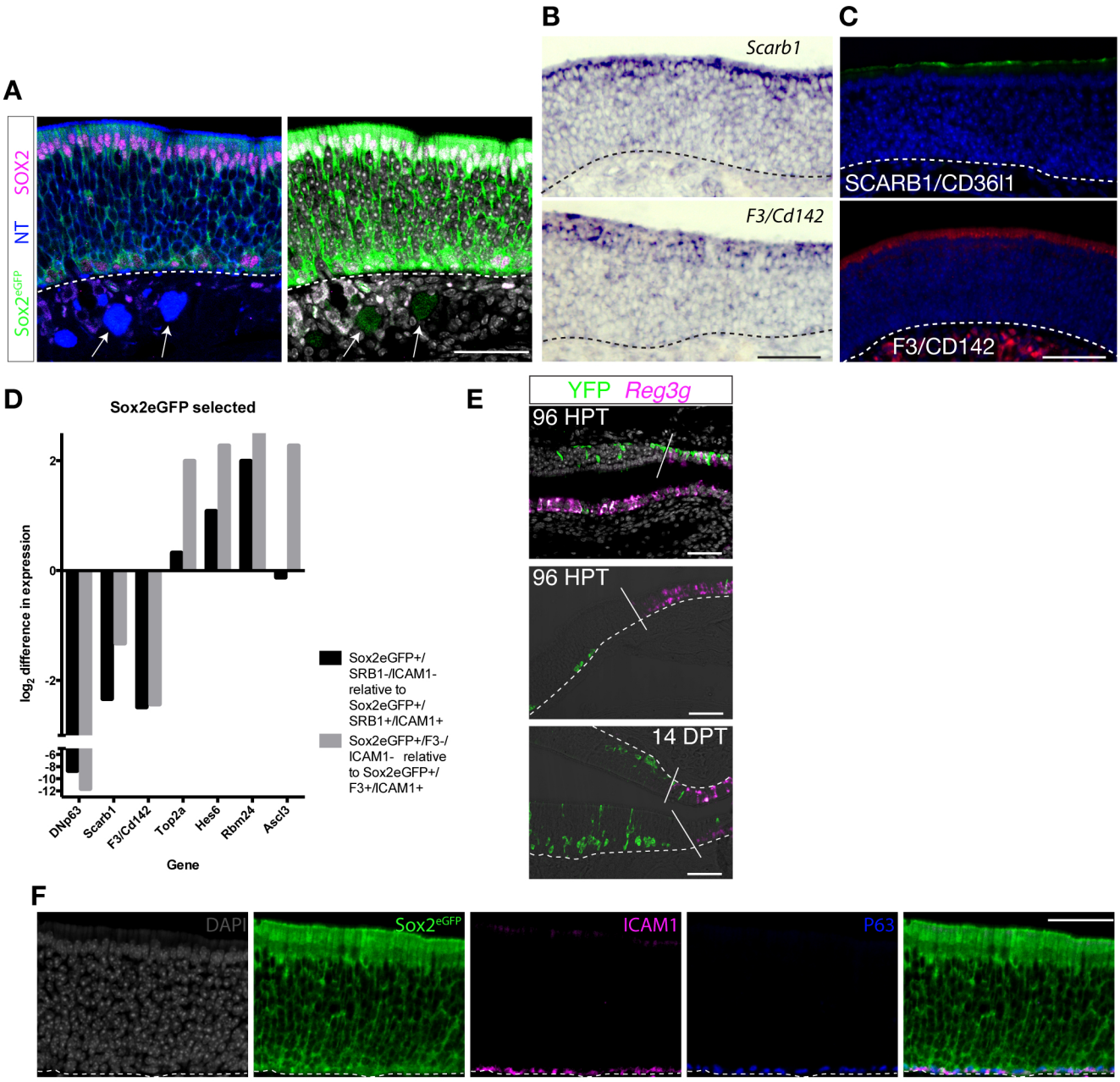

A
SOX2
NT
Sox2eGFP
B
Scarb1
F3/Cd142
C
SCARB1/CD36l1
F3/CD142
D
Sox2eGFP selected
log2 difference in expression
2
0
-2
-6
-8
-10
-12
DNp63
Scarb1
F3/Cd142
Top2a
Hes6
Rbm24
Ascl3
Gene
Sox2eGFP+/ SRB1-/ICAM1- relative to Sox2eGFP+/ SRB1+/ICAM1+
Sox2eGFP+/F3-/ ICAM1- relative to Sox2eGFP+/ F3+/ICAM1+
E
YFP Reg3g
96 HPT
96 HPT
14 DPT
F
DAPI
Sox2eGFP
ICAM1
P63

Figure S1

Figure S1. Related to Figure 1

(A) Sox2eGFP co-labels with neuronal tubulin (NT); overexposure reveals expression in the sub-mucosal axon fascicles (arrows). **(B,C**) Both *Scarb1* and *F3* RNA (B) and protein (C) localize to sustentacular cells. **(D)** RT-qPCR of FACS-purified Sox2-eGFP-positive cells demonstrates that cell depletion with SCARB1 or F3 antibodies enriches for neural progenitors (*Hes6*, *Rbm24*, and the cell cycle gene *Top2a*) and microvillous cells (*Ascl3*) (n=1 for each; table with oligonucleotide sequences in Table S6). **(E)** *Reg3g* is expressed in the non-sensory regions of the epithelium, with no detectable expression in the *Krt5-CreER*; *Trp63*$^{lox/lox}$*; Rosa26*^{eYFP} lineage-traced tissue at any stage examined (96 hours post tamoxifen (HPT), 14 days post tamoxifen (DPT) or 48HPT (not shown)); solid lines mark the boundary between sensory and non-sensory epithelium. Dashed lines mark the boundary between the epithelium and submucosa; scale bar = 50 microns. **(F)** All HBCs express *p63*, *Icam1*, and *Sox2*: immunohistochemistry for the Sox2eGFP transgene, P63, and ICAM1 shows co-labeling. Scale bars, 50 microns.

