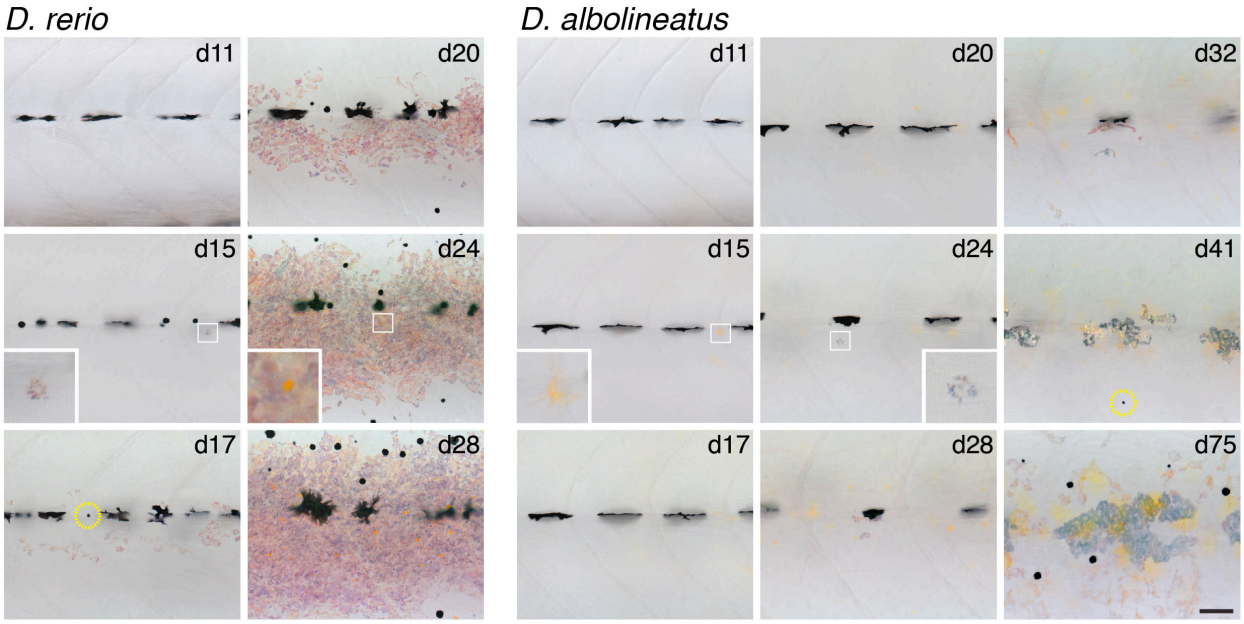
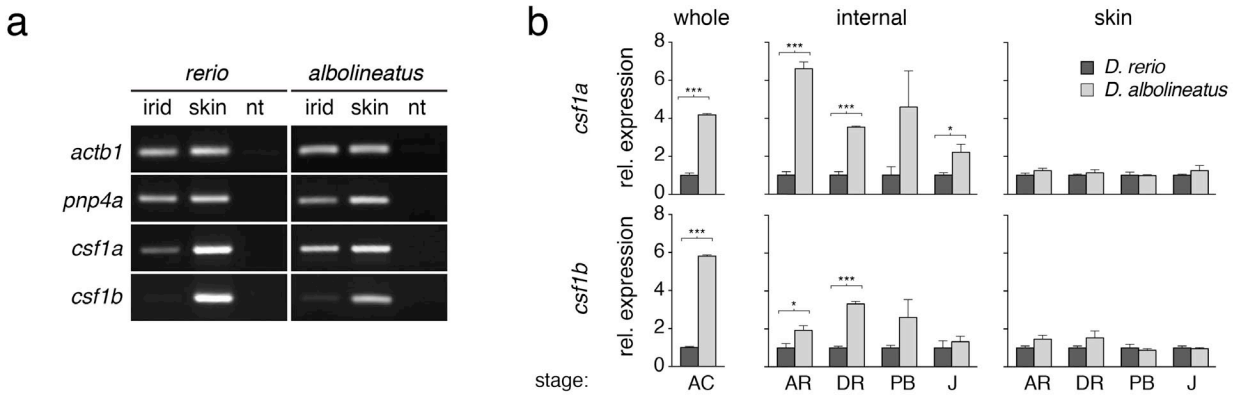
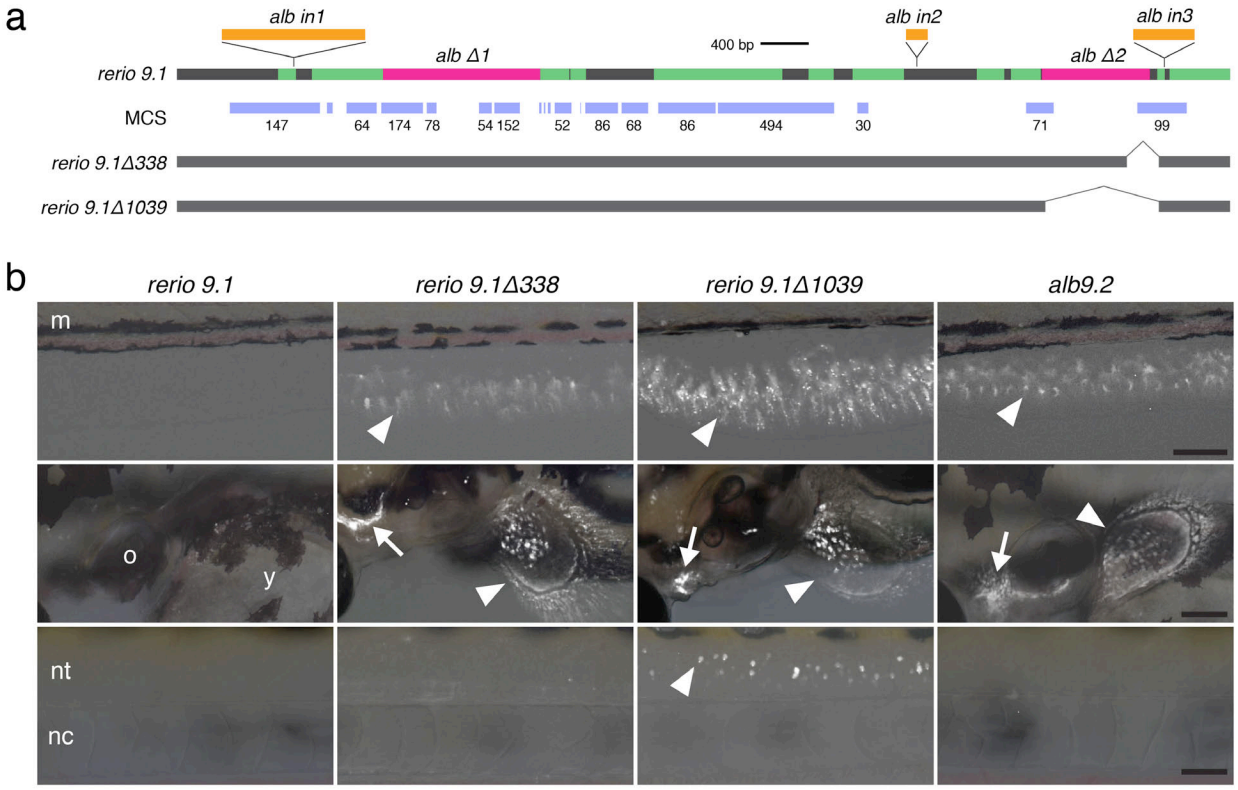


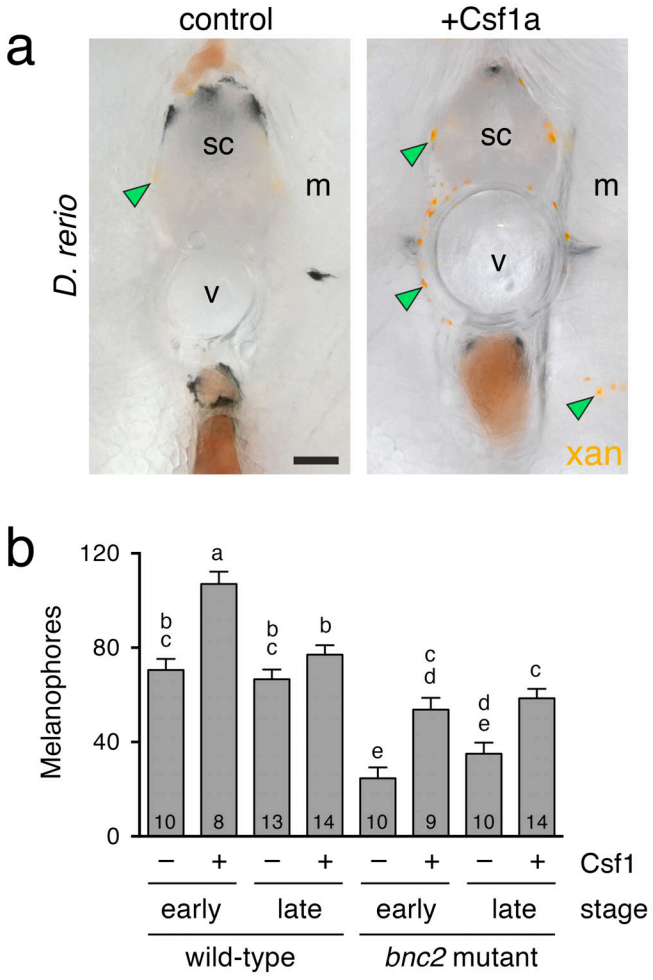

**Supplementary Figure 1. Shifts in the timing of adult pigment cell differentiation.**
First appearance of the three pigment cell classes in *D. rerio* and *D. albolineatus.* Panels show representative individual imaged daily through adult pigment pattern formation (stages AC to PR[42]; dpf in upper right of frames). Prior to the onset of adult pigment pattern development, embryonic/early larval melanophores were found along the