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Supplemental information

Centrosome-dependent microtubule modifications set the conditions for axon formation

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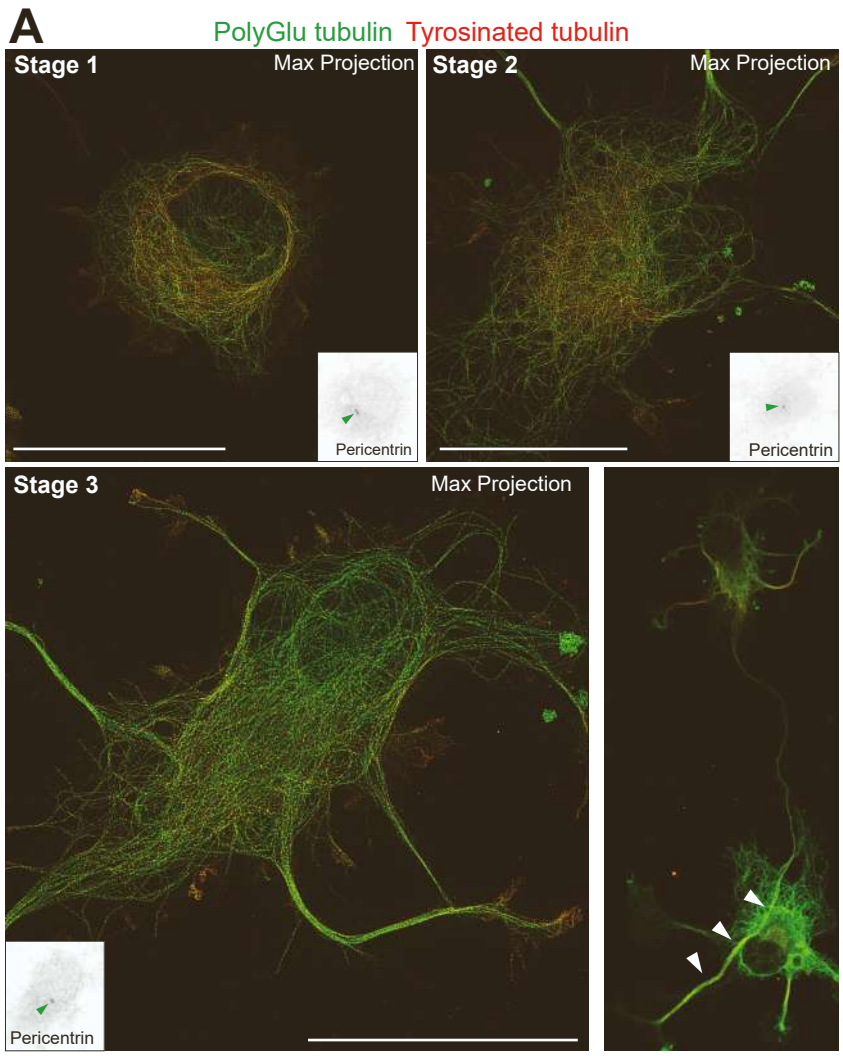
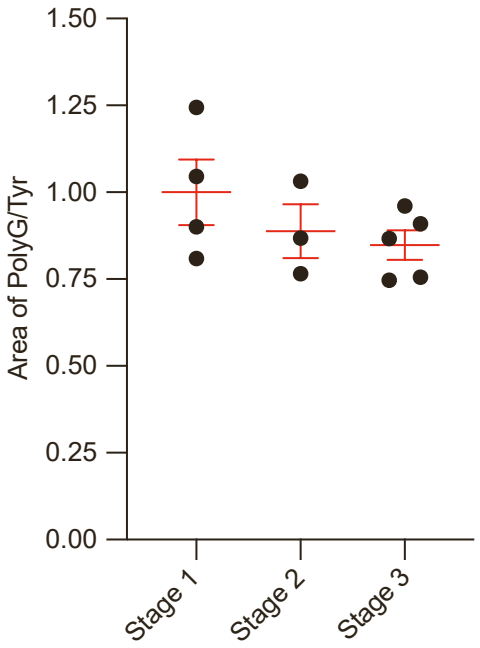
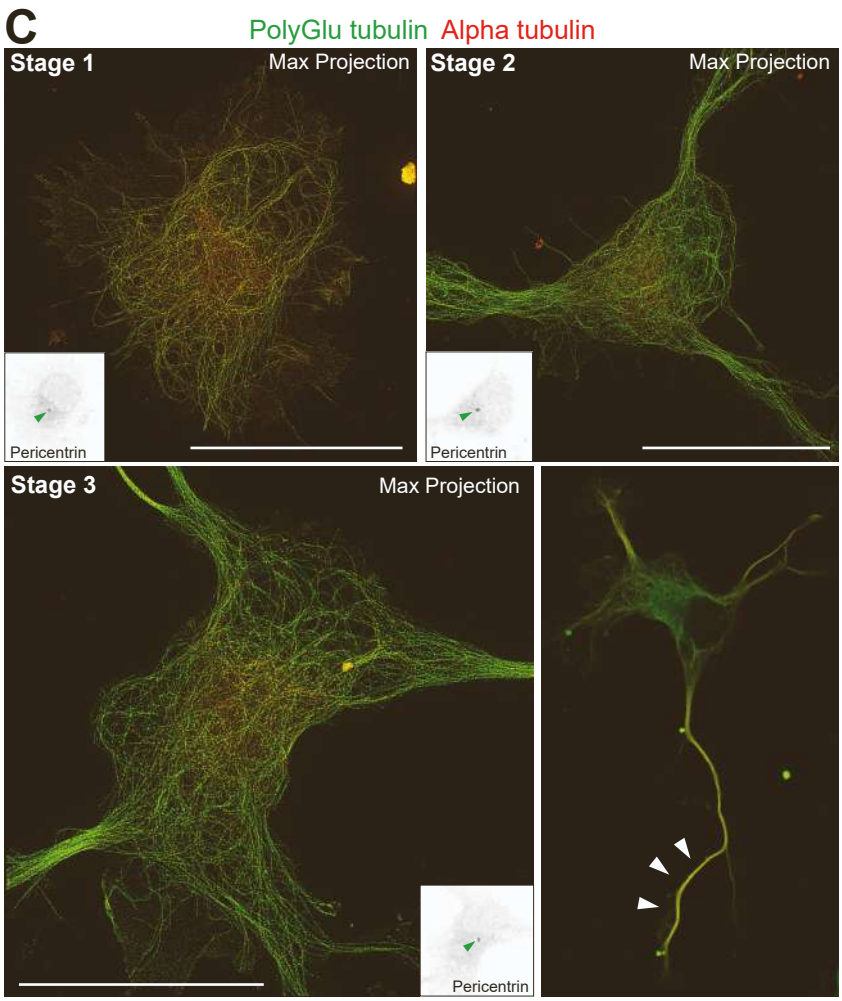
Figure S1


A
PolyGlu tubulin Tyrosinated tubulin
Stage 1
Max Projection
Pericentrin
Stage 2
Max Projection
Pericentrin
Stage 3
Max Projection
Pericentrin


B
Area of PolyG/Tyr
1.50
1.25
1.00
0.75
0.50
0.25
0.00
Stage 1
Stage 2
Stage 3


C
PolyGlu tubulin Alpha tubulin
Stage 1
Max Projection
Pericentrin
Stage 2
Max Projection
Pericentrin
Stage 3
Max Projection
Pericentrin

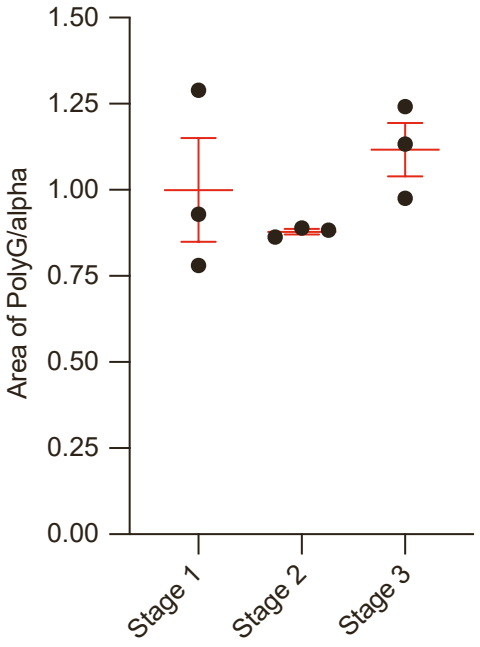

D
Area of PolyG/alpha
1.50
1.25
1.00
0.75
0.50
0.25
0.00
Stage 1
Stage 2
Stage 3

Figure S1. STED imaging analysis of polyglutamylated MTs distribution in relation to tyrosinated and α-tubulin around the centrosome in the soma of developing neurons. (related to Figure 1)

