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Figures and figure supplements

Sox10-dependent neural crest origin of olfactory microvillous neurons in zebrafish

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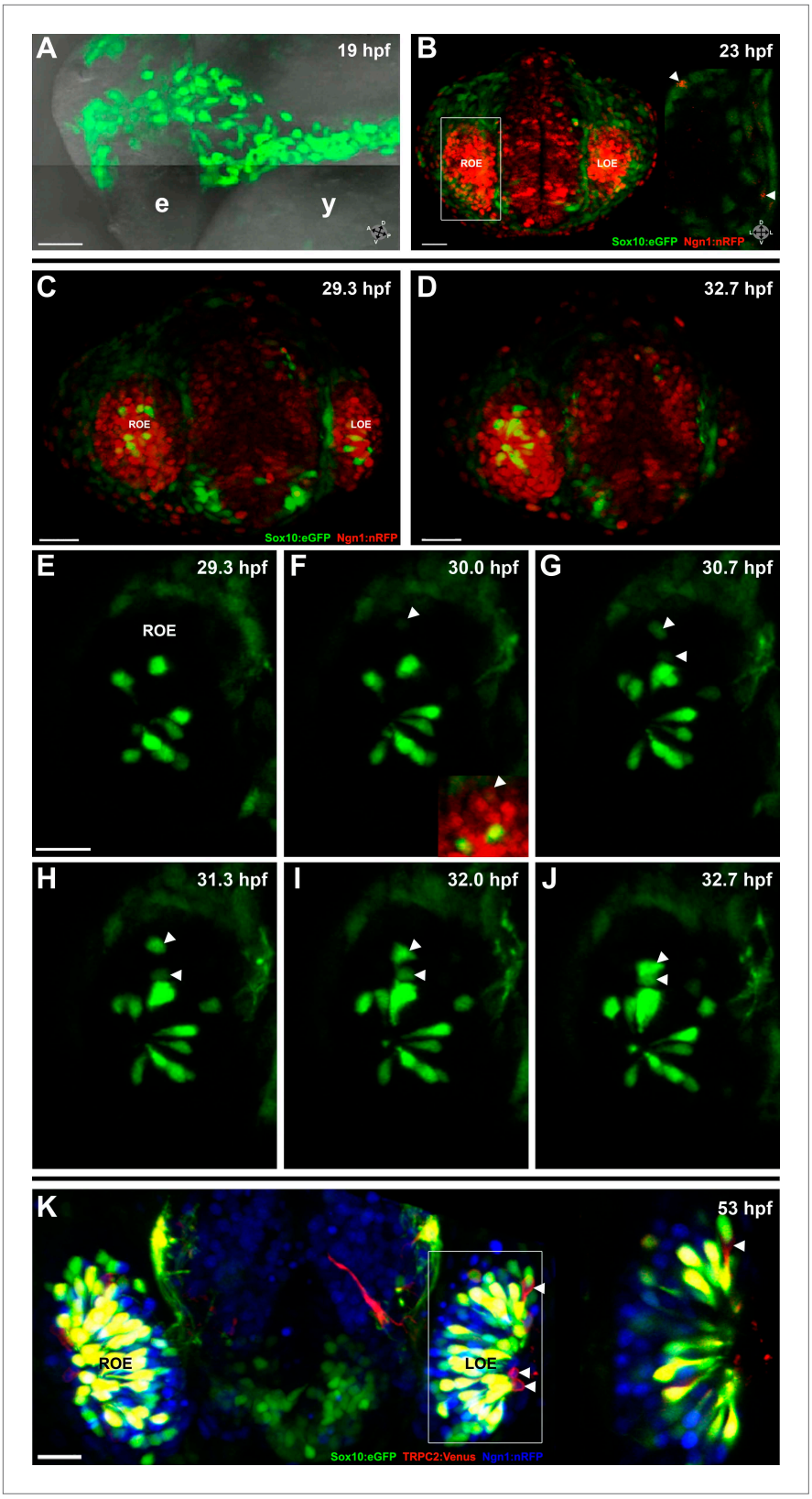

Figure 1. Sox10:eGFP^{+}/Ngn1:nRFP^{+} cells migrate and differentiate into microvillous neurons. (**A**) Sox10:eGFP^{+} neural crest migrates dorsal to the eye and toward the olfactory region at 19 hpf as the olfactory placode is first

Figure 1. Continued on next page

Figure 1. Continued

apparent. (**B**)–(**D**) Time-lapse confocal microscopy of live embryos (Sox10:eGFP^{+}; Ngn1:nRFP$^{+)}$ demonstrates that a subset of nasal cavity cells that express Ngn1:nRFP (**B**, boxed area arrowheads; z = 3.5 μm) ingress into the olfactory epithelium. (**E**)–(**J**) Selected time point and z-plane excerpts from full z stacks are shown from ~29 hpf (**C**) to ~33 hpf (**D**) and are 40' apart; z = 17.5 μm (consisting of five 3.5 μm slices). Arrowheads indicate two ingressing cells, one from the top and the other directly behind the olfactory epithelium. All ingressing cells express Ngn1:nRFP (**F**, inset arrowhead). Sox10:eGFP, green; Ngn1:nRFP, red. (**K**) At 53 hpf, all Sox10:eGFP^{+} cells in the olfactory epithelium are TRPC2:Venus^{+} microvillous neurons. (because eGFP signal bleeds into the Venus channel, overlap was confirmed via image processing; see ***Figure 1—figure supplement 1***). Only a small number of microvillous neurons are not Sox10:eGFP^{+} (arrowheads). A single 3.5-μm-thick z-plane slice of the boxed area more clearly shows colocalization. Sox10:eGFP: green; TRPC2:Venus: red; Ngn1:nRFP: blue. e: eye; y: yolk; LOE: left olfactory epithelium; ROE: right olfactory epithelium. Orientation arrows: A: anterior; P: posterior; D: dorsal; V: ventral; L: lateral. Scale bars: 50 μm (**A**); 30 μm (**B**–**D**); 20 μm (**E**–**K**). See also ***Figure 1—figure supplements 1 and 2*** and ***Videos 1 and 2***.

