Supplementary Information

Adaptive cell invasion maintains lateral line organ homeostasis in response to environmental changes

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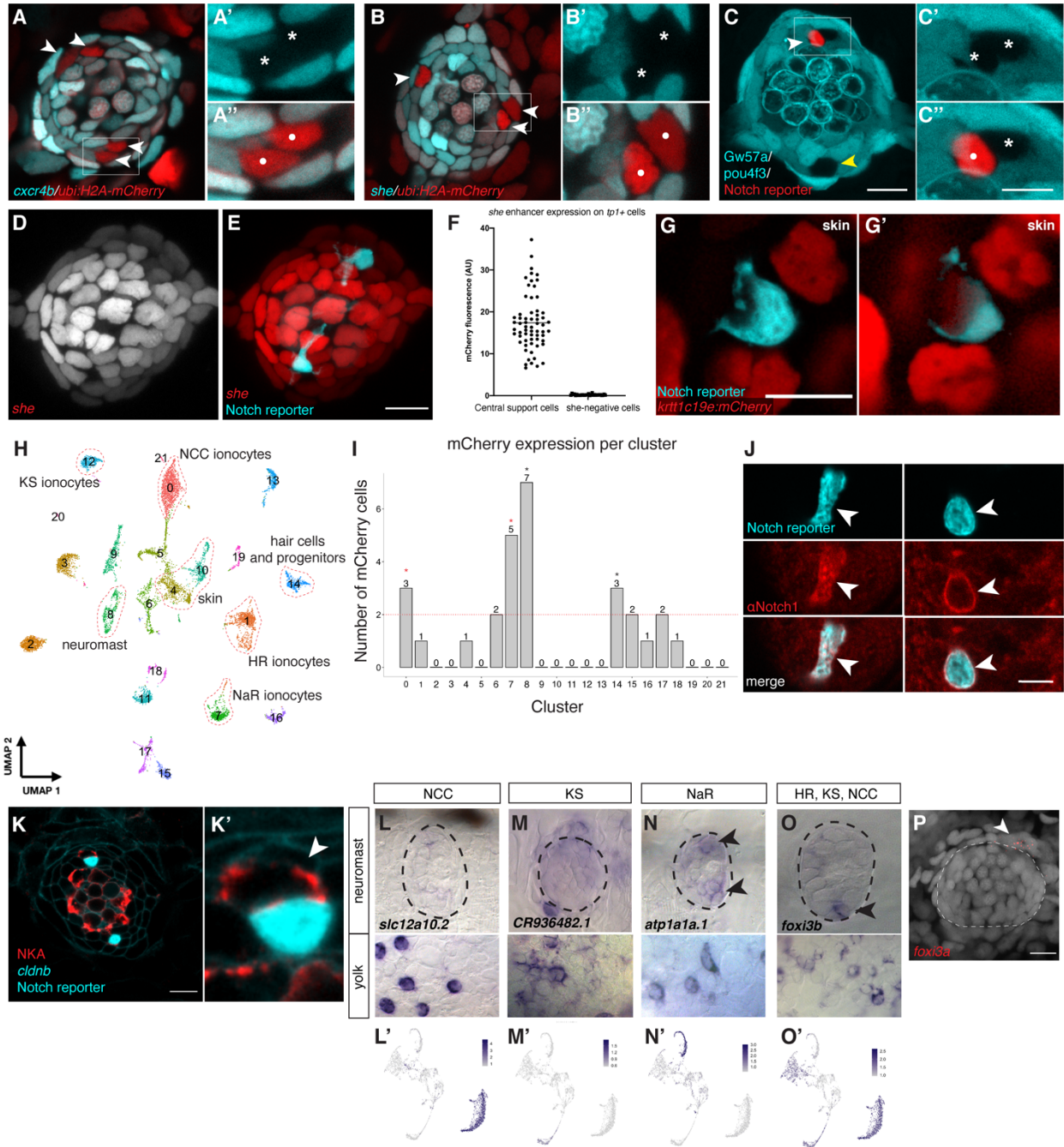*Correspondence: pio@stowers.org

Figure S1

Fig. S1: Non-lateral line cells do not express neuromast markers but share molecular features with ionocyte subpopulations, Related to Figure 1.

