

Supplemental material

Verma and Maresca, <https://doi.org/10.1083/jcb.201904114>

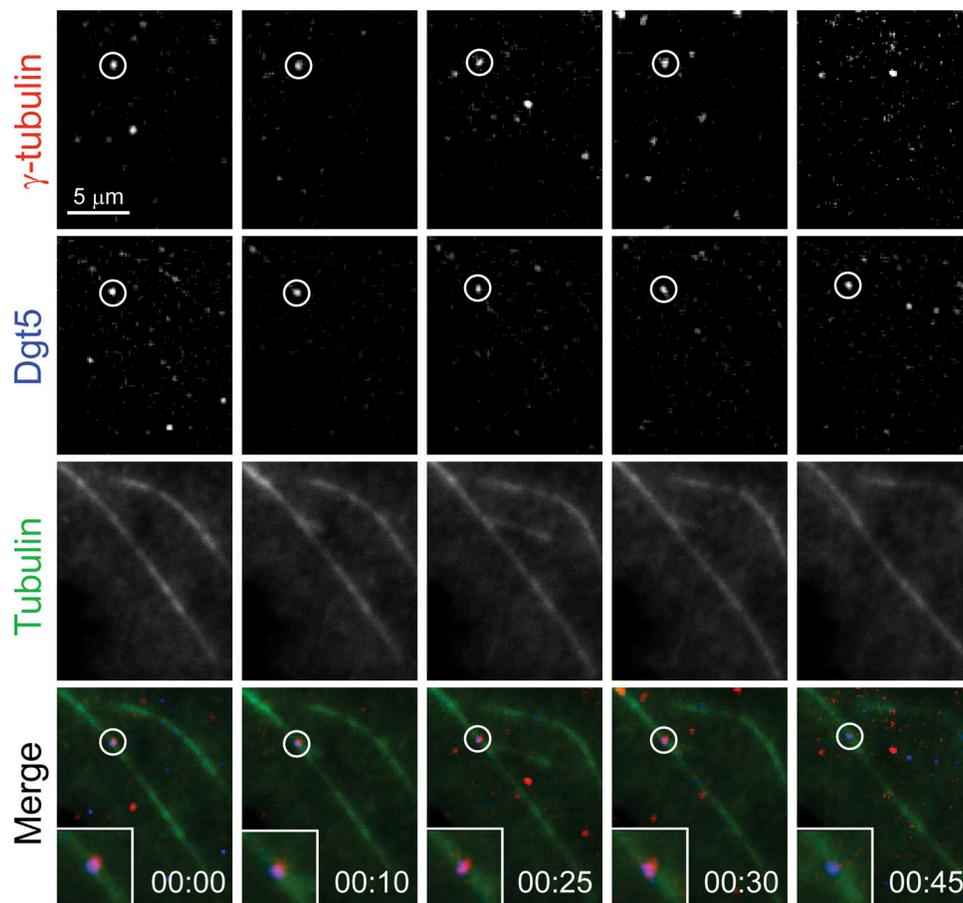
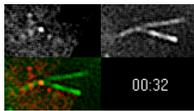
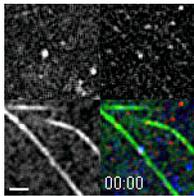


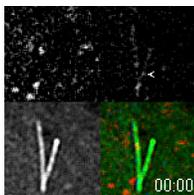
Figure S1. **An MT branching event during anaphase in a cell coexpressing GFP- α -tubulin (green), mTurquoise2-Dgt5 (blue), and γ -tubulin-TagRFP-T (red).** Colocalized Dgt5 and γ -tubulin (time point 00:00) nucleates an MT branching event at 00:10 s. Subsequent frames show polymerization (00:10–00:25) and depolymerization (00:30–00:45) of the daughter MT. Time: min:s. Scale bar, 5 μ m.



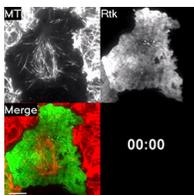
Video 1. **Direct observation of γ -tubulin and MTs during an MT branching event.** Time-lapse TIRF microscopy showing an MT branching event in an anaphase *Drosophila* S2 cell coexpressing EGFP- α -tubulin (green) and γ -tubulin-Tag-RFP-T (red). The mother MT and daughter MT both contact the plasma membrane. In the montage, γ -tubulin-Tag-RFP-T is in the upper left panel, EGFP- α -tubulin is in the upper right panel, and the merge is in the lower left panel. Time: min:s. Scale bar, 1 μ m.



Video 2. **Direct visualization of augmin-mediated MT branching.** Time-lapse TIRF microscopy showing an MT branching event in an anaphase *Drosophila* S2 cell coexpressing EGFP- α -tubulin (green), γ -tubulin-Tag-RFP-T (red), and mTurquoise2-Dgt5 (blue). In the montage, γ -tubulin-Tag-RFP-T is in the upper left panel, mTurquoise2-Dgt5 is in the upper right panel, EGFP- α -tubulin is in the lower left panel, and the merge is in the lower right panel. Time: min:s. Scale bar, 1 μ m.



Video 3. **Direct visualization of augmin-mediated MT branching.** Time-lapse TIRF microscopy showing two MT branching events in a cell coexpressing EGFP- α -tubulin (green), γ -tubulin-Tag-RFP-T (red), and mTurquoise2-Dgt5 (blue) in an anaphase *Drosophila* S2 cell. Two Dgt5 puncta bind to a mother MT (one indicated by an arrow, \leftarrow , and another by a left caret, $<$, in the Dgt5 channel). One Dgt5 puncta ($<$) recruits a γ -tubulin (left caret, $<$, in RFP channel, indicated by 1) at 00:20; however, this γ -tubulin dissociates from Dgt5 at 00:50 without nucleating a daughter. The second Dgt5 puncta (\leftarrow) recruits γ -tubulin at 00:50 (indicated by an arrow, \leftarrow , and designated 2) and nucleates a daughter MT at 01:20. The first Dgt5 puncta ($<$) remains localized to the mother and recruits another γ -tubulin (left caret, $<$, in RFP channel, indicated by 3) at 01:30, which nucleates a daughter MT at 01:40 shortly before the mother MT depolymerizes (01:50–02:10). In the montage, γ -tubulin-Tag-RFP-T is in the upper left panel, mTurquoise2-Dgt5 is in the upper right panel, EGFP- α -tubulin is in the lower left panel, and the merge is in the lower right panel. Time: min:s. Scale bar, 1 μ m.



Video 4. **Branched MT arrays amplify RhoA activation near the cortex.** Time-lapse TIRF microscopy of an anaphase *Drosophila* S2 cell coexpressing Tag-RFP-T- α -tubulin and Rhotekin-EGFP (a marker for active RhoA). Enriched Rhotekin signal is observed near the branched MT arrays; the nearby region devoid of MT plus ends does not exhibit Rhotekin enrichment. In the montage, Tag-RFP-T- α -tubulin is in the upper left panel, Rhotekin-EGFP is in the upper right panel, and the merge is in the lower left panel. Time: min:s. Scale bar, 5 μ m.