Supplemental Materials

Molecular Biology of the Cell

Qu et al.

Supplementary Figures, Tables and Movies

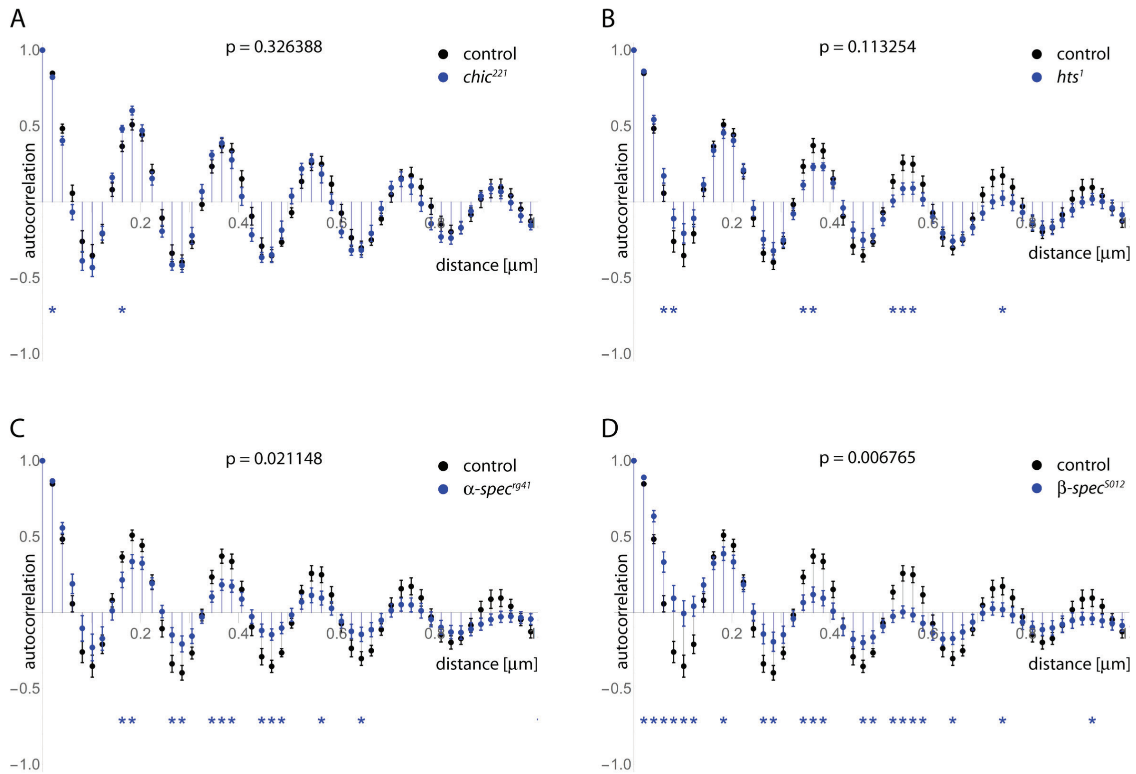

Fig. S1. Statistical comparisons of autocorrelation plots. The autocorrelation plots of four different mutant conditions including *chic*^{221} (A), *hts*^{1} (B), α-spec^{rg41} (C) and ß-spec^{S012} (D) are shown in blue and compared to wild type at 10DIV (black). Each plot shows the mean ± SEM of 15 autocorrelation curves. A Mann-Whitney test is performed at each point, and those points which are statistically significantly different ($p<0.05$) are indicated with stars. The integrated statistical significances are shown above each graph.

