Supplemental Information

Supplemental Figures and Figure Legends

Figure S1. Related to Figure 1.

Figure S2. Related to Figure 2.

Figure S3. Related to Figures 3 and 4.

Figure S4. Related to Figure 4.

Figure S5. Related to Figures 4 and 5.

Figure S6. Related to Figure 6.

Figure S7. Related to Figures 6 and 7.

Supplemental Tables

Table S1. Clonal Lineage-Tracing Data. Related to Figure 1.

Table S2. SCONE Performance Metrics. Related to Figures 2 and 7.

Table S3. Differential Gene Expression Between Transitional and Activated HBCs, WT regeneration clusters, TFs, HBC*1 DE. Related to Figures 3, 4, S4, S5, Table S5.

Table S4. Gene Set Enrichment Analysis Results. Related to Figure 3.

Table S5. Transcription Factor Binding Motif Analysis of the Promoters of Genes Enriched in HBC*1. Related to Figures 4, S4, S5.

Table S6. Differential Gene Expression Analysis of Wild Type vs. *Sox2* Conditional Knockout HBCs. Related to Figures 7 and S7.

Table S7. Oligos Used to Synthesize RNA In Situ Hybridization Probes and Cell Cycle Gene Set. Related to Figure S3 and 3.

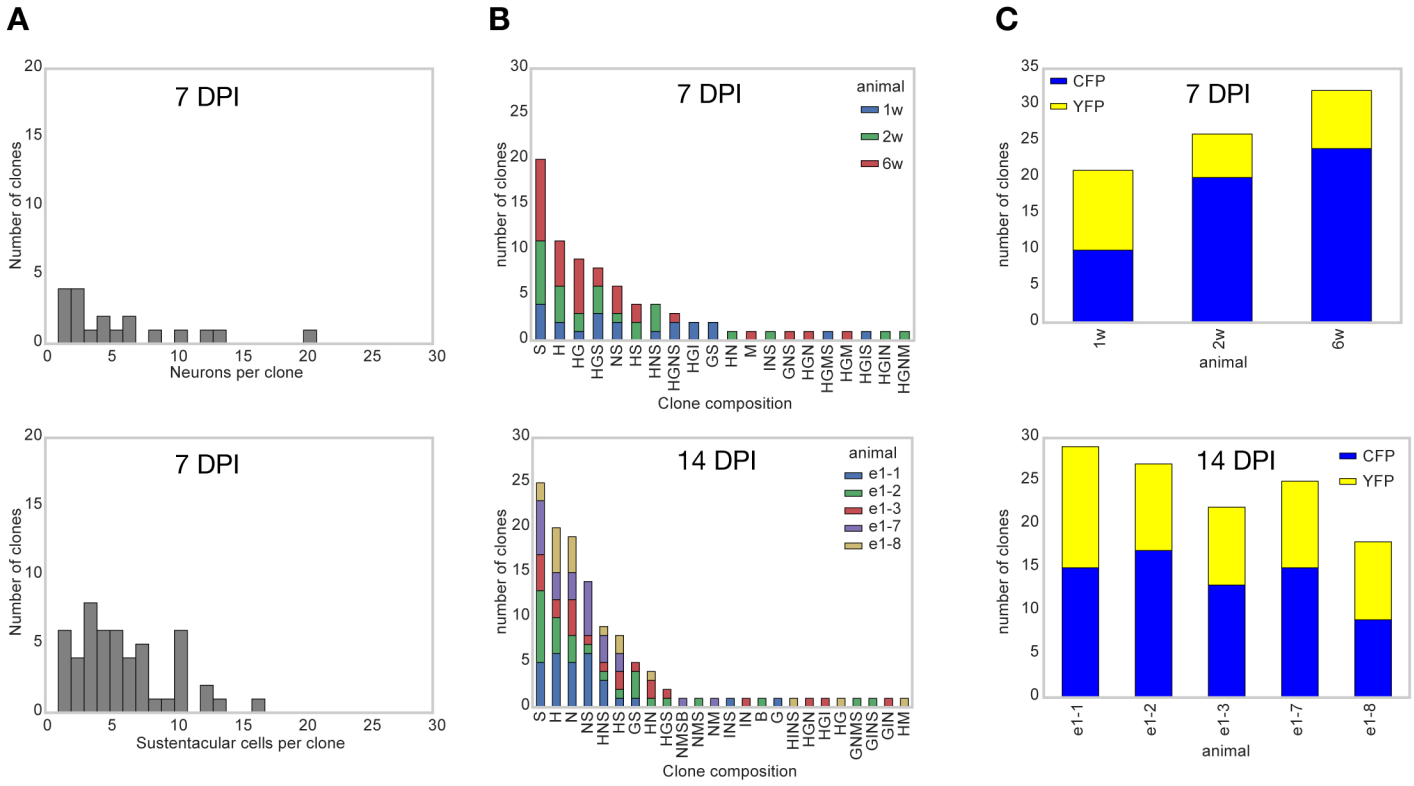

A
B
C
7 DPI
Number of clones
Neurons per clone
7 DPI
Number of clones
Sustentacular cells per clone
7 DPI
animal
1w
2w
6w
number of clones
Clone composition
S H HG HGS NS HS HNS HGNS HGI GS HN M INS GNS HGN HGMS HGM HGIS HGIN HGNM
14 DPI
animal
e1-1
e1-2
e1-3
e1-7
e1-8
number of clones
Clone composition
S H N NS HNS HS GS HN HGS NMSB NMS NM INS IN B G HINS HGN HGI HG GNMS GINS GIN HM
7 DPI
CFP
YFP
number of clones
1w
2w
6w
animal
14 DPI
CFP
YFP
number of clones
e1-1
e1-2
e1-3
e1-7
e1-8
animal

Figure S1

Figure S1. Related to Figure 1.
(A) Distributions of neurons per clone (top) and sustentacular cells per clone (bottom) in clones possessing at least one of each cell type, respectively, at 7 DPI. **(B)** Histogram of clone compositions at 7 DPI (top) and 14 DPI (bottom), according to the presence of at least one cell of a given cell type. Key for cell types: H, HBC; G, GBC; I, immediate neuronal precursor; N, neuron; M, microvillous cell; S, sustentacular cell; B, Bowman's gland. **(C)** Prevalence of clones lineage-traced by membrane CFP (blue) or cytoplasmic YFP (yellow), by animal, at 7 DPI (top) and 14 DPI (bottom).

