Supporting Information

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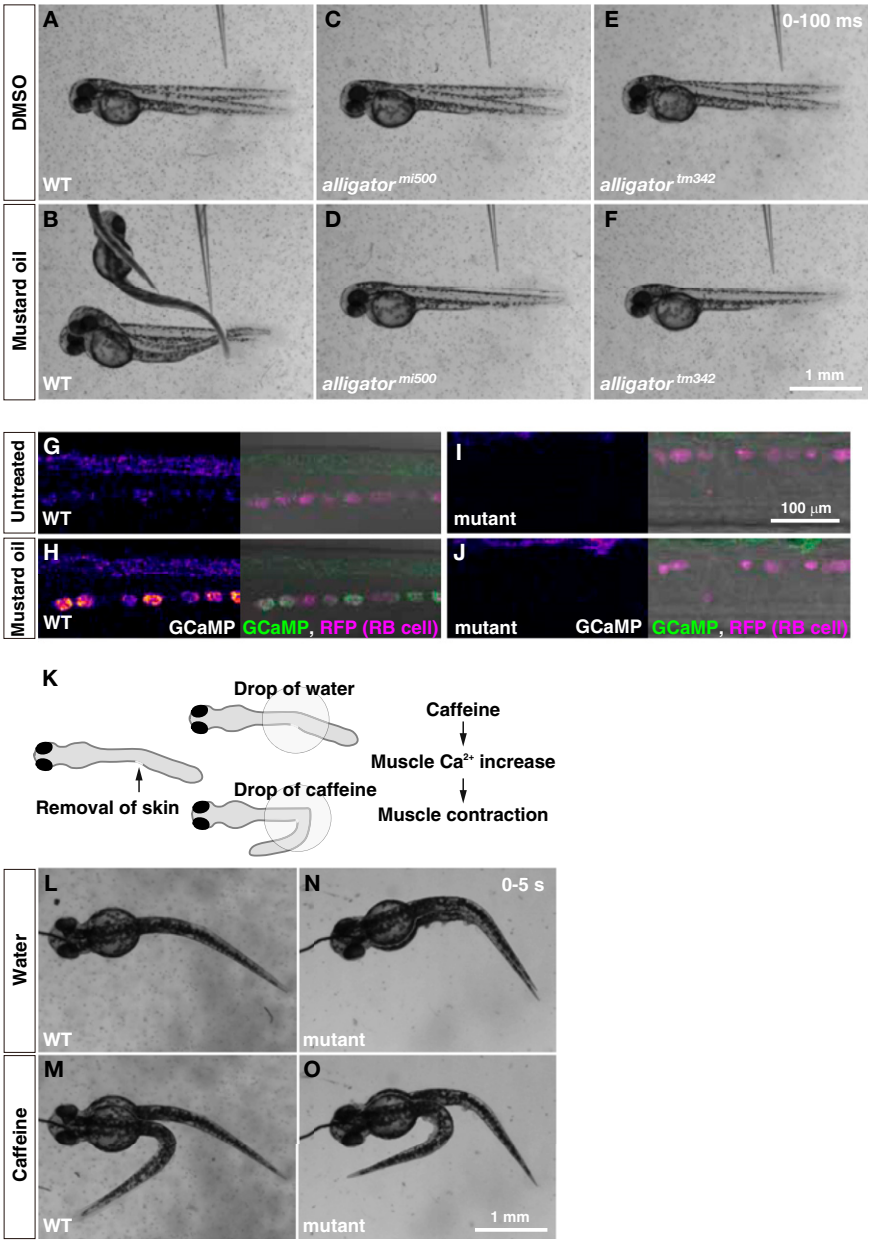

Fig. S1. (*A–F*) *alligator* mutant alleles *mi500* and *tm342* have a defect in sensorimotor coupling. A puff of 1% DMSO to the tail of WT (*A*), *mi500* mutant (*C*), or *tm342* mutant (*E*) fails to evoke a motor response. A similar puff of mustard oil in 1% DMSO evokes an escape response in a WT larva (*B*), but not in *mi500* (*D*) or *tm342* (*F*) mutant larva. The images are 100 ms of superimposed video stills of larval movement following the stimulus. (*G–J*) Activation of RBs as indicated by an increase in Ca^{2+} from a *Tg*(*SAIGFF213A*;*UAS*:*GCaMP7a*;*UAS*:*RFP*) transgenic WT and mutant larvae. RBs in untreated WT (*G*) and mutant (*I*) larvae showed no Ca^{2+} increase in RB cells. Exposure to mustard oil caused an increase in Ca^{2+} in WT (*H*) but not mutant (*J*) RBs. (*K–O*) Forced muscle contraction by exposure to caffeine. (*K*) Schematic of caffeine experiment. The skin was removed on the left side of the trunk to enhance penetration of caffeine to the left muscle. This procedure causes the trunk of the larvae to bend to the left. The application of caffeine triggers Ca^{2+} release, causing muscle contraction on the left side in both WT (*M*) and mutant larva (*O*). A drop of water failed to evoke muscle contractions in WT (*L*) and mutant larva (*N*). The images are superimposed video stills of larval movement from 5 s of assay.

