

Supplementary information

Vacuolar-ATPase-mediated muscle acidification caused muscular mechanical nociceptive hypersensitivity after chronic stress in rats, which involved extracellular matrix proteoglycan and ASIC3

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Supplementary Table 1

Comparison between two groups

Treatment, measurementt	Figure No.	Normality test	Equal variance	analysis method	n	p values
pH of the muscle (control vs RCS)	Fig. 2	P = 0.2168, 0.3277 (normality assumed, Shapiro-Wilk test)	P= 0.0314 (equal variance not assumed, F test)	unpaired t-test with Welch's correction	n=12 each	P= 0.0245
Comparison of %MPE of bafilomycin A1 40 nmol/kg and PF3716556 2.5 µmol/kg	In the text	P < 0.706, p < 0.8799 (normality assumed, Shapiro Wilk test)	p = 0.0176 (Equal variance not assumed)	Unpaired t-test with Welch's correction	n = 6 each	p = 0.0017
pH of the muscle (control animal, DMSO or BafilomycinA1 inj)	Fig. 5A	P = 0.7204, 0.1073 (normality assumed, Shapiro-Wilk test)	P = 0.1051 (equal variance assumed, F test)	Unpaired t-test with Welch's correction	n=7 each	P = 0.9461
pH of the muscle (RCS animal, DMSO or BafilomycinA1 inj)	Fig. 5B	P = 0.9412, 0.1243 (normality assumed, Shapiro-Wilk test)	P= 0.8582 (equal variance assumed, F test)	Unpaired t-test with Welch's correction	n = 10 (DMSO) n = 9 (bafilomycin A1)	P = 0.0244
Basic characteristics of recorded fibers (Conduction velocity)	In the text	P = 0.087, <0.001 (normality not assumed, Shapiro-Wilk test)	P = 0.269 (equal variance assumed, Levene's test)	Mann-Whitney U test	n = 21 (control) n = 47 (RCS)	P = 0.094
Basic characteristics of recorded afferents (background discharges)	In the text	P <0.001, <0.001 (normality not assumed, Shapiro-Wilk test)	P = 0.951 (equal variance assumed, Levene's test)	Mann-Whitney U test	n = 21 (control) n = 47 (RCS)	P = 0.746
Baseline mechanical response of thin-fibres (response magnitude)	Fig. 6C upper graph	P = 0.005, 0.189 (normality not assumed, Shapiro-Wilk test)	P = 0.519 (equal variance assumed, Levene's test)	Mann-Whitney U test	n = 21 (control) n = 47 (RCS)	P = 0.015
Baseline mechanical response of thin-fibres (response threshold)	Fig. 6C lower graph	P = 0.00025, <.00001 (normality not assumed, Shapiro-Wilk test)	P = 0.011 (equal variance not assumed, Levene's test)	Unpaired t-test with Welch's correction	n = 21 (control) n = 47 (RCS)	P = 0.034

Supplementary Table 1 (continued)

Comparison between two groups

Treatment, measurementt	Figure No.	Normality test	Equal variance	analysis method	n	p values
Mechanical response magnitude of RCS fibres before drug application	In the text	P = 0.311, 0.956 (normality assumed, Shapiro-Wilk test)	P=0.116 (equal variance assumed, Levene's test)	Unpaired Student t-test	n = 26 (bafilomycin) n = 12 (DMSO)	p = 0.257
Mechanical response threshold of RCS fibres before drug application	In the text	P <.001, <.001 (normality not assumed, Shapiro Wilk test)	P = 0.248 (equal variance assumed, Levene's test)	Mann-Whitney U test	n = 26 (bafilomycin) n = 12 (DMSO)	P = 0.376
TRPV1 mRNA	Fig. 10B	P = 0.551(normality assumed, Shapiro-Wilk test)	P = 0.775 (equal variance assumed, Brown-Forsythe test)	Unpaired Student t-test	n = 6 each	P = 0.953
ASIC3 mRNA	Fig. 10B	P = 0.136 (normality assumed, Shapiro-Wilk test)	P = 0.290 (equal variance assumed, Brown-Forsythe test)	Unpaired Student t-test	n = 6 each	P =0.0150
ASIC3 protein	Fig. 10D	P < 0.4578, <0.5089 (normality assumed, Shapiro Wilk test)	P = 0.9563 (equal variance assumed, F test)	Unpaired Student t-test	n= 6 each	P = 0.0405

Supplementary Table 2.

