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Supplemental Information

Five Alternative Myosin Converter Domains Influence Muscle Power, Stretch Activation, and Kinetics

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SUPPLEMENTAL MATERIALS

SUPPLEMENTAL METHODS

Quantification of myosin

Individual thoraces from 2-3 day old *Drosophila* were bisected sagittally and homogenized in 30 µl Laemmli sample buffer on ice. Samples were boiled for 7 minutes and then centrifuged for 1 minute at 10,000 rpm. 10 µl of supernatant was loaded onto a 10% polyacrylamide gel. Gels were stained with Coomassie Blue and the ratio of myosin to actin was analyzed using Image J (National Institutes of Health, Bethesda, Maryland).

EM

Skinned fibers from 3 day old flies were prepared, split and T-clipped as for the mechanics experiments. Individual samples were mounted onto the muscle mechanics rig in EM buffer (pH 6.8, 20 mM MOPS, 10 mM $MgCl_2$, 5 mM NaN_3, 5 mM EGTA, 12 mM MgATP) and stretched until just taut. Temperature was maintained at 10-11°C. The length between the T-clips was measured and the fiber was stretched 10%. EM buffer was completely exchanged with tannic acid fix solution (pH 6.8, same as EM buffer with 0.02% tannic acid), and the fiber fixed for 30 minutes. Fibers were washed with EM buffer, removed from the mechanics rig and transferred to post-fixative solution (pH 6.8, 4% formaldehyde, 2.5% glutaraldehyde in EM buffer without MgATP) for 4 hours at room temperature. Fixed fibers were transferred and stored in EM buffer at 4°C and shipped overnight in EM buffer to the Marine Biological Laboratory (MBL) Central Microscopy Facility. At the MBL, samples were washed twice in EM buffer and once in 0.1M phosphate buffer, pH 6.8, for 10 minutes each. Post-fixation in 1% Osmium in 0.1M phosphate buffer was carried out overnight at 4°C. After three 10 minute washes in 0.1M phosphate buffer,

dehydration was performed sequentially in solutions of 30, 50, 70, 85, 95, and 100 % ethanol for 10 minutes in each solution. Fibers were quickly rinsed in propylene oxide, infiltrated with 50, 75, 85, 95 and 100% Alardite 502/EmBed-812 (EMS cat.#13940) for 4-8 hours, and transferred to a flat embedding mold containing fresh 100% plastic and cured at 60°C for a minimum of two days. The resulting sample blocks were cut on a Reichert-Jung Ultracut E microtome. Thin sections were collected on copper grids (EMS cat.#FCF100H-CU), stained with 2% uranyl acetate in water for 7 minutes followed by Reynold's Lead Citrate for 3 minutes. Sections were imaged using a JEM -200CX JEOL transmission electron microscope.

Optimized work loops

The maximum mechanical power output of IFM fibers at approximate *in vivo* length changes and oscillation frequencies was determined using the work loop technique as previously described (38). In brief, a muscle fiber was oscillated in a sinusoidal pattern starting at 25 Hz and 0.5% amplitude. Frequency was increased by 25 Hz until power output peaked and then started to decrease. Next, the ML amplitude was increased (0.75%, 1%, 1.25% and 1.5% 1.75, 2.0 and 2.25% ML) and frequency again varied to determine maximum power output at the given ML amplitude. Negative work absorbed by the fiber during lengthening (calculated as $W = \int F \mathrm{d}L$, where F is tension and $\mathrm{d}L$ is fiber length change) was subtracted from positive work produced by the fiber during shortening to obtain net work production per cycle. Net work is multiplied by frequency to calculate power.

