Supplemental Text and Figures

Figure S1 (related to Figure 1). Characterization of gene expression in ICAM1(+) vs. ICAM1(-) FACS-purified cell populations. (A) Microarray-based gene expression data for representative genes differentially expressed in FACS-purified ICAM1(+) vs. ICAM1(-) cells. Transcripts known to be expressed in the HBCs (*Krt14, Icam1, Krt5, Itgb4*) were enriched (blue) in the ICAM1-positive cells whereas markers of other cell types – *Ascl1, Ngn1* (GBCs); *Gap43* (immature olfactory sensory neurons); *Omp* (mature olfactory sensory neurons) – were depleted (red). Ratio of gene expression is shown both as M-value ($\log_2$[ICAM1(+)/ICAM1(-)]) and fold-difference (i.e., the corresponding difference in linear space). **(B)** qRT-PCR analysis of selected marker genes in a pair of ICAM1(+) and ICAM1(-) cell samples obtained from a single FACS purification run. *Ost* is a marker of sustentacular cells. **(C)** qRT-PCR showing that p63 – and specifically *ΔNp63* –is highly enriched in ICAM1(+) vs. ICAM1(-) cells.

Figure S2. Proliferation of p63(+) cells during injury-induced regeneration (related to Figure 2). Quantitation of the number of p63(+)/Ki67(+) cells **(A)** and p63 (+) cells **(B)** per 100 μm olfactory epithelium in 12 micron-thick tissue sections, and the percentage of p63(+) cells that are Ki67(+) **(C)**. Olfactory epithelium was analyzed from control, uninjured animals (UI) or from methimazole-treated animals at 1, 2, and 3 days post-injury (dpi). Bars represent mean values +/- standard deviations.

Figure S3 (related to Figure 3). Additional control experiments for the p63 conditional knockout in regenerating and uninjured olfactory epithelium. Analysis of *Krt5-CrePR;Rosa26*^{YFP} mice in the p63$^{+/+}$ and *p63*$^{lox/+}$, *p63*$^{lox/lox}$ backgrounds, and in *p63*$^{lox/lox}$ animals lacking the *Krt5-crePR* transgene. Olfactory epithelium from these genotypes

was analyzed at different times following methimazole-induced injury as well as in the uninjured steady state. These experiments were conducted to ensure that the observed phenotypes are caused by excision of the $p63^{lox/lox}$ by cre recombinase expressed under the control of the *Krt5-crePR* transgene, and not due to effects of cre recombinase expression or the unexcised $p63^{lox}$ allele. **(A-D)** Expression of olfactory cell type-specific markers in heterozygous $p63^{lox/+}$ mice is similar to that observed in wild type controls, six days after methimazole injury (compare with panels E-H; see also Figure 3). **(E-H)** Olfactory epithelium from $p63^{lox/lox}$ mice in the absence of cre recombinase (left-hand column) or $p63^{+/+}$ mice in the presence of cre recombinase (right-hand column) shows normal expression of olfactory cell type-specific markers. **(I-P)** p63 and Krt14 expression was analyzed in olfactory epithelium from mice with the indicated genotypes two days following methimazole treatment. p63- and Krt14-positive cells are reduced in the *Krt5-CrePR;Rosa26*^{YFP}*;p63*$^{lox/lox}$ background (O,P), compared to the heterozygous and wild type controls shown in panels I-N. **(Q-T)** In uninjured olfactory epithelium, expression of p63 and Krt14 is indistinguishable between $p63^{lox/lox}$ mice lacking cre recombinase and *Krt5-CrePR;Rosa26*^{YFP}*;p63*$^{+/+}$ animals. Images were captured from the septum in the middle and ventral regions of the olfactory epithelium. Bar = 50μm.

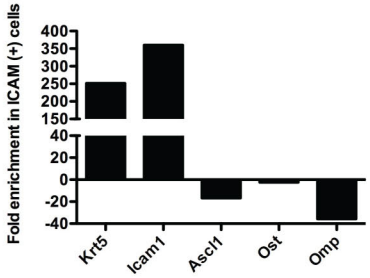
Figure S4 (related to Figure 5). The YFP-labeled HBC lineage in uninjured olfactory epithelium from *Krt5-CrePR;Rosa26*^{YFP} mice in the $p63^{+/}$ and $p63^{lox/lox}$ backgrounds was assessed for formation of mature olfactory sensory neurons by immunohistochemistry to OMP at P12 and then later at P28. **(A)** Although neurons are formed by P12 in the $p63^{lox/lox}$ background (see Figure 5), we only rarely observe YFP lineage-traced cells co-labeled with OMP (arrowhead), a marker of fully mature olfactory receptor neurons. We attribute this to the fact that the transgene becomes active at P3 and full maturation

of olfactory neurons from HBCs requires 10-14 days. **(B)** In the occasional *Krt5-CrePR; $p63^{lox/lox}$; $Rosa26^{YFP}$* animal that survives beyond three weeks of age (here at P28), extensive co-labeling of YFP lineage-traced cells with OMP can be observed, indicating that in the absence of p63, HBCs fully differentiate into mature olfactory sensory neurons. **(C)** Low power view of a coronal section with olfactory epithelium (OE) and olfactory bulb (OB) of a *Krt5-CrePR; $p63^{lox/lox}$; $Rosa26^{YFP}$* mouse. Note the extensive YFP/OMP co-labeling of sub-mucosal axon fascicles and olfactory nerve processes (arrows) that ultimately innervate the glomerular layer of the olfactory bulb. Images in (A) and (B) were captured from the septum in the middle and ventral regions of the olfactory epithelium. Bar = 50μm.

