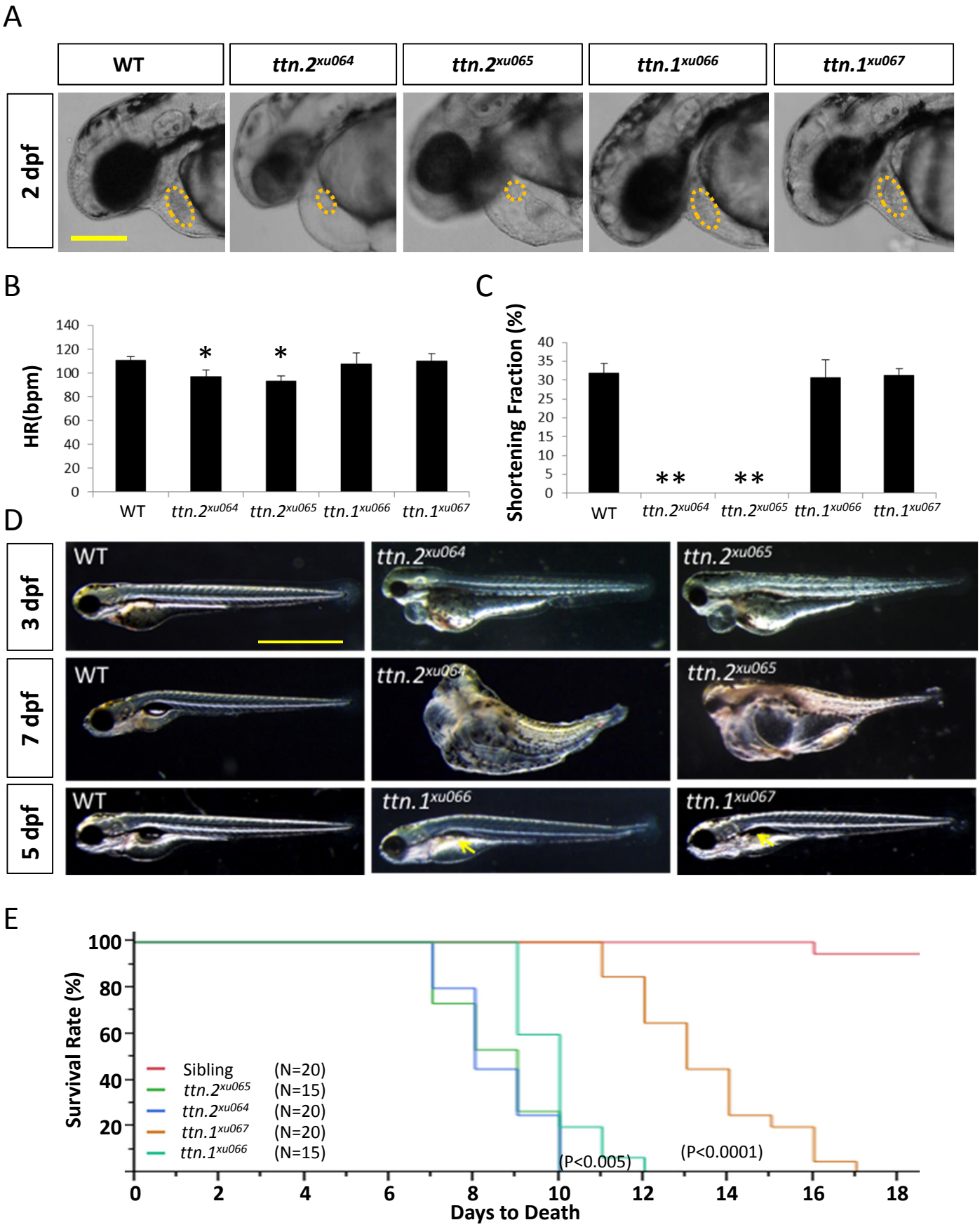

Figure S1.

