Dynamic microtubule association of Doublecortin X (DCX) is regulated by its C-terminus Maryam Moslehi, Dominic C.H. Ng, and Marie A. Bogoyevitch Key to Supplementary Movies

Movie S1

GFP-α-tubulin



File: supp movie 1 A for GFP-tubulin.avi

File: supp movie 1 A for Myc-DCX WT and GFP-tubulin.avi

GFP-DCX WT



File: supp movie 1 B for GFP-DCX WT.avi

Movie S1: FRAP assessments of GFP- α -tubulin or GFP-DCX associations with the microtubule network. COS-1 cells were transfected to express (A) GFP- α -tubulin only or GFP- α -tubulin and myc-DCX WT to induce microtubule bundling and (B) GFP-DCX WT. S1A showed the fluorescence recovery of GFP- α -tubulin in the absence (left) and presence (right) of myc-DCX WT. S1B showed the fluorescence recovery of full-length GFP-DCX WT. In these movies, we monitored the fluorescence recovery of the mentioned constructs for 60 s (1 frame/s). Scale bars represent 10 μ m. See Figure 1 for quantitative analyses.

+ myc-DCX WT

Movie S2

GFP-DCX WT

(A)

+ Nocodazole





File:supp movie 2 A for GFP-DCX WT.avi

File: supp movie 2 A for Nocodazole-treated GFP-DCX WT.avi

GFP-DCX ΔC



File: supp movie 2 A for Taxol-treated GFP-DCX WT.avi











File: supp movie 2 B for GFP-DCX delta C.avi

File: supp movie 2 B for Nocodazole-treated GFP-DCX delta C.avi

File: supp movie 2 B for Taxol-treated GFP-DCX delta C.avi

Movie S2: Deletion of the DCX C-terminus slows DCX dynamics of association with microtubule bundles and decrease DCX's sensitivity to microtubule conformational changes. COS-1 cells were transfected to express (A) GFP-DCX WT and (B) GFP-DCX Δ C. (A) Fluorescence recovery was monitored for non-treated GFP-DCX WT (left), nocodazole-treated GFP-DCX WT (middle) and taxol-treated GFP-DCX WT (right) for 60 s. (B) Fluorescence recovery was monitored for non-treated GFP-DCX Δ C (middle) and taxol-treated GFP-DCX Δ C (left), nocodazole-treated GFP-DCX Δ C (right) for 60 s (1 frame/s). Scale bars represent 10 μ m. See Figures 3 and 4 for quantitative analyses.



File: supp movie 3 B for GFP-DCX delta C.avi

File: supp movie 3 B for Sorbitol-treated GFP-DCX delta C.avi

Movie S3: Osmotic stress decreases DCX dynamic association with microtubules while DCX Δ C shows no difference in kinetics under basal and osmotic stress conditions. COS-1 cells were transfected to express (A) GFP-DCX WT and (B) GFP-DCX Δ C. Cells were treated with sorbitol (0.5M for 1 h) to induce hyperosmotic stress. (A) Fluorescence recovery was monitored for non-treated GFP-DCX WT (left) and sorbitol-treated GFP-DCX WT (right) for 60 s. (B) Fluorescence recovery was monitored for non-treated GFP-DCX Δ C (right) for 60 s (1 frame/s). Scale bars represent 10 μ m. See Figures 5 and 6 for quantitative analyses

Movie S4



File: supp movie 4 A for GFP-tubulin.avi



File: supp movie 4 B for Sorbitol-treated GFP-tubulin.avi

File: supp movie 4B for Sorbitol-treated Myc-DCX WT and GFP-tubulin.avi

File: supp movie 4B for Sorbitoltreated Myc-DCX deltaC and GFPtubulin.avi

Movie S4: Osmotic stress conditions affect tubulin exchange dynamics. COS-1 cells were transfected to express GFP- α -tubulin, together with myc-DCX WT or myc-DCX Δ C as indicated. (B) Cells were treated with sorbitol (0.5M for 1 h) as indicated to induce hyperosmotic stress. Fluorescence recovery was monitored for 60 s (1 frame/s). Scale bars represent 10 μ m. See Figures 7 and 8 for quantitative analyses