Supplementary Data:

Figure S1 and S2

Movies S1-S7

Supplemental Figure Legends

Figure S1. Reorganization of branched microtubule by motor proteins (related to Figure 1)

(A) Branching nucleation in the presence of a constitutively active Ran mutant (Ran Q69L). EB1-GFP (green) was added to follow the microtubule plus ends and identify locations of new microtubule growth; alexa568-labelled bovine brain tubulin (red) was added to visualize microtubules. In the absence of the dynein inhibitor vanadate, branched daughter microtubules are pulled away from their origin and reorganized into higher order structures by motor proteins. However, branching events are still clearly evident. The asterisks for indicated a region that is enlarged in the bottom panels. Large arrows indicate nucleated microtubules that emerge a branch from the template microtubule. Short arrows indicate nucleated microtubules that grow along the length of the template microtubule. Scales bars, 5 μm. See Movie S4. **(B)** Microtubule nucleation in an extract containing both RanQ69L and TPX2. Branched, fan-like structures are visible at early time points in the presence of motor gliding, but are pulled apart and reorganized shortly after. Scale bar, 5 μm. See Movie S5. **(C)** Microtubule nucleation in an extract containing RanQ69L, TPX2 and CC1, which inhibits microtubule gliding and preserved branched fan-like structures when substituted for vanadate (see Fig. 1C). Scale bar, 5 μm. **(D)** Branching nucleation is also stimulated in the presence of TPX2 alone, but the fan-shaped

microtubule structures that formed were less dense compared to combined addition of TPX2 and RanQ69L, and more single microtubules formed. Scale bar, 5 μm.

Figure S2. Immunodepletions of TPX2, augmin and γTB from *Xenopus* egg extract (related to Figure 3)

(A) Immunoblot of immunodepletion of total IgG fraction antibodies as a control and TPX2.

(B) Immunoblot of immunodepletion of augmin, γTB, and total IgG fraction antibodies.

Supplemental Movies

Movie S1: Branching microtubule nucleation in *Xenopus* egg extracts without added Ran or TPX2. Green, EB1-GFP (35 nM); Red, Alexa-568-Tubulin. Sodium orthovanadate (500 μM) was added to prevent dynein-mediated sliding of microtubules along the glass and allow better observation of branching microtubules. Elapsed time is shown in seconds. The field of view is 37.5 x 37.5 μm. See also Figure 1A.

Movie S2: Branching microtubule nucleation in the presence of added constitutively active Ran (RanQ69L) in *Xenopus* egg extracts. Sodium orthovanadate (500 μM) was added to prevent dynein-mediated sliding of microtubules along the glass and allow better observation of branching microtubules. Green, EB1-GFP; Red, Alexa-568-Tubulin. The field of view is 54.2 x 54.2 μm. See also Figure 1B.

Movie 3: Branching microtubule nucleation in the presence of added constitutively active Ran (Ran Q69L) and TPX2 in *Xenopus* egg extracts. Sodium orthovanadate (500 μM) was added to prevent dynein-mediated sliding of microtubules along the glass and allow better observation of branching microtubules. Green, EB1-GFP; Red, Alexa-568-Tubulin. Elapsed time is shown in seconds. The field of view is 54.8 x 54.8 μm. See also Figure 1C.

