Supplementary Material
Melissa H. Jorrisch et al. doi: 10.1242/bio.20133707

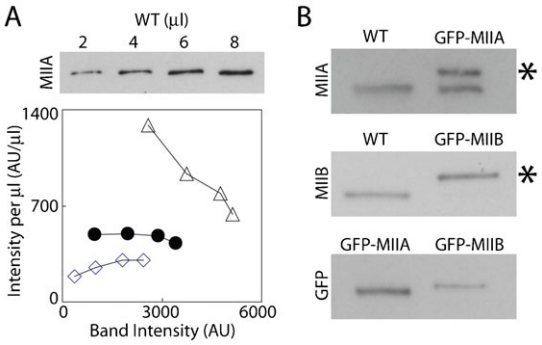

Fig. S1. Quantitative Western blot analysis of myosin II isoforms. (**A**) Determination of the linear range of chemiluminescence detection. By loading different volumes of the same sample, we quantified the band intensities, which were divided by the volume loaded (Intensity per µl). This ratio is constant when the intensity is linearly proportional to the proteins loaded. Under these conditions, the linear range of chemiluminescence bands was between 1000 and 3000 in arbitrary units (see filled circles for the linear range and open triangles and diamonds for non-linear range). (**B**) The ratio of myosin IIA and B protein levels in wild-type MDCK cells. Using myosin IIA antibody, we first determined the relative amount of endogenous myosin IIA in wild type to GFP-myosin IIA (asterisk) in GFP-myosin IIA stable clone (MIIA/GFPMIIA, top blot). The same ratio was obtained for myosin IIB (MIIB/GFPMIIB, middle blot). By quantifying the absolute amount of GFP-myosin isoforms using GFP concentration standards, we can determine the absolute amount of endogenous myosin isoforms. Alternatively, by measuring the relative amount of GFP-tagged myosin isoforms (GFPMIIA/GFPMIIB, bottom blot), the relative amount of endogenous IIA and B was calculated as follows (subscripts denote antibodies used to determine the ratio): $\mathrm{MIIA/MIIB} = (\mathrm{MIIA/GFP-MIIA})_{\mathrm{MIIA}} \times (\mathrm{GFP-MIIB/MIIB})_{\mathrm{MIIB}} \times (\mathrm{GFPMIIA/GFP-MIIB})_{\mathrm{GFP}}$.