

Phosphorylation of cMyBP-C affects contractile mechanisms in a site-specific manner

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SUPPORTING MATERIAL

Due to the possibility of a gender difference in these experiments, we eliminated the data from females (one from WT and one from the SAS group). Data was averaged only in males and plotted in this section. The figure numbers (S2, S4, etc.) correspond to those in the main text (Figs. 2, 4, etc.). Figure numbers S1 and S3 are absent.

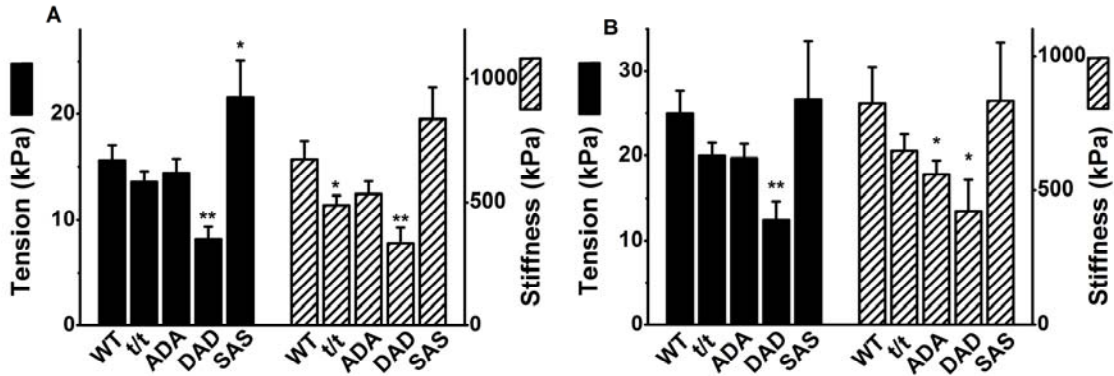


FIGURE S2 **Isometric tension (T_{\max} in Eq. 2: left ordinate) and stiffness (S_{\max} in Eq. 3: right ordinate) in the 8Pi solution (in A) and the 0Pi solution (in B).** The 8Pi solution contained 8 mM Pi and its ionic strength (IS) was 200 mM, whereas the 0Pi solution contained no Pi and its IS was 150 mM. ** $p < 0.01$ and * $p < 0.05$ compared to WT. The number of experiments (n) is in (A), 23 for WT, 28 for t/t, 32 for ADA, 31 for DAD, and 14 for SAS; In (B), $n = 9$ for WT, 14 for t/t, ADA, and DAD, and $n = 5$ for SAS.

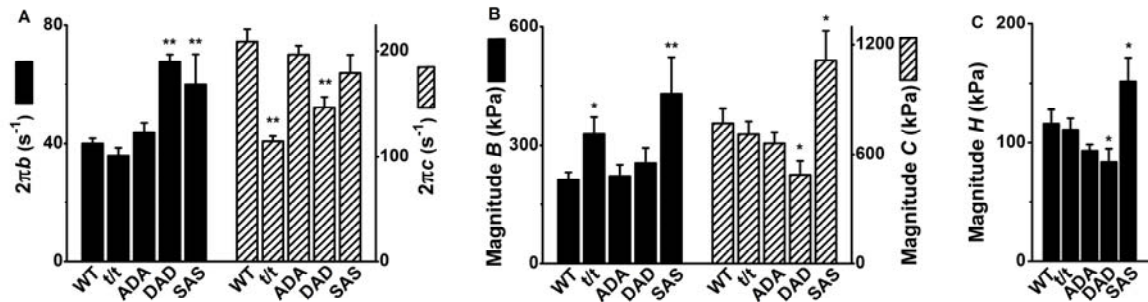


FIGURE S4 **Parameters of exponential processes in the 8Pi solution.** (A) The apparent rate constants $2\pi b$ (left ordinate) and $2\pi c$ (right ordinate). (B) the magnitudes of processes B (left ordinate) and C (right ordinate). (C) magnitude H . Error bars represent SE of the averages of 14-38 experiments carried out in each group (for the number of mice, see Table 1). $n = 23$ for WT, 38 for t/t, 32 for ADA, 31 for DAD, and 14 for SAS. ** $p < 0.01$ and * $p < 0.05$ compared to WT.

