Human Myosin VIIa Is a Very Slow Processive Motor Protein on Various Cellular Actin Structures

Osamu Sato[*], Satoshi Komatsu[*], Tsuyoshi Sakai[*], Yoshikazu Tsukasaki[*†], Ryosuke Tanaka[‡], Takeomi Mizutani[§], Tomonobu M. Watanabe[‖], Reiko Ikebe[*], and Mitsuo Ikebe[*]

From [*]Department of Cellular and Molecular Biology, University of Texas Health Science Center at Tyler, Tyler, Texas 75708; [†]Department of Pharmacology, University of Illinois College of Medicine, Chicago, Illinois 60612; [‡]Graduate School of Information Science and Technology, Hokkaido University, Sapporo 060-0814, Japan; [§]Department of Advanced Transdisciplinary Sciences, Faculty of Advanced Life Science, Hokkaido University, Sapporo 060-0810, Japan; [‖]Laboratory for Comprehensive Bioimaging, RIKEN Quantitative Biology Center, Suita, Osaka 565-0874, Japan.

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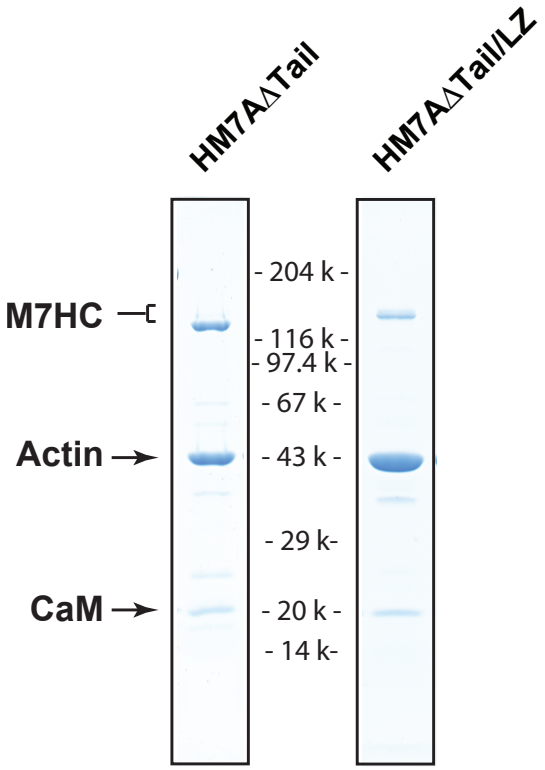
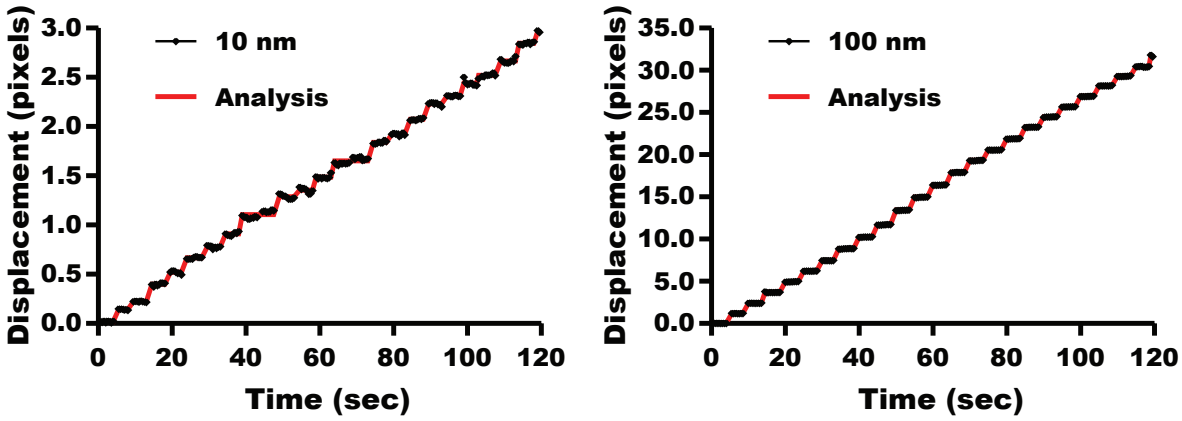


