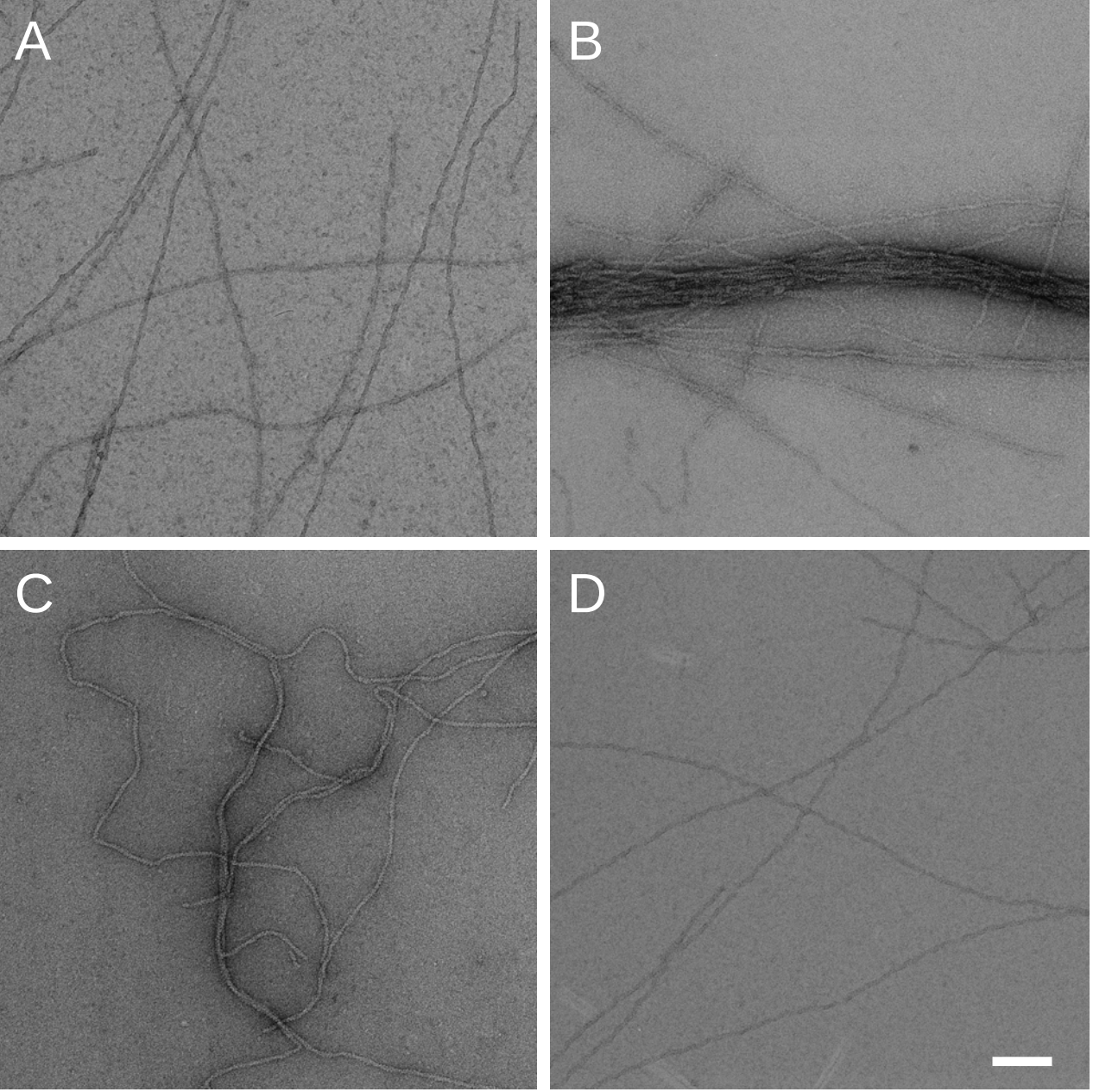

Supporting Figure for Smirnov et al., HP67 does not bundle F-actin filaments. All samples contained 5 uM F-actin. Isolated villin headpiece (HP67, 5 uM) was incubated with F-actin at a 1:1 molar ratio in F-buffer either with or without 5 mM calcium chloride. As a positive control to insure actin was active for bundling, F-actin was incubated with 10 uM D6-HP (1:2 molar ratio) in F-buffer with 5 mM calcium chloride. After incubation for 45 min at room temperature, all samples were loaded on carbon-coated and glow-discharged copper grids (SPI Supplies) for 1min, washed with 10 drops buffer containing 5 mM Tris, 50 mM sodium chloride at pH 7.5 (with an additional 5 mM calcium chloride if the sample mixture has it), and then stained with 1% uranyl acetate. All samples were imaged on a Philips CM12 transmission electron microscope operated at 120 kV with LaB6 filament and recorded on SO-163 EM (Kodak) film at 45,000 X magnification under minimal electron dose conditions. The film was processed with undiluted Kodak D-19 developer for 12 min and Kodak rapid fixer for 5 min. Electronmicrographs were digitized on a Creo IQ Smart2 Scanner (GlobalImaging) at 1270 dpi.

A. F-actin in F-buffer + 5 mM Ca
B. D6-HP w/ F-actin in F-buffer 5 mM Ca
C. HP67 w/ F-actin in F-buffer
D. HP67 w/ F-actin in F-buffer + 5 mM Ca

Magnification bar = 100 nm