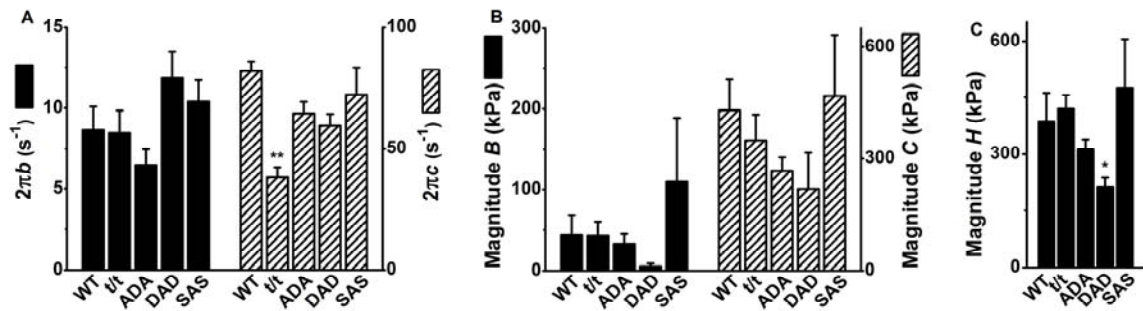


FIGURE S5 **Parameters of exponential processes in the 0Pi solution.** (A) The apparent rate constants $2\pi b$ (left ordinate) and $2\pi c$ (right ordinate). (B) the magnitudes of processes B (left ordinate) and C (right ordinate). (C) magnitude H . $n = 9$ for WT, 14 for t/t , ADA, and DAD, and $n = 5$ for SAS. $**p < 0.01$ and $*p < 0.05$ compared to WT.

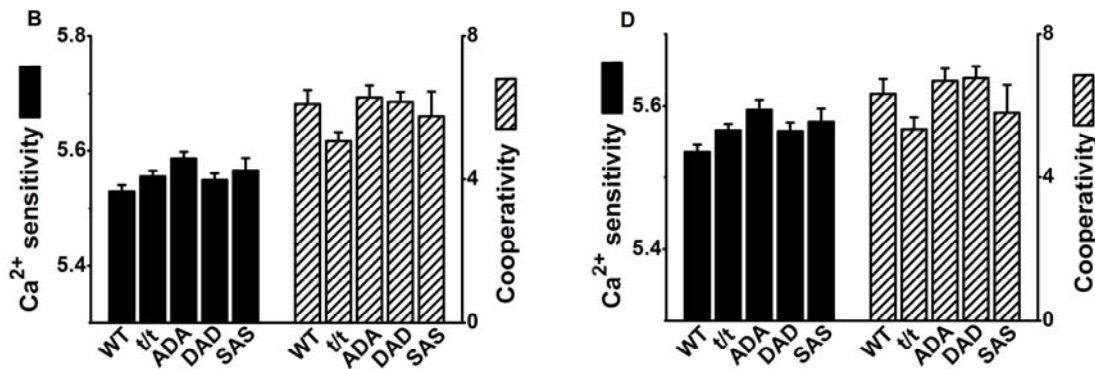


FIGURE S6 **The effect of $[Ca^{2+}]$ in the 8Pi solution in five groups of mice.** (B) pCa_{50} (left ordinate) and n_H (right ordinate), as deduced by fitting the individual data of pCa-tension relationship to Eq. 2, and averaged. (D) pCa_{50s} (left ordinate) and n_{HS} (right ordinate), as deduced by fitting the data of pCa-stiffness relationship to Eq. 3, and averaged. In (B) and (D), no significant difference was found in any of the parameters measured. $n = 9$ for WT, 13 for t/t , 14 for ADA, 18 for DAD, and 5 for SAS.

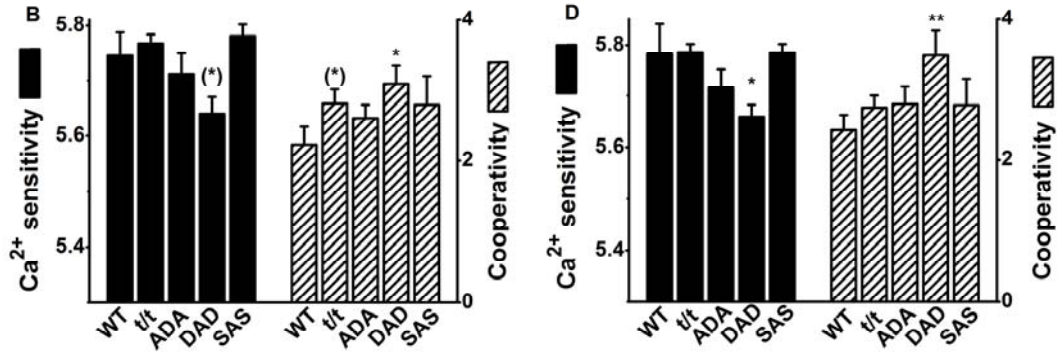


FIGURE S7 **The effect of [Ca²⁺] in the 0Pi solution in five groups of mice.** The same nomenclature and conventions are used as in Fig. S6. $n = 9$ for WT, 14 for t/t, ADA, and DAD, and $n = 5$ for SAS. ** $p \leq 0.01$, * $0.01 < p \leq 0.05$, and (*) $0.05 < p \leq 0.1$ compared to WT.

