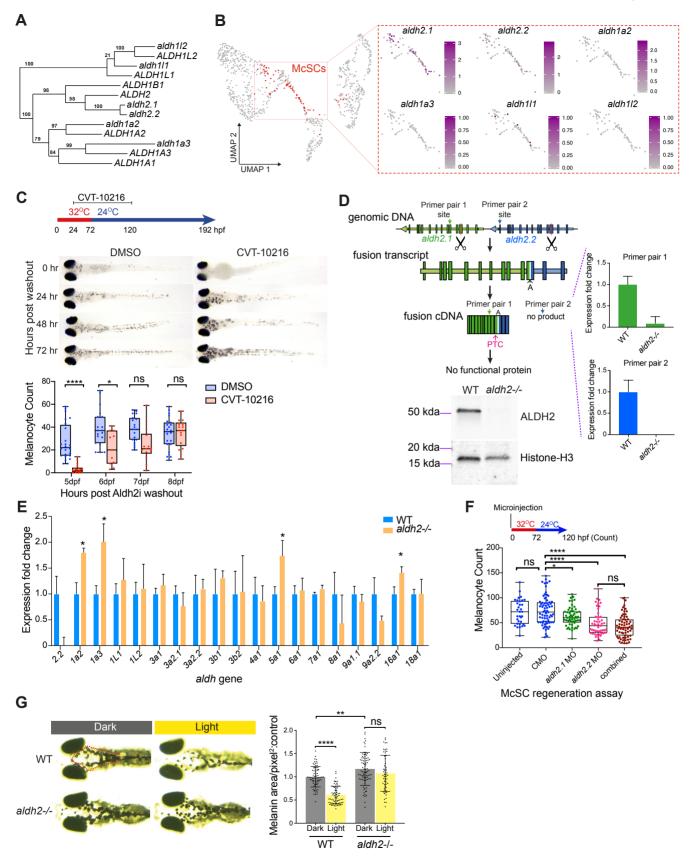
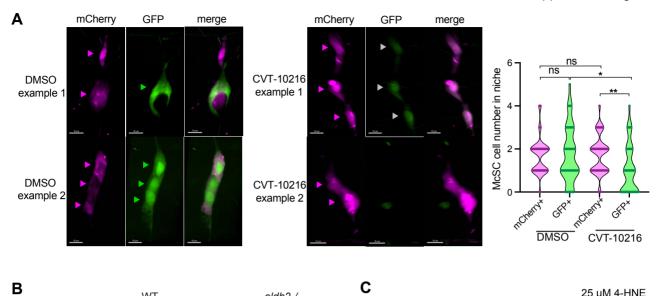
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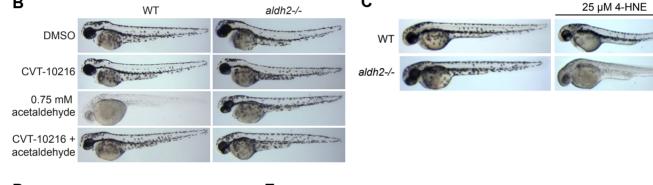


Fig"S1" *aldh gene* expression in the McSC lineage and Aldh2 loss of activity assays

- **A.** Phylogenetic tree showing the relationship between human *ALDH* and zebrafish *aldh* genes from the ALDH1 and ALDH2 families.
- B. As Fig. 1A, but showing expression levels of *aldh1* family genes.
- C. Extended regeneration assay on *mitfa^{vc7}* embryos following CVT-10216 treatment between 24-120hpf. After washout, larvae were imaged and melanocytes quantified each day. Representative images shown. Each datapoint represents a single larva, 3 experimental replicates. *: p<0.0332, ****: p<0.0001, ns: not significant. Kruskal-Wallis test with Dunn's multiple comparisons.</p>
- D. sgRNAs targeting exon 3 of *aldh2.1* and *aldh2.2* were co-injected with Cas9, causing deletion of the intergenic region and creation of a fusion transcript, containing a 1 bp insertion and premature termination codon (PTC). RT-qPCR was performed with primers targeting Primer site 1, persisting in the truncated fusion transcript, and Primer site 2 (excised), see Methods. Housekeeping control: *β-actin.* 3 experimental replicates. Error bars = mean±s.e.m. Western blot analysis of ALDH2 protein in *aldh2-/-* and WT embryos confirms a lack of Aldh2 protein in mutants. Loading control: Histone-H3.
- E. RT-qPCR showing *aldh2.2* expression levels relative to other *aldh* genes in 72hpf WT or *aldh2-/-* mutant embryos, normalized to β-*actin.* 3 experimental replicates. Error bars = mean±s.e.m. Asterisks mark *aldh* genes upregulated >1.5-fold in *aldh2-/-* mutants.
- F. Regeneration assay on *mitfa^{vc7}* embryos injected with 6 ng of standard control morpholino (CMO), or morpholinos against either/both *aldh2.1* and *aldh2.2*. Regenerated melanocytes are quantified, each datapoint represents a single embryo, 3 experimental replicates. * p<0.0332, **** p<0.0001, ns: not significant. One-way ANOVA with Tukey's multiple comparisons.</p>
- G. Representative images shown of embryos after adaptation to dark or light surroundings. Melanin coverage within the red outlined area was quantified. N=3 biological replicates. Error bars = mean±s.d. **: p<0.0021, ****: p<0.0001. One-way ANOVA with Tukey's multiple comparisons.</p>