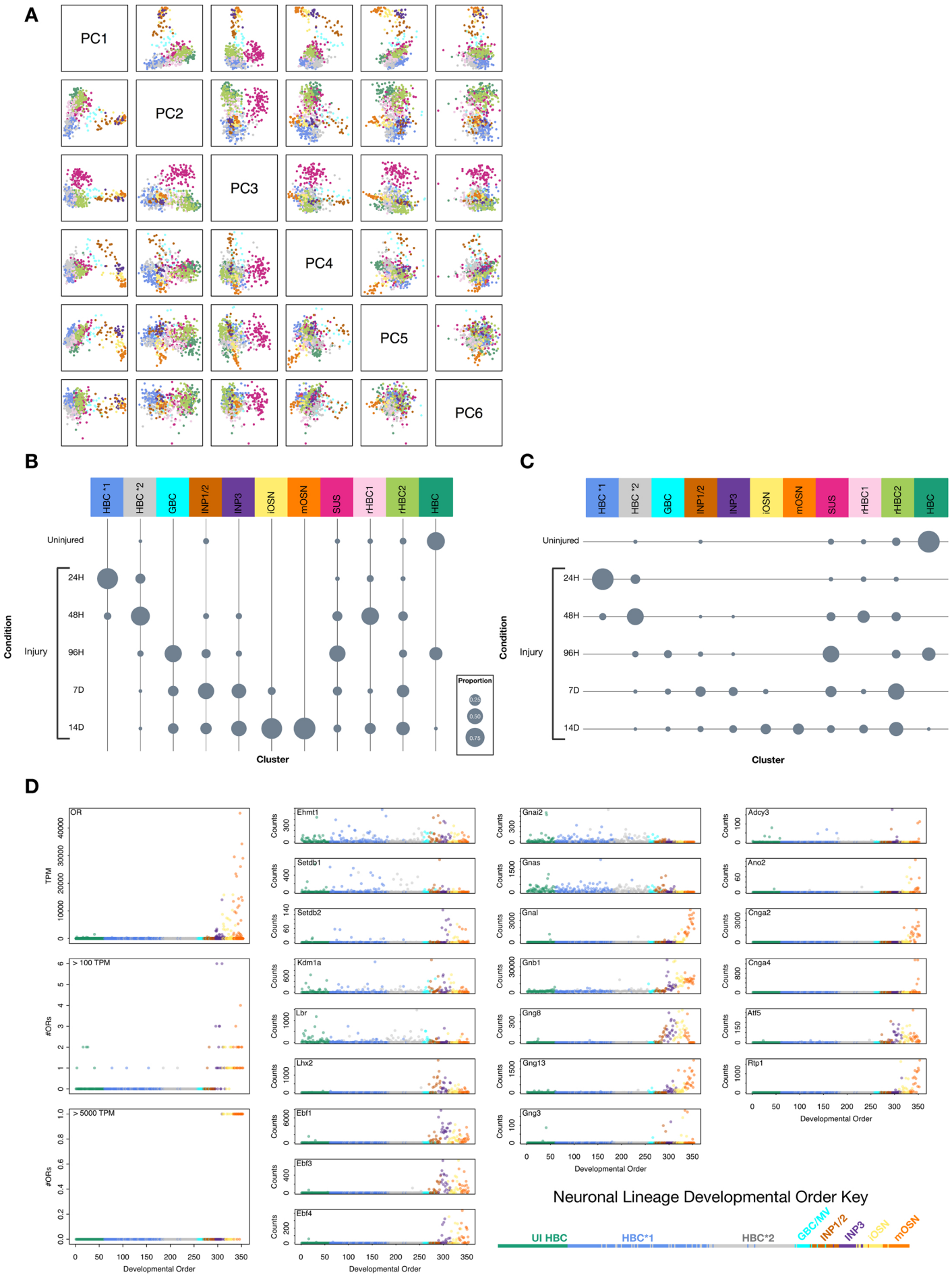
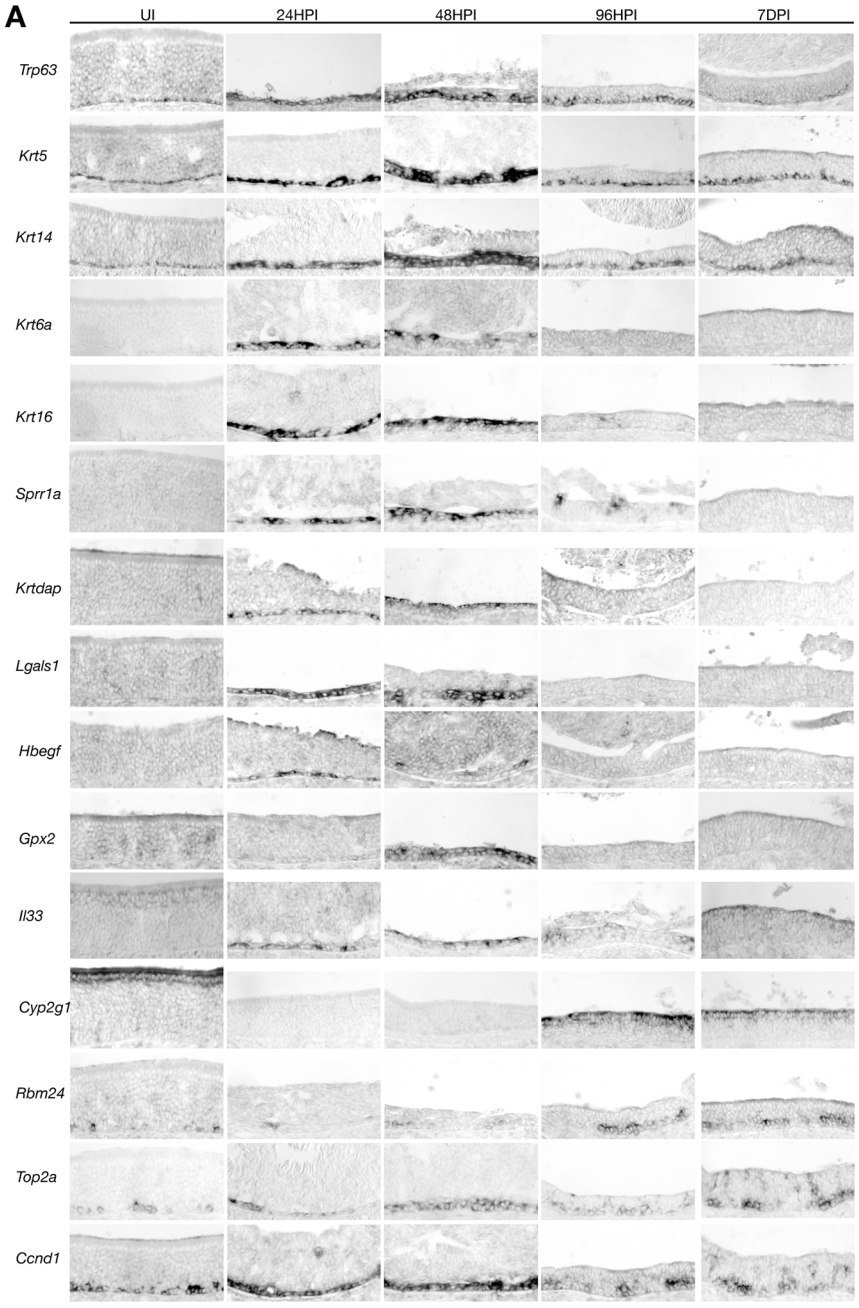
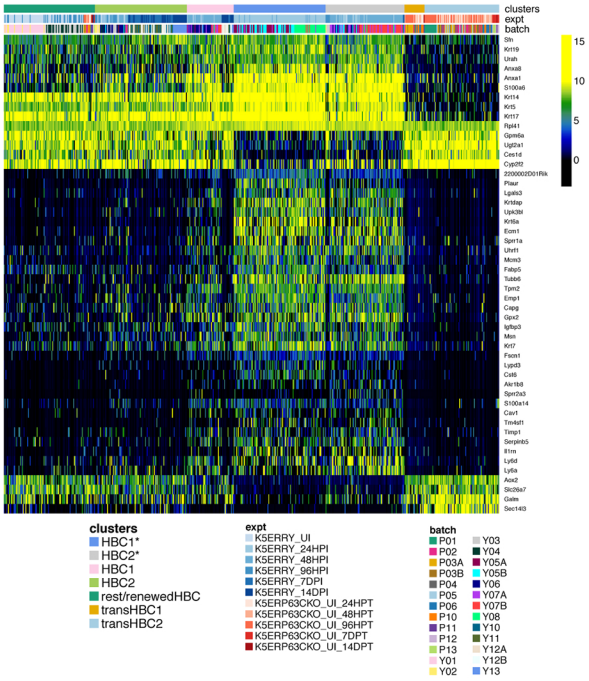