SUPPLEMENTAL TABLES

	Protein amount relative to *yw*
yw	1.00 ± 0.03 [9]
IFI-11a line 5	**0.97 ± 0.05** [9]
IFI-11a line 6	0.90 ± 0.06 [5]
IFI-11b line 14	**0.93 ± 0.03** [5]
IFI-11b line 6	0.97 ± 0.02 [5]
IFI-11c line 1	**0.96 ± 0.09** [5]
IFI-11c line 26	1.02 ± 0.07 [5]
IFI-11d line 270	**0.93 ± 0.04** [5]
IFI-11e line 3	**0.96 ± 0.06** [5]
IFI-11e line 9	0.99 ± 0.04 [5]
Mhc^{10}	0.13 ± 0.02*[9]

Supplemental Table 1: Myosin expression levels for the two independent transgenic fly lines that were generated for each version of the converter. One Way ANOVA did not identify any statistical differences between the duplicate lines of each converter. Thus, we only used one line in this study. The line identified in bold font was used for EM and muscle mechanics experiments. All values are mean ± S.E.M. Brackets indicate number of fly thoraces tested. *One Way ANOVA with Holm-Sidak pairwise multiple comparisons indicated a significant difference between the amount of myosin present in the Mhc^{10} null line compared to all other lines.

	Flight Index 15°C (0 - 6)	WBF 15°C (Hz)
IFI-11a line 5	**3.18 ± 0.10 [107]**	**156.01 ± 1.42 [23]**
IFI-11a line 6	3.19± 0.18 [106]	152.46 ± 1.71 [18]
IFI-11b line 14	**1.56 ± 0.14 [100]**	**138.84 ± 1.27 [30]**
IFI-11b line 6	1.81 ± 0.15 [125]	136.50 ± 1.83 [27]
IFI-11c line 1	**2.11 ± 0.16 [104]**	**142.53 ± 1.42 [18]**
IFI-11c line 26	2.26 ± 0.16 [108]	148.76 ± 2.84 [16]
IFI-11e line 3	**2.24 ± 0.06 [108]**	**135.33 ± 1.65 [20]**
IFI-11e line 9	2.19 ± 0.06 [115]	133.96 ± 2.94 [15]

Supplemental Table 2: Flight results for the two independent transgenic fly lines that were generated for each version of the converter. Flight index and wing beat frequency (WBF) were measured from 2-3 day old female flies at 15°C. The two lines for each converter were compared to make sure there were no differences in flight characteristics. One Way ANOVA did not identify any statistical differences between the two lines for each converter. IFI-11d lines are not shown in the supplemental tables because two IFI-11d lines were previously generated and shown to be identical (12, 18). The line identified in bold font was used for EM and muscle mechanics experiments. All values are mean ± S.E.M. Brackets indicate number of flies tested.

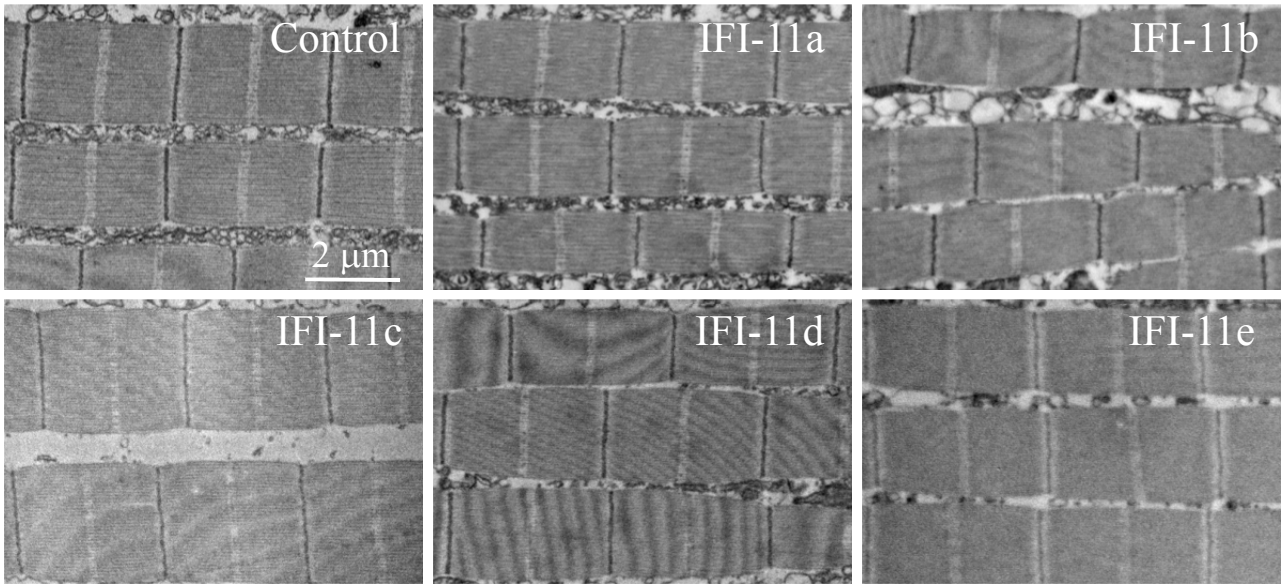
	Power (W/m^3)	f_{max} (Hz)
IFI-11a line 5 [13]	**177 ± 20**	**196 ± 6**
IFI-11a line 6 [9]	200 ± 32	207 ± 6
IFI-11b line 14 [10]	**88 ± 11**	**150 ± 7**
IFI-11b line 6 [6]	73 ± 9	157 ± 5
IFI-11c line 1 [13]	**138 ± 10**	**179 ± 3**
IFI-11c line 26 [8]	125 ± 21	173 ± 7
IFI-11e line 3 [14]	**63 ± 7**	**107 ± 4**
IFI-11e line 9 [11]	65 ± 8	105 ± 6

