Supplementary materials and methods

In-Situ Hybridization

Tissue Preparation:

In this study, E16.5 heads were used to detect the expression of core PCP genes *Vangl2*, *Fzd3*, *Celsr1* and *Celsr3*. The heads were fixed overnight at 4^0C in 4% PFA (Electron Microscopy Sciences, Catalog No. 15714) prepared in 1X PBS (which is prepared in DEPC (Fisher scientific, Catalog No. 50-213-289) treated autoclaved water). Heads were cryopreserved by passing through a 30% sucrose solution made in 1X PBS (DEPC treated) and embedded in Neg-50 (Thermofisher Scientific, Catalog No. 6506). The sections were cut at 20 µm using a Leica 3050 Cryostat.

Day 1: Hybridization

Sections were brought to room temperature (RT) and then dried in oven for 15 minutes at 50^0C. Sections were fixed in ice cold 4% paraformaldehyde solution (Electron Microscopy Sciences, 15714) prepared in PBS for 10 minutes. Sections were washed twice with PBS-TritonX-100 (0.1%) for 5 minutes each at RT with gentle shaking. Sections were fixed again in ice cold 4% PFA for 10 minutes. Sections were washed twice with PBS-TritonX-100 (0.1%) for 5 minutes each at RT with gentle shaking. Sections were treated with 1 µg/ml Proteinase K (Invitrogen, Catalog No. AM2546) in PBS for 10 minutes at RT, with gentle shaking. Sections were washed twice with PBS-TritonX-100 (0.1%) for 5 minutes each at RT with gentle shaking. Sections were acetylated in 0.1M Triethanolanamine/0.25% acetic anhydride (Sigma Aldrich, Catalog Nos. T58300 and 242845 respectively) for 15 minutes at RT, with gentle shaking. Sections were washed twice with PBS-TritonX-100 (0.1%) for 5 minutes each at RT with gentle shaking. Sections were then incubated with Pre-hybridization solution for 4 hours at 60^0C. Probes were added to hybridization solution to a final concentration of 200-500 ng/ml. The hybe-probe mixture was warmed at 85^0C for 3 minutes before applying to the sections. Sections were covered with cover slips and incubated at 60^0C for a minimum of 16 hours.

Day 2: Post-hybridization washes and antibody labeling

Coverslips were removed gently and sections were washed with pre-warmed 5X SSC at 65C for 10 minutes with gentle shaking. Sections were washed with 50% formamide/1X SSC at 65C for 30 minutes with gentle shaking. Sections were washed with 2X SSC at 65 C for 20 minutes with gentle shaking. Sections were washed twice in 0.2X SSC at 65 C for 20 minutes each with gentle shaking. Sections were washed twice with MABT at

RT for 5 minute each, with gentle shaking. Sections were blocked in MABT/20% heat inactivated sheep serum (HISS, Sigma-Aldrich, Catalog No. S2263) for 1 hour or more at RT, with gentle shaking. Sections were incubated overnight at 4C in anti-digoxigenin (1:2000, Roche, Catalog No. 11093274910) prepared in MABT/10% HISS.

Day 3: Post-antibody washes and detection

Sections were rinsed with MABT and then washed twice in MABT for 5 minutes each with gentle shaking. Sections were washed one more time in MABT for 10 minutes, with gentle shaking. Sections were washed in alkaline phosphatase buffer (AP) for 10 minutes with gentle shaking. AP was replaced with BM Purple (Roche, Catalog No. 11442074001) and sections were incubated at RT in dark without shaking. (Sections were checked periodically for color development.) After the color development, reaction was stopped by rinsing the slides couple of times with stop buffer. Sections were fixed with 4% PFA for 10 minutes. Sections were rinsed with 1X PBS and then washed in 1X PBS for 5 minutes with gentle shaking. Sections were mounted and stored at 4C for imaging.

