Figure S1. 3MB-PP1 inhibits TbPLK^{as} in vitro, but has no effect on wild-type TbPLK. Recombinant TbPLK^{as} and TbPLK were used in ^{32}P kinase assays with TbCentrin2 as a substrate and a range of concentrations of 3MB-PP1 or DMSO as a vehicle control. A portion of each assay was fractionated on SDS-PAGE gels, stained with Coomassie, dried, and exposed to film. The upper panel (^{32}P-TbCentrin2) shows the ^{32}P incorporation into TbCentrin2. The lower panel (Coomassie) shows the total TbCentrin2 loaded in each lane. The concentration of 3MB-PP1 in micromolar used in each assay is listed above the lane, with "Ctr" referring to the vehicle control.

Figure S2. Cells expressing a single TbPLK allele are not affected by 3MB-PP1 treatment. The single allele TbPLK knockout cell line was treated with different concentrations of 3MB-PP1 or a vehicle control and its growth was monitored with a cell counter for 12 hours. Error bars represent the standard deviation of three biological replicates.

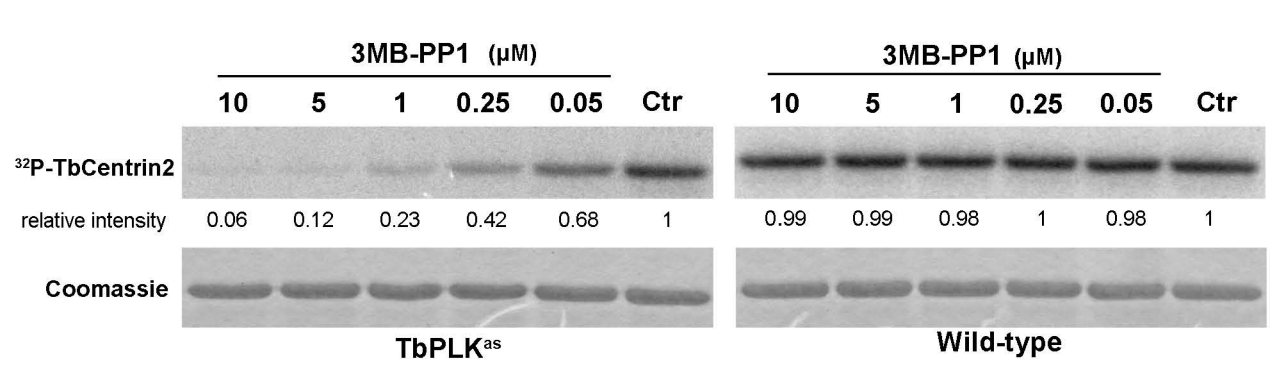
Figure S3. Wild-type cells do not develop defects in DNA, FAZ or bilobe duplication after 3MB-PP1 treatment. Wild-type cells were treated with 3MB-PP1 or vehicle control for 9 hours and then fixed. The cells were stained with DAPI to label DNA and either anti-TbCentrin4 or anti-FAZ1 to label the basal body and bilobe or FAZ, respectively. The DNA content of the cells and the status of the bilobe and FAZ were determined by fluorescence microscopy. (A) The DNA state of wild-type cells is not altered by 3MB-PP1 treatment. Aberrant DNA states such as 2N1K were not present in either experimental condition. (B) Bilobe duplication is not perturbed by 3MB-PP1 treatment of wild-type cells. (C) New FAZ assembly is not blocked by 3MB-PP1 treatment of wild-type cells. Error bars represent the standard deviation for three biological replicates. 200 cells were counted per condition. Ns- not significant.