A

gene	M value	p value	fold difference
Trp63	8.27	1.57E-06	308
Krt14	8.05	1.20E-06	265
Icam1	7.24	2.40E-09	151
Krt5	6.62	5.38E-09	98.3
Itgb4	4.74	2.41E-07	26.7
Ascl1	-3.34	5.00E-07	0.0987
Omp	-4.03	1.19E-06	0.0612
Ngn1	-4.09	2.04E-09	0.0587
Gap43	-4.39	5.94E-08	0.0477

B



C

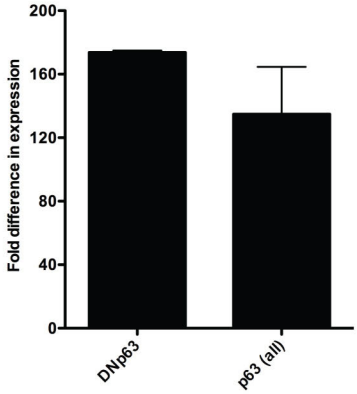


Figure S1

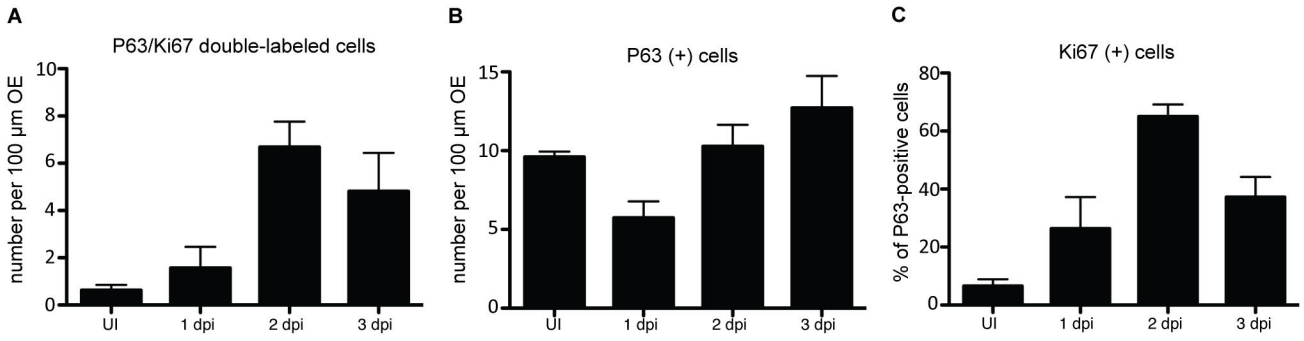

A
P63/Ki67 double-labeled cells
number per 100 µm OE
10
8
6
4
2
0
UI
1 dpi
2 dpi
3 dpi
B
P63 (+) cells
number per 100 µm OE
15
10
5
0
UI
1 dpi
2 dpi
3 dpi
C
Ki67 (+) cells
% of P63-positive cells
80
60
40
20
0
UI
1 dpi
2 dpi
3 dpi

