Supplementary Data: des Georges et al.

Mal3, the *S. pombe* homolog of EB1, changes the microtubule lattice

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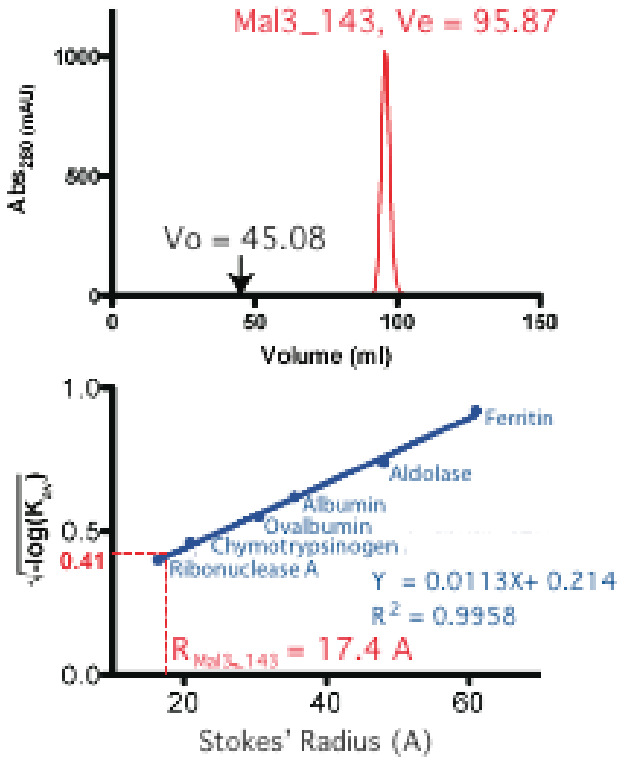
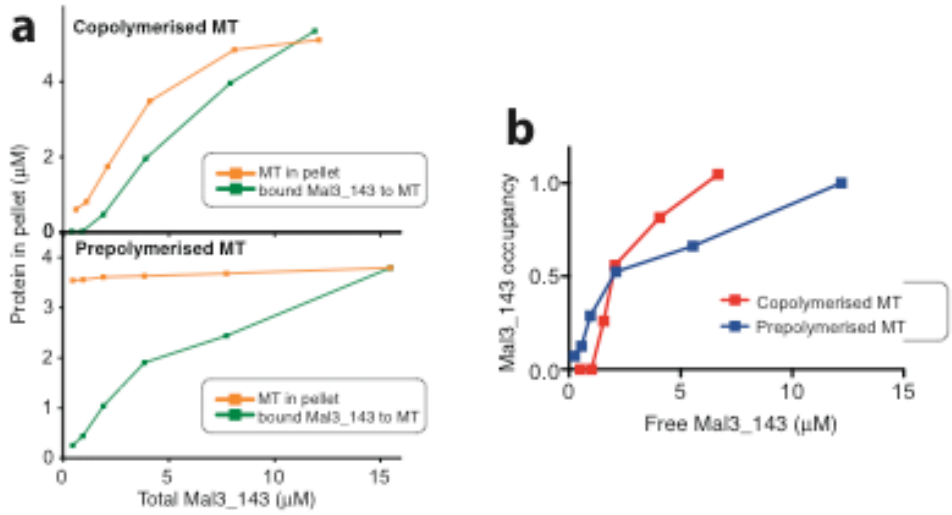
Figure 1 | Gel filtration of Mal3_143



