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Supplemental Information

Modeling the Actin.myosin ATPase Cross-Bridge Cycle for Skeletal and Cardiac Muscle Myosin Isoforms

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SUPPORTING MATERIAL

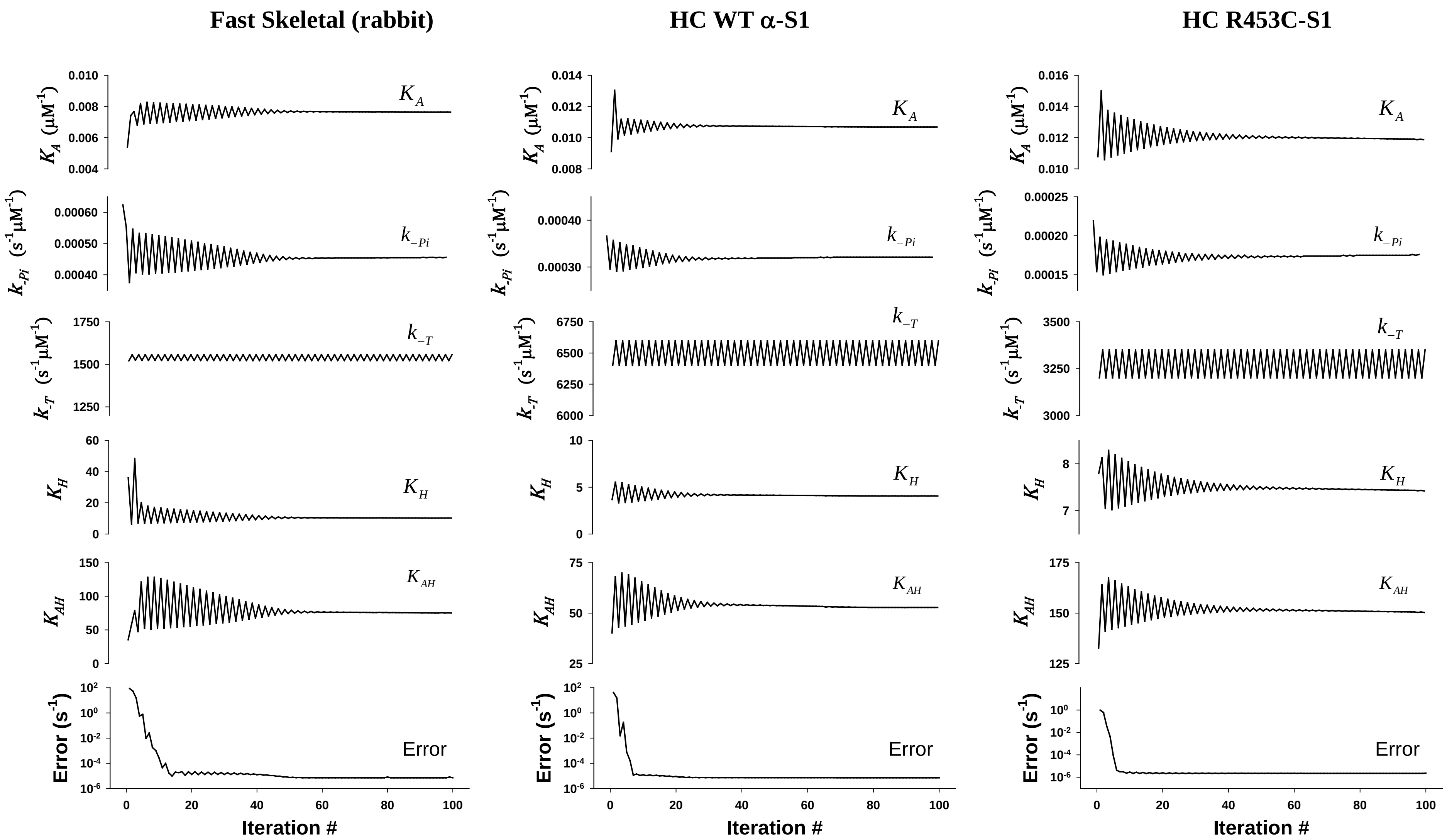

FIGURE S1 Convergence of fitted parameters for rabbit fast skeletal, human cardiac α-S1 and human cardiac β-R453C-S1

