

Supplementary material

The structure of TRAF7 coiled-coil trimer provides insight into its function in zebrafish embryonic development

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Supplementary Tables

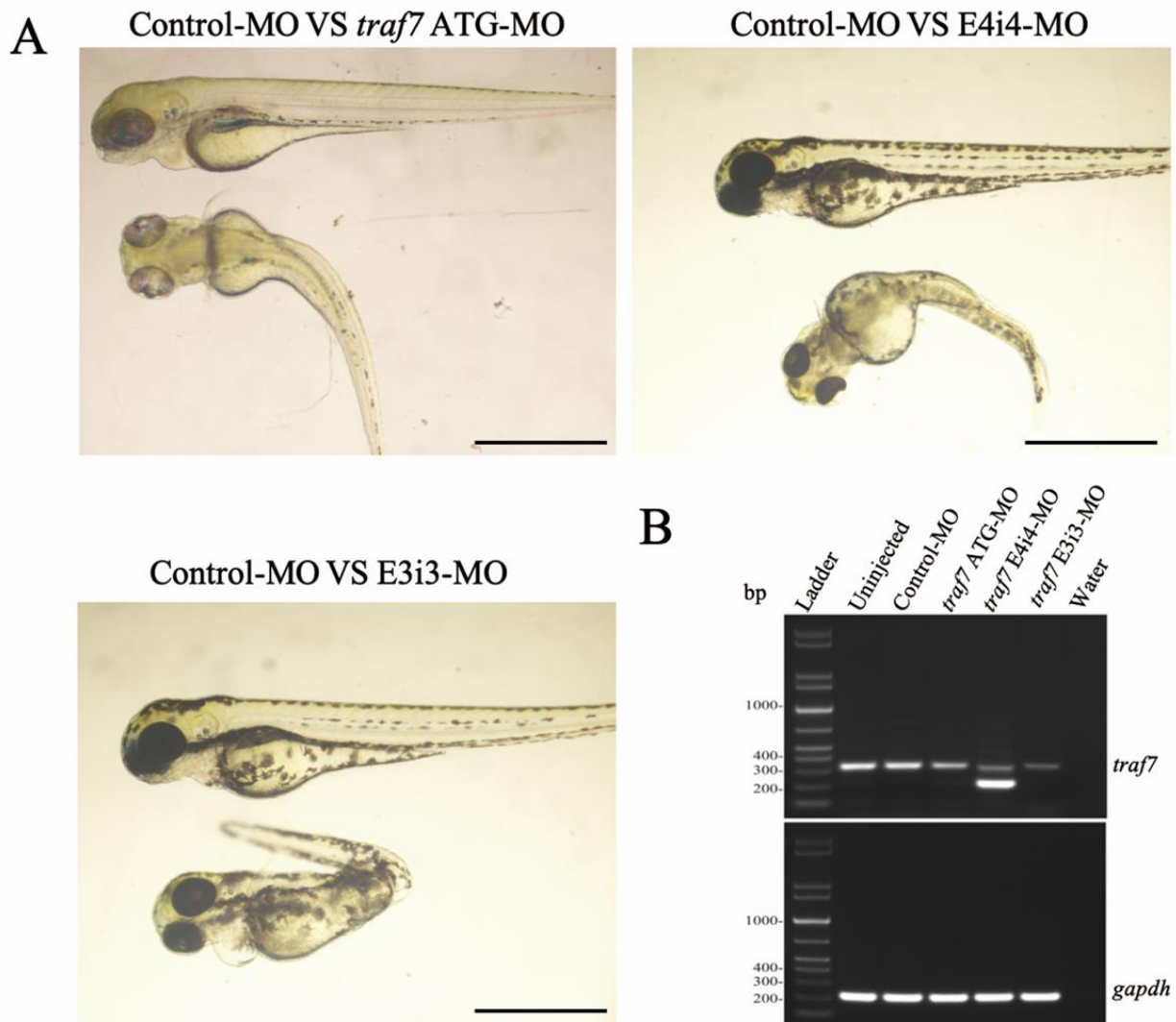
Supplementary Table S1 Primer sequences used in zebrafish experiments.

