SUPPLEMENTARY MATERIAL

Late endosomal Rab7 regulates lysosomal trafficking of endocytic but not biosynthetic cargo in *Trypanosoma brucei*

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Running Title: Rab7 and late endosomes in African trypanosomes

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Figure S1. Expression of Ty-tagged TbRab7 in BSF trypanosomes. The TbRab7:Ty reporter was constitutively expressed in cultured BSF trypanosomes as described in Experimental Procedures. As indicated extracts of the transgenic TbRab7:Ty and parental untransformed cells were fractionated by SDS-PAGE (10^7 cell equivalents/lane) and subjected to immunoblotting with anti-Ty antibody (left panel). The membrane was then stripped and reblotted with anti-HSP70 as a loading control (right panel).

Figure S2. Immunofluorescence localization of TbRab7 in PCF trypanosomes. PCF trypanosomes were formaldehyde-fixed and NP-40 permeabilized as described in (Sevova & Bangs, 2009). Cells were stained with rat anti-TbRab7 (green) and mouse anti-p67 (red). Costaining with DAPI was done to reveal the nucleus (n) and kinetoplast (k). Three representative 3-channel summed stack projections of deconvolved z series are presented. The TbRab7$^+$ late endosome (l.e.) and the p67$^+$ lysosome (ly) are indicated (left panel only). Cell outlines were traced from matched DIC images.

Figure S3. Effect of TbRab7 silencing in BSF trypanosomes. The BSF TbRab7 RNAi cell line was cultured without (A) or with (B) tetracycline to induce specific knockdown. Cells were then MeOH/acetone fixed and stained with rat anti-TbRab7 (green) and mouse anti-p67 (red).

Co-staining with DAPI was done to reveal the nucleus (n) and kinetoplast (k). Representative 3-channnel epifuourescence images are presented. The images were acquired at identical exposure times, were not deconvolved, and were contrast enhanced identically. The TbRab7^{+} late endosome (l.e.) and the p67^{+} lysosome (ly) are indicated (left panels only). Cell outlines were traced from matched DIC images.

Figure S4. Effect of TbRab7 silencing on lysosomal ultrastructure in BSF trypanosomes. The BSF TbRab7 RNAi cell line was pulse loaded (2 hr, 37ºC) with TL:colloidal gold (15 nm) and then re-cultured for 28 hours without (A) or with (B & C) tetracycline to induce specific knockdown. Cells were fixed and stained for electron microscopy as described in Experimental Procedures. The region of the gold-containing lysosome (white squares) is enlarged in the inserted images. Black bars, 1.0 μm; white bars, 0.2 μm. Nuclei (n), kinetoplasts (k) and flagellar pocket (fp) are indicated as appropriate.