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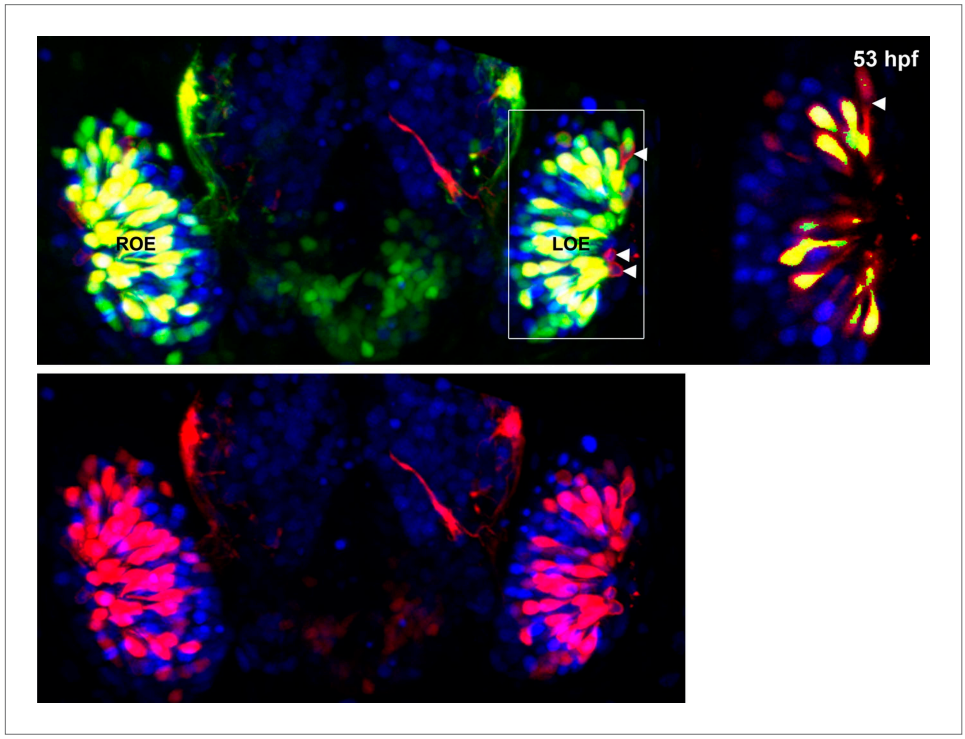

Figure 1—figure supplement 1. Panel (K) from ***Figure 1*** with Sox10:eGFP (green) channel removed (bottom) to better illustrate the large population of ciliated neurons present basally that are not Sox10:eGFP^{+} but are Ngn1:nRFP^{+} (blue). High magnification inset (right) has Sox10:eGFP signal artificially underexposed and decreased to better view the colocalization with membrane TRPC2:Venus expression.

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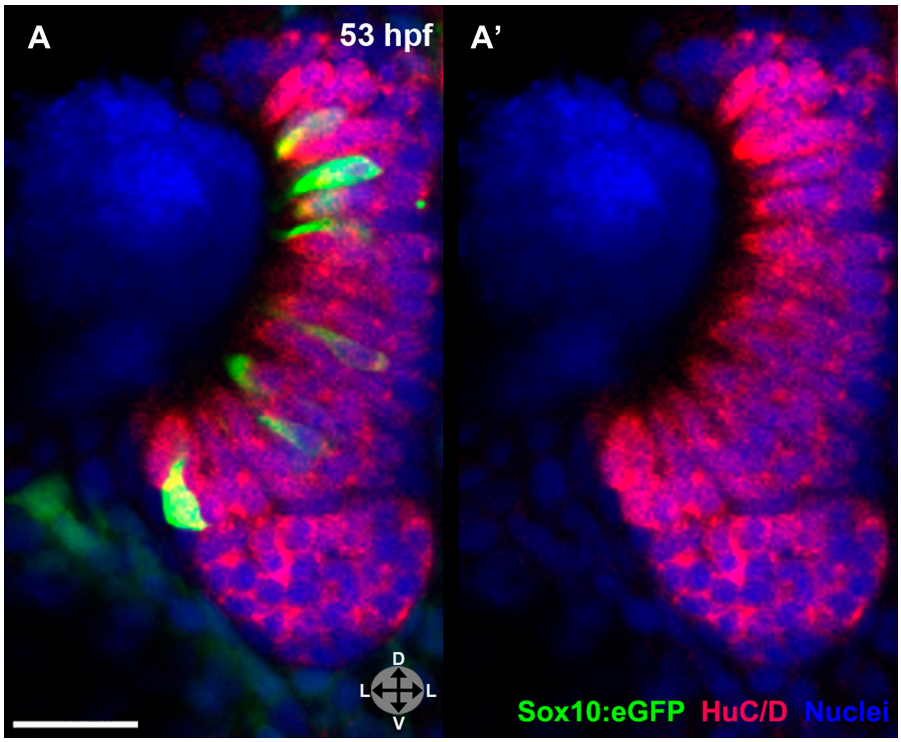

Figure 1—figure supplement 2. (A) and (A') Sox10:eGFP^{+} microvillous neurons in fixed embryos stained with anti-GFP antibody colocalize with anti-HuC/D antibody staining at 53 hpf, confirming post-mitotic neuronal identity. Sox10:eGFP: green; HuC/D: red; nuclear stain: blue. Orientation arrows: D: dorsal; V:ventral; L: lateral. z = 2.5 μm. Scale bar: 20 μm.

