

Figure S1. Median and rostrolateral PPE cells present different mitotic rates. A) Schematic representation of a HH5/6 embryo with the PPE in pink and the analysed median and rostrolateral PPE areas are represented as grey and blue boxes. B-C') Confocal dorsal views of embryos hybridized *in toto* with a probe for the placodal marker *Tfap2a* and immunostained for BrdU. B' and C' are a high magnification views in B, C. D) Quantification of the number of BrdU positive cells in median (n embryos =8) and rostrolateral (n=8) PPE at HH5/6 (* p< 0.05, Student's *t*-test; error bars

indicate mean \pm s.e.m.).

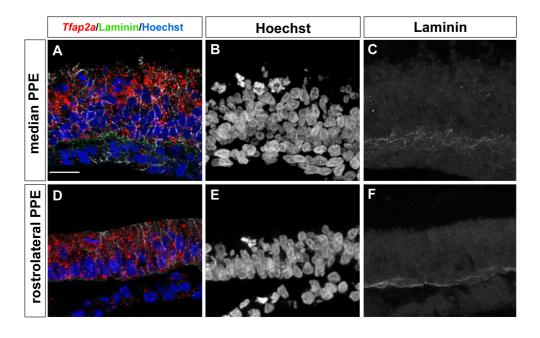
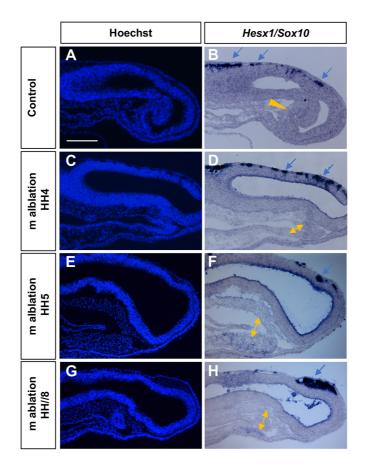


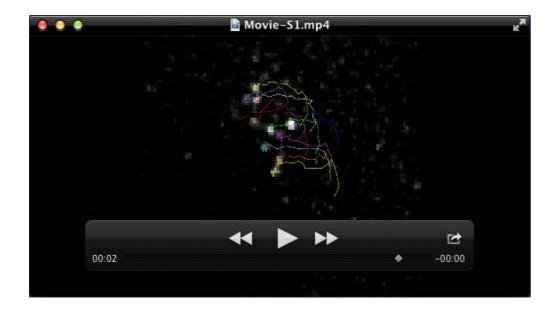
Figure S2

Figure S2. The median PPE is superimposed to the mesendoderm. Frontal sections of HH6/7 embryos (n=6) hybridized *in toto* with a probe for *Tfap2a* and immunostained with antibodies against laminin (A,D). Note the absence of signal underneath the AHP prospective region (A-C). Immunostaining is instead prominent at the level of the rostrolateral PPE (D-F). Scale bar: 20 μm.

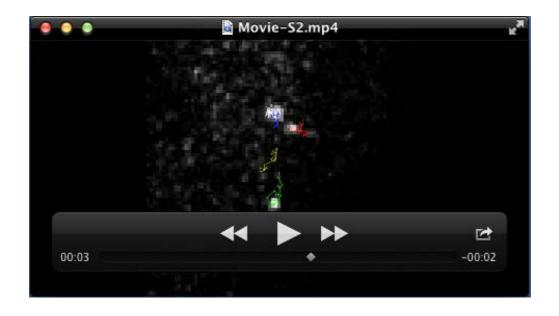


Supplementary Figure 3

Figure S3. Ablations of the median PPE leaves the underlying mesendoderm intact. Sagittal sections of HH10/11 embryos with (C-H) or without (A, B) median PPE ablations performed at the stages indicated in the panels. Embryos were hybridized with probes specific for *Sox10* and *Hesx1* to detect the presence of the neural crest and mesendoderm, respectively. Blue arrows indicate *Sox10* positive neural crest cells, double yellow arrows point to the *Hesx1*-positive mesendoderm. Note that ablation of the median PPE does not affect these tissues. Scale bar:50 μm.



Movie 1: Cells in the rostolateral PPE undergo medio-lateral intercalating movements. The movie shows an example of an embryo in which a plasmid carrying the photo-convertible *Kaede* sequence was electroporated at gastrula stage in the rostrolateral PPE domain. After four hours, few kaede-expressing cells positioned in the rostrolateral PPE were hit with a laser beam to photoconvert the kaede to red. The behaviour of red-labelled cells was recorded for the next few hours until the embryo reached stage HH5+/6. Note the medio-lateral movements of the cells.



Movie 2: Cells in the medial PPE hardly move from their position. The movie shows an example of an embryo in which a plasmid carrying the photo-convertible *Kaede* sequence was electroporated at gastrula stage in the median PPE domain. After four hours, few kaede-expressing cells positioned in the median PPE were hit with a laser beam to photoconvert the kaede to red. The behaviour of red-labelled cells was recorded for the next few hours until the embryo reached stage HH8/9.

Table S1. List of the labelling experiments performed to obtain the fate map of adenohypophysis placode precursors. The table reports the parameters used to position the initial and final position of the labelled cells for each one of the performed experiment (case). The final position of labelled cells is color coded: adenohypophysis placode (red), adenohypophysis and olfactory (yellow), olfactory only (light green); olfactory and lens (bright green); lens only (blue).

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Table S2. List of the genes enriched in either the medial or rostrolateral placodal explants. The table shows all the genes found enriched in either the medial or the rostro-lateral placodes expressed in Log2 (fold change) and the related p and q values and proposed function of the genes

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