Supplementary information for:

The single ENTH domain protein of trypanosomes; functional and evolutionary relationship with the epsins

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Legends to supplementary figures and tables

Table S1: Accessions for Epsin and EpsinR candidates retrieved from various databases and as used in the phylogenetic analysis. See methods for sources of these data.

Figure S1: Phylogenetic analysis of Epsin/EpsinR families. The topologies generated from three different algorithms are shown; A; MrBayes, B; RaxML and C; PhyML. In each case numbers against the internodes indicate statistical support, either posterior probability (A) or bootstrap values (B and C).

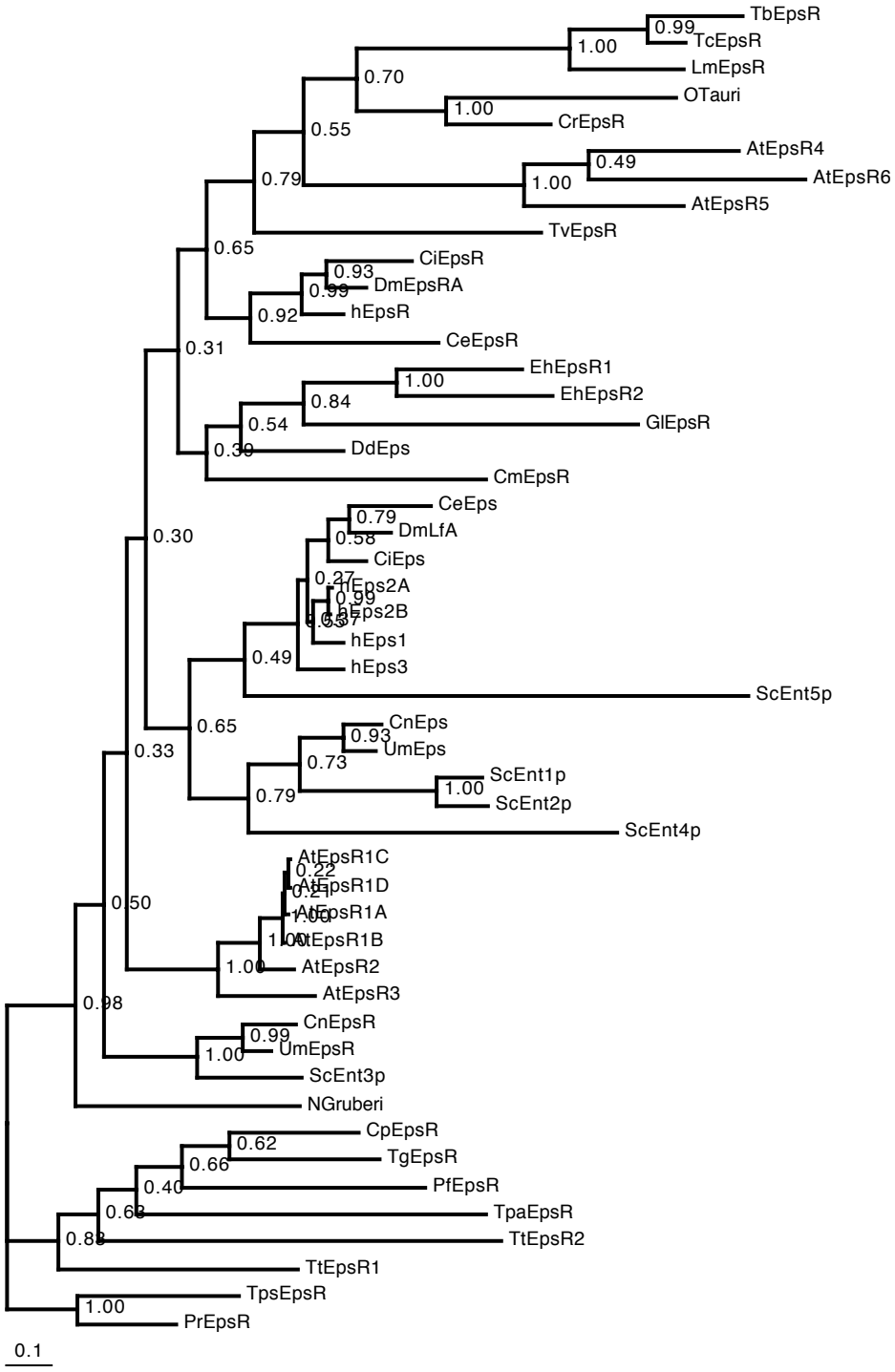
Figure S2: TbEpsinR antibody verification. BSF cell lysate and purified recombinant GST-TbEpsinR from *E.coli* were fractionated by SDS-PAGE in duplicate, and analysed by Western blotting with anti-TbEpsinR antibody (left panels), or anti-TbEpsinR antibody pre-incubated with recombinant purified GST-TbEpsinR (right panels). Note that the signal is lost when the antibody is preincubated with the recombinant protein.

