Supplementary Information

Pediatric and adult-onset HCM mutations in the myosin motor domain have similar properties

Carlos D Vera[1,#], Chloe A Johnson[2,#], Jonathan Walklate[2], Arjun Adhikari[3], Zoltan Ujfalusi[4], Marina Svicevic[5], Srba M Mijailovich[6], Ariana Combs[1], Stephen J. Langer[1], Kathleen M. Ruppel[7], James A. Spudich[7], Leslie A. Leinwand[1,*], and Michael A. Geeves[2,*]

From the [1]BioFrontiers Institute and Department of Molecular, Cellular and Developmental Biology, University of Colorado Boulder, Boulder CO 80309 USA; [2]School of Biosciences, University of Kent, Canterbury UK, CT2 7NJ; [3]Rumi Scientific, San Francisco CA 94107 USA; [4]Department of Biophysics, University of Pécs, Medical School, Szigeti Street 12, H-7624 Pécs, Hungary, [5]Faculty of Science, University of Kagujevac, Serbia; [6]Department of Biology, Illinois Institute of Technology, Chicago IL 60616 USA; [7]Stanford University School of Medicine, Department of Biochemistry, Stanford CA 94305 USA.

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*To whom correspondence should be addressed: Michael A. Geeves School of Biosciences, University of Kent, Canterbury, UK, CT2 7NJ m.a.geeves@kent.ac.uk; Leslie A. Leinwand BioFrontiers Institute and/or Department of Molecular, Cellular and Developmental Biology, University of Colorado, Boulder CO 80309 leslie.leinwand@colorado.edu

Table S1. This table describes a set of queries regarding the clinical frequency of HCM linked or associated mutations in MYH7 assessed using the NCBI tool ClinVar. (https://www.ncbi.nlm.nih.gov/clinvar/).

Mutation in MYH7	ClinVar Variation ID	Has the mutation been described in more than one person?	Has the mutation been seen in multiple families?	Has the mutation affected associated family members of the patient?	Is it predicted to be pathogenic?	Is it present in large population databases?	Identified as early onset?
H251N	n/a	No	No	No	No	No	Yes
D382Y	237432	No	No	No	No, VUS	No	Yes
R403Q	14087	Yes	Yes	Yes	Yes	No	No
R453C	14089	Yes	Yes	Yes	Yes	No	No
F540L	407186	Yes	No	No	Yes	No	Yes
P710R	n/a	No	No	No	No	No	Yes
R719W	14104	Yes	Yes	Yes	Yes	No	No
R723G	42885	Yes	Yes	Yes	Yes	No	No
G741R	42890	Yes	Yes	Yes	Yes	No	No
V763M	177642	No	No	No	No, VUS	No	Yes

Table S2. Fitted Rate and Equilibrium Constants of the ATPase cycle. Highlighted to show which fitted & which measured and which derived from assumption or detailed balance.

A. Equilibrium Constants

	Units	*β-WT*	*H251N*	*D382Y*	*F540L*	*P710R*	*V763M*	*R719W*	*R723G*	*G741R*
K_{app}	μM	58	60	64	15.5	24	27	34	30	23
k_{cat}	s^{-1}	4.5	5.6	4.3	6.5	2.8	4.5	5.4	3.2	4.6
K_A	μM^{-1}	0.0088	0.0088	0.0071	0.020	0.032	0.017	0.0076	0.014	0.014
K_{pi}	mM	100	100	100	100	100	100	100	100	100
K_{D*}		0.167	0.167	0.167	0.167	0.167	0.167	0.167	0.167	0.167
K_D	μM	36	36	36	36	36	36	36	36	36
K_T	μM^{-1}	0.0036	0.0076	0.0035	0.0019	0.0062	0.0037	0.0080	0.0049	0.0056
K_{T*}		154	66.6	130	189.7	110.7	117.6	67.6	55.6	94
K_{T**}	μM	1000	1000	1000	1000	1000	1000	1000	1000	1000
K_H		7.3	10.6	6.0	8.8	7.3	6.2	5.7	3.8	5.2
K_{AH}		51.7	75.9	42.6	57.9	52.5	44.0	40.3	26.9	36.6

B. Forward Rate Constants

	Units	*β-WT*	*H251N*	*D382Y*	*F540L*	*P710R*	*V763M*	*R719W*	*R723G*	*G741R*
k_A	$\mu M^{-1}s^{-1}$	8.807	8.779	7.071	19.9	31.8	16.4	7.606	13.9	13.6
k_{Pi}	s^{-1}	10.2	11.9	11.3	24.7	4.3	12.1	25.4	9.9	18
k_{D*}	s^{-1}	59	56.7	64.3	55	69	68	96.8	106.8	71.1
k_D	s^{-1}	1000	1000	1000	1000	1000	1000	1000	1000	1000
k_T	$\mu M^{-1}s^{-1}$	10.15	10.00	10.15	10.03	10.23	10.14	10.01	10.05	10.01
k_{T*}	s^{-1}	1543	666	1300	1897	1106.5	1175	676	556	940
k_{T**}	s^{-1}	1000	1000	1000	1000	1000	1000	1000	1000	1000
k_H	s^{-1}	10.2	14.9	8.4	11.5	10.3	8.7	7.9	5.3	7.2
k_{AH}	s^{-1}	10.3	15.2	8.5	11.6	10.5	8.8	8.1	5.4	7.3