FIGURE S1. Calmodulin content of myosin VIIa. To determine the numbers of CaM bound to HM7AΔTail and HM7AΔTail/LZ, we co-precipitated HM7AΔTail or HM7AΔTail/LZ with F-actin, and the pellets were subjected to SDS-PAGE as described in "Experimental Procedures." Shown are typical SDS-PAGE images of HM7AΔTail and HM7AΔTail/LZ (stained with Coomassie Brilliant Blue G-250). The positions of M7HC (HM7AΔTail or HM7AΔTail/LZ heavy chain), actin, and CaM bands were shown in the left. The positions of molecular weight markers were shown between the gel images.

A



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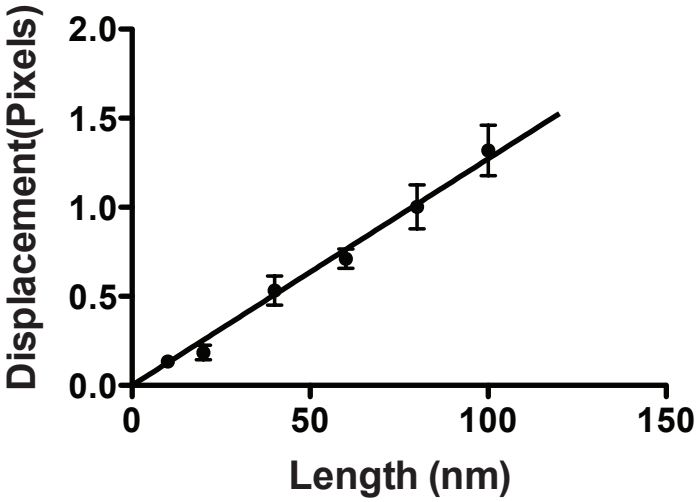

FIGURE S2. Calibration of TIRF microscope. (A) Typical data for the stage calibration. **(Left)** 10 nm and **(Right)** 100 nm were shown. The position of FluoSpheres sulfate (0.2 μm) fluorescence was captured with TIRF microscope, and repeatedly moved the Proscan III stage at 10 nm and 100 nm with 5 seconds interval. **(B)** Length vs. pixel relationship. Experiment was done as in A at 10 nm (n=22), 20 nm (n=18), 40 nm (n=23), 60 nm (n=23), 80 nm (n=24), and 100 nm (n=24) and pixel vs. length relationship was plotted. The error bars are s.d.

