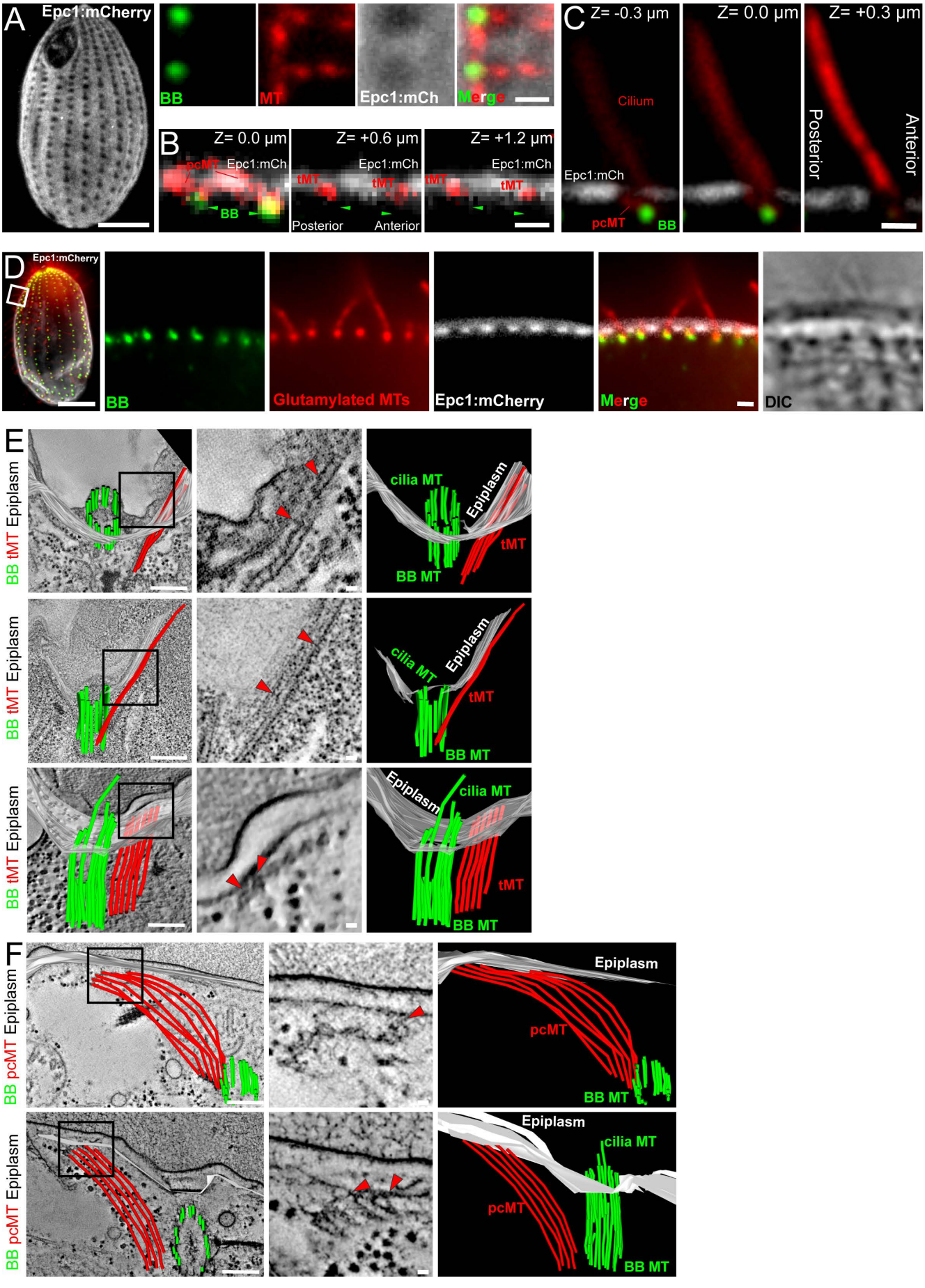


Figure S1: *Tetrahymena* cortical cytoskeleton. (A) Left and top panels, Epc1:mCherry (grayscale) labeled cell with BBs (Centrin, green) and BB-appendage microtubules (polyglycylated tubulin, red). Scale bars, 10 and 1 (inset) µm. (B) Single Z-plane images of longitudinal sections through BB units showing the relative proximity of tMTs and the epiplasm (Centrin, green; acetylated-tubulin, red; Epc1:mCherry, grayscale). Scale bar, 1 µm. (C) Single Z-plane images of longitudinal sections through BBs show the relative proximity of pcMTs to the epiplasm (Centrin, green; polyglycylation, red; Epc1:mCherry, grayscale). Scale bar, 1 µm. (D) Left panel, BBs (Centrin, green), cilia and BB-appendage microtubules (glutamylated tubulin, red), and epiplasm (Epc1:mCherry, grayscale). Scale bar, 10µm. Right panels, single Z-plane images of a longitudinal section through BB units. Scale bar, 1 µm. (E) Left panels, 3D models of epiplasm (white), BB (green) and tMTs (red) projected on tomographic slices. The boxed regions highlight tMT bundles running directly below the epiplasm. Scale bar, 200 nm. Middle panels, tomographic slices from the boxed regions show tMT connections with the cortical epiplasm (red