C. Backward Rate Constants

	Units	***β-WT***	***H251N***	***D382Y***	***F540L***	***P710R***	***V763M***	***R719W***	***R723G***	***G741R***
k_{-A}	s^{-1}	1000	1000	1000	1000	1000	1000	1000	1000	1000
k_{-Pi}	$mM^{-1}s^{-1}$	0.102	0.119	0.113	0.247	0.043	0.121	0.254	0.099	0.18
k_{-D*}	s^{-1}	353.29	339.52	385.03	329.34	413.17	407.19	579.64	639.52	425.75
k_{-D}	$\mu M^{-1}s^{-1}$	27.78	27.78	27.78	27.78	27.78	27.78	27.78	27.78	27.78
k_{-T}	s^{-1}	3385	1311	2941	5271	1650	2740	1258	2050	1787
k_{-T*}	s^{-1}	10	10	10	10	10	10	10	10	10
k_{-T**}	$\mu M^{-1}s^{-1}$	1	1	1	1	1	1	1	1	1
k_{-H}	s^{-1}	1.4	1.4	1.4	1.4	1.4	1.4	1.4	1.4	1.4
k_{-AH}	s^{-1}	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2

Table S3. Predicted cross-bridge cycle parameters at three actin concentrations from the modelled ATPase cycle.

Isoform	*[Actin] (μM)*	*ATPase (s^{-1})*	*Detached*	*Weakly attached*	*Strongly attached*	*Duty Ratio*	*Velocity (μm/s)*
β-WT							
	K_{app} = 58	2.253	0.706	0.238	0.056	0.056	0.202
	3 K_{app} = 174	3.378	0.529	0.388	0.084	0.084	0.202
	20 K_{app} = 1160	4.287	0.240	0.653	0.107	0.107	0.199
	3 K_{app} + Load	1.480	0.448	0.465	0.087	0.087	0.085
H251N							
	K_{app} = 60	2.809	0.674	0.252	0.074	0.074	0.190
	3 K_{app} = 180	4.218	0.485	0.404	0.111	0.111	0.190
	20 K_{app} = 1200	5.384	0.206	0.650	0.144	0.144	0.187
	3 K_{app} + Load	1.787	0.415	0.475	0.110	0.110	0.081
D382Y							
	K_{app} = 64	2.154	0.737	0.212	0.051	0.051	0.213
	3 K_{app} = 192	3.230	0.567	0.356	0.076	0.076	0.212
	20 K_{app} = 1280	4.099	0.258	0.644	0.099	0.099	0.209
	3 K_{app} + Load	1.483	0.487	0.432	0.081	0.081	0.091
F540L							
	K_{app} = 15.5	3.255	0.776	0.140	0.084	0.084	0.194
	3 K_{app} = 46.5	4.883	0.652	0.222	0.126	0.126	0.194
	20 K_{app} = 310	6.188	0.456	0.384	0.160	0.160	0.193
	3 K_{app} + Load	2.364	0.553	0.301	0.147	0.147	0.080

P710R							
	K_{app} = 24	1.408	0.635	0.334	0.032	0.032	0.222
	3 K_{app} = 72	2.112	0.443	0.509	0.048	0.048	0.222
	20 K_{app} = 480	2.676	0.226	0.713	0.061	0.061	0.220
	3 K_{app} + Load	0.825	0.371	0.586	0.043	0.043	0.097
V763M							
	K_{app} = 27	2.254	0.752	0.197	0.051	0.051	0.221
	3 K_{app} = 81	3.381	0.609	0.314	0.077	0.077	0.220
	20 K_{app} = 540	4.290	0.368	0.534	0.099	0.099	0.218
	3 K_{app} + Load	1.562	0.512	0.407	0.081	0.081	0.096
R719W							
	K_{app} = 34	2.703	0.826	0.123	0.051	0.051	0.264
	3 K_{app} = 102	4.054	0.709	0.214	0.077	0.077	0.262
	20 K_{app} = 680	5.144	0.429	0.470	0.101	0.101	0.255
	3 K_{app} + Load	2.137	0.63	0.284	0.085	0.085	0.126
R723G							
	K_{app} = 34	1.601	0.795	0.175	0.029	0.029	0.272
	3 K_{app} = 102	2.402	0.668	0.287	0.045	0.045	0.269
	20 K_{app} = 680	3.048	0.408	0.532	0.060	0.060	0.255
	3 K_{app} + Load	1.176	0.573	0.383	0.044	0.044	0.134
G741R							
	K_{app} = 23	2.300	0.810	0.139	0.051	0.029	0.225
	3 K_{app} = 69	3.447	0.700	0.228	0.077	0.045	0.224
	20 K_{app} = 460	4.376	0.461	0.440	0.099	0.060	0.221
	3 K_{app} + Load	1.740	0.601	0.311	0.088	0.044	0.099

Table S4. Predicted Occupancy of the 8 states during the ATPase cycle.

