

An epigenetic mechanism for cavefish eye degeneration

Aniket V. Gore^{1,*}, Kelly A. Tomins¹, James Iben², Li Ma³, Daniel Castranova¹, Andrew E. Davis¹, Amy Parkhurst¹, William R. Jeffery³ and Brant M. Weinstein^{1,*}

Supplementary Information Guide

Supplementary figures 1-7

Supplementary figure legends 1-7

Supplementary data 1: Differentially up and down regulated genes from surface and cavefish eyes at 54hpf by RNA seq analysis

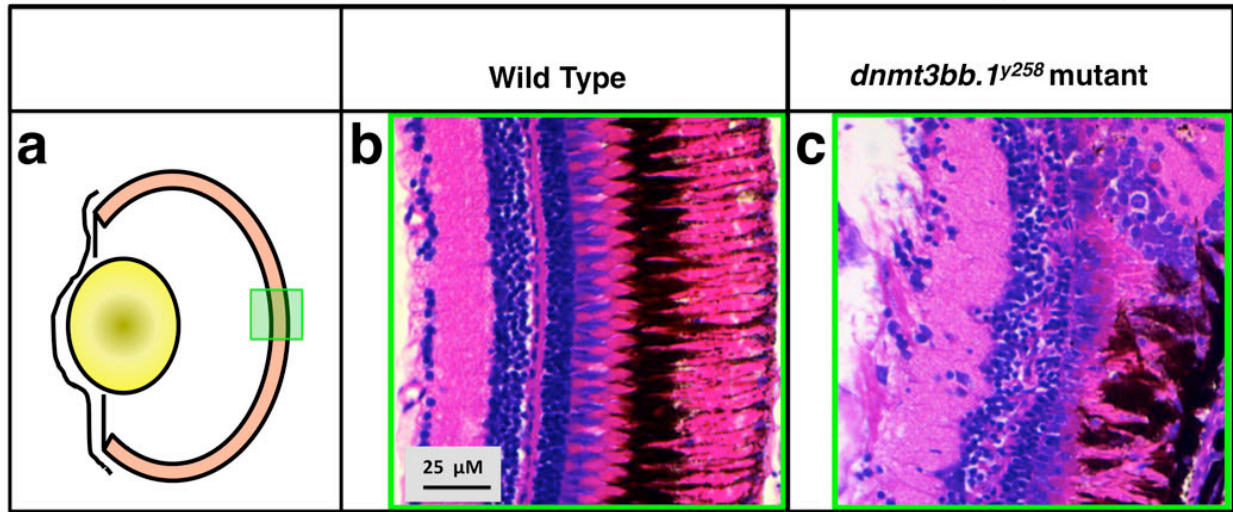
Supplementary data 2: Cavefish genes with significant promoter hypermethylation and reduced gene expression

Supplementary data 3: Cavefish genes with substantial promoter hypermethylation and reduced gene expression and their linked human disease phenotypes

