SHORT REPORT

Figures and figure supplements

Gill developmental program in the teleost mandibular arch

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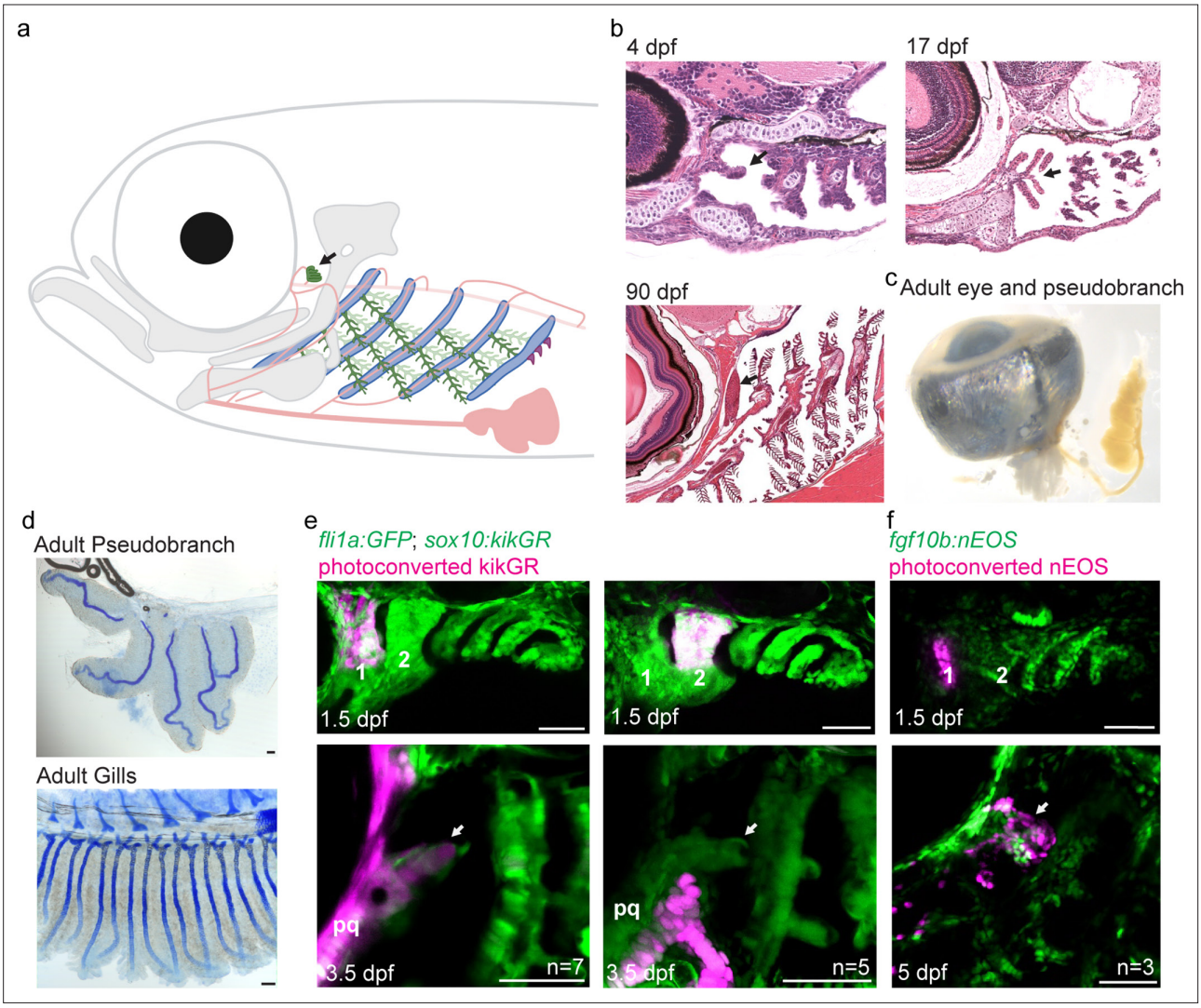

