

# SUPPLEMENTARY INFORMATION

## **Tubb3 expression levels are sensitive to neuronal activity changes and determine microtubule growth and kinesin-mediated transport**

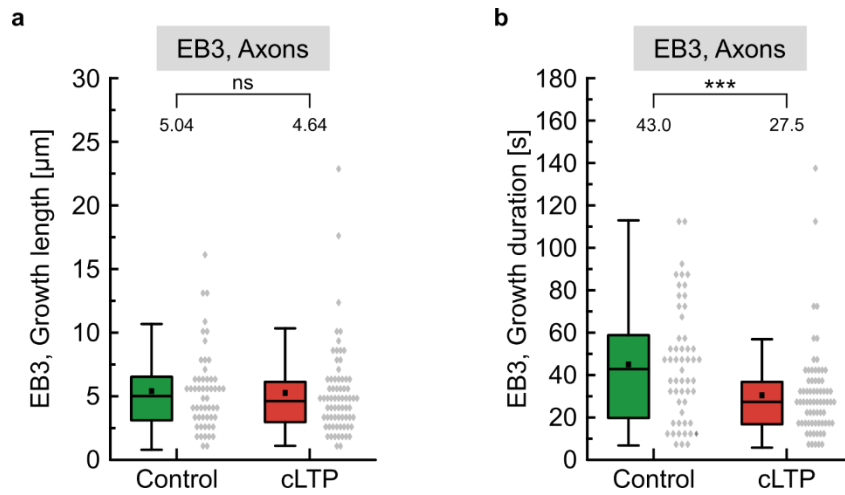
Cellular and Molecular Life Sciences

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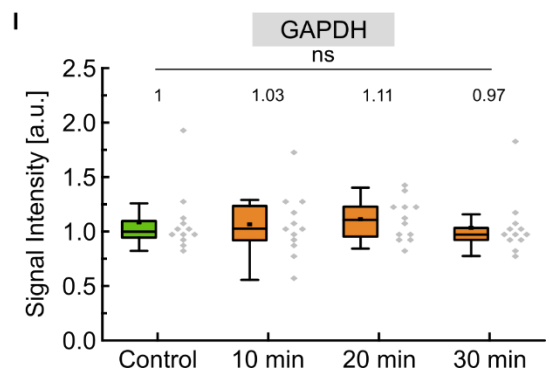
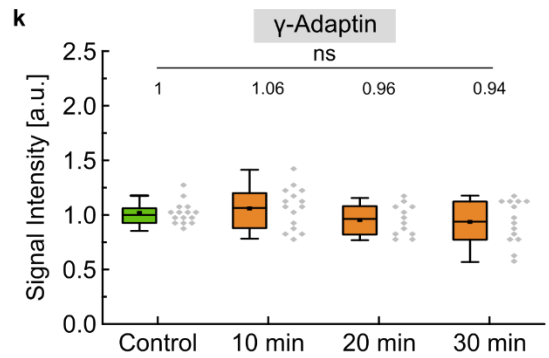
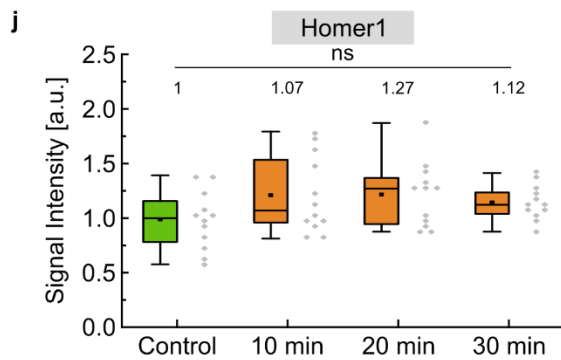
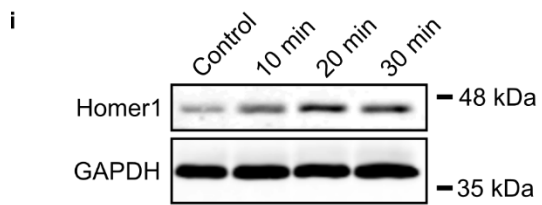