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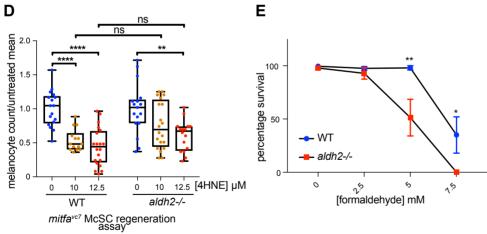


Fig. S2. Results of aldehyde screen on *aldh2-/-* mutant zebrafish

- A. Left: Static images from Movie S1 and S2 (bottom row) plus two additional example McSC niches showing individual *crestin:mCherry* and *mitfa:GFP*-expressing cells (arrowed) during regeneration in embryos treated with either DMSO or CVT-10216, scale bars 10 μm. GFP fluorescence is fainter (gray arrows) or missing after CVT-10216 treatment. Right: Quantification of mCherry+ and GFP+ cells in each niche (3 niches imaged in 12 embryos per condition). Each dot represents a single niche. **: p<0.0021, *:p<0.03. Kruskall-Wallis test with Dunn's multiple comparisons.</p>
- B. Representative images of 72hpf WT and aldh2-/- mutant embryos treated with 0.75 mM acetaldehyde with or without CVT-10216. Unexpectedly, Aldh2 loss or deficiency confers resistance to acetaldehyde. 3 experimental replicates, >8 embryos per condition.
- C. Representative images of 72hpf WT and *aldh2-/-* mutant embryos treated with 25 μM 4-HNE, showing whole body sensitivity in the mutant. 2 experimental replicates, 15 embryos per condition.
- D. mitfa^{vc7} melanocyte regeneration assay and subsequent quantification of embryos treated with increasing doses of 4-HNE, showing no significant difference between controls and aldh2-/-; mitfa^{vc7} embryos in terms of reduction in regeneration potential after 4-HNE treatment. For comparison between genotypes, melanocyte numbers were normalized to the average untreated condition for each genotype. 2 experimental replicates, each datapoint represents a single embryo. ** p<0.0021, **** p<0.0001, ns not significant. One-way ANOVA with Tukey's multiple comparisons.</p>
- E. Survival percentage of WT and *aldh2-/-* mutant embryos treated with various concentrations of formaldehyde. 6 experimental replicates, 20 embryos per condition. Error bars = mean±s.e.m. ** p<0.0021. Two way ANOVA with Sidak's multiple comparisons.

Α В clusters 2 ~ r foxd3 0 Ś 6 1 9 sox10 橙 柳 gstp1 prdx1 gch2 gch2 txn akr1b1.1 -222l21.1 si:ch211 nhox2hh phox2bb hoxb5b hand2 :110425 elov11a fxyd1 phda2 snai1b pax3a zgo 쟿 phida2 ada2b anxa4 aqp3a sic2a11b si:ch211-251b21.1 CABZ01021592.1 IMAR Early NC UMAP-1 pax7a apodb anxa5b aqp1a.1 aox5 mvp17 aqp ra. r crip1 tyrp1b pmela dct zgc:91968 GFP si:dkey-197i20.6 akap12a JMAP-JMAF Iridophore Xanthop UMAP-1 ey-197120.6 akap12a zgc:110789 fhl3a lypc mef2cb UMAPpou3f1 elavl3 sox4a.1 fabp7a krt18a.1 mitfa mki67 progenitor cenpf si:dkey-108k21.10 hist1h4l.11 Schwann Cell NC - neural UMAP-С Saunders et al., (2019) cluster IDs Farnsworth et al., (2020) cluster IDs Cranial ganglia Epidermis FMB differentiating neuron Enteric Neuron foxd3+ Progenitor 10 10 Iridophore Iridoblasts Melanophore Neural Progenitor Kidney Macrophage Melanoblasts:early ~ 2 (Pigment Progenitor 0 UMAP UMAP PNS neuron Melanoblasts:late Schwann Cell Muscle progenitor NCNeural:early Schwann Cell Precursor NCNeural:late unassigned -10 -10 Other Xanthophore Unassigned Xanthoblasts:early Xanthoblasts:late . 10 -10 -10 ċ 5 -5 5 -5 10 UMAP 1 UMAP 1 Pigment cell reclustering Farnsworth et al., (2020) Pigment cell reclustering D mapped cell cluster ID new clusters original clusters 9 Early NC Neural Early Melanoblasts Late Melanoblasts crestin+ mitfa-high Early NC Neural Iridoblasts Late NC Neural Early Xanthoblasts McSC/ Melanoblast progenitor Late Melanoblasts 2 2 Late Xanthoblasts Muscle progenitor Mel/irid progenitor 11 6 Iridoblasts 11 Epithelial Unassigned Other Early Xanthoblasts Late Xanthoblasts crestin+ mitfa-low UMAP-1 JMAP-3 Ε Geneset Enrichment plot: De Novo Purine Biosynthesis Enrichment Score crestin+;mitfa-low -0.4 -0.5 -0.6 -0.7

