

Figure S1 Altered NMJ morphology in unc-104^{bris}/- larvae. Analysis of NMJ 4, Segment A2 of mid third instar Drosophila wild-type, unc-104^{bris}/+, unc-104^{bris}, and unc-104^{bris}/- larvae. Non-normalized raw data of NMJ length **(A)**, NMJ size **(B)**, bouton number **(C)** is shown. Data normalized by (D) the muscle length or area is shown in Figure 8 B,C,D. The SEM is shown as a box, the SD as a black line. ** p<0.01.

File S1

Sequence raw data for genes listed in Figure 2B

unc-104^{bris} R561H

CGCTGCTCAGAAACGATGGTGTGGAGCATGCGCGGGATTCTCACCGAAGAAGAACACCCGCATTTGGTCAACCTAAACGAGGATCCCAATC TGTCTGAGTGTCTGCTTTACTACATCAAGGAGGGTCTAACTCGGTTGGGTACCCATGAAGCAAATGTGCCCCAGGACATTCAGCTCTCG GATCGCACATCCTCAAGGAGCACTGCACCTTTGAGAACAAGAACAAGAACAGCACGGTTACATTGCTGCCACAAAGGATGCTATCATCTATGTAA ATGGACGCAAGTTGGTTGAACCGGAGGTTCTTAAGACCGGTTCCACGTGATCCTCGGAAAGAACCACGTGTTCCGCTTTACCAATCCAG AACAGGCACGCGAATTACGGGATAAGATCGAGACCGAAAATGAGGCTGAGAACGAAGTGGAGAAGACCAGACAGCCCAGCAGGTGGACT GGAACTTTGCCCAGTGCGAATTGCTCGAGAAGCAAGGCATGGATCTTAAAAGCTGAAAAGGAGAGAGCGGTTTAGACAACTTGGAGGAACA GTACAAGCGGGAGAAACTTCAGGCCGATCAGCAATTCGAGGAGCAGCGCGCAAAACGTACGAAGGCTCGCATCGATGCTTTGCAAAAACAG GTTAGAAGACAAATCCAATGAA

unc-104^{bris} V772L

unc-104^{bris} D1073E

unc-104^{bris} V1170M

unc-104^{bris} A1405V

parental R561R

parental V772L

parental D1073E

parental V1170M

parental A1405V

Table S1 Statistical tests used in this study

Figure:	2E	
Number:	Genotype:	n:
1	w ¹¹¹⁸ ;; (WT)	5
2	;unc-104 ^{bris} /unc-104 ^{bris} (unc-104 ^{bris})	6

Test: Student's two-tailed t-test

p-Value:



Figure: 2F

Number:	Genotype:	n:
1	elav ^{C155} -Gal4 /+; ;UAS-unc-104 ^{mCherry} /+ (control)	9
2	;unc-104 ^{bris} /unc-104 ^{d11024} ;UAS-unc-104 ^{mCherry} /+ (unc-104 ^{bris} /-)	10
3	elav ^{C155} -Gal4/+;unc-104 ^{bris} /unc-104 ^{d11024} ;UAS-unc-104 ^{mCherry} /+ (rescue)	9

Test: One-Way ANOVA followed by Tukey's Multiple Comparison test

p-Value:

-	1	2	3
1		***	n.s.
2			***
3			

Figure: 4A

Number:	Genotype:	n:
1	w ¹¹¹⁸ ;; (WT)	6
2	;unc-104 ^{bris} /unc-104 ^{bris} (unc ^{bris})	8
3	;unc-104 ^{bris} /unc-104 ^{d11024} (unc-104 ^{bris} /-)	8
4	elavX-Gal4/+;;UAS-unc-104 ^{mCherry} /+ (control)	6
5	elavX-Gal4 /+ ;unc-104 ^{bris} /unc-104 ^{d11024} ;UAS-unc-104 ^{mCherry} /+ (rescue)	6

Test: One-Way ANOVA followed by Tukey's Multiple Comparison test



Figure: 6C

Number:	Genotype:	n:
1	elav ^{C155} -Gal4 /+; ;UAS-unc-104 ^{mCherry} /+ (control)	9
2	;unc-104 ^{bris} /unc-104 ^{d11024} ;UAS-unc-104 ^{mCherry} /+ (unc-104 ^{bris} /-)	10
3	elav ^{C155} -Gal4/+;unc-104 ^{bris} /unc-104 ^{d11024} ;UAS-unc-104 ^{mCherry} /+ (rescue)	9

