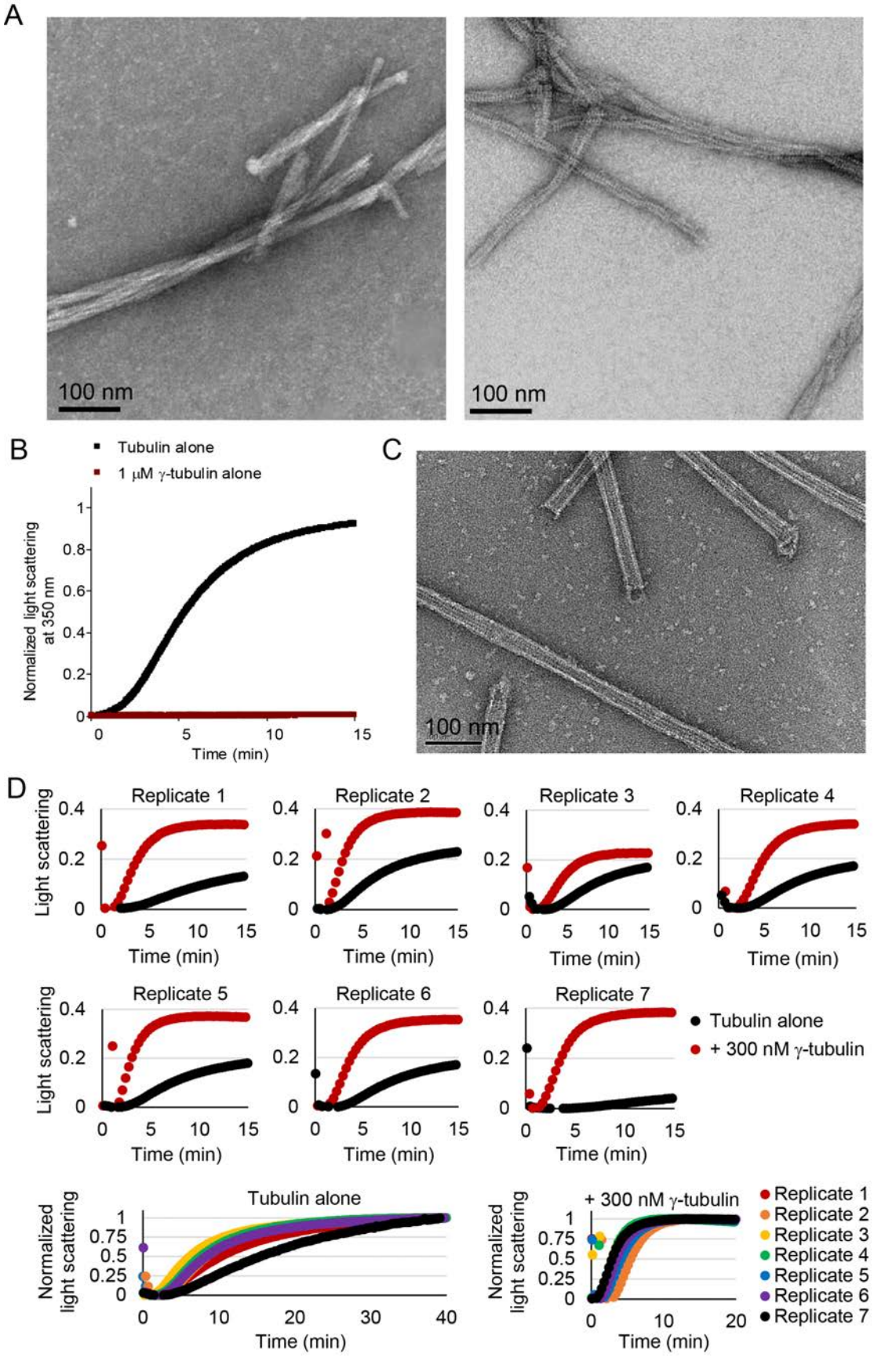
Supplemental Materials

Molecular Biology of the Cell

King *et al*.


A
100 nm
100 nm
B
Tubulin alone
1 μM γ-tubulin alone
Normalized light scattering at 350 nm
Time (min)
C
100 nm
D
Replicate 1
Replicate 2
Replicate 3
Replicate 4
Replicate 5
Replicate 6
Replicate 7
Light scattering
Time (min)
Tubulin alone
+ 300 nM γ-tubulin
Normalized light scattering
Tubulin alone
+ 300 nM γ-tubulin
Time (min)
Replicate 1
Replicate 2
Replicate 3
Replicate 4
Replicate 5
Replicate 6
Replicate 7

Supplementary Figure 1. γ-tubulin arrays promote microtubule formation in a light scattering assay.