A. STED images of polyglutamylated and tyrosinated tubulin immunostaining in stage 1, stage 2, and stage 3 hippocampal neurons. White arrowheads point polyglutamylated tubulin enrichment in stage 2-3 cell and stage 3 growing axons. Insets: Centrosome labelled by pericentrin antibody staining pointed by green arrowhead. Scale bar: 10 μm

B. Quantifications from STED super resolution images show ratio of polyglutamylated to tyrosinated tubulin signal area in the soma of stage 1 (n = 4), stage 2(n = 3) and stage 3 (n = 5) neuronal soma. Mean ± SEM values for stage 1 neurons = 1.000 ± 0.0947 and stage 2 neurons = 0.8886 ± 0.07745, and stage 3 neurons = 0.8478 ± 0.0422. P = 0.3117 by one-way ANOVA, post hoc Tukey's test.

C. STED images of polyglutamylated and α-tubulin immunostaining in stage 1, stage 2, transition stage 2-3 cell and stage 3 hippocampal neurons. White arrowheads point polyglutamylated tubulin enrichment in stage 2-3 cell and stage 3 growing axons. Insets: Centrosome labelled by pericentrin antibody staining pointed by green arrowhead. Scale bar: 10 μm

D. Quantifications from STED super resolution images show ratio of polyglutamylated to α-tubulin signal area in the soma of stage 1 (n = 3), stage 2 (n = 3) and stage 3 (n = 3) neurons. Mean ± SEM values for stage 1 neurons = 1.000 ± 0.1512 and stage 2 neurons = 0.8785 ± 0.0079, and stage 3 neurons = 1.117 ± 0.0774. P = 0.3004 by one-way ANOVA, post hoc Tukey's test.

Figure S2

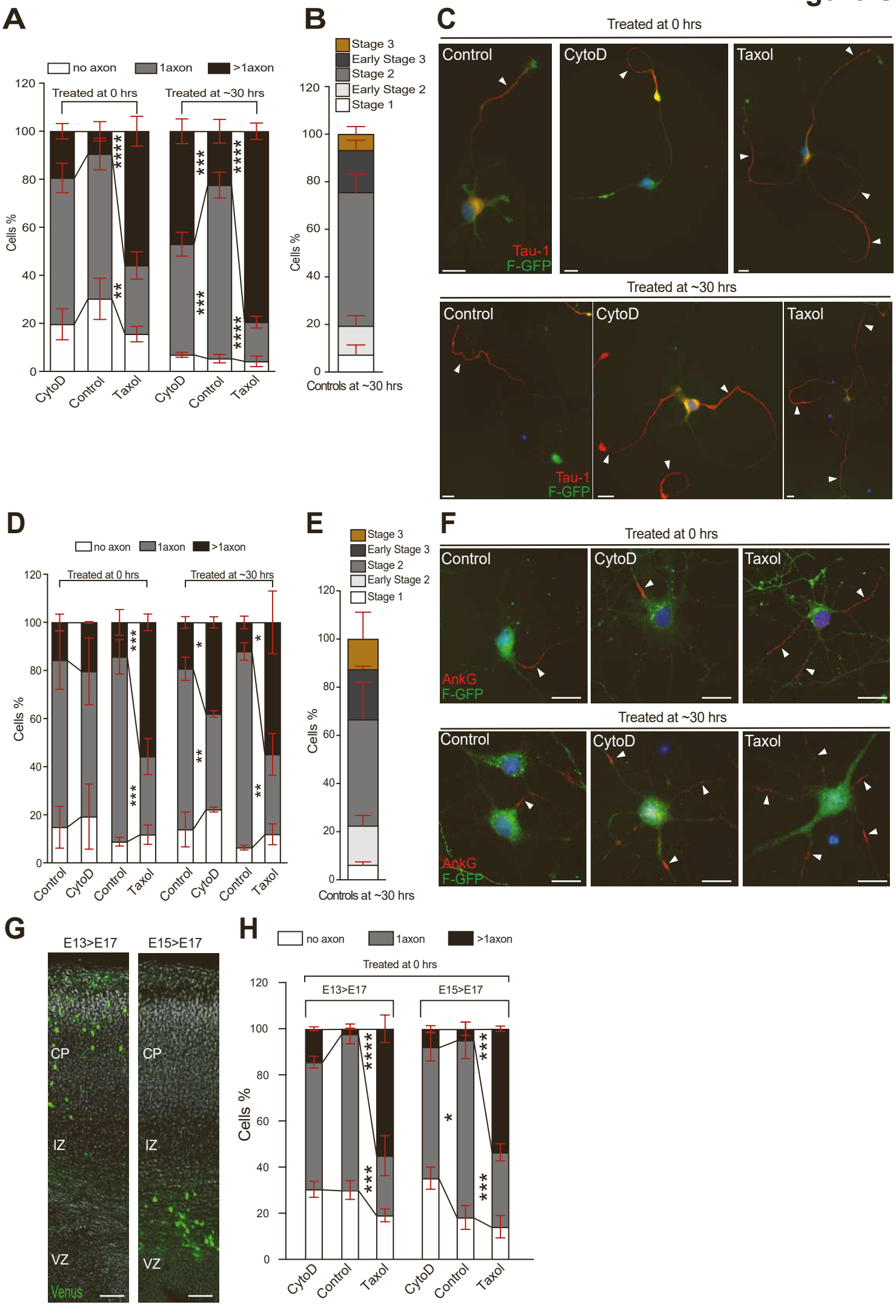

A
no axon
1axon
>1axon
Treated at 0 hrs
Treated at ~30 hrs
Cells %
CytoD
Control
Taxol
B
Stage 3
Early Stage 3
Stage 2
Early Stage 2
Stage 1
Controls at ~30 hrs
C
Treated at 0 hrs
Control
CytoD
Taxol
Tau-1
F-GFP
Treated at ~30 hrs
D
E
F
AnkG
F-GFP
G
E13>E17
E15>E17
CP
IZ
VZ
Venus
H

Figure S2. Contrary to MT stabilization (by Taxol), actin disruption (by CytoD) induced multipolarity is time-dependent – confirmed by Tau-1 and Ankyrin-G immunostaining. (related to Figure 3)

A. Quantifications show the percentage of neurons that were either treated immediately (0h)or ~ 30h of plating in vitro with DMSO or 2μM CytoD or 5nM Taxol for 48h differentiated to have no axon or 1 axon or more than 1 axon. Mean ± SEM values for percentage of cells treated at 0h with DMSO: no axons = 30.22 ± 8.554, 1 axon = 60.32 ± 6.606, more than 1 axon = 9.463 ± 3.979; CytoD: no axons = 19.65 ± 6.441, 1 axon = 60.94 ± 6.129, morethan 1 axon = 19.42 ± 3.226; Taxol: no axons = 15.56 ± 3.195, 1 axon = 28.54 ± 5.698, more than 1 axon = 55.91 ± 6.196; treated at ~ 30h with DMSO: no axons = 5.333 ± 1.781, 1 axon = 72.23 ± 5.354, more than 1 axon = 22.44 ± 4.914; CytoD: no axons = 6.898 ± 1.021, 1 axon = 46.09 ± 4.951, more than 1 axon = 47.02 ± 5.115; Taxol: no axons = 4.180 ± 2.181, 1 axon = 16.34 ± 2.419, more than 1 axon = 79.48 ± 3.403. α = 0.05 by two-way ANOVA, post hoc Tukey's test, **** P < 0.0001, *** P < 0.001, ** P < 0.01. Data is obtained from 4 different hippocampal cultures.

B. Quantifications show the percentage of neurons (untreated) after ~ 30h of plating in vitro in different stages of development, stage 1 to stage 3.

C. Images of Farnesylated-GFP (F-GFP) transfected neurons that were either treated immediately (0h) or ~ 30h of plating in vitro with DMSO or 2μM CytoD or 5nM Taxol for 48h. The cells are then PFA fixed for post hoc Tau-1 (shown in red) immunostainings to confirm axonal (indicated by white arrowheads) identity of the neurites. Scale bar: 10 μm.