arrowheads). Scale bar, 20 nm. Right panels, 3D model of the BB units derived from EM tomographic reconstructions. (F) Left panels, 3D models of epiplasm (white), BB (green) and pcMTs (red) projected on tomographic slices. Boxed regions highlight pcMT bundles running directly below the epiplasm. Scale bar, 200 nm. Middle panels, tomographic slices from the boxed regions show pcMT-Epiplasm interfaces (red arrowheads). Scale bar, 20 nm. Right panels, 3D models of the BB units derived from EM tomographic reconstructions.

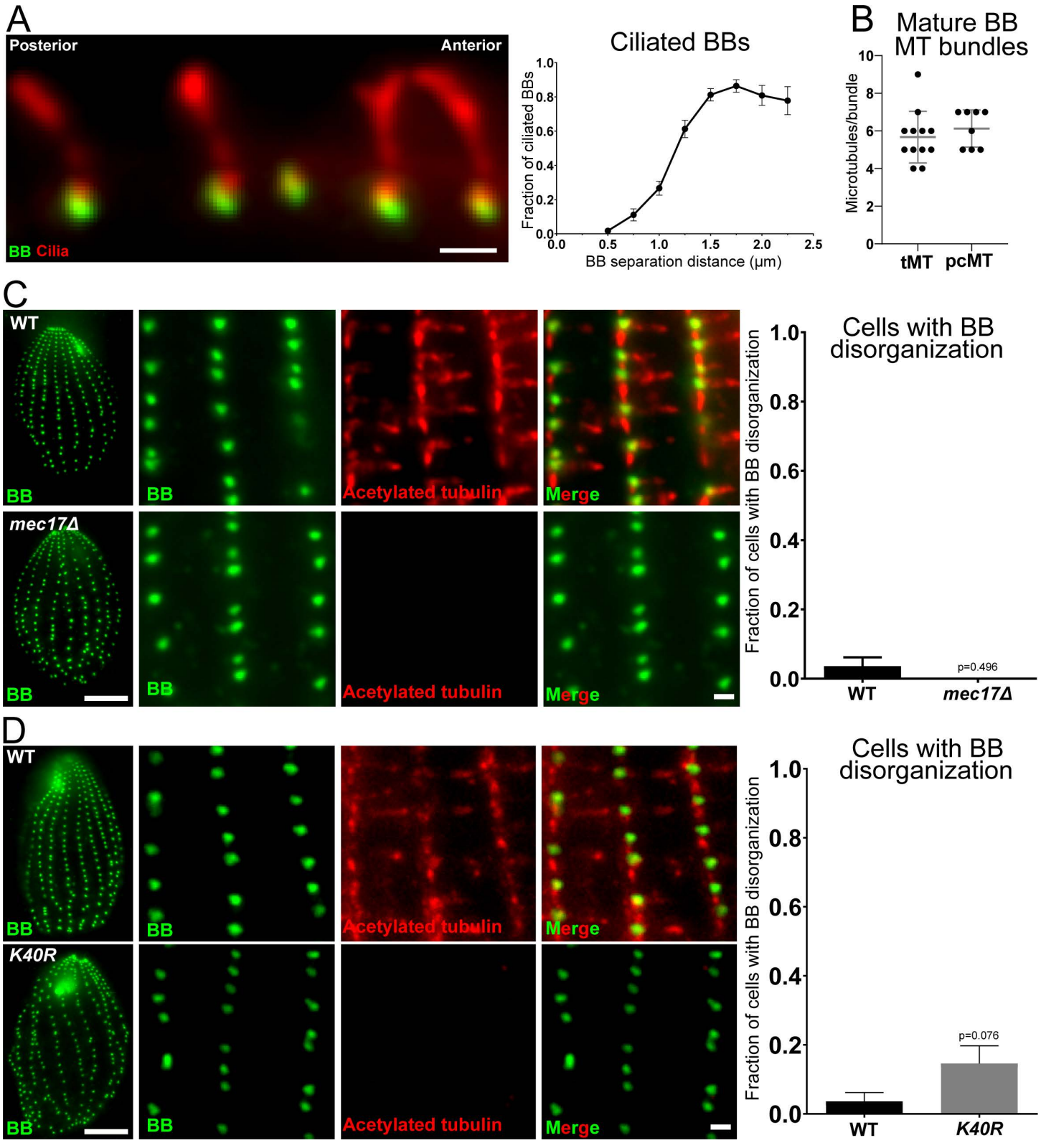


Figure S2: Microtubule acetylation is not required for BB organization. (A) Left panel, *Tetrahymena* cilia (glutamylated-tubulin, red) and BBs (Centrin, green). Scale bar, 1 µm. Right panel, the frequency of ciliated new BBs increases with separation distance from mother BBs (n = 52 cells, 648 ciliated BB pairs). (B) Quantification of the number of microtubules in tMT and pcMT bundles of mature BBs. (C) Left panels, BBs (Centrin, green) in WT and *mec17Δ* cells. Scale bar, 10µm. Middle panels, insets of WT and *mec17Δ* BBs (Centrin, green) and BB-appendage microtubules (acetylated-tubulin red). Scale bar, 1 µm. Right panel, BB disorganization does not increase in *mec17Δ* cells