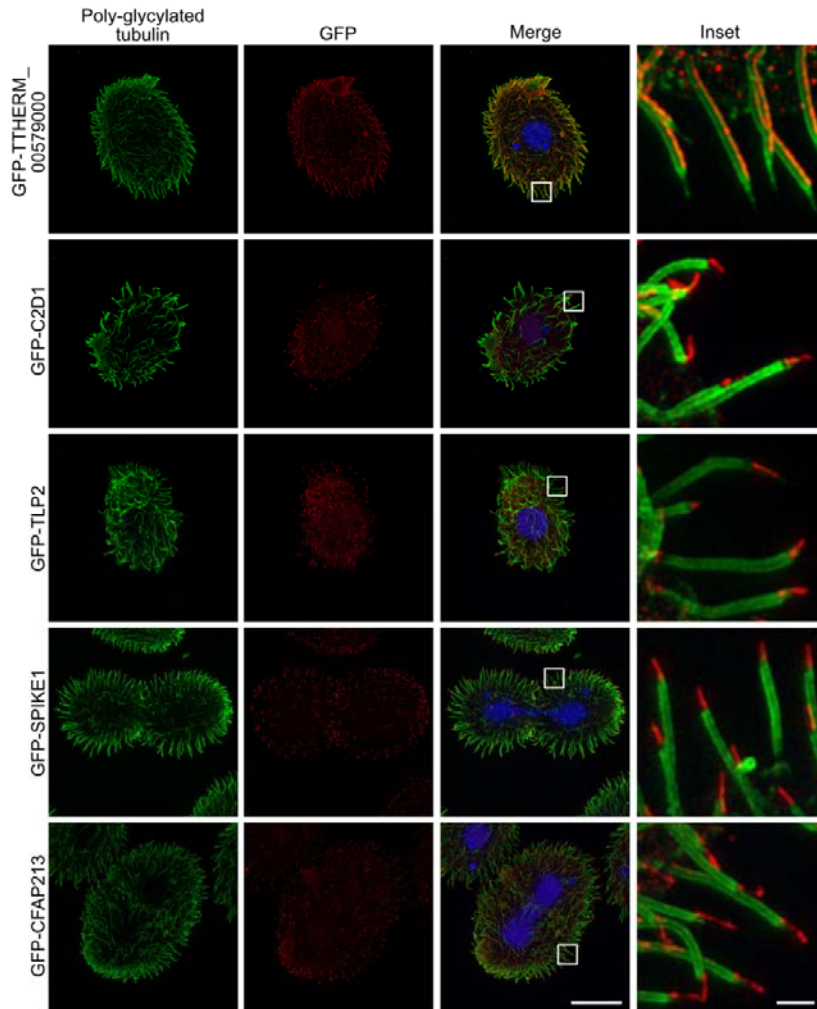
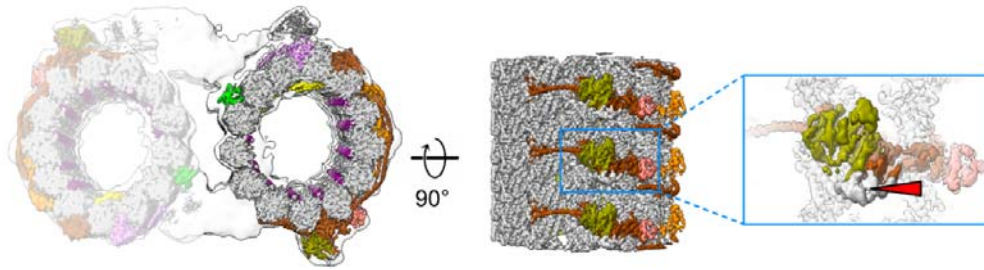


Supplementary Figure 1: (A) Cryo-EM processing workflow of the tip CP. (B) Fourier shell correlation curves of the local refinements used in the composite map of the tip CP. (C) Densities specific to C1 (blue) and C2 (orange) highlight the asymmetry between the two microtubules. (D) Mask (purple) used for the 3D classification of the helical bundle and the 16 nm feature (green) identified. (E) Densities segmented for use with DomainFit (grey) and best fit of the predicted model for each density (colour). (F) AlphaFold2-predicted dimers of three ciliary DPY-30-containing proteins (UniProt IDs: I7LWQ5, Q237C4, and Q22W95).



Supplementary Figure 2: Representative images of the colocalization of polyglycylated tubulin (green) and GFP-tagged THERM_00579000, TLP1, C2D1, TLP2, SPIKE1 and CFAP213 (red) and DNA (blue). The last column shows merged images. Scale bar: 20 μm . Insets zoomed in on representative cilia from the cell shown in the merged image. Scale bar: 1.5 μm .