Figure 1. The zebrafish pseudobranch derives from mandibular arch mesenchyme and first pouch epithelia. (**a**), Schematic showing the pseudobranch (arrows), gill filaments (branched green structures) connected to gill bars (blue), teeth (purple), vasculature (pink), and jaw and jaw-support skeleton (gray). (**b**) Hematoxylin and Eosin-stained sections show emergence of the pseudobranch bud at 4 dpf (adapted from https://bio-atlas.psu.edu/zf/view.php?atlas=5&s=41), five filaments at 17 dpf (adapted from https://bio-atlas.psu.edu/zf/view.php?atlas=65&s=1738), and the fused pseudobranch at 90 dpf (adapted from https://bio-atlas.psu.edu/zf/view.php?atlas=29&s=312). (**c**) Dissected adult pseudobranch shows the ophthalmic artery connecting it to the eye. (**d**) Alcian staining shows five cartilage rods in the pseudobranch and similar cartilage in gill primary filaments. (**e**) Photoconverted kikGR-expressing mesenchyme (red) from the dorsal first arch (numbered) at 1.5 dpf contributes to the palatoquadrate cartilage (pq) and pseudobranch mesenchyme (arrow) at 3.5 dpf. Photoconverted dorsal second arch cells do not contribute to the pseudobranch. In green, *fli1a:GFP* labels the vasculature and neural crest-derived mesenchyme, with mesenchyme also labeled by unconverted *sox10:kikGR*. (**f**) In *fgf10:nEOS* embryos, photoconversion of first pouch endoderm (numbered) at 1.5 dpf labels the pseudobranch epithelium (arrow) at 5 dpf. n numbers denote experimental replicates in which similar contributions were observed. Scale bars, 50 μm.