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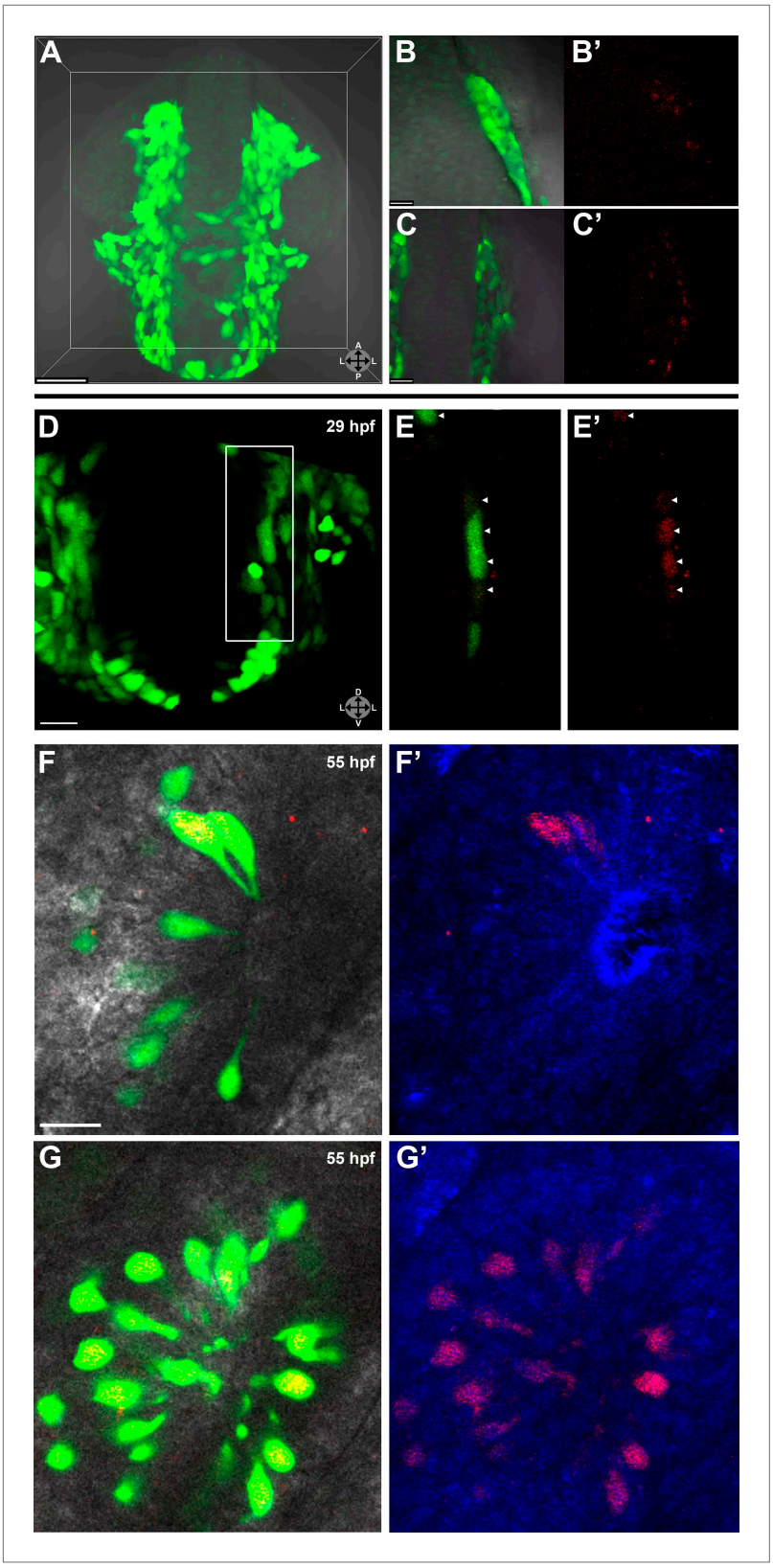

Figure 2. Lineage tracing by photoconversion demonstrates neural crest origin of microvillous neurons. (**A**) Nuclear Dendra2 (low level green) was photoconverted in Sox10:eGFP^{+} (bright green) neural crest cells at 14–16 hpf

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Figure 2. Continued

(10–14 somite stage) in live embryos. (**B**)–(**C'**) Representative example z-planes show unilateral photoconversion of a few cells (**B** and **B'**) or a large number of cells (**C** and **C'**). (**B** and **C**) show ubiquitous Dendra2 (low level green) and Sox10:eGFP (bright green), and (**B'** and **C'**) show photoconverted Dendra2 (red). (**D**) Photoconverted cells are visible in the contralateral nasal cavity at 29 hpf; (**E** and **E'**) show a 2.5-μm-thick z-plane slice of the boxed area with photoconverted cells (red, arrowheads). (**F** and **F'**) At 55 hpf, photoconversion of a small number of cells (**B** and **B'**) results in a subset of Sox10:eGFP^{+} microvillous neurons being labeled by photoconverted Dendra2 on the contralateral side. (**G** and **G'**) Large-scale photoconversion (**C** and **C'**) labels most Sox10:eGFP^{+} microvillous neurons. Directly adjacent ciliated neurons are never labeled. (**F**–**G'**) z = 2.5 μm; Sox10:eGFP: green; Dendra2$_{Green}$: blue; Dendra2$_{Red}$: red; histology in brightfield. Orientation arrows: A: anterior; P: posterior; D: dorsal; V: ventral; L: lateral. Scale bars: 50 μm (**A**); 20 μm (**B**–**D**); 10 μm (**F**–**G'**). See also ***Figure 2—figure supplement 1***.

