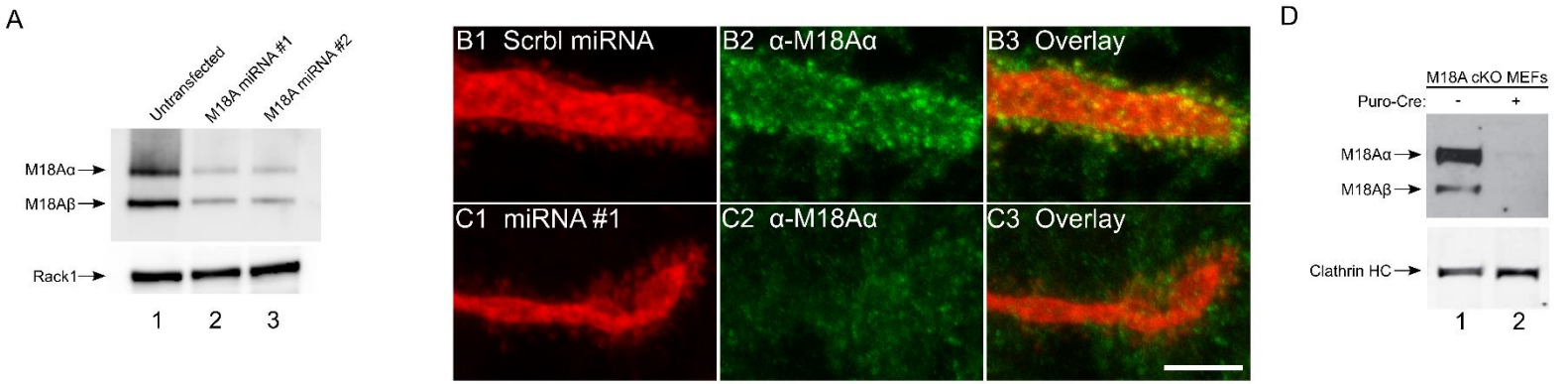

Figure S5. miRNA-mediated knockdown of myosin 18Aα. (A) Representative Western blot of whole cell extracts of mouse NIH 3T3 mouse fibroblasts that were either untransfected (lane 1) or transfected with two different miRNAs directed against myosin 18Aα (miRNA #1, lane 2; miRNA #2, lane 3). Rack1 was used as a loading control. Densitometry indicated that the level of myosin 18Aα was reduced by 81 ± 10% and 84 ± 9% by miRNAs #1 and #2, respectively (N=3). Because 3T3 fibroblasts (unlike Purkinje neurons) also express myosin 18Aβ (which shares with myosin 18Aα both miRNA targeting sequences), its levels were also reduced by both miRNAs by ~80%. (B1-B3) Shown is a portion of a dendrite from a representative cultured Purkinje neuron (DIV 18) that was expressing a scrambled control miRNA and that was fixed and stained for mCherry (to amplify the signal from the miRNA-associated mCherry volume marker) (B1) and for myosin 18Aα (B2); Overlay (B3). (C1-C3) As in Panels B1-B3, except the Purkinje neuron was expressing myosin 18Aα miRNA #2. Control and knockdown neurons were imaged on the same day with identical settings on a Zeiss 780 confocal microscope so that the intensities for myosin 18Aα can be directly compared. (D) Representative Western blot of whole cell extracts of mouse embryo fibroblasts (MEFs) isolated from a conditional myosin 18A knockout mouse (M18 cKO; see Methods) that were (lane 2) or were not (lane 1) transfected with a puromycin-resistant plasmid harboring Cre recombinase (Puro-Cre). The expected positions for the heavy chains of myosin 18Aα and myosin 18Aβ are shown. Clathrin heavy chain (HC) was used as a loading control. Scale bar: 5 μm (B3).