

Supplementary Information for:

CryoET reveals actin filaments within platelet microtubules

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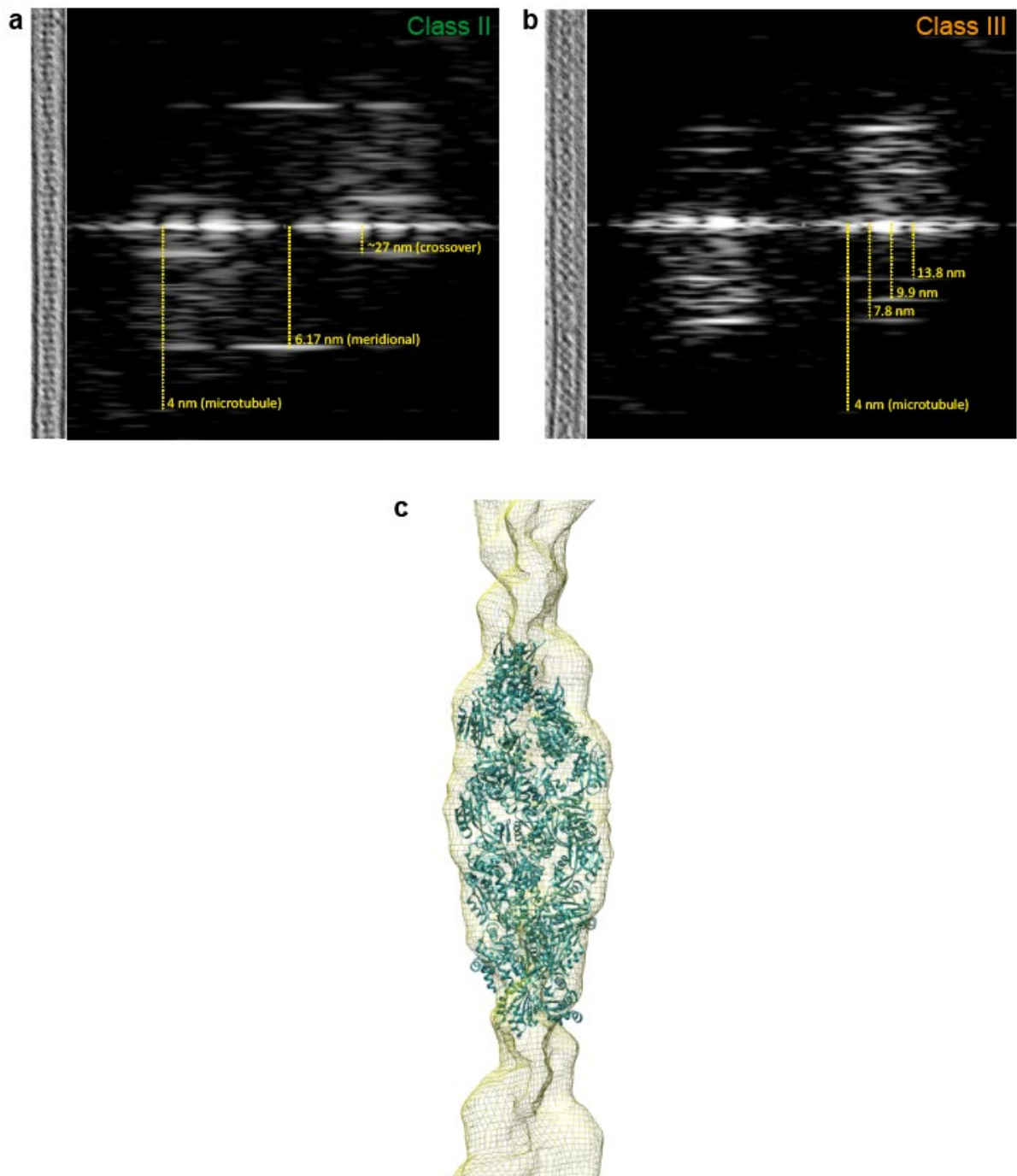
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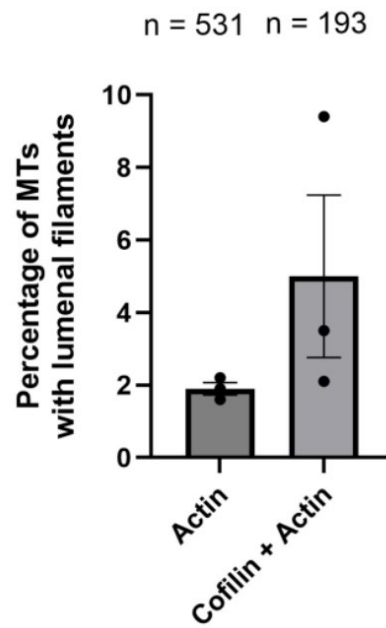
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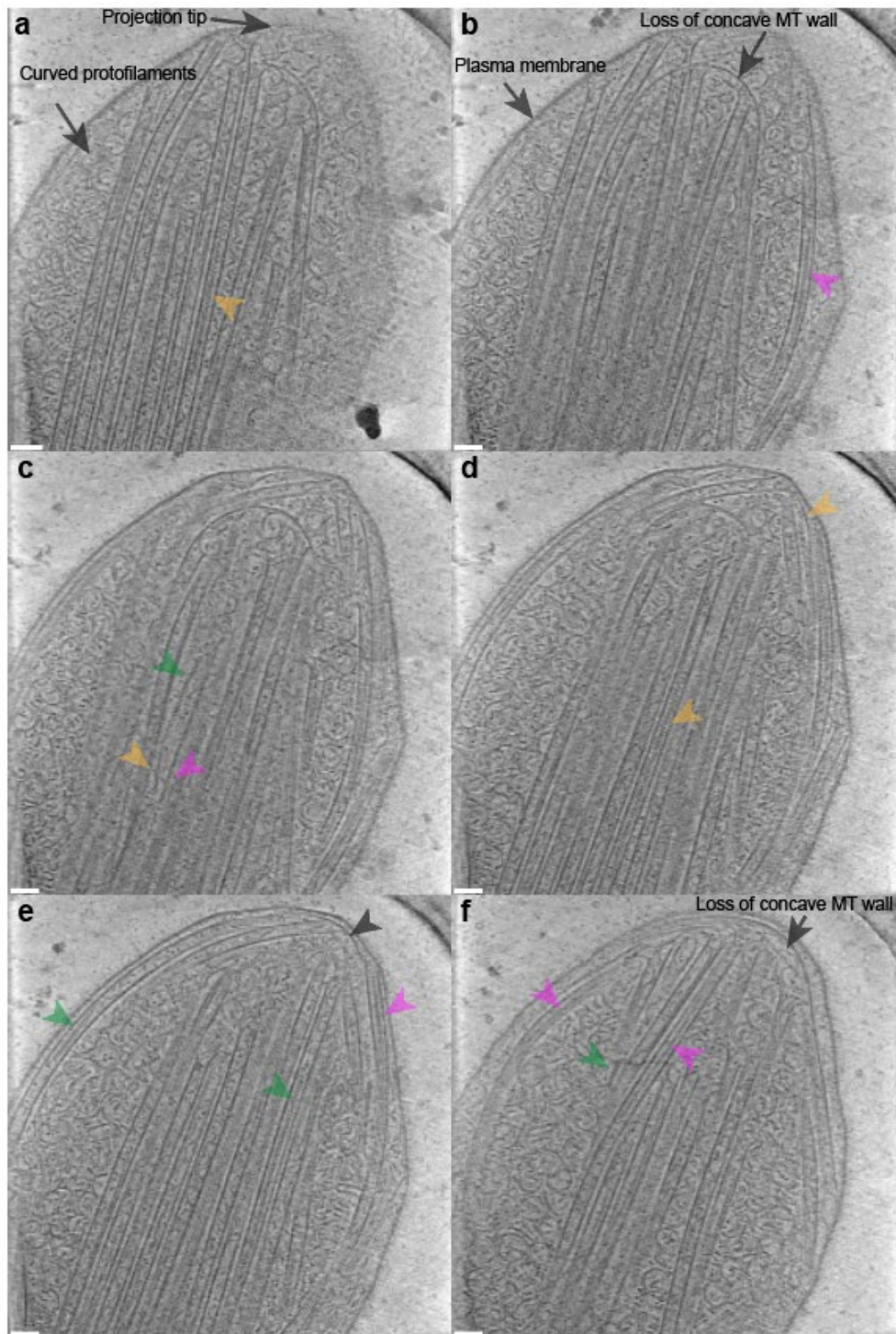
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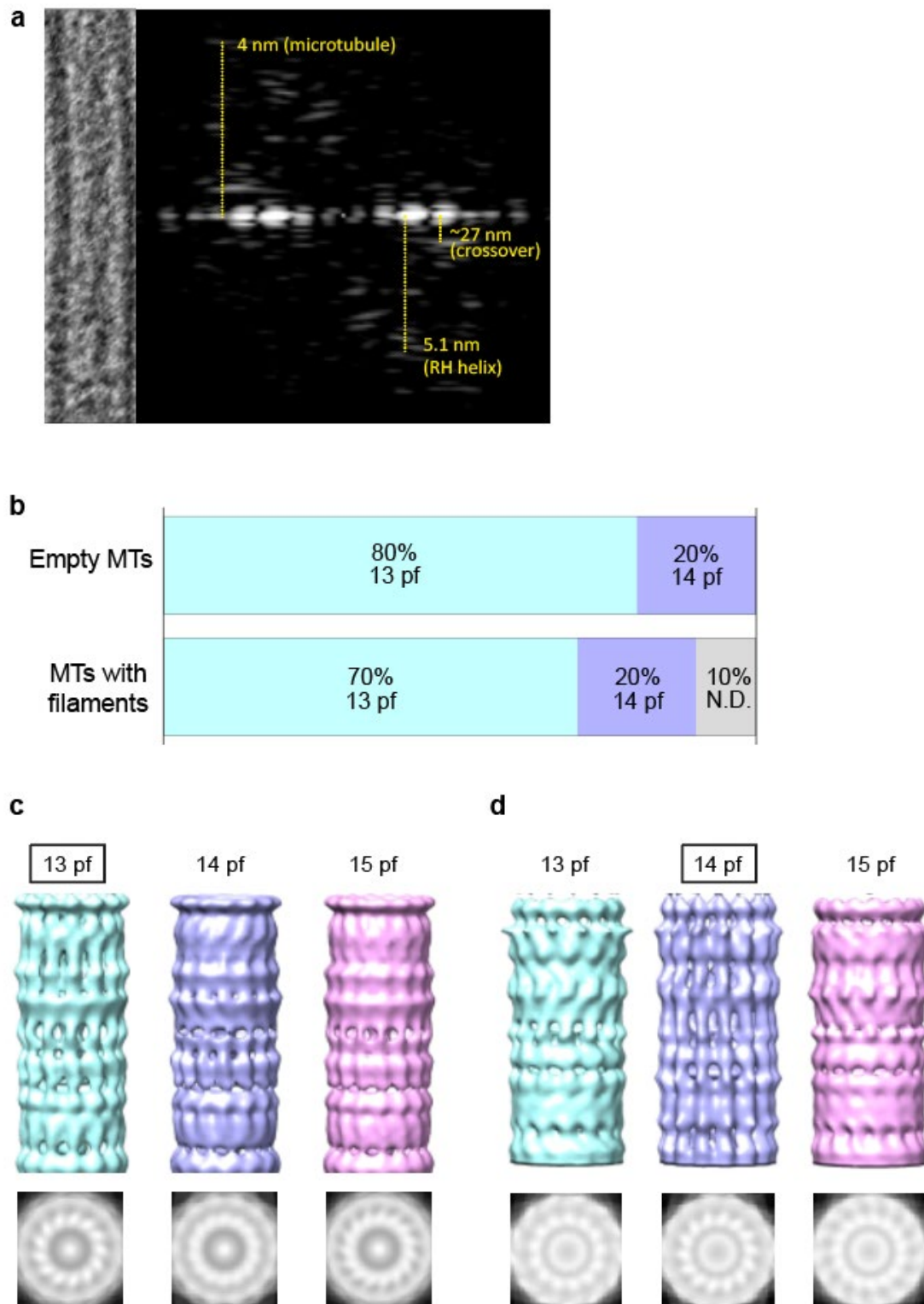
Supplementary Fig. 1. Characterisation of luminal filaments. (a,b) Power spectra of Class II and Class III filaments annotated with real space distances. **(c)** Docking of cofilin-actin structure PDB:8OH4 into map shown in Fig. 1H.



Supplementary Fig. 2. Quantification of *in vitro* reconstitution data. Three polymerisation experiments were performed for each condition (tubulin and actin, or tubulin and actin plus cofilin) and microtubules (n = 531 and n=193 respectively, in total) were counted. Error bars show S.E.M. There was no significant difference using a 2-tailed t-test (p=0.1).



Supplementary Fig. 3. Microtubules are under structural and mechanical stress when they are extruded from HAP1 cells. Tomogram slices showing a series of images along the Z-axis of the tip of a projection (a-f). The three filament classes are highlighted by coloured arrows (magenta – Class I, green Class II, orange, Class III). A video showing this Z-series is provided in Supplementary Movie 1. Scale bar = 50 nm.



Supplementary Fig. 4 Characterisation of microtubules and luminal filaments in platelets. (a) Power spectrum of a luminal Class I filaments in platelet microtubules annotated with real space distances. (b) Quantification of the protofilament number for 10 microtubules in platelets that do and do not contain filaments. (c, d) Representative examples of cross sections of rotationally averaged segments of microtubules and their associated 3D models viewed on UCSF Chimera with C13, C14 or C15 symmetries. The example in (c) was identified as a 13 protofilament microtubule whereas (d) was identified as a 14 protofilament microtubule.