lacking acetylated-tubulin (WT: n = 55 cells, *mec17Δ*: n = 51 cells). (D) Left panels, WT and *K40R* BBs (Centrin, green). Scale bar, 10µm. Middle panels, insets of WT and *K40R* BBs (Centrin, green) and BB-appendage microtubules (acetylated-tubulin, red). Scale bar, 1 µm. Right panel, BB disorganization does not increase in *K40R* cells (WT: n = 55 cells, *K40RΔ*: n = 48 cells).

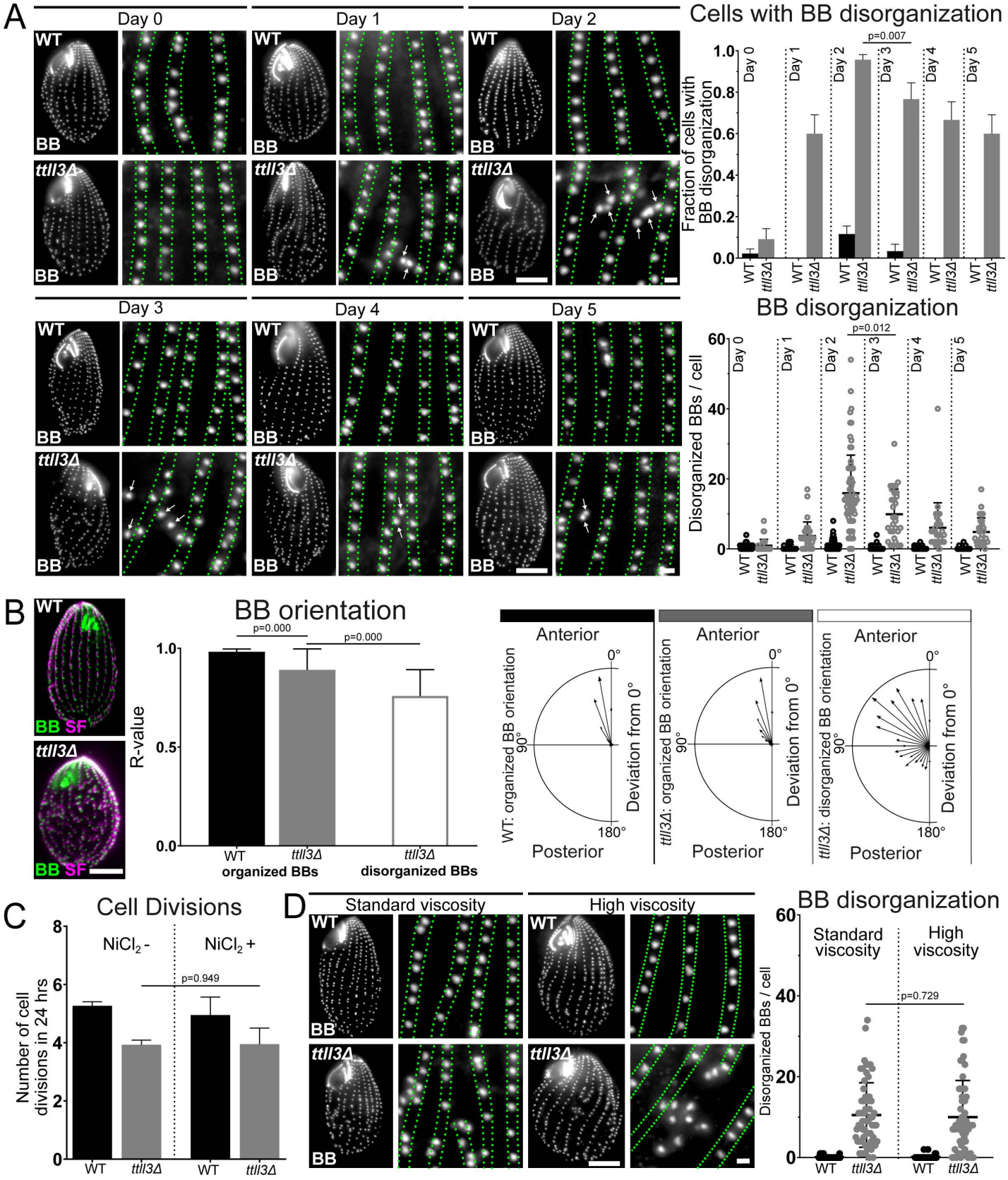


Figure S3: Glycylation promotes BB organization against ciliary forces. (A) Left panels, WT and *ttll3Δ* BBs (Centrin, grayscale) in cells grown 0, 1, 2, 3, 4 and 5 days post knockout at 30ºC. Scale bars, 10 and 1 (inset) µm. Green lines denote boundaries of BB rows. Arrows indicate disorganized BBs. Top right panel, fraction of cells with BB disorganization decreases after Day 2 in *ttll3Δ* cells (WT day 0: n = 46 cells, *ttll3Δ* day 0: n = 33, WT day 1: n = 30, *ttll3Δ* day 1: n = 30, WT day 2: n = 69, *ttll3Δ* day 2 : n = 70, WT day 3: n = 30, *ttll3Δ* day 3: n = 30, WT day 4: n = 30, *ttll3Δ* day 4: n = 30, WT day 5: n = 30, *ttll3Δ* day 5: n = 30). Bottom right panel, the number of disorganized BBs per *ttll3Δ* cell decreases after Day 2 (WT day 0: n = 46, *ttll3Δ* day 0: n = 33, WT day 1: n = 