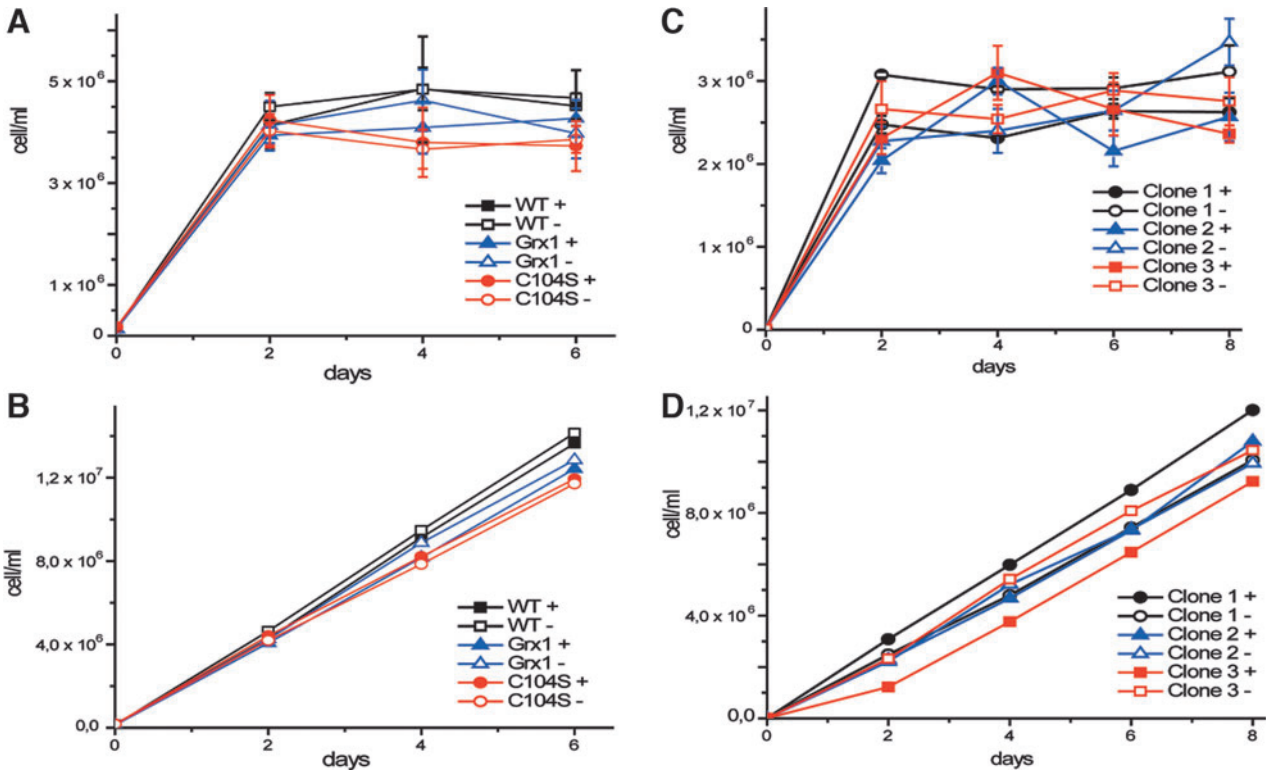
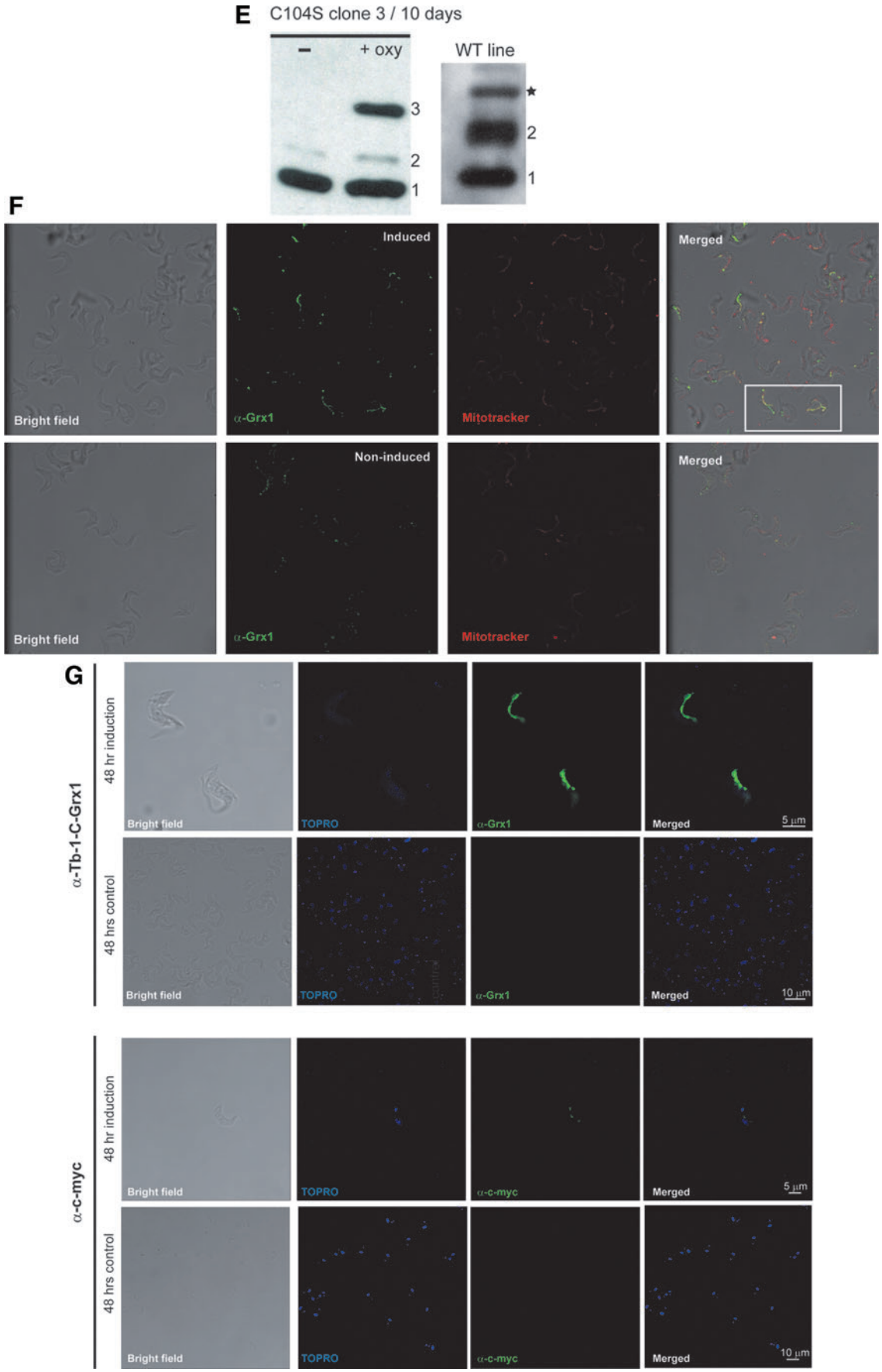


SUPPLEMENTARY FIG. S5. Phenotypic analysis of infective trypanosomes overexpressing C104S *Tb*1-C-Grx1. (A, B) Comparative growth of *T. brucei* parental WT strain and cell lines for inducible overexpression of WT (Grx1) and C104S mutant (C104S) of *Tb*1-C-Grx1 (3) in the presence (+) and absence (−) of 10 μg/ml oxytet. Cells were inoculated at 10^5 cells/ml in a culture medium containing or not oxytet, counted every 48 h, and diluted to the initial cell density with replenishment of inducer every 24 h. Cell growth **(A)** and cumulative cell density **(B)** are shown. **(C, D)** Growth curves of clones 1, 2, and 3 from cell line C104S. Cells were inoculated at 10^4 cells/ml in a culture medium with (+) or without (−) 10 μg/ml oxytet, counted every 48 h, and seeded at the same density with replenishment of an inducer every 24 h. Cell growth **(C)** and the cumulative cell density **(D)** are shown. Error bars in *panel* **(A)** and **(C)** represent the standard deviations from the mean corresponding to four independent measurements. The error bars in **(B)** and **(D)** are omitted for better visualization. The differences in the growth rates between induced and noninduced trypanosomes were not statistically significant (two-tailed Student's *t*-test). **(E)** Representative Western blot showing the expression levels of the ectopic copy of C104S in the cell line used for the infection experiment (clone 3; Fig. 10A, B) under induced (10 days of continued induction with 10 μg/ml oxytet; +oxy) and noninduced conditions (−). Total cell extracts from 6×10^6 parasites were loaded per lane on an SDS-12% PAGE, and the blot was revealed as described in Figure 9. Similar results were obtained for all three