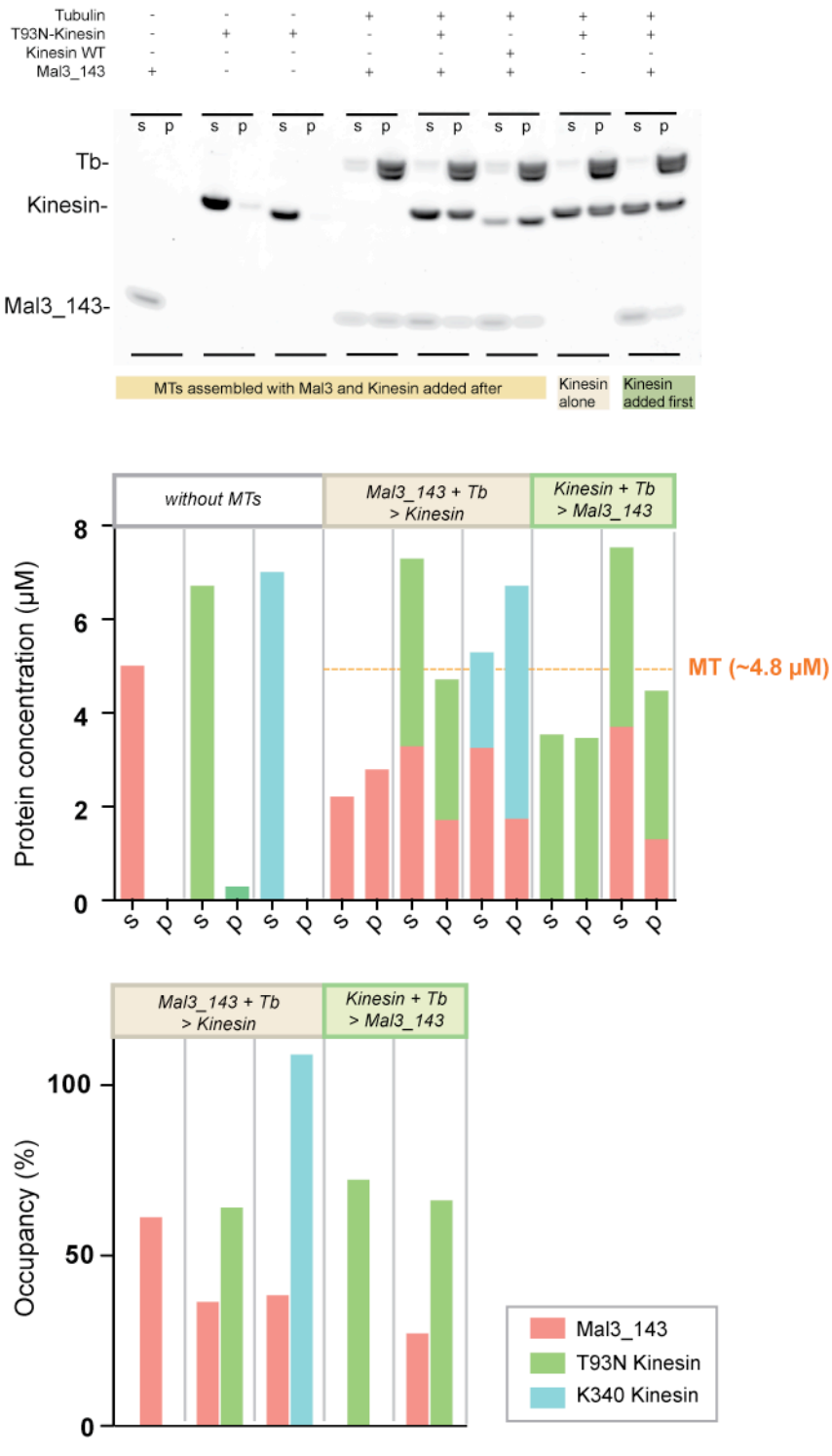
Mal3_143 was analysed by gel permeation chromatography on a Superdex 200 16/60 pg column (GE Healthcare) in an Akta purifier 10 system (GE Healthcare) in 20 mM Tris pH 7.4, 400 mM NaCl at 4ºC. The column was calibrated using protein standards of known Stokes' radius (GE Healthcare) and a standard curve of $(-\log Kav)^{1/2}$ plotted against Stokes' radius. Mal3_143 eluted as a single peak in a volume equivalent to a Stokes' radius of 17.4 Å. This is close to the Stokes' radius of 20.5 Å predicted for the calculated molecular weight of Mal3_143 (mw 17, 486) using the relationship determined by Uversky (1993 Biochemistry 32:13288-13298) for a range of native proteins, and is consistent with Mal3_143 protein being a monomer.