D. Quantifications show the percentage of neurons that were either treated immediately (0h)or ~ 30h of plating in vitro with DMSO or 2μM CytoD or 5nM Taxol for 7 days differentiated to have no axon or 1 axon or more than 1 axon. For CytoD treatment group cells, fresh medium was exchanged after 16 hours to prevent cells from dying otherwise when incubated with CytoD for 7days. Mean ± SEM values for percentage of cells treated at 0h with DMSO (CytoD controls): no axons = 14.84 ± 8.690, 1 axon = 69.45 ± 12.10, more than 1 axon = 15.72 ± 3.405; CytoD: no axons = 19.24 ± 13.53, 1 axon = 60.42 ± 13.87, more than 1 axon = 20.35 ± 0.345; with DMSO (Taxol controls): no axons = 8.805 ± 1.805, 1 axon = 76.85 ± 7.150, more than 1 axon = 14.35 ± 5.350; Taxol: no axons = 11.74 ± 4.050, 1 axon = 32.50 ± 7.500, more than 1 axon = 55.76 ± 3.450. Mean ± SEM values for percentage of cells treated at ~ 30h Mean ± SEM values for percentage of cells treated at 0h with DMSO (CytoD controls): no axons = 13.90 ± 7.230, 1 axon = 66.82 ± 4.850, more than 1 axon = 19.29 ± 2.385; CytoD: no axons = 22.18 ± 1.030, 1 axon = 39.77 ± 1.305, more than 1 axon = 38.05 ± 2.335; with DMSO (Taxol controls): no axons = 6.380 ± 0.9700, 1 axon = 81.54 ± 3.600, more than 1 axon = 12.09 ± 2.625; Taxol: no axons = 11.92 ± 4.365, 1 axon = 33.20 ± 8.665, more than 1axon = 54.89 ± 13.03. α = 0.05 by two-way ANOVA, post hoc Sidak's test, *** P < 0.001, ** P < 0.01, ** P < 0.05. Data is obtained from 2 different hippocampal cultures.

E. Quantifications show the percentage of neurons (untreated) after ~ 30h of plating in vitro in different stages of development, stage 1 to stage 3.

F. Images of Farnesylated-GFP (F-GFP) transfected neurons that were either treated immediately (0h) or ~ 30h of plating in vitro with DMSO or 2μM CytoD or 5nM Taxol for 48h. The cells are then PFA fixed for post hoc Ankyrin-G (shown in red) immunostainings to confirm axonal (indicated by white arrowheads) identity of the neurites. Scale bar: 10 μm.

G. E17 mouse brain cortices that were either electroporated at E13 or E15 via IUE with Venus plasmids shows that the E13>E17 brains have more differentiated neurons mostly in cortical plate (CP), the E15>E17 brains have less developed neurons residing in the lower intermediate zone (IZ). Scale bar: 100 μm.

H. Quantifications show the percentage of cortical neurons from E13 versus E15 IUE brains transfected with Venus plasmid cultured at E17 that were treated immediately (0h) after plating with DMSO or 2μM CytoD or 5nM Taxol for 2 days differentiated to have no axon or 1 axon or more than 1 axon. Axonal identities in the PFA-fixed cells are confirmed by Tau-1 immunostainings.

Mean ± SEM values for percentage for E13 IUE Control group – CytoD (n = 111 cells): no axons = 30.41 ± 3.490, 1 axon = 55.10 ± 2.595, more than 1 axon = 14.49 ± 0.8900; untreated controls (n = 91 cells): no axons = 30.08 ± 4.075, 1 axon = 67.71 ± 4.295, more than 1 axon = 2.220 ± 0.2200; Taxol (n = 119 cells): no axons = 19.13 ± 2.770, 1 axon = 25.88 ± 8.675, more than 1 axon = 55.00 ± 5.905.

Mean ± SEM values for percentage for E15 IUE Control group – CytoD (n = 111 cells): no axons = 35.22 ± 4.785, 1 axon = 56.92 ± 6.120, more than 1 axon = 7.860 ± 1.340; untreated controls (n = 123 cells): no axons = 18.22 ± 5.180, 1 axon = 76.79 ± 7.990, more than 1 axon = 4.985 ± 2.815; Taxol (n = 128 cells): no axons = 14.18 ± 4.875, 1 axon = 32.29 ± 3.715, more than 1 axon = 53.54 ± 1.160.

α = 0.05 by two-way ANOVA, post hoc Tukey's test, **** P < 0.0001, *** P < 0.001, * P < 0.05. Data is obtained from cortical cultures of 3 or more IUE embryos from 2 different mothers.