horizontal myoseptum; no adult pigment cells were present and the two species were practically indistinguishable. By 15 dpf, the first iridophore differentiated in *D. rerio* (inset) and the first xanthophore has differentiated in *D. albolineatus* (inset). In *D. rerio*, melanophores were the next cell type to appear (yellow circle, d17) and xanthophores (inset, day 24) differentiated about a week later, after iridophores had filled the primary interstripe. In *D. albolineatus*, xanthophores were the first pigment cell type to develop (green circle and inset, d15), and did so independently of iridophores, which differentiated later (inset, d24) but did not fill the interstripe region as in *D. rerio*. Melanophores developed still later (yellow circle, d41).
Scale bar, 60 $\mu$m.



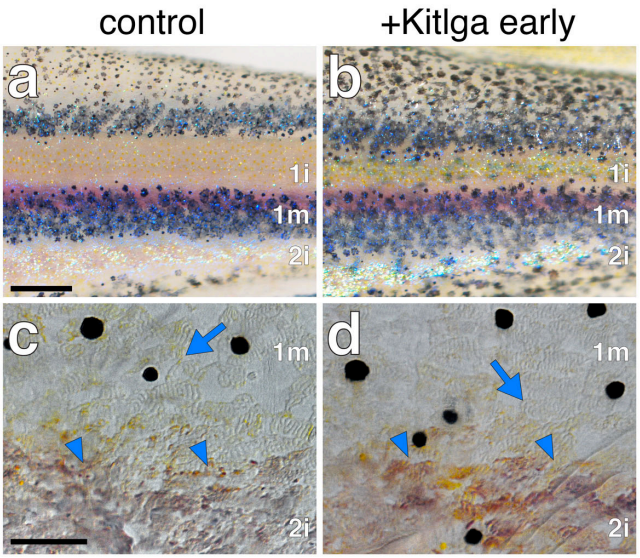

**Supplementary Figure 2. Expression of *csf1a* and *csf1b*.**
(**a**) In both species *csf1a* and *csf1b* were expressed in skin whereas *csf1a* was predominantly expressed by iridophores. *actb1*, *actinb1*, a loading control; *pnp4a*, *purine nucleoside phosphorylase 4a*, a marker of iridophores[22] (note that iridophores were also present in skin); nt, no-template control.
