Fig. S1. The stiffness of F-actin networks can be tuned by varying the number of motors or cross-linkers per actin filament. These parameters can be tuned either by variation of [FLNa] and [myosin] at a fixed filament length (blue circles) or by variation of filament length at fixed concentrations of actin, FLNa, and myosin (green squares). The average filament length is controlled by varying the concentration of gelsolin, which nucleates and caps actin filaments. The actin concentration is 23.8 µM, and the ATP concentration is 5 mM.
Fig. S2. The elasticity of active F-actin networks increases strongly with increasing actin concentration, both in the case of long filaments (blue circles; no gelsolin, 15-μm average length) and in the case of short filaments (red squares; [actin]/[gelsolin] = 555, 1.5-μm average length). The molar ratios [FLNa][actin] = 0.010 and [myosin][actin] = 0.020 are fixed, and the ATP concentration is 5 mM.
Electron microscopy images of reconstituted F-actin networks reveal signatures of contractile foci. Contractile foci remain on the bottom coverslip in areas where the surrounding F-actin network has detached (23.8 μM actin, [myosin]/[actin] = 0.02, [FLNa]/[actin] = 0.005). (Scale bar: 1 μm.)
Fig. S4. Internal prestress and estimated force level, calibrated by comparison with passive networks under a constant external shear stress. Plot on the left shows dependence on the molar ratio of myosin to actin for two FLNa concentrations as indicated; plot on the right shows dependence on the molar ratio of FLNa to actin for two myosin concentrations as indicated. Right y axis displays corresponding force level (assuming isotropic application of stress by myosin), and top x axis displays number of myosin thick filaments or FLNa dimers per actin filament.
Fig. S5. Myosin activity has little or no stiffening effect on networks rigidly cross-linked by scrin. (A) Myosin (at a molar ratio to actin of 0.02) has a maximal stiffening effect of a factor 2 for the highest [scrin]/[actin] ratio of 0.13 where actin–scrin networks still display no bundles. [Actin] = 23.8 μM, and [ATP] = 5 mM. Solid symbols are storage modulus; open symbols are loss modulus. (B) Corresponding stress-stiffening curves for the active and passive actin–scrin network.