TABLE S1 Rate and equilibrium constants for the 3 myosin isoforms and one β-cardiac myosin mutant. At both 100 and 25 mM KCl

	Units	Fast Skeletal		HC WT β		HC WT* α	HC WT α	β-R453C*	β-R453C
		25 mMKCl	100 mM KCl	25 mM KCl	100 mM KCl	25 mM KCl	100 mM KCl	25 mM KCl	100 mM KCl
K_A	mM^{-1}	**7.08**	**2.0**	**10.7**	**4.0**	**?**	**11.30**	**?**	**14.35**
K_{Pi}	mM	**100**	**?**	**100**	**?**	**?**	**100**	**?**	**100**
K_{D^*}	–	50	50	0.1667	0.14	50	83.33	0.05	0.046
K_D	μM	100	120	36	170	197	152	33	100.00
K_T	mM^{-1}	5	1.92	3	0.88	4.1	1.6	3.2	1.1
K_{T^*}	–	1507	1250	154.30	144	150.00	150.00	150	124.80
$K_{T^{**}}$	μM	1000	1000	1000	1000	1000	1000	1000	1000
K_H	–	**5**	**5.5**	**8.91**	**5.6**	**5**	**8.52**	**1.8**	2.86
K_{AH}	–	**61.77**	**?**	**63.14**		**63.3**	**99.60**	**?**	124.43
K_m	μM	**101.0**[1]	-	**39.55**[3]	-	**67.8**		**28**[4]	
V_{max}	s^{-1}	**29.3**[1]	-	**5.94**[3]	-	**18**		**5**[4]	
Forward Rate Constants									
k_A	$\mu M^{-1}s^{-1}$	**3.54**	**1**	**10.75**	**4**	**?**	**11.30**	**?**	**14.35**
k_{Pi}	s^{-1}	**49.67**	**?**	**15.95**	**?**	**?**	**27.20**	**?**	**14.69**
k_{D^*}	s^{-1}	300	300	59.00	93	100	100.00	39.69	63.00
k_D	s^{-1}	1000	1200	1000	1000	1970	1520	1000	1000
k_T	$\mu M^{-1} s^{-1}$	**7.7**	**10**	**10.00**	**3.33**	**27.2**	**10.51**	**10**	**3.3**
k_{T^*}	s^{-1}	1507	1250	1543	1445	1800	1500	1500	1248
$k_{T^{**}}$	s^{-1}	1000	1000	1000	1000	1000	1000	1000	1000
k_H	s^{-1}	**70**	**110**	**12.47**	**14**	**95.1**	**143.16**	**2.52**	**4**
k_{AH}	s^{-1}	**123.54**	**?**	**12.63**	**?**		**149.40**		**8.71**
Backward Rate Constants									
k_{-A}	s^{-1}	500	500	1000	1000		1000	?	1000
k_{-Pi}	$mM^{-1} s^{-1}$	**0.5**	**0.5**	**0.15**			**0.27**	**?**	**0.15**
k_{-D^*}	s^{-1}	6	6	354	650		1.20	794	1369
k_{-D}	$\mu M^{-1} s^{-1}$	10	10	27.8	5.9	10	10	30.3	10
k_{-T}	s^{-1}	**1540**	**5208**	**3349**	**3784**	**6500**	**6541.53**	**3125**	**3000**
k_{-T^*}	s^{-1}	1	1	10	10	12	10	10	10.00
$k_{-T^{**}}$	$\mu M^{-1} s^{-1}$	1	1	1	1	1	1	1	1
k_{-H}	s^{-1}	14	20	1.4	2.5	19	16.8	1.4	1
k_{-AH}	s^{-1}	2	?	0.2	?	?	1.5	?	0.07

For the ATPase assays

[ATP] = 5mM , [ADP] = [Pi] = 0 mM (standard initial rate ATPase conditions).