(**b**) Results of quantitative RT-PCR for *csf1a* and *csf1b* (mean±SE relative abundances) across stages. Differences were observed in whole young larvae (stage AC, anal fin condensation), before internal and skin tissues could be easily separated. Later, differences through juvenile (J) stage were observed for both loci but only in internal samples, which included myotome-associated tissue adjacent to the hypodermis. Within each stage, expression of the *D. rerio* locus is set to 1. Plot for internal *csf1a* expression is the same as in Figure 2a. Shown are means±s.e.m.; $n$=3 biological replicates for all samples.
***$P$<0.0001; *$P$<0.05.



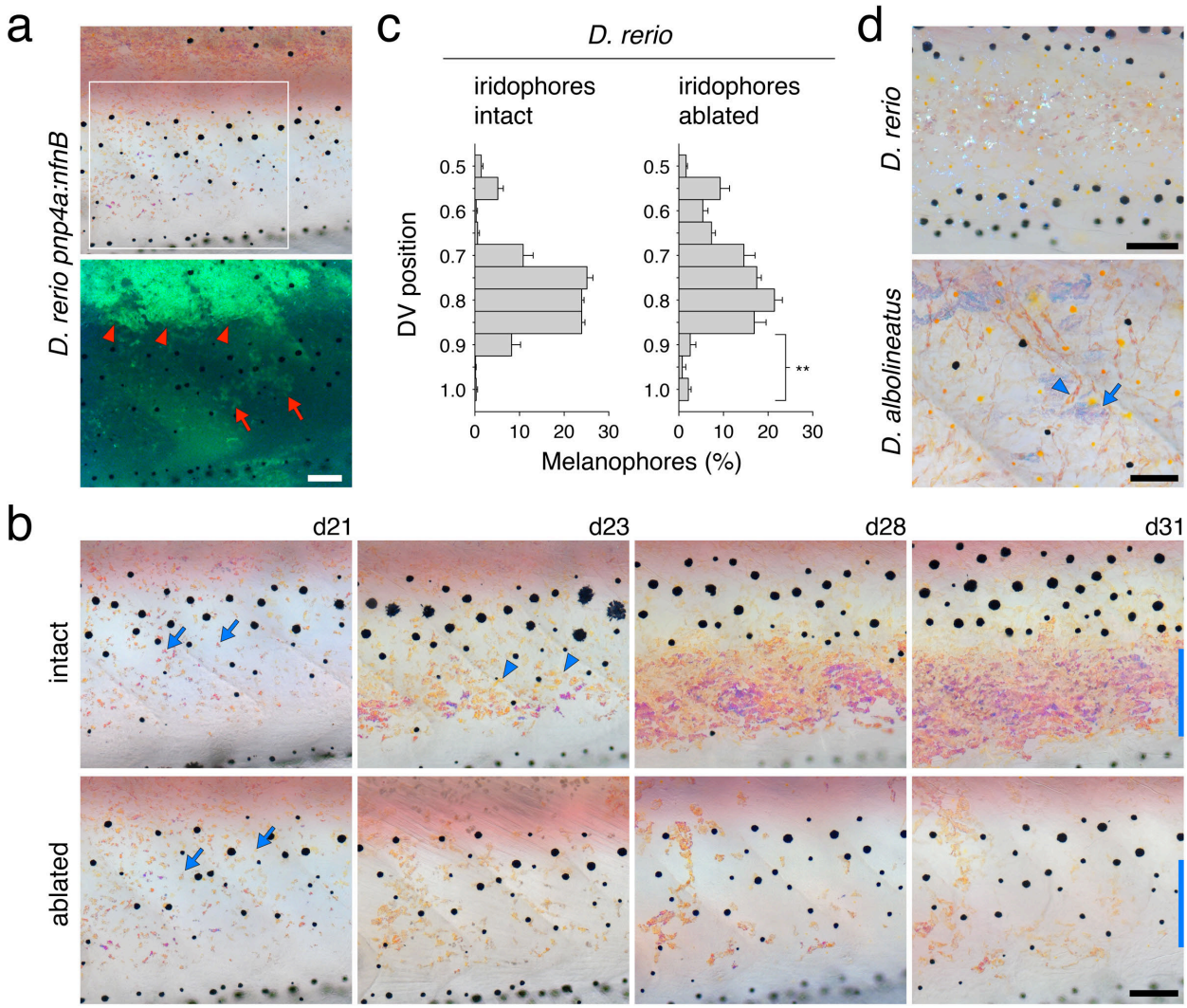

**Supplementary Figure 3. Analyses of *csf1a* regulatory region.**
(**a**) Conservation and divergence within ~9 kb *csf1a* regulatory regions of *D. rerio* and *D. albolineatus*. *rerio9.1*, indicates regions with >75% nucleotide conservation between species (green), locations of two large regions missing in *D. albolineatus* (magenta, *alb Δ1-2*) and three large insertion in *D. albolineatus* (orange, *alb in1–3*), mapped onto the *D. rerio* 9.1 kb regulatory region; widespread smaller insertions and deletions in *D. albolineatus* are not shown. MCS, multi-species conserved sequences identified in the *D. rerio* relative to other vertebrates (*Oryzias latipes*, *Gasterosteus aculeatus*, *Tetraodon nigroviridis*, *Takifugu rubpribes*, *Xenopus