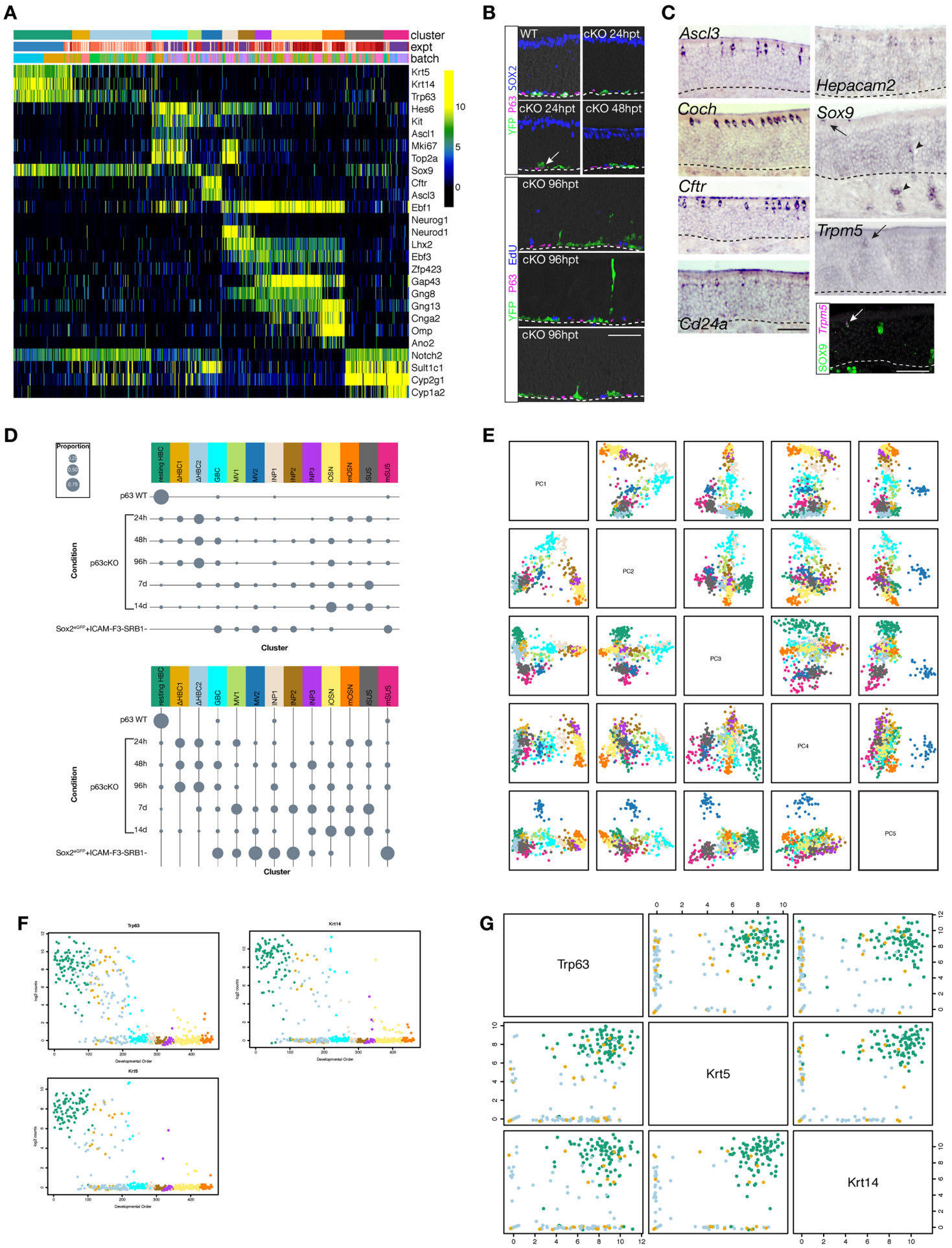

A
cluster
expt
batch
Krt5
Krt14
Trp63
Hes6
Kit
Ascl1
Mki67
Top2a
Sox9
Cftr
Ascl3
Ebf1
Neurog1
Neurod1
Lhx2
Ebf3
Zfp423
Gap43
Gng8
Gng13
Cnga2
Omp
Ano2
Notch2
Sult1c1
Cyp2g1
Cyp1a2
10
5
0
B
WT
cKO 24hpt
SOX2
P63
YFP
cKO 24hpt
cKO 48hpt
cKO 96hpt
EdU
cKO 96hpt
YFP
P63
cKO 96hpt
C
Ascl3
Hepacam2
Coch
Sox9
Cftr
Trpm5
Cd24a
Trpm5
SOX9
D
Proportion
resting HBC
ΔHBC1
ΔHBC2
GBC
iMV1
iMV2
iNP1
iNP2
iNP3
iOSN
mOSN
iSUS
mSUS
p63 WT
Condition
p63cKO
24h
48h
96h
7d
14d
Sox2nGFP+ICAM-F3-SRB1-
Cluster
E
PC1
PC2
PC3
PC4
PC5
F
Trp63
Krt14
Krt5
log2 counts
Developmental Order
G
Trp63
Krt5
Krt14