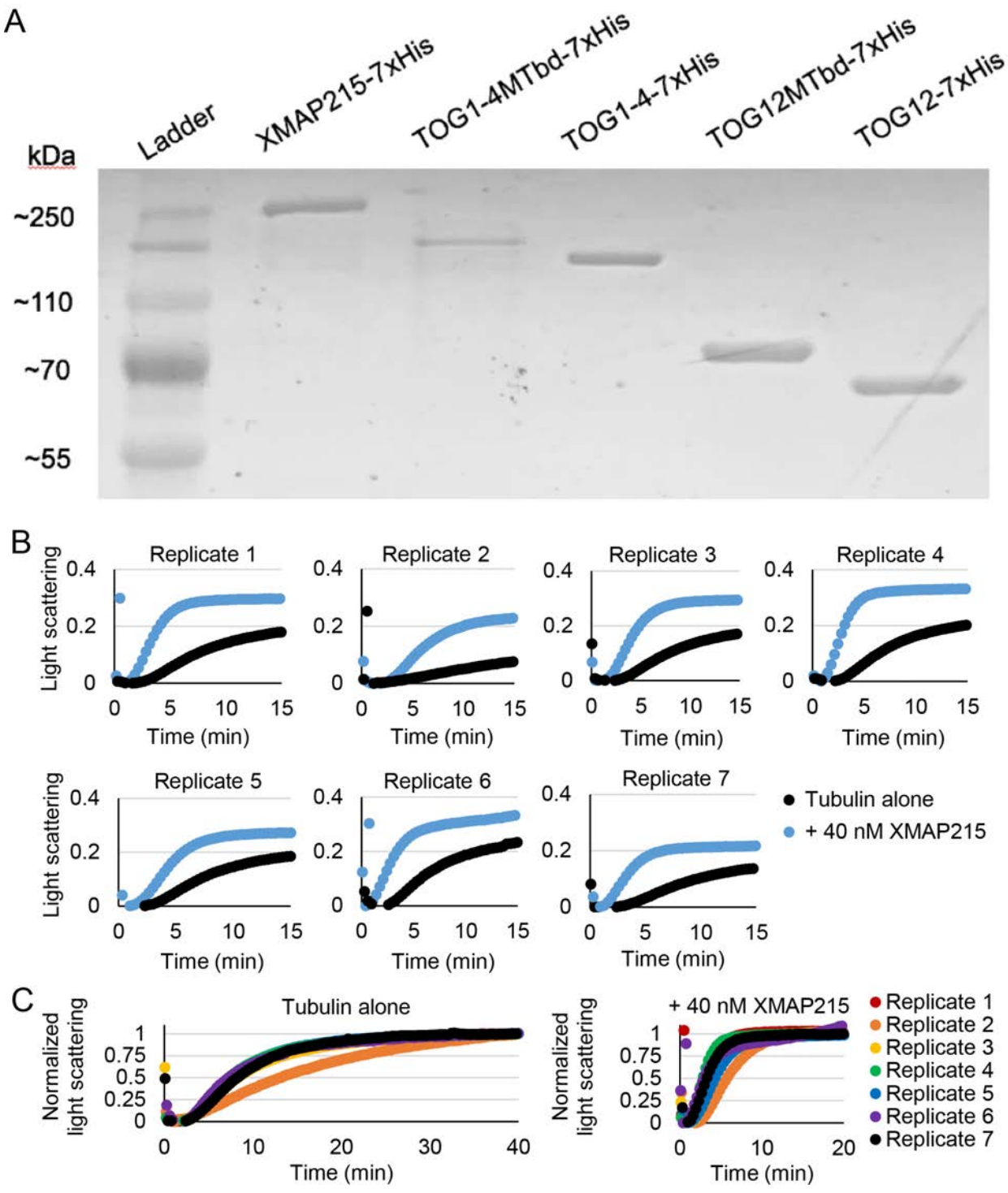
(A) γ-tubulin oligomerizes to form detectable arrays at 300 nM (left) and 1.1 μM (right). At 300 nM γ-tubulin, 6/10 grids had filaments for an average of 0.5 μm length of filament/μm^2 of grid. At 80 nM, no filaments were detected in 10 grids.

(B) Turbidity assay at 12 μM free αβ-tubulin or 1 μM γ-tubulin, showing that γ-tubulin arrays alone do not scatter light under our experimental conditions. Evidently, they do not scatter light at the low concentrations used (≤ 1μM).

(C) Negative-stain electron microscopy image of a sample from a light scattering assay using 1 μM γ-tubulin and 5 μM αβ-tubulin, showing that γ-tubulin arrays support the formation of microtubules, as opposed to other types of tubulin polymers.