	Forward primer	Reverse primer
RT-PCR test	CAGCAGTCCCACAAGTAGCA	GCAGAATGTGTGTCCACAGG
pCS2+ zebrafish traf7-res	TAGCGAATTCGCCGCCACCCatggatgctggtttggatc	TAGCCTCGAGGtagcacgtccacacctttac
pCS2+ human TRAF7-res	tagcGAATTCgccaccatgagctcaggcaagagtgccgc	tagCTCGAGGtagcaagtccaaaccttcacagtgc
pCS2+ human K346E TRAF7-res	F1: gacgaaaaccagagcagctcagcaggacct F2: TACAAGCTACTTGTCTTTTTGCAGGA TCCgccaccatgagctcaggcaagagtgcc	R1: ggcctcgtgagctcgtctggtttcgtcc R2: TTCTAGAGGCTCGAGAGGCCTTGAAT TCtagcaagtccaaaccttcacagt
pCS2+ human R371G TRAF7-res	F1: tcccacatcaacgcggggctgaacatgggcat F2: TACAAGCTACTTGTCTTTTTGCAGGA TCCgccaccatgagctcaggcaagagtgcc	R1: tgcccatgttcagccccgcgttgatgtgggac R2: TTCTAGAGGCTCGAGAGGCCTTGAAT TCtagcaagtccaaaccttcacagt
pCS2+ human CCD TRAF7-res	F1: tacgggtgcacgttcacatctacaccctcagcagatctcaag F2: GCAGGATCCCATCGATTCGAATTCgcc accatgagctcaggcaagagtgccgc	R1: gtcgtagatgaacgtgcaccgtacttgga R2: TCACTATAGTTCTAGAGGCTCGAGGtag caagtccaaaccttcacagt
pClone007- traf7-WISH	CCTCCATTGCTCACTATGAA	AGTAGATCGCCTGTCTGAATA

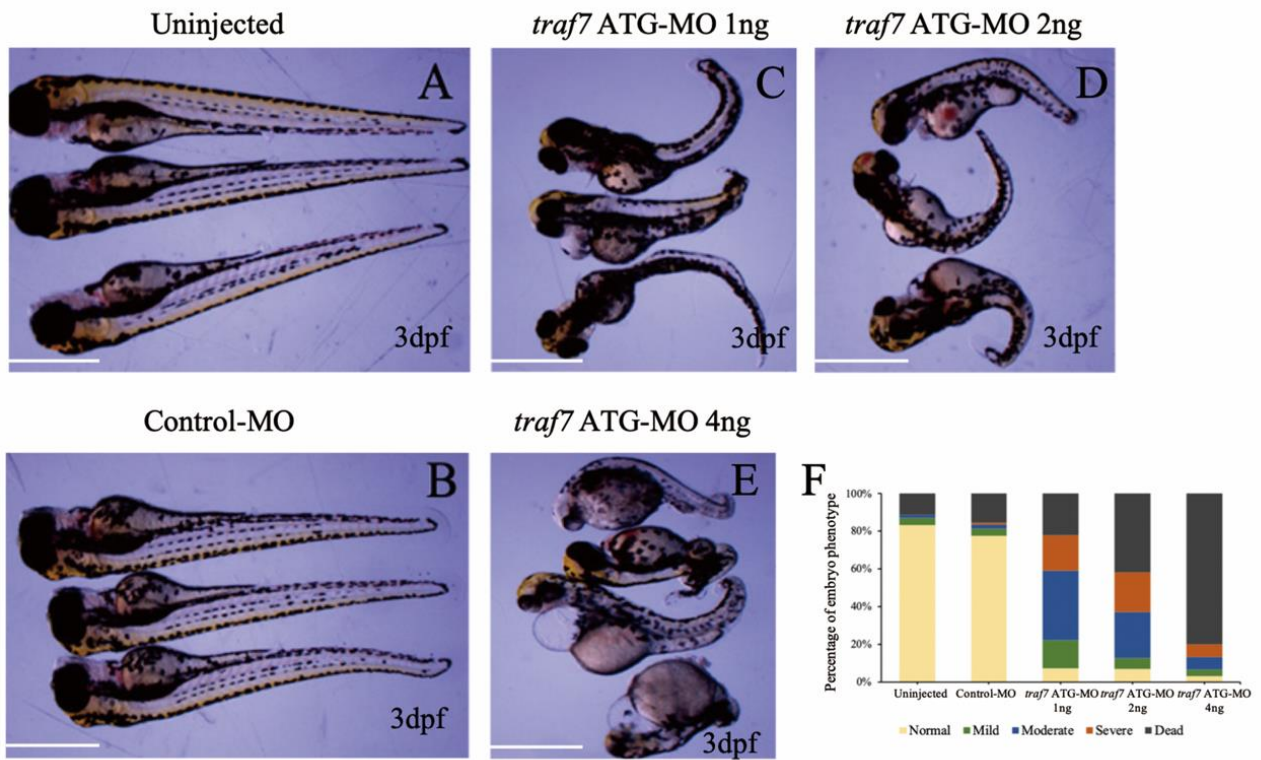
Supplementary Table S2 Sequences of morpholino injected in zebrafish larvae.