Figure S3

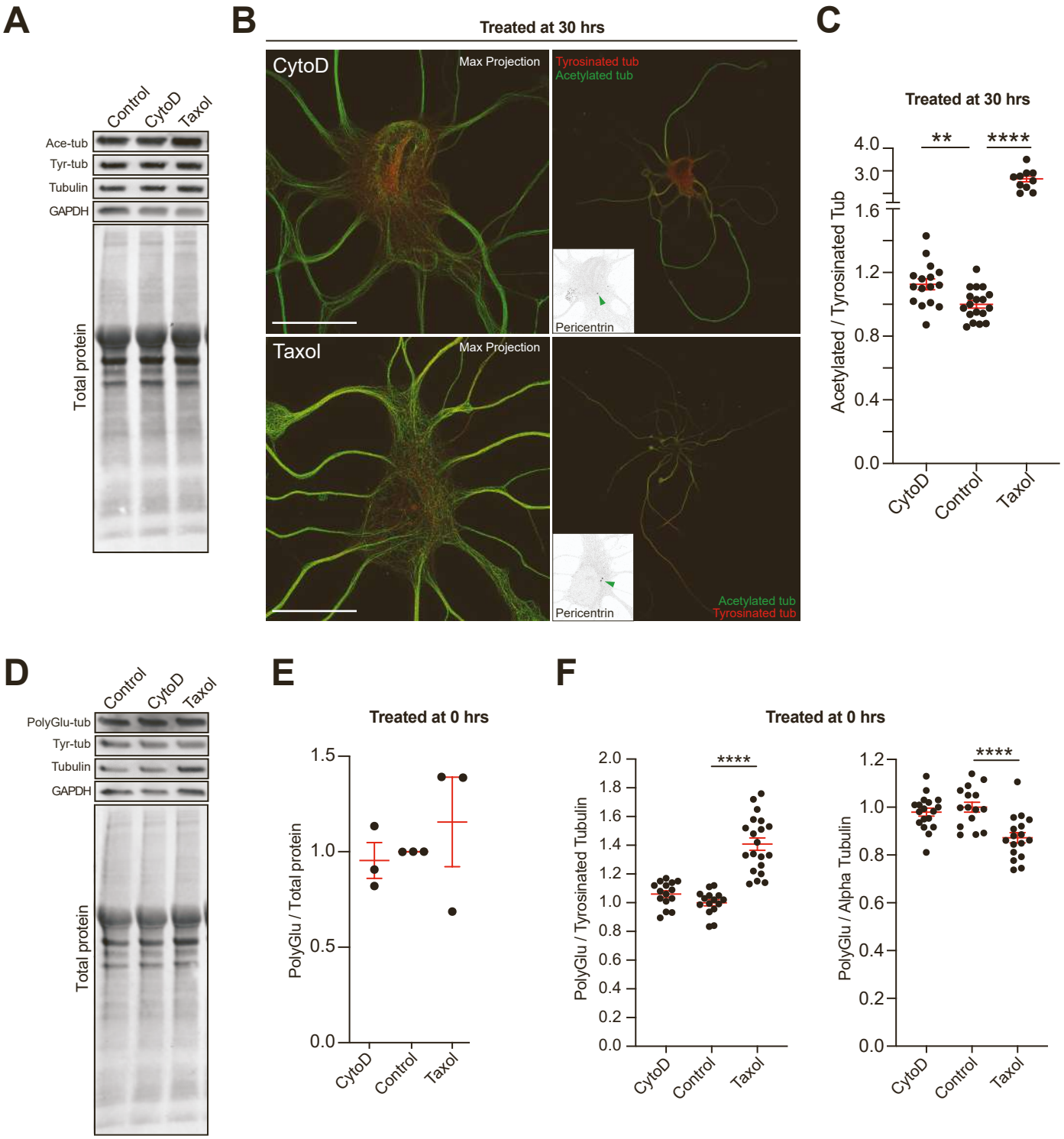

A
Control
CytoD
Taxol
Ace-tub
Tyr-tub
Tubulin
GAPDH
Total protein
B
Treated at 30 hrs
CytoD
Max Projection
Tyrosinated tub
Acetylated tub
Pericentrin
Taxol
Max Projection
Pericentrin
Acetylated tub
Tyrosinated tub
C
Treated at 30 hrs
**
****
Acetylated / Tyrosinated Tub
4.0
3.0
1.6
1.2
0.8
0.4
0.0
CytoD
Control
Taxol
D
Control
CytoD
Taxol
PolyGlu-tub
Tyr-tub
Tubulin
GAPDH
Total protein
E
Treated at 0 hrs
PolyGlu / Total protein
1.5
1.0
0.5
0.0
CytoD
Control
Taxol
F
Treated at 0 hrs
****
PolyGlu / Tyrosinated Tubulin
2.0
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
CytoD
Control
Taxol
****
PolyGlu / Alpha Tubulin
1.2
1.0
0.8
0.6
0.4
0.2
0.0
CytoD
Control
Taxol

Figure S3. Differential regulation of Tubulin PTMs upon CytoD and Taxol treated developing primary neurons. (related to Figure 3)

A. Western blot images of tubulin PTMs (acetylated, tyrosinated), total tubulin, GAPDH and Revert loading (for labeling total protein) from rat cortical neuron lysates treated with 2μM CytoD and 5nM Taxol immediately (0h) after plating and cultured for 18h. Same blots were shown in **Figure 3F** without Revert loading (for labeling total protein).

B. STED images of rat hippocampal neurons treated with 2μM CytoD and 5nM Taxol immediately (30h) after plating, and PFA-fixed 48h later, immunostained for tubulin PTMs (acetylated and tyrosinated). Scale bar: 10 μm.

C. Quantifications compare ratio of acetylated to tyrosinated tubulin signal intensities in the soma of untreated, 2μM CytoD and 5nM Taxol treated neurons ~ 28h after plating, and PFA-fixed 18h later. Mean ± SEM values of Control cells = 1.000 ± 0.02338, CytoD treated cells = 1.126 ± 0.03439, Taxol treated cells = 2.646 ± 0.1255. $P < 0.0001$ by one-way ANOVA, post hoc Tukey's test, **** $P < 0.0001$ for Control vs. Taxol groups. ** $P = 0.0041$ by unpaired Student's t-test for Control vs. CytoD groups. n = 18 in Control, 16 in CytoD and 12 in Taxol groups.

D. Western blot images of Tubulin PTMs (polyglutamylated, tyrosinated), total tubulin, GAPDH and Revert loading (for labeling total protein) from rat cortical neuron lysates treated with 2μM CytoD and 5nM Taxol immediately (0h) after plating and cultured for 18h

E. Quantifications compare normalized polyglutamylated to total tubulin levels from rat cortical neuron lysates from untreated, treated with 2μM CytoD and 5nM Taxol immediately (0h) after plating, and cultured for 18h Cell lysates are obtained from three different experiments.

F. Quantifications compare ratio of polyglutamylated to tyrosinated tubulin signal (left) and polyglutamylated to α-tubulin signal (right) intensities in the soma of untreated, 2μM CytoD and 5nM Taxol treated neurons 0h after plating, and PFA-fixed 18h later.

Polyglutamylated to tyrosinated tubulin: Mean ± SEM values of Control cells = 0.9998 ± 0.0214, CytoD treated cells = 1.061 ± 0.02252, Taxol treated cells = 1.408 ± 0.0435. $P < 0.0001$ by one-way ANOVA, post hoc Tukey's test, **** $P < 0.0001$. n = 15 in Control, 15 in CytoD and 20 in Taxol groups.

Polyglutamylated to α-tubulin: Mean ± SEM values of Control cells = 1.000 ± 0.02103, CytoD treated cells = 0.9794 ± 0.01698, Taxol treated cells = 0.8721 ± 0.02129. $P < 0.0001$ by one-way ANOVA, post hoc Tukey's test, **** $P < 0.0001$. n = 15 in Control, 18 in CytoD and 17 in Taxol groups.

Figure S4

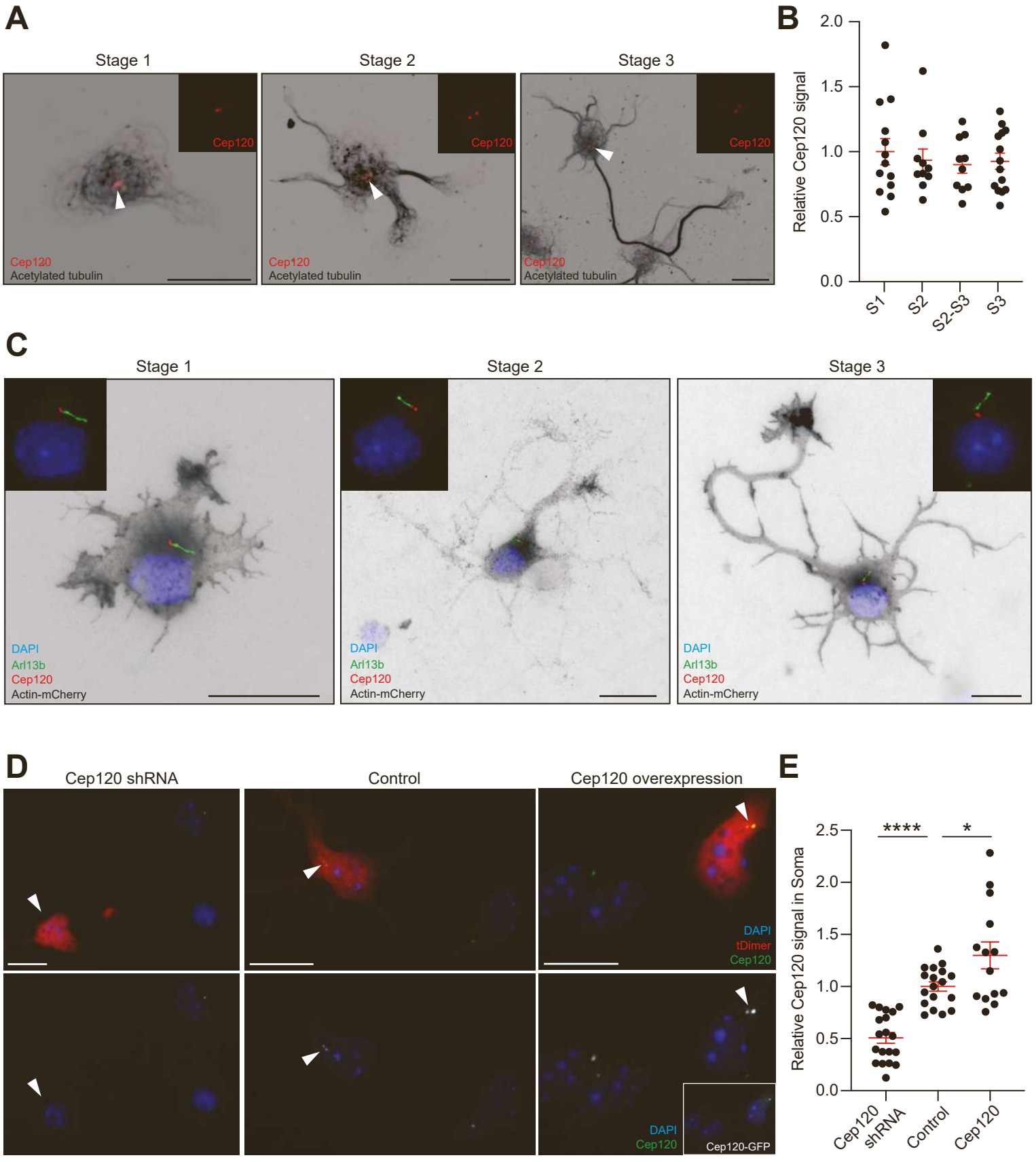

A
Stage 1
Stage 2
Stage 3
Cep120
Acetylated tubulin
B
Relative Cep120 signal
2.0
1.5
1.0
0.5
0.0
S1
S2
S2-S3
S3
C
Stage 1
Stage 2
Stage 3
DAPI
Arl13b
Cep120
Actin-mCherry
D
Cep120 shRNA
Control
Cep120 overexpression
DAPI
tDimer
Cep120
Cep120-GFP
E
Relative Cep120 signal in Soma
2.5
2.0
1.5
1.0
0.5
0.0
****
*
Cep120 shRNA
Control
Cep120

Figure S4. Characterization of Cep120 levels in developing primary neurons and efficient knockdown in Cep 120 knockdown and overexpression. (related to Figure 4)

A. Cep120 localization in developing rat hippocampal neurons, stage 1, stage 2 and stage 3 neurons, co-stained with acetylated tubulin. Insets and white arrowheads point the localization of Cep120 staining at the centrioles. Scale bar: 10 μm.