A
PC1
PC2
PC3
PC4
PC5
PC6
B
HBC *1
HBC *2
GBC
INP1/2
INP3
iOSN
mOSN
SUS
rHBC1
rHBC2
HBC
Uninjured
24H
48H
96H
7D
14D
Injury
Condition
Cluster
Proportion
0.25
0.50
0.75
C
D
OR
TPM
> 100 TPM
#ORs
> 5000 TPM
Developmental Order
Ehmt1
Setdb1
Setdb2
Kdm1a
Lbr
Lhx2
Ebf1
Ebf3
Ebf4
Gnai2
Gnas
Gnal
Gnb1
Gng8
Gng13
Gng3
Adcy3
Ano2
Cnga2
Cnga4
Atf5
Rtp1
Counts
Neuronal Lineage Developmental Order Key
UI HBC
HBC*1
HBC*2
GBC/MV
INP1/2
INP3
iOSN
mOSN

Figure S2

Figure S2. Related to Figure 2.
(A) The first five principal components of the expression matrix of all detected genes effectively separate the clusters and serve as input for Slingshot. **(B, C)** Like steady-state differentiation (Fletcher et al. 2017), regeneration is generally asynchronous, as demonstrated by the partial overlap of experimental condition and cell cluster identity, but cells from early time-points populate the activated HBC clusters. In (B), the size of the circles reflects the proportion of cells from each cluster derived from each experimental condition. In (C), the size of the circles reflects the proportion of cells from each experimental condition that contribute to each cluster. Most experimental conditions contribute to multiple clusters, while the uninjured HBCs predominantly populate the resting HBC cluster. **(D)** Expression of odorant receptors (ORs) in the neuronal lineage, with cells sorted by their developmental order. On the left, the top plot reveals significant upregulation of OR expression in mature olfactory sensory neurons (mOSN; orange); the second and third plots show the number of OR genes expressed per cell at different thresholds of expression. Multiple ORs are detected at the third immediate neuronal precursor stage (INP3; purple); as cells mature, the pattern shifts to high level expression of one OR per cell, starting in the immature olfactory sensory neuron (iOSN) stage (yellow). The remaining plots display selected transcription factors, chromatin modifiers and other signaling molecules relevant to OR gene expression.





C

Gene Set	P-value, enrichment in: resting HBC cluster	P-value, enrichment in: HBC*1 cluster	P-value, enrichment in HBC*2 cluster
HALLMARK_EPITH_MESENCH_TRANSITION	1.00E-07	0.0097035	0.0020842
HALLMARK_TNFA_SIGNALING_VIA_NFKB	1.00E-07	0.0001157	0.0287206
REACTOME_INTEGRIN_CELL_SURFACE_INTERACTIONS	1.00E-07	0.0047554	0.0183624
KEGG_ECM_RECEPTOR_INTERACTION	2.00E-07	0.1019771	0.0206633
PID_MYC_REPRESS_PATHWAY	2.00E-07	0.1384512	0.0186585
HALLMARK_MYC_TARGETS_V1	0.9849404	1.00E-07	1.00E-07
HALLMARK_MYC_TARGETS_V2	0.9539475	1.00E-07	2.00E-07
KEGG_RIBOSOME	0.0071245	1.00E-07	6.60E-06
REACTOME_TRANSLATION	0.0456086	1.00E-07	1.00E-07
HALLMARK_MTORC1 SIGNALING	0.0692145	1.00E-07	1.00E-07
HALLMARK_E2F_TARGETS	0.9999113	0.0148733	1.00E-07
REACTOME_CELL_CYCLE	0.9999997	0.0212022	1.00E-07
REACTOME_MITOTIC_M_M_G1_PHASES	0.9999985	0.000796	1.00E-07
REACTOME_DNA_REPLICATION	0.9999979	0.0003819	1.00E-07
REACTOME_CELL_CYCLE_CHECKPOINTS	0.9999943	7.00E-07	1.00E-07

