```
                        10        20        30        40        50        60
SpCdc13/1-482   1 MTTRRLTRQHLLANTLGNNDENHPSNHIARAKSSLHSSENSLVNGKKATVSSTNVPKKRHALD 63
GiCycB/1-341    1 MP------------------------------------------------------------ 2

                   70        80        90       100       110       120
SpCdc13/1-482  64 DVSNFHNKEGVPLASKNTNVRHTTASVSTRRALEEKSIIPATDDEPASKKRRQPSVFNSSVPS 126
GiCycB/1-341    3 ----------------------------------------L-DDESQSVHTFEQPV--DNVEE 22

                  130       140       150       160       170       180
SpCdc13/1-482 127 LPQHLSTKSHSVSTHGVDAFHKDQATIPKKLKKDVDERVVSKDIPKLHRDSVESPESQDWDDL 189
GiCycB/1-341   23 MPQALINFRHANP-------------------------------------DKETDILDI 44

                 190       200       210       220       230       240       250
SpCdc13/1-482 190 DAEDWADPLMVSEYVVDIFEYLNELEIETMPSPTYMDRQKELAWKMRGILTDWLIEVHSRFRL 252
GiCycB/1-341   45 DLIDKDDIQACAEYVREIYEYLLQSEGKYMLGSDFLSNQIDFDAEDRTLLVDWISRCHTRFNL 107

                 260       270       280       290       300       310
SpCdc13/1-482 253 LPETLFLAVNIIDRFLSLRVCSLNKLQLVGIAALFIASKYEEVMCPSVQNFVYMADG------ 309
GiCycB/1-341  108 ANESFFHCIAIFDSYLSRVVVPKASNRLVGICCLFIAAKYEQSCIPSLRHFVEALTQVDPEFL 170

                 320       330       340       350       360       370
SpCdc13/1-482 310 ---------------GYDEEEILQAERYILRVLEFNLAYPNPMNFLRRISKADFYDIQTRTVAK 358
GiCycB/1-341  171 VNCKCEGNRSGPCYRCSREAIIDTEESILHTLNFQLAMPTILTFLRRYARISEMKNRDRYIAF 233

                 380       390       400       410       420       430
SpCdc13/1-482 359 YLVEIGLLDHKLLPYPPSQQCAAAMYLAREMLGRGPWNRNLVHYSGYEEYQLISVVKKMINYL 421
GiCycB/1-341  234 YISELSCLTTSIFKFKPSEIAAGCIAMSRRLTRKPTWDETLEKYSGYTESDTKKVMAEISTIM 296

                 450       460       470       480       490       500
SpCdc13/1-482 422 QK----PVQHEAFFKKYAS-KKFMKASLFVRDWIKKNSIPLGDDADEDYTFHKQKRIQHDMKD 479
GiCycB/1-341  297 KKYACNPKAR-YIRRKYSQEDRFYGVSLMIETVVTQ-ALTLN--------------------TK 338
```

**Fig. 1. Sequence alignment of *Giardia* cyclin B with *S. pombe* cdc13.** The ORFs for the mitotic cyclin in *Giardia* (GiCycB) and fission yeast *Schizosaccharomyces pombe* (SpCdc13) were aligned using the Tcoffee program (http://tcoffee.vital-it.ch/cgi-bin/Tcoffee/tcoffee_cgi/index.cgi). Conserved residues are highlighted in blue.

