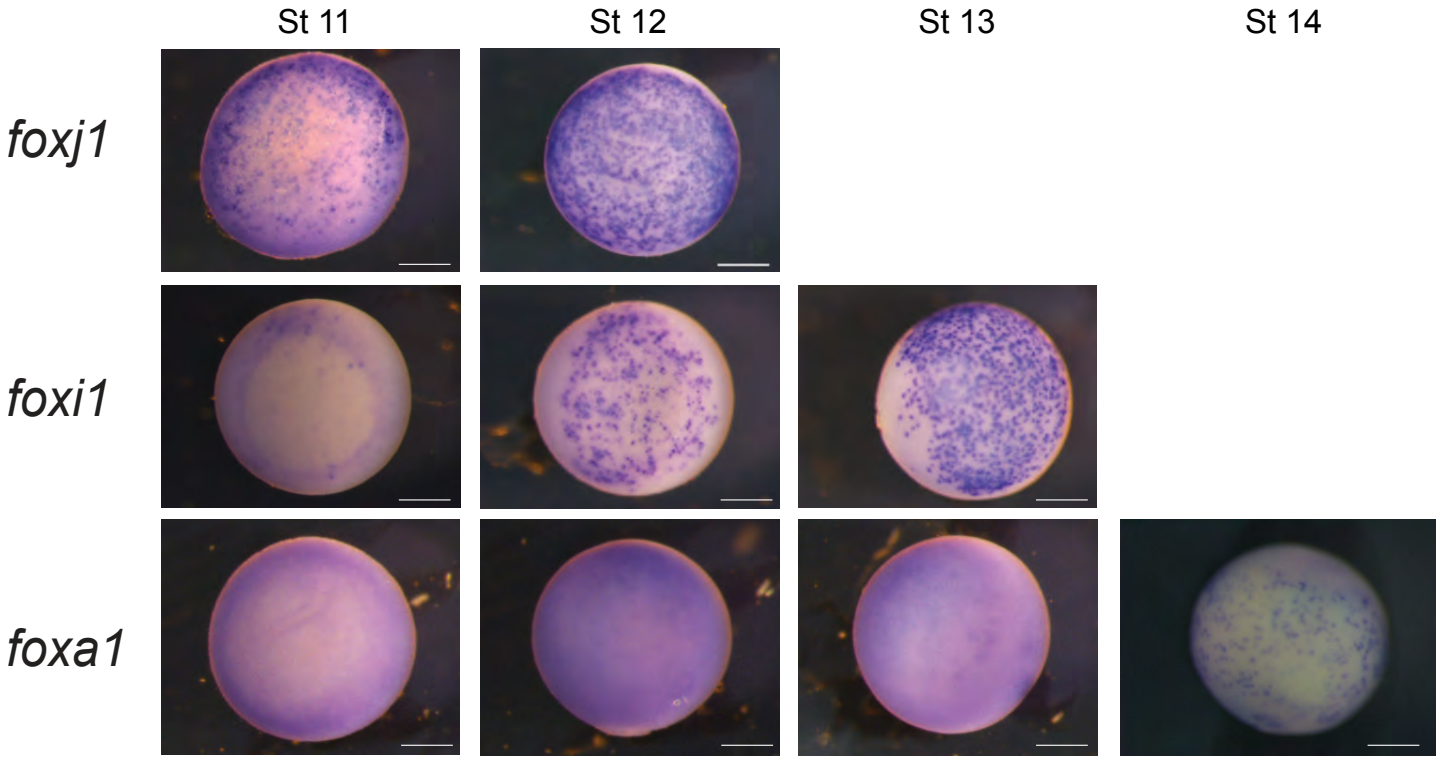


Fig. S1. Time course comparison and expression pattern of new cell type. Time course of expression for early markers of ciliated cells (*foxj1*), ionocytes (*foxi1*) and the new cell type (*foxa1*). Ciliated cells arise first in the epidermis appearing at stage 11, ionocytes next appear at stage 12 and finally the new cell type is expressed in the epidermis in a scattered distribution between stages 13-14. Scale bars: 300 μm.