Leading edge genes. adss1, ak2, taf9, atic, ppat, prps1a, impdh1b,

paics, pfas, gmps

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lits

Fig. S3. Identification of transcriptionally distinct scRNA-seq clusters during McSC regeneration

- **A.** Heatmap showing top 5 cluster-defining genes per selected clusters.
- B. UMAP of the combined dataset showing gene expression of early neural crest markers, and non-pigment clusters marked by *pou3f1* (Schwann Cell Progenitors), *mcamb* and *elavl3* marking NC-derived neural cells, *mvp17* marking iridophores, and *aox5* marking xanthophores.
- **C.** UMAP of this scRNA seq data mapped with cell identity annotation from Farnsworth et al (2020) and Saunders et al (2019).
- D. Left: UMAP of the combined dataset subsetted by the pigment cells and reclustered into 12 clusters. Center: UMAP annotated with the original clusters, showing the *crestin+;mitfa*high and *crestin+;mitfa*-low McSCs and split cluster 11. Right: overlap with Farnsworth clusters, showing a portion of original cluster 11 containing a range of neural and pigment cell identities.
- E. GSEA enrichment plot of the *de novo* purine biosynthesis signature upregulated in clusters 2,6,12 in CVT-10216 treated embryos compared to control, generated using the Deseq2 output ranked by the Deseq2 test statistic. The green line and y-axis represent the enrichment score of the pathway, that is, the extent of correlation between the DMSO dataset to *de novo* purine synthesis, relative to the Aldh2i dataset. Individual genes within this pathway are represented as vertical black lines, with genes contributing most towards the result NES -1.18, FDR <25% (Kolganov Smirnov test) boxed and listed to the right.</p>



Fig. S4. Validation of Mtx treatment

A. Mtx treated embryos (96 hpf) have lost reflective iridophore pigments (clearly observed in the eye) and yellow xanthophore pigments.

Table S1. scRNA-seq: top 30 cluster markers

Click here to download Table S1

Table S2. scRNA-seq: metrics, clustering information and cell states

Click here to download Table S2

Table S3. Differential expression analysis of crestin+ mitfa-low vs crestin+ mitfa-high cells

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Table S4. Differential expression analysis of crestin+ mitfa-low cells, DMSO vs CVT-10216

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Table S5. Differential expression analysis of crestin+ mitfa-high cells, DMSO vs CVT-10216

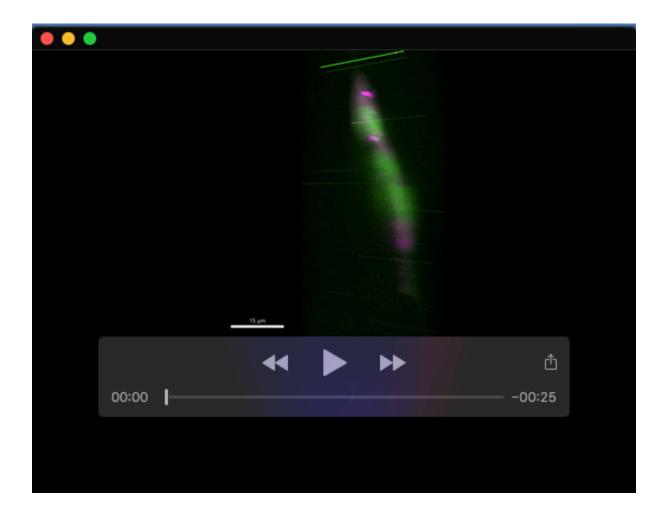
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Table S6. Differential expression analysis of iridophore cluster 9, DMSO vs CVT-10216