Test: Kruskal-Wallis H-test followed by Mann-Whitney pairwise comparison test



Figure: 6D

Number:	Genotype:	n:
1	elav ^{C155} -Gal4 /+; ;UAS-unc-104 ^{mCherry} /+ (control)	9
2	;unc-104 ^{bris} /unc-104 ^{d11024} ;UAS-unc-104 ^{mCherry} /+ (unc-104 ^{bris} /-)	10
3	elav ^{C155} -Gal4/+;unc-104 ^{bris} /unc-104 ^{d11024} ;UAS-unc-104 ^{mCherry} /+ (rescue)	9

Test: Kruskal-Wallis H-test followed by Mann-Whitney pairwise comparison test

p-Value:	(Bonferroni	corrected)	
		•	

	1	2	3
1		**	n.s.
2			*
3			

Figure: 7B

Number:	Genotype:	n:
1	elav ^{C155} -Gal4 /+;; (control)	9
2	elav ^{C155} -Gal4 /+;unc-104 ^{bris} /unc-104 ^{d11024} (unc-104 ^{bris} /-)	8
3	elav ^{C155} -Gal4 /+;unc-104 ^{bris} /unc-104 ^{d11024} ;UAS-Brp (unc-104 ^{bris} /-; Brp个)	8

Kruskal-Wallis H-test followed by Mann-Whitney pairwise comparison test Test:

(Bonferroni corrected) p-Value:



Figure: 7C

Number:	Genotype:	n:
1	elav ^{C155} -Gal4 /+;; (control)	9
2	elav ^{C155} -Gal4 /+;unc-104 ^{bris} /unc-104 ^{d11024} (unc-104 ^{bris}/-)	8
3	elav ^{C155} -Gal4 /+;unc-104 ^{bris} /unc-104 ^{d11024} ;UAS-Brp/+ (unc-104 ^{bris}/-; Brp ↑)	8

Kruskal-Wallis H-test followed by Mann-Whitney pairwise comparison test Test:

p-Value:	(Bonferroni		
	1	2	3
1		**	n.s.
2			**
3			

Figure: 7D

Number:	Genotype:	n:
1	elav ^{C155} -Gal4 /+;; (control)	9
2	elav ^{C155} -Gal4 /+;unc-104 ^{bris} /unc-104 ^{d11024} (unc-104 ^{bris} /-)	8
3	elav ^{C155} -Gal4 /+;unc-104 ^{bris} /unc-104 ^{d11024} ;UAS-Brp/+ (unc-104 ^{bris} /-; Brp ↑)	8

Kruskal-Wallis H-test followed by Mann-Whitney pairwise comparison test Test:

p-Value:	(Bonferroni corrected)
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	1	2	3
1		**	**
2			*
3			

Figure: 8B

Number:	Genotype:	n:
1	w ¹¹¹⁸ ;; (WT)	7
2	;unc-104 ^{bris} /+ (unc-104 ^{bris} /+)	9
3	;unc-104 ^{bris} /unc-104 ^{bris} (unc-104 ^{bris})	7
4	;unc-104 ^{bris} /unc-104 ^{d11024} (unc-104 ^{bris} /-)	9

Kruskal-Wallis H-test followed by Mann-Whitney pairwise comparison test Test:

p-Value:	-Value: (Bonferroni corrected)				
	1	2	3	4	
1		n.s.	*	**	
2			n.s.	**	
3				n.s.	
4					

Figure: 8C

Number:	Genotype:	n:
1	w ¹¹¹⁸ ;; (WT)	7
2	;unc-104 ^{bris} /+ (unc-104 ^{bris} /+)	9
3	;unc-104 ^{bris} /unc-104 ^{bris} (unc-104 ^{bris})	7
4	$(unc-104^{bris}/unc-104^{d11024})$	9

Test: Kruskal-Wallis H-test followed by Mann-Whitney pairwise comparison test

p-Value:	(Bonferroni corrected)				
	1	2	3	4	
1		n.s.	n.s.	n.s.	
2			n.s.	n.s.	
3				n.s.	
4					

Figure: 8D

Number:	Genotype:	n:
1	w ¹¹¹⁸ ;; (WT)	7
2	;unc-104 ^{bris} /+ (unc-104 ^{bris} /+)	9
3	;unc-104 ^{bris} /unc-104 ^{bris} (unc-104 ^{bris})	7
4	;unc-104 ^{bris} /unc-104 ^{d11024} (unc-104 ^{bris} /-)	9

Test: Kruskal-Wallis H-test followed by Mann-Whitney pairwise comparison test

p-Value:	(Bonferroni corrected)				
	1	2	3	4	
1		n.s.	*	**	
2			*	*	
3				n.s.	
4					

Figure: 8F

Number:	Genotype:	n:
1	w ¹¹¹⁸ ;; (WT)	8
2	;unc-104 ^{bris} /+ (unc-104 ^{bris} /+)	10
3	;unc-104 ^{bris} /unc-104 ^{bris} (unc-104 ^{bris})	10
4	;unc-104 ^{bris} /unc-104 ^{d11024} (unc-104 ^{bris} /-)	10

