

Fig. S1. Morphological changes during the initiation of sensory placode invagination.

(A–F) Cryo-sections of the developing chick otic, lens, and olfactory placodes indicating the morphological movements during placodal invagination. Nuclei are detected with DAPI staining. Scale bar: 100µm.

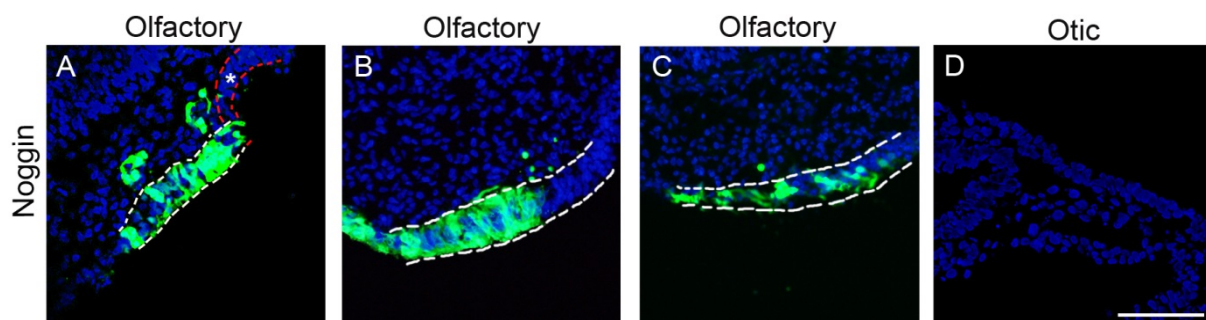


Fig. S2. Partial invagination or lack of placode formation after BMP inhibition.

(A, B, C) Stage 11/12 chick electroporated with GFP (green) and Noggin in the olfactory placodal region, and cultured to approximately stage 20. (D) *Ex ovo* culture of stage 7 embryo cultured to stage 12 in the presence of Noggin. (A) Few embryos with partial electroporation lack placode invagination (n=5/21). (B) A partial invagination of the olfactory placode was observed in GFP negative areas of the epithelium, indicated by a white asterisk and red dotted lines (n=1/21). (C, D) After inhibition of BMP activity, a few embryos lacked placode formation in the olfactory region (n=3/21) and otic region (n=2/17). (A-D) Nuclei are detected with DAPI. Scale bar: 100µm.