Treatment	Figure No.		analysis method	factors	F values	p values	Note (Shapiro-Wilk test was used for normality test, and Brown–Forsythe test was used for equal variance test)
Effect of bafilomycin A1 on MMWT	Fig 3A	1 w aft RCS	2-way ANOVA with repeated measures				normality test $p = 0.730$, equal variance test $p = 0.095$
				time	$F(4, 92) = 168,481$	$P < 0.001$	
				drug	$F(4, 23) = 6.703$	$P < 0.001$	
				time x drug interaction	$F(16, 92) = 8,047$	$P < 0.001$	
	Fig. 3B	2 w aft RCS	same as above				sphericity not assumed, degree of freedom was adjusted with Geisser-Greenhouse's epsilon.
				time	$F(2.76601574, 27.6601574) = 53.0221$	$P < 0.0001$	
				drug	$F(1, 10) = 24.1450923$	$P = 0.0006$	
				time x drug interaction	$F(4, 40) = 9.65186043$	$P < 0.0001$	
	Fig. 3C	3 w aft RCS	same as above				normality test $p=0.808$, equal variance test $p = 0.997$
				time	$F(4, 40) = 68.506$	$P < 0.001$	
				drug	$F(1, 10) = 1.061$	$P = 0.327$	
				time x drug interaction	$F(4, 40) = 4.056$	$P = 0.007$	
	Fig 3D	Control (no RCS)	same as above				normality test $p = 0.387$, equal variance test $p = 0.409$
				time	$F(3,30) = 0.437$	$P = 0.728$	
				drug	$F(1, 10) = 0.0111$	$P = 0.918$	
				time x drug interaction	$F(3, 30) = 0.460$	$P = 0.712$	

Supplementary Table 3

Treatment	Figure No.		analysis method	factors	F values	p values	note
Effect of PF	Fig. 4		2-way ANOVA with repeated measures				normality test $p = 0.739$, equal variance test $p = 0.666$
				time	$F(4,80) = 303.858$	$P < 0.001$	
				drug	$F(3, 20) = 1.574$	$P = 0.227$	
				time x drug interaction	$F(12, 80) = 2.751$	$P = 0.004$	
Single fiber recording	Fig.7A	response magnitude	same as above				sphericity assumed
				time	$F(4,144) = 8.44$	$P < 0.001$	
				drug	$F(1,36) = 4.66$	$P = 0.038$	
				time x drug interaction	$F(4,144) = 3.09$	$P = 0.018$	
	Fig 7B	response threshold	same as above				sphericity not assumed, degree of freedom was adjusted with Geisser-Greenhouse's epsilon.
				time	$F(2.84, 102.15) = 0.88$	$P = 0.451$	
				drug	$F(1, 36) = 5.58$	$P = 0.024$	
				time x drug interaction	$F(2.84,102.15) = 3.04$	$P = 0.035$	

