Supplemental Materials

Molecular Biology of the Cell

Chrystelle et al.

SUPPLEMENTAL MATERIAL

Supplemental figure legends.

Figure S1. αΔ3-tubulin is present in all regions of adult mouse brain. Equal quantities of proteins extracted from various regions of neonate mouse brain were subjected to immunoblot for analysis of αΔ3-tubulin presence (with 3EG antibody). Protein levels were controlled using αtot antibody.

Figure S2. Immunoblots for quantitative analysis of α-tubulin variants in mouse brain. Proteins extracts from neonate and adult mice brains were co-analyzed with extracts from HEK293T cells transfected with mCherry-α-tubulin variants, including tyrosinated, detyrosinated, αΔ2 and αΔ3. α tot antibody was used to control loading of cell extracts and mCherry tubulin variant (95kDa) expression levels.

Figure S3. Cleavage of mCherry-αdeTyr-tubulin by CCP1 and CCP5 enzymes. Immunoblots of protein extracts from HEK293T cells co-expressing mCherry-αdeTyr-tubulin and the CCP enzymes. αtot antibody was used to control the expression level of mCherry-αdeTyr-tubulin (95kDa) in cells. mCherry tubulin variants produced from the detyrosinated form were analyzed.

Figure S4. Presence of the lower band stained by 3EG in purified tubulin preparations. Immunoblot of tubulin purified from adult and neonate brains and the corresponding Coomassie-stained gel (bottom). Tubulin monomers are indicated by arrows. Tubulin preparation was performed by tissue homogeneisation in 100 mM Pipes, 1 mM $MgCl_2$, 1 mM EGTA followed by a cycle of microtubule assembly and disassembly.

Figure S5. βΔ4-tubulin evidenced in fibroblasts. Immunocytochemistry of murine embryonic fibroblasts using 3EG and βtot antibodies. Scale bar, 10 μM.

Figure S6. Distribution of αtubulin variants in 2DIV hippocampal neurons. (A) Immunofluorescence images of microtubules bearing αΔ3 and βΔ4-tubulin (3EG-positive microtubules), αΔ2-, αdeTyr- and αΔTyr-tubulin in the neurons. Scale bar = 10 μm. (B) Quantitative analysis (mean ± SEM) of immunofluorescence signals of microtubules from axon shafts and from growth cones. Variant signal (Fvariant) was normalized to αtot signal (Fαtot, which is an index of the remaining microtubules) and plotted in gray values (***): $P<0.001$, (*): $P<0.1$with a *t* test. The 3EG immunoreactivity, including both αΔ3- and βΔ4-tubulin, is bright throughout hippocampal neurons. It is even intense on microtubules present in the growth cone, like the αTyr staining (see growth cone's magnifications). In contrast, αdeTyr- and αΔ2-microtubular staining significantly decrease at the transition zone between axon and beginning of growth cone (see quantifications in B). Growth cones contain however detectable amounts of αΔ2-tubulin which seem not associated to microtubules. Comparable results were already described for αTyr-, αdeTyr- and αΔ2-signals with rat cerebellar neurons (Arregui *et al.*, 1991; Paturle-Lafanechere *et al.*, 1994).

Supplemental Tables

Table S1. Quantitative analysis of figure 1A. Western-blots of mCherry-tubulin variants expressed in HEK293T were analyzed using ImageJ. Each mCherry-tubulin variant signal was normalized to αtot signal and was then plotted as percent (with the sum of the variants signals corresponding to 100%). Signals obtained with the mCherry-αdeTyr extract using αTyr and αΔ2 antibodies very probably correspond to processing of the expressed protein in HEK293T cells and not to cross-reactions of antibodies. (*) Signal observed with mChe-αdeTyr extract using αTyr antibody is due to the addition of a tyrosine on the expressed protein by the endogenous TTL of HEK293T cells, generating mCherry-αTyr variant. αTyr antibody was indeed shown to be highly specific using purified proteins (Bosson *et al.*, 2012). (**) Signal observed with mCherry-αdeTyr extract using αΔ2 antibody also very probably results from processing of the last glutamate of the expressed protein by an endogenous CCP of HEK293T cells, generating mCherry-αΔ2 variant.

