SUPPLEMENTAL INFORMATION

Figure S1. Related to Figure 1.
(A-G) H&E staining in frontal sections through the nasal cavity.
(A) Nasal pit (NP) at E10.5.
(B) Turbinates n1 and n2 at E12.5. n3 appears at this stage and can be found in more posterior sections (data not shown). * indicates site of future c1 development. VNO, vomeronasal organ.
(C) Turbinates n1, c1, and n2 at E14.5. n1 and n2 are connected at this section. c2, n3, and n4 appear at this stage and can be found in more posterior sections (data not shown).
(C') Magnification of the boxed region in (C). c1 appears as a mesenchymal condensation (M) between the OE and the nasal cavity wall (N).
(D-F) All six turbinates in anterior (D), mid (E), and posterior (F) sections at E17.5.
(E') Magnification of the boxed region in (E). Condensed mesenchymal cells have differentiated into chondrocytes. N, nasal cavity wall; T, turbinate cartilage; M, mesenchyme.
(G) All six turbinates at P7.
(G') Magnification of the boxed region in (G). Cartilage have been mostly replaced by eosinophilic ossified bone. * indicates remaining hypertrophic chondrocytes. N, nasal cavity wall; T, ossified turbinate; M, mesenchyme.
(H) Diagram of a frontal section through the nasal cavity at perinatal stages, showing three layers of tissue: OE, mesenchyme (mes), and cartilage. Inset: zoom showing "neck" and "tip" regions of c1.
(I) H&E staining in anterior (1), mid-anterior (2), mid-posterior (3), and posterior (4) frontal sections through the mouse nasal cavity at P30. Individual turbinates (n1, c1, n2, c2, n3, n4) are outlined. OB, olfactory bulb.
(I') Diagram of a mid-sagittal adult mouse nasal cavity showing locations of the four sections in (I).
S, nasal septum. D, dorsal; M, medial. Scale bars, 100 μm (A-C, C', E', G'), 500 μm (D-G), 1 mm (H).

Figure S2. Related to Figure 2.
(A) $Fgf20^{GFP-Cre}$ expression and EdU incorporation in $Fgf20^{GFP-Cre/+}$ c1 OE at E17.5. OE overlying the "neck" and "tip" regions of the developing turbinate are outlined.
(B) Quantification of proliferating cells in c1 OE overlying "neck" and "tip" regions per 1000 μm^2 of OE.
(C,D) $Fgf20^{GFP-Cre}$ and OMP expression in anterior (C) and posterior (D) sections at E17.5. RE, respiratory epithelium. Arrowheads indicate negatively-curved "neck" OE.
(E) Linear plot of OMP$^+$ ORN and $Fgf20^+$ cell distribution along c1 OE (dashed outline in (C)) at E17.5. Length: 1.27 mm.
(F) Pde2a expression in c1 dorsal "neck" or cul-de-sac region at P30.
(G) $Fgf20^{GFP-Cre}$ and (lack of) Pde2a expression in c1 dorsal "neck" region at E17.5.
(H) $Fgf20^{GFP-Cre}$; $ROSA^{mTmG}$ lineage (mG) in the vomeronasal organ (VNO, outlined).
(I) $Fgf20^{GFP-Cre}$; $ROSA^{mTmG}$ lineage (mG) in the septal organ (SO, outlined).
Dashed line, epithelial-mesenchymal boundary. DAPI, nuclei. Scale bars, 500 μm (C, H), 100 μm (A, F, G). Error bars, mean ± SD.

Figure S3. Related to Figure 3
(A,B) H&E staining in anterior (A) and posterior (B) sections through the nasal cavity in control ($Fgf20^{GFP-Cre/+}$) and $Fgf20$-KO ($Fgf20^{GFP-Cre/βgal}$) mice at P0. S, nasal septum.
(A') Magnification of boxed region in (A). N, nasal cavity wall; T, turbinate cartilage; M, mesenchyme.
(C-F) Quantification of c1 cartilage volume (C), OE surface area (D), mesenchyme thickness (E), and OE thickness (F) at P0. n = 7 control, 8 $Fgf20$-KO, Student's t-test.
(G) $Fgf20^{GFP-Cre}$ and Sox2 expression in c1 at P0. BC, basal cells; Sus, sustentacular cells.

