Figure S1. In DYF1Δ cells, the *neo3* cassette is integrated at the expected position within the *DYF1* locus. A. A scheme showing the predicted organization of the wildtype and disrupted *DYF1* loci. The grey boxes show portions of the locus subcloned onto the targeting fragment. Arrows mark the positions of diagnostic primers used for amplification of the junction between *neo3* and the flanking region of the *DYF1* locus that is positioned outside of the targeting fragment. B. A PCR analysis of the genomic DNA of wild-type and DYF1Δ cells using either a pair of primers marked in A or a pair of control primers designed to amplify a non-targeted *NRK1* locus. As expected, a 2.7 kb amplification product is seen in the DYF1Δ sample but not in wild-type cells indicating that the *neo3* cassette is integrated at the desired position.

Figure S2. An extended cell cycle cannot explain hyperglutamylation of axonemal microtubules. A-B3. Wild-type and PF20Δ cells were stained side-by side with the monoclonal anti-polyglutamylated tubulin antibody, ID5 (A) and polyclonal anti-tubulin antibodies SG (A1). A2 shows a merged image. Wildtype cells were fed with black India ink and are identified by phase contrast (A3). B. A growth curve graph of wild-type and PF20Δ grown in MEPP medium. Bars represent 10 μm.

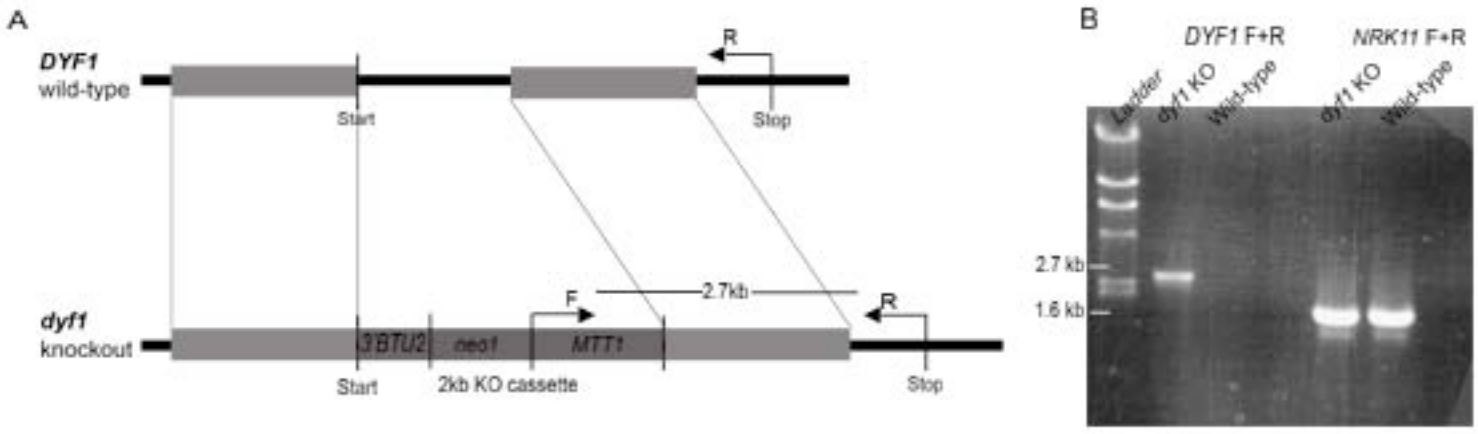
A
DYF1
wild-type
Start
R
Stop
dyf1
knockout
F
2.7kb
R
3'BTU2
neo1
MTT1
Start
2kb KO cassette
Stop
B
DYF1 F+R
NRK11 F+R
Ladder
dyf1 KO
Wild-type
dyf1 KO
Wild-type
2.7 kb
1.6 kb