	mChe-αTyr	**mChe-αdeTyr**	**mChe-αΔ2**	**mChe-αΔ3**
αTyr	**84.08**	**15.91***	**-**	**0.01**
αdeTyr	**-**	**95.22**	**0.53**	**4.25**
αΔ2	**0.13**	**10.46****	**89.37**	**0.04**
αΔ3	**-**	**0.17**	**1.66**	**98.17**

Table S2 - Primary antibodies used in this study

Antibody name	**Antigen or epitope**	**Dilutions**		**Type**	**Provider**
		immuno blotting	**immuno cyto-chemistry**		
α tot (α3a)	Epitope: DMAALE (see figure 1B)	1:10,000	1:8000	mouse monoclonal	(Erck et al., 2005) Kind gift of L Lafanechère
β tot (β3a)	Epitope: EYQQYQ (see figure 4C)	1:10,000	1:1,000	mouse monoclonal	Kind gift of L Lafanechère
Anti-Tyr tubulin (YL1/2)		1:10,000	1:5,000-1:6000	rat monoclonal	(Wehland and Willingham, 1983)
anti-deTyr-tubulin	KLH-CGEEEGEE-COOH*	1:10,000	1:500	rabbit polyclonal or guinea pig polyclonal	our own production, (Paturle-Lafanechere et al., 1994)
anti-Δ2-tubulin	KLH-CEGEEEGE-COOH*	1:10,000	1:800	rabbit polyclonal	our own production, (Paturle-Lafanechere et al., 1994)
3EG (anti-αΔ3 and β-truncated tubulins)	KLH-CEEGEEEG-COOH*	1:10,000	1:500-1:1,000	rabbit polyclonal	see Material and Methods
anti-GFP	GFP	1:5,000		rabbit polyclonal	Invitrogen
Anti-mCherry	mCherry		1:1,000	mouse monoclonal	Clontech
H3-ser10P	Histone H3 (phospho S10)	1:2,500		rabbit polyclonal	Abcam

*KLH: keyhole limpet hemocyanin

Table S3 - Primers used to mutagenize α1B tubulin cDNAs fused to mCherry.
Forward primer was GGCCATCTAT**GACATCTGTC**G and reverse primers are listed below. Restriction sites used for the cloning are in bold, substituted nucleotides are underlined, non-coding sequences are in lower case and stop codon is in red.

cDNA	Reverse PCR primer
mCherry α tub	Template plasmid - α1B tubulin cloned into pcDNA3.1-mCherry Gift from F. Saudou
mCherry α deTyr	cgcccttcga**ggatcc**ttaTTCCTCTCCTTCTTCCTCACC
mCherry α Δ2	cgcccttcga**ggatcc**ttaCTCTCCTTCTTCCTCCCCCT
mCherry αΔ3 (GEEEG)	cgcccttcga**ggatcc**ttaTCCTTCTTCCTCACCCTCTCC
mCherry αΔ3-GE<u>D</u>EG	cgcccttcga**ggatcc**ttaTCCTTC<u>A</u>TCCTCACCCTCTCCTTCAAC
mCherry αΔ3-GE<u>N</u>EG	cgcccttcga**ggatcc**ttaTCCTTC<u>G</u>T<u>T</u>CTCACCCTCTCCTTCAACAG
mCherry αΔ3-G<u>A</u>EEG	cgcccttcga**ggatcc**ttaTCCTTCTTC<u>AG</u>CACCCTCTCCTTCAACAGAAT
mCherry αΔ3-G<u>DD</u>EG	cgcccttcga**ggatcc**ttaTCCTTC<u>A</u>TC<u>A</u>TCACCCTCTCCTTCAACAGAA
mCherry αΔ3-<u>A</u>EEEG	cgcccttcga**ggatcc**ttaTCCTTCTTCCTCA<u>G</u>CCTCTCCTTCAACAGAATCCA
mCherry αΔ3-<u>E</u>EEEG	cgcccttcga**ggatcc**ttaTCCTTCTTCCTC<u>TT</u>CCTCTCCTTCAACAGAATCCA

Supplemental references.

Arregui, C., Busciglio, J., Caceres, A., and Barra, H.S. (1991). Tyrosinated and detyrosinated microtubules in axonal processes of cerebellar macroneurons grown in culture. Journal of neuroscience research *28*, 171-181.

