Supplemental Materials

Molecular Biology of the Cell

Ishihara *et al.*

Supplementary Material

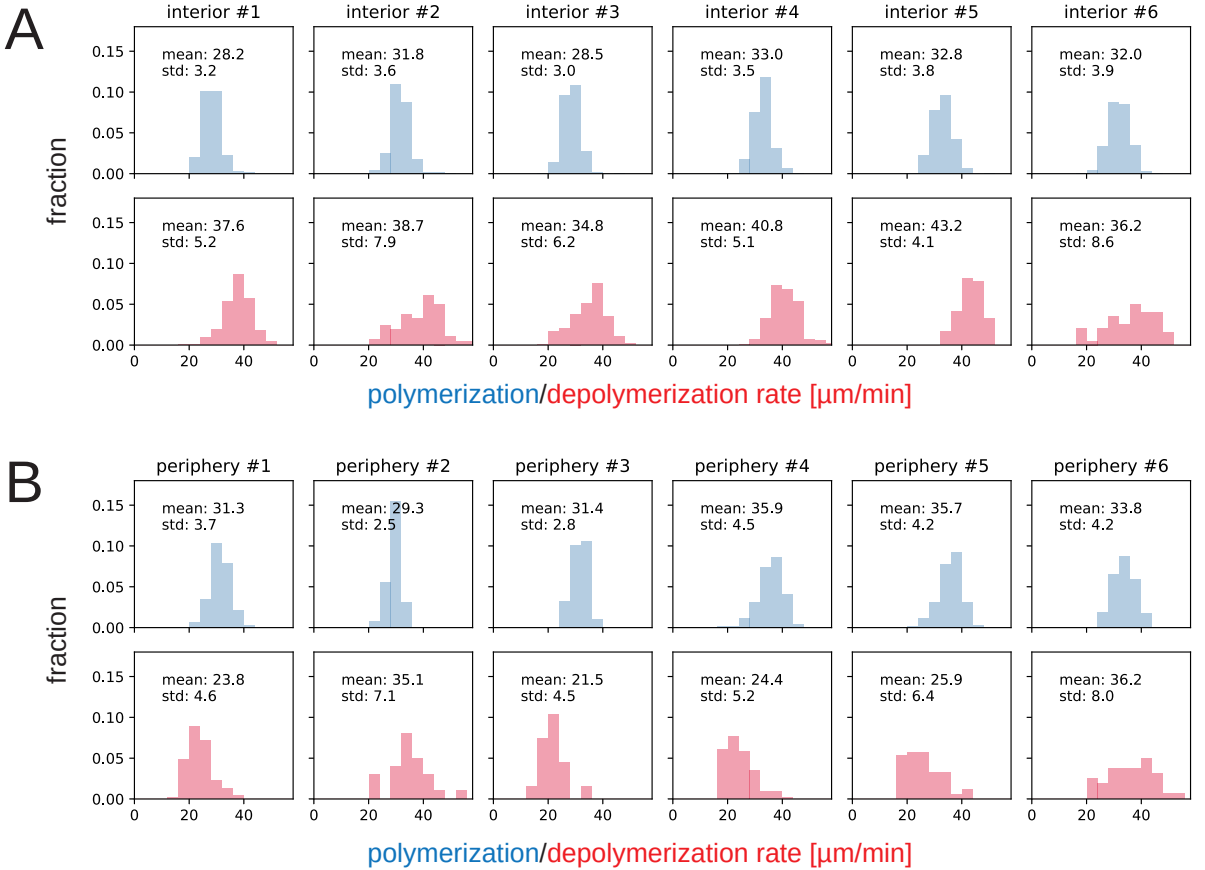
Supplemental Figure 1. Tubulin intensity difference based analysis reports polymerization and depolymerization rates. (A) Six individual field of views from the aster interior. (B) Six individual field of views from the aster periphery. Std=standard deviation.