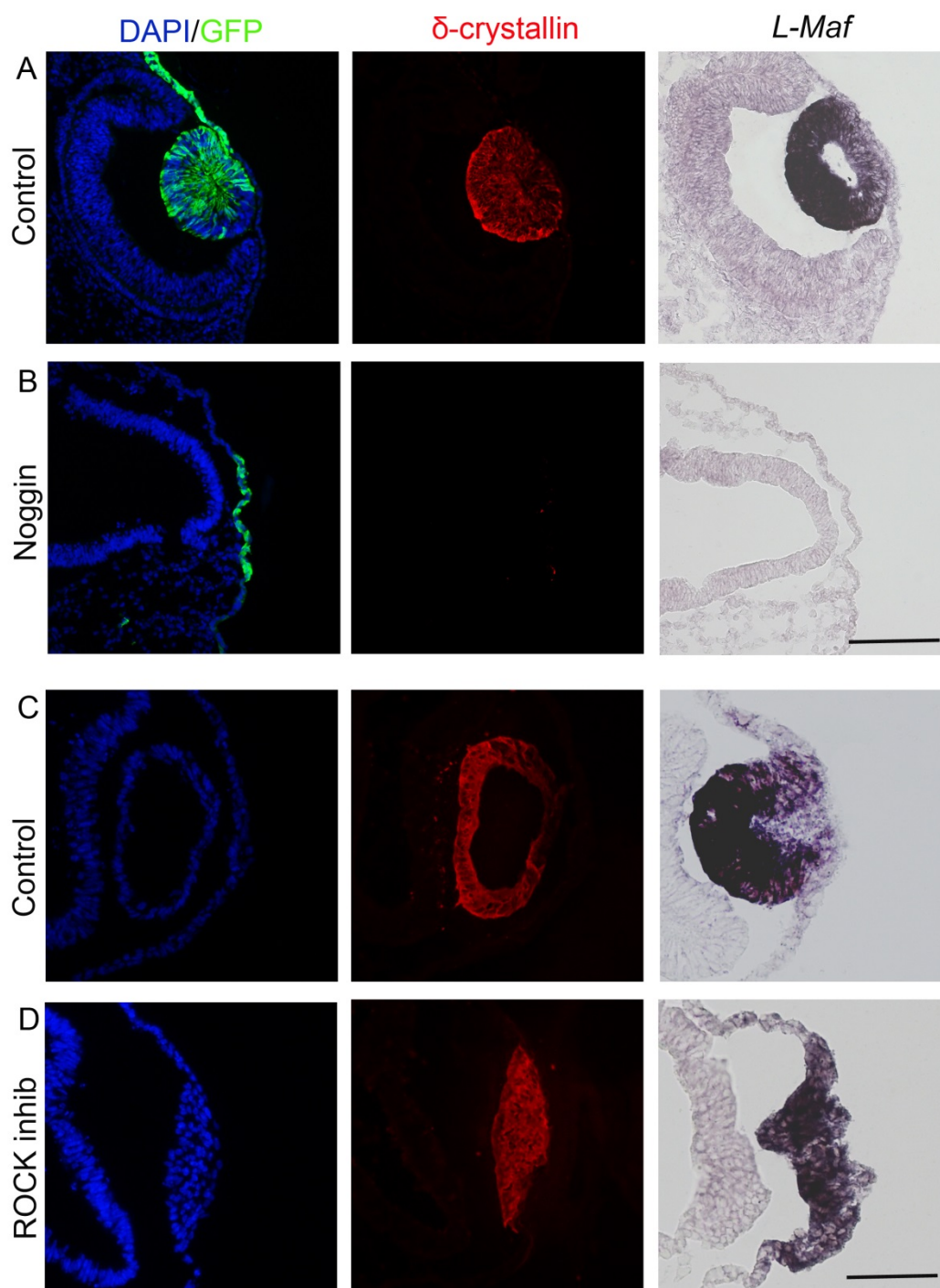


Fig. S3. The specification of lens fiber cells is BMP- but not ROCK-dependent.

(A, B) In ovo electroporation of stage 10/11 embryos in the prospective lens ectoderm region using GFP alone (n=3) or together with Noggin (n=3) and cultured to approximately stage 16. (A) Control GFP-electroporated embryos exhibited normal lens morphology and normal expression pattern of δ -crystallin and *L-Maf* (n=3/3). (B) In Noggin-electroporated embryos,

no δ -crystallin or *L-Maf* expression was detected. (C,D) In vivo cultures of stage 5/6 embryos alone (C, n=3) or together with the ROCK inhibitor Y27632 (D, n=3), and cultured to stage 18. Both control (C) and ROCK inhibited (D) embryos generate *L-Maf*⁺ and δ -crystallin⁺ lens fiber cells. Scale bar: 100 μ m.