(H) OMP expression in c1 at P0.
(I) Quantification of c1 OMP$^+$ ORNs, Sus cells, and Sox2$^+$ BCs per 100 µm OE at P0. n = 4, Student's t-test.
(J) EdU incorporation in c1 OE Sox2$^+$ cells at E14.5. M, mesenchyme.
(K-M) Quantification of c1 OE thickness (K), Sox2$^+$ BCs per 100 µm (L), and percent of EdU-incorporating Sox2$^+$ BCs (M) at E14.5. n = 7, Student's t-test.
Dashed line, epithelial-mesenchymal boundary. DAPI, nuclei. Scale bars, 500 µm (A), 100 µm (A', G, H, J). Error bars, mean ± SD.

Figure S4. Related to Figure 3
(A-E) EdU incorporation in control (*Fgf20$^{GFP-Cre/+}$*) and *Fgf20*-KO (*Fgf20$^{GFP-Cre/βgal}$*) mice at E17.5.
(A) *Fgf20$^{GFP-Cre}$* expression and 1 h EdU incorporation in c1 at E17.5. N, nasal cavity wall; T, turbinate cartilage; M, mesenchyme.
(A') Magnification of boxed region in (A). M, mesenchyme. Dashed line, epithelial-mesenchymal boundary. Solid line, cartilage-mesenchyme boundary.
(B-E) Quantification of 1 h (n = 6) and 4 h (n = 3 control, 4 *Fgf20*-KO) EdU incorporation in c1 "neck" region mesenchymal cells (B), "tip" region mesenchymal cells (C), "neck" and "tip" chondrocytes (D), and FEP cells (E) at E17.5. Student's t-test.
(F-L) EdU incorporation and *Dusp6* in situ hybridization in control (*Fgf20$^{GFP-Cre/+}$*; *ROSA$^{rtTA/+}$*) and Fgf9-OA (*Fgf20$^{GFP-Cre/+}$*; *ROSA$^{rtTA/+}$*; TRE-Fgf9-IRES-eGfp) mice at E14.5.
(F) EdU incorporation in c1 condensed and diffuse mesenchymal cells at E14.5. Dashed outline, c1 mesenchyme (M), including both condensed (Sox9^{hi}) and diffuse cells. N, nasal cavity wall. Brackets, "OE adjacent cells" within 69 µm below OE.
(G) In situ hybridization at E14.5 showing c1 mesenchymal *Dusp6* expression in control and increased expression in Fgf9-OA embryos (arrows). Data is representative of 4 control and 4 Fgf9-OA embryos. N, nasal cavity wall.
(H-L) Quantification of c1 normalized condensed cell number (H), normalized diffuse cell number (I), ratio of condensed cells to diffuse cells (J), and percent of EdU-incorporating condensed cells (K) and EdU-incorporating diffuse cells (L) at E14.5. n = 4 control and 6 Fgf9-OA, Student's t-test.
DAPI, nuclei. Scale bars, 100 µm. Error bars, mean ± SD.

Figure S5. Related to Figure 5
(A) *Fgf20$^{GFP-Cre}$* and Sox2 expression in control (*Fgf20$^{GFP-Cre/+}$*; *βCat$^{fl(ex2-6)/+}$*), βFF-CKO (*Fgf20$^{GFP-Cre/+}$*; *βCat$^{fl(ex2-6)/fl(ex2-6)}$*), and βDF-CKO (*Fgf20$^{GFP-Cre/+}$*; *βCat$^{DM/fl(ex2-6)}$*) mice at P0, with two βFF-CKO phenotype examples (shapes 2 and 3). S, nasal septum. Inset, 2x magnification of the boxed region; arrows indicate presence of FEP cells (*Fgf20* and Sox2 double-positive in control; Sox2 single-positive in βFF-CKO, shape 2).
(B) Left: H&E staining in control, βFF-CKO, and βDF-CKO mice at P30. S, nasal septum. Right: 10x magnification of boxed regions showing OMP expression on an adjacent frontal section from the same sample. Region a, magnification of c1 "tip" OE; region b, magnification of c1 "neck" OE. Arrowhead indicates area with extremely thin or absent OE, as indicated by lack of OMP expression. Ax, axon bundles. Dashed line, epithelial-mesenchymal boundary.
(C) In situ hybridization at E14.5 showing c1 mesenchymal *Dusp6* expression in control and loss of in βFF-CKO embryos (arrows). Data is representative of 4 control and 6 βFF-CKO embryos. N, nasal cavity wall.
(D) H&E staining in control (*Fgf20$^{GFP-Cre/+}$*; *βCat$^{fl(ex2-6)/+}$*) and βDF-CKO (*Fgf20$^{GFP-Cre/+}$*; *βCat$^{DM/fl(ex2-6)}$*) mice at P0. S, nasal septum.
(D') Magnification of boxed region in (D). N, nasal cavity wall; T, turbinate cartilage; M, mesenchyme. Inset, 2x magnification of the OE at the turbinate tip. Dashed line, epithelial-mesenchymal boundary.