(D) Raw data for each of 7 replicates at 15 μM αβ-tubulin. Each experimental reaction with 300 nM γ-tubulin was run simultaneously with a tubulin alone control. Light scattering for each curve was normalized to the maximum plateau value. Nucleation lag was quantified using the corresponding tubulin alone control.

(E) Normalized light scattering data for each replicate of tubulin alone and + 300 nM γ-tubulin.



Supplementary Figure 2. XMAP215 characterization.

(A) SDS-polyacrylamide gel of aliquots (1 μg) of purified XMAP215 constructs.

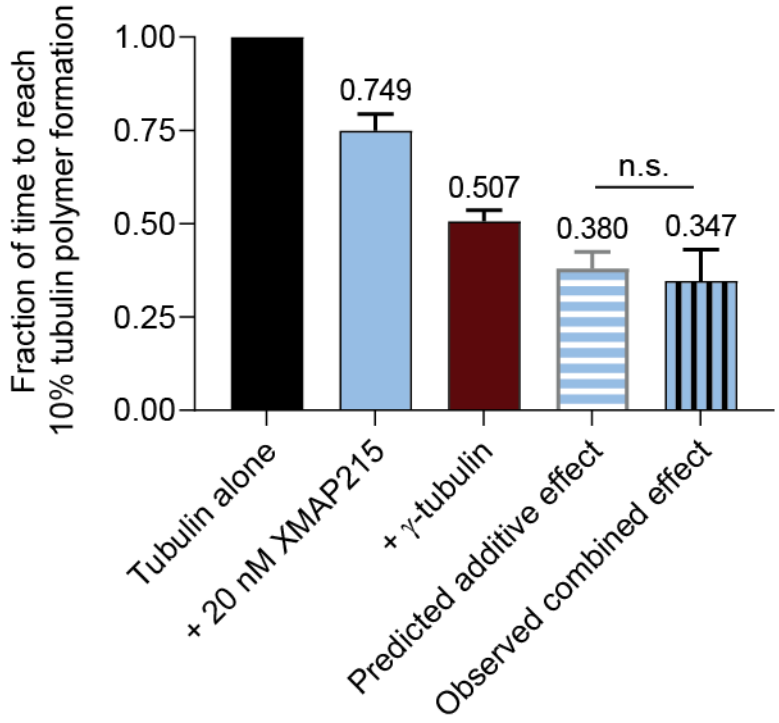
(B) Raw data for each of 7 replicates at 15 μM αβ-tubulin. Each experimental reaction with 40 nM XMAP215 was run simultaneously with a tubulin alone control. Light

scattering for each curve was normalized to the maximum plateau value. Nucleation lag was quantified using the corresponding tubulin alone control.

(C) Normalized light scattering data for each replicate of tubulin alone and + 40 nM XMAP215.

Condition	Normalized nucleation lag
Tubulin alone	1
+ 300 nM γ-tubulin	0.507
+ 20 nM XMAP215	0.749

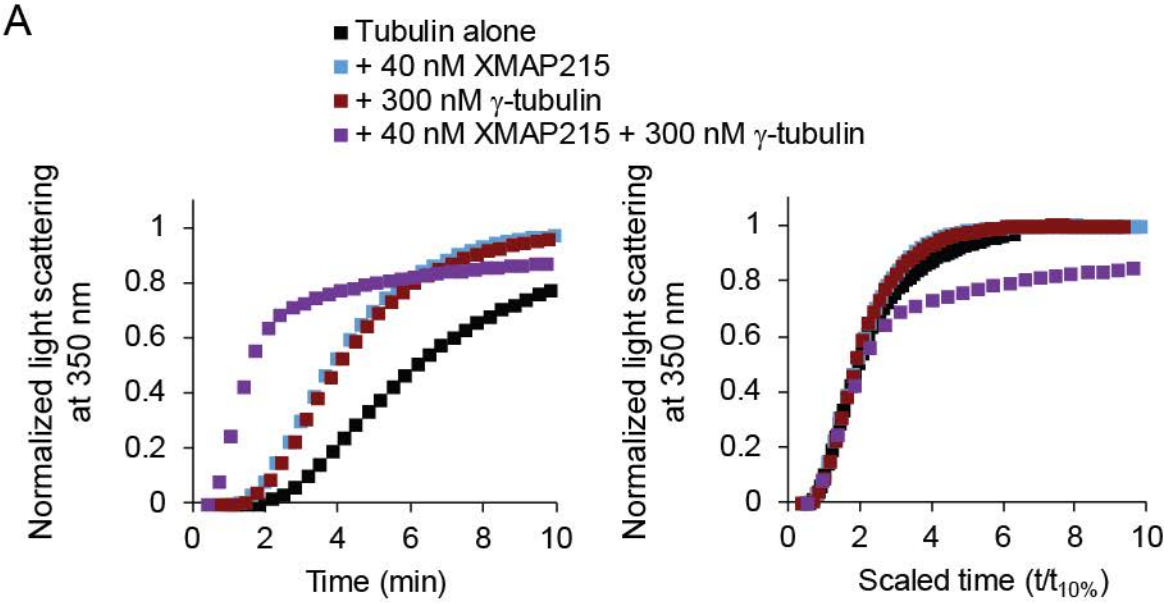
Normalized nucleation lag with 300 nM γ-tubulin alone:	0.507
	x
Normalized nucleation lag with 20 nM XMAP215 alone:	0.749
Predicted additive effect:	0.380