[S1] = 1 μM with varied [actin].

Conditions: 25 mM KCl, 20 mM MOPS, 5 mM $MgCl_2$ unless otherwise stated.

Source of Data

Bold allowed to vary in the fit to ATPase data

X Assumed diffusion limited:

X Assumed min/max value

X From detailed balance $K_i = k_i/k_{-i}$

X From transients

X from ATPase data reference:

NB the data for HC α WT and R453C at 25 mM KCl is estimated from the 100 mM data and the KCl dependence of the valued for the rabbit fast and HC-β muscle isoforms.

1: Swenson, A.M., Trivedi, D.V., Rauscher, A.A., Wang, Y., Takagi, Y., Palmer, B.M., Málnási-Csizmadia, A., Debold, E.P., Yengo, C. M. Magnesium modulates actin binding and ADP release in myosin motors. The Journal of Biological Chemistry, 2014, 289 (34) pp 23977-91.

IIa from rabbit

2: Nyitrai, M., Rossi, R., Adamek, N., Pellegrino, M.A., Bottinelli, R., Geeves, M. A. What limits the velocity of fast-skeletal muscle contraction in mammals? Journal of Molecular Biology, 2006, 355 (3) 432-42

This work gives values of k_2 (= 740 s^{-1}) and $1/K_T$ (= 520 µM) at 12 C. The value of $1/K_T$ is independent of temperature so the value of k_2 at 20 C can be estimated from the $K_T k_2$ value at 20 C. Thus $K_T k_2$= 2.4 $uM^{-1}s^{-1}$ where K_T = 0.00192 µM and k_2 = 1,248 s^{-1}.

3: Nag, S., Sommese, R.F., Ujfalusi, Z., Combs, A., Langer, S., Sutton, S., Leinwand, L. A., Geeves, M. A., Ruppel, K. M., Spudich, J. A. Contractility parameters of human β-cardiac myosin with the hypertrophic cardiomyopathy mutation R403Q show loss of motor function. Sci Adv. 2015 Oct 9;1(9):e1500511. doi: 10.1126/sciadv.1500511

4: Sommese R.F., Sung J., Nag S., Sutton S. Deacon J.C.Choe E., Leinwand L.A., Ruppel K., Spudich J.A. Molecular consequences of the R453C hypertrophic cardiomyopathy mutation on human β-cardiac myosin motor function. Proc Natl Acad Sci U S A. 2013 Jul 30;110(31):12607-12. doi: 10.1073/pnas.1309493110

Table S2 State occupancies of intermediates in the crossbridge cycle for the four myosins used here as a function of actin concentration and load.