Figure 2 | Binding difference between Mal3_143 co-assembled or pre-assembled with *S. pombe* tubulin



Mal3_143 (0 – 12 μM) and 8 μM of *S. pombe* tubulin were copolymerized in the presence of 1 mM GTP and pelleted (pellets and supernatants are analyzed in the SDS gel shown in Fig. 2b of main article).
Alternatively, Mal3_143 was added to prepolymerised MTs and pelleted.
More tubulin assembles when more Mal3 is added and more Mal3 is also pelleted until the MT dimer lattice becomes fully saturated (**a**). Copolymerised MTs become saturated more readily (**b**), as in the case of brain MTs (Fig. 2d of main article).

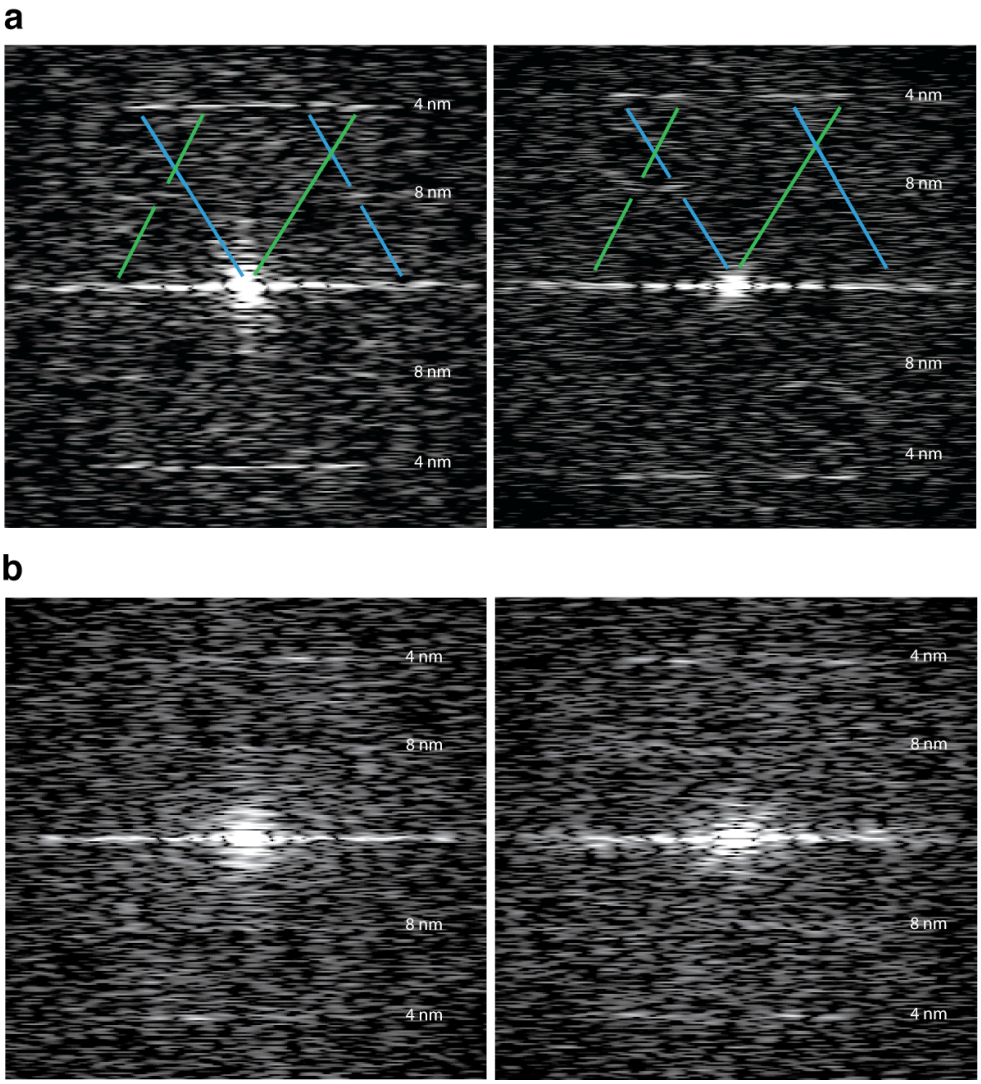
Figure 3 | Competition assay between Kinesin and Mal3_143



