

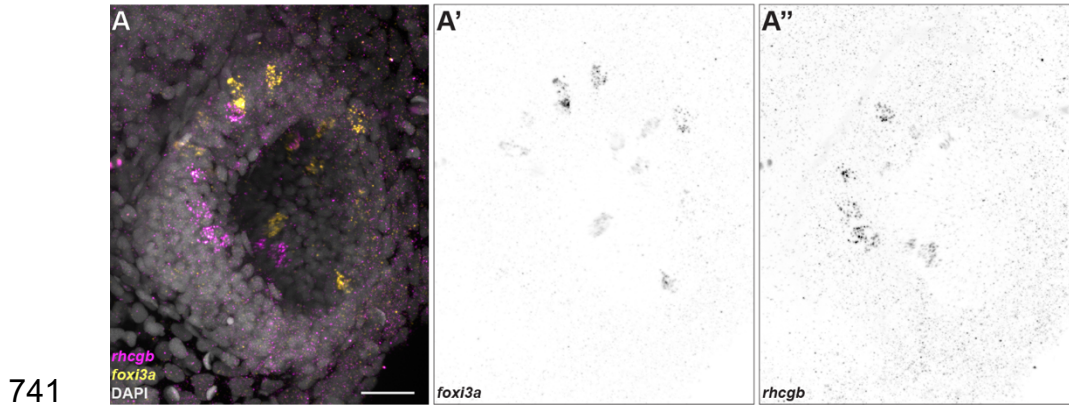
733

734 **Figure S1, related to Figure 1**

735 **(A)** Heatmap showing top 5 highest expressing genes based on LogFC on the
736 clusters obtained in the dataset from dissected adult zebrafish olfactory organs. **(B)**
737 Feature plots of *trpc2b* and **(C)** *ompb*. **(D)** Violin plot showing the percentage of
738 mitochondrial genes in the dataset.

739

740



742 **Figure S2, related to Figure 2**

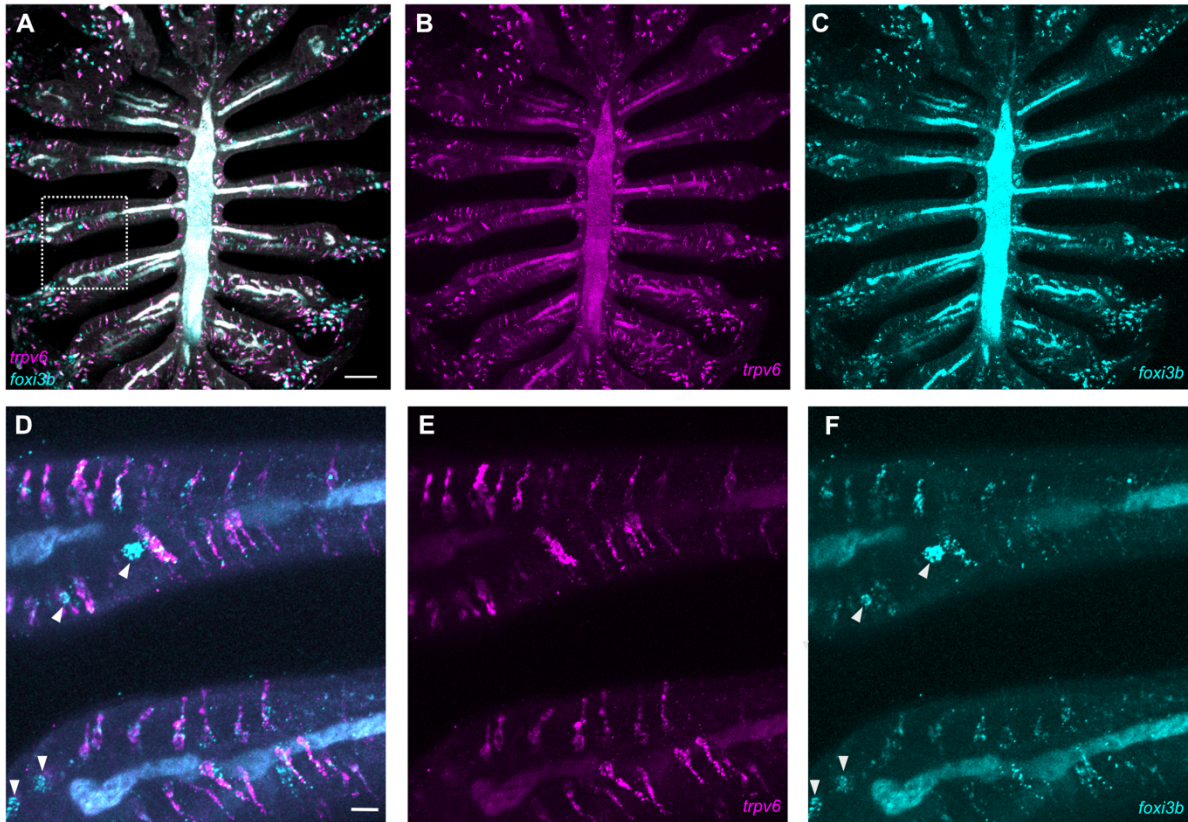
743 (A) Maximum intensity projection of a confocal image showing HCR RNA-FISH
744 signals for *foxi3a* (yellow) and *rhcgb* (magenta) with DAPI stain (grey). (A') Individual
745 channels for *foxi3a* and (A'') *rhcgb*. Scale bar: 20 μm.

