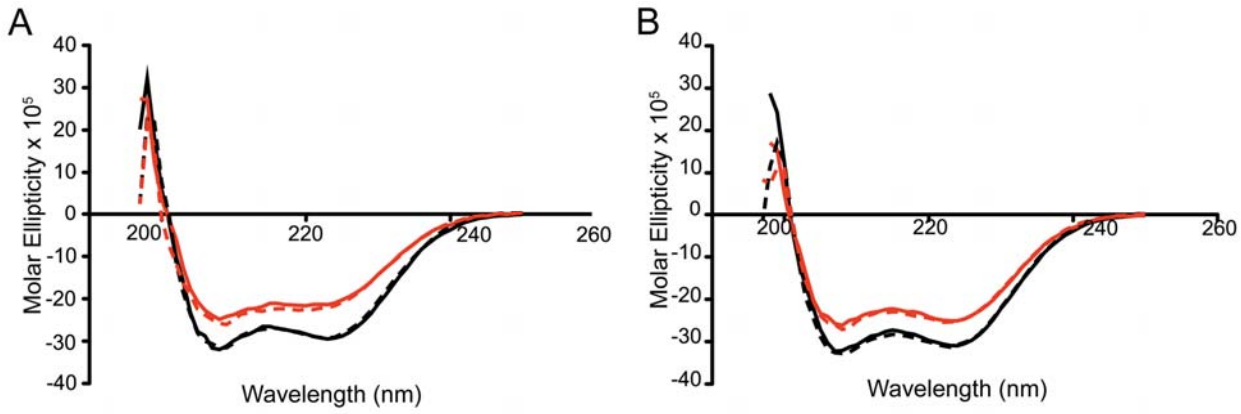
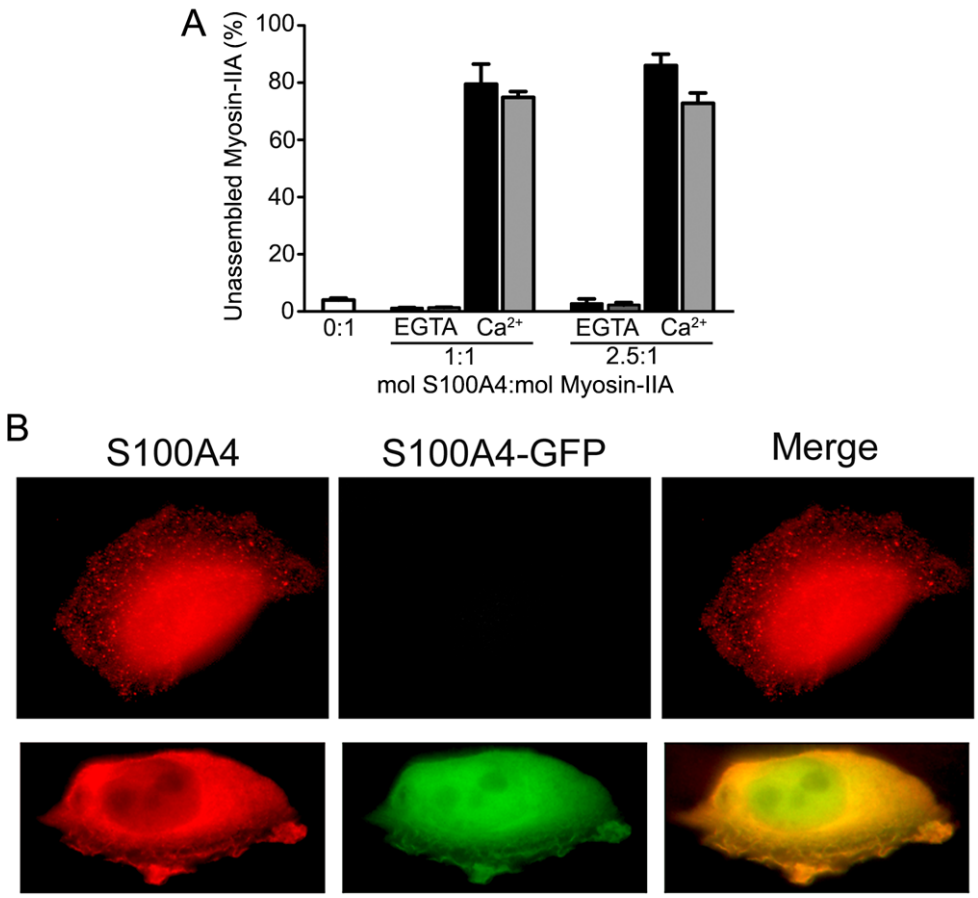
Supporting Information

Supplemental Figure 1: Far UV circular dichroism spectra of sc-S100A4 EF-hand mutant proteins.



Circular dichroism spectra of sc-S100A4 mutant proteins. (A) sc-S100A4-5M1 (black); sc-S100A4-5M2 (red). (B) sc-S100A4-10M1 (black); sc-S100A4-10M2 (red). Solid and dotted lines correspond to spectra in the presence of 2 mM EGTA or 0.3 mM $CaCl_2$, respectively. The alanine substitutions do not affect the overall secondary structure of the sc-S100A4 proteins.

Supplemental Figure 2. Characterization of S100A4-GFP.



S100A4-GFP regulates the assembly of myosin-IIA. (A) S100A4-GFP depolymerizes myosin-IIA filaments in a Ca^{2+}-dependent manner similar to the wild-type, untagged S100A4. MIIA alone (white bars), wild-type S100A4 (black bars); S100A4-GFP (gray bars). Values represent the mean ± standard error of the mean for 3 independent experiments. (B) Colocalization of S100A4-GFP and endogenous S100A4 in HCT116 cells. Upper panel: Localization of endogenous S100A4 in parental HCT116 cells. S100A4 localizes to the leading edge and exhibits a diffuse perinuclear distribution. Lower panel: Localization of endogenous S100A4 and exogenously expressed S100A4-GFP. Parental HCT116 cells were transfected with

S100A4-GFP and 48 hrs post transfection both the endogenous S100A4 and S100A4-GFP were detected using a S100A4 primary antibody (1:100 dilution Abcam) and Alexa 555 secondary antibody (1:500 Molecular Probes). The S100A4-GFP localization pattern is comparable to the total S100A4 localization pattern, demonstrating that the endogenous and exogenously expressed S100A4 exhibit the same overall distribution. Some S100A4-GFP is present in the nucleus due to protein overexpression.