Figure S1. Phenotypes of *ttn.2* and *ttn.1* mutants. A, Cardiac edema with decreased ventricle size (dashed circles) was noted in both *ttn.2*^{xu064} and *ttn.2*^{xu065}, but not in *ttn.1*^{xu066} and *ttn.1*^{xu067}. Shown are bright-field images at 2 dpf. Scale bar, 0.05 mm. B and C, In *ttn.2*^{xu064} and *ttn.2*^{xu065}, the heart rate was slightly decreased (B), and the ventricular shortening fraction was reduced to zero (C). * and ** indicates $p < 0.05$ and $p < 0.01$, respectively . Means ±S.D. N=5. D, Bright-field images demonstrated that the cardiac edema was still present in both *ttn.2*^{xu064} and *ttn.2*^{xu065} at 3 dpf, but muscle disarray was more obvious in *ttn.2*^{xu065}. Both *ttn.2* mutants had whole-body edema at 7 dpf. *ttn.1*^{xu066} and *ttn.1*^{xu067} showed deflation of the swim bladder at 5 dpf (arrows). Scale bar, 1 mm. E, *ttn.2*^{xu064} and *ttn.2*^{xu065} mutants started to die at 7 dpf and could survive up to 10 dpf; *ttn.1*^{xu066} could survive until 12 dpf; and *ttn.1*^{xu067} started to die at 11 dpf and could live up to 17 dpf.

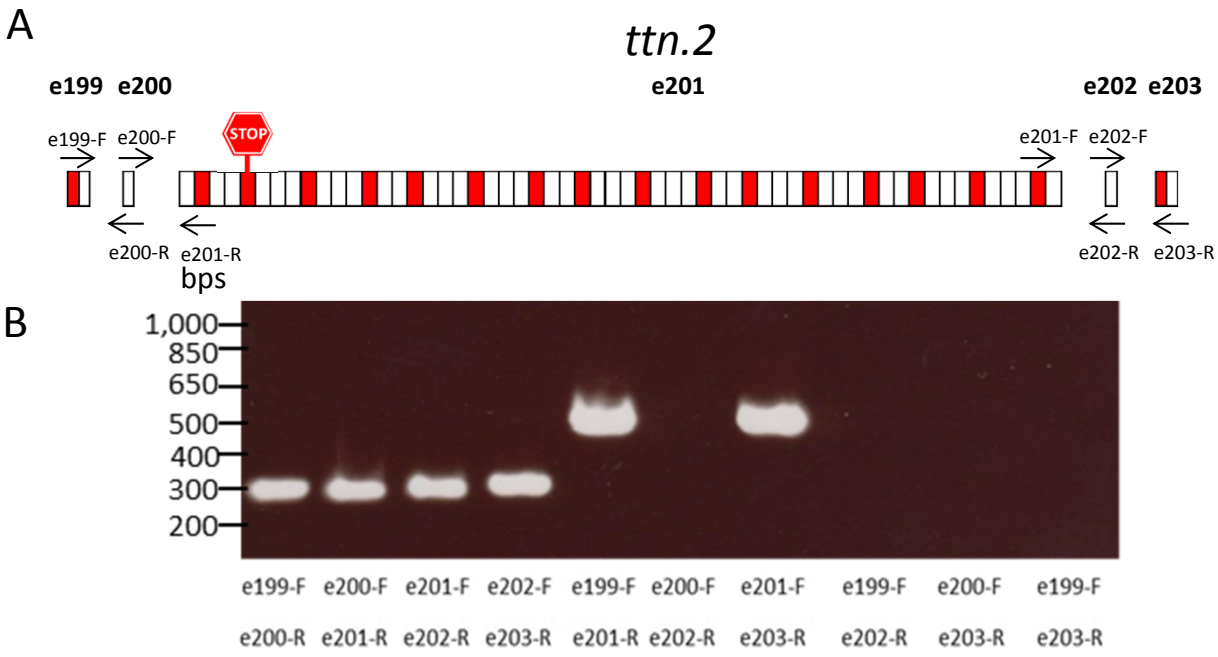

Figure S2. No Exon Skipping of Exon 201 of *ttn.2*. A, RT-PCR using different combinations of forward and reverse primers targeted to exons around 199-203 of *ttn.2*. B, RT-PCR using different combinations of primers showed no exon-skipping event.