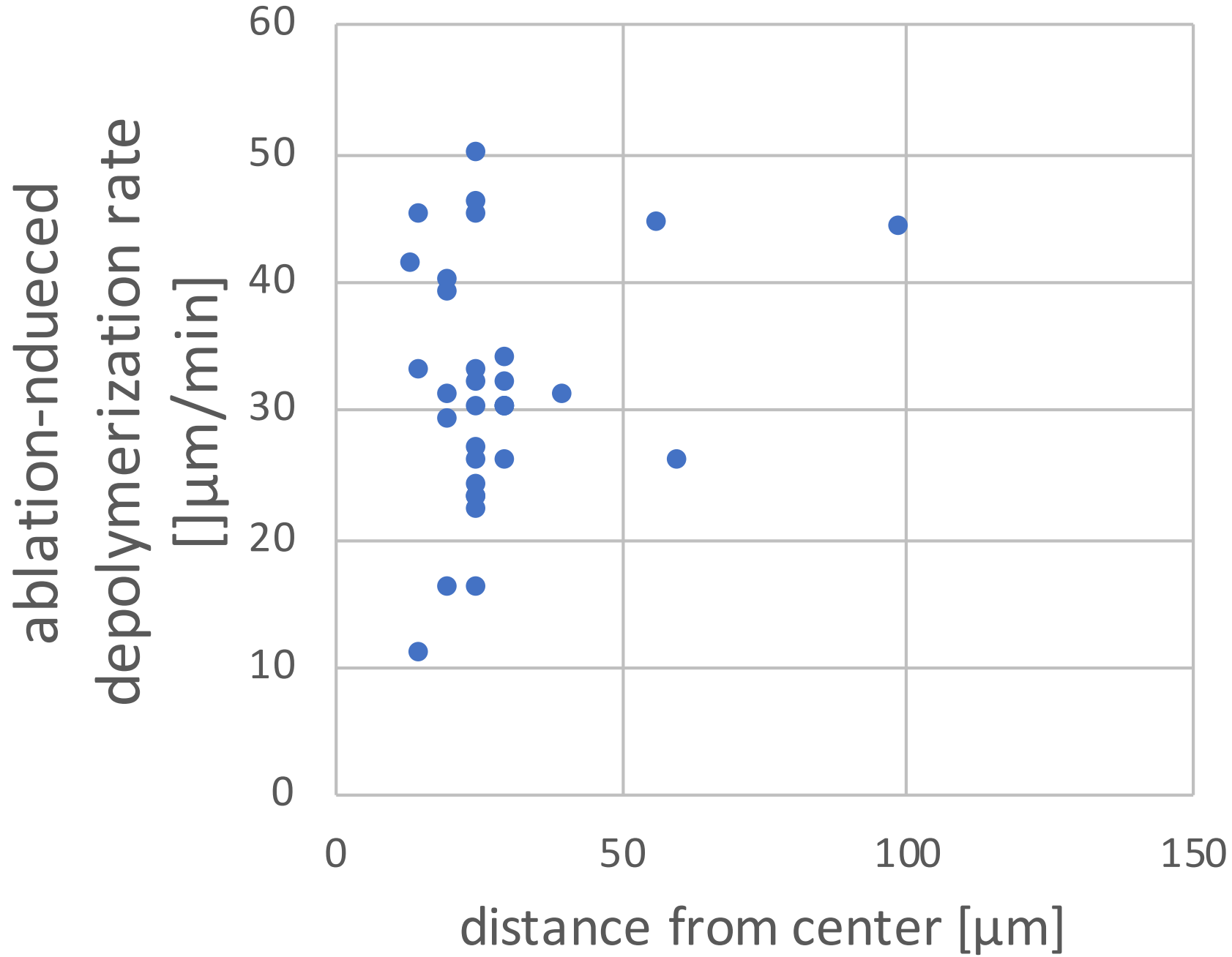
Supplemental Figure 2. The rate of depolymerization following laser ablation as a function of distance from the center. Depolymerization rates obtained in this method showed great variability and did not show correlation with distance.

Supplemental Figure 3. Fluorescent intensity profile of Tau-mCherry. (A) Wide field images of a growing aster visualized with Alexa647-labelled tubulin, EB1-GFP, and Tau-mCherry. For all images, the contrast is adjusted to emphasize the zone of low fluorescence intensity. (B) Quantification of the fluorescence intensity profiles averaged over the quadrant and normalized to the intensity outside the aster. This analysis suggests that the spatial variation of soluble MAPs is a general phenomenon, regardless of whether a MAP associates with the growing plus ends (i.e. EB1-GFP) or all over the microtubule lattice (i.e. Tau-mCherry). Scale bar, 100 μm.