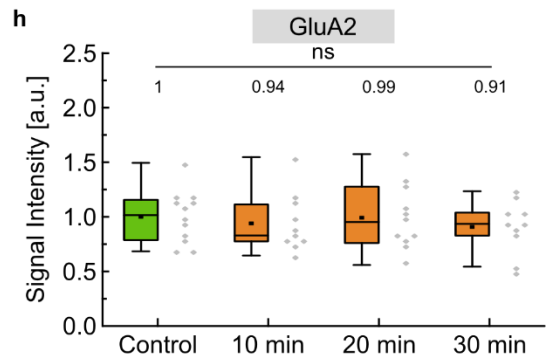
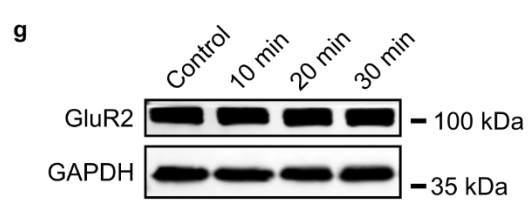
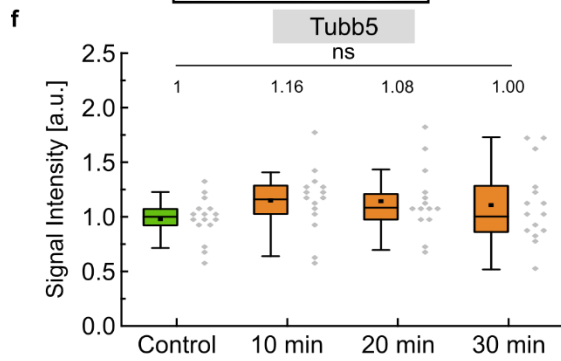
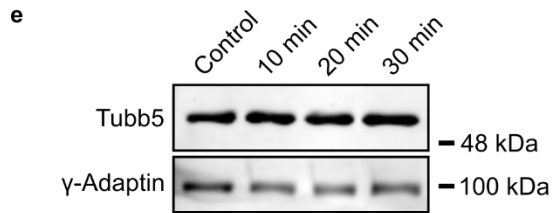
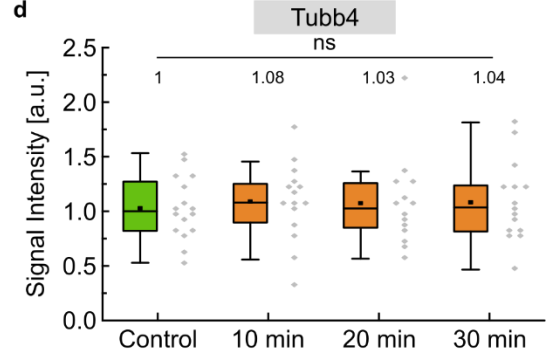
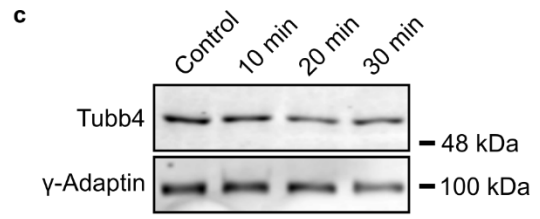
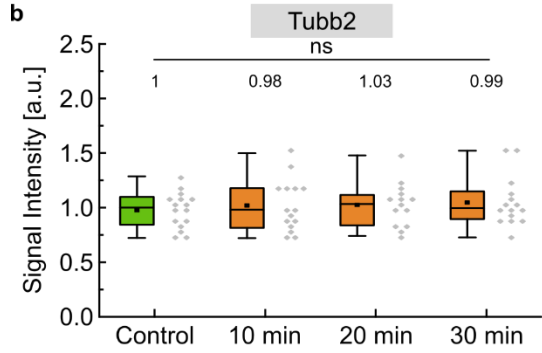
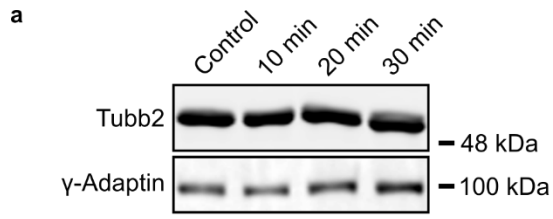
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<sup>3</sup>Correspondence: [matthias.kneussel@zmnh.uni-hamburg.de](mailto:matthias.kneussel@zmnh.uni-hamburg.de)