Test: One-Way ANOVA followed by Tukey's Multiple Comparison test

p-Value: (Bonferroni corrected)

	-	-	3	4
1		***	***	***
2			***	***
3				n.s.
4				

Figure: 8G

Number:	Genotype:	n:
1	w ¹¹¹⁸ ;; (WT)	8
2	;unc-104 ^{bris} /+ (unc-104 ^{bris} /+)	10
3	;unc-104 ^{bris} /unc-104 ^{bris} (unc-104 ^{bris})	10
4	:unc-104 ^{bris} /unc-104 ^{d11024} (unc-104 ^{bris} /-)	10

Test: One-Way ANOVA followed by Tukey's Multiple Comparison test

p-Value:	(Bonferroni corrected)				
	1	4			
1		**	***	***	
2			***	***	
3				n.s.	
4					

Figure: 8I

Number:	Genotype:	n:
1	w ¹¹¹⁸ ;; (WT)	10
2	;unc-104 ^{bris} /+ (unc-104 ^{bris} /+)	10
3	;unc-104 ^{bris} /unc-104 ^{bris} (unc-104 ^{bris})	10
4	;unc-104 ^{bris} /unc-104 ^{d11024} (unc-104 ^{bris} /-)	10

Test: Kruskal-Wallis H-test followed by Mann-Whitney pairwise comparison test

p-Value:	(Bonferroni corrected)			
	1	2	3	4
1		n.s.	**	**
2			**	**
3				**
4				

Figure: 8J

Number:	Genotype:	n:
1	w ¹¹¹⁸ ;; (WT)	10
2	;unc-104 ^{bris} /+ (unc-104 ^{bris} /+)	10
3	;unc-104 ^{bris} /unc-104 ^{bris} (unc-104 ^{bris})	10
4	;unc-104 ^{bris} /unc-104 ^{d11024} (unc-104 ^{bris} /-)	10

Test: Kruskal-Wallis H-test followed by Mann-Whitney pairwise comparison test

p-Value:	(Bonferroni corrected)
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	1	2	3	4
1		n.s.	*	*
2			n.s.	*
3				n.s.
4				

Figure: 8K

Number:	Genotype:	n:
1	w ¹¹¹⁸ ;; (WT)	8
2	;unc-104 ^{bris} /+ (unc-104 ^{bris} /+)	10
3	;unc-104 ^{bris} /unc-104 ^{bris} (unc-104 ^{bris})	10
4	$(unc-104^{bris}/unc-104^{d11024})$	10

Test: Kruskal-Wallis H-test followed by Dunn's Multiple Comparison test

p-Value:

	1	2	3	4
1		n.s.	*	***
2			*	***
3				n.s.
4				

Figure: 9B

Number:	Genotype:	n:
1	elav ^{C155} -Gal4 /+; ;UAS-unc-104 ^{mCherry} /+ (control)	9
2	;unc-104 ^{bris} /unc-104 ^{d11024} ;UAS-unc-104 ^{mCherry} /+ (unc-104 ^{bris} /-)	11
3	elav ^{C155} -Gal4/+;unc-104 ^{bris} /unc-104 ^{d11024} ;UAS-unc-104 ^{mCherry} /+ (rescue)	8

Test: Kruskal-Wallis H-test followed by Mann-Whitney pairwise comparison test

p-Value: (Bonferroni corrected)

	1	2	3
1		**	n.s.
2			**
3			

Figure: 9C

Number:	Genotype:	n:
1	elav ^{C155} -Gal4 /+; ;UAS-unc-104 ^{mCherry} /+ (control)	9
2	;unc-104 ^{bris} /unc-104 ^{d11024} ;UAS-unc-104 ^{mCherry} /+ (unc-104 ^{bris} /-)	11
3	elav ^{C155} -Gal4/+;unc-104 ^{bris} /unc-104 ^{d11024} ;UAS-unc-104 ^{mCherry} /+ (rescue)	8

Test: Kruskal-Wallis H-test followed by Mann-Whitney pairwise comparison test

p-Value:	(Bonferroni corre	cted)
	1	2

,	1	2	3
1		n.s.	n.s.
2			n.s.
3			

Figure: 9D

Number:	Genotype:	n:
1	elav ^{C155} -Gal4 /+; ;UAS-unc-104 ^{mCherry} /+ (control)	9
2	;unc-104 ^{bris} /unc-104 ^{d11024} ;UAS-unc-104 ^{mCherry} /+ (unc-104 ^{bris} /-)	11
3	elav ^{C155} -Gal4/+;unc-104 ^{bris} /unc-104 ^{d11024} ;UAS-unc-104 ^{mCherry} /+ (rescue)	8