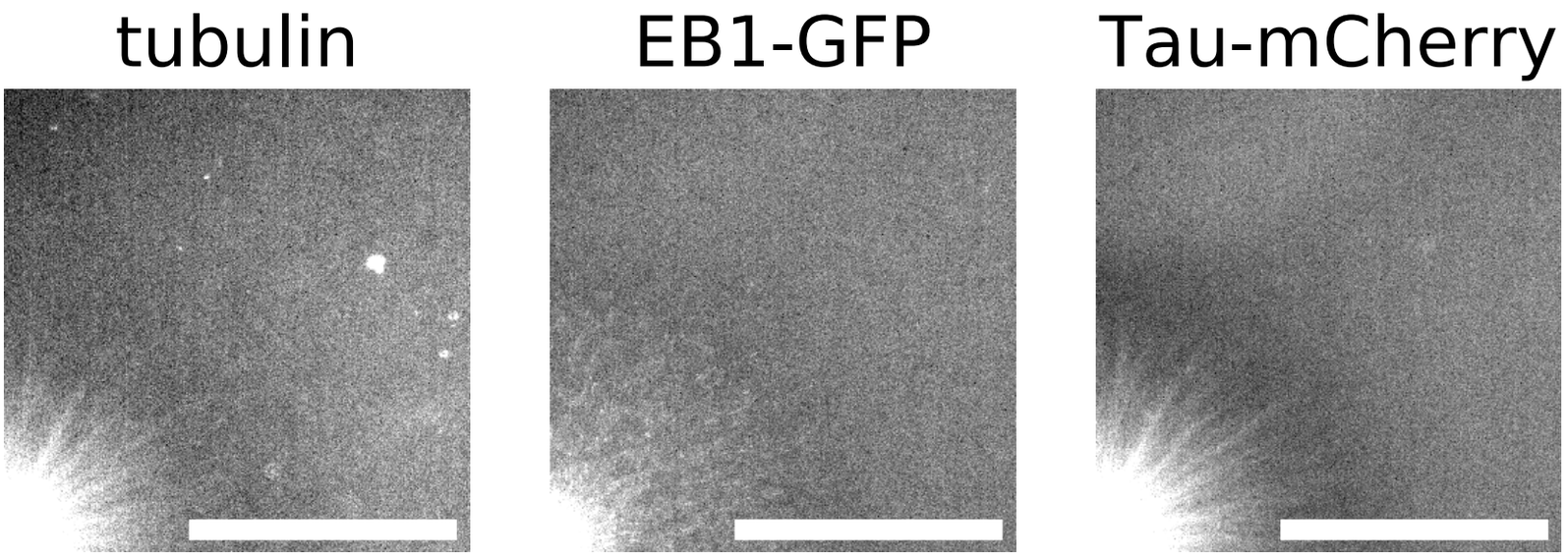
Supplemental Figure 4. Fluorescent intensity profile of 10k Da Dextran. (A) Wide field images of a growing aster visualized with fluorescently labelled tubulin and Alexa568-labelled 10k Da Dextran. For all images, the contrast is adjusted to emphasize the zone of low fluorescence intensity. (B) Quantification of the fluorescence intensity profiles averaged over the quadrant and normalized to the intensity outside the aster. Unlike the profiles of tubulin and MAPs, fluorescently labelled Dextran shows a flat intensity profile serving as a control for a soluble species that does not show a spatial variation in this assay. Scale bar, 100 μm.

Supplemental Table 1. Expanded table of microtubule associated proteins (MAPs) that may increase or decrease microtubule stability based on the literature. Protein abundance in the frog egg is from proteome data in (Wühr et al. 2014).

