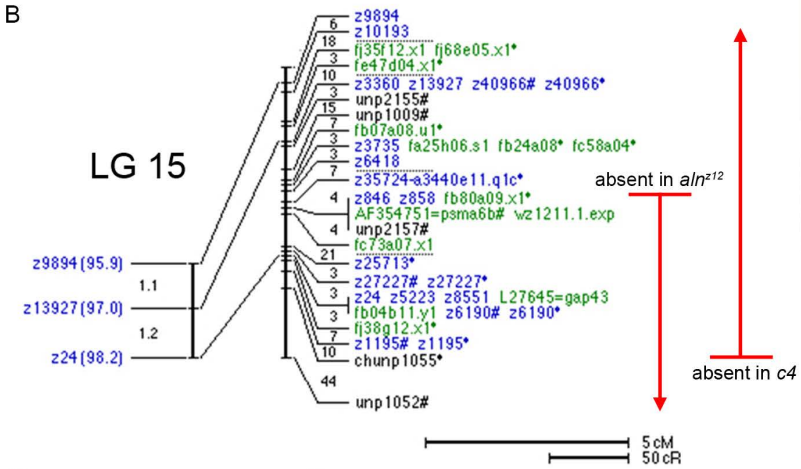
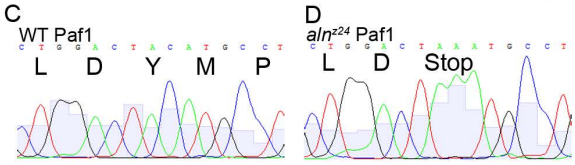
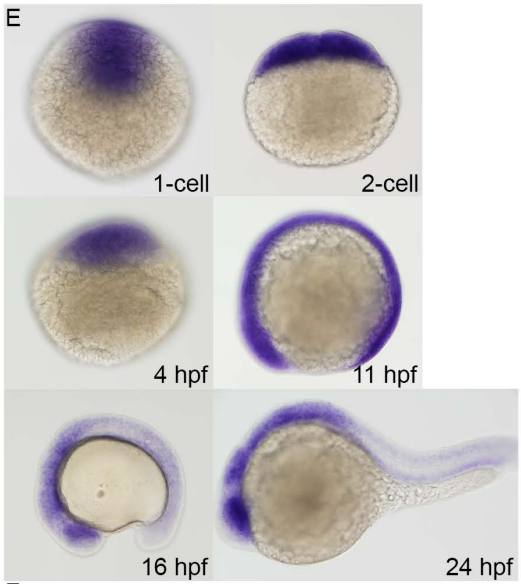
A

Present in *aln*^{z12}/*c4*	Absent in *aln*^{z12}/*c4*
z13927	z846
z40966	z858
z3735	z5223
z6418	z6190
z25713	z1195
z27227	*gap43*
z37	*lsamp*
psma6b	*phox2a*
ship2	fa25h06
otx5	fb80a09
dmnn9	zeh0667
map4k	wz10529
stard10	wz13579
sfrp2	wz6078
kbtb3	wz1221
cent	
qik	
fb07a08	







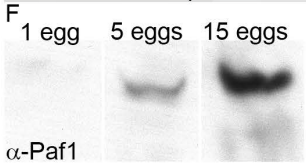


Figure S1.

(A) SSRs, genes, and ESTs that are present or absent in *aln*^{z12}*/c4* genomic DNA as determined by PCR analysis. (B) Meiotic map (left) and physical map (center) of LG15 genomic regions affected by the *aln*^{z12} and *c4* mutations. Red arrows indicate approximate genomic regions removed by each mutation. Region absent in *aln*^{z12}*/c4* transheterozygous embryos defines the *aln* 'critical region'. Partial sequence chromatogram of *paf1* coding sequence from +/+ (C) and *z24/z24* (D) embryos. The *z24* mutation results in a C>A transversion at base pair 843 of the *paf1* coding sequence, which causes a premature stop codon. (E) *paf1* mRNA expression in WT embryos. *paf1* transcripts are maternally supplied as detected by WISH of *paf1* at the 1-cell and 2-cell stages. *paf1* transcripts are broadly expressed during early cleavage (4 hpf) through the beginning of segmentation stages (11 hpf / 2 somite). As development continues (16 hpf and 24 hpf), *paf1* expression is most highly maintained in the anterior portion of the CNS. (F) Immunoblot analysis indicates Paf1 protein is maternally loaded into unfertilized eggs. 1-4 hpf images, animal pole up; 11-24 hpf images, lateral views with anterior to the left.