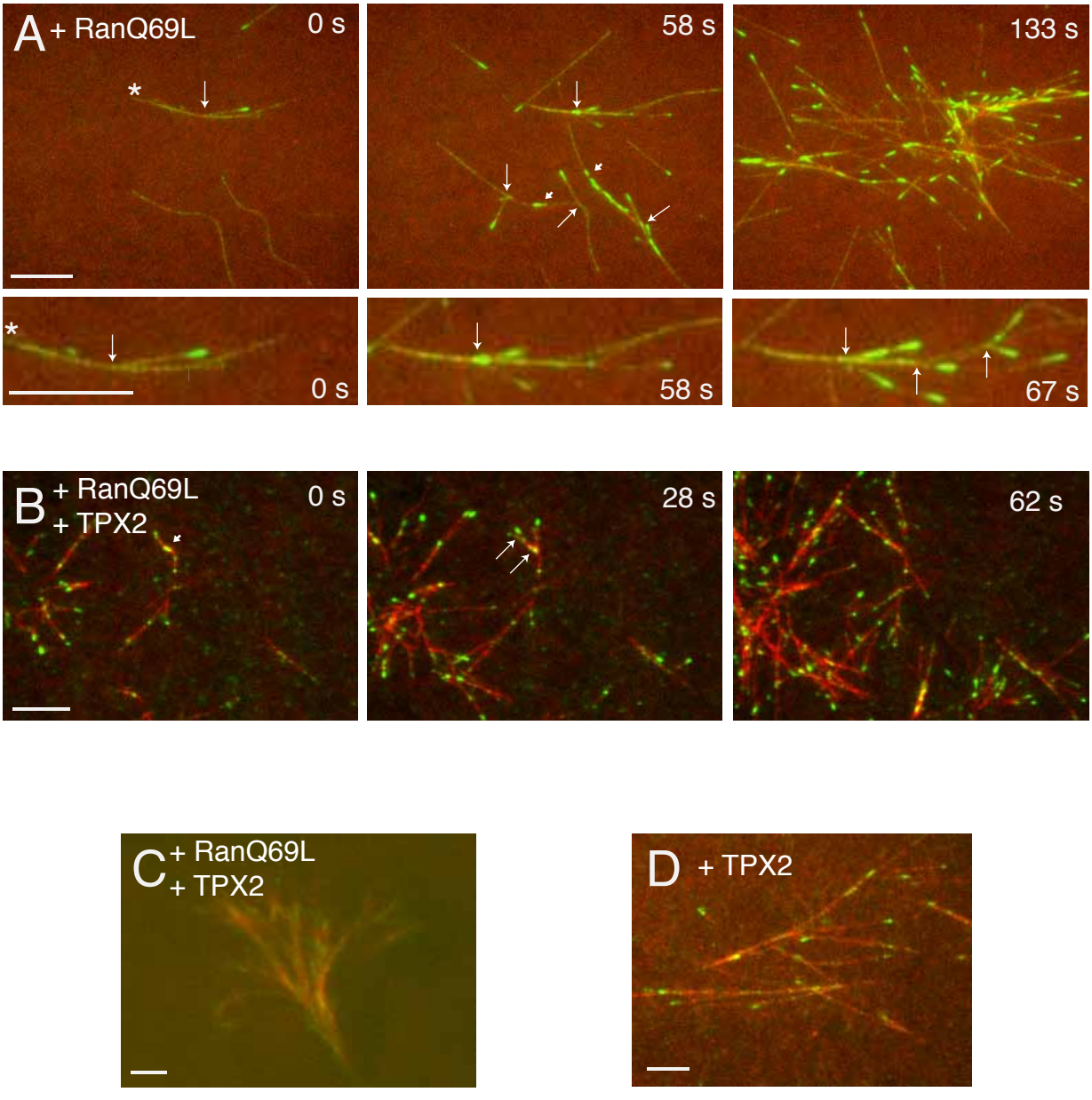
Movie S4: Branching microtubule nucleation in the presence of added constitutively active Ran (RanQ69L) in *Xenopus* egg extracts. Green, EB1-GFP; Red, Alexa-568-Tubulin. Elapsed time is shown in seconds. The field of view is 54.8 x 54.8 μm. See also Figure S1A.

Movie S5: Branching microtubule nucleation in the presence of added constitutively active Ran (Ran Q69L) and TPX2 in *Xenopus* egg extracts. Green, EB1-GFP; Red, Alexa-568-Tubulin. Elapsed time is shown in seconds. The field of view is 54.2 x 54.2 μm. See also Figure S1B.

Movie S6: Branching microtubule nucleation assayed in extract after immunodepletion of a control IgG (top left), γTB (bottom left), augmin (top right), and TPX2 (bottom right). Branching microtubule nucleation was abolished after depletion of augmin and TPX2, whereas there was virtually no microtubule nucleation in the absence of γ-TB (first part of movie). Occasionally, a branching microtubule structure with few, but long MTs was observed after γ-TB was depleted (second part of movie). Green, EB1-GFP; Red, Alexa-568-Tubulin. Elapsed time is shown in seconds. The field of view of each of the four squares is 54.6 x 54.6 μm. See also Figure 3.

Movie S7: Add-back experiments with purified TPX2 constructs after immunodepletion of TPX2. Full length-TPX2 was added to control depleted extract (top left). Full-length TPX2 (top right), NT-TPX2 (bottom left), and CT-TPX2 (bottom right) were added to TPX2 depleted extract. Only full-length and CT TPX2 could restore branching microtubule nucleation and the formation of branched microtubule structures, whereas add-back of the NT TPX2 resembled the TPX2 depletion phenotype. Green, EB1-GFP; Red, Alexa-568-Tubulin. Elapsed time is shown in seconds. The field of view of each of the four squares is 82.2 x 82.2 μm. See also Figure 5.

Fig. S1


A
+ RanQ69L
0 s
58 s
133 s
0 s
58 s
67 s
B
+ RanQ69L
+ TPX2
0 s
28 s
62 s
C
+ RanQ69L
+ TPX2
D
+ TPX2