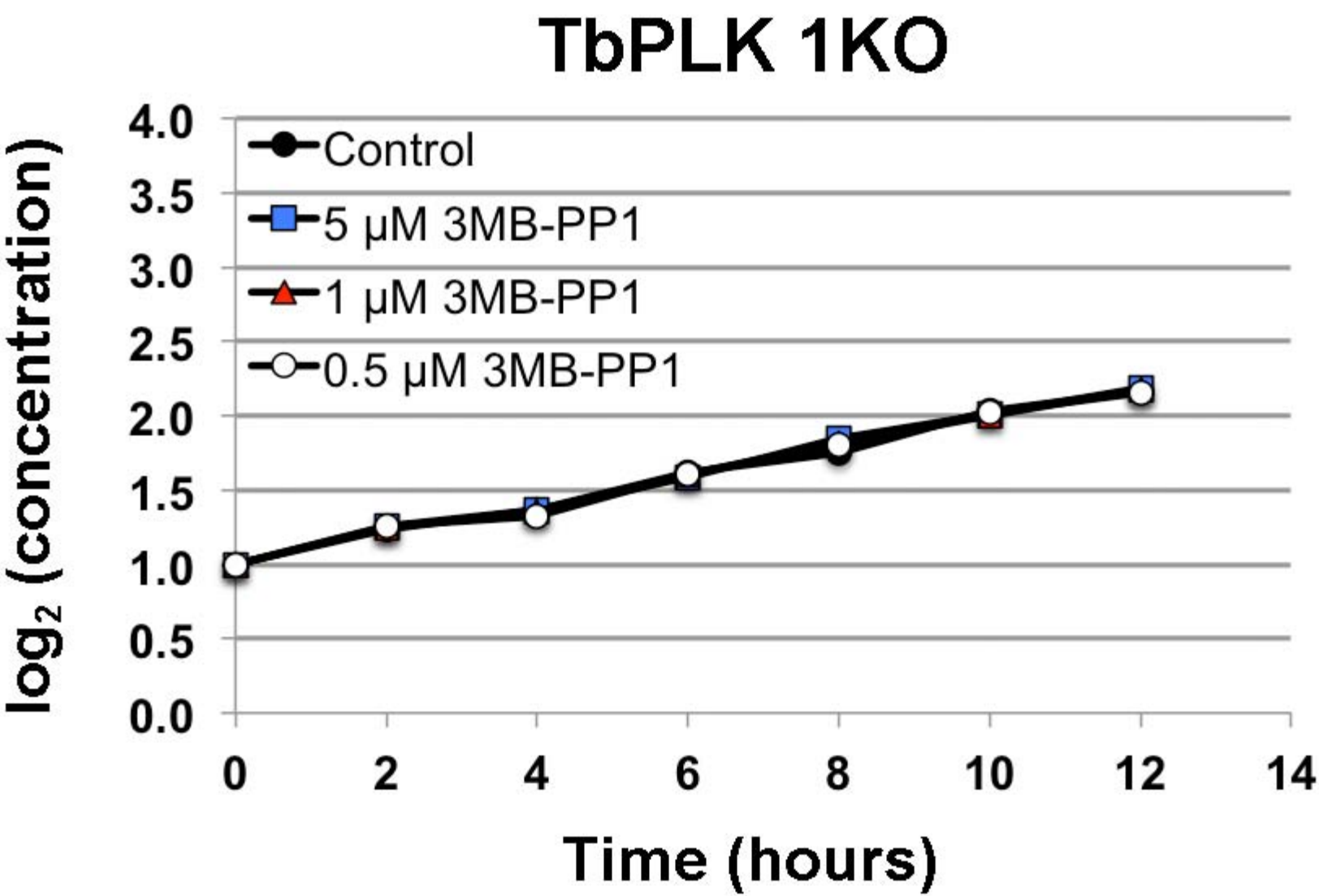
Figure S4. Double cut elutriation yields cells synchronized early in G1. TbPLK^{as} cells were elutriated, selecting for the largest cells, which were late in the cell cycle and about to undergo cytokinesis. These large cells were put back into culture for 1.5 hours, then the resultant population was subjected to a second round of elutriation, selecting for the smallest cells, which had just completed division. These small cells were put back into culture and their cycle progression was monitored by propidium iodide staining followed by flow cytometry. Control cells from an asynchronous culture are shown, followed by cells just after the second round of elutriation (t = 0 hours), and selected time points thereafter. Cells at t = 0 were highly enriched for G1 cells, which progressed to G2 between t = 3 and t = 5 hours, emerging back into G1 at t = 8 hours.