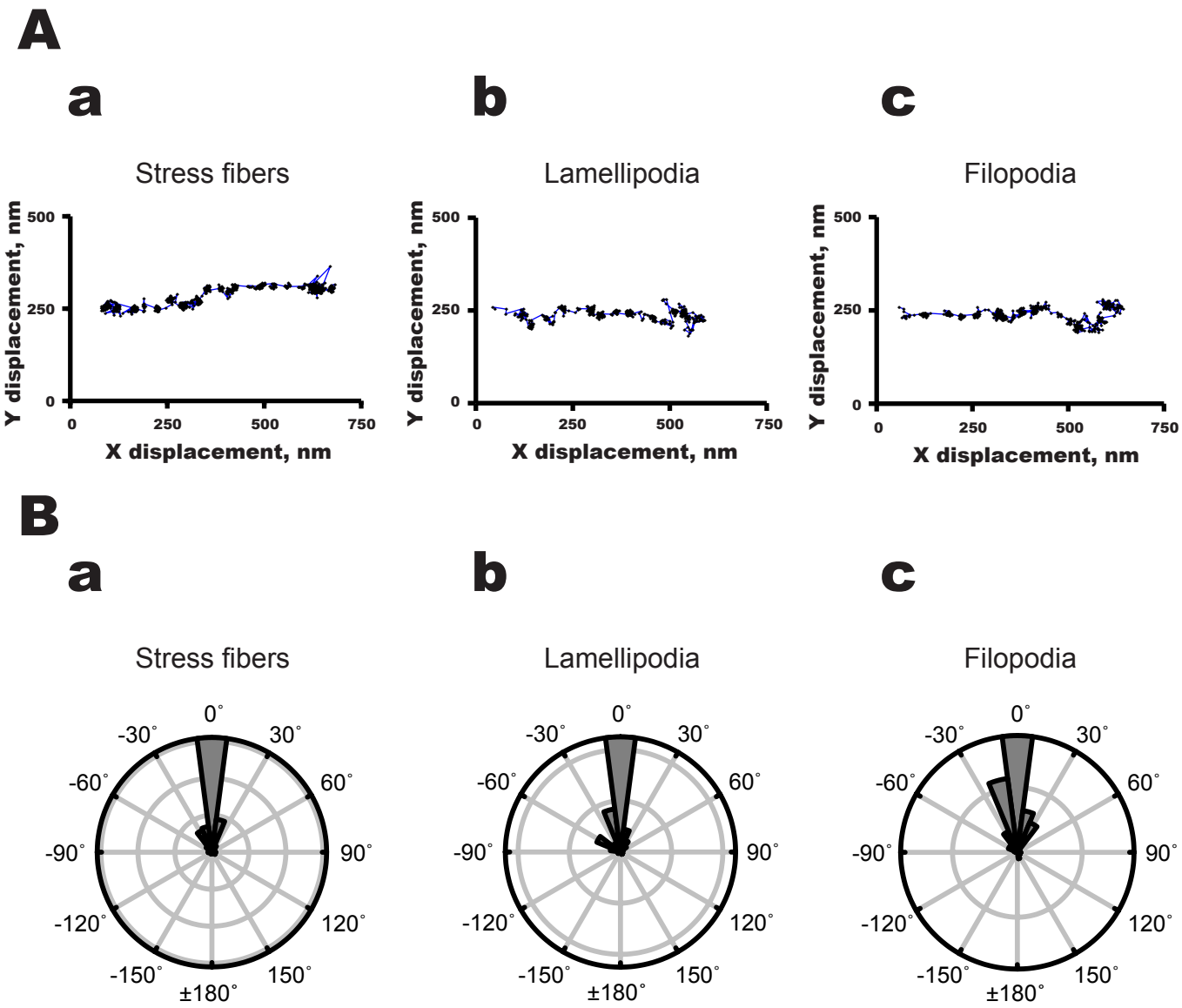

FIGURE S3. Stepping orientation of myosin Va movements. (A) The typical stepping traces of myosin Va movement in **(a)** stress fibers, **(b)** lamellipodia, and **(c)** filopodia. **(B)** The polar plots of the individual stepping orientation of myosin Va HMM on **(a)** stress fibers, **(b)** lamellipodia and **(c)** filopodia. The stepping orientation of mouse myosin Va was measured and plotted as described in "Experimental Procedures." The 0º is the orientation of individual myosin Va steppings, parallel to the moving direction.