746

747

748

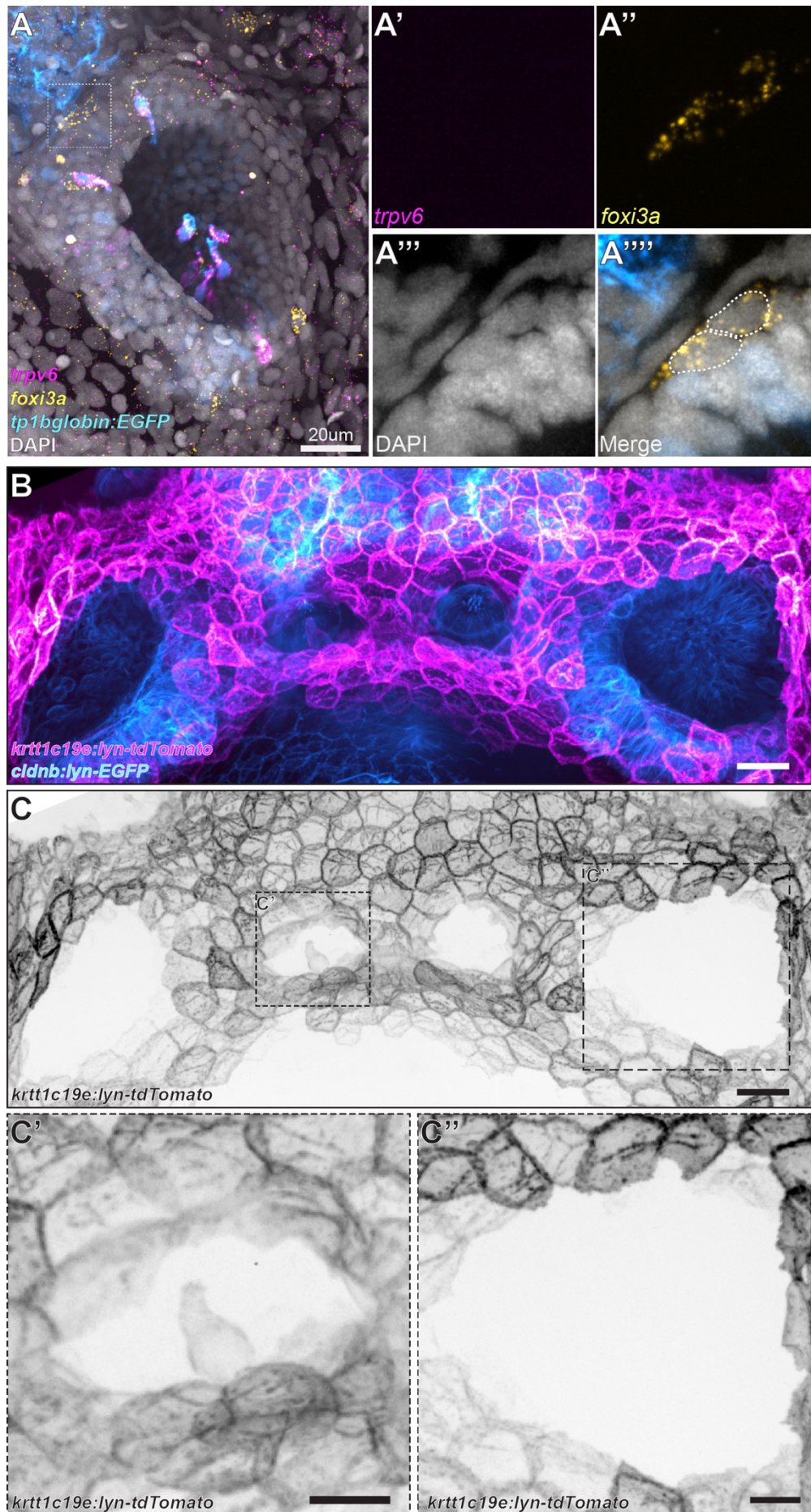
749



750

751 **Figure S3, related to Figure 3**

752 **(A-C)** Overview of an adult olfactory rosette, showing expression of *trpv6* **(B)** and
753 *foxi3b* **(C)**. **(D-F)** High magnification view of the region outlined in panel A. The
754 arrowheads indicate solitary NCC-like ionocytes, which express *foxi3b* and have a
755 rounded shape. These are distinct from elongated pairs of cells that express either
756 *foxi3b* or *trpv6*. Scale bar: 50 μ m in panel A, 20 μ m in panel D.