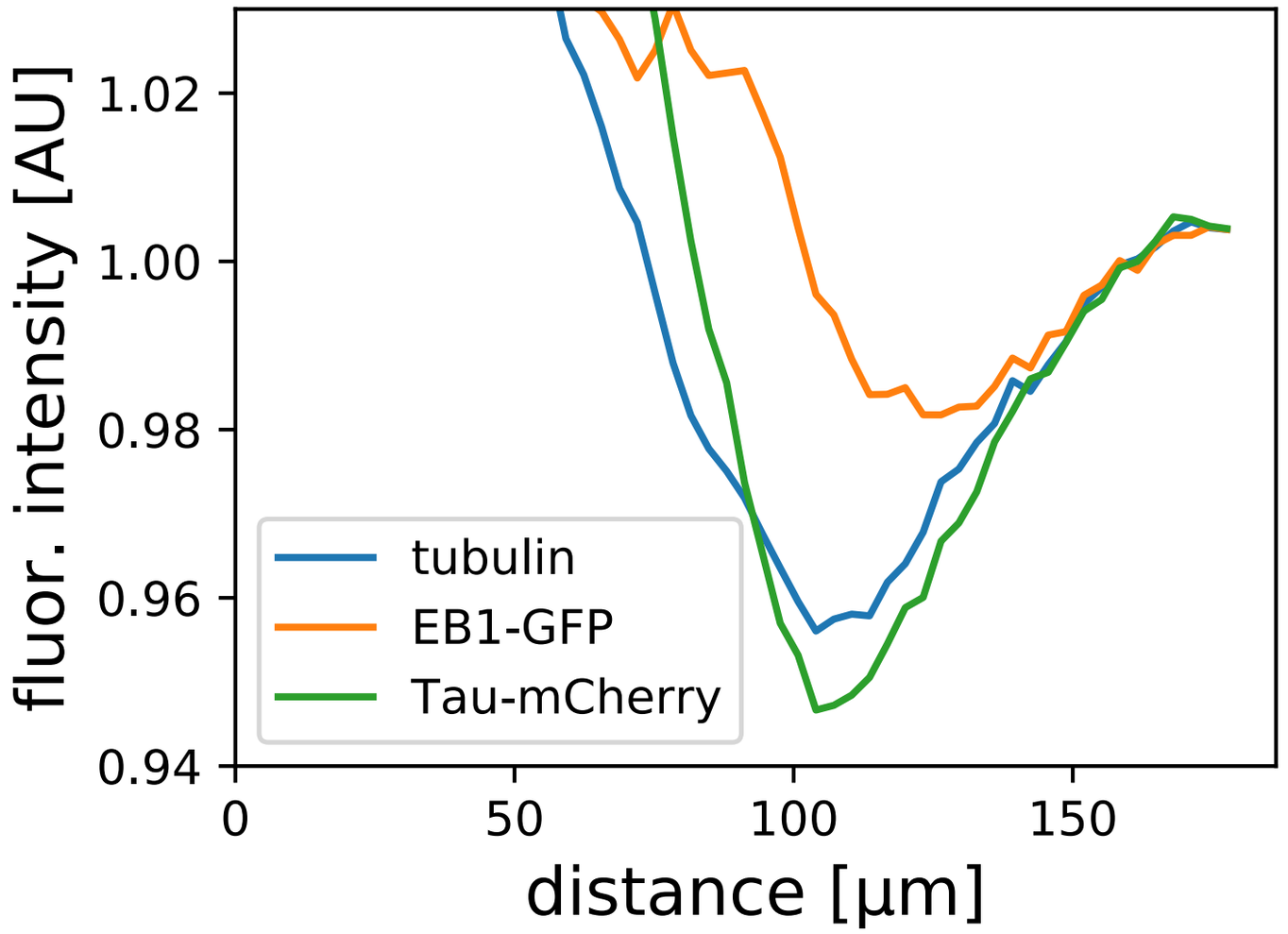

A
interior #1
mean: 28.2
std: 3.2
interior #2
mean: 31.8
std: 3.6
interior #3
mean: 28.5
std: 3.0
interior #4
mean: 33.0
std: 3.5
interior #5
mean: 32.8
std: 3.8
interior #6
mean: 32.0
std: 3.9
mean: 37.6
std: 5.2
mean: 38.7
std: 7.9
mean: 34.8
std: 6.2
mean: 40.8
std: 5.1
mean: 43.2
std: 4.1
mean: 36.2
std: 8.6
fraction
0.15
0.10
0.05
0.00
0
20
40
polymerization/depolymerization rate [μm/min]
B
periphery #1
mean: 31.3
std: 3.7
periphery #2
mean: 29.3
std: 2.5
periphery #3
mean: 31.4
std: 2.8
periphery #4
mean: 35.9
std: 4.5
periphery #5
mean: 35.7
std: 4.2
periphery #6
mean: 33.8
std: 4.2
mean: 23.8
std: 4.6
mean: 35.1
std: 7.1
mean: 21.5
std: 4.5
mean: 24.4
std: 5.2
mean: 25.9
std: 6.4
mean: 36.2
std: 8.0
fraction
polymerization/depolymerization rate [μm/min]