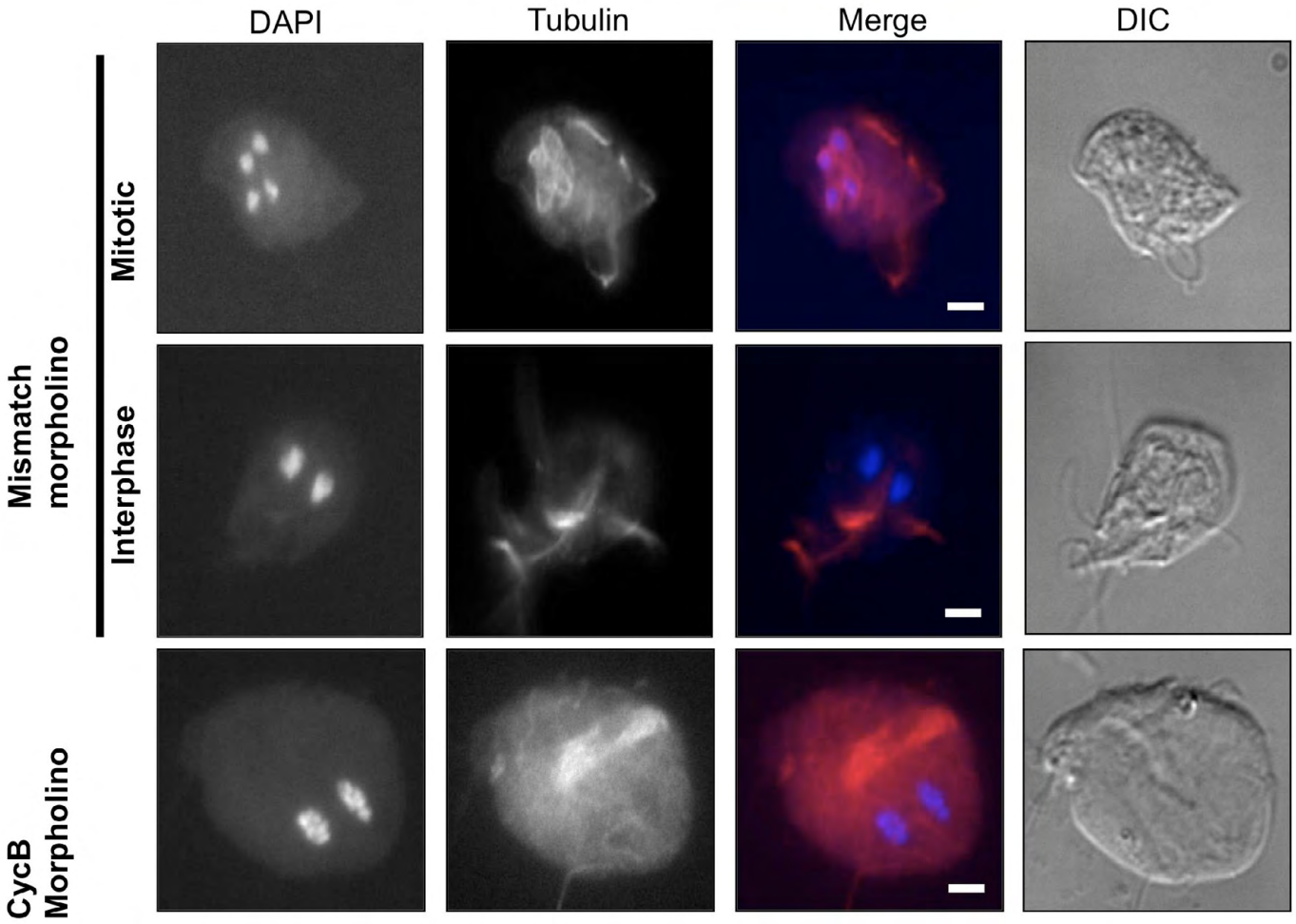



**Fig. S2. No mitotic spindles can be observed in cyclin B-depleted *Giardia* cells.** Control and cyclin B depleted cells were fixed and stained with Tat1 anti-tubulin antibody. DAPI was used to stain nuclei. An example of each of Mitotic, Interphase and cyclin B depleted cells is shown. Bar=2μm

