Supplemental Material

Table S1 – oligonucleotides used in this work

Oligonucleotide	**Sequence**
*Tc*CyP19BamHIfow	5’-GGATCCATGTCGTACAAGCCGCATCACG
*Tc*CyP19HAXbaIrev	5’-CCTCTAGATTAAGCGTAATCTGGAACATCG
*Tc*CyP19_199_sgRNA1	5’AAGCTAATACGACTCACTATAGGCTGGCACATGAACT GGGGGATGTTTTAGTACTCTGGAAACAGAATC
REVSaCas_scaffold_WDR	5’AAAAAAATCTCGCCA ACAAGTTGAC
FOW5'UTR _sgRNA1_*Tc*CyP19	5’TCTGTAACATTTTTCTCAAGAGTAGCTCAGTGAGCATT TTACATCTTTCTAAAAGGGAATTAATTTATTAACAAC ACACAATGGCCAAGCCTTTGTCTCA
REVpos-PAM_sgRNA1_TcCyP19	5’GCCTTGCCAGCAAAGGACTCGTCGGCAAATTTCTCAC CGTAGATCGACCTGCCGCCAGTGCCGTTGTGATTGGT GAAGTCTTAGCCCTCCCACACATAAC
(P1) – Check Fow 5’UTR Cyp19	5’ TCCTTCATGTAAGCCTGCGT
(P2) - Cyp19 Rev Xba	5’TCTAGATTAAAGTTGACCAGAGGCC
(P3) –BSD Fow SgRNA1	5’GGGAATTAATTTATTAACAACACACAATGGCCAAGCC TTTGTCTCA