	A-MDPi	AMD	AM-D	AM	AMT	A-MT	MT	MDPi	ATPase (s^{-1})	Detach.	Weak Att	Strong Att	Duty ratio	Δ% AMD with load	Velocity (μm/s)
Fast Skeletal															
[A]= Km = 101 μM	0.3234	0.0494	0.0147	0.0008	0.0098	0.0244	0.1260	0.4515	14.7353	0.5775	0.3478	0.0747	0.0747		0.9862
[A]= 3Km	0.4848	0.0741	0.0221	0.0012	0.0147	0.0534	0.1269	0.2229	22.0896	0.3498	0.5382	0.1120	0.1120		0.9860
[A]= 20Km	0.6152	0.0940	0.0280	0.0015	0.0187	0.1377	0.0639	0.0410	28.0331	0.1049	0.7529	0.1422	0.1422		0.9857
[A]= 3Km - loaded	0.5611	0.0857	0.0085	0.0004	0.0057	0.0255	0.0649	0.2481	8.5232	0.3130	0.5866	0.1004	0.1004	15.75	0.4245
HC WT β - S1															
[A]= Km = 39.55 μM	0.1865	0.0683	0.0030	0.0002	0.0020	0.0138	0.2779	0.4484	2.9744	0.7263	0.2002	0.0734	0.0734		0.2023
[A]=3Km	0.2797	0.1024	0.0045	0.0003	0.0032	0.0444	0.3423	0.2232	4.4614	0.5655	0.3241	0.1103	0.1103		0.2021
[A]=20Km	0.3550	0.1299	0.0057	0.0004	0.0050	0.2056	0.2562	0.0422	5.6619	0.2984	0.5606	0.1410	0.1410		0.2008
[A]=3Km - loaded	0.3707	0.1357	0.0020	0.0001	0.0014	0.0224	0.1744	0.2933	1.9720	0.4677	0.3930	0.1393	0.1393	32.52	0.0708
HC WT α - S1															
[A]= Km = 67.8 μM	0.2801	0.0901	0.0046	0.0003	0.0051	0.0212	0.1988	0.3997	9.0033	0.5985	0.3013	0.1001	0.1001		0.4497
[A]=3Km	0.4201	0.1352	0.0069	0.0005	0.0078	0.0475	0.1833	0.1987	13.5034	0.3820	0.4676	0.1504	0.1504		0.4489
[A]=20Km	0.5333	0.1716	0.0087	0.0006	0.0105	0.1399	0.0982	0.0373	17.1395	0.1355	0.6732	0.1914	0.1914		0.4477
[A]=3Km - loaded	0.4829	0.1554	0.0026	0.0002	0.0030	0.0253	0.1058	0.2247	5.1737	0.3305	0.5082	0.1612	0.1612	14.94	0.1605
HC R453C β - S1															
[A]= Km = 28 μM	0.1423	0.1130	0.0025	0.0002	0.0017	0.0105	0.2904	0.4394	2.5013	0.7298	0.1528	0.1174	0.1174		0.1065
[A]=3Km	0.2134	0.1696	0.0038	0.0002	0.0027	0.0334	0.3584	0.2185	3.7525	0.5769	0.2468	0.1763	0.1763		0.1064
[A]=20Km	0.2707	0.2151	0.0048	0.0004	0.0043	0.1682	0.2953	0.0412	4.7601	0.3365	0.4389	0.2246	0.2246		0.1060
[A]=3Km - loaded	0.2835	0.2253	0.0017	0.0001	0.0012	0.0168	0.1837	0.2877	1.6619	0.4714	0.3003	0.2283	0.2283	32.84	0.0364

TABLE S3: Resolution matrices for Fast Skeletal (Rabbit), HC WT α-S1 and HC R 452C β-S1

HF-Sk S1			K_A	k_{Pi}	k_{-T}	K_H	K_{AH}
K_A	**0.008**	–	**0.98105**	0.02946	-0.00102	-0.09029	-0.09531
k_{-Pi}	**0.452**	$mM^{-1}\ s^{-1}$	0.02946	**0.95335**	0.00107	0.14446	0.15064
k_{-T}	**1557**	$s^{-1}\mu M^{-1}$	-0.00102	0.00107	**2.5×10^{-6}**	0.00024	0.00030
K_H	**10.22**	μM^{-1}	-0.09029	0.14446	0.00024	**0.84917**	-0.46562
K_{AH}	**75.15**	μM^{-1}	-0.09531	0.15064	0.00030	-0.46562	**0.81309**

HC WT-α S1			K_A	k_{Pi}	k_{-T}	K_H	K_{AH}
K_A	**0.011**	–	**0.99340**	0.01164	-0.00051	-0.04754	-0.05355
k_{-Pi}	**0.321**	$mM^{-1}\ s^{-1}$	0.01164	**0.99653**	-0.00018	0.01166	0.00802
k_{-T}	**6597**	$s^{-1}\mu M^{-1}$	-0.00051	-0.00018	**2.3×10^{-6}**	0.00097	0.00103
K_H	**4.061**	μM^{-1}	-0.04754	0.01166	0.00097	**0.95986**	-0.02654
K_{AH}	**52.76**	μM^{-1}	-0.05355	0.00802	0.00103	-0.02654	**0.98087**

