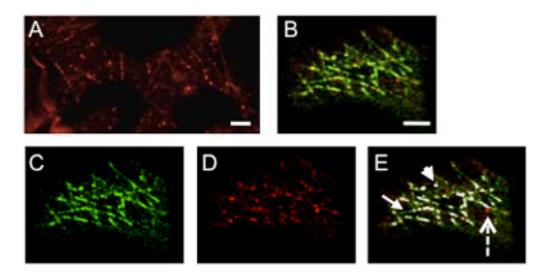
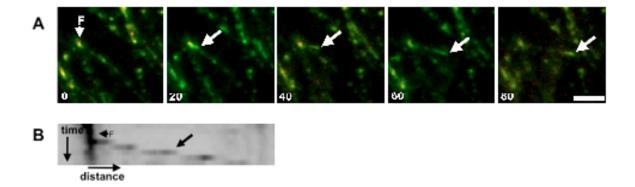
Supplemental Data. Chan et al. (2009). *Arabidopsis* cortical microtubules are initiated along, as well as branching from, existing microtubules.



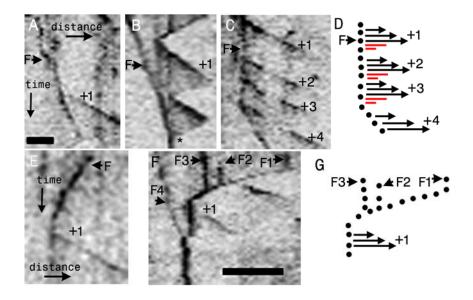
## Supplemental Figure 1: AtEB1a-GFP foci co-localize with spots of NEDD1-RFP along microtubules

(A) Cortical plane of a leaf epidermal cell (*N. benthamiana*) transiently expressing NEDD1-RFP alone. (B) Cortical view of *N. benthamiana* leaf epidermal cell coexpressing EB1a-GFP and NEDD1-RFP. (C and D). The separated green and red channels from (B). (E) A co-localization map was obtained using the ImageJ plugin "Colocalization Finder"; this shows that some EB1a-GFP and NEDD1-RFP co-localize (white spots and arrowhead) at foci along microtubule bundles. Some EB1a-GFP foci (arrow) and NEDD1-RFP spots do not co-localize (dashed arrow). Scale bars A = 10  $\mu$ m, B = 5  $\mu$ m. The scale bar in B applies for C-E.



## Supplemental Figure 2: Microtubules emerge from foci double-labelled with AtEB1a-GFP and NEDD1-RFP

(A) Frames from a time-lapse series showing the emergence of a single microtubule (arrow) from a focus (F) that is doubly-labelled with EB1a-GFP and NEDD1-RFP (and is, hence, yellow) in a leaf epidermal cell (N. benthamiana) (B) Kymograph analysis along the axis of the microtubule (arrow) emerging from the focus (F), shown in panel A. Scale bar  $A = 2 \mu m$ . Time is indicated in seconds.

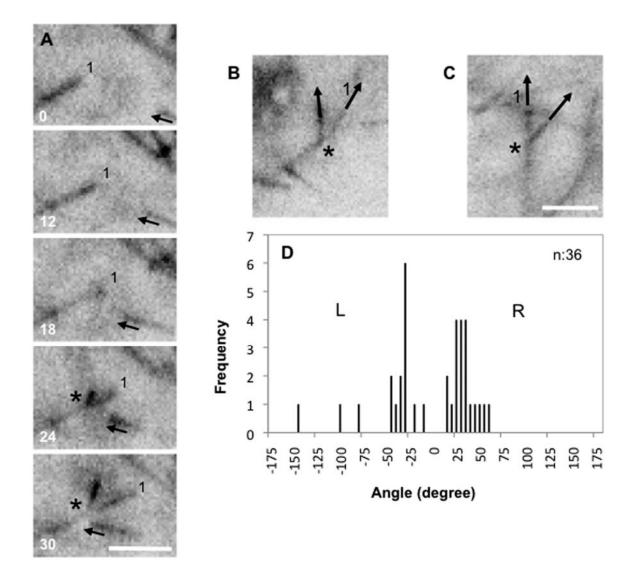


Supplemental Figure 3. Plus- and minus end directed movements of cortical sites of microtubule emergence.

(A) Kymograph showing a focus (F) displaying plus end-directed motility as a comet (+1) is initiated from it. (B) A focus (F) moving towards the plus end of a microtubule undergoing catastrophe and rescue (+1). Note the plus-end eventually depolymerises back to the line marking the focus (asterisk). (C) A focus that displays multiple initiations of comets (+1-4) then moves towards the growing plus ends as shown by change of slope of the focus line. (D) A schematic showing the events in (C). Key: the focus F is marked by a black dot, a growing microtubule by the arrow, and a red line denotes a depolymerising microtubule. (E) A focus (F) is moving in the opposite direction to the AtEB1a-GFP comet (+1) that subsequently emerges from it. This is therefore interpreted as minus end motility of the focus. Note that the focus then becomes stationary after microtubule growth. (F) Multiple foci (F1-4) move in a minus-end direction (as in E) and gather to become a single focus during microtubule depolymerization. Note a comet (+1) initiates from the focus following the gathering of foci 1-3. (G) A schematic showing the events in (F). Key: the focus F is marked by a black dot, a growing microtubule by the arrow, and a line without an arrowhead denotes a depolymerising microtubule. Scale

bars: A-C: x-axis = 2  $\mu$ m, y-axis = 50 sec ; E-F: x-axis = 5  $\mu$ m, y-axis = 114 sec.

The scale bar shown in A applies to B and C. The bar in F applies to E.



Supplemental. Figure 4: Microtubules branch to left and right in ProSPR1:SPR1-GFP plants

(A) Time-lapse series showing the emergence of a single microtubule (asterisk) from a mother microtubule (1) growing into an empty area. Note that the second microtubule marked with an arrow is not branching from microtubule 1 but growing towards it. Scale bar = 3  $\mu$ m. Time in seconds. (B) Projection showing a microtubule branch to the left of a mother microtubule (1) from a branch point (asterisk). (C) Projection showing a microtubule branch in the opposite direction, towards the right of the mother microtubule (1). (D) Frequencies of branching

angles relative to the axis defined by the plus end of the mother microtubule. Note that microtubules predominantly show forward-facing branching. Scale bar =  $3 \mu m$ .