Supplementary Information

Chase-and-run between adjacent cell populations promotes coordinated directional collective migration

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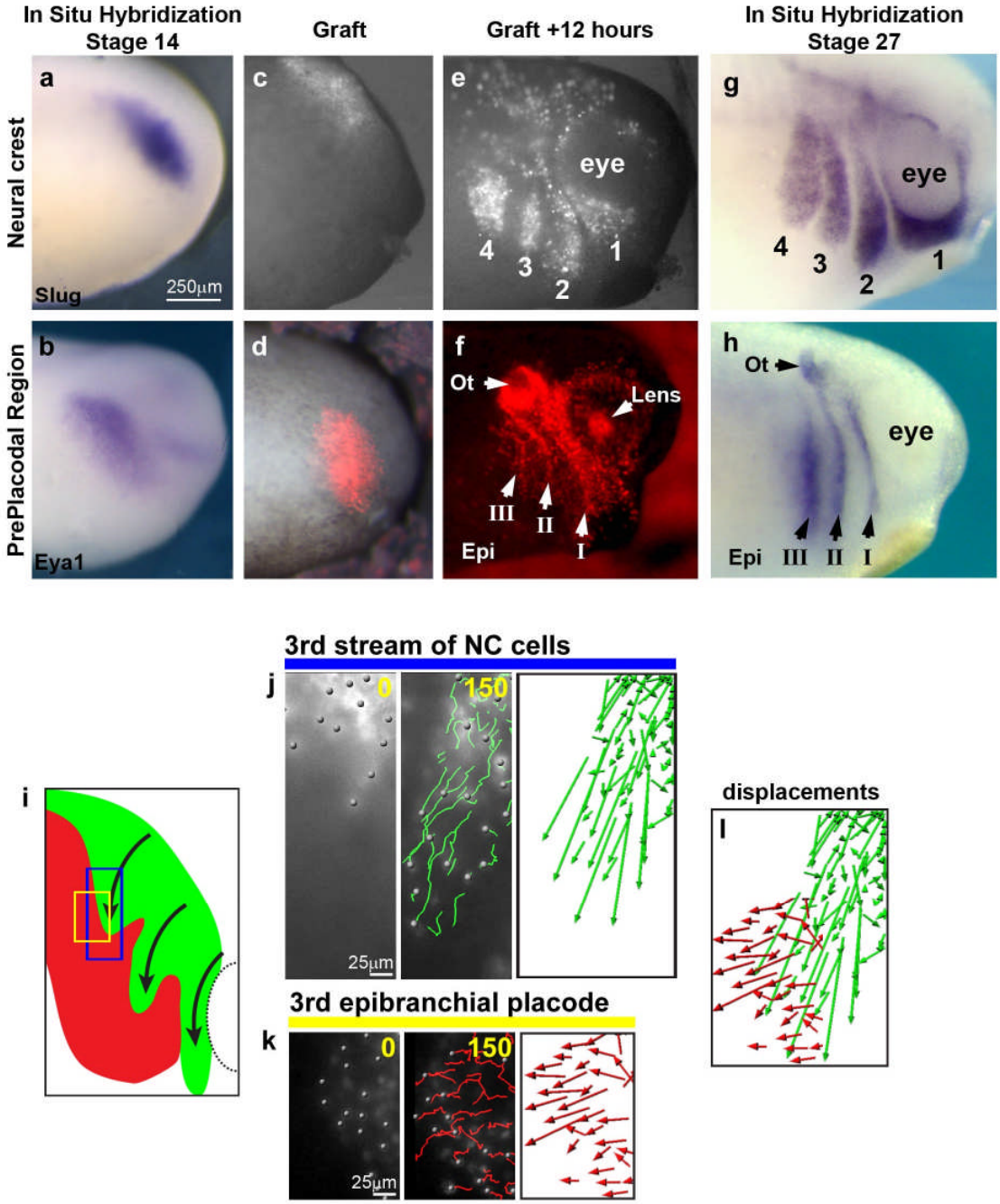
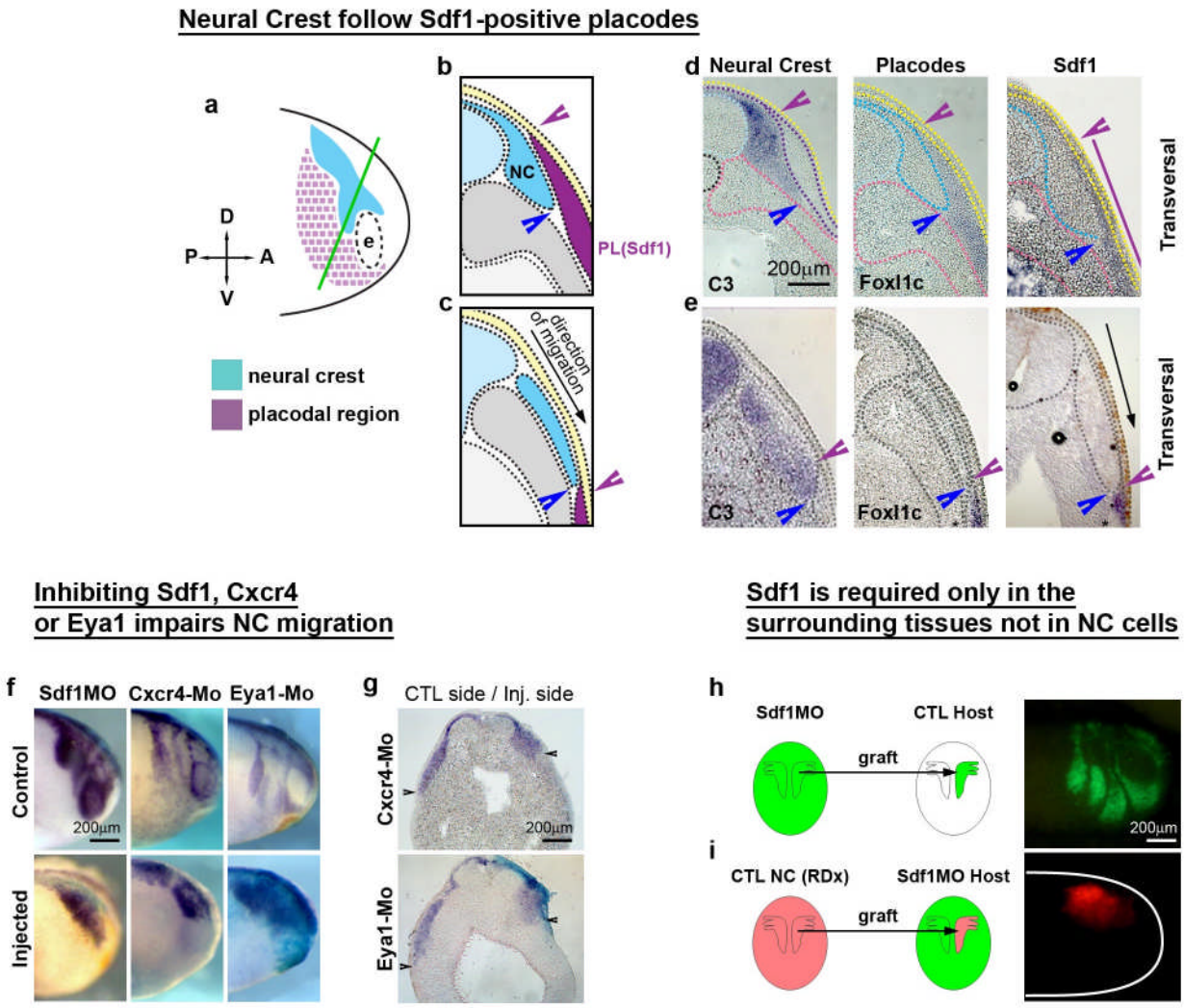
This PDF contains