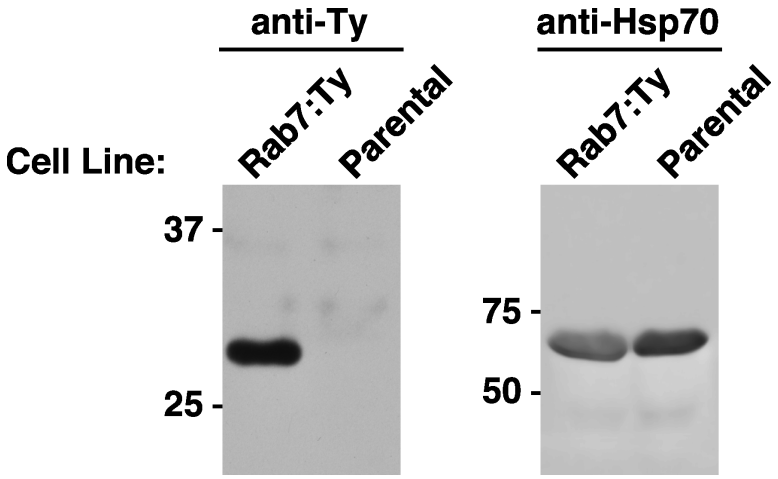

Figure S1

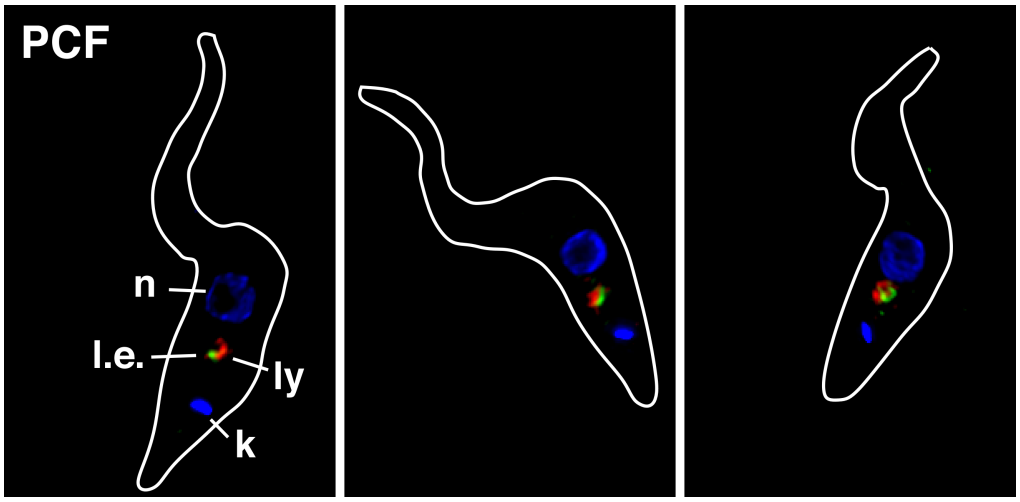

Figure S2

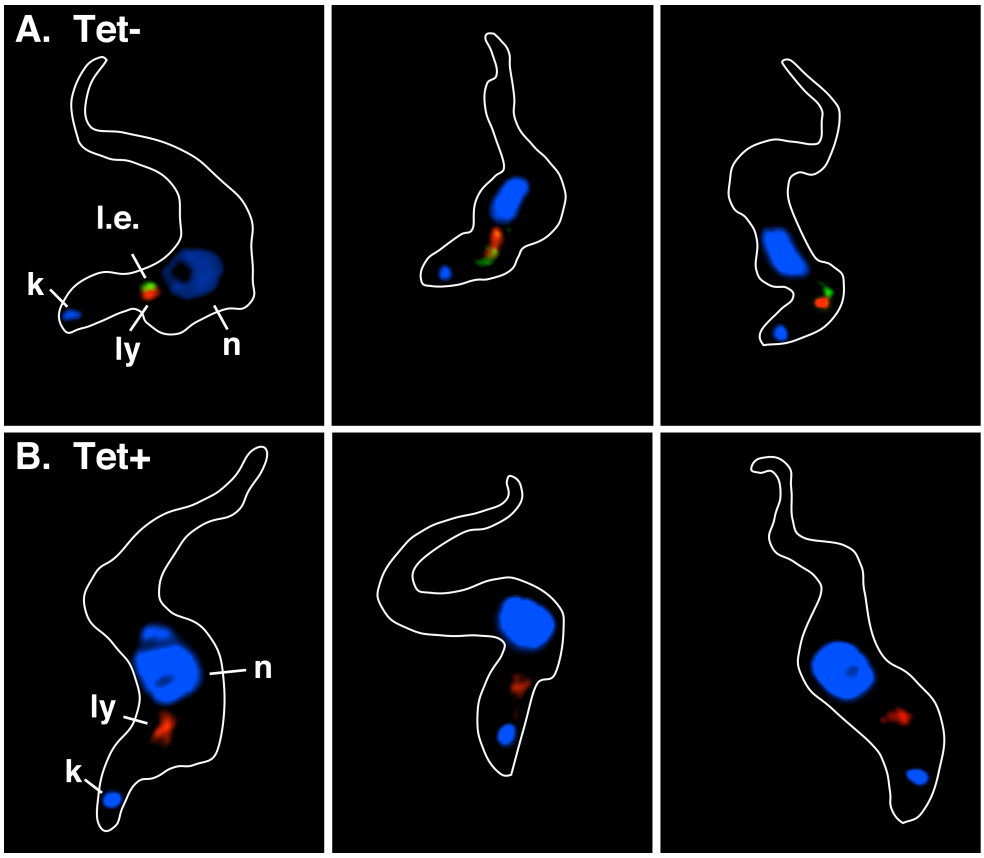

Figure S3

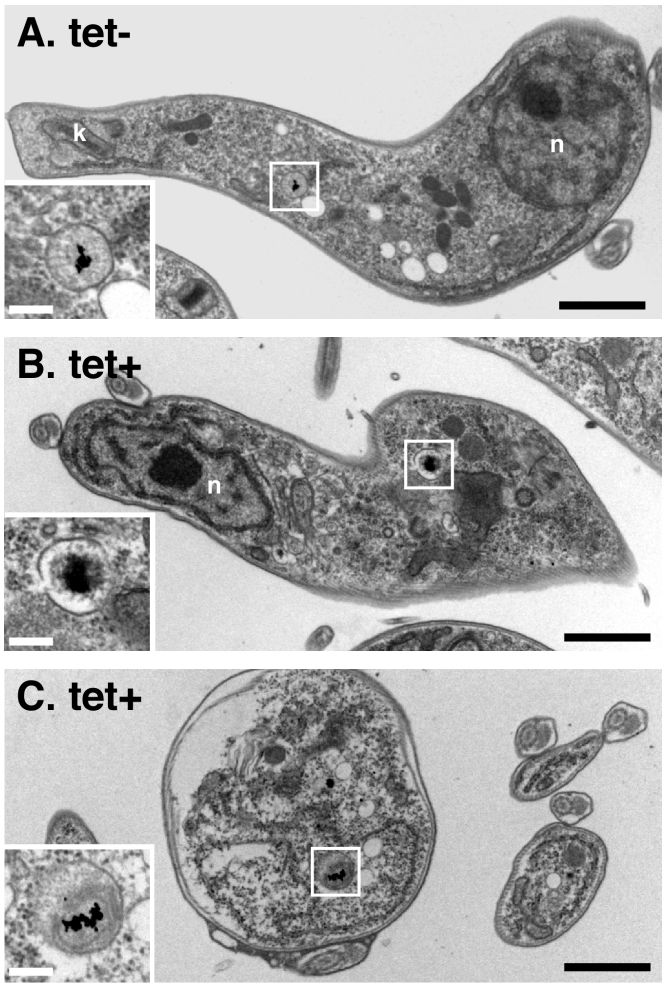

Figure S4