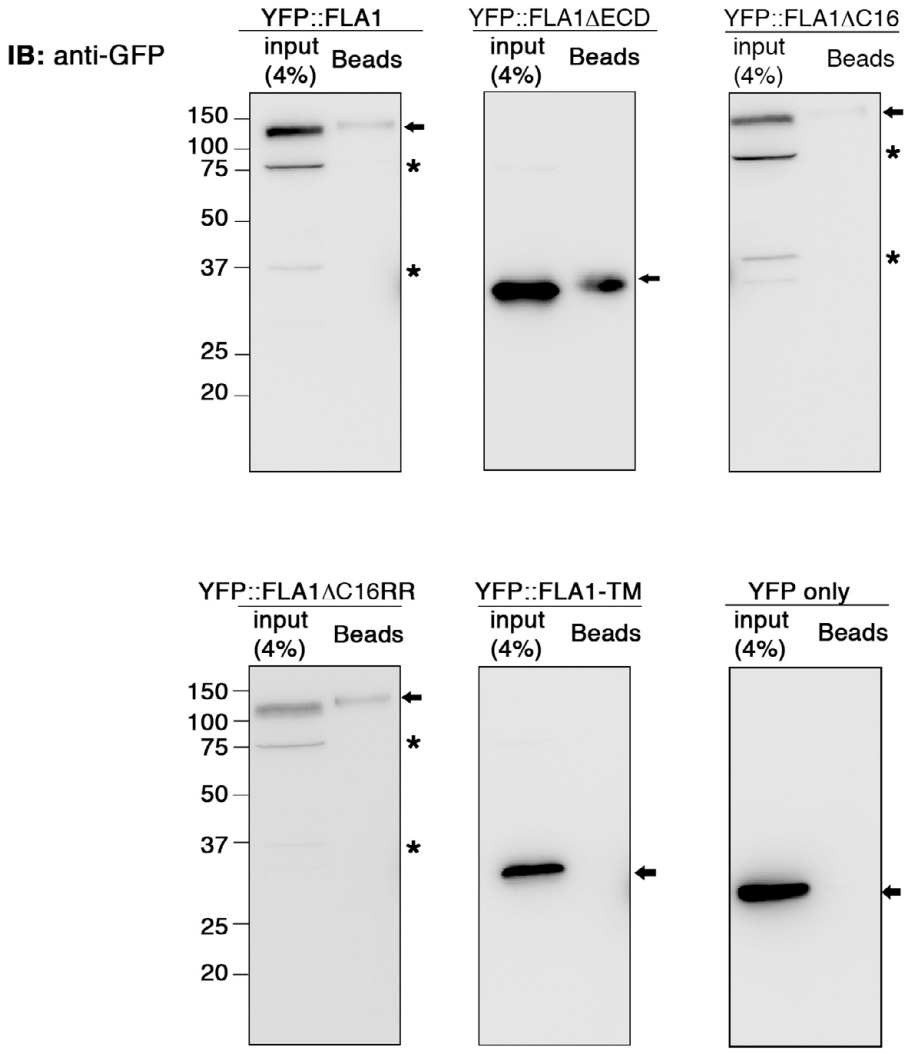

Fig. S1. Surface biotinylation analyses of YFP::FLA1 and truncation mutants. Live cells stably expressing YFP::FLA1, YFP::FLA1ΔECD, YFP::FLA1ΔC16, YFP::FLA1ΔC16RR, YFP::FLA1-TM or YFP alone were surface biotinylated as described in Materials and Methods. Cleared cell lysates were then incubated with streptavidin-coated beads for affinity purification of biotinylated proteins, which were separated on SDS-PAGE and immunoblotted with anti-GFP. Arrows indicate the YFP fusion proteins present in cell lysate input and bound to streptavidin beads. Asterisks mark nonspecific bands labeled by anti-GFP, mostly found in blots that were exposed for longer time to detect the weakly expressed YFP::FLA1, YFP::FLA1ΔC16 and YFP::FLA1ΔC16RR.