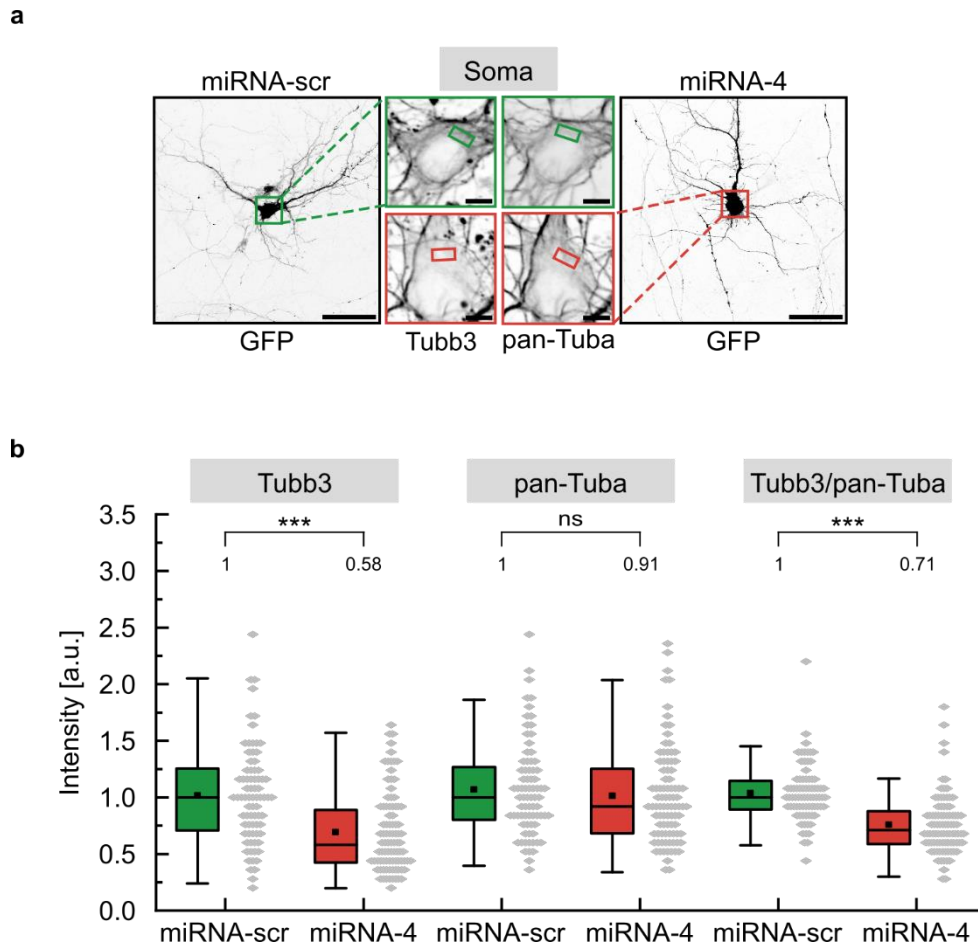
**Figure S1. Supplemental Data to Figure 1a, b.**

Quantification of microtubule growth lengths and growth durations in axons, depicting median values (N=3 experiments). \*\*\*  $p < 0.001$ , ns: not significant. Each data point represents one EB3 comet; control (n=54), cLTP (n=70). For graphical representation of the distribution, data are binned with a bin size of (a)  $0.75 \mu\text{m}$  and (b) 5 s. Statistics: Mann-Whitney Rank Sum Test.