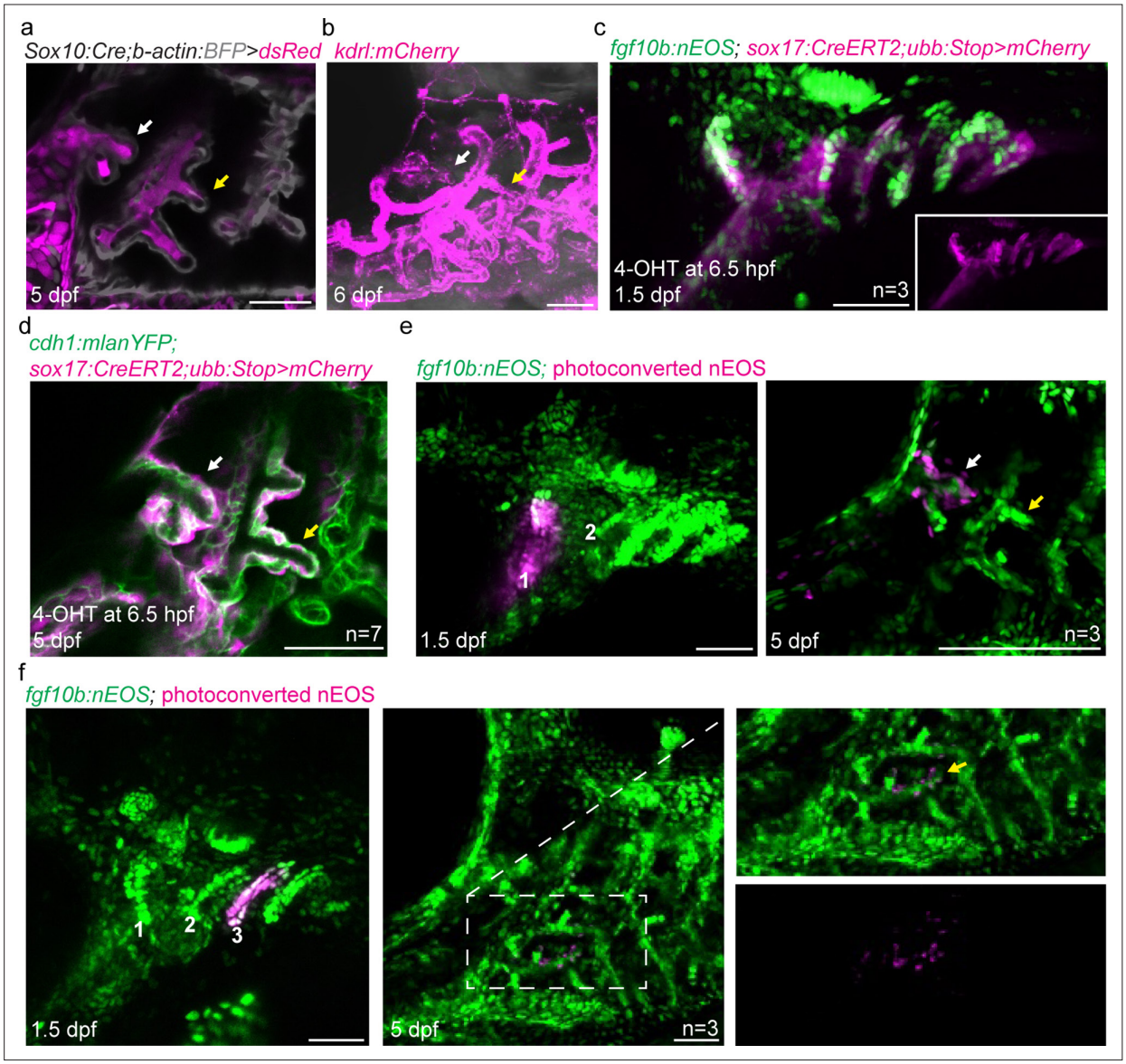

Figure 1—figure supplement 1. Development of zebrafish pseudobranch and lineage analysis of gill filament epithelia. (**a**) In *Sox10:Cre; acta2:loxP-BFP-Stop-loxP-dsRed* fish at 5 dpf, the developing pseudobranch (white arrow) and gill buds (yellow arrow) consist of Cre-converted dsRed+ neural crest-derived mesenchyme (magenta) and unconverted BFP+ epithelia (gray). (**b**) At 6 dpf, *kdrl:mCherry* labeling of vasculature reveals a branch of the first aortic arch in the position of the pseudobranch (white arrow), and branches of the posterior aortic arches in the positions of the gills (yellow arrow). (**c**) Endoderm is labeled in red by adding 4OH-tamoxifen to *sox17:CreERT2; ubb:loxP-Stop-loxP-mCherry* fish at 6.5 hr post-fertilization to induce Cre recombination that removes the Stop cassette and allows mCherry expression (mCherry channel alone shown in inset). Co-localization shows *fgf10b:nEOS* expression (green) in endodermal pouches. (**d**), Endoderm labeling by addition of 4OH-tamoxifen to *sox17:CreERT2; ubb:loxP-Stop-loxP-mCherry* fish at 6.5 hr post-fertilization results in contribution to *cdh1:mlanYFP+* pseudobranch epithelium at 5 dpf. (**e,f**) In *fgf10:nEOS* embryos, photoconversion of first pouch endoderm (and some more ventral mandibular cells) at 1.5 dpf labels pseudobranch (white arrow) but not gill epithelia (yellow arrow) at 5 dpf in (**e**), and conversion of third pouch cells labels the first gill filament epithelium (yellow arrow, boxed region magnified to right and shown in merged and red-only channels) in (**f**). Scale bars, 50 μM.

