INTRODUCTION

DNA-damaging ultraviolet (UV) radiation from the sun is absorbed by the black pigment, melanin (Herrling et al., 2008). Mammalian melanocyte cells produce melanin during melanogenesis. Melanogenesis depends upon multiple enzymes. Microphthalmia transcription factor (MITF) controls tyrosinase (TYR), tyrosinase-related protein 1 (TYRP1), and dopachrome tautomerase (DCT) expression (Kawakami & Fisher, 2017). The latter three enzymes convert tyrosine into melanin (Pillaiyar et al., 2017). Melanogenesis is an adaptive, conserved process regulated by homologous genes across animal taxa (Hoekstra, 2006).

Dysfunctional melanogenesis causes the heterogenic disorder, albinism (Garrido et al., 2021). People with albinism have visual impairments and increased skin cancer risk (Inena et al., 2020; Izquierdo et al., 1995). Albinism can be caused by defects in melanocyte specification and differentiation or can be non-syndromic or syndromic (Federico & Krishnamurthy, 2020;...
Pigment development at 24 hours postfertilization (hpf) and con-
tinued survival via autophagy. This model can be used to better un-
derstand the molecular mechanisms of disease in HPS10
and AP-3 complex function.

2.3 | Isolation and mapping of crasher

crasher originated from an ENU mutagenesis screen at Steve
Johnson’s Laboratory (Washington University). The crasher
mutation was mapped to chromosome 22 from larvae at 3 days post-
fertilization (dpf) via bulk segregant and fine mapping analysis
described previously (Clancey et al., 2013). See Methods S1.

2.4 | Clustered regularly interspaced short
palindromic repeats (CRISPR) gene knockout

Two CRISPR guide DNA oligos per candidate gene were designed
using CRISPR scan (Moreno-Mateos et al., 2015). Highest-scoring
guides were selected if they targeted an early exon with no predicted
off-targets. Guides were annealed to Cas9 interacting scaffold
(gatccgcaccgactcggtgccactttttcaagttgataacggactagccttattttaactt-
gatatctagctctaaaac). Guide RNA (gRNA) was synthesized then
purified and injected into embryos at the 1–2 cell stage. Detailed
description in Methods S1. Embryos at 3 dpf were fixed in 4% para-
formaldehyde (PFA; Sigma-Aldrich) overnight at 4°C. Experiments
replicated three times.

2.5 | In situ hybridization

Embryos at 24 and 30 hpf were dechorionated, fixed in 4% PFA for
6 hours at room temperature, and dehydrated and stored in metha-
onal at −20°C until used. In situ hybridization protocol was derived
from Thisse et al., 1993 with modifications (Thisse et al., 1993). No
proteinase K was used. Hybridization and washes were performed
at 65°C. Digoxigenin-labeled probes for faxd3 (Odenthal & Nusslein-
Volhard, 1998), mitfa (Lister et al., 1999), dct (Kelsh et al., 2000), tyr
(Camp & Lardelli, 2001), and tyrp1b (Braasch et al., 2009), were pre-
viously described. Experiments replicated three times unless other-
wise stated.

2.6 | Bafilomycin A1 autophagy drug treatments

2.5 dpf crasher mutant and wild-type (WT) sibling larvae were treated
with 50nM bafilomycin A1 (Enzo Life Sciences) in EM containing 1%

Significance

We have characterized a new zebrafish model for
Hermansky-Pudlak syndrome type 10. Using this model,
we have shown ap3d1 is important for melanophore sur-

METHODOLOGY

2 | Ethics statement

Research protocols using animals and recombinant DNA/RNA are
approved by the Washington State University Institutional Animal
Care and Use Committee (ASAF 3848 and 6777) and the Institutional
Biosafety Committee (BAF 1107).

Fish were fed dry and live foods, maintained on a recirculat-
ing water system at 28–30°C on a 14-h light/10-h dark cycle
and checked daily for abnormal behavior. *AB zebrafish are a wild-
type strain. The crasher mutant allele was maintained in heterozy-
gous adults. oca2 were also maintained as heterozygotes (Beirl
et al., 2014). Embryos were raised in E3 embryo media (EM) at 28°C
(Westerfield, 2007). Embryos were staged according to Kimmel
et al. (1995).