Isoform	*[Actin] (μM)*	*A·M*	*A·M·T*	*A-M·T*	*M·T*	*M·D·Pi*	*A-M·D·Pi*	*A·MD*	*A·M-D*
β-WT									
	K_{app} = 58	0.000132	0.001574	0.01754	0.267297	0.438699	0.220839	0.051667	0.002253
	3 K_{app} = 174	0.000209	0.002557	0.056648	0.309971	0.218574	0.33118	0.077483	0.003378
	20 K_{app} = 1160	0.000323	0.004287	0.232348	0.198731	0.041363	0.420322	0.098338	0.004287
	3 K_{app} + Load	0.000093	0.00115	0.029492	0.162755	0.285732	0.43521	0.084088	0.00148
H251N									
	K_{app} = 60	0.000173	0.004454	0.015668	0.218803	0.455634	0.236087	0.066372	0.002809
	3 K_{app} = 180	0.00027	0.007079	0.04972	0.257486	0.227158	0.354427	0.099642	0.004218
	20 K_m = 1200	0.000397	0.011051	0.197517	0.162741	0.043205	0.452493	0.127211	0.005385
	3 K_{app} = Load	0.000116	0.00305	0.024418	0.127765	0.287013	0.450574	0.105276	0.001787
D382Y									
	K_{app} = 64	0.000148	0.001824	0.021742	0.309623	0.427462	0.190643	0.046403	0.002154
	3 K_{app} = 192	0.000239	0.003028	0.070592	0.354479	0.213007	0.285848	0.069576	0.00323
	20 K_{app} = 1280	0.000389	0.005313	0.28076	0.21808	0.0403	0.362762	0.088298	0.004099
	3 K_{app} + Load	0.000113	0.001437	0.038579	0.19503	0.291638	0.393646	0.078075	0.001483
F540L									
	K_{app} = 15.5	0.00025	0.001759	0.008259	0.33211	0.443935	0.131768	0.078665	0.003255
	3 K_{app} = 46.5	0.000382	0.002703	0.024552	0.431499	0.220288	0.197679	0.118014	0.004883
	20 K_{app} = 310	0.000541	0.003963	0.133042	0.414746	0.041443	0.250519	0.149559	0.006188
	3 K_{app} + Load	0.000186	0.001316	0.013164	0.236567	0.315961	0.287292	0.14315	0.002364

P710R									
	K_{app} = 24	0.00007	0.001328	0.006069	0.197117	0.437646	0.327518	0.028844	0.001408
	3 K_{app} = 72	0.000108	0.002073	0.018174	0.22613	0.217052	0.4911	0.04325	0.002112
	20 K_{app} = 480	0.000157	0.003237	0.090651	0.185307	0.040951	0.622223	0.054798	0.002676
	3 K_{app} + Load	0.000043	0.000829	0.00919	0.116939	0.25453	0.576841	0.040804	0.000825
V763M									
	K_{app} = 27	0.000153	0.002009	0.010817	0.322274	0.429578	0.186273	0.046642	0.002254
	3 K_{app} = 81	0.000238	0.003173	0.034973	0.395032	0.213763	0.279462	0.069976	0.003381
	20 K_{app} = 540	0.000364	0.005173	0.17926	0.327191	0.040396	0.354548	0.088777	0.00429
	3 K_{app} + Load	0.000111	0.00149	0.018962	0.217227	0.294713	0.387664	0.078269	0.001562
R719W									
	K_{app} = 34	0.00016	0.004239	0.016205	0.402397	0.423751	0.10643	0.044114	0.002703
	3 K_{app} = 102	0.000252	0.0068	0.054283	0.497848	0.211017	0.159595	0.066151	0.004054
	20 K_{app} = 680	0.000393	0.011566	0.267449	0.389183	0.039778	0.202536	0.08395	0.005144
	3 K_{app} + Load	0.000134	0.003642	0.032533	0.301177	0.32898	0.252376	0.07902	0.002137
R723G									
	K_{app} = 30	0.000159	0.003123	0.013507	0.400951	0.394325	0.16175	0.024583	0.001601
	3 K_{app} = 90	0.000257	0.005121	0.044512	0.471752	0.196445	0.242636	0.036875	0.002402
	20 K_{app} = 600	0.000449	0.009515	0.224245	0.370936	0.037177	0.307845	0.046786	0.003048
	3 K_{app} + Load	0.000129	0.002596	0.026698	0.285736	0.287157	0.356425	0.040083	0.001176
G741R									
	K_{app} = 23	0.000137	0.002561	0.011178	0.391861	0.418379	0.127549	0.046039	0.002296
	3 K_{app} = 69	0.000214	0.004057	0.036646	0.486628	0.208406	0.191487	0.069117	0.003447
	20 K_{app} = 460	0.000328	0.006748	0.196699	0.421612	0.039383	0.243105	0.087749	0.004376
	3 K_{app} + Load	0.000109	0.002079	0.021418	0.288297	0.31256	0.289969	0.083828	0.00174

Table S5. Resolution matrices.

β-WT	K_A	k_{pi}	k_{-T}	K_H	K_{AH}
K_A	0.78958	0.23154	-0.00011	-0.22247	-0.23324
k_{pi}	0.23154	0.73303	0.00010	0.26596	0.26647
k_{-T}	-0.00011	0.00010	0.00000	0.00010	0.00010
K_H	-0.22247	0.26596	0.00010	0.72312	-0.26164
K_{AH}	-0.23324	0.26647	0.00010	-0.26164	0.73076

H251N	K_A	k_{pi}	k_{-T}	K_H	K_{AH}
K_A	0.83786	0.17829	-0.00014	-0.21500	-0.22464
k_{pi}	0.17829	0.79644	0.00015	0.25242	0.25465
k_{-T}	-0.00014	0.00015	0.00000	0.00009	0.00013
K_H	-0.21500	0.25242	0.00009	0.67606	-0.31222
K_{AH}	-0.22464	0.25465	0.00013	-0.31222	0.67846