Morpholino	Sequence
<i>traf7</i> ATG-MO	CATTTTGGCCTTGTTGGTGATGTCC
<i>traf7</i> E3i3-MO	TGGTCCAACAGTTAATACTGACCTT
<i>traf7</i> E4i4-MO	CAGAAGATTGCTGACACTCACCGTG
<i>traf7</i> 5MIS-MO	CATTaTcGCgTTGTTGcTcATGTCC

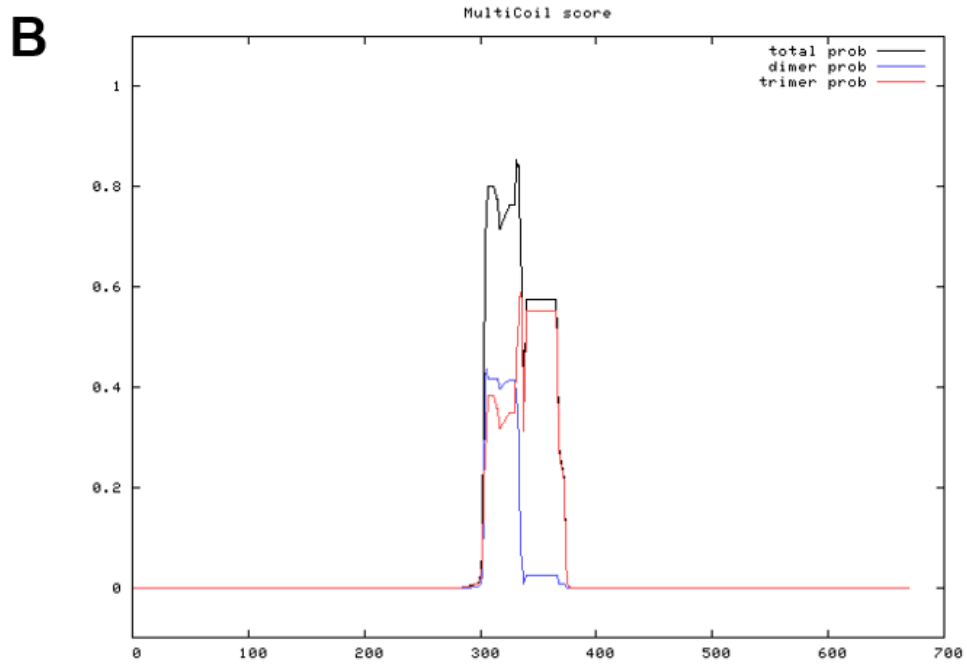
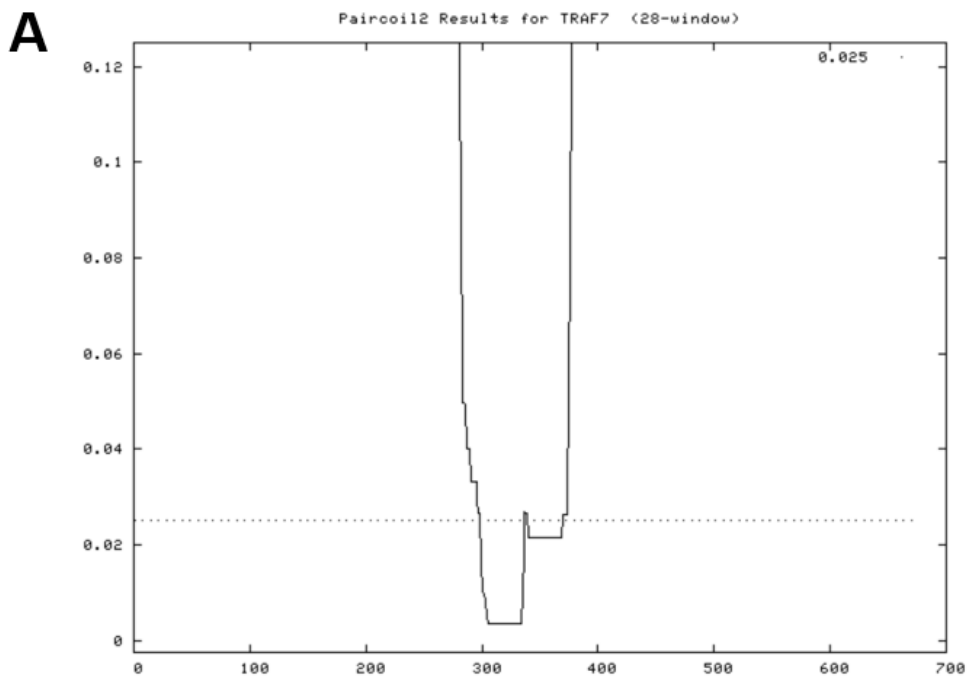
Supplementary Figures