Supplemental Table 3. Small amplitude power. Power was measured under small amplitude conditions, 0.125% ML, for the two independent transgenic fly lines that were generated for each version of the converter. IFI-11d lines are not shown in the supplemental tables because two IFI-11d lines were previously generated and shown to be identical (12, 18). Values are for the run that generated maximum power. Brackets indicate number of fibers tested. One Way ANOVA did not identify any statistical differences between the two lines for each converter. The line identified in bold font was used for EM muscle mechanics experiments in the main paper.

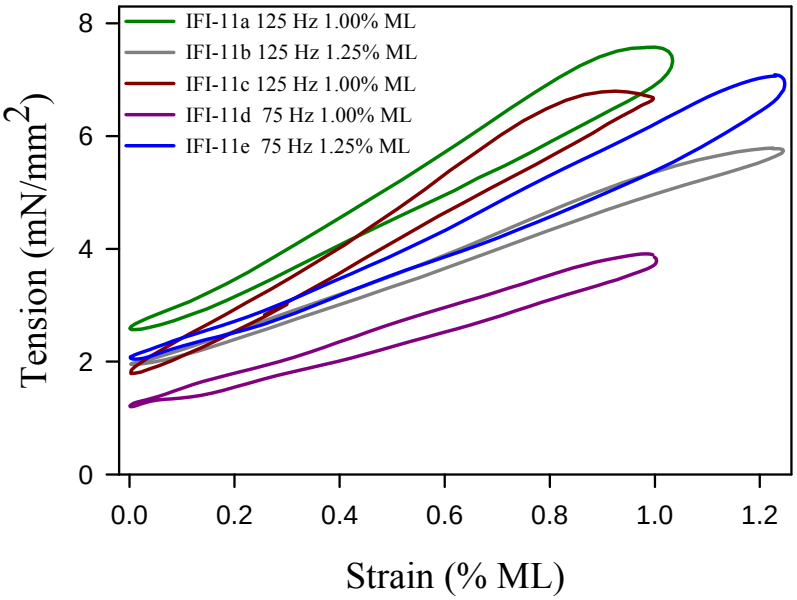
	0.5 ms length step			3.5 ms length step		
	A_{SA}	P_{SA}	F_{SA}	A_{SA}	P_{SA}	F_{SA}
IFI-11a line 5 [13]	**5.59 ± 0.68**	**3.18 ± 0.33**	**2.41 ± 0.39**	**5.49 ± 0.63**	**3.18 ± 0.33**	**2.31 ± 0.33**
IFI-11a line 6 [8]	4.71 ± 0.88	3.26 ± 0.50	1.45 ± 0.39	4.57 ± 0.85	3.28 ± 0.49	1.28 ± 0.36
IFI-11b line 14 [9]	**4.62 ± 0.44**	**2.79 ± 0.27**	**1.83 ± 0.19**	**4.65 ± 0.45**	**2.85 ± 0.28**	**1.80 ± 0.19**
IFI-11b line 6 [5]	4.00 ± 0.44	2.44 ± 0.26	1.57 ± 0.32	3.96 ± 0.44	2.52 ± 0.25	1.46 ± 0.29
IFI-11c line 1 [12]	**4.90 ± 0.43**	**2.69 ± 0.24**	**2.21± 0.31**	**4.70 ± 0.41**	**2.79 ± 0.20**	**1.92 ± 0.23**
IFI-11c line 26 [8]	5.58 ± 0.50	3.50 ± 0.30	2.09 ± 0.35	5.52 ± 0.51	3.45 ± 0.30	2.11 ± 0.31
IFI-11e line 3 [11]	**4.77 ± 0.39**	**2.68 ± 0.33**	**2.08 ± 0.17**	**4.54 ± 0.39**	**2.71 ± 0.34**	**1.83 ± 0.16**
IFI-11e line 9 [11]	4.58 ± 0.80	2.45 ± 0.36	2.17 ± 0.45	4.37 ± 0.73	2.51 ± 0.37	1.90 ± 0.36