A

ttn.2		*ttn.1*	
e4/e5	98.9%	e4/e5	91.8%
e200/e201	93.4%	e164/e165	94.9%
N2B	98.1%	N2B	98%
N2A	98.1%	N2A	98.8%
M	91.7%	M	95.2%

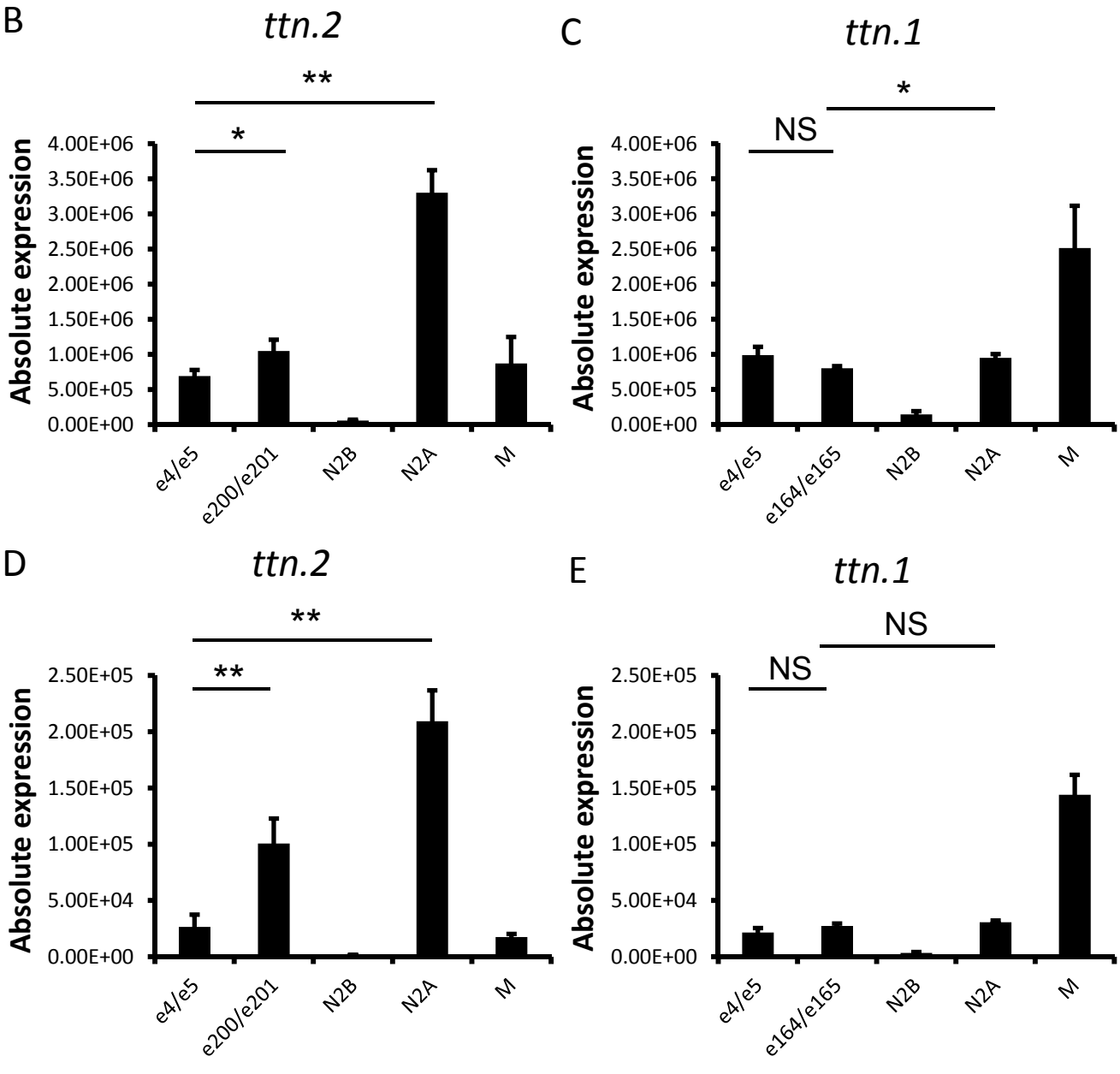

Figure S3.

Figure S3. Usage of Different Exons in *ttn.2* and *ttn.1* in 5 dpf and 9 dpf WT embryos. A, Efficiency of the primers in *ttn.2* and *ttn.1* was obtained by qPCR using standard curve. B and C, Exon usage was quantified using absolute qPCR with primers targeting exons in *ttn.2* (B) and *ttn.1* (C) in 5 dpf WT embryos. Means ±S.D. N=9. D and E, Usage of exons of *ttn.2* (D) and *ttn.1* (E) in 9 dpf WT embryos. Means ±S.D. N=9. * indicates $p < 0.05$, ** indicates $p < 0.01$, NS indicates not statistically significant ($p > 0.05$).

