

**Supplemental Figure 1.** Live confocal imaging showing the presence of MTs in dendritic spines. Hippocampal neurons at DIV21 expressing mOrange and GFP- $\alpha$ -tubulin were examined using a Nikon C1 confocal. One of the spines was found to contain a polymerized microtubule (arrows), which is also shown in the magnified panels on the right (2X). There are several spines that also appear to contain microtubules (arrowheads), but not in as distinct a form as the one indicated by the arrows.



**Supplemental Figure 2.** Immunostaining of microtubules in spines. Cultured hippocampal neurons were labeled with anti-class III  $\beta$ -tubulin (Tuj1, green in overlay image) and phalloidin (labeling actin filaments, red in overlay image) (a-b) Representative images of DIV21 hippocampal neurons stained by rhodamine-phalloidin (a) and immunostaining of MTs (b). The low concentration of phalloidin staining highlights spines along dendritic processes. The region outlined by red rectangles is shown at higher magnification in (c). Arrows indicate a mushroom-shaped spine that contains microtubules. (d-h) Images of spines from different neurons showing MTs in spines. Scale bar in (a-b) = 5  $\mu$ m. Scale bars in (c-h) = 1  $\mu$ m.



**Supplemental Figure 3.** Immunostaining of EB3 in hippocampal neurons. Cultured hippocampal neurons were transfected with GFP at DIV14 and labeled with rabbit anti-EB3 antisera at DIV 21. (a) Representative images of hippocampal neurons. Dendritic spines on one neuron are highlighted by GFP fluorescence. The region outlined by rectangles is shown at higher magnification in (b). Numerous EB3 signals (red in overlay image) exist along dendrites, as wells as in spines. Arrows indicate EB3 signals (red) in one spine. (c) Images from different neurons showing EB3 signal in spines. Scale bar = 5  $\mu$ m.