Supplementary data 4: Primer sequences used in this study

Supplementary Figure 1

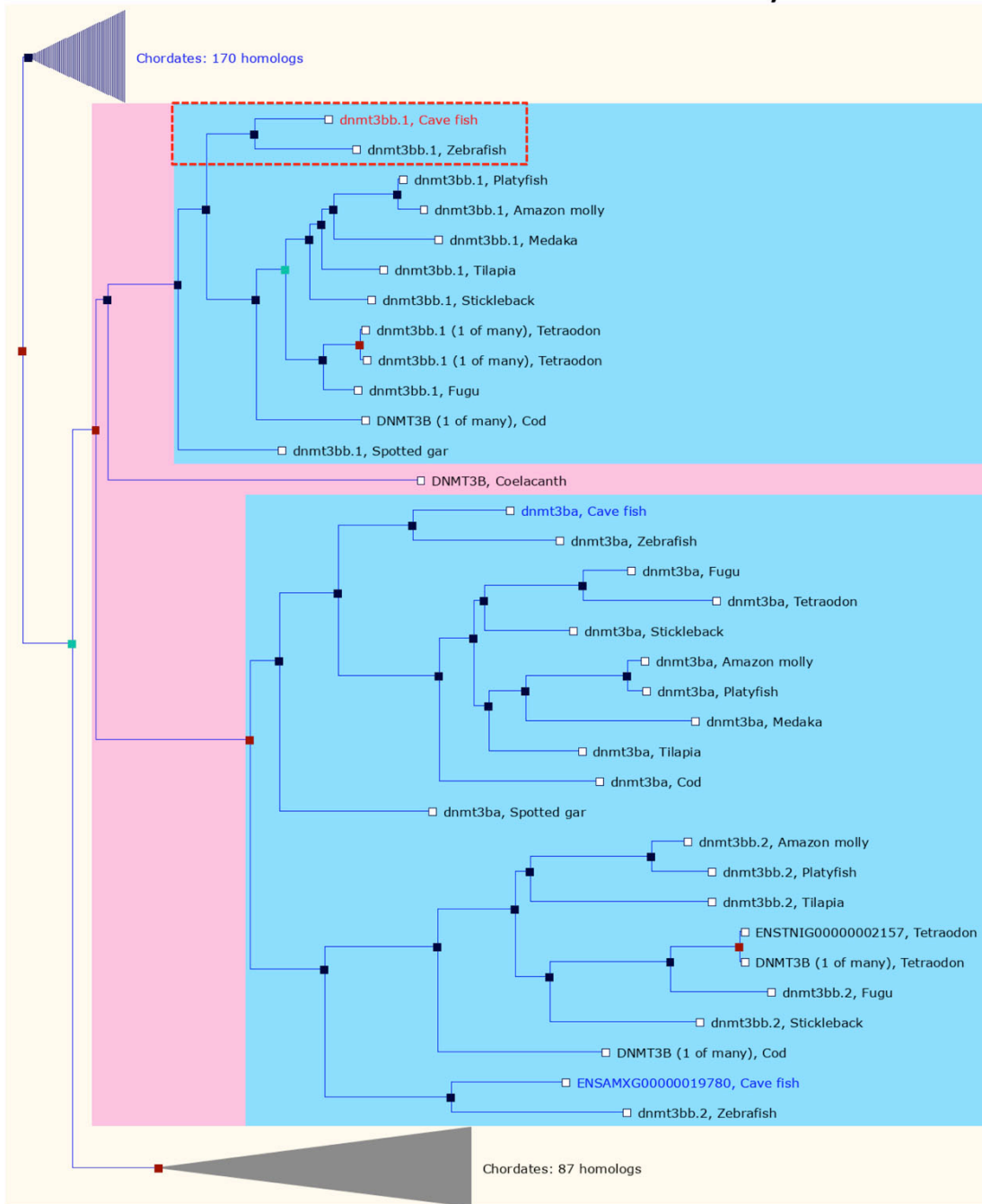
Zebrafish *Danio rerio* Eyes



Eye phenotypes in in zebrafish *Danio rerio* wild type and *dnmt3bb.1^{y258}* mutant animals. **a**, Schematic diagram of a transverse section through the adult fish eye, with approximate area of the eye shown in panels b and c noted by the green box. **b,c**, H&E-stained transverse sections through adult wild type (b) and *dnmt3bb.1^{y258}* mutant (c) eyes. The wild type retina (b) contains well-organized normal layers while hyperplasia and abnormal dysmorphic layers are noted in *dnmt3bb.1^{y258}* mutants (n=2). Scale bar 25 μ M in b for b,c.