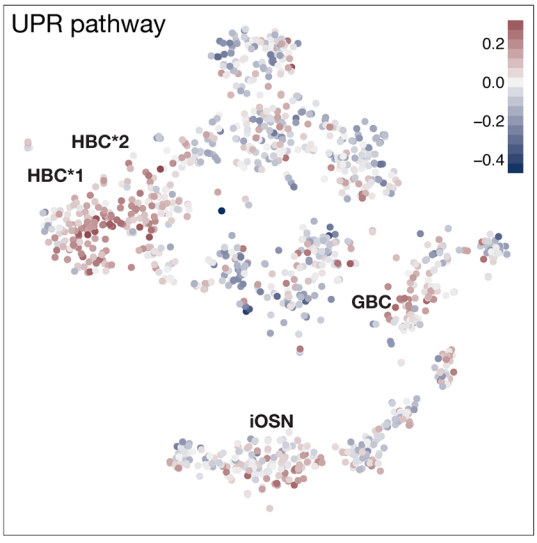


Figure S3

Figure S3. Related to Figures 3 and 4.
(A) RNA in situ hybridizations for selected marker genes in uninjured olfactory epithelium (UI) and at 24 hours, 48 hours, 96 hours and 7 days following injury with methimazole. Beginning at 24 HPI, activated HBCs express markers associated with wound response and proliferation, which become more apically restricted after 48 HPI. **(B)** Heatmap of the top 50 differentially expressed genes between the activated HBCs from regeneration and the transitional HBCs from uninjured differentiation. The top color bar indicates the type of HBC cluster/celltype. The experimental condition/timepoint for each cell is indicated in by the color bar labeled “expt”. Batch is discussed in the STAR Methods and refers to the biological replicate FACS run for each experiment. **(C)** Examples of enriched gene sets for the resting HBCs, HBC*1, and HBC*2, identified by gene set enrichment analysis (GSEA) of the wildtype regeneration data. **(D)** This t-SNE plot indicates the enrichment of the UPR gene set identified by GSEA in the cells of the wildtype regeneration experiment.