Reagent preparation

DEPC water treatment	1ml DEPC is added to 1 L ddH_2O and let it sit overnight in fume hood. Autoclave and use.
PBST	100 ml 10XPBS (DEPC treated) 1 ml TritonX-100 X-100 Bring to volume via DEPC ddH2O
4% PFA	5 ml 32% EMS PFA 35 ml 1X PBS (DEPC Treated)
STOP buffer	1% SDS (Bio-Rad Catalog No. 161-0418) 20mM EDTA 20mM Tris pH 7.5 100mM NaCl Bring to volume with autoclaved ddH2O **For 10 ml** 500 µl of 20% SDS 400 µl of 0.5mM EDTA 200 µl of Tris pH 7.5 200 µl of 5M NaCl
1 M TEA pH 8.0	**For 500 mL**

<table>
<tr><td>(TriEthanolAmine)</td><td>300 mL DEPC H2O
66.5ml TEA (Sigma T58300, stock is 7.5M)
pH 8.0 with HCl
Final volume 500 mL with DEPC ddH2O</td></tr>
<tr><td>0.1M TEA/0.25% acetic anhydride</td><td><u>For 10 mL</u>
5 ml 1M TEA (pH 8.0)
45 ml DEPC ddH2O
25 µl acetic anhydride</td></tr>
<tr><td>20X SSC</td><td><u>For 1L</u>
3M NaCl (175.3 g)
0.3M C6H5Na3O7.2H2O (dihydrous sodium citrate) (88.23g)
pH 7.0 via HCL
Final volume 1 L with autoclaved ddH2O
DO NOT AUTOCLAVE</td></tr>
<tr><td>5X MABT</td><td><u>For 1L</u>
58 g Maleic acid
43.5g NaCl
pH 7.5 with Tris-Base (need ~ 100 g)
Final Volume 1 L with autoclave ddH2O</td></tr>
<tr><td>Alkaline Phosphatase (AP) buffer</td><td>100mM Tris pH 9.5 (1ml of 1M)
50mM MgCl2 (500 µl of 1M)
100mM NaCl (200µl of 5M)
0.1% Tween 20 (100 µl of 10%)
Final volume 10 mL with autoclaved ddH2O</td></tr>
<tr><td>Pre-Hybridization buffer</td><td>10mM Tris pH 7.5
600mM NaCl
1mM EDTA
0.25% SDS
1X Denhardt's (Sigma Aldrich, Catalog No. D2532)
50% Formamide (Sigma Aldrich, Catalog No. F7503)
300 µg/ml Yeast tRNA (Invitrogen Catalog No. 15401029)

<u>For 50 mL</u>
<table>
<tr><td>Item</td><td>Stock</td><td>Final</td><td>Unit Quantity</td><td>Quantity for 50 mL</td></tr>
<tr><td>Tris pH 7.5</td><td>1M</td><td>10mM</td><td>10 µl</td><td>500 µl</td></tr>
<tr><td>NaCl</td><td>5M</td><td>600mM</td><td>120 µl</td><td>6 mL</td></tr>
<tr><td>EDTA</td><td>.5M</td><td>1mM</td><td>2 µl</td><td>100 µl</td></tr>
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<tr><td>SDS</td><td>20%</td><td>0.25%</td><td>12.5 µl</td><td>625 µl</td></tr>
<tr><td>Denhardt's</td><td>50X</td><td>1X</td><td>20 µl</td><td>1 mL</td></tr>
<tr><td>Formamide</td><td>100%</td><td>50%</td><td>500 µl</td><td>25 mL</td></tr>
<tr><td>Yeast tRNA</td><td>10 mg/mL</td><td>300 µg/mL</td><td>30 µl</td><td>1.5 mL</td></tr>
<tr><td>DEPC ddH_2O</td><td></td><td></td><td>305.5</td><td>15.275 mL</td></tr>
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<tr><td>Hybridization buffer</td><td>Hybridization buffer is prepared same way as pre-hybridization buffer except that the plain DEPC treated water is replaced with 25% Dextran sulphate (Sigma Aldrich, Catalog No. D8906) (Dextran sulphate is prepared in DEPC treated water.)</td></tr>
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