2.2 | Fish husbandry

2 | METHODOLOGY

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2.6 | Bafilomycin A1 autophagy drug treatments

2.5 dpf crasher mutant and wild-type (WT) sibling larvae were treated
with 50nM bafilomycin A1 (Enzo Life Sciences) in EM containing 1%
dimethylsulfoxide (DMSO) (Sigma-Aldrich) or with 1% DMSO in EM as a control. Each day, 50% of treatment solution was replaced with fresh EM/DMSO solution with or without drug. At 5 dpf, fish were fixed in 4% PFA. Experiments replicated four times.

2.7 | Imaging

Fish were visualized and imaged under a Nikon SMZ1500 microscope and a Digital Sight DSRi1 camera. Live fish were anesthetized with tricaine (Western Chemical Inc) and transferred to an EM droplet with methylcellulose on a glass slide. After imaging, fish recovered in fresh EM. Fixed CRISPR and bafilomycin A1 experiment fish were imaged in 50% glycerol/PBS on a glass slide. Fixed in situ experiment fish were imaged in 50% glycerol/PBS using a bridged coverslip. Images were white balanced and cropped using Adobe Photoshop Elements 2020.

2.8 | Cell counts

Cells were counted under a Nikon SMZ1500 microscope. For crasher mutant and CRISPR-edited fish, melanophores were counted throughout the body. For in situ cell counts, in situ signal+ cells were counted over the whole body (except eyes). The dorsal stripe melanophores were counted looking top-down, and ventral stripe melanophores were counted on one side of bafilomycin A1-treated fish. The dorsal and ventral stripe signal were counted by observing the fish ventrally for the mitfa+ 30 hpf dorsal/ventral ratio in situ. Experiments replicated three times unless otherwise stated. Statistical analyses were performed using the packages devtools (v2.3.2; Wickham et al., 2020), tidyverse (Wickham et al., 2019), rstatix (v.0.7.0; Kassambara, 2021), car (Fox & Weisberg, 2019), nortest (v1.0-4; Gross & Ligges, 2015) and R base packages (v.4.0.4; R Core Team, 2021).

2.9 | RNA-Seq and quantitative reverse transcription PCR (qRT-PCR)

2.9.1 | Sample preparation and sequencing

Three samples each of 50 crasher homozygous recessive mutants and 50 *AB line fish were collected at 3 dpf and stored at −80°C in TRIzol (ThermoFisher Scientific). RNA was extracted and single-end sequenced using an HiSeq3000 (Illumina, HiSeq) at a read length of 100 bp at Oregon State University’s Center for Genome Research and Biocomputing (Corvallis, OR). Detailed description in Methods S1.

2.9.2 | Data preparation

Sequencing quality was determined using FASTQC and adaptor sequences trimmed using Trimmomatic (Andrews, 2019; Bolger et al., 2014). Genome alignment and indexing to Ensembl Danio rerio genome version 99 was performed using STAR to create a sequence alignment map (SAM) file (Dobin et al., 2013; Yates et al., 2020).

2.9.3 | RNA-Seq data analysis

STAR alignment was performed to generate gene counts using R then converted into Transcripts Per Million. Generally Applicable Gene-set Enrichment (GAGE) for pathway analysis was performed.

Figure 1: crasher mutants have fewer and lighter melanophores. (a, a’) Lateral (a) and dorsal (a’) view of crasher siblings with typical the wild-type (WT) phenotype at 3 days postfertilization (3 dpf). (b, b’) Lateral (b) and dorsal (b’) view of homozygous recessive (mutant) phenotype. Arrowheads indicate punctate melanophores. (c) Melanophore count boxplot at 3 dpf. Mutants average 25% fewer melanophores (μ = 188, SD = 20.4) than WT siblings (μ = 255, SD = 20.9). (Student’s t-test, t[28] = −8.87, p = 1.27×10−9***). (d) Iridophore count boxplot at 3 dpf. Mutants vary more (median = 25, interquartile range [IQR] = 23 to 29.5), but have similar iridophore numbers as WT siblings (median = 28, IQR = 22–29) (Mann–Whitney U test, W = 11.05×103, p = 0.95). Scale bar is 1 mm.
in R (Luo et al., 2009; Luo & Brouwer, 2013; R Core Team, 2021; Yates et al., 2020). Single-gene expression data was called using the median value within 3 samples per genotype for each gene using the BiomaRt package (Durinck et al., 2005).