Figure S1

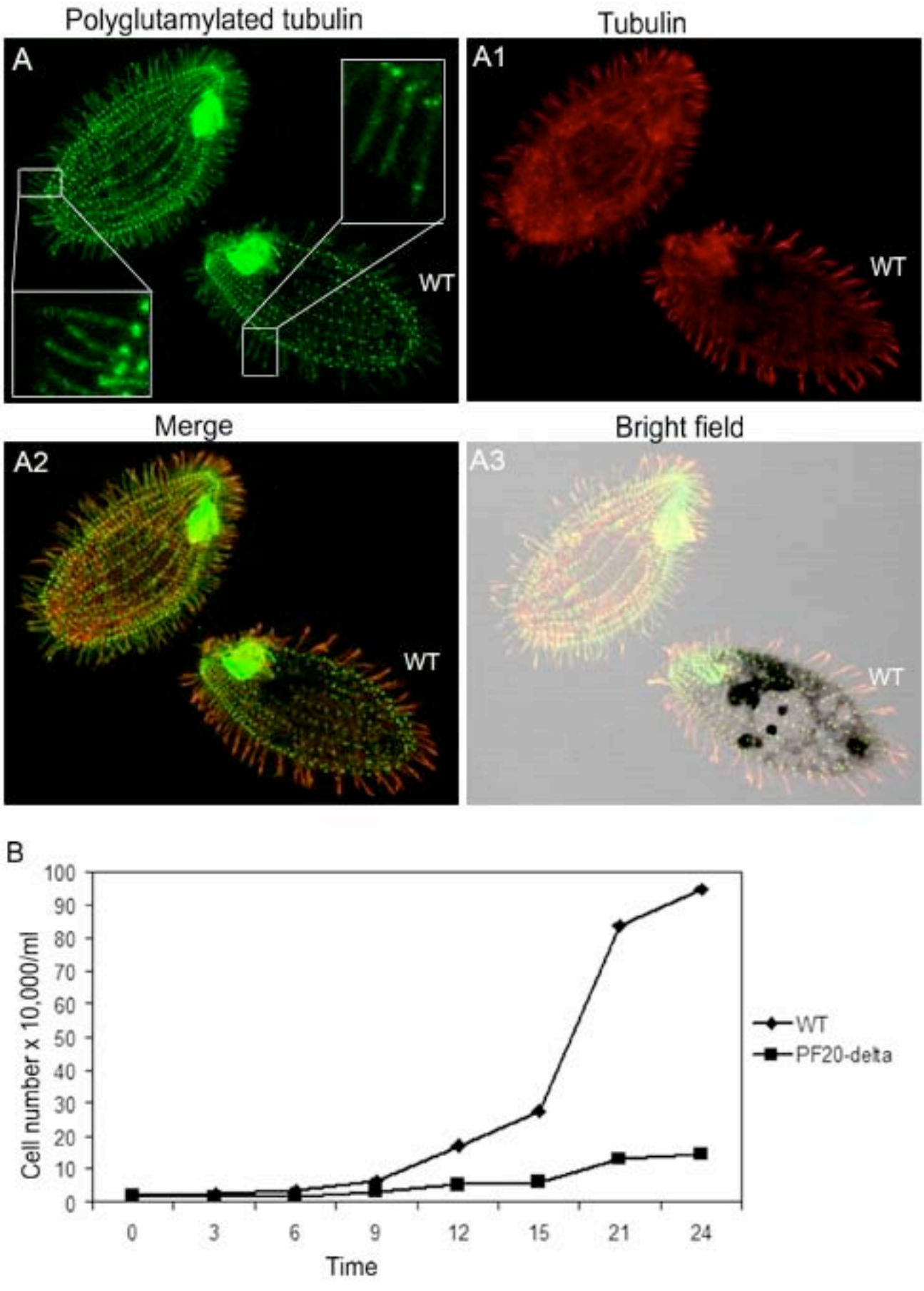
Polyglutamylated tubulin
A
WT
Tubulin
A1
WT
Merge
A2
WT
Bright field
A3
WT
B
Cell number x 10,000/ml
100
90
80
70
60
50
40
30
20
10
0
0
3
6
9
12
15
21
24
Time
WT
PF20-delta

Figure S2