Bosson, A., Soleilhac, J.M., Valiron, O., Job, D., Andrieux, A., and Moutin, M.J. (2012). Cap-Gly proteins at microtubule plus ends: is EB1 detyrosination involved? PLoS One *7*, e33490.

Erck, C., Peris, L., Andrieux, A., Meissirel, C., Gruber, A.D., Vernet, M., Schweitzer, A., Saoudi, Y., Pointu, H., Bosc, C., Salin, P.A., Job, D., and Wehland, J. (2005). A vital role of tubulin-tyrosine-ligase for neuronal organization. Proceedings of the National Academy of Sciences of the United States of America *102*, 7853-7858.

Paturle-Lafanechere, L., Manier, M., Trigault, N., Pirollet, F., Mazarguil, H., and Job, D. (1994). Accumulation of delta 2-tubulin, a major tubulin variant that cannot be tyrosinated, in neuronal tissues and in stable microtubule assemblies. Journal of cell science *107 (Pt 6)*, 1529-1543.

Wehland, J., and Willingham, M.C. (1983). A rat monoclonal antibody reacting specifically with the tyrosylated form of alpha-tubulin. II. Effects on cell movement, organization of microtubules, and intermediate filaments, and arrangement of Golgi elements. The Journal of cell biology *97*, 1476-1490.