B. Quantifications showing relative Cep120 levels in stage 1 (n = 13), stage 2 (n = 10), stage 2-3 (n = 10) and stage 3 (n = 14) rat hippocampal neurons. Mean ± SEM values for stage 1 neurons = 1.000 ± 0.1005 and stage 2 neurons = 0.9338 ± 0.08733, stage 2-3 neurons = 0.9012 ± 0.06636 and stage 3 neurons = 0.9264 ± 0.06257. P = 0.2740 by one-way ANOVA, post hoc Tukey's test.

C. Cep120 and Arl13b (primary cilia marker) localization in developing rat hippocampal neurons, stage 1, stage 2 and stage 3 neurons transfected with Actin-mCherry. Scale bar: 10 μm.

D. Images showing efficient knockdown and overexpression of Cep120 in utero electroporated with Cep120sh and Cep120-GFP, respectively, compared to control mouse cortical neurons. White arrowheads point the localization of Cep120 staining at the centrioles in the Control and Cep120-GFP transfected cells, but not in the Cep120sh cells. Scale bar: 10 μm.

E. Quantifications showing relative Cep120 levels in control (n = 18), Cep120sh (n = 19) and Cep120-GFP (n = 14) expressing mouse cortical neurons. Mean ± SEM values for Control neurons = 1.000 ± 0.04436 and Cep120sh neurons = 0.5072 ± 0.0524, and Cep120-GFP neurons = 1.299 ± 0.1286. P < 0.0001 by one-way ANOVA, post hoc Tukey's test, **** P < 0.0001, * P = 0.0238.

Figure S5

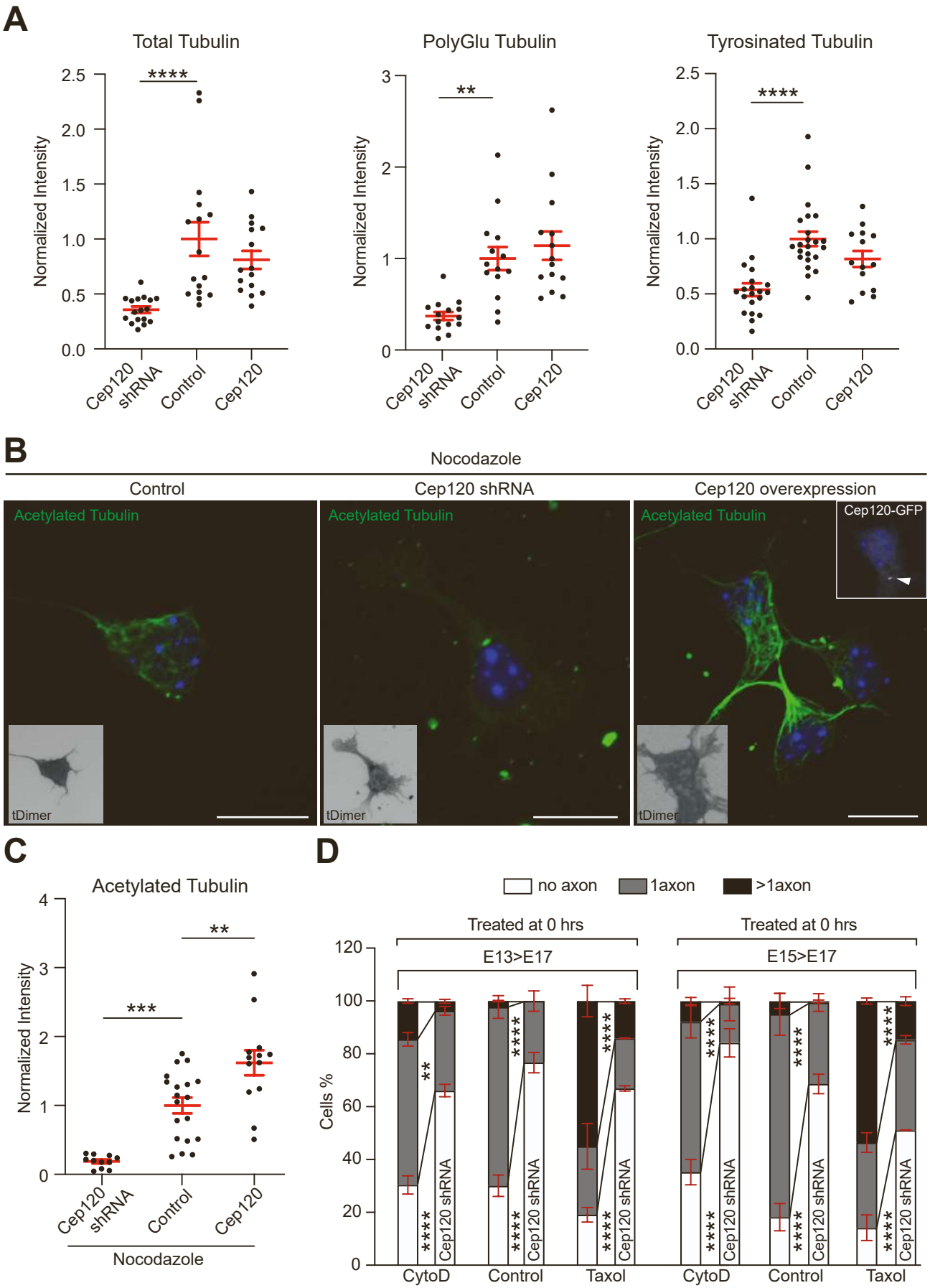

A
Total Tubulin
PolyGlu Tubulin
Tyrosinated Tubulin
Normalized Intensity
****
**
Cep120 shRNA
Control
Cep120
B
Nocodazole
Control
Cep120 shRNA
Cep120 overexpression
Acetylated Tubulin
Cep120-GFP
tDimer
C
Acetylated Tubulin
***
**
Nocodazole
D
no axon
1axon
>1axon
Treated at 0 hrs
E13>E17
E15>E17
Cells %
CytoD
Control
Taxol

Figure S5. Effect of Cep 120 knockdown and overexpression on tubulin PTMs and axon formation. (related to Figure 4)

A. Quantifications (left to right) compare normalized Total tubulin, polyglutamylated and tyrosinated tubulin intensities in the soma of neurons expressing control, Cep120 shRNA, and Cep120-GFP.

Total tubulin: Mean ± SEM values of control cells = 1.000 ± 0.1525, Cep120 shRNA cells = 0.3575 ± 0.0285 and Cep120-GFP cells = 0.8107 ± 0.0817. P = 0.0001 by one-way ANOVA, post hoc Tukey's test, **** P < 0.0001, ** P = 0.0071. n = 16 control, 17 Cep120sh and 15 Cep120-GFP cells

Poly-glutamylated tubulin: Mean ± SEM values of control cells = 1.000 ± 0.1264, Cep120 shRNA cells = 0.3721 ± 0.0437 and Cep120-GFP cells = 1.142 ± 0.1150. P < 0.0001 by one-way ANOVA, post hoc Tukey's test, **** P < 0.0001, ** P = 0.0012. n = 14 control, 15 Cep120sh and 14 Cep120-GFP cells

Tyrosinated tubulin: Mean ± SEM values of control cells = 1.000 ± 0.0658, Cep120 shRNA cells = 0.5385 ± 0.0575 and Cep120-GFP cells = 0.8175 ± 0.0732. P < 0.0001 by one-way ANOVA, post hoc Tukey's test, **** P < 0.0001, * P = 0.0193. n = 23 control, 20 Cep120sh and 14 Cep120-GFP cells.