Figure S2

Figure S2. Related to Figure 2

(A) The heatmap shows expression of selected marker genes (rows) in cells (columns) organized by cluster (indicated by colored coded bars across the top, as in Figure S1). Experimental condition and batch are indicated by the middle and bottom color bars. Experimental condition colors correspond to the time-points indicated in panel (D). **(B)** At 24 hours post tamoxifen (hpt), some HBCs still express Trp63 (P63) while others no longer express detectable levels (arrow). By 48hpt, Trp63 is no longer detectable by immunohistochemistry. There is asynchrony in the differentiation of HBCs upon conditional knockout (cKO) of *Trp63*, as the three examples demonstrate. **(C)** *Ascl3*, *Coch*, *Cftr*, *Cd24a*, *Hepacam2* label one type of microvillous cell. We also observe that *Sox9* is expressed in a different class of microvillous cells (arrow) in addition to the Bowman's gland (arrowheads). *Trpm5* is also expressed in this MV cell sub-type (arrow). **(D)** Differentiation is asynchronous, as evidenced by the partial overlap of experimental condition and cell cluster identity. In the top plot, the percentage of cells from each experimental condition that contribute to each cluster is indicated by the size of the circle. Note that most experimental conditions contribute to multiple clusters, while the *Trp63*$^{+/+}$ HBCs predominantly populate the resting HBC cluster. In the bottom plot, the size of the circles reflects the proportion of cells from each cluster derived from each experimental condition. These plots reveal that clusters defined by more differentiated cell types are populated by cells with later experimental time points. HBCs from *Trp63* wild type animals constitute most of the resting HBCs. Sox2eGFP-positive cells comprise mostly mature sustentacular cells and neuronal precursors. Persistence of GFP expression late in the neuronal lineage is due to perdurance of GFP following its initial expression in GBCs. **(E)** The first five principal components of the expression matrix of all detected genes effectively separate the clusters and serve as input for *Slingshot*. **(F)** Expression of *Trp63*, *Krt5*, and *Krt14*, established markers of resting HBCs (green), in the neuronal lineage and organized by developmental order. **(G)** Pairwise comparisons of *Trp63*, *Krt5*, and *Krt14* gene expression in the resting (green) and transitional HBCs (ΔHBC1, gold; ΔHBC2, light blue). All three are highly correlated in resting HBCs, but their expression is no longer tightly coordinated in the transition states.

