### **Supplementary Information**

### Figure S1. Effect of EB1 on microtubule sheet length.

Microtubules were polymerized in different conditions: 45  $\mu$ M tubulin without or with 1.5  $\mu$ M EB1, and 90  $\mu$ M tubulin. Sheet length distributions are given for different assembly time points (elongation, overshoot and plateau). L<sub>avg</sub>, average length; El., elongation; Ov., overshoot; Pl., plateau.

## Figure S2. EB1 induces the closure of tubulin sheets assembled in the presence of GMPCPP and DMSO.

(a) and (b) Negative-staining electron microscopy of microtubules assembled from 20  $\mu$ M tubulin in BRB80 buffer supplemented with 1 mM GMPCPP and 5 % DMSO, in the absence (a) and in the presence (b) of 2.0  $\mu$ M EB1. S, sheet; T, tube. Scale bar, 50 nm. (c) Percentages of tubes and sheets obtained for the control and with 2.0  $\mu$ M EB1. Results are the mean of two experiments. The total measured length was 120  $\mu$ m for the control and 147  $\mu$ m with EB1.

# Table S1. Microtubule types assembled at 45 $\mu$ M tubulin, 90 $\mu$ M tubulin and 45 $\mu$ M tubulin with 1.5 $\mu$ M EB1.

El., elongation;  $F_{trans}$ , transition frequency; Ov., overshoot; Pl., plateau; MT, microtubule; 13(s), skewed 13-protofilament microtubule.

### Supplementary Methods. Microtubule assembly with GMPCPP and DMSO.

To test the effect of EB1 on tubulin sheet closure, 20  $\mu$ M tubulin was mixed at 4 °C in BRB80 with 5 % DMSO and 1 mM GMPCPP, in the absence or in the presence of 2.0  $\mu$ M EB1, according to published procedures<sup>9</sup>. Samples were incubated 10 min at 36 °C, diluted 10 times in BRB80 and prepared for electron microscopy using the negative staining method. A 4  $\mu$ l sample was applied onto a collodion/carbon-coated grid, blotted and stained with 1 % uranyl acetate for 0.5 min. Grids were observed using a Philips CM12 electron microscope operating at 120 kV (FEI Company, Eindhoven, The Netherlands). Images were recorded at a magnification of 22,000 X and ~2.5  $\mu$ m underfocus. Negatives were digitized using a NIKON Super CoolScan 9000 ED scanner at 2540 dpi (NIKON-France, Champigny sur Marne, France). Sheet and tube lengths were measured using Image J (National Institute of Health, MD, USA).