B. Confocal maximum projection images of mouse cortical neurons co-transfected at E15 via IUE with tDimer and Cep120 shRNA, or control or Cep120-GFP (indicated by arrowhead in the inset) cultured at E17 for 48h were treated with 6μM Nocodazole for 30min before fixing the cells with 4%PFA for 2min followed by 3min ice-cold Methanol immunostained with acetylated tubulin antibody. Scale bar: 10 μm.

C. Quantifications compare normalized acetylated tubulin intensities in the soma of Nocodazole-treated neurons expressing, control (n = 19), Cep120 shRNA (n = 11), and Cep120-GFP (n = 13) as shown in b. Mean ± SEM values of control cells = 1.000 ± 0.1134, Cep120 shRNA cells = 0.1879 ± 0.0279 and Cep120-GFP cells = 1.620 ± 0.1803. P < 0.0001 by one-way ANOVA, post hoc Tukey's test, **** P < 0.0001, *** P = 0.0002, ** P = 0.0030.

Data shown in **A-C** is obtained from cortical cultures of 3 or more IUE embryos from 2 different mothers.

D. Quantifications show the percentage of cortical neurons from E13 versus E15 IUE brains co-transfected with Cep120sh and Venus plasmid cultured at E17 that were treated immediately (0h) after plating with 2μM CytoD or 5nM Taxol for 2 days differentiated to have no axon or 1 axon or more than 1 axon. Axonal identities in the PFA-fixed cells are confirmed by Tau-1 immunostainings.

Mean ± SEM values for percentage for E13 IUE Control group – CytoD (n = 111 cells): no axons = 30.41 ± 3.490, 1 axon = 55.10 ± 2.595, more than 1 axon = 14.49 ± 0.8900; untreated controls (n = 91 cells): no axons = 30.08 ± 4.075, 1 axon = 67.71 ± 4.295, more than 1 axon = 2.220 ± 0.2200; Taxol (n = 119 cells): no axons = 19.13 ± 2.770, 1 axon = 25.88 ± 8.675, more than 1 axon = 55.00 ± 5.905.

Mean ± SEM values for percentage of cells in E13 IUE Cep120 shRNA group – CytoD (n = 82 cells): no axons = 66.19 ± 2.385, 1 axon = 30.24 ± 1.665, more than 1 axon = 3.580 ± 0.7200; untreated controls (n = 121 cells): no axons = 76.74 ± 3.860, 1 axon = 23.26 ± 3.860, more than 1 axon = 00.00 ± 00.00; Taxol (n = 100 cells): no axons = 66.93 ± 0.9700, 1 axon = 19.03 ± 0.1250, more than 1 axon = 14.05 ± 0.8450.

Mean ± SEM values for percentage for E15 IUE Control group – CytoD (n = 111 cells): no axons = 35.22 ± 4.785, 1 axon = 56.92 ± 6.120, more than 1 axon = 7.860 ± 1.340; untreated controls (n = 123 cells): no axons = 18.22 ± 5.180, 1 axon = 76.79 ± 7.990, more than 1 axon = 4.985 ± 2.815; Taxol (n = 128 cells): no axons = 14.18 ± 4.875, 1 axon = 32.29 ± 3.715, more than 1 axon = 53.54 ± 1.160.

Mean ± SEM values for percentage of cells in E15 IUE Cep120 shRNA group – CytoD (n = 148 cells): no axons = 84.23 ± 5.375, 1 axon = 14.73 ± 6.425, more than 1 axon = 1.050 ± 1.050; untreated controls (n = 175 cells): no axons = 68.71 ± 3.705, 1 axon = 30.90 ± 3.305, more than 1 axon = 0.450 ± 0.450; Taxol (n = 172 cells): no axons = 51.11 ± 0.090, 1 axon = 34.23 ± 1.575, more than 1 axon = 14.67 ± 1.665.

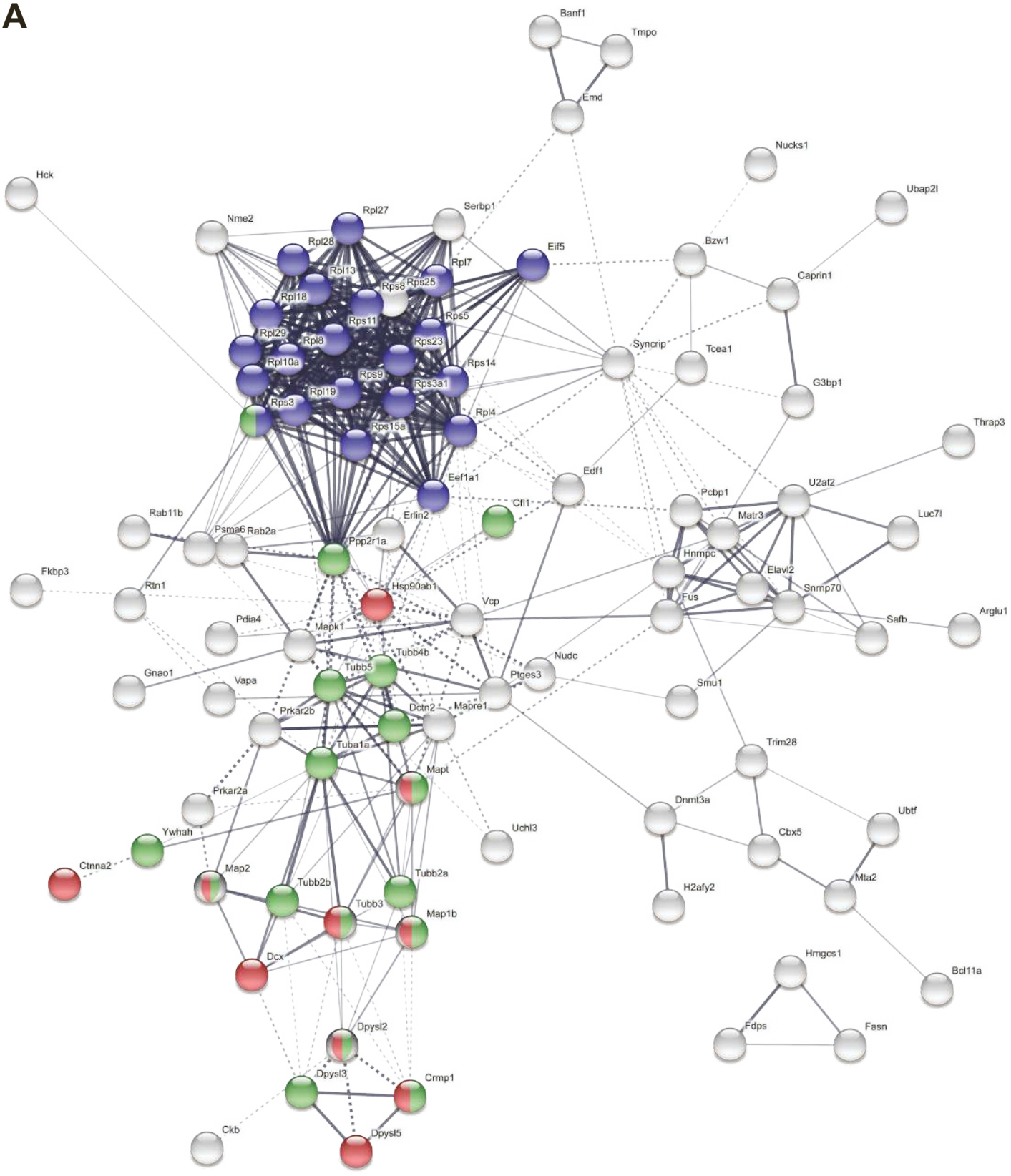
α = 0.05 by two-way ANOVA, post hoc Tukey's test, **** P < 0.0001, *** P < 0.001, * P < 0.05.

Data is obtained from cortical cultures of 3 or more IUE embryos from 2 different mothers.

Note: Experiments in **Figure S2G**, **S2H** and **Figure S5D** were done at the same time. So, neurons in the E13 and E15 IUE Control transfection group (untreated controls, CytoD and Taxol) cells were the same as the ones shown in **Figure S2H**.