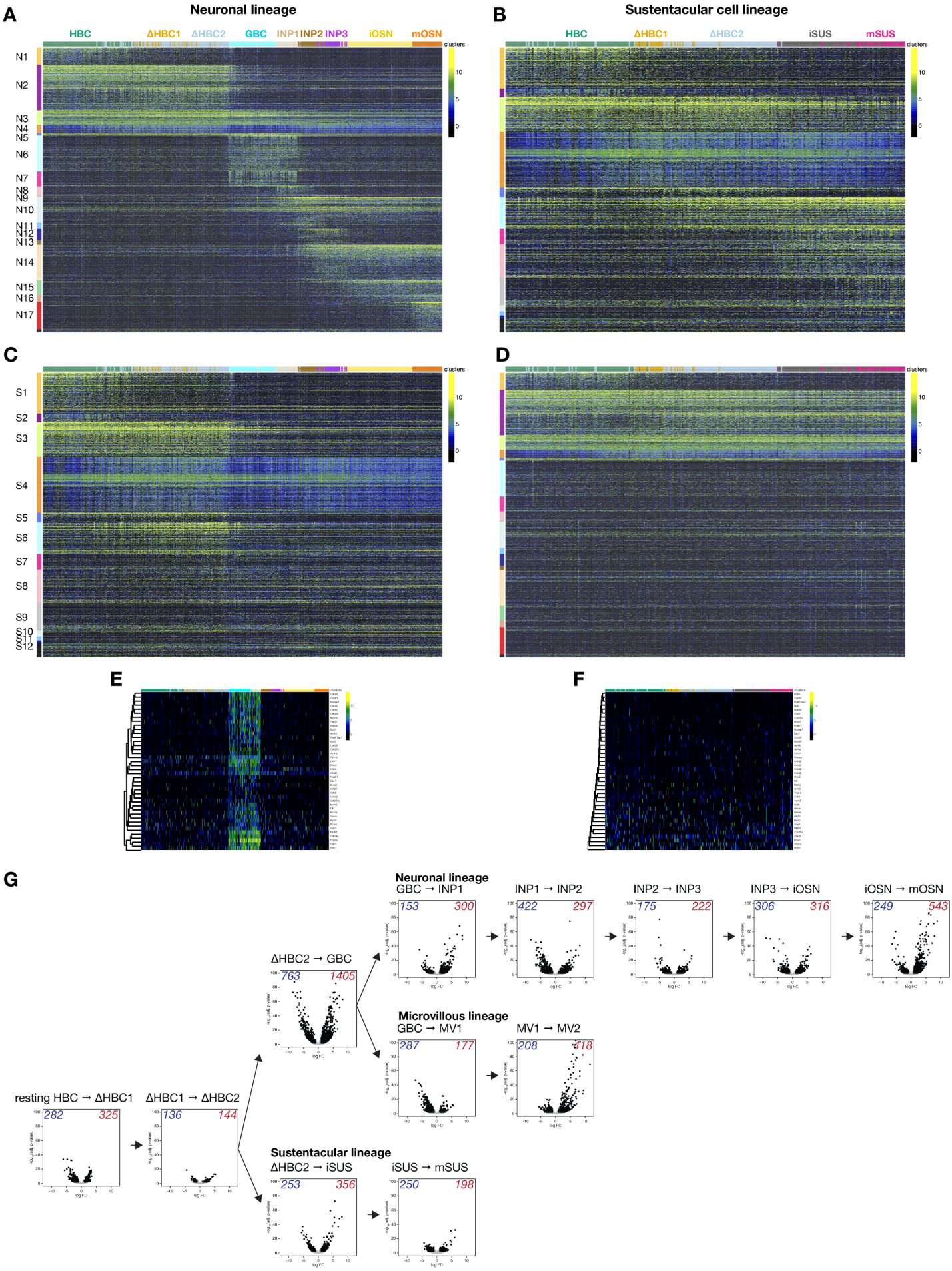

A
Neuronal lineage
HBC
ΔHBC1
ΔHBC2
GBC
INP1
INP2
INP3
iOSN
mOSN
clusters
N1
N2
N3
N4
N5
N6
N7
N8
N9
N10
N11
N12
N13
N14
N15
N16
N17
B
Sustentacular cell lineage
HBC
ΔHBC1
ΔHBC2
iSUS
mSUS
clusters
C
clusters
S1
S2
S3
S4
S5
S6
S7
S8
S9
S10
S11
S12
D
clusters
E
F
G
Neuronal lineage
GBC → INP1
INP1 → INP2
INP2 → INP3
INP3 → iOSN
iOSN → mOSN
153
300
422
297
175
222
306
316
249
543
ΔHBC2 → GBC
763
1405
Microvillous lineage
GBC → MV1
MV1 → MV2
287
177
208
418
resting HBC → ΔHBC1
ΔHBC1 → ΔHBC2
282
325
136
144
Sustentacular lineage
ΔHBC2 → iSUS
iSUS → mSUS
253
356
250
198
log FC

Figure S3

Figure S3. Related to Figure 3

(A) Expression (log2 normalized counts) of all 2327 differentially expressed genes used in the gene clustering for the neuronal lineage, ordered by gene cluster (vertical axis) and neuronal lineage developmental order (horizontal axis). **(B)** Expression of all 1622 differentially expressed genes used in the gene clustering for the sustentacular cell lineage, ordered by gene cluster (vertical axis) and sustentacular cell lineage developmental order (horizontal axis). **(C)** Expression of the genes used in the sustentacular cell lineage gene clustering, as they are expressed in the neuronal lineage. **(D)** Expression of the genes used in the neuronal lineage gene clustering, as they are expressed in the sustentacular cell lineage. Note that the heatmap shows genes that are enriched in both resting and transitional HBCs as well as immature and mature sustentacular cells, highlighting the similarity of sustentacular cells and their HBC precursors. **(E, F)** Expression of the cell cycle genes used in the cell cycle gene set heatmap in Figure 3, in the neuronal (E) and sustentacular cell lineages (F). **(G)** Volcano plots of –log10 adjusted p-value (adj. p-value) versus log2 fold change (log2FC) between clusters. The plots are arranged to represent the lineage trajectory map and display the number of differentially expressed genes between the clusters at each transition in the lineage (genes with adj. p-value < 0.01 and log2FC > 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 multiplicity using the Benjamini-Hochberg procedure (see Star Methods). See Table S2.