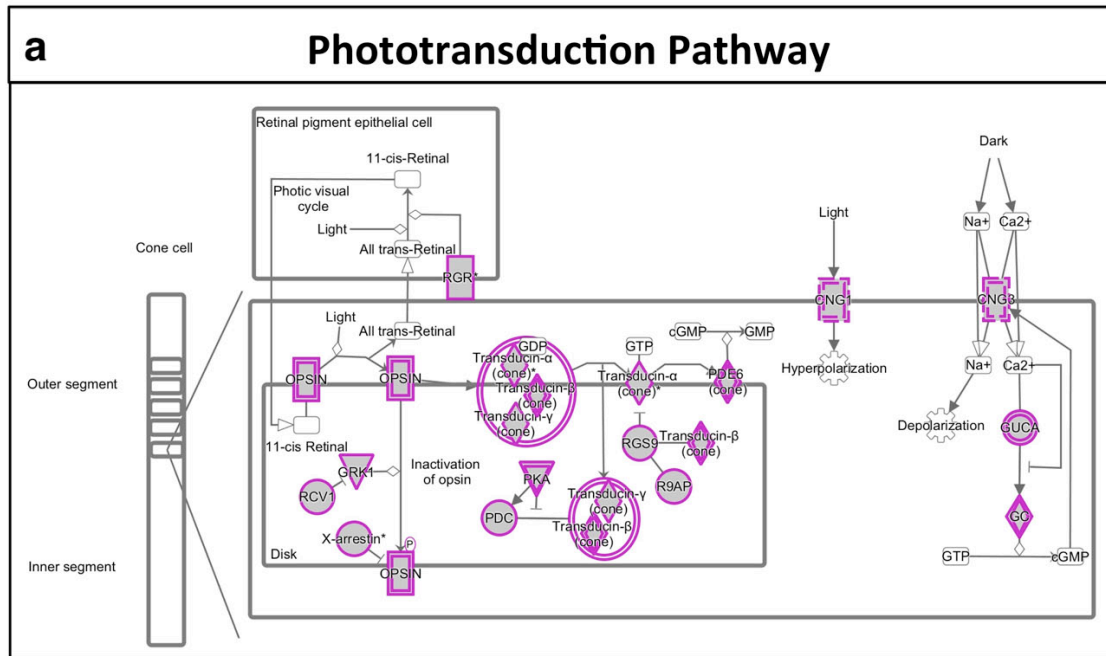
Supplementary Figure 2

D. rerio and *A. mexicanus* *dnmt3bb.1* are closely related



Astyanax mexicanus *dnmt3bb.1* homology to zebrafish. Phylogenetic tree showing *A. mexicanus* Dnmt3bb.1 homology to different teleost fish. Its closest homolog is zebrafish Dnmt3bb.1 (dotted red box).