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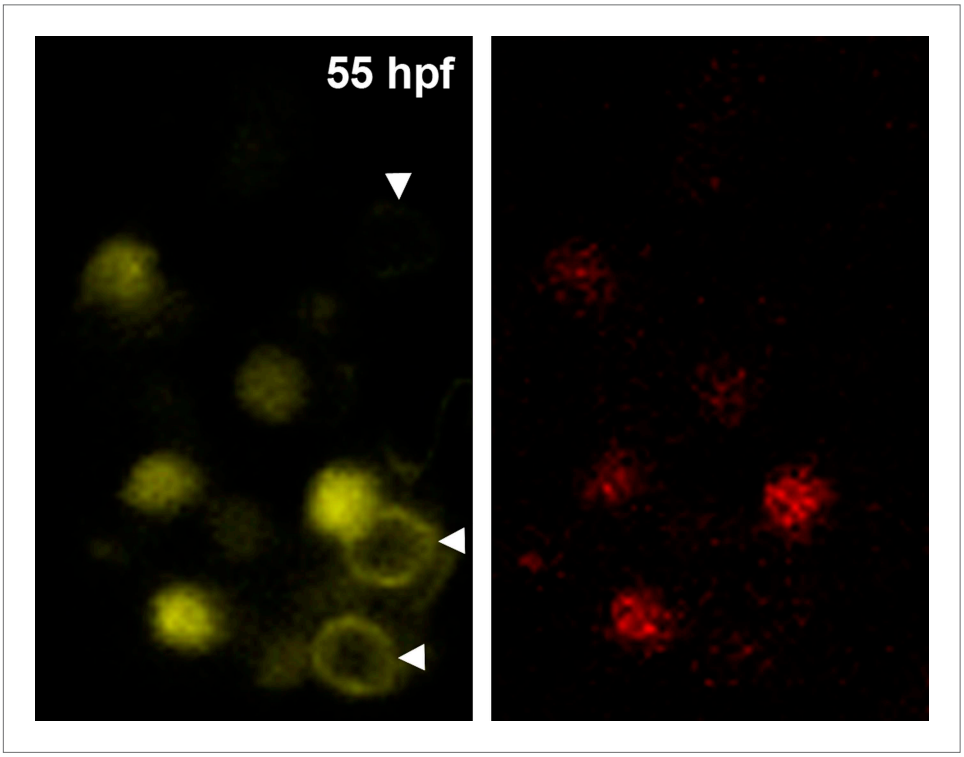

Figure 2—figure supplement 1. Shown are a few 55 hpf microvillous neurons that are Sox10:eGFP negative (arrowheads) and were not photoconverted, suggesting a possible non-neural crest origin.

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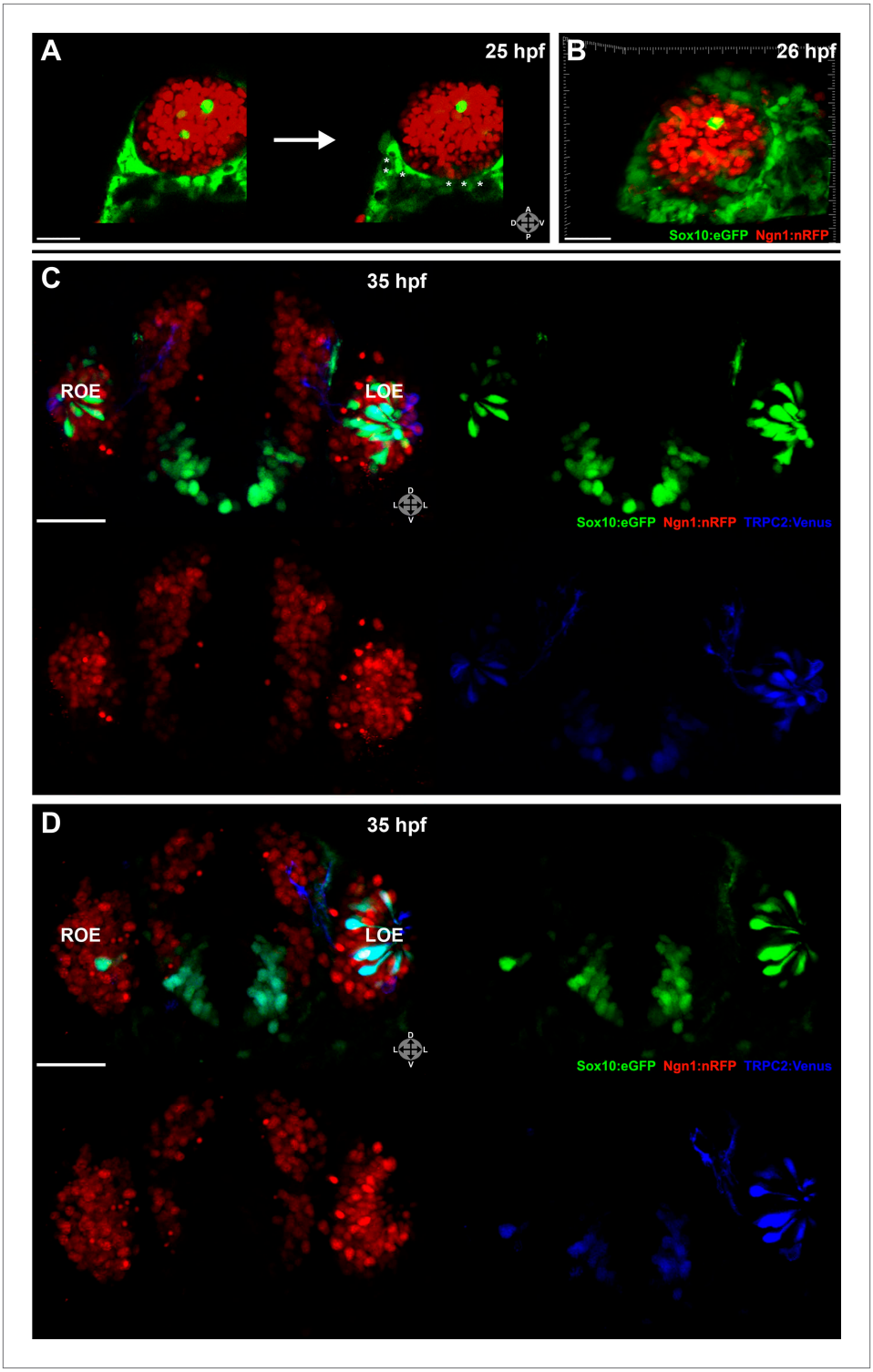