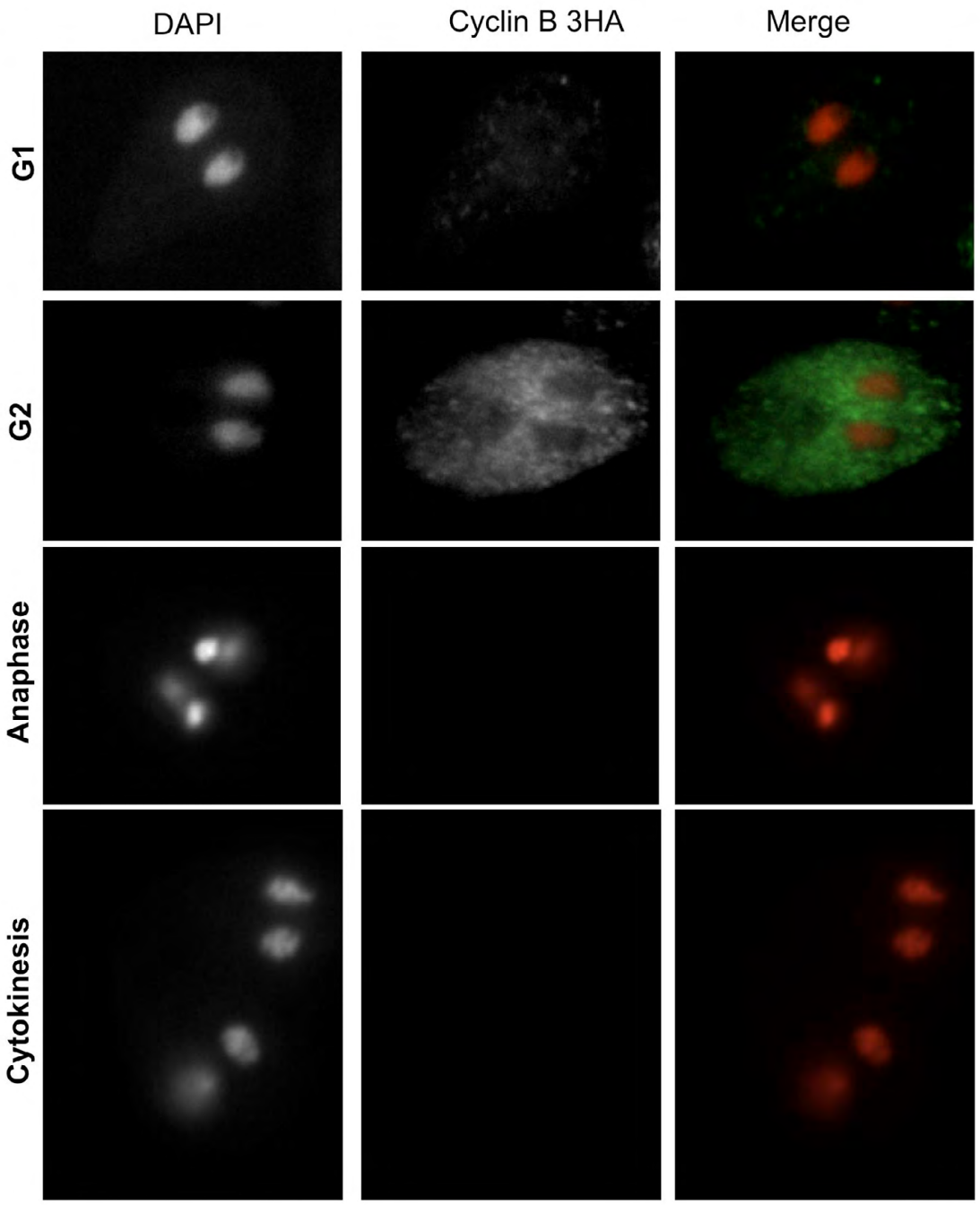
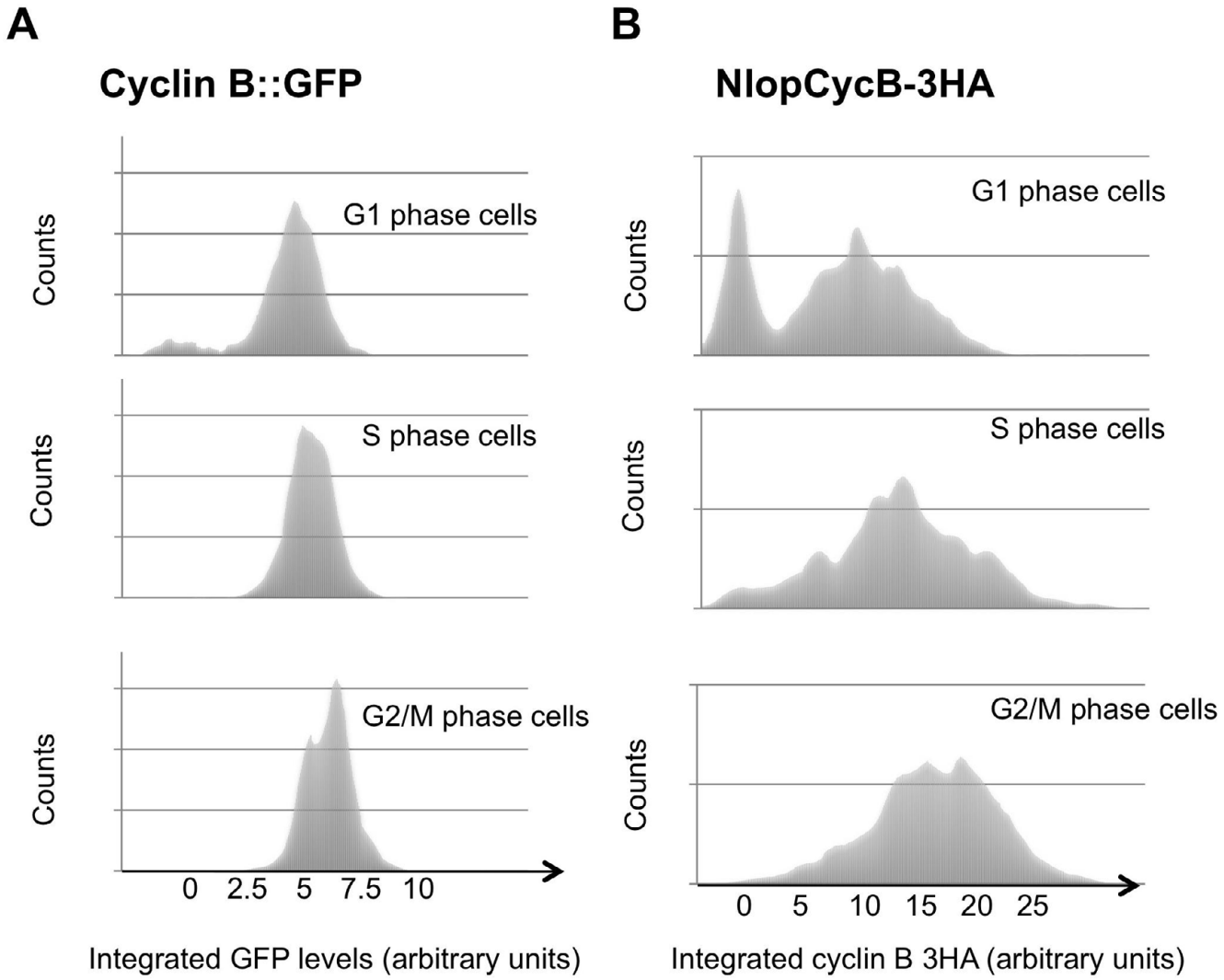