a, 5 μM Mal3_143 and 5 μM *S. pombe* tubulin were mixed in BRB80 containing 4 mM GMPCPP , 1mM DTT, 2 mM AMPPNP on ice for 5 min then incubated at 30ºC for 10 min. 7 μM rat kinesin-1 K340 (wild-type or T93N) was added, and incubation continued for 5 min before centrifugation. Alternatively, the kinesin and tubulin were mixed first on ice for 5 min then incubated at 30ºC for 10 min before

addition of Mal3_143 and incubated for 5 min before centrifugation of 50 μl samples in TLA100 rotor , 5 min, 50000 rpm, 30ºC . After separation by SDS-PAGE the quantities of Mal3_143, kinesin and tubulin were measured. *S* and *P* indicate supernatant and pellet fractions, respectively. **b,** Upper histogram shows the tubulin, Mal3_143 and kinesin content of the pellet and supernatant fractions. The lower histogram shows the percentage occupancy of the MT lattice by Mal3_143 and kinesin in each sample, assuming one Mal3_143 and one kinesin binding site per tubulin heterodimer in the MT pellet. The partial displacement of Mal3_143 by kinesin binding suggests that the binding sites of both proteins either partially overlap or are close enough for kinesin to cause steric hindrance of Mal3_143 binding.

Figure 4 | Diffraction patterns from brain MTs assembled with Mal3_143

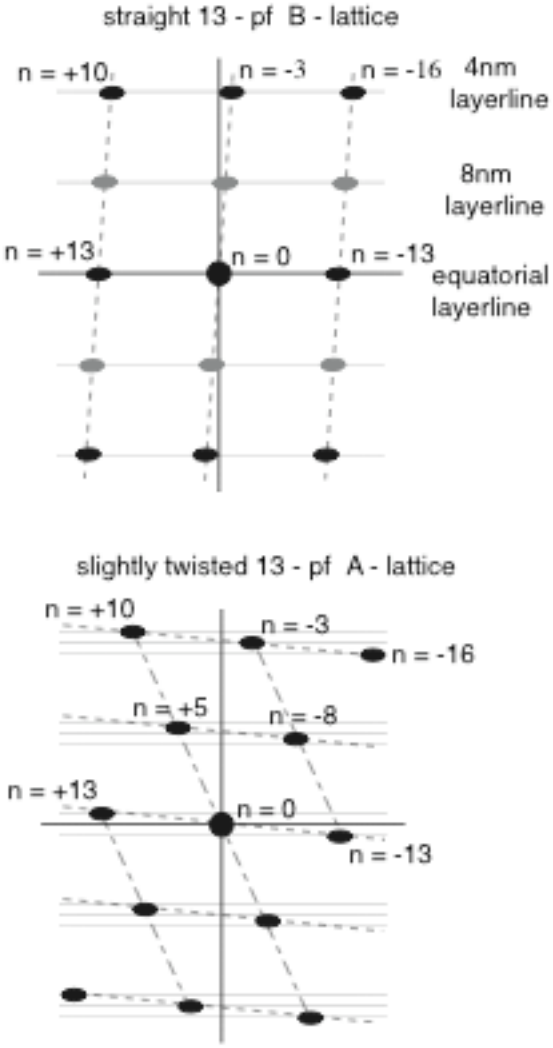


Computed diffraction patterns of cryo-EM image of pig brain MTs copolymerised with Mal3. Blue and green lines show the A-lattice contributions to the diffraction pattern. Blue lines, contribution of the near side of the MT. Green lines, contribution of the far side of the MT.

a: Pig brain MT diffraction patterns with predominantly A-lattice reflections on the 8-nm layerline.

b: Pig brain MT diffraction patterns with mixed lattice reflections on the 8-nm layerline.