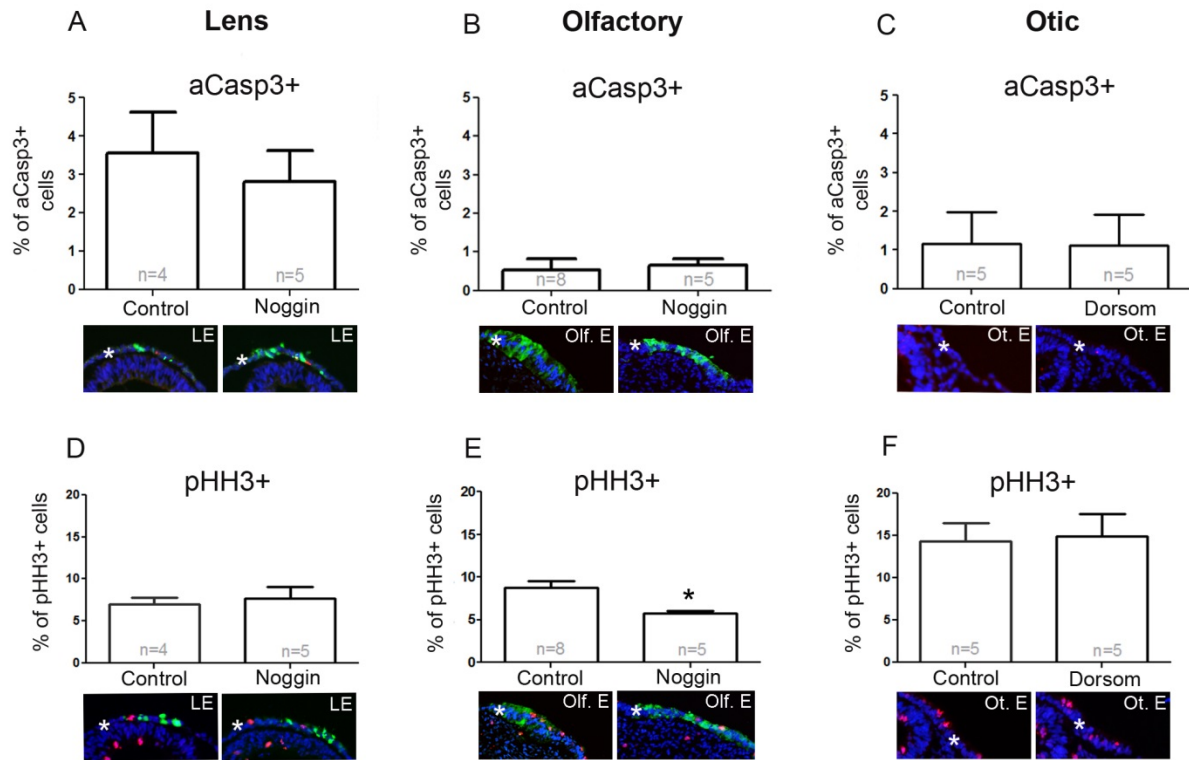


Fig. S4. Statistical analyses of cell death and cell proliferation after BMP inhibition.

(A-F) Analyses of aCaspase3⁺ apoptotic cells and pHistoneH3⁺ proliferative cells by immunohistochemistry. (A-C) BMP inhibition did not change the number of aCaspase3⁺ apoptotic cells in the lens (A), olfactory (B) or otic (C) placodes. (D-F) BMP inhibition did not change the number of pHistoneH3⁺ proliferative cells in the lens (D) or otic (F) placodes, but significantly decreased the number of pHistoneH3⁺ proliferative cells in the olfactory placode (E). White asterisks indicate lens, olfactory and otic ectoderm. Abbreviations: LE - lens ectoderm; Olf. E - olfactory ectoderm; Ot. E - otic ectoderm. Error bars represent mean \pm s.e.m, *P<0.05.