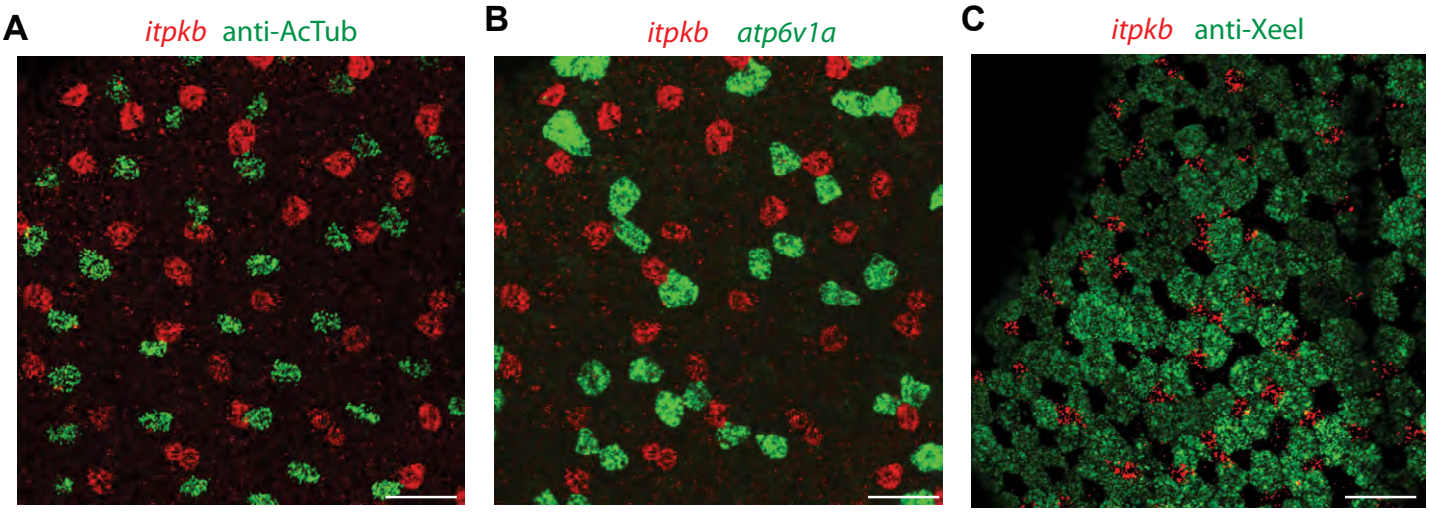


Fig. S2. *Itpkb* is not found in ciliated cells, ionocytes or goblet cells. *Itpkb* shows staining in a cell type independent of ciliated cells (A), ionocytes (B) and goblet cells (C) by fluorescent *in situ* hybridization combined with antibody staining. Ciliated cells are marked with the anti-acetylated α-tubulin (AcTub) antibody, ionocytes with an *atp6v1a* probe, and goblet cells with an anti-Xeel antibody. Note that (A) and (B) represent the same embryo and staining for *itpkb* but with anti-AcTub and *atp6v1a*, respectively. Scale bars: 50 μm.