ablation-nduceced depolymerization rate [ ]µm/min]
60
50
40
30
20
10
0
0
50
100
150
distance from center [µm]

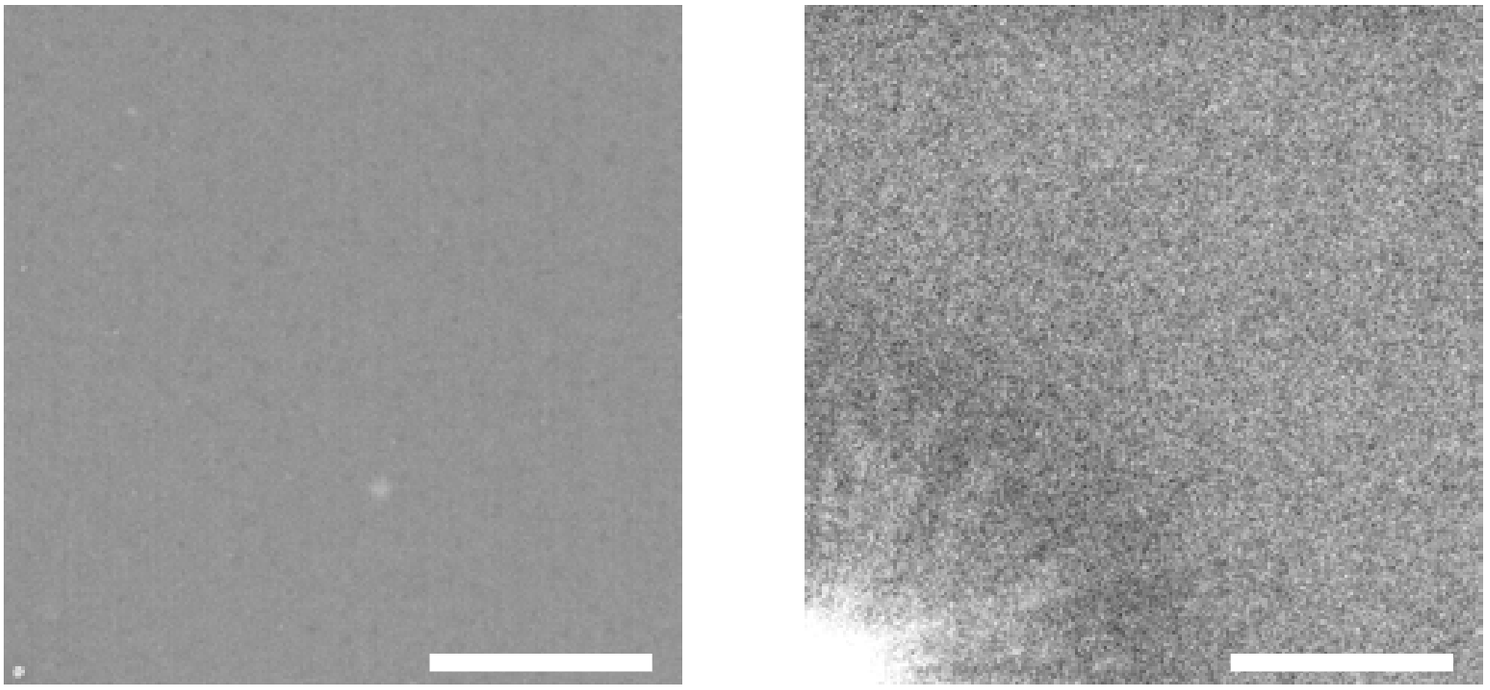
A


tubulin
EB1-GFP
Tau-mCherry

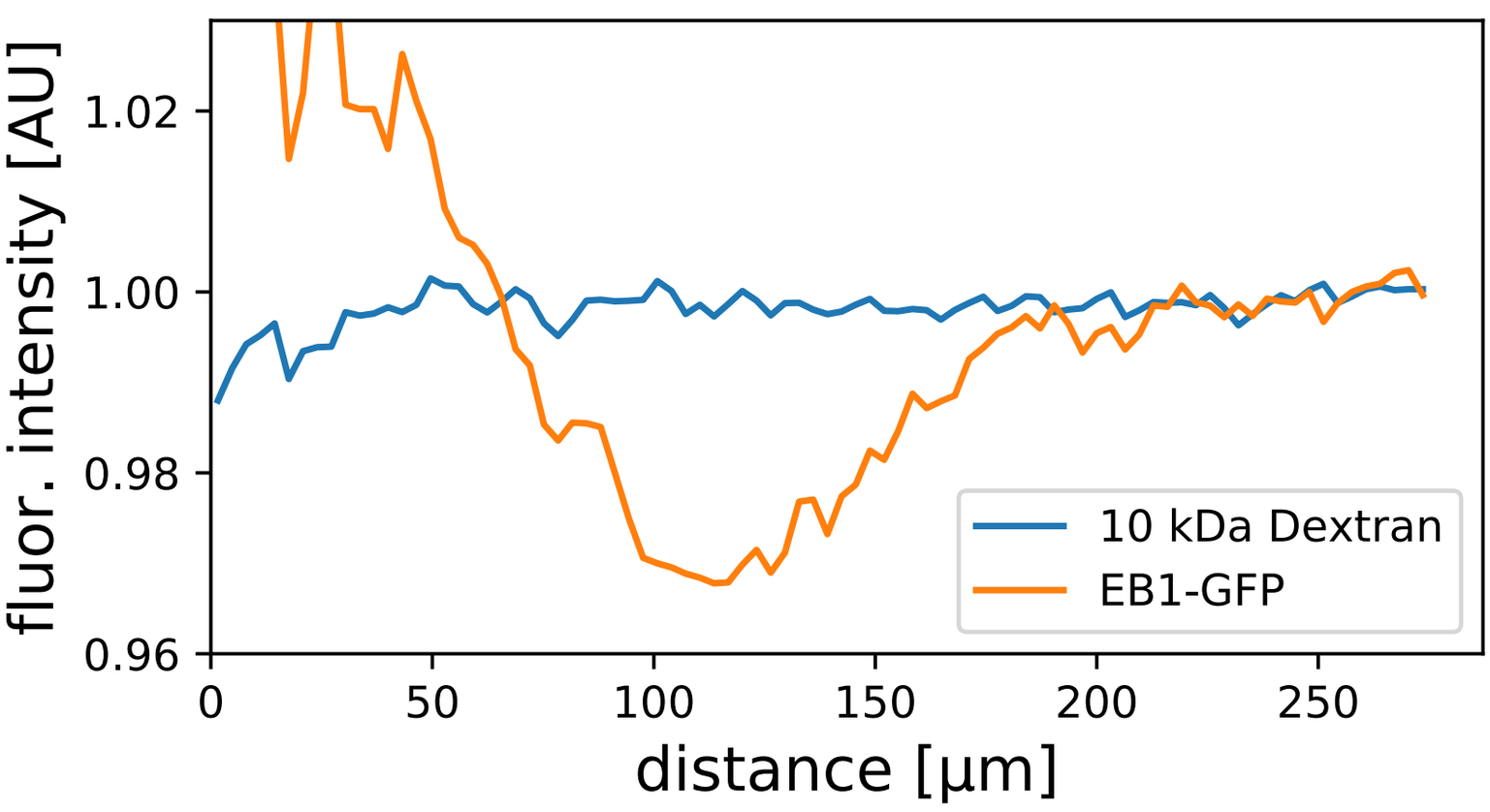
B


fluor. intensity [AU]
1.02
1.00
0.98
0.96
0.94
0
50
100
150
distance [µm]
tubulin
EB1-GFP
Tau-mCherry

A


10 kDa Dextran-A568
EB1-GFP

B


fluor. intensity [AU]
1.02
1.00
0.98
0.96
0
50
100
150
200
250
distance [µm]
10 kDa Dextran
EB1-GFP