HC R452C-β S1			K_A	k_{Pi}	k_{-T}	K_H	K_{AH}
K_A	**0.012**	–	**0.96580**	0.35804	-0.00010	-0.21072	-0.21505
k_{-Pi}	**0.176**	$mM^{-1}\ s^{-1}$	0.35804	**0.90917**	0.00009	0.23395	0.23350
k_{-T}	**3349**	$s^{-1}\mu M^{-1}$	-0.00010	0.00009	**0.15×10^{-6}**	0.00014	0.00015
K_H	**7.419**	μM^{-1}	-0.21072	0.23395	0.00014	**0.85566**	-0.13811
K_{AH}	**150.3**	μM^{-1}	-0.21505	0.23350	0.00015	-0.13811	**0.85850**

TABLE S4: Sensitivity analysis of the fitted parameters to change of +20% or -20% fixed parameters k_{-D}, K_{D^*} , $k_{D^*}$and K_{-T^*} for HC WT β - S1.

	Units	Estimated constants	Fixed k_{-D}		Fixed K_{D^*}		Fixed k_{-D^*}		Fixed K_{-T^*}		Fixed $k_{-T^{**}}$	
			K_D +20%	K_D -20%	k_{D^*} +20%	k_{D^*} -20%	k_{D^*} +20%	k_{D^*} -20%	k_{-T^*} +20%	k_{-T^*} -20%	$K_{-T^{**}}$ +20%	$K_{-T^{**}}$-20%
K_A	μM^{-1}	**0.01075**	**0.47%**	**-2.33%**	**2.33%**	**-5.12%**	**3.26%**	**-6.98%**	**0.93%**	**-1.40%**	**3.26%**	**-0.47%**
K_{Pi}	mM	100	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
K_T	μM^{-1}	0.003	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
K_H	–	**8.9136**	**-1.48%**	**0.30%**	**-2.16%**	**1.04%**	**-2.55%**	**1.83%**	**-0.51%**	**0.20%**	**0.97%**	**-0.47%**
K_{AH}	–	**63.145**	**-1.48%**	**0.33%**	**-2.18%**	**1.06%**	**-2.57%**	**1.86%**	**-0.63%**	**0.20%**	**0.98%**	**-0.41%**
Forward Rate Constants												
k_A	$\mu M^{-1}s^{-1}$	**10.75**	**0.47%**	**-2.33%**	**2.33%**	**-5.12%**	**3.26%**	**-6.98%**	**0.93%**	**-1.40%**	**3.26%**	**-0.47%**
k_{Pi}	s^{-1}	**15.95**	**-0.31%**	**2.19%**	**-2.19%**	**5.33%**	**-2.82%**	**6.58%**	**-0.31%**	**0.94%**	**-3.45%**	**0.31%**
k_T	$\mu M^{-1}s^{-1}$	**10.048**	**0.00%**	**0.00%**	**0.00%**	**0.00%**	**0.00%**	**0.00%**	**0.00%**	**0.00%**	**0.01%**	**0.00%**
k_H	s^{-1}	**12.479**	**-1.48%**	**0.32%**	**-2.16%**	**1.04%**	**-2.55%**	**1.83%**	**-0.51%**	**0.20%**	**0.97%**	**-0.47%**
k_{AH}	s^{-1}	**12.629**	**-1.49%**	**0.33%**	**-2.18%**	**1.06%**	**-2.57%**	**1.86%**	**-0.63%**	**0.20%**	**0.98%**	**-0.41%**
Backward Rate Constants												
k_{-A}	s^{-1}	1000	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
k_{-Pi}	$mM^{-1}s^{-1}$	**0. 159**	**-0.31%**	**2.19%**	**-2.19%**	**5.33%**	**-2.82%**	**6.58%**	**-0.31%**	**0.94%**	**-3.45%**	**0.31%**
k_{-T}	s^{-1}	**3349.37**	**0.00%**	**0.00%**	**0.00%**	**0.00%**	**0.00%**	**0.00%**	**0.00%**	**0.00%**	**0.01%**	**0.00%**
k_{-H}	s^{-1}	1.4	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
k_{-AH}	s^{-1}	0.2	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%