(A), (A') and (A'') Neuromast of a 5 dpf *cxcr4b-H2B-EGFP;ubi:H2A-mCherry* larva with two pairs of cells (A, arrowheads; A'', white dots) not labeled by the neuromast marker *cxcr4b* (A', asterisks). Boxed area in (A) is shown at higher magnification in (A') and (A''). **(B), (B') and (B'')** Neuromast of a 4 dpf *she:H2B-EGFP;ubi:H2A-mCherry* larva containing one cell and one pair of cells (B, arrowheads; B'', white dots) that are not labeled by the neuromast transgenic '*she*' line

(B', asterisks). Boxed area is shown at higher magnification in (B') and (B''). **(C), (C') and (C'')** Neuromast of a 5 dpf *Gw57a:EGFP;pou4f3:GFP;tp1bglobin:mCherry* larva with gaps in the fluorescence (C, C', asterisks). One gap contains a cell labeled by the Notch reporter (C, white arrowhead; (C''), white dot), whereas the other gap does not contain a Notch+ cell due to mosaicism of the Notch reporter line (C, yellow arrowhead). Boxed area is shown in higher magnification in (C') and (C''). Scale bar of overview image equals 10 μm, scale bar in magnified image equals 5 μm. **(D)** Lateral line neuromast labeled by *she:H2A-mCherry.*