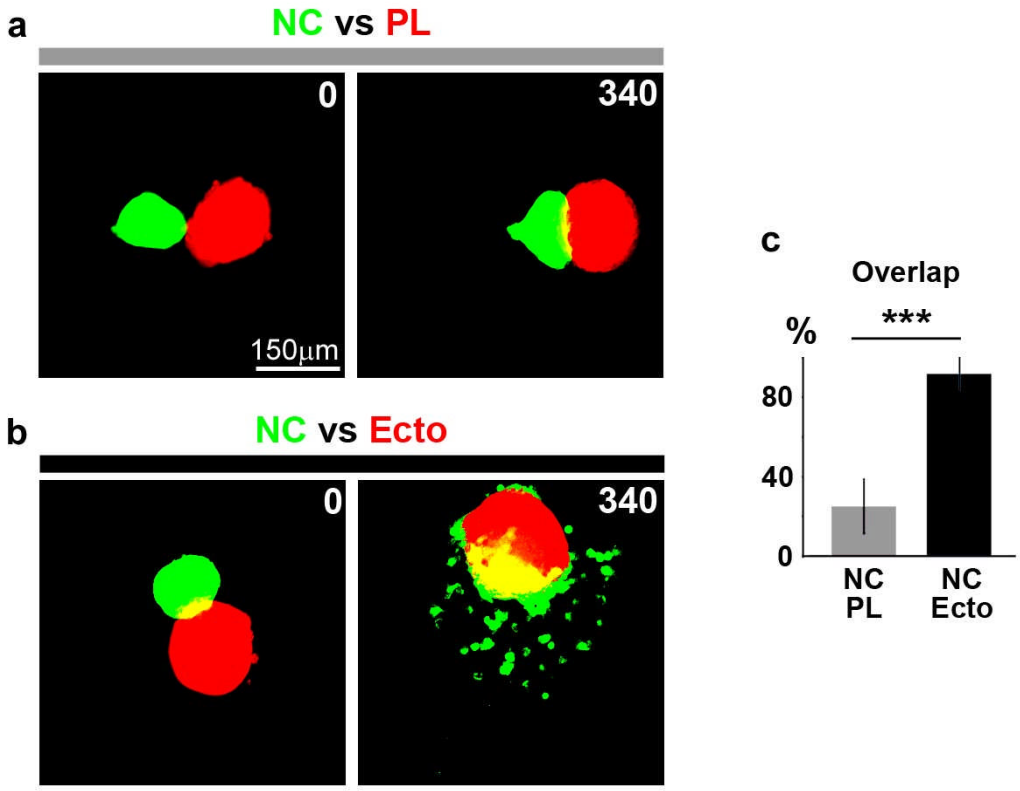
Supplementary Figures



Supplementary Figure 1. Early Neural Crest and placodes are in direct apposition and NC migration triggers placodes migration.
(**a-b**) In situ hybridization showing early NC cells (a, *Slug*) and early placodal cells (b, *Eya1*) in close proximity. (**c-f**) These two regions can be easily dissected and grafted into unlabelled host embryos. After 12 hours grafted NC cells (e) and placodes (f) distribute as expected in a control embryo. (**g-h**) In situ of *Twist* (g) and *Eya1/Foxi1c* (h) showing the distribution of NC and placodes at later stages to compare with the distribution obtained from the grafts. (**i-l**) In vivo cell migration of NC and placodes. Cells were labelled with nuclear-mCherry prior to the graft. Note that placodal cells move away from the NC but that the actual direction of movement depends on the relative position of NC and placodes. NC cells from the third NC stream (j) and placodes from the third epibranchial region (k). An overlay of both datasets is presented in **l.** See supplementary Movies 1 and 2.