Figure S3: Absence of morphological abnormalities following TbRab5A RNAi. Cells were induced for RNAi of TbRab5A as described in methods. Cells were prepared for thin section trasmission EM as descibed (25). Scale bars are 2um (panel A) and 5um (panels B - D). Apart from the apparent compression of internal membrane-bound compartments, there is no evidence for proliferation, enlargement or other abnormal endosomal structures under these conditions.

Figure S4: TbEpsinR expression levels in TbRab5A RNAi cells. TbRab5A RNAi was induded in cells with 1μg ml^{-1} tetracycline for 24h and whole cell lysates analyzed by Western blotting for TbEpsinR and TbRab5A. TbBiP was used as a loading control. Histograms show quantitation of total protein, filled bars are expression levels in uninduced cells and open bars levels in induced cells.

Figure S5: Cell-cycle analysis of TbEpsinR knockdown cells. p2T7•TbEpsinR cells were induced for 24h with tetracycline, fixed with 4% paraformaldehyde and stained with DAPI. Panel A: To determine position in the cell-cycle, the numbers of nuclei and kinetoplasts per cell were counted for at least 200 cells for uninduced and induced cultures. Panel B: Light microscopy was used to determine incidence of cells with abnormal morphologies and cells presenting the Big Eye phenotype; at least 200 cells were counted.