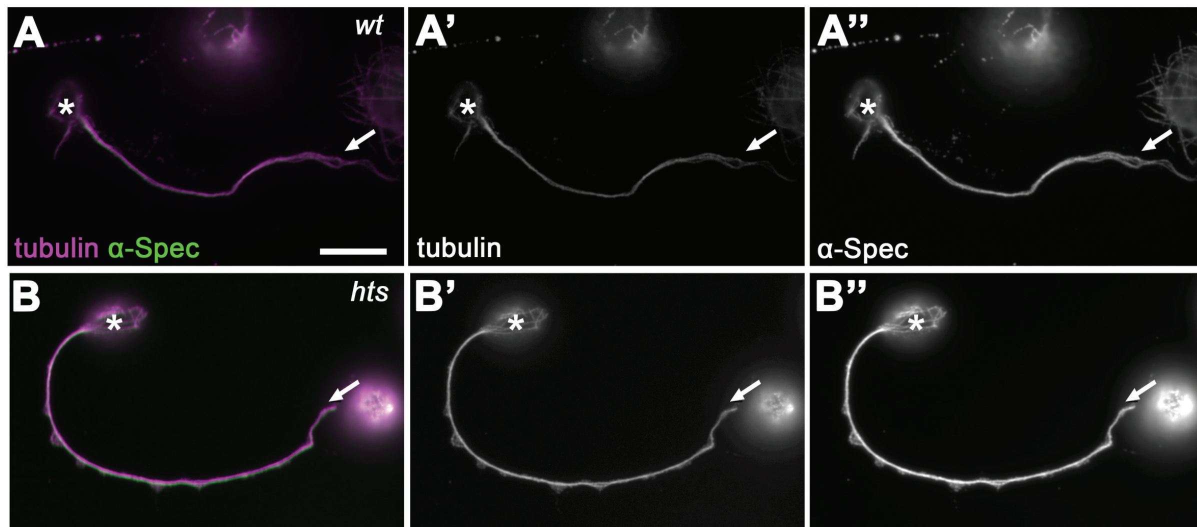

Fig. S2. Axonal α-spectrin staining is unaffected in *hts*[1] mutant neurons. **A-B'')** α-spectrin staining in wildtype primary neurons (A-A''), or *hts*[1] mutant neurons cells (B-B''), all double-labelled for tubulin (magenta) and α-spectrin (green) and shown as single channel images in middle and right column; scale bar in A represents 10µm in all images.

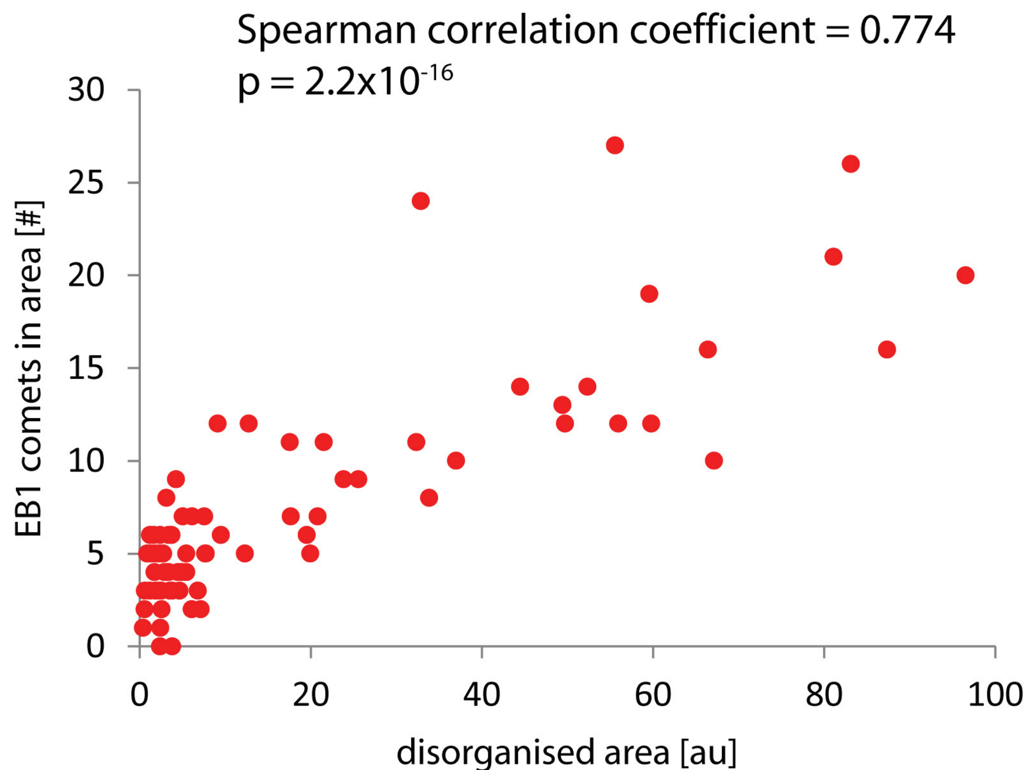

Fig.S3. The correlation of MDI with EB1::GFP comet numbers. Stills of movies with EB1::GFP in *shot*[3] mutant neurons (Figure 7) were analysed with respect to area of disorganisation (equivalent to MDI; plotted on the abscissa) and number of comets found within this area (plotted on the ordinate). The Spearman correlation coefficient is given above the graph.