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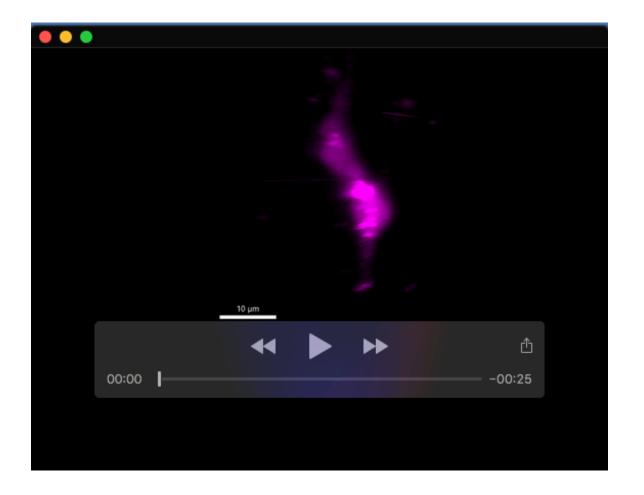
 Table S7. Oligonucleotide sequences

Click here to download Table S7



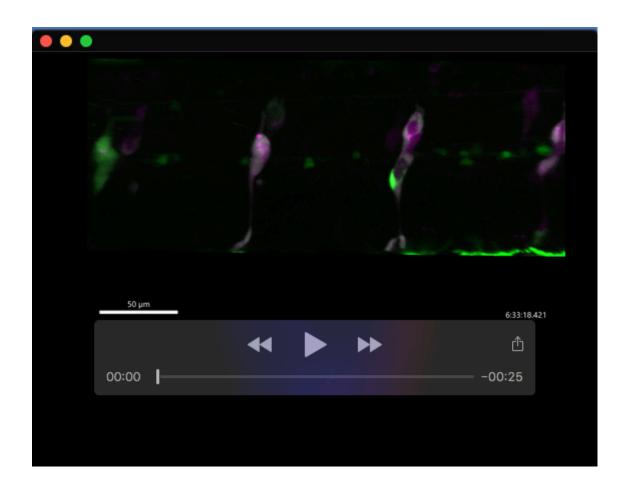
Movie 1. McSCs in a regenerating embryo (DMSO control)

Movie showing 3D rendering of a representative McSC cluster during regeneration. Here, an embryo expressing transgenes *crestin:mCherry* and *mitfa:GFP* embryo is imaged at the onset of regeneration following MoTP treatment. In this example, three cells in the McSC niche express both *GFP* and *mCherry*. See **Fig. S2**, DMSO example 2



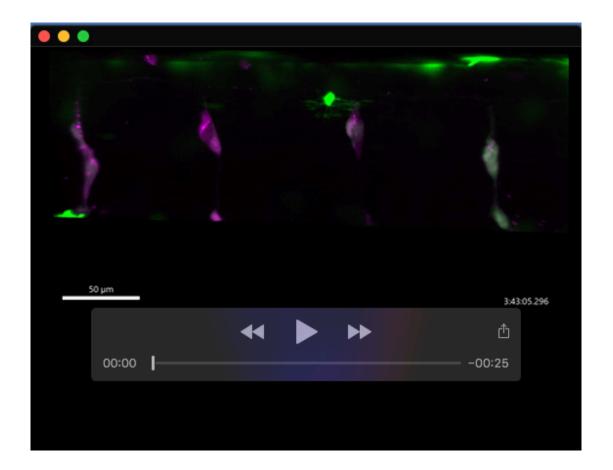
Movie 2. McSCs in a regenerating embryo following ALDH2 inhibitor (CVT-10216) treatment.

Movie showing 3D rendering of a representative McSC cluster during regeneration. Here, an embryo expressing transgenes *crestin:mCherry* and *mitfa:GFP* embryo is imaged at the onset of regeneration during CVT-10216 treatment and following MoTP treatment. In this example, two cells express *mCherry*, but while GFP is detected in a neighbouring cell, it is not expressed in this McSC niche. See **Fig. S2**, CVT-10216 example 2.



Movie 3. McSCs generate progeny

Time-lapse video of a *Tg(crestin:mCherry;mitfa:GFP)* embryo during McSC regeneration (DMSO control). Embryos were treated with MoTP to kill differentiated melanocytes and initiate melanocyte regeneration. McSCs were followed for over 14 hours (post MoTP washout). McSCs are *crestin+ mitfa-low*, but then during or shortly after cell division, a cell strongly expresses GFP+ to become a *mitfa-high* cell, which then leaves the McSC compartment and migrates towards the epidermis.



Movie 4. Aldh2i inhibits McSCs ability to generate progeny

Time-lapse video of a *Tg(crestin:mCherry;mitfa:GFP)* embryo during McSC regeneration in the presence of ALDH2i. Embryos were treated with CVT-10216 to inhibit Aldh2, and co-treated with MoTP to kill differentiated melanocytes, and initiate melanocyte regeneration. McSCs were followed for over 14 hours (post MoTP washout, but in the presence of ALDH2i).