Figure S6: TbEpsinR protein copy number. Lysates of 10^7 BSF cells, and aliquots of purified GST-TbEpsinR equivalent to 10^{11}, 10^{12} and 10^{13} copies of recombinant protein, estimated based on protein concentration, were analyzed by Western blotting with anti-TbEpsinR antibody. The TbEpsinR copy number in BSF was estimated to be between 10^4 and 10^5 copies per cell.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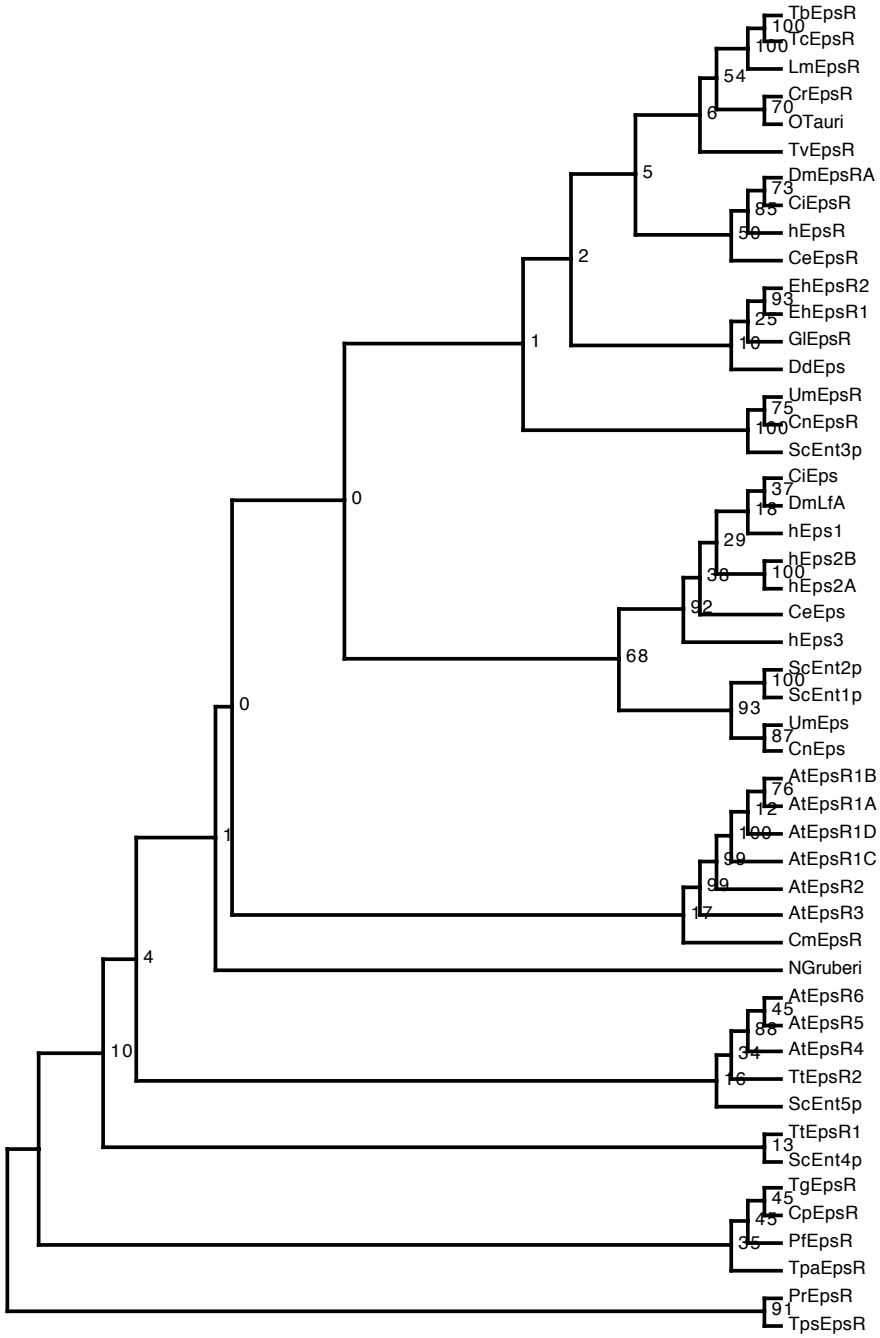
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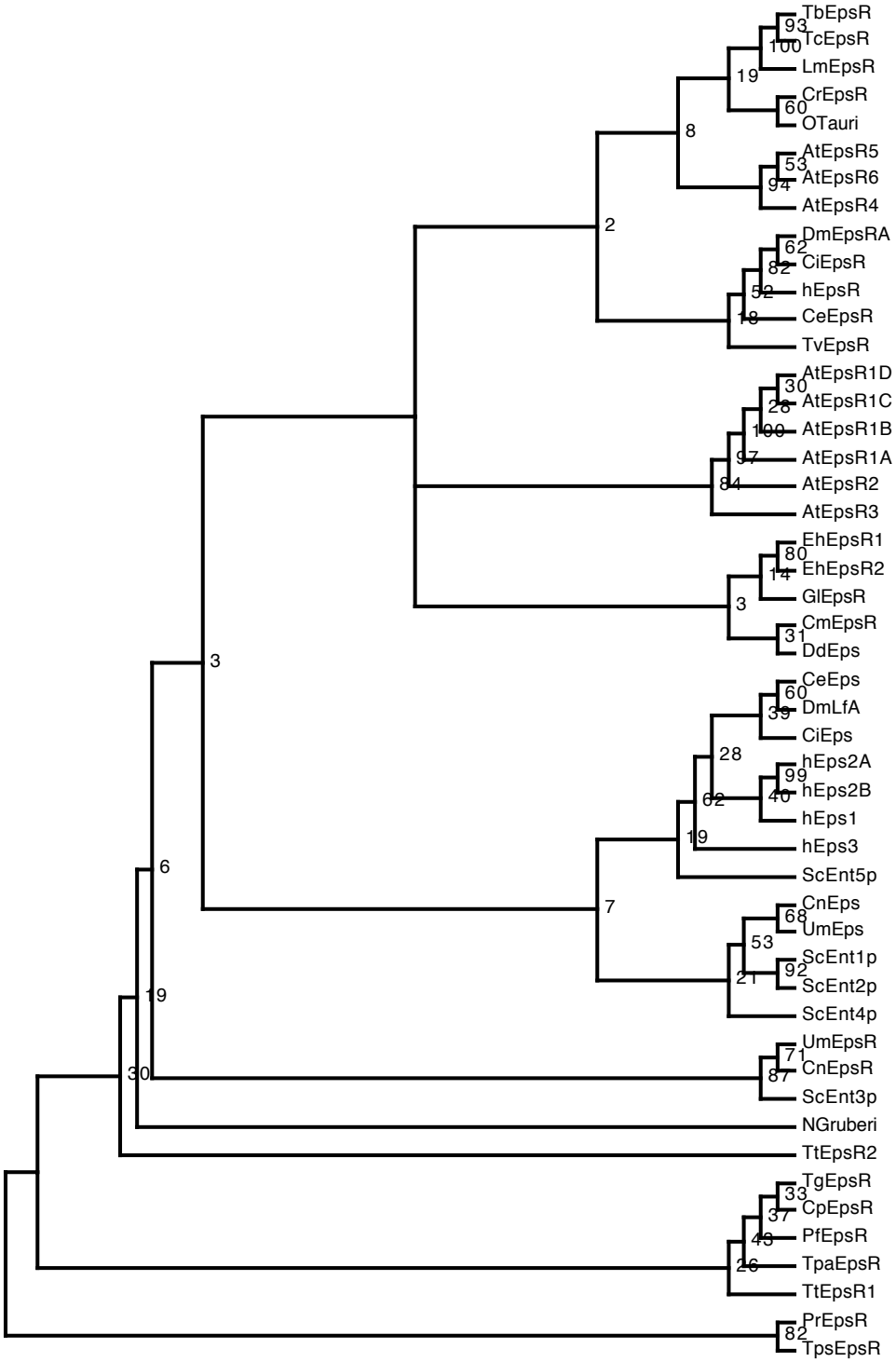
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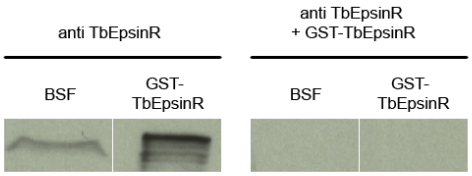
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GST-
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GST-
