

### Description of Additional Supplementary Files

File Name: Supplementary Movie 1

Description: **An HFF labeled with a low dose of SiR-actin.** Left: the whole cell is imaged every 2 s for 120 s total. The scale bar is 10  $\mu\text{m}$ . Right: the two boxed regions of the cell on the left, magnified 6x. The scale bar is 1  $\mu\text{m}$ .

File Name: Supplementary Movie 2

Description: **Speckle tracking by QFSM and subpixel localization.** An HFF labeled with SiR-actin, imaged every 2 s for 120 s total, overlaid with tracked speckles from QFSM (circles). Localizations which pass subpixel localization are shown in green; those that fail are shown in magenta (see Methods, Actin tracking analysis for details). Video has been denoised using noise2void.

File Name: Supplementary Movie 3

Description: **A HUVEC labeled with a low dose of SiR-actin.** The labeled cell is imaged every 2 s for 3 min 16 s total. The scale bar is 10  $\mu\text{m}$ .