757

758 **Figure S4, related to Figure 4**

759 **(A–A''')** *foxi3a*⁺ cells (yellow) at the edge of an olfactory pit, as shown by HCR
760 RNA-FISH. **(B)** Maximum intensity projection of a confocal image from *Tg(-*
761 *8.0cldnb:lyn-EGFP)^{zf106Tg};Tg(krtt1c19e:lyn-tdTomato)^{sq16}* transgenic larva shows a
762 pair of Nm ionocytes in the neuromast, but no tdTomato⁺ cells in the olfactory pit. **(C)**
763 Single channel image of *krtt1c19e:lyn-tdTomato*. **(C')** Enlargement of a neuromast
764 containing tdTomato⁺ Nm ionocytes. **(C'')** Enlargement of an olfactory pit containing
765 no tdTomato⁺ cells.

766 **Supplementary Material**

767 **Supplementary Table 1. Differentially expressed genes in dissected adult**

768 **zebrafish olfactory organ scRNA-seq dataset.** This spreadsheet contains the
769 differentially expressed gene list (cluster markers) of olfactory cell types from the
770 integrated adult dataset via Seurat::FindAllMarkers function with default parameters.
771 Ionocytes are on cluster 18.

772 **Supplementary Table 2. Differentially expressed genes in larval 5 dpf olfactory**

773 **cell subset.** This spreadsheet contains the differentially expressed gene list (cluster
774 markers) of olfactory cell types from the subsetted larval dataset via
775 Seurat::FindAllMarkers function with default parameters.

776 **Movie 1. 3D rendering of an embryonic olfactory pit depicting HR- and NaR-like**

777 **ionocytes.** HCR RNA-FISH for *trpv6* (magenta) and *foxi3a* (yellow), combined with
778 the Notch reporter *tp1bglobin:EGFP* (cyan), shows spatial distribution of ionocyte
779 pairs in the larval 5 dpf olfactory pit. Initial image is a frontal view of the left olfactory
780 pit, with dorsal to the top and lateral to the right.

781 **Movie 2. 3D rendering of an embryonic olfactory pit showing NCC-like**

782 **ionocytes.** HCR RNA-FISH for *slc12a10.2* (yellow) and *foxi3b* (magenta), combined
783 with the Notch reporter *tp1bglobin:EGFP* (cyan), showing the spatial distribution of
784 ionocyte pairs in the larval 5 dpf olfactory pit. Initial image is a frontal view of the left
785 olfactory pit, with dorsal to the top and lateral to the right.

786 **Movie 3. New olfactory ionocytes do not express skin transgenes.** Time lapse

787 from *Tg(-8.0cldnb:lyn-EGFP)^{zf106Tg};Tg(krtt1c19e:lyn-tdTomato)^{sq16}* transgenic

788 zebrafish larva showing pairs of tdTomato⁺ Nm ionocytes (arrow, middle panel)

789 invading neuromasts, but no positive cells in the olfactory epithelium.

790 **Movie 4. Differentiation of ionocyte pairs in a larval olfactory pit and**

791 **neuromast.** Time lapse from a 3 dpf transgenic zebrafish larva (*Tg(dld:hist2h2l-*

792 *EGFP)^{psi84}*) showing a pair of ionocytes (cyan and yellow dots; visible at the start of

793 the recording) invading the neuromast. At ~90 hours ionocytes are visible in the

794 olfactory pit (red and cyan dots). No invasion was detected.

795 **Movie 5. Ionocyte pair development in a larval olfactory pit.** Time lapse from a 3

796 dpf transgenic zebrafish larva (*Tg(dld:hist2h2l-EGFP)^{psi84}*) showing the appearance

797 of a pair of olfactory ionocytes (red and cyan dots). The pair is visible at

798 approximately 85 hours, and move around together.

799 **Movie 6. 3D reconstruction of an HR-like/NaR-like ionocyte pair from a 7 dpf**

800 **wild-type olfactory pit.** 360° rotation of Fig. 6A. Red, HR-like cell; cyan, NaR-like

801 cell.

802 **Movie 7. 3D reconstruction of the tight junctions of an HR-like/NaR-like**

803 **ionocyte pair.** 360° rotation of Fig. 6G; compare to Movie 9. Green, shallow tight

804 junction between the two ionocytes; yellow, deep tight junction between NaR-like

805 ionocyte and olfactory supporting cell; orange, deep tight junction between NaR-like

806 ionocyte and multiciliated cell; blue, deep tight junction between HR-like ionocyte

807 and olfactory supporting cell.

808 **Movie 8. 3D reconstruction of a 3-cell ionocyte complex from a 7 dpf wild-type**

809 **olfactory pit.** 360° rotation of Fig. 6L. Red, HR-like cell; cyan, NaR-like cell; dark

810 blue, possible second NaR-like cell; yellow, ciliated OSN (not part of the ionocyte
811 complex, but included for context and scale).

812 **Movie 9. 3D reconstruction of the tight junctions between the HR-like (red) and**
813 **NaR-like (cyan) ionocytes in Fig. 6L.** 360° rotation of Fig. 6P; compare to Movie 7.
814 Green, shallow tight junction between the two ionocytes; yellow, deep tight junction
815 between NaR-like ionocyte (cyan) and olfactory supporting cell; blue, deep tight
816 junction between HR-like ionocyte (red) and olfactory supporting cell.