30, *ttll3Δ* day 1: n = 30, WT day 2 : n = 69, *ttll3Δ* day 2 : n = 70, WT day 3: n = 30, *ttll3Δ* day 3: n = 30, WT day 4: n = 30, *ttll3Δ* day 4: n = 30, WT day 5: n = 30, *ttll3Δ* day 5: n = 30). B) Left panels, BB (Centrin, green) and SF (α-striated fiber, magenta) orientation in WT and *ttll3Δ* cells. Middle panel, organized and disorganized *ttll3Δ* BBs are disoriented (WT: n = 58 cells, WT organized BBs and SFs = 1235, *ttll3Δ* cells: n = 64 cells, *ttll3Δ* organized and disorganized BBs and SFs = 1567). Right panel, arrow graphs represent the frequency of angular deviation from the cell anterior-posterior axis observed in disoriented BBs. (C) $NiCl_2$ does not affect the number of WT or *ttll3Δ* cell divisions in 24 hrs (WT $NiCl_2$-: n = 3, WT $NiCl_2$+: n = 3, *ttll3Δ* $NiCl_2$-: n = 3, *ttll3Δ* $NiCl_2$+: n = 3). (D) Left panels, WT and *ttll3Δ* BBs (Centrin, grayscale) in cells cultured at 30ºC in standard and viscous media. Scale bars, 10 and 1 (inset) µm. Green lines denote BB rows. Right panel, BB disorganization does not increase in *ttll3Δ* cells in high viscosity media (WT standard viscosity: n = 46 cells, *ttll3Δ* standard viscosity: n = 62 cells, WT high viscosity: n = 49 cells, *ttll3Δ* high viscosity: n = 62 cells).

