID: 32750316)
AT2
SNO
Other
Primed AT2
Lyz2
Sftpc
Etv5
Abca3
Cebpa
Cpam
Nr4a1
Expression
% cells expressing gene
J
Comparison of Native, Transplanted, and Organoid AT2 Cells in the SCA1- scRNA-Seq data
Hopx
Ager
Expression
Remove AT1 cells from the SNO cluster
Differential Expression Analysis
Transplanted cells
Native cells
Organoid cells
Top DE genes per cluster
Genes
Relative Expression
Transplanted cells
Native cells
Organoid cells
K
DE analysis using Transplated and Native cells in the SCA1- scRNA-Seq data
Relative Expression
Transplanted cells
Native cells
L
Enriched GO Biological Process 2021 Terms in Transplanted and Native SCA1- cells
cytoplasmic translation (GO:0002181)
cotranslational protein targeting to membrane (GO:0006613)
SRP-dependent cotranslational protein targeting to membrane (GO:0006614)
protein targeting to ER (GO:0045047)
nuclear-transcribed mRNA catabolic process, nonsense-mediated decay (GO:0000184)
nuclear-transcribed mRNA catabolic process (GO:0000956)
peptide biosynthetic process (GO:0043043)
translation (GO:0006412)
cellular macromolecule biosynthetic process (GO:0034645)
cellular protein metabolic process (GO:0044267)
gene expression (GO:0010467)
rRNA metabolic process (GO:0016072)
rRNA processing (GO:0006364)
ribosome biogenesis (GO:0042254)
ncRNA processing (GO:0034470)
ribosomal small subunit biogenesis (GO:0042274)
ribonucleoprotein complex biogenesis (GO:0022613)
ribonucleoprotein complex assembly (GO:0022618)
ribosome assembly (GO:0042255)
ribosomal large subunit biogenesis (GO:0042273)
Native
-log10(Adjusted P-value)
mitochondrial ATP synthesis coupled electron transport (GO:0042775)
aerobic electron transport chain (GO:0019646)
ubiquitin-dependent protein catabolic process (GO:0006511)
cellular protein modification process (GO:0006464)
proteasome-mediated ubiquitin-dependent protein catabolic process (GO:0043161)
mitochondrial electron transport, NADH to ubiquinone (GO:0006120)
modification-dependent protein catabolic process (GO:0019941)
endoplasmic reticulum to Golgi vesicle-mediated transport (GO:0006888)
neutrophil mediated immunity (GO:0002446)
neutrophil degranulation (GO:0043312)
neutrophil activation involved in immune response (GO:0002283)
protein transport (GO:0015031)
protein ubiquitination (GO:0016567)
mitochondrial respiratory chain complex assembly (GO:0033108)
COPII-coated vesicle budding (GO:0090114)
protein polyubiquitination (GO:0000209)
mitochondrial respiratory chain complex I assembly (GO:0032981)
NADH dehydrogenase complex assembly (GO:0010257)
regulation of autophagy (GO:0010506)
proteasomal protein catabolic process (GO:0010498)
Transplanted SNO
-log10(Adjusted P-value)

Figure S2. scRNA-Seq reveals that SNO transplanted cells are transcriptionally similar to native alveolar epithelial cells, Related to Figure 2.
(A) Clustering of transcriptomes using UMAP. Cells are colored based on batch ID.
(B) Histogram showing batch distribution in each Leiden cluster.
(C) Histogram showing group distribution in each Leiden cluster.
(D) Top differentially expressed genes in each Leiden cluster, visualized with a dot plot.
(E) RNA velocity analysis of SNO cells from the SCA1- scRNA-Seq dataset. Organoid cells were subset and visualized by UMAP on the left. Z-scores for AT2 and AT1 cell signatures are visualized on the right.
(F) RNA velocity plus expression of AT1 (*Hopx, Ager*), AT2 (*Sftpc, Lyz2*), Club (*Scgb1a1, Scgb3a2*), and Ciliated (*Foxj1, Cd24a*) in the scRNA-Seq SNO cluster only.
(G) Expression of AT1 (*Hopx, Ager*), AT2 (*Sftpc, Lyz2*), Club (*Scgb1a1, Scgb3a2*), and Ciliated (*Foxj1, Cd24a*), Basal (*Trp63*), Goblet (*Muc5ac*), and AEP (*Axin2, Tm4sf1*) marker genes in the SCA1- "Other" cluster.
(H) Hierarchically clustered heatmap of individual cells in the SCA1- scRNA-Seq dataset (x-axis) using z-scores of indicated cell signatures (y-axis). Grouped annotations and Leiden clusters are indicated.
(I) Expression of "Primed AT2" marker genes *Lyz2*, *Sftpc*, *Etv5*, *Abca3*, *Cebpa*, *Cpam*, and *Nr4a1* in each Leiden cluster, visualized with a dot plot.
(J) Differential expression analysis comparing AT2 populations in the SCA1- scRNA-Seq dataset. The AT2 and SNO clusters were subset and organoid cells with high AT1 marker expression (*Hopx, Ager*) were removed to allow direct comparison of AT2 cells. Expression of the top differentially expressed gene in each cluster were visualized as a heatmap.
(K) Differential expression analysis comparing native AT2 and transplanted SNO cells in the SCA1- scRNA-Seq dataset. Expression of the top differentially expressed gene in each cluster were visualized as a heatmap.
(L) Gene Ontology Biological Process 2021 analysis using differentially expressed genes in native AT2 cells and transplanted SNO cells.