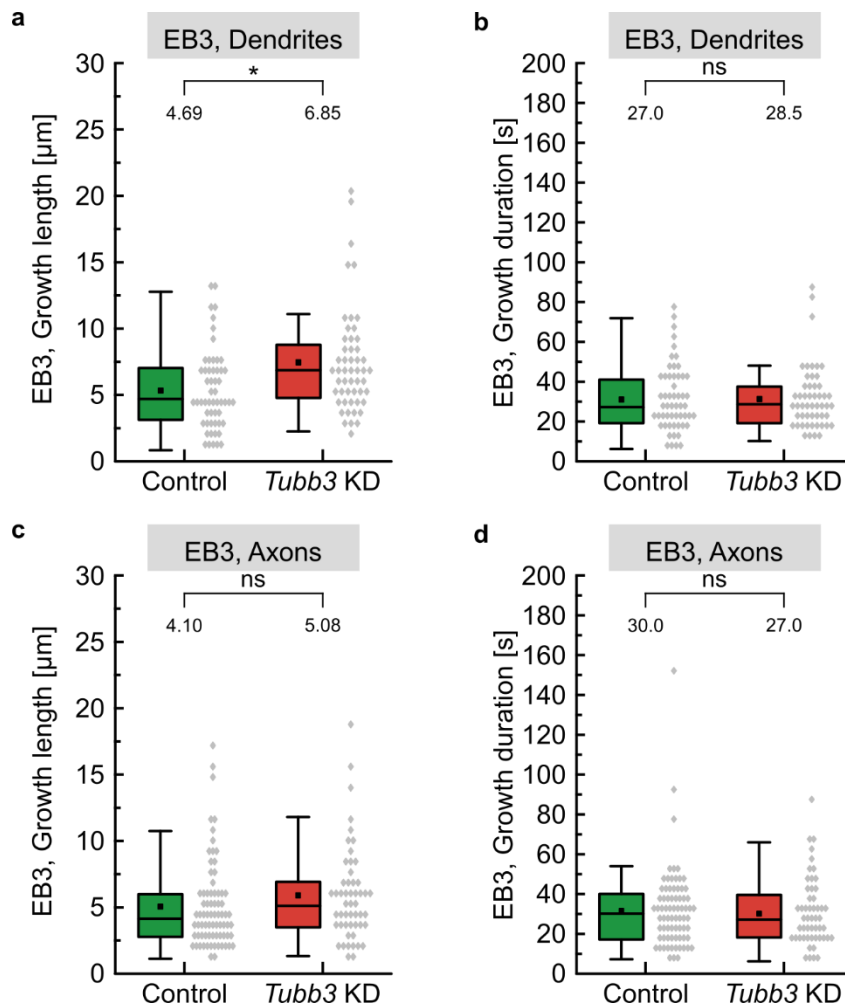
**Figure S2. Supplemental Data to Figure 1c-j.**

Western blot analysis and quantification of tubulin isotype expression in acute hippocampal slices, following chemical induction of long-term potentiation (cLTP). (a, b) Tubb2, (c, d) Tubb4, (e, f) Tubb5, (g, h) GluA2, (i, j) Homer1, (k) gamma-Adaptin, (l) GAPDH. Median values are depicted. Black dots represent mean values. For graphical representation of the distribution, data were binned with a bin size of 0.05 a.u. Statistics: One way ANOVA. (ns: not significant).