SAM files were compressed into binary alignment map (BAM) files using SAMtools (Li et al., 2009). Mutations were called using somatic calling in VarScan2 with *AB fish defined as “normal” and crashe mutants or oca2 homozygous recessive mutants defined as “tumor” (Koboldt et al., 2012).

Exon read counts for each candidate gene were normalized to RPKM and read ratios between *AB and crashe mutants determined by dividing normalized crashe read number at each exon by normalized *AB read number at each exon.

2.9.4 | Quantitative Reverse Transcription PCR (qRT-PCR)

qRT-PCR was performed to quantify ap3d1 transcripts as detailed in the Methods S1. Transcripts were normalized to actb1 as a reference gene using the Pfaffl method (Pfaffl, 2001). Statistical analyses were performed using the packages dev.tools (v2.3.2; Wickham et al.,

![Figure 2: crasher mutant phenotype recapitulation using CRISPR knockout.](image-url)

(a) The crasher mutation maps to a ~375 kb region on chromosome 22. R = recombination event. (b–g) CRISPR ap3d1 knockout in *AB lines recapitulates the crasher phenotype. (b) Uninjected *AB control. (c) tyr gRNA injected *AB fish have mosaic tyrosinase activity. (d) crasher mutant with slight lightening of the retinal pigmented epithelium. (e) ap3d1 gRNA injected *AB fish display light, punctate crasher-like melanophores and a lighter retinal pigmented epithelium than crasher mutant. (f) Melanophore count boxplot in uninjected and injected tyr and ap3d1 fish. Melanophore number is reduced in both populations injected with gRNA (two-way ANOVA, guide F(1, 56) = 5.50, p = <0.02; injection status F(1, 56) = 247.50, p = <0.22 × 10⁻¹⁵; interaction F(1, 56) = 23.68, p = <9.68 × 10⁻⁶), Tukey HSD performed to determine group differences, p = <0.001***). (g) Boxplot shows iridophore number is unchanged between uninjected and injected for both guides (two-way ANOVA, injection status F(1, 56) = 0.02, p = 0.88; guide F(1, 56) = 46.05, p = 7.80 × 10⁻⁹; interaction F(1, 56) = 0.89, p = 0.35, Tukey HSD performed to determine group differences, p = <0.001***). Scale bar is 500 μm. 78 eggs injected for tyr; 58 eggs injected for ap3d1.
RESULTS

3.1 | crasher homozygous recessive mutants have fewer and lighter melanophores

The crasher phenotype is discerned at 2.5–3 days postfertilization (dpf), and inherited in a single-gene, autosomal recessive pattern. Similar to mocha mouse melanocytes, melanophores were lighter, indicative of a melanin synthesis problem (Figure 1a–b; Kantheti et al., 1998). Some mutant melanophores appear punctate. Total melanophore number was significantly reduced at 3 dpf in mutants when compared to their wild-type (WT) siblings (Figure 1c). Interestingly, mutants appear to have less yellow pigment (Figure 1a–b'). Silver iridophore number was unchanged at 3 dpf (Figure 1d). Mutants were lethargic and survived ~9–11 dpf. The observed albinism and early-death phenotype is shared with HPS10 patients (Ammann et al., 2016; Mohammed et al., 2019).

3.2 | CRISPR ap3d1 knockout recapitulates the mutant phenotype and crasher mutants overexpress exon 14 in ap3d1

crasher mutation mapping analysis revealed several “candidate genes” (Figure 2a). Only AP-3 complex subunit delta 1 (ap3d1) is associated with albinism (Ammann et al., 2016; Kantheti et al., 1998; Mohammed et al., 2019). We hypothesized disrupting ap3d1 function via CRISPR within wild-type *AB zebrafish could recapitulate the crasher phenotype by disrupting melanogenesis enzyme cargo transport to the melanosome (Richmond et al., 2005). Positive control CRISPR tyrosinase guide injection caused pigment loss (Figure 2c). ap3d1 guide injection recapitulated lighter and punctate crasher-like melanophores (Figure 2d,e). A two-way ANOVA tested injection status (injected or uninjected) and guide type (ap3d1 or tyr) main effects on melanophore number, and were statistically significant, as was the interaction effect. A post hoc Tukey’s Honest Significant Difference (HSD) test showed differences between uninjected and injected melanophore counts for both guide types were statistically significant (Figure 2F; Table S1). Two additional replicates were analyzed by two-factor permutations to handle unequal variance between treatment groups (Howell, 2009). Injection status was always significant. Guide and interaction effects were inconsistent across replicates (Table S2).