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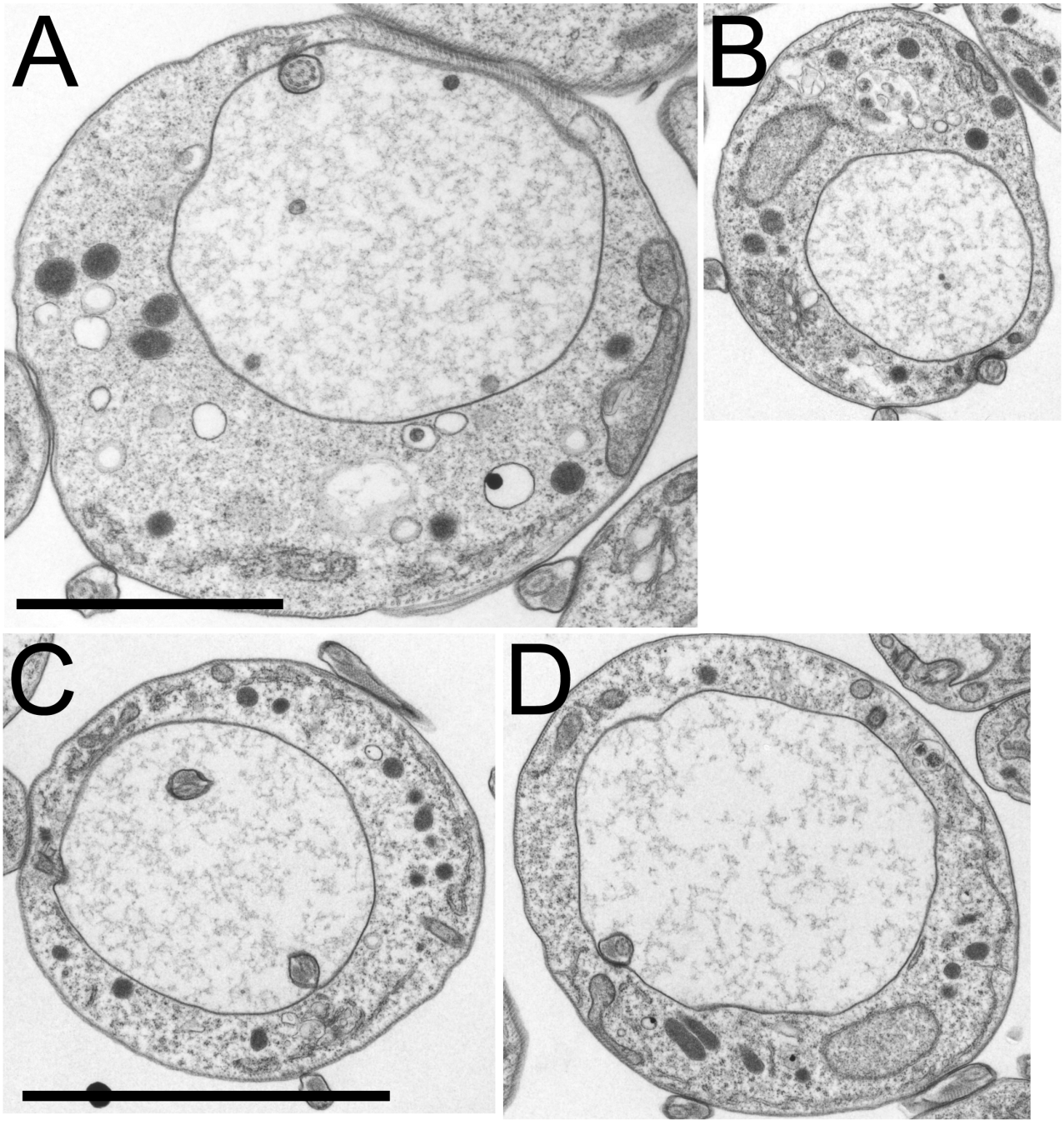
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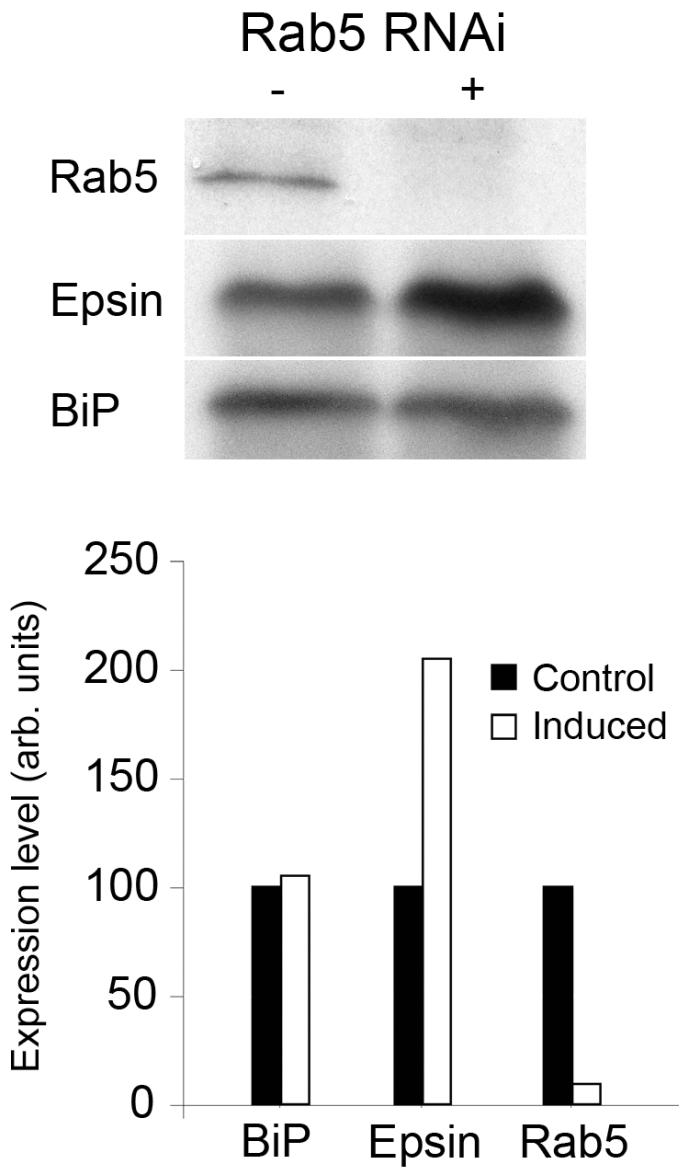
B
C
D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+
Rab5
Epsin
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Induced
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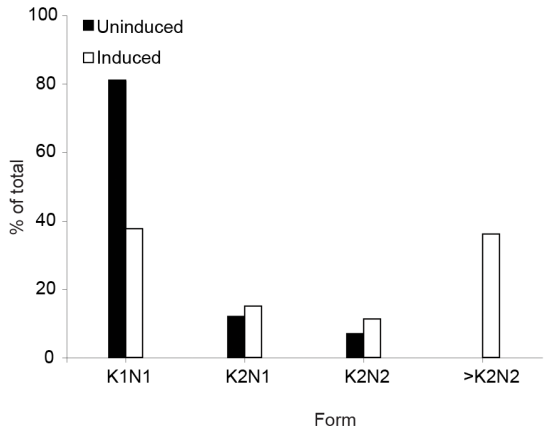
Epsin
Rab5