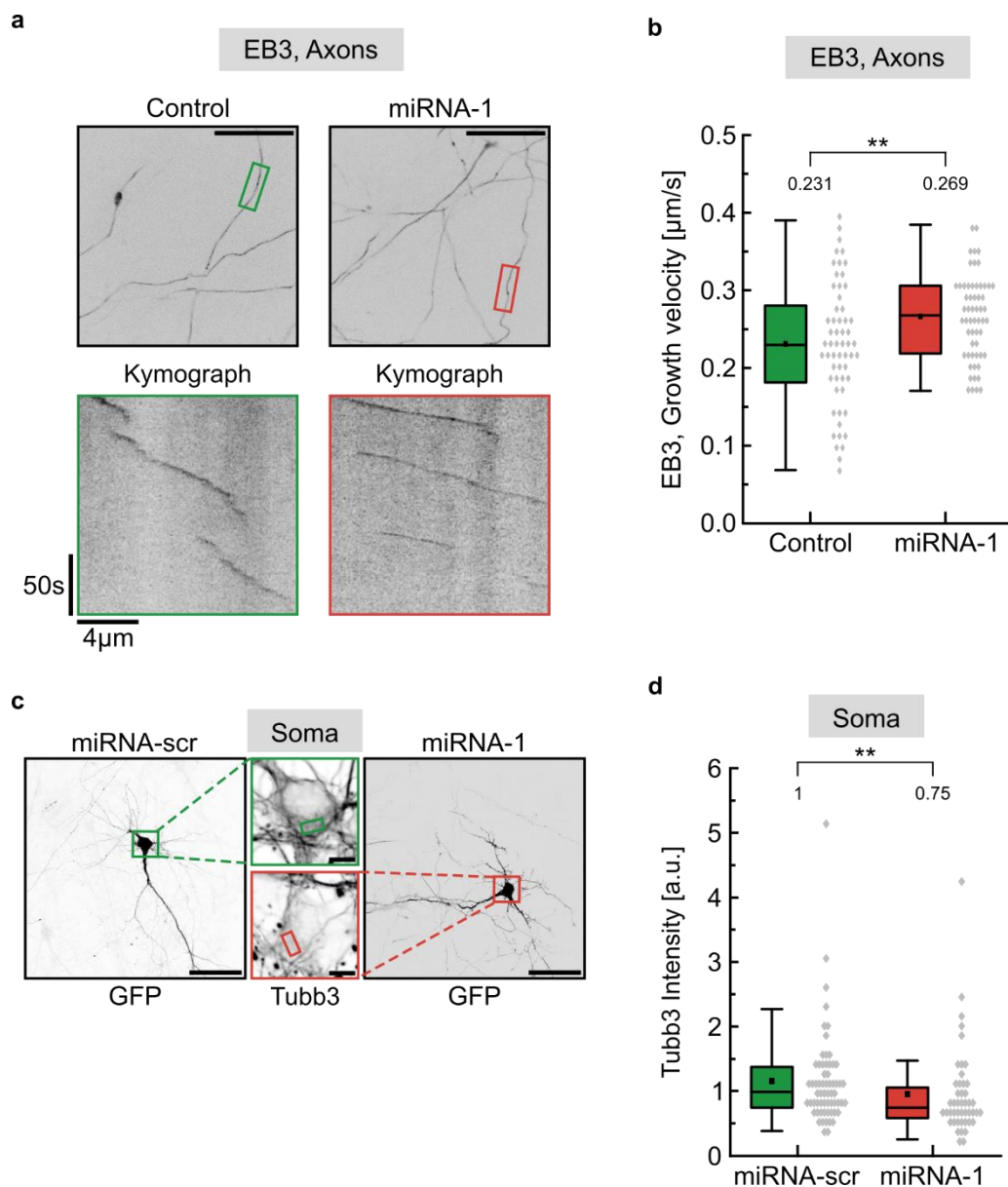
**Figure S3. Supplemental Data to Figure 2.**

(a) Cultured hippocampal neurons transfected with miRNA-scr or miRNA-4, respectively. Scale bar, 50  $\mu\text{m}$ . Somata in boxed regions are enlarged in the middle (green: control, red: knockdown). Tubb3 and pan-Tuba intensities were measured at DIV12 inside the soma, indicated by the small colored boxes. Scale bar, 5  $\mu\text{m}$ . (b) Quantification of a, depicting median values (N=3 experiments). On the right Tubb3 data were normalized to pan-Tuba. Each data point represents one cell; miRNA-scr (n = 78), miRNA-4 (n = 91). Statistics: Mann-Whitney Rank Sum Test. For graphical representation of the distribution, data are binned with a bin size of 0.08 a.u.



**Figure S4. Supplemental Data to Figure 3.**

(a, b) Quantification of microtubule growth lengths and growth durations in dendrites, depicting median values (N=3 experiments). \*  $p < 0.05$ , ns: not significant. Each data point represents one EB3 comet; control (n=55), knockdown (n=52). For graphical representation of the distribution, data are binned with a bin size of (a)  $0.8 \mu\text{m}$  and (b) 5s. (c, d) Quantification of microtubule growth lengths and growth durations in axons depicting median values (N=3 experiments). ns: not significant. Each data point represents one EB3 comet; control (n=75), knockdown (n=52). For graphical representation of the distribution, data are binned with a bin size of (c)  $0.8 \mu\text{m}$  and (d) 5 s. Statistics: Mann-Whitney Rank Sum Test.