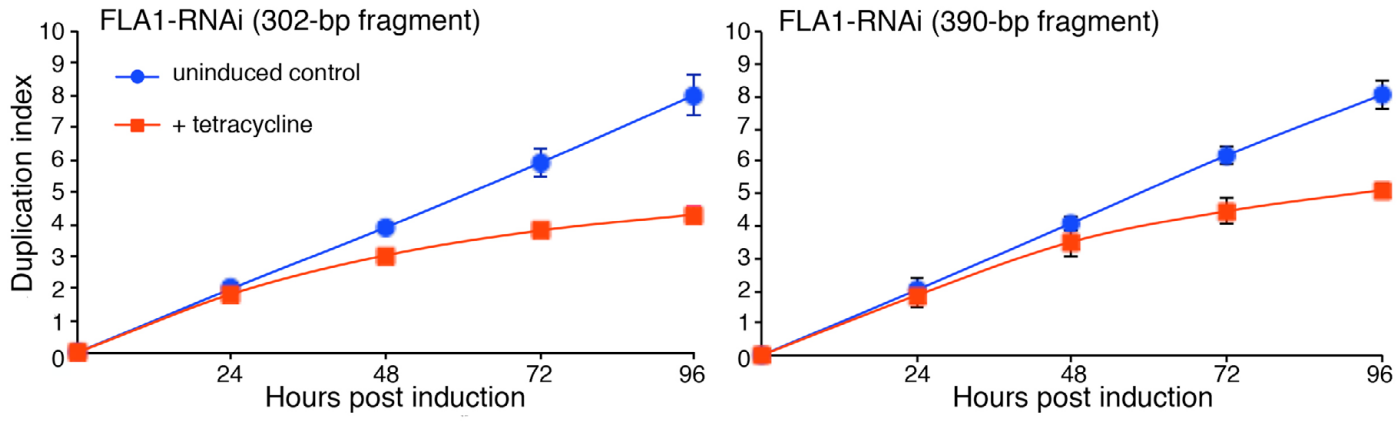

Fig. S2. FLA1-RNAi using two different fragments both lead to inhibited cell duplication. FLA1-RNAi using either a 302-bp fragment (nucleotides 715-1016) or a 390-bp fragment (nucleotides 1364-1641 of the coding sequence and 112 nucleotides in 3′-untranslated region) specific to FLA1 produced similar inhibitory effects on cell proliferation. For the calculation of duplication index, see Materials and Methods.

A

```
FLA1R  1354    CCATGGACCTGGACGCGCATGTTTCTAGAGGAGGTCTCCCAACAGGAACAATGGAGCCAT
FLA1   1354    CCATGGACGTGGACCCGGATGTTCCTTGAAGAACTGTCGCAGCAAGAGCAGTGGAGTCAC
                ProTrpThrTrpThrArgMetPheLeuGluGluLeuSerGlnGlnGluGlnTrpSerHis

FLA1R  1414    CTCTTACCCCTGTGTATGGTCAAATGCGTCGACGACTGTCAACATATAACGTTGGCAGAA
FLA1   1414    CTGTTGCCGCTCTGCATGGTGAAGTGTGTGGATGATTGCCAGCACATTACCTTAGCTGAG
                LeuLeuProLeuCysMetValLysCysValAspAspCysGlnHisIleThrLeuAlaGlu

FLA1R  1474    TCGGTTTGTGGTGACGATTCTCGACTTTCAAGGTGTGATGGAGTCTGCCTGGGAGGAATA
FLA1   1474    TCCGTATGCGGAGATGACGCACGTCTATCTAGATGCGACGGGGTGTGTCTCGGTGGTATC
                SerValCysGlyAspAspAlaArgLeuSerArgCysAspGlyValCysLeuGlyGlyIle

FLA1R  1534    GTATCGTCGGCTCTACTAGGAGCCGTTGCAATAGTTCTACTATTTCTCATGGTCGTTTGA
FLA1   1534    GTTTCCTCCGCACTTCTTGGTGCGGTAGCTATCGTACTTCTTTTCCTGATGGTGGTATGA
                ValSerSerAlaLeuLeuGlyAlaValAlaIleValLeuLeuPheLeuMetValVal *
```

B

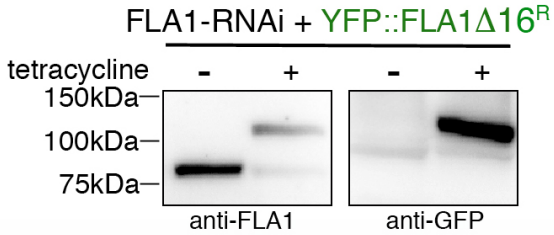

Fig. S3. Sequence comparison of native and RNAi-resistant FLA1 (nucleotides 1360-1590). (**A**) To construct RNAi-resistant FLA1 mutants (denoted with '^{R}'), the native coding sequence was recoded, with changed nucleotides shown in red. The resulting FLA1^{R} sequence encoded the same peptide but was resistant to FLA1-RNAi that targeted the region shown in the blue box. (**B**) Inducible expression of the RNAi-resistant YFP::FLA1ΔC16^{R} in FLA1-RNAi cells was detected by both anti-FLA1 and anti-GFP.

