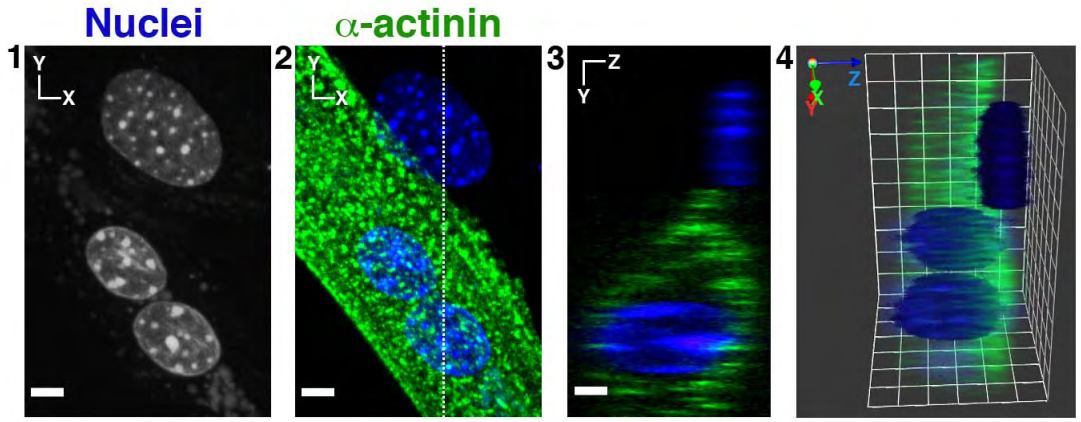

Fig. S1. Comparison of nuclear shape in mononucleated myoblasts and multinucleated myotubes. The nuclei in myotubes adopt a spherical shape whereas the nuclei in myoblasts are wider and flatter. Cells were transfected with YFP-α-actinin to show development of myofibrils in myotubes and nuclei are stained with Hoechst dye. Panels 1 and 2 show maximum projections of a confocal z-series of a nucleus in a myoblast (top) and two nuclei in a neighboring myotube. Panel 2 includes the YFP-α-actinin signal to denote the myotube. The dotted line indicates the location of the single YZ plane shown in panel 3. In this cross-section, the difference in the height of the nuclei in the myoblast and myotube can be appreciated. Panel 4 is a three-dimensional rendering of these nuclei to again emphasize the spherical vs. flattened shape of the myotube and myoblast nuclei, respectively. For panels 1-3, scale bar: 5 μm.

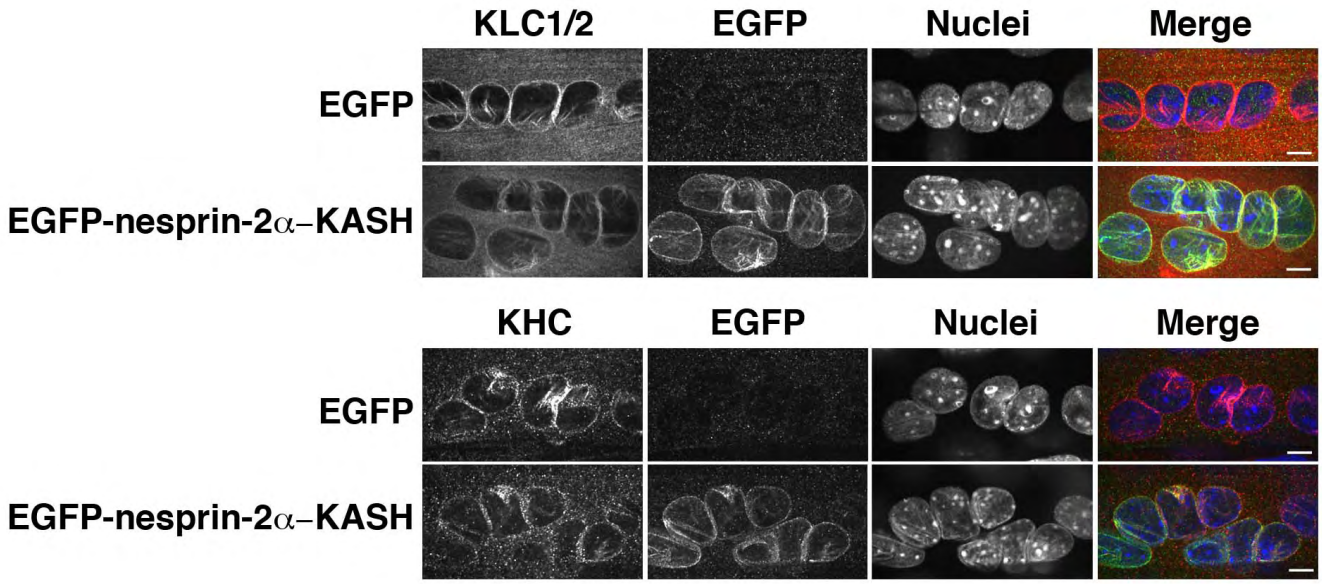

Fig. S2. EGFP-nesprin2α-KASH does not displace all of the kinesin-1 motor from the nuclear envelope. Myotubes were transfected with either EGFP or EGFP-nesprin-2α-KASH and stained for subunits of the plus-end directed microtubule motor kinesin-1 [kinesin light chains 1/2 (KLC1/2); kinesin heavy chain (KHC)] and for EGFP. Myotubes were fixed with methanol, DNA was labeled with Hoechst dye. Scale bar: 10 μm.