Supplementary Figure 3



b Ingenuity Pathway Analysis

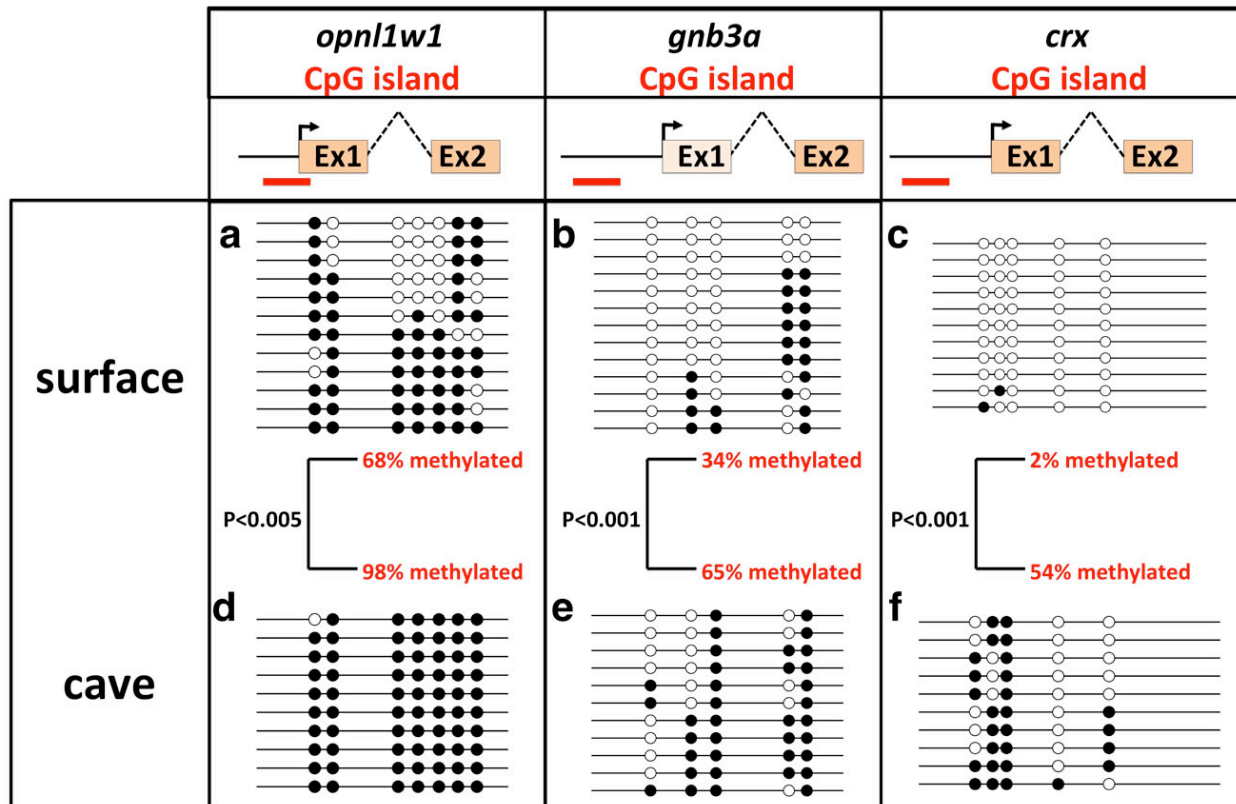
Categories	Diseases or Function Annotations	p-Value
Nervous System Development and Function	electrophysiology of eye	2.81E-37
Developmental Disorder, Hereditary Disorder, Ophthalmic Disease, Organismal Injury and Abnormalities	retinal dystrophy	2.38E-36
Hereditary Disorder, Ophthalmic Disease, Organismal Injury and Abnormalities	Hereditary Eye Disease	3.6E-35
Visual System Development and Function	vision	2.13E-31
Ophthalmic Disease, Organismal Injury and Abnormalities	retinal degeneration	7.97E-28
Nervous System Development and Function, Visual System Development and Function	electrophysiology of retinal rods	1.69E-27
Embryonic Development, Organ Development, Organ Morphology, Organismal Development, Tissue Development, Visual System Development and Function	morphology of eye	2.83E-23
Embryonic Development, Organ Development, Organismal Development, Tissue Development, Visual System Development and Function	formation of eye	9.59E-23
Cell Morphology, Embryonic Development, Organ Development, Organ Morphology, Organismal Development, Tissue Development, Visual System Development and Function	Cell Morphology, Embryonic Development, Organ Development, Organ Morphology, Organismal Development, Tissue Development, Visual System Development and Function	1.03E-20
Nervous System Development and Function, Visual System Development and Function	electrophysiology of retinal cone cells	4.91E-17

Ingenuity Pathway Analysis (IPA) of differentially expressed genes in cave and surface fish.

a, IPA suggests the phototransduction pathway is one of the key signaling pathways affected in cavefish eyes. Genes highlighted in purple are significantly downregulated. **b**, Developmental processes most significantly affected in cavefish, as predicted by IPA analysis.

Supplementary Figure 4

Targeted Bisulfite Sequencing of *opn1w1*, *gnb3a*, and *crx* CpG Islands from surface or cave morphs of *Astyanax mexicanus*



Targeted bisulfite sequencing of eye genes from cave and surface morphs. a-f, Targeted bisulfite sequencing analysis of DNA methylation in CpGs isolated from the *opn1w1* (a,d), *gnb3a* (b,e) and *crx* (c,f) promoter regions of 54-60 hpf surface (a-c) or cavefish (d-f) *Astyanax mexicanus* eyes. Methylation of all three genes is increased in cave compared to surface fish. p values are calculated using paired t-test.