Iridophore counts were analyzed via two-way ANOVA in fish injected with tyr or ap3d1 guide (Figure 2g). Guide type main effect was statistically significant, but not injection status and interaction effects. Iridophore number was significantly reduced between

![FIGURE 3](image) ap3d1 gene expression is reduced overall in crasher mutants, but exon 14 of ap3d1 is overexpressed. (a) RNA-Seq data bar graph shows no reduction in any candidate gene in crasher mutants as compared to *AB to a log2 fold difference of 1. Log2 fold difference is the difference in mutant and *AB expression. However, ap3d1 expression is reduced the most. Negative values indicated reduced expression in crasher mutants as compared to *AB. A difference of 1 is considered of import and equivalent to a twofold difference. Significance testing was not performed. (b) ap3d1 expression is reduced in crasher mutants according to qRT-PCR (Student’s t-test, t[4] = 2.94, p = 0.04*, n = 3). Error bars are standard deviation. (c) Exon 14 overexpression in ap3d1 line graph. The normalized reads ratio is defined as the normalized (transcripts per million) number of crasher reads at that exon divided by the normalized number of *AB reads at that exon.
embryos injected with ap3d1 or tyr guide. However, ap3d1 guide injection did not reduce iridophore number compared to uninjected siblings (Table S3). Injection status effects were not significant in three replicates. Notably, iridophore development was not affected by ap3d1 knockouts, which is an expected finding as iridophore development is not affected in crasher mutants at 3 dpf.

Baseline variances were observed for the wild-type line and crasher mutant line in the exome of candidate genes, but did not reveal a strong candidate causing the mutant phenotype (Table S4). We asked if candidate gene expression changed in crasher mutants. Since we performed RNA-Seq in whole larvae, we included mitfa expression to rule out changes due to reduced numbers of

VarScan analysis compared nucleotides at each position in the *AB wild-type line and crasher mutant line in the exome of candidate genes, but did not reveal a strong candidate causing the mutant phenotype (Table S4). We asked if candidate gene expression changed in crasher mutants. Since we performed RNA-Seq in whole larvae, we included mitfa expression to rule out changes due to reduced numbers of

**Figure 4** Specification and early differentiation markers are unchanged in crasher (ap3d1 mutants). (a and b) Melanoblast/iridoblast bipotent precursor marker, foxd3, expression is unchanged in progeny of crasher heterozygotes. (c–h) mitfa expression is not altered in crasher heterozygote progeny. Melanoblast marker, mitfa, expression at 24 h postfertilization (hpf) in *AB control (c) and crasher heterozygote progeny (d) is not different. (e–h) Representative early differentiated melanophore marker, mitfa, expression at 30 hpf in *AB control (e) and crasher heterozygote progeny (f) from 2 replicates is not different. (g) mitfa + cell counts are not significantly different for early differentiating melanophores at 30 hpf between progeny of *AB parents and crasher heterozygote parents (Student’s t-test, t(49) = 0.36, p = 0.72). (h) crasher heterozygote progeny have similar mitfa + signal, but less mitfa + signal on the ventral side. Ratio is mitfa + dorsal cell number divided by mitfa + ventral cell number (Student’s t-test, t(45) = −0.94, p = 0.35). Plots are violin box plots. Scale bars are 500 μm. Images labeled 2crasher are representative fish from the clutch.