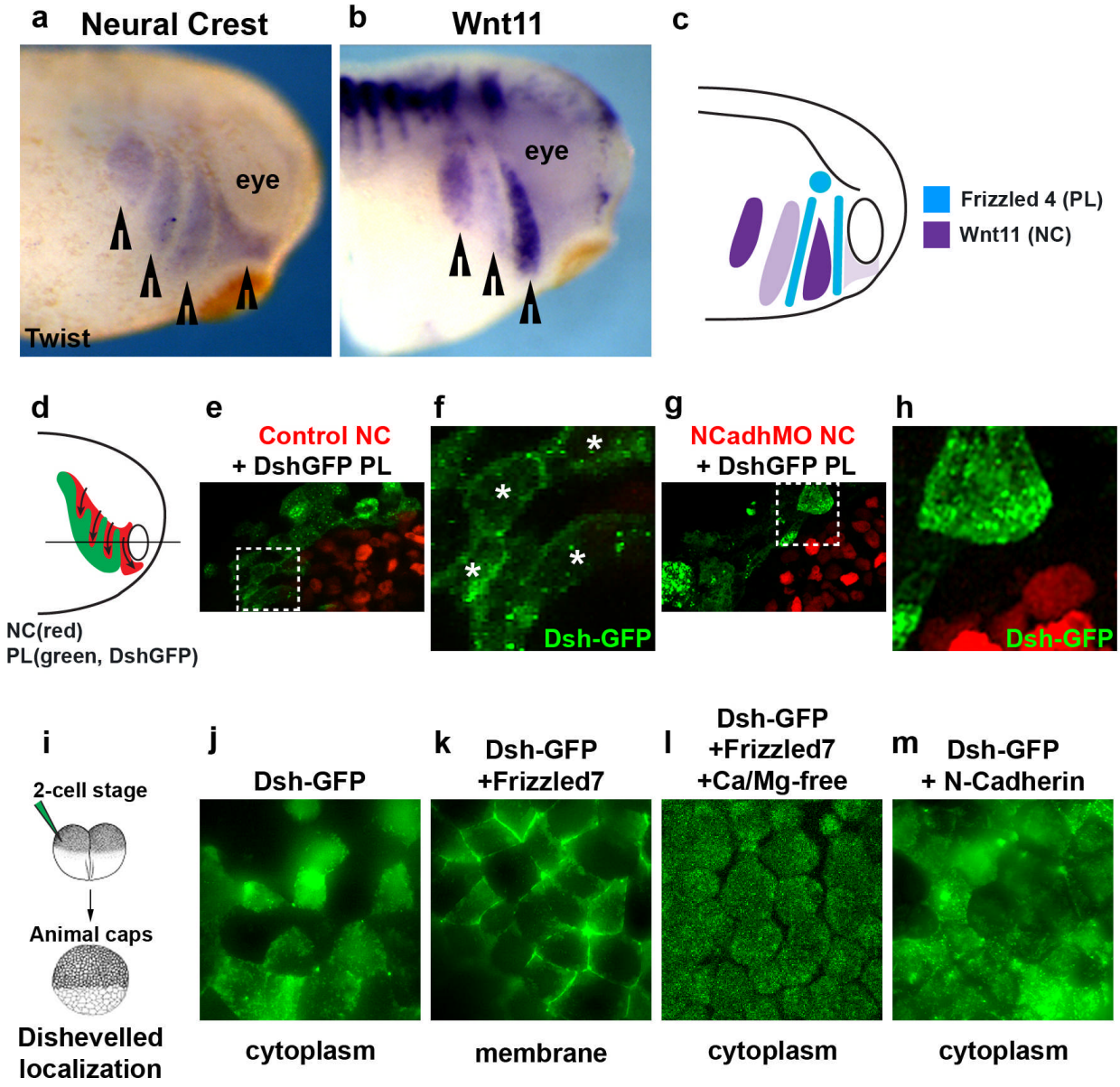


Supplementary Figure 2. Neural Crest cell migration requires Sdf1-positive placodes. (**a-e**) NC cells follow Sdf1-positive placodes. (**a**) Diagram of a *Xenopus* head showing the orientation of the sections shown in b-e. NC cells are located dorsally to a region of deep ectoderm co-expressing the placodal marker *FoxI1c* and *Sdf1*. (**b, d**) Onset of NC cell migration. (**c, e**) Late stages of NC migration. Note that placodes and NC cells are moving ventrally and that placodal cells accumulate at the tip of the NC stream. Blue arrowheads indicate the leading edge of the NC stream. Purple arrowheads indicate the dorsal limit of placodal marker and Sdf-1 expression. (**f**) Injection of Sdf1MO, Cxcr4MO and Eya1MO all inhibit NC cells migration in vivo. *Eya1* is expressed in the placodes and required for their development. (**g**) Sections from embryos shown in **f**. Note that blocking chemotaxis (Cxcr4MO) or placode development (Eya1MO) equally blocks NC migration, arrowheads indicate the front of NC migration on control and injected side. (**h**) Graft of NC cells injected with Sdf1MO into a control host, NC migration proceeds normally, indicating that Sdf-1 is not required in the NC. (**i**) Graft of control NC cells (Red) into a host injected with Sdf1MO, NC migration is impaired. This indicates that Sdf1 is not required in the NC cells but only in the surrounding tissues.