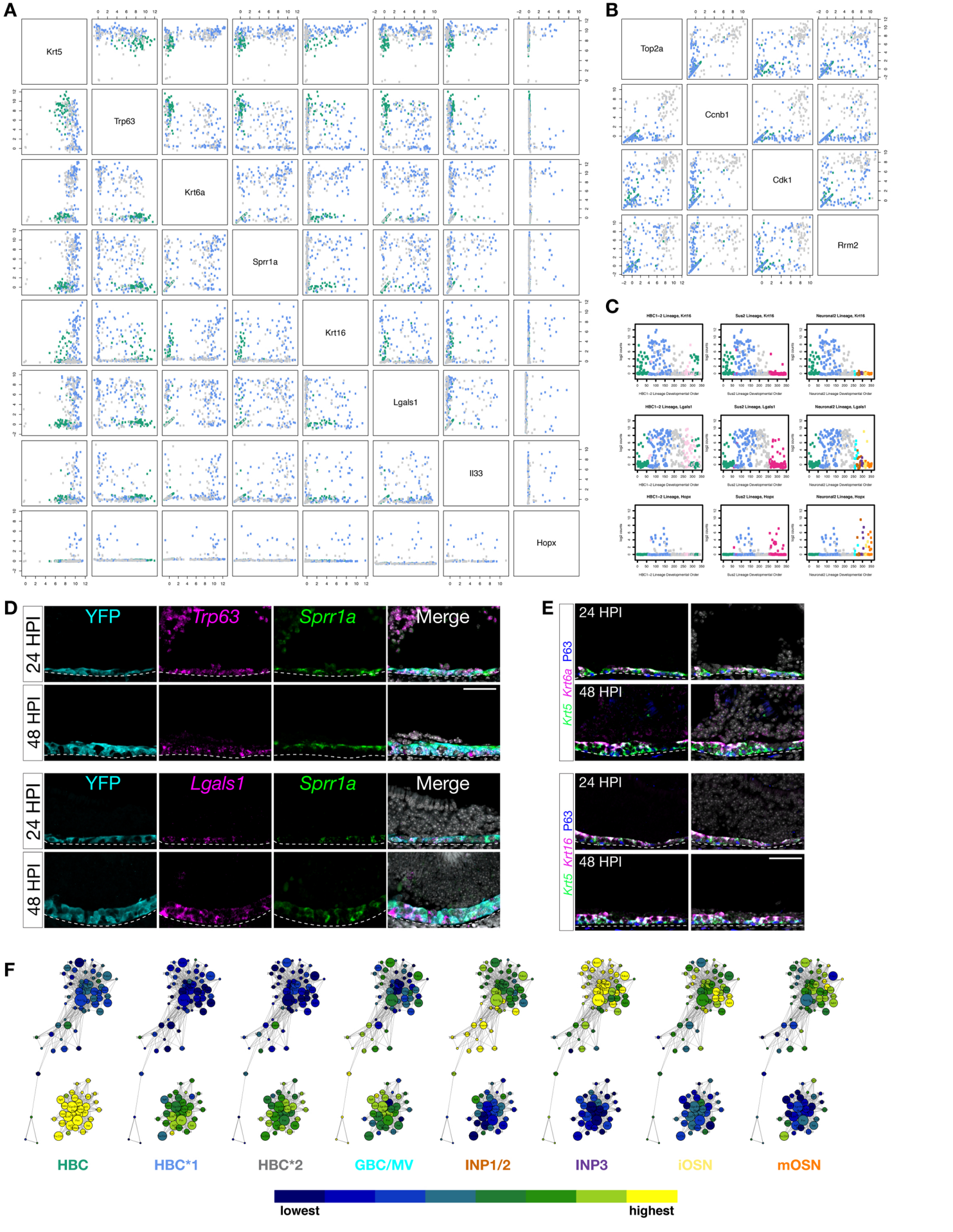

A
Krt5
Trp63
Krt6a
Sprr1a
Krt16
Lgals1
Il33
Hopx
B
Top2a
Ccnb1
Cdk1
Rrm2
C
HBC1-2 Lineage, Krt16
Sus2 Lineage, Krt16
Neuronal2 Lineage, Krt16
HBC1-2 Lineage, Lgals1
Sus2 Lineage, Lgals1
Neuronal2 Lineage, Lgals1
HBC1-2 Lineage, Hopx
Sus2 Lineage, Hopx
Neuronal2 Lineage, Hopx
D
YFP
Trp63
Sprr1a
Merge
24 HPI
48 HPI
YFP
Lgals1
Sprr1a
Merge
24 HPI
48 HPI
E
24 HPI
48 HPI
Krt5 Krt6a P63
24 HPI
48 HPI
Krt5 Krt16 P63
F
HBC
HBC*1
HBC*2
GBC/MV
INP1/2
INP3
iOSN
mOSN
lowest
highest

Figure S4

Figure S4. Related to Figure 4.
(A) Co-expression of selected marker genes in resting and activated HBCs. Although *Krt5* expression remains high through all HBC stages, there is heterogeneity in expression of other stem cell markers and wound response genes. **(B)** Cell cycle-associated genes show more coordinated expression in the HBC*2 cluster (grey), in comparison to other HBC clusters. **(C)** *Krt16*, *Lgals1*, and *Hopx* are enriched in a subset of activated HBCs. Color scheme of cluster assignments in panels A-C as in Figure 2. **(D)** Heterogeneity of activated HBCs demonstrated by partial overlap of markers at 24 and 48 HPI. YFP was detected by immunohistochemistry using an anti-GFP antibody and marker genes were detected by RNA in situ hybridization. **(E)** *Krt6a* and *Krt16* mark a subset of *Krt5*+ HBCs, which have variable levels of P63 expression. P63 was detected by immunohistochemistry using an anti-P63 antibody and other marker genes were detected by RNA in situ hybridization. **(F)** Connectivity graph for differentially expressed transcription factors in the neuronal lineage, colored by ranked average expression with respect to other clusters in the lineage. See also Table S5.