tropicalis*, *Mus musculus*, *Homo sapiens*; excluding *D. albolineatus*) and selected log odds (lod) ratio scores, indicating extent of conservation, available through the UCSC genome browser[59] (https://genome.ucsc.edu/). Deletion *alb Δ1* affects MCS lod=71 and lod=99, with the latter also interrupted by insertion *alb in3*. *rerio9.1Δ338* and *rerio9.1Δ1039* indicate regions deleted from the *D. rerio* sequence in additional transgenic reporter lines.
(**b**) Examples of derepressed and dysregulated mCherry expression in *csf1a* deletion lines generated in *D. rerio* at 3 days post fertilization; fluorescence with brightfield overlay. Top, expression was not detectable in the larval fin fold for *rerio9.1* but was apparent (arrowhead) for *rerio9.1Δ338*, *rerio9.1Δ1039* and *alb9.2.* m, myotome. Middle, mCherry was similarly expressed in cranial ganglia (arrows) and pectoral fins of *rerio9.1Δ338*, *rerio9.1Δ1039* and *alb9.2.* o, otic vesicle; y, yolk. Bottom, among the unique domains of expression in deletion lines were cells within the neural tube (nt) of *rerio9.1Δ1039* (arrowhead). nc, notochord. mCherry was expressed in 2 of 3 lines for *rerio9.1Δ338* and 6 of 7 lines for *rerio9.1Δ1039.* Deletion constructs did not recapitulate the specifically flank hypodermal expression of *csf1*$^{alb9.2}$ at later stages, suggesting additional *cis* regulatory differences not uncovered here.
Scale bar, 40 μm.