D382Y	K_A	k_{pi}	k_{-T}	K_H	K_{AH}
K_A	0.72416	0.30169	-0.00006	-0.21910	-0.22924
k_{pi}	0.30169	0.65641	0.00008	0.25910	0.25865
k_{-T}	-0.00006	0.00008	0.00000	0.00007	0.00011
K_H	-0.21910	0.25910	0.00007	0.79385	-0.19167
K_{AH}	-0.22924	0.25865	0.00011	-0.19167	0.80255

F540L	K_A	k_{pi}	k_{-T}	K_H	K_{AH}
K_A	0.62203	0.40143	-0.00012	-0.18730	-0.19048
k_{pi}	0.40143	0.57004	0.00006	0.20191	0.20355
k_{-T}	-0.00012	0.00006	0.00000	0.00021	0.00020
K_H	-0.18730	0.20191	0.00021	0.90401	-0.09509
K_{AH}	-0.19048	0.20355	0.00020	-0.09509	0.90286

P710R	K_A	k_{pi}	k_{-T}	K_H	K_{AH}
K_A	0.93500	0.05948	-0.00008	-0.11846	-0.14659
k_{pi}	0.05948	0.92038	0.00007	0.18357	0.18806
k_{-T}	-0.00008	0.00007	0.00000	0.00003	0.00002
K_H	-0.11846	0.18357	0.00003	0.54305	-0.42043
K_{AH}	-0.14659	0.18806	0.00002	-0.42043	0.53847

V763M	K_A	k_{pi}	k_{-T}	K_H	K_{AH}
K_A	0.71253	0.31248	-0.00011	-0.22232	-0.22899
k_{pi}	0.31248	0.65100	0.00007	0.25277	0.25430
k_{-T}	-0.00011	0.00007	0.00000	0.00012	0.00011
K_H	-0.22232	0.25277	0.00012	0.81263	-0.18252
K_{AH}	-0.22899	0.25430	0.00011	-0.18252	0.81265

R719W	K_A	k_{pi}	k_{-T}	K_H	K_{AH}
K_A	0.57587	0.44828	-0.00007	-0.13908	-0.14345
k_{pi}	0.44828	0.51994	0.00011	0.15156	0.15283
k_{-T}	-0.00007	0.00011	0.00001	0.00012	0.00012
K_H	-0.13908	0.15156	0.00012	0.95001	-0.04746
K_{AH}	-0.14345	0.15283	0.00012	-0.04746	0.95048

R723G	K_A	k_{pi}	k_{-T}	K_H	K_{AH}
K_A	0.68987	0.26278	-0.00015	-0.09877	-0.14615
k_{pi}	0.26278	0.76206	0.00006	0.09866	0.12967
k_{-T}	-0.00015	0.00006	0.00000	0.00009	0.00007
K_H	-0.09877	0.09866	0.00009	0.95055	-0.05082
K_{AH}	-0.14615	0.12967	0.00007	-0.05082	0.92613

G741R	K_A	k_{pi}	k_{-T}	K_H	K_{AH}
K_A	0.60262	0.41547	-0.00016	-0.16199	-0.17080
k_{pi}	0.41547	0.55769	0.00007	0.17552	0.18082
k_{-T}	-0.00016	0.00007	0.00001	0.00016	0.00013
K_H	-0.16199	0.17552	0.00016	0.92789	-0.07077
K_{AH}	-0.17080	0.18082	0.00013	-0.07077	0.92479