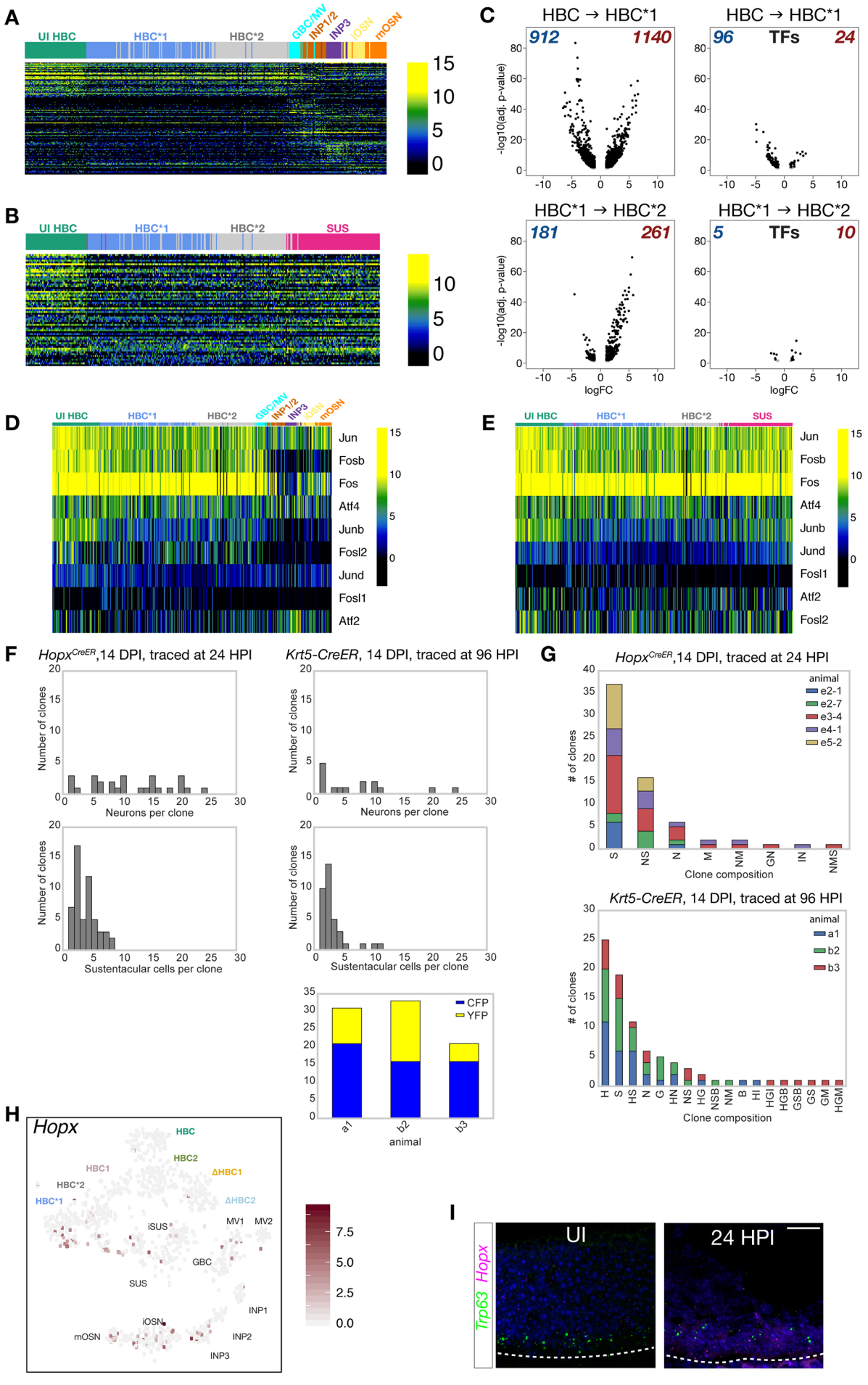

A
UI HBC
HBC*1
HBC*2
GBC/MV
INP1/2
INP3
iOSN
mOSN
15
10
5
0
B
UI HBC
HBC*1
HBC*2
SUS
10
5
0
C
HBC → HBC*1
912
1140
HBC → HBC*1
96
TFs
24
HBC*1 → HBC*2
181
261
HBC*1 → HBC*2
5
TFs
10
-log10(adj. p-value)
logFC
D
UI HBC
HBC*1
HBC*2
GBC/MV
INP1/2
INP3
iOSN
mOSN
Jun
Fosb
Fos
Atf4
Junb
Fosl2
Jund
Fosl1
Atf2
E
UI HBC
HBC*1
HBC*2
SUS
Jun
Fosb
Fos
Atf4
Junb
Jund
Fosl1
Atf2
Fosl2
F
HopxCreER,14 DPI, traced at 24 HPI
Krt5-CreER, 14 DPI, traced at 96 HPI
Number of clones
Neurons per clone
Sustentacular cells per clone
CFP
YFP
animal
a1
b2
b3
G
HopxCreER,14 DPI, traced at 24 HPI
animal
e2-1
e2-7
e3-4
e4-1
e5-2
# of clones
Clone composition
S
NS
N
M
NM
GN
IN
NMS
Krt5-CreER, 14 DPI, traced at 96 HPI
animal
a1
b2
b3
H
HS
G
HN
HG
NSB
B
HI
HGI
HGB
GSB
GS
GM
HGM
H
Hopx
HBC
HBC2
ΔHBC1
HBC1
HBC*2
HBC*1
ΔHBC2
MV1
MV2
iSUS
GBC
SUS
INP1
iOSN
mOSN
INP2
INP3
7.5
5.0
2.5
0.0
I
Trp63
Hopx
UI
24 HPI

Figure S5

Figure S5. Related to Figures 4 and 5.
(A, B) Differentially expressed transcription factors in the neuronal (A) and sustentacular cell (B) lineages. As in steady state differentiation, the neuronal lineage during regeneration exhibits step-like transitions in between cell types. **(C)** Volcano plots of –log10 adjusted p-value (-log10(adj. p-value)) versus log2 fold-change (logFC) between early HBC clusters. The plots display the number of differentially expressed genes between the clusters at each transition in the lineage (genes with adj. p-value < 0.01 and logFC > 1 are shown in black). Up-regulated genes are shown in red text and down-regulated in blue. *Limma* was used for differential expression, and the p-values were adjusted for multiple testing using the Benjamini-Hochberg procedure (see STAR Methods). The step from resting HBCs to activated HBCs is marked by both downregulation and upregulation of many genes, while fewer changes occur between the HBC*1 and HBC*2 states. In both cases, relatively few of these differentially expressed genes are transcription factors. See Table S3. **(D, E)** Expression of AP-1 transcription factors in the neuronal (D) and sustentacular cell (E) lineages. **(F)** Distributions of neurons per clone (top) and sustentacular cells per clone (middle) in clones possessing at least one of each cell type, respectively, in the *Hopx*^{CreER} and *Krt5-CreER* late-tracing experiments. Data for the number of CFP and YFP clones in each animal for the latter experiment are presented (bottom). **(G)** Histogram of clone compositions in the *Hopx*^{CreER} (top) and *Krt5-CreER* late-tracing (bottom) experiments, according to the presence of at least one cell of a given cell type. Key for cell types: H, HBC; G, GBC; I, immediate neuronal precursor; N, neuron; M, microvillous cell; S, sustentacular cell; B, Bowman's gland. **(H)** This t-SNE plot shows expression of *Hopx* in the combined dataset that includes cells from uninjured differentiation and regeneration. Clusters are labeled as in Figure 3. **(I)** RNAscope in situ hybridization was used to detect *Hopx* and *Trp63* mRNA. *Hopx* is expressed at 24 HPI in a subset of activated HBCs but is essentially undetectable in resting HBCs prior to injury.