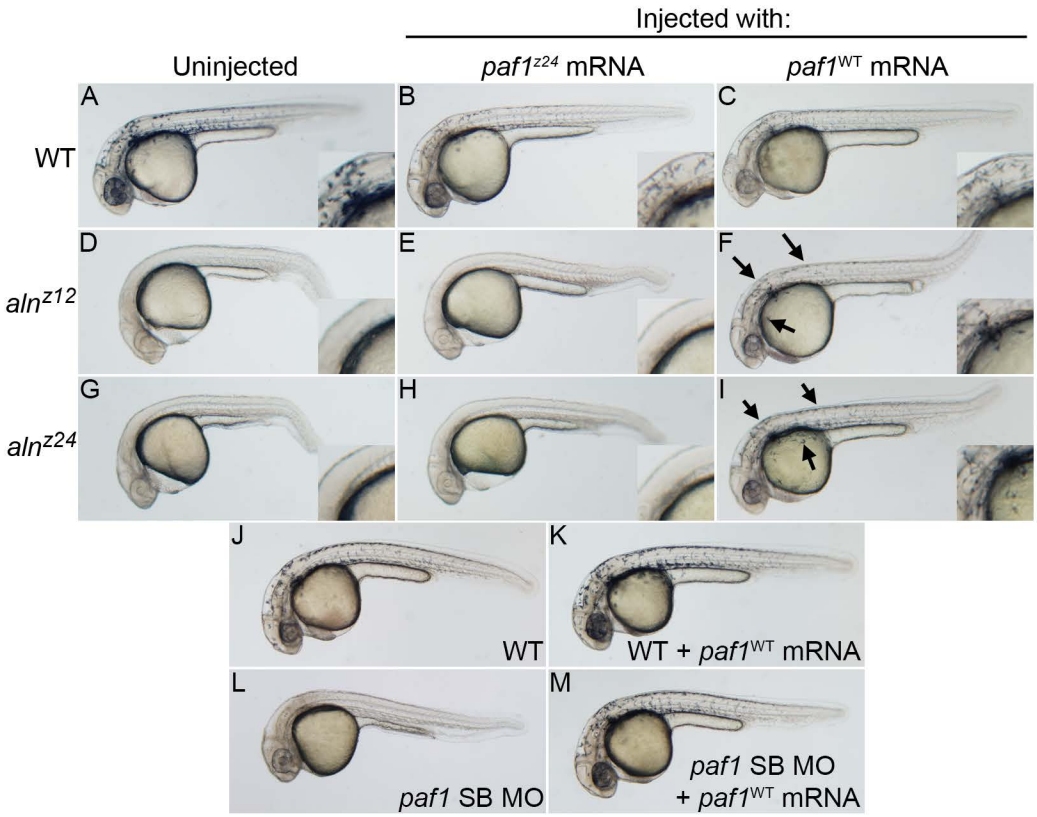


Figure S2.

Loss of the *paf1* gene is responsible for the *aln* phenotype. The phenotypes of uninjected WT (A), *aln*^{z12} (D), and *aln*^{z24} (G) 48 hpf embryos. One-cell WT or *aln* mutant embryos were injected with either mutant *paf1* (encoding Paf1^{T281X}) (B, E, and H) or WT *paf1* (C, F, and I) mRNA. Injection of *paf1* RNA into WT embryos does not affect melanophore formation. Injection of WT but not mutant *paf1* mRNA rescues melanophore formation in *aln*^{z12} or *aln*^{z24} embryos (F, I). Insets show higher magnification of the area near the ear. Arrows indicate examples of rescued melanophore. (J – M) Embryos injected with splice blocking (SB) MO. (J, K) Injection of SB MO into 1-cell WT embryos produces phenocopies of the *aln* mutant. (L, M) WT *paf1* mRNA injection can rescue the SB MO-induced phenotype.