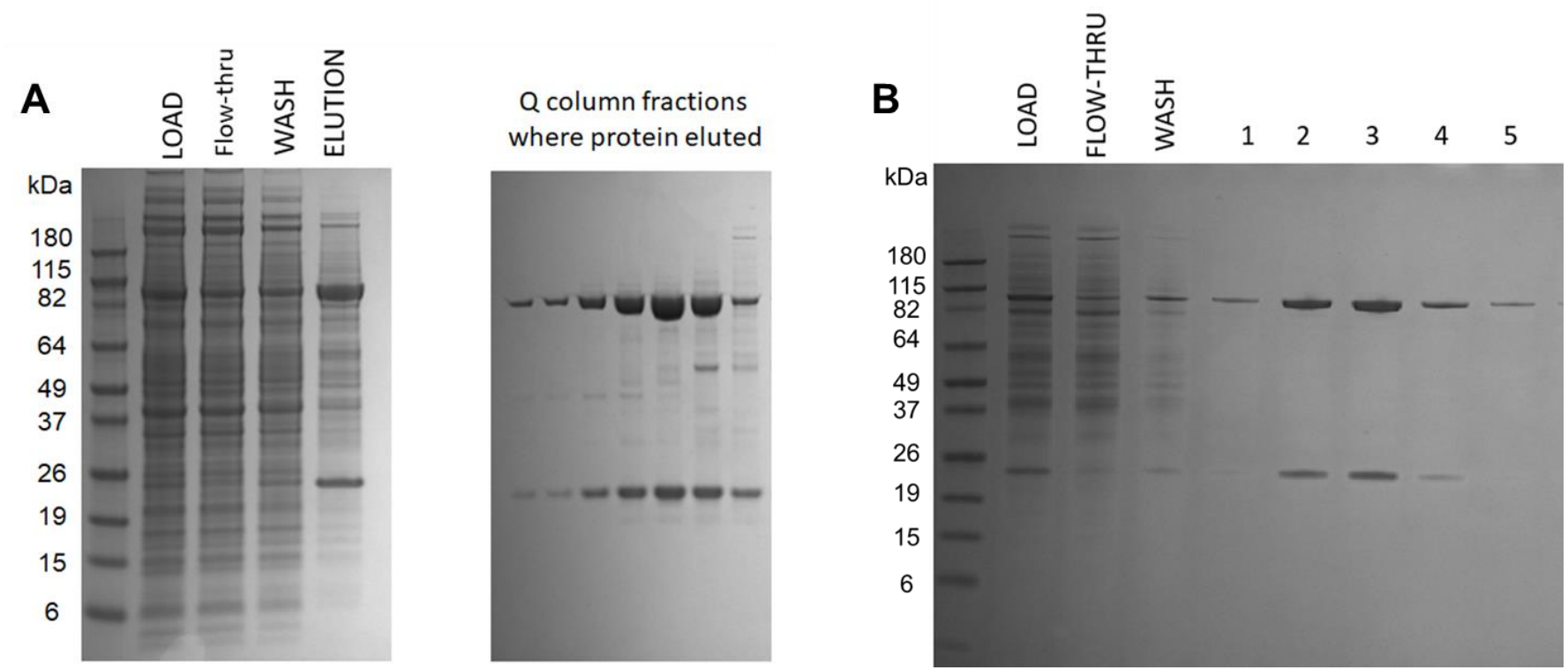

Figure S1. (A) Representative gels from a purification of β-R719W. Left is a His-NTA column by gravity flow starting with the lysate and ending with the imidazole eluted sS1. On the right is part of a gel with the sS1 containing fractions. (B) is a representative gel from a purification of β-V763M. After a gravity His column, the solution was loaded on a PDZ column, where the collected fractions also resulted in clean sS1 (sS1: 93 kDa, MYL3: 25 kDa).

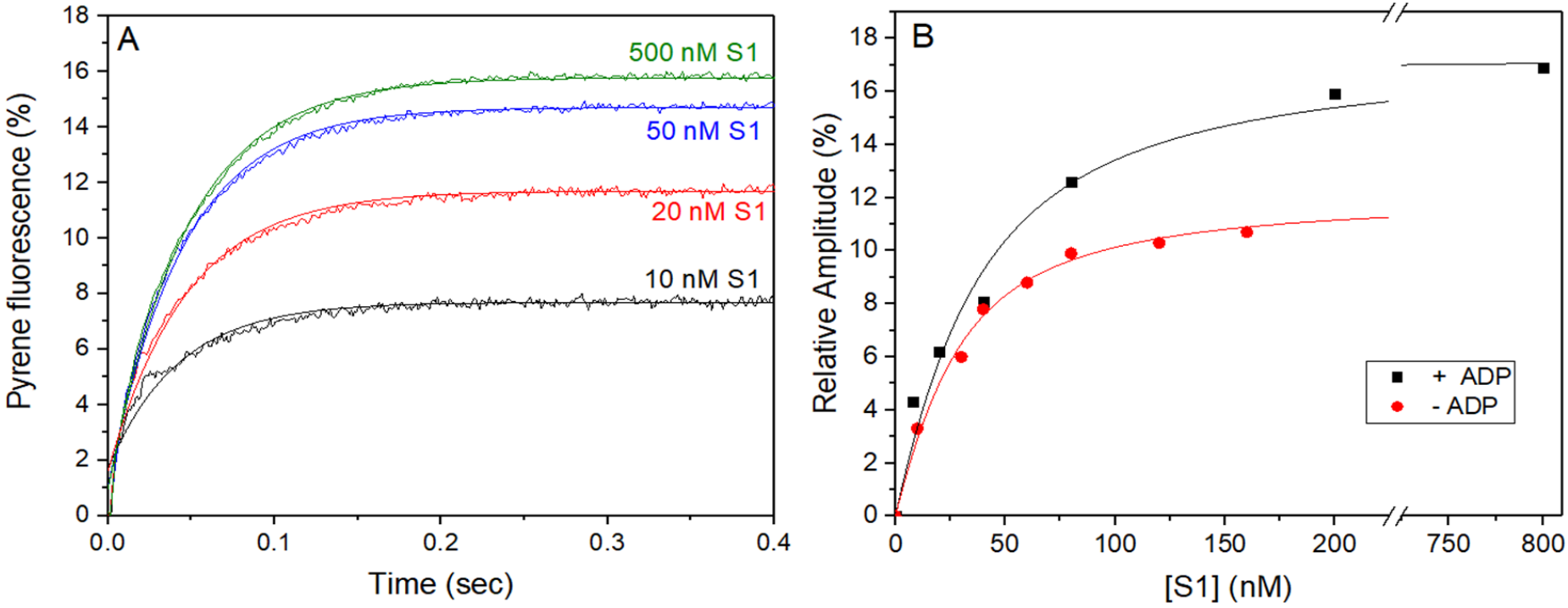

Figure S2. H251N sS1 affinity for actin in the presence and absence of ADP. (A) Example traces of the increase in pyrene fluorescence when 10 μM ATP is mixed with 15 nM pyr-actin, 200 uM ADP and varying concentrations of sS1 (10-500 nM). The pyr-actin was treated with hexokinase and Ap_5A to ensure no ATP was present in the protein before mixing in the stopped-flow. (B) Fluorescence amplitude plotted as a function of sS1 concentration can be described by a quadratic fit resulting in a K_{DA} of 65.6 ± 13 in the presence of ADP, and a K_A of 11.5 ± 1.8 nM when in the absence of ADP. The average values from 3 independent measurements are given in Table 1.