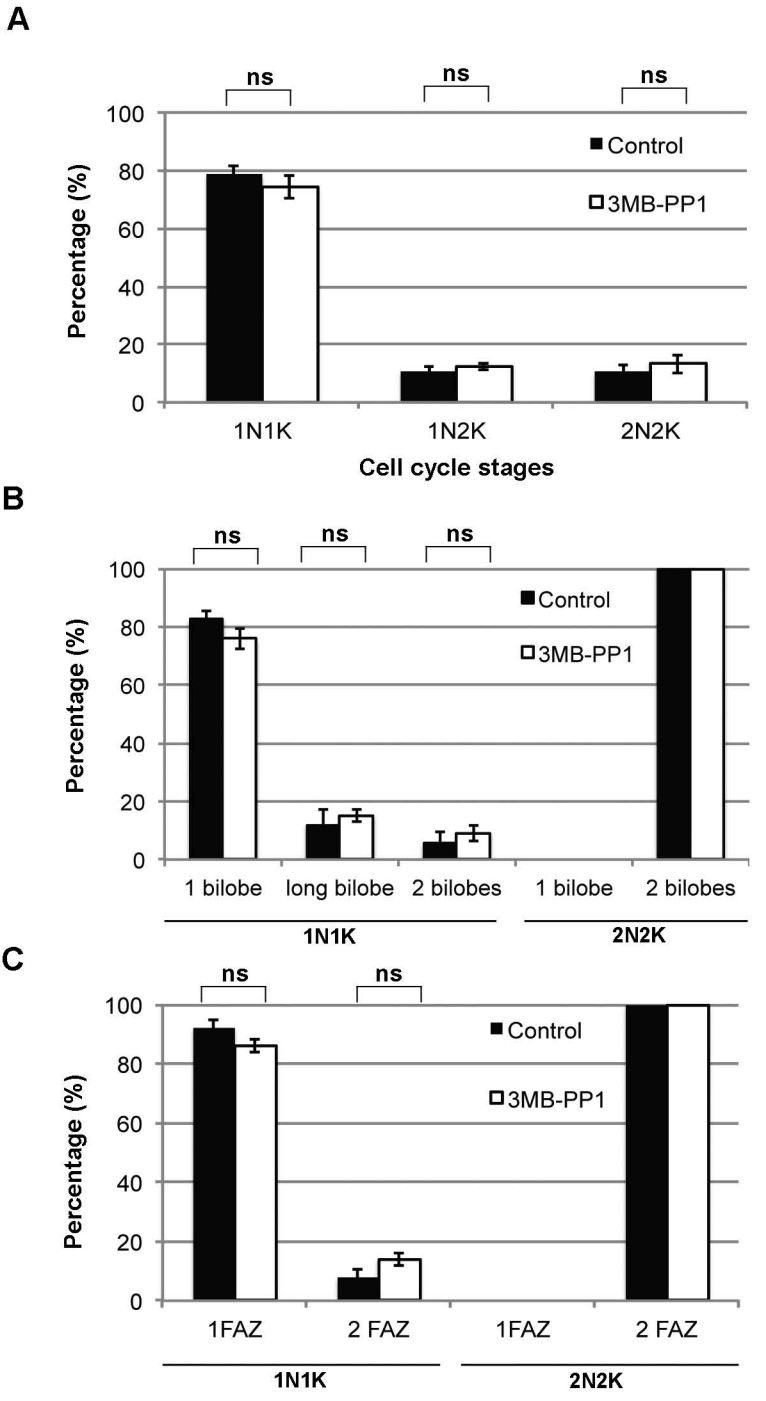
Figure S5. Treatment of synchronized TbPLK^{as} cells with 3MB-PP1 at different points in the cell cycle generates distinct phenotypes. TbPLK^{as} cells were treated with 3MB-PP1 or vehicle control at t = 0, 1.5, 3.5, or 5.5 hours after synchronization by double cut elutriation, then fixed at t = 7.5 hours. The cells were stained with DAPI to label DNA and either anti-TbCentrin4 or anti-FAZ1 antibodies to label the bilobe or FAZ, respectively. (A) DNA state of the cells fixed treated as mentioned above. Diminished 1N1K and the appearance of aberrant 2N1K cells was evident in samples treated with 3MB-PP1 at t = 0 and t = 1.5 hours. (B) Bilobe state of cells fixed as mentioned above. Bilobe duplication was effectively blocked by 3MB-PP1 treatment at t = 0 and t = 1.5 hours. (C) FAZ state of cells fixed as mentioned above. FAZ duplication was effectively blocked by 3MB-PP1 at t = 0 and t = 1.5 hours, while treatment at t = 3.5 hours gave an intermediate phenotype. Error bars represent the standard deviation of three biological replicates. 200

cells were counted per condition and time point. Double asterisks denote a P value less than 0.01, single asterisk less than 0.05, ns- not significant.