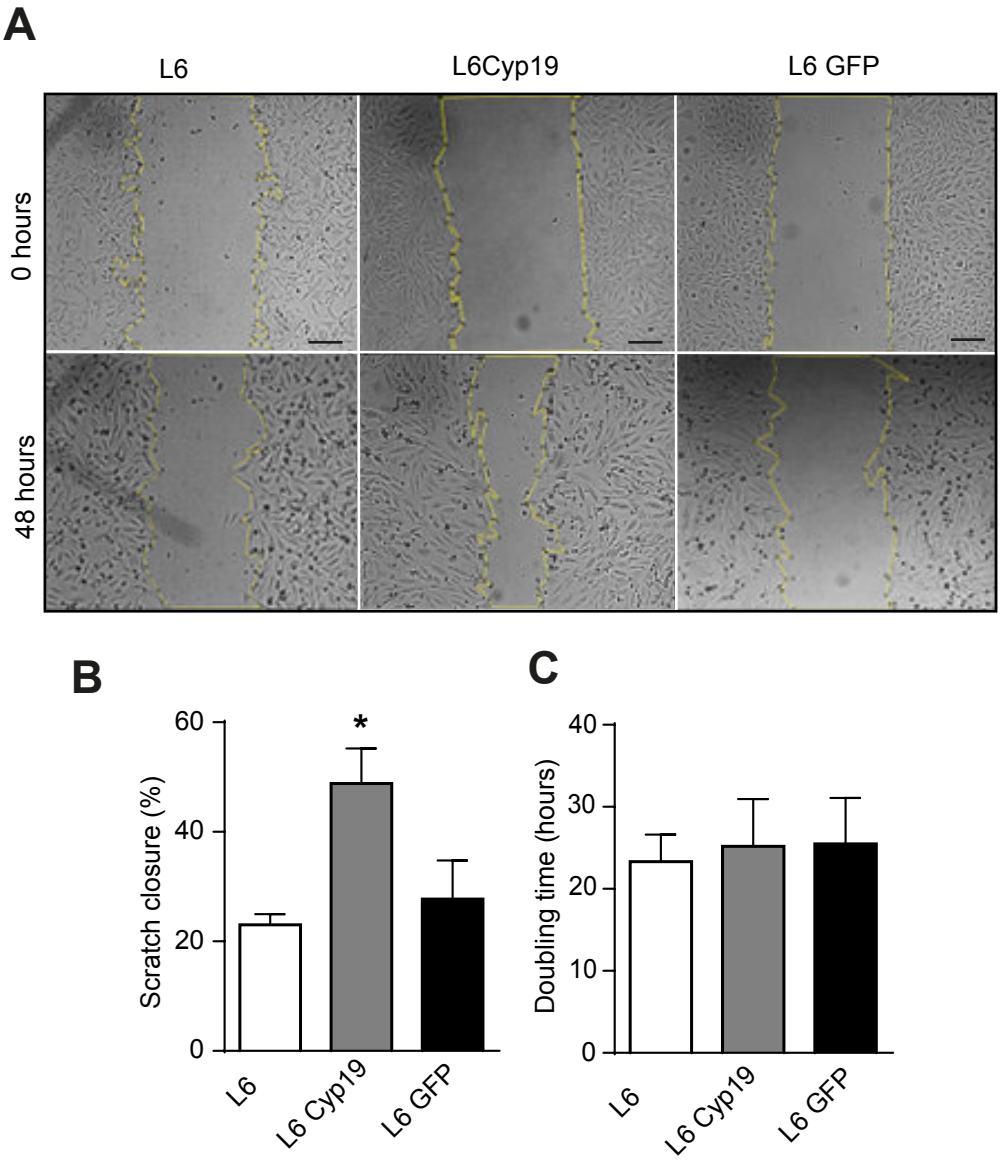
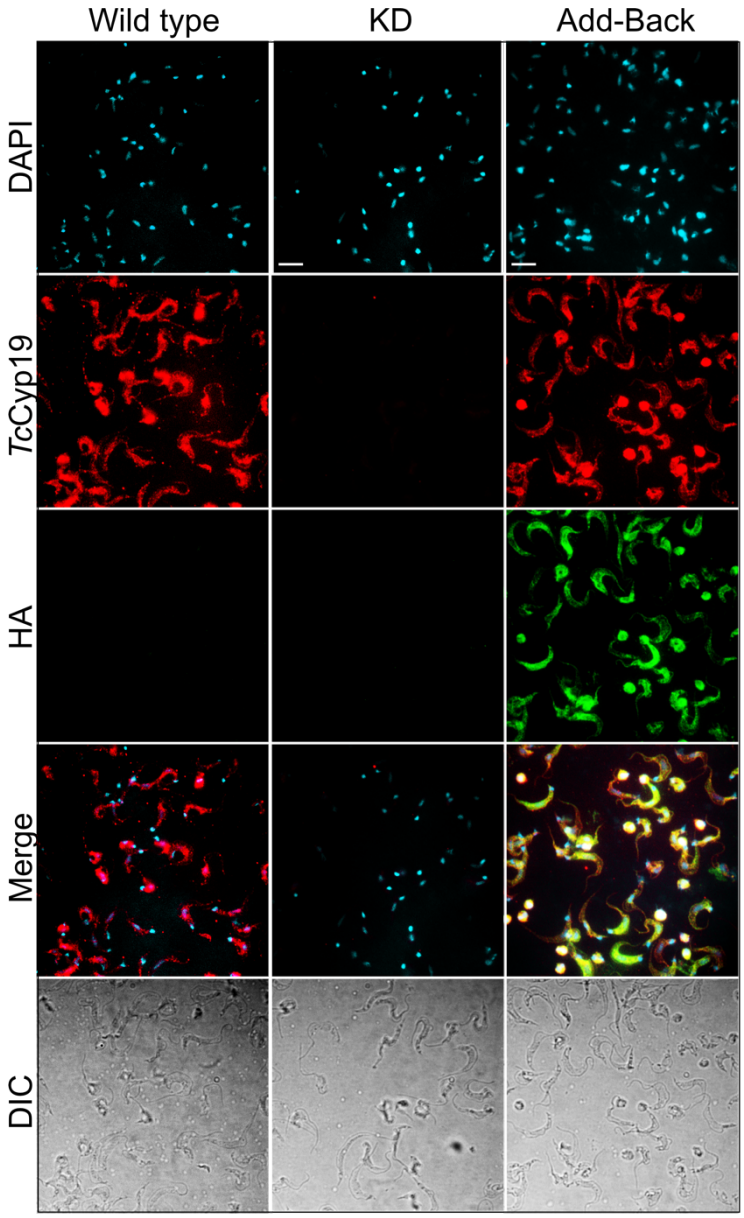


Fig. S1. Effect of *Tc*Cyp19 expression on cell migration and replication.
(A) Images of the indicated cells before scraping, and 48 hours after scraping. Yellow lines mark the beginning of the determined wound area. Bars = 300 μm. (B) Percentage of the closed area by the cells in each case by using ImageJ software (version 2.0). The bars are means of triplicate ± standard deviation and the asterisk indicates significant p value = 0.0035, calculated by two-way ANOVA analysis with Dunnett's multiple comparison tests between the migration rate L6 CyP19-HA compared to L6 or L6 GFP. (C) The graph indicates the cells doubling time of means of three independent experiments, each done in triplicates. There is no significant difference comparing the different cell lines.



