Figure S1. Specificity of Bchs antibody and lack of effect on transport of mitochondria in *bchs*.

(A) Confocal images of WT (top left) and bchs1/Df (top right) stage 15/16 embryonic fillets labeled with rabbit polyclonal antiserum against Bchs, and detected with fluorescent secondary antibody. Images were obtained using identical confocal settings in the same imaging session.

Blue cheese antibody on Western blot (bottom) of larval brain extracts. Extracts of 3.5 larval brains were loaded per lane for each of the genotypes, left to right: *yw1118*, *C155(elav)>EP2299*, $bchs^{1}$*/Df(2L)c17*, *bchs58/Df(2L)c17*. No Bchs product is detected in *bchs* brains.

(B) Representative kymographs of GFP-labeled mitochondria (mito-GFP) in RP2 or aCC motor axons. A 53.5μm (x-axis) length of axon from wild type (left) and $bchs^{58}$*/Df(2)c17* (right) larvae are shown. The kymographs are arranged with the cell body to the left and the axon terminals to the right. In both wild type and $bchs^{58}$ larvae, mitochondrial movement has a strong anterograde bias despite frequent pauses and temporary changes in direction. Time is represented on the y-axis, over a period of 200s for both kymographs.

C) Effects of *bchs* on mitochondrial mobility. Quantification of moving and stationary vesicles in 20-second segments is shown (see Methods) (N=128 for WT, 154 for $bchs^{58}$*/Df(2)c17*). The slight decrease in mitochondrial mobility in *bchs* mutants is not highly significant (P value = 0.03, Student's t-test)

D) *bchs* mutant has no effect on net transport direction. The number of anterograde, retrograde and bidirectional moving vesicles were counted in 20-second segments from

kymographs (as in B, and Methods) and expressed as a fraction of the total number of moving vesicles. There were no significant differences observed between the number of anterograde, retrograde or bidirectional vesicles in wild type and *bchs* mutants. WT denotes wild type, and bchs58 denotes $bchs^{58}/Df(2)c17$ in the figure.

WT ant: 0.75±0.03, ret: 0.19±0.04, bi: 0.06±0.005

bchs58 ant: 0.7±0.008, ret: 0.22±0.02, bi: 0.079±0.02

Movie S1. Wide-field fluorescence movie of spinster-GFP vesicles in motoraxons of a wild type third instar larva (corresponding to Figure 8A, WT panel; genotype is RRa/RRa>spinster-GFP). Most vesicles can be seen being transported smoothly in the anterograde direction (top left to bottom right). Single images were acquired every 0.6-1sec continuously under 10% transmission of fluorescent light with a 63X/1.42NA lens for this and all subsequent movies. Movies were assembled at 30 fps.

Movie S2. Wide-field fluorescence movie of spinster-GFP vesicles in motoraxons and termini of another *bchs* mutant larva (genotype is $bchs^{1}/Df(2L)c17$; *RRa/RRa>spinster-GFP*). One prominent vesicle can be seen moving rapidly in the retrograde direction from the top right to the lower left of the frame.

Movie S3. Wide-field fluorescence movie of spinster-GFP vesicles in motoraxons of a *bchs* mutant larva (genotype is $bchs^{58}/Df(2L)c17$; *RRa/RRa>spinster-GFP*). Little directed movement can be seen. Anterograde direction is from the bottom right to the upper left of the frame.

Movie S4. Wide-field fluorescence movie of spinster-GFP vesicles in motoraxons of a Bchs-overexpressing larva (genotype is *EP(2)2299/+; RRa/RRa>spinster-GFP*). Most vesicles are stationary. Anterograde direction is from the top to the bottom of the frame.