Supplementary Table 4

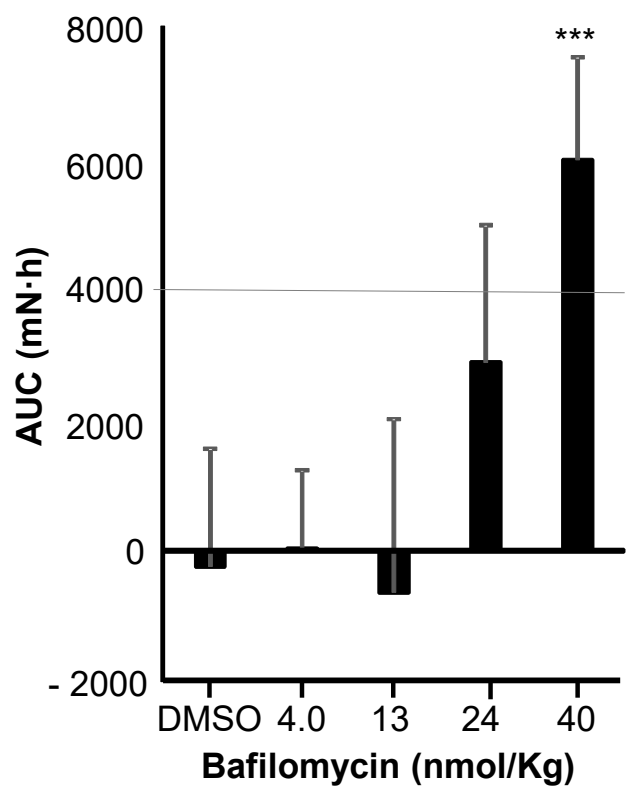
Treatment	Figure No.		analysis method	factors	F values	p values	note
Effects of chondroitin sulfate	Fig. 8A	up to 6 hs	2-way ANOVA with repeated measures				Normality Test $p = 0.519$, Equal Variance Test $p = 0.124$
				time	$F(4,80) = 137.522$	$p < 0.001$	
				drug	$F(3,20) = 14.223$	$P < 0.001$	
				time x drug interaction	$F(12,80) = 12.91$	$p < 0.001$	
	Fig.8B	longer time	same as above				Normality Test $p = 0.753$ Equal Variance Test $p = 0.297$
				time	$F(5,100) = 165.502$	$p < 0.001$	
				drug	$F(3, 20) = 11.586$	$p < 0.001$	
				time x drug interaction	$F(15,100) = 16.691$	$p < 0.001$	
Effects of chondroitin ase ABC	Fig 9.		same as above				sphericity not assumed, degree of freedom was adjusted with Geisser-Greenhouse's epsilon.
				time	$F(2.28928329, 57.2320821) = 95.4719715$	$P < 0.0001$	
				drug	$F(4, 25) = 6.89310696$	$P = 0.0007$	
				time x drug interaction	$F(16, 100) = 5.87206422$	$P < 0.0001$	

Supplementary Table 5

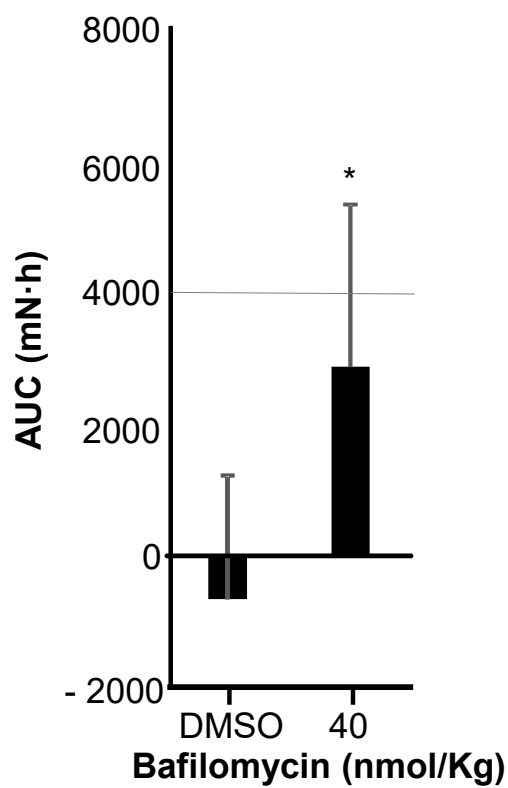
Treatment	Figure No.		analysis method	factors	F values	p values	note
Effect of APETx2 on MMWT	Fig 10A, left (APETx2)		2-way ANOVA with repeated measures				sphericity not assumed, degree of freedom was adjusted with Geisser-Greenhouse's epsilon.
				time	F (1.87284114, 26.2197760) = 144.715122	P <0.0001	
				drug	F (2, 14) = 5.89997609	P =0.0139	
				time x drug interaction	F (4, 28) = 6.63304939	P =0.0007	
Effect of ruthenium red on MMWT	Fig 10A, right (RR)		same as above				same as above
				time	F (1.96942453, 27.5719435) = 204.885677	P <0.0001	
				drug	F (2, 14) = 1.23684694	P =0.3202	
				time x drug interaction	F (4, 28) = 1.27967896	P =0.3016	

