

Fig. S1. Phylogenetic analysis of Tyrosinase amino acid sequences. Alignment and phylogenetic analysis of nine deuterostome Tyrosinase sequences were performed in MEGA6, using the WAG model of protein evolution for Maximum Likelihood tree construction. A 250 replicate bootstrap test was performed; the scores for this analysis are found at the nodes in the tree. Sequence accession numbers are as follows: *Branchiostoma floridae* Tyrosinase: XM_002598584, *Canis lupis familiaris* Tyrosinase: NP_001002941.1, *Ciona intestinalis* Tyrosinase: XM_002123004, *Danio rerio* tyrosinase: AAN17339.1, *Gallus gallus* Tyrosinase: NP_989491.1, *Homo sapiens* TYROSINASE: NP_000363.1, *Mus musculus*: NM_011661, *Petromyzon marinus* Tyrosinase: KR150760, *Saccoglossus kowalevskii* Tyrosinase-like: XP_006817095.1.

A	<i>Tyr g2</i>	<p>GC GG GCTTCCCTTCGCGGGAGTCGACGACCGGAGCTCTGGCCACGGCTTCTACTCGCTGTGCGCTGCCGGGCCAACTACTGGGGCCA GC GG GC-----ACGACTGCGGCA GC GG GCTTCCCTTCGCGGGAGTCGACGACCGGAGCTCTGGCCACGGC-----TGTGCGCTGCCGGGCCAACTACTGGGGCCA GC GG GCTTCCCTTCGCGGGAGTCGACGACCGGAGCTCTGGCCACGGCTTCT(+28)-----GTGCGCTGCCGGGCCAACTACTGGGGCCA GC GG GCTTCCCTTCGCGGGAGTCGACGACCGGAGCTCTGGCCACGGCTTCTAC-----GTGCA GC GG GCTTCCCTTCGCGGGAGTCGACGACCGGAGCTCTGGCCACGGCTTCTAC-----GCGCTGTGCGCTGCCGGGCCAACTACTGGGGCCA</p>
B	<i>FGF8/17/18 g1</i>	<p>GGCGGCGCGGATTTTACGACGACGTGGAGGCGCAGCTCCAGCGACGACGCGCGTGAGCCGCAAGCACATCCGCTCCTATCAGCTCTACAGCC GGCG-----CCGACGCGGTGAGCCGCAAGCACATCCGCTCCTATCAGCTCTACAGCC GGCGGCGCGGA-----CGCGTGAGCCGCAAGCACATCCGCTCCTATCAGCTCTACAGCC GGCGGCGCGGATTTTACGACGACGTGGAGGCGCAGCTCCAG-----ACCGACGCGGTGAGCCGCAAGCACATCCGCTCCTATCAGCTCTACAGCC</p>
C	<i>FGF8/17/18 g2</i>	<p>GCGACCGGACGCGGTGAGCCGCAAGCACATCTCTCTATCAGCTCTACAGCCGACGAGCGGGAAGCACGTGCAGATCGTGGCGAGCGCATCA GCGACCGGACGCGGTGAGCCGCAAGCAC-----TATCAGCTCTACAGCCGACGAGCGGGAAGCACGTGCAGATCGTGGCGAGCGCATCA GCGACCGGGAAGCGGTGAGCCGCAAGCACAT-----ACGCGCATCA GCGACCGGACGCGGTGAGCCGCAAGCACATCCG-----GCATCA</p>

Fig. S2. Genotyped loci of targeted genes. All sequences show the sense strand in 5' to 3' orientation with respect to the corresponding gene. Forward target sites and PAMs are shown in yellow and purple, respectively, while the reverse strand target and PAM are shown in orange and red, respectively, for *FGF8/17/18* gRNA 2. Deletions relative to the wt sequence are shown as red dashes, while polymorphisms and inferred insertions are shown as red letters. (A) wt (top sequence) and 5 example mutant sequences are shown for *Tyr* gRNA 2 target site; "(+28)" indicates a 28 bp insertion. (B) wt (top sequence) and 3 mutant sequences returned for genotyping of the *FGF8/17/18* gRNA 1 target site. (C) wt (top sequence) and 3 mutant sequences returned for genotyping of the *FGF8/17/18* gRNA 2 target site.