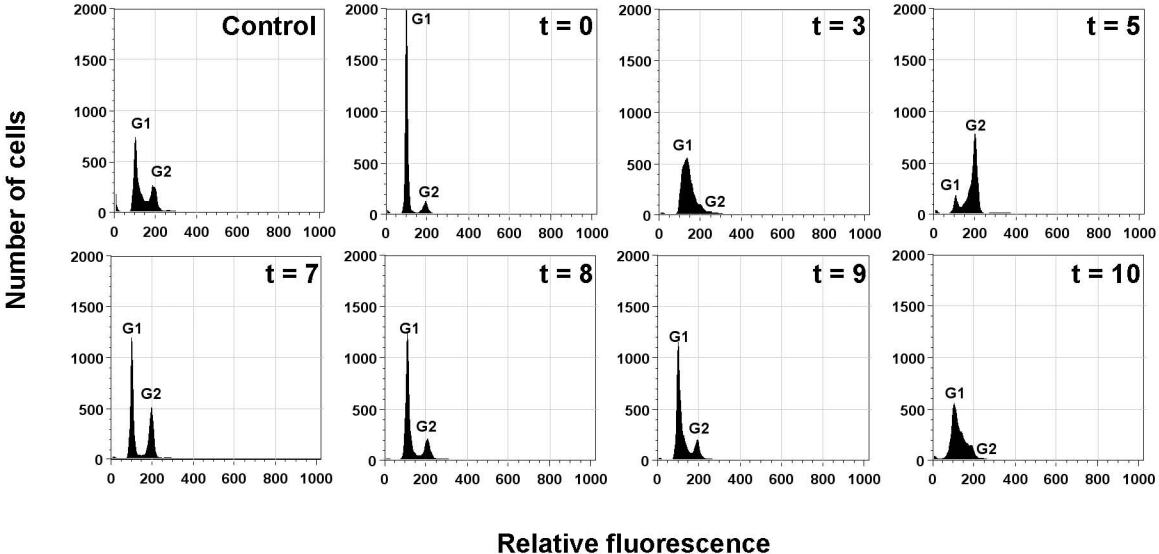
Figure S6. Additional images of synchronized TbPLK^{as} cells treated with 3MB-PP1 at t = 0 where basal body rotation has not occurred. Synchronized TbPLK^{as} cells were treated as in Figure 6, then fixed and stained for negative stain EM. The cells in A-D represent cells where basal body rotation has not occurred and show how this phenotype persists even as the new flagellum increases in length. The old basal body is identified with an empty arrowhead and the new basal body is shown with a filled arrowhead. The scale bar is 5 microns.