Supplementary Figure 3: Spike density coloured near the predicted model of *SPIKE1* (green) and *TLP2* (brown). The red arrowhead points to a density whose protein was not identified (gray).

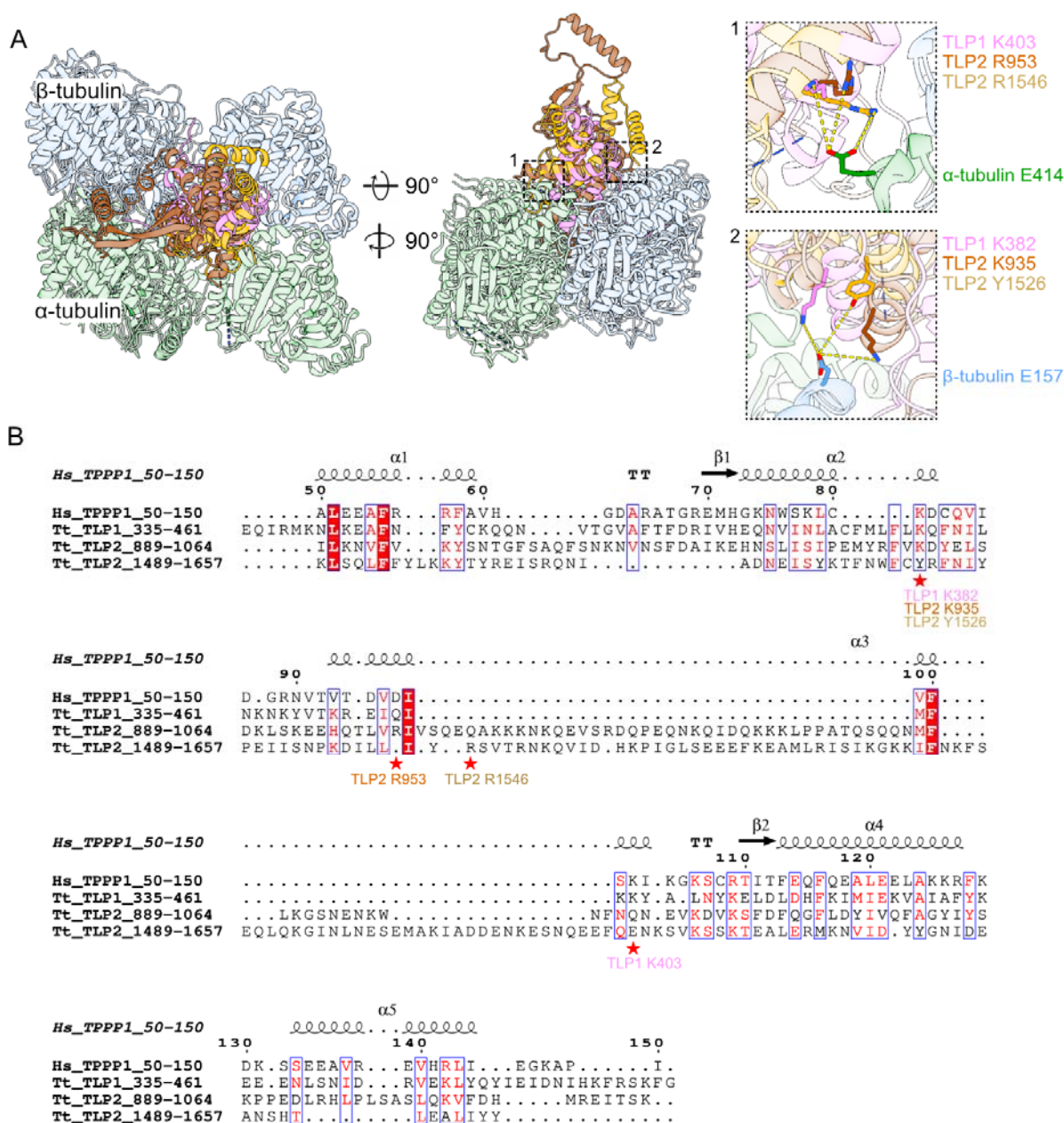


Figure S4: (A) Models of TPLP-like domains of TLP1 and TLP2 interacting with tubulin. (B) Alignment of the human TPLP1 (UniProtID O94811) and TPLP-like domains of TLP1 and TLP2. The amino acids shown in panel A are indicated by a red star.