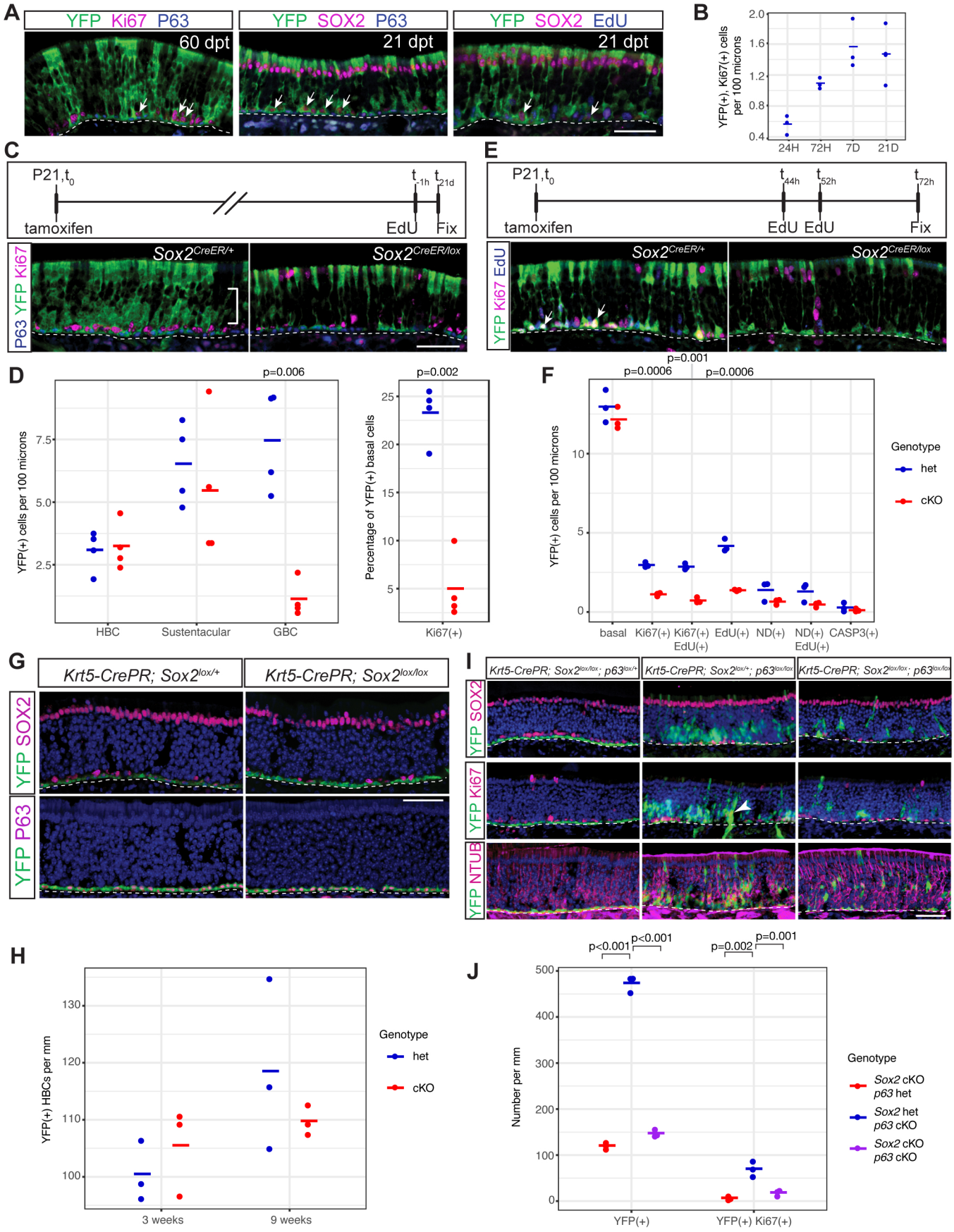

A
YFP Ki67 P63
60 dpt
YFP SOX2 P63
21 dpt
YFP SOX2 EdU
21 dpt
B
YFP(+), Ki67(+) cells per 100 microns
24H 72H 7D 21D
C
P21,t0
tamoxifen
EdU Fix
P63 YFP Ki67
Sox2CreER/+
Sox2CreER/lox
E
P21,t0
tamoxifen
EdU EdU Fix
YFP Ki67 EdU
Sox2CreER/+
Sox2CreER/lox
D
p=0.006
p=0.002
YFP(+) cells per 100 microns
HBC Sustentacular GBC
Percentage of YFP(+) basal cells
Ki67(+)
F
p=0.001
p=0.0006
p=0.0006
YFP(+) cells per 100 microns
basal Ki67(+) Ki67(+) EdU(+) EdU(+) ND(+) ND(+) EdU(+) CASP3(+)
Genotype
het
cKO
G
Krt5-CrePR; Sox2lox/+
Krt5-CrePR; Sox2lox/lox
YFP SOX2
YFP P63
I
Krt5-CrePR; Sox2lox/lox; p63lox/+
Krt5-CrePR; Sox2lox/+; p63lox/lox
Krt5-CrePR; Sox2lox/lox; p63lox/lox
YFP SOX2
YFP Ki67
YFP NTUB
H
YFP(+) HBCs per mm
3 weeks 9 weeks
Genotype
het
cKO
J
p<0.001
p<0.001
p=0.002
p=0.001
Number per mm
YFP(+) YFP(+) Ki67(+)
Genotype
Sox2 cKO p63 het
Sox2 het p63 cKO
Sox2 cKO p63 cKO

Figure S6

Figure S6. Related to Figure 6.
(A) *Sox2*$^{CreER/+}$*; Rosa26*^{eYFP} lineage-traced cells were labeled at P21 by tamoxifen injection and analyzed at 60 or 21 DPT. Even at 60 days post labeling, the Sox2(+) lineage contains actively cycling suprabasal progenitors (arrows). **(B)** The number of lineage-traced proliferating progenitors over time is plotted; at least three animals were examined per time-point. **(C, D)** *Sox2*$^{CreER/+}$*; Rosa26*^{eYFP} and *Sox2*$^{CreER/lox}$*; Rosa26*^{eYFP} lineage-traced cells were analyzed at 21 DPT. There is a marked reduction in the number of GBCs and neurons in the *Sox2* conditional knockout in the steady state uninjured olfactory epithelium (compare region of epithelium demarcated by bracket in C); four replicates were examined per genotype. **(E, F)** *Sox2*$^{CreER/+}$*; Rosa26*^{eYFP} and *Sox2*$^{CreER/lox}$*; Rosa26*^{eYFP} animals were injected with tamoxifen at P21, then injected with EdU at 44 and 52 HPT to label proliferating cells, and fixed at 72 HPT. The cells from the conditional knockout fail to continue to proliferate and maintain progenitor cells (indicated with arrows in the *Sox2*$^{CreER/+}$ background); three replicates were examined per genotype. **(G)** The *Krt5-CrePR* transgene, which is constitutively active in HBCs from approximately P3 onward, was used to conditionally ablate *Sox2* in HBCs; this has little, if any effect on homeostatic maintenance in the OE. **(H)** The number of lineage-traced HBCs in the *Sox2* conditional knockout is not significantly different from the heterozygote. **(I, J)** Knockout of *p63* in HBCs in a *Sox2* heterozygous background causes HBCs to differentiate and produce both sustentacular cells and neurons. Knockout of both *Sox2* and *p63* in HBCs causes HBCs to differentiate but produce only sustentacular cells; few proliferative progenitors are observed; three replicates per genotype. Lineage-traced cells comprising a Bowman's Gland is indicated with an arrowhead. P-values were calculated using a negative binomial regression model, and the Benjamini-Hochberg method was used to adjust for multiple testing; see STAR Methods. Scale bars, 50 μm.