Figure 3. Laser ablation of neural crest in the nasal cavity inhibits microvillous neurogenesis. (**A**) Immediately before and after ablation of Sox10:eGFP^{+}/Ngn1:nRFP^{+} cells in the right nasal cavity in live embryos; example z-plane slice shows six regions of ablation (asterisks). (**B**) 3D z-stack post-ablation of several z-planes with total of ≥20 Sox10:eGFP^{+}/Ngn1:nRFP^{+} ablated cells. (**C**) and (**D**) 35 hpf embryos show a significant decrease in the number of Sox10:eGFP^{+}/TRPC2:Venus^{+} microvillous neurons within the right olfactory epithelium as compared to the unablated left side in both small (**C**) and large (**D**) ablation experiments. Directly adjacent ciliated neurons are only marginally affected. Sox10:eGFP: green; Ngn1:nRFP: red; TRPC2:Venus: blue. LOE: left olfactory epithelium;
Figure 3. Continued on next page

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ROE: right olfactory epithelium. Orientation arrows: A: anterior; P: posterior; D: dorsal; V: ventral; L: lateral. Scale bars: 30 μm (**A** and **B**); 40 μm (**C** and **D**).

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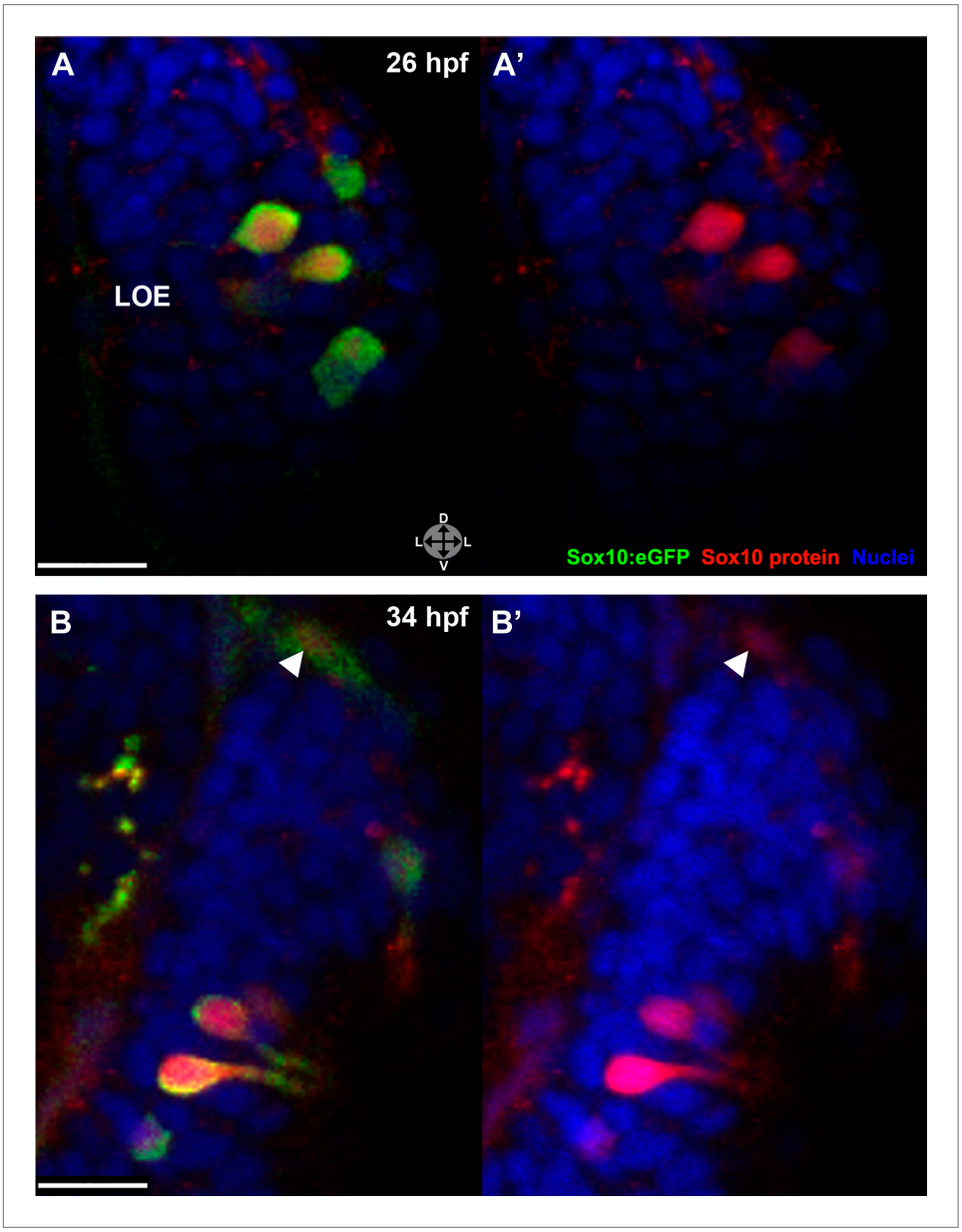

Figure 4. Sox10 protein is preferentially upregulated in neural crest cells that differentiate into microvillous neurons. (**A**)–(**B'**) Sox10:eGFP^{+} differentiating microvillous neurons in fixed embryos stained with anti-GFP antibody colocalize with anti-Sox10 antibody staining at 26 hpf (**A** and **A'**) and 34 hpf (**B** and **B'**). Levels of eGFP and Sox10 mirror each other, both increasing as cells ingress and become neurons. Arrowhead in (**B** and **B'**) indicates a nasal cavity cell likely about to ingress that has increased Sox10 protein expression. Sox10:eGFP: green; Sox10 protein: red; nuclear stain: blue; LOE: left olfactory epithelium. Orientation arrows: D: dorsal; V: ventral; L: lateral. z = 2.5 μm. Scale bars: 15 μm (**A**–**B**); 30 μm (**C**).