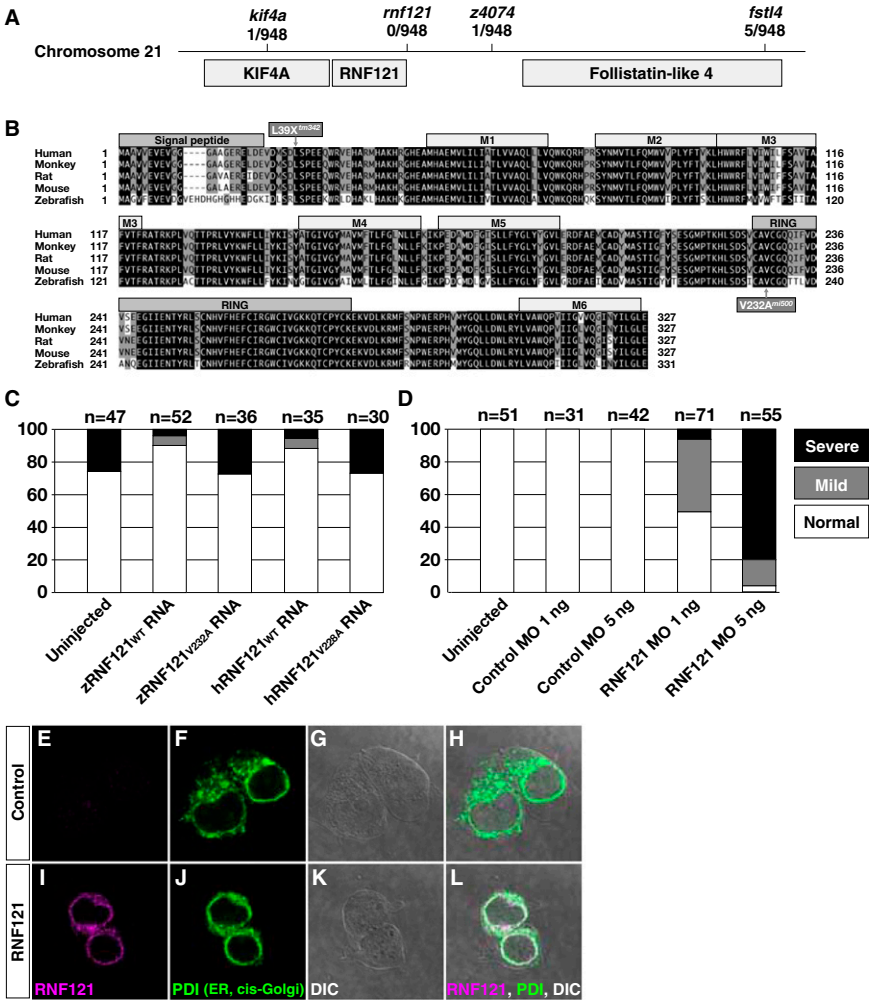

Fig. S2. (*A*) Meiotic mapping of the *alligator* locus between *kif4a* and *z4074* in chromosome 21. Numbers indicate the frequency of recombination observed near the *rnf121* gene. (*B*) Protein alignment of vertebrate RNF121. Shaded residues indicate conserved amino acids. Signal peptide, six membrane domains, and RING-finger motif are indicated. The positions of *mi500* and *tm342* mutations are indicated by arrows. Note that the V232, which is mutated in *mi500*, is conserved in vertebrates. (*C*) The histograms represent the percentage of RNA-injected larvae showing a touch response at 48 hpf. Injection of WT RNF121 RNA rescued touch responsiveness in mutants. However, injection of RNA encoding zebrafish RNF121$_{V232A}$ or human RNF121$_{V228A}$ had no effect on touch responsiveness. The uninjected control is the same as *tm342* × *tm342* in Fig. 1*D*. (*D*) The percentage of touch-responsive larvae following injections of the RNF121 MO. The uninjected control is the same as WT × WT in Fig. 1*D*. (*E*–*L*) Immunofluorescence labeling of RNF121 and protein disulfide isomerase (PDI), which is a marker of ER and *cis*-Golgi compartments. HEK293T cells were transfected with empty vectors (*E*–*H*) or human RNF121$_{WT}$ expression vectors (*I*–*L*).