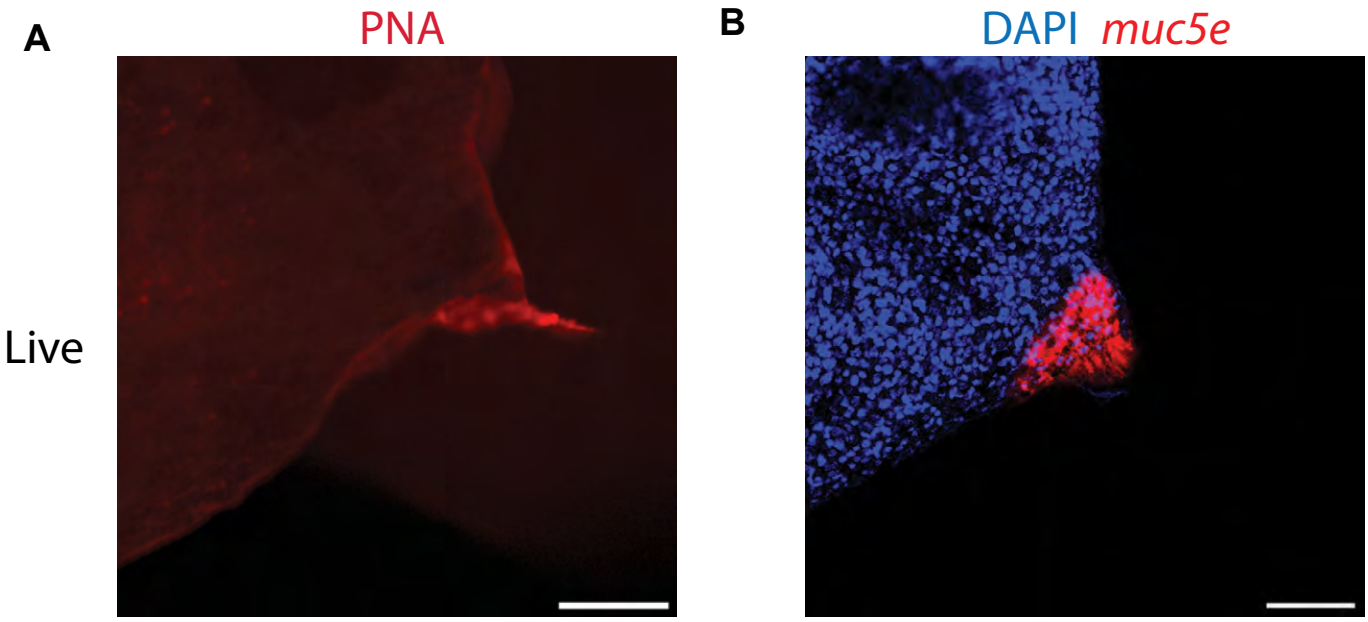


Fig. S3. Muc5e, a cement gland specific Mucin. (A) Addition of PNA-Alexa Fluor-568 to live embryos at stage 32 shows strong staining of the mucus-like material that emanates from the cement gland. (B) Fluorescent *in situ* hybridization for *muc5e* (red) and DAPI (blue) staining of nuclei shows exclusive expression of *muc5e* in the cement gland. Scale bars: 200 µm (A) and 100 µm (B).