Supplemental Table 4. Stretch activated tension for the independent transgenic fly lines for each version of the converter. A_{SA}: total active stretch tension (pCa 5.0), P_{SA}: passive stretch tension (pCa 8.0), F_{SA}: corrected active stretch tension (F_{SA}= A_{SA} - P_{SA}), k_3: rate of tension development for phase 3. All units are mN/mm^2. All values are mean ± S.E.M. Brackets indicate number of fibers tested. One Way ANOVA did not identify any statistical differences between the two lines for each converter. IFI-11d lines are not shown in the supplemental tables because two IFI-11d lines were previously generated and shown to be identical (12, 18). The line identified in bold font was used for mechanics testing in the main paper.

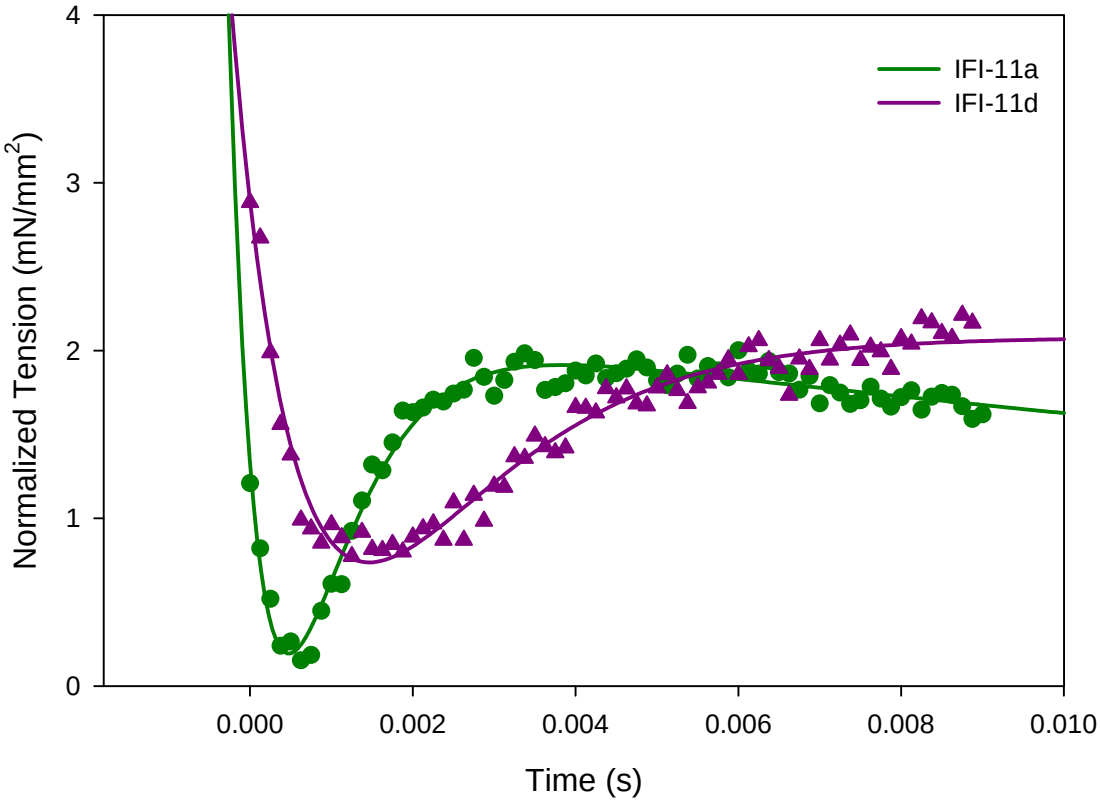
SUPPLEMENTAL FIGURES



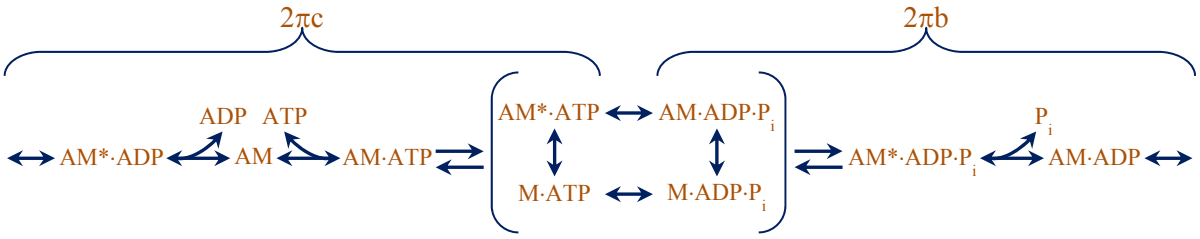
Supplemental Figure 1. IFM Structure. Longitudinal sections of the five converter mutants and control PwMhc2 show normal myofibril and sarcomere ultrastructure. The sections were cut from fixed, skinned IFM fibers. Variations in myofibril diameter are due to the depth at which the individual myofibril was cut. Only remnants of ER and mitochondria are visible due to the removal of membranes during skinning. The additional control, PwMhc2, is a full length transgenic version of *Mhc* (32). Scale bar is 2 μm for all panels.