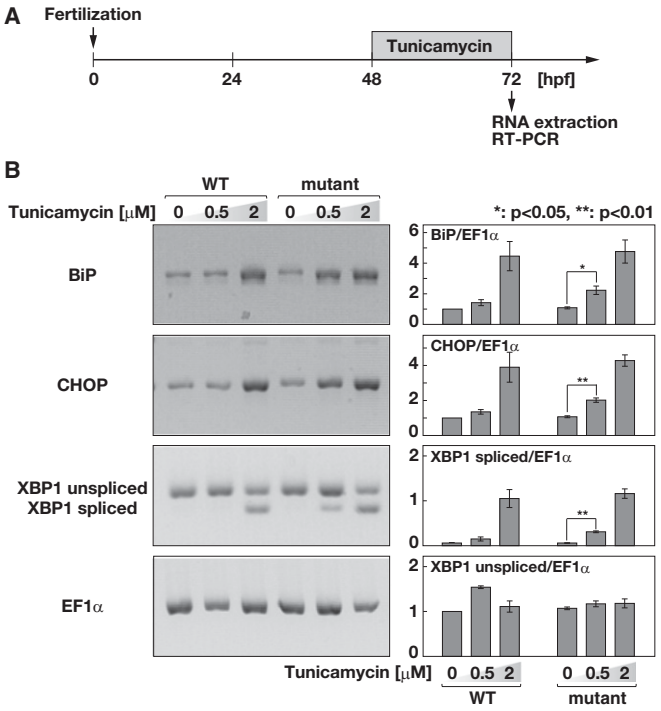

Fig. S3. Mutants are susceptible to ER stress. (*A*) Schematic of tunicamycin treatment. Zebrafish larvae were raised in bath solution containing 0, 0.5, or 2 μM tunicamycin, which is a typical ER stress inducer, from 48 to 72 hpf and subjected to RT-PCR at 72 hpf. (*B*) Exposure to tunicamycin induced transcription of BiP and CHOP in 2 μM tunicamycin-treated WT and mutant larvae, and 0.5 μM tunicamycin-treated mutants, but not in 0.5 μM tunicamycin-treated WT larvae. Likewise, alternative splicing of XBP1 was seen in 2 μM tunicamycin-treated WT and mutant larvae, and 0.5 μM tunicamycin-treated mutants, but not in 0.5 μM tunicamycin-treated WT larvae. EF1α was included as a lane control because it is not affected by ER stress. Histograms represent the statics of four independent experiments. Note that ER stress response was not seen in mutant larvae in the absence of stress inducer.

Table S1. Spontaneous coiling observed at 19 hpf

Spontaneous coiling	WT (n = 51)	Mutant (n = 21)	P value (t test)
Frequency, Hz	0.076 ± 0.046	0.072 ± 0.044	>0.7

Table S2. Touch-evoked contractions observed at 24–25 hpf

No. of touch-evoked coils	WT (n = 51)	Mutant (n = 21)	P value (χ^2 test)
0, %	5.9	9.5	>0.9
1, %	19.6	14.3	
2, %	47.1	38.1	
3, %	21.6	23.8	
4, %	5.9	14.3	

Table S3. Electrophysiological properties of excitable cells

Cell and property	WT	Mutant	*P* value (*t* test)
RB sensory neuron	$n = 10$	$n = 10$	
I_{Na}, pA	3,415 ± 379	48 ± 25	<0.001
I_K, pA	4,497 ± 543	4,755 ± 445	>0.7
Resting membrane potential, mV	−69 ± 3	−67 ± 2	>0.7
Motor neuron	$n = 11$	$n = 7$	
I_{Na}, pA	1,592 ± 162	92 ± 28	<0.001
I_K, pA	2,573 ± 396	2,063 ± 184	>0.3
Resting membrane potential, mV	−63 ± 1	−62 ± 2	>0.3
Skeletal muscle	$n = 5$	$n = 8$	
I_{Na}, pA	4,008 ± 960	1,197 ± 203	<0.005
I_K, pA	5,228 ± 909	3,589 ± 1,759	>0.3
Resting membrane potential, mV	−60 ± 1	−61 ± 2	>0.3

Voltage-gated inward and outward currents were measured in RB sensory neurons, motor neurons, and fast-twitch skeletal muscle at 48–60 hpf.