(E-G)   Quantification of c1 cartilage volume (E, $p < 0.001$, one-way ANOVA), surface area (F, $p < 0.001$, one-way ANOVA), and mesenchyme thickness (G, $p = 0.04$, one-way ANOVA) of $Fgf20^{GFP-Cre/+}$; $\beta Cat^{+/+}$ (n = 7), $Fgf20^{GFP-Cre/+}$; $\beta Cat^{DM/+}$ (n = 5), $Fgf20^{GFP-Cre/+}$; $\beta Cat^{fl(ex2-6)/+}$ (n = 5), and $Fgf20^{GFP-Cre/+}$; $\beta Cat^{DM/fl(ex2-6)}$ (n = 7) mice at P0. * indicates statistically significant at $\alpha = 0.05$ by Tukey's HSD.

DAPI, nuclei. Scale bars, 1 mm (B), 500 µm (A, D), 100 µm (C, D'). Error bars, mean ± SD.

Figure S6. Related to Figure 6

(A)   Sox2 expression in c1 of control ($Fgf20^{GFP-Cre/+}$; $\beta Cat^{fl(ex2-6)/+}$) and βDF-CKO ($Fgf20^{GFP-Cre/+}$; $\beta Cat^{DM/fl(ex2-6)}$) mice at P0. Arrows indicate presence of FEP cells in control mice. Arrowheads indicate absence of FEP cells in βDF-CKO mice. BC, basal cells; Sus, sustentacular cells.

(B)   Quantification of c1 OE thickness in $Fgf20^{GFP-Cre/+}$; $\beta Cat^{+/+}$ (n = 7), $Fgf20^{GFP-Cre/+}$; $\beta Cat^{DM/+}$ (n = 5), $Fgf20^{GFP-Cre/+}$; $\beta Cat^{fl(ex2-6)/+}$ (n = 5), and $Fgf20^{GFP-Cre/+}$; $\beta Cat^{DM/fl(ex2-6)}$ (n = 7) mice at P0. $p < 0.001$, one-way ANOVA. * indicates statistically significant at $\alpha = 0.05$ by Tukey's HSD.

(C)   OMP expression in c1 at P0.

(D)   Quantification of c1 OMP[+] ORNs (n = 4), Sus cells (n = 4 control, 5 βDF-CKO), and Sox2^{+} BCs (n = 4 control, 5 βDF-CKO) per 100 µm OE at P0. Student's t-test.

(E)   EdU incorporation in c1 OE Sox2^{+} cells at E17.5. Arrow indicates presence of FEP cells. T, turbinate cartilage; M, mesenchyme.

(F-H)   Quantification of c1 OE thickness (F), Sox2^{+} BCs per 100 µm OE (G), and percent of EdU-incorporating Sox2^{+} BCs (H) at E17.5. n = 3, Student's t-test.

(I) $Fgf20^{GFP-Cre}$ and OMP expression in c1 at E17.5, with linear plot of OMP[+] ORN and $Fgf20^+$ cell distribution along the c1 OE (dashed outline). Distribution plot length: 1.27 mm, control; 0.787 mm, βDF-CKO (average of n = 3). Arrows indicate $Fgf20$ expression. Arrowheads indicate c1 "neck" region OE. N, "neck"; T "tip".

Dashed line, epithelial-mesenchymal boundary. DAPI, nuclei. Scale bars, 100 µm. Error bars, mean ± SD.

