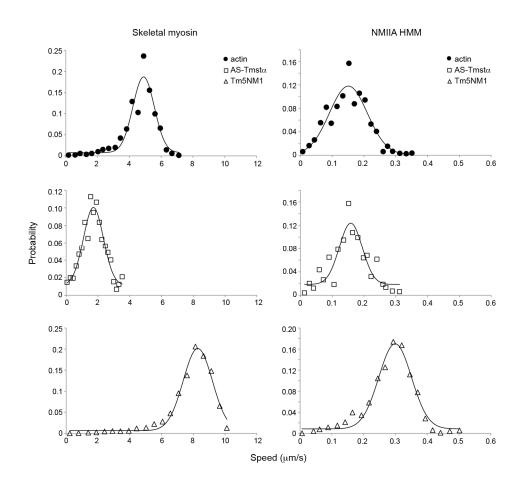
## **Supporting Information for:**

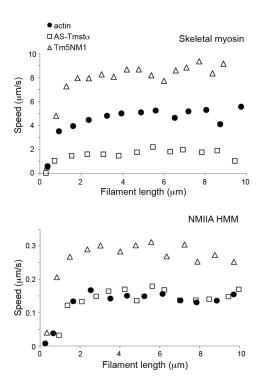
## Regulation of nonmuscle myosin II by tropomyosin

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**FIGURE S1**. Velocity probability distributions for actin, actin-AS-Tmst $\alpha$  and actin-Tm5NM1 on skeletal myosin and NMIIA HMM. Each plot is from a representative experiment. The data was fit to a Gaussian distribution to determine the mean speed  $\pm$  S.D. for each experiment.



**FIGURE S2**. The speeds of individual actin, actin-AS-Tmst $\alpha$  and actin-Tm5NM1 filaments on skeletal myosin and NMIIA HMM as a function of filament length. Each plot is from a representative experiment. The speed is independent of filament length except for very short filaments (<1  $\mu$ m).

## **MOVIE LEGENDS**

**MOVIE S1**. *In vitro* motility assays for actin (A), actin-AS-Tmst $\alpha$  (B) and actin-Tm5NM1 (C) filaments moving on skeletal myosin (40 µg/ml). The video shows the first 500 frames out of a total of 1000 frames at 2x speed (30 fps) of the original recording (15 fps).

**MOVIE S2**. *In vitro* motility assays for actin (A), actin-AS-Tmst $\alpha$  (B) and actin-Tm5NM1 (C) filaments moving on NMIIA HMM (60 µg/ml). The video shows the first 500 frames out of a total of 1000 frames at 5x speed (30 fps) of the original recording (6 fps).