Suppl. Fig.S1

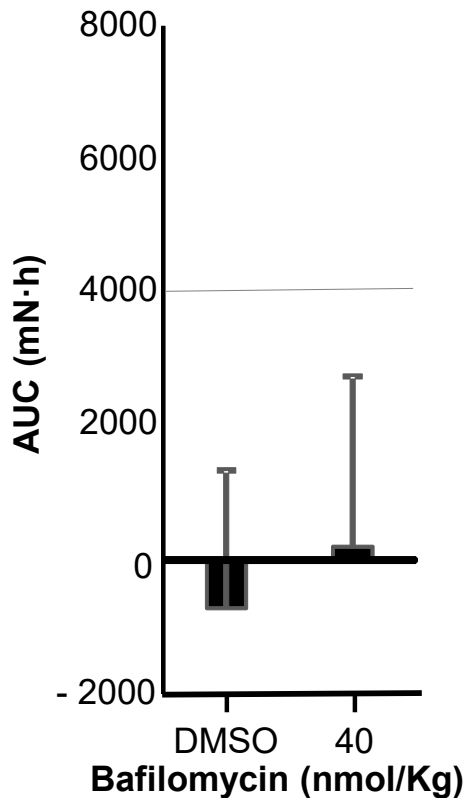
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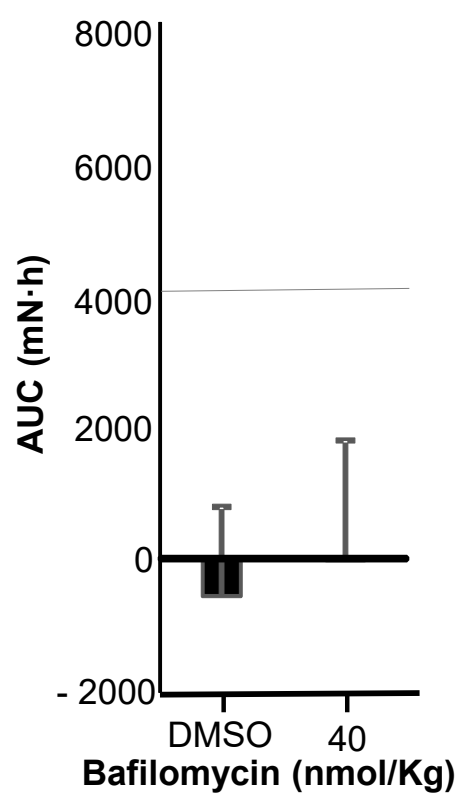
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