Figure S6

A

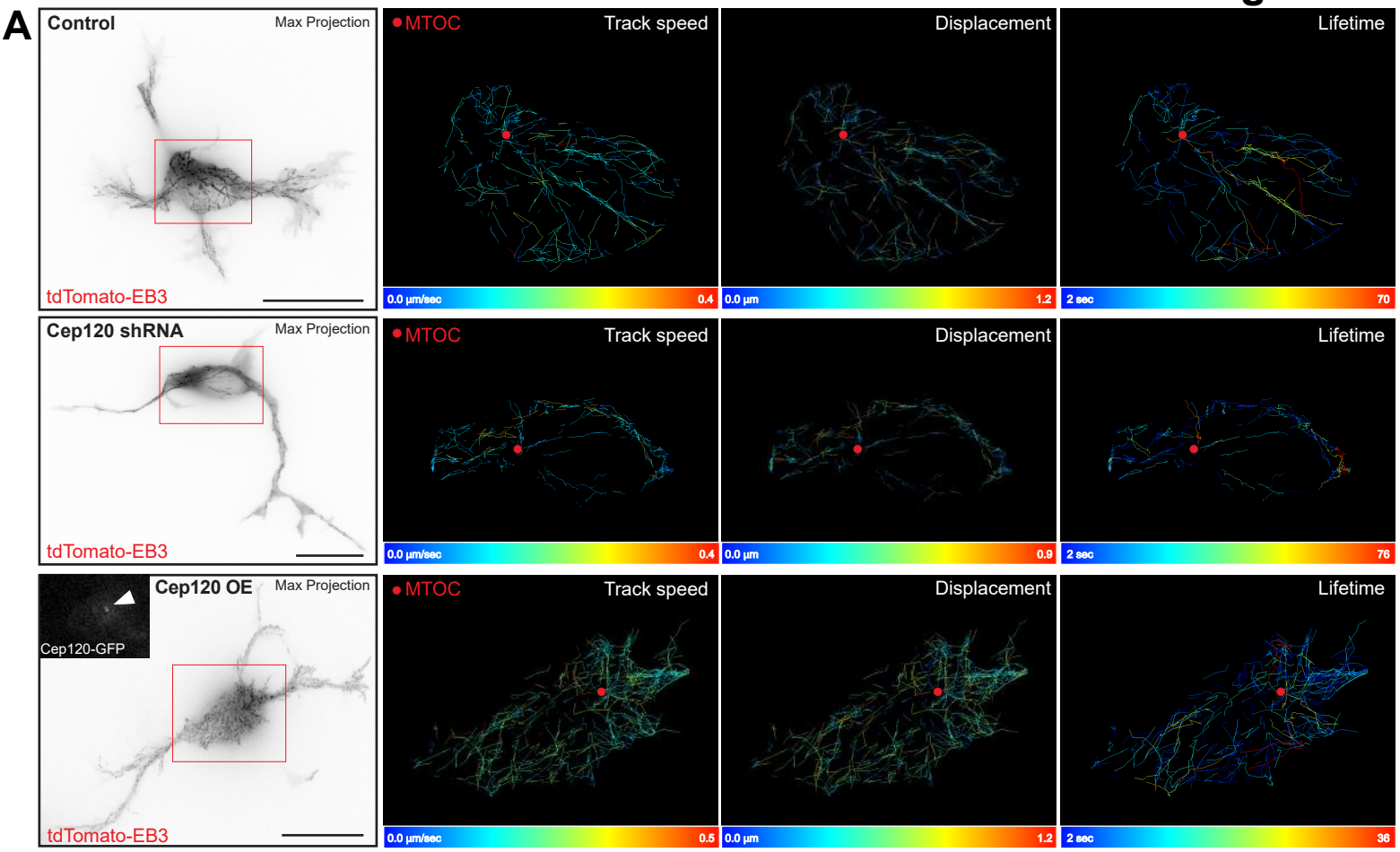


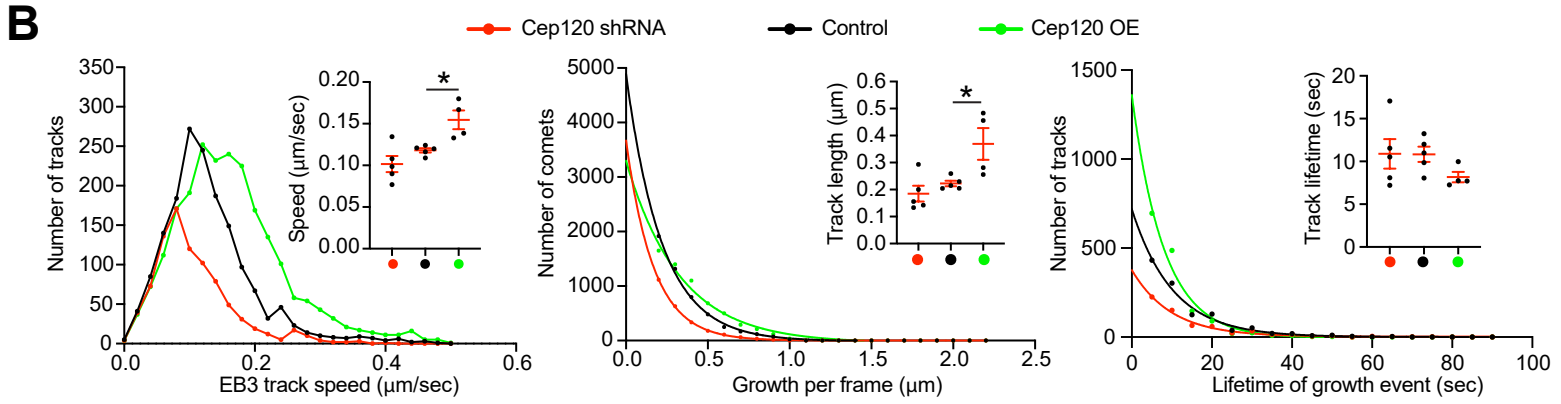
	GO-term	description	count in gene set	false discovery rate
1	GO:0006412	translation	21 of 313	4.28E-15
1	GO:0007010	cytoskeleton organization	17 of 916	4.18E-05
1	GO:0007409	axonogenesis	10 of 310	7.84E-05

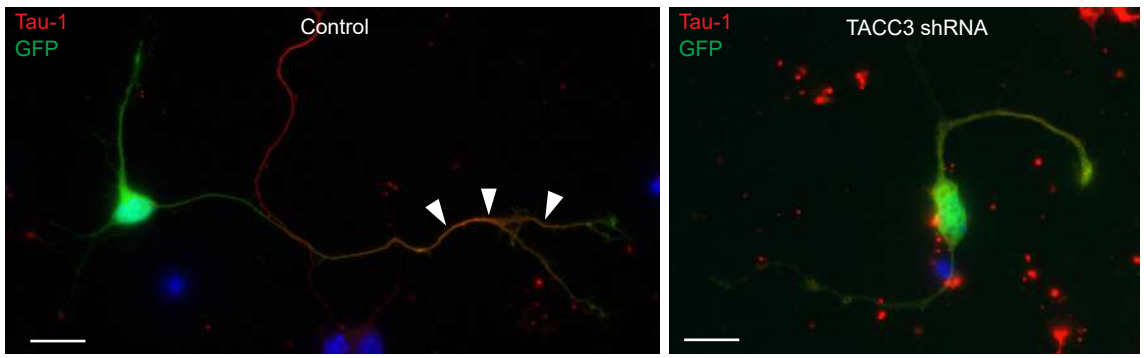
Figure S6. STRING analysis visualization of the proteins that were significantly alteredbetween the control and Cep120 shRNA conditions (P < 0.05). (related to Figure 5)

A. STRING analysis visualization of all the proteins whose levels were significantly changed between the control and Cep120 shRNA conditions (P < 0.05). For simplicity, the disconnected proteins are hidden in the visualization and the "confidence" link visualization is selected (highlighting the strength of data support in each network edge). The three following GO terms are colored and detailed as shown in the legend at the bottom of the figure: GO:0007409-axonogenesis; GO:0007010-cytoskeletal organization and GO:0006412-translation. (See Supplementary excel file 3. LC-MSMS STRING.xlsx for details).