Supplementary 2. Immunofluorescence of *Tc*Cyp19 depleted and *Tc*Cyp19-HA add-back trypomastigotes. A) Parasites were adsorbed to glass slides, fixed, permeabilized, and incubated with anti-*Tc*Cyp19 (red), anti-HA (green) antibodies, and DAPI (blue). The images show individual labeling, the fluorescence signals merged and differential interference contrast (DIC). Bars = 5 μm.

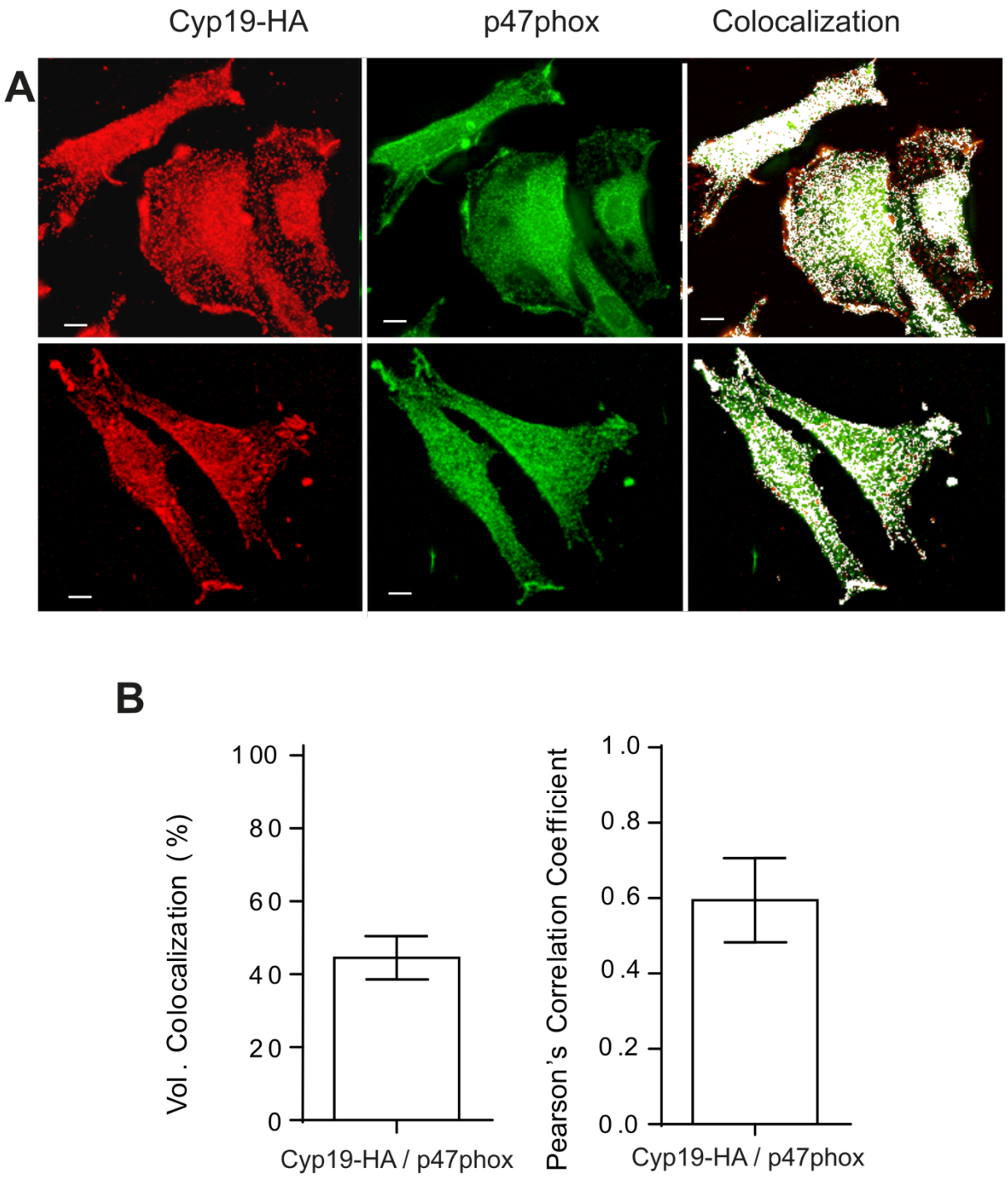


Fig. S3 Colocalization analysis of *Tc*Cyp19-HA and p47^{phox} in L6 myoblasts. (A) Representative immunofluorescence images obtained by staining L6 expressing *Tc*Cyp19-HA with anti-HA (red) and anti-p47^{phox} (green) antibodies. The images show individual labeling and the colocalization signals (white). Bars = 5 µm. (B) Percentage of the volume quantified to be the area of colocalization from *Tc*Cyp19-HA and p47^{phox} labels and the Person's Correlation Coefficient (n=5) performed using ImageJ software.