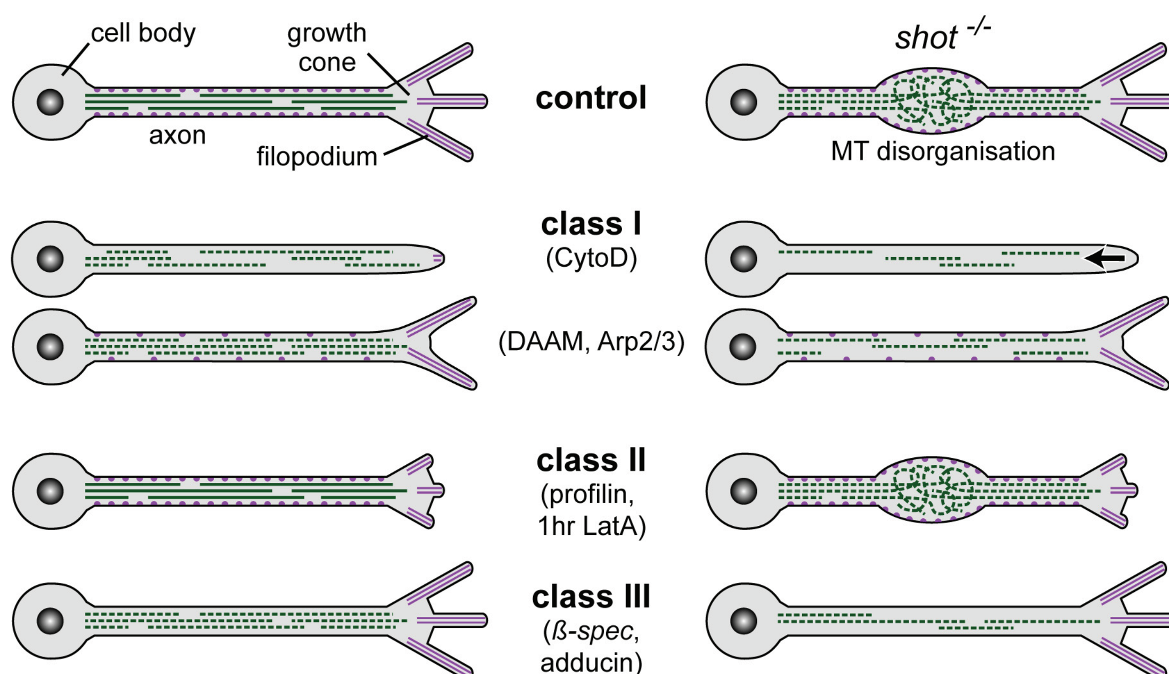

Fig. S4 Illustration of the correlation between PMS abundance and MDI. Actin manipulations in neurons with wildtype background (left) are compared to the same treatments/conditions in *shot* mutant neurons (right); actin filaments are shown as magenta lines, PMS in axons as magenta half-circles, stable MTs as green lines, less stable MTs (due to loss of PMS or Shot) as stippled green lines. Class I phenotype: CytoD affects PMS as well as filopodial length, whereas loss of DAAM or Arp2/3 affect PMS and filopodia numbers in growth cones; class II phenotype: LatA and loss of profilin affect primarily filopodia and far less PMS; class III phenotype: loss of ß-Spectrin or Adducin affects PMS but not filopodial length. When PMS are absent, areas of MT disorganisation in *shot* mutant neurons are not maintained. Other actin manipulations tested here express the class I-III phenotypes with lower penetrance, but their degrees of PMS loss strongly correlate with the degree of MDI reduction in *shot* mutant background (Figure 9).

Tab. S1 Detailed data for the various experiments shown. Data are organised into five separate tables provided as 5 separate pages within this spreadsheet.

Page 1. PMS abundance data. Data shown here accompany Figs.1, 2 and 4. The first column ("conditions") indicates the genotype/treatment/stage of each experiment. For all experiments with actin manipulations (drug treatments or mutants) wildtype controls were measured in parallel (note that experiments performed on the same days have the same wt controls); therefore two rows are shown for each condition of which the respective upper row shows the control counts ["wt (manipulation) stage"] and the lower row the counts for manipulated neurons ["manipulation stage"]; stages are indicated as 6HIV and 10DIV (hours/days in vitro). For each condition the absolute number of counted axon segments (~5µm) is given in the second column ("all axons"), of those the absolute number with PMS in the third column ("with PMS"), and the calculated fraction in the fourth column ("ratio").