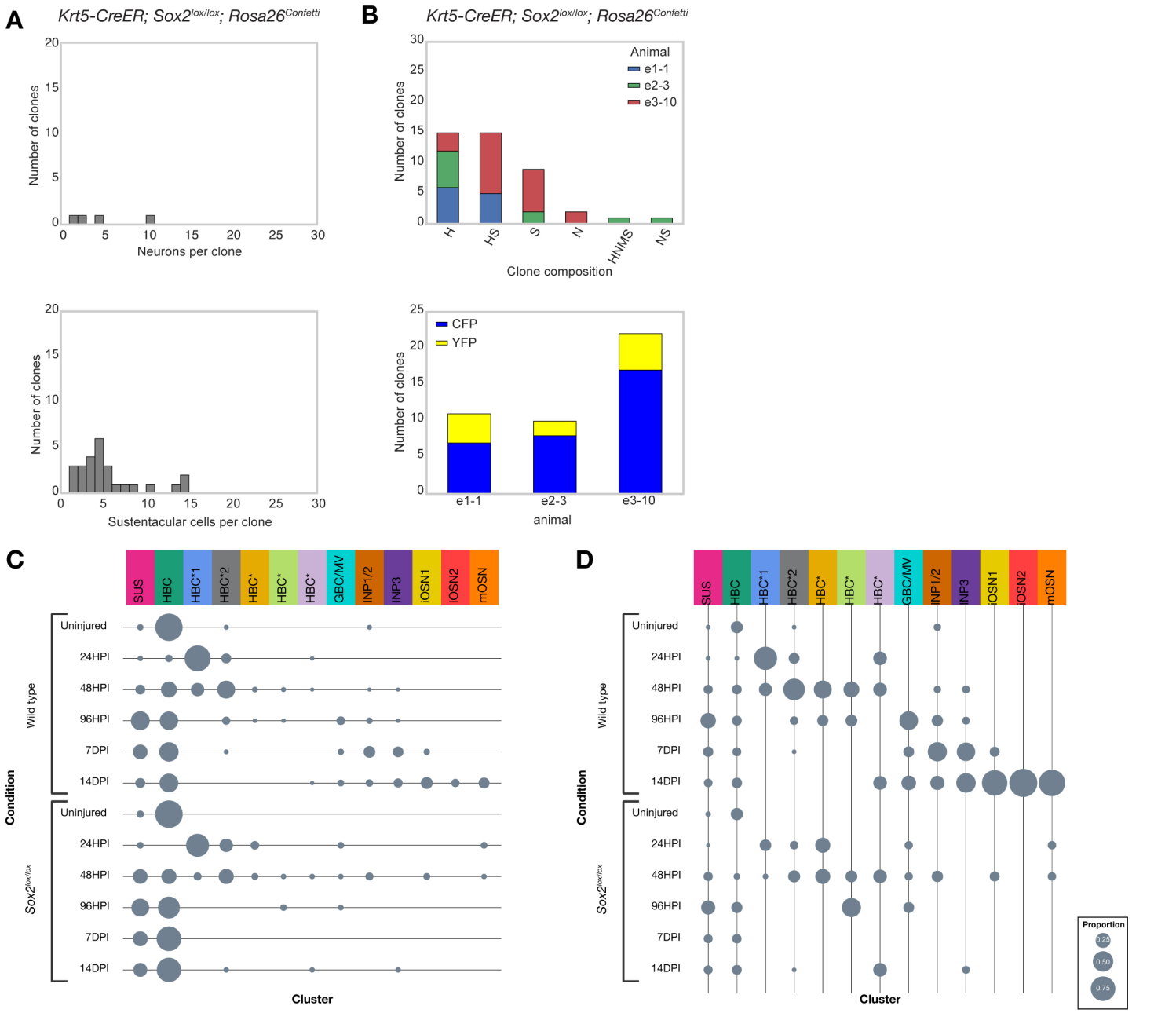

A
Krt5-CreER; Sox2lox/lox; Rosa26Confetti
Number of clones
Neurons per clone
B
Krt5-CreER; Sox2lox/lox; Rosa26Confetti
Animal
e1-1
e2-3
e3-10
Number of clones
H
HS
S
N
HNMS
NS
Clone composition
Number of clones
Sustentacular cells per clone
CFP
YFP
Number of clones
e1-1
e2-3
e3-10
animal
C
SUS
HBC
HBC*1
HBC*2
HBC*
HBC*
HBC*
GBC/MV
INP1/2
INP3
iOSN1
iOSN2
mOSN
Condition
Wild type
Sox2lox/lox
Uninjured
24HPI
48HPI
96HPI
7DPI
14DPI
Cluster
D
Proportion
0.25
0.50
0.75
E
clusters
expt
batch
Pgf
4932438A13Rik.1
Pcp4
Negr1
Kcnk2
Sox2ot
Abca1
Pon3
Trpm3
Nbl1
Hmgn2−ps1
4932438A13Rik
Mettl24
Fam161a
Sox2
1500009L16Rik
Adamdec1
Mettl7a1
Ermn
Serpine2
Sema3d
Ugt2a2
Pla2g7
Pon1
Sod1
F
clusters
expt
batch
15
10
5
0
Experimental condition
Wild-type, uninjured
Wild-type, 24HPI
Wild-type, 48HPI
Wild-type, 96HPI
Wild-type, 7DPI
Wild-type, 14DPI
Sox2 KO, uninjured
Sox2 KO, 24HPI
Sox2 KO, 48HPI
Sox2 KO, 96HPI
Sox2 KO, 14DPI

Figure S7

Figure S7. Related to Figures 6 and 7.
(A) Distributions of neurons per clone (top) and sustentacular cells per clone (bottom) in clones possessing at least one of each cell type, respectively, in the *Sox2* conditional knockout. **(B)** (top) Histogram of clone compositions in the Sox2 conditional knockout, according to the presence of at least one cell of a given cell type. Key for cell types: H, HBC; G, GBC; I, immediate neuronal precursor; N, neuron; M, microvillous cell; S, sustentacular cell; B, Bowman's gland. Data for the number of CFP and YFP clones in each animal in the Sox2 conditional knockout (bottom). **(C, D)** Overlap of experimental condition and cell cluster identity. In (C), the size of the circles reflects the proportion of cells from a given experimental condition that contribute to each cluster. In (D), the size of the circles reflects the proportion of cells in each cluster that are derived from each experimental condition. Most of the neuronal lineage is derived from wild-type cells. **(E, F)** Differences between wild type and *Sox2* knockout HBCs are apparent when examining gene expression within each cluster. Wild-type and knockout resting HBCs exhibit clear differences in gene expression prior to injury (E); following injury, the activated HBCs continue to differentially express some genes according to genotype (F).