(E) Merged image from (D) with *tp1bglobin:EGFP.* **(F)** Quantification of fluorescence intensity of all Notch+ cells in the neuromast shows that Notch+ cells in the poles do not express *she:H2A-mCherry*. **(G) and (G')** Two individual z-slices of the double transgenic *krtt1c19e:H2A-mCherry;tp1bglobin:EGFP* shows a rare pair of Notch+ and Notch- cell in the skin. **(H)** UMAP of all clusters. Highlighted clusters represent different ionocyte populations, skin and lateral line cells. **(I)** *tp1bglobin*:mCherry expression in clusters of (H). 35% of mCherry cells are in central support cells (clusters 8 and 14, black asterisks), and 29% of mCherry cells are in ionocyte clusters (0 and 7, red asterisk). Remaining cells were dispersed in other clusters. **(J)** Notch+ (*tp1bglobin:EGFP)* skin cells are labeled by an anti-Notch1 antibody (arrowheads). Two z-slices of a confocal z-stack depicting the apical extension (left panel) and the cell body (right panel). Scale bar equals 5 μm. **(K) (K')** Na$^+$/K$^+$ ATPase (NKA) immunohistochemistry labels the Notch- cell and hair cells. **(L)** *In situ* hybridization of the NCC ionocyte cluster marker *slc12a10.2*. No staining is detectable in the neuromast (upper panel), but ionocytes on the yolk are labeled (lower panel). **(L')** Feature plot of *slc12a10.2.* **(M)** *In situ* hybridization of the KS ionocyte cluster marker *CR936482.1*. No strong staining is detectable in the neuromast (upper panel), but cells outside of the neuromast and ionocytes on the yolk are labeled (lower panel). **(M')** Feature plot of *CR936482.* **(N)** *In situ* hybridization of the NaR ionocyte cluster marker *atp1a1a.1*. Weak staining is detectable in neuromast poles (upper panel, arrowheads), and in presumed NaR ionocytes on the yolk (lower panel). **(N')** Feature plot of *atp1a1a.1.* **(O)** *In situ* hybridization of the ionocyte marker *foxi3b* shows expression in the ventral pole of the neuromast (arrowhead). **(O')** Feature plot of *foxi3b*. **(P)** HCR shows a *foxi3a*+ cell in the neuromast vicinity (arrowhead).
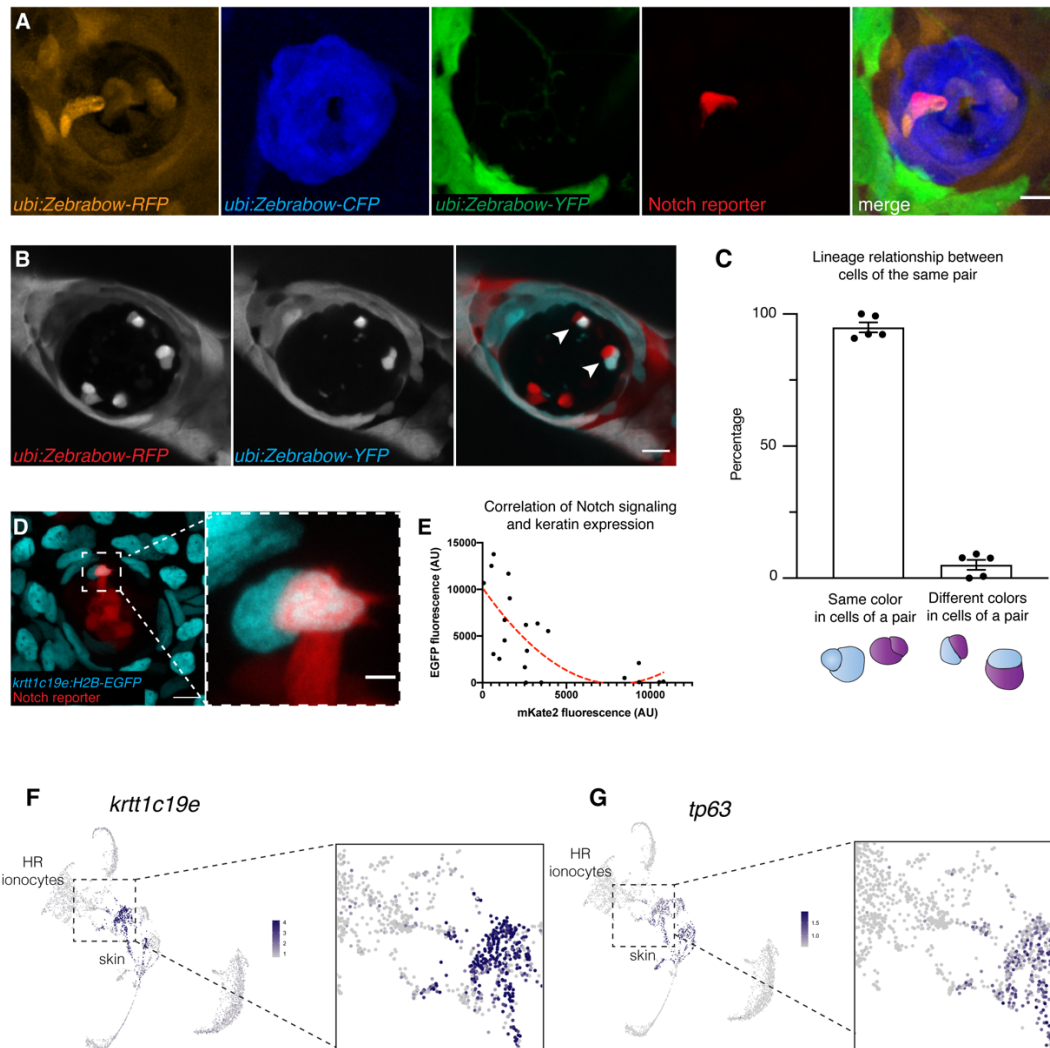
Figure S2