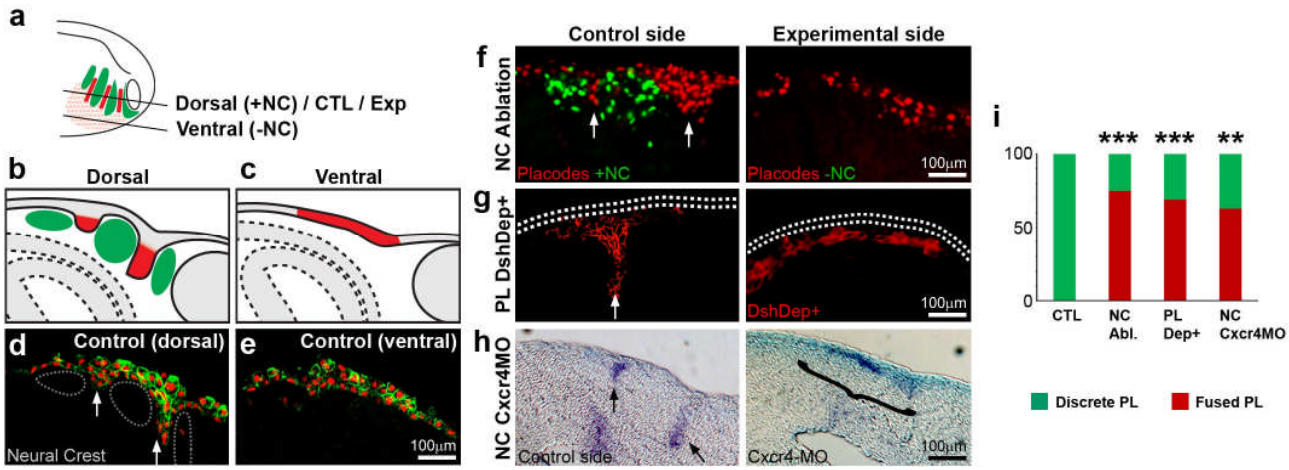


Supplementary figure 3. Co-culture of NC cells and placodal cells or Sdf1-positive non placodal ectoderm .

(a-c) Invasion assay with control NC (green) and control placode (red) explants (a, 25 explants from 3 independent experiments) or control NC (green) and Sdf1-positive non-placodal ectoderm (red) (b, 21 explants from 3 independent experiments). (c) Average overlap for each condition. Note that both ectoderm explants endogenously express Sdf1 but that NC cells invade only the non-placodal tissue (b) and that directional movement is seen only with NC cells and placodes.



Supplementary Figure 4. Wnt/PCP between NC and placodes.
(**a-c**) *Twist* (a, NC marker) and *Wnt11* (b) show similar expression patterns. (**c**) Diagram summarizing the distribution of *Wnt11* and that of *Frizzled4* which has been described in cranial placodes in *Xenopus* (Shi and Boucaut, 2000) and Zebrafish (Nikaido et al., 2013). (**d-h**) Localization of Dishevelled-GFP in placodal cells. (**d**) Diagram showing the orientation of the sections shown in e-h. (**e-f**) Dsh is at the cell membrane in control placodes adjacent to migratory NC cells, indicating an activation of the Wnt/PCP signalling in these placode cells. (**g-h**) Dsh becomes localized in the cytoplasm of placodes located next to NC cells injected with NCadherin MO, indicating a loss of Wnt/PCP signalling. (**i-m**) Dishevelled-GFP localization in animal caps. (**i**) Diagram indicating the stages used for injections and analysis. (**j**) Dsh-GFP injected alone localizes in the cytoplasm. (k) Co-injection of Frizzled7 and Dsh-GFP triggers membrane localization of Dsh-GFP. (**l**) Placing cells injected as in k in a low calcium culture medium for 10 minutes prior to confocal imaging abolishes membrane localization of Dsh-GFP suggesting that cell-cell adhesions are required for Wnt/PCP signalling. (**m**) Co-injection of Dsh-GFP and N-Cadherin are not sufficient to target Dsh at the cell membrane.



Supplementary Figure 5. NC-placodes interactions via CIL and chemotaxis are required for discrete placodes formation and NC cell migration in vivo.
(**a-c**) Diagrams showing the orientation of sections in d-h. (**d**) Dorsal level of a control embryo, placodes accumulate in between the NC streams (arrows). (**e**) Ventral level of a control embryo, NC cells have not reached this region yet. Placodes remain superficial. Ablation of NC (**f**), inhibition of Wnt/PCP signalling in the placodes (**g**) and inhibition of chemotaxis in the NC (**h**) leads to impairment in placode segregation. (**i**) Percentages of embryos from 2 independent experiments (46 animals were analyzed) with discrete or fused placodes for the different treatments shown in d –h (Parametric approach for percentages, two-sided test, $T_{NCablation}$=12.69, α=0.001(***); T_{PLdep+}=11.37, α=0.001(***); $T_{NCCxcr4MO}$=9.54, α=0.01(**)).