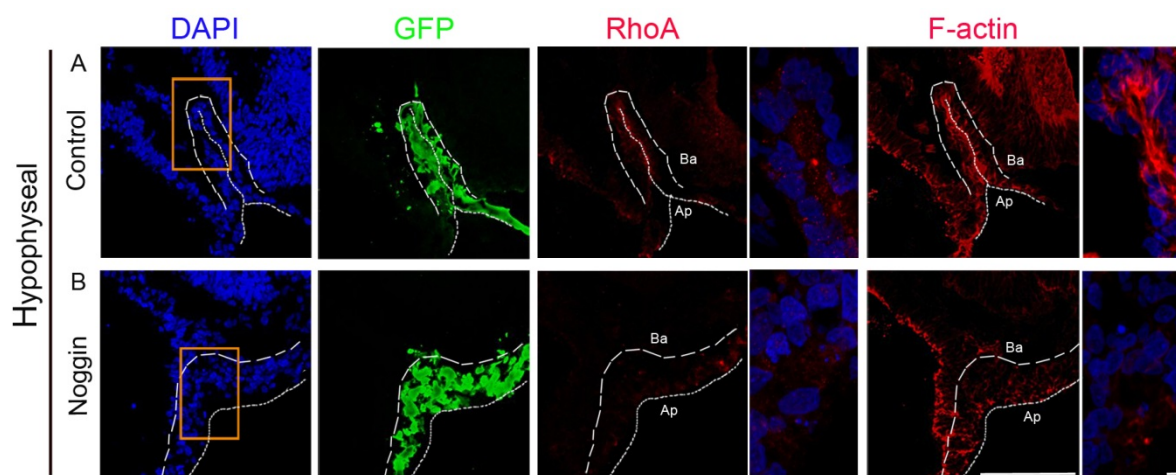


Fig. S5. BMP inhibition disturbs apical accumulation of F-actin and invagination of the hypophyseal placode.

(A, B) *In ovo* electroporation of stage 12/13 embryos electroporated with GFP alone (A) or together with Noggin (B) and cultured to stage 16/17. (A) All control embryos show accumulated expression of F-actin at the apical region of the cells, and an invagination of the hypophyseal epithelium. (B) Noggin-electroporated embryos failed to accumulate F-actin at the apical side of the cells and exhibited disturbed epithelial invagination. (A, B) RhoA is not expressed in the hypophyseal placode at this stage. Boxed regions indicate the areas of higher magnification images. Scale bar: 100µm and 10µm (higher magnifications). Ap: Apical, Ba: Basal.

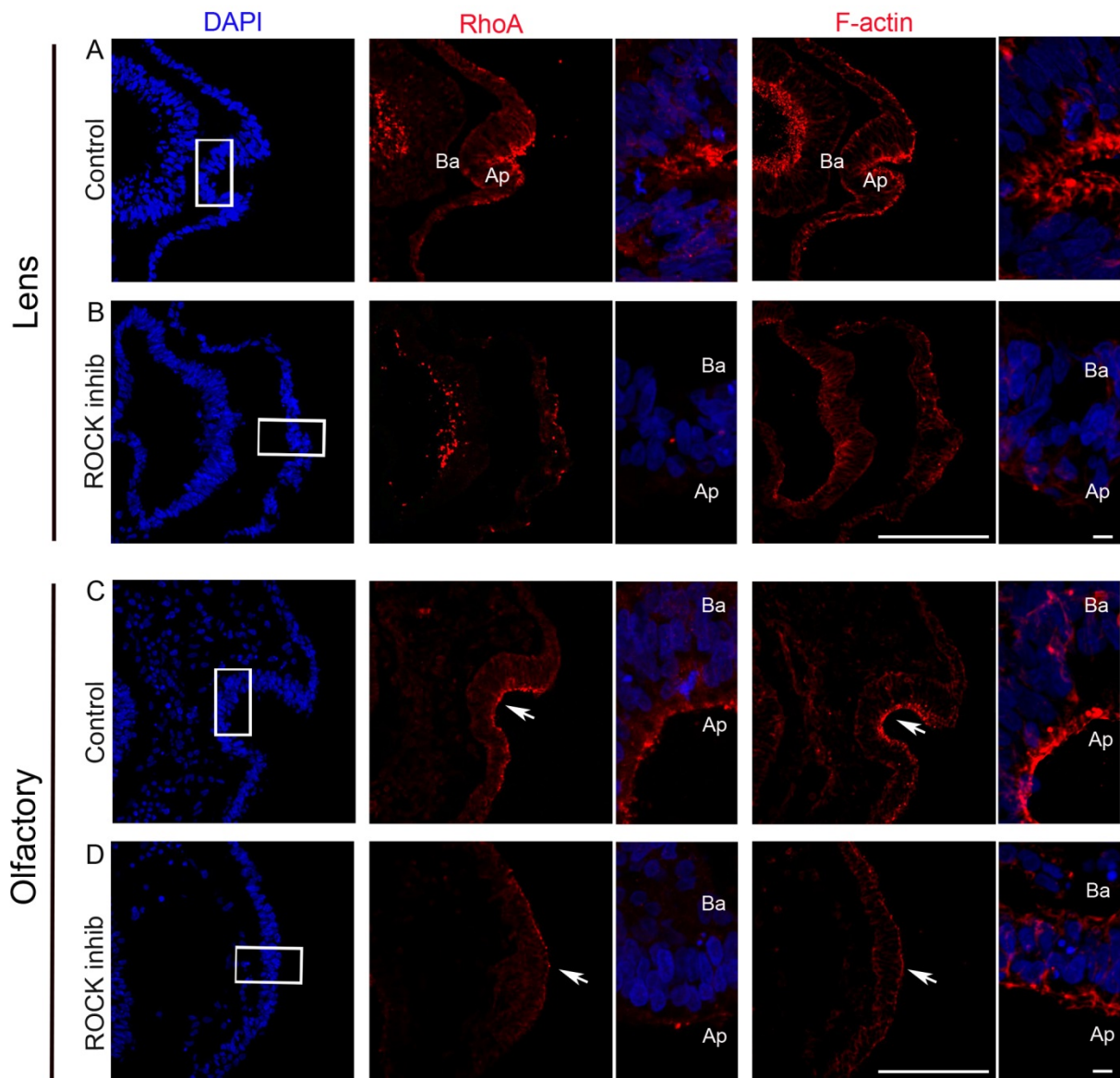
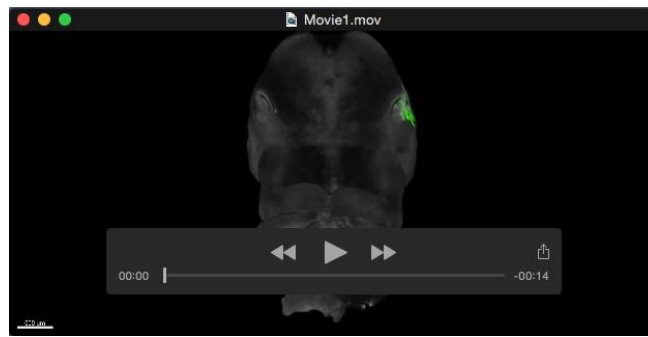