Supplemental Figure 2. Work loop power measurements. Representative work loops generated by fibers when sinusoidal oscillation frequency and ML change are optimized for maximum power generation. All loops are counterclockwise indicating that all 5 lines produced net positive work and power. The representative IFI-11a fiber produced 7.3 J/m^3 work and 910 W/m^3 power at a frequency of 125 Hz and amplitude of 1.0 % ML. IFI-11b generated 3.3 J/m^3 work and 410 W/m^3 power at 125 Hz and 1.25% ML. IFI-11c generated 5.2 J/m^3 work and 660 W/m^3 power at 125 Hz and 1.0% ML. IFI-11d generated 3.1 J/m^3 work and 230 W/m^3 power at 75 Hz and 1.0% ML. IFI-11e generated 6.1 J/m^3 work and 450 W/m^3 power at 75 Hz and 1.25% ML.



Supplemental Figure 3. Representative fits of two stretch activation tension traces. Phases 2-4 of the corrected active trace of an IFI-11a fiber and an IFI-11d fiber were fitted to the sum of three exponential curves to determine the rate of tension redevelopment following stretch (k3). $F(t)=a2\exp(-k2t) + a3[1-\exp(-k3t)] + a4\exp(-k4t) + C$. Constants a2, a3, a4 are amplitudes; k2, k3 and k4 are rate constants and C accounts for non-zero starting values.



Supplemental Figure 4. **Schematic of the cross-bridge cycle**. The brackets enclose steps of the cycle that collectively set muscle apparent rate constants 2πb or 2πc. Cross-bridges steps that produce work primarily influence 2πb while those that absorb work primarily influence 2πc (See Kawai and Brandt 1980 for a full explanation of the model). Actin is represented by A, myosin by M, and an asterisk indicates a second conformational state. Modified from (40).