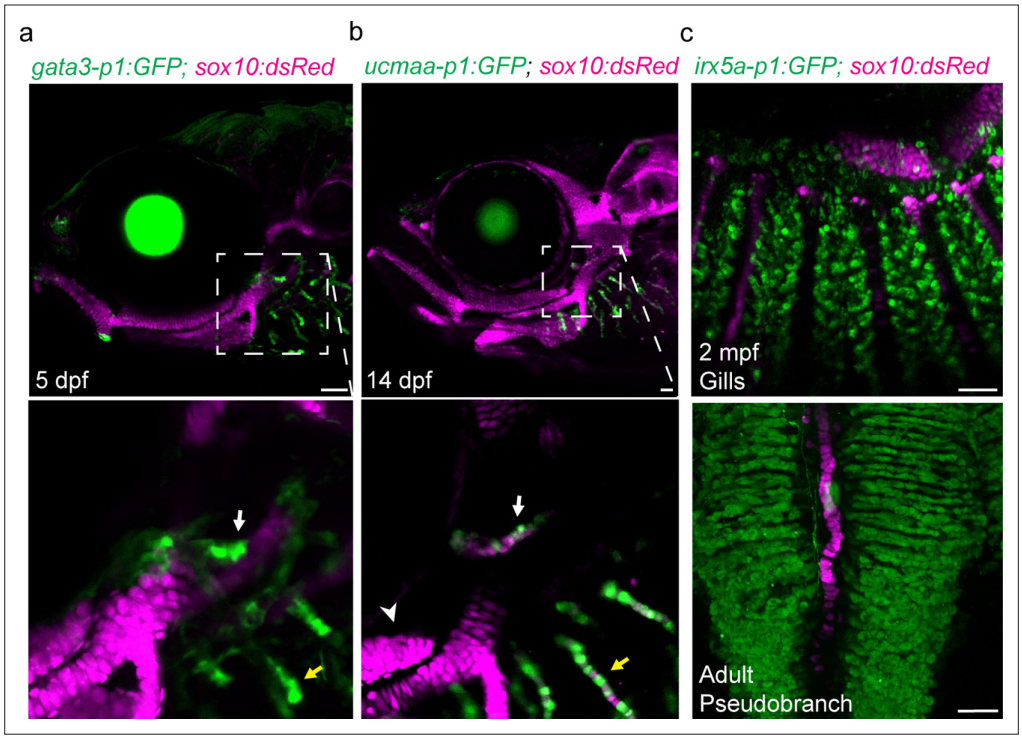

Figure 2. Shared regulatory program for pseudobranch and gill development. (**a-c**) In the pseudobranch (white arrows) and gill filaments (yellow arrows), *gata3-p1:GFP* labels growing buds, *ucmaa-p1:GFP* labels cellular cartilage (distinct from hyaline cartilage, arrowhead), and *irx5a-p1:GFP* labels pillar cells. *sox10:dsRed* labels cartilage for reference. Images in (**b**)and (**c**) are confocal projections, with magnified regions shown below in single sections for *gata3-p1:GFP* and *ucmaa-p1:GFP*. Scale bars, 50 μM.

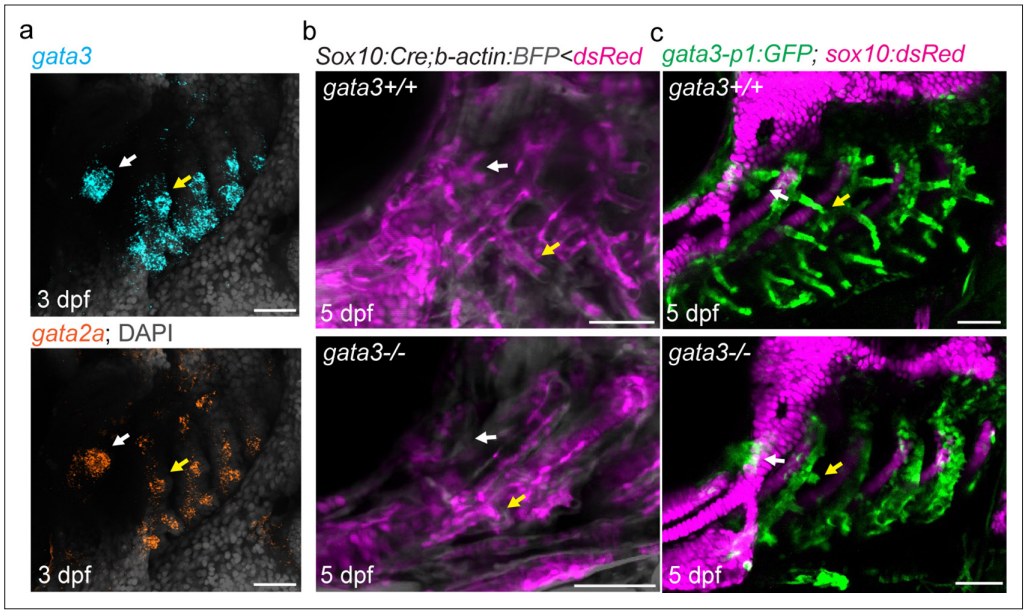

Figure 3. Pseudobranch and gill development requires *gata3* function. (**a**) Similar expression of *gata3* and *gata2a* in developing pseudobranch (white arrows) and gill regions (yellow arrows). (**b**) *Sox10:Cre; acta2:loxP-BFP-Stop-loxP-dsRed* labels Cre-converted dsRed+ neural crest-derived mesenchyme (magenta) and unconverted BFP+ epithelia (gray). (**c**) *gata3-p1:GFP* labels pseudobranch and gill filament buds, and *sox10:dsRed* labels cartilage. For both (**b**) and (**c**), 3/3 *gata3* mutants displayed reduced formation of the pseudobranch (white arrows) and gill filaments (yellow arrows), compared to 3 controls each. Scale bars, 50 μM.