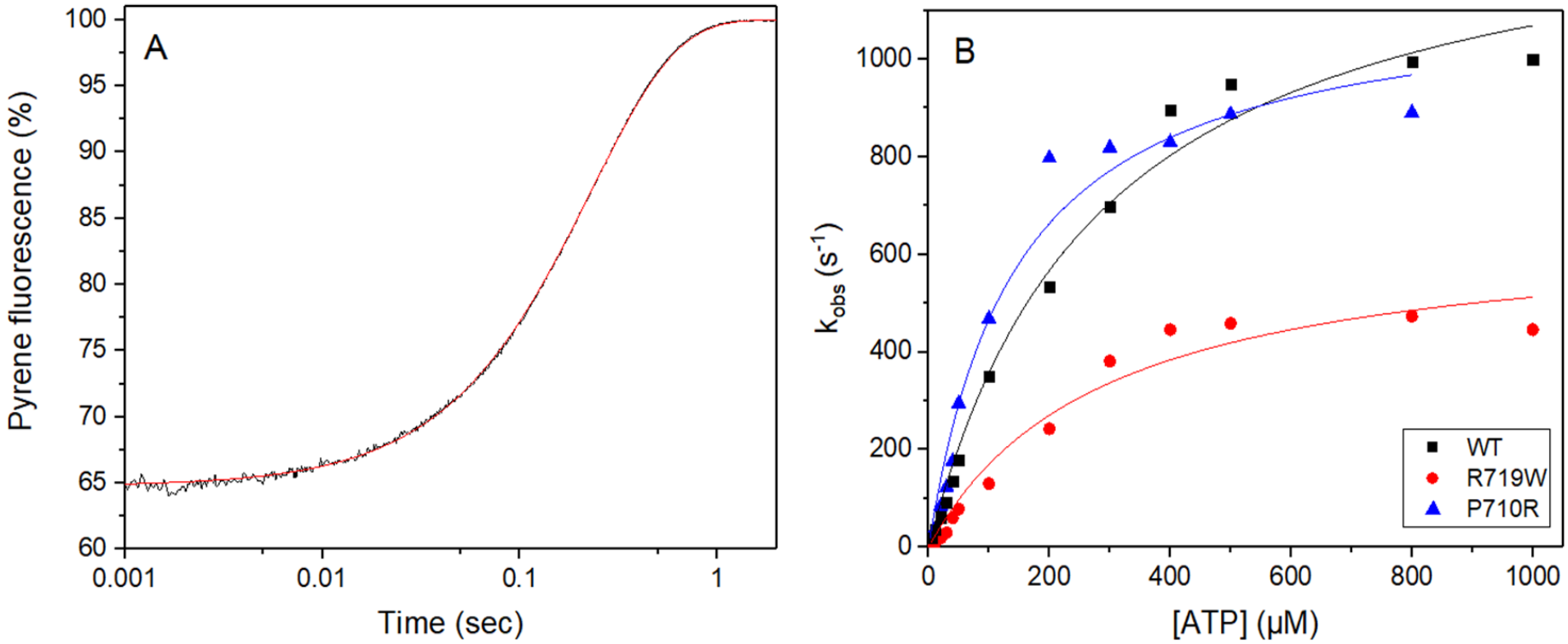

Figure S3. ATP induced-dissociation of acto.sS1. (A) Example stopped-flow trace to measure ATP-induced dissociation of pyr-actin from the WT sS1. 10 μM ATP was mixed with 50 nM pyr-actin.sS1, and the increase in pyrene fluorescence was measured. The experiment was repeated for all constructs and the observed amplitude of the fluorescence changes were similar (within 30%) in each case. (B) The effect of ATP concentration on k_{obs} for ATP-induced dissociation of pyr-actin.sS1 for 3 proteins; WT, R719W and P710R sS1. The gradient of the initial slope generates a second order rate constant of ATP binding, $K_T k_{+T^*}$. The best fit to the hyperbola also yields a maximum $k_{obs} = k_{+T^*}$ and the ATP concentration required for half maximum $k_{obs} = K_T$. Best fit values for 3 independent measurements of all constructs are in Table 1.