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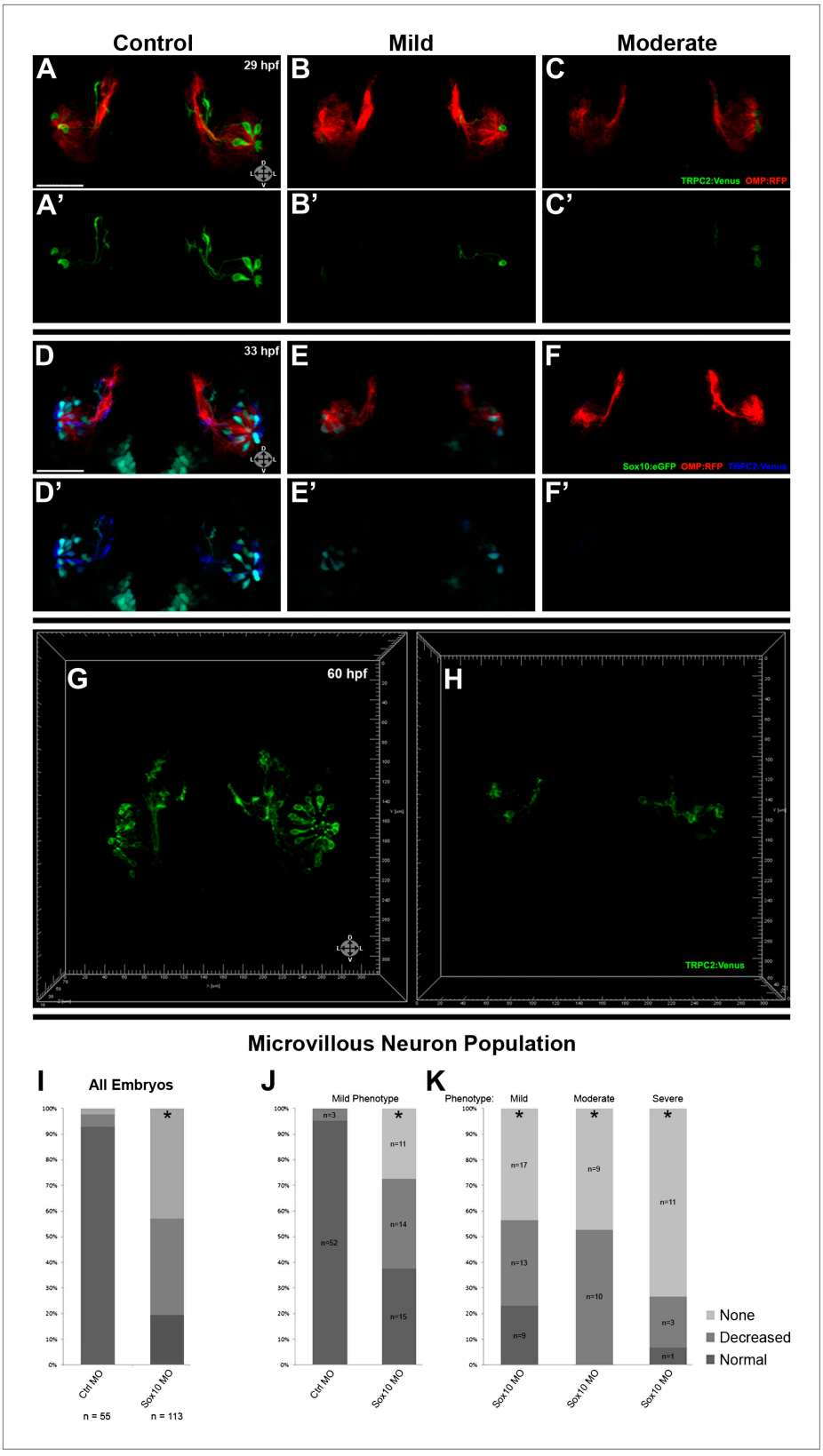

Figure 5. Morpholino knockdown of Sox10 selectively inhibits microvillous neurogenesis. (**A**) and (**A'**) Control morpholino-injected live embryos at 29 hpf have ciliated (red) and microvillous (green) neurons. (**B**)–(**C'**) Embryos

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Figure 5. Continued

with mild and moderate phenotypes (assayed histologically) display only slight changes in ciliated neurons, whereas microvillous neurons are significantly decreased in number and organization. (**D**)–(**F'**) Similar results are seen at 33 hpf, with almost no Sox10:eGFP^{+} (green) cells becoming microvillous (blue) neurons. Ciliated (red) neurons remain relatively unaffected. (**G**) and (**H**) Antibody staining with anti-GFP against TRPC2:Venus in fixed embryos demonstrates a persistent decrease in the number of microvillous cells and disorganization at 60 hpf in Sox10 morpholino-treated embryos (**H**) in comparison to control embryos (**G**). All images were captured at identical settings to facilitate direct comparison of control and experimental embryos. (**I**)–(**K**) All embryos (**I**) were divided into 'high pigmentation' (**J**) or 'low pigmentation' (**K**) to roughly correlate Sox10 levels (higher and lower, respectively) with degree of olfactory phenotype. As expected, all control morpholino-treated embryos have high pigmentation, and all Sox10 morpholino-treated embryos with high pigmentation have only a mild phenotype. In all cases (**J** and **K**), Sox10 morpholino treatment results in most embryos having either no microvillous neurons or a decreased number. Ratios correlate with degrees of pigmentation and phenotype. (**A**–**C'**, **G**, and **H**) TRPC2:Venus: green; OMP:RFP: red. (**D**–**F'**) Sox10:eGFP: green; OMP:RFP: red; TRPC2:Venus: blue. Orientation arrows: D: dorsal; V: ventral; L: lateral. Scale bars: 50 μm. *p<0.001. See also ***Figure 5—figure supplement 1***.