Figure 5 | Reciprocal lattices of 13-protofilament MTs



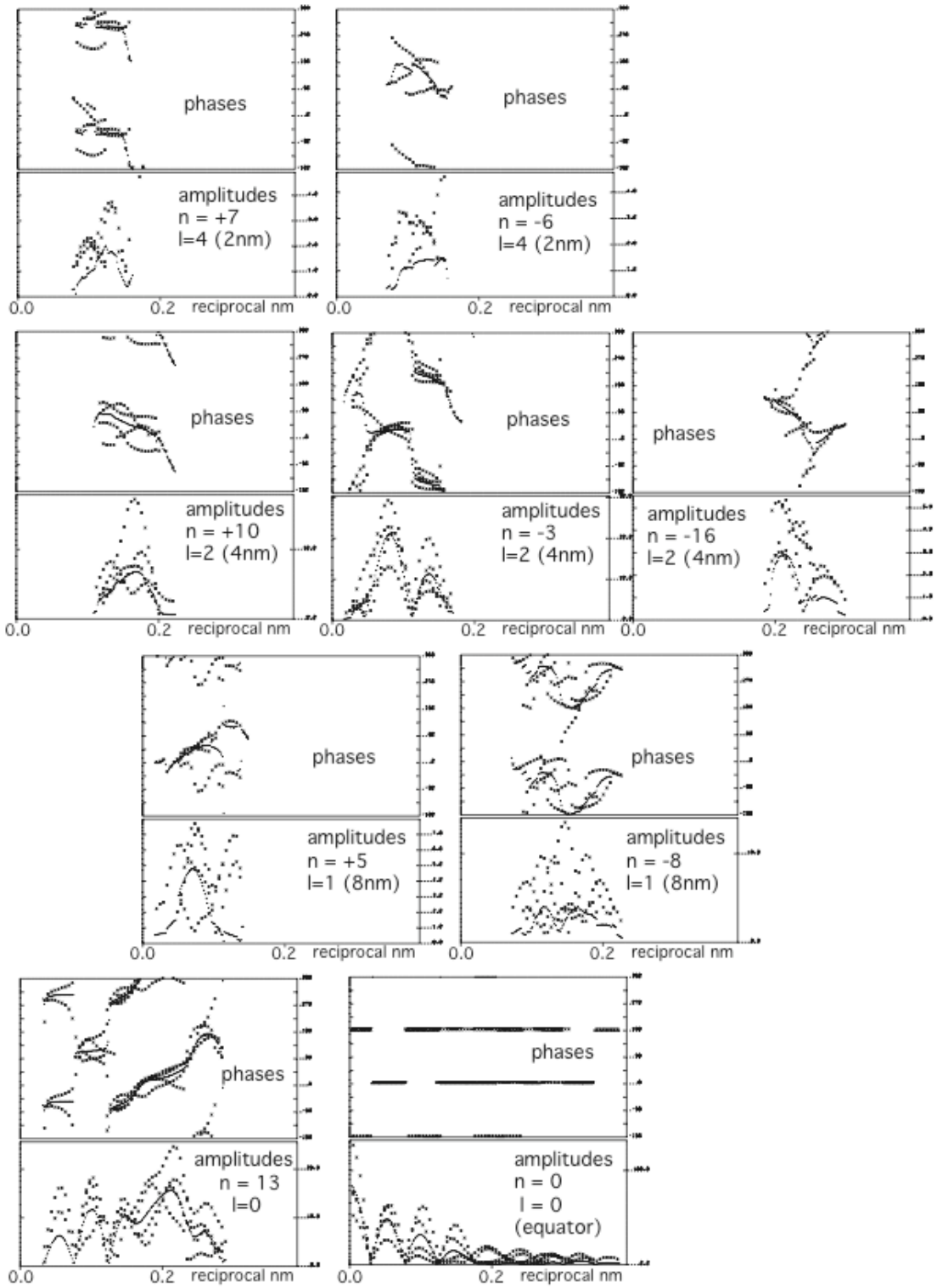
Processing of images of 13-protofilament MTs

Diffraction patterns such as Figure 3i of the main article could be indexed according to the above reciprocal A lattice. Most images had to be rejected, however, as their diffraction patterns showed a mixture of A-lattice and B-lattice peaks.

Amplitudes and phases were extracted for points along each layerline and the values from different images were compared – see plots of data from 4 independent images in Figure 6 (next).

The 3D image shown in Figure 3 of the main article was reconstructed from the averaged data. The plus and minus ends of the reconstructed MT were established by comparing the image with previously published images of kinesin-decorated MTs. However, the current image does not have sufficient resolution to distinguish alpha and beta tubulin subunits.

Figure 6 | Layerline data; values from 4 A--lattice images.



Plots of amplitude and phase of the layerlines from 4 different images are superimposed. The Bessel order (n) and layerline number (l) are shown in each plot.