Test: Kruskal-Wallis H-test followed by Mann-Whitney pairwise comparison test



Figure: 9F

Number:	Genotype:	n:
1	elav ^{C155} -Gal4 /+; ;UAS-unc-104 ^{mCherry} /+ (control)	9
2	;unc-104 ^{bris} /unc-104 ^{d11024} ;UAS-unc-104 ^{mCherry} /+ (unc-104 ^{bris} /-)	10
3	elav ^{C155} -Gal4/+;unc-104 ^{bris} /unc-104 ^{d11024} ;UAS-unc-104 ^{mCherry} /+ (rescue)	9

One-Way ANOVA followed by Tukey's Multiple Comparison test Test:

p-Value:

	1	2	3
1		***	n.s.
2			***
3			

Figure: 9G

Number:	Genotype:	
1	elav ^{C155} -Gal4 /+; ;UAS-unc-104 ^{mCherry} /+	(control)

1	elav ^{C155} -Gal4 /+; ;UAS-unc-104 ^{mCherry} /+ (control)	9
2	;unc-104 ^{bris} /unc-104 ^{d11024} ;UAS-unc-104 ^{mCherry} /+ (unc-104 ^{bris} /-)	10
3	elav ^{C155} -Gal4/+;unc-104 ^{bris} /unc-104 ^{d11024} ;UAS-unc-104 ^{mCherry} /+ (rescue)	9

n:

Test: One-Way ANOVA followed by Tukey's Multiple Comparison test



Figure: 11B

Number:	Genotype:		n:
1	elav ^{C155} -Gal4 /+;unc-104 ^{bris} /unc-104 ^{d11024}	(unc-104 ^{bris} /-)	8
2	elav ^{C155} -Gal4 /+;unc-104 ^{bris} /unc-104 ^{d11024;}	UAS-Brp-RNAi (Brp↓)	8
3	elav ^{C155} -Gal4 /+;unc-104 ^{bris} /unc-104 ^{d11024;}	UAS-Brp (Brp↑)	8

Test: One-Way ANOVA followed by Tukey's Multiple Comparison test

p-Value:



Figure: 11C

Number:	Genotype:	n:
1	elav ^{C155} -Gal4 /+;unc-104 ^{bris} /unc-104 ^{d11024} (unc-104 ^{bris} /-)	8
2	elav ^{C155} -Gal4 /+;unc-104 ^{bris} /unc-104 ^{d11024;} UAS-Brp-RNAi (Brp↓)	8
3	elav ^{C155} -Gal4 /+;unc-104 ^{bris} /unc-104 ^{d11024;} UAS-Brp (Brp ↑)	8

Test: One-Way ANOVA followed by Tukey's Multiple Comparison test

p-Value:

	1	2	3
1		n.s.	n.s.
2			n.s.
3			

Figure: 11D

Number:	Genotype:	n:
1	elav ^{C155} -Gal4 /+;unc-104 ^{bris} /unc-104 ^{d11024} (unc-104 ^{bris} /-)	8
2	elav ^{C155} -Gal4 /+;unc-104 ^{bris} /unc-104 ^{d11024;} UAS-Brp-RNAi (Brp ↓)	8
3	elav ^{C155} -Gal4 /+;unc-104 ^{bris} /unc-104 ^{d11024;} UAS-Brp (Brp ↑)	8

One-Way ANOVA followed by Tukey's Multiple Comparison test Test:

	1	2	3
1		n.s.	n.s.
2			n.s.
3			

Figure: 11E

Number:	Genotype:		n:
1	elav ^{C155} -Gal4 /+;unc-104 ^{bris} /unc-104 ^{d11024}	(unc-104 ^{bris} /-)	8
2	elav ^{C155} -Gal4 /+;unc-104 ^{bris} /unc-104 ^{d11024;}	UAS-Brp-RNAi (Brp↓)	8
3	elav ^{C155} -Gal4 /+;unc-104 ^{bris} /unc-104 ^{d11024;}	UAS-Brp (Brp↑)	8

Test: One-Way ANOVA followed by Tukey's Multiple Comparison test

p-Value:



Figure: 11F

Number:	Genotype:	n:
1	elav ^{C155} -Gal4 /+;unc-104 ^{bris} /unc-104 ^{d11024} (unc-104 ^{bris} /-)	8
2	elav ^{C155} -Gal4 /+;unc-104 ^{bris} /unc-104 ^{d11024;} UAS-Brp-RNAi (Brp ↓)	8
3	elav ^{C155} -Gal4 /+;unc-104 ^{bris} /unc-104 ^{d11024;} UAS-Brp (Brp ↑)	8

Test: One-Way ANOVA followed by Tukey's Multiple Comparison test

	1	2	3
1		n.s.	n.s.
2			n.s.
3			