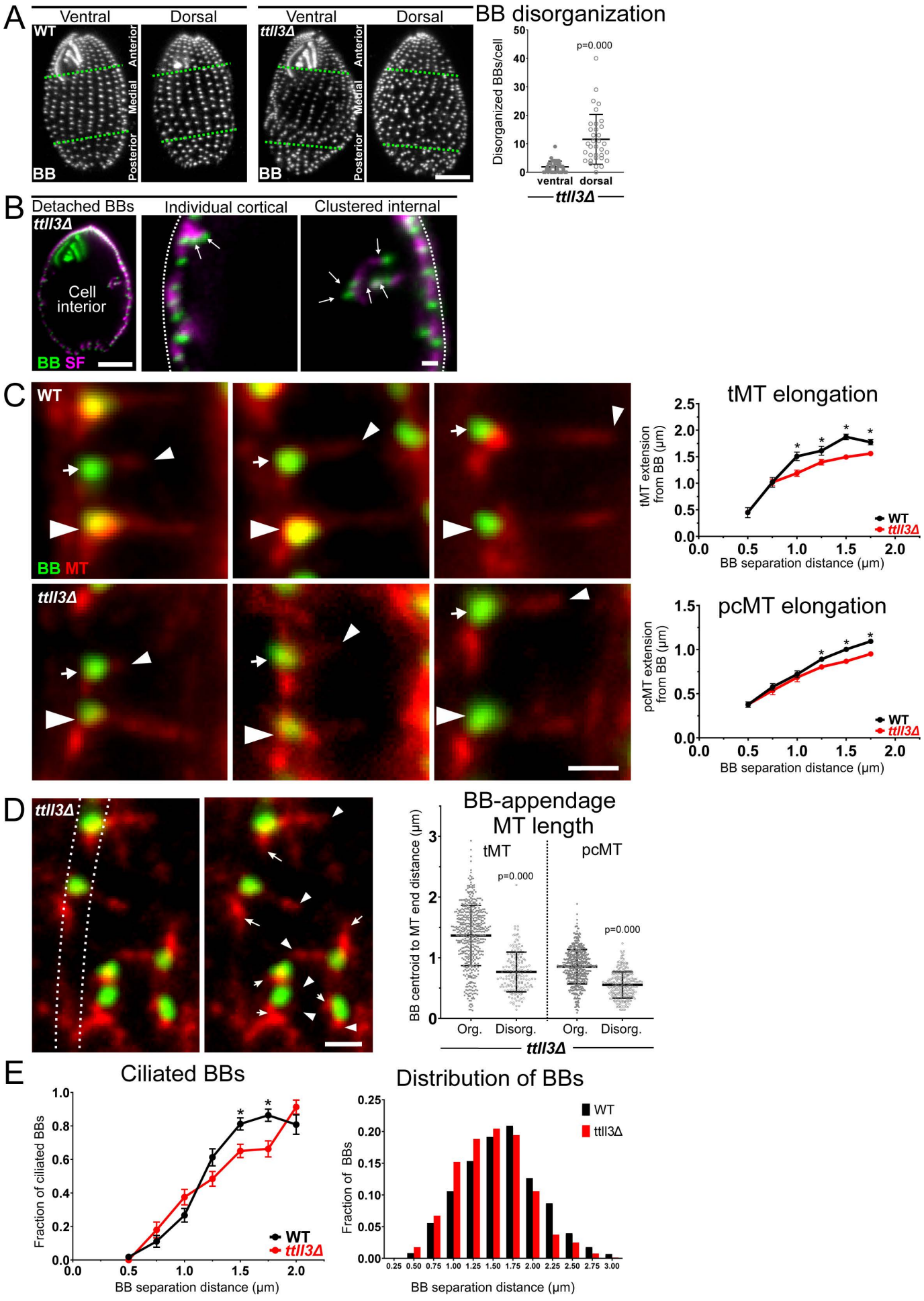


Figure S4: BB disorganization and slower BB-appendage microtubule elongation in ttll3Δ cells. (A) BB (Centrin, grayscale) organization along the ventral and dorsal axis of WT and *ttll3Δ* cells. The medial and posterior regions of the dorsal side of *ttll3Δ* cells exhibited increased BB disorganization. Green lines denote boundaries between the anterior quartile, medial half, and posterior quartile. Scale bar, 10 µm. BB disorganization is elevated on the dorsal side compared to ventral side within the medial half of *ttll3Δ* cell (*ttll3Δ* ventral side: n = 34 cells, *ttll3Δ* dorsal side: n = 34 cells). (B) Longitudinal view of BBs (Centrin, green) and SFs (α-SF, magenta) relative to the cell outline. Detached BBs are either near the cortex or in internal clusters. Scale bars, 10 and 1 (inset) µm. Arrows denote detached BBs. (C) Left panels, BBs (Centrin, green) and BB-appendage microtubules (α-tubulin, red) in WT and *ttll3Δ* cells during BB maturation. Arrowheads denote mother BBs