Figure S7. Related to Figure 7

(A) $Fgf20^{GFP-Cre}$ expression without anti-GFP antibody staining in c1 in control ($Fgf20^{GFP-Cre/+}$; $\beta Cat^{+/+}$) and βEX3-OA ($Fgf20^{GFP-Cre/+}$; $\beta Cat^{fl(ex3)/+}$) mice at E17.5. N, nasal cavity wall; T, turbinate cartilage; M, mesenchyme. Arrowheads indicate faint $Fgf20^{GFP-Cre}$ expression in control. Arrows indicate duct structures in the mesenchyme. Inset, 3x magnification of boxed region. Note: in all other figures except S7A, GFP-Cre expression was detected with an anti-GFP antibody (including S7B, S7C, and S7E).

(B) $Fgf20^{GFP-Cre}$ expression and EdU incorporation in c1 at E17.5. Dashed outline, epithelial-mesenchymal boundary in control, FEP cell clumps in βEX3-OA. Solid line, cartilage-mesenchyme boundary. N, nasal cavity wall; T, turbinate cartilage; M, mesenchyme.

(C) $Fgf20^{GFP-Cre}$ and Sox2 expression in dorsomedial n2 at E17.5. BC, basal cells; Sus, sustentacular cells.

(D)   Sox9 and OMP expression in dorsomedial n2 at E17.5. Note: high Sox9 expression was also found in Bowman's gland and duct cells, which can be seen in the mesenchymal layer and OE, respectively (Packard et al., 2011).

(E) $Fgf20^{GFP-Cre}$ expression and EdU incorporation in c1 at E13.5. M, mesenchyme. Arrowheads indicate a dense clump of FEP cells.

(F-H)   Quantification of c1 OE thickness (F), $Fgf20^+$ cells per 100 µm² of OE (G), and percent of EdU-incorporating $Fgf20^+$ cells (H) at E13.5. n = 4, Student's t-test.

DAPI, nuclei. Dashed line, epithelial-mesenchymal boundary. Scale bars, 100 µm. Error bars, mean ± SD.

A E10.5

NP

B E12.5

n1

n2

*

VNO

C E14.5

c1

n1

n2

C'

N

M

OE

D E17.5 Anterior

n1

c1

n2'

n2"

n3

S

E Mid

n1

c1

n2

c2

n3

S

F Posterior

c1

n2

c2

n3

n4

S

E'

N

T

M

OE

G P7

n1

c1

n2

c2

n3

n4

S

G'

N

*

M

OE

T

H E17.5

n1

c1

n2

S

c2

n3

n4

D

M

OE
Mes
Cartilage

"neck" "tip"

I

1

S

2

S

3

S

4

OB

P30

I'

Turbinates

1 2 3 4

OB

● c1
● c2

● n1
● n2
● n3
● n4

A E17.5
GFP-Cre EdU DAPI
"Neck" "Tip"

B
Proliferative cells/1000 μm^2
"Neck" "Tip"

C E17.5
GFP-Cre OMP DAPI
n1 c1 n2 c2 RE D M

D
c2 n3 n4

E
OMP GFP
c1 "Neck" "Tip" "Neck"

F P30
Pde2a DAPI

G E17.5
GFP-Cre Pde2a DAPI

H P30
mG mT DAPI
VNO

I
SO

A Control P0 — n1, c1, c2, n2, n3, S

A *Fgf20*-KO — n1, c1, n2, n3, S

B Control — c1, n2, c2, n3, n4, S

B *Fgf20*-KO — c1, n2, c2, n3, n4, S

A' Control — N, T, M OE

A' *Fgf20*-KO — N, T, M OE

C Cartilage volume (mm$^3 \cdot 10^{-3}$)
p = 0.009

D OE surface area (mm^2)
p < 0.001

E Mesenchyme thickness (μm)
p = 0.9

F OE thickness (μm)
p = 0.4

G Control P0 — Sus, BC; *Fgf20*-KO — Sus, BC
GFP-Cre Sox2 DAPI

H Control; *Fgf20*-KO
OMP DAPI

I Cells/100 μm
- Control
- *Fgf20*-KO
OMP$^+$ ORNs — p = 0.06
Sus cells — p = 0.2
Sox2$^+$ BCs — p = 0.96