Legends for Supplementary Movies

Supplementary Movie 1. In vivo cell migration of Neural Crest and placodal cells after a double graft. Neural crest cells are in green, placodal cells in red. Note that a gap is generated in the placodal region where NC cells are migrating as marked by red dots. Green arrow corresponds to ventral limit of the migrating NC. 1 picture every 5 minutes. 10x lens. 5 hours.

Supplementary Movie 2. In vivo cell migration of Neural Crest and placodal cells.
Panel 1: placodes from the third epibranchial domain located posterior to the third NC stream.
Panel 2: NC cells from the third NC stream.
Panel 3: NC cells from the second NC stream
Panel 4: placodes from the first epibranchial domain located ventral and anterior to the second NC stream. Note that placodes move away from the migratory NC cells but that the actual direction of their movement depends on the relative position of placodes and NC cells. Diagrams at the top indicate which regions are monitored. Blue squares mark the NC streams whereas yellow squares indicate the placodes. 1picture every 5 minutes, 10x lens. 1h30min.

Supplementary Movie 3. In vivo placodal cell migration. Placodal cells express nuclear-RFP. Time-lapse movie of the placodal region depicted in Supplementary Figure 1i-k.
Left panel: placodal cells in a control embryo before NC cell migration.
Middle panel: display placodal cell movements during NC cell migration.
Right panel: placodes in an embryo where NC cells were removed. Note that directionality is observed only in the control situation (middle panel) in cells that are located to a gap (second NC stream, green circle). 1 picture every 5 minutes. 10x lens. 3 hours.

Supplementary Movie 4. Chase-and-Run: co-culture of NC cells and placodes
First row: control NC cells
Second row: control placodal cells
Third row: co-culture of NC (green) and placodes (red). Note that NC and placodes undergo coordinated cell migration (chase-and-run).
Fourth row: co-culture of Cxcr4MO NC (green) and placodes (red). Note that inhibiting Sdf1 chemotaxis impairs the coordinated migration.
1 picture every 5 minutes. 5 hours.

Supplementary Movie 5. Attraction assay: control NC cells and control placodes (left), Cxcr4MO NC cells and control placodes (middle), control NC cells and Sdf1MO placodes (right). Note that control placodal cells endogenously express Sdf1. Lower panels show the automated tracks for NC cells for each condition.
1 picture every 5 minutes. 5 hours.

Supplementary Movie 6. Invasion assay. Control NC cells are co-cultured with control non-placodal ectoderm from stage 10 Xenopus embryo (left panel) or control placodes (right panel). Both tissues endogenously express Sdf1. Note that NC invade the ectoderm and do not undergo coordinated migration. White cross marks the centre of the placode explants at the beginning of the movie for reference.
1 picture every 5 minutes. 5 hours.

Supplementary Movie 7. Cell-cell interaction at the NC-placodes interface.
This movie shows two examples of the dynamic interactions of NC and placode cells at the interface.

Left panel: NC (green) migrates towards placodes (red) making numerous transient contacts. Note that NC cells are far more active and migratory than placodes in accordance with their respective mesenchymal (NC) and epithelial (placodes) phenotypes. 1 picture every 30 secondes, 10X objective, 1h45minutes.
Right panel: NC (green) and placodes (red) go through cycle of protrusion, contact and retraction. Note that NC cells are more readily reforming cell protrusions than placodes. 1 picture every 10 secondes, 40X objective, 35minutes.
Both examples show how repeated contacts between NC cells progressively force placodes to retreat.

Supplementary Movie 8. Transient accumulation of N-Cadherin-GFP between NC and placodes. Both NC and placodes were transfected with N-Cadherin-GFP. Placodes were also injected with nuclear-mCherry. Top panel shows the merged green (N-Cadherin) and red (nuclear-mCherry) fluorescent channels. Middle panels show the green channel only. Bottom panels show the heat maps of the green channel. Regions of cell-cell contact are highlighted with a white square. Frames are from a single confocal z plan extracted from a stack. 63X objective, digital zoom 2x, 1 picture every 15 seconds, 6 minutes.

