Supplemental Materials

Molecular Biology of the Cell

Clark and Link

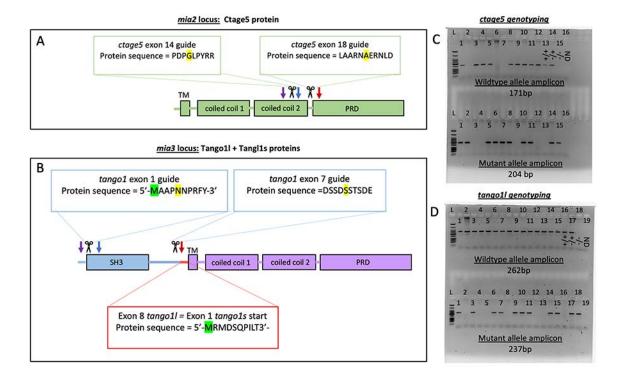


Fig. S1: Description of Ctage5 protein from the *mia2* **locus and Tango11 and Tango1s proteins from the** *mia3* **locus.** (A) Ctage5 is expressed from the *mia2* locus. The two guide RNAs (yellow highlight) cut a segment of DNA out that expresses the coiled coil 2 protein domain. (B) Tango11 and Tango1s are expressed from the mia3 locus. The first guide RNA (yellow highlight) cuts around 4 amino acids after the start site (green highlight). The second guide RNA cuts in exon 7 eliminating the entire luminal domain of Tango11 (blue + purple domains). Exon 1 of Tango1s (red + purple) starts in exon 8 of Tango11 (green highlight) and is not cut by the guide RNAs. tango11 exon 8 is not included in the mature Tango11 protein. In A and B, the arrows represent primer locations (blue=wildtype, red=mutant, purple=shared). (C+D) Agarose gels for *ctage5* (C) and *tango11* genotyping (D) using a low-molecular weight ladder (L) showing wildtype or mutant amplicons from numbered samples using the primers from the schematic in A (ND=no gDNA control).

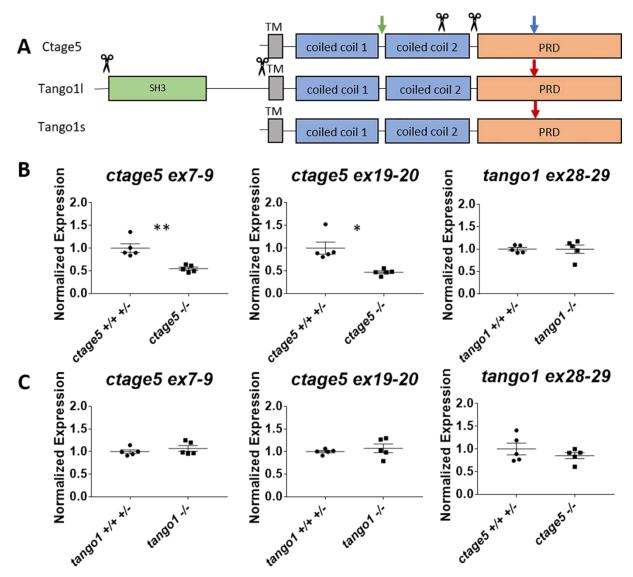


Fig. S2: *ctage5* mutants have decreased transcript: (A) Schematic showing large deletion cut sites (scissors), and qRTPCR primer set locations (green arrow=*ctage5* exon 7-9; blue arrow=*ctage5* exon 19-20; red arrow=*tango1* exon 28-29). (B) qRTPCR t-test results investigating alterations in gene expression in 7 dpf large deletion mutants compared to wildtype or heterozygous siblings using the specified primers. Welch's t-test was used to analyze statistical differences (*ctage5* ex7-9, p=0.0061; ctage5 ex19-20, p=0.0147; *tango1* ex28-29, p=0.9716) (C) qRTPCR results investigating compensation of *ctage5* or *tango1* gene expression in 7dpf *tango1* or *ctage5* large deletion mutants respectively compared to wildtype or heterozygous siblings using the specified primers. Welch's t-test was used to analyze statistical differences (*ctage5* ex7-9, p=0.3762; *ctage5* ex19-20, p=0.4954; tango1 ex28-29, p=0.3534). *=p<0.05**=p<0.01.

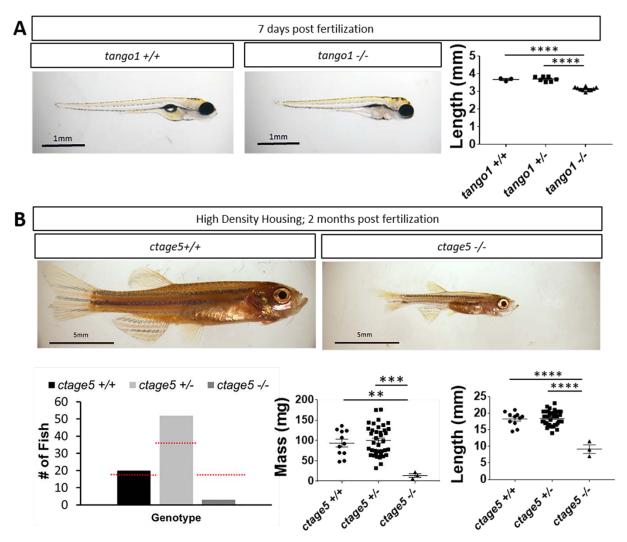


Fig S3: Size differences in *ctage5* and *tango1* mutants. (A) Length measurements for 7dpf embryos (one-way ANOVA, F=55.95, p<0.0001). (B) Representative images of *ctage5* +/+ and *ctage5* -/- 2-month-old zebrafish raised in a high density environment (about 30 fish per tank) and quantification for survival (χ^2 =18.92, p<0.05, n=75), mass (one-way ANOVA, F=8.372, p=0.0007), and length (one-way ANOVA, F=26.69, p<0.0001). Red-dotted lines in represent expected survival, and bars are actual survival.

Table S1: qPCR primer sequences.

ctage5 exon 7-9	
Primer 1	CTCATCTTGGCCGCTTCTAT
Primer 2	CATCGACGGCAGCACTAATA
ctage5 exon 19-20:	
Primer 1	GGCGGAGGCATTGACATTA
Primer 2	AGAGAAGGCTCTGGAGATATGA
tango1 exon 28-29:	
Primer 1	AGAGGTCCAGGCGGAAA
Primer 2	CACACATCGGCCCGTTT