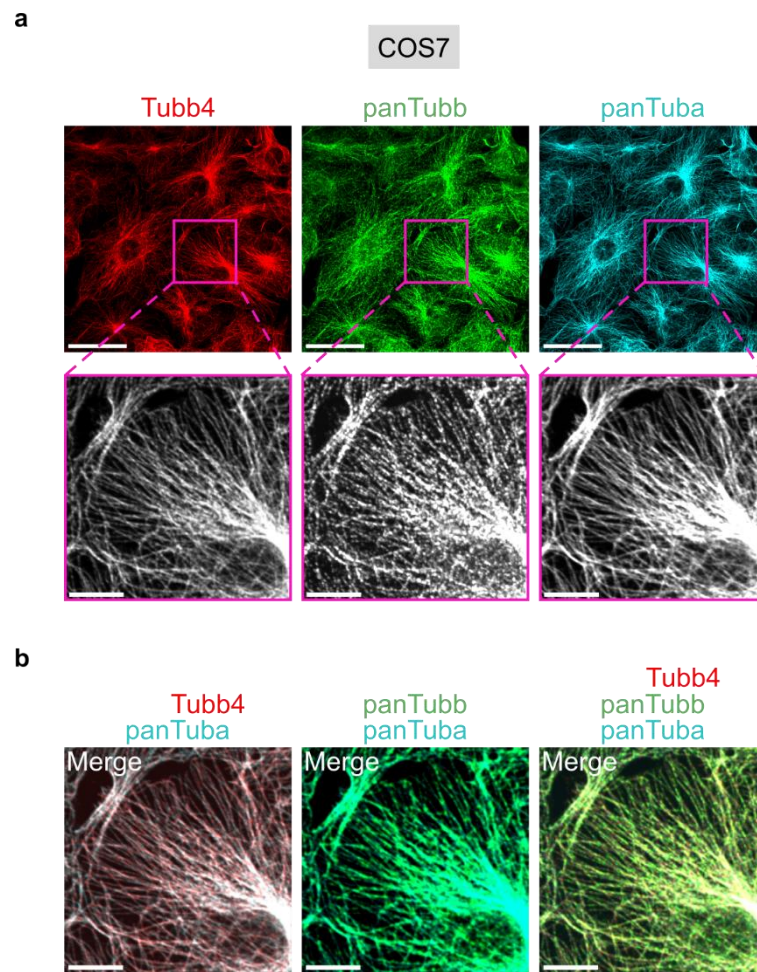
**Figure S5. Supplemental Data to Figure 3.**

(a) Live cell imaging of DIV 12 cultured hippocampal neurons cotransfected with EB3-Tomato and control or *Tubb3* knockdown construct (miRNA-1) in axons. Image acquisition: 1 frame/s over 3 min. Scale bar, 20  $\mu$ m. EB3 comets in axonal segments in colored boxes of overview images (top panel) are visualized by kymographs (bottom panel). (b) Quantification of a, depicting median values (N=3 experiments). \*\*  $p < 0.01$ . Each data point represents one EB3 comet; control (n=58), knockdown (n=58). For graphical representation of the distribution, data are binned with a bin size of 0.015  $\mu$ m/s. Statistics: Welch's t-test. (c) Cultured hippocampal neurons transfected with miRNA-scr or miRNA-1, respectively. Scale bar, 50  $\mu$ m. Somata in boxed regions are enlarged in the middle (green: control, red: knockdown). Tubb3 intensities were measured at DIV12 inside the soma, indicated by the small colored boxes. Scale bar, 5  $\mu$ m. (d) Quantification of c, depicting median values (N=2 experiments). Each

data point represents one cell; miRNA-scr (n = 63), miRNA-1 (n = 46). Statistics: Mann-Whitney Rank Sum Test.

