Supporting Methods

Verification of the in-focus-by-eye Method by Using the Visibility Index. The mean visibility index, V, (11) was calculated from the raw CCD output of a slack test on a typical non activated myofibril containing 12 extensible sarcomeres between the glass needles, analyzing 11 consecutive frames from the moment of release, each separated by 2 ms:

$$V = \sum_{i=1}^{12} \frac{\left(I_{i,\max} - I_{i,\min}\right)}{\left(I_{i,\max} + I_{i,\min}\right)} \Big/ 12$$

where $I_{i,\max}$ and $I_{i,\min}$ are the maximum and minimum intensities corresponding to the ith sarcomere. The in-focus-by-eye method suggested that the myofibril was first in focus after release at frame 55, which was seen to correspond very well with visibility estimations (Fig. 7); the intercept of the high-V plateau (myofibril in focus, calculated from the mean of the final four V values) with the linear fit between the low-V and high-V plateaus (calculated from the V values of frames 52-55 inclusive) is a measure of the position at which the myofibril is first in focus. This result was extrapolated to be equivalent to frame 55.4. In general, it was concluded that the in-focus-by-eye method agreed with the visibility-index method to within one frame, and was therefore a reliable approach for determining the position at which the myofibril is first in focus.

Modeling the Recoil of I-Band Titin. The motion of I-band titin with extension $x(t)$ and force $F(x(t))$ at a time, t, after release of the stretched sarcomere was treated as a spring-dashpot system characterized by a frictional drag coefficient, g, and pulling a total effective mass, m:

$$F(x) + g\dot{x} + m\ddot{x} = 0.$$

Estimations of the mass of the myofibril suggested that the maximum value of the inertial term was 6-7 orders of magnitude smaller than the other terms and therefore could be neglected, as has been observed for similar biopolymer systems (2). Substituting in the term for fractional extension $z(t) = x(t)/L_c$ in the above expression, where L_c is the total contour length of the I-band titin, implied a simple integral for the total reuptake time t_r:

$$t_r = -\int_{x(0)}^{x(t_r)} \frac{g\,dx}{F(x)} = L_c \int_{z(t_r)}^{z(0)} \frac{g\,dz}{F(z)}$$

For a sarcomere with initial fractional extension, $z(0)$, released by an amplitude, a, the final fractional extension will be $z(t_r) = z(0) - a/2L_c$ because the change in length of I-band titin is the length change of the half-sarcomere. Therefore:

$$t_r = L_c \int_{z(0)-a/2L_c}^{z(0)} \frac{g\,dz}{F(z)} \qquad \textbf{[1]}$$

For the gelsolin-treated data, the drag coefficient was approximated from the Zimm model (3, 4), which assumes that the polymer reptates (3) within a flexible cylinder of diameter, D, [we used a value of 4nm, which fits the dimensions of titin (5)] surrounded by a solvent of intrinsic viscosity η:

$$g \approx \frac{2\pi\eta L_c}{\ln(L_c/D)} \quad ,$$

which is strictly valid for $L_c - x \ll L_c$ and for flow in an infinitely deep chamber (no walls). If solvent flow is confined between two opposed plates, at a depth, h, there is an increase in effective viscosity, η_{eff}, and the approximation becomes:

$$g \approx \frac{2\pi\eta_{eff} L_c}{\ln(L_c/D)} \cong \frac{2\pi\eta L_c}{\ln(4h/\pi D)} \quad ,$$

where $\eta_{eff} = \alpha\eta_{water}$, and α is an appropriate scaling factor; α incorporates not only finite h (the presence of walls) but also changes to the intrinsic viscosity of the solvent compared to water.

The force exerted by the extended I-band titin was modeled as three independent worm-like chains (6) in series, being comprised of the tandem-Ig regions, the N2B unique sequence, and the PEVK region (7):

$$x = \sum_{i=1}^{3} x_i \quad ,$$

where the extension, x_i, at a force, F, of the ith spring satisfies:

$$F = \frac{k_B T}{L_{pi}}\left(\frac{1}{4(1 - x_i/L_{ci})^2} + \frac{x_i}{L_{ci}} - \frac{1}{4}\right) \quad ,$$

where k_B is the Boltzmann constant, T the absolute temperature, with L_{pi} and L_{ci} the persistence and contour lengths, respectively, of the ith spring. Solutions for the equations were found with a standard numerical interpolation technique by using parameters determined from single-molecule force spectroscopy (7). The I-band region of the N2B isoform was assumed to contain 15 proximal Ig domains and 22 distal Ig domains plus four additional Ig's associated with the N2B region (such that the total number of domains was 41) with 572 and 186 residues in the N2B and PEVK unique sequences, respectively (8). The additional quantity of PEVK for the N2BA isoform was assumed to be ~600 residues (8). This quantity allowed an estimate (based on the apparent molecular size of titin on polyacrylamide gels) of ~20 modules for the central Ig domain insertion (8, 9), assuming 10-12 kDa per domain. The total effective force at each given extension was assumed to be the normalized sum of force due to the N2BA and the N2B isoforms predicted from the three-serial worm-like chain model,which was weighted in proportion to their ratio in muscle of 30:70 measured from gel densitometry

(9). The integral of Eq. **1** was solved numerically for different values of a by using an incremental dz value of 10^{-3}.

Fits were generated to the gelsolin-treated data (Fig. 8, or Fig. 5 in the main text) by using a Nelder–Mead Simplex minimization algorithm (10) written in MATLAB to vary the parameter, α. Best fits were generated with $\alpha = 20 \pm 3$, which was consistent with values deduced from diffusivity measurements of several different proteins in muscle fibers (11). For recoil data involving no gelsolin treatment, the Zimm model alone was inappropriate because the total drag is due not only to the viscosity of the surrounding solution on the recoiling titin but also to the interaction between the titin and thin filament systems, most probably through the PEVK region (12). These data were therefore fitted by varying the total drag coefficient by using the same fitting algorithm, with the simplistic assumption that it will be independent of fractional extension, also shown in Fig. 8. Best fits were generated by using a drag coefficient whose equivalent scaling factor in the Zimm model would have an effective value of $\alpha = 37 \pm 4$. Thus, the presence of the thin filaments increases the total drag coefficient by a factor of approximately two.

This model may also be used to predict reuptake times for different ratios of titin-isoform expression, as have been reported in cases of HH disease (9). Increased expression of longer cardiac titin N2BA isoforms, e.g. in end-stage failing hearts from coronary artery disease patients (9), results in a decrease in PT at equivalent sarcomere lengths. As can be seen from the integral of Eq. **1**, this finding implies an increase in reuptake time for equivalent values of release amplitude, and, therefore, a reduction in passive instantaneous recoil velocity.

As a caveat, if I-band titin force generation was modeled as a Hookean spring (only valid for stretches over a small range of z), namely $F(x) = k_m x$, where k_m is the molecular stiffness, Eq. **1** can be solved exactly:

$$a(t_r) = a(\infty)(1 - \exp(-k_m t_r / g))$$

and, therefore, a (single exponential plus constant) fit is valid.

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