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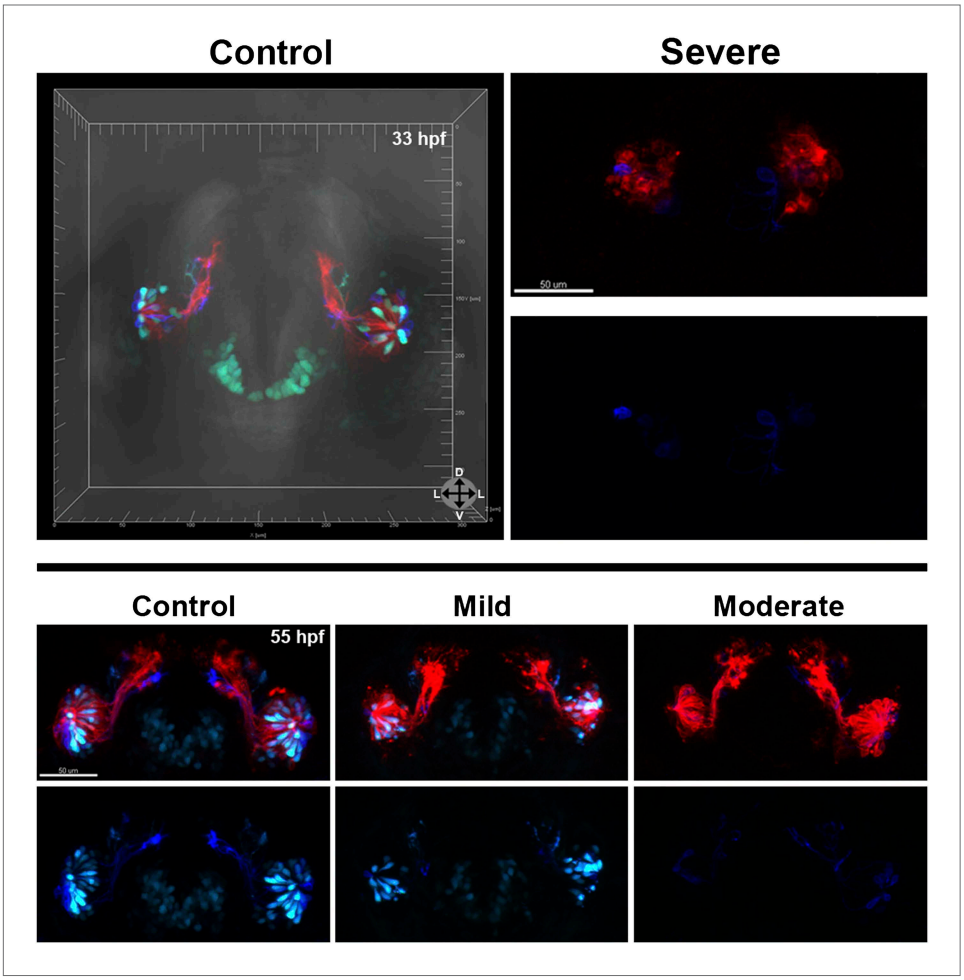

Figure 5—figure supplement 1. At 33 hpf, severe phenotype embryos (top right) have major malformations in both microvillous and ciliated neurons and often perish, likely reflecting non-specific effects. At 55 hpf (bottom) are shown the same embryos as in ***Figure 5 (D–F')***. At 55 hpf (bottom) are shown the same embryos as in ***Figure 5*** (**D**–**F'**). While there is some recovery of microvillous neurons in 'mild' embryos, their numbers remain decreased in comparison to controls. 'Moderate' embryos have near complete lack of neural crest-derived microvillous neurons, whereas ciliated neurons are present and project to the olfactory bulb.

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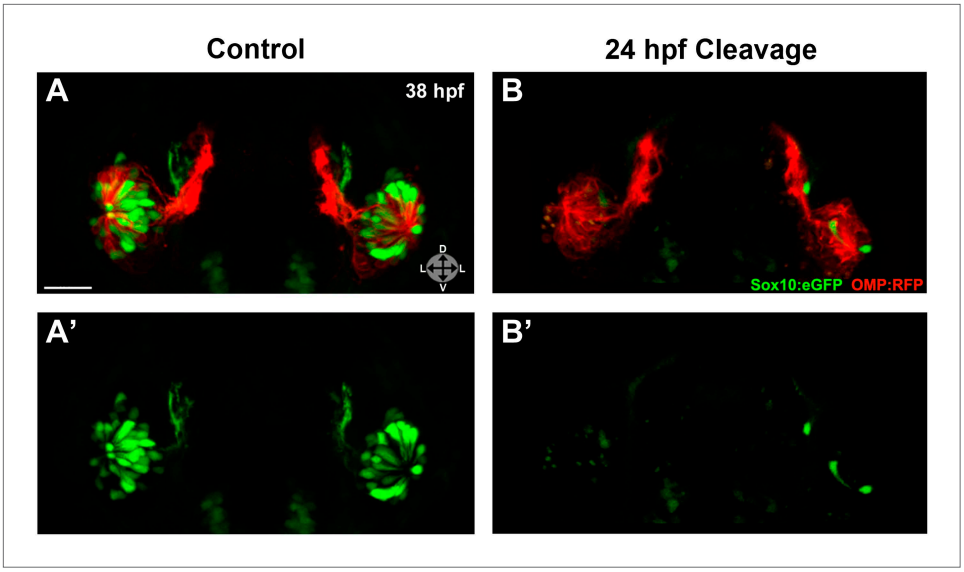