and arrows denote new BBs. Scale bar, 1 µm. Right panels, elongating tMT and pcMT lengths are shorter in *ttll3Δ* cells (WT: n = 67 cells, 258 tMTs, 331 pcMTs; *ttll3Δ*: n = 75 cells, 379 tMTs, 396 pcMTs). Asterisks denote p value <0.05. (D) Left panel, image of BBs (Centrin, green) and BB-appendage microtubules (acetylated-tubulin, red) with BB row demarcated by white line. Middle panel, same image with tMTs indicated by arrowhead and pcMTs indicated by arrows. The distance between BB centroid (Centrin) to tMTs and pcMTs ends (α-tubulin) of disorganized BBs are shorter than those of organized BBs (n = 75 cells, *ttll3Δ* organized BBs: 511 tMTs, 550 pcMTs; *ttll3Δ* disorganized BBs: 166 tMTs, 245 pcMTs). (E) *ttll3Δ* cells exhibit a delay in ciliogenesis compared to WT cells (WT: n=54 cells, 603 BB pairs; *ttll3Δ:* n=48 cells, 629 BB pairs). Asterisks denote p value <0.05. *ttll3Δ* cells have increased BBs that are less separated from their posterior mother BBs (WT: n=69 cells, 737 BB pairs; *ttll3Δ:* n=70 cells, 803 BB pairs).