**Fig. S3. *Giardia* cells in anaphase and cytokinesis do not express cyclin B.** Cyclin B 3HA expressing cells were fixed, and stained for cyclin B using the anti-HA antibody and for nuclei using DAPI. An example of G2 and G1 cells (as indicated and determined by their DNA content) are shown to reflect cells expressing or not expressing cyclin B, respectively. Mitotic cells in anaphase and cytokinesis (determined visually by the aspect and position of the nuclei in the cell) were also stained and processed in the same way.




**C**

| Cell type | Mean fluorescence (Arb Units) |
|---|---|
| G1 cells | 6.54 |
| S cells | 6.94 |
| G2/M cells | 7.14 |
| Wt cells, background | 0.00 |

**D**

| Cell type | Mean fluorescence (Arb Units) |
|---|---|
| G1 cells | 4.17 |
| S cells | 10.93 |
| G2/M cells | 12.85 |
| Wt cells, background | 0.00 |

**Fig. S4. Reporter gene assay in *Giardia*. A,** An asynchronous cell population expressing GFP under the control of the cyclin B gene regulatory elements was fixed, stained with DAPI and analyzed using CellProfiler, for their integrated intensity of both DNA and GFP. Cells were categorized into G1, S or G2/M then a histogram for the levels of GFP per cell was plotted using the same scale for all categories.. **B,** Cells lines carrying a tetracycline-inducible cyclin B gene integrated at a separate genetic locus were generated using previously described techniques (Gourguechon and Cande, 2011). Expression of cyclin B was induced using tetracycline (10µg/ml) for two days to allow it to reach a steady state level, then cells were fixed, stained with anti-HA antibody and DAPI and analyzed using CellProfiler to determine cyclin B and DNA staining per cell as described in figure 3. **C** and **D**, Mean fluorescence values were tabulated for each cell type. In all cases, cells not transfected were used to define background levels.