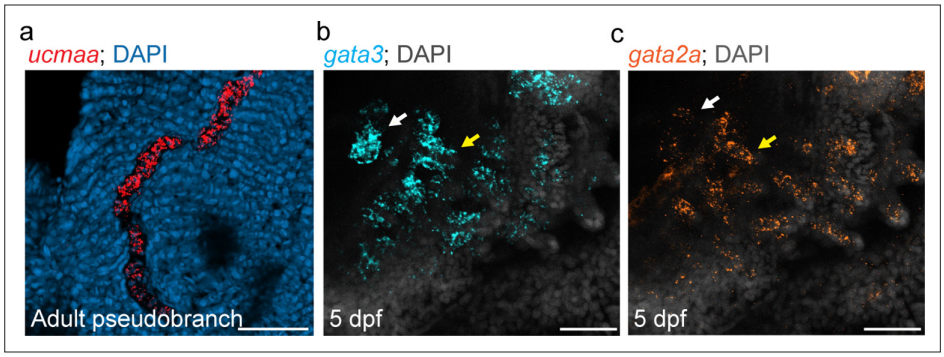

Figure 3—figure supplement 1. Pseudobranch shares gene expression with gill filaments. (**a**) Expression of *ucmaa* in pseudobranch cartilage from one-year-old fish. (**b,c**) Developing pseudobranch (white arrows) and gill buds (yellow arrows) express *gata3* and *gata2a* at 5 dpf. DAPI labels nuclei in blue (**a**) or white (**b,c**). Scale bars, 50 μM.

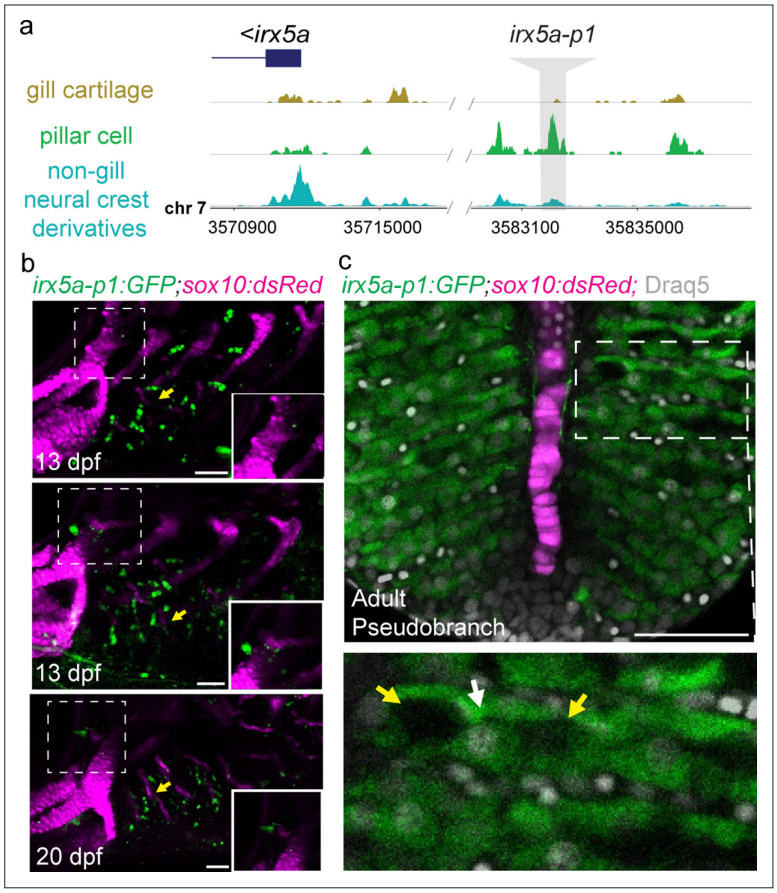

Figure 3—figure supplement 2. The pseudobranch shares *irx5a-p1* pillar cell enhancer activity with gill filaments. (**a**) An intergenic irx5a-p1 region displays accessible chromatin specifically in pillar cells at 60 dpf. (**b**) irx5a-p1 drives GFP expression in pillar cells of the developing gills (yellow arrows) and pseudobranch (insets). (**c,d**) Dissected pseudobranch from a 60 dpf *irx5a-p1:GFP* adult shows GFP-positive pillar cells adjacent to a core of filament cartilage. The boxed region magnified below depicts an individual pillar cell (white arrow) flanked by characteristic lacunae (yellow arrows). Cartilage is labeled by *sox10:dsRed* in (**b**) and (**c**). Scale bars, 50 μM.