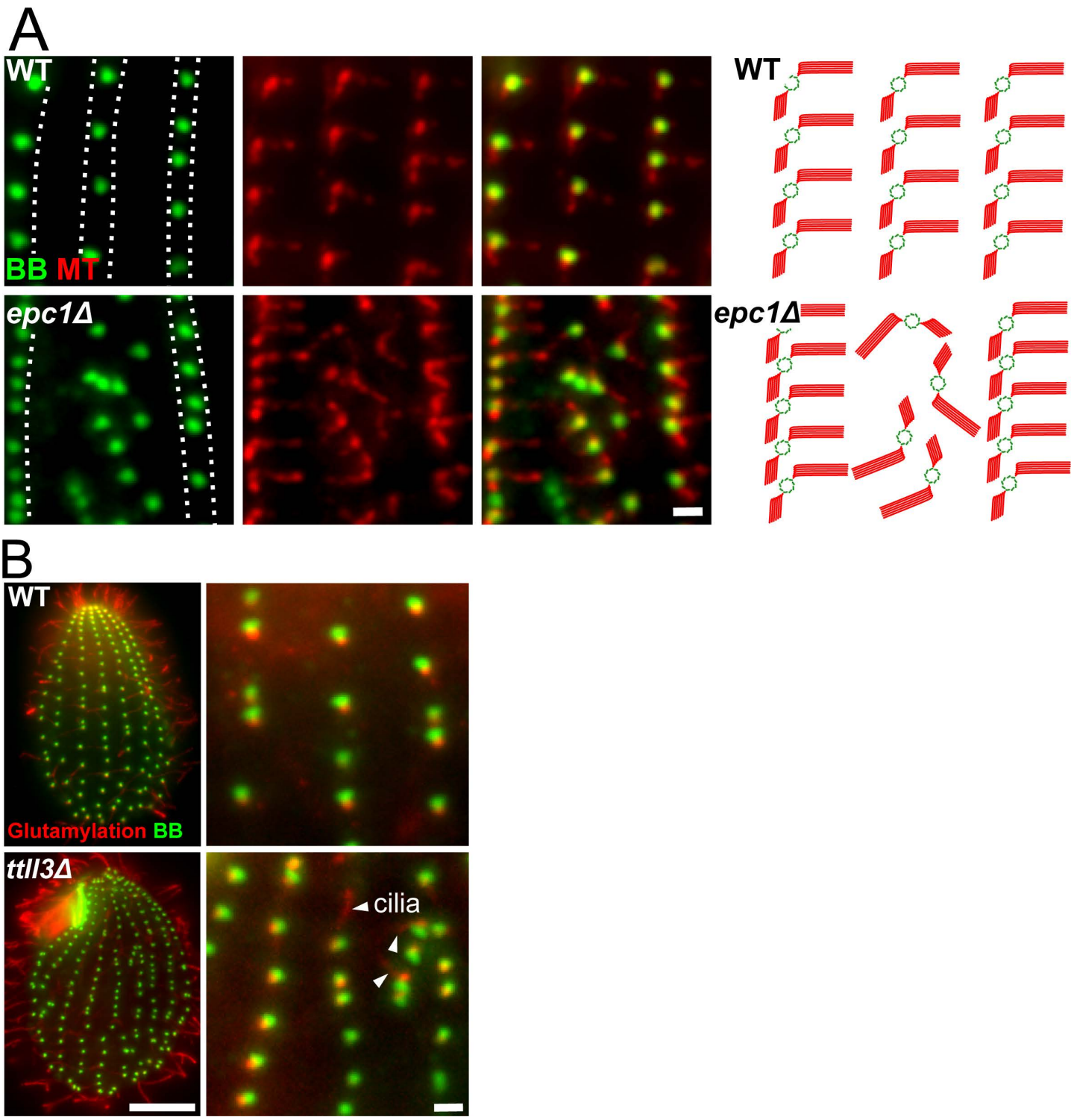
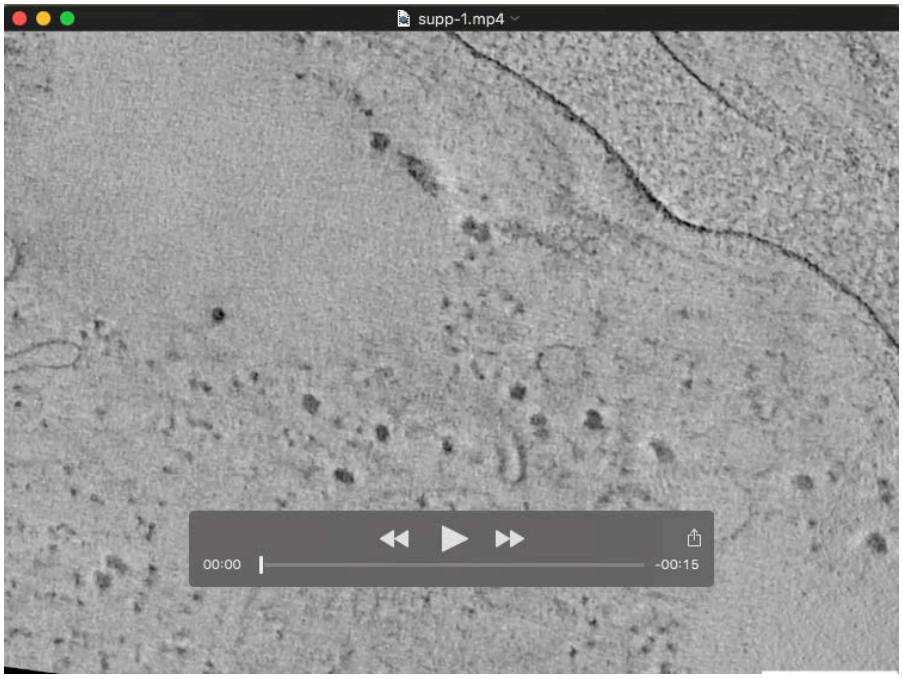
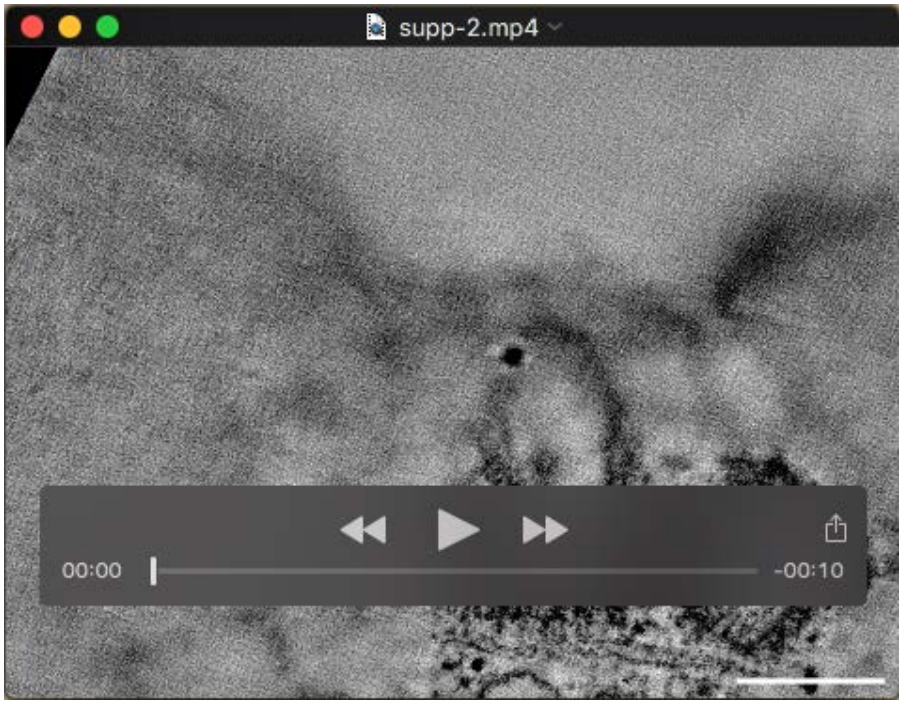


Figure S5: BB and BB-appendage microtubule orientation in epc1*Δ* cells and compensation in *ttll3Δ* cells. (A) Images of BBs (Centrin; green) and BB-appendage microtubules (α-tubulin; red) in WT and epc1*Δ* cells. White lines denote boundaries of BB rows. Scale bar 1 µm. Model of BB organization and BB-appendage microtubule orientation. (B) WT and *ttll3Δ* cells cultured for >30 days at 23ºC, shifted to 30ºC for 24 hrs and stained for BBs (Centrin, green) and glutamylated tubulin (GT335, red). Arrowheads denote glutamylated tubulin stained cilia. Scale bars, 10 and 1 (inset) µm.



Movie 1: tMTs are associated with the cortical epiplasm. EM tomogram of a longitudinal view of tMTs, epiplasm, and BB. First Z series shows the tomogram without modeled structures. The second Z series models the tMTs (red), Epiplasm (white), and BB (green) onto the EM tomographic planes. The third Z series shows a 3-dimensional view of the modeled tMTs, Epiplasm, and BB.



Movie 2: pcMTs are associated with the cortical epiplasm. EM tomogram of a longitudinal view of the pcMTs, epiplasm, and BB. First Z series shows the tomogram without modeled structures. The Z series models the pcMTs (red), Epiplasm (white), and BB (green) onto the EM tomographic planes. The third Z series shows a 3-dimensional view of the modeled pcMTs, Epiplasm, and BB.