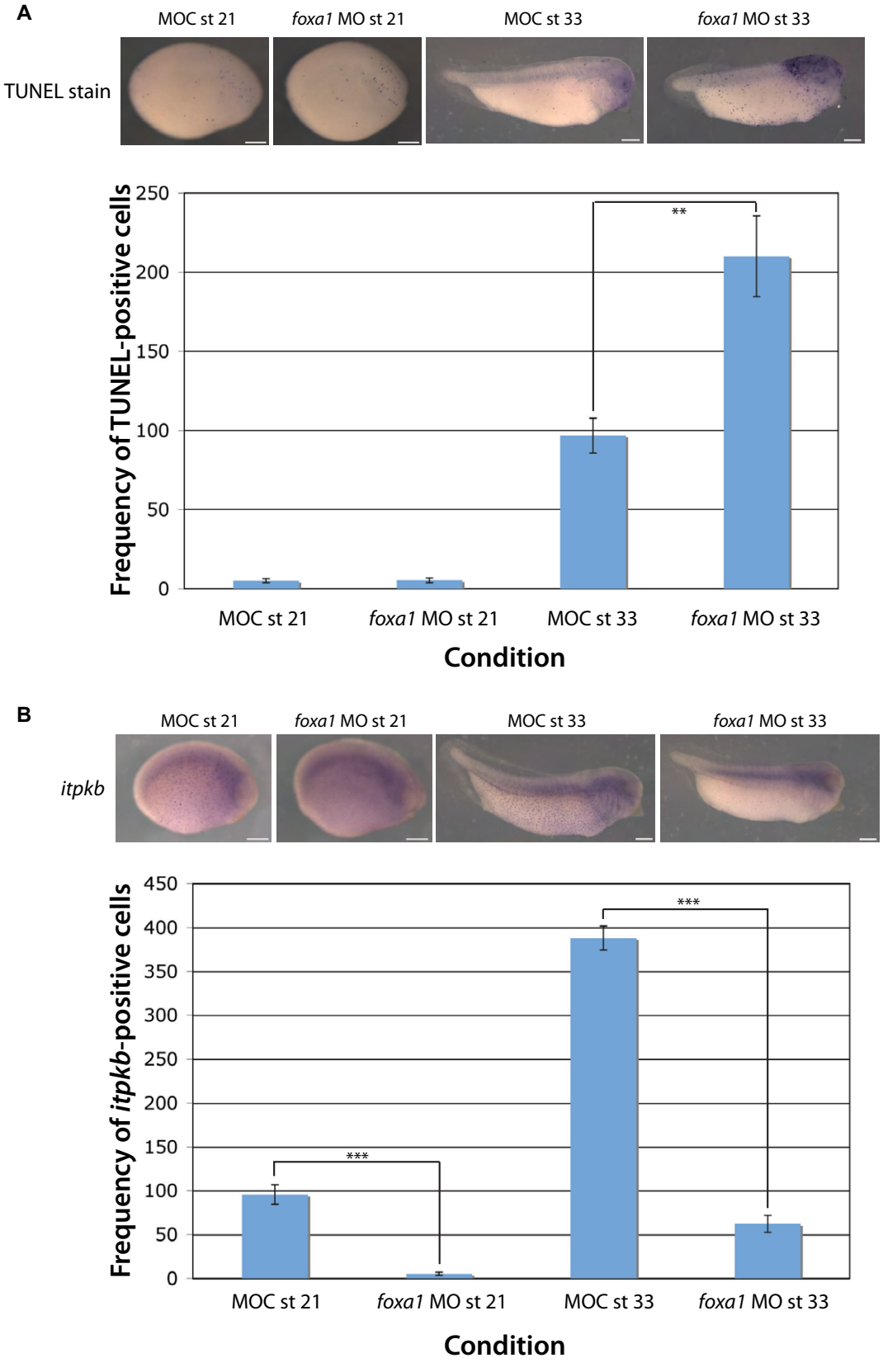


Fig. S4. Analysis of cell death in the epidermis of *foxa1*morphants. (A) TUNEL stains of epidermis in controls and *foxa1* morphants at stage 21 and stage 33. The frequency of cells in a defined area on the flank epidermis was counted and the means determined as shown in the chart. *n*=11 embryos (MOC st. 21), *n*=9 embryos (*foxa1* MO st. 21 and MOC st. 33), *n*=8 embryos (*foxa1* MO st. 33). (B) *In situ* hybridization for *itpkb* in controls and Foxa1 morphants at stage 21 and stage 33. The frequency of cells in a defined area on the flank epidermis was counted and the means determined as shown in the chart. *n*=9 embryos (MOC st. 21 and *foxa1* MO st. 33), *n*=10 embryos (*foxa1* MO st. 21 and MOC st. 33). Error bars represent s.e.m. Student's *t*-test, $P<0.01$ (**) and $P<0.001$ (***). Scale bars: 250 µm.