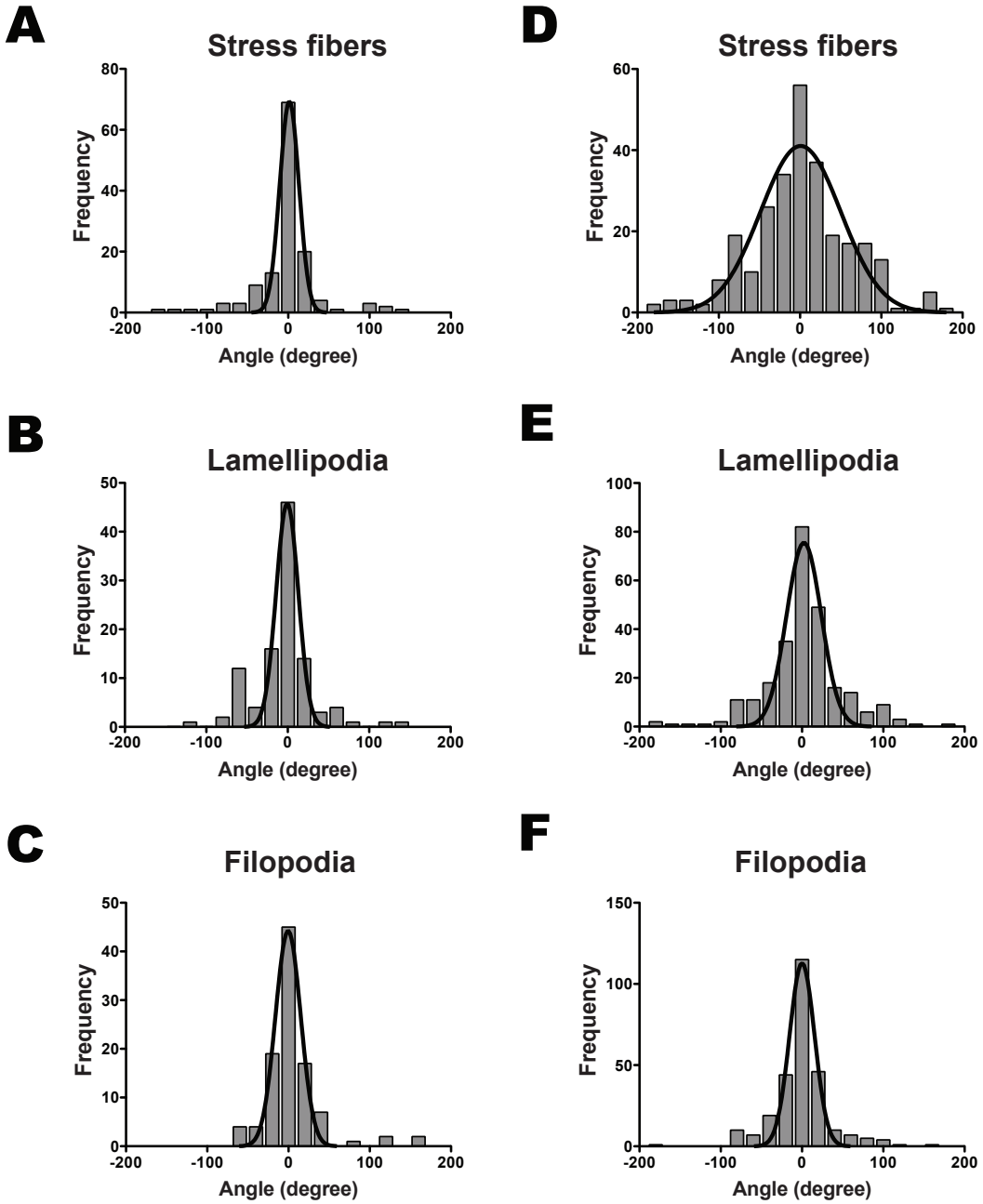

FIGURE S4. Histograms of stepping orientation of myosin Va and VIIa. (A–C) The histograms of stepping orientation of myosin Va HMM on **(A)** stress fibers, **(B)** lamellipodia and **(C)** filopodia. Data in **Fig. S3** were re-plotted to calculate standard deviation. The histogram showed –3.4 ± 40.5º (mean ± s.d., n=132), –5.6 ± 36.7º (n=105) and 2.5 ± 36.1º (n=101) for stress fibers, lamellipodia and filopodia, respectively. The solid lines were best fit to Gaussian distributions. **(D–F)** The histogram of stepping orientation of HM7AΔTail/LZ on **(D)** stress fibers, **(E)** lamellipodia and **(F)** filopodia. Data in **Fig. 5C** were re-plotted, and the histogram showed –1.0 ± 61.8º (mean ± s.d., n=274), 0.5 ± 47.3º (n=263) and –1.9 ± 35.4º (n=270) for stress fibers, lamellipodia and filopodia, respectively.