Tang et al., http://www.jcb.org/cgi/content/full/jcb.201511102/DC1

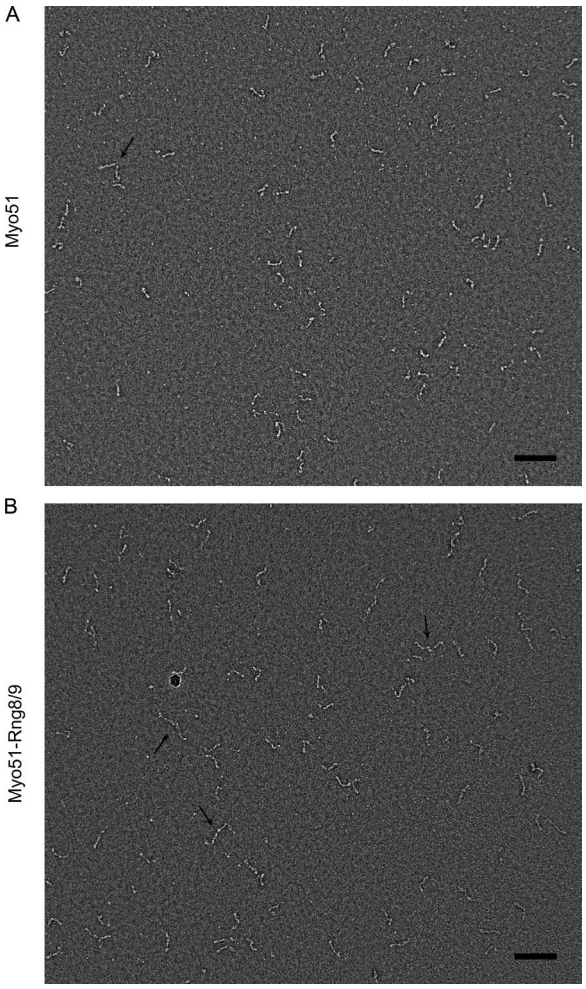


Figure S1. **Myo51 and Myo51–Rng8/9 are single-headed motors as visualized by EM.** Representative fields of negatively stained EM images of Myo51 (A) and Myo51–Rng8/9 (B). Arrows indicate occasional structures that resemble dimeric class V myosin. Bars, 100 nm.

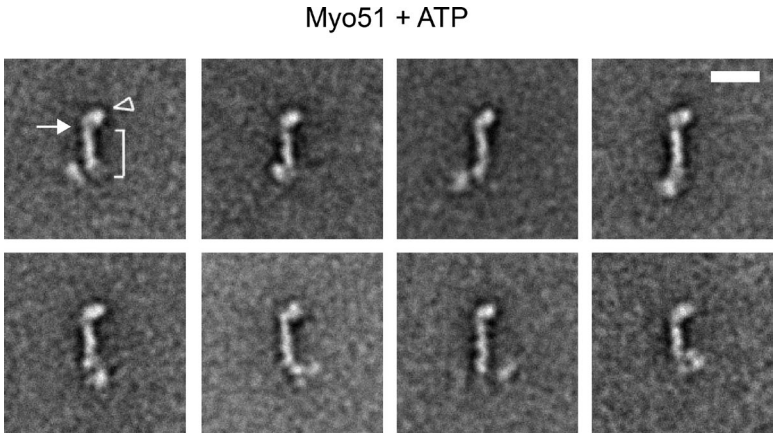


Figure S2. **Representative class average images of Myo51 in the presence of MgATP.** Open triangle, motor domain; single bracket, lever arm. Arrow points to the bend between the globular motor domain and the lever arm. Bar, 20 nm.

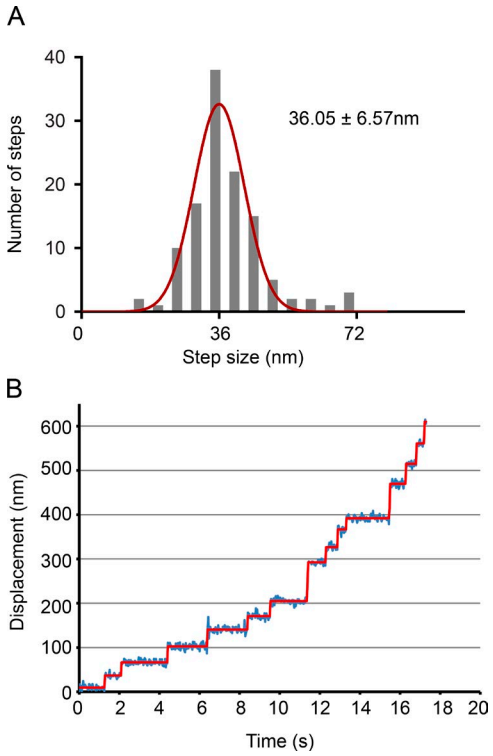


Figure S3. **Step size of vertebrate myoVa-HMM attached by a C-terminal biotin tag to a streptavidin-Qdot.** (A) Histogram of step sizes taken by MyoVa-HMM at 1 µM MgATP. The mean step size ± SD (36.02 ± 6.57 nm) was obtained by fitting to a Gaussian distribution (red curve). *n* = 118. (B) A representative displacement of MyoVa stepping versus time. Red line, the step fit to the displacement trace.