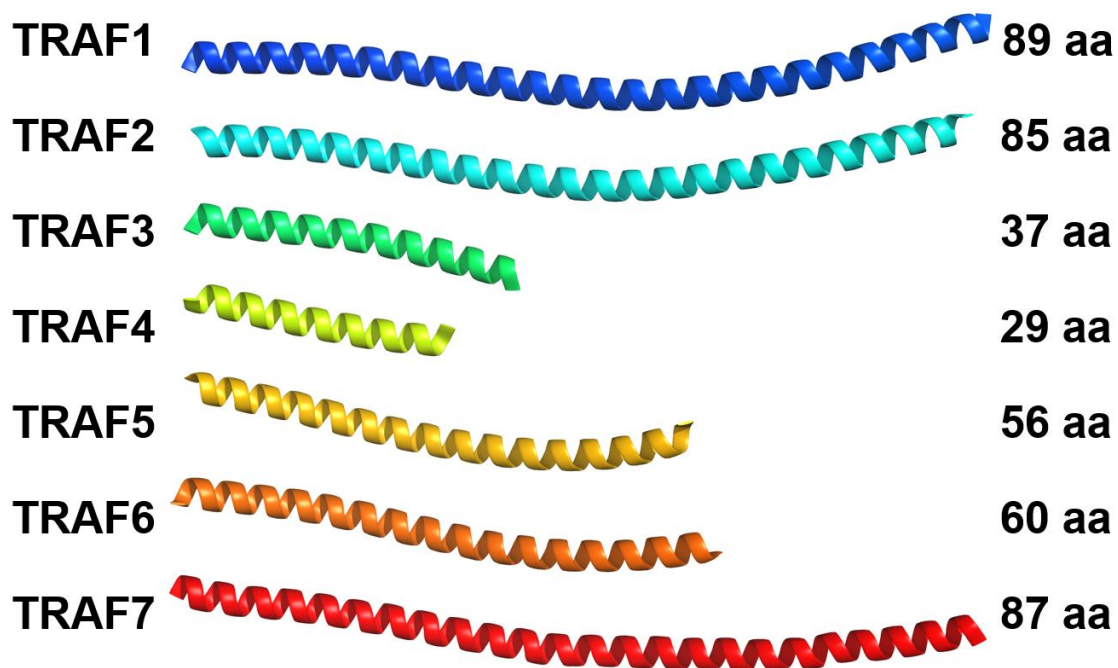
Supplementary Figure S1 Loss of Traf7 in zebrafish induced abnormal embryonic development. (A) Compared with control-MO group, zebrafish showed similar abnormal phenotypes after injection with *traf7* ATG-MO, Splicing-blocking MO *traf7* E3i3-MO or *traf7* E4i4-MO at 3 dpf. Scale bar, 1mm. (B) RT-PCR for *traf7* expression in *traf7* E3i3-MO and *traf7* E4i4-MO injected zebrafish showed *traf7* expression effectively reduced compared to uninjected and Control-MO groups.