**Figure 5** Melanogenesis expression is reduced in crasher (ap3d1) mutants. (a, b and c) dct expression at 24 hpf is reduced in 1/4 of crasher heterozygote progeny (b) as assessed by a chi-square goodness-of-fit test to a 3 wild-type phenotype: 1 mutant phenotype ratio (χ² = 1.08, χ = 3.84, p = 0.30). dct + cell number is significantly reduced in mutant phenotype fish at 24 hpf (Student’s t-test, t(6) = 2.83, p = 0.03*). (c, d and f) dct expression is reduced in the crasher clutch overall by 30 cells on average at 30 hpf (Student’s t-test, t(6) = 2.39, p = 0.02*). (f) crasher clutch cell counts at 30 hpf segregate into a 3:1 ratio (χ² = 1.61, χ = 3.84, p = 0.20). (g–l) tyr is not significantly reduced at 24 hpf [Student’s t-test, t(57) = 0.90, p = 0.38] (g, h and k), but is slightly reduced at 30 hpf in the crasher clutch (Student’s t-test, t(48) = 2.14, p = 0.04*) (i, j and l). (m-v) tyrp2b + cells are reduced at 24 hpf in the crasher clutch (Student’s t-test, t(57) = 3.51, p = 8.73 × 10⁻⁴***), but no obvious mutant phenotype is observed (m, n and q). At 30 hpf, tyrp1b + cells are significantly reduced (Student’s t-test, t(52) = 2.66 p = 0.01**), but no obvious mutant phenotype is observed (o, p, and r). (s) Expression heatmap of melanogenesis genes at 3 dpf using RNA-Seq. tyrp1b is reduced by >twofold (log₂ fold difference of 1), while dct is also reduced. tyr expression is slightly increased. mitfa expression is not altered. (a, b, g, h, m and n) Scale bars are 500 μm. (c, d, i, j, o and p) Scale bars are 1 mm. tyrp1b 24 hpf and tyr 30 hpf experiment repeated twice. dct 30 hpf experiment repeated once. Plots are violin box plots. Images labeled 2crasher are representative fish from the clutch.
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(a) *AB, dct, 24 hpf
(b) crasher, dct, 24 hpf
(c) *AB, dct, 30 hpf
(d) *crasher, dct, 30 hpf
(e) 
(f) 
(g) *AB, tyr, 24 hpf
(h) *crasher, tyr, 24 hpf
(i) *AB, tyr, 30 hpf
(j) *crasher, tyr, 30 hpf
(k) 
(l) 
(m) *AB, tyrip1b, 24 hpf
(n) *crasher, tyrip1b, 24 hpf
(o) *AB, tyrip1b, 30 hpf
(p) *crasher, tyrip1b, 30 hpf
(q) 
(r) 

**Genotype of Parents**

- **Wild-type**
- **Phenotype**
- **Mutant**

**Genotype of Parents**

- ***AB crasher Heterozygote**

**mitfa**
**tyr**
**tyrip1b**
**dct**
pigment cells. Candidate gene expression data did not reach a twofold change threshold; however, crasher mutants have reduced expression in ap3d1 whole transcripts according to qRT-PCR (Figure 3a,b). Next, we asked if exon count in candidate gene mRNA was altered in the mutant. Reads mapping to each exon in each candidate gene in crasher and AB showed the ratio of crasher reads per exon per gene was similar to AB with the exception of a 5.5-fold increase in exon 14 of ap3d1 in mutants (Figure 3c). CRISPR, RNA expression data, and mapping data supported crasher as an ap3d1 mutant.

3.3 Specification and early differentiation markers are not lost in ap3d1 mutants

Using in situ hybridization, we examined neural crest and melanoblast specification markers, foxd3 and mitfa, at 24 hpf (Curran et al., 2010). Because the mutant phenotype is not apparent until 2.5–3 dpf, we analyzed a clutch of 30 embryos from crasher (ap3d1) heterozygous parents, assuming one-quarter of the clutch progeny would be homozygous recessive and cell count distributions would be bimodal (two separate violin plot peaks with one peak at third of the width of the other). foxd3 and mitfa expression were similar between all progeny from crasher heterozygous parents and AB fish suggesting specification was normal in crasher (Figure 4a–d).

We tested if melanophore differentiation failed in crasher mutants by examining differentiating mitfa+ melanophores at 30 hpf (Figure 4e–g). The crasher clutch had a greater proportion of fish with less signal distributed over the ventral side, but this was not statistically significant, nor were the differences in overall cell counts (Figure 4h). No obvious phenotype distinguishing one-quarter of the crasher clutch was observed.