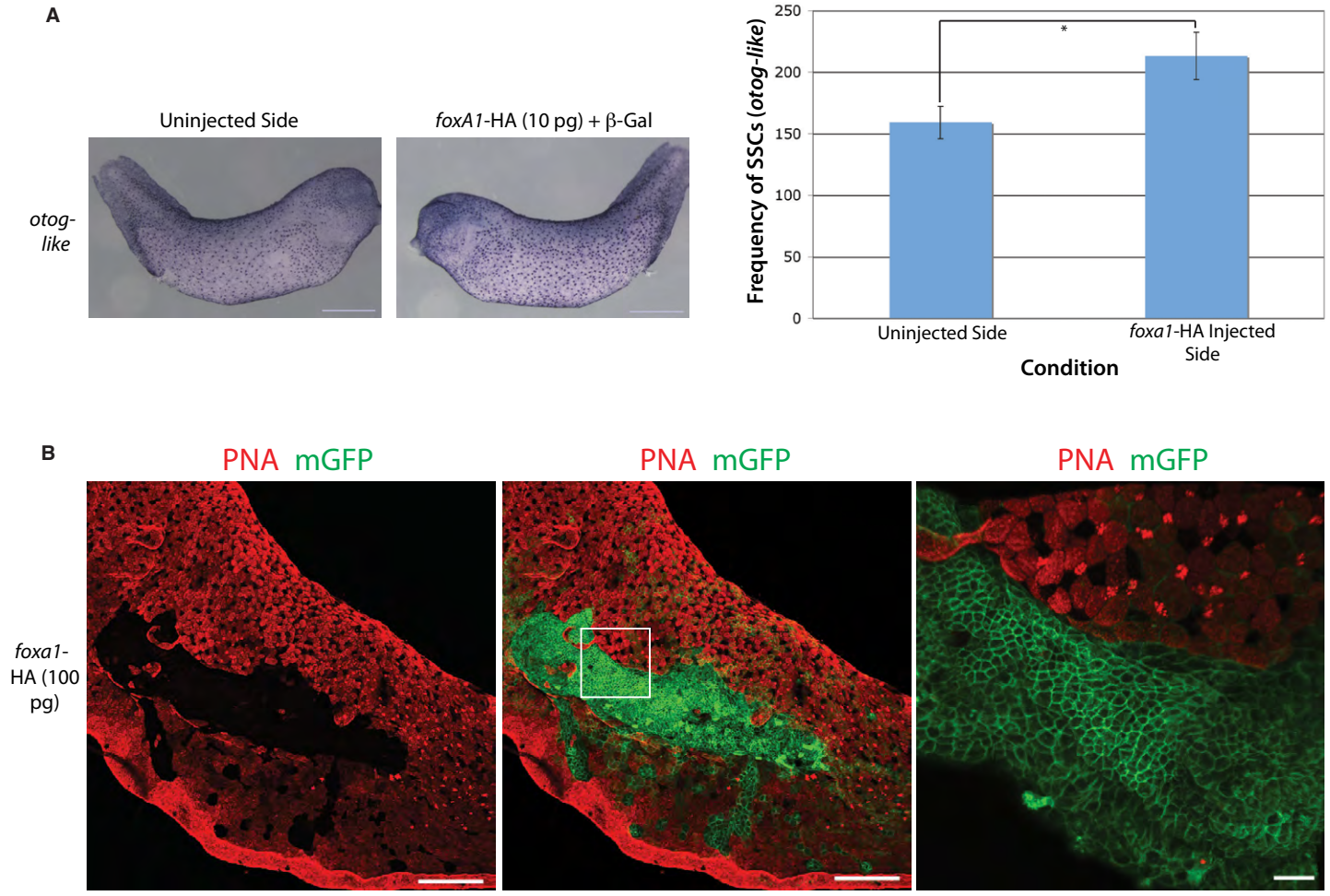
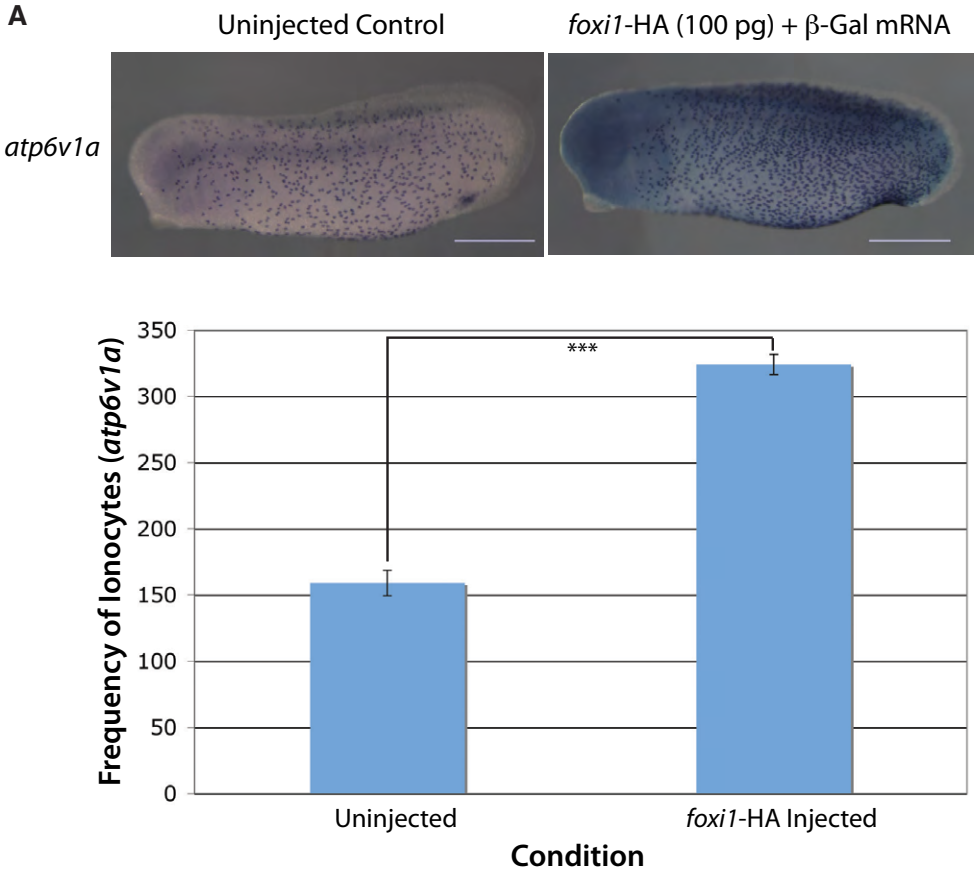