Figure S1

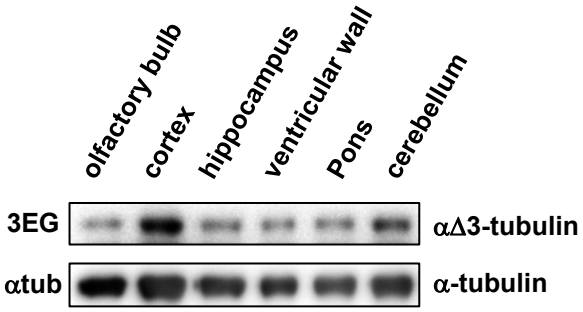


Figure S2

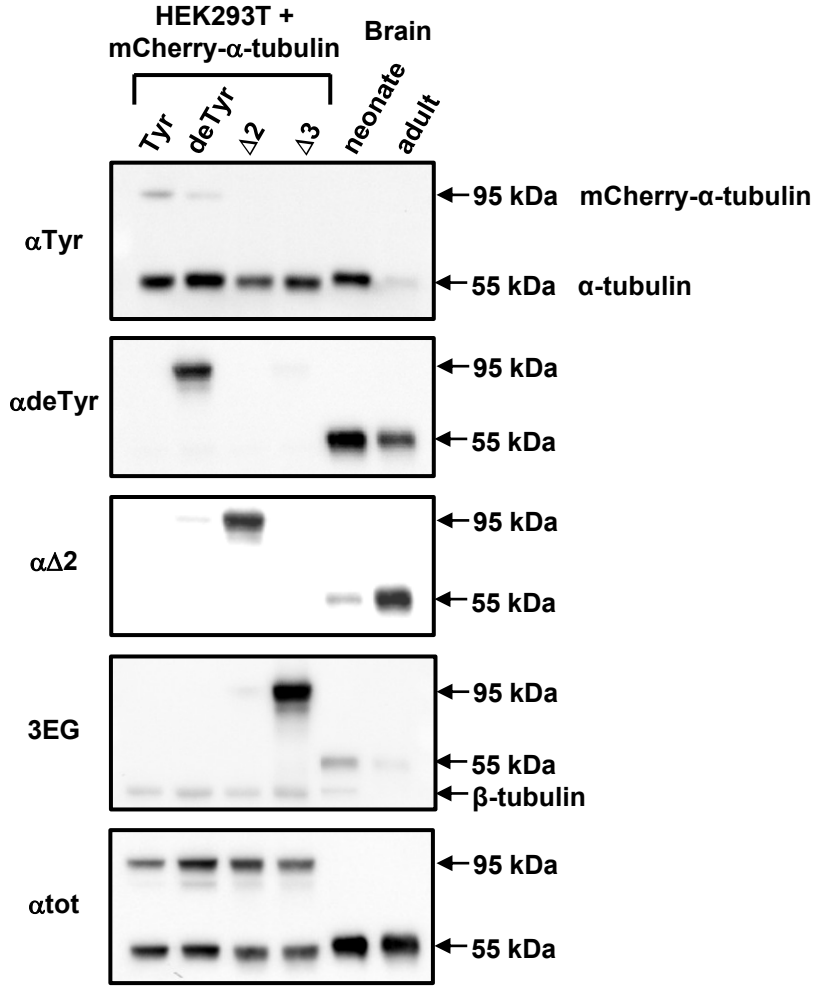


Figure S3

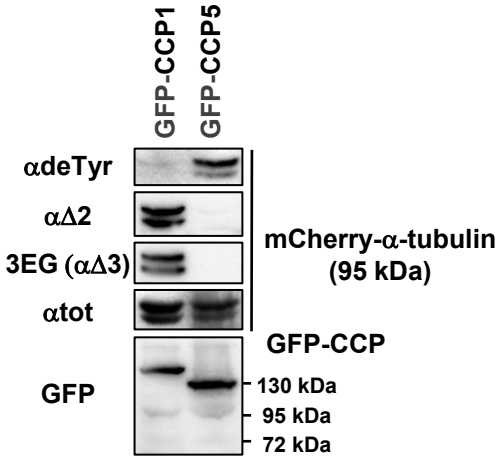


Figure S4