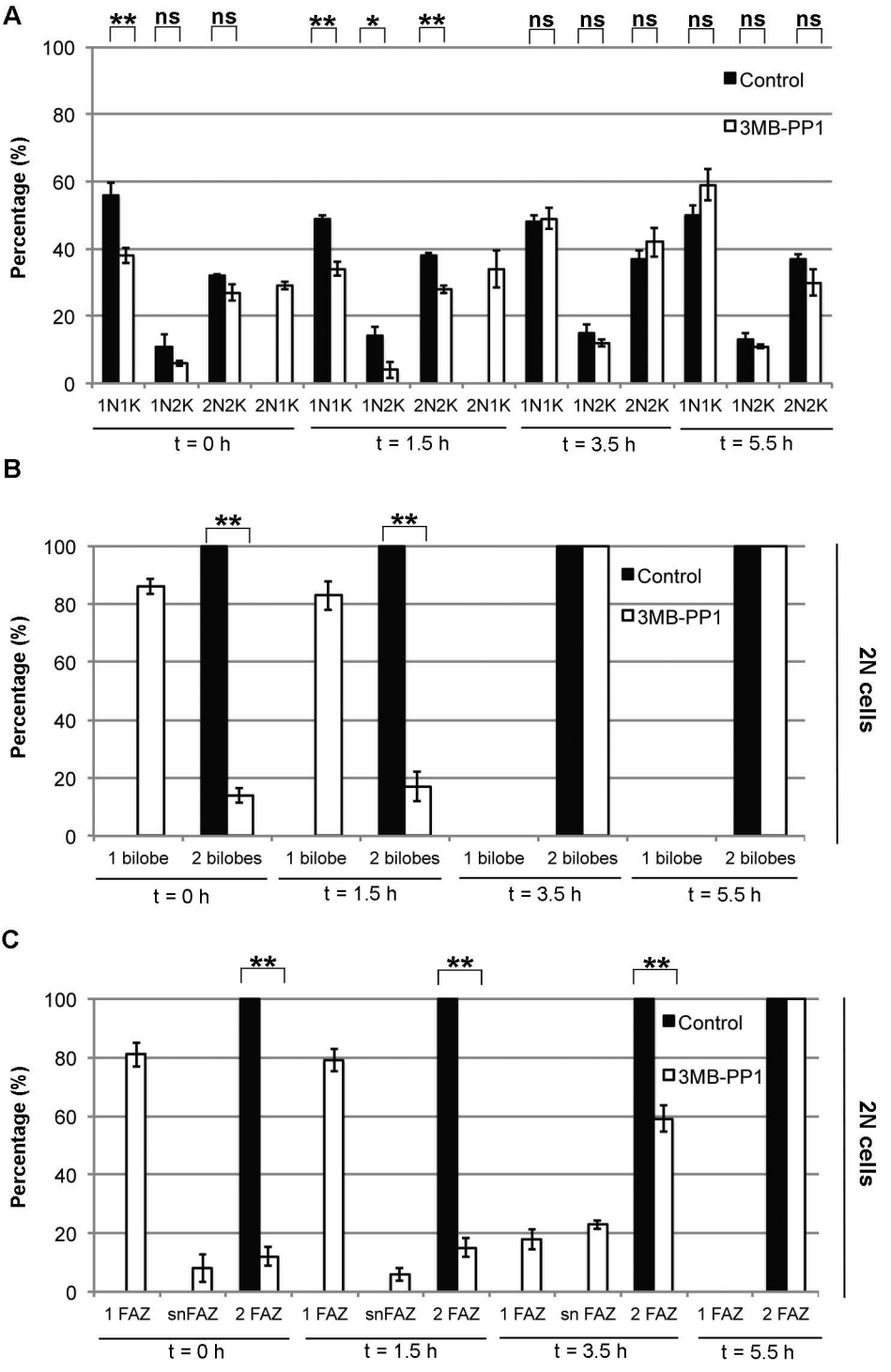

3MB-PP1 (μM)
10
5
1
0.25
0.05
Ctr
32P-TbCentrin2
relative intensity
0.06
0.12
0.23
0.42
0.68
1
Coomassie
TbPLKas
3MB-PP1 (μM)
10
5
1
0.25
0.05
Ctr
0.99
0.99
0.98
1
0.98
1
Wild-type