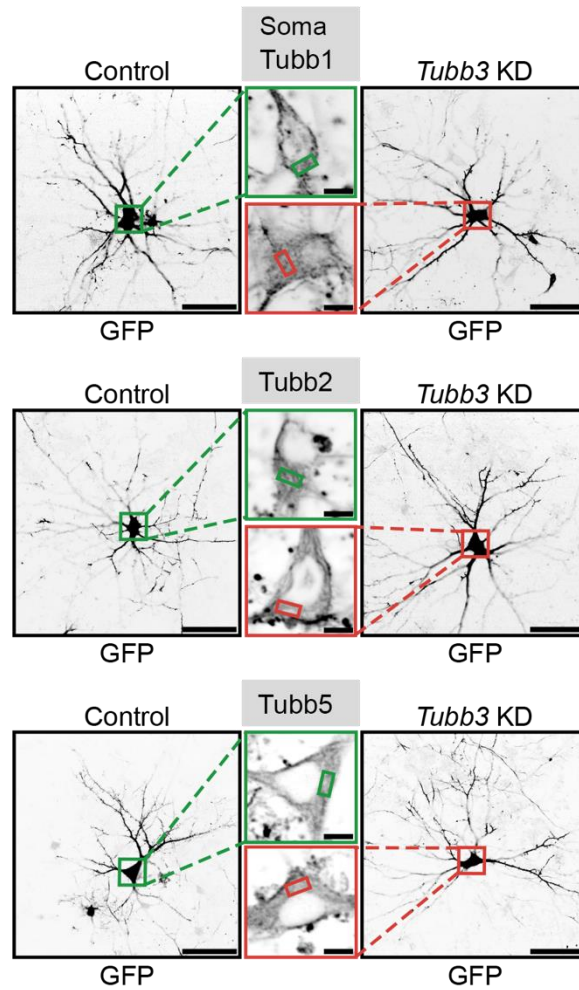
For graphical representation of the distribution, data are binned with a bin size of 0.15 a.u.





**Figure S6. Supplemental Data to Figure 4.**

(a) Cultured COS7 cells immunostained with Tubb4-, panTubb- and panTuba-specific antibodies. Scale bar 40  $\mu\text{m}$ . Enlargements of the boxed regions depict filamentous tubulin. Scale bar 10  $\mu\text{m}$ . (b) Merged images of enlarged regions of a. Scale bar 10  $\mu\text{m}$ .



**Figure S7. Supplemental Data to Figure 4.**

Cultured hippocampal neurons (DIV12) transfected with control or *Tubb3* knockdown constructs. From top down: Detection with a specific Tubb1, Tubb2 and Tubb5 antibody. Scale bar, 50  $\mu\text{m}$ . Middle panels: Enlargements of cell somata boxed regions. Tubulin intensities were measured inside the soma, indicated by the small colored boxes. DAPI staining of the nucleus (not shown) was used to verify that tubulin intensity measurements were performed in the cytoplasm. Scale bar, 5  $\mu\text{m}$ .

G <sub>long</sub> (Tubb3)	G <sub>lat</sub> (Tubb3)	G <sub>long</sub> (Tubb4)	G <sub>lat</sub> (Tubb4)	velocity control	velocity knockdown
in RT	in RT	in RT	in RT	in $\mu\text{m/s}$	in $\mu\text{m/s}$
-9	-1.5	-7	-6.5	0.165	0.204
-9	-0.5	-7	-6.5	0.162	0.207
-8.5	-6.5	-6	-5.5	0.168	0.201
-8.5	-3	-8	-4.5	0.168	0.203
-8.5	-3	-7	-5.5	0.171	0.207
-8.5	-2.5	-6.5	-6	0.169	0.199
-8.5	-2	-7.5	-5	0.164	0.198
-8	-5.5	-6.5	-5.5	0.167	0.199
-8	-2	-8	-5.5	0.171	0.199
-8	-1.5	-7.5	-6	0.173	0.200
-7.5	-7	-6	-6	0.165	0.204
-7.5	-6	-6.5	-6	0.164	0.202
-7.5	-3	-8.5	-4	0.172	0.200
-7.5	-1.5	-8	-4.5	0.168	0.198
-7.5	-1	-7.5	-7	0.171	0.201
-7.5	-1	-7.5	-6.5	0.163	0.204
-7.5	-1	-7.5	-5	0.162	0.209
-7	-6.5	-7.5	-4.5	0.162	0.198
-7	-5	-7	-6	0.173	0.206
-7	-3.5	-9	-3	0.172	0.197
-7	-2.5	-7	-6.5	0.166	0.209
-7	-1.5	-7.5	-6	0.164	0.196
-7	-1	-8	-6	0.173	0.197
-7	-1	-8	-5	0.169	0.197
-7	-1	-7	-7	0.162	0.198
-6.5	-5.5	-7	-6	0.170	0.204
-6.5	-5.5	-7	-5.5	0.172	0.197
-6.5	-5	-7	-6.5	0.169	0.197
-6.5	-4.5	-8.5	-4	0.164	0.202
-6.5	-4.5	-8.5	-3.5	0.169	0.203
-6.5	-4.5	-7.5	-5	0.164	0.195
-6.5	-4	-7	-7	0.170	0.194
-6.5	-3.5	-7.5	-5	0.165	0.202
-6.5	-2	-7.5	-6	0.167	0.197
-6.5	-1	-8.5	-4.5	0.171	0.208
-6.5	-1	-8	-5	0.167	0.202
-6	-4.5	-9	-4	0.168	0.196
-6	-3.5	-8.5	-4	0.169	0.196
-6	-3.5	-8	-5.5	0.166	0.202
-6	-3.5	-7	-5	0.165	0.196
-6	-2.5	-8.5	-4	0.164	0.204
-6	-2.5	-8	-6	0.167	0.205
-6	-2.5	-7.5	-6	0.173	0.205
-6	-2	-9	-4	0.166	0.206