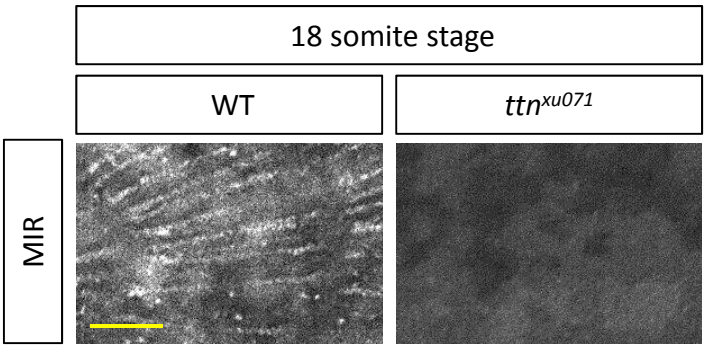

Figure S4. The Assembly of Titin Into Premyofibrils. Titin isoforms were assembled into premyofibrils and became striated at the 12th somite of an 18-somite embryo, as indicated by immunostaining using MIR, an anti-Titin antibody. Loss of Titin in somites in the *ttn*^{xu071} mutant has been seen. Scale bar, 20 μm.

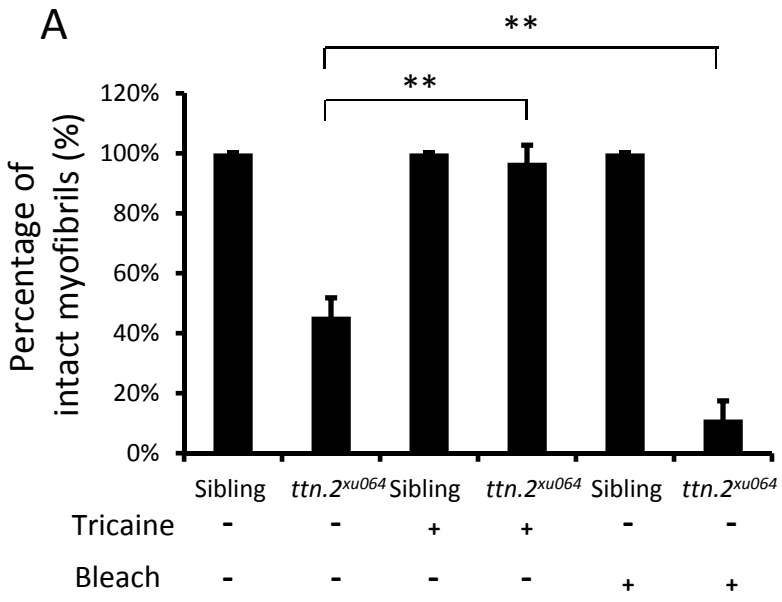

Figure S5. Inhibiting or inducing muscle contraction exert opposite effects on myofibril damage in the slow muscle of *ttn.2*^{xu064}. Shown are percentage of intact myofibrils out of total myofibrils at the 8th to 12th somites of larvae. Representative images are shown in Fig. 8C. Means ±S.D. N=5. ** indicates p <0.01. Tricaine treatment significantly prevented the breakage of myofibrils in *ttn.2*^{xu064}. Conversely, bleach treatment significantly increased the breakage of myofibrils in *ttn.2*^{xu064}. Siblings include both WT and heterozygotes.

Mutants	*ttn.1*^{xu067}	*ttn.1*^{xu066}	*ttn.2*^{xu064}	*ttn.2*^{xu065}	*ttn*^{xu069}	*ttn*^{xu070}	*ttn*^{xu071}
Ttn Expression							
Phenotypes in somites	N.A.	9 dpf	2 dpf (S)	18S (m)	18S	18S	18S
	Sarcomere stability			*de novo* Sarcomere assembly			

Figure S6. Summary of Ttn protein expression and sarcomeric phenotypes in 7 *ttn* mutants. (S) indicates slow muscle–specific phenotypes; 18S, 18-somite stage; N.A., not available; (m), mild phenotypes, as represented by occasional striated structures (See inset in Fig. 2A).