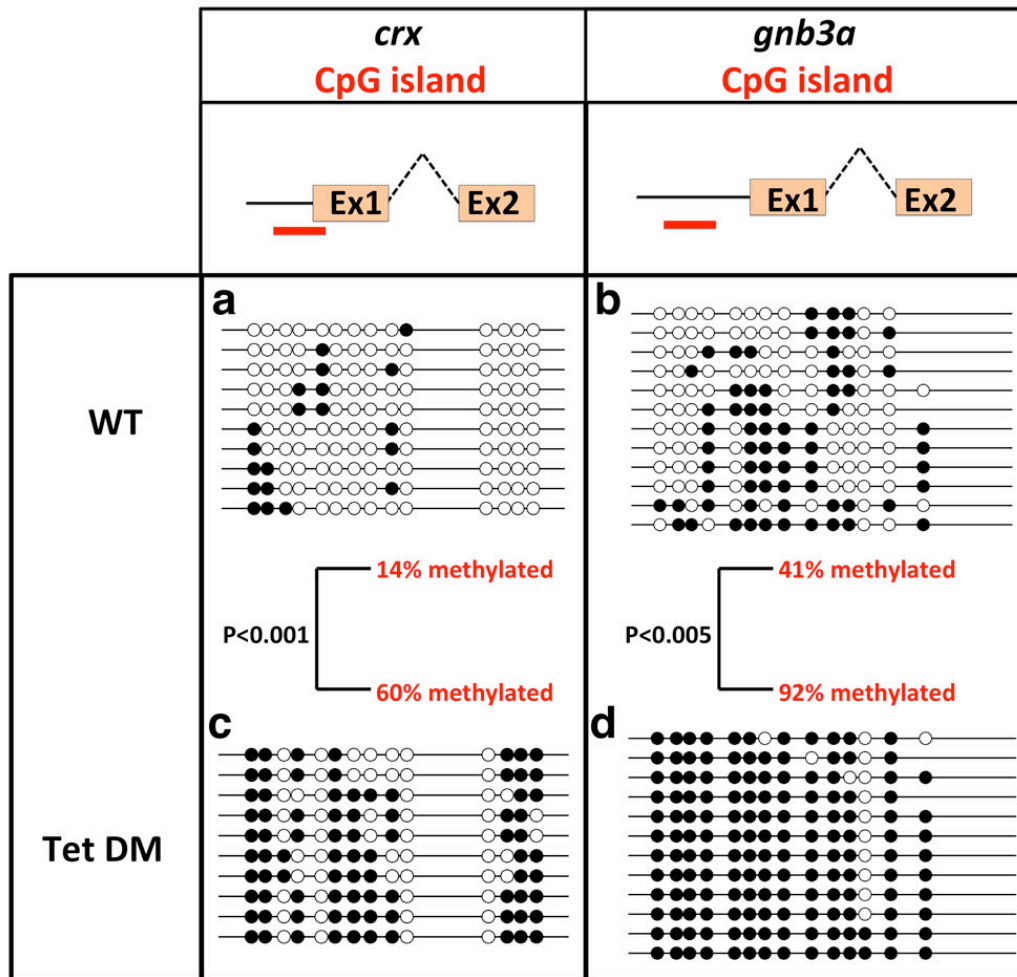
Supplementary Figure 5

Crx target genes (no significant change in methylation in CF vs. SF)			
Gene	Fold reduced Expression (Log ₂ , p<0.05)		Associated human Eye phenotype
	-10	0 10	
NR2E3	■		Enhanced S-cone syndrome
CNGB3	■		Achromatopsia pingelapese
PDE6G	■		Retinitis pigmentosa
TULP1	■		Retinitis pigmentosa
CNGA3	■		Achromatopsia
RDH5	■		Fundus albipunctatus
NRL	■		Retinitis pigmentosa
RCVRN	■		
PDE6B	■		Retinitis pigmentosa
ABCA4	■		Macular dystrophy
RP1	■		Retinitis pigmentosa
PDE6A	■		Retinitis pigmentosa
CNGA1	■		Retinitis pigmentosa
CABP5	■		
GUCA1B	■		Retinitis pigmentosa
SAG	■		Retinitis pigmentosa
LAPTM4B	■		
GNAT2	■		Recessive achromatopsia
GNGT2	■		
ARR3	■		
PDC	■		Familial paroxysmal nonkinesigenic dyskinesia
PDE6C	■		Cone dystrophy
RHO	■		Retinitis pigmentosa
Crx target genes (also hypermethylated in CF vs. SF)			
GNB3	■		Night blindness
PDE6H	■		Achromatopsia
RS1	■		Retinoschisis
PRPH2	■		Retinitis pigmentosa
OPN1LW	■		Macular dystrophy

Reduced expression of Crx target genes in cavefish. RNAseq analysis reveals that twenty-eight known Crx target genes also show reduced expression in 54-60 hpf cave versus surface fish eyes (fold decrease ≤ 1.5 , $p \leq 0.05$), although twenty-three of these twenty-eight genes show no associated changes in DNA methylation. Twenty-three of these genes have also been linked to human eye disorders. Five target genes of Crx are also hypermethylated.