Figure S7


A
Control
Max Projection
tdTomato-EB3
MTOC
Track speed
Displacement
Lifetime
0.0 µm/sec
0.4
0.0 µm
1.2
2 sec
70
Cep120 shRNA
Max Projection
tdTomato-EB3
MTOC
Track speed
Displacement
Lifetime
0.0 µm/sec
0.4
0.0 µm
0.9
2 sec
76
Cep120 OE
Max Projection
Cep120-GFP
tdTomato-EB3
MTOC
Track speed
Displacement
Lifetime
0.0 µm/sec
0.5
0.0 µm
1.2
2 sec
36


B
Cep120 shRNA
Control
Cep120 OE
Number of tracks
EB3 track speed (µm/sec)
Speed (µm/sec)
Number of comets
Growth per frame (µm)
Track length (µm)
Number of tracks
Lifetime of growth event (sec)
Track lifetime (sec)


C
Tau-1
GFP
Control
Tau-1
GFP
TACC3 shRNA

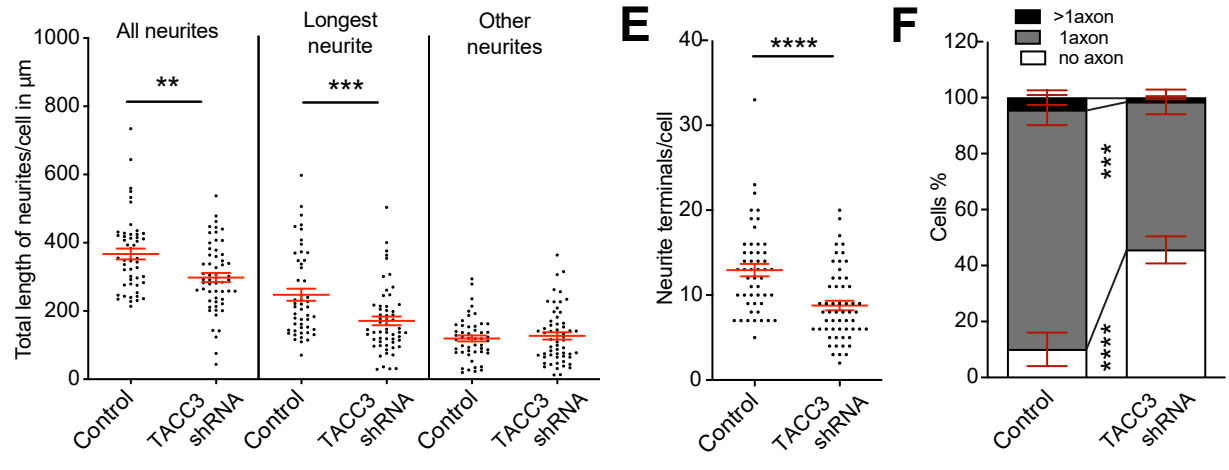

D
All neurites
Longest neurite
Other neurites
Total length of neurites/cell in µm
Control
TACC3 shRNA
E
Neurite terminals/cell
F
>1axon
1axon
no axon
Cells %

Figure S7. MT plus-end (EB3) dynamics are modulated by Cep120. (related to Figure 5 and Figure 7)
A. Images showing maximum intensity projections (black on white images) from a 5 min time- lapse (1 frame every 2 seconds) of stage 2 mouse cortical neurons co-transfected (via IUE at E15) with EB3-tdTomato and control or Cep120 shRNA or Cep120-GFP (indicated by arrowhead in the inset). Speed of EB3 tracks, displacement (per frame) and lifetime of EB3 tracks (analysed by TrackMate Fiji - ImageJ plugin) in the soma from the respective cells were shown as heatmaps. Red dot in the heatmaps indicate MTOC. Scale bar: 10 µm.
B. Left panel: Frequency distribution histogram compares Speed of EB3 tracks (µm/sec) in the soma of Cep120 shRNA, control, and Cep120-GFP expressing neurons. Inset shows values of median speed (µm/sec) obtained from all the three groups. Mean ± SEM values of median speed in the soma of Cep120 shRNA cells = 0.1016 ± 0.009650, control cells = 0.1181 ± 0.002579, Cep120-GFP cells = 0.1546 ± 0.01126. P = 0.0033 by one-way ANOVA, post hoc Tukey's test, * P = 0.0275.
Middle panel: Nonlinear fit histogram compares displacement of EB3 (in µm) per each frame (2sec) in the soma of Cep120 shRNA, control, and Cep120-GFP expressing neurons. Inset shows characteristic growth (λ) per frame, which is the half values of displacement per frame obtained from all the three groups. Mean ± SEM values of the characteristic growth (λ) per each frame in the soma of Cep120 shRNA cells = 0.1850 ± 0.0294, control cells = 0.2225 ± 0.01029, Cep120-GFP cells = 0.3690 ± 0.0585. P = 0.0091 by one-way ANOVA, post hoc Tukey's test, * P = 0.0330.
Right panel: Nonlinear fit histogram compares growth lifetime of EB3 tracks (in sec) in the soma of Cep120 shRNA, control, and Cep120-GFP expressing neurons. Inset shows growth half lifetime (τ) obtained from all the three groups. Mean ± SEM values of growth half lifetime (τ) in the soma of Cep120 shRNA cells = 10.88 ± 1,731, control cells = 10.83 ± 0,8904, Cep120-GFP cells = 8.170 ± 0.6093. P = 0.2840 by one-way ANOVA, post hoc Tukey's test.
For data shown in **B**, EB3 tracks (left and right panels) analyzed per cell in the respective stages are as follows: Control (n=5): cell 1 = 143; cell 2 = 512; cell 3 = 239; cell 4 = 518; cell 5 = 232. Cep120 shRNA (n=5): cell 1 = 237; cell 2 = 139; cell 3 = 252; cell 4 = 165; cell 5 = 86. Cep120 OE (n=4): cell 1 = 404; cell 2 = 498; cell 3 = 659; cell 4 = 668. EB3 comets (middle panel) analyzed per cell in the respective stages are as follows: Control (n=5): cell 1 = 629; cell 2 = 2023; cell 3 = 1210; cell 4 = 2536; cell 5 = 1225. Cep120 shRNA (n=5): cell 1 = 1128; cell 2 = 982; cell 3 = 1178; cell 4 = 721; cell 5 = 473. Cep120 OE (n=4): cell 1 = 1178; cell 2 = 2204; cell 3 = 2298; cell 4 = 1990. Cells are obtained at least from 3 or more IUE embryos obtained from 2 different mothers.
C. Epifluorescence images of mouse cortical neurons transfected via IUE at E15 with control shRNA-GFP or TACC3 shRNA-GFP cultured at E17 for 48 or 72h immunostained with Tau-1 antibody to confirm axonal identity (indicated by white arrow heads). Scale bar: 10 µm.
D. Neurite length quantification in neurons (in µm) control shRNA-GFP (n = 50) and TACC3 shRNA-GFP (n = 57) expressing neurons. Mean ± SEM values for length of all neurites in control cells = 367.1 ± 15.77, TACC3 shRNA-GFP cells = 298.1 ± 13.35. Mean ± SEM values for length of longest neurite in control cells = 247.6 ± 17.60, TACC3 shRNA-GFP cells = 171.0 ± 12.88. Mean ± SEM values for length of other neurites in control cells = 119.4 ± 8.770, TACC3 shRNA-GFP cells = 127.0 ± 10.65. ** P = 0.0011, *** P = 0.0005 by unpaired Student's t- test.
E. Neurite terminals quantification in control shRNA-GFP (n = 50) and TACC3 shRNA-GFP (n = 57) expressing neurons. Mean ± SEM values for neurite terminal per cell in control neurons = 12.94 ± 0.7315, TACC3 shRNA-GFP cells = 8.772 ± 0.5636. **** P < 0.0001 by unpaired Student's t-test.
F. Quantifications show the percentage of neurons expressing control shRNA-GFP (n = 225 cells) and TACC3 shRNA-GFP (n = 243 cells), differentiated to have no axon, 1 axon and more than 1 axon. Mean ± SEM values for percentage of control cells with no axon = 10.08 ± 5.975, 1 axon = 85.50 ± 5.366, more than 1 axon = 4.430 ± 2.623; percentage of TACC3 shRNA cells with no axon = 45.59 ± 4.835, 1 axon = 52.88 ± 4.406, more than 1 axon = 1.533 ± 0.5436.α = 0.05 by two-way ANOVA, post hoc Sidak's test, **** P < 0.0001, *** P < 0.001.
For data shown in **D**, **E** and **F** cells were from cortical cultures prepared from 3 or more IUE embryos obtained from at least 2 different mothers.