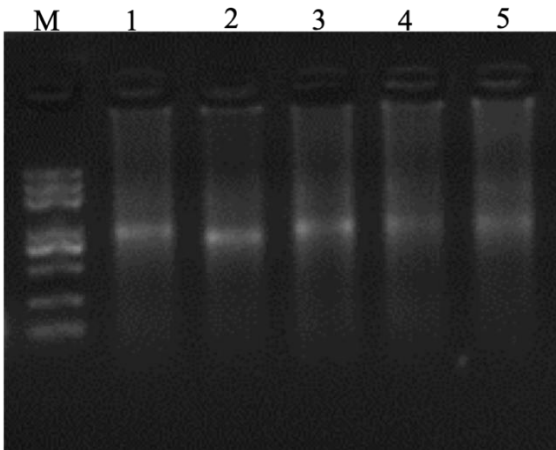
Supplementary Figure S2 Morphology of *traf7* knockdown zebrafish based on *traf7* ATG-MO at 3 dpf. (A–E) The severity of morphology was dose dependent after injection of *traf7* ATG-MO. Scale bar, 1 mm. (F) The phenotypic classification of morphant.



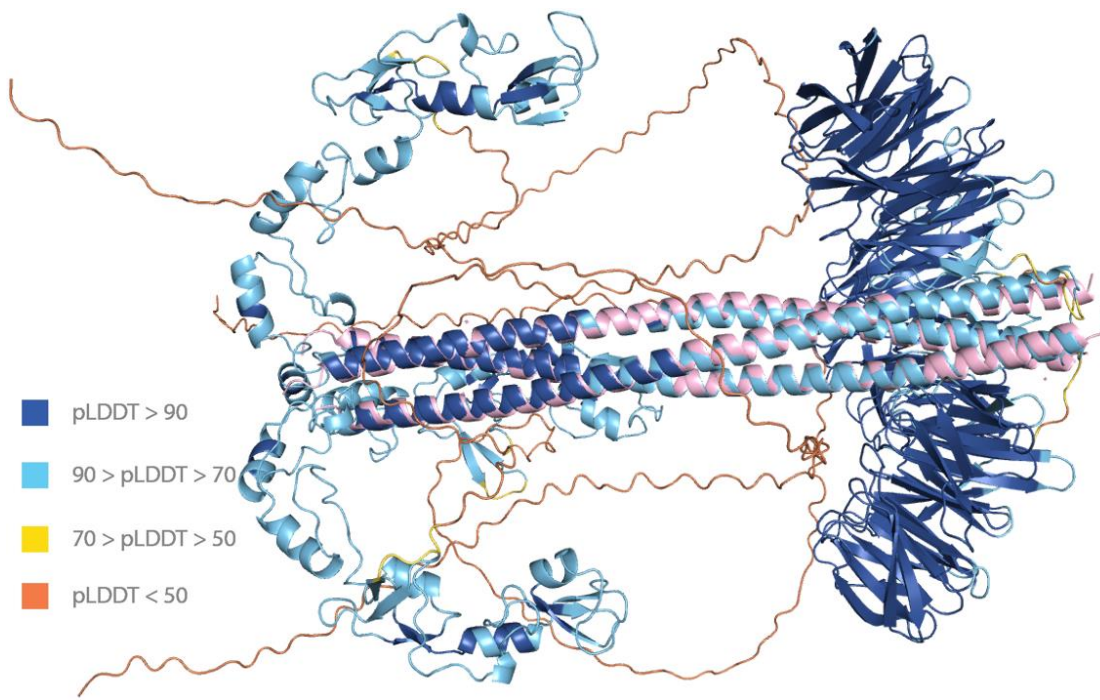
Supplementary Figure S3 Prediction of TRAF7 CC region by Paircoil2 and MultiCoil.



Supplementary Figure S4 Structure overlay of CC domain among paralogs, based on AlphaFold predicted structure. Compared to TRAF1–6, TRAF7 CC (87 aa of each chain) contains many residues similar to TRAF1/2, and in experimental structure TRAF7 CC (91 aa of each chain) is longer than TRAF1-6.



Supplementary Figure S5 Agarose gel electrophoresis identification results of purified mRNA. The first lane M is DNA Marker: 100 bp, 250 bp, 500 bp, 750 bp, 1000 bp, 1500 bp, 2000 bp, 3000 bp, 5000 bp from bottom to top; Lanes named 1–5 were purified hTRAF7-WT, hTRAF7-CCD, hTRAF7-R371G, zTraf7-WT and hTRAF7-K346E mRNA, respectively.



Supplementary Figure S6 Experimental structure of TRAF7 trimer (colored light pink) aligned with AlphaFold2.3.1 computed structure.