A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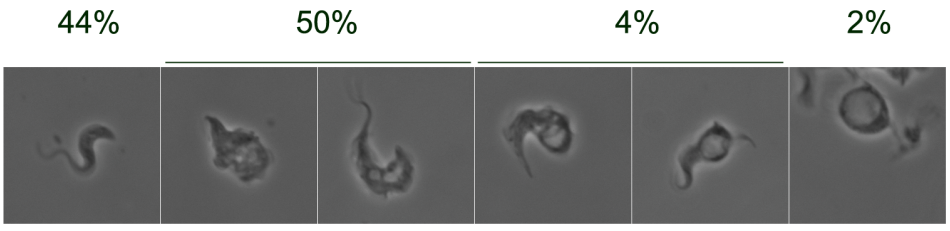
Form

B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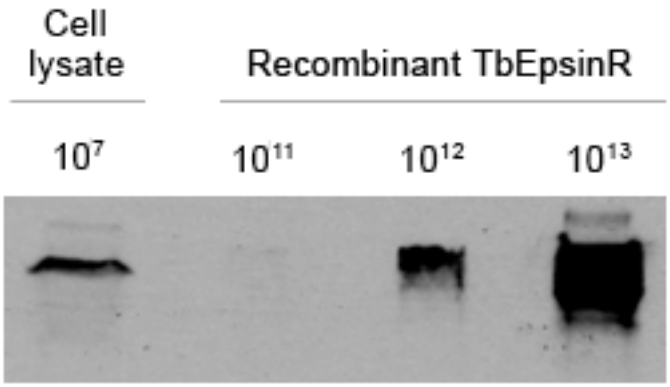
Cell lysate
Recombinant TbEpsinR
10^7
10^{11}
10^{12}
10^{13}