J Control E14.5 — M, OE; *Fgf20*-KO — M, OE
Sox2 EdU DAPI

K OE thickness (μm)
p = 0.04

L Sox2$^+$ BCs/100 μm
p = 0.02

M EdU$^+$ Sox2$^+$ BCs (%)
p = 0.8

A

A'

Control

Fgf20-KO

N   M   OE

T

E17.5

M

M

GFP-Cre   EdU   DAPI

B

EdU$^+$ "neck" mesenchymal cells (%)

p = 0.03

p = 0.003

1 h EdU   4 h EdU

C

EdU$^+$ "tip" mesenchymal cells (%)

p = 0.4

p = 0.2

1 h EdU   4 h EdU

● Control   ● *Fgf20*-KO

D

EdU$^+$ chondrocytes (%)

p = 0.3   p = 0.9

1 h EdU   4 h EdU

E

EdU$^+$ FEP cells (%)

p = 0.8   p = 0.6

1 h EdU   4 h EdU

F

Control

Fgf9-OA

N   M   OE

E14.5

N   M   OE

Sox9   EdU   DAPI

G

N   OE

N   OE

Dusp6

H

Condensed cells (normalized)

p = 0.1

I

Diffuse cells (normalized)

p = 0.01

J

Condensed/ diffuse cell ratio

p = 0.002

K

EdU$^+$ condensed cells (%)

p = 0.3

L

EdU$^+$ diffuse cells (%)

p = 0.4

p = 0.02

● Control   ● Fgf9-OA   ● OE adjacent cells

A Control | ßFF-CKO shape 2 | ßFF-CKO shape 3 | ßDF-CKO

GFP-Cre Sox2 DAPI

B Control | ßFF-CKO | ßDF-CKO

OMP DAPI

C Control / ßFF-CKO

Dusp6

D Control | ßDF-CKO

E Cartilage volume (mm^3·10^{-3})

Fgf20$^{GFP-Cre/+}$;ßCat$^{+/+}$
Fgf20$^{GFP-Cre/+}$;ßCat$^{DM/+}$
Fgf20$^{GFP-Cre/+}$;ßCat$^{fl(ex2-6)/+}$
Fgf20$^{GFP-Cre/+}$;ßCat$^{DM/fl(ex2-6)}$

F OE Surface area (mm^2)

G Mesenchyme thickness (μm)

A Control | ßDF-CKO
Sus
BC
Sus
BC
P0
Sox2 DAPI

B OE thickness (µm)
100
80
60
40
20
0
*

Fgf20^{GFP-Cre/+};
ßCat^{+/+}

Fgf20^{GFP-Cre/+};
ßCat^{DM/+}

Fgf20^{GFP-Cre/+};
ßCat^{fl(ex2-6)/+}

Fgf20^{GFP-Cre/+};
ßCat^{DM/fl(ex2-6)}

C Control | ßDF-CKO
P0
OMP DAPI

D
50
40
30
20
10
0
Cells/100 µm
p = 0.2
p = 0.3
p = 0.03
OMP+ ORNs | Sus cells | Sox2+ BCs

E Control | ßDF-CKO
E17.5
M OE | M OE
T | T
Sox2 EdU DAPI

F OE thickness (µm)
100
80
60
40
20
0
p = 0.04

G Sox2+ BCs/ 100 µm
30
20
10
0
p = 0.3

H EdU+ Sox2+ BCs (%)
80
60
40
20
0
p = 0.01

I Control | ßDF-CKO
E17.5
OMP GFP | OMP GFP
N | N
T | T
N | N
n = 3 | n = 3
GFP-Cre OMP DAPI

Yang et al., Figure S6

A — Control | ßEX3-OA — E17.5 — GFP-Cre (no ab) | DAPI — N, M, T, OE

B — Control | ßEX3-OA — E17.5 — GFP-Cre | EdU | DAPI — N, M, T, OE

C — Control | ßEX3-OA — E17.5 — Sus, BC, n2 — GFP-Cre | Sox2 | DAPI

D — Control | ßEX3-OA — E17.5 — n2 — Sox9 | OMP | DAPI

E — Control | ßEX3-OA — E13.5 — M, OE — GFP-Cre | EdU | DAPI

F OE thickness (µm) — p = 0.004

G *Fgf20*+ cells/ 100 µm^2 — p = 0.02

H Edu+ *Fgf20*+ cells (%) — p = 0.7

Control ßEX3-OA