The ratio normalised to the ratio of control neurons is shown in the fourth column ("rel. to control"). P values were obtained via χ^2 analysis of raw data comparing axon segments with/without PMS and are given in the sixth column ["p(chi^2)"]. Slide average and slide SEM represent normalised average and SEM of independent experimental repeats (independent microscopic slides analysed); n.d., not done.

Page 2 Axons with gaps in the tubulin staining. Data shown here accompany Figure 5. Genotypes and drug treatments of assessed neurons are shown in the first column ("Experimental conditions"), the total number of analysed axons at 6-8HIV in the second column ("total"), of those the number displaying gaps in their tubulin staining in the third column ("neurons with axon"), the ratio in the fourth column {"axons with gaps (%)"], and statistics performed via χ^2 analysis of raw data (comparing numbers of axons with/without gaps under experimental conditions with those of wt controls) in the last column ["p(chi^2) compared to wt"].

Page 3 Neurons without axons. Data shown here accompany Figure 6. Genotypes and drug treatments of assessed neurons are shown in the first column ("Experimental conditions"), the total number of Elav-positive cells (i.e. neurons) at 6-8HIV in the second column ("total"), of those the number carrying an axon (tubulin structure longer than the soma diameter) in the third column ("neurons with axon"), the ratio in the fourth column {"neurons with an axon (%)"], and statistics performed via χ^2 analysis of raw data (comparing numbers of neurons with/without axons under experimental conditions with those of wt controls) in the last column ["p(chi^2) compared to wt"].

Page 4 Data for analyses of MT disorganisation indices (MDI). Data shown here accompany Figure 8. The first column ("experimental conditions") indicates the genotype and/or treatment of each experiment; all actin manipulations (drug treatments or mutation) were performed in *shot*^{3} mutant background, and untreated *shot*^{3} mutant controls were measured in parallel; therefore two rows are shown for each condition of which the respective upper row shows the control counts ["*shot*^{3}"] and the lower row the counts for manipulated neurons ["manipulation + *shot*^{3}"]. The stage of analysed neurons is given in the second column ("culture time"), the total number of analysed neurons in the third column ("n"), the average±SEM normalised to wildtype controls (also run in parallel) is given in the fourth column ("normalised to wt"), the average normalised to respective *shot*^{3} mutant controls in the fifth column ("normalised to *shot*"), the statistics using Mann-Whitney test comparing data to wildtype controls in the sixth column ["P(MW) compared to wt"] and comparing to shot^3 controls in the last column ["P(MW) compared to *shot*"].

Page 5 EB1 comet number and velocity data. Data shown here accompany Figure 7. The first column ("experimental conditions") lists drug treatments (1.6µM CytoD, 800nM CytoD or 200nM LatA), time in culture (6HIV or 3DIV) and genotype (wildtype or *shot*^{3}) for each experiment. The top row ("pre, 5 min, 30 min, 60 min, 90 min, 120 min") indicates the assessed time point (i.e. before or after drug application). For each time point, the left column provides data for "comet number", the right for "comet velocity". Each data field shows sample numbers at the top [for comet number n = number of axons; for comet velocity n = number of axons/number of comets], the normalised comet numbers±SEM or normalised comet velocities±SEM in the middle, and p values for statistical analysis via Mann-Whitney test at the bottom (comparing data at time point post application to data at time point "pre"); n.d., not done.

Movie. S1 Live recordings of wildtype primary neurons expressing EB1::GFP shows that EB1 comets are not reduced after CytoD treatment. The film accompanies Figure 7 and shows the axon (bottom) and growth cone (top) of a wild type *Drosophila* primary neuron at 6HIV which expresses EB1::GFP mediated by the pan-neuronal *elav-Gal4* driver. The changing label (top right: "before, 10 min, 30 min, 1hr") indicates the assessed time point (i.e. before or after drug application). Images were captured every 4s, adding up to 8mins film length.

Movie. S2 Live recordings of *shot* mutant primary neurons expressing EB1::GFP shows that EB1 comets faint away after CytoD treatment. The film accompanies Figure 7 and shows the axon (bottom) and growth cone (top) of a *shot*^{3} mutant *Drosophila* primary neuron at 6HIV which expresses EB1::GFP mediated by the pan-neuronal *elav-Gal4* driver. The changing label (top right: "before, 10 min, 30 min, 1hr") indicates the assessed time point (i.e. before or after drug application). Images were captured every 4s, adding up to 8mins film length. Note that immediately after CytoD treatment, EB1::GFP comets start to show a reduced velocity/oscillating behaviour and then gradually faint away.