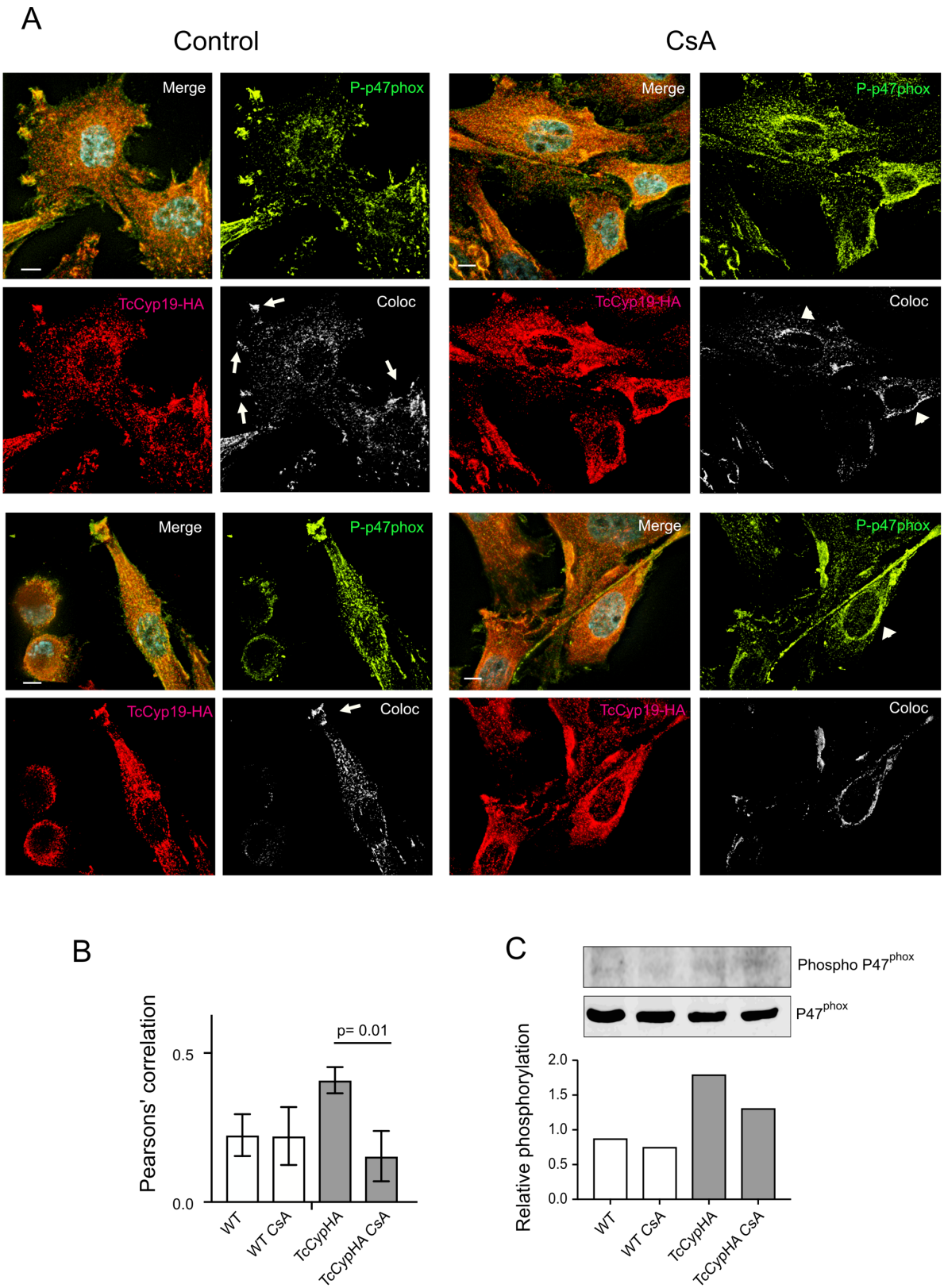


Fig. S4 Phosphorylated p47^{phox} concentration in the cell leading edges and colocalization with *Tc*Cyp19 is affected by CsA. (A) L6 cells expressing *Tc*Cyp19-HA platted on round glass coverslips were incubated 24 hr in cDMEM in absence (Control) or presence of 20 μM CsA. The cells were then fixed, permeabilized and probed with anti-phospho-p47^{phox} (green) and anti-HA (red). The panels illustrate two

fields showing the merged fluorescent signals and the colocalization (white) images. The arrows show the presence of intense colocalization in the migrating edges in the control and arrowheads the colocalization surrounding the cell nucleus. (B) Person's correlation coefficient of 6 different microscope fields containing between 3 and 6 cells each. The values correspond to the mean values and standard deviations. The p value was calculated using Two-way ANOVA with Tukey post-test. (C) Western blot of the same samples probed with antibodies to phospho $p47^{phox}$, and total $p47^{phox}$ with the graph indicating the relative levels of phosphorylation.