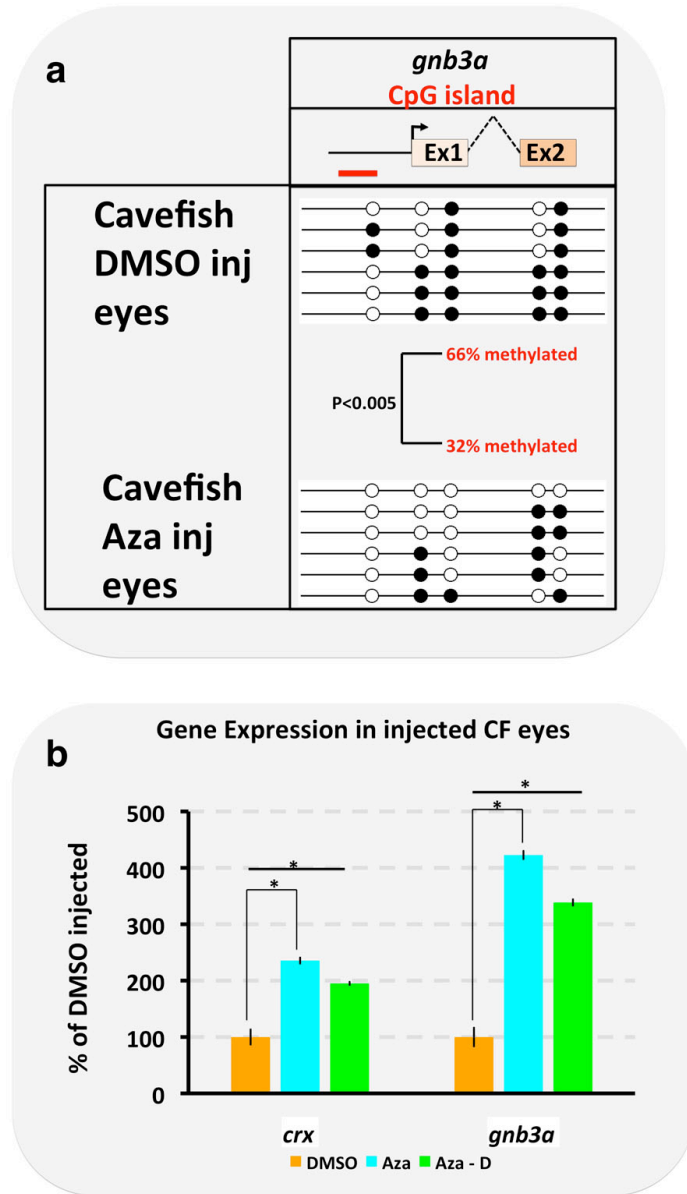
Supplementary Figure 6

Targeted Bisulfite Sequencing of *crx* and *gnb3a* CpG Islands from Wild Type Sibling and *tet*^{2/3} Double Mutant (DM) Zebrafish



Targeted bisulfite sequencing of eye genes from WT and *tet2/3* double mutants. a-f, Targeted bisulfite sequencing analysis of DNA methylation in CpG islands isolated from the *crx* (a,c) or *gnb3a* (b,d) promoter regions of 48 hpf wild type sibling (a,b) or *tet*^{2-/-}, *tet*^{3-/-} double mutant (c,d) zebrafish eyes. Methylation of both genes is increased in *tet*^{2-/-}, *tet*^{3-/-} double mutants compared to their wild type siblings. p values are calculated using paired t-test.

Supplementary Figure 7



Aza or Aza-D eye injections in cavefish embryos block DNA methylation and activate eye gene expression. **a**, Targeted bisulfite sequencing analysis of DNA methylation in CpG islands isolated from the *gnb3a* promoter region of 5 dpf DMSO or Aza injected cavefish embryonic eyes. p values are calculated using paired t-test. **b**, Quantitative RT-PCR analysis of the percent relative expression of *crx* and *gnb3a* in isolated eyes from 5 dpf DMSO (orange columns), Aza (blue columns), or 5-aza-2-deoxycytidine (Aza-D; green columns) injected cavefish embryonic eyes. Histograms show mean, error bars represent s.e.m of three biological replicates; two-tailed t-test, * $p < 0.005$.