**Supplementary Figure 4. Extra-hypodermal xanthophores and Csf1-dependent alterations in melanophore numbers.**
(**a**) Cross sections of heat shocked stage SA *D. rerio* sibling larvae either not carrying the *hsp70l:csf1a* transgene (left), or carrying the transgene to overexpress Csf1. In the control, rare, very lightly pigmented xanthophores (arrowhead) could be seen occasionally in extra-hypodermal locations corresponding to where extra-hypodermal cells expressing xanthophore lineage markers are known to occur[26]. In Csf1 over-expressing fish, well-pigmented extra-hypodermal xanthophores were abundant. “sc” denotes spinal cord; “v” denotes vertebral column; “m” denotes myotome.
(**b**) In addition to effects on xanthophores, Csf1 overexpression increased melanophore numbers. Shown are means±s.e.m. Different letters above bars indicate groups that were significantly different ($P$<0.05) in Tukey Kramer *post hoc* comparisons; sample sizes are shown within each bar. Overall ANOVA revealed a significant overall effect of Csf1 overexpression (treatment; $F_{1,80}$=63.7, $P$<0.0001) in addition to genotype x treatment, stage x genotype and stage x treatment interactions ($F_{1,80}$=13.04, $P$<0.001; $F_{1,80}$=13.02, $P$<0.001; $F_{1,80}$=6.2, $P$<0.05).
Scale bar, 60 $\mu$m.




**Supplementary Figure 5. Iridophore organization persists despite supernumerary melanophores.** (**a, b**) When Kitlga was induced early in *D. rerio* (**b**; stage AR+) additional melanophores developed and partially covered iridophores in the interstripes, yet iridophore organization resembled that of controls (**a**). (**c, d**) Higher magnification images showing scattered iridophores (arrows) and interstripe iridophores (arrowheads). Sample sizes: *n*=10 Kitga transgenic and *n*=10 non-transgenic controls examined. Scale bars, 400 $\mu$m (**a**, for **a, b**); 60 $\mu$m (**a'** for **a', b'**).




**Supplementary Figure 6. Secondary interstripe iridophores terminate stripes in *D. rerio*.**
(**a**) Iridophores expressing Venus in the primary interstripe with scattered Venus$^{+}$ cells extending ventrally in wild-type *D. rerio.* Fluorescence image shows boxed region. Arrowheads, nVenus$^{+}$ iridophores of the primary interstripe. Arrows, scattered nVenus$^{+}$ iridophores in the primary stripe. Fish were treated with epinephrine to contract pigment granules towards cell centers.
(**b**) Development of iridophore and melanophore patterns in control and iridophore-ablated *D. rerio* (detail of individual in **a**) at the level of the secondary interstripe (blue vertical bar). Arrows, iridophores within the primary melanophores stripe. Arrowheads, initial iridophore aggregations in the secondary interstripe.
(**c**) Melanophore distributions ventral to the horizontal myoseptum (means±s.e.m.). Melanophores were more likely to localize in the ventral region of the flank (D–V positions 0.9–1.0) in iridophore-ablated *D. rerio* as compared to control *D. rerio* ($\chi^2$=15.0, d.f.=2, $P$<0.001; $N$=103 melanophores); because some primary interstripe iridophores were ablated as well, melanophores normally confined to the stripe also spread further dorsally, potentially limiting the extent of ventral expansion. Melanophore distributions were less uniform than those observed for Csf1 early-overexpressing *D. rerio* or *D. albolineatus*, suggesting roles for both xanthophore excess and iridophore reduction in *D. albolineatus* (compare to Figure 3i).
(**d**) In adult *D. albolineatus*, iridophores were present but scattered widely. Several morphological sub-classes were evident (e.g., arrowhead, arrow) as in *D. rerio*[18]; assessment of whether some or all of these develop differently in *D. albolineatus* will require subclass-specific molecular markers.
Scale bars, 100 $\mu$m.