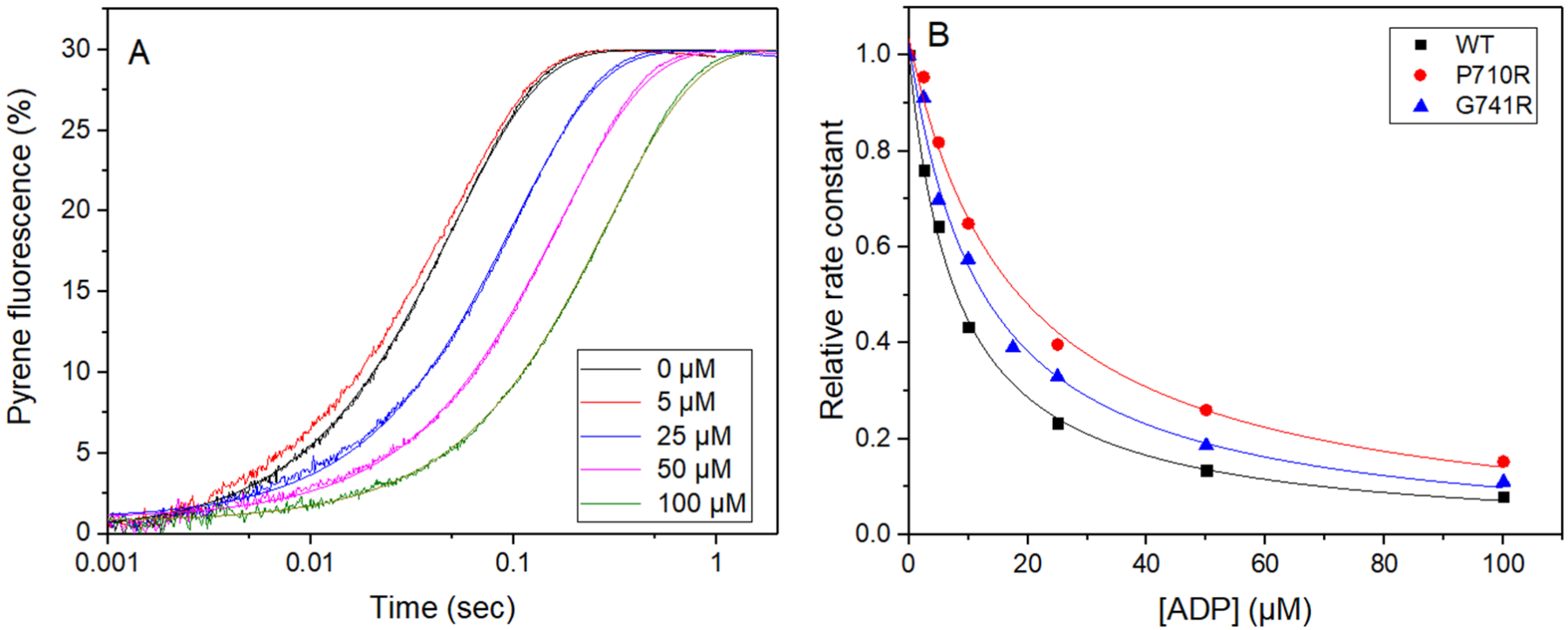

Figure S4. ADP affinity for acto.sS1. (A) Example transients showing an increase in pyrene fluorescence when 100 nM pyr-actin.WT sS1 is mixed with 10 μM ATP and 0-100 mM ADP. (B) k_{obs} plotted as a function of ADP concentration for WT, P710R and G741R sS1 proteins, showing hyperbolic dependence. The value of $((K_{D^*}+1)/K_D K_{D^*})$ is given by the ADP concentration at 50% inhibition.