clones and longer induction times (up to 28 days, not shown). The *right panel* shows a Western blot from the WT parental strain treated with 10 μg/ml oxytet for 48 h. Total cell extracts from 6×10^6 cells were loaded per lane on an SDS-15% PAGE, and the immunoblot conditions adjusted to allow detection of endogenous *Tb*1-C-Grx1 while avoiding saturation for TXN signal (e.g., guinea pig serum α-*Tb*1-C-Grx1: 1/500, goat HRP-conjugated serum anti-guinea pig IgG: 1/5,000, and rabbit serum α-TXN: 1/10,000). The star indicates a nonspecific reaction of the α-*Tb*1-C-Grx1 antibodies that has been previously reported (3). **(F)** Subcellular localization of C104S *Tb*1-C-Grx1 in bloodstream parasites. The immunofluorescence images correspond to cells from clone 3 induced for 48 h with 10 μg/ml oxytet (*upper panel*) or noninduced (*lower panel*) and prepared as described in the Materials and Methods section (main text). From left to right: (i) bright field, (ii) α-guinea pig Alexa-Fluor^{488} signal corresponding to samples incubated with purified guinea pig serum α-*Tb*1-C-Grx1 (green label), (iii) the mitochondrial marker Mitotracker® (Molecular Probes; red label), and (iv) merge image. The image shown in Figure 9B is highlighted with a white box. **(G)** In *both panels*, the upper and lower image strips show photographs from 48-h induced and noninduced (control) cultures. From left to right: (i) bright-field image, (ii) TOPRO staining of nucleic acids (blue), (iii) α-guinea pig Alexa-Fluor^{488} signal from samples incubated with purified guinea pig serum α-*Tb*1-C-Grx1 (*upper panel*; green label) or mouse monoclonal antibodies (clone 9E10) α-C-myc (*lower panel*; green label), and (iv) merge images with scale bars. oxytet, oxytetracycline.



SUPPLEMENTARY FIG. S5. (*Continued*)