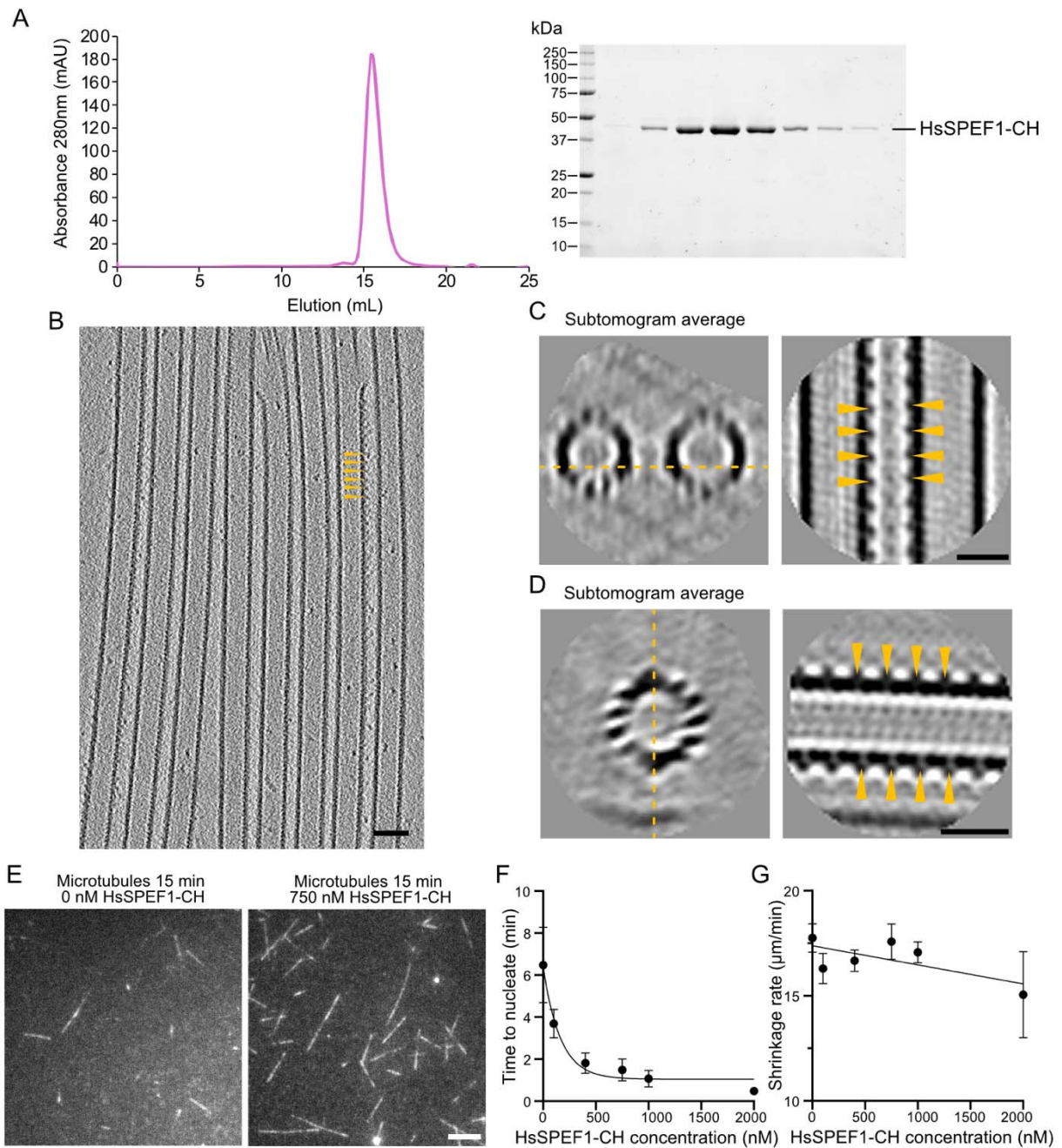


Figure S6: (A) Curve after SEC and the corresponding gel for HsSPEF1-CH. (B) Example tomogram of microtubules with HsSPEF1-CH. Arrowheads point to extradensity binding to the side of a microtubule with an apparent repeat of 8 nm. Scale bar, 40 nm. (C - D) Subtomogram averages of a microtubule pair shown in Fig. 6F and a single microtubule from another tomogram. Arrowheads point to extra densities that repeat every 8 nm. Scale bar 20 nm. (E) Representative TIRF images of the AF546 channel (microtubules) at $t = 15$ min with and without 750 nM HsSPEF1-CH. Scale bar, 4 μ m. (F) Time for microtubules to nucleate vs the HsSPEF1-CH concentration. (G) Microtubule shrinkage rate vs the HsSPEF1-CH concentration.