-6	-2	-8	-4.5	0.162	0.208
-6	-1.5	-7.5	-6	0.172	0.202
-6	-1	-8.5	-4.5	0.166	0.194
-6	-1	-7	-7	0.162	0.194
-5.5	-4.5	-8.5	-4	0.168	0.201
-5.5	-4.5	-7.5	-5	0.169	0.195
-5.5	-3	-8.5	-4.5	0.166	0.202
-5.5	-2.5	-7.5	-7	0.167	0.207
-5.5	-2.5	-7	-7	0.161	0.206
-5.5	-1	-7.5	-7	0.161	0.198
-5	-4.5	-8.5	-4.5	0.168	0.206
-5	-4.5	-8.5	-4	0.166	0.206
-5	-4	-7.5	-5	0.165	0.195
-5	-3.5	-8.5	-4.5	0.172	0.206
-5	-3.5	-7.5	-7.5	0.165	0.208
-5	-3	-8	-5.5	0.163	0.198
-5	-3	-7.5	-6	0.172	0.205
-5	-2.5	-8	-6	0.172	0.195
-5	-2	-7	-7	0.168	0.195
-5	-1.5	-7.5	-7.5	0.167	0.208
-5	-1	-8.5	-4.5	0.172	0.197
-5	-1	-8	-6	0.164	0.193
-4.5	-4.5	-7	-7	0.162	0.193
-4.5	-4	-7.5	-6.5	0.168	0.202
-4.5	-3	-8.5	-4.5	0.168	0.206
-4.5	-3	-8	-5	0.169	0.198
-4.5	-1	-8	-4.5	0.168	0.199
-4	-3.5	-8	-5.5	0.165	0.194
-4	-3	-8	-5.5	0.165	0.201
-4	-3	-7.5	-6.5	0.172	0.194
-4	-2.5	-8	-5	0.172	0.199
-4	-1.5	-7	-6.5	0.162	0.204
-4	-1	-8	-6	0.167	0.205
-4	-1	-7.5	-7	0.163	0.195
-3.5	-3.5	-7.5	-6.5	0.172	0.200
-3.5	-3	-8.5	-4.5	0.167	0.193
-3.5	-3	-8.5	-4	0.162	0.199
-3.5	-2.5	-9	-3.5	0.169	0.198
-3.5	-2	-8	-4.5	0.170	0.194
-3.5	-2	-7.5	-7.5	0.165	0.208
-3	-3	-9	-4	0.169	0.205
-3	-2.5	-8	-7	0.167	0.203
-3	-1.5	-8.5	-5	0.168	0.198
-3	-1.5	-8	-8	0.165	0.197

**Table S1. Energy parameter settings - Supplemental Data to Figure 5.**

For each energy parameter set, one growth velocity value was calculated under control or *Tubb3* knockdown conditions, respectively. Experimental mean values of microtubule growth in axons (compare with Figure 3d) were  $0.167 \pm 0.006 \mu\text{m/s}$  (control) and  $0.201 \pm 0.008 \mu\text{m/s}$  (*Tubb3* knockdown). Energy parameter sets that generated velocities in these ranges were selected. Parameter sets that coincided for control and knockdown conditions were filtered. This resulted in 88 parameter settings that fulfilled the requirements.