Figure 6. Photo-morpholino knockdown of Sox10 demonstrates its necessity during the ingression/differentiation process. (**A**) and (**A'**) Control (antisense morpholino + sense photo-morpholino injected but not photocleaved) live embryos have robust numbers of ciliated (red) and neural crest–derived microvillous (green) neurons at 38 hpf. In contrast, identically injected embryos subjected to photocleavage at 24 hpf go on to develop ciliated neurons but significantly lack microvillous neurons at 38 hpf (**B** and **B'**) in comparison to control embryos. Sox10:eGFP: green; OMP:RFP: red. Orientation arrows: D: dorsal; V: ventral; L: lateral. Scale bars: 30 μm. See also ***Figure 6—figure supplement 1***.

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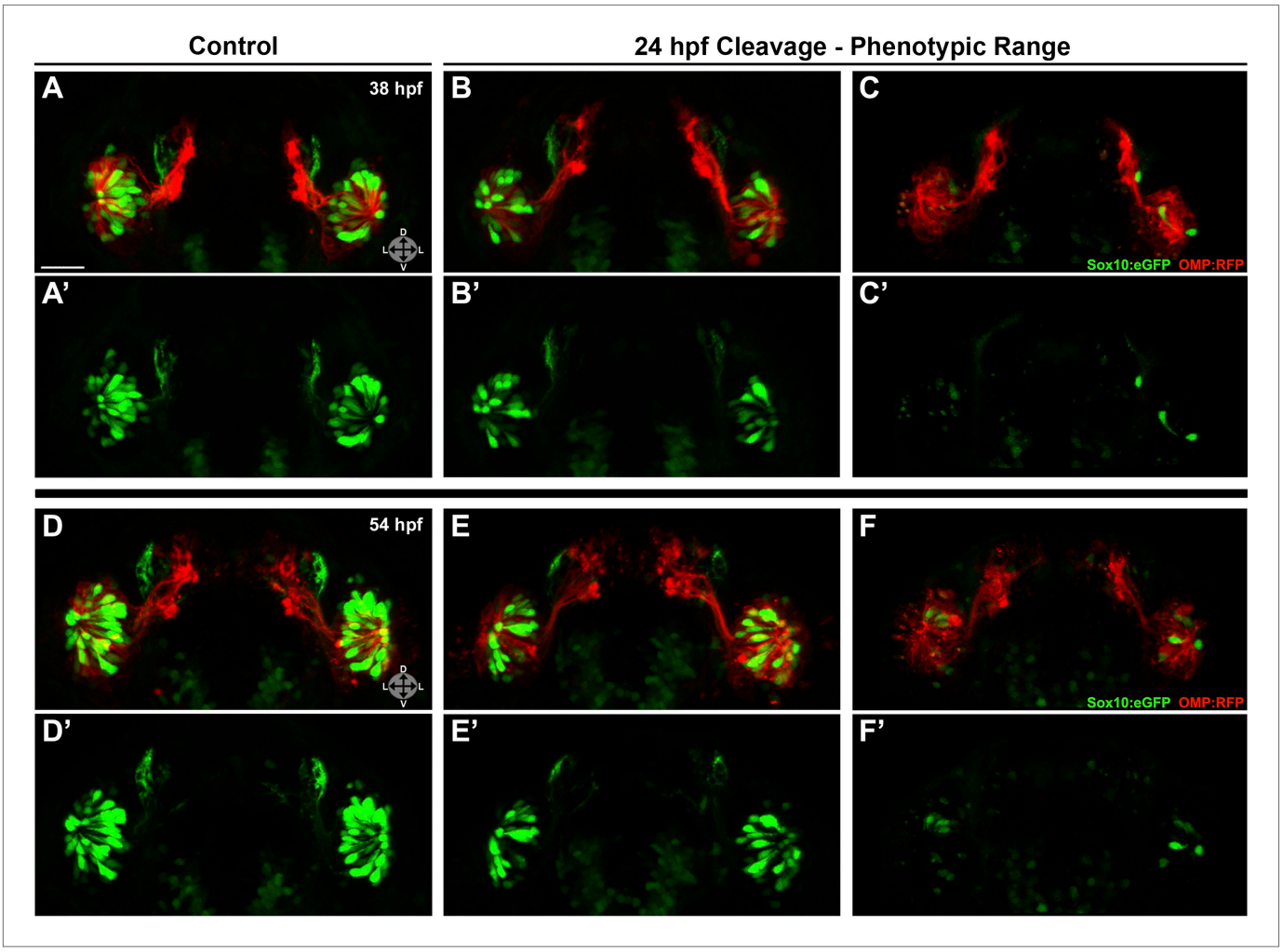

Figure 6—figure supplement 1. Shown are more details of the phenotype in ***Figure 6***: (A and A') control (antisense morpholino + sense photo-morpholino injected but not photocleaved) live embryos have robust numbers of ciliated (red) and neural crest–derived microvillous (green) neurons at 38 hpf. In contrast, identically injected embryos subjected to photocleavage at 24 hpf go on to develop ciliated neurons but have slight (B and B') or, much more commonly, large (C and C') decreases in microvillous neuron numbers and organization in comparison to control embryos. Photocleavage may have been less efficient in some embryos, resulting in a range of phenotypic severity. (**D**–**F'**) similar results are seen in the same embryos at 54 hpf, now with an even greater difference in microvillous neuron numbers between control and cleaved embryos. Sox10:eGFP: green; OMP:RFP: red. Orientation arrows: D: dorsal; V: ventral; L: lateral. Scale bars: 30 μm.

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