Fig. S5. *Foxa1-HA* misexpression. (A) Representative images showing misexpression of 10 pg HA-tagged *foxa1* mRNA (and lineage tracer *β-gal* mRNA) in one side of the embryo and probed by *in situ* hybridization for SSC marker, *otog-like*, at stage 33. Chart shows comparison of mean number of SSCs in a defined area of the flank epidermis (*otog-like* positive) on injected and uninjected sides (n=9 embryos). Error bars represent s.e.m. Student's t-test, $P<0.05$ (*). Scale bars: 500 µm. (B) Representative image showing embryo at stage 33 injected with higher dose (100 pg) of *foxa1-HA* mRNA and lineage tracer, membrane GFP (mGFP, green). PNA (red) is largely absent from areas where mGFP and *foxa1-HA* are misexpressed. The mGFP cells are numerous and form 'masses'. Right panel is a higher magnification image of area enclosed in white box in the middle panel. Scale bars: 200 µm (left and middle panel) and 30 µm (right panel).



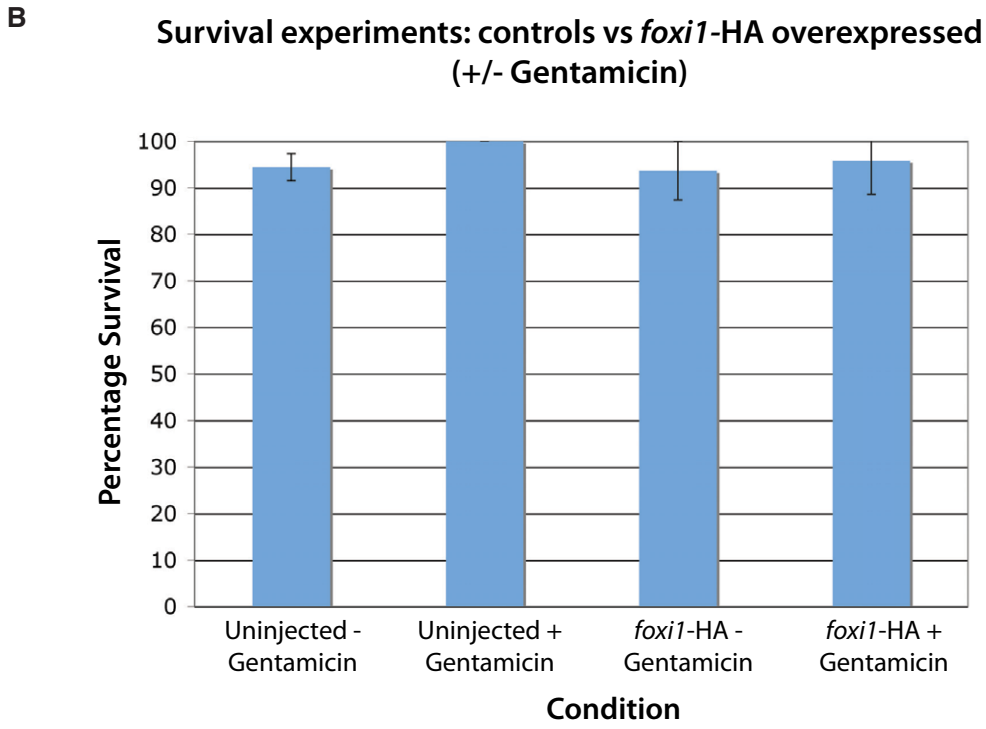


Fig. S6. *Foxi1-HA* misexpression increases ionocytes but does not affect survival. (A) Representative images of an uninjected control and an embryo injected at the one cell stage with *foxi1-HA* mRNA (100 pg) and *β-gal* mRNA. Embryos were fixed at stage 30 and probed for the ionocyte marker gene, *atp6v1a*. Chart shows comparison of mean number of *atp6v1a* positive cells in a defined area on the flank epidermis of controls and *foxi1-HA* overexpressed embryos (n=5 embryos each). (B) Chart showing survival rates of controls and *foxi1-HA* misexpressed embryos both with and without supplementing with the antibiotic, Gentamicin (10 µg/ml). Results of three independent experiments. Error bars represent s.e.m. Student's t-test, P<0.001 (***). Scale bars: 500 µm