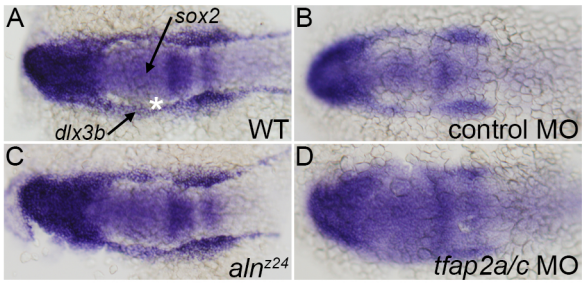


Figure S3.

The NC does not transfate in *aln*^{z24} mutant embryos. (A-D) The NC domain is maintained in *aln*^{z24} mutants, but is absent in *tfap2a/c* deficient embryos. (A) 11-11.5 hpf WT embryo showing expression of *sox2* in the neural plate and *dlx3b* in the preplacodal ectoderm. The white asterisk (*) marks the presumptive NC domain. This domain is readily distinguished in both control MO (B) and *aln*^{z24} mutant embryos (C) as compared with *tfap2a/c* deficient embryos (D), which have an expanded *sox2* domain. A-D, dorsal views with anterior to the left.

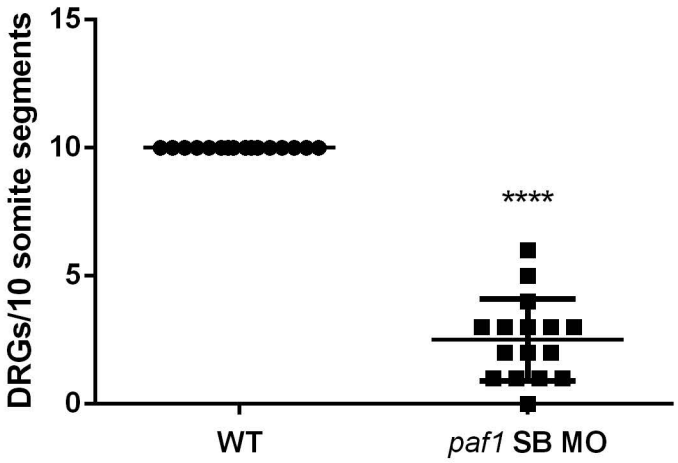


Figure S4

Loss of DRG cell formation in embryos with reduced Paf1 function. The number of DRGs per 10 somite segments were counted in 16 WT and 16 *paf1* SB MO-injected *Tg(isl2b:GFP)* embryos at 72 hpf. Error bars indicate mean ± SD. **** = $p < 0.0001$, determined by an unpaired Student's t-test.