Fig. S2: Nm ionocytes are derived from basal keratinocytes, Related to Figure 2.
(A) A neuromast in a 3-week old *ubi:Zebrabow;ubi:cre^{ERt2};tp1bglobin:mKate2* larva shows that the cells with a different color hue than neuromast cells are the Notch+ cells. **(B)** Neuromast of 2-month old fish shows some cell pairs are composed of cells of different color hues, suggesting they did not originate from the same progenitor cell or the progenitor cell divided before Cre-induced recombination. **(C)** Ratio of ionocyte pairs that consist of cells of same or different color hues (*n* = 5 fish, 194 neuromasts). Error bars indicate SEM. **(D)** Neuromasts in a *tp1bglobin:mKate2;krtt1c19e:H2B-EGFP* larva show a pair of skin-derived cells in the neuromast, one of which is also labeled by the Notch reporter. **(E)** Inverse correlation (quadratic) between *krtt1c19e*-driven EGFP and *tp1bglobin-driven* mKate2 fluorescence suggests skin genes, such as *krtt1c19e* are turned off as Notch signaling increases. Adjusted R-squared: 0.4416 (*n* = 10 fish). **(F-G)** Feature plots for *krtt1c19e* (F) and *tp63* (G) show a possible differentiation trajectory from skin cells to HR ionocytes.
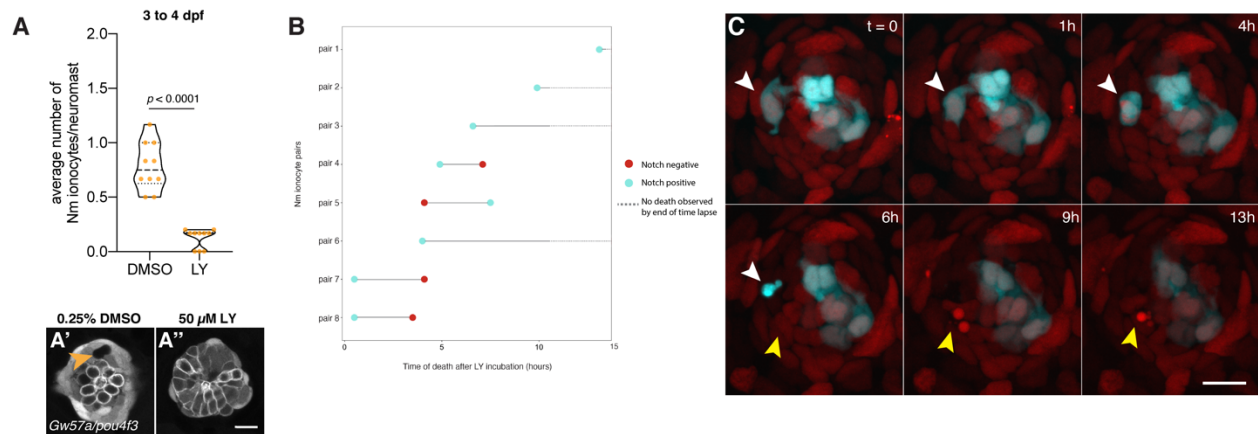
Figure S3

Fig S3: Notch signaling affects Nm ionocyte survival, Related to Figure 3.
(A) Nm ionocyte frequency following inhibition of Notch signaling with 50 µM LY411575 (n = 10 larvae, 60 neuromasts) for 24 h between 3 and 4 dpf compared to DMSO controls (n = 10 larvae, 60 neuromasts; Mann-Whitney test, p < 0.0001). **(B)** Lolipop plot of time lapse recording of eight Nm ionocyte pairs shows Notch+ cells usually die before the Notch- Nm ionocyte after inhibition of Notch signaling (p = 0.023, paired t-test). In four cases the Notch- Nm ionocyte did not undergo cell death by the end of time lapse (15h post LY) **(C)** Maximum intensity projection of a time lapse recording of *ubi:H2A-mCherry; tp1bglobin:EGFP* neuromasts showing the death of one Notch+ Nm ionocyte and an adjacent cell. See also Supplementary Video 9.