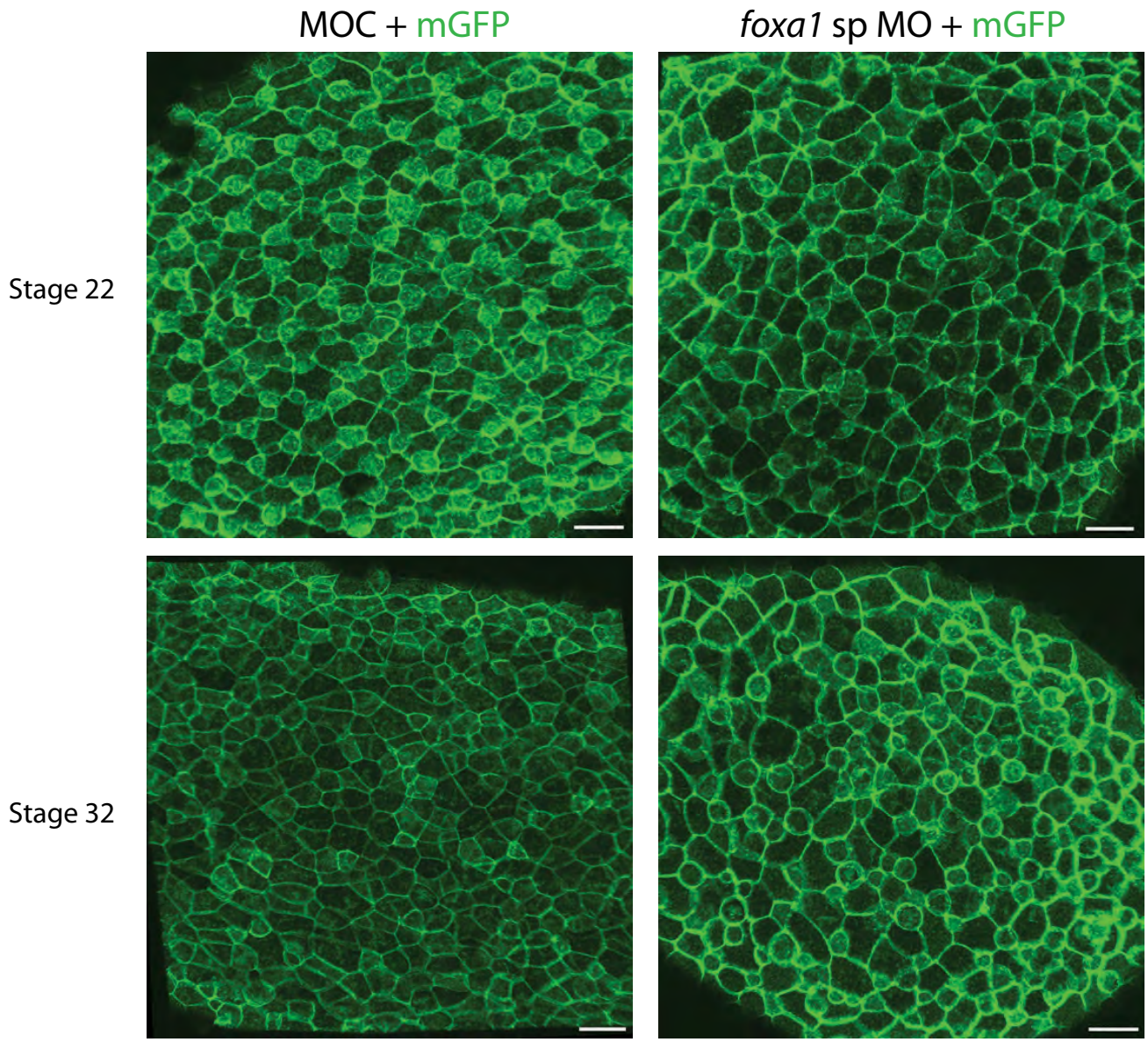


Fig. S7. Impact of *foxa1* knockdown on epidermal integrity. Representative images of MOC-treated and *foxa1* MO-treated embryos expressing membrane GFP at stage 22 and stage 32. Scale bars: 30 μm