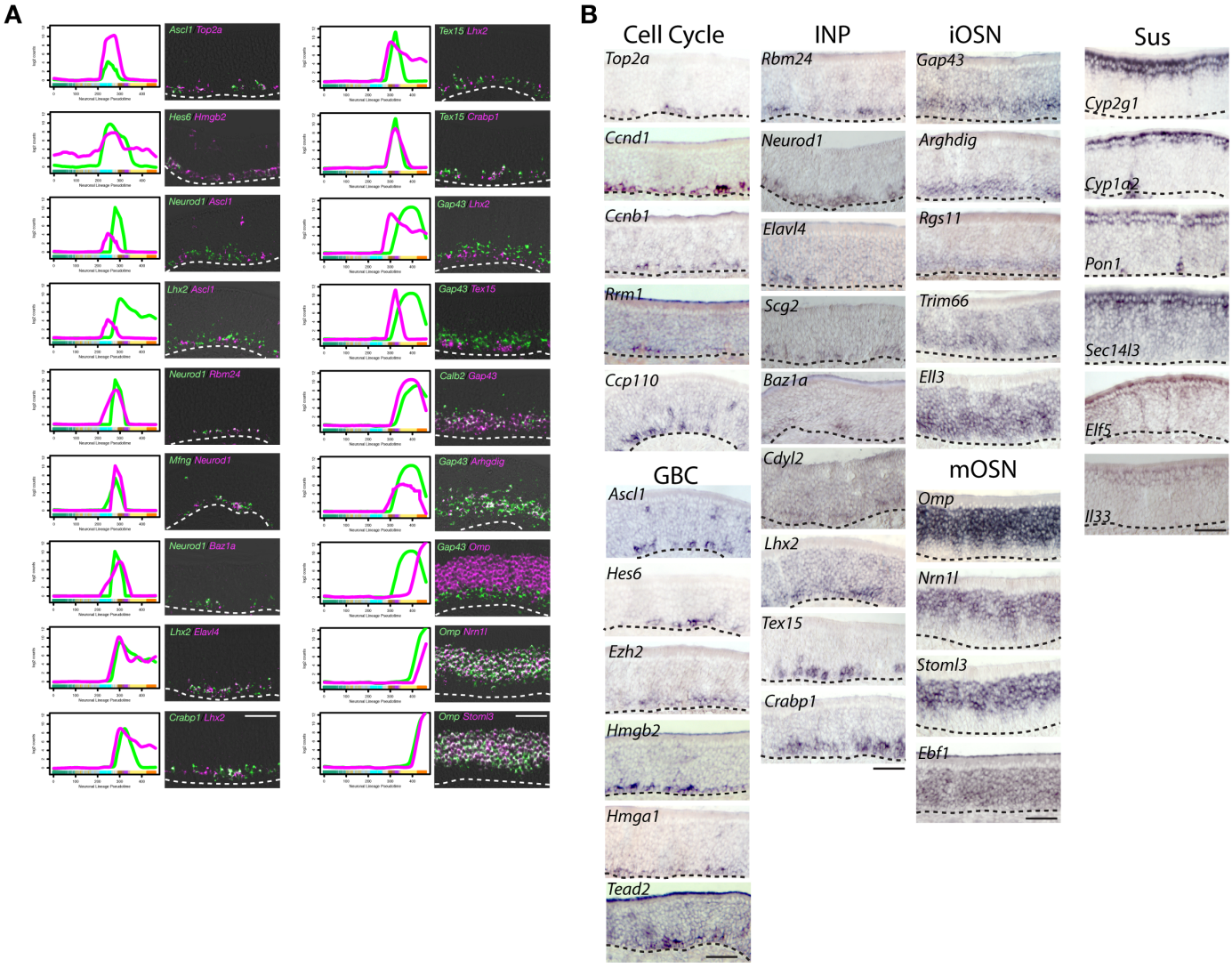
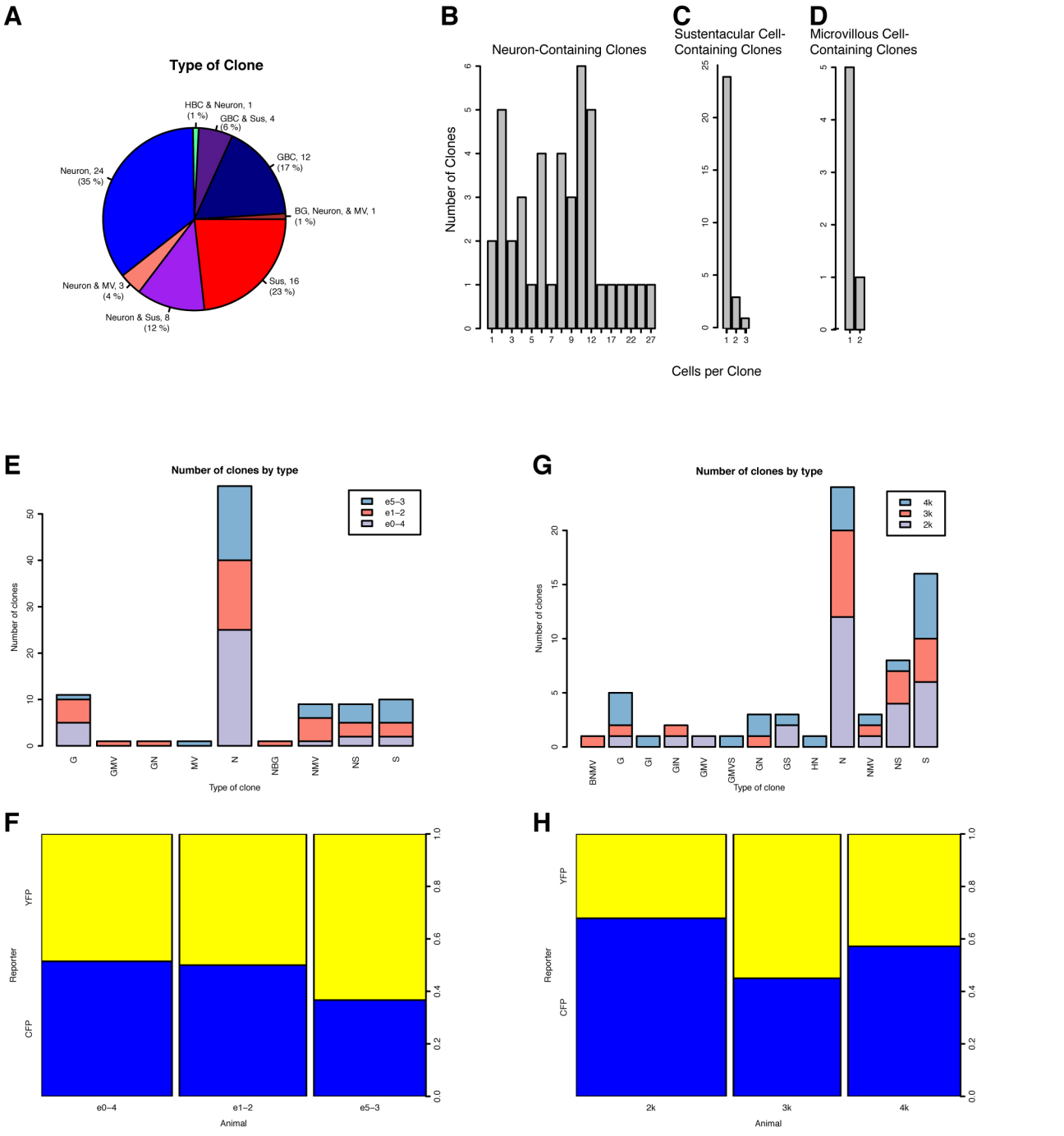

A
Ascl1 Top2a
Hes6 Hmgb2
Neurod1 Ascl1
Lhx2 Ascl1
Neurod1 Rbm24
Mfng Neurod1
Neurod1 Baz1a
Lhx2 Elavl4
Crabp1 Lhx2
Tex15 Lhx2
Tex15 Crabp1
Gap43 Lhx2
Gap43 Tex15
Calb2 Gap43
Gap43 Arhgdig
Gap43 Omp
Omp Nrn1l
Omp Stoml3
B
Cell Cycle
Top2a
Ccnd1
Ccnb1
Rrm1
Ccp110
INP
Rbm24
Neurod1
Elavl4
Scg2
Baz1a
Cdyl2
iOSN
Gap43
Arghdig
Rgs11
Trim66
Ell3
Sus
Cyp2g1
Cyp1a2
Pon1
Sec14l3
Elf5
Il33
GBC
Ascl1
Hes6
Ezh2
Hmgb2
Hmga1
Tead2
Lhx2
Tex15
Crabp1
mOSN
Omp
Nrn1l
Stoml3
Ebf1
C
OR
TPM
> 100 TPM
#ORs
> 5000 TPM
Developmental Order
D
Ehmt1
Setdb1
Setdb2
Kdm1a
Lbr
Lhx2
Ebf1
Ebf3
Ebf4
Gnai2
Gnas
Gnal
Gnb1
Gng8
Gng13
Gng3
Adcy3
Ano2
Cnga2
Cnga4
Atf5
Rtp1
Counts
Developmental Order