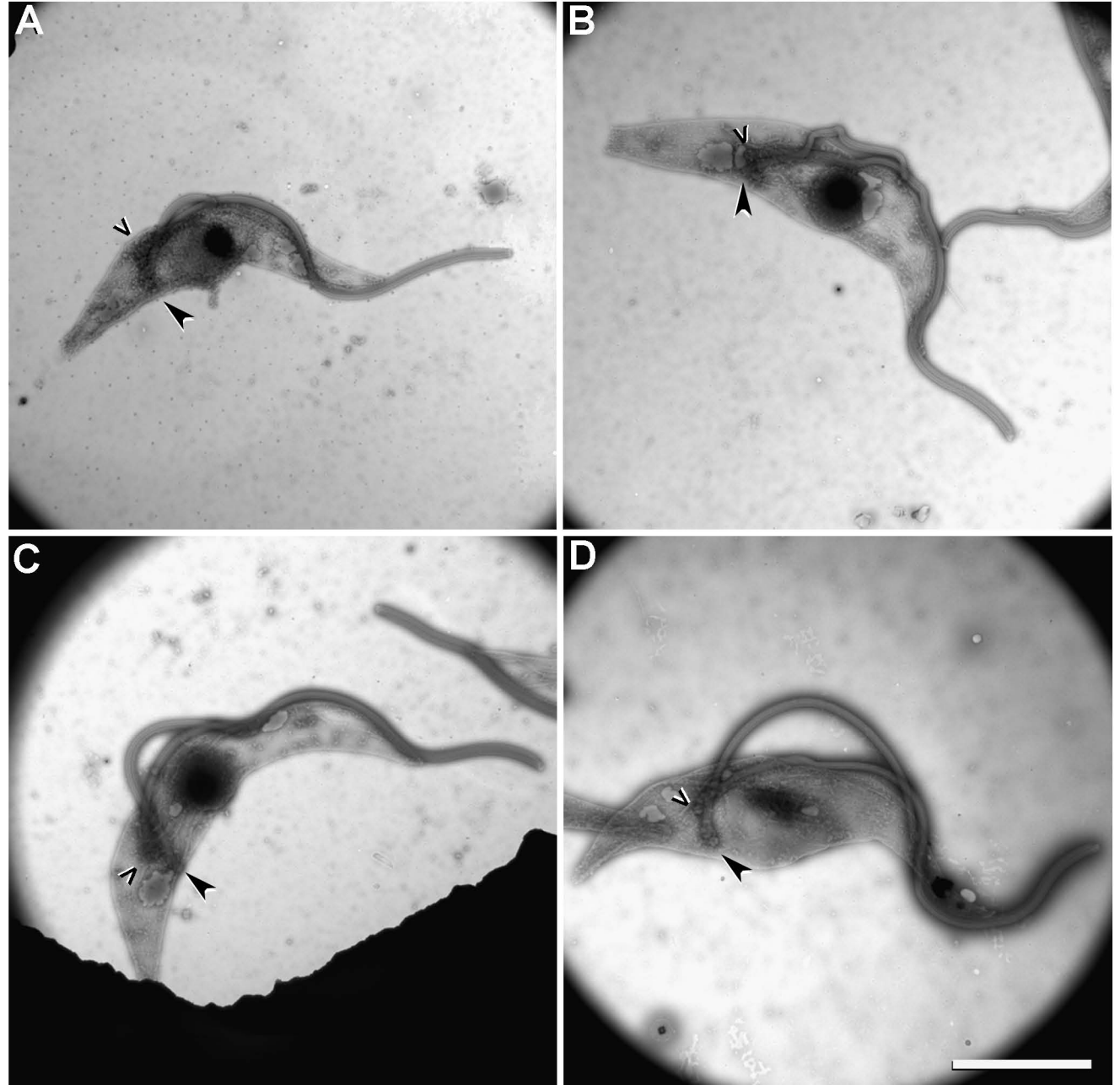

TbPLK 1KO
Control
5 μM 3MB-PP1
1 μM 3MB-PP1
0.5 μM 3MB-PP1
log2 (concentration)
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
0
2
4
6
8
10
12
14
Time (hours)


A
ns
ns
ns
100
80
60
40
20
0
Percentage (%)
Control
3MB-PP1
1N1K
1N2K
2N2K
Cell cycle stages
B
ns
ns
ns
100
80
60
40
20
0
Percentage (%)
Control
3MB-PP1
1 bilobe
long bilobe
2 bilobes
1 bilobe
2 bilobes
1N1K
2N2K
C
ns
ns
100
80
60
40
20
0
Percentage (%)
Control
3MB-PP1
1FAZ
2 FAZ
1FAZ
2 FAZ
1N1K
2N2K

propidium iodide staining




A
** ns ns ** * ** ns ns ns ns ns ns
100
80
60
40
20
0
Percentage (%)
Control
3MB-PP1
1N1K 1N2K 2N2K 2N1K 1N1K 1N2K 2N2K 2N1K 1N1K 1N2K 2N2K 1N1K 1N2K 2N2K
t = 0 h
t = 1.5 h
t = 3.5 h
t = 5.5 h
B
**
**
100
80
60
40
20
0
Percentage (%)
Control
3MB-PP1
2N cells
1 bilobe 2 bilobes 1 bilobe 2 bilobes 1 bilobe 2 bilobes 1 bilobe 2 bilobes
t = 0 h
t = 1.5 h
t = 3.5 h
t = 5.5 h
C
**
**
**
100
80
60
40
20
0
Percentage (%)
Control
3MB-PP1
2N cells
1 FAZ snFAZ 2 FAZ 1 FAZ snFAZ 2 FAZ 1 FAZ sn FAZ 2 FAZ 1 FAZ 2 FAZ
t = 0 h
t = 1.5 h
t = 3.5 h
t = 5.5 h


A
B
C
D