Table S1. Nomenclature of ttn mutants

Click here to Download Table S1

Table S2. Primers list for genotyping (A) and polymerase chain reaction (B).

A.

allele	Forward Primer	Reverse Primer	Restriction Enzyme	Size of PCR product	Size of PCR product after digestion
xu64, xu68, xu69	TGCATTTCATTGGTTCACAG	CCTCACAGGAGAGGGAGACT	EcoRV	406	81, 325
xu65, xu68, xu70, xu71	ACAAACTGGCAGAAATGCTC	GTCGACTTTCTCGAGGTTCA	HindIII	403	304, 99
xu66, xu69, xu71	TGGTTTCACCCAAATTTCAC	CTTTAACAGGCCTCACAGGA	CaC8I	453	120, 333
xu67, xu70	TGGGAAATGCCTCTTATTGA	GATGACTTCGCCCAGAACTA	BglII	480	131, 349

B.

	Primer	**Note**		**Primer**	**Note**
ttn.2-e4-F	CCGCTGACTTTCAGATTGTT	†	ttn.1-M-R	GGACTGCACTTCCTCATAGGT	§ †
ttn.2-e5-R	CGAGTCTGACGAGTTTGTGA	†	gapdh-F	CCACCCATGGAAAGTACAAG	§ #
ttn.2-e200-F	GTTTTGGTGAACCCAGTGAG	† ^	gapdh-R	CTCTCTTTGCACCACCCTTA	§ #
ttn.2-e201-R	AAGCGCACTGACTCTGAAGT	† ^	ttn.2-e2-F	CAGGCACCAACATTTACACA	%
ttn.2-N2B-F	CACAAACCGGTTGTACTTTCA	†	ttn.2-e7-R	GGAGGTCTGGACTTGTGTTG	%
ttn.2-N2B-R	TTCTCTTAGGGGTTGCACTG	†	ttn.1-e2-F	AACATTTACACAGCCGCTTC	%
ttn.2-N2A-F	TGCAGCACTGACTTGAATGT	†	ttn.1-e7-R	AACATAACCCTCCTGCTTCC	%
ttn.2-N2A-R	TCGATAGCTTGAAGGAGACC	†	ttn.2-e199-F	GTCTGGACCAGTCAAAATGG	^
ttn.2-M-F	CTCTTTTGTTGGGCAAGTGT	§ †	ttn.2-e200-R	CTCACTGGGTTCACCAAAAC	^
ttn.2-M-R	AATGATGCCTCTTGCATTGT	§ †	ttn.2-e201-F	GTGGTGCTCCAGTCAAAAAC	^
ttn.1-e4-F	CCGCTGACTTTCAGATTGTT	†	ttn.2-e202-R	CTTCCTCCATCATGTTCTGG	^
ttn.1-e5-R	CCTGGCGAGTCTGTGAAAT	†	ttn.2-e202-F	CCAGAACATGATGGAGGAAG	^
ttn.1-	GTGAACCCTCAATTCCTGTG	† *	ttn.2-	CCAGTAAGGGGGATCTCAAT	^

e164-F			e203-R		
ttn.1-e165-R	ACTTCAGTCCAGCTTCCAAA	† *	ttn.1-e163-F	CTGGTGATGCCTCTCACTCT	*
ttn.1-N2B-F	GCTACAGTGTCACCGAAGGA	†	ttn.1-e164-R	ATGCTCGTGTCCATCTCATT	*
ttn.1-N2B-R	TGCATTTCAAAGATTTCCTCTT	†	ttn.1-e165-F	AGGCCTGGGTATGTGTATCA	*
ttn.1-N2A-F	TTGCTAAAGTCGGTGGTGAT	†	ttn.1-e166-R	TCCACCATCAAACTCTGGTT	*
ttn.1-N2A-R	GGCCCAGACTCTTTCTTCTC	†	ttn.1-e166-F	GACTCAGTGAGCCCAAAGAA	*
ttn.1-M-F	CATGGTTCAGCACATCTTGA	§ †	ttn.1-e167-R	CTAAATCGCTTGCCACACTT	*

§: Primers used in Fig. 1D-E and 4D and 5C
†: Primers used in Fig. 3A-B and Fig. S3
%:Primers used in Fig. 3C
^: Primers used in Fig. S2
*: Primers used in Fig. 3E-F