3.4 Melanogenesis gene expression is altered in ap3d1 mutants

We asked if the crasher mutant’s light melanophores resulted from reduced melanogenesis gene expression. Using in situ hybridization, we evaluated the expression of tyr, dct, and tyrp1b at 24 hpf and 30 hpf. dct expression was strongly reduced at 24 hpf in one-quarter of the crasher clutch. We performed a chi-squared goodness-of-fit test in which the null hypothesis was the ratio of wild-type phenotype fish to crasher phenotype fish was 3:1 (R Core Team, 2021). The null hypothesis was not rejected, and the test confirmed the ratio of wild-type phenotype fish to crasher phenotype fish was 3:1 (Figure 5a,b). dct + cell number was reduced in crasher phenotype fish (Figure 5e). No obvious crasher phenotype stands out in dct + expression at 30 hpf, though we observed an overall reduction in dct + cell number in the crasher clutch (Figure 5c,d,f). However, the distribution of dct + cells was different between the AB clutch and crasher clutch. The lower grouping of cell counts appeared to form a separate group and matched a 3:1 ratio. This group did not have a distinct expression pattern like mutants at 24 hpf. dct expression is possibly induced later in development in crasher mutants than their wild-type siblings, and mutants “catch up” slightly by 30 hpf. To our knowledge, there has not been a link between dct expression and ap3d1 gene function before now.

Tyr expression was examined, but a crasher phenotype was not readily observable in a quarter of the crasher clutch (Figure 5g–l). At 24 hpf, tyr + cells were not reduced, and the distributions of these cell counts were not strongly bimodal (Figure 5k). At 30 hpf, overall

![Image](https://example.com/image.png)

**FIGURE 6** Autophagy inhibition with bafilomycin A1 negatively impacts melanophore survival in crasher (ap3d1) mutants. (a–d) Melanophore morphology of mutant fish treated with 50 nM bafilomycin A1 appears more punctate. (e) Melanophore count boxplot of crasher mutants and wild-type siblings treated with bafilomycin A1 or DMSO. Bafilomycin A1-treated mutants have 39.51% fewer melanophores ($\mu = 39.2$, SD = 4.75) than DMSO-treated mutants ($\mu = 64.8$, SD = 6.76). Bafilomycin A1-treated wild-type siblings have 15.74% fewer melanophores ($\mu = 68.0$, SD = 8.70) than DMSO-treated wild-type siblings ($\mu = 80.7$, SD = 3.39). Two-way ANOVA, phenotype F(1, 20) = 77.03, p = 2.71 x 10\(^{-8}\), treatment F(1, 20) = 56.74, p = 2.92 x 10\(^{-7}\), interaction F(1, 20) = 6.53, p = 0.02, Tukey HSD performed to determine group differences, $p < 0.001$ $***$, $p < 0.01$ $**$, $p < 0.05$ *). Scale bar is 500 μm.
numbers of tyr + cells are reduced, but we did not observe a distinct crasher mutant phenotype (Figure 5i,j,l).

No apparent mutant phenotype in tyrp1b+ cells was observed (Figure 5m–p). At 24 hpf, tyrp1b+ cells did not have a strong bimodal distribution in the crasher clutch. tyrp1b+ cell numbers at 24 hpf and 30 hpf were reduced overall in the crasher clutch (Figure 5q,r).

We pulled melanogenesis pathway expression data from our RNA-Seq dataset and compared expression levels between *AB fish and crasher mutants at 3 dpf (Figure 5s). mifia is highly expressed in differentiated melanophores, and it was unchanged between crasher and *AB clutches, indicating melanophores were differentiating (Higdon et al., 2013). It is less likely changes in other melanogenesis genes are due to reduced melanophore number. tyr expression was slightly increased. This data is in contrast with our in situ data showing crasher clutches had fewer tyr + cells at 30 hpf. dct expression at 3 dpf was decreased in mutants, and mutants had fewer dct + cells at 24 hpf. tyrp1b expression was decreased to a larger degree at 3 dpf compared with dct expression which we did not observe in our in situ data at earlier timepoints. Taken together, dct and tyrp1b expression was most affected in crasher mutants.

3.5 | *ap3d1* promotes survival of melanophores via autophagy

The punctate, crasher melanophore morphology is similar to other pigment-deficient zebrafish mutants. Specifically, vps11 mutants display fragmented cells similar to punctate cells in crasher mutants (Clancey et al., 2013). Autophagy was significantly upregulated in crasher mutants at 3 dpf according to Generally Applicable Gene-set Enrichment analysis of RNA-Seq data (Table S5; Figure S1). We hypothesized crasher mutants upregulate autophagy to maintain melanophore number because of the known role of autophagy in melanophore survival (Clancey et al., 2013). Therefore, inhibiting autophagy would reduce melanophore number in mutants. We treated mutants and WT siblings with autophagy-inhibitor, bafilomycin A1, starting at 2.5 dpf (Figure 6). At 5 dpf, we observed a ~39.51% decrease in dorsal and ventral stripe melanophores in mutants treated with bafilomycin compared to DMSO-treated mutants, and a 15.74% decrease in dorsal and ventral stripe melanophores in WT siblings treated with bafilomycin compared with DMSO-treated WT siblings (Figure 6a–e; Table S6). We suggest autophagy promotes melanophore survival when *ap3d1* is mutated.