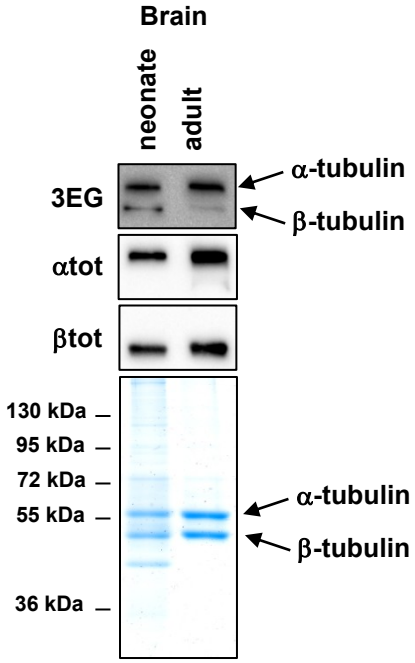


Figure S5

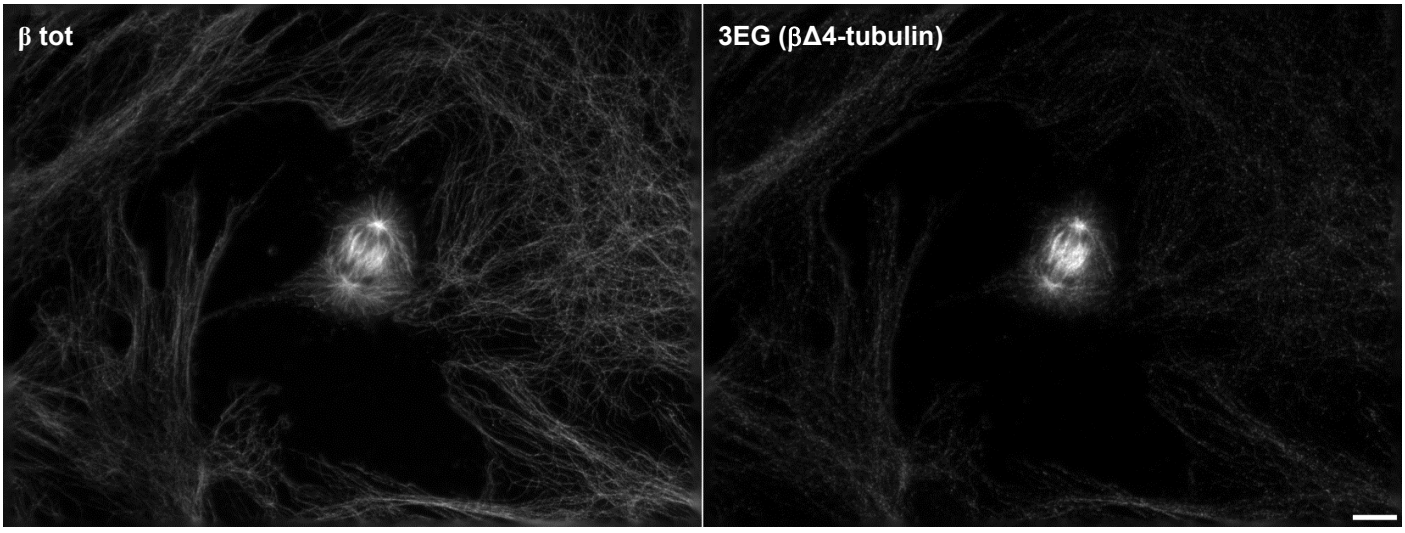
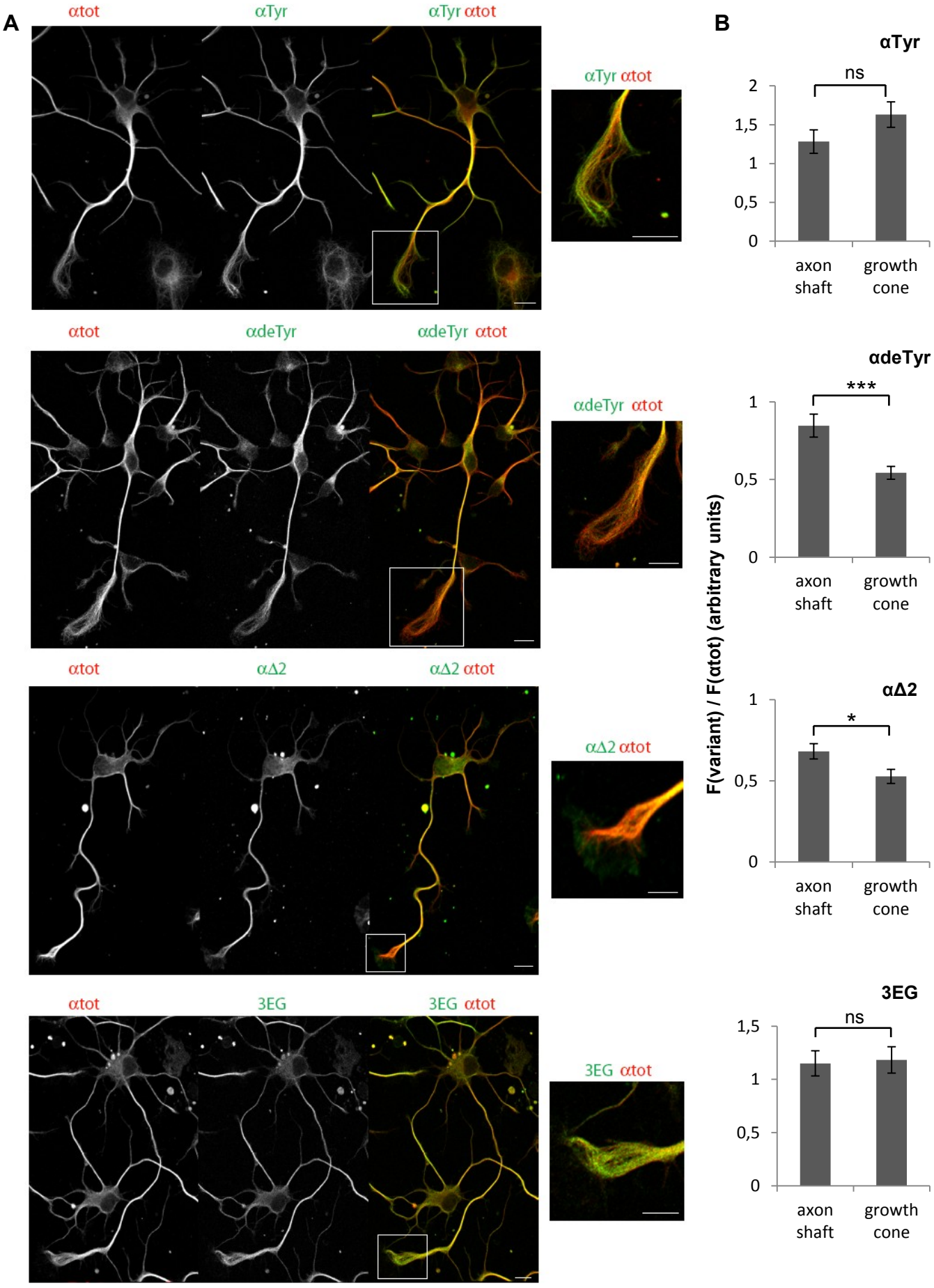


Figure S6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