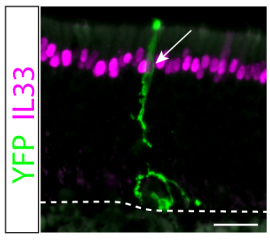
Figure S4

Figure S4. Related to Figure 3

(A) Double fluorescent RNA in situ hybridizations for the indicated genes in each panel. The plots are best-fit curves to the single-cell RNA-seq data from cells in the neuronal lineage, sorted by their developmental order in the lineage. Cells labeled with both probes appear white in these pseudo-colored images. The expression patterns of these selected genes demonstrate the progression of cells through multiple immediate neuronal precursor stages that were inferred by our single-cell RNA-seq analysis. **(B)** Colorimetric RNA in situ hybridizations for known and novel genes of the different olfactory cell types. We identified and validated many novel genes along the neuronal lineage (*Ezh2*, *Hmgb2*, *Hmga1*, *Tead2*, *Elavl4*, *Scg2*, *Baz1a*, *Cdyl2*, *Tex15*, *Crabp1*, *Arghdig*, *Ell3*, *Nrn1l*) and sustentacular cell lineage (*Pon1*, *Sec14l3*, *Elf5*, *Ill33*). Scale bars = 50 microns. **(C)** Expression of odorant receptors (ORs) in the neuronal lineage, sorted by their developmental order in the lineage. The top panel reveals significant up-regulation of OR expression in mature olfactory sensory neurons (mOSN; orange). The second and third panels show the number of OR genes expressed per cell at different thresholds of expression. Multiple ORs are detected at the INP3 stage (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). (**D**) The remaining panels display selected transcription factors, chromatin modifiers and other signaling molecules relevant to OR gene expression.



I



J

Sample	Distance	YFP+	CASP3+	Co-labeled
24H-1	5475	187	20	0
24H-2	4816	118	25	0
24H-3	5037	158	23	1
48H-1	4421	158	11	0
48H-2	5550	258	28	1
96H-1	4245	273	25	0
96H-2	4669	249	10	0
7D-1	4327	245	17	0
7D-2	4990	278	27	1

K

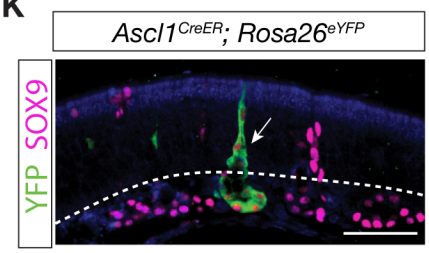


Figure S5

Figure S5. Related to Figure 4

(A-D) *Krt5-CreER*; *Trp63$^{lox/lox}$; Rosa26^{Confetti}* clonal lineage tracing analysis at 7-days post tamoxifen (DPT) induction. A summary of the types of clones obtained is presented in (A). Neuron-containing clones usually contain more than one neuron (mean = 8.8 +/- 1.0 standard error of the mean), whereas sustentacular cells (C) and microvillous cells (D) are almost always limited to one cell of that type per clone (1.2 +/- 0.1 and 1.2 +/- 0.2, respectively). Note that the GBC category in (A) represents all GBC-containing clones that had any type of cell from the neuronal lineage, such as a neuron or microvillous cell. **(E-H)** Plots relating to the 14DPT (E, F) or 7DPT (G, H) clonal lineage tracing. (E, G) The number of clones of each type from each animal in the analysis. **(F, H)** The proportion of clones that were labeled by membrane CFP or cytosolic YFP for each animal. These data demonstrate the consistency of our observations across multiple animals; no single animal skewed the results. G = GBC; GMV = GBC, microvillous cell; GN = GBC, neuron; MV = microvillous cell; N = neuron; NBG = neuron, Bowman's gland; NMV = neuron, microvillous cell; NS = neuron, sustentacular cell; S = sustentacular cell; BNMV = Bowman's gland, neuron, microvillous cell; GI = GBC, neural precursor; GIN = GBC, neural precursor, neuron; GMVS = GBC, microvillous cell, sustentacular cell; GS = GBC, sustentacular cell; HN = HBC, neuron. (**I**) Immunohistochemistry for YFP and IL33 in a *Krt5-CreER*; *Trp63$^{lox/lox}$; Rosa26^{Confetti}* clone demonstrates co-labeling of a sustentacular cell with nuclear IL33 (arrow), consistent with our classification. Scale bar, 25 microns. **(J)** Table of the quantification of the immunohistochemistry of the *Krt5-CreER*; *Trp63$^{lox/lox}$; Rosa26^{eYFP}* samples presented in the main Figure 4. The table displays the animals/samples at each time-point (24-hours, 48-hours, 96-hours, and 7-days) post tamoxifen injection, the length of OE counted in microns, the number of YFP+ lineage traced cells, the number of activated CASPASE3+ cells, and the number of co-labeled cells in each sample **(K)** This micrograph shows a rare Bowman's gland labeled by *Ascl1-CreER; Rosa26^{eYFP}* lineage tracing at 21 DPT. Scale bar, 50 microns.