Figure S4
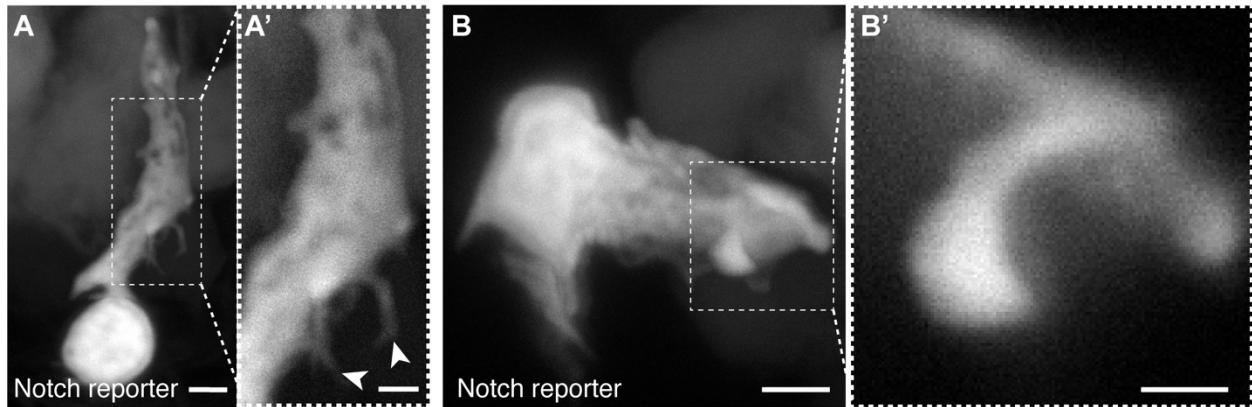
Fig. S4: Nm ionocytes display multiple thin cellular protrusions and a pronounced apical extension, Related to Figure 4.
Maximum intensity projection of the apical extension formed by Notch+ cells (*tp1bglobin:EGFP)*. **(A)** multiple thin cellular protrusions. **(A')** Magnification of boxed area in (A) with white arrowheads pointing at protrusions. **(B)** depicts the most apical part of the extension of the Notch+ cell. **(B')** magnification of the boxed area in (B). Scale bars in overview images = 2 µm, in magnified images = 1 µm. Images were gamma-adjusted.