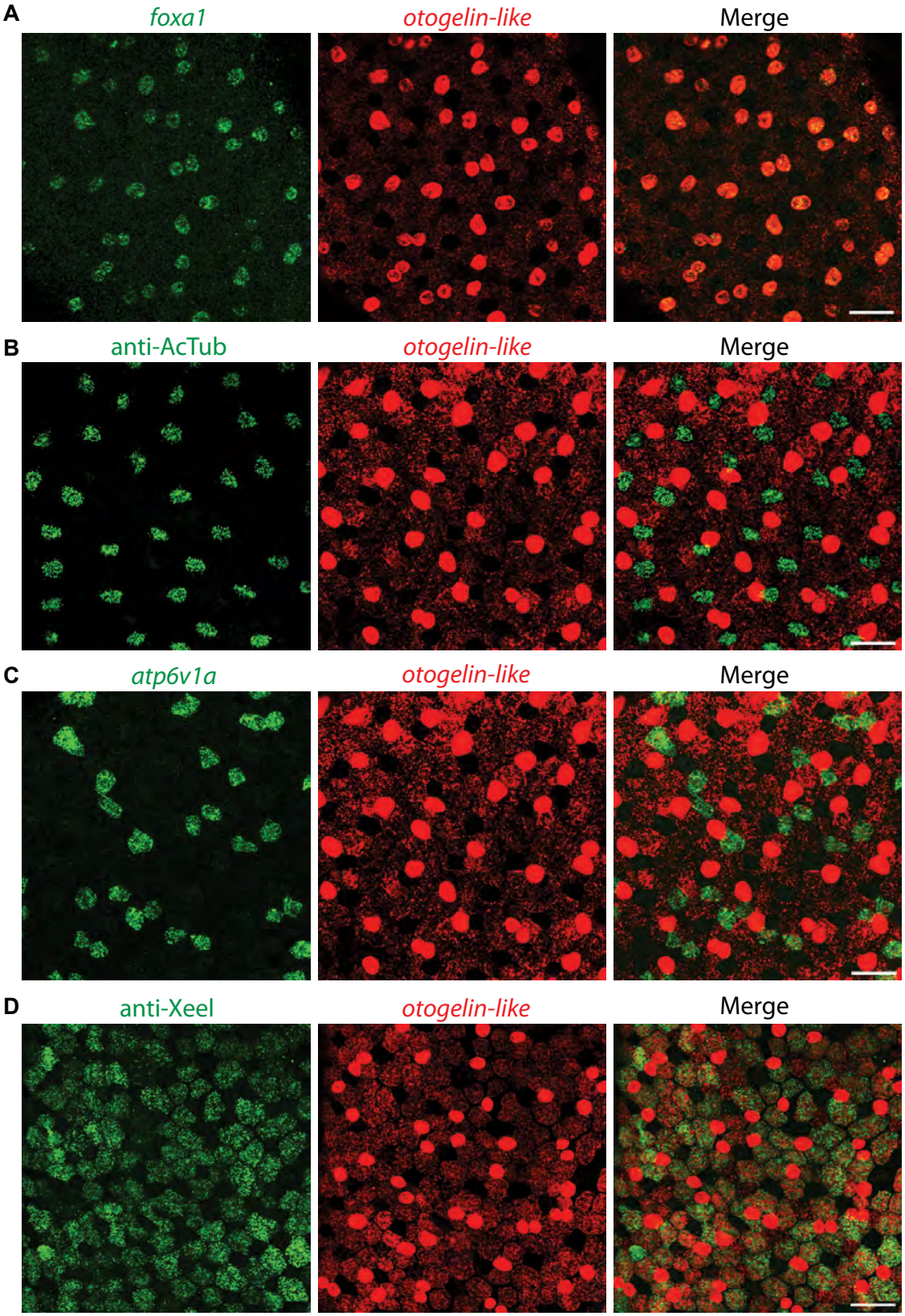


Fig. S8. Comparison of expression of *otogelin-like* with other epidermal markers. (A) Double fluorescent *in situ* hybridization for *foxa1* and *otogelin-like*. *Otogelin-like* co-localizes with *foxa1* in SSCs. (B) Fluorescent *in situ* hybridization for *otogelin-like* and antibody staining for the ciliated cell marker acetylated α-tubulin. *Otogelin-like* is not expressed in ciliated cells. (C) Double fluorescent *in situ* hybridization for *otogelin-like* and the ionocyte marker, *atp6v1a*. *Otogelin-like* shows low-level expression in ionocytes. (D) Fluorescent *in situ* hybridization for *otogelin-like* and antibody staining for the goblet cell marker, Xeel. *Otogelin-like* is expressed in goblet cells but at a lower level than in the SSCs. All images show embryos fixed at stage 32. Scale bars; 50 μm.

Table S1. Wild type with cement gland

Download Table S1