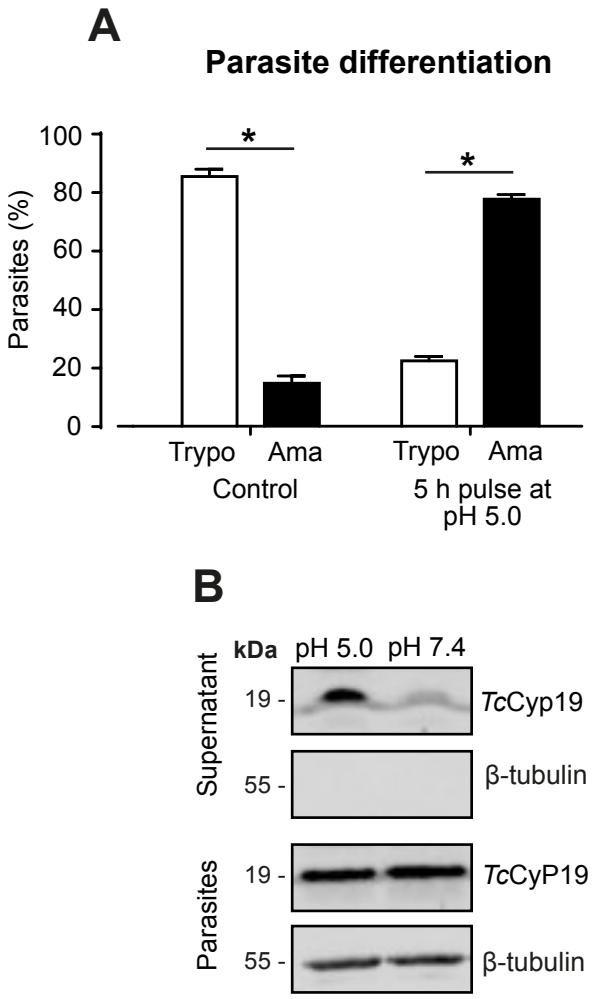


Fig. S5 Trypomastigotes secrete *Tc*CyP19 when induced to differentiate into amastigotes. (A) Tissue culture trypomastigotes were diluted in cDMEM at the indicated conditions for 5 hr and then for another 19 hr, as described in Methods. The percentage of trypomastigotes and axenic amastigotes were then microscopically quantified in each incubation. The data represent the means ± standard deviation of three independent experiments each determined in triplicate measurements. Asterisks indicate statistically significant differences ($p < 0.05$) based on Multiple t-tests, Sidak method was applied. (B) Incubated parasites ($1x10^7$) and the 70 µL of supernatant collected from the last incubation of 24 hr were used to detect *Tc*CyP19 presence in the samples in Western blot probed with anti-*Tc*CyP19 and anti-β tubulin antibodies as loading control.