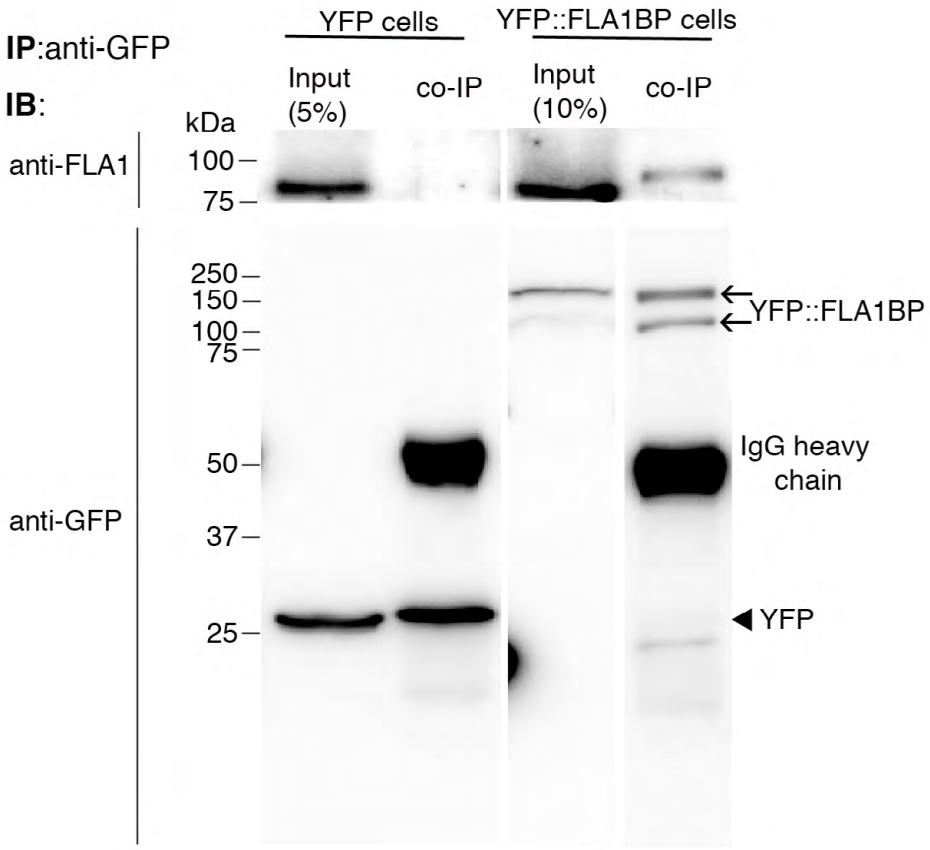

Fig. S4. FLA1 co-immunoprecipitates with YFP::FLA1BP. Cell lysates from parasites stably expressing YFP::FLA1BP or YFP only (negative control) were incubated with Dynabeads conjugated with anti-GFP. Protein eluates were analyzed with immunoblotting analyses using anti-FLA1 and anti-GFP. FLA1 was co-precipitated specifically with YFP::FLA1BP but not YFP only. Note that two bands were recognized by anti-GFP in YFP-FLA1BP expressing cells, consistent with what was observed in Fig. 5.

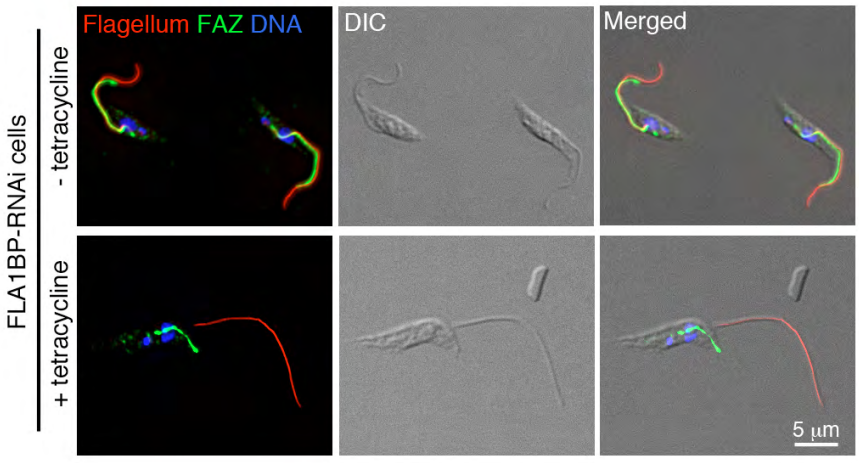

Fig. S5. FAZ1 localizes properly to the truncated FAZ in FLA1BP-depleted cells. FLA1BP-RNAi cells cultured in the absence or presence of tetracycline for 72h were fixed and immuno-labeled with L3B2 for FAZ1 (green), anti-PFR1 for flagellum (red) and DAPI for DNA (blue).