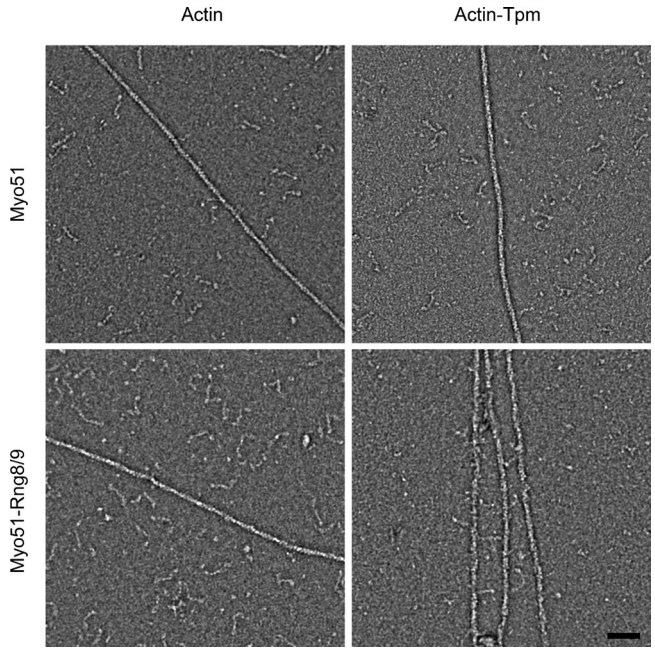
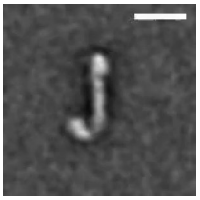
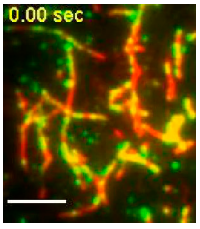


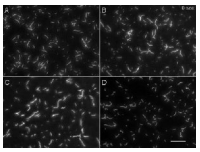
Figure S4. **Representative EM images of Myo51 or Myo51–Rng8/9 with actin or actin-Tpm in the presence of ATP.** Bar, 50 nm.



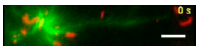
Video 1. **Negatively stained EM image series showing Myo51 motor domain bends relative to the lever arm upon addition of MgATP.** Arrow indicates the position of the bend. Bar, 20 nm. The movie is played at ten frames per second (fps).



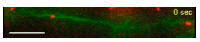
Video 2. **Multiple Myo51–Rng8/9 motors bound to a Qdot do not move on actin-Tpm tracks.** Qdots, green; actin-Tpm, red. Bar, 5 μm. The movie was captured at 20 fps and is played at 20 fps.



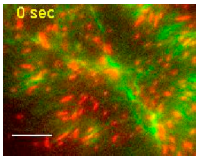
Video 3. **A four-panel movie of actin or actin-Tpm gliding motility by Myo51 or Myo51–Rng8/9 recorded at 1 fps.** The myosins were attached to the neutravidin-coated nitrocellulose surface via their C-terminal biotin tag. (A) Myo51, actin. (B) Myo51, actin-Tpm. (C) Myo51–Rng8/9, actin. (D) Myo51–Rng8/9, actin-Tpm. Bar, 12 μm. The movie is played at 8 fps.



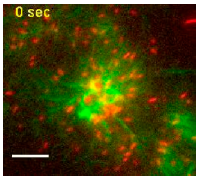
Video 4. **A red actin-Tpm filament traveled along a green actin-Tpm bundle, propelled by Myo51–Rng8/9 in a dual-color motility assay.** The filament paused and resumed motility on the motility trajectory. The movie was captured at 1 fps. The layer of green actin-Tpm adhered to the nitrocellulose surface formed bundles with Myo51–Rng8/9. Bar, 5 μm. The movie is played at 12 fps.



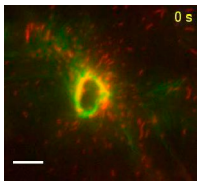
Video 5. **A red actin-Tpm filament traveled along a green actin-Tpm bundle, propelled by Myo51–Rng8/9 in a dual-color motility assay.** The movie was captured at 1 fps. The layer of green actin-Tpm adhered to the nitrocellulose surface formed bundles with Myo51–Rng8/9. Bar, 10 μm. The movie is played at 10 fps.



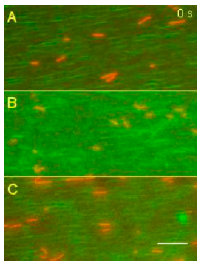
Video 6. **Multiple red actin-Tpm filaments glide toward and along the center of green actin-Tpm bundles, propelled by Myo51–Rng8/9 in a dual-color motility assay.** The movie was captured at 1 fps. The red actin-Tpm filaments were less motile or static (potentially cross-linked with the green actin-Tpm) at the edge of the bundles but highly motile at the center. Bar, 5 μm. The video is played at 10 fps.



Video 7. **Multiple red actin-Tpm filaments glide toward and along the center of green actin-Tpm bundles, propelled by Myo51–Rng8/9 in a dual-color motility assay.** The movie was captured at 1 fps. The red actin-Tpm filaments were less motile or static (potentially cross-linked with the green actin-Tpm) at the edge of the bundles but highly motile at the center. Bar, 5 μm. The video is played at 10 fps.



Video 8. **Multiple red actin-Tpm filaments glide toward and along the center of green actin-Tpm bundles, propelled by Myo51–Rng8/9 in a dual-color motility assay.** The movie was captured at 1 fps. The red actin-Tpm filaments were less motile or static (potentially cross-linked with the green actin-Tpm) at the edge of the bundles but highly motile at the center. Bar, 5 µm. The video is played at 8 fps.



Video 9. **A three-panel movie of dual-color motility by Myo51 or Myo51–Rng8/9 captured at 1 fps.** The green layer of actin or actin-Tpm was adhered to the nitrocellulose-coated coverslip. (A) Myo51, actin. (B) Myo51, actin-Tpm. (C) Myo51–Rng8/9, actin. Bar, 12 µm. The movie is played at 10 fps.