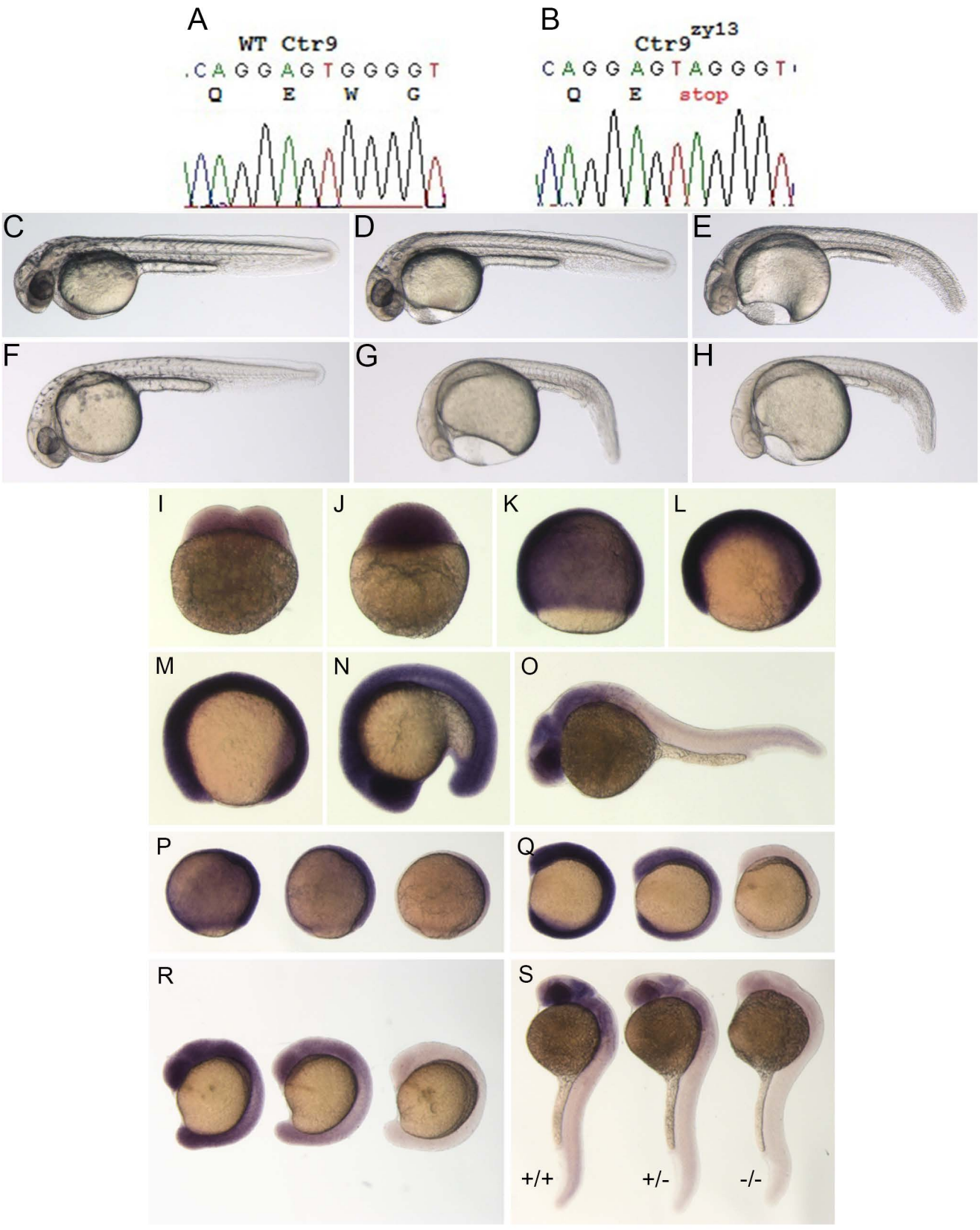


Figure S5.

A premature stop mutation in *ctr9* is responsible for the *zy13* phenotype. Sequencing traces of WT (A) and *zy13* mutant (B) genomic DNA revealed a G to A substitution that causes an in-frame stop at amino acid 580 of the Ctr9 protein ($Ctr9^{W580X}$). (C) Injection of 100 pg WT *ctr9* RNA at the 1-2 cell stage had no effect on the phenotype of WT embryos. However, injection of WT *ctr9* RNA into $ctr9^{zy13}$ mutant embryos (D) substantially rescued the pigmentation defects and ventral flexion of the tail that are hallmarks of uninjected mutant embryos (E). Injection of mutant *ctr9* RNA had no effect on WT embryos (F), and it also provided no rescue to mutant embryos (G), which appeared identical to uninjected mutant siblings (H). Lateral views of embryos: C-E, 38 hpf; F-H, 32 hpf. Expression of WT *ctr9* and $ctr9^{zy13}$ mutant RNA transcripts in developing embryos: WT RNA is broadly expressed, whereas mutant RNA is not detectable presumably due to nonsense-mediated decay. WISH reveals WT *ctr9* RNA is present in blastomeres of early cleavage stage embryos (I) and remains ubiquitously expressed as embryonic development continues through the (J) sphere, (K) 80% epiboly, (L) bud, (M) 5 somite, and (N) 15 somite stages. (O) By 24 hpf *ctr9* expression has been downregulated in much of the embryo, but remains at high levels throughout the central nervous system. Embryos produced from intercrosses among $ctr9^{zy13/+}$ individuals exhibited three different levels of *ctr9* RNA expression at (P) the 90% epiboly stage, (Q) the 5 somite stage, (R) the 15 somite stage, and (S) 24 hpf. At all stages shown the ratio of high:medium:low-expressing embryos was approximately 1:2:1. High, medium, and non-expressing 24 hpf embryos shown in (S) were genotyped and shown to be homozygous WT, heterozygous, and *ctr9* mutants respectively.