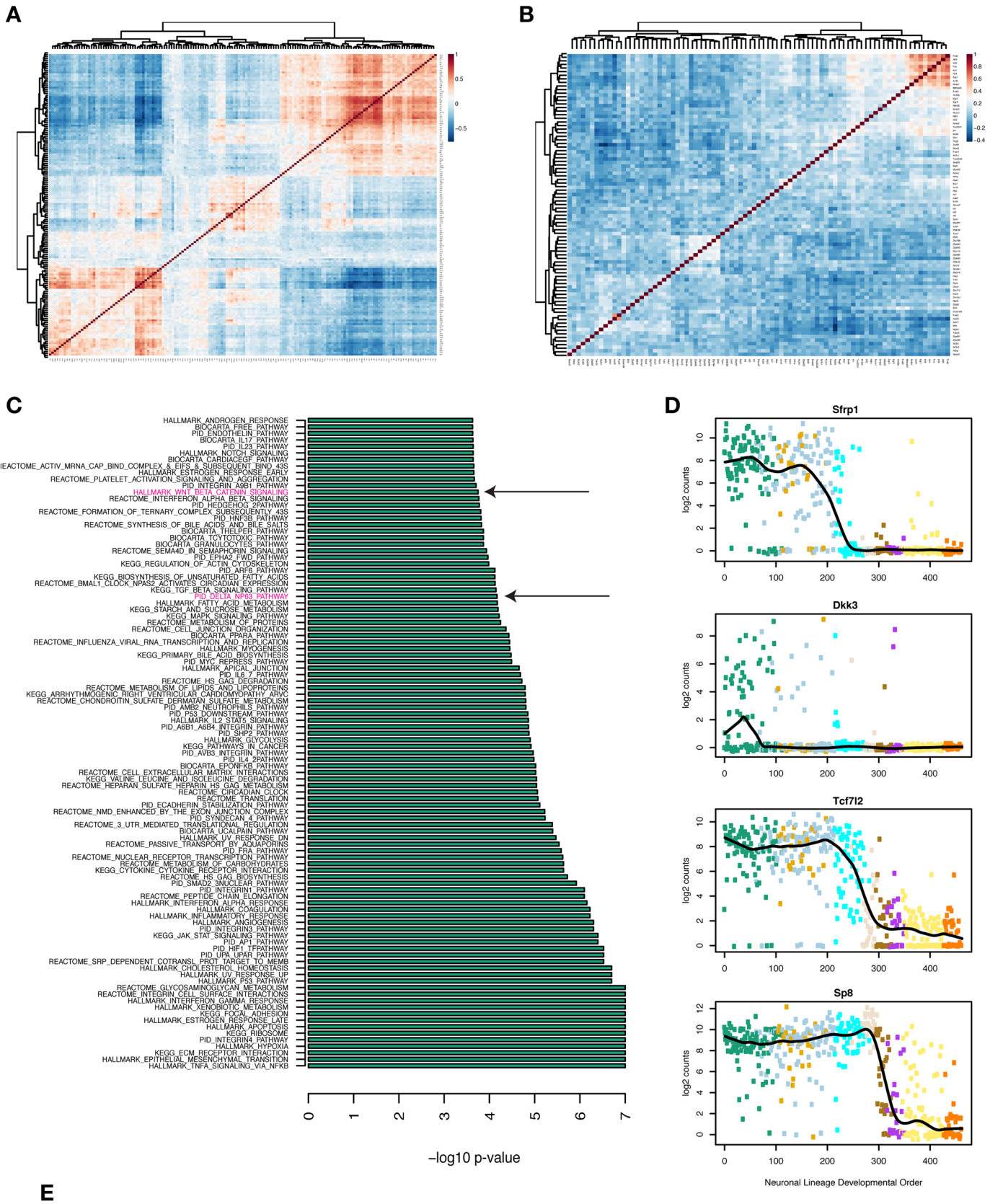

A
B
C
HALLMARK_ANDROGEN_RESPONSE
BIOCARTA_FREE_PATHWAY
PID_ENDOTHELIN_PATHWAY
BIOCARTA_IL17_PATHWAY
PID_IL23_PATHWAY
HALLMARK_NOTCH_SIGNALING
BIOCARTA_CARDIACEGF_PATHWAY
!EACTOME_ACTIV_MRNA_CAP_BIND_COMPLEX_&_EIFS_&_SUBSEQUENT_BIND_43S
HALLMARK_ESTROGEN_RESPONSE_EARLY
REACTOME_PLATELET_ACTIVATION_SIGNALING_AND_AGGREGATION
PID_INTEGRIN_A9B1_PATHWAY
HALLMARK_WNT_BETA_CATENIN_SIGNALING
REACTOME_INTERFERON_ALPHA_BETA_SIGNALING
PID_HEDGEHOG_2PATHWAY
REACTOME_FORMATION_OF_TERNARY_COMPLEX_SUBSEQUENTLY_43S
PID_HNF3B_PATHWAY
REACTOME_SYNTHESIS_OF_BILE_ACIDS_AND_BILE_SALTS
BIOCARTA_THELPER_PATHWAY
BIOCARTA_TCYTOTOXIC_PATHWAY
BIOCARTA_GRANULOCYTES_PATHWAY
REACTOME_SEMA4D_IN_SEMAPHORIN_SIGNALING
PID_EPHA2_FWD_PATHWAY
KEGG_REGULATION_OF_ACTIN_CYTOSKELETON
PID_ARF6_PATHWAY
KEGG_BIOSYNTHESIS_OF_UNSATURATED_FATTY_ACIDS
REACTOME_BMAL1_CLOCK_NPAS2_ACTIVATES_CIRCADIAN_EXPRESSION
KEGG_TGF_BETA_SIGNALING_PATHWAY
PID_DELTA_NP63_PATHWAY
HALLMARK_FATTY_ACID_METABOLISM
KEGG_STARCH_AND_SUCROSE_METABOLISM
KEGG_MAPK_SIGNALING_PATHWAY
REACTOME_METABOLISM_OF_PROTEINS
REACTOME_CELL_JUNCTION_ORGANIZATION
BIOCARTA_PPARA_PATHWAY
REACTOME_INFLUENZA_VIRAL_RNA_TRANSCRIPTION_AND_REPLICATION
HALLMARK_MYOGENESIS
KEGG_PRIMARY_BILE_ACID_BIOSYNTHESIS
PID_MYC_REPRESS_PATHWAY
HALLMARK_APICAL_JUNCTION
PID_IL6_7_PATHWAY
REACTOME_HS_GAG_DEGRADATION
REACTOME_METABOLISM_OF_LIPIDS_AND_LIPOPROTEINS
KEGG_ARRHYTHMOGENIC_RIGHT_VENTRICULAR_CARDIOMYOPATHY_ARVC
REACTOME_CHONDROITIN_SULFATE_DERMATAN_SULFATE_METABOLISM
PID_AMB2_NEUTROPHILS_PATHWAY
PID_P53_DOWNSTREAM_PATHWAY
HALLMARK_IL2_STAT5_SIGNALING
PID_A6B1_A6B4_INTEGRIN_PATHWAY
PID_SHP2_PATHWAY
HALLMARK_GLYCOLYSIS
KEGG_PATHWAYS_IN_CANCER
PID_AVB3_INTEGRIN_PATHWAY
PID_IL4_2PATHWAY
BIOCARTA_EPONFKB_PATHWAY
REACTOME_CELL_EXTRACELLULAR_MATRIX_INTERACTIONS
KEGG_VALINE_LEUCINE_AND_ISOLEUCINE_DEGRADATION
REACTOME_HEPARAN_SULFATE_HEPARIN_HS_GAG_METABOLISM
REACTOME_CIRCADIAN_CLOCK
REACTOME_TRANSLATION
PID_ECADHERIN_STABILIZATION_PATHWAY
REACTOME_NMD_ENHANCED_BY_THE_EXON_JUNCTION_COMPLEX
PID_SYNDECAN_4_PATHWAY
REACTOME_3_UTR_MEDIATED_TRANSLATIONAL_REGULATION
BIOCARTA_UCALPAIN_PATHWAY
HALLMARK_UV_RESPONSE_DN
