



**Figure S4. Myosin 18A $\alpha$ 's N-terminal extension binds F-actin.** (A) SDS-PAGE of an F-actin co-sedimentation assay using a GST-tagged version of myosin 18A $\alpha$ 's N-terminal extension (NT) and the indicated ratios of NT to F-actin (obtained by fixing the concentration of NT at 1  $\mu$ M and increasing the molar ratio of F-actin as indicated). S = supernatant, P = pellet. (B) As in (A) except using a GST-tagged version of myosin 18A $\alpha$ 's N-terminal extension harboring a function blocking mutation in the actin binding site (NT-AB<sup>mut</sup>; residues V117 and L118 both changed to A). (C) Binding isotherms for NT (closed circles) and NT-AB<sup>mut</sup> (open circles) (representative of two independent experiments). The  $K_d$  values shown were determined by fitting the data to a one-site binding hyperbola.