Figure S5

A total neuromast cell number — MilliQ, 0.5x E2, 5x E2
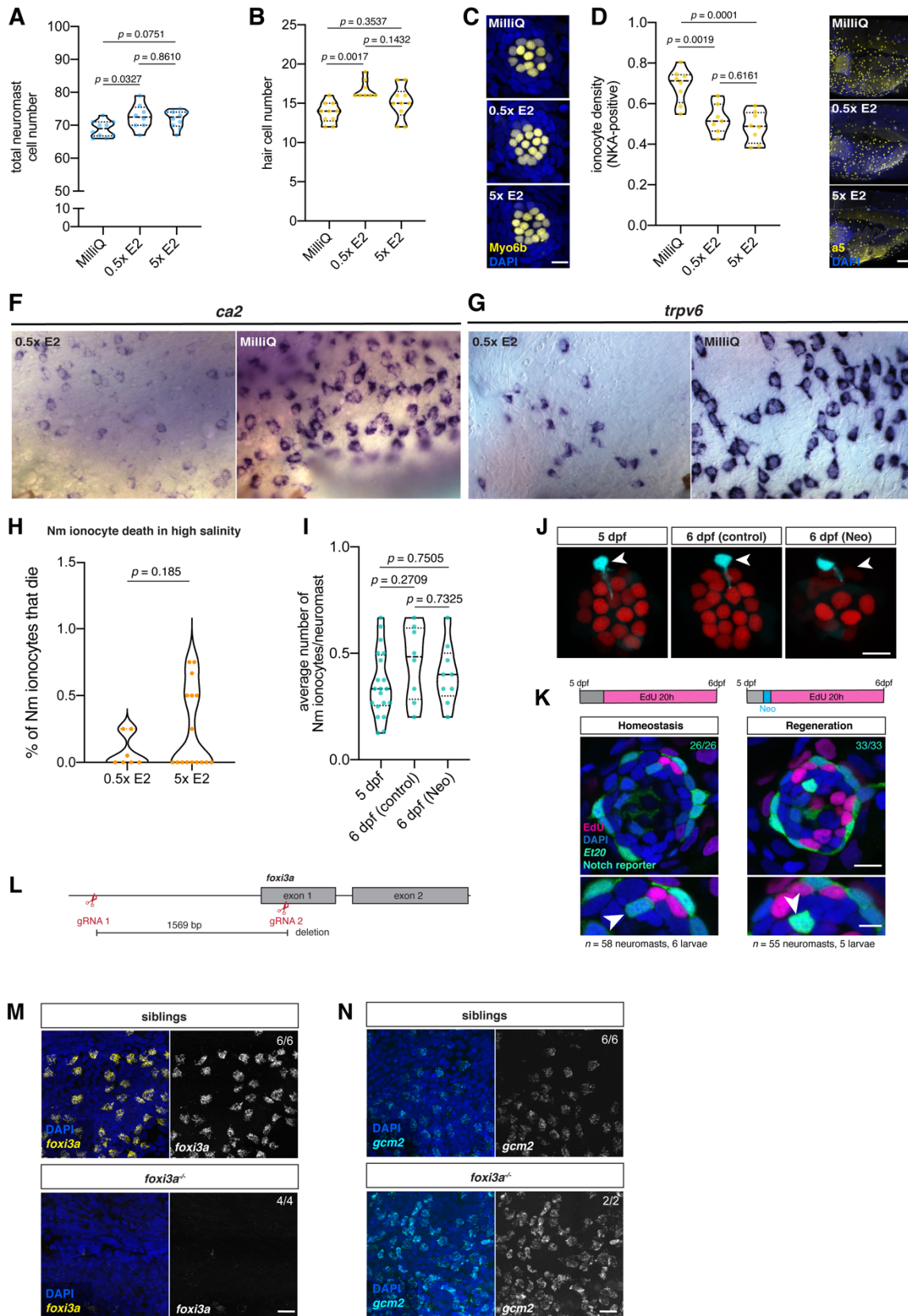p = 0.0751
p = 0.8610
p = 0.0327

B hair cell number — MilliQ, 0.5x E2, 5x E2
p = 0.3537
p = 0.1432
p = 0.0017

C MilliQ / 0.5x E2 / 5x E2
Myo6b
DAPI

D ionocyte density (NKA-positive) — MilliQ, 0.5x E2, 5x E2
p = 0.0001
p = 0.0019
p = 0.6161
MilliQ / 0.5x E2 / 5x E2
a5
DAPI

F *ca2*
0.5x E2 | MilliQ

G *trpv6*
0.5x E2 | MilliQ

H Nm ionocyte death in high salinity
% of Nm ionocytes that die — 0.5x E2, 5x E2
p = 0.185

I average number of Nm ionocytes/neuromast — 5 dpf, 6 dpf (control), 6 dpf (Neo)
p = 0.7505
p = 0.2709
p = 0.7325

J 5 dpf | 6 dpf (control) | 6 dpf (Neo)

K EdU 20h (5 dpf → 6dpf) | Neo, EdU 20h (5 dpf → 6dpf)
Homeostasis | Regeneration
26/26 | 33/33
EdU
DAPI
Et20
Notch reporter
n = 58 neuromasts, 6 larvae | n = 55 neuromasts, 5 larvae