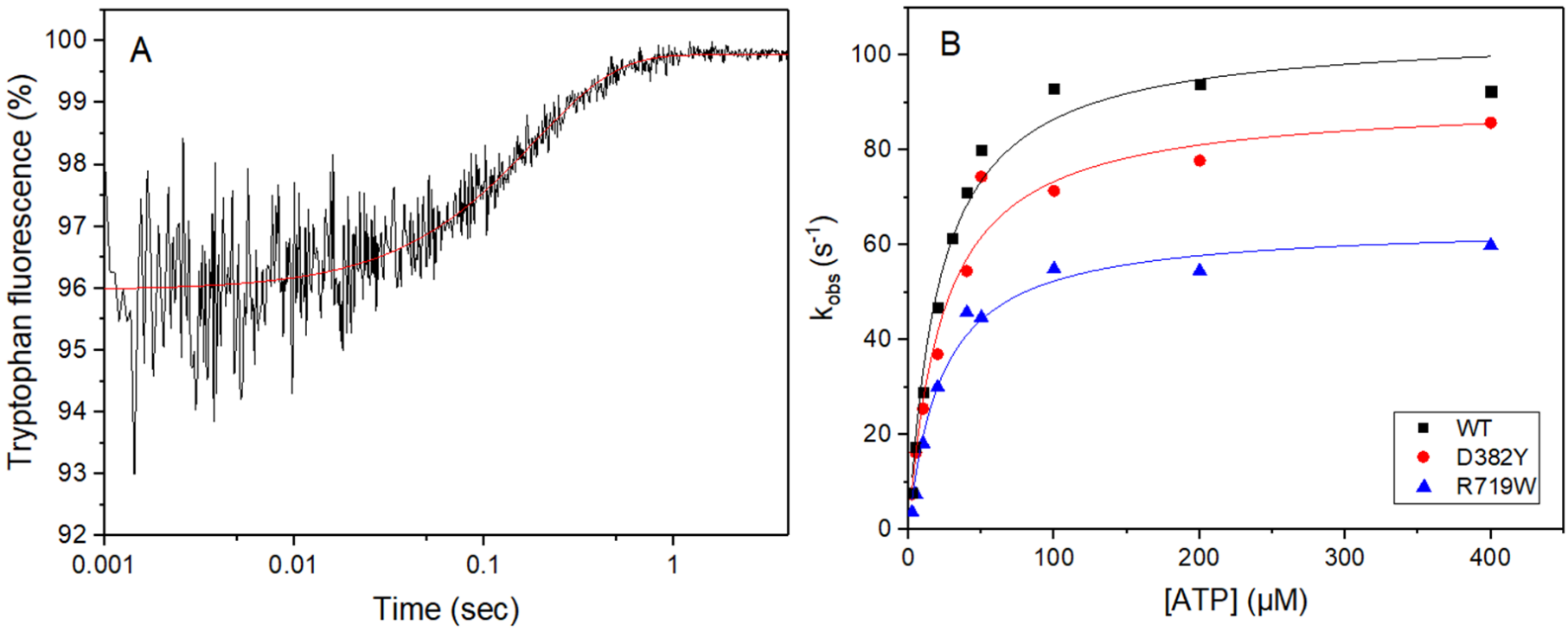

Figure S5. ATP binding to sS1. (A) Example stopped-flow trace to measure ATP binding to WT sS1. 10 μM ATP was mixed with 200 nM sS1, and the increase in tryptophan fluorescence was measured. The sS1 was pre-treated with apyrase to ensure no nucleotide was present before mixing in the stopped-flow. Least squares best fit to a single exponential is shown in red with k_{obs} = 22 s^{-1} and a 4% increase in pyrene fluorescence. (B) The hyperbolic dependence of ATP concentration on k_{obs}. The best fit to a hyperbola yields max k_{obs} = k_{+H} + k_{-H}, and the ATP concentration required for half maximum k_{obs} = $K_{50\%}$. The initial slope defines the second order rate constant of ATP binding to sS1. Best fit values for 3 independent measurements of all constructs are in Table 1.

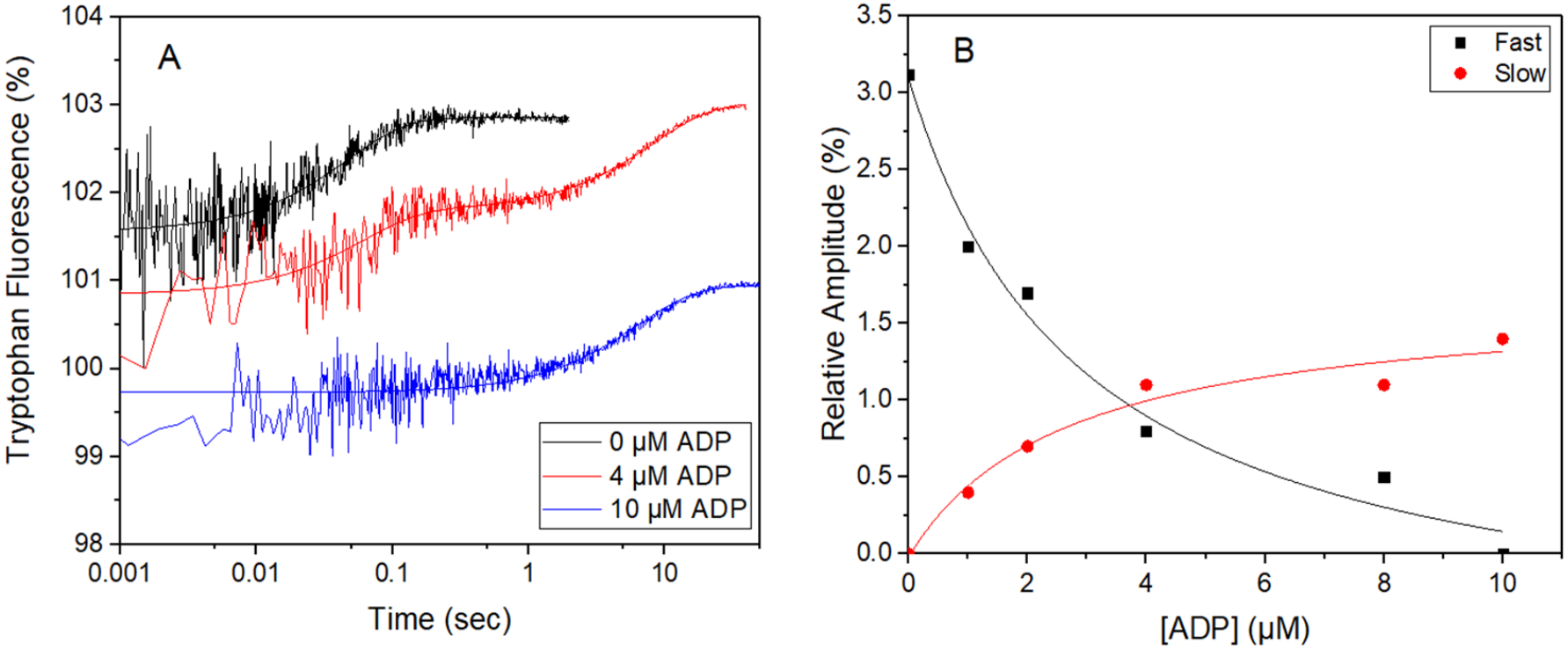

Figure S6. ADP affinity for P710R sS1. (A) Example traces of 50 μM ATP mixed with 200 nM sS1 that is preincubated with ADP. The black trace contains no ADP, and results in a transient best described by a single exponential fit. In the presence of 4 μM ADP, the amplitude of the fast phase decreased as the concentration of free sS1 decreases, and a second slow phase is observed representing the release of ADP from sS1. The transient is best described by a double exponential. At high ADP concentration, the amplitude of the fast phase is lost, and the amplitude of the slow phase can be best described by a single exponential. (B) The relative amplitudes of the fast and slow phases plotted against ADP concentration, which has a hyperbolic dependence. Table 1 lists averages for 3 independent measurements for all constructs.