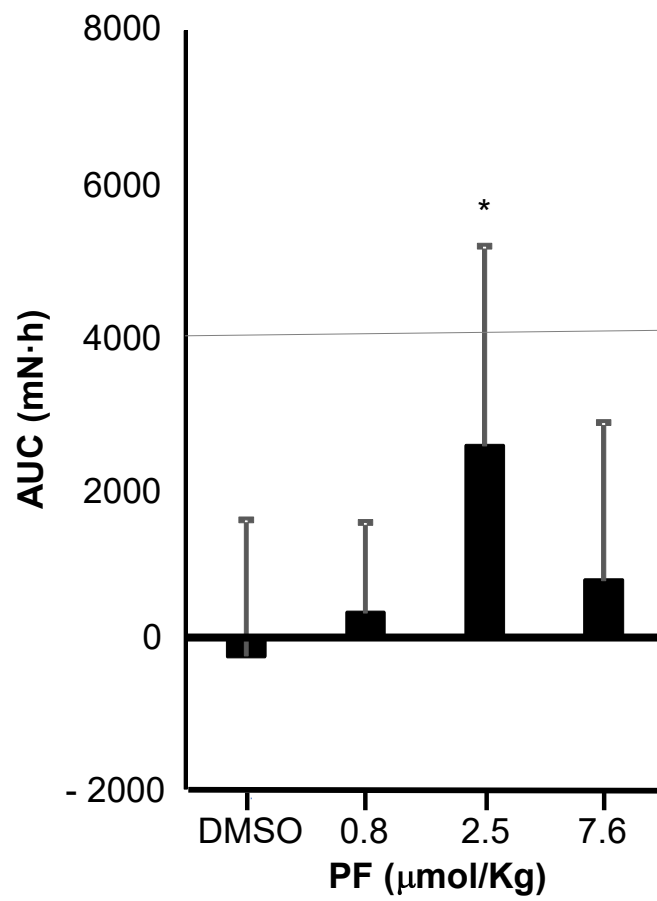
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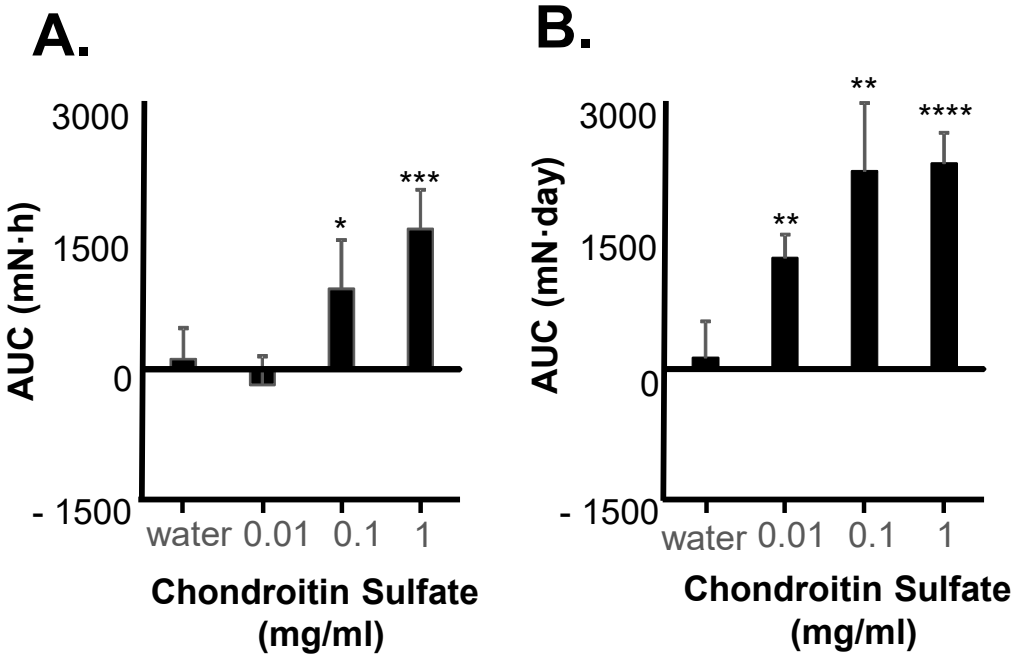
D.



Suppl. Fig. S2



Suppl. Fig. S3.



Suppl. Fig.S4