TABLE S1. The biophysical parameters (** $p < 0.01$ and * $p < 0.05$ compared to WT) including two female mice.

Study	Parameter	WT	t/t	ADA	DAD	SAS
8Pi, Standard activation	$2\pi b$ (s ⁻¹)	40.0 ± 1.4	36.0 ± 2.6	43.7 ± 3.2	67.5 ± 2.5**	55.6 ± 6.2**
	$2\pi c$ (s ⁻¹)	198 ± 10	115 ± 5**	197 ± 8	146 ± 10**	183 ± 16
	Magnitude B (kPa)	221 ± 17	329 ± 42*	221 ± 30	255 ± 39	365 ± 64*
	Magnitude C (kPa)	760 ± 68	711 ± 68	661 ± 59	486 ± 79*	956 ± 111
	Magnitude H (kPa)	116 ± 10	111 ± 10	93 ± 5	84 ± 11*	137 ± 12
	Tension (kPa)	15.4 ± 1.2	13.6 ± 1.0	14.4 ± 1.4	8.2 ± 1.1**	18.1 ± 2.2
	Stiffness (kPa)	655 ± 60	489 ± 38*	534 ± 50	335 ± 63**	729 ± 93
0Pi, Standard activation	$2\pi b$ (s ⁻¹)	8.3 ± 1.4	8.5 ± 1.4	6.5 ± 1.0	11.8 ± 1.6	10.4 ± 1.3
	$2\pi c$ (s ⁻¹)	70.5 ± 5.6	37.9 ± 4.2**	64.5 ± 4.8	59.4 ± 4.7	64.6 ± 6.6
	Magnitude B (kPa)	30.1 ± 16.5	42.8 ± 17.3	33.2 ± 11.9	5.5 ± 3.7	49.7 ± 38.0
	Magnitude C (kPa)	384 ± 63	349 ± 68	267 ± 37	218 ± 98	378 ± 78
	Magnitude H (kPa)	373 ± 48	420 ± 36	312 ± 27	211 ± 25*	429 ± 66
	Tension (kPa)	24.3 ± 2.5	20.0 ± 1.6	19.7 ± 1.7	12.4 ± 2.2**	24.0 ± 3.3
	Stiffness (kPa)	761 ± 101	645 ± 64	558 ± 52*	423 ± 115*	757 ± 109
8Pi, pCa- tension	pCa ₅₀ (Ca ²⁺ sensitivity)	5.562 ± 0.011	5.555 ± 0.010	5.586 ± 0.012	5.548 ± 0.012	5.548 ± 0.015
	n_H (cooperativity)	5.7 ± 0.3	5.1 ± 0.3	6.3 ± 0.3	6.2 ± 0.3	5.9 ± 0.4
8Pi, pCa- stiffness	pCa _{50S} (Ca ²⁺ sensitivity)	5.565 ± 0.012	5.566 ± 0.009	5.595 ± 0.014	5.565 ± 0.012	5.563 ± 0.015
	n_{HS} (cooperativity)	6.2 ± 0.3	5.4 ± 0.3	6.7 ± 0.3	6.8 ± 0.3	5.9 ± 0.5
0Pi, pCa- tension	pCa ₅₀	5.726 ± 0.030	5.768 ± 0.017	5.712 ± 0.038	5.640 ± 0.030 ^(*)	5.714 ± 0.023
	n_H	2.3 ± 0.2	2.8 ± 0.2 ^(*)	2.6 ± 0.2	3.1 ± 0.3*	2.7 ± 0.2
0Pi, pCa- stiffness	pCa _{50S}	5.760 ± 0.041	5.786 ± 0.016	5.715 ± 0.037	5.659 ± 0.023*	5.728 ± 0.022
	n_{HS}	2.4 ± 0.2	2.7 ± 0.2	2.8 ± 0.3	3.5 ± 0.4**	2.6 ± 0.2