Supplementary Movie 9. Placodal cells protrusion dynamics. Spinning disk confocal microscopy. Both cell types were injected with LifeActin-mCherry. NC cells were co-injected with membrane-GFP. Left panel, placodal cells alone; right panel, interface between NC and placodes explants. Asterisks mark the collapsing protrusions. 100x lens. 1 picture per minute. 30 minutes.

Supplementary Movie 10. N-Cadherin affects Placodal cell protrusions.
Panel 1: Placodal cells cultured on Fibronectin.
Panel 2: Placodal cells on FN+1ug/mL of N-Cadherin.
Panel 3: Placodal cells on FN+3ug/mL of N-Cadherin.
Panel 4: Placodal cells on FN+3ug/mL of N-Cadherin in low Calcium/Magnesium conditions.
Panel 5: Placodal cells on FN+3ug/mL of N-Cadherin with placodal cells preincubated in blocking antibody against N-Cadherin (NCD2).
10X lens, digital zoom 2X. 1 picture every 3 minutes. 1 hour.

Supplementary Movie 11. Heterotypic collisions between control NC cells and control placodal cells.
Left panel: single NC cell versus a placodes explant.
Middle panel: single placodal cell versus a NC explant.
Right panel: single placodal cells colliding.
Note that collisions between NC and placodal cells lead to Contact-Inhibition of Locomotion with cells moving away from each other whereas collisions between placodal cells lead to cell clustering.
1 picture every 3 minutes. 10x lens. 30 minutes.

Supplementary Movie 12. Homotypic collisions between NC or placodes.
Top panel: Collisions between NC cells.
Bottom panel: Collisions between placodes.
Consecutive frames were subtracted and colour-coded such that protrusions and retraction appear red and blue respectively. Cell bodies are green. Note that cells collapse protrusions and repolarize upon contact with one another, making new protrusions opposite to the region cell-cell contact. NC cells subsequently move away from each other whereas placodes cluster.
1 picture every 3 minutes, 10x lens, 36 minutes.

Supplementary Movie 13. Heterotypic collisions between NC and placodal cells upon inhibition of N-Cadherin, Dishevelled and dnWnt11.
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Panel 1: Control cells.
Panel 2: Both cell types injected with N-Cadherin Morpholino.
Panel 3: Both cell types expressing Dishevelled dominant-negative (DshDep+).
Panel 4: control placodes (red) and NC cells expressing dominant-negative Wnt11 (dnWnt11, green).
Note that control NC and placodal cells move away from each other. When N-Cadherin, Dsh or dnWnt11 are inhibited NC and placodal cells remain in contact after collision. 1 picture every 5 minutes. 10x lens. 30 minutes.

Supplementary Movie 14. Invasion assay with NC and placodal cells.
Panel 1: Control cells.
Panel 2: Both cell types injected with DshDep+.
Panel 3: Placodal explants treated with N-cadherin antibody (NCD2).
Panel 4: Placodal explants treated with E-Cadherin antibody.
Note that control conditions and E-Cadherin treated explants show a clear chase-and-run behaviour while Dsh and N-Cadherin inhibition lead to overlapping of both explants and no directional movement of the placodal cells. 1 picture every 5 minutes. 10x lens. 5 hours.

Supplementary Movie 15. Co-culture of NC explants
Left panel: Control NC cells alone.
Middle panel: Two control NC explants.
Right panel: A control NC explant (red, nuclear-mCherry) is co-cultured next to a NC explant injected with Sdf1 (green, cytoplasmic Fluorescein-dextran).
Note that overexpressing Sdf1 in one NC explant is sufficient to drive a coordinated migration of the two explants.
1 picture every 3 minutes, 10x lens, 4 hours.

Supplementary Movie 16. In vivo placodal cell migration.
Left panel: Control conditions
Middle and right panels: Placodal cells expressing Dishevelled dominant negative (DshDep+).
Note that coordinated movements can be seen in DshDep+ condition compared to the control placodes (left).
1 picture every 5 minutes. 10x lens. 3 hours.

Supplementary Movie 17. 3D reconstruction of Sox3 in situ hybridization in Zebrafish injected with a control MO or Sdf1 MO. Note that after blocking NC cell migration with Sdf1 MO (right panel) placodal cells remains organized as one domain instead of splitting into subgroups (control embryo, left panel).