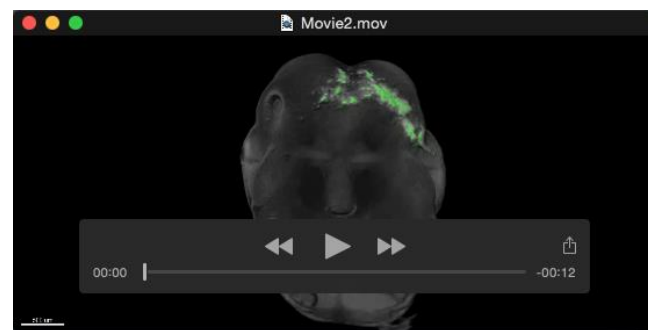
Fig. S6. ROCK inhibition disrupts apical accumulation of RhoA and F-actin in the lens and olfactory placodes.

(A-D) *Ex-ovo* cultures of whole stage 5/6 embryos alone (A, n=3; C, n=3) or together with the ROCK inhibitor Y27632 (B, n=5; D, n=7), and cultured to stage 15/16 for lens assays (A,B) or to stage 18 for olfactory assays (C,D). Y27632 was added to the cultures around embryonic stage 11. (A,C) In control embryos, RhoA was apically localized with apical F-actin polarization. (B,D) Embryos treated with the ROCK inhibitor still generated placodes, but exhibited disturbed apical accumulation of RhoA and F-actin, and failed to invaginate. Boxed regions indicate the areas of higher magnification images. Arrows in C and D indicate the apical side. Scale bar: 100µm and 10µm (higher magnifications). Ap: Apical, Ba: Basal.



Movie 1. 3D-View of Olfactory Pit Formation in Control Embryo.

3D reconstruction and rotation of the head of a control-GFP electroporated embryo. The olfactory pit has formed normally.



Movie 2. 3D-View of Loss of Olfactory Pit Formation after BMP Inhibition.

3D reconstruction and rotation of the head of a Noggin-GFP electroporated embryo. The olfactory pit has clearly failed to form.