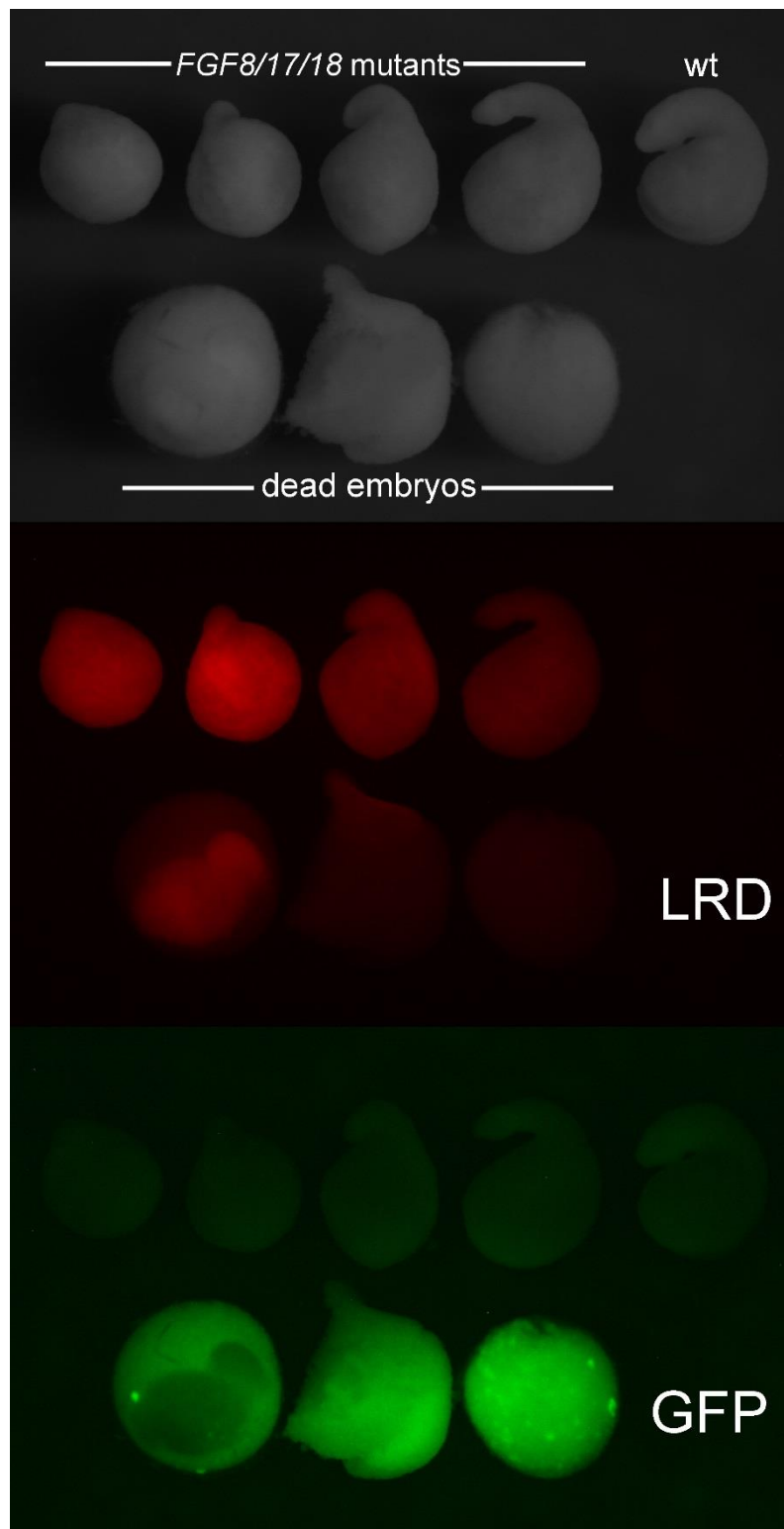


Fig. S3. Severely affected *FGF8/17/18* mutants do not display autofluorescence associated with death. Whole-mount embryos from each of 4 mutant classes, and a wt embryo shown with normal light (top), LRD fluorescence (middle), and on the GFP channel (bottom). Note the high level of autofluorescence exhibited by the dead embryos, whereas even the most affected mutants glow only as strongly as a wt embryo.

Tables S1

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