REACTOME_PASSIVE_TRANSPORT_BY_AQUAPORINS
PID_FRA_PATHWAY
REACTOME_NUCLEAR_RECEPTOR_TRANSCRIPTION_PATHWAY
REACTOME_METABOLISM_OF_CARBOHYDRATES
KEGG_CYTOKINE_CYTOKINE_RECEPTOR_INTERACTION
REACTOME_HS_GAG_BIOSYNTHESIS
PID_SMAD2_3NUCLEAR_PATHWAY
PID_INTEGRIN1_PATHWAY
REACTOME_PEPTIDE_CHAIN_ELONGATION
HALLMARK_INTERFERON_ALPHA_RESPONSE
HALLMARK_COAGULATION
HALLMARK_INFLAMMATORY_RESPONSE
HALLMARK_ANGIOGENESIS
PID_INTEGRIN3_PATHWAY
KEGG_JAK_STAT_SIGNALING_PATHWAY
PID_AP1_PATHWAY
PID_HIF1_TFPATHWAY
PID_UPA_UPAR_PATHWAY
REACTOME_SRP_DEPENDENT_COTRANSL_PROT_TARGET_TO_MEMB
HALLMARK_CHOLESTEROL_HOMEOSTASIS
HALLMARK_UV_RESPONSE_UP
HALLMARK_P53_PATHWAY
REACTOME_GLYCOSAMINOGLYCAN_METABOLISM
REACTOME_INTEGRIN_CELL_SURFACE_INTERACTIONS
HALLMARK_INTERFERON_GAMMA_RESPONSE
HALLMARK_XENOBIOTIC_METABOLISM
KEGG_FOCAL_ADHESION
HALLMARK_ESTROGEN_RESPONSE_LATE
HALLMARK_APOPTOSIS
KEGG_RIBOSOME
PID_INTEGRIN4_PATHWAY
HALLMARK_HYPOXIA
KEGG_ECM_RECEPTOR_INTERACTION
HALLMARK_EPITHELIAL_MESENCHYMAL_TRANSITION
HALLMARK_TNFA_SIGNALING_VIA_NFKB
0 1 2 3 4 5 6 7
–log10 p-value
D
Sfrp1
Dkk3
Tcf7l2
Sp8
log2 counts
0 100 200 300 400
Neuronal Lineage Developmental Order
E
YFP IL33 P63
p63lx/lx; β-catenin lx/lx
p63lx/lx; β-catenin lx/lx

Figure S6

Figure S6. Related to Figure 5

(A, B) Correlation heatmap of the most highly differentially expressed (DE) transcription factors of the neuronal (A) and sustentacular cell (B) lineages. **(C)** Gene Set Enrichment Analysis (GSEA) for the resting HBC cluster, sorted by the –log10 p-value; the top 100 gene sets are displayed. The Wnt signaling pathway and the P63 (Trp63) pathway are highlighted in magenta. See also Table S4. **(D)** Gene expression plots for the secreted Wnt signaling antagonists (*Sfrp1*, *Dkk3*) and key transcription factors involved in Wnt signaling (*Tcf7l2*, *Sp8*). (**E**) Consistent with our contention that HBCs form more sustentacular support cells relative to neurons in the *p63*$^{lox/lox}$*; Beta-catenin*$^{lox/lox}$ double knockout, many of the HBC derived cells in the middle and apical regions of the epithelium express IL33, a gene we validated as being expressed in sustentacular cells and Bowman's gland, and not neurons (see Figure S4).