4 | DISCUSSION

We characterized a novel syndromic albinism zebrafish model named crasher. Mutations in *ap3d1* caused aberrant pigmentation in zebrafish, as seen in HPS patients, *ap3d1* mutant mocha mice, and other melanogenesis enzyme trafficking zebrafish mutants, such as vps11 (Ammann et al., 2016; Clancey et al., 2013; Kantheti et al., 1998). Among HPS10 animal models, crasher uniquely overexpressed exon 14 of *ap3d1*. Though the etiology of the allele is still unknown, it could be possible that exon 14 is left in a greater proportion of alternatively spliced transcripts in mutants than WT fish. Furthermore, the mutation may lie in a promoter as evidenced by reduced *ap3d1* expression, leading to decreased protein availability. Further studies will clarify the genetic nature of exon overexpression in crasher and its impact on *ap3d1* protein activity.

Crasher mutants do not survive, and cause of death is unknown. Patients with HPS10 die of infection (Ammann et al., 2016; Mohammed et al., 2019). According to GAGE analysis, crasher showed decreased expression in ribosome, oxidative phosphorylation, proteasome, and cardiac muscle contraction pathways, possibly indicating systemic stress, but these pathway reductions require further mechanistic studies to determine their impact on organismal health (Table S5). Deficiencies in these pathways are not necessarily linked to syndromic albinism disorders. crasher mutants have upregulated immune signaling pathways which may not parallel the immune dysfunction observed in patients with HPS10 (Ammann et al., 2016; Mohammed et al., 2019). RNA-Seq was performed at 3 dpf, 6 to 8 days before mutants die, and may not reflect immune dysfunction present later.

Specified and early differentiated melanophores were not affected by *ap3d1* mutations. Melanogenesis was affected in crasher mutants, likely due to changes in the melanogenesis gene expression (*dct* and *tyrp1b*) in addition to melanogenesis enzyme trafficking defects (Richmond et al., 2005). AP-3 regulates zinc in mouse brains (Kantheti et al., 1998). DCT and TYRP1 rely on zinc as a cofactor, but whether AP-3 directly or indirectly mediates melanogenesis gene expression requires further investigation (Lai et al., 2017; Solano et al., 1996). Interestingly, LROs traffic mRNA cleavage protein, Ago. Garnet flies mutated in the AP-3 subunit delta have increased RNA silencing and decreased pigmentation (Harris et al., 2011). *Crasher* is an attractive model to study *dct* and *tyrp1b* expression and cell trafficking effects on the expression of melanogenesis enzyme mRNA.

Autophagy is a cellular process involved in melanocyte loss and survival (Clancey et al., 2013). Inhibiting autophagy negatively impacted crasher melanophore survival, suggesting autophagy up-regulation occurs to prevent further melanophore loss in crasher. The AP-3 complex may interact with PI(4)KIIα, involved in autophagosome maturation, and ATG9A, an autophagy sorting signal, to transport cargo from the Golgi to the autophagosome (De Tito et al., 2020). If AP-3 is needed for autophagosome maturation, then loss of AP-3 would be expected to inhibit autophagy. However, AP-3 complex loss is associated with increased LC3-II activation in a cell specific manner, and LC3-II activation is correlated with autophagosome formation (Kabeya et al., 2000; Mantegazza et al., 2017). Additionally, treatment of vps11 zebrafish mutants with bafilomycin A1 increases melanophore number, indicating autophagy and/or pH regulation play different roles during melanophore development (Clancey et al., 2013). Further studies on autophagy in crasher and vps11 mutants are needed to understand the context in which autophagy impacts melanophore survival.

Crasher is a novel zebrafish model for human HPS10. Whether mutants display the neurological and immunological symptoms in
HPS10 remains to be tested. In any case, crasher will be an excellent model to better understand AP-3 complex, specifically the δ subunit, function in pigment development and disease.

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DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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