## **Supporting Information**

## Shimamoto et al. 10.1073/pnas.0813288106



**Fig. S1.** Frequency of sarcomeres that "yield" against various loading rates at ADP activation. (*A*) The distributions of  $\Delta SL$  at various loading rates. The loading rate is shown in each histogram. (*B*) The proportion of "yielding" sarcomeres against the loading rate. The sarcomeres with  $\Delta SL$  over 150 nm were classified as "yielding" (distributions to the right of the dashed line in *A*). The curve was drawn by eye. Data were obtained from 3 myofibrils at  $\Delta P$  approximately 0.1  $P_0$ .



**Fig. S2.** A typical response observed at maximal Ca<sup>2+</sup>-activation. The responses of tension (*Top*), total length of the myofibril (*Middle*), and length of individual sarcomeres (*Bottom*) to  $\Delta P = 0.15 P_0$  are shown. The myofibril was stretched at time 0. The loading rate was approximately 0.02  $P_0$ /ms. Out of 28 sarcomeres in which A-bands were clearly observable in the phase-contrast image, 10 consecutive sarcomeres in the central part of the myofibril were analyzed. The cross-sectional area of the myofibril was determined to be 2.58  $\mu$ m<sup>2</sup>.



**Fig. S3.** A typical response under relaxing conditions. The responses of tension (*Top*), total length of the myofibril (*Middle*), and length of 15 individual sarcomeres (*Bottom*) to  $\Delta P = 2.7 P_0$  are shown. The loading rate was approximately 0.04  $P_0$ /ms. The myofibril was stretched at time 0. The cross-sectional area of the myofibril was determined to be 3.51  $\mu$ m<sup>2</sup>.

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Fig. S4. Relationship between the initial and the maximal SLs following stretch at ADP activation. The maximum SLs following stretch were plotted against their initial SLs. The dashed line corresponds to the same SL before and after the stretch. Colors correspond to each stretch of the untreated myofibril in Fig. 4A.





**Fig. S5.** Localization of the anti- $\alpha$ -actinin antibody at the Z-line. (A) The phase-contrast image (green) and the fluorescence image of Alexa555-Fab fragment conjugated to an anti- $\alpha$ -actinin antibody (red) are merged. (Scale bar, 10  $\mu$ m.) (B) The intensity profile of a part of the myofibril in A. Colors correspond to those in A.

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**Fig. S6.** A typical response of sarcomeres treated with anti- $\alpha$ -actinin antibody at ADP activation. The responses of tension (*Top*), total length of the myofibril (*Middle*), and length of individual sarcomeres (*Bottom*) to  $\Delta P = 0.15 P_0$  are shown. Data correspond to the first stretch of the antibody-treated myofibril in Fig. 4A. For clarity, only the responses of sarcomeres with even numbers are shown. The myofibril was stretched at time 0. The cross-sectional area of the myofibril was determined to be 3.94  $\mu$ m<sup>2</sup>.

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**Movie S1.** Response of sarcomeres to  $\Delta P = 0.02 P_0$  at ADP activation. This movie corresponds to Fig. 2*A*. The needles on the left and right were the stiff and the flexible, respectively.

Movie S1

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Movie S2

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Movie S3

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