L *foxi3a*
gRNA 1 — 1569 bp — gRNA 2
exon 1 | exon 2
deletion

M siblings
DAPI / *foxi3a* | *foxi3a* — 6/6
foxi3a⁻/⁻
DAPI / *foxi3a* | *foxi3a* — 4/4

N siblings
DAPI / *gcm2* | *gcm2* — 6/6
foxi3a⁻/⁻
DAPI / *gcm2* | *gcm2* — 2/2

Fig. S5: Salinity changes lead to tissue-specific phenotypes, Related to Figure 5.
(A) Incubation of larvae in MilliQ water (n = 5 fish, 10 neuromasts), but not 5x E2 (n = 5 fish, 10 neuromasts) from 3 dpf to 5 dpf significantly decreases the total neuromast cell number based on DAPI staining, compared to standard 0.5 x E2 media (n = 4 fish, 8 neuromasts; One-Way ANOVA with Tukey's post hoc test). **(B)** Incubation of larvae in MilliQ water but not 5x E2 from 3 dpf to 5 dpf significantly decreases hair cell numbers compared to standard 0.5 x E2 media (n = 4 fish, 8 neuromasts; Kruskal-Wallis ANOVA with Dunn's post hoc test). **(C)** Images of hair cell numbers in *Myo6b:H2B-Scarlet-I* larvae after incubation in media with different salinities, quantified in (B). Scale bar equals 10 μm. **(D)** The density of yolk ionocytes detected with an anti-Na$^+$-K$^+$-ATPase (NKA) antibody increases after incubation in MilliQ water (n = 8 fish) but does not decrease significantly in 0.5x E2 (n = 7 fish) compared to 5x E2 media (n = 8 fish; One-Way ANOVA with Tukey's post hoc test). **(E)** NKA+ ionocytes on the yolk in conditions quantified in (D). Scale bar equals 100 μm. **(F)** *In situ* hybridization depicting increased cell numbers of *ca2*-expressing ionocytes on the yolk after incubation in MilliQ water for 48 h (right panel) compared to controls (left panel). **(G)** *In situ* hybridization depicting increased cell numbers of *trpv6*-expressing ionocytes on the yolk after incubation in MilliQ water for 48 h (right panel) compared to controls (left panel). **(H)** Percentage of Nm ionocytes that undergo cell death in control conditions and following incubation with 10-fold concentrated E2 media **(I)** Average Nm ionocyte numbers per fish do not significantly increase within 24 h after killing hair cells with the antibiotic neomycin (n = 20 fish, 305 neuromasts at 5 dpf; n = 8 fish, 119 control neuromasts at 6 dpf; n = 9 fish, 137 neuromasts at 6 dpf 24 h after neomycin treatment; One-Way ANOVA with Tukey's post hoc test). **(J)** Neuromasts of *tp1bglobin:EGFP;Myo6b:H2B-Scarlet-I* larvae at 5 dpf before neomycin treatment (top panel), as well as 6 dpf control larvae (middle panel) and 6 dpf larvae 24 h post neomycin (lower panel). Scale bar equals 10 μm. **(K)** EdU incorporation analysis of controls (n = 6 fish, 58 neuromasts with 26 Notch+ Nm ionocytes) and Neomycin-treated neuromasts (n = 5 fish, 55 neuromasts with 33 Notch+ Nm ionocytes) of *tp1bglobin:EGFP;Et20* larvae stained with DAPI. Notch+ cells of Nm ionocytes do not divide during the first 20 h after neomycin. **(L)** *foxi3a* promoter deletion strategy using CRISPR/Cas12a. **(M)** HCR of *foxi3a* on the yolk in *foxi3a$^{-/-}$* mutants (lower panel, n = 4 fish) and their siblings (upper panel, n = 6 fish). Scale bar equals 20 μm. **(N).** HCR of *gcm2* on the yolk in *foxi3a$^{-/-}$* mutants (lower panel, n = 3 fish) and their siblings (upper panel, n = 12 fish). Scale bar equals 20 μm.