Numbers in bold (black) were obtained from fits to the ATPase data shown in Fig 2A & 3A. The rest of the values were assigned as described in the methods section. Bold gray numbers are calculated from fitted values in bold black.

TABLE S5: The effect of change of k_H by ±20% on estimated parameters including k_{Pi}

	Units	Estimated constants	Fixed k_{-H}		Fixed k_{-H}	
			k_H +20%	k_H -20%	k_H +20%	k_H -20%
K_A	μM^{-1}	**0.01075**	**0.00758**	**0.0128**	**-29.51%**	**19.23%**
K_{Pi}	mM	100	100	100	0.00%	0.00%
K_T	μM^{-1}	0.003	0.003	0.003	0.00%	0.00%
K_H	–	**8.9136**	**7.1257**	**10.696**	**20.00%**	**-20.00%**
K_{AH}	–	**63.145**	**50.4133**	**75.888**	**-20.16%**	**20.18%**
Forward Rate Constants						
k_A	$\mu M^{-1}s^{-1}$	**10.75**	**7.578**	**12.82**	**-29.51%**	**19.23%**
k_{Pi}	s^{-1}	**15.950**	**23.290**	**13.129**	**46.02%**	**-17.77%**
k_T	$\mu M^{-1}s^{-1}$	**10.048**	**10.042**	**10.047**	**0.00%**	**0.00%**
k_H	s^{-1}	12.479	9.976	14.975	-20.00%	20.00%
k_{AH}	s^{-1}	**12.629**	**10.083**	**15.178**	**-20.16%**	**20.18%**
Backward Rate Constants						
k_{-A}	s^{-1}	1000	1000	1000	0.00%	0.00%
k_{-Pi}	$mM^{-1}s^{-1}$	**0. 159**	**0.233**	**0.131**	**46.48%**	**-17.51%**
k_{-T}	s^{-1}	**3349.37**	**3349.45**	**3349.17**	**0.00%**	**0.00%**
k_{-H}	s^{-1}	1.4	1.4	1.4	0.00%	0.00%
k_{-AH}	s^{-1}	0.2	0.2	0.2	0.00%	0.00%
Error	s^{-1}	$1.43x10^{-6}$	$7.60x10^{-7}$	$1.76x10^{-6}$		

Numbers in bold (black) were obtained from fits to the ATPase data shown in Fig 2A & 3A. The rest of the values were assigned as described in the methods section. Bold gray numbers are calculated from fitted values in bold black.

Table S6 Change of state occupancies of intermediates in the crossbridge cycle for HC WT β - S1 as a function of +/- 20% k_H at actin concentration Km= 39.55 μM.

	A-MDPi	AMD	AM-D	AM	AMT	A-MT	MT	MDPi	ATPase (s^{-1})	Detach	Weak Att	Strong Att	Duty ratio	Velocity (μm/s)
[A]= K_m	0.1865	0.0682	0.0029	0.0002	0.0020	0.0137	0.2777	0.4484	2.9750	0.7262	0.2002	0.0734	0.0734	0.2026
[A]=3K_m +20% k_H	0.2265	0.0681	0.0029	0.0002	0.0020	0.0119	0.2320	0.4561	2.9717	0.6881	0.2385	0.0733	0.0733	0.2026
[A]=20K_m –20% k_H	0.1275	0.0681	0.0029	0.0002	0.0020	0.0164	0.3452	0.4374	2.9714	0.7826	0.1439	0.0733	0.0733	0.2025