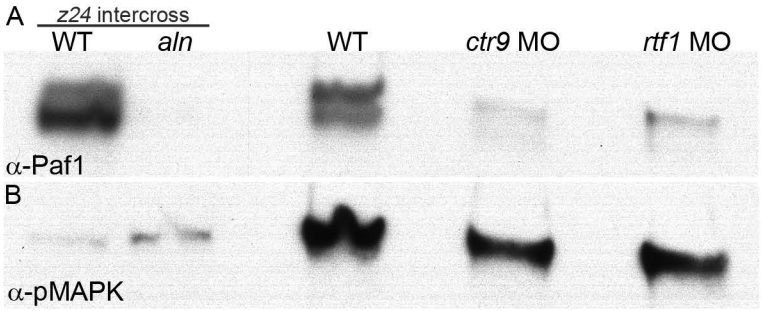


Figure S6.

Paf1 protein expression is dependent on other Paf1C members. (A, B) Immunoblot analysis of Paf1 expression in WT sibling, *aln*^{z24}, WT control, Ctr9-, and Rtf1-depleted (MO-injected) embryos. (A) Analysis of 36 hpf progeny from an intercross of *aln*$^{z24/+}$ animals indicates Paf1 protein is absent from *aln*^{z24} mutant embryos. Paf1 protein is also reduced in extracts prepared from 16-18 hpf *ctr9* and *rtf1* MO-injected embryos relative to the amount present in control WT samples. (B) pMAPK expression serves as a loading control.

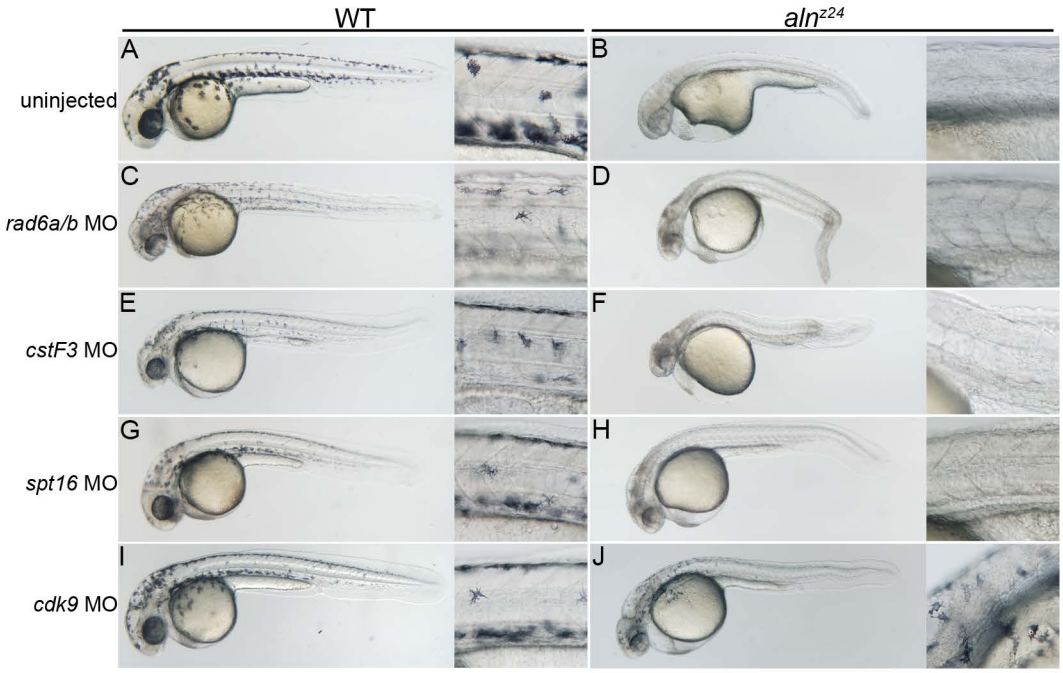


Figure S7.

Loss of Cdk9 function in *aln* rescues melanophore development. (A-J) Effects of knockdown of factors known to functionally interact with the Paf1C during transcription. Injection of *rad6a/b* (C and D), *cstF3* (E and F), *spt16* (G and H), or *cdk9* (I and J) MOs into *aln*^{z24} intercross embryos. Only MO depletion of Cdk9 rescues melanophore formation in *aln*^{z24} mutant embryos (compare A, B, I, and J). Melanophores present in *cdk9* MO-injected *aln*^{z24} mutant embryos migrate throughout the embryo and assume WT morphology.