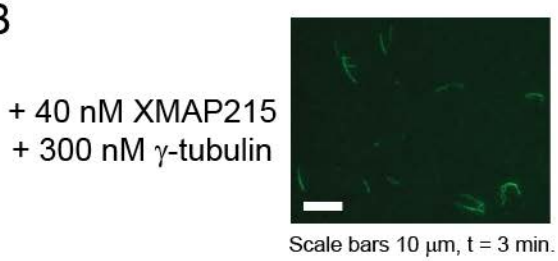
Supplementary Figure 3. Example calculation for predicted additive effect.

Data plotted are mean values ± SEM (N = 12 for tubulin alone, N = 4 for all other conditions). Significance was tested, two-sided t-test.

A

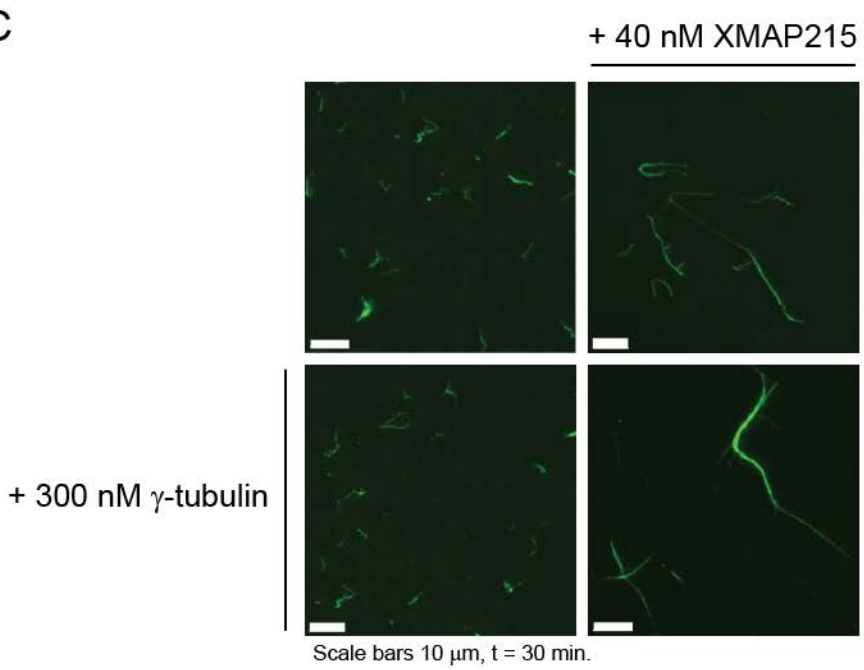


B



Scale bars 10 μm, t = 3 min.

C



Scale bars 10 μm, t = 30 min.

Supplementary Figure 4. XMAP215 and γ-tubulin in combination promote microtubule bundling.

Historically, the mechanism of spontaneous tubulin assembly has been explored using the turbidity data across a range of initial tubulin concentrations [3]. Flyvbjerg and coworkers normalized light scattering to the maximum value at which the data plateaus and normalized time to the time at which turbidity reaches one-tenth its maximum. Finding that the curves were identical following these normalizations, they determined that tubulin follows a single mechanism during assembly, regardless of initial concentration.

We applied this type of analysis to explore the mechanism of assembly in the presence of γ-tubulin and XMAP215. When γ-tubulin is added at 300 nM in combination with XMAP215 at sufficiently high concentration (~40 nM), light scattering increases indefinitely. Normalization of the light scattering of this condition results in a curve with differing shape, specifically with a distinct linear increase in light scattering after turbidity reaches 60% of its maximum value, after approximately 2 min (Supplementary Figure 4A).

To determine if the species of microtubules were different when both γ-tubulin and XMAP215 were present, we performed microtubule pelleting at various time points. By the earliest time point of 3 min, small (≤ 10 microns) microtubule bundles were rare but visible (Supplementary Figure 4B). By the latest time point of 30 min, most microtubules were present in bundles (Supplementary Figure 4C).

The combination of γ-tubulin and XMAP215 in purified solutions promotes bundling of existing microtubules, as noted previously [25]. Comparisons for the effect on nucleation are taken when turbidity reaches 10% of its maximum value, well before bundling occurs.