Figure S3


A
Batch contributions
Transplanted Mouse – Transplanted Cells 1
Transplanted Mouse – Transplanted Cells 2
Organoid Cells 1
Organoid Cells 2
Organoid Cells 3
Wildtype Mouse – Native Cells 1
Wildtype Mouse – Native Cells 2
Wildtype Mouse – Native Cells 3
UMAP2
UMAP1
B
Batch contribution to cluster
Leiden clusters
Cell type in each cluster (%)
C
Group contribution to cluster
Organoid Cells
Native Cells
Transplanted Cells
D
Top Differentially Expressed Genes
Clusters
Relative Expression
% cells expressing gene
E
Hierarchical clustering of the SCA1-scRNA-Seq data using lung epithelial cell signatures
Legend
z-score
Grouped Annotation
Leiden Clusters
AT1 Cells
AT2 Cells
Club Cells
Goblet Cells
Ciliated Cells
Basal Cells
F
Hierarchical clustering of the transplanted SPO cells lung epithelial cell signatures and program scores
Grouped Annotation
Transplanted Cells
Leiden Clusters
Ciliated Cells
Transitional Cells 1
Transitional Cells 2
AT2 Cells
Ciliated Cells
G
Hallmark Wnt
Hallmark Notch
Hallmark TGF-b
GO: Lung Epithelial Dev
H
Lung epithelial marker gene expression in the subset SPO cluster
Transplanted Cells
Native Cells
Organoid Cells
Subset organoid cells
Organoid Cells 1
Organoid Cells 2
Organoid Cells 3
AT2 genes
Sftpc
Sftpb
Lyz2
AT1 genes
Hopx
Pdpn
Ager
Ciliated genes
Foxj1
Cd24a
Tubb4a
Basal genes
Trp63
Krt5
Krt14
Ngfr
NE genes
Syp
Uchl1
Chga
Club genes
Scgb1a1
Cyp2f2
BASC genes
Ly6a
Axin2+ and AEP genes
Axin2
Tm4sf1
α6β4+ SPC-Alveolar Progenitor genes
Itga6
Itgb4
Goblet genes
Muc5b
Muc5ac
Spdef
Expression

Figure S3. scRNA-Seq captures transitional progenitor cell states following transplantation of SPO cells, Related to Figure 3.

(A) Clustering of transcriptomes using UMAP. Cells are colored based on batch ID.

(B) Histogram showing batch distribution in each Leiden cluster.

(C) Histogram showing group distribution in each Leiden cluster.

(D) Top differentially expressed genes in each Leiden cluster, visualized with a dot plot.

(E) Hierarchically clustered heatmap of individual cells in the SCA1+ scRNA-Seq dataset (x-axis) using z-scores of indicated cell signatures (y-axis). Grouped annotations and Leiden clusters are indicated.

(F) Hierarchically clustered heatmap of individual cells from the transplanted SPO cell group (x-axis) using z-scores of indicated cell signatures (y-axis). Grouped annotations and Leiden clusters are indicated.

(G) Hierarchically clustered heatmap of individual cells from the transplanted SPO cell group (x-axis) using z-scores of Hallmark signatures and Gene Ontology signatures scores (y-axis). Grouped annotations and Leiden clusters are indicated.

(H) Expression of epithelial marker genes in the SPO cluster. SPO cells were subset from the SCA1+ scRNA-Seq data then re-analyzed. Batch distribution and the expression of marker genes for various lung epithelial cell types were visualized on the UMAP.

