Vignjevic et al., http://www.jcb.org/cgi/content/full/jcb.200603013/DC1

Supplemental materials and methods

Fascin-binding assay

B16F1 cells were extracted in extraction solution (1% Triton X-100, 4% polyethylene glycol [40,000 kD], 100 mM PIPES, pH 6.9, 1 mM $MgCl_2$, and 1 mM EGTA) supplemented with 2 μM Alexa Fluor 488–phalloidin (Invitrogen) for 2 h at room temperature. Bacterially expressed fascin proteins (WT, S39A, and S39E) were purified as previously described (Vignjevic et al., 2003, 2006) and applied to the extracted cells at a final concentration of 1.5 μM. After incubation for 1 min, the samples were fixed with 0.2% glutaraldehyde for 20 min and treated with $NaBH_4$. The subsequent methanol treatment and antibody reaction were described in the Materials and methods section. TRITC-conjugated anti–mouse IgG (Jackson ImmunoResearch Laboratories) was used as the secondary antibody.

Fascin-bundling assay

12.5 μM F-actin was incubated with 1.2 μM of purified fascin in KME buffer (50 mM KCl, 10 mM imidazole-HCl, pH 7.0, 1 mM $MgCl_2$, and 1 mM EGTA) for 1 h at room temperature. For sedimentation assays, the reactions were centrifuged at 12,000 *g* for 10 min. The resulting supernatants and pellets were analyzed by SDS-PAGE. For fluorescence microscopic assay, tetramethylrhodamine-labeled actin was incorporated into F-actin. The reaction was observed as previously described (Vignjevic et al., 2003).