Figure S2

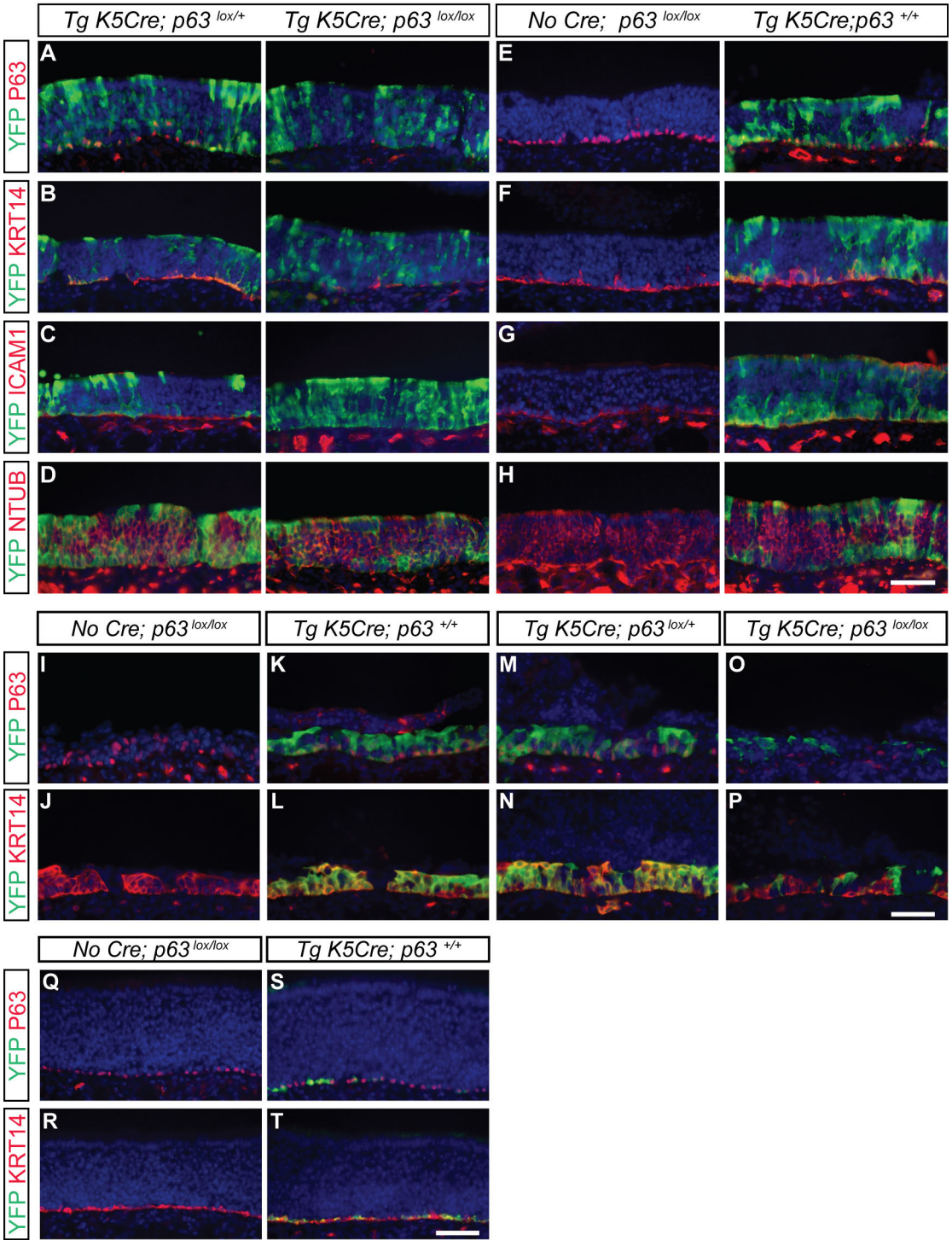

Tg K5Cre; p63 lox/+
Tg K5Cre; p63 lox/lox
No Cre; p63 lox/lox
Tg K5Cre;p63 +/+
YFP P63
YFP KRT14
YFP ICAM1
YFP NTUB
A
B
C
D
E
F
G
H
No Cre; p63 lox/lox
Tg K5Cre; p63 +/+
Tg K5Cre; p63 lox/+
Tg K5Cre; p63 lox/lox
I
J
K
L
M
N
O
P
No Cre; p63 lox/lox
Tg K5Cre; p63 +/+
Q
R
S
T

Figure S3

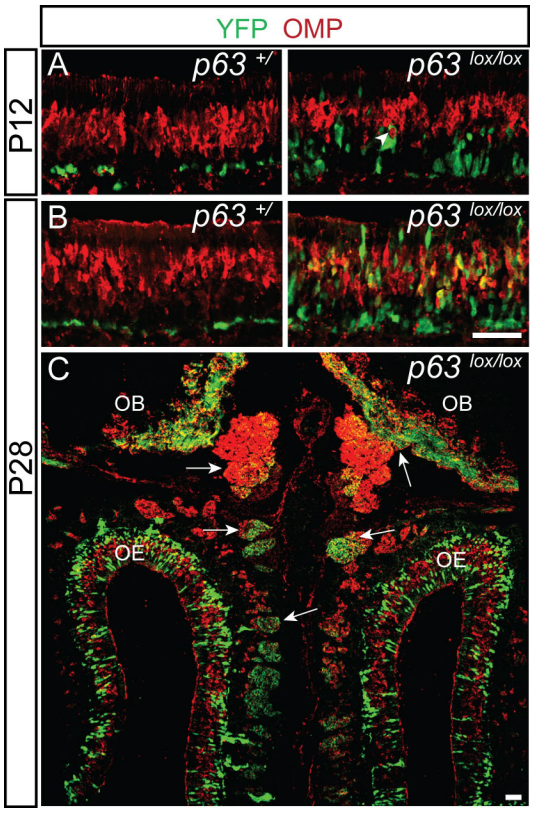

YFP OMP
P12
P28
A
p63 +/
p63 lox/lox
B
p63 +/
p63 lox/lox
C
p63 lox/lox
OB
OB
OE
OE

Figure S4