Figure S4

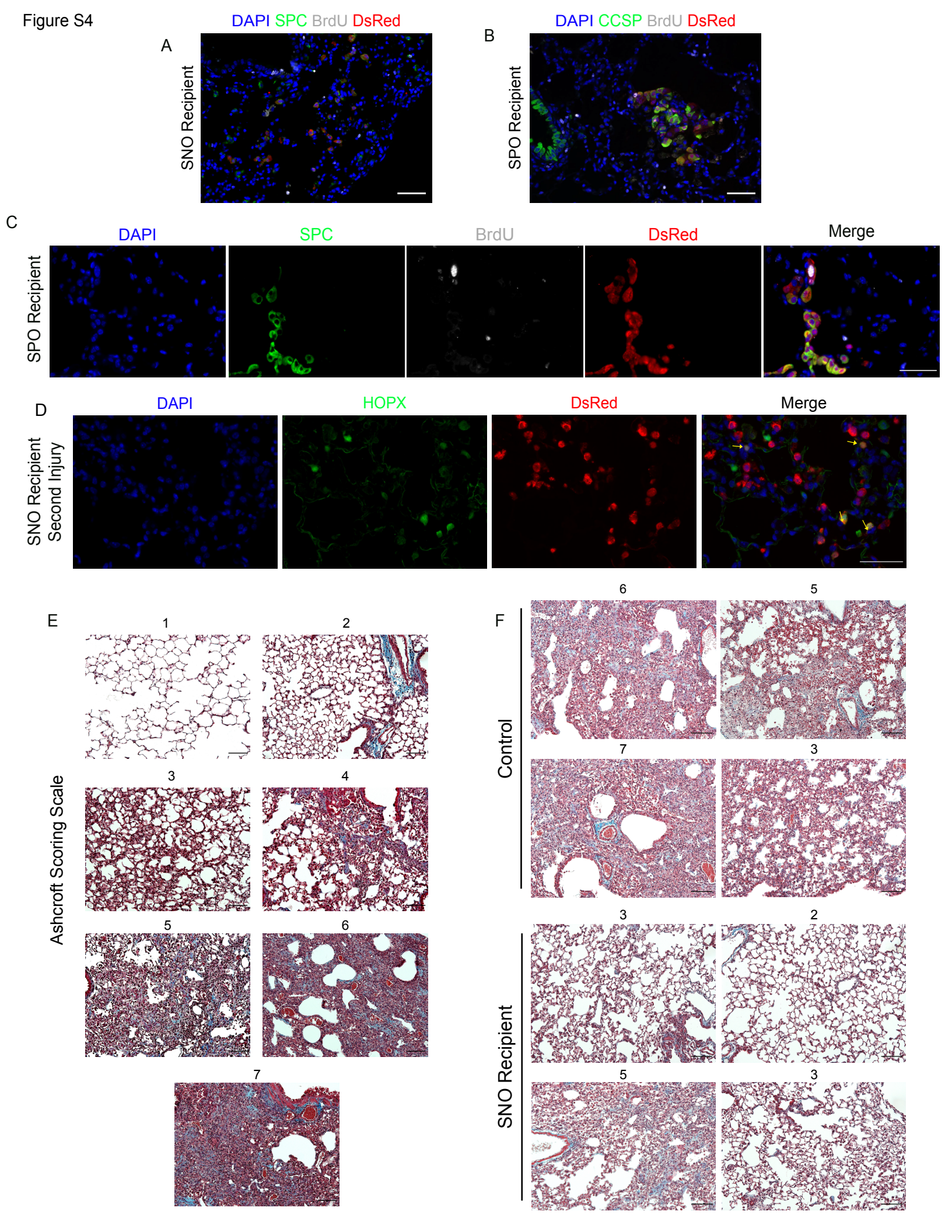
A
DAPI SPC BrdU DsRed
SNO Recipient
B
DAPI CCSP BrdU DsRed
SPO Recipient
C
DAPI
SPC
BrdU
DsRed
Merge
SPO Recipient
D
DAPI
HOPX
DsRed
Merge
SNO Recipient
Second Injury
E
Ashcroft Scoring Scale
1
2
3
4
5
6
7
F
Control
SNO Recipient
6
5
7
3
3
2
5
3

Figure S4. Transplanted cells retain progenitor cell potential *in vivo* and *in vitro*, Related to Figure 4.
(A) (B) Representative pictures of SNO and SPO transplanted mice that received a second bleomycin injury. Engrafted areas are shown. **(A)** Blue, DAPI; Green, SPC; White, BrdU; Red, DsRed. **(B)** Blue, DAPI; Green, CCSP; White, BrdU; Red, DsRed. Scale bar = 100 μm.
(C) Representative pictures of IF staining on mouse lungs that were transplanted with SPO cells that had received a subsequent injury and BrdU incorporation. An engrafted area is shown. Blue, DAPI; Green, SPC; Grey, BrdU; Red, DsRed. Scale bar = 50 μm.
(D) Representative pictures of IF staining on mouse lungs that were transplanted with SNO cells that had received a subsequent bleomycin injury and injected with BrdU. Arrowheads point to cells that dually express HOPX and DsRed. Blue, DAPI; Green, HOPX; Red, DsRed. Scale bar = 50 μm.
(E) Representative pictures of Masson's Trichrome staining on mouse lung following bleomycin injury used for Ashcroft scoring. Representative images show a scale of Ashcroft scores 1 to 7. Scale bar = 200 μm.
(F) Additional pictures of Masson's Trichrome staining of control or SNO recipient mouse lungs following bleomycin injury. Ashcroft scores for each image are indicated. Scale bar = 200 μm.